UNIVERSITY OF CALIFORNIA, SAN DIEGO

SAN DIEGO STATE UNIVERSITY

The Rearranging Chromosomes of Host-specific *Salmonella enterica* Serovars

A dissertation submitted in partial satisfaction of the
requirements for the degree Doctor of Philosophy

in

Biology

by

Thomas Davidson Matthews

Committee in charge:

University of California, San Diego

Professor Donald Guiney
Professor Joseph Pogliano

San Diego State University

Professor Stanley Maloy, Chair
Professor Kathie McGuire
Professor Moselio Schaechter
Professor Anca Segall

2009

The Dissertation of Thomas Davidson Matthews is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

_____

_____

_____

_____

_____

_____
Chair

University of California, San Diego

San Diego State University

2009

DEDICATION

This dissertation is dedicated to my wonderful, loving wife Pia Matthews. Without her love and support this work would not have been possible.

EPIGRAPH

Nothing in this world can take the place of persistence. Talent will not; nothing is more common than unsuccessful people with talent. Genius will not; unrewarded genius is almost a proverb. Education will not; the world is full of educated derelicts. Persistence and determination alone are omnipotent.

Calvin Coolidge

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES CONTINUED

LIST OF TABLES

ACKNOWLEDGMENTS

Without the help and support of my committee, friends, and family, this dissertation would not have been possible. I would like to start by acknowledging and thanking my advisor and committee chair, Stanley Maloy. Not only is Stanley an inspiration through his knowledge, intelligence, and work ethic, but also his extraordinary patience. I am extremely grateful for his advice and support while working with him over the years. I would also like to thank Anca Segall for inspiring me to continue my education towards a doctoral degree, her advice and support of my project, which was "close to her heart", as well as for "adopting" me into her lab during my last year. I want to acknowledge and thank my other committee members also for their advice and support: Moselio Schaechter; Kathie McGuire; Don Guiney; and Joe Poligano.

To work successfully in an academic lab, one must depend upon their fellow lab mates. While I am grateful to all the previous members of the lab, I want to specifically thank Shingo Tsuji and Gerardo Perez for their help, advice, encouragement, and friendship. I also want to acknowledge and thank all the undergrads that have worked with me in the lab, particularly Nick Daniel, Daniel Camp, Leslie Hernandez, and Michele McKeand.

Without the help of my collaborators, significant parts of this project would not have been possible. I am deeply indebted to Rob Edwards for writing the PERL script used to calculate replichore balance. This script was an integral part of Chapter 2 and the arrangement type analysis in Chapter 4. I am also grateful to Allen Helm for previously setting the stage for my project with his doctoral research. In addition to Allen, I also

want thank Abraham Eisenstark and Wolfgang Rabsch for giving me the opportunity to analyze the Typhi strains isolated from human carriers described in Chapter 4.

The love and support of my family was integral to my success as a graduate student. I want to acknowledge my brother Mike for inspiring me to go back to graduate school by deciding to further his own education. I want to thank Mom and Dad for all their love and support, not only as a graduate student, but in life. Finally I want to thank and acknowledge my wonderful wife Pia for all of her hard work, her love and support, and for just being there after those days when nothing seemed to work.

VITA

Education

B.S. in Biology, concentration in Molecular Biology; minor in Chemistry. Awarded May 1989. San Jose State University, San Jose, CA

M.S. in Biology, Molecular Biology program area. Thesis: Identification of Genes Involved In Phenotypic Phase Shifting of *Sphingomonas sp.* ATCC 53159. Awarded January 2005. San Diego State University, San Diego, CA

Ph.D. in Biology. Awarded December 2009. Dissertation: The Rearranging Chromosomes of Host-specific *Salmonella enteric* serovars. University of California, San Diego/San Diego State University Joint Doctoral Program, San Diego, CA

Research Experience

9/03-present	University of California, San Diego/San Diego State University Joint Doctoral Program, San Diego, CA
9/02-9/03	Biotechnology Internship Program, The Molecular Biology Institute, San Diego State University, San Diego, CA
12/97-5/02	CP Kelco/Kelco Biopolymers/Monsanto, San Diego, CA
6/96-12/97	Quidel Corporation, San Diego, CA
8/86-9/95	Novartis Crop Protection, Inc. (formerly Sandoz Agro, Inc.) Palo Alto, CA

Teaching Experience

9/03-12/08	Teaching Assistant, Department of Biology San Diego State University

Professional Presentations

"Chromsomal Rearrangements in *Salmonella enterica* sv. Typhi Strains Isolated from Human Carriers." Poster presentation at American Society of Microbiology General Meeting, Philadelphia, PA; May, 2009.

"Evolution In Action: The Rearranging Chromosomes of Host-specific *Salmonella enterica* Serovars." Poster presentation at the San Diego Microbiology Group Annual Meeting, San Diego, CA; May 2008.

"Chromosomal Rearrangements in Salmonella enterica sv. Typhi Strains Isolated from Human Carriers." Poster presentation at the CSU Biotechnology Symposium, Oakland, CA; January 2008.

"Chromosomal Rearrangements in Host-specific Serovars of *Salmonella enterica.*" Oral presentation at the San Diego Microbiology Group Annual Meeting, San Diego, CA; May 2007.

"Chromosomal Rearrangement Frequencies of Generalist and Host-specific Serovars of *Salmonella enterica.*" Poster presentation at the American Society of Microbiology General Meeting, Orlando, FL; May 2006.

"Chromosome Rearrangements in *Salmonella enterica*: Do They Play a Role in the Development of Host-Specificity?" Poster presentation at the International Union of Microbiological Societies XI International Congress of Bacteriology and Applied Microbiology, San Francisco, CA; July 2005.

"Identification of Genes Involved in Phenotypic Phase Shifting of *Sphingomonas sp.* ATCC 53159." Oral presentation at the San Diego Microbiology Group Annual Meeting, San Diego, CA; May 2005.

"Conditionally "Naked" Bacteria: A Potential Useful Tool for Studying Integral Membrane Proteins *in vivo*." Poster presentation at the San Diego Microbiology Group Annual Meeting, San Diego, CA; May 2003.

Patents, Papers, and Publications

Chromosomal Rearrangements in Host-specific serovars of *Salmonella enterica*. Review in preparation.

Chromosomal Rearrangements occurring *in vivo* in Asymptomatic Carriers of *Salmonella enterica* sv. Typhi. In preparation.

Laboratory Maintenance of *Salmonella enterica*. Chapter in preparation for *Current Protocols in Microbiology*. John Wiley & Sons, Inc., Tom Downey series editor.

Product and process for transformation of Thraustochytriales microorganisms. Roessler, P. G., Matthews, T. D., Ramseier, T.M., and J. G. Metz. U. S. Patent # 7,001,772.

Professional Memberships

American Society of Microbiology
American Society for the Advancement of Science

ABSTRACT OF THE DISSERTATION

The Rearranging Chromsomes of Host-specific *Salmonella enterica* Serovars

by

Thomas Davidson Matthews

Doctor of Philosophy in Biology

University of California, San Diego, 2009
San Diego State University, 2009

Professor Stanley Maloy, Chair

Broad host range serovars of *Salmonella enterica* have a conserved chromosomal gene order, or arrangement type, similar to that of the close relative *E.coli*. In contrast, host-specific serovars nearly always have large-scale rearrangements with endpoints in rRNA, or *rrn* operons. As these rearrangements are an important but poorly understood evolutionary process, two hypotheses for what causes these rearrangements were tested. One hypothesis proposes that horizontal gene transfer of phages and pathogenicity islands imbalances DNA replication, and rearrangements then occur to restore balance. Another hypothesis suggests that aspects of the host-specific lifestyle, such as the ability to establish a chronic carrier state in the host, either reduces selective pressure to maintain gene order or increases the rearrangement frequency.

The arrangement types that occur naturally were compared to the theoretical possibility, and the amount of imbalance of each arrangement type was estimated using a PERL script. Out of 1,440 theoretical arrangement types, only ~50 were found to occur naturally. While most natural arrangement types were well-balanced, the majority of theoretical arrangement types were very imbalanced. Furthermore, the most common types of rearrangements that were identified did not affect balance.

The relative fitness of *Salmonella* strains having transposon-held duplications of varying size that introduced up to 23° of imbalance was determined. The sizes of the duplications were similar to many of the horizontally transferred genetic elements in *Salmonella*. While no correlation was found between the amount of introduced imbalance and fitness, the duplication of some chromosomal regions was detrimental to fitness.

The arrangement types of serovar Typhi strains isolated from human carriers over time were also analyzed. Strains isolated from the same carrier almost always had different arrangement types. Furthermore, various arrangement types were identified in colonies isolated from archived slants of these strains; however, rearrangements were not detected in colonies derived from standard culturing conditions. Finally rearrangements occurring over time did not improve balance.

Taken together these results refute the hypothesis that imbalanced replication causes the rearrangements, while supporting the hypothesis that aspects of lifestyle are responsible for the rearrangements found in host-specific serovars of *Salmonella enterica*.

Chapter 1. Chromosomal Rearrangements in Host-specific *Salmonella enterica* Serovars

Introduction

The order of genes on the bacterial chromosome was once thought to be conserved within a particular species, particularly within the Enterobacteriaciae. This assumption was based on the early linkage maps obtained for members of this genus, in particular laboratory strains of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium (50, 60). As shown in Figure 1.1, these maps suggested that except for an inversion in the terminus region (11, 60), the order of genes on the *E. coli* chromosome is very similar to that found on the Typhimurium chromosome in spite of the more than one hundred million years since the two species diverged from a common ancestor (14, 50, 51). This observation is quite remarkable when one considers that both bacteria frequently undergo chromosomal rearrangements in culture.

Rearrangements can occur by homologous recombination between direct or inverted repeats, resulting in duplications, inversions, or levitations and translocations (Figure 1.2). Duplications typically occur at frequencies between 10^{-3} and 10^{-4}, but can be as high as 10^{-2} when the endpoints are within rRNA (*rrn*) operons (5). Furthermore, recombination between *rrn* operons has also been shown to invert as well as translocate the intervening chromosomal region (24, 25). Inversions occur when the *rrn* operons are on opposite replichores, or halves of the chromosome between the origin and terminus of DNA replication, while translocations occur when the *rrn* operons are on the same replichore. One would then expect that these types of rearrangements are commonly found in the environment; however this is not the case. While examples of chromosomal

rearrangements involving recombination between *rrn* operons have been found in naturally-occurring bacterial strains, in the enterics some type of selection prevents strains with these types of chromosomal rearrangements from becoming fixed within most populations (33, 60). One major exception to this observation occurs in the host-specific serovars of *Salmonella enterica*.

Rearranging the Chromosome through Recombination

Most of the intra-species large scale chromosomal rearrangements that have been identified so far in bacteria occur by recombination between *rrn* operons and/or recombination between insertion sequence (IS) elements (Table 1.1). Rearrangements occurring by *rrn* recombination were first described in 1981, when Hill and Harnish constructed *E. coli* strains having large inversions between either *rrnB* or *rrnE* and *rrnD*, as well discovering the *rrnD/E* inversion in *E. coli* strain W3110 (24). Recombination between *rrn* operons in the α-proteo-bacterium *Brucella suis* has resulted in biovars not only having chromosomes of various size, but also different chromosome numbers, the first time this was observed within a bacterial species (30). In the closely related opportunistic human pathogen *Ochrobactrum intermedium*, strains isolated from the same patient revealed a 150-kb chromosomal deletion that had occurred by *rrn* recombination over a one year period (69).

While the large size of *rrn* operons makes them excellent targets for intrachromosomal homologous recombination, the number of *rrn* operons in a particular bacterial genome varies between one (3, 6-7) and at least 13 (74), which limits the potential number of syntenic blocks available for rearrangement. IS elements on the other

hand are usually present at much higher numbers in most genomes, allowing for more diversity of rearrangements. Recombination between the hundred IS*Ftu1* and IS*Ftu2* IS elements present in the chromosomes of *Francisella tularensis* strains accounted for 49 out of 51 rearrangements found between two different virulent subspecies, while the remaining rearrangements were due to recombination between two of the three rRNA operons (55). A genomic comparison of *Bordetella bronchiseptica* and *Bordetella pertussis* indicated that 88% of about 150 rearrangements found between the two species were between IS elements, primarily IS*481* of which there are 238 copies in *B. pertussis* (52).

Another consequence of the increased copy number of IS elements compared to *rrn* operons is the potential for an increased rearrangement frequency from having more homologous recombination sites on the genome. In the yersiniae, *Yersinia pestis* has undergone an extensive IS element expansion since diverging from *Y. pseudotuberculosis* 1,500-20,000 years ago (1). Genome sequence analyses of several *Y. pestis* strains have revealed numerous chromosomal rearrangements thought to be due to recombination between the more than 100 IS elements present on the *Y. pestis* chromosome. In contrast, IS-mediated rearrangements are rarer in *S. enterica* in spite of frequent duplication formation due to unequal recombination between four of the six chromosomal IS*200* elements in the Typhimurium serovar (19). The Typhi serovar has twenty-five IS*200* elements, but only one IS*200*-mediated inversion between two of the IS*200* elements has been identified, and was present in all the analyzed strains (2).

Rearrangements can also occur by recombination between other homologous genetic elements present in multiple copies on the chromosome. A study of 38 strains of

Bartonella henselae, the causative agent of cat scratch fever, revealed numerous inversions in the terminus region (34). Most of these inversions had endpoints near genomic islands containing numerous inverted repeats, while 12% contained an inversion due to recombination between duplicated *tuf* genes. Recombination can also occur through pairs of prophages, leading to large-scale chromosomal rearrangements. In the plant pathogen *Xylella fastidio*sa rearrangements of three large chromosomal regions were identified that had a phage-related region at one of the endpoints (71). In *E. coli* O157:H7 strain EDL933 five types of large-scale inversions were found with endpoints within homologous prophages or phage-like regions (29). A strain of *S. enterica* sv. Paratyphi C has also recently been shown to have a large inversion with endpoints within the homologous regions of the Gifsy-1 and Gifsy-2 prophages (42, 43).

As shown in the above examples, aligning genome sequences from bacteria that are closely related but differ in virulence or host-specificity has revealed numerous types of rearrangements, suggesting a role for rearrangements in the evolution of pathogens (1, 12, 13, 29, 32, 34, 52, 53, 55, 69). Different types of changes can occur to the chromosomal DNA sequence during the evolution of bacterial genomes. Changes that are vertically inherited, such as acquired point mutations, duplications, and deletions are classical examples and provide genetic diversity within a population. Horizontal gene transfer of large pieces of DNA into the genome also plays a major role in bacterial genome evolution, and these new genes often encode virulence factors in many pathogens including *Salmonella* (18, 31, 58). While the role of chromosomal rearrangements in regards to virulence and host-specificity is unknown, it is possible that

these rearrangements optimize expression and/or stability of recently acquired virulence genes in their new genomic environment.

Chromosomal Rearrangements in *Salmonella enterica*

Salmonella enterica and *E. coli* diverged from a common ancestor over 100 million years ago (14, 50, 51). About 40-60 million years ago another split in the *Salmonella* lineage occurred, resulting in the two recognized *Salmonella* species: *enterica* and *bongori* (46). Currently there are over 2,500 recognized serovars of *Salmonella enterica* divided among seven subspecies (Figure 1.3) (46, 57). Most of these serovars are thought to have a broad host range and are capable of causing disease in a variety of reptilian, avian, and mammalian species. Serovars that fall into this class include the well-studied Typhimurium as well as other serovars implicated in outbreaks of food borne disease. A small number of serovars are host-specific and can only cause disease in one species or in closely-related species. The causative agent of typhoid fever, the human-specific Typhi serovar falls into this class as do the Paratyphi serovars, which cause a typhoid fever-like disease. Other host-specific serovars include the fowl-specific Gallinarum serovar (consisting of the Pullorum and Gallinarum biovars) as well as the horse-specific serovar Abortusequi. A few serovars are known to be host-adapted: while they are capable of causing disease in different animal species, they usually only infect one particular species. Examples of host-adapted serovars are Dublin and Choleraesuis, which primarily infects cattle and swine respectively. Finally, even within a serovar there may be strains that are associated with a particular host; for example Typhimurium

strains with phage types DT2 and DT99 are pigeon-associated and cause a usually lethal systemic disease (reviewed in (59)).

The pathology of the disease states caused by broad host range and host-specific *Salmonella* serovars also differ. Upon infection of a suitable host, the broad host range serovars usually cause a self-resolving gastroenteritis, but also rarely cause septicemia depending on the serovar and the infected host. The host-specific serovars on the other hand typically cause a systemic disease in their hosts that can last for weeks. In humans, typhoid fever causes a recurring fever and malaise, and has a mortality rate up to 30% without medical intervention that usually involves antibiotics (27, 28, 54). Furthermore up to 5% of untreated survivors become carriers of the Typhi bacteria (70), and act as sources for new rounds of outbreaks of the disease. Pullorum disease and fowl typhoid are caused by the Pullorum and Gallinarum biotypes of the Gallinarum serovar respectively. Pullorum disease usually infects young chicks and poults, causing diarrhea and is often fatal, whereas fowl typhoid is a chronic systemic infection that occurs in adults (56, 68). These pathological differences reflect the host-specific serovars' ability to recruit and survive within macrophages for systemic dissemination (28, 73). Establishment of the carrier state occurs when various organs are chronically colonized with small numbers of bacteria (28, 72, 73). In contrast, in cases of gastroenteritis, bacteria passing through the epithelial layer into the lamina propria set off an inflammatory response that recruits neutrophils (70) that help prevent the spread of as well as clear the infection.

The capability of the *Salmonella enterica* chromosome to undergo large-scale rearrangements was first demonstrated by Anderson and Roth. They showed that tandem

duplications by unequal recombination between *rrn* operons occur frequently by selecting for merodiploid strains carrying two selectable alleles of the same gene (4). In nature the formation of such duplications could possibly provide a selective advantage to the strains carrying them under certain environmental conditions. Without selection however these duplications also collapse with around the same frequency that they are formed. This allows populations to change the copy number of genes as conditions warrant, since environmental selective sweeps would determine the duplication status within a population.

Inversions of large regions of the chromosome were once thought to be extremely rare (24, 46, 64) and were first constructed experimentally in *Salmonella* using portable regions of homology (65, 66), and subsequently using P22-mediated transduction (47). These studies identified regions of the chromosome that were "non-permissive" for inversions. Some of these "non-permissive" inversions were thought to be due either to a mechanistic problem with the RecBCD recombination system, a bias against the exchange of flanking sequences during Holliday junction resolution at these sites, or the nucleoid structure preventing contact of the recombining regions, as strains carrying "non-permissive" inversions could be constructed using transduction. Other "non-permissive" intervals may be due to poor viability of strains carrying these inversions, as other large inversions have been shown to be detrimental to the cell (17, 23, 45). However, inversions of large chromosomal regions are now known to occur naturally within the salmonellae, as shown by the previously mentioned inversion found in Paratyphi C RKS4594 (42, 43) and the inversion present in the terminus region of numerous Typhi strains due to recombination between IS*200* elements (2).

Large-scale chromosomal rearrangements in naturally-occurring *Salmonella* strains were first identified in the mid 1990's by Liu and Sanderson (37-41). They physically mapped the chromosomes of numerous strains from both broad host range and host-specific serovars by subjecting partial I-*Ceu*I digests of chromosomal DNA to pulsed field gel electrophoresis. I-*Ceu*I is an endonuclease that recognizes a conserved 23 base pair sequence in the 23S rRNA gene. By comparing banding patterns, they found that in contrast to the broad host range strains which have the conserved arrangement type (Figure 1.4) also found in *E. coli*, host-specific strains almost always have rearrangements with endpoints within the seven *rrn* operons on the *Salmonella* chromosome. This suggested that the rearrangements were the result of recombination between the *rrn* operons.

While there are 1,440 possible arrangement types due to recombination between the seven *rrn* operons, out of 220 strains analyzed to date only about fifty types have been observed. Furthermore, each host-specific serovar appears to have a preferred arrangement type (Table 1.2). The most common arrangement type identified in strains of Typhi has a translocation of Region 4 into the *rrnE* operon and an inversion of Region 1 by recombination between the *rrnG* and *rrnH* operons (Figure 1.4), while the next most common arrangement lacks the inversion. However most of the Typhi strains as well as all of the Paratyphi A strains analyzed so far have the *rrnG*/*rrnH* inversion, suggesting that inversion of this chromosomal region may offer some kind of selective advantage. The most common arrangement type observed in analyzed Pullorum and Gallinarum strains also have Region 4 translocated into the *rrnE* operon and the *rrnG*/*rrnH* inversion, but also has a second inversion between *rrnD* and *rrnE*. This results in Regions 2 and 7

switching to opposite replichores as well as putting Region 1 back into its original orientation. In Paratyphi C the most common arrangement type has a translocation of Region 6 into the *rrnD* operon. Interestingly a small number of Gallinarum and Paratyphi C strains as well as all five analyzed strains of Paratyphi B have the conserved arrangement type, which indicates that the rearrangements themselves are not responsible for causing host specificity.

Why Do the Host-specific *Salmonella* Serovars Have Chromosomal Rearrangements?

One question these data brings up is what is causing the rearrangements in the host-specific serovars? One hypothesis proposed by Liu suggests that horizontal gene transfer of a relatively large piece of DNA into the chromosome, for example a pathogenicity island or prophage, makes one replichore longer than the other which results in imbalanced DNA replication. Rearrangements are then induced to reestablish a balanced state (35, 36, 39). Circumstantial evidence that supports this hypothesis includes the observation that most observed arrangement types are relatively balanced.

However, one problem with this hypothesis has to do with how the termination of DNA replication is believed to occur. In *E. coli* there are 10 known *ter* sites spread around the terminal half of the chromosome (reviewed in (16, 49)). When bound with the Tus protein these *ter* sites pause replication forks by inhibiting DnaB, the major replicative DNA helicase, in an orientation-dependent manner (48, 49); replication forks traveling toward the origin of replication are blocked while forks traveling towards the terminus are not. The termination of DNA replication is believed to occur mostly

between *terC* and *terA*, the two *ter* sites in the terminus region that flank the *dif* site (15, 26). The *dif* site is a 28-bp sequence recognized by the site-specific recombinase XerCD during the resolution of chromosome dimers that can form during the termination of DNA replication (26). The GC skew also switches strands at *dif*, defining the terminal region border between replichores (8, 10, 44). The *Salmonella* chromosome shares 7 *ter* sites with *E. coli* and has two additional putative sites based on homology to other *ter* sites, one within the *amyA* gene (26) and one within the *bcfC* gene (Figure 1.5). The *terC*-*terA* replication fork trap is almost 200 kb in size in *S. enterica*, comprising over 4% of the chromosome. As the size of genetic material horizontally transferred is usually smaller than this, the replication fork trap could act as a buffer to alleviate any replichore imbalance effects. Another problem with the replichore balance hypothesis is that chromosomal rearrangements themselves have a greater propensity to imbalance the replichores through asymmetrical inter-replichore inversions and inter-replichore translocations than horizontally transferred genes. While the frequency of these extremely imbalanced strains in nature is much lower than strains with balanced rearrangements, they do exist. If replichore imbalance does drive the observed rearrangements, then one could predict that these imbalanced strains would have a higher rearrangement frequency than balanced strains; however this has not been tested directly.

An alternative hypothesis that explains these chromosomal rearrangements suggests that lifestyle differences either induce the rearrangements or allows them to be better tolerated within host-specific serovar natural populations. One lifestyle difference between the host-specific and broad host range *Salmonella* serovars is the ability of the host-specific serovars to establish a chronic carrier state within their hosts. One way this

carrier state is established is for the bacteria to reside within special vacuoles within macrophages. This situation allows long-term exposure to the oxidative bursts released from NADPH oxidase which can result in DNA damage. If the SOS response is activated to repair the damage, a hyper-recombination state could result in a higher rearrangement frequency within the host. Bacteria residing in the carrier state within their specific host are also not subject to the same selective pressures as shown by the increased number of pseudogenes in host-specific serovars. Selective pressures are also affected by differences in genetic bottleneck size between broad host range and host-specific serovars during transmission to a new host. These changes in selective pressure may also allow chromosomal rearrangements to persist within a population.

Proposed Questions and Experiments Performed to Test the Hypotheses:

Question 1

Which arrangement types occur naturally versus the theoretical possibility? Previously arrangement types were classified as genome types based on the order of I-*Ceu*I fragments BCDEF and the relative orientations of the A and C fragments from naturally-occurring strains only. However these studies did not take into account arrangement types that do not occur naturally but are theoretically possible. By determining which arrangement types do not occur naturally, aspects of chromosomal architecture that limit plasticity may be revealed.

The total number of possible arrangement types was determined and compared to both naturally-occurring arrangement types determined previously as well as during the course of this study. In contrast to the well-characterized Typhi serovar (>150 strains

analyzed), the arrangement types from only 21 serovar Gallinarum strains have been previously determined. To better understand the arrangement type diversity within this serovar, the arrangement types of 22 strains belonging to the Gallinarum and Pullorum biovars of serovar Gallinarum were determined using a PCR assay. The results from this analysis showed that while arrangement type diversity exists within each host-specific serovar, each serovar also has its own unique most common arrangement type.

Question 2

Does replichore balance determine which arrangement types occur naturally? If the balanced replichore hypothesis is correct, then most naturally-occurring arrangement types should be well-balanced and rearrangements should lead to a more balanced state.

To test this, the replichore balance of all possible arrangement types was estimated using a Perl script calculator and compared to the estimated balance of naturally-occurring arrangement types. The balance estimates from the calculator were validated using previous physical balance data of Typhi strains with known arrangement types. While this analysis agreed with previous results showing that most naturally-occurring arrangement types are well balanced, most possible arrangement types have very imbalanced replichores, which suggests that random rearrangements will lead to less balanced replichores.

Question 3

Does replichore balance affect fitness? Another explanation for why most naturally-occurring arrangement types are balanced is that strains with balanced replichores are more fit and will dominate within a population while arrangement types that are imbalanced are rapidly lost.

To answer this question, the relative fitness of a set of *Salmonella* strains containing transposon-held duplications varying in size up to 729 kbp was determined. Relative fitness was based on growth rate, and the ability to compete against a tagged isogenic strain lacking a duplication. Depending on the size of the duplication, up to 23° of imbalance was introduced. The results showed that duplications of this size do not have a significant effect on fitness as far as replichore balance is concerned. However fitness was affected by duplication location. The strain carrying a duplication of the region next to the origin of replication between *ilvA* and *purA* grew more slowly in rich media and competed poorly. Loss of the duplication restored these defects and occurred more often than duplication loss in the other analyzed strains.

Question 4

Do rearrangements occur over time *in vivo* after establishment of a carrier state? The lifestyle hypothesis would predict that rearrangements occur readily within the host over time, but not outside the host.

The arrangement types of Typhi strains isolated from four human carriers over a 22 year period were determined using PCR. Many strains isolated from the same carrier at different time points had different arrangement types. However, individual colonies isolated off archived slants had different arrangement types as well, suggesting that rearrangements also occur *in vitro* over time. The results suggest that conditions both found *in vivo* as well as on the archived slant, such as slow growth and low competition over an extended period of time, allows rearrangements to accumulate within a population by relaxing selection.

Question 5

Is there a difference in rearrangement frequency between host-specific and broad host range *Salmonella* serovars? This question can be expanded to include the frequency of rearrangements under certain conditions, such as *in vivo*, *in vitro*, under various forms of stress, and varying degrees of replichore imbalance. Thus the ability to measure rearrangement frequency allows direct testing of a variety of conditions that affect rearrangement frequency, as well as the two hypotheses that explain the rearrangements found in the host-specific serovars.

To this end strains were constructed that had one of six *rrn* operons tagged by replacing the 5S rRNA gene in that operon with a promoterless *lacZ* gene. The strategy required that the native promoters of the tagged *rrn* operons be inactivated so that rearrangements occurring through recombination of the tagged operon will swap in functional promoters driving *lac* expression. Cells with rearrangements can then be selected by demanding growth on lactose as a sole carbon source. However multiple attempts at inactivating the promoters of *lac*-tagged *rrn* operons were unsuccessful.

Summary

In conclusion, the chromosomal rearrangements observed in host-specific *Salmonella* serovars reveals an important evolutionary process that is presently occurring. While the ancestral conserved arrangement type may be selected for in the broad host range serovars to help maintain their host range, the host-specific serovars may become better adapted to their specific host by rearranging the order of genes on their chromosomes. For example, chromosomal rearrangements could optimize expression of

virulence or other genes necessary for survival within the specific host. Based on the chromosomal rearrangements observed in other bacteria, one could predict that over time rearrangements could become more frequent and pronounced in host-specific serovars due to increases in IS copy number. Even though chromosomal rearrangements in *Salmonella* due to recombination between IS elements are relatively rare, genomic sequence analysis has revealed more IS elements in the Typhi chromosome than in the Typhimurium chromosome. Increases in IS copy number results in more chromosomal targets for homologous recombination. This can result in rearrangements that can drastically shuffle gene order as well as genome reduction due to deletion of chromosomal regions no longer needed for survival within the specific host.

Further analysis of arrangement types found in host-specific strains isolated from carriers as well as direct measurement of *in vitro* and *in vivo* rearrangement frequencies would allow more thorough testing of the two hypotheses that explain why there are chromosomal rearrangements in host-specific *Salmonella* serovars. Determining arrangement types of carrier-derived strains could show whether rearrangements occur *in vivo* over time and whether or not these rearrangements lead to a more balanced replichore state. Previous work has shown that both broad host range and host-specific serovars undergo inversions between *rrnG* and *rrnH* at very similar frequencies *in vitro* (20); however limitations to this approach preclude analysis of rearrangements involving other *rrn* operons as well as *in vivo* analysis. Direct measurement of rearrangement frequencies at each *rrn* operon would resolve these issues as well as determine if there is a difference in rearrangement frequency between strains with imbalanced replichores versus balanced replichores.

References

1. **Achtman, M., K. Zurth, G. Morelli, G. Torrea, A. Guiyoule, and E. Carniel.** 1999. *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. Proc Natl Acad Sci U S A **96:**14043-8.

2. **Alokam, S., S. L. Liu, K. Said, and K. E. Sanderson.** 2002. Inversions over the terminus region in *Salmonella* and *Escherichia coli*: IS200s as the sites of homologous recombination inverting the chromosome of *Salmonella enterica* serovar Typhi. J Bacteriol **184:**6190-7.

3. **Amikam, D., S. Razin, and G. Glaser.** 1982. Ribosomal RNA genes in *Mycoplasma*. Nucleic Acids Res **10:**4215-22.

4. **Anderson, P., and J. Roth.** 1981. Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (*rrn*) cistrons. Proc Natl Acad Sci U S A **78:**3113-7.

5. **Anderson, R. P., and J. R. Roth.** 1979. Gene duplication in bacteria: alteration of gene dosage by sister-chromosome exchanges. Cold Spring Harb Symp Quant Biol **43 Pt 2:**1083-7.

6. **Andersson, S. G., A. Zomorodipour, H. H. Winkler, and C. G. Kurland.** 1995. Unusual organization of the rRNA genes in *Rickettsia prowazekii*. J Bacteriol **177:**4171-5.

7. **Bercovier, H., O. Kafri, and S. Sela.** 1986. Mycobacteria possess a surprisingly small number of ribosomal RNA genes in relation to the size of their genome. Biochem Biophys Res Commun **136:**1136-41.

8. **Blattner, F. R., G. Plunkett, 3rd, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao.** 1997. The complete genome sequence of *Escherichia coli* K-12. Science **277:**1453-62.

9. **Bulach, D. M., R. L. Zuerner, P. Wilson, T. Seemann, A. McGrath, P. A. Cullen, J. Davis, M. Johnson, E. Kuczek, D. P. Alt, B. Peterson-Burch, R. L. Coppel, J. I. Rood, J. K. Davies, and B. Adler.** 2006. Genome reduction in *Leptospira borgpetersenii* reflects limited transmission potential. Proc Natl Acad Sci U S A **103:**14560-5.

10. **Capiaux, H., F. Cornet, J. Corre, M. I. Guijo, K. Perals, J. E. Rebollo, and J. M. Louarn.** 2001. Polarization of the *Escherichia coli* chromosome. A view from the terminus. Biochimie **83:**161-70.

11. **Casse, F., M. C. Pascal, and M. Chippaux.** 1973. Comparison between the chromosomal maps of *Escherichia coli* and *Salmonella typhimurium*. Length of the inverted segment in the *trp* region. Mol Gen Genet **124:**253-7.

12. **Chain, P. S., E. Carniel, F. W. Larimer, J. Lamerdin, P. O. Stoutland, W. M. Regala, A. M. Georgescu, L. M. Vergez, M. L. Land, V. L. Motin, R. R. Brubaker, J. Fowler, J. Hinnebusch, M. Marceau, C. Medigue, M. Simonet, V. Chenal-Francisque, B. Souza, D. Dacheux, J. M. Elliott, A. Derbise, L. J. Hauser, and E. Garcia.** 2004. Insights into the evolution of *Yersinia pestis* through whole-genome comparison with *Yersinia pseudotuberculosis*. Proc Natl Acad Sci U S A **101:**13826-31.

13. **Deng, W., V. Burland, G. Plunkett, 3rd, A. Boutin, G. F. Mayhew, P. Liss, N. T. Perna, D. J. Rose, B. Mau, S. Zhou, D. C. Schwartz, J. D. Fetherston, L. E. Lindler, R. R. Brubaker, G. V. Plano, S. C. Straley, K. A. McDonough, M. L. Nilles, J. S. Matson, F. R. Blattner, and R. D. Perry.** 2002. Genome sequence of *Yersinia pestis* KIM. J Bacteriol **184:**4601-11.

14. **Doolittle, R. F., D. F. Feng, S. Tsang, G. Cho, and E. Little.** 1996. Determining divergence times of the major kingdoms of living organisms with a protein clock. Science **271:**470-7.

15. **Duggin, I. G., and S. D. Bell.** 2009. Termination structures in the *Escherichia coli* chromosome replication fork trap. J Mol Biol **387:**532-9.

16. **Duggin, I. G., R. G. Wake, S. D. Bell, and T. M. Hill.** 2008. The replication fork trap and termination of chromosome replication. Mol Microbiol **70:**1323-33.

17. **Esnault, E., M. Valens, O. Espeli, and F. Boccard.** 2007. Chromosome structuring limits genome plasticity in *Escherichia coli*. PLoS Genet **3:**e226.

18. **Gal-Mor, O., and B. B. Finlay.** 2006. Pathogenicity islands: a molecular toolbox for bacterial virulence. Cell Microbiol **8:**1707-19.

19. **Haack, K. R., and J. R. Roth.** 1995. Recombination between chromosomal IS200 elements supports frequent duplication formation in *Salmonella typhimurium*. Genetics **141:**1245-52.

20. **Helm, R. A., A. G. Lee, H. D. Christman, and S. Maloy.** 2003. Genomic rearrangements at *rrn* operons in *Salmonella*. Genetics **165:**951-9.

21. **Helm, R. A., S. Porwollik, A. E. Stanley, S. Maloy, M. McClelland, W. Rabsch, and A. Eisenstark.** 2004. Pigeon-associated strains of *Salmonella enterica* serovar Typhimurium phage type DT2 have genomic rearrangements at rRNA operons. Infect Immun **72:**7338-41.

22. **Hill, C. W., R. H. Grafstrom, B. W. Harnish, and B. S. Hillman.** 1977. Tandem duplications resulting from recombination between ribosomal RNA genes in *Escherichia coli*. J Mol Biol **116:**407-28.

23. **Hill, C. W., and J. A. Gray.** 1988. Effects of chromosomal inversion on cell fitness in *Escherichia coli* K-12. Genetics **119:**771-8.

24. **Hill, C. W., and B. W. Harnish.** 1981. Inversions between ribosomal RNA genes of *Escherichia coli*. Proc Natl Acad Sci U S A **78:**7069-72.

25. **Hill, C. W., and B. W. Harnish.** 1982. Transposition of a chromosomal segment bounded by redundant rRNA genes into other rRNA genes in *Escherichia coli*. J Bacteriol **149:**449-57.

26. **Hill, T. M.** 1996. Features of the chromosomal terminus region, p. 1602-1614. *In* F. C. Neidhardt, Curtis III, R., Ingraham, J. L., Lin, E.C.C., Low, K. B., Magasanik, B., Reznikoff, W.S., Riley, M., Schaechter, M., and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella* Cellular and Molecular Biology. ASM Press, Washington, D.C.

27. **House, D., A. Bishop, C. Parry, G. Dougan, and J. Wain.** 2001. Typhoid fever: pathogenesis and disease. Curr Opin Infect Dis **14:**573-8.

28. **Huang, D. B., and H. L. DuPont.** 2005. Problem pathogens: extra-intestinal complications of *Salmonella enterica* serotype Typhi infection. Lancet Infect Dis **5:**341-8.

29. **Iguchi, A., S. Iyoda, J. Terajima, H. Watanabe, and R. Osawa.** 2006. Spontaneous recombination between homologous prophage regions causes large-scale inversions within the *Escherichia coli* O157:H7 chromosome. Gene **372:**199-207.

30. **Jumas-Bilak, E., S. Michaux-Charachon, G. Bourg, D. O'Callaghan, and M. Ramuz.** 1998. Differences in chromosome number and genome rearrangements in the genus *Brucella*. Mol Microbiol **27:**99-106.

31. **Kelly, B. G., A. Vespermann, and D. J. Bolton.** 2009. Horizontal gene transfer of virulence determinants in selected bacterial foodborne pathogens. Food Chem Toxicol **47:**969-77.

32. **Kothapalli, S., S. Nair, S. Alokam, T. Pang, R. Khakhria, D. Woodward, W. Johnson, B. A. Stocker, K. E. Sanderson, and S. L. Liu.** 2005. Diversity of genome structure in *Salmonella enterica* serovar Typhi populations. J Bacteriol **187:**2638-50.

33. **Lehner, A. F., and C. W. Hill.** 1980. Involvement of ribosomal ribonucleic acid operons in *Salmonella typhimurium* chromosomal rearrangements. J Bacteriol **143:**492-8.

34. **Lindroos, H., O. Vinnere, A. Mira, D. Repsilber, K. Naslund, and S. G. Andersson.** 2006. Genome rearrangements, deletions, and amplifications in the natural population of *Bartonella henselae*. J Bacteriol **188:**7426-39.

35. **Liu, G. R., A. Rahn, W. Q. Liu, K. E. Sanderson, R. N. Johnston, and S. L. Liu.** 2002. The evolving genome of *Salmonella enterica* serovar Pullorum. J Bacteriol **184:**2626-33.

36. **Liu, G. R., W. Q. Liu, R. N. Johnston, K. E. Sanderson, S. X. Li, and S. L. Liu.** 2006. Genome plasticity and *ori-ter* rebalancing in *Salmonella typhi*. Mol Biol Evol **23:**365-71.

37. **Liu, S. L., A. Hessel, H. Y. Cheng, and K. E. Sanderson.** 1994. The *Xba*I-*Bln*I-*Ceu*I genomic cleavage map of *Salmonella paratyphi* B. J Bacteriol **176:**1014-24.

38. **Liu, S. L., and K. E. Sanderson.** 1995. The chromosome of *Salmonella paratyphi* A is inverted by recombination between *rrnH* and *rrnG*. J Bacteriol **177:**6585-92.

39. **Liu, S. L., and K. E. Sanderson.** 1996. Highly plastic chromosomal organization in *Salmonella typhi*. Proc Natl Acad Sci U S A **93:**10303-8.

40. **Liu, S. L., and K. E. Sanderson.** 1998. Homologous recombination between *rrn* operons rearranges the chromosome in host-specialized species of *Salmonella*. FEMS Microbiol Lett **164:**275-81.

41. **Liu, S. L., and K. E. Sanderson.** 1995. I-*Ceu*I reveals conservation of the genome of independent strains of *Salmonella typhimurium*. J Bacteriol **177:**3355-7.

42. **Liu, W. Q., Y. Feng, Y. Wang, Q. H. Zou, F. Chen, J. T. Guo, Y. H. Peng, Y. Jin, Y. G. Li, S. N. Hu, R. N. Johnston, G. R. Liu, and S. L. Liu.** 2009. *Salmonella paratyphi* C: genetic divergence from *Salmonella choleraesuis* and pathogenic convergence with *Salmonella typhi*. PLoS One **4:**e4510.

43. **Liu, W. Q., G. R. Liu, J. Q. Li, G. M. Xu, D. Qi, X. Y. He, J. Deng, F. M. Zhang, R. N. Johnston, and S. L. Liu.** 2007. Diverse genome structures of *Salmonella paratyphi* C. BMC Genomics **8:**290.

44. **Lobry, J. R., and J. M. Louarn.** 2003. Polarisation of prokaryotic chromosomes. Curr Opin Microbiol **6:**101-8.

45. **Louarn, J. M., J. P. Bouche, F. Legendre, J. Louarn, and J. Patte.** 1985. Characterization and properties of very large inversions of the *E. coli* chromosome along the origin-to-terminus axis. Mol Gen Genet **201:**467-76.

46. **McQuiston, J. R., S. Herrera-Leon, B. C. Wertheim, J. Doyle, P. I. Fields, R. V. Tauxe, and J. M. Logsdon, Jr.** 2008. Molecular phylogeny of the salmonellae: Relationships among *Salmonella* species and subspecies determined from four housekeeping genes and evidence of lateral gene transfer events. J Bacteriol **190:**7060-67.

47. **Miesel, L., A. Segall, and J. R. Roth.** 1994. Construction of chromosomal rearrangements in *Salmonella* by transduction: inversions of non-permissive segments are not lethal. Genetics **137:**919-32.

48. **Mulugu, S., A. Potnis, Shamsuzzaman, J. Taylor, K. Alexander, and D. Bastia.** 2001. Mechanism of termination of DNA replication of *Escherichia coli* involves helicase-contrahelicase interaction. Proc Natl Acad Sci U S A **98:**9569-74.

49. **Neylon, C., A. V. Kralicek, T. M. Hill, and N. E. Dixon.** 2005. Replication termination in *Escherichia coli*: structure and antihelicase activity of the Tus-Ter complex. Microbiol Mol Biol Rev **69:**501-26.

50. **Ochman, H., and A. C. Wilson.** 1987. Evolution in bacteria: evidence for a universal substitution rate in cellular genomes. J Mol Evol **26:**74-86.

51. **Ochman, H., and A. C. Wilson.** 1987. Evolutionary History of Enteric Bacteria, p. 1649-1654. *In* F. C. Neidhardt, Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: Cellular and Molecular Biology. American Society of Microbiology, Washington, DC.

52. **Parkhill, J., M. Sebaihia, A. Preston, L. D. Murphy, N. Thomson, D. E. Harris, M. T. Holden, C. M. Churcher, S. D. Bentley, K. L. Mungall, A. M. Cerdeno-Tarraga, L. Temple, K. James, B. Harris, M. A. Quail, M. Achtman, R. Atkin, S. Baker, D. Basham, N. Bason, I. Cherevach, T. Chillingworth, M. Collins, A. Cronin, P. Davis, J. Doggett, T. Feltwell, A. Goble, N. Hamlin, H. Hauser, S. Holroyd, K. Jagels, S. Leather, S. Moule, H. Norberczak, S. O'Neil, D. Ormond, C. Price, E. Rabbinowitsch, S. Rutter, M. Sanders, D. Saunders, K. Seeger, S. Sharp, M. Simmonds, J. Skelton, R. Squares, S. Squares, K. Stevens, L. Unwin, S. Whitehead, B. G. Barrell, and D. J. Maskell.** 2003. Comparative analysis of the genome sequences of *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella bronchiseptica*. Nat Genet **35:**32-40.

53. **Parkhill, J., B. W. Wren, N. R. Thomson, R. W. Titball, M. T. Holden, M. B. Prentice, M. Sebaihia, K. D. James, C. Churcher, K. L. Mungall, S. Baker, D. Basham, S. D. Bentley, K. Brooks, A. M. Cerdeno-Tarraga, T. Chillingworth, A. Cronin, R. M. Davies, P. Davis, G. Dougan, T. Feltwell, N. Hamlin, S. Holroyd, K. Jagels, A. V. Karlyshev, S. Leather, S. Moule, P. C. Oyston, M. Quail, K. Rutherford, M. Simmonds, J. Skelton, K. Stevens, S. Whitehead, and B. G. Barrell.** 2001. Genome sequence of *Yersinia pestis*, the causative agent of plague. Nature **413:**523-7.

54. **Parry, C. M., T. T. Hien, G. Dougan, N. J. White, and J. J. Farrar.** 2002. Typhoid fever. N Engl J Med **347:**1770-82.

55. **Petrosino, J. F., Q. Xiang, S. E. Karpathy, H. Jiang, S. Yerrapragada, Y. Liu, J. Gioia, L. Hemphill, A. Gonzalez, T. M. Raghavan, A. Uzman, G. E. Fox, S. Highlander, M. Reichard, R. J. Morton, K. D. Clinkenbeard, and G. M. Weinstock.** 2006. Chromosome rearrangement and diversification of *Francisella tularensis* revealed by the type B (OSU18) genome sequence. J Bacteriol **188:**6977-85.

56. **Pomeroy, B. S.** 1984. Fowl typhoid, p. 79-90. *In* M. S. Hofstad, Barnes, H.J., Calneck, B.W., Reid, W.M., Yoder, H.W. (ed.), Diseases of poultry, 8th ed. Iowa State University Press, Ames, IA.

57. **Popoff, M. Y., J. Bockemuhl, and L. L. Gheesling.** 2004. Supplement 2002 (no. 46) to the Kauffmann-White scheme. Res Microbiol **155:**568-70.

58. **Porwollik, S., and M. McClelland.** 2003. Lateral gene transfer in *Salmonella*. Microbes Infect **5:**977-89.

59. **Rabsch, W., H. L. Andrews, R. A. Kingsley, R. Prager, H. Tschape, L. G. Adams, and A. J. Baumler.** 2002. *Salmonella enterica* serotype Typhimurium and its host-adapted variants. Infect Immun **70:**2249-55.

60. **Riley, M., and A. Anilionis.** 1978. Evolution of the bacterial genome. Annu Rev Microbiol **32:**519-60.

61. **Sanderson, K. E.** 1976. Genetic relatedness in the family Enterobacteriaceae. Annu Rev Microbiol **30:**327-49.

62. **Sanderson, K. E.** 1971. Genetics of the Enterobacteriaceae. A. Genetic homology in the Enterobacteriaceae. Adv Genet **16:**35-51.

63. **Sanderson, K. E., and C. A. Hall.** 1970. F-prime factors of *Salmonella typhimurium* and an inversion between *S. typhimurium* and *Escherichia coli*. Genetics **64:**215-28.

64. **Schmid, M. B., and J. R. Roth.** 1983. Selection and endpoint distribution of bacterial inversion mutations. Genetics **105:**539-57.

65. **Segall, A., M. J. Mahan, and J. R. Roth.** 1988. Rearrangement of the bacterial chromosome: forbidden inversions. Science **241:**1314-8.

66. **Segall, A. M., and J. R. Roth.** 1989. Recombination between homologies in direct and inverse orientation in the chromosome of *Salmonella*: intervals which are nonpermissive for inversion formation. Genetics **122:**737-47.

67. **Shu, S., E. Setianingrum, L. Zhao, Z. Li, H. Xu, Y. Kawamura, and T. Ezaki.** 2000. I-*Ceu*I fragment analysis of the *Shigella* species: evidence for large-scale chromosome rearrangement in *S. dysenteriae* and *S. flexneri*. FEMS Microbiol Lett **182:**93-8.

68. **Snoeyenbos, G. H.** 1984. Pullorum disease, p. 66-79. *In* M. S. Hofstad, Barnes, H.J., Calneck, B.W., Reid, W.M., Yoder, H.W. (ed.), Diseases of Poultry, 8th ed. Iowa State University Press, Ames, IA.

69. **Teyssier, C., H. Marchandin, M. Simeon De Buochberg, M. Ramuz, and E. Jumas-Bilak.** 2003. Atypical 16S rRNA gene copies in *Ochrobactrum intermedium* strains reveal a large genomic rearrangement by recombination between *rrn* copies. J Bacteriol **185:**2901-9.

70. **Tukel, C., M. Raffatellu, D. Chessa, R. P. Wilson, M. Akcelik, and A. J. Baumler.** 2006. Neutrophil influx during non-typhoidal salmonellosis: who is in the driver's seat? FEMS Immunol Med Microbiol **46:**320-9.

71. **Van Sluys, M. A., M. C. de Oliveira, C. B. Monteiro-Vitorello, C. Y. Miyaki, L. R. Furlan, L. E. Camargo, A. C. da Silva, D. H. Moon, M. A. Takita, E. G. Lemos, M. A. Machado, M. I. Ferro, F. R. da Silva, M. H. Goldman, G. H. Goldman, M. V. Lemos, H. El-Dorry, S. M. Tsai, H. Carrer, D. M. Carraro, R. C. de Oliveira, L. R. Nunes, W. J. Siqueira, L. L. Coutinho, E. T. Kimura, E. S. Ferro, R. Harakava, E. E. Kuramae, C. L. Marino, E. Giglioti, I. L. Abreu, L. M. Alves, A. M. do Amaral, G. S. Baia, S. R. Blanco, M. S. Brito, F. S. Cannavan, A. V. Celestino, A. F. da Cunha, R. C. Fenille, J. A. Ferro, E. F. Formighieri, L. T. Kishi, S. G. Leoni, A. R. Oliveira, V. E. Rosa, Jr., F. T. Sassaki, J. A. Sena, A. A. de Souza, D. Truffi, F. Tsukumo, G. M. Yanai, L. G. Zaros, E. L. Civerolo, A. J. Simpson, N. F. Almeida, Jr., J. C. Setubal, and J. P. Kitajima.** 2003. Comparative analyses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. J Bacteriol **185:**1018-26.

72. **WHO.** 2003. Background document: the diagnosis, treatment and prevention of typhoid fever. Genenva: WHO, WHO/V&B/03.07.

73. **Wigley, P., A. Berchieri, Jr., K. L. Page, A. L. Smith, and P. A. Barrow.** 2001. *Salmonella enterica* serovar Pullorum persists in splenic macrophages and in the reproductive tract during persistent, disease-free carriage in chickens. Infect Immun **69:**7873-9.

74. **Wilkinson, S. R., and M. Young.** 1995. Physical map of the *Clostridium beijerinckii* (formerly *Clostridium acetobutylicum*) NCIMB 8052 chromosome. J Bacteriol **177:**439-48.

75. **Wu, K. Y., G. R. Liu, W. Q. Liu, A. Q. Wang, S. Zhan, K. E. Sanderson, R. N. Johnston, and S. L. Liu.** 2005. The genome of *Salmonella enterica* serovar Gallinarum: distinct insertions/deletions and rare rearrangements. J Bacteriol **187:**4720-7.

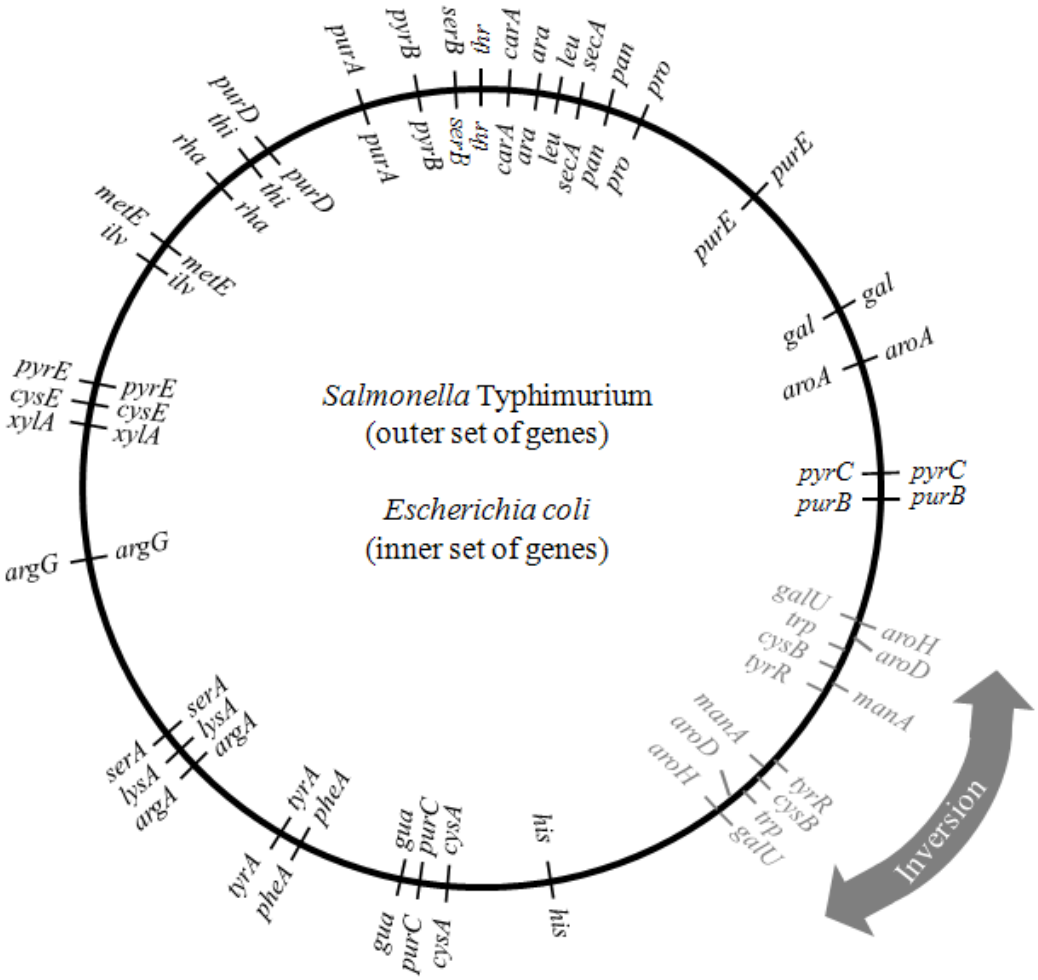

Figure 1.1. Genetic map of *S. enterica* serovar Typhimurium and *E. coli* chromosomes showing that except for an inversion in the Terminus region, the order of shared genes is highly conserved.

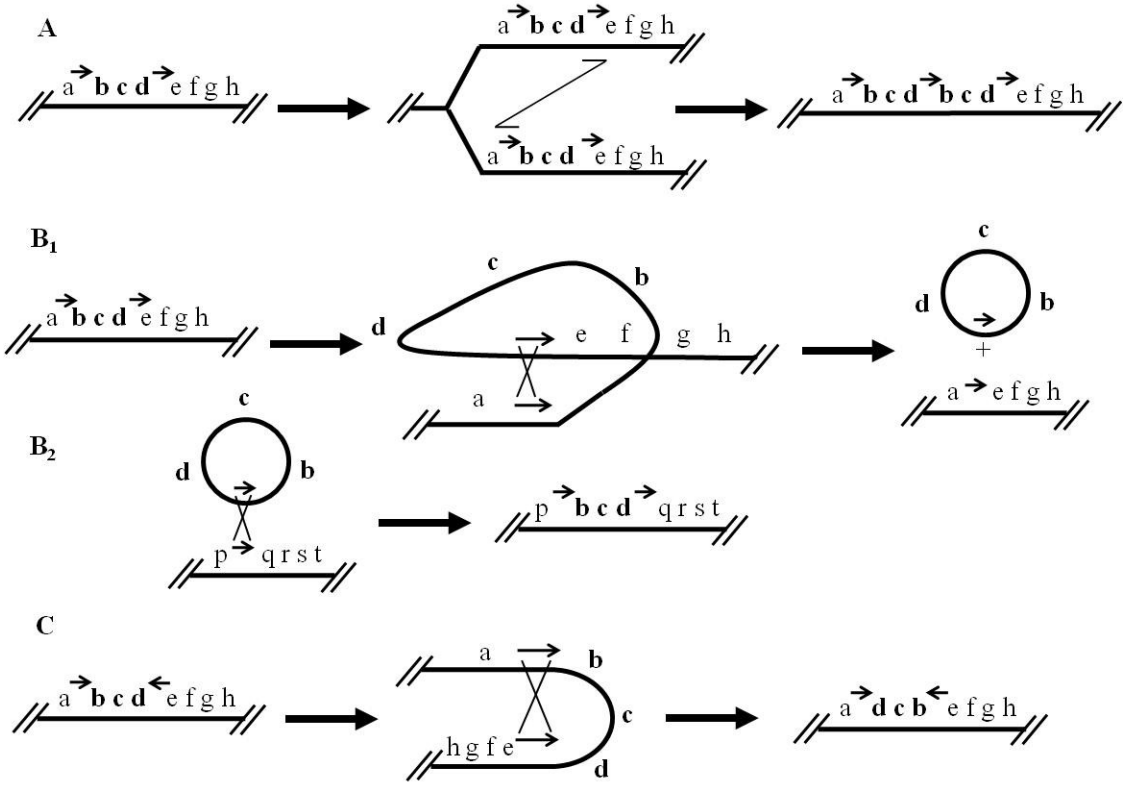

Figure 1.2. Chromosomal rearrangements through homologous recombination between direct and indirect repeats. A) Unequal exchange between direct repeats on sister chromosomes results in one sister chromosome containing a duplication, and the other containing a deletion (not shown). B) Recombination between direct repeats on the same chromosome results in a levitation of the intervening region (B_1). A translocation occurs when recombination with a homologous repeat somewhere else on the chromosome integrates the levitating region back into the chromosome (B_2). C) Recombination between inverted repeats results in an inversion of the intervening region.

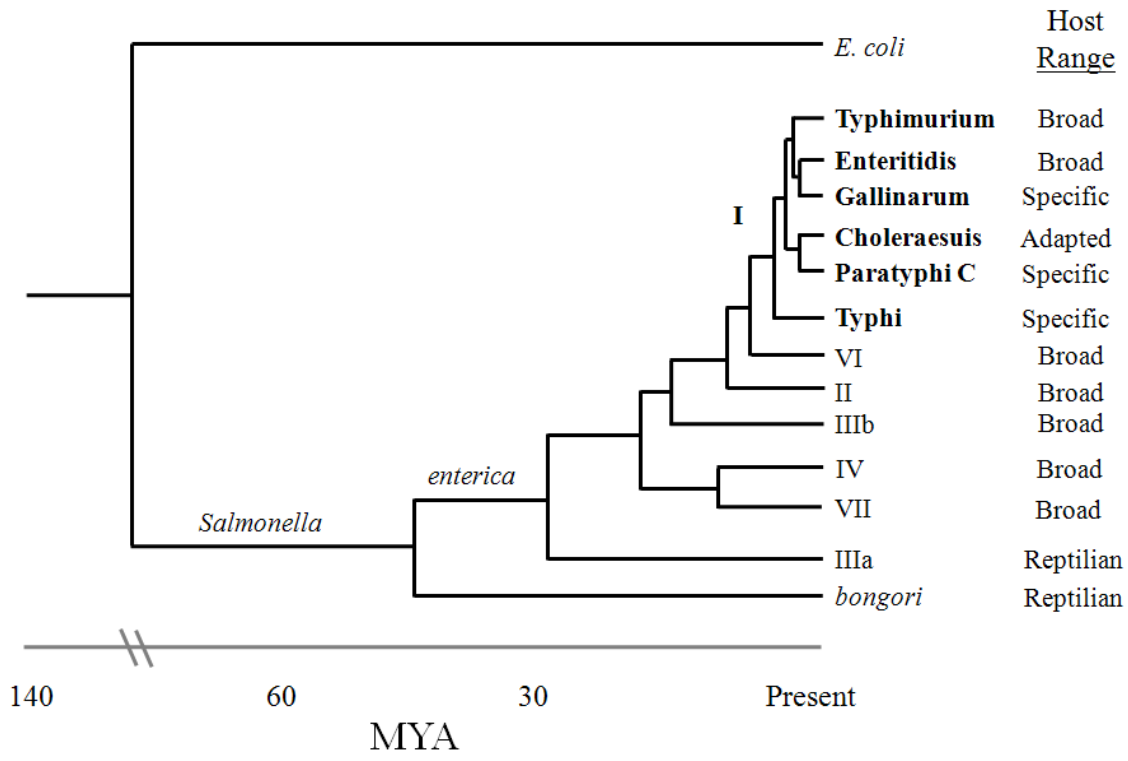

Figure 1.3. Evolutionary tree showing approximate date of divergence and phylogenetic relationship of the *Salmonella* lineage (from 46). The host range of subspecies (Roman numerals) as well as representative serovars belonging to subspecies I (in bold) are also indicated.

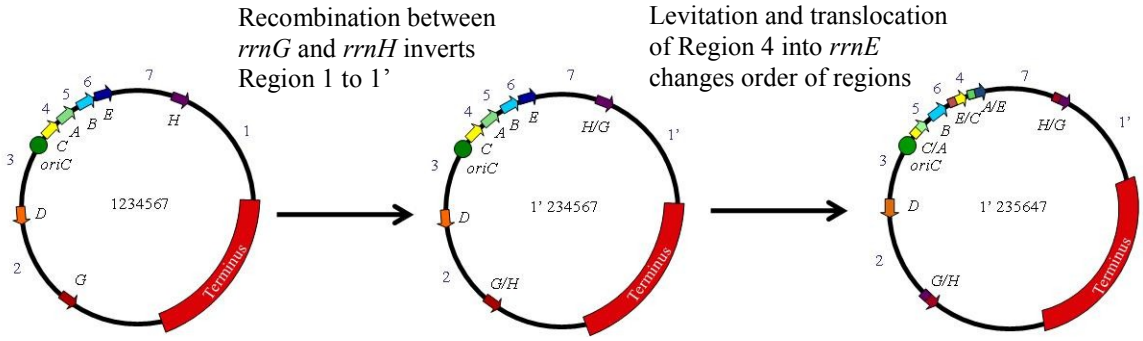

Figure 1.4. Recombination events that change the arrangement type from the conserved arrangement type (1234567) to the most common arrangement type observed in strains belonging to the Typhi serovar (1' 235647). Recombination between the *rrnG* and *rrnH* operons inverts Region 1 containing the terminus region to 1'. Levitation of Region 4 leaves behind the hybrid *rrnC/A* operon, and translocation of Region 4 into the *rrnE* operon forms the hybrid *rrnE/C* and *rrn A/E* operons.

Table 1.1. Recombination mechanisms used in large-scale bacterial chromosomal rearrangements.

Species	Mechanism of Chromosomal Rearrangement	Reference
Bartonella henselae	Genomic island and *tuf* recombination	(31)
Brucella suis biovars	*rrn* recombination	(27)
Bordetella species	Insertion sequence recombination	(39)
Escherichia coli	Insertion sequence, *rrn*, and prophage recombination	(2, 17, 19, 21, 22, 26)
Francisella tularensis	Insertion sequence and *rrn* recombination	(42)
Leptospira borgpetersenii	Insertion sequence recombination	(7)
Ochrobactrum intermedium	*rrn* recombination	(54)
Salmonella enterica	Insertion sequence, *rrn*, and prophage recombination	(2, 17, 18, 29, 53, 61)
Shigella species	*rrn* recombination	(51)
Yersinia pestis	Insertion sequence recombination	(10, 11, 40)
Xylella fastidiosa	Prophage recombination	(56)

Table 1.2. Chromosome arrangement types observed in *Salmonella enterica* serovars.

Serovar	Host Range	Arrangement Type	Observed Frequency	Reference
Chorerasuis	Adapted	1 2 3 4 5 6 7	Always	(53)
Dublin	Adapted	1 2 3 4 5 6 7	Always	(53)
Enteriditis	Broad	1 2 3 4 5 6 7	Always	(53)
Paratyphi A	Specific	1’ 2 3 4 5 6 7	Always	(53)
Paratyphi B	Specific	1 2 3 4 5 6 7	Always	(53)
Paratyphi C	Specific	1 2 6 3 4 5 7	Most common	(53)
Pullorum/Gallinarum	Specific	1 7 3 5 6 4 2	Most common	(53, 61)
Typhi	Specific	1’ 2 3 5 6 4 7	Most common	(29, 53)
Saintpaul	Broad	1 2 3 4 5 6 7	Always	(53)
Typhimurium	Broad	1 2 3 4 5 6 7	Always	(53)
Typhimurium	Associated	1 2 3 4 5 6 7	Most common	(18)
Typhimurium	Associated	1’ 7 3 4 5 6 2	Rarely	(18)

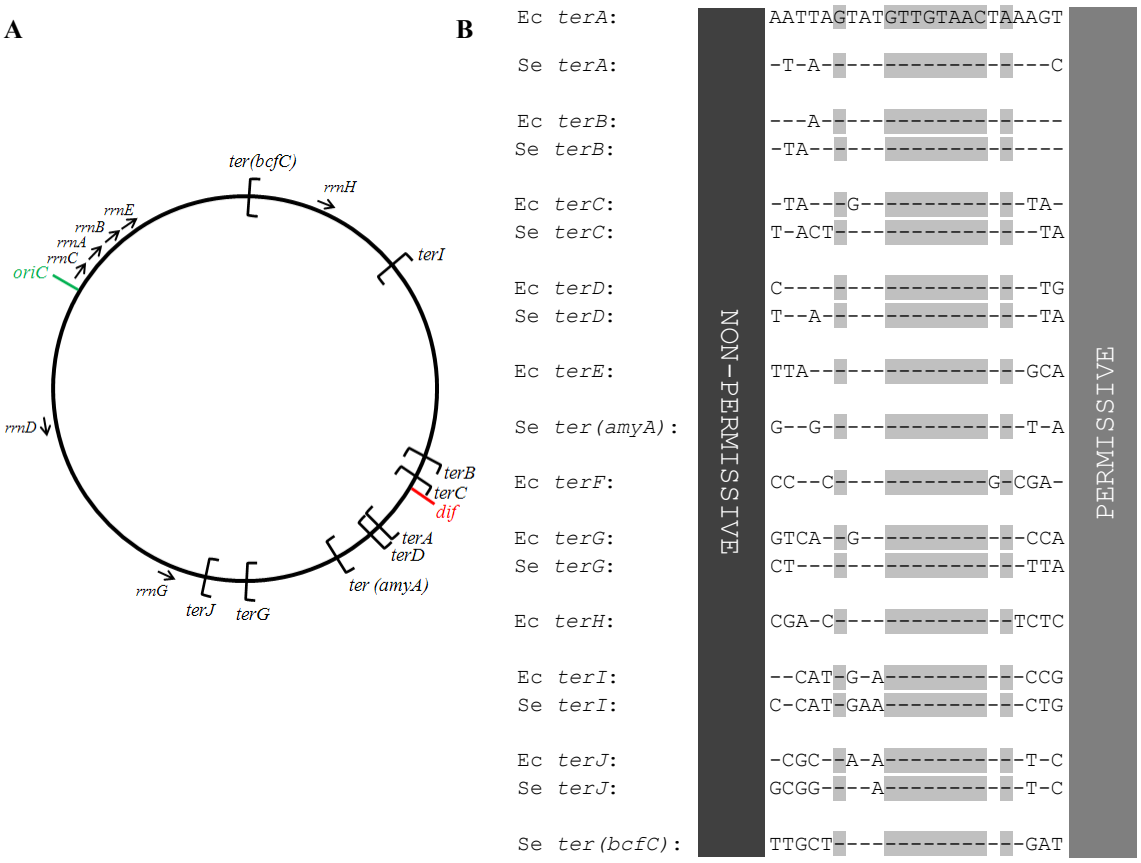

Figure 1.5. *ter* sites from the chromosome of *S. enterica* sv. Typhimurium strain LT2. Genetic map showing the location of nine *ter* sites (brackets showing direction of blocked replication forks) and seven *rrn* operons (arrows). The *oriC* and *dif* sites are shown for reference and define the endpoints of each replichore. B) Comparison of known Ter site sequences from *E. coli* (Ec) and *S. enterica* (Se). Sequence analysis indicated that *terE*, *terF*, and *terH* present in *E. coli* are missing in *S. enterica*. *ter(amyA*) was previously characterized and a novel *ter* site, *ter(bcfC)* was identified base on high homology to other *ter* sites. Dashes show bases conserved with the *E. coli terA* site and greyed bases are conserved in all *ter* sites.

Chapter 2. Chromosomal Rearrangements Formed by *rrn* Recombination Do Not Improve Replichore Balance in Host-specific *Salmonella enterica* Serovars

Introduction

Point mutations, insertions, and deletions account for the majority of genetic differences between strains of the same bacterial species, and reflect the dynamic nature of the bacterial chromosome. These variations in nucleotide sequence and DNA content can alter the relative fitness of a particular strain, allowing the environment to either select for or against it, and accounts for the differences in virulence observed between strains from the same pathogenic species. However, these differences have not been able to account for the host specificity observed in a number of bacterial pathogens. Comparative analyses of bacterial genomes have revealed at least three differences between host-specific and broad host range bacteria belonging to the same species or closely-related species: variation in gene content; more pseudogenes, and large-scale chromosomal rearrangements (4, 6, 11, 13, 29, 53, 55, 64). Changes in gene content are known to affect virulence, and pseudogene formation due to niche exclusion can lead to genome reduction. The evolutionary role of large-scale chromosomal rearrangements, however, is not clear.

Numerous examples of large-scale chromosomal rearrangements between different strains of the same species or closely related species have been identified (1, 2, 4, 10, 22, 25, 27, 28, 30, 32, 36, 53-55, 60, 66). These rearrangements change the order of genes around the chromosome by translocating and inverting chromosomal regions. In addition, some rearrangements are not tolerated, demonstrating there are selective forces that limit genome plasticity. Some features of chromosome organization that affect

plasticity include the frequency of multiple homologous sequences on the chromosome, gene location and dosage (3, 8, 31, 59, 63), orientation of polarized sequence motifs such as *ter* sites and KOPS (used to terminate DNA replication and direct DNA shuffling by FtzK repectively) (16, 21, 47, 65), and the organization of chromosomal macrodomains (16, 65).

An aspect of chromosomal organization that may limit plasticity is replichore balance (9, 37). In most sequenced bacterial chromosomes, the replichores, or opposite sides of the chromosome between the origin of DNA replication and the terminus, are equal in length and represent 180° around the chromosome (49, 62). When replichores are of equal length, DNA replication is balanced. Many types of chromosomal rearrangements can make one replichore longer than the other, altering the amount of time required to replicate each replichore. Strains having imbalanced replichores are rare because unbalanced replication is thought to affect fitness (9, 16, 26). However, the observed amount of replichore imbalance required to affect fitness varies on how the imbalance was introduced. One method utilized the integrative suppression of temperature-sensitive *dnaA* mutants by a plasmid-derived origin of replication. These plasmid-derived origins were placed at various chromosomal locations, and artificially make one replichore longer by changing the location of the beginning of replication relative to the terminus. When plasmid integration occurs close to the terminus region, growth becomes rich media sensitive due to under initiation of replication relative to cell mass synthesis (46). While most naturally-occurring interreplichore inversions are symmetrical (14), another method introduced asymmetrical interreplichore inversions using a site-specific recombination system to alter replichore balance (65). Under these

conditions, fitness was significantly affected only when imbalance was greater than 50° (16). Duplications, deletions, and insertions can also alter replichore length, but these events often affect fitness independent of replichore balance. In spite of the limits to genome plasticity, the amount of replichore balance varies even within the same species (9, 10, 37).

Salmonella enterica is an excellent model system for studying both host-specificity and large-scale chromosomal rearrangements in bacterial pathogens. While most of the >2,500 *Salmonella* serovars have a broad host range and are capable of infecting a wide variety of animal species, a small number of serovars are host-specific and can only cause disease in one species or in closely related species (57, 58). Extensive analyses of the genomes of *Salmonella* strains representing broad host range and host-specific serovars has revealed at least two differences between these serovar types: host-specific serovars have a higher number of pseudogenes and their chromosomes are often rearranged (11, 13, 37, 44, 64). Since the endpoints of these rearrangements mapped to *rrn* operons, they were proposed to occur via homologous recombination between the seven *rrn* operons (41-43). The rearrangements are caused by inversions and levitations/translocations of the chromosomal regions between the operons, changing their order from the conserved order found in the broad host range serovars (Figure 2.1).

Previous work has resolved the genome types of a number of host-specific serovar strains (33, 38, 42, 45, 64, 70). The genome types were defined as the order of restriction fragments (lettered A through G) representing the chromosomal regions between the *rrn* operons, and determined by either physical mapping of partial I-*Ceu*I digest fragments using pulsed field gel electrophoresis, or by analysis of PCR products synthesized using

primers specific to the 5' and 3' flanking regions of each *rrn* operon. I-*Ceu*I cuts within the 23S rRNA gene and by comparing the size of the partial and complete digest products, 25 naturally-occurring genome types were determined. These genome types were further divided based on the relative orientation of the I-*Ceu*I fragments A (containing the terminus) and C (containing the origin of replication).

It is not clear why the host-specific serovars have rearrangements in contrast to the conserved arrangement type found in the broad host range serovars. One hypothesis proposed by Liu suggests that the insertion of large DNA fragments into the genome, such as pathogenicity islands or prophages, imbalances the replichores, inducing chromosome rearrangements in attempts to restore balance (37, 38, 41). An alternative hypothesis proposes that aspects of the host-specific serovars' lifestyle, such as the ability to establish chronic long-term infections, either induces the rearrangements or allows them to be tolerated (23).

To test these two hypotheses, the frequency and estimated replichore balance of naturally-occurring arrangement types was compared to the theoretical possibility. Natural arrangement types were identified by compiling data from previous studies, as well as resolving the arrangement type of an additional 24 fowl-specific serovar Gallinarum strains. The naturally-occurring arrangement types were then compared to all possible theoretical arrangement types to answer the question: out of the theoretically possibile arrangement types, which ones naturally occur and which ones do not? While naturally-occurring arrangement types can be physically mapped, theoretical arrangement types can not. This problem was circumvented by developing a replichore balance calculator that estimates the balance of both theoretical and naturally-occurring

arrangement types. Naturally-occurring arrangement types were further classified depending on which types of rearrangements took place. The estimated replichore balance of natural and theoretical arrangement types was examined and grouped into four classes based on how much imbalance was estimated. The results support the hypothesis that rearrangements occur in host-specific *Salmonella* serovars as a consequence of lifestyle and not from replichore imbalance.

Materials and Methods

Strains, growth conditions, and characterization. Strains used in this study are described in Table 2.1. Bacteria were cultured using Luria-Bertani (LB) medium at 30ºC. Solid LB plates were prepared by adding agar to 1.5% (w/v). Motility was assessed as described in (7). Serological identification was performed using Salmonella O antiserum for Group D_1 Factors 1, 9, &12 (Difco, Detroit, MI, USA) preabsorbed to *S. enterica* Typhimurium LT2 to remove the α-Factor 1 and α-Factor 12 antibodies. Pullorum and Gallinarum biovars were distinguished by the ability to decarboxylate ornithine. Pullorum strains can rapidly decarboxylates ornithine whereas Gallinarum strains can not (18).

Isolation of chromosomal DNA. Chromosomal DNA was isolated using the Wizard® Genomic DNA purification kit as described by the manufacturer (Promega U. S., Madison, WI, USA).

PCR conditions. Reactions were performed in HotStart 50 tubes (Molecular BioProducts, San Diego, CA, USA) and consisted of 200 µM dNTPs and 1µM each primer (in the bottom layer), and 1X PCR buffer (20 mM Tris-HCL, pH=8.4; 50 mM KCl; 0.8% Nonidet P-40), 1.25 mM $MgCl_2$, 5% dimethylsulfoxide, Taq DNA polymerase isolated from *E. coli* harboring a plasmid with an inducible *taq* gene (12), and chromosomal DNA (in the top layer). Primer sequences and combinations for detecting specific *rrn* combinations were previously described (24). Reactions were heated to 94ºC for 3 min followed by 30 cycles of 94ºC for 1 min, 60ºC for 1 min, and 72ºC for 5 min, followed by a final step at 72ºC for 7 min. Presence of *rrn* PCR products were determined by running 10 µl of each reaction out on a 0.8% agarose/1X TBE gel, followed by detection using ethidium bromide staining.

PERL calculator to estimate replichore balance. To estimate replichore balance, the size used for each chromosomal region between the *rrn* operons was the mean length of each region in the sequenced Typhi strains Ty2 and CT18, with the origin of replication and the *dif* site were used as the replichore endpoints. The replichore balance calculator is available on the internet at http://edwards.sdsu.edu/cgi-bin/replichores.cgi.

Results

rrn Arrangement Types

Due to the factorial aspect of the total number of arrangements possible by recombination between *rrn* operons, it is easier to describe the rearrangements in terms of numbers (arrangement types) instead of letters (genome types). For example, the

conserved chromosome arrangement type found in the broad host range serovars is described as arrangement type 1234567 instead of genome type 1 with an I-*Ceu*I fragment order of BCDEFG and an A+/C+ orientation. This description of the arrangement type is based on the relative order of the chromosomal regions between the *rrn* operons, starting with Region 1 (containing the terminus) and proceeding clockwise around the chromosome.

To determine the number of possible arrangement types, the limitations of rearrangements due to *rrn* recombination must be considered. As intrareplichore inversions are restricted by the direct repeat nature of the *rrn* operons on each replichore, the number of possible arrangement type combinations is: 6! X 2 = 1,440. This number is based on the combinations possible when rearranging the order of the six chromosomal regions surrounding Region 3 (containing the origin of replication) plus the same number with Region 1 inverted (designated 1').

Naturally-occurring Arrangement Types

Previously the genome types of 136 Typhi strains were determined (33). After converting the genome types of these strains to arrangement types, 33 arrangement types were found to occur naturally in Typhi. The most common Typhi arrangement type identified was 1'235647, followed by 1235647 (Figure 2.2). These two arrangement types, which all had a translocation of Region 4 into the *rrnE* operon, were found in 40% of the strains. The most-common rearrangement found in 73% of the strains analyzed was the inversion of Region 1 by recombination between the *rrnH* and *rrnG* operons. Eight strains were found to have another inversion due to recombination between the *rrnD* and

rrnE operons. This inversion results in Regions 2 and 7 switching replichores as well as inverting Region 1 (three strains having this inversion as well as Region 1 in the conserved orientation underwent both of the inversions described above). Almost all the other rearrangements involved translocation of Regions 4, 5, and/or 6 to either one of the *rrn* operons bordering these regions, or to *rrnD* on the opposite replichore next to Region 3. Translocations that moved these regions next to Region 1 were only observed in one strain. In 20 strains unique arrangement types were found.

Other human-adapted *Salmonella* serovars also cause enteric fever and have chromosomal rearrangements due to *rrn* recombination. A small number of strains belonging to the Paratyphi A serovar were found to all contain an inversion of Region 1 due to *rrnG*/*rrnH* recombination, but no other types of rearrangements were noted (40). The most common arrangement type observed in 8 out of 23 strains of Paratyphi C was 1263457, most likely as a result of an interreplichore translocation of Region 6 (45). Almost all the rest of the Paratyphi C strains had an intrareplichore translocation of Regions 4, 5, or 6. One exception was a strain that had the unusual arrangement type of 1423657, probably due to an interreplichore translocation of Region 4 in between Regions 1 and 2. Paratyphi B was found to have the conserved arrangement type; however only one strain from this serovar has been characterized (39).

Strains belonging to other host-specific *Salmonella* serovars have also been analyzed to determine their chromosomal arrangement types. The two biovars of the fowl-specific Gallinarum serovar, Pullorum and Gallinarum, cause either pullorum disease or fowl typhoid respectively. Pullorum disease usually infects young chicks and poults, causing diarrhea, and is often fatal, whereas fowl typhoid is a chronic systemic

infection that occurs in adults (56, 61). Nineteen strains belonging to the Pullorum biovar have been previously analyzed to determine their arrangement types (24, 38, 42). Reanalysis of the data showed that 68% of the strains had the arrangement type 1735462, with the remaining strains having unique arrangement types. All of the strains appeared to have an inversion between *rrnD* and *rrnE* resulting in regions 2 and 7 switching replichores. Two Gallinarum biovar strains have been characterized previously. One had an arrangement type of 1'245637 (42, 70), and the other had an arrangement type of 1'734652 (64).

To determine if other arrangement types occur in these fowl-specific *Salmonella* biovars, the arrangement types of eight Gallinarum and fourteen Pullorum strains were determined using PCR (Table 2.2). All the strains were shown to be of the Gallinarum serovar as all were non-motile, in contrast to other *Salmonella* serovars, and agglutinated preabsorbed antiserum specific for O antigen Group D_1 Factor 9. All the Pullorum biovar strains were able to decarboxylate ornithine while all the Gallinarum biovar strains did not. Interestingly six of the Gallinarum strains each had a unique arrangement type and two strains had the conserved arrangement found in the broad-host range serovars. One strain, TYT3316, appeared to have Region 2 integrated into the chromosome by recombination not involving *rrn* operons. The orientation of Region 1 in TYT3335 could not be ascertained from the PCR results. The results from the PCR analysis of the Pullorum strains showed that half of the strains had the previously observed most common arrangement type of 1735462 and half had unique arrangement types. One strain, TYT3345, also contained a duplication of Region 4. These data show that different biovars of the same serovar can differ in their most common arrangement type.

The 48 naturally-occurring arrangement types were then organized into 11 rearrangement groups based on the most likely types of rearrangements that occurred during their formation from the conserved arrangement type (Table 2.4). Intrareplichore translocations of Regions 4-6 were the most common type of rearrangement, followed by the inversion of Region 1 through *rrnG*/*rrnH* recombination. Almost two-thirds of all analyzed strains had either one or both of these types of rearrangements. Interreplichore translocations, which can alter replichore balance, were less frequent and occurred in conjunction with intrareplichore translocations and/or inversions. Two types of inversions occur in naturally-occurring strains; the above mentioned inversion of Region 1 and the inversion of Regions 1, 2, and 7 by recombination through the *rrnD* and *rrnE* operons. A number of strains, mostly from the Gallinarum serovar, have both types of inversions, which returns Region 1 to its original orientation even though the flanking *rrn* operons are hybrids.

While each of the analyzed host-specific serovars has a most common arrangement type, a number of strains in each serovar have unique arrangement types. However, out of the 1,440 possible *rrn* arrangement types that can occur, only 48 have been identified so far in naturally-occurring strains of host-specific *Salmonella* serovars. While determining the arrangement types of more strains may reveal more diversity, there does appear to be some selective force that prevents certain arrangement types from becoming fixed within a population. If replichore balance is a major selective force in determining naturally-occurring arrangement types, most natural arrangement types would be predicted to have well-balanced replichores, and arrangement types with imbalanced replichores would be rare. While physical mapping data supports this idea

(37), to fully understand how much of a selective force replichore balance truly is, one must know the putative replichore balance of arrangement types not observed in isolated strains.

Estimated Replichore Balance of All Possible Arrangment Types

To determine the replichore balance of arrangement types not occurring in nature, as well as to quickly estimate the replichore balance of strains with established arrangement types, a replichore balance calculator was written in PERL by Robert Edwards. While physical mapping can detect strain-specific differences in replichore length due to various insertions and deletions (37), the variability in replichore balance between strains with the same arrangement type has not been determined. The calculator described here allows a rapid estimation of replichore balance for all arrangement types, both natural and theoretical.

To compensate for the variation in the size of the chromosomal regions between the *rrn* operons, an average of region sizes from sixteen sequenced *Salmonella* strains representing both broad host range and host-specific serovars was used in the replichore balance calculations (Table 2.3). The origin of replication and *dif* were used as replichore endpoints. The origin of replication was placed 16 kilobasepairs (kbp) upstream of *rrnC*, between the *gidA* and *mioC* genes. The *dif* site was identified in each strain based on homology to the *E. coli dif* site (accession number S62735; (34)). The distance between the 3' end of *rrnG* and *dif* ranged from ~550 kbp in Paratyphi C RKS4594 to 1,245 kbp in Typhi CT18, and averaged 1,117 kbp. The smaller distance observed in Paratyphi C RKS4594 is due to an inversion between the Gifsy phages, making this strain highly

imbalanced (44). Since this rearrangement skews the normal distance between the 3' end of *rrnG* and *dif*, the data from this strain was not used. The mean distance between the 3' end of *rrnG* and *dif* used in the replichore balance calculations was 1,155 kbp.

To validate the calculator, balance estimates were generated using the average region sizes of the sequenced Typhi strains CT18 and Ty2 (11, 51) and compared to the balance calculated from the physical mapping data from 29 naturally-occurring arrangement types (37) (Figure 2.3). Replichore imbalance in these arrangement types varied from 1-55°. While the calculator slightly underestimated the physical balance, mostly due to strain-specific increases in region size from insertions, the balance estimates from the calculator statistically agree ($p < 0.01$, paired student's t-test; Pearson's correlation = 0.982).

The replichore balance of 48 naturally-occurring arrangement types was estimated (Figure 2.4). Most naturally-occurring arrangement types have well-balanced replichores, with 29 arrangement types having ≤15° imbalance. This group includes the conserved arrangement type with an estimated 0.2° imbalance. Another 11 naturally-occurring arrangement types have an imbalance of 16-30°, and 5 arrangement types have between 31-45° imbalance. Only 3 naturally-occurring arrangement types have an estimated replichore balance >45°. The 48 naturally-occurring arrangement types analyzed here were identified from 212 host-specific strains, representing mostly the Typhi serovar but also the Gallinarum, Paratyphi A, and Paratyphi C serovars. Well-balanced replichores (≤ 15° imbalance) were estimated in 184 of these strains, and 20 strains had an imbalance between 16-30°. Five strains had between 31-45° imbalance, and only 3 strains had >45°

imbalance. These results agree with previous results that naturally-occurring strains of bacteria often have well-balanced replichores (9, 37).

On the other hand, when the replichore balance of all 1,440 possible arrangement types was estimated, only 204 arrangement types were well-balanced (≤15°). Most possible arrangement types appeared to be very imbalanced, with 348 arrangement types having between 31-45° imbalance and 648 having >45°, with a third of these having an imbalance >60°. Another 240 arrangement types had an imbalance between 16-30°. These results show that rearrangements would most likely lead to a less balanced state, and that there are 175 balanced arrangement types that were not found among the naturally-occurring arrangement types. Over 90% of the non-natural, well-balanced arrangement types had at least one region translocated between Region 1 and 2 and/or Region 7 and 1. This is in contrast to only 3 naturally-occurring arrangement types with this configuration. These data suggest that other factors such as chromosomal location of a region can limit genome plasticity in addition to, if not more than, replichore balance.

Discussion

In contrast to the conserved arrangement type observed in host-generalist serovars of *S. enterica*, strains belonging to host-specific serovars almost always have chromosomal rearrangements from recombination between *rrn* operons. The Liu hypothesis proposes that rearrangements occur to reestablish replichore balance after horizontal gene transfer events (37, 38, 41). To test this hypothesis, an analysis of which arrangement types naturally occur versus the theoretical possibility was performed, and the replichore balance of these arrangement types was estimated.

As predicted by the physical mapping data (37), most arrangement types and almost all host-specific strains have well-balanced replichores. This observation has been suggested to be due to rearrangements reestablishing balance after insertions and deletions, as predicted by the Balanced Replichore hypothesis (37). Wavelet analysis and bipartition modeling of numerous sequenced bacterial strains showed a strong tendency towards balanced replichores (49, 62), and it has also been suggested that imbalanced replication is detrimental to fitness and strains with this defect are selected against and lost from the population (9, 16, 26). This argues that most strains are well-balanced because selective forces remove imbalanced strains versus imbalanced strains undergoing rearrangements to become balanced.

The most common rearrangement found was an intrareplichore translocation of Regions 4, 5, and/or 6. However, this type of rearrangement has no effect on replichore balance. Furthermore, both the inversion of Region 1, the other common rearrangement, as well as the inversion from recombination between *rrnD* and *rrnE*, are symmetrical and have a negligible effect on balance. These observations that most rearrangements do not affect balance do not support the Balanced Replichore hypothesis.

Only 48 out of 1,440 arrangement types have been found to occur naturally. While other arrangement types will probably be found in the future as more strains are analyzed, over 95% of theoretical arrangement types have not been observed. One explanation for this is that almost half of theoretical arrangement types are very imbalanced (>45°), and 70% have >30° imbalance. If most arrangement types are imbalanced, how probable is it that a rearrangement would increase balance, especially if the initial imbalance was caused by an insertion as proposed by the Balanced Replichore

hypothesis? The size of the large insertions into the *Salmonella* chromosome can vary from 15-140 kbp, often as pathogenicity islands or prophages. This type of horizontal gene transfer would introduce up to 10° imbalance. However the chromosomal rearrangements occurring in host-specific *Salmonella* would most likely decrease replichore balance rather than increase it. This observation also does not support Liu's hypothesis.

Most DNA replication forks in *Salmonella* are presumed to terminate in the replication fork trap between the *terC* and *terA* sites. As the fork trap is almost 200 kbp in size, imbalance should be buffered up to 15°. Even slightly higher amounts of imbalance would be buffered by the *terD* and *terB* sites flanking the primary fork trap. As the amount of imbalance introduced from horizontal transfer of pathogenicity islands and prophages is buffered by the size of the fork trap, and rearrangements such as interreplichore translocations and asymmetrical inversions can easily introduce >15° imbalance, rearranging the chromosome in an attempt to correct this amount of imbalance would most likely introduce more imbalance.

In addition to replichore balance, gene location can influence genome plasticity. Positional effects on genome plasticity independent of replichore balance are evident in the well-balanced, non-natural arrangement types. Only 15 out of 175 such arrangement types have Region 1 flanked by Regions 2 and 7. However, 92% of naturally-occurring arrangement types have this configuration. This observation suggests a strong selection against arrangement types where Regions 4, 5, or 6 flank Region 1.

One selective force affected by gene location is gene dosage. Rearrangements can change the dosage of genes in Regions 4, 5, and 6 by moving them farther from the origin

of replication, for example into *rrnG* or *rrnH* flanking Region 1. Many genes in these regions encode proteins involved in the transcription and translation machineries, and gene dosage effects on expression have been suggested to limit their chromosomal location close to the origin of replication (8). Within the observed naturally-occurring arrangement types, only five have Region 4, 5, or 6 flanking Region 1, and these arrangement types are only represented by one strain.

Location may also limit certain arrangement types from naturally occurring if macrodomain organization is perturbed. Recent studies in *E. coli* have described the structure of the chromosome in terms of four macrodomains and two non-structured regions that are spatially and temporally separated within the cell (Figure 2.5) (16, 17, 50, 65). Regions 4, 5, and 6, as well as part of Region 3 and most of Region 7 lie within the Ori domain. Analyzed inversions between the Ori and Left domains are interreplichore, asymmetrical, and introduce significant imbalance, which may mask the effect of mingling macrodomain-specific sequence. Intrareplichore inversions with endpoints in the Ori and Right macrodomains do not change balance but do often cause growth defects by interfering with nucleoid management and septum formation. If the *Salmonella* chromosome has a similar macrodomain structure, does that play a role in limiting the arrangement types observed? Since the *rrnH* operon is in the right non-structured region, rearrangements should be tolerated there. However as the *rrnG* operon is in the Left domain, translocation of Ori domain regions may be selected against. Only one strain analyzed in this study, a Paratyphi C strain, had such a translocation.

The number of recombination steps required to obtain certain arrangement types may also limit which arrangement types naturally occur. However, any of the 1,440

theoretical arrangement types can be obtained with a minimum of 3 recombination events, including many naturally-occurring arrangement types.

When naturally-occurring arrangement types were separated into their respective serovars, it was found that each serovar had its own most common arrangement type. In the most common Typhi arrangement type region 4 translocated between Regions 6 and 7 and Region 1 was inverted. The next most common arrangement type had the same translocation of Region 4, but lacked the inversion and was more balanced. In Paratyphi C, the most common arrangement type had an interreplichore translocation of Region 6, which slightly altered balance about 3°. The two biovars of the Gallinarum serovar differed in respect to most common arrangement type. While the Pullorum biovar had a most common arrangement type of 1735462, none of the analyzed Gallinarum biovar strains did, and possibly due to the sample size, no most common arrangement type was observed. Interestingly two Gallinarum strains had the conserved arrangement type, which is very rare in host-specific *Salmonella* strains. Multilocus enzyme electrophoresis (35) and comparative genome analysis (64) have suggested that Gallinarum is a recent descendant of the Enteriditis serovar, which also has the conserved arrangement type. While the Pullorum and Gallinarum biovars are closely related (35, 51), the observed arrangement types in Gallinarum are more ancestral than the ones found in the Pullorum. Furthermore, the 1735462 arrangement type has undergone two inversions, one between *rrnG* and *rrnH* and one between *rrnD* and *rrnE*. The *rrnD-rrnE* inversion is interesting because the same inversion in *E. coli* is rapidly overgrown in culture by revertants, suggesting that it causes a fitness defect (26). In Pullorum this inversion has not only persisted, but appears to be preferred. In spite of having a most common arrangement

type, unique arrangement types were found in many strains, showing that diversity in arrangement types does occur within a serovar.

In conclusion, the results of this study do not support the hypothesis proposed by Liu that replichore imbalance drives the chromosomal rearrangements in host-specific *Salmonella* serovars. The effects on fitness due to changes in replichore balance from horizontal gene transfer is negligible because the replication fork trap where DNA replication terminates is large enough to buffer the imbalance introduced by known horizontal gene transfer events. Also most natural rearrangements in host-specific *Salmonella* do not significantly alter replichore balance while most theoretical arrangement types are very imbalanced. Therefore it seems unlikely that these types of rearrangements would increase balance over time.

Another possibility is that lifestyle differences of the host-specific serovars are either inducing the rearrangements, selecting for rearrangements, or allowing the rearrangements to be tolerated. One lifestyle difference is that host-specific serovars often establish a chronic carrier state within their hosts, and in addition to other cells types often reside within macrophages. Macrophages kill bacterial pathogens with bursts of reactive oxygen and nitrogen species produced by phagocyte NADPH oxidase and iNOS (inducible nitric oxide synthase) respectively. These bursts of reactive species have been shown to kill or inhibit intracellular *S. enterica* sv. Typhimurium *in vitro*, and are required for host resistance to infection (48, 67, 68). However, the protein effectors encoded in *Salmonella* pathogenicity island-2 (SPI-2) enable intracellular *Salmonella* to resist these bursts of reactive species by preventing colocalization of the NADPH oxidase and iNOS with the *Salmonella*-containing vacuole (SCV) (5, 20, 69). While the SPI-2

effectors provide protection from the reactive species bursts, over time in the carrier state the bursts are likely to occasionally hit the intracellular *Salmonella*. If DNA damage occurs and the DNA repair systems are induced, the rearrangements could be the result of increased recombination frequency. Although transcriptional profiling has shown the SOS response to be induced in Typhimurium cells isolated from infected J774-A.1 murine macrophage-like cells (15), in Typhi cells isolated from human THP-1 macrophages there did not appear to be any upregulation (19). Bacteria in the carrier state would also not be under the same selective pressures to maintain gene order. This may allow rearrangements to be more tolerated or selected for. Selection for certain arrangement types by the host may explain why each host-specific serovar has a most common arrangement type. Finally bottlenecks that occur during transmission to a new host are much narrower for host-specific strains than for host generalist strains. These differences in lifestyle may also explain the rearrangements observed in strains belonging to host-specific *Salmonella* serovars and need to be further scrutinized.

References

1. **Alokam, S., S. L. Liu, K. Said, and K. E. Sanderson.** 2002. Inversions over the terminus region in *Salmonella* and *Escherichia coli*: IS200s as the sites of homologous recombination inverting the chromosome of *Salmonella enterica* serovar Typhi. J Bacteriol **184:**6190-7.

2. **Bulach, D. M., R. L. Zuerner, P. Wilson, T. Seemann, A. McGrath, P. A. Cullen, J. Davis, M. Johnson, E. Kuczek, D. P. Alt, B. Peterson-Burch, R. L. Coppel, J. I. Rood, J. K. Davies, and B. Adler.** 2006. Genome reduction in *Leptospira borgpetersenii* reflects limited transmission potential. Proc Natl Acad Sci U S A **103:**14560-5.

3. **Carpentier, A. S., B. Torresani, A. Grossmann, and A. Henaut.** 2005. Decoding the nucleoid organisation of *Bacillus subtilis* and *Escherichia coli* through gene expression data. BMC Genomics **6:**84.

4. **Chain, P. S., E. Carniel, F. W. Larimer, J. Lamerdin, P. O. Stoutland, W. M. Regala, A. M. Georgescu, L. M. Vergez, M. L. Land, V. L. Motin, R. R. Brubaker, J. Fowler, J. Hinnebusch, M. Marceau, C. Medigue, M. Simonet, V. Chenal-Francisque, B. Souza, D. Dacheux, J. M. Elliott, A. Derbise, L. J. Hauser, and E. Garcia.** 2004. Insights into the evolution of *Yersinia pestis* through whole-genome comparison with *Yersinia pseudotuberculosis*. Proc Natl Acad Sci U S A **101:**13826-31.

5. **Chakravortty, D., I. Hansen-Wester, and M. Hensel.** 2002. *Salmonella* pathogenicity island 2 mediates protection of intracellular *Salmonella* from reactive nitrogen intermediates. J Exp Med **195:**1155-66.

6. **Champion, M. D., Q. Zeng, E. B. Nix, F. E. Nano, P. Keim, C. D. Kodira, M. Borowsky, S. Young, M. Koehrsen, R. Engels, M. Pearson, C. Howarth, L. Larson, J. White, L. Alvarado, M. Forsman, S. W. Bearden, A. Sjostedt, R. Titball, S. L. Michell, B. Birren, and J. Galagan.** 2009. Comparative genomic characterization of *Francisella tularensis* strains belonging to low and high virulence subspecies. PLoS Pathog **5:**e1000459.

7. **Clegg, S., and K. T. Hughes.** 2002. FimZ is a molecular link between sticking and swimming in *Salmonella enterica* serovar Typhimurium. J Bacteriol **184:**1209-13.

8. **Couturier, E., and E. P. Rocha.** 2006. Replication-associated gene dosage effects shape the genomes of fast-growing bacteria but only for transcription and translation genes. Mol Microbiol **59:**1506-18.

9. **Darling, A. E., I. Miklos, and M. A. Ragan.** 2008. Dynamics of genome rearrangement in bacterial populations. PLoS Genet **4:**e1000128.

10. **Deng, W., V. Burland, G. Plunkett, 3rd, A. Boutin, G. F. Mayhew, P. Liss, N. T. Perna, D. J. Rose, B. Mau, S. Zhou, D. C. Schwartz, J. D. Fetherston, L. E. Lindler, R. R. Brubaker, G. V. Plano, S. C. Straley, K. A. McDonough, M. L. Nilles, J. S. Matson, F. R. Blattner, and R. D. Perry.** 2002. Genome sequence of *Yersinia pestis* KIM. J Bacteriol **184:**4601-11.

11. **Deng, W., S. R. Liou, G. Plunkett, 3rd, G. F. Mayhew, D. J. Rose, V. Burland, V. Kodoyianni, D. C. Schwartz, and F. R. Blattner.** 2003. Comparative genomics of *Salmonella enterica* serovar Typhi strains Ty2 and CT18. J Bacteriol **185:**2330-7.

12. **Desai, U. J., and P. K. Pfaffle.** 1995. Single-step purification of a thermostable DNA polymerase expressed in *Escherichia coli*. Biotechniques **19:**780-2, 784.

13. **Edwards, R. A., G. J. Olsen, and S. R. Maloy.** 2002. Comparative genomics of closely related salmonellae. Trends Microbiol **10:**94-9.

14. **Eisen, J. A., J. F. Heidelberg, O. White, and S. L. Salzberg.** 2000. Evidence for symmetric chromosomal inversions around the replication origin in bacteria. Genome Biol **1:**RESEARCH0011.

15. **Eriksson, S., S. Lucchini, A. Thompson, M. Rhen, and J. C. Hinton.** 2003. Unravelling the biology of macrophage infection by gene expression profiling of intracellular *Salmonella enterica*. Mol Microbiol **47:**103-18.

16. **Esnault, E., M. Valens, O. Espeli, and F. Boccard.** 2007. Chromosome structuring limits genome plasticity in *Escherichia coli*. PLoS Genet **3:**e226.

17. **Espeli, O., R. Mercier, and F. Boccard.** 2008. DNA dynamics vary according to macrodomain topography in the *E. coli* chromosome. Mol Microbiol **68:**1418-27.

18. **Ewing, W. H.** 1986. Chapter 9. The Genus *Salmonella*, p. 181-245, Edwards and Ewing's Identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co., Inc., New York.

19. **Faucher, S. P., S. Porwollik, C. M. Dozois, M. McClelland, and F. Daigle.** 2006. Transcriptome of *Salmonella enterica* serovar Typhi within macrophages revealed through the selective capture of transcribed sequences. Proc Natl Acad Sci U S A **103:**1906-11.

20. **Gallois, A., J. R. Klein, L. A. Allen, B. D. Jones, and W. M. Nauseef.** 2001. *Salmonella* pathogenicity island 2-encoded type III secretion system mediates exclusion of NADPH oxidase assembly from the phagosomal membrane. J Immunol **166:**5741-8.

21. **Guijo, M. I., J. Patte, M. del Mar Campos, J. M. Louarn, and J. E. Rebollo.** 2001. Localized remodeling of the *Escherichia coli* chromosome: the patchwork of segments refractory and tolerant to inversion near the replication terminus. Genetics **157:**1413-23.

22. **Haack, K. R., and J. R. Roth.** 1995. Recombination between chromosomal IS200 elements supports frequent duplication formation in *Salmonella typhimurium*. Genetics **141:**1245-52.

23. **Helm, R. A., A. G. Lee, H. D. Christman, and S. Maloy.** 2003. Genomic rearrangements at *rrn* operons in *Salmonella*. Genetics **165:**951-9.

24. **Helm, R. A., and S. Maloy.** 2001. Rapid approach to determine *rrn* arrangement in *Salmonella* serovars. Appl Environ Microbiol **67:**3295-8.

25. **Hill, C. W., R. H. Grafstrom, B. W. Harnish, and B. S. Hillman.** 1977. Tandem duplications resulting from recombination between ribosomal RNA genes in *Escherichia coli*. J Mol Biol **116:**407-28.

26. **Hill, C. W., and J. A. Gray.** 1988. Effects of chromosomal inversion on cell fitness in *Escherichia coli* K-12. Genetics **119:**771-8.

27. **Hill, C. W., and B. W. Harnish.** 1981. Inversions between ribosomal RNA genes of *Escherichia coli*. Proc Natl Acad Sci U S A **78:**7069-72.

28. **Hill, C. W., and B. W. Harnish.** 1982. Transposition of a chromosomal segment bounded by redundant rRNA genes into other rRNA genes in *Escherichia coli*. J Bacteriol **149:**449-57.

29. **Holt, K. E., N. R. Thomson, J. Wain, G. C. Langridge, R. Hasan, Z. A. Bhutta, M. A. Quail, H. Norbertczak, D. Walker, M. Simmonds, B. White, N. Bason, K. Mungall, G. Dougan, and J. Parkhill.** 2009. Pseudogene accumulation in the evolutionary histories of *Salmonella enterica* serovars Paratyphi A and Typhi. BMC Genomics **10:**36.

30. **Iguchi, A., S. Iyoda, J. Terajima, H. Watanabe, and R. Osawa.** 2006. Spontaneous recombination between homologous prophage regions causes large-scale inversions within the *Escherichia coli* O157:H7 chromosome. Gene **372:**199-207.

31. **Jeong, K. S., J. Ahn, and A. B. Khodursky.** 2004. Spatial patterns of transcriptional activity in the chromosome of *Escherichia coli*. Genome Biol **5:**R86.

32. **Jumas-Bilak, E., S. Michaux-Charachon, G. Bourg, D. O'Callaghan, and M. Ramuz.** 1998. Differences in chromosome number and genome rearrangements in the genus *Brucella*. Mol Microbiol **27:**99-106.

33. **Kothapalli, S., S. Nair, S. Alokam, T. Pang, R. Khakhria, D. Woodward, W. Johnson, B. A. Stocker, K. E. Sanderson, and S. L. Liu.** 2005. Diversity of genome structure in *Salmonella enterica* serovar Typhi populations. J Bacteriol **187:**2638-50.

34. **Kuempel, P. L., J. M. Henson, L. Dircks, M. Tecklenburg, and D. F. Lim.** 1991. *dif*, a recA-independent recombination site in the terminus region of the chromosome of *Escherichia coli*. New Biol **3:**799-811.

35. **Li, J., N. H. Smith, K. Nelson, P. B. Crichton, D. C. Old, T. S. Whittam, and R. K. Selander.** 1993. Evolutionary origin and radiation of the avian-adapted non-motile salmonellae. J Med Microbiol **38:**129-39.

36. **Lindroos, H., O. Vinnere, A. Mira, D. Repsilber, K. Naslund, and S. G. Andersson.** 2006. Genome rearrangements, deletions, and amplifications in the natural population of *Bartonella henselae*. J Bacteriol **188:**7426-39.

37. **Liu, G. R., W. Q. Liu, R. N. Johnston, K. E. Sanderson, S. X. Li, and S. L. Liu.** 2006. Genome plasticity and *ori-ter* rebalancing in *Salmonella typhi*. Mol Biol Evol **23:**365-71.

38. **Liu, G. R., A. Rahn, W. Q. Liu, K. E. Sanderson, R. N. Johnston, and S. L. Liu.** 2002. The evolving genome of *Salmonella enterica* serovar Pullorum. J Bacteriol **184:**2626-33.

39. **Liu, S. L., A. Hessel, H. Y. Cheng, and K. E. Sanderson.** 1994. The *Xba*I-*Bln*I-*Ceu*I genomic cleavage map of *Salmonella paratyphi* B. J Bacteriol **176:**1014-24.

40. **Liu, S. L., and K. E. Sanderson.** 1995. The chromosome of *Salmonella paratyphi* A is inverted by recombination between *rrnH* and *rrnG*. J Bacteriol **177:**6585-92.

41. **Liu, S. L., and K. E. Sanderson.** 1996. Highly plastic chromosomal organization in *Salmonella typhi*. Proc Natl Acad Sci U S A **93:**10303-8.

42. **Liu, S. L., and K. E. Sanderson.** 1998. Homologous recombination between *rrn* operons rearranges the chromosome in host-specialized species of *Salmonella*. FEMS Microbiol Lett **164:**275-81.

43. **Liu, S. L., and K. E. Sanderson.** 1995. Rearrangements in the genome of the bacterium *Salmonella typhi*. Proc Natl Acad Sci U S A **92:**1018-22.

44. **Liu, W. Q., Y. Feng, Y. Wang, Q. H. Zou, F. Chen, J. T. Guo, Y. H. Peng, Y. Jin, Y. G. Li, S. N. Hu, R. N. Johnston, G. R. Liu, and S. L. Liu.** 2009. *Salmonella paratyphi* C: genetic divergence from *Salmonella choleraesuis* and pathogenic convergence with *Salmonella typhi*. PLoS One **4:**e4510.

45. **Liu, W. Q., G. R. Liu, J. Q. Li, G. M. Xu, D. Qi, X. Y. He, J. Deng, F. M. Zhang, R. N. Johnston, and S. L. Liu.** 2007. Diverse genome structures of *Salmonella paratyphi* C. BMC Genomics **8:**290.

46. **Louarn, J., J. Patte, and J. M. Louarn.** 1982. Suppression of *Escherichia coli dnaA46* mutations by integration of plasmid R100.1. derivatives: constraints imposed by the replication terminus. J Bacteriol **151:**657-67.

47. **Louarn, J. M., J. P. Bouche, F. Legendre, J. Louarn, and J. Patte.** 1985. Characterization and properties of very large inversions of the *E. coli* chromosome along the origin-to-terminus axis. Mol Gen Genet **201:**467-76.

48. **Mastroeni, P., A. Vazquez-Torres, F. C. Fang, Y. Xu, S. Khan, C. E. Hormaeche, and G. Dougan.** 2000. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. II. Effects on microbial proliferation and host survival *in vivo*. J Exp Med **192:**237-48.

49. **Morton, R. A., and B. R. Morton.** 2007. Separating the effects of mutation and selection in producing DNA skew in bacterial chromosomes. BMC Genomics **8:**369.

50. **Niki, H., Y. Yamaichi, and S. Hiraga.** 2000. Dynamic organization of chromosomal DNA in *Escherichia coli*. Genes Dev **14:**212-23.

51. **Olsen, J. E., M. N. Skov, J. P. Christensen, and M. Bisgaard.** 1996. Genomic lineage of *Salmonella enterica* serotype Gallinarum. J Med Microbiol **45:**413-8.

52. **Parkhill, J., G. Dougan, K. D. James, N. R. Thomson, D. Pickard, J. Wain, C. Churcher, K. L. Mungall, S. D. Bentley, M. T. Holden, M. Sebaihia, S. Baker, D. Basham, K. Brooks, T. Chillingworth, P. Connerton, A. Cronin, P. Davis, R. M. Davies, L. Dowd, N. White, J. Farrar, T. Feltwell, N. Hamlin, A. Haque, T. T. Hien, S. Holroyd, K. Jagels, A. Krogh, T. S. Larsen, S. Leather, S. Moule, P. O'Gaora, C. Parry, M. Quail, K. Rutherford, M. Simmonds, J. Skelton, K. Stevens, S. Whitehead, and B. G. Barrell.** 2001. Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. Nature **413:**848-52.

53. **Parkhill, J., M. Sebaihia, A. Preston, L. D. Murphy, N. Thomson, D. E. Harris, M. T. Holden, C. M. Churcher, S. D. Bentley, K. L. Mungall, A. M. Cerdeno-Tarraga, L. Temple, K. James, B. Harris, M. A. Quail, M. Achtman, R. Atkin, S. Baker, D. Basham, N. Bason, I. Cherevach, T. Chillingworth, M. Collins, A. Cronin, P. Davis, J. Doggett, T. Feltwell, A. Goble, N. Hamlin, H. Hauser, S. Holroyd, K. Jagels, S. Leather, S. Moule, H. Norberczak, S. O'Neil, D. Ormond, C. Price, E. Rabbinowitsch, S. Rutter, M. Sanders, D. Saunders, K. Seeger, S. Sharp, M. Simmonds, J. Skelton, R. Squares, S. Squares, K. Stevens, L. Unwin, S. Whitehead, B. G. Barrell, and D. J. Maskell.** 2003. Comparative analysis of the genome sequences of *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella bronchiseptica*. Nat Genet **35:**32-40.

54. **Parkhill, J., B. W. Wren, N. R. Thomson, R. W. Titball, M. T. Holden, M. B. Prentice, M. Sebaihia, K. D. James, C. Churcher, K. L. Mungall, S. Baker, D.**

Basham, S. D. Bentley, K. Brooks, A. M. Cerdeno-Tarraga, T. Chillingworth, A. Cronin, R. M. Davies, P. Davis, G. Dougan, T. Feltwell, N. Hamlin, S. Holroyd, K. Jagels, A. V. Karlyshev, S. Leather, S. Moule, P. C. Oyston, M. Quail, K. Rutherford, M. Simmonds, J. Skelton, K. Stevens, S. Whitehead, and B. G. Barrell. 2001. Genome sequence of *Yersinia pestis*, the causative agent of plague. Nature **413:**523-7.

55. **Petrosino, J. F., Q. Xiang, S. E. Karpathy, H. Jiang, S. Yerrapragada, Y. Liu, J. Gioia, L. Hemphill, A. Gonzalez, T. M. Raghavan, A. Uzman, G. E. Fox, S. Highlander, M. Reichard, R. J. Morton, K. D. Clinkenbeard, and G. M. Weinstock.** 2006. Chromosome rearrangement and diversification of *Francisella tularensis* revealed by the type B (OSU18) genome sequence. J Bacteriol **188:**6977-85.

56. **Pomeroy, B. S.** 1984. Fowl typhoid, p. 79-90. *In* M. S. Hofstad, Barnes, H.J., Calneck, B.W., Reid, W.M., Yoder, H.W. (ed.), Diseases of poultry, 8th ed. Iowa State University Press, Ames, IA.

57. **Popoff, M. Y., J. Bockemuhl, and L. L. Gheesling.** 2004. Supplement 2002 (no. 46) to the Kauffmann-White scheme. Res Microbiol **155:**568-70.

58. **Popoff, M. Y., L. E. Le Minor.** 2005. *Salmonella*, p. 764-799. *In* G. M. Garrity (ed.), Bergey's Manual of Systematic Bacteriology, 2 ed, vol. 2. Springer Science + Business Media, Inc., New York, NY.

59. **Schmid, M. B., and J. R. Roth.** 1987. Gene location affects expression level in *Salmonella typhimurium*. J Bacteriol **169:**2872-5.

60. **Shu, S., E. Setianingrum, L. Zhao, Z. Li, H. Xu, Y. Kawamura, and T. Ezaki.** 2000. I-*Ceu*I fragment analysis of the *Shigella* species: evidence for large-scale chromosome rearrangement in *S. dysenteriae* and S. *flexneri*. FEMS Microbiol Lett **182:**93-8.

61. **Snoeyenbos, G. H.** 1984. Pullorum disease, p. 66-79. *In* M. S. Hofstad, Barnes, H.J., Calneck, B.W., Reid, W.M., Yoder, H.W. (ed.), Diseases of Poultry, 8th ed. Iowa State University Press, Ames, IA.

62. **Song, J., A. Ware, and S. L. Liu.** 2003. Wavelet to predict bacterial *ori* and *ter*: a tendency towards a physical balance. BMC Genomics **4:**17.

63. **Sousa, C., V. de Lorenzo, and A. Cebolla.** 1997. Modulation of gene expression through chromosomal positioning in *Escherichia coli*. Microbiology **143 (Pt 6):**2071-8.

64. **Thomson, N. R., D. J. Clayton, D. Windhorst, G. Vernikos, S. Davidson, C. Churcher, M. A. Quail, M. Stevens, M. A. Jones, M. Watson, A. Barron, A. Layton, D. Pickard, R. A. Kingsley, A. Bignell, L. Clark, B. Harris, D. Ormond, Z. Abdellah, K. Brooks, I. Cherevach, T. Chillingworth, J. Woodward, H. Norberczak, A. Lord, C. Arrowsmith, K. Jagels, S. Moule, K. Mungall, M. Sanders, S. Whitehead, J. A. Chabalgoity, D. Maskell, T. Humphrey, M. Roberts, P. A. Barrow, G. Dougan, and J. Parkhill.** 2008. Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. Genome Res **18:**1624-37.

65. **Valens, M., S. Penaud, M. Rossignol, F. Cornet, and F. Boccard.** 2004. Macrodomain organization of the *Escherichia coli* chromosome. Embo J **23:**4330-41.

66. **Van Sluys, M. A., M. C. de Oliveira, C. B. Monteiro-Vitorello, C. Y. Miyaki, L. R. Furlan, L. E. Camargo, A. C. da Silva, D. H. Moon, M. A. Takita, E. G. Lemos, M. A. Machado, M. I. Ferro, F. R. da Silva, M. H. Goldman, G. H. Goldman, M. V. Lemos, H. El-Dorry, S. M. Tsai, H. Carrer, D. M. Carraro, R. C. de Oliveira, L. R. Nunes, W. J. Siqueira, L. L. Coutinho, E. T. Kimura, E. S. Ferro, R. Harakava, E. E. Kuramae, C. L. Marino, E. Giglioti, I. L. Abreu, L. M. Alves, A. M. do Amaral, G. S. Baia, S. R. Blanco, M. S. Brito, F. S. Cannavan, A. V. Celestino, A. F. da Cunha, R. C. Fenille, J. A. Ferro, E. F. Formighieri, L. T. Kishi, S. G. Leoni, A. R. Oliveira, V. E. Rosa, Jr., F. T. Sassaki, J. A. Sena, A. A. de Souza, D. Truffi, F. Tsukumo, G. M. Yanai, L. G. Zaros, E. L. Civerolo, A. J. Simpson, N. F. Almeida, Jr., J. C. Setubal, and J. P. Kitajima.** 2003. Comparative analyses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. J Bacteriol **185:**1018-26.

67. **Vazquez-Torres, A., and F. C. Fang.** 2001. Oxygen-dependent anti-*Salmonella* activity of macrophages. Trends Microbiol **9:**29-33.

68. **Vazquez-Torres, A., J. Jones-Carson, P. Mastroeni, H. Ischiropoulos, and F. C. Fang.** 2000. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages *in vitro*. J Exp Med **192:**227-36.

69. **Vazquez-Torres, A., Y. Xu, J. Jones-Carson, D. W. Holden, S. M. Lucia, M. C. Dinauer, P. Mastroeni, and F. C. Fang.** 2000. *Salmonella* pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. Science **287:**1655-8.

70. **Wu, K. Y., G. R. Liu, W. Q. Liu, A. Q. Wang, S. Zhan, K. E. Sanderson, R. N. Johnston, and S. L. Liu.** 2005. The genome of *Salmonella enterica* serovar Gallinarum: distinct insertions/deletions and rare rearrangements. J Bacteriol **187:**4720-7.

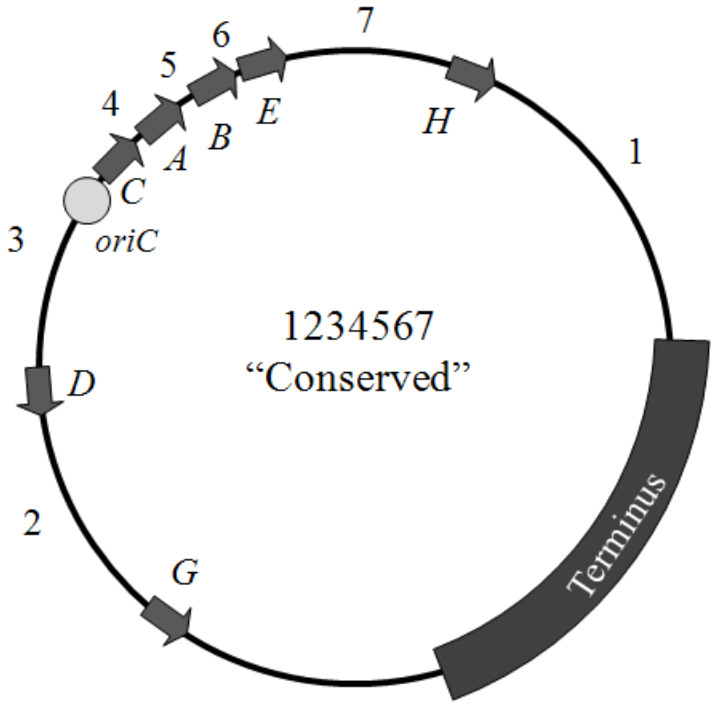

Figure 2.1. The conserved *rrn* arrangement type 1234567 found in the broad host range serovars of *Salmonella enterica*. The seven *rrn* operons are lettered while the regions in between the operons are numbered.

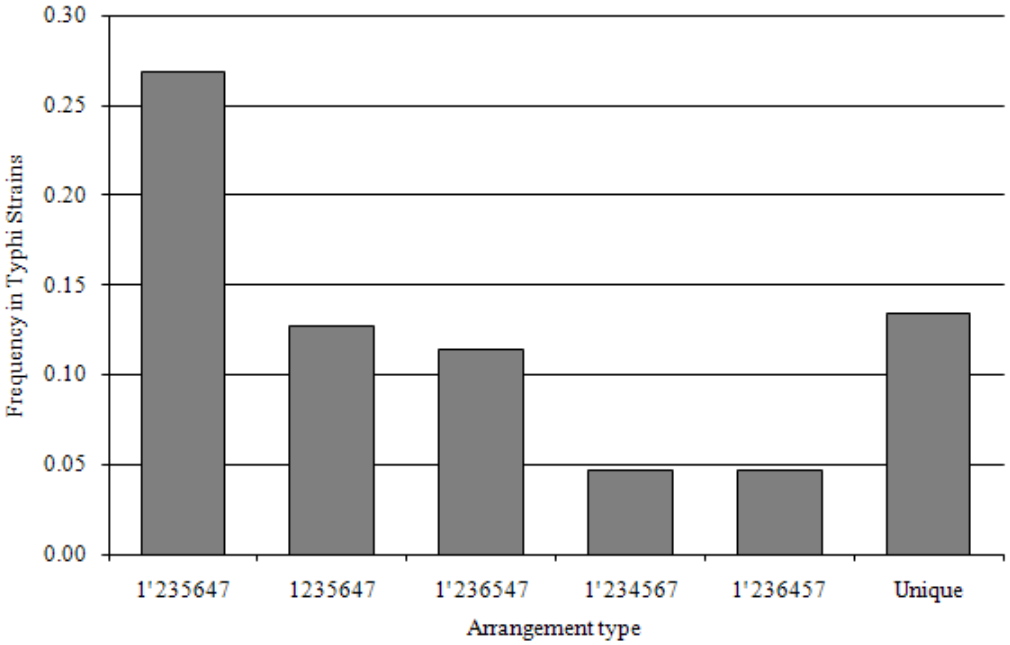

Figure 2.2. Frequency of most common and unique arrangement types in of *Salmonella enterica* sv. Typhi. Data compiled from ~140 Typhi strains. 1' indicates that region 1 is in the inverted orientation.

Table 2.1. *Salmonella enterica* sv. Gallinarum strains used in this study.

Strain	Alias	Biovar	Source[a-e]
TYT3313	RKS 4994	Gallinarum	SGSC
TYT3314	RKS 5079	Pullorum	SGSC
TYT3315	RKS 5021	Gallinarum	SGSC
TYT3316	SA 4404	Gallinarum	SGSC
TYT3325	SA 1684	Gallinarum	SGSC
TYT3326	SA 1685	Pullorum	SGSC
TYT3328	SA 1687	Pullorum	SGSC
TYT3329	SA 1688	Pullorum	SGSC
TYT3331	SA 1689	Pullorum	SGSC
TYT3335	TK619 ISM 1357	Gallinarum	ISU
TYT3339	X3796	Gallinarum	WU
TYT3340	X3544	Pullorum	WU
TYT3341	X3799	Pullorum	WU
TYT3342	X3539	Pullorum	WU
TYT3343	SEPRL #99	Pullorum	SEPRL
TYT3345	SEPRL #92	Pullorum	SEPRL
TYT3349	JEO 1911	Gallinarum	CGM
TYT3350	JEO 1909	Gallinarum	CGM
TYT3352	JEO 2555	Pullorum	CGM
TYT3353	JEO 2600 R9	Pullorum	CGM
TYT3354	JEO 2617	Pullorum	CGM
TYT3355	JEO 2614	Pullorum	CGM

[a] Salmonella Genetic Stock Center, University of Calgary, Calgary, Alberta, Canada
[b] Iowa State University, Ames, IA
[c] Washington University, Saint Louis, MO
[d] US Department of Agriculture, Southeast Poultry Research Laboratory, Athens, GA
[e] Centre de Génétique Moléculaire, Gif-sur-Yvette Cedex, France

Table 2.2. Chromosomal arrangement types of Gallinarum serovar strains.

Gallinarum Biovar		Pullorum Biovar	
Strain	**Arrangement Type**	**Strain**	**Arrangement Type**
TYT3313	1234657	TYT3314	1’234657
TYT3315	1’253467	TYT3326	1735462
TYT3316	1’34657 + 2	TYT3328	1735462
TYT3325	1267354	TYT3329	1735462
TYT3335	1?753246	TYT3331	1’263547
TYT3339	1263457	TYT3340	1736542
TYT3349	1234567	TYT3341	1735462
TYT3350	1234567	TYT3342	1234657
		TYT3343	1735462
		TYT3345	17354462
		TYT3352	1’756342
		TYT3353	1735462
		TYT3354	1267534
		TYT3355	1735462

Table 2.3. Size of regions between *rrn* operons of sequenced *Salmonella* strains.

	Region size in basepairs							
Serovar	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Total**
Typhimurium LT2	2504388	770324	535983	96088	155071	43424	752154	4857432
Choleraesuis	2533708	707552	508771	95087	155208	42757	712617	4755700
Typhi CT18	2430061	705901	508423	134412	149001	42215	839024	4809037
Typhi Ty2	2397285	716333	515429	134577	148993	42055	837289	4791961
Paratyphi A: CP000026	2326185	758888	496761	99133	150592	42103	711567	4585229
Paratyphi A: FM200053	2321680	759028	496762	99139	150595	42104	712489	4581797
Paratyphi B	2525347	715196	514103	96762	151034	43014	813431	4858887
Paratyphi C	2515255	704163	527042	94942	155197	42761	793720	4833080
Gallinarum	2453569	686501	516254	94715	159261	43323	705074	4658697
Arizonae	2404145	709018	456966	93506	140141	45956	751068	4600800
Enteritidis	2459619	699795	516880	94413	158527	43454	713160	4685848
Agona	2416162	772925	529816	97977	154169	42905	784706	4798660
Dublin	2590573	732200	515919	94350	159145	42915	707806	4842908
Heidelburg	2512927	776445	523741	94412	143500	42144	795599	4888768
Newport	2512812	749841	512410	94381	181958	42061	734178	4827641
Schwarzengrund	2462704	715597	506261	98279	143900	42341	739993	4709075
Average:	**2460401**	**729982**	**511345**	**100761**	**153518**	**42846**	**756492**	**4755345**
Standard deviation:	74977	29790	18024	13293	9462	971	47527	104318

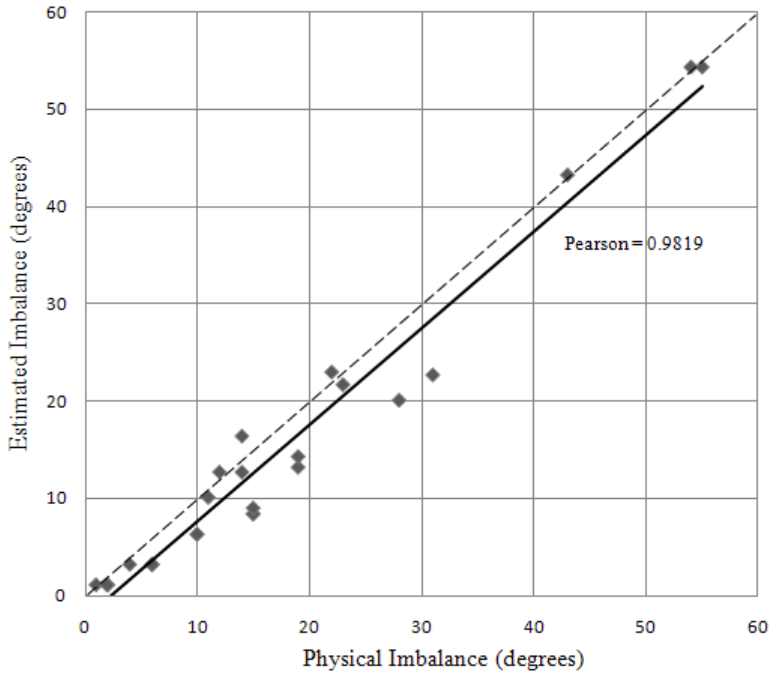

Figure 2.3. Estimated vs. physical replichore balance. While the calculator slightly underestimated replichore balance (dashed line=perfect fit), the estimates were statistically the same.

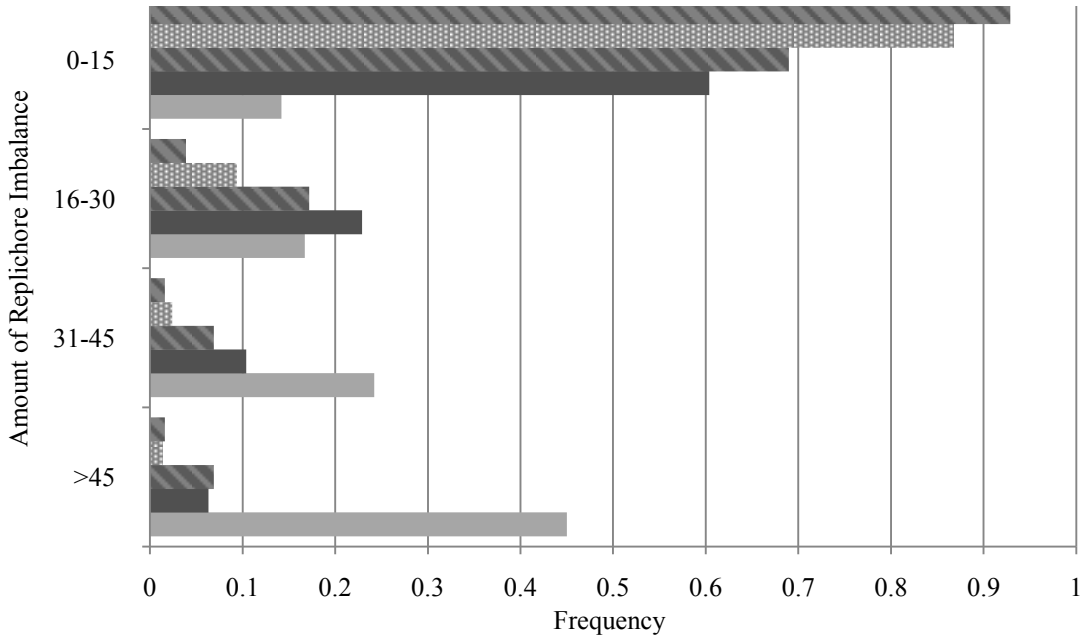

Figure 2.4. Physical and estimated replichore imbalance frequencies. The physical ( ) and estimated ( ) replichore imbalance of host-specific strains, genome types ( ), natural arrangement types ( ), and theoretical arrangement types ( ) were divided into four groups, each with increasing 15° of imbalance.

Table 2.4. Rearrangement groups of naturally-occurring arrangement types.

Rearrangement Group	Type of Rearrangement	# of Arrangement Types	# of Strains	Range of Estimated Replichore Imbalance
1	Intrareplichore translocation	4	42	0.2°
2	Intra- & interreplichore translocations	10	19	3.4-60.7°
3	G/H inversion & intrareplichore translocation	6	83	11.6°
4	G/H inversion & interreplichore translocation	4	4	22.5-34.1°
5	G/H inversion, intra- & interreplichore translocations	6	8	19.2-43.7°
6	D/E inversion	1	3	13.6°
7	D/E inversion & intrareplichore translocation	3	7	13.6°
8	D/E inversion & interreplichore translocation	3	11	21.2-28.5°
9	D/E inversion, intra- & interreplichore translocations	1	1	16.8°
10	Double inversion & intrareplichore translocation	3	22	7.8-17.1°
11	Double inversion & interreplichore translocation	6	7	5.4-21.5°

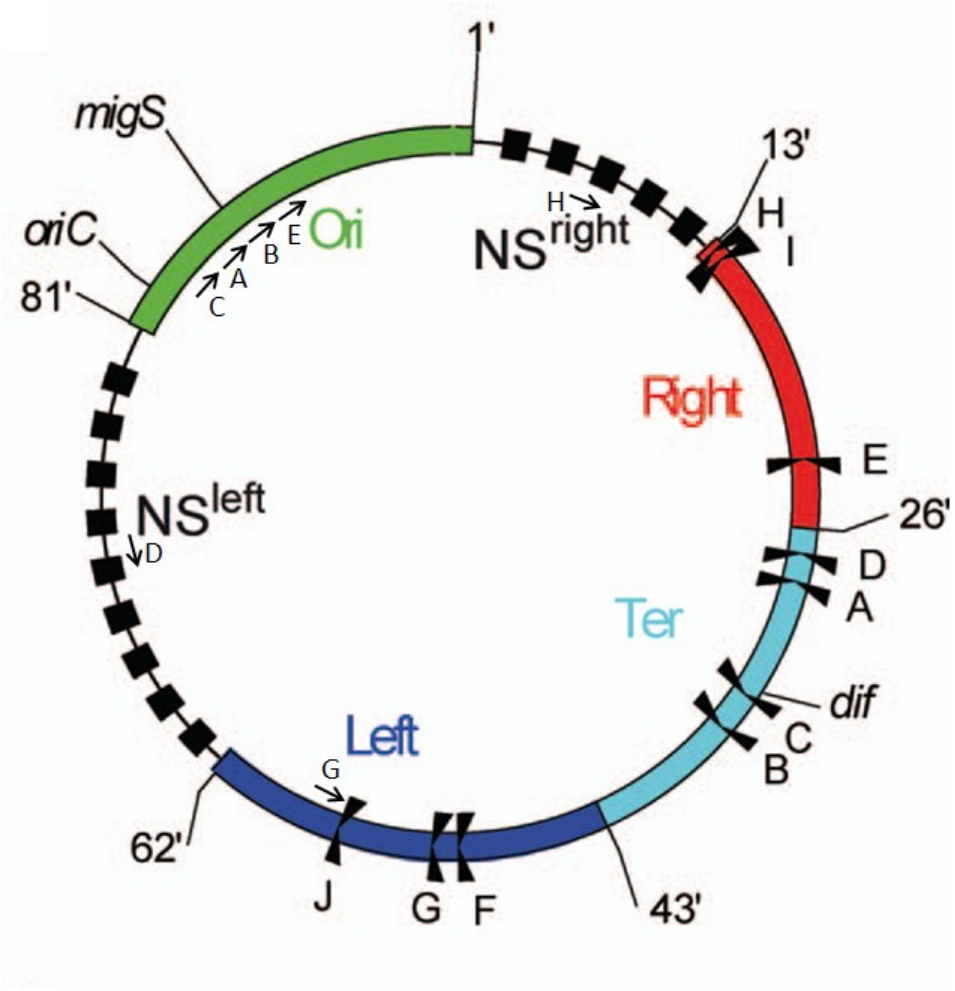

Figure 2.5. Map of *E. coli* chromosome showing location of macrodomain and non-structural regions, *ter* sites, and *rrn* operons. Figure modified from (16).

Chapter 3. Effects of Replichore Imbalance Introduced by Chromosomal Duplications on Fitness

Introduction

The bacterial chromosome is a dynamic molecule that undergoes various types of changes during the course of evolution. Mutations can alter the expression or function of a protein by changing regulatory or coding sequences. New genes can be inserted into the chromosome by horizontal gene transfer while others are lost through deletion. Regions of the chromosome can also undergo various types of rearrangements such as inversions, translocations, and duplications. These rearrangements alter the gene order and may perturb various aspects of chromosomal structure such as replichore balance. Replichores are defined as the halves of the chromosome having a leading strand G/C skew between the origin of replication and the terminus (4). Chromosomal rearrangements can make one replichore longer than the other resulting in imbalanced DNA replication, which may affect fitness.

When DNA replication is balanced, the replication forks travelling along each replichore collide in the Terminus region of the chromosome, approximately 180° across from the origin of replication. Within the Terminus region are polarized sequences termed *ter* sites. These *ter* sites have been studied for over 30 years, especially in *Escherichia coli* and *Bacillus subtilis*, and function by binding a replication terminator protein (Tus in *E. coli* and RTP in *B. subtilis*) that stalls replication forks coming from one direction but not the other (reviewed in (6)). The *ter* sites are aligned so that replication forks passing through the *dif* site and into the other replichore are blocked. Most replication forks are thought to collide within a replication fork trap formed by the

ter sites immediately flanking the *dif* site. In *E. coli* and *Salmonella enterica*, the size of the trap is 4-5% of the total size of the chromosome, or 269 kbp and 197 kbp respectively. Changes in replichore length beyond the size of the trap cause imbalanced replication that could affect the growth rate of the cells.

Various studies over the years have investigated the affect of imbalanced DNA replication on fitness in bacteria. Initial studies utilized *E. coli* strains with a temperature-sensitive mutation in *dnaA* that was suppressed by the integration of a plasmid into specific sites on the chromosome (18). As plasmids were inserted closer to the terminus, strains became rich medium sensitive at the non-permissive temperature and appeared to die from under initiation of DNA synthesis relative to mass increase. Interestingly, the authors concluded that this suggested the terminus had a silencing effect on the firing of the plasmid-derived origin in contrast to an effect due to imbalanced replication. In fact this study showed that some replication forks could eventually bypass the trap, and start replicating around the other replichore towards the origin. However, subsequent work showed that large asymmetrical inversions around the *ori-Ter* axis from recombination between two inverted IS5 elements could correct the rich medium sensitivity at the non-permissive temperature (19). Strains carrying these inversions instead became sensitive at the permissive temperature, which suggested that reorienting the *ter* sites relative to the origin being utilized was responsible for the observed deregulation between DNA and cell mass synthesis.

Recent work examined *E. coli* strains that contained asymmetrical interreplichore inversions (9). The strains were constructed using a λ-based site-specific recombination system, and the inversions introduced varying amounts of replichore imbalance

depending on the chromosomal location of inserted *att* sites. Growth defects were observed in strains when imbalance was at least 50° but were negligible up to 36° imbalance. In strains with an imbalance of greater than 70°, RecBC and RecA were required for viability. However the RecA requirement was suppressed in *tus* mutants, suggesting that replication forks stopped at *ter* sites were responsible for the observed lethality. While these studies have demonstrated that strains with imbalanced replichores are selected against, the methods used to introduce imbalance are not natural.

Rearrangements from recombination between the ribosomal RNA-encoding *rrn* operons occur frequently in culture, occur in natural populations, and can invert large regions of the chromosome. A subline of *E. coli* K-12, starting with W2637 and including the common lab strains W3110 and W3102, contain a natural inversion by recombination between the *rrnD* and *rrnE* operons (13). This particular inversion increases the amount of replichore imbalance from 6.5° to almost 16°. While strains carrying this inversion did not appear to have a growth defect, revertants to the non-inverted orientation occurred at high frequency in culture and rapidly overgrew the parental strain carrying the inversion (12). Furthermore a strain constructed to contain an asymmetric inversion between *rrnE* and *rrnG*, which would introduce significant imbalance, suffered from severe growth defects (12). The fitness loss associated with such asymmetric inversions may also explain the observation that many naturally-occurring inversions are symmetric (7).

Horizontal transfer of large DNA fragments can also cause imbalance by increasing the length of one replichore relative to the other. However the fitness cost from replichore imbalance introduced by horizontal gene transfer has not been studied and is confounded by the fitness cost or benefit of the transferred genes themselves. It has

been hypothesized that the imbalance introduced by the transfer of large pieces of DNA such as pathogenicity islands or prophages drive the chromosomal rearrangements found in strains of host-specific *S. enterica* serovars in attempts to reestablish balance (15-17). While most host specific strains that have been analyzed have balanced replichores (15, 20), which supports the idea that the rearrangements improve balance, another possibility is that strains with imbalanced replichores are less fit and lost from the population.

Duplications are another type of chromosomal rearrangement that can affect replichore balance and form when unequal recombination occurs between two direct repeats. Duplications play important evolutionary roles, as they can increase gene copy number, as well as being a source for new genes. Typically duplications occur at frequencies between 10^{-3} and 10^{-5} (3), but can be as high as 10^{-2} when the endpoints are within *rrn* operons (2). Duplications can also collapse at frequencies similar to their formation, and are lost from a population if they do not provide a selective advantage. However the effect of duplications on fitness in relation to replichore balance has not been investigated.

A major hurdle in studying the effects of duplications on fitness is that if the duplication is detrimental, collapsed revertants will outgrow the parental strain with the duplication and selectively sweep through the population. To circumvent this problem, a set of 11 isogenic *Salmonella enterica* strains containing transposon-held duplications were utilized (5). The strains were constructed by P22-mediated double-lysate transduction using lysates grown on auxotrophic donors containing a Mu*d*P or Mu*d*Q element conferring chloramphenicol resistance in the desired endpoints of the duplication. By selecting for prototrophy and resistance to chloramphenicol, strains

containing the desired duplication with the Mu*d* element at the joint point of the duplicated region were obtained. The sizes of the duplications varied between 146-729 kbp (Table 3.1), and were located around the chromosome except for the terminus region (Figure 3.1). Culturing these strains in the presence of chloramphenicol "locks" the duplications in place because if the duplications collapse, the Mu*d* element is lost and the cells become chloramphenicol sensitive.

The relative fitness of these strains was investigated by growth curve analysis and a mixed culture assay using an isogenic competitor strain. Since the size of the duplications in these strains varies, so does the amount of introduced replichore imbalance. The smallest duplication introduces 5° imbalance whereas the largest duplication introduces 23° imbalance. As duplications of similar size are located around the chromosome, fitness effects due to the location of the duplication versus introduced imbalance can also be discerned.

Materials and Methods

Bacterial Strains and Culture Conditions. Bacterial strains of *S. enterica* serovar Typhimurium and their sources are shown in Table 3.1. Strains were maintained in 5 M glycerol at -70°C, and single colony isolates were used to inoculate broth cultures. The strains were grown in either E medium (23) supplemented with 0.2% glucose (Minimal), Luria-Bertani (LB) medium, or LB supplemented with 1X E-salts and 0.2% glucose (LBEDO). Media were supplemented with chloramphenicol (20 mg/ml) and/or X-gal (40 mg/ml) as required. Solid media contained 1.5% agar.

Growth Curves. Strains were grown in triplicate at 37°C with aeration. Readings were taken at 600 nm every 15-30 minutes on a Spec20 spectrophotometer. Timepoints in early exponential phase were used to calculate the generation time using the following formula:

$$= 1/((\log(OD_{600} \text{ at } t_2) - \log(OD_{600} \text{ at } t_1))/(0.301 \text{ X } (t_2-t_1))$$

Competitor Strain Construction. The competitor strain was constructed by moving *rrfH*::pCE36 from TYT4480 into MST1529 (*srl203*::Tn*10d*(Cam)) using P22 HT *int* transduction. pCE36 confers resistance to kanamycin and contains a promoterless *lacZ* gene (8) driven by the *rrnH* promoter in this strain. The Tn*10d*(Cam) marker confers chloramphenicol resistance. Both markers were then back-crossed in to LT2 resulting in MST5198.

Mixed culture assay. Strains were grown to saturation overnight, and 10^{-4} dilutions were used to inoculate mixed cultures containing one of the duplication-bearing strains or the wild-type strain and the competitor strain. Samples were taken at t=0 (input) and other timepoints as appropriate (output), diluted, and spot-plated in triplicate onto LB plates supplemented with X-gal and chloramphenicol. The wild-type and duplication-bearing strains formed white colonies, while the competitor strain formed blue colonies in the presence of X-gal. After counting colony forming units (CFUs), the competition index (C. I.) was determined as follows:

$$\text{C. I.} = \frac{(\text{\# of output white CFUs}/\text{\# of output blue CFUs})}{(\text{\# of input white CFUs}/\text{\# of input blue CFUs})}$$

Results

Growth curves. The generation times of the duplication-bearing strains and LT2 were determined in minimal medium, LB, and LBEDO (Figure 3.2). In minimal medium, most strains had generation times of around 40 minutes, except for MST3813 and MST3823, which had generation times over 60 minutes, and MST3819 with a generation time slightly over 50 minutes. The average generation time for LT2 in LB was 22 minutes, while all the duplication-bearing strains had slightly longer generation times of up to 30 minutes. MST3819 and MST3823 had even longer generation times of 35 minutes. When grown in LBEDO, all strains had generation times of 11-15 minutes. Observed differences in growth rate were not due to lower viability of MST3819 or MST3823, as 0 hr plate counts for these strains were similar to 0 hr counts of MST3815, MST3828, and MST1. No correlation between duplication size and increased generation time was observed in the three media.

Mixed culture assays. Competition indices were obtained from the mixed culture assays containing the competitor strain MST5198 and either the wild-type or one of the duplication-bearing strains, and compared to determine if duplication size affected the ability of a strain to compete. While data is shown for 6 and 12 hours, readings were taken at various other time points up to one week. The results showed no correlation between the duplication size and the competition index (Figure 3.3). Most duplication-bearing strains competed as well or slightly better than the wild-type against the competitor strain. Two exceptions were MST3819 and MST3823, both of which competed significantly more poorly against the competitor strain than the wild-type strain

($p<0.05$; student's t-test). While both of these strains contain the largest duplications, the size of the duplication in MST3819 was close to the size of the duplications in MST3817 and MST3818. These two strains were either similar or slightly better competitors than the wild-type, demonstrating that the size of the duplications in these strains did not affect fitness.

The MST5198 competitor strain also did not compete as well as expected against the wild-type strain. Both strains are in a LT2 background and the markers in the competitor strain were not expected to affect fitness under the experimental conditions used.

Duplication collapse. To confirm that the duplications in MST3819 and MST3823 were responsible for the observed growth defects, isolates of each strain with collapsed duplications were obtained by growing cultures of each strain without chloramphenicol and screening for chloramphenicol-sensitive colonies. The loss of the duplication from either strain restored wild-type growth (Figure 3.4). The frequency of chloramphenicol resistant cells in cultures of MST3818 and MST3819 grown without chloramphenicol was also determined (Figure 3.5). As these two strains have duplications of similar size, the collapse rate should be similar. While the fraction of chloramphenicol resistant MST3818 cells were maintained or increased during growth, the fraction of chloramphenicol resistant MST3819 cells was less than 0.1 by the time the culture was in exponential growth. Only 2-3% of MST3819 cells were chloramphenicol resistant after the culture reached stationary phase.

Discussion

The effect on fitness from the amount of replichore imbalance introduced by transposon-held duplications of various sizes was measured in a set up *S. enterica* strains by performing growth curve analyses in various media and mixed culture assays. The sizes of the duplications were in the range of naturally-occurring duplications as well as pathogenicity islands and phages commonly transferred horizontally between *S. enterica* strains. It has been hypothesized that the amount of imbalance introduced by these types of events is driving the chromosomal rearrangements observed in host-specific *S. enterica* serovars back to a more balanced state (15-17). One prediction of this hypothesis is that most host-specific strains with rearrangements have balanced replichores, and this appears to be the case (15). However, another explanation for this observation is that naturally-occurring strains with imbalanced replichores are less fit and lost from the population. By determining the relative fitness of these strains, this study addresses that possibility.

The growth rates of all the strains varied depending on the nutritional content of the medium the strains were grown in. If fitness effects from replichore imbalance were substantial, a decrease in growth rates as duplication size increased would be predicted, and would be more pronounced as the nutritional value of the medium increased. However, as duplications increased in size, lower growth rates were not observed even in the richest medium. Some strains did have growth defects independent of the size of the duplication. MST3813 had a significantly longer generation time than the wild-type in minimal and LB medium, but had the same generation time in LBEDO. Both MST3819 and MST3823 generally had longer generation times regardless of the medium used.

The results of the mixed culture assays demonstrated that duplication location, not size, affected the ability of a strain to compete for resources. Only MST3819 and MST3823 consistently competed poorly. While these strains carry the largest duplications, the duplication in MST3819 is similar in size to the duplications in MST3817 and MST3818. This suggests that the location or content of the duplication in MST3819 is responsible for the growth defect. The duplicated region in MST3819 lies next to the origin of replication and contains 3 of the *rrn* operons as well as genes encoding various ribosomal proteins. These genes are tightly regulated and can be expressed at high levels. Furthermore, their chromosomal position subjects them to gene dosage effects. By increasing the copy number of these genes, the duplication may be disturbing their regulation resulting in the growth defects.

Recent work has suggested that the *E.coli* chromosome is structurally divided into macrodomains, and intermingling of these macrodomains can be detrimental to fitness (9, 10, 21, 22). Since *E. coli* and *S. enterica* are closely related and share many aspects of chromosome structure, the *Salmonella* chromosome is also expected to have macrodomains. The duplication in MST3819 contains the majority of the Ori macrodomain, including *migS*, the putative bacterial centromere (24). A deficiency in DNA segregation could also explain the growth defect, and microscopic analysis of this strain did reveal filamented cells with diffuse DNA. Surprisingly MST3818, the strain carrying a duplication of the origin of replication, did not have any growth defects and competed well in mixed culture assays.

MST3823 contained the largest duplication, which introduced over 23° of replichore imbalance. This strain consistently grew slower, more due to having a longer

lag phase than a slower growth rate, and competed poorly. While replichore imbalance may be affecting the fitness of this strain, the duplication location can also be affecting fitness. The Right macrodomain is duplicated in this strain, including some flanking non-structural and Ter macrodomain sequences. Previous studies have shown that inversions between the Right and Ter macrodomains can be detrimental to fitness by perturbing chromosome structure and segregation (9, 11). It is possible that a large duplication in this region also has an affect on chromosome structure.

In conclusion, the results of this study show that up to 16° of replichore imbalance introduced by transposon-held duplications in the chromosome does not have a measurable affect on the fitness of *S. enterica*. A duplication that introduced 23° of replichore imbalance did have a growth defect, but whether the defect is from replichore imbalance or the gene content of the duplication could not be distinguished. This suggests that fitness effects from naturally-occurring duplications as well as horizontally-transferred genes are most likely not due to the replichore imbalance introduced by these events, but due to gene content and chromosomal location.

References

1. **Altman, E., Roth, J. R., Hessel, A., and K. E. Sanderson.** 1996. Transposons currently in use in genetic analysis of *Salmonella* species, p. 2613-2626. *In* F. C. Neidhardt, Curtis III, R., Ingraham, J. L., Lin, E.C.C., Low, K. B., Magasanik, B., Reznikoff, W.S., Riley, M., Schaechter, M., and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella* Cellular and Molecular Biology. ASM Press, Washington, D.C.

2. **Anderson, P., and J. Roth.** 1981. Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (*rrn*) cistrons. Proc Natl Acad Sci U S A **78:**3113-7.

3. **Anderson, R. P., and J. R. Roth.** 1979. Gene duplication in bacteria: alteration of gene dosage by sister-chromosome exchanges. Cold Spring Harb Symp Quant Biol **43 Pt 2:**1083-7.

4. **Blattner, F. R., G. Plunkett, 3rd, C. A. Bloch, N. T. Perna, V. Burland, M. Riley, J. Collado-Vides, J. D. Glasner, C. K. Rode, G. F. Mayhew, J. Gregor, N. W. Davis, H. A. Kirkpatrick, M. A. Goeden, D. J. Rose, B. Mau, and Y. Shao.** 1997. The complete genome sequence of *Escherichia coli* K-12. Science **277:**1453-62.

5. **Camacho, E. M., and J. Casadesus.** 2001. Genetic mapping by duplication segregation in *Salmonella enterica*. Genetics **157:**491-502.

6. **Duggin, I. G., R. G. Wake, S. D. Bell, and T. M. Hill.** 2008. The replication fork trap and termination of chromosome replication. Mol Microbiol **70:**1323-33.

7. **Eisen, J. A., J. F. Heidelberg, O. White, and S. L. Salzberg.** 2000. Evidence for symmetric chromosomal inversions around the replication origin in bacteria. Genome Biol **1:**RESEARCH0011.

8. **Ellermeier, C. D., A. Janakiraman, and J. M. Slauch.** 2002. Construction of targeted single copy *lac* fusions using lambda Red and FLP-mediated site-specific recombination in bacteria. Gene **290:**153-61.

9. **Esnault, E., M. Valens, O. Espeli, and F. Boccard.** 2007. Chromosome structuring limits genome plasticity in *Escherichia coli*. PLoS Genet **3:**e226.

10. **Espeli, O., R. Mercier, and F. Boccard.** 2008. DNA dynamics vary according to macrodomain topography in the *E. coli* chromosome. Mol Microbiol **68:**1418-27.

11. **Guijo, M. I., J. Patte, M. del Mar Campos, J. M. Louarn, and J. E. Rebollo.** 2001. Localized remodeling of the *Escherichia coli* chromosome: the patchwork of segments refractory and tolerant to inversion near the replication terminus. Genetics **157:**1413-23.

12. **Hill, C. W., and J. A. Gray.** 1988. Effects of chromosomal inversion on cell fitness in *Escherichia coli* K-12. Genetics **119:**771-8.

13. **Hill, C. W., and B. W. Harnish.** 1981. Inversions between ribosomal RNA genes of *Escherichia coli*. Proc Natl Acad Sci U S A **78:**7069-72.

14. **Lilleengen, K.** 1948. Typing of *Salmonella typhimurium* by means of bacteriophage. Acta Pathol Microbiol Scand Suppl **77:**11-125.

15. **Liu, G. R., W. Q. Liu, R. N. Johnston, K. E. Sanderson, S. X. Li, and S. L. Liu.** 2006. Genome plasticity and *ori-ter* rebalancing in *Salmonella typhi*. Mol Biol Evol **23:**365-71.

16. **Liu, G. R., A. Rahn, W. Q. Liu, K. E. Sanderson, R. N. Johnston, and S. L. Liu.** 2002. The evolving genome of *Salmonella enterica* serovar Pullorum. J Bacteriol **184:**2626-33.

17. **Liu, S. L., and K. E. Sanderson.** 1996. Highly plastic chromosomal organization in *Salmonella typhi*. Proc Natl Acad Sci U S A **93:**10303-8.

18. **Louarn, J., J. Patte, and J. M. Louarn.** 1982. Suppression of *Escherichia coli dnaA46* mutations by integration of plasmid R100.1 derivatives: constraints imposed by the replication terminus. J Bacteriol **151:**657-67.

19. **Louarn, J. M., J. P. Bouche, F. Legendre, J. Louarn, and J. Patte.** 1985. Characterization and properties of very large inversions of the *E. coli* chromosome along the origin-to-terminus axis. Mol Gen Genet **201:**467-76.

20. **Morton, R. A., and B. R. Morton.** 2007. Separating the effects of mutation and selection in producing DNA skew in bacterial chromosomes. BMC Genomics **8:**369.

21. **Niki, H., Y. Yamaichi, and S. Hiraga.** 2000. Dynamic organization of chromosomal DNA in *Escherichia coli*. Genes Dev **14:**212-23.

22. **Valens, M., S. Penaud, M. Rossignol, F. Cornet, and F. Boccard.** 2004. Macrodomain organization of the *Escherichia coli* chromosome. Embo J **23:**4330-41.

23. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J Biol Chem **218:**97-106.

24. **Yamaichi, Y., and H. Niki.** 2004. *migS*, a cis-acting site that affects bipolar positioning of *oriC* on the *Escherichia coli* chromosome. Embo J **23:**221-33.

Table 3.1. Genotype, duplication size, introduced replichore imbalance, and source of *S. enterica* strains used in this study.

Strain	Alias	Genotype	Duplication Location (min)	Duplication Size (kbp)	Replichore Imbalance	Source
MST1	LT2	Wild-type	---	---	1.9°	(13)
MST3813	SV4200	Dup [*trp248**Mu*d*P**hisD9953*]	38-44	332.1	11.5°	(5)
MST3814	SV3193	Dup [*hisH9962**Mu*d*P**cysA1586*]	44-53	399.7	13.7°	(5)
MST3815	SV4015	Dup [*cysA1586**Mu*d*P**purG2149*]	53-56	158.4	5.7°	(5)
MST3816	SV4193	Dup [*purG2149**Mu*d*P**argA9001*]	56-64	430.7	14.7°	(5)
MST3817	SV4194	Dup [*argA9000**Mu*d*P**cysG1573*]	64-75	484.6	16.3°	(5)
MST3818	SV1601	Dup [*cysG1573**Mu*d*P**ilvA2642*]	75-85	486.4	16.4°	(5)
MST3819	SV4195	Dup [*ilvA2648**Mu*d*P**purA1881*]	85-95	495.3	16.7°	(5)
MST3820	SV4142	Dup [*purA1881**Mu*d*P**thr469*]	95-0	248.7	8.8°	(5)
MST3821	SV1604	Dup [*thr469**Mu*d*P**proA692*]	0-8	367.1	12.6°	(5)
MST3822	SV1603	Dup [*proA692**Mu*d*Q**purE2164*]	8-12	230.2	8.1°	(5)
MST3823	SV1611	Dup [*purE2514**Mu*d*P**purB1879*]	12-27	723.2	23.3°	(5)
MST1529	TT11183	*srl203*::Tn*10d*(Cam)	---	---	---	(1)
TYT4480	---	*rrfH*::pCE36	---	---	---	This work
MST5198	---	*rrfH*::pCE36 *srl-203*::Tn*10d*(Cam)	---	---	---	This work

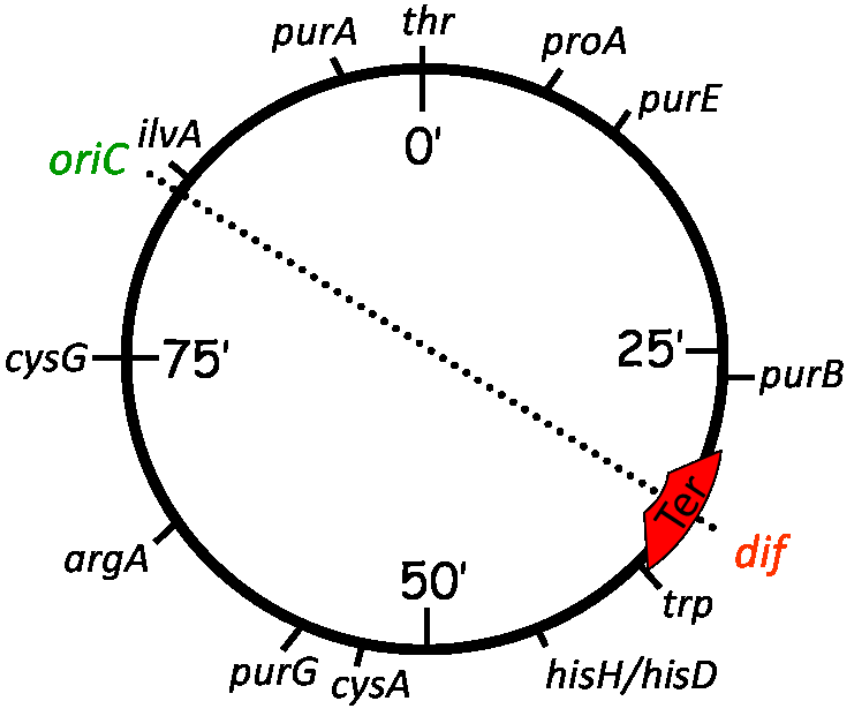

Figure 3.1. Genetic map of *S. enterica* serovar Tyhpimurium LT2 showing genes used as endpoints in constructing transposon-held duplications of the regions between each of the genes. Balanced replichores are indicated by the symmetry of the *oriC*-Ter axis. Duplications increase the length of one replichore relative to the other, imbalancing axis symmetry.

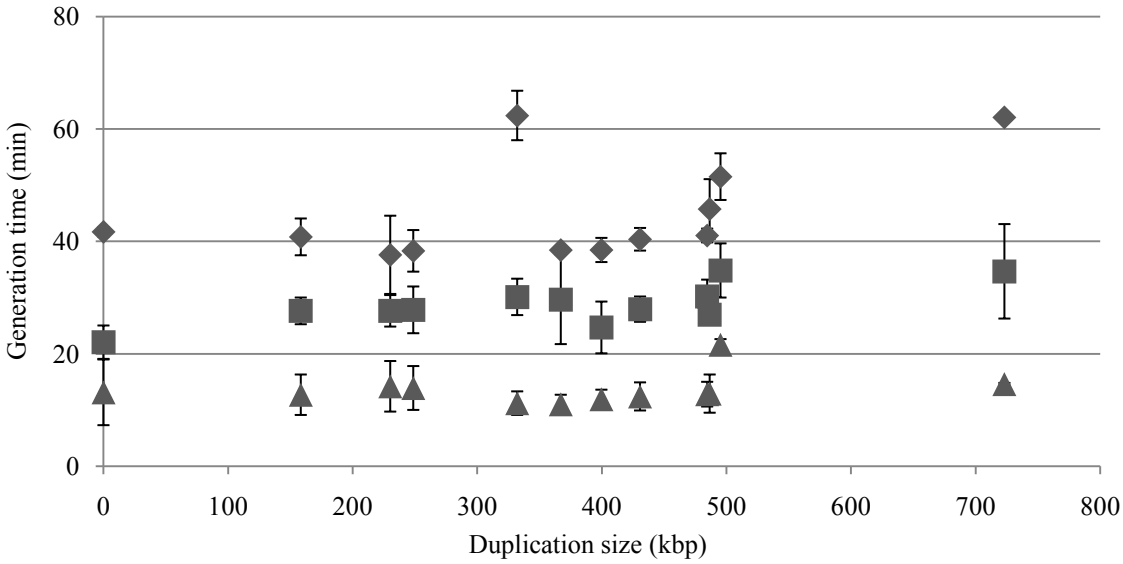

Figure 3.2. Generation times of strains as a function of duplication size. Strains were grown in either Minimal (◆), LB (■), or LBEDO (▲) media aerobically at 37°C. Error bars=standard deviation.

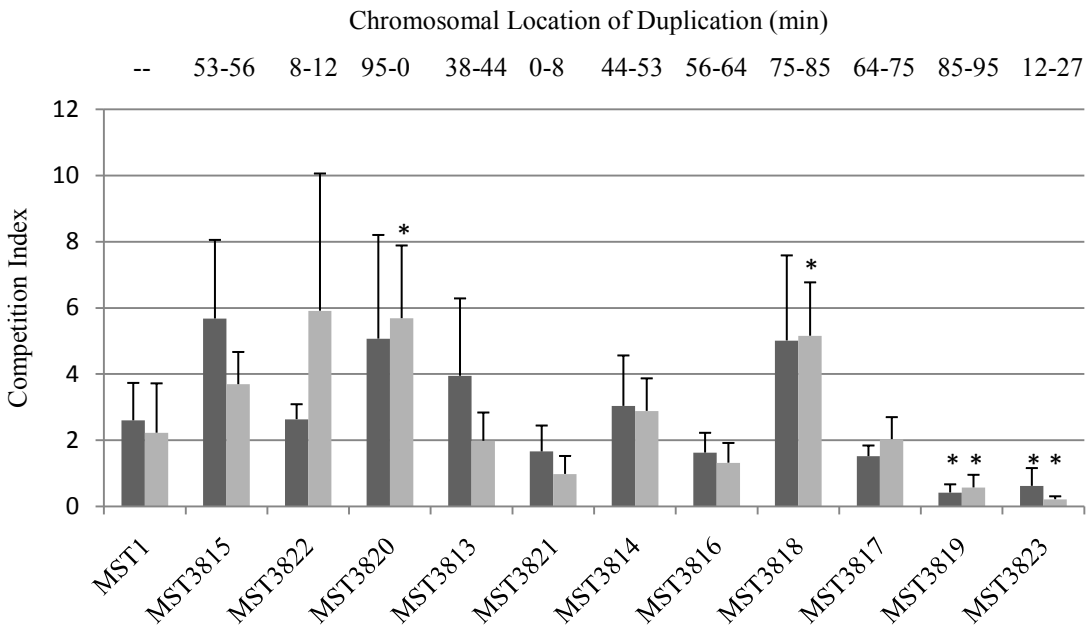

Figure 3.3. Competition Indices of the wild-type and duplication-bearing strains after 6 (■) and 12 hrs (■) of growth in mixed culture with MST5198 (n=6). Strains are in order of increasing duplication size, with location of duplication indicated. Indices statistically different (p<0.05) than the wild-type strain (MST1) are marked (*). Error bars= standard deviation.

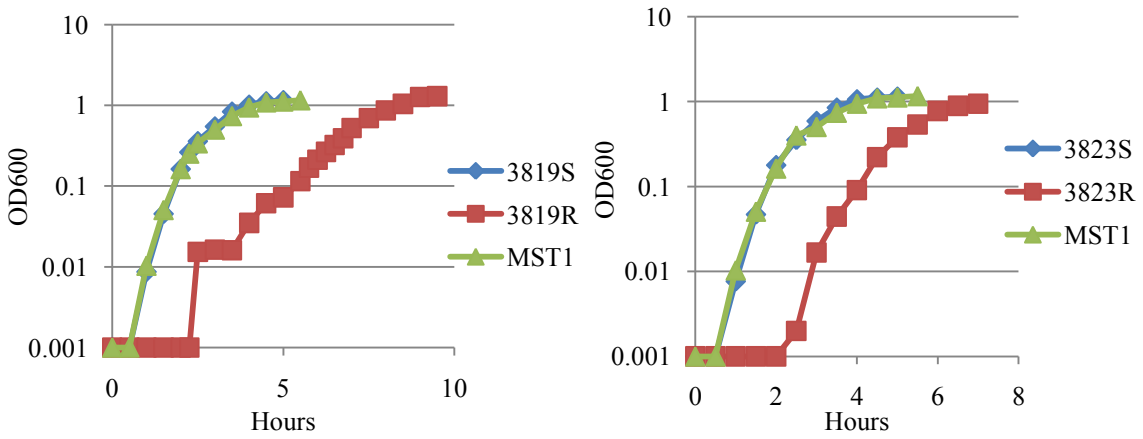

Figure 3.4. Growth curves of chloramphenicol-resistant (R) and chloramphenicol-sensitive (S) isolates of MST3819 and MST3823 compared to MST1. The cultures were grown aerobically in LB at 37°C, with cultures of chloramphenicol-resistant isolates supplemented with 20 µg/ml chloramphenicol.

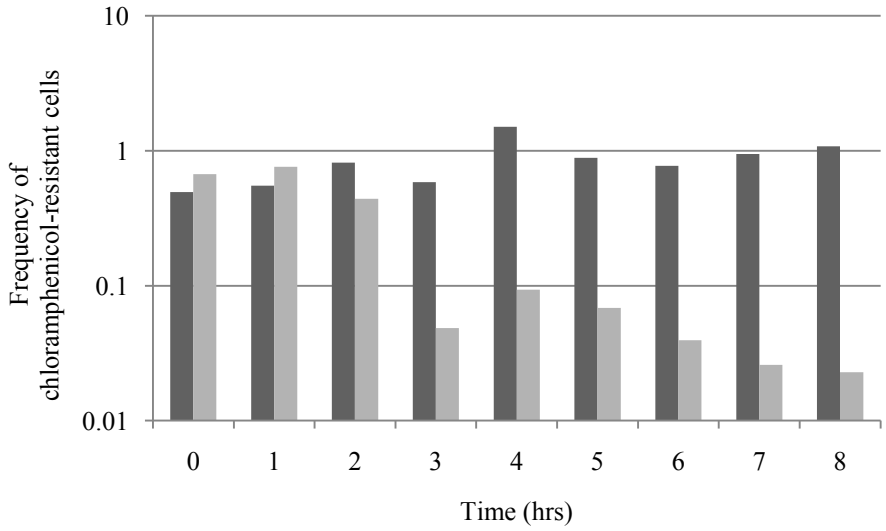

Figure 3.5. Frequency of chloramphenicol-resistant cells over time in cultures of MST3818 (■) and MST3819 (■) grown aerobically in LB at 37°C. Samples were taken hourly, diluted, and plated onto either LB or LB+chloramphenicol. The frequency was determined by taking the average CFU/ml on LB+chloramphenicol divided by the average CFU/ml on LB. Data from one representative experiment.

Chapter 4. Chromosomal Rearrangements in *Salmonella enterica* sv. Typhi Strains Isolated from Asymptomatic Human Carriers

Introduction

More than 2,500 serovars of the gram-negative enteric bacterial species *Salmonella enterica* have been identified (36). Most *Salmonella* serovars are pathogenic and can infect of variety of animal species, leading to pathologies ranging from a self-resolving gastroenteritis to a life-threatening systemic infection, depending on the particular serovar-host interaction. For instance, infecting mice with the Typhimurium serovar causes a systemic disease that is usually lethal, but ingestion of Typhimurium by humans typically results in gastroenteritis that usually self-resolves within a week. While most *S. enterica* serovars have a broad host range and are capable of infecting numerous animal species (generalists), a small number of serovars have become either host-adapted or host-specific. Host-adapted serovars are capable of infecting various animal species but commonly infect a preferred host. For example, serovar Choleraesuis primarily infects swine but can also cause bacteremia in humans. Host-specific serovars such as the fowl-specific serovar Pullorum and serovar Typhi, the etiological agent of typhoid fever in humans, only cause disease in one species or closely-related species.

The genomes of a number of generalist and host-specific serovars have been sequenced (4, 5, 29-31, 33, 39). A comparative analysis indicates that mutual house-keeping genes are $\geq$96% identical and the major pathogenicity islands are shared between serovars (6). Genetic differences between serovars, as well as strains within the same serovar, include small insertions and deletions and varying repertoires of *Salmonella*

Pathogenicity Islands (SPIs) and prophages. While specific genetic differences are known to affect the virulence of a particular strain (7-9, 13, 38), they have not been shown to affect host-specificity; in other words the genetic mechanisms of host-specificity are unknown. Host-specific strains are under less stringent selection for a variety of genetic functions as seen in the higher number of pseudogenes. The human-specific serovars Typhi and Paratyphi A have at least 204 and 173 pseudogenes respectively vs. 39 in the generalist serovar Typhimurium (5, 30, 31, 33). These findings support the hypothesis that during specialization to the human host, the genome of these *Salmonella* serovars undergoes degradation (30). Such genome reduction has also been observed in other bacteria that have specialized to a unique niche. For example *Yersinia pestis* (2, 3) and *Shigella flexneri* (14, 32, 40), as well as in the human-specific β-bacteria *Bordetella pertussis* (34) all have smaller genomes than their broad host range relatives.

Chromosomal rearrangements by homologous recombination between multiple copies of sequences, such as IS elements and rRNA (*rrn*) operons, have also been shown to occur in these and other niche restricted bacteria (2, 16, 35, 40), and may play an important role in the evolution of bacterial pathogens (14, 19, 32, 40, 42). These rearrangements result in inversions, translocations, duplications, or deletions of various sized regions of the genome. The host-specific *Salmonella* serovars almost always have chromosomal rearrangements that occur by recombination between the seven *rrn* operons on the chromosome (15, 19, 24-26, 28). The generalists on the other hand almost always have the same *rrn* arrangement (26, 27), the "conserved" arrangement type 1234567 (Figure 4.1) also found in almost all strains of the close relative *E. coli*. Rearrangements in generalist strains of *Salmonella* are extremely rare, and have only been found in strains

stored in stab vials for decades (37) and in pigeon-associated Typhimurium strains (12). These recombination events can either invert or levitate/translocate the chromosomal regions between the operons, resulting in their reordering. However, out of the 1,440 possible arrangements, only 35 have been observed in the ~150 Typhi strains analyzed to date (10, 15, 25, 26, 28). These data suggest that there may be a selective force against certain arrangements types.

One hypothesis to explain why these *rrn* rearrangements take place in host-specific *S. enterica* serovars proposes that horizontal gene transfer events, such as transfer of phages or SPIs, make one replichore longer than the other (replichores are the chromosomal halves on either side of the *ori*-*dif* axis). This imbalances DNA replication around the chromosome, and the rearrangements occur in attempts to rebalance the replichores (15, 18, 19, 25, 28). However, Tus-*ter* complexes in the terminus region of the chromosome would act as a buffer for such minor replichore imbalances. An alternative hypothesis suggests that some aspect of the lifestyle of the host-specific serovars may some how induce the *rrn* rearrangements (10, 28). For example, strains of many host-specific serovars can induce a carrier state, persisting for months to years within an animal host. As *S. enterica* is a facultative intracellular pathogen that resides within macrophages, repair of DNA damage due to long-term exposure to bursts of oxygen and nitrogen radicals could induce a hyper recombination state allowing these rearrangements to occur more frequently compared to the generalists. Intracellular bacteria in the carrier state also have less competition and grow slowly, which may allow rearrangements to be better tolerated. While previously it has been shown that both

generalist and host-specific serovars undergo *rrn* rearrangements at similar frequencies *in vitro* (10), the frequencies of *rrn* rearrangements *in vivo* have not been determined.

The most common chromosome arrangement types found in Typhi are 1'235647 and 1235647 (1' designates an inversion of region 1 relative to the conserved arrangement) (10, 15, 25). In addition to the region 1 inversion, these strains also have a translocation of region 4 into the *rrnE* operon between regions 6 and 7. These two arrangement types have been found in ~40% of the characterized Typhi strains, with the majority having the region 1 inversion. Other arrangement types observed in Typhi include 1'236547 and 1'236457 via translocation of region 6 next to region 3, and 1'234567, which only has the region 1 inversion and no translocations. Other rarer arrangements occur by additional inversions and translocations.

Two techniques have been used to determine the *rrn* chromosomal arrangement. Liu and Sanderson discovered these rearrangements by running pulse field gels of partial I-*Ceu*I digests of chromosomal DNA (20-23). A major limitation of this technique was the inability to detect inversions of region 1, the most common type of rearrangement. A PCR-based technique was subsequently developed (11) that utilized unique sequences flanking each *rrn* operon. The PCR approach allows rapid determination of the *rrn* arrangement, including the orientation of Region 1. Using this PCR technique, we describe here the arrangement types of multiple colonies from nineteen Typhi strains isolated from four asymptomatic carriers at different time points. The replichore balance of each arrangement type was then estimated using a PERL script. The findings support the hypothesis that aspects of lifestyle are responsible for the chromosomal

rearrangements in host-specific *Salmonella* serovars, and not replichore imbalance from horizontal gene transfer.

Materials and Methods

Strains and growth conditions. The strains used in this study were isolated from fecal samples taken between 1976 and 1999 from four asymptomatic female Typhi carriers living in eastern Germany (Table 4.1). The isolates were streaked on agar slants and stored in a controlled ambient temperature room at the Robert Koch Institut, Wernigerode, Germany. Swabs from the slants were streaked for single colony isolation and colonies were Vi phage typed. Bacteria were routinely cultured using Luria-Bertani (LB) medium at 37ºC. Solid LB plates were prepared by adding agar to 1.5% (w/v). Generation times were calculated by growing strains in triplicate at 37°C with aeration. Readings were taken every 30 minutes on Klett-Summerson colorimeter using a 540 nm filter. Timepoints in early exponential phase were used to calculate the generation time using the following formula:

$$= 1/((\log(OD_{600} \text{ at } t_2) - \log(OD_{600} \text{ at } t_1))/(0.301 \text{ X } (t_2-t_1))$$

Isolation of chromosomal DNA. Chromosomal DNA was isolated from overnight cultures using either the Wizard® Genomic DNA purification kit as described by the manufacturer (Promega U. S., Madison, WI, USA), or the CTAB-based bacterial genomic DNA miniprep protocol in (41).

PCR conditions. Reactions were performed as follows:

1) In HotStart 50 tubes (Molecular BioProducts, San Diego, CA, USA) with 200 µM dNTPs and 1µM each primer in the bottom layer, and 1X PCR buffer (20 mM Tris-HCL, pH=8.4; 50 mM KCl; 0.8% Nonidet P-40), 1.25 mM $MgCl_2$, 5% dimethylsulfoxide, Taq DNA polymerase, and chromosomal DNA in the top layer. Reactions were heated to 94ºC for 3 min followed by 30 cycles of 94ºC for 1 min, 60ºC for 1 min, and 72ºC for 5 min, followed by a final step at 72ºC for 7 min.

2) Using Platinum® *Taq* DNA polymerase High Fidelity according to the manufacturer's instructions (Invitrogen Corp., Carlsbad, CA, USA). Reactions were heated to 94ºC for 2 min followed by 30 cycles of 94ºC for 30 sec, 55ºC for 30 sec, and 68ºC for 7 min, followed by a final step at 68ºC for 7 min.

Primer sequences and combinations for detecting specific *rrn* combinations were previously described (11). The presence of *rrn* PCR products was determined by running 10 µl of each reaction out on a 0.8% agarose/1X TBE gel, followed by detection using ethidium bromide staining.

PERL script to estimate replichore balance. To estimate replichore balance, the size used for each chromosomal region between the *rrn* operons was the mean length of each region determined from the sequenced Typhi strains Ty2 and CT18, and the origin of replication and the *dif* site were used as the replichore endpoints (Chapter 2).

Results

Arrangement types were obtained for up to seven independent colonies of each strain isolated from the four carriers. The PCR results showed that while some slants contained cells with different arrangement types, others had cells with identical arrangement types (Table 4.1).

Carrier 1. Four strains isolated between 1981 and 1998 were analyzed. All strains had a noncharacteristic or untypeable Vi phage type. Colonies from the 1981 strain exhibited four different arrangement types. Four colonies had arrangement types that had a translocation of Region 6 into the *rrnC* operon, and two colonies had an additional inversion of Region 1 by recombination between the *rrnG* and *rrnH* operons. The two other colonies had unusual arrangement types containing a duplication of Region 5; one had a tandem duplication, while the other had an interreplichore translocation of the duplicated region. Four arrangement types were also observed in colonies from the 1993 strain. Two colonies had an interreplichore translocaton of Region 4 into *rrnD* as well as the Region 6 translocation and Region 1 inversion described above. One colony had an additional inversion by recombination between the *rrnA/D* and *rrn B/E* hybrid operons. Four colonies contained duplications of Region 6; three colonies had copies of Region 6 on both replichores, while one colony had a tandem duplication as well as a second inversion that switched the replichore locations of Regions 2 and 7. The set of colonies from the 1996 isolate had five arrangement types. Three colonies contained interreplichore translocations of Regions 4, 5, and 6. One colony only had an interreplichore translocation of Region 6 and another only had interreplichore

translocations of Regions 4 and 5. Two colonies had tandem duplications. One had a tandem duplication of Region 6 that had undergone an interreplichore translocation, and one had a tandem duplication of Region 4. All seven colonies from 1996 contained two inversions that switched replichore locations of Regions 2 and 7 as well as placing Region 1 into its native orientation. Only two arrangement types were found in colonies from the 1998 isolate. All six colonies had intrareplichore translocation of Regions 4 and 6, with Region 4 recombining into the hybrid *rrnH/G* operon. One of the colonies had a second inversion that switched replichore locations of Regions 2, 4, and 7 and placed Region 1 into its native orientation. None of the arrangement types identified in colonies from a strain isolated in one year were found in other years.

Carrier Two. Six strains isolated between 1981 and 1999 were analyzed and all had the 46 Vi phage type. All of the colonies analyzed contained an intrareplichore translocation of Region 6 into *rrnC*. Four out of six colonies from the 1981 strain contained tandem duplications of Region 6, and all of the colonies had Region 1 in its native orientation. However, in colonies from the rest of the strains isolated between 1985 and 1998, Region 1 was in the inverted orientation, except for one colony from the first 1985 strain. This colony had a second asymmetrical inversion that returned Region 1 to its native orientation as well as switching replichore locations of Regions 2, 4, 5, and 7. The only colony analyzed from 1999 had the same arrangement type as the 1981 colonies lacking the Region 6 duplication.

Carrier 3. Three strains isolated between 1977 and 1985 were analyzed and all had the F1 Vi phage type. All the strains contained two inversions by recombination between *rrnG* and *rrnH*, and between *rrnD* and *rrnE* that placed Region 1 in its native orientation and switched the replichore locations of Regions 2 and 7. Two of six colonies from 1977 and all seven colonies from 1998 had an arrangement type that involved intrareplichore translocations of Regions 4, 5, and/or 6 that inverted the region order, as well as a tandem duplication of Region 6. Three colonies from 1977 lacked the Region 6 duplication, and one strain had the same order but a tandem duplication of Region 4. All seven colonies from the 1983 strain had an interreplichore translocation of Region 6 into the hybrid *rrnH/G* operon between Regions 1 and 7. Furthermore, one 1983 colony had a tandem duplication of the translocated Region 6.

Carrier 4. Seven strains isolated between 1976 and 1991 were analyzed and all had an E1A Vi phage type. The only rearrangement present in all seven colonies from the 1981, 1983, 1987, and 1989 strains, and four out of seven colonies from 1976, was the inversion of Region 1. The other three 1976 colonies, and four out of six 1986 colonies had a second inversion by recombination between *rrnD* and *rrnE* that had Regions 2 and 7 switch replichores. The other two 1986 colonies had either a tandem duplication of Region 5, or an interreplichore translocations of Regions 5 and 6. Four arrangement types were observed in the seven 1991 colonies. Besides having the two inversions observed in 1976 and 1986, one colony had an intereplichore translocation and tandem duplication of Region 6. Five colonies had interreplichore translocations of Regions 4, 5, and 6. One

colony had interreplichore translocations of Regions 2, 4, 5, and 7 as well as Region 1 inverted.

Rearrangements occur infrequently during culturing. Three strains from Carriers 1, 2, and 4, and two strains from Carrier 3 were also analyzed to determine if rearrangements occurred often during culturing (Table 4.2). From the original slants, single colonies were cultured and frozen stocks of each strain were prepared. Subsequently single colonies were isolated and six were used to inoculate broth cultures for genomic DNA isolation. All six colonies isolated from each frozen stock had identical arrangement types, suggesting that rearrangements do not occur frequently enough under standard culturing conditions to be detected by the PCR screen.

Estimated replichore balance. While most observed arrangement types had well-balanced replichores, variation was observed in colonies isolated from the same slant depending on the differences in arrangement type (Table 4.1). Most colonies derived from Carrier 1 strains isolated in 1981and 1998 had up to only 3° estimated imbalance. Colonies from the 1993 strain had more replichore imbalance, with one colony having an arrangement type with an estimated imbalance of 23°. Due to the presence of a second inversion, colonies derived from the 1996 strain had even more imbalance, estimated up to 37°. In contrast, all the Carrier 2 colonies had at most 3° estimated imbalance except for one colony derived from the first 1985 sample, which again had undergone a secondary inversion and had an estimated imbalance of 34°. The colonies derived from the Carrier 3 strains all had an estimated imbalance of around 15°, but as many colonies

contained a duplication, the balance estimate was confounded. Finally, all the colonies derived from the 1981, 1983, 1987, and 1989 Carrier 4 strains, and four out of seven colonies from 1976, were nearly balanced, with an estimated imbalance of 1°. The other colonies from 1976 as well as colonies from the 1986 had greater amounts of replichore imbalance, up to 13°, while colonies derived from the 1991 strain had up to 37° estimated replichore imbalance.

Growth rates. The growth rates of strains isolated in 1983 and 1985 from Carrier 3, and strains isolated in 1987, 1989, 1991 from Carrier 4 was determined by growing triplicate cultures of each in LB medium. The set of strains tested here vary in arrangement types, including strains with duplications, as well as replichore balance. The arrangement types of the colonies used to inoculate the cultures were 16673542 and 17366542 for the 1983 and 1985 Carrier 3 strains respectively, 1’234567 for the 1987 and 1989 Carrier 4 strains, and 1’543267 for the 1991 Carrier 4 strain. Each of the strains tested had identical growth rates regardless of the carrier they were isolated from, their arrangement type, or estimated replichore balance.

Discussion

The different arrangement types identified in colonies derived from the same slant implies that rearrangements occurred on the slants over time. Furthermore, the pattern of observed arrangement types, in particular the arrangement types found in the strains from Carriers 1 and 4, also suggests that the carriers were infected with cells having different arrangement types and/or rearrangements occurred over time *in vivo*. In addition, the

replichore balance estimations did not show a trend towards more balanced replichores over time. Finally, colonies with arrangement types having either tandem or interreplichore duplications were found in at least one strain from each carrier. These observations support the hypothesis that aspects of lifestyle, and not replichore imbalance, are responsible for the chromosomal rearrangements found in host-specific *Salmonella* serovars.

Strains isolated from the same carrier always had the same Vi phage type, which suggests that strains from the same carrier are from a single infection. However, the arrangement types of strains isolated from the same carrier at different time points varied depending on the carrier. Two explanations for this observation are that the carrier was originally infected with bacteria having different arrangement types, and that rearrangements are occurring within the carrier over time. Furthermore, these two possibilities are not exclusive, and may be explained by both the lifestyle of Typhi cells in the carrier state, and how Typhi is transmitted from a carrier to a new host. Chromosomal rearrangements occur at the same relatively low frequency in both generalist and host-specific *Salmonella* serovars (10), but do not become fixed within generalist *Salmonella* populations because cells harboring them are thought to be less fit and lost from the population. However, many Typhi cells in the carrier state are intracellular and are not under the same types of selective pressures the generalist *Salmonella* experience. In addition, as Typhi is host-specific to humans, and has no known environmental reservoir, a strict genetic bottleneck occurs during human-to-human transmission. In contrast the generalist *Salmonella* can not only infect multiple host species, but can survive well in the environment outside a host. These processes that

lack selective pressure could explain how chromosomal rearrangements become fixed with Typhi populations as well as populations of other host-specific *Salmonella* serovars.

The replichore balance estimations showed that most observed arrangement types were very well-balanced (<15° imbalance), and the estimated balance of colonies derived from strains isolated during a particular year were similar if not identical. Two exceptions were arrangement types containing duplications, and strains where all the colonies tested had arrangement types estimated to have imbalance. For example, the Carrier 1 strains isolated in 1993 and 1996, and the Carrier 4 strain isolated 1991 are estimated to have replichores that are up to 37° imbalanced. Other imbalanced arrangement types were relatively rare, occurring only once within a set of colonies, as in the 1998 strain from Carrier 1 and the first 1985 strain from Carrier 2. Replichore balance also did not appear to improve over time, but seemed to be random depending on the strain isolated in a particular year, best exemplified by strains isolated from Carrier 4 (Table 4.1).

Rearrangements are known to be infrequent during standard culturing conditions, as described in (10) and shown by the lack of rearrangements in colonies streaked from frozen stocks. Therefore the observation that rearrangements occurred over time on the slants was surprising. However these types of rearrangements are not unprecedented in archived *Salmonella* strains (17, 37). Duplications were regularly observed in colonies isolated from the original slants. Most duplications were tandem duplications of Regions 4, 5, or 6. Such duplications can occur by unequal recombination between *rrn* operons and can occur at frequencies as high as 10^{-2} (1). Other rearrangements that changed the order or inverted the chromosomal regions between *rrn* operons were also observed in colonies derived from the same slant. While duplications may provide a selective

advantage in nutritionally-limited environments such as those found on stored slants, other rearrangements probably are not. Another possibility that explains both types of rearrangements is that during long term growth on the slant selection is relaxed, allowing rearrangements to become fixed within the slant population.

In conclusion, chromosomal rearrangements were observed in archived slants of Typhi strains isolated from human carriers. These rearrangements not only occurred over time during storage of the slants, but also may have occurred during carriage *in vivo* as well as being acquired by the carrier during the infection process. These results support the hypothesis that aspects of lifestyle are responsible for these rearrangements in Typhi and other host-specific *Salmonella*.

References

1. **Anderson, P., and J. Roth.** 1981. Spontaneous tandem genetic duplications in *Salmonella typhimurium* arise by unequal recombination between rRNA (*rrn*) cistrons. Proc Natl Acad Sci U S A **78:**3113-7.

2. **Chain, P. S., E. Carniel, F. W. Larimer, J. Lamerdin, P. O. Stoutland, W. M. Regala, A. M. Georgescu, L. M. Vergez, M. L. Land, V. L. Motin, R. R. Brubaker, J. Fowler, J. Hinnebusch, M. Marceau, C. Medigue, M. Simonet, V. Chenal-Francisque, B. Souza, D. Dacheux, J. M. Elliott, A. Derbise, L. J. Hauser, and E. Garcia.** 2004. Insights into the evolution of *Yersinia pestis* through whole-genome comparison with *Yersinia pseudotuberculosis*. Proc Natl Acad Sci U S A **101:**13826-31.

3. **Chain, P. S., P. Hu, S. A. Malfatti, L. Radnedge, F. Larimer, L. M. Vergez, P. Worsham, M. C. Chu, and G. L. Andersen.** 2006. Complete genome sequence of *Yersinia pestis* strains Antiqua and Nepal516: evidence of gene reduction in an emerging pathogen. J Bacteriol **188:**4453-63.

4. **Chiu, C. H., P. Tang, C. Chu, S. Hu, Q. Bao, J. Yu, Y. Y. Chou, H. S. Wang, and Y. S. Lee.** 2005. The genome sequence of *Salmonella enterica* serovar Choleraesuis, a highly invasive and resistant zoonotic pathogen. Nucleic Acids Res **33:**1690-8.

5. **Deng, W., S. R. Liou, G. Plunkett, 3rd, G. F. Mayhew, D. J. Rose, V. Burland, V. Kodoyianni, D. C. Schwartz, and F. R. Blattner.** 2003. Comparative genomics of *Salmonella enterica* serovar Typhi strains Ty2 and CT18. J Bacteriol **185:**2330-7.

6. **Edwards, R. A., G. J. Olsen, and S. R. Maloy.** 2002. Comparative genomics of closely related salmonellae. Trends Microbiol **10:**94-9.

7. **Figueroa-Bossi, N., and L. Bossi.** 1999. Inducible prophages contribute to *Salmonella* virulence in mice. Mol Microbiol **33:**167-76.

8. **Figueroa-Bossi, N., E. Coissac, P. Netter, and L. Bossi.** 1997. Unsuspected prophage-like elements in *Salmonella typhimurium*. Mol Microbiol **25:**161-73.

9. **Figueroa-Bossi, N., S. Uzzau, D. Maloriol, and L. Bossi.** 2001. Variable assortment of prophages provides a transferable repertoire of pathogenic determinants in *Salmonella*. Mol Microbiol **39:**260-71.

10. **Helm, R. A., A. G. Lee, H. D. Christman, and S. Maloy.** 2003. Genomic rearrangements at *rrn* operons in *Salmonella*. Genetics **165:**951-9.

11. **Helm, R. A., and S. Maloy.** 2001. Rapid approach to determine *rrn* arrangement in *Salmonella* serovars. Appl Environ Microbiol **67:**3295-8.

12. **Helm, R. A., S. Porwollik, A. E. Stanley, S. Maloy, M. McClelland, W. Rabsch, and A. Eisenstark.** 2004. Pigeon-associated strains of *Salmonella enterica* serovar Typhimurium phage type DT2 have genomic rearrangements at rRNA operons. Infect Immun **72:**7338-41.

13. **Ho, T. D., and J. M. Slauch.** 2001. Characterization of *grvA*, an antivirulence gene on the Gifsy-2 phage in *Salmonella enterica* serovar typhimurium. J Bacteriol **183:**611-20.

14. **Jin, Q., Z. Yuan, J. Xu, Y. Wang, Y. Shen, W. Lu, J. Wang, H. Liu, J. Yang, F. Yang, X. Zhang, J. Zhang, G. Yang, H. Wu, D. Qu, J. Dong, L. Sun, Y. Xue, A. Zhao, Y. Gao, J. Zhu, B. Kan, K. Ding, S. Chen, H. Cheng, Z. Yao, B. He, R. Chen, D. Ma, B. Qiang, Y. Wen, Y. Hou, and J. Yu.** 2002. Genome sequence of *Shigella flexneri* 2a: insights into pathogenicity through comparison with genomes of *Escherichia coli* K12 and O157. Nucleic Acids Res **30:**4432-41.

15. **Kothapalli, S., S. Nair, S. Alokam, T. Pang, R. Khakhria, D. Woodward, W. Johnson, B. A. Stocker, K. E. Sanderson, and S. L. Liu.** 2005. Diversity of genome structure in *Salmonella enterica* serovar Typhi populations. J Bacteriol **187:**2638-50.

16. **Lindroos, H., O. Vinnere, A. Mira, D. Repsilber, K. Naslund, and S. G. Andersson.** 2006. Genome rearrangements, deletions, and amplifications in the natural population of *Bartonella henselae*. J Bacteriol **188:**7426-39.

17. **Liu, G. R., K. Edwards, A. Eisenstark, Y. M. Fu, W. Q. Liu, K. E. Sanderson, R. N. Johnston, and S. L. Liu.** 2003. Genomic diversification among archival strains of *Salmonella enterica* serovar Typhimurium LT7. J Bacteriol **185:**2131-42.

18. **Liu, G. R., W. Q. Liu, R. N. Johnston, K. E. Sanderson, S. X. Li, and S. L. Liu.** 2006. Genome plasticity and ori-ter rebalancing in *Salmonella typhi*. Mol Biol Evol **23:**365-71.

19. **Liu, G. R., A. Rahn, W. Q. Liu, K. E. Sanderson, R. N. Johnston, and S. L. Liu.** 2002. The evolving genome of *Salmonella enterica* serovar Pullorum. J Bacteriol **184:**2626-33.

20. **Liu, S. L., A. Hessel, H. Y. Cheng, and K. E. Sanderson.** 1994. The *Xba*I-*Bln*I-*Ceu*I genomic cleavage map of *Salmonella paratyphi* B. J Bacteriol **176:**1014-24.

21. **Liu, S. L., A. Hessel, and K. E. Sanderson.** 1993. Genomic mapping with I-*Ceu* I, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc Natl Acad Sci U S A **90:**6874-8.

22. **Liu, S. L., A. Hessel, and K. E. Sanderson.** 1993. The *Xba*I-*Bln*I-*Ceu*I genomic cleavage map of *Salmonella enteritidis* shows an inversion relative to *Salmonella typhimurium* LT2. Mol Microbiol **10:**655-64.

23. **Liu, S. L., A. Hessel, and K. E. Sanderson.** 1993. The *Xba*I-*Bln*I-*Ceu*I genomic cleavage map of *Salmonella typhimurium* LT2 determined by double digestion, end labelling, and pulsed-field gel electrophoresis. J Bacteriol **175:**4104-20.

24. **Liu, S. L., and K. E. Sanderson.** 1995. The chromosome of *Salmonella paratyphi* A is inverted by recombination between *rrnH* and *rrnG*. J Bacteriol **177:**6585-92.

25. **Liu, S. L., and K. E. Sanderson.** 1996. Highly plastic chromosomal organization in *Salmonella typhi*. Proc Natl Acad Sci U S A **93:**10303-8.

26. **Liu, S. L., and K. E. Sanderson.** 1998. Homologous recombination between *rrn* operons rearranges the chromosome in host-specialized species of *Salmonella*. FEMS Microbiol Lett **164:**275-81.

27. **Liu, S. L., and K. E. Sanderson.** 1995. I-*Ceu*I reveals conservation of the genome of independent strains of *Salmonella typhimurium*. J Bacteriol **177:**3355-7.

28. **Liu, S. L., and K. E. Sanderson.** 1995. Rearrangements in the genome of the bacterium *Salmonella typhi*. Proc Natl Acad Sci U S A **92:**1018-22.

29. **Liu, W. Q., Y. Feng, Y. Wang, Q. H. Zou, F. Chen, J. T. Guo, Y. H. Peng, Y. Jin, Y. G. Li, S. N. Hu, R. N. Johnston, G. R. Liu, and S. L. Liu.** 2009. *Salmonella paratyphi* C: genetic divergence from *Salmonella choleraesuis* and pathogenic convergence with *Salmonella typhi*. PLoS One **4:**e4510.

30. **McClelland, M., K. E. Sanderson, S. W. Clifton, P. Latreille, S. Porwollik, A. Sabo, R. Meyer, T. Bieri, P. Ozersky, M. McLellan, C. R. Harkins, C. Wang, C. Nguyen, A. Berghoff, G. Elliott, S. Kohlberg, C. Strong, F. Du, J. Carter, C. Kremizki, D. Layman, S. Leonard, H. Sun, L. Fulton, W. Nash, T. Miner, P. Minx, K. Delehaunty, C. Fronick, V. Magrini, M. Nhan, W. Warren, L. Florea, J. Spieth, and R. K. Wilson.** 2004. Comparison of genome degradation in Paratyphi A and Typhi, human-restricted serovars of *Salmonella enterica* that cause typhoid. Nat Genet **36:**1268-74.

31. **McClelland, M., K. E. Sanderson, J. Spieth, S. W. Clifton, P. Latreille, L. Courtney, S. Porwollik, J. Ali, M. Dante, F. Du, S. Hou, D. Layman, S. Leonard, C. Nguyen, K. Scott, A. Holmes, N. Grewal, E. Mulvaney, E. Ryan, H. Sun, L. Florea, W. Miller, T. Stoneking, M. Nhan, R. Waterston, and R. K. Wilson.** 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature **413:**852-6.

32. **Nie, H., F. Yang, X. Zhang, J. Yang, L. Chen, J. Wang, Z. Xiong, J. Peng, L. Sun, J. Dong, Y. Xue, X. Xu, S. Chen, Z. Yao, and Q. Jin.** 2006. Complete Genome Sequence of *Shigella flexneri* 5b and Comparison with *Shigella flexneri* 2a. BMC Genomics **7:**173-182.

33. **Parkhill, J., G. Dougan, K. D. James, N. R. Thomson, D. Pickard, J. Wain, C. Churcher, K. L. Mungall, S. D. Bentley, M. T. Holden, M. Sebaihia, S. Baker, D. Basham, K. Brooks, T. Chillingworth, P. Connerton, A. Cronin, P. Davis, R. M. Davies, L. Dowd, N. White, J. Farrar, T. Feltwell, N. Hamlin, A. Haque, T. T. Hien, S. Holroyd, K. Jagels, A. Krogh, T. S. Larsen, S. Leather, S. Moule, P. O'Gaora, C. Parry, M. Quail, K. Rutherford, M. Simmonds, J. Skelton, K. Stevens, S. Whitehead, and B. G. Barrell.** 2001. Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. Nature **413:**848-52.

34. **Parkhill, J., M. Sebaihia, A. Preston, L. D. Murphy, N. Thomson, D. E. Harris, M. T. Holden, C. M. Churcher, S. D. Bentley, K. L. Mungall, A. M. Cerdeno-Tarraga, L. Temple, K. James, B. Harris, M. A. Quail, M. Achtman, R. Atkin, S. Baker, D. Basham, N. Bason, I. Cherevach, T. Chillingworth, M. Collins, A. Cronin, P. Davis, J. Doggett, T. Feltwell, A. Goble, N. Hamlin, H. Hauser, S. Holroyd, K. Jagels, S. Leather, S. Moule, H. Norberczak, S. O'Neil, D. Ormond, C. Price, E. Rabbinowitsch, S. Rutter, M. Sanders, D. Saunders, K. Seeger, S. Sharp, M. Simmonds, J. Skelton, R. Squares, S. Squares, K. Stevens, L. Unwin, S. Whitehead, B. G. Barrell, and D. J. Maskell.** 2003. Comparative analysis of the genome sequences of *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella bronchiseptica*. Nat Genet **35:**32-40.

35. **Petrosino, J. F., Q. Xiang, S. E. Karpathy, H. Jiang, S. Yerrapragada, Y. Liu, J. Gioia, L. Hemphill, A. Gonzalez, T. M. Raghavan, A. Uzman, G. E. Fox, S. Highlander, M. Reichard, R. J. Morton, K. D. Clinkenbeard, and G. M. Weinstock.** 2006. Chromosome rearrangement and diversification of *Francisella*

tularensis revealed by the type B (OSU18) genome sequence. J Bacteriol **188:**6977-85.

36. **Popoff, M. Y., J. Bockemuhl, and L. L. Gheesling.** 2004. Supplement 2002 (no. 46) to the Kauffmann-White scheme. Res Microbiol **155:**568-70.

37. **Porwollik, S., R. M. Wong, R. A. Helm, K. K. Edwards, M. Calcutt, A. Eisenstark, and M. McClelland.** 2004. DNA amplification and rearrangements in archival *Salmonella enterica* serovar Typhimurium LT2 cultures. J Bacteriol **186:**1678-82.

38. **Stanley, T. L., C. D. Ellermeier, and J. M. Slauch.** 2000. Tissue-specific gene expression identifies a gene in the lysogenic phage Gifsy-1 that affects *Salmonella enterica* serovar typhimurium survival in Peyer's patches. J Bacteriol **182:**4406-13.

39. **Thomson, N. R., D. J. Clayton, D. Windhorst, G. Vernikos, S. Davidson, C. Churcher, M. A. Quail, M. Stevens, M. A. Jones, M. Watson, A. Barron, A. Layton, D. Pickard, R. A. Kingsley, A. Bignell, L. Clark, B. Harris, D. Ormond, Z. Abdellah, K. Brooks, I. Cherevach, T. Chillingworth, J. Woodward, H. Norberczak, A. Lord, C. Arrowsmith, K. Jagels, S. Moule, K. Mungall, M. Sanders, S. Whitehead, J. A. Chabalgoity, D. Maskell, T. Humphrey, M. Roberts, P. A. Barrow, G. Dougan, and J. Parkhill.** 2008. Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. Genome Res **18:**1624-37.

40. **Wei, J., M. B. Goldberg, V. Burland, M. M. Venkatesan, W. Deng, G. Fournier, G. F. Mayhew, G. Plunkett, 3rd, D. J. Rose, A. Darling, B. Mau, N. T. Perna, S. M. Payne, L. J. Runyen-Janecky, S. Zhou, D. C. Schwartz, and F. R. Blattner.** 2003. Complete genome sequence and comparative genomics of *Shigella flexneri* serotype 2a strain 2457T. Infect Immun **71:**2775-86.

41. **Wilson, K.** 1994. Preparation of Genomic DNA from Bacteria. Curr. Protoc. Mol. Biol. **27:**2.4.1-2.4.5.

42. **Wu, K. Y., G. R. Liu, W. Q. Liu, A. Q. Wang, S. Zhan, K. E. Sanderson, R. N. Johnston, and S. L. Liu.** 2005. The genome of *Salmonella enterica* serovar

gallinarum: distinct insertions/deletions and rare rearrangements. J Bacteriol **187:**4720-7.

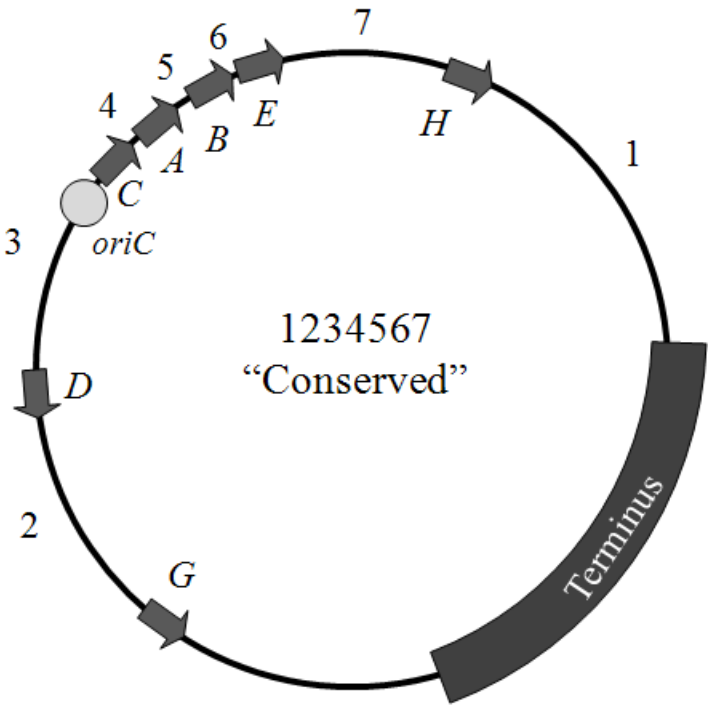

Figure 4.1. The chromosomal organization of the seven *rrn* operons found in generalist serovars of *Salmonella enterica*. The numbering of chromosomal regions between the operons clockwise starting with the region containing the terminus gives the *rrn* arrangement type 1234567.

Table 4.1. Arrangement type, Vi phage type, and estimated imbalance of colonies isolated from slants inoculated with Typhi strains isolated from human carriers.

Carrier	Year Isolated	Vi Phage Type[+]	Colony #	Arrangement Type*	Estimated Imbalance (°)
1	1981	nc	1	1236457	3.2 CCW
			2	1236457	3.2 CCW
			3	17543652	27.5 CCW
			4	1' 236457	1.1 CW
			5	1' 6234557	3.4 CW
			6	1' 236457	1.1 CW
	1993	nc	1	1' 243657	9.0 CCW
			2	17566342	28.4 CCW
			3	1' 2643657	10.5 CCW
			4	1' 2643657	10.5 CCW
			5	1' 2643657	10.5 CCW
			6	1743652	22.8 CCW
			7	1' 243657	9.0 CCW
	1996	nc	1	1763542	15.8 CCW
			2	1745362	34.0 CCW
			3	17665432	17.3 CCW

Table 4.1 Continued.

Carrier	Year Isolated	Vi Phage Type[+]	Colony #	Arrangement Type*	Estimated Imbalance (°)
1	1996	nc	4	1764532	37.1 CCW
			5	17653442	21.4 CCW
			6	1764532	37.1 CCW
			7	1764532	37.1 CCW
	1998	ut	1	1' 236574	1.1 CW
			2	1' 236574	1.1 CW
			3	1475632	37.1 CCW
			4	1' 236574	1.1 CW
			5	1' 236574	1.1 CW
			6	1' 236574	1.1 CW
2	1981	46	1	12366457	1.6 CCW
			2	12366457	1.6 CCW
			3	12366457	1.6 CCW
			4	1236457	3.2 CCW
			5	12366457	1.6 CCW
			6	1236457	3.2 CCW
	1985A	46	1	1' 236457	1.1 CW
			2	1754362	34.0 CCW
			3	1' 236457	1.1 CW
			4	1' 236457	1.1 CW
			5	1' 236457	1.1 CW
	1985B	46	1	1' 236457	1.1 CW
			2	1' 236457	1.1 CW
			3	1' 236457	1.1 CW
			4	1' 236457	1.1 CW
			5	1' 236457	1.1 CW
			6	1' 236457	1.1 CW
	1994	46	1	1' 234567	1.1 CW
	1998	46	1	1' 236457	1.1 CW
			2	1' 236457	1.1 CW
			3	1' 236457	1.1 CW
			4	1' 236457	1.1 CW
			5	1' 236457	1.1 CW
			6	1' 236457	1.1 CW
			7	1' 236457	1.1 CW
	1999	46	1	1236457	3.2 CCW
3	1977	F1	1	17365442	7.4 CCW
			2	1736542	12.7 CCW
			3	1736542	12.7 CCW
			4	17366542	11.0 CCW
			5	17365442	7.4 CCW
			6	1736542	12.7 CCW
	1983	F1	1	16673542	17.3 CCW
			2	1673542	15.8 CCW
			3	1673542	15.8 CCW
			4	1673542	15.8 CCW
			5	1673542	15.8 CCW
			6	1673542	15.8 CCW
			7	1673542	15.8 CCW

Table 4.1 Continued.

Carrier	Year Isolated	Vi Phage Type[+]	Colony #	Arrangement Type*	Estimated Imbalance (°)	
3	1985	F1	1	17366542	11.0	CCW
			2	17366542	11.0	CCW
			3	17366542	11.0	CCW
			4	17366542	11.0	CCW
			5	17366542	11.0	CCW
			6	17366542	11.0	CCW
			7	17366542	11.0	CCW
4	1976	E1A	1	1'234567	1.1	CW
			2	1734562	12.7	CCW
			3	1'234567	1.1	CW
			4	1734562	12.7	CCW
			5	1'234567	1.1	CW
			6	1'234567	1.1	CW
			7	1734562	12.7	CCW
	1981	E1A	1	1'234567	1.1	CW
			2	1'234567	1.1	CW
			3	1'234567	1.1	CW
			4	1'234567	1.1	CW
			5	1'234567	1.1	CW
			6	1'234567	1.1	CW
			7	1'234567	1.1	CW
	1983	E1A	1	1'234567	1.1	CW
			2	1'234567	1.1	CW
			3	1'234567	1.1	CW
			4	1'234567	1.1	CW
			5	1'234567	1.1	CW
			6	1'234567	1.1	CW
			7	1'234567	1.1	CW
	1986	E1A	1	1734562	12.7	CCW
			2	17345562	6.9	CCW
			3	1734562	12.7	CCW
			4	1734562	12.7	CCW
			5	1'265347	13.2	CCW
			6	1734562	12.7	CCW
	1987	E1A	1	1'234567	1.1	CW
			2	1'234567	1.1	CW
			3	1'234567	1.1	CW
			4	1'234567	1.1	CW
			5	1'234567	1.1	CW
			6	1'234567	1.1	CW
			7	1'234567	1.1	CW
	1989	E1A	1	1'234567	1.1	CW
			2	1'234567	1.1	CW
			3	1'234567	1.1	CW
			4	1'234567	1.1	CW
			5	1'234567	1.1	CW
			6	1'234567	1.1	CW
			7	1'234567	1.1	CW
	1991	E1A	1	1'543267	33.2	CW
			2	1765432	37.1	CCW

Table 4.1 Continued.

Carrier	Year Isolated	Vi Phage Type[+]	Colony #	Arrangement Type*	Estimated Imbalance (°)
4	1991	E1A	3	1765432	37.1 CCW
			4	1765432	37.1 CCW
			5	17356642	11.0 CCW
			6	1765432	37.1 CCW
			7	1765432	37.1 CCW

[+] nc/ut = non-characteristic or untypeable.

* 1' indicates that Region 1 is in the inverted orientation.

Table 4.2. Typhi strains assayed for rearrangements during culturing.

Carrier	Year Isolated	Arrangement Type*
1	1993	1' 243657
1	1996	1763542
1	1998	1' 236574
2	1994	1' 234567
2	1998	1' 236457
2	1999	1236457
3	1985	17366542
3	1983	16673542
4	1987	1' 234567
4	1989	1' 234567
4	1991	1' 543267

* 1' indicates that Region 1 is in the inverted orientation.

Chapter 5. Summary and Future Directions

Summary

In contrast to the broad host range serovars of *Salmonella enterica*, strains belonging to host-specific serovars almost always have large scale chromosomal rearrangements as a result of recombination between rRNA (*rrn*) operons. These rearrangements change the order of the chromosomal regions between the *rrn* operons from the conserved arrangement type (1234567) found in the broad host range serovars to one of at least fifty arrangement types identified so far. These rearrangements are interesting because the conserved arrangement type, and therefore the order of shared genes around the chromosome, is similar that of *E. coli* in spite of over 100 million years since the two species diverged. This observation suggested that even though rearrangements can occur through recombination, a selective force must exist that preserves the gene order in these two bacterial species. The large scale rearrangements found in the host-specific serovars showed that this was not the case, and similar types of rearrangements found in other bacterial species suggest that large scale chromosomal rearrangements are an important, but poorly understood, evolutionary process.

The main question that this work addresses is: why are rearrangements so prevalent in host-specific *S. enterica serovars* but are extremely rare in broad host range serovars? Two proposed hypotheses were tested. One hypothesis suggests that horizontal gene transfer of pathogenicity islands and prophages makes one replication arm, or replichore, longer than the other. This causes imbalanced DNA replication, and the

rearrangements occur to reestablish balance. An alternative hypothesis proposes that aspects of lifestyle of the host-specific serovars either induces the rearrangements, or allows them to be tolerated.

To test these two hypotheses, the replichore balance of all theoretical arrangement types (natural and non-natural) was estimated using a calculator, the relative fitness of strains with varying degrees of replichore imbalance was determined, and the arrangement types of serovar Typhi strains isolated from human carriers were ascertained. A global analysis of naturally-occurring arrangement types was performed and compared to the theoretical arrangement types. Out of 1,440 theoretical possibilities, only ~50 arrangement types were found to naturally occur. Furthermore, while variation in arrangement type was observed in strains of each host-specific serovar, there was also a distinct most common arrangement type for each host-specific serovar. The replichore calculator estimated that while most naturally-occurring arrangement types have well-balanced replichores ($\leq$15° imbalance), most theoretical arrangement types have >30° imbalance. This observation demonstrated that rearrangements from recombination between *rrn* operons would most likely introduce more imbalance than correct the imbalance introduced by horizontally-transferred genes. One possible explanation for why most naturally-occurring arrangement types have well-balanced replichores is that strains harboring them are more fit than strains with imbalanced replichores. To test this, the relative fitness of strains with transposon-held duplications that introduce up to 23° imbalance was determined. The results showed no correlation between duplication size and fitness, indicating that fitness is not adversely affected by the amount of imbalance introduced not only by the duplications in these strains, but also by the size of DNA that

is typically transferred horizontally in *Salmonella*. The analysis of arrangement types from Typhi strains isolated from human carriers demonstrated that carriers harbor bacteria having different arrangement types, and that rearrangements occur during long-term storage on agar slants. The results from this work do not support the hypothesis that the rearrangements in host-specific *Salmonella* serovars occur in attempts to reestablish replichore balance after horizontal gene transfer, but do support the hypothesis that lifestyle is responsible for the rearrangements.

If aspects of lifestyle are responsible for the chromosomal rearrangements in the host-specific *Salmonella* serovars, what are they? One major life style difference between broad host range and host-specific *Salmonella* serovars is that the host-specific serovars establish a chronic carrier state within their hosts. In the carrier state, *Salmonella* bacteria evade the host's immune system by residing intracellularly within various cell types, including macrophages. After phagocytosis of bacterial pathogens, macrophages release bursts of oxygen and nitrogen radicals produced by NADPH oxidase and inducible nitric oxide synthase (iNOS) respectively. While these bursts of reactive species have been shown to kill or inhibit intracellular *S. enterica* sv. Typhimurium *in vitro*, and are required for host resistance to infection (7, 12, 13), the protein effectors encoded in *Salmonella* pathogenicity island-2 (SPI-2) enable intracellular *Salmonella* to resist these bursts of reactive species by preventing colocalization of the NADPH oxidase and iNOS with the *Salmonella*-containing vacuole (SCV) (1, 5, 14). However, over time in the carrier state the bursts are likely to occasionally hit the intracellular *Salmonella*. If DNA damage occurs and the DNA repair systems are induced, the rearrangements could be the result of increased recombination frequency. Although transcriptional profiling has

shown the SOS response to be induced in Typhimurium cells isolated from infected J774-A.1 murine macrophage-like cells (3), in Typhi cells isolated from human THP-1 macrophages there did not appear to be any upregulation (4).

The higher number of pseudogenes found in the genomes of host-specific *Salmonella* serovars compared to the genomes of broad host range serovars show that the two serovar types are not under the same types of selective pressures (2, 6, 8-11). This is most likely due to the host serovars' ability to establish a carrier state as well as having an intracellular lifestyle. Under these conditions, genes may no longer be under positive selection, allowing mutations that inactivate them to become fixed within a population. The differences in selective pressure experienced by host-specific serovars may also relax the forces that maintain gene order, allowing rearrangements to be tolerated. A similar lack of selective pressure to maintain gene order can also explain the different arrangement types found in the individual slants of Typhi strains isolated from human carriers. In both cases, the bacteria are not actively growing and are under similar nutrient-limiting conditions.

Population bottlenecks that occur during transmission to a new host are much narrower for host-specific serovars than for broad host range serovars. Host-specific strains have a smaller effective population because they must infect a new specific host animal to start a new round of infection in contrast to the broad host range strains that are capable of infecting a variety of animal species to initiate further outbreaks of disease. In addition, broad host range serovars can survive better outside a host while host-specific serovars have no known environmental reservoir. As rearrangements slowly occur over time, the strict bottleneck that host-specific strains pass through during each round of

infection can also allow rearrangements to become fixed within a population, even if they are slightly deleterious to fitness.

Future Directions

Various approaches are possible to better understand why chromosomal rearrangements are so prevalent in host-specific *Salmonella* serovars and so rare in broad host range serovars. One approach would be to expand the type of study described in Chapter 4 to include strains isolated from additional carriers, as well as strains belonging to other host-specific serovars isolated from humans or other host animals. Analysis of multiple isolates from the same host animal (human or other) isolated at both the same and at different points in time would show definitively whether rearrangements occur *in vivo* over time. Another approach would be to use an animal model system, for example chickens and a strain of the fowl-specific serovar Pullorum with a known arrangement type. After establishment of the carrier state in these animals, isolates obtained over time can be easily screened for rearrangements using PCR.

Furthermore, strains that allow the direct selection of cells that have rearrangements would be invaluable in such a study. Even though the construction of these strains was not accomplished (Appendix B), eventual completion would also permit the measurement of rearrangement frequencies under various *in vitro* and *in vivo* conditions, and allow for direct testing of both the lifestyle and balanced replichore hypotheses.

References

1. **Chakravortty, D., I. Hansen-Wester, and M. Hensel.** 2002. *Salmonella* pathogenicity island 2 mediates protection of intracellular *Salmonella* from reactive nitrogen intermediates. J Exp Med **195:**1155-66.

2. **Deng, W., S. R. Liou, G. Plunkett, 3rd, G. F. Mayhew, D. J. Rose, V. Burland, V. Kodoyianni, D. C. Schwartz, and F. R. Blattner.** 2003. Comparative genomics of *Salmonella enterica* serovar Typhi strains Ty2 and CT18. J Bacteriol **185:**2330-7.

3. **Eriksson, S., S. Lucchini, A. Thompson, M. Rhen, and J. C. Hinton.** 2003. Unravelling the biology of macrophage infection by gene expression profiling of intracellular *Salmonella enterica*. Mol Microbiol **47:**103-18.

4. **Faucher, S. P., S. Porwollik, C. M. Dozois, M. McClelland, and F. Daigle.** 2006. Transcriptome of *Salmonella enterica* serovar Typhi within macrophages revealed through the selective capture of transcribed sequences. Proc Natl Acad Sci U S A **103:**1906-11.

5. **Gallois, A., J. R. Klein, L. A. Allen, B. D. Jones, and W. M. Nauseef.** 2001. *Salmonella* pathogenicity island 2-encoded type III secretion system mediates exclusion of NADPH oxidase assembly from the phagosomal membrane. J Immunol **166:**5741-8.

6. **Liu, W. Q., Y. Feng, Y. Wang, Q. H. Zou, F. Chen, J. T. Guo, Y. H. Peng, Y. Jin, Y. G. Li, S. N. Hu, R. N. Johnston, G. R. Liu, and S. L. Liu.** 2009. *Salmonella paratyphi* C: genetic divergence from Salmonella choleraesuis and pathogenic convergence with *Salmonella typhi*. PLoS One **4:**e4510.

7. **Mastroeni, P., A. Vazquez-Torres, F. C. Fang, Y. Xu, S. Khan, C. E. Hormaeche, and G. Dougan.** 2000. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. II. Effects on microbial proliferation and host survival *in vivo*. J Exp Med **192:**237-48.

8. **McClelland, M., K. E. Sanderson, S. W. Clifton, P. Latreille, S. Porwollik, A. Sabo, R. Meyer, T. Bieri, P. Ozersky, M. McLellan, C. R. Harkins, C. Wang, C. Nguyen, A. Berghoff, G. Elliott, S. Kohlberg, C. Strong, F. Du, J. Carter, C. Kremizki, D. Layman, S. Leonard, H. Sun, L. Fulton, W. Nash, T. Miner, P. Minx, K. Delehaunty, C. Fronick, V. Magrini, M. Nhan, W. Warren, L. Florea, J. Spieth, and R. K. Wilson.** 2004. Comparison of genome degradation in Paratyphi A and Typhi, human-restricted serovars of *Salmonella enterica* that cause typhoid. Nat Genet **36:**1268-74.

9. **McClelland, M., K. E. Sanderson, J. Spieth, S. W. Clifton, P. Latreille, L. Courtney, S. Porwollik, J. Ali, M. Dante, F. Du, S. Hou, D. Layman, S. Leonard, C. Nguyen, K. Scott, A. Holmes, N. Grewal, E. Mulvaney, E. Ryan, H. Sun, L. Florea, W. Miller, T. Stoneking, M. Nhan, R. Waterston, and R. K. Wilson.** 2001. Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. Nature **413:**852-6.

10. **Parkhill, J., G. Dougan, K. D. James, N. R. Thomson, D. Pickard, J. Wain, C. Churcher, K. L. Mungall, S. D. Bentley, M. T. Holden, M. Sebaihia, S. Baker, D. Basham, K. Brooks, T. Chillingworth, P. Connerton, A. Cronin, P. Davis, R. M. Davies, L. Dowd, N. White, J. Farrar, T. Feltwell, N. Hamlin, A. Haque, T. T. Hien, S. Holroyd, K. Jagels, A. Krogh, T. S. Larsen, S. Leather, S. Moule, P. O'Gaora, C. Parry, M. Quail, K. Rutherford, M. Simmonds, J. Skelton, K. Stevens, S. Whitehead, and B. G. Barrell.** 2001. Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18. Nature **413:**848-52.

11. **Thomson, N. R., D. J. Clayton, D. Windhorst, G. Vernikos, S. Davidson, C. Churcher, M. A. Quail, M. Stevens, M. A. Jones, M. Watson, A. Barron, A. Layton, D. Pickard, R. A. Kingsley, A. Bignell, L. Clark, B. Harris, D. Ormond, Z. Abdellah, K. Brooks, I. Cherevach, T. Chillingworth, J. Woodward, H. Norberczak, A. Lord, C. Arrowsmith, K. Jagels, S. Moule, K. Mungall, M. Sanders, S. Whitehead, J. A. Chabalgoity, D. Maskell, T. Humphrey, M. Roberts, P. A. Barrow, G. Dougan, and J. Parkhill.** 2008. Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. Genome Res **18:**1624-37.

12. **Vazquez-Torres, A., and F. C. Fang.** 2001. Oxygen-dependent anti-*Salmonella* activity of macrophages. Trends Microbiol **9:**29-33.

13. **Vazquez-Torres, A., J. Jones-Carson, P. Mastroeni, H. Ischiropoulos, and F. C. Fang.** 2000. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages *in vitro*. J Exp Med **192:**227-36.

14. **Vazquez-Torres, A., Y. Xu, J. Jones-Carson, D. W. Holden, S. M. Lucia, M. C. Dinauer, P. Mastroeni, and F. C. Fang.** 2000. *Salmonella* pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. Science **287:**1655-8.

Appendix A. Estimated Replichore Balance of Arrangement Types

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 2 3 4 5 6 7	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 4 5 7 6	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 4 6 5 7	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 4 6 7 5	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 4 7 5 6	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 4 7 6 5	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 5 4 6 7	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 5 4 7 6	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 5 6 4 7	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 5 6 7 4	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 5 7 4 6	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 5 7 6 4	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 6 4 5 7	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 6 4 7 5	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 6 5 4 7	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 6 5 7 4	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 6 7 4 5	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 6 7 5 4	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 7 4 5 6	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 7 4 6 5	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 7 5 4 6	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 7 5 6 4	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 7 6 4 5	2358.1	2442.4	176.8	183.2	-3.2
1 2 3 7 6 5 4	2358.1	2442.4	176.8	183.2	-3.2
1 2 4 3 5 6 7	2223.6	2576.9	166.8	193.2	-13.2
1 2 4 3 5 7 6	2223.6	2576.9	166.8	193.2	-13.2
1 2 4 3 6 5 7	2223.6	2576.9	166.8	193.2	-13.2
1 2 4 3 6 7 5	2223.6	2576.9	166.8	193.2	-13.2
1 2 4 3 7 5 6	2223.6	2576.9	166.8	193.2	-13.2
1 2 4 3 7 6 5	2223.6	2576.9	166.8	193.2	-13.2
1 2 4 5 3 6 7	2074.6	2725.9	155.6	204.4	-24.4
1 2 4 5 3 7 6	2074.6	2725.9	155.6	204.4	-24.4
1 2 4 5 6 3 7	2032.5	2768	152.4	207.6	-27.6
1 2 4 5 6 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 4 5 7 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 2 4 5 7 6 3	1194.3	3606.2	89.6	270.4	-90.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 2 4 6 3 5 7	2181.5	2619	163.6	196.4	-16.4
1 2 4 6 3 7 5	2181.5	2619	163.6	196.4	-16.4
1 2 4 6 5 3 7	2032.5	2768	152.4	207.6	-27.6
1 2 4 6 5 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 4 6 7 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 2 4 6 7 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 4 7 3 5 6	1385.4	3415.1	103.9	256.1	-76.1
1 2 4 7 3 6 5	1385.4	3415.1	103.9	256.1	-76.1
1 2 4 7 5 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 2 4 7 5 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 4 7 6 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 2 4 7 6 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 5 3 4 6 7	2209.1	2591.4	165.7	194.3	-14.3
1 2 5 3 4 7 6	2209.1	2591.4	165.7	194.3	-14.3
1 2 5 3 6 4 7	2209.1	2591.4	165.7	194.3	-14.3
1 2 5 3 6 7 4	2209.1	2591.4	165.7	194.3	-14.3
1 2 5 3 7 4 6	2209.1	2591.4	165.7	194.3	-14.3
1 2 5 3 7 6 4	2209.1	2591.4	165.7	194.3	-14.3
1 2 5 4 3 6 7	2074.6	2725.9	155.6	204.4	-24.4
1 2 5 4 3 7 6	2074.6	2725.9	155.6	204.4	-24.4
1 2 5 4 6 3 7	2032.5	2768	152.4	207.6	-27.6
1 2 5 4 6 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 5 4 7 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 2 5 4 7 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 5 6 3 4 7	2167	2633.5	162.5	197.5	-17.5
1 2 5 6 3 7 4	2167	2633.5	162.5	197.5	-17.5
1 2 5 6 4 3 7	2032.5	2768	152.4	207.6	-27.6
1 2 5 6 4 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 5 6 7 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 2 5 6 7 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 5 7 3 4 6	1370.9	3429.6	102.8	257.2	-77.2
1 2 5 7 3 6 4	1370.9	3429.6	102.8	257.2	-77.2
1 2 5 7 4 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 2 5 7 4 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 5 7 6 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 2 5 7 6 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 6 3 4 5 7	2316	2484.5	173.7	186.3	-6.3
1 2 6 3 4 7 5	2316	2484.5	173.7	186.3	-6.3
1 2 6 3 5 4 7	2316	2484.5	173.7	186.3	-6.3
1 2 6 3 5 7 4	2316	2484.5	173.7	186.3	-6.3

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 2 6 3 7 4 5	2316	2484.5	173.7	186.3	-6.3
1 2 6 3 7 5 4	2316	2484.5	173.7	186.3	-6.3
1 2 6 4 3 5 7	2181.5	2619	163.6	196.4	-16.4
1 2 6 4 3 7 5	2181.5	2619	163.6	196.4	-16.4
1 2 6 4 5 3 7	2032.5	2768	152.4	207.6	-27.6
1 2 6 4 5 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 6 4 7 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 2 6 4 7 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 6 5 3 4 7	2167	2633.5	162.5	197.5	-17.5
1 2 6 5 3 7 4	2167	2633.5	162.5	197.5	-17.5
1 2 6 5 4 3 7	2032.5	2768	152.4	207.6	-27.6
1 2 6 5 4 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 6 5 7 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 2 6 5 7 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 6 7 3 4 5	1477.8	3322.7	110.8	249.2	-69.2
1 2 6 7 3 5 4	1477.8	3322.7	110.8	249.2	-69.2
1 2 6 7 4 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 2 6 7 4 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 6 7 5 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 2 6 7 5 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 7 3 4 5 6	1519.9	3280.6	114.0	246.0	-66.0
1 2 7 3 4 6 5	1519.9	3280.6	114.0	246.0	-66.0
1 2 7 3 5 4 6	1519.9	3280.6	114.0	246.0	-66.0
1 2 7 3 5 6 4	1519.9	3280.6	114.0	246.0	-66.0
1 2 7 3 6 4 5	1519.9	3280.6	114.0	246.0	-66.0
1 2 7 3 6 5 4	1519.9	3280.6	114.0	246.0	-66.0
1 2 7 4 3 5 6	1385.4	3415.1	103.9	256.1	-76.1
1 2 7 4 3 6 5	1385.4	3415.1	103.9	256.1	-76.1
1 2 7 4 5 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 2 7 4 5 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 7 4 6 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 2 7 4 6 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 7 5 3 4 6	1370.9	3429.6	102.8	257.2	-77.2
1 2 7 5 3 6 4	1370.9	3429.6	102.8	257.2	-77.2
1 2 7 5 4 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 2 7 5 4 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 7 5 6 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 2 7 5 6 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 7 6 3 4 5	1477.8	3322.7	110.8	249.2	-69.2
1 2 7 6 3 5 4	1477.8	3322.7	110.8	249.2	-69.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 2 7 6 4 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 2 7 6 4 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 2 7 6 5 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 2 7 6 5 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 3 2 4 5 6 7	3069.2	1731.3	230.2	129.8	50.2
1 3 2 4 5 7 6	3069.2	1731.3	230.2	129.8	50.2
1 3 2 4 6 5 7	3069.2	1731.3	230.2	129.8	50.2
1 3 2 4 6 7 5	3069.2	1731.3	230.2	129.8	50.2
1 3 2 4 7 5 6	3069.2	1731.3	230.2	129.8	50.2
1 3 2 4 7 6 5	3069.2	1731.3	230.2	129.8	50.2
1 3 2 5 4 6 7	3069.2	1731.3	230.2	129.8	50.2
1 3 2 5 4 7 6	3069.2	1731.3	230.2	129.8	50.2
1 3 2 5 6 4 7	3069.2	1731.3	230.2	129.8	50.2
1 3 2 5 6 7 4	3069.2	1731.3	230.2	129.8	50.2
1 3 2 5 7 4 6	3069.2	1731.3	230.2	129.8	50.2
1 3 2 5 7 6 4	3069.2	1731.3	230.2	129.8	50.2
1 3 2 6 4 5 7	3069.2	1731.3	230.2	129.8	50.2
1 3 2 6 4 7 5	3069.2	1731.3	230.2	129.8	50.2
1 3 2 6 5 4 7	3069.2	1731.3	230.2	129.8	50.2
1 3 2 6 5 7 4	3069.2	1731.3	230.2	129.8	50.2
1 3 2 6 7 4 5	3069.2	1731.3	230.2	129.8	50.2
1 3 2 6 7 5 4	3069.2	1731.3	230.2	129.8	50.2
1 3 2 7 4 5 6	3069.2	1731.3	230.2	129.8	50.2
1 3 2 7 4 6 5	3069.2	1731.3	230.2	129.8	50.2
1 3 2 7 5 4 6	3069.2	1731.3	230.2	129.8	50.2
1 3 2 7 5 6 4	3069.2	1731.3	230.2	129.8	50.2
1 3 2 7 6 4 5	3069.2	1731.3	230.2	129.8	50.2
1 3 2 7 6 5 4	3069.2	1731.3	230.2	129.8	50.2
1 3 4 2 5 6 7	3069.2	1731.3	230.2	129.8	50.2
1 3 4 2 5 7 6	3069.2	1731.3	230.2	129.8	50.2
1 3 4 2 6 5 7	3069.2	1731.3	230.2	129.8	50.2
1 3 4 2 6 7 5	3069.2	1731.3	230.2	129.8	50.2
1 3 4 2 7 5 6	3069.2	1731.3	230.2	129.8	50.2
1 3 4 2 7 6 5	3069.2	1731.3	230.2	129.8	50.2
1 3 4 5 2 6 7	3069.2	1731.3	230.2	129.8	50.2
1 3 4 5 2 7 6	3069.2	1731.3	230.2	129.8	50.2
1 3 4 5 6 2 7	3069.2	1731.3	230.2	129.8	50.2
1 3 4 5 6 7 2	3069.2	1731.3	230.2	129.8	50.2
1 3 4 5 7 2 6	3069.2	1731.3	230.2	129.8	50.2
1 3 4 5 7 6 2	3069.2	1731.3	230.2	129.8	50.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 3 4 6 2 5 7	3069.2	1731.3	230.2	129.8	50.2
1 3 4 6 2 7 5	3069.2	1731.3	230.2	129.8	50.2
1 3 4 6 5 2 7	3069.2	1731.3	230.2	129.8	50.2
1 3 4 6 5 7 2	3069.2	1731.3	230.2	129.8	50.2
1 3 4 6 7 2 5	3069.2	1731.3	230.2	129.8	50.2
1 3 4 6 7 5 2	3069.2	1731.3	230.2	129.8	50.2
1 3 4 7 2 5 6	3069.2	1731.3	230.2	129.8	50.2
1 3 4 7 2 6 5	3069.2	1731.3	230.2	129.8	50.2
1 3 4 7 5 2 6	3069.2	1731.3	230.2	129.8	50.2
1 3 4 7 5 6 2	3069.2	1731.3	230.2	129.8	50.2
1 3 4 7 6 2 5	3069.2	1731.3	230.2	129.8	50.2
1 3 4 7 6 5 2	3069.2	1731.3	230.2	129.8	50.2
1 3 5 2 4 6 7	3069.2	1731.3	230.2	129.8	50.2
1 3 5 2 4 7 6	3069.2	1731.3	230.2	129.8	50.2
1 3 5 2 6 4 7	3069.2	1731.3	230.2	129.8	50.2
1 3 5 2 6 7 4	3069.2	1731.3	230.2	129.8	50.2
1 3 5 2 7 4 6	3069.2	1731.3	230.2	129.8	50.2
1 3 5 2 7 6 4	3069.2	1731.3	230.2	129.8	50.2
1 3 5 4 2 6 7	3069.2	1731.3	230.2	129.8	50.2
1 3 5 4 2 7 6	3069.2	1731.3	230.2	129.8	50.2
1 3 5 4 6 2 7	3069.2	1731.3	230.2	129.8	50.2
1 3 5 4 6 7 2	3069.2	1731.3	230.2	129.8	50.2
1 3 5 4 7 2 6	3069.2	1731.3	230.2	129.8	50.2
1 3 5 4 7 6 2	3069.2	1731.3	230.2	129.8	50.2
1 3 5 6 2 4 7	3069.2	1731.3	230.2	129.8	50.2
1 3 5 6 2 7 4	3069.2	1731.3	230.2	129.8	50.2
1 3 5 6 4 2 7	3069.2	1731.3	230.2	129.8	50.2
1 3 5 6 4 7 2	3069.2	1731.3	230.2	129.8	50.2
1 3 5 6 7 2 4	3069.2	1731.3	230.2	129.8	50.2
1 3 5 6 7 4 2	3069.2	1731.3	230.2	129.8	50.2
1 3 5 7 2 4 6	3069.2	1731.3	230.2	129.8	50.2
1 3 5 7 2 6 4	3069.2	1731.3	230.2	129.8	50.2
1 3 5 7 4 2 6	3069.2	1731.3	230.2	129.8	50.2
1 3 5 7 4 6 2	3069.2	1731.3	230.2	129.8	50.2
1 3 5 7 6 2 4	3069.2	1731.3	230.2	129.8	50.2
1 3 5 7 6 4 2	3069.2	1731.3	230.2	129.8	50.2
1 3 6 2 4 5 7	3069.2	1731.3	230.2	129.8	50.2
1 3 6 2 4 7 5	3069.2	1731.3	230.2	129.8	50.2
1 3 6 2 5 4 7	3069.2	1731.3	230.2	129.8	50.2
1 3 6 2 5 7 4	3069.2	1731.3	230.2	129.8	50.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 3 6 2 7 4 5	3069.2	1731.3	230.2	129.8	50.2
1 3 6 2 7 5 4	3069.2	1731.3	230.2	129.8	50.2
1 3 6 4 2 5 7	3069.2	1731.3	230.2	129.8	50.2
1 3 6 4 2 7 5	3069.2	1731.3	230.2	129.8	50.2
1 3 6 4 5 2 7	3069.2	1731.3	230.2	129.8	50.2
1 3 6 4 5 7 2	3069.2	1731.3	230.2	129.8	50.2
1 3 6 4 7 2 5	3069.2	1731.3	230.2	129.8	50.2
1 3 6 4 7 5 2	3069.2	1731.3	230.2	129.8	50.2
1 3 6 5 2 4 7	3069.2	1731.3	230.2	129.8	50.2
1 3 6 5 2 7 4	3069.2	1731.3	230.2	129.8	50.2
1 3 6 5 4 2 7	3069.2	1731.3	230.2	129.8	50.2
1 3 6 5 4 7 2	3069.2	1731.3	230.2	129.8	50.2
1 3 6 5 7 2 4	3069.2	1731.3	230.2	129.8	50.2
1 3 6 5 7 4 2	3069.2	1731.3	230.2	129.8	50.2
1 3 6 7 2 4 5	3069.2	1731.3	230.2	129.8	50.2
1 3 6 7 2 5 4	3069.2	1731.3	230.2	129.8	50.2
1 3 6 7 4 2 5	3069.2	1731.3	230.2	129.8	50.2
1 3 6 7 4 5 2	3069.2	1731.3	230.2	129.8	50.2
1 3 6 7 5 2 4	3069.2	1731.3	230.2	129.8	50.2
1 3 6 7 5 4 2	3069.2	1731.3	230.2	129.8	50.2
1 3 7 2 4 5 6	3069.2	1731.3	230.2	129.8	50.2
1 3 7 2 4 6 5	3069.2	1731.3	230.2	129.8	50.2
1 3 7 2 5 4 6	3069.2	1731.3	230.2	129.8	50.2
1 3 7 2 5 6 4	3069.2	1731.3	230.2	129.8	50.2
1 3 7 2 6 4 5	3069.2	1731.3	230.2	129.8	50.2
1 3 7 2 6 5 4	3069.2	1731.3	230.2	129.8	50.2
1 3 7 4 2 5 6	3069.2	1731.3	230.2	129.8	50.2
1 3 7 4 2 6 5	3069.2	1731.3	230.2	129.8	50.2
1 3 7 4 5 2 6	3069.2	1731.3	230.2	129.8	50.2
1 3 7 4 5 6 2	3069.2	1731.3	230.2	129.8	50.2
1 3 7 4 6 2 5	3069.2	1731.3	230.2	129.8	50.2
1 3 7 4 6 5 2	3069.2	1731.3	230.2	129.8	50.2
1 3 7 5 2 4 6	3069.2	1731.3	230.2	129.8	50.2
1 3 7 5 2 6 4	3069.2	1731.3	230.2	129.8	50.2
1 3 7 5 4 2 6	3069.2	1731.3	230.2	129.8	50.2
1 3 7 5 4 6 2	3069.2	1731.3	230.2	129.8	50.2
1 3 7 5 6 2 4	3069.2	1731.3	230.2	129.8	50.2
1 3 7 5 6 4 2	3069.2	1731.3	230.2	129.8	50.2
1 3 7 6 2 4 5	3069.2	1731.3	230.2	129.8	50.2
1 3 7 6 2 5 4	3069.2	1731.3	230.2	129.8	50.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 3 7 6 4 2 5	3069.2	1731.3	230.2	129.8	50.2
1 3 7 6 4 5 2	3069.2	1731.3	230.2	129.8	50.2
1 3 7 6 5 2 4	3069.2	1731.3	230.2	129.8	50.2
1 3 7 6 5 4 2	3069.2	1731.3	230.2	129.8	50.2
1 4 2 3 5 6 7	2223.6	2576.9	166.8	193.2	-13.2
1 4 2 3 5 7 6	2223.6	2576.9	166.8	193.2	-13.2
1 4 2 3 6 5 7	2223.6	2576.9	166.8	193.2	-13.2
1 4 2 3 6 7 5	2223.6	2576.9	166.8	193.2	-13.2
1 4 2 3 7 5 6	2223.6	2576.9	166.8	193.2	-13.2
1 4 2 3 7 6 5	2223.6	2576.9	166.8	193.2	-13.2
1 4 2 5 3 6 7	2074.6	2725.9	155.6	204.4	-24.4
1 4 2 5 3 7 6	2074.6	2725.9	155.6	204.4	-24.4
1 4 2 5 6 3 7	2032.5	2768	152.4	207.6	-27.6
1 4 2 5 6 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 2 5 7 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 4 2 5 7 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 2 6 3 5 7	2181.5	2619	163.6	196.4	-16.4
1 4 2 6 3 7 5	2181.5	2619	163.6	196.4	-16.4
1 4 2 6 5 3 7	2032.5	2768	152.4	207.6	-27.6
1 4 2 6 5 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 2 6 7 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 4 2 6 7 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 2 7 3 5 6	1385.4	3415.1	103.9	256.1	-76.1
1 4 2 7 3 6 5	1385.4	3415.1	103.9	256.1	-76.1
1 4 2 7 5 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 4 2 7 5 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 2 7 6 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 4 2 7 6 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 3 2 5 6 7	2934.7	1865.8	220.1	139.9	40.1
1 4 3 2 5 7 6	2934.7	1865.8	220.1	139.9	40.1
1 4 3 2 6 5 7	2934.7	1865.8	220.1	139.9	40.1
1 4 3 2 6 7 5	2934.7	1865.8	220.1	139.9	40.1
1 4 3 2 7 5 6	2934.7	1865.8	220.1	139.9	40.1
1 4 3 2 7 6 5	2934.7	1865.8	220.1	139.9	40.1
1 4 3 5 2 6 7	2934.7	1865.8	220.1	139.9	40.1
1 4 3 5 2 7 6	2934.7	1865.8	220.1	139.9	40.1
1 4 3 5 6 2 7	2934.7	1865.8	220.1	139.9	40.1
1 4 3 5 6 7 2	2934.7	1865.8	220.1	139.9	40.1
1 4 3 5 7 2 6	2934.7	1865.8	220.1	139.9	40.1
1 4 3 5 7 6 2	2934.7	1865.8	220.1	139.9	40.1

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 4 3 6 2 5 7	2934.7	1865.8	220.1	139.9	40.1
1 4 3 6 2 7 5	2934.7	1865.8	220.1	139.9	40.1
1 4 3 6 5 2 7	2934.7	1865.8	220.1	139.9	40.1
1 4 3 6 5 7 2	2934.7	1865.8	220.1	139.9	40.1
1 4 3 6 7 2 5	2934.7	1865.8	220.1	139.9	40.1
1 4 3 6 7 5 2	2934.7	1865.8	220.1	139.9	40.1
1 4 3 7 2 5 6	2934.7	1865.8	220.1	139.9	40.1
1 4 3 7 2 6 5	2934.7	1865.8	220.1	139.9	40.1
1 4 3 7 5 2 6	2934.7	1865.8	220.1	139.9	40.1
1 4 3 7 5 6 2	2934.7	1865.8	220.1	139.9	40.1
1 4 3 7 6 2 5	2934.7	1865.8	220.1	139.9	40.1
1 4 3 7 6 5 2	2934.7	1865.8	220.1	139.9	40.1
1 4 5 2 3 6 7	2074.6	2725.9	155.6	204.4	-24.4
1 4 5 2 3 7 6	2074.6	2725.9	155.6	204.4	-24.4
1 4 5 2 6 3 7	2032.5	2768	152.4	207.6	-27.6
1 4 5 2 6 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 5 2 7 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 4 5 2 7 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 5 3 2 6 7	2785.7	2014.8	208.9	151.1	28.9
1 4 5 3 2 7 6	2785.7	2014.8	208.9	151.1	28.9
1 4 5 3 6 2 7	2785.7	2014.8	208.9	151.1	28.9
1 4 5 3 6 7 2	2785.7	2014.8	208.9	151.1	28.9
1 4 5 3 7 2 6	2785.7	2014.8	208.9	151.1	28.9
1 4 5 3 7 6 2	2785.7	2014.8	208.9	151.1	28.9
1 4 5 6 2 3 7	2032.5	2768	152.4	207.6	-27.6
1 4 5 6 2 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 5 6 3 2 7	2743.6	2056.9	205.7	154.3	25.7
1 4 5 6 3 7 2	2743.6	2056.9	205.7	154.3	25.7
1 4 5 6 7 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 5 6 7 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 4 5 7 2 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 4 5 7 2 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 5 7 3 2 6	1947.5	2853	146.0	214.0	-34.0
1 4 5 7 3 6 2	1947.5	2853	146.0	214.0	-34.0
1 4 5 7 6 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 5 7 6 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 4 6 2 3 5 7	2181.5	2619	163.6	196.4	-16.4
1 4 6 2 3 7 5	2181.5	2619	163.6	196.4	-16.4
1 4 6 2 5 3 7	2032.5	2768	152.4	207.6	-27.6
1 4 6 2 5 7 3	1194.3	3606.2	89.6	270.4	-90.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 4 6 2 7 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 4 6 2 7 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 6 3 2 5 7	2892.6	1907.9	216.9	143.1	36.9
1 4 6 3 2 7 5	2892.6	1907.9	216.9	143.1	36.9
1 4 6 3 5 2 7	2892.6	1907.9	216.9	143.1	36.9
1 4 6 3 5 7 2	2892.6	1907.9	216.9	143.1	36.9
1 4 6 3 7 2 5	2892.6	1907.9	216.9	143.1	36.9
1 4 6 3 7 5 2	2892.6	1907.9	216.9	143.1	36.9
1 4 6 5 2 3 7	2032.5	2768	152.4	207.6	-27.6
1 4 6 5 2 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 6 5 3 2 7	2743.6	2056.9	205.7	154.3	25.7
1 4 6 5 3 7 2	2743.6	2056.9	205.7	154.3	25.7
1 4 6 5 7 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 6 5 7 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 4 6 7 2 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 4 6 7 2 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 6 7 3 2 5	2054.4	2746.1	154.1	205.9	-25.9
1 4 6 7 3 5 2	2054.4	2746.1	154.1	205.9	-25.9
1 4 6 7 5 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 6 7 5 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 4 7 2 3 5 6	1385.4	3415.1	103.9	256.1	-76.1
1 4 7 2 3 6 5	1385.4	3415.1	103.9	256.1	-76.1
1 4 7 2 5 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 4 7 2 5 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 7 2 6 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 4 7 2 6 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 7 3 2 5 6	2096.5	2704	157.2	202.8	-22.8
1 4 7 3 2 6 5	2096.5	2704	157.2	202.8	-22.8
1 4 7 3 5 2 6	2096.5	2704	157.2	202.8	-22.8
1 4 7 3 5 6 2	2096.5	2704	157.2	202.8	-22.8
1 4 7 3 6 2 5	2096.5	2704	157.2	202.8	-22.8
1 4 7 3 6 5 2	2096.5	2704	157.2	202.8	-22.8
1 4 7 5 2 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 4 7 5 2 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 7 5 3 2 6	1947.5	2853	146.0	214.0	-34.0
1 4 7 5 3 6 2	1947.5	2853	146.0	214.0	-34.0
1 4 7 5 6 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 7 5 6 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 4 7 6 2 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 4 7 6 2 5 3	1194.3	3606.2	89.6	270.4	-90.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 4 7 6 3 2 5	2054.4	2746.1	154.1	205.9	-25.9
1 4 7 6 3 5 2	2054.4	2746.1	154.1	205.9	-25.9
1 4 7 6 5 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 4 7 6 5 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 5 2 3 4 6 7	2209.1	2591.4	165.7	194.3	-14.3
1 5 2 3 4 7 6	2209.1	2591.4	165.7	194.3	-14.3
1 5 2 3 6 4 7	2209.1	2591.4	165.7	194.3	-14.3
1 5 2 3 6 7 4	2209.1	2591.4	165.7	194.3	-14.3
1 5 2 3 7 4 6	2209.1	2591.4	165.7	194.3	-14.3
1 5 2 3 7 6 4	2209.1	2591.4	165.7	194.3	-14.3
1 5 2 4 3 6 7	2074.6	2725.9	155.6	204.4	-24.4
1 5 2 4 3 7 6	2074.6	2725.9	155.6	204.4	-24.4
1 5 2 4 6 3 7	2032.5	2768	152.4	207.6	-27.6
1 5 2 4 6 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 2 4 7 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 5 2 4 7 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 2 6 3 4 7	2167	2633.5	162.5	197.5	-17.5
1 5 2 6 3 7 4	2167	2633.5	162.5	197.5	-17.5
1 5 2 6 4 3 7	2032.5	2768	152.4	207.6	-27.6
1 5 2 6 4 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 2 6 7 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 5 2 6 7 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 2 7 3 4 6	1370.9	3429.6	102.8	257.2	-77.2
1 5 2 7 3 6 4	1370.9	3429.6	102.8	257.2	-77.2
1 5 2 7 4 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 5 2 7 4 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 2 7 6 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 5 2 7 6 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 3 2 4 6 7	2920.2	1880.3	219.0	141.0	39.0
1 5 3 2 4 7 6	2920.2	1880.3	219.0	141.0	39.0
1 5 3 2 6 4 7	2920.2	1880.3	219.0	141.0	39.0
1 5 3 2 6 7 4	2920.2	1880.3	219.0	141.0	39.0
1 5 3 2 7 4 6	2920.2	1880.3	219.0	141.0	39.0
1 5 3 2 7 6 4	2920.2	1880.3	219.0	141.0	39.0
1 5 3 4 2 6 7	2920.2	1880.3	219.0	141.0	39.0
1 5 3 4 2 7 6	2920.2	1880.3	219.0	141.0	39.0
1 5 3 4 6 2 7	2920.2	1880.3	219.0	141.0	39.0
1 5 3 4 6 7 2	2920.2	1880.3	219.0	141.0	39.0
1 5 3 4 7 2 6	2920.2	1880.3	219.0	141.0	39.0
1 5 3 4 7 6 2	2920.2	1880.3	219.0	141.0	39.0

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 5 3 6 2 4 7	2920.2	1880.3	219.0	141.0	39.0
1 5 3 6 2 7 4	2920.2	1880.3	219.0	141.0	39.0
1 5 3 6 4 2 7	2920.2	1880.3	219.0	141.0	39.0
1 5 3 6 4 7 2	2920.2	1880.3	219.0	141.0	39.0
1 5 3 6 7 2 4	2920.2	1880.3	219.0	141.0	39.0
1 5 3 6 7 4 2	2920.2	1880.3	219.0	141.0	39.0
1 5 3 7 2 4 6	2920.2	1880.3	219.0	141.0	39.0
1 5 3 7 2 6 4	2920.2	1880.3	219.0	141.0	39.0
1 5 3 7 4 2 6	2920.2	1880.3	219.0	141.0	39.0
1 5 3 7 4 6 2	2920.2	1880.3	219.0	141.0	39.0
1 5 3 7 6 2 4	2920.2	1880.3	219.0	141.0	39.0
1 5 3 7 6 4 2	2920.2	1880.3	219.0	141.0	39.0
1 5 4 2 3 6 7	2074.6	2725.9	155.6	204.4	-24.4
1 5 4 2 3 7 6	2074.6	2725.9	155.6	204.4	-24.4
1 5 4 2 6 3 7	2032.5	2768	152.4	207.6	-27.6
1 5 4 2 6 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 4 2 7 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 5 4 2 7 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 4 3 2 6 7	2785.7	2014.8	208.9	151.1	28.9
1 5 4 3 2 7 6	2785.7	2014.8	208.9	151.1	28.9
1 5 4 3 6 2 7	2785.7	2014.8	208.9	151.1	28.9
1 5 4 3 6 7 2	2785.7	2014.8	208.9	151.1	28.9
1 5 4 3 7 2 6	2785.7	2014.8	208.9	151.1	28.9
1 5 4 3 7 6 2	2785.7	2014.8	208.9	151.1	28.9
1 5 4 6 2 3 7	2032.5	2768	152.4	207.6	-27.6
1 5 4 6 2 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 4 6 3 2 7	2743.6	2056.9	205.7	154.3	25.7
1 5 4 6 3 7 2	2743.6	2056.9	205.7	154.3	25.7
1 5 4 6 7 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 4 6 7 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 5 4 7 2 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 5 4 7 2 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 4 7 3 2 6	1947.5	2853	146.0	214.0	-34.0
1 5 4 7 3 6 2	1947.5	2853	146.0	214.0	-34.0
1 5 4 7 6 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 4 7 6 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 5 6 2 3 4 7	2167	2633.5	162.5	197.5	-17.5
1 5 6 2 3 7 4	2167	2633.5	162.5	197.5	-17.5
1 5 6 2 4 3 7	2032.5	2768	152.4	207.6	-27.6
1 5 6 2 4 7 3	1194.3	3606.2	89.6	270.4	-90.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 5 6 2 7 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 5 6 2 7 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 6 3 2 4 7	2878.1	1922.4	215.8	144.2	35.8
1 5 6 3 2 7 4	2878.1	1922.4	215.8	144.2	35.8
1 5 6 3 4 2 7	2878.1	1922.4	215.8	144.2	35.8
1 5 6 3 4 7 2	2878.1	1922.4	215.8	144.2	35.8
1 5 6 3 7 2 4	2878.1	1922.4	215.8	144.2	35.8
1 5 6 3 7 4 2	2878.1	1922.4	215.8	144.2	35.8
1 5 6 4 2 3 7	2032.5	2768	152.4	207.6	-27.6
1 5 6 4 2 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 6 4 3 2 7	2743.6	2056.9	205.7	154.3	25.7
1 5 6 4 3 7 2	2743.6	2056.9	205.7	154.3	25.7
1 5 6 4 7 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 6 4 7 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 5 6 7 2 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 5 6 7 2 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 6 7 3 2 4	2039.9	2760.6	153.0	207.0	-27.0
1 5 6 7 3 4 2	2039.9	2760.6	153.0	207.0	-27.0
1 5 6 7 4 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 6 7 4 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 5 7 2 3 4 6	1370.9	3429.6	102.8	257.2	-77.2
1 5 7 2 3 6 4	1370.9	3429.6	102.8	257.2	-77.2
1 5 7 2 4 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 5 7 2 4 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 7 2 6 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 5 7 2 6 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 7 3 2 4 6	2082	2718.5	156.1	203.9	-23.9
1 5 7 3 2 6 4	2082	2718.5	156.1	203.9	-23.9
1 5 7 3 4 2 6	2082	2718.5	156.1	203.9	-23.9
1 5 7 3 4 6 2	2082	2718.5	156.1	203.9	-23.9
1 5 7 3 6 2 4	2082	2718.5	156.1	203.9	-23.9
1 5 7 3 6 4 2	2082	2718.5	156.1	203.9	-23.9
1 5 7 4 2 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 5 7 4 2 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 7 4 3 2 6	1947.5	2853	146.0	214.0	-34.0
1 5 7 4 3 6 2	1947.5	2853	146.0	214.0	-34.0
1 5 7 4 6 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 7 4 6 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 5 7 6 2 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 5 7 6 2 4 3	1194.3	3606.2	89.6	270.4	-90.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 5 7 6 3 2 4	2039.9	2760.6	153.0	207.0	-27.0
1 5 7 6 3 4 2	2039.9	2760.6	153.0	207.0	-27.0
1 5 7 6 4 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 5 7 6 4 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 6 2 3 4 5 7	2316	2484.5	173.7	186.3	-6.3
1 6 2 3 4 7 5	2316	2484.5	173.7	186.3	-6.3
1 6 2 3 5 4 7	2316	2484.5	173.7	186.3	-6.3
1 6 2 3 5 7 4	2316	2484.5	173.7	186.3	-6.3
1 6 2 3 7 4 5	2316	2484.5	173.7	186.3	-6.3
1 6 2 3 7 5 4	2316	2484.5	173.7	186.3	-6.3
1 6 2 4 3 5 7	2181.5	2619	163.6	196.4	-16.4
1 6 2 4 3 7 5	2181.5	2619	163.6	196.4	-16.4
1 6 2 4 5 3 7	2032.5	2768	152.4	207.6	-27.6
1 6 2 4 5 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 2 4 7 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 6 2 4 7 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 2 5 3 4 7	2167	2633.5	162.5	197.5	-17.5
1 6 2 5 3 7 4	2167	2633.5	162.5	197.5	-17.5
1 6 2 5 4 3 7	2032.5	2768	152.4	207.6	-27.6
1 6 2 5 4 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 2 5 7 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 6 2 5 7 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 2 7 3 4 5	1477.8	3322.7	110.8	249.2	-69.2
1 6 2 7 3 5 4	1477.8	3322.7	110.8	249.2	-69.2
1 6 2 7 4 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 6 2 7 4 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 2 7 5 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 6 2 7 5 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 3 2 4 5 7	3027.1	1773.4	227.0	133.0	47.0
1 6 3 2 4 7 5	3027.1	1773.4	227.0	133.0	47.0
1 6 3 2 5 4 7	3027.1	1773.4	227.0	133.0	47.0
1 6 3 2 5 7 4	3027.1	1773.4	227.0	133.0	47.0
1 6 3 2 7 4 5	3027.1	1773.4	227.0	133.0	47.0
1 6 3 2 7 5 4	3027.1	1773.4	227.0	133.0	47.0
1 6 3 4 2 5 7	3027.1	1773.4	227.0	133.0	47.0
1 6 3 4 2 7 5	3027.1	1773.4	227.0	133.0	47.0
1 6 3 4 5 2 7	3027.1	1773.4	227.0	133.0	47.0
1 6 3 4 5 7 2	3027.1	1773.4	227.0	133.0	47.0
1 6 3 4 7 2 5	3027.1	1773.4	227.0	133.0	47.0
1 6 3 4 7 5 2	3027.1	1773.4	227.0	133.0	47.0

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 6 3 5 2 4 7	3027.1	1773.4	227.0	133.0	47.0
1 6 3 5 2 7 4	3027.1	1773.4	227.0	133.0	47.0
1 6 3 5 4 2 7	3027.1	1773.4	227.0	133.0	47.0
1 6 3 5 4 7 2	3027.1	1773.4	227.0	133.0	47.0
1 6 3 5 7 2 4	3027.1	1773.4	227.0	133.0	47.0
1 6 3 5 7 4 2	3027.1	1773.4	227.0	133.0	47.0
1 6 3 7 2 4 5	3027.1	1773.4	227.0	133.0	47.0
1 6 3 7 2 5 4	3027.1	1773.4	227.0	133.0	47.0
1 6 3 7 4 2 5	3027.1	1773.4	227.0	133.0	47.0
1 6 3 7 4 5 2	3027.1	1773.4	227.0	133.0	47.0
1 6 3 7 5 2 4	3027.1	1773.4	227.0	133.0	47.0
1 6 3 7 5 4 2	3027.1	1773.4	227.0	133.0	47.0
1 6 4 2 3 5 7	2181.5	2619	163.6	196.4	-16.4
1 6 4 2 3 7 5	2181.5	2619	163.6	196.4	-16.4
1 6 4 2 5 3 7	2032.5	2768	152.4	207.6	-27.6
1 6 4 2 5 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 4 2 7 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 6 4 2 7 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 4 3 2 5 7	2892.6	1907.9	216.9	143.1	36.9
1 6 4 3 2 7 5	2892.6	1907.9	216.9	143.1	36.9
1 6 4 3 5 2 7	2892.6	1907.9	216.9	143.1	36.9
1 6 4 3 5 7 2	2892.6	1907.9	216.9	143.1	36.9
1 6 4 3 7 2 5	2892.6	1907.9	216.9	143.1	36.9
1 6 4 3 7 5 2	2892.6	1907.9	216.9	143.1	36.9
1 6 4 5 2 3 7	2032.5	2768	152.4	207.6	-27.6
1 6 4 5 2 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 4 5 3 2 7	2743.6	2056.9	205.7	154.3	25.7
1 6 4 5 3 7 2	2743.6	2056.9	205.7	154.3	25.7
1 6 4 5 7 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 4 5 7 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 6 4 7 2 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 6 4 7 2 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 4 7 3 2 5	2054.4	2746.1	154.1	205.9	-25.9
1 6 4 7 3 5 2	2054.4	2746.1	154.1	205.9	-25.9
1 6 4 7 5 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 4 7 5 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 6 5 2 3 4 7	2167	2633.5	162.5	197.5	-17.5
1 6 5 2 3 7 4	2167	2633.5	162.5	197.5	-17.5
1 6 5 2 4 3 7	2032.5	2768	152.4	207.6	-27.6
1 6 5 2 4 7 3	1194.3	3606.2	89.6	270.4	-90.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 6 5 2 7 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 6 5 2 7 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 5 3 2 4 7	2878.1	1922.4	215.8	144.2	35.8
1 6 5 3 2 7 4	2878.1	1922.4	215.8	144.2	35.8
1 6 5 3 4 2 7	2878.1	1922.4	215.8	144.2	35.8
1 6 5 3 4 7 2	2878.1	1922.4	215.8	144.2	35.8
1 6 5 3 7 2 4	2878.1	1922.4	215.8	144.2	35.8
1 6 5 3 7 4 2	2878.1	1922.4	215.8	144.2	35.8
1 6 5 4 2 3 7	2032.5	2768	152.4	207.6	-27.6
1 6 5 4 2 7 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 5 4 3 2 7	2743.6	2056.9	205.7	154.3	25.7
1 6 5 4 3 7 2	2743.6	2056.9	205.7	154.3	25.7
1 6 5 4 7 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 5 4 7 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 6 5 7 2 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 6 5 7 2 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 5 7 3 2 4	2039.9	2760.6	153.0	207.0	-27.0
1 6 5 7 3 4 2	2039.9	2760.6	153.0	207.0	-27.0
1 6 5 7 4 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 5 7 4 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 6 7 2 3 4 5	1477.8	3322.7	110.8	249.2	-69.2
1 6 7 2 3 5 4	1477.8	3322.7	110.8	249.2	-69.2
1 6 7 2 4 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 6 7 2 4 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 7 2 5 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 6 7 2 5 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 7 3 2 4 5	2188.9	2611.6	164.2	195.8	-15.8
1 6 7 3 2 5 4	2188.9	2611.6	164.2	195.8	-15.8
1 6 7 3 4 2 5	2188.9	2611.6	164.2	195.8	-15.8
1 6 7 3 4 5 2	2188.9	2611.6	164.2	195.8	-15.8
1 6 7 3 5 2 4	2188.9	2611.6	164.2	195.8	-15.8
1 6 7 3 5 4 2	2188.9	2611.6	164.2	195.8	-15.8
1 6 7 4 2 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 6 7 4 2 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 7 4 3 2 5	2054.4	2746.1	154.1	205.9	-25.9
1 6 7 4 3 5 2	2054.4	2746.1	154.1	205.9	-25.9
1 6 7 4 5 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 7 4 5 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 6 7 5 2 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 6 7 5 2 4 3	1194.3	3606.2	89.6	270.4	-90.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 6 7 5 3 2 4	2039.9	2760.6	153.0	207.0	-27.0
1 6 7 5 3 4 2	2039.9	2760.6	153.0	207.0	-27.0
1 6 7 5 4 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 6 7 5 4 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 7 2 3 4 5 6	1519.9	3280.6	114.0	246.0	-66.0
1 7 2 3 4 6 5	1519.9	3280.6	114.0	246.0	-66.0
1 7 2 3 5 4 6	1519.9	3280.6	114.0	246.0	-66.0
1 7 2 3 5 6 4	1519.9	3280.6	114.0	246.0	-66.0
1 7 2 3 6 4 5	1519.9	3280.6	114.0	246.0	-66.0
1 7 2 3 6 5 4	1519.9	3280.6	114.0	246.0	-66.0
1 7 2 4 3 5 6	1385.4	3415.1	103.9	256.1	-76.1
1 7 2 4 3 6 5	1385.4	3415.1	103.9	256.1	-76.1
1 7 2 4 5 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 7 2 4 5 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 2 4 6 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 7 2 4 6 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 2 5 3 4 6	1370.9	3429.6	102.8	257.2	-77.2
1 7 2 5 3 6 4	1370.9	3429.6	102.8	257.2	-77.2
1 7 2 5 4 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 7 2 5 4 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 2 5 6 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 7 2 5 6 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 2 6 3 4 5	1477.8	3322.7	110.8	249.2	-69.2
1 7 2 6 3 5 4	1477.8	3322.7	110.8	249.2	-69.2
1 7 2 6 4 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 7 2 6 4 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 2 6 5 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 7 2 6 5 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 3 2 4 5 6	2231	2569.5	167.3	192.7	-12.7
1 7 3 2 4 6 5	2231	2569.5	167.3	192.7	-12.7
1 7 3 2 5 4 6	2231	2569.5	167.3	192.7	-12.7
1 7 3 2 5 6 4	2231	2569.5	167.3	192.7	-12.7
1 7 3 2 6 4 5	2231	2569.5	167.3	192.7	-12.7
1 7 3 2 6 5 4	2231	2569.5	167.3	192.7	-12.7
1 7 3 4 2 5 6	2231	2569.5	167.3	192.7	-12.7
1 7 3 4 2 6 5	2231	2569.5	167.3	192.7	-12.7
1 7 3 4 5 2 6	2231	2569.5	167.3	192.7	-12.7
1 7 3 4 5 6 2	2231	2569.5	167.3	192.7	-12.7
1 7 3 4 6 2 5	2231	2569.5	167.3	192.7	-12.7
1 7 3 4 6 5 2	2231	2569.5	167.3	192.7	-12.7

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 7 3 5 2 4 6	2231	2569.5	167.3	192.7	-12.7
1 7 3 5 2 6 4	2231	2569.5	167.3	192.7	-12.7
1 7 3 5 4 2 6	2231	2569.5	167.3	192.7	-12.7
1 7 3 5 4 6 2	2231	2569.5	167.3	192.7	-12.7
1 7 3 5 6 2 4	2231	2569.5	167.3	192.7	-12.7
1 7 3 5 6 4 2	2231	2569.5	167.3	192.7	-12.7
1 7 3 6 2 4 5	2231	2569.5	167.3	192.7	-12.7
1 7 3 6 2 5 4	2231	2569.5	167.3	192.7	-12.7
1 7 3 6 4 2 5	2231	2569.5	167.3	192.7	-12.7
1 7 3 6 4 5 2	2231	2569.5	167.3	192.7	-12.7
1 7 3 6 5 2 4	2231	2569.5	167.3	192.7	-12.7
1 7 3 6 5 4 2	2231	2569.5	167.3	192.7	-12.7
1 7 4 2 3 5 6	1385.4	3415.1	103.9	256.1	-76.1
1 7 4 2 3 6 5	1385.4	3415.1	103.9	256.1	-76.1
1 7 4 2 5 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 7 4 2 5 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 4 2 6 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 7 4 2 6 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 4 3 2 5 6	2096.5	2704	157.2	202.8	-22.8
1 7 4 3 2 6 5	2096.5	2704	157.2	202.8	-22.8
1 7 4 3 5 2 6	2096.5	2704	157.2	202.8	-22.8
1 7 4 3 5 6 2	2096.5	2704	157.2	202.8	-22.8
1 7 4 3 6 2 5	2096.5	2704	157.2	202.8	-22.8
1 7 4 3 6 5 2	2096.5	2704	157.2	202.8	-22.8
1 7 4 5 2 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 7 4 5 2 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 4 5 3 2 6	1947.5	2853	146.0	214.0	-34.0
1 7 4 5 3 6 2	1947.5	2853	146.0	214.0	-34.0
1 7 4 5 6 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 4 5 6 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 7 4 6 2 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 7 4 6 2 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 4 6 3 2 5	2054.4	2746.1	154.1	205.9	-25.9
1 7 4 6 3 5 2	2054.4	2746.1	154.1	205.9	-25.9
1 7 4 6 5 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 4 6 5 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 7 5 2 3 4 6	1370.9	3429.6	102.8	257.2	-77.2
1 7 5 2 3 6 4	1370.9	3429.6	102.8	257.2	-77.2
1 7 5 2 4 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 7 5 2 4 6 3	1194.3	3606.2	89.6	270.4	-90.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 7 5 2 6 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 7 5 2 6 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 5 3 2 4 6	2082	2718.5	156.1	203.9	-23.9
1 7 5 3 2 6 4	2082	2718.5	156.1	203.9	-23.9
1 7 5 3 4 2 6	2082	2718.5	156.1	203.9	-23.9
1 7 5 3 4 6 2	2082	2718.5	156.1	203.9	-23.9
1 7 5 3 6 2 4	2082	2718.5	156.1	203.9	-23.9
1 7 5 3 6 4 2	2082	2718.5	156.1	203.9	-23.9
1 7 5 4 2 3 6	1236.4	3564.1	92.7	267.3	-87.3
1 7 5 4 2 6 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 5 4 3 2 6	1947.5	2853	146.0	214.0	-34.0
1 7 5 4 3 6 2	1947.5	2853	146.0	214.0	-34.0
1 7 5 4 6 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 5 4 6 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 7 5 6 2 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 7 5 6 2 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 5 6 3 2 4	2039.9	2760.6	153.0	207.0	-27.0
1 7 5 6 3 4 2	2039.9	2760.6	153.0	207.0	-27.0
1 7 5 6 4 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 5 6 4 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 7 6 2 3 4 5	1477.8	3322.7	110.8	249.2	-69.2
1 7 6 2 3 5 4	1477.8	3322.7	110.8	249.2	-69.2
1 7 6 2 4 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 7 6 2 4 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 6 2 5 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 7 6 2 5 4 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 6 3 2 4 5	2188.9	2611.6	164.2	195.8	-15.8
1 7 6 3 2 5 4	2188.9	2611.6	164.2	195.8	-15.8
1 7 6 3 4 2 5	2188.9	2611.6	164.2	195.8	-15.8
1 7 6 3 4 5 2	2188.9	2611.6	164.2	195.8	-15.8
1 7 6 3 5 2 4	2188.9	2611.6	164.2	195.8	-15.8
1 7 6 3 5 4 2	2188.9	2611.6	164.2	195.8	-15.8
1 7 6 4 2 3 5	1343.3	3457.2	100.7	259.3	-79.3
1 7 6 4 2 5 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 6 4 3 2 5	2054.4	2746.1	154.1	205.9	-25.9
1 7 6 4 3 5 2	2054.4	2746.1	154.1	205.9	-25.9
1 7 6 4 5 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 6 4 5 3 2	1905.4	2895.1	142.9	217.1	-37.1
1 7 6 5 2 3 4	1328.8	3471.7	99.6	260.4	-80.4
1 7 6 5 2 4 3	1194.3	3606.2	89.6	270.4	-90.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1 7 6 5 3 2 4	2039.9	2760.6	153.0	207.0	-27.0
1 7 6 5 3 4 2	2039.9	2760.6	153.0	207.0	-27.0
1 7 6 5 4 2 3	1194.3	3606.2	89.6	270.4	-90.4
1 7 6 5 4 3 2	1905.4	2895.1	142.9	217.1	-37.1
1' 2 3 4 5 6 7	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 4 5 7 6	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 4 6 5 7	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 4 6 7 5	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 4 7 5 6	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 4 7 6 5	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 5 4 6 7	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 5 4 7 6	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 5 6 4 7	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 5 6 7 4	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 5 7 4 6	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 5 7 6 4	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 6 4 5 7	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 6 4 7 5	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 6 5 4 7	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 6 5 7 4	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 6 7 4 5	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 6 7 5 4	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 7 4 5 6	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 7 4 6 5	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 7 5 4 6	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 7 5 6 4	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 7 6 4 5	2415.2	2385.3	181.1	178.9	1.1
1' 2 3 7 6 5 4	2415.2	2385.3	181.1	178.9	1.1
1' 2 4 3 5 6 7	2280.7	2519.8	171.0	189.0	-9.0
1' 2 4 3 5 7 6	2280.7	2519.8	171.0	189.0	-9.0
1' 2 4 3 6 5 7	2280.7	2519.8	171.0	189.0	-9.0
1' 2 4 3 6 7 5	2280.7	2519.8	171.0	189.0	-9.0
1' 2 4 3 7 5 6	2280.7	2519.8	171.0	189.0	-9.0
1' 2 4 3 7 6 5	2280.7	2519.8	171.0	189.0	-9.0
1' 2 4 5 3 6 7	2131.7	2668.8	159.9	200.1	-20.1
1' 2 4 5 3 7 6	2131.7	2668.8	159.9	200.1	-20.1
1' 2 4 5 6 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 2 4 5 6 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 4 5 7 3 6	1293.5	3507	97.0	263.0	-83.0
1' 2 4 5 7 6 3	1251.4	3549.1	93.8	266.2	-86.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 2 4 6 3 5 7	2238.6	2561.9	167.9	192.1	-12.1
1' 2 4 6 3 7 5	2238.6	2561.9	167.9	192.1	-12.1
1' 2 4 6 5 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 2 4 6 5 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 4 6 7 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 2 4 6 7 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 4 7 3 5 6	1442.5	3358	108.2	251.8	-71.8
1' 2 4 7 3 6 5	1442.5	3358	108.2	251.8	-71.8
1' 2 4 7 5 3 6	1293.5	3507	97.0	263.0	-83.0
1' 2 4 7 5 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 4 7 6 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 2 4 7 6 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 5 3 4 6 7	2266.2	2534.3	169.9	190.1	-10.1
1' 2 5 3 4 7 6	2266.2	2534.3	169.9	190.1	-10.1
1' 2 5 3 6 4 7	2266.2	2534.3	169.9	190.1	-10.1
1' 2 5 3 6 7 4	2266.2	2534.3	169.9	190.1	-10.1
1' 2 5 3 7 4 6	2266.2	2534.3	169.9	190.1	-10.1
1' 2 5 3 7 6 4	2266.2	2534.3	169.9	190.1	-10.1
1' 2 5 4 3 6 7	2131.7	2668.8	159.9	200.1	-20.1
1' 2 5 4 3 7 6	2131.7	2668.8	159.9	200.1	-20.1
1' 2 5 4 6 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 2 5 4 6 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 5 4 7 3 6	1293.5	3507	97.0	263.0	-83.0
1' 2 5 4 7 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 5 6 3 4 7	2224.1	2576.4	166.8	193.2	-13.2
1' 2 5 6 3 7 4	2224.1	2576.4	166.8	193.2	-13.2
1' 2 5 6 4 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 2 5 6 4 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 5 6 7 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 2 5 6 7 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 5 7 3 4 6	1428	3372.5	107.1	252.9	-72.9
1' 2 5 7 3 6 4	1428	3372.5	107.1	252.9	-72.9
1' 2 5 7 4 3 6	1293.5	3507	97.0	263.0	-83.0
1' 2 5 7 4 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 5 7 6 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 2 5 7 6 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 6 3 4 5 7	2373.1	2427.4	178.0	182.0	-2.0
1' 2 6 3 4 7 5	2373.1	2427.4	178.0	182.0	-2.0
1' 2 6 3 5 4 7	2373.1	2427.4	178.0	182.0	-2.0
1' 2 6 3 5 7 4	2373.1	2427.4	178.0	182.0	-2.0

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 2 6 3 7 4 5	2373.1	2427.4	178.0	182.0	-2.0
1' 2 6 3 7 5 4	2373.1	2427.4	178.0	182.0	-2.0
1' 2 6 4 3 5 7	2238.6	2561.9	167.9	192.1	-12.1
1' 2 6 4 3 7 5	2238.6	2561.9	167.9	192.1	-12.1
1' 2 6 4 5 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 2 6 4 5 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 6 4 7 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 2 6 4 7 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 6 5 3 4 7	2224.1	2576.4	166.8	193.2	-13.2
1' 2 6 5 3 7 4	2224.1	2576.4	166.8	193.2	-13.2
1' 2 6 5 4 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 2 6 5 4 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 6 5 7 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 2 6 5 7 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 6 7 3 4 5	1534.9	3265.6	115.1	244.9	-64.9
1' 2 6 7 3 5 4	1534.9	3265.6	115.1	244.9	-64.9
1' 2 6 7 4 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 2 6 7 4 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 6 7 5 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 2 6 7 5 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 7 3 4 5 6	1577	3223.5	118.3	241.7	-61.7
1' 2 7 3 4 6 5	1577	3223.5	118.3	241.7	-61.7
1' 2 7 3 5 4 6	1577	3223.5	118.3	241.7	-61.7
1' 2 7 3 5 6 4	1577	3223.5	118.3	241.7	-61.7
1' 2 7 3 6 4 5	1577	3223.5	118.3	241.7	-61.7
1' 2 7 3 6 5 4	1577	3223.5	118.3	241.7	-61.7
1' 2 7 4 3 5 6	1442.5	3358	108.2	251.8	-71.8
1' 2 7 4 3 6 5	1442.5	3358	108.2	251.8	-71.8
1' 2 7 4 5 3 6	1293.5	3507	97.0	263.0	-83.0
1' 2 7 4 5 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 7 4 6 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 2 7 4 6 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 7 5 3 4 6	1428	3372.5	107.1	252.9	-72.9
1' 2 7 5 3 6 4	1428	3372.5	107.1	252.9	-72.9
1' 2 7 5 4 3 6	1293.5	3507	97.0	263.0	-83.0
1' 2 7 5 4 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 7 5 6 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 2 7 5 6 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 7 6 3 4 5	1534.9	3265.6	115.1	244.9	-64.9
1' 2 7 6 3 5 4	1534.9	3265.6	115.1	244.9	-64.9

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 2 7 6 4 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 2 7 6 4 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 2 7 6 5 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 2 7 6 5 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 3 2 4 5 6 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 4 5 7 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 4 6 5 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 4 6 7 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 4 7 5 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 4 7 6 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 5 4 6 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 5 4 7 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 5 6 4 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 5 6 7 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 5 7 4 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 5 7 6 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 6 4 5 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 6 4 7 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 6 5 4 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 6 5 7 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 6 7 4 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 6 7 5 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 7 4 5 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 7 4 6 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 7 5 4 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 7 5 6 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 7 6 4 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 2 7 6 5 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 2 5 6 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 2 5 7 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 2 6 5 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 2 6 7 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 2 7 5 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 2 7 6 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 5 2 6 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 5 2 7 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 5 6 2 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 5 6 7 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 5 7 2 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 5 7 6 2	3126.3	1674.2	234.4	125.6	54.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 3 4 6 2 5 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 6 2 7 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 6 5 2 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 6 5 7 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 6 7 2 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 6 7 5 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 7 2 5 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 7 2 6 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 7 5 2 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 7 5 6 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 7 6 2 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 4 7 6 5 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 2 4 6 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 2 4 7 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 2 6 4 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 2 6 7 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 2 7 4 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 2 7 6 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 4 2 6 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 4 2 7 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 4 6 2 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 4 6 7 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 4 7 2 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 4 7 6 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 6 2 4 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 6 2 7 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 6 4 2 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 6 4 7 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 6 7 2 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 6 7 4 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 7 2 4 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 7 2 6 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 7 4 2 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 7 4 6 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 7 6 2 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 5 7 6 4 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 2 4 5 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 2 4 7 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 2 5 4 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 2 5 7 4	3126.3	1674.2	234.4	125.6	54.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 3 6 2 7 4 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 2 7 5 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 4 2 5 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 4 2 7 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 4 5 2 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 4 5 7 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 4 7 2 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 4 7 5 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 5 2 4 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 5 2 7 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 5 4 2 7	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 5 4 7 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 5 7 2 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 5 7 4 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 7 2 4 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 7 2 5 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 7 4 2 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 7 4 5 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 7 5 2 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 6 7 5 4 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 2 4 5 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 2 4 6 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 2 5 4 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 2 5 6 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 2 6 4 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 2 6 5 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 4 2 5 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 4 2 6 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 4 5 2 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 4 5 6 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 4 6 2 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 4 6 5 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 5 2 4 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 5 2 6 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 5 4 2 6	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 5 4 6 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 5 6 2 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 5 6 4 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 6 2 4 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 6 2 5 4	3126.3	1674.2	234.4	125.6	54.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 3 7 6 4 2 5	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 6 4 5 2	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 6 5 2 4	3126.3	1674.2	234.4	125.6	54.4
1' 3 7 6 5 4 2	3126.3	1674.2	234.4	125.6	54.4
1' 4 2 3 5 6 7	2280.7	2519.8	171.0	189.0	-9.0
1' 4 2 3 5 7 6	2280.7	2519.8	171.0	189.0	-9.0
1' 4 2 3 6 5 7	2280.7	2519.8	171.0	189.0	-9.0
1' 4 2 3 6 7 5	2280.7	2519.8	171.0	189.0	-9.0
1' 4 2 3 7 5 6	2280.7	2519.8	171.0	189.0	-9.0
1' 4 2 3 7 6 5	2280.7	2519.8	171.0	189.0	-9.0
1' 4 2 5 3 6 7	2131.7	2668.8	159.9	200.1	-20.1
1' 4 2 5 3 7 6	2131.7	2668.8	159.9	200.1	-20.1
1' 4 2 5 6 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 4 2 5 6 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 2 5 7 3 6	1293.5	3507	97.0	263.0	-83.0
1' 4 2 5 7 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 2 6 3 5 7	2238.6	2561.9	167.9	192.1	-12.1
1' 4 2 6 3 7 5	2238.6	2561.9	167.9	192.1	-12.1
1' 4 2 6 5 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 4 2 6 5 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 2 6 7 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 4 2 6 7 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 2 7 3 5 6	1442.5	3358	108.2	251.8	-71.8
1' 4 2 7 3 6 5	1442.5	3358	108.2	251.8	-71.8
1' 4 2 7 5 3 6	1293.5	3507	97.0	263.0	-83.0
1' 4 2 7 5 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 2 7 6 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 4 2 7 6 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 3 2 5 6 7	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 2 5 7 6	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 2 6 5 7	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 2 6 7 5	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 2 7 5 6	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 2 7 6 5	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 5 2 6 7	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 5 2 7 6	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 5 6 2 7	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 5 6 7 2	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 5 7 2 6	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 5 7 6 2	2991.8	1808.7	224.4	135.6	44.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 4 3 6 2 5 7	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 6 2 7 5	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 6 5 2 7	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 6 5 7 2	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 6 7 2 5	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 6 7 5 2	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 7 2 5 6	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 7 2 6 5	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 7 5 2 6	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 7 5 6 2	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 7 6 2 5	2991.8	1808.7	224.4	135.6	44.4
1' 4 3 7 6 5 2	2991.8	1808.7	224.4	135.6	44.4
1' 4 5 2 3 6 7	2131.7	2668.8	159.9	200.1	-20.1
1' 4 5 2 3 7 6	2131.7	2668.8	159.9	200.1	-20.1
1' 4 5 2 6 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 4 5 2 6 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 5 2 7 3 6	1293.5	3507	97.0	263.0	-83.0
1' 4 5 2 7 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 5 3 2 6 7	2842.8	1957.7	213.2	146.8	33.2
1' 4 5 3 2 7 6	2842.8	1957.7	213.2	146.8	33.2
1' 4 5 3 6 2 7	2842.8	1957.7	213.2	146.8	33.2
1' 4 5 3 6 7 2	2842.8	1957.7	213.2	146.8	33.2
1' 4 5 3 7 2 6	2842.8	1957.7	213.2	146.8	33.2
1' 4 5 3 7 6 2	2842.8	1957.7	213.2	146.8	33.2
1' 4 5 6 2 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 4 5 6 2 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 5 6 3 2 7	2800.7	1999.8	210.0	150.0	30.0
1' 4 5 6 3 7 2	2800.7	1999.8	210.0	150.0	30.0
1' 4 5 6 7 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 5 6 7 3 2	1962.5	2838	147.2	212.8	-32.8
1' 4 5 7 2 3 6	1293.5	3507	97.0	263.0	-83.0
1' 4 5 7 2 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 5 7 3 2 6	2004.6	2795.9	150.3	209.7	-29.7
1' 4 5 7 3 6 2	2004.6	2795.9	150.3	209.7	-29.7
1' 4 5 7 6 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 5 7 6 3 2	1962.5	2838	147.2	212.8	-32.8
1' 4 6 2 3 5 7	2238.6	2561.9	167.9	192.1	-12.1
1' 4 6 2 3 7 5	2238.6	2561.9	167.9	192.1	-12.1
1' 4 6 2 5 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 4 6 2 5 7 3	1251.4	3549.1	93.8	266.2	-86.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 4 6 2 7 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 4 6 2 7 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 6 3 2 5 7	2949.7	1850.8	221.2	138.8	41.2
1' 4 6 3 2 7 5	2949.7	1850.8	221.2	138.8	41.2
1' 4 6 3 5 2 7	2949.7	1850.8	221.2	138.8	41.2
1' 4 6 3 5 7 2	2949.7	1850.8	221.2	138.8	41.2
1' 4 6 3 7 2 5	2949.7	1850.8	221.2	138.8	41.2
1' 4 6 3 7 5 2	2949.7	1850.8	221.2	138.8	41.2
1' 4 6 5 2 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 4 6 5 2 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 6 5 3 2 7	2800.7	1999.8	210.0	150.0	30.0
1' 4 6 5 3 7 2	2800.7	1999.8	210.0	150.0	30.0
1' 4 6 5 7 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 6 5 7 3 2	1962.5	2838	147.2	212.8	-32.8
1' 4 6 7 2 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 4 6 7 2 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 6 7 3 2 5	2111.5	2689	158.3	201.7	-21.7
1' 4 6 7 3 5 2	2111.5	2689	158.3	201.7	-21.7
1' 4 6 7 5 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 6 7 5 3 2	1962.5	2838	147.2	212.8	-32.8
1' 4 7 2 3 5 6	1442.5	3358	108.2	251.8	-71.8
1' 4 7 2 3 6 5	1442.5	3358	108.2	251.8	-71.8
1' 4 7 2 5 3 6	1293.5	3507	97.0	263.0	-83.0
1' 4 7 2 5 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 7 2 6 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 4 7 2 6 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 7 3 2 5 6	2153.6	2646.9	161.5	198.5	-18.5
1' 4 7 3 2 6 5	2153.6	2646.9	161.5	198.5	-18.5
1' 4 7 3 5 2 6	2153.6	2646.9	161.5	198.5	-18.5
1' 4 7 3 5 6 2	2153.6	2646.9	161.5	198.5	-18.5
1' 4 7 3 6 2 5	2153.6	2646.9	161.5	198.5	-18.5
1' 4 7 3 6 5 2	2153.6	2646.9	161.5	198.5	-18.5
1' 4 7 5 2 3 6	1293.5	3507	97.0	263.0	-83.0
1' 4 7 5 2 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 7 5 3 2 6	2004.6	2795.9	150.3	209.7	-29.7
1' 4 7 5 3 6 2	2004.6	2795.9	150.3	209.7	-29.7
1' 4 7 5 6 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 7 5 6 3 2	1962.5	2838	147.2	212.8	-32.8
1' 4 7 6 2 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 4 7 6 2 5 3	1251.4	3549.1	93.8	266.2	-86.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 4 7 6 3 2 5	2111.5	2689	158.3	201.7	-21.7
1' 4 7 6 3 5 2	2111.5	2689	158.3	201.7	-21.7
1' 4 7 6 5 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 4 7 6 5 3 2	1962.5	2838	147.2	212.8	-32.8
1' 5 2 3 4 6 7	2266.2	2534.3	169.9	190.1	-10.1
1' 5 2 3 4 7 6	2266.2	2534.3	169.9	190.1	-10.1
1' 5 2 3 6 4 7	2266.2	2534.3	169.9	190.1	-10.1
1' 5 2 3 6 7 4	2266.2	2534.3	169.9	190.1	-10.1
1' 5 2 3 7 4 6	2266.2	2534.3	169.9	190.1	-10.1
1' 5 2 3 7 6 4	2266.2	2534.3	169.9	190.1	-10.1
1' 5 2 4 3 6 7	2131.7	2668.8	159.9	200.1	-20.1
1' 5 2 4 3 7 6	2131.7	2668.8	159.9	200.1	-20.1
1' 5 2 4 6 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 5 2 4 6 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 2 4 7 3 6	1293.5	3507	97.0	263.0	-83.0
1' 5 2 4 7 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 2 6 3 4 7	2224.1	2576.4	166.8	193.2	-13.2
1' 5 2 6 3 7 4	2224.1	2576.4	166.8	193.2	-13.2
1' 5 2 6 4 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 5 2 6 4 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 2 6 7 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 5 2 6 7 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 2 7 3 4 6	1428	3372.5	107.1	252.9	-72.9
1' 5 2 7 3 6 4	1428	3372.5	107.1	252.9	-72.9
1' 5 2 7 4 3 6	1293.5	3507	97.0	263.0	-83.0
1' 5 2 7 4 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 2 7 6 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 5 2 7 6 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 3 2 4 6 7	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 2 4 7 6	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 2 6 4 7	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 2 6 7 4	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 2 7 4 6	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 2 7 6 4	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 4 2 6 7	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 4 2 7 6	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 4 6 2 7	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 4 6 7 2	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 4 7 2 6	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 4 7 6 2	2977.3	1823.2	223.3	136.7	43.3

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 5 3 6 2 4 7	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 6 2 7 4	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 6 4 2 7	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 6 4 7 2	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 6 7 2 4	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 6 7 4 2	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 7 2 4 6	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 7 2 6 4	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 7 4 2 6	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 7 4 6 2	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 7 6 2 4	2977.3	1823.2	223.3	136.7	43.3
1' 5 3 7 6 4 2	2977.3	1823.2	223.3	136.7	43.3
1' 5 4 2 3 6 7	2131.7	2668.8	159.9	200.1	-20.1
1' 5 4 2 3 7 6	2131.7	2668.8	159.9	200.1	-20.1
1' 5 4 2 6 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 5 4 2 6 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 4 2 7 3 6	1293.5	3507	97.0	263.0	-83.0
1' 5 4 2 7 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 4 3 2 6 7	2842.8	1957.7	213.2	146.8	33.2
1' 5 4 3 2 7 6	2842.8	1957.7	213.2	146.8	33.2
1' 5 4 3 6 2 7	2842.8	1957.7	213.2	146.8	33.2
1' 5 4 3 6 7 2	2842.8	1957.7	213.2	146.8	33.2
1' 5 4 3 7 2 6	2842.8	1957.7	213.2	146.8	33.2
1' 5 4 3 7 6 2	2842.8	1957.7	213.2	146.8	33.2
1' 5 4 6 2 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 5 4 6 2 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 4 6 3 2 7	2800.7	1999.8	210.0	150.0	30.0
1' 5 4 6 3 7 2	2800.7	1999.8	210.0	150.0	30.0
1' 5 4 6 7 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 4 6 7 3 2	1962.5	2838	147.2	212.8	-32.8
1' 5 4 7 2 3 6	1293.5	3507	97.0	263.0	-83.0
1' 5 4 7 2 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 4 7 3 2 6	2004.6	2795.9	150.3	209.7	-29.7
1' 5 4 7 3 6 2	2004.6	2795.9	150.3	209.7	-29.7
1' 5 4 7 6 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 4 7 6 3 2	1962.5	2838	147.2	212.8	-32.8
1' 5 6 2 3 4 7	2224.1	2576.4	166.8	193.2	-13.2
1' 5 6 2 3 7 4	2224.1	2576.4	166.8	193.2	-13.2
1' 5 6 2 4 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 5 6 2 4 7 3	1251.4	3549.1	93.8	266.2	-86.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 5 6 2 7 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 5 6 2 7 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 6 3 2 4 7	2935.2	1865.3	220.1	139.9	40.1
1' 5 6 3 2 7 4	2935.2	1865.3	220.1	139.9	40.1
1' 5 6 3 4 2 7	2935.2	1865.3	220.1	139.9	40.1
1' 5 6 3 4 7 2	2935.2	1865.3	220.1	139.9	40.1
1' 5 6 3 7 2 4	2935.2	1865.3	220.1	139.9	40.1
1' 5 6 3 7 4 2	2935.2	1865.3	220.1	139.9	40.1
1' 5 6 4 2 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 5 6 4 2 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 6 4 3 2 7	2800.7	1999.8	210.0	150.0	30.0
1' 5 6 4 3 7 2	2800.7	1999.8	210.0	150.0	30.0
1' 5 6 4 7 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 6 4 7 3 2	1962.5	2838	147.2	212.8	-32.8
1' 5 6 7 2 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 5 6 7 2 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 6 7 3 2 4	2097	2703.5	157.3	202.7	-22.7
1' 5 6 7 3 4 2	2097	2703.5	157.3	202.7	-22.7
1' 5 6 7 4 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 6 7 4 3 2	1962.5	2838	147.2	212.8	-32.8
1' 5 7 2 3 4 6	1428	3372.5	107.1	252.9	-72.9
1' 5 7 2 3 6 4	1428	3372.5	107.1	252.9	-72.9
1' 5 7 2 4 3 6	1293.5	3507	97.0	263.0	-83.0
1' 5 7 2 4 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 7 2 6 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 5 7 2 6 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 7 3 2 4 6	2139.1	2661.4	160.4	199.6	-19.6
1' 5 7 3 2 6 4	2139.1	2661.4	160.4	199.6	-19.6
1' 5 7 3 4 2 6	2139.1	2661.4	160.4	199.6	-19.6
1' 5 7 3 4 6 2	2139.1	2661.4	160.4	199.6	-19.6
1' 5 7 3 6 2 4	2139.1	2661.4	160.4	199.6	-19.6
1' 5 7 3 6 4 2	2139.1	2661.4	160.4	199.6	-19.6
1' 5 7 4 2 3 6	1293.5	3507	97.0	263.0	-83.0
1' 5 7 4 2 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 7 4 3 2 6	2004.6	2795.9	150.3	209.7	-29.7
1' 5 7 4 3 6 2	2004.6	2795.9	150.3	209.7	-29.7
1' 5 7 4 6 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 7 4 6 3 2	1962.5	2838	147.2	212.8	-32.8
1' 5 7 6 2 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 5 7 6 2 4 3	1251.4	3549.1	93.8	266.2	-86.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 5 7 6 3 2 4	2097	2703.5	157.3	202.7	-22.7
1' 5 7 6 3 4 2	2097	2703.5	157.3	202.7	-22.7
1' 5 7 6 4 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 5 7 6 4 3 2	1962.5	2838	147.2	212.8	-32.8
1' 6 2 3 4 5 7	2373.1	2427.4	178.0	182.0	-2.0
1' 6 2 3 4 7 5	2373.1	2427.4	178.0	182.0	-2.0
1' 6 2 3 5 4 7	2373.1	2427.4	178.0	182.0	-2.0
1' 6 2 3 5 7 4	2373.1	2427.4	178.0	182.0	-2.0
1' 6 2 3 7 4 5	2373.1	2427.4	178.0	182.0	-2.0
1' 6 2 3 7 5 4	2373.1	2427.4	178.0	182.0	-2.0
1' 6 2 4 3 5 7	2238.6	2561.9	167.9	192.1	-12.1
1' 6 2 4 3 7 5	2238.6	2561.9	167.9	192.1	-12.1
1' 6 2 4 5 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 6 2 4 5 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 2 4 7 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 6 2 4 7 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 2 5 3 4 7	2224.1	2576.4	166.8	193.2	-13.2
1' 6 2 5 3 7 4	2224.1	2576.4	166.8	193.2	-13.2
1' 6 2 5 4 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 6 2 5 4 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 2 5 7 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 6 2 5 7 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 2 7 3 4 5	1534.9	3265.6	115.1	244.9	-64.9
1' 6 2 7 3 5 4	1534.9	3265.6	115.1	244.9	-64.9
1' 6 2 7 4 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 6 2 7 4 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 2 7 5 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 6 2 7 5 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 3 2 4 5 7	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 2 4 7 5	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 2 5 4 7	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 2 5 7 4	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 2 7 4 5	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 2 7 5 4	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 4 2 5 7	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 4 2 7 5	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 4 5 2 7	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 4 5 7 2	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 4 7 2 5	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 4 7 5 2	3084.2	1716.3	231.3	128.7	51.3

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 6 3 5 2 4 7	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 5 2 7 4	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 5 4 2 7	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 5 4 7 2	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 5 7 2 4	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 5 7 4 2	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 7 2 4 5	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 7 2 5 4	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 7 4 2 5	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 7 4 5 2	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 7 5 2 4	3084.2	1716.3	231.3	128.7	51.3
1' 6 3 7 5 4 2	3084.2	1716.3	231.3	128.7	51.3
1' 6 4 2 3 5 7	2238.6	2561.9	167.9	192.1	-12.1
1' 6 4 2 3 7 5	2238.6	2561.9	167.9	192.1	-12.1
1' 6 4 2 5 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 6 4 2 5 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 4 2 7 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 6 4 2 7 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 4 3 2 5 7	2949.7	1850.8	221.2	138.8	41.2
1' 6 4 3 2 7 5	2949.7	1850.8	221.2	138.8	41.2
1' 6 4 3 5 2 7	2949.7	1850.8	221.2	138.8	41.2
1' 6 4 3 5 7 2	2949.7	1850.8	221.2	138.8	41.2
1' 6 4 3 7 2 5	2949.7	1850.8	221.2	138.8	41.2
1' 6 4 3 7 5 2	2949.7	1850.8	221.2	138.8	41.2
1' 6 4 5 2 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 6 4 5 2 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 4 5 3 2 7	2800.7	1999.8	210.0	150.0	30.0
1' 6 4 5 3 7 2	2800.7	1999.8	210.0	150.0	30.0
1' 6 4 5 7 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 4 5 7 3 2	1962.5	2838	147.2	212.8	-32.8
1' 6 4 7 2 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 6 4 7 2 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 4 7 3 2 5	2111.5	2689	158.3	201.7	-21.7
1' 6 4 7 3 5 2	2111.5	2689	158.3	201.7	-21.7
1' 6 4 7 5 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 4 7 5 3 2	1962.5	2838	147.2	212.8	-32.8
1' 6 5 2 3 4 7	2224.1	2576.4	166.8	193.2	-13.2
1' 6 5 2 3 7 4	2224.1	2576.4	166.8	193.2	-13.2
1' 6 5 2 4 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 6 5 2 4 7 3	1251.4	3549.1	93.8	266.2	-86.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 6 5 2 7 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 6 5 2 7 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 5 3 2 4 7	2935.2	1865.3	220.1	139.9	40.1
1' 6 5 3 2 7 4	2935.2	1865.3	220.1	139.9	40.1
1' 6 5 3 4 2 7	2935.2	1865.3	220.1	139.9	40.1
1' 6 5 3 4 7 2	2935.2	1865.3	220.1	139.9	40.1
1' 6 5 3 7 2 4	2935.2	1865.3	220.1	139.9	40.1
1' 6 5 3 7 4 2	2935.2	1865.3	220.1	139.9	40.1
1' 6 5 4 2 3 7	2089.6	2710.9	156.7	203.3	-23.3
1' 6 5 4 2 7 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 5 4 3 2 7	2800.7	1999.8	210.0	150.0	30.0
1' 6 5 4 3 7 2	2800.7	1999.8	210.0	150.0	30.0
1' 6 5 4 7 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 5 4 7 3 2	1962.5	2838	147.2	212.8	-32.8
1' 6 5 7 2 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 6 5 7 2 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 5 7 3 2 4	2097	2703.5	157.3	202.7	-22.7
1' 6 5 7 3 4 2	2097	2703.5	157.3	202.7	-22.7
1' 6 5 7 4 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 5 7 4 3 2	1962.5	2838	147.2	212.8	-32.8
1' 6 7 2 3 4 5	1534.9	3265.6	115.1	244.9	-64.9
1' 6 7 2 3 5 4	1534.9	3265.6	115.1	244.9	-64.9
1' 6 7 2 4 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 6 7 2 4 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 7 2 5 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 6 7 2 5 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 7 3 2 4 5	2246	2554.5	168.4	191.6	-11.6
1' 6 7 3 2 5 4	2246	2554.5	168.4	191.6	-11.6
1' 6 7 3 4 2 5	2246	2554.5	168.4	191.6	-11.6
1' 6 7 3 4 5 2	2246	2554.5	168.4	191.6	-11.6
1' 6 7 3 5 2 4	2246	2554.5	168.4	191.6	-11.6
1' 6 7 3 5 4 2	2246	2554.5	168.4	191.6	-11.6
1' 6 7 4 2 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 6 7 4 2 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 7 4 3 2 5	2111.5	2689	158.3	201.7	-21.7
1' 6 7 4 3 5 2	2111.5	2689	158.3	201.7	-21.7
1' 6 7 4 5 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 7 4 5 3 2	1962.5	2838	147.2	212.8	-32.8
1' 6 7 5 2 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 6 7 5 2 4 3	1251.4	3549.1	93.8	266.2	-86.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 6 7 5 3 2 4	2097	2703.5	157.3	202.7	-22.7
1' 6 7 5 3 4 2	2097	2703.5	157.3	202.7	-22.7
1' 6 7 5 4 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 6 7 5 4 3 2	1962.5	2838	147.2	212.8	-32.8
1' 7 2 3 4 5 6	1577	3223.5	118.3	241.7	-61.7
1' 7 2 3 4 6 5	1577	3223.5	118.3	241.7	-61.7
1' 7 2 3 5 4 6	1577	3223.5	118.3	241.7	-61.7
1' 7 2 3 5 6 4	1577	3223.5	118.3	241.7	-61.7
1' 7 2 3 6 4 5	1577	3223.5	118.3	241.7	-61.7
1' 7 2 3 6 5 4	1577	3223.5	118.3	241.7	-61.7
1' 7 2 4 3 5 6	1442.5	3358	108.2	251.8	-71.8
1' 7 2 4 3 6 5	1442.5	3358	108.2	251.8	-71.8
1' 7 2 4 5 3 6	1293.5	3507	97.0	263.0	-83.0
1' 7 2 4 5 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 2 4 6 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 7 2 4 6 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 2 5 3 4 6	1428	3372.5	107.1	252.9	-72.9
1' 7 2 5 3 6 4	1428	3372.5	107.1	252.9	-72.9
1' 7 2 5 4 3 6	1293.5	3507	97.0	263.0	-83.0
1' 7 2 5 4 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 2 5 6 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 7 2 5 6 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 2 6 3 4 5	1534.9	3265.6	115.1	244.9	-64.9
1' 7 2 6 3 5 4	1534.9	3265.6	115.1	244.9	-64.9
1' 7 2 6 4 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 7 2 6 4 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 2 6 5 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 7 2 6 5 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 3 2 4 5 6	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 2 4 6 5	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 2 5 4 6	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 2 5 6 4	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 2 6 4 5	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 2 6 5 4	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 4 2 5 6	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 4 2 6 5	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 4 5 2 6	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 4 5 6 2	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 4 6 2 5	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 4 6 5 2	2288.1	2512.4	171.6	188.4	-8.4

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 7 3 5 2 4 6	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 5 2 6 4	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 5 4 2 6	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 5 4 6 2	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 5 6 2 4	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 5 6 4 2	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 6 2 4 5	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 6 2 5 4	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 6 4 2 5	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 6 4 5 2	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 6 5 2 4	2288.1	2512.4	171.6	188.4	-8.4
1' 7 3 6 5 4 2	2288.1	2512.4	171.6	188.4	-8.4
1' 7 4 2 3 5 6	1442.5	3358	108.2	251.8	-71.8
1' 7 4 2 3 6 5	1442.5	3358	108.2	251.8	-71.8
1' 7 4 2 5 3 6	1293.5	3507	97.0	263.0	-83.0
1' 7 4 2 5 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 4 2 6 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 7 4 2 6 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 4 3 2 5 6	2153.6	2646.9	161.5	198.5	-18.5
1' 7 4 3 2 6 5	2153.6	2646.9	161.5	198.5	-18.5
1' 7 4 3 5 2 6	2153.6	2646.9	161.5	198.5	-18.5
1' 7 4 3 5 6 2	2153.6	2646.9	161.5	198.5	-18.5
1' 7 4 3 6 2 5	2153.6	2646.9	161.5	198.5	-18.5
1' 7 4 3 6 5 2	2153.6	2646.9	161.5	198.5	-18.5
1' 7 4 5 2 3 6	1293.5	3507	97.0	263.0	-83.0
1' 7 4 5 2 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 4 5 3 2 6	2004.6	2795.9	150.3	209.7	-29.7
1' 7 4 5 3 6 2	2004.6	2795.9	150.3	209.7	-29.7
1' 7 4 5 6 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 4 5 6 3 2	1962.5	2838	147.2	212.8	-32.8
1' 7 4 6 2 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 7 4 6 2 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 4 6 3 2 5	2111.5	2689	158.3	201.7	-21.7
1' 7 4 6 3 5 2	2111.5	2689	158.3	201.7	-21.7
1' 7 4 6 5 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 4 6 5 3 2	1962.5	2838	147.2	212.8	-32.8
1' 7 5 2 3 4 6	1428	3372.5	107.1	252.9	-72.9
1' 7 5 2 3 6 4	1428	3372.5	107.1	252.9	-72.9
1' 7 5 2 4 3 6	1293.5	3507	97.0	263.0	-83.0
1' 7 5 2 4 6 3	1251.4	3549.1	93.8	266.2	-86.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 7 5 2 6 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 7 5 2 6 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 5 3 2 4 6	2139.1	2661.4	160.4	199.6	-19.6
1' 7 5 3 2 6 4	2139.1	2661.4	160.4	199.6	-19.6
1' 7 5 3 4 2 6	2139.1	2661.4	160.4	199.6	-19.6
1' 7 5 3 4 6 2	2139.1	2661.4	160.4	199.6	-19.6
1' 7 5 3 6 2 4	2139.1	2661.4	160.4	199.6	-19.6
1' 7 5 3 6 4 2	2139.1	2661.4	160.4	199.6	-19.6
1' 7 5 4 2 3 6	1293.5	3507	97.0	263.0	-83.0
1' 7 5 4 2 6 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 5 4 3 2 6	2004.6	2795.9	150.3	209.7	-29.7
1' 7 5 4 3 6 2	2004.6	2795.9	150.3	209.7	-29.7
1' 7 5 4 6 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 5 4 6 3 2	1962.5	2838	147.2	212.8	-32.8
1' 7 5 6 2 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 7 5 6 2 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 5 6 3 2 4	2097	2703.5	157.3	202.7	-22.7
1' 7 5 6 3 4 2	2097	2703.5	157.3	202.7	-22.7
1' 7 5 6 4 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 5 6 4 3 2	1962.5	2838	147.2	212.8	-32.8
1' 7 6 2 3 4 5	1534.9	3265.6	115.1	244.9	-64.9
1' 7 6 2 3 5 4	1534.9	3265.6	115.1	244.9	-64.9
1' 7 6 2 4 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 7 6 2 4 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 6 2 5 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 7 6 2 5 4 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 6 3 2 4 5	2246	2554.5	168.4	191.6	-11.6
1' 7 6 3 2 5 4	2246	2554.5	168.4	191.6	-11.6
1' 7 6 3 4 2 5	2246	2554.5	168.4	191.6	-11.6
1' 7 6 3 4 5 2	2246	2554.5	168.4	191.6	-11.6
1' 7 6 3 5 2 4	2246	2554.5	168.4	191.6	-11.6
1' 7 6 3 5 4 2	2246	2554.5	168.4	191.6	-11.6
1' 7 6 4 2 3 5	1400.4	3400.1	105.0	255.0	-75.0
1' 7 6 4 2 5 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 6 4 3 2 5	2111.5	2689	158.3	201.7	-21.7
1' 7 6 4 3 5 2	2111.5	2689	158.3	201.7	-21.7
1' 7 6 4 5 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 6 4 5 3 2	1962.5	2838	147.2	212.8	-32.8
1' 7 6 5 2 3 4	1385.9	3414.6	103.9	256.1	-76.1
1' 7 6 5 2 4 3	1251.4	3549.1	93.8	266.2	-86.2

Arrangement Type	Left Replichore Lengh (kbp)	Right Replichore Length (kbp)	Balance (°)		Degrees Off
1' 7 6 5 3 2 4	2097	2703.5	157.3	202.7	-22.7
1' 7 6 5 3 4 2	2097	2703.5	157.3	202.7	-22.7
1' 7 6 5 4 2 3	1251.4	3549.1	93.8	266.2	-86.2
1' 7 6 5 4 3 2	1962.5	2838	147.2	212.8	-32.8

Appendix B. Construction of *Salmonella* Strains for Directly Measuring Recombination between *rrn* Operons

Introduction

Large-scale chromosomal rearrangements occur in many bacterial species by recombination between multiple copies of homologous sequences present on the chromosome. Examples of such sequences include rRNA (*rrn*) operons, insertion sequence (IS) elements, and prophages (1, 5, 8, 15, 20, 23-26, 28, 29, 38, 39, 41, 45, 48). Recombination between homologous sequences can either duplicate or translocate the intervening region between directly repeated sequences, or if the sequences are inverted relative to each other, invert the intervening region. While the evolutionary role of large-scale chromosomal rearrangements in bacteria is not clear, their consequence can change gene location and dosage (6, 12, 27, 42, 46), replichore balance (13, 30), as well as the orientation of polarized sequence motifs such as *ter* sites and KOPS (17, 19, 37, 47) (used to terminate DNA replication and direct DNA shuffling by FtzK repectively). An aspect of rearrangements that is not known is their frequency within a population.

Salmonella enterica is an excellent model organism for studying large-scale chromosomal rearrangements. In contrast to most of the >2,500 *S. enterica* serovars that have a broad host range and conserved chromosome arrangement type, strains belonging to host-specific serovars almost always have rearrangements from recombination between the seven *rrn* operons present on the chromosome (31-36). These rearrangements change the order of the regions between the *rrn* operons from the conserved arrangement type (1234567) (Figure B.1) to one of at least 50 other naturally-occurring arrangement types. Two hypotheses have been proposed to explain these

rearrangements. One hypothesis proposed by Liu (30, 31, 33) suggests that horizontal gene transfer makes one replichore, or half of the chromosome between the origin and terminus of DNA replication, longer than the other. Replication then becomes imbalanced, and the rearrangements occur to reestablish balance. Another hypothesis proposes that aspects of lifestyle either induce the rearrangements or allow them to be tolerated in host-specific serovars (21).

Previous work in *S. enterica* has suggested that duplications with endpoints within *rrn* operons occur as frequently as 10^{-2} (2). Other studies have investigated inversion frequencies using portable regions of homology (18, 43, 44, 47). While some inversions can occur with a frequency of 10^{-5} inversions per cell, inversion frequency can vary due to the distance between the homologous regions, as well as their chromosomal location, which can limit which inversions are permissive. To better understand if there are differences in rearrangement frequencies between host-specific and broad host range *Salmonella* serovars, strains were constructed that allowed indirect selection of cells carrying a common inversion of Region 1 (containing the terminus) from recombination between *rrnG* and *rrnH* (Figure B.1) (21). These strains carried different antibiotic resistance markers on both sides of an operon that would only cotransduce if the flanking sequences were homologous from the inversion. While these strains were useful in showing that both broad host range and host-specific *Salmonella* serovars undergo this particular inversion at the same relatively low frequency *in vitro*, the use of transduction and reliance on homologous flanking regions limited their utility.

In this study, construction of strains for direct measurement of rearrangement frequency not only at different *rrn* operons, but also under various experimental

conditions, was attempted by individually tagging the 3' end of the *rrn* operons with a promoterless *lacZY* operon and knocking out the *rrn* promoters of the tagged operon by insertional mutagenesis. Any rearrangement involving recombination of the tagged, non-functional *rrn* operon should swap in a functional promoter from the other recombining *rrn* operon, driving *lac* expression (Figure B.2). As *Salmonella* is naturally Lac-, cells with rearrangements can be directly selected for by demanding growth on lactose as a sole carbon source. The ability to directly measure the rearrangement frequency at the various *rrn* operons would be an invaluable tool in testing the two above hypotheses that explain why host-specific *Salmonella* have chromosomal rearrangements.

Strain Construction Strategy

The strains were constructed in a *S. enterica* sv. Typhimurium 14028s background. The approach used was to first individually replace the 5S rRNA genes from each *rrn* operon individually using the phage λ Red recombination system (Red-swap) (14). A 1.5 kbp DNA fragment containing a gene conferring kanamycin resistance (*aph*) and flanked by Flippase Recognition Target (FRT) sites, which allow for site-specific recombination by the Flippase recombinase from *Saccharomyces cerevisiae*, was PCR amplified using pKD4 (14) as the template and hybrid primers 5SKan-for and 5SKan-rev (Table B.1) that also anneal to the 5S rRNA genes. The next step was to transform the kanamycin resistant strains with pCP20 (9), a plasmid that contains the gene from the 2μm plasmid encoding Flippase (FLP), which "flips out" *aph* leaving behind a single FRT site. After screening for kanamycin sensitivity, strains were then transformed with pCE36 (16), which contains a promoterless *lacZY* operon immediately downstream from

a FRT site. pCE36 also contains *aph* as well as the conditional origin of replication, *oriR6K*, which requires the π protein to function. Since these strains lack *pir*, which encodes π, the only way to regain kanamycin resistance was for pCE36 to "flip into" the chromosomal FRT site. Kanamycin resistant strains were then assayed for ß-galactosidase activity as well as the ability to grow on lactose as a sole carbon source. While the strains had ß-galactosidase activity, they were not able to grow on NCE medium (4) supplemented with 0.4% lactose without first undergoing a selection process on that medium. The final step was to knock out the two promoters that drive expression of the *lac*-tagged *rrn* operon again using the phage λ Red recombination system. Although multiple attempts using this method and other genetic approaches failed to construct the desired strains, the methods and rationale described will be useful in future efforts to construct these strains.

Results

After the Red-swap of the 5S rRNA genes with PCR-amplified *aph* from pKD4, 34 kanamycin resistant colonies from two independent experiments were screened using PCR and/or transduction to determine which 5S rRNA gene was replaced with *aph*. The PCR assay utilized Kan-for, and primers that hybridize to genes flanking the 3' end of each of the seven *rrn* operons (22) (Table B.1). Transduction using lysates grown on strains with an inserted Tn*10* conferring tetracycline resistance genetically linked to one of the *rrn* operons (Table B.2) was also used to determine or confirm which 5S gene was interrupted by looking for co-transduction of kanamycin sensitivity with tetracycline resistance. However, two tranducing lysates failed to yield transductants, and were

subsequently dropped from use in the screening process. The lysate for detecting tagged *rrnH* strains was prepared from a donor strain with a Tn*10* insertion in *dnaQ*, which encodes the DNA polymerase III epsilon subunit. This strain grew very poorly, which probably explains the meager transduction frequency. The donor strain used for preparing the lysate for screening tagged *rrnE* strains was subsequently found to be a lysogen, which explains why that transduction screen failed.

The distribution of interrupted 5S genes (Figure B.3) showed that the number of strains with interrupted 5S genes in the *rrnA*, *B*, *C*, and *H* operons were similar; however only two strains were found with an interrupted *rrnE* 5S gene, and none were found with an interrupted *rrnG* 5S gene. Furthermore, more than twice the expected number of strains with interrupted *rrnD* 5S genes were obtained; this is probably due to *rrnD* having two 5S rRNA genes, which then provide two targets for Red-swap recombination instead of one target as in the other operons.

To flip-out the *aph* gene, strains with individually tagged *rrnA, B, C, D, E* and *H* operons were transformed with pCP20. The transformants were then selected for on ampicillin, followed by screening for kanamycin sensitivity. More than 95% of the colonies were kanamycin sensitive, indicating that the Flippase expressed from pCP20 easily removed the *aph* gene from the chromosome, leaving behind a FRT scar in place of the 5S rRNA gene in the tagged *rrn* operon.

The flip-in of pCE36 into the FRT scar was confirmed by screening transformants using PCR as above, except the oriR6K primer (Table B.1) was used instead of Kan-for. These strains were then assayed for ß-galactosidase activity and the ability to grow on lactose as a sole carbon source. The strains either did not grow or grew poorly on lactose,

which was explained by the low activity observed in the ß-galactosidase assays (Figure B.4).

Mutants that could grow on lactose were selected for to obtain better expressing strains. These mutants were found to have an order of magnitude increase in ß-galactosidase activity (Figure B.4). PCR-amplified DNA produced using template DNA from four *rrnH*-tagged up-regulated mutants and with primers that amplify the region containing the 3' end of the 23S rRNA gene, the 5' FRT site, and the 5' end of *lacZ* were sequenced. Three mutants contained single base changes 5-6 base pairs upstream of the stem-loop structure formed by the FRT site, and one mutant had a 3 base pair insertion right before the ribosome binding site (Figure B.5). This insertion mutation suggests that the low ß-galactosidase activity was caused by the FRT stem-loop structure occluding the ribosome binding site, and that increasing the distance between the two sequence motifs improves expression by enhancing ribosome binding and initiation of translation. The insertion mutation was then moved into the other *lac*-tagged *rrn* operons using P22-mediated transduction. The resulting transductants were all able to grow well on lactose as a sole carbon source.

Transcription of rRNA is controlled by two different promoters. The upstream promoter, P1, is a much stronger promoter and is regulated by the stringent response (7) as well as other regulatory mechanisms. The downstream promoter, P2, is not as tightly regulated as P1 and provides basal levels of rRNA during stationary phase or in other nutrient-limiting conditions (40). The nascent RNA molecule produced during transcription is processed by specific nucleases into the mature rRNAs and transfer RNAs (tRNAs) (3).

To inactivate these promoters, the *cat* gene, conferring chloramphenicol resistance, and flanked by the *thr* attenuator and *E. coli rrnC* terminator was PCR-amplified using pPC263 as template and hybrid primers rrncatboxA and rrncatboxC (Table B.1). These primers are homologous at their 5' ends to either the Box A or Box C elements of the anti-termination system immediately downstream of the *rrn* promoter region, and to the sequences flanking the multiple cloning site of pTZ19U (the vector portion of pPC263) at their 3' ends, and their design allows recombination into any of the sever *rrn* operons. To differentiate which *rrn* operon's promoter was interrupted, blue-white screening was employed (desired colonies with a *lac*-tagged *rrn* operon and the interrupted promoter will be white on X-gal plates) followed by a confirmatory PCR screen using primers that hybridize to genes flanking the 5' end of each *rrn* operon (22) and cat1 (Table B.1). PCR-amplified *cat* DNA was used in five independent Red-swap experiments using strains with *lac*-tagged *rrnA* or *rrnH* operons. Four experiments resulted in no colonies, and the fifth one resulted in four chloramphenicol resistant white colonies with the *lac*-tagged *rrnH*. However, PCR analysis showed that all four of these colonies did not have the *rrnH* promoter interrupted but the promoters for *rrnD* and *rrnE* instead.

The hybrid primers for amplifying the *cat* gene from pPC263 were redesigned to have longer regions of homology to the *Salmonella* chromosome, specifically to the Box A element described above (rrnboxAcat2) and to the 16S rRNA gene (16Scat) (Table B.1). The Red-swap strategy implemented was the same as before using strains with *lac*-tagged *rrnA* or *rrnH* operons. Two independent experiments only yielded blue

chloramphenicol resistant colonies. PCR analysis of one colony of each *lac*-tagged operon showed that in both cases the *rrnD* promoter was interrupted.

To circumvent the problems interrupting the *rrn* promoters using Red-swap, P22 transduction was attempted to move the interrupted *rrnD* promoter in the *lac*-tagged *rrnA* and *rrnH* operons. As the 5' end of the promoter is not homologous between *rrn* operons, these transductions were performed in either a *recD* or *mutS* background to increase homeologous recombination frequency. Unfortunately only blue transductants with the interrupted *rrnD* promoter were obtained. An attempt was also made to transduce the interrupted *rrnD* promoter into a *lac*-tagged *rrnD* strain; however no white transductants were obtained. Red-swap was also attempted in the *lac*-tagged *rrnA* or *rrnH* strains in either the *recD* or *mutS* background. All chloramphenicol resistant colonies were blue and were not characterized further.

Another approach used to interrupt the promoters of *lac*-tagged *rrn* operons was to Red-swap the *cat* gene into a plasmid containing the cloned *rrn* promoter region from either *rrnA* or *rrnH*. Once incorporated into the plasmid, PCR amplification would allow generation of a product with long stretches of homology to the interrupted promoter region of the specific *rrn* operon, and increase Red-swap recombination efficiency. The TOPO cloning kit (Invitrogen, Carlsbad, CA, USA) was employed to clone the PCR-amplified promoter regions. However only the *rrnH* promoter was cloned as the cloning efficiency of both the *rrnA* and *rrnH* promoters was lower than expected, and the Red-swap of the *cat* gene into the plasmid *rrnH* promoter was not successful.

The last attempt to interrupt the promoters of *lac*-tagged *rrn* operons used the Red-swap approach by amplifying *cat* from pPC263 with a 5' *rrnH*-specific hybrid

primer (rrnHcat) and 16Scat (Table B.1). Amplification of *cat* using 5' *rrnA*-specific (rrnAcat) or *rrnB*-specific (rrnBcat) hybrid primers was also attempted but failed. No colonies were obtained from the *rrnH* Red-swap.

Conclusions

The objective of this study was to construct strains that directly select for cells with chromosomal rearrangements occurring by recombination between *rrn* operons. The strategy utilized here was to construct strains containing a promoterless *lac* operon at the 3' end of a tagged *rrn* operon also containing non-functional promoters. Recombination involving the tagged non-functional *rrn* operon will swap in a functional promoter from the other recombining operon driving *lac* expression. As *Salmonella* is naturally *lac*-, cells with rearrangements can be selected for by demanding growth on lactose as a sole carbon source.

The strategies employed were well-characterized techniques for genetically manipulating bacteria. The phage λ Red recombination system was used to initially tag the operons by replacing the 5S rRNA gene with *aph*, which worked well with six out of seven operons tagged. However multiple attempts using this technique to interrupt the promoters in tagged strains failed. The Flippase recombination system on the other hand was very efficient at both flipping out *aph* and flipping in pCE36 containing the *lac* operon. Other strategies such as transduction were also used successfully to construct the *recD* and *mutS* background strains; however this technique also failed to yield strains with the desired interrupted promoter.

An unexpected result that occurred during the strain construction process was the low ß-galactosidase activity of the tagged strains (Figure B.4). The *rrn* operon promoters should have been highly expressing *lac* mRNA under the assay conditions. Sequencing of the high-expressing *lac* mutants suggested that translation was affected due to the FRT site occluding the ribosome binding site. These results also showed that the ability to grow on lactose as a sole carbon source requires significant *lac* expression, supporting the strategy of using the *lac* system to select cells with rearrangements.

The inability to interrupt the promoter of a *lac*-tagged *rrn* operon was perplexing. The failure of multiple attempts using different approaches suggests that there is a viability issue. However, work in *E. coli* has shown fitness is not significantly affected when up to two *rrn* operons are deleted (10), much less viability. Furthermore many of the attempts were made in the strain containing a *lac*-tagged *rrnH*. The *rrnH* operon is the farthest operon from the origin of replication and so is less subject to gene dosage effects, as shown by the lower *rrnH* expression levels in rich media compared to *rrn* operons closer to the origin (11). Therefore the loss of function of at least this operon should be well-tolerated. Other explanations for why these strains could not be constructed exist. A possible but unlikely one is that the Red-swap functions were unknowingly not expressed correctly. The attempts using transduction most likely failed because recombination frequency is much higher when sequences are homologous vs. homeologous, as when trying to move the interrupted promoter from the untagged *rrnD* operon to another tagged *rrn* operon; however this doesn't explain why attempting to tranduce the interrupted promoter into the *rrnD*-tagged strain also failed. The TOPO cloning of the *rrn* promoters was also difficult, probably because the cloned promoters

were on multi-copy plasmids. The high number of plasmid-derived promoters could have sequestered the transcription machinery and affected the viability of the transformants. Ultimately the reason why these strains could not be constructed is unknown.

Significant progress was made in this study to construct strains for selecting cells with chromosomal rearrangements. While the *lac* system appeared robust enough for selection, screening for rearrangements is also a possibility with this system using flow cytometry and fluorophores for detecting ß-galactosidase. As these strains could prove very useful, and only one step remains to construct them, hopefully they will be completed in the future. Such attempts at finishing these strains could employ other Red-swap or cloning strategies to interrupt the promoters of the *lac*-tagged *rrn* operons. Alternatively new technologies may be developed that allow easier construction of strains for detecting of chromosomal rearrangements.

References

1. **Alokam, S., S. L. Liu, K. Said, and K. E. Sanderson.** 2002. Inversions over the terminus region in *Salmonella* and *Escherichia coli*: IS200s as the sites of homologous recombination inverting the chromosome of *Salmonella enterica* serovar typhi. J Bacteriol **184:**6190-7.

2. **Anderson, R. P., and J. R. Roth.** 1979. Gene duplication in bacteria: alteration of gene dosage by sister-chromosome exchanges. Cold Spring Harb Symp Quant Biol **43 Pt 2:**1083-7.

3. **Apirion, D., and A. Miczak.** 1993. RNA processing in prokaryotic cells. Bioessays **15:**113-20.

4. **Berkowitz, D., J. M. Hushon, H. J. Whitfield, Jr., J. Roth, and B. N. Ames.** 1968. Procedure for identifying nonsense mutations. J Bacteriol **96:**215-20.

5. **Bulach, D. M., R. L. Zuerner, P. Wilson, T. Seemann, A. McGrath, P. A. Cullen, J. Davis, M. Johnson, E. Kuczek, D. P. Alt, B. Peterson-Burch, R. L.**

Coppel, J. I. Rood, J. K. Davies, and B. Adler. 2006. Genome reduction in *Leptospira borgpetersenii* reflects limited transmission potential. Proc Natl Acad Sci U S A **103:**14560-5.

6. **Carpentier, A. S., B. Torresani, A. Grossmann, and A. Henaut.** 2005. Decoding the nucleoid organisation of *Bacillus subtilis* and *Escherichia coli* through gene expression data. BMC Genomics **6:**84.

7. **Cashel, M., Gentry, D. R., Hernandez, V. J., and D. Vinella.** 1996. The Stringent Response, p. 1458-1496. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella* Cellular and Molecular Biology, 2nd ed. ASM Press, Washington, D.C.

8. **Chain, P. S., E. Carniel, F. W. Larimer, J. Lamerdin, P. O. Stoutland, W. M. Regala, A. M. Georgescu, L. M. Vergez, M. L. Land, V. L. Motin, R. R. Brubaker, J. Fowler, J. Hinnebusch, M. Marceau, C. Medigue, M. Simonet, V. Chenal-Francisque, B. Souza, D. Dacheux, J. M. Elliott, A. Derbise, L. J. Hauser, and E. Garcia.** 2004. Insights into the evolution of *Yersinia pestis* through whole-genome comparison with *Yersinia pseudotuberculosis*. Proc Natl Acad Sci U S A **101:**13826-31.

9. **Cherepanov, P. P., and W. Wackernagel.** 1995. Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. Gene **158:**9-14.

10. **Condon, C., D. Liveris, C. Squires, I. Schwartz, and C. L. Squires.** 1995. rRNA operon multiplicity in *Escherichia coli* and the physiological implications of *rrn* inactivation. J Bacteriol **177:**4152-6.

11. **Condon, C., J. Philips, Z. Y. Fu, C. Squires, and C. L. Squires.** 1992. Comparison of the expression of the seven ribosomal RNA operons in *Escherichia coli*. Embo J **11:**4175-85.

12. **Couturier, E., and E. P. Rocha.** 2006. Replication-associated gene dosage effects shape the genomes of fast-growing bacteria but only for transcription and translation genes. Mol Microbiol **59:**1506-18.

13. **Darling, A. E., I. Miklos, and M. A. Ragan.** 2008. Dynamics of genome rearrangement in bacterial populations. PLoS Genet **4:**e1000128.

14. **Datsenko, K. A., and B. L. Wanner.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. Proc Natl Acad Sci U S A **97:**6640-5.

15. **Deng, W., V. Burland, G. Plunkett, 3rd, A. Boutin, G. F. Mayhew, P. Liss, N. T. Perna, D. J. Rose, B. Mau, S. Zhou, D. C. Schwartz, J. D. Fetherston, L. E. Lindler, R. R. Brubaker, G. V. Plano, S. C. Straley, K. A. McDonough, M. L. Nilles, J. S. Matson, F. R. Blattner, and R. D. Perry.** 2002. Genome sequence of *Yersinia pestis* KIM. J Bacteriol **184:**4601-11.

16. **Ellermeier, C. D., A. Janakiraman, and J. M. Slauch.** 2002. Construction of targeted single copy *lac* fusions using lambda Red and FLP-mediated site-specific recombination in bacteria. Gene **290:**153-61.

17. **Esnault, E., M. Valens, O. Espeli, and F. Boccard.** 2007. Chromosome structuring limits genome plasticity in *Escherichia coli*. PLoS Genet **3:**e226.

18. **Garcia-Russell, N., T. G. Harmon, T. Q. Le, N. H. Amaladas, R. D. Mathewson, and A. M. Segall.** 2004. Unequal access of chromosomal regions to each other in *Salmonella*: probing chromosome structure with phage lambda integrase-mediated long-range rearrangements. Mol Microbiol **52:**329-44.

19. **Guijo, M. I., J. Patte, M. del Mar Campos, J. M. Louarn, and J. E. Rebollo.** 2001. Localized remodeling of the *Escherichia coli* chromosome: the patchwork of segments refractory and tolerant to inversion near the replication terminus. Genetics **157:**1413-23.

20. **Haack, K. R., and J. R. Roth.** 1995. Recombination between chromosomal IS200 elements supports frequent duplication formation in *Salmonella typhimurium*. Genetics **141:**1245-52.

21. **Helm, R. A., A. G. Lee, H. D. Christman, and S. Maloy.** 2003. Genomic rearrangements at *rrn* operons in *Salmonella*. Genetics **165:**951-9.

22. **Helm, R. A., and S. Maloy.** 2001. Rapid approach to determine *rrn* arrangement in *Salmonella* serovars. Appl Environ Microbiol **67:**3295-8.

23. **Hill, C. W., R. H. Grafstrom, B. W. Harnish, and B. S. Hillman.** 1977. Tandem duplications resulting from recombination between ribosomal RNA genes in *Escherichia coli*. J Mol Biol **116:**407-28.

24. **Hill, C. W., and B. W. Harnish.** 1981. Inversions between ribosomal RNA genes of *Escherichia coli*. Proc Natl Acad Sci U S A **78:**7069-72.

25. **Hill, C. W., and B. W. Harnish.** 1982. Transposition of a chromosomal segment bounded by redundant rRNA genes into other rRNA genes in *Escherichia coli*. J Bacteriol **149:**449-57.

26. **Iguchi, A., S. Iyoda, J. Terajima, H. Watanabe, and R. Osawa.** 2006. Spontaneous recombination between homologous prophage regions causes large-scale inversions within the *Escherichia coli* O157:H7 chromosome. Gene **372:**199-207.

27. **Jeong, K. S., J. Ahn, and A. B. Khodursky.** 2004. Spatial patterns of transcriptional activity in the chromosome of *Escherichia coli*. Genome Biol **5:**R86.

28. **Jumas-Bilak, E., S. Michaux-Charachon, G. Bourg, D. O'Callaghan, and M. Ramuz.** 1998. Differences in chromosome number and genome rearrangements in the genus *Brucella*. Mol Microbiol **27:**99-106.

29. **Lindroos, H., O. Vinnere, A. Mira, D. Repsilber, K. Naslund, and S. G. Andersson.** 2006. Genome rearrangements, deletions, and amplifications in the natural population of *Bartonella henselae*. J Bacteriol **188:**7426-39.

30. **Liu, G. R., W. Q. Liu, R. N. Johnston, K. E. Sanderson, S. X. Li, and S. L. Liu.** 2006. Genome plasticity and *ori-ter* rebalancing in *Salmonella typhi*. Mol Biol Evol **23:**365-71.

31. **Liu, G. R., A. Rahn, W. Q. Liu, K. E. Sanderson, R. N. Johnston, and S. L. Liu.** 2002. The evolving genome of *Salmonella enterica* serovar Pullorum. J Bacteriol **184:**2626-33.

32. **Liu, S. L., and K. E. Sanderson.** 1995. The chromosome of *Salmonella paratyphi* A is inverted by recombination between rrnH and rrnG. J Bacteriol **177:**6585-92.

33. **Liu, S. L., and K. E. Sanderson.** 1996. Highly plastic chromosomal organization in *Salmonella typhi*. Proc Natl Acad Sci U S A **93:**10303-8.

34. **Liu, S. L., and K. E. Sanderson.** 1998. Homologous recombination between rrn operons rearranges the chromosome in host-specialized species of *Salmonella*. FEMS Microbiol Lett **164:**275-81.

35. **Liu, S. L., and K. E. Sanderson.** 1995. Rearrangements in the genome of the bacterium *Salmonella typhi*. Proc Natl Acad Sci U S A **92:**1018-22.

36. **Liu, W. Q., G. R. Liu, J. Q. Li, G. M. Xu, D. Qi, X. Y. He, J. Deng, F. M. Zhang, R. N. Johnston, and S. L. Liu.** 2007. Diverse genome structures of *Salmonella paratyphi* C. BMC Genomics **8:**290.

37. **Louarn, J. M., J. P. Bouche, F. Legendre, J. Louarn, and J. Patte.** 1985. Characterization and properties of very large inversions of the *E. coli* chromosome along the origin-to-terminus axis. Mol Gen Genet **201:**467-76.

38. **Parkhill, J., M. Sebaihia, A. Preston, L. D. Murphy, N. Thomson, D. E. Harris, M. T. Holden, C. M. Churcher, S. D. Bentley, K. L. Mungall, A. M. Cerdeno-Tarraga, L. Temple, K. James, B. Harris, M. A. Quail, M. Achtman, R. Atkin, S. Baker, D. Basham, N. Bason, I. Cherevach, T. Chillingworth, M. Collins, A. Cronin, P. Davis, J. Doggett, T. Feltwell, A. Goble, N. Hamlin, H. Hauser, S. Holroyd, K. Jagels, S. Leather, S. Moule, H. Norberczak, S. O'Neil, D. Ormond, C. Price, E. Rabbinowitsch, S. Rutter, M. Sanders, D. Saunders, K. Seeger, S. Sharp, M. Simmonds, J. Skelton, R. Squares, S. Squares, K. Stevens, L. Unwin, S. Whitehead, B. G. Barrell, and D. J. Maskell.** 2003. Comparative analysis of the genome sequences of *Bordetella pertussis*, *Bordetella parapertussis* and *Bordetella bronchiseptica*. Nat Genet **35:**32-40.

39. **Parkhill, J., B. W. Wren, N. R. Thomson, R. W. Titball, M. T. Holden, M. B. Prentice, M. Sebaihia, K. D. James, C. Churcher, K. L. Mungall, S. Baker, D. Basham, S. D. Bentley, K. Brooks, A. M. Cerdeno-Tarraga, T. Chillingworth, A. Cronin, R. M. Davies, P. Davis, G. Dougan, T. Feltwell, N. Hamlin, S. Holroyd, K. Jagels, A. V. Karlyshev, S. Leather, S. Moule, P. C. Oyston, M. Quail, K. Rutherford, M. Simmonds, J. Skelton, K. Stevens, S. Whitehead, and B. G. Barrell.** 2001. Genome sequence of *Yersinia pestis*, the causative agent of plague. Nature **413:**523-7.

40. **Paul, B. J., W. Ross, T. Gaal, and R. L. Gourse.** 2004. rRNA transcription in *Escherichia coli*. Annu Rev Genet **38:**749-70.

41. **Petrosino, J. F., Q. Xiang, S. E. Karpathy, H. Jiang, S. Yerrapragada, Y. Liu, J. Gioia, L. Hemphill, A. Gonzalez, T. M. Raghavan, A. Uzman, G. E. Fox, S. Highlander, M. Reichard, R. J. Morton, K. D. Clinkenbeard, and G. M. Weinstock.** 2006. Chromosome rearrangement and diversification of *Francisella tularensis* revealed by the type B (OSU18) genome sequence. J Bacteriol **188:**6977-85.

42. **Schmid, M. B., and J. R. Roth.** 1987. Gene location affects expression level in *Salmonella typhimurium*. J Bacteriol **169:**2872-5.

43. **Segall, A., M. J. Mahan, and J. R. Roth.** 1988. Rearrangement of the bacterial chromosome: forbidden inversions. Science **241:**1314-8.

44. **Segall, A. M., and J. R. Roth.** 1989. Recombination between homologies in direct and inverse orientation in the chromosome of *Salmonella*: intervals which are nonpermissive for inversion formation. Genetics **122:**737-47.

45. **Shu, S., E. Setianingrum, L. Zhao, Z. Li, H. Xu, Y. Kawamura, and T. Ezaki.** 2000. I-CeuI fragment analysis of the *Shigella* species: evidence for large-scale chromosome rearrangement in *S. dysenteriae* and *S. flexneri*. FEMS Microbiol Lett **182:**93-8.

46. **Sousa, C., V. de Lorenzo, and A. Cebolla.** 1997. Modulation of gene expression through chromosomal positioning in *Escherichia coli*. Microbiology **143 (Pt 6):**2071-8.

47. **Valens, M., S. Penaud, M. Rossignol, F. Cornet, and F. Boccard.** 2004. Macrodomain organization of the *Escherichia coli* chromosome. Embo J **23:**4330-41.

48. **Van Sluys, M. A., M. C. de Oliveira, C. B. Monteiro-Vitorello, C. Y. Miyaki, L. R. Furlan, L. E. Camargo, A. C. da Silva, D. H. Moon, M. A. Takita, E. G. Lemos, M. A. Machado, M. I. Ferro, F. R. da Silva, M. H. Goldman, G. H. Goldman, M. V. Lemos, H. El-Dorry, S. M. Tsai, H. Carrer, D. M. Carraro, R. C. de Oliveira, L. R. Nunes, W. J. Siqueira, L. L. Coutinho, E. T. Kimura, E. S. Ferro, R. Harakava, E. E. Kuramae, C. L. Marino, E. Giglioti, I. L. Abreu, L. M. Alves, A. M. do Amaral, G. S. Baia, S. R. Blanco, M. S. Brito, F. S. Cannavan, A. V. Celestino, A. F. da Cunha, R. C. Fenille, J. A. Ferro, E. F. Formighieri, L. T. Kishi, S. G. Leoni, A. R. Oliveira, V. E. Rosa, Jr., F. T. Sassaki, J. A. Sena, A. A. de Souza, D. Truffi, F. Tsukumo, G. M. Yanai, L. G. Zaros, E. L. Civerolo, A. J. Simpson, N. F. Almeida, Jr., J. C. Setubal, and J. P. Kitajima.** 2003. Comparative analyses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. J Bacteriol **185:**1018-26.

Table B.1. PCR primer sequences.

Primer	Sequence (5'→ 3'):
5SKan-for	TAGCGCGGTGGTCCCACCTGACCCCATGCCGTGTAGGCTGGAGCTGCTTC
5SKan-rev	GGCAGTTCCCTACTCTCGCATGGGGAGACCCATATGAATATCCTCCTTAG
oriR6K	GGACAACAAGCCAGGGATG
rrnboxAcat	GCTCTTTAACAATTTATCAGACAATCTGTGCTAATACGACTCACTATAGG
rrnboxCcat	AAGAATCCGTATCTTCGAGTGCCCAGTCACGACGTTGTAAAACGACGGCC
rrnboxAcat2	GCTCTTTAACAATTTATCAGACAATCTGTGTGGGCACTCGAAGATACGGACTAATACGACTCACTATAGGGAAAGC
16Scat	CGTTCAATCTGAGCCATGATCAAACTCTTCAATTTAAAAGTTTGATGCTCGTCACGACGTTGTAAAACGACGGCC
HemG	TCCGTGGCGACTTGACTACTGTGCC
MobB	TGCCTTCATTTTGCGGTGGTTAGAG
MurI	GCGTCGGTGGATTGTCGGTCTATGA
MurB	CCAGGCGCTCAGTAGTTGTTGTTCG
YieP	GCTCCAGGCTAATACGCATCACCAG
YifA	GCTGTTAGGGCACTTCACTTTGGCG
YrdA	GGGTTGCCGTGTGGATTGGATGGAG
AcrF	CGCAGTAGGCACAGGGGTTATGGGG
PurH	CGATAGGGGCGATGTGGTGCTGTTC
MetA	GGAAATCGGCATAGCGTGAGTGTGG
ClpB	CCGTCGCCCTTATTCCGTCATCTTG
KgtP-2	CGCATCTCCCGAGCCTCAGCGTTGT
YaeD	CCATCCGCAGGGCAGCATAGAAGAG
YafB	CGGCAATAGCCTTTTCCATCAACGG
rrnAcat	GGTTGCGCACTTTGCCCGTGAAATAGCCCATTTAACCAACAAATCGTCGGCTAATACGACTCACTATAGGGAAAGC
rrnBcat	ATGGCTTTGAAACGCTCGAAAAACTGCCGGTTTAATGGCGTTTTGGGTAACTAATACGACTCACTATAGGAAAAGC
rrnHcat	GGCGGACTGGGTGTTAAACAGCCTTGCTGACCTGCCATCAGCGATAACTAATACGACTCACTATAGGGAAAGC

Table B.2. Donor strains used for preparing transducing lysates for mapping tagged *rrn* operons.

Strain	Tests for *rrn*:	Gene Tn10 Inserted Into	Distance between Tet^R and 5S rRNA gene	Estimated Co-transduction Frequency
MST4618	A	*polA*	~6.1	0.639
MST2768	B	*argH*	~14.4 kbp	0.304
MST2765	C	*ilvA*	~9.4 kbp	0.486
MST401	D	*rrlD* (16S rRNA)	~1.9 kbp	0.876
MST226	E	*aceA*	~4.4 kbp	0.729
MST274	G	*pheA*	~12.3 kbp	0.374
MST3017	H	*dnaQ*	~9.2 kbp	0.495

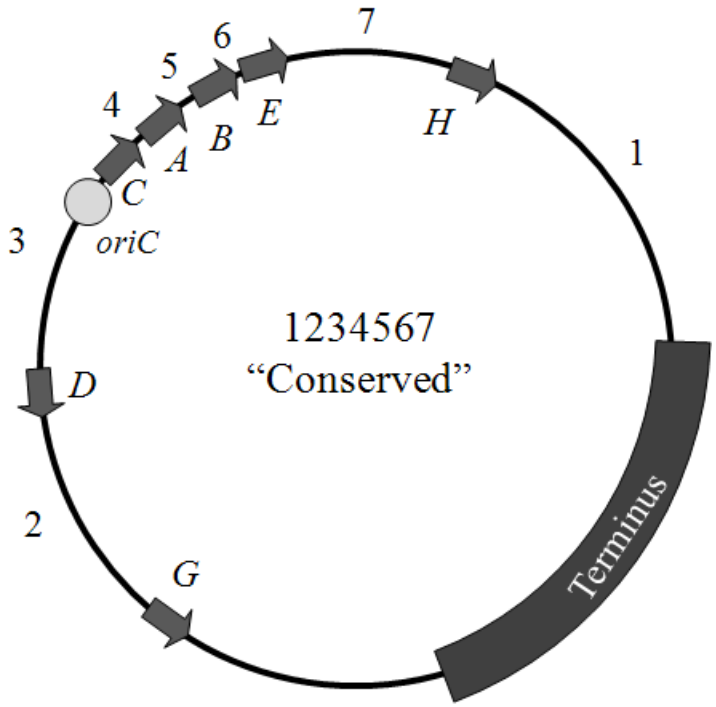

Figure B.1. The conserved *rrn* arrangement type 1234567 found in the broad host range serovars of *Salmonella enterica*. The seven *rrn* operons are lettered while the regions in between the operons are numbered.

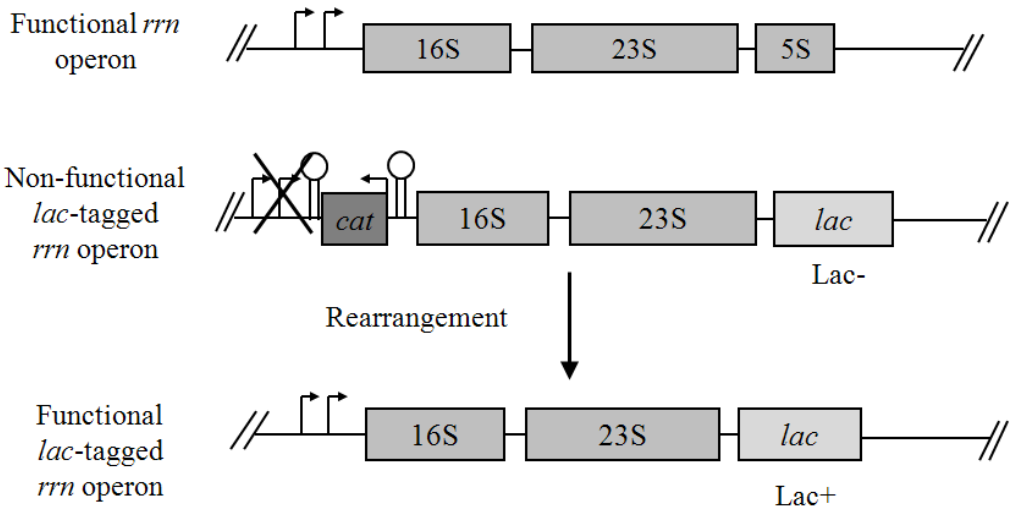

Figure B.2. Strategy for selection of cells that have chromosomal rearrangements by recombination between *rrn* operons. A) A function *rrn* operon. B) A non-functional *lac*-tagged rrn operon. C) Recombination involving the non-functional lac-tagged rrn operon swaps in functional promoters that drive lac expression.

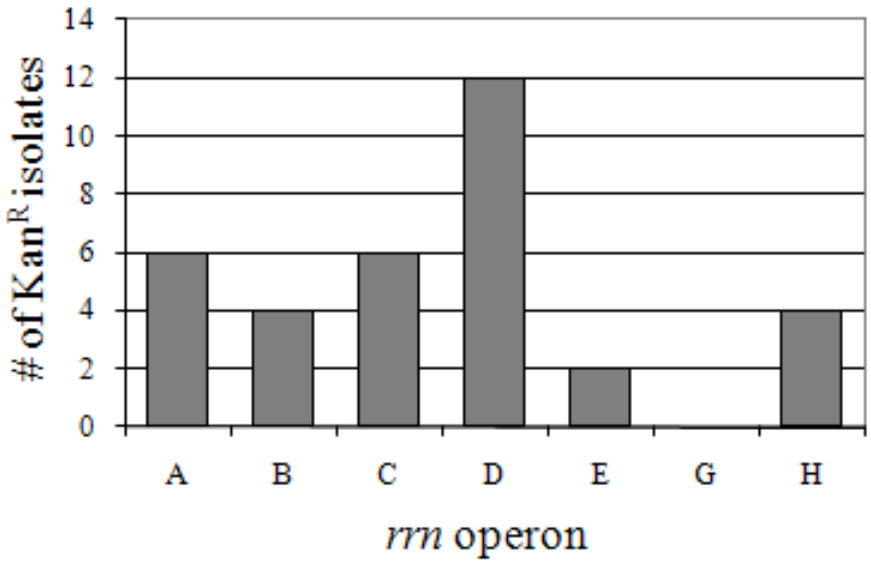

Figure B.3. Distribution of *rrn* operons tagged by replacing the 5S rRNA gene with *aph* conferring kanamycin resistance.

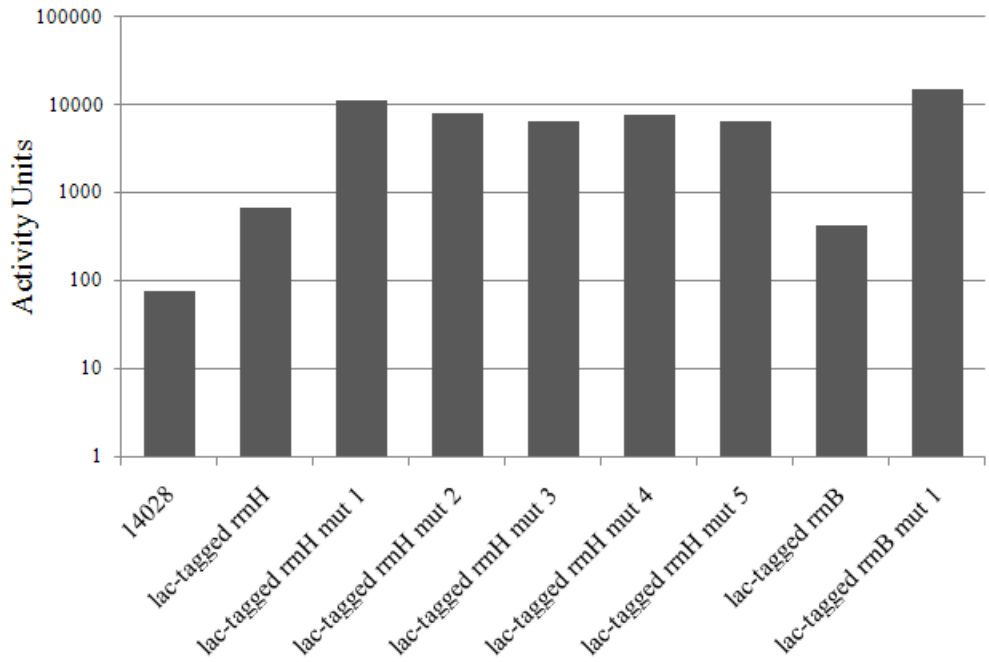

Figure B.4. ß-galactosidase activity of strains with *lac*-tagged *rrnH* or *rrnB*, and mutants selected by demanding growth on lactose as a sole carbon source. *S. enterica* sv. Typhimurium 14028 is the parental strain showing background activity (lacks *lacZ*).

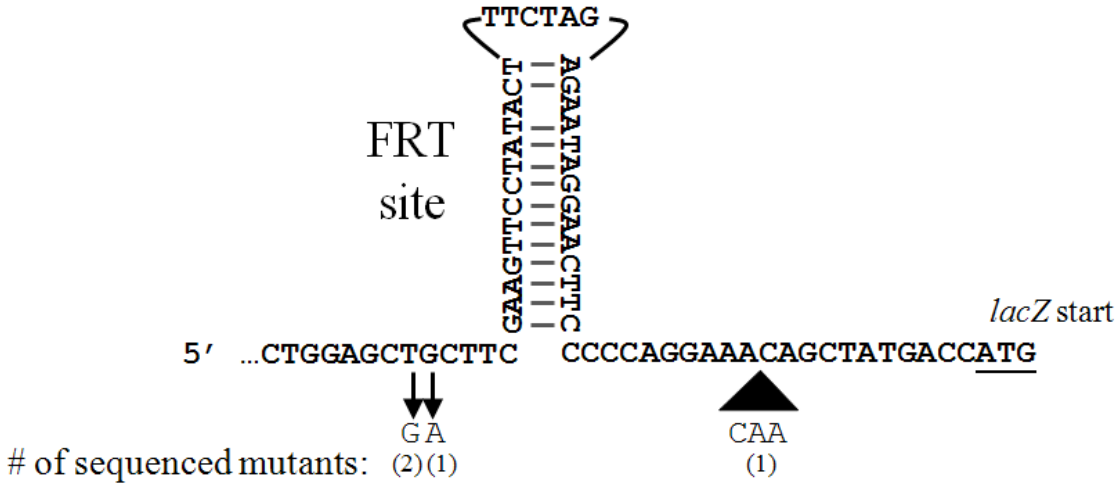

Figure B.5. Location of mutations that increase ß-galactosidase activity. Three mutations were single base changes upstream of the FRT site, and one mutation was a 3 bp insertion between the FRT site and the ribosome binding site of *lacZ*.