THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF CHEMICAL ENGINEERING

ANALYZING THE EFFECTS OF SECONDARY STRUCTURE ON BACTERIAL PERSISTENCE IN SELECTED E. COLI GENES OF INTEREST

BURGES UNWALLA
SPRING 2014

A thesis
submitted in partial fulfillment
of the requirements
for a baccalaureate degree in
Chemical Engineering
with honors in Chemical Engineering

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ABSTRACT

From human teeth to small pebbles in a river bed, biofilms have been found to inhabit nearly every surface imaginable in today's world. Biofilms can be thought of as highly intricate microbial communities in which various bacteria adhere to a solid and thrive under certain conditions. These complex systems undertake a dualistic nature. On the negative side, biofilms can lead to infections within the human body. Conversely, they possess many industrial advantages within wastewater treatment and oil spill cleanup. The overarching desire is to understand the genetic basis of biofilms in order to quell their negative effects and subsequently promote their beneficial characteristics. As a starting point, it is essential to understand bacterial toxin-antitoxin systems as they relate to individual cell physiology within the bacteria of interest, *E. coli* K12 MG1655. The newly discovered type V toxin-antitoxin system serves as the basis for this research as a protein antitoxin is seen to inhibit the toxin by cleaving its mRNA. Further interest lies within the toxin motility quorum sensing regulator (MqsR) as it inhibits protein synthesis through mRNA degradation at preferred guanine-cytosine-uracil (GCU) locations. Each of the 4501genes of *E. coli* were analyzed and the corresponding number of GCU sites for each were documented. Those genes that exhibited either zero, one or two cleavage sites were given special attention as they may shed important light on the ability of MqsR to increase antibiotic persistence. These seemingly important genes were analyzed through the technique of persistence assays.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF TABLES CONTINUED

ACKNOWLEDGEMENTS

None of this research would have been possible if it was not for the opportunity afforded to me by my adviser, Dr. Thomas Wood. A sincere thanks is in order for his guidance throughout the research and the thesis writing stages. I would have never imagined that I could apply my chemical engineering degree to a molecular biology lab in a research area that has enormous potential for growth. Dr. Wood's availability and passion towards my project made the process all the more engaging.

An enormous debt of gratitude is owed to the various graduate students (Brian Kwan, Sabina Islam, and Mike McAnulty) and Post-Doc fellows (Valerie Soo) in the Wood lab family who patiently answered all of my questions on virtually any aspect of the research.

Another thank you is in order for Dr. Darrell Velegol, my academic adviser, who not only helped me throughout the years navigating the chemical engineering curriculum, but also read and provided comments on this thesis.

A final thank you is extended to friends and family members who have supported me throughout these past four years at Penn State and in the Honors College.

CHAPTER 1: RESEARCH BEGINNINGS

INTRODUCTION & LITERATURE REVIEW

Revisiting the starker side of bacterial communities and their negative effects on human life, the bacteria comprising the biofilm work in concert with one another making them highly resistant to antibiotic treatment, a concept called persistence. Persister cells are the few bacteria that withstand antibiotic treatment *without* undergoing any recognizable genetic change[1]. As the antibiotic runs its course, the select few cells withstand the wave by becoming metabolically dormant. Once the treatment has passed, these persister cells are able to spring back up and continue causing havoc to the patient. It is important to note the distinction made when detailing persistence as opposed to resistant cells. Resistant cells are those that grow in the presence of antibiotics, while persister cells do not and are tolerant to antibiotics[2].

A phenomenon called quorum sensing is at work within these microbial communities, with the toxin motility quorum sensing regulator (MqsR) being of high interest for this study. This sensing can be thought of as cell to cell communication that is a transferal of chemical signals and gives these cells an idea about the surrounding environment's conditions[3]. It comes as no surprise that quorum sensing plays a key role in persistence. The timeline for metabolic dormancy (and subsequent toxin attack) is a carefully mediated process between cells through these chemical signals.

The central dogma of molecular biology dictates the flow of genetic information within cells as going from DNA to RNA (through transcription) and finally to protein (through translation). Toxins function by interfering with this process by targeting the intermediate product, RNA and as a result bringing on metabolic dormancy to the cells which is a key

component of persistence. As is common in nature, for every toxin that exists there is often a complementary antitoxin in contest with it. The pair forms toxin-antitoxin systems which are found in all bacteria and give a better understanding to the cell's physiology especially during times of persister cell formation. Previous work has outlined four toxin-antitoxin systems at play in bacteria, each detailing a different way in which antitoxins try to combat the vicious attacks of their counterpart toxins[4].

Type	Classification
I	antitoxin RNA prevents the translation of toxin RNA
II	antitoxin protein binds and inhibits the toxin protein
III	antitoxin RNA binds and inhibits the protein toxin
IV	protein antitoxin interferes with binding of the toxin to its target

Table 1-1. Previously identified toxin/antitoxin (TA) systems

As is apparent, none of the aforementioned four systems target the heart of the central dogma that is the RNA stage, rather, they are concerned with the translation step and targeting the toxin's protein. A newly discovered "Type V" TA system has recently been discovered as the only one that cleaves toxin mRNA and serves as the basis for this research within the bacterium, *E. coli*[5].

The protein YjdO which has since been renamed to GhoT is a protein within *E. coli* that damages the membranes of cells as a lytic protein and increases persistence. GhoT's counterpart has been identified as YjdK (renamed GhoS). Antitoxin GhoS has been found to limit GhoT toxicity by cleaving the protein's mRNA. The previously mentioned, regulator, MqsR is intricately involved with the aforementioned TA system as it degrades mRNA specifically at GCU sites in the transcript. Interestingly enough, MqsR was found to cleave transcripts of

antitoxin GhoS, which led to the activation of its toxin counterpart, GhoT (does not contain the preferred GCU cleavage site as seen in figure 1-1)[5].

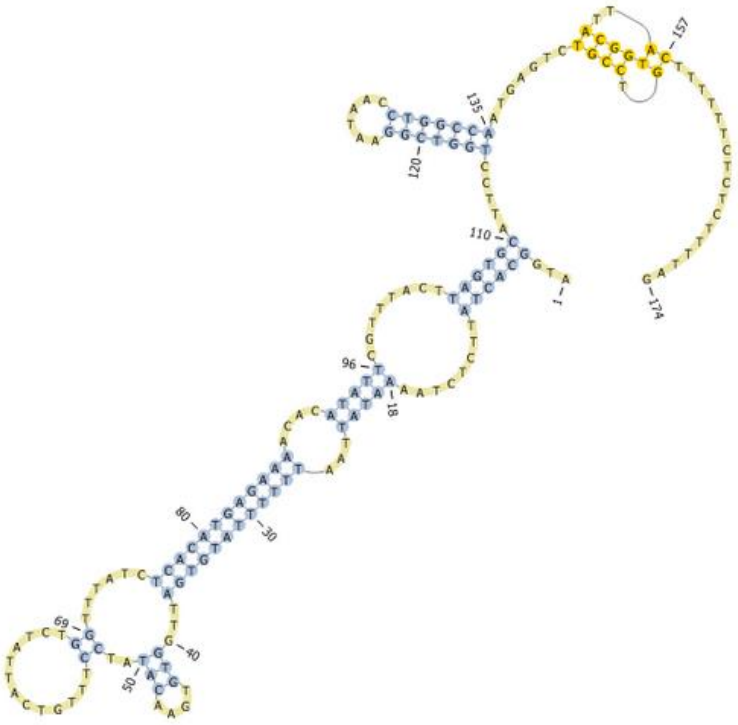

Figure 1-1. Predicted Secondary structure of GhoT with no GCT sites

This toxin activation led to an increase in persister cell formation. To further chase this finding, an initial testing of fourteen separate transcripts that lack GCU sites from the *E. coli* genome were examined in order to evaluate MqsR's ability to increase persistence[6]. As a result, researchers removed or "knocked out" each of the fourteen genes and overproduced MqsR in these strains[5]. The results showed that deleting these singular genes from *E. coli* significantly *reduced* MqsR-mediated persistence.

MOTIVATIONS FOR CURRENT STUDY

The previous work serves as the perfect backdrop for further work as only a small fraction (14 genes) of the large *E. coli* genome (4501 genes) has been examined [5]. This study aims to carefully comb the remainder of the genome and identify the sequences that have minimal GCU sites and see whether or not they exhibit the same behavior as GhoT when exposed to MqsR.

RNA transcripts within the bacterium span a broad spectrum of lengths. Some genes may be only a few hundred nucleotides long while others could number into the thousands[7]. In order to achieve a minimum free energy, the single stranded RNA often folds into secondary structures through hydrogen bonds between its base pairs. These secondary structures can take on many forms such as helices, loops, bulges, or loops[8]. A developed hypothesis suggests that when these strands form elaborate secondary structures, the sections that are stabilized by hydrogen bonds are "masked" from MqsR cleavage. It is believed, therefore, any GCU site that is tied up in one of the aforementioned secondary structures, will behave like a gene with zero GCU sites and will *not* be cleaved by MqsR, hence leading to an increase in persistence. As a result, genes that have one or two GCU sites will be examined and their secondary structure will be modeled using an algorithmic online program entitled, PknotsRG.

Capable of handling up to 800 base pairs, Pknots is a useful prediction tool for folding RNA secondary structures[9]. Pknots differs from its counterparts in the sense that its algorithmic approach takes into account the pseudoknot structure, when folding. Inherent to this structure is the presence of two stem loops in which half of one stem is placed between the halves of another stem (Figure 1-2)[10].

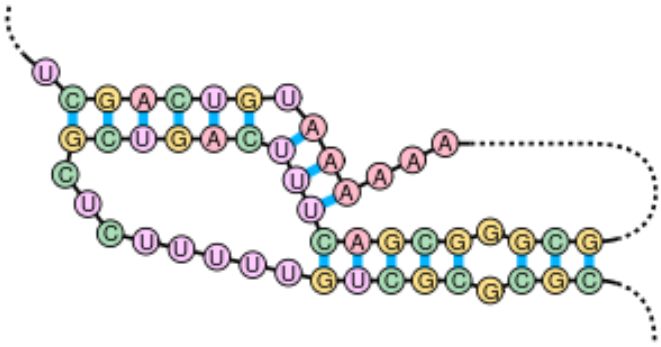

Figure 1-2. Diagram of a pseudoknot structure appearing in RNA.

As is evident, the pseudoknot structure is very important to consider as it shows the single stranded RNA base pairing with its complementary counterparts to form a more stable structure that minimizes free energy. The PknotsRG software was applied to this research and those foldings which suggest the GCU sequence is intertwined and double stranded were analyzed alongside the other, zero GCU genes.

Once the online software yielded plausible secondary structures for research, it was time to obtain them from a genome database for testing purposes. The Keio knockout collection for *E. coli* is a database of 3985 genes that contain a set of single gene, knockout mutants[11]. The fourteen genes that lacked a GCU site were used to analyze MqsR's effects were taken from this compilation to draw conclusions about persistence when the genes were not present, or knocked out. The same approach and collection was used for the suitable candidates as predicted by the folding software. The selected knockout genes tested allowed for conclusions to be drawn about the importance of these genes when they are indeed intact in the complete genome.

CHAPTER 2: EXPERIMENTAL UNDERTAKINGS

EXPERIMENTAL METHODS: BIOINFORMATICS

Often described as a conglomerate of disciplines spanning computer science, engineering, and mathematics, bioinformatics has quickly become an important course of study in recent years. Bioinformatics is the field of science that algorithmically unearths biological data that has been made available through the use of computers. This is where the initial phase for research began rooted in the various *E. coli* databases online. The goal was to uncover genes that had minimal (1 or 2) GCU sequences whose secondary structure may occlude them from being cleaved by MqsR.

A comprehensive detailing of all 4501 genes and their respective number of GCU sites had yet to be performed in previous studies. The website, Ecogene.org, provides the entire genome of *E. coli* K12 MG1655. The given gene name is also listed along with the complete nucleotide sequence[12]. Every single one of the genes was downloaded and their sequences analyzed. A simple search function yielded the amount of times GCT showed up in the gene's sequence (Note: the terms GCU and GCT can be used interchangeably as they simply correspond to the gene's RNA and DNA sequence, respectively). All findings were placed in an accessible database which allows the user to simply search for the gene name and will detail the corresponding number of GCT sites. Results spanned a broad range as there were some lengthy genes identified that had nearly 70-80 GCT sites present. The search yielded very interesting findings that bolstered related work. Wang et. al. had previously identified fourteen *E. coli* transcripts that lacked GCU sites. The comprehensive bioinformatics analysis actually showed 76 sequences which did not contain the aforementioned site; many more than what was previously identified. Furthermore, one gene out of the fourteen (*ygaQ*) actually was found to

contain 17 GCU sites, not zero as identified by the researchers. Interestingly, only a singular gene (outside of the original fourteen already studied) was found to exist within the Keio collection needed for testing on persistence. It is highly possible that since the majority of these 76 genes were shorter transcripts, they were too insignificant for the collection and were not included. Seeing that none of the newly discovered zero GCU site genes showed up in the Keio collection and therefore could not be used for experimentation, the search was increased to unearthing the genes that had a singular GCU site.

According to the bioinformatics performed, there were 113 genes that had one GCU site. Of these candidates, only 50 were found to be contained within the Keio collection. The decision was made to model all one GCU site genes using the PknotsRG software and as a first step, identify candidates that contained a GCU site that was *not* masked by its secondary structure hence made it susceptible to MqsR attack. These few genes identified will serve as a control group as it is suspected that they will be degraded by the aforementioned toxin and not lead to an increase in persistence.

Pknots modeling identified six genes that showed an unprotected GCT site in its secondary structure (*glgS*, *ogrK*, *tdcR*, *yaiZ*, *ydfZ*, *yggL*).

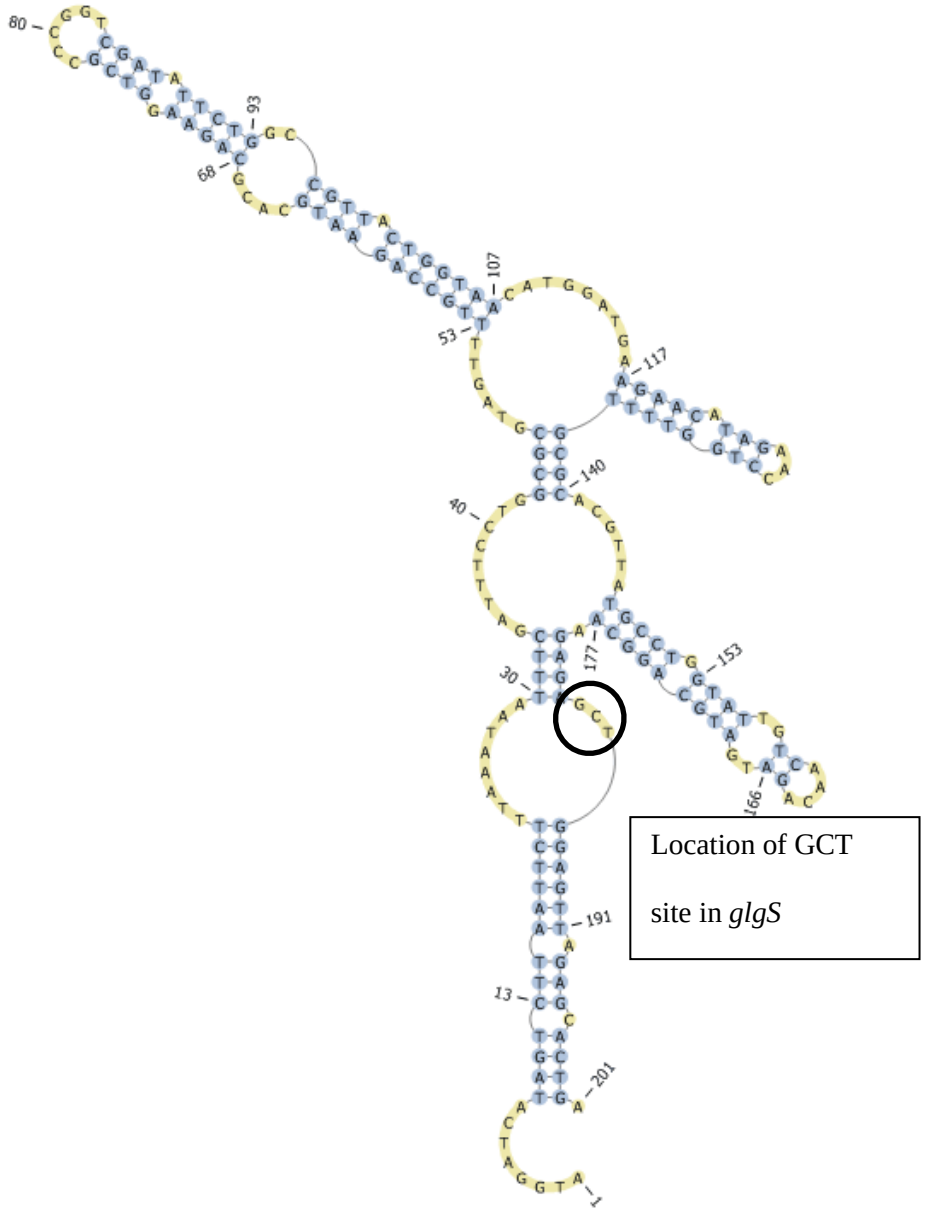

Figure 2-1. Secondary structure of *glgS* with its GCT site identified

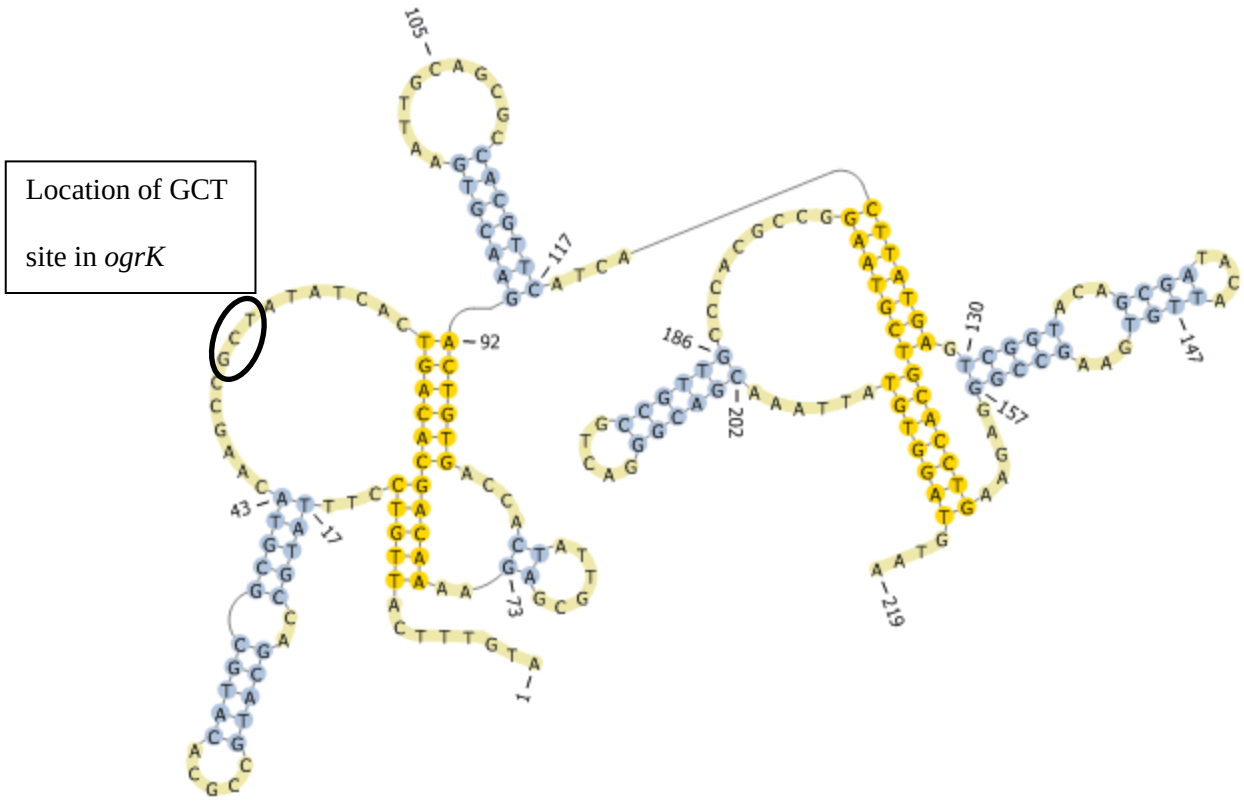

Figure 2-2. Secondary structure of *ogrK* with its GCT site identified

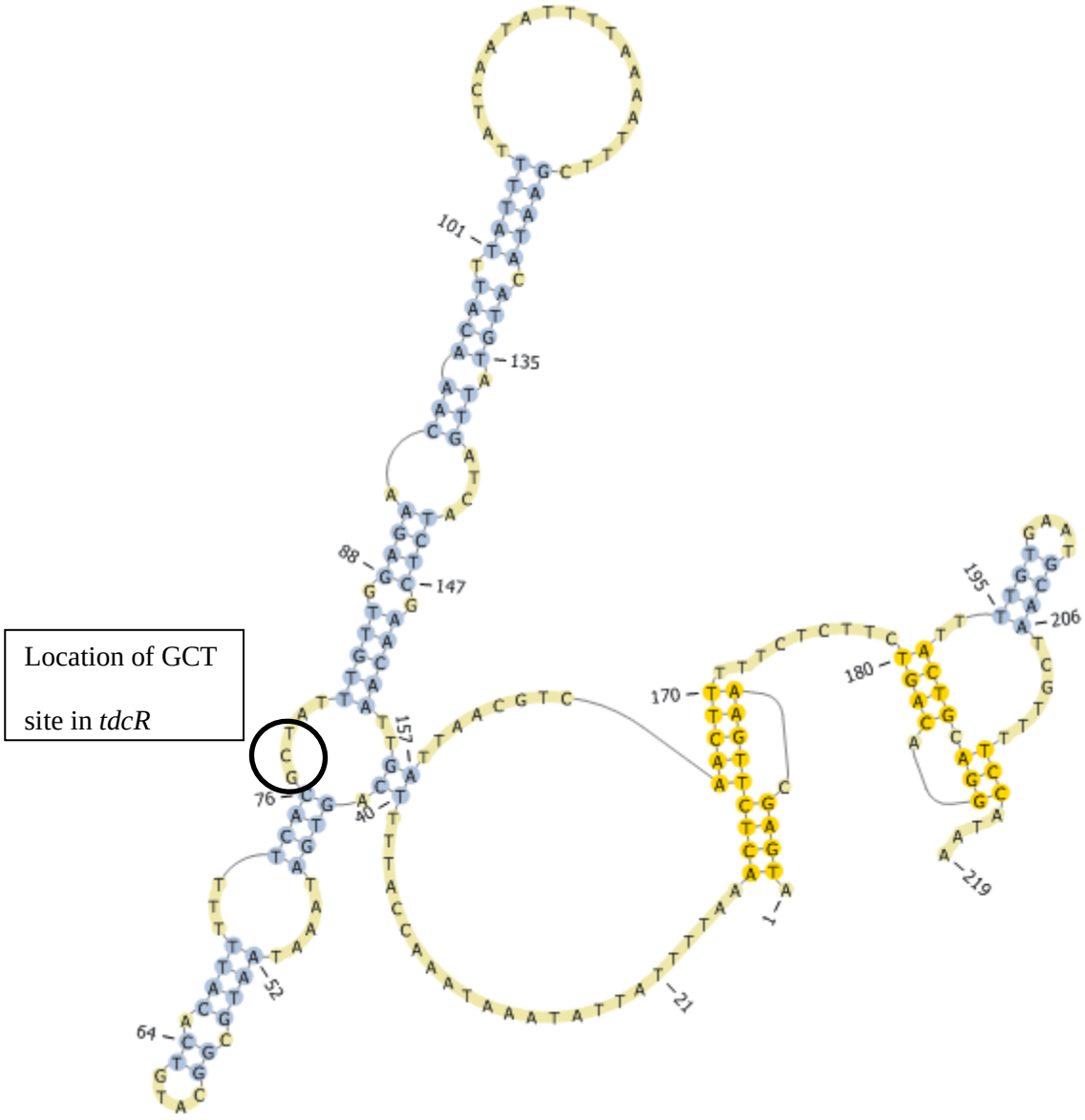

Figure 2-3. Secondary structure of *tdcR* with its GCT site identified

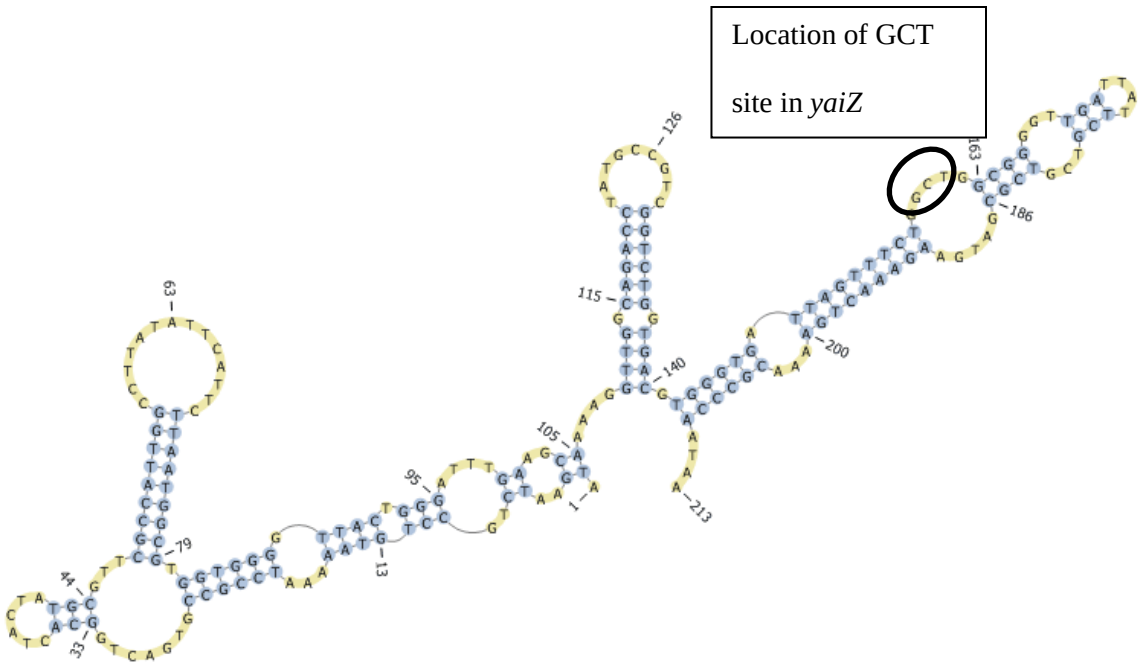

Figure 2-4. Secondary structure of *yaiZ* with its GCT site identified

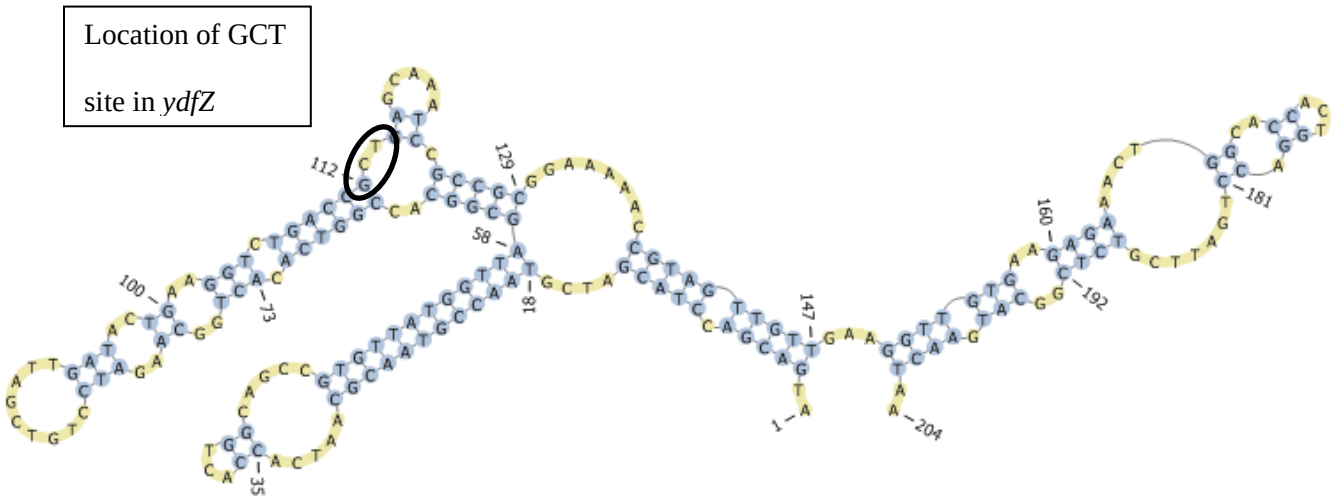

Figure 2-5. Secondary structure of *ydfZ* with its GCT site identified

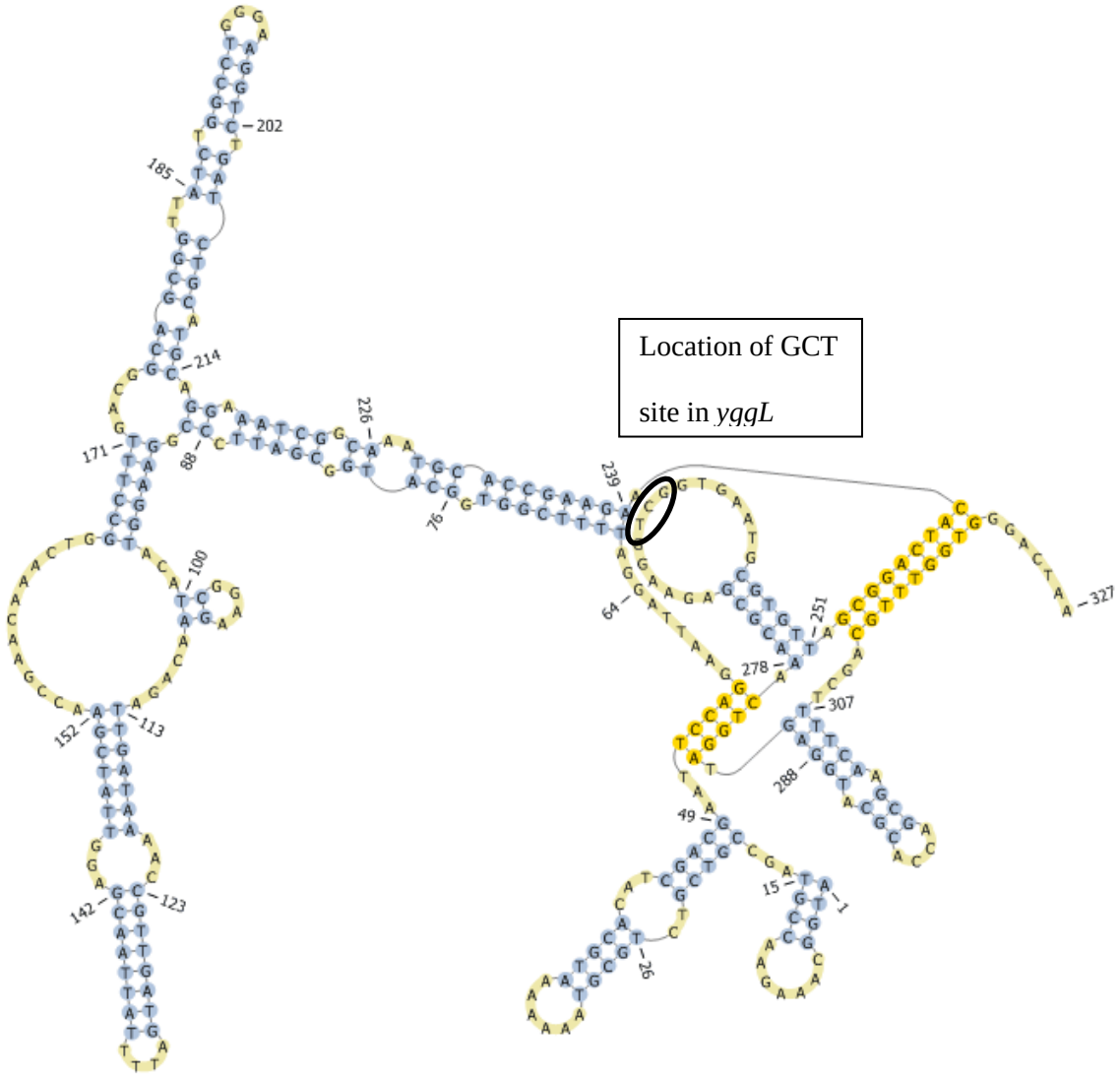

Figure 2-6. Secondary structure of *yggL* with its GCT site identified

EXPERIMENTAL METHODS: PERSISTENCE ASSAYS

Any molecular biology laboratory often makes use of a particular assay in order to quantitatively measure a phenotype of interest. The six genes that were identified as having susceptible GCT sites were studied using persistence assays. Inherent to this assay is selecting genes from the Keio collection that have the gene of interest knocked out. As a result, the main conclusion that can often be drawn from a persistence assay is that if a certain knockout gene is found to show an increase in persistence, then when this gene is intact in the bacterial genome it *may* lower persistence. The converse holds true as well: If a knockout gene shows a decrease in persistence, then when the gene is found in the genome, then it *may* be important to higher persistence. These assays can serve as a good starting point for further studies to eventually draw distinct conclusions on the gene's role with regards to persistence.

With the goal of running the persistence assay, the knockout genes from the Keio collection had to be obtained and grown in appropriate conditions to begin experimentation. All six strains with the genes deleted were taken from the collection and streaked on Luria-Bertani (LB) agar plates made on petri dishes (Figure 2-7)[13].

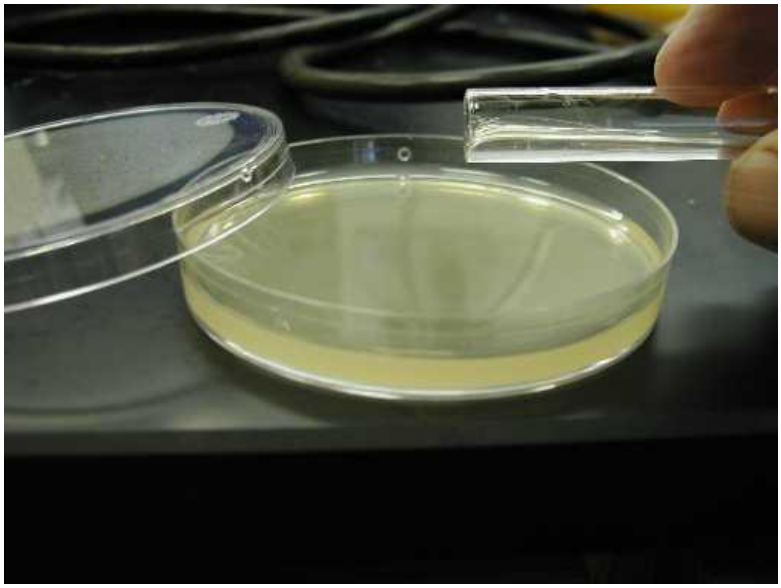

Figure 2-7. Agar plate used for cell colony growth

Once fully streaked, the plates were placed in an incubator to allow cells to grow at 37 ^{o}C which is determined to be the optimum growth temperature for the *E. coli* bacterium. Following overnight growth, the plates were removed and a multitude of colonies were present. Observations indicated that each of the plates was littered with colonies with each colony containing a number of cells. The cells on these plates were only viable for a limited amount of time (two days maximum) and hence had to be harvested and stored for future use and experiments. In order to preserve the integrity of the cells, a colony can be picked and immersed in LB liquid media and glycerol, frozen to a temperature of -80 ^{o}C, and housed in a freezer for future use in persistence assays. These are commonly known as glycerol stocks and are important to maintaining the long term storage of the gene[14].

To accomplish this, 25 mL of LB media was put into six separate flasks along with 25 µL of Kanamycin antibiotic (50 mg/mol stock solution). As bacteria grow, there are always possibilities of mutations occurring in the DNA sequence. Every time a cell multiplies, there are more chances for mutations and standardization of testing decreases. Antibiotics, such as Kanamycin are used to eliminate any unwanted, mutated cells that do not possess the correct strain wanted for testing. The desirable cells withstand the antibiotic and are isolated. Following addition, a wire loop was sterilized with a Bunsen burner flame and cooled. A singular colony was picked from the overnight agar plates with the loop and immediately immersed in the liquid broth of media. The loop was swirled to ensure the proper transfer of the colony. Once this was accomplished for all six genes, the flasks were put in an incubator shaker at 250 RPM, 37 ^{o}C, for 16 hours of growth. The following day, one mL of the culture was transferred to vials containing glycerol. The newly formed solutions were vortexed (mixed) and then placed in the -80 ^{o}C freezer.

With the glycerol stocks available for use, it came time to run the persistence assay experiment. The total process takes four days with each day requiring time sensitive steps that allow for proper cell growth to be used for the experiment. On the first day, the gene of interest that will be tested is isolated and its glycerol stock is removed from the freezer. Whenever a single gene knock out strain is being tested (like one of the six detailed above), it is always important to also test a standard in which results can be compared to. This standard also has its own glycerol stock and is commonly known as Wild Type (WT). Wild type refers to the naturally occurring genetic composition of an organism without any changes to its makeup[15]. In the case of *E. coli*, this refers to an organism with its complete genome intact rather than having one of its genes being knocked out. With the sample and WT standard removed from its cold environment, a sterilizing wire loop is passed under a hot flame, cooled, and then carefully touched on the surface of the glycerol vials. The small contact between the loop and the stock is enough to transfer ample amounts of colonies onto its surface. Immediately following this, the loop is smeared across an LB agar plate. The plate is completely streaked with the gene of interest and then covered. The same procedure is repeated for the WT standard. Both plates are then placed in an incubator oven at 37 ^{o}C for a time period of ~24 hours.

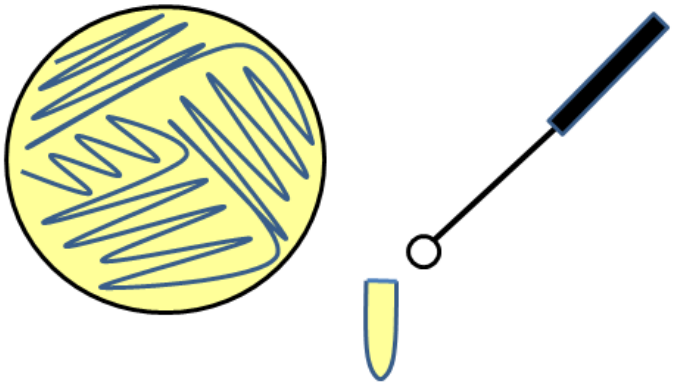

Figure 2-8. Hand drawn picture of glycerol stock, wire loop, and proper streak technique

The following day, the cultures had to be inoculated and its procedure mimics the glycerol stock preparation with some key differences. The inoculation step on the second day of experimentation meant that the researcher had to prepare the samples in the proper liquid media for ample cell growth. The plates from the previous day were removed from the incubator and numerous colonies were observed on the surface at the locations where the wand streaked over. After removal, 25 mL of LB media was drawn into flasks. The general guideline followed was to have two flasks per each strain tested for comparison purposes. In addition to the LB media, the antibiotic Kanamycin was added to achieve a final concentration of 50 µg/mL. Seeing that the numerous colonies on the agar plates represented an identical compilation of cells, any one of these could be picked for the experiment. With the sterilizing loop, a singular colony was picked and swirled in the liquid media. Once the colony transfer was complete, the flasks were labeled and placed in the incubator shaker at 250 RPM, 37 ^{o}C, for 16 hours of growth.

The third day was the most important as the actual persistence assay was performed at this time. Following the 16 hours of growth, the flasks were removed from the shaker and 25 µL from these were removed through a pipet and placed into new flasks of fresh LB media (25 mL). The new flasks were placed in the shaker for an additional three hours of growth. The refreshing of the media allowed for the samples to reach the desired period of rapid growth or commonly referred to as the exponential phase. In order to accurately study the *E. coli* physiology, experiments are often conducted during this growth phase[16]. The exponential phase is described as the rapid division phase as cells have the proteins they already need from the media and divide under the least amount of metabolic stress. There are seemingly unlimited amount of nutrients available. As resources become used up, growth rates often plateau as metabolites are less readily available. This can be described as the stationary phase. After the three hours of

additional growing time, in order for the experiment to begin, samples had to have a uniform cell density when running the assay. This allows for a standardization of samples and limits the amount of variability between trials. Cell density is often quantified using absorbance through a device called a fluorometer. By inserting a one mL sample into the machine, an optical density reading at 600 nanometers (OD_{600}) is produced which ranges anywhere from a value of 0 to 1.

The ideal testing conditions for the persistence assay call for an OD_{600} measurement of 0.8. It is important to note that this number is specific to the type of media the assay is conducted in. Once a sample has reached this threshold, an additional 1 mL is removed and placed in a centrifuge tube for later. Furthermore, this OD measurement signals the time for antibiotic introduction. The underlying premise that was being studied is the fact that cells that survive after antibiotics are called persisters. By taking a before and after snapshot of those cells, it will shed some light on persistence in the various *E. coli* strains.

The two antibiotics used in this experiment were Ampicillin and Ciprofloxacin. In general, there are often two classes of antibiotics used: bacteriostatic and bactericidal. Bacteriostatic are often thought of as bacteria inhibiting drugs, while bactericidal leads to cell death. The two aforementioned antibiotics are bactericidal drugs. These are the best choices when trying to measure persistence and comparing the cells that survived to the ones that died.

Whatever the remaining volume of the flask is dictates the amount of antibiotic added to the samples. The general rule was to follow a thousand fold addition for the Ampicillin (If flask volume equals 22 mL, then add 22 µL of antibiotic). After introduction, the antibiotic ran its course for three hours as all flasks were in the shaker. During this time, the 1 mL samples that were removed in centrifuge tubes *before* being impacted by antibiotics were isolated and tested. For each strain that was removed, seven additional centrifuge tubes were laid out (Figure 2-9).

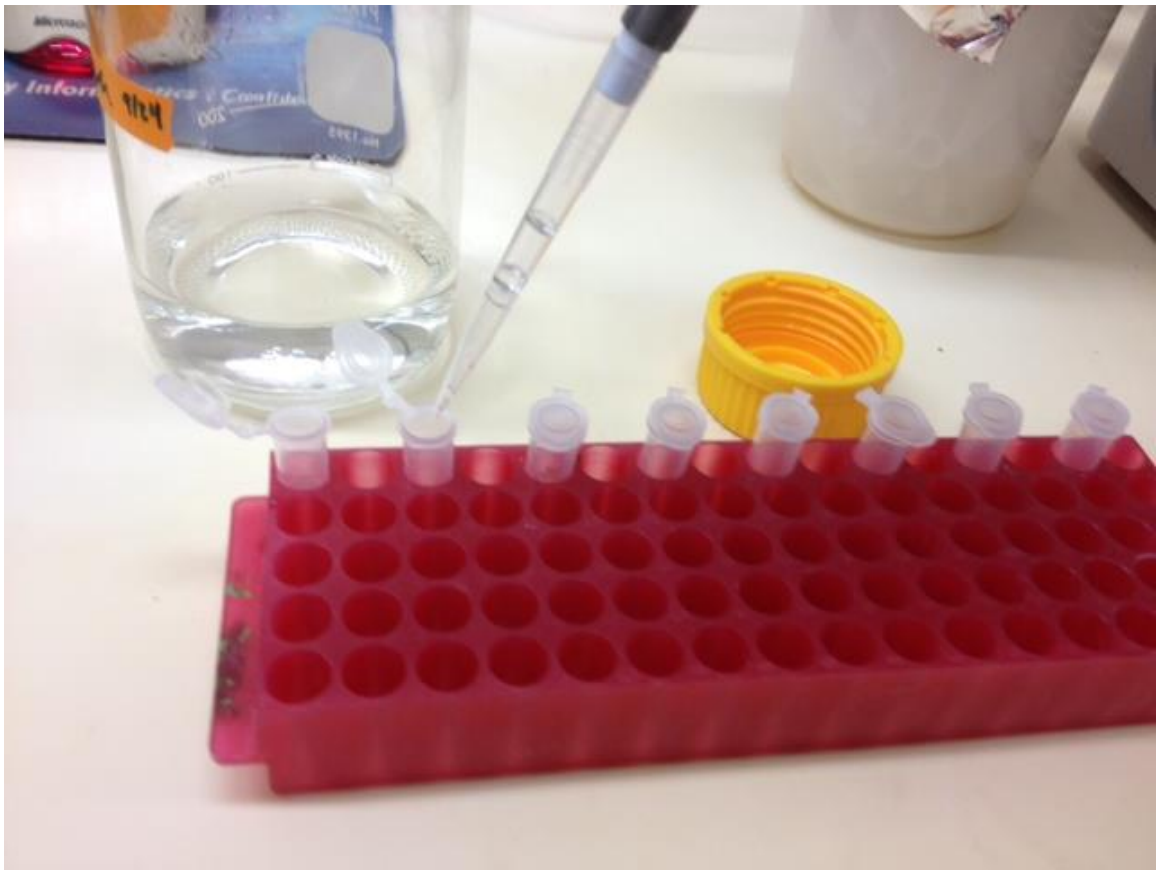

Figure 2-9. Serial dilution being carried out in microfuge tubes.

Each of the remaining tubes was filled with 900 µL of 0.85% NaCl solution. The 1 mL tube containing the sample immersed in the liquid LB was placed in the centrifuge machine at 13,000 RPM for one minute to allow the individual cells to settle at the bottom. The supernatant LB layer was then discarded and the remaining cluster of cells was refreshed with 0.85% NaCl solution.

A serial, tenfold dilution was then performed as 100 µL of the first tube that contained all of the cells was transferred to the second microfuge tube. The second tube was mixed and 100 µL of that sample was transferred to the third tube. This continued all the way down the line for the remaining tubes to give a total of eight needed for plating. An agar plate was then carefully

annotated with a marker (called 0 hour plates) to identify the eight dilution quadrants (Figure 2-10). Three, 10 µL droplets were placed in their respective areas on the plate and were allowed to grow on the surface for a day in the 37 ^{o}C incubator.

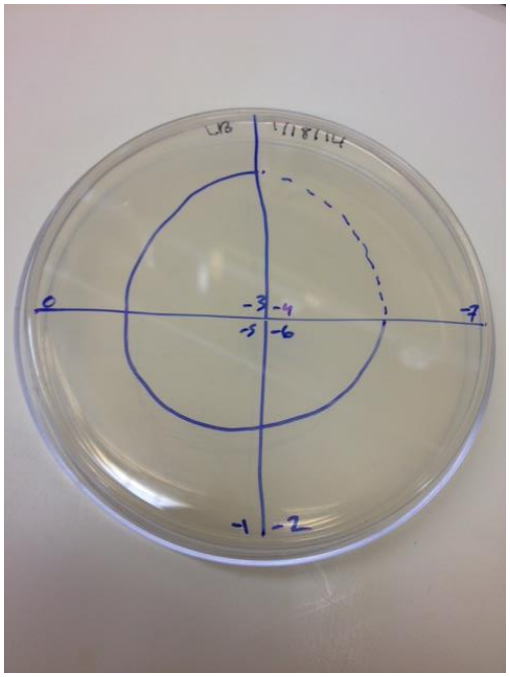

Figure 2-10. Sample LB plate ready for drop plating.

After the Ampicillin had spent its three hours in the flasks, these were also removed and the same series of steps were taken with these samples (called 3 hour plates). The dilutions were carried out and drop plating followed in the same fashion with the plates being placed in the incubator along with its 0 hour counterparts.

Analysis was typically carried out on the fourth day of the experiment as it came time to quantify the number of colonies showing up in each dilution quadrant. The plates were removed from the incubator and various colonies showed up in the locations they were drop plated. Using a marker, the three individual drops were examined and counted with each little dot signifying one colony of cells. Results were then recorded and an average number of colonies for each

dilution was calculated. When performing this for each strain, there were quite a few apparent observations. On quadrants with lower dilutions (0,-1,-2,-3,-4) there were so many colonies that they all blended together and it was indiscernible to count individual ones. At the -5 and -6 dilutions however, there tended to be a wide range of countable colonies with each spot exhibiting anywhere from 1 to 60 colonies. At the last dilution (-7) it was extremely rare to see any formation as the sample was too diluted by this stage.

Before proceeding with further analysis it is important to consider once again the trends that are being studied concerning persistence and the toxin MqsR. Once again, if a gene is knocked out from the *E. coli* genome (as it has been with the strains that have been used in this experiment), and an increase in persistence is observed, then when gene X is in the bacteria, it exhibits lower persistence. If a decrease in persistence is observed with a knockout gene, then when it is intact in the genome, then you have increased persistence. Furthermore, a deletion leading to a decrease in persistence suggests MqsR selectively cleaves less frequently to increase persistence and *may* be important for a cell to survive as a persister. With this information, it came time to quantify whether or not the cell demonstrated an increase or decrease in persistence.

Following colony counting, the number of colony forming units (CFU's) were determined for each spot on the dilution quadrants. For example, if there were 20 colonies counted on the "-5" dilution section, this meant there were $20*(10^8)$ CFU/mL. This number was found by multiplying the 10 µL (volume of individual droplet on plate) by 100 (to get into mL), then multiplying the result by 10^5 as it was the fifth dilution section. The average value for the three droplets was taken and the standard deviation between the spots was calculated as well. The percent survival was then calculated for the strains as this was simply defined as the number

of CFU/mL in the 3 hour antibiotic addition samples divided by the number of CFU/mL in the 0 hour samples that were void of any antibiotic. Lastly, for the knockout strains of interest in the experiment, a fold change was calculated relative to Wild Type standard. This was simply the percent survival of the knockout strain divided by the percent survival for the Wild Type. The fold changes are the best indicator as to whether or not there was an increase or decrease in persistence.

CHAPTER 3: EXPERIMENTAL RESULTS OF SIX INITIAL STRAINS

yaiZ & *yggL* Strains

As mentioned, there were six strains of interest (*glgS*, *ogrK*, *tdcR*, *yaiZ*, *ydfZ*, *yggL*) that were studied to have a singular GCT site which was unprotected as postulated by its secondary structure. Experiments were conducted in sets of two strains coupled with the WT standard. For the first round of testing, *yaiZ* and *yggL* were plated from their glycerol stocks. Table 3-1 shows the measured OD_{600} measurements for the strains before the experiment began.

Strain	Flask 1	Flask 2
MG 1655 Wild Type	0.764	0.744
MG 1655 yaiZ	0.754	0.808
MG 1655 yggL	0.777	0.746

Table 3-1. OD_{600} Readings for *yaiZ* and *yggL*

For persistence assays, 1 mL was removed. Upon removal, 220 µL of 10 mg/mL ampicillin stock was added to the *yggL* samples, 210 µL to WT 2, 200 µL to *yaiZ* samples, and 190 µL to WT 1. The reason for the differing amounts of antibiotic added to the flasks was to maintain a steady concentration of 0.1 mg/mL of ampicillin. The antibiotic ran its course for three hours and 1 mL was removed towards drop plating for the persistence assay.

Table 3-2 provides a summary of the fold changes observed for the two strains. The results showed an interesting behavior for both strains that were tested. First, the *yaiZ* (-3.66 fold) sample demonstrated a decrease in persistence upon exposure to Ampicillin as the antibiotic of choice. The second strain tested, *yggL* (-8.24 fold) saw the same trend in persistence.

Strain	Time	Condition	CFU/mL	stdev	%Survival	Fold Change
MG 1655 Wild Type: Flask 1	0 hour	No Treatment	3.8E+08	2.65E+07		
	3 hour	Amp 10	2.77E+04	3.51E+03	7.28E-03	
MG 1655 Wild Type: Flask 2	0 hour	No Treatment	4.4E+08	1.73E+07		
	3 hour	Amp 10	3.20E+04	3.00E+03	7.27E-03	
BW25113 yaiZ: Flask 1	0 hour	No Treatment	4.23E+08	8.50E+07		
	3 hour	Amp 10	1.07E+04	3.21E+03	2.52E-03	-2.890
BW25113 yaiZ: Flask 2	0 hour	No Treatment	4.27E+08	7.57E+07		
	3 hour	Amp 10	7.00E+03	1.73E+03	1.64E-03	-4.433
BW25113 yggL: Flask 1	0 hour	No Treatment	2.83E+08	3.06E+07		
	3 hour	Amp 10	3.23E+03	404.1452	1.14E-03	-6.380
BW25113 yggL: Flask 2	0 hour	No Treatment	3.33E+08	2.08E+07		
	3 hour	Amp 10	2.40E+03	4.58E+02	7.20E-04	-10.101

Table 3-2. Summary of findings for *yaiZ* and *yggL*

glgS & *ogrK* Strains

Moving on to the second round of testing, *glgS* and *ogrK* were plated from their glycerol stocks. Table 3-3 shows the measured OD_{600} measurements for the strains before the experiment began.

Strain	Flask 1	Flask 2
MG 1655 Wild Type	0.834	0.829
MG 1655 glgS	0.828	0.851
MG 1655 ogrK	0.829	0.798

Table 3-3. OD_{600} Readings for *glgS* and *ogrK*

For persistence assays, 1 mL was removed. Upon removal, 210 µL of 10 mg/mL Ampicillin stock was added to the *glgS* and *ogrK* samples and 200 µL to WT samples. The reason for the differing amounts of antibiotic added to the flasks was to maintain a steady concentration of 0.1 mg/mL of Ampicillin. The antibiotic ran its course for three hours and 1 mL was removed towards drop plating for the persistence assay.

Table 3-4 provides a summary of the fold changes observed for the two strains. The results showed an interesting behavior for both strains that were tested. First, the *glgS* (-1.76 fold) sample demonstrated a decrease in persistence upon exposure to Ampicillin as the antibiotic of choice. The second strain tested, *ogrK* (-4.23 fold) saw the same trend in persistence.

Strain	Time	Condition	CFU/mL	stdev	%Survival	Fold Change
MG 1655 Wild Type: Flask 1	0 hour	No Treatment	3.07E+08	1.15E+07		
	3 hour	Amp 10	2.93E+04	2.08E+03	9.57E-03	
MG 1655 Wild Type: Flask 2	0 hour	No Treatment	3.9E+08	3.00E+07		
	3 hour	Amp 10	2.73E+04	6.03E+03	7.01E-03	
BW25113 glgS: Flask 1	0 hour	No Treatment	3.27E+08	2.08E+07		
	3 hour	Amp 10	1.30E+04	3.00E+03	3.98E-03	-2.40E+00
BW25113 glgS: Flask 2	0 hour	No Treatment	3.4E+08	5.00E+07		
	3 hour	Amp 10	2.13E+04	2.08E+03	6.27E-03	-1.12E+00
BW25113 ogrK: Flask 1	0 hour	No Treatment	4.03E+08	6.81E+07		
	3 hour	Amp 10	8.33E+03	3.06E+03	2.07E-03	-4.63E+00
BW25113 ogrK: Flask 2	0 hour	No Treatment	3.83E+08	3.06E+07		
	3 hour	Amp 10	7.00E+03	0.00E+00	1.83E-03	-3.84E+00

Table 3-4. Summary of findings for *glgS* and *ogrK*

tdcR & *ydfZ* Strains

For the last round of experiments on the six postulated genes, *tdcR* and *ydfZ* were plated from their glycerol stocks. Table 3-5 shows the measured OD_{600} measurements for the strains before the experiment began.

Strain	Flask 1	Flask 2
MG 1655 Wild Type	0.744	0.782
MG 1655 tdcR	0.749	0.776
MG 1655 ydfZ	0.749	0.775

Table 3-5. OD_{600} Readings for *tdcR* and *ydfZ*

For persistence assays, 1 mL was removed. Upon removal, 210 µL of 10 mg/mL Ampicillin stock was added to the WT and *tdcR* samples and 190 µL to *ydfZ* samples. The reason for the differing amounts of antibiotic added to the flasks was to maintain a steady concentration of 0.1 mg/mL of Ampicillin. The antibiotic ran its course for three hours and 1 mL was removed towards drop plating for the persistence assay.

Table 3-6 provides a summary of the fold changes observed for the two strains. The results showed an interesting behavior for both strains that were tested. First, the *tdcR* (+4.97 fold) sample demonstrated an increase in persistence upon exposure to Ampicillin as the antibiotic of choice. The second strain tested, *ydfZ* (-1.44 fold) saw the opposite trend in persistence. The *tdcR* strain presents interesting results as it is the only strain out of the six tested (*yggL, yaiZ, glgS, ogrK, tdcR,* and *ydfZ*) that shows an increase in persistence upon deletion.

Strain	Time	Condition	CFU/mL	stdev	%Survival	Fold Change
MG 1655 Wild Type: Flask 1	0 hour	No Treatment	3.63E+08	3.21E+07		
	3 hour	Amp 10	1.83E+04	2.89E+03	5.05E-03	
MG 1655 Wild Type: Flask 2	0 hour	No Treatment	4.07E+08	2.89E+07		
	3 hour	Amp 10	1.27E+05	3.06E+04	3.11E-02	
BW25113 tdcR: Flask 1	0 hour	No Treatment	3.4E+08	3.61E+07		
	3 hour	Amp 10	1.43E+05	2.52E+04	4.22E-02	8.35E+00
BW25113 tdcR: Flask 2	0 hour	No Treatment	3.87E+08	2.52E+07		
	3 hour	Amp 10	1.90E+05	2.65E+04	4.91E-02	1.58E+00
BW25113 ydfZ: Flask 1	0 hour	No Treatment	3.93E+08	3.51E+07		
	3 hour	Amp 10	1.50E+04	3.61E+03	3.81E-03	-1.32E+00
BW25113 ydfZ: Flask 2	0 hour	No Treatment	3.5E+08	3.61E+07		
	3 hour	Amp 10	7.00E+04	2.65E+04	2.00E-02	-1.56E+00

Table 3-6. Summary of findings for *tdcR* and *ydfZ*

CHAPTER 4: PHASE TWO EXPERIMENTS

Previous work performed had concentrated on six knockout strains of *E.coli* (*yaiZ, yggL, glgS, ogrK, tdcR, and ydfZ)* that contain a singular GCT site that would be potentially cleaved by the toxin MqsR. These genes were first tested due to the fact that they possessed secondary structures showing the availability of the aforementioned GCT site and were not masked by folding. These strains were tested using persistence assays that showed modest fold changes in persistence. The relatively low standard deviations confirm the success of these experiments (refer to Appendix for fold change derivations).

The next task was to reevaluate the compiled list of genes within the bacterium that have one GCT site and look for ones with secondary structures that mask or cover the three letter sequence. Further, it was desired to find candidates from the list that are known toxins according to gene databases such as ecogene.org and ecocyc.com.

The following genes meet the two criteria mentioned above:

1) *yoeB*: The YoeB toxin induces cleavage of translated mRNAs. YoeB can be activated by overproduction of the Lon protease, which is lethal
2) *hicA:* HicA is an mRNA interferase acting as the toxin of the HicA-HicB toxin-antitoxin system. Overexpression of HicA induces mRNA cleavage and growth inhibition, but not cell death

Although there were only two known toxins found in this list, the search was expanded with these other genes. Going back to the original goal, there were plenty of options of genes that have a singular GCT site that is masked by secondary structure. The following strains were chosen to be tested: *ygiW, yhhY, yciH, flgM, and yiaG.* All of these five, alongside the

aforementioned two toxins display double stranded interactions with other segments of the RNA strand that occludes the GCT site. For this second round of testing, the decision was made to use 5µg/mL Ciprofloxacin as the antibiotic of choice to try and see whether or not this would affect the trends in persistence.

Pknots modeling was performed on these seven genes as well to verify the occluded structure of the GCT sites.

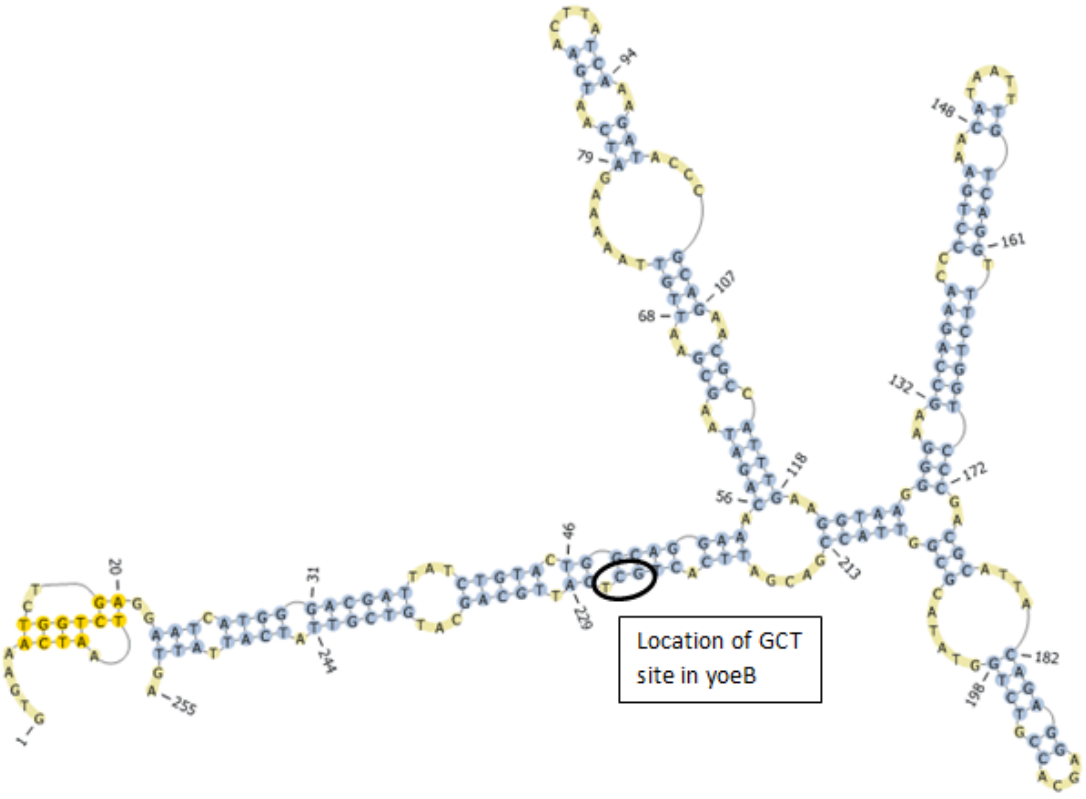

Figure 4-1. Secondary structure of *yoeB* with its GCT site identified

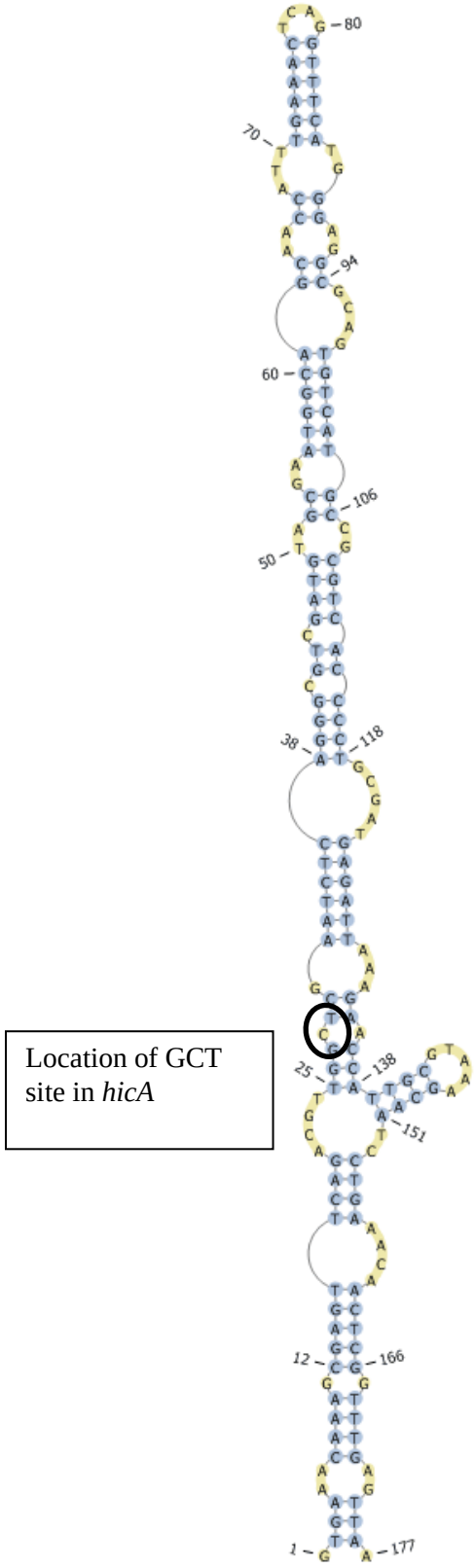

Figure 4-2. Secondary structure of *hicA* with its GCT site identified

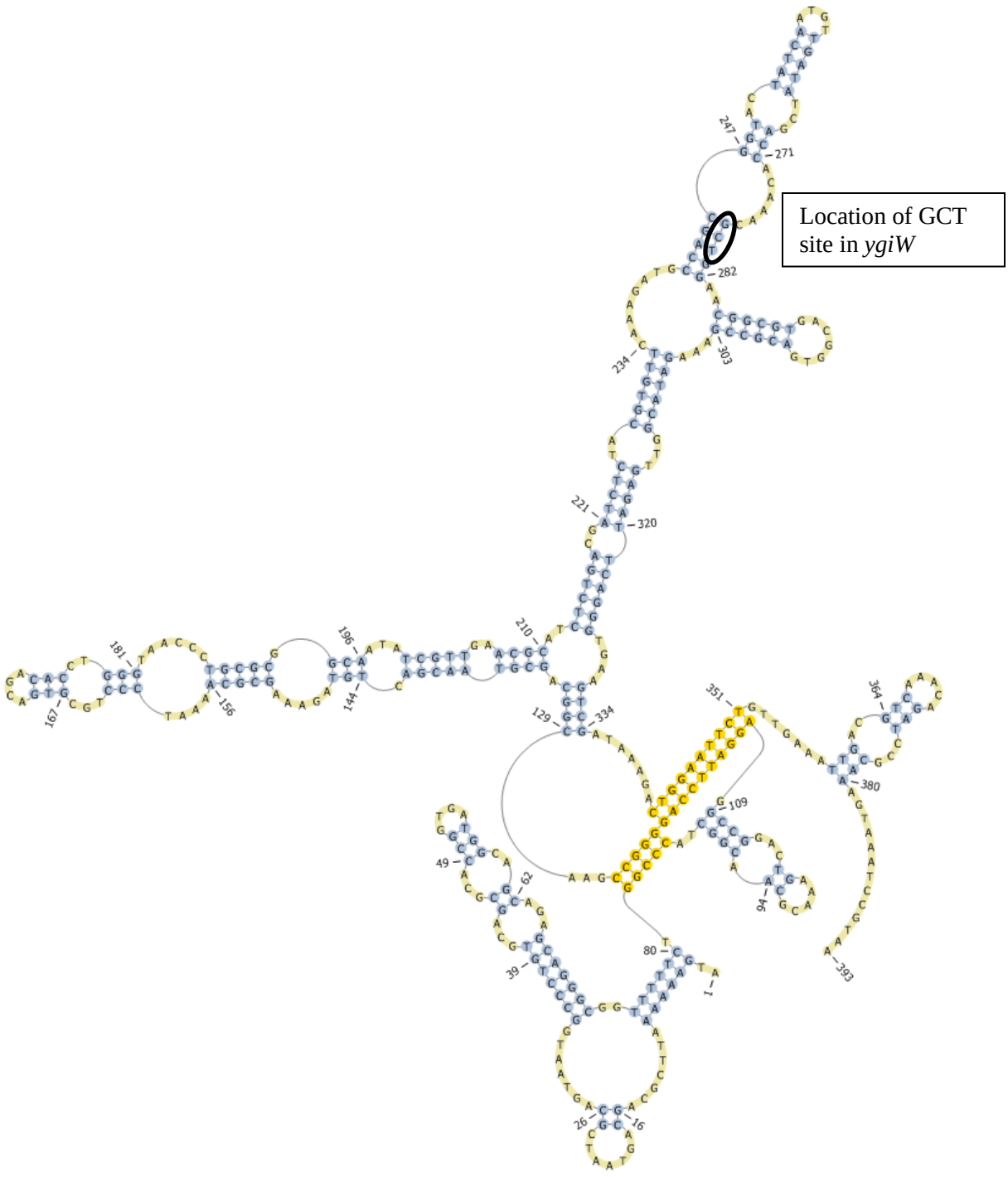

Figure 4-3. Secondary structure of *ygiW* with its GCT site identified

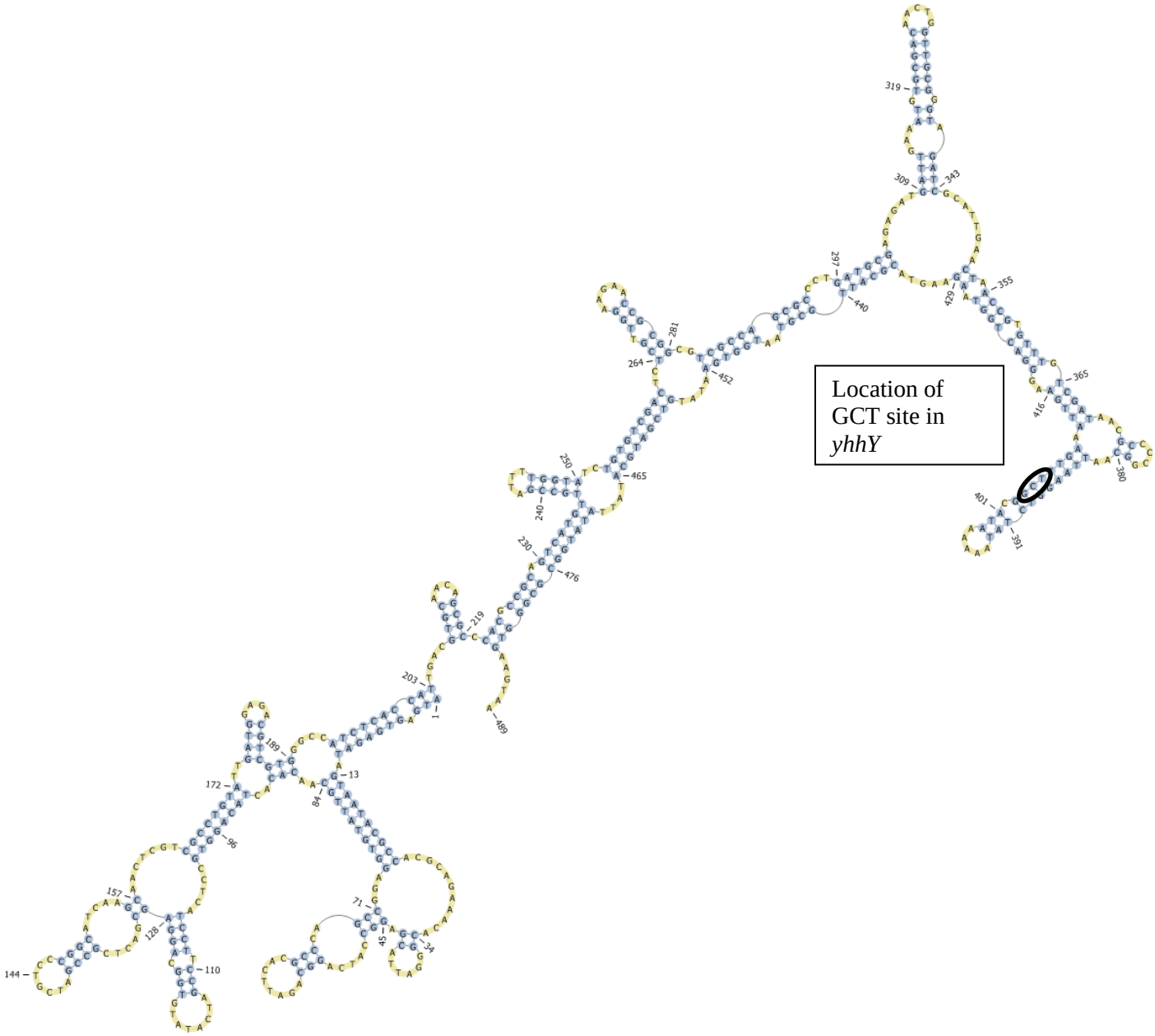

Figure 4-4. Secondary structure of *yhhY* with its GCT site identified

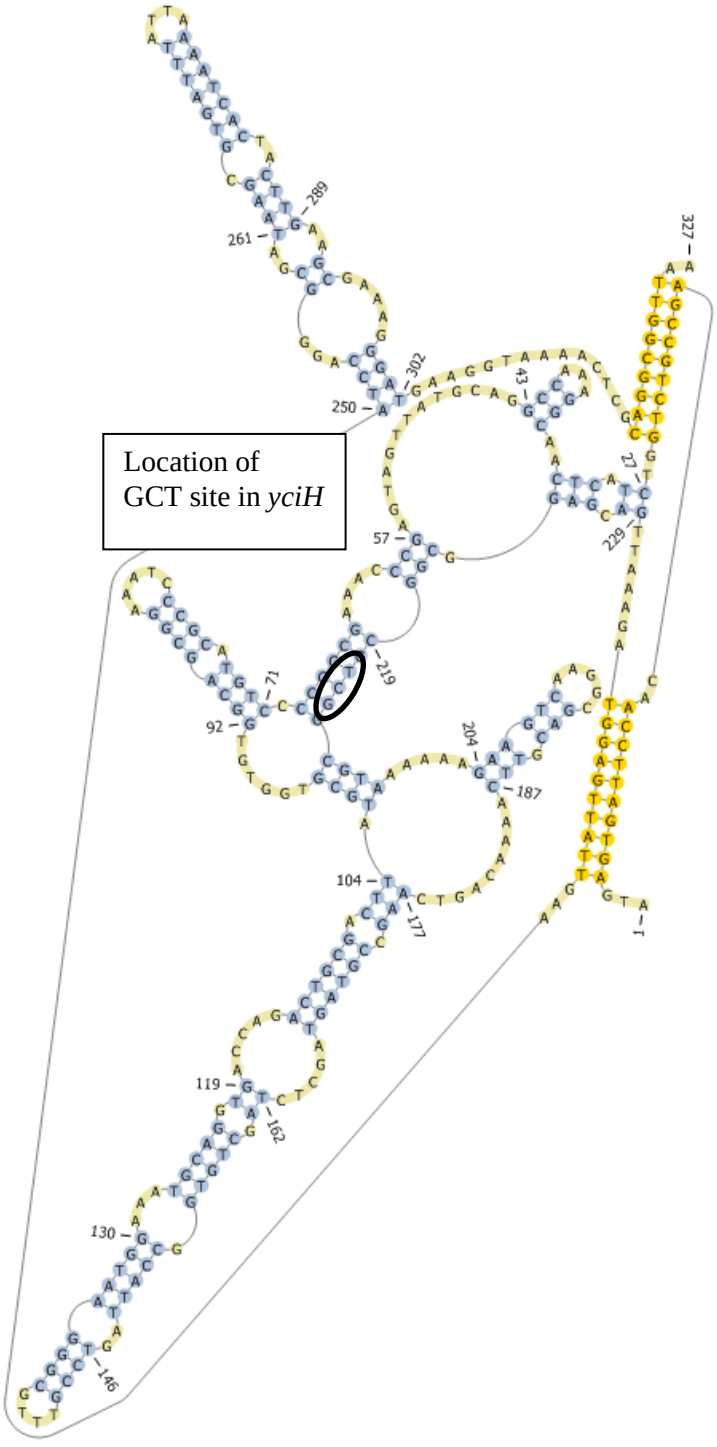

Figure 4-5. Secondary structure of *yciH* with its GCT site identified

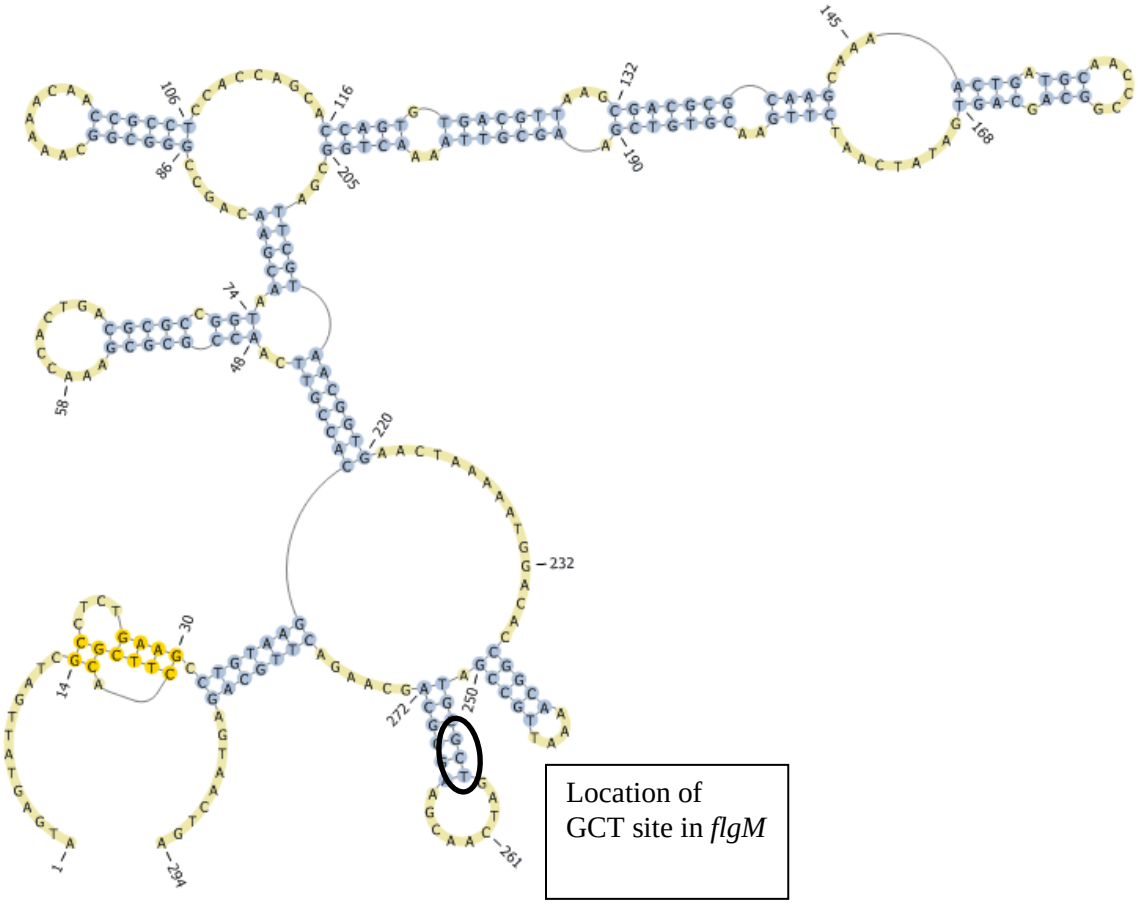

Figure 4-6. Secondary structure of *flgM* with its GCT site identified

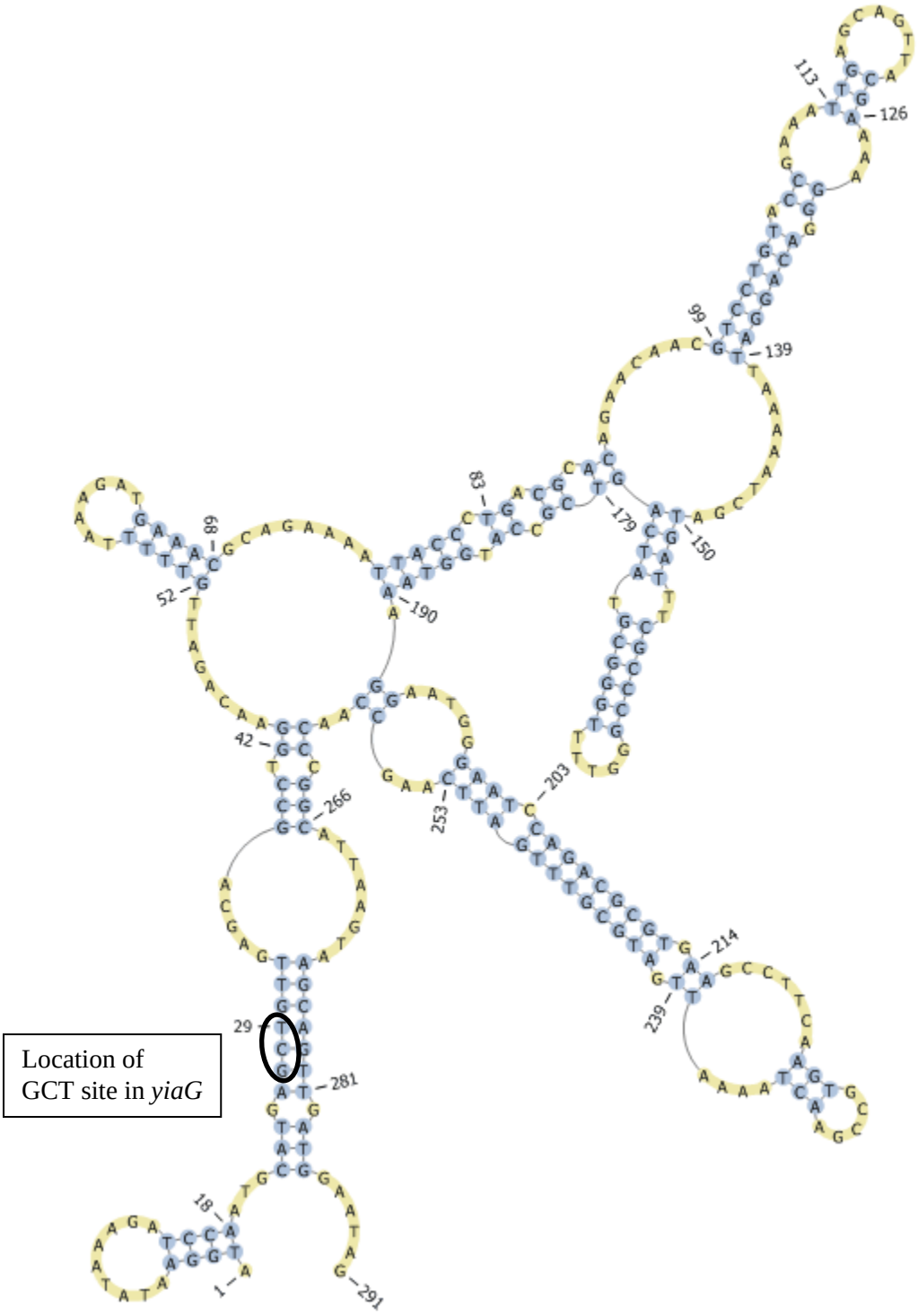

Figure 4-7. Secondary structure of *yiaG* with its GCT site identified

ygiW & *yhhY* Strains

To begin the phase two experiments on *E. coli* genes whose secondary structure seems inaccessible to MqsR cleavage, *ygiW* and *yhhY* were first tested from their glycerol stocks. Table 4-1 shows the measured OD_{600} measurements for the strains before the experiment began.

Strain	Flask 1	Flask 2
MG 1655 Wild Type	0.789	0.815
MG 1655 ygiW	0.746	0.756
MG 1655 yhhY	0.807	0.81

Table 4-1. OD_{600} Readings for *ygiW* and *yhhY*

For persistence assays, 1 mL was removed. Upon removal, 210 µL of 0.5 mg/mL Ciprofloxacin stock was added to the *ygiW 2* and *yhhy 2* samples and 200 µL to the WT samples, *ygiW 1* and *yhhY 1*. The reason for the differing amounts of antibiotic added to the flasks was to maintain a steady concentration of .005 mg/mL of Ciprofloxacin. The antibiotic ran its course for three hours and 1 mL was removed towards drop plating for the persistence assay.

Table 4-2 provides a summary of the fold changes observed for the two strains. First, the *ygiW* (+1.37 fold) sample demonstrated an increase in persistence upon exposure to Ciprofloxacin as the antibiotic of choice. The second strain tested, *yhhY* (+3.43 fold) saw the same trend in persistence. Judging by these results of a positive fold increase, a deletion meant that we observed an increase in persistence.

Strain	Time	Condition	CFU/mL	stdev	%Survival	Fold Change
MG 1655 Wild Type: Flask 1	0 hour	No Treatment	3.6E+08	4.00E+07		
	3 hour	Cipro 5	3.50E+04	2.00E+03	9.72E-03	
MG 1655 Wild Type: Flask 2	0 hour	No Treatment	3.63E+08	1.15E+07		
	3 hour	Cipro 5	3.60E+04	2.00E+03	9.91E-03	
BW25113 ygiW: Flask 1	0 hour	No Treatment	1.93E+08	2.52E+07		
	3 hour	Cipro 5	3.17E+04	4.93E+03	1.64E-02	1.68E+00
BW25113 ygiW: Flask 2	0 hour	No Treatment	3.17E+08	2.08E+07		
	3 hour	Cipro 5	3.30E+04	5.57E+03	1.04E-02	1.05E+00
BW25113 yhhY: Flask 1	0 hour	No Treatment	2.80E+08	4.36E+07		
	3 hour	Cipro 5	1.10E+05	1.00E+04	3.93E-02	4.04E+00
BW25113 yhhY: Flask 2	0 hour	No Treatment	3.93E+08	3.21E+07		
	3 hour	Cipro 5	1.10E+05	2.00E+04	2.80E-02	2.82E+00

Table 4-2. Summary of findings for ygiW and yhhY

flgM & *yciH* Strains

The next couple of genes that were obtained from their glycerol stocks were *flgM* and *yciH*. Table 4-3 shows the measured OD_{600} measurements for the strains before the experiment began.

Strain	Flask 1	Flask 2
MG 1655 Wild Type	0.849	0.753
MG 1655 flgM	0.799	0.775
MG 1655 yciH	0.798	0.791

Table 4-3. OD_{600} Readings for *flgM* and *yciH*

For persistence assays, 1 mL was removed. Upon removal, 220 µL of 0.5 mg/mL Ciprofloxacin stock was added to the *flgM* samples and WT 2. 210 µL was placed in the WT 1 sample, and finally, 200 µL in the *yciH* samples. The reason for the differing amounts of antibiotic added to the flasks was to maintain a steady concentration of .005 mg/mL of Ciprofloxacin. The antibiotic ran its course for three hours and 1 mL was removed towards drop plating for the persistence assay.

Table 4-4 provides a summary of the fold changes observed for the two strains. First, the *flgM* (-1.14 fold) sample demonstrated a decrease in persistence upon exposure to Ciprofloxacin as the antibiotic of choice. The negative fold change signaled that a deletion meant that we observed a decrease in persistence. The second strain tested, *yciH* (+1.59 fold) saw an increase in persistence. Judging by these results of a positive fold change, a deletion meant that there was an increase in persistence.

Strain	Time	Condition	CFU/mL	stdev	%Survival	Fold Change
MG 1655 Wild Type: Flask 1	0 hour	No Treatment	4.03E+08	1.53E+07		
	3 hour	Cipro 5	2.53E+04	5.77E+02	6.28E-03	
MG 1655 Wild Type: Flask 2	0 hour	No Treatment	4.03E+08	3.06E+07		
	3 hour	Cipro 5	2.87E+04	3.79E+03	7.11E-03	
BW25113 flgM: Flask 1	0 hour	No Treatment	3.17E+08	2.52E+07		
	3 hour	Cipro 5	1.93E+04	1.15E+03	6.11E-03	-1.03E+00
BW25113 flgM: Flask 2	0 hour	No Treatment	3.47E+08	3.21E+07		
	3 hour	Cipro 5	1.97E+04	1.15E+03	5.67E-03	-1.25E+00
BW25113 yciH: Flask 1	0 hour	No Treatment	3.07E+08	2.31E+07		
	3 hour	Cipro 5	3.50E+04	1.00E+03	1.14E-02	1.82E+00
BW25113 yciH: Flask 2	0 hour	No Treatment	3.80E+08	4.00E+07		
	3 hour	Cipro 5	3.67E+04	2.08E+03	9.65E-03	1.36E+00

Table 4-4. Summary of findings for *flgM* and *yciH*

yoeB & *hicA* Strains

The next couple of genes that were obtained from their glycerol stocks were *yoeB* and *hicA*. Table 4-5 shows the measured OD_{600} measurements for the strains before the experiment began.

Strain	Flask 1	Flask 2
MG 1655 Wild Type	0.794	0.806
MG 1655 hicA	0.756	0.771
MG 1655 yoeB	0.757	0.769

Table 4-5. OD_{600} Readings for *yoeB* and *hicA*

For persistence assays, 1 mL was removed. Upon removal, 220 µL of 0.5 mg/mL Ciprofloxacin stock was added to the WT and *hicA* samples, while 210 µL was placed in the *yoeB* samples. The reason for the differing amounts of antibiotic added to the flasks was to maintain a steady concentration of .005 mg/mL of Ciprofloxacin. The antibiotic ran its course for three hours and 1 mL was removed towards drop plating for the persistence assay.

Table 4-6 provides a summary of the fold changes observed for the two strains. First, the *hicA* (-1.34 fold) sample demonstrated a decrease in persistence upon exposure to Ciprofloxacin as the antibiotic of choice. The negative fold change signaled that a deletion meant that we observed a decrease in persistence. The second strain tested, *yoeB* (+5.04 fold) saw an increase in persistence. Judging by these results of a positive fold change, a deletion meant that there was an increase in persistence.

Strain	Time	Condition	CFU/mL	stdev	%Survival	Fold Change
MG 1655 Wild Type: Flask 1	0 hour	No Treatment	4E+08	0.00E+00		
	3 hour	Cipro 5	3.53E+04	2.52E+03	8.83E-03	
MG 1655 Wild Type: Flask 2	0 hour	No Treatment	3.8E+08	3.61E+07		
	3 hour	Cipro 5	3.33E+04	4.73E+03	8.77E-03	
BW25113 hicA: Flask 1	0 hour	No Treatment	3.17E+08	1.53E+07		
	3 hour	Cipro 5	2.47E+04	2.08E+03	7.79E-03	-1.13E+00
BW25113 hicA: Flask 2	0 hour	No Treatment	3.63E+08	1.53E+07		
	3 hour	Cipro 5	2.07E+04	1.53E+03	5.69E-03	-1.54E+00
BW25113 yoeB: Flask 1	0 hour	No Treatment	2.93E+08	1.15E+07		
	3 hour	Cipro 5	1.80E+05	1.00E+04	6.14E-02	6.95E+00
BW25113 yoeB: Flask 2	0 hour	No Treatment	3.27E+08	2.31E+07		
	3 hour	Cipro 5	9.00E+04	1.73E+04	2.76E-02	3.14E+00

Table 4-6. Summary of findings for *yoeB* and *hicA*

yiaG Strain

The final strain that was tested and still had its GCT site blocked was *yiaG*. Table 4-7 shows the measured OD_{600} measurements for the strain before the experiment began.

Strain	Flask 1	Flask 2
MG 1655 Wild Type	0.804	0.798
MG 1655 yiaG	0.85	0.773

Table 4-7. OD_{600} Readings for *yiaG*

For persistence assays, 1 mL was removed. Upon removal, 220 µL of 0.5 mg/mL Ciprofloxacin stock was added to the WT samples, while 210 µL was placed in the *yiaG* 1 sample and 200 µL into *yiaG* 2. The reason for the differing amounts of antibiotic added to the flasks was to maintain a steady concentration of .005 mg/mL of Ciprofloxacin. The antibiotic ran its course for three hours and 1 mL was removed towards drop plating for the persistence assay.

Table 4-8 provides a summary of the fold changes observed for the two strains. The *yiaG* (+1.08 fold) sample demonstrated an increase in persistence. Judging by this result of a positive fold change, a deletion meant that there was an increase in persistence. This was the last of the seven strains that were tested which had an occluded GCT site.

Strain	Time	Condition	CFU/mL	stdev	%Survival	Fold Change
MG 1655 Wild Type: Flask 1	0 hour	No Treatment	3.73E+08	3.21E+07		
	3 hour	Cipro 5	2.80E+04	1.73E+03	7.50E-03	
MG 1655 Wild Type: Flask 2	0 hour	No Treatment	3.97E+08	5.77E+06		
	3 hour	Cipro 5	2.60E+04	2.00E+03	6.55E-03	
BW25113 yiaG: Flask 1	0 hour	No Treatment	3.10E+08	1.73E+07		
	3 hour	Cipro 5	2.33E+04	2.31E+03	7.53E-03	1.00E+00
BW25113 yiaG: Flask 2	0 hour	No Treatment	3.10E+08	2.65E+07		
	3 hour	Cipro 5	2.33E+04	1.53E+03	7.53E-03	1.15E+00

Table 4-8. Summary of findings for *yiaG*

CHAPTER 5: DISCUSSION OF PHASE 1 & 2 RESULTS

All in all, there were a total of thirteen strains from the *E. coli* genome that were tested using the persistence assay technique. Revisiting the hypothesis first postulated before experimentation mentioned that the secondary structure of certain genes from the bacterium would play a role in bacterial persistence in biofilms. The hypothesis stated that those genes identified as susceptible candidates for MqsR attack at their open GCT sites, would be readily degraded by the toxin and *not* lead to an increase in persistence. The opposite was also thought to be true as well wherein candidates whose GCT structure had favorable hydrogen bonding interactions with other nucleotides and sheltered it from MqsR attack would lead to an increase in persistence.

Table 5-1 highlights the trends observed for the six strains tested in the first phase of experiments. The strains of *yaiZ*, *yggL*, *glgS*, *ogrK*, and *ydfZ* all showed a negative fold change which signified a decrease in persistence. The lone gene, *tdcR*, showed the reverse effect as it displayed a positive fold change and signaled an increase in persistence. Although the majority of these strains showed an expected decrease in persistence, their corresponding fold changes did not warrant a conclusive finding. Consulting literature, it is only advisable to further examine a strain if it exhibits *at least* a tenfold change in persistence[17]. Unfortunately none of these strains met the aforementioned criteria.

Strain	Fold Change	Persistence Trend?
yaiZ	-3.66	Decrease
yggL	-8.24	Decrease
glgS	-1.76	Decrease
ogrK	-4.23	Decrease
ydfZ	-1.44	Decrease
tdcR	4.97	Increase

Table 5-1. Summary of Fold Changes for unmasked GCT site strains

Phase two experiments yearned to test the second part of the hypothesis that suggested that the secondary structure of the gene can somehow prevent MqsR from degrading the gene and lead to an increase in persistence. As Table 5-2 suggests, this hypothesis doesn't seem to hold up quite well either. Although the folding algorithm predicted the seven strains listed below would be masked from MqsR degradation, this seemingly wasn't always the case. The fold changes show both increases *and* decreases in persistence for the strains. Furthermore, the magnitude of these changes weren't significant enough to draw any definitive conclusions.

Strain	Fold Change	Persistence Trend?
ygiW	1.37	Increase
yhhY	3.43	Increase
flgM	-1.14	Decrease
yciH	1.59	Increase
hicA	-1.34	Decrease
yoeB	5.04	Increase
yiaG	1.08	Increase

Table 5-2. Summary of Fold Changes for occluded GCT site strains

Before leaving these thirteen strains for future work, there was still a significant interest in the *hicA* and *yoeB* strains. Considering the fact that the recognition site (GCU) was hidden due to their secondary structures, a hypothesis was formed that suggested the overexpression of MqsR in the wild type strains will lead to enrichment of these toxin mRNAs. When exposed to any

stressful conditions, the cell might use one toxin (MqsR) to further up-regulate many more toxins to rapidly change the cell physiology to go into the dormancy state. Furthermore, the overexpression of MqsR was postulated to lead to an increased expression of the toxin counterparts, YoeB and HicA. As a result, the knockout strains, *hicA* and *yoeB* would have a *greater* growth rate compared to wild type strains during this overexpression.

CHAPTER 6: FINAL PHASE GROWTH EXPERIMENT

As mentioned, the toxic nature of *hicA* and *yoeB* made them interesting strains to further study. Although they did not exhibit significant fold change results (table 5-2), it was still important to carry out a final experiment (aside from the persistence assay) to measure the growth rate of these two strains when MqsR is overexpressed.

On the first day of the experiment, the three strains (WT, *hicA*, and *yoeB*) were streaked on separate plates for colony growth. The *hicA* and *yoeB* strains were streaked on LB-Kanamycin (Kan) 50 Chloramphenicol (Cm) 30 plates in order to maintain the deletion and plasmid, respectively. The Wild Type was streaked on a LB-Cm 30 plate. All three plates were placed in the incubator at 37 ^{o}C for overnight growth. The following day, six flasks were prepared for inoculation and contained 25 mL LB media. For the four flasks that contained the *hicA* and *yoeB* strains, 125 µL Kan (10 mg/mL stock) and 22 µL Cm (34 mg/mL stock) in order to maintain a 50 µg/mL Kan concentration and 30 µg/mL Cm concentration. For the Wild Type, again, only Cm was added to the pure LB. All flasks were placed in the shaker at 37 ^{o}C and 250 RPM. On the following day, all six flasks were removed and OD_{600} measurements were taken and recorded. The overnight culture was refreshed into 25 mL LB-Cm 30 in order to obtain an OD_{600} reading ~0.05. The refreshed flasks were put into the shaker under the same conditions and were allowed to grow for an eight hour period. Following one hour of growth, 1mM IPTG was induced into the flasks to overproduce MqsR when an OD_{600} reading of 0.1 was achieved. After induction, growth was monitored on a consistent basis, every hour for a total of 8 hours (table 6-1).

	WT/pCA24N-*mqsR*					Δ*hicA* /pCA24N-*mqsR*					Δ*yoeB* /pCA24N-*mqsR*			
Time (h)	Flask 1	Flask 2	Average	STDEV	Time (h)	Flask 1	Flask 2	Average	STDEV	Time (h)	Flask 1	Flask 2	Average	STDEV
0	0.033	0.04	0.0365	0.00495	0	0.035	0.036	0.0355	0.000707	0	0.039	N/A	0.039	N/A
1	0.097	0.1	0.0985	0.002121	1	0.135	0.1	0.1175	0.024749	1	0.125	N/A	0.125	N/A
2	0.173	0.175	0.174	0.001414	2	0.41	0.264	0.337	0.103238	2	0.234	N/A	0.234	N/A
3	0.242	0.199	0.2205	0.030406	3	0.508	0.329	0.4185	0.126572	3	0.256	N/A	0.256	N/A
4	0.278	0.276	0.277	0.001414	4	0.704	0.47	0.587	0.165463	4	0.35	N/A	0.35	N/A
5	0.297	0.313	0.305	0.011314	5	0.823	0.53	0.6765	0.207182	5	0.388	N/A	0.388	N/A
6	0.326	0.341	0.3335	0.010607	6	0.993	0.613	0.803	0.268701	6	0.461	N/A	0.461	N/A
7	0.42	0.45	0.435	0.021213	7	1.44	1	1.22	0.311127	7	0.64	N/A	0.64	N/A
8	0.51	0.49	0.5	0.014142	8	1.76	1.24	1.5	0.367696	8	0.58	N/A	0.58	N/A

Table 6-1. Growth rates (OD_{600} measurements) charted over an 8 hour period

The second trial of the *yoeB* strain was disregarded as it did not even begin to grow at all until the seven hour mark. Charting the average OD values for the strains against time yielded interesting results into the behavior of the toxins (Figure 6-1).

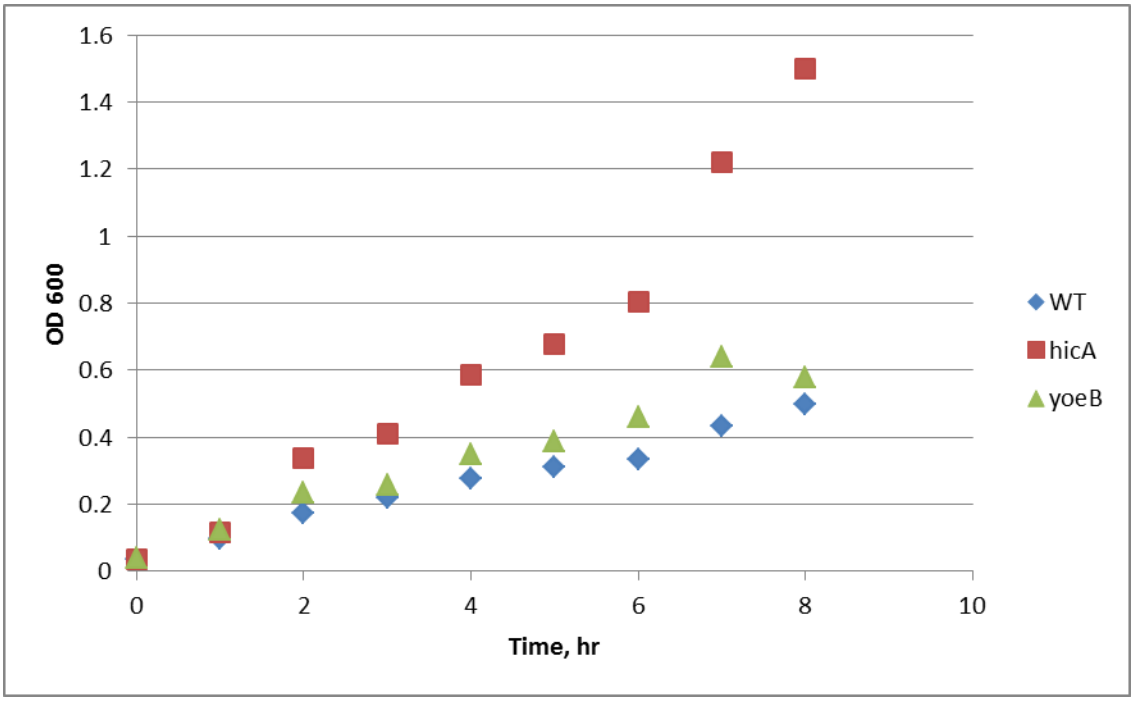

Figure 6-1. Growth curve of strains with IPTG induction at one hour

The *hicA* strain demonstrated the greatest growth rate as it followed an exponential track. Following the introduction of MqsR into the strains at one hour, *hicA* demonstrated rapid growth. The next step was to try and quantify the growth observed by the strains by calculating an

average growth rate and comparing it against the standard WT strain. To do this, a specific growth rate was found for each colony by plotting the ln OD_{600} versus time. The range was then narrowed to ensure a proper fit of data points and that it yielded a straight line. The slope of the line corresponded to the specific growth rate which was then averaged for the two trials of each.

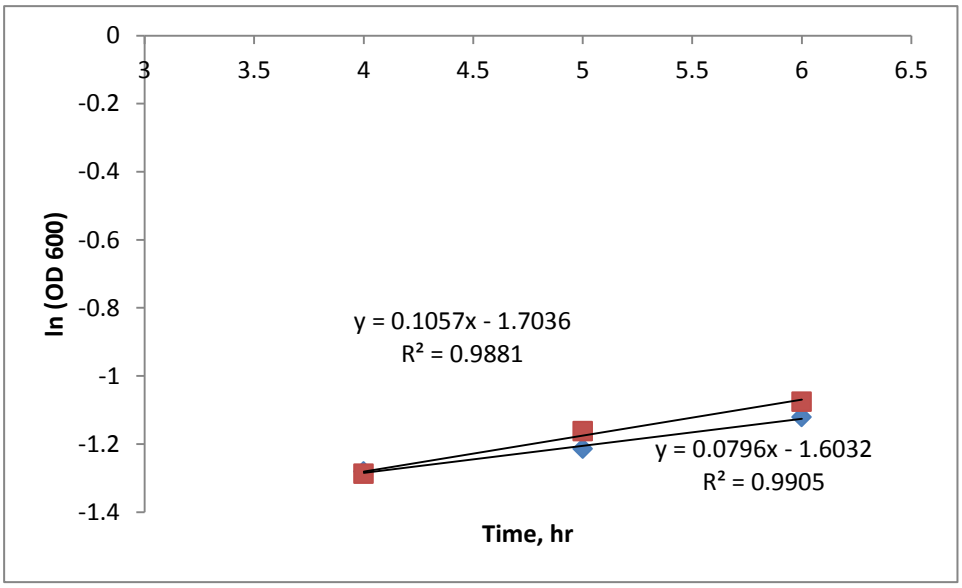

Figure 6-2. Specific growth rate for Wild Type

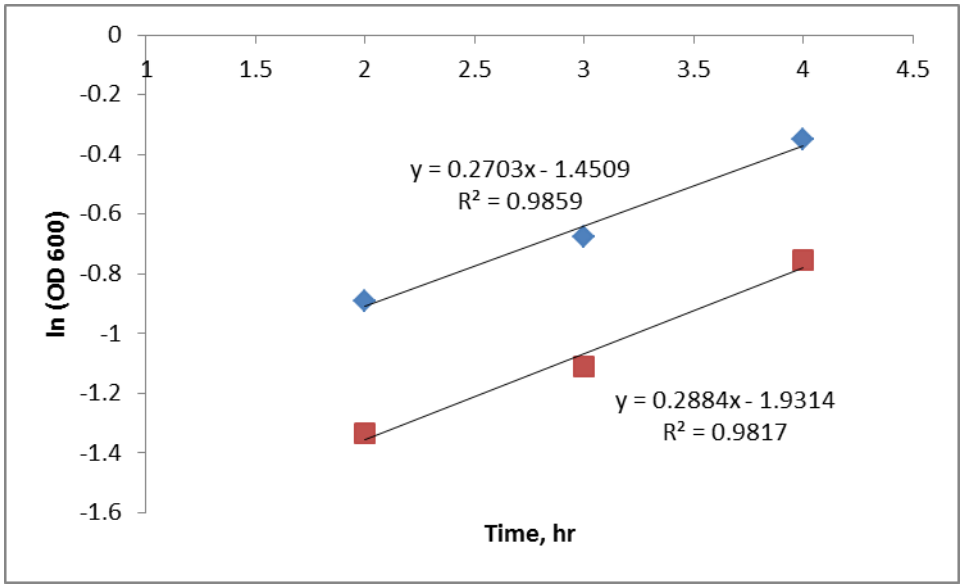

Figure 6-3. Specific growth rate for *hicA*

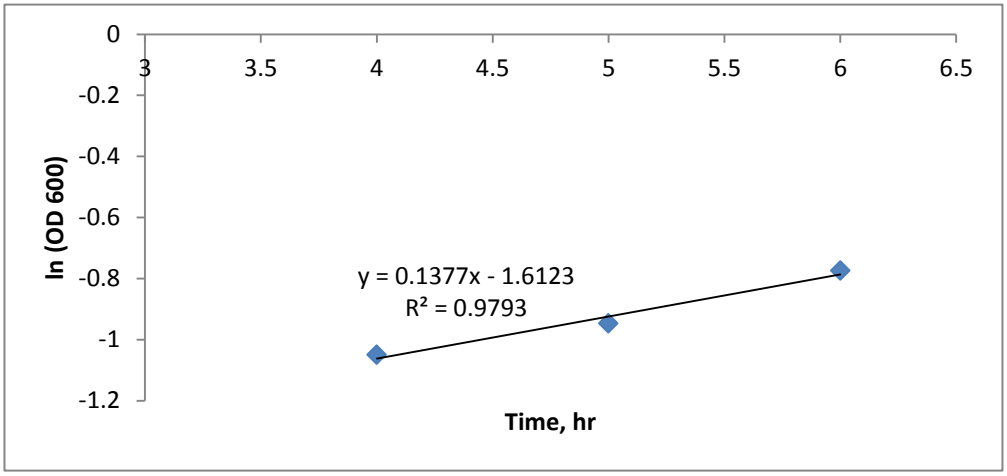

Figure 6-4. Specific growth rate for *yoeB*

	Average Growth	Fold Change
WT/pCA24N-mqsR	0.09265 +/- 0.018	1
ΔhicA/pCA24N-mqsR	0.27935 +/- 0.012	3.015
ΔyoeB/pCA24N-mqsR	0.1377	1.486

Table 6-2. Specific growth rate during MqsR overproduction

Looking at the results displayed in the above table, *hicA* showed a better growth rate (+3.0) compared to the other two strains after exposure of 1mM IPTG. For at least the *hicA* strain, the hypothesis seemed to hold true as it showed that the overexpression of MqsR did indeed lead to an increased expression of the protein HicA. This can be validated by the fact that the knockout strain tested above has a higher growth rate than its wild type counterpart.

CHAPTER 7: CONCLUSIONS AND FUTURE WORK

The starting point and motivations for the research started with the understanding of the protein GhoT. A protein within *E. coli*, GhoT was found to damage the membranes of cells and lead to an increase in persistence. Inherent to this toxin was an antitoxin counterpart, GhoS which was found to limit the toxic effects of GhoT by cleaving the protein's mRNA (a newly identified toxin/antitoxin system). The regulator, MqsR is intricately involved with the aforementioned TA system as it degrades mRNA specifically at GCU sites in the transcript. Interestingly enough, MqsR was found to cleave transcripts of antitoxin GhoS, which led to the activation of its toxin counterpart, GhoT. As GhoT was activated, persister cell formation occurred. Researchers tested an initial set of genes from the *E. coli* genome that were found to lack GCU sites in their mRNA transcripts to determine the role of MqsR plays on increasing persistence. When these genes were knocked out and exposed to MqsR, this reduced persistence. When these genes were not knocked out and were intact in the genome and exposed to MqsR, an increase in persistence was observed. The motivations were to find a similar relationship to the ghoS/ghoT pairing by studying a select number of genes.

With these trends in mind, thirteen additional genes (with a singular GCU site) of interest were examined using the persistence assay technique to test whether or not they exhibited an increase or decrease in persistence when exposed to antibiotics. Six genes were studied in phase one of the experiment as they had an exposed GCU site. In phase two, seven additional genes were examined as they had a GCU site that was occluded by its secondary structure and it was postulated that they would behave synonymously to the candidates tested in previous research that had no GCT sites.

Although none of the strains tested showed many definitive trends in persistence alone when exposed solely to antibiotics (tables 5-1 & 5-2), future considerations may be taken to test the masked versus unmasked GCU phenotype. Previous work has shown that the overexpression of *mqsR* increases the formation of persisters as its toxin counterpart functions by cleaving mRNA at GCU sequences[5]. By exposing the aforementioned thirteen knockout genes to MqsR in large amounts would be a clearer indicator as to their role in persistence by measuring their respective fold changes. Seeing that more 0 GCT site genes were found (Appendix C) than were originally thought of, strains with these deletions should also be analyzed to see whether or not they behave similarly to the original fourteen surveyed by previous researchers. There is even a large abundance of one and two GCT site candidates that have yet to be analyzed. Furthermore, experiments can be carried out using different antibiotics, stress conditions, or cell growth phases (trying stationary versus exponential). The work performed through the technique of persistence assays will hopefully be used as a launching point for future considerations which may show more definitive trends in persistence in these strains of *E. coli*.

Appendix A

Colony Counts for *yaiZ* and *yggL*

0 Hour Colony Counts

	WT 1					yaiZ 2			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	37	5	Nothing	Drop 1	Dense	48	9	Nothing
Drop 2	Dense	41	4	Nothing	Drop 2	Dense	46	6	Nothing
Drop 3	Dense	36	2	Nothing	Drop 3	Dense	34	5	Nothing
	WT 2					yggL 1			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	45	4	Nothing	Drop 1	Dense	25	6	Nothing
Drop 2	Dense	45	3	Nothing	Drop 2	Dense	31	2	Nothing
Drop 3	Dense	42	7	Nothing	Drop 3	Dense	29	3	Nothing
	yaiZ 1					yggL 2			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	42	7	Nothing	Drop 1	Dense	35	7	Nothing
Drop 2	Dense	51	5	Nothing	Drop 2	Dense	34	6	Nothing
Drop 3	Dense	34	5	Nothing	Drop 3	Dense	31	2	Nothing

Table A-1. Colony Counts for *yaiZ* and *yggL* before antibiotic addition

3 Hour Colony Counts

	WT 1					yaiZ 2			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	24	2	Nothing	Drop 1	Dense	9	0	Nothing
Drop 2	Dense	31	3	Nothing	Drop 2	Dense	6	1	Nothing
Drop 3	Dense	28	3	Nothing	Drop 3	Dense	6	2	Nothing
	WT 2					yggL 1			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	35	7	Nothing	Drop 1	30	5	3	Nothing
Drop 2	Dense	29	8	Nothing	Drop 2	37	11	1	Nothing
Drop 3	Dense	32	3	Nothing	Drop 3	30	10	0	Nothing
	yaiZ 1					yggL 2			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	13	1	Nothing	Drop 1	29	6	1	Nothing
Drop 2	Dense	12	2	Nothing	Drop 2	23	1	1	Nothing
Drop 3	Dense	7	2	Nothing	Drop 3	20	6	2	Nothing

Table A-2. Colony Counts for *yaiZ* and *yggL* after 3 hours of antibiotic addition

Colony Counts for *glgS* and *ogrK*

0 Hour Colony Counts									
	WT 1					glgS 2			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	32	2	Nothing	Drop 1	Dense	29	7	Nothing
Drop 2	Dense	30	4	Nothing	Drop 2	Dense	34	12	Nothing
Drop 3	Dense	30	1	Nothing	Drop 3	Dense	39	8	Nothing
	WT 2					ogrK 1			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	36	3	Nothing	Drop 1	Dense	38	8	Nothing
Drop 2	Dense	39	1	Nothing	Drop 2	Dense	48	5	Nothing
Drop 3	Dense	42	4	Nothing	Drop 3	Dense	35	6	Nothing
	glgS 1					ogrK 2			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	31	6	Nothing	Drop 1	Dense	35	10	Nothing
Drop 2	Dense	35	7	Nothing	Drop 2	Dense	41	10	Nothing
Drop 3	Dense	32	2	Nothing	Drop 3	Dense	39	8	Nothing

Table A-3. Colony Counts for *glgS* and *ogrK* before antibiotic addition

3 Hour Colony Counts									
	WT 1					glgS 2			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	31	9	Nothing	Drop 1	Dense	22	3	Nothing
Drop 2	Dense	27	3	Nothing	Drop 2	Dense	19	7	Nothing
Drop 3	Dense	30	4	Nothing	Drop 3	Dense	23	3	Nothing
	WT 2					ogrK 1			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	28	5	Nothing	Drop 1	Dense	9	4	Nothing
Drop 2	Dense	33	6	Nothing	Drop 2	Dense	11	1	Nothing
Drop 3	Dense	21	4	Nothing	Drop 3	Dense	5	1	Nothing
	glgS 1					ogrK 2			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	16	2	Nothing	Drop 1	Dense	7	0	Nothing
Drop 2	Dense	13	3	Nothing	Drop 2	Dense	7	0	Nothing
Drop 3	Dense	10	5	Nothing	Drop 3	Dense	7	1	Nothing

Table A-4. Colony Counts for *glgS* and *ogrK* after 3 hours of antibiotic addition

Colony Counts for *tdcR* and *ydfZ*

0 Hour Colony Counts										
	WT 1					tdcR 2				
Dilution #	**0, -1, -2, -3, -4**		**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense		40	6	Nothing	Drop 1	Dense	41	5	Nothing
Drop 2	Dense		34	3	Nothing	Drop 2	Dense	36	8	Nothing
Drop 3	Dense		35	6	Nothing	Drop 3	Dense	39	8	Nothing
	WT 2					ydfZ 1				
Dilution #	**0, -1, -2, -3, -4**		**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense		39	5	Nothing	Drop 1	Dense	43	8	Nothing
Drop 2	Dense		39	7	Nothing	Drop 2	Dense	36	10	Nothing
Drop 3	Dense		44	7	Nothing	Drop 3	Dense	38	6	Nothing
	tdcR 1					ydfZ 2				
Dilution #	**0, -1, -2, -3, -4**		**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense		37	5	Nothing	Drop 1	Dense	34	7	Nothing
Drop 2	Dense		30	6	Nothing	Drop 2	Dense	32	6	Nothing
Drop 3	Dense		35	5	Nothing	Drop 3	Dense	34	9	Nothing

Table A-5. Colony Counts for *tdcR* and *ydfZ* before antibiotic addition

3 Hour Colony Counts										
	WT 1					tdcR 2				
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**		**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	15	3	Nothing		Drop 1	Dense	Dense	22	Nothing
Drop 2	Dense	20	1	Nothing		Drop 2	Dense	Dense	18	Nothing
Drop 3	Dense	20	3	Nothing		Drop 3	Dense	Dense	17	Nothing
	WT 2					ydfZ 1				
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**		**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	Dense	12	Nothing		Drop 1	Dense	12	Nothing	Nothing
Drop 2	Dense	Dense	16	Nothing		Drop 2	Dense	19	Nothing	Nothing
Drop 3	Dense	Dense	10	Nothing		Drop 3	Dense	14	Nothing	Nothing
	tdcR 1					ydfZ 2				
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**		**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	Dense	12	Nothing		Drop 1	Dense	9	Nothing	Nothing
Drop 2	Dense	Dense	17	Nothing		Drop 2	Dense	8	Nothing	Nothing
Drop 3	Dense	Dense	14	Nothing		Drop 3	Dense	4	Nothing	Nothing

Table A-6. Colony Counts for *tdcR* and *ydfZ* after 3 hours of antibiotic addition

Derivation of Fold Change Calculations for *yaiZ* & *yggL*

0 h	3 h amp10			MG1655 yaiZ 1	0 h	3 h amp10			MG1655 yggL 1	0 h	3 h amp10
3.70E+08	2.40E+04				4.20E+08	1.30E+04				2.50E+08	3.00E+03
4.10E+08	3.10E+04				5.10E+08	1.20E+04				3.10E+08	3.70E+03
3.60E+08	2.80E+04				3.40E+08	7.00E+03				2.90E+08	3.00E+03
3.80E+08	2.77E+04			Mean	4.23E+08	1.07E+04			Mean	2.83E+08	3.23E+03
2.65E+07	3.51E+03			stdev	8.50E+07	3.21E+03			stdev	3.06E+07	4.04E+02
100.0000	0.0073			Survival %	100.0000	0.0025			Survival %	100.0000	0.0011
9.8465	0.0011			stdev rel to 0 h	28.4120	0.0009			stdev rel to 0 h	15.2488	0.0002
				fold change		0.35			fold change		0.16
						-2.889528509					-6.379996383
CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL
0 h	3 h amp10			MG1655 yaiZ 2	0 h	3 h amp10			MG1655 yggL 2	0 h	3 h amp10
4.50E+08	3.50E+04				4.80E+08	9.00E+03				3.50E+08	2.90E+03
4.50E+08	2.90E+04				4.60E+08	6.00E+03				3.40E+08	2.30E+03
4.20E+08	3.20E+04				3.40E+08	6.00E+03				3.10E+08	2.00E+03
4.40E+08	3.20E+04			Mean	4.27E+08	7.00E+03			Mean	3.33E+08	2.40E+03
1.73E+07	3.00E+03			stdev	7.57E+07	1.73E+03			stdev	2.08E+07	4.58E+02
100.0000	0.0073			Survival %	100.0000	0.0016			Survival %	100.0000	0.0007
5.5670	0.0007			stdev rel to 0 h	25.0975	0.0005			stdev rel to 0 h	8.8318	0.0001
				fold change		0.23			fold change		0.10
						-4.432900433					-10.1010101

Table A-7. Calculated fold changes for *yaiZ* and *yggL*

Derivation of Fold Change Calculations for *glgS* & *ogrK*

Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL
MG1655 WT 1	0 h	3 h amp10			MG1655 glgS 1	0 h	3 h amp10			MG1655 ogrK 1	0 h	3 h amp10
	3.20E+08	3.10E+04				3.10E+08	1.60E+04				3.80E+08	9.00E+03
	3.00E+08	2.70E+04				3.50E+08	1.30E+04				4.80E+08	1.10E+04
	3.00E+08	3.00E+04				3.20E+08	1.00E+04				3.50E+08	5.00E+03
Mean	3.07E+08	2.93E+04			Mean	3.27E+08	1.30E+04			Mean	4.03E+08	8.33E+03
stdev	1.15E+07	2.08E+03			stdev	2.08E+07	3.00E+03			stdev	6.81E+07	3.06E+03
Survival %	100.0000	0.0096			Survival %	100.0000	0.0040			Survival %	100.0000	0.0021
stdev rel to 0 h	5.3250	0.0008			stdev rel to 0 h	9.0120	0.0010			stdev rel to 0 h	23.8670	0.0008
					fold change		0.42			fold change		0.22
							-2.403567					-4.629565
Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL
MG1655 WT 2	0 h	3 h amp10			MG1655 glgS 2	0 h	3 h amp10			MG1655 ogrK 2	0 h	3 h amp10
	3.60E+08	2.80E+04				2.90E+08	2.20E+04				3.50E+08	7.00E+03
	3.90E+08	3.30E+04				3.40E+08	1.90E+04				4.10E+08	7.00E+03
	4.20E+08	2.10E+04				3.90E+08	2.30E+04				3.90E+08	7.00E+03
Mean	3.90E+08	2.73E+04			Mean	3.40E+08	2.13E+04			Mean	3.83E+08	7.00E+03
stdev	3.00E+07	6.03E+03			stdev	5.00E+07	2.08E+03			stdev	3.06E+07	0.00E+00
Survival %	100.0000	0.0070			Survival %	100.0000	0.0063			Survival %	100.0000	0.0018
stdev rel to 0 h	10.8786	0.0016			stdev rel to 0 h	20.7973	0.0011			stdev rel to 0 h	11.2709	0.0001
					fold change		0.90			fold change		0.26
							-1.116987					-3.838014

Table A-8. Calculated fold changes for *glgS* and *ogrK*

Derivation of Fold Change Calculations for *tdcR* & *ydfZ*

Culture	CFU/mL	CFU/mL
MG1655 WT 1	0 h	3 h amp10
	4.00E+08	1.50E+04
	3.40E+08	2.00E+04
	3.50E+08	2.00E+04
Mean	3.63E+08	1.83E+04
stdev	3.21E+07	2.89E+03
Survival %	100.0000	0.0050
stdev rel to 0 h	12.5121	0.0009

Culture	CFU/mL	CFU/mL
MG1655 tdcR 1	0 h	3 h amp10
	3.70E+08	1.20E+05
	3.00E+08	1.70E+05
	3.50E+08	1.40E+05
Mean	3.40E+08	1.43E+05
stdev	3.61E+07	2.52E+04
Survival %	100.0000	0.0422
stdev rel to 0 h	14.9971	0.0086
fold change		8.35
		-0.119693

Culture	CFU/mL	CFU/mL
MG1655 ydfZ 1	0 h	3 h amp10
	4.30E+08	1.20E+04
	3.60E+08	1.90E+04
	3.90E+08	1.40E+04
Mean	3.93E+08	1.50E+04
stdev	3.51E+07	3.61E+03
Survival %	100.0000	0.0038
stdev rel to 0 h	12.6268	0.0010
fold change		0.76
		-1.32314

Culture	CFU/mL	CFU/mL
MG1655 WT 2	0 h	3 h amp10
	3.90E+08	1.20E+05
	3.90E+08	1.60E+05
	4.40E+08	1.00E+05
Mean	4.07E+08	1.27E+05
stdev	2.89E+07	3.06E+04
Survival %	100.0000	0.0311
stdev rel to 0 h	10.0389	0.0078

Culture	CFU/mL	CFU/mL
MG1655 tdcR 2	0 h	3 h amp10
	4.10E+08	2.20E+05
	3.60E+08	1.80E+05
	3.90E+08	1.70E+05
Mean	3.87E+08	1.90E+05
stdev	2.52E+07	2.65E+04
Survival %	100.0000	0.0491
stdev rel to 0 h	9.2044	0.0076
fold change		1.58
		-0.63388

Culture	CFU/mL	CFU/mL
MG1655 ydfZ 2	0 h	3 h amp10
	3.90E+08	9.00E+04
	3.20E+08	8.00E+04
	3.40E+08	4.00E+04
Mean	3.50E+08	7.00E+04
stdev	3.61E+07	2.65E+04
Survival %	100.0000	0.0200
stdev rel to 0 h	14.5686	0.0078
fold change		0.64
		-1.557377

Table A-9. Calculated fold changes for *tdcR* and *ydfZ*

Appendix B

Colony Counts for *ygiW* and *yhhY*

0 Hour Colony Counts										
	WT 1					ygiW 2				
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	40	6	Nothing	Drop 1	Dense	31	5	Nothing
Drop 2	Dense	32	3	Nothing	Drop 2	Dense	30	5	Nothing
Drop 3	Dense	36	0	Nothing	Drop 3	Dense	34	3	Nothing
	WT 2					yhhY 1			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	37	3	Nothing	Drop 1	Dense	30	3	Nothing
Drop 2	Dense	35	3	Nothing	Drop 2	Dense	31	8	Nothing
Drop 3	Dense	37	7	Nothing	Drop 3	Dense	23	2	Nothing
	ygiW 1					yhhY 2			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	17	1	Nothing	Drop 1	Dense	37	9	Nothing
Drop 2	Dense	22	4	Nothing	Drop 2	Dense	43	10	Nothing
Drop 3	Dense	19	2	Nothing	Drop 3	Dense	38	4	Nothing

Table B-1. Colony Counts for *ygiW* and *yhhY* before antibiotic addition

3 Hour Colony Counts										
	WT 1					ygiW 2				
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	35	10	Nothing	Drop 1	Dense	28	5	Nothing
Drop 2	Dense	37	4	Nothing	Drop 2	Dense	32	0	Nothing
Drop 3	Dense	33	6	Nothing	Drop 3	Dense	39	3	Nothing
	WT 2					yhhY 1			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	36	12	Nothing	Drop 1	Dense	Dense	11	Nothing
Drop 2	Dense	38	10	Nothing	Drop 2	Dense	Dense	12	Nothing
Drop 3	Dense	34	3	Nothing	Drop 3	Dense	Dense	10	Nothing
	ygiW 1					yhhY 2			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	35	6	Nothing	Drop 1	Dense	Dense	9	Nothing
Drop 2	Dense	34	3	Nothing	Drop 2	Dense	Dense	13	Nothing
Drop 3	Dense	26	2	Nothing	Drop 3	Dense	Dense	11	Nothing

Table B-2. Colony Counts for *ygiW* and *yhhY* after 3 hours of antibiotic addition

Colony Counts for *flgM* and *yciH*

0 Hour Colony Counts										
	WT 1						flgM 2			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**		**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	42	9	Nothing		Drop 1	Dense	31	4	Nothing
Drop 2	Dense	40	11	Nothing		Drop 2	Dense	37	7	Nothing
Drop 3	Dense	39	10	Nothing		Drop 3	Dense	36	4	Nothing
	WT 2						yciH 1			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**		**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	41	8	Nothing		Drop 1	Dense	32	5	Nothing
Drop 2	Dense	37	6	Nothing		Drop 2	Dense	28	10	Nothing
Drop 3	Dense	43	11	Nothing		Drop 3	Dense	32	10	Nothing
	flgM 1						yciH 2			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**		**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	29	4	Nothing		Drop 1	Dense	38	4	Nothing
Drop 2	Dense	34	7	Nothing		Drop 2	Dense	34	11	Nothing
Drop 3	Dense	32	5	Nothing		Drop 3	Dense	42	13	Nothing

Table B-3. Colony Counts for *flgM* and *yciH* before antibiotic addition

3 Hour Colony Counts										
	WT 1						flgM 2			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**		**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	26	Nothing	Nothing		Drop 1	Dense	19	6	Nothing
Drop 2	Dense	25	Nothing	Nothing		Drop 2	Dense	19	6	Nothing
Drop 3	Dense	25	Nothing	Nothing		Drop 3	Dense	21	4	Nothing
	WT 2						yciH 1			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**		**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	27	10	Nothing		Drop 1	Dense	36	12	Nothing
Drop 2	Dense	26	8	Nothing		Drop 2	Dense	34	9	Nothing
Drop 3	Dense	33	4	Nothing		Drop 3	Dense	35	11	Nothing
	flgM 1						yciH 2			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**		**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	20	1	Nothing		Drop 1	Dense	31	7	Nothing
Drop 2	Dense	20	0	Nothing		Drop 2	Dense	35	11	Nothing
Drop 3	Dense	18	2	Nothing		Drop 3	Dense	36	12	Nothing

Table B-4. Colony Counts for *flgM* and *yciH* after 3 hours of antibiotic addition

Colony Counts for *hicA* and *yoeB*

0 Hour Colony Counts										
	WT 1						hicA 2			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**		**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	40	7	Nothing		Drop 1	Dense	35	4	Nothing
Drop 2	Dense	40	12	Nothing		Drop 2	Dense	36	8	Nothing
Drop 3	Dense	40	10	Nothing		Drop 3	Dense	38	8	Nothing
	WT 2						yoeB 1			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**		**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	39	8	Nothing		Drop 1	Dense	28	5	Nothing
Drop 2	Dense	41	10	Nothing		Drop 2	Dense	30	4	Nothing
Drop 3	Dense	34	8	Nothing		Drop 3	Dense	30	5	Nothing
	hicA 1						yoeB 2			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**		**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	32	8	Nothing		Drop 1	Dense	30	5	Nothing
Drop 2	Dense	30	8	Nothing		Drop 2	Dense	34	7	Nothing
Drop 3	Dense	33	8	Nothing		Drop 3	Dense	34	2	Nothing

Table B-5. Colony Counts for *hicA* and *yoeB* before antibiotic addition

3 Hour Colony Counts										
	WT 1						hicA 2			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**		**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	33	7	Nothing		Drop 1	Dense	21	5	Nothing
Drop 2	Dense	38	8	Nothing		Drop 2	Dense	19	5	Nothing
Drop 3	Dense	35	8	Nothing		Drop 3	Dense	22	3	Nothing
	WT 2						yoeB 1			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**		**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	35	9	Nothing		Drop 1	Dense	Dense	19	Nothing
Drop 2	Dense	37	9	Nothing		Drop 2	Dense	Dense	17	Nothing
Drop 3	Dense	28	9	Nothing		Drop 3	Dense	Dense	18	Nothing
	hicA 1						yoeB 2			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**		**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	24	5	Nothing		Drop 1	Dense	Dense	10	Nothing
Drop 2	Dense	23	9	Nothing		Drop 2	Dense	Dense	10	Nothing
Drop 3	Dense	27	5	Nothing		Drop 3	Dense	Dense	7	Nothing

Table B-6. Colony Counts for *hicA* and *yoeB* after 3 hours of antibiotic addition

Colony Counts for *yiaG*

0 Hour Colony Counts										
	WT 1					yiaG 1				
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	36	10	Nothing	Drop 1	Dense	32	10	Nothing
Drop 2	Dense	41	8	Nothing	Drop 2	Dense	29	5	Nothing
Drop 3	Dense	35	14	Nothing	Drop 3	Dense	32	10	Nothing
	WT 2					yiaG 2			
Dilution #	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**	**Dilution #**	**0, -1, -2, -3, -4**	**-5**	**-6**	**-7**
Drop 1	Dense	40	13	Nothing	Drop 1	Dense	28	4	Nothing
Drop 2	Dense	39	9	Nothing	Drop 2	Dense	33	3	Nothing
Drop 3	Dense	40	11	Nothing	Drop 3	Dense	32	8	Nothing

Table B-7. Colony Counts for *yiaG* before antibiotic addition

3 Hour Colony Counts									
	WT 1					yiaG 1			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	27	7	Nothing	Drop 1	Dense	26	3	Nothing
Drop 2	Dense	30	5	Nothing	Drop 2	Dense	22	3	Nothing
Drop 3	Dense	27	4	Nothing	Drop 3	Dense	22	3	Nothing
	WT 2					yiaG 2			
Dilution #	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**	**Dilution #**	**0**	**-1**	**-2**	**-3,-4,-5,-6,-7**
Drop 1	Dense	28	4	Nothing	Drop 1	Dense	25	2	Nothing
Drop 2	Dense	26	2	Nothing	Drop 2	Dense	22	9	Nothing
Drop 3	Dense	24	3	Nothing	Drop 3	Dense	23	2	Nothing

Table B-8. Colony Counts for *yiaG* after 3 hours of antibiotic addition

Derivation of Fold Change Calculations for *ygiW* & *yhhY*

MG1655 WT 1	0 h	3 h cipro 5			MG1655 ygiW 1	0 h	3 h cipro 5			MG1655 yhhY 1	0 h	3 h cipro 5
	4.00E+08	3.50E+04				1.70E+08	3.50E+04				3.00E+08	1.10E+05
	3.20E+08	3.70E+04				2.20E+08	3.40E+04				3.10E+08	1.20E+05
	3.60E+08	3.30E+04				1.90E+08	2.60E+04				2.30E+08	1.00E+05
Mean	3.60E+08	3.50E+04			Mean	1.93E+08	3.17E+04			Mean	2.80E+08	1.10E+05
stdev	4.00E+07	2.00E+03			stdev	2.52E+07	4.93E+03			stdev	4.36E+07	1.00E+04
Survival %	100.0000	0.0097			Survival %	100.0000	0.0164			Survival %	100.0000	0.0393
stdev rel to 0 h	15.7135	0.0012			stdev rel to 0 h	18.4088	0.0033			stdev rel to 0 h	22.0158	0.0071
					fold change		1.68			fold change		4.04
							-0.593567					-0.247475
Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL
MG1655 WT 2	0 h	3 h cipro 5			MG1655 ygiW 2	0 h	3 h cipro 5			MG1655 yhhY 2	0 h	3 h cipro 5
	3.70E+08	3.60E+04				3.10E+08	2.80E+04				3.70E+08	9.00E+04
	3.50E+08	3.80E+04				3.00E+08	3.20E+04				4.30E+08	1.30E+05
	3.70E+08	3.40E+04				3.40E+08	3.90E+04				3.80E+08	1.10E+05
Mean	3.63E+08	3.60E+04			Mean	3.17E+08	3.30E+04			Mean	3.93E+08	1.10E+05
stdev	1.15E+07	2.00E+03			stdev	2.08E+07	5.57E+03			stdev	3.21E+07	2.00E+04
Survival %	100.0000	0.0099			Survival %	100.0000	0.0104			Survival %	100.0000	0.0280
stdev rel to 0 h	4.4945	0.0006			stdev rel to 0 h	9.2966	0.0019			stdev rel to 0 h	11.5578	0.0056
					fold change		1.05			fold change		2.82
							-0.950792					-0.354295

Table B-9. Calculated fold changes for *ygiW* and *yhhY*

Derivation of Fold Change Calculations for *flgM* & *yciH*

0 h	3 h cipro 5			MG1655 flgM 1	0 h	3 h cipro 5			MG1655 yciH 1	0 h	3 h cipro 5
4.20E+08	2.60E+04				2.90E+08	2.00E+04				3.20E+08	3.60E+04
4.00E+08	2.50E+04				3.40E+08	2.00E+04				2.80E+08	3.40E+04
3.90E+08	2.50E+04				3.20E+08	1.80E+04				3.20E+08	3.50E+04
4.03E+08	2.53E+04			Mean	3.17E+08	1.93E+04			Mean	3.07E+08	3.50E+04
1.53E+07	5.77E+02			stdev	2.52E+07	1.15E+03			stdev	2.31E+07	1.00E+03
100.0000	0.0063			Survival %	100.0000	0.0061			Survival %	100.0000	0.0114
5.3560	0.0003			stdev rel to 0 h	11.2390	0.0006			stdev rel to 0 h	10.6500	0.0009
				fold change		0.97			fold change		1.82
						-1.028783					-0.550335
CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL
0 h	3 h cipro 5			MG1655 flgM 2	0 h	3 h cipro 5			MG1655 ychiH 2	0 h	3 h cipro 5
4.10E+08	2.70E+04				3.10E+08	1.90E+04				3.80E+08	3.90E+04
3.70E+08	2.60E+04				3.70E+08	1.90E+04				3.40E+08	3.50E+04
4.30E+08	3.30E+04				3.60E+08	2.10E+04				4.20E+08	3.60E+04
4.03E+08	2.87E+04			Mean	3.47E+08	1.97E+04			Mean	3.80E+08	3.67E+04
3.06E+07	3.79E+03			stdev	3.21E+07	1.15E+03			stdev	4.00E+07	2.08E+03
100.0000	0.0071			Survival %	100.0000	0.0057			Survival %	100.0000	0.0096
10.7120	0.0011			stdev rel to 0 h	13.1136	0.0006			stdev rel to 0 h	14.8865	0.0012
				fold change		0.80			fold change		1.36
						-1.252837					-0.736589

Table B-10. Calculated fold changes for *flgM* and *yciH*

Derivation of Fold Change Calculations for *hicA* & *yoeB*

0 h	3 h cipro 5			MG1655 hicA 1	0 h	3 h cipro 5			MG1655 yoeB 1	0 h	3 h cipro 5
4.00E+08	3.30E+04				3.20E+08	2.40E+04				2.80E+08	1.90E+05
4.00E+08	3.80E+04				3.00E+08	2.30E+04				3.00E+08	1.70E+05
4.00E+08	3.50E+04				3.30E+08	2.70E+04				3.00E+08	1.80E+05
4.00E+08	3.53E+04			Mean	3.17E+08	2.47E+04			Mean	2.93E+08	1.80E+05
0.00E+00	2.52E+03			stdev	1.53E+07	2.08E+03			stdev	1.15E+07	1.00E+04
100.0000	0.0088			Survival %	100.0000	0.0078			Survival %	100.0000	0.0614
0.0000	0.0006			stdev rel to 0 h	6.8218	0.0008			stdev rel to 0 h	5.5670	0.0042
				fold change		0.88			fold change		6.95
						-1.134009					-0.143951
CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL
0 h	3 h cipro 5			MG1655 hicA 2	0 h	3 h cipro 5			MG1655 yoeB 2	0 h	3 h cipro 5
3.90E+08	3.50E+04				3.50E+08	2.10E+04				3.40E+08	1.00E+05
4.10E+08	3.70E+04				3.60E+08	1.90E+04				3.40E+08	1.00E+05
3.40E+08	2.80E+04				3.80E+08	2.20E+04				3.00E+08	7.00E+04
3.80E+08	3.33E+04			Mean	3.63E+08	2.07E+04			Mean	3.27E+08	9.00E+04
3.61E+07	4.73E+03			stdev	1.53E+07	1.53E+03			stdev	2.31E+07	1.73E+04
100.0000	0.0088			Survival %	100.0000	0.0057			Survival %	100.0000	0.0276
13.4185	0.0015			stdev rel to 0 h	5.9456	0.0005			stdev rel to 0 h	9.9979	0.0056
				fold change		0.65			fold change		3.14
						-1.542162					-0.318389

Table B-11. Calculated fold changes for *hicA* and *yoeB*

Derivation of Fold Change Calculations for *yiaG*

Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL
MG1655 WT 1	0 h	3 h cipro 5			MG1655 yiaG 1	0 h	3 h cipro 5
	3.60E+08	2.70E+04				3.20E+08	2.60E+04
	4.10E+08	3.00E+04				2.90E+08	2.20E+04
	3.50E+08	2.70E+04				3.20E+08	2.20E+04
Mean	3.73E+08	2.80E+04			Mean	3.10E+08	2.33E+04
stdev	3.21E+07	1.73E+03			stdev	1.73E+07	2.31E+03
Survival %	100.0000	0.0075			Survival %	100.0000	0.0075
stdev rel to 0 h	12.1769	0.0008			stdev rel to 0 h	7.9016	0.0009
					fold change		1.00
							-0.996429
Culture	CFU/mL	CFU/mL			Culture	CFU/mL	CFU/mL
MG1655 WT 2	0 h	3 h cipro 5			MG1655 yiaG 2	0 h	3 h cipro 5
	4.00E+08	2.80E+04				2.80E+08	2.50E+04
	3.90E+08	2.60E+04				3.30E+08	2.20E+04
	4.00E+08	2.40E+04				3.20E+08	2.30E+04
Mean	3.97E+08	2.60E+04			Mean	3.10E+08	2.33E+04
stdev	5.77E+06	2.00E+03			stdev	2.65E+07	1.53E+03
Survival %	100.0000	0.0066			Survival %	100.0000	0.0075
stdev rel to 0 h	2.0584	0.0005			stdev rel to 0 h	12.0699	0.0008
					fold change		1.15
							-0.870828

Table B-12. Calculated fold changes for *yiaG*

Appendix C

Gene Name	RNA Type	Function/Description	Amino Acids
tnaC	mRNA	Regulatory leader peptide for tna operon	24
trpL	mRNA	Regulatory leader peptide for trp operon	14
ralR	mRNA	Restriction alleviation gene in Rac prophage	64
ptwF	mRNA	thrW pseudogene, CP4-6 prophage attachment site	N/A – 49 nucleotides
psaA	mRNA	ssrA pseudogene, CP4-57 attachment site duplication; putative defective prophage	N/A – 14 nucleotides
yciG	mRNA	Required for swarming phenotype, function unknownymdF and yciG are paralogs. YciG and YmdF may be related to small stress proteins (shares COG3729 with GsiB of B. subtilis) and appears to be regulated by RpoS.	59
yheV	mRNA	Function unknown	66
yjdO	mRNA	Function unknown	57
ykgR	mRNA	Expressed protein, function unknown	33
ylbI	mRNA	The ylbI internal fragment is N-terminal, but missing the start codon Pseudogene, internal fragment, Rhs family	31
ylcH	mRNA	Immunoblots of chromosomal SPA-tagged YlcH failed to show a band, indicating that if ylcH is a gene, it is not expressed under the conditions tested Function unknown, DLP12 prophage	33
ymdF	mRNA	ymdF and yciG are paralogs. YciG and YmdF are similar to small stress proteins (COG3729) including GsiB of B. subtilis and yciG is regulated by RpoSFunction unknown	57
ymgJ	mRNA	ymgI and ymgJ are adjacent, divergently oriented, and paralogous to one another Function unknown	61
ynbG	mRNA	Expressed protein, function unknown	21
ythA	mRNA	Expressed protein, function unknown	41
yqcG	mRNA	Expressed protein, function unknown	46
ypdK	mRNA	Expressed protein, membrane-associated, function unknown	23
ypdJ	mRNA	YpdJ appears to be a pseudogene fragment encoding the N-terminus (minus the start codon) of an excisionasePseudogene, exisonase fragment, CPS-53/KpLE1 prophage	17
yoeD	mRNA	Pseudogene, CP4-44 putative defective prophage; C-terminal fragment of a putative transposase	42
acrZ (ybhT)	mRNA	accessory factor stabilizing or modulating the acrABTolC efflux pump through AcrB bindingAcrAB-TolC efflux pump accessory protein, membrane-associated	49
azuC	mRNA	Expressed protein, membrane-associated	28
hisL	mRNA	his operon leader peptide	16
ilvX	mRNA	Expressed protein, function unknown	16
iroK	mRNA	3-hydroxypropionic acid resistance peptide	21
kilR	mRNA	Killing function of the Rac prophage during zygotic induction; causes cell division arrest and altered cell morphology, suppressed by ftsZ overexpression	73
mgtL	mRNA	The mgtA gene is regulated by a riboswitch responding to Mg(2+) and the MgtL leader peptide responding to intracellular proline levelsRegulatory leader peptide for mgtA	17
pauD	mRNA	argU pseudogene, DLP12 prophage attachment site	N/A 45 nucleotides
pawZ	mRNA	CPS-53 prophage attachment site attR, argW pseudogene	N/A 15 nucleotides
pheL	mRNA	pheA gene regulatory leader peptide	15
ttcC	mRNA	Pseudogene, prophage Rac integration site ttcA duplicationThe ttcC' pseudogene is a remnant 17 aa displaced from the original chromosomal ttcA gene	17

ybfQ	mRNA	Pseudogene, H repeat-associated protein, RhsC-linked; putative defective transposaseN-terminal domain fragment, matches first 79 residues of paralogs YhhI, YdcC, YbfD, pseudogene YbfL, and the more distant pseudogene paralog YncI. Putative transposase fragment	79
cyst	tRNA	Cysteine tRNA(GCA)	N/A 74 nucleotides
leuW	tRNA	Leucine tRNA(CAA) 3; anticodon has cmo(5)U	N/A 85 nucleotides
leuX	tRNA	Leucine tRNA(UAA) 5 (amber [UAG] suppressor)	N/A 85 nucleotides
glnU	tRNA	Glutamine tRNA(UUG) 1	N/A 75 nucleotides
glnW	tRNA	Glutamine tRNA(UUG) 1	N/A 75 nucleotides
glnV	tRNA	Glutamine tRNA(CUG) 2	N/A 75 nucleotides
glnX	tRNA	Glutamine tRNA(CUG) 2	N/A 75 nucleotides
gltT	tRNA	Glutamate tRNA(UUC) 2	N/A 76 nucleotides
gltV	tRNA	Glutamate tRNA(UUC) 2	N/A 76 nucleotides
gltU	tRNA	Glutamate tRNA(UUC) 2	N/A 76 nucleotides
gltW	tRNA	Glutamate tRNA(UUC) 2	N/A 76 nucleotides
aspV	tRNA	Aspartate tRNA(GUC) 1 triplicated gene	N/A 77 nucleotides
asnU	tRNA	Asparagine tRNA(GUU)	N/A 76 nucleotides
asnW	tRNA	Asparagine tRNA(GUU)	N/A 76 nucleotides
aspT	tRNA	Aspartate tRNA(GUC) 1 triplicated gene	N/A 77 nucleotides
asnT	tRNA	Asparagine tRNA(GUU)	N/A 76 nucleotides
asnV	tRNA	Asparagine tRNA(GUU)	N/A 76 nucleotides
aspU	tRNA	Aspartate tRNA(GUC) 1 triplicated gene	N/A 77 nucleotides
proK	tRNA	Proline tRNA(CGG) 1	N/A 77 nucleotides
proL	tRNA	Proline tRNA(GGG) 2	N/A 77 nucleotides
trpT	tRNA	Tryptophan tRNA(CCA)	N/A 76 nucleotides
tyrU	tRNA	Tyrosine tRNA(GUA) 2	N/A 85 nucleotides
tyrT	tRNA	Tyrosine tRNA(GUA) 1; tandemly duplicated	N/A 85 nucleotides
tyrV	tRNA	Tyrosine tRNA(GUA) 1; tandemly duplicated	N/A 85 nucleotides
micA	sRNA	Induced during stationary phase. RpoE regulon. Hfq-bound MicA antisense sRNA binds the RBS region of ompA mRNA, blocking translation and promoting mRNA degradation.sRNA regulator of ompA, lamB, ompX and phoP, Hfq-dependent	N/A 78 nucleotides
omrB	sRNA	Hfq-dependent omrA and omrB sRNAs downregulate curli gene activator CsgD post-transcriptionally and repress curli formationsRNA downregulating OM proteins and curli; positively regulated by OmpR/EnvZ; binds Hfq	N/A 82 nucleotides
rdlC	sRNA	Antisense sRNA RdlC affects LdrC translation; proposed addiction module in LDR-C repeat, with toxic peptide LdrC	N/A 68 nucleotides
rdlD	sRNA	Antisense sRNA RdlD affects LdrD translation; proposed addiction module in LDR-D repeat, with toxic peptide LdrD	N/A 66 nucleotides
rdlA	sRNA	Antisense sRNA RdlA affects LdrA translation; proposed addiction module in LDR-A repeat, with toxic peptide LdrA	N/A 67 nucleotides
rdlB	sRNA	Antisense sRNA RdlB affects LdrB translation; proposed addiction module in LDR-B repeat, with toxic peptide LdrB	N/A 66 nucleotides
tff	sRNA	Novel sRNA, function unknown	N/A 136 nucleotides
sroH	sRNA	Novel sRNA, function unknown	N/A 161 nucleotides

ryjB	sRNA	Novel sRNA, function unknown	N/A 90 nucleotides
rydC	sRNA	sRNA regulator of yejABEF; over-expression causes a thermosensitive growth phenotype on minimal glycerol, maltose, or ribose media	N/A 64 nucleotides
ryfD	sRNA	Novel sRNA, function unknown	N/A 143 nucleotides
gadY	sRNA	pairs with the 3' end of gadX mRNA to stabilize itgadY also has two short forms: 90 and 59 nt. RpoS regulon. Found only in E. coli.sRNA regulator of gadAB transcriptional activator GadX mRNA	N/A 105 nucleotides
mgrR	sRNA	sRNA affecting sensitivity to antimicrobial peptides; regulated by PhoPQ and Mg(2+)An mgrR deletion confers a 10-fold increase in Polymyxin B resistance, indicating a probable change in LPSygdQ and eptB are negatively regulated by the mgrR sRNAThe sRNA mgrR and the adjacent converging protein gene yneM are conserved and are also expressed in Salmonella	N/A 98 nucleotides
rrfB	rRNA	5S rRNA of rrnB operon	N/A 120 nucleotides
rrfD	rRNA	5S rRNA of rrnD operon	N/A 120 nucleotides
rrfF	rRNA	5S rRNA of rrnD operon	N/A 120 nucleotides
rrfH	rRNA	5S rRNA of rrnH operon	N/A 120 nucleotides
rrfA	rRNA	5S rRNA of rrnA operon	N/A 120 nucleotides
rrfC	rRNA	5S rRNA of rrnC operon	N/A 120 nucleotides
rrfE	rRNA	5S rRNA of rrnE operon	N/A 120 nucleotides
rrfG	rRNA	5S rRNA of rrnG operon	N/A 120 nucleotides

Table C-1. Chart documentation of 0 GCT site genes

Appendix D

Gene Name	RNA Type	Amino Acids	KEIO/ASKA Collection	
yhhY	mRNA	162	K: Plate 35, Well 7C	A: Plate 39, Well 10E
yhfA	mRNA	134	K: Plate 35, Well 5D	A: Plate 43, Well 10G
ygiW	mRNA	130	K: Plate 33, Well 7A	A: Plate 33, Well 2D
yggL	mRNA	108	K: Plate 61, Well 2C	A: Plate 32, Well 12E
yciH	mRNA	108	K: Plate 21, Well 4E	A: Plate 20, Well 3B
iraM (elbA)	mRNA	107	K: Plate 31, Well 8G	A: Plate 46, Well 7A
mqsR (ygiU)	mRNA	98	K: Plate 33, Well 6G	A: Plate 22, Well 6G
flgM	mRNA	97	K: Plate 89, Well 12H	A: Plate 19, Well 9C
yiaG	mRNA	96	K: Plate 35, Well 10H	A: Plate 44, Well 4C
yggU	mRNA	96	K: Plate 69, Well 6G	A: Plate 48, Well 9E
eutN (cchB)	mRNA	95	K: Plate 27 Well 9E	A: Plate 31, Well 10C
ihfB	mRNA	94	K: Plate 1, Well 4F	A: Plate 9, Well 11H
gatB	mRNA	94	K: Plate 85, Well 6G	A: Plate 22, Well 5F
racC	mRNA	91	K: Plate 63, Well 2A	A: Plate 20, Well 5D
yeaC	mRNA	90	K: Plate 11, Well 9D	A: Plate 21, Well 7E
yfhL	mRNA	86	K: Plate 27, Well 4C	A: Plate 31, Well 12H
yoeB	mRNA	84	K: Plate 69, Well 1F	A: Plate 48, Well 7D
ydcY	mRNA	77	K: Plate 13, Well 12D	A: Plate 20, Well 9C
yodD	mRNA	75	K: Plate 77, Well 7A	A: Plate 48, Well 4D
ykfH	mRNA	73	K: Plate 17, Well 9B	A: Plate 3, Well 5G
yiiF	mRNA	72	K: Plate 87, Well 1E	A: Plate 48, Well 3B
ygeI	mRNA	72	K: Plate 79, Well 3B	A: Plate 48, Well 3D
tdcR	mRNA	72	K: Plate 87, Well 6F	A: Plate 48, Well 3C
ogrK	mRNA	72	K: Plate 63, Well 4A	A: Plate 22, Well 5A
imfA	mRNA	72	NONE	A: Plate 9, Well 8B
yaiZ	mRNA	70	K: Plate 75, Well 1F	A: Plate 48, Well 2D
cspH	mRNA	70	K: Plate 85, Well 11F	A: Plate 48, Well 2B
rcbA (ydaC)	mRNA	69	K: Plate 13, Well 9E	A: Plate 20, Well 5B
ydfZ	mRNA	67	K: Plate 15, Well 1G	A: Plate 20, Well 11H
iscX (yfhJ)	mRNA	66	K: Plate 17, Well 5E	A: Plate 31, Well 12A
glgS	mRNA	66	K: Plate 5, Well 3G	A: Plate 33, Well 3C
ydfD	mRNA	63	K: Plate 11, Well 6D	A: Plate 21, Well 2D
hicA (yncN)	mRNA	58	K: Plate 67, Well 10A	A: Plate 47, Well 9H
ymgI	mRNA	57	NONE	
ydcA	mRNA	57	K: Plate 13, Well 11C	A: Plate 20, Well 8D
yciZ	mRNA	57	NONE	
ansB	mRNA	57	K: Plate 77, Well 2E	A: Plate 47, Well 9E
yciX	mRNA	55	K: Plate 79, Well 12A	A: Plate 47, Well 5C
yjiS	mRNA	54	K: Plate 39, Well 5B	A: Plate 46, Well 4A
ynfN	mRNA	51	K: Plate 67, Well 10G	A: Plate 47, Well 8F
ydaF	mRNA	51	K: Plate 13, Well 9G	A: Plate 20, Well 5G
ecnB	mRNA	48	K: Plate 63, Well 11C	A: Plate 47, Well 7C
yobF	mRNA	47	K: Plate 83, Well 2B	A: Plate 22, Well 10C
ykgO	mRNA	46	NONE	
sra	mRNA	45	K: Plate 57, Well 5E	A: Plate 11, Well 6C
blr	mRNA	41	K: Plate 85, Well 9D	A: Plate 47, Well 5B
ymiB	mRNA	34	NONE	
ivbL	mRNA	32	K: Plate 89, Well 6C	A: Plate 44, Well 7G
yneM	mRNA	31	NONE	
ynhF	mRNA	29	NONE	
kdpF	mRNA	29	K: Plate 67, Well 2A	A: Plate 7, Well 9F
uof	mRNA	28	NONE	
leuL	mRNA	28	K: Plate 41, Well 2H	A: Plate 1, Well 9F
ydgU	mRNA	27	NONE	
yqeL	mRNA	26	NONE	
yoaJ	mRNA	24	NONE	
yobI	mRNA	21	NONE	
thrL	mRNA	21	K: Plate 79, Well 11D	NONE
yoeI	mRNA	20	NONE	
ypfM	mRNA	19	NONE	
ibsC	mRNA	19	NONE	
ibsE	mRNA	19	NONE	
yjeV	mRNA	17	NONE	
acrZ	mRNA	121 nt	NONE	
oxyS	mRNA	110 nt	NONE	
yrhA	pseudogene	164	K: Plate 89, Well 1G	A: Plate 49, Well 7E
ykfJ	pseudogene	69	K: Plate 17, Well 8F	A: Plate 22, Well 7F
yibU	pseudogene	66	NONE	
ysaD	pseudogene	64	NONE	
intG	pseudogene	54	K: Plate 67, Well 12H	NONE
yoeH	pseudogene	53	NONE	
ybfI	pseudogene	49	NONE	
yrdF	pseudogene	47	NONE	
ymjC	pseudogene	46	K: Plate 13, Well 8H	A: Plate 20, Well 4F
ysaC	pseudogene	33	NONE	

sokA	pseudogene	30 nt	NONE
ykgP	pseudogene	29	NONE
yeeH	pseudogene	29	NONE
ykgT	pseudogene	26	NONE
ymjD	pseudogene	21	NONE
spf	sRNA	109 nt	NONE
micC	sRNA	109 nt	NONE
mcaS	sRNA	96 nt	NONE
micF	sRNA	93 nt	NONE
omrA	sRNA	88 nt	NONE
chiX	sRNA	84 nt	NONE
ohsC	sRNA	77 nt	NONE
rybB	sRNA	68 nt	NONE
rydB	sRNA	68 nt	NONE
sokE	sRNA	59 nt	NONE
sokB	sRNA	56 nt	NONE
sokC	sRNA	55 nt	NONE
dicF	sRNA	53 nt	NONE
selC	tRNA	95 nt	NONE
serU	tRNA	90 nt	NONE
serX	tRNA	88 nt	NONE
serT	tRNA	88 nt	NONE
serW	tRNA	88 nt	NONE
valW	tRNA	77 nt	NONE
valV	tRNA	77 nt	NONE
proM	tRNA	77 nt	NONE
argU	tRNA	77 nt	NONE
thrW	tRNA	76 nt	NONE
thrU	tRNA	76 nt	NONE
pheU	tRNA	76 nt	NONE
pheV	tRNA	76 nt	NONE
lysY	tRNA	76 nt	NONE
pheV	tRNA	76 nt	NONE
lysY	tRNA	76 nt	NONE
lysV	tRNA	76 nt	NONE
lysT	tRNA	76 nt	NONE
lysZ	tRNA	76 nt	NONE
lysW	tRNA	76 nt	NONE
lysQ	tRNA	76 nt	NONE
argW	tRNA	75 nt	NONE

Table D-1. Chart documentation of 1 GCT site genes

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EDUCATION

The Pennsylvania State University- The Schreyer Honors College — **University Park, PA**
College of Engineering — Class of May 2014
Bachelor of Science in Chemical Engineering

LEADERSHIP EXPERIENCE

Boy Scouts of America — **Worcester, PA**
Eagle Scout; Junior Assistant Scoutmaster, Troop 133 — September 1999 - Present

- Constructed four owl nesting boxes for Mill Grove Sanctuary located in Audubon, PA with the hope of increasing the dwindling Screech and Great Horned Owl populations.
- Compiled a total of 36 total Merit Badges in various fields ranging from chemistry to swimming
- Served as a senior mentor to the newly joined scouts and presented the various intricacies associated in the scouting realm
- Elected into the Order of the Arrow Brotherhood, Scouting's National Honor Society

Society of Distinguished Alumni Mentoring Program — **University Park, PA**
Protégé — Spring 2011 - Present

- Paired up with Mr. Arthur Glenn '56, B.S. Engineering, who was a pioneer of implementing CAT scan and MRI technology within the ever successful company of General Electric.
- Participate in Distinguished Alumni networking discussions in which various alums share their wisdom and experience in their respective fields

WORK EXPERIENCE

Air Liquide Americas - Electronics Division — **Manassas, VA**
Chemical Intern — June 2013 – August 2013

- Worked on the several parameters surrounding the air separation unit's cooling water system, its treatment, and points for improvement
- Developed a site specific procedure detailing not only the operation settings but also testing requirements that must be maintained on the water to meet quality standards
- Performed daily readings of equipment out in the plant that included surveying compressors, expanders, pumps, and filters
- Met the customer's needs by filling cylindrical dewars from a storage vessel with liquid Nitrogen or Argon

Fenske Chemical Engineering Lab — **University Park, PA**
Intern — September 2012 – Present

- Work in the fields of biofilms and understanding their formations within E. coli
- Discerned novel toxin and antitoxin systems and understood their combative nature through gene documentation of E. coli.
- Work will be culminated in a full fledge thesis upon graduation under the guidance of Dr. Thomas Wood.

ArcelorMittal Monessen LLC — **Monessen, PA**
Associate Chemical Intern — May 2012 – August 2012

- Worked on capital improvement projects at the hot idled coke plant outside of Pittsburgh.
- Developed the specifics for a new liquid ammonium sulfate system, a key by product in the coke producing process sold commonly as an effective fertilizer
- Performed numerous material and heat balances on the industrial system as reflected through the development of a process flow diagram
- The findings were fully presented to the plant manager, Paul Champagne, at the end of the summer in a formal presentation

Penn State Abington Kids & Teen College Enrichment — **Abington, PA**
Summer Camp Counselor — Summer 2011

- Led and instructed 15 children aged 9-13 each week in a classroom setting about preliminary scientific concepts.
- Topics of education varied by week and included: Rocketry Principles and Lego Robotics.
- Dedicated 40 hours per week

ACCOMPLISHMENTS

The Society of Omega Chi Epsilon, Beta Alpha Chapter — **University Park, PA**
Member — March 2013-Present

- Nationally recognized society that promotes high scholarship and investigation within Chemical Engineering
- Members must maintain a 3.5 GPA or higher for admittance into the program
- Must complete 10 hours minimum of peer tutoring to fellow undergraduate students in the major

Schreyer Honors College Academic Excellence Scholarship — **University Park, PA**
Recipient — August 2010

- Yearly scholarship of $3,500 bestowed upon the select few individuals who not only are members of Penn State University but also the intimate atmosphere of the Schreyer Honors College.
- Intention of the honors college is to stimulate a multidisciplinary center of academic excellence.