FNR is a Regulator of *Salmonella* Pathogenicity Island 2 in *Salmonella* Typhimurium

A thesis submitted to the University of Dublin in fulfillment of the requirements for the degree Doctor of Philosophy by

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Declaration

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Summary

During infection, *S.* Typhimurium employs *Salmonella* pathogenicity island (SPI)-encoded type three secretion systems (T3SS) 1 and 2 to invade and survive in host cells. However, expression of SPI-2 is seen at the epithelial border prior to host cell invasion in response to an unknown signal. A zone of relative oxygenation adjacent to the gastrointestinal tract mucosa, caused by diffusion of oxygen from the capillary network has been shown to cause priming of *Shigella* and Enterotoxigenic *E. coli* for entry into host cells through the oxygen sensing capabilities of the anaerobic metabolism regulator FNR. However, regulation of SPI-2 by FNR has not been established in *S.* Typhimurium. Here we show using a combination of RNA-seq and ChIP-seq that SPI-2 is highly expressed in an Δ*fnr* mutant under microaerobic conditions, and that FNR is a direct repressor of SPI-2 genes. Our particular focus on FNR regulation of SPI-2 under microaerobic growth in a glycerol/trimethylamine N-oxide/fumarate minimal medium has revealed that, not only does FNR repress the expression of the SPI-2 encoded type three secretion system apparatus proteins, effectors and chaperones through direct regulation of the SPI-2 response regulator SsrB, but it is also involved in the direct repression of a great number of effectors and virulence relevant sRNAs encoded throughout the chromosome and on the *Salmonella* virulence plasmid and that growth under aerobic conditions relieved repression. Furthermore, we have shown that the accurate spatiotemporal expression of SPI-2 is integral for maintenance of bacterial fitness, provided additional evidence that oxygen is an important signalling molecule for the control of bacterial motility and demonstrated that FNR is an important regular in both intra- and extracellular environments. Our results demonstrate that *S.* Typhimurium regulates expression of virulence genes in response to changing oxygen concentrations to prepare for the harsh intracellular environment of host cells using the regulator of anaerobic metabolism FNR. Importantly, analysis of our Δ*fnr* RNA-seq-based transcriptomic data in conjunction with previously published datasets, will provide a more complete picture of mixed regulatory interactions within the cell. We hope that the addition of this data will help in the development of the full understanding of all regulatory interactions and inputs involved in the establishment of *S.* Typhimurium infection.

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Publications and Conferences

Publications

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Kary, S. C., Colgan, A. M., Mogre, A., Hansmeier, N., MacKenzie, K. D., Chao, T. C., Hokamp, K., Dorman, C. J., Hinton, J. C. D., Cameron, A. D. S., and Kröger, C. FNR is a regulator of SPI-2 in *Salmonella* Typhimurium (2018), Oral Presentation at the Canadian Society of Microbiologists (CSM) Student Symposium Competition, Winnipeg, Manitoba, Canada.

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List of Abbreviations

3′UTR	Three prime untranslated region
4/74	*Salmonella enterica* serovar Typhimurium strain 4/74
5′UTR	Five prime untranslated region
Amp	Ampicillin
APS	Ammonium persulfate
B_{max}	Maximum bound ratio
bp	Base pair
cDNA	Complementary DNA
CDS	Coding sequence
CFU	Colony forming unit
ChIP	Chromatin Immunoprecipitation
CI	Competitive index
Cm	Chloramphenicol
dH_2O	Distilled water
Dig	Digoxigenin
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide
EDTA	Ethylenediaminetetraacetic acid
EMSA	Electrophoretic mobility shift assay
ESP	Early stationary phase
FLP	Flippase recombinase
FRT	Flippase recognition site
GFP	Green fluorescent protein
GI	Gastrointestinal
HRP	Horseradish peroxidase
IGB	Integrated Genome Browser
InSPI2	SPI-2 inducing PCN medium
iNTS	Invasive Non Typhoisal *Salmonella*
Kan	Kanamycin
kb	Kilobase pair
K_D	Dissociation constant
LA	Lennox agar

LB	Lennox broth
LEP	Late exponential phase
LSP	Late stationary phase
MCS	Multiple cloning site
MEP	Mid exponential phase
MES	2-(N-morpholino)ethanesulfonic acid
MMA	Minimal medium A
MOPS	3-(N-morpholino)propanesulfonic acid
mRNA	Messenger RNA
NAP	Nucleoid associated protein
NonSPI2	Non-SPI-2 inducing PCN medium
nt	Nucleotide
OD_{600}	Optical density at 600 nm
ORF	Open reading frame
PBS	Phosphate buffered saline
PCN	Phosphate, Carbon, Nitrogen medium
PCR	Polymerase chain reaction
PES	Polyethersulfone
PIPES	Piperazie-N'N'bis(2-ethanesulfonic acid)
qPCR	Quantitative real-time PCR
RBS	Ribosome binding site
RNA	Ribonucleic acid
RNA-seq	High-throughput cDNA sequencing
RNAP	RNA polymerase
RNase	Ribonuclease
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
rpm	Revolutions per minute
rRNA	Ribosomal RNA
RT	Reverse transcriptase/transcription
SCV	*Salmonella* containing vacuole
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate poly-acrylamide electrophoresis
SOB	Super optimal broth
SOC	Super optimal broth with catabolite repression
SPI	*Salmonella* Pathogenicity Island
sRNA	Small RNA
SSC	Saline-sodium citrate
STncXXX	*Salmonella* Typhimurium non-coding
Str	Streptomycin
T3SS	Type three secretion system
TAE	Tris acetate ethylenediaminetetraacetic acid
TBE	Tris borate ethylenediaminetetraacetic acid
TCA	Trichloroacetic acid
TCS	Two component system

TE	Tris Ethylenediaminetetraacetic acid
TEMED	Tetramethylethylenediamine
Tet	Tetracycline
TF	Transcription factor
TMAO	Trimethylamine *N*-oxide
TPM	Transcripts per million
tRNA	Transfer RNA
TSS	Transcription start site
UV	Ultra violet
v/v	Volume per volume
w/v	Weight per volume
WT	wild-type
X	xylose

Chapter 1
General Introduction

1.1 *Salmonella*

1.1.1 Overview of the Salmonella genus

Members of the genus *Salmonella* belong to the *Enterobacteriaceae* family (Ibarra & Steele-Mortimer, 2009; Rhen & Dorman, 2005). They diverged from a common ancestor with *Escherichia coli* (*E. coli*) between 100 and 150 million years ago (Fookes *et al.*, 2011; Sabbagh *et al.*, 2010). *Salmonella* are Gram-negative, rod-shaped, motile flagellates, facultative anaerobes and facultative intracellular pathogens (Fàbrega & Vila, 2013). The nomenclature of *Salmonella* is complex, contentious and still evolving. The current recommended system of nomenclature by the World Health Organization (WHO) Collaborating Centre (Issenhuth-Jeanjean *et al.*, 2014) subdivides the genus by differences in their 16S ribosomal RNA (rRNA) into two species: *Salmonella bongori* (*S. bongori*) and *Salmonella enterica* (*S. enterica*) (Popoff *et al.*, 2004). The type species, *S. enterica,* can be further divided into six subspecies, *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV) and *indica* (VI), based on their genomic relatedness and biochemical properties (Reeves *et al.*, 1999). *S. enterica* subsp. *enterica* is found predominantly in mammals and contributes to the vast majority of *Salmonella* infections in humans and warm-blooded animals. *S. bongori* and the remaining *S. enterica* subspecies are commonly associated with commensalism of cold-blooded vertebrates and isolation from environmental sources, with only a limited number of human infections reported (Desai *et al.*, 2013; Fookes *et al.*, 2011; Sabbagh *et al.*, 2010). *Salmonella* is further subdivided into serovars (serotypes) based on serologic identification of O (lipopolysaccharide) and H (flagellar) antigens with over 2,500 in total, and the *enterica* subspecies encompassing over 1,500 serovars (Grimont & Weill, 2007; Issenhuth-Jeanjean *et al.*, 2014). For simplicity, the species and subspecies of *S. enterica* subsp. *enterica* serovars are commonly omitted, for example *Salmonella enterica* subsp. *enterica* sv. Typhimurium is shortened to *S.* Typhimurium (Brenner *et al.*, 2000).

S. enterica infections, or salmonellosis, result in two major clinical manifestations: gastroenteritis and enteric fever. Enteric fever, also called Typhoid fever is human host-restricted systemic febrile infection caused by *S.* Typhi. A clinically similar but, often less severe disease, Paratyphoid fever, is caused by *S.* Paratyphi A, B and C (Crump & Mintz, 2010; McClelland *et al.*, 2004). *S.* Typhi is a relatively recently evolved serovar of *Salmonella*, as its divergence from a common ancestor is estimated to be only 50,000 years

ago and it has adapted to exclusively infect humans (Kidgell *et al.*, 2002). *S.* Typhi remains relevant today as it causes an estimated 21 million infections annually, resulting in approximately 200,000 – 600,000 deaths primarily in regions with poor economic development in children, adolescents, and the elderly (Buckle *et al.*, 2012; Crump & Mintz, 2010). *S.* Typhi is spread through fecal-oral transmission via contaminated food or water. After exposure, signs and symptoms may initially be absent, as the bacteria can disseminate systemically without triggering a pro-inflammatory response or diarrheal disease. Infected individuals carry *S.* Typhi in their bloodstream and intestinal tract, and they shed bacteria in their stool. After the incubation period, some people may remain asymptomatic. Asymptomatic carriers of *S.* Typhi harbour the bacteria in their gallbladder and, as was demonstrated by the infamous case of Mary Mallon or "Typhoid Mary" in the early 1900s, go on to spread enteric fever through person-to-person transmission (Basnyat & Baker, 2015; Gonzalez-Escobedo *et al.*, 2010). Others will go on to exhibit extreme fatigue, high fever (>39ºC), coughing, vomiting, headache and rapid pulse (Dougan & Baker, 2014).

Strains of non-typhoidal *Salmonella* (NTS) are the second most common causative agent of gastroenteritis after *Campylobacter* species, responsible for an estimated 93 million cases of gastroenteritis annually, as well as more than 155,000 deaths (Majowicz *et al.*, 2010; Murray *et al.*, 2015). The majority of human infections are caused by two *S. enterica* serovars, Typhimurium and Enteritidis. NTS infection begins with an inflammatory response upon invasion of host epithelial cells, with 80% of cases leading to a self-limiting gastroenteritis with symptoms including diarrhea with or without blood, abdominal cramps, nausea and fever (Dougan & Baker, 2014). NTS is typically non-fatal in healthy adults however, infants, young children, elderly people and immunocompromised patients are highly susceptible to NTS infections (Scallan *et al.*, 2011). Invasive NTS (iNTS) has recently emerged as a new pathogenic clade with a distinct genotype in sub-Saharan Africa. This emerging pathogen may have adapted to occupy an ecological and immunological niche provided by HIV, malaria, and malnutrition in Africa (Feasey *et al.*, 2012). iNTS strains of *S.* Typhimurium lead to an estimated 2 to 3 million infections and up to 700,000 deaths per year, with a mortality rate between 20-25% (Ao *et al.*, 2015; Feasey *et al.*, 2012; Gordon, 2008). Unlike Typhoidal serovars, NTS are zoonotic pathogens and have a broad spectrum of warm- and cold-blooded hosts. Thus, NTS are easily disseminated through agriculture resulting in contamination of food such as beef, poultry, eggs, and fresh produce (Stevens *et al.*, 2009). In mice, infection by *S.* Typhimurium results in a typhoid-like systemic fever similar to that of the human host-restricted serovars Typhi and Paratyphi. Therefore, *S.* Typhimurium is

used in murine systemic infections for the study of typhoid fever pathogenesis (Coburn *et al.*, 2006; Hurley *et al.*, 2014; Sabbagh *et al.*, 2010).

In this study *S.* Typhimurium strain 4/74 (GenBank accession numbers CP002487-CP002490) was used for all experiments. Strain 4/74 was originally isolated from a calf with salmonellosis in the UK, it is a virulent isolate and the parent strain of the commonly used lab strain and histidine (*hisG*) auxotroph SL1344. Strain 4/74 differs from SL1344 by only 8 single nucleotide polymorphisms (SNPs) including in *hisG* (Hoiseth & Stocker, 1981; Kröger *et al.*, 2012; Richardson *et al.*, 2011). Strain 4/74 is a prototroph for histidine production that possesses a functional histidine biosynthetic pathway that is important for replication in the vacuolar environment of certain mammalian cells including macrophages (Henry *et al.*, 2005) and is more invasive than another commonly used lab strain ATCC 14028S due to heterogeneity in expression of *Salmonella* pathogenicity island 1 (Clark *et al.*, 2011).

1.1.2 Pathogenesis of S. Typhimurium

Consumption of contaminated food and water is the primary route of infection for *S.* Typhimurium (Ohl & Miller, 2001; Thompson *et al.*, 2006). Following ingestion, *S.* Typhimurium must pass through the gastrointestinal (GI) tract from the mouth, through the esophagus, and through the stomach to reach the small intestine where it encounters a variety of stressful conditions meant to protect the host from infection (**Figure 1.1**). First, there is an upshift in temperature from that of the outside environment to approximately 37ºC in humans. As *S.* Typhimurium reaches the stomach, it must also survive the extreme acidic pH. Response to acid shock and survival in the stomach is facilitated by the acid-tolerance response (ATR) (Álvarez-Ordóñez *et al.*, 2011; Haraga *et al.*, 2008; Ohl & Miller, 2001). The ATR is also responsible for preparing bacterial cells for the acidic intracellular environment later in infection (Foster, 1991; Foster & Hall, 1990). As *S.* Typhimurium travels through the duodenum and jejunum of the small intestine to reach the ileum, its preferred site for invasion, the bacterium encounters increased osmolarity and anaerobiosis, as well as the host intestinal microbiota (Hébrard *et al.*, 2011). To reach the intestinal epithelium, *S.* Typhimurium requires a functional motility and chemotaxis system (Jones *et al.*, 1981). While crossing the intestinal mucosal layer, the bacterium must resist the host innate immune system including such elements as bile salts and antimicrobial peptides in order to access and adhere to the underlying epithelium (Haraga *et al.*, 2008).

Once at the intestinal epithelium, *S.* Typhimurium invades epithelial cells. It preferentially targets microfold (M) cells for translocation and can induce transformation of epithelial cells into M cells to promote host colonization and invasion (Jepson & Clark, 2001; Tahoun *et al.*, 2012). M-cell-mediated uptake of *S.* Typhimurium also allows bacterial cells to invade adjacent epithelial cells from their apical and basolateral surfaces (Haraga *et al.*, 2008). Adherence of bacterial cells to the apical surface of epithelial cells is necessary for the induction of invasion associated genes (Jones *et al.*, 1981) and is accomplished by *Salmonella* surface appendages such as fimbriae and non-fimbrial adhesins (Wagner & Hensel, 2011). Following attachment, *Salmonella* Pathogenicity Island 1 (SPI-1) encoded type III secretion system (T3SS)-dependent translocation of effector proteins into the cytosol of non-phagocytic cells actively induces host cell cytoskeletal rearrangement, membrane ruffling and ultimately uptake of the bacterium into a membrane-derived vacuole (Finlay *et al.*, 1991; Lhocine *et al.*, 2015; Srikanth *et al.*, 2011). Detection of the invading bacteria by components of the host immune system and active translocation of additional bacterial effectors into epithelial cells by *S.* Typhimurium leads to a pro-inflammatory immune response, resulting in the inflammation of the gut, fluid secretion and the symptoms of gastroenteritis (Haraga *et al.*, 2008; Hurley *et al.*, 2014; Thiennimitr *et al.*, 2012). Inflammation promotes shedding of epithelial cells into the lumen of the intestine, depositing bacteria back into the lumen. Bacteria that remain in the intestinal lumen can benefit from the by-products inflammation of the intestine to use as electron acceptors for anaerobic metabolism to outcompete the resident microbiota who rely on fermentation (Thiennimitr *et al.*, 2012; Winter *et al.*, 2013). Enhanced growth in the intestinal lumen promotes transmission of *S.* Typhimurium to new hosts by the fecal–oral route (Lawley *et al.*, 2007).

S. Typhimurium cells that successfully invade host epithelial cells are enclosed in a membrane-derived vesicle termed the spacious phagosome (SP). The SP later fuses with lysosomes, acidifies and shrinks to become adherent around one or more bacteria creating the *Salmonella*-containing vacuole (SCV) (Steele-Mortimer, 2008). Maturation of the SCV and survival of bacterial cells is dependent upon proteins encoded on *Salmonella* pathogenicity island 2 (SPI-2). The SPI-2 T3SS translocates effector proteins across the phagosomal membrane to allow intracellular survival and replication. Formation of the SCV requires altering the host cell endocytic trafficking pathway to avoid normal phagosome maturation and fusion with lysosomes (Rathman *et al.*, 1997). As the SCV matures, *S.* Typhimurium encounters additional stressors including starvation of magnesium, phosphate and iron and a further reduction in pH (Hébrard *et al.*, 2011). The mature SCV migrates to

the Golgi-apparatus and simultaneously *Salmonella*-induced filaments (SIFs) are formed. SIFs are tubular filamentous structures which extend from the SCV and form complex networks throughout the cell to facilitate interactions with host organelles (Knuff & Finlay, 2017; Krieger *et al.*, 2014). *S.* Typhimurium can also proliferate in epithelial cells outside of their SCV. A significant subpopulation damages the SCV membrane, leading to escape from the SCV and extensive proliferation in the cytosol. These bacteria use SPI-1 encoded proteins to evade destruction by autophagic mechanisms of the host epithelial cell and survive to hyper-replicate in the epithelial cytosol (Knodler *et al.*, 2014).

Infection with NTS in healthy human adults, is usually limited to the intestine, however in cases of susceptible hosts and in murine infections, *S.* Typhimurium can go on to persist in the SCV of macrophages. SCVs transcytose to the basolateral membrane of epithelial cells and are subsequently engulfed by phagocytic cells, primarily macrophages. This bacterial internalization ultimately results in an intra-macrophage SCV. The intra-macrophage SCV is similar to the intra-epithelial SCV, and similar host cell pathways are triggered, allowing *S.* Typhimurium to proliferate by preventing phagosome maturation (Fàbrega & Vila, 2013). There are some differences between the SCVs of the epithelial cells and macrophages. While nutrient starvation and acidic pH is a feature of both intracellular vacuoles, the intra-macrophage is more bactericidal in nature (Hautefort *et al.*, 2008). Remodelling of the protein, carbohydrate and membrane components of the bacterial envelope confer resistance to antimicrobial peptides and reactive oxygen and nitrogen species (ROS, RNS) that can damage the bacterial cell (Haraga *et al.*, 2008). Infected macrophages can go on to cause systemic infection by disseminating through the bloodstream to organs such as the spleen and liver (Haraga *et al.*, 2008).

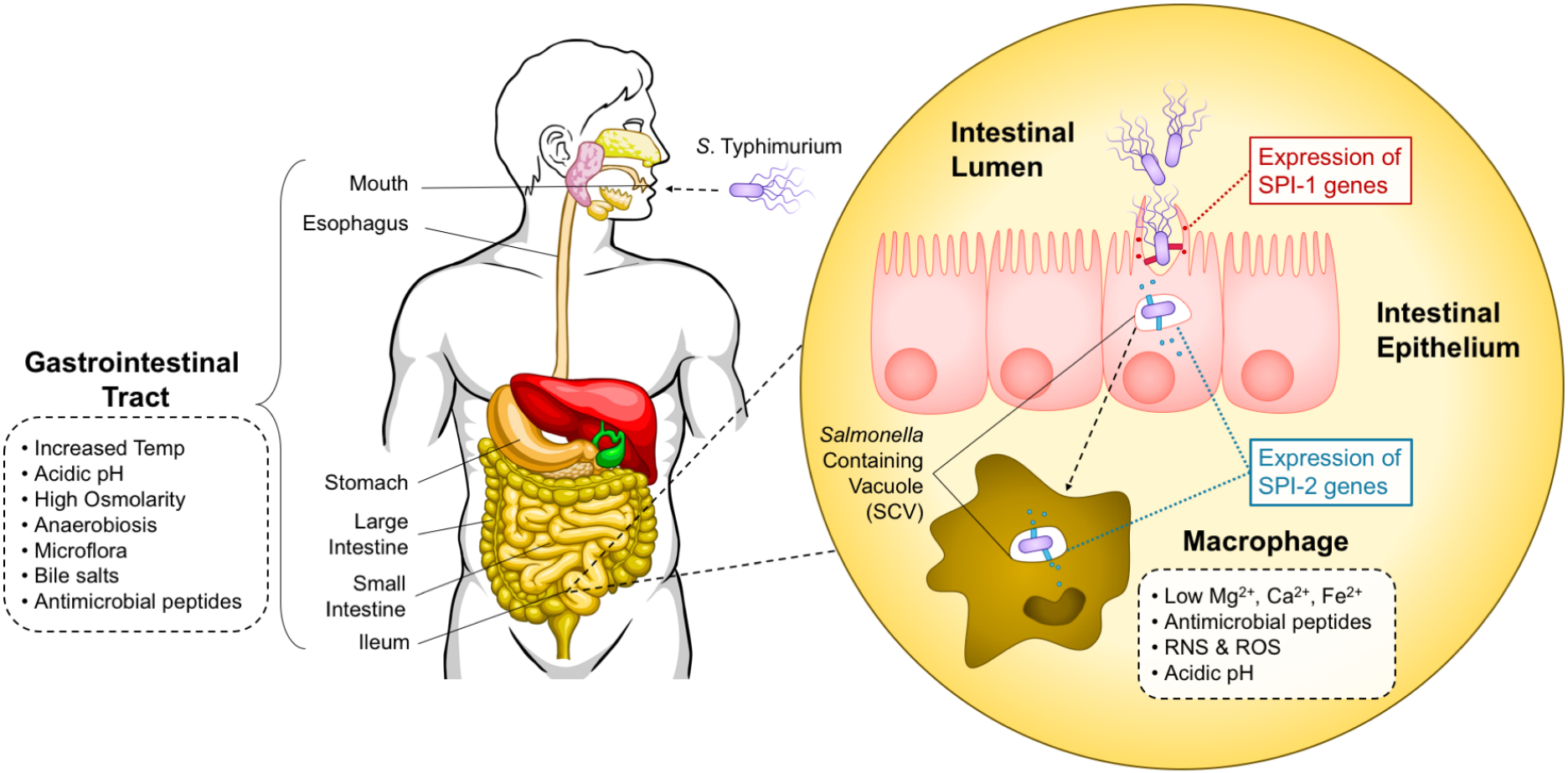

Gastrointestinal Tract
• Increased Temp
• Acidic pH
• High Osmolarity
• Anaerobiosis
• Microflora
• Bile salts
• Antimicrobial peptides
Mouth
Esophagus
Stomach
Large Intestine
Small Intestine
Ileum
S. Typhimurium
Intestinal Lumen
Expression of SPI-1 genes
Intestinal Epithelium
Salmonella Containing Vacuole (SCV)
Expression of SPI-2 genes
Macrophage
• Low Mg2+, Ca2+, Fe2+
• Antimicrobial peptides
• RNS & ROS
• Acidic pH

Figure 1.1 *S.* Typhimurium must circumvent stressful conditions during infection.
S. Typhimurium is ingested via contaminated food or water and passes through gastrointestinal (GI) tract from the mouth, esophagus, and stomach to the small intestine. *S.* Typhimurium encounters several deleterious stress conditions in the GI tract as part of the hosts first line of defense. Magnified panel: Environmental signals within the intestinal lumen induce expression of the *Salmonella* Pathogenicity Island 1 (SPI-1) Type 3 Secretion System (T3SS), which injects effector proteins into the host epithelial cell (in red). These effector proteins trigger intestinal inflammation and bacterial endocytosis of the bacteria and invasion of the intestinal epithelial cells. The intracellular environment of host cells provides the necessary signals for expression of the SPI-2 T3SS and effector proteins (in blue). *S.* Typhimurium survives and proliferates intracellularly within the *Salmonella* containing vacuole (SCV). In susceptible hosts, *S.* Typhimurium becomes engulfed by macrophages and survives and replicates within the SCV, leading to bacterial dissemination and systemic infection. Conditions encountered by *S.* Typhimurium within the macrophage SCV induce expression of SPI-2 genes necessary for survival and proliferation.

1.1.3 Flagella

Flagella are complex motility structures that allow rotational propulsion of bacteria and chemotaxis towards nutrients (Stecher *et al.*, 2008). *Salmonellae* are peritrichously flagellated, meaning they have multiple flagella at random positions on cell. They express between 5-10 flagella at a time (Parker & Guard-Petter, 2001; van Asten & van Dijk, 2005). The synthesis and function of the flagellar and chemotaxis systems involves over 50 genes from at least 17 operons (*flh*, *flg*, *fli*, *flj*, *mot*, *che*, *tar*, *tsr*, and *aer*) that constitute the flagellar regulon (Chilcott & Hughes, 2000). The individual flagella are composed of three distinct substructures: the basal body, a transmembrane motor, a hook that links the motor and the filament, and the filament that acts as a propeller (Aizawa, 1996). The major structural component of the filament is flagellin, a monomeric protein encoded by two different genes, *fljB* and *fliC*. *S.* Typhimurium exhibits phase variation via alternate expression of the two antigenically distinct flagellins (Bonifield & Hughes, 2003). Cytosolic delivery of flagellin by bacterial secretion systems has been shown to cause activation of the host inflammasome and subsequent pro-inflammatory cell death of infected macrophages, thus repression of flagellin in the macrophage is important for intracellular survival. Recently, a trans-acting leader mRNA was discovered to post-transcriptionally regulate *fljB* from the same operon

as a virulence protein, *mgtCBR* during infection (Choi *et al.*, 2017; Miranda-CasoLuengo *et al.*, 2017). Additionally, bacteria expressing FliC-flagella were advantaged in identifying target sites on host cell surfaces and invading epithelial cells and, FliC-expressing *S.* Typhimurium outcompeted FljB-expressing bacteria for intestinal tissue colonisation in gastroenteritis and typhoid murine infection models (Horstmann *et al.*, 2017). Bacterial flagella are required for both bacterial movement and immune detection, therefore production of flagella must be tightly regulated during infection. As previously discussed, flagella play a role in *S.* Typhimurium virulence as motility is necessary for bacterial approach to the epithelial cells in the host ileum, which is a prerequisite for bacterial adhesion and invasion of host cells. Flagella also play a role in providing a competitive advantage to *S.* Typhimurium as mutations affecting flagellar assembly and chemotaxis impaired the fitness of the pathogen in the inflamed intestine, but not in the normal gut. Thus, motility allows *S.* Typhimurium to move towards high energy nutrients and utilize them for enhanced growth in the inflamed gut (Stecher *et al.*, 2008). Aflagellated *Salmonella* were deficient in their ability to upregulate the pro-inflammatory response in a murine enteritis model and caused increased epithelial apoptosis. Therefore, it can be suggested that flagella are involved in the induction of the pro-inflammatory response and inhibition of apoptosis in epithelial cells (Vijay-Kumar & Gewirtz, 2009). Recently a link has been made between SPI-2 and flagellar regulation. The study shows that SsrB, the SPI-2 response regulator, alters a transcriptional network controlling bacterial motility through an SsrB binding site upstream of *flhDC*. This repression through SsrB limits inflammasome activation during host cell infection (Ilyas *et al.*, 2018). Thus, flagella are important virulence factors for *S.* Typhimurium, as the bacterium must move toward nutrients to obtain food, and it simultaneously must evade the host immune system.

1.1.4 Salmonella pathogenicity islands

The ability of *Salmonella* to cause disease is largely dependent on the expression of genes found in the *Salmonella* pathogenicity islands (SPIs). The SPIs are large clusters of genes encoding various virulence factors with low GC content and different codon usage compared to the rest of the chromosome (Groisman & Ochman, 1997). The pathogenicity islands were acquired by horizontal gene transfer and are therefore not found in non-pathogenic relatives (Jacobsen *et al.*, 2011). To date 23 pathogenicity islands have been identified across the *Salmonella* genus (Fookes *et al.*, 2011; Hayward *et al.*, 2014; Sabbagh *et al.*, 2010), and *S.* Typhimurium carries 13 SPIs (Kröger *et al.*, 2012). However, only 5 SPIs are of particular

importance and have been clearly shown to play a role in *S*. Typhimurium virulence (Fabrega & Vila, 2013) (**Table 1.1**). Coordinated regulation of SPI encoded genes involves loci situated both within and outside the islands on the chromosome, and a virulence plasmid (Ellermeier & Slauch, 2007; Fabrega & Vila, 2013). SPI-l and SPI-2 are the best studied pathogenicity islands, both encoding T3SSs that translocate effector molecules into host cells and are required for invasion of epithelial cells and intracellular survival, respectively (Haraga *et al.*, 2008).

1.1.4.1 Salmonella Pathogenicity Island-1

The SPI-1 pathogenicity island is approximately 40 kb and has been shown to be required for invasion of non-phagocytic epithelial cells, induction of intestinal inflammatory responses and diarrhea, as well as colonization of the intestine. Genes encoding the structural components of the SPI-1 T3SS and effector proteins are organised into three operons: *prg*/*org*, *inv*/*spa* and *sic*/*sip* (Ellermeier & Slauch, 2007). The *prg*/*org* and *inv*/*spa* operons encode a needle complex while the *sic*/*sip* operon encodes a translocon which embeds in the host cell membrane for translocation of effector proteins (Haraga *et al.*, 2008; Srikanth *et al.*, 2011). Upon contact with epithelial cells, *S*. Typhimurium initiates the translocation of several effector proteins in a highly co-ordinated fashion to mediate its uptake by endocytosis (Srikanth *et al.*, 2011). SopE, SopE2 and SopB are essential for invasion of *S*. Typhimurium as mutants defective in any of these effectors are incapable of inducing actin cytoskeleton rearrangements (Haraga *et al.*, 2008; Srikanth *et al.*, 2011). The effectors SipA and SipC are responsible for membrane ruffling and engulfment (Srikanth *et al.*, 2011).

Signals integrated by a multitude of SPI-1 encoded and global regulators constitute a complex regulatory network to control the expression of SPI-1. HilA (hyperinvasion locus), encoded by *hilA*, is the master regulator of SPI-1 and ultimately, all forms of regulation act via modulating its expression. Mutants of *hilA* are incapable of invasion and are phenotypically equivalent to SPI-1 mutants (Lee *et al.*, 1992). HilA binds to and activates the promoters of the needle complex operons *prg*/*org* and *inv*/*spa*. Three positive regulators, SPI-1 encoded HilC and HilD, and RtsA control the expression of *hilA* through a feed forward loop. Additionally, whether by direct regulation of *hilA* or indirect regulation via regulation of *hilC*, *hilD* or *rtsA*, SPI-1 expression is controlled by environmental cues which are sensed and integrated by two component regulatory systems such as SirA/BarA, OmpR/EnvZ and PhoP/PhoQ (Ellermeier *et al.*, 2005; Ellermeier & Slauch, 2007).

Furthermore, SPI-1 is regulated by changes in DNA supercoiling and by several global regulators including the nucleoid-associated proteins (NAPs) FIS, H-NS, IHF, Hha and HU (Baños *et al.*, 2009; Cameron *et al.*, 2011; Mangan *et al.*, 2006; 2011; Ono *et al.*, 2005; Troxell *et al.*, 2011).

1.1.4.2 Salmonella Pathogenicity Island-2

The SPI-2 pathogenicity island is in its entirety 40 kb however, it can be separated into two distinct sections. The first section is a 15 kb region that encodes genes necessary for anaerobic respiration using tetrathionate as a terminal electron acceptor (discussed in detail in Section 1.2.2) and other genes of unknown function. The second 25 kb region encodes the T3SS and accessory proteins. These genes have been annotated according to their function: the secretion system regulators are named *ssr*, components of the secretion system apparatus are named *ssa*, secretion system effectors are named *sse*, and secretion system chaperones are named *ssc* (Hensel, 2000). It should be noted that 2 proteins of SPI-2, *ssrA* and *ssaB*, are sometimes called *spiR* and *spiC*, respectively. However, the contemporary literature most frequently refers to them as the former, and so they shall be referred to as *ssrA* and *ssaB* in the remainder of this study. The function of the SPI-2-encoded T3SS and effectors is to mediate the survival of *S.* Typhimurium within the intracellular compartment. SPI-2 secreted effector proteins are translocated into the host cell cytoplasm across the membrane of the SCV to manipulate host cell function and to allow intracellular survival and replication (Abrahams *et al.*, 2006).

The SPI-2 T3SS is essential for the systemic phase of infection and intracellular replication of *S.* Typhimurium. It is activated within the SCV and translocates a complex set of effector proteins into the host cell cytoplasm (Kuhle & Hensel, 2004). The requirement of *S.* Typhimurium to detect the near neutral pH of the host cytosol for effector translocation to occur is debated in the literature as some groups argue that only acidification of the bacterial cytosol is required (Kenney, 2018; Yu *et al.*, 2010; 2018). Regardless, secretion occurs through a hollow needle-like filament that extends from the bacterial cell surface and a translocon pore that is formed in the host cell membrane (**Figure 1.2**). The T3SS is comprised of SsaRSTUV which form the basal body and span the inner membrane to form an export gate that is connected to two additional inner membrane proteins, SsaDJ, and an outer membrane ring, SsaC. SsaV is an essential component of the export gate and interacts in the cytoplasm with a sorting platform consisting of SsaKOQX and an ATPase SsaN. A

cytoplasmic “gatekeeper” complex comprised of SsaBLM is required for translocon protein secretion and to prevent premature secretion of effectors. After assembly of the secretion apparatus, subunits of the needle filament SsaG, inner rod protein SsaI and a molecular “ruler” protein SsaP are secreted. When the filament reaches a defined length, translocon proteins are secreted (Kuhle & Hensel, 2004; Yu *et al.*, 2018).

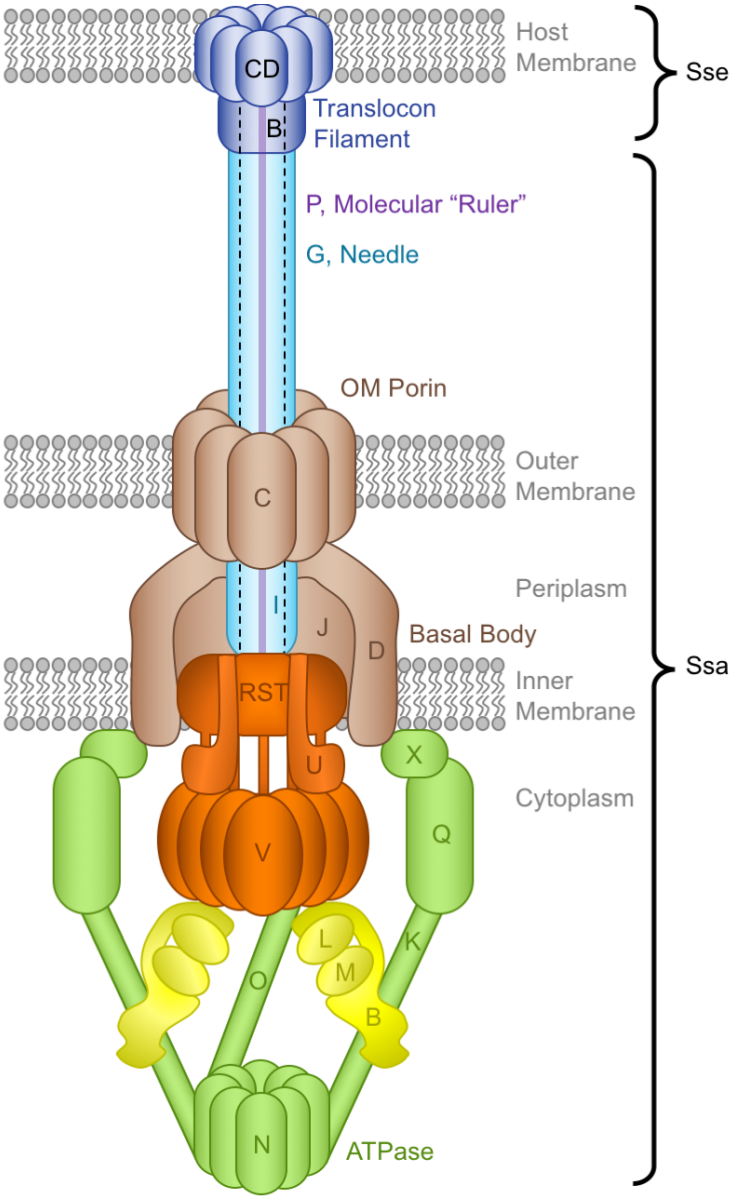


Figure 1.2 The SPI-2 type III secretion system.

Schematic drawing of the SPI-2 T3SS representing the secretion state of translocon proteins. Cytoplasmic sorting platforms are shaded in green, export gates in orange, gatekeepers in yellow, basal bodies in brown, needles and inner rods in light blue, translocon proteins in blue, and molecular “ruler” in purple. Adapted from Yu *et al*., 2018.

SPI-2 regulation, although studied extensively, is still not completely understood and the signals for expression under certain conditions have not been elucidated. The indispensable master regulator of SPI-2, SsrA/SsrB is encoded on the island by two adjacent genes, *ssrA* and *ssrB* (Cirillo *et al.*, 1998). Transcription of *ssrA* and *ssrB* occurs from divergent promoters. The promoter of *ssrA* lies in an extremely AT rich (69.03%) intergenic region and includes 2 transcriptional start sites (TSS) where the primary (1º) TSS is located closest to the *ssrA* open reading frame (ORF), and the 2º is located upstream. The *ssrB* promoter has one TSS internal to the 3′ end of the *ssrA* ORF (Feng *et al.*, 2003; Kröger *et al.*, 2012; Ochman *et al.*, 1996). SsrA/SsrB is a two-component system (TCS) where SsrA is the membrane-bound sensor kinase that phosphorylates the response regulator SsrB upon detection of an exogenous signal. SsrA is reported to respond to extracellular acidification, but no other signals have been identified (Fass & Groisman, 2009; Mulder *et al.*, 2015). Phosphorylated SsrB binds to the promoters and activates the transcription of SPI-2 encoded virulence gene operons and many effectors found outside of the gene island (Fass & Groisman, 2009; Worley *et al.*, 2000). SsrB binds to and regulates transcription from its both its own promoter and of *ssrA*. This is particularly unusual because TCSs with proteins encoded from adjacent genes are typically co-regulated resulting in a polycistronic mRNA from the same promoter. The use of separate promoters has been hypothesized to allow differential regulation of the genes according to the growth conditions, indeed in the absence of SsrA, the unphosphorylated SsrB protein controls expression of genes involved in biofilm biosynthesis (Desai *et al.*, 2016). Additionally, differential RNA-seq analysis has revealed that the *ssrB* promoter is 3.5-fold more active than the *ssrA* promoter within murine macrophages (Srikumar *et al.*, 2015). However, others have reported that a single promoter upstream of *ssrA* can drive transcription of both genes (Bustamante *et al.*, 2008; Fass & Groisman, 2009).

Many transcription factors (TFs) and NAPs have been established as regulators of SPI-2 genes as either repressors or activators in a complex regulatory network (**Figure 1.3**). The NAP H-NS is an important repressor of horizontally acquired genes and is involved in gene silencing at the *ssrAB* locus in accordance with its role in binding to AT-rich sequences (Lucchini *et al.*, 2006; Navarre *et al.*, 2006; Walthers *et al.*, 2007). H-NS binding to DNA leads to the formation of a stiff nucleoprotein filament through oligomerization of H-NS monomers which silences genes by restricting access of RNA polymerase to promoters (Lim *et al.*, 2006; Liu *et al.*, 2010; Winardhi *et al.*, 2015). Repression of SPI-2 promoters can also occur through direct repression by H-NS paralogs Hha and YdgT (Silphaduang *et al.*, 2007).

H-NS can form repressive complexes on SPI-2 promoters with both YdgT and Hha that interfere with SsrB binding and transcriptional activation (Ali *et al.*, 2013; Coombes *et al.*, 2005; Silphaduang *et al.*, 2007). Hha can also repress transcription of SPI-2 genes in an H-NS-independent manner (Solórzano *et al.*, 2015). Interestingly, YdgT is required for full virulence in infected macrophages. Coombes *et al*., postulate that the presence of YdgT might cause dampening of SPI-2 activation and the intracellular growth rate required for sustained infection and persistence, whereas the absence of YdgT ultimately leads to attenuation possibly caused by alterations in intracellular growth and/or fitness (Coombes *et al.*, 2005). Fur, a central regulator of iron utilization, causes repression of SPI-2 genes via direct binding of the *ssrB* promoter in acidic media. Within macrophages, *S*. Typhimurium lacking the Fur protein has earlier induction and ultimately higher expression of SPI-2 (Choi *et al.*, 2014). The Lrp global regulator of metabolism also plays a role in SPI-2 repression. It binds directly to a consensus motif at the *ssrA* promoter which results in down-regulation of SPI-2 genes (Baek *et al.*, 2009). The phosphorelay system RcsCDB plays a dual role in SPI-2 regulation. Microarray data has shown that the RcsCDB system normally functions as a positive regulator of SPI-2, although when highly activated, the system completely represses SPI-2 virulence (Wang *et al.*, 2007). Finally, under invasion-inducing conditions, the SPI-1 activator HilA was found to repress expression of genes encoding a SPI-2 apparatus protein, *ssaH,* and a SPI-2 effector protein, *sseL* (Thijs *et al.*, 2007).

Known signals of SPI-2 induction are acidic pH, limitation of inorganic phosphate (P_i), Mg^{2+} deprivation, high osmolarity, and the presence of antimicrobial peptides. These signals are thought to mimic the environmental cues found in the SCV (Cirillo *et al.*, 1998; Deiwick *et al.*, 1999; Löber *et al.*, 2006). Under SPI-2-inducing conditions, SsrB has a dual function in relieving the H-NS-mediated silencing of *ssrA* expression, as well as classical transcription initiation at SPI-2 genes including at the *ssaB*, *sseA*, *ssaG* and *ssaM* promoters (Walthers *et al.*, 2007; 2011). After SsrB, OmpR is arguably the most important activator of SPI-2. In the EnvZ-OmpR TCS, EnvZ is the sensor kinase and OmpR is the response regulator. OmpR has binding sites at both the *ssrA* and *ssrB* promoters, and directly activates transcription in response to low pH and osmotic stress (Feng *et al.*, 2003). Furthermore, an *ompR* mutant was severely attenuated *in vivo* (Dorman *et al.*, 1989) and the acidification of the *Salmonella* cytoplasm in the macrophage vacuole and subsequent SPI-2 T3SS effector secretion was found to be OmpR dependent (Chakraborty *et al.*, 2015). Additionally, expression of SPI-2 genes does not occur in an *ompR*/*envZ* mutant under SPI-2 inducing condition *in vitro* (Xu & Hensel, 2010). Another TCS involved in SPI-2 regulation is PhoQ-PhoP. Low

extracellular cation concentrations, such as those detected within the SCV, and low pH have been reported to activate the sensor kinase PhoQ, which, in turn, activates the response regulator PhoP (Choi & Groisman, 2016). Experiments have shown that PhoP controls SsrA post-transcriptionally and directly activates the *ssrB* gene by binding to its promoter (Bijlsma & Groisman, 2005). However, in the macrophage and under SPI-2 inducing conditions *phoP* mutants still express SPI-2 genes at low levels (Colgan *et al*., 2016; Fass & Groisman, 2009), furthermore a *phoP* mutant was observed to have severely delayed SPI-2 expression in SPI-2 inducing conditions (Xu & Hensel, 2010), implying that PhoQ-PhoP activation may be necessary for full expression and correct timing of SPI-2 expression. SlyA is required for virulence and survival within macrophages so not surprisingly the *slyA* gene is highly expressed during macrophage infection, and absence of the SlyA protein renders *S*. Typhimurium extremely susceptible to oxidative stress and antimicrobial peptides (Buchmeier *et al.*, 1997; Shi *et al.*, 2004). Additionally, in SPI-2 inducing conditions a *slyA* mutant shows a 2- to 3-fold reduction and delay of SPI-2 expression *in vitro* (Xu & Hensel, 2010). Transcription of *slyA* is activated by PhoP (Norte *et al.*, 2003; Stapleton *et al.*, 2002) which promotes SlyA binding to and directly regulating expression from the *ssrA* promoter (Navarre *et al.*, 2005; Okada *et al.*, 2007). A number of these regulators work together to remove repressive HNS complexes from the *ssrA* promoter. Under inducing conditions, SlyA and HilD or in nutrient limited conditions only SlyA, displace the H-NS complex bound to the promoter upstream of *ssrA*. This allows binding of OmpR that recruits the RNA polymerase on this promoter, which induces the transcription of *ssrAB* (Banda *et al.*, 2019). HilD also mediates cross-talk between SPI-1 and SPI-2 via counter-silencing of H-NS at the *ssrB* promoter (Bustamante *et al.*, 2008; Martinez *et al.*, 2014). Xu & Hensel also observed that a *sirA* mutant had reduced SPI-2 expression (Xu & Hensel, 2010). SirA is the response regulator of the TCS SirA/BarA, which also induces expression of SPI-1 genes. SirA/BarA induction of SPI-2 gene works through HilD. A global regulatory RNA binding protein, CsrA post-transcriptionally regulates *hilD* mRNA; negative regulation of HilD is counteracted by SirA/BarA, which directly activates the expression of the sRNAs *CsrB* and *CsrC* that sequester CsrA (Martínez *et al.*, 2011).

A number of NAPs are also important for induction of SPI-2 genes. FIS is a global regulator of gene expression and chromosome structure. FIS binds directly to the promoters of *ssrA* and *ssaG* and overall expression of SPI-2 genes is downregulated in a Δ*fis* mutant (Cameron *et al.*, 2011; Fass & Groisman, 2009; Kelly *et al.*, 2004). Additionally, low level SPI-2 expression activated by OmpR and FIS has been observed prior to epithelial invasion in the

intestinal lumen, but the signal for induction remains unclear (Brown *et al.*, 2005; Osborne & Coombes, 2011). HU and IHF are homologous histone-like proteins. Several SPI-2 genes, including the *ssrAB* genes, are downregulated in a HU double mutant, similarly loss of IHF causes a down regulation in all SPI-2 genes (Mangan *et al.*, 2006; Mangan *et al*., 2011).

1.1.4.3 Salmonella Pathogenicity Islands 3 through 5

Salmonella pathogenicity islands 3 through 5 play a smaller but still important role in *Salmonella* pathogenesis. SPI-3 encodes 10 open reading frames organized into 6 operons. These include the *mgtCB* operon encoding the macrophage survival protein MgtC and the Mg^{2+} transporter MgtB. The 5′ leader mRNA of *mgtC* is also involved in regulation of the *fljB* flagellin as previously discussed (Choi *et al.*, 2017; Miranda-CasoLuengo *et al.*, 2017). SPI-3 also encodes *misL* and *marT*, where MisL is a fibronectin binding protein important for oral colonization of mice and chicks, and MarT is its positive regulator (Blanc-Potard *et al.*, 2005; Tukel *et al.*, 2007). SPI-4 encodes 6 genes, *siiABCDEF*, termed the *Salmonella* intestinal infection genes which are important for interaction of *S*. Typhimurium with the host intestinal mucosa and help facilitate membrane ruffling and entry of polarized epithelial cells in conjunction with SPI-1 (Gerlach *et al.*, 2008). SPI-4 encodes a 595 kDa non-fimbrial giant adhesin, SiiE, which is secreted by a type I secretory system encoded by *siiC*, *siiD*, and *siiF* (Barlag & Hensel, 2015). SiiE mediates primary contact with host cells, allowing positioning of the SPI-1 T3SS to initiate the translocation of SPI-1 effectors (Barlag & Hensel, 2015). Expression of SPI-4 genes corresponds with that of SPI-1 genes, and the activators and repressors of SPI-1 are involved in its regulation (Main-Hester *et al.*, 2008). SPI-5 encodes SPI-1 T3SS secreted effector protein SopB which modulates host cell exocytosis and its chaperone PipC, and the SPI-2 T3SS secreted effectors PipA and PipB, thought be important in development of systemic infection and PipD a hypothetical secreted peptidase (Perrett & Zhou, 2013; Knodler *et al.*, 2002; Marcus *et al.*, 2000; Morgan *et al.*, 2004).

Overall pathogenicity islands and other horizontally acquired genes are critical for cultivating a niche for *S*. Typhimurium in the host. SPI-1, SPI-4 and SPI-5 all play a role in niche creation as they induce inflammation in the host. That niche can then be colonized with the help of anaerobic metabolism genes found on SPI-2 and a bacteriophage acquired effector, SopE which is secreted through the SPI-1 T3SS to make nitrate available for respiration (Winter *et al.*, 2013). Genes encoded on SPI-2 and SPI-3 are also important for

intracellular survival (Blanc-Potard *et al.*, 1999; Figueira & Holden, 2012). Finally, SPI-2 optimizes the niche by prolonging intestinal inflammation, and thereby making more nutrients available for this pathogen (Winter *et al.*, 2013).

Table 1.1 *Salmonella* pathogenicity islands 1 through 5[a]

Pathogenicity Island	Size (kb)	Secretion System	Description
SPI-1	40	T3SS	T3SS-dependent invasion of intestinal epithelium Encodes effector proteins important for actin cytoskeleton rearrangements & membrane ruffling
SPI-2	40	T3SS	Encodes tetrathionate metabolism proteins T3SS-dependent survival within SCVs of epithelial cells and macrophages Inhibits fusions between lysosomes and SCVs Encodes effector & chaperone proteins
SPI-3	17	–	Intramacrophage survival and persistence Oral colonization of mice and chicks Trans-acting sRNA post-transcriptional repression of FljB flagellin in macrophages
SPI-4	24	T1SS	*Salmonella* intestinal infection genes mediate adhesion to epithelial cells *siiCDF* encode components of a T1SS SiiE, 595 kDa non-fimbrial adhesion protein involved in oral virulence
SPI-5	6.6	–	SPI-1 T3SS effector SopB and its chaperone PipC SPI-2 T3SS effectors PipA and PipB; PipD

[a]Table adapted/updated from Hurley *et al.*, 2014; Wisner *et al.*, 2012.

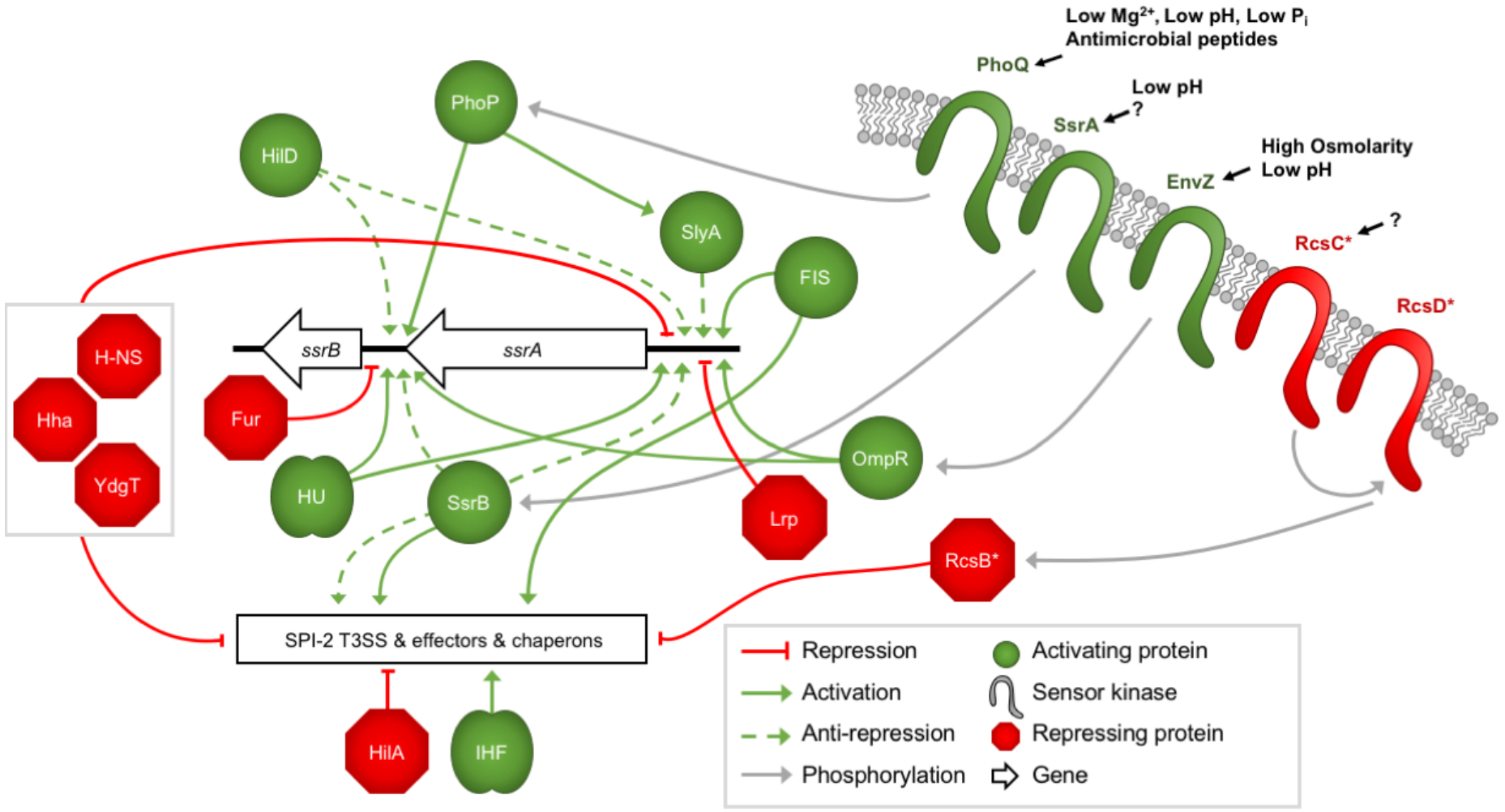

Low Mg2+, Low pH, Low Pi
Antimicrobial peptides
PhoQ
Low pH
SsrA
?
High Osmolarity
Low pH
EnvZ
RcsC*
?
RcsD*
PhoP
HilD
SlyA
FIS
H-NS
Hha
YdgT
ssrB
ssrA
Fur
HU
SsrB
OmpR
Lrp
RcsB*
SPI-2 T3SS & effectors & chaperons
HilA
IHF
Repression
Activation
Anti-repression
Phosphorylation
Activating protein
Sensor kinase
Repressing protein
Gene

Figure 1.3 Regulation at *Salmonella* pathogenicity island 2 involves many transcription factors.

There are many proteins that influence expression of SPI-2 genes. Sensory kinases respond to environmental factors and in turn activate their response regulators. PhoQ-PhoP, EnvZ-OmpR, FIS, HU, IHF, SlyA and HilD cause activation and H-NS, Hha, YdgT, Lrp, HilA and Fur cause repression. The phosphorelay system RcsCDB represses SPI-2 genes when highly induced, but low induction causes activation. SsrA and SsrB the master regulators of SPI-2 are encoded upstream of the SPI-2 T3SS, effector and chaperone genes. SsrA is a sensory kinase and SsrB is a response regulator. SsrB autoregulates at its own promoter and activates the T3SS, chaperones and effector proteins on SPI-2 by relieving repression by H-NS and direct activation. HilD and SlyA also activate SPI-2 by relieving repression of H-NS.

1.2 Metabolism in the gut

1.2.1 Oxygen in the gut

When the concentration of oxygen is lower than the atmospheric concentration (~21%), the environment is referred to microaerobic or hypoxic. In bacteria, aerobic respiration occurs at oxygen concentrations above 0.5%, while anaerobic respiration occurs between 0.1 - 0.5%, and fermentation is initiated at concentrations lower than 0.1% (Unden & Bongaerts, 1997). The lumen of the ileum is a fairly anaerobic environment, and any trace amount of oxygen is readily consumed by facultative anaerobic bacteria, such as members of the *Enterobacteriaceae* family, that constitute approximately 0.1% of the microbiota (Eckburg *et al.*, 2005). The limited availability of oxygen in the lumen limits growth of *Enterobacteriaceae* populations as increasing the level of oxygen increases their relative abundance (Rigottier-Gois, 2013; Winter *et al.*, 2013). For example, under inflammatory conditions, gut luminal oxygen levels rise partly due to elevated blood flow and hemoglobin and the increase of oxygen causes a disruption in anaerobiosis which confers a selective advantage to facultative anaerobes, allowing them to out-compete anaerobes and overgrow (Rigottier-Gois, 2013; Zeng *et al.*, 2016). The ability to proliferate in the lumen is an important part of transmission in *Salmonella* infection (Santos *et al.*, 2009). *Salmonella* can survive in anaerobic environments as well by way of anaerobic respiration but will preferentially choose an aerobic environment when given the choice. Conveniently, within

the GI tract in close proximity to the mucosal surface, there is a 70 mm zone of increased oxygen concentration (Marteyn *et al.*, 2010). This creates a steep oxygen gradient from the lumen to the intestinal epithelium that is reliant on blood flow and is caused by diffusion of oxygen from the capillary network at the villi (**Figure 1.4**) (Marteyn *et al.*, 2010). Oxygen concentration also increases substantially from the tips of the villi to the crypts (Zheng *et al.*, 2015). Oxygen concentrations inside of host tissues also vary dependent on the state of infection, for example in the murine gut oxygen concentrations decreased from ~11% to 2% after *Salmonella* infection (Jennewein *et al*., 2015). The anti-*Salmonella* activity of macrophages is also dependent on available oxygen as antimicrobial molecules such as phagocyte oxidase and nitric oxide synthase require oxygen molecules, however this leaves little free dioxygen inside of cells (Vazquez-Torres & Fang, 2001). Furthermore, low oxygen concentrations found in *Salmonella*-infected gut tissue boost *Salmonella* replication in macrophages by impairing antimicrobial activity and augmenting *Salmonella* virulence (Jennewein *et al*., 2015). In general the tissues of the gut including intestinal epithelial cells have a lower oxygen concentration than atmospheric oxygen and are described as microaerobic or hypoxic (Zheng *et al*., 2015; Zeitouni *et al*., 2016). The flagella of *Salmonella* play a role in movement from the hypoxic lumen to the intestinal epithelium through the mucus layer. Chemotaxis towards host derived electron acceptors is described however, aerotaxis towards the oxygen gradient could also be involved (Marteyn *et al.*, 2010; 2011; Rivera-Chávez *et al.*, 2013; Stecher *et al.*, 2008). The anaerobic state of the lumen is due to the high rates of respiration by host and commensal cells. Therefore, pathogens like *Salmonella* must employ an alternative metabolism to survive the hypoxic environment (Cook *et al.*, 2014).

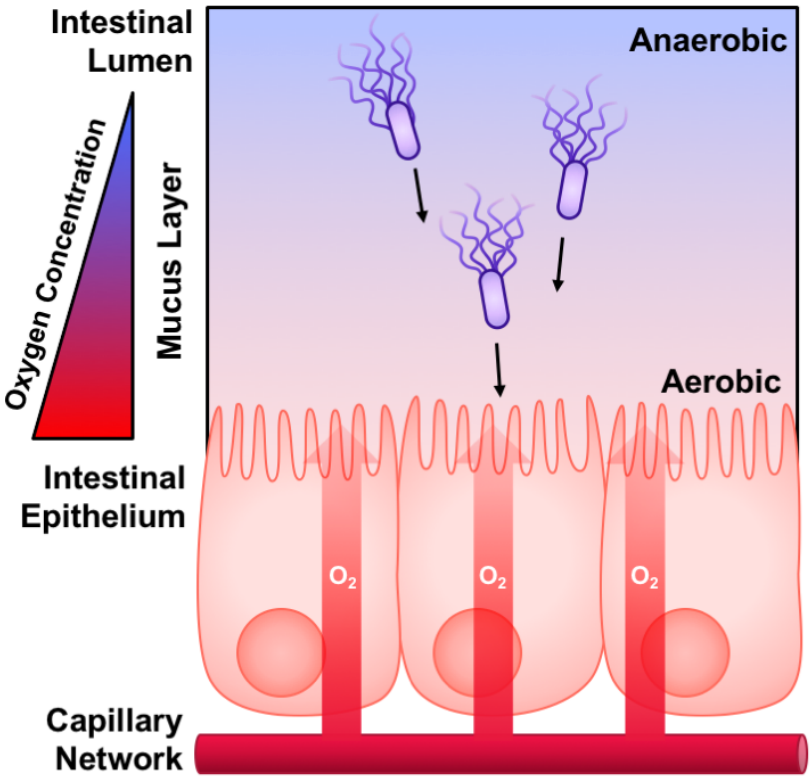


Figure 1.4 The oxygen gradient in the gut.

The intestinal lumen is an anaerobic environment (blue) however, there is a zone of relative oxygenation adjacent to the gastrointestinal tract mucosa (red), caused by diffusion of O_2 (arrows) from the capillary network at the tips of villi. Aerotaxis may occur at this site. Figure adapted from Marteyn *et al*., 2010.

1.2.2 Anaerobic respiration in the gut

To survive in the lumen, facultative anaerobes such as *Salmonella* may employ anaerobic respiration (Rivera-Chávez & Bäumler, 2015). Cellular respiration utilizes highly reduced electron donors such as NADH to establish an electrochemical gradient across a membrane. In aerobic respiration electrons are shuttled along the electron transport chain (ETC) from primary dehydrogenases to the terminal respiratory oxidase and the final electron acceptor oxygen and simultaneously creating a proton gradient across the cytoplasmic membrane (Ingledew & Poole, 1984). The proton motive force drives protons down the gradient through the proton channel of ATP synthase, driving ATP synthesis from ADP and inorganic phosphate (Berg *et al.*, 2002). Simply, anaerobic respiration is respiration using terminal respiratory reductases and electron acceptors other than oxygen as the final electron acceptor

in the ETC. In organisms undergoing anaerobic respiration, oxygen is unavailable, therefore less-oxidizing substances are used as terminal electron acceptors (Neidhart, 1996). These alternative electron acceptors have smaller redox potentials than O_2, meaning that less energy is released per oxidized molecule. Thus, anaerobic respiration is less efficient than aerobic respiration (Ingledew & Poole, 1984; Neidhart, 1996). *S.* Typhimurium have evolved the capability to use a range of alternative electron acceptors and the expression of the terminal reductases is regulated by availability of electron acceptors (Bumann, 2009). As oxygen is the preferred electron acceptor, the presence of O_2 represses expression of the terminal reductases of anaerobic respiration. In the absence of oxygen, nitrate (NO_3^-) or nitrite (NO_2^-) are the preferred terminal electron acceptors (Richardson *et al.*, 2001). The presence of NO_3^-/NO_2^- therefore results in repression of other alternative terminal reductases, followed by the terminal electron acceptors, in order of oxidation/reduction (redox) potential: dimethyl sulfoxide (DMSO), trimethylamine N-oxide (TMAO), fumarate, and tetrathionate (**Table 1.2**). This regulatory pathway favours high ATP yields, resulting in the most efficient production of energy possible in the given environment (Cook *et al.*, 2014).

Table 1.2 Electron acceptors used by *Salmonella* during anaerobic respiration

Electron acceptor[a]	Redox Couple	Redox Potential[d]	Terminal respiratory enzyme	Operon[e]
Nitrate	NO_3^-/NO_2^-	+430 mV	Nitrate reductase	*narGHJI*
			Nitrate reductase	*narZYWV*
			Nitrate reductase	*napFDAGHBC*
Nitrite	NO_2^-/NH_4^+	+350 mV	Nitrite reductase	*nfrABCDEFG*
			Nitrite reductase	*nirBDC*
DMSO	DMSO/DMS[b]	+160 mV	DMSO/TMAO reductase	*dmsABC*
TMAO	TMAO/TMA[c]	+130 mV	TMAO reductase	*torCAD*
Fumarate	fumarate/succinate	+33 mV	Fumarate reductase	*frdABCD*
Tetrathionate	$S_4O_6^{2-}$/ $S_2O_3^{2-}$	+24 mV	Tetrathionate reductase	*ttrBCA*

[a]Electron acceptors are listed in descending order of their redox potential.

[b]Dimethyl sulfoxide/Dimethyl sulfide

[c]Trimethylamine N-oxide/Trimethylamine

[d]Data from Thauer *et al.*, 1977

[e]Data from Cook *et al.*, 2014; Hensel *et al.*, 1999; Unden & Bongaerts, 1997

Terminal reductases are repressed by multiple TFs that regulate according to availability of terminal electron acceptors with the highest redox potential. Nitrate regulation is controlled by the TCSs NarXL and NarQP while oxygen regulates by the TCS ArcAB and FNR (Gilberthorpe & Poole, 2008; Stewart *et al.*, 2003).

ArcAB is the regulator of aerobic metabolism and represses the genes of aerobic metabolism under anaerobiosis (Unden & Bongaerts, 1997). FNR is the regulator of genes required for anaerobic metabolism, and it is discussed in detail in Section 1.3. Most of the genes involved in aerobic and anaerobic metabolism are regulated by more than one of the regulatory proteins, which act in many different combinations and activate or repress as necessary (Constantinidou *et al.*, 2006; Myers *et al.*, 2013). FNR and ArcA regulatory proteins play different roles under microaerobic conditions; FNR functions as an aerobic/anaerobic switch in the range of 0 to 10% air saturation, while ArcA exerts control in the 10 to 20% oxygen range. These two transcriptional regulators coordinate the hierarchial control of respiratory pathway gene expression to ensure the optimal use of oxygen in the cell environment (Tseng *et al.*, 1996) and in response to the availability of electron acceptors (Unden & Bongaerts, 1997). ArcA serves as a transcriptional regulator coordinating aerobic cellular metabolism, flagella biosynthesis, and motility in *S.* Typhimurium. Moreover, ArcA and FNR share regulation of at least 120 genes (Evans *et al.*, 2011). Recently the ArcA response regulator was found to promote intracellular survival in macrophages and neutrophils by promoting resistance to ROS, enabling *S.* Typhimurium to successfully establish a systemic infection (Pardo-Esté *et al.*, 2018).

To outgrow the resident microbiota of the gut, *Salmonella* must defend against host defenses and find nutrients. *S.* Typhimurium use virulence factors to induce intestinal inflammation (Section 1.1.2), which releases specific nutrients not usable by the microbiota (Thiennimitr *et al.*, 2012). One way in which *S.* Typhimurium can take advantage of host defenses by converting nitric oxide (NO) to NO_3^- inside macrophages. This results in a two-pronged advantage to the pathogen, as it eliminates toxic NO and provides *S.* Typhimurium with a favourable terminal electron acceptor for anaerobic respiration (Mills *et al.*, 2008). Host production of ROS and RNS creates a hostile environment in close proximity to the mucosal surface, but as these molecules diffuse towards the gut lumen they contact and oxidize organic sulfides, such as methionine, or tertiary amines, such as trimethylamine, to form S-oxides (DMSO) and N-oxides (TMAO), respectively. DMSO and TMAO can then be used by *S.* Typhimurium as terminal electron acceptors which are reduced by terminal reductases

encoded by *dmsABC* and *torCAD* (Winter *et al.*, 2010; Winter *et al*., 2013). Additionally, when *Salmonella*-induced enteritis causes diarrhea to flush the intestines, epithelial cells shed into the lumen and become a source of membrane lipids. These lipids include phosphatidylcholine and sphingomyelin from which choline can be derived. Degradation of choline to TMA plus oxidation by ROS leads to further availability of TMAO (Winter *et al.*, 2013). Similar to the genes encoding specific virulence factors, horizontal acquisition of genes encoding metabolic functions are advantageous for surviving in the host. The genes encoding tetrathionate reductase, *ttrCBA* and their regulating TCS TtrSR, are found on SPI-2. Tetrathionate is generated when inflammation derived ROS react with endogenous thiosulphate. In contrast to the gut microbiota, *Salmonella* can use tetrathionate as a respiratory electron acceptor providing a unique advantage to the pathogen allowing for anaerobic respiration. As anaerobic respiration is more energy efficient than fermentation, *Salmonella* outcompetes strains of the microbiota in the inflamed gut (Winter *et al.*, 2010).

1.3 Regulation of the aerobic to anaerobic switch

1.3.1 FNR

Facultative anaerobic bacteria prefer an oxygenated environment however, when oxygen concentrations decrease they can reprogram their gene expression to facilitate anaerobic metabolism. That switch is regulated by an oxygen-sensing transcription factor called FNR, named for a mutant defective in "fumarate and nitrate reduction" (Lambden & Guest, 1976). FNR operates the master switch between aerobic and anaerobic metabolism by ensuring that oxygen is used in preference to alternative electron acceptors (Guest *et al.*, 1996). When *E. coli* moves from an aerobic to anaerobic environment, a third of its genes undergo transcriptional change. FNR is responsible for the regulation of half of those either directly or indirectly (Salmon *et al.*, 2003). FNR facilitates adaptation to anoxia by providing alternative pathways for energy generation and is a well-studied global transcriptional regulator in enteric bacteria (Constantinidou *et al.*, 2006; Fink *et al.*, 2007; Myers *et al.*, 2013; Wang *et al.*, 2019). FNR is a homologous protein to the cAMP receptor protein (CRP) (Shaw *et al.*, 1983), and belongs to the CRP-FNR superfamily of transcriptional regulators (Körner *et al.*, 2003). These proteins have an N-terminal sensory domain, a dimerization motif, and a C-terminal helix-turn-helix DNA-binding domain (Körner *et al.*, 2003). FNR primarily works as an activator of anaerobic metabolism genes during oxygen deprivation, however it is known to repress at a limited number of promoters (Green & Marshall, 1999).

1.3.2 Regulation of FNR

Expression of the *fnr* gene occurs under both aerobic and anaerobic conditions, and negative autoregulation and repression by glucose occurs during anaerobiosis (Mettert & Kiley, 2007a; Spiro & Guest, 1987). The concentration of FNR protein stays relatively constant regardless of the presence or absence of oxygen (Sutton *et al.*, 2004). When oxygen concentrations are as low as 0 to 5 mbar (0-0.5%) the FNR protein is active, with DNA binding capabilities (Jervis *et al.*, 2009; Sawers, 1999) (**Figure 1.5A**). Under oxygen limiting conditions, FNR binds an [4Fe-4S] cluster to four cysteine residues in the sensory domain (Green & Guest, 1993). FNR bound by [4Fe-4S] undergoes a conformational change allowing dimerization. In the presence of adequate oxygen concentrations, the [4Fe-4S] cluster is oxidized, and FNR becomes an inactive monomer (Beinert & Kiley, 1999; Green *et al.*, 2014) (**Figure 1.5B**). Importantly, FNR is highly functional over a small range of oxygen concentrations with approximately 95% activity at 0.3 μM O_2 and 50% activity at 6 μM O_2. Surprisingly, an FNR-dependent reporter gene suggests that a chromosomally encoded FNR is 2.5% active under fully aerobic conditions (Jervis *et al.*, 2009). The functional state of FNR during the switch from aerobic to anaerobic environments is not regulated by iron content as only severely Fe^{2+} limiting conditions has any effect on FNR dependent gene expression (Niehaus *et al.*, 1991).

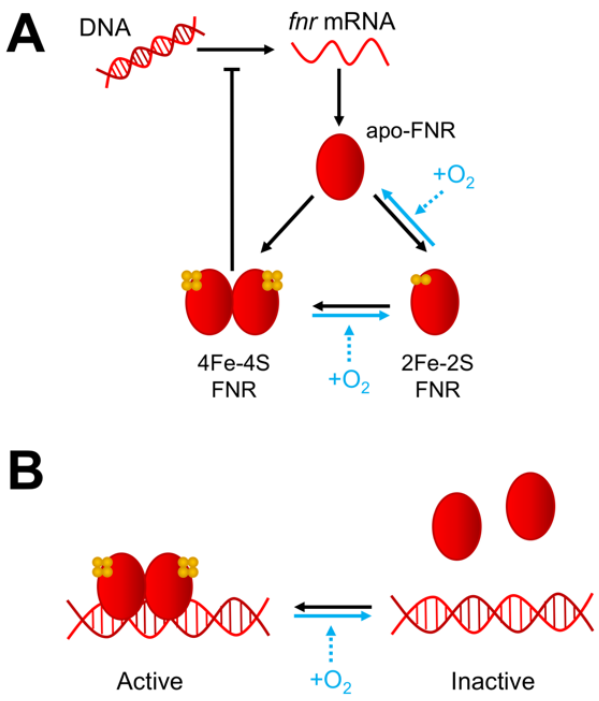


Figure 1.5 FNR activity is directly regulated by oxygen.

A. The *fnr* gene is transcribed, then translated. Cellular concentration of FNR is similar under both anaerobic and aerobic growth, but its activity is regulated directly by oxygen. Under anaerobiosis, the FNR monomer (red oval) acquires a [4Fe-4S] cluster (yellow circles) that causes a conformational change and dimerization of the protein, and it autoregulates at its own promoter. B. Under anaerobic conditions the active FNR dimer can bind to DNA causing activation and repression of many genes, however when oxygen is introduced the dimer dissociates and is no longer able to bind DNA.

1.3.3 Mechanisms of transcriptional regulation by FNR

During anaerobiosis the active FNR homodimer can bind to DNA to regulate transcription either by activation or repression. The active FNR homodimer preferably binds a specific 14 bp palindromic sequence of DNA with the consensus sequence **TTGAT**$N_1N_2N_3N_4$**ATCAA** (Eiglmeier *et al.*, 1989; Gerasimova *et al.*, 2001). Interestingly, it has been shown experimentally that FNR can recognize a consensus CRP-binding site *in vivo* (Sawers *et al.*, 1997).

1.3.3.1 FNR-dependent activation

For activation to occur, FNR interacts directly with RNA polymerase (RNAP) in two ways depending on the architecture of the promoter (Blake *et al.*, 2002). FNR activates the transcription from class I and class II promoters. In class I promoters N_2 and in class II promoters N_3 of the consensus FNR site tend to be an A or T (Scott *et al.*, 2003). At class I promoters, FNR binds to a site centered at -61.5 or further upstream (-71, -82, or -92), then the activating region 1 (AR1) of the downstream subunit of the FNR homodimer directly contacts C-terminal domain of the alpha subunit (αCTD) of RNAP (**Figure 1.6A**) (Blake *et al.*, 2002; Wing *et al.*, 1995). At class II promoters, multiple interactions with RNAP are possible as FNR binds to a site centered at or near -41.5, causing the homodimer to be embedded in the polymerase. Here the αCTD makes contact with the AR1 surface of the upstream subunit of the FNR. The AR3 surface of the downstream subunit of FNR contacts σ70 and AR2 makes contact to the N-terminal domain of the alpha subunit (αNTD), the same interaction that CRP uses with RNAP (**Figure 1.6B**) (Blake *et al.*, 2002). These direct interactions allow proper positioning of the RNAP for transcription initiation.

It has also been suggested that at some promoters FNR acts as a co-activator with other transcription factors such as CRP, NarP and NarL. A number of promoters have distinct FNR and CRP binding sites and are only activated when the conditions of both TFs are met, i.e. under anaerobiosis without glucose (Myers *et al.*, 2013). NarP and NarL are response regulators that respond to the presence of NO_3^- and/or NO_2^-. The operon encoding periplasmic nitrate reductase, *napFDAGHBC*, is maximally expressed only in response to anaerobiosis and nitrate or nitrite and thus, co-activated by FNR and NarP and/or NarL (Myers *et al.*, 2013; Stewart *et al.*, 2009). Co-activation by two TFs is important for precise control of genes under specific conditions.

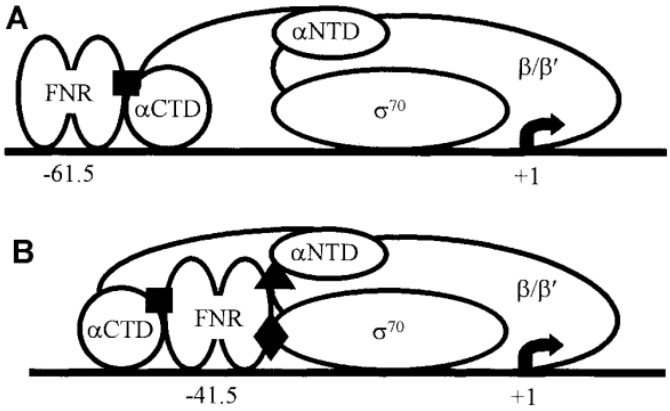


Figure 1.6 Activation of simple class I and class II FNR-dependent promoters. A. At class I promoters, FNR binds to a site centered at –61.5 or further upstream. AR1 of the downstream subunit of the FNR dimer contacts αCTD of RNAP (■). B. At class II promoters, FNR binds to a site centered at or near –41.5 and is thus embedded within RNAP. The AR1 surface of the upstream subunit of the FNR dimer contacts αCTD (■), the AR3 surface of the downstream subunit of FNR contacts σ^{70} (▲), and the AR2 surface makes contact with αNTD (◆). Figure taken from Blake *et al*., 2002.

1.3.3.2 FNR-dependent repression

FNR-dependent repression can be complex and is not fully understood. Because FNR autoregulates at the *fnr* gene, and has 2 FNR binding sites, one centered on the *fnr* TSS (+1.5) and one centered farther upstream centered at -103.5, it is an ideal candidate to examine for a repressive mechanism. Early experiments suggested both FNR binding sites needed to be present for repression of *fnr* under anaerobic conditions (Spiro & Guest, 1987) however, later *in vitro* experiments suggest the downstream binding site alone is sufficient for repression (Mettert & Kiley, 2007b). This suggests that promoter occlusion may be one way in which FNR can cause repression. However, repression by FNR at the *cydAB* (Cytochrome d terminal oxidase) promoter requires binding at both FNR binding sites, one site at the TSS and a second at -53.5 (Cotter *et al.*, 1997). Bernard *et al*. investigated repression by FNR by adding FNR consensus sites to several regions upstream of a semi-synthetic class II promoter based on that of *melR*. Binding of FNR at the -41.5 site activates expression of the promoter, however when an additional consensus site was added -85 and

-95 bp upstream, a sharp repression was observed. Similarly, a class I promoter with a binding site at -61.5 showed strong repression by FNR when a consensus site was placed at -100.5, indicating that upstream binding sites play an important role in FNR repression at some sites and that FNR can cause repression when bound in tandem spaced 44 or 53 bp apart (Barnard *et al.*, 2004). Indeed, repression of the gene encoding respiratory NADH dehydrogenase, *ndh* requires two FNR binding sites, found centered at -50.5 and -94.5 but not at the TSS. Both of binding sites appear to be required for full repression, and in a DNase footprinting assay both FNR and RNAP were able to bind simultaneously, suggesting promoter occlusion is not the mechanism for repression. Instead, FNR may jam the progression of RNAP by forming a complex and may disrupt productive RNAP interactions with other regulators (Meng *et al.*, 1997). Importantly, Williams *et al*. have shown that introducing a semi-synthetic FNR binding site to the -35 region of a promoter can simply block progress of RNAP, suggesting that a single FNR binding site is capable of causing efficient repression (Williams *et al.*, 1998). Finally, it seems that the full 14 bp palindromic binding does not need to be present for FNR binding; Several promoters only have half of the palindromic sequence at their promoters. For example, the glutamyl-tRNA reductase gene *hemA* is modestly repressed by FNR and has only a TTGAT site around -30. This half-site was protected by FNR in a DNase footprinting assay, thus suggesting that FNR can repress when only a half-site is present (Melville & Gunsalus, 1996). Additional transcriptional repression by FNR with only a half-site includes the promoters of *moeAB*, *nrdAB* and *sodA* (Constantinidou *et al.*, 2006). In summary, it appears FNR can cause repression in a multitude of ways including promoter occlusion by binding at the TSS, by blocking RNAP access by binding near the RBS, with interactions between tandem-bound FNR molecules, by jamming RNAP by forming inactive complexes and by disrupting productive RNAP interactions with other regulators.

1.3.4 The FNR regulon

The FNR regulon has previously been examined by microarray analysis in non-pathogenic *E. coli* (Constantinidou *et al.*, 2006; Grainger *et al.*, 2007; Kang *et al.*, 2005; Salmon *et al.*, 2003) and *S.* Typhimurium (Fink *et al.*, 2007) and more recently by RNA-seq in *Shigella flexneri* and Enterotoxigenic *E. coli* (ETEC) (Crofts *et al.*, 2018; Vergara-Irigaray *et al.*, 2014), and proteomic analysis in *S.* Typhimurium (Wang *et al.*, 2019). These studies find that FNR is a regulator either directly or indirectly of approximately 50% of genes that undergo transcriptional change during the switch between aerobic and anaerobic

metabolism. FNR directly regulates a subset of genes while others are affected through FNR regulation of TFs (Myers *et al.*, 2013). Also, many genes originally thought to be directly regulated by FNR but lacked FNR binding have been found to be regulated post-transcriptionally through the FNR-dependent sRNA FnrS (Durand & Storz, 2010).

Regulation of *Salmonella* gene expression is often similar to *E. coli*, and findings from a study of the *S*. Typhimurium FNR regulon were consistent with findings from *E. coli*. FNR regulates of genes involved in aerobic metabolism, NO• detoxification, flagellar biosynthesis, motility, chemotaxis, and anaerobic carbon utilization under anaerobic growth found in both species. However, some genes and operons found in both species were found only to be regulated by FNR in *S*. Typhimurium include those coding for ethanolamine utilization, a universal stress protein, a ferritin-like protein, and a phosphotransacetylase. *Salmonella*-specific genes and operons regulated by FNR were all found to be activated by FNR and were of virulence and motility genes not found in *E. coli* including numerous SPI-1 encoded genes, flagellar genes *mcpAC*, *cheV*, and the virulence/motility operon *srfABC* (Fink *et al.*, 2007). Stecher *et al*. described the role of the *Salmonella* flagella in moving towards the intestinal epithelium through the mucus layer in response to readily available metabolites (Stecher *et al.*, 2008). However, Marteyn *et al*. suggest aerotaxis towards the oxygen gradient of the gut epithelium may also occur, and upregulation of motility and chemotaxis genes under anaerobic conditions fits with this model (Fink *et al.*, 2007; Marteyn *et al.*, 2011). Furthermore, the role of FNR as a positive regulator of motility and flagellar biosynthesis was confirmed by showing that Δ*fnr* is nonmotile and lacks flagella (Fink *et al.*, 2007).

FNR is essential to the enteritis model of infection, plays a weak role in typhoid fever and *fnr* mutants are attenuated in calf and chicken infections (Chaudhuri *et al.*, 2013; Rollenhagen & Bumann, 2006). The anaerobic regulator has been identified as an important regulator of virulence of other pathogens such as *Neisseria meningitides* (Bartolini *et al.*, 2006), ETEC (Crofts *et al.*, 2018), *Shigella flexneri* (Marteyn *et al.*, 2010), and in *Pseudomonas aeruginosa* by an FNR orthologue (Filiatrault *et al.*, 2006). Deletion of *fnr* in ETEC caused a significant increase in expression of all classical virulence factors, including an adhesin operon and enterotoxins, indicating FNR represses these genes under anaerobic conditions (Crofts *et al.*, 2018). Similarly, *Shigella flexneri* undergoes FNR-mediated priming for invasion by expressing extended T3SS needles while reducing effector secretion anaerobically through FNR repression of virulence gene regulators (Marteyn *et al.*, 2010).

Both groups describe a model where virulence gene expression is coordinated with pathogen proximity to the epithelium. In *Salmonella*, FNR has been shown to be positive regulator of pathogenesis. An Δ*fnr* mutant showed a decrease in expression of invasion genes, indicating that FNR can activate the SPI-1 genes *prgKJIH*, *iagB*, *sicA*, *spaPO*, and *invJICBAEGF* and the *srfABC* virulence genes under anaerobiosis. Interestingly, the Δ*fnr* mutant was attenuated in mice and could not survive in macrophages, although no differentially regulated SPI-2 genes were identified (Fink *et al.*, 2007).

1.4 Aims of the Study

The aim of this study is to determine the regulatory role of FNR in the expression *Salmonella* Pathogenicity Island 2 in *S.* Typhimurium.

There is increasing evidence that changes in environmental oxygen play a critical role in the timing and deployment of bacterial virulence factors, and that regulators of metabolism control the expression of genes imperative for causing infection and disease. Oxygen levels have already been shown to be important for interactions between *Salmonella* and host cells. *S.* Typhimurium grown in microaerobic environments have increased adhesive and invasive capacity compared to their aerobically grown counterparts (Lee & Falkow, 1990). Additionally, anaerobically grown *S.* Typhimurium had increased binding to murine enterocytes and intestinal mucus, and increased survival in macrophages (Singh *et al.*, 2000). FNR, the regulator of anaerobic metabolism that responds directly to oxygen concentration, has been established as an activator of genes involved in host-cell invasion such as components of the T3SS and chaperones encoded on SPI-1 (Fink *et al.*, 2007). Furthermore, FNR is required for full virulence in the murine model, and a mutant lacking this transcription factor is rapidly killed by macrophages (Fink *et al.*, 2007). The SPI-2 T3SS and an intact SCV are required for evasion of ROS in macrophages (van der Heijden *et al.*, 2015), and FNR helps to promote resistance against oxidative stress although the mechanism has not been described. Importantly, low-level invasion-independent transcriptional activity of the SPI-2 T3SS in the lumen of the gut was observed in an oral mouse model (Osborne & Coombes, 2011). Thus, FNR regulation of SPI-2 is heavily implied. Moreover, the influence of an oxygen gradient at the point of *S.* Typhimurium infection has not been considered. The effect of oxygen on invasion in *S.* Typhimurium and therefore on regulation of SPI-1 has been investigated, however SPI-2 expression and oxygen have not been linked.

Activity of FNR as a transcriptional regulator directly corresponds to the absence of oxygen, and during infection *Salmonella* encounters a steep oxygen gradient from the anaerobic lumen to a zone of relative oxygenation at the epithelial border, therefore molecular oxygen can be considered a major signal for virulence factors. We propose that direct repression of SPI-2 by FNR occurs in anaerobic and microaerobic environments, and that upon detection of increased oxygen, like at the host epithelial border, repression is lifted. Expression of SPI-2 prior to epithelial invasion could prime cells for the challenges of the intracellular environment. FNR regulation of virulence factors, especially those found within the SPIs is important as it shows the capacity of regulators of metabolism located on the core genome to evolve to regulate horizontally acquired pathogenicity genes.

The major aim of this study is to determine the regulatory role of FNR in the expression SPI-2 in *S.* Typhimurium. More specifically, we aim to determine the mechanism of SPI-2 repression by FNR, and the input for the repression. Additionally, we intend to confirm that FNR, as a regulator of anaerobic metabolism, can control SPI-2 genes in response to oxygen and alternative electron acceptors.

Chapter 2

Material & Methods

2.1 Chemicals and Reagents

Unless otherwise stated, all chemicals and reagents were purchased from Acros Organics, Agilent, Ambion, Applied Biosystems, Bioline, Fisher Scientific, Illumina, Invitrogen, New England Biolabs, Promega, Roche, Sigma-Aldrich, and Thermo Scientific.

2.2 General Microbiological Techniques

2.2.1 Media

Media recipes are shown in **Table 2.1**. All media were made using analytical grade, deionised water (Analar, BDH). Media were sterilized by autoclaving or filtration using 0.2 µm polyethersulfone (PES) membrane filters (Fisher Scientific). To prepare agar plates, Lennox agar (LA) was melted at 95ºC, then cooled to 50ºC before addition of appropriate antibiotics prior to pouring. Agar plates were stored at 4ºC prior to use.

2.2.2 Maintenance of Bacterial Stocks

All strains and plasmids used in this study are listed in **Tables 2.2** and **2.3** respectively. Bacterial strains were prepared for long term storage by combination of overnight cultures in Lennox broth (LB, **Table 2.1**) in 30% (v/v) sterile glycerol and frozen at -80ºC. When required, strains were streaked for single colonies onto LA plates with the appropriate antibiotics and grown at 30ºC or 37ºC. Plates were stored at 4ºC and kept for a maximum of 1 week.

2.2.3 Culture Conditions

2.2.3.1 Growth in Lennox Broth

Bacterial strains were routinely grown on LA plates and incubated at 37ºC. Bacterial cultures were routinely grown from single colonies in 5 mL of LB in glass test tubes at 37ºC in an Innova 40 air-incubator (New Brunswick Scientific) at 200 rpm for 16 h (overnight). For further growth in LB, bacterial cultures were sub-inoculated 1:1000 in 25 mL of LB in a 250 mL Erlenmeyer flask with appropriate antibiotics and grown without agitation or with

agitation at 200 rpm at 37ºC in an Innova 3100 water-bath shaker (New Brunswick Scientific).

2.2.3.2 Growth in Minimal Media

For growth in minimal media (MMA or PCN, **Table 2.1**), overnight cultures were grown as previously described. One mL of culture was harvested by centrifugation at 6,500 × g, washed 3 times in minimal media by centrifugation at 6,500 × g, and sub-inoculated 1:500 in 25 mL of minimal media in a 250 mL Erlenmeyer flask with appropriate antibiotics, and grown without agitation or with agitation at 200 rpm (**Figure 2.1**) at 37ºC in an Innova 3100 water-bath shaker (New Brunswick Scientific).

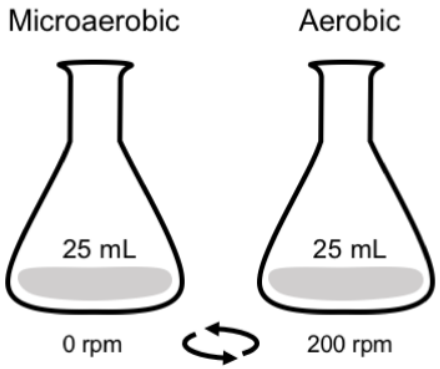


Figure 2.1 Experimental set-up for aerobic and microaerobic growth.
Cultures were grown in 25 mL of media in 250 mL and left static (0 rpm) for microaerobic growth and agitated at 200 rpm for aerobic growth. Under the "microaerobic" condition, bacteria in the initial inoculum quickly deplete the medium of oxygen below the surface, and further oxygen diffusion is limited as the flasks are not aerated.

2.2.4 Monitoring Bacterial Growth

Growth of bacterial cultures was monitored by measuring optical density at a wavelength of 600 nm (OD_{600}) with a Biomate 3S spectrophotometer (Thermo Scientific). Cultures with an OD_{600} of greater than approximately 0.5 were first diluted 10-fold in the appropriate medium before taking a measurement. To calculate viable cell counts, overnight cultures were serially diluted 10-fold in sterile PBS and plated on LA. Cell numbers were calculated as colony forming units per mL (CFU/mL).

2.2.5 Antibiotics

Antibiotic stocks were made at 1000 × concentration and stored in aliquots at -20ºC. Stocks were made in dH_2O and filter sterilized with 0.2 µm PES membrane filters (Fisher Scientific), or in 70% ethanol. The following antibiotics were used in this study; Kanamycin (Kan, 50 µg/mL, dH_20), ampicillin (Amp, 150 µg/mL, dH_2O), and chloramphenicol (Cm, 35 µg/mL, EtOH).

2.2.6 Motility Assays

Swimming motility assays were performed to measure the swimming capacity of bacterial strains under aerobic and anaerobic conditions. Swimming motility assays were performed by stab inoculation with 1 µL of 1 OD unit bacterial culture into swimming agar (**Table 2.1**) and incubated for 6 h at 37ºC in an anaerobic jar with an Oxoid™ AnaeroGen™ 2.5 L Sachet (Thermo Scientific) to remove O_2 for anaerobic assays. Plates for anaerobic assays were left in anaerobic jars with an anaerobic gas sachet overnight prior to inoculation to remove any oxygen from the media. Diameter of swim colonies were measured with a ruler, and plates were photographed.

2.2.7 Growth curves and kinetic gene expression assay

2.2.7.1 Microaerobic and aerobic growth curves

Growth curves were used to measure growth rate of bacterial strains. Bacterial cultures for growth curves were inoculated as previously described (Section 2.2.3) in LB or minimal media as appropriate. Absorbance measurements (OD_{600}) were taken every 1 – 2 h depending on the growth rate of the culture with a Biomate 3S spectrophotometer (Thermo Scientific). Best-fit growth rate and lag time of each strain was determined using GrowthRates (Hall *et al.*, 2014).

2.2.7.2 Anaerobic growth curves

Anaerobic growth curves were performed by Daniel Ryan at The Helmholtz Institute for RNA-based Infection Research, Würzurg, Germany. Overnight cultures were grown as previously described (Section 2.2.3) and subcultured anaerobically with a 1:100 dilution into

LB media which had been kept in a Coy Anaerobic chamber with a gas mix containing 10% H_2, 5% CO_2 and 85% N_2 overnight. Bacterial cultures were grown and absorbance was measured every 20 min in a Synergy HT Microplate reader (Biotek) in the anaerobic chamber.

2.2.7.3 Kinetic gene expression

Gene expression was measured across growth or at OD_{600} of 0.3 to determine expression of *ssaG*, *ssrA*, *ssrB*, *ssaB*, and *ydgT* at different growth phases in different media. Bacteria transformed with plasmids with transcriptional promoter fusions to *luxCDABE* (**Table 2.3**) were inoculated as previously described (Section 2.2.3) in LB or minimal media as appropriate. Absorbance measurements (OD_{600}) were taken every 1 – 2 h depending on the growth rate of the culture using a Biomate 3S spectrophotometer (Thermo Scientific). At the same time points, 200 µL of culture was used to measure luminescence in white-walled 96-well plates (Sarstedt) in a Synergy HT Microplate reader (Biotek). All luminescence values were normalized to the corresponding optical density (at 600 nm) of the culture.

2.2.8 Oxygen gradient gene expression assay

Oxygen gradient gene expression assays were used to assess the repression of SPI-2 by FNR at various oxygen concentrations. Oxygen gradient gene expression assays were carried out by mixing 2 mL liquid MMA soft agar (**Table 2.1**), cooled to 50ºC, with 200 µL of overnight culture in small glass test tubes. The tubes were left to solidify at room temperature, and subsequently incubated at 37°C for 72 h. Within the test tube there is O_2 above the agar and at the surface, O_2 concentration decreases down through the agar. GFP expression was detected via a blue epi-illuminator (460 nm) using the ImageQuant LAS4000 (GE). Photos of the tubes were also taken in natural light to observe regions of turbidity indicating growth of each of the strains.

2.2.9 Relative Fitness Assay

Relative fitness assays were used to determine if bacterial strains with genotypic alterations such as deleted genes, would incur a fitness advantage or disadvantage in mixed populations. Strains used in these assays were used as their original versions or were transduced with the ΔSL1483::*cat* mutation as a selective marker. Strains were competed against themselves

with the selective marker to assess that there was no phenotypic disadvantage to addition of the marker. Cultures were prepared as previously described for sub-culture into minimal media, and flasks were inoculated with 10^7 cells of each strain. Inoculums were plated at 0 h and after 24 h of co-culture. CFU/mL of co-cultures was determined using the exclusion method. Briefly, co-cultures were diluted appropriately onto LA plates and LA plates containing Cm (25 µg/mL) to assess total number of colonies, and number of Cm resistant colonies. Subtraction of Cm resistant CFU from total CFU gives the number of Cm susceptible CFU. The relative fitness was determined by calculating the competitive index (C.I.) by the following formula:

$$C.I. = \frac{\ln\left(\frac{A_f}{A_i}\right)}{\ln\left(\frac{B_f}{B_i}\right)}$$

Where C.I. is competitive fitness, *A* and *B* are the population sizes of the two competitors in CFU/mL, and subscripts *i* and *f* indicate the initial and final time points in the assay, 0 h and 24 h respectively. The natural logarithm, ln, reflects population growth (Wiser & Lenski, 2015). Relative fitness assays were performed by Stefani Kary and Naoise McGarry at Trinity College Dublin, Dublin, Ireland, and data analysis completed by Stefani Kary.

Table 2.1 Media recipes

Medium	*Reagent*	*Concentration*	*Reference*
Lennox Broth (LB)	Bacto-Tryptone	10 g/L	(Lennox, 1955)
	NaCl	5 g/L	
	Bacto-Yeast extract	5 g/L	
Lennox Agar (LA)	Lennox broth		(Lennox, 1955)
	Bacto-Agar	1.5% (w/v)	
Swimming Agar	Lennox broth		
	Bacto-Agar	0.3% (w/v)	
Minimal Medium A (MMA)[ab]	K_2HPO_4	60 mM	(Constantinidou *et al.*, 2006)
	KH_2PO_4	33 mM	
	$(NH_4)_2SO_4$	7 mM	
	Sodium citrate dihydrate	1.7 mM	
	$MgSO_4$	1 mM	
	Glycerol	0.4% (v/v)	
	Fumarate	40 mM	
	TMAO	20 mM	
MMA Soft Agar	MMA		
	Bacto-Agar	1% (w/v)	
PCN (SPI-2-inducing, InSPI2)	MES (pH 5.8)	80 mM	(Löber *et al.*, 2006)
	Tricine	4 mM	
	$FeCl_3$	100 µM	
	K_2SO_4	376 µm	
	NaCl	50 mM	
	K_2HPO_4/KH_2PO_4 (pH 5.8)	0.4 mM	
	Glucose	0.4 % (w/v)	
	NH_4Cl	15 mM	
	$MgSO_4$	1 mM	
	$CaCl_2$	0.01 mM	
	Na_2MoO_4	10 nM	
	Na_2SeO_3	10 nM	
	H_3BO_3	4 nM	
	$CoCl_2$	300 nM	
	$CuSO_4$	100 nM	
	$MnCl_2$	800 nM	
	$ZnSO_4$	1 nM	

[a]variations of this medium without fumarate and/or TMAO were used in some experiments, unless otherwise stated MMA refers to the medium containing both fumarate and TMAO.

[b]variations of this medium with different concentrations of K_2HPO_4 and KH_2PO_4 and pH were used for some experiments, unless otherwise stated MMA refers to the medium with the above recipe and pH of ~7.4.

Table 2.1 Media recipes (continued)

Medium	*Reagent*	*Concentration*	*Reference*
PCN (SPI-2-Non-inducing, NonSPI2)	PCN w/o MES & K_2HPO_4/KH_2PO_4		(Löber *et al.*, 2006)
	MOPS (pH 7.4)	80 mM	
	K_2HPO_4/KH_2PO_4 (pH 7.4)	25 mM	
Green Agar	Bacto-Tryptone	8 g/L	(Smith & Levine, 1967)
	NaCl	5 g/L	
	Bacto-Yeast extract	1 g/L	
	Alizarin yellow	0.625 g/L	
	Aniline blue	0.006% (w/v)	
	Glucose	0.8% (w/v)	
	Bacto-Agar	1.5% (w/v)	
Super Optimal Broth (SOB)	Bacto-Tryptone	20 g/L	(Hanahan, 1983)
	NaCl	5 g/L	
	Bacto-Yeast extract	5 g/L	
	KCl	2.5 mM	
	$MgCl_2$	10 mM	
	$MgSO_4$	10 mM	
SOB with Catabolite Repression (SOC)	SOB		(Hanahan, 1983)
	Glucose	20 mM	

Table 2.2 Bacterial strains used in this study.

Strain Name	*Relevant Genotype*	*Resistance*	*Original Source/Reference*
S. Typhimurium 4/74	wild-type	Str^R	Mark Stevens
	Δ*fnr*::FRT		Jay Hinton, JH3307
	Δ*fnr*::FRT attTn7::*fnr*::*kan*	Kan^R	This Study
	Δ*ssrAB*::FRT		Aoife Colgan
	Δ*fnr*::FRT, Δ*ssrAB*::FRT		OS: Aoife Colgan, *kan* removal: This Study
	ΔrpoE::cat	Cm^R	Aoife Colgan
	Δ*hilA*::FRT		OS: Jörg Vogal, JVS-1195, transduction, *kan* removal: This Study
	Δ*hilD*::FRT		Aoife Colgan
	hns-1::*kan*	Kan^R	Jay Hinton, JH3863
	fnr-3×FLAG::*kan*	Kan^R	C. Kröger
	steC-3×FLAG::*kan*	Kan^R	OS: A. Westermann, transduction: This Study
	Δ*fnr*::FRT *steC*-3×FLAG::*kan*	Kan^R	OS: A. Westermann, transduction: This Study
	Δ*ssrAB*::FRT *steC*-3×FLAG::*kan*	Kan^R	OS: A. Westermann, transduction: This Study
	Δ*hilA*::FRT *steC*-3×FLAG::*kan*	Kan^R	OS: A. Westermann, transduction: This Study
	Δ*hilD*::FRT *steC*-3×FLAG::*kan*	Kan^R	OS: A. Westermann, transduction: This Study
	sopE-3×FLAG::*kan*	Kan^R	OS: A. Westermann, transduction: This Study
	Δ*fnr*::FRT *sopE*-3×FLAG::*kan*	Kan^R	OS: A. Westermann, transduction: This Study
	Δ*ssrAB*::FRT *sopE*-3×FLAG::*kan*	Kan^R	OS: A. Westermann, transduction: This Study
	Δ*hilA*::FRT *sopE*-3×FLAG::*kan*	Kan^R	OS: A. Westermann, transduction: This Study
	Δ*hilD*::FRT *sopE*-3×FLAG::*kan*	Kan^R	OS: A. Westermann, transduction: This Study
	pipB2-3×FLAG::*kan*	Kan^R	OS: O. Steele-Mortimer, transduction: This Study
	Δ*fnr*::FRT *pipB2*-3×FLAG::*kan*	Kan^R	OS: O. Steele-Mortimer, transduction: This Study
	Δ*ssrAB*::FRT *pipB2*-3×FLAG::*kan*	Kan^R	OS: O. Steele-Mortimer, transduction: This Study
	Δ*hilD*::FRT *pipB2*-3×FLAG::*kan*	Kan^R	OS: O. Steele-Mortimer, transduction: This Study

Table 2.2 Bacterial strains used in this study (continued).

Strain Name	***Relevant Genotype***	***Resistance***	***Original Source/Reference***
S. Typhimurium 4/74	ΔSL1483::*cat*	Cm^R	C. Kröger
	Δ*fnr*::FRT ΔSL1483::*cat*	Cm^R	OS: C. Kröger, transduction: This Study
	Δ*ssrAB*::FRT ΔSL1483::*cat*	Cm^R	OS: C. Kröger, transduction: This Study
	Δ*fnr*::FRT Δ*ssrAB*::FRT ΔSL1483::*cat*	Cm^R	OS: C. Kröger, transduction: This Study
	Φ(*ssaG′-gfp*$^+$)1, *cat*	Cm^R	Jay Hinton, JH3009
	Δ*fnr*::*kan* Φ(*ssaG′-gfp*$^+$)1, *cat*	Kan^R, Cm^R	OS: Jay Hinton, JH3009, C. Kröger
	Δ*ssrAB*::*kan* Φ(*ssaG′-gfp*$^+$)1, *cat*	Kan^R, Cm^R	OS: Jay Hinton, JH3009, C. Kröger
E. coli TOP10	F$^-$ *mcrA* Δ(*mrr-hsdRMS-mcrBC*) (φ80*lacZ*Δ*M15* Δ*lacX74* *nupG* *recA1* *araD139* Δ(*ara-leu*)*7697* *galE15* *galK*16 *rpsL*(Str^R) *endA*1 λ^-	Str^R	Invitrogen
E. coli DH5α	F$^-$ *endA*1 *glnV44* *thi-1* *recA1* *relA1* *gyrA96* *deoR* *nupG* *purB20* φ80d*lacZ*Δ*M15* Δ(*lacZYA-argF*)*U169*, *hsdR17*(r_K^- m_K^+), λ^-		Charles Dorman
E. coli DH5α*pir*$^+$	*endA1* *hsdR17* *glnV44* (=*supE44*) *thi-1* *recA1* *gyrA96* *relA1* φ80d*lac*Δ(*lacZ*)*M15* Δ(*lacZYA-argF*)*U169* *zdg-232*::Tn10 *uidA*::*pir*$^+$		Jörg Vogel
E. coli BL21(DE3) (PK22)	*fhuA2* [*lon*] *ompT* *gal* (λ *DE3*) [*dcm*] Δ*hsdS* λ *DE3* = λ *sBamHIo* Δ*EcoRI-B* *int*::(*lacI*::*PlacUV5*::T7 *gene1*) *i21* Δ*nin5* Δ*fnr*Δ*crp*		(Shan *et al.*, 2012b)

OS: Original Strain

Table 2.3 Plasmids used in this study.

Plasmid Name	*Description*	*Resistance*	*Reference*
pBR322	Low copy number cloning vector	Tet^R, Amp^R	(Bolivar *et al.*, 1977)
p*fnr*	*fnr* with 215 bp upstream cloned in pBR322	Amp^R	This Study
p*fnrD154A*	Oxygen insensitive *fnr* (D154A) with native promoter cloned in pBR322	Amp^R	This Study
p(*fnrD154A*)$_2$	Oxygen insensitive *fnr* (D154A)$_2$ from *E. coli* with 6xHis-tag in pET28a	Kan^R	(Shan *et al.*, 2012b)
pCP20	Plasmid with yeast Flp recombinase gene, FLP, and temperature sensitive replication	Cm^R, Amp^R	(Cherepanov & Wackernagel, 1995)
pDIGc	Constitutive expression of GFP in bacteria	Amp^R	(Helaine *et al.*, 2010)
pDEW201	Moderate copy number with a promoterless *luxCDABE*	Amp^R	(Van Dyk & Rosson, 1998)
pDEW201-P_{ssaG}	P_{ssaG} cloned into pDEW201	Amp^R	This Study
pUC18R6K-miniTn7T-PacI	Delivery plasmid for chromosomal integration at *att*Tn7 downstream of *glmS*	Amp^R	(Shivak *et al.*, 2016)
pHSG415-*tnsABCD*	Replication-proficient helper plasmid with temperature-sensitive origin	Amp^R	(Shivak *et al.*, 2016)
pCS26-Kn^R-*luxCDABE*-PacI	Low copy number modular cloning vector with a promoterless *luxCDABE*	Kan^R	(Shivak *et al.*, 2016)
pCS26-Kn^R-PacI	Low copy number modular cloning vector without reporter	Kan^R	This Study
pCS26-Kn^R-*fnr*-PacI	*fnr* and native promoter cloned into pCS26-Kn^R-PacI	Kan^R	This Study
pCS26-Kn^R-*fnr*-pUC18R6K-miniTn7T	pCS26-Kn^R-*fnr*-PacI cloned into pUC18R6K-miniTn7T-PacI	Kan^R, Amp^R	This Study
pCS26-Cm^R-*luxCDABE*-PacI	Low copy number modular cloning vector with a promoterless *luxCDABE*	Cm^R	(Shivak *et al.*, 2016)

Table 2.3 Plasmids used in this study (continued).

Plasmid Name	*Description*	*Resistance*	*Reference*
pCS26-Cm^R-*luxCDABE*-PacI-P_{ssrA1^o}	$P_{ssrA}1^o$ cloned into pCS26-Cm^R-*luxCDABE*-PacI	Cm^R	This Study
pCS26-Cm^R-*luxCDABE*-PacI-P_{ssrA}	P_{ssrA} cloned into pCS26-Cm^R-*luxCDABE*-PacI	Cm^R	This Study
pCS26-Cm^R-*luxCDABE*-PacI-P_{ssrB}	P_{ssrB} cloned into pCS26-Cm^R-*luxCDABE*-PacI	Cm^R	This Study
pCS26-Cm^R-*luxCDABE*-PacI-P_{ssaB}	P_{ssaB} cloned into pCS26-Cm^R-*luxCDABE*-PacI	Cm^R	This Study
pCS26-Cm^R-*luxCDABE*-PacI-*dbpA*	*dbpA* cloned into pCS26-Cm^R-*luxCDABE*-PacI	Cm^R	This Study

2.3 General molecular techniques

2.3.1 Isolation of chromosomal S. Typhimurium 4/74 DNA for use in PCR

Overnight cultures were prepared as previously described (Section 2.2.3). 400 µL of the culture was harvested by centrifugation at 8,000 × g for 1 minute at room temperature. The cell pellet was resuspended in 200 µL of sterile nuclease free H_2O and boiled at 100ºC for 5 min, and subsequently vortexed for 30 s to ensure complete lysis. Cell debris was pelleted by centrifugation at 8,000 × g for 5 min, and supernatants were added to 1 volume chloroform and vortexed for 30 s. Organic and aqueous phases of the solution were separated by centrifugation at 8,000 × g for 10 min at 4ºC. 75% of the aqueous layer was transferred to a new 1.5 mL tube to eliminate chloroform contamination. DNA contained in the aqueous phase was measured with the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific) and the concentration was adjusted to 100 ng/mL.

2.3.2 Isolation of plasmid DNA

Plasmid DNA was extracted and purified using the GeneJet Plasmid Miniprep Kit (Thermo Scientific) according to the manufacturers' instructions except plasmids were eluted into dH_2O. For extremely low copy plasmids, such as pCS26 derivatives, up to 50 mL of culture was harvested and multiple minipreps were combined into 50 µL of dH_2O.

2.3.3 Polymerase chain reaction (PCR)

PCR amplifications were carried out in a SimpliAmp Thermal Cycler (Applied Biosystems, Thermo Scientific). Routine PCR and colony screening were carried out using Taq polymerase (New England Biolabs) according to the manufacturers' specifications. Q5 polymerase (New England Biolabs) was used for applications requiring high fidelity amplification and RANGER Mix (Bioline) was used for products larger than 10 kbp according to the manufacturers' specifications. As a template for PCR, 100 ng chromosomal DNA, 1-10 ng of plasmid DNA, or single colony resuspended in 200 µL of H_2O was used.

2.3.4 Agarose gel electrophoresis

Agarose gel electrophoresis was used for size separation of DNA and RNA molecules. DNA samples were mixed with DNA loading dye (40% (v/v) glycerol, 60 mM EDTA, 10 mM Tris-HCl pH 7.6, 0.25% (w/v) bromophenol blue pH 8.0, 0.25% (w/v) xylene cyanol FF, 0.25% (w/v) orange G) to a final concentration of 1 × DNA loading dye and routinely electrophoresed in a 1% to 2.5% (w/v) agarose gel in 1 × Tris Acetate Ethylenediaminetetraacetic acid (TAE) buffer at 90 V. RNA samples were mixed with RNA loading dye (0.025% (w/v) xylene cyanol FF, 0.025% (w/v) bromophenol blue, 18 mM EDTA pH 8.0, 0.025% (w/v) SDS, 95% (v/v) formamide) to a final concentration of 1 × RNA loading dye and heat denatured at 65ºC for 5 min. RNA was routinely electrophoresed in a 2.5% (w/v) agarose gel in 1 × Tris Borate EDTA (TBE) buffer at 90 V. Gels were stained with SafeView Nucleic Acid Stain (NBS Biologicals) prior to visualization under ultra violet light in the ImageQuant™ LAS 4000 (GE Healthcare Life Sciences). The molecular size of electrophoresed DNA fragments was estimated by comparison with HyperLadder™ I (Bioline) or O'GeneRuler™ 1 kb DNA Ladder (Thermo Scientific).

2.3.5 Restriction digestion

All enzymes were purchased from Thermo Scientific (FastDigest enzymes), New England Biolabs, or Roche and used according to the manufacturers' specifications. Restriction digestions were carried out in a SimpliAmp Thermal Cycler (Applied Biosystems, Thermo Scientific) and digestion products were analyzed by agarose gel electrophoresis as previously described.

2.3.6 Purification of PCR products

If required for downstream applications, PCR products were purified to remove primers, enzymes, and other impurities using the Monarch® PCR & DNA Cleanup Kit (New England Biolabs) or the High Pure PCR Product Purification Kit (Roche) according to the manufacturers' specifications. In order to concentrate samples, multiple PCR reaction products were combined and spun through a single spin column.

2.3.7 Precipitation of DNA

In order to increase the concentration of low concentration DNA samples Pellet Paint® Co-Precipitant (Millipore) was used to facilitate alcohol precipitation of nucleic acids. 1 µL of Pellet Paint® and 1 volume of 3 M sodium acetate were added to the sample and mixed briefly. 2 volumes of ethanol were then added followed by briefly vortexing the sample, and a 2 min incubation at room temperature. Sample was centrifuged at 8,000 × g for 5 min and washed in 70% ethanol. DNA pellet was then resuspended in desired volume of H_2O.

2.3.8 Extraction of DNA from agarose gels

DNA digests or PCR products that required precise purification of a product of interest were electrophoresed as previously described. DNA fragments were visualized briefly under ultra violet light and the specific DNA band was excised with a scalpel. The excised DNA in agarose was weighed and dissolved in gel dissolving buffer at 55ºC with occasional vortexing. Once dissolved, 3 M sodium acetate pH 5.2 was added to lower the pH of the sample to increase binding to the column membrane. Monarch® DNA Gel Extraction Kit or High Pure PCR Product Purification Kit (Roche) were used to spin column purify the DNA according to the manufactures' specifications.

2.4 Preparation and transformation of competent bacteria

2.4.1 Preparation of electro-competent S. Typhimurium and electroporation

Overnight cultures of *S*. Typhimurium were subcultured 1:100 in 50 mL LB with appropriate antibiotics and grown with agitation at 200 rpm at 30ºC or 37ºC to exponential phase (OD_{600} 0.5). Cultures were then incubated on ice for 20 min. Cells were harvested by centrifugation at 2,880 × g at 4ºC for 10 min and resuspended in 40 mL ice-cold sterile 10% (v/v) glycerol and incubated on ice for 20 min. The glycerol wash was repeated two additional times with resuspensions in 25 mL, then 10 mL of ice-cold 10% (v/v) glycerol. After a final centrifugation at 2,880 × g and 4ºC, the pellet was resuspended in 300 µL of ice-cold 10% (v/v) glycerol. Cells were either stored at -80ºC or immediately electroporated. 40 µL of electro-competent cells were added to 2 mm electroporation cuvettes with up to 5 µL of plasmid DNA and electroporated with the GenePulser Xcell electroporator (Biorad) at 2.5 kV, 200 Ω, 25 µF. 1 mL of SOC (**Table 2.1**) was added to cells, and the cells were recovered

at 37ºC for 1 h or at 30ºC for 2 h with agitation at 200 rpm. For small and high copy plasmids 150 µL of cells was plated on LA plates with appropriate antibiotics and incubated at 30ºC or 37ºC overnight. For large and low copy plasmids, cells were harvested by centrifugation at 6,500 × g and resuspended in 150 µL of SOC. The entire resuspension was plated on LA plates with appropriate antibiotics and incubated at 30ºC or 37ºC overnight.

2.4.2 Preparation and transformation of chemically competent E. coli

2.4.2.1 $CaCl_2$ chemically competent E. coli

Overnight cultures of *E. coli* were diluted 1:100 in 50 mL LB with appropriate antibiotics with agitation at 200 rpm to exponential phase (OD_{600} 0.4 – 0.8). Cells were then incubated on ice for 20 min. Cells were harvested by centrifugation at 2,880 × g and 4ºC and resuspended in 40 mL sterile ice-cold 100 mM $CaCl_2$ and incubated on ice for 20 min. Cells were harvested as before and resuspended in 10 mL sterile ice-cold 100 mM $CaCl_2$. Sterile glycerol was added to a final concentration of 10%, and cells were incubated on ice for an additional 20 min. Cells were either stored at -80ºC in 300 µL aliquots or immediately transformed. $CaCl_2$ competent cells were used for the transformation of small plasmids.

2.4.2.2 PIPES chemically competent E. coli

Overnight cultures of *E. coli* were diluted 1:100 in 100 mL LB with appropriate antibiotics with agitation at 200 rpm to exponential phase (OD_{600} 0.4 – 0.8). Cells were then incubated on ice for 20 min. Cells were harvested by centrifugation at 2,880 × g and 4ºC and resuspended in 30 mL sterile ice-cold 100 mM $MgCl_2$ and incubated on ice for 20 min. Cells were harvested as before and resuspended in 30 mL sterile ice-cold PIPES buffer (60 mM $CaCl_2$, 10 mM PIPES pH 7.6, 15% (v/v) glycerol). Cells were harvested as before and resuspended in 5 mL PIPES buffer. Cells were either stored at -80ºC in 300 µL aliquots or immediately transformed. PIPES competent cells were used for the transformation of large (>10 kb) plasmids.

2.4.2.3 Chemical transformation of E. coli

100 – 300 µL of chemically competent cells was mixed with up 20 µL of plasmid DNA or ligation mixture and incubated on ice for 20 min. The cells were then heat shocked in a 42ºC

water-bath for 90 – 120 s, followed by a 2 min incubation on ice. 1 mL of SOC was added to each transformation, and cells were recovered at 37ºC with agitation at 200 rpm for 1 h. Cells were harvested by centrifugation at 6,500 × g and resuspended in 150 µL of SOC. The entire resuspension was plated on LA plates with appropriate antibiotics and incubated at 37ºC overnight.

Table 2.4 Table of oligonucleotides

Primer	*Sequence (5' – 3')*	*Description*
FNR_com_F215	TTTAAGCTTAAGGCTATCTTTTATTATG	To amplify *fnr* with 215 bp upstream, adds HindIII restriction site
FNR_com_R	TTTGTCGACAGTTTGTAACTAAAGAGTAACTC	To amplify *fnr*, adds SalI
D145A_FNR_F	*ATTAAAGGCGCTCAGGATATGA	To introduce D154A mutation to *fnr* on a plasmid
D145A_FNR_R	TTCACCGCTCATCAGACGCAT	To introduce D154A mutation to *fnr* on a plasmid
pBR_seq_4	TGCCACCTGACGTCTAAG	Sequencing primer for pBR322
pDEW201_PssaG_F	CCGGAATTCTTACTCGCTTCGGTATGG	To amplify P_{ssaG}, adds EcoRI restriction site
pDEW201_PssaG_R	TTCCGGATCCAATATCCATAATGCTTTTCC	To amplify P_{ssaG}, adds BamHI restriction site
luxC_R	CCAGTACTATCAGCGC	Verify insertion in pDEW201
qPCR_sseA_F	TCACCAAATCCGGGCTAAG	To amplify *sseA* for qPCR
qPCR_sseA_R	GCAACGCCTTGTGGAAATAG	To amplify *sseA* for qPCR
qPCR_ssaH_F	GCGTTAACCATAGCCTGATTTC	To amplify *ssaH* for qPCR
qPCR_ssaH_R	CCAACAATAATGCCAGACATACC	To amplify *ssaH* for qPCR
qPCR_fnr_F	ATTGGCAGCGGTCATCAT	To amplify *fnr* for qPCR
qPCR_fnr_R	TGACGCAGGTTAGGCATTT	To amplify *fnr* for qPCR
hemX_RT_F	CGCCTGACGGTATGTTTCTT	To amplify *hemX* for qPCR, control
hemX_RT_R	CCCAACCAGGACGTCTATTTAC	To amplify *hemX* for qPCR, control
fnr_EMSA_F	AGGCTATCTTTTATTATG	To amplify P_{fnr} for EMSA
fnr_EMSA_R	CAAAGCTGGCTGATACTGC	To amplify P_{fnr} for EMSA
FnrS_EMSA_F	ATAATAAGGTCAAAAGACAGCTC	To amplify P_{FnrS} for EMSA
FnrS_EMSA_R	CAAAAGCGCTTTTCAGACC	To amplify P_{FnrS} for EMSA
ydgT_EMSA_F	ATTAAATATAATGCCAACGGAG	To amplify P_{ydgT} for EMSA
ydgT_EMSA_R	TCCGTCCAGAAGAAGTAAGC	To amplify P_{ydgT} for EMSA
ssrA_EMSA_F	TATATAACCCAGTCGATGAC	To amplify P_{ssrA} for EMSA
ssrA_EMSA_R	ATTCACAATTACATTTTCAGC	To amplify P_{ssrA} for EMSA
ssrA2_EMSA_F	CTGATTACTAAAGATGTTTGCAG	To amplify P_{ssrA} for EMSA
ssaB_EMSA_F	GGCTTTTTACGGATGTGG	To amplify P_{ssaB} for EMSA

Table 2.4 Table of oligonucleotides (continued)

Primer	*Sequence (5' – 3')*	*Description*
ssaB_EMSA_R	AGAAATAGAAAATGCTTCTGAG	To amplify P_{ssaB} for EMSA
ssrB_EMSA_F	CCGTTAATGATGATTTCATGATCGTCT	To amplify P_{ssrB} for EMSA
ssrB_EMSA_R	AAATGCCGTATCGGCTGGA	To amplify P_{ssrB} for EMSA
dbpA_EMSA_F	CGATCATTTTAATAGCCGTACC	To amplify *dbpA* for EMSA
dbpA_EMSA_R	CTCAAGCTGAACTGGCTGAA	To amplify *dbpA* for EMSA
FnrS_P1	GCAGGTGAATGCAACGTCA	To amplify DNA template for FnrS riboprobe
FnrS_P2	GAATTAATACGACTCACTATAGCCGACTAATCTAAGTCGG	To amplify DNA template for FnrS riboprobe
5S_P1	GCGGCACTAGCGCGGTGGTC	To amplify DNA template for 5S riboprobe
5S_P2	GAATTAATACGACTCACTATAGCATGGGGAGACCCCACACT	To amplify DNA template for 5S riboprobe
pZE.05	AATCATCACTTTCGGGAA	Verify insert insertion in pCS26
pCS26-For	TAGCAACACCAGAACAGCC	Amplify pCS26 without origin of replication
pCS26-Rev	ATCACTATACCAATTGAGATGGGC	Amplify pCS26 without origin of replication
pUC18R6K-checkF	ATCCGCCGCTAGGAGCTTG	Verify insertion of pUC18R6K-miniTn7T
pUC18R6K-checkR	TTGGCCTGCAAGGCCTTCG	Verify insertion of pUC18R6K-miniTn7T
lux-check60	TATATGCGCGAGCGCTTGATCC	Verify lux insertion
cm-check60	TGTGACCGTGTGCTTCTCAAATGC	Verify Cm^R insertion
kn-check60	TACCCGTGATATTGCTGAAGAGCTTGG	Verify Kn^R insertion
glmSdetectFor	AACCACCCGTTCAGGCTGGCTA	Verify insertion downstream of *glmS*
glmSdetectRev	ACGTTGACCAGCCGCGTAAC	Verify insertion downstream of *glmS*
fnrcom_XhoI_F	TTTCTCGAGAGTTTGTAACTAAAGAGTAACTC	To amplify *fnr* with promoter, adds XhoI site
fnrcom_BamHI_R	TTTGGATCCAAGGCTATCTTTTATTATG	To amplify *fnr* with promoter, adds BamHI site
PssrA_XhoI_F	TTTCTCGAGATTCACAATTACATTTTCAGC	To amplify primary *ssrA* TSS, adds XhoI site
PssrA2_XhoI_F	TTTCTCGAGGAATCCCTCCTCAGACATAAA	To amplify entire *ssrA* promoter, adds XhoI site
PssrA_BamHI_R	TTTGGATCCCTTTGGCACTTGATCACTA	To amplify P_{ssrA} promoter, adds BamHI site
PssrB_XhoI_F	TTTCTCGAGGCTGGCTGATATTGAAAATGC	To amplify P_{ssrB}, adds XhoI site
PssrB_BamHI_R	TTTGGATCCGCCGTTAATGATGATTTCATG	To amplify P_{ssrB}, adds BamHI site
PssaB_XhoI_F	TTTCTCGAGTTATCGGAAAATCCGAATGATAG	To amplify P_{ssaB}, adds XhoI site

Table 2.4 Table of oligonucleotides (continued)

Primer	***Sequence (5′ – 3′)***	***Description***
PssaB_BamHI_R	TTTGGATCCAGAAATAGAAAATGCTTCTGAG	To amplify P_{ssaB}, adds BamHI site
dbpA_XhoI_F	TTTCTCGAGCGATCATTTTAATAGCCGTACC	To amplify *dbpA*, adds XhoI site
dbpA_BamHI_R	TTTGGATCCCTCAAGCTGAACTGGCTGAA	To amplify *dbpA*, adds BamHI site
sopE_check	ATCAGGAAGAGGCTCCGC	Verify insertion of 3×FLAG with Kan^R at *sopE*
steC_check	ATCTGTAGCGAATGTGCCC	Verify insertion of 3×FLAG with Kan^R at steC

* represents a 5′ phosphorylation modification

Restrictions sites are underlined

SNPs are double underlined

2.5 Genetic manipulations

2.5.1 P22 Phage transduction

2.5.1.1 Preparation of bacteriophage lysates

Bacteriophage P22 HT 105/1 *int-201* was used introduce desired mutations linked to antibiotic markers for all transductions. To generate P22 phage lysates, first a donor strain was subcultured 1:1000 in 10 mL of LB with appropriate antibiotics and grown to an OD_{600} of 0.1. The culture was then inoculated with 20 µL of P22 phage from wild-type 4/74 cells and incubated with agitation at 200 rpm for 4 h. All remaining bacterial cells were killed with the addition of 500 µL of chloroform, gentle mixing, and incubation at room temperature for 10 min. Cellular debris was removed by collecting supernatants after centrifugation at 2,880 × g for 20 min at room temperature. Supernatants were filtered using 0.2 µm PES membrane filters (Fisher Scientific). Stocks of bacteriophage P22 were stored in 5 mL volumes supplemented with 10 µL chloroform at 4ºC.

2.5.1.2 Transduction by bacteriophage P22

Recipient strains were grown overnight as previously described (2.2.3.1). Transductions were performed by combination of 100 µL of stationary phase recipient strain with 100 µL of neat, 10^{-1}, and 10^{-2} dilutions of phage, and stationary incubation at 37ºC for 1 h. Negative controls of recipient strain alone and phage alone samples were also incubated at 37ºC. The entire transduction reaction was plated onto appropriate antibiotic LA plates and incubated at 37ºC overnight. If possible, colonies were chosen from plates with the highest phage dilution to prevent the occurrence of double transductants. Green agar contains a low pH indicator whereby reduction in pH of lysed cells is detected by the presence of dark green colonies containing unstable pseudolysogens. To eliminate the possibility of phage contamination or the presence of pseudolysogens, pale colonies were passaged twice on green agar containing the relevant antibiotic (**Table 2.1**). Finally, a pale colony was chosen, verified by colony PCR and custom sequencing, and stored as a glycerol stock.

2.5.2 Removal of antibiotic resistance cassettes

To ensure that the presence of antibiotic resistance cassettes did not have polar effects on surrounding genes, and to facilitate additional genetic manipulations, resistance genes were removed from mutant *S.* Typhimurium strains using the pCP20 plasmid (Cherepanov & Wackernagel, 1995; Datsenko & Wanner, 2000). The pCP20 plasmid encodes the yeast FLP recombinase and has a temperature sensitive origin of replication. FLP targets FLP recombinase target sites (FRT sites) found flanking antibiotic resistance cassettes amplified from pKD3, pKD4 and pSUB11. FLP mediated site-specific recombination of FRT sites causes removal of the antibiotic resistance cassette, leaving a scar sequence of 82 – 85 nt. Finally, cells were cured of the pCP20 plasmid by passaging cells at 42ºC. To ensure both the resistant cassette and pCP20 plasmid were lost, cells were streaked on LA plates, and LA plates containing appropriate antibiotics. Colonies that grew only on LA plates without antibiotics were verified by PCR and stored in glycerol stocks.

2.5.3 Modular Tn7-based plasmid system

A modular Tn7-based system (Shivak *et al.*, 2016) was used for construction of the *fnr*$^{+}$ complementation (**Figure 2.2**). First, a kanamycin resistant pCS26 plasmid (pCS26-Kn^{R}-*luxCDABE*) was digested with NotI to remove the *luxCDABE* operon. The digested fragment was gel extracted and re-circularized with T4 DNA Ligase (Thermo Scientific). The new pCS26-Kn^{R} and a PCR amplified *fnr* with its promoter were digested with BamHI and XhoI and ligated with T4 DNA Ligase. The resulting pCS26-*fnr*-Kn^{R} plasmid and pUC18R6K-miniTn7T-PacI plasmid were digested with PacI and ligated with T4 DNA Ligase. The resulting pCS26-*fnr*-Kn^{R}-pUC18R6K-miniTn7T plasmid was electroporated into Δ*fnr* cells already containing the pHSG415-*tnsABCD* plasmid, cells were recovered at 30ºC for 2 h to allow for transposition of *fnr* and plated on LA plates containing kanamycin and grown overnight at 37ºC. Colonies were then streaked on kanamycin and ampicillin containing plates to ensure acquisition of *fnr* and loss of both plasmids. Colonies with growth on only kanamycin-containing LA were then verified by PCR and sequencing.

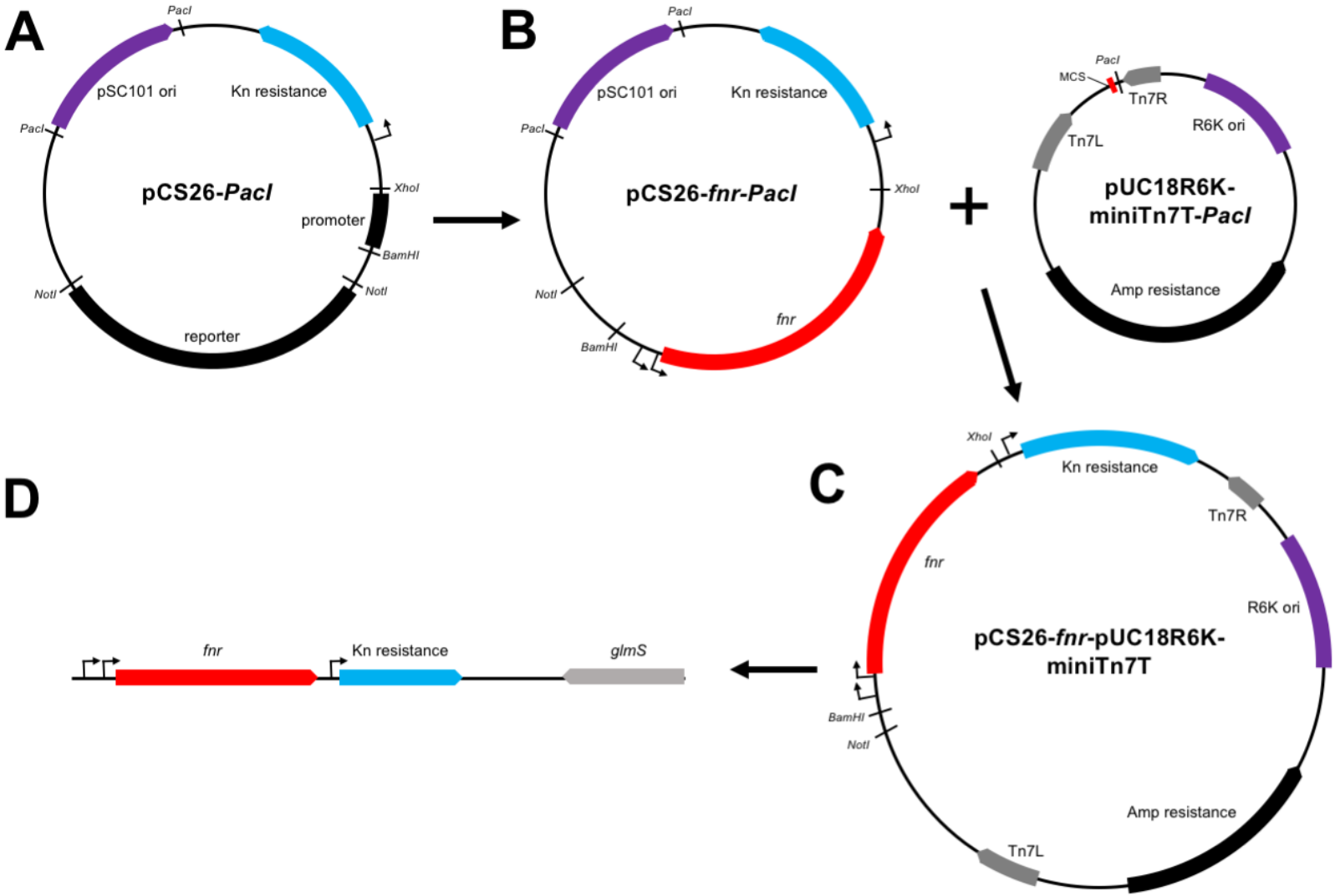

A
PacI
pSC101 ori
Kn resistance
PacI
pCS26-PacI
XhoI
promoter
BamHI
NotI
NotI
reporter
B
PacI
pSC101 ori
Kn resistance
PacI
pCS26-fnr-PacI
XhoI
NotI
BamHI
fnr
+
PacI
MCS
Tn7R
R6K ori
Tn7L
pUC18R6K-
miniTn7T-PacI
Amp resistance
C
XhoI
Kn resistance
Tn7R
fnr
R6K ori
pCS26-fnr-pUC18R6K-
miniTn7T
BamHI
NotI
Amp resistance
Tn7L
D
fnr
Kn resistance
glmS

Figure 2.2 Construction of the *fnr*$^+$ complementing strain.

A. A pCS26-PacI reporter plasmid with an kanamycin resistance cassette was digested with NotI, BamHI and XhoI and ligated to a XhoI and BamHI digested PCR product containing *fnr* with its native promoter to create pCS26-*fnr*-PacI. B. pCS26-*fnr*-PacI amplified to remove the pSC101origin of replication and was SLiCE cloned into the pUC18R6K-miniTn7T-*PacI* plasmid between the Tn7L and Tn7R sites to create C. pCS26-*fnr*-pUC18R6K-miniTn7T. This plasmid was then transformed into Δ*fnr*::FRT containing the pHSG-*tnsABCD* plasmid. D. Cells were recovered at 30ºC to allow transposition at the *att*::Tn7 site upstream of *glmS*. Figure adapted from Shivak *et al*., 2016.

2.6 Analysis of RNA

2.6.1 Extraction of RNA from S. Typhimurium 4/74

Cultures were grown as previously described. Transcription was stopped, and RNA was stabilized by addition of 2/5 volume "stop solution" (5% phenol pH 4.3, 95% ETOH) and incubation on ice for 30 min. Cells were harvested by centrifugation at 2,880 × g and 4ºC for 10 min. Pellets were either stored at -80ºC or RNA was immediately extracted.

Total RNA was extracted from bacterial cultures using TRIzol® (Ambion) for Northern Blots and RNA sequencing (RNA-seq). For TRIzol® RNA extraction, extracted pellets were resuspended on ice in 1 mL TRIzol®. Samples were then transferred to 2 mL Heavy Phase-Lock tubes (5PRIME), 400 µL chloroform was added, samples were mixed by inversion for 10 s, and incubated at room temperature for no longer than 5 min. Aqueous and organic phases were separated by centrifugation at 20,000 × g for 15 min at room temperature. The aqueous phase was then transferred to a new 1.5 mL tube containing 300 µL isopropanol, and RNA was precipitated for 30 min at room temperature. Samples were subsequently centrifuged at 20,000 × g for 30 min at room temperature, and RNA pellets were washed with 350 µL of 70% ethanol and centrifuged for a further 10 min at 20,000 × g. The supernatant was discarded, and RNA pellets were air dried to remove residual ethanol, then resuspended in DEPC treated H_2O. Samples were intermittently vortexed and mixed at 65ºC, 900 rpm on a ThermoMixer® (Eppendorf) for 5 minutes. RNA samples were then quantified using the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific), and quality was

assessed using agarose gel electrophoresis or with the 2100 Bioanalyzer using the RNA 6000 Nano Kit (Agilent), and stored at -80ºC.

Total RNA extracted using the SV Total RNA Isolation System (Promega) for RT-qPCR according to the manufactures' specifications. RNA samples were then quantified using the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific), and quality was assessed using agarose gel electrophoresis, and stored at -80ºC.

2.6.2 DNase I digestion

Total RNA samples were DNase I digested with the TURBO DNA-*free*™ Kit (Ambion) according to the manufacturers' instructions. DNase I digested samples were quantified with the NanoDrop ND-1000 Spectrophotometer (Thermo Scientific) and stored at -80ºC.

2.6.3 Reverse transcription

Reverse transcription (RT), or cDNA synthesis, of DNase I digested total RNA was completed using the GoScript™ Reverse Transcription System (Promega) according to the manufacturers' specifications. 400 ng of each RNA sample was processed in a 5 µL reaction volume, and following RT, was added to 100 µL of nuclease free H_2O for a final concentration of 3.33 ng/µL. For each sample a "No RT" control was also included that contained no RT enzyme to confirm the absence of genomic DNA in real-time quantitative PCR. All cDNA samples were stored at -80ºC.

2.6.4 Real-time quantitative PCR

Real-time quantitative PCR (RT-qPCR) was used to quantify gene expression of *S.* Typhimurium cells. The PowerUp™ SYBR™ Green Master Mix (Applied Biosystems) was used for all RT-qPCR reactions in 20 µL volumes including 8 µL of cDNA, "No Reverse Transcriptase" RNA or genomic DNA as template. cDNA and "No Reverse Transcriptase" templates were diluted 5-fold to a final concentration of 0.67 ng/µL. Standard curves were generated with six 10-fold serial dilutions of wild-type *S.* Typhimurium 4/74 genomic DNA. RT-qPCR reactions were set up in duplicate in MicroAmp Fast Optical 96-well reaction plates (Applied Biosystems) and run on the ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems). Default RT-qPCR settings were used for each run as well as for melt

curves for each new primer set. All primers used for RT-qPCR are listed in **Table 2.4**. Data analysis was done using the StepOne™ software and Prism 6 GraphPad. PCR products were quantified relative to *hemX.* The *hemX* gene encodes a putative uroporphyrinogen III C-methyltransferase and was used as a reference gene as it showed low variation in gene expression across RNA-seq transcripts from the wild-type and Δ*fnr* mutant.

2.6.5 Generation of Digoxigenin-labelled riboprobes

Digoxigenin (DIG)-labelled single-strand RNA molecules ("riboprobes") were generated for Northern Blotting by *in vitro* transcription using T7 RNA polymerase and the DIG Northern Starter Kit (Roche). A linear DNA template including the T7 promoter sequence (GAATTAATACGACTCACTATA) was generated through PCR with Q5 polymerase (New England Biolabs) and primers found in **Table 2.4**. DNA templates were purified through gel extraction as previously described, and 200 ng of DNA was used as template in each 20 µL reaction (1 × labelling mix, 1 × transcription buffer, 40 U T7 RNA polymerase). Labelling transcription reactions were incubated at 42ºC for 1 h in a SimpliAmp Thermal Cycler (Applied Biosystems, Thermo Scientific). Template DNA was removed by incubation with DNase I at 37ºC for 15 min, and reactions were stopped by addition of 400 mM EDTA pH 8.0. Riboprobes were stored at -20ºC.

Labelling efficiency of labelled riboprobes was tested by a dot-blot of 10-fold serial dilutions of riboprobes on a positively charged nylon membrane. Membranes were UV-crosslinked at 120 mJ (Peqlab crosslinker), rinsed with washing buffer, incubated at room temperature in 1 × blocking buffer for 30 min, and subsequently incubated with a 1:10,000 dilution of the Anti-digoxigenin-AP, Fab fragments in 1 × blocking buffer for 30 min. The membrane was washed two times in washing buffer, equilibrated in detection buffer for 5 min, then covered with CDP-star (Applied Biosystems). CDP-star acts as the chemiluminescent substrate, where enzymatic dephosphorylation of CDP-star by the alkaline phosphatase conjugated anti-DIG produces light which was detected using the ImageQuant™ LAS 4000 (GE Healthcare Life Sciences). All buffer and solution recipes are listed in **Table 2.5**.

2.6.6 Northern blotting

Total DNaseI treated RNA was electrophoresed through 7% acrylamide, 8.3 M urea, 1 × TBE gels. 5 µg of RNA was mixed with 2 × RNA loading dye and heat denatured at 65ºC for 10 min prior to loading into gels. Gels were electrophoresed at 90 V in 1 × TBE for 1 h and 15 min. RNA-containing gels, positively charged nylon membranes (Roche), and filter paper were equilibrated in ice-cold 1 × TBE. RNA was transferred to the membrane using the Biometra Fastblot B43 semi-dry blotting apparatus at 125 mA for 30 min at 4ºC. RNA was UV-crosslinked to membrane at 120 mJ (Peqlab crosslinker). The membrane was then pre-hybridized in pre-warmed DIG Easy Hyb buffer (Roche) in a rotating oven at 62ºC for 1 h. Approximately 1.25 µg (5 µL) of riboprobe was boiled at 95ºC for 5 min, then incubated on ice for 5 min before being added to pre-hybridization buffer. Hybridization of RNA with the riboprobe of interest was incubated in a rotating oven at 62ºC overnight. Membranes were washed twice for 10 min in Stringency Wash Buffer I pre-warmed to 62ºC, then washed twice for 30 min in Stringency Wash Buffer II at room temperature on a rocker. The membranes were blocked for non-specific sites with 1 × blocking buffer at room temperature for 30 min, and subsequently incubated with a 1:10,000 dilution of the Anti-digoxigenin-AP, Fab fragments in 1 × blocking buffer for 30 min. The membrane was washed two times in washing buffer for a total of 10 min, equilibrated in detection buffer for 5 min, then covered with CDP-star (Applied Biosystems). Chemiluminescence was detected as previously described using the ImageQuant^{TM} LAS 4000 (GE Healthcare Life Sciences). To determine if RNA samples were equally loaded onto gels, membranes were re-probed as previously described with a 1:1000 diluted 5S riboprobe. All buffer and solution recipes are listed in **Table 2.5**.

2.6.7 cDNA preparation of RNA and RNA-seq

Total non-depleted RNA was sent to Vertis Biotechnologie AG (Freising Germany) for cDNA library preparation and RNA-seq.

Table 2.5 Buffers and solutions for Northern blotting

Name	*Reagent*	*Concentration*
RNA loading dye	xylene cyanol FF	0.025% (w/v)
	bromophenol blue	0.025% (w/v)
	EDTA pH 8.0	18 mM
	SDS	0.025% (w/v)
	formamide	95% (v/v)
20 × SSC	NaCl	3 M
	Sodium Citrate	0.3 M
Stringency wash I	SSC	2 ×
	SDS	0.1% (w/v)
	H_2O	
Stringency wash II	SSC	0.5 ×
	SDS	0.1% (w/v)
	H_2O	
Maleic acid buffer pH 7.5	Maleic acid	0.1 M
	NaCl	0.15 M
	*adjust pH with NaOH pellets	
Washing buffer	Maleic acid buffer	1 ×
	Tween-20	0.3% (v/v)
1 × Blocking buffer	Casein Blocking Solution (Roche)	1 ×
	Maleic acid buffer	1 ×
Detection buffer pH 9.5	Tris-HCl	0.1 M
	NaCl	0.1 M

2.7 Analysis of proteins

2.7.1 Preparation of whole cell lysates for analysis of cellular proteins

Cultures were grown as previously described. 1 OD_{600} unit of cells were harvested for PCN and LB cultures, while MMA cultures required 5 OD_{600} units. Cells were harvested by centrifugation at 4ºC for 8 min at 2,880 × g. Cell pellets were washed once in 1 mL PBS then resuspended in 20 mL nuclease free H_2O and 20 mL 2 × Laemmli buffer (0.1 M Tris-HCl pH 6.8, 6% (w/v) SDS, 20% (v/v) glycerol, 10% (v/v) β-mercaptoethanol, 0.1% (w/v) bromophenol blue). Samples were boiled for 5 min at 100ºC, then centrifuged for 3 min at 6,500 × g at 4ºC. Supernatants were moved to new 1.5 mL tubes and stored at -20ºC.

2.7.2 Preparation of culture supernatants for analysis of secreted proteins

Cultures were grown as previously described (Section 2.2.3). 10 OD_{600} units of cells were harvested for PCN and LB cultures, while MMA cultures required 50 OD_{600} units. Cells were centrifuged at 4ºC for 40 min at 2,880 × g, and supernatants were filtered through 0.2 µm PES membrane filters (Fisher Scientific) into sterile tubes. Ice-cold trichloroacetic acid (TCA) was added to a final concentration of 10% (v/v) to precipitate proteins, and proteins were harvested by centrifugation at 4ºC for 40 min at 2,880 × g. Protein pellet were washed in 1 mL ice-cold acetone, transferred to new 1.5 mL tubes and centrifuged at 4ºC for 10 min at 6,500 × g. Pellets were resuspended in 20 µL nuclease free H_2O and 20 µL 2 × Laemmli buffer. Samples were boiled at 99ºC for 5 min prior to storing at -20ºC.

2.7.3 SDS polyacrylamide gel electrophoresis (SDS-PAGE)

SDS polyacrylamide gels (SDS-PAGE) were used for size separation of denatured proteins based on molecular weight using a discontinuous gel system. Resolving gels were made to a final concentration of 10 to 12.5% from a 40% acrylamide/bis-acrylamide (37:5:1) stock, with 0.375 M Tris-Cl (pH 8.8), 0.1% (w/v) SDS, and were polymerized by 0.1% (w/v) ammonium persulfate (APS) and 0.01% (v/v) tetramethylethylenediamine (TEMED). Once poured, resolving gels were overlaid with isopropanol until polymerization was complete. Isopropanol was removed before overlaying the resolving gel with the stacking gel. Stacking gels were comprised of 5% from a 40% acrylamide/bis-acrylamide (37:5:1) stock, with

0.082 M Tris-Cl (pH 6.8), 0.1% (w/v) SDS, and were polymerized with 0.1% (w/v) APS and 0.01% (v/v) TEMED. Samples were electrophoresed alongside the PageRuler^{TM} Plus Prestained Protein Ladder (Thermo Scientific) to help monitor progress of the gel and estimate protein band sizes. Gels were run in 1 × running buffer (25 mM Tris, 0.19 M glycine, 0.1% (w/v) SDS) at 90 V until the dye front surpassed the stacking gel, then the voltage was increased to 180 V until the dye front reached the bottom of the gel. Gels were either analyzed by Coomassie staining or 108 immunoblotting.

2.7.4 Coomassie staining of polyacrylamide gels

SDS-PAGE gels were Coomassie stained using InstantBlue^{TM} (Expedeon). Gels were rinsed with dH_2O then submerged in InstantBlue^{TM} for 15 min on a rocker. Gels were then washed in dH_2O overnight and proteins were visualized using white light using the ImageQuant LAS4000 (GE).

2.7.5 Western Immunoblotting

Western immunoblotting was used to detect specific proteins from whole extracts or culture supernatants separated by size on SDS-PAGE gels. Proteins were transferred from gels to nitrocellulose membranes using a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). Gels were rinsed with dH_2O then equilibrated in 1 × transfer buffer (25 mM Tris, 0.19 M glycine). Transfer "sandwiches" were assembled in gel holder cassettes in a dish containing 1 × transfer buffer as follows: a fiber pad, 3 pieces of 3 mm filter paper (Whatman), the polyacrylamide gel, a 0.45 µm PROTRAN nitrocellulose membrane (Whatman), and 3 additional pieces of 3 mm filter paper and fiber pad. Cassettes were loaded into transfer tanks with membranes towards the anode, filled with 1 × transfer buffer and an ice-pack. Proteins were transferred at 300 mA for 90 min on ice.

To verify that proteins had efficiently transferred, membranes were briefly incubated in Ponceau S solution (Sigma) until bands were visible. Membranes were imaged under white light using the ImageQuant LAS4000 (GE), then rinsed with water until staining was no longer visible.

To reduce non-specific protein binding, membranes were blocked for 90 min at room temperature in blocking buffer (5% skimmed milk powder, PBS, 0.05% Tween-20). The

primary antibodies, monoclonal anti-FLAG M2 (Sigma) and monoclonal anti-DnaK (*E. coli*, Enzo Life Sciences) derived from mice, were diluted in blocking buffer 1:10,000 and 1:30,000 respectively, and incubated with membranes at 4ºC overnight on a rocker. DnaK served as a loading control for whole cell extracts and as a cell lysis control for supernatants. DnaK was used as a control for protein levels as a cytosolic control for Western blotting. This allowed us to determine if general protein levels were expressed evenly between different samples. Membranes were washed in PBST (PBS, 0.05% Tween-20) four times for a total of 40 min before incubation with the secondary antibody, anti-mouse IgG horseradish peroxidase (HRP, Santa Cruz Biotechnology), diluted 1:6,000 in blocking buffer for 90 min at room temperature on a rocker. Membranes were washed an additional 3 times in PBST for a total of 30 min and once in PBS for 15 min. ECL chemiluminescent HRP substrate (Pierce) was used to detect chemiluminescent signal. A 1:1 ratio of luminol and peroxide were mixed and used to flood the membrane. Membranes were sealed in plastic and incubated in the dark for 5 min. Light emission occurs as a by-product of the oxidation of luminol by HRP and was detected using the ImageQuant LAS4000 (GE).

2.7.6 Proteomic analysis by mass spectrometry

Protein sample preparation was done by Dr. Nicole Hansmeier and all subsequent processing and analysis as described here were completed by Dr. Tzu-Chiao Chao at the University of Regina, Regina, Saskatchewan, Canada. Cultures were grown in MMA as previously described. Protein isolation was conducted according to the manufacturers' instructions for TRIzol protein extraction (Life Technologies) with minor modifications. Approximately 10^{10} bacterial cells were harvested and washed 4 × with PBS, the resulting cell pellet was resuspended in 750 µL of TRIzol and incubated for 5 min on ice. Subsequently, 200 µL chloroform was added and incubated for 3 min on ice. The samples were centrifuged for 15 min at 12,000 × g at 4°C. The phenolic phase was transferred into a new tube and the proteins were precipitated with isopropanol. The protein pellet was collected by centrifugation at 12,000 × g at 4°C and washed twice with 70% ethanol, followed by 100% ethanol incubation for 20 min before air drying. Protein pellets were resolubilized in 50 mM ammonium bicarbonate buffer containing 0.1% SDS. The resulting protein samples were reduced and alkylated with 5 mM DTT and 20 mM iodoacetamide (45 min incubation at RT each) and rebuffered into 50 mM ammonium bicarbonate buffer on filter columns (MWCO 10 kDa, Amicon, Millipore). Protein concentration of the resulting protein solution was determined by Pierce BCA protein assay kit (Thermo Fisher Scientific) and 50 µg protein was

subsequently digested with trypsin gold (protein/enzyme ratio 50:1) according to the manufacturers' instructions (Promega). Digested proteins were acidified with 0.1% formic acid and dried. Dried digested samples were resuspended in running solution (0.1% formic acid, 3% acetonitrile) and centrifuged at 12,000 × g for 10 min to remove insoluble material. The supernatant (0.5 µg/µl protein) was spiked with Phos B standard (Waters) for quantitation before LC-MS analyses. Each analysis injection contained 40 fmol/µl of Hi3 Ecoli standard (Waters).

Each sample was analyzed on a NanoAcquity system (Waters) coupled to a Synapt G2 HDMS (Waters). 0.5 µg spiked digests were loaded onto a NanoAcquity UPLC Symmetry C18 trap column (180 µm × 20 mm, dp: 5 µm, Waters) to desalt and chromatographically focus peptides for 3 min (5 µL/min flow rate) prior to elution onto an Acquity UPLC M-class HSS T3 analytical column (75 µm × 200 mm, dp:1.8 µm, Waters). The separation was conducted with a 120 min gradient from 3% acetonitrile/0.1% formic acid to 45% acetonitrile/0.1% formic acid at a flow rate of 0.35 µL/min. The eluted peptides were analyzed in a Synapt G2 HDMS QTof (Waters) in positive MS^E resolution mode with a 1 s scan time. In low energy MS mode, data were collected at constant collision energy of 4 eV. High energy collision energy was ramped between 18 and 42 V. Leucine enkephaline was measured as lock mass every 30 s to maintain mass accuracy throughout the run.

The resulting raw spectral data were processed with the ProteinLynx Global Server (PLGS) v. 3.02 with Identity (Waters). Data were extracted and searched against a protein sequence database built with the UniProt *Salmonella enterica* sv. Typhimurium strain LT2 reference proteome (accessed January 2016), appended with the sequences of the Hi3 Ecoli standard (Waters) for quantitation. The following settings were used as search parameters: mass tolerance of 8 ppm, trypsin specificity, 1 missed cleavage; stable modification carbamidomethyl(C); variable modification methionine oxidation, false discovery rate: 4%.

2.8 Chromatin Immunoprecipitation

2.8.1 Preparation of cross-linked lysates

Chromatin immunoprecipitation (ChIP) was carried out as previously described (Dillon *et al.*, 2010) with some modifications. All ChIP buffer recipes are listed in **Table 2.6**. Cultures of *fnr*::3×FLAG were grown in MMA as previously described. 40 OD units were harvested

by room temperature centrifugation at 2,880 × g for 8 min. Cells were resuspended in 50 mL of 37ºC PBS. Crosslinking of protein-DNA complexes was done by slowly adding molecular grade formaldehyde (Sigma) to a final concentration of 1% with gentle mixing on a Belly Dancer® for 30 min. Cross-linking was stopped by addition of ice-cold glycine to a final concentration of 0.125 M, and incubation for 5 min with gentle mixing. Cross-linked cells were centrifuged at 4ºC, at 2,880 × g for 8 min. Cell pellets were resuspended in 600 µL of lysis buffer and incubated on ice for 30 min. Cells were further diluted in 1.4 mL of dilution buffer before sonication. Chromatin was sonicated to achieve an average fragment length of 500 bp using the MSE Soniprep sonicator (Sanyo). Sonication was performed in 10 to 15, 30 s bursts at an amplitude of 10 µM with 1 min of incubation on ice between bursts. A sample of sonicated chromatin was verified for sonication efficiency by agarose gel electrophoresis as previously described (Section 2.3.4). Cellular debris was removed by centrifugation of samples at 4ºC, at 8,000 × g for 10 min. Supernatants containing sheared chromatin were added to 1 mL of dilution buffer and stored at -80ºC.

2.8.2 Immunoprecipitation

To eliminate non-specific binding of the antibody, chromatin was pre-cleared using 50 µg of normal rabbit IgG (Santa Cruz Biotechnology). Chromatin was incubated at 4ºC on a rotating wheel for 1 h, followed by addition of 100 µL of homogeneous protein G-agarose bead suspension (Roche), and incubated as before for an additional 3 h. Beads were pelleted by centrifugation at 4ºC, at 2,880 × g for 4 min. Chromatin-containing supernatants were carefully transferred to new 2 mL tubes to avoid disruption of beads. The “Input” sample, 200 µL of pre-cleared chromatin, was stored at -20ºC for later analysis. The remaining pre-cleared chromatin was split into 1350 µL aliquots for Immunoprecipitation (IP) reactions. The “Mock” IP reaction was carried out using 10 µg of normal mouse IgG (Santa Cruz Biotechnology). Mock IP reactions were used to measure the background levels of DNA binding. The experimental IP reaction, referred to hereafter as “FLAG,” was carried out using 10 µg of monoclonal mouse anti-FLAG M2 antibody (Sigma). Both IP reactions were incubated overnight at 4ºC on a rotating wheel, followed by addition of 50 µL of homogeneous protein G-agarose bead suspension and a further 3 h of incubation.

2.8.3 Washing the Protein-G agarose beads and elution of DNA

The protein-G agarose beads were bound to antibody-protein-DNA complexes and were carefully washed in several steps. First, beads were pelleted by centrifugation at 6,000 × g at 4ºC for 2 min, beads were incubated on ice for 1 min before discarding supernatants. For each wash, 750 µL of the appropriate pre-chilled wash buffer was added, followed by vortexing and centrifugation at 6,000 × g at 4ºC for 2 min and a 1 min incubation on ice. Beads were washed in Wash Buffer I, transferred to new 1.5 mL tubes and washed again in Wash Buffer I. Beads were then washed once in Wash Buffer II and twice in TE Buffer. Finally, beads were resuspended in 225 µL of room temperature Elution Buffer, vortexed and pelleted to elute antibody-protein-DNA complexes from the beads. The elution step was performed twice, and both eluates were combined into a new 1.5 mL tube.

2.8.4 Reversal of cross-links and DNA extraction

To reverse crosslinking, Input, Mock and FLAG samples were treated with 5ng/µL RNase A (Sigma) and 0.3 M NaCl and incubated at 65ºC for a minimum of 6 h. Samples were then treated with 9 µg of Proteinase K and incubated at 45ºC for a minimum of 3 h.

DNA extraction of all samples was accomplished using standard phenol-chloroform extraction followed by ethanol precipitation. Input samples were co-precipitated with glycogen, and both Mock and FLAG samples were co-precipitated with yeast tRNA and glycogen. Input DNA was ultimately resuspended in 100 µL nuclease free H_2O and Mock and FLAG DNA was resuspended in 50 µL nuclease free H_2O at 37ºC and 900 rpm on a ThermoMixer® (Eppendorf) for 1 h.

Input and IP DNA were diluted 1:50 and 1:5 respectively for RT-qPCR analysis as previously described (section 2.6.4). The quantity of immunoprecipitated DNA is relative to specific protein binding of that region and was calculated as a fraction of the starting amount of DNA (Input). The mock IP DNA was subtracted from the experimental IP DNA and compared to a control region which was negative for specific transcription factor binding according to the following formula:

$$\frac{Experimental\ IP\ of\ promoter\ X}{Experimental\ Input\ of\ promoter\ X} - \frac{Mock\ IP\ of\ promoter\ X}{Mock\ Input\ of\ promoter\ X}$$

Table 2.6 Buffers for ChIP

Name	Reagent	Concentration
Lysis Buffer	Tris-HCl, pH 8.1	50 mM
	EDTA	10 mM
	SDS	1% (w/v)
	Protease inhibitor tablet stock	1 ×
Dilution Buffer	Tris-HCl, pH 8.1	20 mM
	NaCl	150 mM
	EDTA	2 mM
	Triton X-100	1% (v/v)
	SDS	0.01% (w/v)
IP Wash Buffer I	Tris-HCl, pH 8.1	20 mM
	NaCl	50 mM
	EDTA	2 mM
	Triton X-100	1% (v/v)
	SDS	0.1% (w/v)
IP Wash Buffer II	Tris-HCl, pH 8.1	10 mM
	LiCl	250 mM
	EDTA	1 mM
	NP-40	1% (v/v)
	Deoxycholic acid	1% (w/v)
TE Buffer, pH 8.0	Tris	10 mM
	EDTA	1 mM
	*adjust pH with HCl	
Elution Buffer	$NaHCO_3$	100 mM
	SDS	1% (w/v)

2.9 DNA sequencing

2.9.1 Preparation of DNA libraries

DNA was quantified using the Qubit™ dsDNA HS Assay Kit and a Qubit™ Fluorometer (Thermo Scientific). Sequencing libraries were constructed using the NEBNext® Ultra™ II DNA Library Prep Kit (New England Biolabs) according to the manufacturers' specifications. Libraries were prepared without additional DNA fragmentation as DNA is already fragmented through the chromatin immunoprecipitation protocol and the 400 – 500 bp size selection step was used. TruSeq® DNA Adaptors (Illumina) were ligated to DNA fragments using 6 amplification cycles for Input samples, and 10 cycles for Mock and FLAG samples according to the manufacturers' specifications. Samples verified for quality using the 2100 Bioanalyzer and High Sensitivity DNA Kit (Agilent).

2.9.2 Sequencing with Illumina MiSeq

DNA libraries were prepared for sequencing with the MiSeq Reagent Kit v3 (Illumina) and run on a MiSeq System (Illumina) according to the manufacturers' specifications. The first libraries and sequencing runs were constructed and performed by Stefani Kary, with additional replicates by Dr. Keith MacKenzie at the University of Regina, Regina, Saskatchewan, Canada.

2.10 Bioinformatic analysis

2.10.1 Mapping of RNA-seq data and differential expression analysis

Data from RNA-seq was analyzed by Karsten Hokampf and Carsten Kröger at Trinity College Dublin, Dublin, Ireland. READemption (Forstner *et al.*, 2014) was used for computational evaluation of the RNA-seq data. Reads obtained from RNA-seq experiments were mapped to the 4/74 reference genome using the Segemehl mapping software (Hoffmann *et al.*, 2009). Reads that do not map in a single chromosomal location (uniquely mapped reads) were truncated from the 3′ end in stepwise manner by removing one nucleotide at a time until the read is mapped uniquely or until the read length reaches 20 nucleotides. Remaining reads were discarded. Data were normalised using the transcripts per million (TPM) method (Wagner *et al.*, 2012). This method of measuring transcript

abundance from high throughput sequencing data is closely-related to the widely used reads per kilobase per million method (RPKM) (Mortazavi *et al.*, 2008), but removes the bias, of normalisation to the total number of reads mapped in each sequencing run, that the RPKM method introduces. Instead the TPM method measures transcript abundance by calculating the number of transcripts of a particular gene by dividing the number of nucleotides mapped to that gene by the length of the gene (t_g). All of these numbers are summed to get the total number of transcripts represented by all the mapped reads (T). The transcript abundance for each gene is then calculated as number of transcripts per million transcripts according to the following formula:

$$TPM = \frac{t_g}{T} 10^6$$

A TPM value of 2 is the suggested cut-off value for determining if a gene is expressed or not (Wagner *et al.*, 2013), however a more conservative cut-off of TPM = 10 was chosen for this study based on TPM values of indicator genes which were previously shown not to be expressed under a particular condition, as previously described (Kröger *et al.*, 2013). Differential expression of genes between WT and Δ*fnr* was calculated from TPM values.

2.10.2 ChIP-seq analysis

Data from ChIP-seq was analyzed by Aalap Mogre and myself, at Trinity College Dublin, Dublin, Ireland. Qualities of sequenced reads were assessed using FastQC (Andrews, 2010). Reads were mapped to the *S. enterica* subsp. *enterica* serovar Typhimurium str. 4/74 chromosome and plasmids combined reference sequences (chromsome: NC_016857.1, TY474p1: NC_016858.1, TY474p2: NC_017675.1, TY474p3: NC_016859.1) using the Burrows-Wheeler Aligner (Li & Durbin, 2009). SAMtools was used to sort aligned reads by the reference sequence and remove unmapped and low mapping quality reads (mapping quality < 30) (Li *et al.*, 2009). Some samples had large numbers of unmapped reads that were found to map to the *S. cerevisiae* genome. Using qPCR with primers specific to *S. cerevisiae* we identified that these came from yeast gDNA contamination in the yeast tRNA used as a co-precipitant intended to increase DNA yields in the ChIP protocol. Since this contaminant gDNA was introduced in the final stages of the experiment, they do not affect the outcome of the ChIP experiment apart from reducing the coverage of the sample. We decided to not remove duplicate reads as the coverage of some of the samples was low and

peak calling was less effective after removal of duplicate reads. Model-based analysis of ChIP-Seq 2 (MACS2) (Zhang *et al.*, 2008) was used to call FNR peaks from the sorted BAM files using the mock as control. Due to differing fragment sizes among the replicates, different mock replicates were used as control for different sample replicates: mock replicate 1 was used as control for ChIP replicate 1 (fragment size ~250 nt), mock replicate 3 was used as control for ChIP replicates 2 and 3 (fragment sizes ~300 nt). Peak lists thus generated by MACS2 were roughly annotated with the names of neighbouring genes and sRNAs using a R script (R Core Team, 2013) and are available as both Excel and .gff files. Overlaps of peaks between different samples were analysed using DiffBind (Stark & Brown, 2011). Most peaks in replicate 1 overlapped with peaks in replicate 2. This list of high confidence peaks annotated roughly using a R script are available as both Excel and .gff files. Sequences of peaks from this list were used to generate the FNR motif using MEME-ChIP (Machanick & Bailey, 2011). This resulting motif was used to find all possible motifs in the genome, bound or not according to our experiment, using Find Individual Motif Occurrences (FIMO) (Grant *et al.*, 2011). deepTools was used to create bigWig (.bw) files, for display of dense, continuous data, from sorted BAM (binary SAM files) files containing coverage information scaled to the library size and multiplied by a factor (1×10^6) for each sample that could be viewed using Integrated Genome Browser (IGB) (Nicol *et al.*, 2009) along with the .gff peak lists. All scripts used in the analysis can be accessed from GitLab (https://gitlab.com/aalap.mogre/fnr-chip-seq).

Table 2.7 Table of programs and online tools for bioinformatic analysis in this study

Name of Program/Tool	*Description*	*Reference or URL*
BLAST	Basic Local Alignment Search Tool, for local sequence alignment and identification of regions of sequence similarity.	(Altschul *et al.*, 1990)
Benchling	Online tool for *in silico* manipulation of DNA sequences.	http://benchling.com
BWA	Burrows-Wheeler Aligner, a software package for mapping low-divergent sequences against a large reference genome.	(Li & Durbin, 2009)
Cytoscape	An open source software platform for visualizing molecular interaction networks and biological pathways and integrating these networks with annotations, gene expression profiles and other state data.	www.cytoscape.org
deepTools	A suite of python tools particularly developed for the efficient analysis of high-throughput sequencing data, such as ChIP-seq.	(Ramírez *et al.*, 2016)
DiffBind	For differential binding analysis of ChIP-seq peak data.	(Stark & Brown, 2011)
FastQC	A quality control tool for high throughout sequence data.	(Andrews, 2010)
FIMO	Find Individual Motif Occurrences, for scanning for occurrences of a given motif.	(Grant *et al.*, 2011)
GenBank	The NIH genetic sequence database, an annotated collection of all publicly available DNA sequences.	(Benson *et al.*, 2012)
GrowthRates	For calculating the best-fit growth rates, the lag times and the maximum OD from growth curves.	(Hall *et al.*, 2014)
ImageJ	Densitometry analysis of EMSA, Northern blot, and Western blot signals.	(Schneider *et al.*, 2012)

Table 2.7 Table of programs and online tools for bioinformatic analysis in this study (continued)

Name of Program/Tool	*Description*	*Reference or URL*
Inkscape	An open source professional vector graphics editor.	www.inkscape.org
IGB	Integrated Genome Browser, for visualization of RNA-seq and ChIP-seq reads, and other genomic features.	(Nicol *et al.*, 2009)
JBrowse	Visualisation of RNA-seq reads and other genome features.	(Skinner *et al.*, 2009)
Ligation Calculator	For calculation of amount of required insert DNA for a given molar ratio for ligation reactions.	http://www.insilico.uni-duesseldorf.de/Lig_Input.html
MEME-ChIP	Multiple Em for Motif Elicitation, for ChIP a motif analysis of large DNA datasets.	(Machanick & Bailey, 2011)
MACS2	Model-based analysis of ChIP-seq 2, for improved spatial resolution of predicted binding sites of ChIP-seq data.	(Zhang *et al.*, 2008)
Multiple Primer Analyzer	For analyzing and comparing multiple primer sequences simultaneously.	ThermoFisher
NEB Tm Calculator	For estimating the optimal annealing temperature for PCR.	https://tmcalculator.neb.com/
Prism GraphPad v6.0h	Statistical analysis and graphing software.	https://www.graphpad.com/scientific-software/prism/
R	A language and environment for statistical computing.	(R Core Team, 2013)
READemption	An RNA-Seq analysis pipeline.	(Forstner *et al.*, 2014)

Table 2.7 Table of programs and online tools for bioinformatic analysis in this study (continued)

Name of Program/Tool	***Description***	***Reference or URL***
SalCom	*S.* Typhimurium mRNA and sRNA expression profiles based on RNA-seq data from 20 environmental conditions.	(Kröger *et al.*, 2013)
SalComMac	*S.* Typhimurium mRNA and sRNA expression profiles based on RNA-seq data within murine macrophages.	(Srikumar *et al.*, 2015)
SalComRegulon	*S.* Typhimurium mRNA and sRNA expression profiles based on RNA-seq data from 18 regulatory systems.	(Colgan *et al*., 2016)
Segemehl	For mapping sequencer reads to a reference genome.	(Hoffmann *et al.*, 2009)
SAMTools	Sequence Alignment/Map format and tool for implementing various utilities for post-processing alignments in the SAM format, such as indexing, variant caller and alignment viewer.	(Li *et al.*, 2009)
Venny v2.1	An interactive tool for comparing lists with Venn's diagrams	http://bioinfogp.cnb.csic.es/tools/venny/index.html

2.11 DNA binding analysis

2.11.1 Large scale induction of protein production

Overnight cultures were set up in 50 mL of LB with kanamycin of PK22 cells with the p(*fnrD154A*)$_2$ plasmid. Strains were subcultured 1:50 in 1 L LB with kanamycin in a 2 L baffled flask, and grown at 37ºC, 200 rpm to between OD_{600} 0.4-1.0. Cultures were induced with 1 mM IPTG and grown at 16ºC, 200 rpm overnight. Cells were harvested by centrifugation at 8,000 × g for 10 min at 4ºC. Pellets were transferred to 50 mL falcon tubes with an EDTA-free protease inhibitor tablet and stored at -20ºC.

2.11.2 6×His-Tag protein purification

Bacterial pellets were thawed in an ice-water slurry, resuspended in ice-cold PBS, and lysed using a French pressure cell press at 1000 psi. Lysates were centrifuged at 15,000 × g for 20 min at 4ºC. DNase was added to a final concentration of 5 µg/mL gently mixed and lysates were filtered through a 0.2 µm PES membrane filter. A 5 mL HiTrap affinity column was used with a peristaltic pump. The column was washed with dH_2O, followed by 3 to 5 column volumes of Nickel Buffer A (4 mM Tris, 0.1 M NaCl, pH 7.9). The filtered lysate was then loaded onto the column and collected for downstream analysis as the "Unbound" fraction. Nickel Buffer B (4 mM Tris, 0.1 M NaCl, 0.2 M Imidazole, pH >7.9) was diluted with Nickel Buffer A using a gradient of concentrations from 10% to 100%. The column was then washed with 10 column volumes of 10% Nickel Buffer B in Nickel Buffer A to remove further unbound protein and collected as the "Wash" fraction for downstream analysis. The protein was then eluted from the column using the gradient of Nickel Buffer B concentrations collecting each fraction. The imidazole in Nickel Buffer B competes with the 6×His-tagged (FNRD154A)$_2$ protein for binding to the metal-charged resin in the column, thus eluting the recombinant protein. The fractions were analyzed by SDS-PAGE and Coomassie staining as described previously. Fractions containing the (FNRD154A)$_2$ protein were pooled and dialyzed against PBS at 4ºC overnight. Proteins were then concentrated using an Amicon® Ultra-15 Centrifugal Filter (Sigma). Protein concentration was determined using a Pierce™ BCA Protein Assay Kit (Thermo Scientific) according to the manufacturers' specifications.

2.11.3 Electrophoretic mobility shift assay

Electrophoretic mobility shift assays (EMSA) were used to investigate *in vitro* binding of FNR at promoters of interest. Varying concentrations of $(FNRD154A)_2$ were combined with 0.5 pmol of probe DNA (**Table 2.7**) in EMSA Binding Buffer (20 mM Tris, 50 mM NaCl, 10 mM EDTA, 4 mM DTT, 5% (v/v) glycerol, 0.5 mg/mL BSA, 1 µg/mL poly-d[I-C], pH 7.2) in a 15 µL reaction volume, and incubated at 37ºC for 1 h. Binding reactions were electrophoresed using 7% acrylamide, 2% glycerol, 1 × TBE gels in 1 × TBE at 150 V for 75 min on ice and at 4ºC. Gels were stained with SafeView Nucleic Acid Stain (NBS Biologicals) prior to visualization under ultra violet light in the ImageQuant™ LAS 4000 (GE Healthcare Life Sciences). The molecular size of electrophoresed DNA fragments was estimated by comparison with O'GeneRuler™ 1 kb DNA Ladder (Thermo Scientific). Band shifts were quantified using ImageJ and the bound ratio was calculated using the following formula:

$$B_R = \frac{D_b}{(D_b + D_u)}$$

Where B_R is the bound ratio, D is the band intensity of DNA in pixels, and the subscripts b and u represent the bound (DNA bound by protein) and unbound (DNA only) bands. Equilibrium DNA binding curves were fitted by nonlinear regression and the dissociation constant and maximum bound ratio, K_D and B_{max}, respectively, were determined by one site specific binding with Hill slope analysis.

2.12 Atomic force microscopy imaging

For Atomic force microscopy (AFM) analysis, 20 mL of bacterial culture grown in LB as previously described, were spotted onto coverslips (Hartenstein) and incubated at room temperature for 1 h before rinsed with ultrapure water and subsequently mounted onto glass microscope slides for immediate AFM imaging. Measurements were taken using the NanoWizard II AFM system (JPK Instruments AG). To visualize the sample, the AFM was driven in soft contact mode using silicon nitride AFM probes with a nominal force constant of 0.06 N/m (SiNi, Budget Sensors). Scan rates were set to 1 Hz and images were acquired with a resolution of 512 × 512 pixel. For each sample, topographic overview images were taken before magnification was performed. Representative images are XY tilt corrected,

polynomial fitted, and unsharpened mask filtered to remove noise using JPK data processing software (JPK Instruments AG). All images are displayed in false-color. Atomic force microscopy imaging was completed by Nicole Hansmeier at Universität Osnabrück, Osnabrück, Germany.

2.13 Infection of murine macrophages

RAW 264.7 murine macrophages were grown in Dulbecco's Modified Eagle Medium (DMEM, Sigma) with fetal bovine serum (FBS, Sigma) and seeded into 24 well plates at a concentration of 4 x 10^5 cells per well. Macrophages were inoculated with *S.* Typhimurium strains that had been grown overnight in LB or MMA, at a multiplicity of infection (MOI) of 10:1 in DMEM. Macrophages were left to phagocytose bacterial cells for 30 min at 37°C in 5% CO_2 before treatment with 100 µg/mL gentamycin (Sigma) for 1 h at 37°C in 5% CO_2. The macrophages were washed with sterile PBS, lysed with ice-cold nuclease free water, and plated at appropriate dilutions on LA. In the remaining wells, media was replaced with DMEM with FBS and 10 µg/mL gentamycin and incubated at 37°C in 5% CO_2 for the remainder of the infection. Cells were routinely sampled at 1 h, 4 h, 8 h, and 16 h post-infection.

Chapter 3

Transcriptional regulation in Δ*fnr*

3.1 Introduction

3.1.1 Overview of the Study

To establish and maintain a successful infection in the host, *Salmonella* must integrate a multitude of environmental inputs by co-ordinating regulatory proteins in a complex transcriptional network (Osborne *et al.*, 2009; Yoon *et al.*, 2009). *S.* Typhimurium host cell invasion, survival and proliferation inside of host cells has been thoroughly studied (Ellermeier & Slauch, 2007; Fass & Groisman, 2009; Haraga *et al.*, 2008) however, priming of cells prior to invasion is relatively underexplored (Brown *et al.*, 2005; Osborne & Coombes, 2011). Anaerobic and microaerobic growth of *Salmonella* has long been known to increase adherence and invasion of mammalian cells (Lee & Falkow, 1990; Schiemann & Shope, 1991). More recently, the regulator of anaerobic metabolism, FNR has been shown to be important for priming cells prior to host cell invasion as a regulator of virulence in response to changing oxygen concentrations in other species including *E. coli* and *Shigella flexneri* (Crofts *et al.*, 2018; Marteyn *et al.*, 2010) and has been shown to activate SPI-1 and motility/chemotaxis genes in *S.* Typhimurium (Fink *et al.*, 2007). Fink et al. also showed that FNR was necessary for full virulence in murine macrophages but were unable to link FNR and SPI-2. With the observation that low-level SPI-2 expression occurs at the intestinal epithelial border prior to invasion (Brown *et al.*, 2005; Osborne & Coombes, 2011) and that an oxygen gradient is present in the same location (Marteyn *et al.*, 2010), it follows that a transcription factor (TF) that responds directly to changes in oxygen, FNR, would be the prime candidate as a regulator of SPI-2 under these conditions. To our knowledge, there have been no large-scale investigations identifying the involvement of FNR in controlling SPI-2 expression in *S.* Typhimurium.

The aim of this chapter is to investigate the global changes in gene expression in an Δ*fnr* mutant with a focus on *Salmonella* Pathogenicity Island 2, flagella and motility in *S.* Typhimurium. We have used RNA-seq to investigate the FNR regulon under microaerobic growth in MMA, a minimal medium with a glycerol carbon source and the alternative electron acceptors fumarate and TMAO and verified these results with additional molecular techniques. A general workflow of the main experimental procedure is outlined in **Figure 3.1**.

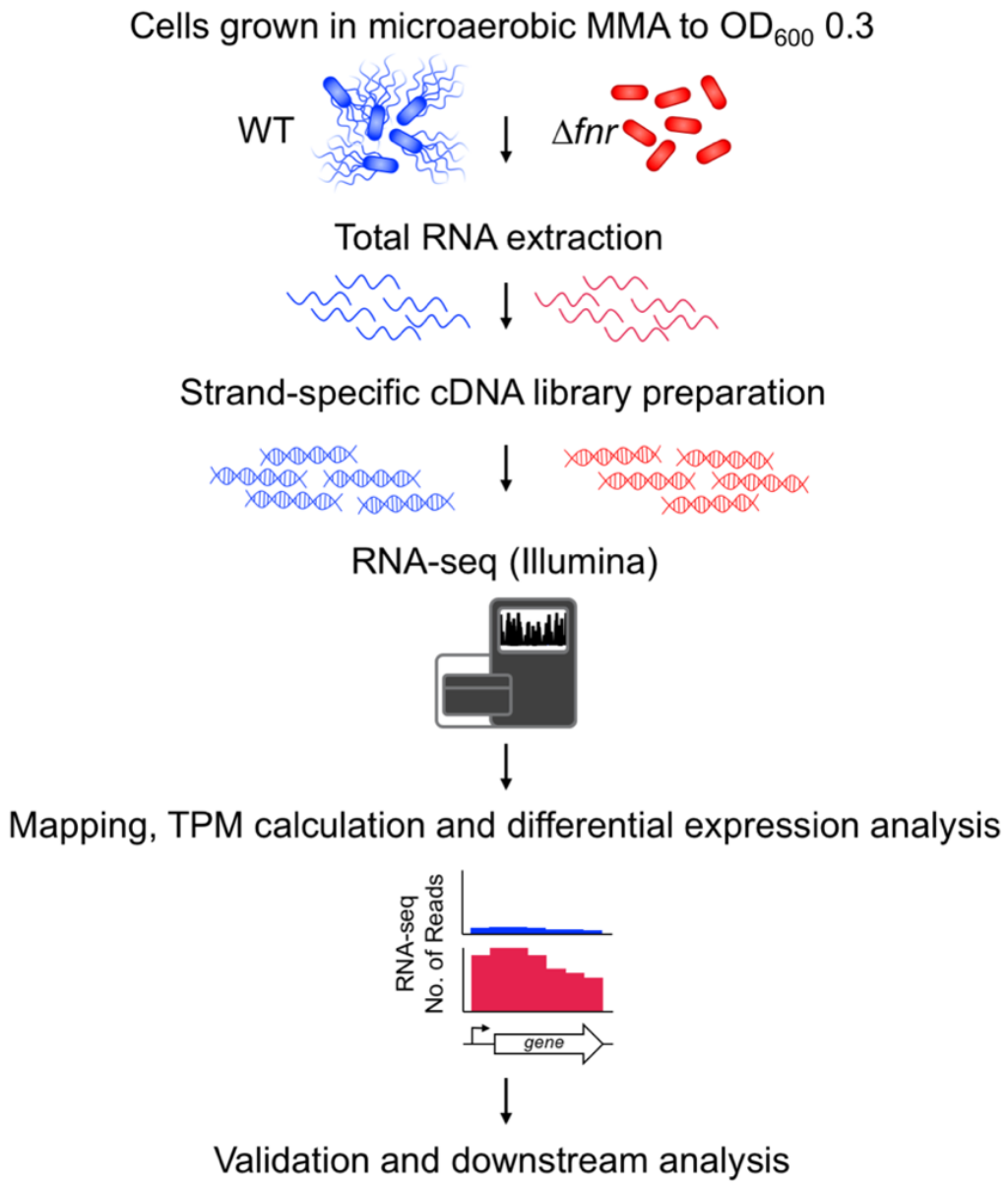


Figure 3.1 Workflow of general experimental procedures for investigating the regulation of SPI-2.

Wild-type (WT) and Δ*fnr* cells were grown under microaerobic conditions to OD_{600} 0.3 in MMA and total RNA was extracted by Dr. Aoife Colgan for replicate 1 and by me for replicate 2. cDNA library preparation and sequencing were performed by Vertis Biotechnologie AG. Mapping of sequence reads to the reference 4/74 genome and calculation of TPM values were performed by Dr. Karsten Hokamp and Dr. Carsten Kröger (Trinity College Dublin). Differential gene expression analysis, validation and downstream analyses were performed by me.

3.1.2 Bacterial transcriptome analysis using RNA-seq

RNA sequencing uses next-generation sequencing (NGS) to reveal the presence and quantity of RNA in a given condition using high-throughput sequencing of cDNA libraries. Although direct sequencing of yeast RNA on an array of nanopores has recently been demonstrated (Garalde *et al.*, 2018), cDNA sequencing or RNA-seq has been shown to generate highly reproducible and reliable data for identifying and quantifying which genes are being actively transcribed within *Salmonella* on a global scale (Colgan *et al*., 2016; Kröger *et al.*, 2012; 2013). This technology provides a highly sensitive and dynamic method of transcriptome profiling at single nucleotide resolution (Ozsolak & Milos, 2010). The cDNA libraries used for RNA-seq in this study were generated from total RNA samples by Vertis Biotechnologie AG, Freising, Germany and RNA-seq was performed using an Illumina Hiseq 2000 platform. No methods of RNA depletion were used in this study, but analysis was limited to the sequencing reads that mapped to a single location on the chromosome (uniquely mapped reads) and reads mapping to many rRNA genes or other paralogous genes were removed. A systematic analysis of the effects of sequencing depth on discovery of rare transcripts demonstrated that sequencing of 5-10 million reads of non-rRNA fragments provides sufficient coverage of a bacterial transcriptome and is adequate to detect most expressed genes (Haas *et al.*, 2012). RNA-seq analysis pipelines and the use of TPM values to quantify gene expression (Section 2.10.1) allowed identification of differentially expressed genes in Δ*fnr* (Forstner *et al.*, 2014; Wagner *et al.*, 2012).

The use of RNA-seq to investigate the transcriptome of Δ*fnr* is helping to expand our knowledge of the FNR regulon and how this regulator of anaerobic metabolism can also play a role in regulation of *Salmonella* virulence-associated genes. Absolute expression values (in TPM) for every chromosomal *S.* Typhimurium 4/74 gene in Δ*fnr* and wild-type comparator strain, sequenced in this study, are available in **Table S1** in Appendix I.

3.2 Results

3.2.1 Strategy for experimental design

Mutants in *fnr* are either unable to grow anaerobically or grow far slower than the parental strain in the presence of nonfermentable carbon sources and most terminal electron acceptors (Lambden & Guest, 1976). This presents a problem when comparing the Δ*fnr* mutant to the WT strain, as differences are due to direct effects of FNR and of growth rate. Past studies have used glucose as a carbon source (Kang *et al.*, 2005; Salmon *et al.*, 2003), however glucose represses expression from some FNR-activated genes due to catabolite repression, (Browning *et al.*, 2005; Lambden & Guest, 1976) and replacement of glucose by a less repressing fermentable carbohydrate would decrease these effects but to an unknown extent. To alleviate these problems Fink *et al*. used MOPS buffered LB with xylose (Fink *et al.*, 2007). LB is known to induce SPI-1 expression from late exponential phase (OD_{600} 1.0, LEP) to early stationary phase (OD_{600} 2.0, ESP) and by oxygen shock (Kröger *et al.*, 2013), but it can be problematic in physiological studies due to intrinsic differences in complex components (Sridhar & Steele-Mortimer, 2016), and xylose is a fermentable carbon source for *Salmonella* species (Rosenberg, 1980). Constantinidou *et al*. found that using MMA with fumarate and TMAO as alternative electron acceptors and glycerol as a non-fermentable non-repressing carbon source allowed WT and an Δ*fnr* mutant in *E. coli* to have similar growth rates (Constantinidou *et al.*, 2006). We chose to use the same medium and growth conditions, MMA and static growth, as Constantinidou *et al*. but, found that in *S.* Typhimurium, growth rates of the WT and an isogenic Δ*fnr* mutant were not the same (**Figure 3.2A & B**). The Δ*fnr* mutant grew significantly slower than the WT and a Δ*ssrAB* mutant. The growth rate of a mutant missing the *ssrAB* and *fnr* ORFs, Δ*ssrAB/fnr*, was not significantly different than WT. In a Δ*ssrAB* mutant, all SPI-2 expression is turned off (Colgan *et al*., 2016; Xu & Hensel, 2010) thus, the difference in growth between the WT and Δ*fnr* could be due to expression of SPI-2 genes. These differences in growth and fitness will be explored further in Chapter 5. Unless otherwise indicated, samples for all subsequent experiments were taken when cultures reached an OD_{600} of 0.3. A Northern blot was used to show that our microaerobic growth condition was sufficiently oxygen limited for active FNR protein to be present. WT and Δ*fnr* cultures were grown in MMA under aerobic (aerated flasks) and microaerobic (static flasks) conditions; the FNR-dependent sRNA *FnrS* (Durand & Storz, 2010) was induced in the WT under microaerobic growth, but not aerated conditions or in Δ*fnr* (**Figure 3.2 C**).

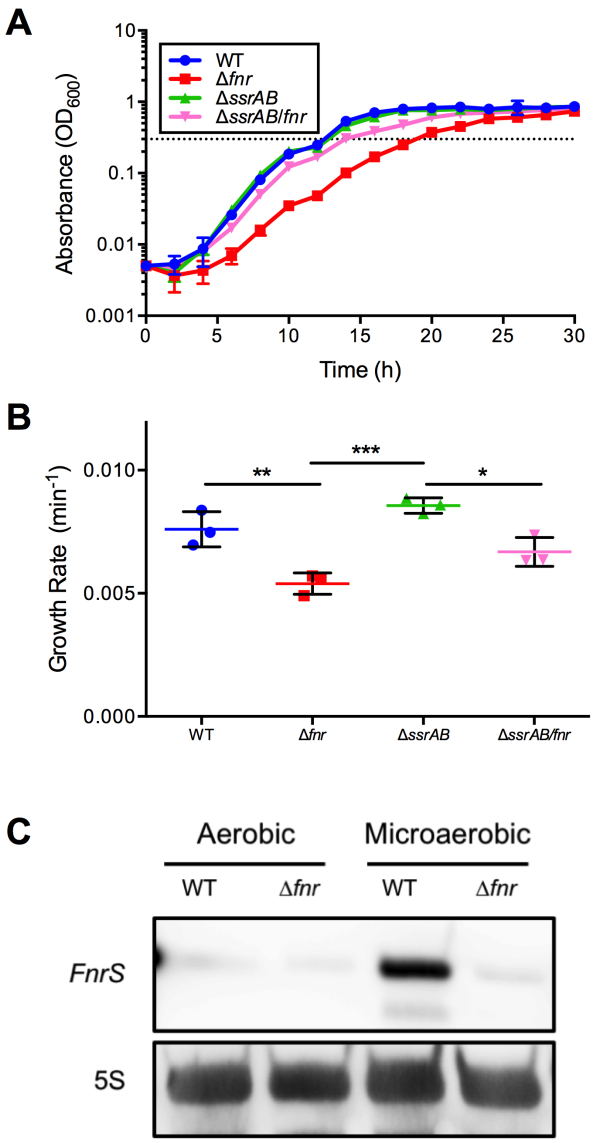


Figure 3.2 Growth of *4/74* and isogenic mutants and expression of *FnrS* under microaerobic conditions in MMA.

A. Growth curve of WT, Δ*fnr*, Δ*ssrAB* and Δ*ssrAB*/*fnr*. Strains were grown and sampled as previously described (Section 2.2.3.2). The dotted line indicates OD_{600} 0.3, where samples were taken for all subsequent experiments. B. Best-fit growth rate determined from growth curves in A. Statistical significance was determined by an Ordinary One-Way ANOVA with Tukey's multiple comparisons test, only significant differences are labeled, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, the error bars represent standard deviation. C. Northern blot showing *FnrS* expression in MMA under aerobic and microaerobic conditions. 5S RNA was probed as loading control.

3.2.2 Reproducibility of RNA-seq data

Inherent biological variability and random variation, such as that introduced during sample preparation, are features of any assay used in microbiology. Replication of experiments is necessary to identify and minimise the variation in experimental systems (Quackenbush, 2002), and to provide statistical significance for the acquired data. RNA-seq data has been previously shown to be highly reproducible, even for independent samples (Colgan *et al*., 2016; Kröger *et al.*, 2013). The sequencing data used in this study were acquired in two separate sequencing runs using independent RNA samples. Additionally, it was important to show that our microaerobic growth condition was reproducible. It was, therefore, important to quantify the reproducibility of the results between sequencing runs to compare gene expression across both replicates. Independent biological replicates of WT and Δ*fnr* grown to OD_{600} 0.3 in microaerobic MMA were generated by Dr. Aoife Colgan and myself. The reproducibility and robust nature of the RNA-seq-based transcriptomic data set and the reproducibility of the microaerobic environment were confirmed using correlative analysis of the independent biological replicates. WT and Δ*fnr* replicates had high values coefficient of determination or R^2 of 0.9209 and 0.8844, respectively (**Figure 3.3**). During downstream analyses, independently extracted RNA was used to validate many RNA-seq-based findings by northern blot and RT-qPCR.

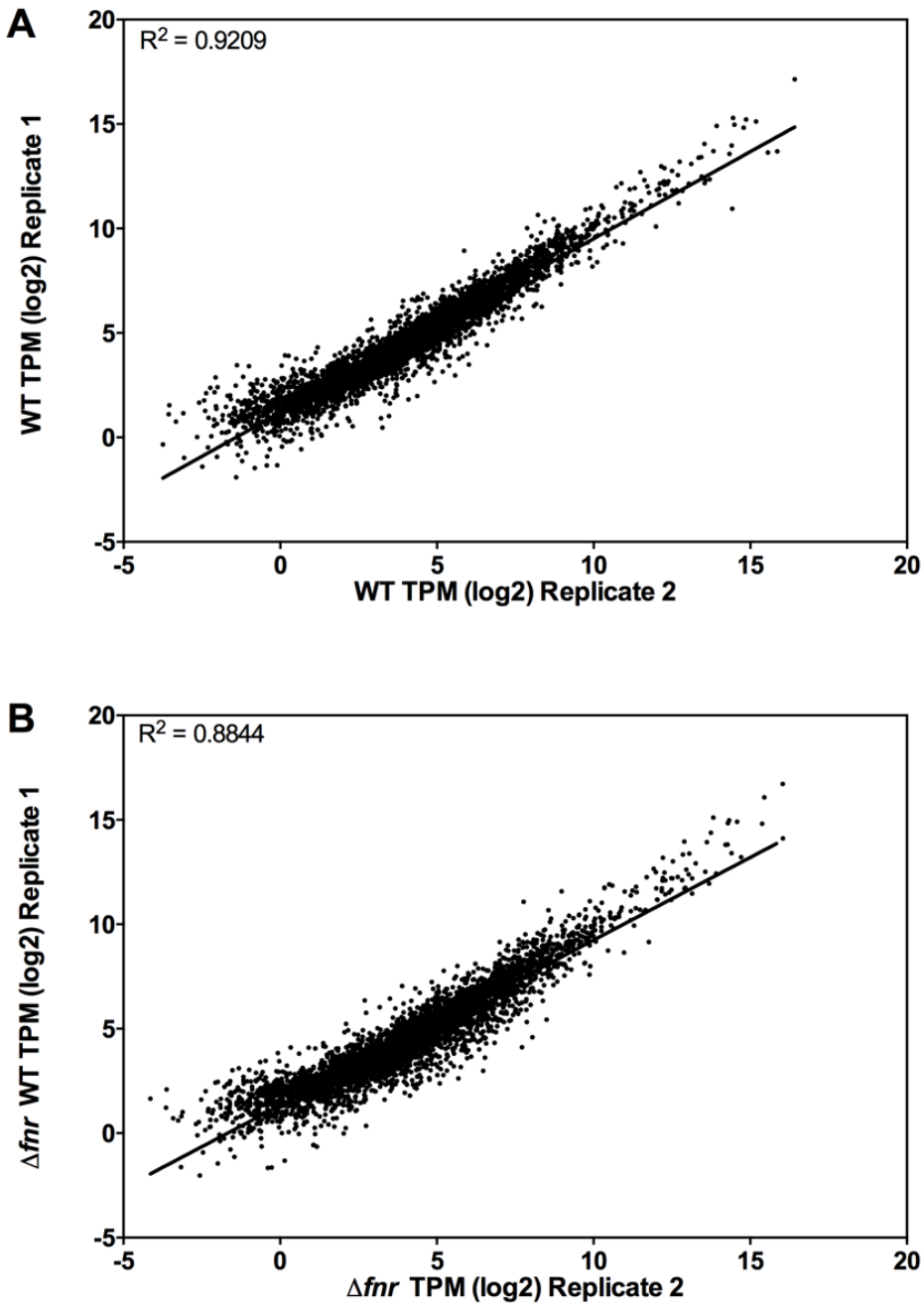


Figure 3.3 High correlation of RNA-seq data from independent biological replicates. RNA-seq-based transcriptomic data comparing log2 transcripts per million (TPM) values from (A) wild-type and (B) Δ*fnr* cells grown to OD_{600} 0.3 in MMA in microaerobic conditions show a high level of correlation with an independent biological replicate sequenced in a separate sequencing run. Media preparation, culture growth and RNA isolation for replicate 1 was performed by Dr. Aoife Colgan, media preparation, culture growth and RNA isolation for replicate 2 was performed by me.

3.2.3 Absence of polar effects in Δfnr

A polar mutation affects expression of downstream genes or operons and it is not desirable in a strain to be used for determining differences in gene expression. The isogenic *fnr* mutant used in this study was constructed by Dr. Aoife Colgan using the Datsenko and Wanner method of gene deletion (Datsenko & Wanner, 2000). This method involves replacing the gene of interest with an antibiotic resistance cassette by homologous recombination. The recombination event is mediated by the plasmid-encoded recombinase system of Bacteriophage λ (Murphy, 1998). The resistance cassette is amplified from a plasmid using oligonucleotides which also contain sequences homologous to the DNA on either side of the gene to be deleted. FLP recombinase target (FRT) sites flank the plasmid-encoded resistance gene to allow for removal of the antibiotic resistance gene from the resultant mutant strain using the pCP20 plasmid which encodes the yeast FLP recombinase (Cherepanov & Wackernagel, 1995). Removal of the antibiotic resistance gene leaves an 82–85 bp scar which contains an idealised ribosome binding site and a start codon to allow for expression from the downstream gene. To avoid polar effects, the mutant was transduced into a clean genetic background and then the kanamycin resistance cassette was removed. **Figure 3.4** shows RNA-seq data from the region surrounding *fnr* in the WT and Δ*fnr* (visualised using the Integrated Genome Browser IGB) (Nicol *et al.*, 2009). In the WT, *fnr* is expressed under our growth condition, while no reads have mapped to the ORF of *fnr* in the Δ*fnr* mutant, indicating that the deletion was successful. The genes immediately up- and downstream of *fnr* are *ogt* and *ydaA*. Expression of *ogt* was only 1.34 fold up-regulated in Δ*fnr*, and it is expressed from its own promoter and lies upstream of the deletion, indicating the small difference is unlikely a polar effect. The *ydaA* gene is also encoded from its own promoter but, lies downstream of the *fnr* gene. Expression of *ydaA* was reduced 2.18-fold in the absence of FNR. The *ydaA* gene encodes for the universal stress protein UspE in *S.* Typhimurium, and expression of *ydaA* is highest under anaerobic shock and during anaerobic growth in WT cells (Kröger *et al.*, 2013). It is possible that the *ydaA* gene is regulated by FNR under microaerobic and/or anaerobic conditions, resulting in the decrease in *ydaA* expression in the Δ*fnr* mutant. In *E. coli* there is a regulatory link between FNR and *ydaA* via GadX (Constantinidou *et al.*, 2006; Hodges *et al.*, 2010). There is no *gadX* orthologue in *S.* Typhimurium but, it may be possible that an alternative regulatory link exists between FNR and *ydaA* in *Salmonella*. Therefore, there were no polar effects from the deletion of the *fnr* gene. Additionlly, an fnr^+ complementation was created to corroborate the absence of polar effects of the Δ*fnr* mutant.

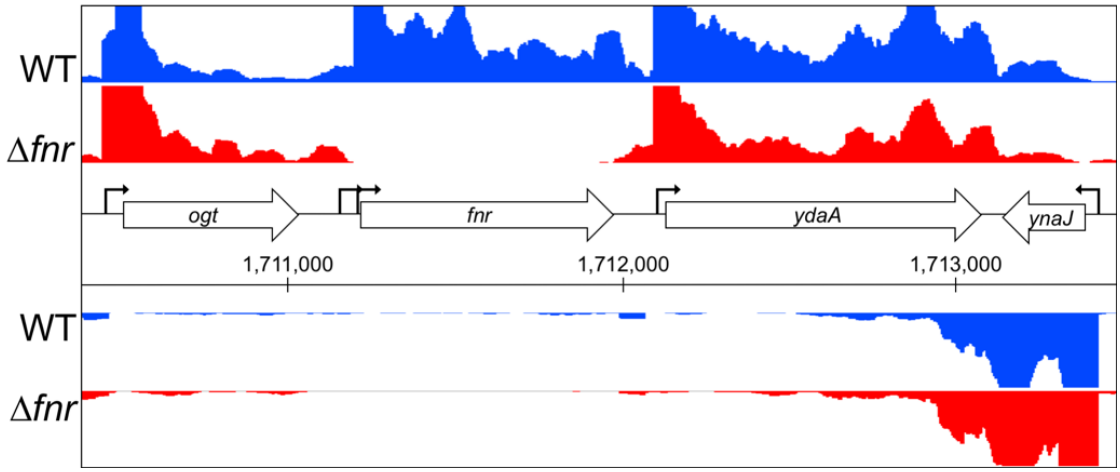


Figure 3.4 Confirmation of *fnr* chromosomal deletion by RNA-seq.
Visualization of sequenced reads in the surrounding region of the deleted *fnr* open reading frame in the mutant strain and WT, grown under microaerobic conditions in MMA, in the Integrated Genome Browser. The tracks for the WT and Δ*fnr* mutant are blue and red, respectively. The colours of each track represent the sequencing reads which map to that locus and the height of the normalised reads is directly proportional to the level of expression at that locus, the scale is 0–100 normalized reads for each sample. The tracks demonstrate that *fnr* was expressed in the WT strain, and that no sequencing reads mapped to the deleted region in Δ*fnr*. Neighbouring genes were generally not affected by polar mutations. White arrows with a black outline denote protein-coding genes. Black bent arrows indicate TSS. All arrows indicate the direction of transcription. The Δ*fnr* deletion mutant was constructed by Dr. Aoife Colgan.

3.2.4 Complementation of the Δfnr mutant

Complementation of a mutant strain is important to show that any observed phenotype can be rescued and therefore, that the observed phenotype is due to the mutation in question and not a polar effect or additional unintended mutation. The Δ*fnr* mutant was complemented both by cloning of *fnr* and its native promoter (215 nt upstream) onto the pBR322 plasmid, p*fnr*, and by introducing the same cloned fragment onto the chromosome downstream of *glmS* to create *fnr*$^{+}$. The plasmid p*fnr* overexpressed *fnr* 36.8-fold over WT levels, but rescued *FnrS* expression to approximately normal levels in the mutant (**Figure 3.5**). However, in later experiments including motility assays and macrophage infections, p*fnr* performed poorly, therefore the chromosomal complementation was constructed (See **Figure 2.1** in section 2.5.3). The advantage of a chromosomal complementation is that there

is only a single copy of the gene, compared to plasmid complementation where multiple copies are present in the cell, even when low-copy plasmids have been used. The chromosomal complementation *fnr*$^{+}$, expressed *fnr* to levels slightly lower but not significantly different from WT when examined by RT-qPCR, and rescued *FnrS* expression on a Northern blot. A growth curve was performed in a microplate reader under anaerobic conditions which showed *fnr*$^{+}$ was able to restore WT growth (**Figure 3.6**).

Interestingly, Baek *et al*. discovered a 66 nt unannotated ORF, named *mia*-44, in the *fnr* promoter region beginning with a start codon 218 bp from the *fnr* start codon in *S.* Typhimurium 14028s (Baek *et al.*, 2017). After many failed attempts at cloning *fnr* with 400 bp upstream of the *fnr* start codon new oligonucleotides were designed that eliminated the putative ribosome binding site and ATG, leaving 215 bp of sequence upstream of the *fnr* start codon. A cloning attempt with the shorter sequence was immediately successful. This finding indicates that overexpression of *mia*-44 may be toxic in *E. coli*, as cloning was attempted in *E. coli* TOP10.

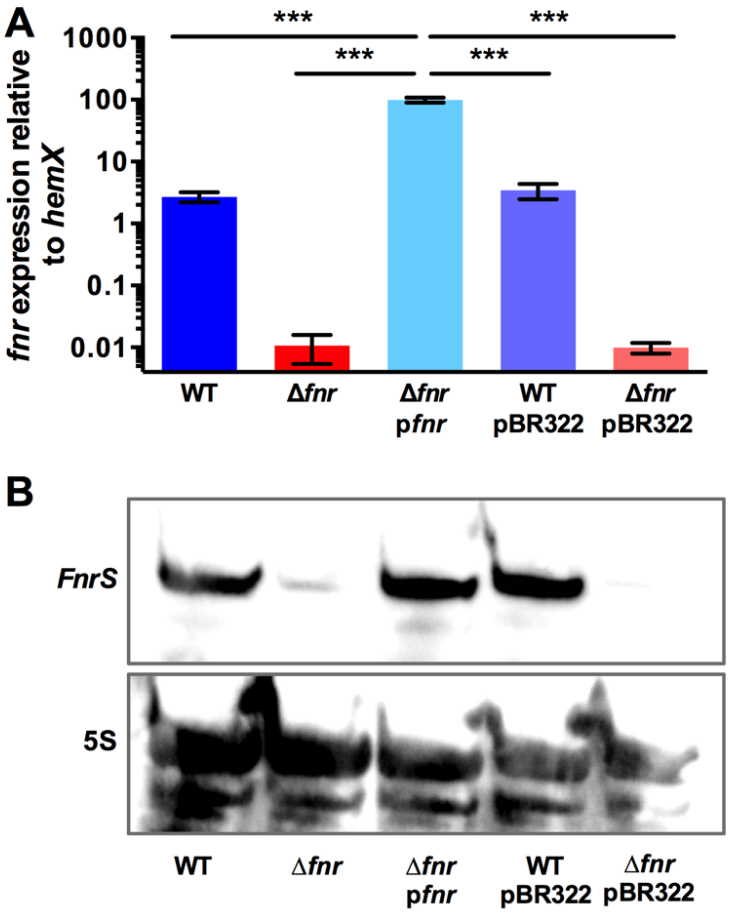


Figure 3.5 p*fnr* overexpresses *fnr* but recues *FnrS* expression in Δ*fnr*.

RNA was extracted from cells grown to OD_{600} 0.3 in microaerobic MMA. A. RT-qPCR data showing *fnr* expression relative to *hemX*. Statistical significance was determined by an Ordinary One-Way ANOVA with Tukey's multiple comparisons test, only significant differences are labeled, *** = $p \leq 0.001$, the error bars represent standard deviation. B. Northern blot showing *FnrS* expression is rescued by p*fnr* in the Δ*fnr* mutant. 5S RNA was probed as loading control.

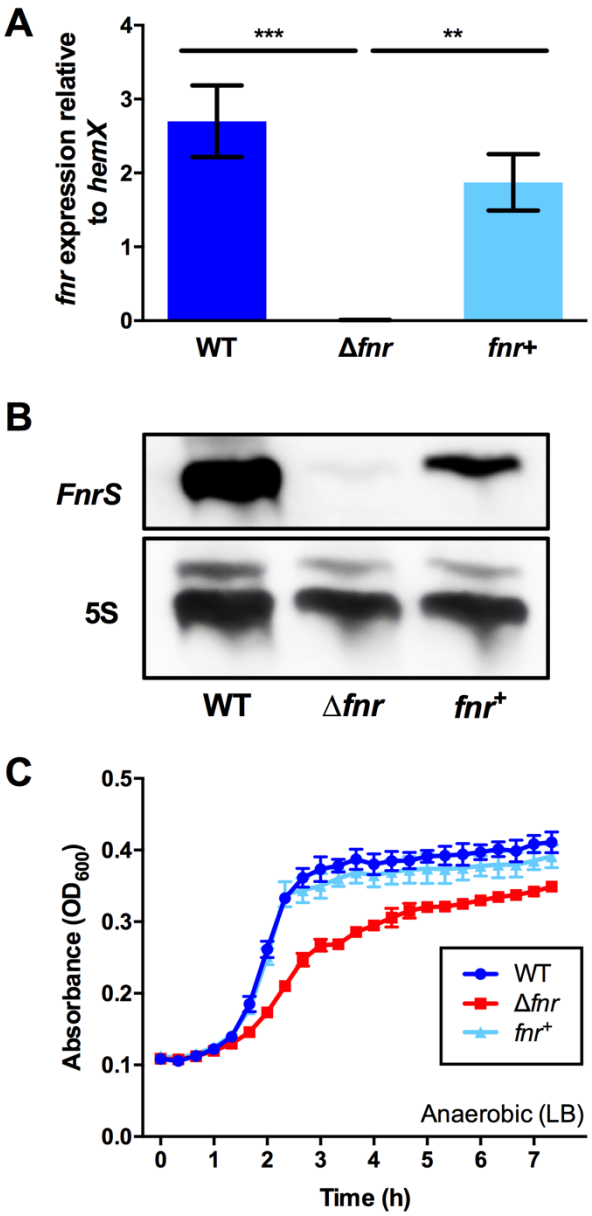


Figure 3.6 *fnr*$^{+}$ rescues *FnrS* and *fnr* expression under microaerobic & anaerobic conditions.

RNA was extracted from cells grown to OD_{600} 0.3 in microaerobic MMA for A and B. A. RT-qPCR data showing *fnr* expression is shown relative to *hemX*. Statistical significance was determined by an Ordinary One-Way ANOVA with Tukey's multiple comparisons test, only significant differences are labeled, ** = $p \leq 0.01$, *** = $p \leq 0.001$, the error bars represent standard deviation. B. Northern blot showing *FnrS* expression is partially recovered by the complementing strain *fnr*$^{+}$ under microaerobic conditions in MMA. 5S RNA was probed as loading control. C. Growth curve showing *fnr*$^{+}$ rescues growth rate of the mutant in LB under anaerobic conditions performed by Dr. Daniel Ryan.

3.2.5 RNA-seq determines differentially expressed genes in Δfnr

The relative expression levels during microaerobic growth of all genes found in *S.* Typhimurium 4/74 on the chromosome and its three plasmids, pColIb9, pRSF1010 and pSLT can be observed in **Figure 3.7**. The log2 ratios of Δ*fnr* mutant TPM over WT TPM were plotted against their location on the chromosome or plasmid. Genes which were below the 2-fold cut-off (or between log2 of -1 to 1) were not considered to be differentially expressed (DE). DE genes in the Δ*fnr* mutant when compared to WT were organized into clusters of orthologous groups (COGs) according to the gene list in **Table S2** in Appendix II with the addition of virulence genes, sRNAs and unknown genes (**Figure 3.8**). In total, 482 genes and sRNAs were regulated directly or indirectly by FNR under microaerobic conditions in MMA. 286 genes were activated by FNR and 196 were repressed. Not surprisingly, FNR activated many genes categorized into anaerobic metabolism. Additionally, many genes from energy production and conversion were FNR activated. Approximately 2/5 of these genes overlap with genes of anaerobic metabolism, and the majority of the remaining genes in this category encoded NADH dehydrogenase subunits. The vast majority of genes activated by FNR fell into the chemotaxis and motility, surface structures, and cell motility and secretion categories which have a high degree of redundancy. There were no genes categorized into drug analogues and resistance, protein transport, or translation, ribosomal structure and biogenesis. Many unknown genes could not be categorized as they had either unknown function, were poorly characterized or had only a general predicted function. In total 56 unknown genes were activated by FNR and 86 were repressed. The majority of genes found to be repressed by FNR did not fall into the typical COG categories; These were virulence associated genes and sRNAs. The virulence genes up-regulated in the Δ*fnr* mutant were predominantly SPI-2 encoded or associated genes.

The most interesting feature of this data set is the up-regulation of genes encoding SPI-2 regulators, T3SS apparatus proteins, effector proteins, and chaperones (**Figure 3.9**). The 15 kb portion of the pathogenicity island with genes for tetrathionate reductase and of unknown function (Hensel, 2000) was not differentially expressed in Δ*fnr* due to the lack of tetrathionate in the growth medium. Notably, many genes which are not encoded in SPI-2, but that are induced in SPI-2 inducing conditions (Kröger *et al.*, 2013) and many of which are effectors that are secreted through the SPI-2 T3SS were also up-regulated in the Δ*fnr* mutant. These included effectors such as *pipB2*, *sifA*, *sifB*, *sopD2*, *sseJ*, *sseK2*, *sseL*, *steA*, and *steC*.

Also up-regulated in Δ*fnr* were the genes from the *spv* (*Salmonella* plasmid virulence) locus found on the low-copy *Salmonella* virulence plasmid pSLT. The *spv* locus encodes the *spvABCD* operon and a positive regulator *spvR*. SpvA is an outer membrane protein that can dampen the expression of the operon, while SpvB, SpvC and SpvD are translocated into the host cell by the SPI-2 T3SS. Genes from this locus are required for full virulence in mice (Grabe *et al.*, 2016; Guiney & Fierer, 2011; Passaris *et al.*, 2018). Five PhoP-activated genes (*pag*) were up-regulated in Δ*fnr* including the SPI-11 encoded outer membrane virulence proteins *pagC* and *pagD* which are involved in intramacrophage survival (Gunn *et al.*, 2000; Sabbagh *et al.*, 2010), the bacteriophage encoded virulence protein *pagK*, flagella-independent surface motility genes *pagM*, and inner membrane protein *pagO* (Gunn *et al.*, 2000; Park *et al.*, 2015). All of these genes are upregulated under SPI-2 inducing conditions and in macrophages (Kröger *et al.*, 2013; Srikumar *et al.*, 2015).

Genes from SPI-1, SPI-3 and SPI-4 were not DE in Δ*fnr* and had low absolute expression in both the WT and Δ*fnr* (**Figure 3.10A, B, & C**). The most down-regulated locus in our data set was the SPI-5 encoded gene *orfX* which was 62-fold down-regulated in Δ*fnr* (**Figure 3.10D**). Very little is known about the specific function of *orfX* however it appears to be important for infection of chicken, pigs and calves, but not in mice (Chaudhuri *et al.*, 2013). Also encoded in SPI-5, the effector protein *pipB* was up-regulated 8.5-fold in Δ*fnr* (**Figure 3.10D**). Similar to *orfX*, very little is known about the function of *pipB* however it is secreted via the SPI-2 T3SS (Jennings *et al.*, 2017).

DE genes which were down-regulated in Δ*fnr* included metabolism operons that were likely upregulated in the WT because of the nutrients that were either available or missing in the growth medium. These genes included proteins for cobalamin (vitamin B_{12}) biosynthesis (*cbiFJKLMQT*) and propanediol degradation (*pduBDEGHJKMNQSV*). Previously, CRP and ArcAB were shown to regulate these operons when cells were provided a poor carbon source under aerobic conditions and anaerobic conditions (Ailion *et al.*, 1993). This data suggested that FNR may also play a role in the activation of these operons, and that since there is no added propanediol in MMA, that *S.* Typhimurium could be converting glycerol to propanediol. Interestingly, a recent proteomic study of Δ*fnr* in SL1344 showed that under anaerobic growth in MOPS buffered LB with xylose, the propanediol utilization proteins were up-regulated (Wang *et al.*, 2019), suggesting a dual role for FNR in the regulation of propanediol utilization dependent on available nutrients. We also saw down-regulation of the glycerol metabolism operon *glpABC*, which has previously been described to be under

the indirect control of Fur, but Fur binding sites were not found in the promoter (Troxell *et al.*, 2011). Expectedly, anaerobic metabolism genes necessary for nitrate, TMAO and fumarate reduction, *narJ*, *dmsABC* and *frdABCD*, respectively were also down-regulated. Genes encoding proteins for hydrogenase maturation *hypBCDEO* and *hybABCDEFG* encoding a hydrogenase, HYD2, that is responsible for uptake of hydrogen as an electron donor during anaerobic respiration, with fumarate serving as an electron acceptor were both down regulated in the absence of FNR. NADH hydrogenase I (NDH-I) catalyzes the first step of electron transport by the oxidation of NADH and is the preferred NADH hydrogenase under oxygen limited conditions as it is "energy conserving" (Price & Driessen, 2010). NDH-I subunits are encoded by *nuoABCEFGHIJKLMN* and were downregulated in Δ*fnr*. Finally, two outer membrane porins (OMPs) *ompD* and *ompW* were down-regulated in the mutant. These OMPs are required for the efflux of methyl viologen, a charged quaternary ammonium compound that generates ROS under aerobic growth conditions (Gil *et al.*, 2007). OmpW was also down-regulated in an Δ*fnr* mutant recent study grown under anerobic conditions in MOPS-buffered LB with xylose (Wang *et al.*, 2019). The gene encoding OmpW was highly down-regulated approximately 21.5-fold. These results suggest that FNR is involved either indirectly or directly in the regulation of many different categories of genes.

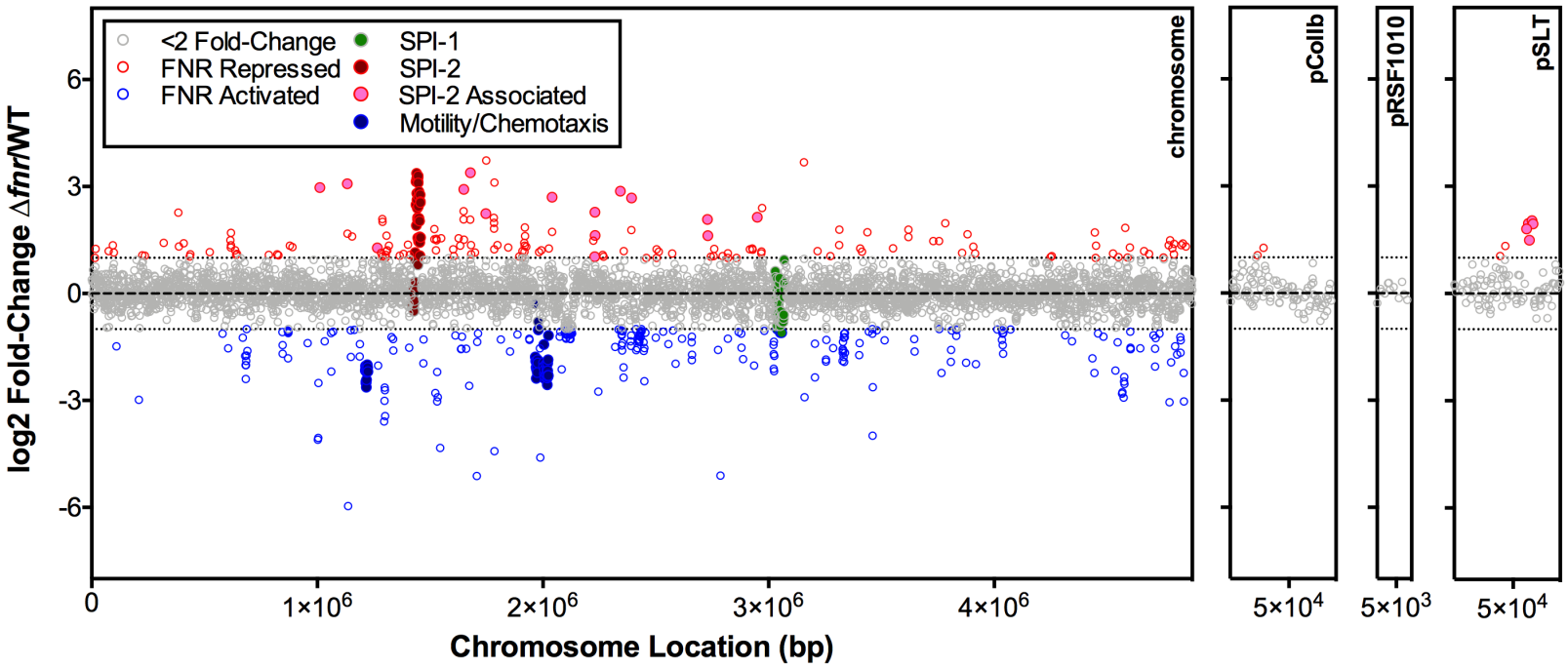


Figure 3.7 Relative gene expression across the *S.* Typhimurium chromosome and plasmids.

Comparing the transcriptome of Δ*fnr* over WT shows genes that are activated and repressed by FNR under microaerobic conditions in MMA. The first panel shows transcripts from the chromosome followed by the three plasmids present in *S.* Typhimurium 4/74: pColIb, pRSF1010, and pSLT. Circles with a grey outline were less than 2-fold (log2 ratio between 1 to -1) different, circles with a red outline were upregulated in Δ*fnr* and circles with blue outline were down-regulated in Δ*fnr*, implying that FNR is a repressor or activator, respectively. Circles filled with green belong to SPI-1, dark red-filled circles belong to SPI-2, pink-filled circles represent SPI-2 associated genes and dark blue-filled circles belong to motility and chemotaxis genes/operons. A log2 value of 0 indicates no change in expression, and we used a cut-off of 1 to -1, denoted by dotted lines, to indicate differential expression.

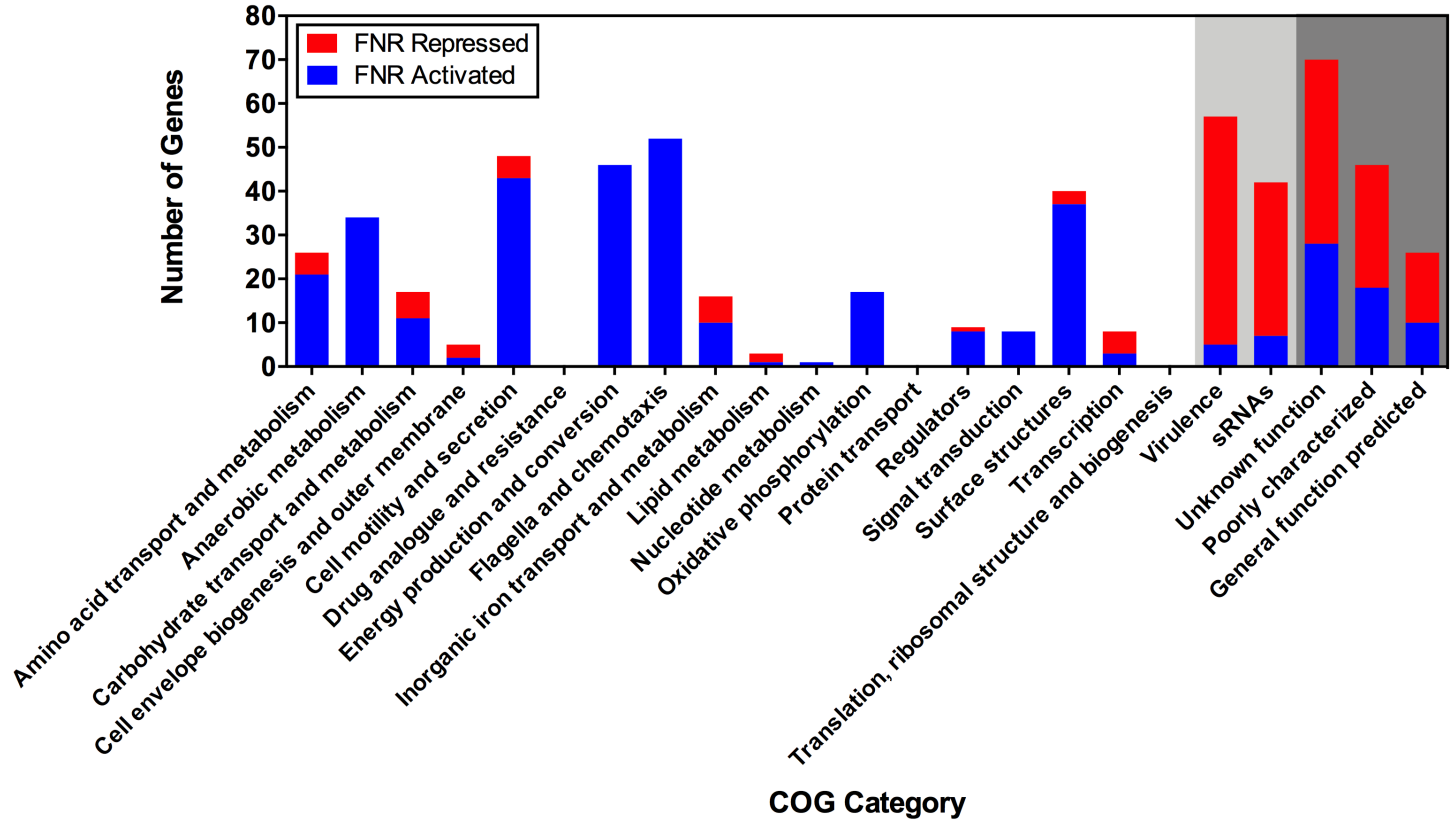


Figure 3.8 Classification of FNR-regulated genes according to COGs.

Genes which were ≥2-fold differentiated were organized into COG categories according to (white panel) **Table S2** in Appendix II, (light grey panel) virulence associated genes and transcripts encoding sRNAs, and (dark grey panel) unknown categories. Red and orange bars indicate FNR repressed and activated genes, respectively. The complete list of genes in each category can be found in **Table S3** in Appendix II.

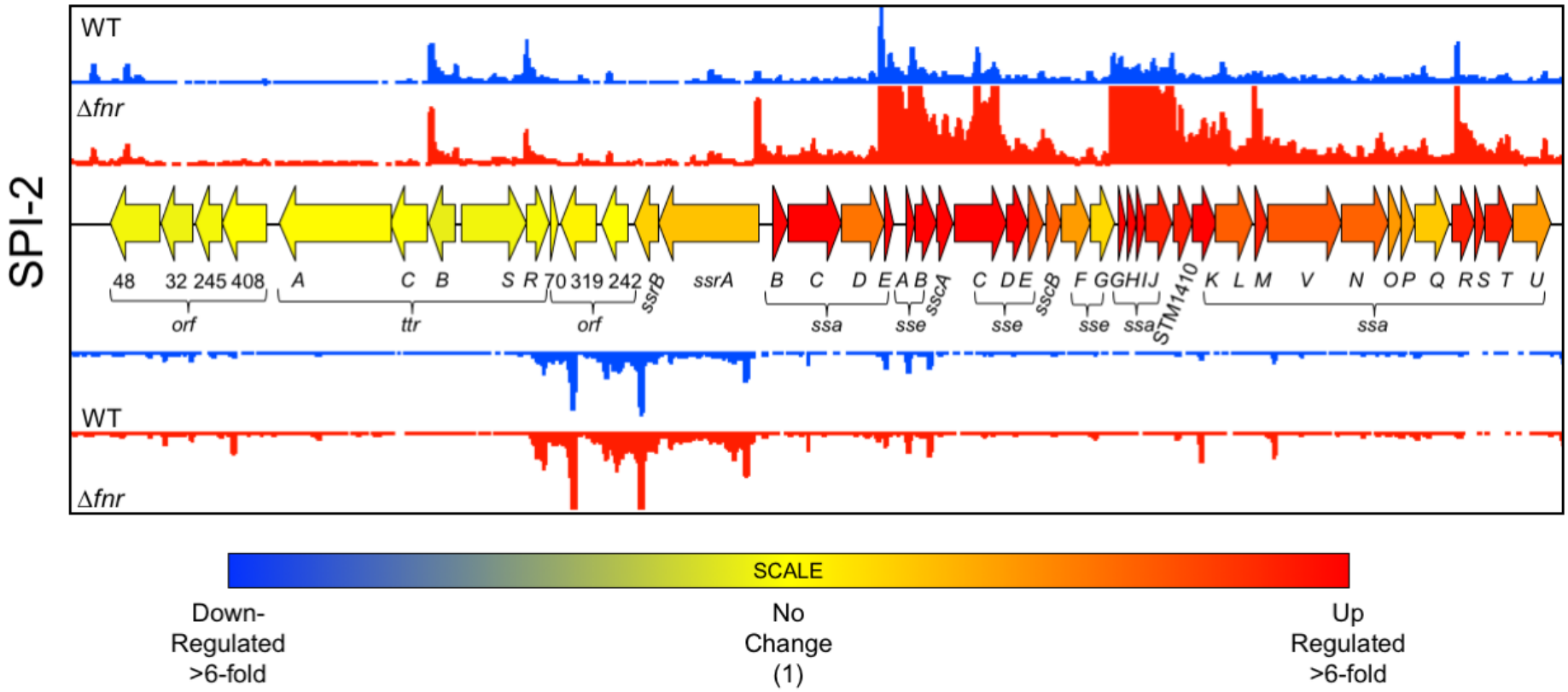


Figure 3.9 SPI-2 expression is highly up-regulated in the absence of FNR.

Visualization of relative gene expression and sequenced reads at SPI-2 in the Δ*fnr* mutant and WT, grown under microaerobic conditions in MMA. Each arrow represents a gene and the colour represents the relative gene expression according to the scale bar. The IGB tracks for the WT and Δ*fnr* mutant are blue and red, respectively. The colours of each track represent the sequencing reads which map to that locus and the height of the normalised reads is directly proportional to the level of expression at that locus, the scale is 0–100 normalized reads for each sample.

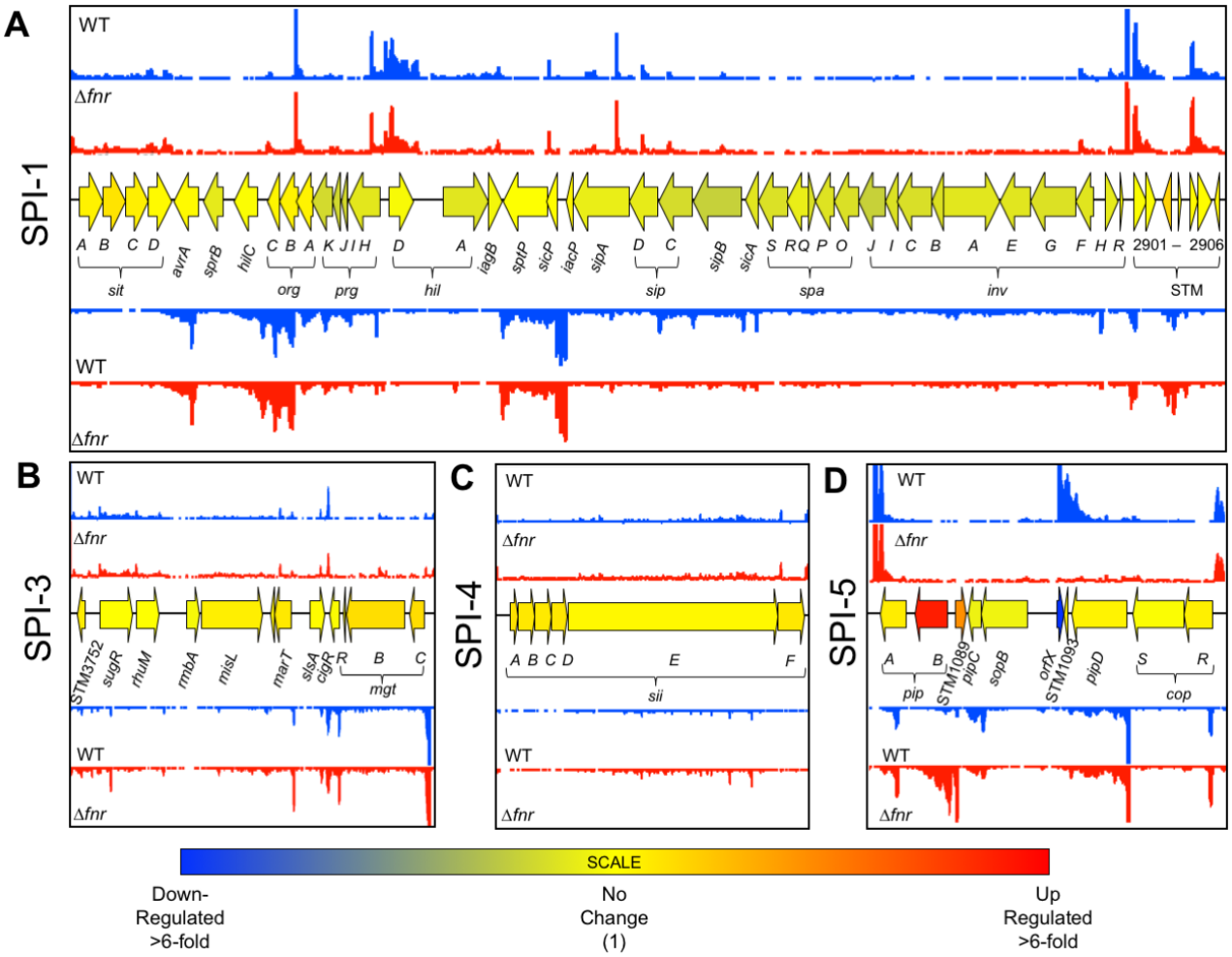


Figure 3.10 Low level absolute and relative expression in SPI-1, 3, 4 and 5. Visualization of relative gene expression and sequenced reads at A. SPI-1, B. SPI-3, C. SPI-4 and D. SPI-5 in the Δ*fnr* mutant and WT, grown under microaerobic conditions in MMA. Each arrow represents a gene and the colour represents the relative gene expression according to the scale bar. The IGB tracks for the WT and Δ*fnr* mutant are blue and red, respectively. The colours of each track represent the sequencing reads which map to that locus and the height of the normalised reads is directly proportional to the level of expression at that locus and the scale is 0–100 normalized reads for each sample.

3.2.6 SPI-2 is derepressed in Δfnr under microaerobic conditions in MMA

SPI-2 genes were some of the most up-regulated genes in the Δ*fnr* mutant according to RNA-seq. Recently, the SPI-2 protein SsaH, encoded by *ssaH*, was found to regulate the secretion of an inner rod and early substrate, SsaI, and to form a heterodimer with the chaperone protein SseA, to switch to secretion of the needle protein SsaG (Takaya *et al.*, 2019). SseA, encoded by *sseA*, is essential for SPI-2-mediated translocation of effector proteins (Coombes *et al.*, 2003). The *ssaH* and *sseA* genes were 9.6- and 9.4-fold up-regulated in Δ*fnr* according to RNA-seq; These data were validated by RT-qPCR of independent biological replicates. Expression of *ssaH* in Δ*fnr* showed a 3.5-fold increase over WT ($P < 0.01$) (**Figure 3.11A**), and expression of *sseA* increased 2-fold in Δ*fnr* over WT ($P < 0.01$) (**Figure 3.11B**). Expression of *ssaH* and *sseA* in *fnr*$^+$ was not significantly different from WT, expression of *ssaH* in WT was not significantly different from the negative control *ssrAB*, and expression of *sseA* in WT was significantly higher than in Δ*ssrAB* (**Figure 3.11A & B**). When WT, Δ*fnr* and Δ*ssrAB* were grown in microaerobic LB there was little expression of *ssaH* or *sseA*, and these values were not significantly different (**Figure 3.11C & D**). Gene expression was normalized to expression of the gene *hemX*, that has the same level of expression in WT and Δ*fnr* in MMA FT according to RNA-seq data. These RT-qPCR data confirm that FNR represses expression of SPI-2 in WT and *fnr*$^+$ *S.* Typhimurium cells grown in MMA under microaerobic conditions.

To demonstrate the ability of FNR to block SPI-2 expression at decreasing O_2 concentrations, we developed an assay using a soft MMA agar (1% agar w/v) that creates an oxygen gradient within a test tube and strains with transcriptional fusion of the *ssaG* promoter with *gfp* (Hautefort *et al.*, 2003). Encoded by *ssaG*, SsaG proteins make up the needle structure of the SPI-2 T3SS (Diepold & Wagner, 2014). Because FNR is only active in oxygen-limited environments, cells growing at the surface of the agar would have inactive or apo-FNR proteins, while cells growing deeper in the agar experience microaerobic and anaerobic environments where FNR would form active homodimers (**Figure 3.12A**). WT, *fnr*$^+$ and Δ*ssrAB* cells grown in the MMA soft agar could proliferate throughout the media, while Δ*fnr* cells could only grow near the surface due to the lack of O_2 deep in the agar and the absence of FNR in the cell preventing anaerobic respiration. The point at which Δ*fnr* cells stopped growing is indicated with an arrow (**Figure 3.12B**). In WT and *fnr*$^+$ cells, *ssaG-gfp*$^+$ was expressed only at the surface of the agar, while in Δ*fnr* cells *ssaG-gfp*$^+$ expression

was observed deeper into the agar, to the boundary of cell growth (**Figure 3.12B**). Active FNR protein homodimers in WT and *fnr*$^{+}$ repressed SPI-2 expression in the microaerobic and anaerobic portions of the media; In Δ*fnr*, The FNR protein is absent therefore, SPI-2 was not repressed. The Δ*ssrAB* mutant was used as a negative control for *ssaG-gfp*$^{+}$ expression, and WT transformed with the pDIGc plasmid, which expresses GFP from a strong promoter driving the expression of the ribosomal protein RpsM, was used as a positive control for GFP expression to show how deep into the media GFP fluoresces (**Figure 3.12B**).

Because cells can differ phenotypically when grown in agar versus in broth cultures, it was important to verify that SPI-2 expression was derepressed in WT cells in aerobically grown MMA broth cultures. WT, Δ*fnr* and Δ*ssrAB* strains transformed with pDEW201-P_{ssaG}, which express *luxCDABE* from the *ssaG* promoter, were grown in MMA to OD_{600} 0.3 under aerobic and microaerobic conditions. Expression of *ssaG* was significantly higher in aerated WT samples than in static growth ($p < 0.001$): expression was 1.8 fold higher (**Figure 3.13A & B**). In the Δ*fnr* mutant, microaerobic cultures had significantly higher expression than in aerated samples ($p < 0.05$) however, they were less than 1-fold different, and there was no expression in Δ*ssrAB* under either growth condition (**Figure 3.13A & B)**. Interestingly, expression of *ssaG* in aerated WT cultures was not as high when compared to aerated Δ*fnr* cultures, indicating there may be another factor involved.

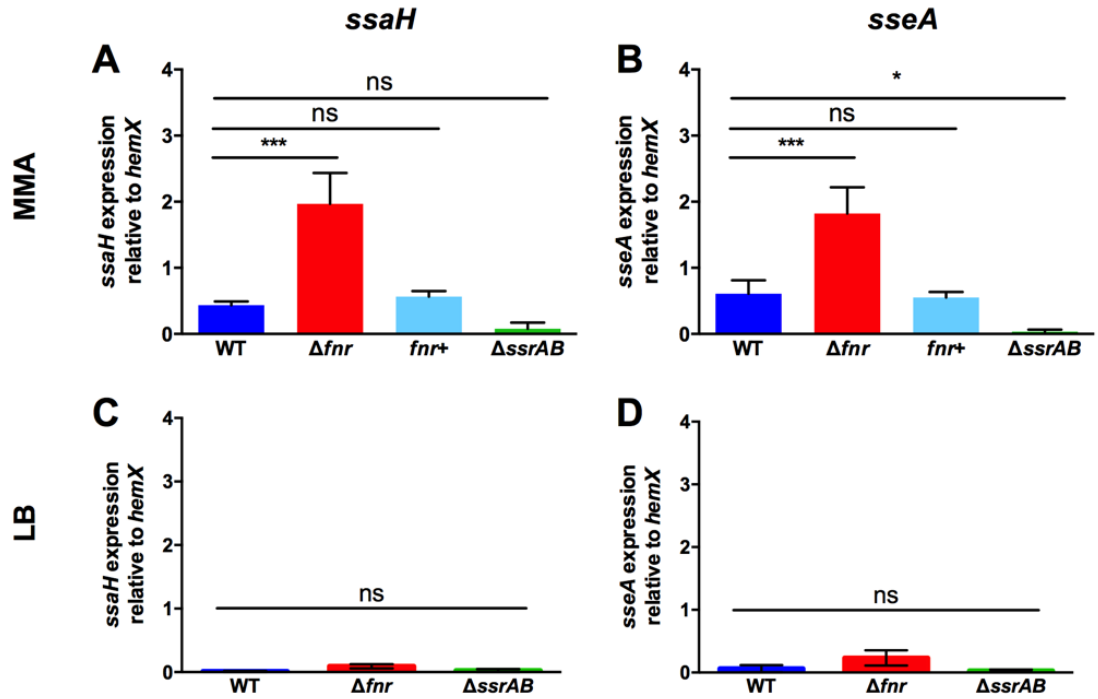


Figure 3.11 SPI-2 apparatus and effector expression is up-regulated in Δ*fnr* under microaerobic conditions in MMA.

RT-qPCR data showing that cells grown under microaerobic conditions in MMA had A. *ssaH* and B. *sseA* expression relative to *hemX* that was significantly higher in Δ*fnr* than WT and *fnr*$^{+}$. RT-qPCR data showing that cells grown under microaerobic conditions in LB did not have significantly different expression relative to *hemX* of C. *ssaH* and D. *sseA*. Statistical significance determined by Ordinary one-way ANOVA with Tukey's multiple comparisons test compared to WT, ns = $p > 0.05$, * = $p \leq 0.05$, *** = $p \leq 0.001$, error bars represent standard deviation.

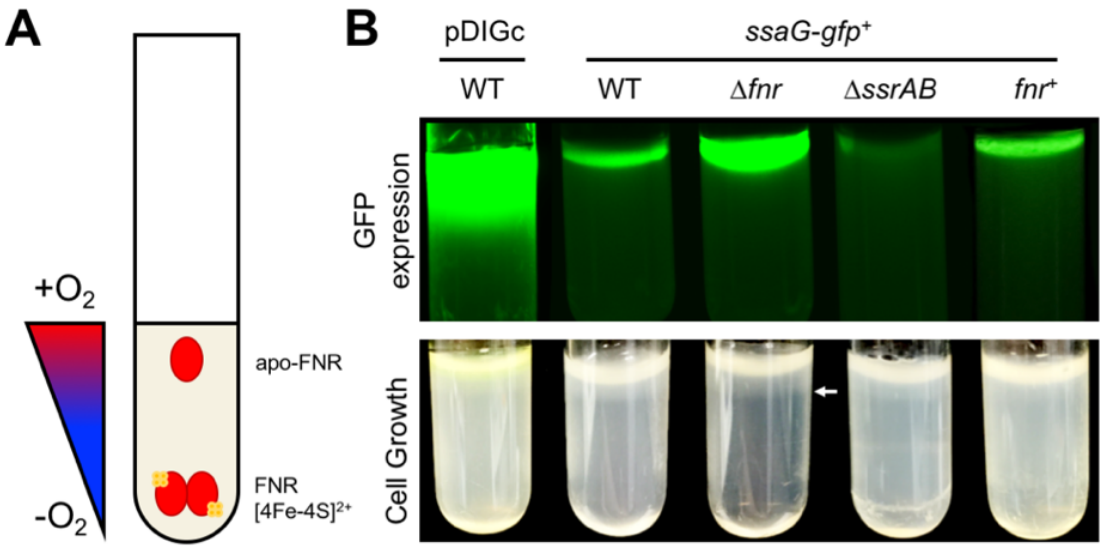


Figure 3.12 FNR blocks *ssaG* expression under low O_2 conditions.

A. Schematic explaining the presence or absence of active FNR dimers according to an oxygen gradient formed by 1% agar in MMA. B. Oxygen gradient gene expression assay demonstrating the ability of FNR to block *ssaG-gfp*$^+$ expression under low O_2 concentrations after 72 h. The top panel shows GFP expression in cells grown in MMA. The bottom panel shows bacterial growth of the same cultures in the 1% agar. The white arrow indicates the point at which the Δ*fnr* mutant can no longer grow due to low O_2 concentration.

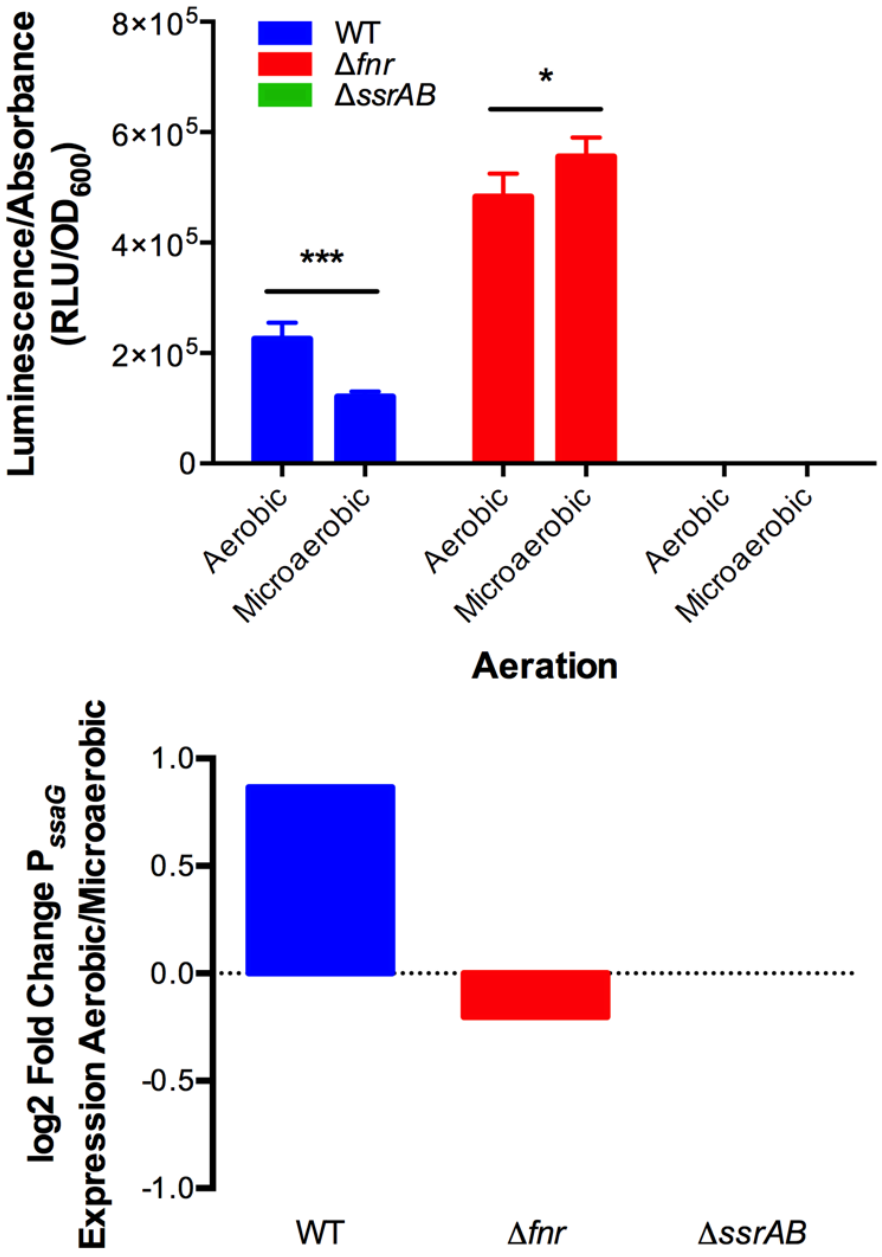


Figure 3.13 SPI-2 expression is increased in WT under aerobiosis.

Cells were grown in MMA under aerobic and microaerobic conditions to OD_{600} 0.3; A. P_{ssaG} expression is shown in relative luminescence units over absorbance (RLU/OD_{600}) for WT, Δ*fnr* and Δ*ssrAB*. Statistical significance was determined between aerobic and anaerobic cultures using a two way ANOVA and Sidak's multiple comparisons test, only significant differences are shown; * = $p \leq 0.05$, *** = $p \leq 0.001$, error bars represent standard deviation. B. log2 fold change in average aerobic over microaerobic P_{ssaG} expression.

3.2.7 Expression and secretion of effector proteins

The *S.* Typhimurium chromosome encodes for two type III secretion systems, T3SS-1 and T3SS-2, encoded on the pathogenicity islands SPI-1 and SPI-2, respectively. Several effector proteins are secreted through these systems into host cells. The cellular location of effectors examined by Western blot in this study are SPI-2 effectors PipB2 and SteC, and SPI-1 effector SopE. PipB2 is described as a "core" effector of SPI-2 although it is encoded outside of the SPI-2 locus (Jennings *et al.*, 2017). PipB2 is responsible for recruiting the host protein kinesin-1, a heterotetrameric, plus-end-directed microtubule motor protein, to the SCV as part of the process of *Salmonella*-induced tubule formation (Henry *et al.*, 2005; Jennings *et al.*, 2017). PipB2 is typically expressed in conjunction with SPI-2 encoded effector proteins although, it is secreted through both the T3SS-1 and T3SS-2 at different time points during infection (Baisón-Olmo *et al.*, 2012). The effector SteC is not encoded within the SPI-2 locus, but it is exclusively expressed under SPI-2 inducing conditions, inside epithelial cells and macrophages, and is secreted through T3SS-2 (Jennings *et al.*, 2017). SteC is found within the majority of intestinal serovars of *S.* Typhimurium; it is a kinase that manipulates the actin cytoskeleton by inducing assembly of the F-actin meshwork around the SCV inside of host cells (Jennings *et al.*, 2017). SopE is a guanidyl exchange factor, is translocated by the T3SS-1 upon host cell contact and promotes entry through triggering of actin-dependent ruffles (Galan & Zhou, 2000). Following host cell entry, SopE undergoes proteasomal degradation however, a subset of SopE may be important for replication in the early SCV (Vonaesch *et al.*, 2014).

Strains with 3×FLAG-tagged PipB2 and SteC proteins were grown in microaerobic MMA and aerobic SPI-2 inducing PCN (InSPI2) as a control. PipB2 levels were indistinguishable between WT, Δ*fnr* and *hilD* mutants in MMA, while there was no expression in Δ*ssrAB* (**Figure 3.14A**). Surprisingly, there was higher PipB2 expression in Δ*fnr* when grown in aerated InSPI2, and a small amount of expression in Δ*ssrAB* (**Figure 3.14A**). When grown in microaerobic MMA, SteC expression was clearly higher in Δ*fnr* than the other strains, but showed similar levels between WT, Δ*fnr* and Δ*hilD* when aerobically grown in InSPI2 (**Figure 3.14B)**.

T3SS delivery is a contact-dependent process characterized by the formation of a pore, or translocon, at the point of contact with the eukaryotic membrane and through which effectors are delivered into the host cell. Effector secretion into broth during *in vitro* culturing has

been observed, usually under acidic conditions which mimic the acidification of the SCV (Beuzón *et al.*, 2002). We hypothesized that effectors would not be secreted in MMA due to the neutral pH of the media. We attempted to see if SPI-2 effectors were secreted in MMA media. Whole cell lysates and culture supernatants were loaded on the same gel and probed with antibody on the same membrane. **Figure 3.15A** and **B** show that although there was high levels of PipB2 expression in both MMA and inSPI-2 cultures, we were unable to detect secreted protein by ponceau staining or Western blotting despite loading 50 OD units of culture supernatant. It is not clear if this was due to this protein not being secreted under these culturing conditions or if precipitation of the protein was not effificient. We did confirm, in agreement with RNA-seq data, that SPI-1 effector SopE was not expressed and therefore not secreted in microaerobic MMA (**Figure 3.15C & E**). As expected SopE was highly expressed in WT, Δ*fnr* and Δ*ssrAB* and not expressed in Δ*hilD* in aerated LB Miller and secreted from the strains in which it was expressed (**Figure 3.15D**). To verify that there was no expression of SopE in microaerobic MMA, whole cell lysates from MMA cultures and aerated LB Miller cultures were loaded onto the same gel and probed on the same membrane for the 3×FLAG and DnaK as a control. Interestingly, Δ*hilA* strains with 3×FLAG-tagged *steC* and *sopE* were constructed for these experiments as controls under SPI-1 and SPI-2 inducing conditions; However, the Δ*hilA* strains did not increase in OD_{600} over 48 h in microaerobic MMA. Therefore, Δ*hilD* strains were constructed and used as a substitute.

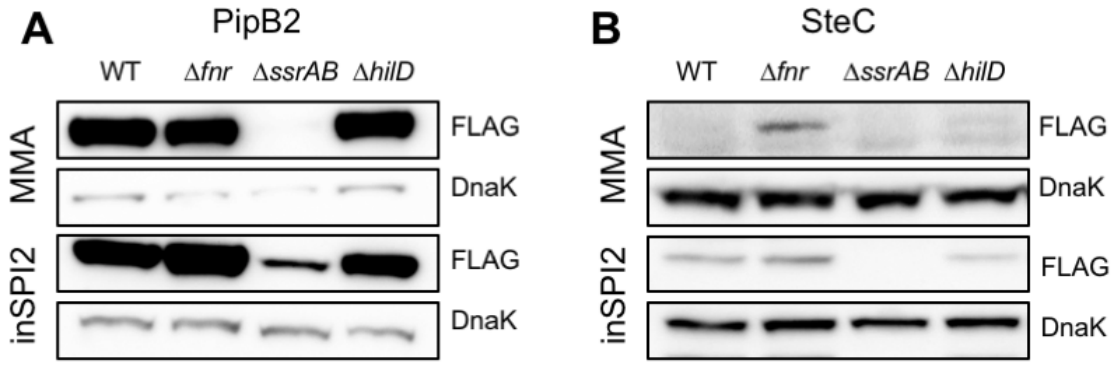


Figure 3.14 Expression of SPI-2 effector proteins.

Cells with a A. *pipB2*-3×FLAG::kan and B. *steC*-3×FLAG::kan were grown to OD_{600} 0.3 in microaerobic MMA or to OD_{600} 1.0 in aerated inSPI-2. 1 and 5 OD unit of lysed cells from inSPI-2 and MMA cultures, respectively, were loaded into each lane of the SDS-PAGE gel. Membranes were probed with anti-FLAG M2 and anti-DnaK antibodies.

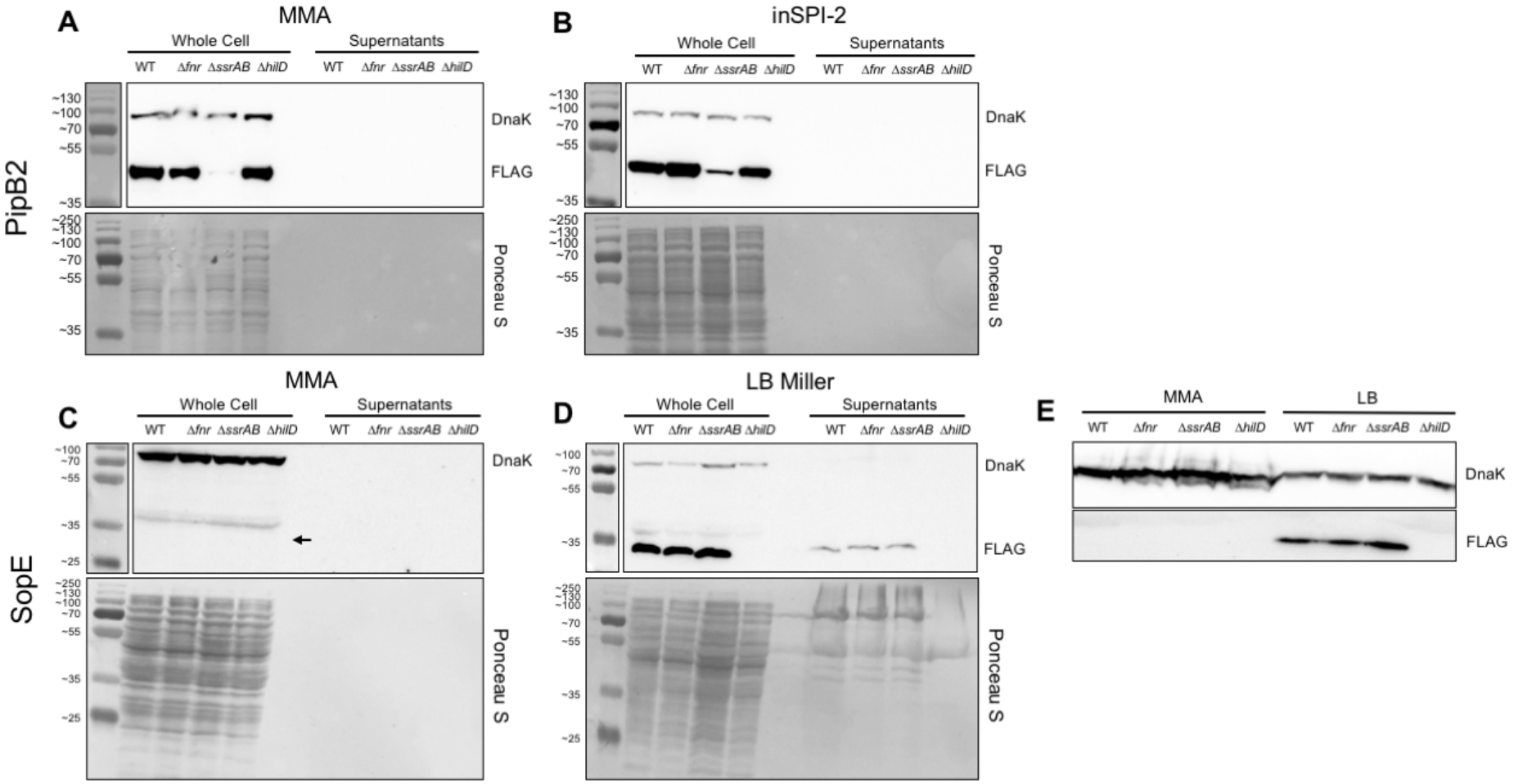

A
MMA
Whole Cell
Supernatants
WT
Δfnr
ΔssrAB
ΔhilD
PipB2
DnaK
FLAG
Ponceau S
B
inSPI-2
C
SopE
D
LB Miller
E
LB

Figure 3.15 Secretion of SPI-1 and SPI-2 effector proteins.

Cells with *pipB2*-3×FLAG::kan were grown in A. MMA and B. InSPI2 media, and cells with *sopE*-3×FLAG::kan were grown in C. MMA and D. LB Miller to OD_{600} 0.3 in microaerobic MMA or to OD_{600} 1.0 in aerated InSPI2 and LB Miller. From MMA cultures 5 and 50 OD units were loaded for whole cell lysates and supernatants respectively; From rich media cultures 1 OD unit was loaded for whole cell lysates, and from inSPI-2 and LB Miller supernatants 50 and 10 OD units were loaded, respectively. An arrow indicates the would be location of SopE-3×FLAG in C. E. Show strains with *sopE*-3×FLAG::kan whole cell lysates only of MMA and LB Miller cultures. Membranes were probed with anti-FLAG M2 and anti-DnaK antibodies.

3.2.8 Repression of motility in Δfnr

The ability of *S.* Typhimurium to thrive in the gut is critical for transmission to new hosts. Using chemotaxis and flagella, they seek out nutrients and host cells. Flagellated subpopulations of *S.* Typhimurium accumulate proximal to the gut mucosa, the location of an oxygen gradient (Marteyn *et al.*, 2010; 2011; Stecher *et al.*, 2008). Fink et al. have shown under anaerobic growth conditions in MOPS-LB-X that chemotaxis, motility and flagellar genes were down-regulated (Fink *et al.*, 2007). Our RNA-seq data show that motility, chemotaxis and flagellar gene expression is also repressed in the Δ*fnr* mutant under microaerobic conditions in MMA (**Figure 3.16**). All genes encoding proponents of the bacterial chemotaxis pathway were down-regulated ≥2-fold except *cheC*, *cheD* and *cheV*. Four of the methyl-accepting chemotaxis proteins (MCPs) *aer*, *cheM*, *trg* and *tsr* were also down regulated ≥2-fold in Δ*fnr* (**Figure 3.17A**). MCPs are the receptors at the beginning of the chemotaxis signal transduction cascade that process environmental and intracellular sensory (input) signals and alter the activity of the CheAY TCS (Wuichet *et al.*, 2007). All genes encoding components of the flagellum were down-regulated ≥2-fold including the motor, the C- and MS-ring of the cytoplasmic membrane, proximal and distal rods, the P- and L-rings, the hook, filament and hook-filament junction, as well as the filament cap (**Figure 3.17B**).

Therefore, strains were compared for swimming motility. Under aerobic conditions WT and Δ*fnr* were equally motile (**Figure 3.18A**). Under anaerobic conditions, WT was motile while Δ*fnr* exhibited limited motility. The p*fnr* and fnr^{+} strains complemented motility under anaerobic conditions in the mutant approximately 69% and 94%, respectively (**Figure 3.18A & B**). Thus,

the activating activity of FNR only motility only occurs under microaerobic and anaerobic conditions, as expected. The *hns-1* mutant is known to be non-motile but not deficient in growth, as H-NS is positive regulator of *flhDC* (Donato & Kawula, 1999) and was used as a negative control for swimming motility. To determine whether the reduced motility of the Δ*fnr* strain was due to lack of flagella, WT and Δ*fnr* cells were examined by AFM for the presence of flagella. The distinction between the WT and mutant was clear, WT cells had abundant peritrichous flagella, while Δ*fnr* had very few flagella present (**Figure 3.18C**). The atomic force microscopy (AFM) images also allowed us to examine the cell morphology; WT and Δ*fnr* cell were found to not be significantly different in size (**Figure 3.18D**). When cells grown microaerobically in MMA where examined using the hanging drop method, WT cells were motile while Δ*fnr* cells exhibited only Brownian movement. Under anaerobic conditions, Δ*fnr* had reduced motility on swimming plates and in microaerobic MMA broth.

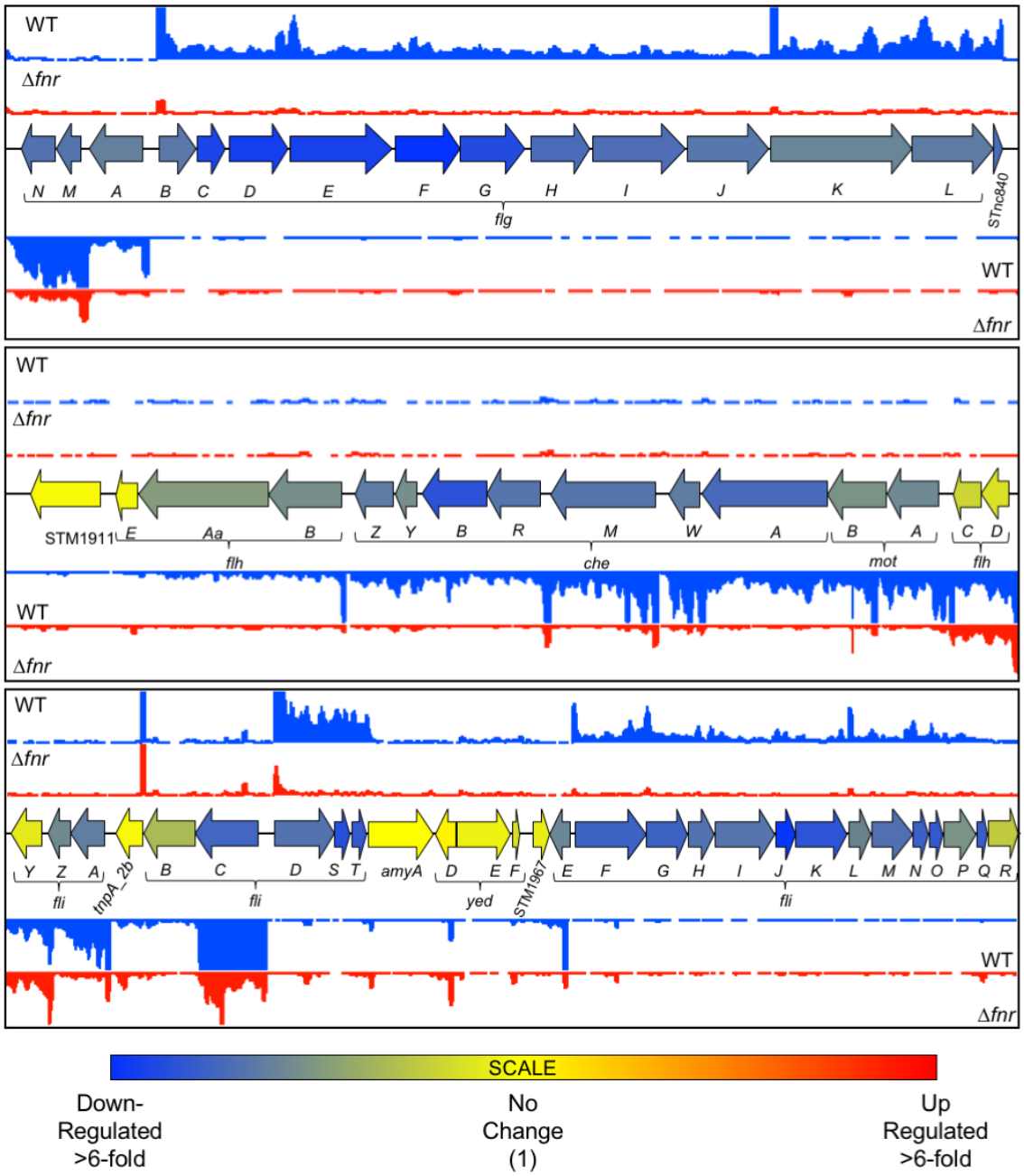


Figure 3.16 Down regulation of genes at major chemotaxis and flagellar loci.
Visualization of relative gene expression and sequenced reads at 3 chemotaxis, motility and flagellar gene loci in the Δ*fnr* mutant and WT, grown under microaerobic conditions in MMA. Each arrow represents a gene and the colour represents the relative gene expression according to the scale bar. The IGB tracks for the WT and Δ*fnr* mutant are blue and red, respectively. The colours of each track represent the sequencing reads which map to that locus and the height of the normalised reads is directly proportional to the level of expression at that locus.

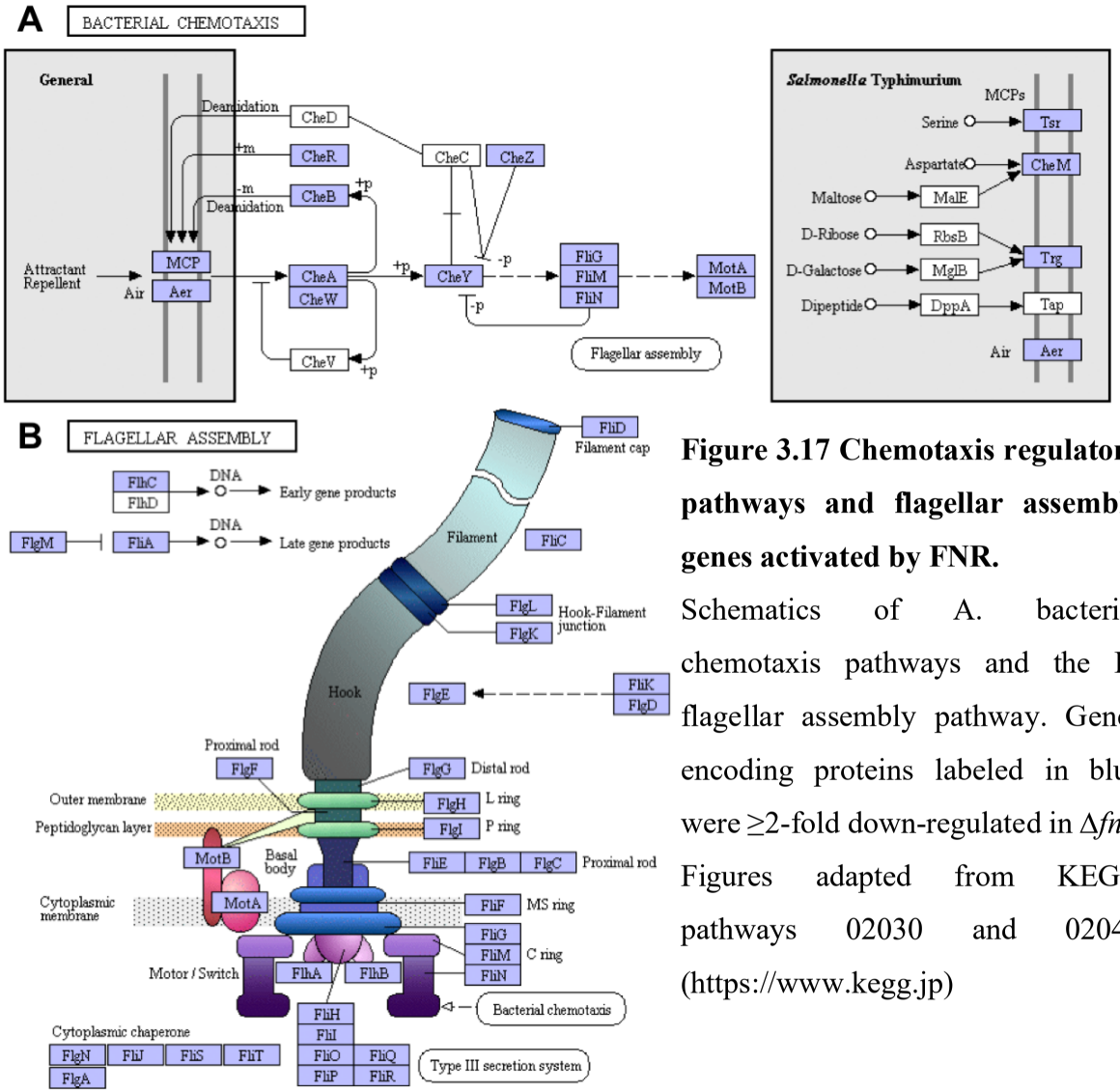


Figure 3.17 Chemotaxis regulatory pathways and flagellar assembly genes activated by FNR.

Schematics of A. bacterial chemotaxis pathways and the B. flagellar assembly pathway. Genes encoding proteins labeled in blue were ≥2-fold down-regulated in Δ*fnr*. Figures adapted from KEGG pathways 02030 and 02040 (https://www.kegg.jp)

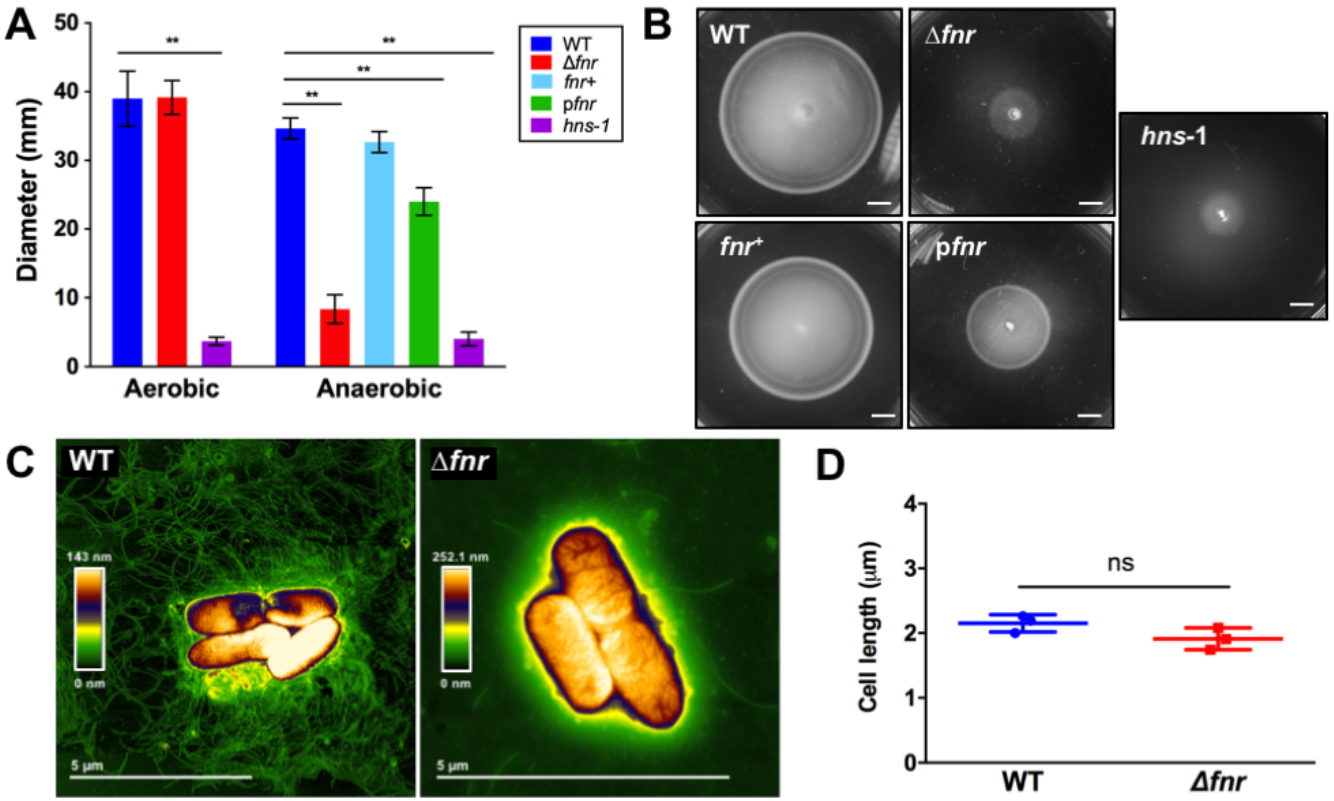


Figure 3.18 Motility is repressed and flagella are absent in Δ*fnr*.

A. Diameters of swimming plates under aerobic and anaerobic conditions. An *hns*-1 mutant was used as a negative control. Significance determined by nonparametric one-way ANOVA with Dunnet's multiple comparison test to WT of aerobiosis and anaerobiosis, respectively; only significant differences are labeled, ** = $p \leq 0.01$. B. Representative photos of anaerobic swimming plates. The scale bar represents 5 mm. C. AFM images of WT and Δ*fnr* cells taken after growth in LB to OD_{600} 2.0. The scale bar represents 5 µm. D. Cell lengths in µm of WT and Δ*fnr* cells measured from AFM images. Significance was determined by a two-tailed t test, ns = $p > 0.05$. Error bars represent standard deviation.

3.2.9 Regulation of small RNAs

Gene expression is carefully co-ordinated in response to environmental, spatial and temporal stimuli for the survival and successful infection inside the host. This must all occur without incurring fitness costs as a result of inappropriate gene expression. Gene expression is controlled at all levels from transcription initiation to translation. Post-transcriptional control of gene expression is important and often overlooked; This gene regulation is often mediated by small, regulatory non-coding RNAs (sRNAs). In our RNA-seq data set 42 sRNAs were identified as DE: 35 were up-regulated and 7 were down-regulated in the Δ*fnr* mutant (**Table 3.1 & 3.2**).

The 7 down-regulated sRNAs from our dataset can be seen in **Table 3.1**. Not surprisingly, *FnrS*, the FNR dependent sRNA was the most down regulated, and this decrease in expression was consistent with data from cells under oxygen shock. The FNR dependency of FnrS had previously only been shown in *E. coli* (Boysen *et al.*, 2010; Durand & Storz, 2010); the regulation appears to be conserved in our study. Another sRNA both down regulated under oxygen shock and in Δ*fnr* was STnc1340, an uncharacterized 82 nt sRNA. All but 1 of the down-regulated RNAs had unsuccessful transposon insertion from TraDIS data. STnc1560 is also an uncharacterized sRNA and is activated by RpoS (Lévi-Meyrueis *et al.*, 2014). Surprisingly, 2 sRNAs which were downregulated in Δ*fnr*, STnc070 and STnc4180, are up-regulated when there is an oxygen shock (Kröger *et al.*, 2013).

Of the 35 up-regulated sRNAs in the FNR regulon, 3 have previously been reported to have a SPI-2 like pattern of expression (**Table 3.2**) (Colgan *et al*., 2016). *IsrH_1_2*, *PinT* and STnc1480 had a high degree of correlation (>0.8) with the *ssaG* pattern of expression (Colgan *et al*., 2016). *IsrH_1_2* is comprised of two overlapping sRNAs, *IsrH-1* and *IsrH-2*, which are 480 nt and 250 nt, respectively. *IsrH-1* shares an overlapping sequence at the 5′ end of *sseL* and is expressed inversely to the SPI-2 effector, and *IsrH-2* overlaps the 3′ end of *glpC,* (encoding the Glycerol-3-phosphate dehydrogenase small subunit) of the *glpABC* operon and is induced under anaerobic conditions (Padalon-Brauch *et al.*, 2008). *PinT* is a 80 nt PhoP-activated sRNA, which upon bacterial internalization temporally controls the transition from invasion (SPI-1) to intracellular survival (SPI-2) (Westermann *et al.*, 2016). STncl480 is a 400 nt intergenic sRNA which, in a similar fashion to *ssrAB* promoters, is silenced by H-NS, and repression of the sRNA is countered by SlyA and PhoP (Colgan *et al*., 2016). All three sRNAs are highly upregulated in SPI-2 inducing conditions

as well as in macrophage and activated by important regulators of SPI-2: SsrB, OmpR, PhoP and SlyA (Colgan *et al*., 2016; Kröger *et al.*, 2013; Srikumar *et al.*, 2015). The most highly upregulated sRNA was *GcvB*. *GcvB* is a globally acting sRNA that regulates a post-transcriptional that influences approximately 1% of the *Salmonella* genome including the global regulator Lrp (Sharma *et al.*, 2007; 2011). Interestingly, the *GcvB* regulon overlaps with the *DapZ* regulon (Chao *et al.*, 2012), another sRNA that was up-regulated in Δ*fnr*. These riboregulators transiently downregulate amino acid and peptide transporters, which may be important for efficient host cell invasion (Miyakoshi, 2019).

To investigate if any of the up-regulated sRNAs play an important role during *S.* Typhimurium infection, published data from a transposon-directed insertion site sequencing (TraDIS) study was interrogated. In the study, high-throughput sequencing of insertion sites of pools of *S.* Typhimurium 4/74 transposon mutants, following oral infections of chicken, pigs and calves were compared to an input inoculum (Chaudhuri *et al.*, 2013). The ratio of input to output reads was used to calculate the fitness score of each mutant. Transposon insertions that result in significant attenuation of the mutant strain were located in the three SPI2-like sRNAs as well as three others: STnc1330, STnc1920 and *tpke70* (**Table 3.2**). STnc1330 is a 141 nt sRNA regulated by RpoS and PhoPQ (Perez-Sepulveda & Hinton, 2018), STnc1920 is a 98 nt uncharacterized sRNA, and *tpke70* is predicted to regulated nitrate reductase genes *napG* and *napD* in *E. coli* (Ishchukov *et al.*, 2014).

3.2.9.1 FNR regulation of sRNAs in the context of a transcriptional network

Many sRNAs are still uncharacterized therefore, it is difficult to gain insight to their importance from a single RNA-seq experiment. For this reason, the sRNAs from our data were compared with those found in the regulons of 18 regulatory proteins in *S.* Typhimurium. Relative expression values of sRNAs found in the FNR regulon were taken from SalComRegulon, and DE sRNAs with fold-change of ≥2 were plotted (**Figure 3.19**). Of the 42 sRNAs found to be DE in Δ*fnr*, only 4 were regulated by FNR alone: *tpke70*, *IsrM*, *IsrN* and STnc350. All four of these sRNAs were found to be putatively repressed. *IsrM* is important for invasion of epithelial cells, intracellular replication inside macrophages, virulence and colonisation in mice (Padalon-Brauch *et al.*, 2008). It targets the SopA and HilE mRNAs, virulence factors essential for bacterial invasion (Gong *et al.*, 2011). *IsrN* has been shown to be important for the early stages of intracellular survival (Padalon-Brauch *et al.*, 2008). As described previously, *tpke70* is also important for virulence in pigs and calves

(Chaudhuri *et al.*, 2013). STnc350 is an uncharacterized sRNA. Many of the sRNAs regulated by FNR are also regulated by other TFs including 24 sRNAs in common with Fur, 23 with RpoS, 20 with Hfq, 18 with SlyA, 16 with SsrA/B and 15 in common with both OmpR/EnvZ and HilD. This substantial overlap with important regulators of *S.* Typhimurium virulence regulators provides support that FNR is also intrinsically embedded in the complicated regulatory network controlling virulence (**Figure 3.19**).

Table 3.1 sRNAs down-regulated in Δ*fnr*

sRNA	Strand	Start	End	Δ*fnr*/WT	Oxygen Shock[a]	TraDIS attenuation[b]
STnc400	+	4072457	4072530	0.498	0.64	–
STnc070	+	669642	669793	0.424	2.36	–
STnc4180	-	109243	109322	0.358	1.95	–
STnc1340	-	2319956	2320037	0.353	0.18	–
STnc840	+	1224244	1224316	0.219	0.69	–
STnc1560	+	2449075	2449183	0.181	0.54	No
FnrS	-	1706784	1706905	0.029	0.01	–

[a]Data from Kröger *et al*. 2013
[b]Data from Chaudhuri *et al*., 2013

Table 3.2 sRNAs up-regulated in Δ*fnr*

sRNA	Strand	Start	End	Δ*fnr*/WT	InSPI2[a]	Macrophage[b]	SPI-2 like[c]	TraDIS attenuation[d]
IsrH_1_2	-	2392019	2392469	3.44	8.15	5.2	Yes	Yes
PinT	+	4580486	4580566	3.58	24.48	8.31	Yes	Yes[e]
STnc1480	+	1316874	1317268	2.54	28.07	11.26	Yes	Yes
STnc150	-	1282521	1282677	2.21	2.91	5.97	No	–
STnc3150	-	3273499	3273824	2.07	2.45	2.09	No	No
STnc3750	-	2503726	2503803	2.36	6.29	2.93	No	–
STnc980	-	889558	889714	2.46	1.77	2.21	No	–
GcvB	+	3156779	3156979	12.85	0.95	0.09	No	–
IsrI	-	2759018	2759265	2.38	1.63	1.66	No	–
IsrM	+	2927616	2927944	2.4	1	1	No	–
IsrN	+	2929501	2929643	2.35	1	–	No	–
RprA	-	1401682	1401790	2.58	0.4	0.08	No	–
RyeB	-	1925621	1925723	2.48	3.2	0.28	No	–
RyhB-2	-	1309727	1309937	2.31	0.06	85.5	No	–
SraL	-	4526163	4526303	2.18	13.18	0.87	No	–
sRNA12	-	3755045	3755125	2.78	1.37	1.39	No	–
STnc1150	+	2222979	2223134	2.79	0.98	0.54	No	–
STnc1220	-	1448718	1448790	2.75	1.68	0.29	No	–
STnc1330	+	2268274	2268414	2.05	2.3	0.58	No	Yes[e]
STnc1700	+	386381	386476	2.5	2.47	0.72	No	–
STnc1870	+	617317	617426	2.54	1.62	1	No	–
STnc1920	+	2756549	2756646	2.08	0.74	0.77	No	Yes
STnc2030	+	1784673	1784725	8.65	1	0.33	No	–
STnc3000	+	4591419	4591506	2.02	0.74	0.27	No	–
STnc310	-	3413972	3414032	2.26	1.63	0.41	No	–
STnc3120	+	4249943	4250101	2.03	1.68	0.17	No	–
STnc3210	+	17025	17111	2.38	3.52	1.42	No	–
DapZ	+	74847	74924	2.28	2.4	0.18	No	–
STnc3440	+	1303294	1303380	2.11	4.55	0.48	No	–
STnc350	-	3782696	3782857	3.91	1.92	0.21	No	No
STnc380	-	3906945	3907052	2.48	3.51	1.92	No	–
STnc4000	+	3619657	3619755	3.47	0.3	2.9	No	–
STnc4010	+	3729767	3729899	2.77	1	0.72	No	–
STnc890	+	14720	14783	2	1.26	0.27	No	No
tpke70	-	2513324	2513722	2.25	1	1	No	Yes[e]

[a]Data from Kröger *et al*. 2013
[b]Data from Srikumar *et al*., 2014
[c]Data from Colgan *et al*., 2016
[d]Data from Chaudhuri *et al*., 2013
[e]Attenuated in pig and calf only

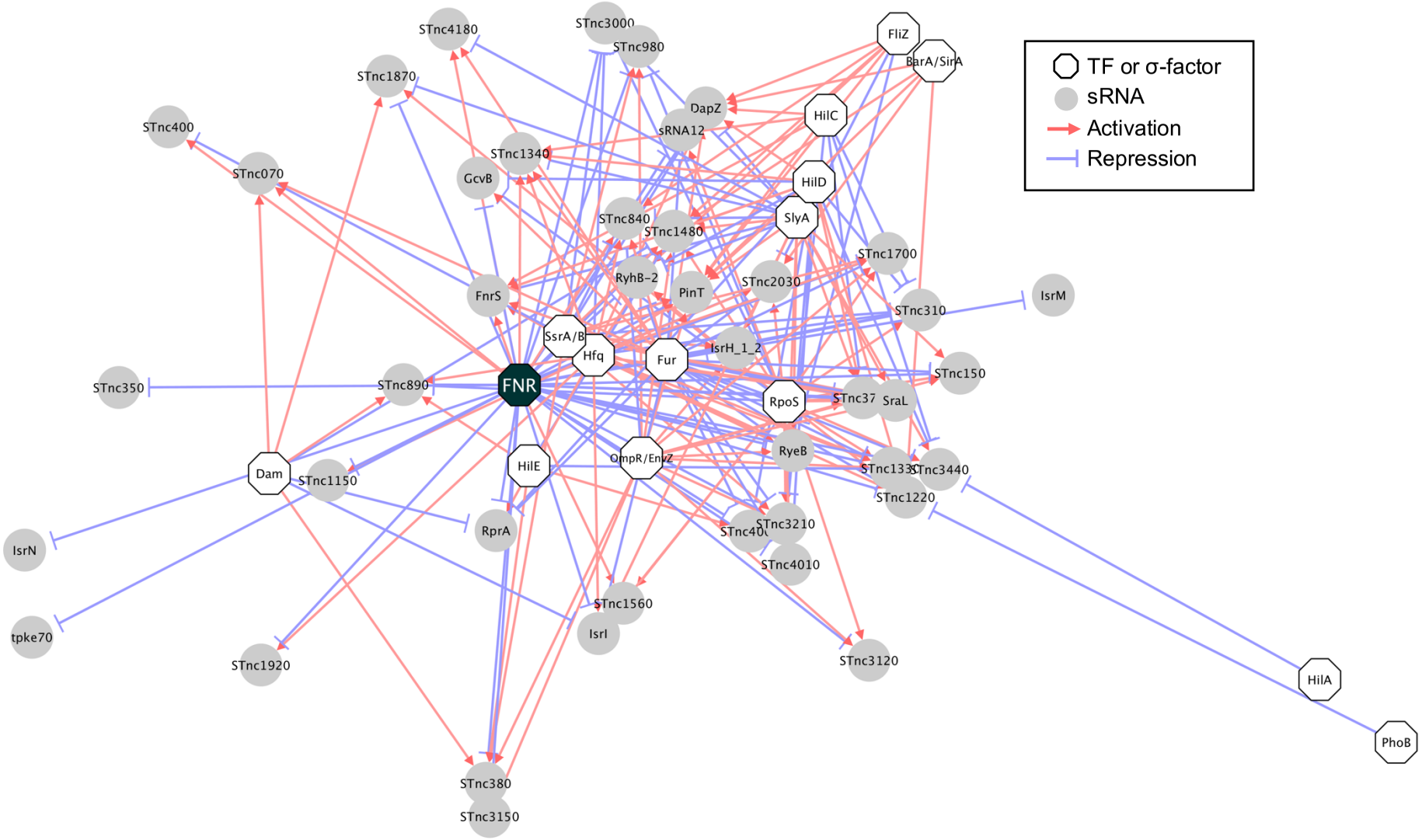

TF or σ-factor
sRNA
Activation
Repression
FNR
Fur
Hfq
SsrA/B
FnrS
RyhB-2
PinT
GcvB
HilD
HilC
HilE
HilA
SlyA
RpoS
Dam
OmpR/EnvZ
PhoB
FliZ
BarA/SirA
DapZ
sRNA12
RyeB
SraL
IsrM
IsrH_1_2
IsrN
IsrI
RprA
tpke70
STnc4180
STnc3000
STnc980
STnc1870
STnc400
STnc070
STnc1340
STnc840
STnc1480
STnc1700
STnc2030
STnc310
STnc150
STnc350
STnc890
STnc37
STnc1150
STnc1330
STnc3440
STnc1220
STnc3210
STnc40
STnc4010
STnc1560
STnc3120
STnc1920
STnc380
STnc3150

Figure 3.19 Regulatory network of FNR regulated sRNAs.

Regulatory network was generated using software from www.cytoscape.org. Regulatory interactions are based on mutant RNA-seq data from this study and Colgan *et al*., 2016. DE sRNA genes that are down-regulated in a mutant strain lacking a TF or σ-factor (regulatory protein) are putatively activated by that regulatory protein, and the interaction is represented using a red arrow from the source node (regulatory protein) to the target node (sRNA). DE sRNA genes that are up-regulated in a mutant lacking a certain regulatory protein are putatively repressed by that regulatory protein, and the interaction is represented using a blue T-shaped line from the source to the target node. The length of each line is representative of the strength of the putative interaction: nodes representing sRNAs which show higher fold-increase or decrease in a mutant strain are located closer to that regulatory protein. Regulatory proteins are hexagonal in shape and white except FNR which is black, sRNAs are circular and grey.

3.3 Discussion

To our knowledge, the work presented here is the first large scale transcriptomic study that has been carried out in an Δ*fnr* mutant of *S.* Typhimurium using high throughput next generation sequencing. We were able to corroborate existing data associating FNR as an activator of anaerobic metabolism, cell motility and chemotaxis, and reveal FNR as a newly identified repressor of *Salmonella* pathogenicity island 2. The use of RNA-seq rather than microarray-based technology has also allowed us to identify differentially expressed small non-coding RNAs in an Δ*fnr* background.

3.3.1 SPI-2 expression in MMA

It was unusual to see SPI-2 expression in the MMA media as it possesses nearly the opposite signals that are typically necessary for SPI-2 induction (Deiwick *et al.*, 1999; Löber *et al.*, 2006; Xu & Hensel, 2010). The medium contains high concentrations of Mg^{2+} and P_i compared to the SPI-2 inducing media that has been used in the past, as well as neutral pH. However, we see minimal SPI-2 expression in the WT under microaerobic conditions, and those level increase nearly 2-fold when the media is aerated; in the absence of FNR we see very high induction of SPI-2 regardless of the aeration (**Figure 3.13**). This indicates that there may be another factor involved as we expected SPI-2 expression in aerated WT cultures to reach the same level of expression. However, it is unclear if OD_{600} of 0.3 in the WT and Δ*fnr* are the same stage of growth as in microaerobic cultures therefore, a growth curve should be completed under aerated conditions in MMA. Perhaps a more informative experiment would be to grow cultures microaerobically to an OD_{600} of 0.3 and then perform an oxygen shock whilst measuring gene expression throughout. Also, it is apparent that another metabolic signal, in addition to oxygen, is important for regulation of SPI-1 or SPI-2 as expression of these genes are differentially expressed in Δ*fnr* in different growth media (**Figure 3.9**, **Figure 3.10A**) (Fink *et al.*, 2007). We may also see expression of SPI-2 in microaerobic MMA, as the expression we measured is from across the gradient in the flasks as the samples taken are a mixture of the culture. It would be informative to be able to measure the concentration of oxygen precisely of our cultures. Although the measurements that have been taken were consistent over different biological replicates (**Figure 3.3**) it would be ideal to have a more homogeneous culture from which to take measurements. The oxygen gradient gene expression assay (**Figure 3.12**) could also be improved to eliminate the confounding factor of differences in density of cell growth throughout the medium.

Because of the nature of GFP, it folds more slowly under low oxygen conditions, therefore to have adequate growth in the medium the assays were left to incubate for 72 h. If the assays were redone with our luminescent pDEW201-P_{ssaG} containing strains we could likely measure P_{ssaG} expression much earlier as the *lux* operon requires less posttranslational modifications and luminescences earlier than GFP fluoresces under low oxygen conditions. This would reduce the accumulation of cells at the surface of the agar and allow us to more easily visualize which cells are expressing SPI-2. The other problem with this assay is that again we cannot measure the exact concentration of oxygen throughout the medium, but it still provides a simple visualization of expression under a gradient of oxygen concentrations.

3.3.2 Motility and chemotaxis

Although there are some major differences between the Fink *et al*. FNR regulon and the one reported here, these are almost certainly due to the difference in growth conditions between the studies, and differences between the *S.* Typhimurium strains 4/74 (used in this study) and 14028S (Clark *et al.*, 2011; Hoiseth & Stocker, 1981; Kröger *et al.*, 2012; Richardson *et al.*, 2011). Here we have grown cells in microaerobic conditions in a minimal medium (MMA) with a glycerol carbon source, whereas the Fink et al. study grew cells anaerobically in MOPS buffered LB with added xylose (Fink *et al.*, 2007). Core anaerobic metabolism genes were found to be DE in both studies because their expression is more dependent on the availability of oxygen than other nutrients. Interestingly, flagellar, motility and chemotaxis genes were also found to be highly down-regulated in both studies. This may indicate that for expression at these loci, the availability of oxygen is more important than other nutrients or environmental signals.

3.3.3 Regulation of sRNAs by FNR

Many of sRNAs regulated by FNR are also regulated by Fur (**Figure 3.19**). Although the functional state of FNR during aerobic/anaerobic switch is not regulated by iron content and reversible binding of Fe^{2+} under physiological conditions, and FNR does not communicate with the iron pool regulating the Fur protein (Niehaus *et al.*, 1991), they share many genes in their regulons including flagellar genes and SPI-1 genes (Colgan *et al*., 2016). In the Colgan *et al*. study the Fur regulon was investigated under ESP growth in LB, which is closer to the growth conditions found in the Fink *et al*. microarray study (Colgan *et al*., 2016; Fink

et al., 2007). If grown in MMA medium, it is possible that absence of Fur may result in up-regulation of SPI-2 genes, based on the similarities of the regulons.

There were 3 sRNAs upregulated in Δ*fnr* that have been previously described as SPI-2-like as well as may other which are upregulated in SPI-2 inducing conditions and macrophages, and 6 sRNAs that have been linked to attenuation of virulence in food animals (**Table 3.2**); additionally, important virulence sRNAs *IsrM* and *IsrN* were found to be regulated by only FNR. Furthermore, IsrM is differentially expressed *in vivo*, with higher expression in the ileum than in the spleen (Gong *et al.*, 2011), the site where we propose FNR exerts control over virulence genes. In a recent study, deletion of *FnrS* led to increased HilD production under low aeration conditions, and *FnrS* could bind to the *hilD* mRNA 5' UTR, resulting in translational repression (Kim *et al.*, 2018). The authors discuss the importance of *FnrS* on SPI-1 expression however fail to mention the potential impact of HilD-mediated crosstalk between SPI-1 and SPI-2 (Bustamante *et al.*, 2008). Our data reveal that *FnrS* expression is dramatically decreased in Δ*fnr* and in aerated WT cultures (**Figure 3.2C**), and SPI-2 expression is increased under these conditions (**Figure 3.13**); in the absence of *FnrS*, HilD levels may be increased, contributing to the SPI-2 induction phenotype that we have observed. These data confirm that FNR is an important regulator of sRNAs involved in virulence.

3.3.4 Comparison of FNR regulons

While comparisons to the FNR regulon in *E. coli* can be useful for widely conserved genes, *Salmonella* specific genes are obviously missed. The only existing study identifying the FNR regulon in *S.* Typhimurium through transcriptomic data was published in 2007 and was accomplished using a DNA microarray (Fink *et al.*, 2007). The drawbacks to using microarrays for transcriptomic analysis include low resolution with high background due to non-specific hybridisation between cDNA and probes (Aikawa *et al.*, 2010) and the dynamic range of microarrays is limited by the use of fluorescently-labelled DNA, detection of which can be saturated and subtle changes in gene expression may not be detected. RNA-seq allows all transcription to be studied without bias and without prior knowledge of the DNA sequences that are being transcribed, and with no limits on the dynamic range (Croucher & Thomson, 2010). The use of RNA-seq in this study as well as a different growth condition allows us to expand the existing FNR regulon to include new genes as well as not previously identified sRNAs.

In this study, the FNR regulon was examined under microaerobic conditions in MMA and analysed by RNA-seq. In **Figure 3.20** the FNR regulon as determined by this study was compared to the FNR regulon under anaerobic conditions in MOPs buffered LB with xylose as determined by microarray analysis (Fink *et al.*, 2007). Only genes with ≥2.5-fold change in expression in the Δ*fnr* mutant compared to WT were included in the comparison between the two studies. In total 75 genes were found in common in both regulons (**Figure 3.20A**). Sixty-nine genes in common were down-regulated in both studies (**Figure 3.20B**). These comprised mostly of genes which encode components of flagella biosynthesis, motility and chemotaxis regulons (discussed in more detail in Section 3.2.8) and genes involved in anaerobic systems, including *aer*, *dmsABC, dmsA1, dmsA2, nrdD, nrfA*, STM2530, and STM4305 – 4307. The *Salmonella* specific virulence proteins encoded by *srfABC* were also found to be down regulated in both studies. These SsrB-regulated factors are horizontally acquired genes and as the name would indicate, regulated by the SPI-2 encoded SsrB protein, but are expressed in SPI-1 inducing conditions and have been found to be repressed by PhoP and RcsB (Garcia-Calderon *et al.*, 2007; Worley *et al.*, 2000).

Interestingly, 3 genes found to be up-regulated in the microarray study, were down-regulated in our data set: *dcuB*, *fumB* and *nuoJ*. DcuB functions primarily as a fumarate:succinate antiporter during anaerobic fumarate respiration (Six *et al.*, 1994) and FumB is one of three fumarase isozymes participating in the TCA cycle (Woods *et al.*, 1988). These two proteins are involved in anaerobic metabolism and are induced under anaerobic conditions in *E. coli*, consistent with our data. NuoJ is part of the inner membrane component of NADH dehydrogenase I and is typically repressed by FNR (Leif *et al.*, 1995), which is consistent with data from Fink et al. Additionally, one gene that was down-regulated in Fink et al. was upregulated in our study. This was the *osmY* gene, that encodes for the periplasmic chaperone protein OsmY, that is induced under conditions of hyperosmotic stress (Yim & Villarejo, 1992). These discrepancies between the regulons indicate FNR can activate and repress expression of genes depending on the growth conditions.

Also identified in this study were 42 sRNAs which were DE in Δ*fnr*. None of these sRNA genes could be detected by the microarray analysis. Included in this group is the sRNA *FnrS*, which is an anaerobically-inducible, FNR-dependent sRNA conserved in *E. coli* (Boysen *et al.*, 2010; Durand & Storz, 2010). Recently, *FnrS* was found to regulate SPI-1 expression by

repression of *hilD* translation (Kim *et al.*, 2018). These will be discussed further in Section 3.2.9.

Many of the genes which are only DE in the microarray-based study included genes encoding SPI-l regulators, components of the T3SS and effector proteins. In our data set, expression of SPI-1 genes in Δ*fnr* generally trended downward however their differential expression was less than 2.5-fold and the genes were either not or only lowly expressed in the WT and Δ*fnr* under microaerobic growth in MMA (**Figure 3.10A**). It is unclear from the Fink *et al*. study whether SPI-l-associated genes are directly regulated by FNR or if the FNR-dependence of these genes is due to FNR-mediated down-regulation of the genes encoding FliZ and FliA, which play roles in the regulation of SPI-l (Lucas & Lee, 2001). Another explanation may be that the disruption in anaerobic metabolism in the absence of FNR leads to induction of *hilA* via BarA/SirA, due to accumulation of fatty acid metabolites, such as acetate (Lawhon *et al.*, 2002). As mentioned previously, *FnrS* which was not identified in the Fink *et al*. study has recently been implicated in repression of *hilD* translation (Kim *et al.*, 2018), and thus may have played a role down-regulation of SPI-1 under the growth conditions in Fink *et al*. (Fink *et al.*, 2007). The differences in oxygen levels and thus in differential anaerobic metabolism gene expression may account for the variation in SPI-l gene expression between the two studies. Another difference in the data sets were the significant number of genes encoding proteins for ethanolamine utilization in Fink *et al*. study that were not identified in our dataset. Ethanolamine is available in the host and can be used by *S*. Typhimurium as a source of carbon, nitrogen, and energy to outcompete host microbiota but, is not necessary for virulence in mice (Thiennimitr *et al.*, 2011). The lack of expression at this operon could be explained by the absence of ethanolamine in MMA, or the expression may have to do with a component of LB as *eut* genes are upregulated in LB in anaerobic growth (Kröger *et al.*, 2013).

The most striking difference between the two datasets is the increased expression of genes encoding SPI-2 regulators, components of the T3SS apparatus and effector proteins in this study (**Figure 3.7** & **Figure 3.9**). These genes are not up-regulated in Δ*fnr* in the study by Fink *et al*, except for *sopD2* and *sseL*, which are common to both datasets (**Figure 3.20C**). In the microarray study *sopD2* and *sseL* were up-regulated in Δ*fnr* 3.0- and 3.5-fold respectively, in this study, they were up-regulated 7.9- and 6.4-fold. These effectors proteins are expressed in SPI-2 inducing conditions and in macrophages but, are not encoded within SPI-2 (Kröger *et al.*, 2013; Srikumar *et al.*, 2015). SopD2 is important for formation of

Salmonella-induced filaments and interference with endosome to lysosome trafficking, while SseL induces late macrophage cell death (Jennings *et al.*, 2017). Fink *et al*. did not detect differential gene expression of any other of SPI-2 genes, however they found that the Δ*fnr* mutant is attenuated for survival and replication within murine macrophages. This result would be consistent with FNR regulation of SPI-2 gene expression but was not detected under the conditions used in the microarray-based study.

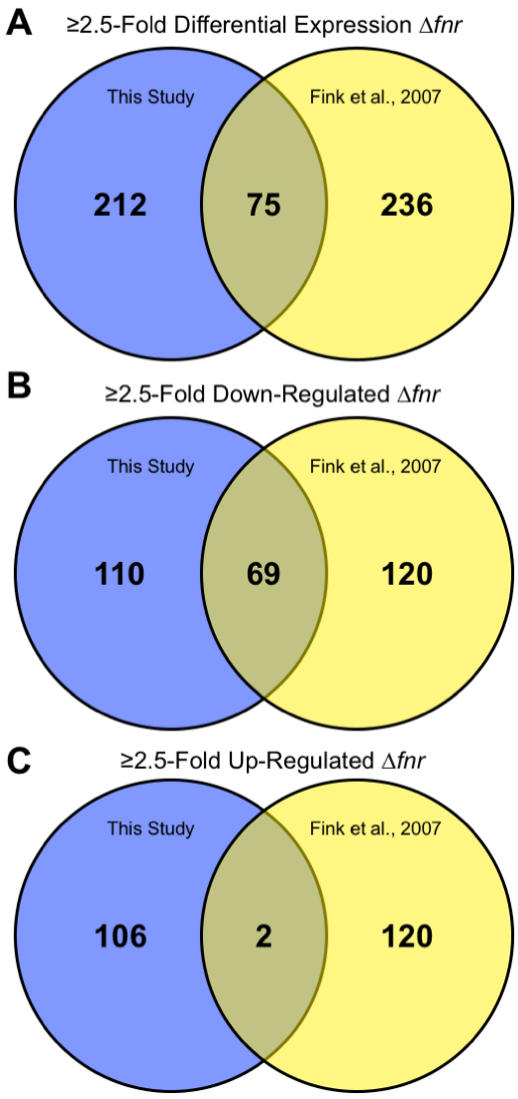


Figure 3.20 Comparison of FNR regulons.

FNR regulon under micro-aerobic conditions, as analysed by RNA-seq (this study, blue circles), compared to the FNR regulon under anaerobic conditions as determined by microarray (Fink *et al*., 2007, yellow circles). Comparisons were made between genes that were >2.5-fold DE in an Δ*fnr* mutant where A. compares the entire regulon, B. compares down-regulated genes, and C. compares up-regulated genes.

3.3.5 The benefits and limitations of an RNA-seq-based approach for the investigation of bacterial regulons

RNA-seq provides convenient and precise method for studying gene expression at single nucleotide resolution. Large amounts of data can be generated rapidly and simply, relative to more laborious protocols such as DNA microarrays, RT-qPCR or transcriptional fusions. Extraction of good quality total or depleted RNA is important, and conveniently most sequencing facilities offer cDNA library preparation services to ensure consistent high-quality samples for sequencing. Sequencing technologies themselves are improving and advancing regularly, resulting in the generation of large amounts of good quality data, within a short timescale. RNA-seq technology is more accessible than ever to researchers, meaning that high throughput next generation sequencing and RNA-seq are contributing to the expansion of our knowledge base. The development of tools for bioinformatics and data handling programs permit rapid and convenient downstream analyses, allowing us to distil the important information from the massive data sets that are produced using RNA-seq. We have shown that the use of RNA-seq to determine the FNR regulon provides accurate information that agrees with what has been previously shown using transcriptional fusions and microarrays in *E. coli* and *S.* Typhimurium, but the increased sensitivity and dynamic range of RNA-seq allowed identification more subtle changes in gene expression, as well as to detect expression of non-coding sRNA species, thereby increasing our understanding of complex regulatory pathways.

However, there are some limitations to the approach used in this study. RNA is extracted from a population of bacteria for use in RNA-seq; this provides an average view of the transcriptomes of millions of cells. Additionally, our growth condition is heterogenous in nature, meaning we likely have very different expression in subpopulations throughout the oxygen gradient of the cultures. Genetically identical cells can often be phenotypically different and subpopulations of cells or outlier cells play important roles in disease outcome or antibiotic tolerance (Diard *et al.*, 2013; Helaine *et al.*, 2014). Single-cell RNA sequencing can reveal complex and rare cell populations, uncover regulatory relationships between genes. Single-cell transcriptomic analysis will allow the detection of even more subtle, and often biologically significant, changes in gene expression that could be masked at the level of whole bacterial populations (Hwang *et al.*, 2018).

RNA-seq of strains and their isogenic mutants can reveal a multitude of changes in gene expression however, it cannot distinguish between direct and indirect regulation. The complexity of bacterial regulatory networks and the interplay between different regulators means that many of the putative interactions that are detected in the absence of a TF are as a result of perturbations in regulatory cascades rather than direct control of gene expression. Therefore, it is important to combine RNA-seq with techniques which uncover direct regulation of genes such as chromatin immunoprecipitation sequencing and will be covered in detail in Chapter 4.

RNA-seq provides important clues about gene expression and regulatory interactions but important findings must be validated using additional molecular biological approaches. To provide a better understanding of the inherent complexity of biological systems, complete descriptions of biological molecular networks, including important environmental stimuli, transcription factors, regulatory proteins, sRNAs, and all other components (Golubeva *et al.*, 2012; Hébrard *et al.*, 2011). Complete descriptions of biological systems can be achieved through the integration of information from a combination of datasets, including transcriptomics, epigenomics, proteomics, global ChIP studies, and others. Great resources are available for *E. coli* including RegulonDB, EcoCyc, GenExpDB and UniProt which provide curated information on *E. coli* gene and protein expression from thousands of transcriptomic experiments (Hébrard *et al.*, 2011; The UniProt Consortium, 2017), and much information from *S.* Typhimurium is available through SalCom, SalComMac and SalComRegulon (Colgan *et al.*, 2016; Kröger *et al.*, 2013; Srikumar *et al.*, 2015). Despite decades of research, the complexities of the *E. coli* and *S.* Typhimurium genomes are only becoming realized in recent times thanks to NGS technologies. Our hope is that this transcriptomic analysis of *S.* Typhimurium FNR regulon and its role in regulation of virulence will contribute to the overall understanding of *S.* Typhimurium infection.

3.3.6 Summary

The aim of this chapter was to investigate the global changes in gene expression in an Δ*fnr* mutant with a focus on *Salmonella* Pathogenicity Island 2, flagella and motility in *S.* Typhimurium. We have shown using a wide variety of techniques that under microaerobic conditions in MMA that the absence of the FNR protein causes SPI-2 encoded and associated genes and sRNAs to have increased expression and flagellar and motility genes to have decreased expression when compared to the WT. These results indicate that when *S.*

Typhimuirum cells are exposed to oxygen (like at the epithelial border in the gut), and FNR dimers are no longer active in the cell, that SPI-2 expression increases and motility and flagellar gene expression decreases, likely in preparation for the harsh intracellar environment of host epithelial cells.

Chapter 4

Promoter occupancy of FNR

4.1 Introduction

4.1.1 Identification of global FNR binding through ChIP-seq

Transcription factors that bind to specific DNA sequences are essential for the correct regulation of gene expression and identification of the specific DNA sequences that each factor binds helps to elucidate regulatory networks within bacteria (Browning & Busby, 2004; Myers *et al.*, 2015). Traditional methods for determining the specificity of DNA-binding proteins such as electrophoretic mobility shift assays (EMSA) are important for showing direct binding but are performed under *in vitro* conditions and thus disregard any other factors which may be important for binding *in vivo*. They are also too slow and laborious to investigate an entire genome. In contrast, a high-throughput method such as chromatin immunoprecipitation sequencing (ChIP-seq) can provide global *in vivo* binding information rapidly (Stormo & Zhao, 2010). Regulatory mutants can be used to investigate bacterial regulons using RNA-seq, and the technique is able to provide candidate genes that a transcription factor is likely to regulate however, it is unable to discriminate whether regulation is direct or indirect. In Chapter 3, we identified FNR as a repressor of SPI-2 genes under microaerobic conditions in MMA, using RNA-seq, and here we used another large-scale investigative technique, ChIP-seq, to identify whether FNR repression of SPI-2 genes is direct or indirect. A general workflow of the main experimental procedure is outlined in **Figure 4.1**.

DNA-protein binding experiments, such as EMSA, were traditionally used to demonstrate that a protein could directly bind a specific fragment of DNA, but more recently ChIP has become the method of choice to provide evidence of direct protein binding *in vivo*. Originally, ChIP experiments were followed by RT-qPCR to determine protein binding within particular sequences of interest by examining enrichment of DNA from the immunoprecipitated sample, or by ChlP-chip where protein-associated DNA is hybridized to a tiling microarray to determine all DNA binding sites of a particular protein. Within the last decade, ChlP-chip has been replaced with ChIP-seq which has a much higher resolution and signal-to-noise ratio than ChIP-chip and involves the use of deep sequencing technology to determine all DNA molecules bound by DNA-associated proteins (Cai & Huang, 2012; Myers *et al.*, 2015). Sequencing-based approaches have increased sensitivity and reduced bias compared to microarray-based technology. ChlP-seq also allows for the identification of transcription factor binding site sequences at the level of the individual nucleotide and

can be used to accurately determine transcription factor binding motifs. Motif analysis can predict direct protein binding sites and provide focused data on highly likely candidate gene targets. The combination of transcriptomic data from WT and Δ*fnr*, with FNR occupancy data obtained under the same growth conditions can associate the changes in gene expression to binding and direct regulation by FNR. To our knowledge, this study provides the first large scale investigation into DNA-protein interactions of the oxygen sensitive transcription factor FNR in *S.* Typhimurium.

The aim of this chapter is to use the global approach of ChIP-seq to identify candidate regulators of SPI-2 in the case of indirect regulation of SPI-2 through FNR or to identify FNR as a direct regulator of genes encoded on the pathogenicity island, and to verify those results with other molecular techniques.

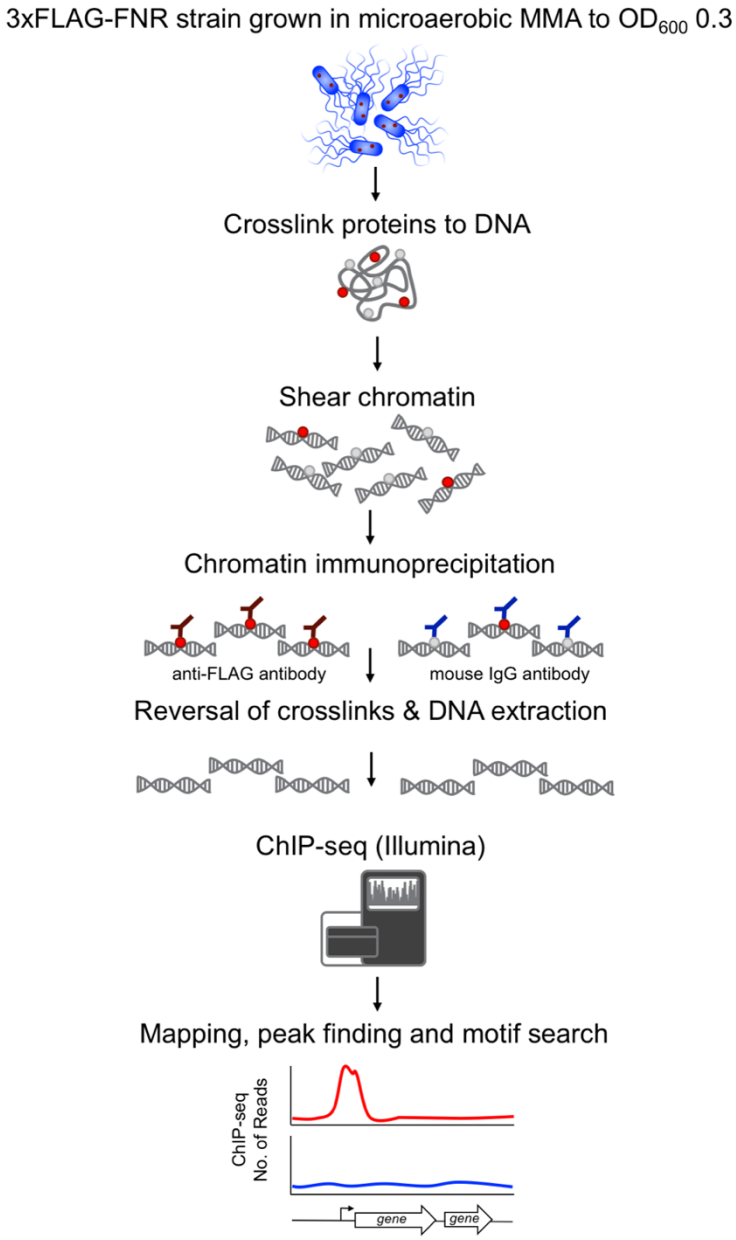


Figure 4.1 Workflow of chromatin immunoprecipitation sequencing to identify FNR binding sites.

A 3×FLAG-FNR strain was grown under microaerobic conditions to OD_{600} 0.3 in MMA. Proteins were crosslinked to DNA, chromatin was sheared then immunoprecipitated by and anti-FLAG antibody to identify specific FNR binding and by a mouse IgG antibody for background non-specific binding. Crosslinks were reversed, and DNA was extracted and prepared for sequencing. Library preparation and sequencing for replicate 1 was performed by me and replicate 2 was performed by Dr. Keith MacKenzie at the University of Regina, Regina, Saskatchewan, Canada. Bioinformatic analyses were performed by Dr. Aalap Mogre at Trinity College Dublin, Dublin, Ireland.

4.2 Results

4.2.1 Verification of 3×FLAG-FNR

For ChIP-seq experiments a strain with a 3×FLAG-tagged FNR was used (originally constructed by Dr. Aoife Colgan). To ensure that the 3×FLAG-tag did not interfere with the binding capability of the FNR protein a northern blot was performed looking at expression of the FNR dependent small RNA *FnrS* (**Figure 4.2A**). There was high expression of *FnrS* in the 3×FLAG-tagged strain although slightly lower than WT levels, it was higher than in the Δ*fnr* mutant. A preliminary ChIP-RTqPCR experiment was performed to determine enrichment of the ChIP DNA of a positive control and a negative control, the *fnrS* promoter and *hemX* respectively (**Figure 4.2B & C**). The amplified ChIP DNA was normalized to the amplified Input DNA, which was extracted prior to immunoprecipitation. Normalised ChIP-RTqPCR data from two independent biological replicate experiments and demonstrates that there is strong enrichment (approximately 16-fold) of the *fnrS* promoter region in the experimental ChIP DNA, compared to the background "Mock" ChIP DNA. The negative control gene, *hemX*, showed negligible enrichment (approximately 1-fold) in the experimental ChIP DNA sample, compared to the mock ChIP DNA sample (**Figure 4.2B**). Following subtraction of the Mock ChIP DNA, the *fnrS* promoter DNA was approximately 31-fold enriched for FNR binding, compared to the negative control region, *hemX* (**Figure 4.2C**). These data show that FNR specifically binds within the *fnrS* promoter region, FNR is a direct activator of *FnrS* transcription, and the 3×FLAG-tagged FNR can be confidently used for ChIP-seq experiments.

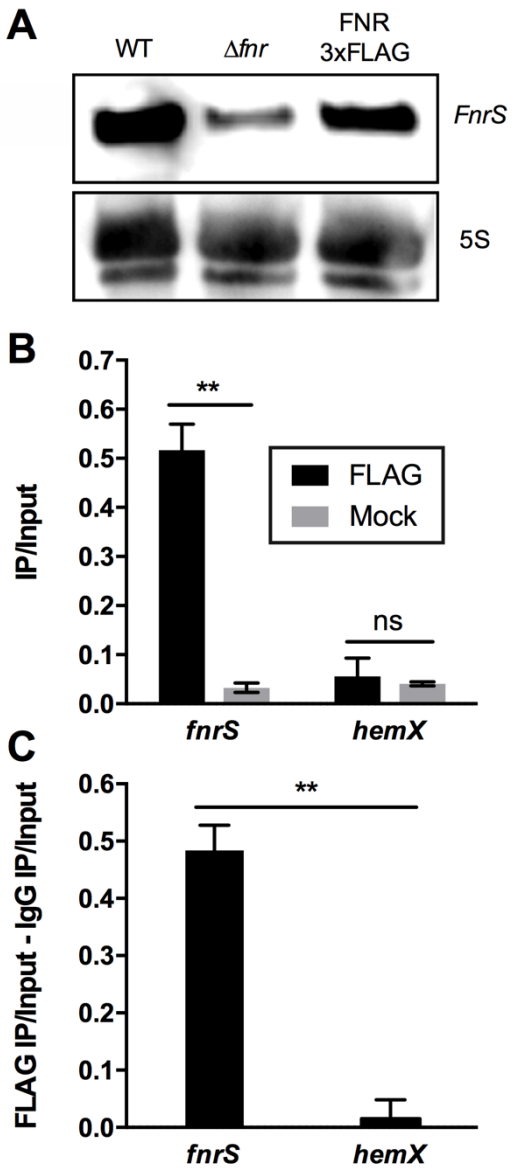


Figure 4.2 FnrS is expressed in FNR-3×FLAG and the *fnrS* gene is bound by FNR-3×FLAG.

A. Northern blot showing FnrS expression is not affected in a strain expressing FLAG-tagged FNR under microaerobic conditions in MMA. 5S rRNA was probed as loading control. B. qPCR data of a representative ChIP assay from 2 independent experiments demonstrating direct binding of FNR to the *fnrS* gene under microaerobic conditions in MMA. Experimental (FLAG) and mock ChIP DNA from *fnrS* and the negative control *hemX*, was normalised to the starting amount of DNA (IP/Input). Statistical significance determined by t-test, $\alpha = 5\%$ C. qPCR data of a representative ChIP assay from 2 independent experiments shows enrichment for FNR binding of *fnrS*, compared to the negative control region *hemX* following subtraction of background mock ChIP DNA (as described in section 2.8.4). Statistical significance determined by Unpaired two-tailed t-test, $\alpha = 5\%$. * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, error bars represent standard deviation.

4.2.2 Overview of FNR binding using ChIP-seq

Chromatin Immunoprecipitation is the method of choice to provide evidence for direct protein binding *in vivo*. ChIP-seq involves the use of deep sequencing technology to determine all DNA molecules bound by DNA-associated proteins (Cai & Huang, 2012). Here, we have used ChIP-seq to determine FNR binding under microaerobic conditions in MMA with a strain containing 3×FLAG-tagged FNR. **Figure 4.3** provides an overview of all the predicted FNR binding sites (blue lines and boxes) and FNR binding motifs (red lines) across the *S*. Typhimurium chromosome and two plasmids, pSLT and pColIB9 from two independent ChIP-seq experiments. There were no FNR binding sites on the pRSF1010 plasmid which was anticipated as there were no DE genes found on the plasmid (see Section 3.2.5). Binding of the global transcription factor FNR was uniform across the chromosome. Due to contamination of *S. cerevisiae* yeast genomic DNA from the tRNA used as a co-precipitant intended to increase DNA yields in the ChIP protocol the coverage of ChIP (FLAG and Mock) samples were reduced. Additionally, coverage of the FLAG 1 sample was lower than of FLAG 2 (**Figure 4.4A**) However, the coverage was sufficient to recover a similar number of peaks from both samples, and there was a large of overlap of 288 peak locations (**Figure 4.4B**).

Approximately 19% of differentially expressed genes in this study were associated with peaks from ChIP-seq (**Figure 4.4C**). As anticipated, the *fnr* promoter was bound by FNR. Also, *fnrS* was associated with FNR binding, as were promoters of *nrdD,* the anaerobic ribonucleoside-triphosphate reductase, *nirB,* the large nitrite reductase subunit, and *nuoA*, NADH dehydrogenase I chain A. The extremely downregulated SPI-5 encoded gene *orfX* and the minor curli fimbrae subunit *csgB* were also associated with FNR binding. These genes are therefore directly activated by FNR under microaerobic growth in MMA. Notable up-regulated genes with FNR binding sites included the sRNA *GcvB*, which was highly upregulated in Δ*fnr* and controls approximately 1% of the *Salmonella* genome (Hébrard *et al.*, 2012; Sharma *et al.*, 2007; 2011), and PhoP activated genes *pagC*, *pagD* and *pagO*. FNR occupancy was also observed at the promoter of the plasmid encoded virulence gene and regulator, *spvR*. Many effector proteins which are secreted by the SPI-2 T3SS were associated with peaks including *sseK2*, *sifB*, SPI-5 encoded *pipB*, effectors important for SCV maturation *sifA*, *sseJ* and *sopD2*, and *Salmonella* translocated effectors *steA*, *steB* and *steC*. The important virulence sRNA *IsrM* was also found to be bound by FNR. Within the SPI-2 locus, specific T3SS apparatus genes associated with FNR binding included *ssaH*,

ssaI, *ssaJ* and *ssaU*, but most importantly an FNR binding peak was found near the promoter of the SPI-2 response regulator *ssrB*. Additionally, a long binding region was identified in one replicate at the *ssrA*/*ssaB* promoter region. These results indicate that FNR may repress SPI-2 under microaerobic conditions by direct repression of several effectors and other virulence associated genes, and especially through *ssrB*.

Approximately 81% of DE genes were not associated with FNR occupancy (**Figure 4.4C**) and, therefore, indirectly regulated by FNR under these conditions. DE genes not associated with FNR binding included the majority of flagellar and chemotaxis genes. However, *fliC* and *fliD* were associated with a peak, as well as *aer*, the gene encoding Aer the signal transducer for aerotaxis. FNR therefore, may indirectly activate certain chemotaxis and flagellar motility through direct activation of *aer*. Additionally, a recent study found that SsrB can repress *S.* Typhimurium motility through direct binding to a region upstream of *flhDC* (Ilyas *et al.*, 2018). Thus, much of the down-regulation of motility, especially of early gene products can be explained by FNR activation of *ssrB*, leading to SsrB repression of motility. Other genes which seem to be indirectly regulated by FNR include the propanediol degradation and cobalamin (vitamin B12) biosynthesis operons, SPI-1 genes *sipB* and *invJ*, and many of the T3SS apparatus and effector genes encoded within SPI-2.

Interestingly, many peaks were identified at genes not found to be DE in our study. This was also the case in an *E. coli* ChIP-seq from a previous study (Myers *et al.*, 2013). Genes of particular interest were all six SPI-4 encoded *sii Salmonella* intestinal invasion genes and SPI-1 encoded genes *avrA*, *sprB*, *hilC*, *prgH*, *hilD*, *hilA*, *iagB*, *sptP*, *sicP*, *invB*, *invA*, *invH*, *InvR*, STM2902, and STM2903. Regulation by FNR may be masked by other more significant factors that also regulate transcription at these loci positively or negatively. The full list of ChIP-seq peaks and ChIP peaks associated to DE genes can be found in **Tables S4**, **S5**, and **S6** in Appendix III.

4.2.3 FNR consensus binding motif

Peak sequences from ChIP-seq revealed a palindromic FNR binding motif with a length of 14 bp (**Figure 4.5**). The consensus motif was, TTGATNTRSATCAA, where A is adenine, C is cytosine, G is guanine, T is thymine, N is any nucleotide, R is a purine, and S is guanine or cytosine. This consensus motif is almost identical to the FNR consensus binding motifs found in *E. coli* (Myers *et al.*, 2013; Spiro & Guest, 1990) and similar to the previously

described *S.* Typhimurium FNR binding motif. The letter-probability matrix generated by MEME-ChIP was used to find all possible FNR binding sites in the *S.* Typhimurium chromosome using FIMO. The letter-probability matrix of the *S.* Typhimurum FNR binding motif and the top 100 FNR binding motifs found in the *S.* Typhimurium 4/74 chromosome can be found in **Tables S7** and **S8** in Appendix III. A conserved and experimentally confirmed FNR binding site can be found in the *ndh* (NADH dehydrogenase) promoter region in *E. coli*, whereby binding of FNR leads to activation (Green & Guest, 1994). We searched for this site as a positive control for our FNR motif and FIMO found an FNR binding site in the *S.* Typhimurium *ndh* promoter region centered around -48 from the *ndh* TSS (**Figure 4.5**). The two sites with the closest to consensus binding motifs were found in the promoters of *nirB* and *orf70* centered around -44 and -41, respectively. The large subunit of the NADH nitrite reductase is encoded by *nirB*, and FNR is known to activate at this site. Indeed, in our dataset *nirB* is down-regulated in Δ*fnr* and a ChIP peak was observed for FNR binding at this site. Interestingly, *orf70* is a gene found in the non-T3SS section of SPI-2. A BLAST search revealed that *orf70* shares 99% identity with a fumarase encoding gene, *fumD* (Kronen & Berg, 2015). Under our growth condition, *orf70* was not differentially expressed but the promoter region was enriched in ChIP-seq. As expected, the *fnr* and *fnrS* promoter regions contained FNR binding motifs at +1 and -42, respectively.

Although there was enrichment for FNR binding across most of SPI-2 in our FLAG 2 sample and peaks called in both replicates at the *ssrB* promoter, no full 14 bp motifs were detected. However, when half of the consensus motif was used as a search query many putative sites were detected in the SPI-2 promoters. FNR half-sites have previously been confirmed to be sufficient for FNR binding and regulation (Constantinidou *et al.*, 2006; Melville & Gunsalus, 1996). The most likely candidate half sites for the *ssrB* and *ssrA*/*ssaB* promoter regions, based on their location are shown (**Figure 4.5**). Interestingly, both a full FNR binding motif and a half binding motif were found at the promoter of *ydgT*. As previously described, YdgT is a known repressor of SPI-2 (Coombes *et al.*, 2005). Again, the *ydgT* gene was not found to be differentially expressed in our data set, however, an enrichment for FNR binding was found in the ChIP data. These half-sites represent putative binding sites for FNR which may explain regulation of SPI-2 under microaerobic conditions.

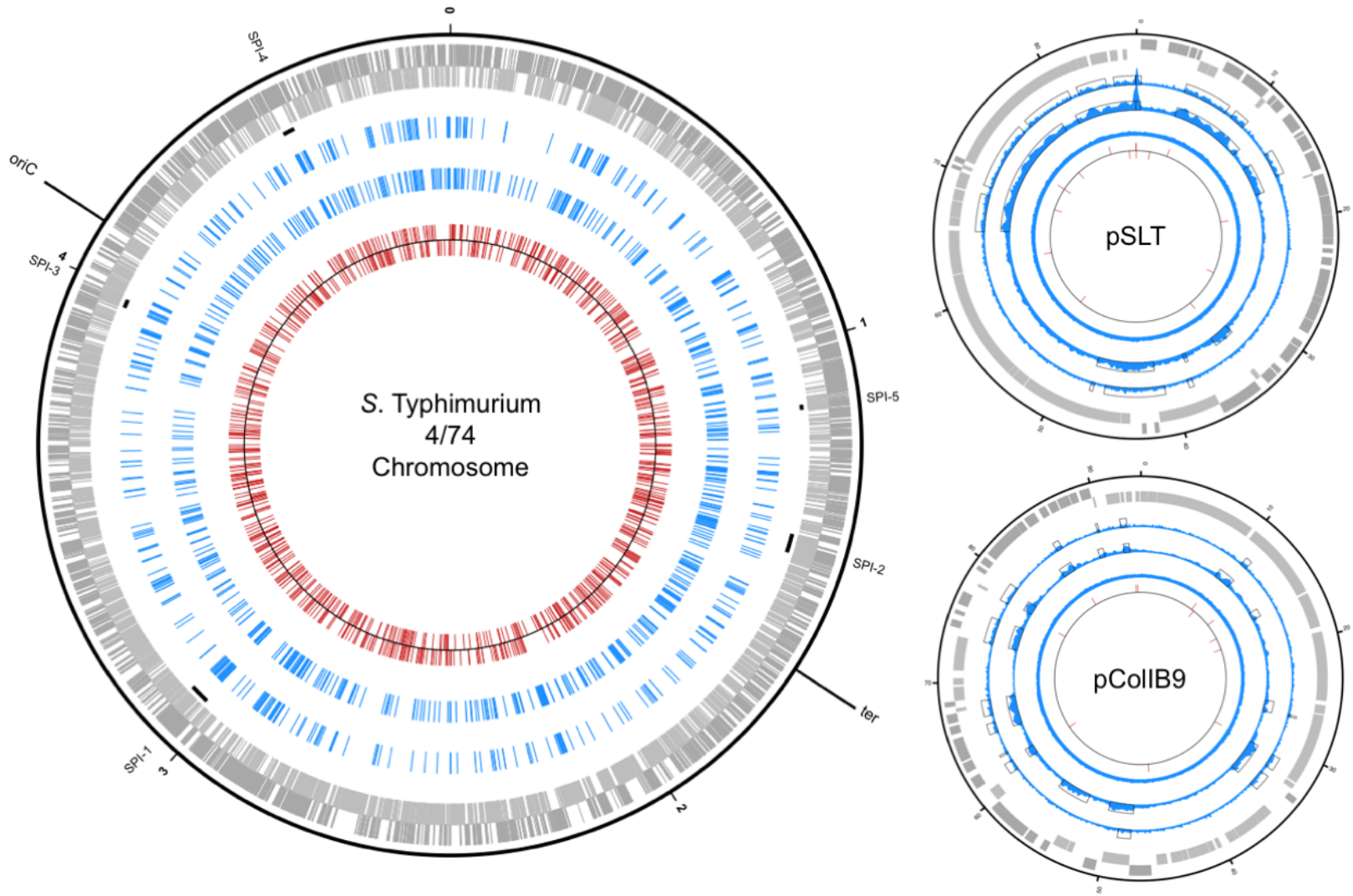

S. Typhimurium
4/74
Chromosome
oriC
ter
SPI-1
SPI-2
SPI-3
SPI-4
SPI-5
0
1
2
3
4
pSLT
pColIB9

Figure 4.3 FNR ChIP peaks across 2 replicates on the 4/74 chromosome and plasmids. Peaks representing putative FNR binding sites were determined by MACS2 across two independent replicates on the *S.* Typhimurium chromosome and pSLT and pColIB9 plasmids. From outside to inside: coordinates are denoted, on the chromosome SPIs 1 through 5 are labeled; plus strand genes are dark grey; minus strand genes are light grey; replicate 1 peaks are blue, represented by lines on the chromosome and coverage trace for the plasmids; replicate 2 peaks are blue; on plasmids only, mock coverage trace is in blue; FIMO predicted FNR binding sites based on the consensus motif on plus strand are in red; and the minus strand in red. There were no peaks on the pRSF1010 plasmid.

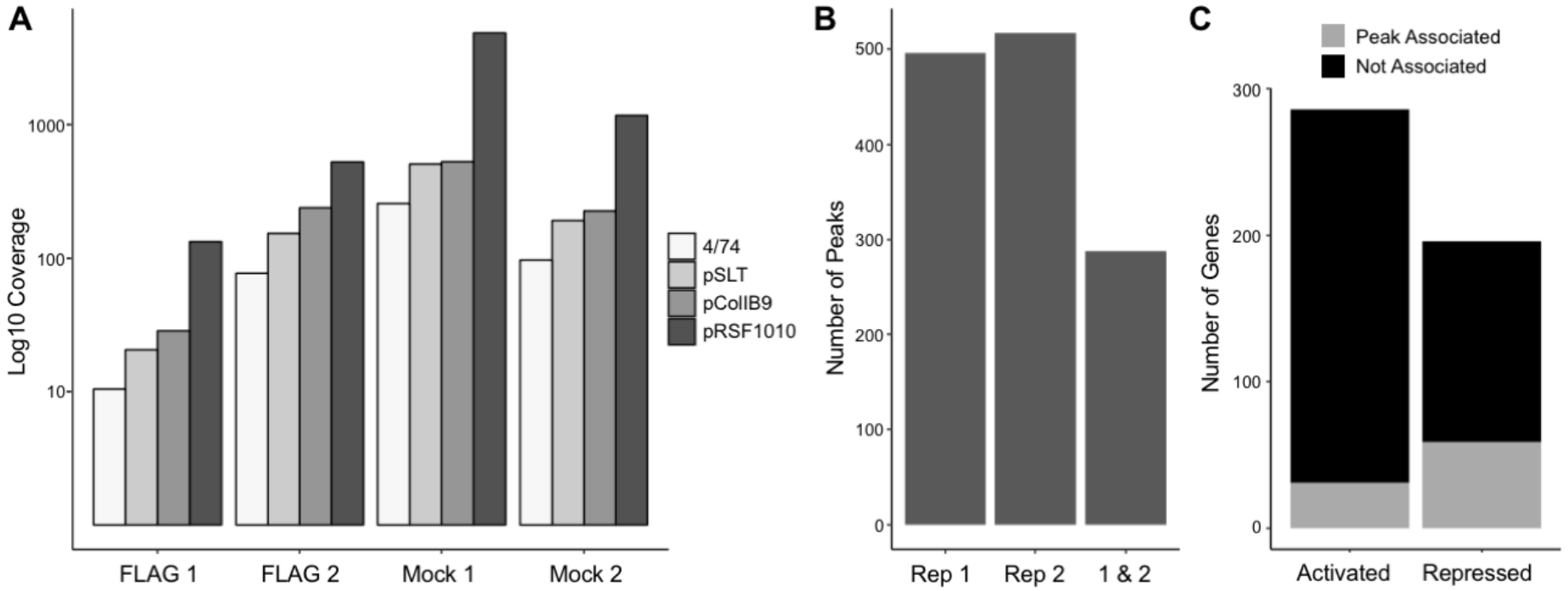


Figure 4.4 Overview of two independent ChIP-seq replicates.

A. Sequencing coverage for each ChIP FLAG and mock replicates divided between the 4/74 chromosome and plasmids. B. Total number of peaks found in each of the ChIP FLAG replicates 1 and 2 and the number of peaks that were common between the two replicates. C. Number of differentially expressed genes in Δ*fnr* from transcriptomic data associated with peaks from ChIP-seq analysis.

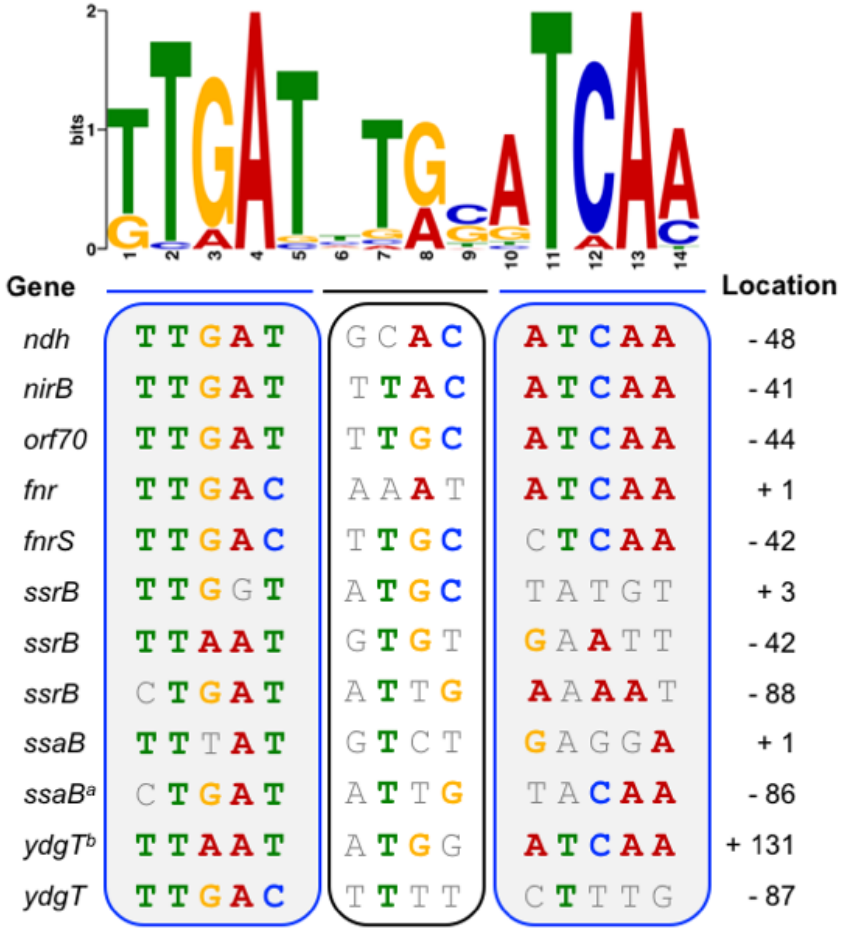


Figure 4.5 FNR binding motifs.

FNR position weight matrix was constructed from the FNR ChIP-seq peak sequences from 288 peaks common to 2 independent ChIP-seq replicates. The height (y-axis) of the letters represents the degree of conservation at that position within the aligned sequence set (in bits), with perfect conservation being 2 bits. The x-axis shows the position of each base (1–14) starting at the 5′ end of the motif. Under the consensus motif, a sample of full motifs and half sites were found using the 14 bp and 7 bp of the MEME-ChIP generated motif matrix in FIMO, respectively. Only statistically significant binding sites with $p < 0.0001$ for full motifs and < 0.01 for half sites were interrogated. [a]This half motif overlaps the 2° *ssrA* TSS. [b]This motif is found overlapping a TSS of an antisense transcript found in the *ydgT* promoter region.

4.2.4 Verification of FNR binding

To verify putative FNR binding sites binding experiments, such as electrophoretic mobility shift assays (EMSA), have been used to demonstrate that a protein can directly bind a specific fragment of DNA. However, the native FNR protein cannot be easily used for *in*

vitro experiments, as atmospheric oxygen levels render the protein inactive (Beinert & Kiley, 1999; Green *et al.*, 2014); therefore, a modified FNR must be used. Bates *et al*. originally showed that a mutation resulting in a switch from aspartic acid to alanine at position 154 (D154A) in the dimerization domain of the FNR monomer, resulted in a mutant FNR protein which could be purified as a dimer at atmospheric oxygen levels in *E. coli* (Bates *et al.*, 1995). We introduced the same mutation into our plasmid-borne *S*. Typhimurium *fnr* to create p*fnrD154A*. The WT and Δ*fnr* mutant were transformed with both p*fnr* and p*fnrD154A*, grown in MMA under aerobic and microaerobic conditions, and *FnrS* expression was used to determine the activity of the FNR or FNRD154A protein. We saw similar levels of *FnrS* expression in cells with an FNR or FNR154A protein under microaerobic conditions, while there was a very limited expression of *FnrS* under aerobic conditions in p*fnr* cells, expression of *FnrS* was near WT microaerobic levels in the presence of FNRD154A (**Figure 4.6**). The expression in p*fnr* under aerobic conditions might be explained by the high copy number of the plasmid. Shan *et al*. were able to construct a more oxygen tolerant FNR variant by covalently linking two FNRD154A monomers in *E. coli* (Shan *et al.*, 2012a). $(FNRD154A)_2$ recognizes an FNR-dependent promoter under aerobic conditions and maintains its promoter specificity and conformational stability (Shan *et al.*, 2012a). Conveniently, the FNR protein is extremely well conserved at the amino acid level with the *Enterobacteriaceae* family. The amino acid sequences of FNR in *S*. Typhimurium and *E. coli* share 99.2% identity and 99.6% similarity (**Figure 4.7**). Serine (S) and asparagine (N) are neutral polar amino acids, and this substitution has no effect. Alanine (A) is a small non-reactive amino acid, a change to glutamine (Q) is significant as glutamine is much larger and polar neutral amino acid (Betts & Russell, 2003) however, the 2 amino acid differences are found near the C-terminus outside of the DNA binding region. Dr. Aixin Yan from the Shan *et al*. study provided us with a plasmid containing the $(FNRD154A)_2$ protein; this was used for subsequent binding experiments. To test that the *E. coli* $(FNRD154A)_2$ protein was active under aerobic conditions, Δ*fnr* was transformed with the plasmids, and we carried out the same experiment using *FnrS* expression to determine activity of the protein. **Figure 4.8A** shows that Δ*fnr* cells containing p$(fnrD154A)_2$ had higher expression of *FnrS* under aerobic than microaerobic conditions. Subsequently, we His-tag purified the protein as a dimer (**Figure 4.8B**) and concentrated the sample (**Figure 4.8C**) for use in EMSA. The same protein was recently used with success in an EMSA analysis on DNA promoters from SL1344 (Wang *et al.*, 2019).

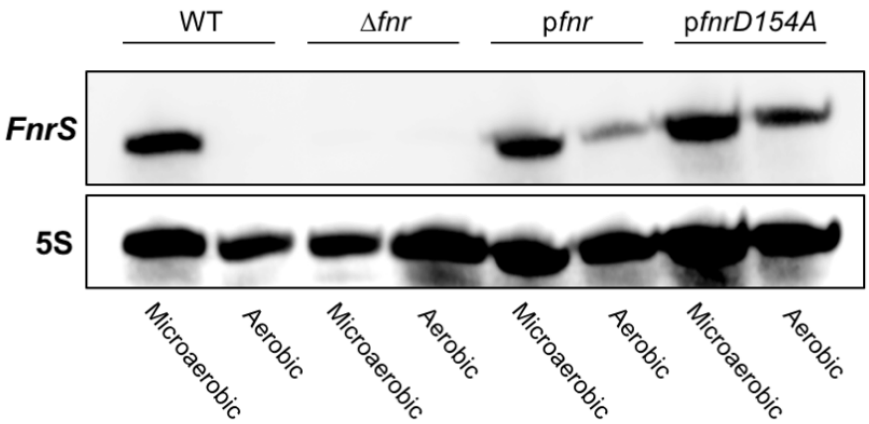


Figure 4.6 FNRD154A is active under aerobic conditions.

Northern blot showing *FnrS* was expressed under aerobic conditions in an Δ*fnr* mutant expressing the FNRD154A protein from the pBR322 plasmid.

```
S.   1   MIPEKRIIRRIQSGGCAIHCQDCSISQLCIPFTLNEHELDQLDNIIERKK  50
         ||||||||||||||||||||||||||||||||||||||||||||||||||
E.   1   MIPEKRIIRRIQSGGCAIHCQDCSISQLCIPFTLNEHELDQLDNIIERKK  50

S.  51   PIQKGQTLFKAGDELKSLYAIRSGTIKSYTITEQGDEQITGFHLAGDLVG 100
         ||||||||||||||||||||||||||||||||||||||||||||||||||
E.  51   PIQKGQTLFKAGDELKSLYAIRSGTIKSYTITEQGDEQITGFHLAGDLVG 100

S. 101   FDAIGSGHHPSFAQALETSMVCEIPFETLDDLSGKMPNLRQQMMRLMSGE 150
         ||||||||||||||||||||||||||||||||||||||||||||||||||
E. 101   FDAIGSGHHPSFAQALETSMVCEIPFETLDDLSGKMPNLRQQMMRLMSGE 150

S. 151   IKGDQDMILLLSKKNAEERLAAFIYNLSRRFAQRGFSPREFRLTMTRGDI 200
         ||||||||||||||||||||||||||||||||||||||||||||||||||
E. 151   IKGDQDMILLLSKKNAEERLAAFIYNLSRRFAQRGFSPREFRLTMTRGDI 200

S. 201   GNYLGLTVETISRLLGRFQKSGMLAVKGKYITIENSDALAALAGHTRNVA 250
         ||||||||||||||||||||||||||||||||||:|||||.|||||||||
E. 201   GNYLGLTVETISRLLGRFQKSGMLAVKGKYITIENNDALAQLAGHTRNVA 250
```

Figure 4.7 Alignment of *S.* Typhimurium and *E. coli* FNR protein sequences.

FNR amino acid (aa) sequences of *S.* Typhimurium 4/74 (*S.*) and *E. coli* K-12 MG1655 (*E.*) were aligned using the BLOSUM62 matrix with a gap penalty of 10 and extend penalty of 0.5. The sequences are both 250 aa in length and have 99.2% identity and 99.6% similarity. The 4 cystine residues important for binding 4Fe-4S clusters are underlined, the dimerization region is underlined with a dash line, the DNA binding region is in bold. Identical aa are connected with a |, differences in amino acids are labeled in red with a : and blue with a . indicating changes in aa which are strongly similar and weakly similar in their properties, respectively.

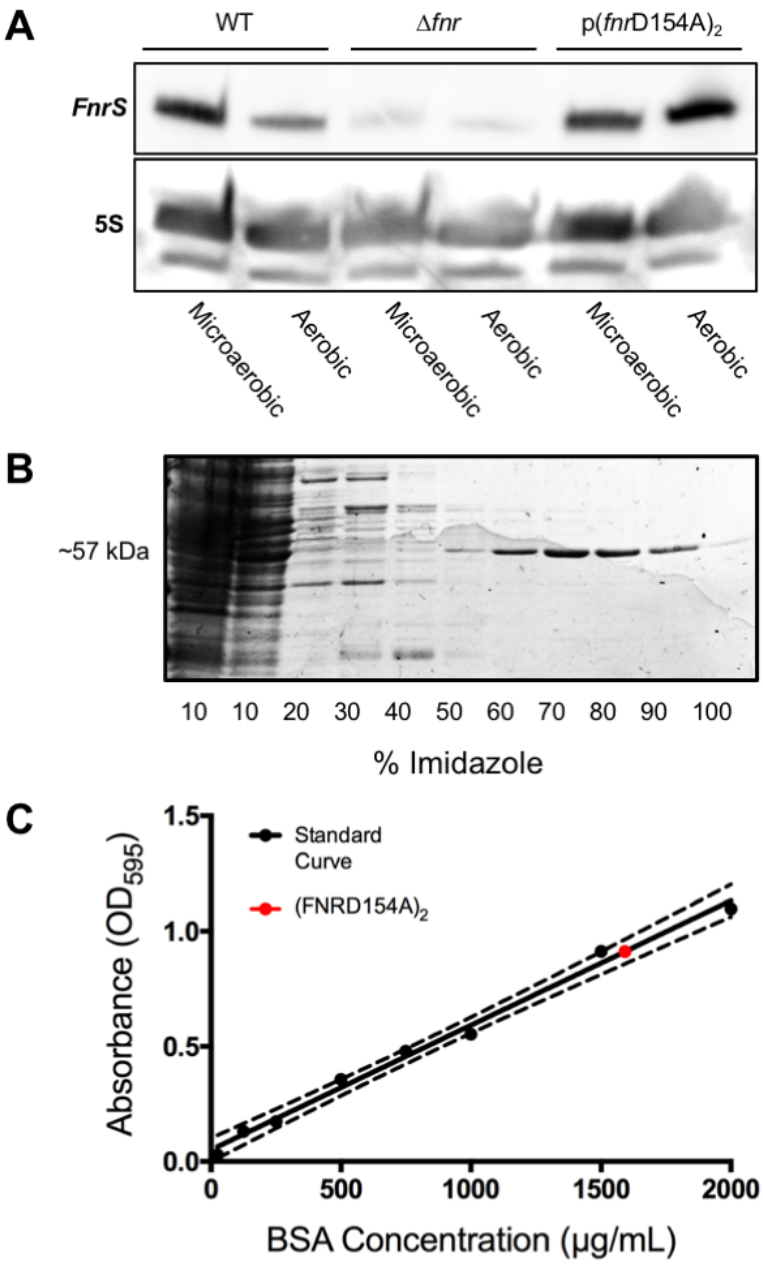


Figure 4.8 $(FNRD154A)_2$ is active and can be purified under aerobic conditions.

A. Northern blot showing *FnrS* was expressed under aerobic conditions in an Δ*fnr* mutant expressing the *E. coli* $(FNRD154A)_2$ protein. B. Coomassie stained SDS-PAGE of protein fractions from His-Tag purification of $(FNRD154A)_2$ showing that the protein was isolated in its dimeric form (Approximately 57 kDa with 6His-Tag). C. BCA assay of $(FNRD154A)_2$ sample after purification and concentration of protein samples, the dotted lines represent a 95% confidence interval of the linear regression of the standard curve.

4.2.5 FNR binding to control regions

Based on the expression of SPI-2 regulators, either SsrA and/or SsrB were the most likely candidates to explain the increased SPI-2 expression seen in Δ*fnr* grown in MMA under microaerobic conditions, as they were the most differentially expressed of SPI-2 regulators in Δ*fnr* (**Figure 4.9**). ChIP-seq data also revealed enrichment for FNR binding in 2 independent replicates and motif analysis uncovered 3 potential FNR half-sites at the *ssrB* promoter. Peaks at the *ssrA*/*ssaB* promoter region were only identified in the second replicate. To verify these findings, we used electrophoretic mobility shift assays. First, as positive controls, the *fnr* and *fnrS* promoters were assayed. A site down stream of *fnrS* in the ORF of *dbpA* was chosen as a negative control as there was no FNR binding as determined by ChIP-seq and FNR would be unlikely to bind at the 3′ end of an ORF that did not contain a TSS. The peaks called in ChIP-seq analysis and the coverage trace is shown in **Figure 4.10 A** & **B** and is accompanied by a schematic showing the location of sequence used for the EMSAs. As expected, P_{fnr} and P_{fnrS} EMSAs showed a band shift with a dissociation constant (K_D) of approximately 9.3×10^{-7} M and 1.5×10^{-6} M confirming that $(FNRD154A)_2$ binds to the *fnr* and *fnrS* promoters (**Figure 4.10 C**, **D**, **F** & **G**). The smaller the K_D, the more tightly bound the ligand (in this case DNA) is, or the higher the affinity between DNA and protein. The B_{max} is the maximum ratio of bound to unbound DNA; $(FNRD154A)_2$ bound to 84.9% and 92.9% of P_{fnr} and P_{fnrS}, respectively. $(FNRD154A)_2$ did not bind to *dbpA* DNA (**Figure 4.10 E** & **H**).

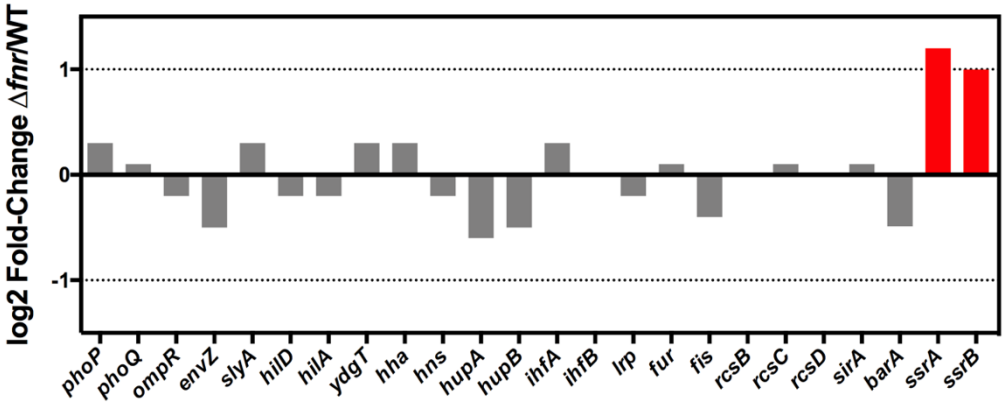


Figure 4.9 The genes encoding SsrA and SsrB are the most differentially expressed of SPI-2 regulators.

RNA-seq data showing log2 fold-change of important SPI-2 regulators. All regulators in grey were not differentially expressed (>-1 and < 1) in Δ*fnr*. The *ssrA* and *ssrB* genes (in red) had the highest fold-change of all regulators with known involvement in SPI-2 regulation.

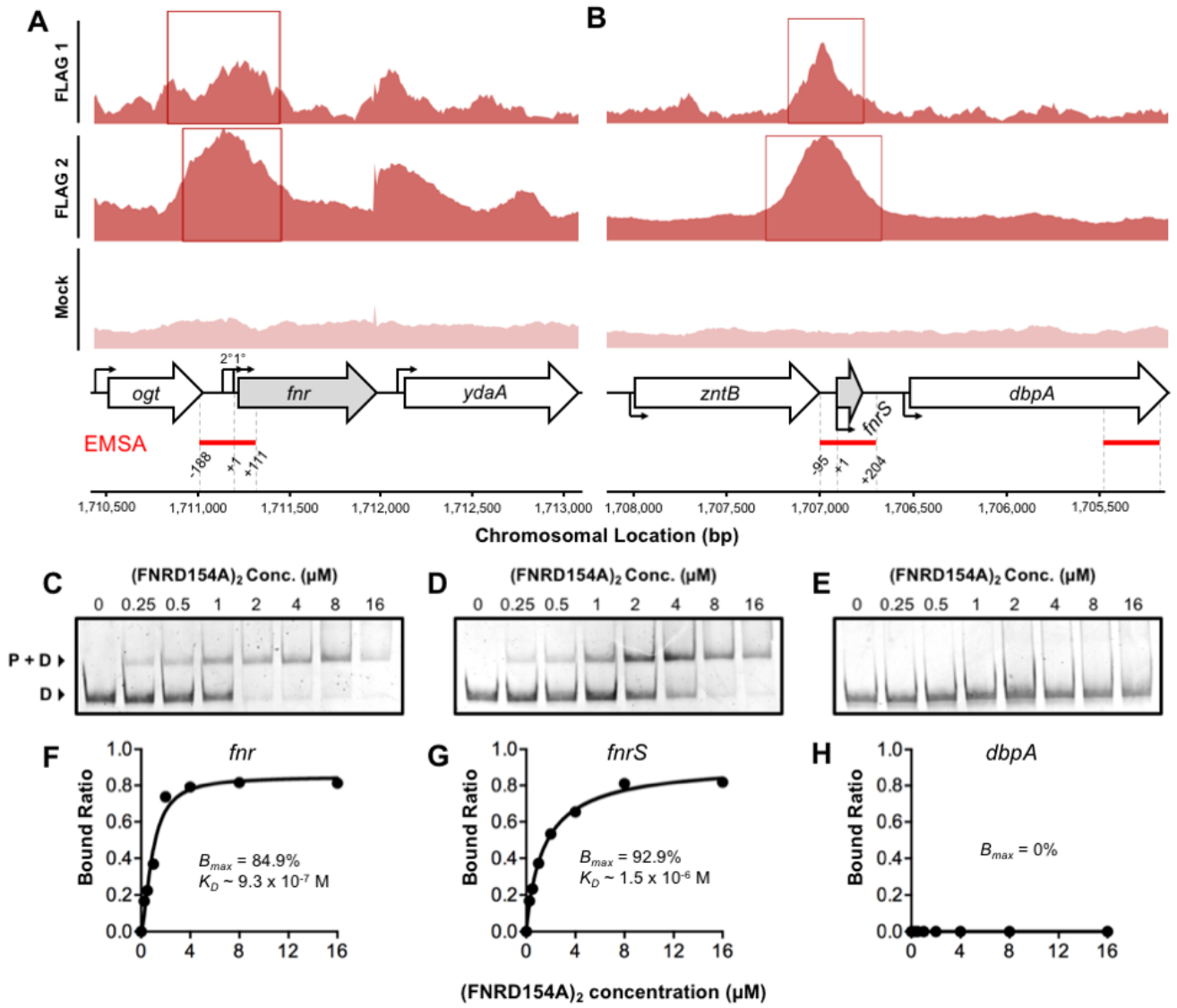
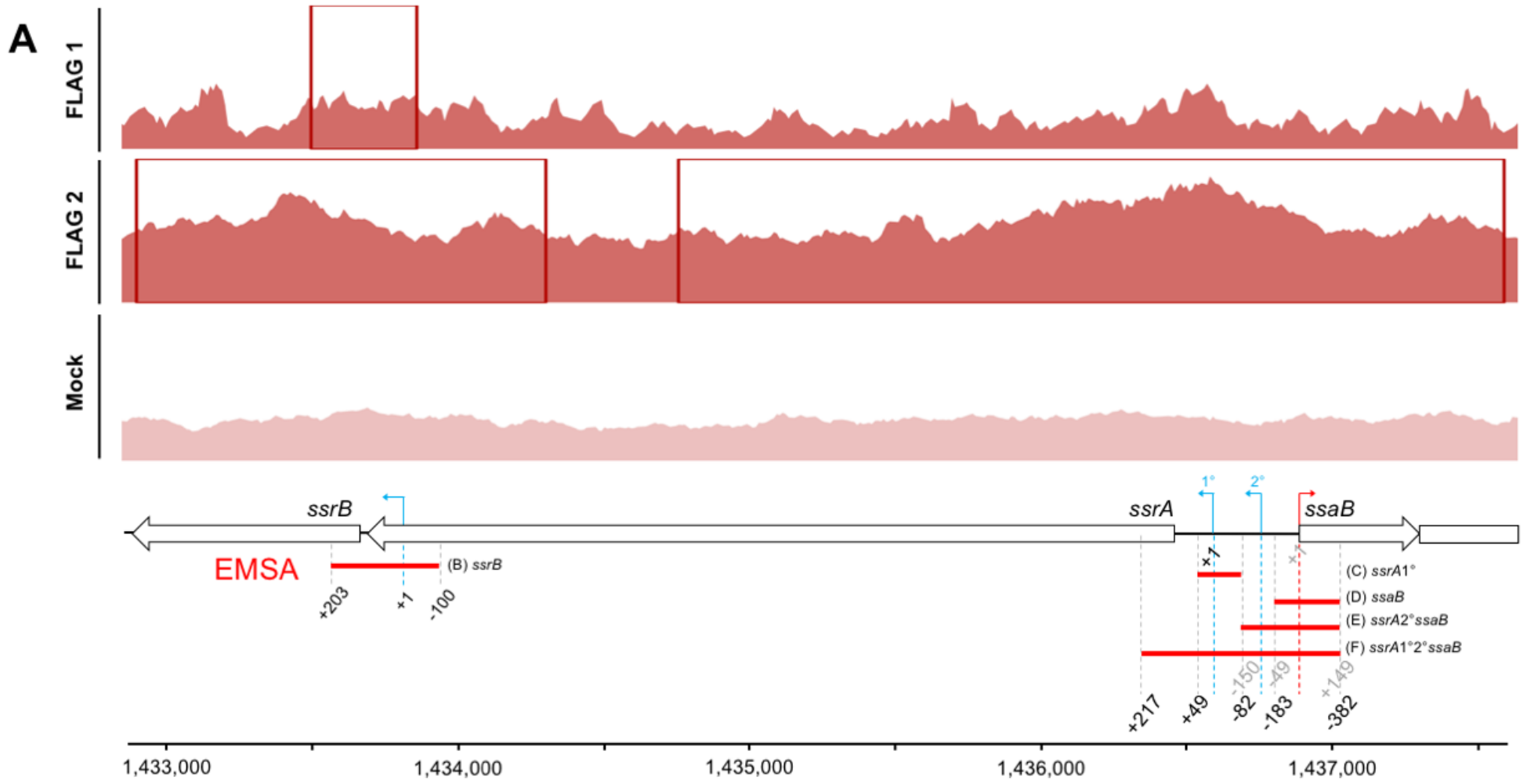

A
B
FLAG 1
FLAG 2
Mock
2°
1°
ogt
fnr
ydaA
zntB
fnrS
dbpA
EMSA
-188
+1
+111
-95
+1
+204
1,710,500
1,711,000
1,711,500
1,712,000
1,712,500
1,713,000
1,708,000
1,707,500
1,707,000
1,706,500
1,706,000
1,705,500
Chromosomal Location (bp)
C
D
E
(FNRD154A)2 Conc. (μM)
0 0.25 0.5 1 2 4 8 16
P + D
D
F
G
H
fnr
fnrS
dbpA
Bound Ratio
Bmax = 84.9%
KD ~ 9.3 x 10-7 M
Bmax = 92.9%
KD ~ 1.5 x 10-6 M
Bmax = 0%
(FNRD154A)2 concentration (μM)

Figure 4.10 FNR binding at the P_{fnr} and P_{fnrS} promoters and a negative control.
A, B. ChIP-seq coverage (y-axis) for FLAG 1 and 2 replicates in red and Mock in light red. Boxes indicate putative FNR binding sites called by MACS2. Schematic shows location of *fnr* and *fnrS* as grey arrows and surrounding genes including *dbpA* as white as arrows and shows the location (red lines) of the sequence used for EMSA relative to the respective TSSs (black bent arrows). C – E. Representative EMSAs using purified $(FNRD154A)_2$ with the P_{fnr} and P_{fnrS} promoters and *dbpA*, respectively; "P + D" is protein bound to DNA, and "D" is unbound DNA. F – H. Equilibrium DNA binding curves for the P_{fnr} and P_{fnrS} promoters and *dbpA*, respectively; fitted by nonlinear regression and K_D and B_{max} were determined by one site specific binding with Hill slope analysis. Promoters P_{fnr} and P_{fnrS} were used as positive controls, and *dbpA* as a negative control for EMSAs.

4.2.6 FNR binding to SPI-2 promoters

Next, we investigated the promoters of *ssrB*, *ssrA* and *ssaB*. The P_{ssrB} is comprised of the intergenic region between *ssrB* and *ssrA* ORFs and extends to overlap a portion of the 3′ end of *ssrA* (**Figure 4.11A**). $(FNR154A)_2$ had a weaker binding affinity to P_{ssrB} than positive controls ($K_D \sim 2.1 \times 10^{-6}$ M, **Figure 4.11B** & **G**). The intergenic region between the *ssrA* and *ssaB* genes is extremely AT rich (69.0% AT content) compared to the rest of the chromosome (47.8% AT content) (Papanikolaou *et al.*, 2009) and contains two TSSs for *ssrA* and one for *ssaB* (**Figure 4.11A**) and the *ssrA* mRNA is transcribed from the 1° TSS. $(FNR154A)_2$ was unable to cause a band shift in the region surrounding (49 bp downstream and 82 bp upstream) the 1° TSS of *ssrA*; the unbound probe appears to reduce in intensity at 16 µM of $(FNR154A)_2$ however, there is no clear shifted band (**Figure 4.11C** & **H**). $(FNR154A)_2$ had a stronger binding affinity to the *ssaB* promoter ($K_D \sim 1.3 \times 10^{-6}$ M) than to P*ssrB*, but nearly the same affinity when the DNA probe was extended to also include the 2° *ssrA* TSS ($K_D \sim 2.0 \times 10^{-6}$ M) (**Figure 4.11D**, **E**, **I**, & **J**). EMSA with the entire intergenic region between *ssrA* and *ssaB*, $P_{ssrA}1°2°_{ssaB}$, showed a higher binding affinity than any of the smaller fragments ($K_D \sim 6.2 \times 10^{-7}$ M) as DNA started shifting at a lower concentration of $(FNR154A)_2$ however, similarly to the $P_{ssrA}1°$ EMSA, the binding at 16 µM becomes unclear; this larger DNA probe contains the $P_{ssrA}1°$ sequence, and so this result indicates there may be non-specific binding at the highest concentration of $(FNR154A)_2$ used in these assays (**Figure 4.11 F** & **K**). Non-specific binding by FNR at this site could be due to the extremely high AT concentration found at this locus. A higher concentration of the

$(FNRD154A)_2$ protein may be required shift a higher proportion of the DNA of these promoters, as 16 μM of the protein resulted in B_{max} values below 50% (**Figure 4.11G–K**). These results indicate that FNR binds with low affinity to P_{ssrB} and P_{ssaB} likely due to the presence of FNR half sites at these locations, and FNR may also bind non-specifically in near the P_{ssrA} due to the high AT content of this region (~69%) and high AT content of the FNR consensus binding motif (~64%).


A
FLAG 1
FLAG 2
Mock
ssrB
ssrA
ssaB
1°
2°
EMSA
(B) ssrB
+203
+1
-100
+1
+1
(C) ssrA1°
(D) ssaB
(E) ssrA2°ssaB
(F) ssrA1°2°ssaB
+217
+49
-150
-82
-49
-183
+149
-382
1,433,000
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1,435,000
1,436,000
1,437,000

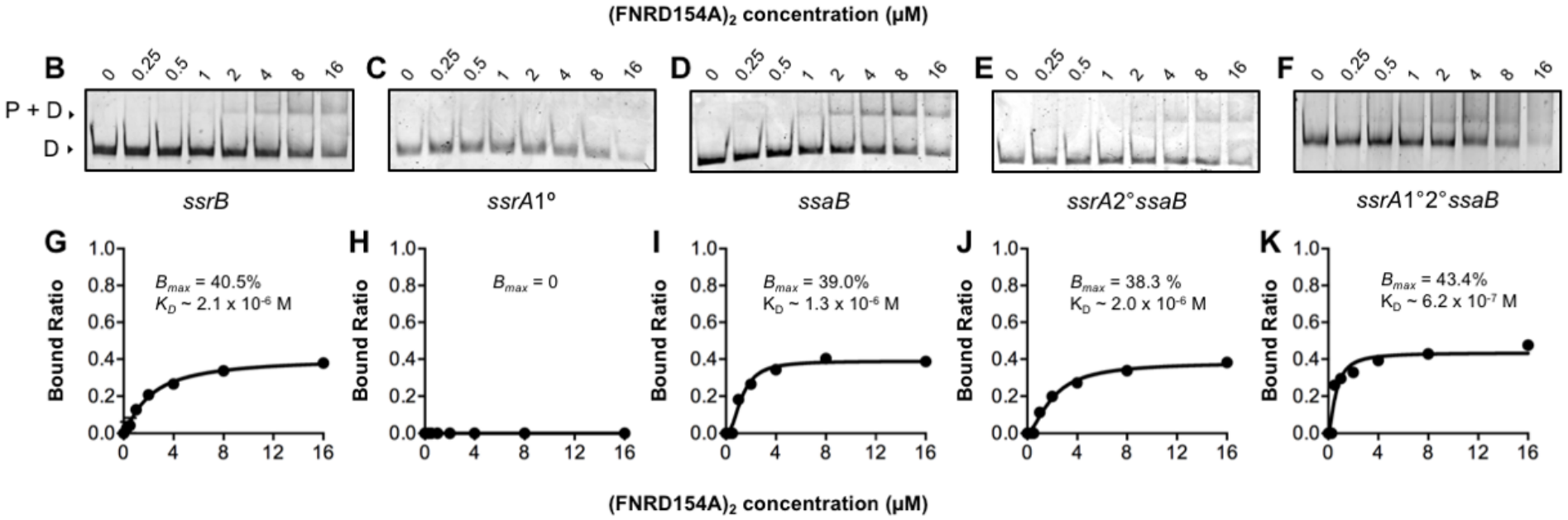

Figure 4.11 FNR binding at the P_{ssrB}, P_{ssrA} and P_{ssaB} promoters.

A. ChIP-seq coverage (y-axis) for FLAG 1 and 2 replicates in red and Mock in light red. Boxes indicate putative FNR binding sites called by MACS2. Schematic shows location of the *ssrB, ssrA* and *ssaB* genes in white as arrows, and shows the location (red lines) of the sequence used for EMSA relative to the respective TSSs (blue and red bent arrows); in the *ssrA*/*ssaB* intergenic region of the schematic, grey numbers are bp relative to the *ssaB* TSS and black numbers are bp relative to the *ssrA* 1° TSS. B – F. Representative EMSAs using purified $(FNRD154A)_2$ with the $P_{ssrB,}$ $P_{ssrA}1°$, P_{ssaB}, $P_{ssrA}2°_{ssaB}$ and $P_{ssrA}1°2°_{ssaB}$ promoters, respectively; "P + D" is protein bound to DNA, and "D" is unbound DNA. G – K. Equilibrium DNA binding curves for the $P_{ssrB,}$ $P_{ssrA}1°$, P_{ssaB}, $P_{ssrA}2°_{ssaB}$ and $P_{ssrA}1°2°_{ssaB}$ promoters, respectively; fitted by nonlinear regression and K_D and B_{max} were determined by one site specific binding with Hill slope analysis.

4.2.7 FNR binding to the ydgT promoter

Finally, we examined binding at the *ydgT* promoter region. As previously discussed, YdgT is a known repressor of SPI-2. Although there was little difference in expression of *ydgT* between WT and Δ*fnr* (**Figure 4.9**), ChIP-seq showed binding near the P_{ydgT}. However, the peaks have the highest coverage at the TSS of an unannotated antisense transcript found in the *ydgT* promoter region (**Figure 4.12A**). The EMSA sequence does contain 5 bp of the 14 bp binding motif found at the antisense TSS at the edge of the DNA probe as well as the half motif centered at -87 from the *ydgT* TSS. While binding of $(FNRD154A)_2$ caused a shift beginning at 0.25 μM, higher concentrations do not reveal a clear shifted band (**Figure 4.12B & C**); this could be due to transient binding at the partial antisense TSS. More experimentation is required to elucidate the binding affinities specifically for each of the antisense TSS and the P_{ydgT} half-site. Nevertheless, *ydgT* was not differentially expressed in Δ*fnr*, indicating it likely does not play a role in FNR control over SPI-2 expression under these conditions.

4.2.8 Expression from SPI-2 promoters

To investigate the effect of Δ*fnr* on individual promoters, the promoters of *ssrB*, *ssrA* (partial promoter, $P_{ssrA}1°$, and full promoter) and *ssaB* and a negative control sequence from *dbpA* were cloned into the pCS26-*luxCDABE* low copy plasmid. **Figure 4.13A** shows the locations of promoter sequences taken from *ssrB*, *ssrA* and *ssaB* promoter regions relative to the TSS; the *ssrA* locations are shown relative to the primary TSS. Expression from P_{ssrB} was significantly higher (approximately 1.8-fold) in Δ*fnr* than WT which was consistent with RNA-seq data; P_{ssrB} expression in WT was also significantly higher than in Δ*ssrAB* (**Figure 4.13B**). Neither $P_{ssrA}1°$ or P_{ssrA} expression was significantly different in any strain (**Figure 4.13C & D**). The result from $P_{ssrA}1°$ is consistent with our data showing that FNR is unlikely to cause repression at this location however, the expression from the full *ssrA* promoter was very low. We had expected to see higher expression, especially in the Δ*fnr* mutant. The strains carrying this plasmid were also grown in SPI-2 inducing PCN medium as a control and showed the same low expression therefore, thus there is an unknown problem with this plasmid. As anticipated, expression from P_{ssaB} was significantly higher (approximately 18-fold) in Δ*fnr* than WT (**Figure 4.13E**). There was no expression detected in the *dbpA* negative control (**Figure 4.13F**). These results, especially the invariable

expression from $P_{ssrA}1°$, highlight *ssrB* as the major target for FNR for regulation of SPI-2 expression.

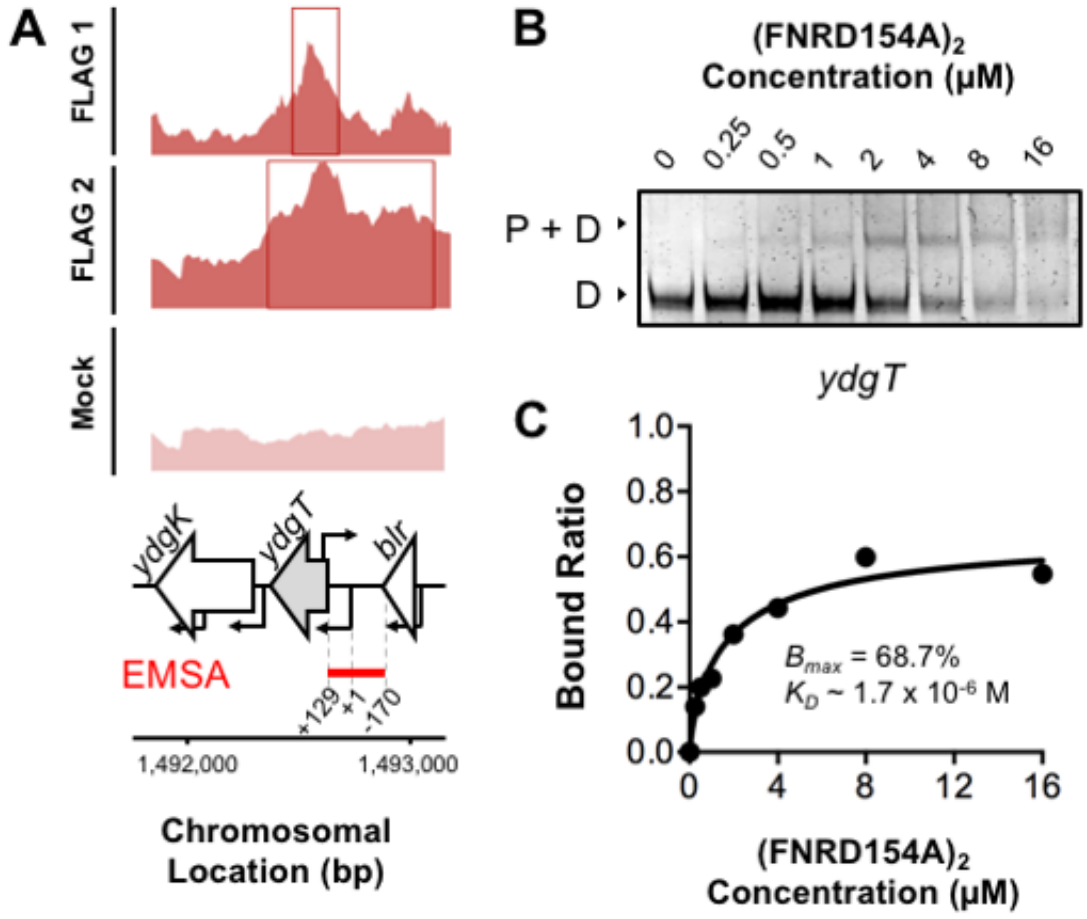

Figure 4.12 FNR binding at the P_{ydgT} promoter.

A. ChIP-seq coverage (y-axis) for FLAG 1 and 2 replicates in red and Mock in light red. Boxes indicate putative FNR binding sites called by MACS2. Schematic shows location of the *ydgT* gene in grey and surrounding genes in white as arrows and shows the location (red line) of the sequence used for EMSA relative to the *ydgT* TSS (black bent arrow). B. Representative EMSA using purified $(FNRD154A)_2$ with the P_{ydgT} probe; "P + D" is protein bound to DNA, and "D" is unbound DNA. C. Equilibrium DNA binding curve fitted by nonlinear regression and K_D and B_{max} determined by one site specific binding with Hill slope analysis.

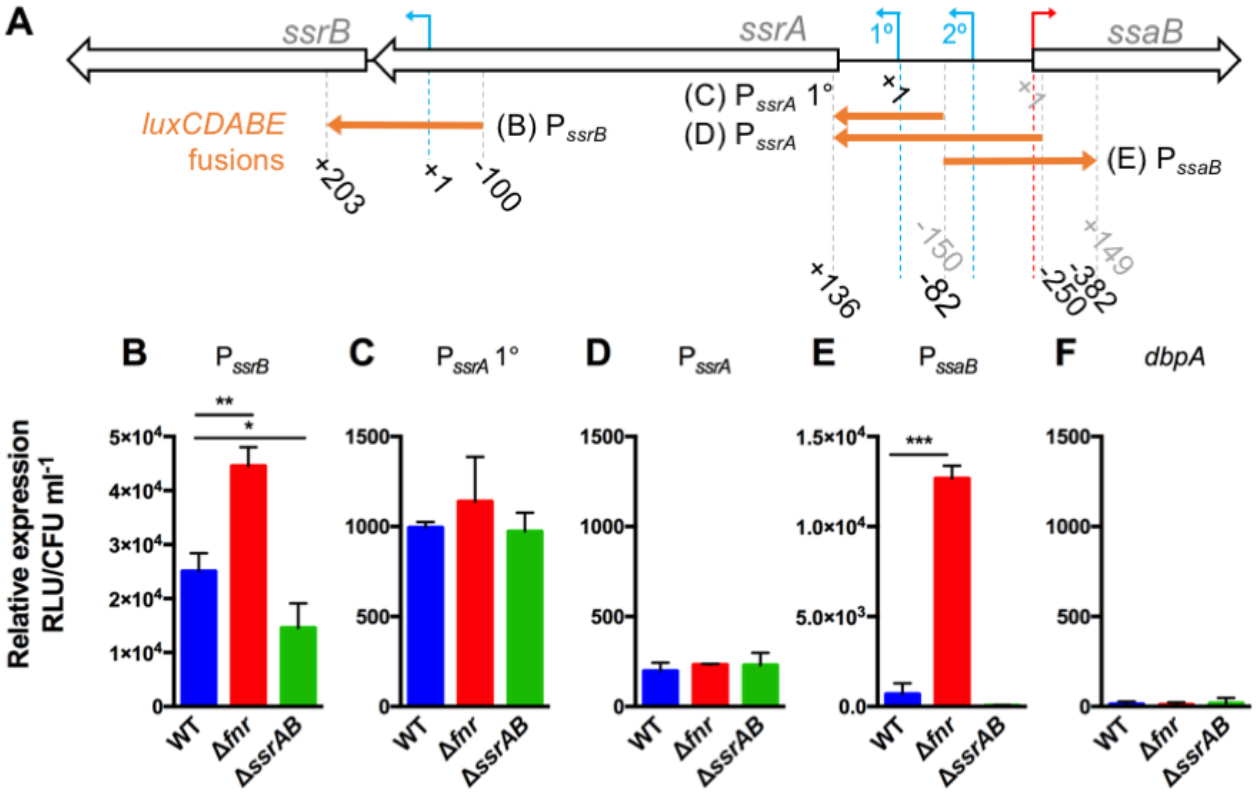

Figure 4.13 Promoter expression from SPI-2 promoters.

A. Schematic showing the locations of promoter sequences cloned into the pCS26-*luxCDABE* plasmid (orange arrows); in the *ssrA*/*ssaB* intergenic region grey numbers are bp relative to the *ssaB* TSS and black numbers are bp relative to the *ssrA* 1° TSS. Relative promoter expression of the B. *ssrB* promoter, C. truncated *ssrA* promoter, D. full *ssrA* promoter, E. *ssaB* promoter, and F. a section of the *dbpA* gene (as in Figure 4.10) as a negative "no promoter" control. Significance determined by nonparametric one-way ANOVA with Dunnet's multiple comparison test to WT for each promoter; only significant differences are labeled, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$, error bars represent standard deviation.

4.3 Discussion

To our knowledge, the work presented here is the first large scale DNA-protein binding study that has been carried out for the FNR protein in *S*. Typhimurium using high throughput next generation sequencing. The use of ChIP-seq has allowed us to identify 288 putative FNR binding sites across the entire *S*. Typhimurium chromosome and plasmids. We were able to validate through ChIP-seq and EMSA that FNR acts as a direct repressor of *Salmonella* pathogenicity island 2 through direct repression of the response regulator *ssrB* and the apparatus gene *ssaB*. Consequently, FNR indirectly represses the remainder of SPI-2 genes through the repression of *ssrB*. ChIP-seq also revealed FNR binding sites at SPI-2 associated genes encoded outside of the island, which could be validated in the future.

4.3.1 Low binding affinity at SPI-2

ChIP-seq revealed occupancy of FNR in long binding regions at SPI-2 promoters, and in a low coverage sample, binding was difficult to distinguish from background (**Figure 4.11A**). However, we were able to show that FNR binds to these sequences, albeit weakly, *in vitro* using EMSA (**Figure 4.11 B–K**). The weak binding affinity found at these sites is likely due to the presence of FNR half-sites as opposed to full binding motifs (**Figure 4.5**). The quality of the binding site in a given promoter may govern the degree of anoxia required to induce that promoter (Spiro & Guest, 1990). Additionally, because our microaerobic growth condition is heterogeneous, there are likely different subpopulations of cells in the flask where binding occurs (at the bottom), and another where it cannot occur due to higher oxygen concentrations (at the surface). An experiment that would provide insight to binding of FNR to SPI-2 promoters is DNase I footprinting. DNase I footprinting is advantageous because it determines the boundaries of a transcription factor binding site and is informative for evaluating or optimizing binding motif analysis (Myers *et al.*, 2015). Although, the drawback is that DNase I footprinting is performed *in vitro*. Weak binding or failure to observe binding *in vitro* does not rule out binding *in vivo* and could signify an interesting regulatory mechanism. For example, some binding events require cooperative interactions with other TFs (Lee & Groisman, 2012; Melville & Gunsalus, 1996; Park *et al.*, 2013) and may require specific cellular conditions that are difficult to replicate *in vitro* (Myers *et al.*, 2015).

4.3.2 The role of ydgT

Although *ydgT* was not differentially expressed in the Δ*fnr* mutant, FNR was found to bind at this promoter (**Figure 4.12**). Motif analysis revealed a full binding motif as well as a half motif in the *ydgT* promoter region. While FNR may regulate *ydgT* through binding at the half motif, the full FNR motif is positioned at the TSS of an unannotated antisense transcript in the *ydgT* promoter region, and not in a position relative to the *ydgT* TSS that is compatible with either activation or repression of the gene. Further investigation into this antisense transcript is required but, we can speculate that it is directly regulated by FNR due to the presence of the near consensus FNR binding motif and up-regulation in Δ*fnr*, and its position relative to *ydgT* makes it a prime candidate as a cis-acting sRNA which may post-transcriptionally repress *ydgT* under aerobic conditions.

4.3.3 The benefits and limitations of ChIP-seq for investigations of in vivo protein-DNA interactions

ChIP-seq technology is becoming more accessible to researchers and producing more large-scale *in vivo* DNA-protein interaction studies which contribute to the expansion of our knowledge base. The development of tools to analyze ChIP-seq data in bacteria is an evolving pipeline and, as such, new approaches and algorithms are being introduced frequently. These bioinformatic tools allow us to distil the important information from the massive data sets that are produced using ChIP-seq. We have shown that the use of ChIP-seq provides accurate information that agrees with existing FNR binding and motif data from *E. coli*, and here in combination with RNA-seq has helped to determine the direct and indirect control exerted by the FNR regulon, particularly as it relates to SPI-2 expression.

Like other high throughput sequencing techniques such as RNA-seq, ChIP-seq also comes with limitations. As discussed in Chapter 3, examination of populations of cells gives an average view of gene regulatory events. Currently there are no studies using single-cell ChIP-seq in bacteria or eukaryotic cells (Nakato & Shirahige, 2017), and bacteria present an even larger barrier to single-cell ChIP as the small size of individual bacterial cells means they contain far less DNA and protein than eukaryotic cells. DNA sequencing coverage is a major limitation of single-cell ChIP-seq, as the analysis requires large amounts of starting material (Nakato & Shirahige, 2017).

DNA sequencing coverage can also pose a problem to existing ChIP-seq methods. In this study, the first replicate had lower sequencing coverage than replicate 2 (**Figure 4.4A**). This made peak calling more difficult, especially in the low copy replicate and in regions where FNR is suspected to bind but, at lower affinity due to the quality of FNR binding sites present (**Figure 4.5**). Peak calling algorithms, such as MACS2 (used in this study) are important because they remove the subjective bias from visual peak calling and provide a statistical basis for determining areas of enrichment (Myers *et al.*, 2015), but had problems identifying peaks in the lower coverage sample, especially in AT rich regions where FNR occupancy occurred as a long binding region. The ChIP protocol used for this study was originally designed for use with ChIP-chip and not sequencing (Dillon & Dorman, 2010). Yeast tRNA is used in the protocol as a co-precipitant intended to increase DNA yields during immunoprecipitation. However, some samples had large numbers of reads which did not map to the *S.* Typhimurium genome but, were found to map to the *S. cerevisiae* genome. Dr. Aoife Colgan who was performing similar ChIP experiments during the analysis of ChIP data in this study, used qPCR with primers specific to *S. cerevisiae* and identified that the reads came from yeast gDNA contamination in the tRNA. Since this contaminant gDNA was introduced in the final stages of the experiment, they do not affect the outcome of the ChIP experiment apart from reducing the coverage of the sample. Therefore, if the FNR-ChIP were to be repeated, eliminating yeast tRNA as a co-precipitant is advised.

Successful chromatin immunoprecipitation requires that proteins be crosslinked with DNA to trap interactions inside actively growing cells. Formaldehyde fixation is the method of choice as it allows captures of weaker and transient protein-DNA interactions (Davis *et al.*, 2011; Myers *et al.*, 2015; Schmidt *et al.*, 2009). However, more recent studies have shown that variation in ChIP signal can be attributed to cross-linking efficiency and that extended cross-linking time may introduce false positives (Baranello *et al.*, 2016; Myers *et al.*, 2013). It may be desirable to repeat the ChIP experiment in this study and reduce cross-linking time to help eliminate any binding artifacts. However, when considering FNR binding at SPI-2 promoters, we were able to show that FNR is able to bind *in vitro* to these sequences using EMSA (**Figure 4.11**).

Correlation of FNR ChIP-seq peaks with transcriptomic data showed that the minority of FNR-regulated operons could be attributed to direct FNR binding, the same as in *E. coli* (Myers *et al.*, 2013). Conversely, FNR bound some promoters, such as at SPI-1 and SPI-4

without regulating expression. These genes likely require additional regulatory input by other condition-specific transcription factors, especially given the fact it has been shown that FNR leads to differential expression of SPI-1 genes in MOPS buffered LB with xylose (Fink *et al.*, 2007; Wang *et al.*, 2019). Nuanced regulation by multiple transcription factors may allow *S.* Typhimurium to respond rapidly to environmental changes and confer an advantage in the gut which fluctuates in availability of nutrients and oxygen concentration. However, binding of a TF to a specific DNA sequence does not necessarily result in control of gene expression of nearby genes (Stormo & Zhao, 2010). Many FNR binding sites are also occupied by nucleoid associated proteins (NAPs) such as H-NS, IHF and Fis in *E. coli*, which restrict access of FNR to the genome (Myers *et al.*, 2013). This is important for this study as many NAPs also occupy the AT rich promoter regions of SPI-2 genes (**Figure 4.14**). However, SPI-2 repression by TFs such as H-NS appears not to occur in MMA under microaerobic or aerobic conditions (**Figure 3.14**), further experimentation is required to determine exactly which transcription factors bind to SPI-2 promoters under these growth conditions.

An additional control which may add confidence to our results would be to perform ChIP with either a control strain lacking FNR (Δ*fnr*) or containing an untagged version of FNR to reveal any non-specific enrichment due to aberrant binding of the monoclonal anti-FLAG M2 antibody used in this study, thus allowing removal of any false positive areas of enrichment (Myers *et al.*, 2015). Furthermore, studies using two biological replicates have shown to be sufficient for identification of highly enriched DNA binding locations (Myers *et al.*, 2013; Park *et al.*, 2013) however, three biological replicates can aid in identifying less enriched regions and also provide greater statistical power (Myers *et al.*, 2015). Mutational analysis of putative FNR binding sites could also give greater insight into binding at SPI-2 promoters. The rearrangement of half motifs followed by either ChIP-RTqPCR or EMSA could determine if loss of the FNR motif abolished FNR binding.

Nevertheless, the combination of transcriptomic analysis, occupancy data and DNA-protein binding analysis of the *S.* Typhimurium FNR regulon and its role in regulation of virulence through direct repression of *ssrB* and SPI-2 effectors presented here, will contribute to the overall understanding of *S.* Typhimurium infection.

4.3.4 Summary

The aim of this chapter was to use the global approach of ChIP-seq to identify candidate regulators of SPI-2 under microaerobic conditions. Here we have shown that FNR binds weakly to SPI-2 promoters, particularily to the promoters of *ssrB* and *ssaB*. This may partially explain the repression of SPI-2 genes in WT under microaerobic conditions in MMA as SsrB is a necessary activator of all SPI-2 genes including those encoded outside of the genomic island. We have also shown that FNR binds strongly at the *FnrS* promoter under these conditions, and we know from transcriptomic data that *FnrS* expression is drastically decreased when FNR is absent. In a recent study, deletion of *FnrS* led to increased HilD production under low aeration conditions, and *FnrS* could bind to the *hilD* mRNA 5' UTR, resulting in translational repression (Kim *et al.*, 2018). As HilD is an important activator of SPI-2 and thus FNR could indirectly repress HilD through *FnrS* post-transcriptional repression. Overall, oxygen is an important signalling molecule for low level induction of SPI-2 genes and this induction works directly and possibly indirectly through FNR.

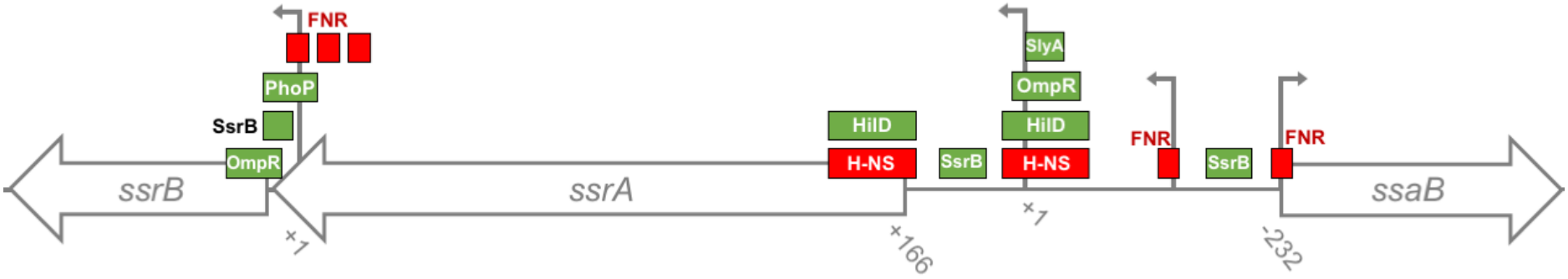

Figure 4.14 Putative FNR binding sites overlap with TF binding at the *ssrB*/*ssrA*/*ssaB* locus.

Putative binding sites are FNR half-sites (Figure 4.9) present in locations where there was a positive band shift (Figure 4.11) SPI-2 encoded genes are represented by white arrows, TSSs are represented by bent black arrows, and transcription factor binding sites are represented by boxes filled according to the binding sites legend. Activation sites are green and repression sites are red. Numbers represent bp in the *ssrA*/*ssaB* intergenic region and are relative to the *ssrA* 1° TSS. Schematic including putative FNR binding sites adapted from Banda et al., 2018.

Chapter 5

Effects of Δ*fnr* on virulence in macrophages and bacterial fitness

5.1 Introduction

5.1.1 Investigation of virulence and bacterial fitness in Δfnr

Virulence factors enhance the fitness of a pathogen under specific conditions such as in the host and genes involved in metabolism can provide a niche for bacteria during infection which allows them to out compete resident microbiota (Thiennimitr *et al.*, 2012; Winter *et al.*, 2013). After entering the gastrointestinal tract on the way to the site of infection, *S.* Typhimurium encounters a remarkable diversity in environmental conditions between the stomach, small intestine and large intestine. Moreover, even within the small intestine, there are differences in the microenvironments of the lumen, mucous layer and epithelial surface. Surviving passage through the vastly different environments encountered in the host is accomplished through appropriate coordination of virulence gene expression in response to environmental cues at different stages of infection. Both under and over-expression of virulence factors can be detrimental to bacterial fitness (Coombes *et al.*, 2005). Through previous work and this study, it is clear that FNR is an important regulator of virulence in *S.* Typhimurium (Fink *et al.*, 2007; Wang *et al.*, 2019). Here we investigate the effect of the *fnr* mutation in different media to attempt to elucidate what signals besides oxygen might be important for SPI-2 regulation in an Δ*fnr* mutant. We also wanted to investigate the role of FNR during macrophage infection, as previous studies have revealed that Δ*fnr* mutants are attenuated *in vivo*. Additionally, we looked at the relative bacterial fitness of Δ*fnr* compared to WT and other isogenic mutant of strain 4/74 under different conditions in an attempt to tease apart if SPI-2 expression was a burden to cell fitness. Finally, we used a proteomic approach (**Figure 5.1**) to investigate the energetics of WT and Δ*fnr* during growth in microaerobic MMA.

The aim of this chapter is to investigate the role of FNR during intracellular replication in murine macrophages and to investigate the bacterial fitness of the Δ*fnr* mutant in MMA and other *in vitro* growth conditions.

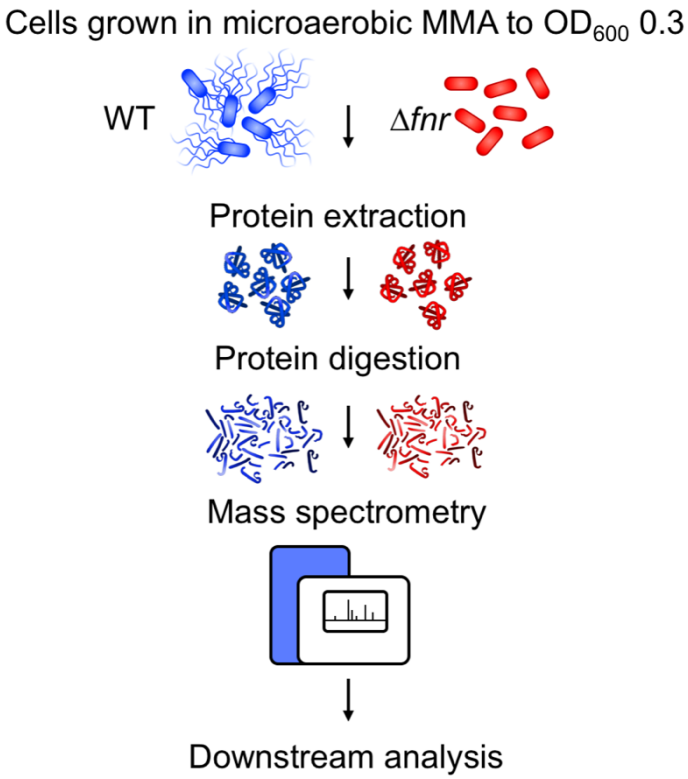

Figure 5.1 Workflow of general experimental procedures for investigating expressed proteins.

Wild-type (WT), Δ*fnr* strains were grown under microaerobic conditions to OD_{600} 0.3 in MMA and protein was extracted. Protein was digested and prepared for processing by mass spectrometry. Protein sample preparation was done by Dr. Nicole Hansmeier and all subsequent processing and analysis was completed by Dr. Tzu-Chiao Chao at the University of Regina, Regina, Saskatchewan, Canada. Downstream analysis was performed by Dr. Tzu-Chiao Chao and me.

5.2 Results

5.2.1 SPI-2 expression in vitro

Although SPI-2 expression has not previously been directly linked to FNR, previous studies have shown that an Δ*fnr* mutant is attenuated during infection. The mutant was recently shown to be attenuated in a *C. elegans* infection model (Wang *et al.*, 2019), and FNR was found to be essential during an enteritis model of infection and to play a weak role during a typhoid model of infection in BALB/c mice (Rollenhagen & Bumann, 2006). Fink *et al*. have also shown that the Δ*fnr* mutant was 100% attenuated after both oral and intraperitoneal infection of C57BL/6 mice, and that the mutant was attenuated in an *ex vivo* infection of peritoneal macrophages from C57BL/6 mice (Fink *et al.*, 2007). They have also shown that Δ*fnr* was attenuated in mice and macrophages deficient in production of reactive nitrogen species (RNS) but not in mice and macrophages deficient in production of reactive oxygen species (ROS). They explain that the reason for attenuation of the mutant is due to the inability of the Δ*fnr* mutant to mount a defense against ROS. However, during growth in PCN SPI-2 inducing medium (InSPI2), we observed that the Δ*fnr* mutant had reduced growth rate compared to WT (**Figure 5.2**), similar to what we had previously seen in MMA (**Figure 3.2**), except that in InSPI2 WT growth was also slowed when compared to strains deficient in SPI-2 expression, Δ*ssrAB* and Δ*ssrAB*/*fnr*. Also, PipB2 and SteC were were slightly higher in Δ*fnr* than WT in InSPI2 at OD_{600} 1.0 (**Figure 3.15**). Curiously, these cultures were all grown under aerated conditions therefore, we would not expect FNR to have an effect. We reasoned that intracellular and growth attenuation of Δ*fnr* may have to do with unrestrained expression of SPI-2 due to a lack of repression by FNR–a phenotype that can be observed in Δ*hns* mutants where the absence of the NAP leads to uncontrolled expression of SPIs and a loss in fitness (Ali *et al.*, 2014; Lucchini *et al.*, 2006; Martinez *et al.*, 2014; Navarre *et al.*, 2006; Sturm *et al.*, 2011). WT, Δ*fnr* and Δ*ssrAB* strains transformed with pDEW201-P_{ssaG}, which expresses *luxCDABE* from the *ssaG* promoter, were grown in LB, InSPI2 and Non-Inducing PCN (NonSPI2) under aerobic and microaerobic conditions and growth and *ssaG* expression where measured until stationary phase was reached (**Figure 5.3**). Throughout these assays, Δ*ssrAB* pDEW201-P_{ssaG} was used as a negative control for SPI-2 expression. There was little to no *ssaG* expression in any strain in LB regardless of oxygen concentration (**Figure 5.3A** & **D**) which is consistent with previously pubished data (Fink *et al.*, 2007; Wang *et al.*, 2019). Aerobic growth in InSPI2 showed that *ssaG* expression was marginally higher in WT than Δ*fnr*, and there was no expression in Δ*ssrAB*

(**Figure 5.3B**). Surprisingly, expression of *ssaG* in microaerobic InSPI2 was much higher in WT than Δ*fnr* during late exponential to early stationary phase (**Figure 5.3E**). This suggests that under *in vitro* microaerobic SPI-2 inducing conditions FNR is involved in activating SPI-2 genes, the opposite of what we have previously obserbed in MMA. This suggests that FNR may play a dual role in the control of SPI-2 which is dependent on environmental signals other than oxygen. Cells were also grown in NonSPI2 as a control, and regardless of aeration, there was minimal *ssaG* expression in both WT and Δ*fnr* from lag phase to early exponential (**Figure 5.3C** & **F**), which could be partially explained by low level SPI-2 expression carry over from overnight growth in LB (Kröger *et al.*, 2013; Rice *et al.*, 2015).

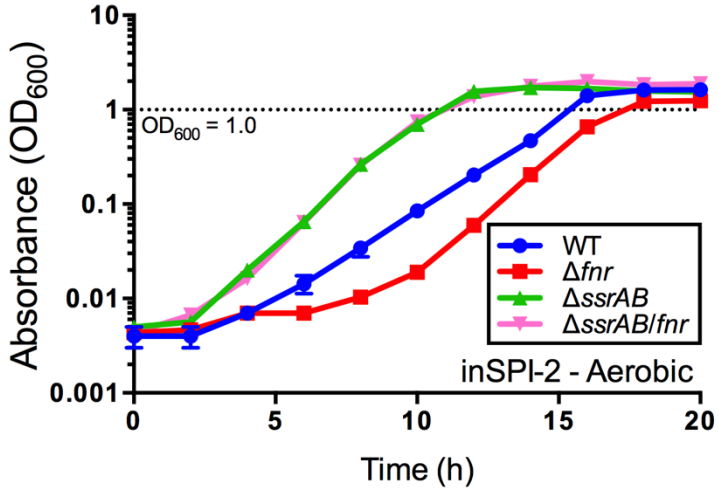

Figure 5.2 Growth of 4/74 and isogenic mutants in SPI-2 inducing media (InSPI2). Growth curve of WT, Δ*fnr*, Δ*ssrAB* and Δ*ssrAB*/*fnr* in aerobic InSPI2. Strains were grown as described in Section 2.2.3.2. The dotted line indicates OD_{600} 1.0.

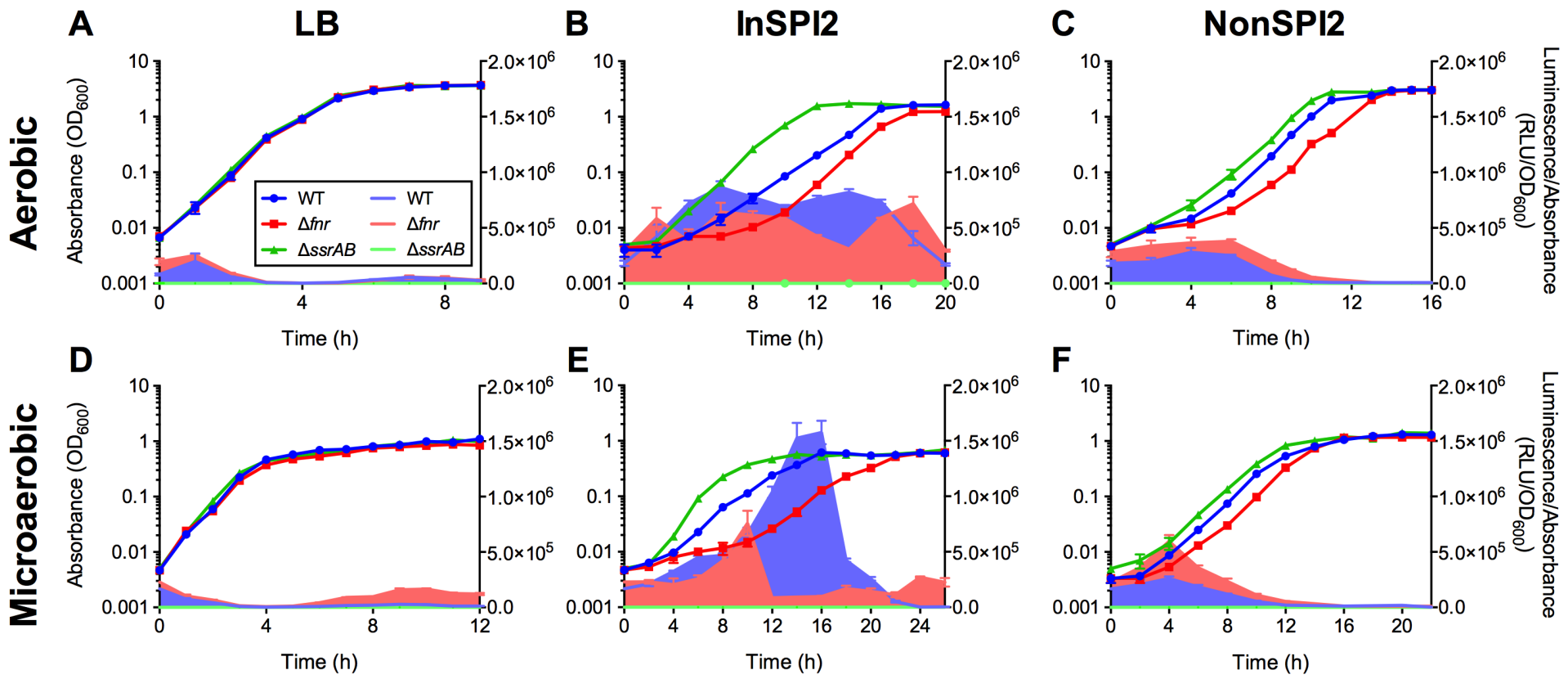

Figure 5.3 Aerobic and microaerobic growth and *ssaG* expression in 4/74 and isogenic mutants over time in LB, inSPI2 and NonSPI.

Cultures were grown as previously described (Sections 2.2.3.1 and 2.2.3.2) and sampled every 1 to 2 h depending on the growth rate of the culture. Samples were measured for optical density (600 nm) and luminescence (RLU). Aerobic cultures were grown in A. LB, B. InSPI2 and C. NonSPI2 and microaerobic cultures were grown in D. LB, E. InSPI2, and F. NonSPI2. For all graphs, optical density is represented by connected points (bright colours) and correspond to values on the left y-axis and *ssaG* expression is represented by filled areas (light colours) and correspond to values on the right y-axis. Error bars represent standard deviation.

5.2.2 Intramacrophage survival of S. Typhimurium

As previously described, Δ*fnr* has been found to be attenuated in mice, murine macrophages and in *C. elegans* (Fink *et al.*, 2007; Rollenhagen & Bumann, 2006; Wang *et al.*, 2019). In this study we infected RAW 264.7 murine macrophages at a multiplicity of infection of 10:1 with *S.* Typhimurium 4/74 and isogenic mutants. The Δ*rpoE* mutant was used as a negative control for intramacrophage replication for all infection experiments (Helaine *et al.*, 2010). Bacterial uptake between strains was not significantly different at 1 h post infection (**Figure 5.4A** & **C**), Δ*fnr* was attenuated at 4 h and 8 h post infection, and WT and *fnr*$^{+}$ were not significantly different (**Figure 5.4 B** & **D**). Interestingly, there were fewer total CFU/mL in the Δ*fnr* mutant at 16 h post infection, but the fold replication for WT and Δ*fnr* was not significantly different (**Figure 5.4D**). This was interesting when compared to the result obtained by Fink et al., as they saw attenuation of the mutant to 20 h post infection (Fink *et al.*, 2007). However, they used primary murine macrophages and the *S.* Typhimurium strain 14028s while we used RAW 264.7 murine macrophages and strain 4/74. These differences may account for the differences seen here.

Next, we measured intramacrophage *ssaG* expression from cells infected with WT and Δ*fnr* cells transformed with the pDEW201-P$_{ssaG}$ plasmid (**Figure 5.5**). While cells harbouring plasmids were taken up by macrophages at the same level as non-transformed cells and showed similar levels of replication at 4 h post infection (**Figure 5.5A**), replication of the pDEW201-P$_{ssaG}$-harbouring cells was significantly lower at 8 and 16 h post infection (**Figure 5.5A** & **B**). The WT strain with the pDEW201 empty vector had significantly lower replication only at 16 h post infection, and not nearly to the extent of the pDEW201-P$_{ssaG}$ harbouring cells (**Figure 5.5B**). This is due to the inherent fitness cost associated with maintaining plasmids and high levels of luminescent proteins in the cell (Knodler *et al.*, 2005). While there was very little detectable signal due to low cell concentration at 1 and 4 h post infection, Δ*fnr* had significantly higher *ssaG* expression at 8 h post infection. Interestingly, although there were the most cells present at 16 post infection, there was very little signal detected (**Figure 5.5C**). These results indicate that at 8 h post infection, while Δ*fnr* is attenuated, there is also increased SPI-2 expression. Interestingly, this does not agree with *in vitro* results where SPI-2 was more highly expressed in WT cells (**Figure5.3**). Possibly, over expression of SPI-2 in murine macrophages is energetically disadvantageous for cells.

Finally, we were interested to see what effect growth of cells in microaerobic MMA prior to infection would have on intramacrophage survival. Firstly, the macrophage uptake of WT and Δ*fnr* were approximately 22- and 15-fold lower, respectively in cells grown in microaerobic MMA (**Figure 5.6A** & **C**). Cells grown in MMA were unable to reach the number of CFU/mL attained by those grown in LB by 16 h post infection (**Figure 5.6A**), however the Δ*fnr* mutant was attenuated (**Figure 5.6B** & **E**). Interestingly, although starting from smaller numbers, the fold replication of MMA grown cells was significantly higher in WT and was similar in Δ*fnr* by 16 h post infection (**Figure 5.6D**). Although macrophages phagocytose bacterial cells, up-regulated SPI-1 probably increases the number of infected cells (Drecktrah *et al.*, 2005). The most substantial difference between the LB and MMA grown cells is that LB grown cells have increased SPI-1 expression, which we have seen was at similar levels in WT and Δ*fnr* from Western blots of SopE (**Figure 3.16**). Additionally, cells are stressed after growth in a minimal medium as compared to a rich growth medium such as LB which may have initially impaired their ability to replicate *in vivo*. Overall, we see that the Δ*fnr* is attenuated *in vivo* which could be attributed to previously reported inability to protect against ROS (Fink *et al.*, 2007) but also over-expression of SPI-2 resulting in a reduction of fitness.

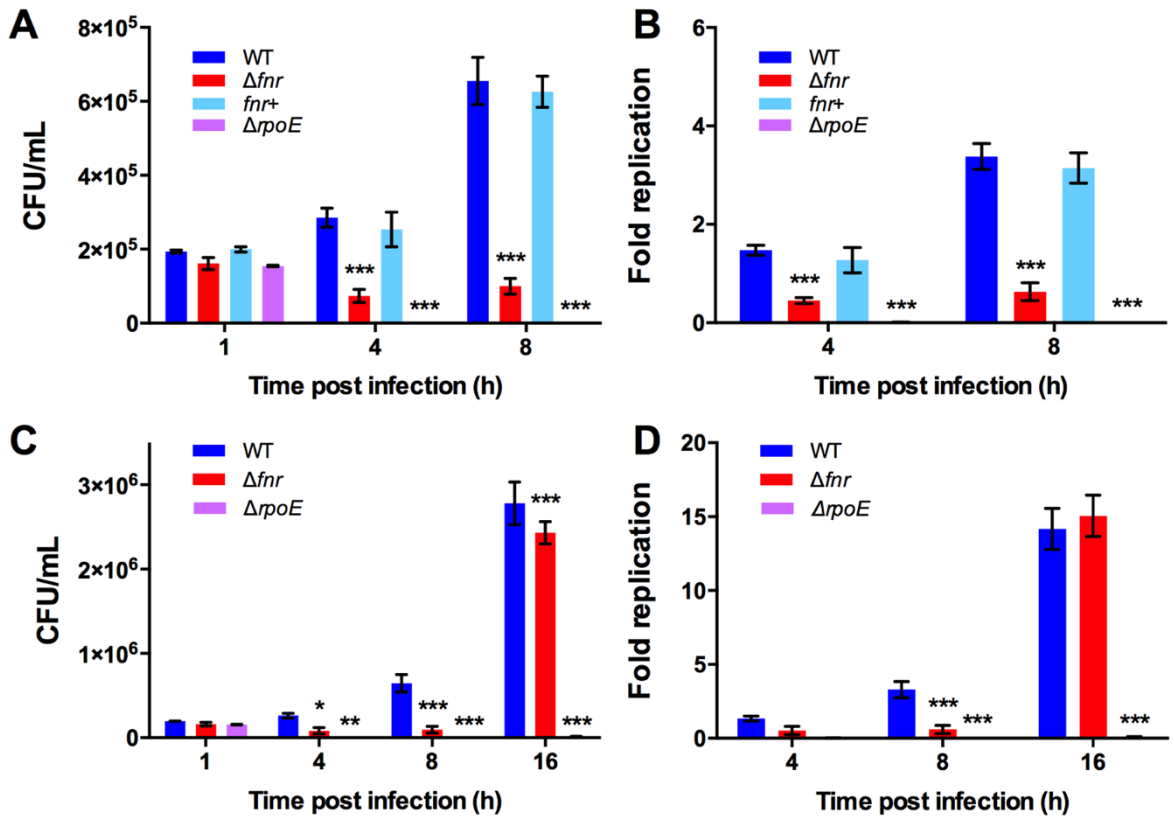

Figure 5.4 The Δ*fnr* mutant is attenuated in murine macrophages at early time points. Survival of WT, Δ*fnr*, *fnr*$^{+}$ and Δ*rpoE* up to 8 h post infection. A. Total viable cells post infection and B. the number of viable cells expressed as fold replication over 1 h post infection. Survival of WT, Δ*fnr* and Δ*rpoE* up to 16 h post infection. C. Total viable cells post infection and D. the number of viable cells expressed as fold replication over 1 h post infection. RAW 264.7 macrophages were infection with stationary phase *S.* Typhimurium cells with an MOI of 10:1. Statistical significance determined by Two-way ANOVA with Dunnett's multiple comparisons test to WT; Only statistically significant differences are shown; * = $p \leq 0.05$, ** = $p \leq 0.01$, and *** = $p \leq 0.001$; Error bars represent standard deviation.

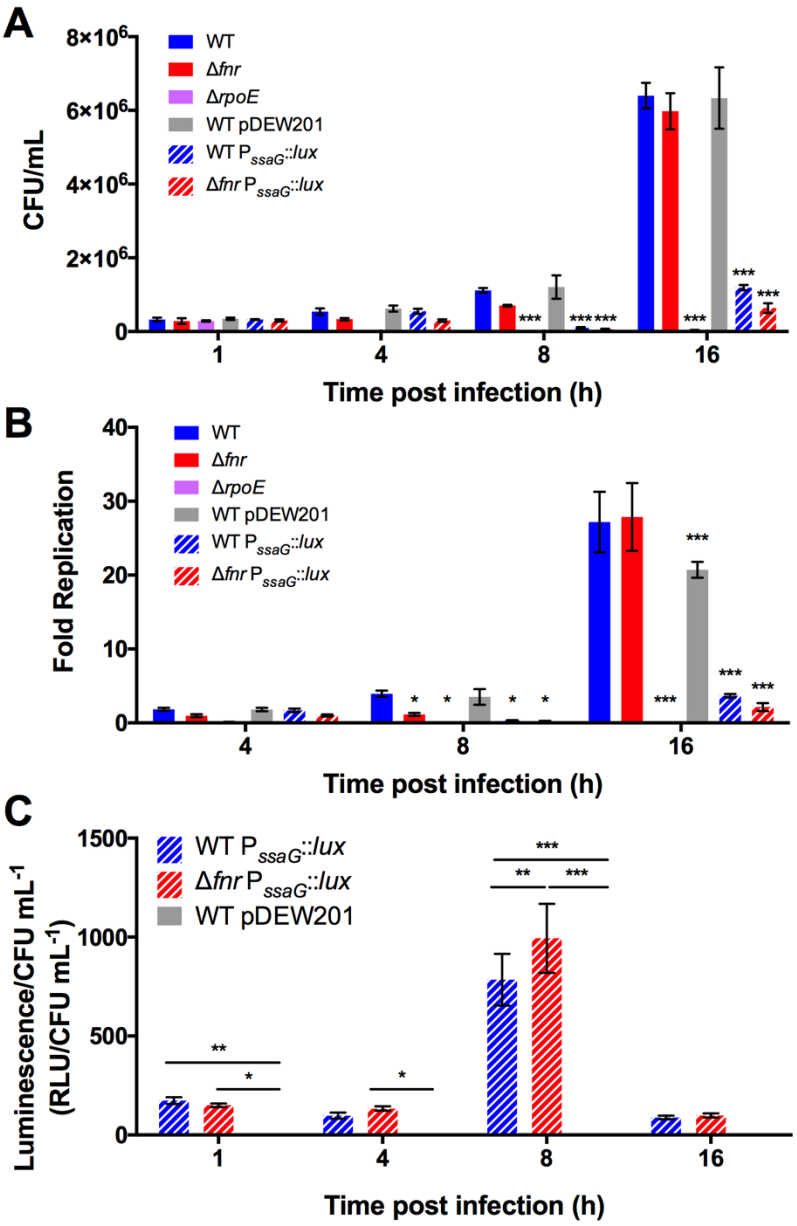

Figure 5.5 Plasmid carriage reduced intramacrophage replication and SPI-2 expression was elevated at early time points in Δ*fnr* during infection.

Survival of WT, Δ*fnr*, Δ*rpoE*, WT pDEW201, WT P$_{ssaG}$::*lux* and Δ*fnr* P$_{ssaG}$::*lux* up to 16 h post infection. A. Total viable cells post infection and B. the number of viable cells expressed as fold replication over 1 h post infection. C. Intramacrophage expression of *ssaG*. Statistical significance determined for A & B by Two-way ANOVA with Dunnett's multiple comparison's test to WT, and for C by Two-way ANOVA with Tukey's multiple comparisons test; Only statistically significant differences are shown; * = $p \leq 0.05$, ** = $p \leq 0.01$, and *** = $p \leq 0.001$; Error bars represent standard deviation.

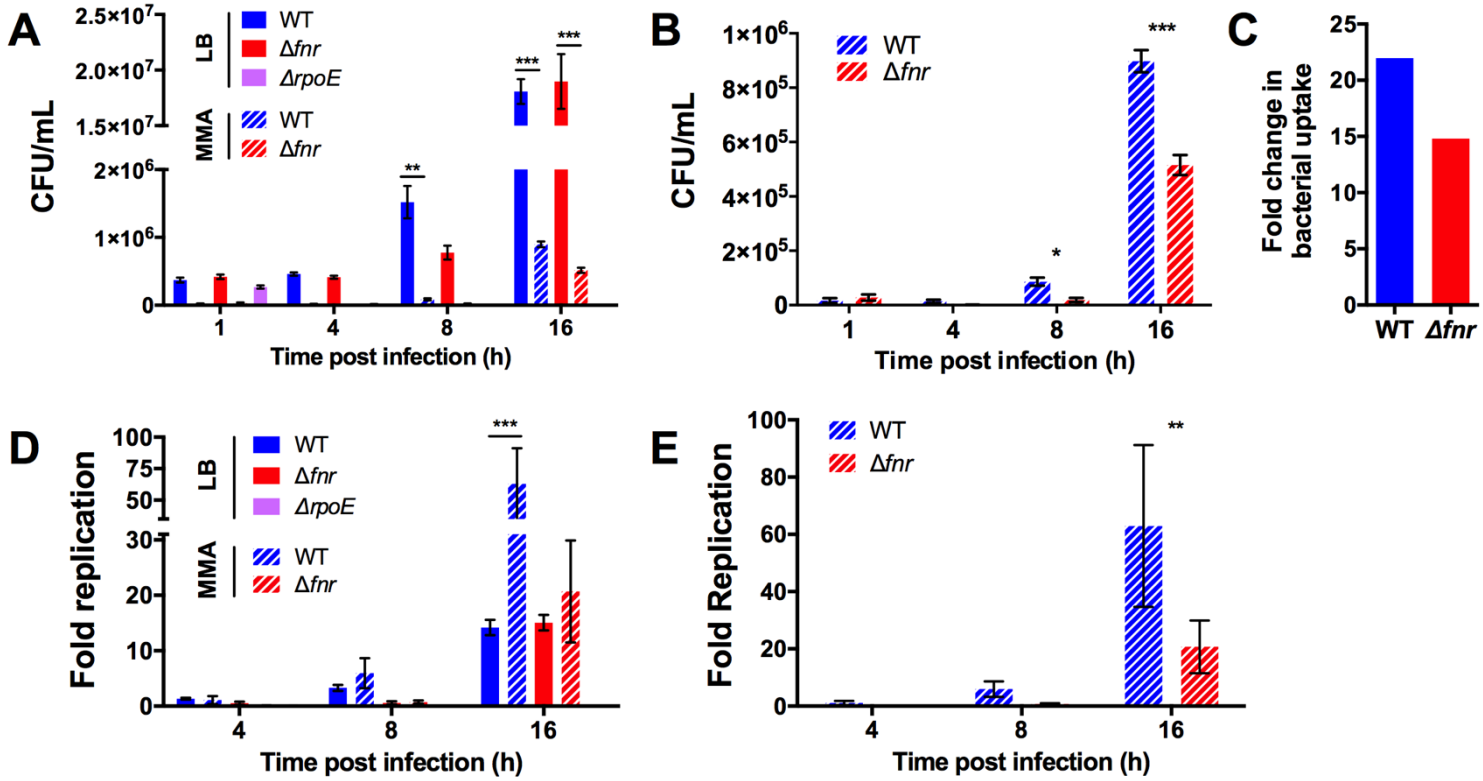

Figure 5.6 Growth in MMA prior to infection reduces bacterial uptake but increases fold replication in WT.

Survival of WT, Δ*fnr*, Δ*rpoE* up to 16 h post infection when inoculum was grown in LB compared to MMA. A. Total viable cells post infection, B. Total viable cells post infection, MMA inoculum only, C. fold change in bacterial uptake in WT and Δ*fnr* from cells grown in LB compared to MMA, D. the number of viable cells expressed as fold replication over 1 h post infection, E. the number of viable cells expressed as fold replication over 1 h post infection, MMA inoculum only. Statistical significance determined by Two-way ANOVA with Sidak's multiple comparisons test; Only statistically significant differences are shown; * = $p \leq 0.05$, ** = $p \leq 0.01$, and *** = $p \leq 0.001$; Error bars represent standard deviation.

5.2.3 Contribution of alternative electron acceptors to growth and SPI-2 expression

In this study we primarily examined expression of SPI-2 when cells reached an OD_{600} of 0.3. To determine if expression was present throughout growth, kinetic gene expression assays were conducted over 28 h. Cells were transformed with pDEW201-P*ssaG*, a low copy plasmid that produces luciferase upon activation of the *ssaG* promoter. Throughout these assays, Δ*ssrAB* pDEW201-P_{ssaG} was used as a negative control for SPI-2 expression. When grown under microaerobic conditions in MMA, *ssaG* expression in Δ*fnr* was >2-fold higher than WT over 28 h, with the highest expression (>1 × 10^6 RLU) at 6 h, decreasing to approximately 5.5 × 10^5 RLU in mid-exponential growth, and the lowest in stationary phase, 3.5 × 10^5 RLU (**Figure 5.7A**). The growth of WT and Δ*ssrAB* was the same, while Δ*fnr* grew considerably slower (**Figure 5.7A**). To test whether addition of alternative electron acceptors had an influence on SPI-2 expression we grew cells in MMA without fumarate and TMAO (MMA^-), with only fumarate (MMA^{+F}), and only TMAO (MMA^{+T}). In MMA^-, *ssaG* expression in WT was comparable when grown in MMA, however, in Δ*fnr*, *ssaG* expression remained higher than WT but less than 5 × 10^5 RLU for 28 h (**Figure 5.7B**), and the lack of electron acceptors slowed the growth of all strains. When supplemented with only fumarate (MMA^{+F}), cells had similar growth to that of MMA, however *ssaG* expression in Δ*fnr* remained much lower (**Figure 5.7C**). Growth in MMA^{+T} yielded increased expression of *ssaG* in WT and a small increase in Δ*fnr* compared to MMA, with the highest expression for both strains in early exponential phase (**Figure 5.7D**). However, when grown in MMA^{+T} all strains grew slower than in MMA. The MMA experiments showed that expression of SPI-2 in Δ*fnr* is aided by addition of TMAO as an alternative electron acceptor, while fumarate may act as an electron acceptor and as an additional carbon source. Overall, the growth of Δ*fnr* was always slower in MMA regardless of the addition of alternative electron acceptors, which is correlated with increased SPI-2 expression.

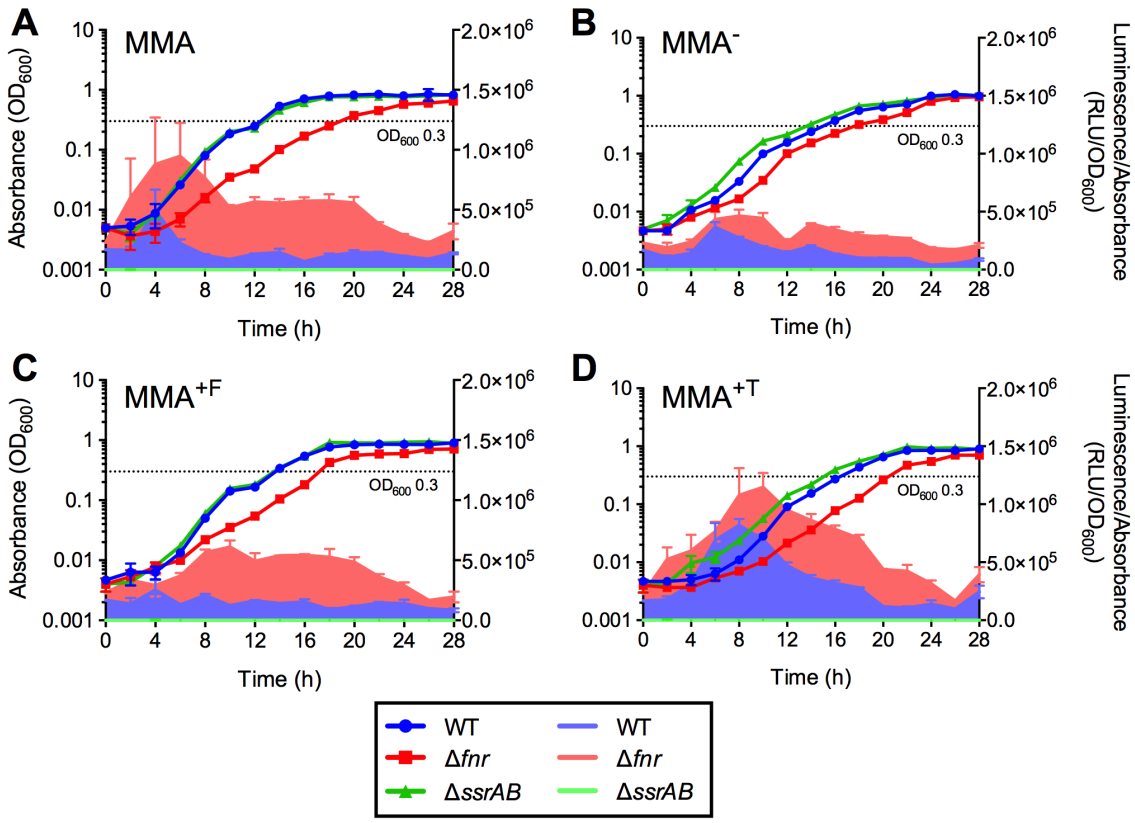

Figure 5.7 Growth and *ssaG* expression in MMA with and without alternative electron acceptors.

Cultures were grown as previously described (Section 2.2.3.2) and sampled every 1 to 2 h depending on the growth rate of the culture. Samples were measured for growth and luminescence. Cultures were grown in A. MMA with both electron acceptors fumarate and TMAO, B. MMA without alternative electron acceptors, C. MMA with only fumarate added and D. MMA with only TMAO added. For all graphs growth is shown by connected points bright colours) and correspond to values on the left y-axis and *ssaG* expression is shown by filled areas (light colours) and correspond to values on the right y-axis. Error bars represent standard deviation.

5.2.4 Relative fitness in vitro

The correct spatial and temporal programming for gene expression is necessary to avoid incurring fitness costs. This is especially important during pathogenesis, when *S.* Typhimurium encounters a series of stressful environments and must out-compete the host microbiota to establish successful infection. By removal of specific regulatory proteins gene expression is altered under certain growth conditions. In the following experiments isogenic mutants of *S.* Typhimurium 4/74 were grown in co-culture to determine which strain

possessed a fitness advantage. The strains WT, Δ*fnr*, Δ*ssrAB* and Δ*ssrAB*/*fnr* were transduced with a chloramphenicol resistance cassette (Cm^R) replacing the SL1483 gene, a transposase pseudogene which was unlikely to cause a reduction in fitness by removal. These strains will be referred to as WT-Cm^R, Δ*fnr*-Cm^R, Δ*ssrAB*-Cm^R and Δ*ssrAB*/*fnr*-Cm^R for brevity. The competitive index (CI) was determined as described in Section 2.2.9, but briefly a CI of 1 indicates there was no difference between strains, while a CI above 1 indicates a fitness advantage and below 1 a disadvantage. Strains were competed against themselves (e.g. WT versus WT-Cm^R) to determine if the introduction of the ΔSL1483::Cm^R was detrimental to fitness. In the graphs below the y-axis shows the log2 CI value, but data points are labeled with non-log adjusted average CI values.

First, we compete Δ*fnr* against each of Δ*ssrAB*, WT and Δ*fnr*/*ssrAB* in microaerobic MMA and the introduction of ΔSL1483::Cm^R did not seem to be detrimental to strains under this condition (**Figure 5.8**). As expected, due to the serious growth defect in Δ*fnr* in microaerobic MMA it was outcompeted by all strains. In Δ*fnr* versus Δ*fnr*/*ssrAB* we see that the abolishment of SPI-2 expression resulted in a fitness advantage to Δ*fnr*/*ssrAB*, suggesting that the expression of SPI-2 is detrimental under these growth conditions (**Figure 5.8C**). Next we performed a competition between WT and Δ*ssrAB* in SPI-2 inducing media. Unfortunately, under these conditions the ΔSL1483::Cm^R mutation in WT was detrimental to fitness (**Figure 5.9**). However, we could still gain insight from the WT versus Δ*ssrAB*-Cm^R, as the mutation had no effect on the fitness of Δ*ssrAB* under these conditions. Here we see a similar result as the Δ*fnr* versus Δ*fnr*/*ssrAB* MMA competition, where the WT strain expressing SPI-2 was outcompeted by the non-expressing Δ*ssrAB* strain (**Figure 5.9**). Finally, we performed competitions in microaerobic LB, a condition in which there is little SPI-2 expression and no growth defects between the mutants (**Figure 5.3D**). Interestingly, the WT and Δ*ssrAB* both outcompeted Δ*fnr* (**Figure 5.10A** & **B**). This is likely due to the ability of WT and Δ*ssrAB* to express anaerobic metabolism genes, however Δ*fnr*/*ssrAB*, also outcompeted Δ*fnr* (**Figure 5.10C**), suggesting that the small amount of SPI-2 expression seen at late stationary phase may be sufficient to cause a detriment to fitness of Δ*fnr* in microaerobic LB. When we competed WT against Δ*ssrAB*, neither strain was more fit (**Figure 5.10D**). These results provide insight into the energetic burden caused by expression of SPI-2 genes. Previously it has been shown that expression of SPI-1 genes imposed a growth penalty *in vitro* (Sturm *et al.*, 2011), and in an Δ*hns* mutant, deletion of SPI-1 particularly, and SPI-2 to a lesser extent restored growth (Ali *et al.*, 2014). These data

suggest that expression of SPI-2 can also impose a fitness disadvantage under *in vitro* conditions where it is not necessary for expression of the virulence genes.

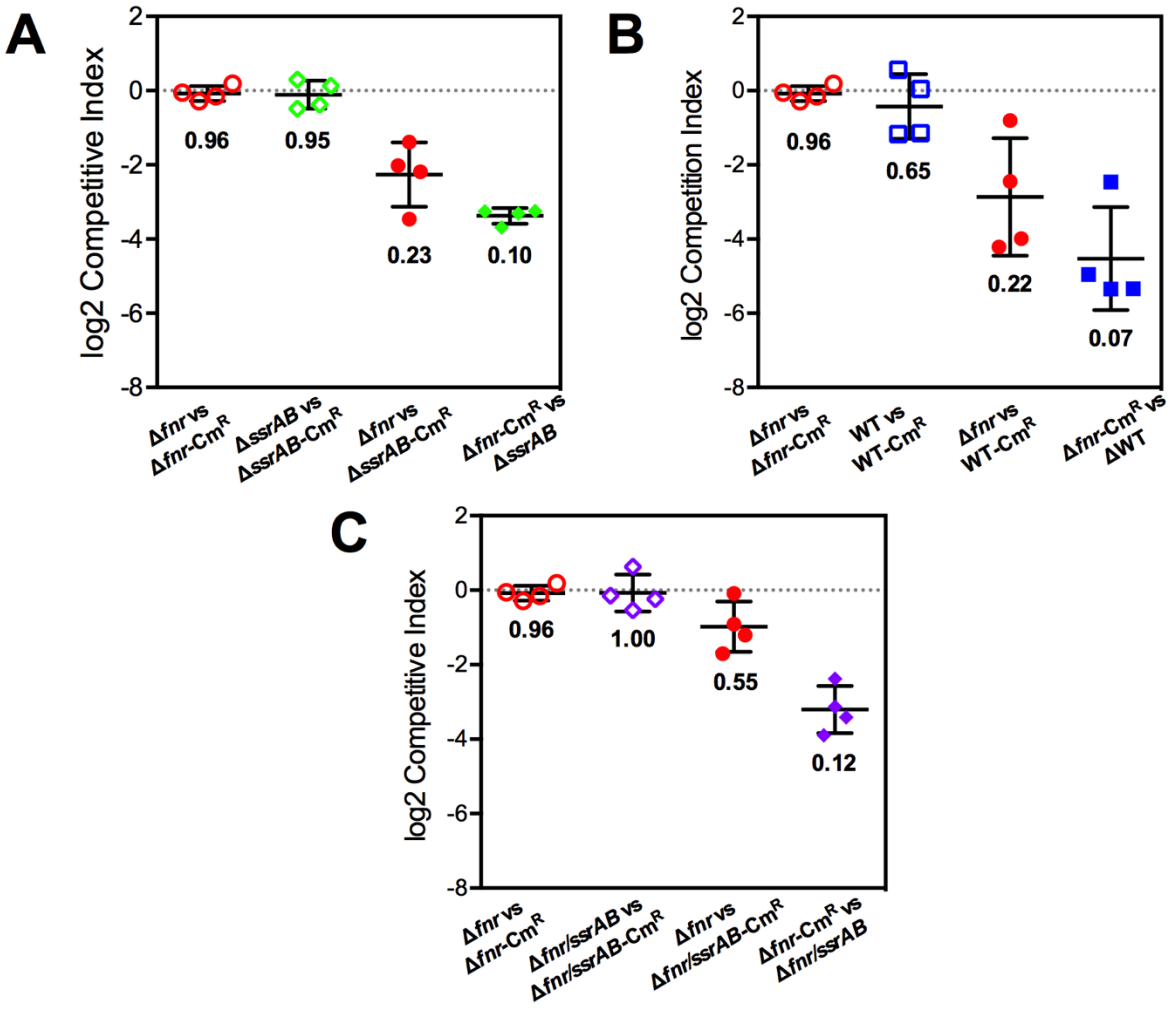

Figure 5.8 The Δ*fnr* mutant has reduced fitness in microaerobic MMA.

Relative fitness assays showing *in vitro* co-culture competitions in microaerobic MMA between A. Δ*fnr* and Δ*ssrAB*, B. Δ*fnr* and WT, and C. Δ*fnr* and Δ*fnr/ssrAB*. The y-axis shows log2 transformed competitive indices, and each competition is labeled with the CI (non-log transformed). The dotted grey line indicates a CI of 1, where there is no difference between strains. Error bars represent standard deviation.

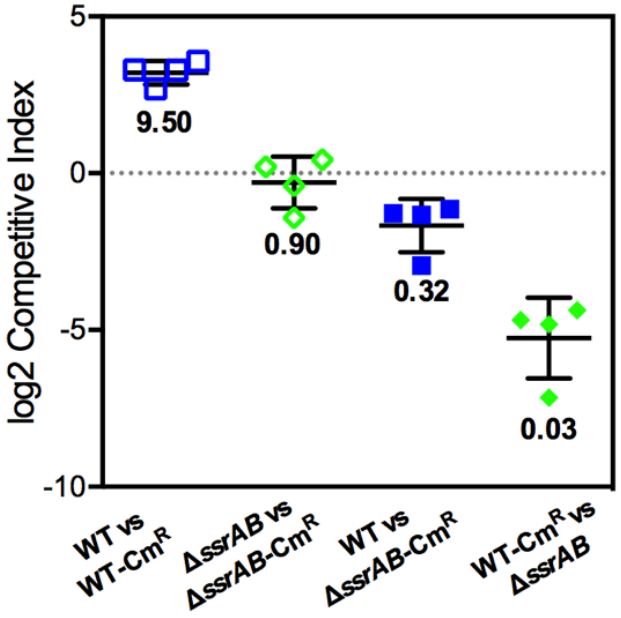

Figure 5.9 In SPI-2 inducing conditions the Δ*ssrAB* mutant outcompetes WT.
Relative fitness assays showing *in vitro* co-culture competition in aerobic inSPI2 between WT Δ*fnr/ssrAB*. The y-axis shows log2 transformed competitive indices, and each competition is labeled with the CI (non-log transformed). The dotted grey line indicates a CI of 1, where there is no difference between strains. Error bars represent standard deviation.

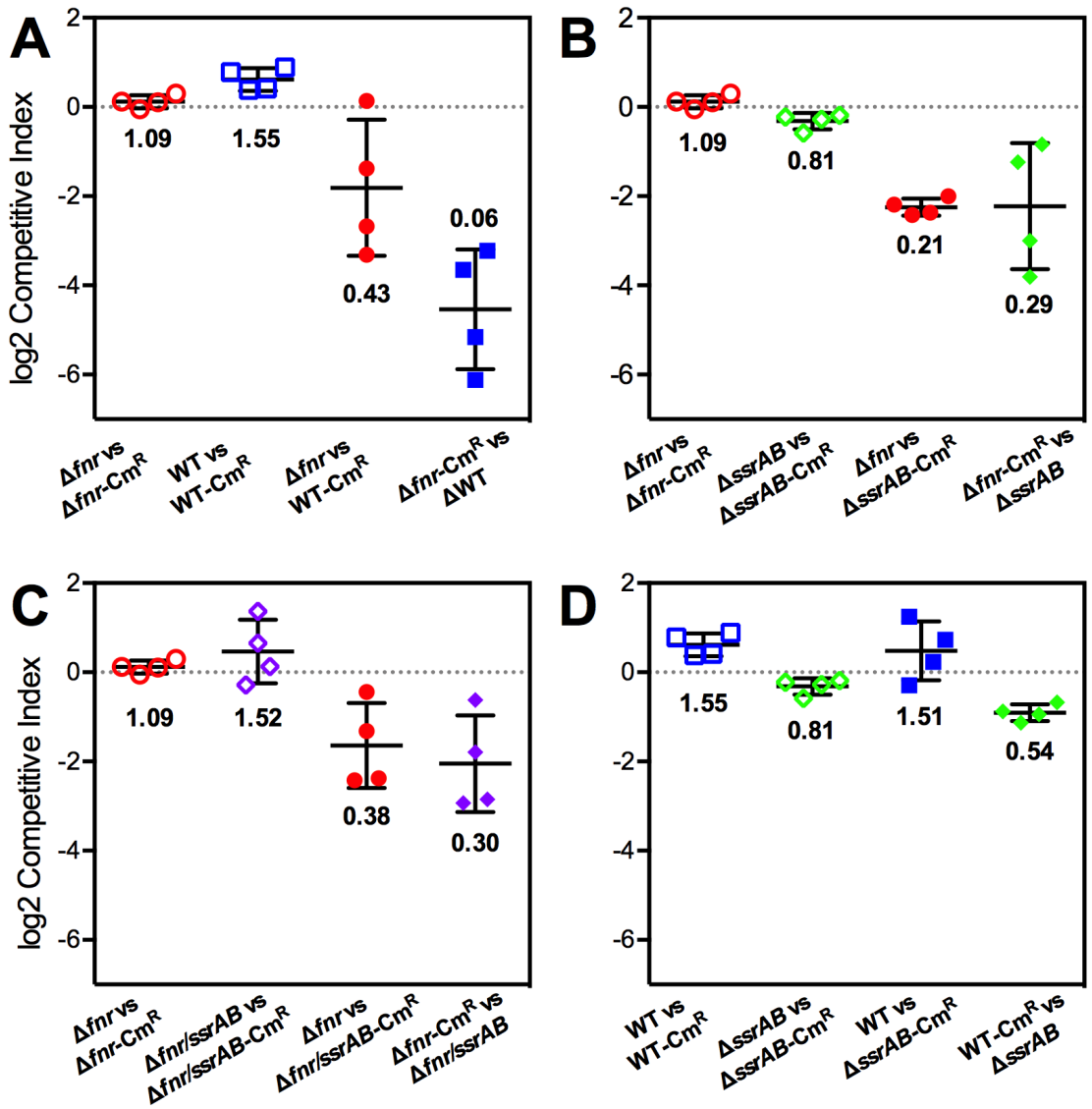

Figure 5.10 The Δ*fnr* mutant has reduced fitness and WT and the Δ*ssrAB* are equally "fit" in microaerobic LB.

Relative fitness assays showing *in vitro* co-culture competitions in aerobic LB between A. Δ*fnr* and WT, B. Δ*fnr* and Δ*ssrAB*, C. Δ*fnr* and Δ*fnr/ssrAB*, and D. WT vs Δ*ssrAB*. The y-axis shows log2 transformed competitive indices, and each competition is labeled with the CI (non-log transformed). The dotted grey line indicates a CI of 1, where there is no difference between strains. Error bars represent standard deviation.

5.2.5 Proteomic study of Δfnr

The Δ*fnr* mutant has been studied primarily from a transcriptomic point of view. Only one very recent study has attempted to elucidate the FNR regulon at a proteomic level (Wang *et al.*, 2019), however they have grown cells in anaerobic MOPS buffered LB with xylose, a medium shown not to allow expression of SPI-2 in Δ*fnr* (Fink *et al.*, 2007). In this study, we have used mass spectrometry to investigate the energy metabolism of WT and Δ*fnr* in microaerobic MMA and to identify abundant proteins which may occur only in the mutant. In the WT, 841 proteins were detected and in the Δ*fnr*, mutant 617 proteins were detected, and 524 proteins were common between the two (**Figure 5.11**). All detected proteins and absolute and relative quantities can be found in **Tables S9** and **S10** in Appendix IV.

The proteomic data identify abundant proteins found in the cytoplasm, however, proteins missing from the data set are not necessarily absent from cells, instead their abundance may be too low to be detected. Detected proteins included highly abundant proteins such as ribosomal subunits (Rpl, Rpm, Rps), RNA polymerase subunits (RpoA, RpoB, RpoC, and RpoZ), sigma factor RpoE. RpoE is a sigma factor typically associated with membrane stress but has been shown to be involved in upregulation of genes when transiting from aerobic to microaerobic environments in *E. coli*, which might explain its presence here (Partridge *et al.*, 2007). RpoD was only found in the Δ*fnr* mutant, however this is likely due to an issue in relative abundance of proteins in the WT. Proteins detected in both WT and Δ*fnr* also included a wide variety of nucleotide and amino acid biosynthesis proteins, which was expected as minimal media does not provide these nutrients. Nucleoid associated proteins and transcriptional regulators are highly abundant proteins in the cell cytoplasm (Cai & Inouye, 2002; Dillon & Dorman, 2010). Found in both the WT and Δ*fnr* proteomes were proteins including H-NS, both IHF subunits, HU alpha subunit, LRP, Dps and OmpR. Curiously, the HU beta subunit and CRP were only found in the WT. Indeed, *hupB* and *crp* were downregulated 0.73 and 0.80-fold in Δ*fnr* respectively according to RNA-seq data, therefore their abundance may have been too low to be detected. Fis was not present in either strain which is likely due to the poor nutrient content of the minimal medium. Although cells were taken at an OD_{600} of 0.3, in MMA FT, cells would not be considered to be at early exponential growth. WT and Δ*fnr* cultures inoculated at a starting OD_{600} of 0.005 take approximately 12 h and 20 h to reach OD_{600} 0.3 respectively. This may explain the presence of Dps and absence of Fis. This finding is contradictory with the literature which finds that Fis is required for *ssaG* expression or full SPI-2 expression in *S.* Typhimurium (Fass &

Groisman, 2009; Lim *et al.*, 2006) as there is highly induced expression of *ssaG* and 37 other SPI-2 encoded genes and effectors in this condition. Other highly abundant proteins found in both WT and Δ*fnr* included outer membrane proteins (OmpA, OmpC, OmpD, OmpF, OmpS, and OmpX), and chaperonin proteins GroEL/GroES, and DnaK. FNR was not detected in WT cells, and ArcA and ArcB were not at detectable concentrations in either strain.

We categorized the detected proteins into clusters of orthologous groups (COGs) according to the COGs as defined at http://www.ncbi.nlm.nih.gov/COG (**Figure 5.12**). They are presented as percentage of detected protein. In general, the growth of both the WT and Δ*fnr* were comparable to stationary phase cultures even though samples were taken at OD_{600} 0.3, and the *fnr* mutation did not seem to have any major effects when compared to WT. Energy production and conversion, cell wall and to a lesser extent post-translational modification were the most differentially detected protein categories. Under energy metabolism, as expected anaerobic proteins were produced less in Δ*fnr*. This includes expected candidates, such as fumarate reductase, pyruvate lyase, but also a number of propanediol utilization genes, which are expressed in the intestine (Faber *et al.*, 2017; Jakobson & Tullman-Ercek, 2016). Interestingly, the Wang et al., study found that FNR directly repressed propanediol degradation (Wang *et al.*, 2019) however, our results suggest that it indirectly activates these genes thus resulting in increased protein in the WT. This suggests that FNR plays a dual role in the regulation of propanediol degradation depending on available nutrients. The slower growth of the mutant may be due to decreased anaerobic metabolism proteins as Δ*fnr* struggles to produce energy efficiently. Slower growth of the mutant may also be due to the increased proportion of chaperones and proteases, since this can result in depleted protein synthesis under energy deficiency. Although, the *fnr* mutation generates an energy shortage under these conditions the WT is also already disadvantaged due to the poor growth conditions provided by MMA and low oxygen concentration, so the effects in Δ*fnr* are moderate.

While this proteomics study provides valuable information into the energetics of the Δ*fnr* mutant compared to WT, because of the number and abundance of metabolic proteins we were unable to detect many SPI-2 encoded proteins. We were able to detect the highly expressed SPI-2 effector proteins proteins SseA and SifB in the Δ*fnr* mutant and not the WT. Transcriptomic data previously revealed that *sseA* and *sifB* were upregulated 9.4-fold and 7.5-fold above WT, respectively. The presence of abundant SseA and SifB effector proteins

potentially indicates that the remaining upregulated genes identified by RNA-seq may also be translated and present in Δ*fnr* cells. Many of the other most highly upregulated SPI-2 genes are SPI-2 T3SS apparatus proteins and would likely be localized at the cell envelope, meaning they would be excluded from the cytoplasmic preparation for this experiment. In addition to SPI-2 effectors, SopB, a SPI-5 encoded, SPI-1 regulated effector was also detected in only the Δ*fnr* mutant. However, there is no difference in transcription of *sopB* between the WT and Δ*fnr* mutant, and the absolute expression was low.

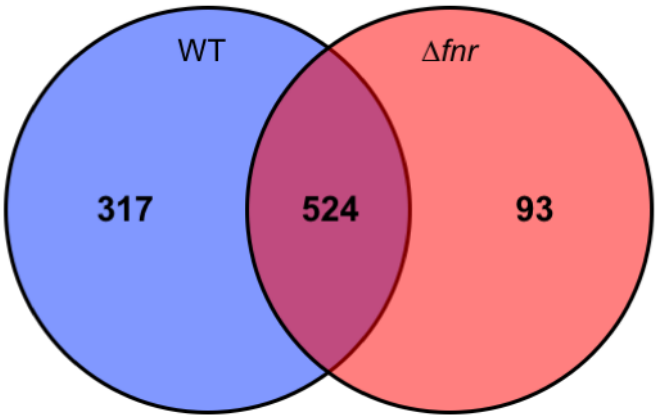

Figure 5.11 Venn diagram representing proteins detected by mass spectrometry in WT and Δ*fnr*.

The blue circle represents the total number of proteins detected in the WT and the red circle represents the total number of proteins detected in the Δ*fnr* mutant. The overlapping region indicates the number of proteins that were the same between both samples.

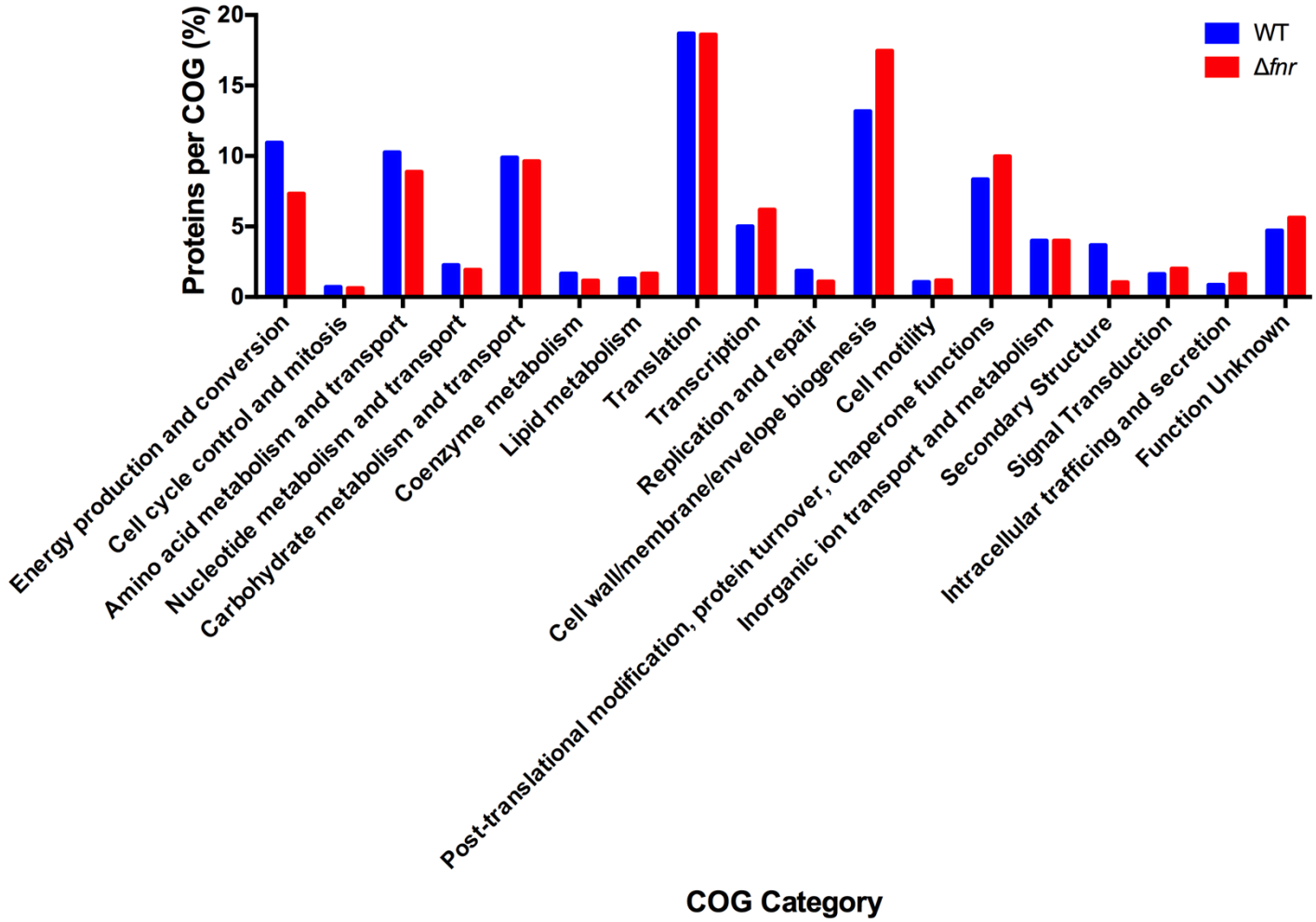

Figure 5.12 Classification of abundant proteins from WT and Δ*fnr* into COG categories.

Proteins detected in WT and Δ*fnr* cells were sorted into COG categories as defined by http://www.ncbi.nlm.nih.gov/COG. This graph represents the percentage of detected proteins which fit into each COG category.

5.3 Discussion

5.3.1 SPI-2 regulation by FNR in vitro and in vivo

We have shown that in microaerobic MMA SPI-2 is directly repressed by FNR in response to changes in oxygen concentration. Evidently, the addition of alternative electron acceptors fumarate and TMAO enhance that expression (**Figure 5.7**). Previous studies show that SPI-2 expression is not regulated by FNR in MOPS buffered LB with xylose under anaerobic conditions (Fink *et al.*, 2007; Wang *et al.*, 2019), and we see minimal expression of SPI-2 in microaerobic LB (**Figure 5.3D**). Surprisingly, in microaerobic InSPI2 media, FNR acted as an activator of SPI-2. This suggests that FNR may play a dual role in the regulation of SPI-2 in accordance with exogenous environmental signals besides oxygen. However, when we measured SPI-2 expression in infected macrophages, SPI-2 expression was significantly higher in Δ*fnr* (**Figure 5.5C**). This exposes a fundamental difference between *in vitro* and *in vivo* conditions. Further work is certainly required to elucidate the role of FNR in *vivo*, and to eliminate the problems of plasmid carriage and expression vectors it would be favourable to use a technique such as dual RNA-seq (Westermann & Vogel, 2018).

5.3.2 Attenuation of Δfnr in murine macrophages

Findings from Fink *et al*. and in this study suggest that FNR is important inside the phagosome of murine macrophages (Fink *et al.*, 2007). While this environment is not completely anaerobic, we have shown that FNR is active under low oxygen conditions, not only anaerobiosis, *in vitro*. Additionally, in *E. coli*, expression of FNR activated operons such as *frd* and *dms* are expressed at up to 10% O_2 saturation (Shalel Levanon *et al.*, 2005; Tseng *et al.*, 1996). The partial pressure of oxygen (pO_2) of the macrophage phagosome of *ex vivo* murine macrophages has also been observed at levels as high as 79.8 ± 1.6 to 32.6 ± 1.7 μM, depending on the density of the macrophages (James *et al.*, 1995). Furthermore, the production of reactive oxygen species (ROS) and oxygen consumption by macrophages and phagocytosed *S.* Typhimurium are expected to further decrease the pO_2 in the phagosome. Moreover, the pO_2 inside *ex vivo* macrophages almost certainly different from the pO_2 of *in vivo* macrophages (Atkuri *et al.*, 2005). A decrease in oxygen concentration in the murine gut from ~11% to ~2% upon infection with *S.* Typhimurium has been observed, and hypoxia enhanced SPI-2 transcription, translocation of SPI-2-encoded virulence proteins and intramacrophage replication *in vivo* (Jennewein *et al.*, 2015). More investigation into the

exact oxygen concentrations of macrophages both *ex vivo* and *in vivo* is required to elucidate the exact mechanism of FNR inside of host cells.

Despite the lack of information concerning the exact oxygen concentrations and mechanisms of intramacrophage FNR activity, it is certain that SPI-2 is necessary for the survival and intracellular replication of *S.* Typhimurium. YdgT has been previously identified as a repressor of SPI-2 expression, and negative control over this virulence system is central to systemic pathogenesis because *ydgT* mutants overexpressing virulence genes are ultimately attenuated during infection (Coombes *et al.*, 2005). As we have shown earlier, FNR binds to the promoter of *ydgT* and to a probable cis-acting sRNA. Potentially, FNR is able to indirectly modulate SPI-2 expression *in vivo* through control of YdgT. Another possibility of FNR influence on macrophage survival is through regulation of ethanolamine genes. In the Fink et al. study, FNR activated ethanolamine utilization genes (Fink *et al.*, 2007). A recent study found that EutH, an ethanolamine permease, is required for ethanolamine utilization at low pH and vacuole adaptation during macrophage infection (Anderson *et al.*, 2018). Thus, FNR activation of *eutH* during infection may also explain the attenuation of Δ*fnr* in macrophages.

5.3.3 Fitness of Δ*fnr*

While the proteomics investigation in this study provides valuable information into the energetics of the Δ*fnr* mutant compared to WT and helps us to understand the growth defects in Δ*fnr*, because of the number and abundance of metabolic proteins in both WT and Δ*fnr* we were unable to detect many SPI-2 encoded proteins. A more recent proteomics study used the approach of fractionation of proteins by SDS-PAGE prior to in-gel protein digestion and mass spectrometry which allowed them to identify more than just the most abundant protein in the cell (Wang *et al.*, 2019). Another useful approach might be to use two-dimensional difference gel electrophoresis (2D DIGE) to recognize differentially expressed proteins prior to identification by mass spectrometry in order to specifically look at those proteins with the most difference between samples (Joshi & Patil, 2017; Marouga *et al.*, 2005). This would add another layer of information to our data sets and help identify where there may be post-translational differences in regulation from what is seen in transcriptomic data.

Chapter 6
General Discussion

6.1 Importance of this study

6.1.1 Context of the study

Salmonella enterica is a medically important zoonotic pathogen that causes a range of diseases from gastroenteritis to Typhoid fever. Non-host adapted serovars of *Salmonella enterica*, such as *S*. Typhimurium, are considered successful pathogens as these serovars can colonize a wide range of hosts, survive a series of stressful events during the infection process, find nutritionally compatible niches inside the host, avoid, subvert, and/or evade the host innate and adaptive immune responses, replicate using resources found in the host, exit and transmit to new hosts and, in susceptible hosts, disseminate and cause systemic infection (Fabrega & Vila, 2013) (**Figure 1.1**).

S. Typhimurium establishes infection through the integration of a multitude of environmental signals through regulatory molecules including transcription factors and small RNAs. This allows the coordination of gene expression at the transcriptional and post-transcriptional levels in a precise spatiotemporal manner. The careful coordination of gene expression by regulatory molecules ensures that *S*. Typhimurium expresses only the genes and proteins necessary for survival and proliferation under each specific environmental condition, while avoiding loss of bacterial fitness due to inappropriate or wasteful gene expression (Groisman & Mouslim, 2006; Sengupta & Bhadauria, 2014). An understanding of the complex signals, regulatory inputs and pathways that result in the transcriptional activation or repression of virulence-associated genes provides insight into the steps necessary to establish an infection. Many studies have focussed on the signals which control the expression of virulence factors through transcriptional regulators particularly as they relate to the intracellular environment or media which mimic intracellular conditions *in vitro* (Colgan *et al*., 2016; Cirillo *et al.*, 1998; Fass & Groisman, 2009; Hébrard *et al.*, 2011; Knuff & Finlay, 2017; Kröger *et al.*, 2013; Lee *et al.*, 1999; Löber *et al.*, 2006; Martínez *et al.*, 2011; Srikumar *et al.*, 2015; Xu & Hensel, 2010; Yoon *et al.*, 2009) (**Figure 1.3**). But no studies have determined the signals or regulation which lead to low level SPI-2 gene expression prior to invasion of epithelial cells (Osborne & Coombes, 2011). There is increasing evidence that changes in environmental oxygen play a critical role in the timing and deployment of bacterial virulence factors, and that regulators of metabolism control the expression of genes imperative for causing infection and disease (Crofts *et al.*, 2018;

Marteyn *et al.*, 2010). The transcription factor FNR is a global regulator of anaerobic metabolism and responds directly to oxygen concentration (**Figure 1.4**). FNR has been established as a regulator of genes involved in host-cell invasion such as components of the T3SS and chaperones encoded on SPI-1 (Fink *et al.*, 2007; Wang *et al.*, 2019). Furthermore, FNR is required for full virulence in the murine model, and a mutant lacking this transcription factor is rapidly killed by macrophages (Fink *et al.*, 2007; Rollenhagen & Bumann, 2006). The SPI-2 T3SS and an intact SCV are required for evasion of ROS in macrophages (van der Heijden *et al.*, 2015), and FNR helps to promote resistance against oxidative stress although the mechanism has not been described (Fink *et al.*, 2007).

In this study, we used an RNA-seq-based transcriptomic approach combined with ChIP-seq to expand the regulon of FNR, under microaerobic conditions in a glycerol/trimethylamine N-oxide/fumarate minimal medium (MMA) (Constantinidou *et al.*, 2006), in *S.* Typhimurium. The use of this medium and RNA-seq has allowed identification of SPI-2 and other virulence-associated genes and sRNAs as targets for FNR gene regulation. RNA-seq provides an unbiased picture of all genes which are being actively transcribed, with no requirement for previous knowledge of the sequence which is being transcribed (Croucher & Thomson, 2010; Ozsolak & Milos, 2010), while ChIP-seq enabled mapping of FNR binding sites in a global and high-throughput fashion (Bonocora & Wade, 2015; Galagan *et al.*, 2012; Myers *et al.*, 2015). We also investigated the role of FNR in murine macrophages and the relative bacterial fitness of the Δ*fnr* mutant.

6.1.2 Global analysis of FNR-mediated regulation in S. Typhimurium

To our knowledge, the work presented here is the first large scale study to combine transcriptomic and global protein-DNA binding that has been carried out in an Δ*fnr* mutant of *S.* Typhimurium using high throughput next generation sequencing. In total, 482 genes and sRNAs were regulated directly or indirectly by FNR under microaerobic conditions in MMA. 286 genes were activated by FNR and 196 were repressed (**Figure 3.7**). We were able to corroborate existing data associating FNR as an activator of anaerobic metabolism (**Figure 3.8**), cell motility and chemotaxis (**Figure 3.17**, **3.18** & **3.19**), and reveal FNR as a newly identified repressor of *Salmonella* pathogenicity island 2 (**Figure 3.9** & **4.11**). The use of RNA-seq rather than microarray-based technology has also allowed us to identify 42 differentially expressed small non-coding RNAs in an Δ*fnr* background (**Table 3.1**, **3.2**, & **Figure 3.20**). ChIP-seq allowed identification of 288 putative FNR binding sites across the

entire *S.* Typhimurium chromosome and plasmids under microaerobic growth conditions in MMA (**Figure 4.4B**). Approximately 19% of differentially expressed genes in this study were associated with peaks from ChIP-seq (**Figure 4.4C**). We were able to validate through ChIP-seq and EMSA that FNR acts as a direct repressor of *Salmonella* pathogenicity island 2 through direct repression of the response regulator *ssrB* and the apparatus gene *ssaB*. Consequently, FNR indirectly represses the remainder of SPI-2 genes through the repression of *ssrB*. ChIP-seq also revealed FNR binding sites at SPI-2 associated genes encoded outside of the island, which could be validated in the future.

Under oxygenated conditions, we see moderate levels of SPI-2 expression in WT and Δ*fnr* (**Figure 3.13** & **3.14**). Furthermore, it is apparent that an additional metabolic signal, other than oxygen, is important for regulation of SPI-1 or SPI-2, as expression of these loci in Δ*fnr* is different dependent on growth media and conditions (**Figure 3.9, 3.10A**, **5.3**, **5.5C**) (Fink *et al.*, 2007; Wang *et al.*, 2019). However, this was not the case for expression of motility and chemotaxis genes, which are universally down-regulated in Δ*fnr* regardless of media composition (**Figure 3.17** & **3.19**) (Fink *et al.*, 2007). What this study highlights, is that the control of virulence expression is more complex and nuanced than originally conceived, and expression can vary dependant on numerous environmental factors which are integrated by multiple transcriptional and post-transcriptional regulators, many of which have not yet been identified.

In the context of *S.* Typhimurium infection, oxygen has previously been shown to be an important signalling molecule, but primarily for adhesion, invasion and SPI-1 expression (Ernst *et al*., 1990; Lee & Falkow, 1990; Schiemann & Shope, 1991; Fink *et al*., 2007). Our results show that the SPI-2 priming of *S.* Typhimurium cells *in vivo* (Brown *et al*., 2005, Osborne & Coombes, 2011) could be a result of cells detecting oxygen at the epithelial border of the intestine through a mechanism involving FNR. Here we describe how can FNR act directly as a transcriptional repressor at SPI-2 promoters, especially *ssrB* and *ssaB*, to cause repression under oxygen-limited conditions, and for that repression to be lifted when oxygen concentrations are increased and allow expression of SPI-2 encoded and associated genes (**Figure 6.1**).

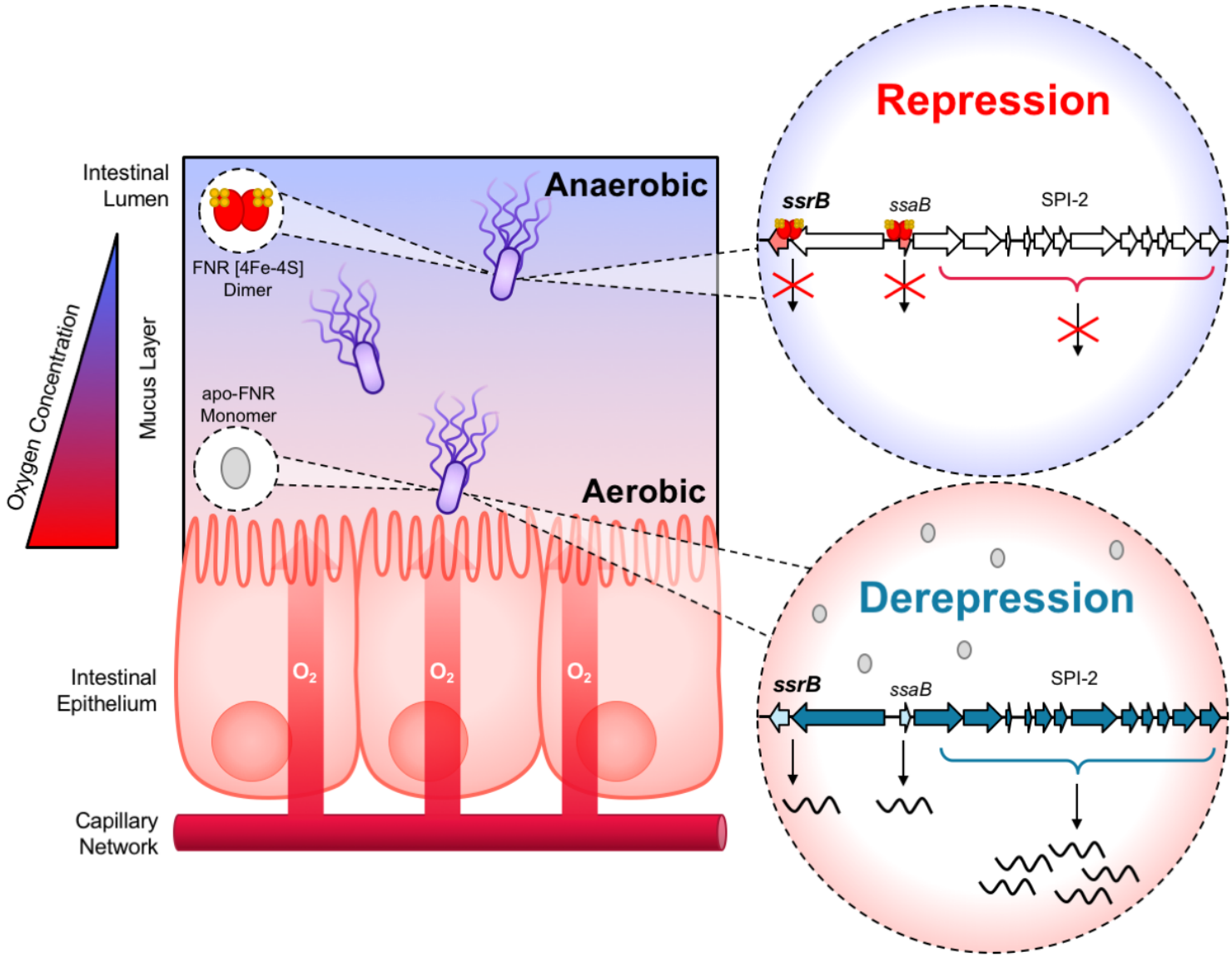
Repression
Intestinal Lumen
Anaerobic
ssrB
ssaB
SPI-2
FNR [4Fe-4S] Dimer
Oxygen Concentration
Mucus Layer
apo-FNR Monomer
Aerobic
Derepression
Intestinal Epithelium
O2
O2
O2
ssrB
ssaB
SPI-2
Capillary Network

Figure 6.1 Model of SPI-2 repression by FNR under changing oxygen conditions.
The intestinal lumen is an anaerobic environment (blue) however, there is a zone of relative oxygenation adjacent to the gastrointestinal tract mucosa (red), caused by diffusion of O_2 (arrows) from the capillary network at the tips of villi. Under anaerobic and microaerobic conditions the active FNR dimer can bind to DNA, however when sufficient oxygen is detected by iron-sulphur clusters bound to FNR, the dimer dissociates to form apo-FNR and is no longer able to bind DNA. Under low oxygen conditions FNR binds directly to and represses expression from the promoter of the SPI-2 response regulator *ssrB*, *ssaB* and promoters of SPI-2 genes. Under aerobic conditions the virulence genes are derepressed allowing preemptive synthesis of important virulence factors in preparation for the harsh intracellular environment of host cells.

6.2 Future prospects

There are many possibilities for the development of this work in the future. The long term aim for this study is that the data presented here will contribute to the complete description of molecular biological networks within *S.* Typhimurium. Below, several avenues for future research are discussed using this work as a starting point.

6.2.1 Signals for expression of virulence

As previously discussed, it is unclear what component in MMA allows for SPI-2 expression as the medium does not incorporate signals which have typically been required for SPI-2 expression (Deiwick *et al.*, 1999; Löber *et al.*, 2006; Xu & Hensel, 2010). However, it may be in part due to the presence of alternative electron acceptors such as fumarate and TMAO (**Figure 5.7**). We identified a near consensus FNR binding motif in the promoter region of a potential class I fumarase homologue *orf70* (*fumD*) also encoded within SPI-2 (**Figure 4.5**). This mesaconase/fumarase enzyme has not been studied in *S.* Typhimurium, however the location of *orf70* would suggest that mesaconate or fumarate may be important signalling molecules for SPI-2 (Kronen & Berg, 2015). Further investigation into other alternative electron acceptors, such as tetrathionate, nitrate or thiosulphate, as signals for virulence regulation is also warranted. These molecules are found in the gut in response to bacteria-induced inflammation (Thiennimitr *et al.*, 2012; Winter *et al.*, 2010; 2013) and interestingly, it has been demonstrated that FNR is involved in regulation of tetrathionate and nitrate

metabolism (Prince-Carter et al., 2001). Moreover, the genes encoding tetrathionate respiration proteins, *ttrRSBCA*, are encoded within SPI-2 (Hensel *et al.*, 1999). Tetrathionate also shares another link with FNR as the molecule supports B12-dependent anaerobic growth of *S.* Typhimurium on ethanolamine or 1,2-propanediol (Price-Carter *et al.*, 2001). Fink et al., showed that FNR activates expression of ethanolamine utilization genes and a recent study found that EutH, an ethanolamine permease, is required for ethanolamine utilization at low pH and vacuole adaptation during macrophage infection (Anderson *et al.*, 2018; Fink *et al.*, 2007). Thus, FNR activation of *eutH* during infection may also explain attenuation of Δ*fnr* in macrophages. In another recent study, propanediol degradation proteins were up-regulated in Δ*fnr* under anaerobic growth in MOPS buffered LB with xylose (Wang *et al.*, 2019), and our study revealed that the *pdu* and *cbi* operons were down-regulated in Δ*fnr*, and Pdu proteins were more abundant in WT. These links between the metabolism of molecules commonly found in the gut and virulence should certainly be investigated further.

6.2.2 sRNAs, the missing link?

Through our transcriptomic investigation we found that there were multiple virulence-associated sRNAs which were differentially expressed in Δ*fnr* (**Table 3.1**, **3.2**, & **Figure 3.20**). Previously, sRNAs had been overlooked in large-scale investigations into the regulatory pathways involved in controlling gene expression in bacteria (Altuvia & Wagner, 2000). The discovery of vast numbers of sRNAs, with diverse mechanisms of action and physiological functions, in many studied bacterial species, highlight the integral role played by sRNAs in bacterial gene regulation, usually at the post-transcriptional level (Kröger *et al.*, 2013; Ryan *et al.*, 2017; Vogel, 2009). While more work is still required to determine the roles of the majority of sRNAs in *S.* Typhimurium, there are a few specific links with FNR which warrant more investigation.

The largest sRNA regulon belongs to *GcvB*, a sRNA which controls expression of ~1% of the *S.* Typhimurium genome (Hébrard *et al.*, 2012; Sharma *et al.*, 2007; 2011). Noteably, in our data set *GcvB* was the most highly upregulated transcript (approximately 13-fold) in Δ*fnr* (**Table 3.2**). While the role of *GcvB* seems to be involved primarily in the downregulation of amino acid and peptide transporters (Sharma *et al.*, 2011), these processes have recently been suggested to be important for efficient host cell invasion (Miyakoshi, 2019). Additionally, *GcvB* negatively regulates the global regulator Lrp (Sharma *et al.*, 2011). This is of particular interest as Lrp is a known repressor of SPI-2. It binds directly to a consensus

motif at the *ssrA* promoter which results in down-regulation of SPI-2 genes (Baek *et al.*, 2009). Thus, there may be additional control of SPI-2 through a regulatory pathway where under low oxygen conditions FNR represses *GcvB*, which then allows repression of SPI-2 through Lrp, and conversely under aerobic conditions, repression of *GcvB* is lifted causing a downregulation in *lrp* mRNA and expression of SPI-2.

Another potential link of FNR to virulence genes expression through an sRNA exists within *FnrS*. In our study, *fnrS* was the most down regulated (approximately 35-fold) sRNA in Δ*fnr* (**Table 3.1**). In a recent study, deletion of *FnrS* led to increased HilD production under low aeration conditions, and *FnrS* could bind to the *hilD* mRNA 5' UTR, resulting in translational repression (Kim *et al.*, 2018). The authors discuss the importance of *FnrS* on SPI-1 expression however fail to mention the potential impact of HilD-mediated crosstalk between SPI-1 and SPI-2 (Bustamante *et al.*, 2008). Our data reveal that *FnrS* expression is dramatically decreased in Δ*fnr* and in aerated WT cultures (**Figure 3.2C**), and SPI-2 expression is increased under these conditions (**Figure 3.14**). Thus, in the absence of *FnrS*, HilD levels may be increased, contributing to the SPI-2 induction phenotype that we have observed.

When we examined the binding of FNR at the *ydgT* promoter region, ChIP-seq data showed a putative binding site near the P_{ydgT}. However, the peaks had the highest coverage at the TSS of an unannotated antisense transcript found in the *ydgT* promoter region (**Figure 4.12A**). A motif search also revealed a near consensus FNR binding motif at the 5′ end of the antisense transcript (**Figure 4.5**). Further investigation into this antisense transcript is required but, we can speculate that it is directly regulated by FNR due to the presence of the near consensus FNR binding motif and up-regulation in Δ*fnr*, and its position relative to *ydgT* makes it a prime candidate as a cis-acting sRNA which may post-transcriptionally repress *ydgT* under aerobic conditions.

There were no SPI-1 like RNAs found in the FNR regulon under microaerobic conditions in MMA. This is not surprising as SPI-1 genes in general were not highly expressed under these conditions (**Figure 3.10A**). The Fink et al. and Wang et al. studies used conditions conducive to SPI-1 expression however their approaches to FNR regulon identification left them unable to detect sRNAs (Fink *et al.*, 2007; Wang *et al.*, 2019). However, if the growth conditions used in the aforementioned studies were used and the regulon were assessed by RNA-seq, it is possible that SPI-1 like sRNAs may emerge as targets for FNR control.

6.2.3 Regulation of motility

It has been demonstrated that under very different growth conditions, FNR was an activator of motility and chemotaxis under low oxygen and anaerobic conditions (**Figure 3.17**, **3.18** & **3.19**) (Fink *et al.*, 2007). However, we have shown that the regulation by FNR is indirect for genes with the exception of *fliC*, *fliD*, and *aer*, the gene encoding Aer the signal transducer for aerotaxis. Interestingly, a recent study demonstrated that SsrB can repress *S*. Typhimurium motility through direct binding to a region upstream of *flhDC* (Ilyas *et al.*, 2018). Thus, much of the down-regulation of motility, especially of early gene products can be explained by FNR activation of *ssrB*, leading to SsrB repression of motility. This link makes sense as FNR is intracellularly active, possibly leading to increased expression of *ssrB*, and thus down regulation of flagella which, has been observed in macrophage as a mechanism to hide from host defenses (**Figure 5.4**, **5.6**) (Ibarra & Steele-Mortimer, 2009). Information on the intracellular role of FNR is lacking, and further investigation of this and of a possible link through SsrB to flagellar regulation is required.

6.2.4 Differences in virulence gene expression in Δfnr based on strain

During the preparation of this thesis, a new study emerged with the goal of investigating the FNR regulon through a proteomics approach (Wang *et al.*, 2019). The same growth conditions as the Fink *et al*. study were used (anaerobic growth in MOPS buffered LB with xylose), but surprisingly Wang *et al*., found contrasting results (Fink *et al.*, 2007; Wang *et al.*, 2019). In the microarray study SPI-1 was activated by FNR while in the proteomic study SPI-1 was found to be repressed by FNR. These results were explained by the difference in strain used in the studies. In the microarray study *S*. Typhimurium strain 14028S was used while strain SL1344 was used for the proteomic study. Interestingly, SL1344 is more invasive than 14028S due to heterogeneity in expression of *Salmonella* pathogenicity island 1 and the absence of the SPI-1 effector SopE in 14028S (Clark *et al.*, 2011). While the growth condition in our study is quite different, we found that expression of SPI-1 was minimally activated by FNR, in agreement with the Fink *et al* study (Fink *et al.*, 2007). In this study we have used strain 4/74, a strain which harbours *sopE* and differes from SL1344 by only 8 SNPs. Therefore, there may be another difference between the Fink *et al*. and Wang *et al*. studies which has not been accounted for. Strain 4/74 is a prototroph for histidine production that possesses a functional histidine biosynthetic pathway missing from SL1344

that is important for replication in the vacuolar environment of certain mammalian cells including macrophages (Henry *et al.*, 2005) which may help to explain differences in SPI-2 expression but not SPI-1. Clearly more work is needed on elucidating the specific difference between commonly used lab strains of *S.* Typhimurium especially as it pertains to mechanisms of virulence.

6.2.5 Limitations of our approach and existing technologies

As previously discussed, there are some limitations to the approach used in this study. Samples for RNA- and ChIP-seq are taken from a population of bacteria, which provide an average view of the transcriptomes of millions of cells. Genetically identical cells can often be phenotypically different and subpopulations of cells or outlier cells play important roles in disease outcome or antibiotic tolerance (Diard *et al.*, 2013; Helaine *et al.*, 2014). Additionally, our growth condition is heterogenous in nature, meaning we likely have very different expression in subpopulations throughout the oxygen gradient of the cultures. Single-cell RNA sequencing can reveal complex and rare cell populations, uncover regulatory relationships between genes and single-cell transcriptomic analysis will allow the detection of even more subtle, and often biologically significant, changes in gene expression that could be masked at the level of whole bacterial populations (Hwang *et al.*, 2018). For ChIP-seq, there are currently no studies using single-cell ChIP-seq in bacteria or eukaryotic cells (Nakato & Shirahige, 2017) and bacteria present an even larger barrier to single-cell ChIP as the small size of individual bacterial cells means they contain far less DNA and protein than eukaryotic cells. DNA sequencing coverage is a major limitation of single-cell ChIP-seq, as the analysis requires large amounts of starting material (Nakato & Shirahige 2017).

Additionally, to provide proof of principle it would be ideal to sequence RNA extracted from an *in vivo* infection where we specifically look at transcription of cells in the gut lumen and at the epithelial border prior to host cell invasion, however, this is not yet possible. Recombinase-based *in vivo* expression technology (RIVET) is a sensitive reporter of gene expression which involves the construction of a transcriptional fusion to a site-specific recombinase, which mediates the loss of a selectable genetic marker. This technique was fundamental for finding SPI-2 expression at the epithelial border (Brown *et al.*, 2005) however, it can only detect expression of individual genes. Nonetheless, other interesting experiments can be done while new technologies and technique develop which could be very

informative. The plasmids that were constructed in this study for examining promoters of SPI-2 genes (**Figure 4.13**) where constructed on a pCS26 plasmid which can be incorporated onto the chromosome in the same method used to build our *fnr*$^{+}$ complementation strain (**Figure 2.1**) (Shivak *et al.*, 2016). In this way, we could measure kinetic *in vivo* expression from SPI-2 promoters with the problems associated with plasmid carriage. We are also interested in performing *in vivo* competitive fitness assays in mice with WT, Δ*fnr* and with strain constructed with an oxygen insensitive FNR. These assays would help to further elucidate the role of FNR in *Salmonella* virulence and determine if Δ*fnr* mutant is attenuated in various tissues of the gut and to see what effect an oxygen insensitive FNR has on virulence.

Furthermore, as can be observed from the Colgan *et al*. study, the targets of transcription factors may change depending on the growth condition, indeed the HilD regulon changes depending on the growth condition (Colgan *et al*., 2016). Similarly, we see that the growth condition in the Fink *et al*. study is extremely different, and thus yielded a different FNR regulon from the current study (Fink *et al.*, 2007). Therefore, it is important to investigate regulons of TFs under more than one condition, especially in reference to TFs who play a role in virulence. Westermann *et al*. have recently shown that dual-RNA-seq can give important insight into the *ex vivo* transcriptome (Westermann *et al.*, 2016), and the group have recently made advances in toward cell type-specific *in vivo* dual-RNA-Seq (Frönicke *et al.*, 2018) which will be very useful techniques for precisely understanding *in vivo* transcriptional regulation and expression in the future.

6.3 Concluding remarks

Overall, we have used a combination of NGS techniques to expand the scope of the FNR regulon in *S.* Typhimurium and have established FNR as a direct repressor of SPI-2. This study demonstrates that oxygen is an important environmental signal and plays a critical role in the timing and deployment of *S.* Typhimurium virulence factors, and that FNR as a regulator of anaerobic metabolism, can also control the expression of genes critical for causing infection and disease. We have also shown that it is imperative to investigate bacterial regulons under a multitude of conditions to encompass the full capacity of a transcriptional regulator. Our particular focus on FNR regulation of SPI-2 under microaerobic conditions has revealed that, not only does FNR repress the expression of the SPI-2 encoded type three secretion system apparatus proteins, effectors and chaperones through direct regulation of the SPI-2 response regulator SsrB, but it also involved in the

direct repression of a great number of effectors and virulence relevant sRNAs encoded throughout the chromosome and on the *Salmonella* virulence plasmid. Furthermore, aerobic conditions relieved repression allowing for expression of virulence factors likely in preparation for the harsh intracellular environment of host cells (**Figure 6.1**). Moreover, we have provided additional evidence that oxygen is an important signalling molecule for the control of bacterial motility and that FNR is an important regular in both intra- and extracellular environments. Additionally, we have shown that the accurate spatiotemporal expression of SPI-2 is integral for maintenance of bacterial fitness. Importantly, analysis of our Δ*fnr* RNA-seq-based transcriptomic data in conjunction with previously published datasets (Colgan *et al*., 2016), will provide a more complete picture of mixed regulatory interactions within the cell. We hope that the addition of this data will help in the development of the full picture of all regulatory interactions and inputs involved in establishment of *S*. Typhimurium infection.

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Appendix I

Table S1. RNA-seq data: Absolute and relative expression.

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
repY	440	520	0.85	ST4-74_pColIb	378	467	+
repZ	41	49	0.85	ST4-74_pColIb	455	1486	+
yacA	88	72	1.23	ST4-74_pColIb	2395	2664	+
yacB	24	19	1.24	ST4-74_pColIb	2661	2942	+
yacC	31	28	1.12	ST4-74_pColIb	2982	3830	+
yadA	69	63	1.08	ST4-74_pColIb	3971	4420	+
yaeA	274	189	1.45	ST4-74_pColIb	4555	4788	+
yaeB	19	19	1.03	ST4-74_pColIb	4897	5166	-
yafA	29	30	0.99	ST4-74_pColIb	5163	5657	-
yafB	39	35	1.11	ST4-74_pColIb	5713	6315	-
yagA	23	24	0.95	ST4-74_pColIb	6669	8015	+
cib	38	27	1.38	ST4-74_pColIb	8294	10174	+
imm	75	43	1.76	ST4-74_pColIb	10192	10539	-
ybaA	44	25	1.77	ST4-74_pColIb	10658	10977	+
ybbA	4	3	1.52	ST4-74_pColIb	11076	11759	+
ybfA	12	13	0.95	ST4-74_pColIb	12400	12852	+
resA	5	5	1.02	ST4-74_pColIb	12854	13639	+
SLP2_0018	14	14	1.05	ST4-74_pColIb	13893	14096	+
parA	34	37	0.92	ST4-74_pColIb	14223	14855	+
SLP2_0020	44	39	1.15	ST4-74_pColIb	14855	15229	+
stbA	53	52	1.01	ST4-74_pColIb	15468	16442	+
stbB	29	31	0.95	ST4-74_pColIb	16446	16838	+
impC	36	43	0.84	ST4-74_pColIb	16979	17101	-
yccA	8	6	1.34	ST4-74_pColIb	17236	17481	+
yccB	9	6	1.56	ST4-74_pColIb	17495	18421	+
ycdA	3	4	0.81	ST4-74_pColIb	18418	18730	+
ycdB	3	2	1.24	ST4-74_pColIb	18806	19489	+
yceA	2	1	1.48	ST4-74_pColIb	19490	19711	+
yceB	2	2	0.77	ST4-74_pColIb	19725	20159	+
ycfA	4	4	1.19	ST4-74_pColIb	20205	20981	+
ycgA	8	9	0.88	ST4-74_pColIb	20956	21426	+
ycgB	4	2	1.68	ST4-74_pColIb	21399	21824	+
ycgC	8	4	1.99	ST4-74_pColIb	21871	22293	+
ychA	5	6	0.93	ST4-74_pColIb	22606	22898	+
ssb	6	3	2.09	ST4-74_pColIb	23250	23777	+
ycjA	6	4	1.45	ST4-74_pColIb	24121	26085	+
psiB	10	9	1.09	ST4-74_pColIb	26140	26574	+
psiA	10	8	1.29	ST4-74_pColIb	26625	27290	+
SLP2_0039	9	7	1.24	ST4-74_pColIb	27290	27883	+
yddA	62	66	0.93	ST4-74_pColIb	27955	28425	+
ard	8	3	2.41	ST4-74_pColIb	28331	28846	+
ydfA	4	2	1.69	ST4-74_pColIb	29575	30009	+
ydfB	4	4	1.02	ST4-74_pColIb	30103	30369	+
ydgA	305	195	1.57	ST4-74_pColIb	30311	31360	+
ydhA	6	6	1.03	ST4-74_pColIb	32007	32342	+
ydiA	8	6	1.37	ST4-74_pColIb	32467	33315	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
SLP2_0047A	219	203	1.08	ST4-74_pColIb	33401	33736	-
nikA	39	46	0.84	ST4-74_pColIb	33969	34301	+
nikB	14	15	0.91	ST4-74_pColIb	34312	37011	+
trbC	13	10	1.27	ST4-74_pColIb	37048	39339	-
trbB	12	9	1.27	ST4-74_pColIb	39332	40402	-
trbA	8	7	1.18	ST4-74_pColIb	40421	41629	-
pndC	3086	3748	0.82	ST4-74_pColIb	41782	42066	+
pndA	5309	6352	0.84	ST4-74_pColIb	41942	42073	+
SLP2_0054A	14106	14363	0.98	ST4-74_pColIb	42696	42992	+
exc	426	425	1.00	ST4-74_pColIb	43906	44568	-
traY	15	13	1.22	ST4-74_pColIb	44633	46864	-
traX	7	6	1.03	ST4-74_pColIb	46892	47476	-
traW	4	4	0.97	ST4-74_pColIb	47505	48707	-
traV	7	7	1.04	ST4-74_pColIb	48674	49288	-
traU	8	7	1.12	ST4-74_pColIb	49288	52332	-
traT	6	5	1.14	ST4-74_pColIb	52422	53222	-
traS	3	3	1.12	ST4-74_pColIb	53206	53394	-
traR	13	17	0.77	ST4-74_pColIb	53458	53862	-
traQ	7	8	0.88	ST4-74_pColIb	53913	54440	-
traP	16	17	0.91	ST4-74_pColIb	54440	55144	-
traO	9	10	0.93	ST4-74_pColIb	55144	56433	-
traN	6	7	0.87	ST4-74_pColIb	56436	57419	-
traM	6	7	0.87	ST4-74_pColIb	57430	58122	-
traL	5	8	0.67	ST4-74_pColIb	58119	58466	-
sogL	6	6	1.02	ST4-74_pColIb	58484	62251	-
nuc	5	7	0.67	ST4-74_pColIb	62341	62892	-
traK	6	10	0.60	ST4-74_pColIb	62907	63197	-
traJ	5	6	0.92	ST4-74_pColIb	63194	64342	-
traI	5	5	0.95	ST4-74_pColIb	64339	65157	-
traH	8	8	1.04	ST4-74_pColIb	65154	65612	-
traG	9	8	1.11	ST4-74_pColIb	66007	66591	-
traF	21	29	0.71	ST4-74_pColIb	66651	67853	-
traE	13	17	0.77	ST4-74_pColIb	67938	68762	-
rci	15	13	1.19	ST4-74_pColIb	68913	70067	-
shfB	8	6	1.33	ST4-74_pColIb	70132	70383	+
shfB	39	22	1.79	ST4-74_pColIb	70385	70630	-
shfC	21	17	1.22	ST4-74_pColIb	70653	71006	+
pilV	10	10	0.97	ST4-74_pColIb	71003	72295	-
pilU	5	5	0.95	ST4-74_pColIb	72295	72951	-
pilT	8	11	0.77	ST4-74_pColIb	72936	73496	-
pilS	22	27	0.83	ST4-74_pColIb	73506	74120	-
pilR	9	10	0.90	ST4-74_pColIb	74138	75235	-
pilQ	7	9	0.84	ST4-74_pColIb	75248	76801	-
pilP	6	10	0.58	ST4-74_pColIb	76812	77264	-
pilO	6	8	0.75	ST4-74_pColIb	77251	78546	-
pilN	6	8	0.79	ST4-74_pColIb	78539	80221	-
pilM	4	7	0.64	ST4-74_pColIb	80235	80672	-
pilL	5	7	0.82	ST4-74_pColIb	80672	81739	-
pilK	4	4	0.94	ST4-74_pColIb	81773	82363	-
pilJ	2	2	0.70	ST4-74_pColIb	82413	82865	-
pilI	6	7	0.89	ST4-74_pColIb	82908	83162	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
trcD	34	36	0.95	ST4-74_pColIb	83430	83996	-
traC	38	35	1.08	ST4-74_pColIb	84010	84693	-
traB	27	22	1.19	ST4-74_pColIb	84947	85480	-
traA	41	47	0.86	ST4-74_pColIb	85922	86209	-
sulII	332	402	0.83	ST4-74_pRSF1010	30	842	-
SLP3_0002	1171	1080	1.08	ST4-74_pRSF1010	854	1075	-
repC	17	17	1.03	ST4-74_pRSF1010	1153	1998	-
repA	29	29	0.99	ST4-74_pRSF1010	1991	2827	-
SLP3_0005	133	109	1.21	ST4-74_pRSF1010	2860	3063	-
SLP3_0006	271	221	1.23	ST4-74_pRSF1010	3068	3277	-
mobA	14	13	1.07	ST4-74_pRSF1010	3341	5467	-
mobB	17	16	1.13	ST4-74_pRSF1010	4309	4719	-
mobC	66	73	0.91	ST4-74_pRSF1010	5666	5947	+
SLP3_0012	71	57	1.25	ST4-74_pRSF1010	6504	7040	+
strB	102	104	0.98	ST4-74_pRSF1010	7018	7860	-
aph(3")-	219	250	0.87	ST4-74_pRSF1010	7854	8654	-
finO	33	28	1.19	ST4-74_pSLT	21	584	-
traX	9	8	1.15	ST4-74_pSLT	639	1334	-
traI	8	7	1.03	ST4-74_pSLT	1399	6657	-
trbH	8	8	1.04	ST4-74_pSLT	6654	7373	-
traD	7	6	1.22	ST4-74_pSLT	7373	9535	-
traT	312	373	0.84	ST4-74_pSLT	9912	10643	-
traS	521	488	1.07	ST4-74_pSLT	10679	11173	-
traG	85	68	1.26	ST4-74_pSLT	11189	14011	-
traH	6	6	0.90	ST4-74_pSLT	14008	15390	-
trbB	6	4	1.75	ST4-74_pSLT	15380	15928	-
traQ	14	12	1.14	ST4-74_pSLT	15915	16208	-
traF	7	5	1.31	ST4-74_pSLT	16422	17171	-
trbE	9	8	1.14	ST4-74_pSLT	17183	17398	-
traN	6	6	1.04	ST4-74_pSLT	17409	19235	-
trbC	4	3	1.18	ST4-74_pSLT	19688	20326	-
PSLT093	6	8	0.79	ST4-74_pSLT	20311	20619	-
traU	5	5	0.89	ST4-74_pSLT	20638	21630	-
traW	2	3	0.73	ST4-74_pSLT	21627	22259	-
trbI	4	3	1.31	ST4-74_pSLT	22256	22642	-
traC	5	3	1.45	ST4-74_pSLT	22617	25265	-
PSLT087	9	8	1.16	ST4-74_pSLT	25661	26128	-
traR	2	1	1.81	ST4-74_pSLT	26121	26342	-
traV	5	6	0.90	ST4-74_pSLT	26477	26992	-
trbD	6	7	0.79	ST4-74_pSLT	26989	27234	-
traP	6	7	0.94	ST4-74_pSLT	27212	27808	-
traB	6	4	1.28	ST4-74_pSLT	27798	29219	-
traK	4	5	0.80	ST4-74_pSLT	29219	29959	-
traE	3	4	0.90	ST4-74_pSLT	29946	30512	-
traL	7	10	0.67	ST4-74_pSLT	30534	30845	-
traA	16	22	0.73	ST4-74_pSLT	30860	31222	-
traY	101	83	1.22	ST4-74_pSLT	31264	31584	-
traJ	8	6	1.33	ST4-74_pSLT	31585	32271	-
traM	36	26	1.39	ST4-74_pSLT	32464	32844	-
finP	49	49	0.99	ST4-74_pSLT	33259	33729	+
psiA	3	2	1.66	ST4-74_pSLT	35368	36105	-
psiB	12	13	0.93	ST4-74_pSLT	36102	36536	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
PSLT068	3	2	1.17	ST4-74_pSLT	36576	38573	-
PSLT067	4	3	1.61	ST4-74_pSLT	38642	38884	-
ssbB	2	1	2.07	ST4-74_pSLT	38939	39457	-
PSLT064	24	18	1.34	ST4-74_pSLT	40158	40481	-
PSLT063	3	4	0.78	ST4-74_pSLT	40717	41151	-
PSLT062	5	5	1.05	ST4-74_pSLT	41218	41652	-
SLP1_0044A	4	3	1.58	ST4-74_pSLT	41666	42115	-
PSLT061	2	1	1.39	ST4-74_pSLT	42148	42885	-
SLP1_0047	4	2	1.92	ST4-74_pSLT	42984	43397	-
PSLT060	2	1	2.51	ST4-74_pSLT	43453	43674	-
PSLT059	3	2	1.44	ST4-74_pSLT	43674	44354	-
PSLT057	4	5	0.90	ST4-74_pSLT	44736	45092	-
PSLT056	5	4	1.19	ST4-74_pSLT	45085	45555	-
samA	25	25	1.02	ST4-74_pSLT	46066	46488	+
samB	7	5	1.42	ST4-74_pSLT	46488	47762	+
parB	63	66	0.96	ST4-74_pSLT	47844	48821	-
parA	53	54	0.97	ST4-74_pSLT	48818	50023	-
samB	151	142	1.06	ST4-74_pSLT	50438	51379	+
PSLT049	11	12	0.88	ST4-74_pSLT	52553	52969	-
tlpA	10	13	0.82	ST4-74_pSLT	53055	54170	-
PSLT047	62	43	1.43	ST4-74_pSLT	54429	54917	+
PSLT046	534	374	1.43	ST4-74_pSLT	55572	56312	+
rlgA	249	245	1.01	ST4-74_pSLT	56519	57079	+
rlgA	42	48	0.86	ST4-74_pSLT	57063	58727	+
PSLT043	44	49	0.91	ST4-74_pSLT	58660	59685	+
PSLT042	5	4	1.21	ST4-74_pSLT	59874	60869	-
spvR	12	3	3.50	ST4-74_pSLT	61520	62413	+
spvA	51	13	3.88	ST4-74_pSLT	62925	63692	+
spvB	28	10	2.81	ST4-74_pSLT	63874	65649	+
spvC	34	8	4.11	ST4-74_pSLT	66047	66655	+
spvD	24	6	3.88	ST4-74_pSLT	66916	67566	+
PSLT036	43	52	0.82	ST4-74_pSLT	67693	67956	-
PSLT035	1046	1203	0.87	ST4-74_pSLT	68174	68524	+
PSLT034	1	2	0.77	ST4-74_pSLT	68591	69151	-
SLP1_0073A	2	2	0.89	ST4-74_pSLT	69028	69687	-
PSLT033	3	5	0.62	ST4-74_pSLT	69880	70365	-
PSLT032	17	16	1.05	ST4-74_pSLT	71034	71585	-
rsdB	19	18	1.04	ST4-74_pSLT	71594	72376	-
PSLT030	16	17	0.91	ST4-74_pSLT	72411	72932	-
PSLT029	20	23	0.87	ST4-74_pSLT	72929	73219	-
ccdB	29	37	0.79	ST4-74_pSLT	73221	73526	-
ccdA	72	79	0.90	ST4-74_pSLT	73528	73746	-
PSLT026	186	183	1.02	ST4-74_pSLT	74422	74943	+
PSLT025	28	18	1.50	ST4-74_pSLT	75427	75723	+
repA2	112	101	1.11	ST4-74_pSLT	76217	77206	+
PSLT020	32	28	1.14	ST4-74_pSLT	78078	78287	-
pefB	7	11	0.62	ST4-74_pSLT	79178	79480	+
pefA	5	6	0.90	ST4-74_pSLT	79755	80273	+
pefC	4	3	1.09	ST4-74_pSLT	80612	82909	+
pefD	3	4	0.78	ST4-74_pSLT	82902	83594	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
orf5	9	8	1.22	ST4-74_pSLT	83648	84166	+
orf6	151	124	1.22	ST4-74_pSLT	84419	85294	+
pefI	44	36	1.21	ST4-74_pSLT	85726	85938	+
orf7	8	5	1.75	ST4-74_pSLT	85923	86273	+
srgA	12	8	1.56	ST4-74_pSLT	86518	87171	+
srgB	5	5	1.10	ST4-74_pSLT	87304	88203	+
rcK	13	11	1.17	ST4-74_pSLT	88293	88850	+
srgC	33	22	1.54	ST4-74_pSLT	89117	89875	+
PSLT007	10	8	1.23	ST4-74_pSLT	89972	90112	-
repA	72	78	0.92	ST4-74_pSLT	91053	91934	-
tap	283	306	0.93	ST4-74_pSLT	91915	91992	-
repA3	5625	4088	1.38	ST4-74_pSLT	91997	92119	-
repC	825	742	1.11	ST4-74_pSLT	92242	92487	-
PSLT002	35	30	1.17	ST4-74_pSLT	92687	93130	-
PSLT001	36	35	1.03	ST4-74_pSLT	93464	93748	-
thrL	5855	4483	1.31	ST4-74	169	255	+
thrA	50	51	0.99	ST4-74	337	2799	+
thrB	72	57	1.26	ST4-74	2801	3730	+
thrC	63	65	0.97	ST4-74	3734	5020	+
yaaA	32	32	0.99	ST4-74	5114	5887	-
yaaJ	36	23	1.58	ST4-74	5966	7396	-
talB	105	126	0.84	ST4-74	7665	8618	+
mog	33	30	1.09	ST4-74	8729	9319	+
yaaH	7	9	0.74	ST4-74	9376	9942	-
htgA	3	2	1.21	ST4-74	10092	10805	-
yaaI	11	8	1.36	ST4-74	10841	11245	-
dnaK	102	105	0.98	ST4-74	11593	13509	+
tpke11	80	68	1.18	ST4-74	13507	13591	+
dnaJ	61	49	1.24	ST4-74	13595	14734	+
STnc890	402	201	2.00	ST4-74	14720	14783	+
STM0014	9	7	1.32	ST4-74	15014	15961	+
STM0015	6	4	1.44	ST4-74	16088	16432	+
STnc3200	15	9	1.69	ST4-74	16445	16493	-
STM0016	1	2	0.78	ST4-74	16493	17026	-
STnc3210	89	37	2.38	ST4-74	17025	17111	+
STM0017	1	1	1.24	ST4-74	17043	17486	-
chiA	5	5	1.10	ST4-74	18083	19966	+
STM0019	4	3	1.35	ST4-74	20058	23054	+
STM0020	8	8	0.99	ST4-74	23335	24039	+
bcfA	5	4	1.40	ST4-74	24469	25011	+
bcfB	1	1	1.44	ST4-74	25112	25798	+
bcfC	4	4	0.92	ST4-74	25803	28424	+
bcfD	3	2	1.30	ST4-74	28425	29432	+
bcfE	1	1	1.01	ST4-74	29433	29978	+
bcfF	2	1	1.28	ST4-74	29994	30512	+
bcfG	2	3	0.91	ST4-74	30505	31209	+
bcfH	10	11	0.94	ST4-74	31274	32119	+
SL1344_0029	9	7	1.39	ST4-74	32116	32445	+
STM0029	1	1	1.13	ST4-74	32545	32994	-
STM0030	4	3	1.25	ST4-74	33364	34368	+
STM0031	2	2	0.84	ST4-74	34376	34816	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM0032	6	5	1.27	ST4-74	35339	37057	+
STM0033	12	12	1.06	ST4-74	37103	38674	-
STM0034	4	3	1.18	ST4-74	38773	39534	-
STM0035	8	7	1.21	ST4-74	40131	41624	+
STnc1010	53	52	1.03	ST4-74	41629	41708	+
STM0036	5	5	0.96	ST4-74	41723	42913	+
STM0037	3	2	1.25	ST4-74	42932	44185	+
STM0038	13	9	1.44	ST4-74	44312	46027	+
nhaA	54	49	1.11	ST4-74	46190	47356	+
nhaR	31	30	1.05	ST4-74	47418	48317	+
STM0041	4	4	1.00	ST4-74	48372	50411	-
STM0042	3	4	0.88	ST4-74	50451	51824	-
rpsT	2329	2073	1.12	ST4-74	52280	52543	-
yaaY	43	33	1.29	ST4-74	52649	52864	+
ribF	35	41	0.85	ST4-74	52872	53810	+
ileS	29	36	0.81	ST4-74	53855	56689	+
lspA	65	69	0.94	ST4-74	56689	57189	+
fkpB	27	37	0.72	ST4-74	57344	57793	+
ispH	24	30	0.78	ST4-74	57796	58746	+
STM0050	12	14	0.85	ST4-74	58946	60148	+
rihC	10	13	0.76	ST4-74	60164	61084	+
citB2	6	6	0.89	ST4-74	61106	61792	-
citA2	16	11	1.47	ST4-74	61794	63413	-
STnc3220	102	76	1.35	ST4-74	61807	62034	+
STM0054	3	3	1.24	ST4-74	63548	64849	-
STM0055	2	2	1.00	ST4-74	64862	66637	-
oadG	2	2	1.01	ST4-74	66654	66893	-
STnc4160	131	254	0.51	ST4-74	66912	67051	-
STnc3090	48	37	1.29	ST4-74	66916	67048	+
STM0057	4	4	0.93	ST4-74	67052	68392	-
citC2	3	4	0.79	ST4-74	68637	69683	+
citD2	4	4	1.17	ST4-74	69713	70006	+
citE2	2	3	0.73	ST4-74	70003	70872	+
citF2	5	5	0.87	ST4-74	70883	72403	+
citX2	2	3	0.72	ST4-74	72403	72954	+
citG2	4	3	1.46	ST4-74	72947	73840	+
dapB	219	276	0.79	ST4-74	74020	74841	+
DapZ	3249	2929	1.11	ST4-74	74847	74924	+
STnc3230	194	85	2.28	ST4-74	74894	75015	-
STM0065	4	4	1.05	ST4-74	75091	75438	+
carA	78	121	0.64	ST4-74	75883	77031	+
carB	30	59	0.52	ST4-74	77050	80277	+
caiF	134	160	0.84	ST4-74	80551	80946	+
caiE	3	3	1.20	ST4-74	81023	81619	-
caiD	2	2	0.93	ST4-74	81728	82513	-
caiC	4	3	1.46	ST4-74	82567	84120	-
caiB	3	4	0.82	ST4-74	84183	85454	-
caiA	7	8	0.87	ST4-74	85512	86654	-
caiT	2	3	0.66	ST4-74	86689	88206	-
fixA	3	4	0.78	ST4-74	88687	89457	+
fixB	2	2	0.76	ST4-74	89473	90414	+

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fixC	2	2	0.97	ST4-74	90464	91750	+
fixX	3	3	0.93	ST4-74	91747	92034	+
yaaU	10	9	1.12	ST4-74	92181	93521	+
STM0080	90	35	2.55	ST4-74	93610	93840	+
STM0081	85	52	1.61	ST4-74	94134	94550	+
STnc470	1034	633	1.63	ST4-74	94653	94741	-
STM0082	82	70	1.17	ST4-74	94774	95064	-
STnc4170	100	104	0.97	ST4-74	95102	95188	+
SL1344_0083A	50	33	1.50	ST4-74	95134	95226	-
STM0084	5	5	1.01	ST4-74	95962	97851	+
SL1344_0085	16	7	2.22	ST4-74	98056	98121	-
yabF	30	17	1.78	ST4-74	98258	98788	+
kefC	12	9	1.30	ST4-74	98781	100643	+
folA	43	37	1.16	ST4-74	100842	101321	+
STnc3240	34	23	1.44	ST4-74	101322	101483	-
apaH	37	27	1.41	ST4-74	101427	102275	-
apaG	24	33	0.73	ST4-74	102286	102663	-
ksgA	46	44	1.05	ST4-74	102666	103487	-
pdxAa	114	80	1.42	ST4-74	103484	104473	-
surA	79	87	0.90	ST4-74	104473	105759	-
imp	63	66	0.96	ST4-74	105813	108173	-
djlA	40	39	1.04	ST4-74	108427	109239	+
STnc4180	10	27	0.36	ST4-74	109243	109322	-
rluA	11	12	0.94	ST4-74	109334	109993	-
hepA	6	7	0.87	ST4-74	110005	112911	-
polB	10	7	1.28	ST4-74	113085	115436	-
STM0098	18	16	1.11	ST4-74	115480	116100	-
SL1344_0099A	10	11	0.92	ST4-74	116195	116254	-
STM0100	6	5	1.10	ST4-74	116819	117238	+
araD	13	19	0.70	ST4-74	117244	117939	-
araA	3	3	1.02	ST4-74	118080	119582	-
araB	2	1	1.32	ST4-74	119593	121302	-
araC	10	15	0.69	ST4-74	121643	122488	+
yabI	21	18	1.18	ST4-74	122607	123374	+
thiQ	11	12	0.88	ST4-74	123413	124120	-
thiP	22	21	1.01	ST4-74	124104	125714	-
tbpA	26	26	0.97	ST4-74	125690	126673	-
SroA	517	666	0.78	ST4-74	126713	126802	-
yabN	4	5	0.85	ST4-74	126851	128509	-
SgrS	318	226	1.41	ST4-74	128577	128808	+
sgrT	0	0	–	ST4-74	128602	128724	+
leuD	31	35	0.90	ST4-74	129060	129665	-
leuC	15	22	0.69	ST4-74	129676	131076	-
leuB	19	25	0.74	ST4-74	131079	132170	-
leuA	20	28	0.73	ST4-74	132170	133741	-
leuL	8772	13911	0.63	ST4-74	133829	133915	-
leuO	5	4	1.45	ST4-74	134572	135516	+
ilvI	24	32	0.75	ST4-74	135901	137562	+
ilvH	42	51	0.82	ST4-74	137565	138056	+
STnc1500	119	121	0.99	ST4-74	138100	138274	-
fruR	46	59	0.78	ST4-74	138340	139344	+

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mraZ	235	184	1.28	ST4-74	139950	140408	+
mraW	119	83	1.44	ST4-74	140410	141351	+
ftsL	158	126	1.26	ST4-74	141348	141713	+
ftsI	290	205	1.42	ST4-74	141729	143495	+
murE	36	36	1.01	ST4-74	143482	144969	+
murF	32	30	1.09	ST4-74	144966	146324	+
mraY	54	48	1.11	ST4-74	146318	147400	+
murD	48	44	1.09	ST4-74	147403	148719	+
ftsW	45	44	1.03	ST4-74	148806	149963	+
murG	36	39	0.94	ST4-74	149960	151027	+
murC	37	38	0.96	ST4-74	151146	152621	+
ddl	74	78	0.95	ST4-74	152614	153534	+
ftsQ	82	77	1.07	ST4-74	153536	154366	+
ftsA	78	76	1.03	ST4-74	154363	155625	+
ftsZ	73	85	0.86	ST4-74	155686	156837	+
lpxC	222	262	0.85	ST4-74	156938	157855	+
yacA	28	31	0.90	ST4-74	158203	158631	+
secA	25	30	0.81	ST4-74	158693	161398	+
mutT	17	13	1.26	ST4-74	161550	161945	+
yacG	27	29	0.93	ST4-74	162267	162458	-
yacF	18	19	0.93	ST4-74	162468	163211	-
coaE	35	39	0.91	ST4-74	163211	163831	-
guaC	74	72	1.03	ST4-74	164057	165100	+
pilC	2	1	1.34	ST4-74	165131	166333	-
pilB	1	1	1.27	ST4-74	166323	167708	-
pilA	3	2	1.12	ST4-74	167718	168155	-
nadC	7	8	0.87	ST4-74	168377	169123	-
ampD	29	28	1.03	ST4-74	169358	169921	+
ampE	26	28	0.93	ST4-74	169918	170772	+
STM0148	7	6	1.14	ST4-74	170864	171814	-
STM0149	6	6	0.98	ST4-74	171814	173220	-
aroP	69	100	0.69	ST4-74	173384	174757	-
pdhR	64	78	0.82	ST4-74	175320	176084	+
tp2	4	5	0.82	ST4-74	176086	176242	-
aceE	35	54	0.64	ST4-74	176244	178907	+
aceF	29	45	0.65	ST4-74	178922	180811	+
lpdA	86	106	0.81	ST4-74	181011	182435	+
STM0155	2	3	0.79	ST4-74	182724	183008	+
STM0156	2	2	1.20	ST4-74	183046	183837	-
yacH	3	2	1.33	ST4-74	183850	185464	-
acnB	42	48	0.87	ST4-74	185853	188450	+
STM0159	118	75	1.57	ST4-74	188511	189353	-
yacL	74	68	1.08	ST4-74	189599	189961	+
kdgT	5	5	0.96	ST4-74	190196	191149	+
STM0162	4	4	0.99	ST4-74	191146	192417	+
pdxAb	33	33	1.01	ST4-74	192407	193390	+
STM0164	32	36	0.89	ST4-74	193400	194167	+
speD	27	29	0.94	ST4-74	194197	194991	-
speE	20	24	0.85	ST4-74	195012	195872	-
yacC	118	100	1.18	ST4-74	195979	196326	-
cueO	10	14	0.70	ST4-74	196528	198138	+

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gcd	27	14	1.92	ST4-74	198216	200606	-
hpt	61	61	1.00	ST4-74	200812	201348	+
yadF	140	115	1.21	ST4-74	201406	202068	-
yadG	11	10	1.03	ST4-74	202177	203103	+
yadH	13	12	1.06	ST4-74	203100	203870	+
stiH	3	3	1.16	ST4-74	203987	205066	-
stiC	1	1	1.38	ST4-74	205075	207621	-
stiB	1	1	0.90	ST4-74	207648	208331	-
stiA	2	2	1.25	ST4-74	208379	208918	-
yadI	9	68	0.13	ST4-74	209289	209729	+
yadE	21	20	1.01	ST4-74	209791	211020	+
STnc4190	28	36	0.79	ST4-74	209868	209971	-
panD	151	172	0.88	ST4-74	211027	211407	-
panC	11	21	0.51	ST4-74	211502	212356	-
panB	14	21	0.65	ST4-74	212482	213273	-
folK	9	9	0.96	ST4-74	213394	213873	-
pcnB	33	36	0.92	ST4-74	213870	215288	-
yadB	10	11	0.99	ST4-74	215429	216370	-
dksA	337	356	0.95	ST4-74	216394	216849	-
sfsA	75	44	1.70	ST4-74	217026	217730	-
ligT	56	47	1.19	ST4-74	217747	218277	-
hrpB	16	16	0.97	ST4-74	218305	220779	+
mrcB	33	37	0.88	ST4-74	220920	223442	+
STnc3250	88	48	1.81	ST4-74	223451	223676	-
fhuA	34	16	2.10	ST4-74	223735	225924	+
fhuC	6	4	1.52	ST4-74	225973	226770	+
fhuD	10	5	1.86	ST4-74	226770	227660	+
fhuB	9	5	1.65	ST4-74	227657	229714	+
stfA	3	2	1.17	ST4-74	230653	231213	+
stfC	3	3	1.01	ST4-74	231299	233956	+
stfD	1	0	1.42	ST4-74	233974	234726	+
stfE	3	3	0.99	ST4-74	234790	235257	+
stfF	2	2	1.06	ST4-74	235254	235730	+
stfG	3	1	2.08	ST4-74	235730	236260	+
STM0201	15	10	1.47	ST4-74	236260	237099	+
STnc1510	79	56	1.41	ST4-74	237091	237187	+
hemL	34	35	0.96	ST4-74	237207	238487	-
clcA	30	24	1.24	ST4-74	238657	240078	+
yadR	229	267	0.86	ST4-74	240160	240504	+
yadS	10	10	1.00	ST4-74	240605	241228	-
btuF	8	10	0.81	ST4-74	241265	242065	-
mtn	30	32	0.93	ST4-74	242058	242756	-
dgt	22	18	1.22	ST4-74	243051	244358	+
htrA	30	38	0.79	ST4-74	244488	245915	+
STnc3100	91	86	1.06	ST4-74	245838	245954	+
cdaR	16	11	1.43	ST4-74	246068	247225	+
yaeH	364	526	0.69	ST4-74	247314	247700	-
STM0212	49	34	1.42	ST4-74	248223	249248	+
STnc3110	159	107	1.48	ST4-74	249153	249274	+
dapD	129	180	0.71	ST4-74	249285	250109	-
glnD	8	10	0.85	ST4-74	250139	252811	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
map	54	53	1.02	ST4-74	253048	253842	-
t44	465	619	0.75	ST4-74	254126	254264	+
rpsB	251	345	0.73	ST4-74	254293	255018	+
tsf	174	234	0.74	ST4-74	255276	256127	+
pyrH	39	51	0.76	ST4-74	256272	256997	+
frr	185	172	1.07	ST4-74	257144	257701	+
sRNA1	228	325	0.70	ST4-74	257725	257790	+
dxr	18	18	0.97	ST4-74	257842	259038	+
STnc920	257	281	0.92	ST4-74	259116	259164	+
uppS	57	67	0.85	ST4-74	259351	260109	+
cdsA	63	67	0.94	ST4-74	260122	260979	+
yaeL	103	105	0.98	ST4-74	260991	262343	+
yaeT	98	109	0.90	ST4-74	262375	264789	+
hlpA	202	200	1.01	ST4-74	264912	265397	+
lpxD	128	151	0.85	ST4-74	265401	266426	+
fabZ	99	109	0.91	ST4-74	266532	266987	+
lpxA	62	71	0.88	ST4-74	266991	267779	+
lpxB	19	24	0.80	ST4-74	267779	268927	+
rnhB	24	29	0.83	ST4-74	268924	269520	+
dnaE	26	32	0.82	ST4-74	269544	273026	+
accA	41	52	0.79	ST4-74	273039	273998	+
STM0233	8	7	1.12	ST4-74	274178	275941	+
ldcC	16	13	1.20	ST4-74	276017	278158	+
yaeR	28	20	1.42	ST4-74	278214	278603	+
mesJ	10	10	1.05	ST4-74	278747	279958	+
rof	205	197	1.04	ST4-74	280042	280296	-
yaeP	577	493	1.17	ST4-74	280289	280507	-
yaeQ	23	21	1.08	ST4-74	280687	281232	+
yaeJ	70	50	1.38	ST4-74	281229	281651	+
cutF	37	28	1.31	ST4-74	281683	282384	+
proS	24	30	0.81	ST4-74	282458	284176	-
yaeB	15	17	0.86	ST4-74	284287	284994	-
rcsF	67	75	0.90	ST4-74	284991	285395	-
metQ	167	227	0.74	ST4-74	285514	286329	-
yaeE	101	135	0.75	ST4-74	286368	287021	-
abc	86	94	0.92	ST4-74	287014	288045	-
yaeD	35	31	1.14	ST4-74	288235	288801	+
dkgB	11	9	1.15	ST4-74	295060	295863	+
yafC	6	5	1.14	ST4-74	295884	296798	-
STM0257	5	5	1.11	ST4-74	296903	298078	+
yafD	38	43	0.89	ST4-74	298231	299010	+
yafE	12	11	1.14	ST4-74	299088	299858	+
dniR	224	296	0.76	ST4-74	299914	301281	-
gloB	8	10	0.79	ST4-74	301353	302108	-
yafS	19	18	1.07	ST4-74	302143	302865	+
rnhA	35	28	1.27	ST4-74	302862	303329	-
dnaQ	49	43	1.13	ST4-74	303393	304124	+
sciA	3	2	1.20	ST4-74	304656	305711	-
sciB	2	1	1.30	ST4-74	305722	306717	-
sciC	2	2	1.22	ST4-74	306714	308597	-
sciD	3	3	1.06	ST4-74	308613	309107	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
sciE	3	3	0.90	ST4-74	309104	309928	-
sciF	4	3	1.24	ST4-74	309915	310817	-
sciG	2	2	0.98	ST4-74	311185	313824	+
sciH	1	1	0.85	ST4-74	313924	314466	+
sciI	2	2	1.15	ST4-74	314490	315983	+
sciJ	4	4	1.25	ST4-74	316169	316615	+
sciK	1	1	0.89	ST4-74	316869	317354	+
STM0277	5	5	1.02	ST4-74	317659	318144	+
STM0278	1	1	1.11	ST4-74	318129	318512	+
sciK1	2	2	1.03	ST4-74	318655	319140	+
sciN	1	0	2.68	ST4-74	319207	319743	+
sciO	2	1	1.19	ST4-74	319747	321090	+
sciP	2	2	0.95	ST4-74	321087	322391	+
sciQ	3	3	1.22	ST4-74	322396	323169	+
sciR	3	2	1.62	ST4-74	323372	323803	+
sciS	3	3	1.05	ST4-74	323837	327706	+
sciT	4	4	1.05	ST4-74	327706	328494	+
sciU	4	5	0.85	ST4-74	328491	328907	+
sciV	9	9	0.99	ST4-74	328931	329452	+
vgrS	2	2	1.27	ST4-74	329849	332038	+
sciW	1	1	0.60	ST4-74	332062	332508	+
STM0291	26	19	1.39	ST4-74	332521	336123	+
SL1344_0286A	71	45	1.56	ST4-74	336117	336380	+
STM0292	101	84	1.20	ST4-74	336396	337313	+
sciX	33	29	1.14	ST4-74	337307	337753	+
sciY	5	3	1.46	ST4-74	338265	338753	+
IsrA	3	2	1.16	ST4-74	338816	339238	+
STM0295	12	12	0.96	ST4-74	339522	339830	+
STM0296	5	4	1.19	ST4-74	339867	340180	-
STM0297	6	7	0.83	ST4-74	340233	340373	+
STM0298	1	2	0.80	ST4-74	340510	340975	+
safA	39	31	1.25	ST4-74	341687	342199	+
safB	5	5	1.03	ST4-74	342307	343020	+
safC	9	8	1.16	ST4-74	343044	345554	+
safD	11	11	1.00	ST4-74	345576	346046	+
ybeJ	6	6	0.99	ST4-74	346452	347273	+
sinR	26	24	1.06	ST4-74	348088	349035	+
STM0305	8	6	1.33	ST4-74	349152	349367	+
pagN	213	139	1.53	ST4-74	349371	350090	-
sciZ	15	18	0.84	ST4-74	350421	350828	-
STnc1030	470	673	0.70	ST4-74	350892	351095	+
STnc1040	480	633	0.76	ST4-74	351053	351100	-
yafV	21	20	1.06	ST4-74	351199	351966	-
fadE	18	17	1.05	ST4-74	352075	354519	-
gmhA	56	54	1.04	ST4-74	354759	355337	+
yafJ	23	24	0.92	ST4-74	355550	356317	+
yafK	51	52	0.98	ST4-74	356288	357028	-
dbh	15	13	1.20	ST4-74	357278	358333	+
STM0314	8	7	1.07	ST4-74	358540	359684	+
prfH	5	5	0.94	ST4-74	359681	360295	+
pepD	47	53	0.89	ST4-74	360451	361908	-

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gpt	84	88	0.95	ST4-74	362157	362615	+
frsA	32	25	1.27	ST4-74	362704	363948	+
crl	185	165	1.12	ST4-74	364006	364407	+
phoE	6	5	1.14	ST4-74	364458	365510	-
proB	35	42	0.83	ST4-74	365793	366896	+
proA	22	24	0.90	ST4-74	366908	368158	+
STM0325	5	4	1.27	ST4-74	368675	369475	-
dhaF	2	2	0.85	ST4-74	369393	370107	+
STM0327	330	397	0.83	ST4-74	370217	370540	+
STM0328	4	3	1.44	ST4-74	370836	372119	+
leuC2	2	2	1.07	ST4-74	372251	373465	+
leuD2	2	1	1.46	ST4-74	373674	374300	+
STM0331	8	6	1.29	ST4-74	374315	375193	+
STM0332	8	7	1.15	ST4-74	375193	376107	+
STnc3270	69	50	1.39	ST4-74	376115	376194	+
STM0333	14	11	1.21	ST4-74	376141	377106	+
STM0334	13	13	0.94	ST4-74	377051	377323	-
STM0335	8	6	1.38	ST4-74	378014	378412	+
stbE	9	8	1.10	ST4-74	378459	379217	-
stbD	9	7	1.32	ST4-74	379183	380508	-
stbC	5	5	1.07	ST4-74	380513	383080	-
stbB	1	1	0.81	ST4-74	383064	383825	-
stbA	1	0	4.83	ST4-74	383887	384423	-
STM0341	17	11	1.50	ST4-74	385057	385821	+
STM0342	16	11	1.44	ST4-74	385794	386294	+
STnc1700	46	19	2.50	ST4-74	386381	386476	+
STM0343	11	9	1.19	ST4-74	386594	388168	+
STM0344	40	38	1.04	ST4-74	388268	389011	+
STM0345	23	21	1.07	ST4-74	388984	389472	+
STM0346	4	4	1.06	ST4-74	389874	390398	+
STM0347	3	1	1.78	ST4-74	390542	391183	+
STM0348	3	4	0.92	ST4-74	391194	391418	+
STM0349	7	7	1.05	ST4-74	391476	391835	+
STM0350	2	2	1.10	ST4-74	391905	393362	-
STM0351	2	2	1.40	ST4-74	393379	396546	-
STM0352	1	1	1.28	ST4-74	396543	397553	-
STM0353	3	3	1.03	ST4-74	398046	400334	+
STM0354	4	4	1.02	ST4-74	400346	400810	+
STM0355	23	29	0.78	ST4-74	400888	401082	+
STM0356	8	9	0.89	ST4-74	401375	402628	+
mod	20	20	1.01	ST4-74	402817	404775	+
res	12	11	1.06	ST4-74	404782	407757	+
STM0359	450	169	2.66	ST4-74	408034	408135	+
STM0360	38	22	1.70	ST4-74	408252	409655	+
STM0361	43	29	1.48	ST4-74	409652	410662	+
STM0362	15	10	1.52	ST4-74	410679	410903	+
STM0363	14	11	1.22	ST4-74	410999	412003	+
foxA	6	5	1.24	ST4-74	412149	414257	+
yahN	99	90	1.10	ST4-74	414298	414930	-
yahO	263	201	1.31	ST4-74	415162	415437	+
prpR	7	7	0.97	ST4-74	415573	417198	-

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prpB	2	1	1.74	ST4-74	417463	418350	+
STnc3280	42	37	1.14	ST4-74	418391	418463	-
prpC	2	2	1.14	ST4-74	418473	419642	+
prpD	2	2	1.09	ST4-74	419682	421133	+
prpE	2	3	0.88	ST4-74	421174	423060	+
hemB	21	23	0.93	ST4-74	423164	424138	-
yaiU	21	19	1.13	ST4-74	424651	427587	+
yaiV	17	13	1.27	ST4-74	427673	428296	+
ampH	21	20	1.03	ST4-74	428341	429471	-
sbmA	17	14	1.16	ST4-74	429947	431065	+
yaiW	9	9	1.07	ST4-74	431080	432174	+
yaiY	14	7	1.91	ST4-74	432212	432520	-
yaiZ	273	252	1.09	ST4-74	432788	433003	+
ddlA	41	38	1.09	ST4-74	433029	434123	-
STM0381	18	9	2.06	ST4-74	434201	434914	+
STM0382	9	6	1.43	ST4-74	435083	436294	+
yaiB	761	542	1.40	ST4-74	436585	436851	+
STnc4150	43	27	1.61	ST4-74	436953	437082	-
psiF	112	53	2.14	ST4-74	437203	437523	+
adrA	20	14	1.41	ST4-74	437615	438727	+
proC	22	21	1.02	ST4-74	438742	439551	-
yaiI	16	16	1.04	ST4-74	439691	440146	+
aroL	54	78	0.68	ST4-74	440331	440876	+
yaiA	325	301	1.08	ST4-74	440903	441094	+
aroM	56	54	1.03	ST4-74	441345	442022	+
yaiE	113	133	0.85	ST4-74	442093	442377	+
rdgC	24	24	0.99	ST4-74	442415	443326	-
yajF	21	17	1.22	ST4-74	443452	444360	+
araJ	11	11	1.00	ST4-74	444382	445554	-
sbcC	4	5	0.79	ST4-74	445727	448867	-
sbcD	7	8	0.86	ST4-74	448864	450066	-
phoB	67	58	1.14	ST4-74	450281	450970	+
phoR	21	18	1.15	ST4-74	451040	452335	+
brnQ	71	73	0.97	ST4-74	452744	454063	+
proY	22	19	1.16	ST4-74	454138	455508	+
malZ	7	7	1.07	ST4-74	455670	457487	+
tsaA	154	249	0.62	ST4-74	457559	458161	-
yajB	48	50	0.96	ST4-74	458371	458952	-
queA	19	18	1.06	ST4-74	459046	460110	+
tgt	76	91	0.83	ST4-74	460307	461434	+
yajC	206	306	0.67	ST4-74	461457	461789	+
secD	80	100	0.80	ST4-74	461817	463664	+
secF	77	102	0.75	ST4-74	463675	464646	+
STM0409	19	20	0.95	ST4-74	464794	465159	+
STM0410	9	14	0.65	ST4-74	465185	465877	+
yajD	57	67	0.84	ST4-74	466047	466394	+
STnc1060	1413	1468	0.96	ST4-74	466718	466783	-
tsx	19	31	0.62	ST4-74	467023	467886	-
yajI	9	5	1.67	ST4-74	468186	468725	-
nrdR	170	115	1.49	ST4-74	468877	469326	+
ribD	36	32	1.13	ST4-74	469330	470433	+

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ribH	136	135	1.01	ST4-74	470522	470992	+
nusB	136	143	0.95	ST4-74	471013	471432	+
thiL	16	17	0.91	ST4-74	471511	472488	+
pgpA	20	19	1.03	ST4-74	472466	472981	+
yajO	23	16	1.40	ST4-74	473085	474059	-
dxs	17	21	0.82	ST4-74	474116	475978	-
ispA	26	28	0.95	ST4-74	476002	476901	-
xseB	102	103	0.99	ST4-74	476902	477144	-
thiI	21	19	1.06	ST4-74	477354	478802	+
phnV	5	5	0.99	ST4-74	478846	479643	-
phnU	3	3	0.85	ST4-74	479646	480506	-
phnT	3	3	1.23	ST4-74	480509	481618	-
phnS	3	3	0.92	ST4-74	481624	482568	-
phnR	10	10	0.98	ST4-74	482835	483554	-
phnW	2	2	0.98	ST4-74	483694	484797	+
phnX	2	2	0.76	ST4-74	484804	485616	+
thiJ	13	12	1.06	ST4-74	485713	486303	-
apbA	30	22	1.36	ST4-74	486266	487177	-
yajQ	182	185	0.99	ST4-74	487285	487794	+
yajR	22	19	1.13	ST4-74	487841	489205	-
SL1344_0430A	35	23	1.52	ST4-74	489507	489698	-
STM0437	12	10	1.22	ST4-74	489742	491253	+
STM0438	10	8	1.29	ST4-74	491475	492758	+
cyoE	93	70	1.32	ST4-74	492840	493730	-
cyoD	116	86	1.35	ST4-74	493742	494071	-
cyoC	97	67	1.43	ST4-74	494071	494586	-
cyoB	83	67	1.24	ST4-74	494675	496666	-
cyoA	165	122	1.36	ST4-74	496677	497633	-
ampG	12	13	0.91	ST4-74	498086	499561	-
yajG	41	46	0.88	ST4-74	499605	500237	-
bolA	351	238	1.48	ST4-74	500487	500804	+
tig	129	142	0.91	ST4-74	501151	502449	+
clpP	133	149	0.89	ST4-74	502696	503319	+
clpX	226	207	1.09	ST4-74	503571	504842	+
SraA	13	11	1.20	ST4-74	504870	504976	-
lon	88	80	1.11	ST4-74	505028	507382	+
hupB	1743	2379	0.73	ST4-74	507591	507863	+
cypD	58	64	0.90	ST4-74	508154	510025	+
comE1	12	13	0.87	ST4-74	510175	510549	+
ybaW	12	9	1.25	ST4-74	510653	511051	+
ybaX	44	41	1.06	ST4-74	511157	511852	-
ybaE	5	6	0.89	ST4-74	511917	513617	-
cof	8	6	1.48	ST4-74	513718	514536	+
STnc930	78	59	1.32	ST4-74	514484	514578	+
STM0458	7	6	1.15	ST4-74	514586	515641	-
ybaO	35	32	1.11	ST4-74	515754	516212	+
mdlA	10	9	1.05	ST4-74	516253	518025	+
mdlB	8	10	0.82	ST4-74	518018	519799	+
glnK	17	10	1.68	ST4-74	520012	520350	+
amtB	19	12	1.54	ST4-74	520382	521668	+
tesB	19	16	1.21	ST4-74	521768	522628	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
ybaY	108	48	2.24	ST4-74	522843	523412	+
ybaZ	153	129	1.19	ST4-74	523445	523756	-
ylaB	25	22	1.14	ST4-74	524065	525615	-
rpmE2	114	63	1.81	ST4-74	525840	526100	+
rpmJa	215	145	1.48	ST4-74	526163	526246	+
ylaC	102	92	1.11	ST4-74	526302	526772	-
maa	99	86	1.16	ST4-74	526889	527440	-
hha	114	92	1.24	ST4-74	527619	527837	-
ybaJ	162	110	1.47	ST4-74	527865	528239	-
acrB	42	51	0.82	ST4-74	528735	531884	-
acrA	29	32	0.90	ST4-74	531907	533100	-
acrR	41	45	0.92	ST4-74	533242	533895	+
aefA	21	20	1.08	ST4-74	534014	537376	+
STM0479	23	22	1.04	ST4-74	537418	538353	-
ybaM	24	16	1.55	ST4-74	538423	538590	-
priC	21	15	1.42	ST4-74	538604	539119	-
apt	43	45	0.94	ST4-74	539730	540281	+
dnaX	39	39	1.00	ST4-74	540395	542323	+
ybaB	36	49	0.73	ST4-74	542369	542698	+
recR	20	31	0.62	ST4-74	542698	543303	+
htpG	35	47	0.74	ST4-74	543414	545288	+
STnc3130	120	175	0.69	ST4-74	545302	545385	-
adk	76	85	0.89	ST4-74	545529	546173	+
hemH	27	24	1.14	ST4-74	546402	547364	+
aes	3	3	0.99	ST4-74	547361	548332	-
gsk	19	18	1.04	ST4-74	548485	549789	+
ybaL	40	38	1.03	ST4-74	549838	551514	-
fsr	9	11	0.85	ST4-74	551729	552949	-
ushA	39	46	0.85	ST4-74	553122	554774	+
ybaK	34	35	0.99	ST4-74	554891	555370	-
ChiX	42639	30555	1.40	ST4-74	555450	555566	+
ybaP	19	19	0.96	ST4-74	555572	556366	-
STM0497	12	9	1.39	ST4-74	556452	557279	-
copA	26	33	0.79	ST4-74	557433	559934	-
cueR	88	84	1.05	ST4-74	560044	560460	+
ybbJ	16	14	1.11	ST4-74	560461	560913	-
ybbK	37	32	1.16	ST4-74	560910	561827	-
ybbL	9	9	1.09	ST4-74	561974	562651	+
ybbM	16	13	1.21	ST4-74	562638	563417	+
ybbN	35	40	0.87	ST4-74	563503	564357	-
ybbO	16	18	0.91	ST4-74	564417	565187	-
tesA	28	26	1.07	ST4-74	565342	565956	-
ybbA	13	17	0.78	ST4-74	565927	566613	+
ybbP	23	21	1.11	ST4-74	566610	569024	+
STnc950	14	19	0.77	ST4-74	569023	569074	+
STM0509	13	11	1.13	ST4-74	569210	570343	+
sfbA	83	77	1.08	ST4-74	570600	571430	+
sfbB	41	44	0.95	ST4-74	571467	572483	+
sfbC	72	61	1.17	ST4-74	572476	573135	+
ybbB	18	21	0.86	ST4-74	573174	574268	-
ybbS	5	5	1.12	ST4-74	574338	575264	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
allA	25	21	1.19	ST4-74	575491	575973	+
allR	40	45	0.88	ST4-74	576052	576870	+
gcl	2	2	0.85	ST4-74	576955	578736	+
gip	4	3	1.11	ST4-74	578749	579525	+
garRa	1	2	0.46	ST4-74	579626	580504	+
STM0520	3	2	1.21	ST4-74	580570	581817	+
allP	2	1	1.44	ST4-74	581913	583367	+
allB	3	2	1.36	ST4-74	583446	584807	+
ybbY	3	3	1.06	ST4-74	584866	586164	+
glxK	3	2	1.15	ST4-74	586187	587335	+
STnc480	137	82	1.68	ST4-74	587347	587412	-
ylbA	13	12	1.10	ST4-74	587415	588200	-
allC	3	3	0.84	ST4-74	588211	589446	-
allD	2	3	0.53	ST4-74	589468	590517	-
fdrA	3	4	0.83	ST4-74	590848	592512	+
ylbE	1	1	1.13	ST4-74	592522	593781	+
ylbF	2	2	1.12	ST4-74	593793	594602	+
arcC	3	3	1.12	ST4-74	594606	595499	+
purK	28	38	0.73	ST4-74	595540	596607	-
purE	43	72	0.59	ST4-74	596604	597113	-
lpxH	6	8	0.83	ST4-74	597231	597953	-
ppiB	216	291	0.74	ST4-74	597956	598450	-
cysS	62	54	1.15	ST4-74	598623	600008	+
STM0538	5	4	1.06	ST4-74	600052	600876	-
STM0539	4	6	0.68	ST4-74	600873	601310	-
ybcI	6	6	1.03	ST4-74	601303	601848	-
ybcJ	28	30	0.93	ST4-74	601976	602188	-
folD	42	45	0.93	ST4-74	602190	603056	-
fimA	11	15	0.72	ST4-74	603618	604160	+
fimI	2	4	0.67	ST4-74	604236	604769	+
fimC	1	2	0.34	ST4-74	604813	605505	+
fimD	2	2	1.17	ST4-74	605536	608148	+
fimH	4	4	0.94	ST4-74	608163	609170	+
fimF	10	7	1.47	ST4-74	609180	609698	+
fimZ	8	7	1.16	ST4-74	609744	610376	-
fimY	1	2	0.69	ST4-74	610980	611702	-
STM0551	3	3	0.94	ST4-74	611721	612032	-
fimW	11	11	0.97	ST4-74	612194	612790	-
STM0555	9	7	1.21	ST4-74	613262	613552	-
STM0557	29	10	2.82	ST4-74	614292	615944	-
gtrBa	17	7	2.44	ST4-74	615925	616851	-
gtrAa	73	23	3.25	ST4-74	616848	617210	-
STnc1870	32	13	2.54	ST4-74	617317	617426	+
STM0561	7	7	1.03	ST4-74	618078	618407	-
cusA	14	16	0.85	ST4-74	618456	618692	+
ykgD	6	5	1.21	ST4-74	618746	619600	-
ykgC	7	6	1.15	ST4-74	619816	621141	+
STM0565	4	3	1.28	ST4-74	621298	621534	+
ykgB	4	3	1.51	ST4-74	621547	622107	+
STM0567	16	24	0.66	ST4-74	622186	623361	-
pheP	40	38	1.05	ST4-74	623687	625081	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
ybdG	13	18	0.74	ST4-74	625123	626253	-
apeE	14	15	0.90	ST4-74	626681	628651	+
STM0571	20	22	0.93	ST4-74	628818	631547	+
STM0572	2	2	0.68	ST4-74	631675	632661	-
STM0573	1	1	0.77	ST4-74	632728	633771	-
manZ	1	1	1.00	ST4-74	633804	634658	-
manY	2	2	1.48	ST4-74	634661	635335	-
manX	1	0	1.75	ST4-74	635419	635889	-
STM0577	1	1	2.31	ST4-74	635867	636316	-
nfnB	26	29	0.91	ST4-74	636516	637169	-
ybdF	9	9	0.98	ST4-74	637266	637634	-
ramR	39	37	1.05	ST4-74	637634	638215	-
ramA	13	12	1.06	ST4-74	638504	638845	+
ybdJ	24	17	1.36	ST4-74	638851	639099	-
ybdK	7	5	1.52	ST4-74	639166	640284	-
entD	11	5	2.11	ST4-74	640296	641000	-
fepA	8	5	1.65	ST4-74	641046	643301	-
fes	8	4	2.15	ST4-74	643490	644704	+
ybdZ	3	2	1.31	ST4-74	644735	644953	+
entF	4	4	1.26	ST4-74	644950	648834	+
fepE	45	43	1.04	ST4-74	649084	650220	+
fepC	4	3	1.14	ST4-74	650273	651067	-
fepG	4	3	1.26	ST4-74	651064	652053	-
fepD	8	4	1.94	ST4-74	652053	653060	-
ybdA	7	6	1.19	ST4-74	653171	654415	+
fepB	7	4	1.65	ST4-74	654478	655434	-
entC	4	3	1.24	ST4-74	655743	656918	+
entE	3	2	1.41	ST4-74	656928	658538	+
entB	3	2	1.51	ST4-74	658552	659409	+
entA	4	3	1.07	ST4-74	659409	660164	+
ybdB	5	3	1.98	ST4-74	660167	660580	+
cstAa	85	121	0.70	ST4-74	660762	662867	+
ybdD	109	122	0.89	ST4-74	662931	663128	+
ybdH	90	78	1.15	ST4-74	663164	664252	-
ybdL	49	51	0.95	ST4-74	664379	665539	+
ybdM	10	10	0.99	ST4-74	665540	666157	-
ybdN	14	13	1.08	ST4-74	666130	667365	-
ybdO	20	20	1.01	ST4-74	667531	668433	-
STnc3180	27	22	1.19	ST4-74	668611	668719	+
dsbG	14	15	0.90	ST4-74	668750	669496	-
STnc070	831	1959	0.42	ST4-74	669642	669793	+
ahpC	208	299	0.70	ST4-74	669937	670500	+
ahpF	24	33	0.75	ST4-74	670742	672307	+
STM0610	2	2	1.22	ST4-74	672639	673199	+
STM0611	3	3	1.20	ST4-74	673192	675471	+
STM0612	6	4	1.42	ST4-74	675468	676025	+
STM0613	22	20	1.08	ST4-74	676025	676792	+
ybdQ	697	505	1.38	ST4-74	676860	677288	-
ybdR	12	7	1.83	ST4-74	677511	678749	+
rnk	43	42	1.03	ST4-74	678832	679242	-
sRNA10	240	160	1.50	ST4-74	679315	679410	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
rna	187	132	1.42	ST4-74	679477	680283	-
citT	12	40	0.30	ST4-74	680393	681856	-
citG	4	16	0.25	ST4-74	681894	682790	-
citX	5	26	0.19	ST4-74	682762	683313	-
citF	4	17	0.25	ST4-74	683317	684846	-
citE	3	10	0.27	ST4-74	684856	685764	-
citD	4	14	0.30	ST4-74	685761	686057	-
citC	20	39	0.50	ST4-74	686054	687130	-
citA	27	26	1.04	ST4-74	687515	689176	+
citB	13	19	0.71	ST4-74	689145	689825	+
STnc3550	37	42	0.88	ST4-74	689844	689990	-
dcuC	5	14	0.33	ST4-74	689879	691132	-
pagP	540	424	1.28	ST4-74	691643	692215	+
cspE	9609	8771	1.10	ST4-74	692400	692612	+
ccrB	114	86	1.33	ST4-74	692670	693053	-
STM0631	13	12	1.08	ST4-74	693144	693932	+
tatE	232	316	0.73	ST4-74	694061	694264	+
lipA	31	31	0.99	ST4-74	694350	695315	-
ybeF	55	36	1.55	ST4-74	695521	696474	-
lipB	27	25	1.10	ST4-74	696776	697417	-
ybeD	273	297	0.92	ST4-74	697517	697711	-
dacA	54	49	1.12	ST4-74	697889	699100	-
rlpA	22	25	0.87	ST4-74	699240	700307	-
mrdB	36	34	1.05	ST4-74	700318	701430	-
mrdA	21	23	0.92	ST4-74	701433	703334	-
ybeA	12	17	0.71	ST4-74	703365	703832	-
ybeB	28	34	0.83	ST4-74	703836	704153	-
cobC	14	12	1.16	ST4-74	704482	705090	-
cobD	7	11	0.64	ST4-74	705187	706281	+
nadD	10	12	0.83	ST4-74	706256	706897	-
holA	21	24	0.86	ST4-74	706899	707930	-
rlpB	66	64	1.03	ST4-74	707930	708520	-
leuS	50	48	1.03	ST4-74	708535	711117	-
STM0649	3	2	1.39	ST4-74	711405	711695	+
STM0650	4	4	1.08	ST4-74	711712	712884	+
STM0651	20	21	0.93	ST4-74	713003	713887	+
STM0652	19	20	0.95	ST4-74	713955	715883	+
ybeL	161	123	1.31	ST4-74	715976	716449	+
ybeQ	12	9	1.36	ST4-74	716488	717483	-
ybeR	4	4	1.08	ST4-74	717608	718315	+
ybeS	3	2	1.41	ST4-74	718312	719745	+
ybeU	5	4	1.30	ST4-74	719757	720453	+
ybeV	7	4	1.51	ST4-74	720450	721922	+
hscC	4	8	0.52	ST4-74	721944	723623	-
STM0660	6	6	0.93	ST4-74	723699	724937	-
rihA	9	11	0.84	ST4-74	724969	725904	-
gltL	15	19	0.75	ST4-74	726021	726746	-
gltK	20	24	0.86	ST4-74	726746	727420	-
gltJ	16	18	0.87	ST4-74	727420	728160	-
SroC	228	158	1.44	ST4-74	728172	728324	-
gltI	127	95	1.34	ST4-74	728320	729246	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
lnt	29	29	1.00	ST4-74	729583	731121	-
ybeX	75	69	1.09	ST4-74	731141	732019	-
ybeY	92	81	1.13	ST4-74	732177	732650	-
phoL	63	56	1.12	ST4-74	732647	733732	-
miaB	25	24	1.05	ST4-74	733898	735322	-
ubiF	24	23	1.04	ST4-74	735466	736641	+
STM0672	24	16	1.56	ST4-74	736708	737283	-
STnc3290	108	99	1.10	ST4-74	738320	738476	+
asnB	19	21	0.92	ST4-74	738350	740014	-
nagD	21	21	1.00	ST4-74	740304	741056	-
nagC	12	14	0.86	ST4-74	741103	742323	-
nagA	27	27	0.99	ST4-74	742328	743482	-
nagB	28	32	0.87	ST4-74	743542	744342	-
nagE	17	30	0.58	ST4-74	744669	746621	+
glnS	50	51	0.98	ST4-74	746832	748499	+
STnc3300	109	77	1.42	ST4-74	748530	748639	-
ybfM	29	39	0.75	ST4-74	748945	750351	+
ybfN	25	41	0.61	ST4-74	750401	750733	+
STM0689	11	8	1.37	ST4-74	750782	752086	-
STM0690	2	2	1.02	ST4-74	752137	753276	-
STM0691	3	3	1.21	ST4-74	753263	754666	-
STM0692	12	13	0.96	ST4-74	754764	755690	-
fur	295	280	1.05	ST4-74	755805	756257	-
sRNA9	337	284	1.19	ST4-74	756377	756436	-
fldA	187	179	1.04	ST4-74	756539	757069	-
ybfE	45	43	1.05	ST4-74	757232	757513	-
ybfF	23	24	0.94	ST4-74	757647	758417	-
seqA	76	77	0.99	ST4-74	758602	759144	+
pgm	40	46	0.86	ST4-74	759169	760809	+
STM0699	6	4	1.32	ST4-74	760924	761409	-
potE	4	4	1.09	ST4-74	761474	762793	-
speF	3	2	1.70	ST4-74	762790	764988	-
kdpE	10	8	1.23	ST4-74	765750	766427	-
kdpD	11	9	1.26	ST4-74	766424	769108	-
kdpC	7	6	1.05	ST4-74	769105	769689	-
kdpB	2	2	1.08	ST4-74	769698	771746	-
kdpA	3	3	1.19	ST4-74	771767	773446	-
kdpF	3	2	1.88	ST4-74	773446	773535	-
ybfA	333	227	1.47	ST4-74	773872	774078	+
phrB	13	8	1.70	ST4-74	774189	775610	+
ybgH	14	17	0.83	ST4-74	775649	777130	-
ybgI	41	41	1.01	ST4-74	777459	778202	+
ybgJ	12	14	0.87	ST4-74	778218	778874	+
ybgK	11	9	1.18	ST4-74	778868	779800	+
ybgL	17	17	0.99	ST4-74	779790	780524	+
STM0715	12	9	1.27	ST4-74	780848	780979	+
fimB	29	22	1.30	ST4-74	781039	781605	+
STM0717	5	5	0.97	ST4-74	782065	782400	+
STM0718	4	2	1.84	ST4-74	782402	783142	+
STM0719	2	2	1.27	ST4-74	783428	784579	+
STM0720	3	1	2.12	ST4-74	784576	785472	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM0721	3	2	1.62	ST4-74	785485	786618	+
STM0722	5	4	1.33	ST4-74	786747	787517	+
STM0723	3	2	1.43	ST4-74	787521	788231	+
STM0724	3	3	1.31	ST4-74	788325	790103	+
STM0725	3	2	1.30	ST4-74	790296	791129	+
STM0726	3	3	1.22	ST4-74	791187	793082	+
STM0727	59	54	1.09	ST4-74	793150	793287	+
nei	15	18	0.86	ST4-74	793336	794127	+
abrB	9	7	1.33	ST4-74	794182	795144	-
gltA	65	78	0.83	ST4-74	795365	796648	-
STM0731	6	5	1.08	ST4-74	796623	796994	+
sdhC	101	118	0.86	ST4-74	797403	797792	+
sdhD	69	81	0.85	ST4-74	797786	798133	+
sdhA	30	40	0.75	ST4-74	798133	799899	+
sdhB	60	74	0.81	ST4-74	799913	800632	+
sucA	33	42	0.80	ST4-74	801156	803957	+
sucB	39	55	0.70	ST4-74	803972	805180	+
sucC	37	44	0.84	ST4-74	805322	806488	+
sucD	60	69	0.87	ST4-74	806488	807357	+
RybD	122	185	0.66	ST4-74	807346	807429	+
cydA	152	125	1.22	ST4-74	808940	810508	+
cydB	131	102	1.29	ST4-74	810524	811663	+
ybgT	362	226	1.60	ST4-74	811678	811791	+
ybgE	269	209	1.29	ST4-74	811928	812209	+
ybgC	68	60	1.15	ST4-74	812438	812842	+
tolQ	28	33	0.83	ST4-74	812839	813531	+
tolR	25	29	0.86	ST4-74	813535	813963	+
tolA	30	32	0.94	ST4-74	814028	815251	+
tolB	113	123	0.92	ST4-74	815375	816667	+
pal	206	289	0.71	ST4-74	816702	817226	+
ybgF	86	101	0.85	ST4-74	817236	818024	+
nadA	22	24	0.91	ST4-74	819489	820532	+
pnuC	36	39	0.92	ST4-74	820557	821276	+
ybgR	20	17	1.19	ST4-74	821273	822211	-
ybgS	114	53	2.14	ST4-74	822322	822708	-
aroG	127	105	1.21	ST4-74	823028	824080	+
STM0761	29	42	0.70	ST4-74	824174	824719	-
STM0762	22	38	0.58	ST4-74	824734	825579	-
STM0763	27	32	0.84	ST4-74	825709	826599	+
STM0764	10	11	0.93	ST4-74	826600	827526	-
STM0765	3	1	2.10	ST4-74	827806	829074	+
dcoC	4	4	1.16	ST4-74	829124	829369	+
dcoA	2	1	1.07	ST4-74	829385	831160	+
dcoB	3	3	1.04	ST4-74	831173	832474	+
STM0769	5	5	1.00	ST4-74	832593	833291	+
STM0770	6	6	1.13	ST4-74	833671	834750	+
STM0771	7	7	1.00	ST4-74	834750	835526	+
gpmA	309	243	1.27	ST4-74	835617	836369	-
galM	12	13	0.92	ST4-74	836593	837633	-
galK	14	15	0.94	ST4-74	837627	838775	-
galT	5	5	1.18	ST4-74	838778	839824	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
galE	19	15	1.26	ST4-74	839835	840851	-
STM0777	4	5	0.88	ST4-74	841074	841982	-
modF	30	33	0.92	ST4-74	842153	843628	-
modE	18	16	1.09	ST4-74	843696	844484	-
ybhT	813	567	1.43	ST4-74	844613	844762	+
modA	50	105	0.47	ST4-74	844929	845702	+
modB	13	42	0.31	ST4-74	845702	846391	+
modC	10	28	0.37	ST4-74	846394	847452	+
ybhA	20	16	1.21	ST4-74	847453	848271	-
ybhE	26	21	1.22	ST4-74	848435	849430	+
ybhC	22	24	0.93	ST4-74	849574	850857	-
hutI	16	17	0.94	ST4-74	851095	852318	+
hutG	8	10	0.82	ST4-74	852315	853256	+
hutC	11	12	0.84	ST4-74	853349	854026	+
hutU	4	3	1.24	ST4-74	854223	855908	+
hutH	3	3	0.88	ST4-74	855910	857430	+
ybhB	87	67	1.30	ST4-74	857515	857991	-
bioA	69	45	1.52	ST4-74	858049	859338	-
bioB	110	81	1.36	ST4-74	859425	860465	+
bioF	45	41	1.10	ST4-74	860462	861619	+
bioC	31	31	0.99	ST4-74	861603	862358	+
bioDa	29	27	1.06	ST4-74	862351	863037	+
STnc3340	72	63	1.15	ST4-74	863329	863391	-
uvrB	21	21	1.03	ST4-74	863688	865709	+
slrP	23	15	1.49	ST4-74	866199	868496	+
ybhK	13	27	0.49	ST4-74	868588	869496	-
moaA	18	65	0.28	ST4-74	869893	870882	+
moaB	14	30	0.46	ST4-74	870904	871416	+
moaC	8	18	0.47	ST4-74	871419	871904	+
moaD	13	27	0.48	ST4-74	871891	872142	+
moaE	11	21	0.50	ST4-74	872144	872596	+
ybhL	176	164	1.07	ST4-74	872644	873348	+
ybhM	4	3	1.42	ST4-74	873495	874202	+
STM0809	4	6	0.69	ST4-74	874226	874834	+
STM0810	4	2	1.80	ST4-74	874842	875402	+
ybhN	18	11	1.61	ST4-74	875405	876367	-
ybhO	7	5	1.46	ST4-74	876367	877608	-
ybhP	20	8	2.53	ST4-74	877605	878363	-
ybhQ	403	358	1.13	ST4-74	878496	878906	+
ybhR	16	14	1.17	ST4-74	878868	879974	-
ybhS	14	11	1.29	ST4-74	880090	881220	-
ybhF	7	7	1.05	ST4-74	881213	882949	-
STM0818	8	8	0.99	ST4-74	882942	883937	-
ybiH	19	16	1.20	ST4-74	883937	884611	-
rhlE	7	5	1.23	ST4-74	884841	886205	+
dinG	13	11	1.11	ST4-74	886512	888557	+
ybiB	12	12	0.98	ST4-74	888587	889561	+
STnc980	44	18	2.46	ST4-74	889558	889714	-
ybiJ	12	8	1.45	ST4-74	889717	889977	-
ybiI	110	58	1.89	ST4-74	890262	890528	-
ybiN	9	10	0.86	ST4-74	890733	891665	+

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ybiO	11	8	1.29	ST4-74	891662	893884	-
glnQ	36	37	0.96	ST4-74	894003	894725	-
glnP	34	40	0.84	ST4-74	894722	895381	-
glnH	126	134	0.94	ST4-74	895525	896271	-
dps	1063	692	1.54	ST4-74	896748	897251	-
ybiF	11	10	1.05	ST4-74	897554	898441	-
ompX	975	596	1.64	ST4-74	898795	899310	+
ybiP	10	10	0.99	ST4-74	899373	900953	-
RybA	597	429	1.39	ST4-74	901301	901397	-
STM0835	51	49	1.04	ST4-74	901532	902005	+
ybiR	13	12	1.10	ST4-74	902002	903114	+
ybiS	40	35	1.14	ST4-74	903158	904078	-
ybiT	39	33	1.16	ST4-74	904297	905889	+
STM0839	7	6	1.20	ST4-74	906498	907241	+
ybiV(2)	1	1	0.93	ST4-74	907270	907800	-
ybiU	3	2	1.37	ST4-74	908194	909459	-
ybiV(1)	31	22	1.41	ST4-74	909721	910530	+
pflF	1	2	0.79	ST4-74	910608	913040	-
pflE	2	3	0.52	ST4-74	913046	913945	-
moeB	8	10	0.83	ST4-74	914223	914972	-
moeA	12	13	0.96	ST4-74	914972	916213	-
ybiK	65	71	0.91	ST4-74	916410	917351	+
yliA	22	25	0.87	ST4-74	917362	919233	+
yliB	22	27	0.82	ST4-74	919266	920804	+
yliC	18	20	0.89	ST4-74	920865	921785	+
yliD	20	17	1.18	ST4-74	921788	922699	+
rimO	21	20	1.04	ST4-74	922790	924115	-
bssR	531	543	0.98	ST4-74	924346	924729	+
STnc1200	1244	1655	0.75	ST4-74	924769	924838	-
STM0854	3	2	1.42	ST4-74	925442	925957	+
STM0855	5	4	1.25	ST4-74	925875	926729	+
STM0856	3	3	0.92	ST4-74	926740	927687	+
STM0857	19	19	0.98	ST4-74	927981	929144	+
STM0858	6	5	1.24	ST4-74	929593	931278	+
STM0859	18	12	1.53	ST4-74	931284	932174	-
STnc1710	178	117	1.52	ST4-74	932197	932336	+
STM0860	41	28	1.45	ST4-74	932439	932864	-
yliI	5	4	1.17	ST4-74	933211	933891	+
yliJ	27	29	0.93	ST4-74	933888	934514	-
dacC	68	47	1.42	ST4-74	934758	935960	+
deoR	52	50	1.04	ST4-74	936005	936763	-
ybjG	66	71	0.93	ST4-74	936835	937443	-
mdfA	14	15	0.90	ST4-74	937756	938988	+
STM0867	9	12	0.76	ST4-74	939042	939857	-
STM0868	9	9	0.94	ST4-74	939854	941065	-
STM0869	6	5	1.28	ST4-74	941233	941853	+
RybB	1751	1341	1.31	ST4-74	941815	941893	-
STM0870	22	20	1.12	ST4-74	941970	943655	-
ybjM	28	29	0.98	ST4-74	943926	944303	+
grxA	768	870	0.88	ST4-74	944335	944598	-
ybjC	33	25	1.32	ST4-74	944768	945058	+

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mdaA	16	14	1.16	ST4-74	945105	945763	+
rimK	53	55	0.97	ST4-74	945821	946723	+
ybjN	35	30	1.19	ST4-74	946820	947296	+
potF	28	32	0.88	ST4-74	947645	948757	+
potG	20	24	0.84	ST4-74	948845	949978	+
potH	21	23	0.91	ST4-74	949988	950941	+
potI	32	36	0.90	ST4-74	950938	951783	+
ybjO	81	70	1.15	ST4-74	951857	952330	+
rumB	10	7	1.37	ST4-74	952373	953503	+
STM0884	5	4	1.19	ST4-74	953786	955129	+
STM0885	9	8	1.14	ST4-74	955159	955479	+
STM0886	7	5	1.33	ST4-74	955489	956976	+
artJ	115	302	0.38	ST4-74	957195	957926	-
artM	79	80	1.00	ST4-74	958163	958831	-
artQ	52	70	0.75	ST4-74	958831	959547	-
artI	65	80	0.81	ST4-74	959554	960285	-
artP	185	178	1.04	ST4-74	960303	961031	-
ybjP	64	57	1.12	ST4-74	961261	961776	-
STM0930	114	87	1.31	ST4-74	961904	962227	+
ybjR	31	29	1.09	ST4-74	962224	963054	+
STM0932	19	19	1.00	ST4-74	963051	964064	-
ybjT	10	11	0.94	ST4-74	964160	965593	-
ltaA	53	45	1.17	ST4-74	965604	966605	-
poxB	15	10	1.55	ST4-74	966644	968362	-
hcr	8	8	1.00	ST4-74	968520	969491	-
hcp	5	6	0.78	ST4-74	969500	971152	-
ybjE	26	35	0.74	ST4-74	971296	972087	-
ybjD	14	14	0.99	ST4-74	972397	974055	+
ybjX	362	307	1.18	ST4-74	974052	975020	-
ybjY	51	43	1.17	ST4-74	975148	976266	+
ybjZ	37	33	1.15	ST4-74	976263	978209	+
cspD	952	1539	0.62	ST4-74	978339	978560	-
clpS	244	248	0.98	ST4-74	978884	979204	+
clpA	54	66	0.82	ST4-74	979235	981511	+
STnc490	24696	33984	0.73	ST4-74	981622	981711	-
tnp	244	250	0.98	ST4-74	981703	982161	+
STM0947	6	5	1.26	ST4-74	982624	983307	-
STM0948	17	9	1.92	ST4-74	983478	983879	-
STM0950	9	8	1.18	ST4-74	984216	984893	-
STM0951	5	4	1.24	ST4-74	984913	985773	-
STM0952	7	7	0.90	ST4-74	985882	986793	+
infA	298	337	0.88	ST4-74	986884	987102	-
STM0954	604	529	1.14	ST4-74	987030	987359	-
aat	33	26	1.25	ST4-74	987414	988118	-
cydC	23	31	0.76	ST4-74	988163	989884	-
cydD	26	34	0.78	ST4-74	989885	991651	-
trxB	88	86	1.02	ST4-74	991765	992733	-
lrp	333	380	0.88	ST4-74	993279	993773	+
ftsK	119	136	0.88	ST4-74	993908	997993	+
lolA	82	90	0.91	ST4-74	998133	998747	+
ycaJ	33	37	0.88	ST4-74	998757	1000100	+

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serS	48	70	0.69	ST4-74	1000359	1001651	+
dmsA	3	52	0.06	ST4-74	1001888	1004332	+
dmsB	3	46	0.06	ST4-74	1004343	1004960	+
dmsC	10	57	0.18	ST4-74	1004962	1005825	+
ycaD	22	19	1.14	ST4-74	1006175	1007323	+
ycaM	16	14	1.14	ST4-74	1007541	1008962	+
STnc3310	108	94	1.15	ST4-74	1009199	1009379	-
pflA	34	31	1.07	ST4-74	1009255	1010079	-
STM0971	109	102	1.07	ST4-74	1010171	1010497	-
sopD2	752	96	7.86	ST4-74	1010628	1011587	+
pflB	76	143	0.53	ST4-74	1011663	1013945	-
focA	126	194	0.65	ST4-74	1014005	1014862	-
ycaO	13	11	1.13	ST4-74	1015267	1017027	-
ycaP	25	16	1.59	ST4-74	1017164	1017856	+
serC	133	126	1.05	ST4-74	1018042	1019130	+
aroA	28	34	0.83	ST4-74	1019201	1020484	+
ycaL	19	19	1.04	ST4-74	1020627	1021388	+
cmk	119	103	1.15	ST4-74	1021561	1022244	+
rpsA	297	358	0.83	ST4-74	1022358	1024031	+
himD	267	272	0.98	ST4-74	1024187	1024471	+
STnc3330	13	11	1.22	ST4-74	1024493	1024591	-
rec2	6	5	1.21	ST4-74	1024701	1026965	+
msbA	48	50	0.95	ST4-74	1027002	1028750	+
lpxK	24	31	0.78	ST4-74	1028747	1029724	+
ycaQ	6	8	0.76	ST4-74	1029768	1031000	+
ycaR	122	111	1.11	ST4-74	1031052	1031234	+
kdsB	13	16	0.79	ST4-74	1031231	1031977	+
STM0989	15	38	0.39	ST4-74	1032188	1033081	+
ycbC	6	7	0.83	ST4-74	1033061	1033837	-
smtA	24	27	0.89	ST4-74	1033973	1034776	+
mukF	14	20	0.73	ST4-74	1034769	1036091	+
mukE	21	28	0.74	ST4-74	1036072	1036776	+
mukB	28	31	0.89	ST4-74	1036776	1041242	+
ycbB	38	34	1.12	ST4-74	1041587	1043428	+
ycbK	87	113	0.77	ST4-74	1043688	1044236	+
ycbL	68	95	0.72	ST4-74	1044264	1044911	+
aspC	72	79	0.92	ST4-74	1044973	1046163	-
ompF	299	372	0.81	ST4-74	1046348	1047439	-
STnc3350	92	48	1.92	ST4-74	1047936	1048028	+
asnCa	99	122	0.81	ST4-74	1048046	1049446	-
STM1001	27	36	0.76	ST4-74	1049647	1050108	-
dpaL	4	4	1.12	ST4-74	1050425	1051639	+
STM1003	3	3	0.95	ST4-74	1051884	1053320	+
pncB	30	29	1.03	ST4-74	1053398	1054600	-
STM1005	53	52	1.03	ST4-74	1054795	1056087	-
STnc4200	308	199	1.55	ST4-74	1055773	1055892	+
STM1006	1	1	0.99	ST4-74	1056132	1056380	-
STM1007	3	2	1.69	ST4-74	1056421	1056660	-
STM1008	3	3	0.82	ST4-74	1056703	1057812	-
recEa	1	1	1.26	ST4-74	1057823	1060708	-
IsrB-2	0	0	–	ST4-74	1060740	1060833	-

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IsrB-1	663	1160	0.57	ST4-74	1060741	1060835	+
STM1010	0	1	0.46	ST4-74	1060835	1061134	-
STM2630	0	0	0.00	ST4-74	1061156	1061314	-
STM1011	0	1	0.22	ST4-74	1061307	1061567	-
STM1012	211	178	1.18	ST4-74	1061617	1061958	-
C1	2	2	0.84	ST4-74	1062352	1062726	+
STnc1080	2025	2577	0.79	ST4-74	1062745	1062806	-
STM2626	2	1	1.35	ST4-74	1062811	1063794	+
STM1015	1	2	0.96	ST4-74	1063797	1064546	+
STM2624	1	2	0.79	ST4-74	1064557	1064904	+
STM2623	22	20	1.06	ST4-74	1064901	1065212	+
STM2622	388	315	1.23	ST4-74	1065290	1065580	+
STM1019	80	85	0.94	ST4-74	1065872	1066105	+
STM1020	28	25	1.10	ST4-74	1066521	1067123	+
ninG	1	2	0.83	ST4-74	1067332	1067943	+
SL1344_0960	0	1	0.00	ST4-74	1067940	1068086	+
STM1022	9	7	1.29	ST4-74	1068076	1068873	+
STM1023	135	125	1.07	ST4-74	1068940	1069257	+
STM1024	3	2	1.27	ST4-74	1069431	1069556	+
STM1025	14	29	0.48	ST4-74	1069692	1070141	-
gtgA	31	25	1.24	ST4-74	1070502	1071188	-
STM2613	6	6	0.97	ST4-74	1071458	1071793	+
nucD	8	6	1.39	ST4-74	1071777	1072229	+
STM1029	1	1	0.93	ST4-74	1072226	1072726	+
STM1030	4	5	0.81	ST4-74	1072934	1073467	+
STM1031	4	3	1.18	ST4-74	1073508	1075562	+
STM1032	3	4	0.93	ST4-74	1075762	1077309	+
STM1033	3	3	0.83	ST4-74	1077233	1079314	+
STM1034	2	1	1.93	ST4-74	1079405	1079728	+
STM1035	2	2	0.98	ST4-74	1079721	1080020	+
STM1036	3	2	1.23	ST4-74	1080001	1080567	+
STM1037	3	3	0.92	ST4-74	1080564	1080965	+
STM1038	3	3	0.87	ST4-74	1081040	1081726	+
STM1039	2	2	0.80	ST4-74	1081772	1082170	+
STM1040	3	2	1.12	ST4-74	1082233	1082496	+
STM1041	4	4	1.04	ST4-74	1082510	1085563	+
STM1042	5	5	1.05	ST4-74	1085560	1085892	+
STM1043	23	19	1.23	ST4-74	1085991	1086488	+
sodCa	222	183	1.22	ST4-74	1086605	1087138	-
STM1045	8	9	0.81	ST4-74	1087228	1087923	+
STM1046	2	3	0.93	ST4-74	1087933	1088670	+
STM1047	3	2	1.10	ST4-74	1088568	1089272	+
STM1048	4	3	1.23	ST4-74	1089344	1092694	+
STM1049	3	2	1.25	ST4-74	1093029	1095236	+
STM1050	4	5	0.93	ST4-74	1095236	1095817	+
SL1344_0990	8	6	1.42	ST4-74	1095821	1096036	-
sseI	14	8	1.83	ST4-74	1096293	1097261	+
STM1052	14	11	1.31	ST4-74	1097359	1097777	+
STM1053	4	2	1.76	ST4-74	1097909	1098535	-
STM1054	346	376	0.92	ST4-74	1098604	1098903	-
gtgE	160	108	1.48	ST4-74	1098888	1099574	-

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gtgF	10	7	1.35	ST4-74	1099845	1100036	-
pepN	23	20	1.13	ST4-74	1100463	1103075	+
pyrD	15	20	0.71	ST4-74	1103256	1104293	+
ycbW	93	92	1.01	ST4-74	1104459	1105001	+
STM1060	15	16	0.94	ST4-74	1104998	1106107	-
rlmL	18	18	1.02	ST4-74	1106206	1108314	+
uup	16	17	0.98	ST4-74	1108327	1110234	+
pqiA	23	22	1.04	ST4-74	1110249	1111502	+
pqiB	21	21	0.99	ST4-74	1111507	1113147	+
ymbA	39	29	1.36	ST4-74	1113144	1113707	+
STnc3390	15	18	0.84	ST4-74	1113731	1113815	-
rmf	4912	6233	0.79	ST4-74	1113963	1114130	+
fabA	149	155	0.96	ST4-74	1114230	1114748	-
lonH	18	18	1.03	ST4-74	1114817	1116403	-
ycbG	78	89	0.88	ST4-74	1116763	1117215	+
ompA	523	496	1.05	ST4-74	1117287	1118339	-
sulA	25	24	1.02	ST4-74	1118696	1119205	-
sxy	11	13	0.81	ST4-74	1119422	1120027	+
yccS	17	19	0.89	ST4-74	1120014	1122167	-
yccF	19	18	1.06	ST4-74	1122186	1122632	-
helD	25	24	1.02	ST4-74	1122756	1124810	+
mgsA	51	87	0.59	ST4-74	1124846	1125304	-
yccT	20	11	1.81	ST4-74	1125399	1126061	-
STM1078	47	43	1.09	ST4-74	1126232	1126648	+
yccV	81	83	0.98	ST4-74	1126693	1127010	-
yccW	15	12	1.21	ST4-74	1127068	1128279	-
STM1081	9	10	0.83	ST4-74	1128494	1129042	+
STM1082	17	16	1.06	ST4-74	1129068	1129847	+
yccX	9	10	0.96	ST4-74	1129896	1130177	+
yccK	63	61	1.04	ST4-74	1130174	1130503	-
yccA	260	288	0.90	ST4-74	1130590	1131168	-
pipA	46	33	1.39	ST4-74	1131870	1132550	-
pipB	89	10	8.47	ST4-74	1132772	1133647	-
STM1089	1	0	3.21	ST4-74	1133859	1134185	+
pipC	56	75	0.75	ST4-74	1134195	1134536	-
sopB	14	18	0.77	ST4-74	1134553	1136238	-
orfX	18	1109	0.02	ST4-74	1136570	1136740	+
STM1093	40	31	1.29	ST4-74	1136741	1136851	-
pipD	66	52	1.27	ST4-74	1136960	1138429	-
copS	6	7	0.93	ST4-74	1138602	1139966	-
copR	28	21	1.33	ST4-74	1139959	1140705	-
STM1097	76	79	0.96	ST4-74	1140775	1141185	+
hpaC	5	4	1.11	ST4-74	1141518	1142030	-
hpaB	4	3	1.05	ST4-74	1142048	1143610	-
hpaR	6	6	1.09	ST4-74	1143828	1144268	-
hpaG	2	2	0.92	ST4-74	1144543	1145832	+
hpaE	2	2	1.14	ST4-74	1145829	1147295	+
hpaD	1	2	0.49	ST4-74	1147297	1148148	+
hpaF	1	3	0.29	ST4-74	1148158	1148538	+
hpaH	1	2	0.77	ST4-74	1148681	1149484	+
hpaI	2	2	0.92	ST4-74	1149495	1150286	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
hpaX	4	4	1.08	ST4-74	1150358	1151734	+
hpaA	2	2	0.98	ST4-74	1151744	1152640	+
STM1109	9	8	1.16	ST4-74	1152675	1153592	+
STnc1880	169	115	1.46	ST4-74	1153745	1153841	+
STM1110	20	7	3.04	ST4-74	1154000	1154362	-
STnc3400	40	31	1.29	ST4-74	1154915	1154976	+
yccD	58	50	1.15	ST4-74	1155016	1155321	-
cbpA	32	36	0.90	ST4-74	1155321	1156241	-
scsA	232	189	1.22	ST4-74	1156476	1156838	+
scsB	3	3	0.92	ST4-74	1156887	1158773	+
scsC	3	3	1.23	ST4-74	1158770	1159393	+
scsD	3	3	1.12	ST4-74	1159383	1159889	+
agp	24	27	0.89	ST4-74	1160022	1161263	+
yccJ	218	229	0.95	ST4-74	1161297	1161524	-
wrbA	128	133	0.96	ST4-74	1161545	1162141	-
ymdF	1005	953	1.05	ST4-74	1162526	1162693	+
ycdC	19	20	0.92	ST4-74	1162830	1163468	+
STM1123	70	63	1.11	ST4-74	1163465	1163860	-
putA	6	12	0.49	ST4-74	1163919	1167881	-
putP	15	23	0.65	ST4-74	1168303	1169811	+
phoH	23	18	1.32	ST4-74	1170638	1171492	+
STM1127	33	31	1.07	ST4-74	1171598	1172479	-
STnc500	724	611	1.19	ST4-74	1172561	1172641	-
STM1128	6	7	0.84	ST4-74	1172764	1174260	-
STM1129	4	6	0.66	ST4-74	1174597	1175277	-
STM1130	6	8	0.75	ST4-74	1175783	1176943	+
STM1131	9	11	0.77	ST4-74	1176989	1177681	+
STM1132	11	10	1.05	ST4-74	1177964	1179244	+
STM1133	22	22	1.00	ST4-74	1179255	1180361	+
ycdW	19	21	0.89	ST4-74	1181205	1182143	+
ycdX	77	71	1.10	ST4-74	1182228	1182965	+
ycdY	21	21	1.00	ST4-74	1182989	1183543	+
ycdZ	31	36	0.87	ST4-74	1183653	1184126	+
csgG	12	8	1.46	ST4-74	1184165	1184911	-
csgF	2	2	1.22	ST4-74	1185025	1185441	-
csgE	5	5	1.06	ST4-74	1185468	1185863	-
csgD	9	8	1.05	ST4-74	1185868	1186518	-
csgB	2	8	0.29	ST4-74	1187273	1187728	+
csgA	3	5	0.50	ST4-74	1187770	1188225	+
csgC	33	57	0.57	ST4-74	1188287	1188613	+
ymdA	10	27	0.37	ST4-74	1188744	1189064	+
STM1147	34	30	1.12	ST4-74	1189154	1189693	+
ymdC	27	22	1.23	ST4-74	1189629	1191116	+
mdoC	16	13	1.24	ST4-74	1191133	1192287	-
mdoG	52	48	1.07	ST4-74	1192559	1194094	+
mdoH	42	45	0.93	ST4-74	1194087	1196630	+
yceK	12	10	1.20	ST4-74	1196704	1196931	+
msyB	180	112	1.61	ST4-74	1196932	1197306	-
yceE	7	6	1.16	ST4-74	1197388	1198602	-
STnc3040	12	13	0.95	ST4-74	1198646	1198752	+
htrB	39	36	1.11	ST4-74	1198757	1199677	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
yceA	21	15	1.41	ST4-74	1199897	1200949	+
yceI	19	10	1.84	ST4-74	1201001	1201576	-
yceJ	24	14	1.76	ST4-74	1201573	1202145	-
yceO	24	18	1.34	ST4-74	1202408	1202521	-
solA	17	21	0.85	ST4-74	1202562	1203680	-
bssS	265	233	1.14	ST4-74	1203793	1204050	-
dinI	69	92	0.75	ST4-74	1204337	1204582	-
pyrC	34	44	0.78	ST4-74	1204656	1205702	-
yceB	191	157	1.22	ST4-74	1205807	1206367	-
grxB	167	139	1.20	ST4-74	1206492	1207139	-
yceL	40	41	0.99	ST4-74	1207203	1208411	-
rimJ	72	87	0.83	ST4-74	1208648	1209232	+
yceH	33	46	0.70	ST4-74	1209268	1209915	+
mviM	17	23	0.72	ST4-74	1209917	1210840	+
mviN	17	18	0.95	ST4-74	1211105	1212679	+
flgN	53	236	0.22	ST4-74	1212761	1213183	-
flgM	162	713	0.23	ST4-74	1213188	1213481	-
flgA	21	84	0.24	ST4-74	1213573	1214232	-
flgB	59	251	0.23	ST4-74	1214389	1214805	+
flgC	14	75	0.18	ST4-74	1214809	1215213	+
flgD	15	83	0.18	ST4-74	1215225	1215923	+
flgE	13	71	0.18	ST4-74	1215950	1217161	+
flgF	12	72	0.16	ST4-74	1217182	1217937	+
flgG	12	64	0.19	ST4-74	1217951	1218733	+
flgH	12	57	0.22	ST4-74	1218788	1219486	+
flgI	9	42	0.22	ST4-74	1219498	1220595	+
flgJ	8	35	0.23	ST4-74	1220595	1221545	+
flgK	41	162	0.25	ST4-74	1221610	1223271	+
flgL	37	157	0.24	ST4-74	1223286	1224239	+
STnc840	145	661	0.22	ST4-74	1224244	1224316	+
rne	26	40	0.66	ST4-74	1224496	1227699	-
rluC	29	23	1.25	ST4-74	1228273	1229232	+
STM1188	24	23	1.03	ST4-74	1229388	1230131	+
SL1344_1125	21	25	0.84	ST4-74	1230337	1230561	+
maf-a	30	32	0.93	ST4-74	1230633	1231217	-
SraB	287	251	1.14	ST4-74	1231270	1231378	+
yceD	668	790	0.85	ST4-74	1231415	1231936	+
rpl32	581	802	0.72	ST4-74	1231988	1232161	+
plsX	67	87	0.77	ST4-74	1232295	1233374	+
fabH	35	39	0.89	ST4-74	1233454	1234407	+
fabD	75	71	1.06	ST4-74	1234423	1235352	+
fabG	206	223	0.92	ST4-74	1235365	1236099	+
acpP	5755	6292	0.91	ST4-74	1236255	1236491	+
fabF	54	77	0.71	ST4-74	1236577	1237818	+
pabC	21	20	1.05	ST4-74	1237942	1238751	+
yceG	27	26	1.05	ST4-74	1238820	1239776	+
tmk	18	19	0.98	ST4-74	1239766	1240407	+
holB	12	14	0.85	ST4-74	1240404	1241408	+
ycfH	70	38	1.82	ST4-74	1241419	1242216	+
ptsG	53	61	0.86	ST4-74	1242511	1243944	+
fhuE	4	3	1.59	ST4-74	1244029	1246203	-

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ycfF	152	132	1.15	ST4-74	1246545	1246904	+
ycfL	46	52	0.90	ST4-74	1246907	1247281	+
ycfM	39	39	1.01	ST4-74	1247295	1247933	+
thiK	26	22	1.17	ST4-74	1247914	1248738	+
nagZ	30	48	0.63	ST4-74	1248749	1249774	+
ycfP	56	78	0.72	ST4-74	1249799	1250341	+
ndh	49	27	1.77	ST4-74	1250596	1251900	+
ycfJ	35	18	1.92	ST4-74	1252079	1252618	+
STnc1090	141	103	1.38	ST4-74	1252560	1252626	-
STnc850	123	78	1.58	ST4-74	1252598	1252673	+
ycfQ	83	63	1.33	ST4-74	1252693	1253328	-
ycfR	27	25	1.09	ST4-74	1253570	1253827	+
ycfS	29	35	0.83	ST4-74	1253924	1254889	-
STnc3410	92	115	0.80	ST4-74	1254996	1255077	-
mfd	16	15	1.10	ST4-74	1255037	1258483	-
ycfU	56	55	1.00	ST4-74	1258821	1260020	+
lolD	18	20	0.90	ST4-74	1260013	1260714	+
ycfW	23	28	0.83	ST4-74	1260714	1261958	+
ycfX	35	38	0.92	ST4-74	1261987	1262898	+
cobB	44	49	0.90	ST4-74	1262917	1263738	+
potD	37	42	0.89	ST4-74	1263820	1264866	-
potC	35	35	1.00	ST4-74	1264891	1265670	-
sifA	36	15	2.42	ST4-74	1265986	1266996	-
potB	19	15	1.25	ST4-74	1267325	1268188	-
potA	33	31	1.05	ST4-74	1268172	1269308	-
pepT	18	72	0.25	ST4-74	1269559	1270788	+
STM1228	21	32	0.65	ST4-74	1270792	1272837	+
STM3032	217	230	0.94	ST4-74	1270948	1271457	+
ycfD	39	37	1.05	ST4-74	1272877	1273998	-
phoQ	151	143	1.06	ST4-74	1274079	1275542	-
phoP	618	491	1.26	ST4-74	1275542	1276216	-
purB	22	27	0.81	ST4-74	1276340	1277710	-
ycfC	33	39	0.84	ST4-74	1277714	1278361	-
mnmA	71	66	1.07	ST4-74	1278442	1279548	-
ymfB	8	10	0.81	ST4-74	1279602	1280063	-
STM1236	9	10	0.98	ST4-74	1280075	1280404	-
rluB	53	34	1.55	ST4-74	1280401	1281066	-
icdA	176	194	0.91	ST4-74	1281238	1282488	+
STnc150	235	106	2.21	ST4-74	1282521	1282677	-
STM1239	37	21	1.77	ST4-74	1282936	1284060	+
envF	7	4	1.74	ST4-74	1285042	1285830	-
IsrC	11	12	0.94	ST4-74	1285998	1286285	+
msgA	152	142	1.07	ST4-74	1286319	1286558	-
envE	78	51	1.55	ST4-74	1286749	1287270	-
cspH	102	50	2.05	ST4-74	1287685	1287897	-
pagD	180	42	4.26	ST4-74	1288029	1288292	-
pagC	2814	701	4.02	ST4-74	1289191	1289661	+
STnc3420	37	25	1.49	ST4-74	1289667	1289799	-
STnc520	320	368	0.87	ST4-74	1290590	1290674	-
pliC	945	489	1.93	ST4-74	1290898	1291242	-
STM1250	40	29	1.41	ST4-74	1291945	1292217	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM1251	334	215	1.55	ST4-74	1292369	1292836	+
STnc1760	76	52	1.48	ST4-74	1292835	1292935	-
STnc1210	296	516	0.57	ST4-74	1292956	1293014	+
STM1252	24	16	1.47	ST4-74	1293175	1294218	+
STM1253	60	68	0.88	ST4-74	1294301	1294831	-
STnc1990	228	156	1.46	ST4-74	1294841	1294974	+
STnc3430	38	37	1.03	ST4-74	1294946	1295057	-
STM1254	102	103	0.99	ST4-74	1294980	1295294	-
STM1255	10	115	0.08	ST4-74	1295976	1297610	+
STM1256	6	48	0.12	ST4-74	1297610	1298584	+
STM1257	5	34	0.16	ST4-74	1298574	1299386	+
STM1258	3	21	0.15	ST4-74	1299380	1300177	+
STM1259	3	28	0.09	ST4-74	1300171	1300761	+
STM1260	32	18	1.76	ST4-74	1300843	1301976	-
STM1261	53	33	1.59	ST4-74	1302170	1302496	+
IsrD	2	2	1.08	ST4-74	1302591	1302641	-
STM1263	58	29	2.01	ST4-74	1302693	1303340	+
STnc3440	22	10	2.11	ST4-74	1303294	1303380	+
aadA	20	21	0.99	ST4-74	1303449	1304237	+
STM1265	25	11	2.27	ST4-74	1304382	1304975	+
STM1266	41	36	1.17	ST4-74	1305044	1305892	+
STM1267	956	311	3.07	ST4-74	1306147	1306395	-
STM1268	80	84	0.96	ST4-74	1306474	1306623	-
aroQ	305	225	1.35	ST4-74	1306706	1307251	+
yeaS	153	130	1.18	ST4-74	1307448	1308086	+
yeaR	29	22	1.30	ST4-74	1308259	1308620	+
yoaG	64	37	1.73	ST4-74	1308623	1308805	+
STM1273	12	9	1.41	ST4-74	1309035	1309676	+
RyhB-2	118	51	2.31	ST4-74	1309727	1309937	-
SL1344_1209	358	219	1.64	ST4-74	1309798	1309983	+
yeaQ	421	268	1.57	ST4-74	1309991	1310239	+
yaoF	135	82	1.65	ST4-74	1310672	1310923	+
STM1276	130	113	1.15	ST4-74	1310992	1311303	+
yeaO	40	38	1.05	ST4-74	1311289	1311636	-
yeaN	5	4	1.15	ST4-74	1311755	1312939	-
yeaM	18	15	1.14	ST4-74	1313040	1313834	+
yeaL	11	12	0.90	ST4-74	1313814	1314260	-
yeaK	14	13	1.05	ST4-74	1314570	1315088	-
yeaJ	28	23	1.25	ST4-74	1315138	1316631	-
STnc1480	833	328	2.54	ST4-74	1316874	1317268	+
yeaH	60	41	1.44	ST4-74	1317272	1318558	-
yeaG	61	33	1.85	ST4-74	1318681	1320465	-
mipA	179	174	1.03	ST4-74	1321042	1321788	+
STM1287	5	4	1.12	ST4-74	1322078	1323274	+
STM1288	16	16	0.99	ST4-74	1323371	1324228	+
yeaD	10	13	0.73	ST4-74	1324327	1325211	-
SL1344_1224A	10	18	0.54	ST4-74	1325211	1325465	-
gapA	223	404	0.55	ST4-74	1325546	1326541	-
yeaA	142	145	0.98	ST4-74	1326853	1327296	+
yeaC	133	153	0.87	ST4-74	1327338	1327616	+
nam	11	11	1.05	ST4-74	1327968	1328624	-

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ansA	14	33	0.43	ST4-74	1328651	1329667	-
sppA	22	21	1.02	ST4-74	1329763	1331619	-
ydjA	31	36	0.87	ST4-74	1331793	1332344	+
selD	14	16	0.91	ST4-74	1332462	1333505	+
topB	9	12	0.81	ST4-74	1333510	1335459	+
gdhA	26	62	0.41	ST4-74	1335489	1336832	-
STM1300	122	370	0.33	ST4-74	1337071	1337346	+
STM1301	4	3	1.40	ST4-74	1337306	1337722	-
xthA	20	24	0.84	ST4-74	1337795	1338601	-
argDa	2	2	0.94	ST4-74	1339047	1340273	+
astA	2	2	0.95	ST4-74	1340264	1341298	+
astD	3	2	1.54	ST4-74	1341295	1342773	+
astB	5	3	1.37	ST4-74	1342770	1344113	+
astE	4	3	1.25	ST4-74	1344106	1345074	+
STM1308	42	47	0.89	ST4-74	1345410	1345895	+
STnc1640	358	527	0.68	ST4-74	1345883	1345970	+
STM1309	22	18	1.22	ST4-74	1345970	1346851	-
nadE	43	38	1.12	ST4-74	1346972	1347799	-
osmE	1377	979	1.41	ST4-74	1348018	1348359	+
celA	31	32	0.99	ST4-74	1348660	1348980	+
celB	17	14	1.29	ST4-74	1349063	1350421	+
celC	19	21	0.92	ST4-74	1350472	1350819	+
celD	10	10	0.97	ST4-74	1350908	1351672	+
celF	8	9	0.94	ST4-74	1351797	1353152	+
celG	17	17	1.03	ST4-74	1353165	1353923	+
STnc2010	62	45	1.40	ST4-74	1353832	1353959	+
katE	22	18	1.23	ST4-74	1353967	1356219	-
cedA	56	47	1.20	ST4-74	1356584	1356826	+
ydjN	170	170	1.00	ST4-74	1356928	1358319	-
ydjM	3	2	1.13	ST4-74	1358456	1359055	-
STnc3570	158	130	1.21	ST4-74	1359017	1359183	-
yniC	35	30	1.17	ST4-74	1359193	1359861	-
yniB	77	57	1.34	ST4-74	1360010	1360546	+
STM1324	39	35	1.12	ST4-74	1360589	1361449	-
ydiZ	28	24	1.18	ST4-74	1361556	1361846	-
pfkB	16	20	0.84	ST4-74	1361943	1362875	-
ydiY	37	24	1.54	ST4-74	1363164	1363922	+
STnc3460	236	158	1.50	ST4-74	1363846	1363967	+
STnc4210	175	106	1.65	ST4-74	1363925	1363997	-
STM1328	26	22	1.19	ST4-74	1363971	1364930	-
STM1329	120	58	2.09	ST4-74	1365073	1365387	+
STM1330	80	48	1.67	ST4-74	1365483	1366238	+
STM1331	57	50	1.14	ST4-74	1366411	1366617	+
rfc	41	41	1.00	ST4-74	1366940	1368163	-
thrS	163	176	0.92	ST4-74	1369055	1370983	+
infC	2776	3225	0.86	ST4-74	1370987	1371529	+
rpl35	2531	3010	0.84	ST4-74	1371625	1371822	+
rpl20	1423	1708	0.83	ST4-74	1371873	1372229	+
pheS	26	33	0.81	ST4-74	1372531	1373514	+
pheT	158	131	1.20	ST4-74	1373530	1375917	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
himA	716	577	1.24	ST4-74	1375910	1376221	+
STnc540	178	180	0.99	ST4-74	1376271	1376364	+
btuC	15	15	1.02	ST4-74	1376424	1377404	+
btuE	35	26	1.35	ST4-74	1377496	1378047	+
btuD	10	9	1.00	ST4-74	1378047	1378796	+
nlpC	102	64	1.58	ST4-74	1378873	1379337	+
ydiV	40	21	1.89	ST4-74	1379651	1380364	+
ydiU	22	16	1.35	ST4-74	1380426	1381868	+
ydiE	98	46	2.16	ST4-74	1381903	1382094	-
aroH	25	25	1.00	ST4-74	1382251	1383297	-
ydiA	20	16	1.26	ST4-74	1383453	1384286	-
pps	84	85	1.00	ST4-74	1384581	1387001	+
ydiD	7	7	1.05	ST4-74	1387043	1388683	-
ydiT	2	2	0.87	ST4-74	1388773	1389066	-
ydiS	3	3	0.96	ST4-74	1389063	1390349	-
ydiR	47	31	1.51	ST4-74	1390406	1391341	-
ydiQ	1	1	0.95	ST4-74	1391363	1392127	-
ydiP	2	2	1.26	ST4-74	1392590	1393480	+
ydiO	22	21	1.02	ST4-74	1393556	1394707	-
ydiF	2	2	1.18	ST4-74	1394721	1396316	-
aroD	11	14	0.81	ST4-74	1396470	1397228	-
aroE	10	8	1.22	ST4-74	1397273	1398139	-
ydiN	1	2	0.79	ST4-74	1398221	1399440	-
ydiM	5	3	1.71	ST4-74	1399804	1401015	-
ydiL	8	10	0.80	ST4-74	1401156	1401515	-
RprA	2345	909	2.58	ST4-74	1401682	1401790	-
ydiK	27	23	1.14	ST4-74	1401973	1403091	-
ydiJ	15	20	0.77	ST4-74	1403356	1406412	+
STM1366	18	26	0.68	ST4-74	1406409	1406819	+
ydiH	351	1003	0.35	ST4-74	1406918	1407106	+
RydB	490	769	0.64	ST4-74	1407278	1407371	+
STM1368	14	17	0.82	ST4-74	1407394	1408740	-
sufA	20	10	1.96	ST4-74	1409167	1409535	+
sufB	11	5	2.12	ST4-74	1409544	1411031	+
sufC	11	7	1.57	ST4-74	1411048	1411794	+
sufD	12	7	1.61	ST4-74	1411769	1413040	+
sufS	10	7	1.42	ST4-74	1413037	1414257	+
ynhA	13	9	1.43	ST4-74	1414270	1414686	+
ynhG	35	26	1.37	ST4-74	1414841	1415842	+
lppB	64	51	1.26	ST4-74	1415909	1416148	-
lpp	27604	27516	1.00	ST4-74	1416231	1416467	-
pykF	39	41	0.96	ST4-74	1416778	1418136	-
orf48	5	7	0.81	ST4-74	1418592	1419935	-
orf32	3	4	0.76	ST4-74	1419957	1420850	-
orf245	10	12	0.84	ST4-74	1420909	1421646	-
orf408	10	10	1.05	ST4-74	1421658	1422884	-
ttrA	2	2	1.11	ST4-74	1423207	1426269	-
ttrC	2	2	0.99	ST4-74	1426262	1427284	-
ttrB	1	2	0.69	ST4-74	1427285	1428037	-
ttrS	20	25	0.79	ST4-74	1428201	1429979	+
ttrR	13	15	0.88	ST4-74	1429936	1430574	+

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orf70	133	116	1.15	ST4-74	1430672	1430884	+
orf319	57	45	1.26	ST4-74	1430980	1431939	-
orf242	23	22	1.05	ST4-74	1432112	1432840	-
ssrB	86	44	1.94	ST4-74	1433019	1433657	-
spiR	52	23	2.22	ST4-74	1433688	1436450	-
ssaB	99	11	8.79	ST4-74	1436851	1437252	+
ssaC	37	7	5.61	ST4-74	1437254	1438747	+
ssaD	34	9	3.75	ST4-74	1438728	1439939	+
ssaE	867	84	10.32	ST4-74	1439947	1440189	+
sseA	405	43	9.40	ST4-74	1440510	1440779	+
sseBa	348	50	7.00	ST4-74	1440786	1441376	+
sscA	129	21	6.15	ST4-74	1441373	1441846	+
sseC	211	34	6.13	ST4-74	1441849	1443303	+
sseD	74	14	5.23	ST4-74	1443319	1443906	+
sseE	50	11	4.39	ST4-74	1443909	1444325	+
sscB	75	18	4.20	ST4-74	1444377	1444811	+
sseF	20	7	2.95	ST4-74	1444827	1445609	+
sseG	20	12	1.74	ST4-74	1445606	1446295	+
ssaG	816	93	8.78	ST4-74	1446389	1446604	+
ssaH	500	52	9.57	ST4-74	1446645	1446872	+
ssaI	487	50	9.84	ST4-74	1446884	1447132	+
ssaJ	377	44	8.55	ST4-74	1447129	1447878	+
STM1410	156	29	5.41	ST4-74	1447896	1448444	+
ssaK	112	17	6.61	ST4-74	1448441	1449115	+
STnc1220	50	18	2.75	ST4-74	1448718	1448790	-
ssaL	93	22	4.20	ST4-74	1449081	1450097	+
ssaM	192	26	7.24	ST4-74	1450155	1450523	+
ssaV	54	12	4.33	ST4-74	1450508	1452553	+
ssaN	45	11	4.09	ST4-74	1452543	1453844	+
ssaO	35	12	3.01	ST4-74	1453847	1454224	+
ssaP	46	17	2.69	ST4-74	1454205	1454579	+
ssaQ	36	17	2.09	ST4-74	1454560	1455528	+
ssaR	224	33	6.83	ST4-74	1455596	1456243	+
ssaS	58	10	5.96	ST4-74	1456240	1456506	+
ssaT	67	11	5.84	ST4-74	1456507	1457286	+
ssaU	32	11	2.97	ST4-74	1457283	1458341	+
norM	28	22	1.24	ST4-74	1458814	1460187	-
ribE	64	59	1.09	ST4-74	1460404	1461045	+
cfa	79	60	1.31	ST4-74	1461087	1462235	-
ydhC	6	5	1.15	ST4-74	1462525	1463730	-
ydhB	9	9	1.12	ST4-74	1463843	1464775	+
purR	28	37	0.78	ST4-74	1464772	1465797	-
STnc4220	279	315	0.89	ST4-74	1464776	1464870	-
ynhF	6877	5047	1.36	ST4-74	1466093	1466182	+
sodB	384	547	0.70	ST4-74	1466265	1466846	-
ydhO	51	39	1.31	ST4-74	1466973	1467827	-
ydhD	479	462	1.04	ST4-74	1468129	1468476	+
rnt	51	48	1.06	ST4-74	1468554	1469201	-
gloA	107	118	0.91	ST4-74	1469302	1469709	-
nemA	5	18	0.26	ST4-74	1469778	1470875	-
ydhM	15	36	0.41	ST4-74	1470934	1471533	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
ydhL	94	49	1.92	ST4-74	1471636	1471875	+
ydhF	22	24	0.91	ST4-74	1471926	1472822	+
sodCb	68	58	1.18	ST4-74	1472902	1473423	+
STM1441	23	25	0.89	ST4-74	1473399	1475438	-
ydhJ	9	10	0.89	ST4-74	1475438	1476334	-
ydhI	40	33	1.21	ST4-74	1476301	1476537	-
slyA	304	249	1.22	ST4-74	1476732	1477172	+
slyB	646	611	1.06	ST4-74	1477220	1477687	-
anmK	10	11	0.99	ST4-74	1477959	1479080	+
mliC	48	47	1.03	ST4-74	1479168	1479497	+
pdxH	33	40	0.82	ST4-74	1479556	1480212	+
tyrS	85	98	0.87	ST4-74	1480339	1481613	+
pdxY	9	15	0.64	ST4-74	1481673	1482533	+
gst	56	67	0.84	ST4-74	1482592	1483197	-
tppB	38	20	1.93	ST4-74	1483302	1484807	-
nth	21	18	1.15	ST4-74	1485409	1486044	-
rnfE	17	23	0.73	ST4-74	1486044	1486736	-
rnfG	17	16	1.09	ST4-74	1486739	1487359	-
rnfD	13	15	0.91	ST4-74	1487363	1488421	-
rnfC	12	15	0.80	ST4-74	1488422	1490629	-
rnfB	20	26	0.75	ST4-74	1490622	1491200	-
rnfA	50	47	1.08	ST4-74	1491200	1491781	-
ydgK	132	127	1.04	ST4-74	1491858	1492298	-
ydgT	185	148	1.25	ST4-74	1492387	1492617	-
blr	277	229	1.21	ST4-74	1492900	1493025	-
ydgJ	29	28	1.04	ST4-74	1493233	1494273	+
add	26	28	0.93	ST4-74	1494348	1495349	-
STnc3500	47	49	0.96	ST4-74	1495398	1495512	+
ydgA	41	37	1.13	ST4-74	1496053	1497561	-
manA	21	23	0.90	ST4-74	1497664	1498839	-
fumA	25	31	0.81	ST4-74	1499039	1500685	+
fumC	26	28	0.92	ST4-74	1500853	1502256	+
tus	8	8	1.07	ST4-74	1502253	1503182	-
rstB	17	15	1.11	ST4-74	1503258	1504559	-
STM1472	3	2	1.10	ST4-74	1504670	1506376	-
STnc3510	31	20	1.50	ST4-74	1506482	1506596	-
ompN	6	4	1.32	ST4-74	1506529	1507662	-
rstA	355	292	1.22	ST4-74	1508142	1508873	-
ydgC	17	18	0.95	ST4-74	1509000	1509335	+
ydgI	16	10	1.55	ST4-74	1509397	1510779	-
ydgH	53	62	0.85	ST4-74	1511078	1512022	-
pntA	31	57	0.55	ST4-74	1512549	1514078	+
pntB	42	77	0.54	ST4-74	1514089	1515477	+
tqsA	14	17	0.81	ST4-74	1515652	1516686	-
ydgF	19	15	1.28	ST4-74	1517104	1517466	+
ydgE	4	5	0.98	ST4-74	1517453	1517782	+
STM1484	15	12	1.29	ST4-74	1517822	1518643	-
ydgU	16	4	3.52	ST4-74	1518743	1518826	-
asr	119	41	2.87	ST4-74	1518912	1519196	-
STnc3480	18	21	0.86	ST4-74	1519287	1519547	+
ynfM	21	18	1.18	ST4-74	1519555	1520808	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
ynfL	3	4	0.83	ST4-74	1520928	1521827	+
mlc	36	38	0.93	ST4-74	1521955	1523175	+
bioDb	29	199	0.14	ST4-74	1523301	1523996	+
clcB	13	10	1.26	ST4-74	1523952	1525241	-
STM1491	18	8	2.31	ST4-74	1525377	1526525	-
STM1492	17	11	1.52	ST4-74	1526525	1527172	-
STM1493	11	4	2.90	ST4-74	1527182	1528084	-
STM1494	21	7	2.81	ST4-74	1528113	1528823	-
ynfI	26	37	0.70	ST4-74	1529128	1529742	-
STM1496	16	24	0.66	ST4-74	1529785	1530642	-
STM1497	2	14	0.12	ST4-74	1530644	1531261	-
dmsA2	2	8	0.22	ST4-74	1531272	1533707	-
dmsA1	3	21	0.13	ST4-74	1533806	1536247	-
ynfD	67	65	1.03	ST4-74	1536401	1536709	-
ynfC	33	34	0.99	ST4-74	1536804	1537523	+
speG	46	57	0.81	ST4-74	1537564	1538124	-
ynfB	82	84	0.98	ST4-74	1538160	1538501	-
ynfA	16	14	1.11	ST4-74	1538654	1538980	+
rspA	2	3	0.87	ST4-74	1539102	1540316	+
rspB	2	3	0.87	ST4-74	1540327	1541346	+
ydfJ	5	5	0.84	ST4-74	1541400	1542779	+
ydfI	9	10	0.94	ST4-74	1542923	1544389	+
ydfZ	19	386	0.05	ST4-74	1544456	1544659	-
ydfH	37	33	1.11	ST4-74	1544840	1545526	-
ydfG	108	103	1.05	ST4-74	1545656	1546402	-
dcp	11	12	0.89	ST4-74	1546541	1548583	+
STM1513	818	279	2.93	ST4-74	1548928	1549110	+
ydeJ	15	9	1.78	ST4-74	1549230	1549757	-
ydeI	46	29	1.62	ST4-74	1550184	1550576	+
MgrR	10450	8584	1.22	ST4-74	1550746	1550844	+
ydeE	12	11	1.12	ST4-74	1551267	1552454	-
ydeD	15	14	1.08	ST4-74	1552682	1553584	+
STnc2170	24	16	1.48	ST4-74	1553669	1553737	-
marB	8	8	0.99	ST4-74	1553703	1553918	-
marA	6	6	0.93	ST4-74	1553947	1554336	-
marR	6	5	1.09	ST4-74	1554350	1554784	-
marC	78	49	1.60	ST4-74	1555043	1555708	+
sotB	20	16	1.24	ST4-74	1555755	1556945	-
yneJ	5	6	0.78	ST4-74	1557061	1557933	-
yneI	3	4	0.89	ST4-74	1558036	1559424	+
yneH	10	10	1.02	ST4-74	1559497	1560423	+
yneG	17	16	1.07	ST4-74	1560423	1560782	+
STM1527	29	19	1.53	ST4-74	1561039	1561953	+
STM1528	7	5	1.27	ST4-74	1562009	1562551	-
STM1530	14	10	1.48	ST4-74	1563370	1564386	+
STnc3530	132	80	1.64	ST4-74	1564332	1564503	+
hypA	4	4	0.81	ST4-74	1564527	1564868	-
STM1532	4	4	1.07	ST4-74	1564881	1565768	-
hyaF2	10	9	1.14	ST4-74	1565756	1566817	-
hyaE2	4	7	0.66	ST4-74	1566835	1567245	-
STM1535	6	5	1.25	ST4-74	1567242	1567541	-

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hyaD2	11	12	0.97	ST4-74	1567541	1568149	-
hyaC2	14	13	1.11	ST4-74	1568155	1568898	-
hyaB2	8	9	0.98	ST4-74	1568849	1570651	-
hyaA2	14	13	1.05	ST4-74	1570654	1571757	-
STM1540	17	14	1.21	ST4-74	1572186	1573277	+
STM1541	60	62	0.98	ST4-74	1573443	1574114	+
STM1542	6	4	1.45	ST4-74	1574173	1575198	-
STM1543	8	7	1.21	ST4-74	1575173	1576459	-
pqaA	31	20	1.55	ST4-74	1576636	1578192	-
STM1545	8	6	1.46	ST4-74	1578262	1579503	-
STM1546	2	1	1.33	ST4-74	1579493	1581001	-
STM1547	25	24	1.01	ST4-74	1581046	1581534	+
STM1548	68	34	1.98	ST4-74	1581727	1582806	+
STM1549	18	23	0.77	ST4-74	1582858	1583247	-
STM1550	37	44	0.84	ST4-74	1583418	1583702	-
STM1551	167	142	1.18	ST4-74	1583692	1583940	-
SL1344_1481	11	11	1.03	ST4-74	1584297	1584629	+
STM1552	15	13	1.21	ST4-74	1584772	1585680	+
STM1553	2	2	1.12	ST4-74	1585908	1586495	-
IsrF	21	22	0.98	ST4-74	1586724	1587013	-
STM1554	23	27	0.86	ST4-74	1587258	1588724	-
STM1555	9	9	1.01	ST4-74	1588983	1589990	+
STM1556	7	5	1.21	ST4-74	1590134	1591585	+
STM1557	5	5	1.00	ST4-74	1591598	1592800	+
STM1558	24	15	1.57	ST4-74	1592913	1594988	-
STM1559	10	6	1.63	ST4-74	1595045	1597573	-
STM1560	11	6	1.70	ST4-74	1597570	1599354	-
STM1561	14	8	1.82	ST4-74	1599483	1600268	-
hdeB	33	26	1.27	ST4-74	1600411	1600740	-
osmC	184	117	1.57	ST4-74	1601090	1601521	-
yddX	153	65	2.36	ST4-74	1602034	1602249	+
rpsV	1662	1406	1.18	ST4-74	1602344	1602487	+
sfcA	24	25	0.97	ST4-74	1602664	1604361	+
adhP	26	13	2.07	ST4-74	1604728	1605657	+
STnc3540	161	142	1.14	ST4-74	1605727	1605912	-
fdnI	16	12	1.27	ST4-74	1605748	1606389	-
fdnH	4	7	0.56	ST4-74	1606397	1607281	-
fdnG	4	8	0.51	ST4-74	1607295	1610342	-
yddG	9	11	0.77	ST4-74	1610677	1611501	+
ompD	825	1868	0.44	ST4-74	1611987	1613114	+
STM1573	15	22	0.69	ST4-74	1613198	1613638	+
smvA	8	8	0.92	ST4-74	1613663	1615150	-
STM1575	63	70	0.90	ST4-74	1615267	1615845	+
narU	8	4	1.90	ST4-74	1616060	1617448	+
narZ	4	3	1.24	ST4-74	1617540	1621280	+
narY	3	3	0.89	ST4-74	1621277	1622821	+
narW	4	4	1.08	ST4-74	1622821	1623516	+
narV	13	10	1.30	ST4-74	1623513	1624193	+
yddE	13	12	1.06	ST4-74	1624299	1625192	+
nhoA	12	10	1.17	ST4-74	1625228	1626073	-
steA	326	111	2.93	ST4-74	1626357	1626989	-

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ansP	13	14	0.96	ST4-74	1627502	1628995	+
STM1585	9	7	1.30	ST4-74	1629072	1629293	-
STM1586	25	16	1.50	ST4-74	1629438	1630499	-
yncD	7	6	1.27	ST4-74	1630763	1632883	+
yncC	12	5	2.53	ST4-74	1632950	1633615	-
yncB	78	47	1.67	ST4-74	1633949	1634986	-
yncA	20	28	0.71	ST4-74	1635198	1635713	+
ydcZ	27	35	0.78	ST4-74	1635713	1636162	+
ydcY	113	111	1.02	ST4-74	1636167	1636400	-
srfA	13	38	0.34	ST4-74	1636624	1637943	+
srfB	4	13	0.34	ST4-74	1637948	1640929	+
srfC	6	15	0.43	ST4-74	1640926	1643070	+
ydcX	38	36	1.07	ST4-74	1643115	1643378	-
STnc1110	744	648	1.15	ST4-74	1643465	1643658	+
ydcW	38	23	1.62	ST4-74	1643687	1645132	-
ydcR	29	20	1.46	ST4-74	1645247	1646671	-
pdgL	70	41	1.71	ST4-74	1646807	1647577	-
STM1600	486	115	4.23	ST4-74	1647836	1648066	-
ugtL	1149	231	4.97	ST4-74	1648099	1648497	-
sifB	268	36	7.54	ST4-74	1649034	1649984	+
yncJ	148	52	2.85	ST4-74	1650258	1650488	+
ydcP	16	14	1.10	ST4-74	1650489	1652453	-
ydcN	19	15	1.27	ST4-74	1652532	1653068	-
STM1606	11	13	0.81	ST4-74	1653159	1654337	+
STM1607	59	69	0.86	ST4-74	1654391	1655059	-
tehB	32	37	0.87	ST4-74	1655215	1655811	-
tehA	29	33	0.86	ST4-74	1655798	1656811	-
ydcK	14	11	1.31	ST4-74	1656918	1657898	+
rimL	17	16	1.04	ST4-74	1657895	1658434	-
STM1612	5	7	0.72	ST4-74	1658651	1659769	+
STM1613	2	5	0.34	ST4-74	1659766	1660047	+
STM1614	5	6	0.96	ST4-74	1660058	1661371	+
STM1615	2	2	0.99	ST4-74	1661385	1662191	+
RyjB	2386	3233	0.74	ST4-74	1662190	1662282	-
STM1616	7	9	0.74	ST4-74	1662323	1662760	+
STM1617	2	4	0.51	ST4-74	1662772	1663404	+
STM1618	9	8	1.13	ST4-74	1663420	1664211	+
aac	25	21	1.18	ST4-74	1664211	1664648	+
STM1620	4	3	1.13	ST4-74	1664695	1665897	-
STM1621	4	4	1.17	ST4-74	1665909	1666484	-
ydcG	15	15	1.03	ST4-74	1666680	1668335	-
STM1623	20	15	1.32	ST4-74	1668555	1670063	-
STM1624	42	27	1.57	ST4-74	1670112	1671455	-
ydcI	33	25	1.34	ST4-74	1671748	1672671	+
trg	14	84	0.17	ST4-74	1672732	1674357	-
adhC	17	22	0.76	ST4-74	1674526	1675644	-
STM1628	21	32	0.65	ST4-74	1675676	1675951	-
steB	45	24	1.92	ST4-74	1676474	1676875	+
STM1630	18	4	4.20	ST4-74	1677004	1677720	-
sseJ	83	8	10.48	ST4-74	1678146	1679372	+
STM1632	17	9	1.97	ST4-74	1679493	1679837	-

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STM1633	88	34	2.57	ST4-74	1680744	1681505	+
STM1634	25	14	1.76	ST4-74	1681512	1682159	+
STM1635	7	3	2.15	ST4-74	1682184	1682915	+
STM1636	8	5	1.53	ST4-74	1682912	1683592	+
STM1637	30	19	1.59	ST4-74	1683729	1685279	+
STM1638	135	155	0.87	ST4-74	1685743	1686405	+
RydC	5516	4812	1.15	ST4-74	1686526	1686591	+
cybB	53	52	1.02	ST4-74	1686676	1687206	-
ydcF	12	16	0.76	ST4-74	1687323	1688123	-
hrpA	11	12	0.95	ST4-74	1688187	1692089	-
acpD	10	8	1.27	ST4-74	1692290	1692895	+
STM1643	43	32	1.34	ST4-74	1692892	1693341	-
ydbL	14	12	1.15	ST4-74	1693484	1693807	-
ynbE	19	17	1.14	ST4-74	1693815	1694006	-
ydbH	18	15	1.21	ST4-74	1694006	1696642	-
ldhA	38	31	1.24	ST4-74	1696882	1697871	+
hslJ	17	11	1.58	ST4-74	1697982	1698392	+
STM1649	104	114	0.91	ST4-74	1698436	1698591	-
STM1650	36	16	2.22	ST4-74	1698618	1698917	+
nifJ	10	7	1.44	ST4-74	1698978	1702502	+
MicC	119	111	1.07	ST4-74	1702531	1702639	-
ynaF	418	421	0.99	ST4-74	1702984	1703418	+
STM1653	238	183	1.30	ST4-74	1703468	1703806	-
SL1344_1584	43	35	1.23	ST4-74	1703907	1704110	+
ydaO	20	17	1.14	ST4-74	1704162	1705097	+
dbpA	16	13	1.20	ST4-74	1705141	1706514	-
FnrS	130	4527	0.03	ST4-74	1706784	1706905	-
zntB	33	24	1.35	ST4-74	1707001	1707984	-
STM1657	17	44	0.39	ST4-74	1708130	1709284	-
ydaL	27	35	0.78	ST4-74	1709695	1710258	-
ogt	79	59	1.34	ST4-74	1710516	1711031	+
fnr	1	245	0.00	ST4-74	1711227	1711979	+
ydaA	80	173	0.46	ST4-74	1712130	1713077	+
ynaJ	382	356	1.07	ST4-74	1713132	1713386	-
ynaI	20	20	1.01	ST4-74	1713625	1714656	+
STM1664	20	21	0.94	ST4-74	1714742	1715344	-
STM1665	5	5	0.97	ST4-74	1715388	1716074	+
STM1667	3	2	1.16	ST4-74	1716546	1717103	-
STM1668	4	3	1.50	ST4-74	1717202	1718032	-
STM1669	2	2	1.13	ST4-74	1718053	1720035	-
STM1670	2	2	1.37	ST4-74	1720110	1720955	-
STM1671	4	3	1.48	ST4-74	1721249	1722107	+
STM1672	444	414	1.07	ST4-74	1722495	1723607	+
STM1673	17	17	1.03	ST4-74	1723821	1724075	-
STM1674	7	7	0.91	ST4-74	1724186	1724776	-
STM1675	8	10	0.85	ST4-74	1724854	1725567	+
STM1676	47	39	1.18	ST4-74	1725658	1726527	-
STM1677	9	8	1.05	ST4-74	1726801	1727706	-
STM1678	3	2	1.40	ST4-74	1727825	1728754	+
mppA	32	43	0.74	ST4-74	1728851	1730464	-
ycjI	16	19	0.83	ST4-74	1730654	1731382	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
ycjG	29	21	1.40	ST4-74	1731357	1732322	-
tpx	198	212	0.93	ST4-74	1732438	1732944	+
tyrR	27	24	1.12	ST4-74	1733034	1734575	-
ycjF	11	12	0.90	ST4-74	1734723	1735784	-
ycjX	10	12	0.87	ST4-74	1735781	1737184	-
pspE	179	155	1.15	ST4-74	1737327	1737641	-
pspD	17	17	0.98	ST4-74	1737717	1737935	-
pspC	17	13	1.30	ST4-74	1737958	1738317	-
pspB	26	26	0.99	ST4-74	1738317	1738541	-
pspA	33	38	0.86	ST4-74	1738601	1739269	-
pspF	7	7	0.99	ST4-74	1739440	1740420	+
sapA	23	20	1.11	ST4-74	1740532	1742181	+
sapB	12	15	0.85	ST4-74	1742349	1743143	+
sapC	15	16	0.90	ST4-74	1743130	1744020	+
sapD	12	12	1.00	ST4-74	1744020	1745012	+
sapF	8	9	0.92	ST4-74	1745014	1745820	+
STM1697	48	28	1.70	ST4-74	1745836	1746543	-
steC	88	19	4.71	ST4-74	1747010	1748383	-
STM1698A	64	5	13.29	ST4-74	1748418	1748597	+
ycjE	59	31	1.89	ST4-74	1748572	1748871	+
fabI	146	142	1.03	ST4-74	1748989	1749777	+
yciW	50	49	1.00	ST4-74	1749977	1751110	+
rnb	44	42	1.05	ST4-74	1751199	1753133	+
yciR	13	12	1.10	ST4-74	1753395	1755377	+
SL1344_1635	83	90	0.93	ST4-74	1755466	1755678	+
yciT	21	24	0.87	ST4-74	1755765	1756517	+
osmB	75	58	1.30	ST4-74	1756776	1756994	+
yciH	15	13	1.14	ST4-74	1757114	1757440	-
pyrF	21	19	1.11	ST4-74	1757440	1758177	-
yciM	57	52	1.10	ST4-74	1758368	1759483	-
yciS	179	141	1.27	ST4-74	1759544	1759852	-
pgpB	39	28	1.38	ST4-74	1760002	1760766	-
ribA	42	40	1.05	ST4-74	1760962	1761552	+
acnA	18	14	1.31	ST4-74	1761608	1764283	-
ymiA	730	768	0.95	ST4-74	1764839	1764967	-
cysB	36	38	0.94	ST4-74	1765260	1766234	-
topA	31	34	0.91	ST4-74	1766645	1769242	-
yciN	257	309	0.83	ST4-74	1769645	1769896	+
sohB	54	51	1.07	ST4-74	1769938	1770984	-
yciK	40	58	0.68	ST4-74	1771222	1771983	+
btuR	25	29	0.87	ST4-74	1771980	1772570	+
yciL	31	30	1.03	ST4-74	1772657	1773532	-
yciO	17	20	0.89	ST4-74	1773633	1774253	-
trpH	30	26	1.16	ST4-74	1774261	1775142	-
trpE	12	18	0.65	ST4-74	1775403	1776965	+
trpD	14	18	0.75	ST4-74	1776965	1778560	+
trpC	19	22	0.86	ST4-74	1778564	1779922	+
trpB	17	23	0.76	ST4-74	1779932	1781125	+
trpA	17	20	0.85	ST4-74	1781125	1781931	+
yciG	1187	274	4.34	ST4-74	1782411	1782593	+
yciF	73	21	3.53	ST4-74	1782683	1783186	+

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yciE	44	11	3.94	ST4-74	1783260	1783766	+
katN	37	16	2.37	ST4-74	1783786	1784664	+
STnc2030	47	5	8.65	ST4-74	1784673	1784725	+
ompW	28	612	0.05	ST4-74	1784746	1785384	-
STM1733	55	59	0.94	ST4-74	1785722	1786126	+
yciC	110	84	1.31	ST4-74	1786152	1786895	+
ispZ	67	62	1.08	ST4-74	1786952	1787491	+
yciA	59	51	1.17	ST4-74	1787652	1788053	+
tonB	32	19	1.69	ST4-74	1788113	1788841	-
yciI	115	160	0.72	ST4-74	1789065	1789361	+
STnc1270	568	481	1.18	ST4-74	1789368	1789691	-
cls	14	15	0.91	ST4-74	1789721	1791181	+
yciU	106	110	0.97	ST4-74	1791215	1791544	+
STM1741	18	15	1.17	ST4-74	1791592	1792374	-
oppF	32	42	0.75	ST4-74	1792476	1793480	-
oppD	13	20	0.63	ST4-74	1793477	1794484	-
oppC	17	25	0.68	ST4-74	1794496	1795404	-
oppB	15	26	0.57	ST4-74	1795419	1796339	-
oppA	50	70	0.72	ST4-74	1796461	1798092	-
STM1747	11	4	2.56	ST4-74	1798160	1798474	+
STnc3580	33	32	1.04	ST4-74	1798747	1798826	+
ychE	17	16	1.07	ST4-74	1798857	1799504	-
adh	65	74	0.88	ST4-74	1799981	1802659	+
tdk	50	51	0.96	ST4-74	1802856	1803473	-
hns	4183	4711	0.89	ST4-74	1804136	1804549	+
galU	71	57	1.26	ST4-74	1804681	1805589	-
hnr	34	28	1.21	ST4-74	1805792	1806805	-
ychK	60	48	1.23	ST4-74	1806896	1807801	-
ychJ	42	47	0.88	ST4-74	1807912	1808370	+
purU	36	38	0.94	ST4-74	1808421	1809263	+
STM1760	18	14	1.29	ST4-74	1810579	1812054	-
STnc3600	43	46	0.93	ST4-74	1812121	1812328	+
narI	7	7	1.09	ST4-74	1812350	1813027	-
narJ	1	3	0.41	ST4-74	1813027	1813737	-
narH	2	2	0.70	ST4-74	1813734	1815269	-
narG	2	2	0.64	ST4-74	1815266	1819009	-
STnc2040	2	3	0.65	ST4-74	1819352	1819414	-
narK	3	3	0.85	ST4-74	1819397	1820794	-
narX	13	11	1.10	ST4-74	1821135	1822931	+
narL	11	10	1.04	ST4-74	1822924	1823574	+
ychP	28	18	1.54	ST4-74	1823575	1824999	-
ychN	88	93	0.95	ST4-74	1825176	1825529	+
chaB	443	279	1.59	ST4-74	1825609	1825839	-
chaA	61	58	1.06	ST4-74	1826106	1827206	+
kdsA	33	48	0.69	ST4-74	1827260	1828114	-
ychA	27	33	0.82	ST4-74	1828152	1828961	-
sirC	38	38	1.00	ST4-74	1828965	1829354	-
hemK	12	12	0.97	ST4-74	1829351	1830184	-
prfA	16	18	0.93	ST4-74	1830184	1831266	-
hemA	52	55	0.95	ST4-74	1831307	1832563	-
hemM	17	18	0.96	ST4-74	1832877	1833500	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
ipk	78	105	0.74	ST4-74	1833497	1834348	+
prs	67	95	0.70	ST4-74	1834614	1835561	+
ychM	6	8	0.76	ST4-74	1835710	1837371	+
ychH	245	166	1.48	ST4-74	1837416	1837694	-
pth	22	25	0.87	ST4-74	1837970	1838554	+
ychF	44	53	0.83	ST4-74	1838671	1839762	+
STM1785	18	20	0.90	ST4-74	1839921	1841186	-
hyaA	1	1	1.54	ST4-74	1841681	1842799	+
STM1787	2	2	1.14	ST4-74	1842796	1844589	+
hyaC	3	3	1.05	ST4-74	1844608	1845339	+
hyaD	1	1	1.63	ST4-74	1845336	1845932	+
hyaE	2	1	2.45	ST4-74	1845922	1846326	+
hyaF	1	1	0.75	ST4-74	1846323	1847171	+
appC	3	2	1.30	ST4-74	1847246	1848790	+
appB	5	3	1.83	ST4-74	1848802	1849938	+
STM1794	34	27	1.26	ST4-74	1849951	1850040	+
STnc3610	88	82	1.08	ST4-74	1850045	1850400	-
STM1795	9	6	1.59	ST4-74	1850384	1851709	+
treA	33	30	1.11	ST4-74	1851923	1853635	+
ymgE	158	87	1.80	ST4-74	1853698	1853952	-
ycgR	22	67	0.32	ST4-74	1854121	1854855	+
emtA	18	16	1.16	ST4-74	1854869	1855480	-
ldcA	20	15	1.31	ST4-74	1855651	1856565	+
cvrA	59	64	0.93	ST4-74	1856662	1858395	+
alr-a	7	5	1.26	ST4-74	1858458	1859528	-
dadA	11	8	1.32	ST4-74	1859542	1860840	-
ycgB	62	34	1.84	ST4-74	1861169	1862695	+
fadR	143	126	1.14	ST4-74	1862742	1863461	-
nhaB	23	25	0.93	ST4-74	1863681	1865225	+
dsbB	119	126	0.95	ST4-74	1865367	1865897	+
STM1808	3	2	1.15	ST4-74	1866006	1866347	+
STM1809	825	484	1.70	ST4-74	1866419	1866592	-
STnc3620	42	30	1.41	ST4-74	1866668	1866747	+
STM1810	141	90	1.57	ST4-74	1866784	1866921	-
ycgN	82	62	1.32	ST4-74	1867273	1867734	-
ycgM	24	30	0.79	ST4-74	1867812	1868471	-
ycgL	124	94	1.32	ST4-74	1868521	1868853	-
minC	102	114	0.90	ST4-74	1868940	1869647	+
minD	124	131	0.95	ST4-74	1869671	1870483	+
minE	179	182	0.99	ST4-74	1870487	1870753	+
rnd	6	5	1.17	ST4-74	1870875	1872002	-
tnpA_1a	244	250	0.97	ST4-74	1872138	1872596	-
fadD	14	15	0.96	ST4-74	1872787	1874472	-
yeaY	29	27	1.05	ST4-74	1874677	1875258	-
yeaZ	36	34	1.05	ST4-74	1875330	1876025	-
yoaA	10	9	1.07	ST4-74	1876083	1877993	-
yoaB	343	365	0.94	ST4-74	1878124	1878468	+
yoaH	146	125	1.16	ST4-74	1878474	1878653	-
pabB	22	18	1.25	ST4-74	1878734	1880098	+
yeaB	9	8	1.18	ST4-74	1880102	1880680	+
sdaA	17	12	1.40	ST4-74	1880944	1882308	+

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STM1827	11	8	1.41	ST4-74	1882446	1884047	+
yoaE	9	8	1.15	ST4-74	1884069	1885628	-
STM1830	69	104	0.66	ST4-74	1886101	1887069	+
STM1831	47	104	0.45	ST4-74	1887122	1887922	+
STM1832	85	120	0.71	ST4-74	1887926	1888786	+
STM1833	11	10	1.14	ST4-74	1888844	1889302	+
yebN	30	32	0.93	ST4-74	1889712	1890278	+
rrmA	16	15	1.09	ST4-74	1890275	1891084	-
ftsI2	7	6	1.21	ST4-74	1891150	1892895	-
cspC	1851	1986	0.93	ST4-74	1893115	1893324	-
yobF	5281	5123	1.03	ST4-74	1893337	1893480	-
STnc3630	302	325	0.93	ST4-74	1894039	1894125	+
STM1839	502	323	1.56	ST4-74	1894129	1894416	-
yobG	4475	2172	2.06	ST4-74	1894487	1894630	-
STM1841	28	15	1.85	ST4-74	1894788	1895027	+
kdgR	96	87	1.11	ST4-74	1895239	1896030	-
STM1843	11	9	1.19	ST4-74	1896206	1897579	+
htpX	125	147	0.85	ST4-74	1897627	1898508	-
prc	60	59	1.02	ST4-74	1898702	1900750	-
proQ	102	117	0.87	ST4-74	1900770	1901456	-
yebR	162	123	1.32	ST4-74	1901554	1902138	-
yebS	58	35	1.65	ST4-74	1902180	1903463	+
STM1849	25	21	1.15	ST4-74	1903426	1906065	+
yebU	10	9	1.08	ST4-74	1906143	1907582	+
STM1851	443	276	1.61	ST4-74	1907697	1907936	+
yebW	126	64	1.98	ST4-74	1908047	1908238	+
pphA	20	10	2.03	ST4-74	1908257	1908907	-
STM1854	897	395	2.27	ST4-74	1909131	1909295	-
sopE2	32	34	0.93	ST4-74	1909580	1910302	-
STM1856	16	19	0.86	ST4-74	1910986	1911381	+
SL1344_1786	89	66	1.34	ST4-74	1911711	1912187	+
STM1857	92	55	1.66	ST4-74	1912575	1912994	-
STM1858	40	26	1.49	ST4-74	1913364	1913633	+
STnc1130	190	104	1.81	ST4-74	1913615	1913755	-
STM1859	93	85	1.09	ST4-74	1913799	1914048	+
STM1860	4	3	1.29	ST4-74	1914085	1915279	-
STM1861	3	3	1.06	ST4-74	1915403	1915771	+
STnc3640	45	27	1.68	ST4-74	1915824	1916082	+
SL1344_1792	7	7	1.05	ST4-74	1916257	1916457	-
pagO	130	49	2.68	ST4-74	1917075	1917989	+
STM1863	222	99	2.25	ST4-74	1918122	1918280	+
STM1864	92	36	2.53	ST4-74	1918290	1918904	+
STM1865	34	18	1.91	ST4-74	1919231	1919377	-
pagM	406	113	3.61	ST4-74	1919657	1919839	-
SL1344_1798	12	6	1.99	ST4-74	1920177	1920299	+
pagK	4535	1482	3.06	ST4-74	1920747	1920947	+
mig-3	3	3	1.04	ST4-74	1921044	1921925	-
SL1344_1801	7	6	1.29	ST4-74	1922080	1922343	-
STM1868A	66	51	1.28	ST4-74	1922651	1922818	+
STM1869	1	1	0.89	ST4-74	1923075	1923608	-
STM1869A	2	1	1.13	ST4-74	1923662	1923892	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM1870	3	4	0.77	ST4-74	1923923	1924576	+
STM1871	6	5	1.07	ST4-74	1924594	1925490	+
SraC	434	268	1.62	ST4-74	1925543	1925853	+
SdsR	11807	4767	2.48	ST4-74	1925621	1925723	-
STM1873	26	25	1.02	ST4-74	1925864	1926217	-
STM1874	29	32	0.91	ST4-74	1926234	1927109	-
yobA	48	55	0.86	ST4-74	1927110	1927490	-
holE	69	56	1.25	ST4-74	1927622	1927852	+
STM1877	61	51	1.20	ST4-74	1927960	1928616	+
exoX	82	75	1.09	ST4-74	1928640	1929338	+
opdB	25	22	1.15	ST4-74	1929373	1931424	-
STnc1690	678	966	0.70	ST4-74	1931455	1931546	+
yebE	64	93	0.69	ST4-74	1931637	1932296	-
yebF	198	123	1.62	ST4-74	1932390	1932743	-
yebG	74	73	1.02	ST4-74	1932811	1933101	-
purT	17	29	0.61	ST4-74	1933232	1934410	+
eda	15	20	0.74	ST4-74	1934510	1935151	-
edd	6	10	0.65	ST4-74	1935189	1937000	-
STnc200	23	22	1.04	ST4-74	1937130	1937244	-
zwf	29	32	0.90	ST4-74	1937235	1938710	-
hexR	27	66	0.42	ST4-74	1939052	1939921	+
pykA	29	71	0.40	ST4-74	1940045	1941487	+
msbB	39	31	1.26	ST4-74	1941560	1942531	-
yebA	40	49	0.82	ST4-74	1942648	1943967	-
znuA	85	98	0.86	ST4-74	1943983	1944984	-
znuC	24	18	1.32	ST4-74	1945006	1945761	+
znuB	13	14	0.90	ST4-74	1945758	1946543	+
ruvB	20	18	1.09	ST4-74	1946622	1947551	-
ruvA	41	40	1.01	ST4-74	1947641	1948252	-
STM1896	9	6	1.54	ST4-74	1948585	1949567	+
SL1344_1830A	3	3	1.07	ST4-74	1949628	1950061	-
yebB	8	7	1.20	ST4-74	1950194	1950793	+
ruvC	29	40	0.73	ST4-74	1950795	1951316	-
yebC	54	62	0.88	ST4-74	1951353	1952093	-
ntpA	87	90	0.96	ST4-74	1952123	1952575	-
aspS	49	50	1.00	ST4-74	1952678	1954450	-
yecD	26	21	1.26	ST4-74	1954775	1955341	+
yecE	6	6	0.99	ST4-74	1955338	1956156	+
yecN	101	86	1.17	ST4-74	1956209	1956604	+
yecO	40	34	1.18	ST4-74	1956645	1957388	+
yecP	11	15	0.76	ST4-74	1957385	1958356	+
RyeF	231	188	1.23	ST4-74	1958513	1958819	-
cutC	9	8	1.09	ST4-74	1958592	1959338	-
yecM	27	23	1.19	ST4-74	1959358	1959927	-
argS	27	25	1.06	ST4-74	1960164	1961897	+
STM1910	3	3	1.11	ST4-74	1962086	1963957	+
STM1911	7	9	0.86	ST4-74	1964011	1965150	-
flhE	10	12	0.82	ST4-74	1965387	1965779	-
flhAa	9	30	0.29	ST4-74	1965779	1967857	-
flhB	18	66	0.27	ST4-74	1967850	1969001	-
cheZ	17	71	0.24	ST4-74	1969195	1969839	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
cheY	31	117	0.27	ST4-74	1969850	1970239	-
cheB	13	69	0.19	ST4-74	1970257	1971306	-
cheR	17	72	0.23	ST4-74	1971303	1972169	-
cheM	49	204	0.24	ST4-74	1972323	1973984	-
cheW	67	279	0.24	ST4-74	1974221	1974724	-
cheA	28	130	0.21	ST4-74	1974745	1976760	-
motB	49	178	0.28	ST4-74	1976765	1977694	-
motA	53	203	0.26	ST4-74	1977691	1978521	-
flhC	94	189	0.49	ST4-74	1978703	1979281	-
flhD	114	198	0.58	ST4-74	1979284	1979634	-
yecG	53	49	1.08	ST4-74	1980421	1980849	+
otsA	24	16	1.57	ST4-74	1980867	1982288	-
otsB	58	26	2.23	ST4-74	1982263	1983066	-
araH	9	8	1.11	ST4-74	1983700	1984257	-
ftnB	21	40	0.52	ST4-74	1984808	1985311	+
STM1933	8	9	0.98	ST4-74	1985490	1986128	-
STM1934	21	61	0.34	ST4-74	1986384	1986719	+
ftn	158	238	0.66	ST4-74	1986959	1987456	+
yecH	5	110	0.04	ST4-74	1987502	1987741	-
tyrP	11	12	0.91	ST4-74	1987968	1989179	+
yecA	57	59	0.96	ST4-74	1989254	1989919	-
STM1939	201	138	1.45	ST4-74	1990157	1990462	-
STM1940	40	27	1.47	ST4-74	1990696	1992135	-
STM1941	511	221	2.32	ST4-74	1992348	1992680	-
SL1344_1874A	9	7	1.38	ST4-74	1992726	1992853	-
STnc2180	604	643	0.94	ST4-74	1992863	1992913	-
pgsA	97	111	0.87	ST4-74	1993381	1993929	-
uvrC	16	20	0.77	ST4-74	1993986	1995818	-
sirA	154	141	1.09	ST4-74	1995815	1996471	-
yecF	177	179	0.99	ST4-74	1996935	1997159	+
sdiA	25	41	0.62	ST4-74	1997226	1997948	-
yecC	18	23	0.76	ST4-74	1998180	1998932	-
yecS	13	16	0.79	ST4-74	1998929	1999597	-
dcyD	15	19	0.81	ST4-74	1999618	2000604	-
fliY	65	99	0.66	ST4-74	2000758	2001558	-
fliZ	32	124	0.26	ST4-74	2001705	2002256	-
fliA	67	280	0.24	ST4-74	2002315	2003034	-
tnpA_2b	249	288	0.86	ST4-74	2003303	2003812	-
fliB	10	26	0.37	ST4-74	2003942	2005147	-
fliC	279	1324	0.21	ST4-74	2005226	2006713	-
fliD	92	406	0.23	ST4-74	2006970	2008373	+
fliS	39	205	0.19	ST4-74	2008388	2008795	+
fliT	30	153	0.20	ST4-74	2008795	2009163	+
amyA	8	8	0.97	ST4-74	2009235	2010719	+
yedD	42	33	1.26	ST4-74	2010759	2011184	-
yedE	8	11	0.75	ST4-74	2011310	2012575	+
yedF	11	20	0.56	ST4-74	2012572	2012805	+
STM1967	5	7	0.69	ST4-74	2013070	2013456	+
fliE	57	223	0.25	ST4-74	2013576	2013890	-
fliF	15	71	0.21	ST4-74	2014107	2015789	+
fliG	12	60	0.21	ST4-74	2015782	2016777	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
fliH	12	53	0.22	ST4-74	2016770	2017477	+
fliI	7	32	0.23	ST4-74	2017477	2018847	+
fliJ	8	48	0.17	ST4-74	2018869	2019312	+
fliK	5	27	0.19	ST4-74	2019309	2020526	+
fliL	23	88	0.26	ST4-74	2020631	2021098	+
fliM	12	55	0.22	ST4-74	2021103	2022107	+
fliN	11	54	0.20	ST4-74	2022104	2022517	+
fliO	10	50	0.20	ST4-74	2022517	2022894	+
fliP	9	34	0.28	ST4-74	2022894	2023631	+
fliQ	5	24	0.20	ST4-74	2023641	2023910	+
fliR	4	9	0.44	ST4-74	2023919	2024713	+
rcsA	9	6	1.60	ST4-74	2024995	2025618	+
dsrB	421	246	1.71	ST4-74	2025657	2025851	-
yodD	199	83	2.39	ST4-74	2025980	2026207	+
DsrA	283	213	1.33	ST4-74	2026216	2026302	-
mngB	8	5	1.52	ST4-74	2026517	2027332	+
gcpA	14	12	1.23	ST4-74	2027311	2029023	-
STM1988	645	427	1.51	ST4-74	2029188	2029373	-
yedI	30	26	1.15	ST4-74	2029450	2030361	-
yedA	5	4	1.03	ST4-74	2030537	2031457	+
vsr	8	10	0.80	ST4-74	2031446	2031916	-
dcm	18	19	0.95	ST4-74	2031897	2033327	-
yedJ	7	7	1.05	ST4-74	2033401	2034096	-
STM1994	9	6	1.40	ST4-74	2034188	2034487	-
RseX	25	19	1.35	ST4-74	2034743	2034837	+
ompS	9	9	1.00	ST4-74	2035137	2036333	+
cspB	40	34	1.15	ST4-74	2036793	2037005	-
umuC	7	5	1.35	ST4-74	2037460	2038728	-
umuD	5	5	1.01	ST4-74	2038731	2039150	-
STM1999	1	1	1.57	ST4-74	2039277	2039438	-
SL1344_1927A	57	32	1.78	ST4-74	2039416	2039658	-
STnc1280	8973	8432	1.06	ST4-74	2039984	2040056	+
SL1344_1927B	197	60	3.31	ST4-74	2040072	2040263	-
SL1344_1928	197	30	6.52	ST4-74	2040503	2041510	+
STM2705	9	9	1.03	ST4-74	2041795	2042394	-
SL1344_1930	3	4	0.80	ST4-74	2042364	2043959	-
SL1344_1931	2	3	0.68	ST4-74	2043913	2044500	-
SL1344_1932	3	2	1.59	ST4-74	2044503	2045024	-
SL1344_1933	4	5	0.82	ST4-74	2045059	2045604	-
SL1344_1934	4	3	1.46	ST4-74	2045576	2045989	-
SL1344_1935	3	4	0.89	ST4-74	2045994	2046527	-
SL1344_1936	4	4	0.93	ST4-74	2046527	2047585	-
SL1344_1937	3	3	0.87	ST4-74	2047582	2048922	-
SL1344_1938	5	5	1.09	ST4-74	2048956	2050884	-
SL1344_1939	5	5	0.89	ST4-74	2050969	2051295	-
SL1344_1940	10	10	0.95	ST4-74	2051292	2051648	-
SL1344_1941	3	3	0.78	ST4-74	2051648	2053144	-
SL1344_1942	2	2	1.16	ST4-74	2053302	2053862	-
SL1344_1943	1	1	0.77	ST4-74	2053859	2054377	-
SL1344_1944	1	2	0.64	ST4-74	2054343	2054747	-
SL1344_1945	2	2	0.72	ST4-74	2054744	2055067	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
SL1344_1946	4	4	0.94	ST4-74	2055070	2055270	-
SL1344_1947	3	4	0.67	ST4-74	2055321	2056526	-
SL1344_1948	2	3	0.61	ST4-74	2056541	2057227	-
SL1344_1949	3	3	0.99	ST4-74	2057169	2058410	-
SL1344_1950	3	3	1.01	ST4-74	2058604	2060337	-
SL1344_1951	5	7	0.68	ST4-74	2060334	2060837	-
SL1344_1952	3	4	0.75	ST4-74	2060954	2061304	-
SL1344_1953	30	25	1.22	ST4-74	2061365	2061667	-
SL1344_1954	4	5	0.73	ST4-74	2061887	2062306	-
rzpR	1	2	0.93	ST4-74	2062519	2063004	-
STM0907	2	2	1.00	ST4-74	2063001	2063615	-
STM0906	4	4	0.93	ST4-74	2063618	2063962	-
SL1344_1958	54	51	1.07	ST4-74	2064488	2064847	-
SL1344_1959	4	5	0.78	ST4-74	2064878	2065501	-
SL1344_1960	4	4	0.98	ST4-74	2065512	2066507	-
SL1344_1961	4	4	1.06	ST4-74	2066509	2067414	-
SL1344_1962	2	2	1.13	ST4-74	2067772	2068665	-
SL1344_1963	1	2	0.74	ST4-74	2068665	2069282	-
SL1344_1964	1	1	1.41	ST4-74	2069149	2069967	-
SL1344_1965	4	4	1.13	ST4-74	2070185	2071393	-
STnc1290	17163	18829	0.91	ST4-74	2070801	2070988	-
STnc1300	5945	8257	0.72	ST4-74	2071271	2071409	+
SL1344_1966	3	3	0.81	ST4-74	2071339	2072049	-
STM0898	222	215	1.03	ST4-74	2072244	2072939	+
STnc1310	81	103	0.79	ST4-74	2073265	2073338	+
SL1344_1968	34	51	0.68	ST4-74	2073535	2074125	+
SL1344_1969	2	4	0.43	ST4-74	2074183	2075010	+
SL1344_1970	2	2	1.16	ST4-74	2075147	2075686	+
SL1344_1971	4	4	0.99	ST4-74	2075984	2076499	+
SL1344_1972	1	3	0.48	ST4-74	2076496	2077113	+
SL1344_1973	5	4	1.40	ST4-74	2077110	2077943	+
SL1344_1974	3	3	0.93	ST4-74	2077947	2078516	+
SL1344_1975	3	4	0.65	ST4-74	2078541	2078783	+
SL1344_1976	9	7	1.27	ST4-74	2078785	2079774	+
yeeI	53	60	0.88	ST4-74	2080066	2080863	+
SL1344_1978	4	4	0.99	ST4-74	2081235	2081525	+
STnc2050	297	299	0.99	ST4-74	2082064	2082105	+
STM2005	2	2	1.15	ST4-74	2082173	2082646	+
SL1344_1980	1	3	0.23	ST4-74	2082737	2083009	+
STM2006	3	3	1.32	ST4-74	2083094	2083819	+
STM2007	3	3	1.05	ST4-74	2084162	2086330	+
STM2008	5	4	1.24	ST4-74	2086387	2088117	+
STnc3680	96	98	0.97	ST4-74	2088048	2088167	+
SL1344_1985	11	10	1.03	ST4-74	2088651	2088905	-
amn	17	17	1.02	ST4-74	2089022	2090476	-
STM2011	42	31	1.37	ST4-74	2090672	2090998	-
SL1344_1988	71	55	1.29	ST4-74	2090992	2091378	-
SL1344_1989	115	109	1.05	ST4-74	2091704	2091871	+
yeeO	12	11	1.05	ST4-74	2092092	2093549	-
erfK	46	60	0.76	ST4-74	2094011	2094940	-
cobT	41	78	0.52	ST4-74	2095018	2096088	-

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cobS	42	90	0.46	ST4-74	2096115	2096858	-
cobU	39	73	0.54	ST4-74	2096855	2097400	-
cbiP	53	92	0.58	ST4-74	2097397	2098917	-
cbiO	74	129	0.57	ST4-74	2098914	2099729	-
cbiQ	52	115	0.45	ST4-74	2099738	2100415	-
cbiN	55	109	0.50	ST4-74	2100402	2100683	-
cbiM	71	147	0.48	ST4-74	2100685	2101422	-
cbiL	48	113	0.42	ST4-74	2101419	2102132	-
STnc1530	49	27	1.85	ST4-74	2101640	2101718	+
cbiK	48	116	0.41	ST4-74	2102129	2102923	-
cbiJ	47	103	0.46	ST4-74	2102926	2103717	-
cbiH	103	165	0.63	ST4-74	2103714	2104439	-
cbiG	65	124	0.52	ST4-74	2104439	2105494	-
cbiF	44	97	0.45	ST4-74	2105475	2106248	-
cbiT	59	123	0.48	ST4-74	2106232	2106810	-
cbiE	72	126	0.57	ST4-74	2106800	2107405	-
cbiD	85	142	0.60	ST4-74	2107399	2108538	-
cbiC	82	145	0.57	ST4-74	2108538	2109170	-
cbiB	143	215	0.67	ST4-74	2109181	2110140	-
cbiA	94	150	0.63	ST4-74	2110137	2111516	-
pocR	154	195	0.79	ST4-74	2112114	2112878	-
pduF	426	718	0.59	ST4-74	2113242	2114036	-
pduA	566	1125	0.50	ST4-74	2114561	2114845	+
pduB	209	433	0.48	ST4-74	2114953	2115654	+
dhaB	132	257	0.52	ST4-74	2115673	2117337	+
pduD	122	265	0.46	ST4-74	2117348	2118022	+
pduE	158	383	0.41	ST4-74	2118037	2118558	+
pduG	114	243	0.47	ST4-74	2118568	2120400	+
pduH	306	612	0.50	ST4-74	2120390	2120740	+
pduJ	195	436	0.45	ST4-74	2120759	2121034	+
pduK	189	385	0.49	ST4-74	2121038	2121520	+
pduL	152	279	0.55	ST4-74	2121520	2122152	+
pduM	43	105	0.41	ST4-74	2122149	2122640	+
pduN	97	212	0.46	ST4-74	2122644	2122919	+
pduO	70	132	0.53	ST4-74	2122929	2123939	+
pduP	70	128	0.55	ST4-74	2123936	2125330	+
pduQ	48	105	0.45	ST4-74	2125342	2126454	+
pduS	58	119	0.48	ST4-74	2126451	2127806	+
pduT	53	98	0.54	ST4-74	2127809	2128363	+
pduU	58	92	0.62	ST4-74	2128363	2128713	+
pduV	31	67	0.46	ST4-74	2128718	2129170	+
pduW	207	312	0.66	ST4-74	2129155	2130369	+
pduX	87	167	0.52	ST4-74	2130427	2131329	+
yeeX	740	743	1.00	ST4-74	2131363	2131698	-
STnc3700	77	53	1.45	ST4-74	2131885	2131995	+
yeeA	31	30	1.05	ST4-74	2131943	2133001	-
STnc1650	78	49	1.60	ST4-74	2132959	2133102	+
gyrI	254	308	0.82	ST4-74	2133120	2133587	-
dacD	4	4	0.94	ST4-74	2133740	2134912	-
phsC	7	8	0.79	ST4-74	2135036	2135800	-
phsB	8	7	1.15	ST4-74	2135797	2136375	-

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phsA	4	6	0.74	ST4-74	2136390	2138666	-
SL1344_2042	5	4	1.38	ST4-74	2138911	2139018	-
sopA	4	5	0.93	ST4-74	2139285	2141633	+
sbcB	18	17	1.06	ST4-74	2141976	2143406	+
yeeF	36	41	0.89	ST4-74	2143542	2144906	-
yoeI	94	115	0.82	ST4-74	2144890	2144952	-
yeeY	24	13	1.90	ST4-74	2145186	2146100	-
yeeZ	62	41	1.52	ST4-74	2146146	2146970	-
STnc700	3265	2733	1.19	ST4-74	2147102	2147280	+
hisG	50	48	1.03	ST4-74	2147330	2148229	+
hisD	33	28	1.17	ST4-74	2148332	2149636	+
hisC	25	22	1.13	ST4-74	2149633	2150712	+
hisB	30	22	1.35	ST4-74	2150709	2151776	+
hisH	20	19	1.04	ST4-74	2151773	2152366	+
hisA	22	23	0.96	ST4-74	2152366	2153103	+
hisF	44	38	1.16	ST4-74	2153085	2153861	+
hisI	55	42	1.31	ST4-74	2153855	2154466	+
wzzB	120	97	1.24	ST4-74	2154547	2155530	-
udg	163	129	1.27	ST4-74	2155673	2156839	-
gnd	66	60	1.10	ST4-74	2157076	2158482	-
rfbP	110	77	1.42	ST4-74	2158646	2160076	-
rfbK	74	60	1.24	ST4-74	2160148	2161581	-
cpsB2	90	65	1.38	ST4-74	2161568	2163007	-
rfbN	106	85	1.25	ST4-74	2163008	2163952	-
rfbU	111	78	1.42	ST4-74	2163953	2165014	-
rfbV	152	101	1.51	ST4-74	2165334	2166335	-
rfbX	143	97	1.48	ST4-74	2166340	2167632	-
rfbJ	123	99	1.25	ST4-74	2167714	2168613	-
rfbH	203	177	1.14	ST4-74	2168641	2169954	-
rfbG	172	150	1.15	ST4-74	2169981	2171060	-
rfbF	136	118	1.15	ST4-74	2171065	2171838	-
rfbI	143	113	1.27	ST4-74	2171835	2172827	-
rfbC	154	112	1.37	ST4-74	2172833	2173384	-
rfbA	81	61	1.33	ST4-74	2173385	2174263	-
rfbD	36	35	1.03	ST4-74	2174311	2175210	-
STnc1320	80	55	1.45	ST4-74	2174386	2174470	+
rfbB	63	55	1.13	ST4-74	2175210	2176295	-
galF	139	108	1.29	ST4-74	2176672	2177694	-
wcaM	3	2	1.29	ST4-74	2177743	2179146	-
wcaL	2	2	1.45	ST4-74	2179157	2180377	-
wcaK	1	1	1.09	ST4-74	2180374	2181654	-
wzx	3	2	1.36	ST4-74	2181676	2183154	-
wcaJ	2	1	1.19	ST4-74	2183287	2184681	-
cpsG	1	1	0.69	ST4-74	2184735	2186105	-
cpsB	3	4	0.82	ST4-74	2186216	2187652	-
wcaI	1	2	0.66	ST4-74	2187655	2188878	-
gmm	1	2	0.90	ST4-74	2188875	2189348	-
fcl	2	1	1.66	ST4-74	2189351	2190316	-
gmd	2	2	1.02	ST4-74	2190319	2191440	-
wcaF	1	1	1.77	ST4-74	2191464	2192018	-
wcaE	2	1	1.25	ST4-74	2192034	2192780	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
wcaD	3	3	1.11	ST4-74	2192793	2194007	-
wcaC	2	2	1.22	ST4-74	2193982	2195199	-
wcaB	5	4	1.48	ST4-74	2195196	2195684	-
wcaA	2	2	1.07	ST4-74	2195687	2196529	-
wzc	2	1	1.36	ST4-74	2196616	2198775	-
wzb	2	1	1.84	ST4-74	2198772	2199221	-
wza	2	1	1.36	ST4-74	2199227	2200282	-
yegH	23	21	1.11	ST4-74	2201041	2202621	+
asmA	40	44	0.89	ST4-74	2202707	2204563	-
dcd	27	34	0.79	ST4-74	2204602	2205183	-
udk	34	37	0.92	ST4-74	2205274	2205915	-
yegE	12	11	1.16	ST4-74	2206246	2209236	+
alkA	8	7	1.22	ST4-74	2209204	2210073	-
yegD	5	5	1.00	ST4-74	2210207	2211559	+
RyeC	7136	10986	0.65	ST4-74	2211623	2211760	+
STM2126	4	4	1.04	ST4-74	2212000	2213241	+
yegN	4	3	1.16	ST4-74	2213241	2216363	+
yegO	5	4	1.27	ST4-74	2216364	2219444	+
yegB	11	9	1.24	ST4-74	2219441	2220853	+
baeS	11	10	1.10	ST4-74	2220853	2222256	+
baeR	12	10	1.22	ST4-74	2222253	2222975	+
STnc1150	2434	873	2.79	ST4-74	2222979	2223134	+
STM2133	1	1	1.46	ST4-74	2223562	2224446	+
STM2134	2	2	1.19	ST4-74	2224446	2225162	+
STM2135	4	4	1.16	ST4-74	2225173	2227284	+
yegQ	9	9	0.98	ST4-74	2227400	2228761	+
CyaR	35723	36420	0.98	ST4-74	2228847	2228932	+
sseK2	9	4	2.04	ST4-74	2229212	2230258	+
srcA	80	16	4.87	ST4-74	2230294	2230710	-
STM2139	116	38	3.09	ST4-74	2230832	2231167	-
SL1344_2116	12	12	0.96	ST4-74	2231529	2231645	-
yegS	24	14	1.71	ST4-74	2231729	2232628	+
fbaB	116	72	1.61	ST4-74	2232681	2233733	-
yegT	5	4	1.17	ST4-74	2233987	2235258	+
yegU	7	6	1.21	ST4-74	2235255	2236259	+
yegV	4	4	1.11	ST4-74	2236361	2237221	+
yegW	49	51	0.96	ST4-74	2237195	2237941	-
thiD	32	37	0.86	ST4-74	2237977	2238777	-
thiM	20	22	0.90	ST4-74	2238764	2239561	-
STM2148	34	42	0.81	ST4-74	2239971	2240285	+
STnc740	174	118	1.48	ST4-74	2240283	2240366	-
stcD	7	7	0.95	ST4-74	2240548	2241555	-
stcC	7	6	1.14	ST4-74	2241571	2244060	-
stcB	1	1	1.05	ST4-74	2244074	2244757	-
stcA	3	19	0.15	ST4-74	2244813	2245343	-
yehE	35	40	0.88	ST4-74	2245622	2245903	-
mrp	34	37	0.90	ST4-74	2246181	2247290	-
metG	40	46	0.87	ST4-74	2247455	2249488	+
yehR	11	8	1.32	ST4-74	2249729	2250187	+
STM2156A	13	9	1.48	ST4-74	2250359	2250889	+
STnc1890	26	23	1.12	ST4-74	2250891	2250938	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
yehS	49	43	1.12	ST4-74	2250946	2251413	-
yehT	10	12	0.90	ST4-74	2251460	2252179	-
yehU	18	19	0.95	ST4-74	2252176	2253861	-
yehV	61	42	1.44	ST4-74	2254084	2254815	+
yohO	630	453	1.39	ST4-74	2254875	2254982	+
yehW	13	9	1.39	ST4-74	2254963	2255694	-
yehX	17	11	1.46	ST4-74	2255678	2256625	-
yehY	27	19	1.44	ST4-74	2256618	2257787	-
yehZ	48	36	1.33	ST4-74	2257791	2258708	-
bglX	11	11	0.98	ST4-74	2258889	2261186	-
dld	13	13	0.99	ST4-74	2261453	2263183	+
pbpG	16	15	1.07	ST4-74	2263241	2264149	-
STnc3720	82	58	1.42	ST4-74	2264259	2264328	+
yohC	45	21	2.11	ST4-74	2264354	2264941	-
yohD	15	13	1.18	ST4-74	2265073	2265669	+
yohF	11	8	1.43	ST4-74	2265718	2266479	-
yohG	5	3	1.39	ST4-74	2266545	2267981	-
STnc1330	1078	526	2.05	ST4-74	2268274	2268414	+
yohI	8	7	1.14	ST4-74	2268440	2269378	-
STM2175	11	9	1.16	ST4-74	2269487	2270680	-
STM2176	4	3	1.60	ST4-74	2270695	2271339	-
STM2177	2	2	1.21	ST4-74	2271348	2272049	-
STM2178	1	1	1.09	ST4-74	2272065	2273102	-
STM2179	2	2	0.92	ST4-74	2273114	2274472	-
STM2180	16	14	1.14	ST4-74	2274598	2275506	+
yohJ	60	41	1.48	ST4-74	2275638	2276036	+
yohK	9	9	1.10	ST4-74	2276033	2276728	+
cdd	7	8	0.85	ST4-74	2276882	2277766	+
sanA	62	60	1.03	ST4-74	2277943	2278662	+
b2145	20	18	1.09	ST4-74	2278659	2278904	+
STM2186	17	17	0.96	ST4-74	2279109	2280350	+
yeiA	10	13	0.79	ST4-74	2280344	2281579	+
mglC	10	14	0.74	ST4-74	2281654	2282664	-
mglA	8	12	0.67	ST4-74	2282680	2284200	-
mglB	13	21	0.60	ST4-74	2284334	2285332	-
galS	15	13	1.15	ST4-74	2285831	2286853	-
yeiB	16	17	0.96	ST4-74	2287003	2288145	-
folE	26	25	1.08	ST4-74	2288160	2288828	-
yeiG	78	66	1.20	ST4-74	2289158	2290015	+
STM2195	20	24	0.82	ST4-74	2290004	2290393	-
STM2196	5	6	0.98	ST4-74	2290398	2291765	-
STM2197	1	1	0.77	ST4-74	2291982	2292869	+
STM2198	6	5	1.15	ST4-74	2292902	2294224	+
cirA	4	3	1.27	ST4-74	2294268	2296259	-
lysP	72	77	0.94	ST4-74	2296604	2298073	-
yeiE	25	25	0.98	ST4-74	2298263	2299126	-
yeiH	8	12	0.68	ST4-74	2299247	2300296	+
nfo	15	15	1.01	ST4-74	2300375	2301232	+
fruA	7	9	0.78	ST4-74	2301297	2302985	-
fruK	6	7	0.91	ST4-74	2303002	2303859	-
fruB	5	7	0.64	ST4-74	2303940	2305070	-

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setB	5	6	0.91	ST4-74	2305439	2306620	+
STM2208	10	7	1.40	ST4-74	2306685	2307350	-
STM2209	19	14	1.37	ST4-74	2307352	2307474	-
SL1344_2187	21	21	0.97	ST4-74	2307862	2308116	-
yeiP	38	42	0.92	ST4-74	2308440	2309012	+
yeiR	25	23	1.11	ST4-74	2309225	2310211	+
yeiU	146	138	1.06	ST4-74	2310241	2310960	+
STnc3010	176	177	1.00	ST4-74	2310959	2311126	-
spr	664	1075	0.62	ST4-74	2311374	2311946	+
rtn	30	29	1.06	ST4-74	2312272	2313828	+
yejA	21	22	0.94	ST4-74	2313935	2315740	+
yejB	25	25	1.00	ST4-74	2315750	2316844	+
yejE	10	12	0.90	ST4-74	2316844	2317869	+
yejF	9	10	0.96	ST4-74	2317871	2319460	+
yejG	162	100	1.62	ST4-74	2319464	2319808	-
STnc1340	126	356	0.35	ST4-74	2319956	2320037	-
bcr	15	16	0.91	ST4-74	2320199	2321389	-
STnc1540	316	530	0.60	ST4-74	2321042	2321137	-
rsuA	39	33	1.20	ST4-74	2321417	2322112	-
yejH	4	4	0.90	ST4-74	2322264	2324024	+
rplY	405	553	0.73	ST4-74	2324149	2324433	+
STM2225	51	46	1.11	ST4-74	2324542	2325162	-
yejK	22	23	0.92	ST4-74	2325190	2326197	-
yejL	209	187	1.12	ST4-74	2326377	2326604	+
yejM	36	35	1.02	ST4-74	2326636	2328396	+
STM2230	3	4	0.69	ST4-74	2328677	2329147	-
STM2231	3	2	1.18	ST4-74	2329208	2329498	+
oafA	69	58	1.20	ST4-74	2329846	2331675	+
STM2233	2	3	0.74	ST4-74	2331729	2332172	-
STM2234	4	4	1.13	ST4-74	2332550	2333077	-
STM2235	4	3	1.19	ST4-74	2333080	2334321	-
STM2236	2	2	1.04	ST4-74	2334382	2334900	-
STM2237	1	1	0.50	ST4-74	2334914	2335243	-
STM2238	54	46	1.17	ST4-74	2335540	2336871	+
STM2239	11	10	1.12	ST4-74	2336900	2337268	-
STM2240	6	5	1.15	ST4-74	2337283	2338272	-
sspH2	12	10	1.18	ST4-74	2338601	2340967	-
STM2242	4	2	1.68	ST4-74	2341136	2341339	-
STM2243	5	4	1.21	ST4-74	2341636	2342463	-
IsrG	6	6	0.98	ST4-74	2342448	2342729	+
STM2244	6	4	1.65	ST4-74	2342730	2342933	+
STM2245	125	17	7.30	ST4-74	2343125	2343688	-
narP	23	24	0.99	ST4-74	2344418	2345065	+
ccmH2	3	7	0.48	ST4-74	2345109	2346152	-
ccmG2	7	13	0.58	ST4-74	2346149	2346706	-
ccmF2	7	14	0.49	ST4-74	2346703	2348634	-
ccmE2	6	13	0.44	ST4-74	2348631	2349110	-
ccmD2	4	10	0.45	ST4-74	2349107	2349280	-
ccmC2	11	29	0.36	ST4-74	2349316	2350062	-
ccmB2	5	10	0.51	ST4-74	2350105	2350761	-
ccmA2	5	15	0.33	ST4-74	2350761	2351378	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
napC	5	8	0.70	ST4-74	2351399	2352001	-
napB	2	5	0.38	ST4-74	2352011	2352460	-
napH	5	7	0.72	ST4-74	2352576	2353445	-
napG	2	4	0.48	ST4-74	2353432	2354127	-
napA	3	5	0.70	ST4-74	2354134	2356620	-
napD	1	6	0.24	ST4-74	2356617	2356880	-
napF	2	12	0.19	ST4-74	2356870	2357361	-
eco	6	3	2.28	ST4-74	2357775	2358269	+
yojI	21	17	1.30	ST4-74	2358478	2360121	-
alkB	6	6	1.12	ST4-74	2360197	2360847	-
ada	21	11	1.90	ST4-74	2360850	2361911	-
apbE	7	8	0.81	ST4-74	2361992	2363044	-
ompC	328	443	0.74	ST4-74	2363159	2364127	-
MicF	295	201	1.47	ST4-74	2364629	2364721	+
yojN	35	36	0.99	ST4-74	2365024	2367693	+
rcsB	60	60	1.01	ST4-74	2367710	2368360	+
rcsC	25	23	1.08	ST4-74	2368463	2371309	-
gyrA	62	63	0.98	ST4-74	2371427	2374063	-
STM2273	11	9	1.20	ST4-74	2374473	2375675	-
STM2274	3	3	1.29	ST4-74	2375690	2377015	-
STM2275	9	11	0.78	ST4-74	2377254	2377949	+
ubiG	72	61	1.17	ST4-74	2378032	2378760	+
nrdA	29	25	1.15	ST4-74	2379116	2381401	+
nrdB	21	22	0.95	ST4-74	2381514	2382644	+
yfaE	15	20	0.72	ST4-74	2382644	2382898	+
STM2280	14	14	0.99	ST4-74	2382900	2384090	-
STM2281	10	10	1.05	ST4-74	2384252	2385130	+
glpQ	315	531	0.59	ST4-74	2385246	2386316	-
glpT	345	527	0.66	ST4-74	2386321	2387679	-
glpA	70	177	0.39	ST4-74	2387952	2389580	+
glpB	40	111	0.36	ST4-74	2389570	2390829	+
glpC	64	144	0.44	ST4-74	2390826	2392016	+
IsrH_1_2	19	6	3.44	ST4-74	2392019	2392469	-
sseL	276	43	6.41	ST4-74	2392511	2393464	+
STM2288	28	24	1.15	ST4-74	2393574	2393750	-
STM2289	7	7	1.02	ST4-74	2393756	2394559	-
yfaV	8	8	1.02	ST4-74	2394585	2395874	-
yfaW	8	6	1.42	ST4-74	2395930	2397147	-
yfaX	4	4	1.07	ST4-74	2397149	2397931	-
STM2293	21	19	1.13	ST4-74	2398181	2399377	-
yfaZ	31	29	1.06	ST4-74	2399492	2400058	-
yfaO	58	70	0.83	ST4-74	2400313	2400738	+
ais	59	69	0.85	ST4-74	2400783	2401388	-
yfbE	162	201	0.80	ST4-74	2401669	2402826	+
pmrF	79	109	0.72	ST4-74	2402829	2403812	+
yfbG	49	74	0.65	ST4-74	2403809	2405791	+
STM2300	43	67	0.65	ST4-74	2405788	2406687	+
arnT	49	67	0.73	ST4-74	2406684	2408330	+
STM2302	33	51	0.64	ST4-74	2408405	2408662	+
STM2303	16	25	0.66	ST4-74	2408662	2409039	+
pmrD	1558	1210	1.29	ST4-74	2409034	2409291	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
menE	8	13	0.61	ST4-74	2409389	2410756	-
menC	7	12	0.61	ST4-74	2410753	2411715	-
menB	9	18	0.50	ST4-74	2411715	2412572	-
yfbB	10	18	0.55	ST4-74	2412587	2413345	-
menD	6	19	0.33	ST4-74	2413342	2415012	-
menF	16	17	0.96	ST4-74	2415098	2416393	-
elaB	332	242	1.37	ST4-74	2416494	2416805	-
elaA	20	19	1.05	ST4-74	2416854	2417315	-
elaC	18	16	1.12	ST4-74	2417376	2418293	+
STM2314	41	119	0.35	ST4-74	2418359	2419360	+
yfbK	6	6	1.11	ST4-74	2419401	2421182	-
nuoN	29	72	0.41	ST4-74	2422070	2423527	-
nuoM	37	93	0.39	ST4-74	2423534	2425063	-
nuoL	43	97	0.44	ST4-74	2425377	2427218	-
nuoK	26	64	0.41	ST4-74	2427215	2427517	-
nuoJ	29	73	0.39	ST4-74	2427514	2428068	-
nuoI	45	100	0.45	ST4-74	2428079	2428621	-
nuoH	34	77	0.44	ST4-74	2428636	2429613	-
nuoG	33	71	0.46	ST4-74	2429610	2432336	-
nuoF	22	47	0.47	ST4-74	2432367	2433704	-
nuoE	31	74	0.43	ST4-74	2433701	2434201	-
nuoC	35	72	0.48	ST4-74	2434204	2436006	-
nuoB	37	74	0.50	ST4-74	2436093	2436755	-
nuoA	84	175	0.48	ST4-74	2436771	2437214	-
STM2329	20	15	1.30	ST4-74	2437609	2437761	-
STnc1550	142	223	0.64	ST4-74	2437665	2437806	+
lrhA	55	48	1.13	ST4-74	2437841	2438779	-
yfbQ	15	19	0.77	ST4-74	2439711	2440925	+
STM2332	14	16	0.90	ST4-74	2441019	2441618	+
yfbS	20	25	0.79	ST4-74	2441710	2443536	-
yfbT	37	49	0.75	ST4-74	2443613	2444290	-
yfbU	85	122	0.69	ST4-74	2444283	2444777	-
STM2336	65	76	0.85	ST4-74	2444860	2445315	-
ackA	45	117	0.39	ST4-74	2445655	2446857	+
pta	14	44	0.33	ST4-74	2446934	2449078	+
STnc1560	69	381	0.18	ST4-74	2449075	2449183	+
STnc3730	122	63	1.93	ST4-74	2449087	2449281	-
yfcC	5	14	0.35	ST4-74	2449296	2450816	+
STM2340	11	12	0.87	ST4-74	2450865	2451818	-
STM2341	4	6	0.67	ST4-74	2451811	2452641	-
STM2342	7	11	0.67	ST4-74	2452638	2454029	-
STM2343	3	7	0.47	ST4-74	2454050	2454322	-
STM2344	19	20	0.93	ST4-74	2454403	2454846	-
STM2345	16	19	0.83	ST4-74	2455099	2456118	+
STM2346	68	66	1.04	ST4-74	2456124	2456678	-
yfcE	87	81	1.07	ST4-74	2456735	2457286	-
yfcF	34	29	1.17	ST4-74	2457342	2457986	-
yfcG	14	7	2.00	ST4-74	2458129	2458776	+
yfcH	24	20	1.25	ST4-74	2458902	2459795	+
STnc3740	176	129	1.37	ST4-74	2459768	2459846	+
hisP	24	27	0.90	ST4-74	2459847	2460623	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
hisM	25	30	0.85	ST4-74	2460634	2461341	-
hisQ	34	42	0.82	ST4-74	2461338	2462024	-
hisJ	78	100	0.78	ST4-74	2462207	2462989	-
argT	20	19	1.05	ST4-74	2463227	2464009	-
ubiX	46	37	1.23	ST4-74	2464298	2464867	-
STM2357	22	22	1.00	ST4-74	2464894	2466300	-
STM2358	11	9	1.24	ST4-74	2466364	2467467	-
STM2359	6	6	0.94	ST4-74	2467469	2468890	-
STM2360	4	3	1.22	ST4-74	2468981	2470378	-
STM2361	11	10	1.06	ST4-74	2470589	2472016	+
purF	75	87	0.86	ST4-74	2472052	2473569	-
cvpA	196	270	0.72	ST4-74	2473607	2474095	-
dedD	71	73	0.97	ST4-74	2474406	2475080	-
folC	40	44	0.90	ST4-74	2475070	2476338	-
accD	118	117	1.01	ST4-74	2476406	2477320	-
dedA	33	31	1.06	ST4-74	2477470	2478129	-
truA	49	47	1.04	ST4-74	2478178	2478990	-
usg	20	22	0.90	ST4-74	2478990	2480003	-
pdxB	34	32	1.05	ST4-74	2480071	2481207	-
div	6	6	0.88	ST4-74	2481311	2482312	+
STM2372	16	19	0.81	ST4-74	2482309	2483487	-
STM2373	5	4	1.23	ST4-74	2483667	2484041	-
STM2374	5	4	1.34	ST4-74	2484214	2484462	+
STM2375	2	2	1.10	ST4-74	2484631	2484999	+
STM2376	1	1	0.54	ST4-74	2484999	2485517	+
STM2377	13	12	1.03	ST4-74	2485584	2486240	-
fabB	155	152	1.02	ST4-74	2486338	2487552	-
mnmC	27	30	0.90	ST4-74	2487652	2489712	+
yfcL	77	66	1.16	ST4-74	2489764	2490039	-
yfcM	20	20	1.03	ST4-74	2490072	2490620	-
yfcA	10	12	0.85	ST4-74	2490620	2491429	-
mepA	11	13	0.88	ST4-74	2491429	2492253	-
aroC	18	21	0.89	ST4-74	2492257	2493342	-
yfcB	31	30	1.02	ST4-74	2493378	2494310	-
yfcN	24	20	1.23	ST4-74	2494476	2495027	+
sixA	156	143	1.09	ST4-74	2495127	2495612	-
fadJ	29	20	1.44	ST4-74	2495821	2497968	-
fadI	5	5	0.91	ST4-74	2497968	2499278	-
yfcZ	78	185	0.42	ST4-74	2499456	2499740	-
fadL	22	24	0.93	ST4-74	2500105	2501418	+
vacJ	92	84	1.10	ST4-74	2501479	2502234	-
yfdC	24	13	1.82	ST4-74	2502523	2503464	+
STnc3750	128	54	2.36	ST4-74	2503726	2503803	-
pgtE	47	23	2.00	ST4-74	2503771	2504709	-
pgtA	9	10	0.87	ST4-74	2504977	2506224	-
pgtB	9	12	0.76	ST4-74	2506214	2508220	-
pgtC	12	9	1.30	ST4-74	2508217	2509410	-
pgtP	11	8	1.33	ST4-74	2509846	2511237	+
STM2400	93	85	1.09	ST4-74	2511513	2511755	-
lpxP	7	6	1.24	ST4-74	2512277	2513197	+
tpke70	6	3	2.25	ST4-74	2513324	2513722	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
ypdK	106	85	1.25	ST4-74	2513600	2513671	+
yfdZ	45	36	1.27	ST4-74	2513721	2514959	-
STnc3760	57	55	1.03	ST4-74	2515508	2515633	+
glk	67	66	1.02	ST4-74	2515680	2516645	-
STM2404	8	8	1.04	ST4-74	2516849	2518084	+
STM2405	3	3	1.01	ST4-74	2518104	2519756	-
STM2406	9	6	1.52	ST4-74	2519936	2520934	+
ypeC	20	14	1.42	ST4-74	2521048	2521374	+
mntH	9	9	0.99	ST4-74	2521435	2522676	-
nupC	40	38	1.05	ST4-74	2523019	2524221	+
yfeA	15	17	0.93	ST4-74	2524274	2526463	-
yfeC	151	152	0.99	ST4-74	2527069	2527431	+
yfeD	39	55	0.71	ST4-74	2527433	2527825	+
gltX	40	40	1.00	ST4-74	2527879	2529294	-
xapR	8	6	1.31	ST4-74	2530110	2530994	-
SL1344_2383	7	7	1.02	ST4-74	2531046	2531204	-
xapB	13	10	1.28	ST4-74	2531263	2532519	-
flhAb	4	9	0.47	ST4-74	2532574	2533407	-
yfeN	8	7	1.21	ST4-74	2533663	2534424	+
yfeR	5	5	0.88	ST4-74	2534486	2535412	-
yfeH	19	16	1.15	ST4-74	2535502	2536500	+
STM2426	14	13	1.05	ST4-74	2536497	2536724	-
lig	13	13	0.95	ST4-74	2536717	2538732	-
zipA	148	151	0.98	ST4-74	2538804	2539790	-
cysZ	19	18	1.06	ST4-74	2540022	2540783	+
cysK	280	325	0.86	ST4-74	2540947	2541918	+
ptsH	248	389	0.64	ST4-74	2542302	2542559	+
ptsI	159	212	0.75	ST4-74	2542608	2544335	+
crr	292	322	0.91	ST4-74	2544376	2544885	+
STM2434	5	7	0.79	ST4-74	2545034	2545273	-
pdxK	9	8	1.10	ST4-74	2545270	2546136	-
ptsJ	4	4	1.00	ST4-74	2546219	2547511	+
yfeJ	3	3	0.76	ST4-74	2547526	2548245	+
yfeK	7	9	0.81	ST4-74	2548304	2548684	+
yfeL	42	29	1.47	ST4-74	2548697	2549236	+
cysM	30	32	0.94	ST4-74	2549367	2550278	-
cysA	66	77	0.86	ST4-74	2550346	2551443	-
cysW	108	128	0.84	ST4-74	2551433	2552308	-
cysU	106	121	0.88	ST4-74	2552308	2553141	-
cysP	108	123	0.88	ST4-74	2553141	2554157	-
ucpA	67	82	0.81	ST4-74	2554315	2555106	-
STM2446	18	47	0.37	ST4-74	2555344	2556243	-
yfeY	16	19	0.86	ST4-74	2556338	2556913	-
yfeZ	20	17	1.17	ST4-74	2556975	2557424	-
STM2449	58	64	0.90	ST4-74	2557411	2557836	-
amiA	22	17	1.33	ST4-74	2558049	2558918	+
hemF	6	6	1.04	ST4-74	2558921	2559820	+
STM2452	11	9	1.24	ST4-74	2559841	2560170	+
STM2453	7	6	1.18	ST4-74	2560299	2561576	+
eutR	7	6	1.17	ST4-74	2561776	2562828	-
eutK	4	4	0.82	ST4-74	2562876	2563370	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
eutL	11	10	1.12	ST4-74	2563383	2564042	-
eutC	2	2	1.45	ST4-74	2564052	2564948	-
eutB	1	2	0.66	ST4-74	2564967	2566328	-
eutA	26	28	0.93	ST4-74	2566340	2567743	-
eutH	8	8	0.98	ST4-74	2567740	2568966	-
eutG	1	2	0.66	ST4-74	2569086	2570273	-
eutJ	2	1	1.10	ST4-74	2570263	2571102	-
eutE	2	2	1.43	ST4-74	2571113	2572516	-
eutN	3	3	0.97	ST4-74	2572528	2572827	-
eutM	4	2	2.17	ST4-74	2572928	2573218	-
eutD	1	1	0.63	ST4-74	2573259	2574275	-
eutT	1	1	1.50	ST4-74	2574272	2575075	-
eutQ	4	5	0.72	ST4-74	2575072	2575761	-
eutP	1	1	0.49	ST4-74	2575739	2576218	-
eutS	2	1	1.04	ST4-74	2576231	2576566	-
tnpA_1b	244	250	0.97	ST4-74	2577047	2577505	-
maeB	17	26	0.66	ST4-74	2577695	2579974	-
tal	29	19	1.49	ST4-74	2580246	2581196	+
tktB	17	13	1.31	ST4-74	2581216	2583216	+
STM2475	101	108	0.94	ST4-74	2583279	2583509	-
ypfG	9	5	1.78	ST4-74	2583637	2584680	-
yffH	25	20	1.24	ST4-74	2584805	2585380	-
STM2478	17	16	1.03	ST4-74	2585584	2586882	+
aegA	3	12	0.25	ST4-74	2587331	2589292	-
narQ	6	7	0.86	ST4-74	2589478	2591178	+
acrD	6	6	1.04	ST4-74	2591363	2594476	+
ypfM	1269	780	1.63	ST4-74	2594578	2594637	-
yffB	21	24	0.86	ST4-74	2595050	2595406	+
dapE	25	21	1.20	ST4-74	2595410	2596537	+
STM2484	50	36	1.40	ST4-74	2596565	2596765	+
ypfI	8	8	1.00	ST4-74	2596792	2598810	-
STM2486	39	31	1.26	ST4-74	2598826	2599689	-
purC	40	78	0.52	ST4-74	2599820	2600533	-
nlpB	83	91	0.91	ST4-74	2600708	2601742	-
dapA	168	178	0.94	ST4-74	2601759	2602637	-
gcvR	85	95	0.90	ST4-74	2602783	2603355	+
bcp	70	82	0.85	ST4-74	2603355	2603825	+
STM2492	9	7	1.27	ST4-74	2603872	2604114	-
perM	30	34	0.88	ST4-74	2604412	2605479	-
STM2494	28	27	1.03	ST4-74	2605687	2607150	+
yfgD	31	31	1.00	ST4-74	2607190	2607549	+
yfgE	52	48	1.08	ST4-74	2607576	2608301	-
uraA	11	21	0.53	ST4-74	2608372	2609661	-
upp	54	86	0.62	ST4-74	2609749	2610375	-
purM	76	106	0.72	ST4-74	2610774	2611826	+
purN	53	73	0.72	ST4-74	2611826	2612464	+
ppk	35	32	1.10	ST4-74	2612653	2614719	+
ppx	15	15	0.95	ST4-74	2614724	2616265	+
STM2503	7	18	0.42	ST4-74	2616301	2618514	-
STM2505	4	5	0.82	ST4-74	2618819	2619079	-
STM2506	378	469	0.81	ST4-74	2618927	2619118	+
STM2508	25	20	1.23	ST4-74	2619659	2619988	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM2509	3	3	0.81	ST4-74	2620008	2620364	-
guaA	23	30	0.76	ST4-74	2620521	2622098	-
guaB	34	36	0.94	ST4-74	2622168	2623634	-
xseA	32	20	1.62	ST4-74	2623795	2625144	+
shdA	4	3	1.16	ST4-74	2625306	2631425	-
ratB	6	5	1.04	ST4-74	2632119	2639425	-
ratA	10	7	1.39	ST4-74	2639591	2645188	-
sinI	3	3	1.18	ST4-74	2645308	2646267	-
sinH	2	2	1.02	ST4-74	2646325	2648517	-
yfgJ	23	25	0.92	ST4-74	2648863	2649084	-
engA	21	16	1.30	ST4-74	2649173	2650645	-
yfgL	110	96	1.14	ST4-74	2650764	2651942	-
yfgM	51	51	0.99	ST4-74	2651953	2652573	-
hisS	47	48	0.99	ST4-74	2652587	2653861	-
gcpE	40	42	0.94	ST4-74	2653972	2655090	-
yfgA	57	57	1.00	ST4-74	2655117	2656121	-
yfgB	18	20	0.92	ST4-74	2656413	2657579	-
ndk	98	103	0.95	ST4-74	2657786	2658217	-
STM2527	2	5	0.39	ST4-74	2658338	2659201	-
STM2528	5	11	0.43	ST4-74	2659201	2660010	-
STM2529	2	7	0.27	ST4-74	2660003	2660632	-
STM2530	2	8	0.30	ST4-74	2660629	2663007	-
pbpC	4	5	0.71	ST4-74	2663163	2665478	-
STM2532	20	24	0.85	ST4-74	2665479	2670413	-
STM2533	42	35	1.19	ST4-74	2670622	2671464	+
STM2534	12	9	1.26	ST4-74	2671711	2672379	+
STnc2070	1487	1725	0.86	ST4-74	2672469	2672567	-
RyfA	169	315	0.54	ST4-74	2672651	2672944	+
sseBb	7	10	0.65	ST4-74	2672948	2673733	-
pepB	11	15	0.70	ST4-74	2673834	2675117	-
yfhJ	46	57	0.81	ST4-74	2675364	2675564	-
fdx	36	50	0.71	ST4-74	2675576	2675911	-
hscA	18	29	0.61	ST4-74	2675913	2677763	-
hscB	25	32	0.80	ST4-74	2677776	2678291	-
iscA	110	137	0.80	ST4-74	2678487	2678810	-
nifU	70	85	0.82	ST4-74	2678839	2679225	-
iscS	49	69	0.71	ST4-74	2679253	2680467	-
yfhP	72	86	0.84	ST4-74	2680648	2681142	-
STM2545	160	119	1.35	ST4-74	2681302	2682033	-
suhB	132	105	1.25	ST4-74	2682152	2682955	+
STM2547	5	5	1.08	ST4-74	2683100	2683978	+
asrA	4	6	0.67	ST4-74	2684160	2685203	+
asrB	3	3	0.99	ST4-74	2685207	2686025	+
asrC	4	4	0.99	ST4-74	2686036	2687049	+
STM2551	13	14	0.97	ST4-74	2687050	2688036	-
STM2552	13	11	1.21	ST4-74	2688027	2688665	-
csiE	30	19	1.62	ST4-74	2688791	2690068	+
hcaT	19	19	0.97	ST4-74	2690063	2691202	-
glyA	142	178	0.80	ST4-74	2691398	2692651	-
hmpA	6	6	1.06	ST4-74	2692976	2694166	+
cadC	39	28	1.37	ST4-74	2694348	2695892	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
cadB	2	2	1.18	ST4-74	2696253	2697584	+
cadA	11	11	1.04	ST4-74	2697730	2699811	+
yjdL	7	7	1.05	ST4-74	2699867	2701327	+
glnB	140	148	0.94	ST4-74	2701376	2701714	-
yfhA	14	18	0.79	ST4-74	2701791	2703128	-
yfhG	26	28	0.91	ST4-74	2703125	2703889	-
yfhK	38	34	1.11	ST4-74	2703891	2705333	-
GlmY	8870	10575	0.84	ST4-74	2705381	2705564	-
purG	19	36	0.52	ST4-74	2705971	2709858	-
yfhD	34	22	1.55	ST4-74	2710114	2711658	+
yfhC	26	23	1.16	ST4-74	2711709	2712260	-
yfhB	25	22	1.11	ST4-74	2712285	2712920	-
STM2570	14	14	0.96	ST4-74	2712924	2714285	-
murQ	6	8	0.77	ST4-74	2714296	2715189	-
yfhH	5	5	0.99	ST4-74	2715305	2716153	+
STM2573	11	11	0.97	ST4-74	2716192	2717058	-
STM2574	4	3	1.46	ST4-74	2717131	2718327	-
STM2575	7	6	1.19	ST4-74	2718443	2719369	+
yfhL	115	69	1.67	ST4-74	2719407	2719667	+
acpS	17	22	0.75	ST4-74	2719779	2720159	-
pdxJ	13	16	0.81	ST4-74	2720159	2720890	-
recO	30	34	0.90	ST4-74	2720902	2721630	-
era	34	39	0.86	ST4-74	2721642	2722547	-
rnc	81	66	1.23	ST4-74	2722544	2723146	-
lepB	53	60	0.89	ST4-74	2723498	2724472	-
lepA	50	46	1.10	ST4-74	2724489	2726288	-
gogB	55	38	1.42	ST4-74	2726744	2728186	+
STM2585	357	85	4.21	ST4-74	2729142	2729588	+
STnc1380	538	842	0.64	ST4-74	2729634	2729701	-
SL1344_2548	28	15	1.85	ST4-74	2730253	2730318	-
STM2585A	1233	401	3.08	ST4-74	2730700	2730927	+
STM2586	1	1	0.63	ST4-74	2731024	2731602	-
STM2587	1	1	1.37	ST4-74	2731592	2732416	-
STM2588	3	3	0.91	ST4-74	2732413	2734785	-
SL1344_2552A	2	2	0.72	ST4-74	2734839	2735081	-
STM2589	3	3	0.96	ST4-74	2735120	2738482	-
STM2590	3	3	1.20	ST4-74	2738544	2739191	-
STM2591	3	3	1.00	ST4-74	2739089	2739826	-
STM2592	7	7	1.15	ST4-74	2739833	2740534	-
STM2593	9	9	1.02	ST4-74	2740541	2740870	-
STM2594	4	3	1.07	ST4-74	2740873	2743968	-
STnc3770	84	66	1.27	ST4-74	2743288	2743373	+
STM2595	3	1	2.05	ST4-74	2743940	2744257	-
STM2596	1	1	0.72	ST4-74	2744275	2744670	-
STM2597	5	5	1.06	ST4-74	2744721	2745467	-
STM2598	5	6	0.77	ST4-74	2745475	2745876	-
gipA	128	169	0.76	ST4-74	2745865	2747115	+
STM2600	3	3	0.91	ST4-74	2747164	2747742	-
STM2601	6	4	1.48	ST4-74	2747770	2748153	-
STM2602	5	6	0.95	ST4-74	2748164	2748523	-
STM2603	5	5	0.98	ST4-74	2748581	2749609	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM2604	2	2	0.91	ST4-74	2749664	2750011	-
STM2605	2	1	1.22	ST4-74	2750024	2751472	-
STM2606	3	3	1.11	ST4-74	2751510	2753090	-
STM2607	3	3	1.18	ST4-74	2753087	2753290	-
STM2608	2	2	1.01	ST4-74	2753274	2755205	-
STM2609	2	1	1.20	ST4-74	2755177	2755722	-
STM2610	41	35	1.18	ST4-74	2756009	2756410	+
STnc1920	362	175	2.08	ST4-74	2756549	2756646	+
STM2611	2	2	1.17	ST4-74	2756646	2757119	-
STM2612	8	6	1.47	ST4-74	2757116	2757568	-
STM2614	38	28	1.34	ST4-74	2758157	2758843	+
IsrI	84	36	2.38	ST4-74	2759018	2759265	-
STnc2080	1089	811	1.34	ST4-74	2759410	2759518	-
IsrJ	1020	860	1.19	ST4-74	2759646	2759717	-
STM2616	12	11	1.16	ST4-74	2759753	2760316	-
STnc1160	26385	28610	0.92	ST4-74	2760372	2760451	-
IsrK	604	775	0.78	ST4-74	2760480	2760556	-
STM2617	2	3	0.80	ST4-74	2760589	2761266	-
STM2618	1	1	1.25	ST4-74	2761263	2761403	-
STM2619	2	1	1.11	ST4-74	2761400	2762011	-
STM2620	23	28	0.84	ST4-74	2762220	2762822	-
SL1344_2583	60	53	1.12	ST4-74	2762857	2763105	-
STM2621	53	62	0.85	ST4-74	2763222	2763455	-
SL1344_2585	267	194	1.37	ST4-74	2763714	2763905	-
SL1344_2586	4	4	0.99	ST4-74	2764017	2764298	-
SL1344_2587	3	4	0.68	ST4-74	2764291	2764944	-
SL1344_2588	3	2	1.31	ST4-74	2764947	2765417	-
SL1344_2589	2	2	1.04	ST4-74	2765419	2766084	-
gpP	2	2	1.04	ST4-74	2766099	2766791	-
gpO	1	2	0.81	ST4-74	2766788	2767693	-
STnc1390	919	1156	0.80	ST4-74	2767704	2767767	+
cIIa	2	2	0.73	ST4-74	2767785	2768207	-
SL1344_2593	353	348	1.01	ST4-74	2768450	2768833	+
SL1344_2594	406	393	1.03	ST4-74	2768985	2770070	+
SL1344_2595	134	104	1.29	ST4-74	2770178	2770384	-
STM2629	2	2	0.92	ST4-74	2770636	2770791	+
STnc1400	536	866	0.62	ST4-74	2770951	2771060	-
recEb	2	2	0.93	ST4-74	2771095	2774295	+
STM2633	3	3	0.79	ST4-74	2774306	2775415	+
STM2634	3	2	1.40	ST4-74	2775458	2775697	+
STM2635	1	3	0.33	ST4-74	2775738	2776022	+
STM2636	7	6	1.20	ST4-74	2776000	2777229	-
rseC	17	19	0.89	ST4-74	2777727	2778206	-
rseB	39	39	1.00	ST4-74	2778203	2779159	-
rseA	128	124	1.03	ST4-74	2779159	2779809	-
rpoE	168	148	1.14	ST4-74	2779841	2780416	-
nadB	11	12	0.91	ST4-74	2780841	2782463	+
yfiC	23	22	1.04	ST4-74	2782448	2783185	-
srmB	31	27	1.12	ST4-74	2783316	2784650	+
yfiE	16	13	1.25	ST4-74	2784668	2785567	-
yfiK	10	6	1.75	ST4-74	2785670	2786257	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
yfiD	26	905	0.03	ST4-74	2786319	2786702	-
ung	57	54	1.05	ST4-74	2787021	2787710	+
yfiF	13	14	0.91	ST4-74	2787826	2788863	-
trxC	97	90	1.09	ST4-74	2789067	2789486	+
yfiP	26	24	1.09	ST4-74	2789559	2790239	+
yfiQ	45	40	1.13	ST4-74	2790293	2792953	+
pssA	52	48	1.10	ST4-74	2793068	2794423	+
yfiM	12	12	0.96	ST4-74	2794468	2794791	+
kgtP	14	10	1.38	ST4-74	2794788	2796089	-
STM2655	24	11	2.06	ST4-74	2796193	2796648	-
clpB	425	447	0.95	ST4-74	2802529	2805102	-
yfiH	61	45	1.35	ST4-74	2805232	2805963	-
rluD	180	119	1.50	ST4-74	2805960	2806940	-
yfiO	131	123	1.07	ST4-74	2807072	2807809	+
STnc3160	112	133	0.84	ST4-74	2807810	2807900	-
yfiA	4097	4054	1.01	ST4-74	2808081	2808419	+
STnc2090	17527	9266	1.89	ST4-74	2808306	2808464	+
sRNA3	1428	1110	1.29	ST4-74	2808504	2808645	+
pheA	16	23	0.72	ST4-74	2808670	2809830	+
STM2668	14	10	1.39	ST4-74	2809791	2810699	-
STnc3790	53	30	1.80	ST4-74	2810663	2810737	+
tyrA	37	52	0.70	ST4-74	2810757	2811824	-
aroF	72	110	0.66	ST4-74	2811888	2812958	-
yfiR	30	23	1.29	ST4-74	2813398	2813916	+
yfiN	7	8	0.93	ST4-74	2813909	2815129	+
int	55	53	1.04	ST4-74	2815382	2816431	-
SL1344_2632	67	78	0.86	ST4-74	2816456	2816794	-
SL1344_2633	57	54	1.06	ST4-74	2816803	2817648	-
SL1344_2634	1	1	1.20	ST4-74	2817762	2818115	+
cIIb	1	1	1.60	ST4-74	2818166	2818675	+
STM2735	1	1	0.98	ST4-74	2818683	2818883	+
SL1344_2637	0	1	0.41	ST4-74	2818847	2819185	+
STM2732	1	0	2.18	ST4-74	2819253	2819480	+
SL1344_2639	1	2	0.65	ST4-74	2819480	2819704	+
SL1344_2640	1	0	2.00	ST4-74	2819701	2820222	+
SL1344_2641	2	2	1.31	ST4-74	2820291	2823074	+
gpF	13	7	1.69	ST4-74	2823088	2823573	+
gpD	4	5	0.90	ST4-74	2823570	2824736	+
STnc3800	112	68	1.65	ST4-74	2824672	2825000	-
SL1344_2644	62	68	0.90	ST4-74	2824931	2825620	+
gpB	113	91	1.24	ST4-74	2825697	2825915	+
rpl19	351	404	0.87	ST4-74	2826061	2826408	-
trmD	155	227	0.69	ST4-74	2826449	2827216	-
rimM	173	252	0.69	ST4-74	2827261	2827809	-
rps16	964	1129	0.85	ST4-74	2827828	2828076	-
ffh	37	38	0.96	ST4-74	2828390	2829751	-
corE	57	49	1.16	ST4-74	2829917	2830708	+
corB	24	19	1.23	ST4-74	2830773	2832014	+
STnc4230	155	102	1.52	ST4-74	2831934	2832038	+
STM2680	17	17	1.00	ST4-74	2832135	2832740	+
grpE	242	259	0.93	ST4-74	2832775	2833365	-

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ppnK	19	18	1.09	ST4-74	2833488	2834366	+
recN	9	8	1.05	ST4-74	2834452	2836113	+
smpA	175	182	0.96	ST4-74	2836262	2836600	+
rnfH	52	44	1.18	ST4-74	2836766	2837056	-
yfjG	131	120	1.08	ST4-74	2837046	2837501	-
smpB	125	130	0.96	ST4-74	2837672	2838154	+
bapA	3	2	1.43	ST4-74	2838768	2850242	+
IsrL	31	29	1.07	ST4-74	2850244	2850588	-
STM2690	2	1	1.39	ST4-74	2850307	2851716	+
STnc3070	405	311	1.30	ST4-74	2851166	2851225	-
STM2691	3	2	1.76	ST4-74	2851713	2853893	+
STM2692	3	2	1.54	ST4-74	2853901	2855064	+
STM2694	12	11	1.05	ST4-74	2855616	2855834	-
STM2695	1	1	0.96	ST4-74	2855903	2857003	-
gpU	2	1	1.99	ST4-74	2857000	2857485	-
STM2697	2	2	0.97	ST4-74	2857482	2860289	-
gpE'	1	0	1.85	ST4-74	2860282	2860401	-
gpE	1	1	1.03	ST4-74	2860416	2860718	-
STM2700	2	2	1.21	ST4-74	2860773	2861288	-
STM2701	2	2	0.82	ST4-74	2861298	2862470	-
STM2702	6	5	1.15	ST4-74	2862587	2862790	-
sopE	49	72	0.67	ST4-74	2863004	2863726	+
STM2704	7	6	1.05	ST4-74	2863923	2864330	-
STM2706	3	2	1.14	ST4-74	2864337	2865956	-
gpI	1	2	0.51	ST4-74	2865953	2866558	-
gpJ	2	2	1.26	ST4-74	2866551	2867459	-
gpW	1	2	0.47	ST4-74	2867446	2867805	-
STM2710	3	2	1.21	ST4-74	2867802	2868380	-
STM2711	0	0	0.65	ST4-74	2868449	2868895	-
STM2712	1	1	1.15	ST4-74	2868888	2869319	-
STM2713	1	1	0.75	ST4-74	2869282	2869485	-
STM2714	1	0	1.09	ST4-74	2869415	2869843	-
SL1344_2685	4	3	1.31	ST4-74	2869840	2870214	-
nucD2	1	1	0.87	ST4-74	2870219	2870689	-
nucE2	3	3	0.99	ST4-74	2870709	2870924	-
STM2717	2	2	0.92	ST4-74	2870928	2871131	-
STM2718	2	1	1.54	ST4-74	2871131	2871595	-
STM2719	1	1	0.71	ST4-74	2871689	2872342	-
STM2720	2	1	1.07	ST4-74	2872346	2873428	-
STM2721	0	0	0.77	ST4-74	2873445	2874278	-
STM2722	1	1	1.51	ST4-74	2874421	2876187	+
STM2723	1	1	1.34	ST4-74	2876187	2877218	+
SL1344_2695	49	80	0.61	ST4-74	2877245	2878222	-
SL1344_2696	239	357	0.67	ST4-74	2878212	2878793	-
SL1344_2697	101	221	0.46	ST4-74	2879070	2879447	-
SL1344_2698	79	225	0.35	ST4-74	2879425	2880480	-
STM2727	10	12	0.82	ST4-74	2880640	2880873	-
STM2728	20	20	1.04	ST4-74	2880885	2881073	-
STM2729	3	3	1.11	ST4-74	2881397	2883640	-
STM2730	3	3	0.96	ST4-74	2883631	2884488	-
STM2731	1	1	1.06	ST4-74	2884485	2884712	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM2733	1	1	0.64	ST4-74	2885007	2885348	-
SL1344_2705	6	4	1.47	ST4-74	2885312	2885608	-
cIIc	1	1	1.25	ST4-74	2885953	2886462	-
apl	0	2	0.28	ST4-74	2886495	2886743	-
cI	27	28	0.97	ST4-74	2886920	2887495	+
STM2739	8	8	0.93	ST4-74	2887497	2888522	+
SL1344_2710	15	17	0.88	ST4-74	2888519	2889730	+
STM2740	9	8	1.15	ST4-74	2890073	2891269	+
SL1344_2713	35	56	0.63	ST4-74	2891273	2892970	-
SL1344_2714	61	102	0.60	ST4-74	2892957	2893190	-
SL1344_2715	65	92	0.70	ST4-74	2893177	2893725	-
SL1344_2716	0	1	0.56	ST4-74	2894442	2895008	-
SL1344_2717	3	2	1.43	ST4-74	2895025	2895267	-
SL1344_2718	2	2	0.95	ST4-74	2895264	2896067	-
SL1344_2719	3847	5429	0.71	ST4-74	2896802	2897353	+
SL1344_2720	1	2	0.62	ST4-74	2897350	2897577	+
SL1344_2721	1	1	1.36	ST4-74	2897574	2897894	+
SL1344_2722	3	3	0.90	ST4-74	2897858	2900242	+
STM2740	16	14	1.16	ST4-74	2900734	2901979	+
STM2741	8	7	1.14	ST4-74	2901981	2902619	+
STM2742	38	35	1.07	ST4-74	2903009	2904202	+
STM2743	50	48	1.03	ST4-74	2904537	2905364	-
STM2744	3	2	1.43	ST4-74	2905815	2906030	+
STM2745	1	1	1.12	ST4-74	2906066	2908135	+
STM2746	74	73	1.01	ST4-74	2908638	2909921	+
STM2747	28	34	0.81	ST4-74	2909966	2910784	+
STM2748	156	153	1.02	ST4-74	2910939	2911229	-
STM2749	4	4	0.82	ST4-74	2911390	2911674	+
STM2750	2	2	0.95	ST4-74	2911787	2912308	+
STM2751	2	3	0.93	ST4-74	2912305	2912679	+
STM2752	2	2	1.10	ST4-74	2912676	2913656	+
STM2753	3	5	0.56	ST4-74	2913667	2914680	+
STM2754	8	10	0.80	ST4-74	2914975	2916177	+
hxlA	3	3	0.96	ST4-74	2916251	2916886	-
hxlB	2	1	2.37	ST4-74	2916910	2917473	-
STM2757	2	2	0.99	ST4-74	2917473	2918315	-
STM2758	2	2	1.30	ST4-74	2918445	2919986	-
STM2759	3	2	1.16	ST4-74	2920209	2921888	+
STM2760	2	1	1.42	ST4-74	2923005	2923880	+
STM2761	7	5	1.38	ST4-74	2924046	2925920	+
STM2762	16	12	1.38	ST4-74	2926180	2927463	-
IsrM	8	3	2.40	ST4-74	2927616	2927944	+
STM2763	24	23	1.06	ST4-74	2928000	2928911	+
STM2764	7	8	0.87	ST4-74	2928907	2929495	-
IsrN	4	2	2.35	ST4-74	2929501	2929643	+
STM2765	15	18	0.84	ST4-74	2929642	2929908	-
STM2766	34	34	0.99	ST4-74	2930361	2930999	-
STM2767	79	77	1.03	ST4-74	2930996	2932978	-
STM2768	8	8	1.02	ST4-74	2933257	2933556	+
STM2769	11	9	1.20	ST4-74	2933553	2934419	+
fljA	7	7	1.08	ST4-74	2935199	2935738	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
fljB	54	218	0.25	ST4-74	2935806	2937326	-
hin	28	20	1.39	ST4-74	2937418	2937990	+
iroB	3	2	1.31	ST4-74	2938953	2940068	+
iroC	3	3	1.01	ST4-74	2940149	2943802	+
iroD	2	1	1.38	ST4-74	2943912	2945156	+
iroE	4	3	1.44	ST4-74	2945188	2946123	+
iroN	4	4	1.12	ST4-74	2946165	2948345	-
pipB2	343	78	4.40	ST4-74	2949378	2950430	-
virK	1248	691	1.81	ST4-74	2950949	2951878	+
mig-14	857	501	1.71	ST4-74	2952171	2953067	+
nixA	31	29	1.06	ST4-74	2953789	2954802	-
tctE	7	11	0.62	ST4-74	2954935	2956350	-
tctD	12	14	0.86	ST4-74	2956337	2957011	-
STM2786	18	25	0.73	ST4-74	2957166	2958143	+
STM2787	7	9	0.69	ST4-74	2958155	2958589	+
STM2788	11	16	0.67	ST4-74	2958600	2960114	+
csiD	8	5	1.77	ST4-74	2960408	2961394	+
ygaF	5	3	1.60	ST4-74	2961420	2962688	+
gabD	3	2	1.63	ST4-74	2962710	2964158	+
gabT	4	3	1.58	ST4-74	2964173	2965456	+
gabP	9	6	1.52	ST4-74	2965586	2966986	+
ygaE	8	7	1.15	ST4-74	2967028	2967705	+
ygaU	383	176	2.19	ST4-74	2967727	2968176	-
yqaE	749	330	2.27	ST4-74	2968276	2968434	-
STM2797	13	12	1.06	ST4-74	2968677	2968916	+
ygaP	8	9	0.91	ST4-74	2968926	2969453	+
stpA	295	336	0.88	ST4-74	2969801	2970202	-
STM2800	100	19	5.26	ST4-74	2970901	2971350	+
ygaC	35	19	1.80	ST4-74	2971385	2971735	-
ygaM	253	157	1.62	ST4-74	2971885	2972223	+
STM2803	9	7	1.24	ST4-74	2972307	2973641	-
STM2804	3	3	1.00	ST4-74	2973730	2974161	+
SL1344_2789	1	1	0.64	ST4-74	2974158	2974334	+
STnc3080	12	9	1.39	ST4-74	2974260	2974404	-
nrdH	16	11	1.48	ST4-74	2974433	2974678	+
nrdI	4	2	1.93	ST4-74	2974675	2975085	+
nrdE	4	2	1.80	ST4-74	2975058	2977202	+
nrdF	12	8	1.37	ST4-74	2977213	2978172	+
proV	67	91	0.73	ST4-74	2978527	2979729	+
proW	30	60	0.51	ST4-74	2979722	2980786	+
proX	28	59	0.48	ST4-74	2980856	2981851	+
STM2812	7	7	0.97	ST4-74	2982016	2983200	+
emrR	56	65	0.87	ST4-74	2983697	2984227	+
emrA	17	16	1.04	ST4-74	2984354	2985526	+
emrB	9	10	0.86	ST4-74	2985543	2987081	+
STM2816	5	5	1.02	ST4-74	2987130	2988500	-
luxS	84	85	0.98	ST4-74	2988846	2989361	-
MicA	6889	4862	1.42	ST4-74	2989429	2989502	+
gshA	26	23	1.14	ST4-74	2989511	2991067	-
yqaA	34	35	0.98	ST4-74	2991144	2991569	-
yqaB	73	79	0.92	ST4-74	2991566	2992132	-

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csrA	1495	1166	1.28	ST4-74	2993527	2993712	-
alaS	53	63	0.84	ST4-74	2993947	2996577	-
oraA	21	15	1.36	ST4-74	2996813	2997313	-
recA	55	56	0.97	ST4-74	2997430	2998491	-
ygaD	33	33	0.99	ST4-74	2998576	2999073	-
mltB	30	25	1.21	ST4-74	2999551	3000630	-
srlA	3	4	0.64	ST4-74	3000884	3001447	+
srlE	2	3	0.68	ST4-74	3001444	3002415	+
slrB	1	2	0.66	ST4-74	3002427	3002789	+
gutD	4	5	0.78	ST4-74	3002801	3003580	+
gutM	30	25	1.22	ST4-74	3003656	3004015	+
srlR	15	21	0.74	ST4-74	3004213	3004986	+
gutQ	9	14	0.69	ST4-74	3004979	3005944	+
norR	5	4	1.17	ST4-74	3005941	3007461	-
norV	2	3	0.91	ST4-74	3007647	3009086	+
norW	2	2	1.08	ST4-74	3009083	3010216	+
C0664	2	3	0.74	ST4-74	3010214	3010321	+
hydA	6	7	0.80	ST4-74	3010313	3012553	-
hydN	3	5	0.61	ST4-74	3012699	3013244	-
STM2844	3	5	0.64	ST4-74	3013445	3014254	-
hycI	3	5	0.60	ST4-74	3014281	3014751	-
hycH	3	4	0.91	ST4-74	3014744	3015154	-
hycG	3	3	0.90	ST4-74	3015151	3015918	-
hycF	2	4	0.53	ST4-74	3015918	3016460	-
hycE	3	3	0.97	ST4-74	3016470	3018179	-
hycD	3	3	1.02	ST4-74	3018197	3019120	-
hycC	4	5	0.77	ST4-74	3019123	3020949	-
hycB	2	2	0.83	ST4-74	3020949	3021557	-
hycA	2	5	0.37	ST4-74	3021703	3022164	-
STM2854	20	85	0.23	ST4-74	3022374	3022730	+
hypB	8	26	0.31	ST4-74	3022799	3023671	+
hypC	10	26	0.39	ST4-74	3023662	3023934	+
hypD	4	17	0.22	ST4-74	3023934	3025055	+
hypE	7	24	0.30	ST4-74	3025052	3026062	+
fhlA	7	10	0.69	ST4-74	3026281	3028359	+
ygbA	8	9	0.92	ST4-74	3028421	3028765	-
sitA	11	9	1.30	ST4-74	3028947	3029864	+
sitB	9	6	1.54	ST4-74	3029861	3030682	+
sitC	8	6	1.36	ST4-74	3030679	3031539	+
sitD	15	13	1.13	ST4-74	3031530	3032378	+
avrA	53	46	1.14	ST4-74	3032477	3033382	-
sprB	8	12	0.66	ST4-74	3033543	3034298	-
hilC	35	39	0.91	ST4-74	3034683	3035570	-
orgC	84	69	1.22	ST4-74	3035915	3036367	-
orgB	116	90	1.29	ST4-74	3036364	3037044	-
orgA	67	53	1.27	ST4-74	3037001	3037600	-
STnc4240	192	177	1.08	ST4-74	3037101	3037394	+
prgK	20	38	0.53	ST4-74	3037572	3038330	-
prgJ	20	38	0.52	ST4-74	3038327	3038632	-
prgI	19	34	0.54	ST4-74	3038651	3038893	-
STnc3020	18	14	1.33	ST4-74	3038687	3038786	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
prgH	27	42	0.63	ST4-74	3038918	3040096	-
hilD	97	110	0.88	ST4-74	3040412	3041341	+
hilA	8	13	0.62	ST4-74	3042432	3044093	+
iagB	26	32	0.82	ST4-74	3044111	3044593	+
sptP	58	59	0.98	ST4-74	3044647	3046278	-
sicP	102	100	1.02	ST4-74	3046265	3046657	-
iacP	136	111	1.23	ST4-74	3046988	3047236	-
sipA	11	16	0.69	ST4-74	3047255	3049312	-
STnc1410	142	106	1.35	ST4-74	3048830	3049012	+
sipD	8	12	0.67	ST4-74	3049331	3050362	-
sipC	23	42	0.54	ST4-74	3050433	3051662	-
sipB	12	25	0.47	ST4-74	3051690	3053471	-
sicA	38	61	0.62	ST4-74	3053474	3053971	-
spaS	3	6	0.57	ST4-74	3054109	3055179	-
spaR	7	10	0.69	ST4-74	3055166	3055957	-
spaQ	11	19	0.57	ST4-74	3055961	3056221	-
spaP	7	12	0.60	ST4-74	3056247	3056921	-
spaO	6	11	0.57	ST4-74	3056911	3057822	-
invJ	3	7	0.47	ST4-74	3057822	3058832	-
invI	11	18	0.58	ST4-74	3058832	3059275	-
invC	5	10	0.56	ST4-74	3059253	3060548	-
invB	16	27	0.60	ST4-74	3060545	3060952	-
invA	16	26	0.63	ST4-74	3060976	3063033	-
invE	11	17	0.62	ST4-74	3063058	3064176	-
invG	11	18	0.58	ST4-74	3064173	3065861	-
invF	17	27	0.61	ST4-74	3065858	3066508	-
invH	14	22	0.66	ST4-74	3066965	3067408	+
InvR	1206	1290	0.94	ST4-74	3067500	3067579	+
STM2901	102	109	0.94	ST4-74	3067839	3068288	+
STM2902	21	23	0.91	ST4-74	3068273	3068620	+
STM2903	61	32	1.92	ST4-74	3068893	3069219	-
SL1344_2883	2	1	1.16	ST4-74	3069460	3069567	+
STM2904	98	83	1.19	ST4-74	3069908	3070198	+
STM2905	17	15	1.08	ST4-74	3070195	3070722	+
STM2906	4	3	1.23	ST4-74	3070798	3071012	-
pphB	10	7	1.54	ST4-74	3071347	3072003	+
STM2908	5	3	1.79	ST4-74	3072175	3072696	-
mutS	11	14	0.80	ST4-74	3072897	3075422	+
STM2910	5	4	1.21	ST4-74	3075478	3075858	-
STM2911	3	2	1.34	ST4-74	3075855	3077063	-
STM2912	4	4	0.95	ST4-74	3077151	3078083	+
STM2913	6	6	0.97	ST4-74	3078125	3079621	-
STM2914	4	2	1.68	ST4-74	3079618	3080568	-
hyi	2	2	0.99	ST4-74	3080608	3081369	-
ygbL	2	2	1.02	ST4-74	3081374	3082012	-
ygbK	1	1	1.09	ST4-74	3082009	3083271	-
ygbJ	2	3	0.91	ST4-74	3083265	3084188	-
ygbI	15	15	1.03	ST4-74	3084385	3085149	+
STM2920	33	32	1.03	ST4-74	3085168	3085572	-
STM2921	3	2	1.17	ST4-74	3085743	3086336	+
STM2922	2	1	1.45	ST4-74	3086336	3087763	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM2923	2	3	0.89	ST4-74	3087774	3088010	+
rpoS	372	400	0.93	ST4-74	3088055	3089047	-
nlpD	587	602	0.97	ST4-74	3089110	3090243	-
pcm	26	29	0.90	ST4-74	3090419	3091045	-
surE	28	30	0.94	ST4-74	3091039	3091800	-
truD	23	25	0.93	ST4-74	3091781	3092830	-
ispF	10	12	0.85	ST4-74	3092827	3093306	-
ispD	12	15	0.77	ST4-74	3093306	3094016	-
ftsB	82	77	1.05	ST4-74	3094035	3094346	-
ygbE	44	39	1.14	ST4-74	3094537	3094893	-
cysC	85	88	0.97	ST4-74	3094911	3095516	-
cysN	63	71	0.88	ST4-74	3095503	3096942	-
cysD	212	219	0.97	ST4-74	3096952	3097860	-
iap	8	6	1.31	ST4-74	3098111	3099157	+
ygbF	9	9	0.99	ST4-74	3100256	3100552	-
STM2938	7	6	1.16	ST4-74	3100549	3101469	-
ygcH	4	3	1.12	ST4-74	3101466	3102116	-
STM2940	3	3	0.82	ST4-74	3102098	3102844	-
yghJ	3	4	0.74	ST4-74	3102855	3103913	-
STM2942	3	4	0.75	ST4-74	3103927	3104487	-
STM2943	2	3	0.84	ST4-74	3104484	3106040	-
ygcB	5	6	0.87	ST4-74	3106052	3108715	-
sopD	9	9	1.00	ST4-74	3109160	3110113	+
STnc3140	679	560	1.21	ST4-74	3109885	3109981	-
cysH	52	59	0.89	ST4-74	3110201	3110935	-
cysI	74	84	0.88	ST4-74	3111011	3112723	-
cysJ	58	63	0.92	ST4-74	3112723	3114522	-
ptpS	15	15	0.99	ST4-74	3114946	3115308	+
STM2950	17	18	0.96	ST4-74	3115396	3116193	-
ygcF	86	68	1.28	ST4-74	3118020	3118691	-
eno	193	301	0.64	ST4-74	3118827	3120125	-
pyrG	122	134	0.91	ST4-74	3120208	3121845	-
mazG	21	20	1.08	ST4-74	3122073	3122873	-
SL1344_2934	1	1	1.24	ST4-74	3123527	3123631	+
SL1344_2935	47	35	1.34	ST4-74	3123641	3123898	-
STM2955	247	180	1.38	ST4-74	3123903	3124178	-
relA	53	57	0.93	ST4-74	3124343	3126577	-
rumA	93	74	1.27	ST4-74	3126629	3127924	-
barA	11	15	0.71	ST4-74	3127982	3130738	+
STM2959	9	7	1.32	ST4-74	3130782	3131924	-
gudD	6	5	1.18	ST4-74	3132002	3133342	-
ygcY	7	5	1.42	ST4-74	3133363	3134703	-
gudT	12	7	1.60	ST4-74	3134700	3136058	-
STM2963	50	57	0.88	ST4-74	3136539	3136988	-
yqcB	21	19	1.08	ST4-74	3137007	3137789	-
yqcC	15	19	0.78	ST4-74	3137789	3138118	-
CsrB	24986	23151	1.08	ST4-74	3138160	3138522	-
syd	76	71	1.07	ST4-74	3138730	3139275	-
queF	24	21	1.10	ST4-74	3139344	3140192	+
ygdH	55	64	0.87	ST4-74	3140305	3141669	+
sdaC	26	17	1.50	ST4-74	3142234	3143523	+

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sdaB	13	10	1.37	ST4-74	3143582	3144949	+
exo	15	14	1.09	ST4-74	3145060	3145875	+
fucO	4	4	0.87	ST4-74	3145967	3147115	-
fucA	3	5	0.68	ST4-74	3147132	3147779	-
fucP	3	3	0.89	ST4-74	3148334	3149650	+
fucI	1	2	0.64	ST4-74	3149682	3151457	+
fucK	2	2	0.81	ST4-74	3151558	3152976	+
fucU	2	3	0.91	ST4-74	3152978	3153400	+
fucR	14	21	0.67	ST4-74	3153458	3154168	+
ygdE	34	37	0.90	ST4-74	3154227	3155327	-
ygdD	22	26	0.83	ST4-74	3155320	3155715	-
gcvA	43	34	1.27	ST4-74	3155734	3156651	-
GcvB	2629	204	12.85	ST4-74	3156779	3156979	+
ygdI	1573	801	1.96	ST4-74	3156998	3157225	-
csdA	12	13	0.87	ST4-74	3157418	3158623	+
ygdK	6	8	0.77	ST4-74	3158623	3159066	+
STM2986	4	30	0.13	ST4-74	3159339	3160253	+
ygdL	50	48	1.06	ST4-74	3160305	3161111	-
mltA	58	58	1.01	ST4-74	3161221	3162318	-
amiC	60	56	1.08	ST4-74	3162818	3164071	-
argA	88	139	0.63	ST4-74	3164304	3165635	+
recD	5	6	0.94	ST4-74	3165738	3167573	-
recB	9	11	0.80	ST4-74	3167570	3171115	-
ptr	12	13	0.91	ST4-74	3171108	3173996	-
recC	9	8	1.10	ST4-74	3174174	3177545	-
comQ	8	8	0.99	ST4-74	3177561	3177881	-
comP	4	3	1.11	ST4-74	3177866	3178273	-
comO	5	5	0.90	ST4-74	3178270	3178833	-
comN	4	5	0.89	ST4-74	3178824	3179294	-
thyA	23	21	1.10	ST4-74	3179479	3180273	-
lgt	42	42	1.01	ST4-74	3180280	3181155	-
ptsP	30	31	0.95	ST4-74	3181371	3183617	-
nudH	168	169	0.99	ST4-74	3183630	3184160	-
mutH	29	36	0.81	ST4-74	3184843	3185538	+
ygdQ	47	41	1.16	ST4-74	3185720	3186433	+
STnc3830	58	61	0.96	ST4-74	3186446	3186537	-
ygdR	260	314	0.83	ST4-74	3186603	3186821	+
tas	21	23	0.93	ST4-74	3187012	3188052	+
ygeD	16	17	0.90	ST4-74	3188139	3189341	-
aas	19	19	1.00	ST4-74	3189334	3191493	-
OmrA	192	109	1.77	ST4-74	3191584	3191670	-
OmrB	248	178	1.39	ST4-74	3191785	3191870	-
galR	30	33	0.91	ST4-74	3192086	3193114	+
STM3012	16	17	0.98	ST4-74	3193125	3194147	+
lysA	27	30	0.90	ST4-74	3194157	3195419	-
lysR	4	5	0.84	ST4-74	3195537	3196472	+
ygeA	41	38	1.08	ST4-74	3196444	3197151	-
araE	6	7	0.78	ST4-74	3197264	3198682	-
kduD	9	10	0.86	ST4-74	3199036	3199797	-
kduI	3	4	0.70	ST4-74	3199854	3200690	-
yqeF	15	16	0.94	ST4-74	3201120	3202298	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM3020	17	21	0.81	ST4-74	3202421	3203284	-
STM3021	18	13	1.41	ST4-74	3203361	3203843	+
STM3022	13	11	1.15	ST4-74	3203985	3205214	+
yohL	49	130	0.38	ST4-74	3205245	3205517	-
yohM	5	28	0.20	ST4-74	3205639	3206475	+
STM3025	5	3	1.64	ST4-74	3206685	3207383	-
STM3025.1N	2	2	0.88	ST4-74	3207430	3207942	-
STM3026	4	4	0.91	ST4-74	3208086	3209162	-
stdC	2	1	1.66	ST4-74	3209159	3209902	-
stdB	2	2	1.01	ST4-74	3209943	3212432	-
stdA	3	2	1.28	ST4-74	3212552	3213262	-
STM3030	35	32	1.09	ST4-74	3213855	3214487	-
STM3031	4	3	1.27	ST4-74	3214534	3215070	-
STnc290	460	501	0.92	ST4-74	3215639	3215717	-
STM3033	84	84	1.01	ST4-74	3215876	3216274	-
STM3034	412	365	1.13	ST4-74	3216274	3216501	-
STM3036	21	9	2.28	ST4-74	3217204	3218010	+
STM3038	46	45	1.01	ST4-74	3218295	3219053	-
IsrO	81	48	1.69	ST4-74	3219100	3219300	+
idi	50	36	1.38	ST4-74	3219318	3219863	+
lysS	45	57	0.78	ST4-74	3219939	3221456	-
prfB	67	68	0.99	ST4-74	3221466	3222542	-
recJ	18	20	0.87	ST4-74	3222669	3224402	-
dsbC	29	31	0.92	ST4-74	3224408	3225082	-
xerD	33	33	1.01	ST4-74	3225145	3226041	-
fldB	25	27	0.93	ST4-74	3226154	3226675	+
ygfX	56	58	0.96	ST4-74	3226728	3227141	-
ygfY	110	114	0.96	ST4-74	3227122	3227388	-
ygfZ	29	33	0.89	ST4-74	3227638	3228618	+
yqfA	62	46	1.34	ST4-74	3228734	3229393	-
yqfB	74	98	0.75	ST4-74	3229557	3229868	-
bglA	12	13	0.95	ST4-74	3230027	3231460	+
STM3052	8	7	1.15	ST4-74	3231508	3232401	-
gcvP	10	9	1.13	ST4-74	3232857	3235730	-
gcvH	37	29	1.28	ST4-74	3235893	3236282	-
gcvT	16	12	1.37	ST4-74	3236308	3237402	-
visC	21	22	0.98	ST4-74	3237857	3239059	-
ubiH	23	21	1.09	ST4-74	3239185	3240363	-
pepP	34	32	1.08	ST4-74	3240360	3241502	-
ygfB	58	57	1.01	ST4-74	3241702	3242280	-
ygfE	106	101	1.05	ST4-74	3242447	3242776	+
SsrS	87372	116036	0.75	ST4-74	3242594	3243000	+
ygfA	11	10	1.02	ST4-74	3243142	3243618	+
RygC	7077	8117	0.87	ST4-74	3243669	3243813	+
serA	119	128	0.93	ST4-74	3243999	3245231	-
rpiA	92	99	0.92	ST4-74	3245497	3246156	-
iciA	9	11	0.83	ST4-74	3246310	3247203	+
yggE	59	37	1.60	ST4-74	3247469	3248215	-
yggA	13	8	1.54	ST4-74	3248309	3248944	-
yggB	90	75	1.20	ST4-74	3249144	3250004	-
STnc3030	94	115	0.82	ST4-74	3250106	3250210	+

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fba	99	126	0.78	ST4-74	3250231	3251310	-
pgk	56	76	0.73	ST4-74	3251412	3252575	-
epd	74	83	0.88	ST4-74	3252597	3253643	-
STM3071	6	22	0.27	ST4-74	3254017	3254442	+
STM3072	4	15	0.28	ST4-74	3254468	3255046	+
STM3073	6	11	0.53	ST4-74	3255047	3255754	+
STM3074	3	7	0.41	ST4-74	3255742	3256419	+
STM3075	4	8	0.50	ST4-74	3256413	3257069	+
tktA	39	63	0.62	ST4-74	3257113	3259059	-
yggG	21	17	1.25	ST4-74	3259380	3260138	+
speB	11	12	0.89	ST4-74	3260239	3261159	-
STnc750	297	259	1.15	ST4-74	3261185	3261273	-
STM3079	7	6	1.20	ST4-74	3261364	3262254	-
STM3080	4	3	1.10	ST4-74	3262534	3263007	-
STM3081	6	6	1.13	ST4-74	3263046	3264053	-
STM3082	3	3	0.86	ST4-74	3264067	3265083	-
STM3083	2	1	1.35	ST4-74	3265086	3266558	-
STM3084	83	77	1.09	ST4-74	3267800	3268549	-
STM3085	5	5	1.14	ST4-74	3268754	3269485	-
speA	32	23	1.37	ST4-74	3269629	3271542	-
yqgB	239	148	1.62	ST4-74	3271614	3271745	-
yqgD	52	41	1.25	ST4-74	3272040	3272339	-
metK	150	194	0.77	ST4-74	3272395	3273549	+
STnc3150	27	13	2.07	ST4-74	3273499	3273824	-
galP	27	20	1.34	ST4-74	3274081	3275436	+
sprT	22	15	1.46	ST4-74	3275515	3276012	+
STnc3850	26	26	0.98	ST4-74	3275931	3276099	-
endA	9	7	1.19	ST4-74	3276107	3276814	+
yggJ	33	28	1.19	ST4-74	3276843	3277622	+
gshB	19	19	0.98	ST4-74	3277642	3278589	+
yqgE	37	32	1.16	ST4-74	3278805	3279368	+
yqgF	9	10	0.87	ST4-74	3279368	3279784	+
iclR	13	11	1.19	ST4-74	3279831	3280517	-
pilT	4	4	1.00	ST4-74	3280649	3281629	-
yggS	36	44	0.83	ST4-74	3281647	3282351	+
yggT	28	31	0.89	ST4-74	3282370	3282936	+
yggU	27	33	0.82	ST4-74	3282933	3283223	+
yggV	20	25	0.80	ST4-74	3283231	3283824	+
STnc3860	47	46	1.04	ST4-74	3283748	3283844	-
yggW	10	11	0.92	ST4-74	3283817	3284953	+
yggM	10	9	1.14	ST4-74	3285044	3286051	-
ansB	16	45	0.35	ST4-74	3286184	3287230	-
yggN	48	38	1.26	ST4-74	3287419	3288138	-
yggL	182	164	1.11	ST4-74	3288188	3288514	-
trmB	98	87	1.12	ST4-74	3288514	3289233	-
mutY	28	27	1.04	ST4-74	3289388	3290440	+
yggX	50	61	0.82	ST4-74	3290468	3290743	+
mltC	18	18	0.99	ST4-74	3290856	3291941	+
nupG	10	15	0.69	ST4-74	3292158	3293414	+
speC	8	10	0.78	ST4-74	3293476	3295611	-
yqgA	8	6	1.35	ST4-74	3296036	3296743	+

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STnc3870	146	125	1.17	ST4-74	3296886	3297024	-
STM3117	13	12	1.03	ST4-74	3297083	3297517	-
STM3118	3	3	1.06	ST4-74	3297528	3298853	-
STM3119	5	4	1.35	ST4-74	3298878	3299405	-
STM3120	9	9	1.07	ST4-74	3299429	3300271	-
STM3121	4	5	0.91	ST4-74	3300513	3301241	+
STM3122	13	9	1.39	ST4-74	3301289	3303028	-
STM3123	2	2	1.19	ST4-74	3303097	3304281	-
STM3124	9	9	0.98	ST4-74	3304793	3305476	+
STnc3880	14	8	1.71	ST4-74	3305410	3305515	+
STM3125	12	12	0.97	ST4-74	3305580	3306137	-
STM3126	12	11	1.11	ST4-74	3306115	3307614	-
STM3127	9	11	0.83	ST4-74	3307823	3308197	-
ordL	21	14	1.53	ST4-74	3308212	3309513	-
STM3129	9	7	1.20	ST4-74	3309675	3311159	+
iraD	5	8	0.65	ST4-74	3311295	3311675	-
STM3131	7	10	0.69	ST4-74	3311675	3312160	-
STnc1170	393	245	1.60	ST4-74	3312429	3312525	-
STM3132	86	33	2.58	ST4-74	3312886	3313809	-
STM3133	120	35	3.46	ST4-74	3313848	3314726	-
exuT	8	8	1.04	ST4-74	3315122	3316426	-
uxuA	8	9	0.82	ST4-74	3316831	3318015	+
uxuB	4	4	0.96	ST4-74	3318126	3319598	+
uxaC	13	10	1.27	ST4-74	3319610	3321022	+
STM3138	203	328	0.62	ST4-74	3321322	3322380	-
STnc3890	73	71	1.03	ST4-74	3322951	3323033	+
gsp	11	10	1.18	ST4-74	3323050	3324906	-
yghU	24	21	1.15	ST4-74	3325133	3325999	+
STM3141	8	9	0.94	ST4-74	3326067	3326777	-
STM3142	3	4	0.79	ST4-74	3326777	3327829	-
hybG	21	77	0.27	ST4-74	3327905	3328153	-
hybF	14	43	0.32	ST4-74	3328181	3328522	-
hybE	11	34	0.34	ST4-74	3328515	3329003	-
hybD	20	58	0.34	ST4-74	3328996	3329490	-
hybC	17	45	0.39	ST4-74	3329490	3331193	-
hybB	19	57	0.33	ST4-74	3331190	3332368	-
hybA	13	44	0.29	ST4-74	3332358	3333344	-
hypO	26	61	0.42	ST4-74	3333347	3334465	-
yghW	5	18	0.26	ST4-74	3334655	3334942	-
STM3152	10	21	0.46	ST4-74	3335027	3336670	-
yqhA	48	65	0.74	ST4-74	3336977	3337471	-
STM3154	111	261	0.43	ST4-74	3337751	3338266	-
STM3155	58	124	0.46	ST4-74	3338358	3338768	+
STM3156	11	36	0.32	ST4-74	3338755	3339159	+
yghA	32	31	1.04	ST4-74	3339283	3340167	+
exbD	72	42	1.69	ST4-74	3340280	3340705	-
exbB	61	38	1.60	ST4-74	3340712	3341446	-
STM3160	139	76	1.84	ST4-74	3341439	3341588	-
metC	25	23	1.09	ST4-74	3341698	3342885	+
yghB	79	79	1.00	ST4-74	3343025	3343684	+
yqhC	22	23	0.93	ST4-74	3343748	3344665	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
yqhD	9	12	0.74	ST4-74	3344840	3346003	+
dkgA	41	30	1.35	ST4-74	3346109	3346936	+
STM3166	7	8	0.91	ST4-74	3347286	3348587	+
STM3167	23	26	0.90	ST4-74	3348632	3349087	+
STnc1820	105	73	1.44	ST4-74	3349037	3349187	-
STnc3920	3	2	1.65	ST4-74	3349508	3349727	+
ygiR	20	15	1.26	ST4-74	3349642	3351813	-
STM3169	2	2	1.27	ST4-74	3352301	3353284	+
STM3170	4	4	0.99	ST4-74	3353326	3353808	+
ygiK	7	6	1.21	ST4-74	3353819	3355126	+
sufI	16	21	0.78	ST4-74	3355171	3356583	-
plsC	33	32	1.01	ST4-74	3356657	3357394	-
parC	27	31	0.88	ST4-74	3357651	3359909	-
STM3175	9	15	0.60	ST4-74	3360020	3360886	-
ygiW	34	52	0.64	ST4-74	3360958	3361350	-
ygiX	13	14	0.93	ST4-74	3361502	3362161	+
ygiY	6	6	0.93	ST4-74	3362158	3363507	+
mdaB	12	16	0.76	ST4-74	3363614	3364195	+
ygiN	60	68	0.89	ST4-74	3364227	3364541	+
parE	24	27	0.90	ST4-74	3364666	3366558	-
yqiA	42	41	1.02	ST4-74	3366586	3367167	-
cpdA	57	56	1.03	ST4-74	3367167	3367994	-
yqiB	145	127	1.14	ST4-74	3368019	3368441	-
nudF	17	20	0.83	ST4-74	3368438	3369070	-
tolC	64	63	1.01	ST4-74	3369271	3370746	+
ygiB	236	218	1.08	ST4-74	3370959	3371630	+
ygiC	58	67	0.87	ST4-74	3371636	3372799	+
ygiD	16	17	0.95	ST4-74	3372861	3373691	-
ygiE	22	22	1.04	ST4-74	3373790	3374563	+
assT	3	3	0.78	ST4-74	3375206	3377002	+
STM3193	4	6	0.71	ST4-74	3377022	3377693	+
STM3194	14	12	1.13	ST4-74	3377708	3378385	+
ribB	39	64	0.61	ST4-74	3378556	3379209	-
SroG	841	1383	0.61	ST4-74	3379312	3379412	-
yqiC	150	119	1.26	ST4-74	3379646	3379945	+
glgS	207	175	1.18	ST4-74	3380019	3380228	-
STM3198	5	5	0.96	ST4-74	3380486	3381106	+
yqiK	7	8	0.94	ST4-74	3381125	3382804	+
STnc1420	447	514	0.87	ST4-74	3382981	3383131	+
RygD	4757	4738	1.00	ST4-74	3383000	3383144	-
ibsC	0	0	–	ST4-74	3383044	3383103	+
rfaE	22	27	0.81	ST4-74	3383264	3384697	-
glnE	14	15	0.93	ST4-74	3384745	3387588	-
ygiF	17	19	0.92	ST4-74	3387706	3389007	-
ygiM	44	48	0.91	ST4-74	3389249	3389863	+
cca	29	24	1.19	ST4-74	3389926	3391167	+
bacA	37	33	1.13	ST4-74	3391272	3392096	-
folB	56	41	1.36	ST4-74	3392191	3392553	-
ygiH	158	154	1.03	ST4-74	3392657	3393268	+
gcp	46	36	1.27	ST4-74	3393519	3394532	-
rpsU	4703	4126	1.14	ST4-74	3394760	3394975	+

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dnaG	55	57	0.96	ST4-74	3395211	3396956	+
rpoD	50	60	0.83	ST4-74	3397106	3398953	+
mug	60	45	1.34	ST4-74	3399077	3399583	-
yqjH	15	12	1.24	ST4-74	3399864	3400631	-
yqjI	28	31	0.92	ST4-74	3400863	3401510	+
STM3216	20	65	0.30	ST4-74	3401507	3403072	-
aer	12	33	0.38	ST4-74	3403460	3404980	-
oat	16	7	2.39	ST4-74	3405500	3406789	+
fadH	7	6	1.31	ST4-74	3406960	3408978	+
ygjO	12	12	1.04	ST4-74	3409059	3410195	-
ygjP	13	12	1.07	ST4-74	3410419	3410778	+
ygjQ	4	3	1.23	ST4-74	3410930	3411622	+
ygjR	21	26	0.83	ST4-74	3411711	3412709	+
SraF	890	771	1.15	ST4-74	3412774	3412961	+
ygjT	28	20	1.35	ST4-74	3412982	3413950	+
STnc310	14	6	2.26	ST4-74	3413972	3414032	-
ygjU	64	86	0.75	ST4-74	3414205	3415449	+
yqjA	138	121	1.14	ST4-74	3415899	3416561	+
yqjB	79	63	1.26	ST4-74	3416565	3416948	+
yqjC	344	228	1.51	ST4-74	3417093	3417461	+
yqjE	141	133	1.06	ST4-74	3417811	3418209	+
yqjK	54	58	0.94	ST4-74	3418206	3418505	+
yqjF	2	3	0.86	ST4-74	3418650	3419135	+
yqjG	29	16	1.84	ST4-74	3419203	3420189	+
yhaH	59	49	1.22	ST4-74	3420313	3420678	+
yhaJ	22	21	1.06	ST4-74	3420717	3421613	-
yhaK	15	23	0.65	ST4-74	3421718	3422419	+
yhaL	44	71	0.62	ST4-74	3422476	3422607	+
yhaN	9	7	1.34	ST4-74	3422720	3424030	-
yhaO	5	5	1.02	ST4-74	3424056	3425387	-
tdcG	4	5	0.84	ST4-74	3425703	3427067	-
tdcE	2	3	0.88	ST4-74	3427137	3429431	-
tdcD	3	5	0.65	ST4-74	3429465	3430673	-
tdcC	6	7	0.91	ST4-74	3430739	3432070	-
tdcB	3	5	0.60	ST4-74	3432091	3433080	-
tdcA	15	13	1.10	ST4-74	3433178	3434116	-
garK	10	6	1.56	ST4-74	3435915	3437060	-
garRb	9	8	1.12	ST4-74	3437158	3438048	-
garL	49	15	3.29	ST4-74	3438074	3438790	-
garD	5	4	1.14	ST4-74	3439374	3440945	+
STM3251	12	12	0.97	ST4-74	3441185	3442132	-
agaR	35	29	1.19	ST4-74	3442143	3442976	-
gatY	3	2	1.21	ST4-74	3443310	3444164	+
STM3254	1	1	0.48	ST4-74	3444175	3445089	+
STM3255	2	3	0.77	ST4-74	3445116	3446543	+
STM3256	2	2	1.03	ST4-74	3446530	3447336	+
STM3257	2	1	1.37	ST4-74	3447534	3448805	+
gatA	2	1	1.29	ST4-74	3448820	3449284	+
gatB	3	2	1.18	ST4-74	3449315	3449599	+
gatC	5	5	0.90	ST4-74	3449603	3450976	+
gatD	2	1	1.53	ST4-74	3451020	3452063	+

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gatR	22	25	0.86	ST4-74	3452174	3452947	+
yraL	18	16	1.13	ST4-74	3453105	3453968	-
yraM	31	32	0.96	ST4-74	3454032	3456074	+
yraN	50	54	0.93	ST4-74	3456032	3456427	+
yraO	87	70	1.25	ST4-74	3456449	3457039	+
yraP	37	39	0.94	ST4-74	3457049	3457624	+
yraR	11	7	1.59	ST4-74	3457691	3458359	-
yhbO	42	22	1.91	ST4-74	3458457	3458975	+
yhbP	5	9	0.54	ST4-74	3458955	3459398	-
yhbQ	37	28	1.32	ST4-74	3459436	3459753	+
yhbS	34	67	0.50	ST4-74	3459740	3460243	-
yhbT	76	166	0.46	ST4-74	3460237	3460761	-
yhbU	2	34	0.06	ST4-74	3460978	3461973	+
yhbV	2	12	0.16	ST4-74	3461982	3462860	+
yhbW	7	8	0.81	ST4-74	3463038	3464045	+
STM3277	5	3	1.67	ST4-74	3464023	3464742	-
STM3278	3	2	1.43	ST4-74	3464901	3465431	+
mtr	18	20	0.90	ST4-74	3465492	3466652	-
deaD	17	19	0.88	ST4-74	3466890	3468779	-
yrbN	73	76	0.97	ST4-74	3468772	3468852	-
nlpI	475	478	0.99	ST4-74	3468958	3469842	-
pnp	53	68	0.79	ST4-74	3469952	3472087	-
SraG	562	639	0.88	ST4-74	3472142	3472312	-
rpsO	2177	2346	0.93	ST4-74	3472329	3472598	-
truB	35	35	1.01	ST4-74	3472749	3473693	-
rbfA	18	25	0.74	ST4-74	3473693	3474094	-
infB	46	58	0.80	ST4-74	3474315	3476993	-
nusA	39	49	0.80	ST4-74	3477018	3478520	-
yhbC	54	62	0.88	ST4-74	3478548	3478970	-
argG	37	76	0.49	ST4-74	3479628	3480971	+
STnc3950	28	21	1.34	ST4-74	3480987	3481116	-
STM3291	11	12	0.93	ST4-74	3481057	3482121	-
secG	1077	1119	0.96	ST4-74	3482557	3482889	-
glmM	16	17	0.95	ST4-74	3483112	3484449	-
folP	22	22	0.96	ST4-74	3484442	3485290	-
tnpA_1c	244	250	0.97	ST4-74	3485437	3485895	-
ftsH	212	247	0.86	ST4-74	3486106	3488040	-
ftsJ	250	236	1.06	ST4-74	3488144	3488770	-
yhbY	165	161	1.03	ST4-74	3488897	3489190	+
STnc1660	231	278	0.83	ST4-74	3489125	3489247	-
greA	87	90	0.97	ST4-74	3489346	3489822	-
dacB	26	25	1.04	ST4-74	3490070	3491503	+
STnc3960	12	10	1.28	ST4-74	3491490	3491592	+
obgE	25	24	1.05	ST4-74	3491635	3492807	-
yhbE	39	27	1.41	ST4-74	3492823	3493833	-
rpmA	481	559	0.86	ST4-74	3493918	3494175	-
rplU	1962	1797	1.09	ST4-74	3494195	3494506	-
ispB	69	63	1.11	ST4-74	3494764	3495735	+
nlp	7	5	1.36	ST4-74	3495967	3496254	+
murA	23	23	0.96	ST4-74	3496312	3497571	-
yrbA	46	49	0.94	ST4-74	3497625	3497918	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
yrbB	41	52	0.78	ST4-74	3498067	3498363	-
yrbC	40	44	0.90	ST4-74	3498363	3498998	-
yrbD	29	31	0.94	ST4-74	3499017	3499568	-
yrbE	14	16	0.88	ST4-74	3499573	3500355	-
yrbF	29	31	0.95	ST4-74	3500363	3501175	-
yrbG	35	39	0.88	ST4-74	3501430	3502365	+
yrbH	34	38	0.89	ST4-74	3502379	3503365	+
yrbI	55	56	0.99	ST4-74	3503386	3503952	+
yrbK	37	38	0.97	ST4-74	3503949	3504524	+
yhbN	29	38	0.75	ST4-74	3504493	3505047	+
yhbG	26	31	0.83	ST4-74	3505054	3505779	+
rpoN	88	109	0.81	ST4-74	3505827	3507260	+
yhbH	365	495	0.74	ST4-74	3507283	3507570	+
STnc3970	160	90	1.78	ST4-74	3507628	3507677	-
ptsN	46	62	0.75	ST4-74	3507688	3508179	+
yhbJ	24	31	0.78	ST4-74	3508225	3509079	+
ptsO	11	13	0.81	ST4-74	3509076	3509348	+
yrbL	20	17	1.18	ST4-74	3509597	3510229	+
mtgA	19	26	0.73	ST4-74	3510304	3511032	-
yhbL	34	51	0.67	ST4-74	3511029	3511682	-
ArcZ	16269	16401	0.99	ST4-74	3511800	3511917	+
arcB	27	33	0.80	ST4-74	3511909	3514245	-
yhcC	5	12	0.40	ST4-74	3514339	3515268	-
gltB	29	54	0.54	ST4-74	3515940	3520400	+
gltD	33	60	0.55	ST4-74	3520410	3521828	+
yhcG	3	2	1.42	ST4-74	3522035	3523138	+
codB	30	41	0.75	ST4-74	3523255	3524508	+
codA	20	30	0.67	ST4-74	3524495	3525775	+
yhcH	11	10	1.06	ST4-74	3525858	3526325	-
nanK	12	9	1.29	ST4-74	3526322	3527197	-
nanE	4	6	0.73	ST4-74	3527194	3527883	-
nanT	12	11	1.10	ST4-74	3527930	3529420	-
nanA	3	4	0.92	ST4-74	3529536	3530429	-
yhcK	30	34	0.89	ST4-74	3530564	3531355	-
sspB	77	80	0.97	ST4-74	3531464	3531964	-
sspA	97	109	0.89	ST4-74	3531970	3532608	-
STM3343	12	12	1.02	ST4-74	3532871	3533623	-
STnc3060	42	40	1.05	ST4-74	3533708	3533783	+
rpsI	239	370	0.64	ST4-74	3533823	3534215	-
rplM	324	456	0.71	ST4-74	3534231	3534659	-
yhcM	16	19	0.89	ST4-74	3534962	3536086	-
yhcB	200	224	0.89	ST4-74	3536273	3536677	+
degQ	20	26	0.77	ST4-74	3536834	3538201	+
degS	28	27	1.02	ST4-74	3538294	3539364	+
STM3350	4	3	1.33	ST4-74	3539398	3540129	-
oadB2	3	2	1.39	ST4-74	3540149	3541450	-
oadA2	1	1	0.72	ST4-74	3541466	3543241	-
STM3353	3	4	0.87	ST4-74	3543257	3543511	-
STnc3050	154	171	0.90	ST4-74	3543529	3543654	-
ttdB	4	3	1.32	ST4-74	3543667	3544284	-
ttdA	4	2	1.54	ST4-74	3544284	3545183	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM3356	3	2	1.50	ST4-74	3545216	3546484	-
STM3357	17	18	0.97	ST4-74	3546695	3547360	-
STM3358	52	53	0.99	ST4-74	3547347	3547976	-
mdh	159	233	0.68	ST4-74	3548096	3549034	-
argR	344	300	1.15	ST4-74	3549448	3549918	+
yhcN	9	7	1.22	ST4-74	3550283	3550546	+
STM3362	8	6	1.35	ST4-74	3550650	3550916	+
STnc2100	14	12	1.15	ST4-74	3550903	3550965	+
yhcO	200	83	2.41	ST4-74	3550976	3551248	-
yhcP	7	7	1.11	ST4-74	3551420	3553387	-
yhcQ	6	5	1.22	ST4-74	3553393	3554325	-
yhcR	8	9	0.97	ST4-74	3554333	3554536	-
yhcS	9	10	0.92	ST4-74	3554718	3555647	+
tldD	27	23	1.14	ST4-74	3555770	3557215	-
yhdP	14	17	0.83	ST4-74	3557360	3561073	-
cafA	33	37	0.89	ST4-74	3561267	3562736	-
maf-b	35	34	1.03	ST4-74	3562726	3563319	-
mreD	43	37	1.17	ST4-74	3563328	3563819	-
mreC	28	31	0.93	ST4-74	3563819	3564871	-
mreB	52	63	0.83	ST4-74	3564936	3565979	-
yhdA	22	27	0.81	ST4-74	3566287	3568227	-
yhdH	21	34	0.63	ST4-74	3568431	3569405	+
STM3377	14	20	0.69	ST4-74	3569518	3570522	+
STM3378	30	33	0.90	ST4-74	3570523	3571122	+
accB	70	87	0.80	ST4-74	3571515	3571985	+
accC	66	83	0.79	ST4-74	3572086	3573345	+
yhdT	16	20	0.80	ST4-74	3573454	3573696	+
panF	8	10	0.83	ST4-74	3573686	3575137	+
prmA	8	9	0.85	ST4-74	3575149	3576030	+
yhdG	124	144	0.86	ST4-74	3576691	3577656	+
fis	81	107	0.76	ST4-74	3577682	3577978	+
yhdJ	10	11	0.96	ST4-74	3578064	3578948	+
yhdU	87	78	1.13	ST4-74	3579031	3579195	+
STM3388	8	7	1.15	ST4-74	3579346	3581445	+
envR	2	2	0.82	ST4-74	3581448	3582110	-
acrE	1	1	0.98	ST4-74	3582524	3583681	+
acrF	5	4	1.13	ST4-74	3583693	3586806	+
yhdV	22	23	0.96	ST4-74	3587042	3587263	+
STnc3990	111	61	1.81	ST4-74	3587262	3587429	-
STnc1670	175	98	1.78	ST4-74	3593794	3593896	+
yrdA	43	32	1.32	ST4-74	3593964	3594518	+
yrdB	22	24	0.91	ST4-74	3594494	3594751	-
STM3401	20	18	1.12	ST4-74	3594748	3595566	-
yrdC	16	16	1.01	ST4-74	3595571	3596143	-
yrdD	124	99	1.25	ST4-74	3596148	3596690	-
smg	301	210	1.43	ST4-74	3596717	3597190	-
dprA	51	43	1.20	ST4-74	3597162	3598286	-
def	175	126	1.39	ST4-74	3598418	3598927	+
fmt	36	32	1.15	ST4-74	3598943	3599890	+
fmu	9	9	1.00	ST4-74	3599942	3601231	+
sapG	14	11	1.29	ST4-74	3601253	3602629	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
mscL	420	281	1.49	ST4-74	3602771	3603184	+
STM3411	392	266	1.47	ST4-74	3603120	3603389	-
zntR	54	52	1.03	ST4-74	3603447	3603872	-
yhdN	120	95	1.27	ST4-74	3603883	3604251	-
rplQ	412	549	0.75	ST4-74	3604359	3604742	-
pez	436	594	0.73	ST4-74	3604783	3605772	-
rpsD	498	611	0.81	ST4-74	3605798	3606418	-
rpsK	467	607	0.77	ST4-74	3606452	3606784	-
rpsM	951	1015	0.94	ST4-74	3606858	3607214	-
rpmJb	976	1148	0.85	ST4-74	3607361	3607477	-
prlA	605	774	0.78	ST4-74	3607509	3608840	-
rplO	389	540	0.72	ST4-74	3608848	3609282	-
rpmD	386	501	0.77	ST4-74	3609286	3609465	-
rpsE	341	475	0.72	ST4-74	3609469	3609972	-
rl18	362	478	0.76	ST4-74	3609987	3610340	-
rplF	312	421	0.74	ST4-74	3610350	3610883	-
rpsH	399	505	0.79	ST4-74	3610896	3611288	-
rpsN	324	395	0.82	ST4-74	3611322	3611612	-
rplE	553	671	0.82	ST4-74	3611642	3612181	-
rplX	851	999	0.85	ST4-74	3612196	3612510	-
rplN	1084	1337	0.81	ST4-74	3612521	3612892	-
rpsQ	410	543	0.76	ST4-74	3613056	3613310	-
rpmC	607	710	0.86	ST4-74	3613310	3613501	-
rplP	460	515	0.89	ST4-74	3613501	3613911	-
rpsC	330	438	0.75	ST4-74	3613924	3614625	-
rplV	294	365	0.80	ST4-74	3614643	3614975	-
rpsS	525	667	0.79	ST4-74	3614990	3615268	-
rplB	148	208	0.71	ST4-74	3615285	3616106	-
rplW	196	284	0.69	ST4-74	3616124	3616426	-
rplD	206	274	0.75	ST4-74	3616423	3617028	-
rplC	215	279	0.77	ST4-74	3617039	3617668	-
rpsJ	429	543	0.79	ST4-74	3617701	3618012	-
hopD	6	5	1.07	ST4-74	3618392	3618859	+
bfr	481	319	1.51	ST4-74	3618856	3619332	-
bfd	489	163	2.99	ST4-74	3619405	3619599	-
STnc4000	99	28	3.47	ST4-74	3619657	3619755	+
tufA	563	709	0.79	ST4-74	3619784	3620968	-
fusA	173	211	0.82	ST4-74	3621040	3623154	-
rpsG	269	328	0.82	ST4-74	3623251	3623721	-
rpsL	726	723	1.00	ST4-74	3623817	3624191	-
yheL	162	92	1.75	ST4-74	3624317	3624604	-
yheM	28	33	0.85	ST4-74	3624612	3624968	-
yheN	30	38	0.80	ST4-74	3624968	3625354	-
yheO	86	95	0.91	ST4-74	3625354	3626088	-
fkpA	100	111	0.90	ST4-74	3626353	3627171	-
slyX	73	80	0.91	ST4-74	3627391	3627609	+
slyD	119	132	0.90	ST4-74	3627797	3628387	-
yheV	18	17	1.06	ST4-74	3628482	3628682	-
kefB	19	15	1.27	ST4-74	3628693	3630498	-
yheR	17	12	1.40	ST4-74	3630498	3631049	-
yheS	14	16	0.93	ST4-74	3631315	3633222	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STnc1590	1627	2319	0.70	ST4-74	3633247	3633424	-
SL1344_3427	13	12	1.13	ST4-74	3633385	3633606	+
STM3461	78	97	0.80	ST4-74	3633608	3633919	-
yheT	26	23	1.10	ST4-74	3634117	3635184	+
yheU	25	28	0.91	ST4-74	3635181	3635399	+
prkB	23	21	1.07	ST4-74	3635451	3636320	+
yhfA	99	112	0.88	ST4-74	3636416	3636820	-
crp	111	138	0.80	ST4-74	3637128	3637760	+
yhfK	23	31	0.73	ST4-74	3637809	3639896	+
argDb	74	108	0.68	ST4-74	3639939	3641156	-
pabA	13	14	0.98	ST4-74	3641242	3641805	-
fic	19	13	1.53	ST4-74	3641837	3642439	-
yhfG	118	72	1.64	ST4-74	3642429	3642596	-
ppiA	124	129	0.96	ST4-74	3642705	3643277	-
yhfC	6	6	1.01	ST4-74	3643554	3644735	+
nirB	3	6	0.41	ST4-74	3644998	3647541	+
nirD	6	20	0.32	ST4-74	3647538	3647864	+
nirC	13	16	0.83	ST4-74	3648130	3648939	+
cysG	13	17	0.74	ST4-74	3648951	3650324	+
bigA	3	3	1.08	ST4-74	3650653	3656547	+
tnpA_1d	244	250	0.97	ST4-74	3656679	3657137	-
STnc340	10	14	0.73	ST4-74	3657275	3657403	-
yhfL	83	69	1.20	ST4-74	3657451	3657621	+
trpS	48	51	0.94	ST4-74	3657766	3658770	-
gph	40	39	1.03	ST4-74	3658763	3659521	-
rpe	21	21	1.00	ST4-74	3659514	3660191	-
dam	33	39	0.84	ST4-74	3660209	3661045	-
damX	58	73	0.79	ST4-74	3661226	3662503	-
aroB	56	63	0.88	ST4-74	3662601	3663689	-
aroK	135	149	0.91	ST4-74	3663746	3664267	-
comE	6	6	0.97	ST4-74	3664732	3665970	-
comD	6	4	1.52	ST4-74	3665885	3666286	-
comC	3	4	0.92	ST4-74	3666276	3666749	-
comB	3	2	1.28	ST4-74	3666733	3667272	-
comA	4	3	1.40	ST4-74	3667272	3668051	-
mrcA	22	22	1.00	ST4-74	3668148	3670724	+
nudE	37	32	1.16	ST4-74	3670820	3671383	-
igaA	29	36	0.82	ST4-74	3671706	3673838	+
yrfG	49	36	1.34	ST4-74	3673903	3674571	+
hslR	16	16	0.96	ST4-74	3674582	3674983	+
hslO	12	13	0.93	ST4-74	3675002	3675886	+
yhgE	11	11	1.02	ST4-74	3675996	3677705	-
pckA	116	135	0.86	ST4-74	3678085	3679704	+
envZ	20	29	0.69	ST4-74	3679779	3681014	-
ompR	45	50	0.89	ST4-74	3681128	3681847	-
greB	22	21	1.02	ST4-74	3682073	3682546	+
yhgF	13	13	0.96	ST4-74	3682648	3684975	+
STnc760	463	362	1.28	ST4-74	3684975	3685117	-
feoA	43	31	1.38	ST4-74	3685412	3685639	+
feoB	26	23	1.14	ST4-74	3685658	3687976	+
yhgG	58	46	1.26	ST4-74	3687989	3688225	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM3508	14	15	0.91	ST4-74	3688460	3689374	+
bioH	21	22	0.95	ST4-74	3689410	3690180	-
comF	35	28	1.25	ST4-74	3690218	3690901	+
yhgI	63	70	0.90	ST4-74	3690960	3691535	+
gntT	7	8	0.97	ST4-74	3691960	3693228	+
malQ	11	8	1.36	ST4-74	3693347	3695425	-
malP	11	8	1.27	ST4-74	3695435	3697828	-
malT	36	40	0.91	ST4-74	3698422	3701127	+
STM3516	51	42	1.23	ST4-74	3701215	3701490	-
STM3517	314	229	1.37	ST4-74	3701493	3701753	-
rtcA	5	6	0.83	ST4-74	3701862	3702881	-
rtcB	15	14	1.08	ST4-74	3702885	3704102	-
STM3521	2	2	1.00	ST4-74	3704447	3706000	-
rtcR	5	5	1.05	ST4-74	3706190	3707773	+
glpR	31	32	0.98	ST4-74	3707770	3708528	-
glpG	31	31	1.00	ST4-74	3708622	3709452	-
glpE	98	81	1.20	ST4-74	3709528	3709854	-
glpD	331	313	1.06	ST4-74	3710053	3711561	+
STM3527	7	5	1.37	ST4-74	3711608	3712153	-
STM3528	14	13	1.10	ST4-74	3712163	3713623	-
STM3529	6	5	1.16	ST4-74	3713816	3714925	-
STM3530	4	3	1.35	ST4-74	3715263	3716597	+
STM3531	3	4	0.72	ST4-74	3716594	3718309	+
STM3532	11	8	1.43	ST4-74	3718353	3719258	+
STM3533	37	52	0.72	ST4-74	3719302	3720057	-
glgP	32	33	0.96	ST4-74	3720235	3722682	-
glgA	28	25	1.11	ST4-74	3722702	3724135	-
glgC	27	34	0.78	ST4-74	3724135	3725430	-
glgX	29	24	1.21	ST4-74	3725445	3727421	-
glgB	41	35	1.20	ST4-74	3727418	3729604	-
STnc4010	44	16	2.77	ST4-74	3729766	3729898	+
asd	64	102	0.63	ST4-74	3729951	3731057	-
gntU	7	6	1.17	ST4-74	3731481	3732821	-
gntK	4	3	1.14	ST4-74	3732818	3733351	-
gntR	30	23	1.29	ST4-74	3733490	3734485	-
yhhW	6	6	1.06	ST4-74	3734615	3735310	-
yhhX	16	15	1.10	ST4-74	3735434	3736471	-
RyhB-1	41	31	1.35	ST4-74	3736722	3736817	-
yhhY	21	23	0.93	ST4-74	3736940	3737428	+
STM3547	2	2	0.75	ST4-74	3737960	3738907	+
STM3548	4	3	1.39	ST4-74	3738923	3739684	+
STnc1830	54	44	1.23	ST4-74	3739678	3739897	-
STM3549	3	2	1.19	ST4-74	3739738	3740709	+
STM3550	4	4	1.06	ST4-74	3740706	3741740	+
ggt	17	15	1.16	ST4-74	3741785	3743527	-
yhhA	75	43	1.75	ST4-74	3743653	3744069	+
ugpQ	14	15	0.97	ST4-74	3744082	3744822	-
ugpC	7	7	0.99	ST4-74	3744819	3745889	-
ugpE	7	5	1.39	ST4-74	3745891	3746736	-
ugpA	9	8	1.18	ST4-74	3746733	3747620	-
ugpB	33	27	1.22	ST4-74	3747684	3748976	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
kil	12	11	1.13	ST4-74	3749259	3749627	-
phd	61	63	0.96	ST4-74	3749624	3749851	-
livF	16	31	0.54	ST4-74	3749977	3750690	-
livG	14	27	0.52	ST4-74	3750692	3751459	-
livM	18	34	0.53	ST4-74	3751456	3752733	-
livH	20	33	0.59	ST4-74	3752730	3753656	-
livK	29	53	0.56	ST4-74	3753716	3754825	-
sRNA12	19	7	2.78	ST4-74	3755044	3755124	-
yhhK	26	23	1.11	ST4-74	3755247	3755630	+
livJ	37	74	0.50	ST4-74	3755825	3756928	-
rpoH	147	159	0.92	ST4-74	3757250	3758104	-
ftsX	36	37	0.97	ST4-74	3758350	3759405	-
ftsE	29	30	0.98	ST4-74	3759398	3760066	-
ftsY	35	36	0.97	ST4-74	3760069	3761544	-
rsmD	22	20	1.09	ST4-74	3761670	3762266	+
yhhL	16	15	1.05	ST4-74	3762253	3762525	+
yhhM	19	20	0.91	ST4-74	3762547	3762789	-
yhhN	38	31	1.20	ST4-74	3763060	3763686	+
zntA	4	5	0.82	ST4-74	3763767	3765965	+
tcp	21	97	0.21	ST4-74	3766165	3767808	+
yhhP	186	109	1.70	ST4-74	3767832	3768077	-
yhhQ	39	24	1.63	ST4-74	3768247	3768912	+
STM3580	98	83	1.19	ST4-74	3768985	3769542	+
yhhS	24	22	1.09	ST4-74	3769546	3770763	-
yhhT	16	10	1.57	ST4-74	3770895	3771944	+
acpT	24	20	1.23	ST4-74	3771996	3772574	+
nikR	30	33	0.91	ST4-74	3772666	3773067	+
yhhJ	11	14	0.78	ST4-74	3773157	3774281	-
yhiH	6	8	0.77	ST4-74	3774281	3777022	-
yhiI	15	18	0.83	ST4-74	3777019	3778086	-
STnc770	592	481	1.23	ST4-74	3778180	3778332	-
yhiN	16	17	0.97	ST4-74	3778392	3779588	-
pitA	30	48	0.64	ST4-74	3779942	3781315	+
uspB	162	112	1.45	ST4-74	3781455	3781790	-
uspA	308	341	0.90	ST4-74	3782178	3782612	+
STnc350	45	12	3.91	ST4-74	3782695	3782856	-
yhiP	7	6	1.32	ST4-74	3782937	3784409	+
yhiQ	7	9	0.86	ST4-74	3784501	3785259	-
prlC	16	19	0.82	ST4-74	3785266	3787308	-
STM3595	37	25	1.50	ST4-74	3787490	3788761	-
yhiR	27	25	1.10	ST4-74	3788972	3789814	+
gor	31	32	0.96	ST4-74	3789919	3791271	+
STM3598	10	11	0.92	ST4-74	3791318	3792361	-
STM3599	8	7	1.00	ST4-74	3792420	3793739	-
STM3600	2	3	0.61	ST4-74	3793793	3794638	-
STM3601	13	12	1.10	ST4-74	3794702	3795679	-
STM3602	20	17	1.21	ST4-74	3795855	3796574	-
treF	16	14	1.19	ST4-74	3796900	3798549	+
STM3604	38	72	0.52	ST4-74	3798562	3800151	-
STM3605	4	4	0.91	ST4-74	3800432	3800788	+
yhjB	4	3	1.18	ST4-74	3800794	3801396	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STnc4020	40	42	0.95	ST4-74	3801174	3801265	+
yhjC	9	8	1.03	ST4-74	3802027	3802926	+
yhjD	20	13	1.48	ST4-74	3802999	3804027	+
yhjE	56	74	0.75	ST4-74	3804378	3805700	+
yhjG	21	19	1.10	ST4-74	3805740	3807800	-
yhjH	27	101	0.26	ST4-74	3807910	3808677	-
kdgK	12	37	0.33	ST4-74	3808906	3809835	+
yhjJ	22	27	0.83	ST4-74	3809889	3811376	-
dctA	44	90	0.48	ST4-74	3811596	3812882	-
yhjK	14	13	1.08	ST4-74	3813040	3814959	-
yhjL	18	18	0.99	ST4-74	3815220	3818762	-
bcsC	20	21	0.95	ST4-74	3818744	3819853	-
yhjN	12	15	0.81	ST4-74	3819857	3822157	-
bcsA	15	17	0.92	ST4-74	3822168	3824792	-
yhjQ	13	14	0.92	ST4-74	3824789	3825541	-
yhjR	81	74	1.09	ST4-74	3825542	3825745	-
yhjS	30	21	1.40	ST4-74	3825990	3827561	+
yhjT	20	17	1.21	ST4-74	3827558	3827749	+
yhjU	20	18	1.14	ST4-74	3827746	3829425	+
STnc1430	648	927	0.70	ST4-74	3829427	3829796	-
STnc710	5219	4839	1.08	ST4-74	3829660	3829727	+
STM3624A	23	18	1.34	ST4-74	3829889	3830139	+
yhjV	11	10	1.12	ST4-74	3830257	3831555	+
dppF	15	34	0.44	ST4-74	3831656	3832669	-
dppD	22	44	0.49	ST4-74	3832666	3833649	-
dppC	26	52	0.49	ST4-74	3833660	3834562	-
dppB	36	68	0.52	ST4-74	3834572	3835591	-
dppA	124	244	0.51	ST4-74	3835748	3837304	-
STM3631	4	3	1.08	ST4-74	3837911	3839236	-
STM3632	4	4	1.04	ST4-74	3839294	3840418	-
STM3633	16	19	0.84	ST4-74	3840568	3841575	+
yhjW	21	24	0.88	ST4-74	3841902	3843593	-
lpfE	5	5	0.91	ST4-74	3843811	3844338	-
lpfD	8	8	0.99	ST4-74	3844344	3845413	-
lpfC	2	3	0.85	ST4-74	3845431	3847959	-
lpfB	2	2	1.09	ST4-74	3847982	3848680	-
lpfA	3	4	0.86	ST4-74	3848765	3849301	-
yhjY	26	15	1.65	ST4-74	3849807	3850511	-
tag	16	21	0.78	ST4-74	3850668	3851249	+
yiaC	14	25	0.57	ST4-74	3851227	3851667	+
bisC	15	15	0.97	ST4-74	3851636	3853969	-
yiaD	8	9	0.91	ST4-74	3854155	3854784	+
yiaE	16	21	0.78	ST4-74	3855003	3855977	+
yiaF	129	110	1.17	ST4-74	3856027	3856737	-
yiaG	405	183	2.21	ST4-74	3857176	3857466	+
cspA	242	203	1.19	ST4-74	3857767	3857967	+
STM3650	48	92	0.52	ST4-74	3858264	3858680	+
STnc780	264	136	1.94	ST4-74	3858665	3858992	-
STM3651	17	19	0.88	ST4-74	3859052	3859537	-
STM3652	60	58	1.03	ST4-74	3859525	3859812	-
yafP	17	16	1.05	ST4-74	3859990	3860457	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
glyS	22	26	0.85	ST4-74	3861086	3863155	-
glyQ	30	35	0.85	ST4-74	3863165	3864076	-
STM3657	56	42	1.35	ST4-74	3864215	3864517	-
yiaH	13	10	1.31	ST4-74	3864683	3865678	+
yiaB	17	13	1.28	ST4-74	3865701	3866054	-
xylB	6	6	1.10	ST4-74	3866229	3867683	-
xylA	10	9	1.11	ST4-74	3867783	3869105	-
xylR	11	12	0.90	ST4-74	3869468	3870646	+
bax	225	301	0.75	ST4-74	3870707	3871531	-
malS	2	2	0.79	ST4-74	3871847	3873874	+
avtA	48	60	0.81	ST4-74	3874048	3875298	+
ysaA	16	61	0.26	ST4-74	3875335	3875808	-
yiaJ	31	26	1.20	ST4-74	3875925	3876725	-
yiaK	2	2	0.84	ST4-74	3876955	3877953	+
yiaL	3	2	1.26	ST4-74	3877965	3878429	+
STM3670	4	5	0.96	ST4-74	3878453	3879385	+
yiaM	3	2	1.73	ST4-74	3879524	3879997	+
yiaN	1	1	1.02	ST4-74	3880000	3881277	+
yiaO	1	0	3.16	ST4-74	3881289	3882275	+
lyxK	1	1	1.08	ST4-74	3882279	3883775	+
sgbH	1	1	1.06	ST4-74	3883772	3884434	+
sgbU	1	1	0.63	ST4-74	3884427	3885287	+
sgbE	3	2	1.30	ST4-74	3885305	3885976	+
STM3678	6	9	0.64	ST4-74	3886137	3886952	-
STM3679	3	3	0.97	ST4-74	3887216	3889171	+
aldB	62	63	0.98	ST4-74	3889289	3890827	-
STM3681	20	13	1.54	ST4-74	3890996	3891877	+
selB	10	14	0.73	ST4-74	3892162	3894012	-
selA	20	27	0.75	ST4-74	3894009	3895400	-
yibF	71	50	1.40	ST4-74	3895499	3896107	-
mtlA	26	31	0.84	ST4-74	3896583	3898499	+
mtlD	27	28	0.98	ST4-74	3898720	3899868	+
mtlR	15	15	0.99	ST4-74	3899865	3900455	+
STM3688	64	32	1.97	ST4-74	3900465	3900674	-
yibL	41	43	0.94	ST4-74	3900965	3901327	+
STM3690	1	0	1.50	ST4-74	3901817	3902500	+
sadA	7	4	1.51	ST4-74	3902544	3906929	+
STnc380	55	22	2.48	ST4-74	3906944	3907051	-
lctP	5	5	0.97	ST4-74	3907228	3908883	+
lctR	3	3	1.04	ST4-74	3908880	3909656	+
lctD	3	2	1.28	ST4-74	3909653	3910843	+
yibK	15	16	0.95	ST4-74	3910907	3911380	+
STM3696	22	22	1.01	ST4-74	3911448	3912452	-
STM3697	3	2	1.60	ST4-74	3912771	3913967	+
STM3698	7	8	0.92	ST4-74	3914041	3915219	+
SL1344_3664	13	6	2.17	ST4-74	3915216	3915350	+
cysE	25	39	0.65	ST4-74	3915433	3916254	-
gpsA	83	88	0.94	ST4-74	3916332	3917351	-
secB	253	256	0.99	ST4-74	3917351	3917818	-
grxC	179	196	0.91	ST4-74	3917862	3918113	-
yibN	187	189	0.99	ST4-74	3918200	3918631	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
pmgI	10	41	0.25	ST4-74	3918879	3920423	+
yibP	53	50	1.07	ST4-74	3920433	3921716	+
yigQ	7	8	0.94	ST4-74	3921720	3922682	+
yibD	12	13	0.90	ST4-74	3922669	3923703	-
SL1344_3673A	105	94	1.12	ST4-74	3923989	3924108	-
tdh	35	40	0.87	ST4-74	3924197	3925222	-
kbl	28	31	0.90	ST4-74	3925232	3926428	-
rfaD	70	69	1.01	ST4-74	3926631	3927563	+
rfaF	23	29	0.79	ST4-74	3927566	3928612	+
rfaC	19	20	0.94	ST4-74	3928612	3929565	+
rfaL	48	49	0.98	ST4-74	3929605	3930819	+
rfaK	40	32	1.24	ST4-74	3930876	3931739	-
rfaZ	117	72	1.62	ST4-74	3932122	3932931	-
rfaY	76	60	1.27	ST4-74	3933081	3933635	-
rfaJ	69	59	1.18	ST4-74	3933802	3934812	-
rfaI	83	62	1.33	ST4-74	3934830	3935843	-
rfaB	57	48	1.18	ST4-74	3935849	3936928	-
yibR	43	34	1.27	ST4-74	3936998	3937231	-
rfaP	34	27	1.26	ST4-74	3937263	3938060	-
rfaG	35	32	1.11	ST4-74	3938053	3939177	-
rfaQ	30	29	1.02	ST4-74	3939174	3940208	-
kdtA	38	36	1.04	ST4-74	3940719	3941930	+
coaD	25	34	0.75	ST4-74	3941939	3942418	+
mutM	8	7	1.08	ST4-74	3942446	3943255	-
rpmG	494	600	0.82	ST4-74	3943353	3943520	-
rpmB	1240	1350	0.92	ST4-74	3943541	3943777	-
radC	28	29	0.96	ST4-74	3943995	3944660	-
dfp	31	31	1.01	ST4-74	3944833	3946056	+
dut	28	27	1.01	ST4-74	3946037	3946492	+
slmA	18	24	0.78	ST4-74	3946600	3947196	+
pyrE	11	12	0.86	ST4-74	3947274	3947915	-
rph	50	46	1.09	ST4-74	3947994	3948710	-
yicC	43	38	1.14	ST4-74	3948836	3949699	+
STM3736	10	8	1.26	ST4-74	3949749	3950606	-
STM3737	4	3	1.53	ST4-74	3950725	3951606	+
STM3738	10	8	1.25	ST4-74	3951857	3952474	+
ligB	5	4	1.29	ST4-74	3952471	3954156	-
gmk	101	97	1.04	ST4-74	3954413	3955036	+
rpoZ	175	184	0.95	ST4-74	3955091	3955366	+
spoT	41	46	0.89	ST4-74	3955385	3957496	+
spoU	29	29	1.00	ST4-74	3957501	3958190	+
recG	12	13	0.94	ST4-74	3958196	3960277	+
STM3745	24	30	0.79	ST4-74	3960280	3961131	-
gltS	28	35	0.79	ST4-74	3961134	3962339	-
yicE	35	51	0.68	ST4-74	3962567	3963958	+
yicH	40	44	0.93	ST4-74	3964079	3965788	+
yicI	3	2	1.31	ST4-74	3965891	3968209	-
yicJ	4	4	1.16	ST4-74	3968221	3969603	-
STM3752	23	16	1.42	ST4-74	3970302	3970670	-
sugR	20	20	0.98	ST4-74	3971324	3972861	+
rhuM	14	14	1.01	ST4-74	3973048	3974085	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
rmbA	1	2	0.85	ST4-74	3975384	3976001	+
misL	6	5	1.35	ST4-74	3976076	3978943	+
fidL	16	12	1.28	ST4-74	3979038	3979520	-
marT	8	5	1.47	ST4-74	3979513	3980301	-
slsA	15	13	1.09	ST4-74	3981154	3981834	+
cigR	59	80	0.74	ST4-74	3982063	3982542	-
mgtR	14	15	0.92	ST4-74	3982743	3982835	-
mgtB	10	9	1.09	ST4-74	3982857	3985583	-
AmgR	6	4	1.66	ST4-74	3985659	3986858	+
mgtC	20	12	1.69	ST4-74	3985803	3986498	-
yicL	19	16	1.15	ST4-74	3987007	3987909	+
STM3766	10	9	1.14	ST4-74	3987952	3988893	-
STM3767	6	7	0.83	ST4-74	3989129	3989872	-
STM3768	3	3	0.91	ST4-74	3989859	3990968	-
STM3769	2	3	0.83	ST4-74	3990972	3991829	-
STM3770	4	4	0.97	ST4-74	3991829	3992578	-
STM3771	1	1	1.21	ST4-74	3992724	3993209	-
STM3772	1	1	1.04	ST4-74	3993220	3993645	-
STnc4040	91	62	1.46	ST4-74	3993821	3993944	-
STM3773	19	18	1.06	ST4-74	3993864	3996662	-
STM3774	33	23	1.45	ST4-74	3996859	3997152	+
STM3775	13	9	1.41	ST4-74	3997258	3998640	+
nepI	8	6	1.34	ST4-74	3998700	3999892	-
STM3777	30	26	1.15	ST4-74	4000108	4000467	+
STM3778	13	13	0.97	ST4-74	4000451	4000774	+
STM3779	23	18	1.32	ST4-74	4000865	4001134	-
STM3780	3	3	0.78	ST4-74	4001144	4002004	-
STM3781	6	6	0.97	ST4-74	4002067	4003551	-
STM3782	8	10	0.88	ST4-74	4003544	4004902	-
STM3783	14	16	0.87	ST4-74	4004978	4005265	-
STM3784	27	28	0.98	ST4-74	4005262	4005735	-
STM3785	46	34	1.35	ST4-74	4005753	4006496	-
yicN	36	34	1.05	ST4-74	4006849	4007301	-
uhpT	4	4	1.00	ST4-74	4007493	4008884	-
uhpC	6	6	1.04	ST4-74	4009027	4010355	-
uhpB	7	7	0.92	ST4-74	4010365	4011867	-
uhpA	20	15	1.36	ST4-74	4011867	4012457	-
STM3791	5	7	0.79	ST4-74	4012532	4013545	-
STM3792	4	8	0.50	ST4-74	4013557	4014873	-
STM3793	7	13	0.56	ST4-74	4014904	4015824	-
STM3794	9	9	0.94	ST4-74	4016149	4016934	+
ilvN	15	17	0.90	ST4-74	4016936	4017226	-
ilvB	13	13	0.95	ST4-74	4017230	4018918	-
ivbL	4003	3906	1.02	ST4-74	4019025	4019123	-
IstR-1,2	74	82	0.90	ST4-74	4019328	4019459	-
STM3796A	7	7	0.96	ST4-74	4020012	4020800	+
emrD	3	3	1.22	ST4-74	4021003	4022187	+
STM3799	19	16	1.21	ST4-74	4022114	4023199	-
dsdC	10	11	0.98	ST4-74	4023263	4024186	-
dsdX	6	5	1.12	ST4-74	4024412	4025749	+
dsdA	7	7	1.08	ST4-74	4025767	4027089	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
yidF	26	34	0.78	ST4-74	4027171	4027701	-
yidG	70	70	1.00	ST4-74	4027698	4028060	-
yidH	50	34	1.48	ST4-74	4028050	4028397	-
yidE	35	32	1.09	ST4-74	4028841	4030502	-
STnc2110	12	16	0.76	ST4-74	4030645	4030701	-
hslS	46	49	0.94	ST4-74	4030692	4031126	-
hslT	103	94	1.10	ST4-74	4031230	4031649	-
yidQ	119	102	1.17	ST4-74	4031950	4032285	+
yidR	16	18	0.86	ST4-74	4032287	4033513	-
ccmH1	4	10	0.41	ST4-74	4033635	4034678	-
ccmG1	8	13	0.58	ST4-74	4034675	4035232	-
ccmF1	7	14	0.49	ST4-74	4035229	4037160	-
ccmE1	6	14	0.46	ST4-74	4037157	4037636	-
ccmD1	4	8	0.46	ST4-74	4037633	4037845	-
ccmC1	11	29	0.37	ST4-74	4037842	4038579	-
ccmB1	5	10	0.51	ST4-74	4038631	4039287	-
ccmA1	5	15	0.31	ST4-74	4039287	4039913	-
yhjA	22	47	0.47	ST4-74	4039910	4041310	-
torD	39	35	1.10	ST4-74	4041500	4042132	-
torA	31	34	0.91	ST4-74	4042125	4044677	-
torC	123	108	1.14	ST4-74	4044667	4045851	-
torR	39	48	0.81	ST4-74	4045981	4046673	+
torT	24	27	0.89	ST4-74	4046646	4047686	-
torS	6	6	0.93	ST4-74	4047766	4050501	+
dgoT	4	4	1.13	ST4-74	4050527	4051864	-
dgoD	3	2	1.46	ST4-74	4051949	4053097	-
dgoA	2	2	0.83	ST4-74	4053094	4053711	-
dgoK	12	13	0.94	ST4-74	4053695	4054573	-
dgoR	8	9	0.90	ST4-74	4054570	4055259	-
yidA	21	22	0.95	ST4-74	4055520	4056365	-
STM3832	3	3	1.18	ST4-74	4056671	4057933	+
STM3833	8	8	0.99	ST4-74	4057947	4059140	+
STM3834	31	27	1.16	ST4-74	4059255	4060151	+
gyrB	27	29	0.92	ST4-74	4060170	4062584	-
recF	43	35	1.22	ST4-74	4062613	4063686	-
dnaN	19	25	0.76	ST4-74	4063834	4064934	-
dnaA	55	62	0.90	ST4-74	4064939	4066339	-
rpmH	3994	3735	1.07	ST4-74	4067000	4067140	+
rnpA	66	82	0.81	ST4-74	4067157	4067516	+
STM3841	55	61	0.90	ST4-74	4067480	4067737	+
yidC	46	62	0.74	ST4-74	4067740	4069386	+
STnc4250	118	138	0.86	ST4-74	4069296	4069428	+
thdF	6	6	1.05	ST4-74	4069516	4070892	+
STnc3170	182	100	1.83	ST4-74	4070922	4071037	-
intA	8	7	1.25	ST4-74	4071148	4072365	+
STnc400	18109	36392	0.50	ST4-74	4072456	4072529	+
STM3845	83	76	1.08	ST4-74	4072690	4073652	+
STM3846	37	40	0.94	ST4-74	4073652	4074587	+
yidY	7	6	1.21	ST4-74	4075389	4076576	+
yidZ	8	5	1.55	ST4-74	4076545	4077504	+
yieE	35	30	1.18	ST4-74	4077662	4078417	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
yieF	76	49	1.54	ST4-74	4078417	4079001	+
yieG	31	38	0.80	ST4-74	4079046	4080383	-
yieH	59	62	0.96	ST4-74	4080552	4081217	+
STnc1860	137	187	0.73	ST4-74	4081218	4081302	-
phoU	14	13	1.07	ST4-74	4081312	4082037	-
pstB	17	20	0.84	ST4-74	4082052	4082825	-
pstA	14	12	1.20	ST4-74	4082912	4083802	-
pstC	15	14	1.06	ST4-74	4083802	4084761	-
pstS	20	20	0.99	ST4-74	4084897	4085937	-
STM3858	7	7	1.02	ST4-74	4086104	4087477	-
STM3859	2	2	0.82	ST4-74	4087526	4088344	-
STM3860	6	7	0.88	ST4-74	4088439	4090196	+
glmS	43	56	0.77	ST4-74	4090347	4092176	-
glmU	119	124	0.96	ST4-74	4092365	4093735	-
STM3863	22	16	1.34	ST4-74	4094055	4094753	-
atpC	126	208	0.61	ST4-74	4094983	4095402	-
atpD	69	111	0.62	ST4-74	4095423	4096805	-
atpG	81	139	0.58	ST4-74	4096832	4097695	-
atpA	86	138	0.63	ST4-74	4097746	4099287	-
atpH	125	194	0.64	ST4-74	4099300	4099833	-
atpF	267	417	0.64	ST4-74	4099848	4100318	-
atpE	398	640	0.62	ST4-74	4100378	4100617	-
atpB	160	218	0.74	ST4-74	4100664	4101479	-
atpI	333	374	0.89	ST4-74	4101488	4101868	-
gidB	19	20	0.95	ST4-74	4102484	4103113	-
gidA	24	26	0.92	ST4-74	4103210	4105099	-
mioC	114	115	0.99	ST4-74	4105478	4105921	-
asnCb	23	20	1.14	ST4-74	4106011	4106469	-
asnA	67	78	0.86	ST4-74	4106620	4107612	+
yieM	8	10	0.83	ST4-74	4107617	4109017	-
yieN	25	23	1.09	ST4-74	4109062	4110558	-
kup	19	21	0.89	ST4-74	4110906	4112774	+
rbsD	34	40	0.85	ST4-74	4112971	4113390	+
rbsA	4	5	0.86	ST4-74	4113398	4114903	+
rbsC	12	14	0.86	ST4-74	4114909	4115874	+
rbsB	337	283	1.19	ST4-74	4115899	4116789	+
rbsK	15	21	0.72	ST4-74	4116923	4117852	+
rbsR	11	17	0.70	ST4-74	4117856	4118854	+
yieO	11	9	1.21	ST4-74	4118820	4120243	-
yieP	16	18	0.93	ST4-74	4120255	4120959	-
yifA	24	27	0.88	ST4-74	4127091	4127939	-
yifE	826	598	1.38	ST4-74	4128046	4128384	+
comM	3	3	1.04	ST4-74	4128409	4129929	-
ilvX	237	232	1.02	ST4-74	4130466	4130516	+
ilvG	32	35	0.92	ST4-74	4130519	4132165	+
ilvM	63	66	0.95	ST4-74	4132165	4132425	+
ilvE	43	47	0.90	ST4-74	4132443	4133372	+
ilvD	28	33	0.84	ST4-74	4133533	4135383	+
ilvA	65	78	0.83	ST4-74	4135386	4136930	+
STM3906	51	48	1.06	ST4-74	4137214	4137531	+
STM3907	48	41	1.16	ST4-74	4137528	4137869	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
ilvY	6	7	0.80	ST4-74	4137872	4138759	-
ilvC	200	239	0.84	ST4-74	4138923	4140398	+
ppiC	198	193	1.03	ST4-74	4140490	4140771	-
SL1344_3870A	20	22	0.91	ST4-74	4140776	4141081	+
SL1344_3871	17	17	0.99	ST4-74	4141099	4141209	+
rep	15	13	1.21	ST4-74	4141309	4143333	+
gppA	17	23	0.77	ST4-74	4143373	4144854	-
rhlB	55	63	0.87	ST4-74	4144973	4146238	-
trxA	589	654	0.90	ST4-74	4146382	4146711	+
rho	134	122	1.10	ST4-74	4147130	4148389	+
rfe	79	77	1.02	ST4-74	4148619	4149722	+
wzzE	91	83	1.11	ST4-74	4149734	4150780	+
rffE	26	28	0.93	ST4-74	4150836	4151966	+
rffD	24	27	0.88	ST4-74	4151963	4153225	+
rffG	18	18	0.99	ST4-74	4153240	4154292	+
rffH	21	23	0.92	ST4-74	4154325	4154549	+
rffC	15	16	0.91	ST4-74	4154656	4155204	+
rffA	17	18	0.96	ST4-74	4155209	4156339	+
wzxE	13	14	0.94	ST4-74	4156341	4157591	+
STM3927	9	9	0.94	ST4-74	4157588	4158667	+
rffT	17	18	0.95	ST4-74	4158664	4160022	+
rffM	11	12	0.88	ST4-74	4160019	4160759	+
yifK	48	32	1.51	ST4-74	4160966	4162351	+
GlmZ	3736	3416	1.09	ST4-74	4163192	4163399	+
hemY	34	38	0.90	ST4-74	4163468	4164607	-
hemX	31	35	0.88	ST4-74	4164667	4165836	-
hemD	27	37	0.72	ST4-74	4165858	4166598	-
hemC	73	74	0.99	ST4-74	4166595	4167551	-
cyaA	51	45	1.14	ST4-74	4167913	4170459	+
STM3940	12	9	1.24	ST4-74	4170567	4170920	+
STM3941	5	3	1.82	ST4-74	4171106	4171648	+
STM3942	4	5	0.96	ST4-74	4171660	4172022	+
cyaY	56	76	0.73	ST4-74	4172093	4172413	-
STM3944	3	2	1.26	ST4-74	4172483	4172872	-
STM3945	3	3	1.01	ST4-74	4172860	4173382	-
yifL	52	62	0.84	ST4-74	4173568	4173771	+
dapF	29	35	0.82	ST4-74	4173805	4174632	+
yigA	15	20	0.76	ST4-74	4174629	4175336	+
xerC	12	16	0.70	ST4-74	4175333	4176235	+
yigB	10	12	0.82	ST4-74	4176235	4176951	+
uvrD	18	18	0.98	ST4-74	4177087	4179249	+
corA	21	22	0.98	ST4-74	4179721	4180671	+
yigF	7	4	1.85	ST4-74	4180720	4181100	-
yigG	5	4	1.31	ST4-74	4181116	4181574	-
rarD	23	21	1.11	ST4-74	4181609	4182493	-
yigI	9	6	1.33	ST4-74	4182537	4183022	-
pldA	73	41	1.81	ST4-74	4183292	4184038	+
recQ	28	26	1.09	ST4-74	4184104	4185951	+
rhtC	15	16	0.94	ST4-74	4186015	4186635	+
rhtB	9	10	0.86	ST4-74	4186675	4187295	-
pldB	39	38	1.03	ST4-74	4187406	4188422	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
yigL	20	16	1.21	ST4-74	4188438	4189238	+
yigM	62	64	0.98	ST4-74	4189319	4190218	+
metR	33	38	0.86	ST4-74	4190106	4191059	-
metE	91	115	0.79	ST4-74	4191308	4193572	+
STM3966	5	5	0.94	ST4-74	4193916	4195259	+
dlhH	43	39	1.11	ST4-74	4195339	4196151	-
udp	31	31	1.02	ST4-74	4196410	4197171	+
yigN	14	15	0.93	ST4-74	4197311	4198741	+
ubiE	100	86	1.17	ST4-74	4198837	4199592	+
yigP	34	34	1.00	ST4-74	4199602	4200207	+
aarF	59	54	1.10	ST4-74	4200204	4201844	+
tatA	614	636	0.97	ST4-74	4202050	4202304	+
tatB	109	137	0.80	ST4-74	4202308	4202856	+
tatC	69	77	0.90	ST4-74	4202859	4203638	+
tatD	6	6	0.91	ST4-74	4203668	4204462	+
rfaH	82	72	1.14	ST4-74	4204470	4204958	-
yigC	59	56	1.05	ST4-74	4205144	4206622	+
fre	54	44	1.24	ST4-74	4206708	4207409	+
STM3980	19	14	1.34	ST4-74	4207663	4209486	+
STnc790	44	33	1.32	ST4-74	4209485	4209680	-
fadA	7	7	1.12	ST4-74	4209683	4210846	-
fadB	5	4	1.33	ST4-74	4210856	4213045	-
pepQ	35	36	0.98	ST4-74	4213235	4214566	+
yigZ	34	33	1.05	ST4-74	4214566	4215180	+
trkH	26	26	0.97	ST4-74	4215219	4216670	+
hemG	21	22	0.94	ST4-74	4216682	4217227	+
mobB	19	18	1.09	ST4-74	4223123	4223638	-
mobA	41	34	1.21	ST4-74	4223635	4224219	-
yihD	171	197	0.87	ST4-74	4224289	4224558	+
rodA	99	101	0.98	ST4-74	4224635	4225621	+
dsbA	110	116	0.94	ST4-74	4225638	4226261	+
yihG	22	18	1.23	ST4-74	4226274	4227182	-
polA	20	21	0.94	ST4-74	4227570	4230356	+
Spf	1745	983	1.77	ST4-74	4230504	4230613	+
engB	60	59	1.01	ST4-74	4230691	4231290	-
CsrC	56981	31195	1.83	ST4-74	4231595	4231838	+
yihI	98	79	1.23	ST4-74	4231906	4232421	+
hemN	18	18	1.05	ST4-74	4232610	4233983	+
yshB	737	991	0.74	ST4-74	4234073	4234183	-
glnG	6	6	1.03	ST4-74	4234296	4235705	-
glnL	9	8	1.02	ST4-74	4235714	4236763	-
STnc800	559	331	1.69	ST4-74	4236851	4237035	-
glnA	88	71	1.23	ST4-74	4237038	4238447	-
typA	61	61	1.01	ST4-74	4238823	4240646	+
STM4010	11	9	1.22	ST4-74	4240701	4241435	-
STM4011	6	5	1.18	ST4-74	4241420	4242298	-
STM4012	3	2	1.47	ST4-74	4242295	4243536	-
STM4013	10	6	1.77	ST4-74	4243538	4244401	-
STM4014	2	1	1.47	ST4-74	4244406	4245431	-
STM4015	4	4	0.85	ST4-74	4245444	4246292	-
ompL	2	2	1.00	ST4-74	4246449	4247141	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
yihO	9	8	1.12	ST4-74	4247209	4248630	-
yihP	5	6	0.87	ST4-74	4248676	4250058	-
STnc3120	180	89	2.03	ST4-74	4249942	4250100	+
yihQ	4	4	1.19	ST4-74	4250104	4252140	-
yihR	2	1	1.40	ST4-74	4252184	4253035	-
yihS	1	1	1.08	ST4-74	4253045	4254286	-
yihT	1	1	1.25	ST4-74	4254302	4255180	-
yihU	1	1	2.07	ST4-74	4255203	4256099	-
yihV	4	4	0.91	ST4-74	4256258	4257160	+
yihW	23	24	0.94	ST4-74	4257194	4257997	+
yihX	68	58	1.16	ST4-74	4258188	4258787	+
rbn	58	48	1.23	ST4-74	4258781	4259653	+
yihZ	29	29	1.02	ST4-74	4259650	4260087	+
yiiD	21	19	1.13	ST4-74	4260084	4261073	+
STM4030	23	21	1.12	ST4-74	4261088	4261516	-
STM4031	65	55	1.19	ST4-74	4261531	4261842	-
STM4032	5	6	0.95	ST4-74	4261928	4262857	-
SL1344_3979	102	96	1.07	ST4-74	4263075	4263386	+
STM4033	33	27	1.20	ST4-74	4263387	4263677	+
fdhE	9	15	0.64	ST4-74	4263724	4264653	-
fdoI	36	36	1.00	ST4-74	4264650	4265285	-
fdoH	5	8	0.72	ST4-74	4265282	4266184	-
fdoG	15	20	0.74	ST4-74	4266197	4269247	-
fdhD	10	13	0.80	ST4-74	4269442	4270278	+
STM4039	13	11	1.18	ST4-74	4270546	4271523	-
yiiG	2	2	1.04	ST4-74	4271802	4272860	+
STM4041	14	15	0.93	ST4-74	4273215	4273538	-
STM4042	22	33	0.66	ST4-74	4273538	4274197	-
STM4042A	40	38	1.05	ST4-74	4274280	4274846	+
yiiL	16	18	0.89	ST4-74	4274935	4275249	-
STM4044	5	5	1.02	ST4-74	4275246	4276394	-
rhaD	6	4	1.50	ST4-74	4276521	4277348	-
rhaA	1	1	1.06	ST4-74	4277491	4278750	-
rhaB	1	1	1.23	ST4-74	4278747	4280216	-
rhaS	9	10	0.86	ST4-74	4280504	4281340	+
rhaR	12	13	0.91	ST4-74	4281493	4282341	+
rhaT	4	5	0.99	ST4-74	4282338	4283372	-
STM4051	4	3	1.11	ST4-74	4283991	4284674	+
STM4052	5	5	0.88	ST4-74	4284832	4286139	-
STM4053	2	3	0.79	ST4-74	4286132	4286647	-
STM4054	2	2	0.81	ST4-74	4286666	4287649	-
sodA	103	65	1.58	ST4-74	4287978	4288598	+
yiiM	11	16	0.66	ST4-74	4288668	4289357	+
STM4057	18	26	0.68	ST4-74	4289369	4289764	+
cpxA	23	35	0.67	ST4-74	4289815	4291188	-
cpxR	46	58	0.80	ST4-74	4291185	4291883	-
cpxP	2655	2314	1.15	ST4-74	4292034	4292534	+
STnc870	8576	11967	0.72	ST4-74	4292540	4292597	+
fieF	87	64	1.37	ST4-74	4292682	4293584	+
pfkA	49	72	0.68	ST4-74	4293769	4294731	+
sbp	62	113	0.55	ST4-74	4294935	4295924	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
cdh-a	159	130	1.23	ST4-74	4296025	4296780	+
STM4065	7	8	0.90	ST4-74	4297043	4298377	+
STM4066	5	5	1.02	ST4-74	4298388	4299347	+
STM4067	46	43	1.07	ST4-74	4299357	4300397	+
STM4068	30	35	0.88	ST4-74	4300460	4301182	+
STM4069	26	17	1.49	ST4-74	4301280	4301444	+
cdh-b	7	5	1.25	ST4-74	4301460	4301591	+
STM4071	12	16	0.75	ST4-74	4301681	4301947	-
ydeV	15	12	1.24	ST4-74	4302045	4303637	-
ydeW	22	20	1.10	ST4-74	4303725	4304684	-
ego	12	15	0.79	ST4-74	4304940	4306475	+
ydeY	11	14	0.79	ST4-74	4306469	4307512	+
ydeZ	16	19	0.87	ST4-74	4307509	4308510	+
yneA	11	18	0.63	ST4-74	4308539	4309561	+
yneB	26	29	0.90	ST4-74	4309590	4310465	+
yneC	26	28	0.91	ST4-74	4310545	4310838	+
STM4080	30	30	1.00	ST4-74	4310848	4311612	+
tpiA	117	169	0.69	ST4-74	4311704	4312471	-
yiiQ	48	38	1.26	ST4-74	4312584	4313180	-
yiiR	35	25	1.37	ST4-74	4313281	4313709	+
fpr	17	18	0.95	ST4-74	4313816	4314562	-
glpX	11	24	0.44	ST4-74	4314659	4315669	-
glpK	161	287	0.56	ST4-74	4315781	4317289	-
glpF	226	417	0.54	ST4-74	4317310	4318155	-
yiiU	1019	918	1.11	ST4-74	4318554	4318793	+
menG	261	319	0.82	ST4-74	4319015	4319500	-
menA	18	17	1.06	ST4-74	4319593	4320522	-
hslU	122	69	1.77	ST4-74	4320589	4321920	-
hslV	53	63	0.84	ST4-74	4321930	4322460	-
ftsN	79	77	1.04	ST4-74	4322552	4323526	-
cytR	18	24	0.74	ST4-74	4323620	4324645	-
priA	18	18	1.01	ST4-74	4324800	4326998	-
rpmE	221	329	0.67	ST4-74	4327202	4327414	+
STM4097	6	6	1.07	ST4-74	4327460	4328122	-
IsrP	55	33	1.66	ST4-74	4328157	4328304	+
STM4098	5	3	1.36	ST4-74	4328323	4330113	-
metJ	220	194	1.13	ST4-74	4330570	4330887	-
metB	59	71	0.83	ST4-74	4331152	4332312	+
metL	30	40	0.73	ST4-74	4332315	4334747	+
STnc4070	42	41	1.01	ST4-74	4334757	4334855	-
STM4102	2	3	0.83	ST4-74	4335001	4335720	-
SL1344_4052	2	2	1.24	ST4-74	4335723	4336721	-
STM4103	10	13	0.80	ST4-74	4336956	4338080	-
STM4104	7	6	1.16	ST4-74	4338216	4339772	+
STnc4080	45	33	1.39	ST4-74	4339805	4339929	-
metF	268	273	0.98	ST4-74	4339967	4340857	+
katG	24	33	0.71	ST4-74	4341022	4343202	+
yijF	12	10	1.17	ST4-74	4343260	4343883	-
gldA	11	28	0.40	ST4-74	4344142	4345245	-
talC	14	23	0.61	ST4-74	4345257	4345919	-
ptsA	6	6	1.03	ST4-74	4345930	4348431	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
frwC	13	9	1.40	ST4-74	4348740	4349819	+
frwB	37	25	1.49	ST4-74	4349834	4350154	+
pflD	15	10	1.52	ST4-74	4350253	4352550	+
pflC	25	18	1.42	ST4-74	4352516	4353394	+
frwD	12	8	1.46	ST4-74	4353396	4353749	+
yijO	38	36	1.07	ST4-74	4353736	4354587	-
yijP	48	52	0.91	ST4-74	4354748	4356481	-
STnc1600	285	256	1.12	ST4-74	4356616	4356686	+
ppc	35	49	0.71	ST4-74	4356692	4359343	-
argE	30	45	0.67	ST4-74	4359712	4360863	-
argC	119	184	0.65	ST4-74	4360952	4361956	+
argB	39	77	0.51	ST4-74	4361964	4362740	+
argH	50	89	0.56	ST4-74	4362858	4364234	+
OxyS	27	42	0.63	ST4-74	4364305	4364423	-
oxyR	25	35	0.71	ST4-74	4364519	4365436	+
udhA	24	24	0.98	ST4-74	4365419	4366819	-
yijC	74	79	0.94	ST4-74	4367018	4367653	+
yijD	41	46	0.89	ST4-74	4367669	4368028	+
trmA	18	16	1.11	ST4-74	4368075	4369175	-
btuB	19	23	0.84	ST4-74	4369507	4371393	+
murI	20	22	0.93	ST4-74	4371338	4372189	+
murB	24	20	1.20	ST4-74	4378004	4379032	+
birA	19	19	1.01	ST4-74	4379029	4379991	+
coaA	39	38	1.04	ST4-74	4380026	4380976	-
STM4141	18	11	1.69	ST4-74	4381185	4381358	-
tufB	545	788	0.69	ST4-74	4381935	4383119	+
secE	165	185	0.89	ST4-74	4383349	4383732	+
nusG	72	91	0.80	ST4-74	4383734	4384279	+
rplK	404	406	0.99	ST4-74	4384437	4384865	+
rplA	192	252	0.76	ST4-74	4384869	4385573	+
rplJ	580	731	0.79	ST4-74	4385993	4386490	+
rplL	690	935	0.74	ST4-74	4386557	4386922	+
rpoB	51	72	0.71	ST4-74	4387240	4391268	+
rpoC	53	76	0.69	ST4-74	4391345	4395568	+
STnc3490	21	15	1.40	ST4-74	4395603	4395691	-
STM4155	9	6	1.46	ST4-74	4395610	4395933	+
STM4156	6	5	1.13	ST4-74	4395939	4396283	-
sseK1	59	40	1.48	ST4-74	4396682	4397692	+
STM4158	186	136	1.37	ST4-74	4398015	4398203	+
thiH	32	37	0.85	ST4-74	4398354	4399487	-
thiG	21	27	0.80	ST4-74	4399484	4400254	-
thiS	30	39	0.77	ST4-74	4400256	4400456	-
thiF	23	30	0.76	ST4-74	4400437	4401195	-
thiE	19	23	0.81	ST4-74	4401188	4401823	-
thiC	29	34	0.85	ST4-74	4401823	4403718	-
STnc1460	5179	6313	0.82	ST4-74	4403810	4404049	-
rsd	38	26	1.47	ST4-74	4404082	4404570	-
nudC	13	9	1.33	ST4-74	4404663	4405436	+
hemE	26	20	1.28	ST4-74	4405477	4406541	+
nfi	23	19	1.23	ST4-74	4406551	4407222	+
yjaG	65	66	0.99	ST4-74	4407264	4407854	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
hupA	1467	2155	0.68	ST4-74	4408041	4408313	+
yjaH	17	22	0.75	ST4-74	4408325	4409017	+
zraP	6	7	0.93	ST4-74	4409059	4409514	-
hydH	14	15	0.90	ST4-74	4409768	4411165	+
hydG	11	12	0.95	ST4-74	4411171	4412496	+
purD	10	19	0.52	ST4-74	4412493	4413782	-
purH	16	27	0.61	ST4-74	4413794	4415383	-
yjaB	54	48	1.12	ST4-74	4421537	4421974	-
metA	115	104	1.11	ST4-74	4422131	4423060	+
aceB	5	4	1.15	ST4-74	4423329	4424930	+
aceA	6	5	1.19	ST4-74	4424962	4426266	+
aceK	10	8	1.29	ST4-74	4426368	4428119	+
STM4186	7	7	1.03	ST4-74	4428083	4428517	-
iclR	12	16	0.76	ST4-74	4428501	4429325	-
metH	25	25	1.03	ST4-74	4429629	4433312	+
yjbB	17	17	1.04	ST4-74	4433654	4435210	+
pepE	9	32	0.28	ST4-74	4435286	4435975	-
STM4191	30	24	1.27	ST4-74	4436047	4436148	+
STM4192	74	66	1.13	ST4-74	4436183	4436722	+
yjbC	25	26	0.96	ST4-74	4436769	4437638	+
yjbD	39	44	0.88	ST4-74	4437635	4437907	-
STM4195	16	27	0.60	ST4-74	4438005	4438946	-
STM4196	7	10	0.75	ST4-74	4439208	4439936	-
STM4197	2	3	0.82	ST4-74	4440133	4440423	-
STM4198	5	5	1.02	ST4-74	4440672	4441127	-
STM4199	4	4	0.83	ST4-74	4441124	4441729	-
STM4200	7	7	1.02	ST4-74	4441734	4443479	-
STM4201	1	1	1.06	ST4-74	4443482	4444114	-
STM4202	1	7	0.21	ST4-74	4444107	4445222	-
STM4203	1	0	3.26	ST4-74	4445213	4445572	-
STM4204	11	10	1.08	ST4-74	4445736	4447283	-
gtrBb	9	8	1.08	ST4-74	4447283	4448212	-
gtrAb	53	42	1.25	ST4-74	4448209	4448535	-
STnc4100	97	66	1.47	ST4-74	4448720	4448837	+
STM4207	4	3	1.10	ST4-74	4448899	4449621	-
STM4208	2	1	2.01	ST4-74	4449631	4450674	-
STM4209	5	2	2.53	ST4-74	4450662	4450871	-
STM4210	3	2	1.66	ST4-74	4450871	4451824	-
STM4211	1	1	1.14	ST4-74	4451824	4454178	-
SL1344_4147	3	4	0.83	ST4-74	4454363	4454680	-
STM4212	2	1	1.16	ST4-74	4454732	4455256	-
STM4213	2	2	0.97	ST4-74	4455256	4456683	-
STM4214	1	1	0.63	ST4-74	4456673	4456870	-
STM4215	1	1	1.24	ST4-74	4456867	4457322	-
STM4216	0	0	1.51	ST4-74	4457482	4457796	-
STM4217	1	1	0.64	ST4-74	4457809	4458414	-
STM4218	3	3	0.93	ST4-74	4458417	4458704	-
STM4219	47	43	1.10	ST4-74	4459280	4459627	+
lysC	85	186	0.46	ST4-74	4459760	4461109	-
pgi	52	52	1.00	ST4-74	4461454	4463103	+
yjbE	30	29	1.04	ST4-74	4463547	4463789	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
yjbF	2	2	0.95	ST4-74	4463823	4464491	+
yjbG	1	1	1.28	ST4-74	4464488	4465225	+
yjbH	8	8	1.01	ST4-74	4465225	4467321	+
yjbA	169	91	1.87	ST4-74	4467464	4467874	+
STnc810	37	26	1.41	ST4-74	4467934	4467988	-
malG	3	3	1.06	ST4-74	4468040	4468930	-
malF	3	3	0.86	ST4-74	4468945	4470489	-
malE	3	3	0.83	ST4-74	4470621	4471820	-
malK	2	2	0.94	ST4-74	4472173	4473282	+
lamB	6	6	1.10	ST4-74	4473371	4474729	+
malM	8	7	1.13	ST4-74	4474893	4475810	+
STnc2130	96	105	0.91	ST4-74	4475777	4475867	+
ubiC	20	20	1.00	ST4-74	4475991	4476488	+
ubiA	16	17	0.92	ST4-74	4476502	4477374	+
plsB	30	34	0.88	ST4-74	4477473	4479893	-
dgkA	40	39	1.01	ST4-74	4480064	4480432	+
lexA	129	134	0.96	ST4-74	4480541	4481149	+
dinF	11	12	0.93	ST4-74	4481328	4482653	+
yjbJ	381	204	1.87	ST4-74	4482782	4482994	+
zur	34	29	1.15	ST4-74	4483093	4483608	-
STM4242	17	39	0.43	ST4-74	4483855	4485165	+
yjbN	41	37	1.11	ST4-74	4485253	4486251	+
pspG	24	25	0.96	ST4-74	4486464	4486661	+
STnc880	1381	2241	0.62	ST4-74	4486745	4486823	+
qor	22	19	1.18	ST4-74	4486836	4487819	-
dnaB	23	26	0.87	ST4-74	4487884	4489299	+
alr-b	13	14	0.90	ST4-74	4489331	4490410	+
tyrB	24	29	0.80	ST4-74	4490596	4491789	+
aphA	49	66	0.74	ST4-74	4491976	4492689	+
yjbQ	46	46	0.99	ST4-74	4492818	4493234	+
yjbR	19	17	1.12	ST4-74	4493237	4493593	+
STM4252	3	2	1.63	ST4-74	4493594	4493932	-
STM4253	1	1	1.21	ST4-74	4493919	4494374	-
uvrA	12	12	1.07	ST4-74	4494506	4497331	-
STM4255	8	11	0.76	ST4-74	4497296	4497412	+
ssb	93	85	1.09	ST4-74	4497579	4498109	+
siiA	20	12	1.60	ST4-74	4499150	4499782	+
siiB	13	9	1.48	ST4-74	4499779	4501167	+
siiC	12	9	1.45	ST4-74	4501157	4502476	+
siiD	12	8	1.51	ST4-74	4502473	4503750	+
siiE	10	8	1.17	ST4-74	4503767	4520446	+
siiF	11	8	1.48	ST4-74	4520486	4522552	+
yjcB	12	14	0.87	ST4-74	4522830	4523111	-
yjcC	10	8	1.31	ST4-74	4523674	4525275	+
soxS	21	30	0.71	ST4-74	4525263	4525586	-
soxR	12	12	1.00	ST4-74	4525673	4526131	+
SraL	1784	818	2.18	ST4-74	4526162	4526302	-
STM4267	70	45	1.58	ST4-74	4526424	4527092	+
yjcD	30	40	0.74	ST4-74	4527439	4528788	+
yjcE	16	16	0.99	ST4-74	4528938	4530584	+
STM4270	29	24	1.20	ST4-74	4530679	4531566	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STM4271	10	14	0.69	ST4-74	4531670	4532080	+
STM4272	7	7	1.02	ST4-74	4532073	4532762	+
actP	10	8	1.25	ST4-74	4532801	4534450	-
yjcH	5	5	1.12	ST4-74	4534447	4534761	-
acs	17	17	1.06	ST4-74	4535007	4536965	-
STM4276	89	88	1.01	ST4-74	4537152	4537298	+
nrfA	4	19	0.22	ST4-74	4537397	4538833	+
nrfB	6	11	0.51	ST4-74	4538945	4539511	+
nrfC	6	8	0.70	ST4-74	4539508	4540179	+
nrfD	3	5	0.67	ST4-74	4540176	4541132	+
nrfE	6	5	1.21	ST4-74	4541125	4543347	+
nrfG	1	2	0.54	ST4-74	4543344	4543964	+
gltP	37	39	0.97	ST4-74	4544309	4545619	+
yjcO	40	28	1.43	ST4-74	4545777	4546388	-
SC4B5.11c	7	19	0.39	ST4-74	4546643	4548790	-
lpxO	53	30	1.73	ST4-74	4549145	4550053	-
phnO	130	64	2.03	ST4-74	4550311	4550745	-
phnB	20	15	1.31	ST4-74	4550897	4551340	-
phnA	56	91	0.61	ST4-74	4551460	4551795	-
proP	55	41	1.33	ST4-74	4552263	4553765	+
STnc630	2260	1641	1.38	ST4-74	4553813	4553972	+
basS	33	42	0.77	ST4-74	4553932	4555002	-
basR	53	47	1.12	ST4-74	4555012	4555680	-
yjdB	60	69	0.87	ST4-74	4555677	4557320	-
adiC	7	6	1.08	ST4-74	4557454	4558791	-
adiY	3	3	1.08	ST4-74	4558931	4559692	-
STnc1180	18	18	1.01	ST4-74	4559883	4559977	-
adi	4	3	1.20	ST4-74	4559990	4562260	-
STnc4110	29	24	1.22	ST4-74	4562397	4562461	+
melR	13	11	1.10	ST4-74	4562490	4563422	-
melA	3	3	1.22	ST4-74	4563691	4565046	+
melB	6	4	1.43	ST4-74	4565130	4566560	+
fumB	11	78	0.14	ST4-74	4566658	4568304	-
dcuB	15	104	0.15	ST4-74	4568400	4569740	-
STM4302	565	720	0.78	ST4-74	4569887	4570153	-
dcuR	24	42	0.58	ST4-74	4570470	4571189	-
dcuS	23	30	0.79	ST4-74	4571186	4572817	-
STM4305	8	62	0.13	ST4-74	4573173	4575602	+
STM4306	7	37	0.18	ST4-74	4575616	4576242	+
STM4307	8	39	0.20	ST4-74	4576235	4577008	+
STM4308	12	67	0.17	ST4-74	4577024	4577677	+
STM4309	2	3	0.80	ST4-74	4577761	4579161	-
STM4310	7	7	0.97	ST4-74	4579465	4580370	+
STnc440	16639	4653	3.58	ST4-74	4580485	4580565	+
STM4312	2	4	0.57	ST4-74	4580664	4580933	-
STM4313	16	17	0.95	ST4-74	4580941	4581156	-
rtsB	10	17	0.56	ST4-74	4581179	4581466	-
rtsA	29	50	0.58	ST4-74	4581463	4582338	-
STM4316	3	2	1.40	ST4-74	4582603	4582824	-
STM4317	52	57	0.91	ST4-74	4583141	4583434	+
STM4318	14	12	1.10	ST4-74	4583431	4583922	+

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phoN	187	101	1.84	ST4-74	4584170	4584922	-
SL1344_4256	2	0	–	ST4-74	4584965	4585099	-
SL1344_4257	4	4	1.01	ST4-74	4586132	4586509	-
STM4320	27	18	1.48	ST4-74	4586581	4586913	+
yjdC	106	65	1.64	ST4-74	4587352	4587927	-
dipZ	13	16	0.77	ST4-74	4587964	4589667	-
cutA	22	22	0.99	ST4-74	4589643	4589990	-
dcuA	36	59	0.60	ST4-74	4590111	4591295	-
STnc3000	9	5	2.02	ST4-74	4591418	4591505	+
aspA	160	225	0.71	ST4-74	4591527	4592963	-
fxsA	76	87	0.88	ST4-74	4593265	4593780	+
yjeH	104	63	1.66	ST4-74	4593839	4595080	-
groES	647	861	0.75	ST4-74	4595356	4595649	+
groEL	150	208	0.72	ST4-74	4595693	4597339	+
yjeI	281	327	0.86	ST4-74	4597565	4597936	+
yjeJ	24	18	1.29	ST4-74	4597984	4598841	-
yjeK	27	21	1.29	ST4-74	4599123	4600151	-
efp	200	223	0.90	ST4-74	4600192	4600758	+
ecnA	22	20	1.09	ST4-74	4600777	4600953	+
ecnB	7018	2863	2.45	ST4-74	4601061	4601207	+
ecnR	16	24	0.69	ST4-74	4601238	4601825	-
sugE	91	72	1.26	ST4-74	4602082	4602399	+
blc	45	23	1.97	ST4-74	4602416	4602949	-
frdD	129	270	0.48	ST4-74	4603061	4603420	-
frdC	40	116	0.35	ST4-74	4603431	4603826	-
frdB	50	147	0.34	ST4-74	4603837	4604571	-
frdA	44	130	0.34	ST4-74	4604564	4606354	-
yjeA	21	17	1.22	ST4-74	4606677	4607654	+
yjeM	6	7	0.96	ST4-74	4607880	4609382	+
yjeO	79	70	1.12	ST4-74	4609434	4609748	+
yjeP	17	19	0.86	ST4-74	4609815	4613117	-
psd	39	44	0.89	ST4-74	4613160	4614128	-
yjeQ	46	40	1.16	ST4-74	4614220	4615296	-
orn	57	57	1.00	ST4-74	4615379	4615924	+
STM4351	14	15	0.91	ST4-74	4615986	4616726	-
yjeV	14	14	1.00	ST4-74	4617775	4617828	+
yjeS	17	14	1.22	ST4-74	4617811	4618950	-
yjeF	21	19	1.16	ST4-74	4618949	4620496	+
yjeE	24	24	0.99	ST4-74	4620468	4620929	+
amiB	31	34	0.92	ST4-74	4620946	4622265	+
mutL	24	30	0.82	ST4-74	4622275	4624131	+
miaA	251	262	0.96	ST4-74	4624124	4625074	+
hfq	680	761	0.89	ST4-74	4625157	4625465	+
hflX	115	132	0.87	ST4-74	4625537	4626817	+
hflK	103	114	0.90	ST4-74	4627032	4628291	+
hflC	119	138	0.87	ST4-74	4628294	4629298	+
purA	230	251	0.91	ST4-74	4629677	4630975	+
nsrR	116	98	1.18	ST4-74	4631182	4631607	+
rnr	28	27	1.02	ST4-74	4631645	4634083	+
yjfH	29	38	0.77	ST4-74	4634174	4634905	+
yjfI	4	3	1.26	ST4-74	4635037	4635441	+

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yjfJ	6	5	1.06	ST4-74	4635456	4636154	+
STM4372	3	2	1.30	ST4-74	4636154	4637224	+
yjfK	3	3	1.25	ST4-74	4637197	4637880	+
yjfL	10	9	1.22	ST4-74	4637898	4638296	+
yjfM	4	5	0.94	ST4-74	4638306	4638944	+
yjfC	6	5	1.14	ST4-74	4638947	4640110	+
aidB	13	12	1.13	ST4-74	4640195	4641817	+
yjfN	79	63	1.25	ST4-74	4641862	4642182	-
yjfO	294	222	1.33	ST4-74	4642268	4642570	-
yjfP	13	15	0.81	ST4-74	4642781	4643530	+
yjfQ	20	23	0.86	ST4-74	4643527	4644282	-
yjfR	3	3	0.96	ST4-74	4644387	4645451	-
sgaT	4	3	1.27	ST4-74	4645814	4647211	+
sgaB	1	4	0.34	ST4-74	4647227	4647532	+
ptxA	2	2	0.97	ST4-74	4647542	4648006	+
sgaH	3	3	1.08	ST4-74	4648020	4648670	+
sgaU	4	3	1.28	ST4-74	4648680	4649534	+
sgaE	6	5	1.33	ST4-74	4649534	4650220	+
STnc4120	143	74	1.94	ST4-74	4650287	4650410	-
yjfY	56	30	1.83	ST4-74	4650349	4650624	-
STM4390	355	375	0.95	ST4-74	4650818	4651024	+
rpsF	199	289	0.69	ST4-74	4651095	4651490	+
priB	174	238	0.73	ST4-74	4651497	4651811	+
rpsR	177	262	0.67	ST4-74	4651816	4652043	+
rplI	168	229	0.74	ST4-74	4652085	4652534	+
yifZ	12	9	1.26	ST4-74	4652682	4653608	+
ytfB	62	47	1.33	ST4-74	4653658	4654293	-
fklB	32	34	0.93	ST4-74	4654471	4655133	+
cycA	27	28	0.95	ST4-74	4655429	4656838	+
ytfE	6	5	1.29	ST4-74	4656950	4657612	-
ytfF	9	7	1.27	ST4-74	4657715	4658680	-
ytfG	3	2	1.33	ST4-74	4658761	4659609	-
ytfH	13	15	0.90	ST4-74	4659697	4660083	+
cpdB	7	10	0.74	ST4-74	4660141	4662084	-
cysQ	33	31	1.08	ST4-74	4662352	4663092	+
ytfJ	26	24	1.08	ST4-74	4663082	4663639	-
ytfK	2510	1102	2.28	ST4-74	4663966	4664172	+
ytfL	35	30	1.17	ST4-74	4664257	4665600	-
msrA	44	28	1.55	ST4-74	4665783	4666421	-
ytfM	45	34	1.34	ST4-74	4666634	4668367	+
ytfN	45	42	1.06	ST4-74	4668364	4672143	+
ytfP	100	101	0.99	ST4-74	4672146	4672490	+
STM4412	6	6	1.01	ST4-74	4672544	4673752	-
STM4413	3	4	0.80	ST4-74	4673749	4674912	-
ppa	274	359	0.76	ST4-74	4675323	4675853	-
fbp	34	36	0.95	ST4-74	4676080	4677078	-
mpl	26	26	0.99	ST4-74	4677253	4678632	+
iolR	22	23	0.94	ST4-74	4679123	4679956	+
iolT1	14	13	1.04	ST4-74	4680007	4681377	-
iolT2	11	7	1.46	ST4-74	4681899	4683335	+
STnc1740	72	56	1.28	ST4-74	4683333	4683512	-

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STnc2160	50	38	1.31	ST4-74	4683636	4683699	-
iolB	8	7	1.05	ST4-74	4683688	4684497	-
iolA	6	5	1.19	ST4-74	4684522	4686027	-
STM4422	1	1	2.16	ST4-74	4686043	4686345	-
STM4423	3	3	1.04	ST4-74	4686467	4687291	+
iolE	2	2	1.36	ST4-74	4687566	4688471	+
iolG1	7	6	1.15	ST4-74	4688490	4689500	+
srfJ	4	4	1.08	ST4-74	4689597	4690940	+
iolI1	9	8	1.14	ST4-74	4690941	4691774	+
STM4428	4	4	1.11	ST4-74	4691771	4692937	-
iolC	4	4	1.18	ST4-74	4692998	4694935	-
iolD	4	3	1.24	ST4-74	4695343	4697292	+
iolG2	7	6	1.11	ST4-74	4697473	4698495	+
STM4434	8	7	1.17	ST4-74	4698568	4699794	+
iolI2	3	3	1.18	ST4-74	4699955	4700770	+
iolH	7	6	1.23	ST4-74	4700823	4701707	+
yjgA	54	47	1.15	ST4-74	4701763	4702314	-
pmbA	31	27	1.17	ST4-74	4702410	4703762	+
cybC	215	160	1.34	ST4-74	4703861	4704247	+
SL1344_4369A	22	22	1.00	ST4-74	4704251	4704396	+
STM4440	4	5	0.72	ST4-74	4704525	4704863	+
STM4441	3	2	1.56	ST4-74	4704874	4705236	+
STM4442	1	3	0.45	ST4-74	4705236	4705538	+
STM4443	3	2	1.20	ST4-74	4705561	4706337	+
STM4444	4	4	0.97	ST4-74	4706349	4706993	+
STM4445	2	3	0.76	ST4-74	4707052	4708185	+
STM4446	2	3	0.74	ST4-74	4708169	4709287	+
STM4447	4	5	0.77	ST4-74	4709284	4710024	+
STM4448	8	7	1.02	ST4-74	4710041	4711954	+
relB	38	37	1.04	ST4-74	4712032	4712274	+
relE	33	24	1.40	ST4-74	4712264	4712548	+
nrdG	2	6	0.34	ST4-74	4712552	4713016	-
nrdD	9	37	0.24	ST4-74	4713137	4715275	-
SL1344_4383	3	7	0.42	ST4-74	4715480	4715650	+
treC	3	5	0.54	ST4-74	4715684	4717336	-
treB	15	25	0.61	ST4-74	4717386	4718803	-
treR	5	7	0.70	ST4-74	4718941	4719888	-
mgtA	14	14	1.06	ST4-74	4720272	4722980	+
STM4457	23	27	0.83	ST4-74	4723096	4723542	-
yjgF	84	134	0.63	ST4-74	4723617	4724003	-
pyrI	4	7	0.55	ST4-74	4724080	4724541	-
pyrB	4	7	0.59	ST4-74	4724554	4725489	-
pyrL	339	416	0.82	ST4-74	4725525	4725626	-
STM4463	8	6	1.40	ST4-74	4725716	4726204	-
STM4464	4	3	1.20	ST4-74	4726391	4727794	-
STM4465	1	1	0.82	ST4-74	4727850	4728854	-
STM4466	1	1	0.76	ST4-74	4728966	4729898	-
STM4467	1	1	1.15	ST4-74	4729909	4731129	-
yjgK	17	24	0.70	ST4-74	4731805	4732257	+
argI	108	236	0.46	ST4-74	4732331	4733335	-
yjgD	159	137	1.16	ST4-74	4733501	4733917	+

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miaE	7	7	0.93	ST4-74	4733929	4734741	+
ytgA	11	9	1.20	ST4-74	4734975	4735463	-
yjgM	31	39	0.79	ST4-74	4735570	4736073	-
yjgN	16	16	1.01	ST4-74	4736268	4737455	+
valS	21	28	0.74	ST4-74	4737597	4740452	-
holC	90	96	0.94	ST4-74	4740452	4740895	-
pepA	29	38	0.78	ST4-74	4741032	4742543	-
STM4478	197	199	0.99	ST4-74	4742587	4742706	-
yjgP	50	51	0.99	ST4-74	4742959	4744038	+
yjgQ	36	37	0.98	ST4-74	4744038	4745120	+
idnR	5	5	0.96	ST4-74	4745315	4746313	-
idnT	16	15	1.09	ST4-74	4746377	4747597	-
idnO	3	3	0.92	ST4-74	4747758	4748522	-
idnD	3	2	1.13	ST4-74	4748547	4749578	-
idnK	12	12	0.93	ST4-74	4749795	4750325	+
yjgB	33	17	2.02	ST4-74	4750353	4751372	-
STM4488	2	1	1.94	ST4-74	4751898	4752167	+
STM4489	18	18	1.00	ST4-74	4752547	4756062	+
STM4490	42	40	1.07	ST4-74	4756159	4757148	+
STM4491	19	19	0.98	ST4-74	4757261	4759345	-
STM4492	13	18	0.71	ST4-74	4759356	4761818	-
STM4493	18	23	0.79	ST4-74	4761956	4762777	-
STM4494	26	30	0.87	ST4-74	4762755	4763846	-
STM4495	20	24	0.85	ST4-74	4763846	4767523	-
STM4496	12	16	0.75	ST4-74	4767569	4771210	-
STM4497	18	28	0.63	ST4-74	4771222	4771824	-
STM4498	31	47	0.66	ST4-74	4771821	4772423	-
SL1344_4427A	2	1	2.55	ST4-74	4772932	4773205	+
yeeN	152	129	1.18	ST4-74	4773249	4773974	-
yjhP	4	6	0.71	ST4-74	4774525	4776156	-
STM4501	87	100	0.87	ST4-74	4776161	4776418	-
STM4502	2	16	0.12	ST4-74	4776907	4777515	+
STM4503	142	132	1.07	ST4-74	4778025	4778762	+
STM4504	59	21	2.82	ST4-74	4779038	4779949	+
STM4505	17	14	1.19	ST4-74	4780154	4780615	+
STM4506	12	12	0.99	ST4-74	4780612	4781286	-
uxuR	19	20	0.92	ST4-74	4781636	4782409	+
trpS2	6	5	1.25	ST4-74	4782410	4783423	-
IsrQ	3	2	1.30	ST4-74	4783574	4783741	+
SL1344_4439	4	14	0.26	ST4-74	4783720	4783872	+
STM4509	28	46	0.62	ST4-74	4784225	4784671	+
STM4510	12	17	0.68	ST4-74	4784910	4785644	+
yjiE	9	8	1.05	ST4-74	4785650	4786558	-
iadA	4	6	0.65	ST4-74	4786677	4787849	-
yjiG	4	5	0.78	ST4-74	4787862	4788323	-
yjiH	6	6	0.97	ST4-74	4788320	4788991	-
SL1344_4445A	29	12	2.41	ST4-74	4789349	4789724	-
yjiJ	14	8	1.73	ST4-74	4789864	4791048	-
STnc4260	29	20	1.45	ST4-74	4791124	4791217	+
yjiN	31	18	1.69	ST4-74	4791268	4792539	-
yjiO	7	11	0.62	ST4-74	4792626	4793867	-

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STM4518	17	18	0.98	ST4-74	4794346	4794861	+
STM4519	21	11	1.84	ST4-74	4795042	4796412	+
STM4520	29	14	2.14	ST4-74	4796507	4796650	-
yjiS	69	26	2.62	ST4-74	4796617	4796781	+
STnc4130	31	37	0.83	ST4-74	4796788	4796863	-
STM4522	18	15	1.20	ST4-74	4796856	4797557	-
symE	6	8	0.73	ST4-74	4797631	4797963	-
STnc1470	3885	6462	0.60	ST4-74	4797957	4798031	+
hsdS	8	11	0.68	ST4-74	4798190	4799599	-
hsdM	7	10	0.65	ST4-74	4799596	4801185	-
hsdR	5	13	0.36	ST4-74	4801343	4804852	-
mrr	12	10	1.23	ST4-74	4805050	4805964	+
STM4528	40	39	1.04	ST4-74	4806233	4806520	+
STM4529	10	9	1.19	ST4-74	4806507	4806809	+
yjiA	13	18	0.74	ST4-74	4806884	4807840	-
yjiX	42	43	0.97	ST4-74	4807851	4808054	-
cstAb	5	4	1.14	ST4-74	4808149	4810299	-
tsr	40	131	0.30	ST4-74	4810666	4812327	+
STM4534	14	13	1.04	ST4-74	4812651	4815416	+
STM4535	8	9	0.85	ST4-74	4815518	4815940	+
STM4536	2	3	0.78	ST4-74	4815955	4816416	+
STM4537	3	4	0.71	ST4-74	4816442	4817221	+
STM4538	2	3	0.78	ST4-74	4817211	4818047	+
STM4539	4	5	0.73	ST4-74	4818060	4819121	+
STM4540	8	8	0.99	ST4-74	4819139	4820149	+
mdoB	100	85	1.18	ST4-74	4820283	4822475	-
yjjA	10	14	0.75	ST4-74	4822857	4823318	-
dnaC	12	16	0.72	ST4-74	4823376	4824113	-
dnaT	42	42	1.01	ST4-74	4824116	4824655	-
STM4545	9	21	0.43	ST4-74	4824748	4825221	-
yjjP	15	46	0.32	ST4-74	4825212	4826123	-
yjjQ	0	1	0.40	ST4-74	4826701	4827426	+
bglJ	3	2	1.28	ST4-74	4827387	4828061	+
STM4549	43	40	1.08	ST4-74	4828112	4828570	-
fhuF	39	15	2.57	ST4-74	4828721	4829509	-
STM4551	22	21	1.04	ST4-74	4829630	4830694	-
STM4552	17	11	1.50	ST4-74	4830945	4831181	+
rsmC	13	12	1.14	ST4-74	4831722	4832750	-
holD	70	62	1.12	ST4-74	4832853	4833290	+
rimI	15	16	0.94	ST4-74	4833235	4833681	+
yjjG	12	14	0.90	ST4-74	4833703	4834380	+
prfC	17	18	0.93	ST4-74	4834472	4836061	+
osmY	348	132	2.63	ST4-74	4836463	4837080	+
yjjU	25	20	1.24	ST4-74	4837494	4838567	+
yjjV	7	7	0.97	ST4-74	4838564	4839337	+
yjjW	2	11	0.21	ST4-74	4839370	4840233	-
yjjI	2	16	0.12	ST4-74	4840205	4841752	-
deoC	10	9	1.12	ST4-74	4841994	4842791	+
deoA	10	9	1.13	ST4-74	4842915	4844237	+
deoB	28	25	1.12	ST4-74	4844289	4845512	+
deoD	28	29	0.97	ST4-74	4845722	4846441	+

Name	*Δfnr TPM*	*WT TPM*	*Δfnr/WT*	*Chr*[a]	*Start*	*End*	*Strand*
STnc4140	85	54	1.57	ST4-74	4846458	4846540	-
stjA	28	19	1.51	ST4-74	4846479	4847051	-
stjB	9	7	1.35	ST4-74	4847048	4849456	-
stjC	3	1	2.49	ST4-74	4849470	4850090	-
STM4574	8	5	1.60	ST4-74	4850255	4850944	-
STM4575	5	3	1.62	ST4-74	4850992	4851681	-
lplA	10	10	1.01	ST4-74	4851994	4853010	-
smp	15	15	1.01	ST4-74	4853044	4853688	-
serB	19	18	1.04	ST4-74	4853805	4854773	+
radA	11	11	1.00	ST4-74	4854857	4856239	+
nadR	31	30	1.02	ST4-74	4856363	4857622	+
yjjK	44	47	0.94	ST4-74	4857740	4859407	-
slt	26	27	0.98	ST4-74	4859580	4861553	+
trpR	79	87	0.91	ST4-74	4861611	4861937	+
yjjX	19	17	1.09	ST4-74	4862039	4862554	-
gpmB	33	32	1.03	ST4-74	4862603	4863250	+
rob	147	118	1.25	ST4-74	4863247	4864116	-
creA	70	73	0.96	ST4-74	4864328	4864801	+
creB	23	26	0.87	ST4-74	4864814	4865503	+
creC	9	10	0.94	ST4-74	4865701	4866927	+
creD	8	8	1.12	ST4-74	4866985	4868334	+
sthE	4	3	1.24	ST4-74	4868392	4869477	-
sthD	2	2	1.33	ST4-74	4869518	4870075	-
sthB	5	4	1.30	ST4-74	4870093	4872630	-
sthA	2	1	1.20	ST4-74	4872676	4873359	-
STM4595	3	3	1.01	ST4-74	4873430	4873975	-
STM4596	54	45	1.20	ST4-74	4874314	4874985	-
STM4597	22	19	1.16	ST4-74	4875185	4875684	-
arcA	167	204	0.82	ST4-74	4875951	4876667	-
yjjY	18	14	1.30	ST4-74	4876763	4876903	+
lasT	7	7	1.07	ST4-74	4877303	4877989	+

[a]Chromosome or plasmid

Appendix II

Table S2 A. COG categories.

Amino acid transport and metabolism	*Carbohydrate transport and metabolism*	*Cell envelope biogenesis and outer membrane*	*Cell motility and secretion*	*Energy production and conversion*	*Inorganic iron transport and metabolism*	*Surface Structures*	*Transcription*	*Translation, ribosomal structure and biogenesis*
yneH	*zwf*	*ytfM*	*yrfC*	*ysaA*	*zur*	*stjC*	*zntR*	*yrfH*
yliD	*ytfF*	*ytfG*	*tsr*	*yqhD*	*zunB*	*stjB*	*ytfH*	*yrdC*
yliC	*yraO*	*ytfB*	*trg*	*yneI*	*znuC*	*stiH*	*yqjI*	*yqcB*
yliB	*ynfM*	*yraR*	*stjC*	*yjeS*	*znuA*	*stiC*	*yqhC*	*yohI*
yjfC	*yneB*	*yohK*	*stjB*	*yiaK*	*zntA*	*stiB*	*yqgE*	*yoaB*
yjeM	*yneA*	*yohG*	*stiH*	*yiaE*	*yrbG*	*stiA*	*yoaA*	*ymfC*
yjeK	*yliI*	*yncA*	*stiC*	*yhdH*	*yqjH*	*sthE*	*ynfL*	*yliG*
yjeH	*yjiO*	*ynaI*	*stiB*	*yhbW*	*yncD*	*sthD*	*yneJ*	*yjgF*
yjdL	*yjiJ*	*yjeP*	*stiA*	*ygiR*	*yjcE*	*sthB*	*yncC*	*yjfH*
yifK	*yjgK*	*yibD*	*sthE*	*yfiQ*	*yjbB*	*sthA*	*yjiE*	*yjeA*
yhjV	*yihV*	*yiaD*	*sthD*	*yfhL*	*yiiP*	*stfG*	*yjgM*	*yjbN*
yhiP	*yihT*	*yhjG*	*sthA*	*yfaE*	*yibN*	*stfF*	*yjfQ*	*yjbC*
yhaO	*yihS*	*yhiI*	*stfG*	*ydjA*	*yheN*	*stfE*	*yjfJ*	*yihZ*
ygjU	*yihR*	*yggB*	*stfF*	*ydiT*	*yheM*	*stfD*	*yjeB*	*yibK*
ygiC	*yihQ*	*ygeA*	*stfE*	*ydiS*	*yheL*	*stfC*	*yjdC*	*yhdG*
yfiK	*yihP*	*ygcY*	*stfD*	*ydiR*	*ygjT*	*stfA*	*yjaB*	*yhbY*
yfhB	*yihO*	*yfhD*	*stfC*	*ydiQ*	*ygiE*	*stdC*	*yijO*	*yhbH*
yfdZ	*yigM*	*yfeL*	*stfA*	*ydgQ*	*ygdQ*	*stdB*	*yijC*	*ygjO*
yfbQ	*yifZ*	*yfdH*	*stdC*	*ydgP*	*ygaP*	*stdA*	*yiiD*	*ygcA*
yejA	*yieO*	*yfbG*	*stdB*	*ydgO*	*yfjD*	*stcD*	*yihW*	*yfiF*
yehY	*yidY*	*yfbE*	*stdA*	*ydgM*	*yfgD*	*stcC*	*yifA*	*yfiA*
yehX	*yicM*	*yfaW*	*stcC*	*ydcW*	*yffB*	*stcB*	*yieP*	*yfcB*
yehW	*yicL*	*yehZ*	*stcB*	*yccX*	*yfbS*	*stcA*	*yidZ*	*yeiP*
yeeF	*yicJ*	*yeeZ*	*stcA*	*yajO*	*yeaR*	*stbE*	*yiaJ*	*yebU*
yedO	*yiaO*	*yebA*	*stbE*	*ugpQ*	*yeaN*	*stbD*	*yiaG*	*yciO*
yecS	*yiaN*	*ydiY*	*stbC*	*udhA*	*ydiE*	*stbC*	*yiaC*	*yciL*
yecC	*yiaM*	*ydhO*	*stbB*	*ttrA*	*ydgF*	*stbB*	*yhjC*	*yciH*
yeaS	*yiaL*	*ycjG*	*stbA*	*torC*	*ydgE*	*stbA*	*yhhY*	*ychF*
ydiB	*yhjE*	*ycfW*	*ssaN*	*torA*	*ydcG*	*safD*	*yhcS*	*ybjF*
ydgR	*yhhS*	*ycfU*	*ssaC*	*tdcE*	*ychN*	*safC*	*yhcO*	*yaeJ*
ydgI	*yhfC*	*ycfN*	*spaS*	*tdcD*	*ychM*	*safB*	*yhcK*	*yadB*
ycjI	*yhcH*	*ybjY*	*safC*	*tas*	*ycgO*	*safA*	*yhaJ*	*tyrS*
ycdX	*ygiK*	*ybjT*	*safB*	*sucD*	*yccK*	*lpfE*	*ygbI*	*tufB*
ycdW	*ygeD*	*ybiO*	*ppdD*	*sucC*	*ybiR*	*lpfD*	*ygaE*	*tufA*
ycaM	*ygbM*	*ybdG*	*ppdC*	*sucB*	*ybgR*	*lpfC*	*ygaA*	*tsf*
ybiK	*ygbL*	*yajG*	*ppdA*	*sfcA*	*ybeX*	*lpfB*	*yfiE*	*truB*

Amino acid transport and metabolism	*Carbohydrate transport and metabolism*	*Cell envelope biogenesis and outer membrane*	*Cell motility and secretion*	*Energy production and conversion*	*Inorganic iron transport and metabolism*	*Surface Structures*	*Transcription*	*Translation, ribosomal structure and biogenesis*
ybiB	*yfaV*	*yaiU*	*motB*	*sdhD*	*ybaL*	*lpfA*	*yfhP*	*truA*
ybgK	*yegV*	*yaeT*	*motA*	*sdhC*	*yaeE*	*glpF*	*yfhH*	*trpS2*
ybgJ	*yegT*	*yaeL*	*lrhA*	*sdhB*	*yaeC*	*fliT*	*yfeR*	*trpS*
ybgH	*yegB*	*yabC*	*lpfE*	*sdhA*	*yadF*	*fliS*	*yfaX*	*trmU*
ybdR	*yedA*	*wzzE*	*lpfD*	*qor*	*yabK*	*fliR*	*yejH*	*trmD*
ybdL	*yeaD*	*wzzB*	*lpfC*	*putA*	*trkH*	*fliQ*	*yeiE*	*trmA*
yahN	*ydiN*	*wzc*	*lpfB*	*pta*	*trkA*	*fliP*	*yehV*	*thrS*
yaeR	*ydhC*	*wza*	*lpfA*	*prpC*	*thiI*	*fliO*	*yehT*	*sun*
yaeD	*ydfJ*	*wecG*	*invC*	*prkB*	*tehA*	*fliN*	*yegW*	*spoU*
yaaJ	*ydfI*	*wecE*	*hopD*	*ppc*	*sugE*	*fliM*	*yebK*	*speG*
wcaB	*ydeZ*	*wecC*	*hofC*	*ppa*	*sseA*	*fliL*	*yeaM*	*smpA*
usg	*ydeV*	*wecB*	*hofB*	*pntB*	*sodC*	*fliK*	*ydiP*	*serS*
tyrP	*ydeE*	*wcaL*	*fljB*	*pntA*	*sodB*	*fliJ*	*ydhM*	*selB*
tyrB	*ydeD*	*wcaJ*	*fliR*	*phsC*	*sodA*	*fliI*	*ydhB*	*rsuA*
tyrA	*ydeA*	*wcaI*	*fliQ*	*phsB*	*sitD*	*fliH*	*ydfH*	*rsmC*
trpE	*yddG*	*wcaG*	*fliP*	*phsA*	*sitC*	*fliG*	*ydeW*	*rpsU*
trpD	*yciM*	*wcaC*	*fliO*	*pflF*	*sitB*	*fliF*	*ydcR*	*rpsT*
trpC	*yceL*	*wcaA*	*fliN*	*pflD*	*sitA*	*fliE*	*ydcN*	*rpsS*
trpB	*yceE*	*vacJ*	*fliM*	*pflB*	*sfbC*	*fliD*	*ydcI*	*rpsR*
trpA	*ycaD*	*udg*	*fliL*	*pduW*	*sfbB*	*fliC*	*yciT*	*rpsQ*
thrB	*ybhE*	*tsx*	*fliK*	*pduS*	*sfbA*	*fliA*	*ycfX*	*rpsP*
thrA	*ybhC*	*tonB*	*fliJ*	*pduQ*	*sbp*	*flhD*	*ycfQ*	*rpsO*
tesA	*ybeJ*	*tolC*	*fliI*	*pduP*	*pstS*	*flhC*	*ycdC*	*rpsN*
tdh	*ybdA*	*tolA*	*fliH*	*pckA*	*pstC*	*flgN*	*yccR*	*rpsM*
tdcG	*yajR*	*spr*	*fliG*	*oadG*	*pstB*	*flgM*	*ybiH*	*rpsL*
tdcC	*yadI*	*slyB*	*fliF*	*nuoN*	*pstA*	*flgL*	*ybeF*	*rpsK*
tdcB	*yaaU*	*slt*	*fliE*	*nuoM*	*pspE*	*flgK*	*ybdO*	*rpsJ*
sufS	*xylB*	*slp*	*fliD*	*nuoL*	*ppk*	*flgJ*	*ybdM*	*rpsI*
speF	*xylA*	*rspA*	*fliC*	*nuoK*	*phoU*	*flgI*	*ybbS*	*rpsH*
speE	*xapB*	*rlpB*	*flgN*	*nuoJ*	*phnS*	*flgH*	*ybaO*	*rpsG*
speD	*uhpT*	*rffH*	*flgL*	*nuoI*	*phnA*	*flgG*	*yafC*	*rpsF*
speC	*uhpC*	*rffG*	*flgK*	*nuoH*	*pgtC*	*flgF*	*xylR*	*rpsE*
speB	*ugpE*	*rfe*	*flgI*	*nuoG*	*nxiA*	*flgE*	*xapR*	*rpsD*
solA	*ugpC*	*rfbV*	*flgH*	*nuoF*	*nrfD*	*flgD*	*wecD*	*rpsC*
serB	*ugpB*	*rfbU*	*flgG*	*nuoE*	*nrfA*	*flgC*	*vacB*	*rpsB*
serA	*ugpA*	*rfbP*	*flgF*	*nuoC*	*nirD*	*flgB*	*uxuR*	*rpsA*
selD	*treF*	*rfbN*	*flgE*	*nuoB*	*nirC*	*flgA*	*umuD*	*rpmJ2*
selA	*treC*	*rfbM*	*flgD*	*nuoA*	*nhaB*	*fimZ*	*ttk*	*rpmJ*
sdaC	*treA*	*rfbJ*	*flgC*	*nrfC*	*nhaA*	*fimY*	*trpR*	*rpmI*
sdaB	*tpiA*	*rfbH*	*flgB*	*nirB*	*narK*	*fimW*	*treR*	*rpmH*
sdaA	*torT*	*rfbG*	*flgA*	*nifU*	*napD*	*fimI*	*tdcA*	*rpmG*
sapA	*tktB*	*rfbF*	*fimI*	*nifJ*	*modF*	*fimH*	*srlR*	*rpmF*
rthB	*tktA*	*rfbD*	*fimF*	*nfnB*	*modC*	*fimF*	*sprB*	*rpmE2*

Amino acid transport and metabolism	***Carbohydrate transport and metabolism***	***Cell envelope biogenesis and outer membrane***	***Cell motility and secretion***	***Energy production and conversion***	***Inorganic iron transport and metabolism***	***Surface Structures***	***Transcription***	***Translation, ribosomal structure and biogenesis***
rspB	*talC*	*rfbC*	*fimD*	*nemA*	*modB*	*fimD*	*soxS*	*rpmE*
rhtC	*talB*	*rfbB*	*fimC*	*ndh*	*modA*	*fimC*	*soxR*	*rpmD*
putP	*talA*	*rfbA*	*fimA*	*narZ*	*mntH*	*fimA*	*slyA*	*rpmC*
pudB	*suhB*	*rfaQ*	*cheZ*	*narW*	*mgtB*	*dsbC*	*sinR*	*rpmB*
ptrB	*srlE*	*rfaL*	*cheW*	*narV*	*mdoG*	*dsbB*	*sdiA*	*rpmA*
proY	*srlA*	*rfaK*	*cheR*	*narJ*	*kup*	*csgG*	*rsd*	*rplY*
proX	*smvA*	*rfaJ*	*cheB*	*narI*	*kefC*	*csgF*	*rpoZ*	*rplX*
proW	*slrB*	*rfaI*	*cheA*	*narH*	*kefB*	*csgE*	*rpoS*	*rplW*
proV	*sgbU*	*rfaG*	*bcfG*	*napF*	*kdpC*	*csgD*	*rpoN*	*rplV*
proC	*sgaU*	*rfaF*	*bcfF*	*napC*	*kdpB*	*csgC*	*rpoH*	*rplU*
proB	*sgaH*	*rfaE*	*bcfE*	*napB*	*kdpA*	*csgB*	*rpoE*	*rplT*
proA	*sgaE*	*rfaD*	*bcfD*	*mioC*	*katG*	*csgA*	*rpoD*	*rplR*
prlC	*sgaB*	*rfaC*	*bcfC*	*mdaA*	*katE*	*crl*	*rpoA*	*rplQ*
poxB	*setB*	*rfaB*	*bcfB*	*maeB*	*iroN*	*bcfH*	*rob*	*rplP*
potI	*rpe*	*prc*	*bcfA*	*lpdA*	*iroD*	*bcfG*	*rnk*	*rplO*
potH	*rhaT*	*pqaB*	STM3216	*lldP*	*glpE*	*bcfF*	*rnc*	*rplN*
potG	*rhaD*	*pmrF*	STM3152	*lldD*	*fur*	*bcfE*	*rmbA*	*rplM*
potF	*rhaB*	*pldA*	STM3138	*ldhA*	*ftnB*	*bcfD*	*rho*	*rplL*
potE	*rhaA*	*phoE*	STM1657	*icdA*	*ftn*	*bcfC*	*rhaS*	*rplK*
potD	*rfbK*	*pgtE*		*hypO*	*foxA*	*bcfB*	*rhaR*	*rplJ*
potC	*rbsK*	*pdgL*		*hydN*	*focA*	*bcfA*	*rfaH*	*rplI*
potB	*rbsD*	*pbpG*		*hycI*	*fhuE*		*rcsA*	*rplF*
potA	*rbsC*	*pal*		*hycG*	*fhuD*		*rbsR*	*rplE*
pipD	*rbsB*	*pagC*		*hycF*	*fhuC*		*purR*	*rplD*
phnW	*rbsA*	*ompX*		*hycE*	*fhuB*		*ptsJ*	*rplC*
phnV	*pykF*	*ompW*		*hycD*	*fhuA*		*pspC*	*rplB*
phnT	*pykA*	*ompN*		*hycC*	*fes*		*pspA*	*rplA*
pheP	*ptxA*	*ompC*		*hycB*	*fepG*		*phnR*	*rph*
pheA	*ptsO*	*ompA*		*hybD*	*fepD*		*phnO*	*rnpA*
pepT	*ptsN*	*nmpC*		*hybC*	*fepC*		*pdhR*	*rne*
pepQ	*ptsI*	*nlpD*		*hybA*	*fepB*		*oxyR*	*rnd*
pepP	*ptsH*	*nlpC*		*hmpA*	*fepA*		*orf408*	*rna*
pepN	*ptsG*	*nlpB*		*hemG*	*feoB*		*orf242*	*rmf*
pepE	*ptsA*	*murG*		*hcr*	*feoA*		*nusG*	*rluD*
pepD	*prpB*	*murF*		*hcp*	*dps*		*nusB*	*rluC*
pepB	*proP*	*murE*		*gpsA*	*cysW*		*nusA*	*rluA*
pepA	*pps*	*murD*		*gor*	*cysQ*		*nlp*	*rimM*
pdxB	*pmgI*	*murC*		*gltP*	*cysP*		*nikR*	*rimL*
pduV	*pgtP*	*murB*		*gltA*	*cysN*		*nhaR*	*rimJ*
pduU	*pgm*	*murA*		*glpQ*	*cysJ*		*nanK*	*rbfA*
pabC	*pgk*	*mtgA*		*glpD*	*cysC*		*nagC*	*queA*
pabB	*pgi*	*mscL*		*glpC*	*cysA*		*nadR*	*pth*

Amino acid transport and metabolism	***Carbohydrate transport and metabolism***	***Cell envelope biogenesis and outer membrane***	***Cell motility and secretion***	***Energy production and conversion***	***Inorganic iron transport and metabolism***	***Surface Structures***	***Transcription***	***Translation, ribosomal structure and biogenesis***
pabA	*pfkB*	*msbB*		*glpA*	*cyaY*		*mtlR*	*proS*
orf48	*pfkA*	*mreD*		*galT*	*cutC*		*mlc*	*prmA*
oppF	*pduF*	*mrdA*		*gabD*	*cutA*		*metR*	*prfH*
oppD	*pagO*	*mrcB*		*fumC*	*corA*		*melR*	*prfC*
oppC	*otsB*	*mraY*		*fumB*	*citT*		*marT*	*prfB*
oppB	*otsA*	*mpl*		*fumA*	*cirA*		*marR*	*prfA*
oppA	*nupG*	*mltC*		*fucO*	*chaA*		*marA*	*pnp*
oat	*nanT*	*mltB*		*frdD*	*cboQ*		*malT*	*pheS*
nifS	*nanE*	*mltA*		*frdC*	*cbiO*		*lysR*	*miaB*
nanA	*nagZ*	*misL*		*frdB*	*cbiN*		*lrp*	*miaA*
mtr	*nagE*	*mipA*		*frdA*	*cbiM*		*lrhA*	*metG*
mppA	*nagD*	*mepA*		*fpr*	*btuF*		*lldR*	*map*
metJ	*nagB*	*mdoH*		*fldB*	*bfr*		*lexA*	*lysS*
metH	*nagA*	*mdoB*		*fldA*	*bfd*		*leuO*	*ligT*
metF	*mtlD*	*lspA*		*fixX*	*apaG*		*kdgR*	*leuS*
metE	*mtlA*	*lpxK*		*fixC*	*amtB*		*invF*	*ksgA*
metC	*mrsA*	*lpxD*		*fixB*	*abc*		*ilvY*	*infC*
metB	*mgsA*	*lpxC*		*fixA*	STM3820		*idnR*	*infB*
metA	*mglC*	*lpxB*		*fdx*	STM3528		*iclR*	*infA*
lysP	*mglB*	*lpxA*		*fdrA*	STM3356		*iciA*	*ileS*
lysC	*mglA*	*lppB*		*fdoI*	STM3166		*hutC*	*hemK*
lysA	*melB*	*lpp*		*fdoH*	STM3142		*hpaR*	*glyS*
ltaA	*melA*	*lolB*		*fdoG*	STM3141		*hpaA*	*glyQ*
livM	*mdfA*	*lolA*		*fdnI*	STM3122		*hilD*	*gltX*
livK	*manZ*	*lnt*		*fdnH*	STM3075		*hilC*	*glnS*
livJ	*manY*	*lgt*		*fdnG*	STM3074		*hilA*	*fusA*
livH	*manX*	*kdtA*		*fdhF*	STM3073		*greB*	*ftsJ*
livG	*manA*	*kdsB*		*fdhD*	STM2446		*greA*	*frr*
livF	*malZ*	*kdsA*		*eutE*	STM2404		*gntR*	*fmt*
leuD	*malS*	*imp*		*eutD*	STM1874		*glpR*	*efp*
leuC	*malK*	*iagB*		*dmsB*	STM1808		*gcvA*	*def*
leuB	*malG*	*htrB*		*dmsA*	STM1741		*galS*	*cysS*
ldcC	*malF*	*hlpA*		*dld*	STM1731		*galR*	*cca*
ilvN	*malE*	*gudD*		*dcuC*	STM1656		*fucR*	*cafA*
ilvI	*lyxK*	*gmd*		*dctA*	STM1653		*fruR*	*aspS*
ilvH	*kgtP*	*glmU*		*cyoD*	STM1490		*fliA*	*asnS*
ilvG	*kduI*	*glmS*		*cyoC*	STM1044		*flgM*	*argS*
ilvE	*kdgK*	*gidB*		*cyoB*	STM0771		*fis*	*alaS*
ilvD	*iroB*	*galU*		*cyoA*	STM0770		*fimW*	STM4600
ilvC	*idnT*	*galF*		*cydD*	STM0765		*fhlA*	STM4450
ilvB	*idnK*	*galE*		*cydC*	STM0562		*fadR*	STM2545
ilvA	*hpaX*	*ftsQ*		*cydB*	STM0355		*eutR*	STM1550
idnD	*hpaI*	*ftsI*		*cydA*	STM0084		*envR*	STM1549

Amino acid transport and metabolism	***Carbohydrate transport and metabolism***	***Cell envelope biogenesis and outer membrane***	***Cell motility and secretion***	***Energy production and conversion***	***Inorganic iron transport and metabolism***	***Surface Structures***	***Transcription***	***Translation, ribosomal structure and biogenesis***
hutH	*hcaT*	*flgJ*		*cybC*	STM0035		*emrR*	STM1548
hutG	*gudT*	*fepE*		*cybB*			*ecnR*	
hpaF	*gsk*	*emtA*		*citF2*			*dsdC*	
hisQ	*gpmB*	*dniR*		*citF*			*dpiA*	
hisP	*gpmA*	*dgoA*		*citD2*			*dinG*	
hisM	*gntU*	*dgkA*		*citD*			*dgoR*	
hisJ	*gntT*	*ddlB*		*citC2*			*dcuR*	
hisI	*gntK*	*ddlA*		*caiB*			*cytR*	
hisH	*gnd*	*ddg*		*bisC*			*cysB*	
hisG	*glxK*	*dadX*		*atpI*			*cueR*	
hisF	*glpX*	*dacD*		*atpH*			*cspE*	
hisD	*glpT*	*dacC*		*atpG*			*cspD*	
hisC	*glpF*	*dacB*		*atpF*			*cspC*	
hisB	*glk*	*dacA*		*atpE*			*cspB*	
gsp	*glgX*	*csgG*		*atpD*			*csiE*	
glyA	*glgP*	*cfa*		*atpC*			*csgD*	
gltS	*glgB*	*caiT*		*atpB*			*cobB*	
gltL	*glgA*	*blc*		*atpA*			*celD*	
gltK	*gip*	*asmA*		*astD*			*cdaR*	
gltJ	*ghmA*	*amiC*		*asrC*			*cadC*	
gltI	*gcd*	*amiB*		*asrA*			*asnC*	
gltD	*garL*	*amiA*		*allD*			*araC*	
glpB	*garK*	*alr*		*aldB*			*allR*	
gloA	*garD*	*acrE*		*adhE*			*agaR*	
glnQ	*gapA*	*acrA*		*acnB*			*adiY*	
glnP	*galP*	STM4540		*acnA*			*acrR*	
glnK	*galM*	STM4539		*ackA*			STM4423	
glnH	*fucU*	STM4510		*aceF*			STM4417	
glnB	*fucK*	STM4272		*aceE*			STM4320	
glnA	*fucI*	STM4260		*aceB*			STM4318	
ggt	*fucA*	STM4259		*aceA*			*rtsA*	
gdhA	*fsr*	STM4217		STM4519			*rtsB*	
gcvT	*frwD*	STM4205		STM4421			STM4270	
gcvR	*frwC*	STM4102		STM4306			STM4068	
gcvP	*frwB*	STM3833		STM4305			STM4033	
gcvH	*fruK*	STM3697		STM4044			STM3834	
gabT	*fruF*	STM3601		STM3529			STM3794	
gabP	*fbp*	STM3038		STM3355			STM3785	
fliY	*fbaB*	STM3031		STM3354			STM3778	
eutT	*fba*	STM2914		STM3129			STM3773	
eutS	*eno*	STM2756		STM3118			STM3736	
eutQ	*emrD*	STM2692		STM3081			STM3696	

Amino acid transport and metabolism	*Carbohydrate transport and metabolism*	*Cell envelope biogenesis and outer membrane*	*Cell motility and secretion*	*Energy production and conversion*	*Inorganic iron transport and metabolism*	*Surface Structures*	*Transcription*	*Translation, ribosomal structure and biogenesis*
eutP	*emrB*	STM2690		STM2963			STM3681	
eutL	*ego*	STM2591		STM2840			STM3678	
eutJ	*eda*	STM1940		STM2530			STM3653	
eutH	*dsdX*	STM1910		STM2529			STM3651	
eutC	*dgoT*	STM1836		STM2527			STM3633	
eutB	*dgoK*	STM1540		STM2406			STM3602	
eutA	*deoB*	STM1530		STM1793			STM3533	
dsdA	*crr*	STM1493		STM1792			STM3358	
dppD	*cobC*	STM1260		STM1789			STM3357	
dppC	*citE2*	STM1043		STM1788			STM3262	
dppB	*citE*	STM0932		STM1787			STM3175	
dppA	*citA*	STM0818		STM1786			STM3124	
dcp	*celF*	STM0725		STM1627			STM3121	
dapF	*celC*	STM0721		STM1620			STM3098	
dapE	*celB*	STM0719		STM1556			STM3084	
dapB	*bglX*	STM0573		STM1539			STM3025	
dapA	*bglA*	STM0572		STM1538			STM3020	
dadA	*bcsC*	STM0509		STM1537			STM3012	
cysZ	*bcr*	STM0352		STM1536			STM2955	
cysM	*araJ*	STM0350		STM1533			STM2920	
cysK	*araE*	STM0346		STM1499			STM2912	
cysH	*araD*	STM0306		STM1498			STM2905	
cysE	*amyA*	STM0292		STM1459			STM2803	
cysD	*ampG*			STM1261			STM2797	
cycA	STM4538			STM1253			STM2749	
csdA	STM4537			STM0858			STM2748	
cobD	STM4536			STM0856			STM2744	
carA	STM4535			STM0855			STM2738	
cadB	STM4448			STM0762			STM2575	
cadA	STM4436			STM0761			STM2374	
brnQ	STM4435			STM0691			STM2361	
avtA	STM4434			STM0612			STM2345	
astE	*iolH*			STM0611			STM2281	
astC	*iolI2*			STM0564			STM2275	
astB	*iolG2*			STM0361			STM2230	
astA	*iolD*			STM0360			STM2180	
aspC	*iolC*			STM0057			STM1857	
aspA	STM4428			STM0056			STM1677	
asnB	*iolI1*						STM1674	
asnA	*iolG1*						STM1671	
asd	*iolE*						STM1664	
artQ	*iolA*						STM1619	
artP	*iolB*						STM1618	

Amino acid transport and metabolism	*Carbohydrate transport and metabolism*	*Cell envelope biogenesis and outer membrane*	*Cell motility and secretion*	*Energy production and conversion*	*Inorganic iron transport and metabolism*	*Surface Structures*	*Transcription*	*Translation, ribosomal structure and biogenesis*
artM	*iolT2*						STM1575	
artI	*iolT1*						STM1555	
aroP	STM4412						STM1547	
aroL	STM4080						STM1541	
aroK	STM4066						STM1265	
aroH	STM4065						STM1243	
aroG	STM4054						STM1127	
aroF	STM4053						STM1082	
aroE	STM4052						STM1012	
aroD	STM3858						STM1001	
aroC	STM3832						STM0952	
aroB	STM3793						STM0900	
aroA	STM3792						STM0898A	
argT	STM3784						STM0898	
argR	STM3783						STM0859	
argI	STM3782						STM0835	
argG	STM3781						STM0764	
argE	STM3780						STM0763	
argD	STM3779						STM0692	
argC	STM3775						STM0652	
argB	STM3772						STM0581	
argA	STM3771						STM0580	
arcC	STM3770						STM0571	
ansP	STM3769						STM0410	
ansB	STM3698						STM0363	
ansA	STM3600						STM0354	
allC	STM3547						STM0347	
aegA	STM3260						STM0333	
adi	STM3259						STM0164	
STM4467	STM3258						STM0052	
STM4466	STM3257						STM0031	
STM4465	STM3256						STM0030	
STM4463	STM3255						STM0029	
STM4446	STM3254						STM0017	
STM4431	STM3253						STM0014	
STM4351	STM3251							
STM4042A	STM3170							
STM4042	STM3169							
STM3859	STM3137							
STM3796A	STM3136							
STM3768	STM3134							
STM3598	STM3132							

Amino acid transport and metabolism	*Carbohydrate transport and metabolism*	*Cell envelope biogenesis and outer membrane*	*Cell motility and secretion*	*Energy production and conversion*	*Inorganic iron transport and metabolism*	*Surface Structures*	*Transcription*	*Translation, ribosomal structure and biogenesis*
STM3532	STM3120							
STM3261	STM3083							
STM3128	STM2959							
STM3126	STM2913							
STM3117	STM2911							
STM3082	STM2758							
STM3022	STM2757							
STM2360	STM2755							
STM2359	STM2752							
STM2358	STM2751							
STM2357	STM2750							
STM2197	STM2668							
STM2196	STM2574							
STM2186	STM2570							
STM1795	STM2492							
STM1636	STM2405							
STM1635	STM2372							
STM1634	STM2344							
STM1633	STM2343							
STM1557	STM2341							
STM1542	STM2340							
STM1494	STM2303							
STM1492	STM2302							
STM1491	STM2300							
STM1484	STM2289							
STM1269	STM2280							
STM1259	STM2274							
STM1257	STM2198							
STM1256	STM2179							
STM1255	STM1933							
STM1128	STM1843							
STM1003	STM1617							
STM1002	STM1616							
STM0458	STM1614							
STM0330	STM1613							
STM0329	STM1612							
	STM1560							
	STM1559							
	STM1558							
	STM1545							
	STM1543							
	STM1252							
	STM1132							

Amino acid transport and metabolism	*Carbohydrate transport and metabolism*	*Cell envelope biogenesis and outer membrane*	*Cell motility and secretion*	*Energy production and conversion*	*Inorganic iron transport and metabolism*	*Surface Structures*	*Transcription*	*Translation, ribosomal structure and biogenesis*
	STM1129							
	STM0885							
	STM0868							
	STM0860							
	STM0854							
	STM0723							
	STM0722							
	STM0650							
	STM0649							
	STM0577							
	STM0576							
	STM0575							
	STM0574							
	STM0520							
	STM0382							
	STM0356							
	STM0257							
	STM0212							
	STM0149							
	STM0042							
	STM0041							
	STM0018							

Table S2 B. COG categories.

Drug analogue and resistance	*Anaerobic metabolism*	*Flagella and chemotaxis*	*Lipid metabolism*	*Oxidative Phosphorylation*	*Protein Transport*	*Nucleotide metabolism*	*Regulators*	*Signal transduction*
yggT	*ynfI*	*yeaJ*	*yqeF*	*cyoC*	*secB*	*yicE*	*zur*	*yojN*
ygeD	*tdcD*	*tsr*	*ymdC*	*cyoB*	*secA*	*yggV*	*zntR*	*ynaF*
ygeA	*tdcC*	*trg*	*yihU*	*cyoA*	*secY*	*yfhC*	*yiaJ*	*ylaB*
ybbM	*nrfG*	*tcp*	*yihG*	*cydA*	*secE*	*yfeJ*	*xapR*	*yjjQ*
tehB	*nrfE*	*motB*	*yhbT*	*cydB*	*secG*	*yeiA*	*wzzE*	*yjiY*
tehA	*nrfD*	*motA*	*ygbJ*	*ppk*	*secD*	*ybeK*	*wzzB*	*yjcC*
sbmA	*nrfC*	*lrhA*	*yfjG*	*ppa*	*secF*	*ybbY*	*uxuR*	*yhjK*
sanA	*nrfB*	*lppB*	*yfcY*	*sdhC*	*yajC*	*xapA*	*uhpA*	*yhjH*
pmrD	*nrfA*	*fliZ*	*yeiU*	*sdhD*	*yidC*	*uraA*	*trpR*	*yhjB*
nfnB	*nrdG*	*fliY*	*yegS*	*sdhA*	*lepB*	*upp*	*treR*	*yhdA*
marR	*nrdD*	*fliT*	*ydiO*	*sdhB*	*lspA*	*udp*	*torT*	*ygiY*
marB	*narX*	*fliS*	*ydiF*	*fliI*	*ffh*	*udk*	*torR*	*ygiX*
marA	*narL*	*fliR*	*ydiD*	*nuoN*	*ftsY*	*tmk*	*tdcA*	*ygiM*
ksgA	*napH*	*fliQ*	*yciA*	*nuoM*	*ybeC*	*thyA*	*stpA*	*yfiN*
emrD	*napG*	*fliP*	*ybjG*	*nuoL*	*tatA*	*thiG*	*sspA*	*yfhK*
emrB	*napF*	*fliO*	*ybhO*	*nuoK*	*tatB*	*tdk*	*soxS*	*yfhA*
emrA	*napD*	*fliN*	*yafH*	*nuoJ*	*tatC*	*rihC*	*soxR*	*yfeA*
bcr	*napC*	*fliM*	*ushB*	*nuoI*	*tatE*	*pyrI*	*slyA*	*yehU*
bacA	*napB*	*fliL*	*uppS*	*nuoH*		*pyrH*	*sfsA*	*yecG*
ampG	*napA*	*fliK*	*tesB*	*nuoG*		*pyrG*	*seqA*	*yebR*
ampE	*hybG*	*fliJ*	*sseJ*	*nuoF*		*pyrF*	*rstA*	*yeaJ*
acrR	*hybF*	*fliI*	*sbmA*	*nuoE*		*pyrE*	*rsd*	*yeaG*
acrF	*hybE*	*fliH*	*pssA*	*nuoB*		*pyrD*	*rpoS*	*ydiV*
acrA	*hybD*	*fliG*	*psd*	*nuoA*		*pyrB*	*rnk*	*ydaA*
	hybC	*fliF*	*prpE*	*atpC*		*purU*	*rhaR*	*yciR*
	hybB	*fliE*	*plsX*	*atpD*		*purT*	*rfaH*	*ybiI*
	hybA	*fliD*	*plsC*	*atpG*		*purN*	*rcsF*	*ybdQ*
	glpC	*fliC*	*plsB*	*atpA*		*purM*	*rcsB*	*yaiC*
	glpB	*fliA*	*pldB*	*atpH*		*purK*	*rcsA*	*wzb*
	glpA	*flhE*	*phoN*	*atpF*		*purH*	*rbsR*	*uvrY*
	fumB	*flhD*	*pgsA*	*atpE*		*purG*	*purR*	*uspA*
	frdD	*flhC*	*pgpB*	*atpB*		*purF*	*phoU*	*uhpB*
	frdC	*flhB*	*oafA*	*frdD*		*purE*	*phoP*	*uhpA*
	frdB	*flhA*	*menE*	*frdC*		*purD*	*oxyR*	*typA*
	frdA	*flgN*	*lytB*	*frdB*		*purC*	*osmE*	*ttrS*
	fdnI	*flgM*	*ispF*	*frdA*		*purB*	*oraA*	*ttrR*
	fdnH	*flgL*	*ispD*			*purA*	*nlp*	*torS*
	fdnG	*flgK*	*ipk*			*prsA*	*nikR*	*torR*
	dmsC	*flgJ*	*idi*			*ppx*	*nagC*	*tctE*
	dmsB	*flgI*	*iacP*			*pfs*	*nadR*	*tctD*
	dmsA	*flgH*	*glxR*			*nupC*	*mtlR*	*ssrB*
	dcuB	*flgG*	*gcpE*			*nrdI*	*modE*	*ssrA*

Drug analogue and resistance	*Anaerobic metabolism*	*Flagella and chemotaxis*	*Lipid metabolism*	*Oxidative Phosphorylation*	*Protein Transport*	*Nucleotide metabolism*	*Regulators*	*Signal transduction*
	dcuA	*flgF*	*garR*			*nrdF*	*metR*	*spoT*
	cadC	*flgE*	*fadL*			*nrdD*	*metJ*	*sixA*
	cadB	*flgD*	*fadD*			*nrdB*	*marR*	*rtn*
	cadA	*flgC*	*fadB*			*nrdA*	*marA*	*rtcR*
	asrC	*flgB*	*fadA*			*ndk*	*lytB*	*rstB*
	asrB	*flgA*	*fabZ*			*miaE*	*lysR*	*rstA*
	asrA	*cheZ*	*fabI*			*hpt*	*luxS*	*rseC*
	aer	*cheY*	*fabH*			*guaC*	*lrp*	*rseB*
	STM4307	*cheW*	*fabF*			*guaA*	*lrhA*	*rseA*
	STM4306	*cheR*	*fabD*			*gpt*	*lexA*	*relA*
	STM4305	*cheM*	*fabB*			*gmk*	*ilvY*	*rcsC*
	STM3599	*cheB*	*fabA*			*dut*	*idnR*	*rcsB*
	STM2530	*cheA*	*dxr*			*dgt*	*iclR*	*ptsP*
	STM2529	*aer*	*cls*			*deoD*	*iciA*	*pspF*
	STM2528	STM4258	*cdsA*			*deoC*	*icc*	*prpR*
	STM1497	STM4210	*caiD*			*deoA*	*hycA*	*prpA*
	STM0761	STM3670	*caiC*			*dcd*	*hupB*	*proQ*
	STM0612	STM3152	*apeE*			*cyaA*	*hupA*	*pphB*
		STM3138	*aes*			*cpdB*	*htgA*	*phoR*
		STM2314	*acpS*			*cmk*	*hns*	*phoQ*
		STM1657	*acpD*			*cdd*	*himD*	*phoP*
			accD			*apt*	*himA*	*phoL*
			accC			*allP*	*hha*	*phoH*
			accB			*allB*	*hflK*	*phoB*
			accA			*allA*	*hflC*	*pgtB*
			aas			*adk*	*gutM*	*pgtA*
			STM4032			*add*	*gntR*	*ompR*
			STM3595			STM4104	*glpR*	*narX*
			STM3119			STM3631	*glnK*	*narQ*
			STM1623			STM3334	*glnB*	*narP*
			STM0857			STM3333	*gcvR*	*narL*
			STM0055			STM3167	*gcvA*	*luxS*
						STM1330	*galS*	*kdpE*
						STM0033	*galR*	*kdpD*
							fur	*hydH*
							fruR	*hydG*
							fnr	*hnr*
							fliS	*glnG*
							fliA	*fnr*
							flhD	*fimZ*
							flhC	*envZ*
							fis	*dpiB*
							fadR	*dksA*

Drug analogue and resistance	*Anaerobic metabolism*	*Flagella and chemotaxis*	*Lipid metabolism*	*Oxidative Phosphorylation*	*Protein Transport*	*Nucleotide metabolism*	*Regulators*	*Signal transduction*
							envR	*dcuS*
							emrR	*cstA*
							eco	*csrA*
							dsrB	*crp*
							dgoR	*creC*
							cytR	*creB*
							csrA	*cpxR*
							cspE	*cpxA*
							crp	*copS*
							crl	*copR*
							creB	*cheY*
							cpxP	*bolA*
							cheZ	*bglJ*
							cheY	*basS*
							cheW	*basR*
							cheR	*barA*
							chaB	*baeS*
							caiF	*baeR*
							birA	*arcB*
							bglJ	*arcA*
							basS	*aer*
							basR	*aceK*
							baeR	STM4551
							asnC	STM4534
							argR	STM3388
							allR	STM2731
							agaR	STM2503
							acrR	STM2314
								STM1987
								STM1827
								STM1697
								STM0561
								STM0343
								STM0053

Table S3 A. COG organized DE genes.

Amino acid transport and metabolism	*Anaerobic metabolism*	*Surface structures*	*Cell motility and secretion*	*Energy production and conversion*	*Flagella and chemotaxis*	*Inorganic iron transport and metabolism*
STM1491	STM1497	*stfG*	*stfG*	STM4305	*flgF*	*fhuA*
STM1494	STM4305	*stbA*	*stbA*	*fumB*	*trg*	*fes*
STM1633	*fumB*	*stjC*	*ssaC*	STM4306	*fliJ*	STM0765
STM1635	*dcuB*	*stcA*	*ssaN*	*napF*	*flgD*	*ydiE*
oat	*dmsC*	*flgF*	*stjC*	*citF*	*flgE*	*sseA*
STM1256	STM4306	*fliJ*	*stcA*	*nemA*	*flgC*	*bfd*
STM1257	*napF*	*flgD*	*flgF*	*ysaA*	*flgG*	*nrfA*
pepT	STM4307	*flgE*	*trg*	STM2529	*fliS*	*napD*
aegA	*nrfA*	*flgC*	*fliJ*	*hybA*	*cheB*	*citT*
pepE	*napD*	*flgG*	*flgD*	*citD*	*fliK*	*modB*
hpaF	*nrdD*	*fliS*	*flgE*	STM2530	*fliO*	*nirD*
ansB	*hybG*	*fliK*	*flgC*	*pta*	*fliT*	*modC*
glpB	STM2529	*fliO*	*flgG*	*dcuC*	*fliQ*	STM2446
gdhA	*hybA*	*fliT*	*cheB*	*frdB*	*fliN*	STM3074
ansA	STM2530	*fliQ*	*fliK*	*frdA*	*fliF*	*modA*
argI	*hybF*	*fliN*	*fliO*	*hybD*	*fliG*	*cbiM*
lysC	*hybB*	*fliF*	*fliQ*	*frdC*	*fliC*	
pduV	*hybE*	*fliG*	*fliN*	*napB*	*tcp*	
proX	*frdB*	*fliC*	*fliF*	STM2527	*cheA*	
eutP	*frdA*	*flgH*	*fliG*	*ackA*	*flgH*	
argG	*hybD*	*flgI*	*fliC*	*nuoJ*	*flgI*	
dppC	*nrdG*	*fliM*	*cheA*	*hybC*	*fliM*	
dppD	*frdC*	*flgN*	*flgH*	*nuoM*	*flgN*	
livJ	*glpB*	*fliH*	*flgI*	*glpA*	*fliH*	
	napB	*fliD*	*fliM*	*nuoN*	*fliD*	
	aer	*flgM*	*flgN*	*narJ*	*flgM*	
	hybC	*fliI*	*fliH*	*nuoK*	*fliI*	
	glpA	*flgJ*	*fliD*	*nirB*	*cheR*	
	STM2528	*flgB*	*fliI*	*hypO*	*flgJ*	
	glpC	*flgL*	*cheR*	*nuoE*	*flgB*	
	frdD	*fliA*	*flgB*	*nuoH*	*flgL*	
	napG	*flgA*	*flgL*	*nuoL*	*cheZ*	
		flgK	*cheZ*	*glpC*	*cheM*	
		fliE	*cheW*	*nuoI*	*fliA*	
		fliL	*flgA*	*pduQ*	*cheW*	
		fliP	*fljB*	*nuoG*	*flgA*	
		csgB	*flgK*	*nuoF*	*flgK*	
		fimC	*fliE*	*frdD*	*fliE*	
		fliR	*fliL*	*nuoA*	*fliL*	
		flhC	*motA*	*pduS*	*motA*	
			fliP	*nuoC*	*fliZ*	
			motB	*dctA*	*cheY*	

Amino acid transport and metabolism	*Anaerobic metabolism*	*Surface structures*	*Cell motility and secretion*	*Energy production and conversion*	*Flagella and chemotaxis*	*Inorganic iron transport and metabolism*
			STM3216	*putA*	*flhB*	
			tsr	*nuoB*	*fliP*	
			fimC		*motB*	
			STM1657		*tsr*	
			fliR		STM2314	
			STM3152		*aer*	
					STM1657	
					fliR	
					STM3152	
					flhC	

Table S3 B. COG organized DE genes.

Oxidative phosphorylation	*Regulators*	*Signal transduction*	*Transcription*	*Lipid metabolism*	*Nucleotide metabolism*	*Cell envelope biogenesis and outer membrane*	*Carbohydrate transport and metabolism*
fliI	*eco*	*ssrA*	STM1265	sseJ	*nrdD*	*pagC*	STM0577
frdB	*fliS*	*yhjH*	*yncC*	yihU		STM1493	*pagO*
frdA	*cheR*	*cheY*	*yhcO*	yhbT		*pgtE*	*otsB*
frdC	*cheZ*	STM2314	*yiaG*			*figJ*	STM3132
nuoJ	*fliA*	*aer*	*phnO*				*garL*
nuoM	*cheW*	*yjjQ*	*flgM*				*yiaO*
nuoN	*cheY*	STM2503	*fliA*				*yadI*
nuoK	*hycA*	*ydaA*	*ydhM*				*pmgI*
nuoE	*flhC*						*citE*
nuoH							*kdgK*
nuoL							STM1613
nuoI							*sgaB*
nuoG							*pykA*
nuoF							*glpX*
frdD							STM2343
nuoA							STM3254
nuoB							STM3792

Appendix III

Table S4. ChIP-seq peaks: FNR binding.

		Peak	
Genes associated with peak[a]	***Chr.[b]***	***Start***	***End***
repY, repZ	pCol1B9SL1344	0	484
yaeA, yaeB, yafA	pCol1B9SL1344	4321	5743
yafB, yagA	pCol1B9SL1344	6037	7620
cib	pCol1B9SL1344	7696	8638
imm, ybaA	pCol1B9SL1344	9141	10730
SLP2_0018, *parA*, SLP2_0020	pCol1B9SL1344	13879	14877
stbA	pCol1B9SL1344	15184	16230
trbA, pndC, pndA	pCol1B9SL1344	41332	41922
SLP2_0054A, *exc*	pCol1B9SL1344	42304	46277
traH, traG	pCol1B9SL1344	65594	66734
traF, traE	pCol1B9SL1344	67156	68931
rci, shfB, shfB, shfC	pCol1B9SL1344	69768	71125
pilV, pilU, pilT, pilS, pilR	pCol1B9SL1344	72184	75372
pilQ, pilP	pCol1B9SL1344	76195	77489
pilO	pCol1B9SL1344	78047	78880
pilL, pilK, pilJ	pCol1B9SL1344	81610	82899
trcD	pCol1B9SL1344	83434	84254
traB, traA	pCol1B9SL1344	84936	86908
traT, traS	pSLTSL1344	10573	11429
traV, trbD	pSLTSL1344	26993	27311
traA, traY, traJ, traM	pSLTSL1344	31179	32898
finP	pSLTSL1344	33815	34895
parB, parA, samB	pSLTSL1344	47671	50469
PSLT042, *spvR*	pSLTSL1344	60927	62144
spvC, spvD	pSLTSL1344	65036	67651
ccdA, PSLT026, PSLT025, *repA2*	pSLTSL1344	73726	76279
PSLT020, *pefB*	pSLTSL1344	78271	79352
pefI	pSLTSL1344	85147	85786
repA, tap, repA3, repC	pSLTSL1344	91908	92600
STnc890, STM0014, STM0015, STnc3200	ST4-74	14781	16510
STM0017, *chiA*	ST4-74	17164	18037
STM0020	ST4-74	23025	23494
bcfA	ST4-74	24008	24437
STM0029, STM0030, STM0031, STM0032	ST4-74	32306	35266
STM0034, STM0035	ST4-74	39372	40230
STM0042	ST4-74	51862	52359
leuA, leuL, leuO	ST4-74	133806	134595
yaeH	ST4-74	247525	247933
sciF, sciG	ST4-74	310765	311287
sciK	ST4-74	316285	316974
STM0277, STM0278, *sciK1*	ST4-74	317558	318812
SL1344_0286A, STM0292	ST4-74	335838	336360
sciX, sciY, IsrA	ST4-74	337188	338796
STM0295	ST4-74	339416	339923
safA, safB	ST4-74	341032	342393
ybeJ	ST4-74	346063	346501
sinR	ST4-74	347728	349037
pagN	ST4-74	349382	350495
STM0328	ST4-74	370613	371025
STM0334, STM0335	ST4-74	377298	378623
stbA, STM0341, STM0342, STnc1700, STM0343	ST4-74	384214	386526
STM0345, STM0346, STM0347, STM0348, STM0349	ST4-74	388882	391454

Genes associated with peak[a]	Chr.[b]	Peak	
		Start	End
yaiB, STnc4150	ST4-74	436424	437097
tsx	ST4-74	467672	468094
STM0438	ST4-74	490749	492761
cyoA	ST4-74	497570	498023
rpmE2, rpmJa	ST4-74	525766	526197
maa, hha, ybaJ	ST4-74	527332	528700
STM0497	ST4-74	556592	557400
fimZ	ST4-74	609785	611037
STM0551, *fimW*	ST4-74	611852	612931
STM0557	ST4-74	614288	616208
gtrBa, *gtrAa*, STnc1870	ST4-74	616736	617335
ybdN, ybdO	ST4-74	667293	668470
citC, citA	ST4-74	687093	687718
cspE	ST4-74	692120	692617
STM0719, STM0720, STM0721, STM0722, STM0723, STM0724, STM0725, STM0726, STM0727	ST4-74	783417	793157
gltA, STM0731, *sdhC*	ST4-74	796516	797389
cydA	ST4-74	807541	808981
STM0762, STM0763, STM0764, STM0765	ST4-74	825138	828930
slrP	ST4-74	865818	866532
glnH	ST4-74	896198	896612
STM0854, STM0855, STM0856, STM0857	ST4-74	925176	928619
STM0858	ST4-74	929008	930178
STM0859	ST4-74	931060	932097
pflA, STM0971, *sopD2*	ST4-74	1010045	1011678
focA	ST4-74	1014575	1015326
ompF, STnc3350	ST4-74	1047357	1048016
STM1001, *dpaL*	ST4-74	1050149	1050717
STM1003	ST4-74	1051463	1051983
STM1003	ST4-74	1052815	1053283
STM2622, STM1019, STM1020	ST4-74	1065164	1066458
gtgA	ST4-74	1070419	1071256
STM1054, *gtgE, gtgF*	ST4-74	1098722	1100020
pipA, *pipB*, STM1089, *pipC*	ST4-74	1132644	1134847
sopB, orfX, STM1093	ST4-74	1136105	1137136
STnc1880, STM1110	ST4-74	1153672	1154710
phoH	ST4-74	1169962	1170414
STM1130	ST4-74	1175387	1175950
STM1131, STM1132	ST4-74	1176770	1178094
csgF, csgE, csgD, csgB	ST4-74	1185409	1187428
ndh	ST4-74	1250095	1250801
potC, sifA	ST4-74	1265767	1267189
STnc150, SL1344_misc_feature_6, STM1239, *envF, IsrC*	ST4-74	1282495	1286129
cspH, pagD, pagC	ST4-74	1287849	1289532
STnc520, *pliC*	ST4-74	1290452	1291651
STM1265, STM1266	ST4-74	1304354	1305232
STM1267, STM1268, *aroQ*	ST4-74	1306242	1306852
gapA, yeaA	ST4-74	1326449	1326856
STM1331, *rfc*	ST4-74	1366420	1368646
ydiQ, ydiP	ST4-74	1392171	1392538
ydiN	ST4-74	1399195	1399768
orf408	ST4-74	1422650	1423124
orf70	ST4-74	1430434	1430778
orf242, ssrB	ST4-74	1432932	1434312
ssaH, ssaI, ssaJ	ST4-74	1446497	1447701
ssaU	ST4-74	1457107	1458308
tppB	ST4-74	1484775	1485450
ydgK, ydgT, blr	ST4-74	1492369	1493116

Genes associated with peak[a]	*Chr.*[b]	*Peak*	
		Start	*End*
STM1527	ST4-74	1560687	1561385
STM1528	ST4-74	1562488	1563261
STM1550, STM1551, SL1344_1481, STM1552	ST4-74	1583358	1585430
STM1554, STM1555	ST4-74	1587253	1588927
steA	ST4-74	1626466	1627272
ydcX, STnc1110	ST4-74	1643054	1643588
pdgL, STM1600, *ugtL, sifB, yncJ*	ST4-74	1647612	1650271
STM1628, *steB*, STM1630, *sseJ*, STM1632, STM1633, STM1634	ST4-74	1676041	1681588
STnc580	ST4-74	1706706	1707160
STM1657	ST4-74	1709097	1709520
fnr	ST4-74	1710839	1711458
STM1670, STM1671, STM1672	ST4-74	1720903	1723651
steC, STM1698A, *ycjE*	ST4-74	1747101	1748641
ompW, STM1733	ST4-74	1785237	1785803
STM1741	ST4-74	1791930	1792308
STM1747, STnc3580	ST4-74	1798223	1798850
ychE, adh	ST4-74	1799396	1800115
STM1785	ST4-74	1839808	1841186
STM1794, STnc3610, STM1795	ST4-74	1850017	1850547
ftsI2, cspC, yobF, STnc3630	ST4-74	1892956	1894056
STM1854	ST4-74	1909338	1909738
sopE2, STM1856, SL1344_1786	ST4-74	1910085	1911622
STnc3640, SL1344_1792, *pagO*, STM1863, STM1864	ST4-74	1915516	1919190
STM1868A	ST4-74	1922572	1923061
araH, ftnB	ST4-74	1984296	1984864
STM1940, STM1941, SL1344_1874A	ST4-74	1991892	1992807
fliC, fliD	ST4-74	2006714	2007102
SL1344_1927A, STnc1280, SL1344_1927B, SL1344_1928	ST4-74	2039652	2041523
STM2007	ST4-74	2084789	2085479
cbiA, pocR	ST4-74	2111850	2112415
SL1344_2042, *sopA*	ST4-74	2138880	2140308
wzzB	ST4-74	2155528	2155962
udg	ST4-74	2156399	2157128
rfbP	ST4-74	2158655	2161291
rfbK, cpsB2, rfbN, rfbU, rfbV, rfbX, rfbJ	ST4-74	2161611	2168990
rfbG, rfbF, rfbI, rfbC	ST4-74	2170769	2173903
sseK2, srcA	ST4-74	2229021	2230739
STnc740, *stcD*	ST4-74	2240321	2241685
stcA	ST4-74	2245041	2245645
STM2186	ST4-74	2278779	2279141
STM2208, STM2209	ST4-74	2306623	2307891
yejG, STnc1340	ST4-74	2319741	2320153
oafA	ST4-74	2329411	2331577
STM2238	ST4-74	2335480	2336899
MicF, yojN	ST4-74	2364341	2364931
STM2274, STM2275	ST4-74	2376859	2377793
yfbK	ST4-74	2421134	2421856
nuoA, STM2329, STnc1550	ST4-74	2437181	2437897
yfbQ	ST4-74	2438908	2439821
STM2360, STM2361	ST4-74	2470310	2470673
yfcZ, fadL	ST4-74	2499678	2500162
upp, purM	ST4-74	2610304	2610723
STM2503, STM2505, STM2506	ST4-74	2618396	2619025
STM2508	ST4-74	2619468	2620057
guaB, xseA	ST4-74	2623538	2623973
cadB	ST4-74	2695797	2696235
lepA, SL1344_misc_feature_12, *gogB*, STM2585	ST4-74	2726315	2729493

Genes associated with peak[a]	Chr.[b]	Peak	
		Start	End
STM2585A	ST4-74	2730527	2730972
SL1344_tRNA_44, STnc2080, *IsrJ*	ST4-74	2759387	2759721
SL1344_2583, STM2621, SL1344_2585	ST4-74	2763106	2764161
SL1344_2594	ST4-74	2768759	2770091
yfiD, ung	ST4-74	2786436	2787186
aroF, yfiR	ST4-74	2812917	2813783
int, SL1344_2632	ST4-74	2816377	2816889
STM2680	ST4-74	2832194	2832785
sopE	ST4-74	2863096	2863454
SL1344_2695, SL1344_2696, SL1344_2697, SL1344_2698	ST4-74	2877180	2880718
cIIc, apl, cI	ST4-74	2886539	2887382
SL1344_2710	ST4-74	2888412	2889786
SL1344_2713, SL1344_2714, SL1344_2715	ST4-74	2890959	2894228
STM2742, STM2743	ST4-74	2902737	2905634
STM2746, STM2747	ST4-74	2908404	2911125
STM2754	ST4-74	2914727	2916052
STM2761, STM2762, *IsrM*, STM2763	ST4-74	2923895	2928171
STM2766, STM2767, STM2768	ST4-74	2930105	2933192
fljA	ST4-74	2935245	2935655
hin, iroB	ST4-74	2937936	2938401
stpA, STM2800	ST4-74	2970115	2970994
avrA	ST4-74	3032892	3033544
sprB, hilC	ST4-74	3034140	3036066
prgH, hilD, hilA	ST4-74	3040049	3042597
iagB	ST4-74	3043284	3044745
sptP, sicP	ST4-74	3045926	3047129
invB	ST4-74	3060826	3061358
invA	ST4-74	3062836	3063528
invH, InvR	ST4-74	3067025	3067478
STM2902, STM2903	ST4-74	3068163	3069232
ygcB, sopD, STnc3140	ST4-74	3108568	3110081
gcvA, GcvB	ST4-74	3156570	3157077
STM3026	ST4-74	3208296	3209101
STM3079, STM3080	ST4-74	3262296	3263205
STM3083	ST4-74	3266523	3267135
STnc3870, SL1344_misc_feature_126, STM3117	ST4-74	3296996	3297599
STM3123, STM3124	ST4-74	3304249	3304895
STnc1170	ST4-74	3312267	3312920
STM3133	ST4-74	3314354	3314967
exuT, uxuA	ST4-74	3316288	3316949
STM3138, STnc3890	ST4-74	3321791	3322853
assT	ST4-74	3374699	3375163
STM3193	ST4-74	3376907	3377336
yqjH, yqjI	ST4-74	3400564	3400921
STM3216	ST4-74	3402887	3403314
aer	ST4-74	3404904	3405320
garD	ST4-74	3438955	3439404
agaR, gatY	ST4-74	3442922	3443364
STM3257	ST4-74	3447341	3447711
yhbT, yhbU	ST4-74	3460672	3461019
STM3343, STnc3060	ST4-74	3533405	3533758
mdh, argR	ST4-74	3549059	3549604
yhdG	ST4-74	3576305	3576768
yhdU	ST4-74	3578772	3579240
rpsM, rpmJb	ST4-74	3607149	3607568
bfr, bfd	ST4-74	3618952	3619556
nirB	ST4-74	3644686	3645129
STnc760, *feoA*	ST4-74	3685056	3685447

Genes associated with peak[a]	Chr.[b]	Peak Start	Peak End
STM3529, STM3530	ST4-74	3714927	3715274
STM3547	ST4-74	3737573	3738211
lpfA	*ST4-74*	*3849010*	*3849621*
STnc780	ST4-74	3858684	3859241
STM3652, *yafP*	ST4-74	3859848	3861143
STM3690, *sadA*	ST4-74	3901409	3902504
rfaK, rfaZ, rfaY, rfaJ, rfaI	ST4-74	3931143	3936149
rfaB, yibR	ST4-74	3936491	3937420
rfaQ	ST4-74	3939834	3940571
STM3752, *sugR*	ST4-74	3970142	3971395
rmbA, misL	ST4-74	3974104	3976057
marT, slsA	ST4-74	3980392	3981119
STM3779, STM3780	ST4-74	4000762	4002180
STM3782, STM3783, STM3784, STM3785	ST4-74	4004820	4006722
yhjA	ST4-74	4041092	4041771
STnc400, STM3845, STM3846	ST4-74	4072267	4074711
atpI	ST4-74	4101840	4102667
yifA, yifE	ST4-74	4127850	4128195
comM, ilvX, ilvG	ST4-74	4129882	4130430
rho	ST4-74	4146784	4147226
tatA	ST4-74	4201127	4201684
STM3980	ST4-74	4207528	4208405
yihG, polA	ST4-74	4227132	4227581
STM4015, *ompL*	ST4-74	4245984	4247419
SL1344_3979, STM4033	ST4-74	4263008	4263452
STM4051	ST4-74	4283485	4284359
ydeW, ego	ST4-74	4304575	4305108
SL1344_4052	ST4-74	4336272	4337013
coaA	ST4-74	4379995	4380383
SL1344_rRNA_20	ST4-74	4415485	4415924
aceB	ST4-74	4422971	4423548
STM4196, STM4197	ST4-74	4439177	4440672
STM4204	ST4-74	4446447	4447543
STM4218, STM4219	ST4-74	4458715	4459369
SL1344_misc_feature_129, *siiA, siiB, siiC, siiD, siiE*	ST4-74	4497954	4507001
siiF	ST4-74	4520086	4522699
yjcB, yjcC	ST4-74	4523191	4523870
adiY, STnc1180	ST4-74	4558985	4559993
STM4302	ST4-74	4569882	4570392
STM4313, *rtsB, rtsA*, STM4316	ST4-74	4581119	4582756
phoN, SL1344_4256	ST4-74	4584700	4586042
aspA, fxsA	ST4-74	4592849	4593368
SL1344_tRNA_82, *yjeV*	ST4-74	4617550	4617897
STM4413	ST4-74	4674875	4675360
iolT1, iolT2	ST4-74	4681329	4681883
iolE, iolG1	ST4-74	4687026	4688406
nrdD, SL1344_4383	ST4-74	4715206	4715714
STM4467, yjgK	ST4-74	4731066	4731723
ytgA	ST4-74	4734869	4735507
STM4489	ST4-74	4753746	4754156
STM4495	ST4-74	4764531	4766043
SL1344_4427A, *yeeN*	ST4-74	4773008	4774261
STM4503, STM4504, STM4505	ST4-74	4777524	4780154
IsrQ, SL1344_4439, STM4509	ST4-74	4783586	4784317
yjiH	ST4-74	4789091	4789570
STM4518	ST4-74	4793969	4794278
STM4520, STnc4130, STM4522	ST4-74	4796718	4797610
hsdS	ST4-74	4798610	4799530

Genes associated with peak[a]	Chr.[b]	Peak	
		Start	End
yjjP, yjjQ	ST4-74	4826026	4827024
yjjI, deoC	ST4-74	4841777	4842074
sthB	ST4-74	4870732	4871229
sthB	ST4-74	4872247	4872645
STM4595, STM4596	ST4-74	4873908	4875191
arcA, yjjY, lasT	ST4-74	4876509	4877354

[a]When multiple genes are listed a peak was present across the promoter region or TSS of all listed genes
[b]Chromosome or plasmid

Table S5. ChIP-seq peaks associated with up-regulated DE genes.

Genes associated with peak[a]	Chr.[b]	Peak	
		Start	End
stbA	pCol1B9SL1344	15184	16230
PSLT042, *spvR*	pSLTSL1344	60927	62144
spvC, *spvD*	pSLTSL1344	65036	67651
STnc890, STM0014, STM0015, STnc3200	ST4-74	14781	16510
stbA,STM0341,STM0342,STnc1700,STM0343	ST4-74	384214	386526
STM0557	ST4-74	614288	616208
gtrBa, gtrAa, STnc1870	ST4-74	616736	617335
STM0719, STM0720, STM0721, STM0722, STM0723, STM0724, STM0725, STM0726, STM0727	ST4-74	783417	793157
STM0762, STM0763, STM0764, STM0765	ST4-74	825138	828930
pflA, STM0971, *sopD2*	ST4-74	1010045	1011678
pipA, *pipB*, STM1089, *pipC*	ST4-74	1132644	1134847
STnc1880, STM1110	ST4-74	1153672	1154710
potC, sifA	ST4-74	1265767	1267189
STnc150, SL1344_misc_feature_6, STM1239, *envF*, *IsrC*	ST4-74	1282495	1286129
cspH, pagD, pagC	ST4-74	1287849	1289532
STM1265, STM1266	ST4-74	1304354	1305232
STM1267, STM1268, *aroQ*	ST4-74	1306242	1306852
STM1331, *rfc*	ST4-74	1366420	1368646
orf242, *ssrB*	ST4-74	1432932	1434312
ssaH, *ssaI*, *ssaJ*	ST4-74	1446497	1447701
ssaU	ST4-74	1457107	1458308
steA	ST4-74	1626466	1627272
pdgL, STM1600, *ugtL*, *sifB*, *yncJ*	ST4-74	1647612	1650271
STM1628, *steB*, STM1630, *sseJ*, STM1632, STM1633, STM1634	ST4-74	1676041	1681588
steC,STM1698A,*ycjE*	ST4-74	1747101	1748641
STM1747, STnc3580	ST4-74	1798223	1798850
STM1854	ST4-74	1909338	1909738
STnc3640, SL1344_1792, *pagO*, STM1863, STM1864	ST4-74	1915516	1919190
STM1940, STM1941, SL1344_1874A	ST4-74	1991892	1992807
SL1344_1927A, STnc1280, SL1344_1927B, SL1344_1928	ST4-74	2039652	2041523
sseK2, *srcA*	ST4-74	2229021	2230739
lepA, SL1344_misc_feature_12, *gogB*, STM2585	ST4-74	2726315	2729493
STM2585A	ST4-74	2730527	2730972
STM2761, STM2762, *IsrM*, STM2763	ST4-74	2923895	2928171
stpA, STM2800	ST4-74	2970115	2970994
gcvA, GcvB	ST4-74	3156570	3157077
STM3133	ST4-74	3314354	3314967
bfr, bfd	ST4-74	3618952	3619556
SL1344_4427A, *yeeN*	ST4-74	4773008	4774261
STM4503, STM4504, STM4505	ST4-74	4777524	4780154
STM4520, STnc4130, STM4522	ST4-74	4796718	4797610

[a]When multiple genes are listed a peak was present across the promoter region or TSS of all listed genes
[b]Chromosome or plasmid

Table S6. ChIP-seq peaks associated with down-regulated DE genes.

		Peak	
Genes associated with peak[a]	***Chr.[b]***	***Start***	***End***
citC, citA	ST4-74	687093	687718
sopB, *orfX*, STM1093	ST4-74	1136105	1137136
csgF, csgE, csgD, csgB	ST4-74	1185409	1187428
STnc580	ST4-74	1706706	1707160
STM1657	ST4-74	1709097	1709520
fnr	ST4-74	1710839	1711458
ompW, STM1733	ST4-74	1785237	1785803
fliC, fliD	ST4-74	2006714	2007102
stcA	ST4-74	2245041	2245645
yejG, STnc1340	ST4-74	2319741	2320153
nuoA, STM2329,STnc1550	ST4-74	2437181	2437897
yfcZ, fadL	ST4-74	2499678	2500162
STM2503, STM2505, STM2506	ST4-74	2618396	2619025
yfiD, *ung*	ST4-74	2786436	2787186
SL1344_2695, SL1344_2696, SL1344_2697, SL1344_2698	ST4-74	2877180	2880718
cIIc, apl, cI	ST4-74	2886539	2887382
STM3216	ST4-74	3402887	3403314
aer	ST4-74	3404904	3405320
yhbT, yhbU	ST4-74	3460672	3461019
nirB	ST4-74	3644686	3645129
yhjA	ST4-74	4041092	4041771
STnc400, STM3845, STM3846	ST4-74	4072267	4074711
nrdD, SL1344_4383	ST4-74	4715206	4715714
IsrQ, SL1344_4439, STM4509	ST4-74	4783586	4784317
yjjP, yjjQ	ST4-74	4826026	4827024
yjjI, deoC	ST4-74	4841777	4842074

[a]When multiple genes are listed a peak was present across the promoter region or TSS of all listed genes
[b]Chromosome or plasmid

Table S7. Letter-probability matrix of FNR binding motif (TTGATNTRSATCAA)

Position	***A***	***C***	***G***	***T***
1	0.000000	0.000000	0.250000	0.750000
2	0.000000	0.041667	0.000000	0.958333
3	0.125000	0.000000	0.875000	0.000000
4	1.000000	0.000000	0.000000	0.000000
5	0.000000	0.041667	0.041667	0.916666
6	0.208333	0.250000	0.125000	0.416667
7	0.041667	0.041667	0.083333	0.833333
8	0.333333	0.000000	0.666667	0.000000
9	0.041667	0.458333	0.375000	0.125000
10	0.791666	0.041667	0.125000	0.041667
11	0.000000	0.000000	0.000000	1.000000
12	0.083333	0.916667	0.000000	0.000000
13	1.000000	0.000000	0.000000	0.000000
14	0.750000	0.208333	0.000000	0.041667

Table S8. Top 100 FNR Motif Hits.

Start	*Stop*	*Strand*	*Score*	*p-value*	*Matched Sequence*
1430596	1430609	+	22.2036	2.83E-09	TTGATTTGCATCAA
3312562	3312575	+	21.3772	1.13E-08	TTGATTTACATCAA
3644926	3644939	+	21.3772	1.13E-08	TTGATTTACATCAA
2536558	2536571	+	20.5928	2.84E-08	GTGATTTGCATCAA
4715480	4715493	+	20.5928	2.84E-08	GTGATTTGCATCAA
1250446	1250459	-	20.5329	3.43E-08	TTGATGTGCATCAA
2320090	2320103	+	20.4551	3.69E-08	TTGATATACATCAA
1820854	1820867	-	19.8024	5.98E-08	TTGATTTATATCAA
1560987	1561000	+	19.2874	1.13E-07	TTGATCTGGATCAC
1799743	1799756	+	19.2575	1.16E-07	TTGATTTAGATCAC
2437400	2437413	+	18.9042	1.47E-07	TTAATTTACATCAA
3122008	3122021	-	18.8144	1.62E-07	TTGATTTACGTCAA
2478446	2478459	+	18.6826	1.83E-07	TTGATGTGCATCAC
1785488	1785501	-	18.6647	1.86E-07	TTAATCTGGATCAA
2786813	2786826	+	18.6168	2.00E-07	TTGATTTAAATCAA
2786813	2786826	-	18.6168	2.00E-07	TTGATTTAAATCAA
1492609	1492622	-	18.5389	2.17E-07	TTAATATGGATCAA
2320090	2320103	-	18.1317	2.96E-07	TTGATGTATATCAA
2632223	2632236	+	18.0958	3.13E-07	TTGATTTAGATAAA
914001	914014	+	17.9701	3.49E-07	TTGATGTGCGTCAA
1643343	1643356	-	17.9701	3.49E-07	TTGATGTGCGTCAA
1979902	1979915	-	17.9461	3.58E-07	GTGATCTGCATCAC
1183545	1183558	-	17.9162	3.64E-07	GTGATTTACATCAC
2808003	2808016	+	17.9162	3.64E-07	GTGATTTACATCAC
1820854	1820867	+	17.6946	4.34E-07	TTGATATAAATCAA
3439225	3439238	+	17.6766	4.41E-07	GTGATCTGGATCAC
4794134	4794147	-	17.6647	4.44E-07	TTGATTAACATCAA
3554685	3554698	-	17.6467	4.49E-07	GTGATTTAGATCAC
4041407	4041420	+	17.5329	4.96E-07	TTGATTGGTATCAA
2997911	2997924	-	17.4731	5.27E-07	TTGATTTTCATCAA
1808887	1808900	-	17.4371	5.43E-07	TTGATGGGCATCAA
3685208	3685221	-	17.2934	6.13E-07	TTGATATGGCTCAA
3316630	3316643	-	17.2695	6.19E-07	TTGATCTGTGTCAA
2932463	2932476	-	17.2216	6.43E-07	TTGATACGGATCAA
2610465	2610478	+	17.2156	6.53E-07	TTGACCTGGATCAA
1291100	1291113	-	17.2036	6.61E-07	GTGATTTACGTCAA
4783741	4783754	-	17.1856	6.79E-07	ATGATTTGCATCAA
4270771	4270784	-	17.1737	6.81E-07	TTGATATAGATAAA
3021733	3021746	-	17.1677	6.90E-07	TTAATTTGCGTCAA
1253486	1253499	-	17.1198	7.09E-07	GTGATCTACATCAC
73219	73232	+	17.0898	7.29E-07	TTTATTTGCATCAA
1712203	1712216	+	17.0898	7.29E-07	TTTATTTGCATCAA
261574	261587	+	16.9641	8.00E-07	TTGATTTACGTCAC
3715132	3715145	-	16.9521	8.06E-07	GTGATTTGCTTCAA
3312562	3312575	-	16.9461	8.09E-07	TTGATGTAAATCAA
3644926	3644939	-	16.9461	8.09E-07	TTGATGTAAATCAA
2692936	2692949	+	16.8922	8.46E-07	TTGATATACATCAT
4243812	4243825	+	16.8922	8.46E-07	TTGATATACATCAT
3224429	3224442	-	16.8204	8.94E-07	TTGATGAGCATCAA
510687	510700	-	16.7425	9.74E-07	TTGATAAACATCAA
527520	527533	-	16.7246	9.87E-07	GTGATATAGATCAC
1992348	1992361	+	16.7186	9.89E-07	TTAATTTGCATAAA
4189176	4189189	+	16.7006	1.01E-06	GTGATCGGCATCAA
2656423	2656436	-	16.6707	1.04E-06	GTGATTGACATCAA
1518635	1518648	-	16.6587	1.05E-06	GTGATATGCATAAA
1014923	1014936	+	16.6228	1.08E-06	TTGATATAGATCAT
4650494	4650507	-	16.497	1.20E-06	GTAATCTACATCAA

Start	Stop	Strand	Score	p-value	Matched Sequence
4639386	4639399	-	16.4611	1.23E-06	TTGATCGGCATCAC
4822711	4822724	+	16.4611	1.23E-06	TTGATCGGCATCAC
76793	76806	-	16.3892	1.31E-06	GTGATATGGATAAA
4876945	4876958	-	16.3174	1.37E-06	TTGATATATGTCAA
585659	585672	+	16.2934	1.39E-06	TTTATCTGCATCAA
3856862	3856875	+	16.2934	1.39E-06	TTTATCTGCATCAA
1799743	1799756	-	16.2096	1.47E-06	GTGATCTAAATCAA
975730	975743	-	16.1796	1.50E-06	GTGATTTGCGTCAC
3896424	3896437	-	16.1796	1.50E-06	GTGATTTGCGTCAC
495837	495850	+	16.1437	1.54E-06	TTGATGTACATCAT
1136494	1136507	-	16.0838	1.62E-06	TTGATCTGAGTCAA
2156517	2156530	-	16.0838	1.62E-06	GTGATCAGCATCAA
2618644	2618657	+	16.0539	1.65E-06	TTGATTTAAGTCAA
1428394	1428407	+	16.0479	1.66E-06	TGGATCTGCATCAA
2932463	2932476	+	16.0419	1.67E-06	TTGATCCGTATCAA
1253486	1253499	+	15.976	1.78E-06	GTGATGTAGATCAC
3147919	3147932	+	15.976	1.78E-06	GTGATTCACATCAA
2786866	2786879	+	15.9581	1.82E-06	TTGTTTTACATCAA
2891426	2891439	+	15.9581	1.82E-06	TTGATTTACATCGA
288281	288294	+	15.9401	1.83E-06	TTAATGTGGATCAC
3147919	3147932	-	15.9222	1.86E-06	TTGATGTGAATCAC
1250446	1250459	+	15.9162	1.87E-06	TTGATGCACATCAA
2165224	2165237	-	15.8683	1.94E-06	TTGATATATATAAA
780800	780813	+	15.8084	2.07E-06	TTGATTTACCTCAC
4241561	4241574	-	15.7186	2.23E-06	GTAATTTATATCAA
1428165	1428178	-	15.6886	2.28E-06	TTGTTTTAGATCAA
765537	765550	-	15.6467	2.33E-06	TTGATCTGGTTCAC
1316146	1316159	-	15.6048	2.40E-06	TTGATTTAAATAAA
4288995	4289008	-	15.6048	2.40E-06	GTGACCTGGATCAA
1486853	1486866	-	15.5928	2.44E-06	GTGATTTTGATCAA
2672110	2672123	-	15.5629	2.49E-06	GTGATATAGATAAA
527520	527533	+	15.5449	2.52E-06	GTGATCTATATCAC
1977320	1977333	-	15.5449	2.52E-06	GCGATCTGGATCAA
3605056	3605069	-	15.5329	2.55E-06	GTGATGTACGTCAA
4453798	4453811	+	15.521	2.56E-06	TTGATTGAAATCAA
4599472	4599485	+	15.521	2.56E-06	TTGATATGGTTCAC
947547	947560	+	15.515	2.58E-06	TTCATTTGTATCAA
4829078	4829091	-	15.515	2.58E-06	TTGATGTGCATCAG
869629	869642	-	15.3174	2.88E-06	TTGATATATATCAT
4722369	4722382	-	15.2395	3.03E-06	TTGATATATTTCAA
4593105	4593118	+	15.2036	3.12E-06	GTAATCTGGATCAC
2663061	2663074	-	15.1677	3.22E-06	TTGATAAATATCAA
4715511	4715524	-	15.1617	3.25E-06	TTGTTCTACATCAA

Appendix IV

Table S9. Proteomics: Proteins in WT.

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
accA	3.70E-02	3.65E-02
accB	7.81E-02	3.65E-02
accC	1.60E-01	2.22E-01
accD	3.43E-02	3.22E-02
aceA	8.66E-03	1.15E-02
aceE	2.29E-01	6.39E-01
aceF	9.10E-02	1.68E-01
ackA	1.09E-01	1.33E-01
acnA	1.88E-02	5.15E-02
acnB	2.16E-01	5.68E-01
acpP	2.67E-01	6.43E-02
acrA	7.20E-02	8.50E-02
acrB	4.91E-02	1.56E-01
acs	1.96E-02	3.96E-02
adhE	3.13E-01	8.47E-01
adhP	3.09E-02	3.10E-02
adk	1.45E-01	9.55E-02
aefA	4.58E-02	1.62E-01
agp	5.18E-02	6.64E-02
ahpC	1.88E-01	1.10E-01
ahpF	6.64E-02	1.04E-01
alaS	1.33E-01	3.57E-01
aldB	9.25E-02	1.46E-01
allR	1.75E-02	1.45E-02
anmK	1.51E-02	1.69E-02
apbC	2.64E-02	2.97E-02
aphA	2.64E-02	1.94E-02
argA	6.08E-02	8.42E-02
argB	5.42E-02	4.09E-02
argC	2.46E-02	2.48E-02
argD	1.13E-01	1.39E-01
argG	1.14E-01	1.59E-01
argH	1.19E-01	1.70E-01
argI	5.08E-02	5.25E-02
argS	3.16E-02	5.70E-02
argT	3.55E-02	2.80E-02
arnB	2.82E-02	3.31E-02
arnC	5.17E-02	5.31E-02
aroA	4.54E-02	5.93E-02
aroB	5.37E-02	5.83E-02
aroC	3.48E-02	3.85E-02
aroD	1.38E-02	1.05E-02
aroF	8.14E-02	8.86E-02
aroG	2.10E-02	2.24E-02
artI	8.07E-02	6.11E-02
artJ	6.69E-02	5.00E-02
asd	3.25E-01	3.68E-01
asnB	3.30E-02	5.79E-02
asnS	2.41E-01	3.56E-01
aspA	1.00E-01	1.48E-01
aspC	4.99E-02	6.11E-02
aspS	3.31E-02	6.10E-02
astC	3.88E-02	4.77E-02
atpA	3.93E-01	6.07E-01

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
atpC	1.92E-02	8.06E-03
atpD	5.40E-01	7.59E-01
atpF	4.11E-02	2.00E-02
atpG	7.96E-02	7.04E-02
atpH	9.15E-02	4.99E-02
bamA	8.13E-02	2.03E-01
bamB	8.66E-02	1.01E-01
bcp	1.04E-01	5.18E-02
bepA	1.14E-02	1.70E-02
bfd	4.92E-03	1.05E-03
bfr	7.73E-02	3.96E-02
bioA	2.08E-02	2.77E-02
bioB	5.10E-02	5.59E-02
bioD1	5.65E-02	3.84E-02
btuB	6.99E-03	1.33E-02
btuE	5.69E-02	3.27E-02
cafA	1.66E-02	2.58E-02
carA	1.54E-01	1.81E-01
carB	1.31E-01	4.34E-01
cbiD	2.60E-02	3.00E-02
cbiF	1.64E-01	1.30E-01
cbiG	1.77E-02	1.89E-02
cbiH	7.06E-02	5.15E-02
cbiJ	2.40E-01	1.93E-01
cbiK	2.11E-01	1.74E-01
cbiL	5.59E-02	4.08E-02
cbiP	2.18E-02	3.36E-02
ccmH	1.23E-02	1.32E-02
cdh	5.70E-02	4.54E-02
celD	3.05E-02	2.82E-02
cheA	4.09E-02	8.38E-02
cheB	1.96E-02	2.06E-02
cheY	1.55E-02	6.10E-03
cheZ	4.17E-02	2.79E-02
citD1	2.08E-02	6.27E-03
citE	3.30E-02	3.04E-02
clpA	2.20E-02	5.15E-02
clpB	1.63E-01	4.34E-01
clpX	3.40E-02	4.45E-02
cmk	2.88E-02	1.99E-02
coaA	1.47E-02	1.48E-02
cobT	4.30E-02	4.43E-02
cpdB	2.04E-02	4.05E-02
cptA	2.38E-02	4.44E-02
cra	1.66E-02	1.78E-02
crp	2.69E-02	1.79E-02
crr	6.73E-01	3.43E-01
cspA	2.99E-01	6.19E-02
cspC	6.04E-01	1.25E-01
cspD	4.83E-01	1.07E-01
cspE	2.63E-01	5.47E-02
cspJ	8.90E-02	1.93E-02
cueO	3.41E-02	5.57E-02
cyoA	3.87E-02	3.82E-02
cypD	1.30E-01	2.47E-01
cysA	6.20E-02	7.12E-02
cysE	3.32E-02	2.72E-02
cysH	5.61E-02	4.38E-02
cysI	4.07E-02	7.31E-02

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
cysJ	7.71E-02	1.43E-01
cysK	3.36E-01	3.24E-01
cysM	2.02E-02	1.84E-02
cysN	7.00E-02	1.04E-01
cysP	5.43E-02	5.69E-02
dacA	2.50E-02	3.10E-02
dacC	2.47E-02	3.01E-02
damX	8.83E-02	1.12E-01
dapA	3.52E-02	3.09E-02
dapB	1.14E-01	9.25E-02
dapD	1.14E-01	9.57E-02
dcp	1.95E-02	4.23E-02
degP	1.14E-01	1.57E-01
degQ	1.19E-02	1.57E-02
deoA	2.37E-02	3.12E-02
deoB	5.20E-02	6.45E-02
deoC	1.94E-02	1.50E-02
deoD	9.74E-02	7.13E-02
dgoD	2.26E-02	2.68E-02
dkgA	3.44E-02	3.00E-02
dkgB	1.94E-02	1.59E-02
dksA	9.34E-02	4.63E-02
dlgD	2.04E-02	2.12E-02
dmsA	3.64E-02	9.25E-02
dnaJ	3.68E-02	4.29E-02
dnaK	4.83E-01	9.35E-01
dppA	1.06E-01	1.78E-01
dppD	3.37E-02	3.38E-02
dppF	2.02E-02	2.15E-02
dps	1.79E-01	9.38E-02
dsbA	4.84E-02	3.11E-02
dsbC	6.97E-02	5.11E-02
dut	8.88E-02	3.98E-02
ecnB	3.31E-01	4.49E-02
eda	1.58E-02	9.86E-03
elaA	4.49E-03	2.20E-03
elaB	9.08E-03	2.94E-03
emrR	2.51E-02	1.46E-02
engB	1.29E-02	8.46E-03
eno	1.38E+00	1.77E+00
entB	3.70E-02	3.35E-02
envF	1.91E-02	1.59E-02
erfK	2.78E-02	2.66E-02
erpA	5.26E-02	1.80E-02
exbB	2.21E-02	1.63E-02
fabA	6.37E-02	3.41E-02
fabB	1.58E-01	1.89E-01
fabD	5.58E-02	5.09E-02
fabF	4.76E-02	5.75E-02
fabG	9.48E-02	6.78E-02
fabH	2.13E-02	2.01E-02
fabI	1.27E-01	9.91E-02
fadL	7.27E-02	9.63E-02
fba	7.13E-02	7.83E-02
fbaB	5.85E-01	6.27E-01
fbp	7.30E-02	7.56E-02
fdx	5.76E-02	2.06E-02
feoA	4.52E-03	1.05E-03
fepA	1.41E-02	3.25E-02

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
ffh	3.74E-02	5.21E-02
fhuA	7.49E-02	1.69E-01
fimZ	8.31E-03	5.44E-03
fklB	8.33E-02	5.52E-02
fkpA	6.23E-02	5.04E-02
fldA	4.52E-02	2.50E-02
flgC	8.31E-03	3.25E-03
flgJ	1.22E-02	1.17E-02
flgK	3.74E-02	6.22E-02
fliB	1.82E-02	2.33E-02
fliC	5.88E-01	8.49E-01
fliD	2.88E-02	4.00E-02
fliY	3.80E-02	3.05E-02
fljB	9.04E-02	1.32E-01
folE	2.75E-02	1.90E-02
frdA	1.79E-01	3.31E-01
frdB	2.58E-01	2.01E-01
frr	9.02E-02	5.19E-02
fsa	1.84E-02	1.21E-02
ftn	5.23E-02	2.83E-02
ftsA	4.59E-02	5.85E-02
ftsH	5.29E-02	1.05E-01
ftsI	9.36E-03	1.68E-02
ftsN	1.31E-01	1.34E-01
ftsZ	1.49E-01	1.68E-01
fumA	3.11E-02	5.59E-02
fumB	1.20E-01	2.03E-01
fumC	5.35E-02	7.54E-02
fur	6.53E-02	3.13E-02
fusA	7.85E-01	1.70E+00
gabD	9.11E-03	1.32E-02
galF	3.30E-02	3.05E-02
galK	2.16E-02	2.51E-02
galM	2.55E-02	2.76E-02
galU	1.28E-01	1.19E-01
gapA	2.01E+00	2.01E+00
garR	4.35E-02	3.73E-02
gcvH	3.36E-02	1.30E-02
gcvP	2.89E-02	8.48E-02
gdhA	1.70E-01	2.33E-01
ggt	2.08E-02	3.61E-02
glgA	3.07E-02	4.55E-02
glgP	3.64E-02	9.54E-02
glk	4.97E-02	4.87E-02
glmM	2.14E-02	2.86E-02
glmS	3.98E-02	7.48E-02
glmU	2.21E-02	3.07E-02
glnA	1.91E-01	2.78E-01
glnH	6.95E-02	5.28E-02
glnQ	3.65E-02	2.72E-02
glnS	3.14E-02	5.62E-02
glpA	1.52E-01	2.54E-01
glpB	2.92E-02	3.78E-02
glpC	5.94E-02	7.48E-02
glpD	8.45E-02	1.35E-01
glpK	4.29E-01	6.74E-01
glpQ	2.27E-01	2.56E-01
glpR	5.92E-02	4.61E-02
gltA	9.02E-02	1.22E-01

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
gltB	1.13E-01	5.20E-01
gltD	2.71E-02	4.02E-02
gltI	7.20E-02	6.72E-02
gltX	3.04E-02	4.59E-02
glyA	2.03E-01	2.59E-01
glyQ	7.27E-02	7.11E-02
glyS	3.84E-02	8.23E-02
gmhA	4.81E-02	2.82E-02
gnd	1.17E-01	1.68E-01
gns	8.42E-02	1.52E-02
gor	2.36E-02	3.26E-02
gpmA	4.40E-02	3.49E-02
gpmI	1.54E-01	2.42E-01
gppA	5.41E-03	8.41E-03
gpsA	2.25E-02	2.29E-02
grcA	1.55E-01	6.25E-02
greA	6.10E-02	3.01E-02
groL	1.90E+00	3.05E+00
groS	1.32E+00	3.82E-01
grpE	3.97E-02	2.42E-02
grxA	8.05E-02	2.27E-02
grxB	2.29E-01	1.57E-01
gst	5.77E-02	3.64E-02
guaA	7.22E-02	1.19E-01
guaB	9.42E-02	1.37E-01
gyrA	3.34E-02	9.09E-02
gyrB	3.56E-02	8.93E-02
hemE	6.83E-03	7.48E-03
hemL	7.06E-02	9.04E-02
hemX	3.10E-02	3.66E-02
hemY	6.36E-02	8.08E-02
hflC	9.23E-02	9.68E-02
hflK	1.40E-01	1.78E-01
hisA	2.00E-02	1.46E-02
hisB	6.11E-02	6.92E-02
hisC	3.80E-02	4.26E-02
hisD	4.76E-02	6.15E-02
hisF	2.82E-02	2.25E-02
hisG	4.61E-02	4.31E-02
hisH	1.78E-02	1.09E-02
hisJ	1.64E-01	1.30E-01
hldD	2.59E-02	2.52E-02
hldE	5.17E-02	7.43E-02
hns	8.54E-01	3.72E-01
holD	4.69E-03	2.14E-03
holE	0.00E+00	0.00E+00
hsdM	2.47E-02	4.10E-02
hslU	8.20E-02	1.14E-01
htpG	3.64E-01	7.26E-01
hupA	2.07E+00	5.52E-01
hupB	1.27E-01	3.27E-02
hybC	2.67E-02	4.68E-02
hypB	2.59E-02	2.32E-02
iadA	2.58E-02	2.94E-02
icdA	1.06E-01	1.36E-01
ihfA	2.69E-01	8.54E-02
ihfB	2.25E-02	6.65E-03
ileS	3.84E-02	1.14E-01
ilvA	5.57E-02	8.82E-02

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
ilvC	2.06E-01	3.12E-01
ilvD	1.10E-01	2.04E-01
ilvE	1.06E-01	1.01E-01
ilvH	2.49E-02	1.25E-02
infA	5.27E-02	1.21E-02
infB	9.92E-02	2.71E-01
infC	5.05E-02	2.90E-02
iraP	1.00E-01	2.76E-02
iscR	6.38E-02	3.13E-02
iscS	5.32E-02	6.72E-02
ispG	6.43E-02	7.34E-02
katE	6.90E-02	1.62E-01
katG	2.73E-02	6.09E-02
kbl	2.91E-02	3.51E-02
kdgR	2.96E-02	2.47E-02
kdsA	4.93E-02	4.27E-02
ldhA	7.08E-02	7.28E-02
lepA	2.76E-02	5.15E-02
leuB	8.61E-02	9.56E-02
leuC1	9.06E-02	1.27E-01
leuD1	1.45E-01	9.15E-02
leuS	1.02E-01	2.77E-01
ligA	3.62E-02	7.44E-02
livG	2.22E-02	1.78E-02
livJ	2.31E-01	2.51E-01
livK	2.72E-02	3.01E-02
lolA	2.03E-02	1.27E-02
lolB	3.64E-02	2.41E-02
lolD	6.45E-03	4.64E-03
lon	6.33E-02	1.55E-01
lpdA	8.57E-02	1.22E-01
lpfB	2.57E-02	1.84E-02
lpp1	3.23E-01	7.60E-02
lptD	4.52E-02	1.14E-01
lptE	6.22E-02	3.73E-02
lrp	6.72E-02	3.55E-02
lsrB	4.32E-02	4.45E-02
lsrF	4.49E-02	4.02E-02
ltaA	1.05E-02	1.07E-02
lysC	3.12E-02	4.27E-02
lysS	5.51E-02	8.89E-02
maeA	2.79E-02	4.92E-02
maeB	7.62E-02	1.76E-01
malP	2.97E-02	7.48E-02
manA	4.42E-02	5.27E-02
manX	2.94E-01	2.87E-01
manY	1.00E-01	7.76E-02
mdh	6.80E-01	6.20E-01
mdoD	2.41E-02	4.21E-02
mdoG	3.31E-02	5.35E-02
metA	3.02E-02	3.01E-02
metE	2.89E-01	6.86E-01
metF	2.90E-02	2.70E-02
metG	7.43E-02	1.59E-01
metH	2.77E-02	1.06E-01
metJ	1.60E-02	5.43E-03
metK	5.16E-02	6.08E-02
metL	3.51E-02	8.79E-02
metN1	4.58E-02	4.84E-02

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
metQ	6.21E-01	5.11E-01
mglB	7.77E-02	7.75E-02
mgsA	4.51E-02	2.14E-02
miaB	6.90E-02	1.04E-01
mig-3	1.47E-02	1.30E-02
minC	4.38E-02	3.10E-02
minD	1.80E-01	1.48E-01
mipA	2.55E-01	1.99E-01
moaB	1.01E-01	5.25E-02
modA	2.19E-01	1.69E-01
modF	1.01E-02	1.55E-02
moeA	6.48E-02	8.07E-02
mppA	1.06E-02	1.77E-02
mrcA	1.43E-02	3.77E-02
mrcB	1.04E-02	2.71E-02
mreB	3.97E-02	4.10E-02
msbA	2.95E-02	5.30E-02
msrA	4.18E-02	2.75E-02
mtlA	4.94E-02	9.43E-02
mtnN	2.77E-02	1.91E-02
mukB	2.60E-02	1.23E-01
murA	4.94E-02	6.23E-02
mutM	8.54E-03	7.34E-03
nadE	3.09E-02	2.63E-02
nagB	1.03E-01	8.54E-02
nanE2	1.86E-02	1.26E-02
ndh	3.03E-02	4.04E-02
ndk	5.99E-02	2.61E-02
nemA	4.74E-02	5.22E-02
nfuA	5.40E-02	3.18E-02
nlpB	9.51E-02	9.83E-02
nlpD	5.66E-02	6.28E-02
nrdA	6.38E-02	1.54E-01
nrfC	1.10E-02	7.92E-03
nuoC	3.62E-02	6.96E-02
nuoF	7.08E-02	9.79E-02
nusA	8.67E-02	1.34E-01
nusG	4.36E-02	2.50E-02
ompA	6.47E+00	6.80E+00
ompC	8.01E-01	9.22E-01
ompD	1.60E+00	1.77E+00
ompF	8.36E-01	9.35E-01
ompN	1.27E-02	1.47E-02
ompR	8.69E-02	6.65E-02
ompS	8.98E-02	1.09E-01
ompW	6.61E-02	4.22E-02
ompX	2.90E-01	1.50E-01
oppA	1.04E-01	1.78E-01
oppD	2.09E-02	2.17E-02
oppF	4.35E-02	4.56E-02
osmC	1.08E-01	4.60E-02
osmE	8.32E-02	2.86E-02
osmX	1.05E-02	9.87E-03
osmY	7.82E-01	4.68E-01
oxyR	3.54E-02	3.39E-02
pagC	1.99E-01	1.12E-01
pagN	8.37E-02	6.02E-02
pal	2.51E-01	1.33E-01
parB	2.63E-02	2.71E-02

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
pbpG	4.41E-02	4.23E-02
pckA	1.07E-01	1.78E-01
pdgL	4.49E-02	3.67E-02
pduA	5.05E-01	1.35E-01
pduB	1.49E+00	1.00E+00
pduC	1.21E+00	2.05E+00
pduD	9.91E-01	6.71E-01
pduE	1.90E+00	1.02E+00
pduG	1.22E-01	2.19E-01
pduJ	7.92E-01	2.02E-01
pduL	1.05E-01	6.71E-02
pduN	5.51E-02	1.41E-02
pduO	2.54E-01	2.61E-01
pduP	5.30E-01	7.31E-01
pduQ	7.27E-02	8.08E-02
pduS	1.03E-01	1.40E-01
pduT	3.61E-02	1.95E-02
pduU	4.75E-02	1.66E-02
pduW	6.72E-02	8.33E-02
pdxH	9.33E-03	6.63E-03
pepB	2.85E-02	3.71E-02
pepD	1.29E-01	1.90E-01
pepP	1.70E-02	2.33E-02
pepQ	3.97E-02	5.59E-02
pfkA	9.26E-02	9.12E-02
pfkB	6.41E-02	5.87E-02
pflB	2.28E-01	5.43E-01
pflC	3.42E-02	3.18E-02
pgi	8.28E-02	1.42E-01
pgk	6.09E-01	7.03E-01
pgm	9.45E-02	1.54E-01
pheS	2.56E-02	2.65E-02
pheT	6.19E-02	1.51E-01
phnO	2.41E-02	1.22E-02
phoL	1.76E-02	2.02E-02
phoN	1.75E-01	1.39E-01
phoP	3.86E-01	2.76E-01
phsB	1.23E-02	7.66E-03
pmbA	2.99E-02	4.07E-02
pncB	2.75E-02	3.55E-02
pnp	1.18E-01	2.55E-01
pntA	3.79E-02	5.74E-02
pntB	4.18E-02	5.69E-02
polA	2.13E-02	6.12E-02
potD	4.37E-02	4.77E-02
potF	7.07E-02	8.10E-02
ppa	6.67E-02	3.68E-02
ppc	5.10E-02	1.41E-01
ppiB	2.81E-02	1.43E-02
pps	1.29E-01	3.15E-01
pqiB	2.56E-02	4.36E-02
prc	1.39E-02	3.01E-02
prfA	4.02E-02	4.54E-02
prfB	1.65E-02	1.89E-02
prkB	3.83E-02	3.45E-02
prlC	7.80E-02	1.69E-01
proA	4.16E-02	5.23E-02
proB	2.12E-02	2.32E-02
proC	3.83E-02	3.02E-02

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
proQ	2.53E-02	1.80E-02
proS	3.93E-02	6.96E-02
proX	9.88E-02	1.01E-01
prr	2.03E-02	2.95E-02
prs	9.17E-02	8.81E-02
psd	8.08E-02	8.10E-02
PSLT034	3.92E-02	2.28E-02
PSLT046	2.46E-02	1.84E-02
pssA	3.52E-02	5.21E-02
pstS	7.24E-02	7.45E-02
pta	2.29E-01	4.97E-01
ptsG	6.04E-02	8.56E-02
ptsH	8.33E-01	2.12E-01
ptsI	3.33E-01	5.91E-01
ptsN	8.29E-02	4.19E-02
purA	4.23E-02	5.61E-02
purB	5.64E-02	8.13E-02
purC	1.13E-01	8.48E-02
purD	2.87E-02	3.67E-02
purE	6.41E-03	3.17E-03
purF	6.19E-02	9.81E-02
purH	8.53E-02	1.38E-01
purL	4.75E-02	1.88E-01
purN	6.50E-02	4.26E-02
purT	3.61E-02	4.28E-02
purU	3.90E-03	3.47E-03
putA	5.37E-02	2.18E-01
pygM	1.01E-01	2.56E-01
pykA	1.67E-01	2.41E-01
pykF	1.90E-01	2.71E-01
pyrB	1.52E-02	1.48E-02
pyrC	2.31E-02	2.51E-02
pyrD	2.46E-02	2.53E-02
qor	7.76E-02	7.66E-02
rbfA	6.98E-02	2.95E-02
rbsB	8.67E-02	7.50E-02
rdgB	2.42E-02	1.42E-02
recA	6.85E-02	7.29E-02
recD	1.40E-02	2.61E-02
recG	1.59E-02	3.42E-02
res	1.46E-02	4.61E-02
rfbC	5.18E-02	3.00E-02
rfbH	3.49E-02	4.71E-02
rhlB	3.51E-02	4.62E-02
rho	1.30E-01	1.71E-01
ribB	1.88E-02	1.23E-02
ribD	2.21E-02	2.48E-02
ribE	6.93E-02	4.56E-02
ridA	2.08E-01	7.94E-02
rlmF	8.47E-03	8.24E-03
rlpA	2.96E-02	3.24E-02
rmuC	3.29E-02	5.02E-02
rnb	1.67E-02	3.38E-02
rne	7.05E-02	2.36E-01
rodZ	2.48E-02	2.47E-02
rpiA	1.54E-01	9.89E-02
rplA	1.44E+00	9.95E-01
rplB	4.38E-01	3.66E-01
rplC	2.23E-01	1.39E-01

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
rplD	4.16E-01	2.56E-01
rplE	6.44E-01	3.66E-01
rplF	6.99E-01	3.69E-01
rplI	8.68E-01	3.83E-01
rplJ	5.10E-01	2.54E-01
rplK	5.64E-01	2.35E-01
rplL	6.57E-01	2.26E-01
rplM	8.23E-02	3.68E-02
rplN	5.20E-01	1.99E-01
rplO	3.48E-01	1.45E-01
rplP	1.06E-01	4.47E-02
rplQ	2.05E-01	8.26E-02
rplR	2.01E-02	7.13E-03
rplS	2.39E-01	8.78E-02
rplT	3.27E-02	1.23E-02
rplU	3.63E-01	1.17E-01
rplV	1.50E-01	5.13E-02
rplW	8.33E-02	2.61E-02
rplX	9.71E-02	3.06E-02
rplY	2.32E-02	6.81E-03
rpmA	1.30E-01	3.33E-02
rpmB	8.40E-02	2.13E-02
rpmC	1.12E-01	2.27E-02
rpmD	5.11E-02	9.27E-03
rpmE2	1.06E-01	2.89E-02
rpmF	8.16E-02	1.46E-02
rpmH	2.82E-02	4.22E-03
rpoA	2.08E-01	2.13E-01
rpoB	2.37E-01	1.00E+00
rpoC	3.93E-01	1.71E+00
rpoE	4.40E-02	2.67E-02
rpoZ	6.42E-02	1.84E-02
rpsA	3.24E-01	5.54E-01
rpsB	6.77E-01	5.07E-01
rpsC	6.50E-01	4.71E-01
rpsD	3.10E-01	2.03E-01
rpsE	8.60E-01	4.23E-01
rpsF	1.34E-01	5.70E-02
rpsG	5.87E-02	2.88E-02
rpsH	2.34E-01	9.26E-02
rpsI	1.17E-01	4.84E-02
rpsJ	6.52E-01	2.14E-01
rpsK	1.43E-01	5.58E-02
rpsL	1.55E-01	6.02E-02
rpsM	1.46E-01	5.39E-02
rpsO	5.03E-02	1.43E-02
rpsR	5.32E-02	1.35E-02
rpsT	1.74E-01	4.68E-02
rpsU	1.33E-02	3.17E-03
rraA	2.96E-02	1.45E-02
rseA	2.09E-02	1.41E-02
rsmH	1.07E-02	1.03E-02
rsxG	7.03E-02	4.37E-02
rtcB	1.93E-01	2.43E-01
sbp	1.68E-01	1.72E-01
sdhA	5.47E-02	9.98E-02
secA	1.35E-01	3.84E-01
secB	3.22E-02	1.57E-02
secD	7.69E-02	1.43E-01

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
seqA	2.86E-02	1.61E-02
serA	8.59E-02	1.06E-01
serB	1.53E-02	1.50E-02
serC	1.24E-01	1.39E-01
serS	6.26E-02	8.53E-02
sfbA	3.17E-02	2.64E-02
sitA	1.44E-02	1.35E-02
skp	7.28E-02	3.63E-02
slt	7.55E-03	1.55E-02
slyA	6.32E-02	2.90E-02
slyB	6.54E-01	2.85E-01
smp	6.24E-02	4.22E-02
sodB	2.67E-01	1.60E-01
sodC1	4.90E-02	2.53E-02
solA	2.48E-02	2.83E-02
spy	3.98E-02	2.02E-02
sra	2.36E-01	3.54E-02
ssb	2.52E-02	1.34E-02
sspA	3.46E-02	2.34E-02
sthB	7.12E-03	1.84E-02
STM0032	9.74E-02	1.76E-01
STM0034	1.08E-02	8.82E-03
STM0080	5.08E-02	1.17E-02
STM0164	9.50E-03	7.42E-03
STM0276	3.13E-03	1.55E-03
STM0285	3.49E-02	1.41E-01
STM0327	1.25E-02	4.14E-03
STM0363	7.04E-01	7.43E-01
STM0402	1.02E-01	6.38E-02
STM0564	1.08E-02	1.43E-02
STM0896	2.19E-02	9.11E-03
STM0908	1.34E-02	6.81E-03
STM0918	3.89E-02	1.24E-01
STM1041	9.93E-02	3.02E-01
STM1078	2.44E-02	1.01E-02
STM1119	1.61E-01	9.40E-02
STM1133	2.58E-02	2.93E-02
STM1263	2.51E-01	1.73E-01
STM1541	4.73E-03	3.96E-03
STM1547	5.15E-02	2.62E-02
STM1558	1.36E-02	2.99E-02
STM1560	1.56E-02	2.88E-02
STM1607	3.46E-02	2.37E-02
STM1627	2.10E-02	2.34E-02
STM1633	1.13E-01	9.00E-02
STM1638	2.96E-02	1.30E-02
STM1672	2.63E-02	3.05E-02
STM1676	2.16E-02	1.96E-02
STM1731	9.66E-03	8.59E-03
STM1849	2.56E-02	6.79E-02
STM1857	1.48E-02	6.57E-03
STM1940	1.17E-02	1.78E-02
STM2008	1.22E-02	2.26E-02
STM2243	6.49E-02	5.12E-02
STM2341	1.95E-02	1.67E-02
STM2447	3.20E-02	1.85E-02
STM2475	3.57E-02	8.57E-03
STM2506	1.22E-02	2.54E-03
STM2594	1.49E-02	4.70E-02

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
STM2605	9.36E-03	1.36E-02
STM2722	3.40E-02	6.45E-02
STM2740	5.63E-03	6.58E-03
STM2744	6.10E-03	1.42E-03
STM2748	8.20E-03	3.05E-03
STM2797	3.54E-02	1.09E-02
STM3030	7.03E-03	4.56E-03
STM3138	2.76E-02	3.08E-02
STM3152	1.80E-02	2.96E-02
STM3154	3.63E-02	2.05E-02
STM3155	5.78E-02	2.49E-02
STM3533	1.20E-02	9.35E-03
STM3580	1.02E-01	5.66E-02
STM3766	4.56E-02	4.60E-02
STM3820	3.56E-02	5.18E-02
STM4208	1.83E-02	1.93E-02
STM4211	3.23E-02	7.66E-02
STM4212	3.47E-02	1.89E-02
STM4255	8.06E-03	9.18E-04
STM4302	1.24E-02	3.61E-03
STM4423	5.25E-03	4.62E-03
STM4489	6.68E-02	2.46E-01
STM4519	3.93E-02	5.52E-02
stpA	1.62E-01	7.02E-02
sucA	3.61E-02	1.06E-01
sucB	7.79E-02	9.54E-02
sucC	7.11E-02	8.27E-02
sucD	3.05E-02	2.56E-02
suhB	3.01E-02	2.45E-02
surA	8.65E-02	1.14E-01
surE	9.47E-03	7.15E-03
talA	4.82E-02	4.81E-02
talB	3.75E-01	3.70E-01
tar	9.16E-02	1.53E-01
tatB	2.36E-02	1.29E-02
tbpA	4.94E-02	5.03E-02
tcp	9.32E-02	1.54E-01
tdcE	4.37E-03	1.05E-02
tesA	1.31E-02	8.52E-03
tgt	1.70E-02	2.03E-02
thiC	5.78E-02	1.15E-01
thiD	4.18E-02	3.36E-02
thiE	2.73E-02	1.75E-02
thiG	1.87E-02	1.41E-02
thiM	4.62E-02	3.58E-02
thrA	2.36E-01	5.89E-01
thrC	2.32E-01	3.06E-01
thrS	1.01E-01	2.09E-01
tig	3.30E-01	4.43E-01
tktA	7.68E-02	1.55E-01
tktB	3.40E-02	6.94E-02
tmk	2.03E-02	1.34E-02
tolB	6.50E-02	8.37E-02
tolC	1.07E-01	1.60E-01
tpx	3.53E-01	1.80E-01
traN	3.17E-02	5.92E-02
traT	3.52E-01	2.58E-01
treA	4.34E-02	7.70E-02
trg	3.85E-02	6.27E-02

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
trkA	8.08E-02	1.13E-01
trmJ	4.34E-02	3.25E-02
trpA	2.91E-02	2.35E-02
trpB	4.23E-02	5.08E-02
trpC	1.69E-02	2.33E-02
trxA	4.20E-01	1.40E-01
trxB	2.47E-01	2.41E-01
tsf	4.47E-01	3.80E-01
tsr	4.54E-02	7.63E-02
tufA	5.82E+00	7.07E+00
typA	7.68E-02	1.45E-01
tyrS	3.20E-02	4.26E-02
ubiE	2.57E-02	2.02E-02
ubiG	6.91E-02	5.22E-02
ucpA	3.74E-02	2.93E-02
udp	3.10E-02	2.36E-02
ugpB	3.11E-02	4.21E-02
ung	1.49E-02	1.06E-02
upp	6.60E-02	4.16E-02
uspA	5.76E-02	2.59E-02
uspE	2.17E-02	2.15E-02
uspF	3.25E-01	1.43E-01
uspG	6.03E-02	2.68E-02
uxaC	1.16E-02	1.75E-02
vacB	4.31E-02	1.12E-01
vacJ	2.18E-02	1.73E-02
valS	1.22E-02	3.72E-02
wzzB	3.29E-02	3.33E-02
wzzE	4.11E-02	4.53E-02
xseB	6.51E-02	1.63E-02
yaaA	2.68E-02	2.23E-02
yadF	6.98E-02	4.86E-02
yaeH	1.03E-01	4.36E-02
yahO	2.41E-01	6.68E-02
yajG	2.62E-02	1.67E-02
yajQ	5.26E-02	2.69E-02
ybaB	5.20E-02	1.75E-02
ybaY	1.52E-01	8.27E-02
ybdH	2.84E-02	3.15E-02
ybeL	1.96E-02	1.02E-02
ybgI	6.77E-02	5.12E-02
ybgJ	3.31E-02	2.24E-02
ybhB	4.54E-02	2.16E-02
ybhC	1.65E-02	2.11E-02
ybiS	1.27E-01	1.19E-01
ybiT	2.28E-02	3.82E-02
ybjP	8.78E-02	4.67E-02
ybjY	8.35E-03	9.58E-03
ycbL	4.25E-02	2.87E-02
yceB	3.30E-02	1.92E-02
ycfF	6.89E-02	2.56E-02
ycgQ	3.25E-02	3.05E-02
ychF	6.19E-02	6.92E-02
yciE	4.06E-02	2.15E-02
yciF	4.60E-02	2.41E-02
yciK	6.16E-02	4.84E-02
ydbH	7.08E-03	1.93E-02
ydeI	1.95E-02	7.79E-03
ydfG	1.53E-01	1.16E-01

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
ydfZ	1.48E-02	3.03E-03
ydgA	6.25E-02	9.48E-02
ydgH	1.15E-01	1.10E-01
ydgH	3.95E-02	5.65E-02
ydgI	1.89E-01	4.34E-02
ydgJ	3.14E-02	3.60E-02
ydhD	2.48E-02	9.02E-03
ydhJ	2.70E-02	2.48E-02
ydiR	1.70E-02	1.60E-02
ydjA	7.37E-02	4.18E-02
ydjN	2.82E-02	3.83E-02
yeaG	2.89E-02	6.05E-02
yebC	1.84E-02	1.36E-02
yecM	1.14E-01	6.89E-02
yedD	4.31E-02	1.86E-02
yedF	8.80E-03	2.14E-03
yegS	4.25E-03	3.82E-03
yehZ	8.12E-02	7.45E-02
yeiP	1.38E-02	8.27E-03
yejK	1.72E-01	1.82E-01
yfbU	5.42E-02	2.96E-02
yfcZ	6.96E-02	2.07E-02
yfgD	3.41E-02	1.27E-02
yfgM	8.50E-02	5.26E-02
yfiA	8.24E-02	2.91E-02
ygaM	1.14E-01	3.99E-02
ygaP	5.25E-02	2.73E-02
ygaU	1.95E-01	8.78E-02
ygfZ	5.09E-02	5.14E-02
yggE	2.99E-02	2.20E-02
yggN	2.41E-02	1.78E-02
yggX	4.25E-02	1.30E-02
yghA	9.68E-02	8.54E-02
ygiC	3.08E-02	3.88E-02
ygiM	2.65E-02	1.69E-02
ygjR	3.86E-02	4.03E-02
yhbL	4.50E-02	2.90E-02
yhbN	5.43E-02	3.03E-02
yhbS	6.40E-02	3.31E-02
yhbW	6.71E-03	6.93E-03
yhcB	1.51E-01	6.44E-02
yhdH	3.77E-02	3.67E-02
yhgF	1.46E-02	3.50E-02
yhhA	4.11E-02	1.76E-02
yhjG	2.78E-02	5.81E-02
yhjJ	5.07E-02	7.81E-02
yiaD	1.54E-02	9.71E-03
yiaO	4.73E-03	4.82E-03
yibF	9.34E-02	5.95E-02
yibN	6.04E-02	2.65E-02
yicC	7.09E-02	6.60E-02
yidC	4.15E-02	7.14E-02
yihD	3.38E-02	9.80E-03
yihG	1.02E-02	1.03E-02
yiiG	7.41E-03	8.16E-03
yjeE	3.13E-02	1.48E-02
yjgM	6.29E-03	3.27E-03
yjjK	8.84E-02	1.54E-01
yliJ	1.21E-02	8.02E-03

Gene Name	*Average of Norm. (fmol)*	*Average of Norm. (ng)*
ymbA	1.42E-02	8.15E-03
yncB	3.47E-02	3.83E-02
yncE	3.66E-02	3.93E-02
ynhG	1.65E-02	1.66E-02

Table S10. Proteomics: Proteins in Δ*fnr*.

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
accA	4.61E-02	4.85E-02
accB	8.77E-02	4.29E-02
accC	1.39E-01	2.04E-01
accD	2.90E-02	2.81E-02
aceE	2.06E-01	6.01E-01
aceF	1.26E-01	2.45E-01
ackA	1.37E-01	1.75E-01
acnA	3.98E-02	1.15E-01
acnB	4.46E-02	1.21E-01
acpP	3.34E-01	8.65E-02
acrA	2.04E-01	2.57E-01
acrB	8.67E-02	2.84E-01
acs	4.97E-02	1.08E-01
adhE	3.16E-01	8.94E-01
adhP	1.03E-01	1.09E-01
adk	9.74E-02	6.62E-02
ahpC	1.20E-01	7.32E-02
ahpF	4.51E-02	7.32E-02
alaS	1.65E-01	4.57E-01
aldB	7.27E-02	1.24E-01
apbC	5.07E-02	5.87E-02
argA	7.80E-02	1.12E-01
argB	7.00E-02	5.47E-02
argD	7.26E-02	9.42E-02
argG	7.42E-02	1.08E-01
argH	1.10E-01	1.62E-01
argI	2.66E-02	2.94E-02
argT	4.05E-02	3.39E-02
arnB	4.15E-02	5.03E-02
arnC	5.42E-02	5.86E-02
aroB	5.40E-02	6.32E-02
aroF	1.96E-02	2.21E-02
artI	5.86E-02	4.65E-02
artJ	2.98E-02	2.31E-02
asd	2.01E-01	2.36E-01
asnS	2.11E-01	3.28E-01
aspA	7.34E-02	1.12E-01
aspC	4.59E-02	5.90E-02
aspS	5.27E-02	1.00E-01
atpA	2.32E-01	3.75E-01
atpD	5.32E-01	7.85E-01
atpF	3.57E-02	1.85E-02
atpG	7.65E-02	6.99E-02
atpH	7.78E-02	4.48E-02
bamA	1.29E-01	3.40E-01
bamB	1.30E-01	1.61E-01
bcfG	3.67E-02	2.90E-02
bcp	2.03E-01	1.06E-01
bfr	1.30E-01	7.01E-02
bioA	3.48E-02	4.82E-02
bioB	4.99E-02	5.66E-02

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
bioD1	2.04E-01	1.47E-01
btuE	5.26E-02	3.16E-02
carA	9.47E-02	1.17E-01
carB	1.18E-01	4.14E-01
cbiF	8.71E-02	7.16E-02
cbiL	4.57E-02	3.45E-02
cdh	8.69E-02	7.33E-02
clpB	2.15E-01	6.04E-01
clpX	6.65E-02	9.28E-02
coaE	1.31E-02	8.93E-03
copA	2.15E-02	5.49E-02
cra	2.76E-02	3.04E-02
crl	1.05E-02	5.02E-03
crr	9.20E-01	4.96E-01
cspA	2.77E-01	6.04E-02
cspC	9.22E-01	2.02E-01
cspD	5.11E-02	1.22E-02
cspE	2.62E-01	5.74E-02
cspJ	1.08E-01	2.48E-02
cypD	1.67E-01	3.35E-01
cysA	4.34E-02	5.24E-02
cysE	4.61E-02	4.06E-02
cysI	3.89E-02	7.35E-02
cysJ	4.74E-02	9.32E-02
cysK	2.19E-01	2.21E-01
cysM	1.17E-02	1.10E-02
cysN	5.84E-02	9.14E-02
cysP	5.24E-02	5.80E-02
dacC	2.75E-02	3.47E-02
damX	1.32E-01	1.77E-01
dapA	5.31E-02	4.85E-02
dapB	9.11E-01	7.90E-01
dapD	1.04E-01	9.16E-02
def	4.39E-02	2.55E-02
degP	1.79E-01	2.62E-01
degQ	8.45E-02	1.19E-01
deoA	3.28E-02	4.54E-02
deoB	3.62E-02	4.65E-02
deoD	6.41E-02	4.90E-02
dkgA	8.34E-02	7.71E-02
dkgB	2.20E-02	1.85E-02
dksA	1.29E-01	6.72E-02
dmsA	3.77E-02	1.03E-01
dnaB	4.97E-02	7.56E-02
dnaJ	4.38E-02	5.29E-02
dnaK	6.40E-01	1.31E+00
dnaN	3.36E-02	3.96E-02
dppA	9.44E-02	1.68E-01
dppD	1.71E-02	1.78E-02
dps	2.43E-01	1.34E-01
dsbA	5.86E-02	3.94E-02
dsbC	2.75E-02	2.07E-02
ecnB	9.84E-01	1.40E-01
eda	3.92E-02	2.62E-02
elaB	8.25E-02	2.86E-02
eno	9.55E-01	1.27E+00
entB	4.86E-02	4.61E-02
erfK	4.19E-02	4.29E-02
erpA	6.11E-02	2.25E-02

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
fabA	6.70E-02	3.76E-02
fabB	3.10E-01	3.93E-01
fabD	1.40E-01	1.32E-01
fabF	8.17E-02	1.05E-01
fabG	1.41E-01	1.04E-01
fabH	3.13E-02	3.11E-02
fabI	8.48E-02	6.92E-02
fadL	8.60E-02	1.18E-01
fba	7.39E-02	8.40E-02
fbaB	1.34E+00	1.52E+00
fbp	5.86E-02	6.38E-02
fdhE	8.16E-02	8.28E-02
fdx	6.93E-02	2.56E-02
ffh	4.81E-02	7.01E-02
fhuA	1.86E-01	4.45E-01
fklB	1.34E-01	9.35E-02
fkpA	6.10E-02	5.19E-02
fldA	5.65E-02	3.35E-02
flgE	1.71E-02	2.16E-02
flgK	7.18E-02	1.23E-01
flgL	5.44E-02	5.56E-02
fliC	8.47E-02	1.29E-01
fliE	1.35E-02	4.47E-03
fliY	3.55E-02	3.01E-02
fljB	3.76E-02	5.91E-02
frdA	6.13E-02	1.17E-01
frdB	3.56E-02	2.85E-02
frr	1.51E-01	9.18E-02
ftn	5.53E-02	3.08E-02
ftsH	9.28E-02	1.95E-01
ftsZ	1.94E-01	2.32E-01
fumC	7.48E-02	1.09E-01
fusA	7.47E-01	1.70E+00
gabT	2.59E-02	3.45E-02
galF	7.87E-02	7.53E-02
galK	2.57E-02	3.10E-02
galU	1.25E-01	1.21E-01
gapA	1.36E+00	1.44E+00
gatY	4.91E-02	4.58E-02
gcvH	9.80E-02	4.08E-02
gcvP	5.16E-02	1.60E-01
ggt	2.55E-02	4.54E-02
glgA	4.54E-02	7.09E-02
glk	3.99E-02	4.07E-02
glmM	8.26E-02	1.14E-01
glnA	1.70E-01	2.59E-01
glnH	7.54E-02	6.07E-02
glpA	5.71E-02	9.86E-02
glpD	9.08E-02	1.52E-01
glpK	1.58E-01	2.62E-01
glpQ	1.22E-01	1.47E-01
glpR	6.96E-02	5.62E-02
gltA	6.18E-02	8.78E-02
gltB	7.38E-02	3.50E-01
gltD	4.29E-02	6.79E-02
gltI	7.51E-02	7.54E-02
glyA	2.06E-01	2.77E-01
glyQ	9.50E-02	9.60E-02
glyS	2.12E-01	4.87E-01

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
gnd	1.05E-01	1.59E-01
gpmA	8.47E-02	7.09E-02
gppA	1.23E-01	1.97E-01
greA	8.22E-02	4.36E-02
groL	2.00E+00	3.38E+00
groS	1.77E+00	5.37E-01
grpE	7.57E-02	4.88E-02
grxA	4.20E-02	1.22E-02
grxB	3.00E-01	2.16E-01
grxC	3.20E-02	8.59E-03
gst	3.57E-02	2.36E-02
guaA	8.78E-02	1.53E-01
guaB	1.10E-01	1.69E-01
gyrB	4.06E-02	1.06E-01
hemL	5.83E-02	7.84E-02
hemX	1.07E-01	1.32E-01
hemY	7.41E-02	9.99E-02
hflC	1.22E-01	1.35E-01
hflK	1.73E-01	2.35E-01
hisA	2.86E-02	2.21E-02
hisB	6.82E-02	8.09E-02
hisC	1.18E-01	1.40E-01
hisD	5.71E-02	7.82E-02
hisG	1.33E-01	1.32E-01
hisH	5.48E-02	3.47E-02
hisJ	2.35E-01	1.98E-01
hldD	1.46E-02	1.48E-02
hldE	5.80E-02	8.75E-02
hns	5.94E-01	2.70E-01
hslU	4.45E-01	6.60E-01
htpG	2.78E-01	5.84E-01
hupA	1.33E+00	3.71E-01
icdA	1.30E-01	1.77E-01
ihfA	2.27E-01	7.54E-02
ileS	4.60E-02	1.41E-01
ilvC	1.55E-01	2.48E-01
ilvD	1.12E-01	2.18E-01
ilvE	1.37E-01	1.39E-01
infB	8.75E-02	2.50E-01
infC	4.98E-02	3.08E-02
iraP	3.61E-02	1.07E-02
iroN	5.06E-02	1.20E-01
iscR	1.24E-01	6.39E-02
ispG	7.57E-02	9.06E-02
katE	8.77E-02	2.12E-01
kdgR	4.15E-02	3.65E-02
kdsA	9.04E-02	8.39E-02
kdsD (yrbH)	2.30E-02	2.35E-02
ldhA	5.42E-02	5.76E-02
leuB	4.80E-02	5.65E-02
leuC1	8.10E-02	1.20E-01
leuD1	1.07E-01	7.07E-02
leuS	6.02E-02	1.74E-01
livJ	2.38E-01	2.72E-01
lolB	3.41E-02	2.34E-02
lolD	6.02E-03	4.45E-03
lon	7.64E-02	1.97E-01
lpdA	8.13E-02	1.22E-01
lpfB	4.61E-02	3.52E-02

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
lpp1	8.13E-01	2.03E-01
lpp2	3.15E-03	8.07E-04
lptD	7.24E-02	1.92E-01
lptE	4.08E-02	2.53E-02
lsrB	4.26E-02	4.66E-02
lsrF	3.28E-02	3.03E-02
luxS	9.49E-02	5.54E-02
lysS	8.52E-02	1.44E-01
maeB	7.94E-02	1.90E-01
manA	3.16E-02	3.96E-02
manX	1.60E-01	1.65E-01
manY	2.96E-01	2.41E-01
mdh	6.23E-01	5.99E-01
mdoG	3.80E-02	6.35E-02
metA	4.42E-02	4.61E-02
metE	2.33E-01	5.86E-01
metG	1.02E-01	2.30E-01
metH	2.61E-02	1.03E-01
metK	3.85E-02	4.77E-02
metL	5.18E-02	1.39E-01
metN1	1.04E-01	1.18E-01
metQ	5.95E-01	5.17E-01
mglB	3.69E-02	3.81E-02
minD	1.91E-01	1.67E-01
mipA	2.62E-01	2.15E-01
mlc	2.36E-02	3.03E-02
moaB	4.77E-02	2.57E-02
modA	2.63E-02	2.09E-02
moeA	4.74E-02	6.35E-02
mppA	2.60E-02	4.49E-02
mreB	4.90E-02	5.36E-02
msbA	4.58E-02	8.52E-02
msrA	7.15E-02	4.97E-02
mtlA	5.03E-02	1.01E-01
mukB	3.63E-02	1.79E-01
murA	8.02E-02	1.08E-01
murG	1.02E-02	1.12E-02
mutT	1.03E-02	4.67E-03
nanE2	2.62E-02	1.84E-02
ndk	1.01E-01	4.64E-02
nfuA	5.30E-02	3.24E-02
nifU	1.98E-01	8.30E-02
nlpB	1.66E-01	1.81E-01
nlpD	1.00E-01	1.18E-01
nrdA	2.86E-02	7.14E-02
nrdB	2.99E-02	3.79E-02
nrdI	1.90E-02	8.77E-03
nusA	7.28E-02	1.18E-01
nusG	4.95E-02	3.05E-02
ompA	1.15E+01	1.27E+01
ompC	7.89E-01	9.61E-01
ompD	4.85E-01	5.65E-01
ompF	5.59E-01	6.55E-01
ompR	7.44E-02	5.89E-02
ompX	7.53E-01	4.11E-01
oppA	7.32E-02	1.32E-01
oppF	2.07E-02	2.25E-02
osmC	2.19E-01	9.72E-02
osmE	2.37E-01	8.71E-02

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
osmY	1.34E+00	8.45E-01
pagC	6.15E-01	3.67E-01
pagN	1.54E-01	1.16E-01
pal	3.70E-01	2.06E-01
parB	2.74E-02	2.97E-02
pckA	1.56E-01	2.76E-01
pdgL	8.70E-02	7.50E-02
pduA	2.02E-01	5.63E-02
pduB	4.05E-01	2.87E-01
pduC	2.88E-01	5.13E-01
pduD	2.99E-01	2.13E-01
pduE	3.55E-01	1.99E-01
pduJ	4.78E-01	1.28E-01
pduL	8.78E-02	5.88E-02
pduO	6.67E-02	7.38E-02
pduP	1.29E-01	1.87E-01
pduQ	3.16E-02	3.64E-02
pduS	2.60E-02	3.73E-02
pepA	3.30E-02	5.47E-02
pepD	7.78E-02	1.22E-01
pepQ	5.64E-02	8.50E-02
pfkA	1.67E-01	1.70E-01
pfkB	8.23E-02	7.94E-02
pflB	1.01E-01	2.54E-01
pgi	1.49E-02	2.74E-02
pgk	9.39E-01	1.14E+00
pgl	6.25E-02	6.85E-02
pgm	7.51E-02	1.28E-01
phoN	2.84E-01	2.39E-01
phoP	4.21E-01	3.19E-01
pnp	1.23E-01	2.80E-01
potD	6.69E-02	7.62E-02
potF	9.13E-02	1.10E-01
ppc	3.19E-02	9.15E-02
pphB	1.03E-01	7.78E-02
pps	1.11E-01	2.86E-01
prc	2.51E-02	5.57E-02
prfB	3.81E-02	4.60E-02
prgJ	4.48E-03	1.47E-03
priB	7.03E-02	2.45E-02
prlC	8.30E-02	1.85E-01
proA	4.51E-02	6.08E-02
proC	3.54E-02	3.00E-02
proQ	5.51E-02	4.07E-02
proS	4.21E-02	7.86E-02
proX	4.82E-02	5.23E-02
prr	4.23E-02	6.30E-02
prs	1.59E-01	1.62E-01
pspA	3.20E-02	2.44E-02
pssA	3.80E-02	5.81E-02
pstS	1.40E-01	1.54E-01
pta	7.46E-02	1.67E-01
ptsG	6.63E-02	9.69E-02
ptsH	5.59E-01	1.50E-01
ptsI	2.16E-01	4.02E-01
ptsN	1.10E-01	5.90E-02
purA	5.70E-02	7.83E-02
purB	7.49E-02	1.12E-01
purE	2.42E-02	1.29E-02

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
purH	5.93E-02	9.90E-02
purT	4.90E-02	6.23E-02
purU	4.70E-02	4.50E-02
PYGM	1.91E-01	5.11E-01
pykA	1.21E-01	1.84E-01
pykF	2.85E-01	4.26E-01
pyrC	7.04E-02	8.21E-02
pyrF	5.76E-03	4.57E-03
pyrI	3.67E-02	1.91E-02
qor	1.41E-01	1.45E-01
rbsB	5.16E-02	4.66E-02
rcsB	5.79E-02	4.11E-02
recA	1.17E-01	1.33E-01
rfaQ	2.40E-02	2.70E-02
rfbB	1.66E-02	2.03E-02
rfbC	4.46E-02	2.70E-02
rhaM	1.94E-02	7.18E-03
rho	1.53E-01	2.11E-01
ribE	1.09E-01	7.55E-02
ribH	5.11E-02	2.43E-02
ridA	1.31E-01	5.22E-02
rlmN (yfgB)	3.09E-02	3.92E-02
rne	5.41E-02	1.87E-01
rodZ	4.78E-02	5.04E-02
rof	1.41E-01	4.14E-02
rpiA	1.41E-01	9.49E-02
rplA	1.60E+00	1.17E+00
rplB	5.08E-01	4.50E-01
rplC	3.46E-01	2.27E-01
rplD	4.28E-01	2.77E-01
rplE	7.27E-01	4.36E-01
rplF	7.70E-01	4.29E-01
rplI	8.69E-01	4.03E-01
rplJ	3.52E-01	1.83E-01
rplK	4.76E-01	2.08E-01
rplL	9.12E-01	3.31E-01
rplM	1.15E-01	5.42E-02
rplN	3.83E-01	1.54E-01
rplO	4.47E-01	1.97E-01
rplP	1.37E-01	6.09E-02
rplQ	1.70E-01	7.14E-02
rplS	2.53E-01	9.77E-02
rplU	4.09E-01	1.39E-01
rplV	1.52E-01	5.36E-02
rplW	1.59E-01	5.24E-02
rplX	3.77E-02	1.25E-02
rpmB	6.45E-02	1.70E-02
rpmD	5.29E-02	9.94E-03
rpmE2	5.99E-02	1.70E-02
rpmF	3.51E-02	6.77E-03
rpoA	2.05E-01	2.23E-01
rpoB	3.09E-01	1.37E+00
rpoC	3.67E-01	1.68E+00
rpoD	3.65E-02	7.74E-02
rpoE	5.98E-02	3.76E-02
rpoZ	8.91E-02	2.63E-02
rpsA	4.08E-01	7.36E-01
rpsB	9.60E-01	7.57E-01
rpsC	7.29E-01	5.57E-01

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
rpsD	3.49E-01	2.41E-01
rpsE	7.77E-01	4.00E-01
rpsF	1.19E-01	5.30E-02
rpsG	1.70E-01	8.85E-02
rpsH	4.23E-01	1.73E-01
rpsI	1.03E-01	4.51E-02
rpsJ	6.92E-01	2.40E-01
rpsK	2.02E-01	8.30E-02
rpsL	1.49E-01	6.24E-02
rpsM	1.12E-01	4.36E-02
rpsR	6.46E-02	1.70E-02
rpsU	2.78E-02	7.13E-03
rraA	5.12E-02	2.68E-02
rseB	1.38E-02	1.49E-02
rsmG (gidB)	2.71E-02	1.82E-02
rtcB	2.52E-02	3.29E-02
secB	9.37E-02	4.85E-02
secD	7.45E-02	1.43E-01
secG	4.50E-02	1.48E-02
serA	9.96E-02	1.30E-01
serC	1.02E-01	1.20E-01
serS	3.32E-02	4.69E-02
sifB	1.74E-02	1.84E-02
sitA	9.96E-02	9.95E-02
sitB	2.37E-02	2.05E-02
skp	7.27E-02	3.89E-02
slyB	1.14E+00	5.15E-01
sodA	7.35E-02	5.08E-02
sodB	2.30E-01	1.43E-01
sohB	4.89E-02	5.51E-02
sopB	2.07E-02	3.71E-02
spy	1.71E+00	9.02E-01
sra	1.05E-01	1.67E-02
ssb	1.20E-02	6.86E-03
sseA	3.64E-02	3.24E-02
sspA	1.88E-02	1.37E-02
sspB	3.88E-02	2.12E-02
STM0080	8.74E-02	2.11E-02
STM0098	2.37E-02	1.61E-02
STM0297	7.14E-02	1.54E-02
STM0402	7.84E-02	5.21E-02
STM0856	4.50E-02	4.34E-02
STM0908	2.07E-02	1.10E-02
STM0951	1.88E-02	1.70E-02
STM1119	3.19E-01	1.96E-01
STM1239	9.49E-03	1.18E-02
STM1263	1.25E-01	9.10E-02
STM1267	2.02E-02	5.72E-03
STM1607	3.14E-02	2.22E-02
STM1633	9.86E-02	8.26E-02
STM1672	3.42E-02	4.10E-02
STM1731	5.90E-02	5.55E-02
STM1858	1.57E-02	4.86E-03
STM2243	1.91E-02	1.55E-02
STM2447	6.14E-02	3.73E-02
STM2475	9.17E-02	2.36E-02
STM2506	7.75E-03	1.67E-03
STM2722	7.82E-02	1.52E-01
STM3256	3.58E-02	3.13E-02

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
STM3354 *(ttdB)*	1.62E-02	1.12E-02
STM3580	1.26E+00	7.42E-01
STM3754	7.31E-03	2.96E-03
STM4033	1.46E-02	4.01E-03
STM4057	4.23E-02	1.88E-02
STM4498	2.41E-02	1.58E-02
STM4519	1.01E-01	1.50E-01
STM4520	5.04E-01	7.91E-02
stpA	1.75E-01	7.92E-02
sucA	4.38E-02	1.33E-01
sucB	1.23E-01	1.59E-01
sucC	5.04E-02	6.23E-02
sucD	4.84E-02	4.20E-02
suhB	2.20E-02	1.86E-02
surA	9.40E-02	1.30E-01
surE	1.97E-02	1.54E-02
talA	1.04E-01	1.09E-01
talB	2.44E-01	2.53E-01
tar	2.44E-02	4.20E-02
tbpA	7.83E-02	8.36E-02
tcp	1.47E-02	2.60E-02
thiC	4.89E-02	1.01E-01
thiD	1.98E-02	1.70E-02
thiM	8.70E-02	7.00E-02
thrA	2.84E-01	7.47E-01
thrC	4.49E-01	6.26E-01
tig	4.04E-01	5.72E-01
tktA	1.57E-01	3.40E-01
tktB	9.05E-02	1.98E-01
tolB	1.30E-01	1.77E-01
tolC	1.31E-01	2.07E-01
topA	2.82E-02	7.99E-02
tpx	3.41E-01	1.81E-01
traM	5.98E-02	2.57E-02
traT	2.28E-01	1.75E-01
treA	9.00E-02	1.69E-01
trmJ	2.18E-02	1.69E-02
trpA	4.58E-02	3.81E-02
trxA	3.83E-01	1.35E-01
trxB	2.16E-01	2.21E-01
tsf	4.42E-01	3.95E-01
tufA	4.51E+00	5.73E+00
typA	1.02E-01	2.03E-01
tyrS	5.97E-02	8.41E-02
ubiB (fre)	2.72E-02	4.98E-02
ubiG	7.93E-02	6.29E-02
udp	3.32E-02	2.61E-02
ugpB	4.71E-02	6.71E-02
umuC	2.06E-02	2.88E-02
upp	5.43E-02	3.59E-02
uspF	3.13E-01	1.44E-01
uspG	1.82E-01	8.57E-02
uxaC	1.60E-02	2.49E-02
vacB	3.32E-02	8.88E-02
valS	3.25E-01	1.06E+00
wzzB	5.77E-02	6.15E-02
wzzE	5.89E-02	6.84E-02
yaaA	1.87E-02	1.61E-02
yadF	4.90E-02	3.61E-02

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
yaeH	9.51E-02	4.25E-02
yahO	3.24E-01	9.42E-02
yajG	4.65E-02	3.07E-02
yajQ	1.08E-01	5.82E-02
ybaB	6.71E-02	2.34E-02
ybaY	5.13E-01	2.95E-01
ybgI	1.52E-01	1.19E-01
ybgJ	2.72E-02	1.90E-02
ybhB	4.56E-02	2.30E-02
ybhG	2.73E-02	2.87E-02
ybiS	4.82E-02	4.70E-02
ybiT	2.61E-02	4.50E-02
ybjP	1.01E-01	5.66E-02
yceI	4.23E-02	2.66E-02
ycfF	6.23E-02	2.42E-02
ycfP	1.21E-01	7.73E-02
ycgL	1.25E-02	4.72E-03
ycgQ	5.66E-02	5.48E-02
yciE	2.83E-02	1.55E-02
yciF	4.39E-01	2.43E-01
yciO	1.33E-02	9.29E-03
ydeI	1.40E-02	5.81E-03
ydfG	1.12E-01	8.85E-02
ydfI	1.07E-02	1.67E-02
ydgA	6.16E-02	9.87E-02
ydgH	8.30E-02	8.27E-02
ydgJ	1.79E-02	2.13E-02
ydhD	8.77E-02	3.31E-02
ydjA	9.25E-02	5.50E-02
ydjN	1.19E-01	1.67E-01
yeaG	5.85E-02	1.26E-01
yebE	5.56E-02	3.95E-02
yedD	3.25E-02	1.47E-02
yedF	6.74E-03	1.76E-03
yedJ	4.32E-02	3.28E-02
yehZ	6.70E-02	6.44E-02
yfcZ	9.33E-02	2.99E-02
yfeR	1.69E+00	1.66E+00
yfgD	5.92E-02	2.38E-02
yfgM	8.87E-02	5.80E-02
yfiA	9.84E-02	3.73E-02
yfiO	5.25E-02	4.38E-02
ygaM	1.41E-01	5.14E-02
ygaU	4.73E-01	2.26E-01
ygdI	2.31E-01	5.58E-02
ygfZ	6.04E-02	6.41E-02
yggE	7.20E-02	5.71E-02
yggN	2.55E-02	1.94E-02
yggX	5.44E-02	1.78E-02
yghA	2.03E-01	1.90E-01
ygiC	4.47E-02	5.84E-02
ygiM	2.22E-02	1.47E-02
yhaK	1.45E-02	1.14E-02
yhbG	9.51E-03	7.38E-03
yhbL	7.49E-02	5.20E-02
yhbO	8.64E-02	4.90E-02
yhbS	9.69E-03	5.39E-03
yhcB	1.36E-01	6.07E-02
yhgF	1.35E-01	3.44E-01

Gene Name	*Average of Norm (fmol)*	*Average of Norm. (ng)*
yhhA	3.80E-02	1.68E-02
yhjB	1.66E-02	1.08E-02
yhjG	4.57E-02	9.87E-02
yiaD	9.67E-03	6.47E-03
yibF	2.35E-02	1.54E-02
yibL	3.79E-02	1.51E-02
yibN	1.07E-01	4.84E-02
yicC	7.86E-02	7.60E-02
yidC	9.62E-02	1.76E-01
yieF	5.98E-02	3.74E-02
yigI	3.42E-02	1.69E-02
yjeE	3.57E-02	1.78E-02
yjeJ	2.77E-02	2.63E-02
yjgB	2.61E-02	2.87E-02
yjjK	1.26E-01	2.32E-01
ymbA	6.77E-03	4.18E-03
yncB	3.43E-02	3.95E-02
yncE	4.51E-02	5.10E-02
ynfD	4.72E-02	1.45E-02
yniC	3.75E-02	2.65E-02
yqhD	3.66E-02	4.45E-02
yqiC	5.57E-02	2.32E-02
yqiK	1.57E-01	2.79E-01
yqjC	6.64E-02	2.82E-02
yqjD	3.06E-01	1.02E-01
yraM	3.34E-02	7.01E-02
yraP	9.50E-02	5.65E-02
yrbC	6.66E-02	4.78E-02
yrbD	1.77E-02	1.02E-02
yrfE	1.57E-02	1.02E-02
ysgA	2.36E-02	2.06E-02
ytfM	1.27E-01	2.37E-01
ytfN	3.69E-02	1.45E-01
znuA	3.42E-01	3.66E-01
zwf	1.79E-02	2.90E-02