Investigating the effects of genetic background on the
fitness of quinolone resistance mutations in *Escherichia*
coli

by

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Abstract

Antimicrobial resistance (AMR) is one of the largest threats to public health and puts a serious strain on healthcare systems around the world. Many first line antibiotics can no longer be used to fight infections due to increased resistance. The fitness of AMR strains of bacteria is determined in part by epistasis, whereby resistance mutations may have different effects on different genetic backgrounds. Thus, the objective of this research is to investigate the effects of genotype on the fitness of AMR. Using genetic techniques in *E. coli*, quinolone resistance alleles of the *gyrA* gene (S83L, D87N, and a combination of S83L-D87N) were transferred into a collection of knockout strains, resulting in approximately 12 000 double-mutants. Genetic interactions that affected fitness, both positively and negatively, were common and a variety of synthetic lethal/sick interactions were found. A number of the genes lethal with *gyrA* mutations were involved in DNA synthesis, repair, and replication, much like *gyrA* itself. Using strains that were able to conditionally express the knocked out genes, yield and growth curves were examined over a 24 hour period to validate the synthetic lethal interactions. Results varied for each strain – of 33 strains assayed, 21 showed a deficit in at least one fitness-related phenotype. Overall, more work should be done to further examine these putative synthetic lethal interactions. Once done, looking at all these genetic interactions will help to untangle the functionality of certain genes and, in the context of AMR, identification of synthetic lethal interactions of AMR mutations may lead to drug targets that can specifically kill resistant bacteria.

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Introduction

1. Antibiotic Resistance

In 1928, Alexander Fleming discovered the first antibiotic. Since then, there are now at least seven classes of antibiotics used in medicine, with well over 100 individual drugs. Thus, antibiotics have become the most reliable way to treat a wide array of bacterial infections. However, as more antibiotics were created and discovered, the more humans began to misuse them. In Fleming's Nobel Prize speech, he remarks that, by under-dosing a patient, we risk exposing microbes to a non-lethal amount of penicillin which could lead to resistance (Rosenblatt-Farrell 2009). Antibiotics are frequently over-used and over-prescribed (McGowan 1983). Many patients do not correctly consume them, be it by exaggerating their symptoms for a prescription or not finishing their dose (Pechere 2001). Antibiotics are also used widely in agriculture – animals are often fed antibiotics to prevent infections (Khachatorians 1998). This misuse has resulted in an increase of antimicrobial resistance in which the bacteria is no longer susceptible or killed by the presence of antibiotics (Levy and Marshall 2004).

The emergence of antimicrobial resistance (AMR) has become one of the largest threats to human health and has put a serious strain on the healthcare system (WHO, 2016). An increasing number of bacterial strains are becoming resistant to antibiotics and few new treatments are being discovered to combat this. First-line antibiotics can now no longer be used to fight bacterial infections which results in many untreatable cases (CDDEP 2015; Bryce *et al.* 2015). For example, *Escherichia coli* infections such as urinary tract infections (UTIs) were often treated using antibiotics such as fluoroquinolones. However, *E. coli* has become increasingly resistant to fluoroquinolones

worldwide. In Canadian hospitals, *E. coli* resistant to ciprofloxacin have increased in prevalence from 20% to 29.2% from 2007 to 2011 (Karlowisky *et al.* 2013), with bacteria from UTIs frequently being found to be multidrug resistant (Karlowisky *et al.* 2006). This has led to an increase in mortality for patients with *E. coli* infections (Camins *et al.* 2011). It is thought that by the year 2050, antimicrobial resistance will be the cause of up to 10 million deaths with the potential to have a \$100 trillion impact on the economy worldwide, if nothing is done to counteract resistance (O'Neil 2016). Thus, global public health authorities have stressed the importance of reducing the effects of AMR.

2. Fluoroquinolones

Fluoroquinolones are a class of synthetic antibiotics used to treat a broad spectrum of bacterial infections. The primary target of these antibiotics are DNA gyrase and topoisomerase IV (Drlica and Zhao 1997) which are both important for DNA replication. For *E. coli* and other Gram-negative bacteria, DNA gyrase is the primary target (Smith 1986) and Topoisomerase IV is only a secondary target (Khodursky *et al.* 1995). DNA gyrase is comprised of the GyrA and GyrB subunits. In bacteria, DNA gyrase is important for two processes involved in DNA replication. First, it introduces negative supercoils in DNA which help to initiate DNA replication (Wang 1996). Second, DNA gyrase also removes positive supercoils that accumulate before the replication fork (Wang 1996). Much like DNA gyrase, topoisomerase IV is comprised of two subunits, ParC and ParE. It separates daughter chromosomes which allows for the division of daughter cells (Adams *et al.* 1992). Fluoroquinolones inhibit these enzymes and interfere with the bacteria's ability to synthesize DNA. For both enzymes,

fluoroquinolones interact with the DNA-enzyme complexes (Drlica and Zhao 1997). Gyrase introduces a double-strand break into the DNA and the fluoroquinolone traps the enzyme onto the gyrase-DSB complex on the DNA, forming a physical barrier and interrupting the movement of the replication fork (Low and Scheld 1998). This inhibits their ability to control supercoiling which, at low concentrations, results in impaired DNA replication and, at high concentrations, results in cell death (Ng *et al.* 1996, Drlica *et al.* 2009).

3. Mechanisms of Resistance

Bacteria evolve resistance to antibiotics in a variety of ways. Some bacteria can produce enzymes that destroy or inactivate the antibiotic before it is able to have an effect. For example, *B*-lactamases are able to hydrolyze the *B*-lactam bond of penicillins and cephalosporins (Richmond and Sykes 1973). Similarly, some bacteria resistant to fluoroquinolones produce a variant of the enzyme aminoglycoside acetyltransferase, ACC(6')-Ib. This enzyme reduces the activity of certain fluoroquinolones, like ciprofloxacin, by introducing an acetyl group (Robicsek *et al.* 2006).

Bacteria can also decrease an antibiotics' ability to penetrate the cell. This is done through the down regulation of porins and up regulation of efflux pumps. Both porins and efflux pumps are proteins that allow for the passage and transport of molecules across the cell membrane. Porins create channels to allow the passage of molecules into the cell and efflux pumps are active transporters that expel substrates outside of the cell. When the level of expression of these proteins change, it can affect how much of the antibiotic is

transferred into the cell and how much antibiotic is expelled from the cell, resulting in antibiotic resistance.

Bacteria can also be resistant to antibiotics by interfering with the antibiotic target site by either protecting or modifying the target site. For example, in tetracycline resistant bacteria, the proteins TetM and TetO remove the tetracycline from its binding site on the ribosome. TetM does this by interacting with part of the 16S rRNA (Donhofer *et al.* 2012) and TetO does this by competing for the same spot on the binding site (Li *et al.* 2013). In fluoroquinolone-resistant bacteria, protection of the target site is caused by the protein Qnr (Munita and Arias 2016). Qnr binds to the DNA binding site of Gyrase and Topoisomerase IV, which reduces the ability of fluoroquinolones to complex with the DNA-enzyme (Tran *et al.* 2005).

Target sites can also be modified so that antibiotics are no longer able to interact with them. This can be done through point mutations, alterations in enzymes, and replacement of the original target (Munita and Arias 2016). For example, in rifampin resistant bacteria, rifampin binds to *rpoB*, a structure located in the B-subunit of the RNA polymerase, which blocks transcription (Campbell *et al.* 2001). Resistance to rifampin can occur through point mutations that substitute amino acids in *rpoB* which decrease the affinity of the drug to the target site. The most common way for bacteria to become resistant to fluoroquinolones is through mutations in DNA Gyrase and Topoisomerase IV. In *E. coli*, S83L and D87N mutations in GyrA are common in fluoroquinolone resistant clinical isolates, with the S83L/D87N double mutant conferring particularly high levels of resistance (Basra *et al.* 2018). These mutations affect the target sites of

fluoroquinolones and cause the antibiotic to lose its ability to form enzyme-DNA-drug complexes, resulting in resistance.

4. Epistasis

The fitness of AMR mutants can be influenced by epistasis (Wong 2017).

Epistasis, or genetic interaction, refers to the influence that genetic background has on the fitness effects of a mutation. Epistasis is most often considered in terms of the fitness effects of two mutations whether alone or in combination with each other. Epistasis occurs when the fitness of the double-mutant differs from what is expected given the fitness effects of the single mutations. There are three varieties of epistatic interactions: positive epistasis, where the fitness of the double-mutant is higher than expected, negative epistasis, where the fitness of the double-mutant is lower than expected (Kimura and Maruyama 1966), and sign epistasis which occurs when one mutation has the opposite effect when in the presence of another mutation (Figure 1). Sign epistasis indicates that the direction of selection changes depending on the genetic context (i.e. two individually deleterious mutations become beneficial when combined or two individually beneficial mutations become deleterious when combined) (Weinreich *et al.* 2005).

5. Persistence of AMR

Bacteria with antimicrobial resistance mutations will often endure a fitness cost for having these mutations, and many studies have shown that resistance mutations are selected against in the absence of antibiotics (Melnik *et al.* 2015). This observation thus suggests that resistance may be eliminated through decreasing or stopping the use of

certain antibiotics. However, studies on this effect have had mixed results and in some cases AMR has persisted for months or even years in the absence of antibiotics (Enne 2010, Sundqvist *et al.* 2010).

Epistatic interactions can affect the persistence of AMR in a variety of ways. One way is through compensatory mutations. These are mutations that increase fitness without eliminating resistance (Hall 2013), which would allow for the persistence of AMR even in the absence of antibiotics. Although a given AMR mutation may disrupt key processes in the bacterial cell, compensatory mutations are often able to restore them (Boone *et al.* 2007, de Visser *et al.* 2011). A variety of experiments have shown that these mutations are able to evolve easily in laboratory strains and that it is likely that resistance mutations may have multiple compensatory mutations (Bjorkman *et al.* 2000). It is also thought that AMR mutations and compensatory mutations interact in an epistatic way - compensatory mutations may be beneficial in the presence of an AMR mutation, but neutral or deleterious otherwise. As a demonstration of epistasis between the resistance mutation and the compensatory mutation, Brandis and colleagues (2012) examined mutations in *rpoB*, the RNA polymerase β -subunit, which can cause a high level of resistance to rifampicin. In *Salmonella enterica*, compensatory mutations were found in either *rpoA*, *rpoB*, or *rpoC* (*rpoA* and *rpoC* being other subunits of RNA polymerase) of every lineage evolved through serial passage. These compensatory mutations were shown to alleviate the costs of the mutation in *rpoB* in the presence and absence of antibiotics. However, although these compensatory mutations alleviate the fitness costs of rifampicin resistant mutations, when isolated they either have no effect or are deleterious.

Figure 1. Example of different types of epistasis. Mutations A and B have an individual fitness of 10. Under normal circumstances, their combined fitness would be the additive fitness of the two individual mutations (i.e. 20). However, epistasis causes their fitness to differ than what is expected. Under positive epistasis, the fitness is greater than expected (30) and under negative epistasis, the fitness is lower than expected (10). In the case of sign epistasis, the combined fitness is the opposite of what is expected – the double mutant is now deleterious when even though the individual mutations were beneficial

As another demonstration of epistasis between the resistance mutation and the compensatory mutation, Knopp and Andersson (2015) examined *E. coli* with *ompC* and *ompF* genes deleted, where both genes code major outer membrane proteins that form channels or pores. These pores allow for the diffusion of small molecules across the membrane which can play a role in antibiotic resistance. With the genes deleted, there was a decrease in fitness and an increase in antibiotic resistance in various lineages. These strains also acquired compensations that allowed them to use alternative porins rather than *ompC* and *ompF*.

Another way that epistatic interactions affect the persistence of AMR is through the evolution of multi-drug resistance (MDR). MDR most often occurs when bacteria have accumulated multiple chromosomal and/or plasmid-borne resistance elements. Studies have shown that genetic interactions, specifically positive epistasis where the combined cost is less than expected, between chromosomal mutations and plasmids (i.e. chromosome-chromosome, plasmid-plasmid, and chromosome-plasmid) are common (Trindade *et al.* 2009, Silva *et al.* 2011, and San Millan *et al.* 2014), which may allow for the persistence of MDR. For example, Trindade *et al.* (2009) examined genetic interactions between antibiotic resistance mutations in *E. coli* that conferred resistance to a variety of antibiotics including streptomycin, nalidixic acid, and rifampin. They found that epistasis, especially positive epistasis, was prevalent throughout. Thus, the double mutant is more fit than one or both of the mutation, facilitating the persistence of the double mutant.

5.2 Functional interactions

Epistasis can be leveraged to understand the functional interactions and relationships between genes. By looking at double-mutant or double-knockout organisms in which each mutation disrupts a separate gene, we can understand how those genes interact. For example, using *Saccharomyces cerevisiae*, Tong *et al.* (2001) screened the genetic interactions between 8 deletion mutations – 4 important in cytoskeletal organization, 2 with roles in DNA synthesis and repair, and 2 uncharacterised genes - against a collection of 4700 deletion backgrounds in order to understand the functional relationship between genes and produce a map of gene function. From these screens, they saw 291 genetic interactions in 204 genes. They expand on this study to include 132 different query genes against the same 4700 deletion backgrounds. Tong *et al.* (2004) used large-scale screens in order to analyze genetic interactions between all the deletion pairs to create a genetic network which would show the functional relationship between the genes.

Genetic interactions can also help in ordering a genetic pathway (Avery and Wasserman 1992). For example, using crosses of temperature-sensitive mutations, four steps of DNA synthesis in yeast, *Saccharomyces cerevisiae*, were determined (Hereford and Hartwell 1973). Mutations were introduced into genes *cdc 28*, *cdc 4*, and *cdc 7*, which had been shown to have a part in DNA synthesis in previous studies (Hartwell *et al.* 1973). Each mutation in isolation, or as double mutants, were exposed to two restrictive conditions, high temperature and the presence of α factor, in order to see when DNA synthesis would stop. Understanding the order of steps was done by comparing the phenotypes of the single and double mutant strains.

6. Synthetic Lethality

Synthetic lethal (SL) interactions are a specific type of genetic interaction in which a combination of mutations are lethal on some genetic backgrounds when they are not lethal on their own. One of the first synthetic lethal screens occurred in yeast studying *cdc24* and *cdc42*, genes required for bud formation (Bender and Pringle, 1991). When temperature sensitive mutations are added to these genes, bud formation stops. *msb1* was found to suppress these mutations as well as interact with other genes such as *bem1* and *bem2*. When temperature sensitive mutations are added to *bem1* or *bem2* in conjunction with the *msb1* deletion, mutants die. In *E. coli*, Babu *et al.* (2014) examined interactions between knockouts of 163 query genes and the Keio collection, a set of about 4000 strains of *E. coli* with knockouts of non-essential genes. These query genes were important for essential processes including metabolism, transcription, protein synthesis, and DNA replication and repair. They found both alleviating and lethal interactions. For example, *recABC*, which encodes recombination machinery, and DNA polymerases, which synthesize DNA, were synthetic lethal in combination.

Understanding genetic interactions between resistance mutations and other loci in the genome could allow for the minimization of AMR. It has been suggested that SL interactions, specifically, can be leveraged to help minimize AMR. Grompone *et al.* (2003) identified temperature sensitive *gyrB* mutations which show synthetic lethality when in combination with mutations in *priA*, a protein that encodes part of the primosome which is involved in restarting stalled replication forks. Thus, synthetic lethal interactions can show the susceptibility of different genotypes with AMR mutations and may often lead to finding targets to specifically kill resistant genotypes. Some

experiments have been done in order to identify compounds that target AMR genotypes. For example, Stone *et al.* (2016) identified compounds that select against *E. coli* with tetracycline (TetA) resistant efflux pumps. This was done through a screen of 19 000 compounds against *E. coli* in order to see how the different compounds effected growth. This and other secondary screens found that disulfiram and β -thujaplicin select against tetracycline-resistant *E. coli* and treatment of β -thujaplicin resulted in the loss of resistance through mutations in *tetA*.

In the case of chemotherapeutics, large scale screens have been used to identify synthetic lethal interactions in cancer cells. These screens can use libraries of molecules to identify drugs that inhibit cells. For example, BRCA1 and BRCA2 are genes important for repairing double stranded breaks in DNA. Women with mutations in these genes are predisposed to breast and ovarian cancer. Farmer *et al.* (2005) found that the inhibition of enzyme PARP1, involved in repairing single strand breaks in DNA, in a BRCA1 mutant kills tumours. They acquire DNA breaks and are unable to repair them. Using this knowledge, a variety of antitumor drugs targeting PARP1 have been designed with a number of number moving on to clinical trials (Malyuchenko *et al.* 2015). One of these drugs, olaparib, inhibited tumours with mutations in BRCA1 BRCA2 (Fong *et al.* 2009) and cells without these genes were more sensitive to PARP1 inhibition.

7. Experimental Purpose

I aimed to investigate how genetic background affects the fitness of fluoroquinolone resistant *E. coli*. I constructed genetic interaction maps between gene knockouts of non-essential genes and three common quinolone resistance alleles – S83L, D87N, and the

double mutant S83L-D87N. This will provide data about novel genetic interactions for *gyrA*, which as an essential gene has not been included in previous large scale interactions screens. Given Gyrase's role in DNA replication, I predict that strains with quinolone resistance mutations will be synthetic lethal when in combination with gene knockouts associated with DNA replication and repair. Ultimately, these screens may suggest targets to specifically kill AMR genotypes. If a knockout of particular gene is synthetically lethal with an AMR mutation, then that gene may be a candidate for future drug development.

Materials & Methods

1. Strains and Media

For this experiment, three *gyrase A* mutations were transferred into Hfr BW7623, a high frequency of recombination (Hfr) genotype, cells. The different mutations used were *S83L*, *D87N*, and *S83L-D87N*. The code *S83L* denotes that the 83rd amino acid in the *gyrase A* gene was mutated and the serine was now a leucine. *D87N* denotes that the 87th amino acid in the *gyrase A* gene was mutated and the aspartic acid was now an asparagine. Hfr cells were used to transfer these quinolone resistance mutations into strains in the Keio collection (figure 2), a collection of approximately 4000 strains of *E. coli* that have knock-outs at every non-essential gene which have been replaced by a kanamycin cassette (Baba *et al.* 2006). The Keio collection strains were obtained from NBRP-E.coli at the National Institute of Genetics (NIG) through Dr. Ashkan Golshani's lab. Hfr strain BW7623 (*purK79::Tn10 λ- relA1 spoT1*) is a specialized *E. coli* that has an F-plasmid incorporated into its chromosomal DNA allowing for the transfer of the

Figure 2. P1 transduction and eSGA methods

whole chromosome during conjugation. After these initial screens were conducted, the plasmids from ASKA (-) collection, obtained from NBRP-E.coli at NIG, were used to express genes of interest from the Keio knockout backgrounds (Kitagawa *et al.* 2005).

All strains were grown in Lysogeny Broth (LB), composed of 10g/L tryptone, 5 g/L yeast extract and 10 g/L NaCl, often supplemented with antibiotics as indicated, at 37C.

2. P1 transduction

Initially, a variety of *gyrA* quinolone resistance alleles (S83L, D87N, and a S83L-D87N double mutant allele) were transferred into the Hfr strain BW7623 using P1 transduction. First, a P1 lysate was created using P1vir obtained from Dr. Roberto Balbontin and Dr. Isabel Gordo (Instituto Gulbenkian de Ciencia, Portugal). Glucose, MgSO₄, and CaCl₂ were added to a final concentration of 0.2%, 10mM, and 5mM, respectively, to an overnight culture of a donor strain (*E. coli* with the mutation to be transferred). This was incubated for 30 minutes and then to this a stock of P1 virulent bacteriophage was added. After 3 hours, the culture cleared and the new lysate was stored in chloroform. Each lysate, which would contain some bacteriophage that had acquired DNA with the resistance mutation, were used to infect the Hfr recipient. For the transduction, MgSO₄ and CaCl₂ at a final concentration of 10mM and 5mM, respectively, were added to an overnight culture of the recipient strain BW7623. This mixture was then added to equal parts of the P1 lysate. After 20 minutes of incubation LB, sodium citrate, to the final concentration of 50mM, were added. After an hour and a half, this mixture was spun down and the resulting pellet was suspended in 0.1M sodium citrate (pH 5.5).

Transductants were then selected on ciprofloxacin (25ng/mL) and the *gyrA* genes were sequenced through Genome Quebec in order to confirm that they had the correct mutation.

3. *E. coli* synthetic genetic arrays (eSGA)

Transconjugants of *gyrA* alleles and strains from the Keio collection were created using a modified eSGA protocol (Butland *et al.* 2008) in triplicate. Strains from the Keio collection were grown in liquid LB supplemented with 50ug/mL Kn and Hfr strains with *gyrA* alleles were grown in liquid LB supplemented with 50ng/mL Cip. These two liquid cultures were pinned together onto drug-free LB plates. Here, the Keio strains underwent mating with the Hfr cells over a 24 hour period, allowing the Hfr cells to transfer the mutation of interest to the Keio strains by conjugation. Double mutants were then selected on double antibiotic plates of 50ng/mL ciprofloxacin and 50ug/mL kanamycin. After 24 hours on antibiotic plates, mutants were again pinned onto drug-free LB again and left to grow for another 24 hours. Pictures of these plates were taken using ProtoCOL3 and colony size was used as a proxy for fitness (figure 3). Using this proxy is common in other genetic screens using SGA analysis (Baryshnikova *et al.* 2010).

Image analysis, normalization, and scoring was done using SGATools (Wagih *et al.* 2013). In cases where growth was minimal and SGATools could not properly analyze the plates, the R package gitter was used by examining plate photographs through the use of a reference plate. SGATools analyzes the images and gives colony sizes in return in the form of pixel count. The sizes of the double mutants were then compared to various

Figure 2. Examples of eSGA plates of Keio strains with S83L-D87N (left) and S83L (right).

control colonies including all single-mutants, a Keio pseudogene (a strain in the Keio collection in which the knockout does not affect the growth), and the Hfr cell in order to determine the expected colony size. The epistasis value was calculated using the equation, in which w is the fitness of the colony:

$$\text{Epistasis} = w_{12} - w_1 w_2$$

Particular attention was paid when the eSGA produced synthetic lethal or synthetic sick combinations. Mutants were considered synthetic lethal when their growth was less than the size of the lowest 5% of growth (i.e. 70 pixels). If two of the three replicates did not grow and the third replicate was under this threshold, then the mutants were considered synthetic sick.

4. Validation of synthetic lethals

For a subset of putative sick/lethal interactions from the eSGA screen, we carried out additional testing to confirm synthetic lethality (figure 4). For a given double mutant (*gyrA* allele and Keio knockout), the individual strain from the Keio collection was made chemically competent. This was done by washing cultures of each strains with 100 mM MgCl_2 , 100 mM CaCl_2 , and 15% glycerol. The corresponding expression plasmid from the ASKA (-) collection was then transformed into the knockout mutant by heat-shocking at 42°C. These plasmids include a chloramphenicol resistant marker, Lac promoter region, as well as the gene that is knocked out in the Keio strain. The gene can be turned off and on by the presence or absence of isopropyl β -D-1-thiogalactopyranoside (IPTG). The *gyrA* allele that causes the synthetic lethality was then transferred into the Keio-plasmid strain using λ -red recombineering. First, the *pma7* plasmid, which encodes the key λ -red

Figure 4. Methods for synthetic lethal verification

functions, was electroporated into the target Keio-ASKA strain. The *pma7* plasmid is induced by arabinose and contains an ampicillin resistance marker along with *bet*, which encodes a single stranded DNA annealing protein (Lennen et al. 2016). After, a single stranded oligonucleotide with the mutation of interest, was then electroporated into the strain containing the Keio knockout, the ASKA (-) plasmid, and the *pma7* plasmid. When induced with arabinose, the *pma7* plasmid causes the ss-oligonucleotide to be methylated which allows it to more easily be recombined into the genome. These strains were selected on LB plates supplemented with 100mM IPTG, 50ng/mL ciprofloxacin, 50ug/mL kanamycin, and 30ug/mL chloramphenicol. They were then streaked onto antibiotic plates with 5% sucrose for single colonies. Because of the *sacB* region in the *pma7* plasmid, growth on sucrose cures the strain of *pma7*. Single colonies were split, half were grown on sucrose plates and the other half on 100ug/mL ampicillin plates. If there was growth on sucrose but not on ampicillin, then that colony was cured of its *pma7* plasmid.

Several phenotypes were measured for the validation strains, including growth assays on solid media, and growth curve parameters. For assays on solid media, cultures were grown for 24 hours, and serial dilutions were spot inoculated on antibiotic plates of 50ng/mL ciprofloxacin, 50ug/mL kanamycin, and 30ug/mL chloramphenicol with and without IPTG and colonies were counted. We expected that, for a true SL interaction, there should be no growth in the absence of IPTG - the presence of IPTG allows for the gene that was knocked out to be expressed from the ASKA plasmid, rescuing any SL phenotype. Synthetic sick interactions should result in reduced growth in the absence of IPTG.

With the same strains, growth curves were obtained over a 24 hour period using a plate reader. Strains were inoculated at 1:100 dilutions from overnight cultures and were grown in triplicate in 50ng/mL ciprofloxacin, 50ug/mL kanamycin, and 30ug/mL chloramphenicol LB with and without IPTG. Every 30 minutes, optical density (OD₆₀₀) was measured through the BioTek Gen5 software. Lag phase, maximum density, and growth rates were estimated from the program GrowthRates (Hall *et al.* 2014). Again, reduced or no growth in the absence of IPTG is indicative of synthetic sickness/lethality.

Results

1. Genetic interaction screens for three quinolone resistance alleles

Three large-scale genetic interaction screens were performed using the Keio collection and three *gyrA* alleles: S83L, D87N, and a S83L/D87N double mutant. From these screens, colony sizes of the resultant double-mutations were used as a proxy for fitness. The fitness of the double-mutants were compared to the single mutations and individual Keio strains in order to infer epistasis, the deviation from expected size, given the single mutant effects. Similarity between the replicates of the same screen was high (table 1), with correlation coefficients ranging from 0.4 to 0.8. For comparison, Babu *et al.* (2011) report r of ~0.8 between replicates. Epistasis is also significantly different between the three *gyrA* alleles (S83L-D87N: $t = 4.572$, $df = 7718.9$, $p < 0.001$, S83L-S83L/D87N: $t = 31.072$, $df = 7755.2$, $p < 0.001$, D87N-S87L/D87N: $t = 27.856$, $df = 7763.4$, $p < 0.001$).

For Keio strains with S83L, mean epistasis is 0.105 (+/- 0.389 std), median epistasis is 0.109, and the mode is 0.157. This shows the proportional difference between

Table 1. R and R² values between the three replicates of the *gyrA* alleles.

<i>gyrA</i> allele	Replications Compared	R	R ²
S83L	1-2	0.57	0.33
	1-3	0.40	0.16
	2-3	0.48	0.23
D87N	1-2	0.75	0.56
	1-3	0.80	0.64
	2-3	0.65	0.42
S83L-D87N	1-2	0.57	0.33
	1-3	0.63	0.4
	2-3	0.75	0.57

the double-mutant colonies and the expected fitness. Thus, for S83L strains, the fitness of the double-mutant colonies is mostly what would be expected from looking at the individual mutations (figure 4). However there is a slight skew to the positive side meaning the double-mutants grow more than what is expected. For Keio strains with D87N, mean epistasis is 0.067 (+/- 0.355 std), median epistasis is 0.084, and the mode is -0.386. The fitness of the double-mutant colonies is mostly centered around what would be expected looking at the individual mutants (figure 5). For the Keio strains with the double-mutant S83L-D87N, mean epistasis is -0.161 (+/- 0.367 std), median epistasis is -0.118, and the mode is -0.713. The fitness of the S83L-D87N colonies is thus skewed towards being mostly negative, meaning that the mutants grow less well than would be expected given the individual mutants (figure 6).

1. Synthetic lethal/sick interactions

From the genetic interaction screens, focus was put on the synthetic lethal/sick knockout *gyrA* allele combinations. In this case, synthetic lethality refers to combinations where all three replicates showed no growth. Synthetic sickness refers to combinations where two of the three replicates had no growth, and the third replicate showed growth below the 95% confidence intervals for all double mutants. Looking at these screens, a variety of synthetic lethal and sick combinations occurred (Appendix D, tables 1 and 2), many of which occurred with genes important for DNA synthesis, repair, and replication (Table 2). Overall, a total of 1571 synthetic lethal or sick interactions were observed – 89 in the *S83L*-Keio interaction, 876 in the *D87N*-Keio interaction, and 606 in the combined *S83L-D87N*-Keio interaction. 61 synthetic sick/lethal interactions are shared between

Figure 5. Histogram depicting the proportional difference between the fitness of the double mutant (S83L and gene knockout) and the individual single mutations. Bar on top showing median and 95% confidence intervals of the standard deviation between replicates (lower interval: 0.019, upper interval: 0.802, median: 0.216).

Figure 6. Histogram depicting the proportional difference between the fitness of the double mutant (D87N and gene knockout) and the individual single mutations. Bar on top showing median and 95% confidence intervals of the standard deviation between replicates (lower interval: 0.003, upper: 0.573, median: 0.120).

Figure 7. Histogram depicting the proportional difference between the fitness of the double mutant (S83L and D87N and gene knockout) and the individual single mutations. Bar on top showing median and 95% confidence intervals of the standard deviation between replicates (lower interval: 0, upper: 0.644, median: 0.150).

Table 2. Example of synthetic lethal combinations

Keio gene	Function	S83L	D87N	S83L-D87N
<i>priA</i>	Involved in restart of stall replication forks – binds to DNA at stalled replication fork, opens DNA duplex, and promotes assembly of primosome and loading helicase	X		X
<i>recB</i>	Helicase/nuclease that prepares dsDNA breaks for recombinational DNA repair – binds to DSBs and unwinds DNA	X		
<i>sbmC</i>	Inhibits supercoiling activity of DNA gyrase	X		X
<i>rnhA</i>	Degrades RNA of RNA-DNA hybrids	X		X
<i>dnaT</i>	Required for DNA replication and involved in inducing stable DNA replication	X		X
<i>mutT</i>	Involved in removing an oxidatively damaged form of guanine from DNA and nucleotides	X		
<i>motB</i>	Motility protein required for the rotation of the flagellar motor	X	X	
<i>ymgB</i>	Critical for biofilm formation		X	X

least two of the alleles (22 shared between *S83L* and *D87N*, 17 shared between *S83L* and *S83L-D87N*, 22 shared between *D87N* and *S83L-D87N*, and 2 shared between all three alleles).

A gene ontology (GO) enrichment assay was also conducted. Once corrected for multiple comparisons, there was no significant enrichment overall. For each *gyrA* allele, there were individual biological processes that were significantly different from expected at a non-conservative p-value cut-off (Appendix C, tables 1-3).

2. Confirming synthetic lethal interactions

Synthetic lethal/sick interactions were validated for a subset of double mutants using a conditional expression assay. The mutants picked were both strains of interest, with gene knockouts that were important for DNA repair and replication, as well as other strains with interesting functions and that had strong epistatic interactions. Using the conditionally expressed genes, assays were completed in the presence and absence of IPTG. With IPTG, the strains are able to express the knocked-out gene. Without IPTG, they could not.

Two assays were carried out. First, growth was measured on solid media after 24 hours of growth by colony count (Figure 8; Appendix A figure 1) There was a significant difference between the number of CFUs on IPTG and non-IPTG plates when factoring in the different Keio strains ($F = 1.951$, $df = 32$, $p = 0.005$). Pairwise comparisons of a subset of these results can be found on figure 8 and the full pairwise comparison can be found in figure 1 of appendix A. In this case, if the mutants are synthetic lethal or sick, there should be more colonies in the presence of IPTG as it restores gene expression. For

Figure 8. Bar plot showing the number of colonies counted (+/- SE) on plates with and without IPTG for a selection of double-mutants. Asterisks indicate significant difference ($p < 0.05$) between the IPTG and no IPTG plates.

example, the *priA* strain with the S83L-D87N alleles has more colonies, although not significantly, when grown on IPTG than when grown without. A summary of the results of the assays for all synthetic lethal/sick interactions can be found in the table in Appendix B. Since endpoint assays may be insensitive to differences in lag phase or growth rate, full growth curves in liquid culture were also collected. The amount of time in the lag phase, before exponential growth, was also calculated for the same selection of strains in IPTG and non-IPTG LB (Figure 9; Appendix A figure 2). Across all Keio strains, there was a significant comparison between the amount of time in the lag phase for these two treatments ($F = 2.748$, $df = 32$, $p < 0.001$). A subset of pairwise comparisons are found on figure 9 with the full graph in Appendix A. For potentially synthetic sick/lethal combinations, the time in lag phase should be longer for those grown in the absence of IPTG. For example, the interaction between *motB* and the *gyrA* allele D87N took a significantly longer time in the lag phase when grown without IPTG ($t = -3.138$, $df = 3.7606$, $p\text{-value} = 0.038$).

The maximum optical density after 24 hours of growth was also examined using the same strains (Figure 10; Appendix A figure 3). There was a significance difference between the densities of the strains when grown in IPTG compared to non-IPTG LB ($F = 2.5982$, $df = 32$, $p < 0.001$). A subset of significant pairwise comparisons are labelled on figure 10 with the full graph in Appendix A. In synthetic sick/lethal combinations, OD would be lower without IPTG after 24 hours. For example, the interaction between the gene *dnaT* and the *gyrA* alleles S83L-D87N is synthetic lethal. The optical density after 24 hours is significantly lower when grown without IPTG than when grown in IPTG ($t = 12.739$, $df = 3.066$, $p\text{-value} < 0.001$).

Figure 9. Bar plot showing the time (in minutes, $\pm$ SE) that a selection of double-mutants are in the lag phase while in LB with and without IPTG. Asterisks denote which IPTG comparisons are significant ($p < 0.05$).

Figure 10. Bar plot showing the optical density (OD, +/- SE) after 24 hours of a selection of double-mutant interactions grown without and without IPTG. Asterisks show which IPTG comparisons are significant ($p < 0.05$).

The overall growth rate was also determined after 24 hours for the same strains (Figure 11; Appendix A figure 4). There was also a significant difference in growth rates between strains grown in IPTG and those grown without IPTG ($F = 3.1356$, $df = 32$, $p < 0.001$). A subset of significant pairwise comparisons are labelled on figure 11 with the full graph in Appendix A. In synthetic sick/lethal interactions, growth rate will be higher when grown in LB with IPTG than when grown without IPTG. For example, the Keio strain *motB* with mutation D87N has a growth rate higher when grown in LB with IPTG than without, but not significantly.

Discussion

Antimicrobial resistance is a leading health concern for humans. Although it is thought that reducing the use of antibiotics will lead to reduced resistance, resistance mutations can persist in the absence of antibiotics (Luo *et al.* 2005). Epistasis, or genetic interactions, may allow for different fitness effects of some mutations depending on genetic background (Melnik *et al.* 2015). This can lead to the persistence of AMR through the evolution of compensatory mutations and multi-drug resistance. Many large scale screens have shown epistasis to be quite prevalent in double knockout mutant bacteria and yeast (Wong 2017) but no large scale experiments have shown the genetic interaction between knockouts and point mutations. I aimed to describe the epistatic interactions of *gyrA* alleles that cause fluoroquinolone resistance in *E. coli*. Particular attention was paid to synthetic lethal combinations to find potential drug targets for AMR mutations.

Figure 11. Bar plot showing the growth rate (+/- SE) after 24 hours of a selection of double-mutant interactions grown without and without IPTG. Asterisks show which IPTG comparisons are significant ($p < 0.05$).

From these screens, it was found that epistatic interactions are fairly common amongst different *gyrA* alleles. While the fitness of the double-mutants does not greatly differ from the fitness of the individual mutations, there are obvious instances of both positive and negative epistasis. The double mutants of *S83L* and *D87N* both center mostly around 0 fold difference (figures 4 and 5) but they both, especially *S83L*, are skewed to having a larger number of positive epistatic interactions than negative. For the *S83L-D87N* mutants, although most of the fold differences are centred around 0, there were a larger number of negative epistatic interactions (figure 6).

Epistatic interactions are common in other large-scale genetic screens. Babu *et al.* (2011) looked at ~350 000 double mutants to understand the genetic interactions between of genes involved in cell envelope biogenesis in *E. coli*. They found 54 000 genetic combinations that were epistatic, suggesting that epistasis is very prevalent in bacteria. Positive epistasis seems to be most common between resistance mutations in different genes but negative epistasis has still been seen in pairs of resistance mutations (Trindade *et al.* 2009). Trindade *et al.* (2009) used *E. coli* to examine genetic interactions between mutations in *rpoB*, *rpsL*, and *gyrA*. Of the 103 double mutants created, epistatic interactions were seen in over half of them and 73% of those were positive. Cases of negative epistasis were rarer but occurred in some instances with mutations in *rpsL* and *gyrA*.

In order to better understand the genetic interactions found through the eSGAs, focus was put onto the combinations that had negative epistatic effects, especially those that were synthetic sick or lethal. Overall, between the different *gyrA* alleles, there were a total of 1571 synthetic lethal or sick (89 in the *S83L* double-mutant combinations, 876 in

the *D87N* double-mutant combinations, and 606 in the combined *S83L-D87N* mutant combinations) with 61 synthetic sick/lethal combinations shared between at least two of the alleles (22 shared between *S83L* and *D87N*, 17 shared between *S83L* and *S83L-D87N*, 22 shared between *D87N* and *S83L-D87N*, and 2 shared between all three alleles).

Looking at other synthetic lethal screens can help show how likely these results are. Synthetic lethality percentages differ from experiment to experiment. For example, Loeillet *et al.* (2004) examined the *rad27Δ* gene in yeast, which plays an important role in DNA replication and repair. Using SGA analysis, they examined the genetic interactions between a collection of 4 847 non-essential gene deletions and a mutation in *rad27Δ*. From this screen, they identified 41 interactions, of which 20 were synthetic lethal (0.4%) and 21 (0.4%) were synthetic sick. Also using yeast, Audhya *et al.* (2004) examined the role of PI4,5P₂, a phospholipid component of cell membranes, by combining the *mss4^{ts}* mutation, which partially interrupts the PI4P5-kinase activity, with 4700 deletion mutations. From this, they found 80 (1.7%) synthetic lethal combinations with only half of those confirmed using synthetic lethal checks. In my experiment, approximately 3888 genetic interactions were examined for each *gyrA* allele. The percentage of synthetic lethal and sick interactions compared to all genetic interactions ranged from 2.2% (for the *S83L* interactions) to 22% (for the *D87N* interactions). Thus, from looking at other studies, the amount of synthetic lethal and sick interactions found in the *S83L* screen seems reasonable. However, for the *S83L-D87N* and *D87N* screens, the synthetic lethal interactions seem very high, which may mean there are a number of false positive interactions. False positives may occur due to problems in the mating process and recombination of the chromosome. This could be because the knocked out

genes are involved in recombination such as those in the *recBCD*, *recE*, and *recF* pathways (Clark 1991) or because there are linkages between the *gyrA* mutations and other genes.

Since there were so many synthetic lethal and sick combinations, a selection of these combinations were examined further. Many of the genes chosen to be examined were important in DNA synthesis, repair, and replication including *dnaT*, *priA*, *sbmC*, and *recB*. More specifically, *dnaT* is required for DNA replication, *priA* is involved in restarting stalled replication forks, *sbmC* inhibits supercoiling of the DNA gyrase, and *recB* prepares double-stranded DNA break for repair. These genes were chosen in light of Gyrase's importance to DNA replication. I selected other synthetic lethal/sick genes due to interesting phenotypes and high epistatic values. Strains were made that were able to express the candidate gene conditionally depending on the presence or absence of IPTG, in order to examine the merit of the synthetic lethal/sick verdict.

In order to confirm synthetic lethal interactions, four phenotypes were examined after 24 hours of growth – overall yield (by colony counts), time in the lag phase, growth rate, and maximum density. If synthetic sick/lethal, fitness is expected to be lower in the absence of IPTG. The results of these tests were mixed. Overall, the comparisons between the two conditions – IPTG and non – showed significant differences but individual results differed. A total of 33 validations were completed and 21 of these had at least one instance of showing the expected synthetic lethal phenotype – 2 in colony count, 5 in lag time, 13 in OD, and 9 in growth rate. Not all the synthetic lethal interactions showed the expected phenotypes. It is possible that this could be because the IPTG-inducible *lac* promoter is leaky and the gene could be expressed when it should not

be (Kitagawa *et al.* 2005). This may also be because of the evolution of compensatory mutations which alleviate the cost of the mutation. Evolution of compensatory mutations are common in laboratory experiments (Bjorkman *et al.* 2000) but whole-genome sequencing would need to be done to confirm this.

Genes involved in DNA replication, repair, and synthesis were found to be synthetic lethal in some instances, but not all, and the same genes were not synthetic lethal with all *gyrA* alleles. For example, *priA*, which is important for restarting stalled replication forks, in combination with S83L could be considered lethal when examining results from colony counts, lag phase, and OD but *priA* in combination with S83L-D87N could only be considered synthetic lethal when examining colony counts. On the other hand, some genes were synthetic lethal for multiple alleles. *dnaT*, which is involved in DNA replication, in combination with S83L and S83L-D87N could be considered synthetic lethal when looking at lag phase and OD. Because of this, it is possible that these results could be used to find drug targets for quinolone resistant *E. coli*. Genes that are synthetic lethal in combination with certain *gyrA* alleles could be targeted in order to kill resistant bacteria.

While completing this project, a number of different problems occurred that limited my data and could limit future studies. First, while completing the different eSGAs, a variety of different *gyrA* mutant alleles were tested including *D87G* and *D87Y*. However, only *S83L*, *D87N*, and a combination of *S83L* and *D87N* worked after substantial troubleshooting about proper antibiotic concentrations and growth times. This may be because these alleles confer a higher level of resistance, and thus the selection of double mutants worked better. If this is the case then future studies may be limited to

mutations conferring higher levels of resistance. However, it may be possible, by troubleshooting the eSGA protocol, to obtain a more consistent outcome.

Another problem that occurred was in the process of creating strains with conditionally expressed genes. Attempts to transfer *gyrA* mutants into *polB*, *xapR*, and *mdtK* knockouts were unsuccessful. *polB* encodes for a DNA polymerase that is thought to be involved in DNA repair. Since both *gyrA* and *polB* are involved in DNA repair, having both of these genes compromised may have made it more difficult to transfer in the mutation. Although the ASKA plasmid was supposed to accommodate this, the dosage of expression may matter – either too much or too little of *polB* may be lethal in combination with the *gyrA* mutations. *mdtK* encodes for a multidrug efflux pump and likely functions as a Na/drug antiporter that confers resistance to a variety of drugs such as fluoroquinolones. Perhaps *mdtK* dosage is important for quinolone resistance so if both knockouts and over-expressers have increased sensitivity to fluorquinolones, then it might be harder to select for double-mutants. *xapR* encodes for a regulator of *xapA* and *xapB* which are important in degrading purine nucleosides. However, it is unclear why it did not work.

In the future, it will be important to look at genetic interactions of other chromosomal resistant mutations for the other major classes of antibiotics, especially other clinically relevant AMR mutations. In *E. coli*, resistance to ciprofloxacin has increased from 20% to 29.2% from 2007 to 2011 in Canadian hospitals (Karlowsky *et al.* 2013). Thus, it would be important to examine other fluoroquinolone resistant mutations than just those examined in this research such as other *gyrA* mutants, *gyrB* mutants, and plasmid-mediated resistance. *E. coli* has also increasingly become resistant to multiple

drugs at a time so it would be important to look at mutations that cause these types of resistance, such as mutations in the AcrAB efflux pump (Okusu *et al.* 1996).

It may also be beneficial to try other assays to answer these same questions. One assay that could be used would be transposon sequencing (tn-seq). Transposon sequencing is a different way to determine genetic interactions in whole genomes using massively parallel sequencing, also known as next-generation sequencing (NGS). Unlike the methods used in this research, which required a collection of knockout strains, tn-seq is based on transposon insertion libraries which consist of mutants that have transposon insertions at various genes (van Opijnen *et al.* 2009). For example, if a mutant has a transposon inserted in a gene that is required for growth, then the frequency of that mutant will decrease over time. By comparing the abundance of each mutant, the importance of the genes can be determined. These libraries can be grown under a variety of conditions to test the importance of genes in different conditions. For this research, insertion libraries would be constructed in wildtype and mutant backgrounds. Then loci with different frequencies of insertion would be examined. This method may be more beneficial than eSGAs or other methods of examining genetic interactions as the technique is robust and the data is easily reproducible (van Opijnen *et al.* 2009). As well, it is applicable to organisms/strains for which knockout libraries are not available, including clinical strains of *E. coli* and other bacterial species.

It would also be important to use this research to further examine the potential drug targets that could kill resistant *E. coli*. Although the synthetic lethality validation assays were not consistent for any of the 33 genes across all phenotypes, I think it would be beneficial to look further at *sbmC*. For the synthetic lethality validations, *sbmC*

showed the expected synthetic lethal phenotype in multiple assays. As well, *sbmC* encodes for a DNA gyrase inhibitor. It would be beneficial to further examine the interaction between mutations in *gyrA* and the inhibition of DNA gyrase to see if that could kill resistant *E. coli* on a larger scale.

In conclusion, my results show that epistasis is common in large-scale genetic interaction screens. Synthetic lethal and sick interactions occur frequently, especially when multiple mutations are present, but are harder to confirm due to the potential evolution of compensatory mutations and/or depending on dosage of the gene. Still, it seems that genes important for DNA repair, replication, and synthesis are often synthetic lethal in combination with *gyrA* mutations, which may lead to possible drug leads.

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Appendix A

Figure 1. Bar plot showing the number of colonies counted (+/- SE) on plates with and without IPTG for a selection of double-mutants. Asterisks indicate significant difference ($p < 0.05$) between the IPTG and no IPTG plates.

Figure 2. Bar plot showing the time (in minutes, $\pm$ SE) that a selection of double-mutants are in the lag phase while in LB with and without IPTG. Asterisks denote which IPTG comparisons are significant ($p < 0.05$).

Figure 3. Bar plot showing the optical density (OD, $\pm$ SE) after 24 hours of a selection of double-mutant interactions grown without and without IPTG. Asterisks show which IPTG comparisons are significant ($p < 0.05$).

Figure 4. Bar plot showing the growth rate (+/- SE) after 24 hours of a selection of double-mutant interactions grown without and without IPTG. Asterisks show which IPTG comparisons are significant ($p < 0.05$).

Appendix B

Table 1. Summary of all Keio-*gyrA* interactions examined and the p-values of colony count, lag time, OD, and rate when comparing IPTG vs. non for each

Interaction	p-value comparing IPTG and no IPTG			
	Colony Count	Lag Time	OD	Rate
dnaT + S83L	0.798	0.037	0.248	0.514
dnaT + S83L-D87N	0.483	0.111	< 0.001	0.928
helD + D87N	0.677	0.941	0.255	0.017
leuO + D87N	0.006	0.284	0.082	0.022
leuO + S83L-D87N	0.115	0.716	0.039	0.033
motB + D87N	0.773	0.038	0.537	0.233
motB + S83L	0.440	0.023	0.197	0.639
mutT + S83L	0.129	0.984	0.524	0.434
priA + S83L	0.531	0.168	0.005	0.949
priA + S83L-D87N	0.152	0.053	0.341	0.120
recB + S83L	0.305	0.087	0.048	0.249
rnhA + S83L	0.2120	0.824	0.448	0.677
rnhA + S83L-D87N	< 0.001	0.009	0.276	0.370
rplK + S83L	0.815	0.952	0.014	0.024
rplK + S83L-D87N	0.264	0.301	0.017	0.015
rpoN + D87N	0.742	0.205	0.081	0.356
rpoN + S83L-D87N	0.839	0.502	0.017	0.016
rpoS + S83L	0.697	0.928	0.005	0.101
rpsF + D87N	0.372	0.888	0.759	0.349
rpsF + S83L	0.422	0.107	0.102	0.753
sbmC + S83L	0.723	0.217	0.690	0.455
sbmC + S83L-D87N	0.716	0.172	0.014	0.004
uvrD + S83L	0.192	0.815	0.292	0.522
yafO + D87N	0.121	0.933	0.042	0.110
yafO + S83L-D87N	0.405	0.708	0.787	0.888
ydeP + S83L	0.841	0.118	0.816	0.941
yecC + D87N	0.778	0.164	0.002	0.191
yecC + S83L	0.389	0.667	0.048	0.335
yecC + S83L-D87N	0.412	0.896	0.241	0.647
yfcJ + D87N	0.618	0.131	0.308	0.009
yfcJ + S83L-D87N	0.483	0.439	0.715	0.379
ymgB + D87N	0.921	0.031	0.0396	0.007
ymgB + S83L-D87N	0.161	0.116	0.196	0.369

Appendix C

Table 1. Results from gene ontology assay showing the biological processes of significantly enriched genes in the D87N gene interaction screen.

Biological process	Number	Expected Number	p-value
Amide biosynthesis process	8	22.27	0.00179
Proteolysis	18	8.33	0.00805
Primary metabolic process	206	243.65	0.00826
Nitrogen compound metabolic process	184	219.06	0.0105
Pilus organization	17	7.94	0.011
Transmembrane transport	72	51.71	0.018
Peptide biosynthesis process	6	15.88	0.0127
Small molecular metabolic process	106	134.03	0.014
Organonitrogen compound biosynthetic process	54	75.34	0.0152
Carboxylic acid metabolic process	58	80.18	0.0152
Cellular nitrogen compound metabolic process	124	153.20	0.0154
tRNA metabolic process	6	15.69	0.0177
Translation	5	13.75	0.0181
Cellular nitrogen compound biosynthetic process	59	80.77	0.0186
Organic substance metabolic process	260	295.56	0.0190
Organic cyclic compound metabolic process	116	143.71	0.0195
Metabolic process	290	325.78	0.0196
Cell projection organization	19	9.68	0.0197
Small molecular biosynthesis process	36	53.46	0.0213
Heterocycle metabolic process	110	136.55	0.026
ncRNA metabolic process	12	23.44	0.028
Oxoacid metabolic process	61	81.93	0.0233
Cellular amino acid metabolic process	23	37.57	0.0234
Pilus assembly	8	2.91	0.0235
Amino acid activation	0	4.65	0.0238
Cellular protein catabolic process	6	1.74	0.024
Extracellular polysaccharide metabolic process	6	1.74	0.024
Extracellular polysaccharide biosynthetic process	6	1.74	0.024
Organic acid metabolic process	63	84.06	0.0249
Cellular amid metabolic process	17	29.25	0.0326
Aspartate family amino acid metabolic process	3	9.49	0.0371
Chromosome organization	0	4.07	0.0372
Pyrimidine-containing compound catabolic process	0	4.26	0.0379

Nucleobase-containing compound metabolic process	93	115.44	0.0398
Oxidation-reduction process	31	45.71	0.0403
tRNA aminoacylation for protein translation	0	4.45	0.0404
tRNA aminoacylation	0	4.45	0.0404
Organic acid biosynthetic process	23	35.44	0.0430
Carboxylic acid biosynthetic process	23	35.44	0.0430
Cellular polysaccharide biosynthetic process	22	13.36	0.042
Protein catabolic process	6	2.13	0.047
Organonitrogen compound metabolic process	116	139.65	0.0456
Proteolysis involved in cellular protein catabolic process	5	1.55	0.046
Purine nucleobase transport	5	1.55	0.046
Cellular aromatic compound metabolic process	23	13.95	0.0476
Antibiotic transport	11	5.42	0.0496

Table 2. Results from gene ontology assay showing the biological processes of significantly enriched genes in the S83L gene interaction screen.

Biological process	Number	Expected Number	p-value
Cellular response to stress	19	6.75	0.0000412
Cellular response to DNA damage stimulus	16	5.05	0.0000493
Response to stress	23	10.05	0.000140
DNA-dependent DNA replication	5	0.58	0.000456
Cellular macromolecular biosynthesis process	17	6.91	0.000528
Macromolecule biosynthesis process	17	6.97	0.000581
Response to stimulus	30	16.52	0.000797
DNA replication	5	0.68	0.000873
Cellular response to stimulus	19	8.65	0.000995
Recombinant repair	3	0.20	0.00180
Propionate catabolic process	2	0.04	0.00222
Replication fork processing	2	0.04	0.00222
Propionate catabolic process, 2-methylcitrate cycle	2	0.04	0.00222
DNA repair	6	1.30	0.00245
Response to radiation	7	1.90	0.00354
Short-chain fatty acid catabolic process	2	0.06	0.00365
Regulation of multi-organism process	3	0.30	0.00478
Regulation of single-species biofilm formation	3	0.30	0.00478
Cellular macromolecular metabolic process	23	12.98	0.00583
Macromolecule metabolic process	26	15.52	0.00380

ATP synthesis coupled proton transport	2	0.10	0.00747
Energy coupled proton transport, down electrochemical gradient	2	0.10	0.00747
Protein stabilization	2	0.10	0.00747
Response to abiotic stimulus	11	4.69	0.00792
Lipopolysaccharide biosynthesis process	4	0.74	0.00799
Primary metabolic process	37	25.12	0.00815
Lipopolysaccharide metabolic process	4	0.78	0.00946
Cation transport	8	2.94	0.0103
Organic substance metabolic process	42	30.48	0.0122
Nucleobase-containing small molecule interconversion	3	0.44	0.0122
DNA metabolic process	8	3.04	0.0124
Cellular protein complex disassembly	2	0.14	0.0125
Regulation of protein stability	2	0.14	0.0125
Translational termination	2	0.14	0.0125
Negative regulation of DNA metabolic process	2	0.14	0.0125
Regulation of single-species biofilm formation in inanimate substrate	2	0.14	0.0125
Response to extracellular stimulus	5	1.34	0.0128
Propionate metabolic process	2	0.16	0.0154
DNA-dependent DNA replication maintenance of fidelity	2	0.16	0.0154
DNA duplex unwinding	2	0.16	0.0154
Lipopolysaccharide core region metabolic process	3	0.50	0.0167
Lipopolysaccharide core region biosynthetic process	3	0.50	0.0167
DNA geometric change	2	0.18	0.0186
Protein-containing complex disassembly	2	0.18	0.0186
Cellular component disassembly	2	0.18	0.0186
DNA recombination	4	1.00	0.0206
Lipopolysaccharide metabolic process	4	1.00	0.0206
Short-chain fatty acid metabolic process	2	0.20	0.020
Nucleoside catabolic process	2	0.22	0.0257
Membrane biogenesis	2	0.22	0.0257
Double-stranded break repair	2	0.22	0.0257
Nucleobase-containing small molecular catabolic process	2	0.22	0.0257
Membrane assembly	2	0.22	0.0257
Gram-negative-bacterium-type cell outer membrane assembly	2	0.22	0.0257
Cell envelope organization	2	0.22	0.0257
Carbohydrate derivative biosynthetic process	8	3.52	0.0265
Cellular response to external stimulus	4	1.12	0.0291

Cellular response to extracellular stimulus	4	1.12	0.0291
L-amino acid transport	3	0.64	0.0302
Nucleobase-containing small molecule metabolic process	8	3.65	0.0322
Glycosyl compound catabolic process	2	0.26	0.0337
Cellular nitrogen compound metabolic process	24	15.80	0.0343
Ribonucleoside monophosphate metabolic process	3	0.70	0.0374
Monosaccharide transmembrane transport	3	0.70	0.0374
Pre-replicative complex assembly involved in cell cycle DNA replication	1	0.02	0.0388
Ornithine transport	1	0.02	0.0388
Negative regulation of strand invasion	1	0.02	0.0388
Regulation of strand invasion	1	0.02	0.0388
Response to gamma radiation	1	0.02	0.0388
Replication fork reversal	1	0.02	0.0388
Pre-replicative complex assembly	1	0.02	0.0388
UV protection	1	0.02	0.0388
Uridine metabolic process	1	0.02	0.0388
Protein chromophore linkage	1	0.02	0.0388
Protein-DNA complex subunit organization	1	0.02	0.0388
Pre-replicative complex assembly involved in nuclear cell cycle DNA replication	1	0.02	0.0388
Uridine catabolic process	1	0.02	0.0388
Maintenance of stationary phase	1	0.02	0.0388
Osmosensory signaling via phosphorelay pathway	1	0.02	0.0388
Protein-DNA complex assembly	1	0.02	0.0388
Rolling circle DNA replication	1	0.02	0.0388
Stringent response	1	0.02	0.0388
Transcription initiation from bacterial-type RNA polymerase promoter	1	0.02	0.0388
Negative regulation of DNA recombination	1	0.02	0.0388
Purine nucleoside interconversion	1	0.02	0.0388
Nuclear DNA replication	1	0.02	0.0388
Ribonucleotide metabolic process	4	1.26	0.0413
DNA conformation change	2	0.30	0.0426
Response to external stimulus	5	1.90	0.0452
Response to starvation	3	0.76	0.0453
Nucleoside monophosphate metabolic process	3	0.76	0.0453
Carbohydrate derivative metabolic process	11	5.73	0.0461
Citrate metabolic process	2	0.32	0.0473
Tricarboxylic acid cycle	2	0.32	0.0473
Nitrogen compound metabolic process	31	22.59	0.0479
Nucleoside metabolic process	3	0.78	0.0481

Oligosaccharide biosynthetic process	3	0.78	0.0481
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Table 3. Results from gene ontology assay showing the biological processes of significantly enriched genes in the S83L-D87N gene interaction screen.

Biological process	Number	Expected Number	p-value
Dicarboxylic acid catabolic process	6	1.49	0.0111
Fatty acid catabolic process	8	2.99	0.0213
Negative regulation of DNA replication	4	0.82	0.0235
Drug catabolic process	15	7.47	0.0236
Carboxylic acid catabolic process	31	19.70	0.0240
Lipid catabolic process	8	3.12	0.0258
Cellular lipid catabolic process	8	3.12	0.0258
Short-chain fatty acid metabolic process	5	1.36	0.0260
Organic acid catabolic process	32	20.79	0.0278
Fatty acid metabolic process	14	6.93	0.0311
Negative regulation of DNA metabolic process	4	0.95	0.0336
Purine-containing compound catabolic process	5	1.49	0.0341
Drug metabolic process	37	25.27	0.0343
Purine-containing compound metabolic process	18	10.33	0.0365
Organic acid transmembrane transport	14	7.34	0.0366
Carboxylic acid transmembrane transport	14	7.34	0.0366
Cellular modified amino acid metabolic process	12	5.98	0.0369
Glucarate catabolic process	3	0.54	0.0412
Glucarate metabolic process	3	0.54	0.0412
D-glucarate catabolic process	3	0.54	0.0412
D-glucarate metabolic process	3	0.54	0.0412
Small molecular catabolic process	43	30.57	0.0414
Nucleobase-containing small molecule metabolic process	36	24.86	0.0425
Coenzyme metabolic process	30	19.70	0.0426
Cellular nitrogen compound catabolic process	22	13.72	0.0480
Fatty acid beta-oxidation	6	2.31	0.0485

Appendix D

Table A1. Common synthetic lethal/sick combinations between different *gyrA* mutant alleles.

Keio strains	S83L	D87N	S83L-D87N
xapR	X	X	X
yecC	X	X	X
ihfA	X	X	
hns	X	X	
nfrA	X	X	
motB	X	X	
pup	X	X	
xylG	X	X	
livH	X	X	
cdaR	X	X	
yedZ	X	X	
ydeH	X	X	
ycfJ	X	X	
atpC	X	X	
rfaG	X	X	
ybjQ	X	X	
ydjE	X	X	
trpC	X	X	

modF	X	X	
citC	X	X	
rpsF	X	X	
tatC	X	X	
ynhG	X	X	
ubiX	X	X	
atpG	X		X
clpB	X		X
dnaT	X		X
hycC	X		X
oxyR	X		X
priA	X		X
prpB	X		X
rfaB	X		X
rfaC	X		X
rnhA	X		X
rplK	X		X
sbmC	X		X
surA	X		X
tufA	X		X
udp	X		X
ybhR	X		X
ycfN	X		X

leuO		X	X
prlC		X	X
yhbU		X	X
ynaJ		X	X
yafO		X	X
yfbK		X	X
yfcJ		X	X
mdtK		X	X
ydhD		X	X
mdtH		X	X
yajG		X	X
yadI		X	X
yaaW		X	X
yfiE		X	X
yfcO		X	X
ydfQ		X	X
ydfI		X	X
yneH		X	X
ycaK		X	X
ymgB		X	X
yghG		X	X
yjfK		X	X

Table A2. All other synthetic lethal interactions.

Keio strains	S83L	D87N	S83L-D87N
nuoK	X		
fhuE	X		
ybcN	X		
yfaW	X		
uvrD	X		
hlpA	X		
ruvA	X		
phr	X		
fpr	X		
gspG	X		
fepB	X		
deoD	X		
nrdG	X		
mglA	X		
yegT	X		
puuP	X		
ptsN	X		
yhcE	X		
yjhE	X		
ycdR	X		

wbbI	X		
yeaH	X		
kil	X		
sgbE	X		
prfB	X		
ydhQ	X		
mrcB	X		
hyfA	X		
yifO	X		
ynjB	X		
rpoS	X		
soxS			X
phnF			X
nanR			X
metJ			X
ilvY			X
galS			X
deoR			X
frdC			X
rpiB			X
gpml			X
rnt			X
yhaK			X

creC			X
dcuS			X
ompR			X
qseC			X
qseB			X
yfaS			X
yedD			X
yecJ			X
yebG			X
yebO			X
yeaR			X
ydiV			X
ydgH			X
ynfB			X
ydcA			X
ydcF			X
ynbD			X
ydbH			X
tfaR			X
ydaY			X
ynaK			X
ydaF			X
ydaC			X

ydaL			X
ycjY			X
ymjC			X
ymjA			X
ychQ			X
ycgR			X
ymfR			X
yceH			X
yccJ			X
yccE			X
yehQ			X
yaiI			X
ykfJ			X
yejL			X
rsxA			X
yfhH			X
yphF			X
ypeA			X
ypdB			X
yfcG			X
yegE			X
yddE			X
ydcS			X

yciA			X
ydiF			X
lsrF			X
ybgC			X
ybhM			X
ynjF			X
ydhC			X
ycdQ			X
rluB			X
ygbJ			X
yddH			X
yqcD			X
yqaX			X
yffI			X
dedD			X
uspG			X
ygbI			X
ypjL			X
yfaY			X
yfjT			X
ycbS			X
ydhP			X
folM			X

ydeW			X
ydeS			X
yncD			X
ycbZ			X
nfsA			X
zitB			X
ybfF			X
hscC			X
ydaS			X
ygfH			X
yqfE			X
yjeK			X
actP			X
yjaH			X
yjaG			X
yiiX			X
yiiR			X
brnQ			X
tauC			X
uvrA			X
alkB			X
alkA			X
nudB			X

tus			X
recT			X
recR			X
priC			X
yjjW			X
yjjU			X
yjhP			X
yjgD			X
ytfN			X
yjfY			X
ulaR			X
flgB			X
flgN			X
csgB			X
sfmC			X
sfmA			X
ppiD			X
ddlA			X
crI			X
htrE			X
lplA			X
thiH			X
rraA			X

fre			X
hemX			X
pabA			X
pdxJ			X
menE			X
ccmB			X
paaG			X
paaF			X
pncB			X
moeA			X
moaD			X
moaC			X
bioC			X
panC			X
nadC			X
slt			X
rfbX			X
nrff			X
glcC			X
ybcF			X
dmsC			X
galE			X
galK			X

kefF			X
fixX			X
cysQ			X
phnK			X
tdk			X
pyrC			X
nei			X
rnk			X
purE			X
xseB			X
codA			X
wzxE			X
rffA			X
kefG			X
yciK			X
fabH			X
tesB			X
uxaA			X
gltJ			X
xseA			X
frwC			X
glvB			X
gatC			X

yecS			X
chbB			X
ydgI			X
ddpF			X
ycjO			X
sapB			X
oppD			X
artQ			X
glnH			X
kbaZ			X
ygbN			X
cysZ			X
hisJ			X
aat			X
rimK			X
phnD			X
cutC			X
mngA			X
nfrB			X
ybcC			X
fsr			X
frmB			X
ampD			X

msrA			X
rseB			X
rseC			X
miaA			X
lysU			X
rpmE			X
dtd			X
ppiA			X
slyD			X
prmA			X
pcm			X
alaS			X
frlA			X
nirC			X
elbB			X
greA			X
yhbT			X
csiR			X
garL			X
galP			X
yqgB			X
hcp			X
yihW			X

argB			X
thiG			X
yhbC			X
garR			X
yqjC			X
alx			X
ygjO			X
dkgA			X
yghY			X
yghU			X
yghS			X
yghQ			X
yghF			X
ygfB			X
ygfU			X
yfeW			X
yqeF			X
amiC			X
exo			X
ypjJ			X
had			X
ucpA			X
torI			X

menD			X
bcr			X
yjcS			X
frlB			X
frlR			X
yhjK			X
bcsC			X
yidQ			X
yleL			X
wzzE			X
yigI			X
ydcX			X
ydcO			X
ydcI			X
cybB			X
ydbD			X
paaD			X
rzpR			X
ydaQ			X
ydaM			X
abgA			X
ycjR			X
yciO			X

dhaL			X
dhaH			X
hlyE			X
ycgN			X
ymgG			X
ycgH			X
ycgG			X
ymfT			X
ymfI			X
ymfE			X
ymfA			X
ycfQ			X
yceF			X
yccS			X
ymbA			X
ycbF			X
ycbV			X
ycal			X
ycjI			X
ybiN			X
ybhT			X
ybgQ			X
kdpE			X

ybfH			X
ybfG			X
ybeQ			X
ybeB			X
ybcV			X
rzpD			X
fimZ			X
ybcJ			X
ybbM			X
yaiZ			X
ylcG			X
yhfZ			X
narU			X
sseA			X
tag			X
phnM			X
gudD			X
ydhB			X
malT			X
modA			X
yagL			X
yedS			X
yaiL			X

yagX			X
ydjQ			X
yhhI			X
rhsA			X
ydeR			X
menB			X
astC			X
iaaA			X
yzgL			X
yhcC			X
ndh			X
yobF			X
yhaL			X
yohO			X
arpB			X
cvrA			X
cspH			X
yajL			X
ampH			X
kgtP			X
livF			X
yfdE			X
bacA			X

folX			X
cspE			X
nikR			X
recX			X
lldP			X
nikB			X
manY			X
narK			X
kdpB			X
acrA			X
lacY			X
polB			X
yidL			X
purH			X
purC			X
apt			X
azoR			X
caiA			X
caiB			X
mtlR			X
hybA			X
hybG			X
epd			X

fucA			X
hycA			X
hycB			X
hycD			X
yfcX			X
hokC			X
lipB			X
ivbL			X
ubiG			X
gntU			X
ymdA			X
gpmA			X
thyA			X
gntK			X
atpD			X
pgi			X
nagA			X
rplA			X
pnp			X
parC			X
nusB			X
rpmJ			X
rluD			X

sdhB		X	
cyoB		X	
cyoD		X	
sdiA		X	
rbsR		X	
perR		X	
pdhR		X	
idnR		X	
fadR		X	
dsdC		X	
hha		X	
ypjB		X	
yfgM		X	
ypdI		X	
yfdV		X	
ackA		X	
ppk		X	
glgC		X	
yahL		X	
ykfA		X	
moaA		X	
hrpA		X	
helD		X	

tap		X	
aer		X	
hofQ		X	
wcaA		X	
yfdF		X	
yfcT		X	
rpoN		X	
clpA		X	
dacC		X	
dacA		X	
clpX		X	
pepD		X	
degP		X	
yoaA		X	
lhr		X	
hrpB		X	
hepA		X	
dbpA		X	
yeaT		X	
cybC		X	
gcd		X	
ygeL		X	
yraJ		X	

fimD		X	
fiu		X	
nlpB		X	
viaT		X	
ycdS		X	
yfeZ		X	
yfeY		X	
ypeC		X	
yfdX		X	
yfdR		X	
yfdQ		X	
yfcR		X	
yfcD		X	
iadA		X	
pepE		X	
gspO		X	
mrcA		X	
ggt		X	
zntA		X	
dgoK		X	
yifK		X	
pepQ		X	
hslV		X	

argC		X	
degQ		X	
dacB		X	
sohA		X	
pppA		X	
sprT		X	
yggG		X	
ydeY		X	
iap		X	
recA		X	
pepB		X	
ypdF		X	
ptrB		X	
htpX		X	
ynbE		X	
ycfL		X	
yceD		X	
yceI		X	
ycbJ		X	
ybjD		X	
ybjC		X	
ybjH		X	
ybiU		X	

ybiI		X	
ybiJ		X	
ybgS		X	
ybfQ		X	
ybfC		X	
ybfM		X	
djlB		X	
ybeR		X	
ybdN		X	
ybdD		X	
ybdJ		X	
cusF		X	
ybcH		X	
ybcI		X	
ylbE		X	
yaiY		X	
ybaM		X	
yajD		X	
yaiW		X	
yaiV		X	
yaiS		X	
yahG		X	
yahE		X	

yagZ		X	
yagY		X	
yagW		X	
yagJ		X	
yafZ		X	
ykfB		X	
yneK		X	
frmR		X	
luxS		X	
yfjY		X	
ypfI		X	
sufE		X	
ykfH		X	
frsA		X	
yafK		X	
yafT		X	
yaeP		X	
yaeH		X	
yadL		X	
yacC		X	
yacH		X	
yaaI		X	
yaaX		X	

ygdE		X	
yqcC		X	
yqaA		X	
yfjF		X	
yfhJ		X	
yfeX		X	
yfdG		X	
yfcN		X	
yejK		X	
yeeX		X	
yeel		X	
yedI		X	
yedP		X	
rsxG		X	
ychA		X	
yfdH		X	
yfcH		X	
ymdB		X	
ykfG		X	
yfiA		X	
yciH		X	
ydiM		X	
ydcN		X	

ydbA		X	
ychP		X	
ygdQ		X	
yqcE		X	
ybaS		X	
ybaY		X	
ybaO		X	
yajO		X	
yahK		X	
yahC		X	
dinJ		X	
metQ		X	
yadN		X	
yadC		X	
thiQ		X	
yhhW		X	
cchA		X	
cchB		X	
yhhI		X	
murP		X	
ypdG		X	
flk		X	
dedA		X	

rtn		X	
paaY		X	
ybgA		X	
crcB		X	
citG		X	
cof		X	
prpD		X	
ivy		X	
rof		X	
ygeR		X	
ygcO		X	
ygcN		X	
yfjU		X	
yfjR		X	
yfiB		X	
yfiF		X	
yfeG		X	
yfdZ		X	
yejE		X	
yeiQ		X	
asmA		X	
wzc		X	
tig		X	

hscB		X	
yiiQ		X	
hslJ		X	
ybjP		X	
erfK		X	
yedS		X	
yeaE		X	
ydjG		X	
spy		X	
ydiT		X	
ydiR		X	
lsrD		X	
ydbC		X	
ydbK		X	
yciT		X	
ynaE		X	
elbA		X	
ymfJ		X	
ybcW		X	
ybcK		X	
ygdR		X	
ogt		X	
tpx		X	

gst		X	
gor		X	
ahpF		X	
lpp		X	
hfq		X	
pphA		X	
yeaG		X	
yraK		X	
yraH		X	
garD		X	
yqjD		X	
yqjA		X	
ygjQ		X	
ygjJ		X	
ygjI		X	
htrG		X	
ygiF		X	
yqiK		X	
ygiL		X	
ygiW		X	
yghZ		X	
yqgA		X	
elaB		X	

ycjQ		X	
ybdR		X	
yibL		X	
yibG		X	
yhhN		X	
yhgE		X	
yrfB		X	
yjfl		X	
yjfJ		X	
yjeB		X	
yjeS		X	
yjeO		X	
yjeN		X	
yjeJ		X	
yjdK		X	
yjdJ		X	
yjdI		X	
yjdF		X	
adiC		X	
yjdA		X	
yjcP		X	
yjcH		X	
yjcF		X	

yjcE		X	
yjcC		X	
yjbR		X	
yjbQ		X	
yjbM		X	
yjbL		X	
yjbJ		X	
yjbI		X	
yjbG		X	
yjbE		X	
yjbC		X	
yjaB		X	
yjaG		X	
nudC		X	
yijF		X	
yiiU		X	
yiiD		X	
yihT		X	
yihI		X	
yihG		X	
yihD		X	
yigA		X	
yieI		X	

yieG		X	
yidJ		X	
ade		X	
metN		X	
yjhG		X	
aroF		X	
hisI		X	
astE		X	
paaE		X	
leuC		X	
ddlA		X	
cobT		X	
abgB		X	
moaE		X	
gspE		X	
gspC		X	
secG		X	
ygiS		X	
bcp		X	
otsB		X	
cedA		X	
dicB		X	
osmC		X	

oppF		X	
oppB		X	
minC		X	
chpA		X	
ahpC		X	
dnaJ		X	
fimH		X	
fimF		X	
fimC		X	
cutA		X	
rfaH		X	
rffH		X	
rffG		X	
rfaP		X	
rfaL		X	
yraI		X	
smpB		X	
rfbX		X	
wbbH		X	
fliG		X	
osmE		X	
ydhU		X	
hipA		X	

ydcW		X	
aldA		X	
puuC		X	
fixX		X	
phnG		X	
pyrC		X	
rffA		X	
nagE		X	
nohB		X	
cueR		X	
deoA		X	
hsdR		X	
pyrE		X	
nrdE		X	
purM		X	
purF		X	
nth		X	
corA		X	
rbsC		X	
lysP		X	
tyrP		X	
ftn		X	
araF		X	

znuC		X	
manZ		X	
manX		X	
malX		X	
potA		X	
potF		X	
eutG		X	
yfaV		X	
mrp		X	
fliZ		X	
uvrC		X	
cheZ		X	
cutC		X	
mdoD		X	
feaB		X	
mppA		X	
puuP		X	
pin		X	
trmU		X	
potB		X	
msyB		X	
citE		X	
dsbG		X	

tauA		X	
mhpC		X	
afuC		X	
ilvI		X	
tbpA		X	
yicE		X	
gltS		X	
mtlA		X	
xylH		X	
dppF		X	
pitA		X	
ugpB		X	
yrbF		X	
mtr		X	
borD		X	
emrE		X	
hyi		X	
frmB		X	
ampD		X	
sgcR		X	
rho		X	
pmbA		X	
rplI		X	

miaA		X	
rpmE		X	
truB		X	
yrbA		X	
tdcD		X	
exuR		X	
bglA		X	
iscU		X	
sanA		X	
yebQ		X	
yobH		X	
astD		X	
yniD		X	
ydhX		X	
ynfH		X	
ynfN		X	
ynfO		X	
yneL		X	
yddA		X	
yncK		X	
hokB		X	
paaK		X	
sieB		X	

ymjB		X	
puuA		X	
yciX		X	
tonB		X	
dsbB		X	
plsX		X	
ssuC		X	
dmsA		X	
hcr		X	
ybhF		X	
tolB		X	
gltI		X	
nmpC		X	
sfmF		X	
mdlB		X	
psiF		X	
ykiB		X	
ykgM		X	
ykgM		X	
mmuP		X	
mltD		X	
ligT		X	
caiE		X	

yjhV		X	
rhoL		X	
ilvL		X	
uhpC		X	
zapA		X	
fliE		X	
paaH		X	
yihW		X	
frvB		X	
thiG		X	
nfi		X	
nanK		X	
yhbX		X	
yhbC		X	
yraR		X	
garR		X	
yqjC		X	
alx		X	
ygjO		X	
yghY		X	
yghS		X	
yggU		X	
ygfT		X	

xdhA		X	
ygeM		X	
yqeF		X	
ygfT		X	
torI		X	
yojL		X	
ecnA		X	
dcuA		X	
adiA		X	
eptA		X	
yjcS		X	
ubiC		X	
yjbF		X	
gspI		X	
gspL		X	
gspM		X	
yheO		X	
yhfK		X	
frlB		X	
frlR		X	
yhfU		X	
yhfY		X	
hslO		X	

gntX		X	
yhhT		X	
yhhJ		X	
rbbA		X	
yhiK		X	
yhiQ		X	
yhiD		X	
bcsC		X	
yhjQ		X	
bcsF		X	
eptB		X	
bisC		X	
viaF		X	
viaB		X	
viaN		X	
htrL		X	
yicN		X	
yicO		X	
cbrA		X	
atpI		X	
ilvD		X	
yigI		X	
tatB		X	

ykgA		X	
ykgK		X	
yafD		X	
clcA		X	
yigE		X	
rhtC		X	
tatD		X	
trkH		X	
yhaM		X	
yqiI		X	
ygiQ		X	
glcE		X	
yggP		X	
sgcE		X	
ygfA		X	
ybbK		X	
yjjX		X	
yjjA		X	
mdoB		X	
yjjN		X	
yjiY		X	
yjiH		X	
yjiD		X	

yjgX		X	
yjgN		X	
yjgL		X	
yjgK		X	
ytfT		X	
ytfK		X	
ytfH		X	
flkB		X	
ytfB		X	
ulaA		X	
yjfO		X	
rnr		X	
ydcM		X	
ydcI		X	
rzpR		X	
abgA		X	
ycjR		X	
yciW		X	
yciO		X	
dhaH		X	
hlyE		X	
ymfT		X	
ycdL		X	

yccX		X	
ybfG		X	
ybcV		X	
ybbM		X	
yfbJ		X	
gatR		X	
ung		X	
yciX		X	
yhfZ		X	
narU		X	
rspB		X	
glyS		X	
tag		X	
glpR		X	
ygcK		X	
rhsC		X	
yqiG		X	
nadR		X	
deaD		X	
ygcG		X	
ykiA		X	
ybcL		X	
mdtB		X	

rsxC		X	
yadH		X	
hokD		X	
ren		X	
yfcS		X	
ycdW		X	
xerC		X	
yajL		X	
poxA		X	
yfdE		X	
udk		X	
mhpD		X	
lacA		X	
blr		X	
ssuE		X	
recX		X	
rumA		X	
lldP		X	
nikB		X	
yghK		X	
acrA		X	
ndk		X	
fdoH		X	

epd		X	
ymdA		X	
csgC		X	
gpmA		X	
gntK		X	
folB		X	
rpoD		X	
phnN		X	
atpD		X	
ycbK		X	

Table 3A. All other synthetic sick combinations.

Keio	S83L	D87N	S83L-D87N
sucB	X		
acnB	X		
ydhA	X		
ydfT	X		
betT	X		
ruvC	X		
rfbD	X		
napF	X		
relA	X		
dos	X		
ypdJ	X		
argT	X		
mutT	X		
recB	X		
ynfG	X		
fruR			X
pdhR			X
nac			X
argP			X
ihfB			X

rstA			X
deoB			X
fumB			X
mgo			X
gnd			X
pps			X
pta			X
oppA			X
basS			X
rpoN			X
iadA			X
frvX			X
ydhW			X
yncE			X
yncE			X
ynbE			X
ymfO			X
ymfM			X
ymfL			X
yehS			X
yehM			X
yehK			X
yehI			X

yehE			X
ydeJ			X
yfeO			X
ypdC			X
yfeO			X
ypdC			X
yehZ			X
ybdB			X
yfiK			X
ycjT			X
ychP			X
ydgG			X
yaaA			X
yqjB			X
rtn			X
yfeG			X
yfdZ			X
viaK			X
ybiC			X
hscB			X
yccZ			X
sfmD			X
erfK			X

spy			X
yneJ			X
lsrB			X
yobG			X
ycgI			X
ybjM			X
ybgT			X
ybcK			X
ygiN			X
sufI			X
yjeN			X
yjdI			X
yjbC			X
yijF			X
yihG			X
rffC			X
ade			X
codB			X
yjgR			X
ytfl			X
flgG			X
flgE			X
cobU			X

bioA			X
cobC			X
motA			X
dicB			X
rfaQ			X
yraI			X
gadA			X
glmM			X
ansA			X
hyaC			X
citD			X
malZ			X
mhpF			X
pyrD			X
cmk			X
uvrB			X
rihA			X
rna			X
allB			X
dgt			X
pfs			X
guaC			X
ksgA			X

carB			X
aas			X
eutC			X
atoB			X
cfa			X
fabF			X
caiT			X
caiD			X
uxuA			X
ascB			X
chbA			X
putP			X
glnP			X
fucP			X
gudP			X
srlB			X
acrD			X
atoE			X
nikD			X
gltS			X
rob			X
torD			X
rhIE			X

pmbA			X
rplI			X
rpiR			X
ulaC			X
tdcD			X
pspD			X
ilvG			X
ligB			X
garP			X
pheP			X
yicL			X
ulaF			X
zraP			X
yhbX			X
agaA			X
yhaO			X
yqiH			X
yfgG			X
yicN			X
tatB			X
ydbL			X
ybdF			X
cusS			X

yajQ			X
ompG			X
entA			X
yibI			X
yibF			X
pinH			X
lacA			X
aphA			X
blr			X
ssuE			X
rumA			X
yghK			X
hisQ			X
glpT			X
gatB			X
recD			X
bfd			X
kduD			X
fucU			X
fucO			X
bolA			X
ynfC			X
yciB			X

glxK			X
yaaY			X
ybiM			X
etp			X
ybjG			X
bglJ			X
folB			X
ycbK			X
yhcB			X
lpcA			X
ybeF		X	
zntR		X	
stpA		X	
purR		X	
nac		X	
malI		X	
hupA		X	
cynR		X	
hupA		X	
cynR		X	
yfiN		X	
yfeU		X	
envZ		X	

coaE		X	
yehB		X	
gltA		X	
dcuR		X	
zraS		X	
glnG		X	
clpP		X	
yeiE		X	
yqeI		X	
ompN		X	
yeaY		X	
ompT		X	
pgpB		X	
yfcI		X	
yfbM		X	
yfaH		X	
yfaQ		X	
radA		X	
hflX		X	
yhjJ		X	
hybD		X	
hyfR		X	
mepA		X	

ycdP		X	
yagU		X	
ygcP		X	
yfcZ		X	
arnT		X	
yfbF		X	
ybaW		X	
ybiB		X	
yfhQ		X	
rcsD		X	
yejB		X	
yfgC		X	
wcaK		X	
yccZ		X	
sfmD		X	
dijlA		X	
yeaM		X	
ydjF		X	
sufC		X	
lsrB		X	
ycaN		X	
ybjM		X	
ygeH		X	

ygeG		X	
yhbW		X	
yraO		X	
yggT		X	
yggF		X	
yggD		X	
yibH		X	
viaV		X	
viaJ		X	
mdtE		X	
hdeA		X	
yhiF		X	
yhiP		X	
yhiN		X	
yhhA		X	
yhhY		X	
yhbH		X	
yjcO		X	
actP		X	
yjbA		X	
yjbH		X	
yijP		X	
fieF		X	

yigF		X	
hsrA		X	
cbrC		X	
yidI		X	
yidG		X	
yicS		X	
topB		X	
ytfM		X	
yjfY		X	
yjfP		X	
cysK		X	
flgB		X	
csgE		X	
htrE		X	
grxA		X	
moeA		X	
ccmG		X	
cheW		X	
sapF		X	
sulA		X	
rfaQ		X	
rfaS		X	
amiA		X	

yfcV		X	
rcaA		X	
fliK		X	
flhB		X	
ydeQ		X	
narW		X	
dmsC		X	
frmA		X	
sfsA		X	
phnK		X	
nirD		X	
uxaA		X	
gutM		X	
bglX		X	
dnaQ		X	
sspB		X	
mlrA		X	
purD		X	
xapB		X	
vsr		X	
glvB		X	
glvG		X	
ddpB		X	

ddpD		X	
ydcV		X	
nhaB		X	
potC		X	
ssuB		X	
modE		X	
rumB		X	
araH		X	
dctA		X	
feoB		X	
feoA		X	
yahJ		X	
ampE		X	
rseC		X	
yegW		X	
dgsA		X	
torD		X	
rimI		X	
tufB		X	
rpmG		X	
greA		X	
tehA		X	
yedO		X	

ynfK		X	
ynfP		X	
ycdN		X	
mgsA		X	
yihX		X	
kdgT		X	
yqiC		X	
yghX		X	
yghQ		X	
yghF		X	
yghE		X	
ygfU		X	
exo		X	
yjeI		X	
yjbN		X	
kdgK		X	
yhjK		X	
yafV		X	
yaeF		X	
yacG		X	
yfdI		X	
ytfR		X	
dgoA		X	

dgoR		X	
cpxP		X	
ygfQ		X	
yjjZ		X	
ydbD		X	
ydaW		X	
lar		X	
ydaQ		X	
yohC		X	
yehU		X	
gspD		X	
yfjP		X	
yfdC		X	
yegI		X	
wbbK		X	
sufD		X	
ydeV		X	
phnL		X	
flhE		X	
pabC		X	
folX		X	
hisM		X	
gatZ		X	

yjeF		X	
atpE		X	