Transcriptomic and Proteomic Analysis of Lycopene-Overproducing *Escherichia coli* Strains

by

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ABSTRACT

Systems biology represents a powerful method to describe and manipulate phenotypes of interest by incorporating biological information from various levels of cellular organization. Such an approach is illustrated from a library of both rationally-directed and combinatorial gene knockout strains of *E. coli* recombinantly producing the small molecule lycopene. Global genomic and proteomic expression changes associated with increased lycopene production of mutant *E. coli* constructs were discovered using whole-genome DNA microarrays and a novel LC-MS technique, respectively. While most genes and proteins showed few expression changes, key differences were identified, including targets distal to the non-mevalonate and precursor-supplying pathways. Based upon the expression data sets, it was hypothesized that the following may be associated with lycopene overproduction: histidine biosynthesis (*hisH*); the quinone pool (*wrbA*); acid resistance (*ydeO* and *gadE*); the glyoxylate pathway (*iclR*); NADPH redox balance (*pntB*); growth rate reduction; and membrane composition. In the pre-engineered background strain, deleting *pntB* (~20-25%) and *ydeO* (~30%) each led to moderately increased production; overexpressing *wrbA* led to 50-100% more production at 8 hours and 5-15% more production at later time points; deleting *iclR* caused small production increases (~5-10%); and supplementing media with histidine caused the parental and mutant strains to have similar production.

From these observations, several themes emerged. First, reduced cellular growth and energy conservation appear to be important tradeoffs for increasing lycopene production. Second, reducing overflow metabolism to acetate and corresponding acid stress as well as providing a gluconeogenic flux to increase lycopene precursors appeared beneficial. Next, NADPH availability and balance seemed to be critical production factors. The σ^S factor is known to affect lycopene accumulation, and it was observed to have far-reaching effects on both the transcriptomic and proteomic data sets. While expression changes were not strictly additive between the five mutant strains examined in comparison to the pre-engineered background strain, a number of these common factors appear to be responsible for the high lycopene-production phenotype. This work serves as an important example of incorporating multiple layers of complementary biological information to define a basis for an observed phenotype, demonstrating a powerful paradigm for realizing production increases via systems metabolic engineering.

Thesis Supervisor: Charles L. Cooney
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First, I would like to thank the many people who helped to make my thesis a success. I came to MIT because I wanted to learn from the best, and my thesis advisor and committee members certainly fulfilled this description. My advisor, Prof. Charlie Cooney, was a great help both in my research and in helping me to consider my long-term career plans. To borrow your quote, Charlie, you often helped me to "know where I was so that I could know where I was going and when I had arrived." At times when I didn't know where I was or where I was headed, your advice, extensive knowledge of biochemical engineering, and support were greatly appreciated. Prof. Greg Stephanopoulos was a source of great help as well. Greg, thank you for suggesting the lycopene project to me and for treating me like a member of your research group in your weekly research meetings. Your expertise in metabolic engineering helped to develop my own interest in the subject. Of course, I owe a great deal of thanks to our administrative assistant, Rosangela Dos Santos, as well, who helped to fit me into my advisors' busy schedules. Rosangela, thank you for all of your assistance in these past few years. I would also like to thank my other Thesis Committee members, Prof. Kristala Jones Prather, Prof. Tony Sinskey, and Prof. Leona Samson, for their constructive criticism and advice throughout my graduate career.

Among the many who helped to make my thesis a success at MIT were the members of the Cooney and Stephanopoulos research groups. Dr. Bill Perry from the Cooney lab was especially helpful in teaching me the art of microarrays. In the Stephanopoulos lab, I'd like to thank Dr. Hal Alper for providing the strains and basis for my research; Dr. Joel Moxley for his microarray analytical assistance and general friendship; Dr. Curt Fischer for letting me bounce ideas off of you and for giving me invaluable suggestions based on your extensive metabolic engineering knowledge; Dr. Ajikumar Parayil for teaching me many of the cloning techniques; Christine Santos for providing me with several plasmids; and Dr. Chris Loose for cooking me dinner during our first year (but I always did the dishes).

I'd also like to thank Dr. Jeff Silva and Dr. Johannes Vissers from the Waters Corporation for completing the proteomics experimental work; Dr. Chris Workman from the Denmark Technical University with microarray analytical advice; Dr. Alexi Goranov from the MIT Biology Grossman lab for the microarray experimental protocol; and my funding sources which included the MIT Presidential Fellowship Program (Robert T. Haslam Fellowship), the NIGMS/NIH Interdepartmental Biotechnology Training Program, and the MIT Portugal Program.

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Kathleen, you are the love of my life and my best friend. Thank you for always believing in me, standing by my side, and making me laugh (and laughing at my jokes), burglar.

Finally, I'd like to acknowledge the Lord for seeing me through this time in my life and for helping me to make great strides, both personally and professionally, during these last few years. I am grateful to be able to quote the Psalmist in describing my work and endless curiosity,

Psalm 143:5b "I meditate on all thy works; I muse on the work of thy hands."

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Chapter 1. INTRODUCTION

1.1. Motivation

Mankind has interacted with and even directed nature for thousands of years, but our ability to fundamentally change the genetic programs controlling life at all levels of existence is a new phenomenon. Whereas we could previously only search for pre-existing fuel sources for heating and powering all aspects of society, now we are able to collect "waste" biomass and feed it to microorganisms, allowing them to convert such materials into useful fuel. Before, we could identify that certain natural products found in specific parts of the world had medicinal properties. Now, we can extract the life codes of these exotic organisms and implant them into easily grown microorganisms in order to produce renewable sources of therapeutics for a vast array of disease and medical applications. In the past, we could synthesize valuable chemicals through laborious synthetic steps; today, we can utilize the cellular architecture of life and carefully refine the metabolic pathways for economical production of many important chemical products. These processes have been operating in nature since life began, but mankind's ability to direct the process towards objectives useful for his own needs has constituted a monumental leap forward. We have begun to shape and influence life at the molecular level, with far-reaching benefits from increased food production, fuel and chemical synthesis, and therapeutics. If our ability to engineer small changes to life itself is relatively new, our understanding of how a myriad of molecular species interact and together constitute life at such basic levels is in its infancy, compelling new generations of scientists and engineers to unravel such secrets of existence.

Systems biology is fundamentally concerned with this unraveling of the enormous and amazing complexities of life. Various definitions exist, but Aebersold (2005) has defined it

simply as the study of the dynamic networks of interacting biological elements. It represents a powerful method to describe and manipulate phenotypes of interest by analyzing and incorporating biological information from various levels of cellular organization. Biotechnology is focused upon harnessing the power of living systems in order to benefit mankind. From early human history in making wine or selectively breeding animals and crossing plants, biotechnology has improved society in dramatic ways.

The work of Pastuer and Tyndall identified microorganisms as the critical, active agents in previous fermentation processes and initiated the emergence of microbiology as a scientific discipline (Bailey and Ollis 1986). Further work by Buchner, Neuberg, and Weizmann led to production processes for ethanol, glycerol, and other chemicals in the early 20th century. The birth of the field of biochemical engineering, the engineering of processes using catalysts, feed stocks, and/or sorbents of biological origin (Bailey and Ollis 1986), from the fields of biochemistry, microbial genetics, and engineering came with developments in the 1940s aimed at developing new antibiotics for eradicating disease and suffering of various kinds. From these beginnings, the methods for cultivation of microorganisms and plant and animal cells made possible mass production of chemicals, vaccines, and other useful biological agents. Bioprocessing and biochemical engineering advances enabled the optimization of product yields, largely treating the biological systems themselves as black boxes through descriptive and empirical approaches until more mathematically rigorous approaches were developed in the early 1970s (Bailey 1998).

The first recombinant DNA molecules were formed *in vitro* in 1972 by Paul Berg and colleagues of Stanford University and the University of California (Jackson, Symons *et al.* 1972), and Cohen *et al.* (1973) first demonstrated the biological functionality of such

recombinant DNA molecules *in vivo*. This marked the birth of modern genetic engineering and revolutionized biochemical engineering. Eventually, researchers such as Bailey (1991) and Stephanopoulos (1991) began applying genetic engineering and recombinant DNA technology to directed pathway modifications in the science of metabolic engineering, or the directed improvement of product formation or cellular properties through the modification of specific biochemical reactions or the introduction of new ones with the use of recombinant DNA technology (Stephanopoulos 1999). These advances enabled the black box to be opened, explored, and manipulated.

With the sequencing of the *E. coli* (Blattner, Plunkett *et al.* 1997), human (Lander, Linton *et al.* 2001; Venter, Adams *et al.* 2001), and other genomes and associated developments in putting such massive biological data sets into functional contexts , the "omics" era of biology has arrived. The new fields of systems biology and synthetic biology promise to deliver high biological resolution and artificially designed biological components, respectively, to microorganisms as miniature biological factories. Now, it is hoped that the previous biological black box can be reconstructed and understood at the most basic levels in order to improve system performance and benefit mankind.

With the ability to alter and change such systems, great responsibility comes in applying such tools for good and not evil. As mankind creates new possibilities from existing life, we would be wise to remember the lessons of Frankenstein's monster or Prometheus and not overreach in such endeavors. The Psalmist proclaims "Thou madest him to have dominion over the works of thy hands; thou hast put all things under his feet," but with such dominion comes great responsibility.

With the responsible and creative application of systems biology to the ever-evolving realm of biotechnology, even greater advances in the understanding, engineering, and production with biological systems will be possible. In this spirit, this thesis concentrates on taking a systems biology view of the model organism *Escherichia coli* and its recombinant production of the model isoprenoid lycopene in order to identify transcriptomic and proteomic factors which influence production. Such an approach is illustrated from a library of both rationally-directed and combinatorial gene knockout strains of *E. coli* which have been shown to produce various levels of lycopene when transformed with the pAC-LYC plasmid. Lycopene is an important neutraceutical of the diverse and valuable isoprenoid chemical class, and therefore an improved description of its recombinant production has significant implications to other related target molecules as well.

Previously, Alper *et al.* (Alper, Jin *et al.* 2005) used genome-wide stoichiometric flux balance analysis to predict which *E. coli* genes should be deleted in single or multiple knockout experiments to improve recombinant lycopene production while maintaining acceptable growth and tested these predictions experimentally. Through this stoichiometric modeling approach, they achieved a 40% increase in production over the "pre-engineered," high producing parental strain *E. coli* K12 WS140 PT5-*dxs*, PT5-*idi*, PT5-*ispFD* harboring pAC-LYC (Yuan, Rouviere *et al.* 2006). This strain will be referred to as the parental or "pre-engineered" (PE) background strain throughout the remainder of this thesis. Since the yield of lycopene still fell far below the stoichiometric maximum of about 10% on glucose via this stoichiometric analysis alone, Alper *et al.* (Alper, Miyaoku *et al.* 2005) next searched for possible kinetic or regulatory factors using a global transposon library search in the background of the "pre-engineered" parental strain. After identifying these "combinatorial" targets, they investigated all possible combinations of eight

selected stoichiometric and eight selected combinatorial genotypes to comprehensively generate a lycopene production landscape. Two global maxima were found in this landscape, one strain purely designed from stoichiometric modeling, Δ*gdhA* Δ*aceE* Δ*fdhF*, and one strain that combined two stoichiometrically-derived targets and one combinatorial target, Δ*gdhA* Δ*aceE* Δ_P*yjiD*. Figure 1-1 is modified from Alper *et al.* (Alper, Miyaoku *et al.* 2005) and shows the lycopene production landscape marked with strains of interest to this thesis.

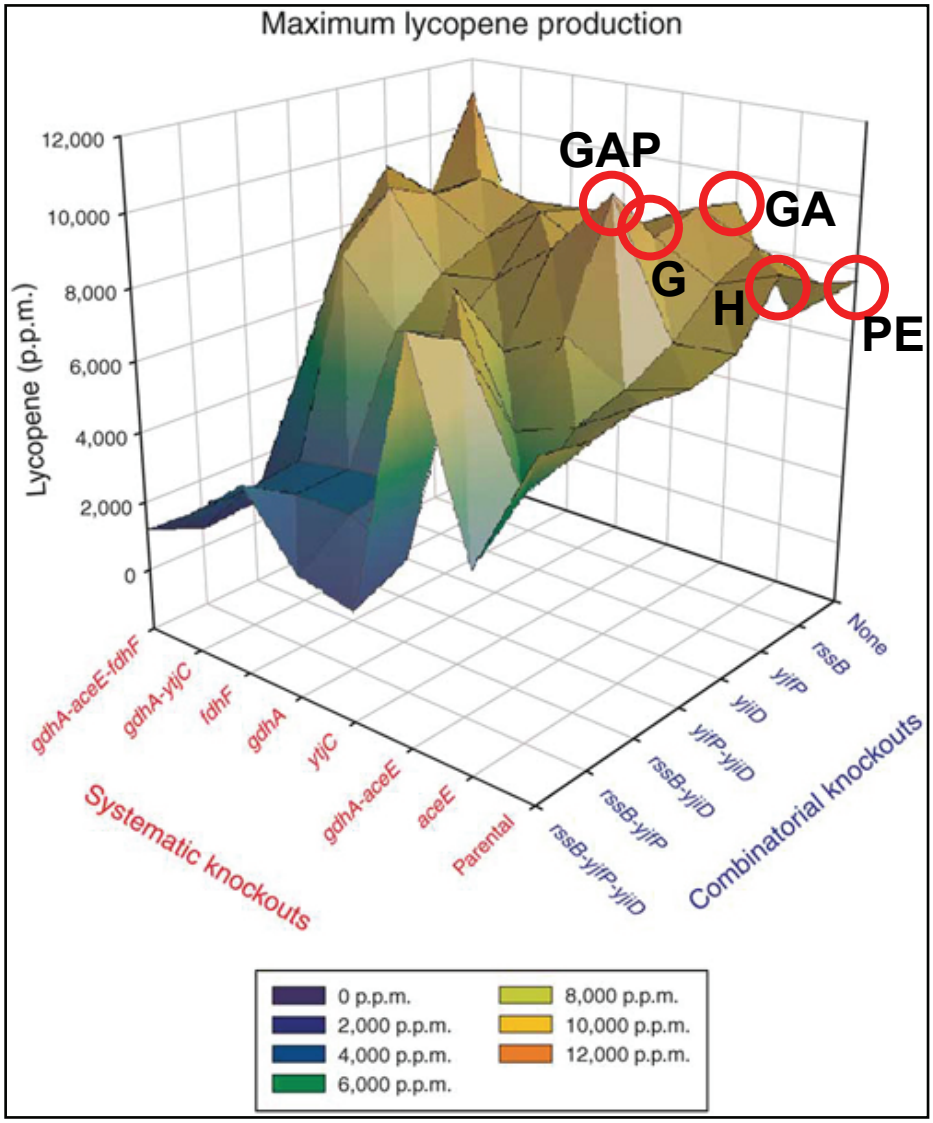

Figure 1-1 Maximum lycopene production in parts per million (PPM) in 48 hours for the parental pre-engineered (PE) background and the five mutant strains of this study grown in 1×M9 media using shake flasks (Δ*hnr* Δ*yliE* not shown). Modified from (Alper, Miyaoku *et al.* 2005). Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ_P*yjiD* (GAP), and Δ*hnr* (H).

Through further transposon mutagenesis in the background of some of these local and global maximum producing strains, Alper *et al.* (2008) found an additional strain, Δ*hnr* Δ*ylie*, which produced more lycopene than any strain they identified previously.

In an alternative extension of this earlier work, Jin and Stephanopoulos (2007) combined gene deletions found previously (Alper, Jin *et al.* 2005; Alper, Miyaoku *et al.* 2005) with gene overexpressions identified via a shotgun approach in order to increase lycopene production. In addition to finding previously identified genes whose overexpression increased lycopene production such as *rpoS*, *appY*, and *dxs*, and *idi*, they also identified new targets using the approach such as *yjiD* and *ycgW*. They identified a maximum producing strain in *E. coli* K12 (PT5-*dxs*, PT5-*idi*, rrnBP-*yjiD*-*ycgW*, Δ*gdhA* Δ*aceE* Δ*fdhF*), which produced 16 mg/g DCW lycopene, a four-fold increase over their parental strain. This strain combines four overexpressions and three knockouts and exemplifies the usefulness of the multi-dimensional search approach.

Alper *et al.* (2006) examined two of these maximally-producing strains, Δ*gdhA* Δ*aceE* Δ*fdhF* and Δ*gdhA* Δ*aceE* Δ_P*yjiD*, in the context of high cell density fermentations and found that despite similarities in overall lycopene production levels, the levels of formate, glutamate, and alanine differed significantly. Thus, the different genotypes lead to similar but slightly different phenotypes. Carbon balances suggested a linkage between glutamate, formate, and alanine levels with lycopene overproduction, while it was previously speculated that NADPH availability was critical to lycopene production based upon the success of the *gdhA* knockout (Alper, Jin *et al.* 2005). Alper *et al.* (2008) also observed overrepresented gateway nodes in gene knockout search trajectories related to lycopene overproduction consisting of glutamate metabolism, the *fdh* operon, and *hnr*.

Despite this collection of work, important information have not been reported concerning the global gene and protein expression programs underlying the lycopene overproduction observed in these high-producing strains. Such information is vital to clarifying more distal effects that may be in common to these strains sharing similar phenotypes but having different genotypes. As Alper *et al.* (2008) has pointed out, desirable perturbations to optimize cellular performance are often highly context-dependent. Thus, finding common strategies for overproduction is a challenge. These general strategies are highly valuable despite the difficulties inherent in finding them across various strains and products. A goal of this thesis is to explore commonalities between strains that share similar phenotypes from gene and protein expression contexts. Additionally, similarities and differences in gene and protein expression between strains differing from each other by single gene deletions can be informative in understanding the effects of individual gene deletions within specific genotype backgrounds. While the mevalonate pathway has been studied for decades and was originally shown to be the source of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) in yeasts and animals by Bloch, Lynen, Cornforth, and co-workers (Cornforth, Hunter et al. 1953; Cornforth, Hunter et al. 1953; Katsuki and Bloch 1967; Lynen 1967), the existence of a second non-mevalonate pathway in microbes has only been discovered recently (Rohmer 1999). Thus, this work provides key insight into this recently-discovered pathway concerning the global characteristics of high lycopene producing strains from the perspectives of gene and protein expression and the effects that the engineered pathways have on the rest of the cellular network.

In this study, a systems biology approach is presented for examining gene and protein expression taken both separately and together as a basis for cellular phenotype. The genomic and proteomic expression of global maxima strains Δ*gdhA* Δ*aceE* Δ$_{\text{P}}$*yjiD* (Alper, Miyaoku *et al.*

2005) and Δ*hnr* Δ*ylie* (Alper and Stephanopoulos 2008) were analyzed along with the associated Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*hnr* strains via DNA microarray analysis and a novel LC-MS method, respectively, to identify a molecular basis for the high production phenotype and to suggest additional metabolic engineering targets for further phenotype improvement. Based upon the resulting data, it was hypothesized that the following may be associated with lycopene overproduction: histidine biosynthesis (*hisH*); the quinone pool (*wrbA*); acid resistance (*ydeO* and *gadE*); the glyoxylate pathway (*iclR*); NADPH redox balance (*pntB*); growth rate reduction; and membrane composition. We report that in the pre-engineered background strain, deleting *pntB* (~20-25%) and *ydeO* (~30%) each led to moderately increased production; overexpressing *wrbA* led to 50-100% more production at 8 hours and 5-15% more production at later time points; deleting *iclR* caused small production increases (~5-10%); and supplementing media with histidine caused the parental and mutant strains to have similar production. Overall, it appears that a number of factors which are small to moderate individually together result in the observed lycopene production phenotypes. More generally, this work serves as an important example of incorporating multiple layers of complementary biological information to define a basis for an observed phenotype, demonstrating a powerful paradigm for realizing production increases via systems metabolic engineering.

1.2. Background

The non-mevalonate (also known as the 2-C-methyl-D-erythritol 4-phosphate (MEP) or 1-deoxyxyulose-5-phosphate (DXP) pathway) of plant plastids and most prokaryotes is one of two isoprenoid biosynthetic pathways from which lycopene can be produced. It was discovered only recently (Rohmer 1999), and only in the last few years have all of the enzymatic steps been described (Rohdich, Hecht *et al.* 2002). Figure 1-2, adapted from Alper *et al.* (Alper, Jin *et al.*

2005), displays an overview of the non-mevalonate pathway in *E. coli*. The non-mevalonate pathway proceeds from the initial condensation of two glycolytic precursors, glyceraldehyde-3-phosphate and pyruvate, and proceeds through seven steps to isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The *dxs*, *ispFD*, and *idi* genes are shown as up-regulated in Figure 1-2 as they are under expression of the constitutively active PT5 promoter in all the strains of this study (Alper, Miyaoku *et al.* 2005). 5-carbon IPP and DMPP are the precursors for additional condensation reactions to form a growing polyprenyl diphosphate chain via the polyisoprenoid pathway. Lycopene ($C_{40}H_{56}$) is formed in *E. coli* by the recombinant expression of the pAC-LYC plasmid containing *crtEBI* genes from the plant pathogen *Pantoea agglomerans* (formerly known as *Erwinia herbicola*) (Cunningham, Sun *et al.* 1994). It can be seen from the chemical equation that the synthesis of one molecule of lycopene has high metabolic costs, requiring 16 NADPH, 8 CTP, and 8 ATP molecules in addition to 8 glceraldehyde-3-phosphate (G3P) and 8 pyruvate (PYR) molecules. However, it is worth the cost as lycopene is a valuable neutraceutical (Lee and Schmidt-Dannert 2002), has potential anti-carcinogenic properties related to its antioxidant activity (Giovannucci 1999), and is related to a number of other valuable isoprenoid products such as artemisinic acid and taxadiene (Chang and Keasling 2006).

Gene knockouts in the PE background to generate the five mutant strains that are the focus of this study are circled or boxed and highlighted in yellow in Figure 1-2. These previously-discovered targets (Alper, Jin *et al.* 2005; Alper, Miyaoku *et al.* 2005; Alper and Stephanopoulos 2008) whose deletion leads to increases in lycopene production include the following: glutamate dehydrogenase (*gdhA*), pyruvate dehydrogenase (*aceE*), the global regulator *hnr* (*rssB*), and two previously uncharacterized genes, *yjiD* and *yliE*. The *yjiD* gene actually has a deletion in its promoter region (Alper, Miyaoku *et al.* 2005), leading to increased

expression of the gene rather than a knockout of gene transcription (Jin and Stephanopoulos 2007). The *yjiD* gene has actually recently been described as coding for the antiadaptor protein *iraD* (Bougdour, Cunning *et al.* 2008; Merrikh, Ferrazzoli *et al.* 2009). As shown in Figure 1-3 adapted from Bougdour *et al.* (2008), the *hnr* deletion or the increased Hnr association with YjiD (IraD) prevents Hnr association with the RNA polymerase subunit σ^S factor and its transfer to the ClpXP degradation complex, leading to increased σ^S levels and increased lycopene production (Becker-Hapak, Troxtel *et al.* 1997; Bougdour, Cunning *et al.* 2008). A large number of expression changes observed in this study thus result from increasing the σ^S global regulator levels, which is essential for multiple stress responses and is normally strongly induced upon entry into stationary phase and/or from multiple stress conditions. As much as 10% of *E. coli* genes have been observed to be directly or indirectly under σ^S control (Weber, Polen *et al.* 2005).

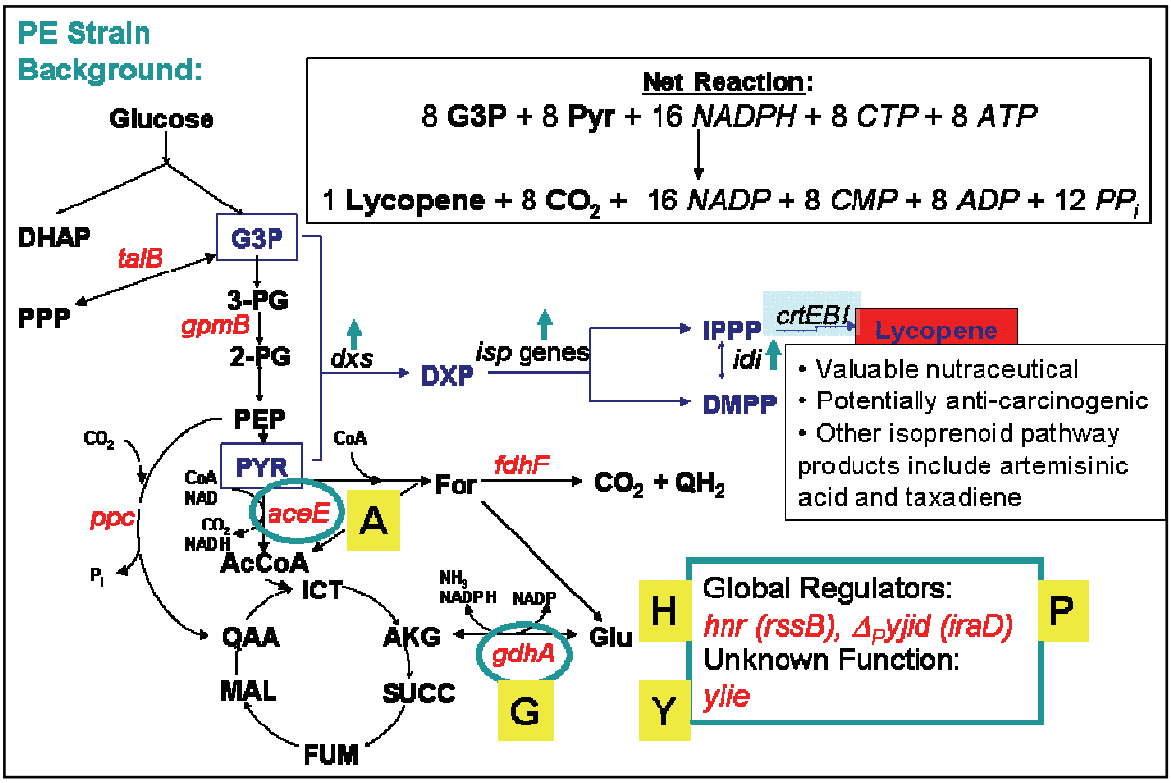

Figure 1-2 Recombinant lycopene biosynthesis via the non-mevalonate isoprenoid pathway in *E. coli*, overall reaction for lycopene biosynthesis, and engineering within the parental pre-engineered (PE) background to generate the five mutant strains. The glycolytic precursors glyceraldehyde-3-phosphate (G3P) and pyruvate (PYR) feed into the non-mevalonate pathway, in which the *dxs*, the *ispFD*, and the *idi* genes are overexpressed under the PT5 promoter. The *crtEBI* genes completing lycopene biosynthesis are present on the pAC-LYC plasmid. Gene knockouts in the PE background to generate the five mutants are circled or boxed and highlighted in yellow. Modified from (Alper, Jin *et al.* 2005). Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY).

Deleting these five genes consecutively in the background of the PE strain, the following strains were generated previously, with symbols used to describe them in the figures shown in parentheses (Alper, Jin *et al.* 2005; Alper, Miyaoku *et al.* 2005; Alper and Stephanopoulos 2008): Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY). The gene and protein expression of these five "mutant" strains compared to the PE strain background are the concentration of this study.

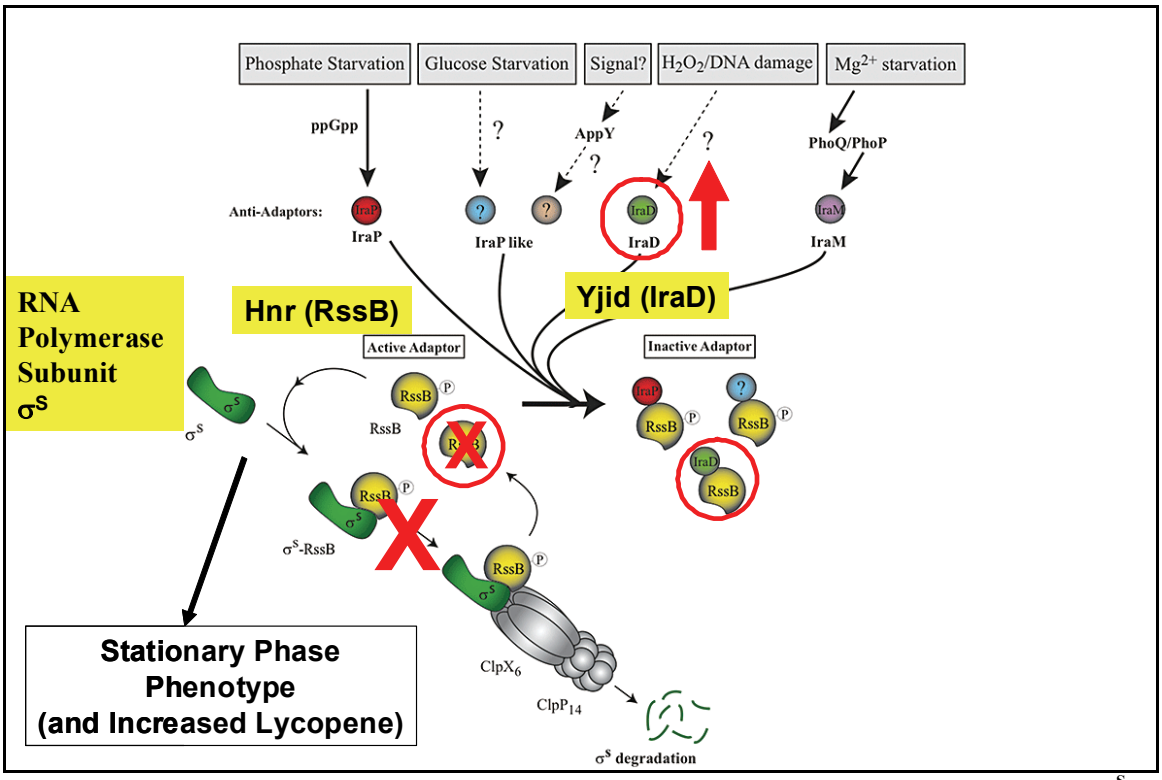

Figure 1-3 Hnr (RssB) and *YjiD* (IraD) protein functions in the context of RNA polymerase subunit σ^S factor degradation. In this study, *yjiD* is actually overexpressed by the deletion in its promoter region, which leads to its increased association with Hnr. The *hnr* deletion of this work or the increased Hnr association with YjiD prevents Hnr association with σ^S and its transfer to the ClpXP degradation complex, leading to increased σ^S levels and increased lycopene production (Becker-Hapak, Troxtel *et al.* 1997). Figure is modified from Bougdour *et al.* (2008).

1.3. Objectives and Approach

Based upon the current challenges and outstanding problems in metabolic engineering and systems biology discussed above, the following objectives were proposed for this thesis with the corresponding approaches:

- Discover and analyze global genomic expression changes associated with increased recombinant lycopene production in the engineered *Escherichia coli* strains Δ*gdhA*, Δ*gdhA* Δ*aceE*, Δ*gdhA* Δ*aceE* Δ*pyjiD*, Δ*hnr*, and Δ*hnr* Δ*yliE* compared with the parental strain PE background via DNA microarray analysis.
- Discover and analyze global proteomic expression changes associated with increased recombinant lycopene production in engineered *Escherichia coli* strains Δ*gdhA*, Δ*gdhA*

Δ*aceE*, Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD*, Δ*hnr*, and Δ*hnr* Δ*yliE* compared with the parental strain PE background via a novel LC-MS approach.

- Examine these transcriptomic and proteomic data sets in an integrated, systems biological analysis to gain further insight into the lycopene production phenotype.
- Based upon these analyses, formulate and test hypotheses concerning the basis of the lycopene production phenotype observed in the engineered *Escherichia coli* strains via metabolic engineering.

The approach taken based upon these objectives is outlined graphically in Figure 1-4. Engineered *E. coli* K12 cells were analyzed for genomic and proteomic expression compared to the PE background both separately and in an integrated manner in order to generate a number of targets that may be correlated with the phenotype of high lycopene production. Hypotheses were formed based upon these targets and tested mainly through metabolic engineering of the PE strain to determine if the high lycopene production phenotype could be recovered through the genetic manipulations. It was desired that such information would lead to increased lycopene production in the PE strain, with potential gains in the mutant and other strains as well. For clarity, the maximum lycopene production of the PE and five mutant strains at 15 and 24 hours grown in 1×M9 media as previously measured by Alper *et al.* (2008) is shown in Figure 1-5. Lycopene production increases with consecutive gene deletions amongst the Δ*gdhA*, the Δ*gdhA* Δ*aceE*, and the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* strains and also amongst the Δ*hnr* and the Δ*hnr* Δ*yliE* strains, with the Δ*hnr* Δ*yliE* strain producing the most lycopene overall and about 47% more than the PE strain at 24 hours. The biological basis of these gains is the focus of this thesis.

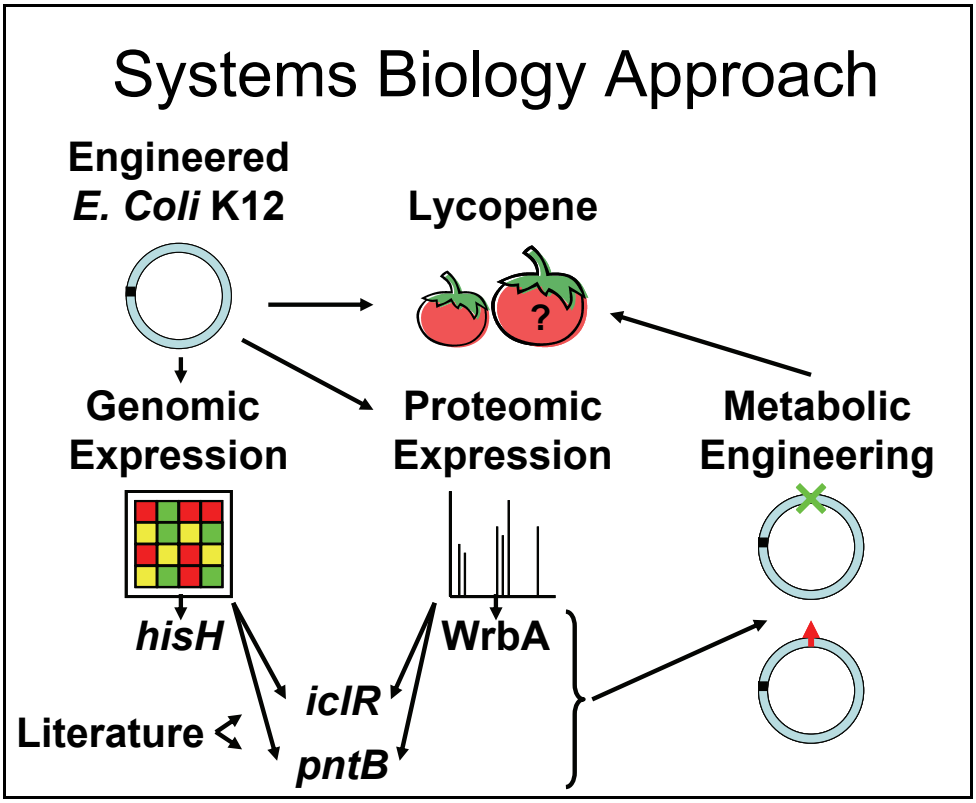

Figure 1-4 Systems biology approach taken in this thesis to study and improve engineered *E. coli* K12 strains metabolically engineered for high lycopene production using genomic and proteomic expression, the literature, and metabolic engineering experiments to test hypotheses driven by the expression data.

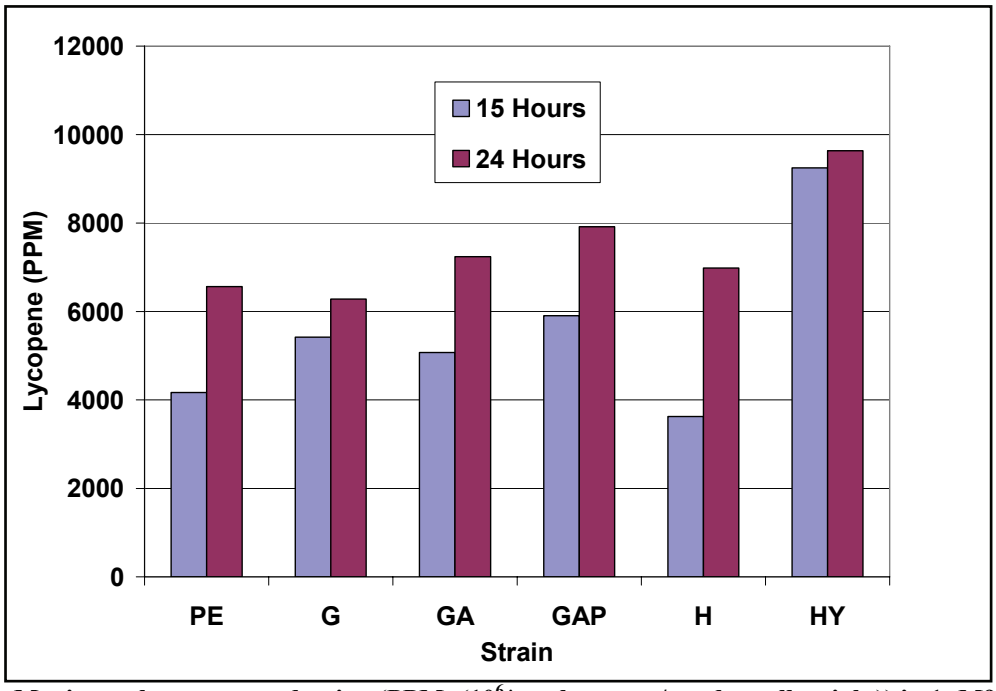

Figure 1-5 Maximum lycopene production (PPM: (10^6*mg lycopene/mg dry cell weight)) in 1×M9 media for the parental pre-engineered (PE) background and the five mutant strains of this study. Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ_P*yjiD* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY). Data are taken from Alper *et al.* (Alper and Stephanopoulos 2008)

1.4. Thesis Organization

This thesis is organized as follows. This introductory chapter has laid out the current challenges in metabolic engineering and systems biology that motivate the overall objectives of the work pertaining to the global genomic and proteomic expression changes that were discovered, analyzed, and tested via further strain engineering in the context of recombinant *E. coli* lycopene production. Chapter 2 gives a literature review focused upon previous metabolic engineering efforts for isoprenoid and lycopene production in *E. coli*. Chapter 3 reviews the systems biology literature as it relates to metabolic engineering and industrial biotechnology. The methods employed in this work follow in Chapter 4, after which the main results and discussion related to the objectives described above are presented. Chapter 5 examines the genomic expression of the five mutant strains compared to the background pre-engineered (PE) strain and analyzes these results. In Chapter 6, the corresponding proteomic expression changes

are presented and discussed in depth. Both data sets are examined together for a manually integrated systems biological view of the lycopene production phenotype in Chapter 7. In all three of these chapters, hypotheses are presented based upon the observed expression changes, and appropriate experiments and metabolic engineering work are presented testing these hypotheses. Finally, the main conclusions and recommendations for future work on these problems are summarized and suggested in Chapter 8. Appendices and a complete bibliography follow in Chapter 9 and Chapter 10 , respectively.

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Chapter 2. METABOLIC ENGINEERING FOR LYCOPENE BIOSYNTHESIS LITERATURE REVIEW

2.1. Introduction to Metabolic Engineering for Natural Product Biosynthesis

There exists a vast array of natural chemical products, and as the industrial and health-related values of these compounds have become more apparent, interest in their production has increased. Plants, animals, and microorganisms can serve as the sources of such chemicals. Many natural products have extremely intricate structures which serve as basis for a wide spectrum of properties. These properties provide the basis for many different biological functions, such as species-specific coloration, photo protection, light harvesting, and hormonal activity (Vershinin 1999). Commercially, they are useful as food colorants, flavoring, fragrances, animal feed supplements, and neutraceuticals for cosmetic and pharmaceutical purposes (Lee and Schmidt-Dannert 2002). Natural products have especially been useful throughout human history for medicinal purposes, today treating conditions such as cancer (vinblastine from Madagascar periwinkle), heart disease (digitalis from purple foxglove), and pain (codeine from opium poppy) (Newman, Cragg *et al.* 2003; Chang and Keasling 2006). These secondary metabolites are usually produced in low abundance naturally and difficult to extract. For example, it would take approximately six 100-year-old Pacific yew trees to produce enough of the cancer drug Taxol (paclitaxel) to treat one cancer patient (Horwitz 1994). In addition to these economic and environmental considerations, newer methods such as combinatorial chemistry and high-throughput screening cannot replicate the diversity and complexity of many natural products. By the year 2030, the projected market for biotechnologically-produced chemicals is expected to be US$400 billion (Lorenz and Zinke 2005; Maury, Asadollahi *et al.* 2005).

Metabolic engineering has been important in attacking the problems associated with natural product production (Stephanopoulos and Sinskey, 1993), and the more recently developed tools of synthetic biology, computational biology, and systems biology including genomics, transcriptomics, proteomics, and metabolomics are just beginning to offer new approaches and solutions (Stephanopoulos, 2004). In effect, microbes can be turned into miniature factories converting low-cost sugar precursors into high-value chemical products (Klein-Marcuschamer, Ajikumar *et al.* 2007). There are a number of amino acids and vitamins currently produced via microbial fermentation (Stephanopoulos, Aristidou *et al.* 1998).

This goal of natural product formation can also be accomplished by transplanting metabolic pathways from natural product organisms into well-characterized microbial hosts such as *E. coli* or *S. cerevisiae* via introduction of heterologous pathway genes missing in the hosts, elimination of native pathways competing with the pathway of interest, and re-engineering of the regulatory networks through either directed or random approaches (Tyo, Alper *et al.* 2007). Complicating these efforts, often the native pathways are multi-step and active only in the presence of certain co-factors and energy carriers such as NADH, NADPH, and ATP (Klein-Marcuschamer, Ajikumar *et al.* 2007). Apart from the genotypes, bioreactor environments of these hosts can be optimized (Kiss and Stephanopoulos 1991; Kiss and Stephanopoulos 1992) in order to achieve the most desirable production phenotypes possible. Successful examples of such xenobiotic production include polyhydroxyalkoanates (Schubert, Steinbuchel *et al.* 1988; Slater, Voige *et al.* 1988; Peoples and Sinskey 1989) and 1,3-propanediol (Nakamura and Whited 2003) in *E. coli*. The largest class of natural products, the isoprenoids, is especially rich with metabolic engineering efforts and successes. This work will focus on heterologous lycopene production in *E. coli*, but Chang and Keasling (2006) have reviewed isoprenoid

production in yeast as well. Lee and Scmidt-Dannert (2002) give several examples of other hosts engineered for carotenoid production, including yeasts (*S. cerevisiae* and *Candida utilis*) and photosynthetic bacteria (*Rhodobacter sphaeroides* and *Synechocystis sp.*). They point out that one of the advantages of yeasts are that they exhibit an efficient isoprenoid metabolism and are capable of accumulating large amounts of the triterpenoid ergosterol in their membranes. By contrast, *E. coli* cells are limited in carotenoid production by having inadequate storage capacity in their membranes for such lipophilic compounds (Sandmann, Albrecht *et al.* 1999). Increasing the storage capacity of carotenoids in *E. coli* or developing sequestering systems for these compounds remains a major outstanding challenge.

2.2. Isoprenoids, Carotenoids, and Lycopene

There are more than 50,000 known isoprenoid compounds representing a vast array of structures (Connolly and Hill 1991). Biological functions of isoprenoids span the following: hormonal activity (steroids, gibberellins, and abscisic acid); membrane fluidity maintenance (steroids); respiration (quinones); photosynthetic light harvesting (carotenoids); and protein targeting and regulation (prenylation and glycosylation) (Chang and Keasling 2006). Among the isoprenoids are the currently utilized and promising pharmaceuticals such as Taxol, vinblastine, artemisinin, and prostratin. Carotenoids are the group of isoprenoids including and descending from phytoene. They are 40-carbon molecules (tetraterpenoids composed of 8 isoprene units) and are synthesized as hydrocarbons (*i.e.* the carotenes, including lycopene, α-carotene, and β-carotene) or their oxygenated derivatives (*i.e.* the xantophylls, including lutein, α-cryptoxanthin, β-cryptoxanthin, zeaxanthin, canthaxanthin and astaxanthin) (Das, Yoon *et al.* 2007). Major functions of carotenoids include protecting against oxidative damage by quenching photosensitizers, interacting with singlet oxygen (Krinsky 1994), and scavenging peroxy radicals

(Conn, Lambert *et al.* 1992), therefore preventing such reactive oxygen species from accumulating. Lycopene is one of the most potent inhibitors of singlet oxygen (Tsen, Tsen *et al.* 2006).

Lycopene ($C_{40}H_{56}$) is found in relatively large quantities in tomatoes, guava, and pink grapefruit and gives these fruits their red hues (Vadali, Fu *et al.* 2005), with tomatoes containing about 8.8 - 42 (μg lycopene/g wet weight) (Rao and Rao 2007) and tomato sauce containing about 63 – 131 (μg lycopene/g wet weight) (Rao, Ray et al. 2006). Typical lycopene levels in tomato plant suspension cell culture have been found to be in the range of 1 -8 (μg lycopene/g) (Lu, Engelmann et al. 2008). As a rough comparison, the dry cell weight normalized lycopene levels of this thesis typically fell in the range of 1 - 8 (mg lycopene/g dry cell weight) (~1000-8000 PPM). Recombinantly producing lycopene in *E. coli* cells could offer potential advantages in increased yields and decreased processing times and costs.

Lycopene has generated considerable attention recently for its potential physiological effects and engineering usefulness. Early evidence suggested that lycopene (Mills, Beeson *et al.* 1989; Tzonou, Signorello *et al.* 1999) levels in blood may be inversely correlated to risks of cancer (Hsing, Comstock *et al.* 1990; Giovannucci, Ascherio *et al.* 1995; Gann, Ma *et al.* 1999; Giovannucci 1999). However, the FDA concluded in 2004 after a comprehensive review of the literature that there was very minimal support for a link between blood lycopene levels and prostrate cancer incidence, with no support found for a link between lycopene levels and other cancers (Kavanaugh, Trumbo *et al.* 2007). A number of studies around this time period have also provided results conflicting with the earlier reports, finding no association between lycopene or tomato consumption and prostrate cancer (Kirsh, Mayne *et al.* 2006; Stram, Hankin *et al.* 2006; Peters, Leitzmann *et al.* 2007). However, limitations of these studies have been pointed

out recently, and it is clear that more research is needed to study the effect lycopene may have upon prostrate cancer prevention and treatment (Giovannucci 2007). For example, lycopene's beneficial effect may depend upon other metabolites being present, bioavailability, or whether the treated population has a specific genotype (Goodman, Bostick *et al.* 2006). Additionally, other potential health benefits of lycopene include the following: enhanced cellular gap junction communication; induction of phase II enzymes through activation of the antioxidant response element (ARE) transcription system; suppression of insulin-like growth factor-1-stimulated cell proliferation by induced insulin-like growth factor binding protein; anti-angiogenesis and inhibition of cell proliferation; and induction of apoptosis (Mein, Lian *et al.* 2008).

Aside from the potential health benefits of lycopene, this compound has served as an important reporting compound for metabolic engineering methods aimed at improving carotenoid production. This is because the screening for lycopene overproduction is simple and straightforward due to its red coloring (Marshall and Wilmoth 1981). Optimization of lycopene production is possibly transferrable to other carotenoids since lycopene is a major precursor to other carotenoids (Sandmann 2002). Indeed, biosynthesis of other valuable isoprenoids can potentially benefit from knowledge gained from lycopene synthesis if the information is more global in nature and transferrable between related pathways. Additionally, since secondary metabolites like lycopene are energetically expensive and complex to synthesize, this pathway provides a good platform for testing pathway optimization procedures and tools (Mijts and Schmidt-Dannert 2003). Significant attention has recently been given to improving recombinant lycopene production.

2.3. Overview of Lycopene Biosynthesis

Biosynthesis of the secondary metabolite lycopene can be roughly divided into three main parts: delivery of upstream substrates and pathway cofactors by the rest of cellular metabolism; the isoprenoid pathway proceeding through either the non-mevalonate or mevalonate route; and the downstream polyisoprenoid (or carotenoid) pathway leading to lycopene.

The isoprenoid universal building blocks of isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) are produced by one of two pathways. The mevalonate (MVA) pathway is used by most eukaryotes to convert acetyl-CoA to IPP, which is then isomerized to DMAPP. The non-mevalonate (2-C-methyl-D-erythritol 4-phosphate (MEP) or 1-deoxyxyulose-5-phosphate (DXP)) pathway of plant plastids and most prokaryotes was discovered more recently (Rohmer 1999), and all of the enzymatic steps have been described only in the past few years (Rohdich, Hecht *et al.* 2002). The non-mevalonate pathway proceeds from the initial condensation of two glycolytic precursors, glyceraldehyde-3-phosphate and pyruvate. Regardless of which pathway is used, the downstream molecules IPP and DMAPP are then converted through a series of condensation reactions to the final lycopene product. Generally, the major obstacles researchers have encountered for lycopene biosynthesis include the following: substrate availability; pathway intermediate accumulation; and restricted storage capacity for products (Sandmann, Albrecht *et al.* 1999).

Since both the MVA and MEP pathways proceed from glycolytic precursors, efforts aimed at increasing upstream flux into these pathways can benefit both approaches, whereas individual pathway efforts are required downstream. This thesis will focus upon studying *E. coli*

cells recombinantly producing lycopene via the non-mevalonate (MEP) pathway, but a description of both pathways and initial attempts to increase their flux follows.

2.4. Metabolic Engineering Strategies for Lycopene Production

2.4.1. Non-Mevalonate (Methylerythritol) Pathway

The non-mevalonate pathway in *E. coli* consists of eight reactions, with six of the associated enzymes structurally characterized. The genes of the pathway are dispersed throughout the genome with no evidence of a common global transcriptional regulator. However, the IspF enzyme binds isoprenoids in a conserved hydrophobic core, raising the possibility of feedback regulation at this step (Hunter 2007).

The reactions of the non-mevalonate pathway have been summarized by Das *et al.* (2007) and are comprised of seven steps. First, DXP synthase (*dxs*) catalyzes the condensation of glyceraldehyde-3-phosphate with pyruvate to form 1-deoxy-D-xylulose-5-phosphate (DXP). DXP, a precursor to non-isoprenoids such as vitamins, then undergoes a rearrangement and is reduced to 2-C-methyl-D-erythritol-4-phosphate (MEP) by DXP reductoisomerase (*dxr*) in an NADPH- and Mn^{2+}-dependent manner (Takahashi, Kuzuyama *et al.* 1998). Next, IspD (4-diphosphocytidyl-2-C-methyl-D-erythritol synthase) catalyzes the reaction of MEP with cytidine 5'-triphosphate to form 4-diphosphocytidyl-2-C-methylerythritol, which is then phosphorylated by IspE in an ATP-dependent manner (Rohdich, Wungsintaweekul *et al.* 1999; Luttgen, Rohdich *et al.* 2000). The product, 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phosphate) is cyclized by IspF to form 2C-methyl-D-erythriol 2,4-cyclodiphosphate. The last two steps are catalyzed by IspG and IspH to form IPP and DMAPP (Hecht, Eisenreich *et al.* 2001; Rohdich, Hecht *et al.* 2002). The IPP and DMAPP products are isomerized by the enzyme encoded by *idi*.

Early work engineering the non-mevalonate pathway for carotenoid production focused on overexpressing the genes coding for enzyme pathways. Under normal growth conditions, isoprenoid synthesis genes are only marginally expressed (Wei, Lee *et al.* 2001; Yuan, Rouviere *et al.* 2006). Farmer and Liao (2000) altered the Ntr regulon in *E. coli* to control the expression of the *idi* and *pps* genes and the flux through the lycopene pathway in response to excess glycolytic flux. Farmer and Liao (2001) also found that properly balancing the precursors pyruvate and glyceraldehyde-3-phosphate feeding into the non-mevalonate pathway is important to maximizing the lycopene yield. In particular, they found that glyceraldehyde-3-phosphate was limiting in the pathway and that modifications that redirected flux from pyruvate to glyceradlehyde-3-phosphate had a positive effect on lycopene production. Similarly, Kim and Keasling (2001) found that balanced overexpression of the *dxs* and *dxr* genes by using the proper promoter strengths and expression vectors was important to optimizing lycopene production while avoiding growth inhibition due to enzyme overexpression. Additionally, inactivation of several competing pathways at the nodes of acetyl-CoA and pyruvate increased lycopene production more than 45% compared to the parental strain (Vadali, Fu *et al.* 2005). In their study, lycopene production from both the non-mevalonate and the mevalonate pathways was shown to increase with these pathway modifications; however, deletion of too many competing pathways led to metabolic imbalance and pyruvate excretion into the media, inhibiting growth. Again, proper balance of the pathways was shown to be important to successful engineering efforts.

Several groups showed that overexpression of the *dxs* gene catalyzing the first step in the non-mevalonate pathway improved lycopene production 2 to 3-fold (Albrecht, Misawa *et al.* 1999; Harker and Bramley 1999; Matthews and Wurtzel 2000). Kim and Keasling (2001) co-

expressed the *dxs* and *dxr* enzymes and further improved production 1.4 to 2-fold. Furthermore, they were able to achieve lycopene production into the stationary phase by overexpressing *dxs*, a result also found by Harker and Bramley (1999) when they overexpressed *dxs* from *Bacillus subtilis* and *Synechocystis sp.* 6803 in *E. coli*. This was in contrast to the reports from Kajiwara *et al.* (1997) and Sandmann *et al.* (1999). Kim and Keasling (2001) reasoned that while the IPP-synthesis pathway producing DXP as a precursor for growth-dependent thiamine (vitamin B1), pyridoxyl (vitamin B6), dolichols (sugar carrier lipids), and respiratory quinones (menaquinone and ubiquinone) may be down-regulated in the stationary phase, the *dxs* overexpression may relieve this bottleneck and allow synthesis into the stationary phase, increasing the overall production level. However, it has also been observed that gratuitous overexpression of the *dxs* gene on a high-copy plasmid represents a major metabolic burden for the cell (Jones, Kim *et al.* 2000), and so low-copy plasmid expression or engineering of the chromosomal promoter is often important for balanced expression and overproduction.

Overexpression of the *idi* gene was seen to have an even more significant effect upon lycopene production, with Kajiwara *et al.* (1997) reporting 3.6 to 4.5-fold more lycopene and 1.5 to 2.7-fold higher β-carotene production when overexpressing the isomerization gene. Furthermore, Sandmann *et al.* (Sandmann, Albrecht *et al.* 1999) found that overexpressing both idi and either dxs or dxr increased zeaxanthin levels to 1.6 mg g DCW-1.

More recently, Yuan *et al.* (2006) replaced the native promoters of the chromosomal isoprenoid genes with the strong bacteriophage T5 promoter (PT5). They found that *E. coli* PT5-*dxs* PT5-*ispDispF* PT5-*idi* PT5-*ispB* strain resulted in high levels of β-carotene production (6 mg/g dry cell weight), emphasizing the importance of these genes in the production pathway.

This strain is the same as the strain used by Alper *et al.* (Alper, Jin *et al.* 2005; Alper, Miyaoku *et al.* 2005) and the strain used in this work except for the *ispB* promoter replacement.

2.4.2. Mevalonate Pathway

The mevalonate pathway has been studied for decades and was originally shown to be the source of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) in yeasts and animals by Bloch, Lynen, Cornforth, and co-workers (Katsuki and Bloch 1967; Lynen 1967; Cornforth 1968; Poulter 2009). All three investigators eventually won Nobel prizes for their work. Their studies formed a basis for later developments of metabolic inhibitors such as pravastatin and related compounds that inhibit HMG-CoA reductase, an intermediate of the mevalonate pathway, for treatment of hypercholesterolemia (Watanabe, Ito et al. 1988; Kuzuyama 2002).

The conversion of acetyl-CoA to IPP in the mevalonate pathway takes six reaction steps. It begins with the conversion of three acetyl-CoA molecules to mevalonate through acetoacetyl-CoA and β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA). Sequential phosphorylation of mevalonate to diphosphomevalonate followed by decarboxylation produces IPP exclusively, making the activity of the IPP-DMAPP isomerase (*idi*) essential (Withers and Keasling 2007). The pathway genes include *atoB*, *mvaA*, *mvaB*, *mvaK1*, *mvaK2*, and *mvaD*.

Because the mevalonate pathway is heterologous to *E. coli*, it is not subject to the same regulatory mechanisms which can hinder flux through the native non-mevalonate pathway. Campos *et al.* (2001) integrated a synthetic operon consisting of yeast 5-diphosphomevalonate decarboxylase, human 5-phosphomevalonate kinase, yeast mevalonate kinase and *E. coli* isopentenyl diphosphate isomerase into the *E. coli* chromosome and observed the synthesis of IPP and DMAPP from exogenously-supplied mevalonate. Martin *et al.* (2003) chose to import

the entire "top" and "bottom" *S. cerevisiae* mevalonate pathway into *E. coli* to supplement endogenous IPP synthesis. They also introduced a codon-optimized amorphadiene synthase gene (Chang, Song *et al.* 2000; Mercke, Bengtsson *et al.* 2000; Martin, Yoshikuni *et al.* 2001), which along with IPP isomerase (*idi*) and FPP synthase (*ispC*) were necessary in order to relieve prenyl diphosphate-associated (IPP, DMAPP, and/or FPP) growth inhibition and toxicity and to achieve high levels of amorphadiene production. A closer examination of this mevalonate pathway showed that the top mevalonate pathway supplying mevalonate to the rest of the pathway was limiting, but efforts to increase flux through the top pathway led to growth inhibition (Pitera, Paddon *et al.* 2007). Through gene titration and metabolite profiling, it was shown that the growth inhibition was due to accumulation of the mevalonate pathway intermediate HMG-CoA. By increasing expression of the enzyme encoding HMG-CoA reductase, they were able to reduce the HMG-CoA accumulation and increase mevalonate production through the top pathway 3-fold compared to their original engineered strain. Furthermore, it was found through the application of DNA microarray analysis and metabolite profiling that the HMG-CoA growth inhibition is due to HMG-CoA inhibiting fatty acid biosynthesis, leading to general membrane stress (Kizer, Pitera *et al.* 2008). This study shares the DNA microarray approach reported in this thesis but applied this tool to strains utilizing the alternate mevalonate pathway instead of the non-mevalonate pathway. In a separate approach, Pfleger *et al.* (2006) were able to overcome the HMG-CoA growth inhibition by library-based engineering of the intergenic regions of the polycistronic operon encoding the top mevalonate pathway. This work built upon the general approach developed by Smolke *et al.* (Smolke, Carrier *et al.* 2000) in which secondary structures were introduced in mRNAs to modulate expression levels of genes under the same promoter, a system which was successfully applied to

varying relative levels of lycopene and β-carotene (Smolke, Martin *et al.* 2001). Pfleger *et al.* (2006) were able to balance gene expression in the top mevalonate operon and achieve a seven-fold increase in the production of mevalonate through the pathway. It was found that the expression of HMG-CoA synthase and HMG-CoA reductase were decreased in the higher producing strains, agreeing with the alternative approach of Pitera *et al.* (2007).

Yoon *et al.* (2006) recently added the mevalonate bottom pathway from *Streptococcus pneumoniae* to *E. coli* for lycopene production and achieved a 3-fold increase when supplementing the rich media with 0.5% glycerol and 3.3 mM mevalonate. In a similar study, they imported the *Streptococcus pneumoniae* mevalonate bottom pathway into *E. coli* and co-expressed the *crtY* and *dxs* genes to achieve high production of β-carotene in rich media containing glycerol and mevalonate (Yoon, Park *et al.* 2007).

2.4.3. Polyisoprenoid Biosynthesis to Lycopene

Following the production of 5-carbon molecules IPP and DMAPP via either the non-mevalonate or the mevalonate pathways, chain elongation next occurs via sequential head-to-tail condensation reactions of IPP to first DMAPP and then to the growing polyprenyl diphosphate chain via the polyisoprenoid (carotenoid) pathway. Prenyl diphosphate synthases synthesize geranyl diphosphate (GPP, C10), farnesyl diphosphate (FPP, C15), and geranylgeranyl diphosphate (GGPP, C20), which are the precursors of the mono-, sesqui-, and di-terpenoids and carotenoids, respectively. In *E. coli*, native *ispA* encodes for FPP synthase, which synthesizes both GPP and FPP. Beyond this point in the pathway to lycopene, the heterologous *crtEBI* genes are required, since *E. coli* naturally directs FPP into the undecaprenyl diphosphate and octaprenyl diphosphate pathways for cell wall lipid and quinone electron carrier synthesis, respectively. The *crtE* gene encodes for GGPP synthase. The colorless 40-carbon phytoene is

produced via the hetrologous phytoene synthase (*crtB*) catalyzing the condensation of two GGPP molecules. Finally, phytoene desaturase (*crtI*) introduces four double bonds into phytoene to produce the red-colored lycopene secondary metabolite. Beyond lycopene, synthesis of the cyclic carotenoids β-carotene, zeaxanthin, canthaxanthin, and astaxanthin requires the products of the *crtY*, *crtZ*, and *crtW* genes (Das, Yoon *et al.* 2007). Additionally, many novel carotenoids can be synthesized in *E. coli* using other heterologous genes (Lee and Schmidt-Dannert 2002).

Wang *et al.* (1999) identified rate-controlling steps in the polyisoprenoid biosynthesis pathway by employing the multifunctional GGPP synthase *gps* gene from *A. fulgidus*. The *gps* gene uniquely combines the functions of the *ispA* and *crtE* genes, directly converting IPP to GGPP. By overexpressing various combinations of the *gps*, *crtE*, *ispA*, and *idi* genes in *E. coli* transformed with astaxanthin biosynthetic genes, they were able to show that GGPP synthase (*crtE*), then IPP isomerase (*idi*), and finally FPP synthase (*ispA*) were rate controlling steps in descending order of importance. Overexpressing these three genes led to a 12-fold increase in astaxanthin production, but overexpressing the *gps* gene with *idi* led to a more than 40-fold yield improvement. Furthermore, they used in vitro evolution on the *gps* gene, which is from a hyperthermophilic organism and is likely suboptimally expressed in *E. coli*, to improve lycopene production 2-fold more (Wang, Oh *et al.* 2000).

However, Yoon *et al.* (2007) found that by overexpressing the *crtEBI* genes from *Pantoea agglomerans* (formerly *Erwinia herbicola*, the source of the carotegenic genes *crtEBI* in this study) instead of from *Pantoea ananatis*, the source of the *crtE* gene for Wang *et al.* (1999), lycopene production was about 2-fold higher. However, when they transformed the *crtE* gene from *Pantoea agglomerans* but the *crtBI* genes from *Pantoea ananatis*, the lycopene production was similar to that using only the Pantoea agglomerans genes. They concluded that the *Pantoea*

agglomerans genes are better for lycopene production in *E. coli* and that the *Pantoea agglomerans crtE* gene was responsible for the difference. Furthermore, they found no difference in lycopene synthesis between *E. coli* harboring the *P. agglomerans crtE* or the *A. fulgidus gps*, suggesting that FPP synthesis wasn't limiting. In light of the results from Wang *et al.* (1999) showing *crtE*-associated limitation with the *crtE* gene from *Pantoea ananatis*, they suggested that whether FPP synthesis is limiting likely depends on the activity of the specific *crtE* gene in *E. coli* (Yoon, Kim *et al.* 2007). This example of sequence optimization of a particular gene illustrates the larger concept that desirable environmental or genetic perturbations are often context-dependent, as Alper *et al.* (2008) also point out. Finding more general strategies that hold across various genetic backgrounds and product targets is challenging.

2.4.4. Combinatorial Engineering Methods

Several combinatorial approaches to exploring and engineering the isoprenoid pathway have been reported. These strategies often allow for a more efficient discovery of factors influencing phenotype which are regulatory, enzymatic, or more distal in nature. Hemmi *et al.* (1998) explored genes involved in the early isoprenoid pathway by first constructing a library of *E. coli* mutants transformed with the *crtEBI* genes for lycopene production and then mutating the cells. Some of the resulting mutated cells could no longer produce lycopene. An *E. coli* genomic library was then transformed into each of these 117 colonies deficient in the biosynthetic pathway of IPP. Within these doubly transformed strains, 29 complementary genes that restored the lycopene production were isolated and analyzed. A number of these genes were associated with ubiquinone and menaquinone biosynthesis. This approach was one of the first to examine genes involved in lycopene biosynthesis in a combinatorial fashion.

Kang *et al.* (2005) used a shotgun approach to discover genes whose overexpression improved lycopene production. They found that in addition to the *rpoS* (Sandmann, Woods *et al.* 1990; Becker-Hapak, Troxtel *et al.* 1997) and *dxs* (Harker and Bramley 1999; Kim and Keasling 2001) targets which had already been described as improving production, the *crl* and *appY* genes encoding regulators controlling the balance between σ^S factor and σ^D factor and anaerobic energy metabolism, respectively, also could be overexpressed to further increase production. By coexpressing *appY* with *dxs*, they were able to achieve about 8 times the amount of lycopene production (4.7 mg/g) versus strains with no overexpressions (0.6 mg/g).

A method for controlling the relative expression of genes in a natural or synthetic operon was also applied to carotenoid production. Five genes encoding for zeaxanthin production from *Pantoea ananatis* were reordered using the ordered gene assembly in Bacillus subtilis (OGAB) method (Tsuge, Matsui *et al.* 2003), resulting in a production increase of about 35% (Nishizaki, Tsuge *et al.* 2007) compared to the operon with the parental gene order. They also found that mRNA levels decreased monotonically with distance from the promoter and that the best operon had the genes ordered according to the order in the metabolic pathway.

Alper *et al.* (Alper, Moxley *et al.* 2006; 2007) applied a novel system for manipulating transcripts globally to lycopene overproduction. They applied random mutagenesis using error-prone PCR to the housekeeping sigma factor (σ^D), improving lycopene yield by up to about 50%. This approach of global transcription machinery engineering (gTME) allowed for the simultaneous perturbation of multiple genes under the control of σ^D. This provided a more efficient method for exploring the metabolic landscape when unknown regulatory and enzymatic factors were limiting, which has appeared to be the case with lycopene overproduction. It was found that a single round of gTME was as effective as multiple rounds of single gene deletion

and overexpression as part of a directed search strategy, and beneficial sigma factor mutations were specific to the background genotypes.

2.4.5. Optimization of Environmental Conditions

Several important observations have been made as to the optimal environmental conditions for lycopene production. Vadali *et al.* (2005) reported that lycopene production was higher when flasks were covered with foil to protect lycopene from light-associated degradation. They also found that growth at 22ºC led to the highest lycopene production, similar to another report suggesting growth between 25 and 28ºC led to higher carotenoid production (Lee, Mijts *et al.* 2004; Kim, Kim *et al.* 2006). Several reports have also indicated that using glycerol as a carbon source instead of glucose can be beneficial (Martin, Yoshikuni *et al.* 2001; Lee, Mijts *et al.* 2004; Yoon, Lee *et al.* 2006; Yoon, Park *et al.* 2007), although the reason for this is not yet known (Das, Yoon *et al.* 2007). Addition of surfactant Tween 80 with sodium dodecyl sulfate (SDS) to media has been reported to prevent cell clump formation presumably caused by the accumulation of hydrophobic lycopene in the cellular membrane (Yoon, Lee *et al.* 2006). Finally, Alper *et al.* (2006) found that high oxygen concentration and a slightly basic pH enhanced lycopene production.

2.5. Summary

Metabolic engineering has found many successes for increasing natural product synthesis. Lycopene is one representative of the diverse isoprenoid molecule class, and it can be synthesized by either the mevalonate or the non-mevalonate (methylerythritol) pathway, the latter of which is native to *E. coli*. The polyisoprenoid synthesis pathway converts the important IPP and DMPP precursors to lycopene via a series of condensation steps. A number of studies have focused upon targets for increasing lycopene biosynthesis in *E. coli*, and much of this work

forms the basis of the background parental PE strain in this thesis. However, application of DNA microarrays and LC-MS for global genomic and proteomic analysis of such lycopene producing strains has yet to be accomplished, highlighting the unique contribution of the current work.

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Chapter 3. Systems Biology Applications to Metabolic Engineering Literature Review

3.1. Introduction

Recent scientific advances have fundamentally changed biology. Integral developments such as high-throughput sequencing, availabilities of whole genomes, and the abilities to study large sets of biological molecules simultaneously have led to a paradigm shift from a general reductionist approach to a desire to understand and manipulate entire biological systems as a whole. Whereas single genes were studied only a few years ago, now entire genomes can be studied for expression changes in response to environmental or genetic perturbations. Similarly, protein, metabolite, and metabolic flux patterns and changes can be identified quickly and at a global scale. This is the essence of systems biology: incorporating multiple layers of complex biological information in order to define a basis for an observed phenotype. This is a lofty goal with many outstanding challenges; however, both the tools and approaches of systems biology are quickly gaining momentum in a quest to better understand the networks of molecules that give rise to all forms of life.

Applications of systems biology are already diverse and growing in number and effectiveness. A natural application is in drug design for combating human disease. Despite the fact that initial expectations for converting "genes to drugs" have been tempered somewhat since the completion of the human genome project (Lander, Linton *et al.* 2001; Venter, Adams *et al.* 2001) by the realizations that disease biology is complex with multiple layers of intricate organization, systems biology is already having a measurable impact upon drug discovery (Butcher, Berg *et al.* 2004). Hypothesis generation and testing in disease models is greatly facilitated by the collection of large-scale genome, proteome, and metabolome measurements, and modeling of organ and system-level responses helps to prioritize drug targets and design

clinical trials. Given the availability of the sequenced genomes and corresponding functional annotation of many microbes such as the model organism *E. coli* (Blattner, Plunkett *et al.* 1997), application of systems biology to the "systems metabolic engineering" of recombinant microbes for production of desired products is growing in importance and popularity as well. A number of successful applications of omics data to bacteria have recently been reviewed (De Keersmaecker, Thijs *et al.* 2006), and a general approach of applying systems biology tools to metabolic engineering has been given by Stephanopoulos *et al.* (2004). This section highlights several areas of large scale "omics" data collection and systems biology approaches to microbial engineering.

The reconstruction of entire genome-wide biochemical networks has become an important task in systems biology (Reed, Famili *et al.* 2006; Feist, Herrgard *et al.* 2009). These *in silico* models can direct biological discovery, enable biologists to reconcile heterogeneous data types, find inconsistencies and systematically generate hypotheses (Covert, Knight *et al.* 2004). However, since such metabolic models are incomplete, experimental approaches are vital to fill in missing links, discover discrepancies, and improve models. Thus, this work sheds additional light on the *E. coli* network for isoprenoid production in addition to providing the immediately practical knowledge of targets that are important to lycopene production and may be valuable for other isoprenoids and products.

3.2. Systems Metabolic Engineering Approach

Park *et al.* (2008) have recently proposed a general three-stage strategy for systems metabolic engineering. In the first stage, a suitable "base strain" is developed for the product of interest. Potential product toxicity to the host strain and product regulatory inhibition are considered. These limitations must be addressed before further efforts can be expected to

improve the production phenotype. Several strategies for addressing toxicity and inhibition have been reported and include site-specific mutagenesis and overexpression of exporter proteins (Lee, Park *et al.* 2007; Park, Lee *et al.* 2007), adaptive evolution to allow for strains to gradually acquire tolerance to a toxic product over successive generations (Guimaraes, Francois *et al.* 2008), and global transcriptional machinery engineering (Alper, Moxley *et al.* 2006; Alper and Stephanopoulos 2007) in which transcription factors are mutagenized to increase global diversity and transformed into hosts which are then screened for the improved phenotype. If rate-limiting steps are known in a pathway that are limiting product formation, the genes encoding for these enzymes can be amplified either on the chromosome or via plasmid transformation. Appropriate promoter strength and plasmid copy number are important considerations. Undesirable byproducts can be eliminated by deleting genes required for their metabolic pathways, and a similar strategy can eliminate or attenuate (Huser, Chassagnole *et al.* 2005) pathways that compete for precursors to the pathway of interest. If pathways are removed, then energy, cofactor, and redox balances need to be considered. If heterologous genes are introduced into the host, then codon usage, ribosome binding sites, and promoter suitability often must be optimized.

After developing a base strain, Park *et al.* (2008) suggest a second round of engineering that takes into consideration a genome-wide analysis. This analysis can help identify non-intuitive and distal genome targets that can be manipulated to further improve strain performance (Lee, Lee *et al.* 2005; Wang, Chen *et al.* 2006). Identifying these targets is a challenging endeavor, and verifying their importance through further metabolic engineering is an important validation step. For example, genes found through transcriptional profiling to be up-regulated may be amplified and those found to be down-regulated may be deleted in order to seek further

increases in production. Whether gene expression, for example, is a result of a given phenotype or a contributing factor to that phenotype is an important distinction that should be addressed through the validation of such targets by further metabolic engineering experiments. Alternatively, transcriptional profiling has been applied to identify genes whose expression is important to a desired production phenotype but that are down-regulated in response to cellular metabolic status (Gasser, Sauer *et al.* 2007; Lee, Park *et al.* 2007; Park, Lee *et al.* 2007). These genes represent additional targets whose overexpression may improve production. Transcriptional, proteomic, metabolomic, and fluxomic information can all be used to identify these types of targets and to help discover unknown regulatory mechanisms that may be limiting further production improvement.

The final suggested step after the genome-wide analysis involves running actual fermentations to detect further potential improvements. Undesired byproducts may be measured at this step, for example, that could negatively affect downstream processing. The strain's behavior at a large scale may be different than the observed behavior at smaller scales, and this step is important to determining whether such issues must be addressed. Such "scale-related" issues are often part of learning that occurs in final production scale up and product launch. To address problems detected at this third stage, the first and second stages of "systems metabolic engineering" may need to be iteratively addressed to realize further production gains.

3.3. Systems Metabolic Engineering Successes

3.3.1. Transcriptomics

Transcriptional profiling has emerged as a powerful tool in metabolic engineering since its introduction. DNA microarrays have quickly become a standard tool for measuring gene expression by quantifying mRNA levels, and exciting developments continue to advance the

technology. Although there are a number of possibilities for array analysis, consensus is emerging upon the most appropriate tools for this purpose (Allison, Cui *et al.* 2006). While regulation of protein abundance in a cell cannot be fully accessed solely by monitoring mRNA transcripts due to translational and post-translational cellular control, virtually all differences in cell type or state correlate with changes in the mRNA levels of a wide number of genes (DeRisi, Iyer *et al.* 1997). Besides transcriptional profiling, there are a growing number of alternative microarray applications including genome-wide location analysis to determine where transcription factors bind (Ren, Robert *et al.* 2000), DNA arrays used to detect single nucleotide polymorphisms (SNPs) (Erdogan, Kirchner *et al.* 2001; Kennedy, Matsuzaki *et al.* 2003), and protein arrays to study protein-protein and protein-small molecule interactions (MacBeath and Schreiber 2000). Even the array configuration itself is undergoing changes as researchers develop microbead-based arrays to overcome space limitations on fixed plates. New applications and developments like these will surely expand the already-impressive capabilities of microarrays, but there remains a vast amount of microarray work to be undertaken in studying gene expression. Improvements in sequencing technologies are currently providing new ways of studying global gene expression, but microarrays remain central tools in this effort.

In a seminal work for microarray analysis, DeRisi *et al.* (1997) used some of the first DNA arrays to find 386 genes whose expression levels change by greater than 4-fold during the diauxic shift from fermentation to respiration in yeast. Interestingly, the genes induced upon the diauxic shift included those converting the products of alcohol dehydrogenase into acetyl-CoA, which in turn is used in respiration to fuel the TCA cycle and the glyoxylate cycle. The genes repressed upon the shift included those responsible for the fermentation of pyruvate into ethanol.

This study demonstrates the power of DNA microarrays in eliciting an understanding of how cells respond to stimuli.

Richmond *et al.* (1999) employed some of the first whole-genome *E. coli* DNA microarrays to identify 5 gene products of the *lac* and *mel* operons that were induced by IPTG addition and 119 genes with differential expression upon heat shock treatment, 35 of which had not been previously assigned a biological role. Other interesting applications of microarray technology to measure *E. coli* stress and environmental change response have included examining reaction to growth in minimal and rich media (Tao, Bausch *et al.* 1999), growth in different carbon sources (Oh and Liao 2000), and response to changes that affect tryptophan metabolism (Khodursky, Peter *et al.* 2000). These studies have allowed for significant advances in understanding the biomolecular networks of *E. coli*.

While early transcriptomic studies made interesting observations, the most powerful approaches use such observations to generate testable hypothesis that are used to guide an inverse metabolic engineering approach (Bailey, Sburlati *et al.* 1996). There are several successful examples of transcriptional profiling applied to microbial strains for improved production. In one study of *Corynebacterium glutamicum*, L-lysine production was increased 40% by comparing the wild-type strain to a mutant strain and identifying that a methyltransferase and an ammonium uptake system should be overexpressed (Sindelar and Wendisch 2007). Hibi *et al.* (2007) used transcriptional profiling to investigate NADPH-dependent recombinant xylitol production in *E. coli*. They found that amongst 56 down-regulated genes in the xylitol-producing condition as compared to a nonproducing condition, disruption of the *yhbC* gene led to the most bioconversion to xylitol, a 2.7-fold increase over the non-disrupted control. Given that the conversion is NADPH-dependent, they inferred that deletion of the *yhbC* gene leads to a

maximum level of NADPH, alleviating NADPH-suppression that the *yhbC* gene product causes. Transcriptomics have been applied to producing human antibody fragments in *Pichia pastoris* (Gasser, Sauer *et al.* 2007). Out of 524 differentially-expressed genes from comparing a strain overexpressing human trypsinogen to a nonexpressing strain, 13 genes were focused upon as potentially important to the secretory machinery and in stress regulation, since inefficient heterologous protein production in yeasts and other eukaryotic hosts is often caused by poor folding and secretion. Through this analysis, 6 novel secretion helper factors were identified that increased human antibody production from 1.4- to 2.5-fold when cloned into a recombinant *Pichia pastoris* strain.

3.3.2. Proteomics

Protoeomic analysis has evolved considerably in the last few years. While 2-dimensional gel electrophoresis has served the field for years now, new mass spectrometry-based proteomic methods have ushered in a new era of increased sensitivity and accuracy in proteomics (Aebersold and Mann 2003; Patterson and Aebersold 2003). Data-independent and label-free mass spectrometry methods have been introduced recently and offer even greater advantages of more accurate quantitation and reduced sample preparation, respectively (Silva, Denny *et al.* 2005; Silva, Denny *et al.* 2006; Silva, Gorenstein *et al.* 2006; Geromanos, Vissers *et al.* 2009; Li, Vissers *et al.* 2009). These latter technologies were applied to the proteomic study of this work.

Proteomic profiling has also been applied successfully to microbial strain development and characterization. Two-dimensional polyacrylamide gel electrophoresis was applied for a proteomic analysis of high-cell density, industrial, phosphate-limited *E. coli* fermentations producing a humanized antibody fragment at the 10-L scale (Aldor, Krawitz *et al.* 2005). Using a false discovery rate (FDR) of 1%, it was found that 25 proteins were differentially-expressed

between the producing and control strains at 72 hours, and 19 proteins were detected only in either one of the strains at this time. The changes were associated with recombinant protein expression. Similarly, 81 proteins were found differentially-expressed between 14 and 72 hours using an FDR of 1%, with 20 uniquely detected proteins to either condition. Physiological changes found for the time course comparison included up-regulation of phosphate starvation proteins and down-regulation of ribosomal and nucleotide biosynthesis proteins. Furthermore, the stress protein phage shock protein A (PspA) was found to be highly correlated with the antibody fragment production, and controlled coexpression of *pspA* during recombinant production led to a higher yield of soluble antibody by about 50%.

There are other examples of successfully applying proteomic studies to increasing recombinant production in *E. coli*. Poly(3-hydroxybutyrate) (PHB) production was increased by following a strategy that included examining proteins using 2d-electrophoresis and mass spectrometry (Han, Yoon *et al.* 2001). Heat shock proteins such as GroEL, GroES, and DnaK were significantly up-regulated, whereas proteins involved in protein biosynthesis were seen to be down-regulated. A few glycolytic enzymes and Eda from the Entner-Doudoroff pathway were also up-regulated, presumably to meet increased cellular demand for coenzyme A and NADPH for use in PHB production. Additionally, it was found that overexpressing a *yfiD* target determined from the proteomic profiling led to increased accumulation of PHB.

The same group applied 2d-electrophoresis to the production of the serine-rich proteins human leptin and interleukin-12 β chain (Han, Jeong *et al.* 2003). Again, they found that heat shock proteins were up-regulated, whereas protein elongation factors, the 30s ribosomal protein, and some amino acid biosynthetic enzymes were down-regulated upon leptin production. In particular, coexpressing enzyme *cysK*, involved in cystein biosynthesis from serine, doubled the

cell growth rate and led to a four-fold increase in the specific leptin productivity. Proteomic analysis of the *cysK* overproducing strains revealed that this strategy led to more protein elongation factor (Ef-tu) existing in soluble form and metabolic flux changes increasing the efficiency of leptin production. Overexpression of *cysK* also led to increased production of interleukin-12 β chain production by 3-fold.

Another study compared the logarithmic and stationary phase proteomic expression in *Mannheimia succinicproducens* producing succinic acid, identifying growth-associated changes (Lee, Lee *et al.* 2006). Proteomic profiling utilizing 2d-electrophoresis and mass spectrometry identified two potential knockout targets, PutA and OadA, that are involved in converting pyruvate into the undesirable byproducts acetate and lactate, respectively, instead of succinic acid.

3.3.3. Multiple Types of Analyses

Systems metabolic engineering approaches have been successfully applied to the production of amino acids. Lee *et al.* (2003) combined genomic, transcriptomic, and proteomic analyses of *E. coli* strains overproducing threonine and found that genes of the glyoxylate shunt, the TCA cycle, and amino acid biosynthesis were significantly up-regulated, whereas ribosomal protein genes were down-regulated. They also found that two mutations in the *thrA* and *ilvA* genes were essential for threonine-overproduction in these strains. Based on this initial data, the group took the following steps to develop a base strain (Lee, Park *et al.* 2007): removing feedback inhibitions of aspartokinase I and III through *thrA* and *lysC* mutations; removing transcription attenuation by replacing the native promoter of the *thrABC* operon with a constituitively-active *tac* promoter; deleting the *lysA*, *metA*, and *tdh* genes to remove pathways competing with threonine production or degrading threonine; and incorporating the *ilvA* mutation

to decrease threonine dehydratase activity. They then further used transcriptional profiling to compare this new base strain with the original control strain to identify more targets for manipulation. In particular, they determined the following improved threonine production based upon the transcriptional data comparison: overexpressing phosphoenolpyruvate carboxylase, *ppc*, at an optimal level, which was down-regulated in the overproducing base strain but actually increased threonine production when overexpressed; deleting *iclR*, the repressor of the glyoxylate pathway, based upon the observed up-regulation of the glyoxylate pathway in the transcriptional data; knocking out a threonine transporter, *tdcC*, involved in the uptake of extracellular threonine back into the cells; and overexpressing a threonine exporter, *rhtC*. Furthermore, they found that upon running a fed-batch fermentation and using an *in silico* flux response analysis, significant acetate accumulation could be reduced by overexpressing the *acs* gene encoding acetyl-CoA synthetase, leading to greater threonine production. These successes were achieved through rational strain design, which is desirable given that unknown genetic mutations can lead to unpredictable strain behavior.

A similar systems metabolic engineering approach was taken to construct an efficient valine-producing *E. coli* strain (Park, Lee *et al.* 2007). Again, a base strain was first constructed by removing known feedback inhibition and transcription attenuation mechanisms via site-specific genome engineering. Competing pathways were also eliminated by deleting corresponding pathway genes. Finally, the *ilvBN* operon involved in the first valine pathway reaction was amplified. Once this base strain was constructed, transcriptional profiling and in silico gene knockout simulations were used to further optimize the strain. Target genes *ilvCED*, *ygaZH*, and *lrp*, encoding valine biosynthetic genes, a valine exporter, and an important global regulator leucine responsive protein, were overexpressed based upon transcriptional profiling

results. Finally, an *in silico* genome scale metabolic model was used to identify a triple knockout mutant (Δ*aceF* Δ*mdh* Δ*pfkA*) that further increased production specifically in the background of the *lrp* and *ygaZH* overexpressions. Since current *in silico* genome modeling does not account for regulatory or export mechanisms, the approach is complementary to transcriptional profiling which can uncover these effects. The final yield of valine was 0.378 g valine/g glucose, compared with a yield of 0.066 g valine/g glucose in the base strain and only minute amounts in the original strain.

A number of additional studies have examined transcriptional data with proteomic data, metabolomic data, or metabolic flux analysis. For example, Workman *et al.* (2006) integrated transcriptional binding profiles with genetic perturbations, mRNA expression, and protein interaction data to reveal both direct and indirect interactions between transcription factors and methyl-methanesulfonate responsive genes in yeast. They were able to generate a highly-interconnected physical map of regulatory pathways supported by binding and deletion-buffering profiles. Another study resulted in a 50% increase in *Aspergillus terreus* lovastatin production by comparing transcriptional and metabolomic data in wild type and recombinant strains and identifying corresponding gene targets for improvement (Askenazi, Driggers *et al.* 2003). Huser *et al.* focused on producing pantothenate in *Cornyebacterium glutamicum* and applied genome-wide transcriptional analysis and metabolic flux analysis to both the pre-engineered and production strain (2005). The production strain was engineered with deletions in the competing pathways of isoleucine and valine biosynthesis and overexpressions in the pantothenate biosynthetic route from pyruvate. It was seen that although the metabolic flux was successfully redirected away from valine biosynthesis, the pantothenate flux did not significantly increase. Instead, the substrate at the branching of these pathways, ketoisovalerate, was found to be

secreted outside the cell along with related byproducts. This suggested that the pantothenate pathway was saturated, and that the *panBC* overexpression may be increased for further production gains. Transcriptional analysis and hierarchical clustering showed enhanced expression of genes involved in leucine biosynthesis, serine and glycine formation, regeneration of methylenetetrahydrofolate, de novo synthesis of nicotinic acid mononucleotide, and acyl coenzyme A conversion. Thus, new and unexpected targets for further strain optimization were discovered through this approach.

3.4. Challenges of Omics Data Integration

Systems biology is concerned with obtaining and integrating data sets from multiple levels of cellular organization in order to gain a more complete understanding of the cellular system. Joyce and Palsson *et al.* (2006) recently reviewed current efforts to integrate multiple omics data sets for systems biology in order to address biological questions that would increase understanding of systems as a whole. These data sets include sequence information, transcriptional expression, protein expression, protein interactions, protein-DNA interactions, metabolic pool and flux measurements, amongst other possibilities. Existing and emerging experimental methods for these measurements include traditional and deep sequencing, DNA microarrays, LC-MS, GC-MS, HPLC, 2-hybrid systems, and chromatin immunoprecipitation chip assays. These different experimental technologies measure different cellular system aspects to varying depths and breadths, and they inherently have high false-positive and high false-negative rates (von Mering, Krause *et al.* 2002; Hwang, Rust *et al.* 2005). Additionally, each technology includes systematic biases of differing natures. Because of these limitations, data integration of multiple data sets of various natures can lead to greater certainty in reducing these false positive and negatives as well as a more complete overall picture of the cellular system.

There are a number of challenges inherent in data integration for systems biology, as explained by Hwang *et al.* (2005). First, the data to be integrated can range from discrete, such as protein localization in particular organelles, to continuous, such as for mRNA or protein expression levels. A transcript or protein may also be found in only one of the samples being compared, preventing the reporting of a particular expression ratio. This was a common occurrence in the current study. Secondly, as mentioned, each experimental technique has a different degree of reliability and different amounts of the various types of error. Homemade DNA microarrays like the ones used in this study are cheaper but may exhibit more variability and yield less information than most current commercially available microarrays. LC-MS proteomic data can be analyzed using various data thresholds and algorithms, as seen in this work, but the different processing strategies can lead to different expression ratios and protein quantification. Third, each data set has its own systematic biases. For example, mass spectrometry approaches tend to preferentially identify the most highly abundant proteins. Fractionation of samples can improve on this tendency, but the overall bias remains. Finally, in addition to high-throughput data, other attractive sources of information such as small-scale experiments, curated databases, and computational predictions may be desirable to incorporate into a biological network being constructed. Although tools and approaches for merging gene and protein abundance data sets together into a comprehensive data set prior to integrated analysis remain limiting factors (Waters, Pounds *et al.* 2006), methods such as those developed by Hwang *et al.* (2005) demonstrate the usefulness of these approaches.

As discussed later in Chapter 7, global correlation between mRNA and protein abundances is often small to nonexistent (Gygi, Rochon *et al.* 1999; Ideker, Thorsson *et al.* 2001; Chen, Gharib *et al.* 2002; Griffin, Gygi *et al.* 2002; Tian, Stepaniants *et al.* 2004; Hwang, Rust *et*

al. 2005; Nie, Wu *et al.* 2006), indicating either the presence of posttranscriptional regulation of various forms or even negative feedback regulation. Such differing trends in the data highlight the complexity of these systems and underscore the need for multiple data sets in systems biology analysis to help uncover hidden regulation and truly define the mechanisms at work.

3.5. Data Integration Example

Ishii *et al.* (2007) provided a good example of integrating multiple layers of expression data describing the intertwined nonlinear and dynamic interactions among large numbers of genes, proteins, and metabolites in *E. coli* undergoing various perturbations. In their systems biology approach, they examined glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle of central carbon metabolism in particular and global responses in general to either changing the growth rate (environmental perturbation) or to knocking out all of these pathway genes individually (genetic perturbations). To accomplish this, they used the following: DNA microarrays and qRT-PCR to measure gene expression; two-dimensional differential gel electrophoresis (2D-DIGE) (Marouga, David *et al.* 2005) and liquid chromatography tandem mass spectrometry (LC-MS/MS) for protein analysis; and capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) (Soga, Ohashi *et al.* 2003; Soga, Baran *et al.* 2006) and metabolic flux analysis for metabolome analysis. This multiple lab effort discovered that metabolite levels remained fairly stable in response to the multiple perturbations, but different cellular strategies to maintain such stable levels seemed to be in effect depending upon the nature of the perturbation. For environmental changes in the concentration of a limiting metabolite controlling growth rate, the gene and protein expressions changed significantly to respond to the environmental stresses and increase the growth rate in order to maintain stable intracellular metabolite levels. For genetic knockout perturbations, however, they found that the

gene and protein expression changes required to keep stable metabolite concentration levels were small. In this case, they hypothesized that the stability was a result of the underlying network redundancy itself. Isozymes and alternative metabolic routes seem to provide structural redundancy in these networks, for the most part limiting enzyme protein and underlying gene expression changes when the cell is faced with genetic perturbations. The cellular regulation and mechanisms leading to the observed robustness were not identified, but this study provides an important basis for incorporating such data on a large scale. From these types of studies, hypotheses can be formulated and tested in order to further clarify the cellular networks leading to the observed phenotypes.

3.6. Summary

The examples of this chapter highlight the fact that a systems metabolic engineering approach incorporating whole genome, proteome, metabolome, or fluxome data can allow for greater production gains than only concentrating on the more intuitive changes aimed at the production pathway itself. Distal genes that are unknown in function or not known to interact with the pathway of interest can be uncovered through these high throughput, data-rich approaches. Even though this can also be accomplished through combinatorial searches where random mutations are introduced and resulting strains are screened for the desired phenotype, the latter approaches can introduce unintended consequences, may not always catch such targets, and will not provide as much information content helping to more completely explain *why* a certain genotype may be leading to the observed phenotype. Such information can be useful in translating general concepts to production challenges outside of the specific pathway of interest, potentially leading to more general metabolic engineering strategies for entire classes of

molecules. Such a systems metabolic engineering approach is illustrated in the next few chapters in the context of lycopene production.

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Chapter 4. MATERIALS AND METHODS

4.1. Strains, Plasmids, and Media

The *E. coli* K12 pAC-LYC PT5-*dxs*, PT5-*idi*, PT5-*ispFD* parental "pre-engineered" (PE) strain, provided by DuPont (Yuan, Rouviere *et al.* 2006) and used by Alper *et al.* (2005), utilizes the low copy plasmid pAC-LYC carrying the genes *crtEBI* from the plant pathogen *Pantoea agglomerans* (formerly known as *Erwinia herbicola*) (Cunningham, Sun *et al.* 1994) in order to synthesize high amounts of lycopene via the non-mevalonate isoprenoid pathway. pAC-LYC also carries the chloramphenicol resistance gene Cm^R. Overexpressions of *dxs*, *idi*, and *ispFD* were chromosomally incorporated previously without an antibiotic marker through promoter delivery (Yuan, Rouviere *et al.* 2006).

Additional gene knockouts have previously been identified either stoichiometrically (Alper, Jin *et al.* 2005) or combinatorially (Alper, Miyaoku *et al.* 2005; Alper and Stephanopoulos 2008) within the PE genetic background to further increase lycopene production. Throughout this report, the following terminology will be used to indicate specific gene deletions: PE: Pre-engineered strain; G: Δ*gdhA* (glutamate dehydrogenase); A: Δ*aceE* (pyruvate dehydrogenase); P: Δ_P*yjid* (hypothetical protein, recently characterized as encoding for the *iraD* antiadaptor protein disrupting *hnr* association with σ^S (Bougdour, Cunning *et al.* 2008; Merrikh, Ferrazzoli *et al.* 2009)); H: Δ*hnr* (global regulator facilitating the degradation of σ^S); and Y: Δ*ylie* (conserved inner membrane protein). The five mutant strains investigated in this thesis are the following: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ_P*yjiD* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY).

Typically, the PE and five mutant strains were grown to mid-exponential phase (OD_{600} = 0.4). Cultures were then harvested for total RNA for either DNA microarray analysis or LC-MS

proteomic analysis. Cultures grown for lycopene production measurement were not harvested but allowed to grow to accumulate lycopene, with the lycopene measured at various time points as described.

Strains were grown at 37 °C with 225 RPM orbital shaking in 1xM9-minimal media (Sambrook and Russell 2001) containing 5 g/L d-glucose and 68 μg/ml chloramphenicol. Strains harboring the *kanR* kanamycin resistance gene were also grown in 20 μg/ml kanamycin. All cultures were grown in 50 ml culture in a 250 ml flask with 1% inoculation from an overnight 5 ml culture grown to stationary phase in M9-minimal media, employing the same growth methods as Alper (2006). All chemicals were from Mallinckrodt (Hazelwood, MO) and Sigma Aldrich (St. Louis, MO). All experiments were performed in replicate to validate data and calculate statistical parameters. Cell density was monitored spectrophotometrically at 600 nm. In some experiments, glucose monitoring was conducted periodically using a YSI2300 glucose analyzer (YSI Incorporated, Yellow Springs, OH) to verify complete usage of glucose.

The strains and plasmids used in this study are described below and in Table 4-1.

Table 4-1 Strains and plasmids used in this study.

Strain/Plasmid	Genotype/Description	Source/Reference
Parental "pre-engineered" (PE) strain	***E. coli* K12 PT5-*dxs*, PT5-*idi*, PT5-*ispFD***	**(Yuan, Rouviere *et al.* 2006)**
G	**PE Δ*gdhA***	**(Alper, Jin *et al.* 2005)**
GA	**G Δ*aceE***	**(Alper, Jin *et al.* 2005)**
GAP	**GA Δ_P*yjiD***	**(Alper, Miyaoku *et al.* 2005)**
H	**PE Δ*hnr***	**(Alper, Miyaoku *et al.* 2005)**
HY	**H Δ*yliE***	**(Alper and Stephanopoulos 2008)**
pAC-LYC	**Contains *crtEBI* operon**	**(Cunningham, Sun *et al.* 1994)**
pZE *wrbA*	**pZE *wrbA***	**(Alper, Fischer *et al.* 2005); This study**
pZE *ydeO*	**pZE *ydeO***	**(Alper, Fischer *et al.* 2005); Christine Santos (2008, unpublished)**
pZE *gadE*	**pZE *gadE***	**(Alper, Fischer *et al.* 2005); Christine Santos (2008, unpublished)**
pZE *gfp*	**pZE *gfp***	**(Alper, Fischer *et al.* 2005)**

4.2. Lycopene Measurement

4.2.1. Lycopene Assay

Intracellular lycopene content was extracted from 1 ml bacterial culture at various time points. The cell pellet was washed and then extracted in 1 ml acetone at 55ºC for 15 min with occasional vortexing. The lycopene content in the resulting supernatant was measured via absorbance at 475 nm (Kim and Keasling 2001) and concentrations were calculated through a standard curve. This process was carried out under low light conditions to prevent photobleaching and degradation. Cell mass was calculated by correlating dry cell weight with OD_{600} for use in parts per million (PPM: (10^6*mg lycopene/mg dry cell weight)). For consistency with previous work, the same standard curves and spectrophotometer equipment was used as employed in previous studies in which the strains were developed and initially measured for production (Alper, Jin *et al.* 2005; Alper, Miyaoku *et al.* 2005; Alper, Miyaoku *et al.* 2006;

Alper 2006; Alper and Stephanopoulos 2008). Typical lycopene yields including growth were on the order of 0.02 g/L or about 0.4% yield on glucose (g lycopene/g glucose). These values and the values measured by Alper *et al.* (Alper, Jin *et al.* 2005) are well below the maximum stoichiometric yield calculated to be approximately 10% yield on glucose (g lycopene/g glucose) (Alper, Jin *et al.* 2005), as would be expected for a secondary metabolite. Thus, there appears to be room for improvement, although other factors such as lycopene storage limitations in the membrane (Albrecht, Misawa et al. 1999) may limit actual production levels well below the theoretical maximum.

4.2.2. Strain Variability for Lycopene Production

It should be noted that the lycopene production profiles presented in this thesis reflect the same trends but were consistently lower compared to those production values displayed by Alper *et al.* (Alper, Jin *et al.* 2005; Alper, Miyaoku *et al.* 2005; Alper, Miyaoku *et al.* 2006; Alper and Stephanopoulos 2008). This is likely due to systematic measurement differences or observed strain variability. For example, Figure 5-14 displays these systematic differences in lycopene production. Since the conclusions of this thesis are dependent upon the relative values of lycopene production between the PE and mutant strains, these differences did not affect the expression analysis work or results.

Variation was observed within the PE strain between various points in this work. In Figure 4-1, two PE flask samples were discarded from the analysis (labeled “August 18- 20 A-3” and “August 18-20 B-2”) while analyzing the effects of the *iclR* and *pntB* deletions because the PE lycopene production varied from the third and fourth replicate flasks by up to 60%, where the production of these third and fourth flasks (labeled “August 18-20 B-3” and “August 18-20 A-2”) were much more consistent with previous PE strain productions observed in this work.

These two strains appear to be outliers based upon the other data and were thus discarded from further analysis. An apparent trend from Figure 4-1 is that $\sigma^2_{day} > \sigma^2_{colony} > \sigma^2_{flask}$. That is, for all the strains, it appears that the particular experimental day is a greater source of variation than the colony selected, which in turn is a greater source of variation than the replicate flasks grown for each colony. Reasons for this are unclear but could be related to strain or plasmid instability. Mathematical models of the intrinsic noise in prokaryotic gene regulation indicate stochastic fluctuations and cell–cell variations in gene regulation can have significant impacts on the mRNA and protein abundance and their relationship with each other (Thattai and van Oudenaarden 2001). However, the fact that these abnormally high production levels were recorded in only two flasks suggests that mutation or instability was the more likely culprit.

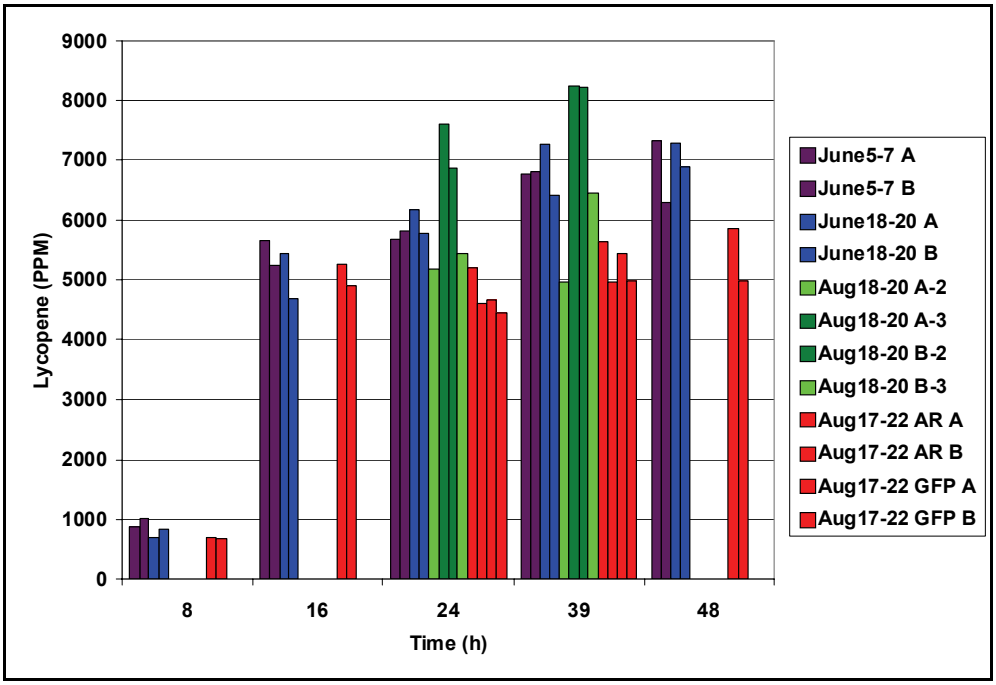

Figure 4-1 Observed variation in lycopene production for the PE strain that has served as a basis for all metabolic engineering in this work.

4.3. DNA Microarrays for Transcriptomic Analysis

DNA microarrays were printed and prepared largely according to Perry (2004), from which much of the scanning and image analysis protocol was taken as well. The experimental

protocol used for DNA microarrays was slightly modified from protocols previously described (DeRisi, Iyer *et al.* 1997; Au, Kuester-Schoeck *et al.* 2005; Goranov, Katz *et al.* 2005). Microarray data analysis and differential expression testing was accomplished via a maximum likelihood method (Ideker, Thorsson *et al.* 2000).

4.3.1. Gene Plate Preparation and DNA Microarray Printing

As described previously (Perry 2004), PCR products used for printing microarrays were the gift of Dr. Susan Lovett (Brandeis University, Waltham, MA). The PCR products were generated by using a primer set (Sigma-Genosys *E. coli* ORFmers) based on the University of Wisconsin annotation for the *E. coli* genome (Blattner, Plunkett *et al.* 1997). This set consists of 4,290 primer pairs that amplify the open-reading frames of each gene. The success of each PCR reaction was checked by running samples on native agarose gels and recorded appropriately. The PCR products were dissolved in 50% (v/v) dimethyl sulfoxide (DMSO), producing concentrations of approximately 67 ng/uL.

DNA microarrays were printed on Corning (Corning, NY) UltraGAPS amino silane coated glass slides. This was accomplished using a BioRobotics MicroGrid II arrayer (MIT BioMicrocenter) utilizing 16 metal pins controlled by a robotic arm. Basically, the pins were dipped into the 384-well plates containing the ORFs, blotted 10 times onto a sample slide to control spot size, and printed onto 60 slides per sample dip by contacting the pins with the slide surface and depositing the DNA onto the slide surfaces. Three wash cycles of 3 s each were completed before and after each printing run of 60 slides in a water bath in order to avoid sample contamination. The 4,290 *E. coli* genes were distributed amongst 12 384-well PCR plates in addition to controls, from which the arrayer sampled and printed onto the slides. One plate was printed twice onto the slide, and otherwise each gene was printed once per slide. During

analysis, the spots printed twice simply had more replicates for analysis. The genes were printed into a square array of 16 total 18x18 spot grids, with 10 spots left empty in each grid. Spots were separated by a 250 μm spacing. Thus, 5,024 spots were printed in total on each microarray slide. These spots included the following control spots that were not analyzed quantitatively but were used to ensure the quality of each microarray hybridization experiment: 50% DMSO; 3xSSC; Hae III-digested *E. coli* K12 genomic DNA; *E. coli* tRNA; *E. coli* rRNA; a yeast library; calf thymus DNA; a human library; viral PCR product; RAP17 C-GlyGly PCR product; pWKS 130 *recJ* D281 plasmid; pWSK2a C552 plasmid; pBSSK plasmid; and pBSSK XSeA plasmid (Perry 2004). Following printing, the slides were stored in a desiccator until use, waiting at least 24 h for the slides to dry. Before microarray experiments, the slides were cross-linked in a UV Stratalinker 2400 (Stratagene, La Jolla, CA) with a dose of 900x100 μJ. Gene array list (GAL) files, which provide the spot location of each gene on the microarray, were generated based upon the printing by the BioRobotics arrayer software.

4.3.2. DNA Microarray Experimental Method and Analysis

4.3.2.1. Bacterial Culture Growth for DNA Microarray Analysis

Strains of the parental pre-engineered (PE) strain or the 5 mutant strains studied, the Δ*gdhA,* the Δ*gdhA* Δ*aceE,* the Δ*gdhA* Δ*aceE* Δ_P*yjiD,* the Δ*hnr,* and the Δ*hnr* Δ*yliE* strains, were grown at 37 °C with 225 RPM orbital shaking in M9-minimal media (Sambrook and Russell 2001) containing 5 g/L d-glucose and 68 μg/ml chloramphenicol. All cultures were grown in 50 ml culture in a 250 ml flask with 1% inoculation from an overnight 5 ml culture grown to stationary phase in M9-minimal media, employing the same growth methods as Alper (2006). All chemicals were from Mallinckrodt (Hazelwood, MO) and Sigma Aldrich (St. Louis, MO). All experiments were performed in replicate to validate data and calculate statistical parameters.

These biological replicates were especially important in array experiments since most genes were only printed once per array, and single microarrays can be susceptible to high rates of false positives and false negatives as high as 10% of the total number of genes (Lee, Kuo *et al.* 2000). Cell density was monitored spectrophotometrically at 600 nm, and cells were harvested for RNA extraction in mid-exponential growth phase at about OD_{600} = 0.4. Based upon cellular growth curves, harvest times were approximated, and final spectrophomteric measurements were taken upon culture harvest.

4.3.2.2. Culture Harvest and Isolation of Total RNA

In all subsequent steps, RNase-free pipette tips and materials were used to avoid ribonuclease-degradation of RNA. Bacterial culture was then added to RNAprotect Bacteria Reagent (Qiagen, Germantown, MD) for stabilization and isolation of total RNA according to the manufacturer's protocol. 5 ml of culture was added to 10 ml RNAprotect Bacteria Reagent. Pellets were either stored at -20 °C for up to 2 weeks or kept at RT to proceed with the protocol. 200 µl of TE buffer containing 1 mg/ml lysozyme (Sigma Aldrich, St. Louis, MO) was next added to the pellet at RT, and the mixture was incubated at RT for 5 min following vortexing. 700 µl Buffer RLT containing 1% β-mercaptoethanol (Pierce, Rockford, IL) to inhibit ribonucleases and 500 µl ethanol were added to the lysate according to the standard protocol.

Isolation of total RNA was accomplished by continuing with the RNeasy Mini kit (Qiagen, Germantown, MD) according to the manufacturer's protocol. Two RNeasy Mini Column loading steps of about 700 µl each were used. A RNase-Free DNase Set (Qiagen, Germantown, MD) was used to digest DNA in the sample and used according to the manufacturer's protocol. 350 ml Buffer RW1 was pipetted into the RNeasy Mini column, and the column was centrifuged for 15 s at over 12,000 x g. After discarding the flow-through, 10 µl

of the prepared DNase I stock solution was added to 70 µl of Buffer RDD, with the mixture added directly onto the center of the RNeasy silica-gel membrane. After a 15 min incubation at RT, 350 µl Buffer RW1 was pipetted onto the RNeasy column and centrifuged as before, discarding the flow-through and collection tube afterwards. The column was then added to a new 2 ml collection tube, and the rest of the protocol was followed according to the manufacturer's instructions. Elution was completed using 50 µl RNase-free water (Ambion, Austin, TX). To obtain a higher total RNA concentration, a second elution step was performed using the first eluate. Resulting total RNA concentrations were measured using a UV/Vis BioPhotometer (Eppendorf, Westbury, NY), which measures and calculates RNA concentration based upon the A_{260} and A_{260}/A_{280} ratios indicating the relative ratio of nucleic acids to proteins. Typical concentrations and relative ratios were 0.6 to 1.3 µg/µl and greater than 1.8, respectively, exhibiting acceptable RNA yields and protein contamination values.

4.3.2.3. Reverse Transcriptase Reaction and RNA Degradation

The remaining experimental procedure for DNA microarrays was slightly adapted from protocols described previously (DeRisi, Iyer *et al.* 1997; Au, Kuester-Schoeck *et al.* 2005; Goranov, Katz *et al.* 2005). The protocol below is taken from Goranov *et al.* (2005) with slight modifications. In the first reverse transcriptase reaction, the mRNA from the bacterial culture samples are used as templates for synthesizing cDNA targets, which are eventually hybridized to the complementary DNA probes attached to the microarrays.

To generate cDNA, RNA from the different experimental conditions was reverse-transcribed in the presence of amino-allyl-dUTP, followed by coupling to Cy5, for all experimental samples, or Cy3, for the parental pre-engineered (PE) strain. In initial experiments determining the method variability and the critical p value for differential expression, Cy5 and

Cy3 were both coupled to the PE strain cDNA. All components were obtained from Invitrogen (Carlsbad, CA) unless otherwise noted.

For reverse-transcriptase reactions, 10 μg of RNA template was mixed with 5.0 μg of random hexamers (in 17.8 μl), incubated at 70°C for 10 min, and placed on ice for 5 min. Reverse-transcription reactions were then started by the addition of a mixture resulting in a final solution of RNA template, random hexamer primers, 300 units of Superscript Reverse Transcriptase III (Invitrogen, Carlsbad, CA), 1× RT buffer, 10 mM DTT, 20 units of RNase Out, and deoxyribonucleoside triphosphates (0.5 mM each dATP, dCTP, and dGTP; 0.1 mM dTTP; 0.4 mM aminoallyl-dUTP (Ambion, Austin, TX)) in a final volume of 30 μl. The labeling reactions were incubated at 25°C for 10 min, at 42°C for 70 min, and then shifted to 70°C for 15 min to stop the reactions. RNA in the reactions was degraded by adding NaOH (33 mM final concentration) and incubating at 70°C for 10 min. HCl (25 mM final concentration) was added to each reaction to neutralize the pH.

4.3.2.4. Cleanup, Dye Coupling, and Quenching

Reactions were purified with Qiagen (Germantown, MD) MinElute kits and eluted in 10-μl volumes of RNase-free water (Ambion, Austin, TX). The manufacturer's protocol was followed except the Buffer PE wash was replaced with a second ethanol wash to avoid free amines in the buffer potentially competing with the dyes during coupling. The purified cDNA was either stored at -20°C or coupling was immediately performed at RT.

In low light conditions, 0.5 μl of 1 M $NaHCO_3$ (pH 9.0) was added to adjust the pH for the coupling reactions. To couple the fluorescent dyes to cDNA, 1 μl of freshly dissolved Cy3 (generally for mutant strains) or Cy5 (generally for PE strain) dye (Amersham Biosciences, Amersham, UK) was added to cDNA and incubated for 1 h in the dark, mixing every 15 min.

Reactions were quenched by incubation with 1.4 M hydroxylamine (Sigma Aldrich, St. Louis, MO) for 15 min. Each mutant (Cy5-labeled fluorescing red) sample was mixed with an aliquot of reference PE cDNA (Cy3-labeled fluorescing green), and mixed samples were purified with Qiagen (Germantown, MD) MinElute kits. Typical dye incorporation was calculated according to the manufacturer's method and found to be about 60-180 pmole/μg nucleic acid.

4.3.2.5. Pre-hybridization, Hybridization, Washing, and Scanning

The labeled samples were mixed with 1 μg of salmon sperm DNA (Sigma Aldrich, St. Louis, MO) and 0.8 μg of yeast tRNA (Invitrogen, Carlsbad, CA), and the volume was adjusted to 14 μl with RNase-free water (Ambion, Austin, TX). The samples were heated to 100°C for 5 min, spun down, mixed with 2× hybridization buffer (0.05% SDS (Ambion, Austin, TX), 5× SSC, 25% formamide (Riedel-de Haan, Seelze, Germany) final concentration, kept at 52°C) and hybridized to DNA probes on the previously prepared microarray slide for at least 16 h at 42°C. Previous preparation for the microarray slide included incubating the slides in prehybridization buffer (5× SSC, 0.1% SDS (Ambion, Austin, TX), 1% BSA (Roche, Indianapolis, IN)) at 42°C for at least 45 min, washing the slide in ddH2O, spinning the array, and drying with nitrogen gas. The hybridization mixture was pipetted to the sides of a water- and ethanol-cleaned, standard size LifterSlip (Erie Scientific, Portsmouth, NH) placed on the array beforehand to ease sample addition and avoid the production of air bubbles under the cover slip. Hybridization was performed within standard size Corning Hybridization Chambers (Corning, NY) with about 10 μl water added to the wells on either side of the chamber. After hybridization, arrays were washed with 1× SSC, 0.2% SDS for 5 min at 42°C (pre-warmed), followed by a 5-min wash with 0.1× SSC, 0.2% SDS at RT and a final 5-min wash in 0.1× SSC at RT. Arrays were spun to remove extra liquid and dried with nitrogen gas.

4.3.2.6. Microarray Scanning and Image Analysis

Arrays were scanned with a GenePix 4000B scanner and analyzed with GenePix 3.0 software (Axon Instruments, Union City, CA). Much of the scanning and image analysis protocol is taken from Perry (2004). The scanning voltage was optimized per microarray in order to gain maximal signal detection while avoiding as much saturated signal as possible (rRNA control sample, for example, was saturated). Slides were scanned at 532 nm (for Cy3) and 635 nm (for Cy5). The four images (low-resolution preview scan, 532 nm image, 635 nm scan, and ratio image) were saved as TIFF files at a 10 μm/pixel resolution.

Image analysis consisted of several steps. Utilizing the GAL file created from the microarray printing step, the GenePix software placed a grid of virtual spots (features) over the image. First, the grid was manually adjusted and aligned to the actual printed features on the array, adjusting the spot diameter as well to fit the image. Following manual grid adjustment, the GenePix Alignment algorithm was utilized to adjust the fit for the entire array. The algorithm was set to adjust the size of the virtual spots between twice and half their original sizes of about 10-15 pixel diameters. The resulting alignment was reviewed in each case, and certain spots were flagged as "Bad" when appropriate. This occurred, for example, if dust or scratches rendered a specific spot unreadable. Virtual spots were aligned manually when appropriate if the Alignment algorithm had missed a particular feature. Less than 1% of spots were altered from the GenePix alignment.

In the above GenePix Alignment algorithm, no threshold value was set to distinguish feature from background pixels, emphasizing spot filtering by visual inspection. Spot filtering was accomplished as follows. First, spots corresponding to ORFs which had failed the initial PCR reaction (208 of the 4,290 reactions failed after two attempts) were removed from analysis.

Next, all control spots not corresponding to *E. coli* K12 genes were removed from analysis. Although such spots were useful in interpreting data quality from a single array, they tended to have very weak or very strong signals and thus were not included in further analysis so as not to skew the data normalization and analysis. The number of discarded spots due to unsuccessful PCR reactions was 332, and 268 control spots were similarly discarded from analysis. Thus, of the 5,024 printed spots, a maximum of 4,424 were analyzed for data on each array. However, those spots which were manually flagged as "Bad" during the alignment step were also removed from consideration because reliable data were not extractable from them, decreasing the number of analyzed spots below 4,424 on a per array basis. Additionally, spots with low signal were subjected to a t-test by GenePix for both Cy3 and Cy5 channels to determine whether the mean pixel intensity in the feature was greater than the mean pixel intensity in the background. Spots determined to have low signal by this method (similar feature and background intensity) were also discarded from further analysis.

Once the feature pixels were defined, the GenePix software defined the background by first defining a concentric circle with three times the diameter of the spot itself. Any pixels within this circle and at least two pixels from the feature were considered background pixels. With the background defined, GenePix calculated statistics such as mean, median, mode, and standard deviation for each spot with both feature and background pixels. Signal was calculated for each spot as follows:

$$S_{Cy3} = (I_{F,532})_{Med} - (I_{B,532})_{Med} \qquad (4.1)$$

$$S_{Cy5} = (I_{F,635})_{Med} - (I_{B,635})_{Med} \qquad (4.2)$$

where S_{Cy3} and S_{Cy5} are the signals for the Cy3 and Cy5 dyes, respectively, and the $(I)_{Med}$ variables represent the median feature intensities for feature (F) and background (B) at 532 nm

and 635 nm. Raw signal ratios $\log_{10}(S_{Cy5}/S_{Cy3})$ could then be assigned to each spot corresponding to the ratio of Cy5-labeled mutant to Cy3-labeled PE expression for a particular mRNA.

4.3.2.7. Microarray Data Normalization and Differential Expression Analysis

Microarray data extracted from the scanned images was next normalized and analyzed for differential expression using a maximum likelihood method (Ideker, Thorsson *et al.* 2000). Unlike simple ratio-based methods of analysis, this method compares a series of repeated measurements of two dye intensities for each gene in order to form estimations of the true gene expression for each sample as well as associated error parameters for these values. This is critical because the uncertainty in the expression ratios is greater for genes that are expressed at low levels than for those that are highly expressed. Additionally, this model explicitly accounts for errors unlike some other microarray analysis models. The method is briefly reviewed here but discussed in detail by Ideker *et al.* (2000).

A distribution of intensities x_{ij} and y_{ij} are considered for each gene i and replicate measurement j. Spot intensities are extracted from the scanned image for each array, filtered, background-subtracted, and normalized using the non-linear method of Workman *et al.* (2002) to have the same medians for both channels x and y. This normalization was done on a per array basis and makes two assumptions. First, it is assumed that although expression of many genes may be changing, the overall changes balance one another such that overall gene expression remains constant. Second, it is assumed that constant overall gene expression translates to constant overall microarray signal. An example of $\log_{10}$(Cy5/Cy3) signal ratios before and after the normalization procedure is given in Figure 4-2. A normal distribution with the same mean and standard deviation is also shown for reference. The raw data for this single array, which has

only been background-subtracted, compares two colonies of the same PE strain and shows that the normalization procedure brings the median log ratio to approximately 0 and a "near"-normal distribution, preserves the signals instead of collapsing all log ratios to 0, and eliminates the heavy "tails" of the raw data distribution.

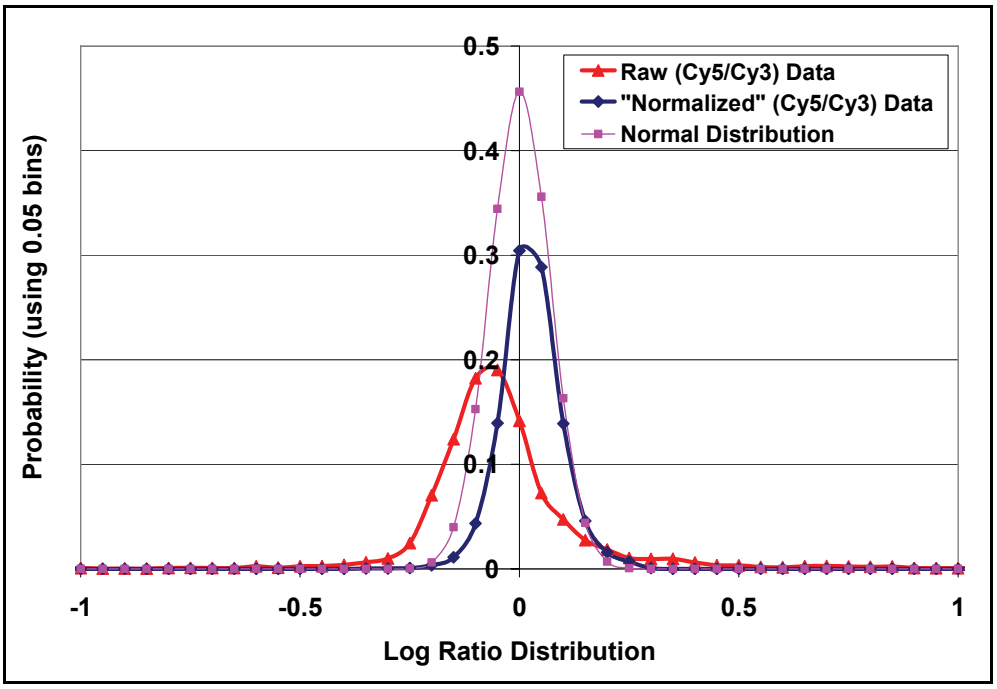

Figure 4-2 Example of $\log_{10}$(Cy5/Cy3) signal log ratios before and after normalization procedure.

Following normalization, a Variability and Error Analysis (VERA) is performed in which relationships between experimental data, true (mean) intensities μ_{ij}, and multiplicative and additive errors ε_{ij} and δ_{ij}, respectively, are formed according to the following equations:

$$x_{ij} = \mu_{x_i} + \mu_{x_i}\varepsilon_{x_{ij}} + \delta_{x_{ij}} \qquad (4.3)$$

$$y_{ij} = \mu_{y_i} + \mu_{y_i}\varepsilon_{y_{ij}} + \delta_{y_{ij}} \qquad (4.4)$$

The errors individually follow bivariate normal distributions, so the samples (x_{ij}, y_{ij}) are also described by such a distribution with parameters μ_{x_i}, μ_{y_i}, σ_{x_i}, σ_{y_i}, and $\rho_{x_i y_i}$, which are the true means, the standard deviations of the signals, and the signal correlation coefficient, respectively.

These parameters can in turn be described by a mean pair per gene, $\boldsymbol{\mu}$, and six gene independent parameters, $\boldsymbol{\beta} = \left(\sigma_{\varepsilon_x}, \sigma_{\varepsilon_y}, \rho_{\varepsilon}, \sigma_{\delta_x}, \sigma_{\delta_y}, \rho_{\delta}\right)$ according to the following equations:

$$\sigma_{x_i} = \sqrt{\mu_{x_i}^2 \sigma_{\varepsilon_x}^2 + \sigma_{\delta_x}^2} \qquad (4.5)$$

$$\sigma_{y_i} = \sqrt{\mu_{y_i}^2 \sigma_{\varepsilon_y}^2 + \sigma_{\delta_y}^2} \qquad (4.6)$$

$$\rho_{x_i y_i} = \frac{\mu_{x_i}\mu_{y_i}\rho_{\varepsilon}\sigma_{\varepsilon_x}\sigma_{\varepsilon_y} + \rho_{\delta}\sigma_{\delta_x}\sigma_{\delta_y}}{\sigma_{x_i}\sigma_{y_i}} \qquad (4.7)$$

These parameters are generally unknown and are estimated by the method of maximum-likelihood estimation (MLE) (Kendall and Stuart 1979).

In the MLE method, likelihood functions for gene i and over all genes are respectively defined according to the following equations:

$$L_i(\beta, \mu_{x_i}, \mu_{y_i}) = \prod_{j=1}^{M} p\left(x_{ij}, y_{ij} \middle| \beta, \mu_{x_i}, \mu_{y_i}\right) \quad (4.8)$$

$$L(\beta, \mu) = \prod_{i=1}^{N} L_i\left(\beta, \mu_{x_i}, \mu_{y_i}\right) \quad (4.9)$$

The MLE parameter values maximizing L, designated $\hat{\boldsymbol{\beta}}$ and $\hat{\boldsymbol{\mu}}$, are estimates of the true parameters underlying the statistical model and are found via standard optimization techniques. $\boldsymbol{\beta}$ and $\boldsymbol{\mu}$ are sequentially fixed at initial or previous values while the other parameter is selected to maximize *L*, and the procedure is repeated until $\boldsymbol{\beta}$ and $\boldsymbol{\mu}$ converge.

Once these model parameters have been determined, the significance of array (SAM) analysis is performed on the values. This tests the alternate hypothesis signifying differential expression, whether $\mu_{x_i} \neq \mu_{y_i}$, by comparing $\max_{\mu} L_i\left(\hat{\boldsymbol{\beta}}, \mu, \mu\right)$ to $\max_{\mu_x, \mu_y} L_i\left(\hat{\boldsymbol{\beta}}, \mu_x, \mu_y\right)$. The first expression is a maximization where the $\mu_{x_i} = \mu_{y_i}$ constraint is imposed, whereas the second

maximization is an unconstrained problem. This comparison is accomplished by the generalized likelihood ratio test (GLRT) (Kendall and Stuart 1979), and a p value is output that describes the likelihood that the particular gene is truly differentially expressed between two tested samples. Using a critical p value as a cutoff, all genes with smaller, *i.e.* more significant, p values can then be selected as exhibiting differential expression. This critical p value can be chosen, for example, as corresponding to the bottom 0.1% of p values in a control experiment hybridizing the same sample against itself (Ideker, Thorsson *et al.* 2000). Using this method, a critical p value of 0.00426 was selected as a critical p value for differential expression. A "relaxed" p value of 0.01 was also applied as indicated in order to determine additional genes that "nearly" exhibited differential expression under the test imposed and may, in fact, be false negatives when using the critical p value cutoff.

False discovery rate (FDR) methods are becoming more prevalent in microarray analysis (Allison, Cui *et al.* 2006), and these methods control the rate of type 1 errors to some defined and acceptable level (Benjamini and Hochberg 1995; Storey and Tibshirani 2003). This is in contrast to family-wise error rate (FWER) control methods such as the Bonferroni correction, which limit the probability of making *any* type 1 errors in multiple testing. These methods are usually performed following the initial differential expression analysis. It should be noted that the maximum likelihood estimate (MLE) method applied in this thesis did not explicitly control for the FDR or apply a Bonferroni correction, the latter of which is usually deemed overly cautious and leads to many false negatives. Instead, though, the VERA and SAM method models the error and determines differential expression based upon the MLE method, an alternative statistical approach. Furthermore, the experimental validation presented in section 4.3.3 using "self-self" (PE-PE) comparison microarrays lends greater confidence to the differential

expression determinations. Further disqualification of differentially expressed genes by VERA-SAM was not pursued given the low numbers of genes identified in the mutant strains and the desire to avoid false negatives.

Actual implementation of the method by Ideker *et al.* (2000) is accomplished by the VERA (Variability and Error Assessment) and SAM (Significance of Array Measurement) tools freely available online for Windows and Unix platforms (http://db.systemsbiology.net/software/VERAandSAM/). The Unix workflow followed for this study is also described in greater detail in Appendix A.2.8 of Dr. Joel Moxley's doctoral thesis (2007). Basically, GPR files corresponding to single microarrays were named in text file tables. A "pre-process" script was used to convert raw intensity data contained in the GPR files into a sorted list of background-subtracted, normalized intensities for each gene on the DNA microarray. A "mergeReps" script was then run to merge biological DNA microarray replicates and compute the average expression ratio of each gene over the replicate measurements. The "VERA" script was then run to estimate the error model parameters from replicated, preprocessed experiments and to use the error model to improve the accuracy of the expression ratio. Next, the "SAM" script was run to assign a p value to each gene indicating the likelihood of differential expression. These latter two tools accomplish the MLA method described above (Ideker, Thorsson *et al.* 2000). Finally, the "mergeConds" script combines multiple condition comparisons (such as multiple mutants compared to the PE strain in this study) and merges them into a single text file containing a list of expression ratios for the genes on the microarray (rows) over the conditions assayed (columns). A "PVALS.logratios" file was generated containing the estimated true expression ratios for the conditions as well as the corresponding p values across all genes.

Before the "pre-process" and "mergeConds" steps, all data from spots corresponding to the unsuccessful PCRs and control spots were discarded so as not to affect the normalization or the VERA-SAM analysis. Additionally, "bad" spots and spots with low signal, as described above, were discarded from further analysis. Spots with saturated signal in one or both fluorescent channels were excluded from the normalization step so as not to affect the global distribution of signal, but they were included for the VERA-SAM analysis to determine differential expression. Since these spots correspond to very high signals, it was reasoned that they may still be exhibiting differential expression. Finally, if signal from only one of the replicate arrays was detected for a particular gene, then the true expression ratio was set equal to the normalized ratio for that gene as an estimate. The Python and PEARL programming languages were utilized to ease the VERA-SAM microarray analysis workflow. Microsoft Excel was used extensively to organize and analyze transcriptional data.

4.3.2.8. Hierarchical Clustering of Transcriptional Data

Normalized $\log_{10}$(Mutant/PE) DNA Microarray gene ratio data were clustered using Cluster version 3.0 (de Hoon, Imoto *et al.* 2004) with complete linkage hierarchical clustering (Eisen, Spellman *et al.* 1998) and a centered Pearson correlation for both "Genes" and "Arrays." The review by D'haeseleer (2005) provides valuable explanatory detail on clustering methods applied to expression data. Data were filtered by requiring that at least 4 of the 5 $\log_{10}$(Mutant/PE) ratios were present for a given gene in order to include it in the clustering. Dendograms were visualized using Java Tree View version 1.1.0 (Saldanha 2004).

4.3.2.9. Pathway Analysis and EcoCyc Omics Viewer

The transcriptional data were overlaid onto the metabolic maps of *E. coli* using the EcoCyc database "Omics Viewer" tool (Keseler, Bonavides-Martinez *et al.* 2009). Additionally,

the EcoCyc database was utilized extensively to retrieve functional annotations and information for the transcriptional data.

4.3.3. DNA Microarray Validation Experiments

4.3.3.1. DNA Microarray Protocol Variation Analysis

Before studying the gene expression changes between the various mutant and PE strains, the variations of the distinct steps of the experimental procedure were analyzed in order to quantify error and better assess later microarray results. Four main sources of variation were identified initially: at the bacterial "colony" level (biological) between various genetically-identical cells from different bacterial colonies; at the "cultivation" level (biological) between various shake flask cultivations of cells from the same original bacterial colony; at the "RNA extraction" level (experimental) for samples from the same colony and cultivation but separate RNA extractions; and at the "microarray" level (experimental) for samples from the same colony, cultivation, and RNA extraction but different microarray hybridizations. The "colony" variability is cumulative in the sense that it encompasses the variability at the other three steps as well. Similarly, the "cultivation" variability is cumulative in that it encompasses the variability for the "RNA extraction" and "microarray" levels as well, and so on. It is of interest to capture the maximal biological variability in this work yet to minimize experimental variability. The latter was significantly reduced via improving experimental technique with experience. An initial hypothesis was formulated that the cumulative variability at the "colony" level would be the largest, whereas the corresponding variability at the "microarray" level would be the smallest. It should be noted that the use of the RNAprotect Bacteria reagent (Qiagen, Germantown, MD) was started after some of these variability microarray experiments had already been completed and is indicated by the "protect" label.

As an example, Figure 4-3 below displays the experimental procedure followed to determine the "colony" variability. Three separate PE bacterial colonies with identical genetic backgrounds were first grown overnight, and each was used to inoculate a separate bacterial cultivation. Each of these three cultivations was then subjected to the "RNA extraction" protocol, after which the first RNA sample was used to generate cDNA labeled with Cy3 dye that was hybridized with each of the other two cDNA samples labeled with Cy5 dye, as shown in Figure 4-3. Since the three samples came from genetically identical PE cells grown under the same conditions, a null hypothesis was made that the gene expression for each gene i on "self-self" (here, PE-PE) arrays 1 and 2 should be such that $x_{i1} = y_{i1} = x_{i2} = y_{i2}$, where x and y refer to the Cy3 and Cy5 channels, respectively. In other words, all feature spots should be yellow in the absence of variance, indicating identical gene expression in the samples compared. Similar PE-PE experiments were completed to quantify the variability at the "cultivation" level, where the same bacterial colony was used to inoculate three separate cultivations, with the resulting cDNA samples being hybridized similar to the setup shown in Figure 4-3. Similar experiments were also completed for the "RNA extraction" and "microarray" levels as well.

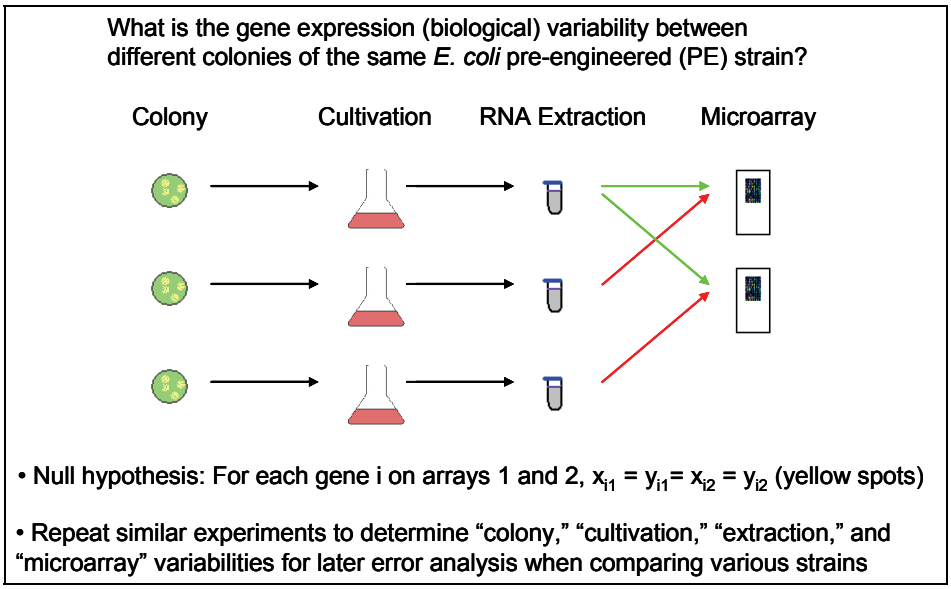

Figure 4-3 Example of variation analysis for the microarray experimental approach. PE-PE ("self-self") arrays are shown for biological colony replicates.

In total, 16 PE-PE microarray experiments were completed for 7 sets in this analysis in the following chronological order: three "extraction variation" arrays; two "microarray variation" arrays; three "cultivation variation" arrays; two "colony-protect variation" arrays; two "cultivation-protect" arrays; two "RNA extraction-protect" arrays; and two "microarray-protect" arrays.

There is a general lack of consensus on how to analyze such microarray reproducibility experiments. Thus, the results of these microarray studies were first analyzed in the following way. The scanned images were subjected to pre-image analysis, background subtraction, and normalization, and the resulting ratios of Cy5 to Cy3 normalized signals were calculated for each gene spot on a particular array. Next, the "errors" were calculated for these ratios by taking the differences between these ratios and 1. These errors were then used to calculate the total root mean square error (RMSE) for each individual microarray. The RMSE values for each of the 16 high quality PE-PE microarrays are shown in Figure 4-4.

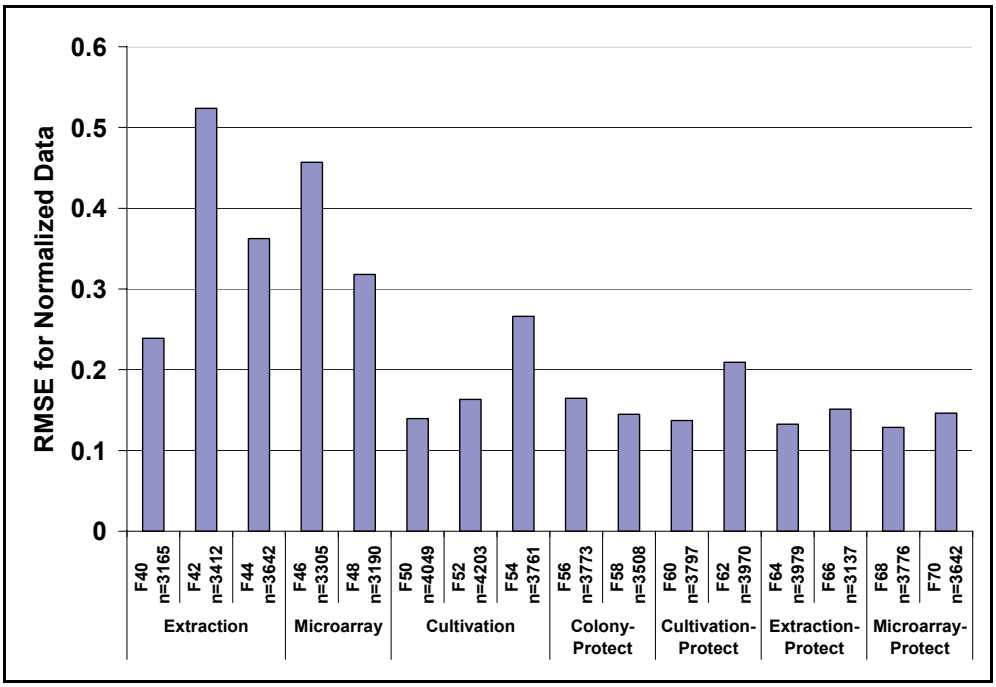

Figure 4-4 RMSE for filtered, background-subtracted, and normalized PE-PE ("self-self") microarrays analyzing variation at the colony, cultivation, RNA extraction, and microarray steps. "Protect" refers to utilizing RNAprotect Bacteria reagent (Qiagen, Germantown, MD).

Figure 4-4 shows that the RMSEs of all six of the "protect" arrays are smaller on average than the non-"protect" arrays. However, the microarrays towards the right of Figure 4-4 were also performed later in time, likely showing the benefit of experience for demanding experimental protocols such as microarrays. Thus, the plot could indicate that more recent microarrays have less error due to either increased skill or the use of the RNAprotect Bacteria reagent (Qiagen, Germantown, MD). For example, a comparison of the "cultivation" RMSE values both with and without the extra reagent seems to indicate only a relatively minor difference, suggesting time may be the dominant factor. This is further supported by the fact that the decreasing RMSE trend with time holds both for the non-"protect" and the "protect" sets taken individually. Nevertheless, the RNAprotect Bacteria reagent (Qiagen) was adopted into the protocol to decrease experimental variability. In any case, it appears that the four "protect" experimental variations are similar in terms of their RMSE statistics, and therefore triplicating

the "colony" level in comparison experiments can be justified to both maximize biological variability and minimize experimental error.

Additionally, Figure 4-5 displays that the Pearson correlation coefficients of the filtered, background-subtracted, and normalized Cy3 PE and Cy5 PE signals are higher than 98.5% for 14 of these 16 arrays and that all are larger than 96%. Examining only the "protect" arrays shows that the correlation coefficient is largest for the "microarray" set, followed by the "extraction," "cultivation," and "colony" sets, respectively. This is logical, since samples that have been split from each other later in the experimental protocol are expected to demonstrate lower variation.

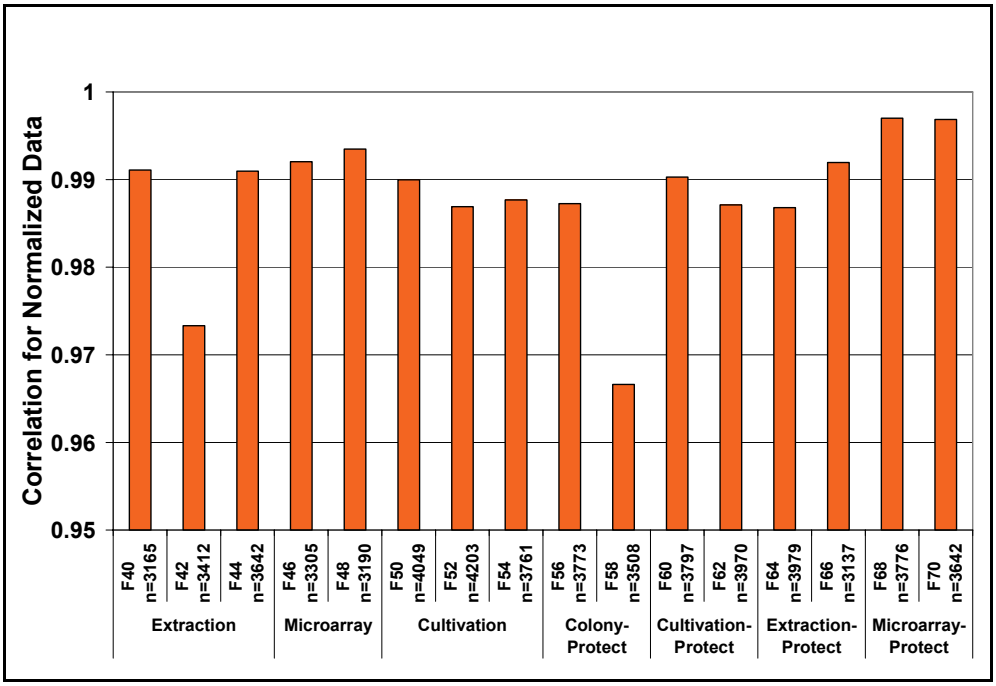

Figure 4-5 Pearson correlation coefficients for filtered, background-subtracted, and normalized PE-PE ("self-self") microarrays analyzing variation at the "colony," "cultivation," "RNA extraction," and "microarray" steps. "Protect" refers to utilizing RNAprotect Bacteria reagent (Qiagen, Germantown, MD).

An alternative in analyzing the variation per experimental step is to examine the ρ_ε parameter, the correlation in the multiplicative errors from the MLA model between the Cy3 and Cy5 channels, for each of the 7 PE-PE data sets described above. This parameter describes the correlation between the microarrays for the various sets. Table 4-2 gives this parameter for the

data sets in addition to a comparison value from Ideker *et al.* (2000), who calculated the mean ρ_ε from 16 separate calculations of the parameter involving 96 genes across 5 separate microarrays. Their comparison value is most analogous to the "microarray" parameter shown here.

Data Set	Colony	Cultivation	RNA Extraction	Microarray	Ideker *et al.* (2000) Microarray Comparison
ρ_ε	--	0.932	0.855	0.866	0.967
Data Set	**Colony-Protect**	**Cultivation-Protect**	**RNA Extraction-Protect**	**Microarray-Protect**	
ρ_ε	0.900	0.876	0.891	0.956	

Table 4-2 Correlation between multiplicative errors between the Cy3 and Cy5 channels for 7 PE-PE ("self-self") data sets and Ideker *et al.* (2000) comparison. The 7 data sets refer to microarrays analyzing variation at the "colony," "cultivation," "RNA extraction," and "microarray" steps as explained in the text. "Protect" refers to utilizing RNAprotect Bacteria reagent (Qiagen, Germantown, MD).

The lowest correlation values are expected for those data sets encompassing more experimental steps for variation (to the left of Table 4-2), and this trend seems to roughly hold for the "protect" data in the bottom row. Additionally, it is unclear why the "cultivation" correlation of 0.932 is better than the "cultivation-protect" correlation of 0.876 since the RNAprotect Bacteria Reagent (Qiagen) was used for the latter sample and is expected to reduce experimental error corresponding to mRNA degradation. The opposite trend is seen for the "RNA extraction" and "microarray" variation experiments. The "microarray-protect" correlation of 0.956 is much more similar to the Ideker *et al.* (2000) value of 0.967 than the "microarray" correlation of 0.866, suggesting the additional value of using the reagent. In any case, the correlations are quite similar to each other, suggesting this background error for mutant to PE strain comparison is quite low and that "colony" triplication is reasonable in comparison experiments.

Although the RMSE may be a useful preliminary statistic for assessing the reproducibility of the various protocol steps, it is also susceptible to distortion due to the fact that the uncertainty in the expression ratios is greater for genes that are expressed at low levels than for those that are highly expressed (Ideker *et al.*, 2000). Thus, an ANOVA analysis of the DNA microarray method was analyzed, and this is presented in the next section.

4.3.3.2. ANOVA Analysis of DNA Microarray Method

In order to investigate which independent variables were influencing the microarray spot intensities, an analysis of variance (ANOVA) was performed on each of 10 factors (array, channel, colony, cultivation, date, extraction, protection solution, RT enzyme, scanning voltage, and strain). ANOVA analysis was performed in R using generalized linear modeling. In summary, 4 of the 10 studied factors were found to have at least one significant group ($p < 0.01$). Of the experimental factors that related to growth, the particular cultivation only once (1 of 11) resulted in a significantly different central tendency. Technical factors such as the individual microarray used (2 of 18), cultivation date (1 of 3), and scanning voltage (1 of 13) also had significant effects on measured data in some cases. However, triplicate cultivations, each from a separate bacterial colony of the same strain, were performed to address variability related to growth. The minor effects of technical factors were noted but not explicitly addressed further.

4.3.4. Exponential Growth Time Course Experiment

In order to compare gene expression throughout the exponential growth phase, samples of the PE strain were taken at OD_{600} = 0.2, 0.4, and 0.8, and microarrays were completed in triplicate comparing the samples from (OD_{600} = 0.2 and OD_{600} = 0.4) and (OD_{600} = 0.8 and OD_{600} = 0.4). For reference, all other microarray results presented in this thesis correspond to samples taken at OD_{600} = 0.4 in mid-exponential growth phase.

Five and two genes, respectively, were determined to be differentially-expressed in the experiments listed above using the standard critical p value determined previously. These low numbers of differentially expressed genes indicate that there is not significant gene expression variation throughout the exponential growth phase for the PE strain. Interestingly, four of the genes from the first experiment comparing samples from (OD_{600} = 0.2 and OD_{600} = 0.4), namely *nuoG*, *ynjE*, *yjjG*, and *frdC*, were also later found to be differentially expressed between the mutants and the PE strain. Based upon the results from this time course experiment with the PE strain, it was decided that all strains would be harvested at about OD_{600} = 0.4 for DNA microarray analysis. In particular, harvesting samples of cells approaching the stationary growth phase was avoided as it was anticipated that such cells would exhibit high variability in their patterns of gene expression given the large numbers of cellular changes associated with this period.

4.4. Proteomic Expression Analysis

4.4.1. Bacterial Culture Growth for Proteomic Analysis

Strains of the parental pre-engineered (PE) strain or the 5 mutant strains studied, the Δ*gdhA,* the Δ*gdhA* Δ*aceE,* the Δ*gdhA* Δ*aceE* Δ_P*yjiD,* the Δ*hnr,* and the Δ*hnr* Δ*yliE* strains, were grown at 37 °C with 225 RPM orbital shaking in M9-minimal media (Sambrook and Russell 2001) containing 5 g/L d-glucose and 68 μg/ml chloramphenicol. All cultures were grown in 50 ml culture in a 250 ml flask with 1% inoculation from an overnight 5 ml culture grown to stationary phase in M9-minimal media, employing the same growth methods as Alper (2006) and as described for the transcriptional profiling experiments. All chemicals were from Mallinckrodt (Hazelwood, MO) and Sigma Aldrich (St. Louis, MO). All experiments were performed in replicate to validate data and calculate statistical parameters. Cell density was monitored

spectrophotometrically at 600 nm, and cells were harvested for RNA extraction in mid-exponential growth phase at about OD_{600} = 0.4. Additionally, the Δ*gdhA* Δ*aceE* Δ_P*yjiD* strain was harvested at about OD_{600} = 0.2 and OD_{600} = 0.8 using separate culture flasks for comparison. Based upon cellular growth curves, harvest times were approximated, and final spectrophomteric measurements were taken upon culture harvest.

4.4.2. Sonication and Total Protein Assay

Upon harvest from liquid media culture, cells were immediately put on ice to minimize protease activity and then resuspended in a 50 mM ammonium bicarbonate (pH = 8.5), 5 mM EDTA buffer. Samples were then sonicated according to a protocol optimized for this study, namely at 70% amplitude on a Branson Digital Sonifier 250 (Branson, Danbury, CT) for four total 60s periods with 60s on ice in between sonication. Pulses of 0.5s were used for every 0.5s rest during the sonication periods. Following centrifugation at 4ºC at 14k RPM for 30 min, the supernatant containing the proteins released from the cellular material was measured for total protein concentration using a total protein assay (Bio-Rad, Hercules, CA) according to the manufacturer's protocol. Samples were diluted 16 and 32-fold in water, and corresponding concentrations were averaged using the BSA standard curve generated. Actual protein concentrations varied from 12-15 mg/ml for the various PE and mutant strains, exceeding the required 10 mg/ml concentration for the LC-MS method. Protein lysate samples were then frozen at -80ºC and shipped to Dr. Jeff Silva and Dr. Johannes Vissers at Waters Corporation (Milford, MA) for analysis using their novel LC-MS^E proteomics method described later.

4.4.3. SDS-PAGE of Total Protein

Total protein samples prepared as described above were first assayed using standard SDS-PAGE (Ornstein 1964). Figure 4-6 below shows a 10% SDS-PAGE gel of the total protein

from the five mutants Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY) in addition to the Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* (GAP) strain harvested at OD_{600} of about 0.2 (early exponential phase) and 0.8 (late exponential phase) and the PE strain. From Figure 4-6, it is apparent that the genetically similar strains all exhibit highly similar protein expression from a global viewpoint. Thus, a more sensitive and accurate technique for measuring the proteomic expression was needed, motivating the use of the LC-MS^E method.

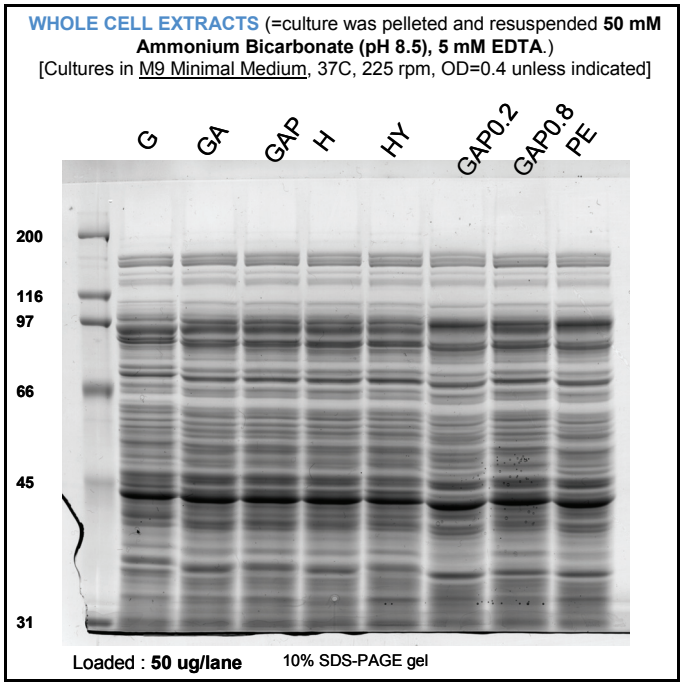

Figure 4-6 SDS-PAGE analysis of mutant and PE strains.

4.4.4. LC-MS^E Protein Expression Analysis

The liquid chromatography-mass spectrometry (LC-MS^E) experimental and analytical method applied in this work has been documented previously (Silva, Denny *et al.* 2005; Silva, Denny *et al.* 2006; Silva, Gorenstein *et al.* 2006; Geromanos, Vissers *et al.* 2009; Li, Vissers *et al.* 2009). It's main advantage is operation of the mass spectrometer in alternating low (precursor detection MS scan) and linearly increasing collision energies (fragment ion detection MS scan) throughout the LC peak, allowing for highly accurate, label-free quantification of

precursor peptides and fragment ions for calculating both relative and absolute quantification of proteins in complex mixtures. Whereas (MS/MS) data-dependent acquisition methods first utilize an MS scan to detect peptides and then serially interrogate a predetermined number of strongest peptide peaks via MS/MS (with fragmentation), spending different amounts of time in the MS and MS/MS modes and potentially missing new precursor peptides while in MS/MS mode, the data-independent LC-MS^E method samples all peptide precursor peaks (*e.g.* between 300-2000 m/z) and spends equal amounts of time in low and higher collision energy modes for sampling throughout the LC peak. This allows for the highly accurate quantification.

First, the protein lysate prepared as described was unfrozen and trypsinized to produce peptides that were partitioned through reverse-phase HPLC on 3 μ C18 columns (300 μ ID x 15 cm), separating the peptides on the basis of hydrophobicity. Peptide elution was afforded by means of an acetonitrile/0.1% formic acid gradient from 1-40% over a 90 min period. Triplicate injections were made for each sample. Eluting peptides were then positively ionized using nanoelectrospray ionization, and data were acquired on a hybrid quadrupole-time of flight mass spectrometer in alternating low and elevated collision energy scanning mode using a reference spray of [Glu]1-fibrinopeptide B and erythromycin (Silva, Gorenstein *et al.* 2006). The proprietary alternating energy function allows for nearly simultaneous acquisition of precursor peptide data from MS operation and corresponding peptide fragment data from MS/MS operation using the low and elevated energy scanning modes, respectively. As a result, highly accurate quantification of the peptides was possible.

Mass/retention time peak detection, charge state reduction, deisotoping, time alignment, databank searching, and absolute quantitation were carried out with IdentityE informatics software (Water Corporation, Milford, MA). Unless otherwise indicated, proteins were counted

as "detected" if they were identified in 2 out of 3 replicate LC-MS runs for all the samples in which the particular protein was identified. Additional data analysis was performed with SpotFire DecisionSite 8.0 (Tibco, Palo Alto, CA), Microsoft Excel (Microsoft, Redmond, WA), and the EcoCyc Omics Viewer and database (Keseler, Bonavides-Martinez *et al.* 2009).

4.4.5. Peptide Thresholds

Two different peptide thresholds were applied to the mass/retention time peak data in order to accurately quantitate proteins (Li, Vissers et al. 2009). Throughout this thesis, these will be referred to "threshold one" and "threshold two," respectively. "Threshold one" data are relative quantification data that resulted from calculating average peptide ratios between the mutant and the PE strain (or the Δ*gdhA* Δ*aceE* Δ*pyjiD* strain sampled at either OD_{600} ~ 0.2 or 0.8 compared to OD_{600} ~ 0.4) for all detected peptides of a particular protein. These ratios were normalized to the ratio of the most highly abundant *E. coli* protein, Ef-Tu, as has been described (Silva, Denny *et al.* 2006). "Threshold two" data are either relative quantification data calculated in the same way as "threshold one" but applying a different peptide threshold or else absolute quantification data that was calculated by dividing the average MS signal responses of the three most intense peptides from each protein by a universal signal response factor resulting from the average MS signal response of internal standard proteins (Silva, Gorenstein *et al.* 2006) It should be noted that the two thresholds differ only in the peptide search engine (Li, Vissers et al. 2009) that was applied to the same proteomic data, and neither threshold is necessarily "superior" to the other. In this way, both sets have been presented in order to complement each other and give a clearer view of the true proteomes of the strains under study. Unless otherwise indicated, the data discussed in this thesis corresponds to the "threshold one" data. The

"threshold two" data were used to gain insight in cases where the "threshold one" data were incomplete or missing and is indicated in the text.

4.4.6. Determination of Differential Protein Expression

Denoting the natural logarithm of the expression ratio by L(Mutant/PE), the probability of up-regulation P(L>0) is calculated via a Bayesian approach. This probability is the posterior distribution for L which corresponds to positive L given the data. That is,

$$P = \int_0^\infty \Pr\left(L \middle| \text{Data}\right) dL \quad . \qquad (4.10)$$

This can be evaluated using Bayes's theorem

$$P\left(L_i \middle| \text{Data,I}\right) = \frac{P(L_i \middle| I) P(\text{Data} \middle| L_i, I)}{P(\text{Data} \middle| I)} \ , \qquad (4.11)$$

where I is the instrument background, $P(L_i| I)$ is the prior based upon the instrument I resolution and calibration, $P(\text{Data}| L_i, I)$ is based upon the response of the instrument and the properties of the tryptic digestion, and the evidence for a particular protein is based on the spectrum from the sample, $d = \{d_1, d_2, \ldots, d_n\}$:

$$P\left(\text{Data} \middle| I\right) = \sum_i P\left(L_i \mid I\right) P\left(\text{Data} \mid L_i, I\right) \quad . \qquad (4.12)$$

The probability of down-regulation is 1-P(L>0).

4.5. Algorithm for Integration of Transcriptomic and Proteomic Data

The metric for integration of the two global data sets was calculated as follows. First, all transcriptomic or proteomic (applying threshold one) ratios corresponding to $\log_{10}$(Mutant/PE) changes greater than ±0.05 (~12%) were counted as either up-regulated or down-regulated on a per enzyme basis. Note that this is not statistical differential expression, but simply meant to

gauge directionalities in the data. For multiprotein complexes, the counts were added from each comprising protein (without taking into account stoichiometries), since the complexes depend upon each component. Isozyme totals were kept separate from each other since they can substitute for each other. For the proteomic data, instances for which the protein was identified only in either the mutant or the PE strain were also counted separately. The magnitudes of the up- and down-regulation $\log_{10}$(Mutant/PE) measurements were then summed separately for each enzyme or enzyme complex across the transcriptional and proteomic data and across all five mutants. The proteomic instances of unique measurement in either the mutant or the PE strain were assigned a $\log_{10}$(Mutant/PE) ratio of 0.39 or -0.39, respectively, where 0.39 was the largest measured numerical $\log_{10}$(Mutant/PE) ratio for the other data. This was justified since measuring a protein only in one condition presumably indicates that the protein is important to the molecular phenotype of that strain. Once the up- and down-regulated measurement magnitudes were summed, a comparison ratio was calculated. For multiprotein complexes, a color was assigned on a consensus of the composing proteins. Although not all composing proteins exhibited the general trend in some cases, an overall trend was assigned for the complex. Colors were assigned according to the following metric. If less than two measurements were up- or down-regulated for a given enzyme or enzyme complex, then that target was assigned a "white" color indicating no change. For all other enzymes, a spectrum of dark green, light green, yellow, light red, and dark red was assigned from most down-regulated to most up-regulated in terms of consistency of directionality and magnitude of expression change. If the calculated comparison ratio indicated at least 3-fold larger up- or down-regulated expression sum as compared to the down- or up-regulated expression sum, respectively, then dark red and dark green were assigned, respectively. If the comparison ratio fell between 2-fold

and 3-fold larger up- or down-regulated expression sum, then light red and light green were assigned, respectively. For those comparison ratios falling between 2-fold larger up-regulated sum and 2-fold larger down-regulated sum, yellow was assigned. In the case that at least two measurements were up- or down-regulated (with $\log_{10}$(Mutant/PE) greater than 0.05 or less than -0.05) but all of the measurements were either up- or down-regulated (corresponding to a 0 or infinity comparison ratio), the dark red and dark green colors were assigned, respectively. Using this metric, the metabolic maps in Figure 7-2 and Figure 7-3 were constructed. The data corresponding to these figures is given in Appendix 9.3, which also includes the proteomic data from applying the second peptide threshold that generally agrees with the proteomic data resulting from the application of the first peptide threshold in directionalities but not completely in magnitudes.

4.6. Metabolic Engineering

4.6.1. Overexpressions of *wrbA*, *ydeO*, and *gadE* in PE Strain

Overexpression of the *wrbA* gene was accomplished by cloning the genes under a pL-tetO promoter and its variants in a pZE plasmid. Briefly, the *wrbA* gene was PCR amplified from genomic *E. coli* K12 MG1655 DNA using the primers (Invitrogen, Carlsbad, CA) listed in Table 4-3. Vector NTI (Invitrogen) was used for primer design. PCR amplification was achieved using Takara LA PCR Kit Version 2.1 (Shiga, Japan) according to the manufacturer's protocol. PCR success was verified by 1% agarose gel electrophoresis. The *wrbA* amplified DNA was purified using the Qiaquick PCR Purification Kit (Qiagen, Germantown, MD) according to the manufacturer's protocol. Purified *wrbA* DNA and the pZE vector were double-digested using the KpnI and MluI restriction enzymes (New England Biolabs, Ipswich, MA) according to the manufacturer's protocol. The resulting digested *wrbA* DNA was purified again

using the Qiaquick PCR Purification Kit, and the pZE vector was purified using the Qiaquick Gel Purification Kit (Qiagen). Purified, digested *wrbA* DNA was then ligated into a digested pZE plasmid containing the *kanR* gene using various promoter strengths as indicated in the text (Alper, Fischer *et al.* 2005). T4 DNA Ligase (New England Biolabs) was used for the ligation step. The ligation product was transformed with DH5α Maximum Efficiency *E. coli* cells (Invitrogen) using the manufacturer's protocol, and cells were plated onto solid LB plates containing 20 μg/ml kanamycin and grown for ~16 hrs. at 37ºC. Three colonies for each promoter strength were picked and transferred to 5 ml liquid LB media containing 20 μg/ml kanamycin. Cultures were grown for ~16 hrs. at 37 ºC at 225 RPM orbital shaking and spun down for 8 minutes at 3000 RPM. Then, the Qiaprep Spin Miniprep Kit (Qiagen) was used to purify the plasmid DNA from the cellular pellets according to the manufacturer's protocol. Plasmid inserts were verified by double digestion (KpnI/MluI) followed by 1% agarose gel electrophoresis and also by sequencing using the primers specified in Table 4-3 at the MIT BioPolymers Lab (Cambridge, MA). Finally, pZE *wrbA* plasmids were transformed into previously prepared PE competent cells (Datsenko and Wanner 2000) via GenePulser electroporation (Bio-Rad, Hercules, CA) using SOC media (Invitrogen). Transformed cells were grown at 37ºC at 225 RPM orbital shaking for 1 hour then plated again onto solid LB plates containing 20 μg/ml kanamycin and 68 μg/ml chloramphenicol for ~16 hrs. growth at 37ºC. Finally, the most prominent and red colonies (indicating lycopene production) were picked from the plates and grown in 5 ml M9 media containing 20 μg/ml kanamycin and 68 μg/ml chloramphenicol for ~16-20 hrs. This culture was then used to inoculate shake flask cultures for lycopene production experiments and to prepare 1:1 30% glycerol stocks for long term storage at -80ºC.

The pZE *ydeO* and pZE *gadE* overexpression plasmids were obtained from Christine Santos (2008, unpublished) and were created using the same techniques as described above. A control pZE *gfp* plasmid was also grown in the acid resistance gene overexpression experiments.

Table 4-3 Gene overexpression primers used in this thesis.

Gene	Strand	Primer 5' to 3'
wrbA	**Sense KpnI**	**CGGGGTACCATGGCTAAAGTTCTGGTGCTTTATTATTCC**
	Antisense MluI	**CGACGCGTGCGTATCCTCCTGTTGAAGATTAGCC**
ydeO	**Sense KpnI**	**CTCGGTACC ATGTCGCTCGTTTGTTCTGTTATATTTATTC**
	Antisense BsaI	**GGTCTCTCTTT TCAAATAGCTAAAGCATTCATCGTGTTGC**
gadE	**Sense KpnI**	**CTCGGTACC ATGATTTTTCTCATGACGAAAGATTCTTTTC**
	Antisense MluI	**CGACGCGT CTAAAAATAAGATGTGATACCCAGGGTGACG**

4.6.2. Deletions of *iclr* and *pntB* in PE Strain

Deletions of the *iclR* and *pntB* genes in the PE strain were accomplished in the following way. Keio collection strains (Baba, Ara *et al.* 2006) of *E. coli* K12 harboring single deletions in these genes (JW3978-2 and JW1594-1, respectively) were obtained from the Yale University (New Haven, CT) *E. coli* Genetic Stock Center. P1vir phage transduction (Moore, Fischer *et al.*; Lennox 1955) was then used to replace the *iclR* and *pntB* PE strain chromosomal genes with the *kanR* gene from the Keio strains. Cells were then plated twice on solid LB plates containing 20 μg/ml kanamycin and 68 μg/ml chloramphenicol for ~16 hrs. at 37ºC each time. Deletions were confirmed by amplifying the regions surrounding the gene deletion and *kanR* insert using colony PCR with 1% gel electrophoresis to verify the size of the inserts. Primers were designed using Vector NTI (Invitrogen) and are listed in Table 4-4. The most prominent and most red colonies (indicating lycopene production) were picked from the plates and grown in 5 ml M9 media containing 20 μg/ml kanamycin and 68 μg/ml chloramphenicol for ~16-20 hrs. This culture was

then used to inoculate shake flask cultures for lycopene production experiments and to prepare 1:1 30% glycerol stocks for long term storage at -80ºC.

Table 4-4 Gene deletion verification primers used in this thesis

Gene	Strand	Primer 5' to 3'
iclR	**Sense**	**AGAATATTGCCTCTGCCCGC**
	Antisense	**CCACCACGCAACATGAGATTTG**
pntB	**Sense**	**AAGAGTGACGGCCTCAGCAGAG**
	Antisense	**TCACCGTGACTCAGCGCATG**

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Chapter 5. Transcriptomic Analysis of Lycopene-Overproducing *Escherichia coli* Strains

5.1. Global Analysis of Differential Gene Expression

Global gene expression of the five mutant strains, Δ*gdhA,* Δ*gdhA* Δ*aceE,* Δ*gdhA* Δ*aceE* Δ_P*yjid,* Δ*hnr,* and Δ*hnr* Δ*yliE,* was first examined relative to the "pre-engineered" (PE) strain in mid-exponential growth phase (OD_{600} = 0.4) via DNA microarray experiments and analysis as described. All measured gene expression values are given in Appendix 9.1, while genes that were determined to be differentially expressed via a maximum likelihood method (Ideker, Thorsson *et al.* 2000) using the previously determined critical p value cutoff of 4.26×10^{-3} are given in Table 5-1. The total number of differentially expressed genes per strain is given in Figure 5-1 for the critical p value determined from the control microarrays in addition to a "relaxed" p value cutoff of 0.01.

Table 5-1 Differential gene expression for the five mutant strains Δ*gdhA,* Δ*gdhA* Δ*aceE,* Δ*gdhA* Δ*aceE* Δ_P*yjid,* Δ*hnr,* and Δ*hnr* Δ*yliE* relative to the PE strain. The Blattner number (Blattner, Plunkett *et al.* 1997), gene name, and annotated gene function according to EcoCyc (Karp, Keseler *et al.* 2007) are given for each differentially expressed gene along with the $\log_{10}$(Mutant/PE) ratios and the associated p values resulting from the applied maximum likelihood method (Ideker, Thorsson *et al.* 2000).

B #	Name	Function	Ratio	p value
Gene			log_10(Mut/PE)	Diff. Exp.
Δ*gdhA*				
b3737	atpE	ATP synthase, F0 complex, c subunit	0.549	1.06E-05
b2187	yejL	conserved protein	0.546	2.62E-03
b1278	pgpB	undecaprenyl pyrophosphate phosphatase [multifunctional]	0.538	2.77E-03
b1549	ydfO	Qin prophage; predicted protein	0.514	2.72E-03
b0763	modA	molybdate ABC transporter	0.496	3.10E-03
b2203	napB	small subunit of periplasmic nitrate reductase, cytochrome c550 protein	0.478	1.60E-04
b3956	ppc	phosphoenolpyruvate carboxylase	0.467	3.23E-03
b1221	narL	NarL-Phosphorylated transcriptional dual regulator	0.452	3.56E-03
b2797	sdaB	L-serine deaminase II	0.434	3.66E-03
b1425	G6740	phantom gene	0.353	6.00E-04
b2128	yehW	YehW/YehX/YehY/YehZ ABC transporter	0.345	6.85E-04
b2434	ypeA	predicted acyltransferase with acyl-CoA N-acyltransferase domain	0.344	6.05E-04
b1578	insD	Qin prophage; predicted transposase	0.318	9.29E-04
b1656	sodB	superoxide dismutase (Fe)	0.287	2.29E-03

B #	Name	Function	Ratio	p value
b3670	ilvN	acetohydroxybutanoate synthase / acetolactate synthase	0.286	2.11E-03
b1510	ydeK	predicted lipoprotein	0.279	3.54E-03
b0339	cynT	carbonic anhydrase monomer	0.274	6.32E-04
b3530	bcsC	oxidase involved in cellulose synthesis	0.261	1.35E-03
b3713	yieF	chromate reductase monomer	0.230	3.87E-03
b1936	intG	predicted defective phage integrase	-0.420	1.12E-03
b2023	hisH	imidazole glycerol phosphate synthase, HisH subunit	-1.041	1.43E-04
Δ*gdhA* Δ*aceE*				
b3737	atpE	ATP synthase, F0 complex, c subunit	0.607	2.75E-07
b4114	eptA	predicted metal-dependent hydrolase	0.430	1.83E-04
b0339	cynT	carbonic anhydrase monomer	0.220	8.17E-04
b3036	ygiA	predicted protein	-0.238	2.17E-03
b1936	intG	predicted defective phage integrase	-0.467	2.75E-05
b1955	yedP	predicted phosphatase	-0.487	2.19E-03
b2023	hisH	imidazole glycerol phosphate synthase, HisH subunit	-1.191	3.51E-07
Δ*gdhA* Δ*aceE* $\Delta_P$yjiD				
b3737	atpE	ATP synthase, F0 complex, c subunit	0.552	1.59E-05
b4114	eptA	predicted metal-dependent hydrolase	0.534	1.61E-04
b3529	yhjK	predicted diguanylate cyclase	0.244	1.64E-03
b4374	yjjG	pyrimidine nucleotidase	0.223	3.43E-03
b1757	ynjE	predicted thiosulfate sulfur transferase	0.204	4.23E-03
b4241	treR	TreR transcriptional repressor	-0.134	1.96E-03
b1882	cheY	chemotaxis regulator transmitting signal to flagellar motor component	-0.149	8.68E-04
b1936	intG	predicted defective phage integrase	-0.427	2.38E-03
b2023	hisH	imidazole glycerol phosphate synthase, HisH subunit	-1.055	7.24E-05
Δ*hnr*				
b3212	gltB	glutamate synthase, large subunit	0.667	2.29E-08
b1757	ynjE	predicted thiosulfate sulfur transferase	0.616	3.32E-08
b4374	yjjG	pyrimidine nucleotidase	0.571	6.15E-08
b0027	lspA	prolipoprotein signal peptidase II	0.566	2.64E-03
b3914	cpxP	(now b4484) regulator of the Cpx resp./ extracytoplasmic stress resp.	0.565	2.37E-03
b0618	citC	citrate lyase synthetase	0.530	6.19E-08
b3529	yhjK	predicted diguanylate cyclase	0.523	8.61E-08
b3534	yhjQ	cell division protein (chromosome partitioning ATPase)	0.494	1.38E-07
b1985	yeeO	YeeO MATE Transporter	0.483	2.72E-06
b3533	bcsA	cellulose synthase, catalytic subunit	0.443	1.56E-04
b3474	yhhT	predicted inner membrane protein	0.432	3.74E-03

B #	Name	Function	Ratio	p value
b2734	pphB	protein-tyrosine-phosphatase / phosphoprotein phosphatase	0.429	4.74E-04
b4073	nrfD	nitrite reductase complex	0.414	4.17E-03
b1786	yeaJ	predicted diguanylate cyclase	0.386	1.11E-06
b3389	aroB	3-dehydroquinate synthase	0.378	8.15E-05
b4332	yjiJ	putative transport protein	0.374	1.21E-03
b0155	clcA	EriC chloride ion ClC channel	0.364	6.46E-04
b2846	yqeH	conserved protein with bipartite regulator domain	0.358	3.55E-06
b3660	yicL	inhibitor of heme biosynthesis	0.357	2.06E-04
b1883	cheB	Chemotactic Signal Transduction System component	0.347	4.30E-11
b1004	wrbA	WrbA monomer	0.344	4.25E-04
b0360	insC-1	IS2 element protein InsA	0.343	2.64E-06
b1848	yebG	conserved protein regulated by LexA	0.343	9.96E-06
b2523	pepB	aminopeptidase B	0.341	4.44E-06
b1884	cheR	chemotaxis protein methyltransferase	0.332	4.61E-11
b4362	dnaT	primosome	0.332	1.22E-05
b0674	asnB	asparagine synthetase B	0.325	1.90E-04
b3582	sgbU	predicted L-xylulose 5-phosphate 3-epimerase	0.309	5.69E-04
b1394	paaG	putative enoyl-CoA hydratase/isomerase of phenylacetate degradation	0.305	8.17E-06
b1881	cheZ	cytosolic phosphatase of the chemotaxis signal transduction complex	0.300	7.65E-10
b3870	glnA	adenylyl-[glutamine synthetase]	0.299	2.67E-03
b3217	ychE_1	predicted protein, N-ter fragment (pseudogene)	0.293	8.53E-05
b3857	mobA	molybdopterin guanine dinucleotide synthase	0.284	2.35E-05
b4119	melA	α-galactosidase monomer	0.284	2.61E-03
b2101	yegW	predicted DNA-binding transcriptional regulator	0.280	4.66E-05
b2513	yfgM	conserved protein	0.272	9.05E-05
b0626	ybeM	predicted C-N hydrolase superfamily, NAD(P)-binding amidase/nitrilase	0.271	3.08E-05
b2722	hycD	hydrogenase 3, membrane subunit	0.269	1.64E-06
b1393	paaF	putative enoyl-CoA hydratase/isomerase of phenylacetate degradation	0.268	2.47E-05
b4352	yjiA	P-loop guanosine triphosphatase	0.268	3.09E-04
b1472	yddL	predicted lipoprotein	0.266	5.98E-05
b2244	yfaD	conserved protein	0.266	2.66E-05
b3112	tdcG	(now b4471) L-serine deaminase III	0.266	2.76E-05
b4176	yjeT	conserved inner membrane protein	0.254	1.61E-03
b1228	ychS	predicted protein	0.249	1.57E-04
b2674	nrdI	conserved protein that may stimulate ribonucleotide reductase	0.249	1.40E-03
b0968	yccX	acylphosphatase	0.247	8.46E-05
b1221	narL	NarL-Phosphorylated transcriptional dual regulator	0.247	2.38E-05
b3498	prlC	oligopeptidase A	0.247	1.60E-03
b1002	agp	3-phytase / glucose-1-phosphatase	0.243	6.78E-05
b3436	gntU	(now b4476) GntU gluconate Gnt transporter	0.241	1.50E-04
b3481	nikR	DNA-binding transcriptional repressor, Ni-binding	0.239	1.58E-04

B #	Name	Function	Ratio	p value
b4109	yjdA	conserved protein with nucleoside triphosphate hydrolase domain	0.238	1.49E-03
b1837	yebW	predicted protein	0.234	3.62E-04
b2708	gutQ	D-arabinose 5-phosphate isomerase	0.233	2.09E-03
b2165	pscG	predicted pseudouridine 5'-phosphate glycosidase	0.232	3.41E-03
b0866	ybjQ	conserved protein	0.231	3.13E-04
b0763	modA	molybdate ABC transporter	0.230	2.22E-03
b2052	fcl	GDP-fucose synthase	0.230	2.71E-04
b2762	cysH	3'-phospho-adenylylsulfate reductase	0.229	2.45E-03
b0801	ybiC	predicted dehydrogenase	0.228	1.76E-04
b1463	nhoA	N-hydroxyarylamine O-acetyltransferase	0.224	3.35E-04
b3111	tdcG	(now b4471) L-serine deaminase III	0.224	1.63E-04
b2075	mdtB	MdtABC-TolC multidrug efflux transport system	0.223	1.94E-04
b1151	ymfO	e14 prophage; conserved protein	0.221	7.19E-04
b1874	cutC	copper homeostasis protein	0.221	2.80E-07
b2659	csiD	predicted protein	0.219	4.39E-04
b0331	prpB	2-methylisocitrate lyase	0.217	5.53E-04
b2316	accD	acetyl-CoA carboxylase	0.217	1.00E-03
b0851	nfsA	NADPH nitroreductase monomer	0.215	2.36E-04
b2073	yegL	conserved protein	0.215	3.50E-04
b3038	ygiC	predicted enzyme	0.213	2.96E-04
b3907	rhaT	rhamnose RhaT transporter	0.211	6.56E-04
b1140	intE	e14 prophage; predicted integrase	0.209	3.90E-04
b2283	nuoG	NADH:ubiquinone oxidoreductase, chain G	0.207	1.68E-05
b4028	yjbG	conserved protein	0.207	8.92E-04
b2765	sscR	6-pyruvoyl tetrahydropterin synthase	0.206	9.68E-04
b0966	hspQ	heat shock protein, hemimethylated DNA-binding protein	0.204	1.26E-03
b2683	ygaH	YgaH L-valine exporter	0.202	8.96E-04
b3671	ilvB	acetohydroxybutanoate synthase / acetolactate synthase	0.202	6.08E-04
b0917	ycaR	conserved protein	0.201	2.55E-03
b1249	cls	cardiolipin synthase	0.196	5.60E-04
b1317	ycjU	β-phosphoglucomutase	0.195	6.83E-04
b0807	rlmF	23S rRNA m6A1618 methyltransferase	0.192	1.99E-03
b2053	gmd	GDP-mannose 4,6-dehydratase	0.187	2.19E-03
b0078	ilvH	acetolactate synthase / acetohydroxybutanoate synthase	0.180	2.15E-03
b1765	ydjA	predicted oxidoreductase	0.178	2.92E-03
b1504	ydeS	predicted fimbrial-like adhesin protein	0.175	3.80E-03
b1964	yedS_1	predicted protein, N-ter fragment	0.175	4.09E-03
b3108	yhaM	(now b4470) conserved protein	0.163	3.95E-03
b2771	ygcS	YgcS MFS transporter	0.159	3.69E-03
b4145	yjeJ	predicted protein	0.159	3.71E-03
b0470	dnaX	DNA polymerase III, γ subunit	0.148	4.75E-04
b1569	dicC	Qin prophage; DNA-binding transcriptional regulator for DicB	0.122	1.06E-03
b2639	ypjL	CP4-57 prophage; predicted inner membrane protein	0.118	2.50E-03
b4075	nrfF	activator of formate-dependent nitrite reductase complex	0.113	2.50E-03

B #	Name	Function	Ratio	p value
b2840	ygeA	predicted racemase	0.106	3.77E-03
b0895	dmsB	dimethyl sulfoxide reductase, chain B	-0.122	3.04E-03
b4152	frdC	fumarate reductase membrane protein	-0.122	2.93E-03
b0721	sdhC	succinate dehydrogenase membrane protein	-0.126	2.76E-03
b3425	glpE	thiosulfate sulfurtransferase	-0.128	2.08E-03
b2321	flk	predicted flagella assembly protein	-0.139	3.05E-03
b1224	narG	nitrate reductase A, α subunit	-0.143	9.42E-04
b1241	adhE	PFL-deactivase / alcohol dehydrogenase / acetaldehyde dehydrogenase	-0.143	7.93E-04
b0652	gltL	GltIJKL glutamate ABC transporter	-0.194	4.08E-03
b1735	chbR	ChbR transcriptional dual regulator	-0.203	2.75E-03
b1325	ycjG	L-Ala-D/L-Glu epimerase	-0.273	2.87E-04
b0581	ybdK	γ-glutamyl:cysteine ligase	-0.303	2.01E-04
b0605	ahpC	AhpC component	-0.336	3.17E-03
b0892	rarA	recombination factor	-0.584	1.31E-04
b2882	ygfO	YgfO NCS2 tranporter	-0.859	2.75E-03
Δhnr ΔyliE				
b3212	gltB	glutamate synthase, large subunit	1.128	2.22E-04
b1757	ynjE	predicted thiosulfate sulfur transferase	0.977	4.16E-05
b3533	bcsA	cellulose synthase, catalytic subunit	0.960	7.54E-04
b4374	yjjG	pyrimidine nucleotidase	0.913	1.10E-05
b3529	yhjK	predicted diguanylate cyclase	0.891	1.38E-05
b3534	yhjQ	cell division protein (chromosome partitioning ATPase)	0.845	5.30E-05
b3660	yicL	inhibitor of heme biosynthesis	0.787	4.32E-04
b1420	mokB	regulatory peptide whose translation enables hokB expression	0.732	6.34E-05
b0618	citC	citrate lyase synthetase	0.699	2.35E-05
b0155	clcA	EriC chloride ion ClC channel	0.600	8.36E-04
b4362	dnaT	primosome	0.590	4.60E-05
b1837	yebW	predicted protein	0.556	7.88E-05
b0917	ycaR	conserved protein	0.492	1.14E-04
b2244	yfaD	conserved protein	0.470	1.50E-04
b3931	hslU	ATPase component of the HslVU protease	0.468	2.98E-03
b2659	csiD	predicted protein	0.437	2.67E-04
b4028	yjbG	conserved protein	0.437	2.21E-03
b1883	cheB	Chemotactic Signal Transduction System component	0.435	1.93E-07
b3112	tdcG	(now b4471) L-serine deaminase III	0.423	3.04E-04
b2101	yegW	predicted DNA-binding transcriptional regulator	0.421	3.63E-04
b1393	paaF	putative enoyl-CoA hydratase/isomerase of phenylacetate degradation	0.419	2.06E-04
b1874	cutC	copper homeostasis protein	0.419	2.27E-07
b0626	ybeM	predicted C-N hydrolase superfamily, NAD(P)-binding amidase/nitrilase	0.418	2.26E-04
b0331	prpB	2-methylisocitrate lyase	0.404	2.65E-04
b0851	nfsA	NADPH nitroreductase monomer	0.404	2.56E-04

B #	Name	Function	Ratio	p value
b3737	atpE	ATP synthase, F0 complex, c subunit	0.403	7.83E-05
b1472	yddL	predicted lipoprotein	0.394	4.62E-04
b2053	gmd	GDP-mannose 4,6-dehydratase	0.386	5.32E-04
b1786	yeaJ	predicted diguanylate cyclase	0.383	1.10E-03
b2674	nrdI	conserved protein that may stimulate ribonucleotide reductase	0.378	4.46E-04
b1881	cheZ	cytosolic phosphatase of the chemotaxis signal transduction complex	0.374	4.64E-07
b1151	ymfO	e14 prophage; conserved protein	0.373	3.57E-04
b3857	mobA	molybdopterin guanine dinucleotide synthase	0.370	5.30E-04
b0966	hspQ	heat shock protein, hemimethylated DNA-binding protein	0.369	3.78E-03
b1228	ychS	predicted protein	0.368	7.68E-04
b2786	barA	sensor protein BarA, sensor kinase-phosphotransferase	0.368	1.20E-03
b3111	tdcG	(now b4471) L-serine deaminase III	0.360	6.78E-04
b2513	yfgM	conserved protein	0.358	4.65E-04
b1394	paaG	putative enoyl-CoA hydratase/isomerase of phenylacetate degradation	0.350	5.21E-04
b0968	yccX	acylphosphatase	0.349	5.10E-04
b1249	cls	cardiolipin synthase	0.344	6.50E-04
b2523	pepB	aminopeptidase B	0.344	8.37E-04
b2165	pscG	predicted pseudouridine 5'-phosphate glycosidase	0.343	9.82E-04
b1847	yebF	predicted protein	0.341	1.24E-03
b2075	mdtB	MdtABC-TolC multidrug efflux transport system	0.339	5.86E-04
b0360	insC-1	IS2 element protein InsA	0.334	8.83E-04
b3687	ibpA	small heat shock protein IbpA	0.334	1.51E-03
b3217	ychE_1	predicted protein, N-ter fragment (pseudogene)	0.323	1.12E-03
b1884	cheR	chemotaxis protein methyltransferase	0.322	1.04E-05
b3122		(deleted from database) formerly thought to be hypothetical protein	0.322	8.90E-04
b2683	ygaH	YgaH L-valine exporter	0.321	9.02E-04
b3038	ygiC	predicted enzyme	0.319	8.53E-04
b1463	nhoA	N-hydroxyarylamine O-acetyltransferase	0.317	1.72E-03
b1140	intE	e14 prophage; predicted integrase	0.314	2.27E-03
b1964	yedS_1	predicted protein, N-ter fragment	0.302	1.18E-03
b2052	fcl	GDP-fucose synthase	0.292	1.31E-03
b1970	yedX	conserved protein	0.288	1.54E-03
b0078	ilvH	acetolactate synthase / acetohydroxybutanoate synthase	0.286	1.67E-03
b2771	ygcS	YgcS MFS transporter	0.285	2.52E-03
b0801	ybiC	predicted dehydrogenase	0.283	1.88E-03
b3481	nikR	DNA-binding transcriptional repressor, Ni-binding	0.277	2.21E-03
b1289	ycjD	conserved protein	0.275	3.67E-03
b4145	yjeJ	predicted protein	0.275	2.21E-03
b1541	ydfZ	conserved protein	0.272	1.93E-03
b0703	ybfO	conserved protein, rhs-like	0.271	2.96E-03
b2330	prmB	N5-glutamine methyltransferase	0.271	1.91E-03
b3792	wzxE	lipid III flippase	0.268	2.16E-03
b4032	malG	maltose ABC transporter	0.264	4.00E-03

B #	Name	Function	Ratio	p value
b0807	rlmF	23S rRNA m6A1618 methyltransferase	0.262	2.54E-03
b2575	yfiC	predicted S-adenosyl-L-methionine-dependent methyltransferase	0.256	3.12E-03
b3246	yhdP	(now b4472) conserved membrane protein, predicted transporter	0.255	3.33E-03
b4066	yjcF	conserved protein	0.253	2.95E-03
b1765	ydjA	predicted oxidoreductase	0.247	3.60E-03
b1317	ycjU	β-phosphoglucomutase	0.246	4.25E-03
b0470	dnaX	DNA polymerase III, γ subunit	0.244	2.43E-04
b1026	insF-4	IS3 element protein InsF	0.242	4.16E-03
b0427	yajR	YajR MFS transporter	0.240	3.57E-03
b3436	gntU	(now b4476) GntU gluconate Gnt transporter	0.236	3.92E-03
b2722	hycD	hydrogenase 3, membrane subunit	0.234	3.24E-04
b0184	dnaE	DNA polymerase III, α subunit	0.231	3.66E-04
b2283	nuoG	NADH:ubiquinone oxidoreductase, chain G	0.219	1.27E-03
b2639	ypjL	CP4-57 prophage; predicted inner membrane protein	0.218	4.11E-04
b3503	arsC	arsenate reductase	0.216	8.91E-04
b3738	atpB	ATP synthase, F0 complex, a subunit	0.202	5.49E-04
b0972	hyaA	hydrogenase 1, small subunit	0.199	9.04E-04
b1569	dicC	Qin prophage; DNA-binding transcriptional regulator for DicB	0.182	6.73E-04
b3513	mdtE	MdtEF-TolC multidrug efflux transport system	0.155	3.02E-03
b2886	ygfS	predicted oxidoreductase, 4Fe-4S ferredoxin-type subunit	0.147	4.25E-03
b1676	pykF	pyruvate kinase I monomer	-0.138	3.85E-03
b0211	mltD	MltD membrane-bound lytic murein transglycosylase D	-0.141	3.73E-03
b4150	ampC	β-lactamase; penicillin resistance	-0.154	1.73E-03
b3425	glpE	thiosulfate sulfurtransferase	-0.159	1.18E-03
b3556	cspA	CspA transcriptional activator	-0.179	1.03E-03
b2321	flk	predicted flagella assembly protein	-0.185	3.51E-03
b3423	glpR	GlpR transcriptional repressor	-0.188	1.91E-03
b0118	acnB	aconitase B	-0.197	1.12E-03
b0733	cydA	cytochrome bd-I terminal oxidase subunit I	-0.211	8.30E-05
b0755	gpmA	phosphoglyceromutase 1 monomer	-0.212	1.04E-04
b1824	yobF	predicted protein	-0.216	4.19E-03
b2457	eutM	predicted carboxysome structural protein, ethanolamine utilization protein	-0.220	4.24E-03
b0882	clpA	ATP-dependent protease specificity component and chaperone	-0.224	3.81E-03
b1411	ynbD	predicted phosphatase, inner membrane protein	-0.226	3.73E-03
b2482	hyfB	hydrogenase 4, component B	-0.226	3.99E-03
b3609	secB	Sec Protein Secretion Complex	-0.226	3.87E-03
b3769	ilvM	acetohydroxybutanoate synthase / acetolactate synthase	-0.231	3.46E-03
b0704	ybfC	predicted protein	-0.240	2.47E-03
b0895	dmsB	dimethyl sulfoxide reductase, chain B	-0.241	3.52E-05
b2817	amiC	N-acetylmuramyl-L-alanine amidase	-0.243	2.23E-03
b0730	mngR	MngR transcriptional repressor	-0.244	1.10E-04
b3891	fdhE	protein that affects formate dehydrogenase-N activity	-0.246	4.28E-04

B #	Name	Function	Ratio	p value
b3768	ilvG_2	acetolactate synthase II, large subunit, C-ter fragment (pseudogene)	-0.247	2.42E-03
b0590	fepD	Ferric Enterobactin Transport System	-0.259	2.00E-03
b0894	dmsA	dimethyl sulfoxide reductase, chain A	-0.268	5.80E-06
b1045	ymdB	conserved protein	-0.278	1.42E-03
b4294	insA-7	KpLE2 phage-like element; IS1 repressor protein InsA	-0.278	2.02E-03
b3636	rpmG	50S ribosomal subunit protein L33	-0.280	2.98E-03
b0615	citF	citrate lyase, citrate-ACP transferase α subunit	-0.284	9.25E-04
b2689	yqaA	conserved inner membrane protein	-0.290	7.66E-04
b0429	cyoD	cytochrome bo terminal oxidase subunit IV	-0.298	5.54E-06
b2307	hisM	lysine/arginine/ornithine ABC Transporter	-0.299	1.15E-03
b1872	torZ	trimethylamine N-oxide reductase III, TorZ subunit	-0.300	1.02E-03
b1224	narG	nitrate reductase A, α subunit	-0.301	1.66E-06
b1882	cheY	chemotaxis regulator transmitting signal to flagellar motor component	-0.302	4.92E-06
b0721	sdhC	succinate dehydrogenase membrane protein	-0.326	1.08E-06
b3138	agaB	Enzyme IIB predicted N-acetylgalactosamine-transp. phosphotransferase sys.	-0.345	3.79E-04
b3842	rfaH	RfaH transcriptional antiterminator	-0.350	1.07E-03
b1241	adhE	PFL-deactivase / alcohol dehydrogenase / acetaldehyde dehydrogenase	-0.403	9.12E-08
b1325	ycjG	L-Ala-D/L-Glu epimerase	-0.422	1.47E-04
b1936	intG	predicted defective phage integrase	-0.445	3.17E-04
b1818	manY	mannose PTS permease	-0.457	1.29E-04
b1817	manX	mannose PTS permease	-0.462	1.97E-03
b2700	ygaD	conserved protein	-0.464	1.14E-04
b2687	luxS	S-ribosylhomocysteine lyase (AI-2 synthesis protein)	-0.488	5.98E-05
b0581	ybdK	γ-glutamyl:cysteine ligase	-0.552	4.08E-05
b3019	parC	topoisomerase IV subunit A	-0.588	1.71E-03
b3458	livK	leucine binding protein of high-affinity branched-chain AA transport system	-0.636	1.84E-03

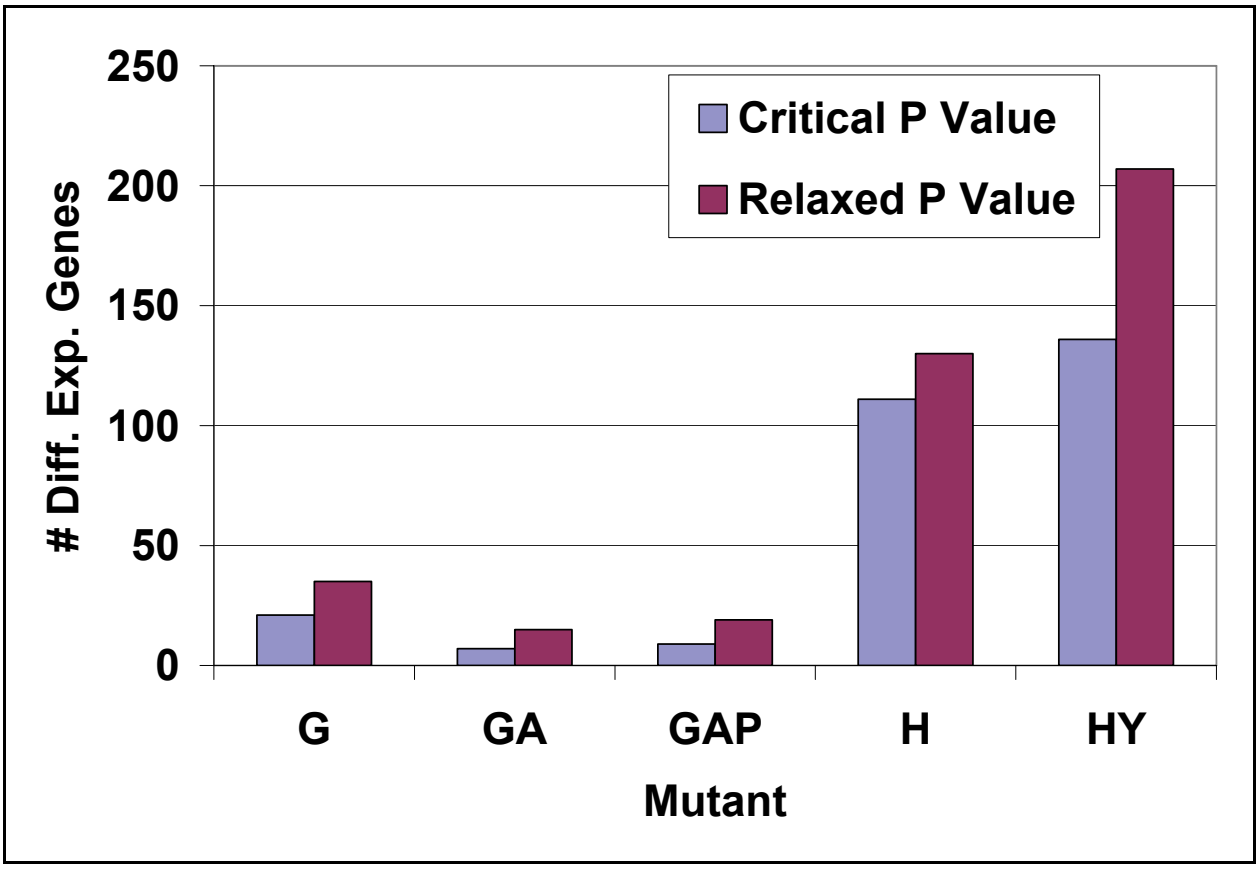

Figure 5-1 Number of differentially expressed genes for the five mutant strains Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY) relative to the PE strain, applying both the determined critical p value (4.26×10^{-3}) and a "relaxed" p value of 0.01.

A number of observations are apparent from Table 5-1 and Figure 5-1. While the three strains sharing the Δ*gdhA* deletion display 7 to 21 differentially expressed genes applying the critical p value, the two strains sharing the Δ*hnr* disruption display about an order of magnitude more differential expression. This relative increase is expected given that the *hnr* gene is a known global regulator controlling the degradation of σ^S factor, which in turn controls many genes, whereas the *gdhA* and *aceE* genes corresponding to glutamate dehydrogenase and a subunit of pyruvate dehydrogenase, respectively, appear in metabolic pathways with more limited effects. This is in agreement with the results of Ishii *et al.* (2007), who similarly found that genetic perturbations in central carbon metabolism led to relatively small changes in gene, protein, and metabolite expression. They attributed this stability to the structural redundancy of

the *E. coli* metabolic network itself, with multiple pathways and isozymes able to accommodate the majority of genetic perturbations. However, they found that environmental perturbations such as a change in the concentration of a limiting substrate led to larger changes in gene and protein expression to provide stability in metabolite levels. Deletion of the global regulator *hnr*, then, is seen to more closely resemble an environmental perturbation in leading to larger changes in gene and protein expression as compared to the deletion of the metabolic pathway targets.

Interestingly, deleting a region of the *yjiD* promoter in the Δ*gdhA* Δ*aceE* background, which has been reported to actually increase the level of *yjiD* expression and increase lycopene production by over 50% in 2xM9 media (Jin and Stephanopoulos 2007), leads to only a marginal change in differential gene expression. YjiD has recently been identified as an antiadaptor protein which interacts with the Hnr (also called RssB) protein and prevents its association with σ^S, thereby blocking the σ^S proteolysis mechanism carried out by ClpXP (Figure 1-3) (Bougdour, Cunning *et al.* 2008). Thus, overexpression of the *yjiD* (renamed as *iraD*) gene by deleting part of the promoter region has a similar σ^S "stabilizing" effect as the *hnr* deletion. When either of the *hnr* gene alone or the *yjiD* promoter region alone was deleted in the PE parental strain, the resulting lycopene production profiles were highly similar (Alper and Stephanopoulos 2008), which is expected given the similar mechanisms by which the two knockouts increase σ^S stability. However, the Δ*gdhA* Δ*aceE* Δ_P*yjid* strain produces more lycopene than the Δ*hnr* strain (Figure 1-5) (Alper and Stephanopoulos 2008), indicating that the Δ*gdhA* Δ*aceE* background is important to increasing production. It is surprising, though, that the relatively high amount of differential expression seen in the Δ*hnr* strain is not reflected in the Δ*gdhA* Δ*aceE* Δ_P*yjid* strain given the similar molecular mechanisms at work. This could indicate that the *hnr* gene deletion has farther-reaching transcriptional effects as a more direct effector of

σ^S stabilization and other functions than the *yjiD* promoter disruption and subsequent *yjiD* overexpression despite the similar σ^S stabilization effects. Otherwise, this could indicate that the Δ*gdhA* Δ*aceE* background interacts with the *yjiD* promoter disruption to suppress higher levels of differential gene expression.

The strains sharing the Δ*gdhA* deletion exhibit a nonlinear response in the number of differentially expressed genes to the subsequent gene deletions, with the double and triple knockouts actually appearing to stabilize in gene expression relative to the Δ*gdhA* strain. On the other hand, the Δ*hnr* and Δ*hnr* Δ*yliE* strains do show an increase in differential expression with both deletions. The differential expression information shown in Figure 5-1 may be especially important in the case of the strains sharing the Δ*gdhA* deletion since the lower amount of differential gene expression in these strains could make the identification of additional important genes for lycopene production less difficult. On the other hand, the overlap of differentially expressed genes between the Δ*gdhA* Δ*aceE* Δ_P*yjid*, the Δ*hnr*, and the Δ*hnr* Δ*yliE* strains may be especially interesting given their shared σ^S stabilization effect. Such common differentially expressed genes could help explain why the σ^S stabilization has a positive effect upon lycopene production.

The function of the *yliE* gene is not annotated beyond being a conserved inner membrane protein based on sequence analysis (Serres, Gopal *et al.* 2001), but it's disruption increases the number of differentially expressed genes by about 25-70, depending upon the p value cutoff applied to the microarray data. Applying the relaxed p value cutoff demonstrates the number of genes that were just excluded from being labeled as "differentially expressed" using the critical p value and which may, in fact, include a number of truly differentially expressed genes amongst a number of true negatives. In general, Figure 5-1 shows that the relaxed p value cutoff increases

the number of differentially expressed genes for the *yliE* deletion the most. Deletion of the *yliE* gene product appears to have more of an effect upon the transcription of other genes than the *gdhA* and *aceE* metabolic targets and also that of the antiadaptor gene *iraD* (*yjiD*) but less of an effect than the *hnr* regulatory gene.

Table 5-1 displays the differentially expressed genes in greater detail along with their associated $\log_{10}$ ratios for the five mutants as compared to the PE pre-engineered strain and the p values resulting from the maximum likelihood method (Ideker, Thorsson *et al.* 2000) indicating the differential expression. The ranges of $\log_{10}$(Mutant/PE) differentially expressed gene expression ratios for the strains were the following: -1.04 to 0.55 for the Δ*gdhA* strain; -1.19 to 0.61 for the Δ*gdhA* Δ*aceE* strain; -1.06 to 0.55 for the Δ*gdhA* Δ*aceE* Δ_P*yjid* strain; -0.86 to 0.67 for the Δ*hnr* strain; and -0.64 to 1.13 for the Δ*hnr* Δ*yliE* strain. Individual differentially expressed genes are discussed more in the following sections.

Table 5-2 and Table 5-3 show the distribution of the differentially expressed genes in the five examined strains as compared to the PE strain, using the critical p value cutoff. Due to the small number of differentially expressed genes, the pairwise overlap was correspondingly small for the strains sharing the Δ*gdhA* deletion. The Δ*hnr* and Δ*hnr* Δ*yliE* strains shared a strong overlap of 71 differentially expressed genes in both strains. Table 5-3 demonstrates that for the Δ*gdhA*, the Δ*hnr*, and the Δ*hnr* Δ*yliE* strains, a strong majority of differentially expressed genes were up-regulated rather than down-regulated, whereas the distribution between up and down regulation was nearly even for the small numbers of differentially expressed genes in the Δ*gdhA* Δ*aceE* and the Δ*gdhA* Δ*aceE* Δ_P*yjid* strains.

Table 5-2 Overlap of differentially expressed genes relative to the PE strain between the five mutant strains. The critical p value cutoff of 4.26×10^{-3} was applied. Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ_P*yjid* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY).

Strain	G	GA	GAP	H	HY	Unique DE
G		4	3	2	1	15
GA			4	0	2	2
GAP				3	6	1
H					71	38
HY						62

Table 5-3 Up and down regulation of the differentially expressed genes for the five mutant strains as compared to the PE strain. The critical p value cutoff of 4.26×10^{-3} was applied. Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ_P*yjid* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY).

Strain	Up DE	Down DE	Total DE
G	19	2	21
GA	3	4	7
GAP	5	4	9
H	97	14	111
HY	88	48	136

It should be noted that although the five mutant strains each contained specific gene deletions, these genes were not among those in Table 5-1 that were found to be differentially down-regulated (or up-regulated for *yjiD* due to the promoter deletion). This was attributed to the fact that these strains were generated by deleting significant regions of the corresponding genes on the chromosome but also leaving from about 30 to over 1,400 base pairs of the original gene sequence in the chromosome (Alper, Miyaoku *et al.* 2005; Alper and Stephanopoulos 2008). Thus, fragments of the genes could still be transcribed and peptides of the complete proteins could also be translated. This would lead to the apparent presence of the genes and proteins, as seen in an apparent lack of down-regulation, despite the facts that the genes (except for *yjiD*) truly are "deleted" and functional proteins are not present. However, the previously reported up-regulation in the *yjiD* transcript level (Jin and Stephanopoulos 2007) was not detected in this study. This may be an artifact of the microarray experimental error.

5.2. Gene Expression by Metabolic Pathways

Using the EcoCyc database and associated Omics Viewer tool (Keseler, Bonavides-Martinez *et al.* 2009), gene expression data were next painted onto the metabolic pathways of *E. coli* to look for trends in gene expression that may be apparent within individual metabolic pathways and other cellular functions. It should be noted that all gene expression $\log_{10}$(Mutant/PE) ratios are painted onto these maps, not simply the differential gene expression that was also determined and shown in the previous section. The comparisons of each of the five mutants with the PE strain appear in Figure 5-2 through Figure 5-6.

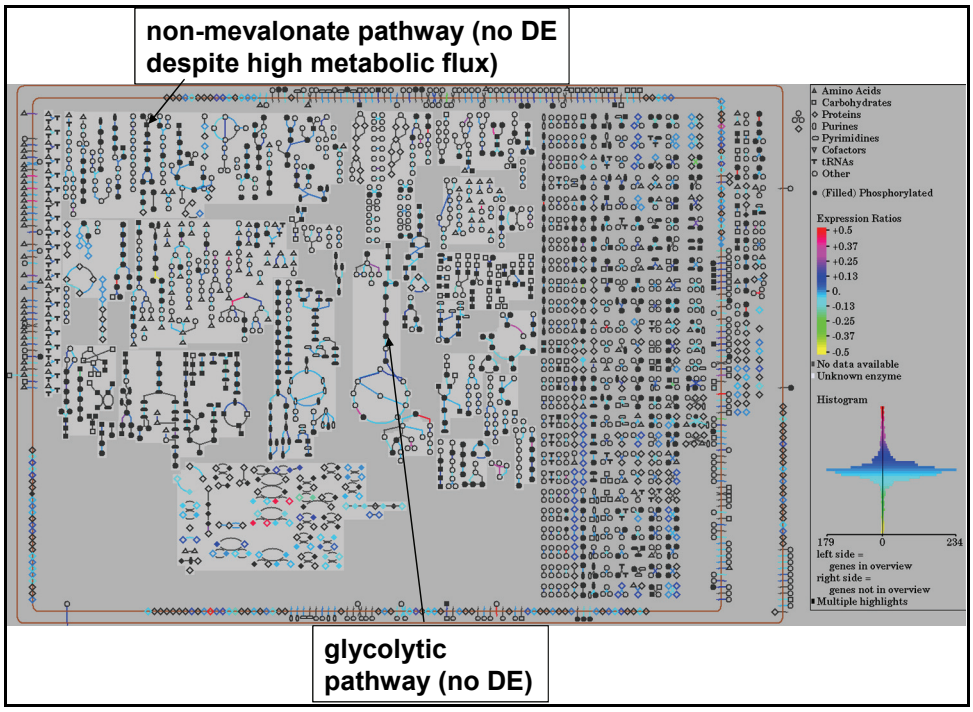

Figure 5-2 Global gene expression of Δ*gdhA* strain compared to PE strain. Log_{10}(Mutant/PE) ratios are shown, with red indicating up-regulation and yellow indicating down-regulation in the Δ*gdhA* strain.

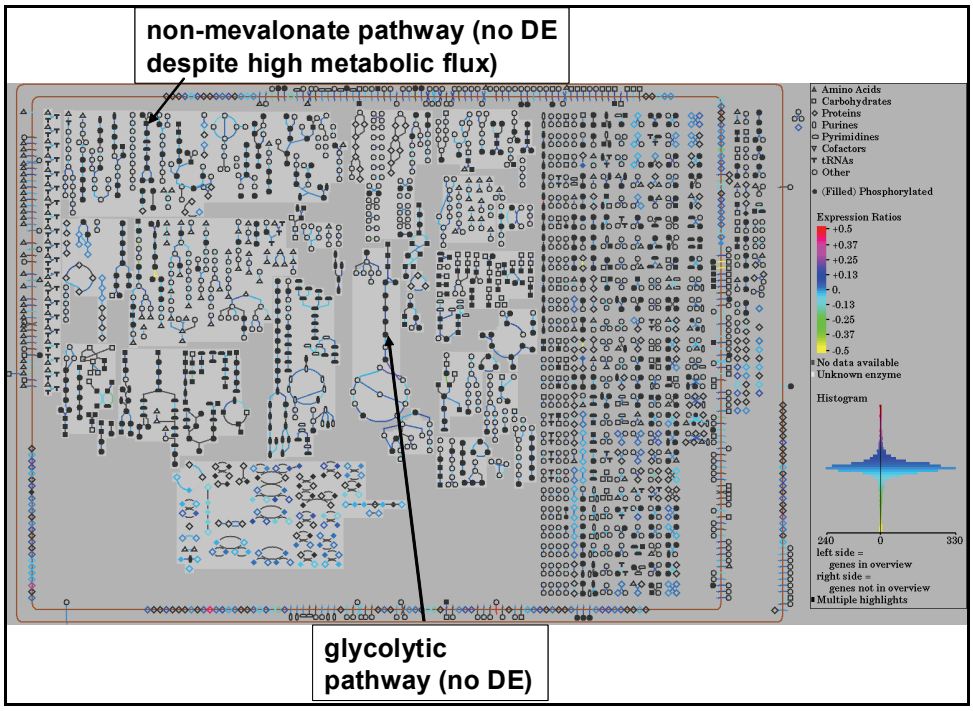

Figure 5-3 Global gene expression of Δ*gdhA* Δ*aceE* strain compared to PE strain. Log_{10}(Mutant/PE) ratios are shown, with red indicating up-regulation and yellow indicating down-regulation in the Δ*gdhA* Δ*aceE* strain.

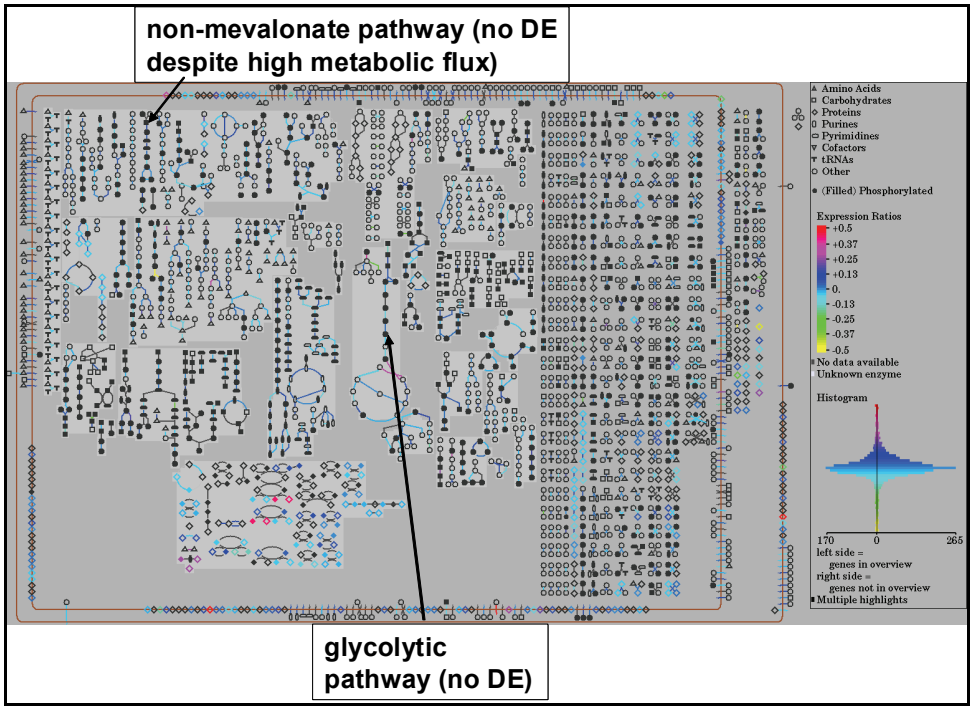

Figure 5-4 Global gene expression of Δ*gdhA* Δ*aceE* Δ_P*yjid* strain compared to PE strain. Log_{10}(Mutant/PE) ratios are shown, with red indicating up-regulation and yellow indicating down-regulation in the Δ*gdhA* Δ*aceE* Δ_P*yjid* strain.

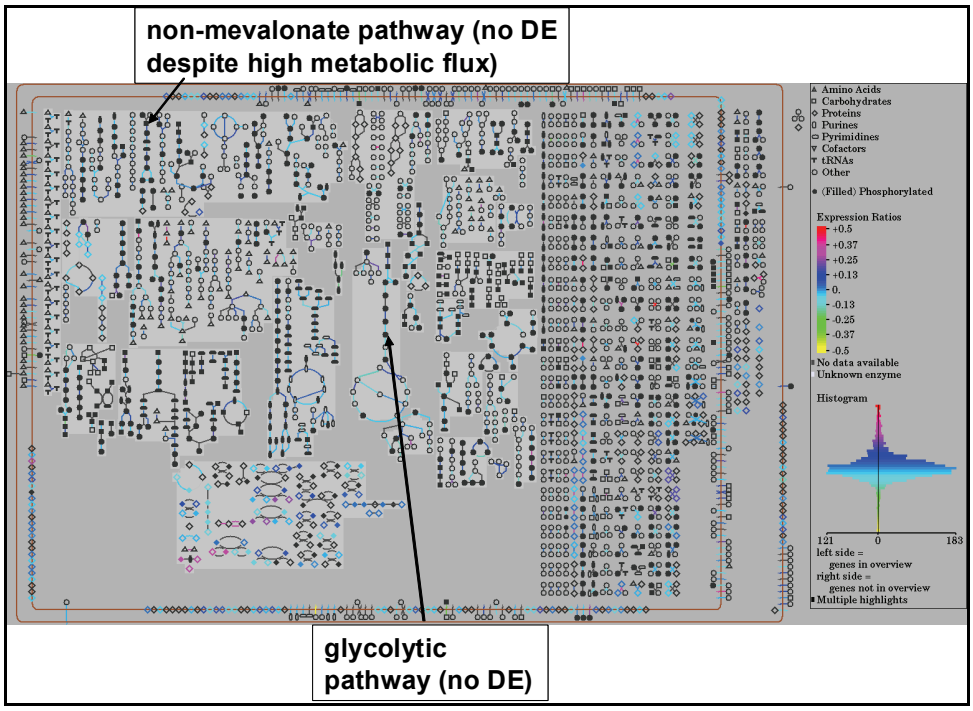

Figure 5-5 Global gene expression of Δ*hnr* strain compared to PE strain. Log_{10}(Mutant/PE) ratios are shown, with red indicating up-regulation and yellow indicating down-regulation in the Δ*hnr* strain.

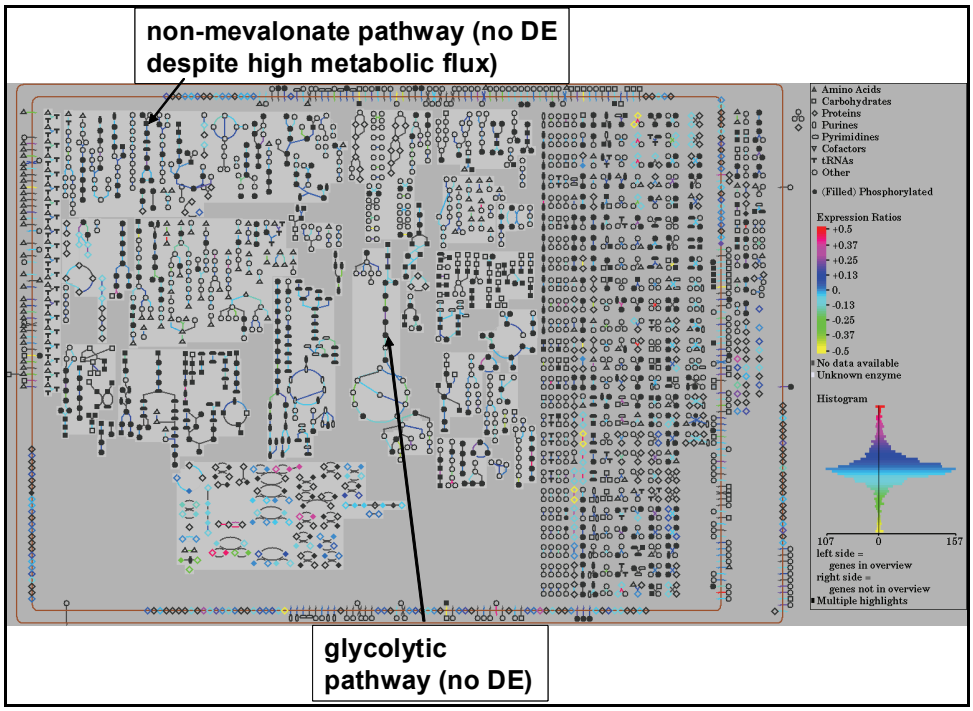

Figure 5-6 Global gene expression of Δ*hnr* Δ*yliE* strain compared to PE strain. Log_{10}(Mutant/PE) ratios are shown, with red indicating up-regulation and yellow indicating down-regulation in the Δ*hnr* Δ*yliE* strain.

Within Figure 5-2 through Figure 5-6, it is apparent that the vast majority of gene expression ratios are painted blue, indicating that the log_{10}(Mutant/PE) ratios for most genes are

nearly 0 and there is little difference between the various mutants and the PE strain. Even from a high level view, no particular pathways appear to exhibit a coordinated program of gene expression with any logical link to the non-mevalonate pathway. This would be expected for the strains sharing the Δ*gdhA* deletion since they exhibited so few differentially expressed genes, but even the strains sharing the Δ*hnr* deletion seem to lack coordination in the increased differential expression that is apparent in Figure 5-5 and Figure 5-6.

Of particular note, neither the glycolytic nor the non-mevalonate pathways themselves display any differential expression, despite the fact that these pathways supply the glyceraldehyde-3-phosphate and pyruvate precursors and convert the precursors into IPP and DMAPP, respectively. The metabolic flux through these pathways must be higher in the mutants compared to the PE strain given that the lycopene production is higher, but the higher flux is not due to coordinate up-regulation of either of these pathways. Indeed, since the *dxs*, *idi*, and *ispFD* genes are overexpressed in the PE background of all of the strains in this study, it would not necessarily be expected that these pathway controlling enzymes are up-regulated even further in the mutant strains. Thus, it appears that the increased mutant lycopene production phenotypes relative to the PE strain are due to more distal factors beyond the glycolytic and the non-mevalonate (or methylerythritol) pathways, an observation which has also been made previously (Alper and Stephanopoulos 2008). These factors may include genes, proteins, cofactors, other metabolites, and sRNAs with previously uncharacterized relationships and interactions with the pathways of interest. In the next section, work aimed at uncovering some of these factors through conserved programs of gene expression across the various mutants is described.

5.3. Conservation of Differential Gene Expression Across Mutants

Differential expression that was conserved between the various mutants was next examined, as it was reasoned that such common factors may reasonably be expected to correlate with the lycopene production phenotype compared with differentially expressed genes that only appear in a single mutant. Especially large changes in differentially expressed genes appearing in only a single mutant are still interesting, but it was decided to mainly focus upon conserved differential expression as a starting point for analysis. As Alper *et al.* (2008) observed, many diverse genotypes can yield a similar phenotype in the context of lycopene production, but a number of key nodes such as glutamate and *hnr* control the search trajectory towards increased production. Figure 5-7 displays the $\log_{10}$(Mutant/PE) ratios for the six total genes differentially expressed in at least 3 of the 5 mutants examined using the critical p value. It can be seen that directionality trends are preserved for all genes in the figure despite some variable magnitudes. It is particularly interesting that *intG*, *atpE*, *ynjE*, *yhjK*, and *yjjG* demonstrate consistent trends even across the distinct mutant families sharing either the Δ*gdhA* or the Δ*hnr* deletions. As seen in Figure 5-8, the gene *hisH* also demonstrates this when the critical p value is relaxed to 0.01, as the Δ*hnr* Δ*yliE* strain shows differential down-regulation as well as the three mutants sharing the Δ*gdhA* deletion. Additional genes that appear in Figure 5-8 include *ybdK*, *citC*, *sdhC*, *yeaJ*, *cheZ*, *cheY*, and *cheB*.

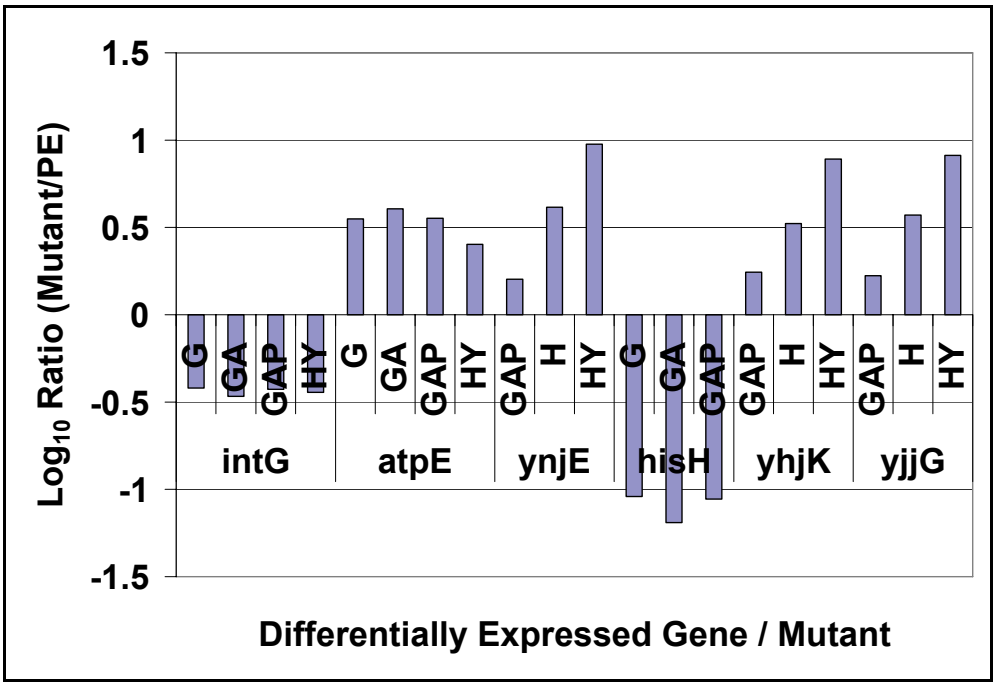

Figure 5-7 Conservation of differential gene expression across mutants. Genes that were differentially expressed using the critical p value cutoff of 4.26×10^{-3} in at least 3 of the 5 mutants are shown with the corresponding $\log_{10}$(Mutant/PE) ratios. Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ_P*yjid* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY).

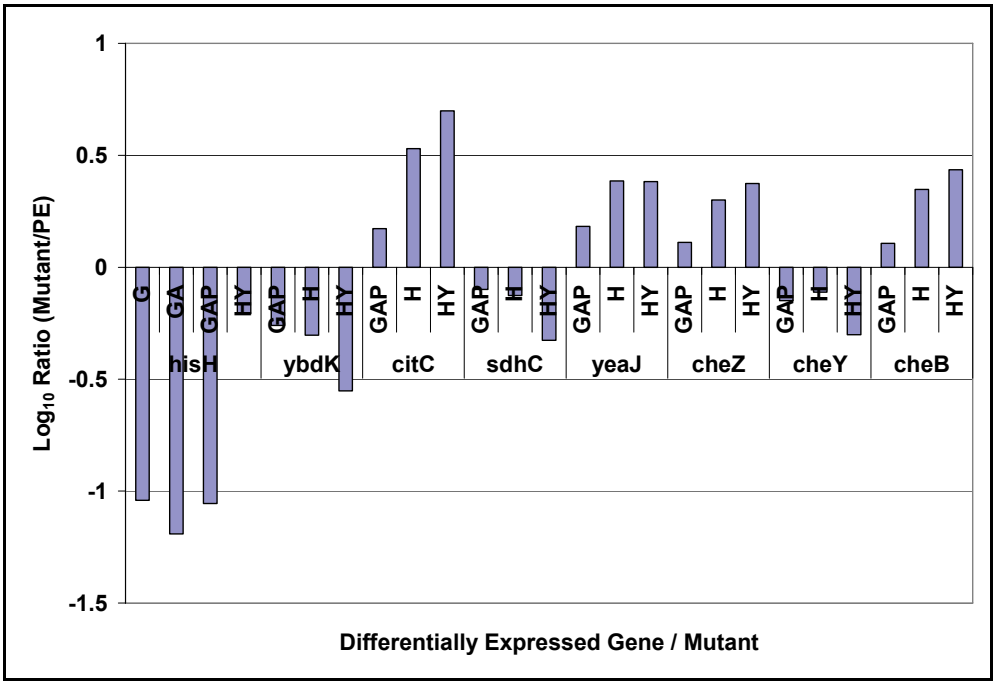

Figure 5-8 Conservation of additional differentially expressed genes across mutants when using a relaxed p value cutoff. Additional genes or mutants not already appearing in Figure 5-7 are displayed that result from applying the relaxed p value cutoff of 0.01 and selecting genes with at least 3 of the 5 mutants differentially expressed. Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ_P*yjid* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY).

The *hisH* gene of the histidine biosynthetic pathway is down-regulated over 10-fold in the three mutants sharing the Δ*gdhA* deletion and nearly 40% in the Δ*hnr* Δ*yliE* strain as seen in Figure 5-7 and Figure 5-8, respectively. This gene encodes for a subunit of the imidazole glycerol phosphate synthase enzyme that catalyzes the branch point reaction and fifth step in histidine biosynthesis from which the products either continue to histidine or purine biosynthesis. The HisFH heterodimer catalyzes the addition of a nitrogen from glutamine to the imidazole ring of phosphoribulosylformimino-AICAR-phosphate to generate aminoimidazole carboxamide ribonucleotide, an intermediate in purine nucleotide biosynthesis, D-erythro-imidazole-glycerol-phosphate, which continues in the pathway towards histidine synthesis, and glutamate (Klem and Davisson 1993). It is interesting that this reaction includes the conversion of glutamine to glutamate in light of the *gdhA* deletion for these mutant strains and the previous identification of glutamate as an important "node" for lycopene biosynthesis strategies (Alper and Stephanopoulos 2008). It is also interesting that it is the *hisG* gene, not *hisH*, that is subject to feedback inhibition in the pathway (Alifano, Fani *et al.* 1996).

The *hisLGDCBHAFI* operon contains all of the genes in the histidine biosynthetic pathway, and the *hisH* gene is in the latter half of the operon. Only the *hisH* gene was seen to be down-regulated, despite the fact that the histidine polycistronic genes are transcribed together. However, ribonucleases and the RNA degradosome complex composed mainly of RNAseE, PNPase, RhlB, and enolase have been documented as being important to *E. coli* RNA degradation and differential expression of polycistronic genes (Rauhut and Klug 1999; Carpousis 2007; Aguena and Spira 2009). This degradative activity seems like the most likely explanation for why only the *hisH* gene was down-regulated. Interestingly, one study found that *his* operon expression was altered by the presence or absence of the ribonuclease RnaseP, which is involved

in the processing of the polycistronic transcript (Li and Altman 2003). The ribose transport *rbs* operon was also found to be affected, suggesting that RNAseP is involved in regulating the levels of metabolic intermediate PRPP which links the histidine, tryptophan, purine, and pyrimidine biosynthetic pathways (Jensen 1969) and is discussed later in this section.

The *atpE* gene encodes for the inner membrane associated and critical c subunit of the F_0 domain of ATP synthase. It is up-regulated over 3.5-fold in the three mutants sharing the Δ*gdhA* deletion and over 2.5-fold in the Δ*hnr* Δ*yliE* strain, as seen in Figure 5-7 and Figure 5-8, respectively. The c subunit is required for linking the membrane associated F_0 and the catalytic F_1 domains of ATP synthase and also for the proton translocation required by this complex for ATP generation (Weber and Senior 2003). The c ring is preferentially composed of 10 c subunits in *E. coli* (Jiang, Hermolin *et al.* 2001), and its rotation is driven by the proton motive force. It has been shown that when the c subunit synthesis is limiting, the stoichiometry of c subunits is preserved in the F_0 domain, fewer complete complexes are produced, and proton translocation is lower (Krebstakies, Aldag *et al.* 2008). Addition of c subunits increases proton translocation again. Thus, it is possible that *atpE* overexpression is a mutant strategy for increasing ATP synthesis via higher levels of the functional ATP synthase if this was a limiting component.

Interestingly, other components of the ATP synthase complex are also up-regulated. The *atpB* gene is about 60% differentially up-regulated in the Δ*hnr* Δ*yliE* strain compared to the PE strain. The *atpA* and *atpD* genes are not statistically differentially up-regulated, but their expression ratios indicate up-regulation of 3.3-fold in the Δ*gdhA* Δ*aceE* Δ_P*yjiD* strain and from 70% to 2-fold in the three strains sharing the Δ*gdhA* deletion, respectively. These data are

consistent with the hypothesis that ATP synthase is up-regulated at the transcriptional level to increase ATP synthesis.

Other genes in Figure 5-7 and Figure 5-8 are of various functions, with a number of unknown functions. *intG* is a predicted defective phage integrase, according to EcocCyc (Karp, Keseler *et al.* 2007), which clustered with chemotaxis, flagellum, and fimbria genes in increased expression after long term adaptation to propionate and acetate (Polen, Rittmann *et al.* 2003). It is down-regulated nearly three-fold in the three strains sharing the Δ*gdhA* deletion as well as the Δ*hnr* Δ*yliE* strain. *citC* encodes for citrate lyase synthetase, which activates citrate lyase by acetylation (Nilekani and SivaRaman 1983), and it is up-regulated over three-fold in the Δ*hnr* and Δ*hnr* Δ*yliE* strains and about 50% in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* strain. *sdhC* is a membrane-associated subunit of the succinate dehydrogenase complex composing the large subunit of cytochrome b_{556} (Nakamura, Yamaki *et al.* 1996). This complex of the TCA cycle couples succinate oxidation to fumarate with the reduction of ubiquinone in the electron transport chain. The association of *sdhC* with ubiquinone, which is composed of polyisoprenoid units like lycopene, is notable. Perhaps the reduction of *sdhC* expression leads to a slight decrease in the amount of ubiquinone required for the electron transport chain, freeing isoprenoid units to be directed towards lycopene synthesis instead. Although the *sdhC* down-regulation is relatively small at only 20-50% for the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid*, Δ*hnr*, and Δ*hnr* Δ*yliE* strains, this possibility is interesting. Indeed, some of the proteomic data resulting from application of the second threshold (Chapter 6 and Appendix 9.1) indicates that the ubiquinone biosynthetic protein UbiB (b3835) is down-regulated about 2.3-fold in the Δ*hnr* Δ*yliE* strain.

Finally, the chemotaxis *cheB*, *cheZ*, and *cheY* genes display differential expression in the Δ*hnr*, the Δ*hnr* Δ*yliE*, and the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* strains, as seen in Table 5-1, and if the p

value cutoff is relaxed to 0.01, the differential expression is conserved amongst all three strains. The *cheB* and *cheZ* genes show similar patterns of up-regulation from about 25-150%, whereas the *cheY* gene displays down-regulation from about 20-50%. The CheZ, CheY, and CheB proteins relate in a complex network of interactions governing *E. coli* locomotion in response to chemical gradients as has been recently reviewed (Manson, Armitage *et al.* 1998). The pattern of gene expression in these three genes seems to favor reducing CheY-P-induced activation of the FliM protein in the motor switch complex to induce counterclockwise flagellar rotation. This would lead to less bacterial "tumbling" in response to concentration gradients and smoother locomotion. Perhaps this behavior is indicative of bacterial cells associating together in biofilm-like communities, where chemotaxis is down-regulated in favor of a more sedentary, multicellular phenotype. This aggregated phenotype prevents oxygen and nutrient transport to all cells, which lowers production. On the other hand, given the high energetic requirement for chemotaxis, this potential switch away from chemotaxis may be advantageous in conserving cellular energy for lycopene production, so there may be a tradeoff.

Despite their partially unknown functions, the remaining genes in Figure 5-7 and Figure 5-8 encode for the following predicted proteins: YnjE is a predicted 3-mercaptopyruvate sulfurtransferase based on sequence similarity (Reed, Vo *et al.* 2003); YhjK is a predicted diguanylate cyclase inner membrane protein with three predicted transmembrane domains (Daley, Rapp *et al.* 2005); YjjG is a monophosphatase with phosphatase activity towards UMP, dUMP, and dTMP and general nucleotidase activity (Proudfoot, Kuznetsova *et al.* 2004); YbdK catalyzes the ligation of glutamate with cysteine at a slow catalytic rate (Lehmann, Doseeva *et al.* 2004); and YeaJ is also a predicted diguanylate cyclase associated with the plasma

membrane, according to the EcoCyc database (Karp, Keseler *et al.* 2007). These genes vary in magnitudes of expression changes from 60% to nearly 10-fold compared to the PE strain.

It is particularly interesting that two of these genes encoding for proteins of "unknown" function, *yhjK* and *yeaJ*, are predicted diguanylate cyclases and are both up-regulated significantly. The yhjK gene is up-regulated about 50% in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* strain and nearly 2.5-fold in the Δ*hnr* the Δ*hnr* Δ*yliE* strains, whereas the yeaJ gene up-regulation spans from about 75% in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* gene to nearly 7.5-fold in the Δ*hnr* Δ*yliE* strain. Diguanylate cyclases catalyze the synthesis of the ubiquitous bacterial second messenger c-di-GMP from two molecules of GTP. A novel signal transduction pathway that is based upon the controlled synthesis and degradation of c-di-GMP is emerging that primarily affects cellular functions involved in the transition between the motile, single-celled state and an adhesive, surface-attached, multicellular biofilm state (Jenal and Malone 2006; Hengge 2009). Low levels of c-di-GMP are associated with cells that move via flagellar motors or retracting pili, whereas increasing concentrations of c-di-GMP promote the expression of adhesive matrix components and result in multicellular behavior and biofilm formation. During the transition from post-exponential to stationary phase, *E. coli* cells grown in complex media have been shown to downregulate the expression of flagella and motility (Pesavento, Becker *et al.* 2008) and to induce adhesive curli fimbriae when grown below 30° C (Olsen, Wick *et al.* 1998). This transition operates by the mutual inhibition of the FlhDC (motility) and σ^S (adhesion) control cascades (Pesavento, Becker *et al.* 2008). At the top levels of these cascades, mutual exclusion operates by competition of sigma subunits of RNA polymerases (σ^{70}, σ^{FliA}, and σ^S) as well as by FliZ, which acts as an inhibitor of σ^S function. At lower levels, c-di-GMP is important to the motile to sedentary switch and is antagonistically controlled by the σ^S-activated diguanylate

cyclase proteins YegE and YedQ and the phosphodiesterase YhjH, which is indirectly controlled by the flagellar master regulator FlhDC (Pesavento, Becker *et al.* 2008).

Thus, it appears likely that the up-regulation of diguanylate cyclases *yhjK* and *yeaJ* is related to the changes in the chemotactic *cheZ*, *cheY*, and *cheB* gene expression described above and could be causing these changes. Although *yhjK* and *yeaJ* have not been previously described as being controlled by σ^S expression, it may be that their up-regulation in the Δ*gdhA* Δ*aceE* Δ_P*yjid* strain, where the *yjiD* (iraD) gene is overexpressed and stabilizing σ^S, and in the Δ*hnr* the Δ*hnr* Δ*yliE* strains, where the *hnr* gene controlling σ^S degradation has been deleted, is due to the increased levels of σ^S. This may be similar to the σ^S control known to exist for diguanylate cyclases YegE and YedQ. This adhesion phenotype of lycopene-producing cells is visually apparent and not ideal for the efficient transfer of dissolved oxygen and nutrients to the producing cells. Notably, *yfcP*, encoding for a predicted fimbrial-like adhesin protein according to the Uniprot Consortium (2009), is up-regulated over 3-fold in the Δ*gdhA* Δ*aceE* Δ_P*yjid* strain. Indeed, Yoon *et al.* (2006) followed the strategy of adding the surfactant Tween80 to lycopene-producing *E. coli* to increase lycopene production by preventing cellular aggregation. The patterns of gene expression observed here help to explain the basis for aggregation of lycopene-producing *E. coli* and why a surfactant strategy can effectively increase production. Since hydrophobic lycopene molecules are stored in the cellular membrane upon production (Fraser and Sandmann 1992) and increased σ^S expression is known to increase lycopene production, it may be that the mutant strains of this study are better able to produce lycopene but are also more prone to aggregation due to the same factors causing increased production. Disrupting these aggregation mechanisms by deleting the diguanylate cyclase genes such as *yhjK* and *yeaJ*, for example, may be an interesting strategy and alternative to using surfactants. On the other hand,

if the gene expression pattern in the *cheZ*, *cheY*, and *cheB* genes truly is indicative of a switch away from chemotaxis, this may have an advantage in conserving energy. Nevertheless, aggregation is not desirable for high production. More experiments should be pursued in this area to clarify the conserved gene expression patterns and determine the effect upon production.

A number of differentially-expressed genes that overlapped between only two of the mutant strains were also found, and lists of these genes appear in Table 5-4 and Table 5-5. The latter is specific to the high number of differentially expressed genes shared by the Δ*hnr* and the Δ*hnr* Δ*yliE* strains and lists all such genes including those differentially expressed in the other mutants. It is interesting that two of the up-regulated genes in Table 5-4 are membrane-associated. *modA* encodes for the periplasmic binding component of the molybdate ABC transporter, and EptA is a predicted metal-dependant hydrolase associated with the inner membrane that forms a complex with ZipA (Stenberg, Chovanec *et al.* 2005), an essential cell division protein. *cynT* encodes for a carbonic anhydrase monomer, and *narL* encodes for a transcriptional dual regulator of anaerobic respiration and fermentation.

Table 5-4 Differential gene expression conserved in exactly two of the five mutants with corresponding log_{10}(Mutant/PE) ratios. Associated Blattner numbers and p values for differential expression can be found in Table 5-1. Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ_P*yjid* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY).

Gene	Mutant	Log_{10} Ratio
cynT	G	0.27
	GA	0.22
eptA	GA	0.43
	GAP	0.53
modA	G	0.50
	H	0.23
narL	G	0.45
	H	0.25

Table 5-5 Differential gene expression conserved between the Δ*hnr* and Δ*hnr* Δ*yliE* strains with corresponding log_{10}(Mutant/PE) ratios. Associated Blattner numbers and p values for differential expression can be found in Table 5-1.

Gene	Function	Δ*hnr*	Δ*hnr* Δ*yliE*
		log_10(Mut/PE)	log_10(Mut/PE)
		Ratio	Ratio
gltB	glutamate synthase, large subunit	0.667	1.128
ynjE	predicted thiosulfate sulfur transferase	0.616	0.977
yjjG	pyrimidine nucleotidase	0.571	0.913
citC	citrate lyase synthetase	0.530	0.699
yhjK	predicted diguanylate cyclase	0.523	0.891
yhjQ	cell division protein (chromosome partitioning ATPase)	0.494	0.845
bcsA	cellulose synthase, catalytic subunit	0.443	0.960
yeaJ	predicted diguanylate cyclase	0.386	0.383
clcA	EriC chloride ion ClC channel	0.364	0.600
yicL	inhibitor of heme biosynthesis	0.357	0.787
cheB	Chemotactic Signal Transduction System component	0.347	0.435
insC-1	IS2 element protein InsA	0.343	0.334
pepB	aminopeptidase B	0.341	0.344
dnaT	primosome	0.332	0.590
cheR	chemotaxis protein methyltransferase	0.332	0.322
paaG	putative enoyl-CoA hydratase/isomerase of phenylacetate degradation	0.305	0.350
cheZ	cytosolic phosphatase of the chemotaxis signal transduction complex	0.300	0.374
ychE_1	predicted protein, N-ter fragment (pseudogene)	0.293	0.323
mobA	molybdopterin guanine dinucleotide synthase	0.284	0.370
yegW	predicted DNA-binding transcriptional regulator	0.280	0.421
yfgM	conserved protein	0.272	0.358
ybeM	predicted C-N hydrolase superfamily, NAD(P)-binding amidase/nitrilase	0.271	0.418
hycD	hydrogenase 3, membrane subunit	0.269	0.234
paaF	putative enoyl-CoA hydratase/isomerase of phenylacetate degradation	0.268	0.419
yfaD	conserved protein	0.266	0.470
tdcG	(now b4471) L-serine deaminase III	0.266	0.423
yddL	predicted lipoprotein	0.266	0.394
nrdI	conserved protein that may stimulate ribonucleotide reductase	0.249	0.378
ychS	predicted protein	0.249	0.368
yccX	acylphosphatase	0.247	0.349
gntU	(now b4476) GntU gluconate Gnt transporter	0.241	0.236
nikR	DNA-binding transcriptional repressor, Ni-binding	0.239	0.277
yebW	predicted protein	0.234	0.556
pscG	predicted pseudouridine 5'-phosphate glycosidase	0.232	0.343
fcl	GDP-fucose synthase	0.230	0.292
ybiC	predicted dehydrogenase	0.228	0.283
tdcG	(now b4471) L-serine deaminase III	0.224	0.360

Gene	Function	Δ*hnr*	Δ*hnr* Δ*yliE*
nhoA	N-hydroxyarylamine O-acetyltransferase	0.224	0.317
mdtB	MdtABC-TolC multidrug efflux transport system	0.223	0.339
cutC	copper homeostasis protein	0.221	0.419
ymfO	e14 prophage; conserved protein	0.221	0.373
csiD	predicted protein	0.219	0.437
prpB	2-methylisocitrate lyase	0.217	0.404
nfsA	NADPH nitroreductase monomer	0.215	0.404
ygiC	predicted enzyme	0.213	0.319
intE	e14 prophage; predicted integrase	0.209	0.314
yjbG	conserved protein	0.207	0.437
nuoG	NADH:ubiquinone oxidoreductase, chain G	0.207	0.219
hspQ	heat shock protein, hemimethylated DNA-binding protein	0.204	0.369
ygaH	YgaH L-valine exporter	0.202	0.321
ycaR	conserved protein	0.201	0.492
cls	cardiolipin synthase	0.196	0.344
ycjU	β-phosphoglucomutase	0.195	0.246
rlmF	23S rRNA m6A1618 methyltransferase	0.192	0.262
gmd	GDP-mannose 4,6-dehydratase	0.187	0.386
ilvH	acetolactate synthase / acetohydroxybutanoate synthase	0.180	0.286
ydjA	predicted oxidoreductase	0.178	0.247
yedS_1	predicted protein, N-ter fragment	0.175	0.302
ygcS	YgcS MFS transporter	0.159	0.285
yjeJ	predicted protein	0.159	0.275
dnaX	DNA polymerase III, γ subunit	0.148	0.244
dicC	Qin prophage; DNA-binding transcriptional regulator for DicB	0.122	0.182
ypjL	CP4-57 prophage; predicted inner membrane protein	0.118	0.218
dmsB	dimethyl sulfoxide reductase, chain B	-0.122	-0.241
sdhC	succinate dehydrogenase membrane protein	-0.126	-0.326
glpE	thiosulfate sulfurtransferase	-0.128	-0.159
flk	predicted flagella assembly protein	-0.139	-0.185
narG	nitrate reductase A, α subunit	-0.143	-0.301
adhE	PFL-deactivase / alcohol dehydrogenase / acetaldehyde dehydrogenase	-0.143	-0.403
ycjG	L-Ala-D/L-Glu epimerase	-0.273	-0.422
ybdK	γ-glutamyl:cysteine ligase	-0.303	-0.552

The differential gene expression shared between the Δ*hnr* and Δ*hnr* Δ*yliE* strains is highly similar in directionality, but the Δ*hnr* Δ*yliE* strain generally appears to have larger magnitude changes for a given differentially expressed gene. A number of the genes in Table 5-5 are transcriptionally-dependent upon σ^S, which is logical given that these strains share the

hnr deletion. In general, these strains exhibit much more additivity in differential gene expression than the strains sharing the Δ*gdhA* deletion, with the Δ*hnr* and Δ*hnr* Δ*yliE* strains having 71 differentially-expressed genes in common.

A particularly interesting gene in Table 5-5 is the *gltB* gene, which encodes for the large subunit of glutamate synthase (GOGAT) and is the most up-regulated gene in both of these strains. This enzyme is part of the nitrogen assimilation pathways of *E. coli*. The primary products of nitrogen assimilation are glutamate and glutamine, the major intracellular nitrogen donors. Figure 5-9 shows the reactions regulated by the enzymatic products of the *gdhA*, *glnA*, *gltB*, and *gltD* genes.

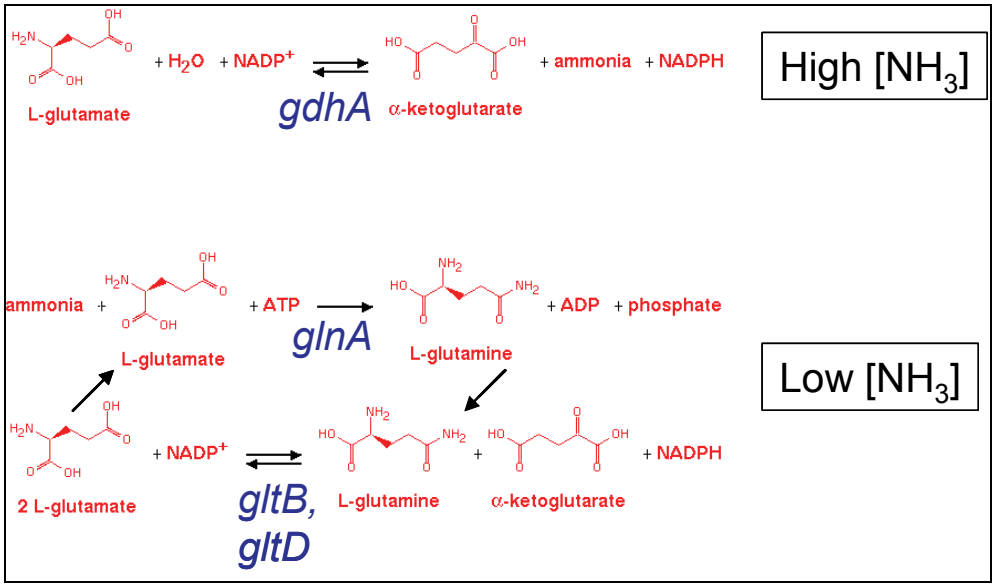

Figure 5-9 Two alternative reactions of glutamate synthesis in *E. coli* (Keseler, Bonavides-Martinez *et al.* 2009).

The *glnA* gene is under control of the Ntr regulon, which is sensitive to [NH_3] (Reitzer 2003). Under energy-limited and high [NH_3] conditions, the glutamate dehydrogenase (*gdhA* product) synthesizes glutamate, whereas ATP-consuming glutamine synthetase (GS: *glnA*) and glutamate synthase (GOGAT: *gltBD*) cooperatively synthesize glutamate under energy-rich and low [NH_3] conditions. It may be expected that following a *gdhA* deletion, the second reaction would

experience increased flux to compensate and potentially be up-regulated genetically. It is thus interesting to observe that amongst the three mutants sharing the Δ*gdhA* deletion, only the Δ*gdhA* Δ*aceE* Δ_P*yjid* mutant "nearly" exhibits differential up-regulation of the *glnA* gene (ratio = 0.273; p value = 0.012). On the other hand, *gltB* is up-regulated for the Δ*hnr* Δ*yliE* strain over 13-fold, and both *glnA* and *gltB* are up-regulated for the Δ*hnr* strain by 2-fold and over 4.5-fold, respectively. More investigation into these nitrogen assimilation pathways is warranted in light of the observed expression patterns.

5.4. Histidine Biosynthetic Pathway and Relationship to Lycopene Production

The strongest differential expression observed in genes of known function, the over 10-fold down-regulation of the *hisH* gene in the histidine biosynthetic pathways of the Δ*gdhA*, the Δ*gdhA* Δ*aceE*, and the Δ*gdhA* Δ*aceE* Δ_P*yjid* strains and the more than 3-fold up-regulation of the ATP synthase *atpE* gene in these same strains, were next examined in detail. A particularly notable observation is that the histidine pathway proceeds from the substrate 5-phosphoribosyl 1-pyrophosphate (PRPP), a derivative of ribose-5-phosphate of the pentose phosphate pathway. PRPP is found primarily in the histidine, tryptophan, purine, and pyrimidine biosynthetic pathways as a precursor or an intermediate (Figure 5-10, Figure 5-11, Figure 5-12, and Figure 5-13, respectively), linking these pathways together (Jensen 1969). Additionally, histidine and tryptophan biosynthesis "cost" 6 and 5 ATPs, respectively, and 1 and 3 NADPHs, respectively, which amount to high metabolic burdens (Stephanopoulos, Aristidou *et al.* 1998). Therefore, it was hypothesized that these three strains are able to synthesize higher amounts of lycopene than the PE strain because they may have more ATP and CTP available for this synthesis than the PE strain. This could be due to down-regulating the energetically-expensive histidine biosynthetic

pathway and conserving PRPP for ADP production. Interestingly, this concept of conserving the PRPP "pool" in light of histidine and tryptophan pathway mutations has been studied in *Salmonella typhimurium* (Henry, Garcia-Del Portillo *et al.* 2005). In the context of this study, increasing ADP levels would also increase the ATP concentration and in turn the CTP concentration because the [ADP] increase would decrease the ATP mass action ratio $[ATP]/[ADP][P_i]$, driving an increase in ADP phosphorylation. The energy charge of the cell is defined as the following:

$$\mathbf{EC}=\frac{\mathbf{[ATP]+[ADP]/2}}{\mathbf{[ATP]+[ADP]+[AMP]}} \quad (5.1)$$

The energy charge for bacteria typically ranges from 0.87 to 0.95 (Stephanopoulos, Aristidou *et al.* 1998), and perhaps conserving and redirecting PRPP leads to an eventual increase in the energy charge. Additionally, up-regulating *atpE* could be related to increasing numbers of ATP synthase in these mutants to increase capacity for producing ATP from ADP. These explanations would be plausible if ATP and CTP are limiting cofactors in the production of lycopene.

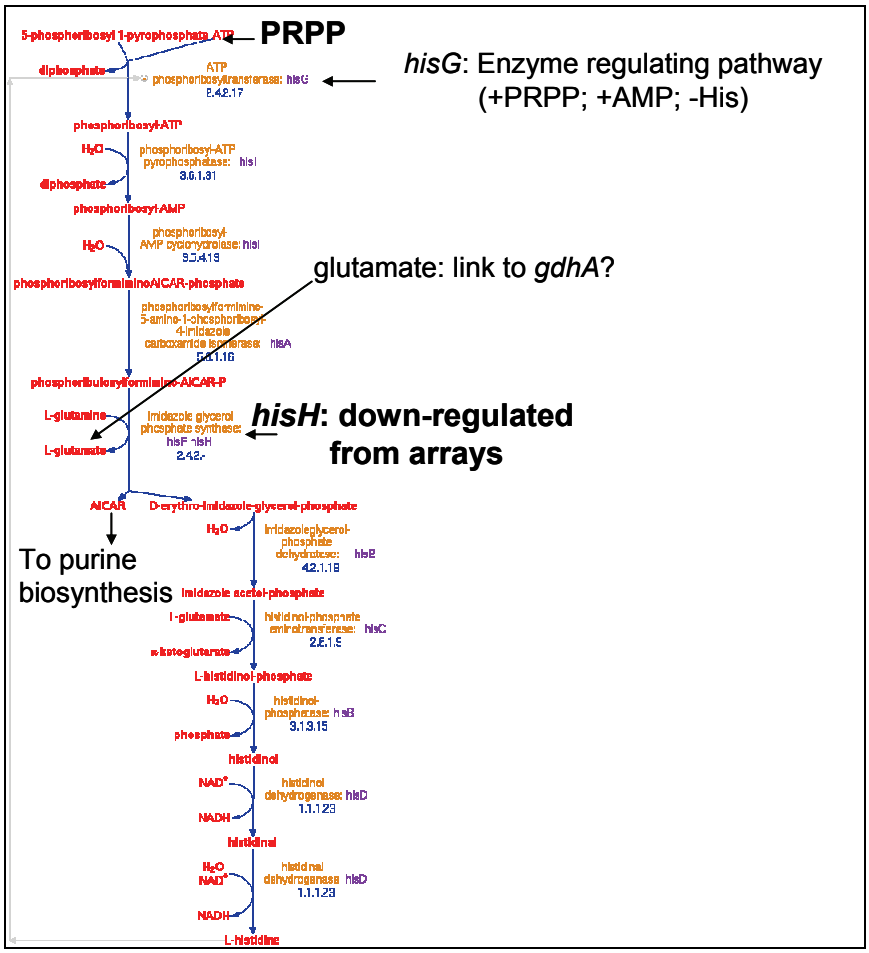

Figure 5-10 Histidine biosynthesis pathway with indicated *hisH* gene and PRPP substrate (Karp, Keseler *et al.* 2007)

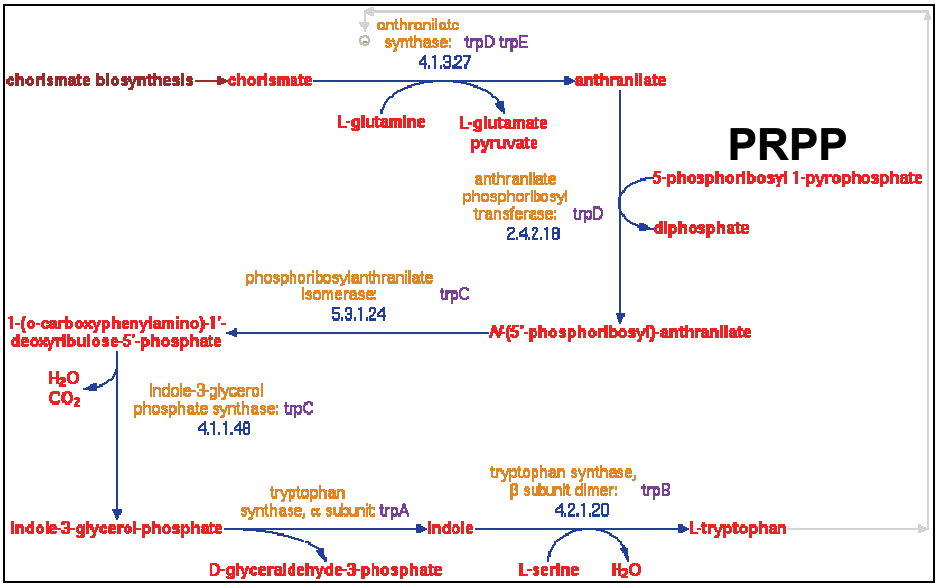

Figure 5-11 Tryptophan biosynthesis pathway with indicated PRPP substrate (Karp, Keseler *et al.* 2007)

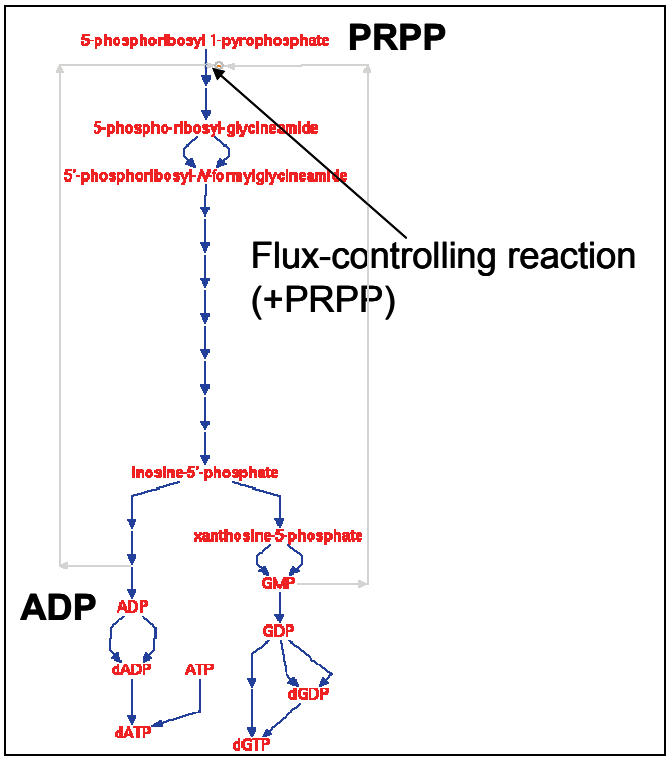

Figure 5-12 Purine biosynthesis pathway with indicated PRPP and ADP substrates (Karp, Keseler *et al.* 2007)

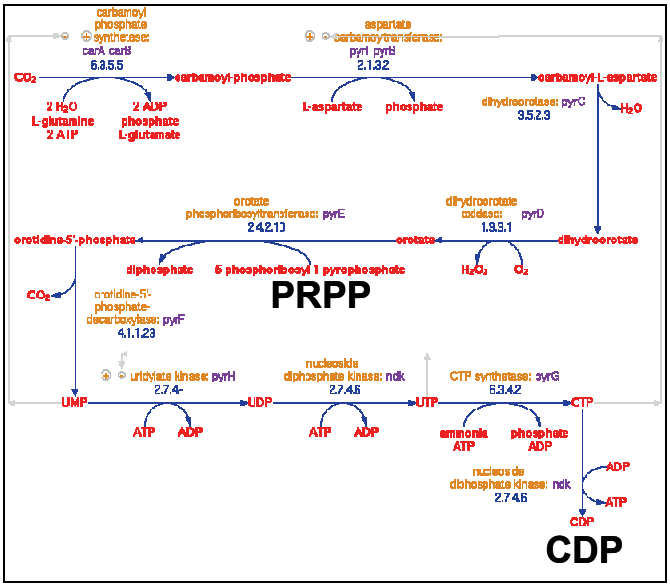

Figure 5-13 Pyrimidine biosynthesis pathway with indicated PRPP and CDP substrates (Karp, Keseler *et al.* 2007)

To test this hypothesis, the PE strain and the Δ*gdhA*, the Δ*gdhA* Δ*aceE*, and the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* strains were grown in the presence and absence of 100 mg/L of histidine and

tryptophan (Sigma Aldrich, St. Louis, MO), and lycopene production levels were determined in a 16 hour time period (gene expression measurements were normally taken in the 6-8 hour time period at OD_{600} = 0.4) and over a 48 hour period separately. The addition of histidine and tryptophan down-regulates the entire *his* and *trp* operons, respectively, via transcription attenuation and feedback inhibition (Alifano, Fani *et al.* 1996), so it was hypothesized that addition of both of these amino acids might conserve PRPP for ATP and CTP biosynthesis, leading to uniformly high levels of lycopene production across all four cell strains. Adding histidine to down-regulate the histidine pathway is experimentally more straightforward than reducing *hisH* gene expression. Since the histidine biosynthetic genes are all transcribed as a polycistronic mRNA within the same operon, simple promoter replacement would affect the entire pathway. Additionally, Δ*hisH* strains cannot grow normally in M9 minimal media (Baba, Ara *et al.* 2006) since they cannot synthesize histidine.

Figure 5-14 and Figure 5-16, respectively, display the lycopene production for the PE, Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*gdhA* Δ*aceE* Δ_P*yjid* strains grown in M9 media without supplementation for the 48 hour time period compared to the previous data using the same strains (Alper, Miyaoku *et al.* 2005) and also for the 16 hour time period. Despite some variance from previous data that may be due in part to experimental deviations, the general trends of lycopene production hold with the PE strain generally trailing the other mutant strains in lycopene production in both figures. It should be noted that at 4 hours, the lycopene variability is high as small amounts of lycopene and cells exist, accentuating measurement error obvious in these figures. By comparison, Figure 5-15 and Figure 5-17 demonstrate the effect of only histidine supplementation over the 48 and 16 hour time periods, respectively. While the absolute levels of production only increase at some time points in comparing Figure 5-14 to Figure 5-15

and Figure 5-16 to Figure 5-17, the PE strain has more similar or greater production that the mutant strains when supplemented with histidine. This is expected from the hypothesis of these experiments, namely that the Δ*gdhA*, the Δ*gdhA* Δ*aceE*, and the Δ*gdhA* Δ*aceE* Δ_P*yjid* strains accomplish higher lycopene production in part due to down-regulation of the histidine pathway, possibly due to the connection of the histidine pathway to purine biosynthesis via PRPP. These three strains already appear to have their histidine pathways down-regulated, though, and there is not any additional increase for them upon addition of histidine to the media. This lends support to the hypothesis that the histidine pathway down-regulation has a positive effect upon lycopene production even though further gains via this route do not appear likely.

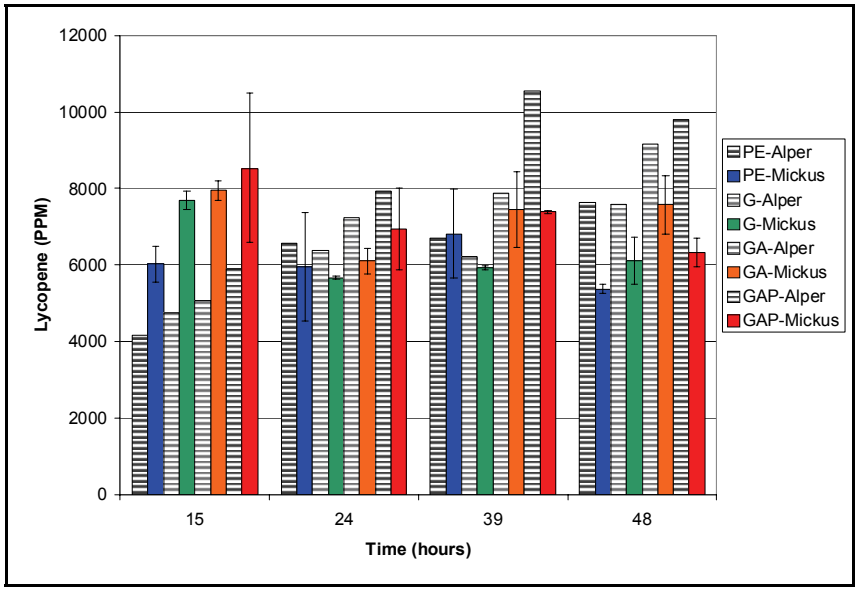

Figure 5-14 Lycopene production comparison with previous data from (Alper, Miyaoku *et al.* 2005) for the PE, Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), and Δ*gdhA* Δ*aceE* Δ_P*yjid* (GAP) strains grown in unsupplemented M9 media.

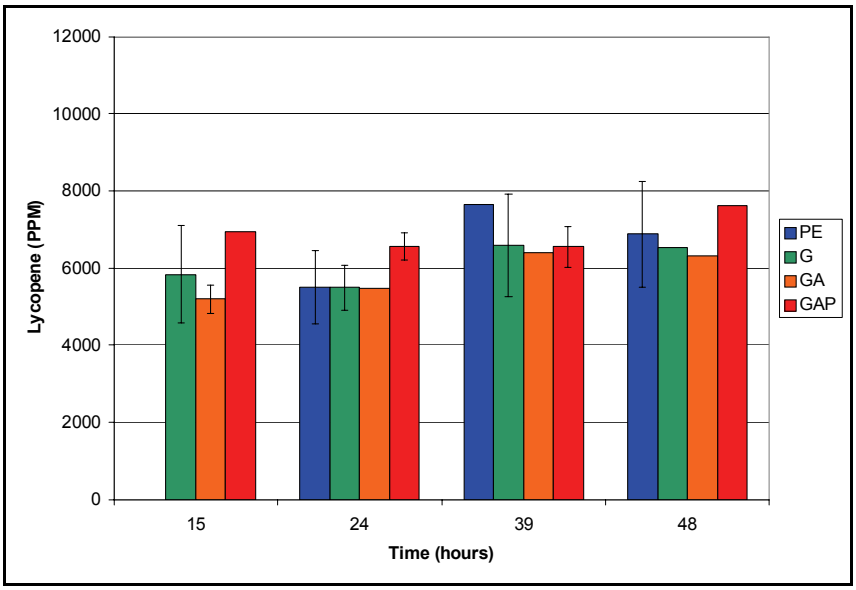

Figure 5-15 Lycopene production for the PE, Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), and Δ*gdhA* Δ*aceE* Δ_{P}*yjid* (GAP) strains grown in media supplemented with histidine for 15-48 hours.

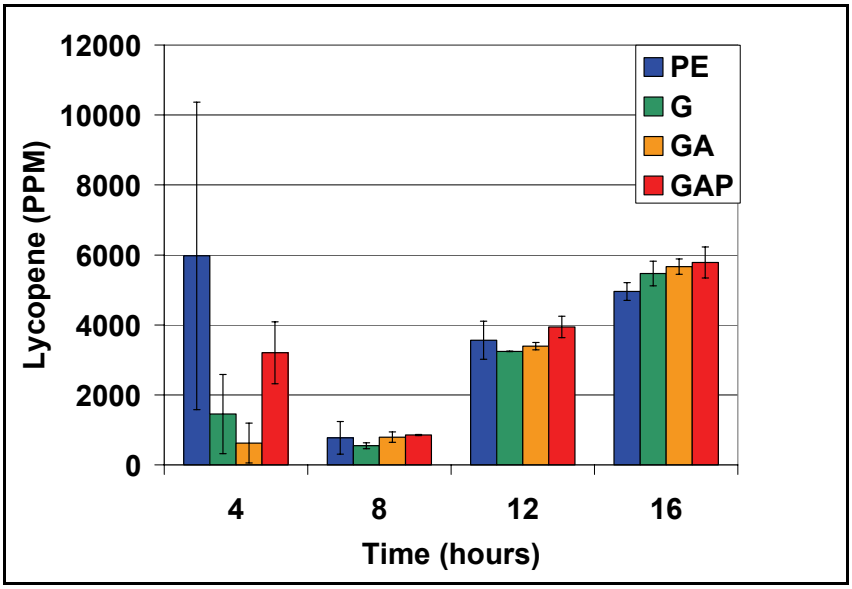

Figure 5-16 Lycopene production for the PE, Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), and Δ*gdhA* Δ*aceE* Δ_{P}*yjid* (GAP) strains grown in unsupplemented M9 media for 4-16 hours.

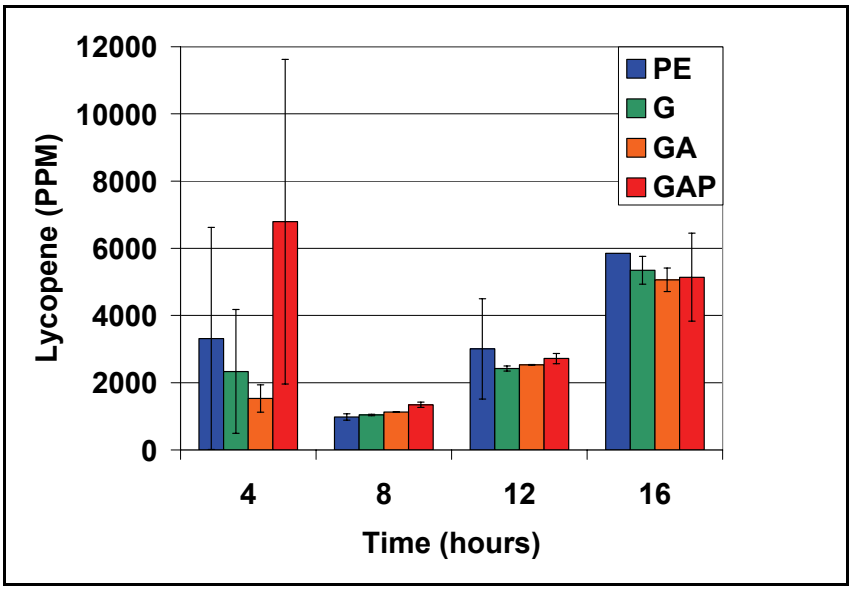

Figure 5-17 Lycopene production for the PE, Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), and Δ*gdhA* Δ*aceE* Δ_P*yjid* (GAP) strains grown in media supplemented with histidine for 4-16 hours.

On the other hand, adding both histidine and tryptophan to the media did not appear to have as large of an effect upon lycopene production at the 16 hour time point despite the fact that the 12 hour time point showed similar production levels, as seen in Figure 5-18. Adding only tryptophan to the media, as seen in Figure 5-19, appears to show the PE strain having similar production levels as the other strains, although the effect is not as strong as for histidine supplementation. It appears that supplementing the media with either histidine or tryptophan has an effect upon production, but the histidine pathway effect may lead to a larger effect. This would be consistent with the observed down-regulation in the histidine pathway. Further investigation of these observations should be pursued.

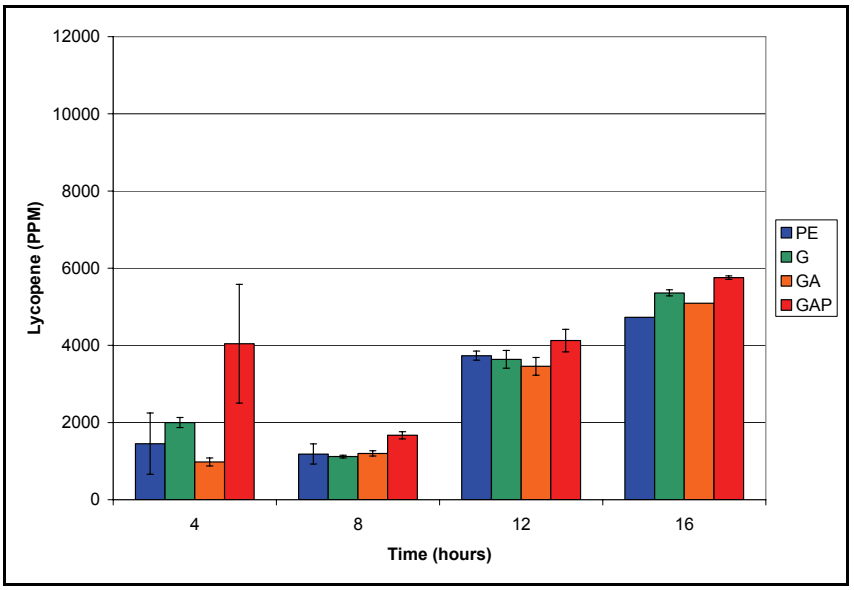

Figure 5-18 Lycopene production for the PE, Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), and Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* (GAP) strains grown in media supplemented with histidine and tryptophan for 4-16 hours.

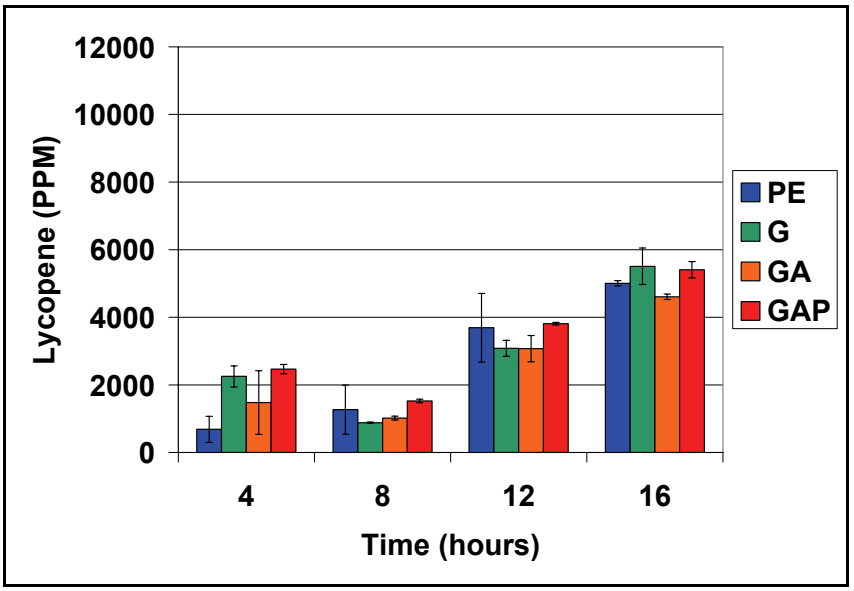

Figure 5-19 Lycopene production for the PE, Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), and Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* (GAP) strains grown in media supplemented with tryptophan for 4-16 hours.

5.5. Hierarchical Clustering of Transcriptional Data

An interesting observation was made from examining the most highly correlated cluster (node correlation > 0.95) from a hierarchical clustering of all of the transcriptional data (Eisen, Spellman *et al.* 1998). Genes with at least 4/5 numerical gene ratios were clustered using a centered Pearson correlation and complete linkage clustering. This cluster of 34 genes contained

an enriched number of genes associated with membrane components (*e.g. yaaH*, *yafU*, *yoaF*, *wcaM*, and *lgt*) or lipid biosynthesis (*e.g. prpB*, *fabH*, and *yhjY*). This is significant because lycopene is stored in the cellular membrane (Fraser and Sandmann 1992), and it has been suggested that membrane storage capacity is one of the limiting factors of lycopene production in *E. coli* (Albrecht, Misawa *et al.* 1999) along with precursor availability (Lee and Schmidt-Dannert 2002) and regulatory control (Yuan, Rouviere *et al.* 2006). In strains that overproduce lycopene, more lycopene must be stored in the membrane; thus, coordinated membrane protein changes accommodating this extra lycopene are logical.

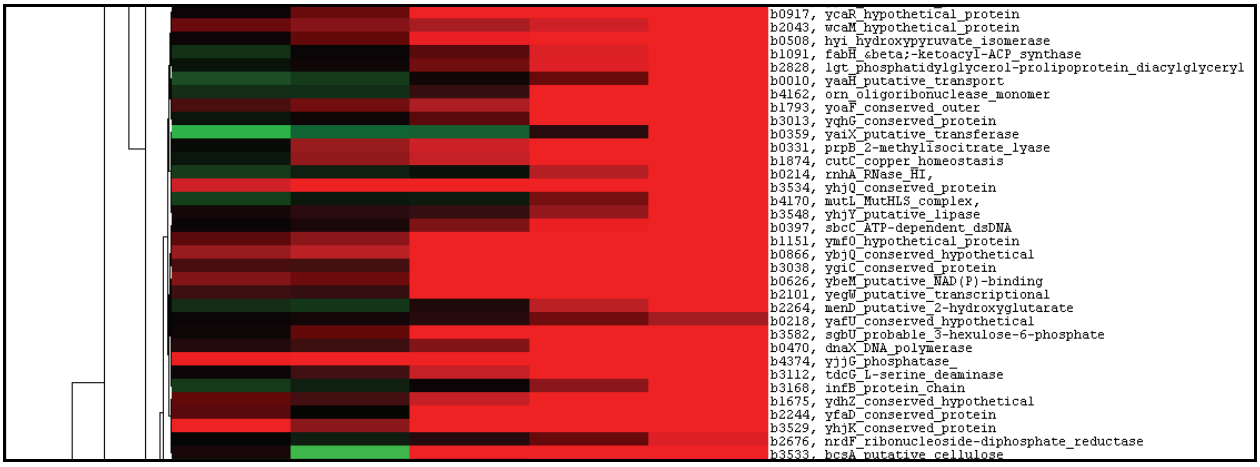

Figure 5-20 Most highly-correlated node (correlation > 0.95) resulting from hierarchical clustering of all the transcriptional data. Only genes with at least 4/5 numerical ratios were clustered using a centered Pearson correlation and complete linkage clustering. This cluster of 34 genes contained an enriched number of genes associated with membrane components (*e.g. yaaH*, *yafU*, *yoaF*, *wcaM*, and *lgt*) or lipid biosynthesis (*e.g. prpB*, *fabH*, and *yhjY*).

Additional information is given in Figure 5-21 in which all genes with at least one mutant strain displaying at least a 3-fold change in expression compared with the PE strain are clustered using a centered Pearson correlation and complete linkage clustering. Genes that cluster together in expression pattern are candidates for co-regulation, and this can be especially useful in determining potential functions for unannotated genes, *i.e.* the genes with names starting in "y" in *E. coli*.

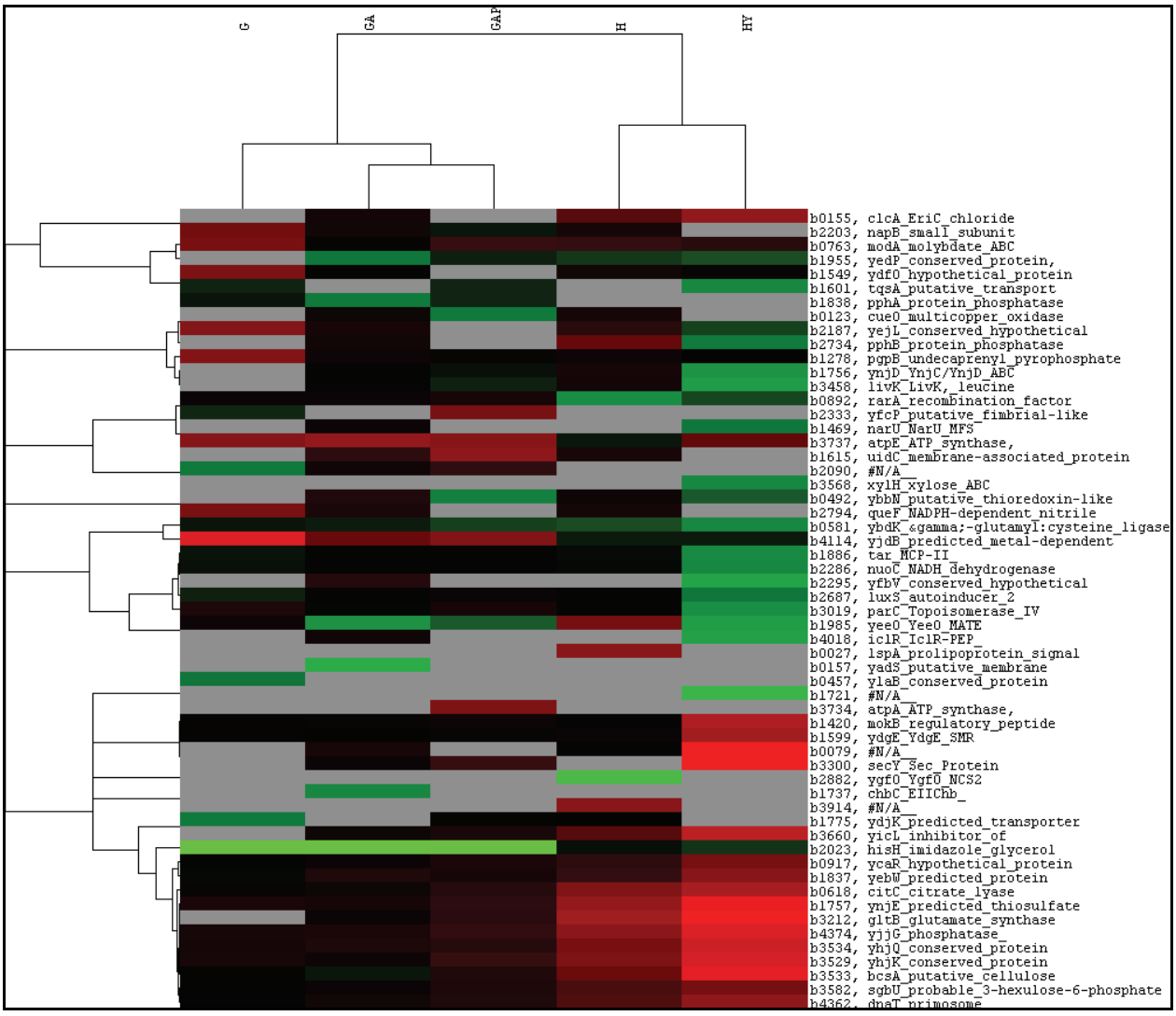

Figure 5-21 Hierarchical clustering for all genes with at least one mutant strain displaying at least a 3-fold change in expression when compared with the PE strain. Genes were clustered using a centered Pearson correlation and complete linkage clustering.

5.6. Summary

- The Δ*hnr* and Δ*hnr* Δ*yliE* strains sharing a deletion in the global regulator *hnr* (*rssB*) exhibit about 5- to 10-fold more differential gene expression compared to the background "pre-engineered" (PE) strain than the mainly stoichiometric gene deletion strains Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid*.

- The non-mevalonate and the glycolytic pathways do not exhibit coordinated differential gene expression. Thus, it appears that more distal factors beyond these pathways producing lycopene and supplying precursors affect the production phenotype.
- Differential gene expression does not appear to be additive for the Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* strains, whereas the Δ*hnr* and Δ*hnr* Δ*yliE* strains display much more additivity in differential gene expression and share 71 differentially expressed genes.
- The differential expression of a few genes is conserved in a majority of the five mutants including *hish*, *atpE*, and genes possibly related to the switch from motility to an aggregative phenotype.
- Supplementing cells with histidine led to similar lycopene production levels in the PE and Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* mutant cells, supporting the hypothesis that down-regulation of the histidine pathway is correlated with lycopene production, possibly via the PRPP link to purine biosynthesis and ADP generation.
- The most highly-correlated node from a hierarchical clustering of the transcriptional data yielded an enriched number of genes associated with membrane components and lipid biosynthesis, reiterating the importance of lycopene storage in the cellular membrane.

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Chapter 6. PROTEOMIC ANALYSIS OF LYCOPENE-OVERPRODUCING *ESCHERICHIA COLI* STRAINS

6.1. Global Analysis of Differential Protein Expression

Using the described LC-MS method, over 500 unique proteins were identified from the Δ*gdhA,* Δ*gdhA* Δ*aceE,* Δ*gdhA* Δ*aceE* Δ$_P$*yjiD,* Δ*hnr,* Δ*hnr* Δ*yliE,* and PE strains harvested from mid-exponential growth phase (OD_{600} = 0.4). The natural logarithms of expression ratios for detected proteins appear in Figure 6-1 for the five strains compared to the PE strain. In addition, the Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* strain harvested earlier (OD_{600} = 0.2) and later (OD_{600} = 0.8) in exponential growth phase is compared to the reference of the Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* strain harvested at the normal mid-exponential growth phase (OD_{600} = 0.4). All measured protein relative expression ratios are given in Appendix 9.1, resulting from either the first or the second peptide thresholds applied to the MS data. For comparison, the absolute quantification of proteins for the various strains is presented in Appendix 9.2 using the alternative quantification method previously described (Silva, Gorenstein *et al.* 2006). Unless otherwise noted, the relative expression ratios from application of the first threshold are discussed throughout this study. In some noted cases, though, the application of the second threshold data helps to clarify the ratios resulting from application of the first threshold. Similarly, the absolute quantification of proteins can be useful in comparing levels between studies or as a check on the relative expression method.

Globally, expression ratios ranged from about 10-fold up to 10-fold down in the mutants as compared to the PE strain. Interestingly, the Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* strain comparisons with time show that the protein expression variations throughout the exponential phase generally appeared to be small and less than the variations associated with the mutant and PE strain comparisons. Similar to the transcriptional results, the Δ*hnr* and Δ*hnr* Δ*yliE* strains again

displayed the greatest variation in protein expression, but the majority of detected proteins were not significantly altered in the mutant strains as compared to the PE strain. Nevertheless, the LC-MS method was sufficiently sensitive to detect the relatively few differentially expressed proteins.

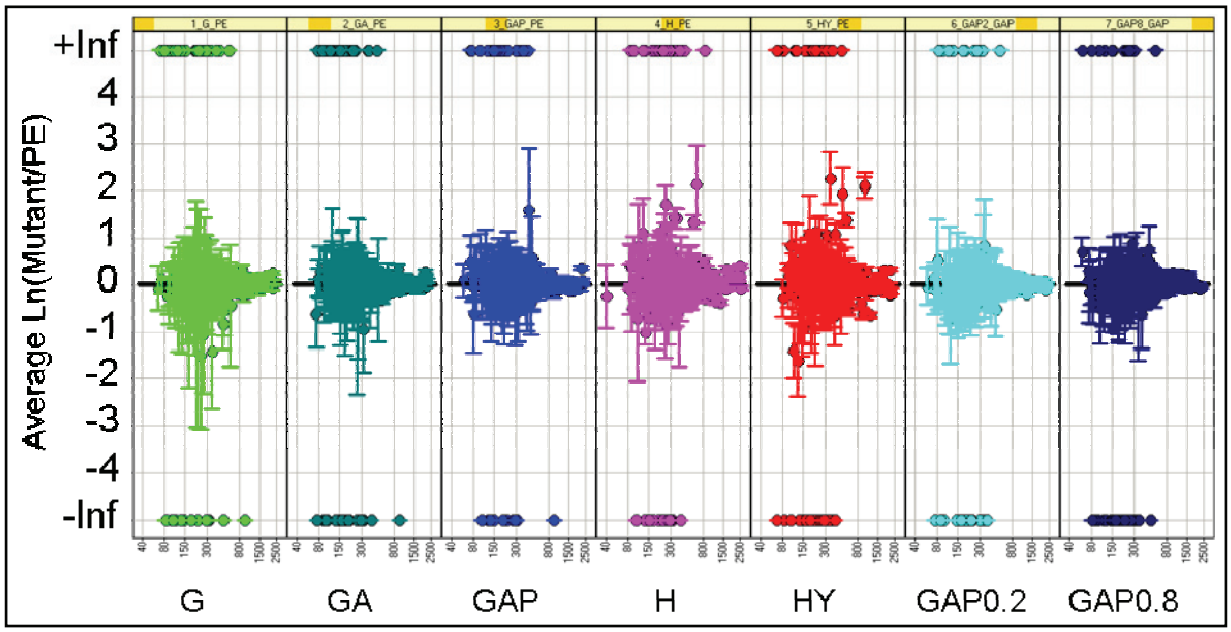

Figure 6-1 Protein expression ratio distribution for the five mutant strains Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* (GAP), Δ*hnr* (H), *and* Δ*hnr* Δ*yliE* (HY) relative to the PE strain, in addition to the Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* strain harvested earlier (OD_{600} = 0.2) and later (OD_{600} = 0.8) in exponential growth phase (GAP0.2 and GAP0.8, respectively) compared to the OD_{600} = 0.4 harvest of all other samples.

The actual number of identified proteins depended upon the different peptide identification thresholds applied to the data, as explained previously. Additionally, using the first threshold, the number of identified proteins depends upon whether "infinite" ratios are counted as identified. As explained previously, proteins with either positive or negative infinity mutant to PE expression ratios simply correspond to those proteins that were identified either only in the mutant or only in the PE strain, respectively. Applying the first threshold and counting infinite ratios, 1,276 unique proteins were identified in all of the mutant and PE strains combined. Applying the first threshold but counting only numerical ratios (*i.e.* proteins identified in both the mutant and PE strain for a given comparison), 482 unique proteins were

identified. Thus, 794 of the unique protein measurements applying the first threshold and counting infinite ratios corresponded to proteins with only infinite ratios. On the other hand, applying the second threshold and protein identification criteria did not lead to "infinite" expression ratios. A total of 700 proteins were uniquely identified using this second threshold. Notice that in comparison to the gene expression data, for which 3,208 unique genes were identified with quantified signal of acceptable quality, far fewer proteins were identified. Figure 6-2 summarizes the numbers of identified expression ratios for transcriptional and proteomic data out of five maximum possible comparisons for each gene or protein.

The number of proteins identified by LC-MS could be improved by fractionating the samples, but such methods were not applied to this study. Thus, the "global" proteomic analysis identified and quantified an impressive number of proteins by current standards, but this number was still only about 10-30% of the 4,252 total protein coding genes in *E. coli*, depending upon the threshold applied to the data. LC-MS methods preferentially detect the most abundant proteins present since these MS signals are the strongest and easiest to detect. The alternating scan LC-MS method employed here helps to improve accuracy and detect some weaker signals as well, but some are still missed. Despite the fact that important weaker signals may be missed, the more abundant proteins constitute a good starting point for proteomic analysis.

Proteins that were determined to be differentially expressed by the described Bayesian probability approach using a probability cutoff of 95% are given in Table 6-1, and the numbers of up- and down-regulated differentially expressed proteins are summarized by mutant in Figure 6-3.

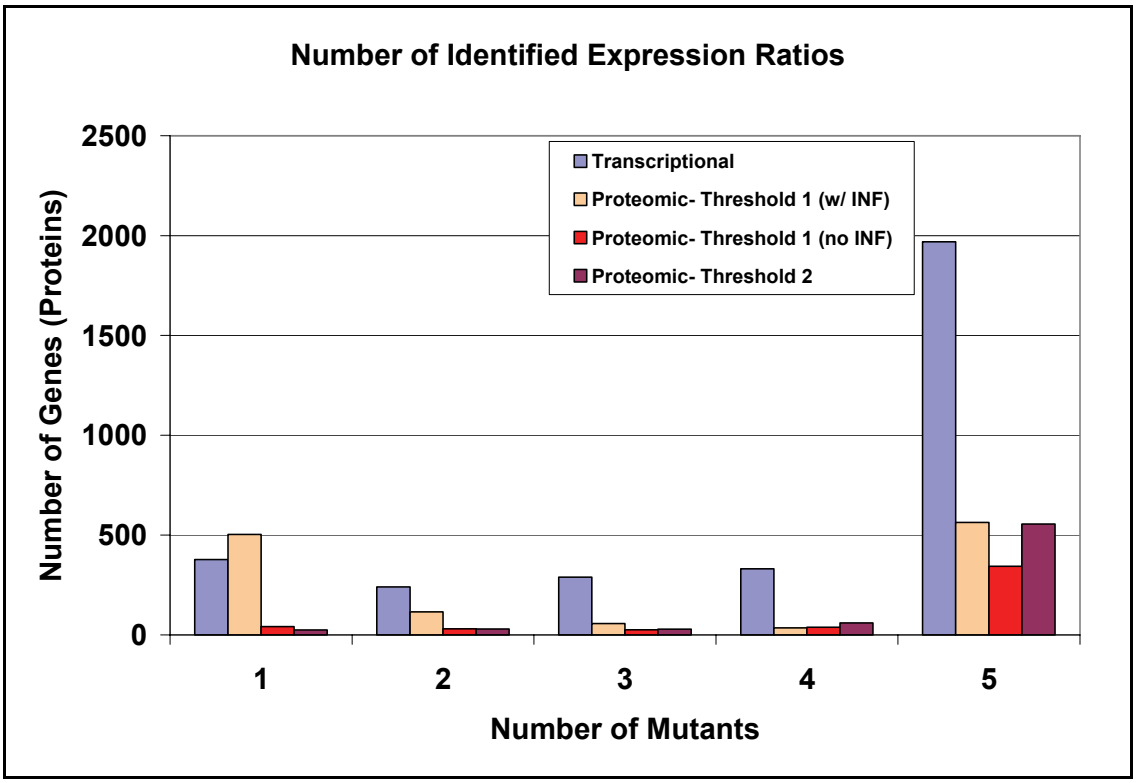

Figure 6-2 Distribution of identified expression ratios for transcriptional and proteomic data out of five maximum possible comparisons for each gene or protein. If a given gene or protein was identified in both one of the five mutants and the PE strain such that a numerical expression ratio could be measured and identified, then that count is added to the other mutants also exhibiting a measured numerical ratio for that gene or protein. The total numbers of genes or proteins exhibiting numerical ratios for each of the possibilities of one through five mutants are plotted. Two different peptide identification thresholds were applied to the proteomic data, as explained previously. The first threshold is shown both with and without measurements in which only one of the mutant or the PE strain was detected (leading to an infinite "INF" ratio).

Table 6-1 Differential protein expression for the five mutant strains Δ*gdhA*, Δ*gdhA* Δ*aceE*, Δ*gdhA* Δ*aceE* Δ$_{p}$*yjiD*, Δ*hnr, and* Δ*hnr* Δ*yliE* relative to the PE strain. The Blattner number (Blattner, Plunkett *et al.* 1997), corresponding gene name, and annotated protein function according to EcoCyc (Karp, Keseler *et al.* 2007) are given for each differentially expressed gene along with the natural logarithm ln(Mutant/PE) ratios, the standard deviations of the ln ratios, the Bayesian probabilities of up-regulation, and an indication of whether the given protein was detected in at least 2 out of 3 replicates for all of the mutant-PE comparisons in which it was detected, not just that particular mutant-PE comparison ("1" indicates "yes" and "0" indicates "no").

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
Protein	Gene		Ln(Mut/PE)	Ln(Mut/PE)		
Δ*gdhA*						
b0237	pepD	peptidase D	0.98	0.44	1.00	1
b3959	argB	acetylglutamate kinase monomer	0.62	0.38	1.00	1
b2569	lepA	elongation factor 4	0.50	0.56	0.96	1
b0104	guaC	GMP reductase	0.47	0.42	0.99	1
b3960	argH	argininosuccinate lyase	0.43	0.43	0.96	1
b2601	aroF	2-dehydro-3-deoxyphosphoheptonate aldolase	0.42	0.50	0.95	1
b2478	dapA	dihydrodipicolinate synthase	0.38	0.29	0.99	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b3870	glnA	adenylyl-[glutamine synthetase]	0.31	0.11	1.00	1
b0095	ftsZ	essential cell division protein FtsZ	0.30	0.26	0.98	1
b0154	hemL	glutamate-1-semialdehyde aminotransferase	0.28	0.30	0.97	1
b4014	aceB	malate synthase A	0.26	0.14	1.00	1
b0026	ileS	isoleucyl-tRNA synthetase	0.24	0.15	1.00	1
b3559	glyS	glycyl-tRNA synthetase, β subunit	0.22	0.19	0.97	1
b4142	groS	chaperone binds to Hsp60 in pres. Mg-ATP, suppressing ATPase activity	0.21	0.13	1.00	1
b0755	gpmA	phosphoglyceromutase 1 monomer	0.19	0.11	1.00	1
b3980	tufB	elongation factor Tu	0.18	0.05	1.00	1
b3339	tufA	elongation factor Tu	0.17	0.03	1.00	1
b0169	rpsB	30S ribosomal subunit protein S2	0.16	0.11	1.00	1
b1324	tpx	thiol peroxidase 2	0.16	0.09	1.00	1
b2913	serA	D-3-phosphoglycerate dehydrogenase	0.16	0.12	0.99	1
b0166	dapD	tetrahydrodipicolinate succinylase subunit	0.15	0.12	1.00	1
b3433	asd	aspartate semialdehyde dehydrogenase	0.15	0.11	0.99	1
b0008	talB	transaldolase B	0.14	0.08	1.00	1
b4015	aceA	isocitrate lyase monomer	0.14	0.06	1.00	1
b2925	fbaA	fructose bisphosphate aldolase monomer	0.12	0.11	0.97	1
b0605	ahpC	AhpC component	0.11	0.09	1.00	1
b3172	argG	argininosuccinate synthase	0.11	0.11	0.99	1
b0004	thrC	threonine synthase	0.10	0.08	0.97	1
b0002	thrA	aspartate kinase / homoserine dehydrogenase	0.10	0.09	0.97	1
b0907	serC	3-phosphoserine aminotransferase	0.08	0.09	0.98	1
b2926	pgk	phosphoglycerate kinase	0.08	0.06	0.98	1
b0420	dxs	1-deoxyxylulose-5-phosphate synthase	0.08	0.08	0.96	1
b3236	mdh	malate dehydrogenase	0.07	0.06	1.00	1
b1136	icd	isocitrate dehydrogenase	0.07	0.06	0.99	1
b3340	fusA	elongation factor G	0.05	0.05	0.97	1
b3829	metE	Cobalamin-independent homocysteine transmethylase	-0.06	0.05	0.98	1
b3212	gltB	glutamate synthase, large subunit	-0.07	0.07	0.95	1
b4203	rplI	50S ribosomal subunit protein L9	-0.08	0.08	0.96	1
b3988	rpoC	RNA polymerase, β' subunit	-0.11	0.11	0.98	1
b1243	oppA	OppA-oligopeptide ABC transporter substrate-binding	-0.12	0.11	0.97	1
b3296	rpsD	30S ribosomal subunit protein S4	-0.13	0.09	1.00	1
b0623	cspE	transcription antiterminator and regulator of RNA stability	-0.13	0.15	0.96	1
b0116	lpd	E3 monomer	-0.13	0.12	0.96	1
b3341	rpsG	30S ribosomal subunit protein S7	-0.14	0.08	1.00	1
b1716	rplT	50S ribosomal subunit protein L20	-0.14	0.13	0.99	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b3305	rplF	50S ribosomal subunit protein L6	-0.15	0.08	1.00	1
b3230	rpsI	30S ribosomal subunit protein S9	-0.15	0.09	1.00	1
b2779	eno	degradosome	-0.15	0.08	0.99	1
b3781	trxA	oxidized thioredoxin	-0.15	0.17	0.95	1
b3303	rpsE	30S ribosomal subunit protein S5	-0.17	0.10	1.00	1
b0911	rpsA	30S ribosomal subunit protein S1	-0.17	0.07	1.00	1
b3314	rpsC	30S ribosomal subunit protein S3	-0.19	0.08	1.00	1
b4202	rpsR	30S ribosomal subunit protein S18	-0.19	0.12	1.00	1
b3732	atpD	ATP synthase, F1 complex, β subunit	-0.19	0.18	0.99	1
b3637	rpmB	50S ribosomal subunit protein L28	-0.20	0.16	1.00	1
b2606	rplS	50S ribosomal subunit protein L19	-0.21	0.12	1.00	1
b0440	hupB	Transcriptional dual regulator HU-β, NS1 (HU-1)	-0.21	0.17	0.99	1
b2609	rpsP	30S ribosomal subunit protein S16	-0.21	0.18	0.97	1
b3301	rplO	50S ribosomal subunit protein L15	-0.25	0.10	1.00	1
b3984	rplA	50S ribosomal subunit protein L1	-0.25	0.12	1.00	1
b3309	rplX	50S ribosomal subunit protein L24	-0.25	0.14	1.00	1
b3319	rplD	50S ribosomal subunit protein L4	-0.26	0.14	1.00	1
b3908	sodA	superoxide dismutase (Mn)	-0.26	0.14	1.00	1
b3317	rplB	50S ribosomal subunit protein L2	-0.27	0.08	1.00	1
b0178	hlpA	periplasmic chaperone	-0.27	0.17	1.00	1
b3734	atpA	ATP synthase, F1 complex, α subunit	-0.27	0.21	0.99	1
b2114	metG	methionyl-tRNA synthetase	-0.29	0.20	1.00	1
b3320	rplC	50S ribosomal subunit protein L3	-0.31	0.12	1.00	1
b3342	rpsL	30S ribosomal subunit protein S12	-0.34	0.20	1.00	1
b1780	yeaD	conserved protein	-0.35	0.37	0.97	1
b2763	cysI	sulfite reductase hemoprotein subunit	-0.36	0.31	0.99	1
b2533	suhB	inositol monophosphatase	-0.43	0.55	0.95	1
b3936	rpmE	50S ribosomal subunit protein L31	-0.44	0.24	1.00	1
b3162	deaD	CsdA, DEAD-box RNA helicase	-0.48	0.59	0.97	1
b2752	cysD	sulfate adenylyltransferase	-0.53	0.45	0.97	1
b3498	prlC	oligopeptidase A	-0.57	0.51	1.00	1
b3315	rplV	50S ribosomal subunit protein L22	-0.57	0.17	1.00	1
b2185	rplY	50S ribosomal subunit protein L25	-0.58	0.33	1.00	1
b3733	atpG	ATP synthase, F1 complex, γ subunit	-0.58	0.57	0.98	1
b1482	osmC	osmotically inducible peroxidase OsmC	-0.59	0.40	0.99	1
b3297	rpsK	30S ribosomal subunit protein S11	-0.62	0.27	1.00	1
b3417	malP	maltodextrin phosphorylase monomer	-0.67	0.69	1.00	0
b2903	gcvP	glycine decarboxylase	-0.81	0.57	1.00	1
b1662	ribC	riboflavin synthase	-1.03	0.72	0.98	0
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase monomer	-1.30	0.85	1.00	0

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b1048	mdoG	periplasmic glucan (MDO) biosynthesis protein	-1.43	1.22	0.99	1
b2898	ygfZ	folate-binding protein	-1.50	1.59	0.97	1
ΔgdhA ΔaceE						
b2905	gcvT	aminomethyltransferase	1.43	1.19	1.00	0
b1817	manX	mannose PTS permease	1.23	0.96	1.00	0
b2818	argA	N-acetylglutamate synthase	0.72	0.54	0.97	0
b0683	fur	Fur-Fe+2 transcriptional dual regulator	0.55	0.55	0.96	1
b3959	argB	acetylglutamate kinase monomer	0.51	0.34	1.00	1
b1236	galU	glucose-1-phosphate uridylyltransferase	0.44	0.27	1.00	1
b3347	fkpA	peptidyl-prolyl cis-trans isomerase; in protein folding	0.36	0.26	1.00	1
b2515	ispG	1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase	0.36	0.33	0.98	0
b0126	can	carbonic anhydrase 2 monomer	0.35	0.31	0.98	1
b3870	glnA	adenylyl-[glutamine synthetase]	0.33	0.11	1.00	1
b0095	ftsZ	essential cell division protein FtsZ	0.31	0.25	1.00	1
b3433	asd	aspartate semialdehyde dehydrogenase	0.29	0.12	1.00	1
b4384	deoD	guanosine phosphorylase [multifunctional]	0.27	0.27	0.97	1
b2697	alaS	alanyl-tRNA synthetase	0.21	0.24	0.96	1
b1677	lpp	murein lipoprotein	0.18	0.18	0.97	1
b2913	serA	D-3-phosphoglycerate dehydrogenase	0.17	0.12	1.00	1
b4014	aceB	malate synthase A	0.14	0.13	0.98	1
b3980	tufB	elongation factor Tu	0.12	0.06	0.99	1
b2309	hisJ	histidine ABC transporter	0.12	0.15	0.95	1
b0002	thrA	aspartate kinase / homoserine dehydrogenase	0.11	0.10	0.98	1
b2476	purC	phosphoribosylaminoimidazole-succinocarboxamide synthase	0.11	0.13	0.96	1
b2925	fbaA	fructose bisphosphate aldolase monomer	0.10	0.09	0.99	1
b0166	dapD	tetrahydrodipicolinate succinylase subunit	0.10	0.10	0.96	1
b4015	aceA	isocitrate lyase monomer	0.09	0.07	1.00	1
b0907	serC	3-phosphoserine aminotransferase	0.09	0.08	0.97	1
b0420	dxs	1-deoxyxylulose-5-phosphate synthase	0.07	0.08	0.98	1
b0008	talB	transaldolase B	0.07	0.07	0.97	1
b1136	icd	isocitrate dehydrogenase	0.06	0.05	1.00	1
b3340	fusA	elongation factor G	0.06	0.05	1.00	1
b1779	gapA	glyceraldehyde 3-phosphate dehydrogenase-A monomer	0.06	0.06	0.95	1
b4143	groL	chaperone Hsp60, peptide-dependent ATPase, heat shock protein	-0.07	0.06	0.98	1
b3341	rpsG	30S ribosomal subunit protein S7	-0.07	0.07	0.96	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b3230	rpsI	30S ribosomal subunit protein S9	-0.09	0.09	0.95	1
b1243	oppA	OppA-oligopeptide ABC transporter substrate-binding	-0.10	0.10	0.97	1
b2417	crr	N-acetylmuramic acid PTS permease	-0.10	0.11	0.96	1
b0116	lpd	E3 monomer	-0.11	0.10	0.99	1
b0727	sucB	SucB-lipoate	-0.11	0.12	0.96	1
b0911	rpsA	30S ribosomal subunit protein S1	-0.12	0.07	1.00	1
b3321	rpsJ	30S ribosomal subunit protein S10	-0.12	0.09	1.00	1
b3314	rpsC	30S ribosomal subunit protein S3	-0.12	0.11	0.98	1
b0755	gpmA	phosphoglyceromutase 1 monomer	-0.12	0.10	0.98	1
b3231	rplM	50S ribosomal subunit protein L13	-0.13	0.14	0.95	1
b3298	rpsM	30S ribosomal subunit protein S13	-0.14	0.14	0.95	1
b3309	rplX	50S ribosomal subunit protein L24	-0.15	0.14	0.99	1
b1716	rplT	50S ribosomal subunit protein L20	-0.15	0.14	0.98	1
b3304	rplR	50S ribosomal subunit protein L18	-0.15	0.17	0.95	1
b3984	rplA	50S ribosomal subunit protein L1	-0.16	0.08	1.00	1
b3308	rplE	50S ribosomal subunit protein L5	-0.16	0.13	0.99	1
b3301	rplO	50S ribosomal subunit protein L15	-0.16	0.12	0.99	1
b3319	rplD	50S ribosomal subunit protein L4	-0.16	0.14	0.98	1
b3305	rplF	50S ribosomal subunit protein L6	-0.17	0.07	1.00	1
b0440	hupB	Transcriptional dual regulator HU-β, NS1 (HU-1)	-0.17	0.17	0.96	1
b1415	aldA	aldehyde dehydrogenase A, NAD-linked	-0.18	0.14	1.00	1
b0178	hlpA	periplasmic chaperone	-0.18	0.19	0.96	1
b3317	rplB	50S ribosomal subunit protein L2	-0.19	0.08	1.00	1
b3637	rpmB	50S ribosomal subunit protein L28	-0.19	0.17	0.97	1
b3164	pnp	polynucleotide phosphorylase monomer	-0.21	0.18	0.99	1
b2425	cysP	thiosulfate ABC transporter	-0.21	0.22	0.95	1
b0726	sucA	subunit of E1(0) component of 2-oxoglutarate dehydrogenase	-0.22	0.15	1.00	1
b3908	sodA	superoxide dismutase (Mn)	-0.23	0.16	1.00	1
b3310	rplN	50S ribosomal subunit protein L14	-0.24	0.17	1.00	1
b3297	rpsK	30S ribosomal subunit protein S11	-0.26	0.16	1.00	1
b3342	rpsL	30S ribosomal subunit protein S12	-0.28	0.20	1.00	1
b2185	rplY	50S ribosomal subunit protein L25	-0.28	0.27	0.97	1
b1852	zwf	glucose 6-phosphate-1-dehydrogenase	-0.30	0.32	0.98	1
b3320	rplC	50S ribosomal subunit protein L3	-0.32	0.09	1.00	1
b2606	rplS	50S ribosomal subunit protein L19	-0.34	0.12	1.00	1
b2752	cysD	sulfate adenylyltransferase	-0.35	0.34	0.96	1
b3315	rplV	50S ribosomal subunit protein L22	-0.36	0.13	1.00	1
b2464	talA	transaldolase A	-0.42	0.50	0.96	1
b3936	rpmE	50S ribosomal subunit protein L31	-0.45	0.25	1.00	1
b3316	rpsS	30S ribosomal subunit protein S19	-0.55	0.54	0.98	0
b3640	dut	deoxyuridine triphosphatase	-0.62	0.71	0.98	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b3939	metB	O-succinylhomoserine lyase / O-succinylhomoserine(thiol)-lyase	-0.62	0.68	0.96	1
b4388	serB	phosphoserine phosphatase	-0.66	0.66	0.96	0
b0632	dacA	D-alanyl-D-alanine carboxypeptidase, fraction A; penicillin-binding protein5	-1.49	0.62	1.00	0
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase monomer	-2.02	1.68	0.99	0
ΔgdhA ΔaceE Δ$_P$yjiD						
b1004	wrbA	WrbA monomer	1.59	1.31	1.00	1
b2905	gcvT	aminomethyltransferase	0.92	0.85	0.97	0
b4376	osmY	hyperosmotically inducible periplasmic protein	0.58	0.15	1.00	1
b1539	ydfG	3-hydroxy acid dehydrogenase monomer	0.51	0.48	0.96	1
b4005	purD	phosphoribosylamine-glycine ligase	0.45	0.39	1.00	1
b3336	bfr	bacterioferritin monomer	0.44	0.44	0.99	1
b2421	cysM	cysteine synthase B	0.44	0.51	0.95	0
b3959	argB	acetylglutamate kinase monomer	0.41	0.29	1.00	1
b2914	rpiA	ribose-5-phosphate isomerase A	0.41	0.40	0.97	1
b2025	hisF	imidazole glycerol phosphate synthase, HisF subunit	0.39	0.39	0.98	1
b3556	cspA	CspA transcriptional activator	0.37	0.39	0.97	0
b1236	galU	glucose-1-phosphate uridylyltransferase	0.36	0.28	0.98	1
b2042	galF	predicted subunit with GalU	0.35	0.46	0.95	0
b3065	rpsU	30S ribosomal subunit protein S21	0.32	0.22	1.00	1
b3870	glnA	adenylyl-[glutamine synthetase]	0.32	0.11	1.00	1
b0767	pgl	6-phosphogluconolactonase	0.30	0.33	0.96	1
b1103	hinT	purine nucleoside phosphoramidase	0.29	0.28	0.98	1
b1482	osmC	osmotically inducible peroxidase OsmC	0.28	0.23	0.99	1
b1740	nadE	NAD synthetase, NH₃-dependent	0.28	0.27	0.96	1
b3433	asd	aspartate semialdehyde dehydrogenase	0.25	0.09	1.00	1
b4025	pgi	phosphoglucose isomerase	0.25	0.21	0.99	1
b3347	fkpA	peptidyl-prolyl cis-trans isomerase; in protein folding	0.24	0.22	0.99	1
b1654	grxD	glutaredoxin 4	0.20	0.18	0.98	1
b0166	dapD	tetrahydrodipicolinate succinylase subunit	0.18	0.11	1.00	1
b0172	frr	ribosome recycling factor	0.17	0.17	0.97	1
b0811	glnH	glutamine ABC transporter	0.17	0.18	0.96	1
b1920	fliY	periplasmic cystine-binding protein	0.16	0.17	0.96	1
b0426	yajQ	nucleotide binding protein	0.15	0.17	0.95	1
b4014	aceB	malate synthase A	0.14	0.12	0.98	1
b2417	crr	N-acetylmuramic acid PTS permease	0.13	0.09	1.00	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b2530	iscS	cysteine desulfurase monomer	0.13	0.16	0.95	1
b0004	thrC	threonine synthase	0.12	0.09	1.00	1
b4226	ppa	inorganic pyrophosphatase	0.12	0.11	0.99	1
b2925	fbaA	fructose bisphosphate aldolase monomer	0.09	0.09	1.00	1
b4015	aceA	isocitrate lyase monomer	0.09	0.06	0.99	1
b0002	thrA	aspartate kinase / homoserine dehydrogenase	0.09	0.09	0.96	1
b0605	ahpC	AhpC component	0.09	0.08	0.96	1
b0907	serC	3-phosphoserine aminotransferase	0.08	0.09	0.96	1
b3339	tufA	elongation factor Tu	0.07	0.04	1.00	1
b3460	livJ	branched chain amino acids ABC transporter	0.07	0.07	0.98	1
b2551	glyA	serine hydroxymethyltransferase	0.07	0.08	0.96	1
b2926	pgk	phosphoglycerate kinase	0.06	0.07	0.97	1
b1136	icd	isocitrate dehydrogenase	-0.06	0.06	0.99	1
b3829	metE	Cobalamin-independent homocysteine transmethylase	-0.07	0.06	0.99	1
b1779	gapA	glyceraldehyde 3-phosphate dehydrogenase-A monomer	-0.07	0.07	0.97	1
b0911	rpsA	30S ribosomal subunit protein S1	-0.09	0.07	1.00	1
b0116	lpd	E3 monomer	-0.09	0.08	0.99	1
b3341	rpsG	30S ribosomal subunit protein S7	-0.09	0.09	0.97	1
b0728	sucC	succinyl-CoA synthetase, β subunit	-0.09	0.08	0.97	1
b2414	cysK	bifunctional CysEK cysteine biosynthesis complex	-0.09	0.08	0.97	1
b3295	rpoA	RNA polymerase, α subunit	-0.09	0.09	0.96	1
b3313	rplP	50S ribosomal subunit protein L16	-0.09	0.12	0.95	1
b3230	rpsI	30S ribosomal subunit protein S9	-0.10	0.08	0.99	1
b3321	rpsJ	30S ribosomal subunit protein S10	-0.10	0.10	0.97	1
b4203	rplI	50S ribosomal subunit protein L9	-0.11	0.07	0.99	1
b4143	groL	chaperone Hsp60, peptide-dependent ATPase, heat shock protein	-0.12	0.05	1.00	1
b3317	rplB	50S ribosomal subunit protein L2	-0.13	0.09	1.00	1
b3305	rplF	50S ribosomal subunit protein L6	-0.13	0.08	1.00	1
b0118	acnB	aconitase B	-0.13	0.07	1.00	1
b1243	oppA	OppA-oligopeptide ABC transporter substrate-binding	-0.13	0.11	0.99	1
b2935	tktA	transketolase I	-0.13	0.15	0.95	1
b3314	rpsC	30S ribosomal subunit protein S3	-0.14	0.06	1.00	1
b3301	rplO	50S ribosomal subunit protein L15	-0.14	0.12	1.00	1
b3320	rplC	50S ribosomal subunit protein L3	-0.14	0.09	0.99	1
b1093	fabG	β-ketoacyl-[acyl-carrier-protein] reductase	-0.14	0.18	0.95	1
b4177	purA	adenylosuccinate synthetase	-0.15	0.10	1.00	1
b1288	fabI	enoyl-ACP reductase (NAD[P]H) [multifunctional]	-0.16	0.12	1.00	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b3732	atpD	ATP synthase, F1 complex, β subunit	-0.16	0.17	0.96	1
b3319	rplD	50S ribosomal subunit protein L4	-0.18	0.14	1.00	1
b0726	sucA	subunit of E1(0) component of 2-oxoglutarate dehydrogenase	-0.18	0.15	1.00	1
b3306	rpsH	30S ribosomal subunit protein S8	-0.18	0.14	0.99	1
b0073	leuB	3-isopropylmalate dehydrogenase	-0.18	0.19	0.95	1
b3984	rplA	50S ribosomal subunit protein L1	-0.19	0.10	1.00	1
b2606	rplS	50S ribosomal subunit protein L19	-0.20	0.15	0.99	1
b2751	cysN	sulfate adenylyltransferase	-0.20	0.32	0.95	1
b3310	rplN	50S ribosomal subunit protein L14	-0.21	0.18	0.97	1
b4200	rpsF	30S ribosomal subunit protein S6	-0.23	0.13	1.00	1
b0727	sucB	SucB-lipoate	-0.23	0.12	1.00	1
b3315	rplV	50S ribosomal subunit protein L22	-0.23	0.11	1.00	1
b1095	fabF	KASII	-0.23	0.29	0.96	1
b3342	rpsL	30S ribosomal subunit protein S12	-0.25	0.17	1.00	1
b2094	gatA	galactitol PTS permease	-0.25	0.23	0.98	1
b3297	rpsK	30S ribosomal subunit protein S11	-0.36	0.17	1.00	1
b0440	hupB	Transcriptional dual regulator HU-β, NS1 (HU-1)	-0.37	0.19	0.98	1
b3316	rpsS	30S ribosomal subunit protein S19	-0.48	0.42	0.99	0
b2898	ygfZ	folate-binding protein	-0.63	0.67	0.96	1
b3863	polA	DNA polymerase I, 3' --> 5' polymerase, 5' --> 3' and 3' --> 5' exonuclease	-0.64	0.59	0.98	0
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase monomer	-2.14	1.46	1.00	0
Δ*hnr*						
b1004	wrbA	WrbA monomer	2.13	0.82	1.00	1
b4383	deoB	phosphopentomutase	2.05	0.67	1.00	0
b3509	hdeB	acid stress chaperone	1.70	0.40	1.00	1
b3517	gadA	glutamate decarboxylase A subunit	1.42	0.21	1.00	1
b1493	gadB	glutamate decarboxylase B subunit	1.40	0.22	1.00	1
b3201	lptB	LptA/LptB/LptC ABC transporter	1.34	0.82	0.99	0
b4376	osmY	hyperosmotically inducible periplasmic protein	1.33	0.15	1.00	1
b3336	bfr	bacterioferritin monomer	1.21	0.29	1.00	1
b0453	ybaY	predicted outer membrane lipoprotein	1.10	0.73	1.00	1
b0683	fur	Fur-Fe+2 transcriptional dual regulator	0.82	0.89	0.97	1
b0237	pepD	peptidase D	0.79	0.52	0.99	1
b0767	pgl	6-phosphogluconolactonase	0.76	0.27	1.00	1
b1482	osmC	osmotically inducible peroxidase OsmC	0.63	0.24	1.00	1
b1103	hinT	purine nucleoside phosphoramidase	0.60	0.31	1.00	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b3114	tdcE	2-ketobutyrate formate-lyase / pyruvate formate-lyase	0.58	0.46	1.00	1
b2266	elaB	conserved protein	0.58	0.22	1.00	1
b4349	hsdM	host modification; DNA methylase M	0.53	0.53	0.98	0
b4401	arcA	ArcA-Phosphorylated transcriptional dual regulator	0.53	0.69	0.96	0
b2962	yggX	protein that protects iron-sulfur proteins against oxidative damage	0.52	0.47	0.99	0
b0854	potF	putrescine ABC transporter	0.52	0.54	0.96	1
b2914	rpiA	ribose-5-phosphate isomerase A	0.51	0.49	0.98	1
b0903	pflB	pyruvate formate-lyase (inactive)	0.50	0.08	1.00	1
b3192	yrbC	predicted ABC-type organic solvent transporter	0.48	0.37	1.00	1
b4025	pgi	phosphoglucose isomerase	0.48	0.13	1.00	1
b2569	lepA	elongation factor 4	0.48	0.41	0.98	1
b1637	tyrS	tyrosyl-tRNA synthetase	0.48	0.65	0.95	1
b1662	ribC	riboflavin synthase	0.45	0.32	1.00	0
b4014	aceB	malate synthase A	0.44	0.12	1.00	1
b2669	stpA	H-NS-like DNA-binding protein with RNA chaperone activity	0.42	0.14	1.00	1
b3959	argB	acetylglutamate kinase monomer	0.41	0.29	1.00	1
b1412	azoR	NADH-azoreductase, FMN-dependent	0.41	0.41	0.98	1
b0812	dps	stationary phase nucleoid protein-sequesters iron, protects DNA damage	0.38	0.14	1.00	1
b4384	deoD	guanosine phosphorylase [multifunctional]	0.37	0.21	0.99	1
b1539	ydfG	3-hydroxy acid dehydrogenase monomer	0.37	0.37	0.96	1
b1714	pheS	phenylalanyl-tRNA synthetase α-chain	0.36	0.21	1.00	1
b0426	yajQ	nucleotide binding protein	0.36	0.18	1.00	1
b0095	ftsZ	essential cell division protein FtsZ	0.36	0.19	1.00	1
b1740	nadE	NAD synthetase, NH₃-dependent	0.36	0.32	0.97	1
b4015	aceA	isocitrate lyase monomer	0.33	0.07	1.00	1
b1262	trpC	indole-3-glycerol phosphate synthase, phosphoribosylanthranilate isomerase	0.31	0.29	0.97	1
b0154	hemL	glutamate-1-semialdehyde aminotransferase	0.30	0.24	0.96	1
b1241	adhE	PFL-deactivase / alcohol dehydrogenase / acetaldehyde dehydrogenase	0.28	0.12	1.00	1
b3919	tpiA	triose phosphate isomerase monomer	0.28	0.19	1.00	1
b2464	talA	transaldolase A	0.28	0.21	0.99	1
b0473	htpG	HtpG monomer	0.27	0.17	0.98	1
b3414	nfuA	iron-sulfur cluster scaffold protein	0.26	0.22	1.00	1
b2400	gltX	glutamyl-tRNA synthetase	0.26	0.29	0.97	1
b0932	pepN	aminopeptidase N	0.26	0.29	0.95	1
b3980	tufB	elongation factor Tu	0.25	0.07	1.00	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b4391	yjjK	YjjK	0.25	0.15	1.00	1
b2747	ispD	4-diphosphocytidyl-2C-methyl-D-erythritol synthetase monomer	0.25	0.18	0.99	1
b3994	thiC	thiamin biosynthesis protein ThiC	0.25	0.23	0.98	1
b4226	ppa	inorganic pyrophosphatase	0.24	0.11	1.00	1
b0172	frr	ribosome recycling factor	0.24	0.16	1.00	1
b2925	fbaA	fructose bisphosphate aldolase monomer	0.24	0.06	1.00	1
b2417	crr	N-acetylmuramic acid PTS permease	0.24	0.08	1.00	1
b2514	hisS	histidyl-tRNA synthetase	0.23	0.22	1.00	1
b0420	dxs	1-deoxyxylulose-5-phosphate synthase	0.23	0.07	1.00	1
b3609	secB	Sec Protein Secretion Complex	0.23	0.22	0.97	1
b3612	gpmM	phosphoglycerate mutase, cofactor independent	0.22	0.19	0.99	1
b2153	folE	GTP cyclohydrolase I monomer	0.22	0.24	0.96	1
b0755	gpmA	phosphoglyceromutase 1 monomer	0.20	0.11	1.00	1
b1850	eda	multifunctional 2-keto-3-deoxygluconate 6-phosphate aldolase and 2-keto-4-hydroxyglutarate aldolase and oxaloacetate decarboxylase	0.20	0.17	0.98	1
b0438	clpX	ClpAXP	0.20	0.24	0.96	1
b2926	pgk	phosphoglycerate kinase	0.17	0.06	1.00	1
b1920	fliY	periplasmic cystine-binding protein	0.17	0.15	0.98	1
b3255	accB	biotinylated biotin-carboxyl carrier protein	0.17	0.17	0.97	1
b2323	fabB	KASI	0.17	0.22	0.96	1
b3433	asd	aspartate semialdehyde dehydrogenase	0.16	0.08	1.00	1
b2942	metK	MetK S-adenosylmethionine synthetase monomer	0.16	0.08	1.00	1
b0014	dnaK	chaperone Hsp70; DNA biosynthesis; autoregulated heat shock proteins	0.15	0.06	1.00	1
b2551	glyA	serine hydroxymethyltransferase	0.14	0.06	1.00	1
b0004	thrC	threonine synthase	0.13	0.08	1.00	1
b2530	iscS	cysteine desulfurase monomer	0.13	0.12	0.98	1
b4142	groS	chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activity	0.13	0.14	0.95	1
b4243	yjgF	conserved protein	0.12	0.11	0.99	1
b3321	rpsJ	30S ribosomal subunit protein S10	0.11	0.08	1.00	1
b3908	sodA	superoxide dismutase (Mn)	0.11	0.10	0.97	1
b1304	pspA	regulatory protein for the phage shock protein operon	0.11	0.12	0.96	1
b3339	tufA	elongation factor Tu	0.10	0.04	1.00	1
b2779	eno	degradosome	0.09	0.06	1.00	1
b3340	fusA	elongation factor G	0.08	0.04	1.00	1
b4244	pyrI	aspartate carbamoyltransferase, PyrI subunit	0.08	0.08	0.96	1
b0114	aceE	subunit of E1p component of pyruvate dehydrogenase complex	0.07	0.07	0.97	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b1676	pykF	pyruvate kinase I monomer	0.06	0.08	0.95	1
b4203	rplI	50S ribosomal subunit protein L9	-0.06	0.06	0.97	1
b3305	rplF	50S ribosomal subunit protein L6	-0.06	0.07	0.96	1
b0033	carB	carbamoyl phosphate synthetase	-0.07	0.08	0.97	1
b3829	metE	Cobalamin-independent homocysteine transmethylase	-0.08	0.04	1.00	1
b3774	ilvC	acetohydroxy acid isomeroreductase	-0.08	0.05	0.99	1
b3303	rpsE	30S ribosomal subunit protein S5	-0.08	0.07	0.98	1
b0436	tig	trigger factor; a molecular chaperone involved in cell division	-0.08	0.06	0.98	1
b0754	aroG	2-dehydro-3-deoxyphosphoheptonate aldolase	-0.09	0.12	0.96	1
b1237	hns	H-NS transcriptional dual regulator	-0.09	0.09	0.95	1
b1324	tpx	thiol peroxidase 2	-0.10	0.07	1.00	1
b4200	rpsF	30S ribosomal subunit protein S6	-0.10	0.10	0.98	1
b0911	rpsA	30S ribosomal subunit protein S1	-0.11	0.06	0.99	1
b0116	lpd	E3 monomer	-0.12	0.07	1.00	1
b0928	aspC	aspartate aminotransferase, PLP-dependent	-0.12	0.08	0.99	1
b3956	ppc	phosphoenolpyruvate carboxylase	-0.13	0.11	1.00	1
b3870	glnA	adenylyl-[glutamine synthetase]	-0.13	0.13	0.96	1
b3314	rpsC	30S ribosomal subunit protein S3	-0.14	0.08	1.00	1
b3985	rplJ	50S ribosomal subunit protein L10	-0.14	0.10	0.99	1
b4177	purA	adenylosuccinate synthetase	-0.14	0.13	0.96	1
b3357	crp	CRP transcriptional dual regulator	-0.16	0.18	0.97	1
b3460	livJ	branched chain amino acids ABC transporter	-0.17	0.07	1.00	1
b3301	rplO	50S ribosomal subunit protein L15	-0.17	0.11	1.00	1
b3458	livK	leucine binding protein of the high-affinity branched-chain amino acid transport system	-0.17	0.17	0.97	1
b2019	hisG	ATP phosphoribosyltransferase	-0.17	0.19	0.95	1
b3984	rplA	50S ribosomal subunit protein L1	-0.18	0.07	1.00	1
b3317	rplB	50S ribosomal subunit protein L2	-0.18	0.07	1.00	1
b3236	mdh	malate dehydrogenase	-0.19	0.05	1.00	1
b3770	ilvE	branched-chain amino-acid aminotransferase	-0.19	0.21	0.98	1
b3732	atpD	ATP synthase, F1 complex, β subunit	-0.19	0.16	0.96	1
b3309	rplX	50S ribosomal subunit protein L24	-0.20	0.12	1.00	1
b0023	rpsT	30S ribosomal subunit protein S20	-0.20	0.21	0.97	1
b3734	atpA	ATP synthase, F1 complex, α subunit	-0.21	0.18	1.00	1
b0729	sucD	succinyl-CoA synthetase, α subunit	-0.22	0.12	1.00	1
b0860	artJ	arginine ABC transporter	-0.22	0.18	0.99	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b1243	oppA	OppA-oligopeptide ABC transporter substrate-binding	-0.23	0.10	1.00	1
b2309	hisJ	histidine ABC transporter	-0.23	0.13	1.00	1
b2935	tktA	transketolase I	-0.23	0.13	1.00	1
b2185	rplY	50S ribosomal subunit protein L25	-0.24	0.22	0.96	1
b3304	rplR	50S ribosomal subunit protein L18	-0.25	0.19	1.00	1
b3306	rpsH	30S ribosomal subunit protein S8	-0.26	0.15	0.99	1
b0439	lon	DNA-binding, ATP-dependent protease La	-0.26	0.25	0.97	1
b3342	rpsL	30S ribosomal subunit protein S12	-0.28	0.16	1.00	1
b3320	rplC	50S ribosomal subunit protein L3	-0.28	0.09	1.00	1
b0720	gltA	citrate synthase monomer	-0.28	0.10	1.00	1
b2022	hisB	imidazoleglycerol-phosphate dehydratase / histidinol-phosphatase	-0.28	0.21	0.99	1
b3310	rplN	50S ribosomal subunit protein L14	-0.29	0.14	1.00	1
b0726	sucA	subunit of E1(0) component of 2-oxoglutarate dehydrogenase	-0.30	0.14	1.00	1
b0728	sucC	succinyl-CoA synthetase, β subunit	-0.30	0.09	1.00	1
b3560	glyQ	glycyl-tRNA synthetase, α subunit	-0.32	0.28	0.98	1
b2606	rplS	50S ribosomal subunit protein L19	-0.33	0.13	1.00	1
b3297	rpsK	30S ribosomal subunit protein S11	-0.34	0.16	1.00	1
b1761	gdhA	glutamate dehydrogenase	-0.35	0.12	1.00	1
b0118	acnB	aconitase B	-0.37	0.07	1.00	1
b4122	fumB	fumarase B monomer	-0.37	0.30	0.99	1
b1136	icd	isocitrate dehydrogenase	-0.38	0.05	1.00	1
b1415	aldA	aldehyde dehydrogenase A, NAD-linked	-0.39	0.19	1.00	1
b3936	rpmE	50S ribosomal subunit protein L31	-0.39	0.28	1.00	1
b3316	rpsS	30S ribosomal subunit protein S19	-0.39	0.38	0.97	0
b0178	hlpA	periplasmic chaperone	-0.40	0.13	1.00	1
b3315	rplV	50S ribosomal subunit protein L22	-0.43	0.12	1.00	1
b3176	glmM	phosphoglucosamine mutase	-0.43	0.46	0.95	0
b1612	fumA	fumarase A monomer	-0.45	0.22	1.00	1
b2415	ptsH	HPr	-0.48	0.28	1.00	0
b3065	rpsU	30S ribosomal subunit protein S21	-0.51	0.48	0.99	1
b0440	hupB	Transcriptional dual regulator HU-β, NS1 (HU-1)	-0.53	0.20	1.00	1
b0727	sucB	SucB-lipoate	-0.57	0.18	1.00	1
b2819	recD	DNA helicase, ATP-dependent dsDNA/ssDNA exonuclease V subunit, ssDNA endonuclease	-0.59	0.69	0.95	0
b2094	gatA	galactitol PTS permease	-0.69	0.23	1.00	1
b0884	infA	protein chain initiation factor IF-1	-0.69	1.38	0.96	1
b2093	gatB	galactitol PTS permease	-0.70	0.25	1.00	1
b0838	yliJ	predicted glutathione S-transferase	-0.81	0.77	0.98	1
b3805	hemC	hydroxymethylbilane synthase	-0.97	0.45	1.00	0

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b2096	gatY	tagatose-1,6-bisphosphate aldolase 2	-1.01	0.27	1.00	1
b3430	glgC	glucose-1-phosphate adenylyltransferase	-1.16	1.53	0.96	0
b0894	dmsA	dimethyl sulfoxide reductase, chain A	-1.40	1.51	0.95	0
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase monomer	-1.84	1.88	0.98	0
Δhnr ΔyliE						
b0660	ybeZ	predicted protein with nucleoside triphosphate hydrolase domain	2.29	1.88	1.00	0
b3509	hdeB	acid stress chaperone	2.26	0.55	1.00	1
b3517	gadA	glutamate decarboxylase A subunit	2.11	0.27	1.00	1
b1493	gadB	glutamate decarboxylase B subunit	2.06	0.22	1.00	1
b1004	wrbA	WrbA monomer	1.92	0.59	1.00	1
b4376	osmY	hyperosmotically inducible periplasmic protein	1.37	0.15	1.00	1
b4383	deoB	phosphopentomutase	1.19	0.83	0.99	0
b2266	elaB	conserved protein	1.12	0.27	1.00	1
b1482	osmC	osmotically inducible peroxidase OsmC	1.08	0.20	1.00	1
b0453	ybaY	predicted outer membrane lipoprotein	1.08	0.81	0.98	1
b3336	bfr	bacterioferritin monomer	1.06	0.31	1.00	1
b1236	galU	glucose-1-phosphate uridylyltransferase	0.90	0.26	1.00	1
b1480	sra	30S ribosomal subunit protein S22	0.83	0.47	1.00	1
b0767	pgl	6-phosphogluconolactonase	0.71	0.30	1.00	1
b0104	guaC	GMP reductase	0.58	0.57	0.96	1
b2400	gltX	glutamyl-tRNA synthetase	0.51	0.34	1.00	1
b2962	yggX	protein that protects iron-sulfur proteins against oxidative damage	0.51	0.64	0.96	0
b1412	azoR	NADH-azoreductase, FMN-dependent	0.50	0.46	0.97	1
b3751	rbsB	ribose ABC transporter	0.49	0.43	1.00	1
b2914	rpiA	ribose-5-phosphate isomerase A	0.48	0.48	0.97	1
b2889	idi	isopentenyl diphosphate isomerase	0.46	0.32	1.00	1
b0095	ftsZ	essential cell division protein FtsZ	0.45	0.26	1.00	1
b1304	pspA	regulatory protein for the phage shock protein operon	0.44	0.12	1.00	1
b3959	argB	acetylglutamate kinase monomer	0.44	0.41	0.97	1
b1740	nadE	NAD synthetase, NH₃-dependent	0.42	0.34	1.00	1
b0812	dps	stationary phase nucleoid protein-sequesters iron, protects DNA damage	0.41	0.17	1.00	1
b4384	deoD	guanosine phosphorylase [multifunctional]	0.40	0.31	1.00	1
b0439	lon	DNA-binding, ATP-dependent protease La	0.40	0.39	0.97	1
b2480	bcp	thiol peroxidase	0.39	0.34	1.00	1
b2464	talA	transaldolase A	0.39	0.25	1.00	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b1712	ihfA	integration host factor (IHF), α subunit	0.38	0.30	1.00	1
b3498	prlC	oligopeptidase A	0.38	0.35	0.98	1
b2296	ackA	propionate kinase / acetate kinase	0.38	0.36	0.98	1
b3417	malP	maltodextrin phosphorylase monomer	0.38	0.39	0.97	0
b2699	recA	DNA strand exchange, recombination protein w/ protease, nuclease activity	0.37	0.17	1.00	1
b0172	frr	ribosome recycling factor	0.37	0.15	1.00	1
b0438	clpX	ClpAXP	0.37	0.26	0.99	1
b2312	purF	amidophosphoribosyl transferase	0.37	0.43	0.97	1
b4025	pgi	phosphoglucose isomerase	0.33	0.19	1.00	1
b4014	aceB	malate synthase A	0.32	0.14	1.00	1
b2153	folE	GTP cyclohydrolase I monomer	0.32	0.20	1.00	1
b2925	fbaA	fructose bisphosphate aldolase monomer	0.32	0.07	1.00	1
b2942	metK	MetK S-adenosylmethionine synthetase monomer	0.31	0.12	1.00	1
b2614	grpE	phage lambda replication; host DNA synthesis; heat shock protein; protein repair	0.31	0.25	1.00	1
b0426	yajQ	nucleotide binding protein	0.30	0.17	1.00	1
b2926	pgk	phosphoglycerate kinase	0.30	0.06	1.00	1
b2417	crr	N-acetylmuramic acid PTS permease	0.30	0.10	1.00	1
b0932	pepN	aminopeptidase N	0.30	0.24	0.99	1
b0863	artI	arginine ABC transporter	0.30	0.29	0.97	1
b2551	glyA	serine hydroxymethyltransferase	0.29	0.08	1.00	1
b3781	trxA	oxidized thioredoxin	0.29	0.18	0.99	1
b4391	yjjK	YjjK	0.28	0.15	1.00	1
b3919	tpiA	triose phosphate isomerase monomer	0.26	0.21	0.99	1
b3302	rpmD	50S ribosomal subunit protein L30	0.26	0.25	0.97	1
b3169	nusA	transcription termination/antitermination L factor	0.25	0.17	1.00	1
b0811	glnH	glutamine ABC transporter	0.24	0.21	0.99	1
b3871	typA	protein possibly involved in LPS biosynthesis and host colonization	0.23	0.20	0.99	1
b0072	leuC	isopropylmalate isomerase	0.22	0.18	0.99	1
b3298	rpsM	30S ribosomal subunit protein S13	0.21	0.09	1.00	1
b1215	kdsA	3-deoxy-D-manno-octulosonate 8-phosphate synthase	0.21	0.23	0.98	1
b0071	leuD	isopropylmalate isomerase	0.20	0.15	0.99	1
b2764	cysJ	sulfite reductase flavoprotein subunit	0.19	0.15	0.99	1
b0903	pflB	pyruvate formate-lyase (inactive)	0.19	0.16	0.99	1
b1654	grxD	glutaredoxin 4	0.19	0.21	0.98	1
b4142	groS	chaperone binds to Hsp60 in pres. Mg-ATP, suppressing its ATPase activity	0.18	0.15	0.97	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b3251	mreB	longitudinal peptidoglycan synthesis/chromosome segregation-directing complex	0.18	0.22	0.95	1
b3908	sodA	superoxide dismutase (Mn)	0.17	0.15	0.98	1
b3231	rplM	50S ribosomal subunit protein L13	0.17	0.14	0.97	1
b0014	dnaK	chaperone Hsp70; DNA biosynthesis; autoregulated heat shock proteins	0.16	0.07	1.00	1
b0623	cspE	transcription antiterminator and regulator of RNA stability	0.15	0.18	0.97	1
b2498	upp	uracil phosphoribosyltransferase	0.14	0.12	0.99	1
b1062	pyrC	dihydroorotase	0.14	0.15	0.97	1
b3304	rplR	50S ribosomal subunit protein L18	0.14	0.16	0.95	1
b3295	rpoA	RNA polymerase, α subunit	0.13	0.11	0.99	1
b0004	thrC	threonine synthase	0.13	0.11	0.98	1
b4226	ppa	inorganic pyrophosphatase	0.13	0.13	0.97	1
b3294	rplQ	50S ribosomal subunit protein L17	0.11	0.10	0.97	1
b0115	aceF	AceF-lipoate	0.10	0.10	0.97	1
b2913	serA	α-ketoglutarate reductase / D-3-phosphoglycerate dehydrogenase	0.09	0.09	0.98	1
b4000	hupA	Transcriptional dual regulator HU-α (HU-2)	0.09	0.10	0.95	1
b3774	ilvC	acetohydroxy acid isomeroreductase	-0.07	0.06	0.98	1
b4245	pyrB	aspartate carbamoyltransferase, PyrB subunit	-0.07	0.07	0.97	1
b0116	lpd	E3 monomer	-0.07	0.08	0.96	1
b3305	rplF	50S ribosomal subunit protein L6	-0.09	0.08	0.97	1
b0605	ahpC	AhpC component	-0.09	0.08	0.96	1
b1779	gapA	glyceraldehyde 3-phosphate dehydrogenase-A monomer	-0.10	0.06	1.00	1
b3317	rplB	50S ribosomal subunit protein L2	-0.10	0.09	0.98	1
b3984	rplA	50S ribosomal subunit protein L1	-0.10	0.09	0.95	1
b3236	mdh	malate dehydrogenase	-0.11	0.06	1.00	1
b0436	tig	trigger factor; a molecular chaperone involved in cell division	-0.12	0.07	1.00	1
b3829	metE	Cobalamin-independent homocysteine transmethylase	-0.14	0.06	1.00	1
b3433	asd	aspartate semialdehyde dehydrogenase	-0.14	0.09	0.99	1
b3315	rplV	50S ribosomal subunit protein L22	-0.16	0.13	0.99	1
b2606	rplS	50S ribosomal subunit protein L19	-0.17	0.13	0.99	1
b3986	rplL	50S ribosomal subunit protein L7	-0.19	0.11	0.99	1
b3956	ppc	phosphoenolpyruvate carboxylase	-0.19	0.15	0.99	1
b3980	tufB	elongation factor Tu	-0.20	0.06	1.00	1
b1324	tpx	thiol peroxidase 2	-0.21	0.10	1.00	1
b3342	rpsL	30S ribosomal subunit protein S12	-0.21	0.18	0.98	1
b0928	aspC	aspartate aminotransferase, PLP-dependent	-0.22	0.10	1.00	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b3544	dppA	dipeptide ABC transporter	-0.22	0.14	1.00	1
b3339	tufA	elongation factor Tu	-0.22	0.04	1.00	1
b0166	dapD	tetrahydrodipicolinate succinylase subunit	-0.23	0.15	1.00	1
b1260	trpA	tryptophan synthase, α subunit	-0.25	0.19	0.99	1
b1612	fumA	fumarase A monomer	-0.26	0.27	1.00	1
b3172	argG	argininosuccinate synthase	-0.28	0.12	1.00	1
b2518	ndk	nucleoside diphosphate kinase [multifunctional]	-0.28	0.19	1.00	1
b3936	rpmE	50S ribosomal subunit protein L31	-0.28	0.30	0.95	1
b1243	oppA	OppA-oligopeptide ABC transporter substrate-binding	-0.29	0.11	1.00	1
b2935	tktA	transketolase I	-0.29	0.20	0.99	1
b3458	livK	leucine binding protein of the high-affinity branched-chain amino acid transport system	-0.31	0.19	0.99	1
b0729	sucD	succinyl-CoA synthetase, α subunit	-0.32	0.15	1.00	1
b2309	hisJ	histidine ABC transporter	-0.33	0.16	1.00	1
b2019	hisG	ATP phosphoribosyltransferase	-0.33	0.20	0.99	1
b3460	livJ	branched chain amino acids ABC transporter	-0.34	0.11	1.00	1
b0727	sucB	SucB-lipoate	-0.35	0.18	1.00	1
b0440	hupB	Transcriptional dual regulator HU-β, NS1 (HU-1)	-0.36	0.15	1.00	1
b0728	sucC	succinyl-CoA synthetase, β subunit	-0.40	0.09	1.00	1
b0720	gltA	citrate synthase monomer	-0.41	0.13	1.00	1
b2021	hisC	histidinol-phosphate aminotransferase	-0.41	0.35	0.99	1
b0726	sucA	subunit of E1(0) component of 2-oxoglutarate dehydrogenase	-0.43	0.19	1.00	1
b4177	purA	adenylosuccinate synthetase	-0.43	0.12	1.00	1
b3560	glyQ	glycyl-tRNA synthetase, α subunit	-0.43	0.40	0.97	1
b0888	trxB	thioredoxin reductase monomer	-0.45	0.47	0.97	1
b1479	maeA	malate dehydrogenase, NAD-requiring	-0.49	0.53	0.96	0
b1656	sodB	superoxide dismutase (Fe)	-0.51	0.26	1.00	1
b0420	dxs	1-deoxyxylulose-5-phosphate synthase	-0.53	0.10	1.00	1
b1415	aldA	aldehyde dehydrogenase A, NAD-linked	-0.53	0.33	1.00	1
b1761	gdhA	glutamate dehydrogenase	-0.54	0.13	1.00	1
b2747	ispD	4-diphosphocytidyl-2C-methyl-D-erythritol synthetase monomer	-0.61	0.67	0.95	1
b0118	acnB	aconitase B	-0.63	0.10	1.00	1
b1136	icd	isocitrate dehydrogenase	-0.65	0.06	1.00	1
b3942	katG	hydroperoxidase I	-0.65	0.66	0.96	1
b1662	ribC	riboflavin synthase	-0.72	0.79	0.95	0
b0882	clpA	ClpAXP	-0.81	0.74	0.99	0
b2094	gatA	galactitol PTS permease	-0.91	0.33	1.00	1

B#	Name	Function	Ratio	StdDev	Prob.	in 2/3
b2674	nrdI	conserved protein that may stimulate ribonucleotide reductase	-0.98	0.93	0.96	0
b2096	gatY	tagatose-1,6-bisphosphate aldolase 2	-1.40	0.60	1.00	1
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase monomer	-1.61	1.30	0.98	0
b2093	gatB	galactitol PTS permease	-1.63	0.76	1.00	1
b4221	ytfN	conserved protein	-1.82	1.72	0.99	0

Overall trends between differentially expressed proteins by mutant in Figure 6-3 and differentially expressed genes by mutant in Figure 5-1 are similar. The three mutant strains sharing the Δ*gdhA* deletion again exhibit less differential expression than the two mutant strains sharing the Δ*hnr* disruption, although for proteins, the difference is less. The three strains sharing Δ*gdhA* range from 77-88 differentially expressed proteins in total, about half of the 141-161 range for the two Δ*hnr* deletion strains. The Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*gdhA* Δ*aceE* Δ_P*yjiD* strains demonstrate a general lack of additivity of differentially-expressed proteins with increasing deletion targets, similar to the case for the differentially-expressed genes for these strains. However, the Δ*hnr* and Δ*hnr* Δ*yliE* strains display a similar lack of additivity with the additional deletion target, which is different than the case for differentially expressed genes. The Δ*gdhA* Δ*aceE* Δ_P*yjiD* strain again shows less than half of the differential protein expression as the two Δ*hnr* strains, similar to the case for differentially expressed genes. This again suggests that even though the *yjiD* (*iraD*) overexpression (via partial promoter deletion) and the *hnr* deletion have similar effects upon stabilizing the σ^S factor, the number and nature of other genes and proteins affected by their perturbations are different. As will be discussed in the next chapter, even though some similar trends hold for overall differential expression by mutants for genes and proteins, the global correlation for targets found in both gene and protein data sets is low.

Table 6-1 displays the following ln(mutant/PE) ranges of differential protein expression: -1.5 to 0.98 for the Δ*gdhA* strain; -2.02 to 1.43 for the Δ*gdhA* Δ*aceE* strain; -2.14 to 1.59 for the Δ*gdhA* Δ*aceE* Δ_P*yjiD* strain; -1.84 to 2.13 for the Δ*hnr* strain; and -1.82 to 2.29 for the Δ*hnr* Δ*yliE* strain. Individual differentially expressed genes are discussed further in the following sections.

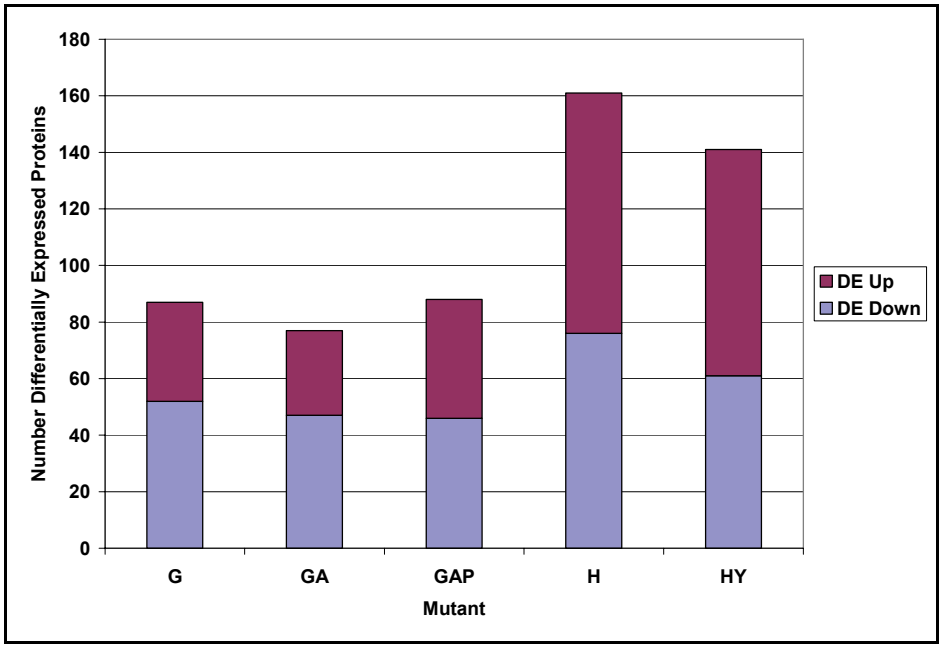

Figure 6-3 Number of up- and down-regulated differentially expressed proteins for the five mutant strains Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ_P*yjiD* (GAP), Δ*hnr* (H), *and* Δ*hnr* Δ*yliE* (HY) relative to the PE strain using a Bayesian probabilistic approach.

6.2. Protein Expression by Metabolic Pathways

Overall proteomic expression and differentially expressed proteins were examined next within the context of cellular metabolism and specific metabolic pathways. Although fewer protein measurements were collected than gene measurements (Figure 6-2), more targets overall were determined to be differentially expressed at the protein level versus the transcript level (Figure 6-3). Thus, examining the differential expression within certain pathways was more insightful for proteomic expression. Accordingly, overall proteomic expression is given in Figure 6-4 through Figure 6-8, and specific pathways of interest are shown in Figure 6-9 through

Figure 6-18. These figures were all generated using the EcoCyc database and associated Omics Viewer tool (Keseler, Bonavides-Martinez *et al.* 2009). In the specific pathway figures, care is taken to distinguish between all measured protein expression and differential protein expression as determined by the Bayesian probabilistic approach explained previously. All ratios plotted are natural logarithms ln(Mutant/PE) comparing the mutant expression with the PE strain expression. This differs from the gene expression data of the previous chapter, in which $\log_{10}$(Mutant/PE) ratios were shown. Given the larger number of differential but relatively small expression changes observed for the proteomic expression, this difference was deemed appropriate. From a high level, it is apparent from Figure 6-4 through Figure 6-8 that most measured protein expression changes are relatively small, similar to the gene expression changes. Again, the Δ*hnr* and Δ*hnr* Δ*yliE* strains demonstrate the most variation in proteomic expression and appear to have the greatest amount of coordination in differentially expressed proteins within specific pathways.

Figure 6-4 Global protein expression of Δ*gdhA* strain compared to PE strain. Natural logarithm ln(Mutant/PE) ratios are shown, with red indicating up-regulation and yellow indicating down-regulation in the Δ*gdhA* strain.

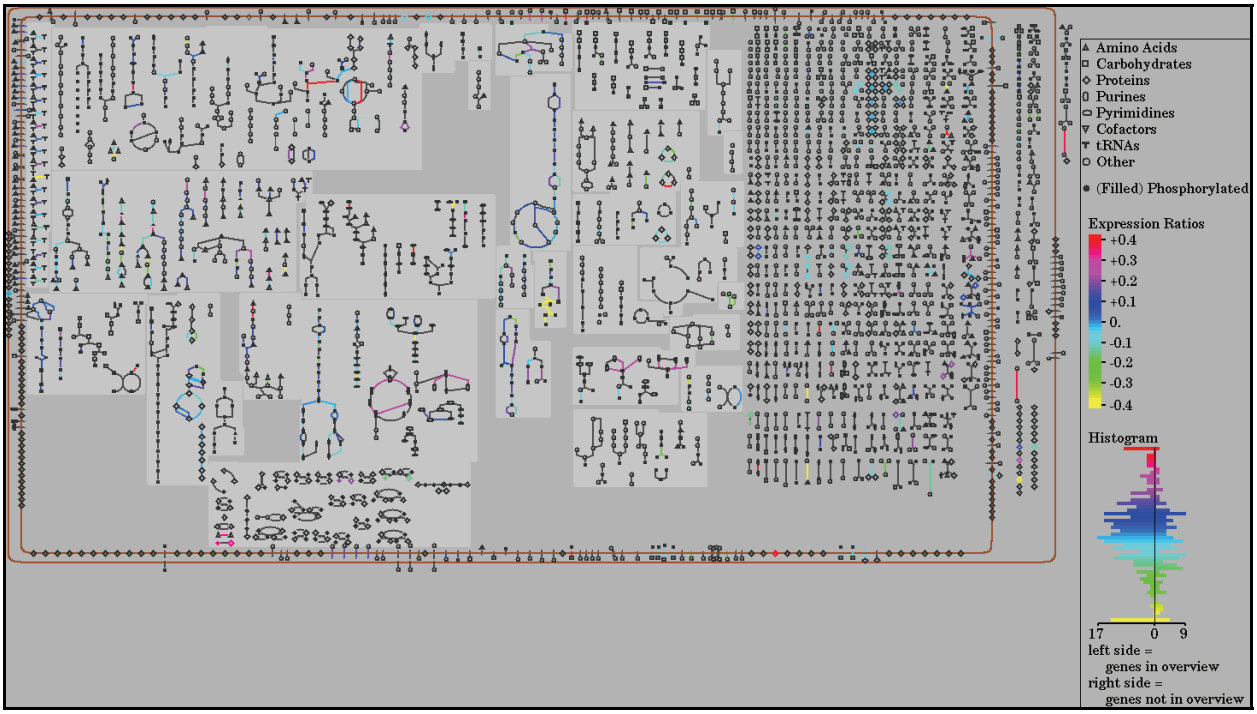

Figure 6-5 Global protein expression of Δ*gdhA* Δ*aceE* strain compared to PE strain. Natural logarithm ln(Mutant/PE) ratios are shown, with red indicating up-regulation and yellow indicating down-regulation in the Δ*gdhA* Δ*aceE* strain.

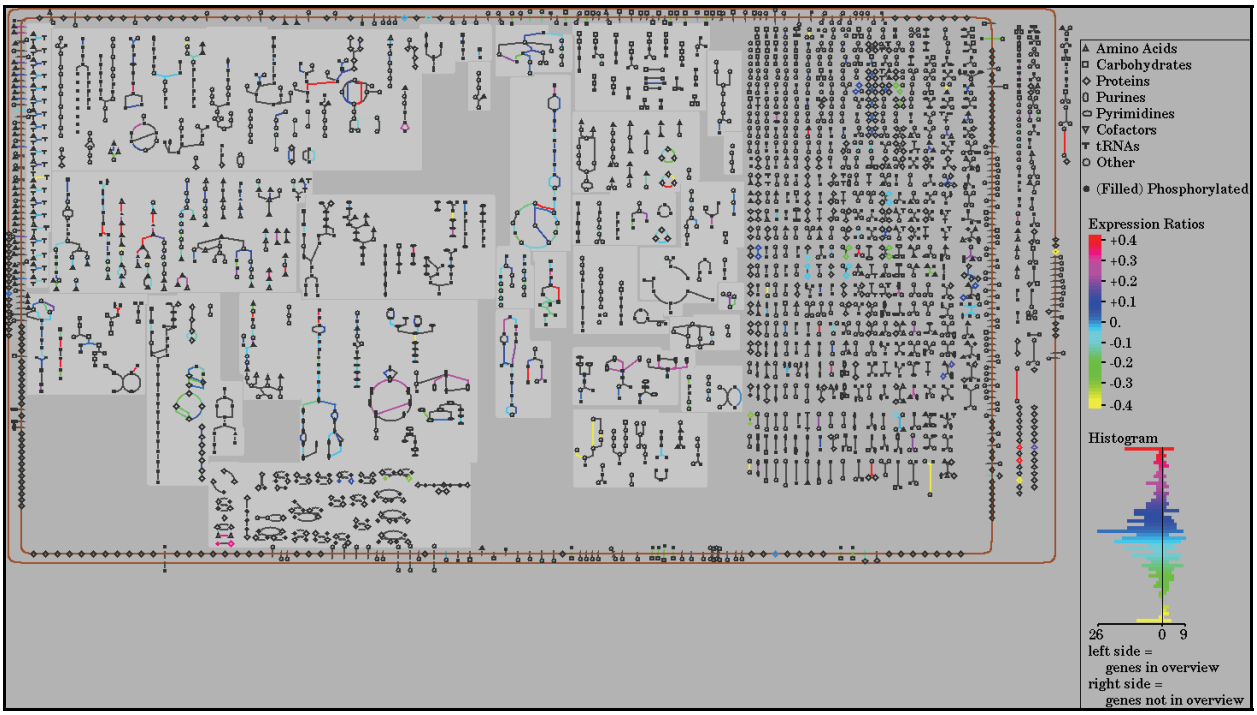

Figure 6-6 Global protein expression of Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* strain compared to PE strain. Natural logarithm ln(Mutant/PE) ratios are shown, with red indicating up-regulation and yellow indicating down-regulation in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* strain.

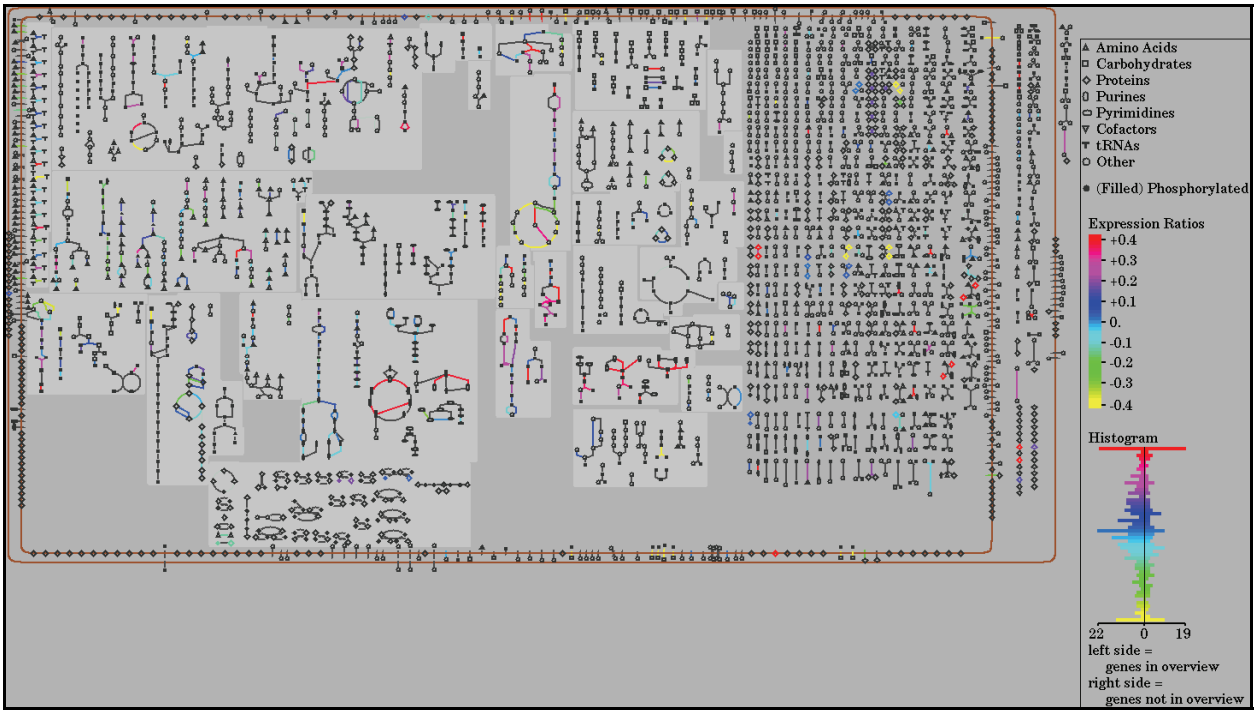

Figure 6-7 Global protein expression of Δ*hnr* strain compared to PE strain. Natural logarithm ln(Mutant/PE) ratios are shown, with red indicating up-regulation and yellow indicating down-regulation in the Δ*hnr* strain.

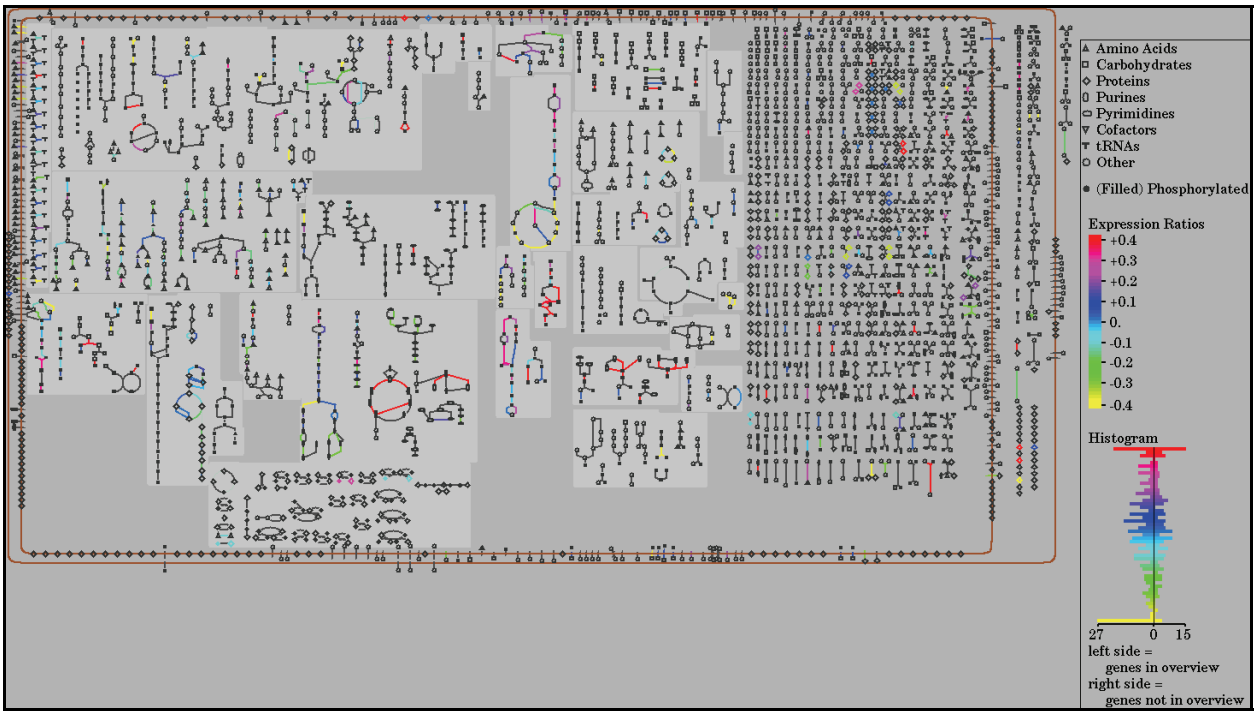

Figure 6-8 Global protein expression of Δ*hnr* Δ*yliE* strain compared to PE strain. Natural logarithm ln(Mutant/PE) ratios are shown, with red indicating up-regulation and yellow indicating down-regulation in the Δ*hnr* Δ*yliE* strain.

Differential expression within the superpathway of glycolysis, pyruvate dehydrogenase, the TCA cycle, and the glyoxylate pathway is shown in Figure 6-9 and Figure 6-10 for the three strains sharing the Δ*gdhA* deletion and the two strains sharing the Δ*hnr* deletion, respectively. Proteins that are differentially expressed are indicated by color according to the same color scale of Figure 6-4 through Figure 6-8, and the ln(Mutant/PE) ratios are given as well. Despite variations, there generally appears to be a slight up-regulation of the glycolytic and the glyoxylate pathways and a slight down-regulation of the TCA cycle in Figure 6-9 for the strains sharing the Δ*gdhA* deletion. These trends appear stronger in the strains sharing the Δ*hnr* deletion in Figure 6-10. These patterns could allow the mutant strains to generally direct carbon towards the non-mevalonate pathway precursors of glyceraldehyde-3-phosphate and pyruvate while also potentially recycling some carbon from the TCA cycle to the non-mevalonate precursors via the glyoxylate shunt. The TCA cycle appears only slightly down-regulated, still allowing for

NADH, ATP, and NADPH generation important for the energy-intensive processes of growth and lycopene production. Running carbon through the glyoxylate pathway instead of the TCA cycle also has an impact upon cellular redox balance, and these effects will be discussed further in the next chapter.

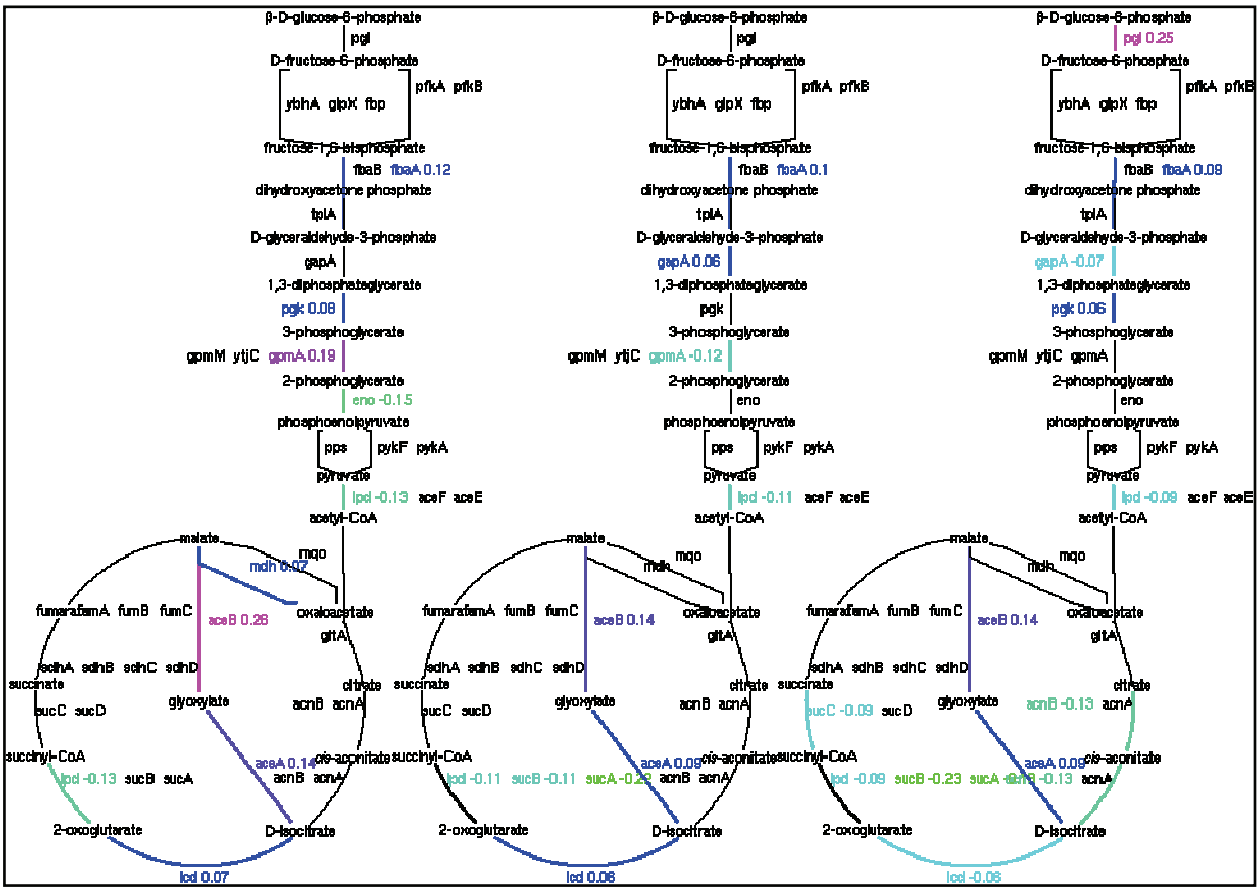

Figure 6-9 Differential protein expression ln(Mutant/PE) ratios in the superpathway of glycolysis, pyruvate dehydrogenase, the TCA cycle, and the glyoxylate pathway for the Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* strains (shown left to right).

All measured protein expression within the non-mevalonate (methylerythritol) pathway itself is shown in Figure 6-11 and Figure 6-12 for the three strains sharing the Δ*gdhA* deletion and the two strains sharing the Δ*hnr* deletion, respectively. Interestingly, proteins corresponding to the genes that are constituitively expressed under the PT5 promoter in both the mutants and the PE strain, Dxs, Idi, and IspD, are among those proteins detected, as would be expected for

high levels of overexpression of these key genes. Whereas the Dxs protein is differentially up-regulated in the Δ*gdhA,* the Δ*gdhA* Δ*aceE,* and the Δ*hnr* strain, the same protein is actually

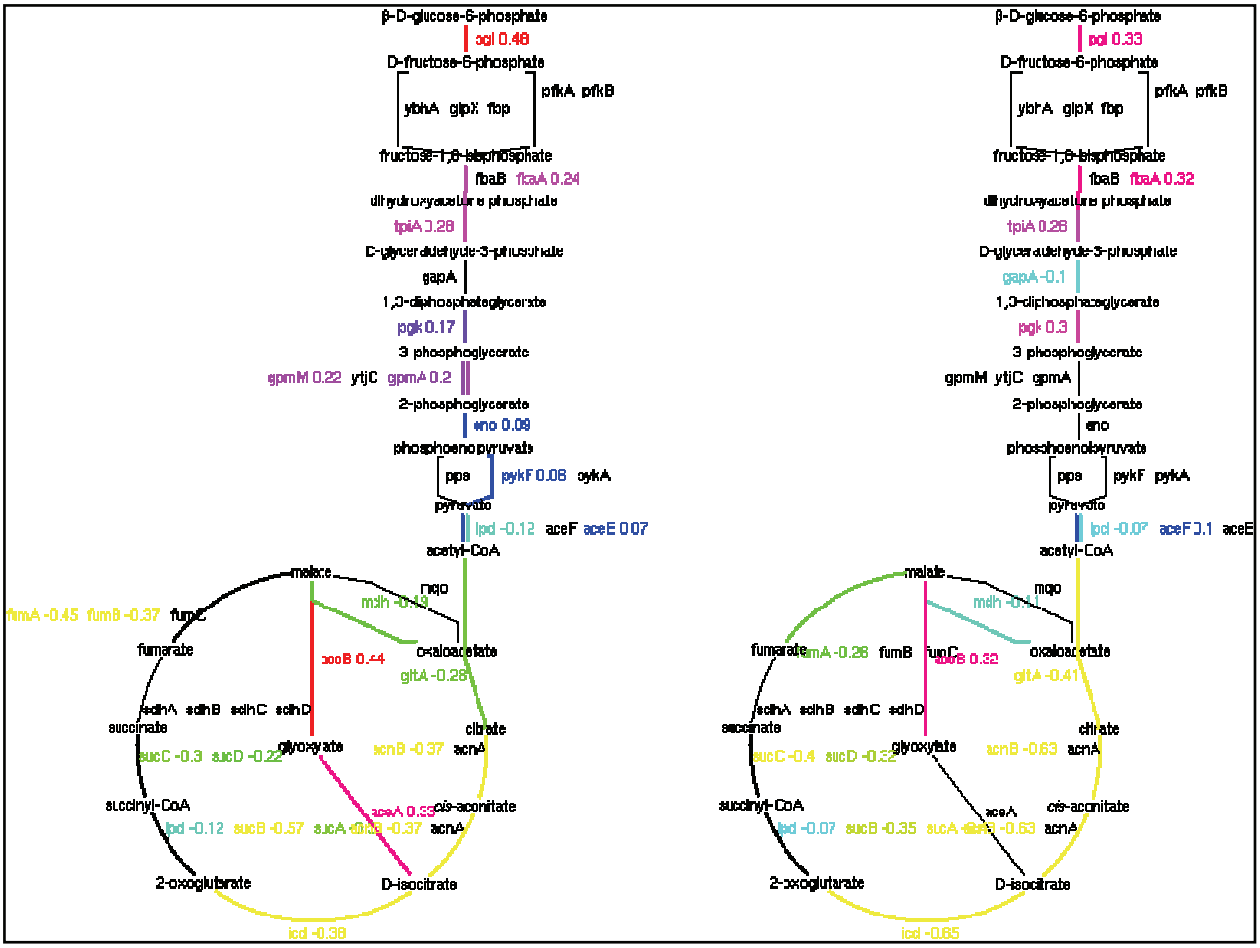

Figure 6-10 Differential protein expression ln(Mutant/PE) ratios in the superpathway of glycolysis, pyruvate dehydrogenase, the TCA cycle, and the glyoxylate pathway for the Δ*hnr* and Δ*hnr* Δ*yliE* strains (shown left to right).

differentially down-regulated in the Δ*hnr* Δ*yliE* strain. The reason for this is not clear. Constitutive overexpression of *dxs* should increase the transcript level in both the mutant and PE strains, but the basis of further increase (or decrease) of Dxs at the protein level in these mutants relative to the PE strain is not known. The IspD protein expression in the Δ*hnr* and Δ*hnr* Δ*yliE* strains is consistent with the Dxs expression, differentially up- and down-regulated, respectively. The other instance of differential expression occurs in the Δ*gdhA* Δ*aceE* strain, where the IspG protein is up-regulated. Overall, it appears that the protein expression in this pathway varies

between the strains. The expression is consistently up-regulated in the Δ*hnr* strain, mixed in the Δ*hnr* Δ*yliE* strain, and showing small decrease and increases in specific proteins for the Δ*gdhA* and the Δ*gdhA* Δ*aceE* and Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* strains, respectively. It is important to note that the metabolic flux through the non-mevalonate pathway must be up-regulated in these mutants compared to the PE strain since the lycopene production is greater, but the protein expression does not necessarily have to be up-regulated within the same pathway to achieve such overproduction.

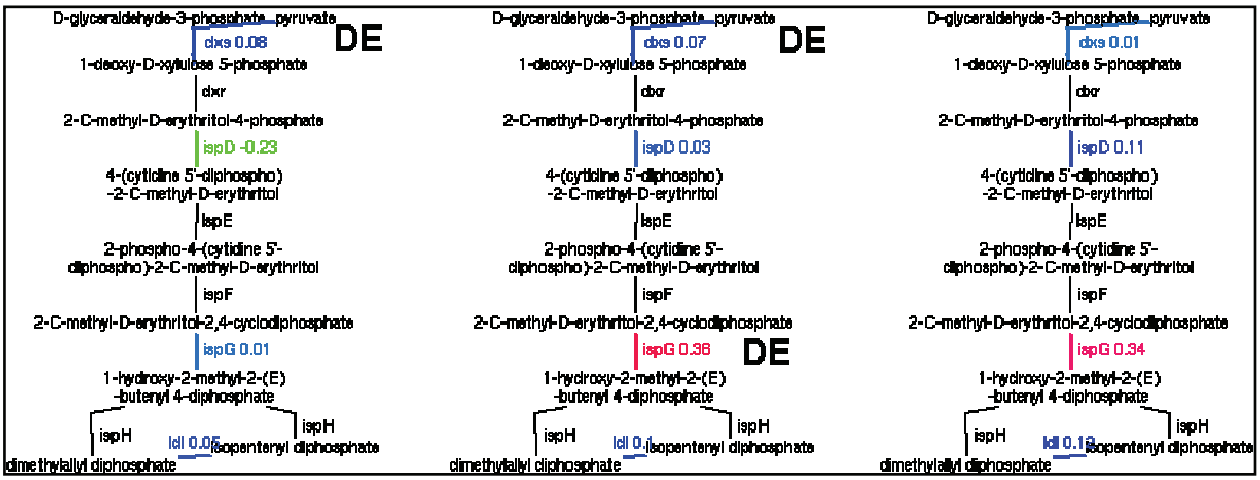

Figure 6-11 All measured protein expression ln(Mutant/PE) ratios in the non-mevalonate (methylerythritol) pathway for the Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* strains (shown left to right). Differentially expressed proteins are indicated.

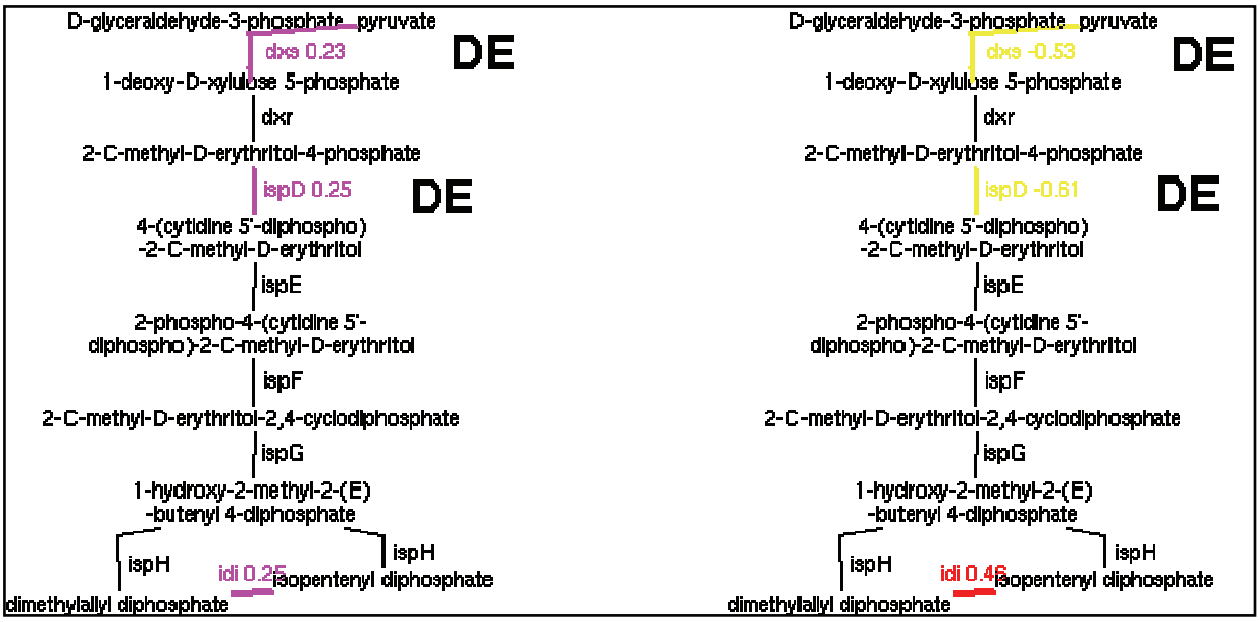

Figure 6-12 All measured protein expression ln(Mutant/PE) ratios in the non-mevalonate (methylerythritol) pathway for the Δ*hnr* and Δ*hnr* Δ*yliE* strains (shown left to right). Differentially expressed proteins are indicated.

Differential protein expression within the pentose phosphate pathway is shown in Figure 6-13 and Figure 6-14. The upper oxidative branch of the pathway producing D-ribose-5-phosphate displays up-regulations in the Pgl and RpiA proteins consistently in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD*, Δ*hnr*, and Δ*hnr* Δ*yliE* strains. This is interesting given that D-ribose-5-phosphate is the immediate precursor to 5-phosphoribosyl 1-pyrophosphate (PRPP), which was discussed in the previous chapter and is a metabolite found in the histidine, tryptophan, purine, and pyrimidine biosynthetic pathways as a precursor or an intermediate. Given the previous discussion and the fact that adding histidine (and tryptophan separately) to the media led to a relative increase in lycopene production in the PE strain relative to the mutant strains, it may be that this RpiA up-regulation is linked to increasing purine ADP levels via the PRPP intermediate, which would lead to an increase in ATP and energy available for lycopene production as well. The Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD*, Δ*hnr*, and Δ*hnr* Δ*yliE* strains show both up- and down-regulation in the lower non-oxidative branch of the pathway. The TktA transketolase 1 protein is negatively regulated

upon entry into stationary phase, and this may be an indirect effect of the σ^S factor (Jung, Phyo *et al.* 2005). Thus, its down-regulation in the Δ*gdhA* Δ*aceE* Δ$_P$*yjiD*, the Δ*hnr* and the Δ*hnr* Δ*yliE* strains is consistent with the stationary phase phenotypes of these cells. Similarly, the TalA transaldolase A protein is known to belong to the σ^S regulon (Weber, Polen *et al.* 2005), and so its up-regulation in the Δ*hnr* and Δ*hnr* Δ*yliE* strains is logical.

The Zwf and Gnd proteins catalyze reactions producing NADPH, a major requirement for lycopene production. It may be expected that any differential expression of these targets would be up-regulation to potentially increase NADPH levels. However, the Zwf protein was detected as differentially down-regulated in the Δ*gdhA* Δ*aceE* strain, and there were no other differential expression detections for these Zwf and Gnd proteins. The reason for the Zwf down-regulation is not clear.

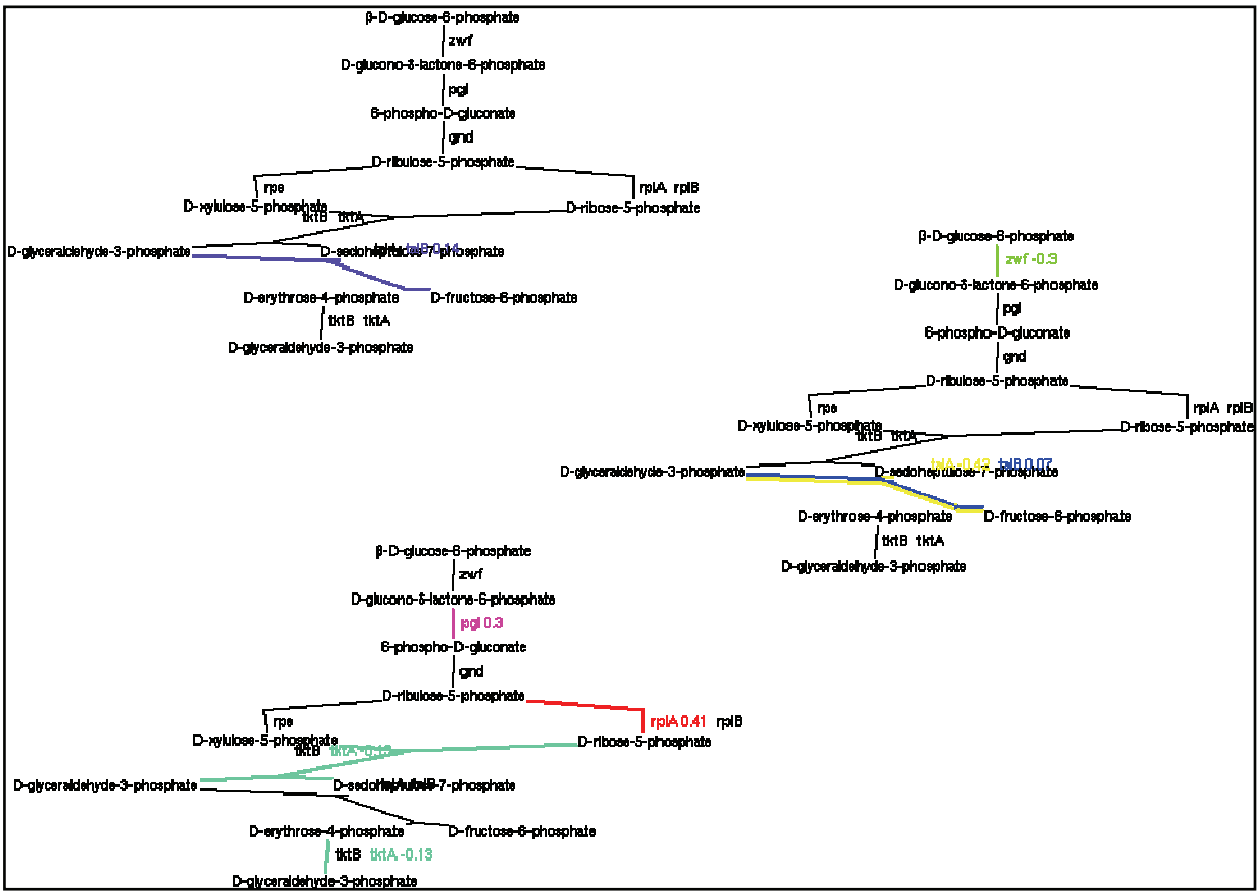

Figure 6-13 Differential protein expression ln(Mutant/PE) ratios in the pentose phosphate pathway for the Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* strains (shown clockwise).

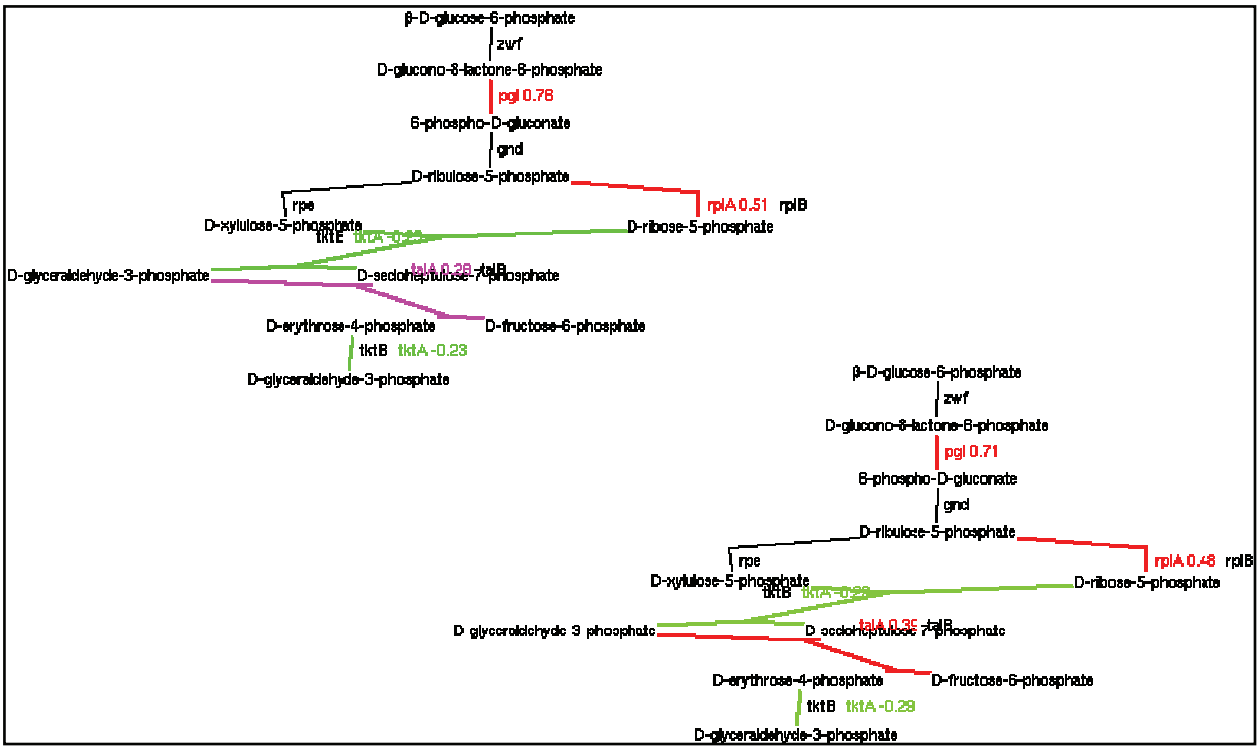

Figure 6-14 Differential protein expression ln(Mutant/PE) ratios in the pentose phosphate pathway for the Δ*hnr* and Δ*hnr* Δ*yliE* strains (shown top to bottom).

A number of additional observations for protein expression were made in the specific pathways shown in Figure 6-15 through Figure 6-18. First, it is interesting that several enzymes in the saturated fatty acid biosynthetic pathway were down-regulated in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* strain as shown in Figure 6-15 (FabF, FabG, FabI). Although the magnitude of down-regulation is not very large, the consistency is interesting especially in light of the gene clustering results shown previously and the fact that lycopene storage in the membrane is considered to be a likely limitation for lycopene production (Fraser and Sandmann 1992; Albrecht, Misawa *et al.* 1999). Second, there appears to be some consistency in the measured protein expression of the histidine biosynthetic pathways in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD,* Δ*hnr,* and Δ*hnr* Δ*yliE* strains shown in Figure 6-16 (differential expression explicitly labeled) with the previously shown down-regulation in the *hisH* gene. Although the HisH protein itself is not differentially expressed, a number of other proteins in the pathway, namely HisG, HisB, and HisC, are differentially down-regulated in the

various mutants. Interestingly, HisF, which forms the imidazole glycerol phosphate synthase complex with HisH, is differentially up-regulated in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* strain.

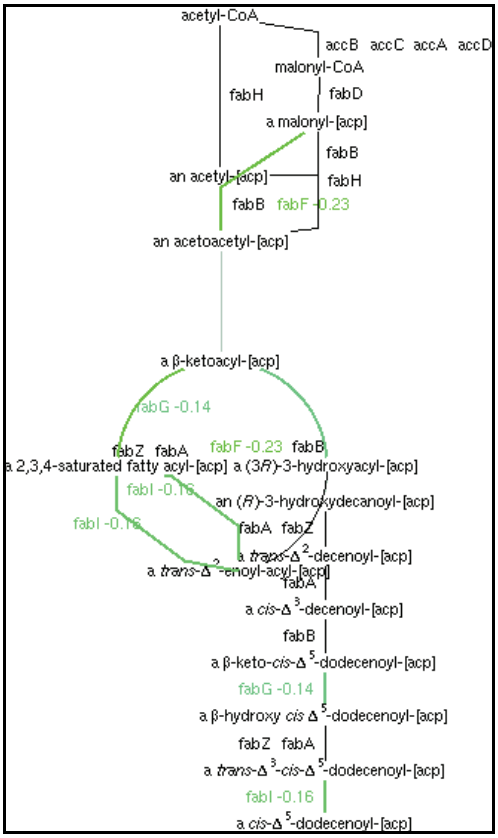

Figure 6-15 Differential protein expression ln(Mutant/PE) ratios in the saturated fatty acid biosynthetic pathway for the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* strain.

Figure 6-17 and Figure 6-18 display differential expression in the Δ*hnr* and Δ*hnr* Δ*yliE* strains for the mixed acid fermentation pathways and the purine deoxyribonucleoside and ribonucleoside degradation pathways, respectively. While the succinate fermentation pathway is down-regulated, it is interesting that the pathway to formate appears up-regulated given that Alper *et al.* (2005) found that the *fdhF* gene is a deletion target for increasing lycopene production in the Δ*gdhA* Δ*aceE* background that produced the maximum amount of lycopene with knockouts from purely rationally-directed knockouts. Acetate is the main product of glucose overflow metabolism, whereas the mixed acid fermentation pathways produce acetate, ethanol, formate, lactate, succinate, carbon dioxide, and dihydrogen under anaerobic conditions.

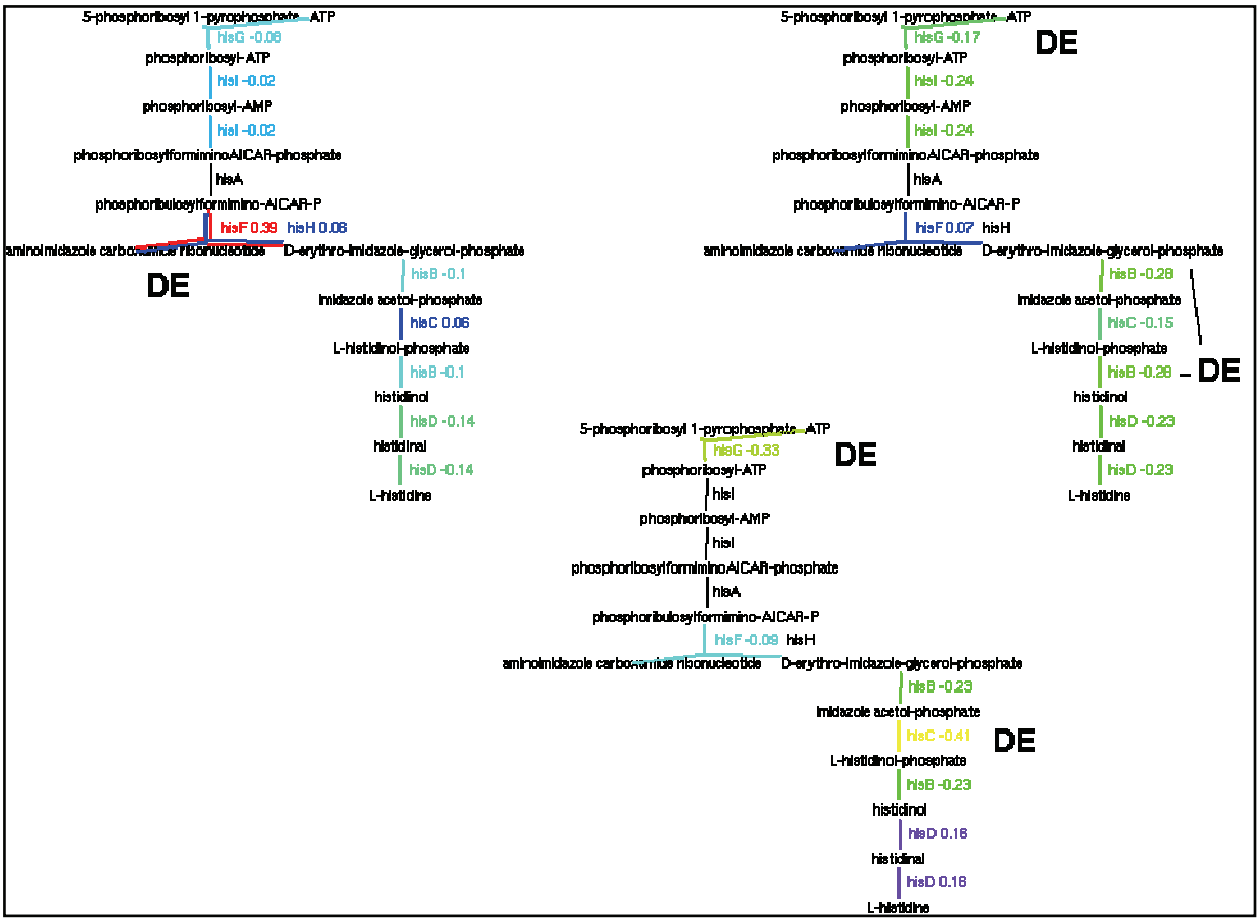

Figure 6-16 All measured protein expression ln(Mutant/PE) ratios in the histidine biosynthetic pathway for the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD*, the Δ*hnr*, and the Δ*hnr* Δ*yliE* strains (shown clockwise). Differentially expressed proteins are indicated.

Thus, the reason for the differential expression pattern in Figure 6-17 is not completely clear. It can be seen that purine deoxyribonucleoside and ribonucleoside degradation in Figure 6-18 lead to the glyceradlehyde-3-phosphate and D-ribose-5-phosphate metabolites. The former is a lycopene precursor, whereas the latter is found within the pentose phosphate pathway and a PRPP precursor, as discussed above. The up-regulation of these salvage pathways via the DeoD, DeoB, and AdhE proteins may lead to greater conservation of these metabolites for boosting lycopene production directly and indirectly, respectively.

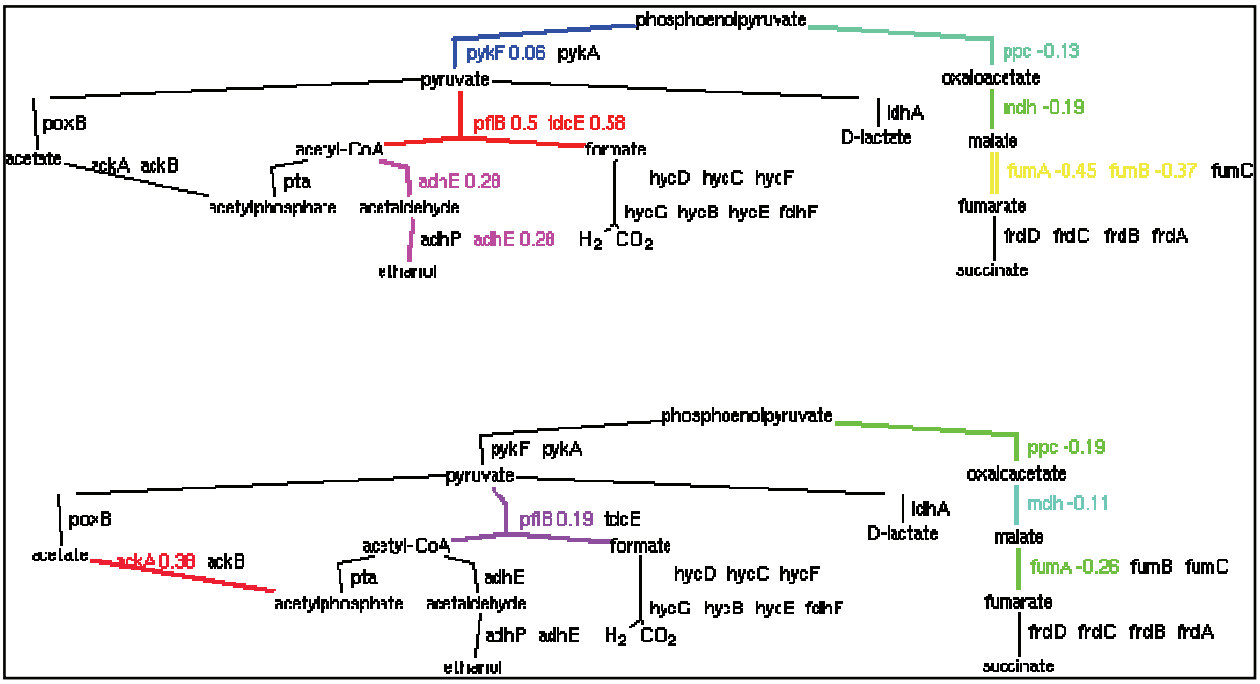

Figure 6-17 Differential protein expression ln(Mutant/PE) ratios in the mixed acid fermentation pathways for the Δ*hnr* and Δ*hnr* Δ*yliE* strains (shown top to bottom).

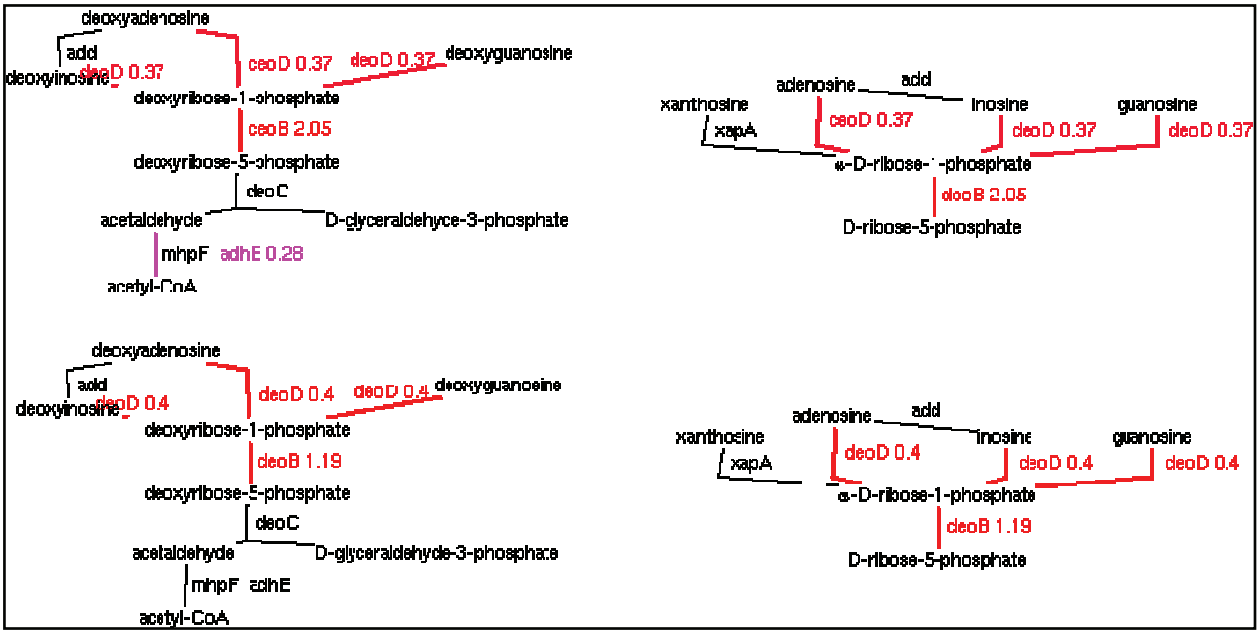

Figure 6-18 Differential protein expression ln(Mutant/PE) ratios in the purine deoxyribonucleoside and ribonucleoside degradation pathways for the Δ*hnr* and Δ*hnr* Δ*yliE* strains (shown top to bottom and left to right).

6.3. Conservation of Differential Protein Expression Across Mutants

Similar to the analysis for conserved differential gene expression, differential protein expression that was conserved between the various mutants was next examined. Concentration

of differentially expressed proteins to specific pathways examined in the previous section is useful to determine expression trends across metabolism and catabolism, but trends across multiple mutants for even single genes can add additional insight into the cellular state associated with the phenotype of interest. Table 6-2 and Table 6-3 both present differentially expressed proteins and overlap to a great extent, but they present the data in two different and useful ways. Table 6-2 lists those proteins that were measured in both the mutant and PE strains for at least 3 of the 5 mutants and also gives the corresponding ln(Mutant/PE) ratios as appropriate. The proteins are listed in decreasing order of number of mutants in which they were differentially expressed. Table 6-3 lists those proteins that were measured in both the mutant and PE strains for at least 2 of the 5 mutants and were in the top 50% of summed absolute values of differential expression per protein, and it also gives the corresponding ln(Mutant/PE) ratios as appropriate. Table 6-3 ranks the proteins according to the sum of the absolute values of their ln(Mutant/PE) ratios ("Sum(Abs)" column). Thus, differentially expressed proteins with either consistently large magnitudes of changes or those with a few very large changes appear near the top of the list. It should be noted that the restriction that these proteins be detected and measured in at least 2 of the 3 LC-MS replicates for all of the mutants quantified was relaxed for these lists. However, the consistency of large measurements across multiple mutants lends greater credibility to these measurements even in the cases where the protein was only detected in 1 of the 3 replicates.

Table 6-2 Conservation of differential protein expression across mutants. Proteins that were measured in both the mutant and PE strains for at least 3 of the 5 mutants are shown, and ln(Mutant/PE) ratios are given as appropriate. Green indicates differential down-regulation, and red indicates differential up-regulation. The "DE" column gives the total number of mutants in which the corresponding protein was differentially expressed. Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY).

Protein	G	GA	GAP	H	HY	G	GA	GAP	H	HY	DE
AceB						0.26	0.14	0.14	0.44	0.32	5
ArgB						0.62	0.51	0.41	0.41	0.44	5

Protein	G	GA	GAP	H	HY	G	GA	GAP	H	HY	DE
Asd					-0.14	0.15	0.29	0.25	0.16		5
FbaA						0.12	0.10	0.09	0.24	0.32	5
HupB	-0.21	-0.17	-0.37	-0.53	-0.36						5
Icd			-0.06	-0.38	-0.65	0.07	0.06				5
Lpd	-0.13	-0.11	-0.09	-0.12	-0.07						5
OppA	-0.12	-0.10	-0.13	-0.23	-0.29						5
PanB	-1.30	-2.02	-2.14	-1.84	-1.61						5
RplA	-0.25	-0.16	-0.19	-0.18	-0.10						5
RplB	-0.27	-0.19	-0.13	-0.18	-0.10						5
RplF	-0.15	-0.17	-0.13	-0.06	-0.09						5
RplS	-0.21	-0.34	-0.20	-0.33	-0.17						5
RplV	-0.57	-0.36	-0.23	-0.43	-0.16						5
RpsL	-0.34	-0.28	-0.25	-0.28	-0.21						5
AceA						0.14	0.09	0.09	0.33		4
Crr		-0.10						0.13	0.24	0.30	4
DapD					-0.23	0.15	0.10	0.18			4
Dxs					-0.53	0.08	0.07		0.23		4
FtsZ						0.30	0.31		0.36	0.45	4
GlnA				-0.13		0.31	0.33	0.32			4
MetE	-0.06		-0.07	-0.08	-0.14						4
OsmC	-0.59							0.28	0.63	1.08	4
Pgk						0.08		0.06	0.17	0.30	4
RplC	-0.31	-0.32	-0.14	-0.28							4
RplO	-0.25	-0.16	-0.14	-0.17							4
RpmE	-0.44	-0.45		-0.39	-0.28						4
RpsA	-0.17	-0.12	-0.09	-0.11							4
RpsC	-0.19	-0.12	-0.14	-0.14							4
RpsK	-0.62	-0.26	-0.36	-0.34							4
SodA	-0.26	-0.23							0.11	0.17	4
SucA		-0.22	-0.18	-0.30	-0.43						4
SucB		-0.11	-0.23	-0.57	-0.35						4
ThrC						0.10		0.12	0.13	0.13	4
TufA					-0.22	0.17		0.07	0.10		4
TufB					-0.20	0.18	0.12		0.25		4
AcnB			-0.13	-0.37	-0.63						3
AhpC					-0.09	0.11		0.09			3
AldA		-0.18		-0.39	-0.53						3
AtpD	-0.19		-0.16	-0.19							3
Bfr								0.44	1.21	1.06	3
DeoD							0.27		0.37	0.40	3
Frr								0.17	0.24	0.37	3
FusA						0.05	0.06		0.08		3
GalU							0.44	0.36		0.90	3

Protein	G	GA	GAP	H	HY	G	GA	GAP	H	HY	DE
GapA			-0.07		-0.10		0.06				3
GatA			-0.25	-0.69	-0.91						3
GlyA								0.07	0.14	0.29	3
GpmA		-0.12				0.19			0.20		3
GroS						0.21			0.13	0.18	3
HisJ				-0.23	-0.33		0.12				3
HlpA	-0.27	-0.18		-0.40							3
LivJ				-0.17	-0.34			0.07			3
Mdh				-0.19	-0.11	0.07					3
NadE								0.28	0.36	0.42	3
OsmY								0.58	1.33	1.37	3
Pgi								0.25	0.48	0.33	3
Pgl								0.30	0.76	0.71	3
Ppa								0.12	0.24	0.13	3
PurA			-0.15	-0.14	-0.43						3
RibC	-1.03				-0.72				0.45		3
RpiA								0.41	0.51	0.48	3
RplD	-0.26	-0.16	-0.18								3
RplI	-0.08		-0.11	-0.06							3
RplN		-0.24	-0.21	-0.29							3
RplR		-0.15		-0.25						0.14	3
RplX	-0.25	-0.15		-0.20							3
RplY	-0.58	-0.28		-0.24							3
RpsG	-0.14	-0.07	-0.09								3
RpsI	-0.15	-0.09	-0.10								3
RpsJ		-0.12	-0.10						0.11		3
RpsS		-0.55	-0.48	-0.39							3
SerA						0.16	0.17			0.09	3
SerC						0.08	0.09	0.08			3
SucC			-0.09	-0.30	-0.40						3
TalA		-0.42							0.28	0.39	3
ThrA						0.10	0.11	0.09			3
TktA			-0.13	-0.23	-0.29						3
Tpx				-0.10	-0.21	0.16					3
WrbA								1.59	2.13	1.92	3
YajQ								0.15	0.36	0.30	3

Table 6-3 Conservation of differential protein expression across mutants, with differentially expressed proteins ranked by the sum of the absolute values of their ln(Mutant/PE) ratios ("Sum(Abs)" column). Proteins that were measured in both the mutant and PE strains for at least 2 of the 5 mutants and were in the top 50% of summed absolute values are shown, and ln(Mutant/PE) ratios are given as appropriate. Green indicates differential down-regulation, and red indicates differential up-regulation. The "DE" column gives the total number of mutants in which the corresponding protein was differentially expressed. Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY).

Protein	G	GA	GAP	H	HY	G	GA	GAP	H	HY	DE	Sum(Abs)
PanB	-1.30	-2.02	-2.14	-1.84	-1.61						5	8.91
WrbA								1.59	2.13	1.92	3	5.64
HdeB									1.70	2.26	2	3.96
GadA									1.42	2.11	2	3.53
GadB									1.40	2.06	2	3.46
OsmY								0.58	1.33	1.37	3	3.28
DeoB									2.05	1.19	2	3.24
Bfr								0.44	1.21	1.06	3	2.71
OsmC	-0.59							0.28	0.63	1.08	4	2.58
GatY				-1.01	-1.40						2	2.41
ArgB						0.62	0.51	0.41	0.41	0.44	5	2.39
GcvT							1.43	0.92			2	2.35
GatB				-0.70	-1.63						2	2.33
RibC	-1.03				-0.72				0.45		3	2.20
YbaY									1.10	1.08	2	2.18
YgfZ	-1.50		-0.63								2	2.13
GatA			-0.25	-0.69	-0.91						3	1.85
Pgl								0.30	0.76	0.71	3	1.77
PepD						0.98			0.79		2	1.77
RplV	-0.57	-0.36	-0.23	-0.43	-0.16						5	1.75
GalU							0.44	0.36		0.90	3	1.70
ElaB									0.58	1.12	2	1.70
HupB	-0.21	-0.17	-0.37	-0.53	-0.36						5	1.64
RpsK	-0.62	-0.26	-0.36	-0.34							4	1.58
RpmE	-0.44	-0.45		-0.39	-0.28						4	1.56
FtsZ						0.30	0.31		0.36	0.45	4	1.42
RpsS		-0.55	-0.48	-0.39							3	1.42
RpiA								0.41	0.51	0.48	3	1.40
Fur							0.55		0.82		2	1.37
RpsL	-0.34	-0.28	-0.25	-0.28	-0.21						5	1.36
AceB						0.26	0.14	0.14	0.44	0.32	5	1.30
SucB		-0.11	-0.23	-0.57	-0.35						4	1.26
RplS	-0.21	-0.34	-0.20	-0.33	-0.17						5	1.25
Icd			-0.06	-0.38	-0.65	0.07	0.06				5	1.22
SucA		-0.22	-0.18	-0.30	-0.43						4	1.13
AcnB			-0.13	-0.37	-0.63						3	1.13
AldA		-0.18		-0.39	-0.53						3	1.10
RplY	-0.58	-0.28		-0.24							3	1.10

Protein	G	GA	GAP	H	HY	G	GA	GAP	H	HY	DE	Sum(Abs)
GlnA				-0.13		0.31	0.33	0.32			4	1.09
TalA		-0.42							0.28	0.39	3	1.09
NadE								0.28	0.36	0.42	3	1.06
Pgi								0.25	0.48	0.33	3	1.06
RplC	-0.31	-0.32	-0.14	-0.28							4	1.05
MalP	-0.67									0.38	2	1.05
GuaC						0.47				0.58	2	1.05
DeoD							0.27		0.37	0.40	3	1.04
YggX									0.52	0.51	2	1.03
Asd					-0.14	0.15	0.29	0.25	0.16		5	0.99
LepA						0.50			0.48		2	0.98
PrlC	-0.57									0.38	2	0.95
Dxs					-0.53	0.08	0.07		0.23		4	0.91
AzoR									0.41	0.50	2	0.91
GdhA				-0.35	-0.54						2	0.89
HinT								0.29	0.60		2	0.89
RplA	-0.25	-0.16	-0.19	-0.18	-0.10						5	0.88
YdfG								0.51	0.37		2	0.88
CysD	-0.53	-0.35									2	0.88
FbaA						0.12	0.10	0.09	0.24	0.32	5	0.87
RplB	-0.27	-0.19	-0.13	-0.18	-0.10						5	0.87
OppA	-0.12	-0.10	-0.13	-0.23	-0.29						5	0.87
IspD					-0.61				0.25		2	0.86
HlpA	-0.27	-0.18		-0.40							3	0.85
RpsU				-0.51				0.32			2	0.83
YajQ								0.15	0.36	0.30	3	0.81
SucC			-0.09	-0.30	-0.40						3	0.79
Dps									0.38	0.41	2	0.79
Frr								0.17	0.24	0.37	3	0.78
Crr		-0.10						0.13	0.24	0.30	4	0.77
SodA	-0.26	-0.23							0.11	0.17	4	0.77
GltX									0.26	0.51	2	0.77
TufB					-0.20	0.18	0.12		0.25		4	0.75
GlyQ				-0.32	-0.43						2	0.75
RplN		-0.24	-0.21	-0.29							3	0.74
RplO	-0.25	-0.16	-0.14	-0.17							4	0.72
PurA			-0.15	-0.14	-0.43						3	0.72

A number of interesting trends are apparent from the differentially expressed proteins listed in Table 6-2 and Table 6-3. A number of the differentially expressed proteins near the top of Table 6-2 are involved in the TCA cycle, the glyoxylate pathway, and glycolysis, as shown

and discussed previously in Figure 6-9 and Figure 6-10. AceB and AceA of the glyoxylate pathway were up-regulated in 5 and 4 of the mutants, respectively, with up-regulations in the Δ*hnr* and Δ*hnr* Δ*yliE* strains ranging from about 40-55%. On the other hand, the TCA cycle branch enzyme isocitrate dehydrogenase Icd was slightly up-regulated in the Δ*gdhA* and Δ*gdhA* Δ*aceE* strains but down-regulated in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD*, Δ*hnr*, and Δ*hnr* Δ*yliE* strains, with the last two strains showing a stronger down-regulation of about 30 and 50%, respectively. Lpd slight down-regulation in all five mutants, on the other hand, is consistent with increasing the amount of pyruvate and glutamate produced in addition to activating the glyoxylate shunt (Li, Ho *et al.* 2006). Similarly, other TCA cycle enzymes are differentially expressed: SucA and SucB are down-regulated in 4 of the mutants; SucC and AcnB are down-regulated in the 3 mutants; SucD and GltA are down-regulated in 2 of the mutants; and Mdh is down-regulated in two mutants and up-regulated in one. Overall, it appears that the TCA cycle has fairly consistent down-regulation, as discussed before.

Within the glycolytic/gluconeogenic pathways, FbaA is up-regulated in 5 mutants; Pgk is up-regulated in 4 mutants; Pgi is up-regulated in 3 strains; GpmA is up-regulated in 2 and down-regulated in 1; and GapA is down-regulated in 2 and up-regulated in 1 strain. The trend for these pathways is up-regulation at the proteomic level, likely feeding carbon to the lycopene precursors glyceraldehyde-3-phosphate and pyruvate as previously discussed. Interestingly, the only slight instance of conserved differential down-regulation in this pathway occurs in the glyceraldehyde-3-phosphate dehydrogenase enzyme (GpmA), which may be slightly discouraging the conversion of glyceraldehye-3-phosphate, the limiting precursor for lycopene production (Farmer and Liao 2001), towards pyruvate. Also notable is that the most highly conserved example of up-regulation in the glycolytic/gluconeogenic pathways, fructose

bisphosphate aldolase class II (FbaA), catalyzes a reaction producing the lycopene precursor glyceradlehyde-3-phosphate in addition to DHAP.

Other trends in the conserved differential protein expression exist. As discussed above, TalA, Pgl, TktA, and RpiA of the pentose phosphate pathway are differentially expressed in 3 mutants each. ArgB, acetylglutamate kinase, is up-regulated in all 5 mutants. This is interesting given its association with glutamate, which has been linked to lycopene production (Alper, Miyaoku *et al.* 2006). The glutamine synthetase (GS) protein GlnA of the Ntr system for nitrogen assimilation is up-regulated in the three mutants sharing the Δ*gdhA* deletion, which is logical given that these strains must assimilate most nitrogen through the GS-GOGAT pathway. This compares to the transcriptional data, where the *glnA* was up-regulated in the Δ*hnr* strain and *gltB* was up-regulated in both the Δ*hnr* and Δ*hnr* Δ*yliE* strains. GadA, GadB, and HdeB of the acid stress response are all near the top of Table 6-3 and show up-regulation, with the first two enzymes glutamate-dependent. SodA and WrbA are both involved in preventing superoxide damage in the cells, an interesting function given the antioxidant properties of lycopene itself. SodA is both up- and down-regulated in 4 total mutants, whereas WrbA is strongly up-regulated in the Δ*gdhA* Δ*aceE* Δ_{P}*yjiD*, Δ*hnr*, and Δ*hnr* Δ*yliE* strains. OsmC, OsmY, and YbaY are proteins taking part in the osmotic stress response that are induced by the σ^{S} factor (Lange, Barth *et al.* 1993; Bouvier, Gordia *et al.* 1998; Weber, Polen *et al.* 2005), and they are mainly up-regulated quite strongly in the Δ*gdhA* Δ*aceE* Δ_{P}*yjiD*, Δ*hnr*, and Δ*hnr* Δ*yliE* strains as shown in Table 6-3. Interestingly, Kizer *et al.* (2008) found significant up-regulation of osmoregulatory genes including *osmC* and *osmY* in lycopene-producing *E. coli* strains utilizing the mevalonate pathway.

Several membrane-associated proteins in these tables are also differentially expressed: GatA and GatB of galactitol PTS permease; OppA of the ATP-dependent oligopeptide transporter complex, Crr of the glucose-specific IIa transporter component; and the GalU extracellular assembly protein. GatA, GatB, and OppA are generally down-regulated, whereas Crr and GalU are generally up-regulated. These changes in the membrane protein concentrations may have an effect upon lycopene storage in the membrane, possibly favoring more storage in the mutant strains. The GalU up-regulation in 3 of the mutants may be related to the general trend of motility to aggregative phenotypes observed in the gene expression data.

Finally, a number of individual proteins appeared interesting. PanB is at the top of Table 6-3 of most changed proteins and is involved in the pantothenate biosynthetic pathway. Pantothenate is a precursor to coenzyme A synthesis, so the consistently strong down-regulation of PanB may have far-reaching effects in the cellular state via coenzyme A effects. CoA is required for membrane-composing fatty acid biosynthesis, for example, and thus PanB down-regulation may have an effect on lycopene membrane storage, similar to the previous observations in the transcriptional data. Dps, a stationary phase nucleoid component that sequesters iron and protects DNA from damage (Martinez and Kolter 1997), is up-regulated in the Δ*hnr* and Δ*hnr* Δ*yliE* strains demonstrating the stationary phase phenotypes.

Finally, RpoE (b2573), the sigma E factor that orchestrates cellular responses to heat shock and other stresses on periplasmic and outer membrane proteins, was observed to be up-regulated over 9.9-fold in the Δ*gdhA* strain in the "threshold two" data (Appendix 9.1). On a related note, the *cpxP* gene (previously b3914, now b4484) encoding for the CpxP protein involved in resistance to extracytoplasmic stresses, was up-regulated in the Δ*hnr* strain by over 3.5-fold. These σ^E and Cpx regulatory pathways function together to provide coordinated yet

distinct responses to cellular envelope stress in *E. coli* (Raivio and Silhavy 1999). Given that lycopene is stored in the cellular membrane (Fraser and Sandmann 1992), these expression observations appear to be evidence of membrane stress resulting from lycopene overproduction.

Perhaps the most obvious trend in the differentially expressed protein data is the number of ribosomal proteins that appear in Table 6-2 and Table 6-3. In fact, 42 of the 55 total 50S and 30S ribosomal proteins in *E. coli* are differentially expressed, with a number of the most altered proteins appearing in Table 6-2 and Table 6-3. 103 out of 112 differential expression measurements for these ribosomal proteins correspond to down-regulation, and this trend of moderate but consistent down-regulation is shown in Figure 6-19. Although the ln(Mutant/PE) ratio distributions for the various mutants are near zero, the averages clearly indicate overall down-regulation, with 22 of the ribosomal proteins exhibiting mutant expression at least 20% different than the PE expression level across the five mutants. Despite the appearance of a few examples of ribosomal protein up-regulation contradicting this trend, the strongest example, RpsV (Sra), which is up-regulated over 2.3-fold in the Δ*hnr* Δ*yliE* strain, is known to be positively regulated by σ^S and increase in the stationary phase (Izutsu, Wada *et al.* 2001). This specific effect may be unrelated to the general trend of down-regulation observed in the other ribosomal proteins. Even small changes in ribosomal protein content may have an effect upon the cellular environment since it has been documented that up to 45% of the mass of rapidly growing *E. coli* cells corresponds to ribosomes (Wittmann 1982).

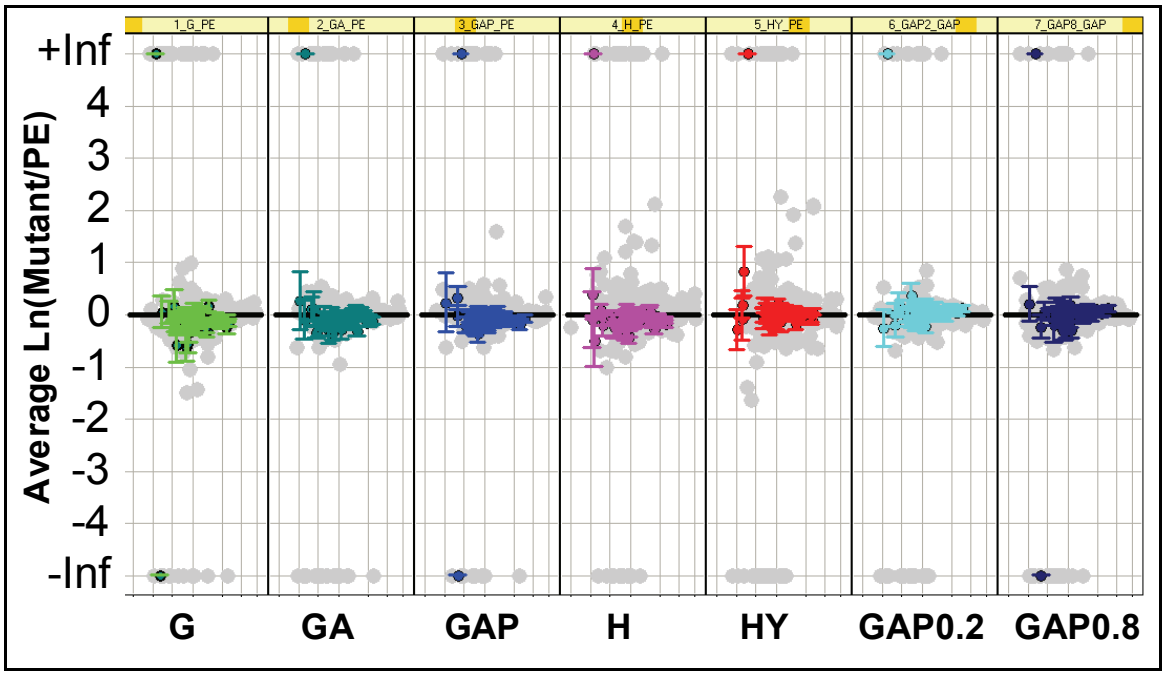

Figure 6-19 Ribosomal protein ln(Mutant/PE) expression ratio distribution for the five mutant strains Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY) relative to the PE strain, in addition to the Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* strain harvested earlier (OD_{600} = 0.2) and later (OD_{600} = 0.8) in exponential growth phase (GAP0.2 and GAP0.8, respectively) compared to the OD_{600} = 0.4 harvest of all other samples. All measured ribosomal proteins are shown in color, whereas the background distribution of the proteome is shown in gray.

This down-regulation of ribosomal synthesis may be a tradeoff of cellular growth for higher lycopene production in the mutant strains. Indeed, it has been shown previously (Alper, Miyaoku *et al.* 2006) and observed in this study that the mutant lycopene strains Δ*gdhA* Δ*aceE* Δ*fdhF* (not examined in this study) and Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* exhibit slower growth compared to the PE strain (~10-30% slower). Partial pH control with only base addition was previously shown to reduce the growth rate of these strains but lead to increased specific lycopene productivity (Alper, Miyaoku *et al.* 2006). Alper *et al.* (2005) performed gene knockout simulations and found an inverse relationship between the stoichiometric maximum lycopene yield and the growth yield, suggesting that reducing the growth yield supports enhanced lycopene production.

While cellular starvation activates the signaling molecule ppGpp, which in turn slows rRNA and ribosomal protein synthesis and stimulates a general stress response via σ^S and a large number of other transcriptional changes (Durfee, Hansen *et al.* 2008), such a full stringent response is not occurring here given that the amino acid biosynthetic pathways, for example, are not universally up-regulated. It is interesting, though, that the *spoT* gene, whose primary physiological role is thought to be ppGpp degradation, is down-regulated about 35-55% in the Δ*hnr* and Δ*hnr* Δ*yliE* strains, although this was not statistically differential down-regulation. Previous studies have documented ribosomal protein down-regulation during recombinant protein production (Rinas 1996; Han, Jeong *et al.* 2003) and threonine overproduction (Lee, Lee *et al.* 2003), for example, and Kizer *et al.* (2008) observed down-regulation at the transcriptional level for lycopene-producing *E. coli* utilizing the mevalonate pathway. Such a shift from cellular maintenance to overproduction appears to be present in this study of small molecule overproduction as well. Glutamate and lysine overproduction have been accomplished in *E. coli* by overexpressing the *relA* gene (Imaizumi, Kojima *et al.* 2006), which encodes ppGpp synthetase, an enzyme synthesizing ppGpp from GDP and ATP. Overexpression of *relA* led to higher intracellular ppGpp levels and higher production of these amino acids. Overproduction of ppGpp has also enhanced secondary metabolite production of antibiotics in *Streptomyces griseus* (Ochi 1987) and *Bacillus subtilis* (Ochi and Ohsawa 1984). Perhaps this cellular strategy of *E. coli* in down-regulating growth-related components and specifically ribosomal proteins is a general production strategy common to many high-producing strains for various target products. Although not pursued in this work, the down-regulation of so many ribosomal proteins suggests that *relA* overexpression may have a positive impact upon lycopene production as well.

6.4. Additional Individual Protein Analysis

While global trends in the proteome, metabolic pathways, and differentially expressed proteins have provided insight into the lycopene production phenotype, a few more individual proteins that were of high interest were examined. For example, similar to the discussion of the previous chapter as to how the Δ*gdhA*, Δ*aceE*, Δ*hnr*, and Δ*yliE* gene deletions still allowed for detection of partial transcripts, some AceE peptides were detected in the Δ*gdhA* Δ*aceE* and Δ*gdhA* Δ*aceE* Δ_{P}*yjiD* strains despite the fact that ln(Mutant/PE) expression ratios were nearly 0. As explained before, this is possible because despite the fact that the gene product is nonfunctional, over 600 and 800 basepairs of the original sequence remain for this AceE protein example at either end of the gene deletion in the center of the AceE gene. The YjiD protein was not detected despite its overexpression resulting from the promoter region deletion, which is possible given that only the most abundant proteins are detected by analyzing unfractionated samples. The Hnr protein was not detected, and the Ylie protein was only detected in the Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*hnr* Δ*yliE* strains applying the first threshold. Again, some peptide fragments were left to allow for this detection in the Δ*hnr* Δ*yliE* strain. The GdhA (b1761) protein was not detected in the three strains sharing the Δ*gdhA* deletion applying the first threshold, but some peptide fragments of the GdhA protein were detected in all five mutants using the second peptide threshold (Appendix 9.1), demonstrating down-regulation. Interestingly, the GdhA protein was differentially down-regulated from 55-70% in the Δ*hnr* and Δ*hnr* Δ*yliE* strains when applying the first threshold, confirming the value of this gene deletion in the other three mutant strains.

Another notable group of proteins was the ATP synthase proteins. Whereas the *atpE* gene was found to be up-regulated over 3-fold, the AtpE protein was not among those detected in

the proteomic measurements. Instead, though, the AtpA, AtpD, and AtpG proteins were differentially down-regulated by about 20% (2 strains), 15% (3 strains), and 45% (1 strain), respectively. These proteins are the α, the β, and the γ subunits of the catalytic site-containing F_1 complex of ATP synthase, respectively, but their changes are significantly less than the over 3-fold up-regulation observed at the transcript level for AtpE. The reason for their down-regulation is not known, but up-regulation of a limiting component of F_0 in *atpE* to such a high level may still have an overall positive effect upon ATP synthesis.

It is notable that the RpoS (σ^S) protein, a known target for increasing lycopene production (Becker-Hapak, Troxtel *et al.* 1997), was up-regulated in the Δ*hnr* and Δ*hnr* Δ*yliE* strains over 9-fold and 6-fold, respectively, applying the second data threshold, since these strains lack the Hnr protein that facilitates and controls the degradation of σ^S. The RpoS protein was only found in the Δ*hnr* and Δ*hnr* Δ*yliE* strains and not detected in the PE strain when the first threshold was applied, explaining why the protein was not listed in the above Table 6-1, Table 6-2, Table 6-3 for differentially expressed proteins using threshold one. Interestingly, the RpoS protein was not one of the most abundant and detected proteins in the Δ*gdhA* Δ*aceE* Δ_P*yjiD* strain, which also disrupts the σ^S degradation as explained previously. Given this strain's similar *hnr* gene inhibition as the strains sharing the Δ*hnr* deletion, it is likely that the RpoS protein was up-regulated in the Δ*gdhA* Δ*aceE* Δ_P*yjiD* strain but simply not detected. Further protein measurement and sample fractionation may reveal this. The expression of σ^S prepares the cells for stationary phase on a global regulatory scale, and many of the individual protein observations of this chapter are related to the up-regulation of σ^S.

6.5. Metabolic Engineering of PE Strain Based on Proteomic Targets

6.5.1. *wrbA* NAD(P)H:Quinone Oxidoreductase Overexpression

A couple of the seemingly most interesting and relevant protein targets were next examined in greater detail, with hypotheses tested via metabolic engineering of the PE strain. It is apparent from Figure 6-20 and Table 6-3 that the WrbA protein, an NAD(P)H:quinone oxidoreductase, displays an upward trend in expression between the Δ*gdhA*, the Δ*gdhA* Δ*aceE*, and the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* mutants that correlates with an increase in lycopene production. Furthermore, WrbA is differentially up-regulated in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD*, the Δ*hnr*, and the Δ*hnr* Δ*yliE* strains from nearly 5-fold to over 8-fold. It is known that WrbA is positively-regulated by σ^S (Yang, Ni *et al.* 1993), and so it's up-regulation in these strains is logical. Data has been shown that are consistent with WrbA reducing the quinone pool to the hydroquinone state in *E. coli* to prevent against the semiquinone interaction with O_2 and subsequent superoxide production (Patridge and Ferry 2006), which is reflected in Figure 6-21 below. The quinone pool of menaquinone and ubiquinone is closely related to the lycopene production pathway from the FPP branch point as shown in Figure 6-21, and so it is proposed that increasing the expression of WrbA could "pull" on the entire polyisoprenoid pathway above by an unknown mechanism, perhaps by eliminating negative feedback or positively activating a limiting enzyme in the pathway. This, in turn, may lead to the observed lycopene overproduction. Alternatively, overproduction of the antioxidant WrbA protein could lead to increased production of the antioxidant lycopene by an unknown mechanism. Yuan and Rouviere *et al.* (2006) found that overexpressing the *ispB* gene (Figure 6-21) led to increased production of β-carotene, which is one reaction downstream of lycopene, but they attributed the increase to the homology between

ispB and *ispU*. Nevertheless, other feedback mechanisms may be affecting the lycopene production via WrbA.

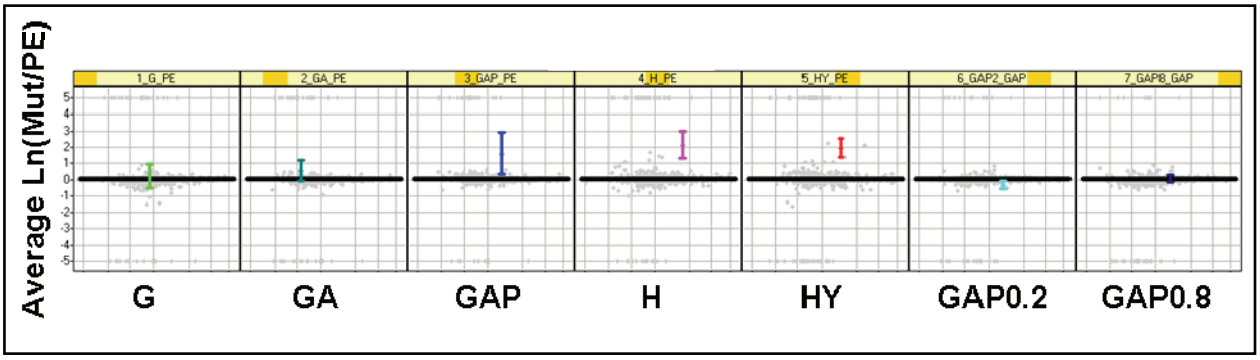

Figure 6-20 Quinone oxidoreductase WrbA protein ln(Mutant/PE) expression ratio distribution for the five mutant strains Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ_P*yjiD* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY) relative to the PE strain, in addition to the Δ*gdhA* Δ*aceE* Δ_P*yjiD* strain harvested earlier (OD_{600} = 0.2) and later (OD_{600} = 0.8) in exponential growth phase (GAP0.2 and GAP0.8, respectively) compared to the OD_{600} = 0.4 harvest of all other samples. The background distribution of the proteome is shown in grey.

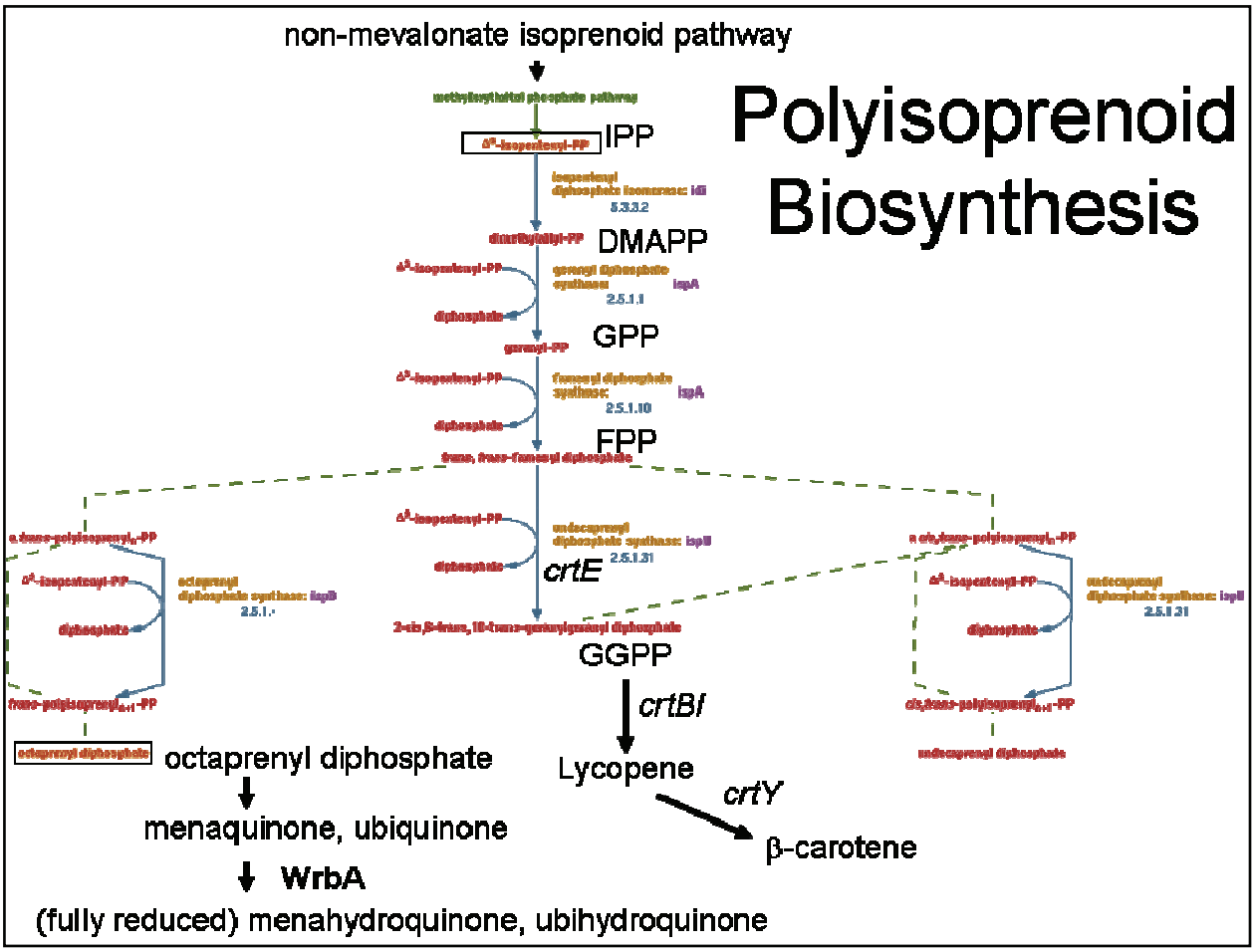

Figure 6-21 Polyisoprenoid biosynthetic pathway in *E. coli* and the relationship between recombinant lycopene production and the WrbA protein. Figure modified from Keseler *et al.* (2009).

It was hypothesized that *wrbA* gene overexpression might lead to lycopene production increases. To test this hypothesis, the pZE21-*gfp* plasmid was modified to replace the *gfp* gene with the *wrbA* gene for overexpression under the control of five different promoters of varying strength (Alper, Fischer *et al.* 2005). These five types of pZE21-*wrbA* plasmids were transformed into the PE strain, and the resulting lycopene production profiles are shown in Figure 6-22.

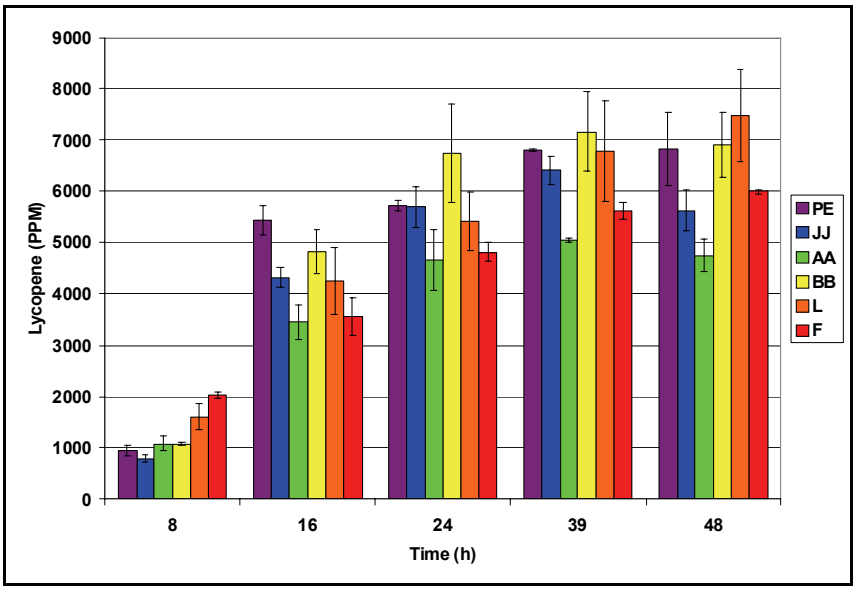

Figure 6-22 Lycopene production for PE strain transformed with the pZE21-*wrbA* plasmid overexpressing *wrbA* gene under control of promoters JJ, AA, BB, L, and F (in increasing order of strength) (Alper, Fischer *et al.* 2005). The purple bars indicate the untransformed PE strain only containing the pAC-LYC plasmid, which all samples contain for lycopene production.

It is apparent from Figure 6-22 that overexpression of *wrbA* in the PE strain has a highly positive effect upon lycopene production at 8 hours, which is close to the times at which the strains were sampled for expression analysis corresponding to OD_{600} = 0.4. Lycopene production is loosely correlated with degree of *wrbA* overexpression at this time point, and the highest level of overexpression (*wrbA* under F promoter) is approximately double that of the PE strain then. Overexpression of *wrbA* thus appears to significantly increase the lycopene productivity.

At later time points, the BB and possibly L promoter strength levels of *wrbA* overproduction appear to have a slightly positive effect upon lycopene production (~5-15%). Given that *wrbA* overexpression has such a beneficial effect at 8 hours but only leads to a marginal production increase at later time points, it may be advantageous to overexpress the *wrbA* gene only at earlier times. At later times, the *wrbA* gene is up-regulated as part of the σ^S-regulated stationary phase response in the PE strain as well, likely erasing any earlier advantage

and actually identifying the slight disadvantage of the increased metabolic burden of the pZE plasmid. Such fine control over gene expression through time is a current limitation of metabolic engineering, and synthetic regulatory networks capable of such control will become valuable in systems like the *wrbA* overexpression of this study once they are available.

In these experiments, the PE strain without a transformed pZE plasmid (but with the pAC-LYC plasmid common to all lycopene-producing strains) was used as the control. However, since the pZE plasmid introduces an additional metabolic burden upon the cells (Bentley, Mirjalili *et al.* 1990; Birnbaum and Bailey 1991), the lycopene production is likely increased even further in the *wrbA* overexpression strains compared to a PE control that contains an empty pZE plasmid as well, such as the control introduced in Figure 6-25. Chromosomal overexpression of the *wrbA* gene would therefore likely be a better long term strategy, although this was not pursued in this study.

Kang *et al.* (2005) observed that overexpressing the *appY* gene increased lycopene accumulation. Noting that ubiquinone levels were lower in lycopene overproducing *E. coli* and that ubiquinone is produced from the isoprenoid pathway as well, they hypothesized that lycopene overproduction drawing from the isoprenoid flux may lead to the ubiquinone decrease. This, in turn, could lead to insufficient energy production. Thus, they reasoned that the *appY* gene, a transcriptional activator of two energy operons induced by anaerobiosis, may help rescue these strains from energy insufficiency due to lower ubiquinone levels.

By a similar argument, the *wrbA* gene overexpression may serve to restore more flux towards essential ubiquinone synthesis in lycopene-overproducing cells, balancing cellular energy requirements with overproduction of the energy-intensive lycopene product. By giving up some flux towards ubiquinone synthesis, the overall flux towards lycopene biosynthesis could

actually increase if energy limitations were preventing further production. The *wrbA* overexpression therefore might either increase the flux towards lycopene by relieving energy limitations in lycopene production or else by some unknown regulatory feedback mechanism. Especially in light of the *atpE* gene up-regulation, the *hisH* down-regulation, ribosomal protein down-regulation, and the high requirement of energetic cofactors for lycopene production, this energy explanation seems feasible. More work is required to investigate these possibilities.

6.5.2. Acid Stress Response Overexpression

Another group of proteins was examined in detail due to their collective expression profiles. GadA and GadB, glutamate decarboxlyase acid resistance isozymes, and HdeB, a periplasmic acid-stress chaperone, were up-regulated as shown in Table 6-3 and Figure 6-23. In the Δ*hnr* and Δ*hnr* Δ*yliE* strains, the proteins were differentially up-regulated from about 4-fold to over 9.5-fold, some of the strongest proteomic expression changes measured.

GadA and GadB are part of the glutamate-associated stress response known as "AR2" (Ma, Gong *et al.* 2003; Foster 2004), and HdeB is part of the periplasmic stress response preventing the aggregation of proteins in the periplasm denatured under extremely acidic conditions (Gajiwala and Burley 2000). It is interesting that one of the 13 genes affecting lycopene accumulation found by Kang *et al.* (2005) using a shotgun approach was *ymgB* (*ariR*), which is now known to have a critical role in *E. coli* acid resistance (Lee, Page *et al.* 2007). While *ymgB* overexpression alone did not affect lycopene levels, it's overexpression with *ymgA* and *ycgZ* more than doubled production in that study. The glutamate-dependent acid response is especially notable given Alper's (2006) implication of glutamate in lycopene production and the fact that the three of the strains of this study share the Δ*gdhA* deletion. Growth experiments of the PE and Δ*gdhA* Δ*aceE* Δ_P*yjiD* strains have shown that after 24 hours, the pH typically falls

from about 7 to approximately 6.5-6.7, indicating slight acid stress. However, this network is also activated by the σ^S up-regulation that is especially present in the Δ*gdhA* Δ*aceE* Δ$_P$*yjiD*, Δ*hnr*, and Δ*hnr* Δ*yliE* strains (Ma, Gong *et al.* 2003). Since these acid response proteins are not differentially expressed in the strains sharing the Δ*gdhA* deletion, it is likely that it is the σ^S up-

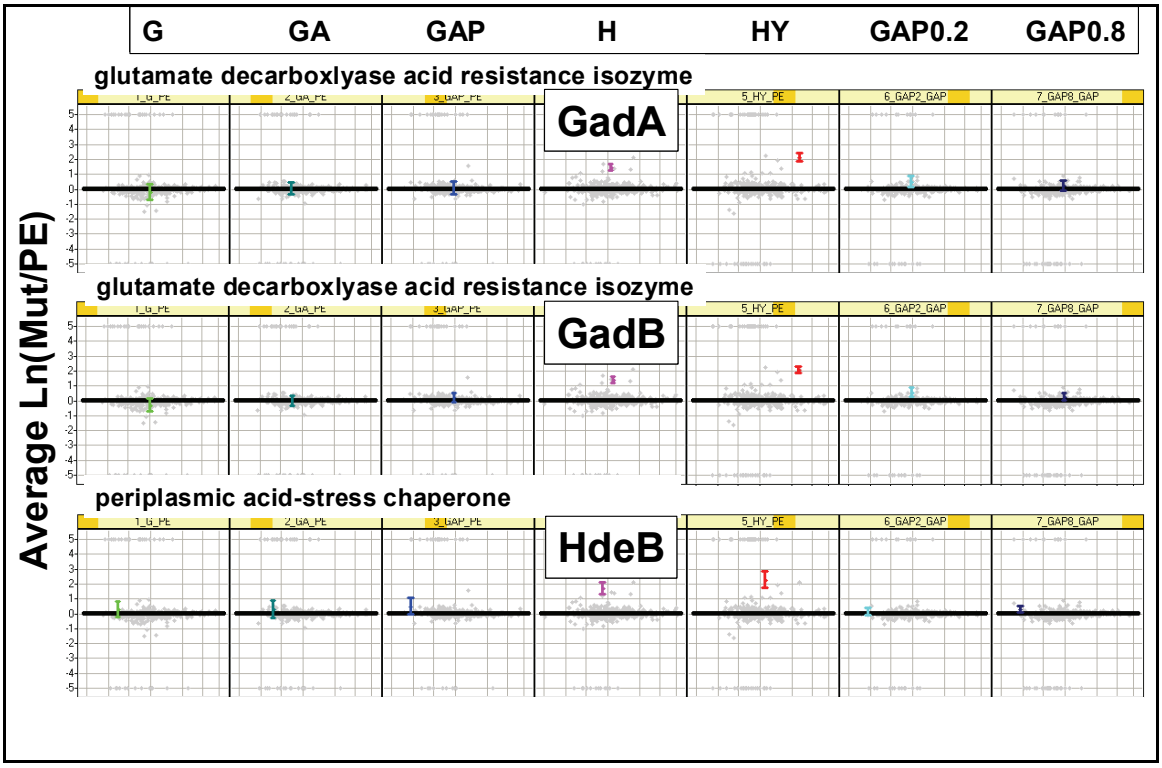

Figure 6-23 Acid stress response protein ln(Mutant/PE) expression ratios for the GadA, GadB, and HdeB proteins in the five mutant strains Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY) relative to the PE strain, in addition to the Δ*gdhA* Δ*aceE* Δ$_P$*yjiD* strain harvested earlier (OD_{600} = 0.2) and later (OD_{600} = 0.8) in exponential growth phase (GAP0.2 and GAP0.8, respectively) compared to the OD_{600} = 0.4 harvest of all other samples. The background distribution of the proteome is shown in grey.

regulation that is mainly responsible for their up-regulation in the other strains. It has been shown that *gadA*, *gadB*, and *hdeB* are among σ^S-regulated genes significantly up-regulated upon acetate exposure (Arnold, McElhanon *et al.* 2001), which is interesting given the role of acetate in *E. coli* overflow metabolism. Other σ^S-regulated proteins found up-regulated in this thesis that have previously been linked to acetate stress as well include AdhE, Dps, OsmC, OsmY, PflB, and TalA (Arnold, McElhanon *et al.* 2001). This theme of acetate stress will be discussed

further in the next chapter. Regardless of the cause, it was of interest whether acid stress response up-regulation in the PE strain would have any effect upon lycopene production.

Masuda and Church (2003) have proposed the main two activators for this complex acid resistance pathway are *ydeO* and *gadE*, and it is shown in Figure 6-24. YdeO and GadE activate a large number of other genes including *gadA*, *gadB*, and *hdeB*. Accordingly, pZE *ydeO* and pZE *gadE* overexpression plasmids were obtained from Christine Sanots (2008, unpublished work) with both genes under control of two promoters of different strengths, named Y or P_L, where the P_L promoter is stronger (Alper, Fischer *et al.* 2005). These four plasmid types were transformed into the PE strain as described previously, and lycopene production was tested in M9 media. As a further control, the pZE *gfp* plasmid was transformed into the PE strain and similarly tested for lycopene production. This second control was only measured for lycopene at the 24 and 39 hour time points in Figure 6-25, though.

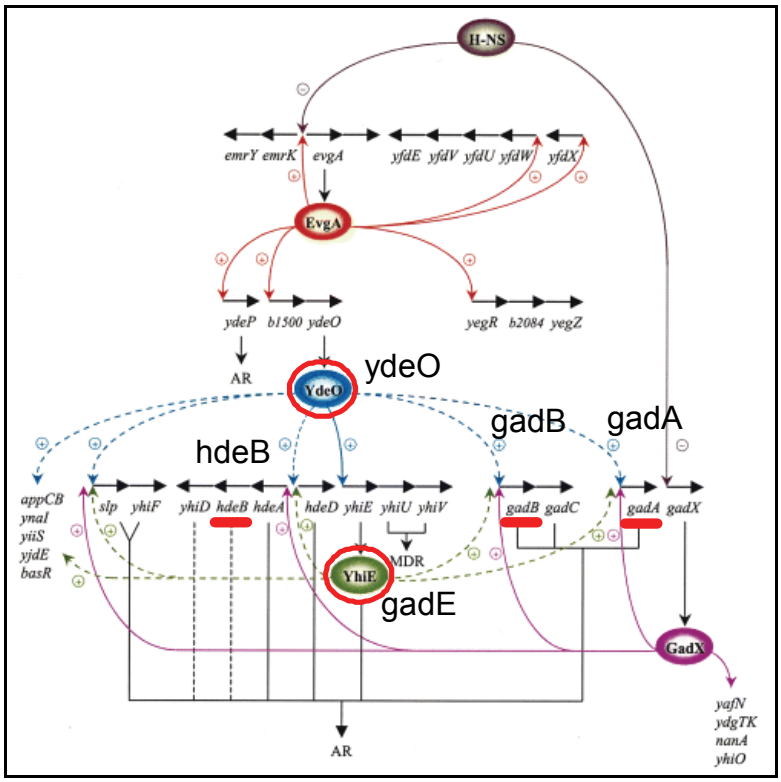

Figure 6-24 Proposed acid resistance regulatory network in *E. coli* showing the prominent roles of *ydeO* and *gadE* in controlling *gadA*, *gadB*, and *hdeB*, among other genes (modified from (Masuda and Church 2003)).

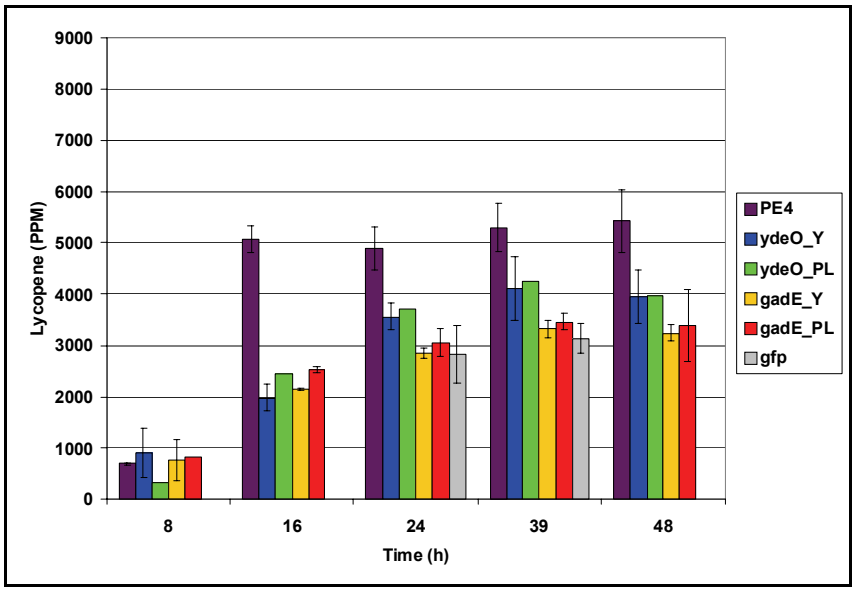

Figure 6-25 Lycopene production for PE strain transformed with the pZE21-*ydeO* plasmid, the pZE21-*gadE* plasmid, or the pZE21-*gfp* control plasmid, where the *ydeO* and *gadE* genes are under control of the Y or the P_L strength promoters and P_L is stronger (Alper, Fischer *et al.* 2005). The *gfp* gene is under control of the P_L promoter.

The data in Figure 6-25 reveals that overexpressing *ydeO* in the PE strain increases lycopene production about 30% at the Y and P_L promoter strength levels compared to the pZE

gfp plasmid control. However, overexpression of the second plasmid pZE decreases the lycopene production in all of the strains tested, likely due to the slightly increased metabolic burden of the strains carrying the pZE plasmids (Bentley, Mirjalili *et al.* 1990; Birnbaum and Bailey 1991). Again, chromosomal overexpression of the *ydeO* gene may lead to higher lycopene production compared to the PE strain, although this was not pursued in this study. Interestingly, the *ydeO* overexpression appears to makeup for some of the lost production associated with the plasmid introduction. Cell growth was generally higher in the *gadE*-overexpressing strains than in the PE strain control or the *ydeO*-overexpressing strain, although the lycopene production did not increase proportionately on a specific cell mass basis.

6.6. Summary

- Over 500 unique proteins were identified ranging from approximately +10 to -10-fold changes in expression of the five mutants compared to the PE strain, and the LC-MS^E method was sufficiently sensitive to detect differentially-expressed proteins.
- The Δ*hnr* and Δ*hnr* Δ*yliE* strains exhibit the greatest protein expression variation, similar to the gene expression results, although the Δ*gdhA*, Δ*gdhA* Δ*aceE*, and Δ*gdhA* Δ*aceE* Δ_P*yjiD* strains exhibit greater numbers of differentially expressed proteins than genes.
- Neither the strain group sharing the Δ*gdhA* deletion nor the strain group sharing the Δ*hnr* deletion exhibit additivity in their numbers of differentially expressed proteins with consecutive gene deletions, but a number of differentially expressed proteins were identified in common between the various strains.
- The Δ*gdhA* Δ*aceE* Δ_P*yjiD* strain again shares similarities to the strains sharing the Δ*hnr* deletion in protein differential expression, but differences are apparent that suggest that *yjid* overexpression and *hnr* deletion have different effects despite similar mechanisms.

- Metabolic pathways were examined for consistency in differential protein expression. Among the most interesting results, it was observed that the glycolytic and glyoxylate pathways are slightly up-regulated, whereas the TCA cycle is generally down-regulated in the various strains and especially in the Δ*hnr* and Δ*hnr* Δ*yliE* strains. Additionally, the upper oxidative pathway of the pentose phosphate pathway appears generally up-regulated in the Δ*gdhA* Δ*aceE* Δ_P*yjiD*, Δ*hnr*, and Δ*hnr* Δ*yliE* strains, but the lower non-oxidative pathway exhibits both up- and down-regulation.
- Of the 55 ribosomal proteins, 42 were measured to be differentially-expressed amongst the five mutant strains. Of the 112 total differential expression measurements for these proteins, 103 corresponded to differential down-regulation.
- RpoS (σ^S), a known target for increasing lycopene production (Becker-Hapak, Troxtel *et al.* 1997), was up-regulated in the Δ*hnr* and Δ*hnr* Δ*yliE* strains over 9-fold and 6-fold, respectively.
- WrbA, a NAD(P)H:quinone oxidoreductase that is known to reduce the quinone pool (Patridge and Ferry 2006), was one of the most up-regulated differentially expressed proteins. Overexpressing *wrbA* in the PE strain led to a doubling of the lycopene production at 8 hours and an approximate 5-15% increase at later time points as compared to the PE strain alone.
- Up-regulation of the GadA, GadB, and HdeB proteins suggested that the acid stress response may be important to the lycopene phenotype. Accordingly, *ydeO* overexpression led to an approximately 30% increase in lycopene production in the PE strain as compared to a control transformed with the blank plasmid containing the *gfp* gene.

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Chapter 7. MANUALLY INTEGRATED TRANSCRIPTOMIC AND PROTEOMIC ANALYSIS OF LYCOPENE-OVERPRODUCING *ESCHERICHIA COLI* STRAINS

7.1. Global Correlation of Transcriptomic and Proteomic Data Sets

While the genomic and proteomic expression data sets are individually useful for defining changes that are correlated with the lycopene overproduction phenotype, the true focus in biological research recently has been to integrate these 'omics' data sets into a systems understanding of the cell (Joyce and Palsson 2006). Currently, DNA microarrays allow for many more gene expression measurements than protein expression measurements detected by LC-MS proteomic platforms. Thus, analyzing the data sets individually as was done in the previous chapters is still highly valuable. Changes in the individual data sets may be significant and either not detected in other 'omics' data sets due to unique errors or limitations of each detection method, or else molecular changes may uniquely occur at one level of cellular organization only as a result of regulatory mechanisms in the cells. However, the greatest amount of information results from a complete picture of DNA, mRNA, proteins, and other molecules with the associated regulatory systems. Manually integrating the genomic and proteomic data sets in this study is a step in this direction. As discussed previously, this data integration task is accompanied by many challenges, and automatic data integration methods continue to be a limiting factor in systems biological analyses (Waters, Pounds *et al.* 2006). Methods such as those by Hwang *et al.* (2005) may be useful for such integration, although these were not pursued in this study. Nevertheless, a combined transcriptomic and proteomic approach to the data were pursued in this study as a starting point for such systems biology analysis of the Δ*gdhA,* the Δ*gdhA* Δ*aceE,* the Δ*gdhA* Δ*aceE* Δ_P*yjiD,* the Δ*hnr,* the Δ*hnr* Δ*yliE,* and the PE strains.

The genomic and proteomic data collected from the DNA microarrays and the LC-MS^E method, respectively, were first compared, and the result is shown in Figure 7-1. An overall Pearson product moment correlation coefficient of r ~ 0.02 was observed for the 1,498 gene and protein detections across all five of the mutant strains for which both macromolecules were detected. For the five individual strains alone, the correlation coefficients ranged from -0.03 for the Δ*gdhA* and Δ*hnr* Δ*yliE* strains to 0.17 for the Δ*hnr* strain. This indicates that there is generally no correlation between the mRNA and protein levels at these global levels and implies the presence of significant posttranscriptional and posttranslational regulation.

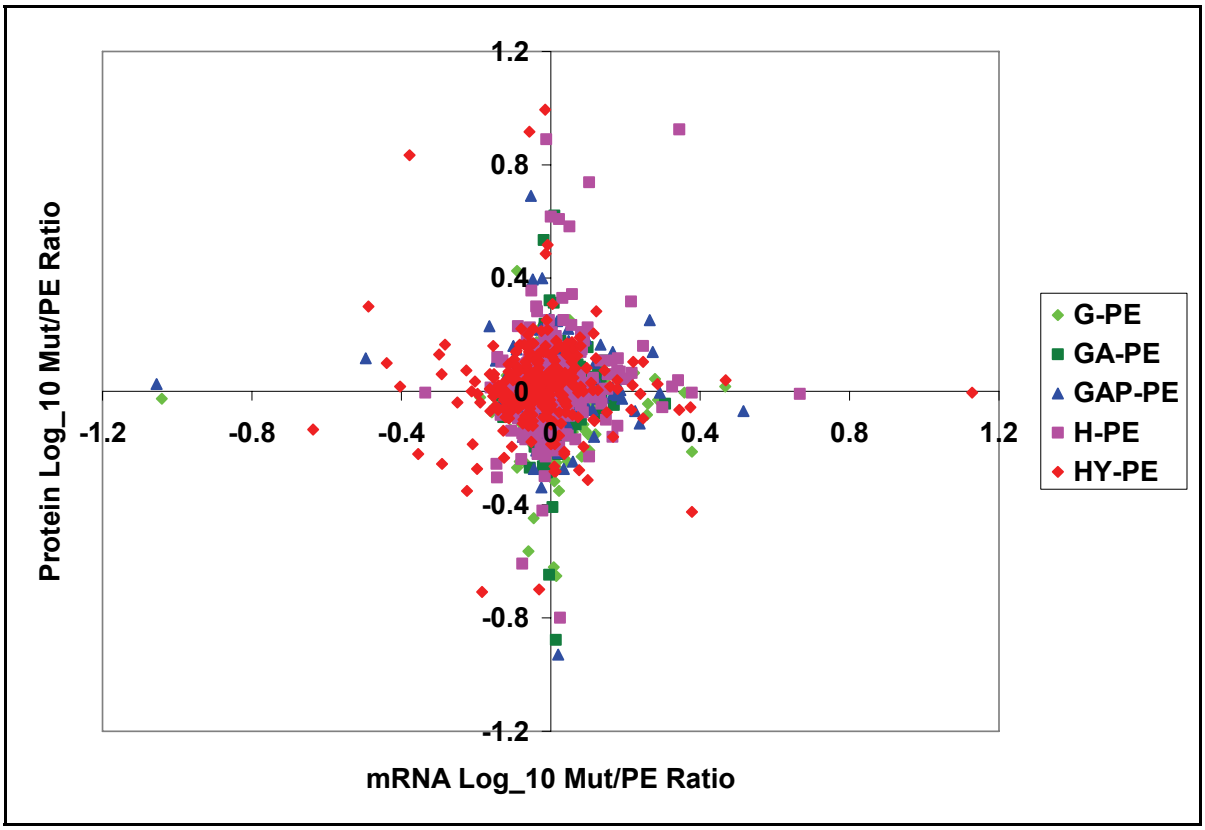

Figure 7-1 Correlation of mRNA to protein expression for 1,498 gene and protein detections across all five mutants for which both were detected. An overall Pearson product moment correlation coefficient of r ~ 0.02 was observed. Symbols are used as follows: Δ*gdhA* (G), Δ*gdhA* Δ*aceE* (GA), Δ*gdhA* Δ*aceE* Δ$_P$*yjid* (GAP), Δ*hnr* (H), and Δ*hnr* Δ*yliE* (HY).

Similarly, the correlation between gene and protein expression for differentially expressed targets was examined to determine if this subset of the data would exhibit greater

agreement. All targets were examined for which at least one of the gene or protein expression ratios was determined to yield differential expression. For a given target in this subset, if the second gene or protein expression ratio corresponding to the differentially expressed ratio was either not measured or not a numerical ratio (only measured in one of the mutant or PE strains, leading to an "infinite" $\log_{10}$ expression ratio), then that target was discarded from the set. An exception to this rule was possible for the proteomic data, however. If a proteomic ratio resulting from application of the first peptide threshold corresponding to a differentially expressed gene was either not measured or numerical, then the measured ratio resulting from the second peptide threshold was used instead if the latter met the desired criteria. The final subset of targets for which at least one ratio is indicative of differential expression and for which numerical ratios exist for both data sets appears in Table 7-1.

Again, it can be seen from the Pearson product moment correlation coefficients listed in Table 7-1 and ranging from -0.049 for the Δ*gdhA* Δ*aceE* strain to 0.190 for the Δ*hnr* strain (0.043 overall correlation) that there is no difference in correlation for this set of differentially expressed targets versus the larger transcriptomic and proteomic data sets. There is little to no correlation for each. Interestingly, the Δ*hnr* strain does appear to exhibit slightly more correlation than the other mutants. It is apparent from Table 7-1 that while some gene and protein expression ratios agree in terms of directionality and even roughly in terms of magnitude, a large number of pairs also show no correlation and even negative correlation. For example, the following (gene $\log_{10}$(Mutant/PE), protein $\log_{10}$(Mutant/PE)) pairs can be seen for the Δ*hnr* Δ*yliE* mutant: positive agreement for *yebF* (0.341, 0.776); negative agreement for *livK* (-0.636, -0.135); one significantly changing and the other not changing for *gltB* (1.128, -0.004); and disagreement for *luxS* (-0.488, 0.300).

Table 7-1 Targets for which at least one of the gene or protein expression log_{10}(Mutant/PE) ratios is indicative of differential expression and for which numerical ratios exist for both gene and protein expression data sets. Targets are sorted first by mutant and then by descending order of gene expression ratios. Positive ratios are colored red and negative ratios are colored green. Protein expression data corresponds to application of the first peptide threshold unless indicated by a "+" in the last column, in which the protein expression ratio corresponding to application of the second peptide threshold was used instead, as described in the text (Chapter 4). The Pearson product moment correlation coefficient is given for each mutant data set in addition to an overall value at the bottom of the table.

B#	Name	Function	Gene	Protein	
	Gene		Log10(Mut/PE) Ratios		
Δ*gdhA*					
b3956	ppc	phosphoenolpyruvate carboxylase	0.467	0.017	
b1656	sodB	superoxide dismutase (Fe)	0.287	-0.030	
b3670	ilvN	acetohydroxybutanoate synthase / acetolactate synthase	0.286	-0.037	+
b3530	bcsC	oxidase involved in cellulose synthesis	0.261	0.078	+
b3732	atpD	ATP synthase, F1 complex, β subunit	0.258	-0.083	
b3713	yieF	chromate reductase monomer	0.230	0.075	+
b3433	asd	aspartate semialdehyde dehydrogenase	0.223	0.065	
b0605	ahpC	AhpC component	0.150	0.048	
b3988	rpoC	RNA polymerase, β' subunit	0.130	-0.048	
b1780	yeaD	conserved protein	0.100	-0.152	
b3236	mdh	malate dehydrogenase	0.100	0.030	
b0178	hlpA	periplasmic chaperone	0.087	-0.117	
b2752	cysD	sulfate adenylyltransferase	0.083	-0.230	
b0623	cspE	transcription antiterminator and regulator of RNA stability	0.068	-0.056	
b3498	prlC	oligopeptidase A	0.045	-0.248	
b2903	gcvP	glycine decarboxylase	0.022	-0.352	
b2185	rplY	50S ribosomal subunit protein L25	0.015	-0.252	
b2898	ygfZ	folate-binding protein	0.015	-0.651	
b2913	serA	D-3-phosphoglycerate dehydrogenase	0.015	0.069	
b0440	hupB	Transcriptional dual regulator HU-β, NS1 (HU-1)	0.014	-0.091	
b3781	trxA	oxidized thioredoxin	0.014	-0.065	
b1482	osmC	osmotically inducible peroxidase OsmC	0.010	-0.256	
b1048	mdoG	periplasmic glucan (MDO) biosynthesis protein	0.008	-0.621	
b0104	guaC	GMP reductase	0.001	0.204	
b0166	dapD	tetrahydrodipicolinate succinylase subunit	0.000	0.065	
b0911	rpsA	30S ribosomal subunit protein S1	-0.001	-0.074	
b4014	aceB	malate synthase A	-0.003	0.113	
b1324	tpx	thiol peroxidase 2	-0.004	0.069	
b3303	rpsE	30S ribosomal subunit protein S5	-0.004	-0.074	
b0169	rpsB	30S ribosomal subunit protein S2	-0.006	0.069	
b3301	rplO	50S ribosomal subunit protein L15	-0.006	-0.109	

B#	Name	Function	Gene	Protein	
b3908	sodA	superoxide dismutase (Mn)	-0.007	-0.113	
b2606	rplS	50S ribosomal subunit protein L19	-0.008	-0.091	
b0026	ileS	isoleucyl-tRNA synthetase	-0.013	0.104	
b4015	aceA	isocitrate lyase monomer	-0.018	0.061	
b3341	rpsG	30S ribosomal subunit protein S7	-0.020	-0.061	
b3980	tufB	elongation factor Tu	-0.020	0.078	
b0002	thrA	aspartate kinase / homoserine dehydrogenase	-0.023	0.043	
b3320	rplC	50S ribosomal subunit protein L3	-0.025	-0.135	
b2601	aroF	2-dehydro-3-deoxyphosphoheptonate aldolase	-0.026	0.182	
b0755	gpmA	phosphoglyceromutase 1 monomer	-0.030	0.083	
b0907	serC	3-phosphoserine aminotransferase	-0.030	0.035	
b2763	cysI	sulfite reductase hemoprotein subunit	-0.030	-0.156	
b0008	talB	transaldolase B	-0.033	0.061	
b2569	lepA	elongation factor 4	-0.033	0.217	
b1716	rplT	50S ribosomal subunit protein L20	-0.034	-0.061	
b3305	rplF	50S ribosomal subunit protein L6	-0.040	-0.065	
b3559	glyS	glycyl-tRNA synthetase, β subunit	-0.044	0.096	
b3637	rpmB	50S ribosomal subunit protein L28	-0.044	-0.087	
b1136	icd	isocitrate dehydrogenase	-0.045	0.030	
b1662	ribC	riboflavin synthase	-0.046	-0.447	
b2478	dapA	dihydrodipicolinate synthase	-0.046	0.165	
b0116	lpd	E3 monomer	-0.047	-0.056	
b3829	metE	Cobalamin-independent homocysteine transmethylase	-0.047	-0.026	
b3936	rpmE	50S ribosomal subunit protein L31	-0.047	-0.191	
b0154	hemL	glutamate-1-semialdehyde aminotransferase	-0.048	0.122	
b3296	rpsD	30S ribosomal subunit protein S4	-0.054	-0.056	
b3314	rpsC	30S ribosomal subunit protein S3	-0.055	-0.083	
b3340	fusA	elongation factor G	-0.055	0.022	
b3230	rpsI	30S ribosomal subunit protein S9	-0.059	-0.065	
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase monomer	-0.060	-0.565	
b2925	fbaA	fructose bisphosphate aldolase monomer	-0.062	0.052	
b3339	tufA	elongation factor Tu	-0.063	0.074	
b3342	rpsL	30S ribosomal subunit protein S12	-0.063	-0.148	
b3319	rplD	50S ribosomal subunit protein L4	-0.068	-0.113	
b3733	atpG	ATP synthase, F1 complex, γ subunit	-0.069	-0.252	
b3984	rplA	50S ribosomal subunit protein L1	-0.069	-0.109	
b2114	metG	methionyl-tRNA synthetase	-0.072	-0.126	
b3317	rplB	50S ribosomal subunit protein L2	-0.077	-0.117	
b4202	rpsR	30S ribosomal subunit protein S18	-0.079	-0.083	
b3315	rplV	50S ribosomal subunit protein L22	-0.083	-0.248	
b0237	pepD	peptidase D	-0.090	0.426	
b3297	rpsK	30S ribosomal subunit protein S11	-0.090	-0.269	

B#	Name	Function	Gene	Protein	
b0004	thrC	threonine synthase	-0.100	0.043	
b1936	intG	predicted defective phage integrase	-0.420	0.133	+
b2023	hisH	imidazole glycerol phosphate synthase, HisH subunit	-1.041	-0.026	
Pearson product moment correlation coefficient r ~ -0.007					
Δ*gdhA* Δ*aceE*					
b1415	aldA	aldehyde dehydrogenase A, NAD-linked	0.130	-0.078	
b2515	ispG	1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase	0.099	0.156	
b1716	rplT	50S ribosomal subunit protein L20	0.051	-0.065	
b0116	lpd	E3 monomer	0.046	-0.048	
b0727	sucB	SucB-lipoate	0.042	-0.048	
b1136	icd	isocitrate dehydrogenase	0.031	0.026	
b4143	groL	chaperone Hsp60, peptide-dependent ATPase, heat shock protein	0.030	-0.030	
b4015	aceA	isocitrate lyase monomer	0.025	0.039	
b4014	aceB	malate synthase A	0.023	0.061	
b2606	rplS	50S ribosomal subunit protein L19	0.020	-0.148	
b3908	sodA	superoxide dismutase (Mn)	0.020	-0.100	
b0002	thrA	aspartate kinase / homoserine dehydrogenase	0.018	0.048	
b2913	serA	D-3-phosphoglycerate dehydrogenase	0.018	0.074	
b4384	deoD	guanosine phosphorylase [multifunctional]	0.017	0.117	
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase monomer	0.014	-0.877	
b3164	pnp	polynucleotide phosphorylase monomer	0.014	-0.091	
b2905	gcvT	aminomethyltransferase	0.010	0.621	
b3314	rpsC	30S ribosomal subunit protein S3	0.010	-0.052	
b2818	argA	N-acetylglutamate synthase	0.008	0.313	
b2185	rplY	50S ribosomal subunit protein L25	0.007	-0.122	
b3347	fkpA	peptidyl-prolyl cis-trans isomerase; in protein folding	0.005	0.156	
b3231	rplM	50S ribosomal subunit protein L13	0.000	-0.056	
b3308	rplE	50S ribosomal subunit protein L5	-0.001	-0.069	
b0008	talB	transaldolase B	-0.002	0.030	
b0726	sucA	subunit of E1(0) component of 2-oxoglutarate dehydrogenase	-0.002	-0.096	
b3301	rplO	50S ribosomal subunit protein L15	-0.002	-0.069	
b1779	gapA	glyceraldehyde 3-phosphate dehydrogenase-A monomer	-0.003	0.026	
b0166	dapD	tetrahydrodipicolinate succinylase subunit	-0.004	0.043	
b0632	dacA	D-alanyl-D-alanine carboxypeptidase, fraction A; penicillin-binding protein5	-0.004	-0.647	
b3321	rpsJ	30S ribosomal subunit protein S10	-0.004	-0.052	
b2417	crr	N-acetylmuramic acid PTS permease	-0.006	-0.043	
b3984	rplA	50S ribosomal subunit protein L1	-0.009	-0.069	

B#	Name	Function	Gene	Protein	
b1852	zwf	glucose 6-phosphate-1-dehydrogenase	-0.010	-0.130	
b3433	asd	aspartate semialdehyde dehydrogenase	-0.010	0.126	
b4388	serB	phosphoserine phosphatase	-0.010	-0.287	
b0755	gpmA	phosphoglyceromutase 1 monomer	-0.011	-0.052	
b0440	hupB	Transcriptional dual regulator HU-β, NS1 (HU-1)	-0.013	-0.074	
b3340	fusA	elongation factor G	-0.013	0.026	
b0178	hlpA	periplasmic chaperone	-0.015	-0.078	
b3341	rpsG	30S ribosomal subunit protein S7	-0.015	-0.030	
b0683	fur	Fur-Fe+2 transcriptional dual regulator	-0.016	0.239	
b2476	purC	phosphoribosylaminoimidazole-succinocarboxamide synthase	-0.016	0.048	
b3316	rpsS	30S ribosomal subunit protein S19	-0.016	-0.239	
b0907	serC	3-phosphoserine aminotransferase	-0.017	0.039	
b3959	argB	acetylglutamate kinase monomer	-0.017	0.221	
b3319	rplD	50S ribosomal subunit protein L4	-0.018	-0.069	
b3637	rpmB	50S ribosomal subunit protein L28	-0.018	-0.083	
b1817	manX	mannose PTS permease	-0.019	0.534	
b3939	metB	O-succinylhomoserine lyase / O-succinylhomoserine(thiol)-lyase	-0.019	-0.269	
b3870	glnA	adenylyl-[glutamine synthetase]	-0.022	0.143	
b3980	tufB	elongation factor Tu	-0.023	0.052	
b0126	can	carbonic anhydrase 2 monomer	-0.025	0.152	
b2925	fbaA	fructose bisphosphate aldolase monomer	-0.025	0.043	
b3230	rpsI	30S ribosomal subunit protein S9	-0.026	-0.039	
b3342	rpsL	30S ribosomal subunit protein S12	-0.031	-0.122	
b3297	rpsK	30S ribosomal subunit protein S11	-0.034	-0.113	
b0911	rpsA	30S ribosomal subunit protein S1	-0.037	-0.052	
b2464	talA	transaldolase A	-0.041	-0.182	
b2697	alaS	alanyl-tRNA synthetase	-0.041	0.091	
b3298	rpsM	30S ribosomal subunit protein S13	-0.043	-0.061	
b3936	rpmE	50S ribosomal subunit protein L31	-0.043	-0.195	
b3317	rplB	50S ribosomal subunit protein L2	-0.046	-0.083	
b3320	rplC	50S ribosomal subunit protein L3	-0.054	-0.139	
b3315	rplV	50S ribosomal subunit protein L22	-0.055	-0.156	
b3640	dut	deoxyuridine triphosphatase	-0.056	-0.269	
b2752	cysD	sulfate adenylyltransferase	-0.061	-0.152	
b3304	rplR	50S ribosomal subunit protein L18	-0.062	-0.065	
b3305	rplF	50S ribosomal subunit protein L6	-0.078	-0.074	
b2425	cysP	thiosulfate ABC transporter	-0.127	-0.091	
b1936	intG	predicted defective phage integrase	-0.467	0.097	+
b2023	hisH	imidazole glycerol phosphate synthase, HisH subunit	-1.191	0.055	+
Pearson product moment correlation coefficient r ~ -0.049					

B#	Name	Function	Gene	Protein	
Δ*gdhA* Δ*aceE* Δ$_P$*yjiD*					
b3870	glnA	adenylyl-[glutamine synthetase]	0.273	0.139	
b4376	osmY	hyperosmotically inducible periplasmic protein	0.265	0.252	
b3732	atpD	ATP synthase, F1 complex, β subunit	0.226	-0.069	
b1740	nadE	NAD synthetase, NH₃-dependent	0.160	0.122	
b2551	glyA	serine hydroxymethyltransferase	0.118	0.030	
b0728	sucC	succinyl-CoA synthetase, β subunit	0.072	-0.039	
b4143	groL	chaperone Hsp60, peptide-dependent ATPase, heat shock protein	0.069	-0.052	
b4226	ppa	inorganic pyrophosphatase	0.051	0.052	
b3959	argB	acetylglutamate kinase monomer	0.048	0.178	
b1539	ydfG	3-hydroxy acid dehydrogenase monomer	0.047	0.221	
b2898	ygfZ	folate-binding protein	0.035	-0.274	
b3984	rplA	50S ribosomal subunit protein L1	0.027	-0.083	
b0002	thrA	aspartate kinase / homoserine dehydrogenase	0.026	0.039	
b3433	asd	aspartate semialdehyde dehydrogenase	0.022	0.109	
b0004	thrC	threonine synthase	0.020	0.052	
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase monomer	0.020	-0.929	
b3065	rpsU	30S ribosomal subunit protein S21	0.017	0.139	
b3301	rplO	50S ribosomal subunit protein L15	0.017	-0.061	
b0605	ahpC	AhpC component	0.013	0.039	
b0767	pgl	6-phosphogluconolactonase	0.013	0.130	
b3230	rpsI	30S ribosomal subunit protein S9	0.013	-0.043	
b1136	icd	isocitrate dehydrogenase	0.010	-0.026	
b4015	aceA	isocitrate lyase monomer	0.006	0.039	
b2025	hisF	imidazole glycerol phosphate synthase, HisF subunit	0.003	0.169	
b3314	rpsC	30S ribosomal subunit protein S3	0.003	-0.061	
b4014	aceB	malate synthase A	0.002	0.061	
b2421	cysM	cysteine synthase B	0.001	0.191	
b0073	leuB	3-isopropylmalate dehydrogenase	0.000	-0.078	
b4005	purD	phosphoribosylamine-glycine ligase	-0.004	0.195	
b0726	sucA	subunit of E1(0) component of 2-oxoglutarate dehydrogenase	-0.005	-0.078	
b1482	osmC	osmotically inducible peroxidase OsmC	-0.005	0.122	
b2925	fbaA	fructose bisphosphate aldolase monomer	-0.005	0.039	
b3306	rpsH	30S ribosomal subunit protein S8	-0.007	-0.078	
b3295	rpoA	RNA polymerase, α subunit	-0.013	-0.039	
b3297	rpsK	30S ribosomal subunit protein S11	-0.016	-0.156	
b3829	metE	Cobalamin-independent homocysteine transmethylase	-0.019	-0.030	
b0907	serC	3-phosphoserine aminotransferase	-0.020	0.035	
b1779	gapA	glyceraldehyde 3-phosphate dehydrogenase-A monomer	-0.022	-0.030	
b3342	rpsL	30S ribosomal subunit protein S12	-0.022	-0.109	

B#	Name	Function	Gene	Protein	
b1654	grxD	glutaredoxin 4	-0.023	0.087	
b2905	gcvT	aminomethyltransferase	-0.023	0.400	
b3347	fkpA	peptidyl-prolyl cis-trans isomerase; in protein folding	-0.023	0.104	
b0116	lpd	E3 monomer	-0.024	-0.039	
b2606	rplS	50S ribosomal subunit protein L19	-0.031	-0.087	
b3315	rplV	50S ribosomal subunit protein L22	-0.037	-0.100	
b0166	dapD	tetrahydrodipicolinate succinylase subunit	-0.038	0.078	
b3319	rplD	50S ribosomal subunit protein L4	-0.038	-0.078	
b0118	acnB	aconitase B	-0.039	-0.056	
b3316	rpsS	30S ribosomal subunit protein S19	-0.039	-0.208	
b3313	rplP	50S ribosomal subunit protein L16	-0.040	-0.039	
b3317	rplB	50S ribosomal subunit protein L2	-0.040	-0.056	
b3321	rpsJ	30S ribosomal subunit protein S10	-0.041	-0.043	
b3341	rpsG	30S ribosomal subunit protein S7	-0.044	-0.039	
b2530	iscS	cysteine desulfurase monomer	-0.052	0.056	
b1004	wrbA	WrbA monomer	-0.053	0.691	
b2751	cysN	sulfate adenylyltransferase	-0.054	-0.087	
b4177	purA	adenylosuccinate synthetase	-0.056	-0.065	
b0426	yajQ	nucleotide binding protein	-0.057	0.065	
b1288	fabI	enoyl-ACP reductase (NAD[P]H) [multifunctional]	-0.060	-0.069	
b3320	rplC	50S ribosomal subunit protein L3	-0.060	-0.061	
b3339	tufA	elongation factor Tu	-0.070	0.030	
b0440	hupB	Transcriptional dual regulator HU-β, NS1 (HU-1)	-0.075	-0.161	
b0911	rpsA	30S ribosomal subunit protein S1	-0.082	-0.039	
b3305	rplF	50S ribosomal subunit protein L6	-0.090	-0.056	
b0172	frr	ribosome recycling factor	-0.099	0.074	
b3556	cspA	CspA transcriptional activator	-0.100	0.161	
b2417	crr	N-acetylmuramic acid PTS permease	-0.118	0.056	
b4025	pgi	phosphoglucose isomerase	-0.147	0.109	
b1936	intG	predicted defective phage integrase	-0.427	0.094	+
b2023	hisH	imidazole glycerol phosphate synthase, HisH subunit	-1.055	0.026	
Pearson product moment correlation coefficient r ~ 0.011					
Δ*hnr*					
b3212	gltB	glutamate synthase, large subunit	0.667	-0.009	
b3389	aroB	3-dehydroquinate synthase	0.378	-0.004	
b1004	wrbA	WrbA monomer	0.344	0.925	
b2523	pepB	aminopeptidase B	0.341	0.039	
b4362	dnaT	primosome	0.332	0.270	+
b0674	asnB	asparagine synthetase B	0.325	0.017	
b3870	glnA	adenylyl-[glutamine synthetase]	0.299	-0.056	
b3498	prlC	oligopeptidase A	0.247	0.161	

B#	Name	Function	Gene	Protein	
b2762	cysH	3'-phospho-adenylylsulfate reductase	0.229	0.131	+
b0801	ybiC	predicted dehydrogenase	0.228	-0.084	+
b2316	accD	acetyl-CoA carboxylase	0.217	0.065	
b2073	yegL	conserved protein	0.215	0.317	
b2283	nuoG	NADH:ubiquinone oxidoreductase, chain G	0.207	0.043	
b3671	ilvB	acetohydroxybutanoate synthase / acetolactate synthase	0.202	0.043	
b0917	ycaR	conserved protein	0.201	0.091	+
b3433	asd	aspartate semialdehyde dehydrogenase	0.185	0.069	
b0078	ilvH	acetolactate synthase / acetohydroxybutanoate synthase	0.180	0.117	
b2022	hisB	imidazoleglycerol-phosphate dehydratase / histidinol-phosphatase	0.179	-0.122	
b1765	ydjA	predicted oxidoreductase	0.178	0.074	
b4122	fumB	fumarase B monomer	0.165	-0.161	
b0440	hupB	Transcriptional dual regulator HU-β, NS1 (HU-1)	0.103	-0.230	
b3509	hdeB	acid stress chaperone	0.103	0.738	
b0854	potF	putrescine ABC transporter	0.099	0.226	
b3959	argB	acetylglutamate kinase monomer	0.089	0.178	
b3192	yrbC	predicted ABC-type organic solvent transporter	0.086	0.208	
b3458	livK	leucine binding protein of the high-affinity branched-chain amino acid transport system	0.086	-0.074	
b1740	nadE	NAD synthetase, NH₃-dependent	0.084	0.156	
b4025	pgi	phosphoglucose isomerase	0.081	0.208	
b3908	sodA	superoxide dismutase (Mn)	0.079	0.048	
b3303	rpsE	30S ribosomal subunit protein S5	0.070	-0.035	
b1415	aldA	aldehyde dehydrogenase A, NAD-linked	0.066	-0.169	
b0237	pepD	peptidase D	0.057	0.343	
b3612	gpmM	phosphoglycerate mutase, cofactor independent	0.055	0.096	
b0154	hemL	glutamate-1-semialdehyde aminotransferase	0.051	0.130	
b4384	deoD	guanosine phosphorylase [multifunctional]	0.051	0.161	
b3201	lptB	LptA/LptB/LptC ABC transporter	0.050	0.582	
b0426	yajQ	nucleotide binding protein	0.046	0.156	
b0473	htpG	HtpG monomer	0.044	0.117	
b3984	rplA	50S ribosomal subunit protein L1	0.042	-0.078	
b3829	metE	Cobalamin-independent homocysteine transmethylase	0.038	-0.035	
b2551	glyA	serine hydroxymethyltransferase	0.034	0.061	
b3114	tdcE	2-ketobutyrate formate-lyase / pyruvate formate-lyase	0.034	0.252	
b0767	pgl	6-phosphogluconolactonase	0.031	0.330	
b1324	tpx	thiol peroxidase 2	0.027	-0.043	
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase monomer	0.025	-0.799	
b1493	gadB	glutamate decarboxylase B subunit	0.022	0.608	
b3609	secB	Sec Protein Secretion Complex	0.018	0.100	

B#	Name	Function	Gene	Protein	
b2747	ispD	4-diphosphocytidyl-2C-methyl-D-erythritol synthetase monomer	0.017	0.109	
b1412	azoR	NADH-azoreductase, FMN-dependent	0.016	0.178	
b1662	ribC	riboflavin synthase	0.014	0.195	
b3314	rpsC	30S ribosomal subunit protein S3	0.014	-0.061	
b4244	pyrI	aspartate carbamoyltransferase, PyrI subunit	0.012	0.035	
b3774	ilvC	acetohydroxy acid isomeroreductase	0.011	-0.035	
b4226	ppa	inorganic pyrophosphatase	0.011	0.104	
b3315	rplV	50S ribosomal subunit protein L22	0.007	-0.187	
b2569	lepA	elongation factor 4	0.003	0.208	
b3339	tufA	elongation factor Tu	0.001	0.043	
b3517	gadA	glutamate decarboxylase A subunit	0.000	0.617	
b2153	folE	GTP cyclohydrolase I monomer	-0.002	0.096	
b3317	rplB	50S ribosomal subunit protein L2	-0.002	-0.078	
b0014	dnaK	chaperone Hsp70; DNA biosynthesis; autoregulated heat shock proteins	-0.003	0.065	
b3316	rpsS	30S ribosomal subunit protein S19	-0.004	-0.169	
b1136	icd	isocitrate dehydrogenase	-0.005	-0.165	
b2266	elaB	conserved protein	-0.005	0.252	
b3994	thiC	thiamin biosynthesis protein ThiC	-0.006	0.109	
b2415	ptsH	HPr	-0.008	-0.208	
b3357	crp	CRP transcriptional dual regulator	-0.008	-0.069	
b4015	aceA	isocitrate lyase monomer	-0.009	0.143	
b3340	fusA	elongation factor G	-0.010	0.035	
b3306	rpsH	30S ribosomal subunit protein S8	-0.011	-0.113	
b0726	sucA	subunit of E1(0) component of 2-oxoglutarate dehydrogenase	-0.012	-0.130	
b4383	deoB	phosphopentomutase	-0.012	0.890	
b0932	pepN	aminopeptidase N	-0.014	0.113	
b3342	rpsL	30S ribosomal subunit protein S12	-0.014	-0.122	
b0884	infA	protein chain initiation factor IF-1	-0.016	-0.300	
b0911	rpsA	30S ribosomal subunit protein S1	-0.016	-0.048	
b1637	tyrS	tyrosyl-tRNA synthetase	-0.017	0.208	
b0812	dps	stationary phase nucleoid protein- sequesters iron, protects DNA damage	-0.020	0.165	
b2606	rplS	50S ribosomal subunit protein L19	-0.020	-0.143	
b3320	rplC	50S ribosomal subunit protein L3	-0.020	-0.122	
b3805	hemC	hydroxymethylbilane synthase	-0.022	-0.421	
b2417	crr	N-acetylmuramic acid PTS permease	-0.023	0.104	
b3304	rplR	50S ribosomal subunit protein L18	-0.025	-0.109	
b3176	glmM	phosphoglucosamine mutase	-0.027	-0.187	
b2185	rplY	50S ribosomal subunit protein L25	-0.031	-0.104	
b2779	eno	degradosome	-0.032	0.039	
b3321	rpsJ	30S ribosomal subunit protein S10	-0.032	0.048	

B#	Name	Function	Gene	Protein	
b0720	gltA	citrate synthase monomer	-0.035	-0.122	
b2464	talA	transaldolase A	-0.035	0.122	
b3065	rpsU	30S ribosomal subunit protein S21	-0.035	-0.221	
b1262	trpC	indole-3-glycerol phosphate synthase, phosphoribosylanthranilate isomerase	-0.037	0.135	
b2400	gltX	glutamyl-tRNA synthetase	-0.038	0.113	
b3956	ppc	phosphoenolpyruvate carboxylase	-0.039	-0.056	
b3305	rplF	50S ribosomal subunit protein L6	-0.040	-0.026	
b2925	fbaA	fructose bisphosphate aldolase monomer	-0.042	0.104	
b0116	lpd	E3 monomer	-0.047	-0.052	
b3301	rplO	50S ribosomal subunit protein L15	-0.047	-0.074	
b3919	tpiA	triose phosphate isomerase monomer	-0.050	0.122	
b0683	fur	Fur-Fe+2 transcriptional dual regulator	-0.052	0.356	
b1761	gdhA	glutamate dehydrogenase	-0.052	-0.152	
b1850	eda	multifunctional 2-keto-3-deoxygluconate 6-phosphate aldolase and 2-keto-4-hydroxyglutarate aldolase and oxaloacetate decarboxylase	-0.055	0.087	
b2962	yggX	protein that protects iron-sulfur proteins against oxidative damage	-0.056	0.226	
b4177	purA	adenylosuccinate synthetase	-0.057	-0.061	
b0928	aspC	aspartate aminotransferase, PLP-dependent	-0.059	-0.052	
b3414	nfuA	iron-sulfur cluster scaffold protein	-0.059	0.113	
b2323	fabB	KASI	-0.060	0.074	
b2514	hisS	histidyl-tRNA synthetase	-0.063	0.100	
b4014	aceB	malate synthase A	-0.064	0.191	
b0004	thrC	threonine synthase	-0.066	0.056	
b3936	rpmE	50S ribosomal subunit protein L31	-0.068	-0.169	
b0118	acnB	aconitase B	-0.073	-0.161	
b1676	pykF	pyruvate kinase I monomer	-0.073	0.026	
b0894	dmsA	dimethyl sulfoxide reductase, chain A	-0.076	-0.608	
b3297	rpsK	30S ribosomal subunit protein S11	-0.076	-0.148	
b2530	iscS	cysteine desulfurase monomer	-0.085	0.056	
b0729	sucD	succinyl-CoA synthetase, α subunit	-0.088	-0.096	
b4349	hsdM	host modification; DNA methylase M	-0.088	0.230	
b0755	gpmA	phosphoglyceromutase 1 monomer	-0.095	0.087	
b0114	aceE	subunit of E1p component of pyruvate dehydrogenase complex	-0.103	0.030	
b3560	glyQ	glycyl-tRNA synthetase, α subunit	-0.106	-0.139	
b0754	aroG	2-dehydro-3-deoxyphosphoheptonate aldolase	-0.111	-0.039	
b3980	tufB	elongation factor Tu	-0.129	0.109	
b0172	frr	ribosome recycling factor	-0.132	0.104	
b3236	mdh	malate dehydrogenase	-0.134	-0.083	
b1241	adhE	PFL-deactivase / alcohol dehydrogenase / acetaldehyde dehydrogenase	-0.143	0.122	
b2093	gatB	galactitol PTS permease	-0.144	-0.304	

B#	Name	Function	Gene	Protein	
b2819	recD	DNA helicase, ATP-dependent dsDNA/ssDNA exonuclease V subunit, ssDNA endonuclease	-0.145	-0.256	
b0605	ahpC	AhpC component	-0.336	-0.004	
Pearson product moment correlation coefficient r ~ 0.190					
Δ*hnr* Δ*yliE*					
b3212	gltB	glutamate synthase, large subunit	1.128	-0.004	
b4362	dnaT	primosome	0.590	0.116	+
b0917	ycaR	conserved protein	0.492	0.098	+
b3931	hslU	ATPase component of the HslVU protease	0.468	0.039	
b2674	nrdI	conserved protein that may stimulate ribonucleotide reductase	0.378	-0.426	
b1881	cheZ	cytosolic phosphatase of the chemotaxis signal transduction complex	0.374	-0.056	
b2523	pepB	aminopeptidase B	0.344	-0.065	
b1847	yebF	predicted protein	0.341	0.776	+
b0078	ilvH	acetolactate synthase / acetohydroxybutanoate synthase	0.286	0.026	
b0801	ybiC	predicted dehydrogenase	0.283	-0.079	+
b0811	glnH	glutamine ABC transporter	0.247	0.104	
b1765	ydjA	predicted oxidoreductase	0.247	-0.096	
b2283	nuoG	NADH:ubiquinone oxidoreductase, chain G	0.219	0.022	
b3513	mdtE	MdtEF-TolC multidrug efflux transport system	0.155	0.128	+
b3908	sodA	superoxide dismutase (Mn)	0.144	0.074	
b1662	ribC	riboflavin synthase	0.099	-0.313	
b2699	recA	DNA strand exchange, recombination protein w/ protease, nuclease activity	0.080	0.161	
b3959	argB	acetylglutamate kinase monomer	0.078	0.191	
b1324	tpx	thiol peroxidase 2	0.073	-0.091	
b2551	glyA	serine hydroxymethyltransferase	0.071	0.126	
b2764	cysJ	sulfite reductase flavoprotein subunit	0.066	0.083	
b3294	rplQ	50S ribosomal subunit protein L17	0.061	0.048	
b4384	deoD	guanosine phosphorylase [multifunctional]	0.057	0.174	
b3295	rpoA	RNA polymerase, α subunit	0.049	0.056	
b3781	trxA	oxidized thioredoxin	0.049	0.126	
b0932	pepN	aminopeptidase N	0.041	0.130	
b3984	rplA	50S ribosomal subunit protein L1	0.039	-0.043	
b1479	maeA	malate dehydrogenase, NAD-requiring	0.036	-0.213	
b1656	sodB	superoxide dismutase (Fe)	0.036	-0.221	
b2606	rplS	50S ribosomal subunit protein L19	0.023	-0.074	
b0440	hupB	Transcriptional dual regulator HU-β, NS1 (HU-1)	0.022	-0.156	
b1654	grxD	glutaredoxin 4	0.016	0.083	
b0812	dps	stationary phase nucleoid protein- sequesters iron, protects DNA damage	0.015	0.178	

B#	Name	Function	Gene	Protein	
b3942	katG	hydroperoxidase I	0.013	-0.282	
b3169	nusA	transcription termination/antitermination L factor	0.011	0.109	
b3560	glyQ	glycyl-tRNA synthetase, α subunit	0.011	-0.187	
b1136	icd	isocitrate dehydrogenase	0.010	-0.282	
b2747	ispD	4-diphosphocytidyl-2C-methyl-D-erythritol synthetase monomer	0.010	-0.265	
b0767	pgl	6-phosphogluconolactonase	0.005	0.308	
b0726	sucA	subunit of E1(0) component of 2-oxoglutarate dehydrogenase	0.001	-0.187	
b3774	ilvC	acetohydroxy acid isomeroreductase	-0.002	-0.030	
b3339	tufA	elongation factor Tu	-0.005	-0.096	
b1412	azoR	NADH-azoreductase, FMN-dependent	-0.008	0.217	
b4383	deoB	phosphopentomutase	-0.008	0.517	
b1062	pyrC	dihydroorotase	-0.009	0.061	
b0104	guaC	GMP reductase	-0.011	0.252	
b2266	elaB	conserved protein	-0.014	0.486	
b2312	purF	amidophosphoribosyl transferase	-0.014	0.161	
b0660	ybeZ	predicted protein with nucleoside triphosphate hydrolase domain	-0.015	0.995	
b4014	aceB	malate synthase A	-0.020	0.139	
b0605	ahpC	AhpC component	-0.022	-0.039	
b3751	rbsB	ribose ABC transporter	-0.025	0.213	
b4000	hupA	Transcriptional dual regulator HU-α (HU-2)	-0.026	0.039	
b0134	panB	3-methyl-2-oxobutanoate hydroxymethyltransferase monomer	-0.031	-0.699	
b2417	crr	N-acetylmuramic acid PTS permease	-0.034	0.130	
b4226	ppa	inorganic pyrophosphatase	-0.034	0.056	
b3871	typA	protein possibly involved in LPS biosynthesis and host colonization	-0.035	0.100	
b3231	rplM	50S ribosomal subunit protein L13	-0.037	0.074	
b2464	talA	transaldolase A	-0.047	0.169	
b2480	bcp	thiol peroxidase	-0.047	0.169	
b2962	yggX	protein that protects iron-sulfur proteins against oxidative damage	-0.047	0.221	
b2518	ndk	nucleoside diphosphate kinase [multifunctional]	-0.048	-0.122	
b0623	cspE	transcription antiterminator and regulator of RNA stability	-0.051	0.065	
b2021	hisC	histidinol-phosphate aminotransferase	-0.052	-0.178	
b3829	metE	Cobalamin-independent homocysteine transmethylase	-0.055	-0.061	
b0426	yajQ	nucleotide binding protein	-0.056	0.130	
b3517	gadA	glutamate decarboxylase A subunit	-0.057	0.916	
b2925	fbaA	fructose bisphosphate aldolase monomer	-0.058	0.139	
b3936	rpmE	50S ribosomal subunit protein L31	-0.060	-0.122	
b2889	idi	isopentenyl diphosphate isomerase	-0.061	0.200	
b4245	pyrB	aspartate carbamoyltransferase, PyrB subunit	-0.062	-0.030	

B#	Name	Function	Gene	Protein	
b0166	dapD	tetrahydrodipicolinate succinylase subunit	-0.065	-0.100	
b3980	tufB	elongation factor Tu	-0.068	-0.087	
b1260	trpA	tryptophan synthase, α subunit	-0.069	-0.109	
b2913	serA	α-ketoglutarate reductase / D-3-phosphoglycerate dehydrogenase	-0.069	0.039	
b1712	ihfA	integration host factor (IHF), α subunit	-0.071	0.165	
b3317	rplB	50S ribosomal subunit protein L2	-0.074	-0.043	
b1779	gapA	glyceraldehyde 3-phosphate dehydrogenase-A monomer	-0.079	-0.043	
b2400	gltX	glutamyl-tRNA synthetase	-0.079	0.221	
b2296	ackA	propionate kinase / acetate kinase	-0.083	0.165	
b4025	pgi	phosphoglucose isomerase	-0.091	0.143	
b0071	leuD	isopropylmalate isomerase	-0.092	0.087	
b2153	folE	GTP cyclohydrolase I monomer	-0.093	0.139	
b3298	rpsM	30S ribosomal subunit protein S13	-0.093	0.091	
b2498	upp	uracil phosphoribosyltransferase	-0.098	0.061	
b3919	tpiA	triose phosphate isomerase monomer	-0.098	0.113	
b0928	aspC	aspartate aminotransferase, PLP-dependent	-0.112	-0.096	
b0072	leuC	isopropylmalate isomerase	-0.113	0.096	
b3342	rpsL	30S ribosomal subunit protein S12	-0.121	-0.091	
b1761	gdhA	glutamate dehydrogenase	-0.125	-0.235	
b0116	lpd	E3 monomer	-0.126	-0.030	
b0729	sucD	succinyl-CoA synthetase, α subunit	-0.127	-0.139	
b1676	pykF	pyruvate kinase I monomer	-0.138	0.000	
b3433	asd	aspartate semialdehyde dehydrogenase	-0.140	-0.061	
b3304	rplR	50S ribosomal subunit protein L18	-0.152	0.061	
b0172	frr	ribosome recycling factor	-0.154	0.161	
b3315	rplV	50S ribosomal subunit protein L22	-0.162	-0.069	
b3556	cspA	CspA transcriptional activator	-0.179	-0.070	+
b2093	gatB	galactitol PTS permease	-0.184	-0.708	
b3305	rplF	50S ribosomal subunit protein L6	-0.189	-0.039	
b0118	acnB	aconitase B	-0.197	-0.274	
b4177	purA	adenylosuccinate synthetase	-0.209	-0.187	
b0755	gpmA	phosphoglyceromutase 1 monomer	-0.212	0.000	
b0882	clpA	ATP-dependent protease specificity component and chaperone	-0.224	-0.352	
b3609	secB	Sec Protein Secretion Complex	-0.226	0.074	
b0590	fepD	Ferric Enterobactin Transport System	-0.259	0.075	+
b3498	prlC	oligopeptidase A	-0.283	0.165	
b2926	pgk	phosphoglycerate kinase	-0.299	0.130	
b1004	wrbA	WrbA monomer	-0.378	0.834	
b1241	adhE	PFL-deactivase / alcohol dehydrogenase / acetaldehyde dehydrogenase	-0.403	0.017	
b1936	intG	predicted defective phage integrase	-0.445	0.177	+

B#	Name	Function	Gene	Protein	
b1817	manX	mannose PTS permease	-0.462	-0.036	+
b2687	luxS	S-ribosylhomocysteine lyase (AI-2 synthesis protein)	-0.488	0.300	
b3458	livK	leucine binding protein of high-affinity branched-chain AA transport system	-0.636	-0.135	
Pearson product moment correlation coefficient r ~ -0.013					
Overall Pearson product moment correlation coefficient r ~ 0.043					

This lack of correlation between mRNA and protein levels when integrating such data sets in systems biology studies has become a familiar feature. Gygi *et al.* (1999) first examined the correlation between mRNA and protein abundance in yeast, specifically focusing on a group of 106 genes. They found that when they excluded the 11 most highly abundant proteins and focused on the 40 to 95 lowest-expressed proteins, the Pearson product moment correlation coefficient fell to r ~ 0.1 to 0.4, with protein expression coded for by mRNA of comparable abundance varying up to 30-fold and mRNA levels coding for proteins of comparable expression levels varied by as much as 20-fold. Ideker and Thorsson *et al.* (2001) found a Pearson product moment correlation coefficient of r ~ 0.61 (289 proteins) between mRNA and protein levels in yeast for specific pathways related to galactose metabolism, a relatively higher correlation. In a related study, though, Griffin *et al.* (2002) found only a 0.21 Spearman rank correlation value for 245 proteins in yeast, although correlation within galactose utilization and glycolysis were higher. Hwang *et al.* (2005) also studied yeast galactose metabolism and found that the correlation coefficients between gene expression, protein-DNA interaction, and protein-protein interaction data sets were all < 0.3. Similarly, Nie *et al.* (2006) found that only about 20-30% of the total variation in protein abundance could be explained by mRNA abundance alone in *Desulfovibrio vulgaris*. Indeed, because of different noise characteristics of each measurement technology, p values demonstrating significant expression may be low enough to conclude significance in one measurement but not in another. For real high-throughput data, highly

correlated true positives comprise a small proportion of the data, and variable true negatives are not highly correlated. Thus, data sets from different technologies will be largely uncorrelated (Jansen, Yu *et al.* 2003).

Other studies in higher eukaryotes have affirmed this assertion that protein abundance cannot readily be inferred from mRNA levels alone. For example, comparison of mRNA and protein levels for 98 genes across 76 neoplastic and normal lung tissues resulted in concordance in expression for only 17% of the genes and an overall correlation of $r \sim -0.025$ taking the average levels of mRNA or protein among all samples (Chen, Gharib *et al.* 2002). Tian *et al.* (2004) found an overall correlation between mRNA and protein of 0.54 for 144 total data points, but when only significant changes in the mRNA or protein levels were considered, 79% of the changes occurred in only one of the data sets and not both. Among 150 proteins determined to be altered, 76% changed in the same direction as the corresponding gene. However, they concluded that only 40% of the changes in protein abundance could be attributed to differential gene expression.

There are multiple explanation for the low correlation between mRNA and protein abundance (Waters, Pounds *et al.* 2006). First, there are many sources of variability with the global measurements of genes and proteins such as differences in sensitivities, dynamic ranges, and identification methods. Analytical noise is strongly dependent upon the expression level, generally decreasing with increasing mRNA abundance (Tu, Stolovitzky *et al.* 2002). Coefficients of variation for technical replicates of LC-MS-based analyses, though, can be comparable to the reproducibilities of current microarray technologies (Adkins, Monroe *et al.* 2005). From a biological standpoint, protein abundance relies not only on mRNA levels, but also on various factors such as mRNA stability, translational control, and protein degradation. If

the mRNA and protein expression changes show a high degree of correlation, this is likely indicative of transcriptional control. If the expression changes demonstrate low or even negative correlation, this may be indicative of posttranscriptional regulation and even possibly negative feedback regulation. Ribonucleases and the RNA degradosome complex have been documented as being important to *E. coli* mRNA degradation (Rauhut and Klug 1999; Carpousis 2007). Translational initiation is emerging as a far-reaching mechanism for regulating protein levels (Pradet-Balade, Boulme *et al.* 2001), and translational efficiencies have been seen to be a great contributor to noise in gene regulatory systems, especially for highly expressed proteins (Thattai and van Oudenaarden 2001). Protein stability and proteolysis also play highly important roles in regulating protein levels (Gottesman 2003), keeping basal levels of regulatory proteins low and rapidly removing proteins when they are no longer needed. Furthermore, mRNAs and proteins have quite different half-lives of 0.1-10 h and 0.5-500 h, respectively (Waters, Pounds *et al.* 2006). All of these factors help to explain the observed discrepancy between mRNA and protein abundances.

The correlation between transcriptomic and proteomic data in this study is likely lower than some of the above examples since the genes are distributed across all different functional groups instead of focused upon specific pathways or groups of interest. Indeed, studies such as those by Greenbaum *et al.* (2002) and Cox *et al.* (2005) have described greater agreement between mRNA and protein abundance in specific structural or functional categories than in global data sets. Also, similar to the observation of Tian *et al.* (2004), directionality may be conserved in mRNA and protein expression changes even when the data are not highly correlated. As such, mRNA and protein "agreement" in terms of directionality within specific pathways was manually examined, and these results are presented in the next section.

As a side note, there are a few examples of targets that were selected as differentially expressed in both the genomic and proteomic expression data, and they are listed in Table 7-2. Again, it can be seen that there are some targets for which the mRNA and protein expression ratios agree and some that exhibit discrepancies. The *wrbA* target, for example, exhibits strong up-regulation in both data sets in the Δ*hnr* strain, lending more support for the decision to overexpress this target as discussed in the previous chapter. This agreement appears to indicate that the *wrbA* target is controlled at the transcriptional level; however, the Δ*hnr* Δ*yliE* strain shows $\log_{10}$(Mutant/PE) ratios of -0.38 and +0.834, respectively. Given that the WrbA protein is also up-regulated in the Δ*gdhA* Δ*aceE* Δ*pyjiD* strain, it seems possible that the low transcriptional measurement could be due to error. More experimental verification of this is required. As for the other targets in Table 7-2, directionality trends also hold for the *acnB*, *clpA*, and *livK* targets, but there is negative correlation for the *adhE*, *glnA*, and *nrdl* targets. Reasons for these specific agreements and differences are likely related to those outlined in the above discussion.

Table 7-2 Targets for which both the gene or protein expression log_10(Mutant/PE) ratios are indicative of differential expression. Positive ratios are colored red and negative ratios are colored green. Protein expression data corresponds to application of the first peptide threshold as explained previously.

B#	Name	Function	DE Gene	DE Protein
	Gene		Log10(Mut/PE) Ratios	
Δ*hnr*				
b1004	wrbA	WrbA monomer	0.344	0.925
b1241	adhE	PFL-deactivase / alcohol dehydrogenase / acetaldehyde dehydrogenase	-0.143	0.122
b3870	glnA	adenylyl-[glutamine synthetase]	0.299	-0.056
Δ*hnr* Δ*yliE*				
b0118	acnB	aconitase B	-0.197	-0.274
b0882	clpA	ClpAXP	-0.224	-0.352
b2674	nrdI	conserved protein that may stimulate ribonucleotide reductase	0.378	-0.426
b3458	livK	leucine binding protein of the high-affinity branched-chain amino acid transport system	-0.636	-0.135

7.2. Integrated Genomic and Proteomic Analysis

7.2.1. "Consensus" Expression Analysis

While each level of cellular information is important, it is clear from the low correlations in the previous section that multiple hierarchical levels are generally required for an adequate descriptor of biological response. Transcriptionally controlled targets for which the mRNA and protein expression changes are highly correlated may present an exception, but the often-encountered scenarios of low or even negative correlations indicating posttranscriptional control and potentially negative feedback regulation, respectively, require multiple data sets and additional experimentation to uncover the mechanisms at work. While specifically uncovering regulatory mechanisms is not the focus of this work and comprehensive integration of the two data sets is not pursued here, integration of the data were pursued on a smaller scale as a starting point for this type of analysis. Since the most abundant proteins are generally measured by the applied LC-MS approach and some metabolic pathways only include a few measured proteins, it was more difficult to calculate meaningful correlations for specific pathways using the global proteomic data. Nevertheless, central carbon metabolism consisting of the TCA cycle, the glyoxylate pathway, glycolysis and gluconeogenesis, and reactions around pyruvate and phosphoenolpyruvate were examined in greater detail for their agreement in the two expression data sets. These pathways were specifically selected given that the lycopene precursors are supplied by the glycolytic pathway, energy requirements are relatively high for lycopene biosynthesis, and initial patterns in the directionality of the expression data were observed that were presented in the previous chapter.

An integrated analysis of most of central carbon metabolism across the five mutant strains compared to the PE strain was pursued in order to determine whether a consensus of pathway expression could be ascertained between the transcriptomic and proteomic data sets. By

identifying some agreement between the mRNA and protein expression levels, metabolic pathway regions in which transcriptional control is likely dominant can more likely be manipulated in a relatively straightforward manner through gene knockout or overexpression. It was reasoned that targets generated from this analysis would stand a significant chance of influencing the lycopene production phenotype. Distal targets that may relate to the production phenotype via unknown or poorly categorized regulatory interactions have been explored in the previous two chapters stemming from the individual data sets, and studies such as Alper *et al.* (2005) have explored central carbon metabolism's relationship to lycopene production via rationally directed stoichiometric modeling. The integrated expression analysis of this work, however, provides insight into what is actually occurring in these central pathways in the generated mutants, more fully characterizing them and potentially uncovering factors related to production. Of course, a greater challenge of systems biology is to integrate multiple molecular data sets for a full description of cellular phenotype and to uncover regulatory mechanisms even in pathways for which the various hierarchy levels show differing trends. This more modest integrated analysis was pursued first as a starting point for future work in this area.

A "consensus" expression level was sought from the transcriptomic and proteomic data for each of the enzymes and multiprotein complexes in the TCA cycle, the glyoxylate pathway, and glycolysis and gluconeogenesis. A metric was devised to reflect both consistency in direction of expression (up- or down-regulation) as well as magnitude of expression changes, without specifically considering only differential expression. Thus, normalized $\log_{10}$ ratios were used to indicate up- or down-regulation without applying the stringency of statistical selection criteria. While this obviously exposes the analysis to higher levels of experimental and biological noise, this was deemed acceptable as consistency in expression across various mutants

or moderately large expression changes (that may or may not be "statistically significant") across a few measurements in both the genomic and proteomic expression data can potentially serve as a substitute for interesting biological phenomenon. Given that the purpose of this analysis was target generation to be followed by experimental validation, potential false positives were accepted in the process of discovering patterns. In this sense, the plot is similar to the widely-used database EcoCyc's Omics Viewer tool (Keseler, Bonavides-Martinez *et al.* 2009), which also colors reactions according to expression data. The Omics Viewer handles several genes and proteins mapping to the same reaction by displaying the maximum up- or down-regulation result, whereas a "consensus" expression across mutants and data sets is plotted here. The metric is presented in Chapter 4, whereas the resulting metabolic maps are given in Figure 7-2 and Figure 7-3. Data on which these figures are based is given in Appendix 9.3.

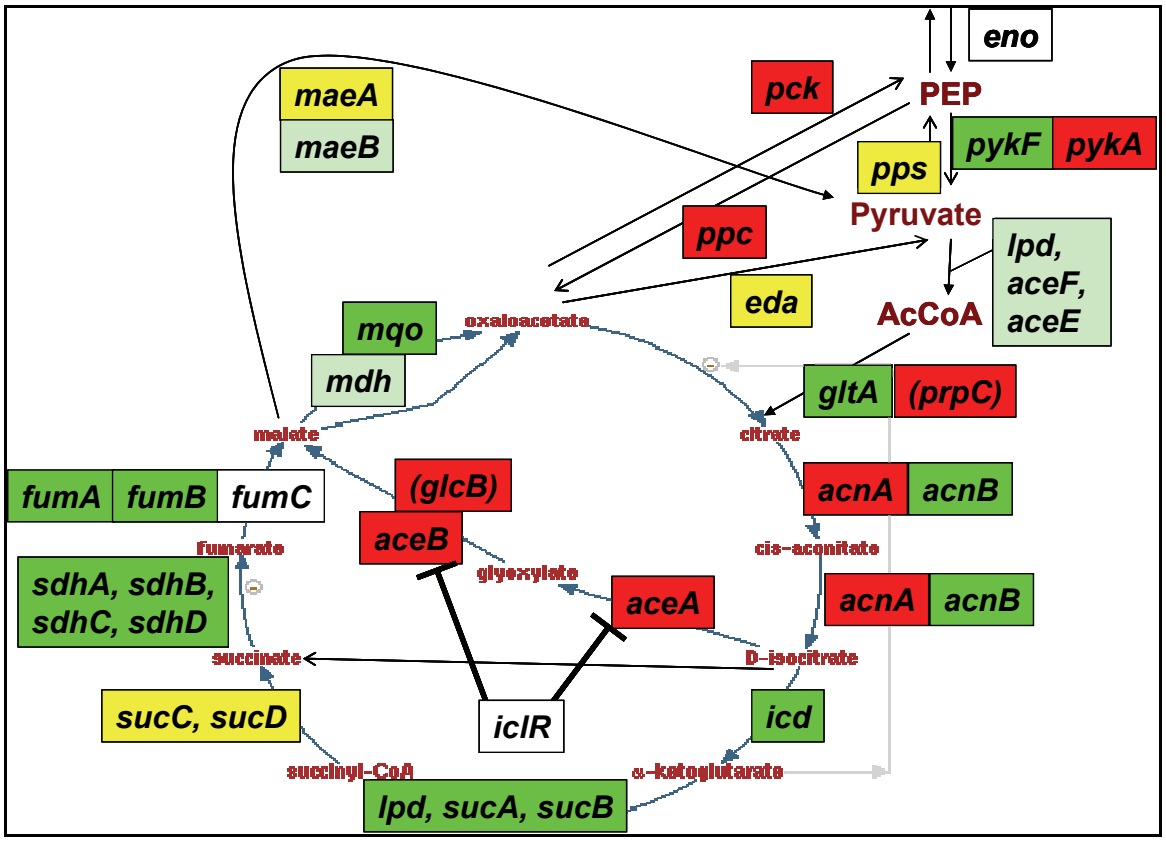

Figure 7-2 Manually integrated transcriptomic and proteomic analysis across all five mutant strains compared to the PE strain of the central carbon metabolic pathways of the TCA cycle, the glyoxylate pathway, and glycolytic and gluconeogenic reactions around phosphoenolpyruvate (PEP) and pyruvate. Genes encoding for enzymes appear in boxes, with multiprotein complexes containing multiple genes within the same boxes and isozymes for the same reactions appearing in separate boxes next to each other. "Consensus" coloring based on directionality and magnitude of expression was determined as explained in the text, with red indicating up-regulation, green indicating down-regulation, and yellow indicating mixed expression. Light green and light red indicate weaker down- or up-regulation, respectively.

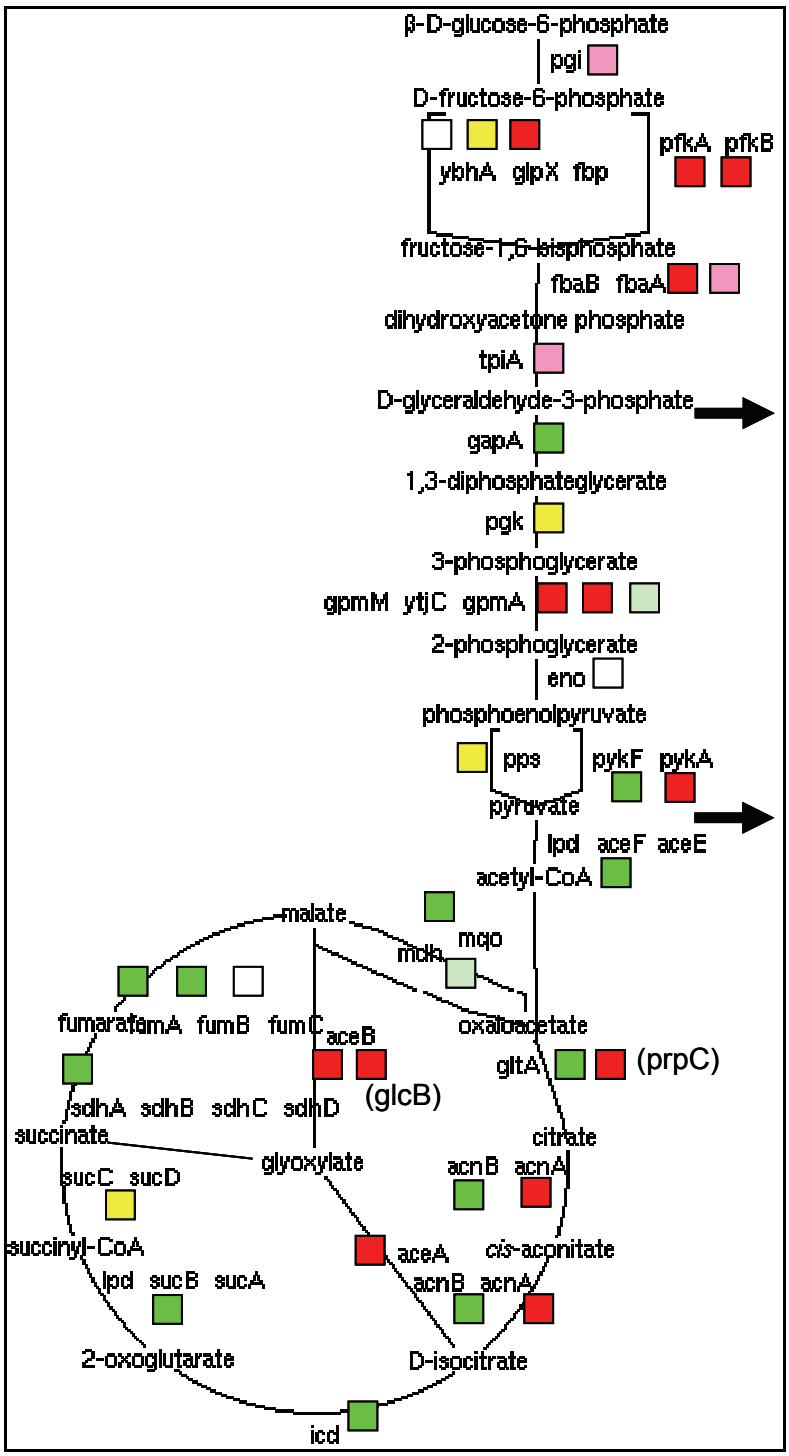

Figure 7-3 Manually integrated transcriptomic and proteomic analysis across all five mutant strains compared to the PE strain of the central carbon metabolic pathways of the TCA cycle, the glyoxylate pathway, and glycolysis and gluconeogenesis. Genes encoding for enzymes for particular reaction steps (including isozymes and multiprotein complexes) appear next to color-coded boxes. "Consensus" coloring based on directionality and magnitude of expression was determined as explained in the text, with red indicating up-regulation, green indicating down-regulation, and yellow indicating mixed expression. Light green and light red indicate weaker down- or up-regulation, respectively.

The general trends of Figure 7-2 and Figure 7-3 reveal that the TCA cycle is generally down-regulated, the glyoxylate pathway is somewhat up-regulated, and a majority of the enzymes in the glycolytic and gluconeogenic pathways appear to be up-regulated. Again, this "up" and "down" regulation is not completely at the level of statistical significance, although similar trends were observed in the proteomic differential expression presented in the previous chapter. Instead, the directionalities and magnitudes of expression measurements across the transcriptomic and proteomic data sets indicate these patterns. The data in Appendix 9.3 reveals that these trends are based more on the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD,* the Δ*hnr,* and the Δ*hnr* Δ*yliE* strains, although the Δ*gdhA,* the Δ*gdhA* Δ*aceE* strains show similar changes as well. Down-regulation of the TCA cycle is more dependent upon the proteomic data, as presented in the previous chapter, but the up-regulation in the glycolytic pathway is seen in both the transcriptomic and the proteomic data.

7.2.2. Glyoxylate Pathway and TCA Cycle

Three main explanations are possible to explain the significance of the glyoxylate pathway up-regulation and the TCA cycle down-regulation and how this might relate to lycopene overproduction. Of course, energy requirements are high for lycopene production and normal cellular growth and maintenance, so the TCA cycle is certainly required to drive oxidative phosphorylation and ATP production. However, Alper *et al.* (2006) observed that acetate secretion fell about 33% in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD* strain during high cell density fermentation as compared to the PE strain. Since the glyoxylate pathway normally functions during acetate growth to provide gluconeogenic precursors (Gui, Sunnarborg *et al.* 1996), it seems likely that the moderate up-regulation of the glyoxylate pathway could be responsible for this observed decrease in acetate levels measured in the media. Some overflow metabolism directed towards

acetate could instead be channeled through the glyoxylate cycle, and once glucose has been exhausted later during stationary phase and catabolite repression has been lifted, some acetate in the media might be re-utilized via the glyoxylate pathway. Such prevention of overflow metabolism to acetate by up-regulation of the glyoxylate cycle during glucose growth has been suggested before in the context of a PEP carboxykinase *E. coli* mutant which operated the glyoxylate pathway and the TCA cycle simultaneously (Yang, Hua *et al.* 2003) and a low acetate producing strain (Noronha, Yeh *et al.* 2000).

Acetate is a well-known growth inhibitor that is widely accepted as resulting from an imbalance between glycolytic flux and the cell's actual requirements for metabolic precursors and energy (Stephanopoulos, Aristidou *et al.* 1998). Despite the high glycolytic flux required for high lycopene production, perhaps the mutant strains are better able to utilize this flux in the proper precursor balance of glyceradlehyde-3-phosphate and pyruvate being shunted into the non-mevalonate pathway instead of being lost to acetate or CO_2. Secreted acetic acid can re-enter the cell and dissociate in the relatively high pH of the intracellular environment, leading if unchecked to the destruction of the ΔpH portion of the proton motive force (Slonczewski, Rosen *et al.* 1981; Diazricci, Hitzmann *et al.* 1990). This may help explain the observed glutamate-dependent acid stress response (GadA, GadB, HdeB) that was observed up-regulated in the proteomic data. As mentioned previously, these three targets in addition to AdhE, Dps, OsmC, OsmY, PflB, and TalA are all σ^S-regulated and were observed to be up-regulated in the proteomic data of this thesis. These targets have previously been observed to be up-regulated at the transcriptional level upon acetate exposure (Arnold, McElhanon *et al.* 2001). Acetate stress may also help explain why overexpression of the *ydeO* acid stress response regulator increased lycopene production by about 30% as compared to the *gfp* overexpression control. A number of

studies have shown that the acetate threshold that influences recombinant production is usually lower than the threshold that causes notable growth inhibition. For example, Jensen and Carlsen (1990) showed that increasing the media acetate level to 100 mM led to a 70% *E. coli* biomass reduction but a 2-fold decline in recombinant human growth hormone production. Furthermore, Vadali *et al.* (2005) deleted the acetate production pathway in *E. coli* and found that recombinant lycopene production rose about 45%. These facts support the hypotheses that reduction of acetate production in the mutant strains benefits lycopene production.

A slight down-regulation of the TCA cycle and up-regulation of the glyoxylate pathway in the mutant strains as compared to the PE strain may allow for some carbon to be channeled back towards pyruvate and especially glyceraldehyde-3-phosphate, the lycopene precursors, rather than being lost as acetate due to overflow metabolism or CO_2 in the TCA cycle. Indeed, in addition to the consensus up-regulation seen in the *aceA*, *aceB*, and *glcB* targets of the glyoxylate pathway, the PEP carboxykinase *pck* target leading to PEP from oxaloacetate is up-regulated as well. *Eda* and *pps* are mixed in their expression changes, whereas *ppc* and *pykA* leading away from PEP and pyruvate, respectively, also have a consensus of up-regulation.

Interestingly, it has been shown that α-ketoglutarate levels are increased in the Δ*gdh1* mutant in *S. cerevisiae* (DeLuna, Avendano *et al.* 2001), where *gdh1* is a homolog to *gdhA* in *E. coli*, which is consistent with decreased expression of downstream TCA cycle enzymes observed in this work for the three mutant strains sharing the Δ*gdhA* deletion. As observed in the previous chapter, the Δ*hnr* and Δ*hnr* Δ*yliE* strains also demonstrate GdhA protein down-regulation, perhaps helping to explain TCA cycle down-regulation in these strains as well.

Farmer and Liao (2001) found that increasing the gluconeogenic flux increased lycopene production via the non-mevalonate pathway in *E. coli*. Specifically, overexpressing *pps* or *pck*

and deleting *pykFA* increased lycopene production, whereas deleting *ppc* decreased production. Alterations promoting a gluconeogenic flux from pyruvate to glyceraldehyde-3-phosphate generally increased lycopene production, suggesting that glyceraldehyde-3-phosphate may be a limiting in lycopene biosynthesis under certain conditions. Furthermore, the authors did not detect growth defects as signs of futile cycles when they overexpressed gluconeogenic enzymes in the strains. In this work, Figure 7-3 shows that a number of glycolytic/gluconeogenic enzymes have consensus up-regulation such as in the targets *pgi*, *pfkA*, *pfkB*, *fbaA*, *fbaB*, *tpiA*, *gpmM*, and *ytjC*. Interestingly, though, the *gapA* target has consensus down-regulation, perhaps indicating agreement with Farmer and Liao (2001) that flux in the direction of glyceraldehyde-3-phosphate from pyruvate is preferred for lycopene production. Again, reduction in acetate production or CO_2 loss in the TCA cycle coupled with increasing glyceradlehyde-3-phosphate availability may explain some of the improvement in lycopene production of the mutants as compared to the PE strain.

On a related note, the carbon storage regulator *csrA* was seen to be down-regulated in all five of the mutant strains in direction but not at the level of statistical significance. The *csrA* gene was down-regulated about 30% in the Δ*gdhA*, Δ*hnr,* and the Δ*hnr* Δ*yliE* strains and is known to positively regulated glycolysis enzymes while negatively regulating gluconeogenic enzymes (Sabnis, Yang *et al.* 1995) as well as many other cellular activities such as biofilm formation (Jackson, Suzuki *et al.* 2002). Slight down-regulation of *csrA* may be important to a shift towards some gluconeogenic flux to feed the lycopene precursor pools in conjunction with the more pronounced effect of the glyoxylate pathway up-regulation. Easing *csrA* suppression of biofilm formation could be related to the previous discussion on the aggregative expression patterns observed in the transcriptomic data as well.

A third potential explanation for the TCA cycle down-regulation and the corresponding glyoxylate pathway up-regulation is related to the cellular redox state. Normally during *E. coli* growth on acetate, the glyoxylate cycle exhibits high activity as the anaplerotic reaction supplying precursors for biosynthesis while the TCA cycle also exhibits a high flux for the production of reducing power via isocitrate dehydrogenase and ATP for biosynthesis (Zhao and Shimizu 2003). Additionally, there is normally PEP/pyruvate formation from oxaloacetate/malate. However, Fischer and Sauer (2003) were able to measure glyoxylate flux during slow growth on glucose. The pathways connecting PEP resulting from glycolysis to the glyoxylate pathway and then back to PEP via oxaloacetate form an alternative cycle to the TCA cycle, which Fischer and Sauer (2003) denote as the "PEP-glyoxylate" cycle. This is shown in Figure 7-4 reproduced from their work. Stoichiometrically, the net result of running the PEP-glyoxylate cycle versus the TCA cycle for glucose growth is +1 NADH, -1 NADPH, and -1 ATP per mole of PEP entering either "cycle." Thus, running the alternative PEP-glyoxylate pathway alters the cellular redox balance, which seems especially important in a system producing lycopene with such a high NADPH metabolic cost. It is more intuitive that creating excess NADPH would increase lycopene production, supporting the precursor conservation and acetate reduction hypotheses for the glyoxylate pathway up-regulation, but perhaps the proper balance of NADPH and NADH for both growth and lycopene production is struck through glyoxylate pathway activation in the mutant strains.

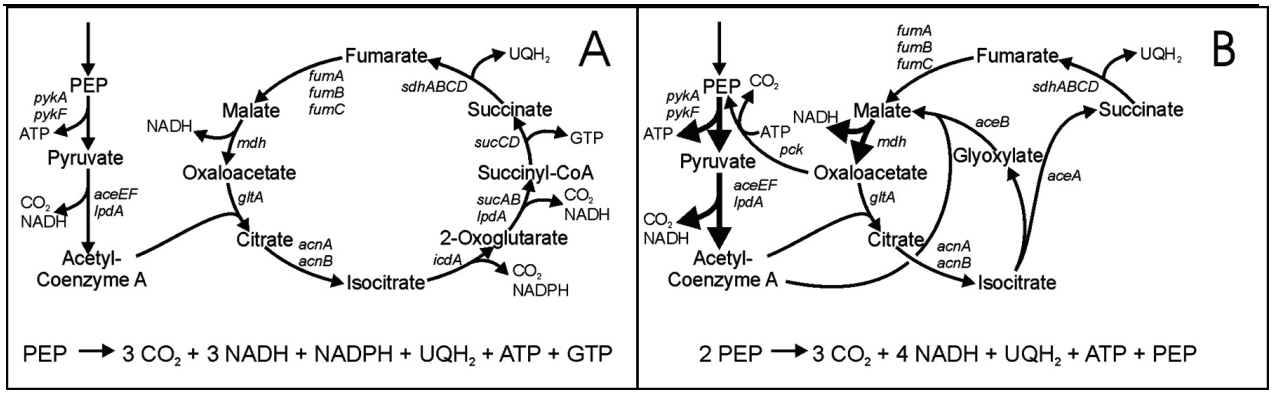

Figure 7-4 TCA cycle (A) and the PEP-glyoxylate cycle (B) reproduced from (Fischer and Sauer 2003).

The *aceBAK* operon encoding two of the three glyoxylate pathway enzymes shown in Figure 7-2 is regulated by the repressor *iclR*. In Δ*iclR* strains, the glyoxylate pathway has been found to be constitutively active (Maloy and Nunn 1982). Furthermore, there are metabolic engineering examples of deleting *iclR* to increase production of certain compounds. Sanchez *et al.* (2005) successfully knocked out *iclR* to boost the glyoxylate pathway flux for succinate overproduction, and Lee *et al.* (2007) observed *aceBA* up-regulation in threonine-overproducing *E. coli*, deleted *iclR*, and recorded 30% higher threonine production. Additionally, the *iclR* gene has been observed to be down-regulated greater than 4-fold in the Δ*hnr* Δ*yliE* strain of this study, although the p value for significance was 2.5×10^{-2}, greater than the 0.00426 critical p value cutoff for statistical significance. Thus, it was hypothesized that deleting the *iclR* repressor in the PE strain and activating the glyoxylate pathway to imitate the mutants may increase lycopene production, whether a positive effect in the mutants is due to reduction of acetate secretion, lycopene precursor conservation, or cellular redox balancing.

7.2.3. NADPH Availability and the Proton-Translocating Transhydrogenase

Martinez *et al.* (2008) recently found that replacing the native NAD-dependent glyceraldehyde-3-phosphate dehydrogenase gene *gapA* with an NADP-dependent version from

Clostridium acetobutylicum, *gapC*, led to about 2.5-fold higher levels of lycopene productivity and accumulation in *E. coli* WS110, demonstrating the clear benefit of increasing NADPH availability on lycopene production. Since the glyoxylate pathway possibly involved in cellular NADPH balance was observed up-regulated in the mutants, other related strategies for altering NADPH availability were considered as well. This direction towards cellular redox effects was also encouraged by reports that when the glutamate dehydrogenase *GDH1* gene, a homolog of the *gdhA* gene in *E. coli*, was knocked out in *S. cerevisiae*, the requirement for NADPH in connection with cellular growth was found to be reduced by over 40% (Nissen, Kielland-Brandt *et al.* 2000). Furthermore, when a highly correlated metabolic subnetwork of this mutant strain was examined, 10 genes in a 34-gene subnetwork encoded for oxidoreductive reactions involving the cofactors NADPH/NADH, clearly demonstrating the effect of the GDH1 gene on redox metabolism (Patil and Nielsen 2005). The NADPH/NADH balance is clearly important to lycopene production, and so a simple experimental strategy was devised to alter this balance and determine the effect upon lycopene production.

The major sources of cellular NADPH during growth on glucose include the pentose phosphate pathway and the Entner-Doudoroff (ED) (pathway genes *zwf* and *gnd*) as well as isocitrate dehydrogenase (gene *icd*) (Fuhrer and Sauer 2009). The actual rate of NADPH production depends on the carbon fluxes through these metabolic pathways, which can vary significantly with environmental conditions. Anabolic demand for NADPH, however, is coupled to the rate of biomass formation (Neidhardt, Ingraham *et al.* 1990) and to the reduction of thioredoxin for maintaining a balanced redox state (Arner and Holmgren 2000). When NADPH requirements are especially high, though, as in the case of high biomass growth, NADPH production through catabolic pathways may be insufficient to meet this demand (Sauer,

Canonaco *et al.* 2004). Similarly, when extensive catabolic fluxes are present, the NADPH produced through these pathways may be in excess of the anabolic demand.

There are two basic methods by which bacteria may balance their NADPH (Fuhrer and Sauer 2009). In the first type of method, the cells may avoid imbalance in the first place by expressing the appropriate catabolic pathways or by differentially expressing isozymes with different cofactor specificities. On the other hand, catabolic NADPH formation and anabolism can be decoupled from each other through either the mechanisms of transhydrogenases, NAD(H) kinases (Kawai, Mori *et al.* 2001), or redox cycles such as the PEP-glyoxylate cycle discussed above (Sauer, Canonaco *et al.* 2004). There exist in *E. coli* both a membrane-bound, proton-translocating transhydrogenase (encoded by genes *pntAB*) (Clarke, Loo *et al.* 1986) and a soluble, energy-independent transhydrogenase (encoded by *udhA*) (Boonstra, French *et al.* 1999).

The soluble and membrane-bound pyridine nucleotide transhydrogenase enzymes catalyzing the reversible transfer of a hydride ion equivalent between NAD and NADP have been thought to adjust the catabolic (CRC) and anabolic (ARC) reduction charges that are defined as follows and normally in the ranges of ~0.03-0.08 and ~0.7-1.0, respectively (Ingraham, Maaloe *et al.* 1983):

$$\mathrm{CRC} = \frac{[\mathrm{NADH}]}{[\mathrm{NADH}]+[\mathrm{NAD}]} \sim 0.03\text{-}0.08 \quad (7.1)$$

$$\mathrm{ARC} = \frac{[\mathrm{NADPH}]}{[\mathrm{NADPH}]+[\mathrm{NADP}]} \sim 0.7\text{-}1.0 \quad (7.2)$$

However, Sauer *et al.* (2004) found that PntAB supplies a large amount (35-45%) of NADPH during *E. coli* glucose growth and speculated that PntAB (membrane-bound form) may increase NADPH levels. On the other hand, they found that UdhA was required under conditions of excess NADPH formation, such as growth on acetate with high NADPH levels from Icd or else a

phosphoglucose isomerase Δ*pgi* mutant that catabolized glucose through the pentose phosphate pathway. Indeed, when *pntAB* were overexpressed in Corynebacterium glutamicum for the production of lysine, a product demanding sufficient NADPH supply, production was increased from 10-300% depending upon the carbon source (Kabus, Georgi *et al.* 2007). Interestingly, the PntB protein was down-regulated about 20% in the Δ*gdhA* strain, 35% in the Δ*gdhA* Δ*aceE* Δ_P*yjid* strain, and 50% and 45% in the Δ*hnr* and Δ*hnr* Δ*yliE* strains, respectively, applying the second peptide threshold. This down-regulation of PntB is not intuitive given the high NADPH requirement for lycopene biosynthesis and will be discussed further in the next section. Based on this data, it was hypothesized that the deletion of the major NADPH source gene *pntB* would affect lycopene production levels.

7.2.4. Metabolic Engineering of *iclR* and *pntB* Targets

To test both of these hypotheses, the *iclR* and *pntB* genes were separately deleted in the PE strain as described in Chapter 4. The resulting lycopene production profiles are shown in Figure 7-5 and Figure 7-6 and indicate small (~5-10%) and moderate (~20-25%) gains in lycopene production for the Δ*iclR* and Δ*pntB* strains, respectively.

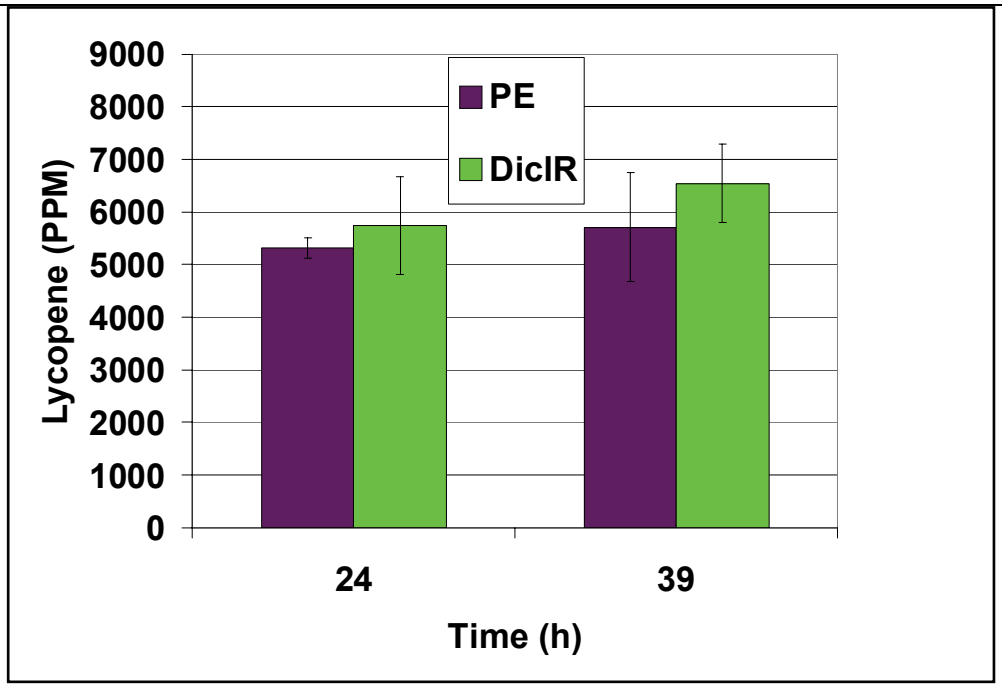

Figure 7-5 Lycopene production for PE strain with Δ*iclR* deletion for the glyoxylate pathway transcriptional repressor. The purple bars indicate the PE strain background.

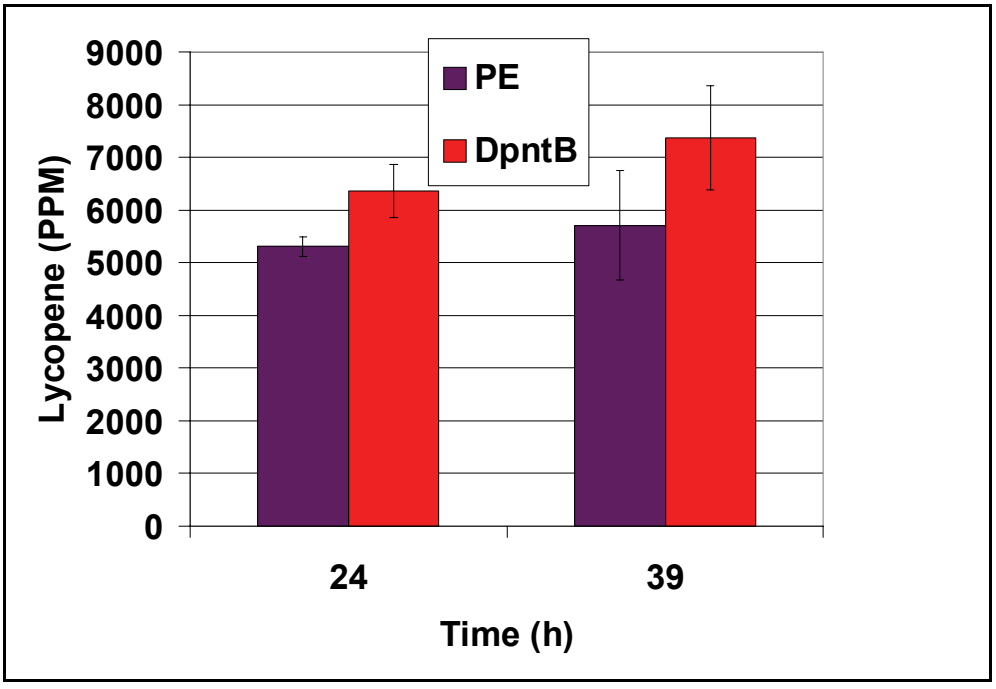

Figure 7-6 Lycopene production for PE strain with Δ*pntB* deletion for the membrane-bound pyridine nucleotide transhydrogenase. The purple bars indicate the PE strain background.

The modest gains in lycopene production for the *iclR* knockout may be rationalized in the following context. Although deleting the *iclR* transcriptional repressor frees the glyoxylate

pathway enzymes for transcription, the so-called "branch point" effect still determines that the flux through the glyoxylate pathway is modest relative to the TCA cycle on glucose growth. This is because isocitrate dehydrogenase has a much greater affinity for isocitrate than isocitrate lyase of the glyoxylate pathway, with isocitrate lyase having a 75-fold larger K_m than isocitrate dehydrogenase (Stephanopoulos, Aristidou *et al.* 1998). Isocitrate dehydrogenase is rapidly dephosphorylized in the presence of glucose, activating it to compete with isocitrate lyase of the glyoxylate shunt. Although deleting *iclR* may lift the repression of the glyoxylate pathway transcription including isocitrate lyase, only modest increases in flux through the glyoxylate pathway and resulting effects upon lycopene production might be expected given the continued dominance of isocitrate dehydrogenase in scavenging isocitrate. Indeed, this may be best from a lycopene production point of view. Some glyoxylate pathway flux may be important to reducing overflow metabolism, increasing gluconeogenic flux towards lycopene precursors, or else affecting the NADPH/NADH balance, but continued TCA cycle flux is important from an energetic point of view. Later, when glucose is depleted and catabolite repression has been lifted, continued increased flux through the glyoxylate pathway due to the *iclR* deletion may increase acetate utilization and further production of lycopene. This would be interesting to test in future experiments.

There are a few possible explanations for the lycopene increase upon *pntB* knockout as well. The membrane-bound transhydrogenase reaction proceeds according to the following reaction in Equation 7.3, shown graphically in Figure 7-7:

$$H^+_{out} + NADH + NADP^+ \rightleftarrows H^+_{in} + NAD^+ + NADPH \quad (7.3)$$

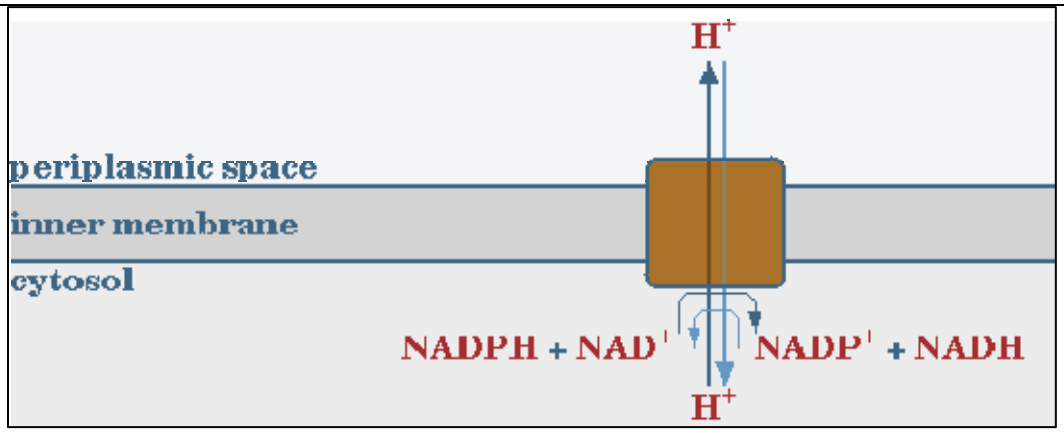

Figure 7-7 Membrane-bound, proton-translocating pyridine nucleotide transhydrogenase (PntAB) reaction linking the cellular energetic and redox states. Figure reproduced from the EcoCyc database (Keseler, Bonavides-Martinez *et al.* 2009).

Pyridine nucleotide transhydrogenase (PntAB) catalyzes the reversible transfer of a hydride ion equivalent between NAD+ and NADP+. While the hydride transfer between NADH and NADP+ is a near-equilibrium reaction, in the presence of a proton motive force Δp, the forward reaction is increased 5-10-fold whereas the reverse reaction is inhibited, increasing the apparent equilibrium constant from about 1 to nearly 500 (Bizouarn, Althage *et al.* 2002). This can help to maintain a high redox level of NADPH required for biosynthesis, regulation, and detoxification. In eukaryotic beef heart membrane preparations, though, the membrane bound transhydrogenase has been observed to be reversible (Hoek and Rydstrom 1988). The forward direction of reaction 7.3 was found to depend upon (NADPH/NADP+) ratio, whereas the reverse reaction was found to depend upon the combined effects of the (NADH/NAD+) ratio and the Δp proton motive force. Fuhrer and Sauer (2009) estimated the $\Delta_r G'$ Gibbs energy of reaction for *E. coli* and seven other bacterial species based upon thermodynamic parameters from Kummel *et al.* (2006), and they estimated the ranges to be from -6.40 to -30.78 kJ/mol for membrane-bound PntAB and -0.84 to -3.82 kJ/mol for the soluble UdhA for intracellular conditions. They rule out a reverse flux from NADPH to NADH through the membrane-bound transhydrogenase based on

$\Delta_r G'$ ranges of -4 to –41 kJ/mol for the other bacterial species, concluding that the soluble transhydrogenase UdhA could possibly fulfill this role in *B. subtilis* and *P. versutus*.

If the 7.3 reaction proceeds in the forward direction in the PE strain of this study, then it may be that deleting the *pntB* gene actually prevents production of NADPH, perhaps balancing an excess of NADPH produced from other cellular sources such as the pentose phosphate pathway and isocitrate dehydrogenase. This would be similar to the explanation proposed above for the PEP-glyoxylate cycle (Sauer, Canonaco *et al.* 2004) actually leading to decreased levels of NADPH to balance the cellular redox state with NADH and ATP as well. However, given the high NADPH lycopene biosynthetic requirement and the results of Martinez *et al.* (2008), it seems unlikely that deleting an NADPH-producing reaction would increase lycopene production. The glyoxylate pathway has been proposed as a fine-tuning balance of NADPH (Fischer and Sauer 2003), whereas the transhydrogenases have been seen to have larger effects on the redox balance (Sauer, Canonaco *et al.* 2004).

Alternatively, it may be that in the PE strain and possibly also the mutant strain examined in this study, the transhydrogenase reaction 7.3 operates in the reverse direction. The estimates proposed by Fuhrer and Sauer (2009) are based on *E. coli* K12, not the unique redox and energetic state of the PE strain. This may especially be true given that the upper range of estimates for the Gibbs energy of reaction of ~ -6 kJ/mol is not far from reversibility. In fact, controversy over the physiological role of these nicotinamide nucleotide transhydrogenases has existed in the literature since their discovery (Sauer, Canonaco *et al.* 2004). If the 7.3 reaction operates in the reverse direction in the PE strain, similar to observations with beef heart membranes (Hoek and Rydstrom 1988), then the *pntB* deletion may be actually conserving NADPH for use in lycopene production instead of using it to reduce NAD+. This may be a

favorable reaction direction if the Δp proton motive force was relatively low for the PE cells, conserving the proton gradient for use in ATP energy production. Indeed, since observations were made in the transcriptomic data of *atpE* and other ATP synthase component up-regulation as well as a possible link of down-regulation of histidine biosynthesis to purine biosynthesis via PRPP, the data are consistent with energetic requirements being highly demanding in the context of lycopene production. Spending the Δp on NADPH production may not be favorable in such an energy-constrained system.

Finally, Δ*pntB E. coli* K12 strains were examined by Sauer *et al.* (2004). They observed higher fluxes into the oxidative pentose phosphate pathway to PEP, lower flux through glycolysis to 3P-glycerate, lower TCA activity, and an increased anaplerotic flux from PEP to oxaloacetate via PEP carboxylase. Interestingly, the proteomic data for the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid*, the Δ*hnr*, and the Δ*hnr* Δ*yliE* strains demonstrated some up-regulation in the Pgl and RpiA enzymes of the oxidative pentose phosphate pathway, and the lower TCA activity is consistent with the observed TCA cycle expression down-regulation of the five mutant strains in this study. It is possible that deleting *pntB* in the PE strain leads to a similar shift in NADPH production from the PntAB transhydrogenase (assuming 7.3 operates in the forward direction) to an increased flux through the pentose phosphate pathway, which may lead to higher overall NADPH production for lycopene biosynthesis.

While the specific reason for increased lycopene production is not obvious, there are several possibilities that warrant further investigation. In any case, it is apparent from the 30% production increase in deleting the *pntB* gene that NADPH availability is highly important to lycopene production and may offer a source of further improvement.

It is important to note that the results from disrupting the *iclR* and *pntB* genes stem from an examination of both the transcriptomic and proteomic data together rather than either data set alone. While either data set alone suggests TCA cycle down-regulation, glyoxylate pathway up-regulation, and glycolytic/gluconeogenic enzyme up-regulation, the consensus diagrams in Figure 7-2 and Figure 7-3 integrating both data sets presents a clearer, more comprehensive picture. Furthermore, using the integrative approach in conjunction with the literature to identify the pathways involved and potential biological explanations suggested metabolic engineering targets for which expression data alone would have proven inconclusive. Limited expression data were obtained for *iclR*, and while the PntB proteomic data showed some down-regulation, the level of down-regulation did not make it an obvious target. It is expected that further advances in easing "omics" data integration from multiple levels of system structure will lead to increased abilities to make such valuable observations, improving resulting hypotheses, subsequent experiments, and eventual biological insight and production strategies.

7.3. Summary

- There was no ($r \sim 0.02$) global correlation of the transcriptomic and proteomic data sets and no ($r \sim 0.04$) correlation for those targets that were differentially expressed in at least one of the strains in at least one of the data sets. This was consistent with similar observations made in the literature and indicates the presence of significant post-transcriptional regulation.
- However, an analysis integrating the transcriptomic and proteomic data was pursued for the specific pathways of central carbon metabolism except for the pentose phosphate pathway. A "consensus" of the transcriptomic and proteomic data was observed to show

slight up-regulation in the glyoxylate pathway, slight down-regulation in the TCA cycle, and increased expression in the glycolytic/gluconeogenic pathways.

- The importance of the slight up-regulation in the glyoxylate pathway could be due to reducing overflow metabolism and acetate production, channeling carbon back to glyceraldehyde-3-phosphate via a gluconeogenic flux, or potentially by balancing the redox state of the cell through the PEP-glyoxylate cycle.
- Deleting the *iclR* transcriptional repressor of the glyoxylate pathway led to modest lycopene production increases of about 10%. Such a modest increase is consistent with simultaneous albeit slightly reduced expression of the TCA cycle and the isocitrate dehydrogenase enzyme with stronger affinity for isocitrate than the isocitrate lyase enzyme of the glyoxylate pathway.
- Deleting the *pntB* subunit of the membrane-bound, proton-translocating pyridine nucleotide transhydrogenase led to lycopene production increases of about 25%, providing strong evidence that NADPH availability has an important effect upon production. The mechanism by which production was increased requires further study but may be related to the following: preserving the Δp proton motive force dissipated by the membrane-bound transhydrogenase reduction of NADP+ if the cellular energetic state is limiting for production; preventing NADPH loss if levels are limiting and the reverse oxidation of NADPH is the preferred transhydrogenase reaction direction; or by increasing the flux of the pentose phosphate pathway and potentially allowing for higher levels of NADPH for lycopene production.

- The integrated analysis approach was important for generating the *iclR* and *pntB* deletion targets in the PE strain, and it is expected that improved integration methods will lead to greater improvements.

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Chapter 8. CONCLUSIONS AND RECOMMENDATIONS

8.1. Conclusions

This thesis demonstrated a systems biology approach to describing several high small molecule-producing microbial strains in terms of transcriptomic and proteomic data and incorporating this information for a more complete view of these desirable cellular production systems. Specifically, whole-genome DNA microarrays and a novel LC-MS technique were applied to study genomic and proteomic expression changes of deletion mutant *E. coli K12* strains producing high levels of lycopene generated by Alper *et al.* (Alper, Jin *et al.* 2005; Alper, Miyaoku *et al.* 2005; Alper and Stephanopoulos 2008) using both a rationally-directed and a combinatorial approach to the metabolic engineering. While specific conclusions were listed at the end of each of the chapters on transcriptomics, proteomics, and an integrated analysis of the data, several main conclusions are summarized here.

The lycopene-producing non-mevalonate pathway did not exhibit consistent and coordinated differential expression compared to the PE parental strain background, so more distal factors affecting the production phenotype were explored. While a majority of genes and proteins showed few expression changes, key differences were identified. Based upon the expression data sets, it was hypothesized that the following may be associated with lycopene overproduction: histidine biosynthesis (*hisH*); the quinone pool (*wrbA*); acid resistance (*ydeO* and *gadE*); the glyoxylate pathway (*iclR*); NADPH redox balance (*pntB*); and membrane composition. In the pre-engineered background strain, deleting *pntB* (~20-25%) and *ydeO* (~30%) each led to moderately increased production; overexpressing *wrbA* led to 50-100% more production at 8 hours and 5-15% more production at later time points; deleting *iclR* caused small production increases (~5-10%); and supplementing media with histidine caused PE and mutant

strains to have similar production. A number of these modifications led to similar magnitude increases in lycopene production as the original gene deletions made by Alper *et al.* (Alper, Jin *et al.* 2005; Alper, Miyaoku *et al.* 2005; Alper and Stephanopoulos 2008).

Overall, it appears that a number of factors which are small to moderate individually may together result in the observed lycopene production phenotypes. The following general conclusions can be drawn from this work:

- Reduced cellular growth and energy conservation appear to be important factors for increasing lycopene production. The *hisH* gene encoding for an enzyme at a branch point between the histidine biosynthesis pathway and purine biosynthesis was down-regulated over 10-fold in the Δ*gdhA,* the Δ*gdhA* Δ*aceE,* and the Δ*gdhA* Δ*aceE* Δ_{P}*yjiD* strains. When the strains were supplemented with histidine, effectively shutting down the histidine pathway, the PE strain experienced a relative increase in lycopene production levels as compared to the other three strains. It was reasoned that this histidine pathway down-regulation may be energetically advantageous for conserving the metabolite PRPP, which is also an intermediate in purine biosynthesis and could boost ADP and thus ATP levels. In light of the over 3-fold up-regulation of the critical *atpE* ATP synthase gene in addition to up-regulations observed in the *atpB* as well as up-regulated directionality for the *atpA* and *atpD* genes, ATP and energetic requirements seem to be critical for lycopene production. Given that each lycopene molecule synthesized requires 8 ATP and 8 CTP molecules, this requirement is logical. Additionally, 42 of the total 55 ribosomal proteins were differentially down-regulated across the five mutant strains, indicating reduced translation and slower growth that was observed for the mutants compared to the

PE strain by ~10-30%. This tradeoff of growth is likely advantageous for redirecting cellular resources to lycopene biosynthesis and is consistent with the literature on recombinant production.

- The data are consistent with strategies of reducing overflow metabolism to acetate and the corresponding acid stress as well as providing a gluconeogenic flux to increase lycopene precursors. The glyoxylate pathway appears to be up-regulated at the proteomic level and from the analysis integrating transcriptomic with proteomic data, whereas the TCA cycle appears to be slightly down-regulated. The glycolytic/gluconeogenic pathway enzymes are also up-regulated for the most part at both the transcriptional and proteomic levels. The integrated analysis was critical to determining these small but consistent effects across the multiple strains and the two global data sets. While there are several possible explanations for these observations, it appears most likely that simultaneous operation of the glyoxylate pathway and the TCA cycle allows for reduction of acetate excretion caused by metabolic overflow and instead directs a gluconeogenic flux towards the critical and limiting substrate of lycopene production, glyceraldehyde-3-phosphate (Farmer and Liao 2001). A net effect on NADPH and NADH redox balance may also occur due to a PEP-glyoxylate cycle (Sauer, Canonaco *et al.* 2004). Deleting the *iclR* gene led to an increase in lycopene production of about 10%. The advantage of reducing acetate stress on the cells is corroborated by the fact that the GadA, GadB, and HdeB proteins previously linked to acetate stress (Arnold, McElhanon *et al.* 2001) showed up-regulation and the overexpression of a

glutamate-dependent acid stress response regulator ydeO led to a 30% increase in lycopene production compared to a control plasmid.

- NADPH availability and balance is a critical factor for lycopene production. Deleting the *pntB* membrane-bound, proton-translocating pyridine nucleotide transhydrogenase led to an approximately 25% increase in lycopene production. It is likely that this increase was due to increasing NADPH levels, either by preventing the loss of NADPH to reducing NADP+, or else by transferring NADPH production to the pentose phosphate pathway instead of from the membrane-bound transhydrogenase. Interestingly, some proteins of the upper oxidative pentose phosphate pathway were up-regulated in the Δ*gdhA* Δ*aceE* Δ_P*yjiD*, the Δ*hnr* and the Δ*hnr* Δ*yliE* strains, supporting this view. Additionally, this latter strategy of not producing NADPH via the PntAB complex would conserve the proton motive force Δp, which may be especially important given the energetic constraints previously discussed.

- The sigma S factor σ^S has far-reaching effects on both the transcriptional and proteomic expression data sets. The Δ*gdhA* Δ*aceE* Δ_P*yjiD*, the Δ*hnr* and the Δ*hnr* Δ*yliE* strains all exhibited the most differentially expressed genes and proteins, presumably due to their shared disruption of the system responsible for the degradation of σ^S, which includes Hnr (RssB) and YjiD (IraD) as central players. The RpoS protein itself, a known target for increasing lycopene production, was found to be up-regulated in the Δ*hnr* and the Δ*hnr* Δ*yliE* strains by over 9-fold and 6-fold, respectively. One protein known to be up-regulated by σ^S, WrbA, was one of the most up-regulated differentially expressed

proteins that was up-regulated from 5-fold to over 8-fold in the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjiD*, the Δ*hnr* and the Δ*hnr* Δ*yliE* strains. Overexpressing the *wrbA* gene in the PE parental strain background led to increased lycopene productivity at early time points, doubling the production at 8 hours compared to the PE strain.

Other interesting observations included evidence of membrane compositional changes and a transcriptional switch to an adhesive phenotype in addition to the up-regulation of glutamate-related targets such as ArgB, GlnA (and the *glnA* gene), and *gltB*. While gene and protein expression changes were not strictly additive between the Δ*gdhA,* the Δ*gdhA* Δ*aceE,* the Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid,* the Δ*hnr,* and the Δ*hnr* Δ*yliE* strains, a number of these common factors appear to be responsible for the high lycopene-production phenotype.

8.2. Recommendations for Future Work

While the experimental data and analysis provided by this thesis was extensive and useful in drawing a number of important and interesting conclusions, more work is suggested that build upon these observations. These recommendations are as follows:

8.2.1. Metabolic Engineering

- Combine the various successful overexpression and deletion strategies such as *wrbA* and *ydeO* overexpressions and *iclR* and *pntB* deletions to determine if the effects are additive in the PE strain.
- Examine the various successful deletion and overexpression strategies in the backgrounds of the highest producing Δ*gdhA* Δ*aceE* Δ$_{P}$*yjid* and Δ*hnr* Δ*yliE* mutant strains to test if

even greater lycopene production is possible or if maximums have been reached for these backgrounds and the improvements are specific to the PE strain.

- Pursue knockout and overexpression strategies for additional up- and down-regulated genes and proteins observed in this study. These targets include overexpressing *atpE* encoding for ATP synthase, overexpressing *relA* in the ppGpp biosynthetic pathway, and deleting potentially adhesion-associated diguanylate cyclase genes such as *yhjK* and *yeaJ*. Such strategies aimed at genes of unknown function such as the latter two may help to clarify their functions and relations to lycopene biosynthesis.
- Perform other manipulations to test observations in this study related to *iclR*, *pntB*, and the glyoxylate pathway. Specifically, test whether *pntB* overexpression decreases lycopene production, *iclR* deleted strains exhibit less acetate production during exponential as well as stationary phase, and delete the *aceAB* genes of the glyoxylate pathway to determine the effect upon production.
- Test the overproduction and deletion targets discovered in this thesis in the production of other isoprenoids and a wider array of cellular products of interest. It may be interesting to combine these targets with other previously discovered targets affecting lycopene synthesis such as those increasing gluconeogenic flux (Farmer and Liao 2001).

8.2.2. Biochemical Experiments

- Measure relative NADPH/NADP+ levels in the PE and five mutant strains of this study with and without the pAC-LYC plasmid containing the lycopene production genes. It would be interesting to observe if the mutant strains exhibit higher NADPH levels than the PE strain when they do not contain the pAC-LYC plasmid. Additionally, it would be interesting to see if NADPH levels are similar in the strains that do contain the pAC-LYC

plasmid as increased NADPH levels in those strains may be spent on lycopene biosynthesis.

- Complete follow-up experiments to measure ATP, PRPP, and quinone levels in order to further investigate the proposed energetic limitation for lycopene. This was based upon *atpE* up-regulation, *hisH* down-regulation, and WrbA up-regulation as it relates to ubiquinone levels.
- Probe the two glutamate synthesis and nitrogen assimilation pathways, the glutamate dehydrogenase and GS-GOGAT pathways, further in light of the *gdhA* deletion and the observed pattern of up-regulation of the *glnA* and *gltB* genes in some but not all of the strains. ^{15}N labeling experiments and enzymatic assays could be used for this work.

8.2.3. Omics Data Integration

- Utilize more sophisticated and computational systems biology data integration methods such as those employed by Ideker *et al.* (2001), Ishi *et al.* (2007), Covert *et al.* (2004), and Hwang *et al.* (2005) in order to test the conclusions of this work that were generated by a simpler integration of transcriptomic and proteomic data. Such approaches may also reveal additional information not reveled by the current study.

8.2.4. Omics Experiments

- Compare gene and protein expression of other high lycopene or high isoprenoid-producing strains discussed in the literature review. For example, the Δ*gdhA* Δ*aceE* Δ*fdhF* mutant of Alper *et al.* (Alper, Jin *et al.* 2005) that was also identified as a global maximum producer for lycopene would provide an interesting comparison given its relationship to the Δ*gdhA* Δ*aceE* Δ_P*yjid* strain. This would allow for a comparison of the

overlap and test common themes found amongst the five mutant strains that were investigated in this thesis. Additionally, the lowest lycopene producing strains resulting from the combination of stoichiometrically and combinatorially selected gene deletions (Alper, Miyaoku *et al.* 2005) would provide an insightful contrast to the high producing strains. Including the *E. coli* K12 strain transformed with the pAC-LYC plasmid could highlight differences in expression between this strain and the PE strain. The *E. coli* K12 strain would likely display many more expression changes compared to the mutant strains than the PE strain.

- Complete metabolic flux analysis of global maxima (and minima) producing lycopene strains for further strain characterization.
- Apply these transcriptomic, proteomic, and metabolomic strategies to the analysis of the modified strains (such as the five deletion mutant strains in this study) to better characterize how these strains differ from the PE strain due to the metabolic engineering approaches taken.
- Investigate the membrane-associated proteomic expression changes of the mutants compared to the PE strain. Due to the experimental design used, membrane-associated proteins were not examined along with the cytosolic proteins. This additional information could lend more insight into membrane changes observed in the transcriptional and proteomic data sets of this work as well as the aggregative phenotype observed in the transcriptional data. Some microorganisms are capable of accumulating up to 75% of their own biomass as lipids (Beopoulos, Chardot et al. 2009), and comparing the membrane compositions of such microorganisms with the membrane changes from the lycopene-producing strains of this thesis could be highly interesting.

Insight into optimizing lycopene storage in the cellular membrane might be gained as well.

8.3. Outlook

This thesis has provided an important example of examining and integrating diagnostic molecular data to better define and improve upon biological production systems. Taking such a systems biology approach, metabolic engineering successes in one production system can potentially be applied to other similar systems with greater understanding. In the context of lycopene production, the improvements noted in this work could be tested in the production of other related isoprenoids such as taxadiene or artemisinin. General trends such as reducing growth to boost production, preventing overflow metabolism, and manipulating cofactor availability can be pursued more readily in various hosts with the specific molecular information provided by this work. The method of examining "consensus" trends for data integration employed here serves as a starting point for improved integration methods. It is also expected that a number of additional important trends and factors may exist in the transcriptomic and proteomic data presented here that were not yet detected. Further examination of expression trends in light of additional omics data or experimental evidence could provide new insights.

This is an exciting time for the nascent field of systems biology. As approaches such as the one presented in this thesis are systematically refined and applied to more cellular systems to improve and guide metabolic engineering efforts, greater successes are expected. Timeless challenges such as curing disease, providing fuel, and growing food will become ever more pressing as world populations expand and political instabilities threaten more disastrous consequences. In light of such challenges, these scientific advancements combining systems

biology with metabolic engineering will enable mankind to better harness the power of living systems in order to confront and overcome some of our most demanding problems.

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Chapter 9. APPENDICES

9.1. All Transcriptomic and Proteomic Data

The following appendix gives all measured transcriptomic and proteomic data as $\log_{10}$(Mutant/PE) ratios for the 5 examined mutant strains, including the relative quantification proteomic data resulting from both thresholds ("T#1" and "T#2") as described in section 4.4.5. Changes corresponding to at least a 2-fold up or down change (simple change not considering statistical significance) are highlighted by red and green, respectively. Ratios for peptide threshold one data that appear as +2.171 or -2.171 are an artifact of the data processing and correspond to positive (only Mutant strain protein detection) and negative (only PE strain protein detection) "infinite" ratios, respectively. Genes are organized by Blattner number (Blattner, Plunkett et al. 1997), and gene annotations can be found using the EcoCyc database (Keseler, Bonavides-Martinez et al. 2009).

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0002	thrA	-0.023	0.018	0.026	0.132	0.080	0.043	0.048	0.039	-0.009	0.017	0.051	0.034	0.018	0.001	0.054
b0003	thrB	-0.067	0.117	0.037	-0.013	-0.032	-0.048	0.035	0.156	0.135	0.065	-0.057	-0.011	-0.032	-0.087	0.018
b0004	thrC	-0.100	0.002	0.020	-0.066		0.043	0.030	0.052	0.056	0.056	-0.006	0.013	0.026	0.066	0.029
b0006	yaaA	-0.032	0.021	0.011	-0.150	-0.018										
b0007	yaaJ	-0.022	0.000	-0.029	-0.058	-0.094										
b0008	talB	-0.033	-0.002	0.005	-0.027	-0.014	0.061	0.030	0.009	-0.013	-0.013	0.090	0.038	0.007	0.007	0.054
b0009	mog	-0.044	-0.017	-0.044	-0.061	-0.093										
b0010	yaaH	-0.031	-0.024	0.007	0.043	0.124										
b0011	yaaW	-0.030	0.002	-0.026	-0.041	-0.111										
b0013	yaaI	-0.014	0.012	0.019	-0.055	0.004						0.368	-0.035	0.182		0.466
b0014	dnaK		0.020	0.029	-0.003		-0.004	-0.004	-0.009	0.065	0.069	0.040	0.023	0.011	0.052	0.080
b0015	dnaJ	-0.112	0.002	0.050	0.038	0.072		2.171			2.171					
b0017		-0.024	-0.012	-0.029	-0.042	0.025										
b0018	mokC		0.018	0.131	-0.005	0.089										
b0020	nhaR		0.170													
b0021	insB-1									2.171						
b0022	insA-1		0.059													
b0023	rpsT		0.053	0.178			0.017	0.043	-0.013	-0.087	-0.126	0.024	-0.006	-0.045	-0.030	-0.045
b0025	ribF	0.036	0.036	-0.081	0.010	-0.006	-2.171	-2.171	-2.171	-2.171	-2.171		-0.040	-0.005	-0.074	-0.065
b0026	ileS	-0.013	-0.010	-0.029	-0.094	-0.107	0.104	0.048	-0.004	0.048	-0.017	0.043	0.014	0.009	0.012	0.048
b0027	lspA				0.566											
b0028	fkpB		0.008	0.066					2.171							
b0029	ispH	0.033	0.027	-0.006	-0.049	-0.018									0.015	
b0030	rihC	-0.009	-0.011	0.022	0.002	0.062										
b0031	dapB	-0.059	-0.077	-0.053	-0.023	-0.081	2.171	2.171	2.171		2.171	0.061	0.039	0.022	0.108	0.168
b0032	carA						0.052	0.056	0.030	0.004	0.043	-0.050	-0.063	-0.019	-0.010	-0.010
b0033	carB						-0.004	-0.009	0.004	-0.030	0.004	0.002	-0.046	-0.017	-0.014	0.007
b0034	caiF		0.021	0.012	0.010	0.052										
b0035	caiE			0.182												
b0036	caiD		0.032	-0.026		0.049										
b0037	caiC		0.021		0.079					2.171						
b0038	caiB	-0.068	-0.043	-0.019	-0.010	-0.020										
b0039	caiA					0.304										
b0040	caiT		-0.032	0.054		0.022										
b0041	fixA		0.018	0.022												
b0042	fixB							2.171								
b0043	fixC	-0.010	0.052	-0.015	-0.041	0.006	-2.171	-2.171	-2.171	-2.171	-2.171					
b0045	yaaU	0.011	0.030	-0.089												
b0046	kefF	-0.012	0.005	-0.039	0.002	-0.037										
b0047	kefC									2.171						
b0048	folA	0.003	-0.011	-0.008	-0.030	-0.038										
b0049	apaH	0.007	0.004	-0.009	-0.037	-0.024										
b0050	apaG	-0.058	0.015	0.015	-0.084	0.055										
b0051	ksgA	-0.017	0.026	0.049	-0.016	0.071	-2.171	-2.171	-2.171	-2.171	-2.171					
b0052	pdxA	-0.006	0.028	0.010	-0.003	0.024	2.171									
b0053	surA	-0.004	-0.015	-0.021	-0.020	-0.111	0.052	0.065	0.009	0.056	0.065	0.049	-0.005	0.027	0.021	0.043
b0055	djlA	0.020	0.008	0.006	-0.049	-0.030										
b0056	yabP	0.031	-0.004	0.002	-0.020	0.029										
b0057	yabQ	-0.051	0.078	0.094	0.035	0.139										
b0058	rluA	-0.045	-0.035	-0.018	-0.042	0.007										
b0059	hepA								2.171							
b0060	polB		-0.013													
b0061	araD		0.019					2.171								
b0062	araA						-2.171	-2.171	-2.171	-2.171	-2.171					
b0063	araB								2.171							
b0066	thiQ		0.164													
b0067	thiP		0.049	0.045												
b0068	tbpA	-0.064	-0.020	0.012	-0.009	-0.039	2.171									
b0069	sgrR	0.040	0.012	-0.009		0.035										
b0070	setA		0.089													
b0071	leuD	-0.052	0.002	0.018		-0.092	0.035	-0.004	-0.026	-0.035	0.087	0.025	0.026	-0.029	-0.019	0.100
b0072	leuC	0.063	-0.008	-0.056	-0.058	-0.113	0.056	0.000	0.004	-0.013	0.096	0.037	0.017	-0.008	-0.034	0.067
b0073	leuB	-0.033	0.006	0.000	0.023	-0.029	-0.026	0.013	-0.078	0.009	0.030	0.013	0.030	-0.034	0.014	0.012
b0074	leuA			0.292			-0.004	0.043	-0.004	-0.009	0.026	0.027	0.031	0.019	0.041	0.138
b0077	ilvI	-0.011	-0.054	-0.049	-0.019	-0.099	2.171					0.010		-0.057	-0.005	0.211
b0078	ilvH	0.072	0.012	0.036	0.180	0.286	-0.104	0.117	0.004	0.117	0.026	0.025	-0.064	0.018	-0.144	0.146
b0079			0.099		-0.023	1.453										
b0080	fruR							2.171				0.087		0.065	0.110	0.162
b0081	mraZ	0.008	-0.049	0.003	-0.085	-0.090										
b0083	ftsL	0.079	-0.007	0.025	-0.011	-0.075										
b0084	ftsI								2.171		2.171					
b0085	murE	0.216	-0.025	-0.042	0.019	0.048		2.171	2.171			0.014		0.039	0.158	
b0086	murF				0.033	0.148										
b0087	mraY	0.021	-0.004	-0.018	0.029	0.027										
b0088	murD						2.171									
b0092	ddlB	0.034	0.058	-0.044	-0.008	-0.077										
b0094	ftsA		-0.019	0.087							2.171					

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0095	ftsZ			0.150			0.130	0.135	0.065	0.156	0.195	0.045	0.087	0.036	0.101	0.131
b0096	lpxC	-0.074	-0.035	-0.097	-0.050	-0.056										
b0097	secM	0.021	0.028	0.106	0.033	0.098	-2.171	-2.171	-2.171	-2.171	-2.171					
b0098	secA	-0.035	-0.009	-0.023			-0.013	-2.171	-0.174	-0.087	-0.022	0.000	-0.076	-0.015	-0.007	0.015
b0100		0.130	0.008		0.004	0.043										
b0101	yacG	-0.068	0.034	0.010	-0.104	-0.007	2.171									
b0102	yacF	-0.074	0.026	-0.100	-0.072	-0.024										
b0103	coaE		0.068	-0.033	0.108											
b0104	guaC	0.001	0.026	0.004	0.002	-0.011	0.204	0.178	0.117	0.200	0.252	0.001	-0.027	-0.029	-0.065	0.004
b0106	hofC	0.004	0.047													
b0107	hofB		0.049	-0.072												
b0108	ppdD	-0.084	0.033	0.031	0.058											
b0109	nadC	-0.048	-0.018	-0.004	-0.018	-0.029										
b0110	ampD		0.025	0.128	0.028	0.022					2.171					
b0114	aceE	-0.035	-0.034	-0.055	-0.103	-0.124	0.022	-0.026	-0.026	0.030	0.004	0.019	-0.013	0.003	0.028	0.008
b0115	aceF	0.075	0.016	0.030			-0.030	-0.026	-0.013	0.013	0.043	0.013	-0.025	-0.008	0.011	0.028
b0116	lpd	-0.047	0.046	-0.024	-0.047	-0.126	-0.056	-0.048	-0.039	-0.052	-0.030	-0.048	-0.024	0.000	-0.055	0.018
b0117	yacH	-0.016	0.031	-0.013	-0.085	-0.010										
b0118	acnB	-0.063	-0.038	-0.039	-0.073	-0.197	-0.017	-0.009	-0.056	-0.161	-0.274	-0.037	-0.021	-0.064	-0.110	-0.230
b0119	yacL	0.045	0.099	0.129						2.171						
b0120	speD	0.091	0.026	0.062	0.121	0.165	-2.171	-0.091	-2.171	-2.171	-2.171	0.059	-0.130	-0.354	0.022	
b0121	speE		-0.017		0.214											
b0123	cueO		0.055	-0.495	0.097		-0.187	-2.171	0.117	-0.004	-2.171	-0.098	0.048	0.074	0.140	0.121
b0124	gcd						-2.171	-2.171	-2.171	0.187	-0.087					
b0125	hpt									2.171	2.171					
b0126	can	0.011	-0.025	-0.017	0.009	-0.040	-0.069	0.152	-0.017	0.061	0.109	0.059	0.184	0.092	0.055	0.189
b0127	yadG		0.087						2.171		2.171					
b0128	yadH	-0.057	0.033	-0.010	-0.078	-0.029										
b0129	yadI	0.011	0.004	-0.022	-0.001	-0.097										
b0130	yadE	-0.005	-0.004	-0.015	-0.019	-0.002			2.171							
b0131	panD	-0.033	-0.021	0.012	0.028	0.015		2.171								
b0132	yadD	-0.034	0.070	0.002	-0.009	0.024										
b0133	panC	-0.006	-0.014	-0.018	0.027	0.040	0.013	0.009	-0.048	-0.004	-0.035	0.053	0.014	-0.057	-0.046	0.057
b0134	panB	-0.060	0.014	0.020	0.025	-0.031	-0.565	-0.877	-0.929	-0.799	-0.699	-0.077	0.065	-0.670	-0.109	-0.150
b0135	yadC			0.161												
b0137	yadL	0.049	0.024	0.012												
b0138	yadM	0.028	0.046	0.036	0.068	0.090										
b0139	htrE	-0.020	-0.028	-0.194	-0.045	-0.140										
b0140	ecpD	-0.083	-0.015													
b0141	yadN	0.000	0.006	0.025	0.191											
b0143	pcnB	-0.004	-0.016	-0.028	-0.039			2.171	2.171							
b0144	yadB		-0.135	-0.054		0.002										
b0145	dksA		-0.143				-0.030	-0.013	-0.009	0.043	0.126	-0.055	0.047	-0.079	-0.058	-0.081
b0146	sfsA							2.171								
b0147	ligT	-0.062	0.037	0.025	-0.010	0.049										
b0148	hrpB	-0.058	-0.019	-0.066	-0.094	-0.085										
b0149	mrcB	0.032	0.000	-0.043	-0.105	0.069			2.171	2.171					0.064	
b0152	fhuD	0.017	0.094	0.066	-0.009	0.017										
b0153	fhuB	-0.077	-0.033	-0.057	-0.069	-0.129										
b0154	hemL	-0.048	0.009	-0.012	0.051	0.096	0.122	0.113	0.013	0.130	0.083	-0.013	0.051	0.009	0.113	0.069
b0155	clcA		0.084		0.364	0.600										
b0156	erpA	0.002	0.038	0.038	0.084	0.005	2.171	2.171	2.171	2.171		0.033	0.039	0.035	0.077	0.006
b0157	yadS		-0.705													
b0158	btuF	-0.037	0.016	0.015	0.076	0.104										
b0159	mtn	0.058	-0.023	0.018												
b0160	dgt	-0.046	-0.015	0.015	-0.083	-0.075										
b0161	degP	0.003	0.011	0.011	0.019	0.028	0.078	-0.013	-0.026	0.056	0.039	0.041	-0.007	0.044	0.056	0.122
b0162	cdaR		0.045		-0.024											
b0163	yaeH	0.016	0.055	-0.005	-0.003	0.071	2.171	2.171		2.171	2.171					
b0164	yaeI	0.104	-0.044	0.037	0.037	-0.019										
b0165		0.043	-0.022	0.020	0.037	0.093										
b0166	dapD	0.000	-0.004	-0.038	-0.061	-0.065	0.065	0.043	0.078	0.022	-0.100	0.080	0.046	0.097	0.000	-0.041
b0167	glnD	-0.036	-0.025	-0.050	-0.044	-0.033			2.171	2.171		-0.021	0.002	-0.040	-0.021	0.025
b0168	map		-0.038	-0.045	-0.088					2.171						
b0169	rpsB	-0.006	0.043	0.001	0.059	0.126	0.069	0.030	0.026	0.022	0.004	0.098	0.037	0.038	0.021	0.044
b0170	tsf	-0.040	-0.016	-0.081	-0.058	-0.051	-0.013	0.000	0.009	0.013	0.043	-0.003	-0.010	0.041	0.005	0.043
b0171	pyrH	-0.092	0.002	-0.006	0.073	0.011	0.004	0.022	-0.052	0.061	0.143	0.066	0.029	-0.069	0.028	0.105
b0172	frr	-0.061	-0.041	-0.099	-0.132	-0.154	0.035	0.052	0.074	0.104	0.161	-0.050	-0.007	0.056	0.080	0.146
b0173	dxr		0.131	0.084	0.083	0.011										
b0174	ispU	-0.137	0.051	0.077												
b0175	cdsA	-0.032	0.029	-0.006	-0.008	-0.015										
b0176	rseP	-0.030	0.007	0.001	-0.034	0.027										
b0177	bamA	0.052	0.018	-0.019	-0.032	0.028				2.171						
b0178	hlpA	0.087	-0.015	-0.077		-0.073	-0.117	-0.078	-0.065	-0.174	0.009	-0.082	-0.060	-0.048	-0.115	0.060
b0179	lpxD	-0.035	-0.020	0.019	0.015	0.039										
b0180	fabZ	0.000	-0.023	-0.004	0.048	-0.070										
b0181	lpxA						2.171		2.171	2.171		-0.089	0.038		0.128	0.111

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0182	lpxB											-0.003	-0.129	0.012	0.115	
b0183	rnhB		-0.176	0.033	0.089	0.156										
b0184	dnaE	0.022	-0.020	-0.005	0.148	0.231			2.171							
b0185	accA	-0.060	-0.025	-0.046	-0.012	-0.054	-0.043	-0.022	-0.035	-0.026	-0.039	0.010	-0.019	0.001	-0.049	-0.004
b0186	ldcC	-0.062	-0.003	-0.004	-0.060	-0.104										
b0187	yaeR	-0.084	-0.048	-0.063	-0.048	-0.020										
b0188	tilS		-0.014	0.010		0.087	-2.171	-2.171	-2.171	-2.171	0.000					
b0189	rof	0.017	-0.017	0.065	0.096	0.179										
b0191	yaeJ	0.231	0.017	-0.263												
b0192	nlpE	0.025	0.038	0.042	-0.152											
b0193	yaeF	0.001	0.047	0.016	-0.019	-0.112										
b0194	proS	-0.079	0.020	-0.023	-0.060	-0.051	-0.009	-0.009	-0.004	-0.048	-0.022	-0.005	-0.026	-0.011	-0.024	0.005
b0195	yaeB	0.045	0.047	0.044	0.011	0.034										
b0198	metI	0.047	0.022	-0.104	-0.072	0.004										
b0199	metN								2.171							
b0200	gmhB	-0.025	0.013	0.058	0.075	0.052										
b0207	dkgB	0.248	0.026	0.038	0.157											
b0208	yafC							2.171								
b0210	yafE	0.052	0.018	0.001	0.014	0.023										
b0211	mltD	-0.001	0.041	0.000	-0.027	-0.141										
b0212	gloB	0.043	0.026	0.035	0.068	0.176				2.171						
b0213	yafS	-0.048	-0.034	-0.041	-0.010	-0.092										
b0214	rnhA	-0.024	-0.012	-0.006	0.075	0.161										
b0217	yafT	0.054	-0.012	-0.012	0.030	-0.158	-2.171	-2.171	-2.171	-2.171	-2.171					
b0218	yafU	0.003	0.008	0.018	0.046	0.067										
b0219	yafV	-0.061	-0.062	-0.069	0.001	-0.060										
b0220	ivy	-0.028	0.005	-0.025	-0.001	-0.017										
b0221	fadE	0.297	0.093	0.004	0.033	0.137										
b0222	lpcA	-0.189	-0.011		-0.040	-0.022	-0.022	0.091	-0.056	-0.148	-0.052	0.004	0.052	-0.023	0.028	0.042
b0224	yafK	0.110	0.021	0.046	0.000	-0.104										
b0225	yafQ	0.198	0.093													
b0226	dinJ	-0.003	0.039	0.027	-0.053	-0.048										
b0227	yafL	-0.224	0.034	-0.197		0.088										
b0228	yafM		0.154													
b0229					0.106											
b0230		0.019	0.017	0.023	0.031	0.155										
b0231	dinB	-0.002	0.054	0.022	0.035	0.027										
b0232	yafN	-0.033	-0.016	-0.022	-0.125	-0.017										
b0233	yafO	0.150	-0.023	-0.053	-0.095	-0.043										
b0235		0.066	-0.003	0.119	0.012	-0.086										
b0236		-0.193	-0.056	0.172	-0.003											
b0237	pepD	-0.090	0.004	0.006	0.057	0.004	0.426	-2.171	0.022	0.343	0.122	0.012	0.081	0.042	0.022	0.128
b0238	gpt	0.003	0.018	-0.006	-0.033	-0.033										
b0239	frsA				-0.140	-0.059	-2.171	-2.171	-2.171	-2.171	-2.171	-0.081	0.064	0.089	0.047	0.083
b0240	crl						2.171	2.171	2.171		2.171	-0.008	-0.097	0.010	0.061	-0.001
b0241	phoE								2.171							
b0242	proB	0.000	-0.011	-0.059	-0.066	-0.026				2.171						
b0243	proA	-0.015	-0.061	-0.049	-0.013	-0.074	2.171	2.171	2.171	2.171	2.171	0.015	0.000	-0.147	0.060	-0.017
b0245	ykfI	-0.061	-0.024	-0.014	-0.132	-0.043										
b0246	yafW	0.059														
b0247	ykfG	-0.096	-0.047	-0.046	0.057	-0.042										
b0249	ykfF	-0.008	-0.060	-0.004	-0.015	0.097										
b0251	yafY		-0.137	-0.138	-0.022	0.099		2.171								
b0253	ykfA	-0.029	0.019	0.169												
b0255	insN-1	-0.053	-0.073	-0.103	-0.090	-0.123										
b0259	insH-1	-0.004	0.091	-0.029												
b0260	mmuP	0.022	0.033	0.175	0.011	0.022										
b0261	mmuM		0.078													
b0262	afuC	0.098														
b0264	insB-2									2.171						
b0266	yagB	0.206	0.031			0.211										
b0267	yagA	0.025	0.059	0.094	-0.012	-0.181										
b0268	yagE		0.090	0.247				2.171								
b0272	yagI			0.237												
b0273	argF	-0.013	0.009	0.000	-0.033	-0.049	-0.069	0.000	-0.013	-0.035	-0.083					
b0274	insB-3	-0.009	0.013	-0.090		0.013				2.171						
b0276	yagJ				0.160	0.190										
b0279	yagM	0.023	0.014	-0.050	0.058	0.031										
b0280	yagN	0.007	-0.035	0.009	-0.052	-0.042										
b0282	yagP				-0.025	-0.005										
b0283	yagQ				0.044											
b0286	yagT	0.013	0.003	0.014	0.104	0.200										
b0287	yagU		0.070													
b0288	ykgJ						-2.171	-2.171	-2.171	-2.171	-2.171					
b0291	yagX						-0.104	-2.171	-2.171	-2.171	-2.171					
b0292	matC									2.171						
b0293	matB	-0.028	0.006	0.084	0.083	0.104										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0294	matA		-0.099		0.066											
b0296	ykgM	-0.011	0.031	-0.008	-0.021	-0.124										
b0297									2.171							
b0298	insE-1	0.217	0.170													
b0300	ykgA	0.128			0.060											
b0303	ykgI					-0.167										
b0304	ykgC	0.143	0.010	0.027	0.059	0.012										
b0305	ykgD		0.042													
b0306	ykgE				0.083						2.171					
b0307	ykgF	0.029	-0.059	0.001												
b0308	ykgG	-0.023	0.027	-0.011	0.012	-0.030	-2.171	-2.171	-2.171	-2.171	-2.171					
b0310	ykgH	-0.046	-0.143	-0.191	-0.030	-0.070										
b0311	betA	0.003	0.031	0.009	0.016	0.023										
b0312	betB	-0.006	0.013	-0.005	-0.001	-0.006	0.208	-2.171	-2.171	-0.074	-2.171					
b0313	betI	-0.035	-0.003	-0.026	0.007	0.010	2.171				2.171					
b0314	betT	-0.013	-0.040	-0.102	-0.134	-0.136										
b0315	yahA						2.171									
b0318	yahD	0.084	0.015	-0.040												
b0322	yahH	0.275	-0.001	0.024		0.040										
b0323	yahI	-0.051	-0.015	-0.012	-0.009	0.036										
b0324	yahJ		0.077	0.076		0.088										
b0325	yahK							2.171	2.171	2.171	2.171	-0.004	-0.036	0.096	0.468	0.379
b0326	yahL	-0.029	-0.012	0.056	-0.008	0.012										
b0327	yahM	0.033	-0.025	-0.067	-0.098	-0.066										
b0328	yahN	-0.081	-0.014	-0.035	-0.044	-0.037										
b0329	yahO	-0.004	-0.039	-0.049	0.022	-0.042			2.171	2.171	2.171	0.061	0.115	0.268	0.478	0.520
b0330	prpR						-2.171	-2.171	-2.171	-2.171	-2.171	0.011	-0.028	-0.047	-0.104	-0.027
b0331	prpB	-0.004	0.063	0.086	0.217	0.404										
b0333	prpC	0.132	0.175	0.295	0.137	0.024										
b0335	prpE	-0.110	-0.033	-0.049	0.007	0.059		2.171								
b0336	codB		-0.005	0.041	0.017	0.161										
b0337	codA	-0.002	-0.041	-0.038	0.003	0.025	-0.122	0.061	-0.083	-0.126	-2.171	-0.056	0.007	-0.046	-0.008	-0.032
b0339	cynT	0.274	0.220	0.075	0.007	-0.010										
b0340	cynS	0.032	0.078	0.035	0.070	0.064										
b0341	cynX		0.175	0.027												
b0342	lacA						2.171									
b0343	lacY	-0.094	0.085	-0.010	0.068	0.047										
b0344	lacZ		-0.146				-2.171	-2.171	-2.171	-2.171	-2.171					
b0346	mhpR	-0.059	0.033	-0.053	-0.159		-2.171	-2.171	-2.171	-2.171	-2.171	-0.058	-0.101	-0.048	-0.021	0.169
b0348	mhpB										2.171		0.024		0.096	-0.049
b0349	mhpC		-0.069	-0.012	-0.086				2.171		2.171					
b0350	mhpD		-0.026	0.042												
b0353	mhpT	-0.003	0.002	-0.004	-0.012	-0.018										
b0355	frmB	-0.002	0.012	-0.021	-0.028	0.027										
b0356	frmA	-0.009	0.039	-0.041	0.021	0.071										
b0357	frmR	-0.063	-0.042	0.089												
b0358	yaiO	-0.106	0.063	-0.009	-0.163	0.109										
b0359	yaiX	-0.074	-0.041	-0.039	0.018	0.109										
b0360	insC-1	0.064	0.024	0.147	0.343	0.334			2.171		2.171					
b0361	insD-1			-0.025												
b0362	yaiF	0.058	0.006	0.009	-0.036	0.053										
b0363	yaiP	0.048	-0.032	-0.025	-0.038	-0.290										
b0365	tauA	0.116	0.042	0.003	0.023	0.029	-2.171	-2.171	-2.171	-2.171	-2.171					
b0366	tauB		0.106													
b0367	tauC		0.086		-0.043	0.018										
b0368	tauD	0.006	-0.049	0.009	-0.023	0.055										
b0369	hemB	-0.002	0.004	0.022	0.018	0.003				2.171	2.171					
b0370			0.031	0.154	0.095	-0.004										
b0371	yaiT	-0.055	0.010	0.030	-0.050	0.020										
b0373	insE-2	0.065	-0.125		0.109	-0.052										
b0374	yaiU	-0.012	0.020	-0.032	-0.109	-0.069										
b0376	ampH						2.171	2.171		2.171						
b0377	sbmA		0.141	0.040												
b0378	yaiW	-0.012	0.053	0.013	-0.043	0.081										
b0379	yaiY	-0.027	0.011	0.084	0.083	0.116										
b0380	yaiZ	-0.054	-0.002	-0.045	-0.070	-0.075										
b0381	ddlA								2.171							
b0382	iraP	-0.028	0.018	0.009	-0.060	-0.046										
b0383	phoA		-0.044	0.005												
b0384	psiF	0.005	-0.060	0.088	0.084	0.015										
b0385	adrA	-0.042	-0.013	-0.011	-0.091	-0.052										
b0386	proC									2.171						
b0388	aroL	-0.009	-0.014	-0.032	-0.032	-0.142										
b0389	yaiA	-0.091	-0.083	-0.152	-0.127	-0.105										
b0390	aroM	0.001	-0.011	-0.064	-0.078	-0.050										
b0391	yaiE	0.055	0.010	0.037	-0.015	0.032										
b0392	ykiA	-0.069	0.002	0.005	-0.025	0.009										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0393	rdgC	-0.042	0.040	-0.050	-0.076	0.012		2.171		2.171	2.171					
b0394	mak	-0.054			0.234	0.161	2.171	2.171		2.171	2.171					
b0395			-0.045		-0.008											
b0396	araJ	0.354	0.046													
b0397	sbcC	0.004	0.011	0.052	0.099	0.177										
b0398	sbcD	-0.060	0.010	-0.038	-0.162	-0.014										
b0399	phoB	-0.020	0.022	-0.039		-0.087										
b0401	brnQ	-0.029	-0.022	-0.029	-0.032											
b0402	proY	0.051	-0.006	-0.072	-0.088	-0.061										
b0403	malZ	0.029	0.000	0.035	0.140	0.172										
b0404	acpH								2.171							
b0405	queA	-0.060	-0.028	-0.036	-0.039	-0.035										
b0406	tgt	0.042	0.016	-0.090		-0.024	-2.171	-2.171	-2.171	0.104	-2.171					
b0408	secD		-0.002	-0.023	0.075	-0.066	2.171									
b0409	secF		0.069	0.052	-0.015	0.098			2.171							
b0410	yajD	-0.032	0.005	-0.031	0.001	0.017										
b0412	yajI	-0.049	0.003	-0.099	0.012	-0.084										
b0413	nrdR									2.171						
b0414	ribD	-0.024	-0.026	-0.064	-0.050	-0.022	2.171									
b0415	ribE	0.025	0.083	0.041	0.066	0.054	0.026	-0.035	-0.013	-0.022	0.065	0.131	0.054	-0.001	0.004	0.235
b0416	nusB	-0.022	-0.027	-0.015	0.001	0.010						-0.026	0.068	0.048	0.010	
b0417	thiL									2.171		0.111	-0.071	-0.024	-0.043	0.016
b0418	pgpA		0.022	-0.066	0.035	0.123										
b0419	yajO		-0.015	0.207												
b0420	dxs						0.035	0.030	0.004	0.100	-0.230	0.091	0.073	0.039	0.109	-0.124
b0421	ispA	-0.028	-0.015	-0.031	0.064	0.029										
b0422	xseB	-0.008	0.008	-0.003	-0.043	-0.013							-0.050	0.017	0.039	0.112
b0423	thiI							2.171		2.171	2.171					
b0424	yajL					0.283			2.171							
b0425	panE				0.025											
b0426	yajQ	0.010	-0.091	-0.057	0.046	-0.056	0.065	0.035	0.065	0.156	0.130	0.049	0.049	0.043	0.080	0.111
b0427	yajR	0.009	0.015	0.024	0.148	0.240										
b0428	cyoE	0.244	0.135	-0.078												
b0429	cyoD	-0.064	-0.036	-0.044	-0.102	-0.298										
b0430	cyoC	-0.085	-0.032	-0.057	-0.105	-0.271										
b0431	cyoB	-0.104	0.005	-0.034	-0.064	-0.118										
b0432	cyoA			0.088			-2.171	-2.171	-2.171	-2.171	-2.171	-0.009		-0.096	-0.241	
b0434	yajG	0.038	-0.003	0.052		0.083					2.171					
b0435	bolA										2.171					
b0436	tig						0.004	0.017	0.004	-0.035	-0.052	0.012	0.018	0.003	-0.027	-0.054
b0437	clpP	0.015	-0.037	-0.009	-0.157	-0.189										
b0438	clpX						-0.052	-0.035	0.022	0.087	0.161	-0.011	0.014	0.046	0.044	0.095
b0439	lon						-0.022	-0.026	-0.061	-0.113	0.174	-0.018	0.045	0.000	0.014	0.076
b0440	hupB	0.014	-0.013	-0.075	0.103	0.022	-0.091	-0.074	-0.161	-0.230	-0.156	-0.059	-0.155	-0.004	0.040	-0.055
b0441	ppiD							2.171								
b0442	ybaV	-0.029	-0.019	0.017	-0.057	-0.120										
b0443	ybaW	-0.006	0.028	0.031	-0.037	-0.087										
b0444	queC		-0.130		-0.010											
b0445	ybaE				-0.146						2.171	0.245		0.126		
b0446	cof	0.019	0.063	-0.010	-0.053	-0.017			2.171		2.171					
b0447	ybaO					0.006										
b0448	mdlA		-0.027	-0.039	-0.033	-0.012										
b0449	mdlB							2.171								
b0450	glnK	0.054	-0.010	0.063	-0.019	-0.001										
b0451	amtB	0.122	-0.006	0.093												
b0452	tesB	-0.003	0.007	-0.037	0.094	0.033										
b0453	ybaY							-2.171	0.052	0.478	0.469	0.002	-0.104	0.161	0.519	0.745
b0454	atl	-0.037	-0.056	-0.051	-0.092	-0.060										
b0456	ybaA	-0.046	0.012	-0.026	-0.069	0.008										
b0457	ylaB	-0.479														
b0458	ylaC	0.037	0.054	0.035	0.040	0.052										
b0459	maa	0.029	-0.009		0.262	-0.012										
b0460	hha	-0.088	-0.028	-0.077	-0.127	-0.193										
b0462	acrB		0.032	0.052	0.006				2.171	2.171	2.171					
b0464	acrR		0.047	0.087	0.034	0.167										
b0465	kefA		0.051			0.033										
b0467	priC	-0.036	-0.005	-0.005												
b0468	ybaN	0.142	0.087		0.100											
b0469	apt	-0.011	-0.028	-0.034	0.001	0.052	-2.171	-2.171	0.043		-2.171					
b0470	dnaX	0.015	0.027	0.054	0.148	0.244										
b0472	recR	-0.013	0.007	0.076		0.102										
b0473	htpG	-0.023	-0.025	-0.009	0.044	0.035	0.043	0.043	0.022	0.117	0.048	0.023	0.022	-0.018	0.084	0.066
b0474	adk	-0.030	0.045	-0.010	0.008	0.031	-0.048	-0.022	0.009	0.013	0.030	0.003	0.029	0.091	0.042	0.107
b0475	hemH	-0.010	0.064	0.133	-0.023	0.009										
b0476	aes	0.101	0.013	0.005												
b0477	gsk	-0.037	-0.069	-0.092	-0.013	0.050	-2.171	-2.171	-2.171	-2.171	-2.171					
b0478	ybaL	-0.047	0.007	0.019	0.015	-0.066										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0479	fsr	-0.047	-0.044	-0.038	-0.041	0.006										
b0480	ushA	-0.050	-0.066	-0.020	-0.042	-0.026					2.171					
b0481	ybaK									2.171	2.171					
b0482	ybaP	-0.031	0.009	-0.045	0.023	0.008										
b0483	ybaQ					-0.257										
b0484	copA	0.028	0.024	0.013	0.035	0.035										
b0485	ybaS			0.079		-0.152										
b0486	ybaT	0.104	0.056	0.030	0.073	0.124										
b0487	cueR		0.050	0.003	0.038											
b0488	ybbJ	-0.009	0.025	-0.003	-0.018	-0.042										
b0489	qmcA		0.037	0.044	-0.039											
b0490	ybbL	-0.027	-0.005	-0.008	-0.056	0.023			2.171							
b0491	ybbM		0.055													
b0492	ybbN		0.143	-0.525	0.056	-0.355	0.091	-2.171	-2.171	-2.171	-0.221	0.167	0.012	0.007	0.028	0.136
b0493	ybbO	-0.012	0.032			-0.056										
b0494	tesA	-0.029	0.007	0.029	0.150	0.113										
b0495	ybbA				0.013	0.129										
b0496	ybbP								2.171							
b0497	rhsD						-2.171	-2.171	-2.171	-2.171	-2.171	-0.009	-0.026	-0.002	-0.010	-0.011
b0499	ylbH		-0.076													
b0500	ybbD	0.001	-0.019	-0.035	-0.049	-0.114										
b0501		0.001	-0.023	-0.020	-0.032	-0.042										
b0502	ylbG	-0.056	-0.080	-0.031	-0.046	-0.176										
b0505	allA	-0.017	-0.005	-0.050	-0.069	0.001										
b0507	gcl	0.037	0.020	0.018	-0.085	-0.010										
b0508	hyi	0.004	0.041	0.098	0.165	0.348										
b0509	glxR	-0.003	0.006	-0.019	0.000	-0.003										
b0510	ybbV	0.124	0.012	0.009	-0.034	-0.002										
b0511	ybbW		-0.005	-0.065	-0.020	0.045										
b0512	allB	-0.107	-0.012	-0.008	0.048	-0.035				2.171						
b0513	ybbY	-0.064	0.034	0.077	0.121	0.044										
b0514	glxK	0.041	0.068	0.010	0.009	0.039										
b0516	allC		-0.019	0.025												
b0517	allD	-0.011	0.038	-0.010	-0.045	-0.055										
b0518	fdrA	0.004	0.021	0.056	0.037	0.051										
b0520	ylbF	-0.024	-0.066	-0.086	-0.060	-0.135										
b0521	ybcF										2.171					
b0522	purK	0.024	0.021	-0.005	-0.025	-0.026										
b0523	purE						-0.334	-0.113	-0.074	-0.065	-0.122	-0.111	-0.069	-0.021	0.065	0.101
b0524	lpxH				0.033					2.171						
b0525	ppiB	-0.100	0.073	0.035	-0.057	0.016					2.171	-0.013	0.014	-0.049	0.095	0.174
b0526	cysS	-0.089	0.008	0.006	-0.010	-0.003	2.171	2.171	2.171	2.171		0.026	-0.009	0.035	-0.039	0.079
b0529	folD	0.038	-0.005	-0.035	0.144											
b0530	sfmA		0.073	0.040	0.040	0.039										
b0533	sfmH	-0.052	-0.029	-0.057	-0.025	-0.118										
b0535	fimZ			-0.453												
b0537	intD	-0.066		-0.139	-0.163											
b0538						-0.225										
b0539	ybcC	-0.111	-0.039	-0.048	-0.132	-0.033										
b0540	insE-3				0.132											
b0541	insF-3				0.093											
b0542	renD			-0.017	-0.026											
b0545	ybcL	0.011	-0.013	-0.091	-0.079	-0.076										
b0546	ybcM	0.006	-0.023	-0.039	-0.043	-0.144		2.171								
b0547	ybcN		0.120													
b0548	ninE	-0.010	-0.019	-0.028	-0.021	-0.016										
b0549	ybcO	-0.055	-0.088	-0.137	-0.062	-0.097										
b0550	rusA	0.017	-0.080	-0.075	-0.074	-0.013										
b0551	ybcQ		0.013	-0.070												
b0553	nmpC	-0.114		0.090	0.087	-0.039										
b0554	essD				0.116											
b0555	ybcS	-0.063	0.008	0.020	0.005	-0.055										
b0556	rzpD	-0.062	-0.157	-0.166	-0.146	-0.283										
b0557	borD	0.035	-0.005	0.095	0.084	0.206										
b0559	ybcW	-0.148	-0.003		-0.035	0.020										
b0560	nohB												0.095			
b0561	tfaD	0.046	0.008	0.014	-0.137	-0.172										
b0562	ybcY	-0.318	0.005	0.038	-0.107	-0.023										
b0563	tfaX		0.052			-0.058										
b0564	appY	-0.075	0.004	-0.025	-0.020	0.012				2.171						
b0568	nfrA						2.171									
b0569	nfrB									2.171	2.171					
b0570	cusS						2.171									
b0572	cusC	-0.029	0.025	-0.047	-0.069	-0.020										
b0573	cusF	-0.090	0.017		-0.078	-0.277								-0.208	-0.500	
b0575	cusA								2.171							
b0576	pheP	-0.061	0.062	0.071	-0.014	-0.055										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0577	ybdG					-0.094										
b0578	nfsB	-0.043	0.058	-0.026	0.016	-0.248	0.026	0.152	0.135	-2.171	-2.171	-0.031	0.065	-0.001	0.027	0.021
b0581	ybdK	-0.073	-0.102	-0.260	-0.303	-0.552	2.171			2.171						
b0583	entD	-0.028	0.006	-0.039	-0.063	-0.032			2.171							
b0584	fepA	-0.027	0.055	-0.036	-0.105	-0.065	-2.171	-2.171	-2.171	-2.171	-2.171					
b0585	fes	-0.158	-0.002	0.002	0.037											
b0586	entF	0.057	0.043	0.101		0.086	2.171							0.072		
b0587	fepE	0.016	0.067	0.087	-0.089	-0.053										
b0588	fepC		-0.085													
b0589	fepG	-0.029	-0.009	-0.070	-0.068	-0.198										
b0590	fepD	0.041	-0.057	-0.157	-0.097	-0.259	-2.171	-2.171	-2.171	-2.171	-2.171	-0.002	0.026	0.038	0.081	0.075
b0591	entS	0.113	-0.008	-0.048	-0.016											
b0593	entC	-0.014	0.006	0.023	0.029	0.027			2.171		2.171					
b0594	entE	0.046	0.061	0.046	0.080	0.105										
b0595	entB	0.015	-0.011	-0.009	0.024	0.087		2.171								
b0596	entA	-0.082	0.033	0.048	0.115	0.045										
b0599	ybdH	-0.132	-0.017	-0.095	0.067	0.014										
b0600	ybdL	-0.128	-0.002	-0.049	0.027	0.017			2.171							
b0601	ybdM					0.169										
b0602	ybdN	0.014	-0.002	0.026	-0.007	0.110										
b0604	dsbG	0.052	-0.046	-0.078			-2.171	-2.171	-2.171		0.287	0.047		-0.082	0.046	0.196
b0605	ahpC	0.150	0.017	0.013	-0.336	-0.022	0.048	0.022	0.039	-0.004	-0.039	0.018	0.002	0.032	-0.018	-0.007
b0606	ahpF		0.099				0.009	-0.009	0.052	-0.004	-0.017	0.020	-0.023	0.022	-0.007	-0.007
b0607	uspG					0.084	-2.171	-2.171	-2.171	-2.171	-2.171					
b0609		0.056	0.059	0.071	0.062	0.061										
b0610	rnk						-2.171	-2.171	-2.171	-2.171	-2.171					
b0611	rna	-0.033	0.020	-0.060	-0.104	-0.064										
b0612	citT	-0.034														
b0613	citG	-0.037	-0.026	0.101	0.003	-0.134										
b0614	citX	-0.055	-0.053	-0.075	-0.043	-0.004										
b0615	citF	-0.010	0.008	-0.026	0.028	-0.284					2.171					
b0616	citE	0.001	-0.006	0.001	-0.120	-0.017	-2.171	-2.171	0.039	-2.171	-2.171		0.051	-0.030	0.002	0.061
b0617	citD	-0.010	0.079	0.040	-0.051	-0.074										
b0618	citC	0.021	0.053	0.172	0.530	0.699										
b0621	dcuC		-0.041													
b0622	pagP	-0.062	-0.001	0.025	-0.081	-0.055										
b0623	cspE	0.068	-0.005	-0.003	-0.029	-0.051	-0.056	-0.043	0.022	-0.043	0.065	-0.040	-0.005	0.034	-0.007	0.072
b0625	ybeH	-0.028	0.008	0.032	-0.044	-0.049										
b0626	ybeM	0.054	0.045	0.116	0.271	0.418				2.171						
b0627	tatE	-0.030	-0.011	0.013	-0.033	0.038										
b0628	lipA	-0.062	-0.038	-0.001	0.019	-0.138										
b0629	ybeF	-0.032	-0.031	-0.004	-0.040	-0.027										
b0630	lipB	-0.069	0.016	0.031												
b0631	ybeD		0.100				-0.004	0.048	-0.096	0.009	0.178	0.008	-0.061	-0.051	0.054	0.122
b0632	dacA		-0.004	-0.006		-0.068	-2.171	-0.647	-2.171	0.096	0.104	0.040	0.046	-0.004	0.027	0.028
b0633	rlpA	-0.038	-0.001	-0.059	-0.029	-0.133										
b0635	mrdA	-0.062	0.080					2.171								
b0637	ybeB	0.427	0.013	0.000	0.081	0.105										
b0638	cobC	-0.018	-0.006	0.003	0.027	0.015										
b0640	holA		-0.012													
b0641	lptE													0.188		0.317
b0642	leuS	-0.009	0.011	-0.008	-0.035	-0.005	-0.030	0.030	0.000	-0.022	-0.009	0.024	0.000	-0.024	0.023	0.018
b0643	ybeL												0.070	0.258	-0.015	0.019
b0644	ybeQ	-0.092	0.003			0.057					2.171					
b0645	ybeR	-0.032	0.013	-0.023	-0.060	-0.175										
b0646	djlB	0.049	0.058	-0.004	-0.047	-0.057			2.171							
b0648	ybeU	-0.030	0.028	-0.020	0.050	0.029										0.040
b0649	djlC	-0.050	-0.017	0.032	-0.042	-0.097										
b0650	hscC	-0.030	0.028	0.036	-0.038	-0.009										
b0651	rihA	-0.047	-0.017	-0.016	-0.002	-0.024										
b0652	gltL	-0.060	0.053	0.027	-0.194	-0.240										
b0653	gltK	0.115	0.047	0.004	-0.068	-0.031										
b0655	gltI						0.004	0.074	0.022	-0.126	0.061	0.070	0.110	0.033	-0.069	0.087
b0656	insH-3			-0.073												
b0657	Int			0.342	0.035	0.156										
b0658	ybeX	0.008	-0.007	0.040	-0.011	0.032	2.171									
b0659	ybeY	0.044	-0.124	-0.011		-0.022										
b0660	ybeZ	-0.084	0.033	0.023	-0.022	-0.015	-2.171	-2.171	-0.030	0.009	0.995	0.004	-0.183	0.028	-0.001	0.166
b0661	miaB						-0.048	0.122	0.087	0.000	0.074	0.040	0.061	0.091	0.139	0.106
b0667				0.222												
b0669		-0.089	0.059	-0.035												
b0674	asnB	0.097	0.016	-0.049	0.325	0.008	0.030	0.056	0.104	0.017	-2.171	0.062	0.042	0.026	0.005	0.015
b0675	nagD		-0.039				2.171				2.171	0.212	-0.052		-0.100	-0.122
b0676	nagC		0.105	0.025	0.028											
b0677	nagA	-0.018	0.020	0.007	-0.044	0.109										
b0678	nagB	-0.015	-0.001	0.043												
b0680	glnS	0.139	-0.011	-0.049		-0.163	-0.039	0.043	0.026	-0.004	0.009	-0.015	-0.118	-0.033	-0.030	-0.004

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0681	ybfM						-0.274	-2.171	-0.178	-2.171	-2.171		-0.280	-0.085	-0.212	-0.047
b0682	ybfN	-0.177	-0.058	-0.044	-0.028	-0.192										
b0683	fur	-0.006	-0.016	0.031	-0.052	-0.048	-0.013	0.239	0.022	0.356	-0.104	-0.063	0.047	0.045	0.084	0.124
b0684	fldA	0.027	0.036	0.005	-0.102		-0.065	0.030	0.048	0.065	0.139	-0.123	0.014	0.070	0.089	0.177
b0685	ybfE	-0.048	-0.077	-0.021	-0.034	0.025										
b0686	ybfF	-0.082	0.007	-0.061	-0.042	-0.200		2.171								
b0687	seqA	0.059	0.083	0.049	-0.011	-0.087	-0.143	-2.171	-0.017	-2.171	-2.171				0.015	0.271
b0688	pgm						-2.171	0.056	0.022	0.069	0.017	-0.047	0.049	0.071	0.024	0.068
b0689	ybfP	-0.024	-0.009	0.002	-0.011	-0.073										
b0690		-0.122	-0.007	0.007	0.013											
b0691		-0.005	-0.059	-0.109	-0.121	-0.210										
b0692	potE	0.018	0.025	0.020	-0.039	-0.052										
b0693	speF	-0.061	-0.021	-0.056	-0.078	-0.116										
b0694	kdpE								2.171							
b0695	kdpD		0.072													
b0696	kdpC		-0.095													
b0697	kdpB					-0.184	2.171	2.171			2.171					
b0699	ybfA				0.287											
b0700	rhsC	0.028	0.028	0.015	0.035	-0.026										
b0701			-0.049	-0.098		0.042										
b0702	ybfB	-0.031	0.025	0.066	0.079	0.000										
b0703		0.050	-0.046		0.129	0.271										
b0704	ybfC	-0.068	-0.061	-0.075	-0.051	-0.240										
b0706	ybfD	0.011	-0.007													
b0707	ybgA			0.291												
b0708	phr	-0.031	0.018	-0.056	-0.036	-0.020			2.171							
b0709	ybgH	0.028	0.039	0.032	-0.007	0.004										
b0710	ybgI			0.082	0.029	0.136			2.171			0.091	0.073	0.010	0.102	0.062
b0712	ybgK	0.098	0.039	0.030	0.040	0.063										
b0715	abrB	-0.032	-0.057	-0.092	-0.091	-0.120										
b0716	ybgO	0.065	-0.087		0.042	0.063										
b0718	ybgQ	0.019	0.034	0.031	-0.010	-0.013										
b0719	ybgD	-0.121	0.026	-0.028	-0.029	0.014										
b0720	gltA		-0.017	-0.060	-0.035		0.030	0.000	-0.013	-0.122	-0.178	0.037	-0.029	-0.049	-0.122	-0.117
b0721	sdhC	-0.045	-0.052	-0.099	-0.126	-0.326										
b0723	sdhA	-0.042	0.043	0.037	0.011	0.132	-2.171	-2.171	-2.171	-2.171	-2.171	-0.042	-0.028	-0.083	-0.070	-0.048
b0724	sdhB											0.038	-0.136	-0.173	-0.054	-0.144
b0726	sucA	0.009	-0.002	-0.005	-0.012	0.001	-0.026	-0.096	-0.078	-0.130	-0.187	-0.017	-0.056	-0.094	-0.107	-0.044
b0727	sucB		0.042				-0.013	-0.048	-0.100	-0.248	-0.152	-0.040	-0.097	-0.062	-0.162	-0.082
b0728	sucC	0.358	0.049	0.072			-0.004	0.004	-0.039	-0.130	-0.174	-0.001	-0.026	-0.048	-0.128	-0.174
b0729	sucD	-0.006	0.007	-0.052	-0.088	-0.127	-0.009	0.022	-0.017	-0.096	-0.139	0.031	0.036	-0.034	-0.075	-0.069
b0730	mngR	-0.019	-0.011	-0.089	-0.108	-0.244										
b0731	mngA	0.003	0.012	-0.036	-0.122	-0.144										
b0733	cydA	-0.004	0.017	-0.039	-0.092	-0.211										
b0736	ybgC	-0.058	0.020	-0.031	-0.009	-0.022										
b0737	tolQ		-0.061	0.040		-0.113										
b0739	tolA					0.069										
b0740	tolB			0.029			-0.460	-0.195	-0.096	0.009	-0.026	0.044	0.005	0.027	0.015	0.101
b0742	ybgF						2.171		2.171							
b0750	nadA	-0.077	0.070	0.124	-0.003	0.098										
b0752	zitB		0.037	0.184	0.189	0.221										
b0753	ybgS				0.085											
b0754	aroG	-0.044	-0.001	-0.010	-0.111	-0.061	0.035	0.030	0.039	-0.039	-0.017	-0.010	0.027	0.009	-0.012	0.011
b0755	gpmA	-0.030	-0.011	-0.071	-0.095	-0.212	0.083	-0.052	0.000	0.087	0.000	0.028	-0.069	-0.021	0.020	0.016
b0756	galM	-0.009	-0.007	-0.001	-0.052	-0.080		2.171		2.171						
b0758	galT		-0.069													
b0759	galE		-0.183													
b0760	modF	-0.082	-0.019	-0.046		0.062										
b0761	modE		0.140													
b0763	modA	0.496	0.014	0.246	0.230	0.188										
b0764	modB				0.342											
b0765	modC	0.039	0.064	0.050	0.121	0.056										
b0766	ybhA		-0.114													
b0767	pgl	-0.033	-0.020	0.013	0.031	0.005	-0.026	0.043	0.130	0.330	0.308	0.068	0.085	0.155	0.371	0.394
b0769	ybhH		0.037	0.126	0.140	0.344	2.171			2.171						
b0771	ybhJ		0.087													
b0772	ybhC		0.151													
b0773	ybhB	-0.120	-0.048	0.025	0.007	0.073										
b0774	bioA	-0.039	0.047	0.090												
b0775	bioB	-0.056	0.030	0.067	0.022	0.020										
b0776	bioF	-0.086	0.089	0.060	0.011	0.005				2.171			0.069	-0.084	0.014	
b0777	bioC	0.178	0.057	-0.028	-0.031	0.007										
b0778	bioD	-0.023	-0.012	-0.014	0.000	-0.059			2.171	2.171	2.171					
b0779	uvrB	-0.087	-0.034	-0.054	-0.090	-0.118					2.171					
b0781	moaA	0.029	0.006	0.013	0.104	0.127			2.171		2.171	-0.104	0.076	0.108	-0.072	0.079
b0782	moaB	-0.032	-0.013	-0.021	-0.021	-0.018	-0.017	0.061	0.035	-0.104	-0.048	0.065	0.025	0.135	-0.066	0.125
b0783	moaC	0.031	-0.012	0.007	0.069	0.073										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0784	moaD	-0.233	-0.014	-0.010	-0.098	-0.022										
b0785	moaE	0.059	0.004	-0.033	-0.137	0.015										
b0786	ybhL	-0.055	-0.064	-0.056	-0.042	-0.045										
b0787	ybhM		-0.019													
b0789	ybhO	0.002	-0.009	-0.027	-0.087	-0.043		2.171								
b0790	ybhP	-0.149	-0.057	-0.078	0.119											
b0791	ybhQ		0.105		0.057	0.100										
b0794	ybhF	-0.148	-0.110	-0.090	-0.181		2.171									
b0795	ybhG						-2.171	-2.171	-2.171	-2.171	-2.171					
b0797	rhlE	0.065	-0.016	-0.033	-0.027	-0.054										
b0798	ybiA			0.041												
b0799	dinG	-0.066	0.007	0.008	-0.057	-0.050				2.171						
b0800	ybiB						2.171	2.171	2.171	2.171	2.171					
b0801	ybiC	-0.027	-0.031	0.071	0.228	0.283	2.171			2.171		-0.067	-0.006	-0.133	-0.084	-0.079
b0803	ybiI				0.334											
b0804	ybiX	-0.011	0.009	-0.038	-0.045	-0.015					2.171					
b0805	fiu						2.171									
b0806	mcbA				0.002											
b0807	rlmF	-0.023	0.059	0.024	0.192	0.262	2.171									
b0808	ybiO				0.167											
b0809	glnQ	0.052	-0.005	-0.014	0.047	0.118										
b0810	glnP	0.031	0.063	0.091	0.055	0.150										
b0811	glnH		0.053			0.247	-0.030	-0.026	0.074	0.048	0.104	0.017	-0.028	0.027	-0.016	0.131
b0812	dps	0.075	0.000	-0.025	-0.020	0.015	-0.030	0.039	0.048	0.165	0.178	0.000	-0.031	0.038	0.156	0.209
b0815	ybiP	0.029	-0.003	0.012	0.069	-0.021					2.171					
b0816	yliL		0.001			0.049										
b0817	mntR	-0.057	-0.020	-0.123	-0.061	-0.137				2.171		-0.072	-0.112	0.010	-0.144	-0.005
b0819	ybiS						-0.035	-0.048	-0.009	-0.048	0.061	0.009	-0.006	0.001	0.048	0.085
b0820	ybiT	0.084	0.041	0.086		0.039			2.171	2.171	2.171	0.142	0.071	-0.088	0.041	0.014
b0821	ybiU					0.022										
b0822	ybiV				0.038											
b0823	ybiW	-0.017	-0.006	0.015	-0.025	0.006			2.171							
b0824	ybiY	0.019	0.028	-0.002	0.170	-0.154										
b0825	fsaA		0.007	-0.006		0.040										
b0826	moeB	-0.002	0.002	-0.009	-0.066	-0.018										
b0827	moeA	0.013	-0.028	-0.052	-0.022	-0.053		2.171	2.171	2.171	2.171		0.025	0.007	-0.036	0.014
b0828	iaaA	0.014	-0.014	-0.019	0.103	-0.268			2.171							
b0829	gsiA	-0.072	-0.003	-0.069	-0.087	-0.038			2.171	2.171						
b0830	gsiB		0.066	-0.040							2.171					
b0831	gsiC	0.068	0.044	0.081		0.081										
b0832	gsiD	-0.085	0.029	-0.037	-0.067	-0.122										
b0833	yliE	-0.008	-0.003	0.044	0.031	0.119	2.171	2.171			2.171					
b0834	yliF					-0.048	2.171									
b0835	rimO	0.005	0.027	0.005	-0.028	0.026										
b0837	yliI	0.033	0.027	0.095	0.128	0.149										
b0838	yliJ		0.049				0.056	-0.152	-2.171	-0.352	-0.130	-0.078	0.031	-0.005	-0.020	-0.051
b0839	dacC								2.171							
b0840	deoR	0.005	0.005	-0.052	-0.035	0.014										
b0841	ybjG	0.021	0.094		0.032	0.072										
b0842	cmr		0.057													
b0843	ybjH				0.060											
b0844	ybjI		-0.063		-0.023											
b0845	ybjJ		-0.052			0.074										
b0846	ybjK				0.065											
b0847	ybjL	-0.034	-0.040	0.017	-0.023	-0.029										
b0848	ybjM	0.001	0.023			-0.049										
b0849	grxA	0.036	0.020	-0.009	0.017	0.038										
b0851	nfsA	-0.045	0.022	0.063	0.215	0.404				2.171						
b0852	rimK		0.021	0.114	0.129	-0.206										
b0854	potF		0.144		0.099		-0.113	0.109	-0.065	0.226	0.087	-0.100	0.026	0.076	0.099	0.157
b0855	potG	-0.021	0.066	0.036	-0.053	0.046										
b0857	potI	-0.007	0.073	-0.058	-0.025	0.022										
b0859	rumB		0.043		-0.076	-0.049										
b0860	artJ						-0.013	-0.013	0.026	-0.096	-0.039	-0.043	-0.034	0.000	-0.094	-0.039
b0861	artM					0.119										
b0862	artQ	0.206														
b0863	artI		0.009	0.165			-0.039	0.004	0.013	0.056	0.130	0.098	0.010	0.038	0.062	0.193
b0865	ybjP	0.002	0.030	0.021	-0.104	-0.182										
b0866	ybjQ	0.063	0.078	0.155	0.231	0.342										
b0867	amiD	-0.031	0.044	-0.014	-0.030	0.100										
b0868	ybjS	0.094	0.022	0.060	0.006	-0.044										
b0869	ybjT	0.139	-0.004			0.081										
b0870	ltaE	0.032	0.048	-0.021	-0.060	-0.052						0.051	0.014	-0.128	0.305	-0.047
b0871	poxB		0.052													
b0872	hcr			0.000								0.003				0.037
b0873	hcp		0.051					2.171								
b0874	ybjE	0.018	0.050	-0.090												

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0875	aqpZ		-0.170													
b0876	ybjD		-0.196													
b0877	ybjX	0.000	0.033	-0.031	0.016	0.012										
b0878	macA						2.171									
b0879	macB	-0.084	-0.052	-0.058	-0.066	-0.059						0.006	-0.002	-0.085	-0.056	-0.198
b0880	cspD	-0.026	-0.003	-0.029	0.042	-0.043										
b0882	clpA	-0.037	-0.104	-0.164	-0.131	-0.224	-0.043	0.022	0.230	-2.171	-0.352	-0.168	-0.018	0.039	0.042	0.065
b0884	infA	0.070	0.053	0.034	-0.016	0.005	-0.104	-2.171	0.000	-0.300	0.013	-0.109	-0.084	0.024	0.034	0.095
b0885	aat		0.025				-2.171	-2.171	-2.171	-2.171	-2.171	0.068	0.056	0.068	0.102	-0.034
b0886	cydC						2.171									
b0888	trxB						-0.013	0.083	0.013	0.009	-0.195	0.008	-0.030	-0.056	0.033	-0.072
b0889	lrp						-0.013	0.035	0.013	0.026	0.026	0.017	0.077	0.017	0.037	0.047
b0891	lolA						2.171		2.171	2.171						
b0892	rarA	0.039	0.040	0.095	-0.584	-0.281										
b0893	serS	0.024	0.032	0.032	0.120	0.178	0.061	0.061	0.022	-0.022	0.039	0.037	0.059	0.060	0.045	0.023
b0894	dmsA	-0.031	-0.008	-0.058	-0.076	-0.268	-2.171	-2.171	-2.171	-0.608	-2.171	-0.105	-0.341	-0.037	0.049	
b0895	dmsB	-0.021	-0.018	-0.091	-0.122	-0.241										
b0896	dmsC		0.106	0.021	0.151	0.166										
b0897	ycaC	-0.059	0.051	-0.175	-0.069	-0.026										
b0900	ycaN								2.171							
b0902	pflA		0.047		0.029	-0.058										
b0903	pflB						0.000	0.030	0.035	0.217	0.083	-0.001	0.002	0.007	0.126	0.059
b0904	focA	-0.265														
b0905	ycaO										2.171					
b0906	ycaP	0.189	0.050	-0.008	0.043	0.021										
b0907	serC	-0.030	-0.017	-0.020	-0.028	-0.132	0.035	0.039	0.035	0.004	-0.009	0.035	0.025	0.030	-0.010	-0.003
b0908	aroA	-0.098	0.003	-0.007	0.017	-0.144	2.171	2.171	2.171		2.171	0.007	0.017	0.115	-0.014	0.171
b0909	ycaL	-0.102	0.030	0.043	0.025											
b0910	cmk	-0.060	-0.067	-0.050	-0.075	-0.082	2.171	2.171	2.171	2.171	2.171		-0.034	0.066	0.116	0.154
b0911	rpsA	-0.001	-0.037	-0.082	-0.016	-0.026	-0.074	-0.052	-0.039	-0.048	0.022	-0.029	-0.050	-0.042	0.012	0.060
b0912	ihfB						-2.171	0.022	-0.087	0.165	0.078	0.029	-0.002	0.103	0.097	0.196
b0914	msbA	-0.052	-0.064	-0.037	-0.005	-0.003										
b0915	lpxK	0.028	0.057	0.032	0.018	0.005										
b0917	ycaR	0.004	0.044	0.120	0.201	0.492		2.171		2.171		0.009	0.030	0.090	0.091	0.098
b0918	kdsB						-0.291	-2.171	-2.171	0.083	0.022	0.005	-0.018	0.058	0.011	0.067
b0919	ycbJ	0.007	-0.044	-0.093	0.021	-0.004										
b0920	ycbC	-0.040	-0.003	-0.153	0.049	-0.013			2.171							
b0921	smtA	-0.055	-0.007	0.005												
b0922	mukF	-0.157	-0.004	-0.083		-0.040		2.171		2.171		0.012	-0.077	0.060	-0.073	-0.094
b0924	mukB	-0.054	-0.015	-0.090	-0.065	-0.082	-2.171	-0.013	-2.171	-2.171	-2.171	-0.009	-0.055	0.031	0.045	0.029
b0925	ycbB	0.016	0.033	0.059	0.045	0.056			2.171	2.171						
b0926	ycbK	-0.108	0.015	-0.040	-0.031	0.038										
b0927	ycbL	0.054	-0.076	0.026	-0.005	-0.015										
b0928	aspC	-0.047	-0.024	-0.032	-0.059	-0.112	0.004	-0.017	-0.017	-0.052	-0.096	0.002	-0.009	0.013	0.000	-0.028
b0929	ompF	-0.050	-0.016	-0.052			2.171									
b0930	asnS	0.008	0.009	-0.003	-0.038	0.056	0.004	0.004	0.013	0.000	0.026	0.013	0.002	-0.003	0.037	0.040
b0931	pncB	0.120	0.116	0.112	0.023	0.221	-0.152	-2.171	0.035	-0.195	0.104	-0.049	-0.057	-0.011	0.004	0.031
b0932	pepN	0.043	0.019	0.026	-0.014	0.041	-0.152	-0.078	0.061	0.113	0.130	0.043	0.073	-0.071	0.029	0.091
b0934	ssuC		-0.061													
b0935	ssuD		0.033		0.099	0.009										
b0936	ssuA	-0.116	-0.009	-0.071	-0.078	-0.110										
b0940	ycbS	-0.124	-0.013	-0.070												
b0942	ycbU	-0.130	0.019	0.025	0.023	0.297										
b0945	pyrD	-0.079	-0.026	-0.076	-0.033	-0.102		2.171		2.171	2.171	-0.037	-0.138	-0.060	-0.057	-0.022
b0946	ycbW	0.028	0.032	0.039	0.112	0.226						-0.199			-0.191	0.008
b0947	ycbX		0.116		0.053	0.135	-2.171	-2.171	-2.171	-2.171	-2.171					
b0948	rlmL	-0.051	-0.027	-0.069	-0.054	-0.058										
b0950	pqiA	0.073	0.000	0.067	0.012	0.043										
b0951	pqiB	0.006	0.006	-0.015	-0.025	0.004		2.171		2.171	2.171					
b0952	ymbA	0.008	-0.008	-0.052	-0.061	-0.036										
b0953	rmf	-0.001	0.022	-0.024	-0.062	0.025										
b0954	fabA	-0.002	0.026	0.044	0.048	0.150	-0.022	-0.009	0.013	-0.043	-0.074	0.038	0.016	0.035	0.001	0.024
b0955	ycbZ	-0.013	0.070	0.030	-0.057	0.041										
b0956	ycbG	0.051	0.052	0.027		0.066										
b0957	ompA						-0.052	-0.004	0.009	0.022	0.043	0.010	-0.007	-0.021	0.049	0.085
b0958	sulA		0.060													
b0959	sxy		0.147	0.024	0.021				2.171							
b0960	yccS	-0.061	-0.003	-0.044	-0.053	-0.017										
b0962	helD	-0.010	-0.042	-0.024	0.009	0.037	-2.171	-2.171	-2.171	-2.171	-2.171					
b0963	mgsA		0.131	0.373	0.149											
b0964	yccT	0.020	-0.007	0.030	0.022	-0.021										
b0965	yccU	0.056	0.003	0.010	-0.021	0.007										
b0966	hspQ	-0.033	0.058	0.035	0.204	0.369										
b0967	yccW	0.046	-0.028	-0.022	-0.088	0.004										
b0968	yccX	0.030	0.012	0.069	0.247	0.349										
b0969	yccK	-0.110	-0.005	0.009	0.093	0.090										
b0972	hyaA		-0.027	-0.006	0.137	0.199										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b0973	hyaB		0.204			0.065										
b0974	hyaC		-0.217		-0.006	0.094										
b0975	hyaD		0.094	-0.089		0.052										
b0976	hyaE	0.271	0.069	0.042	0.094	0.012										
b0977	hyaF	0.020	0.009	-0.020	-0.016	0.105										
b0978	appC		0.031	-0.003	-0.023	0.037			2.171							
b0980	appA				0.205					2.171						
b0981	etk						-2.171	-2.171	-2.171	-2.171	-2.171					
b0982	etp	-0.007	0.017	0.056	0.108	0.161			2.171							
b0984	gfcD	0.022	-0.015	-0.033	-0.006	-0.038			2.171							
b0986	gfcB		0.050						2.171							
b0987	gfcA				0.093	0.046										
b0988	insB-4									2.171						
b0989	cspH	-0.005	0.025	0.040	0.004	-0.006										
b0990	cspG	-0.053	-0.021	-0.071	-0.086	0.056			2.171							
b0991	ymcE					0.029										
b0992	yccM	0.003	0.008		-0.012	-0.004										
b0993	torS	-0.061	-0.028	-0.028	0.025	-0.031										
b0994	torT	0.044	0.005	-0.012	0.016	0.012										
b0996	torC	0.200	0.061	0.078	0.002	0.088										
b0997	torA	0.210	0.002	0.090	0.033	0.057			2.171							
b0998	torD		0.083							2.171	2.171					
b0999	cbpM	0.011	-0.010	0.009	0.048	0.121										
b1000	cbpA	0.033	0.034	-0.004	0.020	0.010										
b1001	yccE				0.121			2.171			2.171					
b1002	agp	-0.005	0.015	0.077	0.243	0.215										
b1003	yccJ	0.172	-0.053		0.082	0.086				2.171	2.171					
b1004	wrbA		-0.016	-0.053	0.344	-0.378	0.078	0.226	0.691	0.925	0.834	-0.045	-0.037	0.038	0.766	0.122
b1006	rutG		0.001	0.066		0.052										
b1007	rutF				0.030											
b1008	rutE							2.171		2.171						
b1009	rutD	-0.045	-0.067	-0.105	-0.072	-0.076										
b1011	rutB	-0.013	0.009	0.022	-0.032	-0.047										
b1012	rutA		-0.017	0.101		-0.099					2.171					
b1013	rutR	0.119	0.083	-0.122	0.001											
b1014	putA	-0.047	-0.023	-0.034	-0.065	-0.110						-0.072	-0.168	-0.020	0.073	0.026
b1018	efeO	0.009	-0.078	0.006	-0.079	-0.119	2.171	2.171		2.171		-0.086	0.085	0.084	0.013	0.067
b1019	efeB					-0.247										
b1020	phoH	0.041	0.050	0.028	0.033	-0.022										
b1022	pgaC	0.006	0.042	0.043	0.050	0.054										
b1023	pgaB	-0.030	-0.026	-0.004	0.007	0.006		2.171								
b1024	pgaA	0.038	-0.023	0.023	0.092	0.079										
b1026	insF-4	0.103	0.045	-0.042	0.040	0.242										
b1027	insE-4	-0.144	-0.058	0.006	-0.092	-0.186										
b1028		-0.011	0.107		0.007	0.047										
b1029	ycdU	-0.025	0.033	-0.045	-0.034	-0.082										
b1030		-0.031	0.101		-0.073	-0.060										
b1031		0.048	0.054	0.063	-0.008	0.117										
b1033	ghrA	0.016	0.084	0.043	-0.022	-0.004		2.171		2.171						
b1034	ycdX							2.171			2.171					
b1035	ycdY								2.171	2.171	2.171	0.080		0.032	0.153	0.227
b1036	ycdZ	-0.070	0.096	-0.022												
b1037	csgG		0.000	-0.044		-0.065										
b1039	csgE				-0.069											
b1043	csgC	0.011	0.043	0.002	-0.001	0.078										
b1045	ymdB	-0.068	-0.039	-0.067	-0.078	-0.278										
b1046	ymdC	0.026	-0.152	-0.005			2.171									
b1048	mdoG	0.008	0.005	0.012	0.153	0.167	-0.621	-0.408	-0.204	0.061	-0.161	0.013	-0.118	0.010	0.016	0.089
b1049	mdoH	0.148	0.088	0.023	0.045	-0.006										
b1050	yceK	-0.076	0.011	0.023												
b1051	msyB			-0.072					2.171	2.171	2.171	0.116	0.064	0.201	0.504	0.588
b1052		-0.031	-0.060	0.008	-0.001	0.002										
b1054	lpxL	0.219	0.016	0.069	0.026	0.037										
b1055	yceA	-0.015	0.032	0.027	0.022	0.077										
b1056	yceI	-0.035	0.029	0.022	0.067	0.144		2.171								
b1057	yceJ	0.077	-0.014	-0.123	0.100											
b1058	yceO	0.068	-0.041	-0.061	-0.046	-0.060										
b1059	solA	0.019	-0.021	0.005	0.062	0.041	-0.274	-0.061	0.056	-0.022	-2.171	-0.006	0.069	-0.008	0.016	0.120
b1060	bssS	-0.013	0.009	0.089	0.094											
b1061	dinI	-0.030	0.005	-0.043	-0.065	0.010										
b1062	pyrC	0.161	-0.052	-0.030	-0.087	-0.009	0.000	0.048	0.052	0.026	0.061	0.042	0.056	0.051	0.051	0.063
b1064	grxB	0.064	0.072	0.033	-0.009	0.122	-0.009	0.096	-2.171	0.208	0.282	0.052	0.013	0.024	0.089	0.211
b1065	mdtH	-0.007	-0.003	0.128	0.149	0.183										
b1066	rimJ	-0.019	-0.035	-0.066	-0.023	-0.049										
b1067	yceH	-0.038	-0.005	0.009	0.049	0.080										
b1068	yceM		0.000	-0.116		-0.088					2.171					
b1069	yceN		0.022	0.074												

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b1070	flgN		0.073	-0.016		-0.077										
b1071	flgM	-0.127	-0.016	-0.034												
b1073	flgB		0.036	0.073	0.157	0.023										
b1074	flgC		0.081	0.051	-0.042	0.010										
b1075	flgD		-0.006	0.016	0.110	-0.009										
b1076	flgE	0.207	0.044	0.048	0.069	-0.010										
b1077	flgF	0.092	0.020	0.139	-0.038											
b1078	flgG		-0.041	-0.060												
b1079	flgH	0.012	-0.028	-0.056	-0.013	0.085										
b1082	flgK			-0.133												
b1083	flgL		0.108			-0.121	2.171									
b1084	rne						-2.171	0.013	-0.178	-0.026	0.035	0.002	-0.033	-0.022	0.142	0.118
b1085	yceQ	-0.019	0.002	-0.008	0.102	0.043										
b1086	rluC	-0.031	0.016	0.029	0.009	0.070										
b1087	yceF	0.050	0.013	0.016	0.084	0.201					2.171					
b1088	yceD	0.045	-0.012	0.009	0.036	0.030										
b1089	rpmF	-0.049	-0.030	-0.008	-0.060	-0.024										
b1090	plsX	0.002	0.092	0.007	0.022	0.047										
b1091	fabH	-0.020	0.003	0.037	0.091	0.240	0.078	0.065	0.004	0.030	-0.009	0.057	0.034	0.075	0.001	0.114
b1092	fabD		0.078				-0.022	0.009	-0.117	-0.017	-0.069	-0.055	0.010	-0.084	-0.064	-0.012
b1093	fabG						-0.039	-0.039	-0.061	-0.004	-0.039	-0.073	-0.081	-0.055	-0.029	-0.035
b1094	acpP	-0.001	-0.001	-0.037	-0.035	-0.086	2.171	2.171	2.171	2.171	2.171			0.152	-0.019	0.077
b1095	fabF		0.136		0.008		0.091	0.000	-0.100	-0.078	0.013	-0.106	0.004	0.003	-0.036	0.059
b1096	pabC	0.080	0.107	0.068	-0.076	0.119										
b1097	yceG	-0.104	0.024	0.022	0.059	0.008										
b1098	tmk	-0.017	-0.011	-0.043	-0.020	-0.065	2.171									
b1099	holB								2.171							
b1100	ycfH	0.018	0.015	-0.060	-0.028	-0.046			2.171							
b1101	ptsG		0.072				2.171									
b1102	fhuE	0.035	-0.006	-0.004												
b1103	hinT						-0.017	0.056	0.126	0.261	0.165	-0.005	0.011	0.120	0.175	0.377
b1104	ycfL	-0.018	-0.016	0.002	0.068	0.054										
b1105	ycfM	-0.043	0.026	-0.029	0.006	0.048										
b1107	nagZ	-0.009	-0.008	-0.042	-0.059	-0.070						-0.138	0.058	-0.044	-0.131	0.121
b1108	ycfP	-0.024	-0.021	0.001	0.080	0.115	-2.171	-0.026	-2.171	0.139	0.204	0.065	0.022	0.074	0.209	0.282
b1109	ndh		0.016	0.004		0.036										
b1110	ycfJ	-0.018	-0.036	-0.029	-0.053	-0.022										
b1111	ycfQ	-0.012	0.004	-0.019	0.039	0.100										
b1112	bhsA	-0.022	-0.055	-0.056	0.015	-0.143										
b1113	ycfS	-0.115	0.027	0.012	-0.005	0.030										
b1114	mfd	-0.069	-0.045	-0.032	-0.039	-0.074				2.171	2.171					
b1115	ycfT	-0.031	0.036	0.026	0.016	0.057										
b1116	lolC		0.034	-0.045		-0.176										
b1117	lolD	-0.062	-0.087	-0.051	0.007	0.069										
b1118	lolE	0.017	-0.034	0.071	0.065	0.202										
b1119	nagK					0.031										
b1120	cobB	-0.036	0.039	0.009												
b1122	ymfA	0.054	-0.073	-0.055	-0.103	-0.164										
b1123	potD							2.171								
b1124	potC					-0.326										
b1125	potB			0.045		-0.063										
b1126	potA		0.065	0.092	-0.050											
b1127	pepT	0.016	0.002	-0.004	-0.053	-0.035										
b1128	ycfD		0.008	-0.058	0.084	0.017					2.171					
b1129	phoQ						2.171	2.171		2.171		0.012	-0.054	0.000	0.013	0.048
b1130	phoP		-0.036	-0.054	0.005		0.078	0.083	0.026	0.078	0.126	0.012	0.044	0.052	0.003	0.098
b1131	purB	0.000	0.016	0.066	0.089	-0.292	0.052	0.035	-0.013	-0.022	0.061	0.027	0.022	-0.021	0.009	0.070
b1132	hflD	0.023	-0.034	0.034	-0.012	0.045										
b1133	mnmA	0.061	-0.059	-0.007	-0.043	-0.041										
b1134	nudJ	-0.099	-0.013	-0.005		-0.063					2.171					
b1135	rluE	-0.036	0.007	-0.011	-0.002	-0.001						-0.080	-0.162	-0.060	-0.059	0.206
b1136	icd	-0.045	0.031	0.010	-0.005	0.010	0.030	0.026	-0.026	-0.165	-0.282	0.035	0.027	-0.030	-0.108	-0.198
b1137	ymfD	0.031	0.044	-0.005	-0.055	-0.034					2.171					
b1140	intE	-0.019	0.081	0.044	0.209	0.314			2.171							
b1141	xisE	0.025	0.031	-0.047	-0.050	-0.029										
b1142	ymfH	-0.039	0.035	0.087	0.027	0.063										
b1143	ymfI	-0.028	-0.023	0.140	-0.092	-0.090										
b1144	ymfJ	-0.026	-0.021	-0.013	-0.038	-0.015										
b1146	ymfT	0.015	0.004	-0.038	-0.060	-0.039										
b1147	ymfL					0.149										
b1149	ymfN								2.171							
b1150	ymfR					-0.024										
b1151	ymfO	0.038	0.058	0.137	0.221	0.373										
b1152	ymfP	-0.026	0.026	0.020	-0.045	-0.044										
b1153	ymfQ		0.013	0.081		0.227										
b1154	ycfK		0.115													
b1157	stfE		-0.059			0.115										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b1159	mcrA	0.164	0.026	0.011												
b1160	iraM					0.168										
b1163	ycgF				0.419											
b1166	ariR				-0.017	0.148										
b1167	ymgC	0.200	0.022	-0.001	-0.021											
b1168	ycgG	0.057	-0.020	-0.003	-0.038	-0.009										
b1169		-0.033	-0.021	-0.009	-0.079	-0.129										
b1170						0.348										
b1171	ymgD	0.030	-0.025	0.002												
b1172	ymgG	0.057	0.038	-0.017	0.070	0.116										
b1173	ycgI	-0.033	0.011	-0.080	0.045											
b1174	minE	0.014	0.053	-0.031	-0.028	0.005				2.171		-0.126	-0.166	-0.130	-0.027	0.038
b1175	minD		0.073	0.075			0.017	-0.013	-0.043	-0.048	0.004	0.081	0.074	0.099	0.138	0.094
b1176	minC	0.107	0.138		0.259											
b1177	ycgJ		-0.057	0.277												
b1178	ycgK	0.177														
b1180	ycgM	0.052	0.022	-0.010	-0.074	-0.149										
b1182	hlyE						-2.171	-2.171	-2.171	-2.171	-2.171					
b1186	nhaB	-0.130	-0.018	-0.013	-0.056	-0.040										
b1187	fadR	0.090	0.043	-0.008	-0.091	-0.090										
b1189	dadA	-0.004	-0.029	-0.085	-0.063	-0.121							-0.070	0.031	0.034	
b1190	dadX	-0.070	-0.014	-0.072	-0.134	-0.183						0.199	0.094	-0.017	0.068	0.049
b1191	cvrA	0.058	-0.040	0.017		0.111			2.171							
b1192	ldcA	0.056	0.007	0.126	0.090	0.119										
b1193	emtA	0.025	0.014	-0.011	0.061	0.102				2.171						
b1194	ycgR	0.065	0.065	0.123	0.070	0.151										
b1195	ymgE	-0.015	-0.010	-0.036	-0.011	-0.075										
b1196	ycgY	-0.004	-0.001	-0.051	-0.019	-0.075										
b1197	treA	0.039	0.049	0.009	0.008	0.001				2.171					-0.161	0.110
b1198	dhaM	0.019	0.063	-0.033	-0.021	0.028		2.171	2.171							
b1199	dhaL	0.004	0.031	0.007		-0.028										
b1200	dhaK	0.073	0.037	0.042		0.010			2.171		2.171	0.110	0.050	-0.073	-0.006	-0.011
b1201	dhaR					0.083										
b1202	ycgV			-0.029		0.056										
b1203	ychF	0.071	0.053	0.059	0.054	0.179	0.013	-0.156	-0.004	-0.017	0.009	0.004	0.013	0.049	0.069	0.062
b1204	pth	0.015	0.029	0.017	-0.036	0.036										
b1205	ychH	0.048	0.011	0.004	-0.033	0.022										
b1206	ychM	0.088	-0.013	0.039	0.026	0.014										
b1207	prs						-0.017	-0.048	-0.009	-0.017	0.004	0.036	-0.020	0.055	-0.050	0.093
b1208	ispE	0.041	0.010	0.056	-0.074	-0.054										
b1209	lolB	0.100	0.095	0.054	-0.027	0.185										
b1210	hemA	-0.044	-0.013	-0.050	-0.115	-0.090	2.171									
b1211	prfA	0.028	0.002	0.026	-0.076	0.017										
b1212	prmC	0.036	0.084	0.028	-0.024	0.143										
b1213	ychQ	-0.025	0.003	-0.006	-0.055	0.025										
b1214	ychA	0.059	0.078	0.003	-0.087	0.050		2.171								
b1215	kdsA		0.084				0.026	0.043	-0.039	0.009	0.091	0.014	0.013	0.044	0.041	0.094
b1216	chaA	-0.092	-0.032	-0.031	-0.048	-0.093										
b1217	chaB	-0.042	0.033	0.019	0.049	0.028										
b1218	chaC					0.223										
b1219	ychN		0.062	0.168	0.062	0.011		2.171								
b1220	ychO	0.002	0.063	-0.020	-0.041	-0.077										
b1221	narL	0.452	0.107	0.428	0.247	-0.004										
b1222	narX		0.016		0.087											
b1223	narK	0.023	0.014	0.026	0.035	0.021										
b1224	narG	0.017	0.003	-0.003	-0.143	-0.301				2.171						
b1226	narJ	-0.031	0.109	0.117	0.004	0.017										
b1227	narI		0.013	-0.073	0.055	0.114										
b1228	ychS	0.100	0.063	0.127	0.249	0.368										
b1232	purU	-0.061	-0.006	-0.049	0.133	-0.064	-0.113	-0.017	-0.043	0.109	0.035	-0.044	-0.003	0.024	0.001	0.116
b1233	ychJ	0.071	0.046	0.057	0.091	0.235										
b1234	rssA	-0.003	-0.037	-0.050	0.012	-0.004										
b1235	rssB	-0.040	-0.003	-0.010	-0.027	-0.068										
b1236	galU	-0.002					0.113	0.191	0.156	0.091	0.391	0.028	0.094	0.090	0.087	0.316
b1237	hns						0.004	-0.017	-0.013	-0.039	-0.004	0.015	-0.032	-0.037	-0.043	0.019
b1238	tdk	0.015	0.026	0.004	0.002	0.048										
b1239		0.012	0.024	0.005	0.071	0.102										
b1240		-0.034	0.008	-0.013	-0.053	-0.067										
b1241	adhE	-0.030	-0.002	-0.041	-0.143	-0.403	-0.039	-0.022	0.004	0.122	0.017	-0.035	-0.006	-0.017	0.083	0.046
b1243	oppA						-0.052	-0.043	-0.056	-0.100	-0.126	-0.029	-0.037	-0.041	-0.059	-0.094
b1244	oppB			-0.086												
b1245	oppC		0.100	0.199	0.054											
b1246	oppD	-0.027	0.008	-0.037	-0.041	-0.008			2.171	2.171						
b1247	oppF	0.136	0.036	-0.025	-0.080	-0.069										
b1248	yciU	-0.015	-0.014	-0.035	-0.049	0.026										
b1249	cls	0.052	0.057	0.076	0.196	0.344										
b1250	kch	-0.005	0.104	0.034	0.194	0.076	-2.171	-2.171	-2.171	-2.171	-2.171					

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b1251	yciI	0.015	0.036	0.016	0.029	0.043										
b1252	tonB		0.028	0.085	0.129	0.213										
b1253	yciA	-0.057	0.055	0.072	-0.006	0.034										
b1254	yciB	0.011	-0.065	-0.080	-0.048	-0.063										
b1255	yciC	-0.019	-0.033	0.005	-0.015	-0.085										
b1257	yciE	0.032	-0.010	0.011	-0.045	-0.030		2.171								
b1258	yciF	-0.126	-0.047	-0.138	-0.099	-0.122										
b1259	yciG		-0.002													
b1260	trpA	0.016	0.008	-0.022	-0.029	-0.069	-0.022	0.022	0.022	-0.022	-0.109	0.001	0.013	0.008	-0.031	-0.059
b1261	trpB	0.029	-0.003	0.004	-0.028	0.019	0.022	-0.035	-0.083	-0.026	-2.171	0.044	0.037	0.017	-0.035	-0.063
b1262	trpC	-0.021	-0.020	-0.073	-0.037	-0.141	0.100	-0.017	-0.061	0.135	-0.065	0.035	-0.003	-0.006	0.051	-0.056
b1263	trpD	-0.012	0.000	-0.050	-0.058	-0.101		2.171								
b1264	trpE	-0.037	-0.025	-0.014	-0.069	-0.098	0.056	-0.026	-0.026	-0.004	-0.030	0.015	-0.046	-0.049	-0.027	-0.021
b1266	trpH	0.007	0.014	-0.001	-0.076	0.033										
b1267	yciO				0.088	0.183			2.171							
b1268	yciQ	0.013	-0.008	-0.028	-0.118	-0.123		2.171								
b1269	rluB	0.041	-0.083	-0.106	-0.065	-0.116										
b1270	btuR	0.030	0.035	0.043	-0.016	-0.098										
b1271	yciK	-0.045	0.016	0.004		-0.033										
b1272	sohB	-0.025	-0.015	-0.072	-0.045	-0.061					2.171					
b1273	yciN	-0.043	0.014	0.069	-0.019	0.054										
b1274	topA	-0.013	-0.064	0.051			2.171	2.171	2.171		2.171	-0.032	0.000	0.050	0.056	0.096
b1275	cysB	0.038	0.012	0.056	0.061	0.190										
b1276	acnA	-0.037	-0.004	0.063	0.027	0.080	2.171	2.171	2.171	2.171	2.171	0.060	-0.003	0.077	0.156	0.095
b1277	ribA	0.044	0.050	0.047	-0.011	-0.026										
b1278	pgpB	0.538	0.050	0.027	0.051	0.002				2.171						
b1279	yciS	0.054	-0.019	-0.004	-0.155	-0.092										
b1281	pyrF	-0.045	-0.010	-0.019	0.020	0.038	2.171	2.171	2.171	2.171		-0.015	0.038	0.055	0.009	0.101
b1282	yciH	-0.003	-0.014	0.021	-0.077	-0.032						-0.015	-0.064	-0.062		
b1285	gmr	-0.102			-0.091	-0.027										
b1286	rnb	-0.073	0.024	-0.018	0.017	-0.048	0.017	-0.009	-0.030	-0.043	-0.017	0.009	-0.053	0.003	-0.035	-0.004
b1287	yciW	0.000	0.031	0.008	0.046	0.108	2.171									
b1288	fabI	-0.056	-0.012	-0.060	-0.034	-0.057	0.000	0.004	-0.069	0.017	0.009	-0.045	-0.066	-0.047	-0.016	-0.040
b1289	ycjD	0.077	-0.066	0.037	0.210	0.275										
b1291	sapD		0.111						2.171							
b1292	sapC						2.171									
b1293	sapB		-0.034	-0.018												
b1294	sapA					0.315	2.171									
b1295	ymjA	0.056	0.002	-0.059	-0.086	-0.070										
b1297	puuA	0.036	0.049	0.022	-0.048	0.047										
b1298	puuD		0.050	0.009		0.091										
b1299	puuR	-0.002	0.015	0.001	0.034	0.033										
b1301	puuB	0.016	-0.012	0.018	-0.021	-0.115										
b1302	puuE	-0.036	-0.007	-0.002	-0.017	0.017										
b1304	pspA						-0.017	-0.030	-0.009	0.048	0.191	0.036	0.036	-0.050	0.055	0.193
b1305	pspB		0.124		-0.096	0.047		2.171		2.171	2.171	-0.081	-0.001	-0.011	0.051	0.358
b1306	pspC				0.071											
b1308	pspE		-0.131													
b1309	ycjM		-0.048	-0.113			-2.171	-2.171	-2.171	-2.171	-2.171					
b1311	ycjO	-0.115	0.113		-0.065	-0.147										
b1312	ycjP				0.062											
b1313	ycjQ	0.001	0.008	-0.001	-0.046	-0.032										
b1315	ycjS	0.072	0.012	-0.029	-0.091	-0.072										
b1316	ycjT	0.012	0.014	0.002	-0.045	0.049										
b1317	ycjU	-0.043	-0.051	0.118	0.195	0.246										
b1318	ycjV	0.132	0.017													
b1319	ompG		-0.045	0.081	-0.001	0.044	2.171									
b1320	ycjW		0.126	0.000	-0.001											
b1321	ycjX	0.037	0.040	0.042	0.088	0.146					2.171					
b1322	ycjF		0.004			-0.047										
b1323	tyrR							2.171								
b1324	tpx	-0.004	0.086	0.055	0.027	0.073	0.069	0.000	0.004	-0.043	-0.091	0.082	-0.005	-0.004	-0.068	-0.054
b1325	ycjG	0.015	-0.018	-0.116	-0.273	-0.422										
b1326	mpaA	0.008	-0.042	-0.003	0.019	0.117										
b1327	ycjY		-0.075	-0.165	0.072	0.082										
b1328	ycjZ									2.171	2.171					
b1332	ynaJ				0.118											
b1334	fnr	-0.024	-0.043	-0.084	0.022	-0.001										
b1335	ogt		-0.169							2.171						
b1337	abgB				-0.023											
b1338	abgA	0.028	-0.172	-0.043	-0.132	-0.145										
b1340	ydaL								2.171							
b1341	ydaM						0.000	-2.171	-2.171	-2.171	-2.171					
b1342	ydaN				0.100											
b1343	dbpA		0.068													
b1344	ttcA				-0.014	-0.033	2.171		2.171							
b1345	intR				0.027	-0.130										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b1346	ydaQ	0.021	0.017	0.002	-0.078	-0.056										
b1347	ydaC	0.005	0.020	0.025	-0.075	-0.021										
b1348	lar	0.181	-0.014	-0.096	0.132											
b1349	recT	0.022	-0.002	-0.029	0.037	0.038										
b1350	recE	0.003	0.002	-0.017	-0.002	-0.035										
b1353	sieB	0.052	-0.042													
b1354					0.045	-0.020										
b1356	racR				0.122											
b1358	ydaT				0.109											
b1359	ydaU							2.171								
b1360	ydaV	-0.056	0.038	0.012	-0.074	0.012										
b1361	ydaW	0.202	0.028		0.041											
b1362	rzpR					-0.083										
b1363	trkG	-0.005	-0.091	0.023	-0.136	-0.159										
b1366	ydaY	-0.039	-0.058	-0.046	-0.048	-0.074										
b1373	tfaR	0.125	0.091	0.082	-0.028	-0.057										
b1374	pinR		0.303						2.171							
b1375	ynaE	0.016	0.025	-0.025	-0.067	-0.002										
b1376	uspF		-0.045	-0.091	-0.125		0.009	0.126	0.148	-2.171	-2.171	-0.038	0.092	0.094	0.013	0.252
b1378	ydbK	0.042	0.009	-0.050	-0.115	-0.160										
b1379	hslJ					0.040										
b1380	ldhA	-0.027	0.031	-0.012	-0.012	0.124	2.171		2.171	2.171	2.171					
b1381	ydbH		-0.007	0.033	0.009	0.005										
b1382	ynbE		0.050	-0.008	-0.069	-0.007										
b1383	ydbL	-0.004	0.062	0.056	0.037	0.126										
b1385	feaB	0.018	-0.011	0.070	0.192	0.208										
b1386	tynA	0.057	0.035	-0.048		0.035										
b1387	maoC	-0.016	0.030	0.052	0.080	0.105										
b1388	paaA	-0.065	0.031	-0.012	-0.014	-0.039										
b1390	paaC	-0.030	-0.013	-0.081	-0.062	-0.080										
b1391	paaD	-0.013	-0.034	-0.050	-0.004	-0.056										
b1392	paaE	0.339	0.062	0.026	0.012	0.012										
b1393	paaF	0.095	0.045	0.147	0.268	0.419										
b1394	paaG	0.013	0.015	0.155	0.305	0.350										
b1396	paaI	-0.083	-0.007	-0.063	0.005	0.004										
b1397	paaJ	0.000	0.003	0.002	0.015	-0.009										
b1398	paaK	-0.008	-0.029	-0.020	-0.003	-0.018										
b1400	paaY	-0.028	-0.019	-0.031	-0.020	-0.039										
b1401					0.426	0.063										
b1403	insC-2			0.263	0.370	0.118			2.171		2.171					
b1405			0.224		0.137											
b1406	ydbC	0.069	0.060	0.076	0.003	0.113	-2.171	-2.171	-2.171	-2.171	-2.171					
b1407	ydbD	0.055	-0.043	0.014	0.121	0.180								-0.058		
b1408	ynbA	0.206	0.025	-0.021	-0.073	-0.046										
b1409	ynbB	-0.094	-0.034	-0.065	-0.044	-0.068										
b1410	ynbC	0.006	0.019	0.028	0.022	0.129										
b1411	ynbD	-0.015	-0.066	-0.105	-0.240	-0.226										
b1412	azoR	0.057	0.014	-0.043	0.016	-0.008	-2.171	0.004	0.091	0.178	0.217	0.079	0.266		0.052	0.084
b1413	hrpA	-0.071	-0.021	-0.037	-0.095	-0.204	-2.171	-0.265	-2.171	-2.171	-2.171	0.017	-0.038	0.009	0.025	0.059
b1414	ydcF	-0.029	-0.005	-0.043	-0.037	0.040										
b1415	aldA	-0.139	0.130		0.066		-0.052	-0.078	-0.043	-0.169	-0.230	-0.011	-0.020	-0.055	-0.141	-0.188
b1418	cybB		0.147													
b1419	ydcA				0.203											
b1420	mokB	0.011	0.025	0.059	-0.004	0.732										
b1421	trg		-0.021	-0.019												
b1423	ydcJ	-0.004	0.101	-0.031	0.094	0.106										
b1424	mdoD						2.171	2.171				0.045	0.219		0.076	
b1425		0.353	0.092	0.105	0.005	0.003										
b1426	ydcH	0.022	-0.010	0.017	-0.084	0.151										
b1427	rimL	0.012	0.034	0.065	-0.035	0.027										
b1429	tehA	-0.034	-0.016	-0.012	-0.051	-0.036										
b1430	tehB	-0.045	-0.025	-0.052		-0.027						-0.042	-0.055		0.039	
b1431	ydcL	-0.044	0.071	0.119	0.032	0.004										
b1433	ydcO							2.171		2.171						
b1434	ydcN	-0.028	-0.021	-0.048	-0.054	-0.044										
b1435	ydcP	0.106	0.018	-0.031	-0.070	-0.099				2.171						
b1436	yncJ	-0.019	-0.011	-0.021	-0.012	0.002				2.171						
b1437			-0.011		-0.134	-0.069										
b1438	ydcQ					-0.011										
b1440	ydcS	0.032	0.033	0.041	0.033	-0.008										
b1441	ydcT	0.018	0.008	0.030	0.111	0.065				2.171						
b1442	ydcU		0.037													
b1444	ydcW	0.061	-0.012	0.033	-0.133	-0.064										0.585
b1445	ydcX		0.134		0.098											
b1446	ydcY	0.013	-0.019	0.115	0.066	0.200										
b1447	ydcZ	-0.214	-0.061	0.045	-0.055	-0.029										
b1448	yncA	0.022	0.048	0.074	0.205	0.192										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b1449	yncB		0.013							2.171	2.171	-0.131		0.150	0.242	0.194
b1451	yncD						2.171					0.127	0.061		0.095	0.083
b1452	yncE						0.009	-0.052	-0.061	-0.035	-0.087	-0.064	-0.060	-0.043	-0.080	-0.103
b1453	ansP		0.000													
b1454	yncG	0.007	0.047	0.010	-0.020	0.040										
b1455	yncH	-0.029	-0.022	0.043	0.018	0.097										
b1456		0.106	-0.059	0.148	-0.003	0.027										
b1457	ydcD	0.124	-0.002		0.062											
b1460	ydcC		0.159	0.080	0.084	0.164										
b1461	pptA	0.002	0.015	0.037	0.004	0.045										
b1462	yddH	-0.047	-0.028	-0.038	-0.069	-0.021										
b1463	nhoA	0.004	0.058	0.134	0.224	0.317										
b1467	narY				0.100											
b1468	narZ								2.171			-0.294		0.001		0.081
b1469	narU		0.044			-0.485										
b1470	yddJ				0.213											
b1472	yddL	0.122	0.078	0.092	0.266	0.394										
b1473	yddG				0.007											
b1478	adhP	0.021	-0.031	0.010	-0.128	0.011										
b1479	maeA	0.134	-0.052	0.017		0.036	0.100	0.009	0.069	-0.156	-0.213	0.039	-0.008	-0.091	0.102	0.128
b1480	sra						-2.171	0.113	0.100	0.169	0.360	-0.083	0.061	0.088	0.246	0.356
b1482	osmC	0.010	0.058	-0.005			-0.256	-0.052	0.122	0.274	0.469	-0.112	-0.068	0.024	0.132	0.416
b1483	ddpF	-0.032	0.035	-0.041	-0.067	0.006										
b1484	ddpD					-0.112	-2.171	-2.171	-2.171	-2.171	-2.171					
b1485	ddpC		-0.014		-0.082											
b1487	ddpA	-0.014	0.054	0.027	0.052	0.107										
b1489	dos							2.171								
b1490	yddV								2.171							
b1492	gadC	0.005	-0.002	0.017	0.034	0.151					2.171					
b1493	gadB	-0.108	-0.142	-0.086	0.022		-0.135	-0.013	0.078	0.608	0.895	0.055	0.019	0.111	0.667	1.050
b1494	pqqL		-0.476		0.015			2.171		2.171						
b1496	yddA	0.012	0.065	0.036	0.047	0.016										
b1497	ydeM						2.171			2.171						
b1498	ydeN	-0.053	0.047	0.025	-0.024	0.086										
b1499	ydeO	0.139			0.030				2.171	2.171			-0.047	0.026	0.104	0.320
b1501	ydeP	0.227	0.056	0.159							2.171					
b1503	ydeR				0.063											
b1504	ydeS	-0.003	0.028	0.106	0.175	0.260										
b1505	ydeT	0.211	0.040	0.112	0.264	0.102										
b1506	yneL				0.097											
b1507	hipA			0.057	-0.126											0.202
b1509	ydeU			-0.315												
b1510	ydeK	0.279	0.091		0.004					2.171						
b1511	lsrK			-0.254		-0.093			2.171	2.171						
b1512	lsrR								2.171	2.171						
b1513	lsrA		0.070	0.063	0.165	-0.014										
b1515	lsrD	-0.191	-0.013	-0.009	-0.003	-0.028										
b1517	lsrF						-2.171	-2.171	-2.171	-2.171	0.091	-0.130	-0.004	-0.003	-0.033	0.049
b1522	yneF	-0.010	-0.025	-0.091	-0.055	-0.032		2.171								
b1523	yneG							2.171								
b1524	yneH	0.028	-0.004	0.090	0.118	0.153										
b1525	sad	0.014	-0.008	-0.029	-0.094	-0.060										
b1526	yneJ	0.418														
b1527	yneK								2.171							
b1528	ydeA	-0.004	-0.015	-0.041	-0.071	-0.196										
b1529	marC	-0.031	0.090		0.015	0.006										
b1530	marR	0.132	0.087	0.169	0.132	0.359										
b1531	marA		0.244	0.084												
b1532	marB	0.075	-0.031	0.054												
b1533	eamA	-0.051	0.012	-0.050	0.033	-0.061										
b1534	ydeE	0.104	0.027	0.023	0.060	0.159										
b1538	dcp	-0.007	0.019	0.000	0.017	-0.066	0.213	-2.171	-2.171	0.026	0.074	-0.031	0.040	0.019	0.116	0.085
b1539	ydfG	-0.029	0.041	0.047		0.060	0.069	-0.022	0.221	0.161	0.148	0.125	0.038	0.035	0.112	0.053
b1540	ydfH	-0.188	0.138		0.163											
b1541	ydfZ	0.015	0.026	-0.021	0.069	0.272				2.171						
b1542	ydfI	0.005	0.017	-0.010	-0.035	0.008						0.217				
b1543	ydfJ		0.025	0.056	0.044	0.098										
b1544	ydfK	-0.105	0.192		0.012											
b1545	pinQ	-0.044	0.037	0.013	0.055	0.041										
b1546	tfaQ	0.027	0.074	0.021	0.002	0.045										
b1547	ydfN		0.057	0.039		0.063										
b1548	nohA	0.004	0.012		0.012	-0.086				2.171					0.040	
b1549	ydfO	0.514	-0.012		0.068	0.020										
b1550	gnsB	0.012	0.050	0.017		-0.018				2.171		0.062	-0.039			
b1551	ynfN		-0.154		0.070											
b1553	ydfP	0.100	0.021	0.078	-0.032	-0.019										
b1557	cspB	-0.030	0.046	0.021	0.040	0.069										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b1558	cspF	-0.001	0.030	-0.035	-0.085	-0.017										
b1559	ydfT	0.032	-0.016	0.141	-0.029	0.062										
b1561	rem	0.175		-0.220	-0.089											
b1562	hokD	-0.022	0.068	-0.037	0.030	0.015										
b1564	relB		0.010													
b1565	ydfV				-0.003	-0.097										
b1567	ydfW		-0.012		0.020											
b1569	dicC	0.096	-0.009	0.032	0.122	0.182										
b1570	dicA	-0.008	0.038	-0.007	0.012	0.050										
b1571	ydfA	-0.104	-0.097	-0.206	-0.063	-0.083										
b1572	ydfB			-0.423												0.051
b1573	rzpQ	0.137	0.078	0.068	0.136	0.208					2.171					
b1575	dicB	0.258	-0.065	-0.026	-0.031	-0.057										
b1578	insD	0.318		0.064	0.064	0.088										
b1582	ynfA		-0.033		0.062	0.054										
b1583	ynfB				0.096											
b1585	ynfC		0.151													
b1586	ynfD	0.104	0.033		0.127	-0.001										
b1589	ynfG		0.201		0.023											
b1591	dmsD	-0.054	-0.028	0.036	-0.021	-0.009										
b1592	clcB	-0.007	-0.014	0.013	0.007	-0.058										
b1594	dgsA	0.207	0.027													
b1597	asr	0.021	0.024	0.033	-0.068	0.008										
b1598	ydgD	0.155	0.010	0.002	0.045	-0.003										
b1599	mdtI	0.013	0.000	0.027	0.035	0.677										
b1601	tqsA	-0.128		-0.138		-0.554										
b1602	pntB	0.000	0.030	0.003	0.002	0.003	2.171	2.171		2.171		-0.100	0.081	-0.174	-0.293	-0.259
b1603	pntA	-0.110	0.017	0.125	0.023	-0.049	0.026	0.039	-0.026	-0.065	-2.171	-0.069	0.029	-0.127	-0.153	-0.059
b1604	ydgH	-0.055	0.032	0.050	0.008	-0.009	0.043	0.074	-0.017	-0.022	0.043	-0.016	-0.039	-0.057	-0.033	-0.155
b1605	ydgI	-0.037	-0.025	-0.018	-0.063	-0.031										
b1606	folM							2.171								
b1607	ydgC				0.042											
b1610	tus	0.050	0.025	-0.022	0.025	0.079	-2.171	-2.171	-2.171	-2.171	-2.171	0.105	0.011	0.085	0.024	-0.037
b1612	fumA						-0.004	0.030	0.000	-0.195	-0.113					
b1613	manA						0.000	-2.171	-2.171	-2.171	-2.171	-0.067	-0.105	0.007	0.008	-0.013
b1614	ydgA					-0.451	2.171		2.171	2.171						
b1615	uidC		0.198	0.583	0.103											
b1616	uidB		0.029					2.171								
b1617	uidA	-0.020	0.042	0.039	-0.001											
b1619	hdhA								2.171							
b1620	malI		0.285													
b1622	malY		0.009													
b1623	add	-0.012	0.007	-0.020	-0.021	-0.093										
b1626	ydgK				-0.058	-0.117										
b1627	rsxA	-0.041	-0.059	-0.057	-0.072	-0.090										
b1628	rsxB	0.028	-0.050	0.012	-0.051	-0.020										
b1629	rsxC	0.012	0.087	0.028	-0.015	0.030										
b1630	rsxD	-0.009	0.000	-0.280	-0.044	-0.009										
b1631	rsxG	-0.043	-0.022	0.000	0.025	-0.003										
b1632	rsxE	-0.086	-0.071	0.037	0.059	0.106										
b1633	nth	0.082	-0.035	-0.015	-0.108	-0.061										
b1634	tppB	-0.029	0.016	-0.043	0.021	-0.006										
b1635	gst	-0.005	-0.008	0.022	-0.021	0.001	-2.171	-0.087	-2.171	-2.171	-0.117	0.079	0.039	0.089	0.093	0.039
b1636	pdxY		0.011	0.014	0.150	0.178										
b1637	tyrS	0.034	0.079	-0.046	-0.017	0.002	-0.043	0.087	0.061	0.208	-2.171	-0.018	0.149	0.248	-0.071	0.176
b1638	pdxH	-0.012	-0.021	-0.054	-0.007	0.058			2.171			0.044	0.013	0.093	0.097	0.191
b1639	mliC	-0.025	0.009	0.044	0.120	0.155										
b1640	anmK	0.042	-0.027	-0.011	0.034	0.001										
b1641	slyB					0.047		2.171								
b1642	slyA	-0.043	-0.051	-0.070	-0.066	-0.044								-0.016	0.059	
b1643	ydhI	-0.014	-0.037	-0.066	-0.040	-0.070										
b1644	ydhJ	-0.026	0.059	-0.017	0.001	0.042	2.171									
b1645	ydhK	-0.029	0.051	0.149	0.066	0.152										
b1646	sodC	-0.024	0.013	-0.008	0.024	0.061										
b1648	ydhL	-0.024	-0.013	-0.043	-0.017	-0.128										
b1649	nemR	0.029	0.065	0.053	0.093	0.070										
b1650	nemA	-0.045	0.026	-0.031	0.035	0.015			2.171							
b1651	gloA	-0.044	0.023	0.023	-0.033	0.017		2.171			2.171	0.065	0.142	0.039	0.229	0.241
b1652	rnt	-0.015	-0.052	-0.051	0.008	-0.098				2.171						
b1653	lhr	-0.087	-0.015	0.009	-0.097	-0.009				2.171		-0.142	0.025	0.053	0.092	0.038
b1654	grxD	-0.151	-0.039	-0.023	0.053	0.016	-0.056	0.022	0.087	0.022	0.083	-0.016	0.026	0.067	0.077	0.115
b1655	ydhO	0.020	-0.046	-0.061	-0.026	-0.084										
b1656	sodB	0.287	0.018	-0.011	-0.062	0.036	-0.030	0.061	0.078	-0.026	-0.221	-0.053	0.008	-0.008	-0.003	-0.136
b1657	ydhP	-0.057	0.036	0.007	-0.006	0.035										
b1658	purR	0.007	0.001	-0.012	-0.013	0.046	2.171			2.171		-0.043	0.025	-0.059	-0.009	-0.086
b1660	ydhC	0.063	0.066	0.039	0.040	0.082										
b1661	cfa	-0.050	0.014	0.015	-0.029	0.000			2.171							

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b1662	ribC	-0.046	0.002	0.036	0.014	0.099	-0.447	0.069	-2.171	0.195	-0.313	0.017	-0.117	0.105	0.119	0.095
b1663	mdtK	0.109	-0.003	0.058	0.000	0.026										
b1664	ydhQ		0.117		0.170	-0.010										
b1667	ydhR				0.099											
b1668	ydhS	-0.033	0.007	0.011	0.116	0.198				2.171						
b1669	ydhT	-0.002	-0.011	-0.006	0.133	0.235										
b1670	ydhU	-0.039	0.018	0.104	0.154	0.205										
b1671	ydhX	-0.043	-0.025	0.045	0.065	0.085		2.171								
b1672	ydhW	-0.015	0.041	0.040	0.089	0.058										
b1673	ydhV	-0.043	0.035	0.050	0.078	0.134										
b1674	ydhY	0.047	-0.036	-0.040	-0.012	0.041										
b1675	ydhZ	0.041	0.029	0.083	0.116	0.224										
b1676	pykF	-0.038	-0.023	-0.029	-0.073	-0.138	0.030	0.000	0.009	0.026	0.000	0.026	-0.016	-0.027	0.021	-0.003
b1677	lpp						0.052	0.078	-0.035	-0.017	-0.048	0.075	0.105	-0.042	-0.025	0.029
b1678	ynhG		0.165													
b1679	sufE	-0.035	0.033	0.003	0.148	0.265										
b1680	sufS	0.121	0.029	0.094	0.116	0.125										
b1681	sufD			0.233	0.162											
b1682	sufC	-0.053	0.052	0.093	0.059											
b1683	sufB						2.171		2.171							
b1684	sufA	-0.024	-0.086	-0.017	-0.027	-0.022				2.171						
b1685	ydiH	0.004	-0.082	-0.035	0.019	-0.009										
b1686	ydiI				0.055							0.113			-0.046	-0.327
b1687	ydiJ											-0.044	-0.029	-0.014	0.001	0.047
b1688	ydiK	0.096			-0.003	-0.013										
b1689	ydiL			-0.048												
b1690	ydiM		-0.018	0.020	-0.013	-0.013										
b1691	ydiN	0.044	0.001	-0.010	0.029	0.039										
b1692	ydiB	0.001	-0.005	0.026	0.012	0.004										
b1693	aroD		-0.258													
b1694	ydiF	-0.077	-0.019	-0.092	0.024	-0.053										
b1695	ydiO	0.021	-0.044	-0.051	-0.062	-0.040										
b1696	ydiP	0.105		0.087	-0.058	-0.130										
b1697	ydiQ	-0.059	0.012	-0.013	-0.049	-0.128										
b1698	ydiR	0.003	0.021	0.041	0.071	0.099										
b1699	ydiS				0.245		-2.171	-2.171	-2.171	-2.171	-2.171					
b1700	ydiT	0.018	0.038	0.091	-0.067	-0.068										
b1702	pps	0.042	0.042	-0.017	-0.058	-0.028	0.030	0.069	-0.013	-0.043	0.004	0.087	0.063	0.015	0.060	0.034
b1703	ydiA	0.031	0.008	-0.019	0.032	0.136	-2.171	-2.171	-2.171	-2.171	-2.171					
b1704	aroH	-0.030	-0.018	-0.019	-0.015	-0.051	2.171	2.171								
b1705	ydiE	0.036	0.073	0.202	-0.024	0.168	2.171									
b1706	ydiU		0.113				2.171	2.171	2.171							
b1707	ydiV					0.017										
b1708	nlpC		-0.033	-0.064	-0.019	0.149										
b1709	btuD	-0.016	0.043	-0.029	0.037	0.062	2.171									
b1710	btuE	-0.041	0.031	-0.014	-0.123	-0.173			2.171	2.171	2.171		0.097		0.436	0.546
b1711	btuC	-0.080														
b1712	ihfA	0.145	-0.006	-0.030	0.042	-0.071	0.061	0.043	0.009	0.087	0.165	-0.014	0.019	-0.059	0.076	0.162
b1713	pheT	-0.006	0.023	-0.041	-0.058	-0.126	0.035	-0.056	-0.022	-0.022	-0.061	-0.020	-0.045	-0.001	-0.001	0.038
b1714	pheS		0.130				0.039	0.022	0.013	0.156	0.087	0.022	-0.174	0.023	-0.002	0.046
b1716	rplT	-0.034	0.051	0.012	0.038	-0.045	-0.061	-0.065	-0.048	-0.009	-0.022	0.010	-0.010	-0.032	0.111	0.121
b1717	rpmI	-0.051	-0.024	-0.006	-0.059	-0.096	2.171	2.171		2.171	2.171	-0.083	-0.062	0.083	-0.107	-0.223
b1718	infC	-0.044	0.001	0.009	-0.016	-0.014	-0.026	-0.039	-0.022	0.004	0.052	-0.011	0.008	-0.011	0.016	0.045
b1719	thrS						0.030	0.000	-0.022	-0.026	-0.048	0.060	-0.012	0.021	-0.014	-0.038
b1721						-0.795										
b1722	ydiY				0.029											
b1723	pfkB	-0.071	0.052	0.130	0.070	0.170			2.171	2.171	2.171					
b1725	yniA	0.047	-0.186	0.046	-0.007	-0.009										
b1727	yniC	0.123		0.239	0.060	0.036						-0.015	0.084	0.001	-0.073	-0.117
b1729	ydjN		-0.013	-0.017	-0.058	-0.032					2.171					
b1730	ydjO			0.000		-0.128										
b1732	katE		-0.077	0.003	0.111											
b1733	chbG	0.056		-0.157	0.081	-0.001										
b1735	chbR	-0.135	-0.020	-0.183	-0.203	-0.266								0.061		0.124
b1737	chbC		-0.554													
b1738	chbB		0.040													
b1739	osmE									2.171	2.171				0.436	0.386
b1740	nadE		-0.018	0.160	0.084		0.052	0.087	0.122	0.156	0.182	0.057	0.022	0.056	0.175	0.150
b1741	cho	0.056	0.159	0.060	0.112	0.235										
b1742	ves	-0.034	-0.061	-0.160	0.172	0.419										
b1743	spy	0.031	-0.052	-0.003	0.025	-0.010										
b1744	astE	-0.013	0.055	0.025	0.023	0.075										
b1745	astB	0.019	0.000	0.107	-0.087	-0.072										
b1746	astD	0.002	-0.008	-0.001	0.002	0.077										
b1747	astA			-0.129	0.094											
b1748	astC	-0.091	-0.038	-0.029	-0.026	-0.145	2.171									
b1749	xthA	0.032	0.040	-0.003	0.043	0.094	2.171	2.171		2.171						

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b1750	ydjX	0.056	-0.008	0.014		0.080										
b1751	ydjY	0.108	0.049		0.078			2.171								
b1752	ydjZ		0.234	0.109		0.017										
b1754	ynjB	0.081	0.002	0.094	-0.098	-0.067										
b1755	ynjC			-0.087												
b1756	ynjD		0.007	-0.058	0.096	-0.606										
b1757	ynjE	0.122	0.092	0.204	0.616	0.977										
b1758	ynjF	-0.026	-0.019	0.007	0.034	-0.002										
b1759	nudG	-0.028	-0.009	0.011	0.009	-0.011										
b1760	ynjH	0.012	-0.033	-0.029	0.002	-0.022										
b1761	gdhA	-0.040	0.023	0.001	-0.052	-0.125	-2.171	-2.171	-2.171	-0.152	-0.235	-0.486	-0.391	-0.203	-0.089	-0.163
b1762	ynjI	-0.009	-0.011	-0.003	-0.053	-0.013						-0.148	-0.309	-0.028	-0.092	
b1763	topB	0.001	-0.016	-0.031	-0.030	-0.035	2.171					0.067		-0.010	0.057	0.149
b1764	selD	-0.032	-0.008	-0.019	-0.053	-0.022	0.030	0.026	-0.052	-0.035	-0.013	0.070	0.057	0.032	0.058	0.081
b1765	ydjA	-0.014	0.039	0.100	0.178	0.247	-0.017	0.004	-0.061	0.074	-0.096	-0.059	0.001	-0.043	-0.043	0.080
b1766	sppA	-0.083	0.024	-0.006	-0.042	-0.106	2.171									
b1767	ansA	-0.033	-0.022	-0.038	-0.085	-0.113										
b1768	pncA	-0.039	-0.079	-0.078	-0.067	-0.121										
b1769	ydjE		-0.068	0.075	0.038	0.036										
b1771	ydjG		0.057	-0.025	0.018		-2.171	-2.171	-2.171	-2.171	-2.171	0.052	0.057			-0.039
b1772	ydjH									2.171						
b1773	ydjI		0.241													
b1774	ydjJ	0.161	0.086	0.048	0.006	0.035										
b1775	ydjK	-0.494		-0.023	0.000											
b1776	ydjL	-0.016	0.011	0.005	-0.062	-0.024					2.171					
b1777	yeaC	0.028	-0.032	-0.055	0.013	0.022										
b1778	msrB	-0.027	-0.099	-0.159	-0.067	-0.070					2.171		-0.091	0.049	-0.053	0.104
b1779	gapA	-0.099	-0.003	-0.022	-0.074	-0.079	0.013	0.026	-0.030	0.000	-0.043	0.037	-0.005	-0.014	-0.020	-0.034
b1780	yeaD	0.100	-0.013	0.079	0.001	0.032	-0.152	-0.035	-0.048	-0.004	-0.030	0.051	0.001	0.030	-0.040	0.135
b1781	yeaE	0.062	0.055	0.062	0.071	0.009										
b1782	mipA	-0.038	-0.002	-0.072	-0.014	-0.019										
b1783	yeaG	-0.014	-0.002	-0.097	-0.066	-0.120			2.171		2.171	-0.153		0.126	0.226	0.321
b1785	yeaI	-0.033	-0.003	-0.017	-0.015	-0.012										
b1786	yeaJ	0.018	0.070	0.182	0.386	0.383										
b1787	yeaK	0.003	-0.038	-0.036	-0.107	-0.108										
b1788	yoaI			-0.020		-0.136										
b1789	yeaL				0.215											
b1790	yeaM			-0.021						2.171						
b1791	yeaN	0.190	0.180	0.007	-0.027	-0.067										
b1793	yoaF	0.032	0.048	0.072	0.131	0.216										
b1794	yeaP	-0.009	-0.005	-0.006	-0.041	-0.069	-2.171	-2.171	-2.171	-2.171	-2.171	0.034		-0.010	0.003	0.080
b1795	yeaQ				0.282											
b1796	yoaG		0.060		-0.111	-0.090										
b1797	yeaR	-0.009	0.056		0.115											
b1798	leuE		0.178	-0.048	0.179											
b1799	yeaT										2.171					
b1800	yeaU	0.072	0.013	0.023	-0.251	-0.014										
b1801	yeaV		-0.017	0.030	-0.003											
b1802	yeaW		0.028	0.008	-0.037	0.020										
b1803	yeaX	0.012	0.032	0.045	0.044	0.009										
b1804	rnd									2.171						
b1805	fadD	0.007	-0.010	-0.052	-0.041	-0.012										
b1806	yeaY	-0.099	0.059	0.011	-0.021	-0.051										
b1807	yeaZ	0.004	0.008	0.033	-0.081	-0.114										
b1808	yoaA							2.171				0.113	0.013			
b1809	yoaB	0.044	0.010	-0.014	-0.040	0.002										
b1810	yoaC	0.015	0.012	0.002	0.088	0.133										
b1811	yoaH	0.056	0.014	0.066	-0.044	-0.026										
b1812	pabB	0.090	0.022	0.027	-0.007	0.130										
b1813	nudL	0.001	0.052	0.039	-0.034	0.036										
b1814	sdaA	0.001	-0.043	-0.028	-0.042	-0.013										
b1815	yoaD	-0.078	0.006	0.190	0.024	0.090										
b1816	yoaE		-0.035	-0.078	-0.047	-0.029										
b1817	manX	-0.082	-0.019	-0.105	-0.220	-0.462	-0.017	0.534	-2.171	-2.171	-2.171	0.058	-0.036	0.001	-0.100	-0.036
b1818	manY	-0.023	-0.020	-0.036	-0.131	-0.457										
b1819	manZ		-0.044													
b1820	yobD	-0.067	-0.016	0.012	-0.084	-0.127										
b1821	yebN	0.005	0.014	0.062	-0.071	-0.087										
b1822	rrmA	0.087	0.005	-0.012	0.094	0.178										
b1823	cspC	-0.043	0.070	-0.033		-0.009	0.017	0.017	0.022	-0.030	-0.009	-0.038	-0.015	-0.002	-0.028	0.075
b1824	yobF	-0.075	-0.133	-0.106	-0.166	-0.216										
b1825	yebO		0.086	0.021	0.055	0.190										
b1826	mgrB	0.093	-0.114	0.094	0.023											
b1827	kdgR					-0.048	2.171	2.171			2.171	0.059	-0.021	0.009	-0.030	0.017
b1828	yebQ	0.016	-0.010	-0.052	-0.006	-0.094										
b1829	htpX			-0.158	-0.033	-0.021										
b1830	prc								2.171	2.171						

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b1831	proQ		-0.033	-0.136		0.151										
b1832	yebR		0.021	0.124	0.125								-0.005	-0.019	0.111	0.122
b1833	yebS	0.032	-0.002	0.064	0.234											
b1834	yebT	0.084	0.019	-0.126	-0.056	0.002										
b1835	rsmF							2.171								
b1836	yebV	-0.064	-0.012	-0.053	0.098											
b1837	yebW	-0.033	0.135	0.097	0.234	0.556										
b1838	pphA	-0.072	-0.495	-0.138												
b1839	yebY	-0.145	0.048	-0.030	0.002	-0.070										
b1840	yebZ	-0.008	-0.042	-0.086	-0.062	-0.103										
b1841	yobA	-0.078	-0.112	0.032	-0.042											
b1842	holE	0.034	0.023	-0.034	0.033	0.005										
b1843	yobB	0.144	0.000	-0.334												
b1844	exoX	-0.041	0.090	0.045		0.085										
b1846	yebE	-0.011	0.041	-0.057	-0.001	0.138										
b1847	yebF	0.010	0.100	0.016	0.165	0.341	2.171	2.171	2.171	2.171	2.171	-0.228		0.306	0.650	0.776
b1848	yebG	-0.040	-0.074	0.030	0.343	0.265										
b1849	purT	0.003	0.005	-0.047	0.013	0.015	0.083	0.039	0.052	0.052	0.061	0.111	0.025	0.094	0.056	0.005
b1850	eda	-0.014	0.044	0.051	-0.055	-0.134	0.052	0.087	0.083	0.087	0.013	0.021	0.047	0.127	0.134	0.148
b1851	edd		-0.015		-0.035											
b1852	zwf	-0.034	-0.010	-0.018	0.047	-0.032	0.013	-0.130	-0.017	0.004	0.109	0.055	0.005	0.012	0.043	0.076
b1853	yebK	-0.051	0.084	0.004	0.033	0.056										
b1854	pykA	-0.043	0.009	-0.033	-0.008	0.056	0.065	-0.048	0.022	0.004	0.104	0.023	0.037	0.060	0.116	0.102
b1855	lpxM		-0.021	0.021	-0.035	0.077				2.171						
b1856	yebA	-0.002	0.014	0.010	-0.017	0.083										
b1857	znuA	0.002	0.042	0.059	0.044	0.157										
b1858	znuC	0.038	-0.057	-0.067	-0.122	-0.062										
b1859	znuB			-0.161												
b1860	ruvB	0.016	-0.080	0.004	-0.029	0.009										
b1861	ruvA		0.023	0.032	0.118	-0.044						0.045	-0.032			
b1862	yebB	-0.002	-0.008	-0.017	-0.035	-0.013										
b1863	ruvC	0.021	-0.044	-0.058	-0.076	-0.003				2.171						
b1864	yebC	0.097	0.025	-0.062	-0.014	0.028	-0.065	-0.069	-0.117	-0.126	-0.113	-0.081	0.013	0.070	-0.013	0.011
b1865	nudB				0.316											
b1866	aspS	0.017	0.029	0.013	0.136	-0.107	0.043	0.000	-0.013	-0.043	-0.048	0.039	0.022	0.001	0.062	0.035
b1867	yecD				0.035											
b1868	yecE	-0.062	0.009	-0.046	-0.109	-0.136										
b1869	yecN			-0.025	0.052	0.116										
b1870	cmoA			0.288												
b1871	cmoB	0.021	0.035	0.002	0.012	0.053	2.171									
b1872	torZ	-0.016	-0.026	-0.023	-0.092	-0.300										
b1873	torY		0.022		0.034											
b1874	cutC	-0.008	0.060	0.082	0.221	0.419										
b1875	yecM		-0.030	0.001												
b1876	argS	0.027	-0.024	-0.054	-0.022	-0.148	0.022	-0.022	-0.013	0.030	-0.052	-0.016	-0.036	0.036	0.025	0.080
b1877	yecT		0.143	0.056	-0.003	0.030										
b1878	flhE	-0.059	-0.066	-0.091	-0.050	-0.034										
b1880	flhB			0.217	-0.009											
b1881	cheZ	-0.047	0.031	0.111	0.300	0.374	-2.171	-2.171	-2.171	-2.171	-0.056					
b1882	cheY	-0.032	-0.021	-0.149	-0.111	-0.302										
b1883	cheB	0.009	0.029	0.107	0.347	0.435										
b1884	cheR	-0.010	0.038	0.101	0.332	0.322	-2.171	-2.171	-2.171	-2.171	-2.171					
b1885	tap	0.001	0.027	0.011	0.016	0.026										
b1886	tar	-0.069	-0.018	-0.023	-0.043	-0.567						0.088	0.059		0.103	0.271
b1887	cheW		0.005	0.004												
b1888	cheA	0.074	-0.027	-0.034	0.016	-0.095	2.171					-0.061	-0.119	-0.018	-0.038	0.057
b1889	motB	0.023	0.062	0.079	-0.014	-0.020										
b1890	motA	0.078	-0.026	0.006	-0.037	-0.141										
b1893	insB-5									2.171						
b1895	uspC	-0.050	-0.023	-0.073	-0.074	-0.159										
b1896	otsA			-0.277						2.171	2.171	0.121	-0.106	0.133	0.131	0.285
b1897	otsB		0.069	0.092	-0.049	-0.038										
b1898			0.033	0.058	0.098	0.107										
b1899				0.063	0.067	0.210										
b1900	araG	0.241	-0.062													
b1902	ftnB	-0.054	0.013	0.014	-0.012	-0.037	2.171		2.171							
b1903		-0.015	-0.019	-0.024	0.019	0.004										
b1904	yecR	-0.001	-0.019	-0.005	0.024	0.003										
b1905	ftnA	0.003	-0.013	0.008	0.007	0.051										
b1906	yecH		0.045	-0.001		0.055										
b1908	yecA	0.132		-0.026	-0.114											
b1912	pgsA	-0.043	0.016	-0.017	0.021	0.029										
b1913	uvrC	-0.074	-0.075	-0.058	-0.103	-0.032										
b1915	yecF		-0.090	0.028												
b1916	sdiA		0.200	-0.055					2.171			-0.021	-0.224	-0.030	-0.002	-0.224
b1917	yecC	0.124	0.100	0.025	-0.091	-0.099										
b1919	dcyD	0.028	0.027	-0.005		-0.011										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b1920	fliY						-0.004	-0.052	0.069	0.074	0.030	-0.001	-0.025	0.084	0.044	0.071
b1921	fliZ	-0.027	-0.046	0.041	-0.038											
b1925	fliS	-0.033	0.016	-0.054	0.007	-0.173		2.171								
b1928	yedD	0.165	0.031	0.021	0.133	0.197		2.171		2.171		0.046	-0.016		0.020	0.052
b1929	yedE		0.017	0.228	-0.017	0.003										
b1930	yedF	-0.009	0.032	0.031	-0.090	-0.034										
b1931	yedK		0.055	-0.012	-0.054	-0.005					2.171					
b1932	yedL	-0.153	-0.006	0.031	-0.026	-0.105										
b1934		0.041	-0.026	0.035	0.043	0.057										
b1935	yedM	-0.030	0.008	0.071	-0.009	-0.021										
b1936	intG	-0.420	-0.467	-0.427	-0.110	-0.445						0.133	0.097	0.094	0.161	0.177
b1937	fliE		0.025													
b1940	fliH							2.171								
b1941	fliI		0.095	-0.247	-0.161		-2.171	-2.171	-2.171	0.013	-0.026					
b1942	fliJ	0.031	0.033	0.011	-0.074	-0.034										
b1943	fliK		0.126	0.042		0.126		2.171								
b1945	fliM										2.171					
b1952	dsrB	-0.043	-0.002	-0.053	-0.071	-0.086										
b1953	yodD						-2.171	-2.171	-2.171	-2.171	-2.171					
b1955	yedP		-0.487	-0.118	-0.220	-0.306										
b1956	yedQ	-0.045	0.060	0.148	0.092	0.103										
b1957	yodC	0.010	-0.047		-0.198											
b1958	yedI	-0.018	0.040	0.060	-0.040	0.018										
b1959	yedA		-0.074		0.055											
b1960	vsr	0.142	0.039	-0.048	-0.108	-0.147										
b1961	dcm	-0.074	0.020	-0.031	-0.076	-0.109										
b1963	yedR				0.056											
b1964		0.026	-0.283	0.086	0.175	0.302										
b1965		0.067	-0.027	0.107	0.187	0.270										
b1966				-0.106		0.315										
b1967	hchA	0.028	-0.026	0.033	0.005	0.074										
b1968	yedV	0.010	0.050	-0.049												
b1969	yedW	0.036	0.053	0.053	0.036	0.077										
b1970	yedX	0.056	0.070	0.098	0.089	0.288										
b1971	yedY	-0.002	0.042	0.124	0.136	0.233										
b1972	yedZ	0.009	-0.011	-0.035	0.028	0.092										
b1973	zinT	-0.023	-0.053	0.019	-0.075	-0.170										
b1974	yodB	0.091	0.025	0.126	0.101	0.102										
b1976	mtfA	0.053	0.125	0.144	0.090	0.047										
b1978	yeeJ	-0.014	-0.003	0.172		0.052										
b1979				0.264												
b1980		-0.011	0.011	0.002	0.007	-0.077										
b1981	shiA	-0.047		0.106		-0.105										
b1982	amn										2.171					
b1983	yeeN						-2.171	-2.171	-2.171	-2.171	-2.171	-0.035	-0.121	0.001	-0.077	0.235
b1985	yeeO	0.049	-0.591	-0.361	0.483	-0.642										
b1987	cbl						2.171			2.171						
b1988	nac							2.171								
b1990	erfK	0.149		-0.112	-0.145	-0.024										
b1991	cobT	-0.126	-0.019	0.023	-0.018	-0.014										
b1992	cobS	0.019	-0.009	-0.035	-0.033	0.041						0.106	0.017		0.159	0.071
b1993	cobU	0.083	0.051	0.054	-0.057	-0.074										
b1995	yoeA		0.028	0.050												
b1996	insD-3	-0.069	0.014	0.014	-0.087	-0.036										
b1997	insC-3	0.157	0.088	-0.094	0.067	-0.086		2.171								
b1998	yoeE														-0.076	0.049
b1999	yeeP	0.040	0.025	-0.103	-0.013	-0.042										
b2000	flu	0.032	0.013	0.027	0.056	0.189				2.171					1.258	0.112
b2001	yeeR	0.018	-0.047	-0.078	-0.136	-0.209										
b2002	yeeS	-0.005	-0.030	-0.042	0.092	0.084		2.171								
b2003	yeeT	-0.068	-0.015	-0.048	-0.100	-0.103										
b2004	yeeU		-0.030		-0.101	-0.042										
b2005	yeeV	0.043	-0.009	-0.009	-0.033	0.048										
b2006	yeeW	0.057	-0.024	-0.042	-0.042	-0.038										
b2007	yeeX		-0.012			0.215										
b2008	yeeA		-0.108	0.081	-0.007											
b2009	sbmC	-0.051	0.017	0.050	-0.065	-0.030										
b2010	dacD	-0.011	-0.044	-0.055	0.093	-0.033				2.171	2.171					
b2011	sbcB	0.063	-0.068	-0.023	-0.071	0.033										
b2012	yeeD	-0.052	-0.009	0.021	-0.045	-0.067										
b2013	yeeE	0.009	0.007	0.121	-0.010	-0.051										
b2014	yeeF		0.030	0.040	-0.041	-0.008										
b2015	yeeY				-0.061											
b2016	yeeZ	0.054	0.051	0.074		0.026	2.171									
b2017	yefM	-0.062	0.000	-0.060	-0.062	-0.119										
b2019	hisG		0.134	-0.130			0.017	0.052	-0.035	-0.074	-0.143	0.045	0.028	0.014	-0.121	-0.137
b2020	hisD	-0.092	-0.015	0.056	0.148	-0.113	-0.009	-0.061	-0.061	-0.100	0.069	-0.117	-0.044	-0.043	-0.019	0.050

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2021	hisC	-0.059	-0.035	-0.008	-0.055	-0.052	0.026	0.030	0.026	-0.065	-0.178	0.089	0.020	-0.012	-0.083	0.022
b2022	hisB	-0.042	-0.034	-0.007	0.179	-0.073	-0.039	-0.078	-0.043	-0.122	-0.100	0.037	0.005	-0.095	0.041	0.029
b2023	hisH	-1.041	-1.191	-1.055	-0.055	-0.207	-0.026	-2.171	0.026	-2.171	-2.171	-0.101	0.055	-0.120	-0.128	-0.081
b2024	hisA							2.171	2.171	2.171		-0.067	-0.106	-0.302	-0.067	-0.214
b2025	hisF	-0.023	-0.036	0.003	0.031	-0.075	-0.022	-0.022	0.169	0.030	-0.039	0.051	0.037	0.047	-0.013	0.002
b2026	hisI	0.014	0.005	-0.020	-0.010	0.011	-0.139	-0.109	-0.009	-0.104	-2.171	-0.066	0.008	-0.070	-0.068	-0.021
b2027	cld		-0.164	0.146	-0.035	-0.062										
b2028	ugd	-0.085	-0.026	-0.016	0.062	-0.029				2.171						
b2029	gnd	0.108					-0.022	-0.013	0.013	0.013	-0.022	-0.002	-0.019	-0.003	0.034	0.016
b2031		-0.209		-0.050												
b2032	wbbK		0.020	0.017	-0.005	0.015					2.171					
b2033	wbbJ	0.060	0.014	0.013		0.007										
b2034	wbbI	-0.008	0.000	0.029	0.040	0.148		2.171			2.171					
b2035	rfc	-0.129	-0.023	0.039	0.035	0.176										
b2036	glf	0.089	0.073	-0.016	0.028	0.010	-2.171	-2.171	-2.171	-2.171	0.052	-0.022	0.000	-0.008	0.001	-0.005
b2038	rfbC	-0.033	-0.029	-0.013	0.027	-0.151	2.171									
b2039	rfbA	-0.039	-0.012	-0.070	-0.003	-0.077	-0.213	-0.200	-2.171	0.061	-2.171	0.057	0.043	0.004	0.031	0.027
b2040	rfbD						0.069	-2.171	0.109	-2.171	-2.171	0.071	0.035	0.052	0.065	-0.099
b2041	rfbB	-0.158	0.027	-0.011	-0.037	0.021	-0.074	-2.171	-0.178	-0.056		-0.003	0.018	0.016	-0.066	0.025
b2042	galF		0.127				0.156	0.065	0.152	0.135	0.113	-0.043	0.034	0.058	-0.013	0.144
b2043	wcaM	0.044	0.055	0.070	0.087	0.155										
b2044	wcaL	-0.091	0.040	0.212												
b2045	wcaK								2.171							
b2046	wzxC	0.053	0.005	0.018	0.072	0.078										
b2047	wcaJ	0.157	-0.001	-0.021		0.075										
b2048	cpsG										2.171					
b2049	cpsB	0.035	0.046	-0.001	0.070	0.075										
b2050	wcaI	-0.003	-0.027	-0.045	-0.006	-0.021		2.171								
b2051	gmm	0.073	-0.090	0.007	-0.044	-0.008										
b2052	fcl	0.047	0.022	0.063	0.230	0.292					2.171					
b2053	gmd	0.088	0.115	0.129	0.187	0.386										
b2054	wcaF	0.030	0.070			0.083										
b2055	wcaE						2.171									
b2057	wcaC	0.256		0.118	0.058	-0.014			2.171							
b2058	wcaB	-0.050	-0.006	-0.033	-0.043	-0.181										
b2059	wcaA		0.051		-0.177											
b2060	wzc		0.169	-0.139			-2.171	-2.171	-2.171	-2.171	-2.171	-0.044	-0.036	-0.102	0.046	-0.006
b2063	yegH	-0.063	-0.028	-0.051	-0.039	-0.097										
b2064	asmA	0.163	0.073	0.165		-0.064										
b2065	dcd	-0.042	-0.031	-0.065	-0.075	-0.047										
b2066	udk		0.014	-0.035	0.131	-0.192						0.020	-0.085		0.118	0.207
b2068	alkA		-0.170					2.171			2.171					
b2069	yegD		-0.106		0.041	0.020					2.171					
b2070	yegI		0.107	0.176		0.073										
b2071	yegJ		-0.018	0.076		-0.072										
b2072	yegK				-0.036	-0.056										
b2073	yegL	-0.012	0.004	0.105	0.215	0.135	-2.171	-2.171	-2.171	0.317	-2.171	-0.006	0.048		0.134	0.293
b2074	mdtA			0.033	-0.066		0.004	0.069	-2.171	-2.171	-2.171					
b2075	mdtB	-0.043	0.005	0.093	0.223	0.339										
b2077	mdtD	0.128	-0.049	0.063	-0.037	0.075										
b2078	baeS	-0.002	-0.005	-0.034	-0.127	-0.053		2.171								
b2079	baeR	-0.007	0.002	-0.010	-0.038	-0.004										
b2080	yegP		0.076	0.365	-0.080	-0.031										
b2081	yegQ	-0.009	0.037	0.022	-0.034	-0.003										
b2083		-0.013	0.049	0.046	0.030	0.046										
b2084		0.048	0.084	0.044	0.057	-0.045										
b2085	yegR	0.131	0.080	-0.009	0.085											
b2086	yegS	-0.055		0.134	0.015	0.034										
b2088	insE-5		0.055													
b2090	gatR_2	-0.493	0.063	0.205								-0.059	0.018	0.048	0.132	-0.020
b2092	gatC	-0.048	0.071	0.046	-0.168	-0.211										
b2093	gatB	-0.077	0.001	-0.012	-0.144	-0.184	0.030	-0.013	0.013	-0.304	-0.708	0.038	0.055	0.046	-0.266	-0.298
b2094	gatA						-0.026	-0.056	-0.109	-0.300	-0.395	-0.048	0.010	-0.046	-0.148	-0.093
b2095	gatZ						-2.171	0.030	-2.171	-2.171	-2.171	-0.075	0.006	-0.155	-0.200	-0.401
b2096	gatY						0.026	0.039	-0.013	-0.439	-0.608	-0.031	0.003	-0.042	-0.343	-0.315
b2097	fbaB		0.097	0.038	-0.017					2.171						
b2098	yegT	0.223	0.105	0.106	-0.026	0.068										
b2100	yegV	-0.002	0.021	0.277		-0.025										
b2101	yegW	0.027	0.024	0.116	0.280	0.421										
b2102	yegX				0.079											
b2103	thiD	-0.009	0.031	0.019	-0.004	-0.064	0.043	0.109	-2.171	0.065	0.091	-0.008	-0.101	0.078	0.029	0.002
b2104	thiM		0.004	0.016		-0.322				2.171	2.171					
b2105	rcnR					0.151										
b2106	rcnA				0.041	0.062										
b2107	yohN				0.001	0.088										
b2108	yehA										2.171					
b2109	yehB		0.048	0.009	0.040	0.032	0.122	-2.171	-2.171	-2.171	-2.171					

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2110	yehC				0.041		2.171					0.031	0.073			-0.030
b2111	yehD		0.050													
b2112	yehE	-0.040	0.040	0.021	-0.164	-0.092										
b2113	mrp	0.018	-0.006	-0.001	-0.018	0.004										
b2114	metG	-0.072	-0.037	-0.056	0.029	-0.036	-0.126	-0.043	0.000	-0.078	0.009	-0.016	-0.037	-0.016	-0.049	-0.007
b2115		-0.040	0.043	0.007	-0.105	-0.162										
b2117		0.009	-0.011	-0.009	-0.129	-0.027										
b2118	yehI	-0.020	0.020	0.029	-0.049	-0.029										
b2119	yehL	-0.034	-0.053	-0.056	-0.081	-0.094		2.171								
b2121	yehP			0.119	-0.053	-0.048										
b2122	yehQ	-0.055	0.065	-0.092	-0.008	-0.008	-2.171	-2.171	-2.171	-2.171	-2.171			-0.033	0.050	
b2124	yehS	-0.017	-0.076	-0.008	0.057	0.195										
b2125	yehT		-0.179	0.145	0.061	0.072				2.171						
b2126	yehU	0.044	-0.031	0.094	-0.031	-0.019										
b2127	mlrA			-0.085												
b2128	yehW	0.345	0.046	0.076	-0.011	0.041										
b2129	yehX	-0.060	0.015	0.036	-0.037	-0.016										
b2130	yehY	0.208			-0.022	0.012										
b2131	osmF		0.112								2.171					
b2133	dld	0.006	0.003	-0.027	-0.021	-0.012		2.171		2.171	2.171					
b2135	yohC	-0.007	-0.034	0.141	0.027	0.024										
b2137	yohF	0.028	0.011	-0.056	-0.059	-0.050										
b2138	mdtQ		0.145				2.171									
b2139	yohH	-0.444			0.141											
b2140	dusC						2.171									
b2141	yohJ	0.006	-0.168	-0.076	0.094	0.102										
b2142	yohK	0.071	0.302													
b2143	cdd	-0.037	-0.011	-0.034	-0.097	-0.065	2.171									
b2144	sanA	-0.044	0.047	0.037	-0.054	-0.025				2.171						
b2145	yeiS	0.103	-0.230	-0.023	0.016	-0.019										
b2146	yeiT	0.135	0.061	0.067	-0.020	-0.042						0.164	-0.014	0.123	-0.045	-0.043
b2147	yeiA	0.038	0.137	-0.121		0.186										
b2149	mglA	-0.029	-0.034	-0.037	-0.038	-0.042	2.171									
b2150	mglB						-0.039	-0.100	-2.171	-2.171	-2.171	0.053	-0.036	-0.027	-0.108	0.041
b2152	yeiB	-0.007	-0.057	0.047	-0.104	-0.086										
b2153	folE	-0.029	-0.020	-0.057	-0.002	-0.093	0.030	-0.056	0.035	0.096	0.139	-0.153	-0.215	-0.082	0.028	0.124
b2154	yeiG	-0.006	-0.001	-0.007	-0.114	0.003		2.171								
b2155	cirA	-0.034	0.023	-0.020	-0.088	-0.067										
b2156	lysP		-0.061													
b2158	yeiH	-0.288	0.208		0.203	0.262										
b2159	nfo	-0.091	0.000	0.034		0.001		2.171	2.171							
b2160	yeiI					0.072					2.171					
b2162	rihB	-0.068	0.023	0.121	0.197	0.267	-2.171	-2.171	-2.171	-2.171	-2.171					
b2165	pscG	0.053	0.145	0.026	0.232	0.343										
b2166	pscK	0.067	0.044	0.106	-0.032	0.045										
b2168	fruK	-0.129	0.025	-0.011	0.032	0.077										
b2169	fruB									2.171						
b2170	setB		0.079	0.128	0.066	0.071										
b2172	yeiQ						2.171									
b2175	spr	-0.002	0.045	-0.003	0.019	0.124										
b2176	rtn	-0.036	0.016	-0.005	-0.118	-0.076			2.171							
b2177	yejA		-0.080	-0.043	0.037	-0.038				2.171						
b2178	yejB	-0.042	-0.168	-0.067		0.053	-2.171	-2.171	-2.171	-2.171	-2.171	-0.161	-0.152	-0.104		
b2180	yejF			0.110	0.174		-2.171	-2.171	-2.171	-2.171	-2.171	0.080	0.102	0.108	0.173	0.039
b2181	yejG	0.170	-0.017	-0.025	-0.001	0.012										
b2182	bcr	0.056	-0.119	0.024	-0.052	0.004										
b2183	rsuA			-0.009	-0.003	0.021	2.171		2.171	2.171	2.171					
b2184	yejH						-2.171	-2.171	-2.171	-2.171	-2.171					
b2185	rplY	0.015	0.007	-0.017	-0.031	0.019	-0.252	-0.122	-0.052	-0.104	0.009	-0.147	-0.031	-0.034	-0.090	0.095
b2186	yejK		0.019	0.224	0.000	0.006	2.171				2.171					
b2187	yejL	0.546	0.095		0.184	-0.265	2.171									
b2188	yejM	-0.002	-0.034	-0.004	-0.031	-0.023										
b2190	yejO	-0.033	-0.053	-0.089	-0.011	-0.054				2.171						
b2191				0.124	0.003	-0.088										
b2192	insH-8		-0.106													
b2193	narP	-0.047	-0.053	-0.038	-0.015	-0.042			2.171		2.171	-0.058	0.030	-0.122	0.005	-0.063
b2194	ccmH						2.171									
b2195	ccmG	-0.132	-0.032	-0.047		-0.075										
b2196	ccmF	-0.048	0.051	0.019	-0.067	0.012										
b2201	ccmA	0.084	-0.003	-0.041	-0.031	-0.015										
b2202	napC	0.083	0.051	0.223												
b2203	napB	0.478	0.068	-0.078	0.097		-2.171	-2.171	-2.171	-2.171	-2.171					
b2204	napH		-0.073	0.127		-0.002										
b2205	napG		0.091	0.115	-0.023	0.024										
b2206	napA	-0.035	-0.023	-0.029	-0.031	-0.007						0.053		-0.258	0.013	0.042
b2207	napD	-0.005	0.006	-0.024	-0.015	0.018										
b2209	eco	-0.054	-0.044	-0.060	0.002	0.064										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2210	mqo	-0.020	-0.054	-0.048	-0.087	-0.094	-2.171	-2.171	0.395		-2.171					
b2211	yojI		0.085	0.204	0.170	0.291										
b2212	alkB	-0.070	0.021	-0.042	-0.041	-0.058										
b2213	ada	0.023	-0.015	-0.066	-0.060	-0.054				2.171						
b2214	apbE	-0.076	0.034	-0.059	-0.062	-0.087		2.171	2.171							
b2217	rcsB						-0.087	-0.065	-0.130	0.013	-0.039	0.039	-0.008	-0.023	0.050	0.038
b2218	rcsC	-0.164			0.104											
b2220	atoC	-0.014	-0.092	0.010	0.049	0.145	-2.171	-2.171	-2.171	-2.171	-2.171					
b2221	atoD	0.041	0.014	0.018		-0.013				2.171						
b2222	atoA	-0.001	-0.018	-0.025	-0.075	-0.009										
b2223	atoE	0.020	0.017	-0.059	-0.041											
b2224	atoB	0.001	-0.013	-0.037	0.009	-0.008										
b2225	yfaP	0.001	-0.014	-0.037	-0.036	-0.009										
b2226	yfaQ							2.171	2.171		2.171					
b2227		0.029	-0.034	-0.066	-0.040	-0.169										
b2228		-0.001	-0.066	-0.121	-0.050	-0.108										
b2229	yfaT	0.047	0.101	-0.003		-0.103										
b2230	yfaA	-0.048	0.010	-0.033	-0.133	-0.147										
b2231	gyrA		0.049	0.112			-0.074	-0.043	-0.065	0.000	-0.004	0.010	0.039	0.039	0.022	0.022
b2232	ubiG	0.001	0.005	-0.006	0.015	0.105		2.171			2.171	0.026	0.017	-0.063	-0.053	-0.107
b2233	yfaL	-0.059	0.097	0.067	0.015	0.083										
b2234	nrdA	-0.012	-0.013	-0.036	-0.047	-0.020	-0.130	0.035	0.043	0.009	-0.091	-0.063	0.008	0.016	0.113	0.162
b2235	nrdB	0.148	-0.019	-0.046		-0.042				2.171		-0.200	0.016		-0.007	0.224
b2236	yfaE	-0.002		0.140	0.070	0.041										
b2237	inaA	0.018	0.015	0.129	-0.016	-0.037										
b2238	yfaH	0.030	0.002	-0.010	0.104	0.147										
b2239	glpQ	-0.015	-0.041	-0.025	-0.094	0.009										
b2241	glpA	-0.023	0.006	-0.030	-0.042	-0.034	-2.171	-2.171	0.100	-2.171	-0.030	0.056	0.072	0.161	0.134	0.301
b2242	glpB	-0.043	0.006	-0.073	-0.066	-0.058										
b2243	glpC		0.005	0.074	0.030							-0.031	0.083	0.000	-0.032	0.034
b2244	yfaD	0.037	-0.002	0.108	0.266	0.470										
b2245	yfaU		-0.101	0.105		0.074										
b2246	yfaV		0.202		0.200	0.300										
b2247	yfaW	-0.093	-0.008	-0.005		0.120	-2.171	-0.022	-2.171	-0.165	-2.171	-0.212	-0.115	-0.166	-0.264	0.009
b2249	yfaY											-0.025	-0.020		-0.001	0.189
b2250	yfaZ			0.293	0.033											
b2251	nudI	0.283	-0.105	0.052	0.010	0.006										
b2252	ais	-0.020	0.039	0.006	-0.098	-0.053										
b2253	arnB		-0.019			-0.098										
b2254	arnC	-0.007	-0.018	-0.024	-0.071	-0.037										
b2255	arnA	-0.024	-0.015	-0.045	-0.039	0.032	-2.171	-2.171	-2.171	0.300						
b2256	arnD	-0.030	-0.005	0.014	-0.001	0.015				2.171						
b2257	arnT	0.107	-0.022	-0.004												
b2259	pmrD	0.149	-0.033	-0.007	-0.003											
b2260	menE	0.016	-0.004	-0.023	0.024	0.040	2.171									
b2261	menC	-0.011	0.017	-0.013	-0.046	-0.058										
b2262	menB	-0.044	0.034	-0.028	-0.053	-0.028						0.074	-0.043	0.030	0.107	0.082
b2263	menH	-0.038	-0.007	0.022	-0.069	0.058			2.171							
b2264	menD	-0.018	-0.022	0.013	0.078	0.136										
b2265	menF	0.023	0.012	0.014	0.017	-0.074										
b2266	elaB	-0.028	-0.060	-0.096	-0.005	-0.014	-0.113	-2.171	0.035	0.252	0.486	-0.155	-0.116	0.071	0.365	0.556
b2267	elaA			0.320	0.102											
b2269	elaD		0.052	0.064		-0.007	-2.171	-2.171	-2.171	0.004	-2.171					
b2270	yfbK	-0.124	0.060	0.008	-0.037	0.066										
b2272	yfbM	-0.008	0.022	0.055	-0.090	0.031										
b2273	yfbN	-0.028	0.109	0.132	0.116	0.192										
b2274	yfbO	0.031	0.041	0.025	0.091	-0.027										
b2275	yfbP	0.005	0.040	0.098	0.000	-0.027										
b2276	nuoN	-0.090	-0.031	-0.030	-0.036	-0.050										
b2277	nuoM	-0.088	-0.030	-0.050	-0.082	-0.114										
b2278	nuoL	0.005	0.026	0.029	0.020	-0.020										
b2279	nuoK	-0.014	0.009	0.177	-0.076	-0.008										
b2280	nuoJ	-0.037	-0.005	0.029	0.091	0.133										
b2281	nuoI	-0.061	-0.021	-0.247												
b2282	nuoH	-0.104	0.004	-0.010												
b2283	nuoG	-0.051	0.021	0.099	0.207	0.219	0.004	-0.161	-0.017	0.043	0.022	0.004	0.020	0.005	-0.007	0.070
b2284	nuoF	0.015	0.055	0.086	-0.035	0.006	2.171	2.171		2.171		0.116	0.074	0.171	0.074	0.035
b2285	nuoE	0.006	0.068	0.074	0.014	-0.039										
b2286	nuoC	-0.057	-0.008	-0.004	-0.032	-0.558	-2.171	-2.171	-2.171	-0.009	-2.171	-0.029	-0.003	-0.079	0.020	0.132
b2287	nuoB	-0.019	0.031	0.014	-0.060	-0.026										
b2288	nuoA	-0.026	0.046	0.000	-0.058	-0.053										
b2289	lrhA	0.002	0.027	0.048	-0.007	-0.015	2.171									
b2290	yfbQ	0.043	0.047	0.094	-0.012	0.036	2.171	2.171	2.171							
b2291	yfbR		0.037	0.158												
b2293	yfbT	0.061	0.053	0.061	0.032	-0.028										
b2294	yfbU	0.019	-0.032	-0.001	-0.027	-0.062	2.171	2.171	2.171	2.171	2.171	0.151	0.017	0.004	0.113	0.051
b2295	yfbV		0.156			-0.672										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2296	ackA	-0.084	0.016	0.005	-0.095	-0.083	-0.074	0.035	0.087	0.039	0.165	-0.052	0.025	-0.107	0.120	0.090
b2297	pta		0.067				0.065	-0.117	-0.083	-0.022	0.026	0.018	0.011	-0.030	-0.003	0.061
b2298	yfcC	-0.054	-0.014	-0.027	-0.055	-0.012										
b2299	yfcD									2.171						
b2300	yfcE	0.224			0.118											
b2301	yfcF				0.099	0.005										
b2303	folX	-0.012	0.003	0.033	0.050	0.082	2.171		2.171		2.171	0.048	0.053	0.013	-0.160	-0.090
b2304	yfcH	0.011	0.007	-0.030	-0.100	-0.126										
b2305	yfcI		0.062	0.097	0.046	0.135		2.171	2.171	2.171						
b2307	hisM	-0.088	0.029	0.080	-0.084	-0.299										
b2308	hisQ		-0.055			0.271										
b2309	hisJ						0.004	0.052	0.004	-0.100	-0.143	0.056	0.017	0.011	-0.025	-0.042
b2310	argT	0.003	0.039	0.028	-0.012	0.076	-2.171	0.035	0.096	-0.113	-0.278					
b2311	ubiX	-0.003	0.019	0.003	0.000	-0.047										
b2312	purF	-0.001	0.009	-0.016	-0.001	-0.014	0.087	-0.065	0.152	0.117	0.161	-0.025	-0.008	-0.064	0.063	0.054
b2313	cvpA	-0.205														
b2314	dedD	0.147	-0.063	0.165	0.026	0.026										
b2315	folC	-0.004	-0.015	-0.033	-0.031	-0.044		2.171	2.171							
b2316	accD	-0.096	-0.035	-0.039	0.217	-0.156	-2.171	-0.013	0.009	0.065	-2.171	0.090	0.017	-0.074	-0.131	0.008
b2317	dedA		0.059		-0.009	-0.050										
b2318	truA			0.039												
b2319	usg							2.171								
b2320	pdxB	0.008	-0.024	-0.001	0.023	-0.015		2.171			2.171	-0.018	0.031	-0.008	0.056	0.251
b2321	flk	-0.045	0.015	-0.089	-0.139	-0.185				2.171						
b2322	yfcJ		-0.054													
b2323	fabB	-0.012	-0.026	0.019	-0.060	-0.037	0.056	-0.009	0.017	0.074	0.009	-0.007	0.048	-0.073	0.018	-0.010
b2324	mnmC	0.082	0.023	0.095	0.140	0.219										
b2325	yfcL	-0.004	-0.040	0.027	0.017	-0.044										
b2327	yfcA	0.041	-0.032	0.064	0.068	0.177										
b2329	aroC	0.054	-0.019	0.029	-0.035				2.171							
b2330	prmB	0.094	0.027	0.086	0.136	0.271			2.171							
b2331	yfcN	0.009	-0.006	0.010	-0.018	0.020										
b2332	yfcO	-0.030	0.031	0.099	-0.032	-0.003		2.171								
b2333	yfcP	-0.146		0.497							2.171					
b2334	yfcQ	0.148	0.097	0.177	0.027	0.092										
b2335	yfcR	0.088														
b2336	yfcS		0.055	0.006												
b2337	yfcT					-0.418										
b2339	yfcV		-0.262													
b2340	sixA	0.215				0.051										
b2341	fadJ	-0.066	-0.002	-0.030	-0.014	-0.022										
b2342	fadI	-0.043	-0.009	-0.010	-0.017	-0.041										
b2344	fadL	0.041	0.003	0.053	0.056	0.072										
b2345	yfdF	0.079		0.163	-0.025	-0.104			2.171							
b2346	vacJ	-0.066	0.035	-0.005		0.074										
b2349	intS							2.171								
b2350	yfdG					-0.035										
b2352	yfdI	0.070	0.061	0.093	0.019	0.073										
b2353	tfaS					-0.075										
b2354	yfdK				0.087	-0.067										
b2355	yfdL	0.025	-0.013	-0.016	0.025	-0.014										
b2356	yfdM	-0.047	0.089	0.056	0.053	0.098										
b2357	yfdN	0.146	0.195		0.040	0.098				2.171						
b2358	yfdO				0.273	0.012										
b2359	yfdP	0.060	0.058	0.052	0.016	0.033										
b2360	yfdQ	0.187	0.068		0.104	0.062										
b2361	yfdR	0.028	0.037	0.028	-0.026	-0.068										
b2362	yfdS	0.189	-0.215			0.044										
b2363	yfdT	0.083	0.106	0.084	0.024	-0.062										
b2364	dsdC	-0.005	-0.020	0.004	0.077	0.068										
b2366	dsdA	-0.067	-0.036	-0.041	0.036	0.007										
b2367	emrY	0.002	0.038	0.043	0.029	0.004										
b2368	emrK	0.043	0.060	0.004	-0.085	-0.035					2.171					
b2369	evgA	-0.048	0.017	-0.002	-0.062	-0.001										
b2370	evgS	0.046	0.015	0.067		0.043		2.171								
b2371	yfdE	-0.419	0.054	-0.021	0.071	-0.011										
b2373	oxc	-0.020	0.054	0.051	0.039	0.031										
b2374	frc	0.079	0.157		0.100	-0.033										
b2375	yfdX	0.085	0.086	0.138	0.081	0.002										
b2376	ypdI	-0.013	0.025	0.157	0.036	0.064										
b2377	yfdY	0.024	-0.001	0.013	-0.039	-0.069										
b2378	lpxP	0.030	0.007	0.177	0.090	0.143										
b2379	yfdZ	0.280	0.009	0.191	0.304	0.230	2.171					0.017	-0.098		-0.003	0.114
b2381	ypdB				0.025	0.053										
b2383	fryA	0.002	-0.005	-0.050	-0.002	0.028										
b2384	ypdE	0.043	-0.003	-0.016	0.010	-0.079										
b2385	ypdF	-0.030	-0.049	-0.072	-0.026	-0.070		2.171								

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2386	fryC	0.047	0.050	0.039	-0.020	-0.082										
b2387	fryB		0.042	-0.001	0.007											
b2388	glk						-2.171	-2.171	-2.171	0.009	0.178	-0.014	0.075	0.011	0.061	0.147
b2389	yfeO	0.162	0.134	0.202	0.025	0.013										
b2390	ypeC	-0.036	0.099	0.197	-0.020	0.019										
b2391		0.186	0.046		0.156	0.022										
b2392	mntH	0.098	0.036	0.081	-0.030	-0.036										
b2393	nupC	0.007	0.007	0.038	-0.012	0.041										
b2394	insL-3					-0.050										
b2395	yfeA	0.093	0.101	0.117	0.093	0.195										
b2398	yfeC	-0.082	0.021	-0.109	0.314	-0.008										
b2399	yfeD	0.011	0.022	0.008	0.026	0.048										
b2400	gltX	-0.003	0.000	-0.055	-0.038	-0.079	-0.043	-0.004	-0.004	0.113	0.221	-0.022	-0.017	-0.055	-0.005	0.097
b2406	xapB	0.022	-0.004	-0.026	0.007	0.027										
b2407	xapA	0.009	0.033	-0.018	0.038	0.090										
b2408	yfeN	-0.024	0.020	0.010	-0.034	-0.119										
b2410	yfeH	-0.078	-0.026	-0.055	-0.042	-0.020										
b2411	ligA	0.087	0.024	-0.061	-0.011		-2.171	0.039	-0.017	-0.100	-0.122	0.078	-0.117	-0.028	-0.074	0.062
b2412	zipA		0.271													
b2413	cysZ	-0.003	0.002	-0.035	-0.024	-0.017										
b2414	cysK						0.013	-0.026	-0.039	0.009	-0.009	0.043	0.019	-0.001	0.068	0.013
b2415	ptsH	-0.044	-0.063	-0.048	-0.008	0.085	-0.048	-0.030	-0.091	-0.208	-2.171	-0.065	0.187	0.146	-0.121	0.084
b2416	ptsI	0.097	0.085	0.015	-0.062	-0.101	-0.009	0.000	-0.022	0.013	0.039	0.058	0.049	0.028	0.009	0.067
b2417	crr		-0.006	-0.118	-0.023	-0.034	-0.013	-0.043	0.056	0.104	0.130	-0.006	-0.029	0.041	0.104	0.181
b2418	pdxK	0.018	-0.001	-0.018	-0.023	0.007										
b2419	yfeK	0.038	0.053	0.007	-0.095	-0.038										
b2420	yfeS	-0.061	0.026	0.087	-0.061	-0.055										
b2421	cysM	-0.008	0.011	0.001	-0.049	-0.104	-0.096	-0.191	0.191	0.004	-0.195	-0.104	0.009	0.159	-0.012	0.095
b2422	cysA	0.178	0.019	-0.019	-0.020	0.001	2.171									
b2423	cysW	-0.022	0.029	-0.014	-0.067	-0.084										
b2424	cysU	-0.037	-0.015	-0.021	-0.011	-0.055										
b2425	cysP		-0.127				0.039	-0.091	0.013	0.004	0.083	0.050	-0.007	0.017	0.029	0.036
b2426	ucpA	-0.113	0.012	0.029		0.063			2.171							
b2427	yfeT									2.171						
b2429	murP	0.022	-0.001	0.037	-0.061	-0.020										
b2430	yfeW	-0.107	-0.030													
b2431	yfeX			0.061	0.076	-0.003			2.171							
b2432	yfeY	-0.062			0.042	-0.097										
b2433	yfeZ	0.051	0.152		-0.001	-0.011										
b2434	ypeA	0.344	0.243	0.050	0.141	0.022										
b2436	hemF	0.058	0.003	0.015	-0.002	0.030										
b2437	eutR	0.166	0.139	0.156	0.048	-0.036										
b2439	eutL	0.041	-0.033	0.106	0.076	-0.050					2.171					
b2440	eutC										2.171					0.081
b2441	eutB										2.171					
b2443	yffL	0.006	-0.026	0.016	0.012	0.007										
b2444	yffM	0.024	0.033	-0.017	-0.047	-0.060										
b2445	yffN	-0.010	0.027		0.060	0.166										
b2446	yffO	0.081	0.050	0.033	0.041	0.005			2.171							
b2447	yffP	-0.037	-0.086	0.124	-0.037	-0.049										
b2448	yffQ	-0.119	0.060	0.005	-0.043	0.053										
b2449	yffR	0.043	0.078	0.033	-0.040	0.011										
b2450	yffS	-0.082	-0.104	0.012	-0.098	-0.223										
b2451	eutA	0.085		-0.074	0.098	0.123										
b2453	eutG					0.001		2.171								
b2454	eutJ								2.171							
b2455	eutE		-0.006				2.171									
b2456	eutN	-0.058	-0.008	0.039	0.043	0.099										
b2457	eutM	-0.019	-0.046	-0.041	-0.050	-0.220										
b2459	eutT	0.000	-0.010	-0.004	0.081	0.078										
b2460	eutQ	0.162		0.168	0.037	0.043										
b2461	eutP			0.042	-0.005	-0.022										
b2462	eutS				0.125	0.042										
b2463	maeB	-0.034	-0.009	-0.026	-0.002	-0.087	0.135	0.013	-0.065	-0.143	-0.061	-0.016	-0.078	-0.082	-0.049	-0.078
b2464	talA	0.002	-0.041	-0.032	-0.035	-0.047	-0.035	-0.182	-0.065	0.122	0.169					
b2465	tktB	0.011	-0.004	0.020	-0.053	-0.067	0.100	-0.208	0.048	0.139	0.161					
b2466	ypfG	-0.060		-0.022	-0.084	0.029				2.171						
b2467	nudK	0.000	-0.049	0.042	-0.064	-0.065										
b2468	aegA	-0.031	0.039	0.002	-0.094		0.026	-0.022	0.009	-0.035	0.004					
b2469	narQ	-0.053	0.020	0.035	-0.058	-0.097										
b2470	acrD	-0.007	0.052	0.062	0.005	0.116				2.171						
b2471	yffB				0.154	0.186										
b2472	dapE	0.013	-0.022	-0.047	-0.099	-0.074					2.171					
b2473	ypfH		-0.044		0.097	0.044										
b2474	tmcA		-0.168			0.036			2.171							
b2475	ypfJ				0.044	0.081										
b2476	purC	0.006	-0.016	-0.010	-0.025	0.015	0.004	0.048	-0.026	0.022	0.022	0.009	0.053	0.027	0.039	0.093

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2477	bamC	-0.028	0.022	0.007	-0.010	-0.049			2.171							
b2478	dapA	-0.046	-0.046	-0.046	-0.054	-0.140	0.165	0.065	0.061	-0.052	0.022	0.180	0.048	-0.003	0.049	0.127
b2479	gcvR	0.089	0.008	0.085	0.026	0.056										
b2480	bcp	-0.060	0.015	-0.001	0.012	-0.047	0.030	-0.013	0.039	0.100	0.169	-0.021	-0.080	0.036	0.012	0.052
b2481	hyfA	0.005	0.074	-0.067	0.111	-0.022	2.171									
b2482	hyfB	-0.045	0.003	-0.078	-0.108	-0.226										
b2483	hyfC			0.154		-0.066										
b2484	hyfD	0.010	0.029	-0.014	-0.029	-0.049										
b2485	hyfE				0.361											
b2486	hyfF	-0.034	0.022	-0.007	0.002	-0.043										
b2488	hyfH		-0.076		0.061											
b2489	hyfI	0.109	0.270	0.040												
b2490	hyfJ	0.008	0.033	0.029	0.047	0.082										
b2493	yfgO				0.049											
b2494	yfgC	0.044	0.040	0.014	0.013	0.013										
b2495	yfgD	0.019	0.018	0.053	-0.047	-0.021								0.121		0.166
b2496	hda			0.138												
b2498	upp	0.001	-0.050	-0.037	-0.113	-0.098	0.030	0.022	0.004	0.000	0.061	0.019	0.018	-0.003	-0.026	0.074
b2499	purM						-0.043	-0.065	-0.013	0.052	0.074	0.059	0.011	0.036	0.051	0.084
b2500	purN		0.034	0.063	0.052	-0.013										
b2501	ppk						2.171	2.171								
b2502	ppx	-0.010	-0.107	0.034	0.027	0.074										
b2503	yfgF	-0.014	-0.057	0.006	-0.002	-0.090										
b2504	yfgG	0.087	0.056	0.150	0.098	0.100										
b2507	guaA	-0.040	-0.017	-0.008	-0.007	-0.053	0.013	0.017	0.035	0.004	0.009	0.005	0.021	0.003	0.050	0.036
b2508	guaB	0.036	0.054	0.076	0.091	0.159	0.004	-0.009	0.004	0.022	0.017	-0.020	-0.008	-0.037	-0.009	0.028
b2509	xseA	0.177	-0.001	-0.020	-0.114	-0.001										
b2510	yfgJ	0.002	-0.058	0.048	-0.024	-0.046										
b2511	der	-0.081	0.019	0.021	-0.054	0.018	2.171	2.171	2.171	2.171	2.171	0.064	-0.053	-0.045	0.028	0.054
b2512	bamB	0.055	-0.024	-0.005	-0.145	-0.125										
b2513	yfgM	-0.004	0.019	0.169	0.272	0.358										
b2514	hisS	-0.026	-0.081	-0.090	-0.063	-0.153	-0.017	-0.017	0.000	0.100	0.048	0.005	-0.083	-0.043	-0.066	-0.036
b2515	ispG	-0.043	0.099	-0.056	0.140	0.227	0.004	0.156	0.148	-2.171	-2.171	-0.054	-0.054	0.018	0.059	0.005
b2517	rlmN		-0.042	0.103				2.171				0.010	0.010	-0.115	-0.126	-0.180
b2518	ndk	-0.020	0.002	0.033	-0.030	-0.048	0.009	-0.043	-0.017	-0.026	-0.122	0.073	0.016	0.086	0.032	-0.056
b2520	yfhM			0.209	0.008	0.020										
b2521	sseA		0.206		0.160											
b2522	sseB	0.013	-0.027	-0.025	-0.111	-0.128										
b2523	pepB	-0.006	0.010	0.118	0.341	0.344	-0.048	0.043	0.100	0.039	-0.065	0.024	0.034	-0.002	0.042	0.005
b2525	fdx							2.171	2.171		2.171	0.003	0.002	-0.001	0.047	0.081
b2526	hscA	0.122	0.038	0.049	-0.084	-0.009	-2.171	-0.013	-2.171	-2.171	-0.069					
b2527	hscB	0.026		0.091		0.000										
b2528	iscA	-0.004	0.017	0.021	-0.001	-0.005	2.171	2.171	2.171	2.171		0.353		-0.150		
b2529	iscU	-0.077	-0.057	0.095	0.048	-0.055	0.017	0.091	0.187	0.013	0.169	0.193	0.156	0.016	0.110	0.051
b2530	iscS	-0.036	-0.009	-0.052	-0.085	-0.203	0.017	0.022	0.056	0.056	0.035	-0.033	-0.022	0.035	0.009	0.079
b2531	iscR	-0.009	-0.058	0.019	0.016	0.107										
b2532	trmJ	-0.022	-0.023	0.048	-0.067	0.068	0.065	-2.171	0.065	-2.171	-2.171	0.023	-0.074	-0.020	0.005	0.002
b2533	suhB						-0.187	-0.048	0.048	0.030	0.026	-0.049	0.008	0.010	0.009	0.031
b2534	yfhR		0.005	-0.024		0.060										
b2535	csiE	-0.076	-0.022	0.053	0.024	-0.027										
b2536	hcaT	-0.004	0.006	-0.023	0.010	-0.080										
b2538	hcaE	-0.025	-0.028	-0.046	-0.004	0.089		2.171	2.171	2.171		-0.161	-0.236	-0.096	0.074	0.380
b2539	hcaF	-0.061	-0.030	-0.013	0.038	0.013						-0.162		-0.102	0.059	-0.031
b2540	hcaC	-0.043	-0.024	0.003	-0.018	-0.009										
b2541	hcaB	-0.043	-0.020	-0.027	-0.111	-0.024										
b2542	hcaD	0.028	-0.040	-0.052	-0.035	-0.024										
b2543	yphA	-0.002	-0.065	0.074	0.019	-0.049										
b2544	yphB	-0.003	0.060	0.043	0.018	-0.102										
b2545	yphC	0.174	0.000	0.106	-0.138	-0.065										
b2547	yphE										2.171	-0.080	0.098		0.421	0.234
b2548	yphF	-0.008	-0.030	-0.019	0.054	-0.064		2.171				0.228	0.028	-0.091		0.049
b2549	yphG	0.002	0.053	0.004	-0.040	-0.065	-0.039	-2.171	0.056	-2.171	-0.039	0.115	-0.058	-0.034	-0.013	
b2551	glyA	0.153	0.027	0.118	0.034	0.071	0.009	-0.004	0.030	0.061	0.126	0.029	0.003	0.043	0.032	0.098
b2552	hmp	-0.035	0.038	0.029	-0.012	-0.028	-2.171	-2.171	-2.171	-2.171	-2.171					
b2553	glnB	-0.058	-0.022	-0.049	-0.103	0.012										
b2554	yfhA	-0.081	-0.050	-0.035	0.011	-0.045			2.171	2.171						
b2555	yfhG	0.067	0.050	0.061	-0.031	0.036										
b2556	yfhK						-2.171	-2.171	-2.171	-2.171	-2.171					
b2557	purL	-0.017	-0.020	-0.037	-0.025	0.060	-0.004	-0.009	-0.004	0.000	0.026	-0.042	-0.063	-0.049	0.041	0.054
b2558	mltF	0.000	-0.004	0.033	0.057	0.057										
b2559	tadA	0.038	0.025	0.025	0.043	0.103										
b2560	yfhB	-0.035	-0.031	-0.014	0.064	0.129										0.053
b2561	yfhH	-0.060	-0.028	-0.058	-0.053	-0.062					2.171					
b2562	yfhL	-0.002	0.025	0.151	0.074	0.095										
b2563	acpS	-0.046	-0.040	-0.073	-0.045	-0.069	2.171									
b2564	pdxJ	0.002	0.010	-0.003		-0.010										
b2565	recO		0.073	-0.056	-0.043	-0.002										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2566	era					-0.143										
b2567	rnc	-0.013	0.000	-0.077	-0.157	-0.109										
b2568	lepB	0.019	0.025	-0.024	0.051	-0.030										
b2569	lepA	-0.033	-0.040	-0.046	0.003	-0.037	0.217	0.161	0.000	0.208	-2.171	-0.018	-0.061	0.000	0.019	-0.027
b2571	rseB		0.149	-0.279												
b2572	rseA	-0.137	0.022	-0.012	-0.085	0.022										
b2573	rpoE		-0.166							2.171		0.996		-0.106		
b2574	nadB	-0.024	-0.029	-0.016	0.002	-0.019										
b2575	yfiC	0.034	0.032	0.117	0.158	0.256										
b2576	srmB	-0.088	-0.041	-0.030	-0.013	-0.052				2.171						
b2577	yfiE								2.171							
b2579	yfiD			0.191												
b2580	ung		0.097	-0.114			-2.171	-2.171	-2.171	0.200	-0.009					
b2581	yfiF	-0.151	-0.035	-0.022	-0.027	-0.025										
b2583	yfiP	-0.006	0.043	-0.032	-0.028	0.038	2.171									
b2584	yfiQ	0.008	0.027	0.104	0.082	0.168										
b2585	pssA	0.018	0.002	-0.039	-0.010	-0.010		2.171	2.171							
b2586	yfiM	-0.147	-0.114	0.034	-0.037	0.090										
b2587	kgtP	-0.058	-0.020	-0.056	-0.110	-0.077	-2.171	-2.171	-2.171	-2.171	-2.171					
b2592	clpB	-0.011	0.016	-0.019	0.027	-0.070	0.039	0.043	-0.004	-0.022	0.022	0.070	-0.011	0.011	0.025	-0.008
b2593	yfiH	0.062	-0.078	0.051	0.012	-0.009										-0.008
b2594	rluD	-0.004	0.001	-0.013	-0.060	-0.076				2.171						
b2595	bamD	0.186	0.069	0.018	-0.013	0.008	-2.171	-2.171	-0.035	-2.171	-0.109					
b2596					0.027	0.030										
b2597	raiA	-0.008	0.000	0.014		-0.023										
b2599	pheA	0.050	0.022	-0.002	0.014	0.109					2.171					
b2600	tyrA		0.052	0.041	-0.069	-0.041				2.171	2.171					
b2601	aroF	-0.026	-0.060	-0.042	0.041	-0.061	0.182	-2.171	0.026	-0.152	-2.171					
b2602	yfiL	0.064	0.007	-0.023	-0.104	-0.063										
b2603	yfiR	-0.131	-0.003	0.006	-0.065	-0.031										
b2604	yfiN	-0.031	-0.001	-0.019	-0.043	-0.060										
b2605	yfiB	0.040	0.025	-0.032		0.016										
b2606	rplS	-0.008	0.020	-0.031	-0.020	0.023	-0.091	-0.148	-0.087	-0.143	-0.074	-0.095	-0.066	-0.049	-0.102	0.028
b2607	trmD		0.213		0.169											
b2608	rimM						2.171					0.020	-0.067	-0.048	0.002	0.010
b2609	rpsP						-0.091	-0.043	-0.009	0.000	0.026	-0.021	-0.042	0.005	0.024	0.175
b2610	ffh	0.030	-0.025	-0.008	0.025	-0.033										
b2611	ypjD			0.344	0.101	0.023										
b2613					0.075											
b2614	grpE						-0.035	-0.083	-0.078	0.048	0.135	-0.107	-0.030	-0.029	0.017	0.044
b2615	nadK		0.071		0.084	-0.060										
b2616	recN	-0.087	0.087	0.087	0.035				2.171							
b2617	smpA	-0.057	0.045	0.034		0.134										
b2618	yfjF				-0.072											
b2619	yfjG		-0.031	-0.040		0.184										
b2620	smpB				0.106	-0.031								0.007		
b2622	intA		-0.003													
b2623	yfjH							2.171								
b2630	rnlA	0.052	0.031	-0.046	-0.016	-0.035										
b2633	yfjQ		-0.006			-0.232										
b2634	yfjR	-0.052	0.003	0.005	-0.071	-0.085										
b2636	yfjS				-0.073	0.031										
b2637	yfjT				-0.102											
b2638	yfjU				-0.011											
b2639	ypjL	0.010	0.066	0.078	0.118	0.218										
b2641	ypjM				0.069											
b2643	yfjX				0.154											
b2644	yfjY	-0.038	-0.015	-0.040	-0.053	0.027										
b2645	yfjZ				-0.017											
b2646	ypjF		-0.076	-0.051	-0.010											
b2647	ypjA		-0.011	0.030	-0.050											
b2649	ypjB	-0.054	0.075	0.079	-0.039	0.077										
b2653						-0.019										
b2654	ygaQ		-0.004		0.047											
b2656		0.145			0.117	0.019										
b2657		-0.048	-0.026	-0.059	-0.117	-0.041										
b2659	csiD	0.063	0.030	-0.043	0.219	0.437										
b2660	lhgO									2.171						
b2661	gabD	-0.057	-0.096	-0.038	-0.040	0.006			2.171	2.171	2.171		0.010	0.059	0.139	0.192
b2662	gabT	-0.043	0.004	-0.016	-0.140	-0.061	2.171		2.171	2.171	2.171	0.048	0.059	0.020	0.252	0.313
b2664	csiR	-0.019	-0.009	-0.050	-0.094	0.125				2.171						
b2665	ygaU	-0.024	0.094	0.163	-0.013	0.215		2.171								
b2666	yqaE	-0.057	0.076	0.141	0.066	0.151										
b2667	ygaV	0.028	-0.015	0.024	0.000	0.010										
b2668	ygaP	0.168	0.036	0.017	0.074											
b2669	stpA						0.122	0.035	0.030	0.182	0.074	0.068	0.068	-0.028	0.135	0.056
b2672	ygaM	0.018	-0.208	0.011	0.033	0.234										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2674	nrdI	-0.018	0.042	0.180	0.249	0.378	-2.171	-2.171	-2.171	-2.171	-0.426					
b2675	nrdE	-0.058	-0.074	-0.035	-0.022	0.025			2.171							
b2676	nrdF	-0.001	-0.011	0.016	0.043	0.092										
b2677	proV	-0.018	0.009	-0.048	-0.080	-0.179										
b2678	proW		-0.034													
b2679	proX	0.076	0.030	-0.058		-0.100	-2.171	-2.171	0.074	-2.171	-2.171					
b2681	ygaY		0.016	0.068		0.050										
b2682	ygaZ	0.157			0.101	-0.025										
b2683	ygaH	0.051	-0.002	0.061	0.202	0.321										
b2684	mprA	-0.097	0.011	0.025	-0.095	-0.212										
b2685	emrA		0.044													
b2686	emrB		0.107	0.121		0.009										
b2687	luxS	-0.122	-0.013	0.033	-0.014	-0.488	-2.171	0.195	0.061	0.169	0.300	0.033	-0.024	0.052	0.255	0.180
b2688	gshA	-0.019	-0.068	-0.103	-0.068	-0.099	0.135	-0.048	0.056	-2.171	-2.171	-0.122	0.023	0.039	0.090	0.230
b2689	yqaA	-0.098	-0.015	0.016	0.010	-0.290										
b2690	yqaB	-0.064	-0.039	-0.021	-0.028	-0.171										
b2696	csrA	-0.153	-0.086	-0.043	-0.143	-0.127										
b2697	alaS	-0.010	-0.041	-0.043	-0.035	0.019	-0.004	0.091	0.004	0.030	0.043	0.032	0.014	-0.015	-0.016	0.021
b2699	recA	-0.065	0.031	0.166	0.001	0.080	0.022	-0.013	-0.009	0.039	0.161	0.093	0.060	0.045	0.104	0.127
b2700	ygaD	-0.081	0.006	-0.002	-0.028	-0.464										
b2702	srlA		-0.061													
b2703	srlE	-0.058	0.032		-0.013											
b2704	srlB	0.001	-0.049	-0.065	-0.033	-0.100										
b2705	srlD								2.171							
b2707	srlR				0.195											
b2708	gutQ				0.233	-0.161										
b2709	norR						2.171									
b2710	norV	0.017	0.054	0.070	0.079	0.086										
b2711	norW		0.061	0.058												
b2712	hypF									2.171						
b2715	ascF		-0.041													
b2716	ascB								2.171	2.171		0.016	0.014	0.002	0.011	0.163
b2717	hycI	-0.002	0.011	0.101	0.012	0.058										
b2718	hycH	-0.112	-0.018	-0.008	-0.074	-0.053	-2.171	-2.171	-2.171	-2.171	-2.171					
b2719	hycG		0.118													
b2720	hycF	-0.072	0.004	0.008	-0.118	-0.011										
b2721	hycE	0.339	0.012	-0.051		0.066										
b2722	hycD	-0.012	0.054	0.082	0.269	0.234										
b2723	hycC	-0.055	0.029	0.033	0.058	0.094										
b2725	hycA	-0.074	-0.020	0.009	-0.042	0.030										
b2726	hypA	-0.083	-0.019	-0.024	0.006	-0.118										
b2727	hypB	-0.139	-0.010													
b2729	hypD		0.043	0.277	0.046											
b2730	hypE	-0.076	0.022	0.009	0.028	0.085	2.171									
b2731	fhlA	-0.044	0.035	-0.036	-0.037	0.097										
b2732	ygbA		-0.062		0.056											
b2733	mutS	-0.064	-0.020	-0.042	-0.066	0.012										
b2734	pphB		0.068		0.429	-0.497	-2.171	-2.171	-2.171	-2.171	-2.171					
b2736	ygbJ	0.008	0.042	0.023	-0.098	-0.065										
b2737	ygbK	0.043	-0.101		0.073	0.126										
b2738	ygbL		0.101	0.067												
b2739	ygbM			-0.064	-0.008											
b2740	ygbN		-0.027	0.058	0.037	0.101										
b2741	rpoS	0.093	0.023	0.064		0.111				2.171	2.171				0.969	0.799
b2742	nlpD	0.070	0.092	0.092	0.094	0.220										
b2743	pcm	-0.038	0.034	0.056	0.082	0.118										
b2744	surE	-0.063	0.034	-0.014	-0.081	-0.063										
b2745	truD	-0.002	0.047	0.022	0.068	0.118				2.171						
b2746	ispF	-0.013	0.017	0.030	0.091	0.101										
b2747	ispD	0.026	-0.030	0.047	0.017	0.010	-0.100	0.013	0.048	0.109	-0.265	-0.041	-0.099	0.018	0.060	-0.220
b2748	ftsB	0.021	0.030	0.015	-0.035	0.011										
b2749	ygbE		0.094													
b2750	cysC	-0.009	-0.011	0.012	-0.040	-0.017										
b2751	cysN	0.034	-0.018	-0.054	0.072	-0.116	-0.009	-0.039	-0.087	-0.026	-0.009	0.036	-0.082	0.005	0.030	0.073
b2752	cysD	0.083	-0.061	-0.063	0.050	0.003	-0.230	-0.152	-0.069	-0.087	0.026	-0.034	-0.004	-0.023	0.020	0.052
b2753	iap	-0.011	-0.003	-0.043	-0.043	0.000				2.171						
b2754	ygbF	-0.257			0.027	0.056										
b2755	ygbT		0.028	0.118	0.069	0.074										
b2756	ygcH	0.131	0.046	-0.072	0.063	0.015										
b2757	ygcI	0.041	0.072	-0.048	0.041	0.023										
b2758	ygcJ		-0.027	0.002					2.171				-0.073		0.001	-0.048
b2759	ygcK	0.029	-0.004	-0.017	-0.031	0.075										
b2760	ygcL	0.000	-0.026	0.010	-0.115	-0.056										
b2761	ygcB	-0.019	-0.034	0.005	0.005	0.067			2.171							
b2762	cysH	0.058	-0.005	0.109	0.229	0.121	2.171	2.171	2.171	2.171	2.171	0.007	0.043	0.071	0.131	0.220
b2763	cysI	-0.030	-0.058	-0.078	-0.040	-0.003	-0.156	-0.056	-0.074	0.000	0.069	-0.003	0.021	0.018	0.021	0.090
b2764	cysJ	-0.019	0.028	0.049	0.059	0.066	-0.056	-0.013	0.013	0.026	0.083	-0.062	-0.011	-0.006	0.012	0.091

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2765	sscR	-0.038	0.055	0.108	0.206	0.254										
b2766	ygcN	0.002	0.014	0.023	0.000	0.034										
b2767	ygcO									2.171						
b2768	ygcP									2.171						
b2769	ygcQ						2.171									
b2770	ygcR	0.089	-0.017	-0.036	-0.068											
b2771	ygcS	0.016	0.051	0.100	0.159	0.285										
b2772		0.017	0.001	-0.044	-0.037	-0.055										
b2773		-0.022	-0.050	-0.071	-0.023	-0.004										
b2774	ygcW	-0.058	0.030	0.050		-0.062										
b2775	yqcE		0.023	0.042	0.283	0.050										
b2776	ygcE	0.041	0.073	0.076		0.026										
b2777	ygcF			0.071												
b2778	ygcG				0.397											
b2779	eno			0.013	-0.032		-0.065	-0.022	0.000	0.039	-0.013	-0.048	-0.002	-0.013	0.049	0.044
b2780	pyrG	0.040	-0.001	0.029	-0.030	-0.026	-0.056	-0.187	0.113	0.087	0.039	0.003	-0.016	0.011	0.051	0.038
b2781	mazG		0.103		0.054	0.065										
b2782	chpA	0.041	0.046	0.092	0.074	0.207										
b2783	chpR	-0.318	-0.153													
b2784	relA						2.171									
b2785	rumA	-0.014	0.002	-0.044	0.007	-0.031					2.171					
b2786	barA	0.142	0.053	0.073	0.076	0.368										
b2790	yqcA	-0.089	-0.011	0.026	0.053	0.043	-2.171	-2.171	-2.171	-2.171	-0.052					
b2792	yqcC				0.068											
b2793	syd				-0.012											
b2794	queF	0.501	0.109		0.090						2.171					
b2795	ygdH	-0.131	0.043	0.070	0.038	0.055			2.171							
b2796	sdaC			0.187												
b2797	sdaB	0.434	0.044		0.160											
b2798	ygdG	-0.001	0.022	0.038	0.028	0.105	-2.171	-2.171	-2.171	-2.171	-2.171					
b2801	fucP		0.038					2.171								
b2803	fucK	0.054	0.020	0.032	0.025		2.171									
b2806	ygdE	0.065	-0.103	0.035	0.032	0.085	-2.171	-2.171	-2.171	-2.171	-2.171					
b2808	gcvA	-0.055	-0.028	0.045	0.018	0.116			2.171							
b2810	csdA						-2.171	-2.171	-2.171	-2.171						
b2811	csdE														0.362	-0.151
b2813	mltA				0.014											
b2817	amiC	-0.084	-0.071	-0.121	-0.016	-0.243										
b2818	argA	-0.077	0.008		0.144		-2.171	0.313	-0.104	0.009	-0.130	-0.072	0.036	0.027	0.069	0.084
b2819	recD	0.018	0.002	-0.014	-0.145	-0.008	-2.171	-2.171	-2.171	-0.256	-2.171					
b2820	recB	-0.050	0.015	0.015	0.040	0.026						-0.052	-0.044	-0.010	0.052	0.006
b2821	ptrA	-0.007	0.001	-0.019	0.029	-0.014				2.171		0.317	0.026	0.010	0.040	0.096
b2822	recC	-0.032	-0.018	-0.008	-0.041	0.016					2.171					
b2823	ppdC	0.064	0.051	0.028	-0.103	0.018										
b2824	ygdB				-0.050	-0.012										
b2825	ppdB	0.070	0.045	0.059	0.081	0.185										
b2826	ppdA	-0.017	-0.050	-0.052	-0.017	-0.146										
b2827	thyA		0.025	0.081	0.145	0.116			2.171	2.171	2.171	-0.032	0.031	0.123	0.048	0.179
b2828	lgt	-0.007	0.006	0.048	0.092	0.228										
b2830	rppH	-0.008	-0.024		-0.024	0.036										
b2831	mutH	0.019	0.002	-0.036	-0.059	-0.003										
b2832	ygdQ	-0.002	0.050	0.034	-0.036	0.031										
b2833	ygdR	-0.078	0.031	-0.035	-0.044	0.015										
b2834	tas	-0.035	0.003	-0.043	0.034	0.062										
b2835	lplT	0.053	-0.024	0.014												
b2836	aas		0.053	0.181		0.031										
b2838	lysA	0.076	-0.043	-0.058	-0.078	-0.016				2.171	2.171					
b2839	lysR	-0.065	-0.063	-0.060	-0.004	-0.191										
b2840	ygeA	0.009	0.083	0.052	0.106	0.124	2.171	2.171			2.171					
b2842	kduD	-0.015	-0.034	0.028	0.122	0.157										
b2843	kduI							2.171								
b2844	yqeF	0.030	0.032	-0.023	-0.022	-0.031	-2.171	-2.171	-2.171	-2.171	-2.171					
b2846	yqeH	-0.082	0.020	0.098	0.358	0.264										
b2847	yqeI					-0.032										
b2848	yqeJ	-0.035	0.023	0.005	0.012	0.063										
b2849	yqeK	0.020		0.009	-0.021	-0.040										
b2850	ygeF		-0.029	-0.021	-0.062	0.005										
b2851	ygeG			0.046	-0.003	0.014				2.171						
b2852	ygeH	0.078	0.071		-0.060	-0.039	-2.171	-2.171	-2.171	-2.171	-2.171					
b2853	ygeI	-0.013	-0.013	-0.034	-0.113	-0.082										
b2855	ygeK					-0.189										
b2856	ygeL				0.076	0.100										
b2857	ygeM			0.095		0.084										
b2858			0.117			0.021										
b2859	ygeO			0.084												
b2861	insC-4								2.171							
b2862	ygeP				0.034											

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2863	ygeQ	-0.102	-0.179	0.003	0.056	0.041										
b2866	xdhA	-0.004			-0.001	-0.028										
b2867	xdhB	-0.007	0.027	0.094		-0.033										
b2868	xdhC		0.024	0.013												
b2869	ygeV		-0.005													
b2870	ygeW		0.060													
b2871	ygeX	0.163	0.026	0.038	0.025	-0.134	2.171		2.171		2.171	-0.032	-0.074	-0.074	-0.107	-0.094
b2872	ygeY		0.030	0.060												
b2874	yqeA		-0.023	0.200							2.171					
b2875	yqeB	-0.042	-0.050	0.023					2.171							
b2877	ygfJ			-0.265	-0.030											
b2878	ygfK			-0.186												
b2879	ssnA		0.014													
b2881	xdhD	-0.013	-0.012	0.026												
b2882	ygfO				-0.859											
b2883	guaD				-0.001		-0.135	-2.171	-2.171	-2.171	-2.171					
b2884					0.063	0.059										
b2885		-0.009	0.026	-0.035	-0.079	0.037										
b2886	ygfS	-0.027	0.009	0.018	0.056	0.147										
b2887	ygfT		0.058	0.203							2.171					
b2889	idi	-0.008	-0.010	-0.017	-0.033	-0.061	0.022	0.043	0.052	0.109	0.200	0.016	-0.027	0.009	0.080	0.180
b2890	lysS	-0.018	-0.042	0.019	-0.002	-0.048	0.035	0.035	-0.043	0.009	-0.039	-0.005	-0.035	-0.024	-0.020	-0.019
b2891	prfB	-0.025	-0.048	-0.003	0.012	0.049										
b2892	recJ	0.015	0.052	0.020		-0.210			2.171	2.171						
b2893	dsbC										2.171					
b2894	xerD	-0.099	0.025	0.023		0.039						-0.162	-0.014	0.092	0.024	0.024
b2895	fldB		0.009	-0.065	0.091											
b2896	ygfX	0.054	-0.089	-0.016	-0.095	-0.057										
b2897	ygfY			0.097	0.069	0.050										
b2898	ygfZ	0.015	-0.014	0.035	0.055	0.058	-0.651	-0.100	-0.274	0.235	-0.083	0.001	0.058	0.027	0.015	0.045
b2899	yqfA	0.050	-0.008	-0.154		-0.005										
b2900	yqfB	-0.015	-0.031	-0.028	0.024	0.095										
b2901	bglA							2.171				0.044	0.144	0.015	0.059	0.051
b2903	gcvP	0.022	0.026	0.015	0.006	0.066	-0.352	-2.171	-0.169	-2.171	-2.171	-0.028	0.004	0.070	-0.086	0.011
b2904	gcvH	0.077	0.026	-0.003	-0.016	-0.005			2.171	2.171						
b2905	gcvT	-0.008	0.010	-0.023	0.022	0.094	-2.171	0.621	0.400	-2.171						
b2906	visC			-0.105	0.029	0.068										
b2907	ubiH	-0.035	-0.003	0.011	0.050	0.117			2.171							
b2908	pepP	-0.020	-0.016	-0.043	0.088	-0.084										
b2909	ygfB							2.171								
b2912	ygfA	0.090	-0.020	-0.044	0.012	-0.065										
b2913	serA	0.015	0.018	-0.002	0.020	-0.069	0.069	0.074	0.022	-0.030	0.039	0.081	0.039	0.027	0.048	0.047
b2914	rpiA		-0.031				0.074	0.087	0.178	0.221	0.208	0.084	0.053	0.087	0.174	0.275
b2915	yqfE		-0.038	0.198	-0.094	0.095										
b2916	argP	0.048	-0.037	-0.009	-0.043	0.044										
b2917	scpA	-0.015	0.003	-0.025	0.046	0.013			2.171							
b2918	argK						0.382	-2.171	-2.171	-0.022	-0.017	-0.139	-0.068	0.012	0.079	-0.001
b2919	scpB	0.040	0.028	0.014	-0.141	0.117										
b2920	scpC	-0.011	-0.011	0.078	0.096	0.216										
b2921	ygfI		0.021		-0.088											
b2922	yggE			0.025						2.171						
b2923	argO	-0.009	-0.130	-0.203	-0.035	-0.008										
b2924	mscS	0.040	-0.018	0.028	0.014	-0.039						-0.014	-0.151		-0.170	-0.036
b2925	fbaA	-0.062	-0.025	-0.005	-0.042	-0.058	0.052	0.043	0.039	0.104	0.139	0.020	0.028	0.035	0.107	0.139
b2926	pgk					-0.299	0.035	0.004	0.026	0.074	0.130	0.056	0.012	0.000	0.049	0.121
b2927	epd		0.021					2.171				0.001	0.051	0.072	0.042	0.012
b2928	yggC	0.056	0.030	-0.012	-0.005	0.092										
b2930	yggF	0.067	0.027	-0.055	-0.047	0.023										
b2931		-0.005	-0.021	0.067	-0.014	0.090										
b2932		-0.176	0.010	-0.015	-0.074	-0.030										
b2935	tktA						0.022	0.000	-0.056	-0.100	-0.126	0.027	-0.010	-0.046	-0.086	-0.010
b2936	yggG	-0.047	-0.041	-0.106	-0.076	-0.047			2.171							
b2937	speB		0.029			0.084										
b2938	speA		0.106	-0.062		-0.001	2.171	2.171	2.171	2.171	2.171					
b2940	yqgC				0.342											
b2941	yqgD	-0.009	0.047	0.044	-0.018	-0.006										
b2942	metK		-0.012				0.009	-0.043	-0.009	0.069	0.135	0.062	0.009	0.008	0.097	0.093
b2944	yggI	-0.015	-0.016	-0.019	-0.050	-0.027										
b2945	endA	-0.003	-0.131	-0.136	-0.155	-0.101										
b2947	gshB	-0.016	-0.034	-0.024	-0.046	-0.021	0.048	-0.221	-0.109	-2.171	-0.052	0.084	-0.017	-0.035	-0.050	0.053
b2948	yqgE	0.001	-0.064	-0.060	-0.038	-0.034	2.171			2.171						
b2949	yqgF		-0.119	0.006		-0.125										
b2950	yggR	-0.061	-0.004	0.085	-0.077	-0.216					2.171					
b2951	yggS	-0.005	0.002	-0.013	-0.061	-0.034										
b2952	yggT	0.026	0.050	0.020	0.015	0.039										
b2953	yggU	-0.003	-0.058	-0.086	-0.033	-0.137										
b2954	rdgB						2.171			2.171		0.033	0.021	0.102	0.024	0.061

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b2955	yggW	-0.043	0.005	-0.008	-0.067	-0.042										
b2956	yggM								2.171							
b2957	ansB	-0.084	0.030	0.024	0.026	0.068										
b2958	yggN		0.070	-0.073	-0.021	0.014					2.171					
b2959	yggL		0.016	-0.101		0.013										
b2960	trmI				0.045	-0.038						0.047	-0.025		0.022	0.024
b2961	mutY		0.075	0.053		-0.009										
b2962	yggX	0.024	-0.007	-0.048	-0.056	-0.047	0.013	-0.009	-0.069	0.226	0.221	0.014	-0.049	0.044	0.045	0.170
b2963	mltC	0.158														
b2965	speC	-0.017	-0.022	-0.096	-0.054	-0.089		2.171	2.171							
b2969		-0.089	-0.009	-0.004	-0.010	-0.093										
b2970	yghF	-0.048	0.036	-0.058	-0.022					2.171						
b2971	yghG		0.273	-0.289	0.048											
b2972	pppA	-0.022	-0.026	-0.024	-0.071	-0.175										
b2973		-0.122	-0.092	0.024	0.063	-0.010			2.171			0.031	0.029	-0.100	-0.234	-0.100
b2974		-0.016	0.001	-0.005	0.008	0.040										
b2975	glcA	0.006	0.030	-0.010	0.106	0.018										
b2976	glcB	-0.001	-0.001	-0.007	-0.007	-0.046		2.171	2.171	2.171						
b2978							-2.171	-2.171	-2.171	-2.171	-2.171	-0.038	-0.217	-0.144	-0.127	0.043
b2980	glcC			0.170												
b2981	yghO	-0.034	-0.056	-0.071	-0.088	-0.115										
b2983	yghQ	0.068	0.000	0.063	0.036	0.113			2.171							
b2984	yghR	-0.080	0.021	-0.054	0.035	0.050										
b2985	yghS	-0.019	0.098	0.196	0.064	0.035		2.171				0.019	-0.037	0.036	0.160	-0.026
b2986	yghT				0.034	0.061										
b2988	gsp	-0.003	-0.026	-0.002	-0.091	0.002	-2.171	-2.171	-2.171	-2.171	-2.171					
b2989	yghU	-0.113		0.144	-0.003	-0.008					2.171	0.014		0.079	0.353	0.210
b2991	hybF		-0.078	-0.059	-0.065											
b2992	hybE										2.171					
b2994	hybC	-0.045	-0.032	-0.055	-0.027	-0.076										
b2995	hybB	-0.008	0.002	-0.012	-0.021	0.025										
b2997	hybO	-0.008	0.007	-0.068	-0.050	-0.127	2.171									
b2998	yghW	-0.007	-0.057	0.004	0.133	0.194										
b2999		-0.086			0.038	0.069					2.171					
b3000					-0.040	0.001					2.171					
b3001	yghZ	0.048	0.094	0.030	-0.061	0.134										
b3002	yqhA				0.137	0.006										
b3003	yghA	0.013	-0.035	0.045												
b3004		0.003	-0.039	-0.006	-0.004	0.004										
b3007			0.147													
b3008	metC	-0.043	0.010	-0.002	-0.045	-0.072	-0.052	-0.117	-0.048	-0.109	-0.026	0.084	0.096	-0.001	0.025	-0.003
b3009	yghB	-0.034	0.025		-0.063	-0.055										
b3010	yqhC		0.073	-0.035												
b3011	yqhD	0.030	0.091	0.012												
b3012	dkgA			-0.288	0.212	0.151				2.171	2.171					
b3013	yqhG	-0.008	0.006	0.038	0.104	0.224										
b3014	yqhH	0.134				0.162										
b3015		0.208	-0.016	0.006	0.142	0.123										
b3016		-0.015	-0.034	-0.075	-0.031	-0.077	-2.171	-2.171	-2.171	-2.171	-2.171					
b3017	ftsP										2.171					
b3018	plsC	-0.006	0.018	0.011	-0.019	-0.009										
b3019	parC	0.126	-0.026	0.097	-0.022	-0.588										
b3020	ygiS		-0.090		0.061											
b3021	ygiT	0.023	0.049	0.001	-0.058	-0.011										
b3023	ygiV	0.024	-0.035	0.027	0.036	0.038	2.171									
b3024	ygiW	-0.041	-0.086	0.059	0.128	0.137				2.171						
b3025	qseB	-0.018	-0.002	-0.048	-0.095	-0.071	2.171					0.059				
b3026	qseC	-0.003	0.000	0.010	-0.021	0.070										
b3027	ygiZ	-0.004	0.114	0.009	0.005	0.062										
b3028	mdaB	-0.025	-0.020	-0.030	-0.084	-0.104				2.171						
b3029	ygiN	-0.122	-0.056	-0.008												
b3030	parE		-0.031	-0.095	0.084	-0.074						-0.006	0.015	0.058	0.019	-0.017
b3033	yqiB	0.060	0.081	0.012	0.039	0.108										
b3034	nudF				0.131											
b3036	ygiA	-0.199	-0.238	0.051	0.034	0.052										
b3037	ygiB	-0.070	-0.014	0.041	0.064	0.205										
b3038	ygiC	0.031	0.029	0.116	0.213	0.319		2.171								
b3039	ygiD		-0.060	-0.002												
b3041	ribB	-0.043	0.012	0.016	0.003	0.044										
b3042	yqiC	0.025	0.000	-0.137	0.069	0.178		2.171								
b3044	insC-5								2.171							
b3045	insD-5	-0.028	-0.005	-0.047	-0.113											
b3046	yqiG													-0.072		
b3047	yqiH		0.079													
b3049	glgS	0.009	0.007	-0.013	0.026	0.005										
b3050	yqiJ	0.026	0.029	0.018	-0.039	-0.007										
b3051	yqiK	0.047	-0.002	0.099	0.004	0.079										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b3052	rfaE						0.004	0.061	0.161	0.052	-0.022	0.011	-0.035	0.037	0.044	0.078
b3053	glnE		0.038													
b3054	ygiF	-0.004	0.031	-0.028	-0.106	-0.004			2.171	2.171		-0.022	0.008	-0.014	-0.006	0.054
b3055	htrG	-0.051	0.074	-0.085	-0.099	-0.053										
b3056	cca	-0.005	0.015	0.049	0.121	0.121	-0.052	-2.171	-2.171	-2.171	0.117					
b3057	bacA	0.269	0.090	-0.102												
b3058	folB	0.006	-0.033	0.054	0.125	0.221										
b3059	ygiH	-0.054	-0.046	-0.048	-0.060											
b3060	ttdR									2.171		-0.133	-0.099	-0.057	0.065	0.048
b3061	ttdA										2.171					
b3063	ttdT	0.059	-0.022	-0.049	-0.009	-0.035										
b3064	ygjD		-0.062	0.060	0.061	-0.033										
b3065	rpsU	0.016	-0.017	0.017	-0.035	-0.032	0.074	0.043	0.139	-0.221	0.083	-0.081	0.018	0.041	-0.151	0.013
b3066	dnaG	-0.023	-0.022	-0.023	-0.057	-0.040		2.171								
b3067	rpoD	-0.067	-0.024	-0.008	-0.009	0.036	-2.171	0.061	-2.171	0.204	0.148	-0.050	-0.155	-0.042	-0.016	0.103
b3068	mug	-0.024	0.016	-0.032	0.018	0.023										
b3070	yqjH	-0.135	-0.065	-0.023	-0.013	-0.071										
b3071	yqjI	-0.148	0.025	0.085	0.087	0.100										
b3073	ygjG	0.044	0.002	-0.004	-0.038	-0.014										
b3074	ygjH	-0.079	-0.036	-0.098	-0.092	-0.137										
b3078	ygjI	0.006	0.021	0.002	-0.054	-0.001										
b3079	ygjJ	0.007	0.038	0.032	0.080	0.106										
b3080	ygjK	0.049	0.027	0.030	0.023	0.172										
b3082	ygjM	0.006	0.037	-0.042	-0.009	-0.022										
b3083	ygjN		0.022	0.080	0.110	0.074										
b3084	rlmG	0.058	0.034	-0.014	0.006	0.037			2.171							
b3085	ygjP	-0.042	0.007	0.042	-0.057	-0.040										
b3086	ygjQ	0.067	0.036	0.021	0.097	0.176										
b3087	ygjR	0.227	-0.003	0.062	0.030	-0.064										
b3088	alx	0.015	0.011	-0.074	-0.034	-0.110			2.171							
b3089	sstT	0.013	0.075	0.028	0.055	0.076										
b3090	ygjV	-0.037	0.010	-0.012	-0.060	0.002										
b3092	uxaC							2.171								
b3093	exuT	-0.006	0.031	0.061	0.006	0.068										
b3094	exuR		0.136				-2.171	-2.171	-2.171	-2.171	-2.171					
b3095	yqjA	0.053	0.007	0.005	-0.031											
b3096	yqjB	0.046	0.008	0.045		0.004					2.171					
b3097	yqjC	-0.059	0.009	0.016	-0.050	-0.062										
b3098	yqjD	0.108	0.002	0.087		-0.029				2.171	2.171					0.464
b3099	yqjE	-0.058	-0.025	0.038	-0.067	-0.082										
b3100	yqjK	0.324			0.142	-0.135										
b3101	yqjF		-0.003	0.017	-0.017	-0.176										
b3102	yqjG								2.171	2.171						
b3107	yhaL					0.035										
b3108		-0.057	-0.049	0.077	0.163	0.190										
b3109		0.083	0.016	-0.029	-0.077	0.024										
b3111		0.020	0.011	0.162	0.224	0.360										
b3112		0.004	0.028	0.082	0.266	0.423										
b3113	tdcF		-0.056		0.081	0.194										
b3114	tdcE			0.019	0.034		0.139	0.087	0.056	0.252	-2.171					
b3116	tdcC	0.013	-0.007	0.002	0.029	0.060										
b3117	tdcB	-0.006	0.034	0.012	-0.074	-0.037										
b3118	tdcA						2.171									
b3119	tdcR		0.038	-0.076	-0.030											
b3121	yhaC	0.033	-0.004	0.004	-0.005	0.004										
b3122		0.072	0.028	0.128	0.194	0.322										
b3124	garK	0.096	-0.023	0.002	0.077											
b3126	garL					0.033										
b3127	garP		-0.175													
b3128	garD	-0.007	0.071	0.130	-0.004	-0.003	2.171						-0.042		0.033	0.041
b3129	sohA	-0.048	-0.023	-0.053	-0.058	-0.055										
b3130	yhaV	-0.074	0.040		0.038	0.135										
b3131	agaR	-0.041	-0.049	-0.049	-0.124	-0.187										
b3132	kbaZ						-2.171	-0.078	-2.171	-2.171	-2.171	-0.041	-0.015	0.073	0.051	0.043
b3133	agaV	-0.060	0.005	0.025	0.014	0.031										
b3134	agaW	-0.033	0.004	0.017	0.004	0.029										
b3135	agaA	0.007	-0.003	0.047	0.062	0.213										
b3136	agaS	-0.078	0.032	0.008	0.031	0.038										
b3137	kbaY	0.024	0.043													
b3138	agaB	0.004	-0.042	-0.069	-0.110	-0.345										
b3139	agaC	-0.059	-0.062	-0.009	0.009	-0.076										
b3140	agaD	-0.041	-0.018	-0.021	0.029	0.028										
b3141	agaI	-0.086	-0.030	0.033	0.063	0.118										
b3143	yraI					-0.430										
b3144	yraJ	0.063	0.041	-0.013	0.067	-0.013										
b3145	yraK		-0.084								2.171					
b3146	yraL	-0.088	-0.004	0.009	-0.007	-0.015					2.171					

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b3147	yraM					0.023										
b3148	yraN	-0.081	-0.027	-0.022	-0.037	-0.144										
b3149	diaA	-0.026	-0.042	0.124	-0.077	0.025										
b3150	yraP	-0.010	0.055	0.048	-0.007	0.095			2.171							
b3151	yraQ	-0.056	0.002								2.171					
b3152	yraR	0.009	-0.015	0.023	-0.102	0.096										
b3153	yhbO	-0.076	-0.021	0.041	-0.017											
b3154	yhbP	-0.013	-0.031	-0.018	-0.099	-0.068										
b3156	yhbS	-0.089	-0.019	-0.009	-0.009	-0.077										
b3157	yhbT	0.000	-0.009	-0.004	-0.093	-0.097										
b3158	yhbU	-0.086	-0.157	-0.142												
b3159	yhbV	-0.103	-0.148	-0.167	-0.060	-0.113				2.171						
b3160	yhbW	0.008	-0.012	-0.003	0.016	-0.021		2.171		2.171	2.171					
b3162	deaD						-0.208	-0.100	-0.083	-2.171	-0.078	-0.183	-0.211	-0.058	0.011	0.195
b3164	pnp	0.010	0.014	-0.008	0.022	0.019	-0.074	-0.091	-0.026	-0.004	0.078	-0.053	-0.003	0.023	-0.042	0.014
b3165	rpsO	-0.036	-0.031	-0.037	-0.061	-0.039	2.171	2.171	2.171	2.171	2.171	-0.088	-0.016	-0.092	-0.034	-0.015
b3166	truB		0.008	-0.019	0.025	0.007										
b3167	rbfA	0.040	-0.005	0.030	0.008	0.055			2.171	2.171	2.171	0.063	-0.066	0.135	0.040	0.044
b3168	infB	-0.023	-0.012	0.004	0.058	0.113	-0.026	0.000	-0.026	0.043	0.004	-0.033	-0.044	-0.039	0.009	0.037
b3169	nusA	-0.018	0.045	0.000	0.030	0.011	0.056	0.017	0.048	0.043	0.109	0.028	0.000	0.029	0.026	0.050
b3170	yhbC	-0.009	-0.018	0.009	-0.067	-0.120										
b3172	argG						0.048	0.026	0.004	-0.022	-0.122	0.020	0.022	0.023	-0.019	-0.042
b3173	yhbX	-0.116	0.067	0.029	0.083											
b3176	glmM	-0.001	0.017	-0.027	-0.027	-0.036	-0.235	0.139	-2.171	-0.187	-2.171	0.133	-0.026	-0.040	-0.025	0.068
b3177	folP	-0.039	0.035	-0.016	-0.051	0.020										
b3178	hflB	0.008	0.036	0.019	-0.044	0.054	2.171				2.171	0.040	-0.073	-0.001	-0.024	0.123
b3179	rrmJ	-0.048	0.037	-0.033	-0.047	-0.116										
b3180	yhbY	0.050	0.072	0.034	-0.042	0.090							-0.095	-0.079	-0.107	
b3181	greA	-0.031	-0.019	0.001	-0.048	0.003	0.104	0.078	-0.096	-0.030	-0.048	0.007	0.000	0.004	-0.061	-0.045
b3182	dacB			-0.230												
b3183	obgE	0.063	0.001	0.071							2.171	-0.123	-0.130	-0.089	-0.060	0.155
b3184	yhbE	0.010	0.068	0.065	0.041	0.137										
b3185	rpmA	-0.072	-0.001	-0.022	-0.047	-0.064	-2.171	-2.171	-2.171	-2.171	-2.171					
b3186	rplU	-0.042	-0.004	-0.026	-0.002	-0.055	-0.043	-0.013	-0.026	-0.026	-0.009	-0.010	-0.025	-0.088	-0.024	0.033
b3187	ispB	-0.035	0.017	0.006	0.073	-0.179	2.171			2.171						
b3189	murA						-0.069	0.161	0.000	0.087	-0.052	-0.007	-0.021	0.026	0.048	-0.018
b3190	yrbA	-0.096	-0.026	-0.016	-0.054	-0.070										
b3191	yrbB	0.041	-0.018	0.000	-0.025	-0.038	2.171									
b3192	yrbC	0.006	0.052	0.166	0.086	0.294	-2.171	-2.171	0.139	0.208	-2.171	-0.144	0.233	-0.374	0.108	-0.341
b3193	yrbD	0.017	0.046	0.050	-0.001	0.066										
b3195	yrbF								2.171							
b3196	yrbG	0.019	0.035	0.069	-0.041	-0.081										
b3197	kdsD		0.083		-0.014		-2.171	-2.171	-2.171	-0.230	-0.169	-0.207	-0.043	-0.073	0.101	-0.024
b3198	kdsC	-0.016	0.021	-0.030	-0.011	-0.080			2.171		2.171			0.172	0.051	0.202
b3199	lptC	-0.038	0.021	-0.011	-0.089	-0.022										
b3200	lptA	-0.072	-0.021	0.025	-0.029	-0.077										
b3201	lptB	-0.038	0.034	0.000	0.050	0.094	-2.171	-2.171		0.582	-2.171		-0.439			
b3203	hpf	0.002	0.074	0.015	-0.024	-0.008										
b3205	yhbJ	-0.016	-0.043	0.033	-0.042	-0.071			2.171							
b3206	npr		-0.017	0.066		0.001										
b3207	yrbL	-0.045	0.010	0.000	-0.045	0.046										
b3209	elbB	-0.098					2.171					0.046	0.016	0.118	0.015	-0.012
b3210	arcB		-0.085		-0.072	-0.060					2.171					
b3211	yhcC	0.008	0.071	0.004	0.007	0.040										
b3212	gltB		0.038	0.191	0.667	1.128	-0.030	-0.022	-0.026	-0.009	-0.004	0.002	0.002	0.004	0.012	0.039
b3213	gltD	0.035	-0.076	0.057	0.064	0.073	-0.026	-0.004	0.000	-0.009	-0.004	-0.012	-0.007	0.012	0.062	0.036
b3215	yhcA	-0.035	-0.015	0.032	-0.046	-0.090										
b3216	yhcD			0.126												
b3217	ychE_1	-0.029	0.002	0.162	0.293	0.323										
b3219	yhcF	0.071	0.019	0.068	0.062	0.142										
b3220	yhcG	-0.015	-0.009	-0.072	-0.042	0.023										
b3221	yhcH	0.097	0.008	0.035	-0.009	0.050										
b3222	nanK		0.039					2.171								
b3223	nanE	-0.058	-0.232													
b3226	nanR						2.171									
b3228	sspB	-0.073	-0.078	-0.070	-0.002	0.073										
b3229	sspA	-0.086	0.051	-0.119	-0.042	0.083		2.171	2.171	2.171	2.171	0.025	0.053	0.091	0.022	0.068
b3230	rpsI	-0.059	-0.026	0.013	-0.008	-0.139	-0.065	-0.039	-0.043	0.000	0.000	-0.050	-0.013	-0.077	-0.026	0.025
b3231	rplM	-0.020	0.000	-0.054	-0.003	-0.037	-0.039	-0.056	0.013	-0.043	0.074	0.013	0.057	0.029	0.029	0.156
b3232	yhcM	-0.074	-0.039	-0.024	0.031	-0.016										
b3233	yhcB	0.158	0.027	0.016	0.051	0.127						-0.168		0.073		
b3234	degQ	0.007	0.003	0.014	-0.052	0.012										
b3235	degS	-0.017	0.028	0.012	0.005	0.031										
b3236	mdh	0.100	-0.011	-0.065	-0.134		0.030	0.013	0.004	-0.083	-0.048	0.022	-0.008	-0.058	-0.083	-0.031
b3237	argR	-0.060	-0.007	0.006	-0.052	-0.036										
b3238	yhcN	0.051	0.021	0.031	0.028	0.049										
b3239	yhcO	-0.005	-0.070	-0.105	-0.127	-0.127										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b3240	aaeB	0.003	-0.044	0.010	0.091	0.041		2.171	2.171							
b3241	aaeA	0.463	0.073								2.171					
b3242	aaeX	-0.062	-0.012	-0.011	-0.087	-0.004										
b3244	tldD	0.006	-0.046	-0.039	0.004	0.044	-2.171	-2.171	-2.171	-2.171	0.169	0.027	-0.152	-0.041	0.026	-0.100
b3245		-0.004	-0.013	-0.007	-0.012	-0.055					2.171					
b3246		0.044	-0.009	0.067	0.145	0.255										
b3247	rng	0.017	0.058	0.061	0.007	0.044		2.171			2.171					
b3248	yhdE	0.057	0.031	0.060	0.109	0.206					2.171					
b3250	mreC						-2.171	-2.171	-2.171	-2.171	-2.171					
b3251	mreB						-0.022	0.043	0.035	0.004	0.078	0.048	0.021	0.009	0.030	0.059
b3252	csrD	0.025	0.036	-0.051	-0.069	-0.070										
b3253	yhdH	0.420		0.254												
b3255	accB		0.046				0.013	0.022	-0.026	0.074	0.056	0.052	0.005	-0.040	0.031	0.037
b3256	accC		0.046				0.000	-0.078	-0.052	0.039	0.026	-0.001	-0.027	-0.028	0.025	0.042
b3257	yhdT	0.056	-0.062	-0.008	0.029	-0.040										
b3259	prmA	0.070	-0.019	0.067	-0.096	-0.037										
b3260	dusB	0.025	0.051	0.116	0.077	0.146										
b3261	fis	-0.020	0.018	-0.035	0.003	0.000	2.171			2.171	2.171	0.002	0.026		-0.030	0.036
b3262	yhdJ	0.008	-0.008	0.006	0.048	0.155			2.171							
b3263	yhdU	-0.010	-0.018	-0.071		0.022										
b3264	envR			-0.182												
b3266	acrF		-0.179	0.098												
b3267	yhdV	0.001	0.012	-0.028	-0.021	0.067										
b3268	yhdW						2.171					-0.071	-0.069	0.262	-0.133	
b3269	yhdX															
b3270	yhdY	0.033	-0.033	-0.031	-0.018	0.077										
b3271	yhdZ	-0.006	0.021	0.037	0.098	0.001				2.171						
b3279	yrdA		-0.006	0.094							2.171					
b3280	yrdB			0.033												
b3281	aroE		0.059	0.045												
b3282	rimN	0.074	-0.037	-0.075	-0.080	-0.012	2.171					-0.026	0.009	0.079	0.112	0.113
b3284	smg	0.055	0.022	0.055		-0.021										
b3285			0.091		0.265											
b3286		0.125	0.034	0.025	0.032	0.060										
b3287	def	-0.108	0.002	-0.027	-0.084	-0.098		2.171				-0.059	-0.043	-0.028	0.050	0.067
b3288	fmt	-0.030	0.003	-0.036	-0.006	-0.013	-2.171	-2.171	-2.171	-0.052	0.139					
b3289	rsmB	-0.035	-0.006	-0.043	-0.168											
b3290	trkA		0.070	0.052		0.156										
b3293	yhdN	0.062	-0.074	-0.013	0.028	-0.027										
b3294	rplQ	-0.060	-0.001	-0.004	-0.013	0.061	-0.022	-0.017	-0.030	-0.030	0.048	0.015	-0.028	-0.069	-0.071	0.045
b3295	rpoA	-0.023	-0.022	-0.013	0.029	0.049	-0.013	-0.009	-0.039	0.039	0.056	-0.048	-0.052	0.006	0.009	0.007
b3296	rpsD	-0.054	-0.048	-0.072	-0.050	-0.029	-0.056	0.026	-0.009	-0.022	0.013	-0.048	-0.019	-0.090	-0.066	-0.031
b3297	rpsK	-0.090	-0.034	-0.016	-0.076	-0.018	-0.269	-0.113	-0.156	-0.148	-0.048	-0.123	-0.149	-0.122	-0.033	-0.013
b3298	rpsM	-0.070	-0.043	0.012	-0.009	-0.093	-0.013	-0.061	-0.009	0.009	0.091	0.044	-0.189	-0.027	-0.016	0.099
b3299	rpmJ											-0.036	-0.096	-0.084	-0.136	-0.043
b3300	secY		0.045	0.248		1.236										
b3301	rplO	-0.006	-0.002	0.017	-0.047	-0.047	-0.109	-0.069	-0.061	-0.074	0.035	-0.045	-0.041	-0.049	-0.123	0.069
b3302	rpmD	0.260	0.169				-0.043	-0.048	-0.022	-0.039	0.113	-0.074	-0.037	-0.005	-0.095	0.061
b3303	rpsE	-0.004	-0.033	-0.005	0.070	-0.052	-0.074	-0.004	-0.039	-0.035	-0.030	-0.078	-0.030	-0.064	-0.049	-0.002
b3304	rplR	-0.050	-0.062	-0.002	-0.025	-0.152	-0.048	-0.065	-0.052	-0.109	0.061	-0.083	-0.021	-0.048	-0.036	0.092
b3305	rplF	-0.040	-0.078	-0.090	-0.040	-0.189	-0.065	-0.074	-0.056	-0.026	-0.039	-0.033	-0.055	-0.040	-0.013	0.013
b3306	rpsH	-0.026	-0.040	-0.007	-0.011	-0.062	-0.035	0.000	-0.078	-0.113	-0.030	-0.064	-0.046	-0.095	-0.010	-0.022
b3307	rpsN	0.010	0.039	0.038	-0.038	-0.025										
b3308	rplE	-0.076	-0.001	0.031	0.006	-0.034	-0.039	-0.069	-0.017	0.004	0.017	-0.019	-0.051	-0.006	-0.039	0.025
b3309	rplX						-0.109	-0.065	-0.039	-0.087	-0.004	-0.077	-0.071	-0.071	-0.043	0.073
b3310	rplN						-0.061	-0.104	-0.091	-0.126	-0.017	-0.069	-0.060	-0.088	-0.098	0.007
b3311	rpsQ	-0.059	-0.053	-0.016	-0.086	-0.115	0.022	-0.043	-2.171	-0.043	-0.039		-0.068	-0.037	-0.006	0.064
b3312	rpmC	-0.056	-0.031	0.018	-0.021	-0.029	0.013	0.022	0.004	-0.026	-0.017	-0.055	-0.051	-0.074	-0.029	-0.072
b3313	rplP	-0.016	-0.045	-0.040	0.026	-0.030	-0.017	-0.022	-0.039	-0.026	0.000	-0.018	-0.023	0.008	-0.029	-0.005
b3314	rpsC	-0.055	0.010	0.003	0.014	-0.037	-0.083	-0.052	-0.061	-0.061	0.017	-0.055	-0.031	-0.042	-0.018	0.041
b3315	rplV	-0.083	-0.055	-0.037	0.007	-0.162	-0.248	-0.156	-0.100	-0.187	-0.069	-0.150	-0.204	-0.053	-0.173	0.023
b3316	rpsS	-0.024	-0.016	-0.039	-0.004	-0.041	-0.200	-0.239	-0.208	-0.169	-2.171	-0.063	-0.039	-0.170	-0.130	0.015
b3317	rplB	-0.077	-0.046	-0.040	-0.002	-0.074	-0.117	-0.083	-0.056	-0.078	-0.043	-0.105	-0.075	-0.054	-0.083	-0.047
b3318	rplW	-0.013	-0.033	-0.036	-0.015	-0.041	2.171	2.171	2.171			-0.135			-0.020	
b3319	rplD	-0.068	-0.018	-0.038	-0.053	0.000	-0.113	-0.069	-0.078	-0.030	-0.061	-0.184	-0.074	-0.063	-0.265	-0.091
b3320	rplC	-0.025	-0.054	-0.060	-0.020	-0.120	-0.135	-0.139	-0.061	-0.122	-0.013	-0.120	-0.109	-0.077	-0.158	-0.015
b3321	rpsJ	0.008	-0.004	-0.041	-0.032	-0.024	0.043	-0.052	-0.043	0.048	0.013	0.036	-0.040	-0.063	0.009	0.070
b3322	gspB	0.016	0.040	-0.002	-0.022	-0.005										
b3323	gspA	-0.015	0.011	0.044	-0.041	-0.070										
b3324	gspC	0.043	-0.013	0.060	-0.027	-0.013										
b3326	gspE	0.053	-0.026	0.029		-0.027										
b3330	gspI	0.045	0.015	-0.033	-0.022	0.083										
b3331	gspJ	0.051	-0.002	0.000	0.016	0.003										
b3332	gspK	0.013	0.024	0.066	-0.025	-0.060										
b3335	gspO	-0.017	-0.030	-0.020	0.138	0.179										
b3336	bfr						-0.026	0.109	0.191	0.525	0.460	-0.057	0.054	-0.012	0.297	0.311
b3337	bfd	-0.038	-0.010	0.105	-0.023	-0.086										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b3338	chiA							2.171								
b3339	tufA	-0.063	-0.080	-0.070	0.001	-0.005	0.074	-0.009	0.030	0.043	-0.096					
b3340	fusA	-0.055	-0.013	-0.041	-0.010	-0.196	0.022	0.026	0.009	0.035	-0.009	0.007	0.005	-0.011	0.002	-0.025
b3341	rpsG	-0.020	-0.015	-0.044	0.002	-0.067	-0.061	-0.030	-0.039	-0.022	-0.026	-0.084	-0.044	-0.062	-0.049	0.064
b3342	rpsL	-0.063	-0.031	-0.022	-0.014	-0.121	-0.148	-0.122	-0.109	-0.122	-0.091	0.028	0.060	0.085	0.000	0.132
b3343	yheL	-0.007	0.069	0.088	-0.071	-0.036										
b3344	yheM	0.015	-0.038	0.060	0.011	0.089										
b3347	fkpA	0.008	0.005	-0.023	0.009	-0.051	-0.056	0.156	0.104	0.074	0.056	-0.082	-0.037	-0.025	0.023	0.074
b3349	slyD	-0.030	-0.005	-0.029	-0.037	-0.088	-2.171	-0.061	0.217	0.104	-2.171	0.008	-0.052	0.180	-0.023	0.137
b3352	yheS	-0.069	0.046		-0.035	0.136										
b3353	yheT							2.171								
b3354	yheU	-0.030	-0.021	0.033	-0.037	0.042										
b3355	prkB		0.046													
b3356	yhfA	-0.035	-0.006	0.025	0.033	0.072										
b3357	crp	0.048	-0.006	-0.008	-0.008	-0.061	-0.013	0.004	0.000	-0.069	-0.009	-0.009	0.018	-0.050	-0.056	0.032
b3359	argD	-0.032	0.019	-0.003	-0.049	-0.002	0.026	0.048	-0.043	-0.017	-0.013	0.003	0.017	0.053	0.027	0.033
b3360	pabA	0.022	-0.007	-0.026	-0.066	-0.079										
b3361	fic	-0.019	-0.016	-0.021												
b3363	ppiA	-0.014	0.029	0.063												
b3364	tsgA	-0.105	-0.010	-0.048	-0.024	0.017										
b3365	nirB						-2.171	-2.171	-2.171	-0.139	-2.171					
b3367	nirC		0.057	-0.002		-0.437										
b3368	cysG	-0.022	0.094	0.107	0.065	-0.015						-0.035	-0.045	0.045	-0.014	0.099
b3369	yhfL	0.023	-0.041		-0.016	0.062										
b3370	frlA	-0.061	0.051	0.027	-0.011	0.036										
b3371	frlB	0.011	0.070	0.144		-0.004										
b3374	frlD	-0.026	-0.033	-0.038	-0.107	-0.096										
b3377	yhfT		-0.026	-0.379												
b3379	php	0.003	0.017	-0.120												
b3380	yhfW		0.132													
b3383	yhfZ	-0.056	0.002	-0.003	0.009	0.033										
b3384	trpS	0.011	0.020	-0.025	0.014	-0.075	-0.083	-0.221	-0.339	-0.208	-0.113	0.069	0.005	0.031	0.139	0.080
b3385	gph	-0.378														
b3386	rpe	0.004	-0.130		-0.045		2.171	2.171	2.171	2.171		-0.168	0.126	-0.025	0.044	-0.007
b3387	dam	0.006	0.013	-0.007	0.011	0.054										
b3389	aroB		-0.062	0.128	0.378	-0.439	0.048	-0.039	0.026	-0.004	0.100	0.081	-0.013	0.043	0.076	0.052
b3390	aroK		-0.297				2.171	2.171	2.171	2.171		-0.055	-0.010	0.043	-0.164	-0.027
b3395	hofM										2.171					
b3396	mrcA	0.096	-0.020		0.023	0.099										
b3397	nudE				0.020											
b3398	yrfF	0.031	0.012	0.056		-0.031						0.042	-0.050	0.094	0.080	0.007
b3399	yrfG	0.167	-0.014	0.003	0.063	-0.068										
b3401	hslO	0.006		0.011												
b3402	yhgE	0.251	0.039	0.079												
b3403	pck						2.171	2.171								
b3404	envZ		-0.005		-0.040	0.004										
b3405	ompR							2.171								
b3406	greB	-0.165	-0.025	0.063												
b3407	yhgF						-2.171	-2.171	0.248	-2.171	-2.171					
b3410	feoC	0.054	0.028	0.118	-0.077	-0.024										
b3411	yhgA	-0.195	0.041	0.105	0.105	-0.158										
b3412	bioH	0.022	0.028	0.015	0.013	-0.036										
b3413	gntX			0.000	-0.011	0.100										
b3414	nfuA	-0.027	0.005	-0.062	-0.059	-0.110	0.065	0.017	0.052	0.113	0.052	0.031	-0.035	0.030	0.040	0.102
b3415	gntT		0.216		0.113	0.094										
b3417	malP						-0.291	-2.171	0.004	0.035	0.165	-0.028	-0.021	0.018	0.160	0.184
b3418	malT						2.171									
b3419			0.097	-0.018	-0.034	0.064										
b3420		0.020	0.046	0.011	0.002	-0.022	-2.171	-2.171	-2.171	-2.171	-2.171					
b3421	rtcB				0.171											
b3423	glpR	-0.045	0.002	-0.015	-0.106	-0.188										
b3424	glpG	-0.060	-0.028	0.045	-0.015	0.061										
b3425	glpE	0.013	-0.002	-0.013	-0.128	-0.159										
b3426	glpD						-2.171	-2.171	-2.171	-2.171	-2.171	0.022	-0.016	0.019	-0.039	-0.006
b3427	yzgL	-0.004	-0.048	-0.041	0.058	0.150										
b3429	glgA						0.009	-2.171	-2.171	-2.171	-2.171					
b3430	glgC	-0.007	0.037	0.017		0.032	-2.171	-2.171	0.248	-0.504	-0.013					
b3432	glgB	0.093	0.010				-0.139		-2.171	-2.171	-0.317					
b3433	asd	0.223	-0.010	0.022	0.185	-0.140	0.065	0.126	0.109	0.069	-0.061	0.064	0.043	0.072	0.002	-0.044
b3436		-0.008	0.069	0.107	0.241	0.236										
b3437	gntK	-0.120	-0.006	0.024	-0.033	-0.088										
b3438	gntR	-0.040	-0.038	0.145												
b3439	yhhW	0.023	0.017	-0.017	-0.051	0.014										
b3441	yhhY	-0.049	-0.045	-0.067	-0.077	-0.065										
b3442	yhhZ	-0.022	0.002	0.003	-0.031	0.001										
b3443					-0.115											
b3445	insB-6									2.171						

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b3446	yrhB		0.023	0.003	-0.004											
b3447	ggt	-0.045	0.042	0.095	-0.042	0.040			2.171							
b3448	yhhA	0.030	0.048	0.079	0.043	0.022			2.171							
b3449	ugpQ	-0.017	-0.002	0.013	0.008	-0.057										
b3450	ugpC		0.051	0.167	-0.147											
b3453	ugpB					0.016										
b3454	livF	0.067	0.096	0.030	0.067											
b3456	livM	0.382		0.163												
b3458	livK		-0.008	-0.124	0.086	-0.636	0.013	0.013	0.000	-0.074	-0.135					
b3459	yhhK	-0.110	-0.006	-0.089	0.025	0.131										
b3460	livJ						0.000	0.017	0.030	-0.074	-0.148	-0.012	0.006	0.034	-0.077	-0.116
b3461	rpoH	0.010	-0.007	-0.021	-0.044	0.002										
b3462	ftsX	-0.007	-0.030	0.030	0.021	0.067			2.171							
b3463	ftsE	0.056	0.022	-0.026	-0.104	0.000			2.171							
b3464	ftsY	0.015	0.061	-0.021	-0.045	-0.043										
b3465	rsmD	-0.083	0.022	-0.013	-0.013	-0.140										
b3466	yhhL		0.059													
b3467	yhhM	-0.226	-0.042	-0.038	-0.122	-0.071										
b3468	yhhN	-0.074	-0.052	-0.015	-0.073	-0.171										
b3469	zntA			0.274				2.171					-0.135	-0.281	-0.488	-0.226
b3471	yhhQ				-0.024	-0.092										
b3472	dcrB					0.349										
b3473	yhhS	0.133	0.046	-0.001												
b3474	yhhT				0.432											
b3475	acpT		0.002													
b3476	nikA	0.053	0.007	0.042	0.048	0.018										
b3478	nikC				-0.149											
b3479	nikD		-0.010	-0.042												
b3480	nikE	-0.011	0.020	-0.004	0.004	-0.026		2.171								
b3481	nikR	0.061	0.060	0.100	0.239	0.277										
b3482	rhsB	0.015	-0.005	0.017	-0.082	-0.030										
b3483	yhhH	0.027	0.019	0.033	0.045	0.018										
b3485	yhhJ	-0.016	0.011	-0.093	-0.054	0.028		2.171								
b3486	rbbA		-0.019	-0.001	0.045	0.012	-2.171	-2.171	-2.171	-2.171	-2.171					
b3488	yhiJ	-0.156	-0.063	0.057												
b3490	yhiL	0.024	0.021	0.059	0.003	0.162										
b3491	yhiM	-0.061	0.002	-0.045	-0.110	-0.203										
b3492	yhiN	0.030	0.016	0.089	-0.002	-0.013			2.171							
b3493	pitA		0.016		0.006	0.179										
b3494	uspB		-0.005	0.036	-0.163											
b3495	uspA	0.017	0.087	0.001	-0.053	-0.062										
b3496	dtpB	-0.039	0.026	0.006	-0.101	-0.103										
b3497	yhiQ							2.171								
b3498	prlC	0.045	-0.016	0.026	0.247	-0.283	-0.248	0.026	0.087	0.161	0.165	0.075	-0.007	0.059	0.115	0.108
b3499	yhiR	0.016	0.030	0.004	0.151	0.210										
b3500	gor	-0.019	0.016	-0.026	0.019	-0.042	2.171		2.171			-0.060	0.013	-0.040	0.079	0.088
b3501	arsR	-0.063	-0.022	-0.098	0.056	0.087										
b3502	arsB					-0.101										
b3503	arsC	-0.082	0.025	0.029	0.054	0.216										
b3504	yhiS	0.032	-0.080	0.034	0.029	0.003										
b3505	insH-11		0.112	-0.032	-0.080											
b3507	dctR	-0.030	-0.042	-0.084	0.009	-0.014										
b3508	yhiD	0.043	0.004	0.007	0.016	0.071										
b3509	hdeB				0.103		0.122	0.113	0.213	0.738	0.982		0.106	0.189	0.722	0.955
b3510	hdeA			-0.440	-0.001	0.001			2.171	2.171	2.171					
b3511	hdeD	-0.074	0.041	0.095	0.057	0.031										
b3512	gadE	-0.025	-0.061		-0.017	0.039										
b3513	mdtE	-0.029	0.014	-0.024	0.041	0.155		2.171			2.171		0.058			0.128
b3514	mdtF	-0.009	0.001	-0.025	0.041	0.089										
b3516	gadX			-0.117			2.171			2.171						
b3517	gadA	-0.042	-0.011	-0.025	0.000	-0.057	-0.091	0.017	0.030	0.617	0.916					
b3521	yhjC															
b3522	yhjD	-0.148	-0.056	-0.060	0.036	-0.038										
b3523	yhjE	-0.098	0.046	-0.012	-0.003	0.029										
b3524	yhjG	-0.072														
b3525	yhjH				0.335											
b3526	kdgK						-0.039	-2.171	-2.171	-2.171	-2.171					
b3527	yhjJ		-0.001		-0.091	0.009			2.171							
b3529	yhjK	0.106	0.057	0.244	0.523	0.891										
b3530	bcsC	0.261	0.029	-0.005	-0.011	0.081	2.171		2.171	2.171		0.078	0.311	-0.164	-0.130	0.106
b3531	bcsZ		0.048													
b3532	bcsB						-2.171	-2.171	-2.171	0.083	-2.171					
b3533	bcsA	0.010	-0.080	0.134	0.443	0.960		-2.171	-2.171	-2.171	-2.171					
b3534	yhjQ	0.087	0.129	0.170	0.494	0.845										
b3536	bcsE	-0.005	0.033	0.000	-0.037	0.085										
b3537	bcsF				0.101											
b3538	bcsG	0.032	0.026	0.056	-0.002	0.069										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b3539	yhjV	-0.002	0.023	0.067	0.016	-0.092										
b3540	dppF	0.125	-0.057	-0.118	-0.081	-0.092										
b3542	dppC		-0.029													
b3543	dppB	0.100	0.010	0.000	-0.030	-0.061										
b3544	dppA						-0.022	0.026	-0.004	-0.009	-0.096	0.049	0.084	-0.019	0.025	-0.041
b3546	eptB	-0.090														
b3547	yhjX	-0.016	-0.069	-0.062	0.017	-0.032										
b3548	yhjY	0.009	0.019	0.024	0.061	0.121										
b3549	tag	-0.035	0.010	-0.036	-0.026	-0.039										
b3551	bisC	0.048	0.066	0.076	0.066	0.114										
b3553	ghrB		-0.013	0.059	-0.009	0.117	-2.171	-2.171	-0.248	0.030	-2.171	0.043	0.007	0.002	-0.006	0.002
b3554	yiaF	0.068	0.099	0.000	0.019	0.013										
b3556	cspA	-0.023	-0.039	-0.100	-0.041	-0.179	0.022	-2.171	0.161	-2.171	-2.171	-0.093	-0.318	-0.045	-0.064	-0.070
b3559	glyS	-0.044	-0.015	-0.022	0.152	-0.083	0.096	0.030	0.035	0.009	0.000	0.060	0.036	-0.020	-0.023	0.017
b3560	glyQ	0.000	0.005	-0.082	-0.106	0.011	-0.143	-0.056	-0.043	-0.139	-0.187	0.055	0.055	-0.031	-0.128	-0.016
b3562	yiaA	0.135	0.000	0.060												
b3564	xylB		0.013	0.037	0.001	0.091	2.171									
b3565	xylA							2.171								
b3566	xylF				0.306											
b3567	xylG	0.028	0.045	0.048	-0.026	-0.017										
b3568	xylH					-0.558										
b3570	bax	-0.046	-0.020	-0.050	0.004	0.111					2.171					
b3572	avtA	-0.012	0.003	-0.053	-0.031	-0.012										
b3573	ysaA	-0.128	0.011	0.018	0.020	0.174										
b3575	yiaK	-0.029	0.021	-0.010	-0.095	-0.045	2.171	2.171			2.171					
b3576	yiaL	-0.054	0.006	0.098	-0.045	0.001										
b3577	yiaM	0.027	0.012	0.045	0.028	0.087										
b3579	yiaO	0.116	0.061	0.010	0.041	0.071		2.171		2.171						
b3582	sgbU	0.006	0.041	0.128	0.309	0.485										
b3583	sgbE	0.100	0.016													
b3586	yiaV	0.024	-0.013	0.073	-0.018											
b3587	yiaW	0.015	-0.013	-0.027	-0.025	-0.010										
b3589	yiaY	-0.006	0.004	0.017	-0.115	0.005										
b3590	selB	-0.074	-0.010	-0.012	0.054	-0.208										
b3591	selA	-0.020	-0.004	-0.044	-0.041	0.029										
b3592	yibF	-0.003	0.052	0.116	-0.004	0.117	-2.171	-2.171	-0.161	-0.152	-0.104	-0.066	-0.137	-0.109	0.161	0.221
b3593	rhsA	-0.001	-0.008	-0.028	-0.002	-0.007										
b3595	yibJ				0.157											
b3598	yibI	-0.028	-0.046	-0.073	-0.087	-0.139										
b3599	mtlA	0.062	0.001	-0.030	-0.054	-0.030										
b3600	mtlD	0.378	-0.089		0.009	0.032	-0.213	-2.171	-2.171	-2.171	-2.171					
b3602	yibL				0.097											
b3604	lldR	-0.022	-0.002	-0.016	-0.027	-0.020										
b3605	lldD								2.171	2.171						
b3607	cysE	-0.013	0.048	0.012	0.066	-0.099										
b3608	gpsA	0.007	0.027	0.052	-0.007	0.068										
b3609	secB	0.279	0.104	0.069	0.018	-0.226	0.043	0.052	0.009	0.100	0.074	0.069	0.099	-0.022	0.215	0.089
b3610	grxC	-0.038	-0.027	-0.003	-0.002	-0.034	0.174	-2.171	0.256	0.017	-2.171	0.126	0.072	0.011	0.237	0.271
b3611	yibN	-0.005	-0.023	-0.024	-0.061	-0.091										
b3612	gpmM	0.174	0.027	0.066	0.055	0.024	0.052	0.061	0.052	0.096	-0.009	0.030	-0.014	0.021	0.061	-0.032
b3614	yibQ	0.051	0.010	0.016	-0.022	0.066										
b3615	yibD	0.032	0.004	-0.027					2.171							
b3616	tdh	0.081	-0.014	-0.098	0.011	-0.134		2.171								
b3617	kbl	-0.070	-0.067	-0.025	-0.090	-0.094			2.171	2.171		0.198	0.180	0.079		0.553
b3619	rfaD						0.013	-0.030	-0.083	-0.017	0.030	-0.037	0.031	-0.048	0.040	0.000
b3621	rfaC			-0.095												
b3622	rfaL		-0.071	-0.004	0.148											
b3623	waaU	-0.013	-0.002	0.018	0.025	0.021	2.171	2.171	2.171							
b3624	rfaZ		0.015													
b3625	rfaY	0.001	-0.051													
b3626	rfaJ	-0.210														
b3628	rfaB							2.171								
b3635	mutM	0.003	0.009	0.014	0.012	0.115										
b3636	rpmG	-0.107	-0.115	-0.075	0.156	-0.280										
b3637	rpmB	-0.044	-0.018	-0.042	-0.001	0.030	-0.087	-0.083	-0.009	-0.052	0.039	-0.079	-0.068	-0.020	-0.013	-0.004
b3638	yicR	-0.026	0.001	-0.021	0.001	0.001							-0.034	-0.087	-0.129	-0.210
b3639	dfp	-0.019	-0.013	-0.049	-0.011	-0.009										
b3640	dut	0.022	-0.056	-0.045	-0.071	-0.167	-2.171	-0.269	-0.274	-0.004	-2.171					
b3642	pyrE	-0.041	0.013	0.024	0.042	0.068										
b3643	rph		-0.083													
b3644	yicC						-0.096	-2.171	-2.171		-2.171	-0.048	-0.074	0.010	0.064	0.185
b3645	dinD	-0.068	-0.004	-0.008	-0.063	0.023										
b3647	ligB								2.171							
b3648	gmk				0.137											
b3649	rpoZ					-0.126	-0.026	-0.048	-0.104	0.056	0.030	-0.067	-0.113	-0.104	0.014	0.024
b3650	spoT	-0.090	0.065		-0.184	-0.353										
b3651	trmH	-0.027	0.022	0.047	-0.001	0.017			2.171							

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b3652	recG	-0.114	-0.051	-0.056	-0.067	-0.199										
b3653	gltS		0.052	0.047		-0.087										
b3655	yicH					-0.131				2.171						
b3656	yicI											0.037	0.064	0.013	-0.005	0.198
b3657	yicJ	-0.003	0.000	0.042	0.045	-0.051										
b3659	setC	-0.038	-0.084	-0.054		-0.041										
b3660	yicL		0.054	0.112	0.357	0.787										
b3661	nlpA	-0.064	-0.042	-0.065	-0.095	-0.015	2.171									
b3663	yicN	0.009	-0.071	-0.060	0.066	0.158										
b3665	ade				0.069											
b3666	uhpT	0.123	0.028	0.044	-0.100	-0.045										
b3670	ilvN	0.286	-0.008	-0.039	0.137	-0.254			2.171			-0.037	0.032	0.083	-0.069	-0.239
b3671	ilvB	0.044	-0.012	0.085	0.202	0.216	0.013	0.074	0.039	0.043	-0.065	-0.093	0.077	0.069	-0.107	-0.106
b3672	ivbL	-0.332														
b3673	emrD	-0.206	-0.037	0.005	-0.074											
b3674	yidF	-0.052	-0.090		-0.108	-0.002										
b3677	yidI	0.031	0.030	0.032	0.053	0.174										
b3679	yidK	-0.051	0.018	-0.076	-0.124	-0.120		2.171								
b3681	glvG	0.202	0.072				-2.171	-2.171	-2.171	-2.171	-2.171					
b3682	glvB	-0.010	-0.020	-0.058	0.017	0.021										
b3683	glvC								2.171							
b3685	yidE	-0.012	0.019	-0.055	-0.009	-0.048										
b3686	ibpB		0.011	0.032	0.010	-0.021				2.171						
b3687	ibpA		0.025	0.095	0.125	0.334					2.171					
b3688	yidQ	-0.041	0.057	0.061	0.019	0.031										
b3689	yidR	0.273														
b3691	dgoT	-0.065	0.005	-0.057	-0.056	-0.159										
b3692										2.171						
b3693	dgoK				-0.005							-0.080	-1.001		-0.245	-0.125
b3695		0.058	0.071	0.120	0.117											
b3698	yidB		-0.100	0.091	0.098											
b3699	gyrB		0.046	-0.111			-0.117	-0.065	-0.069	-0.061	0.061	-0.059	-0.057	-0.043	-0.033	0.034
b3700	recF		0.008	-0.105	-0.058	0.023	2.171					0.235		0.062	-0.069	
b3701	dnaN	-0.093	-0.046	-0.118							2.171	-0.040	-0.079	-0.055	0.008	0.107
b3702	dnaA	-0.016	0.039	-0.030	-0.058	-0.023				2.171						
b3703	rpmH	-0.043	-0.006	0.010	0.003	0.105										
b3705	yidC													-0.066		-0.015
b3706	mnmE		0.095		-0.069			2.171								
b3708	tnaA	-0.011	0.024	0.004	-0.026	0.047										
b3709	tnaB	0.197	0.066	-0.002	-0.008	-0.081										
b3712	yieE	-0.028	0.029	0.089	-0.094	-0.099										
b3713	yieF	0.230	0.010	0.020	-0.104	-0.104						0.075	-0.064	-0.029	0.232	0.194
b3714	yieG		-0.017	0.000					2.171							
b3715	yieH	-0.012	0.013	0.069	-0.075	0.034										
b3717	cbrC	-0.035	-0.039	0.031		-0.036										
b3719	yieL	-0.017	0.029	0.006		-0.002										
b3720	bglH	-0.053	-0.072	0.000	-0.008	-0.071	2.171									
b3721	bglB				-0.102											
b3723	bglG		0.020	0.000	0.001	-0.005										
b3725	pstB				0.072											
b3726	pstA	0.013	0.000	0.008	0.075	0.110										
b3728	pstS	-0.102	0.021	0.017	0.251				2.171							
b3729	glmS				-0.086		0.030	-0.048	0.004	-0.026	0.035	-0.050	0.000	0.009	0.004	-0.035
b3730	glmU	-0.014	0.016	0.023	-0.013	0.019										
b3731	atpC	0.064	-0.016	-0.020	-0.106	-0.013										
b3732	atpD	0.258	0.306	0.226			-0.083	-0.043	-0.069	-0.083	-0.069	-0.063	0.017	-0.092	-0.081	-0.081
b3733	atpG	-0.069	-0.013	0.013	-0.079	0.026	-0.252	0.013	-0.152	-0.239	-2.171	-0.114	-0.110	-0.056	-0.129	-0.079
b3734	atpA			0.516			-0.117	-0.043	-0.069	-0.091	-0.017	-0.049	0.029	-0.080	-0.113	-0.018
b3735	atpH													-0.012	-0.172	0.102
b3736	atpF						-2.171	-2.171	-2.171	-2.171	-2.171					
b3737	atpE	0.549	0.607	0.552	-0.082	0.403										
b3738	atpB	0.009	-0.001	0.013	0.079	0.202										
b3739	atpI	-0.050	0.038	-0.025	0.018	0.049										
b3741	mnmG	-0.030	0.010	-0.087								0.084	0.056	0.063	0.028	0.034
b3742	mioC	-0.033	0.013	-0.049	-0.008	-0.011										
b3743	asnC	-0.015	-0.033	-0.052	-0.013	-0.018										
b3744	asnA	-0.028	0.022	0.004	-0.079	-0.150	2.171	2.171				0.060	0.021	-0.028	-0.117	0.005
b3745	viaA								2.171							
b3746	ravA	0.001	0.024	0.063	0.019	0.017					2.171					
b3748	rbsD		0.087		-0.037	0.019										
b3749	rbsA											0.007	-0.029	0.098	0.363	0.166
b3750	rbsC	0.049	0.033	0.036	-0.004	0.014										
b3751	rbsB	0.032	0.081	0.030	0.047	-0.025	-0.052	-0.100	-0.009	0.083	0.213	-0.112	-0.128	-0.027	-0.048	0.079
b3753	rbsR		0.269													
b3754	hsrA	0.107	0.012	0.087	0.016	0.052										
b3755	yieP	-0.030	-0.055	-0.018	-0.072	-0.033		2.171								
b3762		0.002	0.005	0.003	0.071	0.045										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b3763		-0.031	-0.022	-0.008	0.000	0.031				2.171	2.171					
b3764	yifE	0.109	-0.015	0.044	0.022	0.038	-0.052	-0.117	-0.113	-0.056	0.065	-0.037	-0.020	-0.029	0.021	0.045
b3765	yifB	-0.012	-0.035	-0.014	0.061	0.028										
b3766	ilvL	0.016	0.014	0.014	0.067	0.128										
b3767		0.005	0.030	-0.008	0.030	0.053				2.171						
b3768		-0.043	-0.038	-0.076	-0.034	-0.247										
b3769	ilvM	-0.027	-0.061	-0.108	-0.127	-0.231										
b3770	ilvE		0.017	0.037			0.017	0.048	-0.052	-0.083	-0.074	0.051	0.038	-0.029	-0.051	0.006
b3771	ilvD	0.082	0.078	0.073	-0.019	0.041	2.171	2.171	2.171	2.171	2.171	0.010	0.041	0.034	0.028	0.032
b3772	ilvA	0.038	0.002	-0.011	0.051	0.022										
b3773	ilvY	0.070	0.039	0.037	-0.032	-0.116										
b3774	ilvC	0.076	0.003	0.042	0.011	-0.002	0.022	0.017	0.004	-0.035	-0.030	0.013	0.036	-0.022	-0.051	-0.044
b3775	ppiC	-0.025	-0.008	-0.046	-0.060	-0.098										
b3776	yifO	0.011	-0.009	0.055	0.024	0.071										
b3777		0.097	0.005	-0.019	-0.072											
b3778	rep		0.048	0.006												
b3780	rhlB	-0.095	-0.008	-0.005	-0.013	-0.027	2.171	2.171								
b3781	trxA	0.014	0.002	0.002	0.059	0.049	-0.065	-0.030	0.048	0.009	0.126	-0.060	0.005	0.053	0.021	0.154
b3782	rhoL		0.112		0.109											
b3783	rho	-0.038	-0.039	-0.050	0.024	-0.083	0.009	-0.069	-0.013	-0.017	0.074	0.003	0.008	0.033	0.005	0.048
b3786	rffE	-0.001	0.006	0.027	0.018	0.048										
b3787	rffD	-0.098	0.026	0.025	0.020	0.013				2.171						
b3788	rffG	0.011	-0.022	-0.002	0.035	0.005										
b3789	rffH	-0.021	0.004	-0.025	0.030	-0.101										
b3790	rffC				0.180				2.171					0.014	-0.039	
b3791	rffA									2.171					0.134	
b3792	wzxE	-0.009	0.033	0.096	0.113	0.268										
b3794	rffM							2.171	2.171		2.171					
b3795	yifK		-0.119	-0.002	0.083											
b3800	aslB	0.033	-0.012	-0.013	-0.035	-0.087										
b3801	aslA	-0.009	0.049	0.014	-0.003	0.044										
b3802	hemY	-0.028	-0.005	0.044	0.011	0.006						0.084	-0.036	0.016	0.054	0.103
b3803	hemX	-0.019	-0.135	0.019	-0.048	-0.007										
b3804	hemD	0.033	-0.020	0.003	-0.057	-0.016										
b3805	hemC	-0.040	0.003	0.019	-0.022	-0.260	-2.171	-2.171	-2.171	-0.421	-2.171					
b3807	cyaY				0.041											
b3808	yzcX	0.005	-0.026	0.018	-0.043	-0.019	-2.171	-2.171	-2.171	-2.171	-2.171					
b3809	dapF	0.002	-0.020	-0.044	0.029	-0.030										
b3810	yigA	0.035	0.051	0.084	0.117	0.160										
b3811	xerC	-0.017	0.016	0.003	-0.115											
b3812	yigB	0.006	0.025	0.097	0.015	0.120										
b3813	uvrD	-0.054	-0.010	-0.020	0.002	0.095		2.171	2.171			0.118	0.060	0.115	-0.073	0.059
b3814		-0.113	0.004	-0.020												
b3815		-0.068	0.049	-0.098	-0.015	0.044										
b3816	corA	-0.040	0.084	-0.020	-0.073	-0.010										
b3817	yigF	0.017	0.017													
b3818	yigG	-0.020	0.014	0.007	0.022	-0.028										
b3819	rarD	0.075	0.006	0.003	-0.022	0.044										
b3820	yigI	-0.014	-0.004	-0.017	0.034	0.006	2.171		2.171		2.171					
b3821	pldA	-0.002	0.042	0.093	0.124	0.205										
b3822	recQ		0.009	-0.119												
b3823	rhtC	0.006	-0.016	-0.029												
b3824	rhtB	0.002	-0.019	-0.053	-0.060	-0.039										
b3825	pldB	-0.005	0.023	-0.012	0.025	0.018										
b3826	yigL	0.028	-0.028	-0.005		-0.026										
b3827	yigM	-0.025	0.011													
b3828	metR	0.062	-0.021	0.021	0.063	0.024										
b3829	metE	-0.047	-0.045	-0.019	0.038	-0.055	-0.026	-0.017	-0.030	-0.035	-0.061	0.008	-0.041	-0.022	-0.042	-0.025
b3830	ysgA		0.053	0.130												
b3831	udp	-0.034	-0.040	0.002	0.002	-0.027	2.171	2.171		2.171	2.171					
b3832	rmuC		-0.053	-0.050												
b3833	ubiE										2.171					
b3834	yigP	-0.004	-0.151													
b3835	ubiB	0.015	-0.010	-0.031	-0.054	-0.051								-0.036	-0.031	-0.358
b3836	tatA	-0.094	-0.035	0.002	-0.111	-0.051										
b3837		-0.012	0.056	-0.056	-0.071	-0.025										
b3838	tatB	0.028	-0.136													
b3839	tatC	-0.032	-0.023	-0.027	-0.050	-0.044										
b3840		-0.118	0.016	0.012	-0.061	-0.010									-0.080	
b3841			0.098	0.040	-0.030	-0.043										
b3842	rfaH	0.059	0.078	-0.163	-0.136	-0.350					2.171					
b3843	ubiD	-0.025	-0.096	-0.123	-0.064	-0.056										
b3844	fre	-0.067	-0.075	-0.017	-0.059	-0.095		2.171		2.171			-0.059		0.068	0.020
b3845	fadA	0.020	0.040	-0.024	-0.083	-0.147										
b3846	fadB	0.008	0.029	-0.043	-0.023	-0.002										
b3847	pepQ	-0.080	0.014	-0.012	-0.094	-0.092		2.171	2.171	2.171	2.171	-0.018	-0.006	0.040	0.019	0.033
b3848	yigZ			-0.004		0.051										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b3849	trkH	-0.137	0.075	-0.027	-0.011	0.057										
b3850	hemG				0.061											
b3856	mobB	-0.015														
b3857	mobA	0.036	0.043	0.118	0.284	0.370										
b3858	yihD													0.025	0.086	0.087
b3859	rdoA	-0.085														
b3860	dsbA	-0.072	-0.065	-0.061	0.017	-0.025	2.171	2.171	2.171			0.003	0.112	0.204	0.084	0.162
b3861	yihF	-0.017	0.023	0.003	-0.086	-0.005										
b3862	yihG	-0.050	0.033	0.076		-0.036										
b3863	polA						-0.156	-0.056	-0.278	-0.022	0.182	-0.019	-0.036	-0.022	0.009	-0.053
b3865	yihA	-0.040	-0.002	-0.017	-0.049	-0.084		2.171	2.171	2.171			-0.110			0.184
b3866	yihI	0.026	0.006	-0.010	-0.051	-0.041				2.171						
b3867	hemN	-0.011	0.017	0.016	0.056	0.088										
b3868	glnG	-0.038	-0.014	-0.028	0.116	-0.133										
b3869	glnL	-0.025	0.034	-0.027	-0.030	-0.128										
b3870	glnA		-0.022	0.273	0.299	-0.250	0.135	0.143	0.139	-0.056	-0.039	0.073	0.058	0.062	-0.038	-0.042
b3871	typA	-0.118	-0.094	-0.088	0.020	-0.035	0.056	0.013	0.052	0.069	0.100	0.063	0.062	0.035	0.046	0.089
b3874	yihN				0.008											
b3876	yihO		0.173													
b3877	yihP	0.046	0.083	0.049	0.024	0.129										
b3878	yihQ	-0.123	0.089													
b3879	yihR	0.029	0.005	0.087		-0.018										
b3880	yihS								2.171							
b3881	yihT						2.171									
b3882	yihU	0.058	-0.009	0.213		-0.120										
b3885	yihX	0.057	0.119	0.189	0.175	0.446										
b3888	yiiD									2.171						
b3889	yiiE	0.001	-0.023	-0.014	0.013	0.090										
b3890	yiiF	-0.018	0.037	0.061	0.001	-0.017										
b3891	fdhE	-0.044	-0.001	-0.047	-0.127	-0.246	2.171									
b3893	fdoH	-0.052	-0.001	-0.041	-0.100	-0.162										
b3894	fdoG	0.000	0.032	0.010	0.014	-0.054										
b3895	fdhD	-0.081	-0.007	-0.003	-0.067	-0.197										
b3896	yiiG	0.045	-0.046	-0.073	-0.074	-0.030										
b3897	frvR	-0.038	0.178	0.061	-0.039											
b3898	frvX	-0.052	0.006	-0.014	0.020	0.052		2.171	2.171			-0.095	-0.025	-0.007	-0.061	0.051
b3900	frvA		-0.040	0.009	-0.064											
b3901	rhaM				0.051											
b3904	rhaB						-2.171	-2.171	-2.171	-2.171	-2.171					
b3906	rhaR				0.013	0.002										
b3907	rhaT	0.057	0.068	0.130	0.211	0.196										
b3908	sodA	-0.007	0.020	0.055	0.079	0.144	-0.113	-0.100	0.009	0.048	0.074	-0.149	-0.075	-0.039	0.111	0.080
b3909	kdgT				-0.163											
b3913					0.139											
b3914					0.565											
b3916	pfkA	0.020	0.065	0.037	0.047	0.011	-0.087	0.065	0.013	0.117	0.087	-0.014	0.017	-0.026	0.052	0.127
b3917	sbp												-0.483	-0.171	0.091	
b3918	cdh	-0.060	0.002	-0.023	-0.022	-0.024										
b3919	tpiA	-0.027	0.002	0.024	-0.050	-0.098	-0.039	-0.035	-0.022	0.122	0.113	0.031	-0.022	0.027	0.128	0.203
b3920	yiiQ	0.153	-0.025	0.008	-0.004	0.059										
b3921	yiiR				0.000											
b3923	uspD								2.171							
b3924	fpr			-0.032												
b3925	glpX	0.146	0.004	0.040	-0.200	-0.030										
b3926	glpK	-0.025	-0.020	-0.033	-0.022	-0.013	2.171						-0.113		-0.042	
b3928	zapB						-2.171	-2.171	-2.171	-2.171	-2.171	0.091	-0.007	-0.082	-0.034	
b3929	rraA	0.031	0.046	0.054	0.055	0.170			2.171	2.171	2.171	-0.049	-0.090	-0.086	0.042	0.039
b3930	menA	-0.031	0.065	-0.018	-0.017	0.027										
b3931	hslU		0.046	0.238	0.163	0.468	-0.043	0.039	-0.113	0.109	0.039	0.002	-0.006	0.021	0.088	0.104
b3932	hslV	-0.001	-0.019	-0.075	0.022	-0.009					2.171					
b3933	ftsN	0.074	0.011	-0.014	0.027	0.000										
b3935	priA	-0.069	-0.020	-0.023				2.171								
b3936	rpmE	-0.047	-0.043	-0.124	-0.068	-0.060	-0.191	-0.195	-0.030	-0.169	-0.122	-0.166	-0.079	-0.059	-0.158	-0.129
b3937	yiiX				-0.006											
b3938	metJ	0.032	-0.013	-0.012	0.084											
b3939	metB	0.103	-0.019	0.020	0.104	-0.291	-0.208	-0.269	-0.217	-2.171	-0.256	0.041	-0.104	0.017	0.042	-0.056
b3940	metL	0.003	0.001	0.002	-0.023	-0.154	-0.195	-0.052	0.243	0.056	-0.017	0.020	0.002	0.034	0.052	0.094
b3941	metF	-0.036	-0.022	0.002	0.014	0.037	0.052	0.017	0.035	0.078	0.043	0.017	0.015	0.045	0.021	-0.027
b3942	katG	-0.049	-0.022	-0.060	-0.016	0.013	-0.035	-0.083	-0.074	-0.026	-0.282	-0.005	-0.029	-0.021	-0.059	-0.161
b3943	yijE	0.021	-0.001	0.056	0.054	0.122										
b3944	yijF	-0.034	0.041	0.043	0.057	0.118										
b3945	gldA	0.002	-0.007	-0.039	0.014	0.064										
b3946	fsaB	-0.018	-0.003	0.025	-0.010	0.107										
b3947	ptsA	-0.008	0.090		0.047				2.171							
b3949	frwC										2.171					
b3951	pflD		-0.017	0.044		-0.093										
b3952	pflC		-0.025	0.064												

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b3955	yijP	-0.039	0.008	0.004	0.004	-0.017				2.171						
b3956	ppc	0.467	0.071	0.141	-0.039		0.017	0.004	-0.022	-0.056	-0.083	0.034	0.031	-0.027	-0.012	-0.009
b3957	argE	0.010	-0.004	0.044	0.113	0.011	-0.317	0.022	0.100	0.048	0.065	0.048	0.062	0.070	0.072	0.094
b3958	argC	0.182	0.005	0.015	0.067			2.171								
b3959	argB		-0.017	0.048	0.089	0.078	0.269	0.221	0.178	0.178	0.191	0.083	-0.013	-0.003	0.047	0.089
b3960	argH						0.187	0.117	0.030	0.026	0.091	0.011	0.008	-0.001	0.158	0.035
b3961	oxyR	0.026	-0.057		-0.071											
b3962	sthA	-0.050														
b3963	fabR	0.073	0.029	0.101	-0.035	0.131	2.171									
b3965	trmA	0.001	0.003	0.038	0.126	0.159										
b3966	btuB		-0.108			-0.004										
b3967	murI	0.152	0.044	0.114	0.140	0.075										
b3972	murB	-0.071	-0.035	-0.335												
b3973	birA	0.001	-0.020	-0.048	-0.092	-0.005										
b3974	coaA	-0.010	0.025	0.024	0.040	0.042										
b3975		-0.039	-0.065			-0.028										
b3980	tufB	-0.020	-0.023	-0.054	-0.129	-0.068	0.078	0.052	0.004	0.109	-0.087	0.038	0.024	0.034	0.032	-0.079
b3982	nusG	-0.052	-0.041	-0.006	-0.097		2.171		2.171			0.004	0.014	-0.030	-0.011	0.110
b3983	rplK	-0.048	-0.044	-0.003	0.022	-0.028	0.000	-0.039	-0.026	0.026	0.009	0.025	-0.008	-0.027	0.043	0.006
b3984	rplA	-0.069	-0.009	0.027	0.042	0.039	-0.109	-0.069	-0.083	-0.078	-0.043	-0.069	-0.083	-0.046	-0.083	-0.028
b3985	rplJ		0.000	0.009			-0.026	-0.004	-0.022	-0.061	0.026	-0.022	-0.005	-0.064	-0.058	-0.051
b3986	rplL						0.004	-0.030	-0.017	0.009	-0.083	0.031	0.070	0.035	-0.021	0.075
b3987	rpoB	0.059	0.039	0.002	-0.058	0.108	-0.017	-0.017	-0.013	0.004	0.030	-0.029	-0.003	0.030	0.034	0.025
b3988	rpoC	0.130	-0.021	0.042			-0.048	-0.039	-0.013	0.009	0.009	-0.046	-0.002	0.003	0.020	0.037
b3990	thiH	0.008	0.035	0.059	-0.010	0.007			2.171							
b3991	thiG	0.004	0.030	0.073	0.120	0.114	-0.043	-0.009	0.056	0.000	-0.100	-0.190	0.014	0.025	-0.024	-0.002
b3992	thiF	0.151	-0.008	-0.003	-0.036	0.048		-2.171	-2.171	-2.171	-2.171	-0.078	-0.014	-0.001	0.047	0.051
b3993	thiE	0.051	-0.003	-0.025	-0.050	0.040	0.252	0.321	0.221	0.165	-0.074	0.026	0.052	0.093	-0.035	-0.005
b3994	thiC	-0.009	-0.011	-0.046	-0.006	-0.003	-0.026	-0.030	0.039	0.109	-0.056	0.045	-0.048	-0.011	-0.003	-0.036
b3996	nudC	-0.033	-0.016	0.002	0.063	-0.131										
b3997	hemE	-0.049	0.047	0.002	0.027	-0.026		2.171								
b3998	nfi	-0.139	-0.063	0.256	0.147											
b3999	yjaG				0.062	-0.454			2.171							
b4000	hupA	0.029	0.001	-0.003	0.010	-0.026	0.043	0.004	0.017	0.004	0.039	0.003	0.001	-0.011	-0.012	0.042
b4001	yjaH	0.058	-0.085	0.010	0.023	-0.107										
b4003	zraS		-0.068	0.066												
b4004	zraR		0.049	0.010	0.045		-2.171	-2.171	-2.171	-2.171	-2.171					
b4005	purD	-0.027	0.031	-0.004	0.003	-0.023	0.078	0.022	0.195	0.061	0.143	-0.009	0.018	-0.024	0.044	0.038
b4006	purH	-0.029	0.020	-0.007	0.029	-0.009	0.035	0.013	-0.009	0.009	0.035	0.001	-0.010	0.005	0.012	0.053
b4011	yjaA				0.257	0.060										
b4012	yjaB	0.049	0.013	-0.006	-0.085	-0.100				2.171						
b4013	metA	-0.073	-0.029	-0.011					2.171	2.171		0.002	-0.071	-0.154	0.100	0.073
b4014	aceB	-0.003	0.023	0.002	-0.064	-0.020	0.113	0.061	0.061	0.191	0.139	0.053	0.046	0.016	0.122	0.052
b4015	aceA	-0.018	0.025	0.006	-0.009	0.063	0.061	0.039	0.039	0.143	0.017	0.069	0.046	0.005	0.076	0.014
b4016	aceK	-0.061	0.054	0.019		0.012										
b4017	arpA	-0.047	-0.054	-0.046	0.012	-0.097					2.171					
b4018	iclR		0.064			-0.657										
b4019	metH	-0.063	-0.070	-0.032	-0.028	-0.150	2.171	2.171		2.171		0.039	0.011	-0.037	0.070	0.032
b4020	yjbB															
b4021	pepE	0.094	0.134	0.054	-0.015	0.032										
b4022	rluF	-0.007	-0.010	0.077	0.065	0.184										
b4023	pagB	-0.020	0.025	0.042	0.019	0.031										
b4024	lysC			0.107			0.056	-0.048	0.026	-0.022	0.000	0.046	0.026	0.031	0.012	0.088
b4025	pgi			-0.147	0.081	-0.091	0.039	0.017	0.109	0.208	0.143	0.059	0.039	0.055	0.144	0.112
b4027	yjbF	-0.049								2.171						
b4028	yjbG	0.130	0.141	0.159	0.207	0.437		2.171	2.171	2.171						
b4029	yjbH	-0.006		0.037	-0.093		2.171					-0.085				
b4032	malG	0.111	0.143	0.099	0.103	0.264										
b4033	malF						-2.171	-2.171	-2.171	-2.171	-2.171					
b4034	malE		0.092	0.130		0.071	2.171		2.171							
b4035	malK	-0.061	-0.075	-0.076	-0.025	-0.016										
b4036	lamB	-0.110	0.015	-0.035								-0.060	0.091	-0.051	0.096	
b4038	yjbI				-0.010					2.171	2.171					
b4039	ubiC	-0.010	0.000	-0.004	-0.030	-0.001										
b4040	ubiA	0.161	-0.014	-0.020	-0.013	-0.011										
b4041	plsB	0.055	0.004	-0.001	-0.030	-0.033				2.171						
b4042	dgkA	-0.027	0.009	-0.020	-0.035	0.018										
b4046	zur		0.017	-0.038	0.019	0.091										
b4047	yjbL		0.000	0.139												
b4051	qor		0.030	0.104	0.037	-0.030										
b4052	dnaB	-0.106	-0.113	-0.039												
b4053	alr	-0.029	-0.028	0.057	-0.024	-0.109										
b4054	tyrB		0.192	0.186			-0.043	0.043	0.004	0.048	0.026	0.011	0.035	0.015	0.036	0.022
b4055	aphA	0.224		0.044												
b4058	uvrA	0.017	-0.018	-0.076	-0.021	0.023		2.171								
b4059	ssb	0.003	0.044	0.006	-0.076	-0.024	2.171	2.171	2.171	2.171		-0.080	-0.121	-0.212	-0.168	
b4060	yjcB		0.030	-0.211	0.012											

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b4061	yjcC											0.039	0.024	-0.030	-0.033	-0.071
b4062	soxS		-0.083		-0.071											
b4064	yjcD	0.069	-0.003	0.121	0.028	0.035										
b4065	yjcE	-0.002	-0.123	-0.059	-0.096	-0.025										
b4066	yjcF	0.028	-0.015	0.066	0.158	0.253										
b4070	nrfA	-0.006	-0.035	-0.042	-0.041	-0.001										
b4071	nrfB		0.045	-0.034		0.047										
b4072	nrfC			-0.274												
b4073	nrfD	0.000	0.024	-0.004	0.414											
b4074	nrfE	-0.040	-0.021	-0.065	-0.054	-0.120										
b4075	nrfF	-0.005	-0.010	0.002	0.113	0.076										
b4076	nrfG	-0.021	0.006	-0.027	0.053	0.080										
b4080	mdtP	-0.020	0.052	0.017	-0.046	-0.007										
b4083	yjcS		0.129		-0.004											
b4086	alsC	0.129	0.063	-0.010	0.024	-0.040										
b4087	alsA		0.138	0.148		0.128		2.171	2.171	2.171						
b4088	alsB		-0.010	0.008	0.045	-0.026				2.171						
b4089	rpiR	-0.031	0.045	0.012	-0.030	0.021										
b4091		0.054	0.012	0.018	-0.061	-0.018										
b4092	phnP		0.032													
b4093	phnO		0.024	0.038	-0.070	0.058										
b4094	phnN	0.001	0.027	-0.009	-0.027	0.069										
b4096	phnL	0.047	0.006	0.022	0.041	0.151										
b4097	phnK	-0.084	-0.034	-0.053	0.011	-0.001				2.171						
b4098	phnJ	0.002	0.004	-0.060	-0.025	0.001										
b4099	phnI	0.019	0.001	-0.002	0.030	-0.006										
b4100	phnH		-0.007	0.076	0.151	0.219										
b4101	phnG	0.053	0.039	0.053	0.123	0.101										
b4102	phnF							2.171								
b4103			0.075													
b4104	phnE		-0.036													
b4105	phnD	0.030	0.048	0.070	-0.033	0.165										
b4106	phnC	-0.126	-0.001	-0.052	-0.030	-0.006										
b4107	yjdN		0.030	0.020	0.013	0.047										
b4108	yjdM	0.023	0.028	0.091	0.000	0.001										
b4109	yjdA	-0.037	0.088	0.140	0.238	0.280			2.171		2.171					
b4110	yjcZ	-0.048	-0.022	-0.073	0.023	-0.013							-0.278		-0.062	-0.030
b4111	proP	-0.012	-0.004	0.075	-0.098	0.180										
b4112	basS	-0.064	0.014	-0.018	-0.015	0.027										
b4113	basR	-0.050	-0.020	-0.029												
b4114	eptA	0.919	0.430	0.534	-0.092	-0.097										
b4115	adiC		0.013		0.026	0.138										
b4117	adiA	-0.044	-0.015	-0.023	-0.010	-0.155					2.171					
b4118	melR						-2.171	-2.171	-2.171	-2.171	-2.171					
b4119	melA				0.284					2.171		0.028	0.067			0.159
b4120	melB				0.136											
b4121	yjdF				0.254											
b4122	fumB		0.028	-0.024	0.165	-0.240	-0.065	0.043	-0.065	-0.161	-2.171	-0.058	-0.061	-0.004	-0.072	-0.100
b4123	dcuB		0.046	0.068	0.052											
b4124	dcuR					-0.173								0.275		
b4126	yjdI	0.287														
b4128	yjdK		0.036	0.114	-0.039	-0.044										
b4129	lysU	-0.019	-0.045	-0.031	-0.058	-0.090	0.004	0.074	-0.009	0.009	0.039					
b4130	yjdL	0.080	0.033	0.016	0.008	-0.058										
b4131	cadA	-0.069	-0.015	-0.023	0.081	-0.047	2.171	2.171				-0.167	-0.034	0.013	0.061	-0.308
b4132	cadB		0.017	0.082	-0.045	0.028										
b4133	cadC	-0.030	-0.034	-0.031	-0.031	-0.023										
b4135	yjdC	-0.049	0.002	0.044	-0.105	-0.050	-2.171	-2.171	-2.171	-2.171	-2.171	0.037	0.013	0.057	0.139	0.089
b4136	dipZ				0.188											
b4138	dcuA		0.102		-0.023											
b4139	aspA		0.146					2.171			2.171					
b4140	fxsA	-0.032	-0.069	-0.002	-0.117	-0.084										
b4141	yjeH		0.185			-0.124										
b4142	groS						0.091	-0.022	0.017	0.056	0.078	-0.027	-0.037	0.036	0.084	0.180
b4143	groL	-0.151	0.030	0.069		-0.137	-0.017	-0.030	-0.052	0.000	-0.004	-0.019	-0.052	-0.065	-0.018	-0.016
b4145	yjeJ	0.035	0.016	0.094	0.159	0.275										
b4146	yjeK	-0.004	0.007	0.030	0.066	-0.008										
b4147	efp	-0.005	-0.063	-0.014	-0.048	-0.095	2.171	2.171	2.171	2.171	2.171	0.110	-0.125	0.058	0.019	0.019
b4148	sugE	0.019	0.011	-0.006	-0.102	0.006										
b4149	blc		-0.029	0.000	-0.005	-0.022			2.171							
b4150	ampC	-0.030	0.008	-0.065	-0.089	-0.154										
b4151	frdD	0.071	0.017	-0.021	-0.071	0.051										
b4152	frdC	0.041	0.019	-0.040	-0.122	0.122										
b4153	frdB	-0.079	-0.011	-0.002	0.004	-0.031							-0.117			
b4154	frdA	0.076	-0.044	-0.159	0.075	-0.027										
b4155	poxA	-0.104	-0.009	0.145	0.046	0.056	-2.171	-2.171	-2.171	-0.156	-2.171					
b4156	yjeM	0.007	0.028	0.016	-0.047	-0.024										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b4157	yjeN		0.082													
b4158	yjeO	-0.012	-0.054	-0.045	0.100	0.089										
b4159	yjeP							2.171								
b4160	psd	-0.020	0.006	-0.061	-0.105	-0.024	-2.171	-2.171	-2.171	-2.171	-2.171					
b4161	rsgA	-0.002	0.027	0.052	0.023	-0.010	2.171									
b4162	orn	-0.018	-0.019	0.025	0.102	0.247					2.171		-0.058	0.000	0.165	0.080
b4166	yjeS	0.023	0.000	-0.028	0.000	-0.150										
b4168	yjeE	0.126	-0.040	0.002	0.102											
b4170	mutL	-0.026	-0.009	-0.010	0.049	0.116				2.171						
b4171	miaA	-0.101	-0.027	-0.040	-0.042	-0.082										
b4173	hflX	-0.048	-0.043	-0.041	-0.037	-0.116		2.171								
b4174	hflK	-0.050	-0.004	-0.023	-0.063	-0.033										
b4176	yjeT	-0.082	-0.010	0.019	0.254	-0.022										
b4177	purA	-0.045	-0.025	-0.056	-0.057	-0.209	0.009	0.000	-0.065	-0.061	-0.187	-0.004	0.028	0.040	-0.037	-0.092
b4178	nsrR	-0.011	0.003	0.085	-0.072	-0.026										
b4179	rnr	0.002	-0.031	0.133	0.081	0.163	0.087	0.035	0.165	-0.048	-2.171	0.023	-0.002	0.020	0.005	-0.091
b4181	yjfI		-0.139		0.057											
b4182	yjfJ			-0.005	0.067	-0.037	-2.171	-2.171	-2.171	-2.171	-2.171			-0.009		
b4183	yjfK		0.108	0.193												
b4184	yjfL	0.005	0.106	0.088	-0.013											
b4186	yjfC	0.000	0.124	-0.071		0.005										
b4187	aidB	0.027	0.031	0.012	-0.047	0.058	2.171									
b4188	yjfN		-0.076		0.033											
b4189	yjfO	-0.047	-0.008	0.002	-0.066	-0.027										
b4190	yjfP	0.075	0.029	0.044	-0.038	-0.010										
b4191	ulaR	0.041	0.074			0.026			2.171							
b4192	ulaG		0.015													
b4193	ulaA	0.030	-0.035	-0.079	-0.037											
b4195	ulaC		0.082	0.086		-0.101										
b4196	ulaD				0.120						2.171					
b4197	ulaE	0.118	0.031	0.242	0.129	0.274			2.171							
b4198	ulaF										2.171					
b4199	yjfY		0.030	0.032	0.011	0.022										
b4200	rpsF						-0.043	-0.043	-0.100	-0.043	-0.035	-0.021	-0.021	-0.061	-0.007	-0.003
b4202	rpsR	-0.079	-0.022	-0.041			-0.083	-0.026	-0.022	0.035	0.022	-0.079	-0.065	-0.081	0.018	0.027
b4203	rplI		-0.003				-0.035	-0.026	-0.048	-0.026	0.004	-0.021	-0.026	-0.049	-0.039	0.011
b4204	yjfZ								2.171							
b4205	ytfA	0.091	-0.065		0.038											
b4206	ytfB	-0.013	0.054	0.028	0.096											
b4207	fklB	0.090	0.008	0.027	-0.093	-0.109	-0.026	-0.013	-0.030	-0.052	-0.074	-0.054	0.024	-0.004	-0.055	-0.015
b4208	cycA	0.030	0.031	0.054	0.028	0.189										
b4209	ytfE	0.039	0.022	0.088	-0.011	0.007										
b4210	ytfF	-0.067	0.002	-0.035	-0.037	-0.207										
b4211	ytfG	-0.005	-0.035	-0.004	-0.109	-0.205				2.171			-0.037	-0.005		
b4212	ytfH	0.090														
b4213	cpdB	0.019	0.001	-0.069	0.172	-0.013		2.171								
b4214	cysQ		0.095	0.252			2.171				2.171	-0.065	-0.108	0.029	0.141	0.338
b4215	ytfI	-0.131	-0.036	-0.069	-0.045	-0.054										
b4218	ytfL		0.020	0.069												
b4219	msrA	-0.047	-0.003	-0.017		-0.033	2.171			2.171	2.171				0.111	0.332
b4220	ytfM	-0.021	-0.018	-0.005	0.024	0.029			2.171							
b4221	ytfN	-0.024	-0.023	-0.009	-0.036		-2.171	-2.171	-2.171	0.282	-0.790	0.034	0.021	0.079	0.096	0.081
b4222	ytfP				0.109											
b4224	chpS	-0.036	-0.006	0.023	0.038	-0.128										
b4225	chpB	0.043	-0.036	0.065		0.019										
b4226	ppa	-0.001	0.028	0.051	0.011	-0.034	0.022	0.000	0.052	0.104	0.056	0.048	-0.013	0.028	0.071	0.125
b4227	ytfQ	-0.044	0.058	-0.012	-0.099	-0.094			2.171							
b4228			0.034	-0.016												
b4229			-0.077	0.082	0.004											
b4230	ytfT	0.086	0.018	-0.025	0.051	0.057										
b4231	yjfF		0.049	0.003	0.029	-0.001										
b4232	fbp	-0.010	-0.010	-0.025	0.021	0.012	2.171		2.171	2.171		0.017	0.026	0.088	0.057	0.092
b4233	mpl							2.171								
b4234	yjgA											-0.048		-0.175		0.042
b4235	pmbA	0.018	0.018	0.039	0.027	0.021				2.171	2.171					
b4236	cybC								2.171			-0.065		-0.001	-0.046	0.000
b4237	nrdG	-0.103	0.004	-0.038	-0.029	-0.036			2.171							
b4238	nrdD	0.018	-0.031	-0.024	-0.020	0.044										
b4240	treB		0.112	0.083	-0.074											
b4241	treR	-0.074	-0.047	-0.134	-0.079	-0.057										
b4243	yjgF	0.005		-0.109			0.022	-0.022	0.013	0.052	0.030	-0.029	-0.016	0.000	0.036	0.078
b4244	pyrI	-0.080	0.072	0.012	0.012	-0.005	0.009	0.000	-0.009	0.035	-0.009	0.046	0.023	-0.012	0.030	0.014
b4245	pyrB	0.006	-0.003	0.007	0.032	-0.062	0.009	-0.009	-0.009	0.022	-0.030	-0.026	-0.006	-0.014	-0.019	-0.045
b4246	pyrL		0.130													
b4247		0.103	-0.112	0.090	-0.047											
b4248	yjgH	0.014	0.042	0.077	0.006	0.088										
b4249	yjgI	0.090	0.016	0.054	0.066	-0.020										

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b4250		-0.058	-0.020	-0.007	0.005	0.043										
b4251	yjgJ	0.058	-0.019	0.007	0.048	0.005										
b4252	yjgK		0.058	0.069	0.125											
b4253	yjgL	0.060	-0.014	-0.006	0.027	0.066		2.171								
b4254	argI	0.024	0.000	0.016	-0.020	-0.083	-0.026	0.022	0.004	-0.035	-0.026	0.003	-0.008	-0.008	-0.002	0.067
b4255	rraB	0.051	0.003	0.079	-0.014	0.032										
b4257	yjgN		-0.005	0.030	0.077											
b4258	valS					-0.163	-0.004	-0.026	0.017	0.043	0.061	0.021	-0.025	-0.013	0.011	-0.006
b4259	holC	0.008	0.015	0.001	0.074	0.166										
b4260	pepA	-0.026	0.003	0.034	0.021	0.008	-2.171	-2.171	-2.171	-0.117	0.056	0.031	-0.033	0.197	0.134	0.055
b4261	lptF		0.051		-0.022											
b4262	lptG		-0.027	0.078	0.084											
b4263	yjgR	0.011	-0.014	-0.024	-0.075	-0.054	-2.171	-2.171	-2.171	0.096	-2.171					
b4266	idnO		-0.018	0.047			-0.083	-2.171	-2.171	-2.171	-2.171					
b4267	idnD	0.018		-0.030												
b4268	idnK						2.171									
b4269	yjgB	0.002	-0.047	0.047	0.060	0.078					2.171					
b4271	intB	0.074	0.083	0.006	-0.011	-0.016										
b4272	insC-6		0.000	0.042	-0.080	-0.038			2.171		2.171					
b4274	yjgW	-0.002	-0.038	-0.069	-0.048	-0.009										
b4276			-0.067													
b4277	yjgZ	0.024	0.046	-0.032	-0.086	-0.068										
b4278	insG											0.008	-0.117	-0.100	-0.184	-0.106
b4279	yjhB	-0.014	0.078	0.093	-0.022	0.091										
b4280	yjhC	-0.034	-0.169	-0.232												
b4282		0.058	0.000	0.053	0.001											
b4283	insN-2		-0.014	-0.140		0.132										
b4286	yjhV		0.037	0.149	0.060	0.048										
b4288	fecD		0.021	0.129												
b4289	fecC	-0.069	-0.012	0.041	0.019	0.156										
b4290	fecB	-0.030	0.044	-0.007	-0.060	-0.121										
b4292	fecR	0.054	0.030	0.046	0.060	0.123										
b4293	fecI	-0.035	-0.029	-0.019	-0.034	-0.091										
b4294	insA-7	-0.079	-0.071	-0.125	-0.127	-0.278										
b4295	yjhU						-2.171	-2.171	-2.171	-2.171	-2.171	0.024	-0.113	-0.185	-0.493	-0.155
b4297	yjhG	-0.044	-0.064	-0.040	-0.049	-0.049										
b4298	yjhH	-0.017	0.009	0.109	0.106	-0.118										
b4299	yjhI		0.086													
b4300	sgcR		0.017	0.052	-0.100	-0.038										
b4302	sgcA	0.240	-0.010	0.070	-0.226											
b4303	sgcQ	-0.105	-0.023	-0.056				2.171								
b4306	yjhP			-0.017					2.171							
b4308	yjhR	0.088	0.049	0.073	0.045	0.161	-2.171	-2.171	-2.171	-2.171	-2.171					
b4309	yjhS		0.043													
b4310	nanM	0.175	-0.022	-0.056	-0.046	0.044		2.171								
b4313	fimE					-0.135										
b4314	fimA			-0.166												
b4315	fimI	0.312	0.106													
b4317	fimD							2.171	2.171							
b4323	uxuB						2.171									
b4324	uxuR		-0.033		-0.001	0.036										
b4325	yjiC	-0.030	-0.002	0.018	-0.053	-0.009										
b4326	iraD	0.000	0.008	-0.085	0.049	-0.059										
b4327	yjiE						2.171									
b4328	iadA	0.004	0.031	0.008	-0.036	-0.053			2.171		2.171					
b4329	yjiG		-0.034	-0.021	0.027											
b4330	yjiH	0.061	0.009	0.024	-0.001	-0.218										
b4331	kptA		-0.211		0.045											
b4332	yjiJ				0.374											
b4333	yjiK	0.008	0.012	0.085	0.119					2.171						
b4334	yjiL	0.059	-0.041	-0.018	0.029	0.036										
b4336	yjiN	-0.090	-0.007	0.029	0.061	-0.037			2.171							
b4338	yjiP										2.171					
b4339	yjiQ			0.376												
b4341	yjiS		-0.001	-0.026	0.204	0.030										
b4343		-0.087	-0.034	0.009	0.141											
b4344		-0.022	-0.051	-0.064	-0.028	-0.079					2.171					
b4345	mcrC	-0.056	-0.046	-0.067	-0.082	-0.156						0.072	-0.099	-0.027	-0.018	-0.025
b4346	mcrB	-0.007	-0.016	-0.034	-0.009	-0.018										
b4347	symE		-0.014	0.048												
b4348	hsdS	-0.012	0.018	-0.018	-0.065	-0.038										
b4349	hsdM	-0.004	-0.007	-0.054	-0.088	-0.027	0.052	-2.171	-2.171	0.230	0.004	-0.023	-0.061	0.030	0.042	0.021
b4350	hsdR	0.006	-0.017	-0.053	-0.078	-0.048		2.171								
b4351	mrr	-0.012	-0.009	-0.045	0.000	-0.027										
b4352	yjiA	0.065	0.031	0.213	0.268	0.114										
b4354	yjiY	0.046	-0.067	-0.007	-0.005	-0.034										
b4355	tsr	-0.069	0.027	-0.073	0.013			2.171								

		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE)): T#1					Proteins (log_10(Mut/PE))-T#2				
B #	Name	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
b4356	yjjL	0.023	0.046	0.061	-0.021	-0.057										
b4357	yjjM						2.171									
b4358	yjjN	-0.008	0.014	-0.012	-0.143	-0.014										
b4360	yjjA		0.040	0.071	0.094	0.083										
b4361	dnaC	-0.063	-0.008	-0.007	0.046	0.123										
b4362	dnaT	-0.008	0.069	0.132	0.332	0.590									0.270	0.116
b4363	yjjB	-0.038	-0.001	-0.038	0.082	0.054										
b4364	yjjP	0.027	-0.077		0.071	0.086										
b4365	yjjQ		-0.007								2.171					
b4366	bglJ	0.104			-0.001											
b4371	rsmC	-0.009	0.012	0.031	0.014	0.035				2.171						
b4372	holD		-0.032	-0.058												
b4373	rimI	-0.018	0.004	-0.047	0.033	-0.035				2.171						
b4374	yjjG	0.099	0.122	0.223	0.571	0.913										
b4375	prfC		0.028		0.031	0.024	0.065	0.065	-0.152	-0.061	0.161	-0.009	-0.085	0.056	0.108	0.119
b4376	osmY			0.265			0.035	0.056	0.252	0.578	0.595	0.033	0.006	0.274	0.638	0.655
b4377	yjjU	0.026	0.011	-0.012	-0.009	0.032										
b4379	yjjW	-0.058	0.006	-0.033	-0.059	-0.035										
b4380	yjjI	0.167	-0.041	0.017	0.147	0.306				2.171						
b4381	deoC	0.024	0.043	0.018	0.083	0.153	-2.171	-2.171	-2.171	0.148	-2.171	-0.004	0.067	-0.028	0.086	0.080
b4382	deoA	0.035	0.056	0.036	-0.031	-0.041						-0.206	0.050	0.029	0.028	-0.078
b4383	deoB	0.048	0.004	-0.022	-0.012	-0.008	-2.171	-2.171	0.026	0.890	0.517	-0.039	0.038	0.064	0.208	0.161
b4384	deoD	-0.021	0.017	0.047	0.051	0.057	0.030	0.117	0.104	0.161	0.174	0.000	-0.036	0.027	0.157	0.111
b4386	lplA	-0.011	0.057	0.022						2.171		-0.158	-0.314		-0.049	-0.042
b4387	ytjB				0.141											
b4388	serB	0.020	-0.010	0.012	0.057	0.088	0.052	-0.287	0.017	-2.171	-0.195					
b4389	radA	-0.057	-0.020	-0.043	-0.040	-0.038										
b4390	nadR	-0.088	-0.007	-0.047	-0.093	-0.074	2.171									
b4391	yjjK			-0.141			-0.004	-0.056	0.009	0.109	0.122	-0.025	-0.034	-0.024	0.061	0.084
b4392	slt	0.028	-0.036	-0.078			2.171		2.171	2.171	2.171	0.037	0.180	-0.040	0.019	0.107
b4393	trpR	-0.025	0.004	-0.034												
b4395	ytjC	-0.073	0.087	0.002	0.126	0.228										
b4396	rob	0.012	-0.029	-0.015	0.022	0.057	-2.171	-2.171	-2.171	-2.171	-2.171					
b4397	creA	-0.079	0.010	0.094	0.053	0.103										
b4398	creB		0.013		-0.091	-0.009										
b4399	creC	-0.018	0.014	0.050	0.060	0.087										
b4400	creD	0.025	-0.049	0.059	-0.112											
b4401	arcA						0.004	-0.065	0.104	0.230	0.213	-0.054	-0.135	0.021	0.166	0.264
b4403	yjtD	-0.017	-0.010	-0.046		0.001										
b4405			0.123		0.237											

9.2. Absolute Proteomic Data

In this section, all absolute proteomic measurements corresponding to threshold two as described in section 4.4.5 are given in fmoles. In addition to the PE and 5 mutant strains examined in this thesis, quantification for the strain sampled at both OD_{600} = 0.2 and OD_{600} = 0.8 (instead of the usual OD_{600} = 0.4) in exponential phase are given. Genes are organized by Blattner number (Blattner, Plunkett et al. 1997), and gene annotations can be found using the EcoCyc database (Keseler, Bonavides-Martinez et al. 2009).

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b0002	ThrA	26.4	33.1	36.0	29.4	25.7	30.5	28.6	29.4
b0003	ThrB	4.5	33.1	3.1	2.7	25.7	11.4	3.1	29.4
b0004	ThrC	38.3	46.6	45.9	40.8	36.5	42.8	45.7	44.8
b0008	TalB	40.5	59.1	53.5	44.0	43.9	40.2	46.3	43.1
b0013	YaaI	0.7	59.1	53.5	0.8	43.9	2.1	46.3	43.1
b0014	DnaK	46.4	52.5	52.1	51.3	53.0	46.9	63.2	56.2
b0023	RpsT	16.5	17.3	22.4	14.5	14.4	11.3	16.5	12.7
b0025	RibF	2.3	17.3	22.4	14.5	5.2	4.9	6.9	12.7
b0026	IleS	14.1	18.6	15.6	14.6	16.8	13.8	15.0	11.3
b0029	IspH	1.8	18.6	15.6	14.6	2.3	3.4	2.3	11.3
b0031	DapB	6.9	7.8	5.4	8.6	10.5	7.7	10.1	11.1
b0032	CarA	21.5	26.8	26.2	22.3	29.1	20.5	22.9	19.6
b0033	CarB	33.3	35.4	32.2	27.5	30.7	30.2	31.3	31.4
b0053	SurA	6.4	8.4	9.4	7.1	7.1	6.2	8.5	9.0
b0071	LeuD	18.5	17.6	16.9	15.8	18.6	16.5	18.2	23.5
b0072	LeuC	14.6	18.4	16.8	14.4	14.1	13.8	14.4	19.2
b0073	LeuB	14.5	19.8	18.0	12.1	16.1	11.5	8.9	17.4
b0074	LeuA	7.9	11.0	9.9	7.9	9.0	8.8	9.3	11.7
b0077	IlvI	2.4	8.2	9.9	7.9	9.0	8.8	9.3	2.7
b0078	IlvH	3.2	2.9	3.2	2.6	3.1	2.9	4.2	1.4
b0080	FruR	1.5	2.9	3.2	2.6	3.1	2.9	1.6	1.4
b0085	MurE	1.9	6.1	3.2	2.6	3.1	2.9	6.5	1.4
b0095	FtsZ	10.2	12.5	13.1	10.4	10.4	9.4	25.2	17.0
b0098	SecA	10.1	7.0	10.2	7.5	7.2	10.0	6.6	3.9
b0104	GuaC	4.5	6.9	7.4	5.6	4.1	3.4	6.2	5.4
b0114	AceE	35.0	38.3	36.9	33.2	37.3	34.6	39.8	36.6
b0115	AceF	24.0	21.1	21.6	20.3	23.1	25.3	24.2	22.9
b0116	Lpd	56.5	52.5	56.7	50.4	55.6	58.7	53.7	56.3
b0118	AcnB	42.8	46.3	47.9	34.5	33.9	34.7	29.8	22.6
b0120	SpeD	4.5	46.3	3.9	3.2	5.7	34.7	3.0	22.6
b0123	CueO	2.7	46.3	3.8	3.2	5.2	4.1	6.1	5.7
b0126	Can	4.3	10.2	7.1	6.2	8.1	5.0	7.6	8.7
b0133	PanC	7.1	6.7	7.7	8.0	7.8	6.6	8.4	7.2
b0134	PanB	4.2	4.7	6.4	7.0	3.7	3.2	1.6	1.3
b0145	DksA	4.5	3.9	5.5	4.2	4.3	3.1	4.8	5.0
b0149	MrcB	4.5	3.9	5.5	4.2	4.3	3.1	5.4	5.0
b0154	HemL	7.6	7.3	7.2	7.5	6.1	8.6	8.0	9.8
b0156	ErpA	5.7	4.2	7.6	5.1	6.1	3.0	4.4	4.0
b0161	DegP	4.4	5.0	7.2	4.9	4.2	5.4	4.6	6.2
b0166	DapD	32.6	40.4	38.3	36.5	37.7	33.7	36.2	26.6
b0167	GlnD	6.6	9.4	38.3	6.0	5.5	33.7	36.2	4.0
b0169	RpsB	59.2	77.6	71.9	64.8	72.8	59.8	66.4	64.1
b0170	Tsf	51.0	56.7	63.1	52.5	55.6	52.8	62.4	63.6
b0171	PyrH	7.0	6.6	6.9	4.3	5.4	6.5	4.5	8.8
b0172	Frr	15.5	22.3	25.0	21.9	19.5	20.8	26.2	21.6
b0178	HlpA	20.7	17.0	16.2	16.4	16.3	22.5	14.9	22.0
b0181	LpxA	3.4	2.0	3.2	1.5	2.0	2.0	2.8	2.6
b0182	LpxB	1.4	2.0	0.8	1.5	2.0	1.5	2.8	2.6
b0185	AccA	8.6	6.6	8.5	8.1	8.2	7.7	8.8	8.8
b0194	ProS	11.8	12.4	12.2	11.7	11.7	11.6	10.7	11.6
b0222	LpcA	4.4	4.6	5.5	4.0	4.0	3.1	3.8	3.8
b0237	PepD	5.6	7.0	5.1	7.8	5.2	6.8	6.4	10.6
b0239	FrsA	5.4	7.0	5.1	7.8	5.1	5.2	4.3	10.6
b0240	Crl	3.4	4.5	2.8	4.2	3.9	2.6	4.9	4.3
b0243	ProA	2.7	6.3	4.5	4.5	2.3	4.0	3.3	3.9
b0325	YahK	2.8	5.6	4.5	8.2	6.3	3.1	15.9	12.5
b0329	YahO	1.0	2.0	2.9	3.6	5.5	5.2	12.0	9.1
b0330	PrpR	2.4	2.0	2.9	3.1	5.5	5.2	12.0	9.1
b0337	CodA	6.9	5.5	8.1	4.2	5.3	6.6	6.7	8.2
b0346	MhpR	3.4	5.5	8.1	4.2	5.6	6.4	3.5	8.2
b0348	MhpB	4.4	5.5	3.2	1.5	4.0	6.4	3.5	1.1
b0415	RibE	6.8	8.4	6.9	5.6	5.4	6.0	5.4	7.2

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b0416	NusB	6.0	1.3	3.3	5.6	5.4	6.0	5.9	7.2
b0417	ThiL	3.8	7.2	4.6	4.8	5.4	5.9	1.4	7.2
b0420	Dxs	54.0	60.5	57.9	54.9	52.6	68.1	70.0	29.7
b0422	XseB	1.1	60.5	1.1	1.6	2.2	2.8	2.7	3.7
b0426	YajQ	13.2	15.1	14.6	14.9	13.7	15.1	19.9	18.9
b0432	CyoA	1.2	15.1	14.6	1.8	13.7	4.7	19.9	18.9
b0436	Tig	60.5	65.2	71.4	58.9	57.7	55.4	56.6	58.8
b0438	ClpX	6.3	7.3	8.0	7.5	9.2	8.3	9.6	12.3
b0439	Lon	9.3	7.7	8.6	9.2	11.0	6.8	8.0	6.7
b0440	HupB	27.8	20.0	23.5	23.5	22.2	22.6	25.0	20.9
b0445	YbaE	27.8	0.4	23.5	1.5	2.7	1.6	25.0	20.9
b0453	YbaY	1.0	4.3	23.5	2.7	2.3	5.3	9.2	13.2
b0473	HtpG	17.9	15.5	16.3	13.6	17.8	18.6	16.1	14.1
b0474	Adk	18.5	19.1	21.5	19.5	17.5	18.6	21.2	20.8
b0492	YbbN	3.5	2.1	4.3	1.2	3.1	2.4	5.8	20.8
b0497	RhsD	7.7	5.8	7.6	6.5	7.4	7.9	6.9	20.8
b0523	PurE	3.1	2.8	3.8	3.6	5.6	5.4	4.3	4.2
b0525	PpiB	5.7	7.5	3.8	3.6	6.2	7.9	6.5	6.6
b0526	CysS	8.8	7.3	4.9	6.2	6.2	8.8	5.2	5.5
b0560	NohB	0.4	7.3	3.0	6.2	4.2	1.6	5.2	5.5
b0573	CusF	1.5	0.4	3.0	6.2	4.2	1.6	5.2	5.5
b0578	NfsB	6.7	8.2	5.4	7.3	3.6	8.5	8.4	8.0
b0586	EntF	4.6	8.2	5.4	7.3	3.6	4.7	8.4	9.1
b0590	FepD	3.6	5.6	5.4	7.3	3.4	5.6	8.4	9.1
b0604	DsbG	3.6	1.0	5.4	1.8	0.8	3.8	1.1	2.1
b0605	AhpC	108.9	133.0	132.4	108.2	133.1	103.6	109.2	115.0
b0606	AhpF	6.5	8.0	9.1	8.2	8.1	7.7	10.7	9.4
b0607	UspG	1.0	8.0	9.1	8.2	1.8	0.5	10.7	9.4
b0616	CitE	5.3	8.0	9.1	4.9	4.4	3.5	10.7	9.4
b0623	CspE	45.2	41.3	46.8	46.8	48.2	37.9	46.5	55.9
b0631	YbeD	3.3	3.9	2.4	3.5	3.0	3.5	3.7	5.0
b0632	DacA	14.8	12.1	2.4	3.5	12.1	9.3	3.7	5.0
b0641	LptE	1.4	12.1	2.4	3.5	12.1	9.3	3.7	5.0
b0642	LeuS	13.6	14.0	17.5	13.6	14.4	12.9	14.5	13.1
b0643	YbeL	1.6	14.0	17.5	1.1	14.4	1.4	5.9	13.1
b0648	YbeU	1.6	14.0	17.5	1.2	2.3	1.4	5.9	1.9
b0655	GltI	5.2	8.3	9.0	5.7	5.9	6.4	5.8	7.6
b0660	YbeZ	4.3	4.0	9.0	5.2	4.6	4.0	5.6	6.0
b0661	MiaB	5.4	5.0	7.3	5.2	4.5	6.2	4.4	8.0
b0674	AsnB	5.0	6.2	6.2	4.3	6.5	4.6	4.7	12.6
b0675	NagD	1.9	2.0	6.2	4.3	6.5	1.7	4.7	4.7
b0680	GlnS	7.0	5.6	8.3	6.2	9.1	8.3	8.7	6.8
b0681	YbfM	3.7	5.6	8.3	2.8	9.1	3.3	8.7	6.8
b0683	Fur	12.0	9.0	7.1	16.3	9.1	3.3	11.0	6.8
b0684	FldA	5.1	5.0	4.7	4.3	4.8	3.9	6.5	7.2
b0687	SeqA	5.1	2.8	1.7	4.3	4.8	3.9	2.5	2.9
b0688	Pgm	4.8	5.9	4.6	6.7	4.4	6.3	7.0	6.7
b0710	YbgI	1.3	5.9	2.7	6.7	4.4	5.8	7.0	6.7
b0720	GltA	47.8	58.1	55.3	46.9	51.9	57.3	40.1	34.7
b0723	SdhA	10.8	6.4	8.9	12.2	5.3	9.9	10.3	3.5
b0724	SdhB	3.5	3.6	3.7	2.2	2.3	5.7	2.0	3.1
b0726	SucA	14.1	14.2	12.1	11.5	11.7	13.8	15.4	10.6
b0727	SucB	33.7	34.7	28.2	27.2	26.7	32.7	20.8	15.3
b0728	SucC	33.3	37.9	46.9	26.6	30.2	26.7	26.6	22.2
b0729	SucD	31.0	33.6	34.4	30.8	25.9	24.3	23.9	22.8
b0740	TolB	6.0	6.3	6.7	7.1	6.3	5.3	6.7	7.2
b0754	AroG	40.4	48.2	47.6	37.2	43.8	40.3	36.7	37.7
b0755	GpmA	52.1	62.0	51.1	51.3	62.4	57.8	66.3	53.0
b0767	Pgl	5.7	5.3	5.1	6.9	5.2	6.1	10.8	11.0
b0776	BioF	6.3	5.3	5.1	3.0	5.2	6.1	10.8	11.0
b0781	MoaA	4.4	2.5	5.1	3.0	3.5	4.4	6.8	5.2
b0782	MoaB	6.8	7.7	5.4	6.6	4.6	3.4	5.9	9.0

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b0801	YbiC	2.9	6.6	4.4	7.3	4.1	5.0	5.6	9.0
b0811	GlnH	16.6	16.0	16.0	17.8	18.5	15.8	19.7	22.7
b0812	Dps	14.4	14.6	19.0	15.5	13.4	18.9	23.4	22.3
b0817	MntR	1.7	14.6	1.3	15.5	2.1	18.9	0.3	22.3
b0819	YbiS	6.1	5.5	4.8	4.9	5.6	4.4	5.0	6.2
b0820	YbiT	6.1	6.8	8.1	5.4	3.8	17.0	14.8	16.5
b0827	MoeA	3.4	5.2	4.0	1.9	3.4	17.0	7.9	3.9
b0838	YliJ	4.2	2.8	3.7	1.9	3.5	3.5	3.0	3.6
b0843	YbjH	0.3	2.8	0.5	1.9	3.5	0.3	3.0	3.6
b0854	PotF	4.7	6.7	7.8	8.9	9.4	3.2	9.0	7.7
b0860	ArtJ	23.7	19.6	24.4	23.9	20.9	18.2	22.0	24.1
b0863	ArtI	9.1	7.6	9.7	7.1	8.5	8.4	10.2	14.1
b0870	LtaE	5.2	7.6	2.5	3.1	3.0	8.4	10.2	3.3
b0872	Hcr	1.4	7.6	2.5	3.1	3.0	1.8	10.2	3.9
b0879	MacB	5.0	7.6	12.3	3.1	3.0	1.8	10.2	3.9
b0882	ClpA	4.5	4.7	7.4	12.4	4.8	1.8	10.2	6.2
b0884	InfA	12.4	10.4	12.0	12.5	10.2	10.1	14.1	16.1
b0885	Aat	5.2	3.1	12.0	12.5	5.9	3.2	14.1	2.1
b0888	TrxB	7.0	8.6	7.8	6.3	7.2	4.8	6.6	4.6
b0889	Lrp	28.8	30.9	35.4	28.7	29.1	28.7	25.5	26.0
b0893	SerS	9.9	13.3	13.7	11.8	11.5	12.4	10.4	11.3
b0894	DmsA	3.5	13.3	13.7	5.6	5.5	12.4	5.0	11.3
b0903	PflB	18.7	22.5	22.3	23.7	23.5	22.1	34.1	26.1
b0907	SerC	37.6	47.4	49.6	42.7	42.7	35.1	40.5	45.1
b0908	AroA	5.7	3.9	7.5	42.7	5.0	4.3	40.5	5.3
b0910	Cmk	1.5	1.6	7.1	42.7	4.4	1.8	3.5	2.9
b0911	RpsA	69.9	66.4	73.3	61.1	72.9	72.0	65.2	76.0
b0912	IhfB	2.9	2.6	1.5	1.9	2.2	3.9	4.4	5.8
b0917	YcaR	2.7	2.4	2.6	4.1	4.1	2.8	2.7	4.2
b0918	KdsB	2.8	7.0	1.8	2.8	5.0	3.0	3.4	6.5
b0922	MukF	6.5	8.1	9.6	2.8	10.0	5.9	6.8	1.9
b0923	MukE	6.5	8.1	2.2	2.8	3.6	5.9	6.8	1.9
b0924	MukB	13.8	14.5	17.6	7.1	9.7	9.6	14.8	19.1
b0928	AspC	46.7	44.6	42.4	35.0	38.5	34.4	39.7	39.3
b0930	AsnS	25.7	27.9	25.4	23.2	24.4	23.2	27.4	25.4
b0931	PncB	2.4	4.7	4.8	3.5	3.2	4.0	3.1	4.7
b0932	PepN	7.4	6.9	6.5	6.9	7.8	7.1	9.6	7.1
b0945	PyrD	3.3	1.8	6.5	5.0	2.9	2.8	9.6	7.1
b0946	YcbW	0.5	1.8	6.5	5.0	2.9	2.8	2.7	7.1
b0954	FabA	13.9	15.9	16.0	14.5	12.6	11.5	14.7	11.5
b0957	OmpA	17.0	14.7	17.1	12.2	14.2	35.7	16.8	17.0
b1004	WrbA	2.7	6.6	5.7	14.6	7.5	13.5	41.7	41.9
b1014	PutA	6.4	4.4	5.7	14.6	6.1	13.5	41.7	41.9
b1018	EfeO	7.0	4.0	7.3	4.6	2.7	4.0	1.9	41.9
b1035	YcdY	7.0	4.9	7.3	4.3	6.1	3.8	7.6	5.0
b1048	MdoG	7.0	4.7	7.3	6.4	4.9	5.3	7.3	5.2
b1051	MsyB	2.6	4.7	5.6	4.9	4.9	4.4	10.1	12.1
b1059	SolA	3.1	4.2	2.1	2.8	3.0	2.3	2.7	4.0
b1062	PyrC	12.4	14.0	15.9	13.2	14.2	13.8	14.2	13.8
b1064	GrxB	4.4	3.3	5.7	5.0	4.0	5.8	6.3	9.6
b1084	Rne	7.2	5.0	4.7	6.2	6.7	5.8	4.1	7.6
b1091	FabH	4.2	6.7	6.9	5.8	5.1	3.7	5.9	6.2
b1092	FabD	14.9	19.9	19.2	15.8	15.3	17.0	17.5	15.8
b1093	FabG	15.9	14.3	15.6	13.4	14.2	13.7	15.5	14.5
b1094	AcpP	27.9	36.8	41.0	37.0	27.9	29.1	33.3	36.9
b1095	FabF	9.6	9.2	7.8	7.7	11.3	8.8	7.5	12.7
b1103	HinT	5.7	7.3	6.3	7.5	4.3	5.8	12.2	7.2
b1107	NagZ	1.5	3.0	6.3	7.5	4.3	1.2	12.2	7.2
b1108	YcfP	3.7	4.5	1.2	4.7	2.3	1.2	4.2	3.8
b1129	PhoQ	13.0	12.9	8.4	5.1	11.8	12.2	9.7	13.0
b1130	PhoP	6.3	7.6	7.3	6.7	6.3	8.0	7.9	7.9
b1131	PurB	12.6	15.7	14.7	12.5	13.2	13.6	13.4	15.9

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b1135	RluE	3.4	15.7	1.3	0.6	13.2	13.6	1.3	15.9
b1136	Icd	157.0	150.4	171.2	130.8	114.9	160.6	110.2	80.2
b1174	MinE	3.5	150.4	1.1	130.8	1.3	1.5	110.2	3.7
b1175	MinD	8.5	12.4	11.9	11.1	9.3	9.8	10.1	11.9
b1189	DadA	4.0	12.4	11.9	1.9	9.3	9.8	2.5	11.9
b1190	DadX	2.3	0.5	11.9	3.0	9.3	1.3	2.5	1.4
b1197	TreA	2.3	0.5	11.9	3.0	3.5	1.3	4.5	3.6
b1200	DhaK	0.8	0.5	2.1	1.4	3.5	1.3	4.5	2.9
b1203	YchF	7.2	8.3	8.7	7.0	7.8	6.3	6.3	7.5
b1207	Prs	16.8	19.0	17.3	18.2	14.6	13.7	17.4	17.5
b1215	KdsA	11.5	20.7	9.0	8.5	8.7	11.8	11.7	14.9
b1232	PurU	6.9	5.7	7.0	6.1	6.0	9.3	9.2	7.2
b1236	GalU	9.3	13.7	14.6	12.9	10.3	11.5	12.7	24.8
b1237	Hns	57.6	55.9	59.4	54.6	55.0	52.1	56.3	56.2
b1241	AdhE	18.9	21.3	17.9	19.2	23.6	20.4	30.2	20.5
b1243	OppA	38.4	34.2	36.6	34.2	31.6	30.8	30.5	30.4
b1260	TrpA	15.2	16.0	16.4	16.5	13.6	13.8	17.5	13.4
b1261	TrpB	10.5	13.0	7.2	9.2	11.2	7.1	8.8	8.7
b1262	TrpC	4.6	4.9	8.4	8.0	4.3	3.8	3.5	3.7
b1264	TrpE	6.9	7.2	7.3	5.7	6.6	6.8	5.4	5.2
b1274	TopA	5.9	7.2	4.8	5.3	8.0	8.6	5.4	5.8
b1276	AcnA	6.8	8.3	8.4	7.6	7.8	9.8	13.2	14.0
b1281	PyrF	1.3	2.4	2.2	4.2	3.0	2.6	2.3	2.0
b1282	YciH	1.6	2.4	2.2	4.2	3.0	2.6	2.3	2.0
b1286	Rnb	7.0	7.0	6.8	5.9	7.2	7.0	7.6	6.9
b1288	FabI	24.8	25.0	26.9	23.8	21.8	18.7	24.1	22.1
b1304	PspA	23.2	23.8	23.5	20.2	25.3	26.0	28.3	35.7
b1305	PspB	1.3	2.7	2.1	1.8	2.2	1.7	3.2	5.9
b1324	Tpx	67.1	84.2	79.5	65.9	61.0	61.0	65.7	58.9
b1376	UspF	1.2	1.4	1.6	1.4	61.0	0.2	1.3	2.1
b1407	YdbD	1.2	1.4	5.2	4.3	61.0	4.1	1.3	3.7
b1412	AzoR	2.6	1.7	3.6	5.5	2.4	3.2	4.1	4.0
b1413	HrpA	5.5	1.7	6.6	6.4	11.0	21.6	5.0	9.3
b1415	AldA	10.4	10.8	9.8	12.4	12.0	8.3	5.6	5.7
b1424	MdoD	0.8	4.1	2.1	12.4	1.6	8.3	4.4	5.7
b1430	TehB	0.6	4.1	3.9	12.4	1.6	8.3	3.2	1.9
b1444	YdcW	3.1	4.1	3.9	12.4	1.6	2.3	3.2	1.9
b1449	YncB	4.4	4.1	3.9	1.9	2.8	2.3	4.6	7.2
b1451	YncD	1.8	4.1	3.9	1.9	2.8	1.3	1.3	7.2
b1452	YncE	12.3	12.8	10.9	10.0	11.9	11.9	8.2	9.2
b1468	NarZ	3.7	12.8	10.9	10.0	11.9	11.9	8.2	9.2
b1479	MaeA	2.5	3.1	3.1	2.7	3.1	2.4	4.4	3.8
b1480	Sra	6.5	3.7	5.0	6.4	4.9	7.4	8.6	12.1
b1482	OsmC	12.2	9.0	11.7	14.5	13.5	12.3	23.1	34.7
b1493	GadB	8.1	7.4	9.3	11.4	20.9	13.6	41.4	83.4
b1499	YdeO	5.8	1.7	5.4	2.6	20.9	1.9	1.9	83.4
b1507	HipA	4.0	1.7	5.4	4.1	6.3	1.9	1.9	4.1
b1517	LsrF	5.3	1.7	1.6	4.1	6.3	1.9	3.2	4.4
b1538	Dcp	8.0	4.9	7.0	8.1	6.3	7.0	9.7	7.6
b1539	YdfG	6.3	7.2	5.8	7.3	6.1	6.3	6.8	7.7
b1542	YdfI	6.3	5.5	5.8	7.3	6.1	1.8	6.8	1.7
b1548	NohA	0.4	5.5	5.8	7.3	9.5	1.8	7.7	1.7
b1550	GnsB	0.4	3.3	3.1	7.3	2.1	1.8	4.7	1.7
b1572	YdfB	0.4	3.3	3.1	7.3	2.1	2.6	4.7	3.1
b1602	PntB	3.4	3.3	9.5	7.3	5.8	10.2	4.7	3.1
b1603	PntA	8.0	9.2	13.2	5.8	6.3	14.4	5.2	3.6
b1604	YdgH	7.9	7.2	7.6	6.3	6.8	6.2	6.4	6.9
b1610	Tus	15.2	6.1	11.2	9.9	1.1	2.7	11.6	6.9
b1613	ManA	4.2	4.7	11.2	5.6	4.1	1.9	2.2	6.9
b1635	Gst	1.8	3.1	1.7	1.9	2.9	1.9	2.0	6.9
b1637	TyrS	5.5	6.8	7.0	5.6	2.2	5.0	3.4	6.9
b1638	PdxH	1.0	6.8	7.0	3.5	1.6	5.0	1.9	6.9

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b1642	SlyA	1.0	6.8	7.0	1.7	2.0	5.0	1.9	6.9
b1651	GloA	0.7	1.7	2.2	1.5	3.9	1.3	1.9	1.9
b1653	Lhr	8.9	1.7	4.9	1.5	6.3	13.1	4.9	14.3
b1654	GrxD	21.7	21.9	25.5	25.3	20.2	17.3	24.7	24.5
b1656	SodB	19.0	20.9	25.6	20.2	17.1	22.4	16.4	11.3
b1658	PurR	3.6	4.9	3.8	3.0	6.5	4.7	6.9	6.0
b1662	RibC	3.7	8.7	3.4	3.4	6.4	3.3	6.6	2.4
b1676	PykF	34.9	38.8	39.8	34.7	37.8	33.9	40.0	38.0
b1677	Lpp	11.1	14.4	15.6	11.4	13.0	20.3	11.4	10.8
b1686	YdiI	1.2	1.6	15.6	11.4	13.0	0.5	11.4	10.8
b1687	YdiJ	20.5	12.6	12.0	7.1	4.6	4.1	18.9	7.6
b1702	Pps	8.6	13.2	9.2	10.6	6.5	9.2	15.0	10.0
b1710	BtuE	8.6	13.2	13.6	5.5	4.6	4.3	8.9	12.4
b1712	IhfA	11.1	10.9	12.4	13.0	10.8	12.1	14.2	16.4
b1713	PheT	11.0	11.4	12.0	9.2	9.6	9.3	11.5	9.3
b1714	PheS	6.6	6.9	7.2	7.5	5.8	6.4	8.4	7.0
b1716	RplT	44.5	44.1	42.8	40.0	44.7	40.7	48.3	43.6
b1717	RpmI	4.3	3.9	4.2	5.6	3.8	3.2	3.8	2.3
b1718	InfC	20.0	19.5	19.2	19.8	19.6	19.9	21.5	22.1
b1719	ThrS	11.0	11.6	12.2	8.4	9.3	8.0	8.5	7.4
b1727	YniC	2.7	11.6	4.8	11.1	9.3	12.1	3.5	7.4
b1735	ChbR	2.7	4.6	1.7	0.7	9.3	1.5	3.5	3.3
b1739	OsmE	2.7	1.7	1.7	0.7	2.5	4.2	6.9	6.1
b1740	NadE	5.3	7.8	9.0	7.4	8.3	6.5	9.4	10.8
b1761	GdhA	26.2	1.5	9.0	7.4	8.3	6.5	19.7	16.0
b1762	YnjI	7.1	1.5	9.0	7.4	0.9	6.5	0.8	16.0
b1763	TopB	6.0	5.9	4.3	7.4	4.4	4.4	3.1	2.6
b1764	SelD	5.1	4.6	4.8	5.3	6.7	3.7	4.6	8.5
b1765	YdjA	4.1	3.8	5.4	5.8	4.4	3.5	4.9	5.0
b1771	YdjG	7.0	7.8	5.6	1.2	1.5	2.1	4.9	5.0
b1778	MsrB	1.2	7.8	2.3	1.7	2.6	2.6	2.9	2.5
b1779	GapA	206.9	254.2	211.7	187.2	181.1	175.4	220.2	195.6
b1780	YeaD	10.1	9.5	10.8	9.9	11.3	10.2	12.1	9.7
b1783	YeaG	3.2	4.5	10.8	9.9	6.0	10.2	4.7	6.1
b1794	YeaP	3.2	1.8	4.0	5.5	1.3	4.8	2.1	2.5
b1808	YoaA	3.2	6.9	6.0	5.5	5.4	4.8	2.1	2.5
b1817	ManX	5.0	4.4	4.3	2.7	2.6	4.9	2.1	2.3
b1823	CspC	125.5	134.7	133.3	125.2	133.1	96.1	99.4	142.0
b1827	KdgR	2.5	5.8	3.2	125.2	7.3	4.3	1.1	3.5
b1832	YebR	2.5	5.8	5.7	3.6	1.6	4.3	4.2	7.5
b1847	YebF	4.1	5.3	4.7	8.2	7.5	8.4	20.2	25.7
b1849	PurT	8.6	10.3	9.2	7.5	8.1	12.9	10.1	9.6
b1850	Eda	13.7	15.9	14.2	14.9	15.6	13.6	16.6	14.2
b1852	Zwf	9.2	9.3	10.1	7.7	9.1	10.2	10.5	11.2
b1854	PykA	10.1	10.1	7.8	7.8	9.3	11.6	10.7	10.2
b1861	RuvA	0.6	1.8	7.8	7.8	9.3	11.6	10.7	10.2
b1864	YebC	6.4	7.3	6.3	6.3	5.1	4.0	6.7	6.0
b1866	AspS	9.3	11.8	10.8	16.2	10.1	9.1	8.6	9.3
b1876	ArgS	11.3	8.4	8.7	7.3	5.5	4.8	6.8	6.5
b1886	Tar	2.1	8.4	8.7	7.3	5.5	4.8	6.8	6.5
b1888	CheA	16.7	10.2	13.4	15.5	13.6	18.8	6.8	6.7
b1896	OtsA	9.4	10.2	1.8	15.5	8.6	6.9	10.0	10.8
b1916	SdiA	3.3	10.2	1.8	15.5	1.1	6.9	0.8	1.9
b1920	FliY	13.9	15.1	18.9	15.9	16.8	12.7	18.0	19.8
b1928	YedD	3.6	3.2	2.9	15.9	16.8	2.9	18.0	3.2
b1936	IntG	0.8	3.2	2.9	15.9	16.8	2.9	18.0	3.2
b1983	YeeN	2.4	2.5	2.9	6.5	1.9	3.3	18.0	1.6
b1992	CobS	5.6	2.5	2.9	6.5	1.9	3.3	18.0	1.6
b1998	YoeE	1.7	2.5	2.9	6.5	1.3	3.3	18.0	1.6
b2000	Flu	1.7	2.5	2.9	6.5	1.3	3.9	12.2	4.1
b2019	HisG	18.1	18.4	18.1	14.0	17.5	15.9	15.9	13.5
b2020	HisD	7.3	9.6	11.8	6.6	5.8	3.2	7.4	5.2

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b2021	HisC	10.4	14.6	13.1	11.9	15.1	10.6	10.9	13.4
b2022	HisB	11.5	11.5	12.4	11.9	12.7	9.5	9.7	11.1
b2023	HisH	6.0	3.2	2.8	2.4	5.2	9.5	3.0	11.1
b2024	HisA	3.0	3.6	4.6	4.3	2.8	2.6	2.7	2.6
b2025	HisF	4.8	10.0	13.4	10.6	10.3	9.3	8.6	5.3
b2026	HisI	2.9	4.9	1.6	2.8	2.5	2.4	2.4	4.1
b2029	Gnd	55.0	59.8	63.7	54.1	56.0	50.4	59.4	52.6
b2036	Glf	3.0	3.6	1.1	1.4	2.7	2.0	4.6	2.4
b2039	RfbA	7.7	5.9	3.8	5.3	5.9	6.6	10.0	9.1
b2040	RfbD	2.4	3.8	2.2	2.2	2.7	2.0	2.2	9.1
b2041	RfbB	8.2	8.8	6.6	6.0	8.9	6.1	5.8	8.8
b2042	GalF	12.4	8.8	11.7	15.8	15.3	13.6	14.3	22.9
b2060	Wzc	3.8	8.8	11.7	15.8	5.0	8.2	14.3	22.9
b2066	Udk	6.6	4.5	5.0	15.8	5.0	8.2	14.3	22.9
b2073	YegL	3.3	6.3	2.6	4.3	5.0	8.2	4.5	22.9
b2090	G_2	2.8	2.9	2.7	0.8	5.0	8.2	4.5	5.7
b2093	GatB	23.3	22.3	20.6	21.6	19.5	16.2	10.2	8.1
b2094	GatA	15.5	13.1	16.5	12.0	15.4	11.8	8.5	6.0
b2095	GatZ	7.3	4.4	9.2	5.8	5.2	6.0	1.6	6.0
b2096	GatY	16.7	15.7	15.9	15.2	12.1	8.0	4.9	3.8
b2103	ThiD	5.0	3.7	4.0	7.0	3.7	3.4	5.1	4.2
b2110	YehC	2.6	3.7	4.0	7.0	3.7	3.4	5.1	4.2
b2114	MetG	14.1	11.3	12.0	9.0	9.7	8.5	9.5	10.5
b2122	YehQ	6.6	11.3	2.9	9.0	7.9	8.0	9.5	10.5
b2146	YeiT	4.6	1.9	4.6	4.1	3.2	8.0	9.5	10.5
b2150	MglB	5.0	4.0	5.6	4.9	3.9	5.3	4.5	2.9
b2153	FolE	12.4	10.1	10.2	8.3	12.6	11.7	12.8	19.8
b2178	YejB	1.5	10.1	10.2	8.3	12.6	11.7	12.8	19.8
b2180	YejF	4.0	10.1	10.2	3.8	4.8	11.7	12.8	5.8
b2183	RsuA	4.0	2.3	1.6	2.3	2.4	2.6	2.5	3.4
b2185	RplY	15.1	7.2	12.7	12.6	13.5	9.7	13.0	14.1
b2193	NarP	3.4	3.9	4.0	5.3	3.6	3.3	3.6	3.9
b2206	NapA	3.0	3.9	4.0	3.9	4.3	2.0	5.2	4.0
b2217	RcsB	3.7	3.0	4.3	2.9	3.4	4.0	3.3	3.7
b2231	GyrA	6.1	11.8	9.5	7.7	8.4	9.9	9.3	8.6
b2232	UbiG	3.6	1.9	1.4	4.7	2.6	9.9	3.3	4.4
b2234	NrdA	6.4	5.0	5.4	5.2	6.6	5.4	10.4	7.1
b2235	NrdB	6.4	2.7	2.7	2.0	4.4	2.9	2.7	5.4
b2241	GlpA	8.0	7.6	9.5	2.0	11.4	5.8	6.7	5.4
b2243	GlpC	5.2	7.6	9.5	2.4	11.4	5.8	6.7	5.4
b2247	YfaW	6.1	7.6	6.9	2.4	5.2	5.6	4.9	6.0
b2249	YfaY	4.9	2.8	5.9	2.4	2.7	5.6	4.9	6.0
b2262	MenB	1.9	1.7	1.8	1.7	2.7	1.2	2.3	6.0
b2266	ElaB	5.6	3.7	3.7	4.9	3.6	14.5	11.4	19.6
b2283	NuoG	7.2	5.6	5.6	6.6	4.4	5.4	8.4	8.1
b2284	NuoF	7.2	4.1	6.2	3.4	5.2	4.9	5.9	2.9
b2286	NuoC	5.6	8.2	6.2	3.4	14.1	4.4	3.8	2.9
b2294	YfbU	2.9	4.4	4.1	2.3	14.1	4.7	4.2	2.8
b2296	AckA	5.3	5.2	3.5	5.7	6.0	4.1	8.3	9.3
b2297	Pta	6.1	7.3	5.6	6.2	7.8	6.7	6.9	8.3
b2303	FolX	6.1	5.9	7.3	5.1	8.8	5.5	4.2	3.9
b2309	HisJ	32.3	34.5	41.0	30.7	31.7	28.7	26.9	24.2
b2312	PurF	3.9	7.5	7.7	3.5	3.5	5.2	5.3	5.0
b2316	AccD	3.1	2.6	3.5	3.0	1.8	3.6	3.4	3.1
b2320	PdxB	3.1	2.6	5.0	3.0	1.8	3.6	3.4	5.0
b2323	FabB	12.5	14.6	15.3	11.8	12.2	12.6	14.1	11.2
b2329	AroC	12.5	14.6	15.3	11.8	2.0	3.0	14.1	11.2
b2379	YfdZ	12.5	2.9	3.3	2.2	3.3	6.3	2.0	3.3
b2388	Glk	2.3	1.4	1.8	1.5	1.8	2.1	2.0	2.5
b2400	GltX	7.3	8.7	7.9	7.3	8.3	8.1	7.3	9.9
b2411	LigA	9.8	15.6	3.8	10.5	10.1	9.3	11.8	8.8
b2414	CysK	49.9	53.5	49.0	46.2	57.4	47.0	55.1	53.8

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b2415	PtsH	8.8	4.5	4.1	6.0	3.0	4.0	5.1	14.2
b2416	PtsI	23.9	26.7	26.4	22.5	23.8	19.8	26.6	26.5
b2417	Crr	47.4	48.4	49.1	52.7	52.3	46.9	61.8	64.4
b2421	CysM	7.8	5.1	7.3	5.7	10.6	11.6	6.7	3.6
b2425	CysP	10.4	13.1	11.4	12.3	12.7	10.6	13.5	13.6
b2440	EutC	10.4	13.1	11.4	12.3	12.7	10.6	13.5	2.0
b2463	MaeB	7.6	7.2	7.3	7.4	6.2	5.9	7.1	3.2
b2476	PurC	30.5	33.6	37.5	25.3	27.3	28.4	35.7	31.6
b2478	DapA	9.2	14.9	13.1	13.6	10.7	11.0	9.8	8.9
b2480	Bcp	10.0	12.6	13.6	13.4	6.3	7.3	16.2	14.1
b2495	YfgD	1.4	12.6	13.6	1.7	6.3	0.5	16.2	5.6
b2498	Upp	33.4	40.1	39.9	32.1	33.8	32.7	36.1	40.7
b2499	PurM	7.6	4.7	6.5	5.1	8.8	6.5	6.5	9.4
b2507	GuaA	21.6	26.6	25.7	24.2	21.9	21.9	23.1	22.5
b2508	GuaB	23.5	27.0	25.4	21.9	22.7	28.9	28.1	26.4
b2511	Der	6.2	5.9	4.8	6.4	6.1	2.8	3.9	8.5
b2514	HisS	7.4	9.9	7.8	8.4	7.3	9.1	7.4	9.4
b2515	IspG	6.9	9.9	7.2	8.4	3.9	4.6	7.4	2.3
b2517	RlmN	3.9	1.3	7.2	3.2	2.5	3.0	1.2	2.3
b2518	Ndk	27.2	30.7	28.1	25.3	23.4	20.4	23.8	18.7
b2523	PepB	6.7	5.6	7.5	6.6	7.0	6.4	7.1	8.1
b2525	Fdx	2.8	2.5	2.6	2.7	3.0	3.2	4.1	4.3
b2528	IscA	1.4	2.5	2.2	2.6	1.9	1.7	1.0	4.3
b2529	IscU	3.3	5.2	6.3	6.9	4.1	1.6	7.0	6.5
b2530	IscS	15.3	18.9	21.3	19.3	18.7	15.1	22.6	18.8
b2532	TrmJ	2.9	18.9	1.5	2.3	3.0	15.1	3.3	18.8
b2533	SuhB	5.0	4.8	7.2	8.1	4.8	4.9	6.4	9.1
b2538	HcaE	1.4	5.1	2.9	7.8	4.3	4.9	2.1	5.0
b2539	HcaF	1.0	0.5	2.9	7.8	4.3	4.9	1.1	5.0
b2547	YphE	3.2	0.5	6.8	7.8	4.3	6.4	5.1	6.9
b2548	YphF	16.8	6.1	9.9	7.8	4.3	3.8	3.4	6.9
b2549	YphG	1.7	9.6	6.3	2.2	4.7	2.7	5.2	6.9
b2551	GlyA	59.2	63.0	56.5	62.2	68.2	70.8	69.8	84.6
b2557	PurL	10.4	11.3	11.0	12.2	9.5	12.2	13.9	9.9
b2560	YfhB	10.4	1.9	11.0	12.2	9.5	12.2	13.9	3.1
b2566	Era	10.4	1.9	11.0	12.2	1.8	12.2	0.9	3.1
b2569	LepA	6.2	5.9	5.8	5.4	4.5	4.4	10.5	4.0
b2573	RpoE	6.2	3.9	1.1	3.4	2.2	4.4	10.5	4.0
b2592	ClpB	12.5	15.0	23.1	14.2	15.4	14.3	12.1	17.4
b2593	YfiH	9.9	15.0	23.1	14.2	15.4	14.3	12.1	1.2
b2606	RplS	77.0	47.3	52.5	50.9	68.2	56.9	46.7	50.1
b2608	RimM	1.5	4.6	52.5	50.9	68.2	56.9	46.7	50.1
b2609	RpsP	49.3	45.8	60.1	53.4	45.8	35.8	54.0	53.6
b2614	GrpE	16.7	12.2	15.9	12.5	15.4	25.2	18.4	16.7
b2620	SmpB	16.7	12.2	15.9	3.8	15.4	25.2	2.6	16.7
b2661	GabD	2.5	12.2	0.6	7.3	5.1	5.7	10.2	10.7
b2662	GabT	2.5	5.2	2.6	3.7	4.5	5.1	8.8	6.4
b2668	YgaP	2.1	5.2	2.6	3.7	4.5	5.1	8.8	6.4
b2669	StpA	16.1	22.3	18.4	15.9	15.6	24.2	24.0	18.5
b2687	LuxS	6.9	6.6	7.4	8.6	8.0	14.3	20.6	16.8
b2688	GshA	4.2	6.6	1.9	4.5	2.8	2.0	20.6	4.9
b2697	AlaS	11.8	16.7	17.6	12.5	11.7	11.9	13.1	10.9
b2699	RecA	7.9	13.5	12.1	11.0	12.6	11.1	14.1	11.5
b2716	AscB	3.0	13.5	8.4	11.0	12.6	11.1	5.8	11.5
b2741	RpoS	2.4	13.5	0.9	11.0	12.6	0.9	9.9	15.2
b2747	IspD	10.4	8.4	9.1	11.1	9.4	11.9	13.7	3.5
b2751	CysN	10.4	11.2	12.5	8.0	11.6	10.8	9.8	13.6
b2752	CysD	9.1	8.0	9.8	7.6	13.5	8.5	9.3	10.4
b2758	YgcJ	1.7	8.0	9.8	7.6	2.4	8.5	9.3	2.2
b2762	CysH	3.5	6.8	7.0	4.4	3.6	3.8	5.1	9.2
b2763	CysI	10.6	9.1	12.2	10.0	11.3	10.7	12.6	14.0
b2764	CysJ	14.3	13.2	15.4	16.3	15.7	13.6	15.4	17.1

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b2779	Eno	88.6	72.0	83.7	80.4	85.3	72.8	98.2	82.7
b2780	PyrG	6.2	6.9	7.4	6.0	11.8	5.8	8.2	11.3
b2811	CsdE	6.2	6.9	7.4	6.0	0.2	5.8	3.6	3.9
b2818	ArgA	7.7	3.3	2.3	3.8	3.3	8.7	5.4	10.4
b2820	RecB	3.7	5.9	2.3	3.8	3.3	6.5	8.3	8.8
b2821	PtrA	6.2	6.8	2.3	7.3	8.2	6.5	5.9	9.7
b2827	ThyA	5.9	2.0	5.6	4.1	1.3	6.5	4.5	5.0
b2871	YgeX	2.5	2.8	1.3	4.1	7.4	3.0	3.3	1.1
b2889	Idi	4.9	4.1	5.4	6.5	6.0	6.9	7.4	9.6
b2890	LysS	10.0	9.3	11.4	12.0	9.3	9.8	10.8	10.1
b2894	XerD	2.3	9.3	11.4	2.1	4.0	9.8	10.8	3.0
b2898	YgfZ	3.3	5.6	6.1	5.9	3.6	3.5	8.9	8.6
b2901	BglA	4.0	2.8	3.7	4.3	3.1	3.1	1.8	3.0
b2903	GcvP	9.0	3.2	5.7	8.5	5.0	3.0	8.1	21.1
b2904	GcvH	2.2	3.2	2.1	2.3	3.4	1.7	1.7	21.1
b2913	SerA	55.6	73.9	76.5	57.9	55.4	47.8	53.6	65.1
b2914	RpiA	2.6	11.3	7.6	9.5	6.2	5.5	12.6	13.7
b2918	ArgK	4.7	11.3	3.8	4.0	6.2	7.5	5.0	13.7
b2924	MscS	4.3	1.1	3.8	4.0	6.2	2.9	5.0	3.8
b2925	FbaA	63.1	64.1	72.0	64.5	69.0	58.3	86.1	85.2
b2926	Pgk	95.4	100.7	97.2	89.2	100.3	89.1	120.0	125.5
b2927	Epd	7.5	100.7	97.2	89.2	100.3	89.1	120.0	125.5
b2935	TktA	20.8	24.1	24.8	20.0	18.0	18.4	14.2	13.6
b2942	MetK	32.0	32.7	30.9	30.7	30.5	33.2	38.3	45.2
b2947	GshB	3.9	4.9	5.6	4.0	4.0	1.9	4.2	5.5
b2954	RdgB	3.9	6.4	2.1	12.4	2.4	4.2	2.5	1.7
b2960	TrmI	1.9	2.5	2.2	12.4	0.2	4.2	5.3	2.5
b2962	YggX	6.8	8.1	8.0	5.9	2.9	6.7	9.2	8.1
b2973	YghJ	4.9	6.0	8.0	5.0	2.9	6.7	9.2	8.1
b2978	GEF	2.1	2.9	8.0	5.0	2.9	6.7	2.0	8.1
b2985	YghS	2.4	2.9	8.0	3.2	2.9	6.7	2.1	8.1
b2989	YghU	2.0	3.7	8.0	1.7	1.4	6.7	1.1	6.2
b3008	MetC	4.1	2.1	5.4	7.4	4.7	5.7	4.9	4.7
b3025	QseB	0.3	0.2	5.4	7.4	4.7	13.1	1.4	4.7
b3030	ParE	3.2	0.2	5.4	3.0	4.3	13.1	1.8	2.0
b3046	YqiG	2.0	0.2	5.4	9.8	4.3	13.1	1.8	2.0
b3052	RfaE	4.2	5.1	5.4	5.6	5.2	3.5	4.9	5.5
b3054	YgiF	7.8	6.4	2.2	4.1	5.2	3.5	1.0	5.9
b3060	TtdR	14.2	6.4	2.2	4.1	7.4	8.7	1.9	3.5
b3065	RpsU	9.4	15.8	3.6	16.1	8.0	14.0	2.8	6.9
b3067	RpoD	4.1	2.3	3.6	2.1	1.8	4.5	6.0	3.3
b3098	YqjD	5.2	2.3	3.6	2.1	1.8	21.3	20.7	24.9
b3128	GarD	4.9	2.3	3.6	2.1	1.8	21.3	3.6	24.9
b3132	KbaZ	4.9	13.1	2.0	2.1	1.8	21.3	8.7	24.9
b3157	YhbT	6.9	13.1	2.0	6.0	1.3	21.3	8.7	1.1
b3162	DeaD	5.7	4.3	6.7	4.5	5.6	3.6	4.8	5.9
b3164	Pnp	15.0	14.9	15.7	15.5	18.8	19.2	17.0	16.9
b3165	RpsO	21.3	19.8	22.8	17.3	17.6	19.8	19.3	21.1
b3167	RbfA	1.4	5.8	3.1	3.7	2.7	9.6	7.8	1.2
b3168	InfB	14.4	13.8	13.0	11.1	13.9	14.0	17.2	14.0
b3169	NusA	14.4	17.1	16.7	15.9	17.8	16.6	17.4	19.3
b3172	ArgG	23.6	39.3	33.6	27.9	31.4	26.6	26.1	23.1
b3176	GlmM	6.3	9.3	9.3	6.4	6.9	5.6	0.9	4.0
b3178	HflB	2.4	4.9	9.3	3.1	6.9	5.3	2.1	4.0
b3180	YhbY	3.2	4.9	9.3	3.1	6.9	5.3	2.1	4.0
b3181	GreA	4.2	3.8	5.4	5.2	4.5	1.7	4.4	2.3
b3183	ObgE	2.1	1.6	4.2	2.5	1.5	1.7	4.4	2.6
b3186	RplU	61.5	58.4	63.8	48.9	51.6	47.8	60.8	59.2
b3189	MurA	2.8	4.7	5.6	6.7	9.1	6.6	8.8	4.0
b3192	YrbC	4.5	4.8	5.9	7.8	5.3	5.5	9.0	7.2
b3197	KdsD	6.4	4.8	6.0	6.7	7.2	3.9	7.5	7.2
b3198	KdsC	1.8	4.8	6.0	6.7	7.2	3.9	1.5	1.2

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b3201	LptB	1.8	4.8	5.4	0.1	7.2	3.9	13.7	1.2
b3209	ElbB	2.4	4.8	2.5	2.4	2.5	3.3	5.9	1.2
b3212	GltB	37.6	38.4	41.1	31.7	39.3	43.9	35.3	35.8
b3213	GltD	27.7	27.6	29.6	27.7	31.8	31.0	26.8	29.3
b3229	SspA	8.0	4.1	10.1	27.7	4.9	8.0	5.2	9.7
b3230	RpsI	60.5	54.1	60.7	49.7	56.6	51.1	60.0	66.2
b3231	RplM	31.8	28.4	29.2	28.2	32.4	26.0	27.2	34.1
b3233	YhcB	31.8	1.5	9.7	8.3	0.7	4.3	2.9	1.1
b3236	Mdh	122.3	125.9	126.9	114.6	107.5	123.9	104.1	107.8
b3244	TldD	7.2	3.1	126.9	4.4	4.3	5.1	1.4	107.8
b3251	MreB	14.3	15.9	16.6	15.0	16.5	14.5	16.9	16.8
b3255	AccB	14.5	16.2	17.5	14.4	14.5	12.6	18.6	15.3
b3256	AccC	8.4	8.1	10.0	8.2	10.8	7.3	8.8	9.2
b3261	Fis	2.1	3.4	3.4	1.9	1.8	1.9	2.6	2.2
b3268	YhdW	4.4	3.4	3.4	3.6	4.1	5.4	2.6	2.2
b3269	YhdX	4.4	3.4	3.4	3.6	6.3	5.4	5.4	2.2
b3282	RimN	4.6	1.3	3.4	3.6	2.2	3.7	1.6	2.2
b3287	Def	3.1	1.3	3.4	2.8	2.5	3.7	3.5	2.2
b3294	RplQ	54.3	57.1	57.4	51.7	54.2	48.5	54.4	55.4
b3295	RpoA	33.5	35.8	37.0	28.1	34.3	31.9	39.2	40.3
b3296	RpsD	73.1	71.9	95.9	71.2	70.1	72.1	73.7	79.6
b3297	RpsK	36.7	16.3	26.0	20.6	23.8	28.4	24.9	31.6
b3298	RpsM	67.7	63.0	56.1	58.0	64.1	51.4	66.3	79.3
b3299	RpmJ	6.5	6.0	7.0	5.2	64.1	51.4	66.3	7.1
b3301	RplO	54.7	46.9	54.4	44.5	51.6	50.1	47.9	63.6
b3302	RpmD	11.0	11.9	11.7	11.5	12.9	6.4	9.8	10.8
b3303	RpsE	80.9	72.9	88.0	69.0	73.3	73.8	73.4	81.4
b3304	RplR	22.0	20.1	21.4	20.1	20.4	19.6	22.7	28.4
b3305	RplF	95.3	86.7	90.8	76.4	82.6	74.7	86.3	85.5
b3306	RpsH	27.8	27.6	31.6	22.0	28.0	28.8	20.8	25.4
b3308	RplE	41.8	30.9	38.6	36.0	37.0	36.5	42.9	40.7
b3309	RplX	34.0	27.2	32.2	34.0	24.9	30.9	30.7	37.6
b3310	RplN	34.1	29.2	22.6	21.1	21.6	21.6	20.9	29.5
b3311	RpsQ	6.1	11.0	5.8	4.8	4.9	5.8	5.3	29.5
b3312	RpmC	23.9	25.6	18.9	21.7	24.9	21.9	21.0	25.8
b3313	RplP	48.9	45.3	50.0	43.1	41.6	40.0	47.9	48.7
b3314	RpsC	96.4	90.4	105.5	87.7	102.2	97.0	91.1	105.1
b3315	RplV	48.6	31.8	34.5	35.0	29.4	25.9	31.5	36.9
b3316	RpsS	11.0	9.3	10.7	7.0	9.5	8.7	8.6	10.8
b3317	RplB	56.4	40.8	42.6	43.3	50.4	51.3	44.4	45.9
b3318	RplW	8.1	12.9	9.5	5.8	5.6	51.3	5.1	5.7
b3319	RplD	33.0	26.4	28.3	24.8	24.7	19.0	22.8	24.9
b3320	RplC	68.8	52.8	54.1	58.6	54.7	53.4	53.7	68.6
b3321	RpsJ	79.3	94.3	81.9	70.0	73.8	66.2	95.5	86.1
b3336	Bfr	5.2	10.4	4.7	6.7	6.3	9.0	17.0	12.6
b3340	FusA	104.3	117.1	122.5	102.8	115.3	94.3	116.9	105.8
b3341	RpsG	95.5	87.8	95.2	81.5	87.8	86.2	90.2	106.6
b3342	RpsL	20.9	22.1	23.4	20.5	24.0	19.3	21.6	24.1
b3347	FkpA	11.3	10.3	13.2	11.3	10.6	9.7	12.3	14.4
b3349	SlyD	4.4	4.9	3.5	5.3	4.3	4.5	6.0	5.5
b3357	Crp	16.5	16.6	18.2	15.0	14.7	13.6	13.9	16.5
b3359	ArgD	15.3	16.2	17.6	13.9	15.3	15.4	14.5	14.4
b3368	CysG	8.3	16.2	17.6	13.9	15.3	15.4	14.5	14.4
b3384	TrpS	6.3	11.7	6.6	13.9	7.0	4.7	7.5	14.4
b3386	Rpe	2.4	2.3	2.0	2.5	2.6	3.1	2.0	3.8
b3389	AroB	3.6	4.0	3.0	3.0	3.7	2.2	3.7	3.9
b3390	AroK	7.8	4.0	7.7	4.6	3.7	9.6	3.1	5.3
b3398	YrfF	6.5	4.0	7.7	4.6	3.7	9.6	3.6	5.3
b3414	NfuA	11.5	13.7	13.4	13.3	11.9	10.3	16.3	14.9
b3417	MalP	8.3	5.3	5.5	6.3	7.1	7.8	9.6	7.5
b3426	GlpD	7.5	7.1	5.5	6.3	7.1	4.8	13.3	7.2
b3433	Asd	68.9	87.2	96.3	77.4	87.4	71.6	73.2	61.3

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b3460	LivJ	72.9	77.0	80.9	73.4	62.6	60.9	63.4	49.7
b3469	ZntA	3.9	77.0	80.9	73.4	62.6	3.6	63.4	49.7
b3498	PrlC	6.1	6.6	9.4	6.0	5.4	5.6	12.3	7.0
b3500	Gor	3.4	2.2	9.4	3.6	3.1	2.3	6.3	7.6
b3509	HdeB	3.6	6.0	8.2	4.9	8.2	9.5	27.0	43.9
b3513	MdtE	3.6	6.0	5.0	4.9	8.2	9.5	27.0	6.8
b3521	YhjC	1.5	2.0	5.0	4.9	3.0	9.5	27.0	6.8
b3530	BcsC	7.8	7.0	1.8	6.9	6.9	3.8	7.1	6.3
b3544	DppA	22.4	24.3	27.6	23.7	23.3	21.2	24.0	18.9
b3553	GhrB	5.5	24.3	4.2	6.7	4.7	4.3	5.8	9.7
b3556	CspA	12.0	14.3	6.2	12.8	27.0	4.3	19.0	13.3
b3559	GlyS	13.9	13.9	14.1	11.2	12.3	12.3	13.2	9.9
b3560	GlyQ	7.9	6.2	6.5	7.4	6.2	6.3	7.5	5.5
b3592	YibF	1.6	6.2	6.5	2.7	1.0	0.8	2.3	2.4
b3609	SecB	18.2	20.1	23.9	16.6	18.6	20.8	16.9	20.3
b3610	GrxC	3.4	4.2	4.8	6.1	0.6	5.0	10.2	6.1
b3612	GpmM	13.3	12.4	14.1	12.5	15.3	14.1	15.4	11.4
b3617	Kbl	3.1	12.4	4.2	7.9	3.5	3.6	15.4	9.2
b3619	RfaD	8.5	8.3	7.6	7.6	7.2	7.2	9.4	9.3
b3637	RpmB	31.7	26.9	28.9	29.5	27.8	21.7	30.1	26.2
b3638	YicR	4.0	2.6	28.9	29.5	4.0	2.0	2.1	26.2
b3644	YicC	4.0	2.6	2.4	4.4	3.5	3.3	5.6	7.3
b3649	RpoZ	4.8	4.2	3.6	3.8	4.3	4.0	4.4	4.1
b3656	YicI	3.7	4.2	3.6	3.8	4.3	9.3	4.4	4.1
b3670	IlvN	3.7	1.7	2.6	1.7	4.3	9.3	1.3	0.4
b3671	IlvB	4.9	10.9	11.1	10.5	9.5	7.5	10.9	5.2
b3693	DgoK	1.7	10.9	11.1	10.5	9.5	7.5	10.9	5.2
b3699	GyrB	5.5	5.1	4.7	5.1	5.7	5.1	5.9	6.6
b3700	RecF	5.5	2.5	4.7	1.3	3.0	5.1	7.1	6.6
b3701	DnaN	4.0	2.5	4.7	1.3	3.0	2.7	7.1	6.6
b3705	YidC	1.1	1.0	4.7	0.7	1.4	2.3	7.1	6.6
b3713	YieF	3.2	1.0	4.7	0.7	1.4	2.3	10.1	6.6
b3729	GlmS	10.7	11.6	12.5	11.5	10.9	11.9	13.7	11.3
b3732	AtpD	20.0	18.5	18.1	17.0	17.3	25.2	15.8	17.6
b3733	AtpG	5.8	5.5	6.5	4.1	2.4	8.4	7.1	5.6
b3734	AtpA	19.4	20.5	17.3	14.6	15.0	29.4	16.9	16.8
b3735	AtpH	0.8	0.3	1.0	1.9	2.5	29.4	5.1	1.6
b3741	MnmG	7.6	0.3	1.0	1.9	2.5	29.4	5.1	1.6
b3744	AsnA	4.7	6.7	5.5	5.0	7.0	3.1	3.1	4.7
b3749	RbsA	5.0	16.5	10.3	19.2	11.3	23.1	18.7	4.7
b3751	RbsB	4.3	6.1	4.7	13.8	8.4	7.3	4.8	15.4
b3764	YifE	6.3	6.5	6.4	4.6	5.9	4.6	6.1	4.0
b3770	IlvE	19.0	21.2	24.8	13.3	17.8	15.7	11.7	16.2
b3771	IlvD	6.6	3.9	8.3	6.8	2.5	5.4	7.0	8.6
b3774	IlvC	100.9	111.3	123.6	108.6	102.3	92.3	104.4	94.7
b3781	TrxA	31.1	29.3	33.5	34.0	29.3	29.7	34.6	43.4
b3783	Rho	12.5	14.6	13.0	13.1	14.1	13.9	14.3	16.1
b3790	RffC	12.5	14.6	1.0	1.3	1.2	1.7	5.6	16.1
b3791	RffA	1.0	14.6	1.0	0.9	1.1	1.5	1.4	2.1
b3802	HemY	10.5	2.5	12.9	4.1	1.0	0.9	0.8	2.1
b3813	UvrD	3.1	3.4	1.1	1.2	1.0	0.9	0.8	2.1
b3829	MetE	262.6	335.5	318.3	267.2	270.1	230.4	318.4	242.6
b3835	UbiB	1.7	335.5	318.3	267.2	270.1	230.4	318.4	242.6
b3840	TatD	1.7	335.5	318.3	267.2	270.1	230.4	1.0	5.2
b3844	Fre	1.7	1.4	3.5	267.2	270.1	230.4	3.3	1.6
b3847	PepQ	4.5	6.6	6.7	6.7	6.7	6.0	9.8	9.5
b3858	YihD	3.0	6.6	1.3	4.2	2.2	6.0	2.2	3.2
b3860	DsbA	5.2	7.7	10.7	12.2	10.5	6.4	3.4	11.7
b3863	PolA	3.9	7.9	5.2	5.4	4.9	5.8	4.6	4.0
b3865	YihA	3.9	7.9	3.6	2.3	3.2	2.1	3.8	2.5
b3870	GlnA	34.1	54.8	51.2	58.3	47.4	39.0	39.3	35.6
b3871	TypA	14.5	16.0	14.8	15.3	14.3	11.6	14.3	17.0

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b3898	FrvX	5.3	16.0	4.0	2.2	1.8	11.6	14.3	3.5
b3908	SodA	49.7	36.3	44.6	50.8	48.4	42.7	64.7	62.1
b3916	PfkA	7.6	5.6	8.6	7.0	6.4	7.0	9.5	7.7
b3917	Sbp	6.9	5.6	8.6	0.3	6.4	9.5	2.7	8.1
b3919	TpiA	13.4	14.7	15.1	14.2	15.0	12.3	21.1	20.1
b3926	GlpK	2.9	14.7	3.0	14.2	4.4	3.1	1.5	20.1
b3928	ZapB	2.2	6.1	1.9	0.7	5.1	2.8	2.0	0.6
b3929	RraA	2.8	3.3	1.9	3.2	2.9	3.9	3.9	4.8
b3931	HslU	5.6	11.0	7.9	5.2	5.8	5.4	8.4	8.6
b3936	RpmE	13.7	12.0	9.0	8.7	10.4	13.9	3.5	8.5
b3939	MetB	5.5	5.7	4.3	2.8	4.0	4.1	3.5	3.5
b3940	MetL	6.3	6.2	20.4	14.5	13.5	11.3	12.4	13.7
b3941	MetF	13.4	15.8	12.9	11.8	16.3	8.5	15.8	14.8
b3942	KatG	4.6	10.9	6.3	9.6	11.8	5.5	7.1	14.8
b3956	Ppc	27.2	32.1	29.0	26.3	29.6	29.3	25.6	25.0
b3957	ArgE	4.3	5.0	5.5	5.7	4.8	4.4	5.7	7.5
b3959	ArgB	11.2	11.6	16.7	14.2	15.3	10.0	15.3	14.3
b3960	ArgH	9.6	13.4	13.8	12.0	11.9	9.3	11.8	11.6
b3980	TufB	789.5	806.5	766.1	680.4	815.5	664.0	805.8	687.6
b3982	NusG	9.7	4.9	4.7	6.0	7.2	6.0	4.6	6.8
b3983	RplK	58.6	63.8	61.8	58.8	57.8	52.7	68.0	65.7
b3984	RplA	116.7	113.9	113.0	99.8	99.7	98.7	112.1	104.9
b3985	RplJ	100.2	108.4	113.5	98.3	96.0	89.8	98.8	105.6
b3986	RplL	104.5	115.2	117.6	111.5	98.5	109.2	119.5	127.2
b3987	RpoB	27.3	31.4	30.4	26.9	29.2	25.6	28.2	34.0
b3988	RpoC	31.6	32.2	32.4	30.0	32.9	29.8	34.2	36.2
b3991	ThiG	7.2	5.7	7.2	5.9	5.9	6.3	6.9	6.5
b3992	ThiF	7.1	3.9	7.0	5.9	5.9	6.3	5.9	6.5
b3993	ThiE	4.0	4.5	4.6	4.4	3.9	1.9	6.5	2.6
b3994	ThiC	9.7	12.1	12.6	10.1	10.6	9.5	13.1	9.5
b4000	HupA	147.1	158.0	164.7	141.5	135.4	141.8	156.6	166.9
b4005	PurD	6.3	7.5	10.8	7.7	7.7	5.9	7.6	8.8
b4006	PurH	17.2	20.2	18.4	16.4	15.5	13.8	18.8	18.6
b4013	MetA	3.4	20.2	18.4	3.9	6.1	10.4	5.1	7.5
b4014	AceB	29.4	38.8	37.0	33.1	27.7	36.5	48.8	35.4
b4015	AceA	99.2	141.7	130.3	117.8	102.7	135.8	175.7	104.3
b4019	MetH	7.0	5.8	5.3	6.3	4.8	5.9	7.2	11.5
b4024	LysC	12.9	16.4	13.7	12.8	13.8	10.4	13.7	11.9
b4025	Pgi	16.4	19.1	19.0	19.0	20.3	21.1	27.2	25.5
b4029	YjbH	16.4	21.3	19.0	19.0	5.0	21.1	27.2	25.5
b4036	LamB	1.4	21.3	2.5	19.0	5.0	21.1	27.2	25.5
b4054	TyrB	6.8	7.0	8.1	8.8	6.7	6.4	5.9	7.9
b4059	Ssb	8.3	6.2	4.1	6.2	7.3	5.5	2.3	4.3
b4061	YjcC	2.6	6.2	2.4	5.7	7.3	5.5	2.3	2.0
b4110	YjcZ	1.6	6.2	2.4	5.7	7.3	5.2	1.2	2.0
b4119	MelA	7.8	6.2	2.4	5.7	7.3	5.2	1.2	2.0
b4122	FumB	6.7	6.9	8.5	7.2	6.4	9.4	9.8	7.0
b4124	DcuR	3.4	6.9	8.5	1.1	6.4	5.3	9.8	7.0
b4131	CadA	5.0	8.9	2.5	1.1	6.4	5.3	13.2	3.9
b4135	YjdC	3.6	8.9	2.5	1.1	4.0	4.2	0.7	1.5
b4142	GroS	25.8	37.5	36.1	34.6	32.9	31.8	30.8	43.2
b4143	GroL	61.4	65.8	66.6	52.1	61.0	61.9	62.7	66.5
b4147	Efp	5.0	6.1	2.7	8.1	7.6	1.9	9.5	8.7
b4153	FrdB	5.0	6.1	0.5	8.1	7.6	1.9	9.5	8.7
b4162	Orn	0.8	6.1	2.4	1.9	7.6	0.8	9.5	7.2
b4177	PurA	36.1	41.0	33.4	30.7	32.5	29.0	29.2	23.6
b4179	Rnr	7.8	8.4	6.4	5.8	4.1	6.0	10.9	23.6
b4182	YjfJ	2.3	8.4	6.4	3.6	4.1	6.0	10.9	23.6
b4200	RpsF	45.3	47.4	46.2	36.0	43.7	41.7	43.6	44.8
b4202	RpsR	58.4	48.3	56.5	48.6	53.8	37.6	60.3	60.0
b4203	RplI	78.3	88.9	88.9	68.6	75.1	63.9	79.2	83.9
b4207	FklB	14.9	16.5	17.4	14.2	13.1	12.9	15.0	14.3

		Protein Abundance (Average fmoles)							
B #	Protein	PE	ΔG	ΔGA	ΔGAP	ΔGAP-0.2	ΔGAP-0.8	ΔH	ΔHY
b4211	YtfG	3.8	16.5	17.4	1.2	4.9	12.9	15.0	0.9
b4214	CysQ	3.0	16.5	17.4	1.2	2.3	1.8	15.0	2.9
b4219	MsrA	0.5	1.8	1.4	2.4	2.2	2.1	1.9	1.7
b4221	YtfN	9.5	18.1	11.1	11.6	8.9	8.5	1.9	1.7
b4226	Ppa	24.8	27.5	26.9	28.0	25.7	21.8	34.9	30.7
b4232	Fbp	6.8	3.6	8.8	4.0	4.3	5.1	5.1	6.3
b4234	YjgA	0.8	3.6	0.7	4.0	1.0	0.8	1.1	1.6
b4236	CybC	8.0	5.1	0.7	4.0	1.0	3.6	1.2	7.9
b4243	YjgF	34.5	34.6	37.6	37.7	35.1	35.3	41.9	41.7
b4244	PyrI	66.0	70.2	75.9	62.5	64.5	68.6	78.2	73.2
b4245	PyrB	57.1	63.3	58.1	55.4	52.3	66.8	60.4	56.7
b4254	ArgI	14.8	14.6	12.7	12.0	14.6	10.4	9.7	11.7
b4258	ValS	9.0	10.7	8.7	8.6	9.5	8.0	10.4	9.6
b4260	PepA	3.8	3.4	4.2	2.6	4.1	4.9	5.3	5.1
b4278	InsG	5.1	8.2	3.1	3.7	7.7	7.9	5.7	4.3
b4295	YjhU	1.9	2.5	3.1	3.7	7.7	0.8	0.9	2.3
b4328	IadA	1.9	2.5	3.1	2.3	4.7	4.1	0.9	2.3
b4345	McrC	3.8	2.6	1.1	1.0	4.7	4.1	1.0	2.3
b4349	HsdM	3.7	2.5	7.5	6.9	5.1	3.1	8.2	8.7
b4362	DnaT	5.1	1.3	7.5	6.9	5.1	3.1	1.6	8.7
b4375	PrfC	3.0	3.4	5.5	3.9	5.8	4.0	4.9	4.0
b4376	OsmY	11.0	15.7	14.3	18.3	21.2	21.8	52.6	43.7
b4381	DeoC	2.6	1.4	14.3	18.3	2.8	4.1	2.8	4.8
b4382	DeoA	3.9	1.4	4.4	2.9	2.8	3.3	4.0	4.8
b4383	DeoB	4.9	5.4	4.4	6.2	10.2	7.7	13.1	10.2
b4384	DeoD	8.9	10.3	11.5	10.3	11.7	12.5	15.7	15.4
b4386	LplA	8.9	9.4	2.6	10.3	3.0	3.9	6.2	4.3
b4391	YjjK	12.6	12.8	11.8	11.5	11.9	12.5	15.7	17.7
b4392	Slt	3.7	3.5	11.8	3.3	11.9	3.3	5.4	17.7
b4401	ArcA	11.6	5.7	11.8	19.7	3.1	2.7	9.7	12.0

9.3. Expression Data for Integrated Transcriptomic and Proteomic Analysis

The following appendix gives transcriptomic and proteomic data for the 5 examined mutant strains that were used to generate Figure 7-2 and Figure 7-3 according to the metric described in section 4.5. Data are given as $\log_{10}$(Mutant/PE) ratios, and proteomic data corresponding to both peptide threshold one and two, as explained in section 4.4.5, are presented. Those $\log_{10}$(Mutant/PE) ratios that are greater than or equal to 0.05 or less than or equal to -0.05 (corresponding to about 12%) are highlighted in red and green, respectively. Ratios for peptide threshold one data that appear as +2.171 or -2.171 are an artifact of the data processing and correspond to positive (only Mutant strain protein detection) and negative (only PE strain protein detection) "infinite" ratios, respectively. Genes are ordered roughly according to the pathways of the TCA cycle, glyoxylate pathway, and glycolysis/gluconeogenesis.

Gene		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE))-Threshold 1					Proteins (log_10(Mut/PE))-Threshold 2				
Name	B #	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
aceF	b0115	0.075	0.016	0.030			-0.030	-0.026	-0.013	0.013	0.043	0.013	-0.025	-0.008	0.011	0.028
aceE	b0114	-0.035	-0.034	-0.055	-0.103	-0.124	0.022	-0.026	-0.026	0.030	0.004	0.019	-0.013	0.003	0.028	0.008
lpd	b0116	-0.047	0.046	-0.024	-0.047	-0.126	-0.056	-0.048	-0.039	-0.052	-0.030	-0.048	-0.024	0.000	-0.055	0.018
gltA	b0720		-0.017	-0.060	-0.035		0.030	0.000	-0.013	-0.122	-0.178	0.037	-0.029	-0.049	-0.122	-0.117
(prpC)	b0333	0.132	0.175	0.295	0.137	0.024										
acnB	b0118	-0.063	-0.038	-0.039	-0.073	-0.197	-0.017	-0.009	-0.056	-0.161	-0.274	-0.037	-0.021	-0.064	-0.110	-0.230
acnA	b1276	-0.037	-0.004	0.063	0.027	0.080	2.171	2.171	2.171	2.171	2.171	0.060	-0.003	0.077	0.156	0.095
icd	b1136	-0.045	0.031	0.010	-0.005	0.010	0.030	0.026	-0.026	-0.165	-0.282	0.035	0.027	-0.030	-0.108	-0.198
sucA	b0726	0.009	-0.002	-0.005	-0.012	0.001	-0.026	-0.096	-0.078	-0.130	-0.187	-0.017	-0.056	-0.094	-0.107	-0.044
sucB	b0727		0.042				-0.013	-0.048	-0.100	-0.248	-0.152	-0.040	-0.097	-0.062	-0.162	-0.082
lpd	b0116	-0.047	0.046	-0.024	-0.047	-0.126	-0.056	-0.048	-0.039	-0.052	-0.030	-0.048	-0.024	0.000	-0.055	0.018
sucD	b0729	-0.006	0.007	-0.052	-0.088	-0.127	-0.009	0.022	-0.017	-0.096	-0.139	0.031	0.036	-0.034	-0.075	-0.069
sucC	b0728	0.358	0.049	0.072			-0.004	0.004	-0.039	-0.130	-0.174	-0.001	-0.026	-0.048	-0.128	-0.174
sdhA	b0723	-0.042	0.043	0.037	0.011	0.132	-2.171	-2.171	-2.171	-2.171	-2.171	-0.042	-0.028	-0.083	-0.070	-0.048
sdhC	b0721	-0.045	-0.052	-0.099	-0.126	-0.326										
sdhD	b0722															
sdhB	b0724											0.038	-0.136	-0.173	-0.054	-0.144
fumB	b4122		0.028	-0.024	0.165	-0.240	-0.065	0.043	-0.065	-0.161	-2.171	-0.058	-0.061	-0.004	-0.072	-0.100
fumC	b1611															
fumA	b1612						-0.004	0.030	0.000	-0.195	-0.113					
(mqo)	b2210	-0.020	-0.054	-0.048	-0.087	-0.094	-2.171	-2.171	0.395		-2.171					
mdh	b3236	0.100	-0.011	-0.065	-0.134		0.030	0.013	0.004	-0.083	-0.048	0.022	-0.008	-0.058	-0.083	-0.031
aceA	b4015	-0.018	0.025	0.006	-0.009	0.063	0.061	0.039	0.039	0.143	0.017	0.069	0.046	0.005	0.076	0.014
aceB	b4014	-0.003	0.023	0.002	-0.064	-0.020	0.113	0.061	0.061	0.191	0.139	0.053	0.046	0.016	0.122	0.052
(glcB)	b2976	-0.001	-0.001	-0.007	-0.007	-0.046		2.171	2.171	2.171						
maeA	b1479	0.134	-0.052	0.017		0.036	0.100	0.009	0.069	-0.156	-0.213	0.039	-0.008	-0.091	0.102	0.128
maeB	b2463	-0.034	-0.009	-0.026	-0.002	-0.087	0.135	0.013	-0.065	-0.143	-0.061	-0.016	-0.078	-0.082	-0.049	-0.078
pck (OA	b3403						2.171	2.171								
ppc (PE	b3956	0.467	0.071	0.141	-0.039		0.017	0.004	-0.022	-0.056	-0.083	0.034	0.031	-0.027	-0.012	-0.009
eda (OA	b1850	-0.014	0.044	0.051	-0.055	-0.134	0.052	0.087	0.083	0.087	0.013	0.021	0.047	0.127	0.134	0.148
pps (Pyr	b1702	0.042	0.042	-0.017	-0.058	-0.028	0.030	0.069	-0.013	-0.043	0.004	0.087	0.063	0.015	0.060	0.034
pykF (PE	b1676	-0.038	-0.023	-0.029	-0.073	-0.138	0.030	0.000	0.009	0.026	0.000	0.026	-0.016	-0.027	0.021	-0.003
pykA (PE	b1854	-0.043	0.009	-0.033	-0.008	0.056	0.065	-0.048	0.022	0.004	0.104	0.023	0.037	0.060	0.116	0.102
pgi	b4025			-0.147	0.081	-0.091	0.039	0.017	0.109	0.208	0.143	0.059	0.039	0.055	0.144	0.112
pfkA	b3916	0.020	0.065	0.037	0.047	0.011	-0.087	0.065	0.013	0.117	0.087	-0.014	0.017	-0.026	0.052	0.127
pfkB	b1723	-0.071	0.052	0.130	0.070	0.170			2.171	2.171	2.171					
fbaB	b2097		0.097	0.038	-0.017					2.171						
fbaA	b2925	-0.062	-0.025	-0.005	-0.042	-0.058	0.052	0.043	0.039	0.104	0.139	0.020	0.028	0.035	0.107	0.139
tpiA	b3919	-0.027	0.002	0.024	-0.050	-0.098	-0.039	-0.035	-0.022	0.122	0.113	0.031	-0.022	0.027	0.128	0.203
gapA	b1779	-0.099	-0.003	-0.022	-0.074	-0.079	0.013	0.026	-0.030	0.000	-0.043	0.037	-0.005	-0.014	-0.020	-0.034
pgk	b2926					-0.299	0.035	0.004	0.026	0.074	0.130	0.056	0.012	0.000	0.049	0.121

Gene		Transcripts (log_10(Mut/PE))					Proteins (log_10(Mut/PE))-Threshold 1					Proteins (log_10(Mut/PE))-Threshold 2				
Name	B #	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY	ΔG	ΔGA	ΔGAP	ΔH	ΔHY
gpmM	b3612	0.174	0.027	0.066	0.055	0.024	0.052	0.061	0.052	0.096	-0.009	0.030	-0.014	0.021	0.061	-0.032
ytjC	b4395	-0.073	0.087	0.002	0.126	0.228										
gpmA	b0755	-0.030	-0.011	-0.071	-0.095	-0.212	0.083	-0.052	0.000	0.087	0.000	0.028	-0.069	-0.021	0.020	0.016
eno	b2779			0.013	-0.032		-0.065	-0.022	0.000	0.039	-0.013	-0.048	-0.002	-0.013	0.049	0.044
ybhA	b0766		-0.114													
glpX	b3925	0.146	0.004	0.040	-0.200	-0.030										
fbp	b4232	-0.010	-0.010	-0.025	0.021	0.012	2.171		2.171	2.171		0.017	0.026	0.088	0.057	0.092

Chapter 10. BIBLIOGRAPHY

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