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Viable But Non-Culturable (VBNC) state in Salmonella Typhimurium

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Viable But Non-Culturable (VBNC) state in *Salmonella* Typhimurium

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Abstract

Salmonella enterica serovar Typhimurium is a dangerous food-borne pathogen that causes severe diseases like gastroenteritis and septicaemia. It causes over 800,000 deaths p.a. globally. The majority of the cases are caused by contaminated food. Culturing is still a gold standard to detect bacteria in food, clinical and other samples and this approach assumes that those bacteria present in the samples, which are capable of causing disease, are culturable. There are numerous reports of bacteria entering the viable but non-culturable (VBNC) state, a state where bacteria exhibit loss of culturability under routine culturing conditions despite being alive, when placed under stress conditions. The VBNC state raises questions about the effectiveness of bacterial culturability as a tool for bacterial surveillance. The mechanism(s) of entry into, and maintenance of, the VBNC state is not very well understood. Bacteria can be 'resuscitated' from this state of lowered metabolic activity in the presence of certain 'cues' thus regaining the lost metabolic activity.

To study the VBNC state in *S. Typhimurium*, a reliable and reproducible method was established to generate VBNC *S. Typhimurium*. The bacteria were subjected to an infection- and environment-relevant oxidative stress, using hydrogen peroxide. The non-culturability of the bacteria was established using routinely used media that support *S. Typhimurium* growth. Further, the viability of the 'non-culturable' population was studied using confocal microscopy by Live/Dead™ staining and flow cytometric analysis. Drug tolerance of the VBNC *S. Typhimurium* was revealed. Finally, the ultrastructure of the VBNC bacteria was compared with actively dividing bacteria using a transmission electron microscopy (TEM).

After confirming and characterising the *S. Typhimurium* in the VBNC state, the transcriptome (by RNA-sequencing analysis) of the culturable and VBNC *S. Typhimurium* were compared. Gene knockouts were generated based on the RNA-seq data and the ability of the mutants to form VBNC cells was tested. The proteome of VBNC *S. Typhimurium* was analysed by mass-spectrometry. Unlike the large number of transcriptional changes, the proteome was relatively unaffected, suggesting that translation was reduced. Experiments

were then conducted to study the ribosomal content, protein synthesis and ribosomal degradation in the VBNC *S. Typhimurium* and a possible mechanism of VBNC entry, based on ribosomal dissolution, was proposed based on these observations.

Lastly, the pathogenicity of the VBNC *S. Typhimurium* was tested by using a highly sensitive murine intravenous infection model. VBNC *S. Typhimurium* were used to infect the mice and their growth phenotype was resuscitated *in vivo*. The infection with VBNC *S. Typhimurium* was not found to be lethal out to 20 days post-infection in C57BL/6 mice, or in two mice immunodeficient mice strains that were tested (RAG2 gamma-c chain double knockout mice and INF- γ knockout mice) indicating a loss of virulence in the *in vivo* resuscitated VBNC bacteria. Unlike the attenuated infection seen in animals inoculated with VBNC, reisolated 'VBNC' *S. Typhimurium* regained their virulence in the subsequent infections, causing mortality.

This study describes a reproducible method to drive *S. Typhimurium* into non-culturability without complete loss of virulence and discusses various characteristics of VBNC *S. Typhimurium*. The transcriptomic and proteomic profiles of oxidative stress induced VBNC *S. Typhimurium* have been examined. A possible ribosome-related mechanism of entry into the VBNC state is proposed based on the transcriptome, proteome and mutagenesis studies. Finally, the virulence of the VBNC *S. Typhimurium* was tested in normal and immune-deficient mice. These observations help in gaining insights into the VBNC formation and virulence in *S. Typhimurium*.

Declaration

I hereby declare that, to the best of my knowledge, this thesis comprises only my original work, except where due acknowledgments have been made. The research contributions in this work are my own and all those whom I worked in collaboration are acknowledged. The material in this thesis is not submitted elsewhere for any other degree(s).

.....

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Preface

Conference abstract

Karthik Pallela, Newton G. Peres, Nancy Wang, Mark Davies, Trevor Lithgow, Richard A. Strugnell (2017). *In vitro* induction of *Salmonella* Typhimurium into viable and non-culturable state, characterisation and its relevance to infection. Lorne Infection and Immunity, Lorne, Victoria, Australia.

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List of abbreviations

<u>Abbreviation</u>	<u>Expanded form</u>
°C	Degrees Celsius
(p)ppGpp	Guanosine penta-phosphate
ppGpp	Guanosine tetra-phosphate
Δ	Indicates gene deletion/knockout
μl	microlitres
AODC	Acridine orange direct counting
AOS	Anti-oxidative stress
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
BHI	Brain-Heart infusion
bp	Basepair
CAA	Chloroacetamide
CFU	Colony forming units
DAPI	4',6-diamidino-2-phenylindole
DEG	Differentially expressed genes
DVC	Direct viable count
DTT	Dithiothreitol
dNTPs	deoxyribonucleotide triphosphates

FA	Fatty acid
FACS	Fluorescent-activated cell sorting
FITC	Fluorescein isothiocyanate
FC	Fold change
FDR	False discovery rate
FSC	Forward scatter
GO	Gene ontology
H ₂ O ₂	Hydrogen peroxide
HRP	Horseradish peroxidase
i.v.	Intravenous
INT	Iodonitrotetrazolium chloride
iNTP	Initiating nucleotide triphosphate
IPTG	Isopropyl β-D-1-thiogalactopyranoside
LB	Lysogeny broth
MH	Muller-Hinton (broth)
ml	millilitres
mM	millimolar
O/N	Overnight
OD	Optical density
ODS	Octadecylsilane

OM	Outer membrane
OMP	Outer membrane protein
PCR	Polymerase chain reaction
PI	Propidium iodide
qRT-PCR	Quantitative RT-PCR
PAGE	Polyacrylamide gel electrophoresis
PMA	Propidium monoazide
PVDF	Polyvinylidene difluoride
RFU	Relative fluorescent units
RIN	RNA integrity number
RPKM	Reads per kilobase million
ROS	Reactive oxygen species
RT-PCR	Reverse transcriptase polymerase chain reaction
rpm	Revolutions per minute
SDS	Sodium dodecyl sulphate
SPI	<i>Salmonella</i> pathogenicity island
ST	<i>Salmonella</i> Typhimurium
SSC	Side scatter
T3SS	Type-3 secretion system
TA	Toxin-antitoxin

TCA	Tricarboxylic acid
TEM	Transmission electron microscope
TCEP	tris(2-carboxyethyl)phosphine
TFE	Trifluoroethanol
TS	Tryptic soy (broth)
v/v	Volume per volume
vPCR	Viability PCR
VBNC	Viable but nonculturable
w/v	Weight per volume
w/w	Weight per weight
WT	Wild Type

CHAPTER 1

General Introduction

1.1 *Salmonella enterica*

Salmonella spp. are Gram-negative, rod-shaped, motile, non-sporulating pathogenic bacteria¹. *Salmonella enterica* has been classified into over 2,600 serovars, with many still understudied regarding their pathogenicity². Serovars like *Salmonella enterica* serovar Typhi cause typhoid fever and *Salmonella enterica* serovar Paratyphi cause paratyphoid fever³. These are referred to as typhoidal *Salmonella* that cause enteric fever⁴. Typhoid fever is highly endemic to most countries in Africa, south-east Asia, China, and countries of the Indian subcontinent, which include India, Pakistan, Bangladesh, Nepal and Sri Lanka^{3,5}. Reports show a strong correlation between disease and water contaminated by human faeces, suggesting that *S. Typhi* is spread through contaminated water, however culturing the pathogen from the environment poses some challenges⁶.

Salmonella enterica serovar Typhimurium (henceforth called *Salmonella* Typhimurium) causes non-typhoidal gastroenteritis in humans⁷ and a newly recognized syndrome, especially in sub-Saharan Africa, called invasive Non-Typhoidal Salmonellosis (iNTS). *Salmonella* Typhimurium typically results in a self-limiting gastroenteritis⁸ causing an estimated 93 million reported cases of infection and 155,000 deaths per year⁹. *Salmonella* Typhimurium is one of the top four agents of diarrheal diseases according to World Health Organisation (WHO) reports¹⁰. *S. Typhimurium* can transmit from person to person via the faecal-oral route¹, and through consumption of contaminated food including chicken and other meats¹¹, fruit and vegetables¹². While food is usually processed and stored to prevent bacterial contamination, the procedures of processing, storage and preservation may have the opposite effect on some pathogens^{13,14}. Pathogens including *Salmonella enterica* have developed mechanisms to survive the physiochemical stresses induced during the food processing and storage procedure¹⁵.

Bacteria can adopt different states of dormancy including sporulation, quiescence or persistence when they encounter stress¹⁶. Some Gram-positive bacteria are known to form bacterial endospores¹⁷. 'Persisters', which are an antibiotic-tolerant population, have been observed among both Gram-positive and Gram-negative bacteria¹⁸⁻²¹. The common

phenotype for both spores and persisters includes a lowered metabolism and decreased cell division^{22,23}. Bacteria are known to adopt these states during environmental stresses such as starvation, antibiotics and desiccation²⁴⁻²⁶.

In 1982, a new state of dormancy was reported in bacteria by Xu *et al.*, which was termed the Viable But Nonculturable state (VBNC)²⁷. This state of dormancy was shown to occur in response to starvation in *Escherichia coli* and *Vibrio cholerae* where the bacteria lost their ability to grow on the conventional media on which they usually grow^{28,29}. Bacteria in the VBNC state did not regain their culturability even when provided favourable culture conditions²⁷. This makes the detection of bacteria in the VBNC state, using relatively cheap and widely used culture-based methods, much more difficult³⁰. Since then, many other bacteria including *Salmonella enterica* were reported to enter the VBNC state, making this phenomenon important in the study of infectious food-borne bacteria³⁰.

1.2 Viable but nonculturable cells: the discovery and importance

Bacteria are one of the oldest life forms and their existence for over 3 billion years in variable ecological niches make them highly successful life forms. Bacteria can survive and thrive in a multitude of environments ranging from high temperature sulphur springs³¹ to places with high ionizing radiation³². It is thought that 99% of all bacterial species are 'unculturable' under laboratory conditions³³.

Some Gram-positive genera can form endospores that can 'germinate' after the provision of more favourable conditions^{34,35}. Sporulation or similar phenomena were not observed in Gram-negative bacteria until the VBNC state was observed and described. Xu *et al.* reported the apparent non-culturability of a portion of the *E. coli* and *V. cholerae* populations losing culturability when exposed to salinity and cold temperature²⁷. This changed the existing paradigm of equating viability with culturability³⁶. Over 80 bacterial species including *Salmonella enterica* serovar Typhi³⁷, *Klebsiella pneumoniae*³⁸, *Legionella pneumophila*³⁹, *Mycobacterium tuberculosis*⁴⁰ enter this state when subjected to unfavourable conditions (Table 1.1).

The VBNC state is not unique to Gram-negative bacteria as Gram-positive bacteria can also become non-culturable⁴¹. VBNC bacteria render culture-based bacterial detection methods that are used for food screening and infection diagnosis less definitive and suggests that bacteria that have entered the VBNC state may pose a significant public health risk^{30,42}.

Table 1.1: Conditions of stress reported to drive various species of bacteria into the viable but nonculturable state.

Stress	Bacteria
Starvation	<i>Acinetobacter calcoaceticus</i> ⁴³ , <i>Aeromonas hydrophila</i> ¹ , <i>Agrobacterium tumefaciens</i> ³⁸ , <i>Arcobacter butzleri</i> ⁴⁴ , <i>Burkholderia pseudomallei</i> ⁴³ , <i>Campylobacter coli</i> ^{45*} , <i>C. jejuni</i> ⁴⁶ , <i>C. lari</i> ^{45*} , <i>Citrobacter freundii</i> ⁴⁷ , <i>Edwardsiella tarda</i> ^{48*} , <i>Enterobacter aerogenes</i> ³⁸ , <i>Enterococcus cloacae</i> ³⁸ , <i>E. faecium</i> ⁴⁹ , <i>E. hirae</i> ⁴⁹ , <i>Escherichia coli</i> ^{50,51* 52*} , <i>Francisella tularensis</i> ^{53,54} , <i>Haemophilus influenzae</i> ⁵⁵ , <i>Helicobacter pylori</i> ⁵⁶ , <i>Klebsiella pneumoniae</i> ³⁸ , <i>Legionella pneumophila</i> ⁵⁷ , <i>Listeria monocytogenes</i> ^{58*} , <i>Micrococcus luteus</i> ⁵⁹ , <i>Mycobacterium smegmatis</i> ^{60*} , <i>M. tuberculosis</i> ⁴⁰ , <i>Pseudomonas aeruginosa</i> ^{61*} , <i>P. putida</i> ⁴³ , <i>Salmonella enteritidis</i> ⁶² , <i>S. Oranienburg</i> ^{63*} , <i>S. Typhi</i> , <i>S. Typhimurium</i> ^{64*} , <i>Shigella dysenteriae</i> ⁶⁵ <i>Vibrio cholerae</i> ⁶⁶ , <i>V. fluvialis</i> ⁶⁷ , <i>V. mimicus</i> ⁶⁸ , <i>V. parahaemolyticus</i> ⁶⁹ , <i>V. vulnificus</i> ⁷⁰ , <i>Yersinia pestis</i> ^{71*}
Low temperature	<i>Brucella melitensis</i> ⁷² <i>Campylobacter coli</i> ⁴⁵ , <i>C. jejuni</i> ⁴⁵ , <i>C. lari</i> ^{45*} <i>Enterococcus faecalis</i> ³⁸ , <i>E. faecium</i> ⁴⁹ , <i>E. hirae</i> ^{49*} <i>Escherichia coli</i> ^{52,73} <i>Listeria monocytogenes</i> ⁷⁴ <i>Pseudomonas aeruginosa</i> ^{61*} <i>Salmonella Typhimurium</i> ^{50*}

	<i>Vibrio cholera</i> ⁷⁵ <i>Vibrio parahaemolyticus</i> ⁷⁶ <i>Yersinia pestis</i> ^{71*}
High temperature	<i>Pantoea agglomerans</i> (formerly <i>Enterobacter agglomerans</i>) ⁵⁰ <i>Escherichia coli</i> ⁵⁰
Low pH	<i>Burkholderia pseudomallei</i> ⁷⁷ <i>Campylobacter coli</i> ⁷⁸ , <i>C. jejuni</i> ⁷⁸ <i>Listeria monocytogenes</i> ⁷⁹ <i>Shigella flexneri</i> ^{80*}
Aerosolization	<i>Cytophaga allerginae</i> ⁸¹ <i>Klebsiella planticola</i> ⁸¹ <i>Serratia marcescens</i> ⁸¹
Chemical agents	<i>Agrobacterium tumefaciens</i> (Copper ⁸²) <i>Escherichia coli</i> (Chlorination ⁸³ , H ₂ O ₂ ⁸⁴) <i>Legionella pneumophila</i> (Bleach ⁸⁵) <i>Listeria monocytogenes</i> (food preservatives ⁷⁹ , chlorine ⁸⁶), <i>Pseudomonas aeruginosa</i> (Copper ⁸⁷) <i>Rhizobium leguminosarum</i> (Copper ⁸²) <i>Salmonella</i> Thompson (Chlorine ⁸⁶), <i>S. Typhimurium</i> (Chlorine ⁸³) <i>Staphylococcus aureus</i> (antibiotics while in biofilms ⁴¹)
Osmotic stress	<i>Burkholderia pseudomallei</i> ⁷⁷ <i>E. coli</i> ⁸⁴ <i>S. Oranienburg</i> ^{63,88} <i>V. parahaemolyticus</i> ⁸⁹
Physical stress	<i>Bacillus cereus</i> (Pulsed electric field ⁹⁰) <i>E. coli</i> (High-pressure CO ₂ ⁹¹) <i>L. monocytogenes</i> (light and pulsed electric field ⁷⁴) <i>S. Typhimurium</i> (UV-C irradiation ^{64*} , UV-A irradiation ⁹²)

Footnotes:

* Bacteria might have been subjected to more than one kind of stress in the referred work e.g. incubation at 4°C in sea water would subject the bacteria to cold stress, starvation and potentially osmotic stress.

1.3 Characteristics of VBNC state

To study and identify VBNC bacteria, it is first necessary to define this state. The initial report of the VBNC state used 1) lack of culturability and 2) response to growth media in the presence of drugs i.e. the Kogure's direct viable count method (DVC)⁹³ as the only two parameters to define VBNC cells²⁷. The total number of bacteria were enumerated by acridine orange stain by the acridine orange direct counting (AODC) method.

Since this initial report in 1982, several stains that can detect various aspects of bacterial viability were reported, and these stains can be used to differentiate between live, dead and VBNC bacteria. These newer stains do not rely completely on the ability of the bacterium to respond to nutrients like in the Kogure's DVC method. Techniques are now available to directly test VBNC bacterial respiration, membrane integrity, adenosine triphosphate (ATP) content and anabolic activities such as transcription and translation⁹⁴. Reports show that VBNC bacteria have an intact cell membrane^{87,95,96} and metabolism within these bacteria may be detectable in some species, but virtually undetectable in other bacteria⁵¹.

One of the characteristics observed in some bacteria that have entered the VBNC state was an altered cellular shape in comparison to their culturable counterparts. Scanning electron microscopy images of *E. coli* in the VBNC state showed a coccoid cellular shape instead of the usual rod shape observed in an active growth phase. This was also observed in *V. cholerae*, *Helicobacter pylori* and *Salmonella enterica*⁹⁷⁻¹⁰¹. The significance of this change of shape in VBNC state is still not completely understood.

Another characteristic of VBNC cells is that they do not return to culturability once the stress that induced them is removed but instead require the presence of specific cues or special conditons⁴². For example, studies showed that *Micrococcus luteus* cells entered the VBNC state after starvation and could be resuscitated when co-cultured with actively growing *M. luteus*⁵⁹. *M. tuberculosis* driven into the VBNC state by oxygen deprivation, could be resuscitated by the resuscitation promoting factor (Rpf) protein¹⁰². A starvation-induced VBNC state in *E. coli* could be resuscitated by the addition of enterobacterial autoinducers⁵⁰. These

studies suggest that bacteria in the VBNC state may need specific signals to regain culturability. The signal may be a specific agent, as in the above examples, or could be factors from an infected host as seen in VBNC *Listeria monocytogenes* where the bacterium was 'resuscitated' by something present within embryonated eggs¹⁰³. VBNC *Salmonella enterica* serovar Oranienburg was resuscitated by passage through the mouse gut⁸⁸. These examples suggest that the cues for resuscitation can be simple or complex but they may well be different for different pathogens and may relate to the stress(es) that drove the vegetative form of the bacterium into the VBNC state.

Lastly, the definition of VBNC excludes the bacteria that are 'unculturable', which means those bacteria that cannot be grown yet in laboratory conditions¹⁰⁴. These are not regarded as VBNC bacteria. Arguments explaining the VBNC state suggest this state could be result of temporarily damage to the bacteria and hence this is a period of 'quasi-dormancy'^{105,106}.

The work presented in this thesis adopts a functional definition of the VBNC state as a lack of culturability on routine laboratory media, coupled with viability of the bacteria, ascertained by conventional stains such as propidium iodide, which is consistent with much of the existing literature^{74,100,107}.

1.4 Persisters and VBNC

Bacterial 'persisters' shares many similar characteristic with VBNC bacteria²⁸. The 'golden era' of antibiotics started in 1928 with the discovery of penicillin and, by 1940, the first penicillinase was described¹⁰⁸. Various mechanisms of resistance to existing antibiotics were discovered and antibiotic resistance has been increasing since the 1960s, when antibiotic use became widespread¹⁰⁹. Meanwhile, another group of antibiotic 'tolerant' bacteria called 'persisters' were discovered in *Staphylococcus aureus* in 1942^{18,110}. Persisters are defined as a subpopulation of bacteria that are metabolically inactive^{18,111}. They are different from the antibiotic-resistant population as persisters are genetically identical to their susceptible counterparts in their ability to resist antibiotics when they are grown in a planktonic form¹⁸.

VBNC bacteria and persisters are regarded as part of the bacterial 'dormancy continuum' where VBNC bacteria are thought to be in a deeper state of dormancy than that of the persisters²⁸. There are many overlapping characteristics between the VBNC state and the persister state such as lowered metabolic activity, antibiotic tolerance, reduced cell division and presence in biofilms^{41,112,113}. Both the persister and VBNC states are thought to form stochastically in any given population^{20,114-116}. Both these states might offer different advantages based on unfavourable conditions. Recent studies propose that both the persisters and VBNC bacteria are generated (or possibly selected by) as a response to stress¹⁹. The mechanisms that induce these two dormancy states can be very similar. The so-called 'stringent response' was shown to play a role in persister formation and VBNC state in various bacteria¹¹⁷. The metabolite (p)ppGpp^{25,118} and proteases^{119,120} play a role in induction of persisters and VBNC bacteria. Lowered ATP levels can induce the formation of persisters¹²⁰ and may result in VBNC bacteria⁹¹.

Despite these similarities, the main difference between persisters and VBNC bacteria is their respective culturability. Persisters resume cell division and become culturable upon removal of stress whereas the VBNC bacteria do not regain their culturability when the stress is removed^{28,96}. There are two major theories that hypothesise the relationship between the persisters and the VBNC bacteria (Figure 1.1). They are:

1. Persisters and VBNC bacteria are not part of two different states of dormancy but represent a single dormancy state¹²¹. The persisters eventually become VBNC under prolonged stress and possibly in a deeper state of dormancy¹¹⁴ and heading towards death^{121,122}, as Kim *et al.* showed in *E. coli*¹²¹.

2. Persisters and VBNC cells are stochastically formed and represent a portion of any bacterial population in a given microcosm. This hypothesis was articulated by Ayrapetyan *et al.* based on an observation made in human serum stressed bacteria^{114,116}. This is called as the 'dormancy continuum' hypothesis. This theory proposes that these populations are part of a survival strategy of bacteria to with-stand current and future threats.

Persisters and VBNC bacteria exist in similar environments. Studies have shown that persisters were present in biofilms, stationary phase cultures and even in infected cells^{123,124,125}. VBNC bacteria were also found in biofilms⁴¹, stationary phase cultures¹²⁶ and in serum¹¹⁶. Orman *et al.* and other studies have shown that VBNC cells were many folds higher in number than persisters in any population^{113,116,127}. There are studies supporting both the first hypothesis¹²¹ and the second 'dormancy continuum' hypothesis⁹⁶. The relationship between persister and the VBNC bacteria is yet to be completely understood.

Finally, it is important to distinguish these two drug-tolerant populations in the design of effective treatments, especially if they share similar characteristics and niches but have different physiologies. That said, if they share a similar physiology, then the study of one of these dormancy states might give clues into the formation and maintenance of the other state.

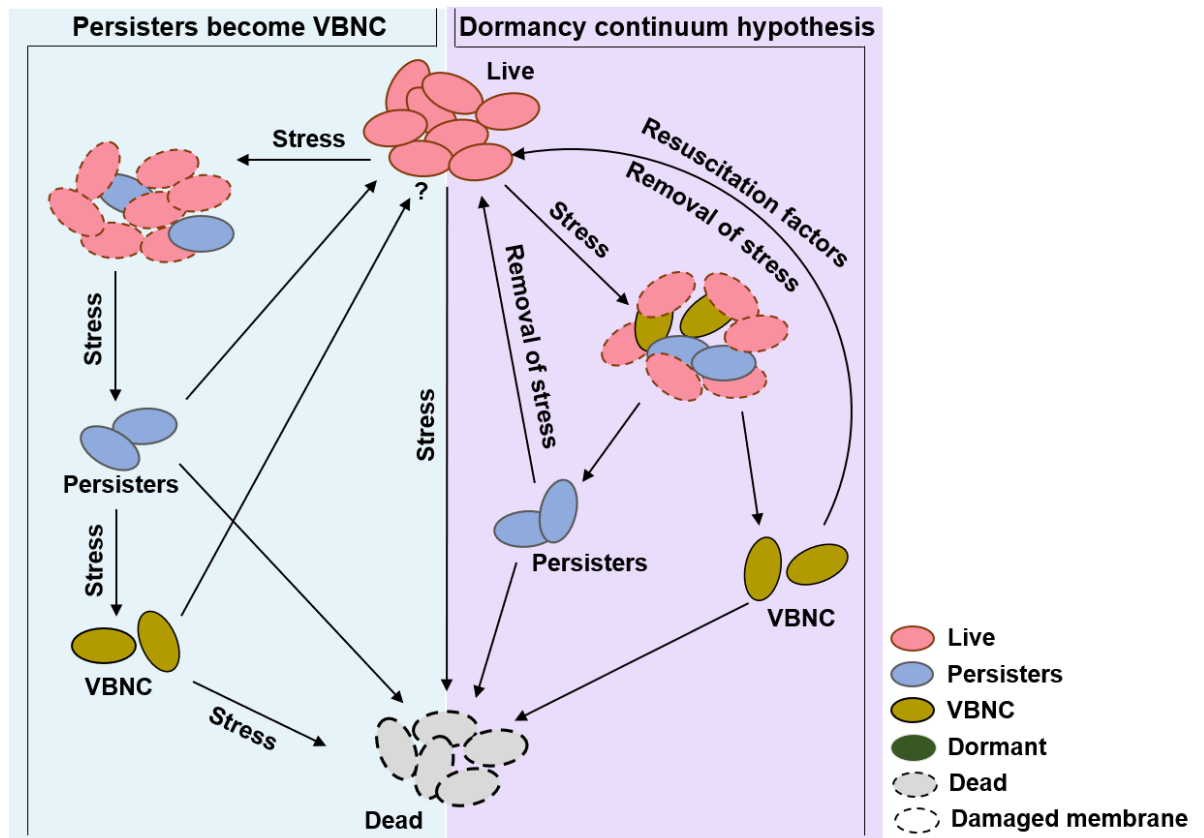


Figure 1.1: Schematic representation of two common models describing the formation of persisters and VBNC bacteria. The first theory (left half) suggests that both persister and VBNC cells could represent a single state of dormancy where persisters upon exposure to stress for longer duration can become VBNC. The 'dormancy continuum' hypothesis (right half), proposes that both persisters and VBNC cells co-exist as part of the dormant bacterial population at a given time in any given microcosm. [Based on data from Ayrapetyan *et al.*, (2005)¹¹⁴]

1.5 Induction into the VBNC state

The characteristics of VBNC bacteria discussed in Section 1.3 can be used as parameters to identify VBNC bacteria. However, it is also necessary to understand the conditions under which the bacteria adopt a state of VBNC. Laam *et al.*⁴² listed over 50 pathogens and various conditions that can induce these bacteria into the VBNC state; to-date, 85 species have been shown to enter the VBNC state including 67 pathogenic species⁴².

The conditions that drive bacteria into the VBNC state are diverse and include physiochemical factors such as salinity, chemicals such as hydrogen peroxide and chlorine, pulsed electric field, UV radiation, pH and starvation (Table 1.1). Nutrient starvation is a condition that drives many other bacteria into the VBNC state, including *Campylobacter coli*⁴⁵, *Campylobacter jejuni*⁴⁶, *Enterobacter aerogenes*³⁸, *Enterococcus faecalis*³⁸, *E. coli*⁵⁰, and *H. pylori*⁵⁶. For example, *V. cholerae* entered the VBNC state at low temperatures in sea water¹²⁸. Oliver *et al.* reported that chlorination, commonly used for disinfection, can drive *E. coli* and *S. Typhimurium* into the VBNC state⁸³. Exposure to various common food preservation methods such as high hydrostatic pressure, pulsed electric fields, and ultraviolet (UV) radiation were shown to induce the VBNC state in bacteria during food processing¹²⁹. For example, *Bacillus cereus* entered the VBNC state when exposed to a pulsed electric field⁹⁰ while another food-borne pathogen, *L. monocytogenes*, entered the VBNC state upon exposure to low pH⁷⁹, sunlight and nutrient starvation^{58,103}.

Common disinfecting agents may also trigger VBNC bacteria formation. *L. pneumophila* entered dormancy upon treatment with sodium hypochlorite or common bleach, as did *S. Typhimurium* upon chlorination treatment³⁹. Morishige *et al.* showed that hydrogen peroxide, another common disinfectant, drove *S. Typhimurium* into the VBNC state^{94,130}. Enterohaemorrhagic *E. coli* O157:F2 and *Pseudomonas syringe* became VBNC after treatment with the oxidising agent hydrogen peroxide and acetylsyringone, respectively^{107,131}. These examples suggest that under certain conditions surface disinfectants instead of eliminating all the bacteria may force some to dormancy.

Chemicals like copper sulphate can cause stress in plant pathogens and key saprophytes such as *Pseudomonas aeruginosa*, *Agrobacterium tumefaciens*, *Rhizobium leguminosarum*, and *Ralstonia solanacearum* become VBNC upon copper treatment^{61,82,95,132}.

Biological factors including the strain and growth phase of the bacteria can also influence VBNC formation¹³³. Bacterial strain, temperature, aeration, growth phase and nutrient starvation were shown to play a role in determining the entry of bacteria into the VBNC state (under laboratory conditions) and changing these five factors can affect, or even block, entry into the VBNC state¹³³. The strain used in any study seems to be very important to the outcome. The *E. coli* O157:F2 strain, after passaging through mice, became stress-susceptible and entered the VBNC state more readily than the bacteria that were not passaged through mice¹³⁴. This suggests that small biological changes can result in differences in response to stress and influence the entry into VBNC state. Reduction in ribosome-associated proteins after passage through mice caused a susceptibility to stress in *E. coli* O157:F2¹³⁴. A similar stress-susceptibility followed by VBNC induction was reported in *S. Oranienburg* when the bacterium acquired a sodium chloride-sensitivity after passage through mice⁸⁸.

Strain-specific induction into the VBNC state has been reported for *Rhodococcus* spp. strains TG13 and TN3⁹⁸. Different *Salmonella* enterica serovars were induced into the VBNC state under various conditions. *S. enterica* serovar Dublin, *S. Oranienburg* and *S. Typhimurium* were shown to take different durations of stress to enter the VBNC state where *S. Dublin* took the longest to become VBNC among the three serovars tested. A single amino acid substitution (D118N) in RpoS of *S. Oranienburg*, and lower levels of RpoS in *S. Typhimurium*, induced the VBNC state in these serovars at a relatively lower level of stress compared to *S. Dublin*. These observations suggest that levels of RpoS, the stress sigma factor, is negatively correlated with entry into VBNC state. The above examples suggest that each bacterial strain might need different conditions to become VBNC and further experiments might be needed to determine and characterise the best conditions for any strain used in

VBNC studies. Table 1.1 lists the bacterial species and their corresponding conditions that induce the VBNC state.

1.6 Methods of bacterial detection and viability

Detection of bacteria, and the assessment of their viability, are fundamental to the management of infectious diseases at the environmental and individual level. There are various methods used to detect bacteria in clinical or environmental samples, and these methods may, or may not be able to assess the viability of the bacteria. In general, the methods of detection can be classified as culture-, microscopy- or molecular-based (Figure 1.2). The viability of bacteria needs to be tested by using proxies such as structural and physiological characteristics. They include cell division, bacterial growth, metabolism, cell membrane integrity. As viability cannot be quantified or qualified in all situations, methods can be used to assess the above mentioned properties that act as indicators to viability or death (Table 1.2).

Traditional culture-based methods include growing bacteria on a suitable solid agar or in broth cultures. These methods are best for detecting actively growing culturable bacteria but are unable to identify unculturable bacteria, including VBNC cells, and bacteria that have yet to be formally cultured on routine laboratory media, e.g. *Treponema pallidum*, the causative agent of syphilis.

Microscopy and molecular methods can help detect and differentiate bacteria in different stages of viability independent of their culturability status. Microscopy-based methods include AODC (acridine orange direct counting) where the samples are treated with dyes such as acridine orange, or by using specific fluorescently-labelled probes in FISH (fluorescent *in situ* hybridisation), or through the use of fluorescent stains that depend on membrane permeability such as propidium iodide, DAPI (4',6-diamidino-2-phenylindole) and SYTO dyes, followed by visualisation using a fluorescent (confocal) microscope. Dyes and stains used to study the membrane potential and membrane integrity of organisms can also be used to analyse the

viability of bacteria using fluorescent microscopy or flow cytometry. For example, propidium iodide (PI), SYTO dyes, and Rhodamine 123¹³⁵ have been used to study cellular properties including membrane permeability¹³⁶, presence of DNA and RNA^{137,138}, and membrane potential¹³⁹, respectively. These stains can demonstrate different physiological and physical aspects of the bacteria, thus providing evidence of their 'viability'. A key characteristic of live bacteria is an intact cell membrane¹⁴⁰. VBNC bacteria have an intact cell membrane^{95,141}, exhibit a highly reduced metabolic activity^{98,142} and show lack of cell division⁹⁶. These characteristics can be used to determine the VBNC population of bacteria. For example, the direct viable count method can be used in combination with membrane sensitive stains (such as PI) to analyse bacterial metabolism and membrane integrity, respectively. In conjunction, these techniques may provide better insights into bacterial viability than when used separately. In addition to routine stains, like the Gram stain, staining methods that can test for various structural and functional aspects of bacteria (e.g. encapsulation) can be used for bacterial detection.

Molecular methods can be categorised into nucleic acid-based methods, that include PCR (polymerase chain reaction), RT-PCR (reverse transcriptase PCR) and vPCR (viability PCR), and metabolism-based methods using chemicals like methylene blue (for respiration), BacTiter-Glo™ (for ATP-adenosine triphosphate quantification). The combination of different methods can assist with an understanding of bacterial culturability, membrane integrity and metabolic activity.

Table 1.2: Methods to assess bacterial viability through different viability properties

<u>Method</u>	<u>Viability property</u>	<u>Examples</u>	<u>Mechanism</u>
<i>Nucleic acid dyes</i>¹⁴³	Presence of DNA and RNA	SYTO9 ¹⁴⁴ , DAPI ¹⁴⁵	Intercalation into nucleic acid strands
<i>Passive dye exclusion</i>¹³⁶	Integrated cell membrane	PI ¹⁴⁴ , PMA ¹⁴⁶ , Ethidium bromide ¹⁴⁷	Impermeable dyes cannot cross intact cell membrane and hence only stain the membrane compromised dead bacteria
<i>Measuring membrane potential</i>¹⁴⁸	Active membrane potential	Membrane dyes such as Rhodamine 123 ¹⁴⁸	Viability is essential to maintain an active membrane potential and dyes used in this method can discriminate determine resting membrane potential
<i>Kogure Direct Viable Counting (DVC)</i>⁹³	Cell division, transcription and translation ¹⁴⁹	Treatment with nalidixic acid ¹⁵⁰	Growth response of bacteria in the presence of quinolones and nutrients
<i>Measuring reduction of substrates</i>¹⁵¹	Enzymatic activity, energy dependent	INT ¹⁵¹ , methylene blue ¹⁵² , fluorescein acetate ¹⁵³	Enzymatic reduction of chemical substrates
<i>Induction of proteins by induction agents</i>	Active transcription and translation	Induction of β -lactamases ¹⁵⁴ , fluorescent proteins ¹⁵⁵	Induction agents like IPTG and arabinose are used to study the transcriptional and translational activity of proteins that contribute to easily observable phenotype

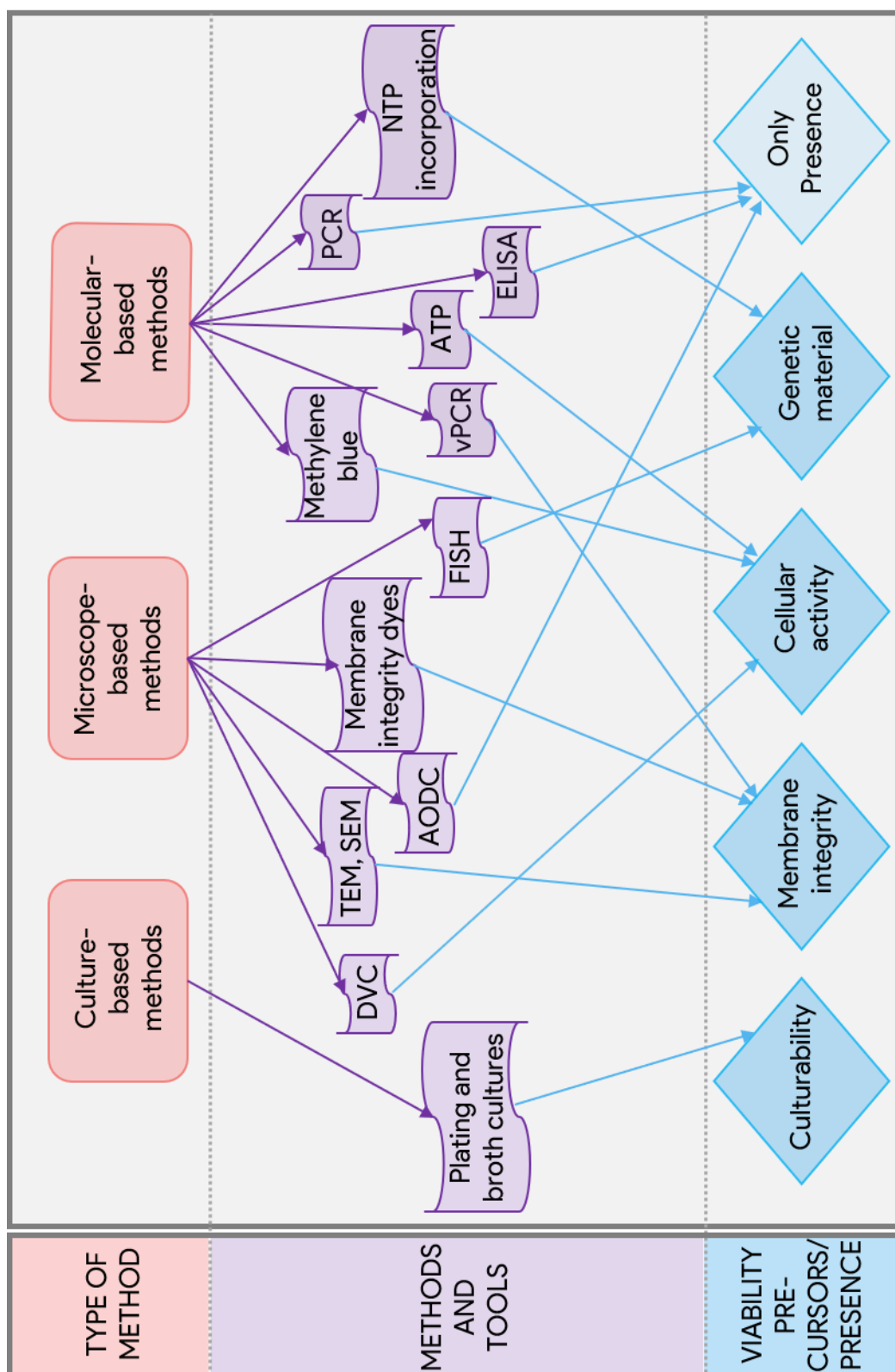


Figure 1.2: Schematic of common methods to study viability and presence of bacterial. A combination of these methods can give reliable evidence for the detection and confirmation of bacteria in VBNC state

PCR analysis of mRNA by RT-PCR, rather than the detection of genomic DNA, has long been proposed as an effective method to identify metabolically active bacteria in a sample, regardless of whether the bacteria are culturable or nonculturable¹⁵⁶. The RT-PCR method to identify metabolically active bacteria is effective because of the shorter mRNA life-span, compared with DNA¹⁵⁷. In the detection of mRNA, researchers have sought for genes that are unique to the pathogen of interest and are abundantly expressed even in a VBNC bacterium. Such genes, in theory, can be used for RT-PCR detection of bacteria, including VBNC bacteria^{158,159}. For example, the presence of mRNA to detect viable bacteria was demonstrated using the *hlyA* gene, that expresses hemolysin A, as a target for RT-PCR. The study revealed a good correlation between *hlyA* mRNA and the number of viable *Listeria* spp. cultured from environmental samples¹⁶⁰.

PCR of bacterial samples obtained from soil and other sources can provide more information than culture-based methods as it confirms the presence of bacteria even when the bacteria are unculturable. However, PCR generally fails to reveal the viability of the bacteria because DNA from dead bacteria can yield a PCR amplicon. To overcome this drawback, vPCR is used where the bacteria are treated with membrane-sensitive molecules like PMA (propidium monoazide), which can covalently intercalate and link double stranded DNA upon UV light exposure¹⁶¹. The covalently bound PMA will prevent the denaturation of the DNA during PCR, thus rendering extracellular DNA, and DNA from dead bacteria, incapable of acting as template for the PCR.

The results from the methods described above can only be interpreted by knowing the various properties that different bacterial populations might exhibit. Table 1.3 and Figure 1.4 summarises common physiological states of the existence of Gram-negative bacteria and the key differentiating properties of those states. In this study, Live/Dead stain (contains SYTO9 and propidium iodide stains) was used for confocal microscopy. Live/Dead staining was combined with Kogure's direct viable counting method of nalidixic acid treatment in the presence of nutrients was used to differentiate dividing *S. Typhimurium* from VBNC

S. Typhimurium. Propidium iodide along with *Salmonella* anti-LPS antibodies were used for a FACS analysis to identify dead bacteria.

State of bacterium	Test	CFU	Staining with PI	PCR	RT-PCR	vPCR	Drug tolerance	Respiration
Active cells		✓	✗	✓	✓	✓	✗	✓
VBNC		✗	✗	✓	✓	✓	✓	✓
Persisters		✓	✗	✓	✓	✓	✓	✓
Dead cells		✗	✓	✓	✗	✗	✗	✗

Table 1.3: Common laboratory tests that define states of bacteria with different viability and culturability characteristics. A tick (✓) indicates a positive result, and cross (✗) indicates a negative result.

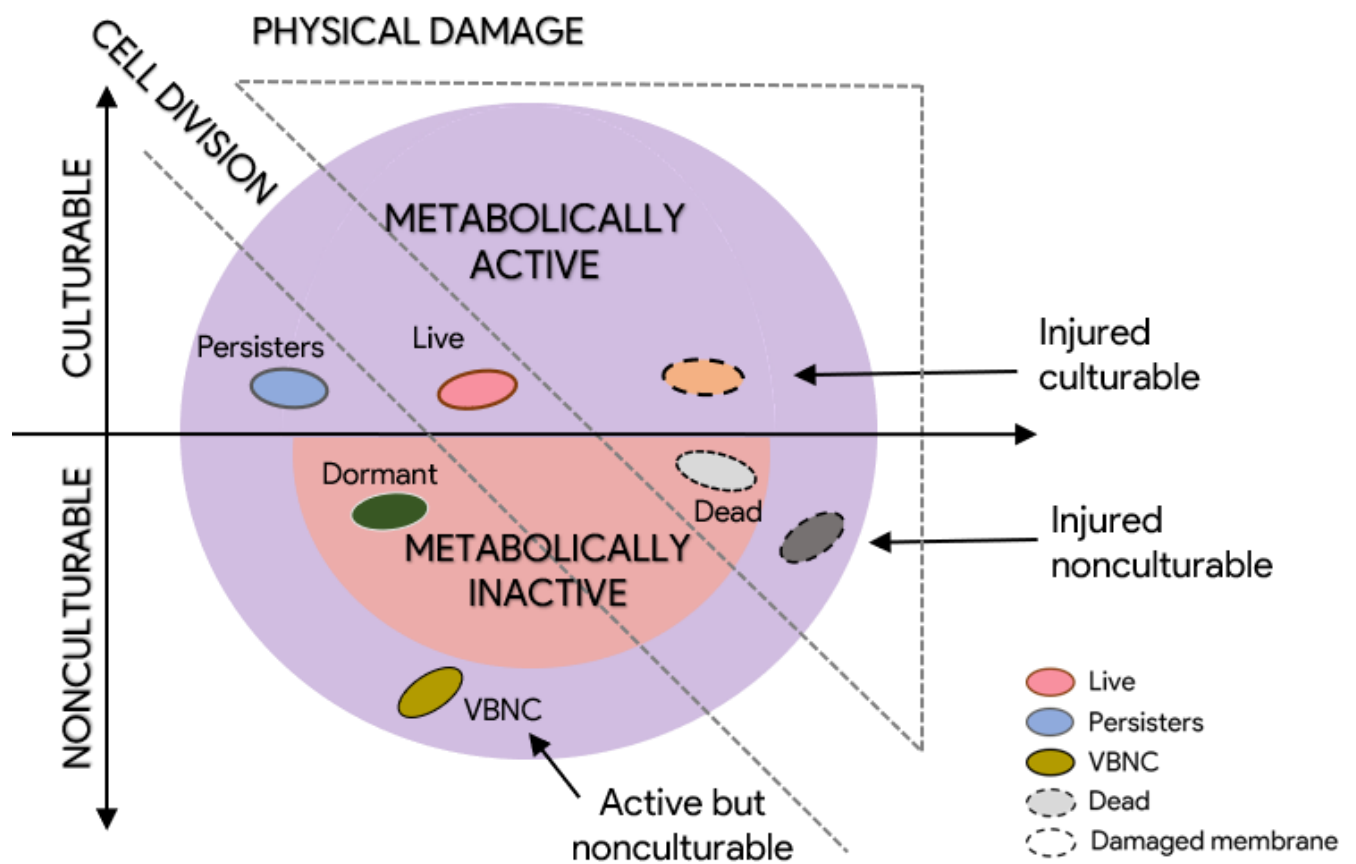


Figure 1.3: Schematic representation of various physiological states of bacteria.

Bacteria can exhibit various states of metabolic activity, culturability and membrane integrity. The various states of existence, e.g. VBNC and persisters, are represented along with the properties they exhibit in these states. Culturability, metabolic activity, physical integrity and cell division are used to represent various population in any given microcosm. [Based on data from Kell *et al.* (2000)¹⁶²].

1.7 VBNC state, pathogenesis and virulence

The first report of VBNC state in *V. cholerae*⁶⁶, a human pathogen, and further reports in other pathogens, focussed on non-culturability and potential to cause disease. Several important pathogens including *P. aeruginosa*⁶¹, *E. aerogenes*³⁸, *Vibrio vulnificus*^{68,70}, *C. jejuni*¹⁶³, *S. Typhimurium*⁸³, *S. aureus*⁴¹ and *E. coli* O157:H7¹⁶⁴ are amongst the 51 pathogens that have currently been reported to enter the VBNC state.

Studies conducted on the virulence of VBNC pathogens have shown variable results that were specific to the pathogen under study. Some pathogens in the VBNC state retained virulence and could successfully infect a host while others lost the ability to infect¹⁶⁵⁻¹⁶⁷. VBNC *V. cholerae* and *V. vulnificus* caused death in mice^{68,168} and culturable bacteria were found in blood and in the peritoneal cavity. VBNC *L. pneumophila* could infect human macrophages and amoeba¹⁶⁹. In some cases, the evidence is less direct. A food poisoning incident involving *E. coli* O157:H7 originated from contaminated salted salmon roe¹⁷⁰. Subsequent studies revealed that *E. coli* O157:H7 can enter the VBNC state in the presence of high salt, which is commonly used in the preparation and preservation of salmon roe¹⁶⁴. Another similar food poisoning incident may have occurred from VBNC *Salmonella* Oranienburg in seafoods. A study showed that *S. Oranienburg* entered the VBNC state when exposed to high salt concentrations, which can occur while preserving dried processed squid⁶³.

Other pathogens lose their virulence after entering the VBNC state but regain full virulence after resuscitation. This can be seen in the cases of *Vibrio alginolyticus* and *V. parahaemolyticus*. These bacteria were resuscitated in mice from the VBNC state and regained virulence only after subsequent passaging through the rat ileal loop¹⁷¹. *L. monocytogenes* lost its ability to cause infection when in the VBNC state, but regained the ability to infect after resuscitation in embryonic eggs¹⁰³. A study by Lindbäch *et al.* on 16 isolates of *L. monocytogenes* collected from the environment, patients and salmon showed that bacteria in the VBNC state were incapable of infecting RAG1^{-/-} mice (i.e. mice lacking both B- and T-lymphocytes)⁷⁴. However, a subsequent study on the same pathogen showed that

VBNC bacteria retained virulence and infected *C. elegans*⁸⁶. This observation may suggest a role for host factors in the virulence of VBNC bacteria.

Aeromonas hydrophila was avirulent in the VBNC state and subsequently regained infectivity only after resuscitation¹⁷². *Edwardsiella tarda*, which failed to cause disease while in the VBNC state, killed inoculated fish within 7 days following resuscitation⁴⁸. Murine infection studies on VBNC *S. Typhimurium* suggested that the bacteria had reduced virulence in the VBNC state, and could not infect BALB/c mice^{83,167}. These examples show that the loss of virulence in VBNC state may be either a permanent or a temporary phenomenon, where the bacteria can still cause infection after resuscitation.

Studies suggested that VBNC pathogens retain partial virulence or exhibited signs of pathogenicity including virulence gene expression (discussed in detail in Section 1.8). For example, *Shigella dysenteriae* in the VBNC state retained the ability to adhere to Henle 407 cells but could not invade them⁶⁵ and VBNC *S. Typhimurium* showed limited invasion of HeLa cells¹²⁶.

Some studies suggest that the VBNC state may contribute to the transient virulence demonstrated for some pathogens³⁰. Passerat *et al.*¹²⁶ proposed that VBNC bacteria only retain virulence for a limited time and argued this transient retention of virulence explains the lack of a consensus on their pathogenicity¹²⁶. Laam *et al.*⁴² reported a 'resuscitation window' for different VBNC species. Beyond this 'resuscitation window' the VBNC bacteria cannot return to culturability, suggesting that bacteria might die after existing in the VBNC state for a prolonged time⁴². The lack of consensus on virulence of VBNC state suggests that this state may represent a continuum between culturability and cell death. The conditions that select for or induce the VBNC state (Table 1.1) may generate different responses within the bacterial population. Different responses of VBNC pathogens induced by a variety of stress conditions might be dissected using modern 'omics' approaches.

1.8 Transcriptomic and protein expression of bacteria in the VBNC state

Cellular physiology is a complex calculus defined by transcription and translation, and the turnover of mRNA and proteins. By studying the gene expression of VBNC bacteria, clues about the mechanism(s) used by bacteria to enter and survive in the VBNC state might be elucidated. As discussed, VBNC cells form in response to unfavourable conditions. Bacteria have different responses to specific stress conditions as well as a very well-studied 'general stress response' that helps bacteria respond to unfavourable conditions¹⁷³. The stress response includes recognition of the stress and subsequent changes in transcription and translation to counter the stress¹⁷⁴⁻¹⁷⁶. Studies have shown the role of the general stress response genes in the entry into, and maintenance of, the VBNC state. For examples, *rpoS*, encoding the stress sigma (σ^{38}), and RelA, which produces the 'alarmone' (p)ppGpp, have been shown to be important for the survival and entry into the VBNC state, respectively^{114,177}.

A complete transcriptome study could give insights into the survival of bacteria in the VBNC state and might provide candidate genes as biomarkers for the VBNC state. There have been several RNA-sequencing based transcriptome studies of bacteria in the VBNC state. Transcriptomic studies might reveal species-specific data on the expression profiles. Stress response genes, ABC transporter proteins, elongation factors, iron-sulphur proteins, siderophores, transcription factors, chaperonins, carbon metabolism-related genes and outer membrane proteins were among the common differentially expressed genes and proteins in VBNC bacteria. Transcriptome studies were conducted on the starvation-induced VBNC *V. cholerae*^{128 178} and VBNC *Rhodococcus biphenylivorans* induced under cold-stress and oligotrophic conditions⁹⁸. The transcriptome of oxidative stress-induced VBNC *Pseudomonas syringae* pv. *syringae*, a plant pathogen, was reported¹⁰⁷ and the transcriptomic profile of *Lactobacillus acetotolerans* from beer brewing by Liu et al.¹⁷⁹ revealed a comprehensive analysis of the gene expression profile of the bacteria in the VBNC state. The findings of these studies will briefly be discussed in this section. A transcriptomic analysis is yet to be completed

for *S. Typhimurium*, *Mycobacterium tuberculosis* and *L. pneumophila*, important human pathogenic organisms that have well-defined conditions for VBNC entry^{39,64,83,102,180}.

Apart from these important more global transcription studies, there have been studies investigating the transcription of virulence-related genes among the pathogens in the VBNC state using RT-PCR. Studies reporting the virulence gene transcription profiles of VBNC pathogens may not be comprehensive but can be useful in identifying and quantifying the response of virulence genes in pathogenic bacteria¹⁵⁹. Studies in *E. coli* O157 showed that the bacteria continue transcription of the *stx*₁ and *stx*₂ genes while in the VBNC state and the mRNA from these genes can be used to identify the bacteria in VBNC state^{181,182}. VBNC *E. coli* O157 transcribes *mobA* (MobA is part of the DNA 'relaxosome'¹⁸³) and *rbfE* (RbfB is a putative ABC transporter) genes¹⁸⁴. *Shigella dysenteriae* Type 1 was shown to maintain transcription of the Shigella toxin, *stx*, while in the VBNC state⁶⁵. VBNC *L. monocytogenes* expressed the hemolysin A toxin gene (*hlyA*) and RT-PCR was used to reliably quantify the bacteria^{74,185}. Similarly, the *cadF* gene, which an outer membrane protein important for host cell adhesion¹⁸⁶, was transcribed by VBNC *C. jejuni*. The *cadf* transcript levels decreased after three weeks into the VBNC state¹⁸⁷ but still retained virulence¹⁶³. Quantitative (q)RT-PCR studies in *V. vulnificus* showed a decrease in RNA transcripts of *tufA* (elongation factor Tu-1), *rpoS*, *vwhA* (hemolysin), capsular genes *wza* and *wzb*, and *katG* (periplasmic catalase) in the VBNC state, over time¹⁸⁸. RpoS plays a role in the maintenance of the VBNC state and decreased translation of *rpoS* might result in death to VBNC bacteria¹¹⁸. Expression of *pbd5* encoding a penicillin-binding protein was detected by RT-PCR for up to three months in *E. faecalis* VBNC cells¹⁸⁹. These examples demonstrate that bacteria in the VBNC state continue to express a number of virulence related genes, suggesting that, although non-culturable, these bacteria may be vehicles for food poisoning through ongoing toxin expression^{158,159}.

While there is evidence of ongoing gene expression in VBNC organisms, there are parallel reports of mRNA degradation under stress and in the VBNC state, which can make

the identification of VBNC bacteria by RT-PCR a challenge. RNA degradation, especially rRNA degradation, by VBNC bacteria was shown by Um *et al.* studying VBNC *R. solanacearum*⁹⁵. Similarly, Smith *et al.*¹⁹⁰ reported a shorter mRNA half-life in *V. vulnificus* in the VBNC state¹⁹⁰. Ribosomal RNA degradation and possible ribosome degradation were observed in some bacteria encountering stress, including nutrient starvation¹⁹¹⁻¹⁹³.

Transcriptome studies:

Unlike gene-targeted mRNA RT-PCR studies, complete transcriptomic studies provide much richer gene expression data and a comprehensive understanding of the VBNC state at the level of transcription. Several RNA-sequencing studies were conducted on VBNC bacteria. Asakura *et al.*¹²⁸ placed *V. cholerae* under cold stress at 4°C in artificial seawater for 70 days and examined the gene expression by microarray. This study showed the expression of siderophores, ABC transporter proteins, *polB* (DNA polymerase II), and flagellar genes *flaC* and *fliG* were significantly increased in comparison with their stationary phase counterparts. Siderophore and other metal ion (and metal ion containing substrates) transportation genes including that of cobalamin were highly upregulated. Genes important in cell envelope physiology including *pilQ* (type IV pilus assembly) and *nlpL* (lipoprotein) were also upregulated. Changes in chitin metabolism and chitin-binding activity suggested a change in the preference of carbon source¹²⁸. Many genes belonging to transport and binding, energy metabolism, cellular processes, DNA metabolism, regulatory functions and amino acid biosynthesis were induced in the VBNC state of *V. cholerae*¹²⁸. Xu *et al.*¹⁷⁸ also studied the gene expression of *V. cholerae* VBNC under same induction conditions as Asakura *et al.*¹²⁸ but used a RNA sequencing approach. The Asakura study made observations similar to the microarray analysis and reported an increase in the expression of chitin utilisation genes. A downregulation of the genes encoding cell division proteins including *ftsZ*, *ftsQ* and *ftsH* was also reported and genes encoding MreBCD were downregulated. It has been speculated that MreBCD play a role in cellular morphology which drive the coccoid shape of *V. cholerae* cells in the VBNC state¹⁷⁸. Interestingly, genes encoding ribosomal proteins *raiA* and *rmf* were

downregulated, suggesting a possible decrease in ribosome assembly, and thus possibly protein synthesis¹⁹⁴. General stress response genes like *rpoE*, *rpoH*, *rpoN* decreased by more than two-fold in the VBNC *V. cholerae*¹⁷⁸. Virulence genes *ctxA* and *tcpA* were expressed at similar levels in VBNC and vegetative cells of *V. cholerae*, suggesting that the bacteria could be virulent¹⁶⁶.

A transcriptomic VBNC *Pseudomonas syringae* was reported by Postnikova *et al.*¹⁰⁷. The bacteria were subjected to a 'mock' oxidative burst using acetosyringone. This study showed that genes responsible for carbohydrate metabolism, cell wall polymers, energy generation and chemotaxis were upregulated in VBNC bacteria. Iron-sulphur proteins such as aconitase (*asnA*) were also upregulated, possibly in response to oxidative stress¹⁹⁵. Similar to VBNC *V. cholerae*, ABC transporters (P syr_1737, P syr_1738) in VBNC *P. syringae* were upregulated. Collectively, these data indicate a trend of ABC transporter upregulation in the VBNC state. Among the upregulated cell wall-related genes in this work, *murG* was notable. MurG is required for cell wall formation and encodes for N-acetylglucosaminyltransferase¹⁹⁶. Previous studies reported that *murG* expression was maintained throughout the VBNC state of *H. pylori*⁵⁴ [unpublished from JD Oliver (2015)]. MurG was necessary for peptidoglycan recombination in VBNC *E. coli*, thus suggesting a role of this protein in the maintenance of the VBNC state^{56,165,197}. Other upregulated genes in VBNC *P. syringae* included a periplasmic binding protein, a CheW-like protein (chemotaxis protein), histidine kinases and NADPH dehydrogenase subunit proteins¹⁰⁷. Twenty-eight of the 40 transcription factors that were differentially expressed (compared to the control) were downregulated in the VBNC *P. syringae*, indicating an overall decrease in cellular transcription. Upregulated transcription factors included the global regulators MarR and LysR, which regulate genes that respond to environmental stresses, antibiotic resistance, and genes related to quorum sensing, virulence, motility and metabolism, respectively^{198,199}. Another transcriptome analysis conducted in *Rhodococcus* spp. strains TG13 and TN3 showed an upregulation of the fatty acid degradation

pathways, citrate cycle and oxidative phosphorylation pathways, which might lead to increased ATP production⁹⁸.

As discussed, the VBNC gene expression profile, the mechanism of maintenance, and entry into the VBNC state might differ from species to species. The stress condition that induces VBNC bacteria can also influence the mechanism and transcriptome of the VBNC bacteria. The species-specific expression of genes in the VBNC state was highlighted by qRT-PCR studies of the gene encoding the elongation factor EFT-Tu (*tufA*). Gene expression levels of *tufA* were reduced in VBNC *E. faecalis* but were unchanged in VBNC *E. coli*^{42,134,200}. EF-Tu has a role in protein synthesis, stress response and cell growth regulation^{201,202}. This species and stress specific response of the bacteria in VBNC state can be a possible challenge in arriving at a common mechanism.

The gene expression studies by RT-PCR, microarray or by RNA sequencing do not always consider whether the transcriptome correlates with the proteome. Studies have argued that the mRNA, including the mRNA of virulence-related genes, is not translated into proteins in VBNC cells. For example, VBNC cells of Enterotoxigenic *E. coli* (ETEC) had mRNA transcripts of genes *eltB* and *estA*, which encode toxins, but did not produce the toxins when a GMI-ELISA study was performed to detect the toxins²⁰³. This suggests that the gene expression in VBNC cells may be poorly correlated with protein expression. The lack of correlation goes both ways - Dinu *et al.*²⁰⁴ showed expression of virulence genes *hly*, *stx₁* and *stx₂* in *E. coli* O157:H7 VBNC, but found very low levels of verotoxin production by these bacteria²⁰⁴.

Proteome studies:

Proteomic analyses have been used to investigate VBNC bacteria. Proteome analysis by 2D electrophoresis in bacteria including *E. coli* O157 and *Vibrio harveyi* was reported¹⁶⁴. In oxidatively-stressed VBNC *E. coli* O157 (stress-sensitive strain), outer membrane protein W (OmpW) was over-expressed along with oxidative-stress response factors AhpC, AhpF and

AceF¹³⁴. Decreased protein levels of ABC transporter proteins were reported in VBNC *V. harveyi*²⁰⁵, in contradiction with their upregulation in *V. cholerae*¹²⁸. Other strongly downregulated proteins in VBNC *V. harveyi* were SecA (protein translocase subunit), EF-Tu (elongation factor Tu), co-chaperonin and glutamine synthetase²⁰⁵. Strongly enhanced proteins in VBNC *V. harveyi* were succinyl-CoA, purine nucleoside phosphorylase, short-chain dehydrogenase and aspartate 1-decarboxylase²⁰⁵.

A possible mechanism based on the observations of the VBNC transcriptome and proteome and will be discussed in the next section (Chapter 1 Section 1.9).

In summary, some pathogens in the VBNC state express virulence genes and may cause pathology. The difference in the expression of genes in the VBNC state among different species indicates that the physiology of the VBNC state in each species could be different. These studies could provide clues into the mechanisms of the induction and survival of bacteria in the VBNC state but so far, a singular mechanism has not been proposed²⁰⁶. More studies are needed especially on the gene expression in the VBNC state of pathogens such as *S. Typhimurium*, where there is a sensitive animal model available to test virulence.

1.9 Possible mechanisms distinct to the general stress response

In the 35 years since the first report of the VBNC state in 1982, there has not been a single definitive mechanism reported to explain the phenomenon²⁰⁶. While no single 'VBNC pathway' has been identified, various processes, genes or gene products/factors that might contribute to the formation and/or maintenance of the VBNC state have been reported. The sections below discuss possible mechanisms involved in the VBNC state in bacteria.

1.9.1 Stringent response

Many general stress response proteins in bacteria were shown to influence the entry and maintenance of the VBNC state. The general stress response in bacteria is led by the 'alarmone' (p)ppGpp¹⁷³. The concentration of (p)ppGpp depends on RelA and SpoT proteins²⁰⁷. The molecule (p)ppGpp plays a role during starvation stress and upregulates the

expression of the stress sigma factor or stationary phase sigma factor, *rpoS* (σ^{38}/σ^s)^{208,209}. The sigma factor RpoS an important key in the general stress response²¹⁰ and essential for the survival of *E. coli* in stationary phase²¹¹. Studies have shown that mutants lacking *rpoS* enter more rapidly into the VBNC state in *E. coli* but died earlier in the VBNC state, compared with the parent strain expressing RpoS¹⁷⁷. The same phenomenon was reported in *S. enterica* where serovars with mutations in *rpoS*, or reduced levels of expression of RpoS, entered the VBNC state faster¹¹⁸. In *V. vulnificus*, *rpoS* expression was shown to be maintained throughout the VBNC state¹⁹⁰ suggesting its importance in maintenance of the VBNC state.

Ayrapetyan *et al.* (2015)¹¹⁴ proposed an alternative theory suggesting (p)ppGpp as a key factor in driving bacteria into the VBNC state¹¹⁴. These two theories contradict each other, because (p)ppGpp increases the expression of *rpoS* and RpoS delays the entry into the VBNC state but is also important for bacterial survival in the VBNC state²⁰⁹. In VBNC *V. cholerae*, the expression of *relA* increased, thus increasing (p)ppGpp levels^{166,212}. Given that (p)ppGpp and *rpoS* are on the frontline of stress response²¹⁰, it is expected that mutants of these genes would show susceptibility to stress conditions used for the induction of VBNC. It is interesting to note that (p)ppGpp was essential for the formation of persisters in the *E. coli* *hipA7* strain, which has a high propensity to form persisters²¹³.

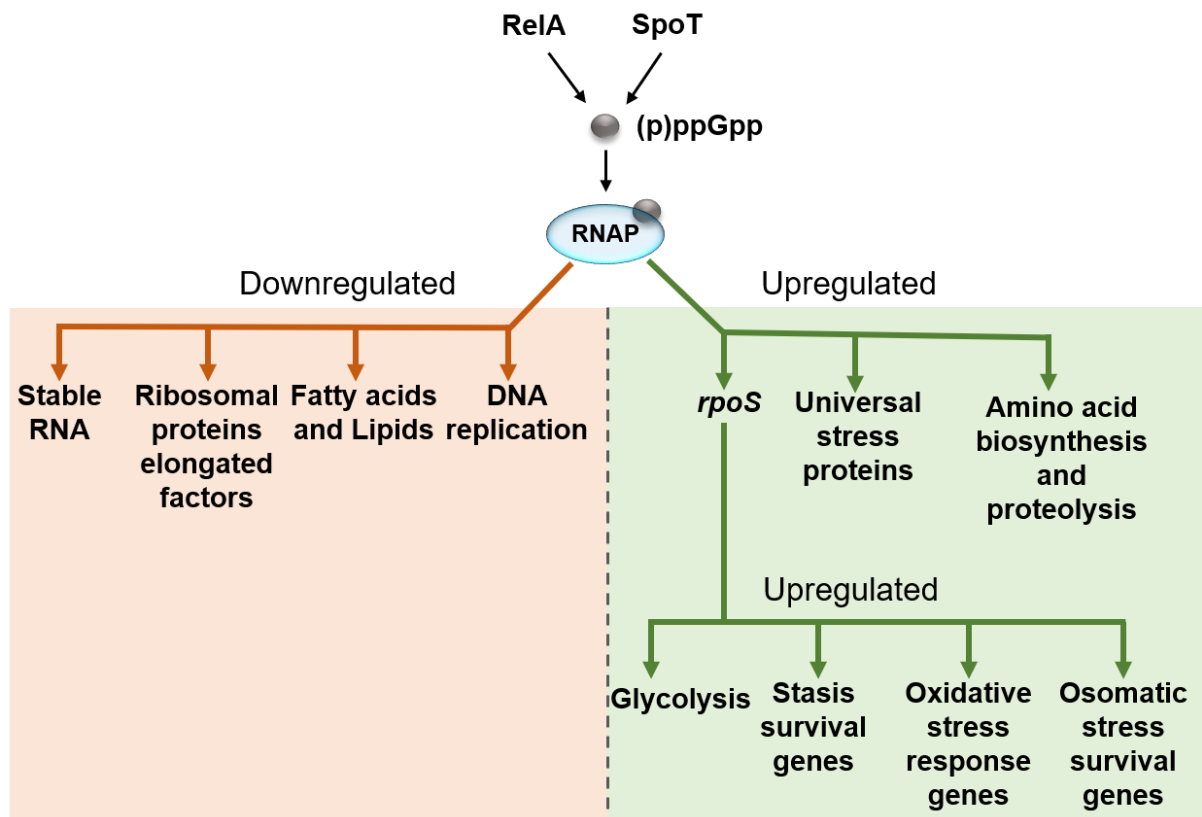


Figure 1.4: Schematic representation of effects of (p)ppGpp on genes. Concentrations of (p)ppGpp is affected by RelA and SpoT. RNAP interacts with (p)ppGpp resulting in downregulation and upregulation of various genes. [Based on data from Magnusson *et al.*²⁰⁹]

1.9.2 Role of catalases and OxyR

Apart from the general stress response genes, oxidative stress response genes play an important role in the formation of the VBNC state in some bacteria under specific conditions. For example, Kong *et al.*²¹⁴ studied the VBNC state in *oxyR* knockouts of *V. vulnificus*²¹⁴. OxyR regulates the response to oxidative stress in bacteria^{195,215}. *V. vulnificus* mutants lacking *oxyR* were found to be susceptible to oxidative stress¹⁴². Data from Smith and Oliver¹⁹⁰ supported the conclusion that VBNC cells in *V. vulnificus* were formed under oxidative stress, as low temperatures induced VBNC formation in the bacterium due to reduced catalase (KatG) activity. This theory was further strengthened by evidence from VBNC *Rhodococcus* spp. where the bacteria was stressed under low temperature conditions. The transcriptomic analysis of the VBNC *Rhodococcus* spp. showed that the catalases were downregulated in the VBNC state. Copper treatment in *Ralstonia solanacearum* caused an increase in intracellular concentrations of hydrogen peroxide, eventually making the bacterium VBNC⁹⁵. This hydrogen peroxide accumulation coincided with downregulation of catalase/peroxidase genes and upregulation of Dps (DNA protection during starvation), suggesting a reduced catalase or peroxidase activity⁹⁵. Cold-stressed VBNC *E. coli* were resuscitated by addition of catalase, suggesting that there was a connection between cold stress and oxidative stress^{216,217}.

1.9.3 ATP and kinases

Transcriptome analysis of VBNC *P. syringae* was performed by Postnikova *et al.*¹⁰⁷. Different mechanisms were proposed to explain the entry and maintenance of this bacterium in the VBNC state, based on the differential gene expression data. One of the unique mechanisms proposed for the induction of the VBNC state of *P. syringae* was based on downregulation of aconitate hydratase. Aconitase has a dual role in the Krebs citric acid cycle (TCA; cycle-tricarboxylic acid cycle) and as an iron-sulphur cluster containing protein that responds to oxidative stress²¹⁸. The TCA cycle is an efficient respiratory pathway important for energy production in bacteria during aerobic growth. The Postnikova *et al.* study suggests

that aconitase could be assisting in the oxidative stress response, induced by metal ion deficiency, thus reducing its role in the TCA cycle. In this theory, the VBNC state would occur as a consequence of reduced TCA cycle activity¹⁰⁷. A similar mechanism was proposed by Zhao *et al.*⁹¹ in *E. coli* O157:H7 where bacteria in the VBNC state were found to be reduced for TCA cycle activity, and instead moved to a less efficient fermentative metabolism⁹¹ resulting in a lower level of ATP production and induction of the VBNC state. Reduction in ATP may also lead to the formation of persisters in Gram-positive bacteria^{21,28}.

Transcriptome studies on VBNC *P. syringae* indicated the role of transcription factors MarR and LysR in triggering the VBNC state formation in the bacterium¹⁰⁷. Transcription factor AsnC might play a role in maintaining the VBNC phenotype in *P. syringae* since ABC transporters and histidine kinases were regulated by the above mentioned transcription factors¹⁰⁷. Kinases, including polyphosphate kinase 1, may play a role in the formation of VBNC *C. jejuni*²¹⁹.

1.9.4 Proteases

Proteases also appear to play an important role in modulating the concentration of proteins that might impact VBNC formation. In *V. cholerae*, regulator ToxR drives the bacterium into the VBNC state under alkaline pH conditions²²⁰. Strains that do not express ToxR, or in strains where ToxR is degraded by protease RspE, were shown to have lost culturability and changed their shape to coccoid, suggesting a VBNC state²²⁰. This may be the case in RpoS-mediated VBNC formation or maintenance since RpoS has a very short half-life of 1.4 minutes, before degradation by the protease ClpXP^{174,221}. ClpX was shown to play a role in the induction of VBNC cells in *Salmonella enterica*²²². Proteases were reported to play a role in persister formation through degradation of anti-toxins as shown in the case of Lon protease. Lon protease degrades anti-toxin HipB leading to persistence^{213,223,224}. This suggests the role of toxin-antitoxin systems (TA systems) in persisters. TA systems have not yet been reported to cause VBNC state²⁸.

All these possible mechanisms were based on experiments made in different bacterial species and so far, a consensus has not been achieved on single mechanism. Molecular studies were usually based on the separate analysis of RNA expression, proteome or knockouts studies, but not by combining all these approaches. A study that combined multiple 'omic' aspects would provide a more comprehensive analysis of the VBNC state.

1.10 Resuscitation

The word 'resuscitation' was first used by Rozak *et al.*⁶² to describe the regained culturability of VBNC *S. enteritidis*. Resuscitation, in this context, defines the recovery of culturability, metabolism and physiology from the VBNC state²²⁵. Many bacteria in the VBNC state appear to need a specific cue to leave dormancy. VBNC states of different bacteria were found to respond to different cues or 'resuscitation factors'^{42,165}. Resuscitation has not been reported in every human pathogen known to enter into the VBNC state. To-date, resuscitation conditions have only been identified for 26 species, or 17 genera out of 50 known human pathogens that have been shown to become viable but non-culturable⁴².

There has been considerable debate regarding "true resuscitation". Simple reversal of conditions or regrowth from residual culturable bacteria are not regarded by most VNBC researchers as 'true resuscitation'²²⁶ and injured and recovered cells might be misidentified as resuscitated VBNC cells⁴⁶. Sublethal damage has been reported to cause temporary loss of culturability in bacteria¹²⁹. Bogosian *et al.*¹⁰⁶ showed how hydrogen peroxide-sensitive cells appear as VBNC bacteria when plated on autoclaved rich media (which usually contains hydrogen peroxide in small quantities²¹⁴) mimicking resuscitation upon addition of catalase¹⁰⁶.

There have been several resuscitation factors and conditions investigated. Sodium pyruvate¹³⁰, catalase⁴⁹, resuscitation promoting factor (Rpf)^{59,63,227} and enterobacterial autoinducers⁵⁰ were some of the chemical and enzymatic resuscitation agents that have been identified. Temperature increases were known to resuscitate bacteria including *E. tarda*⁴⁸, *V. vulnificus*⁶⁸ and *S. Typhimurium*¹⁰⁰. The resuscitation factors can be complex cues

originating from the host. For examples, infecting *Acanthamoeba* could resuscitate VBNC *L. pneumophila*⁸⁵, and passage through the murine gut resuscitated VBNC *V. cholerae*⁶⁶. Thus, resuscitation factors may be simple or complex, and exist as single factors or in composite environments. Careful investigations can reveal the specific factors in the host that can resuscitate VBNC bacteria in a complex host environment as demonstrated by Senoh *et al.*²²⁸ where the VBNC *V. cholerae* were resuscitated when cocultured with eukaryotic cell-line HT-29. The factor that drove resuscitation was identified as human catalase²²⁸.

Resuscitation promoting factor Rpf, is a protein that was first identified in *M. luteus* as a bacteria resuscitation enzyme^{59,229}. Rpf was shown to play a crucial role in resuscitation of *M. tuberculosis*, *S. Oranienburg* and *Tomitella biformata*^{40,63,230}. Rpf belongs to a family of cell-wall lytic enzymes²³¹ which can function when added ectopically. The mechanism of action of Rpf as a resuscitation factor requires further investigation. Rpf is regarded as a potential target to fight drug-tolerant populations and to identify VBNC bacteria in environmental and clinical samples³⁰. Rpf is also known as YeaZ in *S. Typhimurium* and is modelled to have a cytosolic function as a tRNA threonylcarbamoyl adenosine modification enzyme^{232,233}.

1.11 *Salmonella* spp. and VBNC state: the known and unknown

Salmonella spp. have been shown to enter the VBNC state under different stress conditions. Several studies have focused on the induction, resuscitation and virulence of *Salmonella* spp. *S. Typhi* was shown to enter the VBNC state upon cold and copper stress and resuscitation from the VBNC state occurs upon addition of Tween 20 and catalase²³⁴. *S. Oranienburg* entered the VBNC state after osmotic stress and the bacterium can be resuscitated by addition of Rpf from *S. Typhimurium* LT2⁶³. Oxidative stress and chlorination can also drive *S. Typhimurium* into the VBNC state^{83,94}. Morishige *et al.*¹³⁰ reported that sodium pyruvate resuscitated VBNC *Salmonella enterica* serovar Enteritidis that was induced by oxidative stress. All these examples show the induction and resuscitation of various *Salmonella* spp. but do not detail the mechanisms, which have yet to be elucidated.

The work presented in this thesis studies the VBNC state of *S. Typhimurium*. None of the studies to-date have performed a complete proteomic analysis on VBNC *S. Typhimurium*. Murine infection studies on VBNC *S. Typhimurium* were conducted by Caro *et al.*⁶⁴, Oliver *et al.*⁸³ and Smith *et al.*^{92,167}. Though these infection studies have shown that the VBNC *S. Typhimurium* did not cause infection, the type and extent of stress under laboratory conditions and the influence of such factors on the extent of virulence of VBNC bacteria needs further investigation.

There are many unanswered and unsolved questions in understanding the VBNC state in *S. Typhimurium*. The mechanism of entry (and maintenance) of *S. Typhimurium* in the VBNC state is still unknown. A reliable method is required to generate VBNC *S. Typhimurium* to study the mechanism. Transcriptomic and proteomic study of the VBNC state is required to get insights into the mechanism of entry into the VBNC. Finally, the virulence properties of *S. Typhimurium* in VBNC state are still ambiguous. To resolve various aspects of the VBNC state in *S. Typhimurium*, the specific aims of the studies reported in this dissertation were to:

Aim 1: Identify the laboratory conditions that induce the formation of the VBNC state in *S. Typhimurium* and characterise various properties of the VBNC state.

Aim 2: Study transcriptomic and proteomic profiles of *S. Typhimurium* while in the VBNC state and to use this data to identify a potential mechanism(s) of entry and maintenance of VBNC *S. Typhimurium*.

Aim 3: Study the virulence of VBNC *S. Typhimurium* in the murine experimental model of invasive Salmonellosis.

CHAPTER 2

Materials and methods

2.1 Microbial techniques

2.1.1 Culturing of *Salmonella enterica* serovar Typhimurium SL1344

Salmonella enterica serovar Typhimurium SL1344 was obtained from the Strugnell laboratory glycerol stock library (University of Melbourne). Bacteria were resuscitated by streaking a loopful of frozen stock onto LB agar plates supplemented 50µg/ml of Streptomycin and incubated at 37°C overnight until colonies appeared. Single colonies were inoculated into lysogeny broth (LB) supplemented with appropriate antibiotics and grown at 37°C with constant rotation (180 rpm) until the desired growth phase was reached. *S. Typhimurium* mutant strains required the addition 50µg/ml Kanamycin to the growth medium. All the media used in this work were prepared by the Media Preparation Unit (MPU), The University of Melbourne at the Peter Doherty Institute for Infection and Immunity.

2.1.2 Measuring optical density of the bacterial cultures

Optical density of the bacterial cultures as an indication of growth was measured using a BioMate™ 3 series spectrophotometer (Thermo Spectronic). One ml of culture was pipetted into a cuvette and optical density at wavelength 600 nm (OD₆₀₀) was measured. The OD₆₀₀ readings (absorbance values) exceeding the value of 1.0 were further diluted and read again to be within the range of the sensitivity of the spectrophotometer.

2.1.3 Plating and counting of colony forming units (CFU) of *S. Typhimurium*

Colony forming units (CFU) of *S. Typhimurium* were calculated by plating 100µl of serially diluted cultures on LB agar plates containing 50µg/ml of streptomycin. Colonies were counted, and the number multiplied by 10 and by the dilution factor to obtain the number of CFU per millilitre. Plates with only 30-300 colonies were considered as countable.

2.1.4 Ethanol killing of bacteria

Bacteria were treated with ethanol (~96%) to generate dead bacteria. Exponentially growing bacteria (OD₆₀₀) were harvested by centrifugation at 11,500 x *g* for one minute at room temperature in a 1.5ml tube. The pellet was washed with one ml of phosphate buffer saline (PBS – containing 137mM NaCl, 2.6mM KCl, 100mM Na₂HPO₄, 2mM KH₂PO₄, pH 7.4)

and centrifugated at 11500 X *g* for one minute. One ml of ethanol (~96%) was added to the washed bacterial pellet and incubated on ice for at least 20 minutes. The ethanol treated bacteria were harvested by centrifugation at 11500 X *g* for one minute and resuspended in PBS to obtain the required cell density.

2.1.5 Bacterial transformations

Bacteria were transformed either by TSS method of transformation, as described by Chung *et. al.*²³⁵ or by electroporation.

2.1.5.1 Transformation using TSS (Transformation and storage solution) method

TSS was used to prepare chemically competent bacteria for transformation with desired plasmids. The bacteria were cultured until exponential phase and resuspended in TSS LB [LB with 10% (w/v) polyethylene glycol, 5% (v/v) dimethyl sulfoxide and 50mM magnesium chloride at pH 6.5]. An aliquot of 10 µl of plasmid DNA was incubated with 70µl of TSS enhancement buffer [10% (w/v) polyethylene glycol, 5% (v/v) dimethyl sulfoxide and 50mM magnesium chloride at pH 6.5] for 20 minutes on ice. The DNA in TSS-enhancement buffer was added to 100µl of bacteria in TSS LB and incubated on ice of 20 minutes followed by 30 minutes at room temperature and returned on ice for another 30 minutes. Sterile LB (800µl) was added and the bacteria were incubated at 37°C for one hour with shaking at 180 rpm. The transformed bacteria were then plated onto LB agar plates with appropriate antibiotics and incubated at 37°C overnight or until colonies appeared.

2.1.5.2 Electroporation

To prepare electrocompetent bacterial cells for electroporation the bacteria were grown until mid-logarithmic phase and harvested by centrifugation at 1500 X *g* for 10 minutes at 4°C. The bacteria were subsequently washed three times with 10 ml, 5 ml and 3 ml of ice cold Milli-Q water each time resuspending the bacterial gently and placing the tubes on ice. Finally, the bacteria were suspended in 500µl of ice cold Milli-Q water and used for electroporation with at least 100µg of DNA. Electroporation was completed using a Gene Pulser® (Bio-Rad) and a 0.1 cm gap sized electroporation cuvette followed by the addition of 1ml of sterile LB.

Electroporated bacteria were then plated onto LB agar plates with appropriate antibiotics and incubated at 37°C overnight or until colonies appeared.

2.1.6 Direct viable counting

The bacteria to be tested for active cell division were resuspended in LB medium diluted 1:1 using sterile PBS supplemented with 50µg/ml of streptomycin and 60µg/ml of nalidixic acid. The bacteria were cultured at 22°C for 9 hours with shaking (180 rpm) before harvesting by centrifugation (1520 X g) at 4°C for 10 minutes. The bacteria were stained with Live/Dead™ stain (Section 2.2.1) and imaged using a fluorescent confocal microscope (Section 2.2.2).

2.1.7 Disc diffusion assay

S. Typhimurium was cultured overnight in LB at 37°C with shaking at 180 rpm. To create the bacterial lawn, 100µl of the overnight culture was spread onto a LB agar plate. Antibiotic discs of 10, 25, 50, 75 and 100µg/ml concentrations were carefully placed onto the LB agar plate spread with *S. Typhimurium*. The plate was incubated at 37°C overnight until zones of inhibition were observed.

2.1.8 Growth curves

Growth curves presented in this work were done in a clear U-bottomed 96 well plate (Corning Costar®). To obtain the growth curve, an overnight culture was diluted 1:100 or until the OD₆₀₀ was ≥ 0.05 and then 200µl was dispensed into the wells and the plate read in a FLUOstar® microplate reader at OD₆₀₀. Values were graphed, x-axis for time (hrs) and y-axis for OD₆₀₀.

2.1.9 Generation of viable but nonculturable cells (VBNC) in *S. Typhimurium* SL1344 using oxidative stress

LB was inoculated 1 in 100 with an overnight culture and incubated to obtain exponentially growing bacteria with an OD₆₀₀ of 0.6 OD units. This culture was diluted to an OD₆₀₀ of 0.03 using sterile LB. Hydrogen peroxide (30% w/v) was added to obtain a 50mM final concentration. No hydrogen peroxide was added to the control samples. Both the control

sample and the hydrogen peroxide-treated sample were incubated for one hour at 37°C with shaking at 180 rpm.

The stress was stopped by adding 3 volumes of sterile ice-cold PBS to the H₂O₂-treated bacteria as well as the control. The bacteria were then harvested by centrifugation (2846 X *g*, 30 minutes) at 4°C, followed by gentle resuspension in 1 ml of ice-cold PBS and centrifugation for 10 mins to harvest the bacteria. The bacteria were finally resuspended in 1 ml of ice-cold PBS and used for further experimentation and confirmation of the VBNC state.

2.2 Confocal microscopy

2.2.1 Staining for fluorescence confocal microscopy with Live/Dead™ stain

Bacteria were stained using the Live/Dead® BacLight™ bacterial viability kit (Invitrogen/Molecular Probes) for confocal microscopy. The stain was diluted 1:10,000 in PBS and 100µl was added to 10µl of the VBNC or control bacteria (diluted 1:5 in PBS) and incubated for one hour on ice in the dark. The cells were harvested by centrifugation at 1520 X *g* for 10 minutes. The supernatant was gently removed and the stained bacteria were resuspended in 10µl of sterile Milli-Q® water.

0.5% agarose (w/v) was prepared in Milli-Q® water and used to immobilise the bacteria. 3 µl of molten agarose (0.5%) was mixed with 3µl of the resuspended and Live/Dead™-stained bacteria were mixed with a pipette on a glass plate. A coverslip was placed on the droplet of stained bacteria mixed with agarose and gently pressed so that the mixture spreads evenly. The sides were sealed with transparent commercial nail polish. The slides were rested for a minute in dark to let the nail-polish sealing solidify. The slides were then wiped with 80% ethanol (v/v) to remove any surface bacterial contamination before proceeding with the confocal microscopy.

2.2.2 Fluorescence confocal microscopy

A Zeiss LSM710 inverted fluorescence confocal microscope was used for observing fluorescently stained *S. Typhimurium*. The 488nm laser was turned on. The Alexa488 filter

was used to image the bacteria that were stained with SYTO9 (excitation maximum of 485nm and emission maximum of 501nm) and the filter AF647 was used to visualise and image bacteria stained by PI, propidium iodide (excitation maximum of 535nm and emission maximum of 61nm). The pinhole was set to 1 air unit before each acquisition of the image with all the filters used. An oil immersion lens with 67 times (67 X) magnification was used. The images of bacteria emitting green (SYTO9) and red (PI) fluorescence were obtained and then superimposed by the ZEN software used by the Zeiss LSM710 microscope to obtain a single image.

2.3 Fluorescence-activated cell sorting (FACS) analysis

2.3.1 Staining for FACS analysis

Antibody staining for FACS: Flow cytometry analysis of the bacteria was done by staining the *S. Typhimurium* with FITC-labelled, monoclonal antibody against the lipopolysaccharide (LPS) (Anti-ST LPS antibodies). This antibody was developed in the Strugnell lab previously. Both live and dead bacteria stained positively with the anti-ST LPS antibodies. Dead bacteria stained positively with PI. Unstained *S. Typhimurium* was used to separate the bacterial population from background based on size (forward scatter) and complexity (side scatter). Exponentially grown bacteria ($OD_{600} = 0.6$) were stained with anti-ST LPS antibodies and used as a 'compensation control' for anti-ST LPS antibodies. Ethanol-killed *S. Typhimurium* was stained with 100ng/ml of propidium iodide and used as a compensation control for PI staining. *S. Typhimurium* was stained with 1:200 diluted anti-ST LPS antibodies for 20 minutes on ice. Stained bacteria were harvested by centrifugation at $2846 \times g$ at $4^{\circ}C$ for 10 minutes. The harvested bacteria were resuspended in 100ng/ml of PI solution made in PBS.

Live/Dead™ staining for FACS: *S. Typhimurium* was stained with Live/Dead stain as described in section 2.2.1. Compensation controls were generated by staining 1) live, exponentially growing bacteria with SYTO9 in 0.5nM solution and 2) ethanol-killed bacteria

(Section 2.1.4) with 100ng/ml of PI. The test samples and the compensation controls were used for FACS analysis

2.3.2 Flow cytometry

Flow cytometry was conducted using an BD™ LSR II flow cytometer. The flow cytometer was cleaned with 10% bleach, 7% Decon 90 detergent and finally with Milli-Q water. The flow cytometer was then 'primed' once without any tube attached to the sample inlet and once with a Milli-Q water tube attached. The unstained bacterial sample was injected first using the sample inlet to identify the bacterial population. The compensation controls, bacteria stained with individual fluorophores, were then used to correct for any signal overlap from the emissions of the fluorophores. All samples were injected, and the data was collected from the flow cytometer. Finally, the flow cytometer was cleaned by injecting 10% (v/v) bleach, 7% (w/v) Decon 90 and Milli-Q water each for 5 minutes to completely remove any residual contaminants in the instrument. The collected data was analysed using a licenced FlowJo software.

2.4 Sample preparation for Transmission electron microscopy

S. Typhimurium samples were grown and treated as described in sections 2.1.4 and 2.1.9. Bacteria were harvested by centrifugation at 2846 X *g* at 4°C for 10 minutes. The pellets were resuspended in 0.1M sodium cacodylate buffer containing 2.5% glutaraldehyde for 90 minutes. The bacteria were centrifuged at 2846 X *g* for 10 minutes and the pellets were washed in 0.1 M sodium cacodylate buffer. The *S. Typhimurium* were then post-fixed using 1% osmium tetroxide for 90 minutes and washed in distilled water and left overnight at 4°C in 0.3% uranyl acetate. The bacteria were rinsed again in distilled water, dehydrated in a series of acetone, infiltrated and finally embedded with Epon resin. Sections of 70-80nm thickness were cut, stained with 10% (w/v) uranyl acetate and 2.5% (w/v) lead citrate. The resin embedded samples were viewed under a Phillips CM120 Transmission Electron Microscope (TEM) at 120 kV.

2.5 General molecular techniques

2.5.1 Agarose gel electrophoresis

1.2% (w/v) agarose (Sigma) was made using TAE buffer (40mM Tris base, 20mM acetic acid and 1mM EDTA). The agarose was boiled in the microwave until it was completely dissolved. 1µl of 10,000X SYBR® safe (Invitrogen) was added to 100ml of 1.2% agarose before pouring into a DNA casting gel tray. Five µl aliquots of DNA or RNA sample together with 1µl of 6X DNA loading dye (NEB) were loaded into the agarose gel and electrophoresed at 100 volts in 1X TAE buffer until the dye front reached close to the bottom end of the gel. A 1 kb DNA ladder was also loaded to estimate the size of the DNA or RNA separated on the gel. Gels were imaged using the Syngene G:BOX gel documentation imaging system (SYNGENE).

2.5.2 Plasmid isolation

All plasmids were isolated by using a commercially available Wizard Miniprep DNA purification system as per the manufacturer's instructions (Promega). The DNA was quantified using a Nanodrop ND-2000 spectrophotometer (ThermoFisher Scientific). Isolated DNA was frozen at -20°C for future experiments.

2.5.3 DNA isolation from agarose gel

A commercially available DNA isolation kit, Wizard® Gel and PCR Clean-up system (Promega) was used for DNA recovery from agarose gels. DNA fragments were visualised on a Safe Imager™ blue light transilluminator (Invitrogen) and excised from the gel with a sterile scalpel blade. Gel slices were weighed and processed according to the manufacturer's instructions by dissolving in the membrane binding buffer provided in the kit. The DNA was eluted in nuclease-free water and stored at -20°C.

2.5.4 Nucleic acid quantification

DNA and RNA isolated from sources including bacteria, agarose gels and PCR amplifications were quantified using a Nanodrop™ ND-2000 spectrophotometer

(ThermoFisher Scientific). The solute used to dissolve the nucleic acids was used to normalise the machine and the nucleic acids were quantified by selecting the parameters available for DNA and RNA, respectively.

2.5.5 DNA isolation for PCR and restriction digestion reaction

A commercially available Wizard® Gel and PCR clean-up system (Promega) was used to isolate DNA from PCR reactions. Equal volume of membrane binding buffer was added to the PCR product and processed according to the manufacturer's instructions. The product was then quantified and the quality was analysed (Section 2.5.4).

2.5.6 Genomic DNA isolation

Genomic DNA was isolated using 5PRIME Phase Lock Gel Light 15ml tubes (Quantabio). Bacteria were cultured overnight in 10 ml tubes in LB (unless specified otherwise) with appropriate antibiotic for selective growth. The bacterial pellet was washed with 10ml of sterile PBS twice to remove residual components of the growth medium. The bacteria were harvested by centrifugation at 2846 X *g* for 10 minutes after each wash. The washed bacteria were resuspended in 2ml of PBS followed by an addition of 25µl of 20mg/ml Protease K (Promega) and 1.5ml of 20% (w/v) SDS. Bacteria were incubated in this lysis solution for at least 60 minutes at 37°C. The bacterial lysate was transferred to a 15 ml Phase Lock Gel light tube and 3.5 ml of 1:1 ration of (v/v) phenol-chloroform mixture was added. The Phase Lock Gel tubes were mixed gently until a uniform milky solution was formed and then centrifuged at 2846 X *g* for 5 minutes at room temperature resulting in the separation of aqueous and organic phases. The top aqueous phase was carefully removed and transferred to a new Phase Lock Gel tube. Equal volume of chloroform-isoamyl alcohol solution (24:1 v/v) was added and the contents were mixed gently until they turned milky. Phases were again separated by centrifugation at 2846 X *g* for 5 minutes. The top aqueous layer was collected carefully and 1/10 volume of 3M sodium acetate solution (pH 5.2) was added. DNA was precipitated by adding 2.5X volume of ice-cold 95% ethanol. Tubes were gently mixed by inverting them and then frozen at -20°C for at least 2 hours. Precipitated DNA was then collected by centrifugation

at 16,000 X *g* for 10 minutes at 4°C. The supernatant was discarded, and the DNA pellet was washed with 70% ethanol. The pellet was air dried until most of the ethanol had evaporated before finally resuspending in nuclease free water.

2.5.7 SDS-PAGE

Protein samples were analysed using precast gels (Bio-Rad) with 4% acrylamide stacking gel and 12% acrylamide resolving gel. The gel was run with the Tris-glycine buffer (25mM Tris base, 192mM glycine, pH8.4). Gels were initially run at 100 V until the dye-front reached the edge of the resolving gel. The voltage was then increased to 120 V until the samples had reached the bottom of the gel. The gel was then stained using Kang's stain (0.02% Coomassie brilliant blue-G250 in 2% (w/v) phosphoric acid, 5% aluminium sulphate and 10% ethanol) over 6-8 hours and de-stained by Kang's de-stain (2% (w/v) phosphoric acid and 10% ethanol) until the gel background was sufficiently de-stained and the protein bands were visible.

2.6 Polymerase chain reaction (PCR)

Standard PCR was performed using Phusion High Fidelity DNA Polymerase (NEB) for gene amplification and Taq-polymerase (Promega) for confirmation of clones in a Veriti Thermal Cycler (Applied Biosystems). The volume of the PCR was also chosen based on the purpose of the experiment and varied from 20µl to 200µl. For every standard 20µl PCR reaction the following was added: 200 µM of dNTPs (deoxyribonucleotide triphosphates) (Promega), 1µM each of synthesised forward and the reverse primers (IDT), ~10µg template DNA, and 1 Unit of DNA polymerase was used in the recommended buffer from the DNA polymerase manufacturer. Sterile Milli-Q water was used in the PCR reactions when required. An initial denaturation temperature of 95°C, 5 minutes was used followed by an annealing temperature that varied for each reaction. The annealing temperature was estimated using an online tool, NEB Tm calculator, which calculated the primer melting temperature based on the sequence of the primers, the DNA polymerase used and the reaction buffer. The annealing temperature used for a given primer pair was at least 1°C less than the primer melting

temperature (T_m). The extension was done by shifting the temperature to 72°C for 1 minute/kb of the length of the expected product. A final extension was done at 72°C for 5 minutes before stopping the PCR reaction by reducing the temperature to 4°C.

2.6.1 Colony PCR

Colony PCR was performed to confirm the presence or absence of insert DNA in plasmid constructs of transformants and chromosomes of gene knock-out strains. A sterile 200µl pipette tip was used to pick a portion of a selected colony. The pipette tip was then dipped into a small PCR reaction tube with 12µl of sterile Milli-Q water. The same pipette tip was used for 'patching' the remainder of the selected colony onto an agar plate. The agar plate was then incubated overnight for the bacteria to grow (Section 2.1.3) and the PCR tube was boiled using a Thermal Cycler at 95°C for 10 minutes to lyse the bacteria. The PCR tubes were then centrifuged for 5 minutes at 11,500 X *g* to separate the cell debris. Ten µl of the supernatant was used as a template for the PCR reaction (Section 2.6).

2.6.2 Overlap Extension PCR (OE- PCR)

Overlapping PCR was done to generate the fragments for homologous recombination in gene gorging (Section 2.8). Homologous sequences to the target gene were 'stitched' on either side of the Kanamycin cassette using the overlapping PCR. All the primers used for PCR reactions are listed in the Table 2.1. The primer design strategy for OE-PCR is shown in Figure 2.1. Different steps involved in the PCR are discussed under Section 2.8 (Gene-gorging).

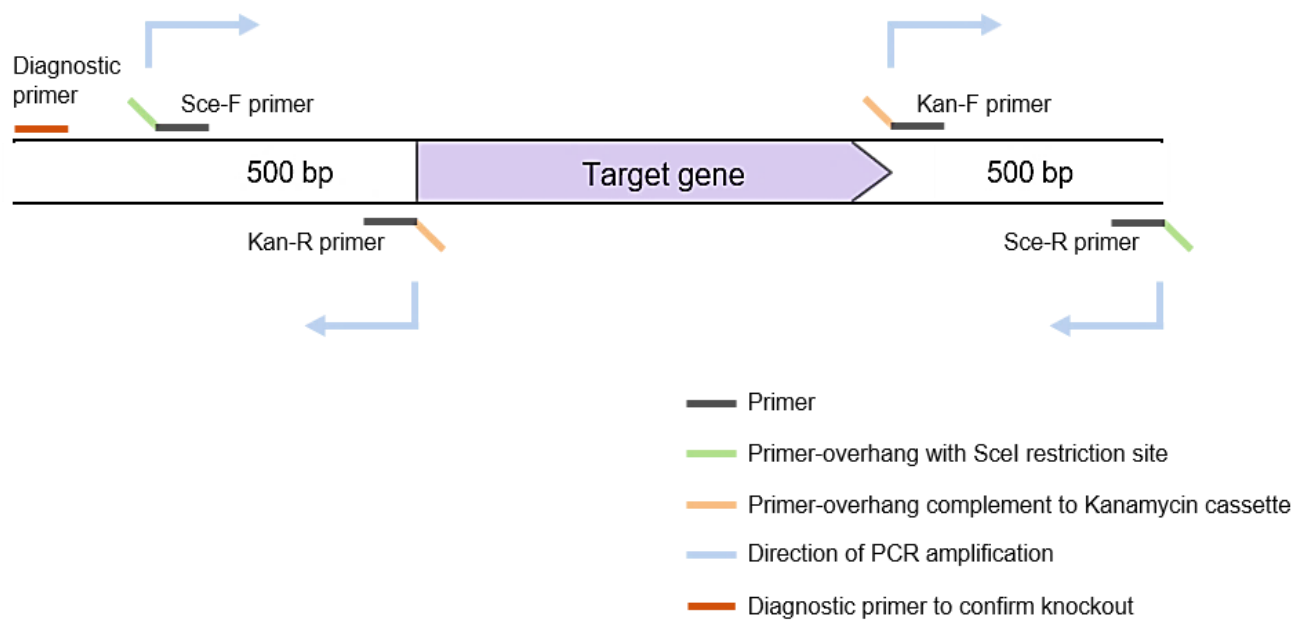


Figure 2.1: Schematic showing primer design strategy for overlapping extension PCR for gene gorging. PCR products obtained from Sce-F/Kan-R and Kan-F/Sce-R primer were used along with Kanamycin resistance cassette for an OE-PCR

Table 2.1: List of all the primers used in the study along with the description of their binding.

Primer name	Sequence (5'-3')	Description
RPFpET30bFor	CCATATGCGAATTCTGGCTATC G	Forward primer with <i>NdeI</i> restriction site to amplify <i>rpf</i> gene from <i>S. Typhimurium</i> SL1344 strain
RPFpET30bRev	CCTCGAGTTCTTTGCCGGAAG -3'	Reverse primer with <i>NdeI</i> restriction site to amplify <i>rpf</i> gene from <i>S. Typhimurium</i> SL1344 strain
T7 promoterFor	TAATACGACTCACTATAGGG	Forward primers that binds to the T7 promoter sequence of pET30 plasmids. Used to detect the insertion of clonal fragments at the multiple cloning site along with T7 terminatorRev
T7 terminatorRev	GCTAGTTATTGCTCAGCGG	Reverse primers that binds to the T7 promoter sequence of pET30 plasmids
cbiA(SceF)	TAGGGATAACAGGGTAATCGGT AAGGATGAGTAGATTAACG	Forward primer binding ~500 bps upstream of <i>S. Typhimurium</i> SL1344 <i>cbiA</i> gene. Primer includes a 5' overhang with a <i>SceI</i> restriction site
cbiA(SceR)	TAGGGATAACAGGGTAATGGTA TTGACGGCTTTGTAGG	Reverse primer binding ~500 bps downstream of <i>S. Typhimurium</i> SL1344 <i>cbiA</i> gene. Primer includes a 5' overhang with a <i>SceI</i> restriction site
cbiA(KanF)	CTAAGGAGGATATTCATATGAAC ACGTTTGCCAGCTATC	Overlapping primer binding to Kanamycin resistant cassette and sequence upstream to the start site of <i>cbiA</i> gene.
cbiA(KanR)	GAAGCAGCTCCAGCCTACACAC TGTGCCTGCAAGAATAAATG	Overlapping primer binding to Kanamycin resistant cassette and sequence downstream of the <i>cbiA</i> gene.

dhaB(SceF)	TAGGGATAACAGGGTAATGAAA CTTGAGAAGCGCTATC	Forward primer binding ~500 bps upstream of <i>S. Typhimurium</i> SL1344 <i>cbiA</i> gene. Primer includes a 5' overhang with a <i>SceI</i> restriction site
dhaB(SceR)	TAGGGATAACAGGGTAATCAAT CTGACGGTAGGTTTCC	Reverse primer binding ~500 bps downstream of <i>S. Typhimurium</i> SL1344 <i>cbiA</i> gene. Primer includes a 5' overhang with a <i>SceI</i> restriction site.
dhaB(KanF)	CTAAGGAGGATATTCATATGGC GGTTAATGACGTCAATGAC	Overlapping primer binding to Kanamycin resistant cassette and sequence upstream to the start site of <i>dhaB</i> gene.
dhaB(KanR)	GAAGCAGCTCCAGCCTACACAC ACTCCTTAACAAAGCCGTC	Overlapping primer binding to Kanamycin resistant cassette and sequence downstream of the <i>dhaB</i> gene.
marR(SceF)	TAGGGATAACAGGGTAATCATT CTGAAGCCGATAAACG	Forward primer binding ~500 bps upstream of <i>S. Typhimurium</i> SL1344 <i>marR</i> gene. Primer includes a 5' overhang with a <i>SceI</i> restriction site
marR(SceR)	TAGGGATAACAGGGTAATCTATT TTGCGCCACAGGTAAC	Reverse primer binding ~500 bps downstream of <i>S. Typhimurium</i> SL1344 <i>marR</i> gene. Primer includes a 5' overhang with a <i>SceI</i> restriction site
marR(KanF)	CTAAGGAGGATATTCATATGGC AATTTGTGAGCAATGTCATC	Overlapping primer binding to Kanamycin resistant cassette and sequence upstream to the start site of <i>marR</i> gene.
marR(KanR)	GAAGCAGCTCCAGCCTACACAG CGGAATGATTTTCATTGAAC	Overlapping primer binding to Kanamycin resistant cassette and sequence downstream of the <i>marR</i> gene.
pduG(SceF)	TAGGGATAACAGGGTAATGCAA TTGAATCGATGGTCC	Forward primer binding ~500 bps upstream of <i>S. Typhimurium</i> SL1344 <i>pduG</i> gene. Primer includes a 5'

		overhang with a <i>SceI</i> restriction site.
pduG(SceR)	TAGGGATAACAGGGTAATCATC TACGGCTGCTTTAACC	Reverse primer binding ~500 bps downstream of <i>S. Typhimurium</i> SL1344 <i>pduG</i> gene. Primer includes a 5' overhang with a <i>SceI</i> restriction site.
pduG(KanF)	CTAAGGAGGATATTCATATGAAC ATTCGCGATATTCCGTTC	Overlapping primer binding to Kanamycin resistant cassette and sequence upstream to the start site of <i>pduG</i> gene.
pduG(KanR)	GAAGCAGCTCCAGCCTACACAG CCAGCTATATATCGCATACG	Overlapping primer binding to Kanamycin resistant cassette and sequence downstream of the <i>pduG</i> gene.
pnp(SceF)	TAGGGATAACAGGGTAATGAAT TAGAGATCGGCGTCCTC	Forward primer binding ~500 bps upstream of <i>S. Typhimurium</i> SL1344 <i>pnp</i> gene. Primer includes a 5' overhang with a <i>SceI</i> restriction site.
pnp(SceR)	TAGGGATAACAGGGTAATCTTG CGCTAACTTATCACGG	Reverse primer binding ~500 bps downstream of <i>S. Typhimurium</i> SL1344 <i>pnp</i> gene. Primer includes a 5' overhang with a <i>SceI</i> restriction site.
pnp(KanF)	CTAAGGAGGATATTCATATGACT ATCTGCAGATGGGTCAGG	Overlapping primer binding to Kanamycin resistant cassette and sequence upstream to the start site of <i>pnp</i> gene.
pnp(KanR)	GAAGCAGCTCCAGCCTACACAC GTACTGGAATTTACGAACGATC	Overlapping primer binding to Kanamycin resistant cassette and sequence downstream of the <i>pnp</i> gene.
pocR-cbiAF	GCTATATTGATGCGCATTTGTCC	Forward primer binding upstream of the <i>cbiA</i> (SceF) primer. Used in pair with pKD4 KanF to confirm the <i>cbiA</i> gene knockout.
pduB-dhaF	GACGCGATGAACTTGAGAAG	Forward primer binding upstream of the <i>dhaB</i> (SceF) primer. Used in pair with pKD4 KanF to confirm the <i>dhaB</i> gene knockout.

pduD-pduGF	CGCATAAGAGCATTCTGCG	Forward primer binding upstream of the <i>pduG</i> (SceF) primer. Used in pair with pKD4 KanF to confirm the <i>pduG</i> gene knockout.
marC-marRF	GAACTGATGATCATTGCGATG	Forward primer binding upstream of the <i>marR</i> (SceF) primer. Used in pair with pKD4 KanF to confirm the <i>marR</i> gene knockout.
truB-pnpF	CGTGTGACGGAAGGTGAAGAC	Forward primer binding upstream of the <i>pnp</i> (SceF) primer. Used in pair with pKD4 KanF to confirm the <i>pnp</i> gene knockout.
pKD4 KanF	TGTGTAGGCTGGAGCTGCTTC	Forward primer to amplify Kanamycin resistant cassette
pKD4 Kan R	CATATGAATATCCTCCTTA	Reverse primer to amplify Kanamycin resistant cassette

2.7 Cloning

Cloning gene fragments included various techniques like PCR amplification, restriction digestion, ligation, colony PCR and DNA isolation. The gene or the DNA fragment to be ligated was amplified using forward and reverse primers using Phusion® high fidelity polymerase (New England Biolabs-NEB).

2.7.1 Restriction digestion

The amplified PCR products (insert) and the plasmids (vector) were restriction digested with 1 unit of the restriction endonuclease enzyme(s) for 1µg of the substrate DNA. PCR products were digested in the Phusion polymerase HF PCR buffer, if the enzymes were compatible and in appropriate buffer recommended by the restriction endonuclease manufacturer (Promega or NEB) when incompatible. The digestion was done by incubating the reaction mixture at 37°C for 1-3 hours. The reaction was stopped by heat inactivation of the enzymes at 65°C for 15 minutes. The restriction digested insert and vector were processed with a Wizard® DNA clean-up kit (Promega) to remove the buffer components and the enzymes (Section 2.5.5).

2.7.2 Ligation

The insert and vector were ligated by using at least 100µg of vector and at least 500µg of the insert in the presence of T4 DNA ligase (Promega) and ligase buffer (Promega) at room temperature for at least 4 hours. A ligation control reaction was set-up using only cut vector and no insert. Products for both the ligation reaction and the vector-only control reaction were used to transform bacteria using TSS transformation method or electroporation (Section 2.1.5).

2.8 Gene gorging method for knocking-out genes

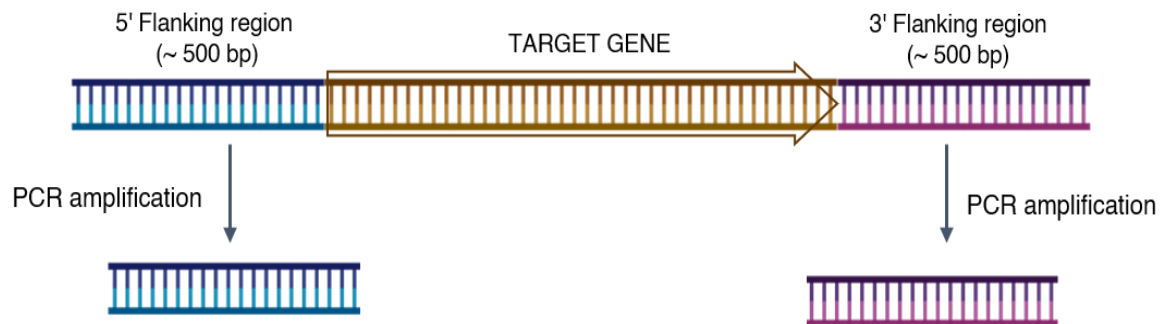
Gene gorging method²³⁶ was used to generate knockouts in *S. Typhimurium*. The method works by the principle of replacing the target gene with a selectable marker (Kanamycin resistance cassette from pKD4, in this case) by homologous recombination.

Using overlap extension PCR DNA fragments were constructed with the Kanamycin resistance cassette flanked by 500 bp long regions with homology to upstream and downstream regions of the target gene (Figure 2.1). Primers used for overlap PCR in gene gorging are listed in the table 2.1. Knockouts were confirmed using a diagnostic primer (Figure 2.1) followed by sequencing the DNA PCR products.

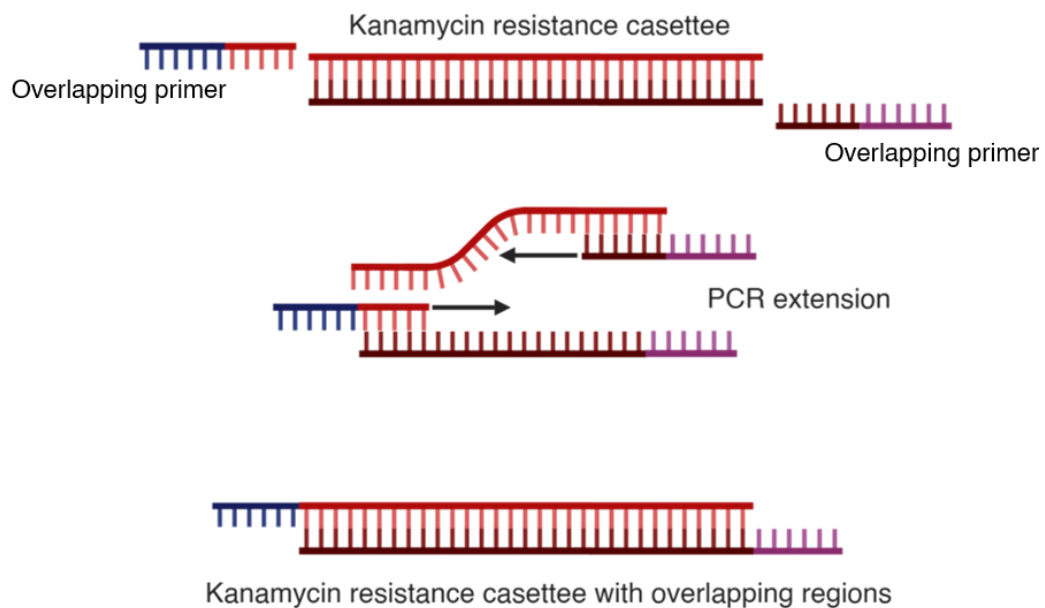
A temperature sensitive mutagenesis plasmid, pACBSR was used to transform *S. Typhimurium* SL1344. The pACBSR plasmid carries a chloramphenicol resistance gene (Cm^R) and contains a restriction endonuclease I *Sce*-I and a gene encoding λ -Red recombinase. I *Sce*-I and λ -Red recombinase can be induced by addition of arabinose. λ -Red recombinase promotes homologous recombination (of the dsDNA) between target gene in the genome and electroporated Kanamycin cassette flanked by homologous regions (Figure 2.2).

The *S. Typhimurium* SL1344 was cultured in the presence of 50 $\mu\text{g/ml}$ of streptomycin and 100 $\mu\text{g/ml}$ of chloramphenicol at 30°C with shaking at 180 rpm overnight. The λ -Red recombinase production was induced by addition of 0.2% (w/v) of L-arabinose. Competent bacteria were prepared for electroporation (Section 2.1.5.2). The 1 μg of kanamycin resistant cassette with homologous sequences to flanking regions of target gene was electroporated into the arabinose induced *S. Typhimurium* with pACBSR plasmid. The bacteria in 1ml of LB were incubated for one hour at 30°C and appropriate dilutions were plated on LB agar containing kanamycin at 37°C overnight. This selects for colonies which retained the kanamycin resistance cassette by homologous recombination. Colonies obtained were selected and replica plated on chloramphenicol + kanamycin containing LB agar plate and only kanamycin plate to select for loss of chloramphenicol resistance from pACBSR. Clones were confirmed by PCR amplification with a 'diagnostic' primer that recognised a sequence outside the homologous region paired with a primer that recognised the kanamycin resistant cassette. The PCR product was sanger sequenced.

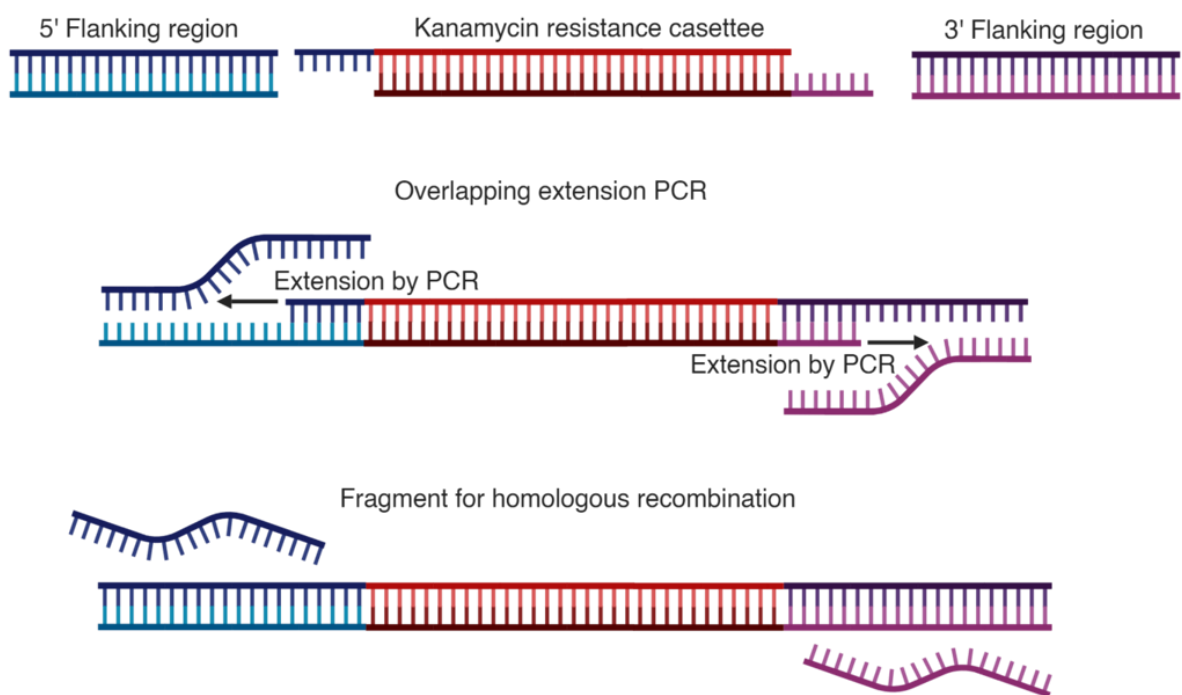
Step 1: PCR amplification of 5' and 3' flanking regions



Step 2: PCR amplification of Kanamycin cassette using overlapping primers that share homology with the flanking regions



Step 3: Overlapping extension PCR with products from step 1 and step 2



Site-directed mutagenesis by λ Red recombinase

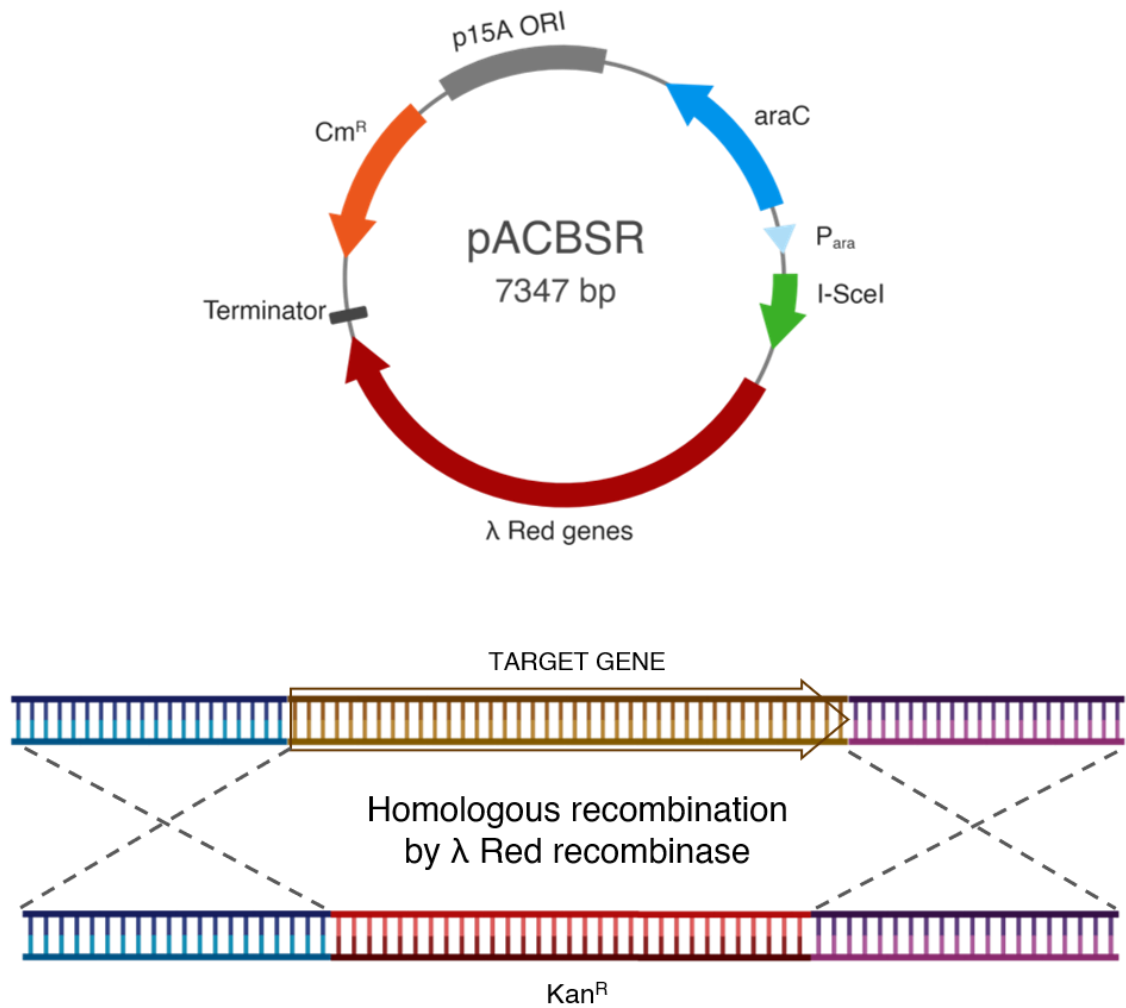


Figure 2.2: Schematic representation of the overlapping PCR used for generating knockouts using gene gorging. Homologous fragments were generated by individual PCR reactions and overlapping PCR was used to combine them to generated a kanamycin resistance cassette containing linear fragment with flanking regions homologous to the target gene. Homologous recombination was initiated upon induction of the λ -Red genes in pACBSR with arabinose.

2.9 RNA isolation

Cultures of *S. Typhimurium* were harvested by centrifugation at 2846 X *g* for 10 minutes at 4°C. The bacteria were washed with RNAprotect® reagent and harvested by centrifugation at 11,500 X *g* for 2 minutes. Five hundred µl of acidic TRIzol™ was added to the bacterial pellet and vortexed for 2 minutes. Equal volume of chloroform was added, mixed gently and centrifuged at 11,500 X *g* for 10 minutes to separate aqueous and organic phases. The top aqueous layer was carefully separated using a filtered tip. One tenth volume of 3M sodium acetate, pH 5.2 together with 2.5 volumes of ice-cold ethanol was added to the separated aqueous phase and RNA was precipitated at -20°C overnight. The RNA pellet was obtained by centrifugation at 11,500 x *g* for 20 minutes at 4°C. The RNA pellet was air-dried and dissolved in 87.5µl of RNA-grade, nuclease-free water.

DNase treatment was performed using the RNase-free DNase set (Qiagen) by adding 10 µl of RDD buffer and 2.5µl of DNase I. . RNEASY mini kit (Qiagen) was used to after the DNase treatment to remove the DNase I from the RNA preparation by following the manufacturer's instructions.

2.10 Nucleic acid sequencing

2.10.1 Whole genome sequencing

Genomic DNA was isolated using Phase Lock Gel Light (5 Prime) kit as described in the section 2.5.6. The whole genome was sequenced using MiSeq system (Illumina) at the MDU sequencing facility, (Microbiology Diagnostic Unit, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity. The sequenced *S. Typhimurium* SL1344 strains were compared with the reference genome *S. Typhimurium* SL1344, EMBL accession number FQ312003 (submitted by Wellcome Trust Sanger Institute Pathogen Genomic Group).

2.10.2 Plasmid and PCR DNA sequencing

Plasmids and PCR products were purified using a Wizard® Gel and PCR Clean-up system (Promega) as described in the section 2.5.3 and section 2.5.5. ABI PRISM Big Dye

Terminators Cycle Sequencing Kit (Applied Biosystems) .The samples were processed for Sanger sequencing at The Centre for Translational Pathology, Department of Pathology, The University of Melbourne.

2.10.3 Sequencing data analysis

The data obtained from the DNA sequencing was analysed using Finch TV (Version 1.4.0) on a computer running Windows 10 operating system. The software was used to analyse the chromatogram files from the sequencing. The DNA sequence was converted into FASTA format using Finch TV and then analysed by BLASTn (Basic local alignment search tool for nucleotides) program on the official website of NCBI (National Centre from Biotechnology Information).

2.10.4 RNA sequencing

RNA from *S. Typhimurium* was isolated using the protocol described in the Section 2.9. The isolated RNA was then separated on an Agilent LabChip Nano as per the manufacturer's instructions to measure the integrity. The whole RNA was then sent to the Ramaciotti Centre for Genomics, The University of New South Wales, Sydney. The ribosomal RNA was removed using a Ribo-Zero® rRNA removal kit (Epicentre) followed by 75 base pair end sequencing using a NextSeq 550 sequencing system (Illumina). The data analysis was done as described in the Figure 4.3.

2.11 Protein expression and purification

Recombinant *S. Typhimurium* resuscitation promoting factor (STrRpf) protein was expressed by ligating the gene *rpf* from *S. Typhimurium* SL1344 into a pET30b plasmid to generate the construct pET30bSTrRpf, which was used to transform *E. coli* Tuner™ (DE3) strain. The bacteria were grown until exponential phase ($OD_{600} \sim 0.6$) and induced with 50μM IPTG. The induced and uninduced control *E. coli* Tuner™(DE3) pET30bSTrRpf were incubated at 37°C shaking at 180 rpm for 3 hours. The bacteria were harvested by centrifugation at 1300 x *g* for 10 minutes, washed with PBS, resuspended in buffer containing 50mM sodium phosphate monobasic (NaH_2PO_4) and 300mM of sodium chloride (NaCl),

binding buffer, for sonication. The sonicated samples were centrifuged at $11,500 \times g$ at 4°C for 10 minutes to separate the insoluble fraction (pellet) and the soluble fraction (supernatant). The pellet was resuspended in the binding buffer. Both the soluble and insoluble fractions from uninduced control and IPTG induced samples were separated on SDS-PAGE to identify any protein induction. STTrPpf was observed in the soluble fraction of the induced samples.

The soluble supernatant was diluted using the above-mentioned binding buffer and loaded onto a column packed and 2 ml of nickel NTA (Ni-NTA) resin. The resin was washed with binding buffer. The diluted protein sample was loaded in small volumes (1-2 ml) onto the column and the flow-through was collected. A wash was given binding buffer containing 20 mM imidazole to remove any non-specific protein binding. STTrPpf was eluted with binding buffer containing 200mM of imidazole and a final elution was carried out in the presence of 500 mM imidazole. Several elution fractions were collected and separated on SDS-PAGE to identify the fraction(s) containing the pure STTrPpf (identified bases on the molecular weight ~ 25 kDa).

2.12 Western blotting

Western blotting was carried out to perform a puromycin incorporation assay (section 2.17). The proteins were quantified using a Bradford assay (section 2.16) and $2\mu\text{g}$ of protein was loaded into each well and the samples were separated by SDS-PAGE (as described in the section 2.5.7). The samples were then transferred on to a PVDF membrane using a NuPAGE® Novex® system (Invitrogen) in the presence of NuPAGE® LDS Sample Buffer (Thermo Fisher Scientific) with an addition of $500\mu\text{l}$ of NuPAGE antioxidant. The proteins were transferred on from the SDS-PAGE gel to the PVDF membrane using 80 volts of electric charge was applied for 60 minutes. The PVDF membrane was blocked with 5% (w/v) skimmed milk for one hour at room temperature on a rocking platform. A 1:1000 dilution of anti-puromycin antibody 12D10 (Merck Millipore) prepared in 5% (w/v) skimmed milk was applied as the primary antibody to the blot for one hour at room temperature on a rocking platform. The PVDF membrane was then washed with PBS containing 0.01% Tween-20, three times

at room temperature on a rocking platform. A 1:5000 dilution of anti-mouse IgG HRP (Horse radish peroxidase) tagged prepared in 5% (w/v) skimmed milk and applied to the blot for one hour followed by three washes with 0.01% Tween-20. Electrochemiluminescence (ECL) western blotting substrate (BioRad) was applied to the blot according to manufacturer's instructions. MF-ChemiBIS gel imaging system was used to visualise the protein bands recognised by anti-puromycin antibody.

2.13 mEoS2 fluorescent tagging on RpsB

A small ribosomal protein RpsB was tagged with mEoS2. A synthetic DNA fragment containing *meos2* was generated with flanking homologous regions to C- terminal end of *rpsB*. Homologous recombination using λ -Red recombinase on pACBSR plasmid was performed following the protocol used for gene-gorging (section 2.8). The knock-in of the *meos2* gene was tested by PCR with primers that bind at to *rpsB* and *meos2* followed by sequencing.

Table 2.2: Sequence of synthetic fragment for mEoS2 fluorescent tag knock-in. The region in green shows the *meos2* sequence attached to the c-terminal end of *rpsB* (red colour). The blue sequence is homologous to the inter-genic region downstream to *rpsB*.

Synthetic fragment for mEoS2 knock-in

```
CTAAACGCGCTGCAAGCGAAGCGGTGAAAGAAGCTGCTAACAGCTGCGACCAGTTCTTCGTG
AACCATCGCTGGCTGGGCGGTATGCTGACTAACTGGAAAACAGTTCGTCAGTCCATCAAACG
TCTGAAAGACCTGGAAACTCAGTCTCAGGACGGTACTTTCGAAAAGCTGACCAAGAAAGAAG
CGCTGATGCGTACTCGTGAGCTTGAGAAACTGGAAAACAGCCTGGGCGGTATCAAAGACATG
GGCGGTCTGCCGGACGCTCTGTTTCGTTATCGATGCTGACCACGAGCACATTGCTATCAAAGA
AGCGAACAACTGGGTATCCCGGTATTTGCTATCGTTGATACCAACTCTGATCCGGATGGCG
TTGACTTCGTTATCCCGGGTAACGATGACGCAATCCGTGCTGTTAGCCTGTACCTGGGCGCT
GTCGCTGCAACCGTTTCGTGAAGGCCGTTCTCAGGATCTGGCTTCCCAGGCGGAAGAAAGCTT
CGTAGAAGCCGAGGGATCCATGAGTGCTATCAAGCCGGACATGAAAATTAATTTACGCATG
GAGGGCAACGTGAATGGTCATCATTTTGTATTGACGGCGATGGTACGGGAAAGCCCTTTG
AAGGAAAGCAATCAATGGACTTGGAAGTTAAAGAGGGAGGTCCGTTGCCCTTCGCTTTTGA
TATCTTGACAACCGCGTTCCATTACGGAAACCGTGTTTTTGCAAAGTATCCTGATAACATTC
AAGACTACTTTAAGCAGTCTTTTCAAAGGGTTACTCATGGGAACGCTCTTTGACATTGAG
GACGGGGGTATCTGCATCGCTCGTAACGATATCACGATGGAAGGCGATACTTTTTACAACA
AAGTGCGCTTCTACGGGACCAATTTCCCGCTAACGGGCCAGTGATGCAGAAGAAGACGC
TGAAGTGGGAGCCCTCGACTGAAAAAATGTATGTCCGGGATGGTGTTTTAACTGGCGATAT
CCACATGGCCTTGTTATTGGAAGGTAATGCGCATTACCGTTGTGACTTTCGTACCACGTACA
AGGCTAAGGAGAAAGGTGTCAAGTTACCAGGTTACCATTTTCGTGACCATTCATTGAGAT
TCTGAGCCACGATAAGGATTATAATAAAGTAAAGTTGTACGAGCATGCCGTAGCGCATTTCG
GGACTGCCGGACAACGCTCGTCGTTAATAAGGCTTGATAACTCCCTAAAATAGTTTCGAGTTG
CAGGAAGGCGGCAAGCTCGAGAATTCGCGGAGCTTACATCAGTAAGTGACCGGGATGAGC
GAGTGAAGGCAACGCGCCTGCGGCTCGAAATATGAAGGGGAGAGCCCTTATAGACCAGGTA
GTATCACGTTTGGTTAGGGGGCCTGTATATGGCCCCCTTTTCACTTTTATATCTGTGCGGTTT
GATGCCGGGCAGATCAAATCTTTCGAGGATTTTGAATGGCTGAAATAACCGCATCCCTGGTA
AAAGAGCTGCGTGAGCGTACCGGCGCAGGCATGATGGATTGCAAAAAGCGCTGACTGAAG
CGAATGGCGACATTGAGCTGGCAATCGAAAACATGCGTAAGTCCGGCGCTATCAAAGCGGC
GAAAAAAGCAGGCAACGTGGCTGCTGACGGCGTAATCAAAACCAAAATCGACGGCAACGTG
CTTTCATTCTGGAAGTTAACTGCCAGACTGACTTCGTGGCTAAAGATGC
```

2.14 Ribosomal fractionation

Culturable and VBNC *S. Typhimurium* were harvested $1500 \times g$ for 10 minutes at 4°C and the pellet was resuspended in 500 μl of sterile PBS. BeadBeater (BioSpec) was used at maximum setting for 40 seconds two times with a short incubation on ice. The cell-lysate was centrifuged at $11,500 \times g$ for 10 minutes at 4°C to remove cell debris. The lysate was then applied gently onto a 70% (w/v) sucrose in a solution containing 0.5M KCl, 20mM of magnesium acetate, 1mM DTT (Dithiothreitol) and 20mM tris-acetate, pH8 in an ultracentrifuge tube. The tube is ultracentrifuged at $790000 \times g$ overnight at 4°C . The ribosomal pellet was very gently resuspended in a buffer containing 1.5M ammonium sulphate, 20mM of magnesium acetate and 20mM tris-acetate, pH8. A sucrose gradient was prepared using a low-density solution containing 10% (w/v) sucrose and high-density 40% (w/v) sucrose in a buffer containing 10mM ammonium sulphate, 15mM of magnesium acetate, 50mM KCl and 20mM tris-acetate, pH 8. The gradient was prepared manually using a peristaltic pump by mixing the low- and high-density sucrose solutions. The resuspended ribosomes were loaded onto the sucrose gradient and centrifuge in a hanging bucket centrifuge at $284061 \times g$ for at least 3 hours at 4°C . The samples were analysed using a Gradient fractionator (Brandel). The UV absorption was monitored at 260nm to identify various ribosomal fractions.

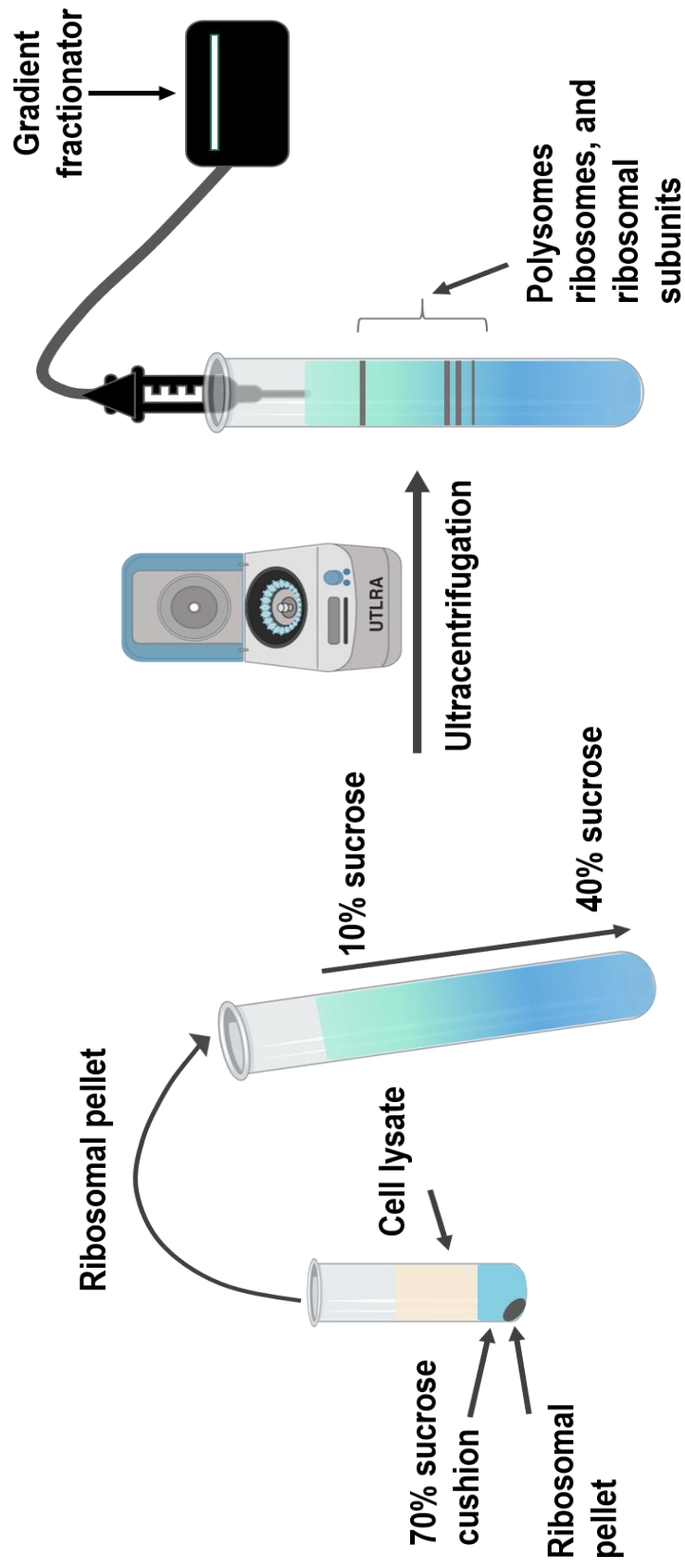


Figure 2.3 Schematic representation of the ribosomal fractionation experiment. Ribosomal concentration in culturable and VBNC *S. Typhimurium* was studied using ribosomal fractionation

2.15 Sample preparation for mass spectrometry

S. Typhimurium samples were prepared in five biological replicates and the cells were harvested by centrifugation at 2850 x *g* for 10 minutes and lysed using a lysis buffer containing 4% (w/v) SDS and 1mM DTT. The bacteria were incubated at room temperature for 30 minutes and centrifuged at 11500 x *g* to remove cell debris. A 100µl fraction of the supernatant was diluted by adding 100 µl of Milli-Q water followed by addition of 800µl of ice-cold acetone for protein precipitation. Samples were incubated at -20°C overnight. Precipitated proteins were collected by centrifugation at 4000 x *g* for 10 minutes at 0°C. Any residual ethanol was air dried.

The samples were processed using for 'in-solution' digestion with trifluoroethanol (TFE). The isolated protein pellet from the last step was resuspended in 100µl of Milli-Q water and 100µl of TFE was added to the sample. Following that, 2µl of 1M TCEP [tris(2-carboxyethyl)phosphine] was added. Freshly made chloroacetamide (CAA) was added to a final concentration of 40mM. The samples were heated at 45°C for 45 minutes and cooled to room temperature. The sample were diluted by adding 100mM Tetraethylammonium bromide (TEAB), pH8.5 and the samples were digested adding modified trypsin (Promega) 50 times the amount of total protein. Samples were digested overnight shaking at 1000 rpm at 37°C.

Peptides were isolated according to the StageTips protocol by Rappsilber *et al.* (2007)²³⁷. Sterile pipette tips packed with ODS (octadecylsilane) or C18 were used to trap digested peptides. The C18 material was prepared by washing three times with a buffer prepared in Milli-Q water containing (all v/v) 0.1% trifluoroacetate, 80% acetonitrile and three times with buffer containing 0.1% trifluoroacetate, 2% acetonitrile. The trypsin digested peptides were acidified by adding formic acid (0.1% final concentration) and loaded into the ODS packed pipette tip and using a syringe. The C18, with the peptides loaded, was washed three times with 0.1% trifluoroacetate, 2% acetonitrile containing buffer. The peptides were eluted two time with 80 µl of 0.1% trifluoroacetate, 80% acetonitrile. The elutes were concentrated using a speed vacuum centrifuge. The dried peptide samples were resuspended

in 0.1% Trifluoroacetate and LC-MS/MS analysis was done on Thermo NanoLC/ OrbiTRAP ELITE ETD mass spectrometer. The data was averaged between the replicates and multi-way ANOVA test was done to identify the statistically significant result.

2.16 Protein quantification

Proteins were quantified using Bradford method. A known concentration of BSA was used to make serial dilutions and a standard curve was generated by using BSA (NEB) in amount ranging from 0-100µg in a volume of 50µl. The assay was performed in a 96 well plate (Corning® Costar®). A volume of 200µl of Bradford reagent (BioRad) was dispensed into each well with the standard curve or the test sample wells. Duplicates were made for both standard curve and the test samples. Presence of proteins would lead to a colour development. Absorbance at 595 nm was measure at the end of the colour development after 5-10 minutes. A standard curve was plotted using the absorbance from the samples with known protein concentrations. The concentration of the unknown test samples was calculated by plotting a linear regression.

2.17 Puromycin incorporation assay

A concentration of 10µg/ml puromycin was added to *S. Typhimurium* cells and incubated for 1, 3, 6 hours or overnight. The treated samples were then lysed using 10% SDS containing solution and boiling for 5 minutes at 95°C. The cell debris was separated by centrifugation at 11,500 x *g* and the amount of protein was quantified using Bradford assay. The samples were separated on SDS-PAGE gel (Section 2.5.7) and processed for Western blotting (2.12).

2.18 Statistical analysis

Statistical analyses were conducted using licenced Graph Pad Prism (Version 8.1 for Windows 10). A two-tailed unpaired t-test was used for comparison between two groups. To compare more than two groups or test parameters multi-way ANOVA analysis was applied with Bonferroni's post-test. A *p*-value of <0.05 was considered as significant in the studies.

2.19 Animal infection

Mice were weighed before infection procedures. The bacterial dose for infection was determined by CFU counts for culturable bacteria and by FACS in case of VBNC bacteria. The mice were infected *via* intravenous injection of bacteria in 200µl. Mice infected with *S. Typhimurium* SL1344 were monitored at least two times every day by weighing. The animals were monitored, weighted and euthanised, when required, in accordance with the animal ethics policies governing the experiment.

2.19.1 Mouse strains

C57BL/6 mice were bred in the animal breeding facility at the Peter Doherty Institute, The University of Melbourne. Both the knockout mice strains used in this study, RAG2^{-/-} common-γ-chain^{-/-} double knockout, and the interferon gamma (IFN-γ^{-/-}) knockout, were generated in the C57BL/6 background.

2.19.2 Intravenous infection

The mice were transferred to a clean animal cage and placed over a warm box with a light bulb for approximately 10 minutes to cause vasodilation. The mouse placed in a restraining chamber and the tail was surface sterilized with 80% (v/v) ethanol. The inoculum was to be injected was prepared in 200µl volume and injected into the tail vein using a 27-gauge needle (Terumo).

2.19.3 Determining the bacterial burden in mice

At the end of the experiment or in accordance to the Animal Ethics requirements (e.g. body weight reduced by 15% compared with the start of the experiment), the mice were euthanised using carbon dioxide (CO₂) asphyxiation. After death, the mice fur was sprayed with 80% ethanol and the liver and spleen were collected into a sterile homogenising bag. The organs were transported on ice and mashed. Five millilitres of sterile cold PBS was added and the organs were homogenised using a Stomacher 80 (Seward Medical) in sterile stomacher bags. Dilutions of the homogenised organs were plated on LB agar containing 50µg of

streptomycin. The plates were incubated at 37°C for 16-18 hours and the colonies were enumerated.

CHAPTER 3

Induction and characterisation of
Salmonella Typhimurium in a viable but
non-culturable state

3.1 Introduction

Salmonella enterica serovar Typhimurium (S. Typhimurium) is a flagellated, facultative anaerobe that can survive in diverse environments. In Australia, 72% of Salmonellosis cases occur via oral ingestion of contaminated food or drinks making this route of infection significantly more common than direct person to person transmission²³⁸. Studies investigating the physiology and infection of S. Typhimurium are typically conducted under standard laboratory conditions, which favour balanced replication and exponential growth. It is assumed that a large proportion of these infections are caused by contamination of foods by vegetative bacterial cells. During transmission via the oral-faecal route, the bacterium is exposed to unfavourable stress conditions such as acidity²³⁹. It is therefore important to study the physiology and pathogenicity of S. Typhimurium under stress conditions.

Bacteria can enter a viable but non-culturable (VBNC) state of metabolic dormancy when subjected to various environmental stresses, during food processing and food preservation^{30,165,240}. For example, it was shown that S. Typhimurium can become VBNC with exposure to chlorine, which is a very common and widely used disinfectant⁸³. High salt conditions that are widely used for food preservation can also induce the VBNC state in *Vibrio*²⁴¹ and *Salmonella*⁶³. *Salmonella enterica* serovar Enteritidis enters the VBNC state when subjected to severe oxidative stress using hydrogen peroxide⁹⁴.

S. Typhimurium is exposed to oxidative stress at different stages during host infection. Soon after entering the stomach, S. Typhimurium is subjected to acid stress and 'gastric oxidative stress'²⁴²⁻²⁴⁴. Following cell entry, the host innate immune system may release a respiratory burst^{245,246} and upon internalisation by macrophages, S. Typhimurium is further subjected to oxidative stress as the *Salmonella*-containing vacuole (SCV) is formed²⁴⁷. Oxidative stress was adopted in this study to drive S. Typhimurium into a VBNC state because it is a stress factor naturally encountered by the bacterium.

The lack of culturability under conventional '*in vitro*' rich growth conditions and proof of viability are two important characteristics of the VBNC state²⁹. To understand and study the physiology of VBNC *S. Typhimurium* SL1344, it is essential to generate sufficient biomass of VBNC bacteria for study, using a reproducible method *in vitro*. A proposed hypothesis called a 'dormancy continuum' states that VBNC bacteria form a fraction of the population in any given environment, including the routine laboratory culture^{28,114}. Techniques to separate the VBNC cells from live and dead cells have been described¹²⁶. Unfortunately, such methods don't generate sufficient VBNC cell biomass or cell number for further molecular analysis, nor provide the necessary control over the extent of the stress. The ability to control *in vitro* stress has the advantage of improving VBNC cell quantity and producing a relatively homogenous population of stressed cells for further study.

The aims of the research presented in this Chapter were 1) to develop a robust method utilising oxidative stress to drive *S. Typhimurium* into a VBNC state and to 2) characterise the VBNC state for viability, antibiotic resistance and cellular ultrastructures using microscopy and FACS. These analyses can help to identify and differentiate the VBNC state from actively dividing bacteria. This further characterisation may assist in the detection of the VBNC bacteria in a mixed population of actively growing, persistent and VBNC bacteria under *in situ* conditions, such as within the SCV.

3.2 Results

3.2.1 *S. Typhimurium* loses culturability following hydrogen peroxide-induced oxidative stress

Hydrogen peroxide (H_2O_2) was used to study the effect of oxidative stress on *S. Typhimurium* and the generation of VBNC cells. Factors including cell density, cell mass, and organic compounds from the media may affect the level of *in vitro* oxidative stress on each bacterium. Hence, various concentrations of H_2O_2 were tested to standardise a method to generate VBNC *S. Typhimurium*. Bacterial cells were cultured in Luria Bertani Broth (LB) until exponential phase was reached (approximately $\text{OD}_{600\text{nm}}$ 0.6). LB, considered as an enriched medium, supports the growth of *Salmonella* spp. very efficiently²⁴⁸. Approximately 10^7 colony forming units (CFUs) were stressed by the addition of H_2O_2 for one hour at 37°C with shaking at 180 rpm. The control sample for the stress conditions was treated similarly but without the addition of H_2O_2 . After incubation, the cells were chilled on ice followed by the addition of ice-cold PBS, and the cells were harvested by centrifugation (Figure 3.1).

Both the unstressed control and the stressed samples were plated onto LB agar plates and incubated overnight or longer at 37°C with colonies counted to determine the effect of oxidative stress on bacterial viability, measured as CFUs (Figure 3.2). The CFUs decreased with increasing concentrations of H_2O_2 above 1 mM. The addition of 5mM H_2O_2 reduced the viable count by approximately 1000-fold. No CFUs were detected after exposing the bacteria to 50mM of H_2O_2 for one hour. (Figure 3.2).

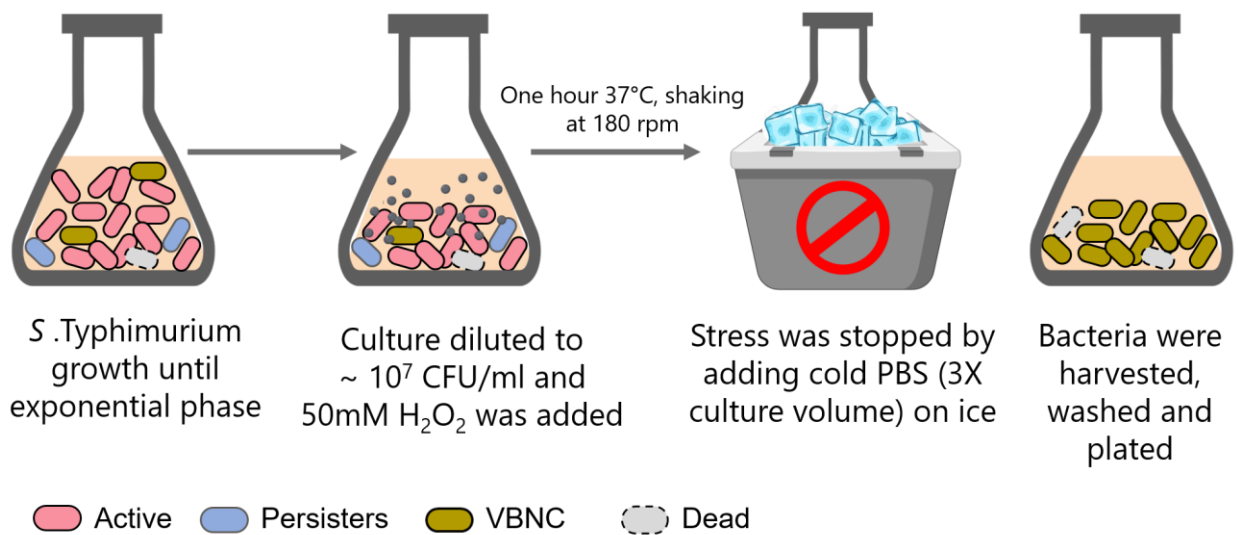


Figure 3.1: Schematic representation of the standardised protocol for the generation of VBNC *S. Typhimurium* using hydrogen peroxide. The detailed method is described in Chapter 2, section 2.1.9.

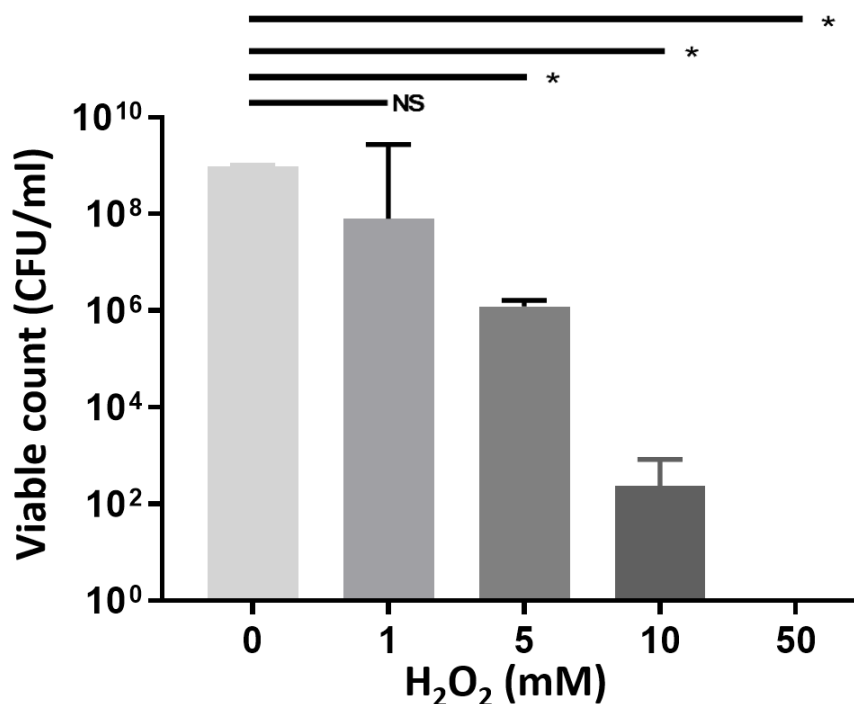


Figure 3.2 Effect of H₂O₂ concentration on *S. Typhimurium* viability. *S. Typhimurium* were incubated with an increasing concentration range of H₂O₂ (0-50mM) for 1 hour at 37°C with shaking at 180 rpm. After incubation, the cells were washed with sterile PBS, serially diluted and spread onto LB agar plates to enumerate the bacteria, expressed as colony forming units (CFUs). Data here represents 2 technical replicates, with error bars representing the standard deviation. Statistical significance ($p < 0.05$) was determined using a one-way ANOVA (* < 0.05).

3.2.2 Non-culturability of oxidative stressed *S. Typhimurium* is independent of routine laboratory media

It has been shown that media alone cannot restore the vegetative growth of the VBNC bacteria; instead, specific cues are necessary for the bacteria to 'resuscitate' from the dormant VBNC state^{3,14,19}. The culturability of 50mM H₂O₂-stressed *S. Typhimurium* was studied in a variety of routine bacteriological media to test whether they were truly dormant even in different nutrient rich and minimal growth media. In addition to LB, five different broth media were used to study the growth of H₂O₂-stressed bacteria. These media were tryptic soy broth (TS), terrific broth (TB), M9 minimal media (M9) supplemented with histidine, Mueller-Hinton broth (MH) and Brain Heart infusion broth (BHI). Unstressed controls were used to determine the normal growth of viable *S. Typhimurium* in these media. The growth curve for each condition was generated over 18 hours at 37°C with shaking.

None of the six media tested supported the growth of the H₂O₂-stressed sample, but the unstressed control samples showed an increase in turbidity, over time, in all culture media tested (Figure 3.3). M9 minimal media supplemented with histidine supported the growth of the untreated control bacteria but had the longest lag phase. This experiment showed that the H₂O₂-stressed bacteria, which lost culturability in LB, did not show any signs of resuscitation or culturability in other media. This suggested that the bacteria may have lost their culturability and their non-culturability was independent of the media tested.

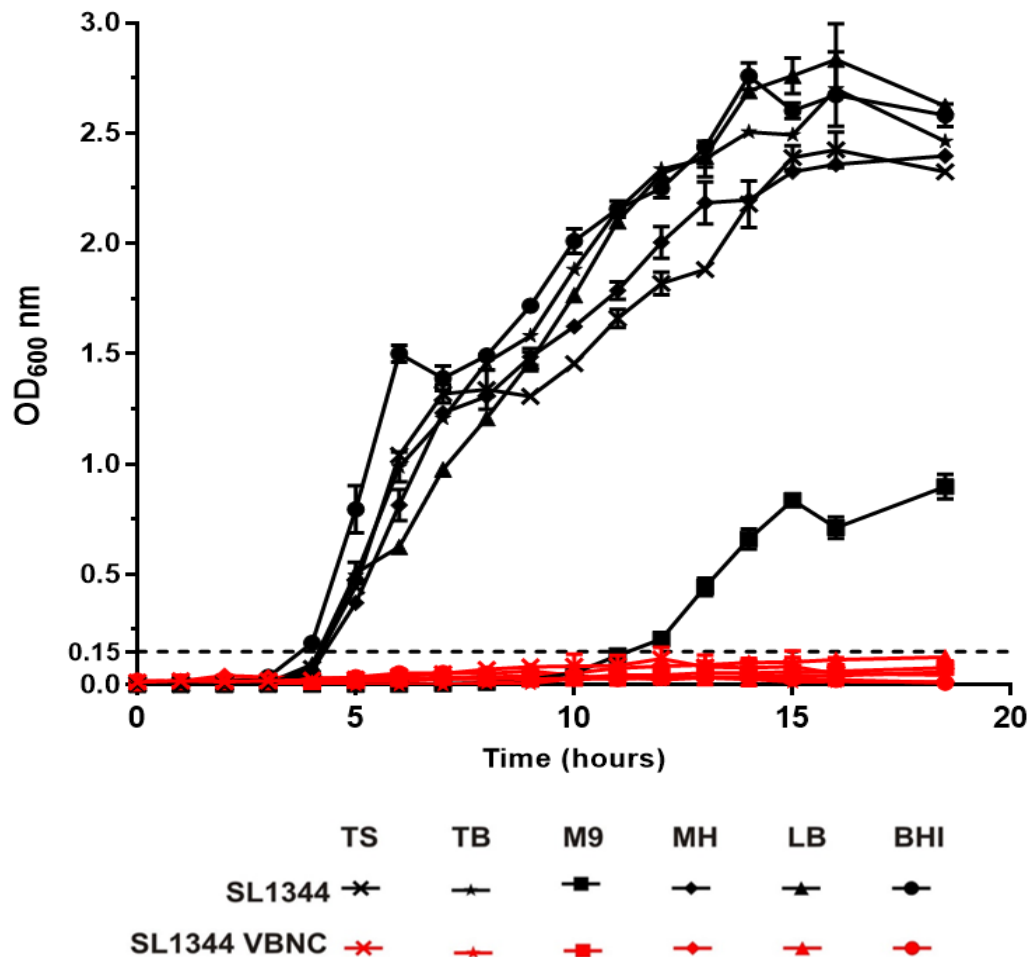


Figure 3.3 Growth of stressed and non-stressed *S. Typhimurium* in various routine media. The unstressed *S. Typhimurium* SL1344 (black) and stressed but nonculturable ‘VBNC’ (red) cells were incubated in five different broth media for 18 hours at 37°C with shaking. The absorbance of the cultures was measured at one-hour intervals as OD₆₀₀. The media used were as follows: Tryptic soy broth (TS); Mueller Hinton broth (MH); Terrific broth (TB); Lysogenic broth (LB); M9 minimal media (M9); and Brain Heart Infusion (BHI). Data here represents 2 technical replicates with the error bars showing standard deviation.

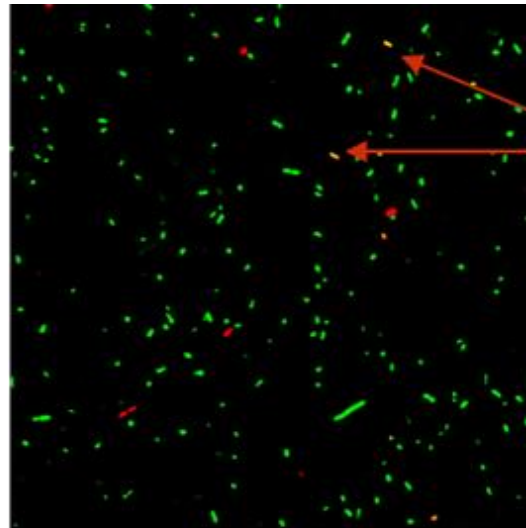
3.2.3 Viability assessment of oxidative stressed, nonculturable *S. Typhimurium* by Live/Dead™ confocal microscopy

While it was determined that H₂O₂ stress induced *S. Typhimurium* could not be revived under various standard laboratory conditions, the viability of the H₂O₂-stressed cells was unknown. To test the viability of the stressed but non-culturable *S. Typhimurium* a Live/Dead stain™, which is a mixture of Propidium Iodide (PI) and SYTO9, was used. Both the stains are nucleic acid binding in nature²⁴⁹, however, PI cannot enter an intact bacterial cell membrane and therefore will only stain bacteria with a non-patent outer envelope, defined as ‘dead’ cells¹³⁶. SYTO9 can penetrate the intact bacterial cell membrane and has a different emission spectrum (498-501 nm) to PI (610-620 nm). Under fluorescent confocal microscopy, the PI positive dead cells appear red in colour and live cells appear green. The relative concentrations of PI and SYTO9 bound by the DNA of the bacteria differentially fluoresce depending on whether the bacteria are dead (red) or alive (green) or damaged (orange)²⁴⁹.

Bacteria in control and stressed samples were treated with Live/Dead stain™ using the protocol provided by the supplier (*BacLight*™ L34856) and visualised using a Zeiss710 fluorescent confocal microscope using channel A488 for SYTO9 and channel A568 for PI. Exponentially growing *S. Typhimurium* were used as a live cell control and 80% (v/v) ethanol treated *S. Typhimurium* as the dead cell control.

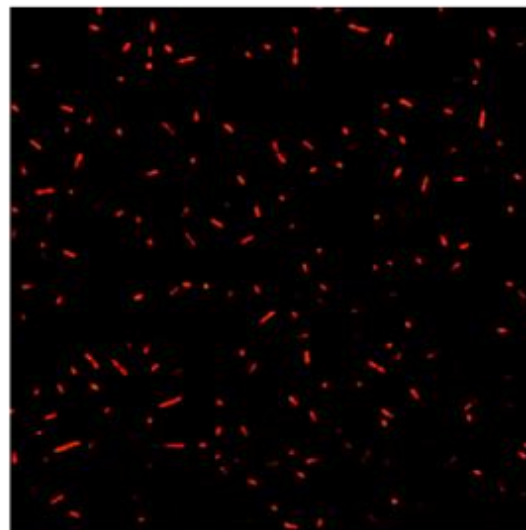
The unstressed live control sample of *S. Typhimurium* was stained with Live/Dead™ *BacLight*® stain and analysed under the fluorescent confocal microscope. Most of the bacteria appeared green, which indicated their viability (Figure 3.4A). All the ethanol-treated *S. Typhimurium* cells were red in colour demonstrating they were stained by PI and hence ‘dead’ (Figure 3.4B). This observation, in conjunction with the lack of CFUs, suggest that the bacteria were truly non-viable. *S. Typhimurium* stressed with 50mM H₂O₂ for one hour carried a mixture of red and green bacteria (Figure 3.4C). The presence of green fluorescent bacteria despite the lack of CFUs indicated that the oxidative stressed *S. Typhimurium* might have entered a viable but non-culturable (VBNC) state.

**A) Exponentially
growing bacteria**

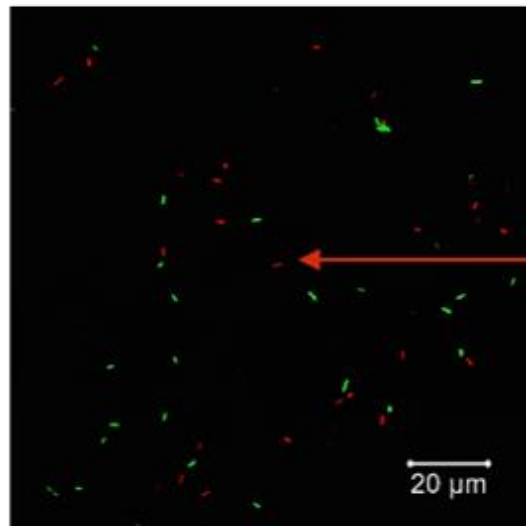


Damaged

**B) Ethanol killed
bacteria**



**C) Oxidative stressed
bacteria**



Damaged

Figure 3.4 Confocal microscopy analysis of stressed and unstressed *S. Typhimurium*.

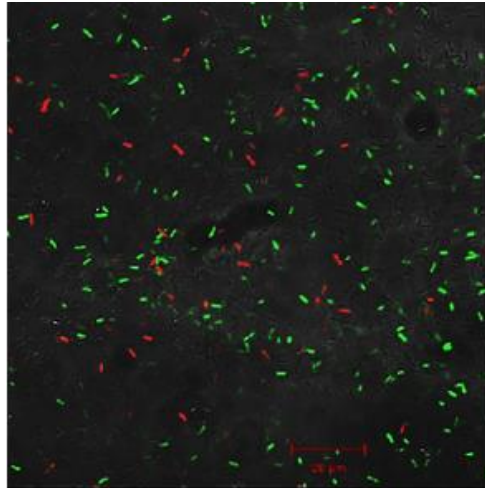
S. Typhimurium cells in different culturable states were harvested by centrifugation and stained with the Live/Dead™ stain containing propidium iodide (PI) and SYTO9 and analysed using a fluorescent confocal microscope. Live cells appeared green, damaged cells were orange and dead cells were red in colour. A) A representative image of the Live/Dead profile of *S. Typhimurium* isolated from an exponential culture. B) A representative image of *S. Typhimurium* cells that were treated with ethanol for 20 mins on ice prior to staining and microscopy analysis. C) A representative image of the Live/Dead populations from unculturable, oxidatively-stressed *S. Typhimurium*.

3.2.4 Direct viable counting (DVC) method using nalidixic acid coupled with Live/Dead™ staining reveals membrane integrity and viability of stressed *S. Typhimurium* cells

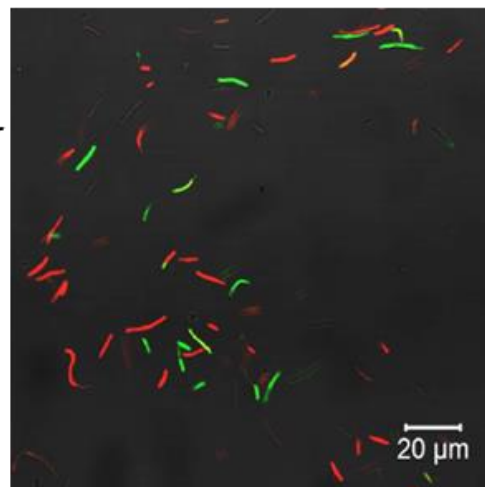
After observing the bacteria using Live/Dead™ stain to study their viability, another common technique employed to define the viability of nonculturable bacteria, the direct viable count (DVC), was used. This method relies on the principle that actively dividing bacteria are nalidixic acid susceptible. These nalidixic acid susceptible bacteria do not divide but will elongate²². This elongation can be visualised using a fluorescent confocal microscope.

Stressed and unstressed cells were incubated with nalidixic acid (60µg/ml) in LB overnight then diluted 1:1 with sterile PBS to obtain the DVC of the bacterial samples. The nalidixic acid treatment was followed by staining using the Live/Dead™ stain for visualisation under a fluorescent confocal microscope. Exponentially growing bacteria untreated with nalidixic acid were used as a control to show the normal size of bacteria (Figure 3.5A). The unstressed bacteria in the presence of nalidixic acid were either stained green ('alive') or red ('dead') but were elongated to about 5 to 10 times longer than bacteria from a logarithmic culture, suggesting that nalidixic acid caused elongation in actively dividing bacteria (Figure 3.5B). In contrast, there was no elongation of the bacteria treated with 50mM H₂O₂ when cultured in the presence of nalidixic acid suggesting the bacteria were not actively dividing by virtue of lack of elongation but were still alive by virtue of their green fluorescence when stained with Live/Dead™ stain (Figure 3.5C). This data provides further support that the unculturable oxidatively stressed bacteria may have entered a VBNC state.

A) Live bacteria- Control



B) Unstressed bacteria after NA treatment



C) Stressed bacteria after NA treatment

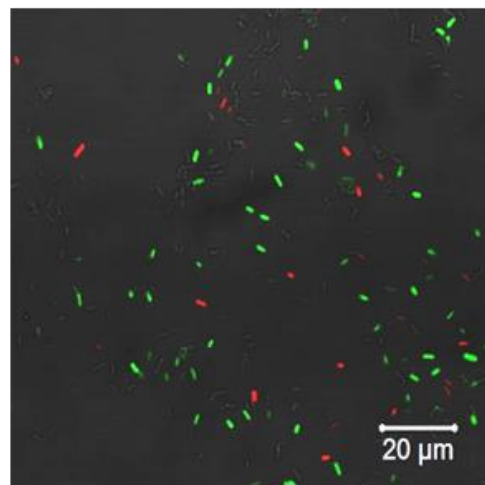


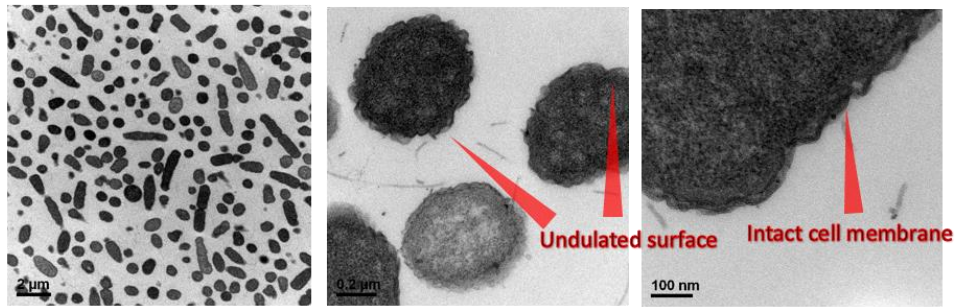
Figure 3.5 Direct viable count of *S. Typhimurium* after nalidixic acid treatment. The direct viable counting method was used in conjunction with Live/Dead™ stain to demonstrate the replication and viability of stressed and unstressed *S. Typhimurium*. A) Unstressed, live *S. Typhimurium* were incubated in LB broth with shaking at 22°C overnight. The cells were subsequently harvested via centrifugation, stained with the Live/Dead™ stain and analysed using fluorescent confocal microscopy. Both B) unstressed and B) oxidatively-stressed *S. Typhimurium* were treated with 60µg /ml of nalidixic acid (NA) in the presence of LB broth and shaking at 22°C overnight followed by analysis using the Live/Dead™ stain in conjunction with fluorescent confocal microscopy. The fluorescence of the bacteria represents live or dead status with red cells defined as 'dead', orange cells as 'damaged', and green as 'viable'.

3.2.5 Transmission electron microscopy of VBNC *S. Typhimurium* shows an intact cell membrane

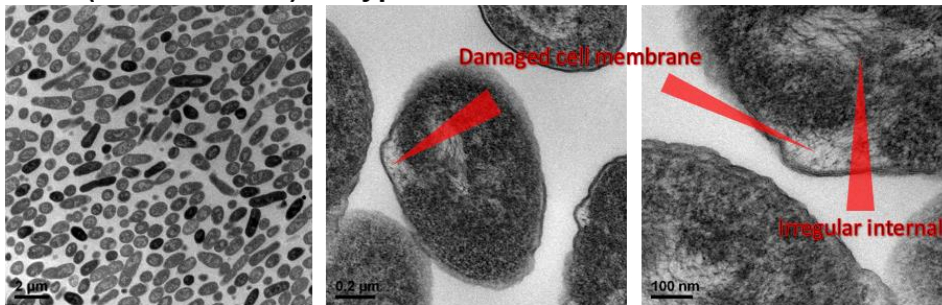
Sustaining cell membrane integrity is a key feature of maintaining bacterial cell viability²⁵⁰. Most staining strategies identify dead bacteria use membrane impermeable stains like propidium iodide (PI) which do not stain bacteria with fully intact membranes¹³⁶. Transmission electron microscopy (TEM) of bacteria that have undergone fixation, resin embedding and thin sectioning may provide additional ultrastructural information about the integrity of the bacterial envelope.

Transmission electron micrographs showed exponentially growing unstressed *S. Typhimurium* to have a complex, undulated surface with an intact cell structure (Figure 3.6A) compared to that of the ethanol killed 'dead' bacteria (Figure 3.6B). The unstressed *S. Typhimurium* (experimental control) has an intact cell membrane and cell structure (Figure 3.6C). The VBNC *S. Typhimurium* showed an intact cell structure (Figure 3.6D) that was comparable to the unstressed control samples (Figure 3.6C). The VBNC bacteria possessed an intact cell membrane but showed a different internal structure to that of vegetatively growing bacteria. The VBNC cells typically have an electron-lucent, pale region in the middle of the bacterial cytoplasm surrounded by a darker electron dense region. This experiment showed that the VBNC cells of *S. Typhimurium* appear to have an intact cell membrane structure providing further support for their viability. This observation aligns with the observations that they were negative for staining with propidium iodide, a stain that typically only stains dead cells with a permeable cell membrane.

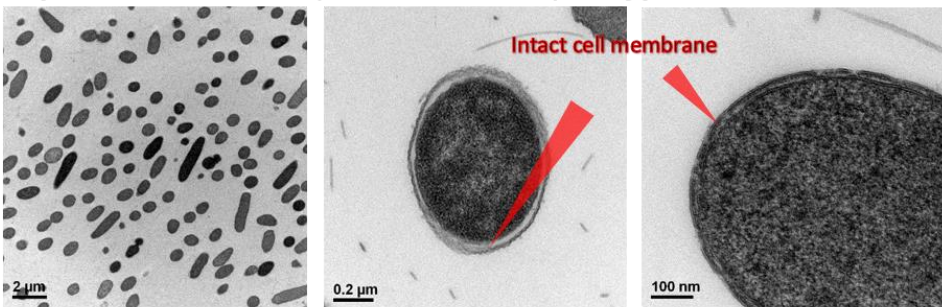
A) Exponentially growing *S. Typhimurium*



B) Dead (ethanol killed) *S. Typhimurium*



C) Experimental control (oxidative stress) *S. Typhimurium*



D) Viable but non-culturable *S. Typhimurium*

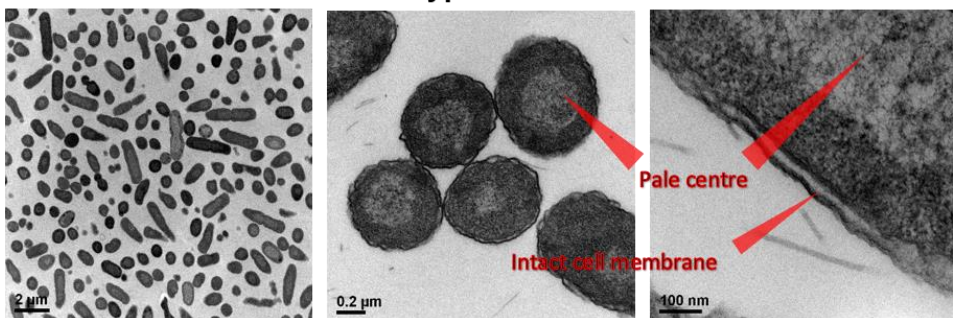


Figure 3.6: Transmission electron micrographs of culturable and VBNC *S. Typhimurium* embedded, sectioned, stained and imaged. A) Exponentially growing *S. Typhimurium* displayed mostly electron dense staining of the cytoplasm with an undulating cell surface and visibly intact cell membranes, B) Ethanol-killed *S. Typhimurium* displayed irregular areas of

electron-lucent staining of the cytoplasm and damaged cell membranes, C) *S. Typhimurium*, which was treated similar to the oxidative-stressed sample but without the addition of hydrogen peroxide displayed mostly electron dense staining of the cytoplasm with intact membranes, and D) VBNC *S. Typhimurium* displayed a cytoplasm with electron-lucent centre surrounded by electron-dense staining and intact cell membranes. The ultrathin sections were stained with 10% (w/v) uranyl acetate and 2.5% (w/v) lead citrate.

3.2.6 Analysis of VBNC *S. Typhimurium* viability by flow cytometry

In order to visualise and quantitatively assess membrane integrity, VBNC formation was analysed using flow cytometry. This method is more sensitive and quantitative than confocal microscopy and is useful in assessing population homology. *S. Typhimurium* bacteria were either unstressed or stressed with 50mM H₂O₂ for one hour, followed by staining with anti-*S. Typhimurium* lipopolysaccharide (LPS) antibodies, specific to *S. Typhimurium*, and PI for analysis by FACS. An aliquot of each culture was spread onto LB agar plates to determine culturability and VBNC state.

As the anti-LPS antibodies available in the laboratory (Raju Mantena, PhD Thesis, The University of Melbourne) were not fluorescent, a fluorophore, fluorescein isothiocyanate (FITC), was conjugated to the anti-*S. Typhimurium* LPS antibodies. This fluorophore had a peak emission spectrum of 515-545 nm. To identify the dead bacteria, PI, which has a peak emission spectrum in the red channel (620 nm), was used. All the bacteria (dead and alive) present in the sample would be positive for the anti-*S. Typhimurium* LPS antibodies (Anti-ST LPS Ab), but only the dead bacteria would be positive for PI staining. The FACS machine can differentiate and quantify the degree of staining (and hence fluorescent emission) of both dyes and can estimate the number and percentage of live vs dead bacteria in each of the samples.

An unstained sample of bacteria was first assessed by two kinds of light scatterings i.e. forward scatter (FSc) and side scatter (SSc) (Figure 3.7, coloured panels). The forward scatter is used as a proxy for bacterial size and the side scatter is used as a measure of their granularity²⁵¹. These parameters can separate the bacterial population from the background

'noise' contributed by particulates present in the growth medium and during analysis of bacterial cultures. The flow cytometer detected a large population of 'bacteria-sized' particles (Figure 3.7A, 3.7B right panels) which, when further analysed, were positive for anti-*Salmonella* LPS staining (Figure 3.7A, 3.7B contour plots left panels). The population of 'live' *S. Typhimurium* was defined by a 'gate' using viable, mid-log phase *in vitro* grown bacteria stained with anti-*Salmonella* LPS antibodies that were not stained by PI (PI-). The 'gate' for the 'dead' population was set using ethanol-killed bacteria that were positive for anti-ST LPS Ab and PI stain (PI+). These gates, which were represented as rectangular boxes over the bacterial population, were used to quantify the percentages of live and dead bacteria in the experimental samples.

The size and the complexity of both unstressed and stressed bacteria was comparable as seen in the FSC and SSC analysis represented in the Figure 3.8 A, 3.8B right panel. The FSC-A and SSC-A gated bacteria were analysed for the fluorescent stains, anti-ST LPS Ab and PI. The majority of *S. Typhimurium* in both the unstressed-culturable (Figure 3.8A) and stressed-nonculturable samples (Figure 3.8B) were determined to be viable (alive) on being PI 'negative' by flow cytometry. By these criteria, more than 85% of the bacteria were 'alive' among the unstressed-culturable bacteria and over 90% of the bacteria were 'alive' in samples from the stressed-nonculturable bacteria.

This FACS analysis validated the observations made using confocal microscopy confirming that the bacteria had entered a viable but non-culturable state. This population that was not culturable in different media, but which was deemed viable by examination using confocal microscope and FACS, will be referred to as *S. Typhimurium* VBNC cells from here-on.

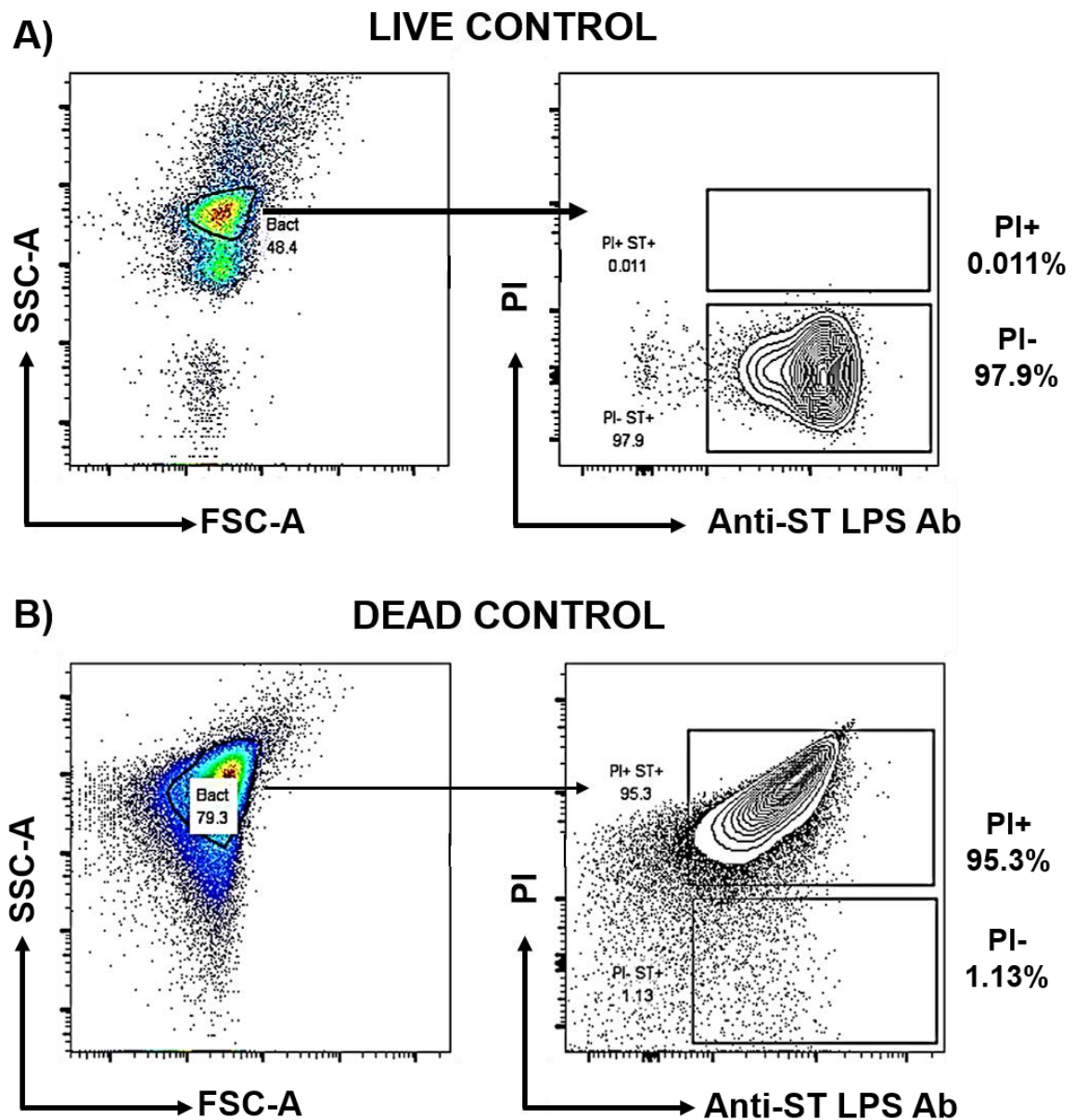


Figure 3.7: Flowcytometry analysis of the viable and dead *S. Typhimurium* populations. Exponentially growing *S. Typhimurium* (A) and ethanol killed dead *S. Typhimurium* (B) were stained with anti-*S. Typhimurium* LPS (Anti-ST LPS Ab) and propidium iodide (PI). The bacterial populations were first separated from background based on the size (FSC-A) and granularity (SSC-A). The identity of the bacterial population was confirmed using Anti-ST LPS Ab and dead cells were positively labelled with PI. Live (PI-, Anti-ST LPS Ab+) and dead bacteria (PI+, Anti-ST LPS Ab+) are shown as percentage of the total bacterial population.

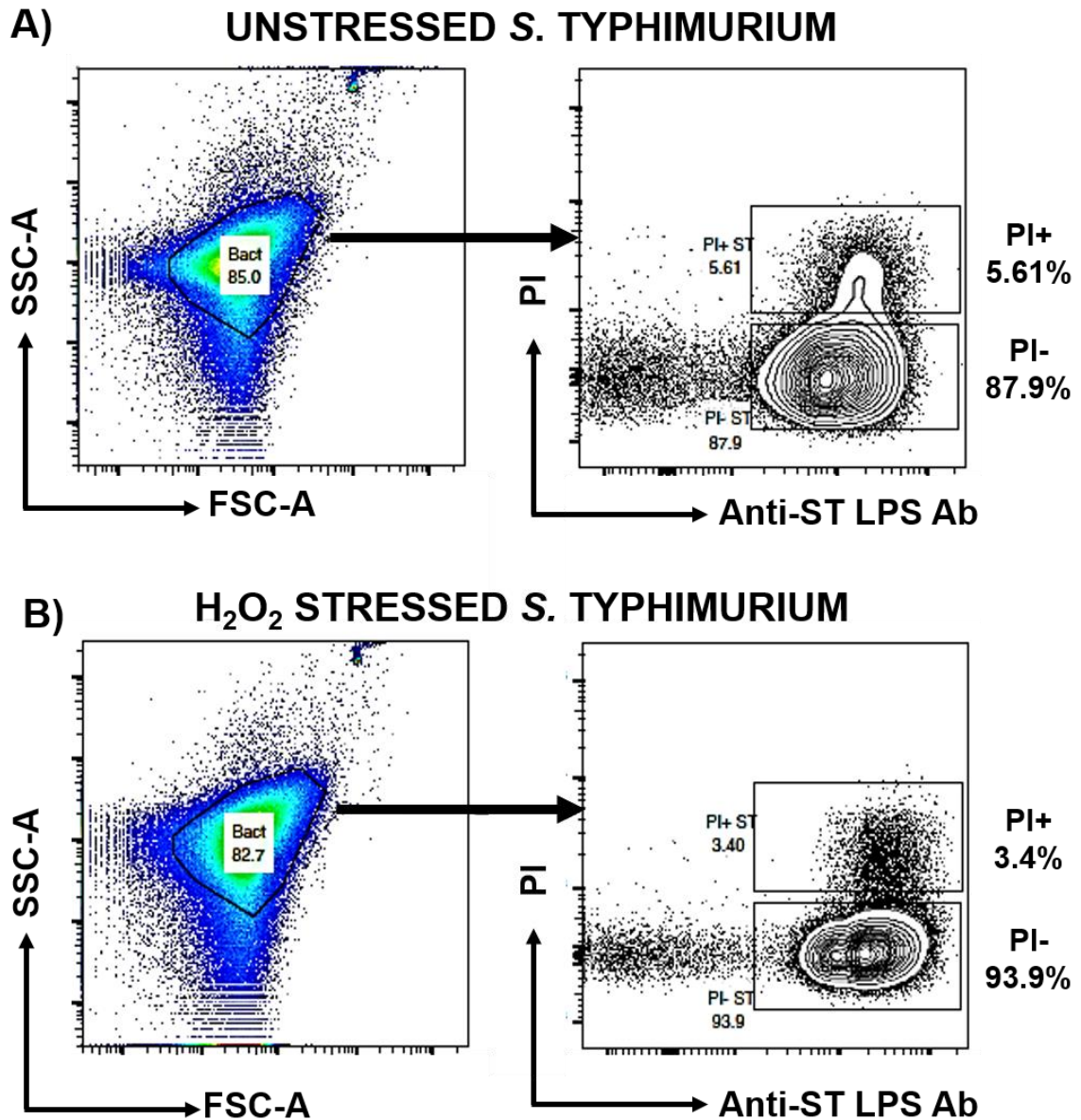


Figure 3.8: Analysis of viability of unstressed, and H_2O_2 -stressed unculturable *S. Typhimurium*, using flow cytometry. Unstressed *S. Typhimurium* (A) and stressed *S. Typhimurium* (B) were stained with anti-*S. Typhimurium* LPS (Anti-ST LPS Ab) and PI. The bacterial populations were first separated from background based on the size (FSC-A) and granularity (SSC-A). All the bacteria in the population were labelled by Anti-ST LPS Ab and only dead cells were positively labelled with both Anti-ST LPS Ab and PI. Dead bacteria are PI+ and Anti-ST LPS Ab+ (upper gate), live and VBNC bacteria are PI- and Anti-ST LPS Ab+ (lower gate). The two populations are shown as percentage of the total bacterial population.

3.2.7 VBNC *S. Typhimurium* are still viable after storage at room temperature for 24 hours

After confirming their viability using confocal microscopy with Live/Dead™ stain and flow cytometry using PI, the stability of the VBNC cells were tested. The viability of *S. Typhimurium* VBNC cells was tested after incubation at room temperature for 24 hours post oxidative stress to evaluate the viability of the VBNC bacteria. Both the unstressed culturable control bacteria and the VBNC bacteria were incubated at room temperature followed by quantitation of viability by FACS analysis. Bacteria were stained with PI and SYTO9. A second control group of bacteria were left on ice for 24 hours and analysed in the same manner. Unstressed and VBNC cells were also incubated at room temperature for 24 hours in PBS the study their viability over time. Exponentially growing bacteria and ethanol killed cells were used as 'live' and 'dead' controls for the flowcytometry analysis (Figure 3.9A and 3.9B).

In this study, the live population of unstressed cells declined to 60% (Figure 3.10A). In contrast, the VBNC cells not only retained their viability, but had a higher percentage of viable cells in the population, at an average of 80% (Figure 3.10B). This suggested that the VBNC *S. Typhimurium* were stable at room temperature for a period of at least 24 hours. Both the unstressed (Figure 3.11A) and VBNC samples (Figure 3.11B) retained a high percentage of viable cells, at over 85%, when stored in PBS on ice for 24 hours.

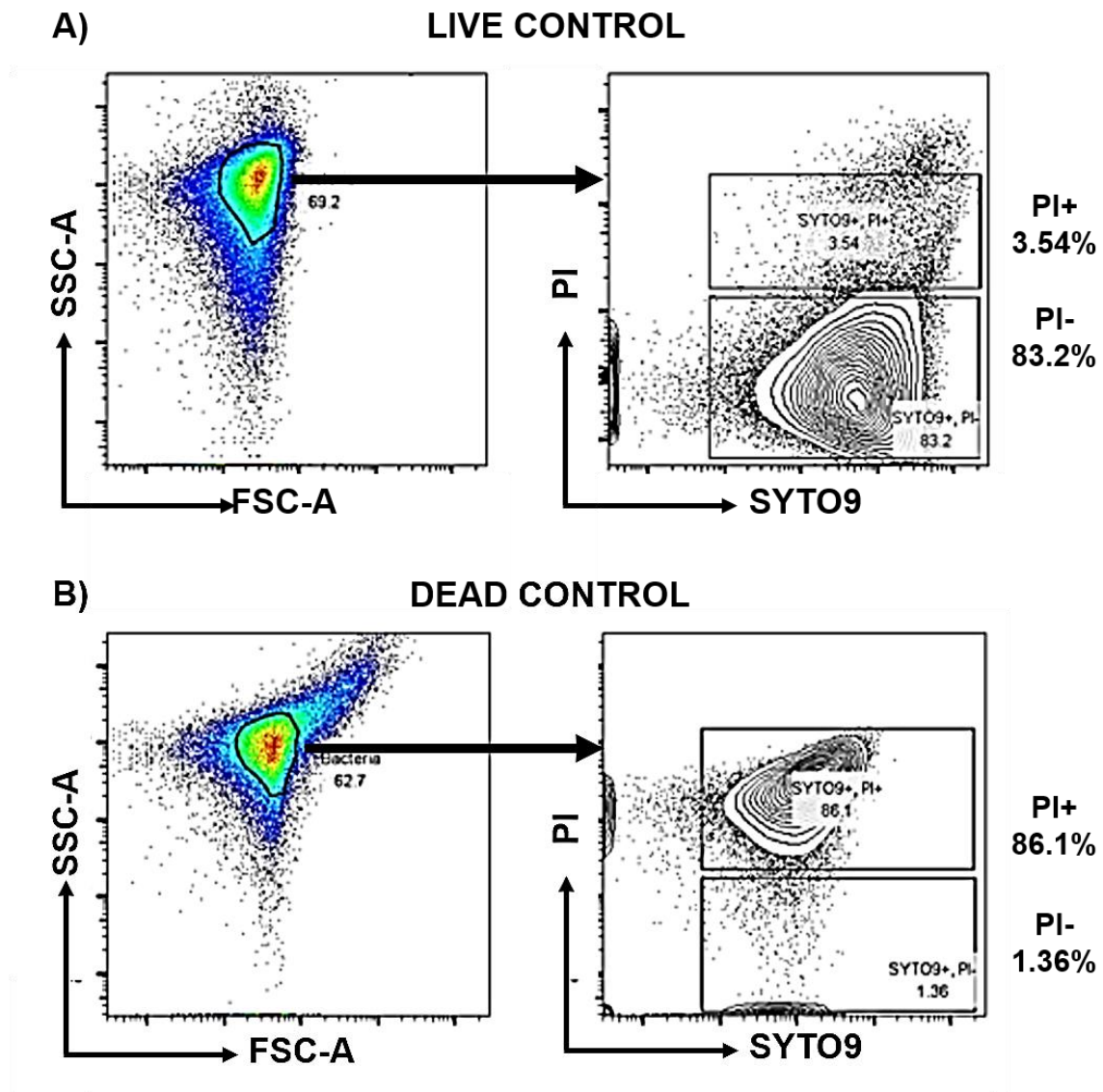


Figure 3.9: Flow cytometry analysis of viable and dead *S. Typhimurium*. Exponentially growing *S. Typhimurium* (A) and ethanol killed dead *S. Typhimurium* (B) were stained with SYTO9 and propidium iodide (PI). The bacterial populations were first separated from background based on the size (FSC-A) and granularity (SSC-A). SYTO9 labelled all the bacteria in the population but both SYTO9 and PI labelled the dead cells. Live (PI-, SYTO9+) and dead bacteria (PI+, SYTO9+) are shown as percentage of the total bacterial population.

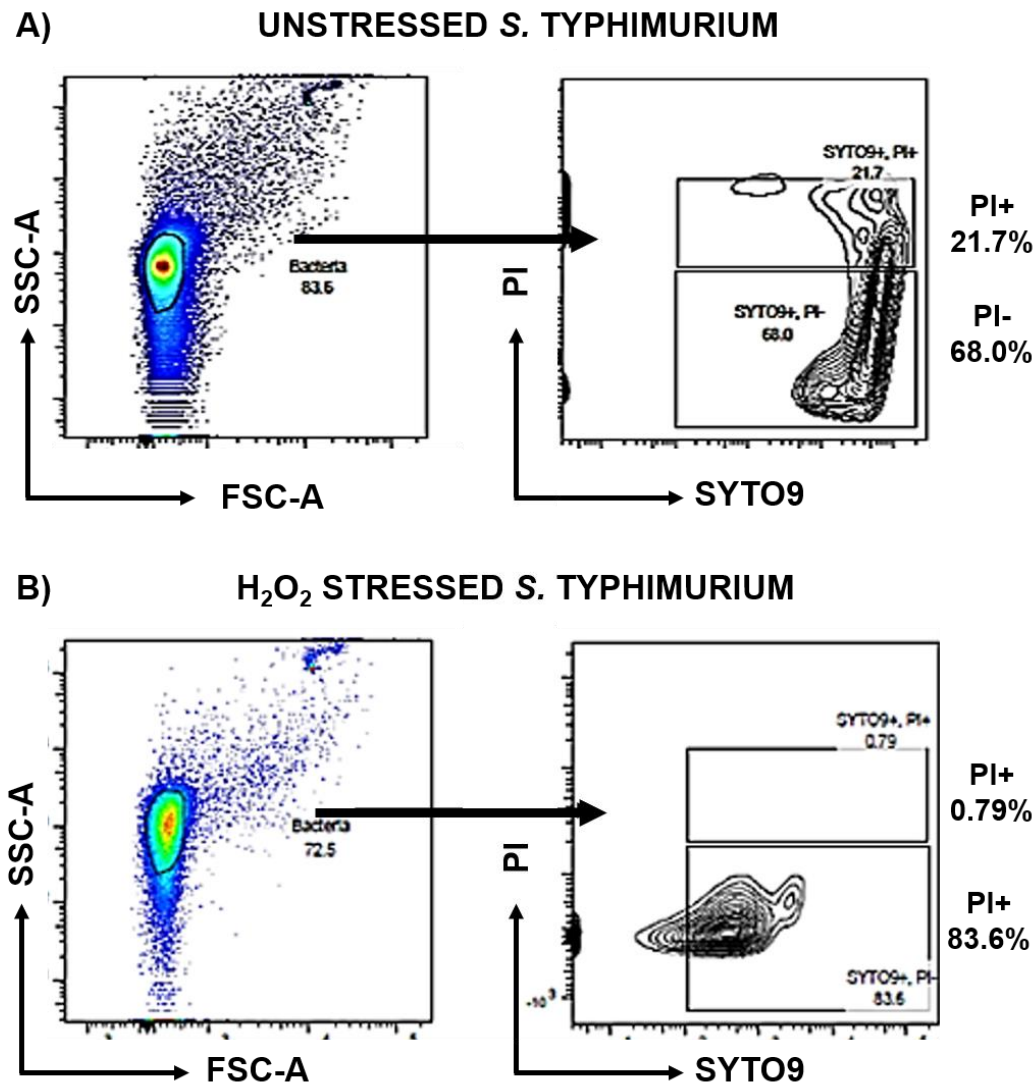


Figure 3.10: Flow cytometry analysis of VBNC *S. Typhimurium* for viability after 24 hours at room temperature in the VBNC state. The VBNC bacteria generated in Figure 3.7B were kept at room temperature for 24 hours. Unstressed *S. Typhimurium* (A) and stressed *S. Typhimurium* (B) were stained with SYTO9 and PI. The bacterial populations were first separated from background based on the size (FSC-A) and granularity (SSC-A). All the bacteria were positive for SYTO9 staining and only dead cells were positively labelled with both SYTO9 and PI. Dead bacteria are PI+ and Anti-ST LPS Ab+ (upper gate), live and VBNC bacteria are PI- and Anti-ST LPS Ab+ (lower gate). The two populations are shown as percentage of the total bacterial population.

3.2.8 VBNC *S. Typhimurium* can tolerate treatment with β -lactam antibiotics

Most existing antibiotics target metabolic activities of bacteria²⁵². Even if these antibiotics are bactericidal in nature, their activity depends upon whether the metabolic pathway, or mechanism they target, is active. For example, β -lactam antibiotics, which inhibit cell wall formation, are most effective against actively dividing bacteria. Antibiotics targeting various vital processes like transcription or translation also require the bacteria to be actively using these processes²⁵³. Unlike actively growing cells, bacterial 'persisters' can tolerate antibiotics, which are otherwise bactericidal or bacteriostatic for the same strain and for the same duration of treatment²⁰. Given that VBNC bacteria do not actively divide, it is likely that they may exhibit tolerance to β -lactam antibiotics. The sensitivity of VBNC bacteria to antibiotics might also provide an indirect measure of the physiology of the bacteria in the VBNC state.

S. Typhimurium is susceptible to β -lactam antibiotics like ampicillin (Figure 3.11). Unstressed control *S. Typhimurium* and VBNC *S. Typhimurium* were treated overnight with 50 μ g/ml of ampicillin at 37°C with shaking at 180 rpm. Cells were harvested and stained for confocal microscopy with Live/Dead™ stain. The ampicillin-treated culturable *S. Typhimurium* were stained with PI and were reduced to debris that appeared as larger particles under the confocal microscope (Figure 3.12A). The majority of VBNC bacteria were intact and alive as determined by the Live/Dead™ stain (Figure 3.12B), indicating that the VBNC *S. Typhimurium* can exhibit ampicillin tolerance.

Subsequently, FACS analysis was used to study the effect of ampicillin on VBNC *S. Typhimurium*. Samples were stained for FACS analysis using anti-*S. Typhimurium* LPS antibodies (anti-ST LPS Ab) and PI. Figure 3.13 shows the live and dead controls used for this flow cytometry analysis. The bacteria were separated from the background based on their size and granularity using the forward (FSC-A) and side (SSC-A) scatter (Figure 3.13, right panels) followed by analysing the fluorescence of anti-*Salmonella* LPS antibodies (x-axis) and PI (y-axis). The controls were used as a reference to identify the PI-positive and anti-ST LPS Ab-positive populations. The unstressed bacteria when treated with ampicillin had a population that was larger and more complex indicated by the FSC-A and SSC-A analysis

(Figure 3.14B). This increased complexity could be a reflection of the aggregation of dead cells and cell debris that was observed under confocal microscopy (Figure 3.12A). The unstressed *S. Typhimurium* were found to be susceptible to the ampicillin treatment as the percentage of live cells decrease from 90% to 42%, as determined by PI staining (Figure 3.14). The VBNC cells showed tolerance to the ampicillin treatment and the percentage of live cells remained unchanged compared to the untreated control sample at approximately 90% (Figure 3.15). These results suggested that *S. Typhimurium* VBNC cells were tolerant to antibiotic treatment that killed vegetative cells.

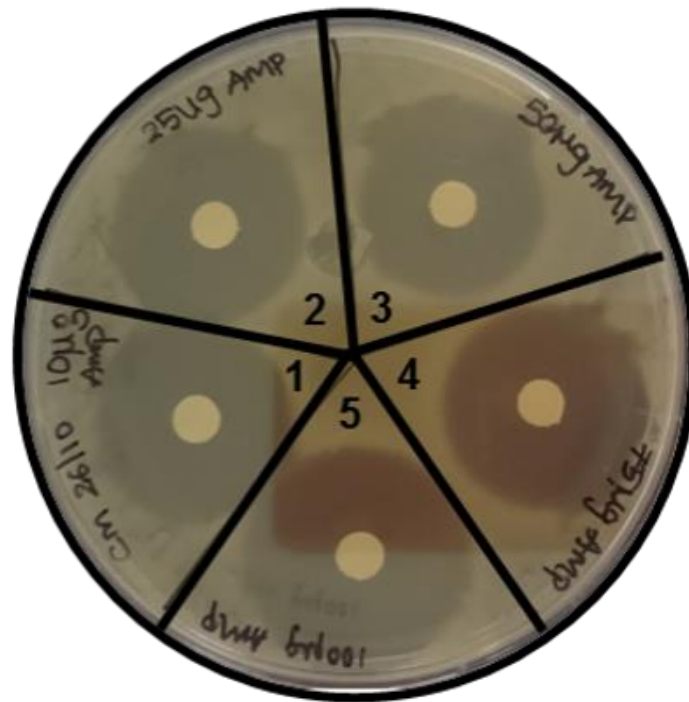


Figure 3.11: Susceptibility of *S. Typhimurium* to β -lactam antibiotics. The Disc diffusion assay was used to test ampicillin susceptibility of wild type *S. Typhimurium* to a range of concentrations. *S. Typhimurium* was incubated in 10 ml LB broth overnight at 37°C with shaking. One hundred μ l of the overnight culture was plated onto LB agar supplemented with 50 μ g/ml of streptomycin. Discs soaked in ampicillin at a concentration of 10, 25, 50, 75 and 100 μ g/ml, (denoted 1,2,3,4, and 5 respectively) were added to the lawn and the plate was incubated overnight at 37°C. Zones of growth inhibition, clearing of the bacterial lawn, are observed around the antibiotic discs.

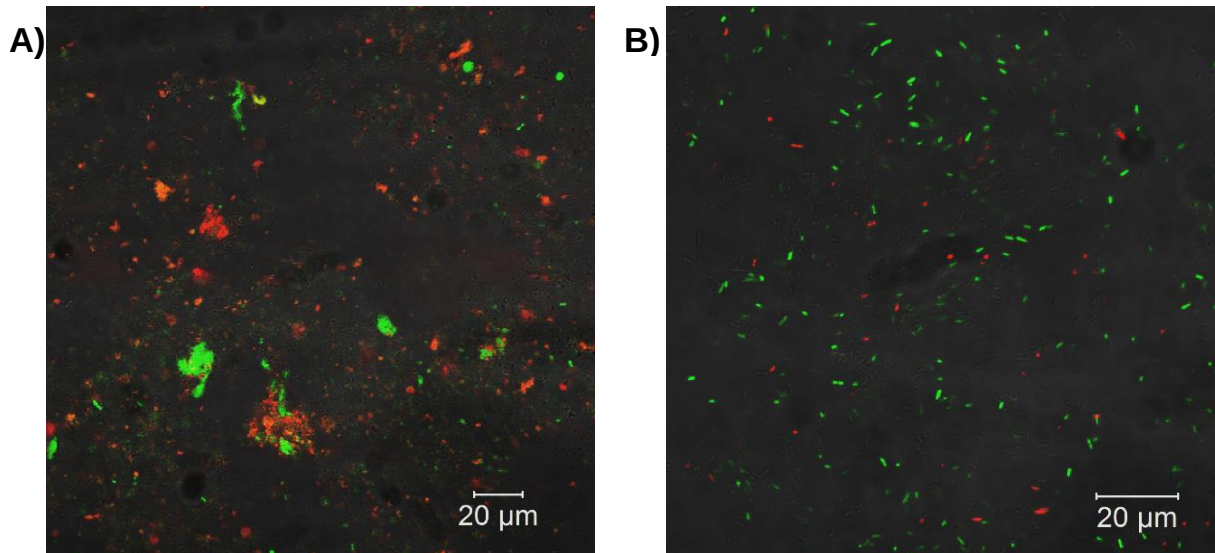


Figure 3.12: Antibiotic sensitivity of H₂O₂-stressed and unstressed *S. Typhimurium*.

A) Unstressed exponentially growing *S. Typhimurium* and B) VBNC *S. Typhimurium* were treated with 50µg/ml of ampicillin overnight in LB broth at 37°C with shaking. Cells were harvested by centrifugation, stained with reagents of the LIVE/DEAD™ BacLight kit, and analysed with fluorescent confocal microscopy. Bacteria fluorescing red are dead, orange are damaged and green are alive.

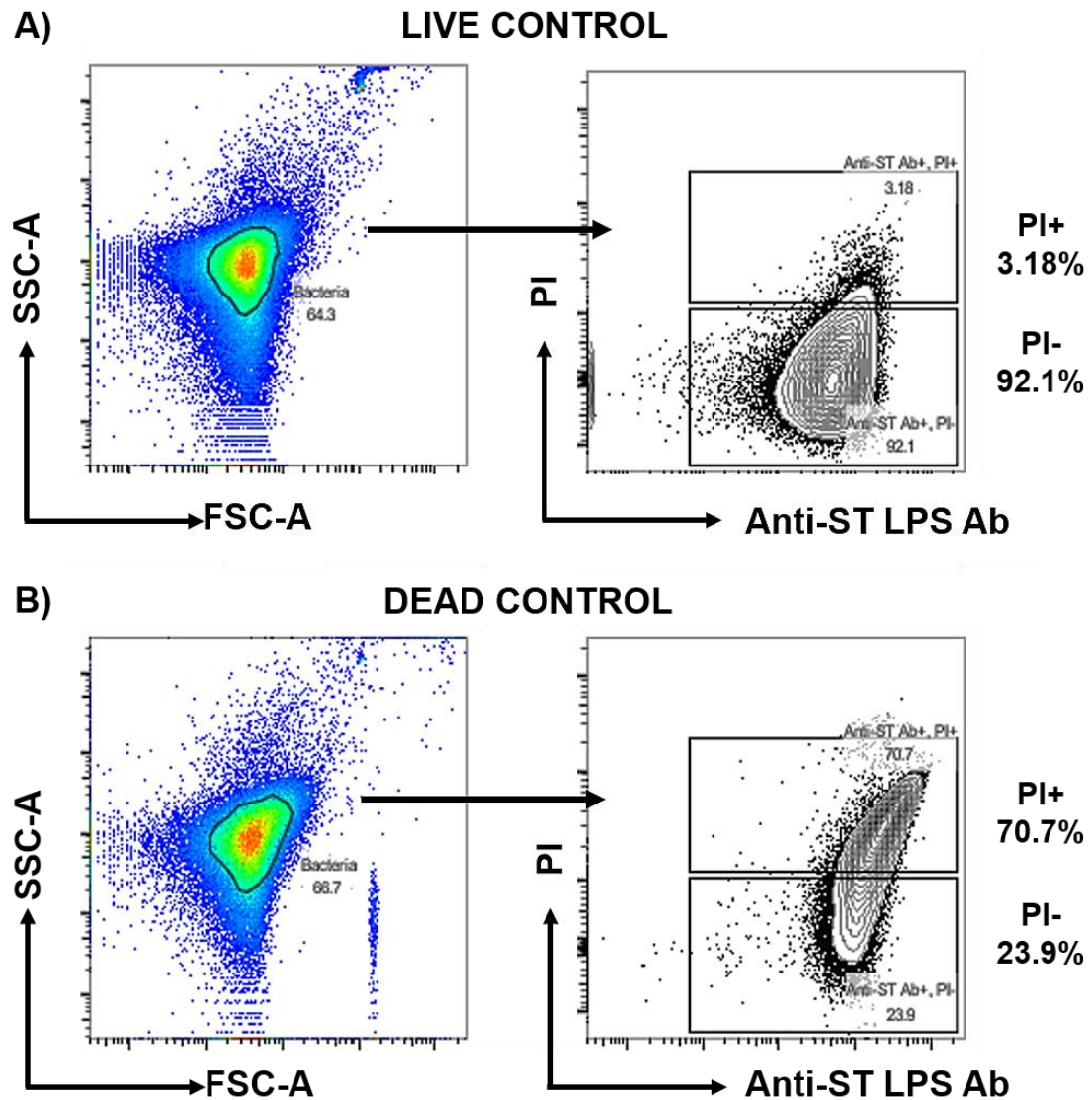
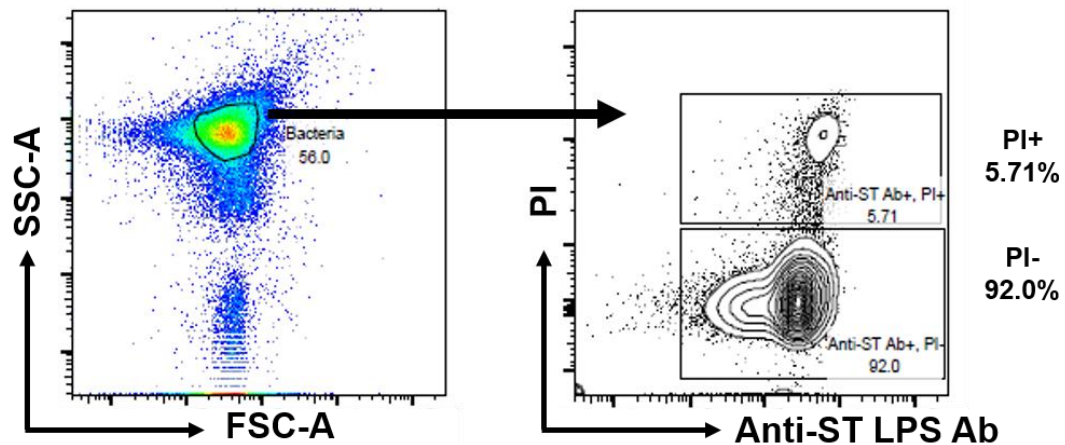


Figure 3.13: Control samples for FACS analysis of ampicillin tolerance of VBNC *S. Typhimurium*. Exponentially growing *S. Typhimurium* (A) and ethanol killed dead *S. Typhimurium* (B) were stained with anti-*S. Typhimurium* LPS (Anti-ST LPS Ab) and propidium iodide (PI). (FSC-A) and (SSC-A) analysis separated bacterial populations from background based on the size and granularity, respectively. Viable bacteria were stained only with Anti-ST LPS Ab whereas both Anti-ST LPS Ab and PI stained dead cells. Live (PI-, Anti-ST LPS Ab+) and dead bacteria (PI+, Anti-ST LPS Ab+) are shown in gates and their percentage in the total bacterial population was calculated.

A) UNSTRESSED *S. TYPHIMURIUM* WITHOUT AMPICILLIN



B) UNSTRESSED *S. TYPHIMURIUM* WITH AMPICILLIN

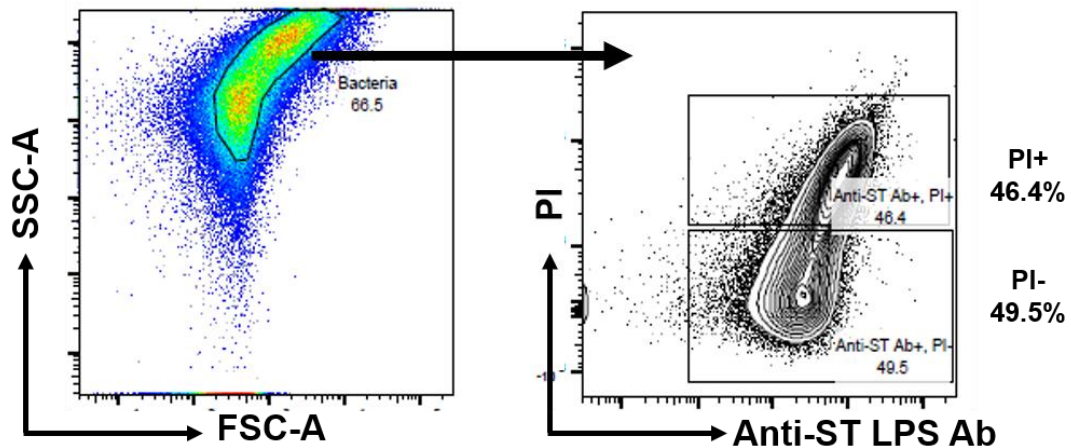


Figure 3.14: FACS analysis of ampicillin treated unstressed *S. Typhimurium*. Unstressed *S. Typhimurium* that was treated with (A) or without (B) ampicillin were stained with anti-*S. Typhimurium* LPS (Anti-ST LPS Ab) and PI. Bacterial populations were identified based on the size (FSC-A) and granularity (SSC-A). The identified bacteria were analysed for labelling by Anti-ST LPS Ab and PI. While all bacteria were labelled with Anti-ST LPS Ab only dead cells were positive for both Anti-ST LPS Ab and PI labels. Dead bacteria are PI+ and Anti-ST LPS Ab+ (upper gate), live and VBNC bacteria are PI- and Anti-ST LPS Ab+ (lower gate). The two populations are shown as percentage of the total bacterial population.

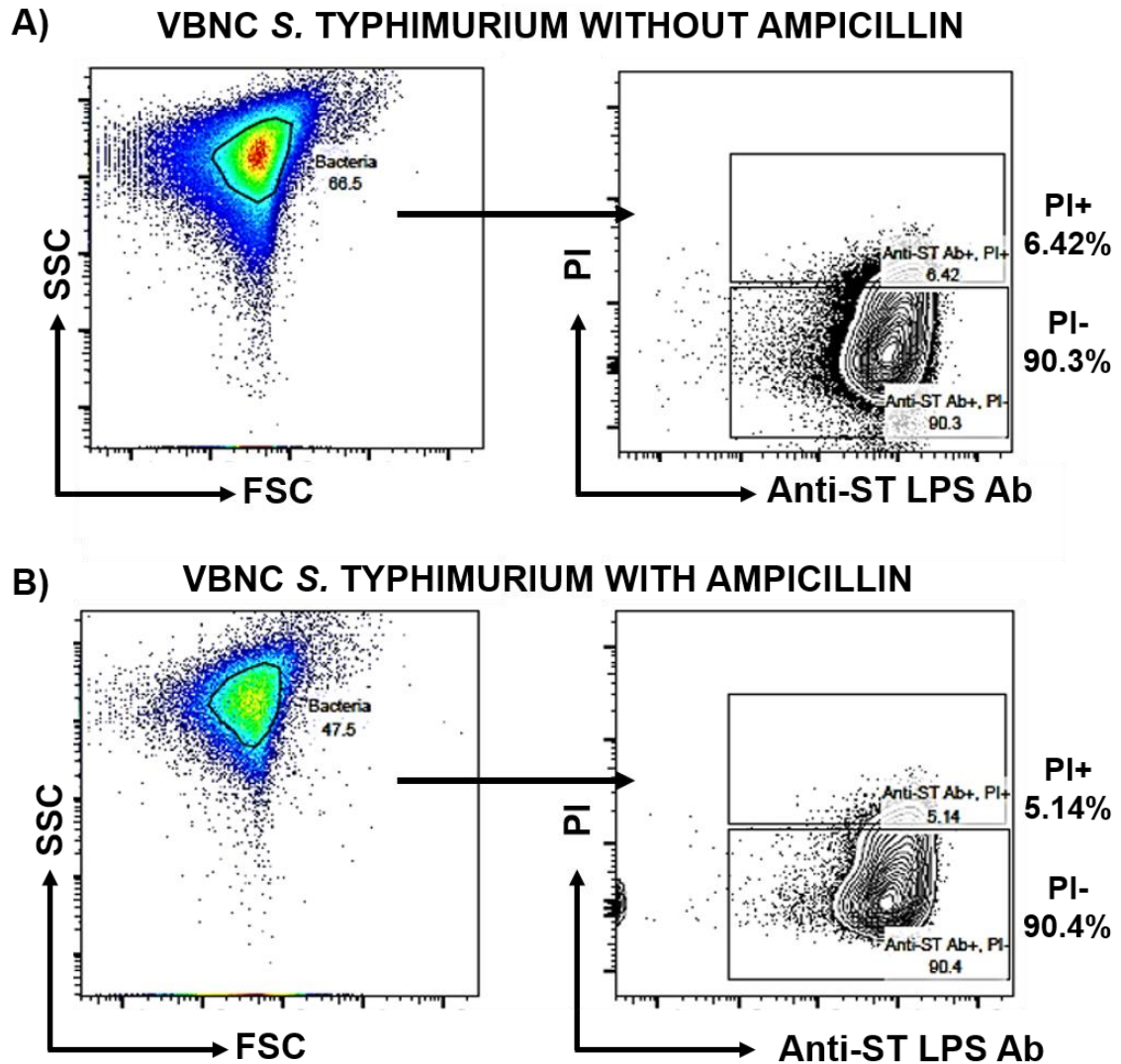


Figure 3.15: FACS analysis of ampicillin treated VBNC *S. Typhimurium*. VBNC *S. Typhimurium* were treated with ampicillin (A) or without ampicillin (B) overnight. The FSC-A and SSC-A analysis was done to separate the bacterial population from the background. The bacteria were selected by labelling with Anti-ST LPS antibodies and only the dead population was stained by PI staining. Live bacteria are PI-/Anti-ST LPS Ab+ and dead are PI+/Anti-ST LPS Ab+. Live and dead bacteria was shown in gates (lower box and upper box, respectively).

3.3 Discussion

The existence of viable but non-culturable (VBNC) state has been an on-going debate since first reported in *E. coli* and *V. cholera* by Xu *et al.* in 1982²⁷. A large number of studies have been published and over 80 different species of bacteria were shown to enter this state of dormancy^{95,107,165,254}. Despite these observations, to-date, there are no standardised methods to generate or detect VBNC bacteria and the mechanisms driving this response are poorly understood⁵⁴. Two defining characteristics of VBNC state are their viability but lack of culturability on routine media^{42,114}.

It is important to define methods for the identification of VBNC pathogens since samples isolated from patients or food are routinely processed for detection of pathogens using standard culturing methods which presently will not detect VBNC bacteria²⁵⁵. Consequently, a failure to identify bacterial pathogens due to their VBNC state may have serious negative consequences for public health.

In this study, VBNC *S. Typhimurium* SL1344 was stably generated under controlled and standardised conditions. The method used for generation of VBNC bacteria used oxidative stress and was adapted from a previous study in *Salmonella enterica* serovar Enteritidis⁹⁴.

Various aspects were considered when generating VBNC bacteria including growth phase and cell numbers. Exponentially growing cultures were used in this study because cells in this phase are more susceptible to oxidative stress than bacteria in stationary phase^{215,256}. Proteins like Dps (DNA binding Protein from Starved cells) are expressed at an elevated level as the cells enter stationary phase²⁵⁷. This elevated protein expression can confer protection against various stress conditions²⁵⁸, including oxidative stress²⁵⁹.

The presence of, and resistance to, the intracellular ROS (reactive oxygen species) generated by host cells when the *Salmonella* containing vacuole (SCV) is formed, is a widely studied phenomenon; the concentration of ROS is generally thought to be in the micromolar range^{247,260}. The complete denaturation of H₂O₂ into hydroxyl radical, which is the functional ROS, is necessary to generate the expected oxidative stress. This denaturation depends on the presence of reducible ions, proteins and their respective redox potential differences to

hydrogen peroxide^{261,262}. Another important factor that can affect the degree of oxidative stress is the number of bacteria. The persistence and effectiveness of ROS is likely inversely proportional to the number of bacteria present. Various enzymatic^{263,264} and non-enzymatic barriers^{216,265,266} produced by bacteria can rapidly quench and/or actively degrade ROS, or prevent ROS from entering the bacterial cell²⁶⁷. The medium in which the cells were grown, and the medium where the *in vitro* stress is performed, are additional variables, given that concentrations of organic and inorganic molecules that are present in the growth media will impact ROS scavenging. This study uses millimolar concentrations of H₂O₂ in line with other previous *in vitro* studies^{94,195,268,269}. Previous studies also showed a rapid decline of potential oxidative stress over time²⁷⁰.

Viability of bacteria has been traditionally defined by their growth under defined conditions of laboratory culture^{162,271}. Subsequently, different staining techniques were developed to test the viability of bacteria. Viability of the bacteria is typically measured by multiplication alone, but the development of staining approaches to assess membrane integrity and other means, including monitoring of metabolic activities, has enabled alternate assessments of cell viability. Live/Dead™ stain became a very popular tool to check the viability of bacteria²⁷². Live/Dead™ stain which contains SYTO9 and PI was used in confocal microscopy. SYTO9 can enter both live and dead bacteria despite their metabolic activity or membrane integrity whereas PI can only enter membrane compromised bacteria. PI has a higher affinity to nucleic acids than SYTO9. Hence, PI can displace already integrated SYTO9. The displacement depends on the relative concentrations of these stains in the cell^{143,249}. The extent of SYTO9 displacement depends on the amount of PI inside the cell which in turn depends on the extent of membrane damage and permeability. Cells with a partial PI permeability are orange in colour due to the emission of both green fluorescence of SYTO9 and red fluorescence of PI stains. Orange coloured cells can be observed in the confocal microscope images among VBNC cells, and even in unstressed populations.

Kogure *et al.* developed a method to identify the actively dividing bacteria in a population by using a microscope and nalidixic acid⁹³. Nalidixic acid inhibits DNA replication and

decatenation by inhibiting the subunit A of the DNA gyrase and topoisomerase IV^{273,274}, thus preventing the formation of daughter cells during the cell division. This results in the elongation of the actively dividing bacteria²⁷⁵. The elongation can differ from bacterium to bacterium depending on their cell division activity²⁷⁶. After treatment with nalidixic acid, bacteria can be as much as 10-times the size of a typical daughter cell²⁷⁶. In this study, DVC was coupled with Live/Dead™ staining to reveal rate of cell division and viability. This experiment shows that the VBNC bacteria were not replicating but still retained viability.

In addition to the Live/Dead stain studies, flow cytometry (FACS) was used to evaluate the oxidatively-stressed *S. Typhimurium*. A modified strategy to that of the Live/Dead™ stain had to be adopted for FACS to decrease background 'noise'. This 'noise' caused by the binding of SYTO9 to debris in the FACS buffers and FACS equipment was difficult to separate from the bacterial population by standard FACS parameters like size (FSC-A) and cellular complexity (SSC-A) when Live/Dead stain was used. Therefore, green fluorescent labelled anti-*Salmonella* LPS antibodies were used instead of SYTO9 together with the PI counter staining for detection of bacteria in the FACS analysis.

Antibiotic tolerance of the VBNC bacteria was studied after the stressed cells were confirmed to be nonculturable but viable by virtue of PI- staining. This experiment confirmed another characteristic of VBNC bacteria, i.e. drug tolerance. Other studies have revealed that a portion of *Salmonella* cells become non-replicating persisters upon internalisation by macrophages¹²⁵. The work on macrophage induction of persisters by Helaine *et al.* revealed that a portion of non-culturable bacteria are present within the slow growing persister population¹²⁵. Like persisters, VBNC cells showed tolerance towards ampicillin treatment probably due to their slow cell or no cell division^{96,277}.

The VBNC state in Gram-negative bacteria is sometimes compared with sporulation in Gram-positive bacteria^{54,129}. It was reported that VBNC cells become coccoid and smaller in size compared with that of their actively dividing counterparts^{48,95,98,278}. There were no observable changes in size between the dividing and VBNC *S. Typhimurium* in the confocal or the TEM studies reported in this Chapter. Decrease in the average length of the glycan

strand was proposed as one mechanism for the coccoid shape in some VBNC bacteria morphology, though the significance of the shape change is not well understood¹⁹⁷. VBNC *S. Typhimurium* showed a different cellular organisation to that of active *S. Typhimurium* when examined under TEM. The VBNC cells had pale, electro-lucent regions in the middle of the bacterial cytoplasm which could be the condensed bacterial 'nucleoid'. This condensation is likely a result of the oxidative stress to which the bacterial cells were exposed. The nucleoid associated proteins and the protein Dps can condense the bacterial nucleoid to protect the DNA from oxidative damage²⁷⁹⁻²⁸¹. This condensation would likely affect the transcription and translation and may contribute towards the VBNC state. Dps, or the DNA-binding protein from starved cells, is involved not only during oxidative stress, but can also confer resistance against starvation and acid stress as well²⁵⁷.

These data show that *S. Typhimurium* can enter into the VBNC state upon encountering oxidative stress and can also show characteristics typical of dormant cells including lack of cell division, antibiotic tolerance and intact cell structure. These VBNC bacteria can possibly be enriched by treatment with β -lactam antibiotics *in situ*. The longevity of *S. Typhimurium* in the VBNC state should be tested to fully estimate the threat food and drinks contaminated with VBNC *S. Typhimurium* can pose. The longevity of VBNC state depends on the species. For example, the resuscitation window for VBNC *Arcobacter butzleri* is 270 days and *Enterococcus faecium* is 7 days⁴². The stability of the VBNC *S. Typhimurium* was tested in this study over a period of 24 hours. The VBNC *S. Typhimurium* were shown to survive better compared to unstressed *S. Typhimurium* at room temperature. A similar 'hardiness' was exhibited by VBNC *L. pneumophila* towards detergents lethal to their culturable counterparts. In the future, it is worth investigating the stability of VBNC *S. Typhimurium* over a much longer period of time. Further studies could be done to identify VBNC bacteria in infected cells like macrophages using methods like fluorescence dilution, which is used to identify persisters or any slow dividing cells. Such studies may determine if VBNC and persister cells form different sub-populations in any given microcosm, as proposed by the 'dormancy continuum hypothesis'.

CHAPTER 4

Transcriptomic and proteomic analysis of VBNC *S. Typhimurium* and gene knock-out studies

4.1 Introduction

The viable and nonculturable state is a poorly understood state of dormancy as the mechanism involved in the entry, maintenance and exit from this state are still unknown²⁰⁶. It is highly likely that changes in transcriptional profile and/or cellular protein quantities of gene products play a role in the conversion of rapidly growing planktonic bacteria into a viable but non-culturable (VBNC) state. Studies presented in Chapter 3 showed that *S. Typhimurium* can enter a VBNC state under oxidative stress. To investigate how this transition from rapid growth to VBNC occurred, transcriptomic studies were conducted to analyse the changes in the messenger RNA (mRNA).

The transcriptome is the sum of all the RNA transcripts in a cell under specified conditions²⁸². Transcriptomics, the study of transcriptomes, can reveal the physiology of bacteria under different conditions including stress and dormancy. Such studies on the transcriptome of VBNC bacteria have previously been performed in *Vibrio cholerae*^{128,178}, *Ralstonia solanacearum*⁹⁵, *Rhodococcus biphenylivorans*⁹⁵ and *Brettanomyces bruxellensis*¹⁴⁰. Studies on *V. cholerae* have shown the changes in transcription of genes related to siderophores and metal ion transporter genes, ABC transporters, catalases, oxidoreductases¹²⁸. RNA studies on *R. biphenylivorans* reported a change in transcription of genes related to citric acid cycle, ATP accumulation, fatty acid degradation⁹⁸. Genes related to outer membrane proteins, elongation factors and ribosomal proteins were found to be differentially expressed in the VBNC state of *R. solanacearum*⁹⁵.

Furthermore, gene knockout or gene mutation studies have shown that various genes play a role in the induction and/or maintenance of the VBNC state in some bacteria. The genes *rpoS* (sigma factor-38), *oxyR* (Hydrogen peroxide-inducible genes activator), *ompW* (outer membrane protein-W), *clpX* (ATP dependent protease) and *relA* (ppGpp synthase), for example, were shown to play a role in the VBNC state of *Salmonella enterica* and *E. coli*^{118,177,222}. Even with the availability of these data, mechanisms could differ from species to species, because the gene expression and protein expression data showed variations from

bacteria to bacteria (discussed in Chapter 1 Section 1.8). Some possible mechanisms of entry into VBNC state were previously proposed based on the gene expression and knockout studies, as discussed in the Chapter 1 Section 1.9. The aims of the studies presented in this Chapter were to assess the gene expression and protein profiles of *S. Typhimurium* in VBNC state. RNA is isolated from VBNC *S. Typhimurium*, mRNA performed and the data analysed (flow chart, Figure 4.3). This study could assist an understanding of the possible mechanism(s) of entry in the VBNC state, and whether VBNC *S. Typhimurium* share physiological similarities with the VBNC states in other bacteria.

4.2 Results

4.2.1 RNA isolated from oxidative stressed VBNC *S. Typhimurium* showed reduced rRNA

RNA from unstressed-culturable *S. Typhimurium* (control) and VBNC *S. Typhimurium*, previously grown in LB until exponential growth phase, was isolated using the TRIzol™ method because it can yield a large amount of total RNA²⁸³. Prior to RNA extraction the control sample was treated with sterile PBS whereas the VBNC sample received 50mM H₂O₂ for 1 hour. One tenth of each bacterial culture was taken to confirm viability using FACS analysis (as described in Chapter 3, Section 3.2.5) and the lack of culturability on LB-agar plates, before isolated RNA was analysed. Remaining cultures were treated with RNAprotect® followed by lysis with TRIzol™ and storage at -20° C. After confirming the absence of CFUs in VBNC sample, the RNA was extracted with chloroform, RNA was precipitated, pelleted, dried and dissolved in RNase free water. Isolated RNA was treated with DNase. Samples were made in technical triplicates and processed simultaneously.

The integrity of the extracted RNA was analysed by Agilent LabChip® Nano 600 (Figure 4.1). The RNA integrity number or RIN score was used as an indication of the RNA quality measured by the Agilent bioanalyzer. RIN is a ratio of the 16S and 23S ribosomal RNA

bands²⁸⁴. The RIN scored on a scale of 1 to 10. RNA carrying a RIN score of less than 5 is generally considered to be of poor quality and is indicative of degraded RNA²⁸⁵. RNA extracted from VBNC bacteria had RIN scores ranging from 1.7 to 1.9 whereas the unstressed bacteria had RIN scores from 6.1 to 6.6 (Figure 4.1B). It was also observed that the RNA from ethanol-killed bacteria had extensive RNA degradation, as seen in the Figure 4.1A (last lane). These results indicated that RNA degradation in VBNC cells may have occurred during the external stress, being the treatment with hydrogen peroxide.

To test if the RNA degradation was a result of a direct hydrogen peroxide damage, RNA from exponentially growing bacteria was treated with different concentrations of hydrogen peroxide over one hour at 37°C. The highest concentration used was 100mM, which was double the concentration used in oxidative stress studies. The results showed that hydrogen peroxide did not degrade the RNA by itself (Figure 4.2). The RNA from the hydrogen peroxide treated sample (Figure 4.2, lanes 2-6) showed intact bands similar to that of the untreated control (Figure 4.2 lane 1) indicating that there was no degradation of RNA when incubated with hydrogen peroxide.

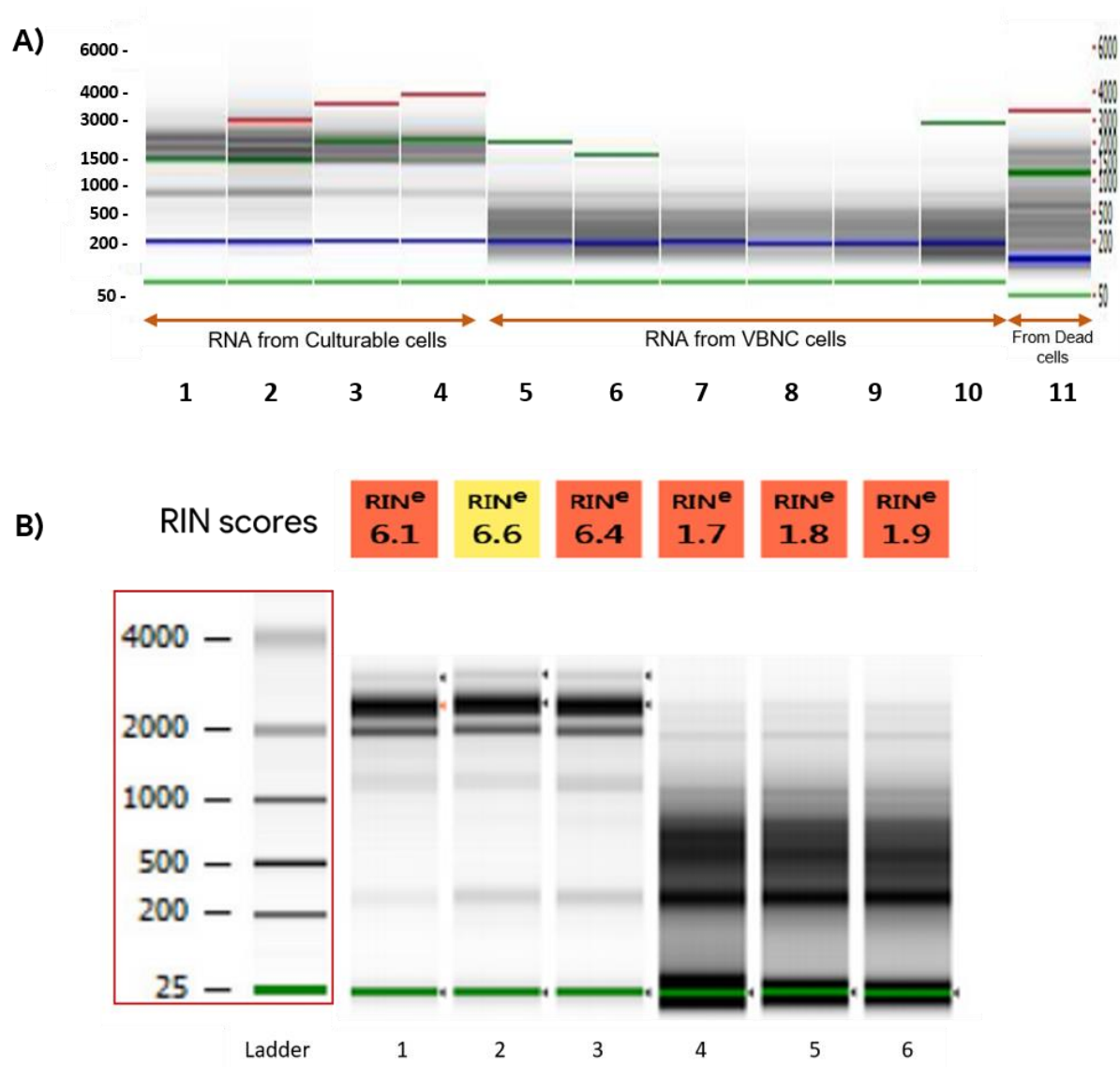


Figure 4.1: RNA integrity analysis (RIN) of RNA isolated from unstressed, VBNC, or ethanol killed (dead) *S. Typhimurium* A) Lanes 1-4 contain RNA from live *S. Typhimurium* cells, the next 6 lanes (5-10) contain RNA extracted from viable but non-culturable (VBNC) *S. Typhimurium*, and lane 12 contains RNA extracted from ethanol killed *S. Typhimurium*. B) RNA analysis of the samples analysed by RNASeq. Lanes 1-3 show the RNA from replicate culturable *S. Typhimurium* and lanes 4-6 show the RNA isolated from the VBNC *S. Typhimurium*. Bands corresponding to 23S rRNA (2.9 kb) and 16S rRNA (1.5kb) were more obvious in live bacteria. These samples generated higher RIN scores (6.1-6.6) than the samples from the VBNC bacteria (1.7-1.9).

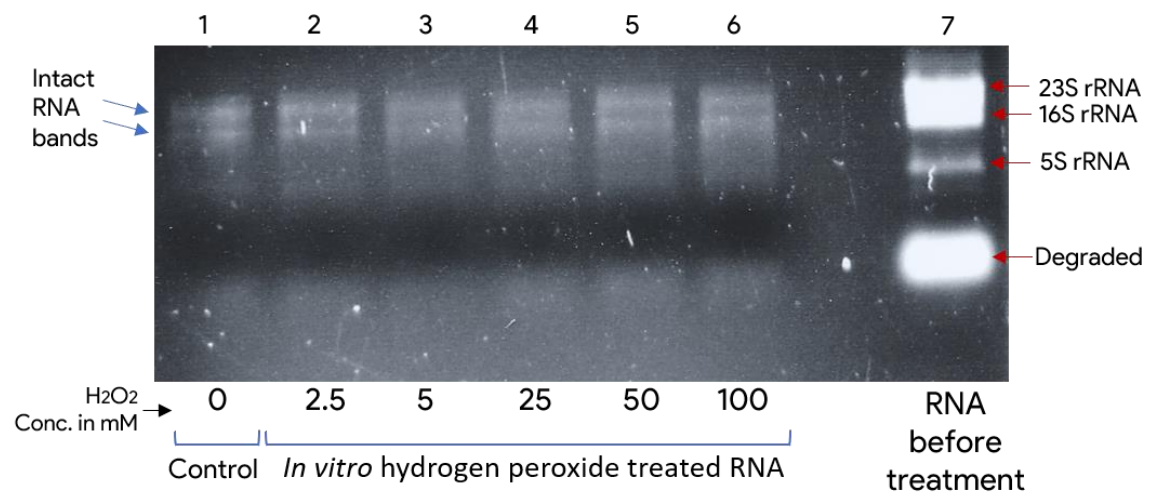


Figure 4.2: *In vitro* treatment of *S. Typhimurium* RNA with hydrogen peroxide. RNA isolated from the exponentially growing cells (lane 7) was treated with different concentrations of hydrogen peroxide (0, 2.5, 5, 25, 50, 100mM) *in vitro* for one hour where after the RNA was precipitated with ice-cold ethanol (2.5 times volume) and separated on a 1.2% agarose gel. Hydrogen peroxide (lanes 2-6) did not degrade the RNA compared to the untreated RNA (lane 1).

4.2.2 VBNC *S. Typhimurium* have a very different gene expression profile when compared to actively dividing *S. Typhimurium*

RNA from unstressed and VBNC *S. Typhimurium* were sequenced using an Illumina Nextseq500 short read (75bp) paired-end sequencing (at the Ramaciotti Centre for Genomics, University of New South Wales, Sydney). The rRNA was depleted from the samples and the resulting samples were converted into cDNA before Illumina sequencing. Illumina sequence reads for each sample were aligned to the complete reference genome *S. Typhimurium* SL1344 sequence²⁸⁶ using the BWA-MEM algorithm. RPKM (reads per kilobase million) were used to normalise the data to account for the depth of sequencing and the length of each gene. Differential gene expression analyses in VBNC relative to unstressed log-phase populations was undertaken using the Deseq2 package²⁸⁷ and visualised using Degust²⁸⁸ (Figure 4.3).

Gene expression data (in triplicate) of VBNC *S. Typhimurium* was compared with that of culturable 'unstressed' *S. Typhimurium*. Comparison of average gene expression data of DEGs in the VBNC state showed extensive changes in VBNC gene profiles (Figure 4.5A and 4.5B). MDS (Multi-dimensional scaling) analysis of VBNC and culturable bacteria revealed clear segregation of two populations (Figure 4.4A). Furthermore, the technical triplicate of each condition clustered relatively closely on their major dimension (x-axis) indicating close concordance between sample preparations. Plotting of all differentially expressed genes (DEGs) from the VBNC and control (unstressed) samples (in triplicate) revealed large changes in the transcriptional profile of the two populations (Figure 4.4B). These data indicate that gene expression profile, and by extension, the physiology of the control and VBNC bacteria were dramatically different, which is not surprising given the contrasting treatments that the two bacterial populations have encountered and the contrasting phenotypes they exhibit.

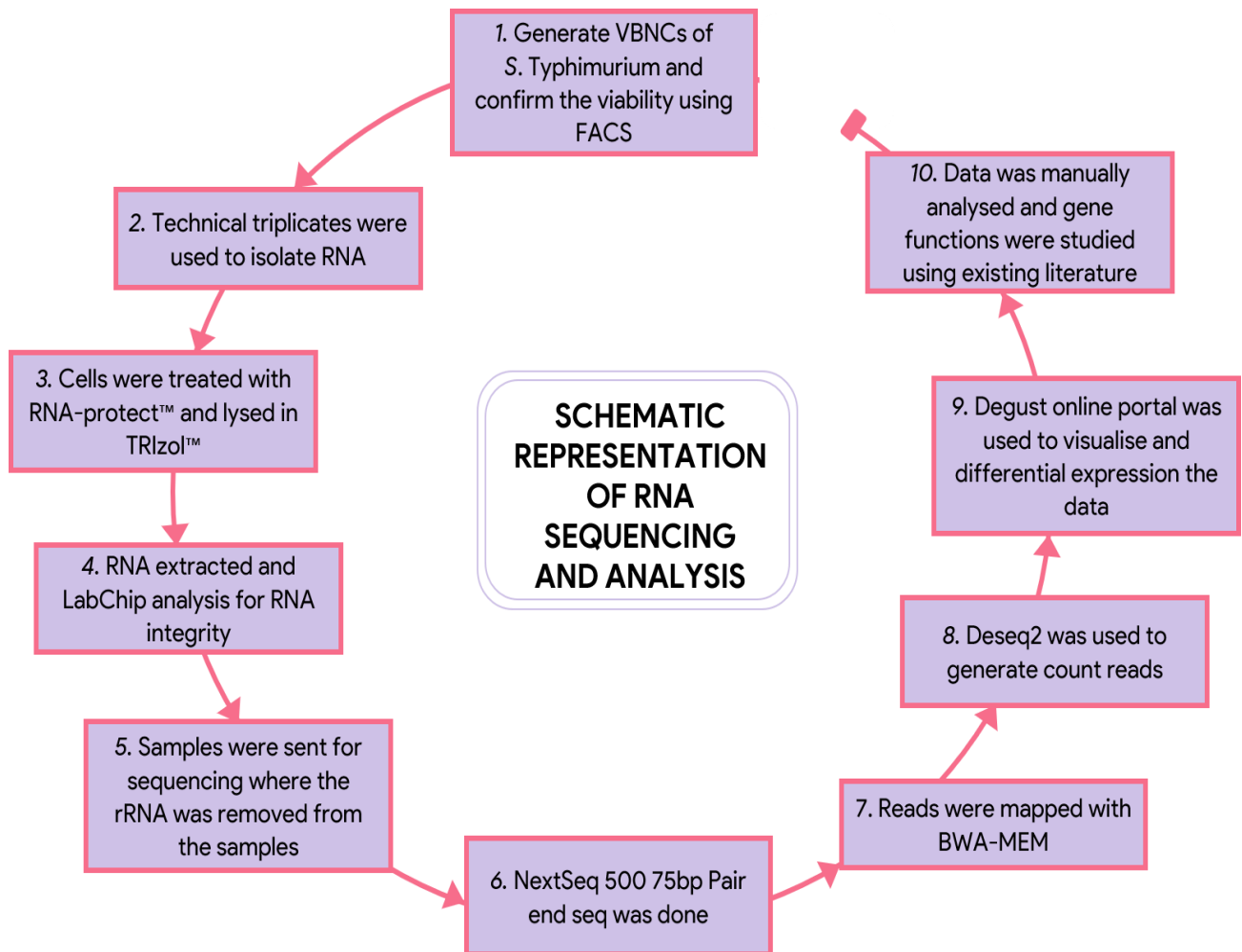


Figure 4.3: Schematic representation of different stages of RNA analysis.

S. Typhimurium was cultured to an OD₆₀₀ of 0.6 and subjected to oxidative stress using 50 mM of hydrogen peroxide. 1. The stressed bacteria were tested to analysed whether they have entered the VBNC state using FACS analysis and plating on LB agar medium with 50 µg of streptomycin. 2, 3. RNA was isolated from the oxidative stressed and control unstressed samples. Samples were prepared in technical triplicates. 4. RNA integrity was analysed using Agilent LabChip Nano. 5. rRNA was depleted and cDNA was synthesised at the RNA sequencing facility. 6. Illumina NextSeq 500 75 bp, pair-end sequencing was used. 7. Sequenced 'reads' were mapped to *S. Typhimurium* SL1344 using BWA-MEM. 8. DeSeq2 was used to generated read counts and to quantify the differentially expressed genes (DEGs) 9. 'Degust' online portal was used to visualise the DEGs. 10. The DEG data was manually analysed and the gene functions were studied based on the existing literature.

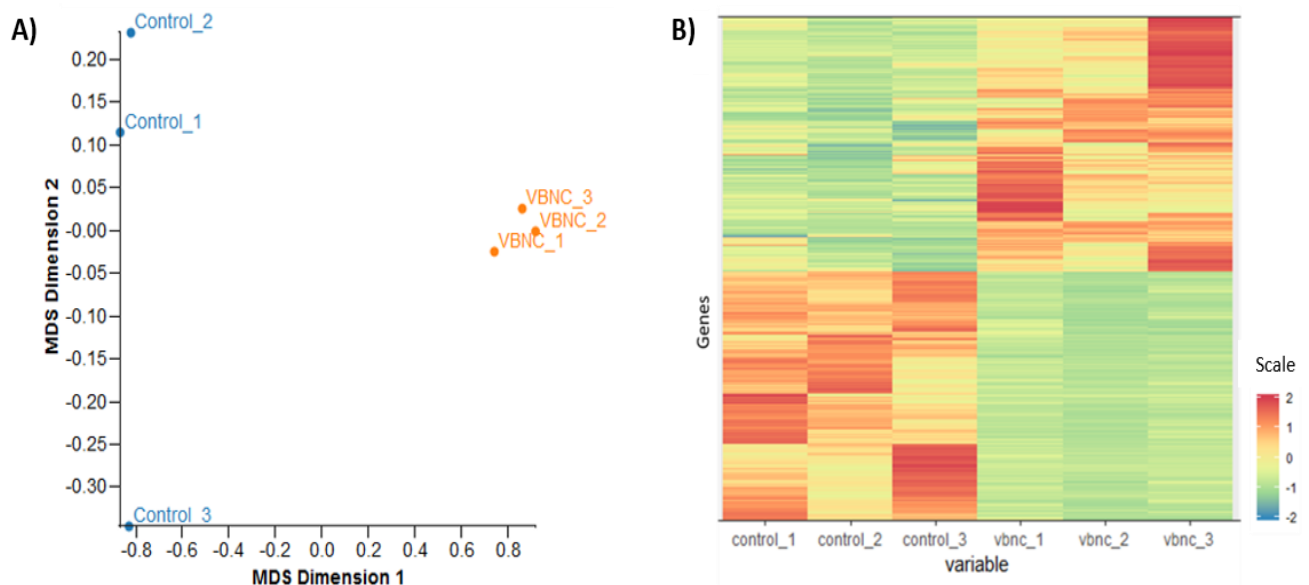


Figure 4.4: Transcriptional profiling of *S. Typhimurium* VBNC compared to unstressed *S. Typhimurium*. A) A multi-dimension scaling MDS analysis showed that the control and VBNC samples were arranged in separate cohorts demonstrating that the control and test samples have more similarities between themselves than with each other in their gene expression profiles. B) Heatmap of differentially expressed genes from the triplicates of the control and VBNC samples. The heatmap shows that the control and the test replicates share similarities in gene expression among themselves than across the samples. All the significantly (p -value < 0.05 and fold change of over Log_2 of 2) differentially expressed genes were arranged to form clusters. All fold changes throughout the thesis were presented in Log_2 values.

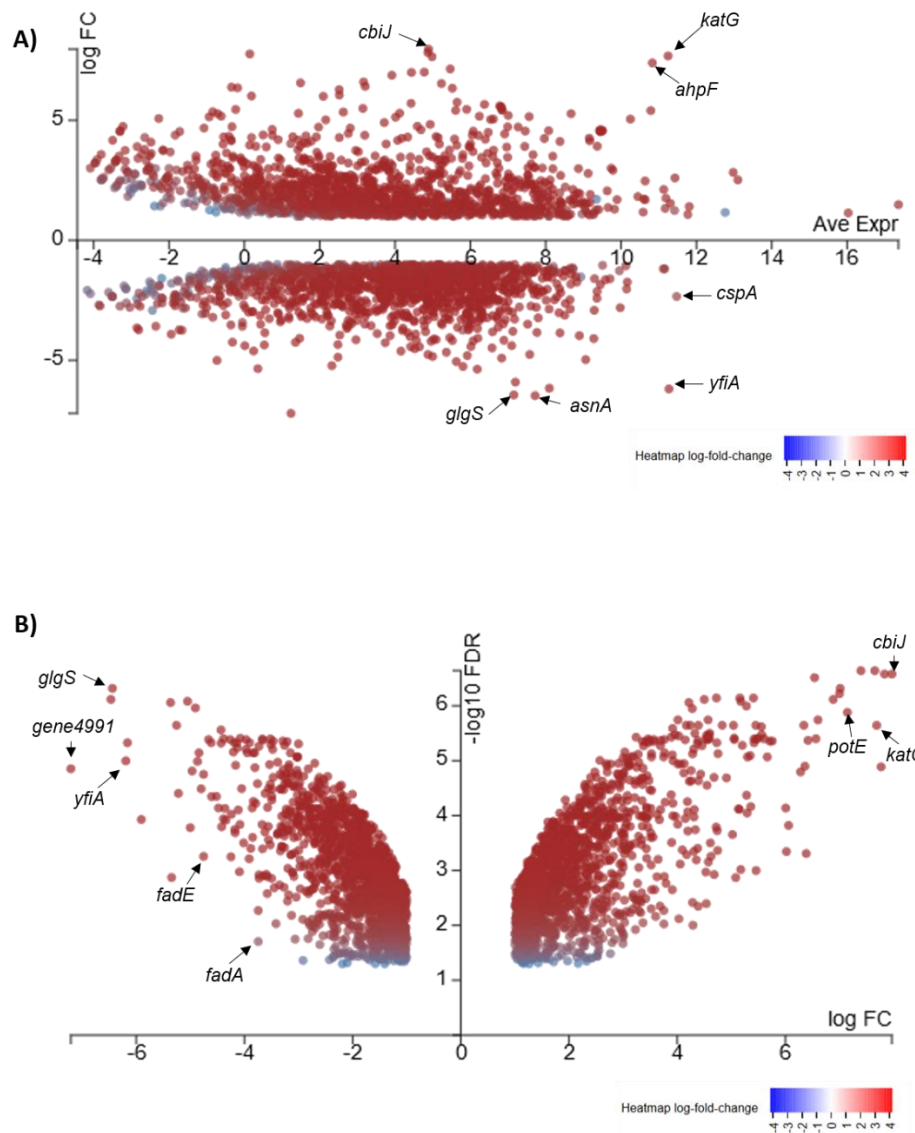


Figure 4.5: Analysis of the differentially expressed genes in VBNC *S. Typhimurium*. MA (log fold change vs average expression) and volcano plot analyses of the DEGs were done on 'Degust' online platform generate interactable graphs. A) MA plot shows the distribution of changes in expression of genes. MA plot showing the differential expression of genes plotting the log ratio and mean average expression of individual genes. The positive y-axis represents the positive log fold change and the negative y-axis represents the negative log fold change of the differentially expressed genes. The x-axis represents the average expression of the genes. MDR cut-off was set at 0.05 and absolute fold change to 1. B) Volcano plot of the data plots the negative p-value vs the log fold change of the individual genes. The genes on the top of the y-axis were of higher significance i.e. with p -value < 0.05 .

The entire differentially expressed genes were further shortlisted based on the 'significance'. Significance was defined as FDR (false discovery rate) value of <0.05 and expression changes over 2-fold (Log_2). Approximately 1990 genes were differentially expressed in the transcriptome VBNC *S. Tyhimurium* compared to the transcriptome of culturable bacteria. Out of the differentially expressed genes, over 400 genes were upregulated significantly (p -value <0.05, fold change $\text{Log}_2 > 2$) while 372 gene were significantly downregulated. MA analysis (plot of log ratio [M] versus abundance [A]²⁸⁹) showed that the upregulated genes included cobalamin biosynthesis genes and oxidative stress response genes, and downregulated genes included ribosomal hibernation factor *yfiA*, fatty-acid degradation related genes and glycogen synthesis related genes (Figure 4.4A). A related volcano plot displaying fold change (Log_2) of each differentially expressed gene on the x-axis versus FDR ($-\text{Log}_{10}$) value of each gene on the y-axis (Figure 4.5B) revealed a similar gene profile. Some of the highly upregulated genes were *ahpF*, *katG* and *oxyS*, which form part of the oxidative stress regulon²⁹⁰.

The top 30 significantly upregulated, significantly down-regulated genes are listed in Table 4.1 and 4.2. The significance was determined by the p -value of less than 0.05 and Log_2 fold change ≥ 2 . The biological functions of the genes were obtained from UniProtKB database²⁹¹. It was observed that the highly upregulated genes in Table 4.1 were related to the oxidative stress response. They included *ahpF*, *ahpC*, *katG* and *dps*. Siderophore genes like *iroC*, *entA*, *entE*, *iroD* were also highly upregulated (Table 4.1). Interestingly, some of the highly upregulated genes were in the *cbi* operon, *dps* and *deaD* were noteworthy upregulated genes. The *dps* was upregulated by 4.8-fold and gene *deaD*, a DEAD-box helicase, by 2.5-fold.

The highly downregulated genes in Table 4.2 (listed in the order of their significance based in the p -value) include a considerable number of hypothetical or uncharacterised genes, which has been seen with previous analyses of VBNC bacterial transcriptomes¹²⁸. Some of the general stress response genes like *yecG/uspC*²⁹² (universal stress protein C),

*bsmA/yifO*²⁹³ were also highly downregulated. The gene *glgS* is one of the highest downregulated genes in terms of log₂ fold-change. The gene encodes GlgS which is involved in glycogen synthesis, a process that is shown to be important in the survival of cells during dormancy. Another well characterised gene that is downregulated is the *yfiA* also known as *raiA* which is known to promote the formation of 70S ribosomal dimers. Decreased expression of *yfiA* can result in the production of 'hibernating' 100S ribosomes²⁹⁴.

The gene *asnA* was also highly downregulated in the VBNC state; *asnA* encodes an asparagine synthase. This was consistent with the observation that there was a general reduction in the expression of the genes involved in the amino acid metabolism, as can be seen in the 'over-representation test' tables in the 4.12. The product of *clpXP*, induced under the conditions of DNA damage, was shown to upregulate *asnA*²⁹⁵. The asparagine transporter proteins encoding genes *glnPQ* and *asnP* were also downregulated^{296,297}. One of the well-known genes that responds to redox potential under oxidative stress, *soxR* was downregulated by 2.47-fold. Paradoxically, *soxS* was slightly upregulated (by 1.47-fold). The expression of *soxS* controls genes like *sodA* (-1.92 FC), *zwf* (1.7 FC), *nfo* (insignificant *p*-value). The expression of *relA*, responsible for the formation of (p)ppGpp, was downregulated in the VBNC *S. Typhimurium*.

In summary, the RNA expression profiles of the culturable and VBNC were markedly different. The top 30 upregulated genes had many oxidative stress response genes including genes encoding catalase and alkyl hydroperoxide reductase, cobalamin biosynthesis genes, siderophore genes, cadaverine transporter proteins (Table 4.1). The list of the top 30 downregulating composed of many uncharacterised genes, metabolism related genes included *glgS*, *asnA*, *glpA* and *rbsD* and ribosomal associated protein *raiA*.

The complete differential read count dataset used for the analyses can be accessed through the Degust platform using the URL:

Table 4.1: Top 30 genes that were upregulated in VBNC *S. Typhimurium*. (Results are arranged in the order of highest to lowest significance according to the *p*-value). Some of the highly upregulated genes are known respondents to oxidative stress, members of cobalamin biosynthesis pathway or siderophores.

Gene	Gene ID	Log2 FC	<i>p</i> -value	Biological function
<i>cbiH</i>	SL1344_2003	7.51	2.49E-190	Anaerobic cobalamin biosynthesis.
<i>ahpF</i>	SL1344_0597	7.26	7.10E-184	Component of alkyl hydroperoxide reductase
<i>cbiJ</i>	SL1344_2002	7.77	2.78E-163	Cobalamin-precorrin-6a reductase. Part of anaerobic cobalamin biosynthesis.
<i>cbiK</i>	SL1344_2001	7.65	5.18E-154	Sirohydrochlorin cobaltochelatase. Part of anaerobic cobalamin biosynthesis.
<i>cbiM</i>	SL1344_1999	6.39	5.46E-137	Cobalt transportation protein.
<i>cbiG</i>	SL1344_2004	6.87	3.25E-118	Anaerobic biosynthesis of cobalamin
<i>cbiL</i>	SL1344_2000	6.81	1.30E-109	Anaerobic biosynthesis of cobalamin
<i>cbiF</i>	SL1344_2005	6.70	2.94E-100	Cobalamin biosynthesis pathway
<i>ybjM</i>	SL1344_0847	4.72	1.04E-90	Predicted inner membrane protein. Unknown function
<i>sufS</i>	SL1344_1307	4.40	2.29E-86	Component of selenocysteine lyase.
<i>sufD</i>	SL1344_1306	4.61	2.80E-86	Forms part of <i>SufBCD</i> complex which protects and generates iron-sulfur clusters under oxidative conditions as they are vulnerable to damage in oxidative environments. <i>SufBCD</i> is important to stabilise the FhuF protein.

<i>potE</i>	SL1344_0682	7.01	1.21E-80	Catalyzes both the uptake and excretion of putrescine. The uptake of putrescine is dependent on the membrane potential and the excretion involves putrescine-ornithine antiporter activity.
<i>iroC</i>	SL1344_2759	4.07	1.21E-79	ABC-transporter protein. Salmochelin siderophore ATP-binding cassette <i>IroC</i>
<i>cbiT</i>	SL1344_2006	6.44	4.15E-79	Anaerobic biosynthesis of cobalamin
<i>cadB</i>	SL1344_2520	5.05	1.17E-76	APC family lysine/cadaverine transport protein. Integral component of membrane. Can confer protection under oxidative stress.
<i>katG</i>	SL1344_4056	7.29	1.40E-75	Bifunctional enzyme with both catalase and broad-spectrum peroxidase activity.
<i>cadA</i>	SL1344_2521	5.11	2.54E-70	Lysine decarboxylase. Carboxy-lyase activity. Cellular amino acid metabolic process.
<i>yjF</i>	SL1344_4057	5.22	7.93E-70	Uncharacterised protein.
<i>ahpC</i>	SL1344_0596	4.66	6.05E-69	Alkyl hydroperoxide reductase subunit C.
<i>ybdB</i>	SL1344_0587	3.77	3.34E-67	Involved in enterobactin synthesis, which is part of Siderophore biosynthesis.
<i>entA</i>	SL1344_0586	3.91	8.72E-65	Enterobactin A. Involved in enterobactin biosynthesis pathway.
<i>trxC</i>	SL1344_2613	4.34	3.10E-59	Oxidoreductase activity. Involved in ribonucleotide reduction.
<i>dmsB</i>	SL1344_0903	5.34	2.35E-56	Dimethylsulphoxide reductase subunit. Plays role during anaerobic respiration. Fe-S cluster acts as its cofactor.
<i>iroD</i>	SL1344_2760	4.06	7.84E-55	Siderophore. Iron ion transport.
<i>dcuB</i>	SL1344_4238	4.25	4.35E-54	Anaerobic C4-dicarboxylate transporter.
<i>cbiP</i>	SL1344_1995	4.92	1.07E-53	Cobalamin biosynthesis pathway. Cobalamin-transporting ATPase activity.
<i>entE</i>	SL1344_0584	4.12	3.06E-53	Biosynthesis of the siderophore enterobactin.
<i>ydhC</i>	SL1344_1360	3.99	3.85E-53	Inner membrane transport protein. Sugar efflux transporter

<i>dps</i>	SL1344_0806	4.83	3.15E-52	DNA binding protein from starving cells. Protects DNA from harmful agents like ROS. Confers resistance to multiple stresses.
<i>fumB</i>	SL1344_4237	3.83	5.94E-52	Fumarate hydratase

Table 4.2: Top 30 downregulated genes in VBNC *S. Typhimurium*. The list is arranged based on the significance of the *p*-value of the differential expression.

Gene	Gene ID	Log2 FC	<i>p</i>-value	Biological function
<i>glgS</i>	SL1344_3170	-6.37	2.39E-107	Predicted glycogen synthesis protein. Glycogen synthesis
<i>asnA</i>	SL1344_3844	-6.37	2.73E-81	Asparagine synthase ATP + L-aspartate + NH ₃ = AMP + diphosphate + L-asparagine.
<i>rbsD</i>	SL1344_3848	-5.27	2.38E-77	Interconversion of beta-pyran and beta-furan forms of D-ribose and conversion of beta-allofuranose and beta-allopyranose as part of Carbohydrate metabolism
<i>ybeL</i>	SL1344_0641	-4.92	1.23E-71	Uncharacterised protein
<i>yecG</i>	SL1344_1861	-5.01	1.81E-53	Also known as <i>uspC</i> , is the universal stress protein C.
<i>SL1344_3743</i>	SL1344_3743	-4.39	4.35E-53	Uncharacterised protein
<i>ypfM</i>	SL1344_2444A	-6.41	8.60E-50	Uncharacterised protein
<i>SL1344_3751</i>	SL1344_3751	-4.70	4.40E-48	Uncharacterised protein
<i>yjfO</i>	SL1344_4312	-4.43	6.84E-47	alternatively known as <i>bsmA</i> , the protein has a role against oxidative stress. Responds to cellular DNA damage
<i>bssS</i>	SL1344_1098	-4.03	2.61E-44	Biofilm regulator in <i>E. coli</i> .
<i>yehH</i>	SL1344_1710	-4.39	3.13E-43	Uncharacterised in <i>S. Typhimurium</i>
<i>ygfA</i>	SL1344_3037	-3.92	7.70E-41	5-formyltetrahydrofolate cyclo-ligase. Associated with nucleotide synthesis

<i>melR</i>	SL1344_4234	-4.12	9.37E-38	Transcription factor. Melibiose operon regulator protein.
<i>glpA</i>	SL1344_2253	-3.89	1.45E-37	Involved in anaerobic respiration. Subunit of anaerobic glycerol-3-phosphate dehydrogenase.
<i>yfiA</i>	SL1344_2624	-6.09	7.02E-37	Renamed as <i>raiA</i> . It prevents the formation of 70S dimer of ribosomes.
SL1344_3013	SL1344_3013	-3.89	4.77E-36	Uncharacterised protein
<i>ygbA</i>	SL1344_2840	-4.72	9.97E-36	Putative cytoplasmic protein
<i>rybA</i>	SL1344_0809	-3.86	1.06E-35	Also known as <i>mntA</i> . Contributes to the manganese availability.
SL1344_0322	SL1344_0322	-4.88	5.48E-32	Uncharacterised protein
<i>glpF</i>	SL1344_4036	-4.06	8.91E-31	Involved in glycerol uptake
<i>zraP</i>	SL1344_4111	-4.77	1.45E-29	Binds to zinc in the periplasm and important for the balance in of zinc in the cell
<i>fadB</i>	SL1344_3936	-6.08	3.27E-29	fatty acid degradation protein that aerobically and anaerobically degrades long-fatty acids
SL1344_3091	SL1344_3091	-4.51	4.48E-29	Uncharacterised protein
<i>uspA</i>	SL1344_3556	-3.96	1.93E-22	Universal stress protein A
<i>nsrR</i>	SL1344_4300	-4.02	5.34E-22	Gene regulator that supresses genes under nitric oxide stress
SL1344_4095	SL1344_4095	-4.39	2.25E-21	Uncharacterised protein
<i>yjfN</i>	SL1344_4311	-4.40	3.38E-21	Uncharacterised protein
<i>ydeE</i>	SL1344_1446	-3.84	7.48E-20	Putative MFS-type (Major Facilitator superfamily) transporter. Integral membrane protein.
<i>siiD</i>	SL1344_4196	-4.47	1.66E-19	Hypothetical type-I secretion protein
<i>siiA</i>	SL1344_4193	-4.09	8.78E-17	Hypothetical type-I secretion protein
<i>fadE</i>	SL1344_0305	-4.72	2.44E-09	Acyl-CoA dehydrogenase

4.2.3 Pathway analysis of differentially expressed genes

Obtaining biological insight from the dataset where over 2000 genes were differentially expressed was a challenge. To investigate pathways of differentially expressed genes (DEGs) from VBNC *S. Typhimurium*, DEGs belonging to various gene ontology pathways and biological processes were analysed. The comparative RNA was isolated from a bacterial population where the majority (over 90%) bacteria in the population were viable, as shown in the Chapter-3, Figures 3.6. The cellular processes presented in the next sections were selected based on literature on formation and/or maintenance of VBNC state (Chapter 1, Section 1.9) previous RNA sequencing studies, or where significant expression changes were observed in the transcriptomic data generated in this study.

4.2.3.1 Oxidative stress and siderophore genes

The VBNC *S. Typhimurium* were generated by subjecting *S. Typhimurium* to oxidative stress using hydrogen peroxide. To understand the bacterial response to this stress, expression of oxidative stress related genes was examined in the VBNC state. Oxidative stress response genes were among the more highly upregulated genes in the VBNC state (Table 4.1). The gene *katG*, encoding catalase, was one of the highly upregulated anti-oxidative stress (AOS) response genes with a fold change of 7.29. The genes *aphF* and *ahpC*, which form parts of alkyl hydroperoxide reductase²⁹⁸, was upregulated by 4.6-fold. The genes *sodCI* and *sodCII*, encoding two Cu/Zn-dependent superoxide dismutases in *S. Typhimurium*, were slightly downregulated^{263,264}. These changes were expected due to the nature of stress used to drive *S. Typhimurium* into VBNC state (i.e. hydrogen peroxide stress).

Another group of genes that were upregulated in *S. Typhimurium* VNBCs were siderophore (ion scavenging) related genes. In particular, the genes *entABEF*, *sufBCDS* and *iroBCDE* genes, which encode for siderophores, were highly upregulated (Figure 4.6B and Table 4.1). The gene transcripts of *entE* and *iroC* were increased by 4-fold. The expression of the *fhuE* (encoding FhuE), another iron acquiring protein, was upregulated 3-fold. Previous gene expression studies have shown the upregulation of siderophore and other iron

acquisition systems following entry into the VBNC state¹²⁸. Siderophores were shown to be under the control of oxidative stress response proteins (in *E. coli*) including Fur²⁶⁵, and the *fur* (ferric uptake regulator) gene was upregulated by 1.28-fold in VBNC *S. Typhimurium*.

4.2.3.2 Fatty acid metabolism

The cell membrane is crucial to maintaining membrane fluidity and patency²⁹⁹. The cell membrane in VBNC *S. Typhimurium* was found to be intact in staining studies (Figure 3.4, Figure 3.6) and appeared to be patent in TEM studies of VBNC (Figure 3.11). Fatty acids form a key part of the hydrophobic portion of the phospholipids that form cell membranes and membrane integrity needs active processes for repair and maintenance^{300, 301}. Hence, the expression of fatty acid biosynthesis genes in the VBNC cells was studied to obtain insights into fatty acid metabolism and thus cell membrane maintenance.

Heatmaps of the DEGs encoding enzymes that are essential for fatty acid metabolism are shown in the Figure 4.6C. Genes in the fatty acid biosynthesis pathway (i.e. *fab*) were systematically upregulated in VBNC *S. Typhimurium* and genes related to fatty acid degradation (*fad*) pathway were highly downregulated (Figures 4.6D). The *fabBDFH* operon was upregulated and *fadABEHIJLR* were all downregulated suggesting an increase in fatty acid synthesis and decrease in fatty acid degradation, as has been reported previously³⁰². Genes involved in phospholipid transport across the inner and outer membrane had small changes in their expression. The genes encoding phospholipid transport proteins *ycfF*, *yrbD* and *vacJ* were downregulated by -1.76 , -0.76, and 0.5-fold respectively; in comparison, *yrbB* was slightly upregulated, by 1.25-fold.

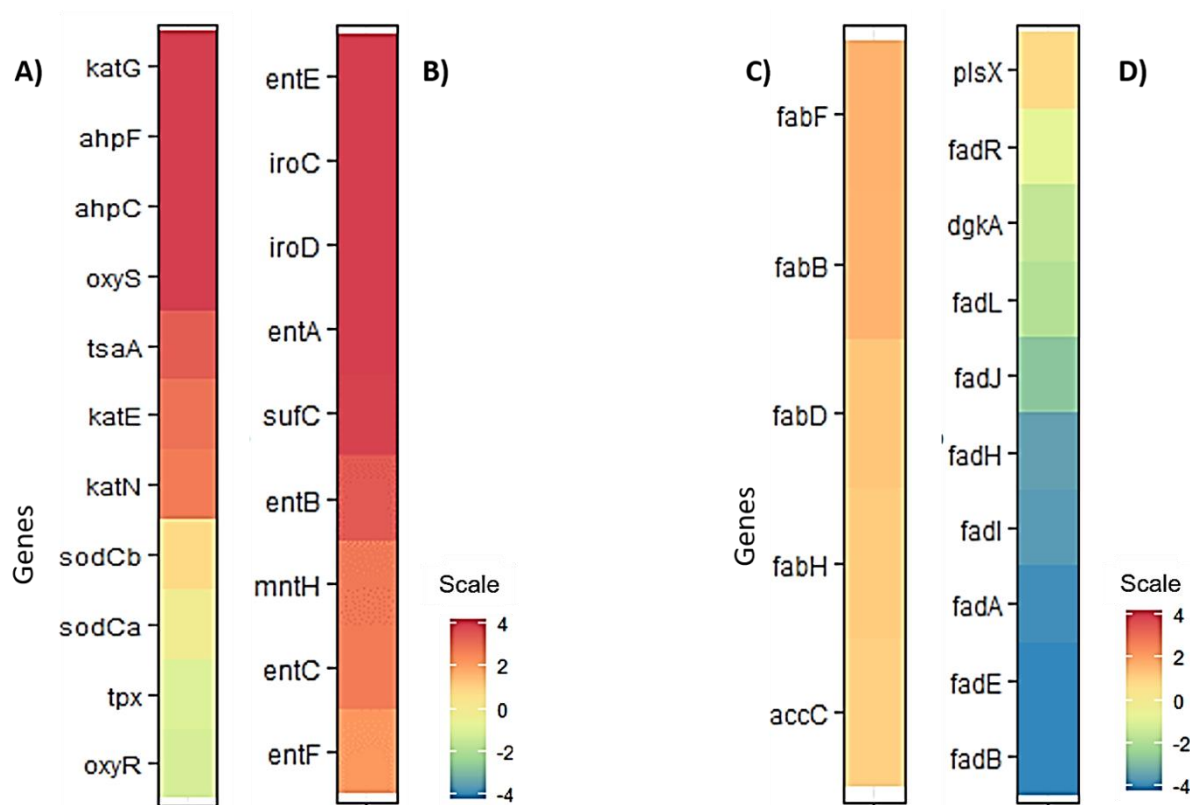


Figure 4.6: Heatmaps of oxidative stress response genes, siderophore genes and fatty acid metabolism genes. Heatmap showing the differential expression of functionally related genes between culturable *S. Typhimurium* and VBNC cells. The heatmaps were coloured such that genes in red (top of the scale) were upregulated, those in blue downregulated. Refer to the scale for all colours and fold changes in between. A) A heatmap of the expression profiles of oxidative stress response genes like catalases, peroxidases and superoxide dismutases. B) A heatmap showing the expression profiles of siderophores in *S. Typhimurium* VBNC vs culturable cells. Siderophores are some of the highest upregulated genes of *S. Typhimurium* in the VBNC state. C) and D) represent the heatmaps of the fatty acid biosynthesis genes and the enzymes involved in the fatty acid degradation respectively.

4.2.3.3 Membrane proteins and LPS

Membrane proteins, LPS, phospholipids and peptidoglycan are the molecules that maintain the structural integrity of the bacterial envelope of Gram-negative bacterial cell³⁰³.

Many membrane protein genes were upregulated in *S. Typhimurium* VBNC (Figure 4.7A). Notably, *ompW* was upregulated by approximately 3.5-fold. LPS genes were also examined. The majority of the genes encoding proteins required for LPS biosynthesis and assembly were upregulated (Figure 4.7B). Lipid A is synthesised in the cytoplasm by *lpx*-pathway, translocated by MsbA, and transported by the LptABCDEFGF protein complex³⁰⁴. Many genes in the *lpx* pathway were upregulated (Figure 4.7B). FtsH protease can negatively regulate the formation of LPS through LpxC³⁰⁵. The levels of *ftsH* transcript were also increased in VBNC *S. Typhimurium* (Figure 4.7C). The *rfp* operon in *S. Typhimurium*, now renamed as *wba*, *wbd* and *wby*, are key to the biosynthesis of O-antigen^{306,307}. These genes were downregulated in the VBNC *S. Typhimurium*, while the LPS core biosynthesis genes including *wzzB*, *wcaJ* and *wzx*³⁰⁶ all these genes were all upregulated, by almost Log₂ 2-fold. Completed LPS is transported across the periplasm by LptABCDEFGF proteins but the transcriptional profile of these genes remains unresolved because the changes observed were not statistically significant through variability. This data indicates likely changes in the LPS profiles but this needs further comparative experimental analysis of the LPS in VBNC *S. Typhimurium*.

4.2.3.4 Proteases

Proteases play an important role in the emergence of spores out from dormancy in Gram-positive bacteria³⁵ and the potential importance of proteases in generating or maintaining the VBNC state was raised in Section 1.9.4 and Chapter 1. Important AAA+ proteases, which are ATP-dependent proteases³⁰⁸, and protease complexes found in Gram-negative bacteria regulate the proteome through controlled protein degradation³⁰⁹. In *Salmonella enterica*, these proteases include ClpXP, ClpAP, ClpCP, Lon, HslUV and FtsH. The AAA+ proteases have a role in dormancy including persistence³¹⁰. As mentioned earlier, Lon protease regulates HipBA toxin-antitoxin³¹¹ system and through this plays a role bacterial persistence²²³.

In the VBNC *S. Typhimurium*, the expression of the gene encoding ClpP was down-regulated (Figure 4.7C). There was no appreciable change in *clpX* which encodes ClpX. ClpX

with ClpP forms ClpXP complex. Protease encoding gene *htrA* was slightly upregulated (1.47-fold). RNA-seq data on *hslUV*, *lon* and *htpX* gene were inconclusive, due to low statistical significance. The gene *htpX* encodes a membrane bound protease that regulates the quality of membrane proteins under stress (in *E. coli*)³¹². The gene *ftsH*, an essential protease, was the upregulated by 2-fold. *ftsH* also has role in regulating LPS biosynthesis³⁰⁵ and in degradation of misassembled proteins³¹³.

4.2.3.5 Sigma factors

Under stress conditions, 'alternate' sigma factors regulate the expression of multiple genes that help bacteria mediate a stress response^{314,315}. Sigma factors are a component of the RNA polymerase (RNAP) complex that bind to the promoter region of bacterial genes and recruit the RNAP complex to the promoter enabling gene expression³¹⁶. Different sigma factors are available under different conditions, and each sigma factor regulates the expression of group of genes or 'regulons'³¹⁷. RpoS or σ^{38} is a sigma factor that plays a key role in the bacterial response to stress conditions, and is sometimes referred to as the stress sigma³¹⁸. A heatmap of some important sigma factors is presented in Figure 4.7D. Transcription of the sigma factor encoding genes did not change significantly (fold change <2) in the VBNC state. There was little change in the transcription of *rpoS* compared with unstressed controls (downregulated by 0.8-fold). The genes *rpoE* and *rpoH* were also slightly downregulated. The highest change observed was in transcription of *rpoN*, which was down-regulated by 1.2-fold.

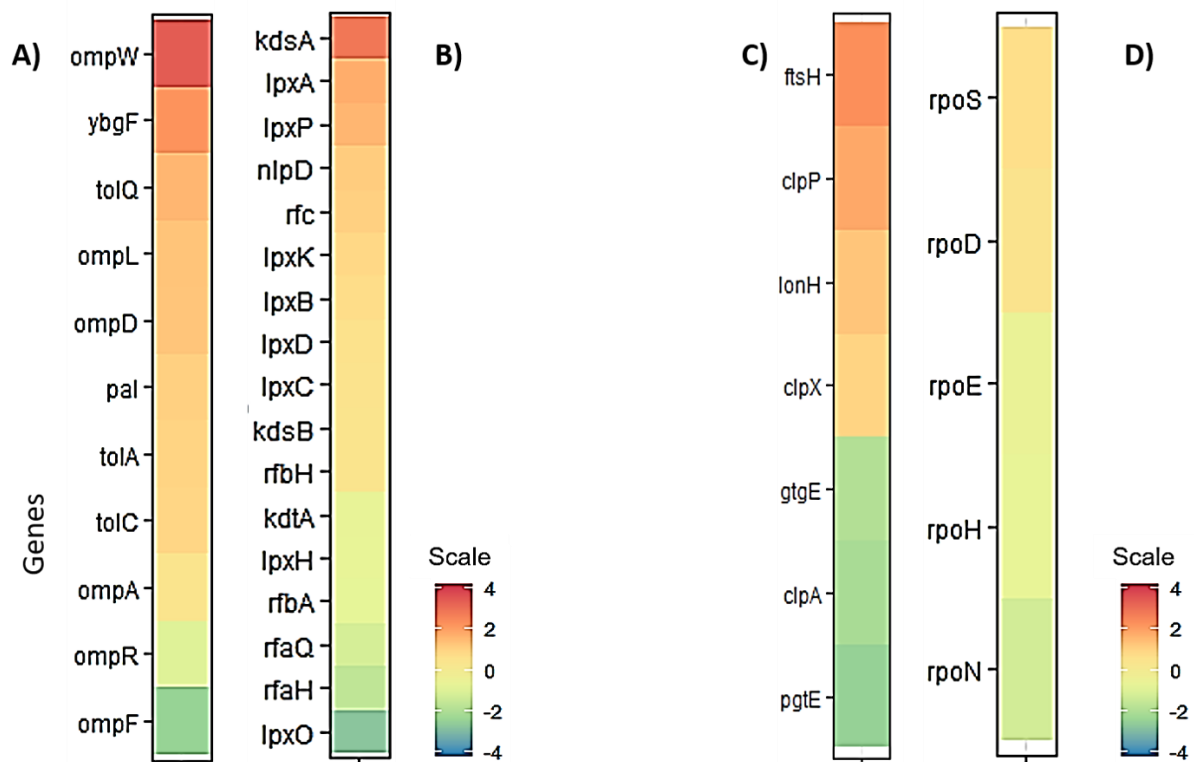


Figure 4.7: Differential expression of genes that encode components of the outer membrane, proteases and sigma factors. Heatmaps showing the differential expression of functionally related genes between culturable *S. Typhimurium* and VBNC cells. The heatmaps were coloured such that genes in red (top of the scale) were upregulated, those in blue down-regulated. Refer to the scale for all colours and fold changes in between. Figures here represent data for genes that encode membrane proteins (A), LPS (B), proteases (C) and (D) sigma factors.

4.2.3.6 Metabolic pathways

Cellular metabolism can influence the physiology of bacteria including cell division. For instance, ATP depletion is a characteristic of persisters in *E. coli* and *S. aureus*^{21,120}. The pentose phosphate pathway generates key substrates in NADPH, ribose 5-phosphate and erythrose 5-phosphate. While NADPH contributes to the energy flux of cells³¹⁹ the latter two metabolites (r5p and e5p) act as precursors for nucleotide biosynthesis and aromatic amino acid synthesis, respectively³²⁰. NADPH can contribute to quenching ROS (reactive oxygen species)³²¹ and help generate ATP. There was an overall upregulation of the pentose phosphate pathway genes in VBNC *S. Typhimurium* (Figure 4.8C). The products from the glycolysis pathway are shunted to the pentose phosphate pathway³²². Multiple genes in the glycolysis and TCA cycle including *glk*, *pfkB*, *gltA*, *mdh*, *pgi* and *kgtP* genes were all downregulated while *fumB*, *sucD* and *pykF* were upregulated (Figure 4.8D).

Analysis of another essential pathway, nitrogen metabolism, showed that the *nar*-operon was upregulated at the pathway level. One of the highest downregulated genes was *asnA*, which is involved in the synthesis of L-aspartate. The *nar*-encoded proteins are induced under anaerobic conditions where they play a role in nitrogen assimilation and electron acceptance³²³. They are attached to the membrane as part of *fnr* regulon that is activated by oxidative stress³²⁴.

4.2.3.7 Flagellar genes

Flagellar proteins of *S. Typhimurium* are highly immunogenic and induces a pro-inflammatory response in infected hosts³²⁵. Regulation of *Salmonella*'s flagellar expression through phase variation³²⁶ is thought to be a strategy used by the organism to avoid recognition of intracellular *Salmonella* by immune T-cells³²⁷. Analysis of the VBNC transcriptome revealed that many genes encoding various flagellar components were downregulated (Figure 4.8B).

The expression of the flagella related genes in VBNC induced by oxidative stress was inconsistent. The genes *motAB*, *fliA* and *flg* genes were downregulated. MotA is involved in

the rotation of flagella and is essential for motility and *fliA* encodes a regulator is a regulator of other flagellar genes³²⁸. The gene *flgM* was highly downregulated with a fold change of -2.66. Other *flg* genes were slightly downregulated but are not represented in the heatmap (Figure 4.8b). RcsCDB was shown to down regulate flagellar genes, especially *fliPQR* and upregulate virulence genes of Salmonella Pathogenicity Island 2 (SPI-2)³²⁹. This downregulation of motility-associated genes and upregulation of SPI-2 genes was also observed in VBNC *S. Typhimurium* as shown in the gene expression heatmaps of the flagellar and SPI-2 genes (Figure 4.8B and Figure 4.9B).

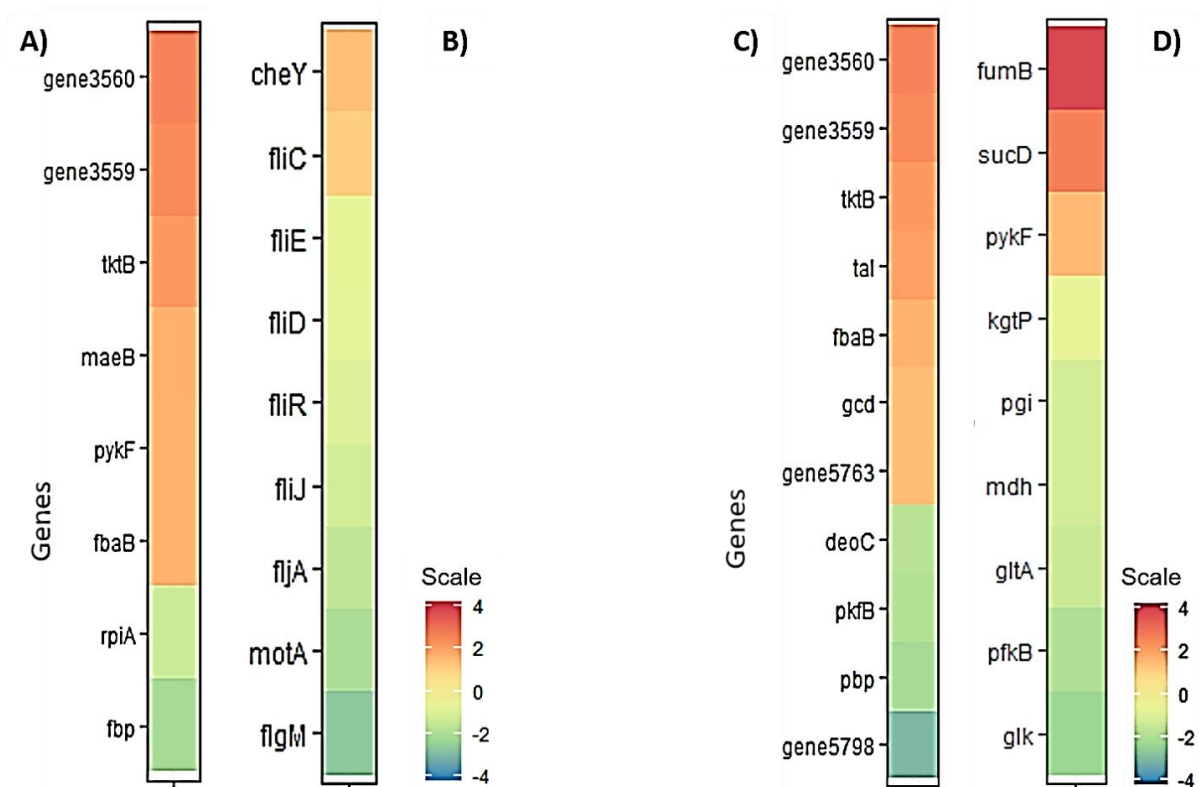


Figure 4.8: Heatmaps of differentially expressed genes in selected metabolic pathways.

Heatmap showing the differential expression of functionally related genes between culturable *S. Typhimurium* and VBNC cells. Colours represent fold change in gene expressions, such that genes in red (top of the scale) were upregulated, those in blue downregulated. Refer to the scale for all colours and fold changes in between. Figures here represent data for genes that encode carbon fixation pathway (A), motility related genes (flagellar) (B), pentose phosphate pathway and (C) glycolysis and TCA cycle (D).

4.2.3.8 Expression of genes from pathogenicity islands SPI-1 and SPI-2 in the VBNC state

Pathogenicity islands play an important role in virulence of pathogenic bacteria including *E.coli* and *Salmonella enterica*³³⁰. There are 11 known SPIs in *S. Typhimurium*³³¹. SPIs encode virulence determinants including secretion systems that are required for *Salmonella* invasion of epithelial cells, and intracellular survival³³². *S. Typhimurium* controls the expression of various pathogenicity related genes using environmental cues detected by the pleiotropic two-component regulator PhoP/Q³³³.

The expression of SPI-1- and SPI-2-encoded genes were typically upregulated in the VBNC state (Figure 4.9). These SPI-1 and SPI-2 loci harbour the type-3 secretion system (T3SS) genes that form the injectosomes and other proteins essential for intestinal epithelial invasion and replication³³⁴. SPI-2 plays an essential role in *Salmonella*-mediated apoptosis of intestinal epithelial cells³³⁵ and in protecting the bacterium against phagocytic killing³³⁶. The *phoP* and *phoQ* genes, that control the expression of the SPI-1 have been found not to be significantly upregulated³³⁷. The SPI-1 locus starts with *ygbA* gene and ends with *pphB*³³⁸ stretching approximately 39.1kb in *S. Typhimurium* SL1344 (base pair coordinates 3028421 to 3067587). The SPI-2 locus extends from *ssaU* till *pykF* in *S. Typhimurium* SL1344. SPI-2 is approximately 41.7kb long (base pair coordinates 1416778 to 1458501). Some secreted effectors sit outside of these loci. Most of the structural *ssa* genes were upregulated in the VBNC transcriptional state (Figure 4.9B) but the *sse* genes were downregulated. *ssaU*, *ssaT*, *ssaS*, *ssaR*, *ssaQ*, *ssaP*, *ssaO*, *ssaN*, *ssaV*, *ssaM*, *ssaL*, *ssaK*, *ssaJ*, *ssaI*, *ssaH*, *ssaG*, *ssaB*, *ssaBa* and *ssaA* genes encode proteins that form part of the T3SS structural apparatus³³⁹. The genes in the *sseGFE* operon encode effector proteins that prevent the aggregation of host endosomes³⁴⁰. *SseDC* are translocon proteins and *sseA* encodes a chaperon³⁴¹.

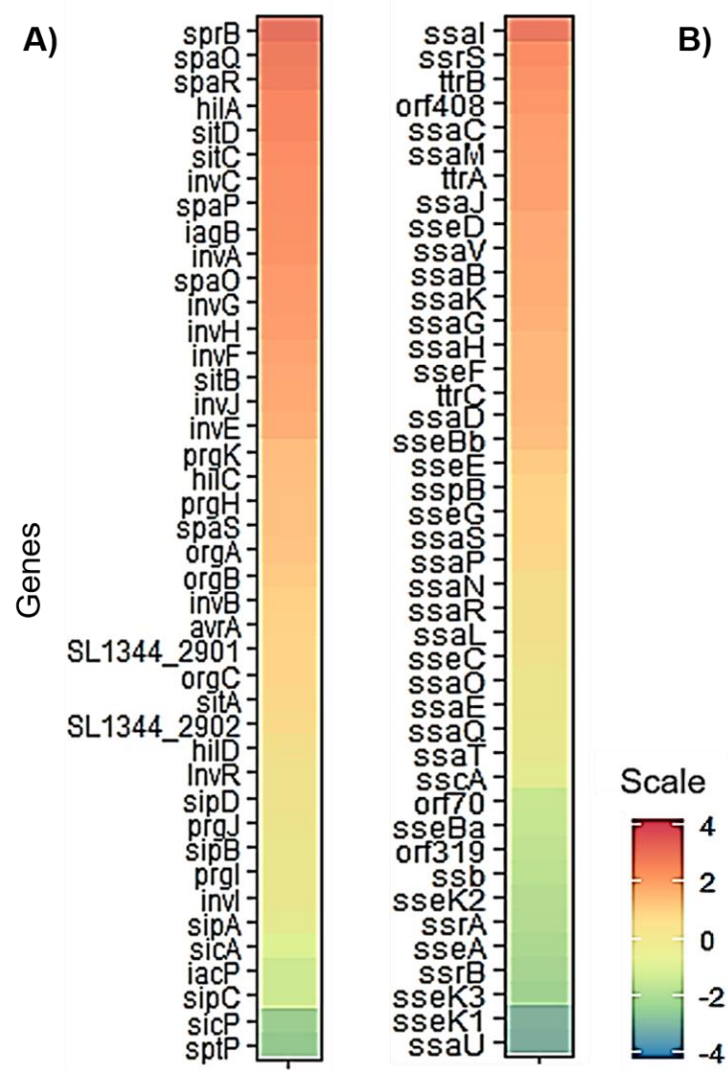


Figure 4.9: The differential expression of virulence related genes. Heatmap of (A) SPI-1 and (B) SPI-2 regulated genes in VBNC state showing an overall upregulation of key effector genes. Colours represent gene transcription, genes in red (top of the scale) were upregulated and those in blue downregulated. Refer to the scale for all colours and fold changes in between.

4.2.4 Functional clustering of differentially expressed genes in the VBNC state based on gene ontology pathways

A gene ontology-based approach was adopted to correlate the DEGs of VBNC *S. Typhimurium* across biological processes. A brief definition of the Gene Ontology terms (GO terms) and the three main categories will be discussed below. These definitions are adopted from the Gene Ontology Consortium and can be found on the organisation's website²³¹. GO terms functionally categorise different genes and the gene products into three main categories of a) their activity, b) their involvement in a biological process and c) if they are structure components in the cell. Thus, providing a GO term analysis can provide overview of gene expression trends but without focusing on a specific activity or function. While GO terms provide functional categorisation of various proteins, PANTHER (www.pantherdb.org) is an online software tool that enables classification of data based on enzyme commission numbers (EC numbers), integrated within the GO database³⁴². Thus, the PANTHER classification system of the *S. Typhimurium* reference genome SL1344 was employed to classify the VBNC differential expressed genes into the three designated categories recommended by the GO consortium i.e. molecular function, biological process and cellular components³⁴³. Specifically, the 'molecular function' refers to the specific 'activity' of the gene product such as binding or catalytic activity. 'Biological process' broadly encompasses molecular activities more than one distinct reaction step, for example, pyrimidine metabolism process. 'Cellular components' include structural (and functional) molecules., for example, cytochrome c falls under a cellular components category as it is a part of the mitochondrial membrane.

Functional pathway clustering of the 1993 VBNC differentially expressed genes showed that GO terms associated with Molecular Function; 'catalytic activity' (31.5%), 'binding' (10.5%) and 'transporter activity', were over-represented in the dataset of genes with altered expression levels (Figure 4.11A). It was noted that randomly selected DEGs in the VBNC *S. Typhimurium*, on average, have an over-representation of genes with activities of 'catalytic activity' 'binding' and 'transporter activity'. The DEGs associated with among the GO terms

'catalytic activity' and 'binding' higher were over-represented by 31.5% and 10.5%, respectively (Figure 4.11A). 'Catalytic activity' includes activities of hydrolase, ligase, lyase, isomerase, transferase and 7 other activities¹²¹. 'Binding' includes binding to drugs, cofactors, metal clusters, nitric oxide, organic cyclic compounds, proteins and 42 other activities¹²¹. Pathway clustering of over-represented genes associated with 'transporter activity' includes lipid transporter activity, transmembrane transporter activity and intermembrane lipid transfer activity.

DEG-encoded Biological Processes were predominantly 'metabolic processes' (31.4%) and 'cellular processes' (27.1%) (Figure 4.11B). Examples of 'metabolic processes' included – ATP metabolic process, demethylation/methylation, oxidation-reduction process, secondary metabolic process, protein synthesis/degradation and many more processes which are both catabolic and anabolic. The GO term 'cellular processes' included cell adhesion molecule production, cell division, cellular detoxification, plasmid maintenance and protein folding. Other over-represented GO terms included 'localisation', 'response to stimulus' and 'cellular component organisation or biogenesis'. Pathway clustering of over-represented genes associated with the third category, Cellular Components, were primarily defined as 'Cell part' (15.6%) and 'membrane' (11.6%) GO terms (Figure 4.11C). The 'membrane' GO terms include envelope, plasma membrane, secretion systems and more than 110 other annotations.

The over-representation of DEGs of cellular components were shown in Figure 4.11C. The cellular component mainly includes genes in the categories of DEGs that are linked as 'structural'. This includes flagellar proteins, ribosomal proteins, membrane proteins including two component systems, ATP synthases etc. Changes in these structural and functional genes are also expected. The membrane proteins and cell parts categories being sharing the high number of DEGs by percentage than other categories.

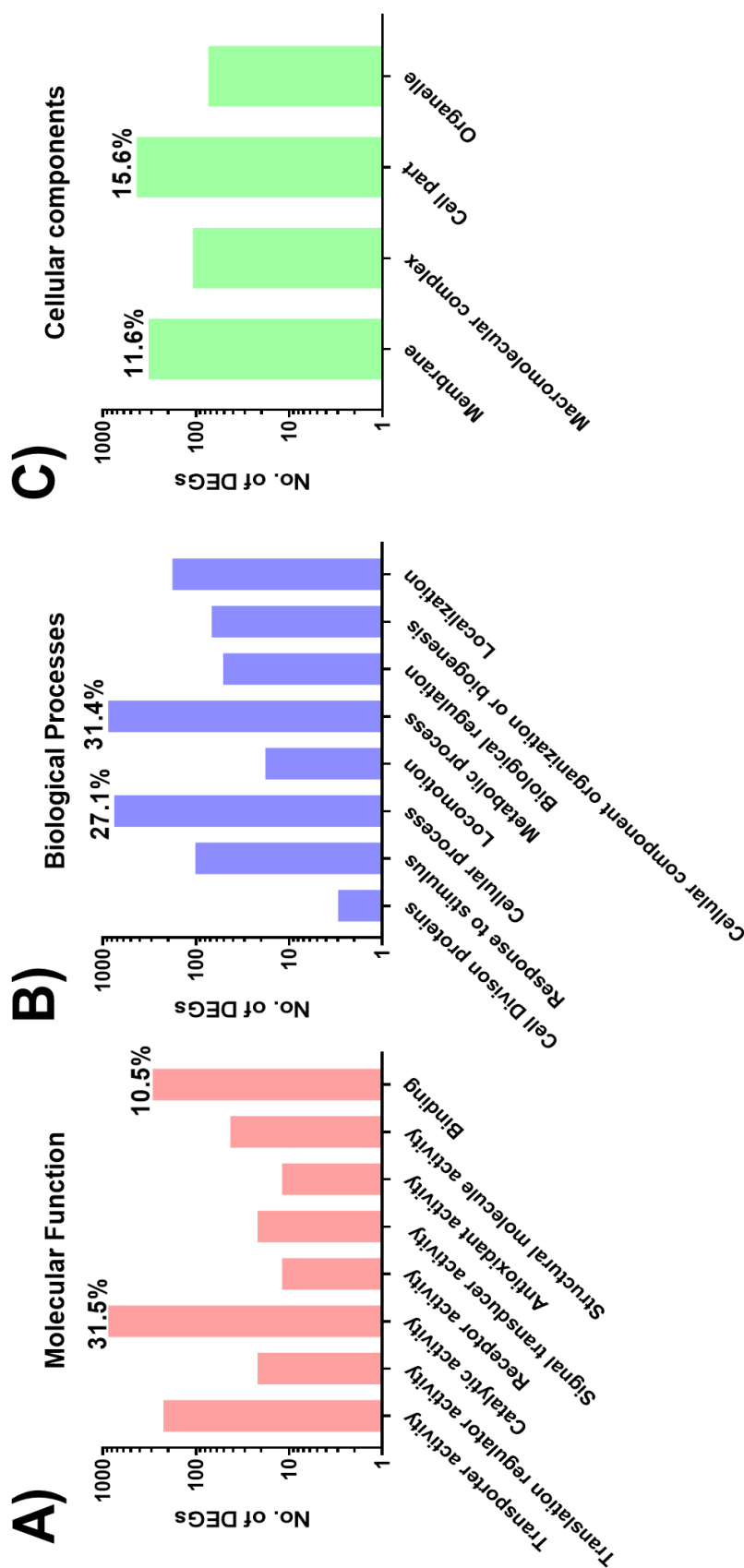


Figure 4.10: Functional clustering of differentially expressed gene (DEGs) in VBNC *S. Typhimurium*. Bar charts represent the over representation test on number of DEGs in the categories of (A) molecular function, (B) biological process and (C) cellular components. The highest over-represented processes have been highlighted with the percentage change on the top of the bars in the graph. The percentage represents the percent of the over-represented genes verse the total number of genes analysed.

4.2.5 Mutagenesis studies of target genes that may play a role in *S. Typhimurium* VBNC formation

Gene knockout studies including those in *S. enterica* and *E. coli* showed the significance of various stress response genes in the VBNC state in bacteria^{30,118,177}. In this study, gene knockouts were made in WT *S. Typhimurium*, by targeting the genes *cbiA*, *dhaB*, *marR*, *pduG*, and *pnp* using the lambda-red recombinase system. These genes were chosen based on the the VBNC transcriptomic data and published literature regarding the movement of bacteria into dormancy. In particular, *cbiA* (cobalamin biosynthesis anaerobic pathway), is the first gene in the cobalamin synthesis operon of *cbiA*, *cbiC*, *cbiD*, *cbiE*, *cbiT*, *cbiF*, *cbiG*, *cbiH*, *cbiJ*, *cbiK*, *cbiL* and *cbiP*³⁴⁴. Most of the genes in the operon were upregulated in VBNC *S. Typhimurium* (Table 4.1) and this operon has previously been shown to be key under anaerobic conditions³⁴⁵. Moreover, cobalamin transport gene (VC2381) was shown to be upregulated in the VBNC *V. cholerae*¹²⁸. The gene *dhaB*, encodes for a glycerol dehydratase that plays a role in anaerobic glycerol metabolism and requires cobalamin as a crucial cofactor for DhaB functionality. The *dhaB* gene was up regulated by more than 5-fold.

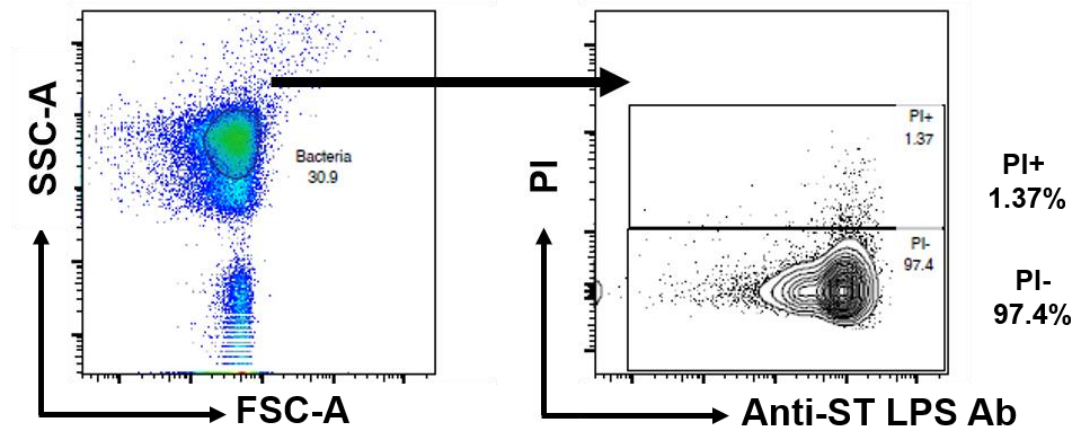
Next, *marR* (multiple antibiotic resistance proteins) is a transcription factor hypothesised to play a role in VBNC formation of *Pseudomonas syringae*¹⁰⁷. MarR activates the oxidative stress genes through *oxyR* and is also involved in sensing the environment¹⁹⁸. MarR was postulated by Postnikova *et al.* to be an early member of a pathway that drives the phenotypical characteristics of the VBNC state¹⁰⁷. The gene *pduG* encodes a glycerol dehydratase and was upregulated over 6-fold in the VBNC state of *S. Typhimurium*. The gene *pduG* has been shown to be upregulated when *S. Typhimurium* transitions from late exponential phase into the early stationary phase²⁴⁸.

The final target gene, *pnp*, encodes the polynucleotide phosphorylase (PNPase) enzyme which is an exonuclease and part of the RNA degradosome. PNPase can also polyadenylate RNA to promote degradation^{346,347}. PNPase was shown to play a role in degrading the oxidatively-damaged RNA³⁴⁸. While *pnp* was not significantly differentially

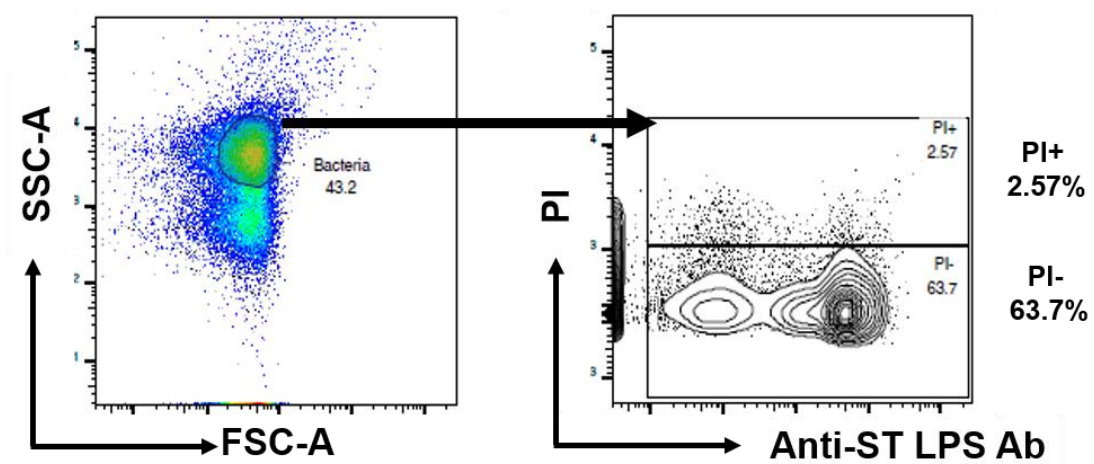
expressed in the VBNC state, a *pnp* mutant was constructed because of the role of PNPase in rRNA degradation (an observation in Figure 4.1A, B), and because of its exonuclease activity under oxidative stress.

In order to assess the contribution of these five *S. Typhimurium* gene mutants to VBNC formation, a FACS analysis of *S. Typhimurium* WT and mutant *S. Typhimurium* was conducted, with and without subjecting the bacteria to oxidative stress, the latter to drive the bacteria into VBNC state. Bacterial population was gated based on the FSC-A and SSC-A parameters that indicate the size and granularity of the bacteria, respectively. The bacteria were identified using anti-*S. Typhimurium* LPS antibodies and the dead bacteria were observed using PI staining. All of the *S. Typhimurium* mutants except the Δpnp had dead bacteria ranging from 0.7% to 4% after stress (Figure 4.11A to 4.11J). Under the conditions that generated VBNC state in WT and other mutants, the Δpnp mutant showed over >15% of cell population were dead, instead of entering the VBNC state, suggesting a possible role of PNPase in the entry or survival of *S. Typhimurium* in VBNC state (Figure 4.11K and 4.11L).

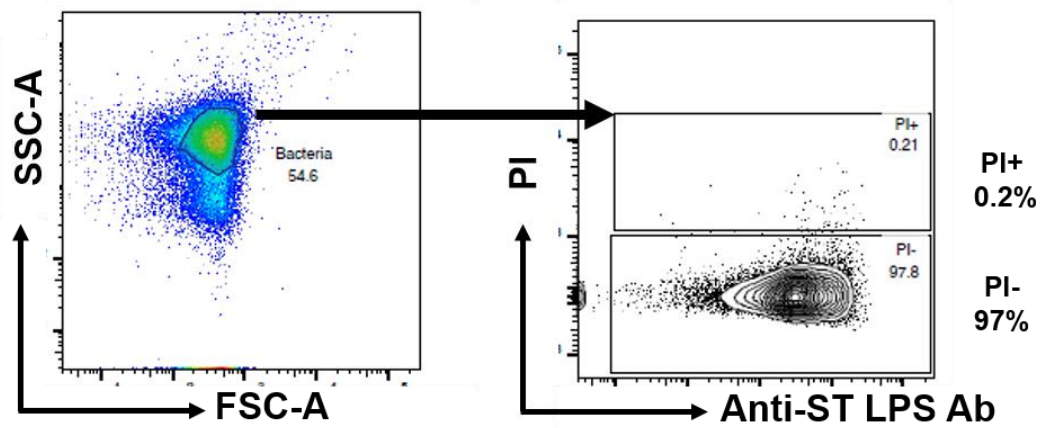
A) UNSTRESSED *S. TYPHIMURIUM* WT



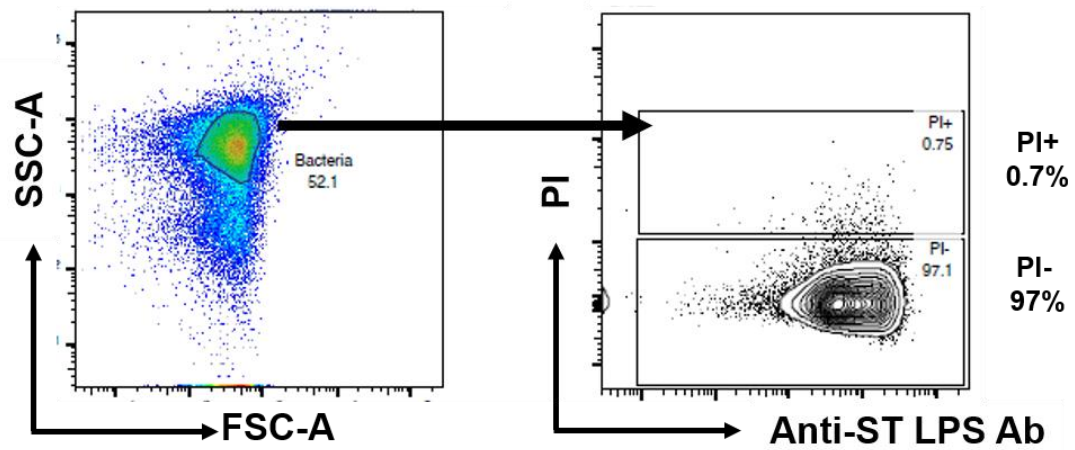
B) STRESSED *S. TYPHIMURIUM* WT



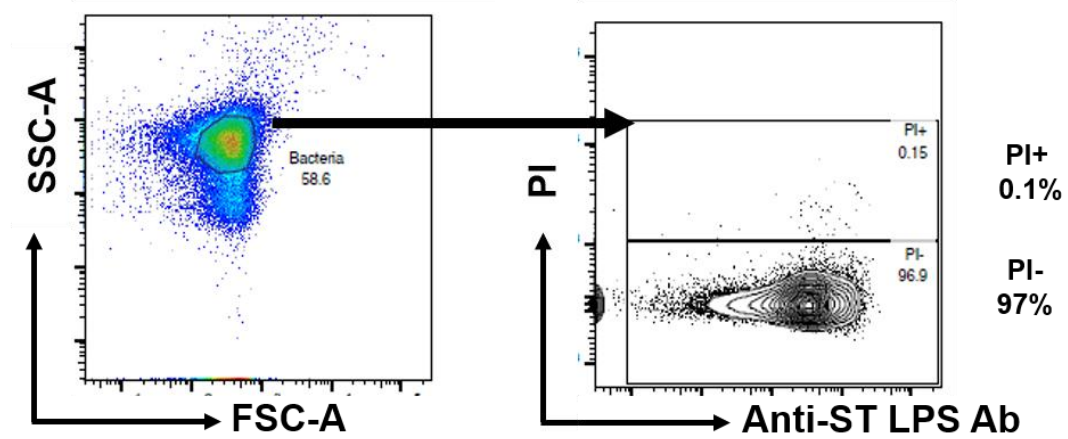
C) UNSTRESSED S. TYPHIMURIUM $\Delta cbiA$



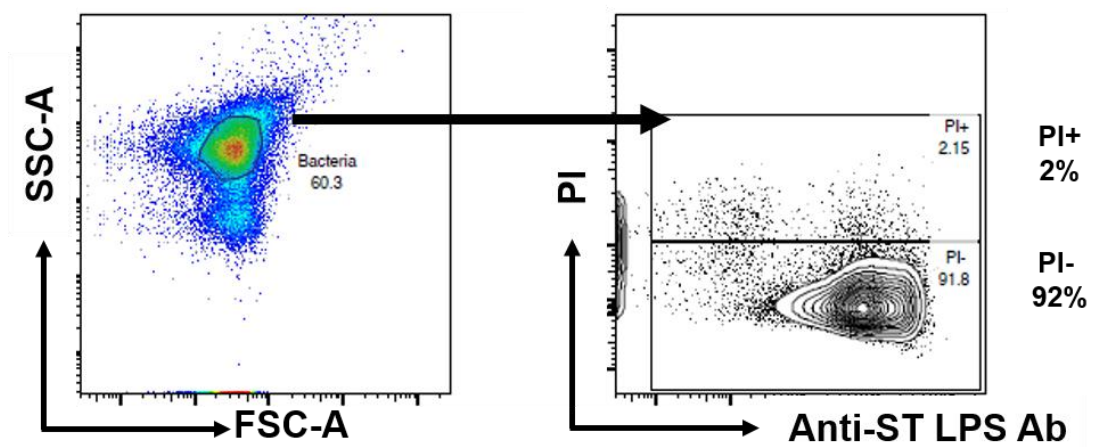
D) STRESSED S. TYPHIMURIUM $\Delta cbiA$



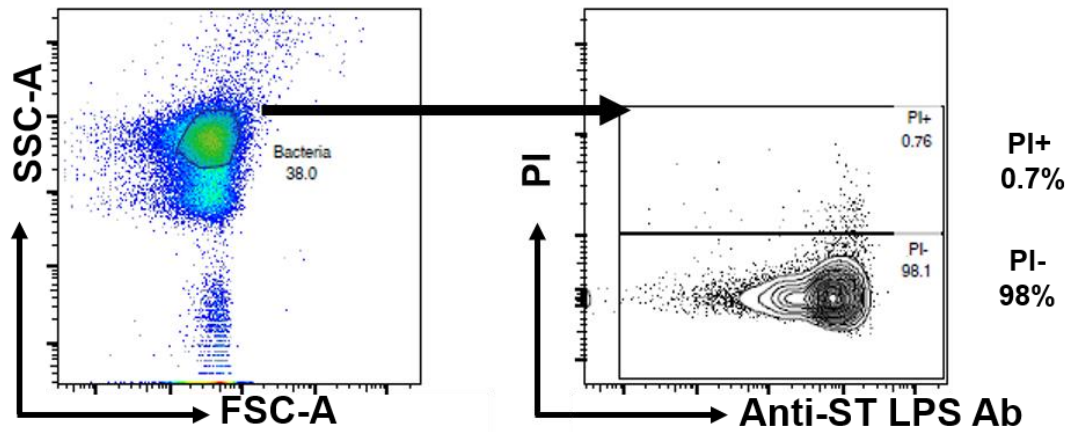
E) UNSTRESSED *S. TYPHIMURIUM* $\Delta dhaB$



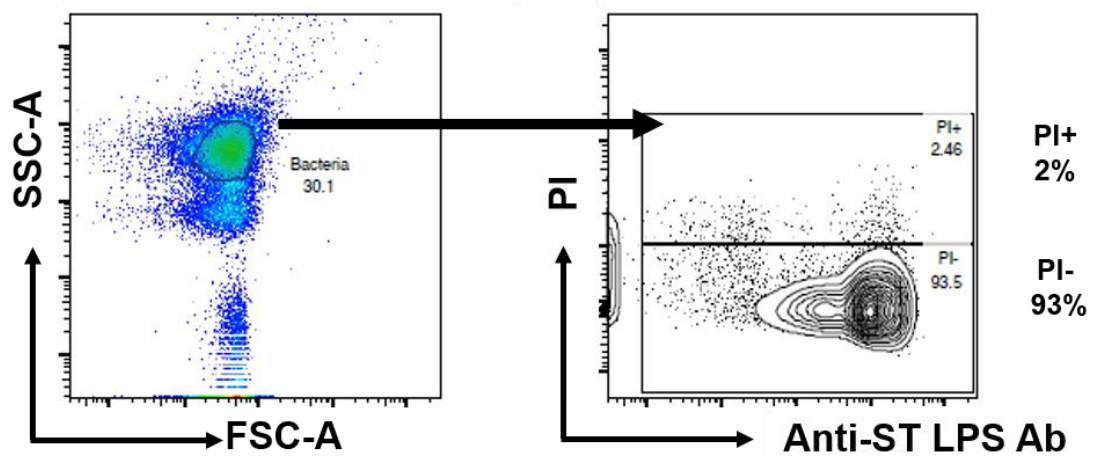
F) STRESSED *S. TYPHIMURIUM* $\Delta dhaB$



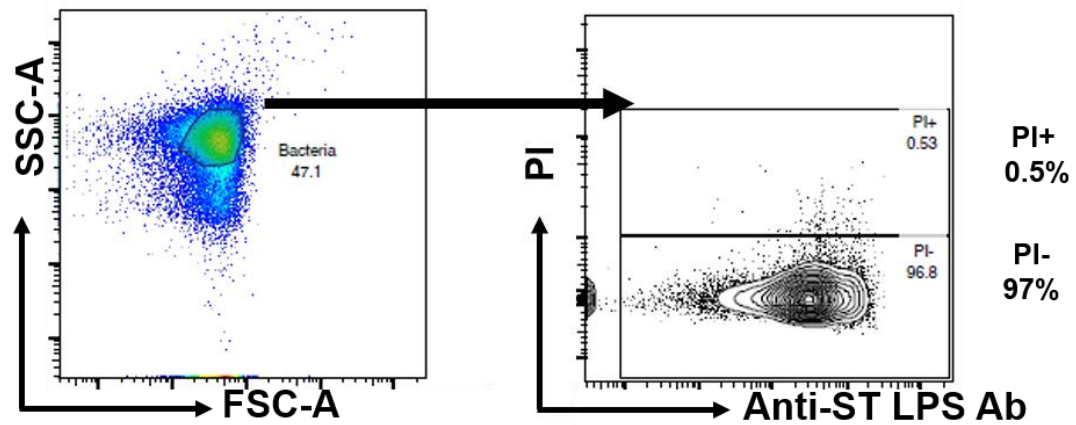
G) UNSTRESSED S. TYPHIMURIUM $\Delta marR$



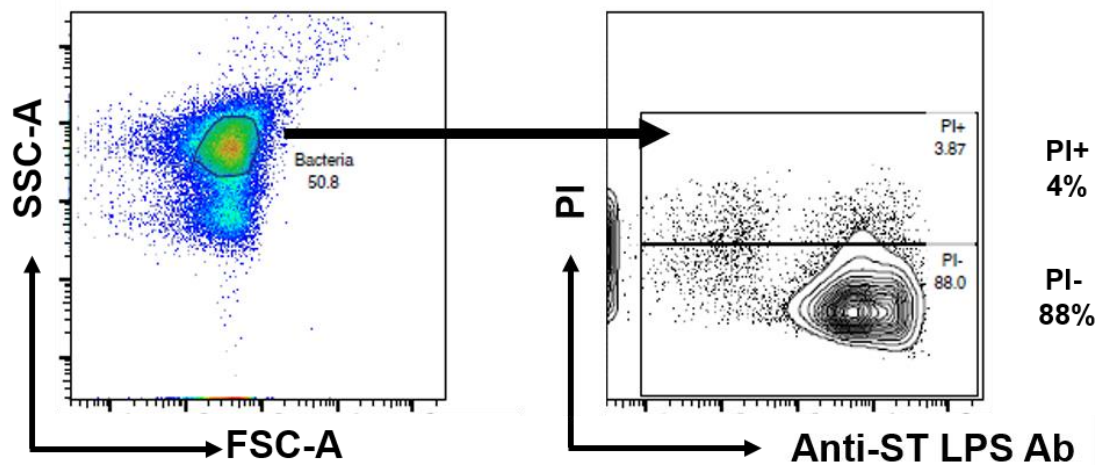
H) STRESSED S. TYPHIMURIUM $\Delta marR$



I) UNSTRESSED *S. TYPHIMURIUM* $\Delta pduG$



J) STRESSED *S. TYPHIMURIUM* $\Delta pduG$



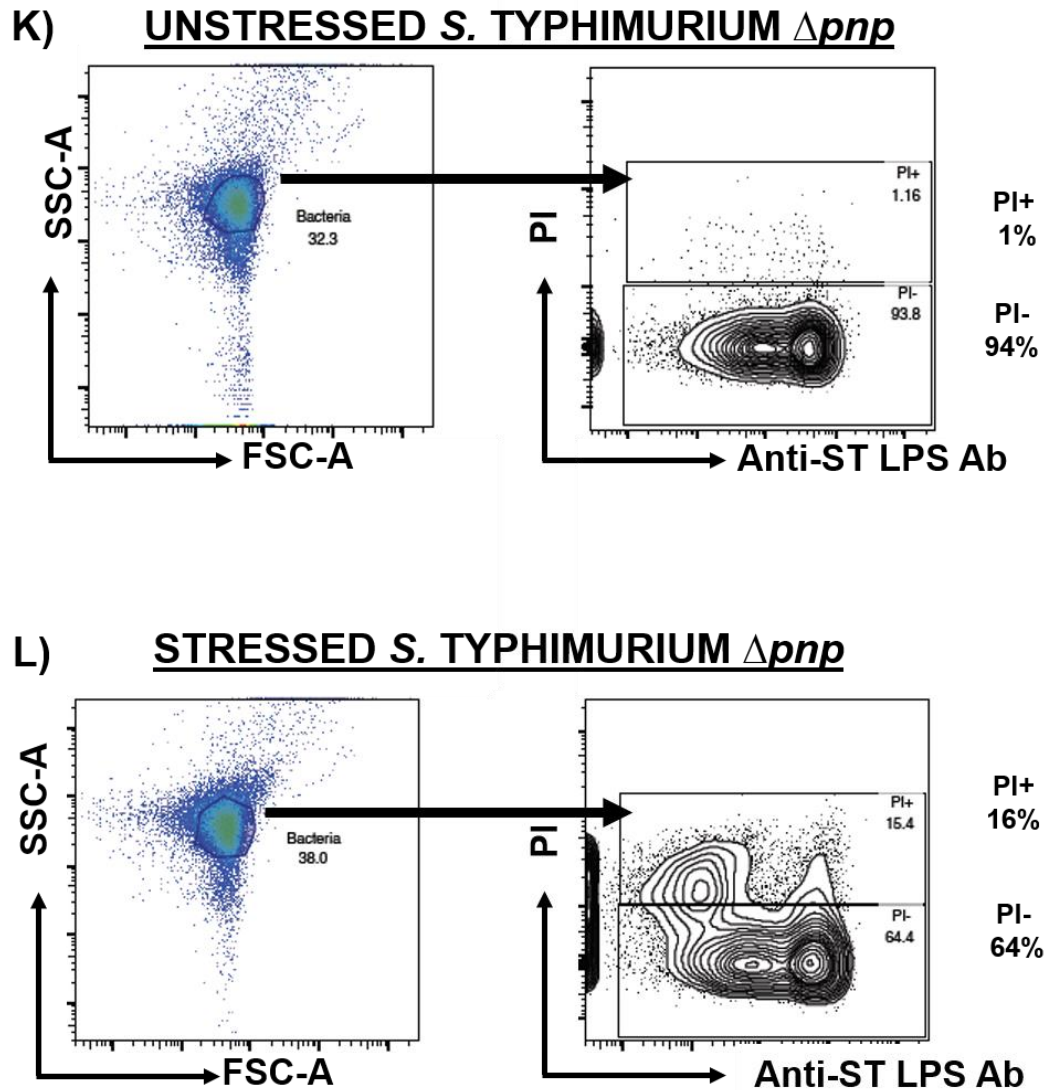


Figure 4.11: Figures A-L show the FACS plots of stressed and unstressed *S. Typhimurium* WT and mutants. WT and mutant *S. Typhimurium* bacteria were first identified in the flow cytometer based on the size (FSC-A) and granularity (SSC-A) of the organisms. The bacterial population was analysed for the markers Anti-ST LPS antibodies (x-axis) and PI (y-axis). Anti-ST LPS antibodies can stain all the bacteria while PI can stain only the dead bacteria. The percentage of dead bacteria (PI+) did not increase significantly after treatment with oxidative-stress that induced VBNC state in WT, $\Delta cbiA$, $\Delta dhaB$, $\Delta marR$ and $\Delta pduG$ mutants (Figure 4.11A-J) and ranged from 0.7% to 4% whereas the Δpnp had higher percentage of dead bacteria at 16% (Figure 4.11L). An increased percentage of the *S. Typhimurium* Δpnp mutants died under stress conditions rather than becoming VBNC.

4.2.5.1 *S. Typhimurium* Δpnp mutant showed a growth deficit compared with WT

The *S. Typhimurium* Δpnp strain was the only mutant that showed a reduced ability to enter VBNC state, shown earlier. A growth curve was plotted to compare the growth kinetics of Δpnp mutant with WT, based on the OD₆₀₀ absorption under static growth conditions at 37°C for 18 hours (Figure 4.12). The Δpnp mutant grew at a much slower rate than the WT with the maximum optical density of the Δpnp mutant being approximately half that of the WT indicating it has a growth defect (Figure 4.12).

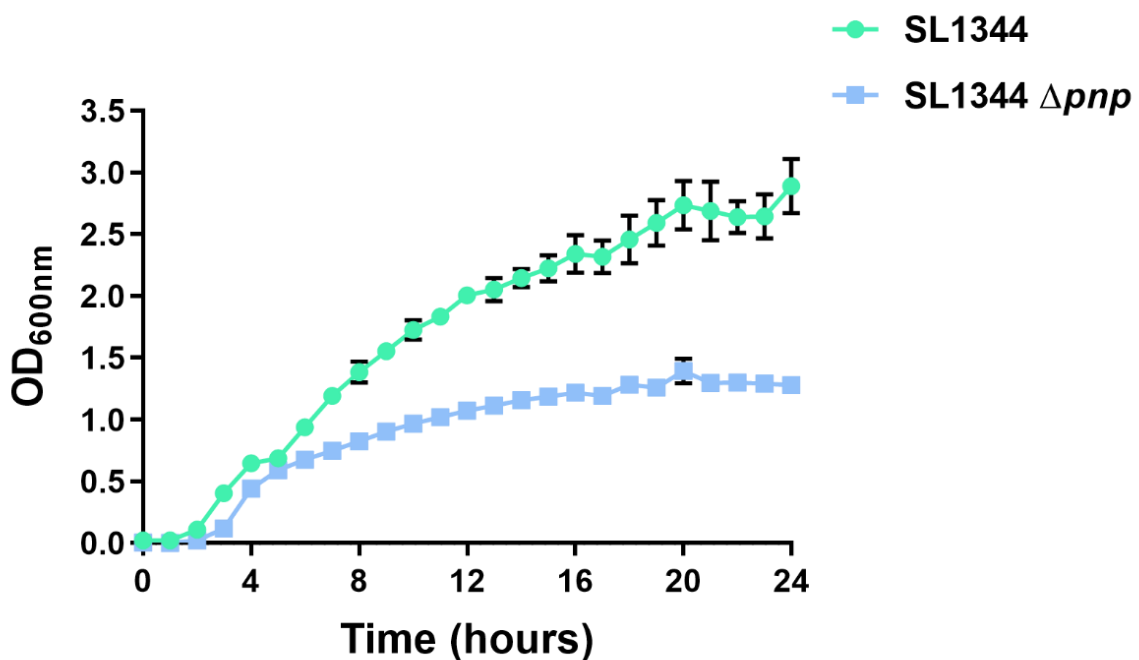


Figure 4.12: Comparison of growth curves of *S. Typhimurium* Δpnp mutant and WT.

S. Typhimurium WT and Δpnp mutant were grown in LB at 37°C with shaking. At each time point the OD₆₀₀ was measured and the data here represents 3 biological replicates, and the error bars show standard deviation. The Δpnp mutant had a growth defect at 37°C and reached a lower optical density than the WT.

4.2.6 Proteomic analysis of VBNC *S. Typhimurium*

Following a transcriptomic study and mutagenesis, the proteome of VBNC *S. Typhimurium* was analysed. Though bacterial transcription and translation are considered to be coupled in bacteria, studies have shown that the correlation between transcriptome and proteome can be poor^{349,350}. Many earlier studies of VBNC bacteria have analysed either RNA or protein expression profiles. To obtain a more complete picture of the physiology of VBNC *S. Typhimurium*, the proteome was studied using mass spectrometry. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for the proteomic analysis. Five biological replicates each of unstressed and VBNC bacterial samples were processed and prepared for mass spectrometry similarly. The results obtained from the biological replicates were averaged and the proteome from VBNC samples was compared to the unstressed samples. ANOVA (Analysis of variance) multiple variable comparison test was conducted to analyse the significance of the findings of the data obtained.

Proteome data of the unstressed and VBNC *S. Typhimurium* did not show as many significant changes as were observed with the transcriptome data. The proteins that were significantly (p -value <0.05) differentially expressed mainly included anti-oxidative stress (AOS) response proteins KatG, AphF (Table 4.3). Oxidative stress response genes were among the highly upregulated genes observed in the transcriptome analysis (Table 4.1). YhjA and CueO were two proteins that were highly expressed in the oxidative-stressed VBNC *S. Typhimurium*. This observation indicated that there may have been very little translational activity except for the AOS proteins. This could be due to various reasons including active degradation of mRNA, tRNA and rRNA and decreased ribosomal concentration in response to the oxidative stress. Ribosomal fractionation was done to analyse the ribosomal content in the unstressed and VBNC *S. Typhimurium*. A decrease in ribosomes in VBNC can lead to lowered protein synthesis³⁵¹ thus potentially leading to a dormancy state.

Table 4.3: Proteins that were significantly enriched in VBNC *S. Typhimurium*

<u>PROTEIN NAMES</u>	<u>PROTEIN</u>	<u>Log₂ FC</u>
Catalase-peroxidase	KatG	7.87
Alkyl hydroperoxide reductase F subunit	AhpF	7.07
Probable cytochrome c peroxidase	YhjA	3.4
Possible multicopper oxidase	CueO	2.24
Low affinity potassium transport system protein	Kup	1.8

4.2.7 Ribosomal Fractionation

Unstressed *S. Typhimurium* and VBNC *S. Typhimurium* were lysed and ribosomes were isolated by ultracentrifugation through a sucrose cushion (70% sucrose solution). The isolated ribosomes were resuspended in a buffer containing Mg^{2+} ions. The resuspended ribosomes were loaded on a sucrose gradient and centrifuged to separate ribosomes, ribosomal subunits and polysomes. Sucrose gradient was then passed through a gradient fractionator that analyses the absorbance at 260nm to detect the ribosomes.

Ribosomal fractionation of unstressed *S. Typhimurium* (Figure 4.13A) showed the presence of absorption peaks corresponding to 70S ribosomes and polysomes. Ribosomal fractionation analysis of VBNC *S. Typhimurium* (Figure 4.13B) did not reveal 70S ribosomes but had peaks indicating polysomes. The presence of polysomes in the VBNC state could suggest some very low translational activity. The results from the ribosomal fractionation, though preliminary, indicated a loss of ribosomal population in the VBNC state (Figure 4.13). Absence of ribosomes in the VBNC state could be due to ribosomal degradation or disassembly of ribosomal proteins. A ribosomal protein, RpsB, was tagged to test for oxidative stress on the ribosomal proteins.

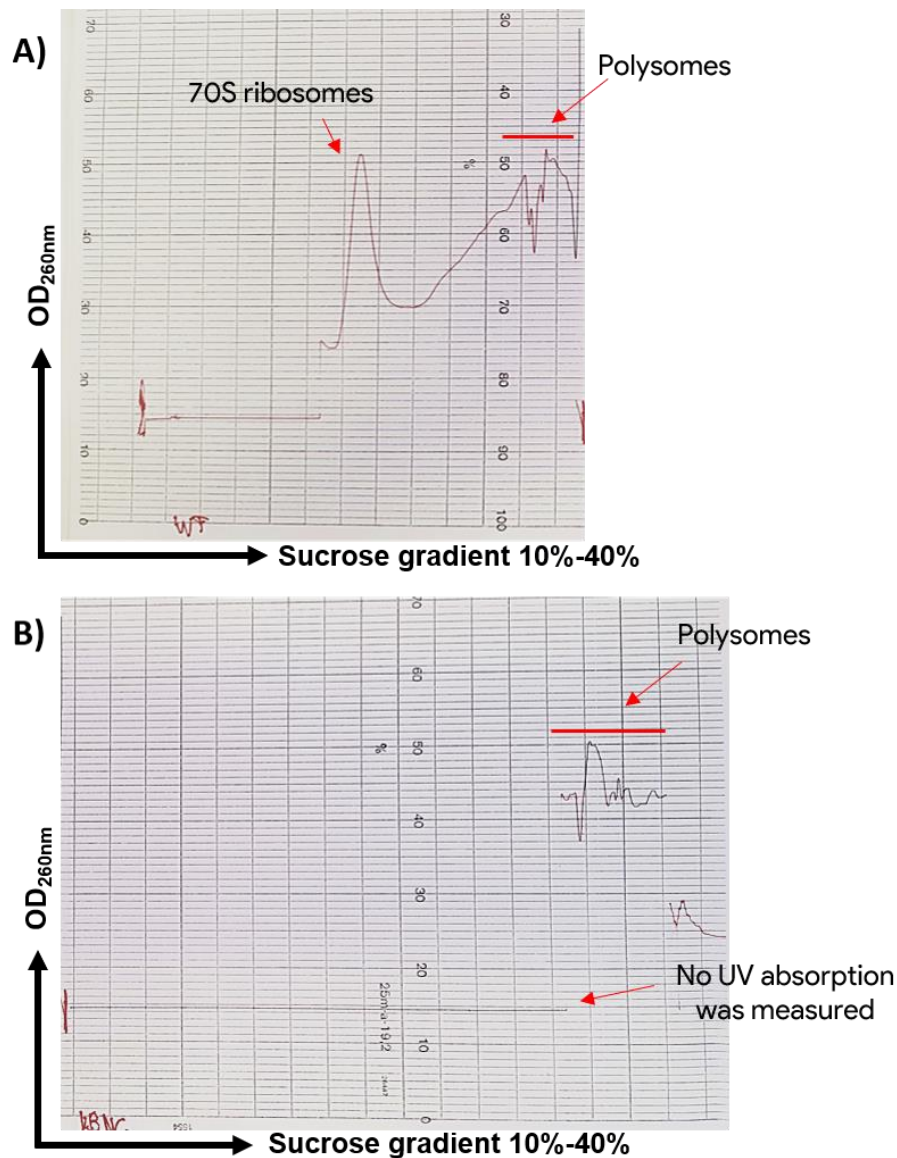


Figure 4.13: Ribosome concentration analysis of culturable and VBNC *S. Typhimurium* sucrose gradients. Ribosomes were isolated from both culturable and VBNC *S. Typhimurium* using a sucrose cushion and separated by high speed centrifugation through a sucrose gradient. The sucrose gradient was analysed using a gradient fractionator with UV absorption (260 nm). The UV absorption indicates the presence of ribosomes. A) The ribosomes from the culturable *S. Typhimurium* showed a large peak indicating the presence of 70S ribosomes (the Mg^{2+} ion concentration was maintained throughout the experiment to prevent the ribosomal subunit dissociation) followed peaks indicative of polysomes. B) VBNC *S. Typhimurium* showed no peaks corresponding to 70S ribosomes, however peaks corresponding to polysomes were found.

4.2.8 Ribosomal subunit RpsB tagged with mEoS2 indicated that the ribosomal proteins were not degraded under oxidative stress

The observations from the proteomic data (Section 4.2.6) and the rRNA degradation seen in VBNC *S. Typhimurium* (Figure 4.1A,B) suggested that there could be ribosomal degradation as the cells entered VBNC state leading to the lack of translational activity in the VBNC state. The degradation of rRNA leads to a degradation of ribosomes as rRNA forms a structural component of the ribosomes³⁵² (Figure 4.1A and 4.1B). To investigate if the ribosomal proteins were degraded in VBNC *S. Typhimurium*, a 30S ribosomal subunit protein called RpsB was tagged with a photoconvertible fluorescent protein mEoS2³⁵³. The RpsB+mEoS2 has been used as a reporter for tracking ribosomes in previous studies³⁵⁴. Tagging RpsB with mEoS2 was done by synthesising a *S. Typhimurium*-codon optimised *rpsB* and mEoS2 gene fragment, where the mEoS2 was tagged to the C-terminal end of the RpsB protein. The synthesised fragment was used for homologous recombination into the *S. Typhimurium* WT.

The *S. Typhimurium* strain with RpsB+mEoS2 (hereon referred to as *S. Typhimurium*-mEoS2) was then analysed for the expression of fluorescent tag by FACS analysis and subjected to hydrogen peroxide stress conditions that induced the VBNC state. Bacteria were collected at various time points (5, 10, 15, 30 and 60 minutes) during oxidative stress and analysed by FACS.

The FACS analysis of untagged *S. Typhimurium* WT strain showed a background mean fluorescence of ~38 relative fluorescent units (RFU) whereas the unstressed *S. Typhimurium*-mEoS2 was 10 times stronger and had a mean fluorescence of over 400 RFU (Figure 4.14A). The oxidative stressed *S. Typhimurium*-mEoS2 bacteria did not show a decrease in their mean fluorescence (Figure 4.14B) suggesting that the ribosomal protein RpsB tagged with mEoS2 has not degraded. This study using the RpsB as a reporter to trace any possible degradation of ribosomal proteins indicate that the ribosomal proteins might get

dis-assembled rather than degraded in VBNC *S. Typhimurium*. A more direct approach is needed to understand the effect of VBNC state on all the ribosomal proteins.

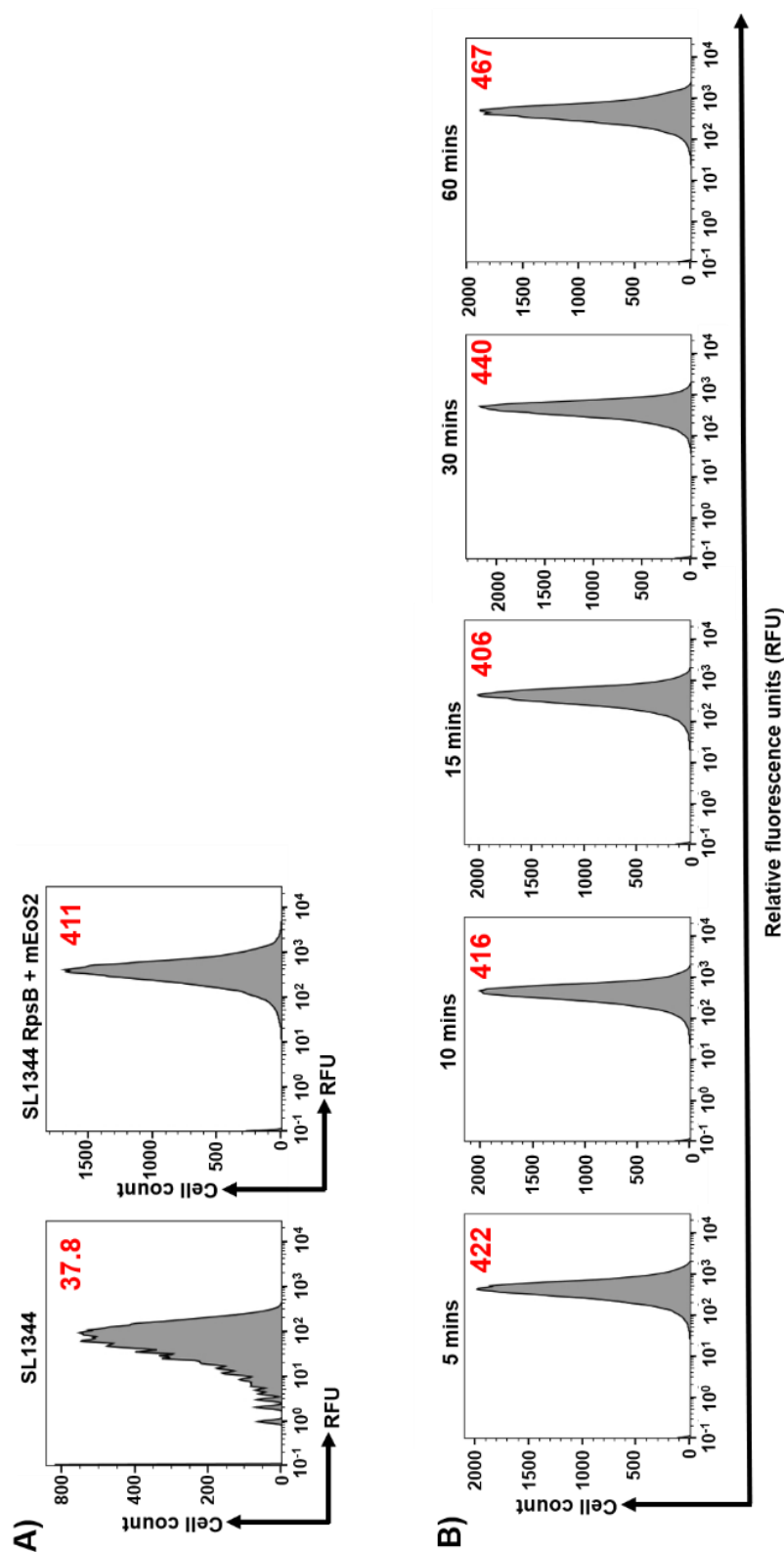


Figure 4.14: Effect of oxidative stress on the ribosomal protein RpsB. The ribosomal protein RpsB was chromosomally tagged with a photoconvertible fluorescent protein mEoS2 to follow the ribosomal degradation. Numbers in red in each histogram represent the mean fluorescence of the mEoS2 tag. (A) *S. Typhimurium* SL1344 without the tag (left) and with the mEoS2 tag were examined for relative fluorescence units (RFU). Exponentially growing cells were isolated and analysed by FACS, measuring cell count (y-axis) and RFU (x-axis). (B) Exponentially growing *S. Typhimurium* SL1344 were stressed with 50 mM H_2O_2 for 1 hour. At regular time points (5, 10, 15, 50 and 60 minutes) post H_2O_2 addition, the cell count and RFU were measured.

4.2.9 Puromycin assay suggests very low translational activity in VBNC cells

Presence of polysomes in VBNC *S. Typhimurium* suggested the possible presence of some very low translational activity. To further understand whether active protein synthesis occurred in VBNC cells, the puromycin incorporation assay was adopted. The assay compared the translational activity of the VBNC bacteria to that of culturable *S. Typhimurium*. Puromycin is an antibiotic that mimics the structure and thus competed with the aminoacyl-tRNA³⁵⁵. Translation is terminated when puromycin is incorporated into the growing amino acid chain³⁵⁵. Following the puromycin treatment, the cellular proteins were isolated and Western blotting was performed using anti-puromycin antibodies.

Puromycin at 10 µg/ml was added to exponentially growing culturable *S. Typhimurium* and incubated at 37° C with shaking at 180 rpm for 1 hour, 3 hours, 6 hours and overnight. The VBNC *S. Typhimurium* were treated with 10 µg/ml of puromycin for overnight (O/N). Cells were harvested using centrifugation and lysed by boiling with SDS containing lysis buffer. The cell debris was removed by centrifugation at >10,000 rpm for >2 minutes. The proteins were quantified using Bradford reagent and (2 µg/lane) whole cell lysate was loaded on NuPAGE gel by mixing with NuPAGE LDS sample reducing buffer. Nu-PAGE gel (12%) was used to separate the proteins.

The proteins were transferred to a PVDF (polyvinylidene difluoride) membrane and probed with anti-puromycin antibodies. The results (Figure 4.15) showed that puromycin was incorporated into the cellular proteins of the culturable bacteria across all time points of incubation. This was indicated by the presence of strong immune-positive bands in the puromycin treated samples (Figure 4.15, lanes 2, 4, 6 and 8) and suggested translational activity. The whole cell lysate obtained from VBNC *S. Typhimurium* after incubation with puromycin overnight (Figure 4.15, lane 10) did not show a difference in protein profile from the puromycin-untreated sample (Figure 4.15, lane 9) when probed with anti-puromycin antibodies. This suggested that there was very low or no translational activity in the VBNC *S. Typhimurium*.

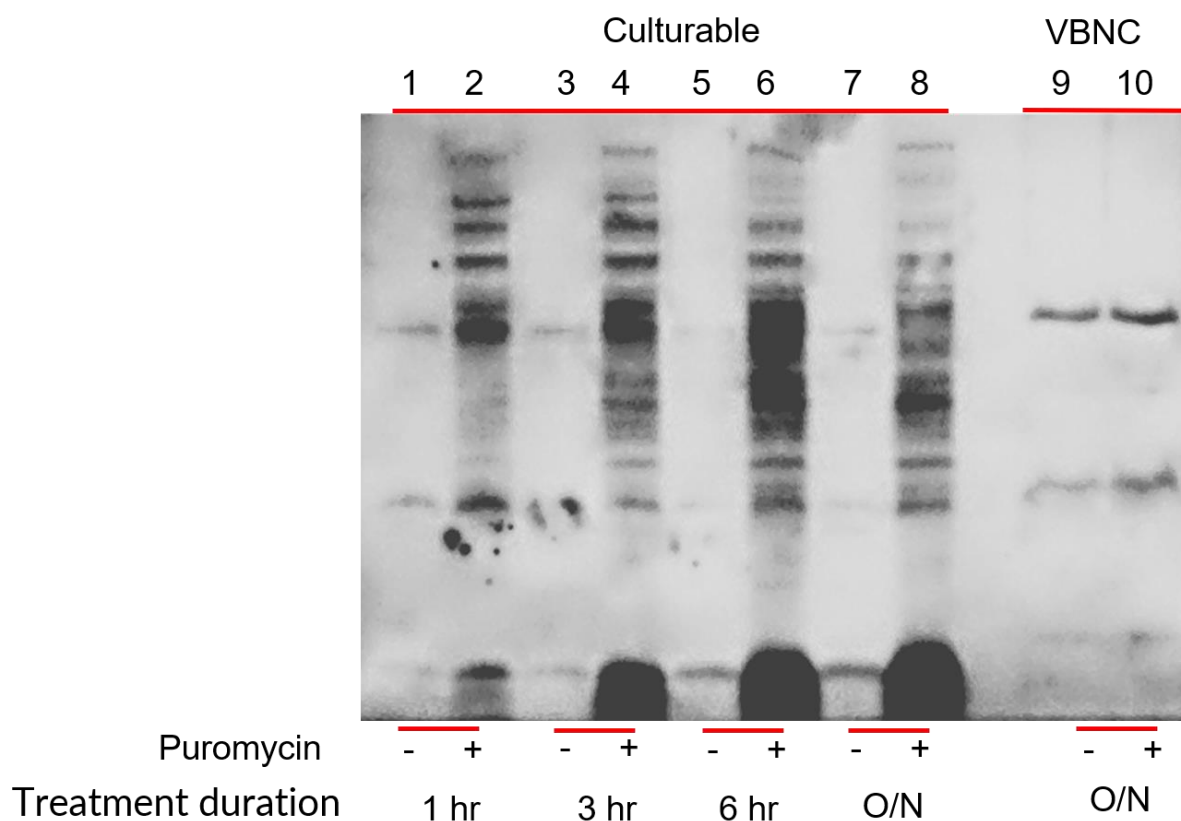


Figure 4.15: Puromycin incorporation analysis of protein synthesis. Ten ml of exponentially growing *S. Typhimurium* was incubated with (+) or without (-) 10µg/ml of puromycin at 37°C for 1, 3, 6 hours and overnight with shaking while the VBNC *S. Typhimurium* was incubated with (+) and without (-) puromycin at 37°C with shaking overnight. After incubation, cells were lysed, and the protein concentrations quantified using a Bradford assay. Each lane was loaded with equivalent concentrations of protein and were analysed by Western immunoblotting using anti-puromycin antibodies. Bands were developed using an ECL Western blotting system and visualised using the ChemiBIS gel imaging system. Lanes 1-8 correspond to culturable *S. Typhimurium* and lanes 9-10 correspond to VBNC *S. Typhimurium*.

4.3 Discussion

Several studies in different pathogens have attempted to identify a molecular signature associated with the VBNC state. There has been no consistent finding that could define a universal mechanism for VBNC formation. The approach undertaken in studies reported in this Chapter included transcriptomic, proteomic and gene knockout studies of VBNC *S. Typhimurium* relative to unstressed *S. Typhimurium*.

One prominent operon that was highly upregulated in VBNC was the *cbi* operon. The *cbi* operon is involved in *de novo* biosynthesis of cobalamin under anaerobic conditions. Cobalamin, or vitamin B₁₂, is an important co-factor, required for a normal functioning of the cell, which is involved in amino acid and DNA synthesis³⁵⁶ and has been shown to play a role in oxidative stress in *Leptospirillum* spp.³⁵⁷ and the VBNC state in *V. cholerae*¹²⁸.

As expected, anti-oxidative stress (AOS) response genes were one of the most highly expressed genes in this experiment. These genes include those that breakdown the ROS and also genes encoding for proteins that help survive under oxidative stress. For example, siderophores were significantly upregulated in VBNC *S. Typhimurium*. Highly oxidative conditions can cause a dearth of soluble metal ions by oxidation of ferrous ions into ferric ions. A report on the proteomic analysis of *S. Typhimurium* inside the host cells showed that the most significantly up regulated proteins were IronCDB, SufBCDS and EntACEF proteins³⁵⁸. The activation of iron-acquiring mechanisms during extreme oxidative stress might reflect evolutionary responses to the low iron environment in the *Salmonella*-containing vacuole (SCV)³⁵⁹. OxyR, under oxidative conditions, upregulates *fur* (Ferric uptake regulator) which further upregulates siderophore production and iron-sulphur proteins in the bacterial defence against oxidative stress²⁹⁰. The gene *potE* which is putrescine transporter, was also upregulated by 7-fold in the data reported here. Putrescine can act as a ROS scavenger like other polyamines such as spermine and spermidine³⁶⁰. Like *potE*, *cadB*, a cadaverine transporter, was highly upregulated (by 5-fold). Cadaverine is also a polyamine and can confer protection against ROS³⁶¹. These transcriptome changes in the oxidative stress response

genes, including those encoding siderophores, are not unexpected given the nature of the applied stress i.e. oxidative stress. Comparison of genome-wide transcriptomic and proteomic profiles of VBNC bacteria formed under different stress conditions could reveal common mechanisms and pathways by firstly eliminating the obvious factors (genes and proteins) responding to the applied stress and focusing on the potential commonalities.

Transcriptomic analysis of VBNC *V. cholerae* has shown an upregulation of siderophores during cold stress-induced dormancy¹²⁸. The authors argued that siderophores were upregulated because catalase activity is decreased at low temperatures²¹⁴; these studies suggest a specific context for VBNC formation and highly nuanced and indirect responses to different environmental stresses. In the experiments reported here, using oxidative stress, the role of AOS response and the expression of genes such as *oxyR* during development of the VBNC state²⁹ was anticipated. Though *oxyR* was downregulated 1.2-fold in VBNC *S. Typhimurium*, *oxyS* was highly upregulated 4.3-fold. *OxyS* is also involved in regulating the levels of hydrogen peroxide in *E. coli*³⁶². *AhpC*, a peroxidase, was also shown to be associated with the formation of VBNC state of *V. parahaemolyticus*³⁶³. *ahpC* was upregulated in the case of VBNC *S. Typhimurium*. *AhpC* works together with *AhpF*, which reduces the oxidised *AhpC* back to its original reduced form after every cycle³⁶⁴. *ahpF* was upregulated 7.26-fold in VBNC *S. Typhimurium*.

The protein encoded by *dps*, DNA binding Protein from Starved cells, is a nucleoid associated protein known to be upregulated as the cells enter stationary phase²⁵⁸. The gene *dps* was upregulated by 4.8-fold in VBNC *S. Typhimurium*. It has been shown to increase the 'hardiness' of bacterial cells and confer protection against various stress conditions including starvation, oxidative stress, acid stress, and metal ion stress^{365,366}. *Dps* also has a ferroxidase activity that can sequester any damaging anions and cations like ferrous out of the nucleoid^{280,367}. *Dps* along with other NAPs (nucleoid associated proteins) causes nucleoid-clumping which can result in the downregulation of transcription³⁶⁸. The gene *soxR* which activates the transcription factor SoxS, which in turn controls other oxidative stress response

genes, was downregulated³⁶⁹. Reduced Fe-S clusters could downregulate *soxR*³⁷⁰. The downregulation of *soxR* could also indicate a restored redox potential inside the cell after one hour of stress. In short, the changes in the expression of AOS genes were expected due to the nature of the stress used, i.e. oxidative stress.

An intact cell membrane can act as a barrier against environmental stress³⁰³. Phospholipids, the fundamental building blocks of the cell membrane, are synthesised by the *fab* and *acc* genes³⁷¹. The *fab* genes were downregulated in the VBNC state. Previous studies have shown that the biosynthesis of fatty acids is negatively regulated by (p)ppGpp through *pIsB*³⁷² and usually proportional to the rate of cell division³⁷¹. Apart from being structurally and metabolically important, fatty acids, especially long chain fatty acids, have been linked with signalling through PhoP/Q³⁷³ in *S. Typhimurium*. Different fatty acids form isoforms, or anteisoforms, that can affect the fluidity of the cell membrane. Pathogens like *L. monocytogenes* were shown to survival during pH stress conditions by changing the balance between these two forms of FAs³⁷⁴. With this current transcriptomic data, we cannot determine which type of fatty acids have been upregulated as there are many genes involved in the producing them, but this data is indicative that the VBNC *S. Typhimurium* upregulates fatty acid biosynthesis. and downregulates the degradation of FAs. This change could help maintain the integrity of the cell membrane which is crucial for cell viability. VBNC *V. vulnificus* has been shown to change the FAs in the cell membrane³⁷⁵. FAs can also be an important source of energy, but the downregulation of the FA degradation suggests that they are not being used as a carbon or energy source.

Membrane proteins also contribute to the structure of the cell membrane. Tol-Pal system which includes *tolB*, *tolR*, *tolQ*, *ygbF*, *ygbC* and *pal* is very important to the membrane integrity of *E. coli* ^{376,377}. The genes *tolAC* and *pal* were upregulated in VBNC *S. Typhimurium*. The Tol/Pal system has been shown to organise close to the cell division ring in dividing Gram-negative bacteria³⁷⁸. Efflux pump systems can also respond to environmental stress. Proteins like TolC which are a part of efflux pump complex with AcrAB could act as a line of defence

against the oxidative radicals entering from outside or being formed inside due to the hydrogen peroxide treatment³⁷⁹. Efflux pumps including MacAB and AcrAB were not upregulated in VBNC *S. Typhimurium*. Most of the LPS biosynthesis and assembling genes were upregulated in VBNC *S. Typhimurium*, the significance of which is yet to be determined. (Figure 4.7B).

Flagella contribute to the motility of the bacteria and appear to be important in cell adhesion, cell invasion and uptake of the bacterium by macrophages³⁸⁰ and hence play a role in virulence. Flagellar genes were found to be downregulated in VBNC. Phase variation in key flagellar genes can turn on or off motility in a *Salmonella* population and the heatmap in Figure 4.8B will be average of all this variation. This averaging effect is true throughout the transcriptomic and proteomic analysis in Chapter-4 but is worthy of a comment. Two of the key genes involved in flagella phase variation are *fliC* and *fljB*³²⁸. While *fliC* gene is negligibly upregulated (1.14 fold) the *fljB* gene's expression data was inconclusive due to a low statistical significance. That said, other flagellar genes- *flgM*, *fljA*, *fliR* were downregulated. Flagella, though motile or not, can contribute to the virulence of *S. Typhimurium* in mice³⁸¹. Schmitt *et al.* showed that *flgM* and *fliA* could regulate virulence factors in *Salmonella enterica*³⁸². Regulation in the context of *Salmonella* virulence is highly complex and incompletely understood – RcsCDB can downregulate flagellar genes while upregulating SPI-2 virulence genes i.e. it is involved in dual-regulation. The reason for this was not completely clear³²⁹ but this effect was observed when the bacteria were grown in nutrient-deprived growth media suggesting this could be a stress and/or dormancy related phenomenon, that could confer an advantage during unfavourable conditions³⁸³. VBNC *S. Typhimurium* under the tested stress condition had shown similarities in gene expression with bacteria in the SCV (*Salmonella*-containing vacuole). For example, the downregulated flagellar gene expression, upregulated SPI2 expression, upregulation of siderophore related genes and other metal ion acquiring genes were observed in *S. Typhimurium* in the SCV^{358,384}.

Universal stress response genes such as sigma factors, were downregulated in VBNC *S. Typhimurium*. In other studies, decreased expression of RpoS was shown to induce the

VBNC state¹¹⁸. RpoS is responsible for the expression of universal stress response genes (Figure 1.4)³⁸⁵. In studies reported here, VBNC *S. Typhimurium* down regulated expression of *rpoH*, *rpoN* and *rpoE* which were also found to be downregulated in VBNC *V. cholerae*¹⁷⁸. RpoS expression was not repressed to any significant extent.

Genes involved in non-essential amino acids biosynthesis were downregulated in VBNC *S. Typhimurium*. The *asnA* gene, which is required for the synthesis of asparagine, was one of the most highly downregulated genes. A similar level of downregulated expression of *acnA*, which is part of the TCA cycle, and genes *rbsK* and *rbsD* genes, which play a role in carbohydrate metabolism and nucleotide synthesis, was observed. These findings are supported by similar studies of VBNC in *V. cholerae*¹²⁸.

FtsH is an ATP dependent metalloprotease which is up regulated in VNBC *S. Tyhimurium*. Its primary target is known to be sigma 32, the heat shock sigma³⁸⁶ but its function to be dependent on Zn²⁺ion. FtsH forms a protease complex with HflKC (*hflK* = 0.84 FC and *hflC* = 1.84 FC) which is attached to the plasma membrane³⁸⁷. It also has a role in the cell division and sporulation initiation in *Bacillus subtilis*³⁸⁸. Proteases also play a role in protein quality control, especially those proteins that are secreted³⁸⁹. Lon protease is important for degrading damaged proteins and also important for the survival of *E.coli* in anaerobic conditions¹¹⁹. But, *lon* gene expression results are inconclusive in this analysis. Upregulation of the proteases can be explained by their role in degradation of damaged proteins.

Transcriptional and proteomic studies are an effective tool for identifying global changes in physiology and to identify potential pathways related to a phenotype, but gene knockout studies are needed to ascertain roles of specific genes in the observed pathways. Given that VBNC cells are usually generated under stress and unfavourable conditions, a role for stress response proteins including RpoS and OxyR would be expected. At the same time, it is difficult to test and quantify the impact of the general stress response genes in the formation of the VBNC cells by knockout studies. This is because such mutations would compromise the survival of the bacteria under stress conditions as studies using gene knockouts of general

stress response genes might be useful in eliminating specific roles for these general stress response proteins in VBNC formation. Previous studies have shown that *rpoS*, *relA* and other key stress response genes are important in the VBNC formation^{118,222} but separating direct from indirect consequences of the mutagenesis is very difficult.

In this Chapter, gene knockout studies have shown a possible role of PNPase in survival of VBNC *S. Typhimurium*. RNAases are important part of regulating the gene expression in *E. coli*³⁹⁰ and *pnp* mutants have an increased mRNA half life³⁴⁷. PNPase was shown to degrade damaged RNA by binding to 8-hydroxyguanine but the protein is not known to have a direct effect on quenching or degrading oxidative radicals³⁴⁸. Translating the oxidatively-damaged RNA might lead to the expression of mutated proteins that can potentially harm the cell. It was hypothesized that PNPase was involved in mRNA degradation and rRNA degradation (leading to ribosomal degradation) to prevent translation, driving the VBNC response. Further analysis on the ribosomal content in the *S. Typhimurium* Δpnp needs to be done to determine the effect of *pnp* on the concentration of ribosomes. Additional experiments on the *S. Typhimurium* Δpnp mutant could be done to understand the ribosomal content and rRNA degradation in this strain. The slow growth rate of the *S. Typhimurium* Δpnp mutant indicates that *pnp* could be important for a normal cellular functioning in addition to any other role it might have in VBNC formation.

RNA isolated from VBNC cells showed degradation, especially the rRNA degradation. It is known that rRNA degradation can affect the ribosomal concentration³⁹¹. 16S rRNA forms a component of the 30S ribosomal subunit and rRNA concentration was shown to be positively correlated with the concentration of ribosomes^{193,392}. The rate of growth also is directly proportional to the content of macromolecules in the cell like RNA and proteins³⁹³. Ribosomes degrade upon bacteria encountering stress conditions including nutrient starvation, and this naturally occurs in stationary phase; this phenomenon has been shown to be even more pronounced in *S. Typhimurium* where more than 90% of the ribosomes are degraded upon entry into stationary phase¹⁹³. Ribosomal degradation can also play a role in dormancy-like

phenotypes^{194,394} and degradation of RNA and ribosomes is postulated as mechanism for rapid adaptation to environmental changes in bacteria³⁹⁰. The RNA degradation observed here in VBNC *S. Typhimurium* appeared to be different from that of ethanol-killed bacteria (Figure 4.1A, lane 11) and hydrogen peroxide did not degrade the RNA by its chemical action (Figure 4.2), suggesting the RNA degradation was an intrinsic product of the action of bacterial RNases, in line with a previous study¹⁹³.

Studies suggest that a coupled analysis of the transcriptomic and proteomic data can provide a reliable and more complete picture than the transcriptomic data alone³⁹⁵. The proteomic data did not correlate with the transcriptomic data, except in the case of several AOS response proteins. This lack of correlation further supports a role for the ribosomes in the VBNC phenotype. The *S. Typhimurium*-mEoS2 suggested that the ribosomal proteins may not have been degraded rather they could be disassembled under stress (Figure 4.12). Lack of significant proteomic changes shown from mass spectrometry also supports the hypothesis that the ribosomal proteins were not degraded and agrees with the mEoS2 ribosomal tagging experiments. As this technique is an indirect method to monitor the ribosomes, ribosomal isolation followed by Western blotting with antibodies specific to ribosomal subunit protein is needed to validate this theory. Puromycin incorporation assay suggested that there was very low translation in the VBNC *S. Typhimurium* and the data from ribosomal fractionation, and the RIN scores of RNA isolated from VBNC bacteria further supported a reduction in the number ribosomal structures, through ribosomal RNA degradation.

The gene *deaD*, a helicase, has a role in mRNA degradation alongside RNaseE³⁹⁶. The *deaD* gene was upregulated Log₂ 2.5-fold in VBNC *S. Typhimurium*. A previous study of VBNC *Ralsotonia solanacearum* reported a similar phenomenon of rRNA degradation along with ribosomal aggregation⁹⁵. A portion of ribosomes could be present in the VBNC *S. Typhimurium* as 'polysomes' as indicated by the data from sucrose density gradient centrifugation of ribosomes (Figure 4.14). Hibernating 100S ribosomes were shown to form in Gamma proteobacteria under stress conditions³⁹⁷. The genes *yfiA*, *yhbH* and *rmf* play a role

in the formation of 100S ribosomes, which are dimers of 70S ribosomes³⁹⁸. The transcriptomic data suggests that there could be formation of 100S ribosomes.

The rRNA genes including *rnbB* P1 have been shown to be under the control of (p)ppGpp mediated stringent response^{192,399} and *spoT* and *relA* genes have been shown to play an important role in driving and maintaining bacteria in the VBNC state²¹². ATP depletion also results in the increase of (p)ppGpp⁴⁰⁰. Depleting ATP has been shown to play a role in persister formation²¹. This could be a possible mechanism in driving the VBNC state. A cellular ATP analysis could be conducted on VBNC *S. Typhimurium* to test this hypothesis.

In conclusion, the observations made in this Chapter, including degradation of RNA in the VBNC state (Figure 4.1A,B), absence of ribosomes (Figure 4.14), lowered levels of translation (Figure 4.15) and possible role of PNPase in entry into VBNC state (which is a RNA exonuclease), point at a mechanism where *S. Typhimurium* entered into dormancy by rapidly downregulating translation through ribosomal RNA degradation and ribosomal hibernation. This could be due to damaged RNA and/or proteins. A comparable mechanism can be observed in persisters, where the HipA toxin causes cellular stasis by reducing translation leading to lowered growth and metabolism^{224,311}. These data indicate that the VBNC state could be a product of an overall change in the physiology of the bacteria rather than a product of a single gene or process.

Future studies can be conducted to compare transcriptomes and proteomes of VBNC cells formed under different stress conditions. This would help eliminate any stress specific molecular responses and can help build a consensus about the gene expression profiles of VBNC *S. Typhimurium* across stress conditions. Ribosomal analysis to identify 100S ribosomes in the VBNC state could be done to investigate the role of ribosomal dormancy and ribosomal degradation in driving *S. Typhimurium* into VBNC state.

CHAPTER 5
Resuscitation of VBNC *Salmonella*
Typhimurium in mice and *in vitro*
resuscitation

5.1 Introduction

Each year over 30,000 case of gastroenteritis are reported in Australia and the number of non-typhoidal invasive *Salmonella* infections are as high as 93 million globally, with over 150,000 deaths^{9,401}. It is estimated that 72% of *Salmonella* infections are food borne rather than through direct person-to-person transmission²³⁸. Regular analysis of foods and beverages is necessary to ensure the absence of harmful pathogens, but the existing food inspection methods are not without their drawbacks. This examination of foods during production is commonly carried out by culture-based methods, seeking to grow any contaminating pathogens⁴⁰². Replicating and culturable bacteria are a prerequisite for culture-based methods to be effective in identifying contamination. To-date, over 80 species of bacteria have been shown to enter a VBNC state which, by definition, would not be detectable by culture-based methods. The presence of VBNC organisms can pose a threat to public health, if these bacteria go undetected and can be resuscitated^{30,165}.

There have been many studies to determine if bacteria in a VBNC state can still cause infection (see, Chapter 1, Section 1.7). It is generally thought that VBNC bacteria cannot cause disease without re-entering vegetative growth¹⁶⁵. This was shown most clearly in the case of *Edwardsiella tarda*⁴⁸ where the bacteria could only cause disease after "resuscitation", but not when they were in the VBNC state. Different VBNC bacteria were tested to determine their pathogenicity but the results so far have not been definitive¹²⁶. Outbreaks of *E.coli* O157 and *Salmonella enterica* serovar Oranienburg have been attributed to the VBNC state^{88,128} and VBNC *Vibrio* spp. caused infections in mice^{64,128,403}. *In vitro*, VBNC *S. Typhimurium* showed signs of virulence when inoculated into HeLa cells, where intracellular growth may signal virulence potential¹²⁶. In contrast to the *in vitro* experiments on pathogenicity, infection with mice showed that the VBNC *S. Typhimurium* were avirulent^{92,167}. This discrepancy between the *in vitro* and *in vivo* experiments needs to be addressed. Resuscitation of VBNCs is still poorly understood albeit a protein, resuscitation promoting factor (Rpf) has been mooted as one trigger for VBNCs to re-enter vegetative growth^{229,404}. Rpf is ectopically secreted enzyme

with 'lysozyme-like' domain^{227,405}. Its putative resuscitation mechanism of action is poorly understood.

It has been suggested that the virulence of the VBNCs might be indirectly proportional to the time spent in this dormant state¹²⁶. This poses the question as to whether virulence of VBNCs is unpredictable and requires an understanding of the period of dormancy.

The virulence of *Salmonella* though complex, is highly regulated and well studied^{1,406}. Virulence is dependent on multiple virulence factors such as the expression of flagella^{381,407}, expression of the SPI-1 and SPI-2 genes, and their secreted effectors³³⁴, leading to invasiveness of the bacteria and survival within phagocytic cells⁴⁰⁸, and the highly sensitive murine model^{409,410} can help dissect the contribution of these virulence attributes. *S. Typhimurium* virulence assays may therefore provide a good test of the ability of VBNCs to "resuscitate" and cause disease.

The aim of the experiments presented in the Chapter are to examine whether VBNC *S. Typhimurium* produced under oxidative stress conditions can be resuscitated *in vitro* and if they can cause morbidity or mortality in C57BL/6 mice and other immunodeficient mice.

5.2 Results

5.2.1 *In vitro* resuscitation with resuscitation promoting factor (Rpf)

Resuscitation is described as VBNC cells regaining culturability after losing all the physiological characteristics that define the VBNC state⁶². VBNC *Salmonella* spp. have been resuscitated using various conditions including the addition of catalase, pyruvate, providing a temperature shift and adding Tween-20^{42,228,234}. One of these studies showed that the resuscitation of osmotic-stressed VBNC *S. Oranienburg* occurred after addition of Rpf from *S. Typhimurium* LT2⁶³. The *rpf* gene encoding Rpf of *S. Typhimurium* LT2 was identified by homology searching using NCBI-BLAST⁶³. The *rpf* gene of LT2 and SL1344 strains is 100% identical (Figure 5.1).

To test the ability of Rpf from *S. Typhimurium* SL1344 to resuscitate oxidative-stressed VBNC *S. Typhimurium*, the *rpf* gene was cloned into the expression vector pET30b using the *Nde*I and *Xho*I sites (Figure 5.2A). The protein is expressed under the control of the T7 promoter with an N-terminal poly-histidine tag which is used to purify the protein. A previous resuscitation study used his-tagged *S. Typhimurium* Rpf protein to resuscitate VBNC *S. Oraneinburg*⁶³. This recombinant form of *S. Typhimurium* Rpf, referred from now on as STrRpf, was expressed in *E. coli* Tuner™(DE3) and the protein purified from the soluble cell fraction under native conditions using immobilised metal affinity chromatography (IMAC) with nickel-charged resin. STrRpf was purified to approximately 85% purity (Figure 5.2B) and used in *in vitro* resuscitation experiments of VBNC *S. Typhimurium*.

The resuscitation of VBNC *S. Typhimurium* using STrRpf was attempted using simple addition of the purified protein, following the method of Panutdaporn *et al.* 2006, and using concentrations of STrRpf ranging from 0.1µg/ml to 100µg/ml⁶³. The maximum concentration was 10 times higher than that used in a previous study⁶³. VBNC *S. Typhimurium* was incubated with STrRPF for 12 hours at 37°C, shaking in BHI broth. No resuscitation was observed, as indicated by no change in the turbidity, when the optical density (OD₆₀₀) was measured (Figure 5.3).

There could be multiple reasons why STrRpf did not resuscitate VBNC *S. Typhimurium* *in vitro* including 1) interference of the poly-histidine tag with protein function, 2) soluble but potentially misfolded STrRpf, 3) degradation of STrRpf at 37°C, and more importantly 4) the type of stress condition used to generate the VBNC state i.e. oxidative versus osmotic stress.

Q7CQE0-LT2	MRILAITATEACSVLWNNGTINAHFELCPREHTQRILPMVQEILAASGASLNEIDALA	60
A0A0H3NC54-SL1344	MRILAITATEACSVLWNNGTINAHFELCPREHTQRILPMVQEILAASGASLNEIDALA	60

Q7CQE0-LT2	FGRGPGSFTGVRIGIGIAQGLALGANLPMIGVSTLATMAQGAWRKTGATRVLAIDARMG	120
A0A0H3NC54-SL1344	FGRGPGSFTGVRIGIGIAQGLALGANLPMIGVSTLATMAQGAWRKTGATRVLAIDARMG	120

Q7CQE0-LT2	EVYWAEYQRDAQGVWQGEETEAVLKPERVGERLKQLSGEWATVGTGWSAWPDLAKECGLT	180
A0A0H3NC54-SL1344	EVYWAEYQRDAQGVWQGEETEAVLKPERVGERLKQLSGEWATVGTGWSAWPDLAKECGLT	180

Q7CQE0-LT2	LHDGEVSLPAAEDMLPIASQKLAAGETVAVEHAEPVYLRNEVAWKKLPGKE	231
A0A0H3NC54-SL1344	LHDGEVSLPAAEDMLPIASQKLAAGETVAVEHAEPVYLRNEVAWKKLPGKE	231

Figure 5.1: Multiple sequence alignment of Rpf protein from *S. Typhimurium* LT2 and SL1344 strains. A multiple sequence alignment of the resuscitation promoting factor (Rpf) from *S. Typhimurium* strain LT2 and SL1344 showed 100% identity.

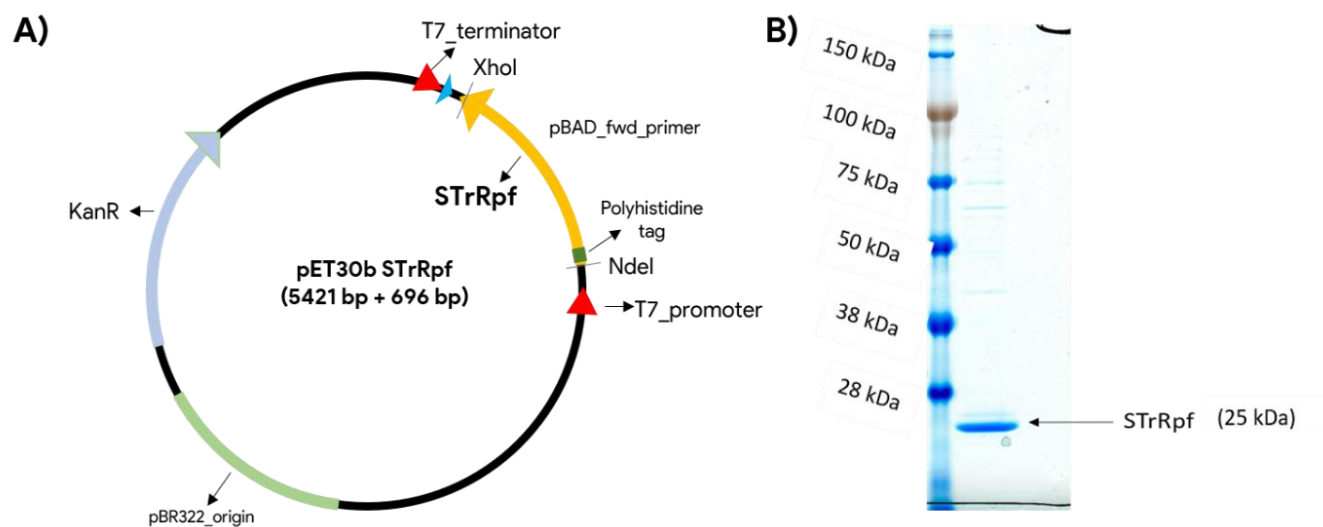


Figure 5.2: STrRpf protein was expressed by cloning the *rpf* gene into the pET30b vector. A) Schematic representation of the vector map pET30b STrRpf. B) Polyhistidine-tagged Rpf was purified using immobilised metal affinity chromatography and eluted with 200 mM of imidazole. Protein purity and yield was assessed by SDS-PAGE using a 12% Bis-Tris gel stained with Colloidal Coomassie G250 blue stain.

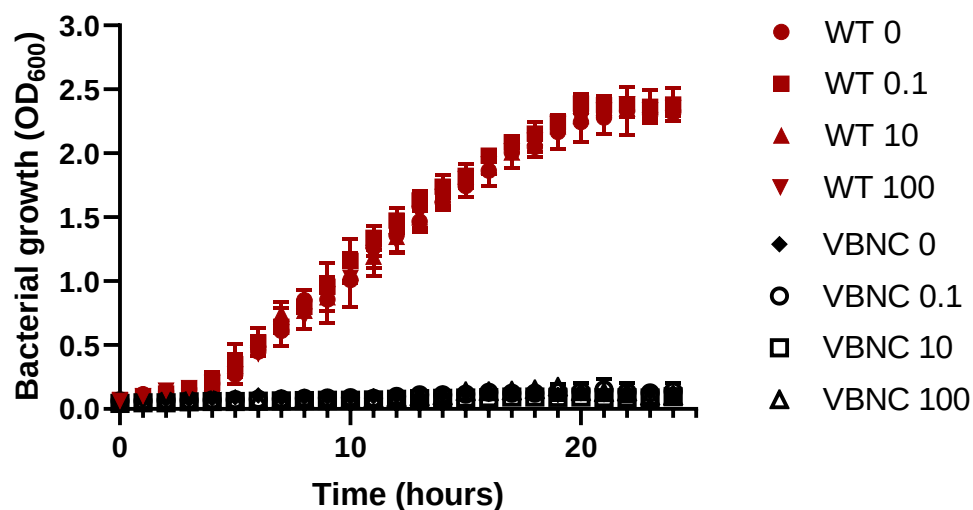


Figure 5.3: Attempts to resuscitate VBNC *S. Typhimurium* using STrRpf. The culturable (WT) and VBNC *S. Typhimurium* were treated with 0, 0.1, 10 and 100 µg/ml of purified STrRpf. A growth curve was plotted while growing the bacteria at 37°C without shaking for 24 hours. ST rRpf did not seem to affect the growth of culturable or VBNC *S. Typhimurium* at these concentrations.

5.2.2 Infection of C57BL/6 mice with VBNC *S. Typhimurium*

The virulence of the VBNC *S. Typhimurium* was studied by infecting C57BL/6 mice. The C57BL/6 mouse is a very sensitive infection model for *S. Typhimurium* with a LD₅₀ (lethal dose 50) as low as 10 bacteria by intravenous (i.v.) infection⁴¹¹. In comparison, an oral infection not only needs a higher bacterial dosage but also includes variables such as the influence of the gut microbiome and any (residual) acidity in the stomach.

Each mouse infected with culturable WT *S. Typhimurium* received a total of ~200 CFU during all the experiments. The VBNC bacteria do not form CFU hence the bacterial number was determined using a flow cytometer. A dosage of 2×10^5 of VBNC *S. Typhimurium* was used for i.v. infection. Before infecting mice with VBNC bacteria, the equivalent of 10^7 VBNC bacteria were plated onto LB agar plates with 50 µg/ml of streptomycin and incubated overnight to ensure no colonies were formed. Experiments were halted if the CFU from the VBNC *S. Typhimurium* were greater than 1 colony/ml, because of the low LD₅₀ of *S. Typhimurium* SL1344 for C57BL/6 mice⁴¹¹.

Infection with ~200 culturable *S. Typhimurium* caused a systemic infection and resulted in high bacterial burden in the liver and spleen by day 5 post infection (Figure 5.4). Mice injected with 2×10^5 VBNC *S. Typhimurium* were colonised, but the bacterial burden in the liver and spleen was very low compared to culturable *S. Typhimurium* WT (Figure 5.4). The number of bacteria that had regained culturability was greater in the liver, than in the spleen ($p < 0.0001$, student t-test).

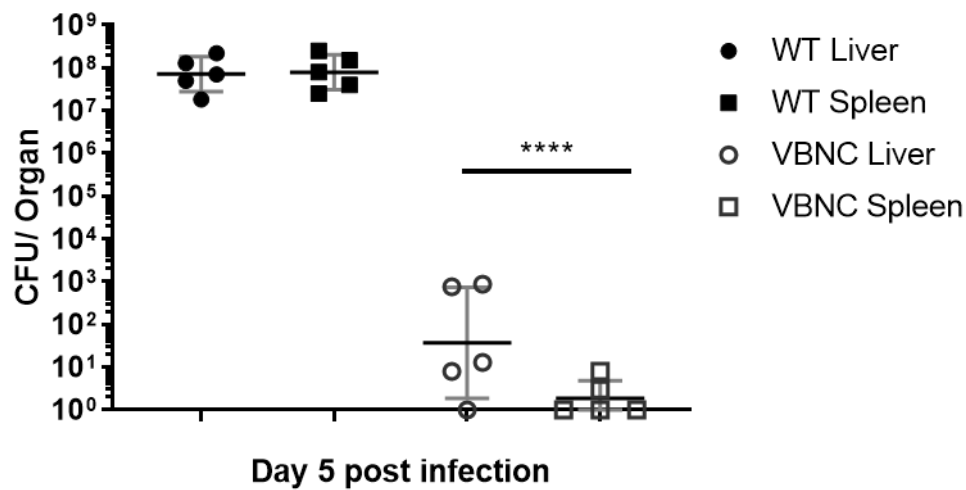


Figure 5.4: Bacterial burden in C57BL/6 mice spleen and liver infected with culturable or VBNC *S. Typhimurium*. C57BL/6 mice were infected with culturable or VBNC *S. Typhimurium* intravenously. ~200 CFUs of culturable bacteria and 2×10^5 of VBNC *S. Typhimurium* (enumerated by FACS) were used for infections. All the mice were culled in day five post infection and the spleens and livers of both the groups were homogenised and plated to identify the culturable bacterial burden.

5.2.3 C57BL/6 mice survive infection with VBNC *S. Typhimurium*

The *S. Typhimurium* SL1344 is a highly virulent strain that can kill infected mice within a week⁴¹². When infected with 200 CFUs of wild type culturable *S. Typhimurium* SL1344, the mice lost >15% of body weight by day 5 (Figure 5.5) and were euthanised in accordance with animal ethics. They exhibited signs of morbidity and systemic infection such as hunching, lack of feeding, inactivity and ruffled fur⁴¹³. The mice infected with VBNC *S. Typhimurium* did not show severe morbidity except for loss of weight of up to 10% on day 1 and 2 post-challenge (Figure 5.5). Mice infected with VBNC *S. Typhimurium* survived for as long as 2 weeks. The mice appeared healthy and gained weight (Figure 5.5) suggesting that they have recovered from the infection possibly due to the low level of replicating bacteria (Figure 5.4). *S. Typhimurium* with a lowered growth rate may have less virulence in infections in mice⁴¹⁴.

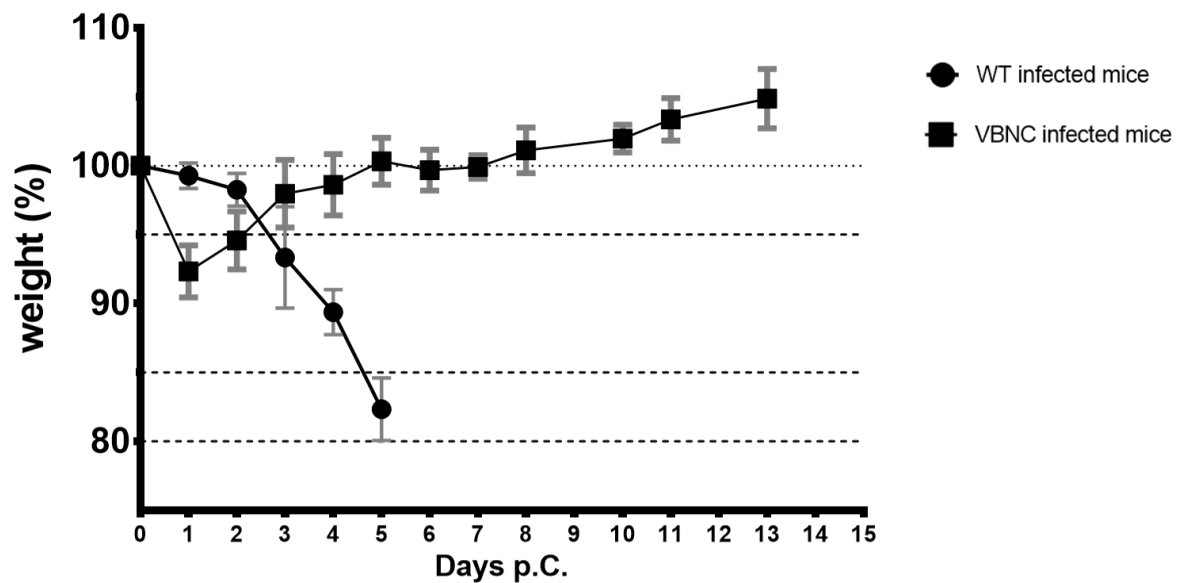


Figure 5.5: Weight loss curve of C57BL/6 mice infected with culturable or VBNC *S. Typhimurium*. C57BL/6 mice were infected with culturable or VBNC *S. Typhimurium* intravenously. The mice were weighed, and the weights were plotted as the percentage of weight before infection with culturable or VBNC bacteria against days of post colonisation.

5.2.4 Resuscitated VBNC *S. Typhimurium* did not show DNA mutations or growth differences

As the VBNC *S. Typhimurium* did not show comparable virulence to that of the culturable *S. Typhimurium* WT, selected colonies obtained from the liver or spleen of VBNC *S. Typhimurium*-infected C57BL/6 mice were analysed by whole genome sequencing. Sequencing the whole genome can determine whether any mutations have occurred during hydrogen peroxide stress, or while in the mice. Hydrogen peroxide can cause DNA damage through the production of reactive oxygen species⁴¹⁵. DNA was extracted at various stages from exponentially growing *S. Typhimurium* SL1344 (unstressed), hydrogen peroxide stressed VBNC *S. Typhimurium* (before mouse infection), and from colonies of resuscitated VBNC *S. Typhimurium* from mouse liver and spleen (Figure 5.4) and sequenced.

Genomic DNA was isolated from the samples using a bacterial DNA extraction kit (Qiagen). The colonies derived from the liver and spleen of two mice (#2.1 and #2.2) were selected for sequencing. The genome sequencing revealed no single nucleotide polymorphisms (SNPs) or 'indels' (insertions/deletions) in VBNC *S. Typhimurium* before mouse infection or after resuscitation. Although the resuscitated VBNC bacteria had no mutations, they may have had reduced growth *in vivo* due to other factors for example epigenetic differences. A growth curve of all 10 resuscitated VBNC *S. Typhimurium* colonies used for genome sequencing was analysed alongside the unstressed culture to observe any changes in growth kinetics. The growth curves identified that all the *S. Typhimurium* colonies show normal growth (Figure 5.6). Collectively, these experiments suggested that the murine infection with VBNC *S. Typhimurium* did not select for any genetic mutants (mutations as a result of hydrogen peroxide treatment) with slower growth rate, as indicated by genomic sequencing. Thus, the low bacterial burden in the spleens and livers of VBNC *S. Typhimurium* infected mice (Figure 5.4) cannot be attributed to slow growing mutants.

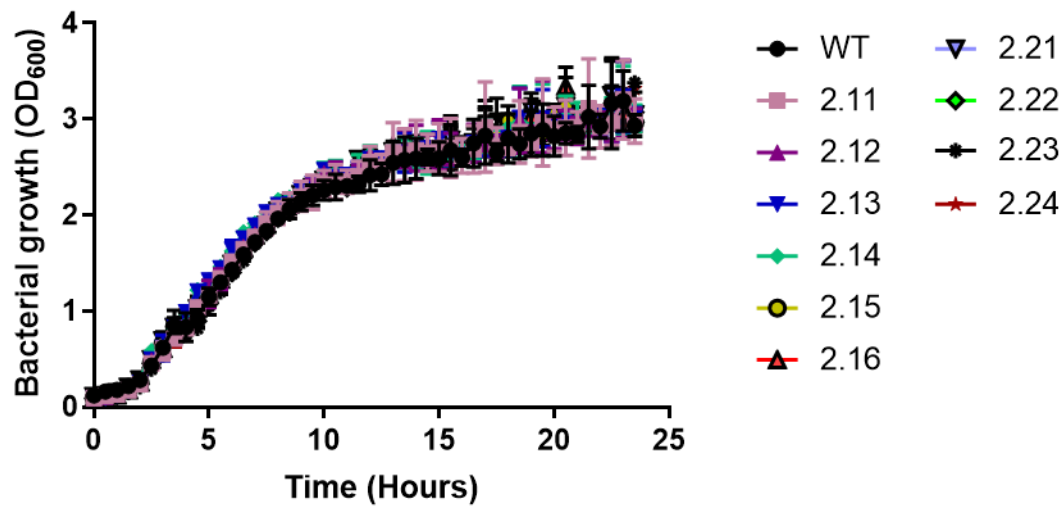


Figure 5.6: Growth curves of colonies of resuscitated VBNC *S. Typhimurium*.

Resuscitated VBNC *S. Typhimurium* colonies from mice were randomly selected and genome sequenced. The same colonies were analysed for any growth defects by plotting a growth curve while growing in LB. The growth curves were plotted based on the changes in bacterial growth (measured as a function of OD₆₀₀ absorption) over 24 hours.

5.2.5 VBNC *S. Typhimurium* did not kill highly immune-compromised mice or immunodeficient mice

The data collected in C57BL/6 mice suggest that VBNC *S. Typhimurium* were not as virulent as the culturable *S. Typhimurium*, based on the bacterial burden in the liver and spleen (Figure 5.4). To determine whether this reduction in virulence of VBNC *S. Typhimurium* (bacterial burden, Figure 5.4) was due to the immune system of C57BL/6 mice, immunodeficient C57BL/6 mice were infected with VBNC *S. Typhimurium*. If the host immune system in the C57BL/6 mouse was suppressing the growth of VBNC *S. Typhimurium*, immunodeficient mouse strains would show more bacterial burden when infected with VBNC *S. Typhimurium*. To test the effect of immune system on VBNC *S. Typhimurium* infection, two immunodeficient mouse strains were used for infection 1) RAG2^{-/-} common γ chain^{-/-} double knockout mice, which are deficient in T-cells, B-cells and NK cells (Natural Killer) cells⁴¹⁶ and 2) IFN γ ^{-/-} (interferon gamma) knockout. The IFN γ ^{-/-} mouse lacks a key cytokine, interferon gamma (γ), required to control the *S. Typhimurium* infection. Interferon gamma is an important bridge between the innate immunity and adaptive immunity⁴¹⁷; it also plays a role in the development and function of CD4⁺ T cells that enhance macrophage activation^{418,419}.

Mice were either infected with ~200 CFU of actively dividing *S. Typhimurium* WT or ~2 x 10⁵ VBNC *S. Typhimurium* via the intravenous route. The mice were monitored for signs of systemic infection. All the mice were sacrificed on day five post infection to compare the bacterial burden after homogenising and plating the liver and spleen samples on LB agar plates.

The results from the study using immunodeficient mice showed that the progress of infection by VBNC *S. Typhimurium* in RAG2^{-/-} common γ chain^{-/-} double knockout mice and IFN γ ^{-/-} knockout mice was similar to that of WT C57BL/6 mice. Both the immunodeficient mice showed lower bacterial burdens in the livers and spleens when infected with VBNC *S. Typhimurium* (Figure 5.7). Culturable *S. Typhimurium* showed more bacterial burden than the VBNC *S. Typhimurium* in the immunodeficient mice (Figure 5.7).

This data suggested that the reduced growth and virulence of VBNC *S. Typhimurium* may not be due to the immune system but probably is an intrinsic incapacity of the VBNC *S. Typhimurium* to establish fulminant infections. To ensure that the *ex vivo* culture result was not from contamination of the culturable bacteria in the VBNC inoculum preparation, the inoculum was left in LB for the length of the mouse experiment; no growth was detected.

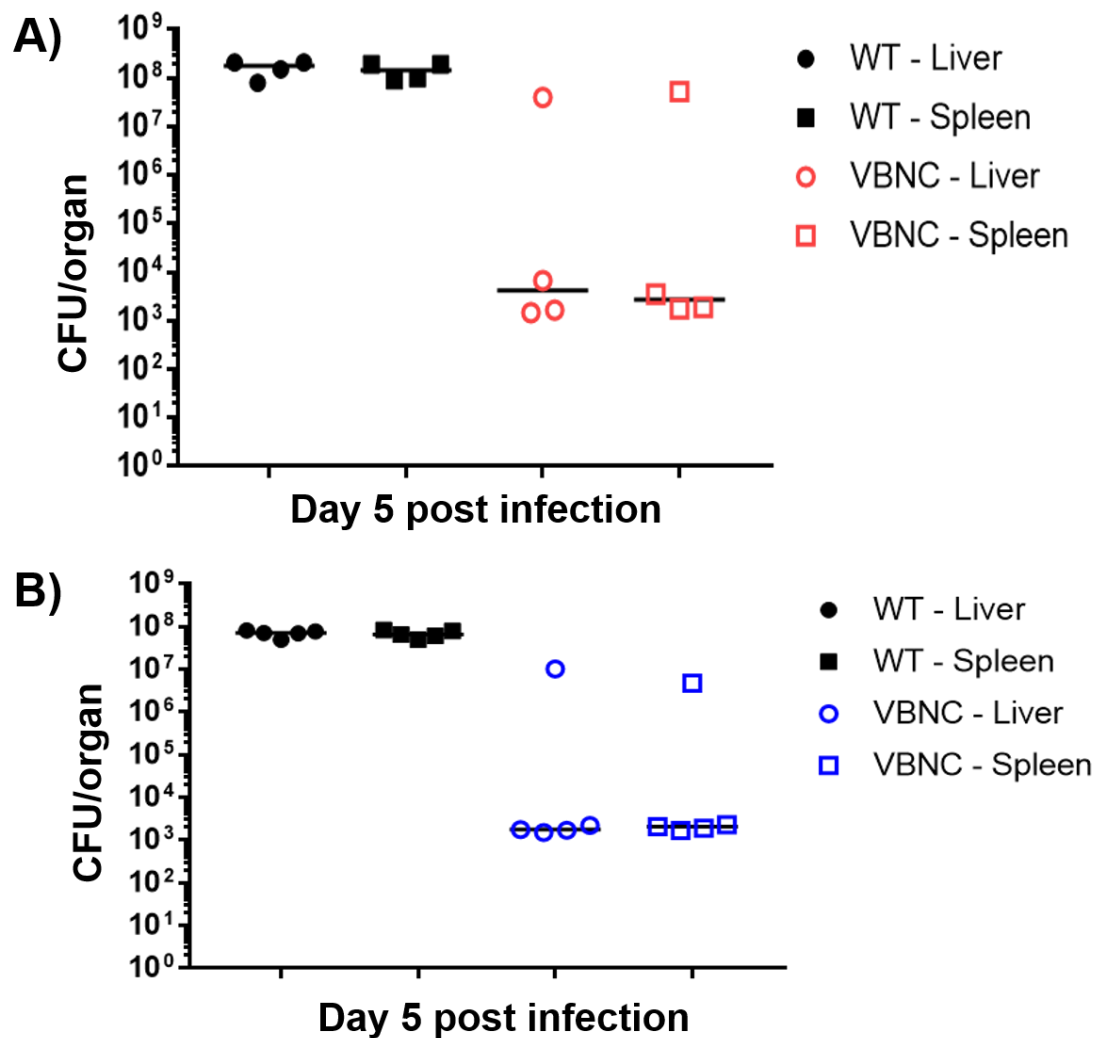


Figure 5.7: Infection of immunodeficient mice with culturable (WT) or VBNC *S. Typhimurium*. Rag2 common γ chain double knockout mice (A) and IFN γ -/- knockout mice (B) were intravenously infected with ~200 CFU of culturable WT or 2×10^5 (FACS enumerated) VBNC *S. Typhimurium*. The organs were harvested on day 5 post infection, homogenised and plated to count CFU. The CFU from the liver and spleen from culturable WT and the VBNC *S. Typhimurium* infected mice were plotted to represent the bacterial burden in the organs.

5.2.6 Resuscitated VBNC *S. Typhimurium* were virulent in C57BL/6 mice

Bacteria pre-exposed to the immune system can have a different infection dynamic to that of unexposed bacteria due to the selection and effect of the immune system^{88,420}. VBNC *S. Typhimurium* passaged through C57BL/6 mouse #2.1 and #2.2, referred to as 'resuscitated' VBNC *S. Typhimurium*, were tested for regained virulence. The resuscitated VBNC *S. Typhimurium* colonies did not exhibit growth defects as previously determined (Figure 5.6) and appeared genetically identical to wildtype SL1344 after murine infection. VBNC *S. Typhimurium* appeared to have persisted in the primary VBNC challenged animals without exhibiting classic wildtype *S. Typhimurium* pathology. To examine whether resuscitated VBNC *S. Typhimurium* regained virulence, ~200 CFU of resuscitated VBNC bacteria were injected intravenously into C57BL/6 mice.

'Resuscitated' *S. Typhimurium* were found to grow within the mice at a growth rate equivalent to that of wildtype culturable *S. Typhimurium*, with all mice exhibiting high bacterial burden and succumbing to infection by day 5 to 6 (Figure 5.8) indicating that virulence was regained in resuscitated VBNC *S. Typhimurium* which previously had displayed an avirulent VBNC state (Figure 5.6).

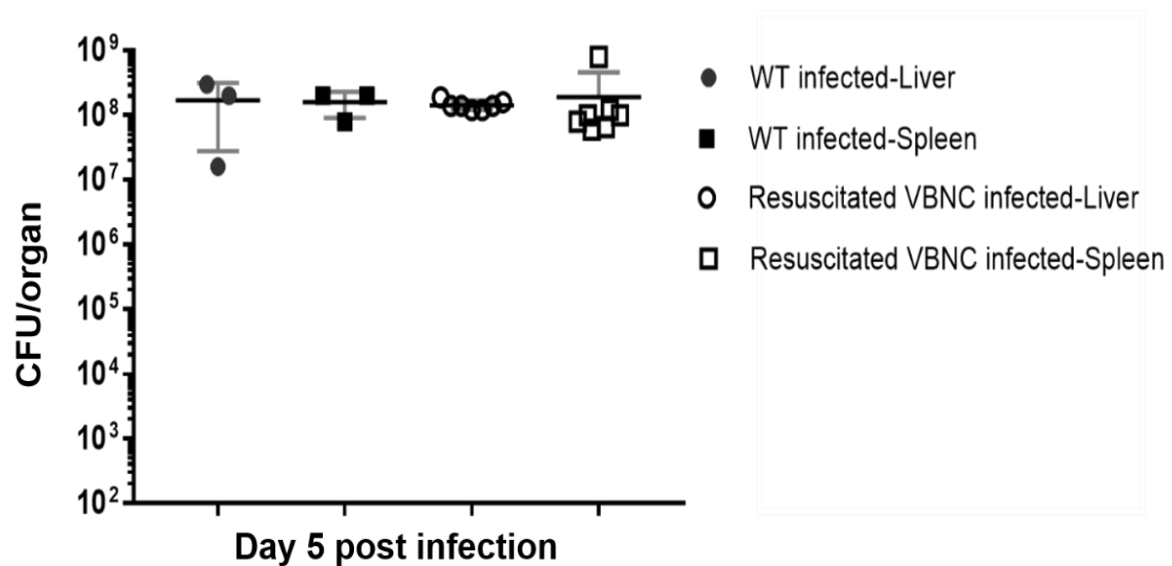


Figure 5.8: Infection studies in C57BL/6 mice with resuscitated VBNC *S. Typhimurium*.

C57BL/6 mice were infected with ~200 CFU of WT bacteria (3 mice) or ~200 CFU of 'resuscitated' VBNC *S. Typhimurium* (7 mice) intravenously. The organs were harvested on day 5 post infection, homogenised and plated to count CFU. CFU calculated from the liver and spleen were plotted to represent the bacterial burden in the organs.

5.3 Discussion

The virulence attributes of VBNC *S. Typhimurium* are not well understood but many studies suggest that the bacterium in this dormancy state is unlikely to be virulent^{64,83,92,167}. However, VBNC from some bacterial species retain virulence (Chapter 1, Section 1.7). Enterohaemorrhagic *E. coli* (EHEC) disease outbreaks in the USA and Japan, where VBNC *E.coli* O157 present in salmon roe were the suspected cause, highlight the importance of understanding the virulence potential of the VBNC bacteria¹⁶⁴.

Roszak *et al.* provided the first report of resuscitation from the VBNC state in 1984⁶². The study showed that the addition of nutrients did not always resuscitate the VBNC cells to a culturable state. VBNC *Salmonella* spp. were resuscitated by catalase, sodium pyruvate, temperature shift and Tween-20. Resuscitation seem to depend on the stress condition and the strain, as much as it does to the environmental 'cue'. For example, Oliver *et al.*, (2005)⁸³ induced both *E. coli* and *S. Typhimurium* into the VBNC state using chlorine, which can produce ROS (reactive oxygen species), but failed to resuscitate them under different conditions like temperature upshift or 1% sodium pyruvate which were previously shown to be effective on VBNC *S. Enteritidis*¹³⁰. Even catalase^{228,421} and agarose which could resuscitate VBNC *V. cholera*²¹⁴ failed to resuscitate *S. Enteritidis*.

Resuscitation promoting factor (Rpf) was proposed to resuscitate VBNC bacteria in Gram-positive bacteria like *M. luteus*^{59,229}. The protein counterpart of Rpf in *S. Typhimurium*, also known as YeaZ or TsaB^{63,422} of *S. Typhimurium*, was shown to be a M22 O-sialoglycoprotein endopeptidase homologue²³³. Further studies of YeaZ (TsaB) have shown that the protein was involved in transferring N6-threonylcarbamoyl group to the 37th adenosine (ANN) of decoding tRNA⁴²². This modification is necessary for bacterial survival thus making the YeaZ/TsaB gene essential in *E. coli*²³² and *S. Typhimurium*. Rpf from *S. Typhimurium* LT2 can be added ectopically to resuscitate VBNC bacteria^{40,59,63}. The reason for the failure of recombinant Rpf in resuscitating VBNC *S. Typhimurium* here could be the poly-histidine tag at the N-terminus of the protein which might have interfered with the normal functioning of the

protein or it may be due to the stress condition used to induce the VBNC state of *S. Typhimurium* i.e. oxidative stress, compared with the osmotic stress used with *S. Oranienburg*. The STRpfp could be degrading when incubated in the LB medium at 37°C. More data needs to be collected to understand the mechanism and function of Rpf from *S. Typhimurium*.

To test the virulence of VBNC *S. Typhimurium* in mice, the intravenous route of infection was chosen as it is very sensitive compared to the more natural oral route of infection⁴²³. While *in vitro* experiments to resuscitate VBNC *S. Typhimurium* SL1344 did not restore the culturable state, the *in vivo* murine challenge model showed evidence of virulence potential of oxidatively-stressed VBNC *S. Typhimurium* (Figure 5.4, 5.7). Only a small portion of bacteria were recovered from the VBNC state after mouse infections and VBNC bacteria failed to cause mortality in the C57BL/6 mouse model (Figures 5.5). The infection studies yielded a paradox. While no bacteria could be cultured from the VBNC inoculum, viable bacteria were recovered by culture from the organs of the VBNC *S. Typhimurium*-infected animals. Given the extreme sensitivity of the C57BL/6 mice to SL1344, where the LD₅₀ by intravenous infection is <10 bacteria, any viable bacteria should have resulted in severe morbidity in the VBNC-inoculated animals^{424,425}. No disease was observed in mice infected with VBNC *S. Typhimurium* even after 2 weeks. As mentioned earlier, the inoculum used for infection was constantly incubated in LB for the duration of the mouse experiments to test for growth of any residual culturable *S. Typhimurium*. VBNC cultures incubated in parallel to the mouse experiments did not increase in optical density over the period the mice were infected suggesting a lack of residual culturable bacteria in the inoculum.

The reason(s) for the recovery of only a small proportion of the infected VBNC bacteria is unclear. Previous studies also indicate that the dormant bacterial populations may not be homogenous⁴²⁶ but could be at different stages of dormancy and recoverability.

Although VBNC *S. Typhimurium* infected mice did not show virulence compared to the culturable *S. Typhimurium* WT, VBNC *S. Oranienburg* can infect BALB/c female mice only

when the mice were treated with morphine⁴²⁷. Dormant *S. Typhimurium* do not infect or colonise female BALB/c mice¹⁶⁷ despite using an infection dose 2 times and 3.5 times higher than the LD₅₀ dose for intraperitoneal and oral routes, respectively. In the present study 1000 times the LD₅₀ dosage was used for VBNC *S. Typhimurium*, delivered via intravenous injection. A dosage of 200 VBNC bacteria did not show any effect in our initial infection dosage optimisation studies. The number of bacteria that can be injected safely, without killing the mouse, was found to be 2×10^5 . Hence, the dosage 2×10^5 was chosen for the experiment.

Even though the level of resuscitation might be low (Figures 5.4 and 5.7), presence of 'resuscitatable' VBNC *S. Typhimurium* bacteria in foods can pose a public health risk as the resuscitated bacteria can multiply and increase in number. The resuscitated VBNC *S. Typhimurium* was shown to regain the lost virulence in the VBNC state. The resuscitated colonies (from Figure 5.4) were cultured in LB before infecting mice. More data on the virulence of VBNC *S. Typhimurium* can be obtained by isolating *S. Typhimurium* directly from the colonised liver and spleen of VBNC *S. Typhimurium* mice. The *S. Typhimurium* isolated from VBNC infected mice can be used directly to infect naïve mice without culturing in a growth medium (like in Figure 5.8). Unsuccessful attempts were made by using a Percoll gradient to isolate the *S. Typhimurium* from liver and spleen on day 5 post infection. Debris from homogenised organs could not be removed completely from the preparation and this caused severe distress to mice upon injection. Future studies can be done on isolating VBNC bacteria from infected mice organs or cell lines and using the isolated *S. Typhimurium* as an inoculum for next round of infections, i.e. without culturing the bacteria in medium. This can help in understanding much more reliably the virulence of 'resuscitated' *S. Typhimurium*.

In future, experiments can be done to isolate and differentiate VBNC bacteria from live and dead bacteria based on their density⁴²⁸. This could help identify VBNC cells from biofilms, planktonic culture, cell cultures and even patient samples. Infection studies with such bacteria which are not artificially stressed into VBNC state can give reliable answers regarding the virulence of these bacteria.

Resuscitation of VBNC *S. Oranienburg* in the host was observed when the bacteria passed through the gut of mice⁸⁸. Similar studies with VBNC *S. Typhimurium* could be conducted. VBNC *S. Typhimurium* can also be used for oral infection followed by examination of culturable *S. Typhimurium* in the faeces.

To conclude, when given in very high dose, the VBNC *S. Typhimurium* SL1344 can colonise and be cultured from murine liver and spleen during a C57BL/6 murine infection challenge but infection does not cause classic immunopathology. However, a murine passage and then challenge with resuscitated *S. Typhimurium* regained the lost virulence and exhibited the classic virulent pathology of a *S. Typhimurium* challenge (Figure 5.8). This implies that primary infection with VBNC *S. Typhimurium* may not cause an immediate virulent phenotype but transmission of resuscitated VBNC *S. Typhimurium* to a new host may lead to successful infection. Experiments to identify culturable *S. Typhimurium* bacteria in faeces from VBNC *S. Typhimurium* mice should be conducted to rule out the possibility of transmission of VBNC *S. Typhimurium* before deeming this state to be non-threatening to public health.

CHAPTER 6

General discussion

6.1 Final Discussion

The viable but nonculturable bacterial state has received its fair share of scepticism since it was first reported in 1982. The reason(s) why genetically competent bacteria fail to grow on conventional media, in the absence of stress, remains unresolved²⁰⁶. While the microbiology community may not yet have answered this question, there is a growing list of bacterial species that are known to enter a VBNC state, and new conditions that drive bacteria into a VBNC state are being reported¹⁶⁵. The absence of clear explanations concerning non-culturability adds impetus into establishing standardised methods to characterise VBNC bacteria and to provide mechanistic insights into the formation and maintenance of the VBNC state and to define the capacity of VBNC pathogens to cause disease¹⁷⁰.

The VBNC state challenged the established paradigm which regarded bacterial culturability as a hallmark of viability. This challenge to dogma triggered a debate around nomenclature for the phenomena, where some researchers prefer to call the VBNC state as ABNC - *active but nonculturable* or propose other alternative names like NIC - *not immediately culturable* or NGMA (*nongrowing but metabolically active*). The name VBNC can be justified by defining non-culturability. The term 'nonculturable' in VBNC bacteria means 'unculturable under previously established conditions (where culture was observed)^{29,226}. This VBNC phenotype is not relevant to naturally "unculturable" bacteria like *T. pallidum* or *Mycobacterium leprae*. The VBNC state does question our understanding of *in vitro* bacterial growth. The differential capacity of a bacterium to grow on laboratory media suggests that *in vitro* growth involves multiple factors other than the growth medium being used, including stochastic physiological variations in a population that can lead to the loss of culturability by some or all members of the population¹¹⁶, the prior exposure of the bacterium to different environmental agents (history-dependent memory), the physical and physiological state of the bacterium, chemical signals from other live bacteria and culturable bacteria^{59,429}. Any or all of these factors could impact bacteria epigenetically^{430,431}. The growing body of evidence concerning collected on VBNC unculturable bacteria¹⁰⁴, phenomenon such as nutrient shock⁴³², co-

culturing studies⁴³³ and biochemical analyses of the VBNC state *per se* suggests that culturability could be a considerably more complex phenotype than it might seem. There is a need for consensus to include other definitions of viability that are independent of culturing methods, while a deeper understanding of culturability is gathered, before culture is used as the important and/or sole yardstick of viability.

The work present in this thesis used oxidative stress as a condition to drive *S. Typhimurium* SL1344 into the VBNC state, and this approach was adapted from a method described by Morishige *et. al.*⁹⁴. The studies in this dissertation confirmed entry of *S. Typhimurium* into the VBNC state by using a Live/dead™ stain in combination with Kogure's direct viable counting methods and flow cytometry^{93,94} (Figure 3.5). The characteristics of the VBNC state described in this work include the lack of culturability, non-replication or cell wall extension as evidence by resistance to β -lactam antibiotics and nalidixic acid, reduced protein synthesis, and cell membrane integrity. These characteristics were tested based on the description of the VBNC state from the literature^{29,96,98,107,434}. The induction method and the attributes described in this thesis can be used to generate and study VBNC *S. Typhimurium* in the future.

VBNC *S. Typhimurium* were examined using TEM alongside the PI staining and these experiments provided evidence of membrane patency, as an indication of viability (Figure 3.11). TEM studies of VBNC *E. coli* by Kim *et al.*, (2018) detected an intact cell membrane but with a highly reduced cytosol when compared to *E. coli* persisters¹²¹. Based on this difference in the ultrastructure between persisters and VBNC bacteria, it was proposed that non-culturability in VBNC *E. coli* was a result of deterioration of persisters, and that these failing persisters were heading towards death¹²¹. The TEM images in this dissertation showed electron sparse areas with fewer the VBNC *S. Typhimurium*, suggesting the presence of an altered cytosol, possibly surrounded by an intact cell membrane. The suggestion that VBNC cells are on a cell death pathway based on the lack of cytosol, may be a misinterpretation. Experiments by Bamford *et. al.*⁹⁶ in *E. coli* suggest that persisters and VBNC bacteria might

co-exist during stationary phase. More experiments are needed on VBNC *S. Typhimurium* to test whether the VBNC state is an extension to state achieved by persisters, or whether they are independent states that co-exist within a *S. Typhimurium* population.

The transcriptome analysis of VBNC *S. Typhimurium* showed some expected and some unexpected differences. The magnitude of the change in overall transcription was very large where approximately half the genome was subjected to differential gene transcription. This drastic change in the transcriptome of VBNC *S. Typhimurium* posed challenges in identifying single genes or pathways that could contribute to the VBNC state. In contrast, the proteomic analysis of VBNC *S. Typhimurium* demonstrated very few changes and did not reflect the observations made in transcriptome, leading to a hypothesis that the immediate response to high level oxidative stress was rapidly reduced translation through ribosome disassembly – this was consistent with the TEM images shown in Figure 3.6. Ribosomal fractionation experiments, the RIN scores of RNA isolated from VBNC *S. Typhimurium*, analyses of tagged ribosomal proteins, and the VBNC behaviour of a Δpnp mutant, supported this hypothesis.

Bacteria, upon encountering high level stress including nutrient starvation³⁹² and oxidative stress⁴³⁵, can rapidly degrade their ribosomes. *Salmonella* spp. were shown to degrade the rRNA to reduce the numbers of ribosomes during entry into stationary phase¹⁹³. Such a phenomenon is universal and can be observed in eukaryotic cells under stress⁴³⁶⁻⁴³⁹. In VBNC *S. Typhimurium*, the reduction in ribosomes was consistent with the hypothesis that stress drove a rapid shut down of protein synthesis. Though bacterial ribosomal degradation under stress has been reported, it was not studied in the context of VBNC state^{191,440}. This hypothesis suggests that *S. Typhimurium* may ‘use’ the stress to increase their chance of survival by entering into a VBNC state that is the consequence of abruptly halted protein synthesis.

Various observations made here on VBNC *S. Typhimurium* supported this hypothesis of entry into dormancy through rapid ribosomal rRNA degradation and ribosomal dissolution. These observations included changes in the ribosomal content by TEM, degradation of rRNA

(Figures 4.1 and 4.2), lack of significant changes in the proteome despite clear differences in the transcriptome (Chapter 4, Section 4.2.6), the absence of ribosomes and ribosomal subunits when examined by gradient centrifugation (Figure 4.14), changed response of *S. Typhimurium* Δpnp to VBNC-inducing conditions (Figure 4.11K and 4.11L) and decreased translation (Figure 4.15). Future studies can examine the effects of other RNases, especially RNase E, on the formation of the VBNC state.

If the VBNC state is created by a cessation of translation through rRNA degradation and ribosomal dissolution, how might resuscitation of VBNC occur? Density gradient analysis of the VBNC ribosomes demonstrated the presence of 'polysomes', i.e. ribosomes attached to an mRNA (Figure 4.13) that might indicate some residual translational activity and these polysomes might play a role in the resuscitation of the VBNC bacteria. In some bacteria, 100S ribosomes or "hibernating ribosomes" can form under stress conditions^{398,441}. These 100S ribosomes are activated by the return of more favourable conditions³⁹⁷.

One interesting consequence of this ribosome dissolution is the impact on genomic identification of actively growing bacteria. Degradation of rRNA may reduce the ability to detect bacteria by RT-PCR analysis of 16S rRNA, which is a common practice to test the presence of active bacteria in various types of samples^{442,443}. Hence, the degradation of rRNA in the VBNC state of *S. Typhimurium* may even lead to unreliable results in detection and estimation of the bacterial burden by the RT-PCR based methods.

The mechanisms which underpin the function of VBNC resuscitating agents that can drive the culturability of VBNC bacteria are not well understood. Ayrapetyan *et al.* held that VBNC bacteria are at a deeper state of dormancy than persisters¹¹⁴. Entering and exiting VBNC state could be a more 'energy expensive' process than persistence. Hence, VBNC bacteria may require multiple cues to revert to growth – these might include autoinducers from other bacteria and factors from host. While resuscitation was not a major focus of the work presented here, there was evidence of VBNC culturability after inoculation into mice. Attempts to resuscitate VBNC *S. Typhimurium* *in vitro* using resuscitation factor, Rpf (Figure 5.3), were

unsuccessful. The data from animal experiments definitively showed that the oxidatively-stressed, ribosome-depleted VBNC could revert to culturable bacteria, with no sequence changes, after passage through mice.

This work also highlights that combined study of the transcriptome and proteome of VBNC *S. Typhimurium* might provide a more complete understanding of the physiology of this state. Physiology of the VBNC state can be misinterpreted based on data from RNA sequencing alone if the proteome is not also analysed. Though this is true for all 'omics'-based studies, it is especially true in the case of VBNC state as the bacteria are induced as a response to stress. The transcription and translation could be highly influenced by bacterial general stress response²⁰⁹, which may occlude the core changes associated with loss of culturability.

It is important to study the metabolic activity of pathogenic bacteria while in the VBNC state and particularly *in situ* (in the environment). Decades of research had provided insights into various properties of bacteria under laboratory conditions and under controlled stress conditions. The complexity of bacterial survival in the environment where organisms would be exposed to a single or a combination of stress conditions, cannot always be simulated in the laboratory. There is not only academic value in answering these questions but also clinical significance in understanding the survival of food borne pathogens while they transit through the environment.

In conclusion, the VBNC state in *S. Typhimurium* could be a potential health risk only if the bacteria resuscitate from their dormant state (Figure 5.8). The mechanism(s) involved in driving the bacterium into the VBNC state could be complex involving different bacterial stress responses such as RNA degradation, ribosomal dimerization or dissolution and/or switching metabolic pathways. Future studies should focus on the factors that resuscitate VBNC *S. Typhimurium* and these may provide mechanistic insights into how bacteria enter VBNC state.

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Appendix

Table A: Transcriptome data showing gene expression changes in VBNC *S. Typhimurium* compared to unstressed, actively dividing *S. Typhimurium*.

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>aac</i>	79.61	0.06	0.83771	<i>allR</i>	647.81	-0.54	0.03911
<i>aadA</i>	292.99	0.39	0.11184	<i>alr-a</i>	952.28	-1.57	0.00183
<i>aarF</i>	3762.54	-0.56	0.01647	<i>alr-b</i>	1204.85	-0.38	0.08282
<i>aas</i>	1194.59	-0.34	0.12674	<i>amiA</i>	2880.05	-0.79	0.03312
<i>aat</i>	602.30	-0.58	0.01900	<i>amiB</i>	1546.57	-0.85	0.00040
<i>abc</i>	1043.60	-1.32	3.10E-06	<i>amiC</i>	1841.60	-0.47	0.02911
<i>abrB</i>	381.13	-0.09	0.73960	<i>amn</i>	2027.55	-1.05	0.00015
<i>accA</i>	3005.40	0.81	0.00099	<i>ampD</i>	1336.57	-1.75	2.81E-13
<i>accB</i>	5145.11	-0.59	0.05006	<i>ampE</i>	791.16	-0.55	0.04005
<i>accC</i>	7233.25	0.98	0.00141	<i>ampG</i>	1095.22	-0.79	0.00092
<i>accD</i>	2199.67	0.05	0.84506	<i>ampH</i>	484.32	-0.33	0.16998
<i>aceA</i>	314.57	-0.20	0.65000	<i>amtB</i>	706.03	0.90	0.00059
<i>aceB</i>	540.43	-1.26	0.01076	<i>amyA</i>	1346.67	-1.78	3.99E-11
<i>aceE</i>	86549.75	1.29	6.16E-07	<i>anmK</i>	633.76	-0.20	0.44341
<i>aceF</i>	37083.54	1.05	8.82E-06	<i>ansA</i>	1199.11	0.43	0.06949
<i>aceK</i>	480.53	-0.36	0.21974	<i>ansB</i>	3439.87	-2.63	1.55E-19
<i>ackA</i>	10801.73	-0.05	0.85994	<i>ansP</i>	3048.19	-1.55	8.24E-12
<i>acnA</i>	28284.54	3.94	3.94E-28	<i>apaG</i>	1497.35	0.98	0.00088
<i>acnB</i>	14615.20	-0.32	0.46990	<i>apaH</i>	1971.23	0.26	0.32175
<i>acpD</i>	1583.17	2.06	1.39E-05	<i>apbA</i>	1715.67	-1.95	1.58E-12
<i>acpP</i>	44668.39	-0.13	0.70744	<i>apbE</i>	814.17	-0.58	0.01109
<i>acpS</i>	369.97	0.28	0.21929	<i>apeE</i>	698.71	0.56	0.02327
<i>acpT</i>	325.60	-0.86	0.00071	<i>aphA</i>	1530.65	-1.44	1.83E-06
<i>acrA</i>	6114.98	0.08	0.74258	<i>apl</i>	207.48	-2.13	1.09E-07
<i>acrB</i>	13116.04	0.84	0.00082	<i>appB</i>	448.95	-0.87	0.00173
<i>acrD</i>	1352.13	1.11	1.78E-06	<i>appC</i>	244.61	1.15	7.41E-05
<i>acrE</i>	197.10	1.68	1.05E-07	<i>apt</i>	897.80	0.71	0.01070
<i>acrF</i>	1697.35	-0.97	0.00016	<i>araA</i>	216.52	0.77	0.01587
<i>acrR</i>	2448.83	-2.39	8.68E-15	<i>araB</i>	203.85	1.02	0.00045
<i>acs</i>	1325.64	-1.62	0.00010	<i>araC</i>	358.62	-1.56	3.45E-06
<i>actP</i>	382.85	0.65	0.00875	<i>araD</i>	81.18	1.43	1.86E-06
<i>ada</i>	210.73	0.81	0.00130	<i>araE</i>	293.06	0.52	0.04550
<i>add</i>	902.15	0.55	0.02530	<i>araH</i>	193.86	0.58	0.03569
<i>adh</i>	6270.06	-0.67	0.02863	<i>araJ</i>	356.24	0.03	0.90457
<i>adhC</i>	883.33	0.71	0.00185	<i>arcA</i>	6739.86	-1.50	8.90E-07
<i>adhP</i>	1613.29	0.28	0.35794	<i>arcB</i>	5833.19	-1.35	1.76E-08
<i>adi</i>	711.60	-0.50	0.08180	<i>arcC</i>	112.47	1.34	0.00014
<i>adiC</i>	440.87	-0.42	0.13840	<i>ArcZ</i>	447.46	-0.93	0.00072
<i>adiY</i>	822.92	-1.15	5.23E-06	<i>argA</i>	667.70	-0.77	0.00375
<i>adk</i>	2804.57	0.33	0.18311	<i>argB</i>	585.32	-1.01	0.00065
<i>adrA</i>	393.87	-1.19	7.54E-06	<i>argC</i>	1180.65	-0.94	0.00138
<i>aefA</i>	5736.53	1.33	9.27E-10	<i>argDa</i>	325.50	-0.94	0.03469
<i>aegA</i>	295.70	1.22	8.60E-07	<i>argDb</i>	902.84	-0.41	0.24221
<i>aer</i>	1642.42	-1.42	0.00012	<i>argE</i>	1171.91	-2.45	2.60E-21
<i>aes</i>	2962.62	3.53	2.03E-36	<i>argG</i>	1267.92	0.15	0.54244
<i>agaR</i>	575.23	-1.81	2.01E-08	<i>argH</i>	1125.15	-0.14	0.53006
<i>agp</i>	923.28	-1.07	0.00072	<i>argI</i>	342.03	0.17	0.71064
<i>ahpC</i>	159228.30	4.66	6.05E-69	<i>argR</i>	2765.17	-1.22	2.72E-06
<i>ahpF</i>	253651.05	7.26	7.10E-184	<i>argS</i>	5628.39	1.22	2.57E-05
<i>aidB</i>	515.74	-0.54	0.15944	<i>argT</i>	1049.09	-1.71	2.92E-05
<i>ais</i>	6172.18	2.46	4.40E-13	<i>arnT</i>	2626.77	2.42	1.97E-29
<i>alaS</i>	18455.66	0.28	0.19406	<i>aroA</i>	1199.34	-0.60	0.00677
<i>aldB</i>	482.92	0.39	0.19670	<i>aroB</i>	5889.96	-0.22	0.33455
<i>alkA</i>	136.18	0.20	0.43398	<i>aroC</i>	1542.38	-0.46	0.03259
<i>alkB</i>	166.96	0.89	0.00081	<i>aroD</i>	374.46	-0.52	0.10052
<i>allA</i>	494.49	-2.15	6.70E-06	<i>aroE</i>	268.25	-0.79	0.01411
<i>allB</i>	267.67	1.93	3.50E-10	<i>aroF</i>	3949.83	-3.34	3.40E-29
<i>allC</i>	149.26	2.53	5.44E-18	<i>aroG</i>	1195.47	0.81	0.00713
<i>allD</i>	91.08	1.33	1.72E-05	<i>aroH</i>	1455.56	0.17	0.45354
<i>allP</i>	133.11	4.12	8.50E-25	<i>aroK</i>	8254.68	-1.79	3.19E-10

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>aroL</i>	1821.37	-2.52	1.85E-25	<i>bioDa</i>	70.91	-0.45	0.18072
<i>aroM</i>	1392.33	-2.15	4.96E-15	<i>bioDb</i>	556.30	-0.65	0.01385
<i>aroP</i>	1304.88	-1.60	3.19E-09	<i>bioF</i>	75.31	2.81	1.81E-15
<i>aroQ</i>	663.96	-2.82	3.52E-14	<i>bioH</i>	727.91	-0.30	0.21120
<i>artI</i>	2251.51	1.11	0.00024	<i>birA</i>	2710.28	-0.74	0.01773
<i>artJ</i>	967.75	-1.16	0.01117	<i>bisC</i>	1410.83	-0.11	0.63928
<i>artM</i>	3055.13	1.67	3.63E-10	<i>blc</i>	953.83	-2.60	1.53E-10
<i>artP</i>	1990.60	0.93	0.00028	<i>blr</i>	320.55	-2.10	2.39E-13
<i>artQ</i>	1284.03	1.94	1.58E-14	<i>bolA</i>	1294.77	-2.07	1.54E-10
<i>asd</i>	3527.08	0.66	0.01527	<i>brnQ</i>	1884.84	-0.85	0.00110
<i>asmA</i>	4364.67	-0.22	0.31134	<i>bssR</i>	1224.61	-2.66	1.78E-14
<i>asnA</i>	23171.07	-6.37	2.73E-81	<i>bssS</i>	25414.78	-4.03	2.61E-44
<i>asnB</i>	3561.67	-1.90	2.01E-09	<i>btuB</i>	3460.70	0.42	0.07932
<i>asnCa</i>	7005.94	-0.85	0.00125	<i>btuC</i>	791.46	0.75	0.00043
<i>asnCb</i>	2302.46	-2.51	1.24E-13	<i>btuD</i>	412.81	1.20	3.47E-06
<i>aspA</i>	55981.21	-0.80	0.01089	<i>btuE</i>	651.48	-0.01	0.98019
<i>aspC</i>	3423.91	0.79	0.00209	<i>btuF</i>	511.89	-0.39	0.13841
<i>aspS</i>	6765.91	0.86	0.00136	<i>btuR</i>	2219.53	0.83	0.00698
<i>asr</i>	459.26	-1.80	9.32E-11	<i>C1</i>	248.20	0.27	0.33795
<i>asrA</i>	312.36	2.18	1.17E-13	<i>cadA</i>	27445.85	5.11	2.54E-70
<i>asrB</i>	110.55	1.33	1.28E-06	<i>cadB</i>	5341.22	5.05	1.17E-76
<i>asrC</i>	394.47	1.18	2.29E-07	<i>cadC</i>	2991.00	0.37	0.19762
<i>assT</i>	307.83	0.95	0.00110	<i>cafA</i>	5345.84	-0.22	0.41375
<i>astA</i>	382.73	-0.77	0.07949	<i>caiA</i>	198.88	1.39	3.70E-07
<i>astB</i>	290.72	0.82	0.03765	<i>caiB</i>	312.65	0.14	0.65215
<i>astD</i>	245.20	0.16	0.74146	<i>caiC</i>	267.32	1.06	0.00024
<i>astE</i>	113.56	1.11	0.00062	<i>caiD</i>	140.65	2.27	6.59E-11
<i>atpA</i>	24818.88	-0.38	0.14013	<i>caiE</i>	127.54	1.57	1.48E-07
<i>atpB</i>	16542.94	-0.46	0.14083	<i>caiF</i>	765.69	-0.36	0.19386
<i>atpC</i>	15288.38	1.80	2.27E-09	<i>caiT</i>	221.88	2.03	4.39E-09
<i>atpD</i>	12540.61	-0.18	0.44137	<i>carA</i>	1473.14	-0.29	0.23983
<i>atpE</i>	7063.23	-1.18	0.00054	<i>carB</i>	3660.87	-0.08	0.78772
<i>atpF</i>	13908.45	0.28	0.32056	<i>cblA</i>	497.69	1.87	6.78E-09
<i>atpG</i>	7628.54	0.14	0.53861	<i>cblB</i>	423.65	2.22	7.00E-13
<i>atpH</i>	6005.99	-1.60	5.46E-10	<i>cblC</i>	411.24	4.60	3.98E-34
<i>atpI</i>	3127.41	-1.92	1.18E-09	<i>cblD</i>	1460.16	4.69	1.18E-39
<i>avrA</i>	3104.14	0.90	0.00262	<i>cblE</i>	588.06	6.17	3.78E-47
<i>avtA</i>	1469.27	-0.73	0.00197	<i>cblF</i>	1759.48	6.70	2.94E-100
<i>b2145</i>	113.91	-0.82	0.00742	<i>cblG</i>	3343.35	6.87	3.25E-118
<i>bacA</i>	1048.21	0.48	0.03991	<i>cblH</i>	4715.59	7.51	2.49E-190
<i>baeR</i>	319.26	0.35	0.15538	<i>cblJ</i>	4926.28	7.77	2.78E-163
<i>baeS</i>	708.67	0.01	0.97773	<i>cblK</i>	4668.79	7.65	5.18E-154
<i>bapA</i>	1393.15	0.96	0.00021	<i>cblL</i>	2611.77	6.81	1.30E-109
<i>barA</i>	1677.95	-0.05	0.83731	<i>cblM</i>	3840.90	6.39	5.46E-137
<i>basR</i>	2720.42	0.67	0.01241	<i>cblN</i>	1272.44	5.19	7.34E-41
<i>basS</i>	3347.92	0.56	0.02110	<i>cblO</i>	3715.12	5.30	1.24E-43
<i>bax</i>	4879.60	-0.51	0.02385	<i>cblP</i>	5963.73	4.92	1.07E-53
<i>bcfA</i>	100.68	1.09	0.00168	<i>cblQ</i>	1808.03	5.64	3.31E-51
<i>bcfB</i>	69.51	0.48	0.16381	<i>cblT</i>	958.32	6.44	4.15E-79
<i>bcfC</i>	526.18	0.19	0.48518	<i>cbpA</i>	1349.42	-1.81	4.93E-09
<i>bcfD</i>	209.43	0.39	0.19159	<i>cca</i>	1783.88	0.85	0.00343
<i>bcfE</i>	70.31	-0.16	0.60564	<i>ccmA1</i>	156.15	2.18	8.99E-15
<i>bcfF</i>	280.87	-3.01	1.61E-22	<i>ccmA2</i>	161.61	1.93	9.26E-14
<i>bcfG</i>	110.49	-0.21	0.47191	<i>ccmB1</i>	203.93	2.26	2.79E-14
<i>bcfH</i>	254.61	1.30	9.24E-07	<i>ccmB2</i>	219.72	2.37	1.04E-18
<i>bcp</i>	847.88	-1.37	6.91E-05	<i>ccmC1</i>	163.78	1.64	6.72E-10
<i>bcr</i>	763.18	0.79	0.00051	<i>ccmC2</i>	166.34	1.96	7.59E-11
<i>bcsA</i>	1513.42	-0.24	0.29259	<i>ccmD1</i>	2.49	1.73	0.06010
<i>bcsC</i>	828.36	0.56	0.05407	<i>ccmD2</i>	3.39	2.65	0.00407
<i>bfd</i>	4275.87	0.39	0.32269	<i>ccmE1</i>	113.64	3.35	8.10E-26
<i>bfr</i>	10362.83	0.30	0.31570	<i>ccmE2</i>	106.57	3.22	9.52E-25
<i>bglA</i>	2329.98	-1.11	2.68E-05	<i>ccmF1</i>	407.48	3.01	1.83E-28
<i>bglJ</i>	139.95	-1.12	7.78E-05	<i>ccmF2</i>	409.41	2.90	1.23E-31
<i>bglX</i>	1531.11	0.74	0.00724	<i>ccmG1</i>	128.35	2.32	6.75E-16
<i>bigA</i>	880.81	0.52	0.09775	<i>ccmG2</i>	135.37	2.44	1.31E-16
<i>bioA</i>	190.57	-0.81	0.00594	<i>ccmH1</i>	315.86	2.47	1.20E-20
<i>bioB</i>	129.14	1.08	0.00149	<i>ccmH2</i>	321.68	0.89	0.00040
<i>bioC</i>	63.24	2.20	8.63E-10	<i>ccrB</i>	206.83	-0.47	0.07337

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>cdaR</i>	7473.21	-3.31	5.05E-23	<i>comA</i>	87.05	-0.23	0.50138
<i>cdd</i>	516.15	-2.44	1.03E-10	<i>comB</i>	55.85	0.17	0.60027
<i>cdh-a</i>	1189.28	-1.30	0.00017	<i>comC</i>	43.25	1.90	1.26E-06
<i>cdh-b</i>	18.40	-0.02	0.96740	<i>comD</i>	51.78	1.48	0.00012
<i>cdsA</i>	4210.98	-0.28	0.23592	<i>comE</i>	407.84	-0.43	0.12135
<i>cedA</i>	354.56	1.36	1.67E-06	<i>comE1</i>	468.08	-0.61	0.00884
<i>celA</i>	2347.57	-1.10	0.00375	<i>comF</i>	213.88	-0.35	0.23449
<i>celB</i>	526.55	0.93	0.01461	<i>comM</i>	633.46	-1.05	0.00049
<i>celC</i>	897.29	0.29	0.58568	<i>comN</i>	80.44	0.28	0.42654
<i>celD</i>	546.24	1.07	0.02801	<i>comO</i>	95.48	0.16	0.60716
<i>celF</i>	1359.76	3.30	2.14E-15	<i>comP</i>	67.56	0.57	0.08664
<i>celG</i>	591.26	3.09	9.43E-13	<i>comQ</i>	65.05	0.28	0.41014
<i>cfa</i>	1328.90	0.17	0.47787	<i>copA</i>	3176.78	-2.08	9.15E-15
<i>chaA</i>	3728.76	1.95	6.59E-15	<i>copR</i>	409.39	-0.52	0.04214
<i>chaB</i>	480.13	-0.17	0.61160	<i>copS</i>	417.54	0.62	0.00610
<i>cheA</i>	10144.07	-0.90	0.00104	<i>corA</i>	2088.75	-1.20	8.22E-07
<i>cheB</i>	2526.80	0.18	0.50921	<i>corB</i>	4297.68	0.58	0.01750
<i>cheM</i>	7217.44	-2.04	6.23E-12	<i>corE</i>	1176.04	-0.48	0.03305
<i>cheR</i>	1443.45	-0.11	0.67442	<i>cpdA</i>	2702.45	-1.08	4.82E-05
<i>cheW</i>	3014.71	0.83	0.01266	<i>cpdB</i>	1231.76	-1.82	9.37E-09
<i>cheY</i>	8263.66	1.45	1.16E-08	<i>cpsB</i>	141.70	2.11	1.06E-11
<i>cheZ</i>	11891.34	1.77	2.02E-09	<i>cpsB2</i>	10731.86	-0.58	0.06673
<i>chiA</i>	544.46	0.20	0.48153	<i>cpsG</i>	133.66	2.64	1.90E-14
<i>ChiX</i>	73.41	-0.66	0.09389	<i>cpxA</i>	2407.62	0.48	0.03795
<i>cl</i>	449.47	-0.85	0.00042	<i>cpxP</i>	22761.53	-1.30	0.00047
<i>cigR</i>	1642.49	-1.44	3.37E-07	<i>cpxR</i>	3041.17	-1.17	0.00014
<i>cIIa</i>	301.38	-1.02	7.46E-05	<i>creA</i>	1277.83	-1.18	1.74E-06
<i>cIIb</i>	34.06	2.24	4.00E-06	<i>creB</i>	448.80	0.33	0.19813
<i>cIIc</i>	147.65	-0.42	0.12044	<i>creC</i>	464.24	0.28	0.26617
<i>cirA</i>	2143.57	1.38	4.03E-07	<i>creD</i>	599.58	0.07	0.80547
<i>citA</i>	338.72	1.01	0.00023	<i>crl</i>	3266.81	-2.55	1.03E-12
<i>citA2</i>	618.21	-1.05	0.00023	<i>crp</i>	9183.23	-3.37	3.79E-32
<i>citB</i>	244.99	1.54	6.18E-09	<i>crr</i>	5933.09	0.57	0.06490
<i>citB2</i>	289.07	-0.38	0.12832	<i>csdA</i>	1183.78	0.45	0.12301
<i>citC</i>	146.00	1.69	2.85E-08	<i>csgA</i>	85.94	0.03	0.93185
<i>citC2</i>	177.53	1.01	0.00414	<i>csgB</i>	28.92	1.10	0.01847
<i>citD</i>	11.77	2.87	6.69E-06	<i>csgC</i>	73.71	-0.39	0.30534
<i>citD2</i>	6.16	1.60	0.02465	<i>csgD</i>	46.75	0.60	0.11914
<i>citE</i>	67.03	2.03	1.17E-07	<i>csgE</i>	33.14	1.38	0.00156
<i>citE2</i>	105.40	3.33	5.60E-20	<i>csgF</i>	22.22	0.60	0.17095
<i>citF</i>	133.81	3.91	8.39E-32	<i>csgG</i>	153.55	0.35	0.24131
<i>citF2</i>	163.39	1.62	7.91E-07	<i>csiD</i>	119.21	0.51	0.19352
<i>citG</i>	100.11	1.77	2.39E-09	<i>csiE</i>	936.79	-1.33	1.04E-06
<i>citG2</i>	159.93	1.61	9.49E-08	<i>cspA</i>	174822.74	-2.09	0.00738
<i>citT</i>	445.06	0.80	0.00269	<i>cspB</i>	35462.05	-1.34	0.00894
<i>citX</i>	44.81	4.30	1.33E-16	<i>cspC</i>	3197.29	1.30	0.00305
<i>citX2</i>	34.79	1.93	5.46E-06	<i>cspD</i>	1275.89	-1.93	7.35E-15
<i>clcA</i>	857.07	0.15	0.53896	<i>cspE</i>	11483.43	-0.99	0.04838
<i>clcB</i>	316.88	0.12	0.63778	<i>cspH</i>	1387.45	-2.74	4.55E-12
<i>clpA</i>	61826.66	1.90	1.26E-07	<i>csrA</i>	4929.50	-1.39	1.24E-05
<i>clpB</i>	11898.49	-0.60	0.04339	<i>CsrB</i>	1532418.86	1.07	9.57E-07
<i>clpP</i>	6503.49	-2.10	5.54E-16	<i>CsrC</i>	266761.68	2.74	5.30E-18
<i>clpS</i>	13922.61	-0.05	0.88124	<i>cstAa</i>	5898.25	-0.35	0.31563
<i>clpX</i>	17034.05	1.04	0.00036	<i>cstAb</i>	974.61	-1.08	8.38E-05
<i>cls</i>	545.16	-0.81	0.00074	<i>cueO</i>	2108.38	-1.52	1.81E-05
<i>cmk</i>	4619.64	0.16	0.52156	<i>cueR</i>	2002.35	-2.22	2.55E-09
<i>coaA</i>	1998.13	-0.49	0.02096	<i>cusA</i>	26.59	1.40	0.00046
<i>coaD</i>	382.01	-0.52	0.05002	<i>cutA</i>	652.09	-2.03	1.53E-10
<i>coaE</i>	513.49	-0.78	0.00276	<i>cutC</i>	203.11	-0.54	0.04623
<i>cobB</i>	1698.41	1.09	3.04E-05	<i>cutF</i>	3070.69	0.35	0.20711
<i>cobC</i>	236.55	-0.17	0.51478	<i>cvpA</i>	1588.58	1.38	4.28E-08
<i>cobD</i>	441.46	-0.92	0.00065	<i>cvrA</i>	3570.31	-1.24	1.22E-06
<i>cobS</i>	2565.71	4.21	4.83E-26	<i>cyaA</i>	9871.18	-0.64	0.00619
<i>cobT</i>	1664.56	3.48	1.38E-21	<i>CyaR</i>	183.52	1.16	0.00119
<i>cobU</i>	934.19	4.19	4.04E-31	<i>cyaY</i>	659.83	-2.28	1.32E-11
<i>codA</i>	1191.62	0.52	0.03335	<i>cybB</i>	635.15	-0.50	0.03170
<i>codB</i>	795.99	-0.76	0.00149	<i>cybC</i>	5084.83	-2.37	3.20E-09
<i>cof</i>	741.87	1.96	4.49E-07	<i>cycA</i>	5085.16	-0.48	0.14858

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>cydA</i>	6166.73	0.57	0.09066	<i>deoD</i>	5206.16	0.95	0.00356
<i>cydB</i>	4814.66	0.89	0.00320	<i>deoR</i>	1389.73	0.67	0.01082
<i>cydC</i>	1625.07	0.47	0.07138	<i>dfp</i>	2294.24	-1.47	4.09E-08
<i>cydD</i>	2249.24	0.17	0.54811	<i>dgkA</i>	2663.42	-1.54	1.45E-09
<i>cyoA</i>	24827.61	-0.93	0.00239	<i>dgoA</i>	58.73	0.70	0.04836
<i>cyoB</i>	9800.65	-0.50	0.06152	<i>dgoD</i>	158.22	1.10	0.00065
<i>cyoC</i>	3413.80	-1.28	1.68E-09	<i>dgoK</i>	230.89	-0.22	0.43201
<i>cyoD</i>	4501.36	-1.47	2.06E-09	<i>dgoR</i>	536.03	-1.69	3.76E-08
<i>cyoE</i>	10264.03	-0.36	0.15477	<i>dgoT</i>	327.68	0.40	0.17673
<i>cypD</i>	6099.15	0.82	0.00063	<i>dgt</i>	2005.73	-1.77	1.89E-08
<i>cysA</i>	408.66	-0.59	0.29589	<i>dhaB</i>	421.37	5.84	5.76E-33
<i>cysB</i>	4572.79	-1.57	9.23E-07	<i>dinF</i>	3011.24	-1.46	3.94E-11
<i>cysC</i>	248.39	-0.79	0.06513	<i>dinG</i>	5382.39	-0.69	0.00650
<i>cysD</i>	1914.96	-1.16	0.20743	<i>dinI</i>	854.11	-3.26	2.53E-12
<i>cysE</i>	1950.01	-0.18	0.50863	<i>dipZ</i>	1320.27	0.07	0.76606
<i>cysG</i>	295.05	0.55	0.02452	<i>div</i>	1436.80	-0.02	0.91047
<i>cysH</i>	207.29	-0.26	0.76222	<i>djlA</i>	3007.80	-1.07	2.70E-06
<i>cysI</i>	735.68	-0.06	0.94267	<i>dkgA</i>	1357.27	-0.22	0.44212
<i>cysJ</i>	587.97	0.10	0.89644	<i>dkgB</i>	2319.41	-1.46	0.00011
<i>cysK</i>	8347.53	-1.23	0.03210	<i>dkSA</i>	14178.87	-1.01	5.19E-05
<i>cysM</i>	541.82	0.19	0.39490	<i>dld</i>	645.32	0.81	0.00237
<i>cysN</i>	1141.01	-1.87	0.00058	<i>dlhH</i>	419.18	-0.76	0.04074
<i>cysP</i>	2430.08	-1.57	0.07699	<i>dmsA</i>	1982.90	2.42	9.61E-11
<i>cysQ</i>	912.32	-1.73	7.00E-09	<i>dmsA1</i>	498.48	0.10	0.70136
<i>cysS</i>	4577.86	-0.05	0.82752	<i>dmsA2</i>	745.62	2.33	2.18E-16
<i>cysU</i>	458.98	-1.04	0.00385	<i>dmsB</i>	653.30	5.34	2.35E-56
<i>cysW</i>	612.33	0.04	0.95794	<i>dmsC</i>	730.14	3.14	6.50E-36
<i>cysZ</i>	1260.43	0.98	9.88E-05	<i>dnaA</i>	5887.59	-0.51	0.08115
<i>cytR</i>	1275.18	-2.06	3.96E-08	<i>dnaB</i>	1547.91	-0.19	0.45045
<i>dacA</i>	4756.57	0.92	0.00040	<i>dnaC</i>	1167.37	-1.42	1.20E-06
<i>dacB</i>	1071.55	-0.33	0.16809	<i>dnaE</i>	10259.06	0.67	0.00971
<i>dacC</i>	932.76	-0.56	0.05777	<i>dnaG</i>	9108.06	0.20	0.43520
<i>dacD</i>	1234.09	0.02	0.91679	<i>dnaJ</i>	4950.08	-0.53	0.14274
<i>dadA</i>	2055.63	-3.01	1.51E-12	<i>dnaK</i>	21491.30	0.00	0.99078
<i>dam</i>	4143.95	0.37	0.15438	<i>dnaN</i>	3151.84	0.42	0.10140
<i>damX</i>	9245.72	-0.81	0.00167	<i>dnaQ</i>	857.34	-0.69	0.00191
<i>dapA</i>	8381.69	-0.65	0.00572	<i>dnaT</i>	942.42	-1.95	4.30E-15
<i>dapB</i>	553.86	-0.22	0.39876	<i>dnaX</i>	4584.50	-0.51	0.03065
<i>dapD</i>	2331.59	-0.24	0.40220	<i>dniR</i>	6581.48	1.93	5.61E-14
<i>dapE</i>	1627.25	0.40	0.09191	<i>dpaL</i>	230.30	1.56	5.55E-06
<i>dapF</i>	1818.52	-1.47	1.04E-06	<i>dppA</i>	1910.98	-1.57	3.21E-05
<i>DapZ</i>	7.80	-0.13	0.82655	<i>dppB</i>	306.88	0.45	0.09400
<i>dbh</i>	994.45	-1.57	5.60E-07	<i>dppC</i>	199.88	2.51	8.03E-16
<i>dbpA</i>	1725.21	0.91	0.00017	<i>dppD</i>	347.70	1.33	4.23E-09
<i>dcd</i>	986.50	-0.51	0.03481	<i>dppF</i>	477.39	0.47	0.02982
<i>dcm</i>	850.27	0.03	0.92836	<i>dprA</i>	452.65	-0.92	0.00119
<i>dcoA</i>	157.58	3.43	4.64E-21	<i>dps</i>	80210.31	4.83	3.15E-52
<i>dcoB</i>	164.91	2.68	1.01E-16	<i>dsbA</i>	12400.69	0.84	0.00593
<i>dcoC</i>	6.74	3.25	4.06E-05	<i>dsbB</i>	1143.34	0.16	0.63159
<i>dcp</i>	842.84	-0.35	0.26773	<i>dsbC</i>	2359.56	-0.49	0.06322
<i>dctA</i>	1871.58	-1.55	1.41E-06	<i>dsbG</i>	1948.01	-0.18	0.59852
<i>dcuA</i>	2190.09	2.18	2.88E-20	<i>dsdA</i>	635.55	-0.11	0.71094
<i>dcuB</i>	3368.99	4.25	4.35E-54	<i>dsdC</i>	429.79	-1.41	1.14E-06
<i>dcuC</i>	280.86	0.81	0.01037	<i>dsdX</i>	383.76	0.54	0.06980
<i>dcuR</i>	292.21	0.47	0.13097	<i>DsrA</i>	11.54	0.68	0.27347
<i>dcuS</i>	734.84	-0.14	0.52569	<i>dsrB</i>	2629.19	-3.40	2.19E-39
<i>dcyD</i>	866.93	0.47	0.05296	<i>dut</i>	781.34	-0.34	0.14089
<i>ddlA</i>	2694.86	-0.45	0.05206	<i>dxr</i>	2848.40	0.91	3.55E-05
<i>ddlB</i>	4054.19	0.81	0.00211	<i>dxs</i>	3189.65	1.11	8.28E-06
<i>deaD</i>	83031.11	2.51	8.00E-19	<i>ecnA</i>	153.64	-1.07	0.00085
<i>dedA</i>	1426.39	1.04	1.04E-05	<i>ecnB</i>	299.14	0.86	0.00566
<i>dedD</i>	1858.58	0.69	0.00264	<i>ecnR</i>	1707.44	-1.80	2.19E-13
<i>def</i>	6215.08	1.86	3.58E-12	<i>eco</i>	3710.66	0.24	0.48235
<i>degQ</i>	2287.96	-1.03	6.98E-05	<i>eda</i>	2222.50	1.57	5.00E-08
<i>degS</i>	794.59	-1.05	4.67E-06	<i>edd</i>	5085.82	0.92	0.00108
<i>deoA</i>	1064.66	-1.04	0.00408	<i>efp</i>	8152.14	0.21	0.39951
<i>deoB</i>	12464.56	0.37	0.15571	<i>ego</i>	606.15	-1.28	0.00017
<i>deoC</i>	2931.37	-2.33	1.26E-17	<i>elaA</i>	415.40	-1.28	7.67E-06

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>elaB</i>	884.81	1.50	3.95E-09	<i>fdhE</i>	1566.53	0.81	0.00069
<i>elaC</i>	355.96	-0.54	0.02746	<i>fdnG</i>	1182.66	0.69	0.06026
<i>emrA</i>	2967.68	-0.24	0.36916	<i>fdnH</i>	324.18	1.51	2.35E-05
<i>emrB</i>	1192.27	-0.09	0.71706	<i>fdnI</i>	2509.97	1.45	0.00180
<i>emrD</i>	657.57	-0.07	0.78649	<i>fdoG</i>	33733.93	-0.38	0.17736
<i>emrR</i>	4303.17	0.11	0.72570	<i>fdoH</i>	6092.12	0.97	0.00217
<i>emtA</i>	479.27	-0.10	0.65724	<i>fdol</i>	5515.60	1.27	8.67E-05
<i>endA</i>	604.00	-0.07	0.78371	<i>fdrA</i>	121.56	2.39	1.68E-13
<i>engA</i>	8304.27	1.53	3.49E-12	<i>fdx</i>	2969.55	1.70	2.50E-11
<i>engB</i>	3669.97	-0.46	0.04093	<i>feoA</i>	254.55	-0.83	0.00381
<i>eno</i>	51795.18	0.30	0.24074	<i>feoB</i>	4171.25	1.39	1.30E-06
<i>entA</i>	1614.36	3.91	8.72E-65	<i>fepA</i>	7007.03	1.48	7.17E-06
<i>entB</i>	3731.96	3.40	1.61E-41	<i>fepB</i>	1352.23	0.51	0.15881
<i>entC</i>	5281.47	2.47	1.79E-11	<i>fepC</i>	599.79	0.97	0.00176
<i>entD</i>	401.87	0.78	0.00312	<i>fepD</i>	515.20	0.91	0.00981
<i>entE</i>	12053.16	4.12	3.06E-53	<i>fepE</i>	751.70	-0.70	0.00204
<i>entF</i>	3465.40	2.10	2.19E-18	<i>fepG</i>	293.39	1.22	1.04E-05
<i>envE</i>	4243.54	0.87	0.00984	<i>fes</i>	1080.59	0.83	0.00927
<i>envF</i>	950.96	-0.79	0.06689	<i>ffh</i>	9214.36	-0.97	1.59E-05
<i>envR</i>	170.57	0.62	0.06694	<i>fhlA</i>	1222.44	0.57	0.09415
<i>envZ</i>	2078.74	0.52	0.03254	<i>fhuA</i>	13270.01	0.98	0.00047
<i>epd</i>	3276.13	-2.02	1.58E-17	<i>fhuB</i>	4126.33	2.53	5.94E-15
<i>era</i>	4417.16	1.04	7.50E-05	<i>fhuC</i>	1215.69	1.57	4.60E-10
<i>erfK</i>	1889.08	-0.09	0.83138	<i>fhuD</i>	1311.79	1.37	2.94E-09
<i>eutA</i>	113.60	3.64	8.74E-22	<i>fhuE</i>	2841.74	3.76	3.65E-32
<i>eutB</i>	220.80	3.27	1.41E-27	<i>fhuF</i>	626.33	-0.76	0.00114
<i>eutC</i>	159.57	4.01	6.95E-34	<i>fic</i>	444.77	0.46	0.08885
<i>eutD</i>	40.03	2.27	7.41E-08	<i>fidL</i>	251.03	-0.17	0.52054
<i>eutE</i>	70.96	3.31	1.38E-18	<i>fieF</i>	5226.55	-1.35	7.34E-10
<i>eutG</i>	85.86	3.80	4.53E-26	<i>fimA</i>	2079.71	2.26	1.61E-09
<i>eutH</i>	142.59	2.45	2.97E-13	<i>fimB</i>	410.14	0.16	0.47496
<i>eutJ</i>	68.89	2.71	4.98E-16	<i>fimC</i>	337.35	3.35	1.32E-16
<i>eutK</i>	137.02	2.98	5.79E-21	<i>fimD</i>	518.40	2.41	2.26E-13
<i>eutL</i>	35.45	2.43	9.87E-09	<i>fimF</i>	34.70	1.17	0.00252
<i>eutM</i>	53.02	4.20	7.79E-20	<i>fimH</i>	346.15	1.72	6.40E-10
<i>eutN</i>	20.07	3.69	5.53E-10	<i>fimI</i>	317.54	2.38	1.75E-09
<i>eutP</i>	28.79	2.36	2.39E-08	<i>fimW</i>	98.98	0.25	0.48716
<i>eutQ</i>	43.65	3.00	1.63E-11	<i>fimY</i>	205.37	-0.62	0.02767
<i>eutR</i>	121.91	1.89	1.53E-08	<i>fimZ</i>	250.41	1.79	1.94E-08
<i>eutS</i>	26.29	2.63	8.42E-08	<i>fis</i>	6715.12	0.56	0.08523
<i>eutT</i>	40.31	2.61	2.76E-11	<i>fixA</i>	168.79	1.98	5.77E-09
<i>exbB</i>	7362.06	0.02	0.95675	<i>fixB</i>	88.02	2.76	1.39E-15
<i>exbD</i>	7731.30	1.78	4.60E-09	<i>fixC</i>	110.35	2.29	6.74E-12
<i>exo</i>	407.40	-0.86	0.00015	<i>fixX</i>	21.97	3.19	5.68E-10
<i>exoX</i>	551.67	-0.98	0.00100	<i>fkIB</i>	2636.92	0.45	0.07840
<i>exuT</i>	3725.05	-3.23	2.49E-29	<i>fkpA</i>	28090.17	-1.17	1.15E-05
<i>fabA</i>	7833.22	2.17	8.94E-13	<i>fkpB</i>	1391.26	0.83	0.00030
<i>fabB</i>	10111.69	1.55	3.63E-07	<i>fldA</i>	5017.55	-0.15	0.70266
<i>fabD</i>	6674.24	1.25	2.23E-08	<i>fldB</i>	1427.90	-1.36	9.22E-09
<i>fabF</i>	39289.37	1.60	1.12E-08	<i>flgA</i>	782.64	1.24	2.73E-05
<i>fabG</i>	17494.56	0.05	0.88083	<i>flgB</i>	2801.83	-0.19	0.50049
<i>fabH</i>	5329.91	1.14	1.57E-05	<i>flgC</i>	579.00	0.58	0.02642
<i>fabI</i>	5417.42	0.75	0.00163	<i>flgD</i>	6754.74	-0.13	0.64215
<i>fabZ</i>	3582.90	1.27	8.50E-08	<i>flgE</i>	11091.72	0.83	0.02051
<i>fadA</i>	5583.51	-3.87	3.40E-05	<i>flgF</i>	5899.34	0.56	0.07914
<i>fadB</i>	19703.71	-6.08	3.27E-29	<i>flgG</i>	7085.78	0.77	0.05292
<i>fadD</i>	3989.97	0.26	0.30791	<i>flgH</i>	1958.47	1.23	0.00019
<i>fadE</i>	18045.17	-4.72	2.44E-09	<i>flgI</i>	3288.19	0.83	0.01785
<i>fadH</i>	2003.56	-3.48	5.26E-12	<i>flgJ</i>	2889.97	0.16	0.63085
<i>fadI</i>	2026.64	-3.61	2.04E-12	<i>flgK</i>	17197.90	-1.13	0.00030
<i>fadJ</i>	2050.20	-2.73	1.18E-07	<i>flgL</i>	8361.45	-0.68	0.01409
<i>fadL</i>	3211.91	-1.87	6.99E-09	<i>flgM</i>	2624.19	-2.66	2.15E-11
<i>fadR</i>	2664.84	-0.77	0.00066	<i>flgN</i>	1900.68	-1.39	0.00032
<i>fba</i>	20305.26	0.82	0.00928	<i>flhAa</i>	4635.50	1.24	0.00021
<i>fbaB</i>	620.20	1.54	1.39E-07	<i>flhAb</i>	92.65	2.28	8.82E-10
<i>fbp</i>	7203.53	-2.15	8.72E-11	<i>flhB</i>	1622.88	1.01	0.00154
<i>fcl</i>	128.29	2.42	1.62E-16	<i>flhC</i>	4316.09	-1.52	2.56E-07
<i>fdhD</i>	831.24	-1.34	1.98E-07	<i>flhD</i>	3759.10	-0.26	0.44693

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>flhE</i>	225.69	0.53	0.11263	<i>ftsY</i>	8655.67	-1.31	2.84E-07
<i>fliA</i>	6642.80	-0.42	0.12903	<i>ftsZ</i>	4413.05	1.06	9.69E-05
<i>fliB</i>	2893.56	0.56	0.06790	<i>fucA</i>	65.64	1.53	1.10E-05
<i>fliC</i>	144901.51	1.14	0.01631	<i>fucl</i>	309.79	3.14	7.75E-30
<i>fliD</i>	13021.77	-0.83	0.00236	<i>fucK</i>	325.87	2.09	1.02E-14
<i>fliE</i>	158.02	-0.76	0.01638	<i>fucO</i>	250.51	1.33	2.53E-06
<i>fliF</i>	3503.61	-0.63	0.03559	<i>fucP</i>	183.80	0.29	0.32474
<i>fliG</i>	1866.40	-0.66	0.04342	<i>fucR</i>	574.25	-0.79	0.00064
<i>fliH</i>	3247.37	-0.37	0.28063	<i>fucU</i>	116.10	1.22	5.70E-05
<i>fliI</i>	1464.51	-0.62	0.07216	<i>fumA</i>	7810.69	-1.32	7.71E-05
<i>fliJ</i>	1257.66	-1.22	0.00018	<i>fumB</i>	7643.28	3.83	5.94E-52
<i>fliK</i>	3582.39	0.37	0.35307	<i>fumC</i>	1765.41	-0.84	0.04622
<i>fliL</i>	398.89	0.05	0.87304	<i>fur</i>	14356.47	1.28	9.47E-05
<i>fliM</i>	3812.53	0.25	0.39429	<i>fusA</i>	131952.08	-0.40	0.07570
<i>fliN</i>	321.23	0.67	0.01959	<i>fxsA</i>	798.05	-0.73	0.01361
<i>fliO</i>	525.45	0.10	0.75579	<i>gabD</i>	160.69	1.44	2.29E-07
<i>fliP</i>	615.41	0.32	0.35701	<i>gabP</i>	193.82	1.42	4.85E-05
<i>fliQ</i>	143.88	0.40	0.24081	<i>gabT</i>	190.62	0.95	0.00059
<i>fliR</i>	578.06	-1.05	0.00213	<i>galE</i>	2126.61	-2.19	8.36E-18
<i>fliS</i>	1383.15	-0.26	0.27811	<i>galF</i>	4514.39	0.34	0.19722
<i>fliT</i>	963.10	-0.29	0.25348	<i>galK</i>	1015.80	-0.08	0.73841
<i>fliY</i>	6557.12	-1.54	8.48E-09	<i>galM</i>	1120.99	-0.25	0.32607
<i>fliZ</i>	6352.18	0.62	0.02355	<i>galP</i>	1728.22	-1.20	9.34E-05
<i>fliJ_A</i>	2739.23	-1.59	9.94E-05	<i>galR</i>	815.18	-1.93	1.29E-12
<i>fliJ_B</i>	330159.92	0.03	0.94156	<i>galS</i>	919.82	1.06	8.73E-06
<i>fmt</i>	10489.44	2.63	1.40E-28	<i>galT</i>	889.86	-1.42	4.86E-07
<i>frnA</i>	7045.89	2.95	2.42E-29	<i>galU</i>	8521.41	0.99	0.00089
<i>fnr</i>	15708.06	-1.03	5.51E-06	<i>gapA</i>	17437.51	0.63	0.01124
<i>focA</i>	1653.46	0.00	0.98791	<i>garD</i>	823.96	-1.24	0.00671
<i>folA</i>	2170.73	0.78	0.01259	<i>garK</i>	685.01	0.25	0.61827
<i>folB</i>	956.68	-1.29	5.32E-06	<i>garL</i>	1693.33	-2.52	8.18E-13
<i>folC</i>	3289.37	0.25	0.29710	<i>garRa</i>	80.90	1.73	1.53E-06
<i>folD</i>	1324.33	-0.46	0.04377	<i>garRb</i>	3257.36	1.01	0.06753
<i>folE</i>	1740.82	1.16	0.00011	<i>gatA</i>	35.39	0.74	0.03463
<i>folK</i>	464.71	-0.45	0.12947	<i>gatB</i>	33.30	1.25	0.00381
<i>folP</i>	2955.36	0.79	0.00026	<i>gatC</i>	227.21	1.96	1.16E-11
<i>foxA</i>	801.58	0.37	0.16289	<i>gatD</i>	227.23	0.57	0.05589
<i>fpr</i>	2679.14	-3.06	7.35E-32	<i>gatR</i>	2233.42	-3.15	1.23E-41
<i>frdA</i>	5352.27	0.73	0.03183	<i>gatY</i>	177.54	1.38	3.35E-06
<i>frdB</i>	3121.57	3.18	2.00E-14	<i>gcd</i>	1676.42	1.42	4.46E-08
<i>frdC</i>	953.91	2.38	8.57E-09	<i>gcl</i>	319.79	1.82	6.48E-10
<i>frdD</i>	886.30	2.40	1.94E-19	<i>gcp</i>	1437.65	-0.32	0.12879
<i>fre</i>	4635.92	-0.87	0.00016	<i>gcpA</i>	663.21	0.99	6.65E-05
<i>frr</i>	7712.05	-0.24	0.36387	<i>gcpE</i>	2205.23	0.21	0.41839
<i>frsA</i>	1451.93	-0.65	0.00286	<i>gcvA</i>	1453.70	-1.13	9.54E-07
<i>fruA</i>	1470.47	-0.12	0.67013	<i>GcvB</i>	13253.49	1.24	0.00273
<i>fruB</i>	966.94	-1.88	2.60E-15	<i>gcvH</i>	952.14	0.96	0.05902
<i>fruK</i>	300.34	-0.81	0.00236	<i>gcvP</i>	2764.44	1.82	3.33E-06
<i>fruR</i>	1472.15	-0.22	0.47856	<i>gcvR</i>	674.58	-1.67	1.91E-09
<i>frwB</i>	184.18	0.05	0.86675	<i>gcvT</i>	991.45	-0.20	0.65092
<i>frwC</i>	234.05	1.04	0.00079	<i>gdhA</i>	1413.18	0.74	0.01055
<i>frwD</i>	203.12	-0.84	0.00804	<i>ggt</i>	583.82	1.36	8.31E-07
<i>fsr</i>	697.48	-0.10	0.66628	<i>gidA</i>	4702.56	-0.05	0.84273
<i>ftn</i>	2729.19	-0.60	0.10404	<i>gidB</i>	1526.16	-0.96	2.31E-05
<i>ftnB</i>	276.52	0.62	0.01197	<i>gip</i>	109.46	1.51	4.43E-07
<i>ftsA</i>	12029.88	-0.06	0.79786	<i>gipA</i>	902.44	-0.55	0.03318
<i>ftsB</i>	1559.46	-0.61	0.05753	<i>gldA</i>	1344.32	1.27	2.95E-07
<i>ftsE</i>	1863.88	0.26	0.32954	<i>glgA</i>	1227.65	1.63	4.01E-08
<i>ftsH</i>	48670.66	2.38	1.10E-23	<i>glgB</i>	1433.60	0.11	0.64509
<i>ftsI</i>	4630.38	-0.05	0.84595	<i>glgC</i>	1281.63	0.45	0.16951
<i>ftsI2</i>	1142.36	-0.07	0.75808	<i>glgP</i>	3561.74	0.99	0.00070
<i>ftsJ</i>	13646.10	0.20	0.45756	<i>glgS</i>	15082.04	-6.37	2.39E-107
<i>ftsK</i>	14816.64	0.96	3.47E-05	<i>glgX</i>	1315.97	0.15	0.61054
<i>ftsL</i>	953.30	-0.69	0.00358	<i>glk</i>	1241.34	-2.33	7.07E-19
<i>ftsN</i>	2039.96	-0.95	8.71E-05	<i>glmM</i>	4275.26	1.15	1.91E-06
<i>ftsQ</i>	3497.52	-0.37	0.10475	<i>glmS</i>	7185.48	-0.85	0.00120
<i>ftsW</i>	3330.21	1.05	0.00017	<i>glmU</i>	4545.76	-1.85	2.17E-11
<i>ftsX</i>	1363.54	0.18	0.41563	<i>GlmY</i>	658.77	1.37	0.00034

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>GlmZ</i>	2221.44	-1.62	2.41E-05	<i>greB</i>	1371.38	-1.82	5.08E-17
<i>glnA</i>	13319.08	2.43	2.22E-20	<i>groEL</i>	35594.92	-0.76	0.02073
<i>glnB</i>	1089.28	-0.09	0.75230	<i>groES</i>	20634.38	-2.09	1.12E-07
<i>glnD</i>	1722.02	-0.36	0.09600	<i>grpE</i>	11889.37	-0.49	0.20713
<i>glnE</i>	1919.44	-0.07	0.72623	<i>grxA</i>	16710.17	4.04	5.24E-27
<i>glnG</i>	1020.84	1.01	3.71E-05	<i>grxB</i>	2738.21	-2.36	2.33E-17
<i>glnH</i>	12135.82	-1.57	3.43E-07	<i>grxC</i>	2138.60	-1.39	3.14E-06
<i>glnK</i>	145.30	0.38	0.23386	<i>gshA</i>	9296.98	-0.65	0.00319
<i>glnL</i>	936.58	0.97	9.62E-05	<i>gshB</i>	1034.74	-0.70	0.01371
<i>glnP</i>	1156.62	-1.50	5.60E-07	<i>gsk</i>	2997.25	0.76	0.00070
<i>glnQ</i>	4220.81	0.03	0.89840	<i>gsp</i>	2081.93	-1.40	8.94E-09
<i>glnS</i>	6033.04	1.10	1.16E-06	<i>gst</i>	3612.88	-1.77	2.00E-12
<i>gloA</i>	1341.70	0.06	0.78797	<i>gtgA</i>	525.97	-0.97	0.00207
<i>gloB</i>	967.33	-1.03	0.00256	<i>gtgE</i>	414.93	-1.88	9.57E-16
<i>glpA</i>	4534.60	-3.89	1.45E-37	<i>gtgF</i>	108.33	0.60	0.15301
<i>glpB</i>	937.84	-0.73	0.17129	<i>gtrAa</i>	82.34	0.00	0.99272
<i>glpC</i>	987.18	0.56	0.28883	<i>gtrAb</i>	63.33	-0.43	0.24511
<i>glpD</i>	48021.09	-0.55	0.06569	<i>gtrBa</i>	219.20	0.84	0.00995
<i>glpE</i>	1104.70	0.45	0.11491	<i>gtrBb</i>	692.56	-0.45	0.12587
<i>glpF</i>	8182.75	-4.06	8.91E-31	<i>guaA</i>	5118.70	1.11	2.52E-05
<i>glpG</i>	5545.35	1.52	4.76E-10	<i>guaB</i>	4627.34	0.72	0.00982
<i>glpK</i>	14440.62	-1.36	0.09025	<i>guaC</i>	3648.30	-1.41	3.17E-08
<i>glpQ</i>	18113.37	1.17	0.02409	<i>gudD</i>	609.89	0.78	0.03723
<i>glpT</i>	18085.30	0.68	0.13818	<i>gudT</i>	805.95	-1.17	0.00224
<i>glpX</i>	988.96	-0.66	0.02684	<i>gutD</i>	1337.33	3.05	2.01E-29
<i>gltA</i>	11947.27	-1.42	0.00026	<i>gutM</i>	196.37	0.52	0.06560
<i>gltB</i>	1986.75	-0.54	0.02959	<i>gutQ</i>	281.37	-0.51	0.04096
<i>gltD</i>	761.24	-0.22	0.34362	<i>gyrA</i>	10371.46	0.67	0.00349
<i>gltI</i>	16576.44	-3.42	5.07E-13	<i>gyrB</i>	14427.95	0.13	0.61000
<i>gltJ</i>	524.10	-1.16	0.00285	<i>gyrI</i>	2257.95	-2.84	1.21E-20
<i>gltK</i>	432.00	-0.76	0.01585	<i>hcaT</i>	946.71	0.37	0.09643
<i>gltL</i>	765.48	-0.18	0.71712	<i>hcp</i>	554.12	-0.47	0.15576
<i>gltP</i>	3006.02	-0.36	0.24096	<i>hcr</i>	326.97	-0.16	0.54616
<i>gltS</i>	1429.64	0.06	0.81387	<i>hdeB</i>	712.68	0.43	0.19334
<i>gltX</i>	5049.92	0.75	0.00059	<i>helD</i>	1489.35	-0.66	0.00684
<i>glxK</i>	220.26	1.75	4.40E-10	<i>hemA</i>	2255.61	0.52	0.03459
<i>glyA</i>	6539.98	-0.80	0.00111	<i>hemB</i>	3186.53	-1.10	1.12E-06
<i>glyQ</i>	891.55	-0.74	0.00383	<i>hemC</i>	3088.47	-1.35	6.94E-08
<i>glyS</i>	6815.04	0.64	0.01319	<i>hemD</i>	1478.60	-0.54	0.04189
<i>gmd</i>	233.14	1.74	4.26E-09	<i>hemE</i>	2414.89	-1.55	9.99E-10
<i>gmhA</i>	2463.26	-1.22	8.20E-06	<i>hemF</i>	458.72	-0.09	0.71764
<i>gmk</i>	3616.91	-1.41	1.10E-07	<i>hemG</i>	1079.75	-0.70	0.00792
<i>gmm</i>	37.17	4.07	8.82E-16	<i>hemH</i>	4227.67	3.31	1.13E-27
<i>gnd</i>	12009.58	0.66	0.02550	<i>hemK</i>	966.44	2.51	3.81E-19
<i>gntK</i>	181.12	-0.22	0.40306	<i>hemL</i>	2065.36	0.64	0.00612
<i>gntR</i>	1523.52	-0.78	0.00088	<i>hemM</i>	941.83	1.38	3.54E-08
<i>gntT</i>	585.88	-0.78	0.00241	<i>hemN</i>	1907.30	-0.10	0.65732
<i>gntU</i>	649.34	-0.26	0.25183	<i>hemX</i>	2118.91	-0.22	0.45409
<i>gogB</i>	1672.44	-1.43	1.19E-09	<i>hemY</i>	2656.70	0.27	0.32707
<i>gor</i>	8859.06	1.36	2.09E-06	<i>hepA</i>	3860.95	-0.55	0.02877
<i>gpB</i>	1073.46	-0.49	0.15254	<i>hexR</i>	1088.66	-2.63	4.60E-19
<i>gpD</i>	346.38	0.01	0.98201	<i>hflC</i>	12580.47	1.84	1.46E-12
<i>gpE</i>	25.85	2.32	7.93E-07	<i>hflK</i>	14721.19	0.85	8.48E-05
<i>gpE'</i>	1.73	-0.19	0.84969	<i>hflX</i>	14782.31	0.93	0.00037
<i>gpF</i>	53.13	-0.01	0.98488	<i>hfq</i>	21616.85	-1.54	2.99E-05
<i>gph</i>	1963.41	0.00	0.98835	<i>hha</i>	3579.42	-2.03	4.42E-08
<i>gpl</i>	94.64	-0.15	0.68254	<i>hilA</i>	5157.10	2.29	1.37E-07
<i>gpJ</i>	123.11	2.01	1.46E-09	<i>hilC</i>	13970.70	1.19	0.00572
<i>gpmA</i>	36257.94	0.01	0.96521	<i>hilD</i>	19898.33	-0.05	0.90898
<i>gpmB</i>	1506.58	0.26	0.22647	<i>himA</i>	11134.44	-0.21	0.47380
<i>gpO</i>	366.06	0.06	0.81240	<i>himD</i>	2654.91	0.58	0.05458
<i>gpP</i>	115.94	1.00	0.00275	<i>hin</i>	360.45	-0.49	0.09302
<i>gppA</i>	4579.14	0.29	0.22853	<i>hisA</i>	207.05	0.36	0.18874
<i>gpsA</i>	3962.41	0.06	0.80295	<i>hisB</i>	703.72	-0.01	0.95543
<i>gpt</i>	1234.47	1.08	0.00672	<i>hisC</i>	427.22	0.26	0.29204
<i>gpU</i>	36.92	1.09	0.00436	<i>hisD</i>	643.32	0.04	0.85191
<i>gpW</i>	7.91	2.03	0.00375	<i>hisF</i>	1093.62	-0.09	0.66812
<i>greA</i>	13124.45	-0.67	0.07590	<i>hisG</i>	972.02	0.05	0.81792

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>hisH</i>	203.22	0.10	0.74206	<i>hyaF2</i>	369.78	1.17	2.12E-07
<i>hisI</i>	741.23	1.08	4.99E-06	<i>hybA</i>	1121.04	2.61	1.19E-28
<i>hisJ</i>	2605.70	-0.09	0.77921	<i>hybB</i>	880.64	2.38	4.61E-19
<i>hisM</i>	255.40	0.89	0.00163	<i>hybC</i>	5759.86	2.62	9.61E-29
<i>hisP</i>	929.44	0.46	0.07828	<i>hybD</i>	2204.07	3.46	3.08E-36
<i>hisQ</i>	580.79	0.72	0.02509	<i>hybE</i>	375.03	2.86	4.61E-22
<i>hisS</i>	8343.78	0.97	3.81E-05	<i>hybF</i>	313.84	2.42	7.37E-15
<i>hlpA</i>	9292.83	-0.33	0.29799	<i>hybG</i>	2868.40	3.64	1.95E-19
<i>hmpA</i>	1205.68	-2.17	2.91E-05	<i>hycA</i>	47.86	1.25	0.00111
<i>hnr</i>	1358.02	-0.74	0.00178	<i>hycB</i>	38.05	2.89	4.09E-11
<i>hns</i>	172011.97	0.99	0.02013	<i>hycC</i>	142.19	2.90	5.13E-20
<i>holA</i>	1364.70	0.13	0.55607	<i>hycD</i>	122.59	2.27	1.81E-10
<i>holB</i>	932.73	1.15	6.04E-06	<i>hycE</i>	196.79	1.79	7.92E-09
<i>holC</i>	445.46	-1.04	0.00053	<i>hycF</i>	92.26	0.94	0.00366
<i>holD</i>	308.18	0.53	0.07490	<i>hycG</i>	75.13	1.59	1.12E-07
<i>holE</i>	392.59	-1.62	6.72E-09	<i>hycH</i>	47.21	0.81	0.01617
<i>hopD</i>	543.00	-1.70	3.48E-12	<i>hycl</i>	103.61	0.31	0.29708
<i>hpaA</i>	95.51	1.58	4.06E-06	<i>hydA</i>	502.40	0.15	0.59553
<i>hpaB</i>	328.75	-0.38	0.22744	<i>hydG</i>	1400.50	-0.92	9.08E-06
<i>hpaC</i>	223.18	-0.40	0.20082	<i>hydH</i>	1639.57	-1.96	9.73E-17
<i>hpaD</i>	86.20	0.61	0.05183	<i>hydN</i>	46.81	0.81	0.02536
<i>hpaE</i>	153.59	0.60	0.07767	<i>hyi</i>	56.29	1.08	0.01115
<i>hpaF</i>	63.51	1.55	4.15E-05	<i>hypA</i>	94.64	0.74	0.01108
<i>hpaG</i>	224.92	-0.43	0.27204	<i>hypB</i>	545.05	1.39	6.77E-07
<i>hpaH</i>	59.61	1.91	5.29E-08	<i>hypC</i>	468.92	1.67	4.71E-10
<i>hpaI</i>	66.14	1.95	3.38E-09	<i>hypD</i>	451.79	2.46	1.60E-21
<i>hpaR</i>	790.74	-2.34	1.76E-13	<i>hypE</i>	1242.61	2.69	1.06E-26
<i>hpaX</i>	201.60	0.64	0.03256	<i>hypO</i>	1060.03	0.69	0.00831
<i>hpt</i>	1984.85	0.38	0.20776	<i>iacP</i>	4058.44	-2.02	9.59E-06
<i>hrpA</i>	6435.15	-0.09	0.68844	<i>iadA</i>	243.72	0.33	0.17853
<i>hrpB</i>	1431.14	-0.11	0.63363	<i>iagB</i>	1304.48	2.09	1.18E-06
<i>hscA</i>	6735.76	2.24	2.74E-20	<i>iap</i>	3931.63	1.51	1.99E-08
<i>hscB</i>	9050.21	2.18	6.38E-15	<i>icdA</i>	22266.41	-0.21	0.50411
<i>hscC</i>	242.09	0.30	0.26369	<i>iciA</i>	2475.41	1.38	1.58E-05
<i>hsdM</i>	2686.24	-0.37	0.15567	<i>iclR</i>	771.39	-0.67	0.01354
<i>hsdR</i>	1596.46	-0.65	0.00725	<i>idi</i>	2083.24	-1.64	9.46E-13
<i>hsdS</i>	2447.55	-1.32	1.27E-05	<i>idnD</i>	213.29	2.23	7.67E-11
<i>hslJ</i>	5822.82	1.93	2.03E-10	<i>idnK</i>	171.97	-1.36	0.00033
<i>hslO</i>	3141.38	1.62	3.89E-12	<i>idnO</i>	63.89	1.22	0.00053
<i>hslR</i>	717.95	0.79	0.00115	<i>idnR</i>	820.94	-1.11	8.97E-07
<i>hslS</i>	2903.07	-2.26	1.15E-07	<i>idnT</i>	270.20	-0.48	0.09866
<i>hslT</i>	3368.43	-2.23	2.71E-09	<i>igaA</i>	5277.05	0.42	0.12110
<i>hslU</i>	6042.21	-0.06	0.82062	<i>ileS</i>	12964.65	0.50	0.03496
<i>hslV</i>	925.07	-0.34	0.26929	<i>ilvA</i>	912.34	0.14	0.56557
<i>htgA</i>	144.31	-0.57	0.05285	<i>ilvB</i>	988.35	-1.54	1.51E-05
<i>htpG</i>	7395.28	-0.43	0.24646	<i>ilvC</i>	2126.66	-1.82	3.44E-07
<i>htpX</i>	6713.60	-0.36	0.14184	<i>ilvD</i>	1328.67	-0.22	0.39218
<i>htrA</i>	5999.40	1.48	0.00031	<i>ilvE</i>	1713.89	0.60	0.01091
<i>htrB</i>	1206.94	0.29	0.25890	<i>ilvG</i>	947.23	-1.84	7.39E-09
<i>hupA</i>	49526.53	-2.81	1.40E-15	<i>ilvH</i>	468.97	-1.18	5.19E-05
<i>hupB</i>	12642.63	-0.01	0.98492	<i>ilvI</i>	714.88	-0.46	0.09070
<i>hutC</i>	599.24	-1.91	1.81E-16	<i>ilvM</i>	96.27	-2.18	1.02E-08
<i>hutG</i>	640.81	-1.40	0.00054	<i>ilvN</i>	57.72	-1.81	3.18E-06
<i>hutH</i>	975.93	-2.03	0.00025	<i>ilvX</i>	64.38	-1.93	4.81E-08
<i>hutI</i>	2562.63	-3.55	1.38E-24	<i>ilvY</i>	213.78	-0.53	0.06569
<i>hutU</i>	2416.31	-3.54	7.44E-11	<i>imp</i>	10495.74	0.02	0.93378
<i>hxlA</i>	106.52	1.51	3.06E-05	<i>infA</i>	186.64	0.53	0.24413
<i>hxlB</i>	30.44	3.88	2.51E-13	<i>infB</i>	60894.98	0.69	0.03096
<i>hyaA</i>	111.56	2.23	1.17E-09	<i>infC</i>	372549.61	-0.57	0.07172
<i>hyaA2</i>	481.67	2.36	3.73E-14	<i>int</i>	3010.88	-1.07	0.00073
<i>hyaB2</i>	837.85	2.61	1.78E-17	<i>intA</i>	718.15	-0.15	0.61506
<i>hyaC</i>	119.04	1.24	0.00016	<i>invA</i>	11346.33	2.05	1.33E-05
<i>hyaC2</i>	300.25	1.02	0.00290	<i>invB</i>	3481.15	0.80	0.11559
<i>hyaD</i>	41.52	2.21	3.81E-09	<i>invC</i>	4128.84	2.15	1.58E-05
<i>hyaD2</i>	348.03	1.99	4.62E-11	<i>invE</i>	3226.44	1.47	0.00053
<i>hyaE</i>	25.48	3.01	6.66E-10	<i>invF</i>	11163.35	1.45	0.00314
<i>hyaE2</i>	159.48	0.88	0.00051	<i>invG</i>	9791.64	1.90	3.04E-05
<i>hyaF</i>	91.05	2.21	7.98E-12	<i>invH</i>	7922.77	1.85	9.31E-07

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>invI</i>	1706.88	-0.28	0.51558	<i>kil</i>	64.45	0.09	0.80783
<i>invJ</i>	3134.39	1.59	0.00046	<i>ksgA</i>	2021.22	0.06	0.81103
<i>InvR</i>	91.27	0.04	0.92156	<i>kup</i>	2166.00	-0.23	0.28216
<i>iolA</i>	371.06	0.58	0.10934	<i>lamB</i>	1505.36	3.15	2.70E-27
<i>iolB</i>	259.73	0.86	0.00091	<i>lasT</i>	785.36	-1.50	3.53E-08
<i>iolC</i>	331.93	1.46	2.61E-07	<i>lctD</i>	453.85	-0.93	0.10015
<i>iolD</i>	323.72	1.10	0.00127	<i>lctP</i>	962.76	-1.82	0.00077
<i>iolE</i>	77.04	2.31	2.39E-07	<i>lctR</i>	379.81	-1.83	0.02355
<i>iolG1</i>	188.10	0.10	0.72064	<i>ldcA</i>	308.08	0.08	0.75088
<i>iolG2</i>	256.42	1.04	0.00042	<i>ldcC</i>	2232.55	-0.69	0.00547
<i>iolH</i>	326.92	0.65	0.01064	<i>ldhA</i>	1890.97	-0.44	0.07560
<i>iolI1</i>	210.45	1.14	0.00073	<i>lepA</i>	11085.24	0.64	0.00497
<i>iolI2</i>	174.03	1.08	0.00170	<i>lepB</i>	4786.80	0.65	0.01629
<i>iolR</i>	3590.78	-0.22	0.37301	<i>leuA</i>	1440.29	-1.82	2.58E-10
<i>iolT1</i>	832.24	-0.27	0.28734	<i>leuB</i>	493.19	-0.59	0.06221
<i>iolT2</i>	562.47	0.89	0.00293	<i>leuC</i>	654.73	-0.03	0.92170
<i>ipk</i>	2728.96	0.94	0.00040	<i>leuC2</i>	241.75	0.60	0.05903
<i>iraD</i>	66.27	0.19	0.57799	<i>leuD</i>	392.29	-0.24	0.44307
<i>iroB</i>	1278.40	2.71	8.46E-26	<i>leuD2</i>	465.50	-2.15	9.81E-09
<i>iroC</i>	4430.91	4.07	1.21E-79	<i>leuL</i>	64.63	-1.45	0.00012
<i>iroD</i>	837.81	4.06	7.84E-55	<i>leuO</i>	137.45	0.72	0.03213
<i>iroE</i>	633.31	3.92	1.59E-31	<i>leuS</i>	9473.18	0.76	0.00063
<i>iroN</i>	1352.38	1.69	5.97E-10	<i>lexA</i>	9027.29	-1.51	9.84E-12
<i>iscA</i>	15836.17	1.51	2.44E-09	<i>lgt</i>	1444.56	0.55	0.01696
<i>iscS</i>	41226.12	1.46	3.85E-05	<i>lig</i>	2689.95	-0.34	0.11370
<i>ispA</i>	1088.70	-0.13	0.60636	<i>ligB</i>	417.77	0.40	0.12044
<i>ispB</i>	1620.44	-0.64	0.00790	<i>ligT</i>	70.00	-0.23	0.45264
<i>ispD</i>	1085.02	0.63	0.00872	<i>lipA</i>	3815.80	1.03	6.05E-06
<i>ispF</i>	804.05	-0.34	0.15076	<i>lipB</i>	821.54	0.18	0.46948
<i>ispH</i>	3344.76	0.74	0.00218	<i>livF</i>	201.88	0.19	0.50209
<i>ispZ</i>	1989.93	0.94	9.33E-05	<i>livG</i>	184.52	-0.11	0.70933
<i>IsrA</i>	64.67	3.57	2.41E-18	<i>livH</i>	174.30	0.73	0.04810
<i>IsrC</i>	201.40	0.18	0.61788	<i>livJ</i>	1572.38	-1.35	1.74E-08
<i>IsrD</i>	3.65	1.61	0.05065	<i>livK</i>	586.88	-1.39	0.00030
<i>IsrF</i>	265.24	1.03	0.00022	<i>livM</i>	275.01	1.12	0.00021
<i>IsrG</i>	11.49	4.00	7.06E-08	<i>Int</i>	2927.33	0.87	0.00022
<i>IsrH_1</i>	18.53	0.30	0.55169	<i>lolA</i>	4251.44	0.37	0.16865
<i>IsrI</i>	489.19	-2.31	6.31E-12	<i>lolD</i>	643.79	1.20	4.29E-07
<i>IsrJ</i>	49.13	-1.08	0.01724	<i>lon</i>	9148.86	-0.22	0.30352
<i>IsrK</i>	0.95	-0.44	0.69329	<i>lonH</i>	3425.06	1.33	3.90E-08
<i>IsrM</i>	209.86	0.05	0.89765	<i>lpdA</i>	34775.13	0.37	0.16389
<i>IsrN</i>	3.60	1.34	0.10528	<i>lpfA</i>	119.15	-0.25	0.42708
<i>IsrO</i>	85.38	-0.67	0.07672	<i>lpfB</i>	87.98	-0.67	0.05287
<i>IsrP</i>	58.91	-0.25	0.48325	<i>lpfC</i>	1181.02	-0.90	0.00176
<i>IsrQ</i>	5.06	0.37	0.60654	<i>lpfE</i>	143.57	-0.58	0.06738
<i>IstR-1,2</i>	311.77	-2.49	3.16E-11	<i>lplA</i>	809.52	-0.45	0.11493
<i>ivbL</i>	261.92	-2.15	9.88E-15	<i>lpp</i>	107311.12	0.58	0.10123
<i>katE</i>	1552.06	2.99	3.39E-20	<i>lppB</i>	1213.28	-0.82	0.01753
<i>katG</i>	382250.78	7.29	1.40E-75	<i>lpxA</i>	3568.55	1.81	4.28E-14
<i>katN</i>	1287.34	2.78	1.39E-11	<i>lpxB</i>	3710.09	0.85	0.00100
<i>kbl</i>	3420.90	-0.95	0.00791	<i>lpxC</i>	19919.05	0.59	0.02433
<i>kdgK</i>	451.99	-0.71	0.02007	<i>lpxD</i>	11806.66	0.69	0.00551
<i>kdgR</i>	2856.12	-1.60	1.43E-07	<i>lpxH</i>	287.18	-0.70	0.01478
<i>kdgT</i>	247.33	0.82	0.00917	<i>lpxK</i>	1823.74	0.95	0.00053
<i>kdpA</i>	186.28	2.28	5.80E-12	<i>lpxO</i>	1421.45	-2.72	1.06E-11
<i>kdpB</i>	193.78	2.11	1.93E-09	<i>lpxP</i>	7079.83	1.63	5.33E-08
<i>kdpC</i>	47.13	0.63	0.08940	<i>lrhA</i>	1505.31	-0.65	0.00580
<i>kdpD</i>	320.82	0.52	0.05547	<i>lrp</i>	11967.43	-0.45	0.10341
<i>kdpE</i>	133.45	-0.70	0.01770	<i>lspA</i>	840.49	0.19	0.43203
<i>kdpF</i>	8.03	1.14	0.07456	<i>ltaA</i>	446.44	-0.58	0.04080
<i>kdsA</i>	9847.43	2.88	2.17E-24	<i>luxS</i>	1576.98	-0.43	0.19228
<i>kdsB</i>	842.44	0.51	0.04319	<i>lysA</i>	1027.42	-2.03	2.76E-07
<i>kdtA</i>	1422.27	-0.67	0.01117	<i>lysC</i>	1002.73	-0.98	0.00497
<i>kduD</i>	173.26	0.91	0.00458	<i>lysP</i>	3411.15	0.81	0.00055
<i>kduI</i>	95.47	0.52	0.18002	<i>lysR</i>	239.49	0.28	0.28361
<i>kefB</i>	3171.20	-1.78	1.77E-09	<i>lysS</i>	6895.27	0.61	0.00815
<i>kefC</i>	843.66	2.08	4.24E-15	<i>lyxK</i>	116.38	2.57	2.24E-13
<i>kgtP</i>	1074.04	-0.68	0.02960	<i>maa</i>	3719.53	-0.69	0.04145

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>maeB</i>	6182.15	1.57	8.05E-06	<i>miaE</i>	398.51	-0.52	0.03291
<i>maf-a</i>	407.67	-0.46	0.06090	<i>MicA</i>	231.26	-2.04	2.17E-06
<i>maf-b</i>	1480.86	-0.59	0.00888	<i>MicC</i>	25.84	-1.46	0.00140
<i>maIE</i>	400.12	2.00	7.46E-16	<i>MicF</i>	23.15	-0.58	0.19848
<i>maIF</i>	351.95	-0.09	0.74141	<i>mig-14</i>	4881.56	-2.01	3.60E-15
<i>maIG</i>	538.06	-0.81	0.00165	<i>mig-3</i>	150.39	0.30	0.44503
<i>maIK</i>	375.74	4.17	3.45E-45	<i>minC</i>	2152.42	-0.18	0.49775
<i>maIM</i>	693.23	2.12	4.80E-15	<i>minD</i>	2566.16	-0.27	0.35198
<i>maIP</i>	1485.30	0.20	0.52008	<i>minE</i>	1647.30	-0.11	0.75406
<i>maIQ</i>	737.48	0.95	0.00054	<i>mioC</i>	9533.64	-2.01	1.94E-11
<i>maIS</i>	371.76	0.90	0.00328	<i>mipA</i>	12563.70	1.16	3.48E-06
<i>maIT</i>	3557.69	-2.63	5.13E-13	<i>misL</i>	990.87	-0.54	0.05451
<i>maIZ</i>	585.58	0.24	0.35098	<i>mlc</i>	744.20	-0.45	0.07652
<i>manA</i>	1316.51	1.19	0.00012	<i>mliC</i>	2499.89	0.67	0.09253
<i>manX</i>	43.11	1.27	0.00084	<i>mltA</i>	2666.63	-0.16	0.52753
<i>manY</i>	62.18	0.71	0.04153	<i>mltB</i>	816.44	-1.09	4.75E-06
<i>manZ</i>	120.64	0.35	0.29396	<i>mltC</i>	1390.43	0.30	0.20357
<i>map</i>	2199.83	0.11	0.69803	<i>mngB</i>	257.33	1.04	0.00012
<i>marA</i>	1890.72	-0.89	3.90E-05	<i>mnmA</i>	7361.65	1.03	2.88E-05
<i>marB</i>	128.17	-0.46	0.14958	<i>mnmc</i>	2512.48	-0.84	0.00033
<i>marC</i>	602.16	-0.46	0.06813	<i>mntH</i>	4255.20	2.83	1.02E-12
<i>marR</i>	762.36	0.29	0.46840	<i>moaA</i>	587.66	-1.23	1.04E-07
<i>marT</i>	460.77	-0.96	6.41E-05	<i>moaB</i>	548.28	-1.18	2.78E-06
<i>mazG</i>	1075.42	0.14	0.53214	<i>moaC</i>	164.27	-0.21	0.46152
<i>mdaB</i>	394.06	-0.40	0.09626	<i>moaD</i>	33.46	-0.20	0.65686
<i>mdfA</i>	1210.62	1.37	1.47E-09	<i>moaE</i>	140.31	-0.27	0.38135
<i>mdh</i>	17376.83	-1.24	0.00531	<i>mobA</i>	880.65	-1.79	1.76E-10
<i>mdlA</i>	1707.54	-0.07	0.76349	<i>mobB</i>	877.80	-1.48	3.43E-07
<i>mdlB</i>	1232.46	-0.08	0.71998	<i>mod</i>	3413.73	-0.32	0.19247
<i>mdoB</i>	4385.77	-1.19	1.57E-05	<i>modA</i>	959.84	-2.09	6.69E-18
<i>mdoC</i>	3104.95	1.54	5.90E-11	<i>modB</i>	355.16	-0.40	0.13589
<i>mdoG</i>	5158.84	-0.14	0.54952	<i>modC</i>	654.83	-0.75	0.00109
<i>mdoH</i>	6343.10	1.35	2.16E-07	<i>modE</i>	633.46	-0.76	0.00071
<i>melA</i>	440.24	3.37	2.54E-36	<i>modF</i>	1440.65	-1.00	8.97E-06
<i>melB</i>	749.76	1.15	5.44E-06	<i>moeA</i>	686.57	0.17	0.53174
<i>melR</i>	2381.60	-4.12	9.37E-38	<i>moeB</i>	340.43	0.60	0.01443
<i>menA</i>	744.91	0.04	0.88709	<i>mog</i>	781.15	-0.08	0.74689
<i>menB</i>	1630.31	2.68	1.10E-27	<i>motA</i>	4715.43	-2.04	1.64E-13
<i>menC</i>	1088.98	2.26	2.97E-16	<i>motB</i>	3619.62	-2.18	9.05E-15
<i>menD</i>	949.61	1.75	1.89E-14	<i>mpl</i>	2840.40	-0.03	0.91484
<i>menE</i>	1546.32	1.87	1.02E-13	<i>mppA</i>	1980.52	-0.78	0.00113
<i>menF</i>	553.61	0.89	0.00020	<i>mraW</i>	5951.91	-0.98	9.25E-05
<i>menG</i>	11462.69	-1.58	1.02E-07	<i>mraY</i>	2559.36	0.97	0.00033
<i>mepA</i>	766.17	-0.52	0.02228	<i>mraZ</i>	5674.59	-1.20	3.76E-06
<i>mesJ</i>	1085.39	-0.18	0.39486	<i>mrcA</i>	2456.30	0.57	0.01801
<i>metA</i>	357.31	-0.73	0.01236	<i>mrcB</i>	8322.25	1.33	2.27E-08
<i>metB</i>	1512.66	-1.57	8.79E-11	<i>mrdA</i>	2090.25	0.03	0.90888
<i>metC</i>	596.75	-0.47	0.05269	<i>mrdB</i>	1470.74	0.25	0.28414
<i>metE</i>	685.83	-0.23	0.36035	<i>mreB</i>	10784.19	1.40	1.14E-07
<i>metF</i>	1233.22	-2.24	3.53E-13	<i>mreC</i>	1224.43	0.33	0.18794
<i>metG</i>	4494.49	0.84	0.00027	<i>mreD</i>	733.38	-0.07	0.72726
<i>metH</i>	1757.45	-0.26	0.28302	<i>mrp</i>	3532.64	0.13	0.61308
<i>metJ</i>	1031.60	-1.98	2.90E-10	<i>mrr</i>	855.43	-1.14	1.18E-05
<i>metK</i>	4275.88	-1.63	3.84E-08	<i>msbA</i>	4005.18	0.33	0.16249
<i>metL</i>	1276.13	-0.15	0.49621	<i>msbB</i>	3400.28	0.92	0.00095
<i>metQ</i>	3318.35	-0.88	0.00099	<i>mscL</i>	3731.69	-1.48	2.57E-05
<i>metR</i>	231.48	-1.00	0.03060	<i>msgA</i>	1614.94	0.35	0.29688
<i>mfd</i>	2787.54	0.39	0.08612	<i>msrA</i>	1300.29	-1.81	1.31E-06
<i>mgIA</i>	584.44	-0.12	0.80425	<i>msyB</i>	478.54	-1.02	0.00013
<i>mgIB</i>	1946.58	-1.28	0.00196	<i>mtgA</i>	397.04	0.09	0.71265
<i>mgIC</i>	232.50	1.47	0.00060	<i>mtlA</i>	1237.66	-1.25	0.00047
<i>mgsA</i>	520.65	-2.78	8.65E-20	<i>mtlD</i>	546.78	-0.65	0.00525
<i>mgtA</i>	2042.64	-0.92	2.44E-05	<i>mtlR</i>	319.51	-0.82	0.00244
<i>mgtB</i>	1240.67	-0.68	0.02111	<i>mtn</i>	857.55	-0.25	0.28502
<i>mgtC</i>	865.40	-2.35	2.66E-11	<i>mtr</i>	848.72	0.70	0.01321
<i>mgtR</i>	21.92	0.14	0.72643	<i>mug</i>	464.08	-0.44	0.07309
<i>miaA</i>	28949.71	-0.45	0.12202	<i>mukB</i>	19746.61	0.57	0.02679
<i>miaB</i>	3304.35	-0.04	0.88191	<i>mukE</i>	1426.34	-0.89	0.00024

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>mukF</i>	1347.80	-0.14	0.55100	<i>nifU</i>	12230.64	0.37	0.19882
<i>murA</i>	6694.54	0.60	0.01776	<i>nikR</i>	406.18	-1.65	2.81E-09
<i>murB</i>	1167.72	-0.93	1.40E-05	<i>ninG</i>	89.53	0.84	0.00750
<i>murC</i>	7241.38	0.60	0.04011	<i>nirB</i>	748.23	0.38	0.12868
<i>murD</i>	4768.42	1.22	0.00022	<i>nirC</i>	245.90	0.93	0.00181
<i>murE</i>	5444.30	0.23	0.33966	<i>nirD</i>	137.11	-0.10	0.74106
<i>murF</i>	4176.16	0.93	0.00092	<i>nixA</i>	1524.48	-2.06	3.89E-11
<i>murG</i>	2808.45	0.71	0.00927	<i>nlp</i>	821.11	-1.23	4.29E-06
<i>murI</i>	643.94	-0.74	0.00136	<i>nlpB</i>	2231.18	0.64	0.00658
<i>murQ</i>	528.24	-1.72	1.12E-09	<i>nlpC</i>	418.03	-1.01	0.00021
<i>mutH</i>	713.27	-0.78	0.00078	<i>nlpD</i>	55174.51	1.19	8.04E-05
<i>mutL</i>	1851.10	-0.87	0.00019	<i>nlpI</i>	59663.71	-0.06	0.82703
<i>mutM</i>	822.34	-0.36	0.19352	<i>norM</i>	828.26	1.46	7.58E-08
<i>mutS</i>	1537.15	-0.18	0.47319	<i>norR</i>	361.19	-0.10	0.75634
<i>mutT</i>	755.38	0.07	0.74867	<i>norV</i>	277.45	-0.04	0.88520
<i>mutY</i>	1684.18	0.11	0.66219	<i>norW</i>	145.24	0.64	0.03132
<i>mviM</i>	392.63	0.20	0.42924	<i>nrdA</i>	14918.36	1.02	7.86E-05
<i>mviN</i>	2277.19	0.93	8.20E-05	<i>nrdB</i>	5278.04	0.95	0.00020
<i>nadA</i>	294.27	-0.28	0.42138	<i>nrdD</i>	873.84	-0.73	0.01953
<i>nadB</i>	502.76	-0.52	0.05751	<i>nrdE</i>	703.70	-0.67	0.01339
<i>nadC</i>	404.79	-0.63	0.00749	<i>nrdF</i>	634.97	-0.57	0.01338
<i>nadD</i>	360.31	-0.21	0.34758	<i>nrdG</i>	201.56	-0.02	0.95250
<i>nadE</i>	2057.09	-2.41	3.02E-15	<i>nrdH</i>	31.29	-0.80	0.04852
<i>nadR</i>	2133.09	-1.39	1.22E-09	<i>nrdI</i>	55.15	-1.07	0.00829
<i>nagA</i>	1551.80	-0.51	0.16377	<i>nrdR</i>	1387.13	-2.05	5.74E-23
<i>nagB</i>	5479.33	-0.02	0.96759	<i>nrfA</i>	327.16	0.49	0.08074
<i>nagC</i>	1146.52	0.05	0.82832	<i>nrfB</i>	101.55	0.65	0.03141
<i>nagD</i>	894.00	-0.39	0.09879	<i>nrfC</i>	81.02	0.87	0.01262
<i>nagE</i>	4291.50	-0.64	0.16292	<i>nrfD</i>	189.82	3.40	1.27E-24
<i>nagZ</i>	1404.10	0.11	0.63065	<i>nrfE</i>	473.36	2.77	1.87E-19
<i>nam</i>	1346.17	0.64	0.00230	<i>nrfG</i>	104.40	-0.58	0.04826
<i>nanA</i>	21240.16	-0.93	0.30318	<i>nsrR</i>	14275.90	-4.02	5.34E-22
<i>nanE</i>	459.82	-0.63	0.44432	<i>nth</i>	1314.54	0.49	0.07233
<i>nanK</i>	286.43	-0.01	0.99185	<i>ntpA</i>	1507.21	0.88	0.00129
<i>nanT</i>	1448.28	-0.54	0.27430	<i>nucD</i>	46.67	0.41	0.24040
<i>napA</i>	664.93	0.09	0.72208	<i>nucD2</i>	27.78	1.89	4.83E-05
<i>napB</i>	108.24	0.33	0.23610	<i>nucE2</i>	41.21	5.05	3.26E-17
<i>napC</i>	189.65	-0.18	0.50273	<i>nudC</i>	845.34	-0.90	0.00070
<i>napD</i>	32.65	-1.10	0.01656	<i>nudE</i>	1858.61	-1.05	4.41E-05
<i>napF</i>	96.24	-0.31	0.49859	<i>nudF</i>	1125.51	-1.91	2.03E-12
<i>napG</i>	91.89	0.23	0.43066	<i>nudH</i>	2458.45	-2.45	2.56E-23
<i>napH</i>	476.68	0.32	0.25729	<i>nuoA</i>	3358.93	-1.81	1.98E-08
<i>narG</i>	376.88	1.45	3.43E-06	<i>nuoB</i>	2654.51	0.63	0.01800
<i>narH</i>	202.75	2.16	1.28E-13	<i>nuoC</i>	8126.22	1.04	6.62E-05
<i>narI</i>	147.57	-0.01	0.98505	<i>nuoE</i>	3934.96	1.07	2.35E-06
<i>narJ</i>	87.36	2.41	2.60E-11	<i>nuoF</i>	3247.60	1.39	4.64E-09
<i>narK</i>	265.01	1.34	2.54E-05	<i>nuoG</i>	11778.03	1.46	6.64E-10
<i>narL</i>	491.94	0.63	0.00661	<i>nuoH</i>	2413.33	1.48	4.82E-09
<i>narP</i>	2088.28	0.03	0.90645	<i>nuoI</i>	2572.78	1.58	8.66E-09
<i>narQ</i>	444.62	0.49	0.03882	<i>nuoJ</i>	3462.88	1.08	1.00E-06
<i>narU</i>	158.84	0.32	0.31217	<i>nuoK</i>	230.04	0.34	0.19023
<i>narV</i>	112.65	0.22	0.48395	<i>nuoL</i>	6860.68	2.12	7.21E-18
<i>narW</i>	49.03	2.01	5.41E-08	<i>nuoM</i>	4651.50	2.38	2.56E-19
<i>narX</i>	1001.26	0.00	0.98314	<i>nuoN</i>	6717.57	2.73	6.56E-28
<i>narY</i>	228.22	1.48	1.97E-06	<i>nupC</i>	1106.88	-0.01	0.96004
<i>narZ</i>	366.82	1.01	0.00030	<i>nupG</i>	2322.06	-2.85	5.42E-20
<i>ndh</i>	11512.42	2.02	5.83E-16	<i>nusA</i>	61852.33	1.43	4.53E-07
<i>ndk</i>	2128.72	0.20	0.45734	<i>nusB</i>	4162.78	2.48	1.99E-17
<i>nei</i>	721.87	0.09	0.69796	<i>nusG</i>	3055.50	0.80	0.00223
<i>nemA</i>	1374.21	0.00	0.99583	<i>oadA2</i>	166.55	2.71	5.45E-16
<i>nfi</i>	347.38	-0.78	0.00241	<i>oadB2</i>	186.04	2.73	1.10E-15
<i>nfnB</i>	4600.45	-0.17	0.43501	<i>oadG</i>	16.19	2.60	1.60E-06
<i>nfo</i>	979.61	0.65	0.01554	<i>oafA</i>	7231.06	-1.44	4.97E-05
<i>nhaA</i>	3573.77	0.09	0.73976	<i>oat</i>	409.85	0.61	0.02796
<i>nhaB</i>	1353.53	-0.11	0.66304	<i>obgE</i>	21521.08	1.19	1.86E-05
<i>nhaR</i>	2667.12	0.35	0.10143	<i>ogt</i>	787.59	-1.38	4.08E-08
<i>nhoA</i>	1099.62	0.36	0.15139	<i>ompA</i>	116803.20	0.51	0.09079
<i>nifJ</i>	3481.49	1.00	4.39E-06	<i>ompC</i>	86916.07	-0.23	0.37649

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>ompD</i>	73879.18	1.33	0.00049	<i>pduJ</i>	57.19	3.21	2.40E-08
<i>ompF</i>	3001.43	-2.44	1.54E-17	<i>pduK</i>	120.24	5.77	1.32E-20
<i>ompL</i>	88.95	1.36	0.00073	<i>pduL</i>	96.74	4.27	1.05E-16
<i>ompN</i>	254.14	-0.24	0.41536	<i>pduM</i>	107.36	4.51	5.86E-17
<i>ompR</i>	3104.20	-0.94	0.00021	<i>pduN</i>	28.86	4.01	9.31E-11
<i>ompS</i>	836.32	-2.81	2.78E-15	<i>pduO</i>	333.30	4.93	2.06E-22
<i>ompW</i>	8065.00	3.44	3.13E-41	<i>pduP</i>	868.63	4.86	1.30E-33
<i>ompX</i>	24516.09	-0.31	0.38586	<i>pduQ</i>	253.47	4.62	4.74E-22
<i>OmrA</i>	33.90	-1.85	1.04E-05	<i>pduS</i>	226.28	4.33	1.29E-17
<i>OmrB</i>	57.04	-0.92	0.04328	<i>pduT</i>	179.27	4.12	1.86E-15
<i>opdB</i>	2994.08	0.73	0.00403	<i>pduU</i>	25.27	3.05	1.91E-07
<i>oppA</i>	2755.07	-0.68	0.08416	<i>pduV</i>	22.31	3.04	1.32E-06
<i>oppB</i>	262.41	0.78	0.00255	<i>pduW</i>	481.62	1.49	0.00086
<i>oppC</i>	680.92	0.61	0.00641	<i>pduX</i>	933.78	-0.56	0.12940
<i>oppD</i>	665.75	1.57	2.40E-08	<i>pdxAa</i>	1199.61	0.14	0.54592
<i>oppF</i>	1132.78	1.13	1.01E-06	<i>pdxAb</i>	233.26	0.13	0.63983
<i>oraA</i>	453.98	-0.80	0.00691	<i>pdxB</i>	1072.51	-0.26	0.28547
<i>ordL</i>	318.94	0.16	0.55778	<i>pdxH</i>	1423.64	0.14	0.60603
<i>orf242</i>	312.70	-0.68	0.00818	<i>pdxJ</i>	534.58	-0.08	0.74006
<i>orf245</i>	325.30	3.66	8.64E-36	<i>pdxK</i>	521.12	-1.48	1.67E-07
<i>orf319</i>	951.61	-1.31	0.00021	<i>pdxY</i>	972.04	0.71	0.00117
<i>orf32</i>	252.00	-0.16	0.57076	<i>pepA</i>	2522.34	-0.70	0.00307
<i>orf408</i>	113.53	2.09	9.04E-09	<i>pepB</i>	5736.03	0.84	0.00118
<i>orf48</i>	948.94	-0.81	0.00805	<i>pepD</i>	4798.04	-0.45	0.13933
<i>orf70</i>	181.20	-1.17	4.47E-06	<i>pepE</i>	558.08	-1.43	5.24E-07
<i>orfX</i>	65.26	-0.72	0.05440	<i>pepN</i>	4764.46	0.06	0.83502
<i>orgA</i>	4050.92	1.02	0.01238	<i>pepP</i>	2613.82	-0.55	0.04675
<i>orgB</i>	5944.03	0.92	0.01016	<i>pepQ</i>	2715.81	-0.62	0.04166
<i>orgC</i>	2946.42	0.63	0.12082	<i>pepT</i>	992.66	-0.70	0.00792
<i>orn</i>	1884.71	-1.30	7.18E-06	<i>perM</i>	1203.89	0.13	0.54445
<i>osmB</i>	5351.88	0.68	0.15900	<i>pez</i>	100078.77	0.11	0.66098
<i>osmC</i>	774.43	1.09	0.00092	<i>pfkA</i>	2845.52	-0.06	0.78758
<i>osmE</i>	3140.54	1.25	0.00059	<i>pfkB</i>	730.94	-1.93	2.61E-10
<i>osmY</i>	14911.39	1.21	0.00115	<i>pflA</i>	1947.30	0.51	0.05884
<i>otsA</i>	909.96	1.37	1.75E-05	<i>pflB</i>	17882.15	2.71	1.34E-24
<i>otsB</i>	815.95	1.40	1.64E-06	<i>pflC</i>	444.76	-0.65	0.01282
<i>oxyR</i>	4554.59	-1.15	6.23E-07	<i>pflD</i>	763.96	-0.94	7.90E-05
<i>OxyS</i>	354.51	4.34	2.37E-43	<i>pflE</i>	164.39	-0.48	0.08511
<i>pabA</i>	267.40	0.06	0.80606	<i>pflF</i>	587.64	0.81	0.00059
<i>pabB</i>	821.58	0.38	0.10264	<i>pgi</i>	9970.04	-1.24	3.34E-05
<i>pabC</i>	1072.66	-0.15	0.48238	<i>pgk</i>	8875.75	-0.18	0.53162
<i>pagC</i>	3079.48	-2.80	2.57E-13	<i>pgm</i>	9804.91	0.74	0.00454
<i>pagD</i>	136.68	-2.55	1.35E-10	<i>pgpA</i>	266.57	0.85	0.00025
<i>pagK</i>	440.58	-0.86	0.00200	<i>pgpB</i>	518.45	-0.35	0.25430
<i>pagM</i>	162.15	1.54	1.21E-07	<i>pgsA</i>	888.47	-1.03	2.29E-05
<i>pagN</i>	602.38	-2.01	3.87E-13	<i>pgtA</i>	1401.12	-1.29	2.41E-07
<i>pagO</i>	376.24	-0.95	0.00232	<i>pgtB</i>	695.00	0.54	0.03381
<i>pagP</i>	1100.47	-1.20	0.00097	<i>pgtC</i>	279.75	-0.98	7.86E-05
<i>pal</i>	7496.95	1.11	4.23E-06	<i>pgtE</i>	1012.33	-2.47	2.80E-16
<i>panB</i>	367.89	0.44	0.06341	<i>pgtP</i>	332.14	0.65	0.04942
<i>panC</i>	804.00	0.66	0.01389	<i>phd</i>	52.26	0.68	0.07253
<i>panD</i>	189.50	0.30	0.29785	<i>pheA</i>	2433.66	-0.24	0.37932
<i>panF</i>	1004.81	-0.06	0.80270	<i>pheP</i>	1391.46	0.51	0.03876
<i>parC</i>	4967.27	-0.04	0.87032	<i>pheS</i>	3453.19	0.42	0.10892
<i>parE</i>	7455.22	0.32	0.15163	<i>pheT</i>	25899.54	0.71	0.00644
<i>pbpC</i>	404.15	1.09	0.00018	<i>phnA</i>	2272.21	0.17	0.66226
<i>pbpG</i>	1076.73	0.17	0.52445	<i>phnB</i>	157.22	0.43	0.12135
<i>pckA</i>	23876.86	-0.70	0.06265	<i>phnO</i>	1193.67	-3.16	2.96E-17
<i>pcm</i>	1507.00	-0.83	0.00034	<i>phnR</i>	240.49	-0.46	0.07252
<i>pcnB</i>	3589.17	0.92	0.00020	<i>phnS</i>	151.13	0.42	0.20927
<i>pdgL</i>	492.58	-1.43	1.96E-06	<i>phnT</i>	189.47	-0.24	0.42521
<i>pdhR</i>	4865.94	0.97	0.00075	<i>phnU</i>	185.12	-0.27	0.49677
<i>pduA</i>	172.92	4.82	1.69E-19	<i>phnV</i>	527.81	-0.59	0.01314
<i>pduB</i>	89.21	6.13	1.19E-19	<i>phnW</i>	84.20	1.53	2.53E-06
<i>pduD</i>	205.27	5.23	5.46E-28	<i>phnX</i>	81.75	1.02	0.00191
<i>pduE</i>	79.09	5.44	1.20E-16	<i>phoB</i>	4567.62	1.18	3.72E-05
<i>pduF</i>	317.34	1.37	2.44E-06	<i>phoE</i>	711.50	-1.15	9.28E-06
<i>pduG</i>	646.82	6.05	2.15E-30	<i>phoH</i>	3055.32	-3.22	2.77E-18

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>phoL</i>	4046.71	-0.16	0.46934	<i>prgl</i>	1727.01	-0.28	0.53309
<i>phoN</i>	2160.28	-1.50	4.28E-10	<i>prgJ</i>	2546.48	-0.10	0.83502
<i>phoP</i>	3776.81	-1.46	1.63E-05	<i>prgK</i>	9363.58	1.14	0.02322
<i>phoQ</i>	4917.60	-0.98	0.00012	<i>priA</i>	2112.80	-0.19	0.37703
<i>phoR</i>	3042.01	2.27	8.00E-21	<i>priB</i>	9003.30	-0.65	0.03737
<i>phoU</i>	3728.96	3.15	1.23E-49	<i>priC</i>	349.28	-0.75	0.00098
<i>phrB</i>	454.64	0.39	0.14629	<i>prkB</i>	998.76	-1.15	6.81E-07
<i>phsA</i>	1838.19	3.55	3.53E-32	<i>prlA</i>	276612.57	-0.02	0.93709
<i>phsB</i>	469.03	3.00	5.88E-30	<i>prlC</i>	6164.90	0.46	0.16785
<i>phsC</i>	1051.45	1.27	3.42E-07	<i>prmA</i>	692.03	1.59	2.14E-10
<i>pilA</i>	109.41	0.97	0.00143	<i>proA</i>	1757.77	0.78	0.00508
<i>pilB</i>	151.24	0.79	0.02303	<i>proB</i>	1639.72	-0.25	0.33001
<i>pilC</i>	315.63	-1.06	0.00013	<i>proC</i>	1966.44	-0.93	0.00247
<i>pilT</i>	190.25	0.64	0.04138	<i>proP</i>	7681.17	1.50	6.77E-09
<i>pipA</i>	4437.78	-3.16	4.80E-15	<i>proQ</i>	5404.84	-0.90	0.00949
<i>pipB</i>	798.09	-2.37	1.02E-12	<i>proS</i>	3934.15	0.93	5.01E-05
<i>pipB2</i>	1545.00	-1.65	1.13E-10	<i>proV</i>	3119.75	-0.59	0.08246
<i>pipC</i>	4422.86	-3.04	1.52E-10	<i>proW</i>	843.55	0.62	0.02859
<i>pipD</i>	2919.85	-3.21	1.54E-15	<i>proX</i>	4617.11	0.37	0.27508
<i>pitA</i>	5501.82	-0.06	0.80822	<i>proY</i>	1719.32	0.65	0.01817
<i>pldA</i>	4936.97	-1.42	1.76E-07	<i>prpB</i>	605.69	3.05	8.93E-15
<i>pldB</i>	1564.65	-1.62	1.70E-12	<i>prpC</i>	497.89	3.16	2.53E-16
<i>pliC</i>	10698.25	0.35	0.20887	<i>prpD</i>	513.62	3.30	4.86E-21
<i>plsB</i>	6071.43	0.09	0.68041	<i>prpE</i>	902.01	2.01	8.41E-10
<i>plsC</i>	1653.55	-0.24	0.38282	<i>prpR</i>	540.71	-1.64	0.00073
<i>plsX</i>	4699.16	0.93	0.00020	<i>prs</i>	12738.56	0.54	0.03748
<i>pmbA</i>	1789.63	-1.14	7.62E-07	<i>psd</i>	1503.44	0.18	0.45575
<i>pmgl</i>	2144.66	-0.60	0.00646	<i>psiF</i>	189.10	-0.49	0.18812
<i>pmrD</i>	1887.44	-3.41	3.88E-15	<i>pspA</i>	4208.42	-3.23	2.41E-31
<i>pmrF</i>	4282.12	2.40	2.33E-27	<i>pspB</i>	251.56	-2.54	1.00E-12
<i>pncB</i>	1044.22	-0.52	0.05127	<i>pspC</i>	950.56	-2.16	2.32E-12
<i>pnp</i>	43861.05	0.60	0.06041	<i>pspD</i>	213.62	-2.56	4.08E-13
<i>pntA</i>	3596.57	-0.12	0.60709	<i>pspE</i>	1102.86	-3.42	2.10E-36
<i>pntB</i>	3566.68	0.22	0.38456	<i>pspF</i>	626.59	-1.84	9.31E-11
<i>pnuC</i>	201.42	0.38	0.18407	<i>pspG</i>	243.80	-0.79	0.00380
<i>pocR</i>	344.28	0.12	0.71924	<i>pssA</i>	4503.48	0.30	0.23240
<i>polA</i>	4836.55	-1.01	0.00138	<i>pstA</i>	2158.87	3.61	4.10E-42
<i>polB</i>	1821.68	-1.46	1.05E-08	<i>pstB</i>	4870.08	3.38	6.40E-42
<i>potA</i>	4433.21	1.09	4.84E-05	<i>pstC</i>	2401.09	3.45	2.73E-45
<i>potB</i>	1243.62	0.62	0.01507	<i>pstS</i>	9724.06	4.25	1.92E-43
<i>potC</i>	601.27	0.29	0.28885	<i>pta</i>	12623.05	1.53	1.53E-08
<i>potD</i>	3317.63	0.90	0.00021	<i>pth</i>	446.74	-1.31	7.75E-09
<i>potE</i>	6035.70	7.01	1.21E-80	<i>ptpS</i>	298.88	0.41	0.19614
<i>potF</i>	1073.54	-0.15	0.51611	<i>ptr</i>	3524.77	-0.15	0.57965
<i>potG</i>	385.85	1.13	4.29E-06	<i>ptsA</i>	859.71	-1.09	0.00018
<i>potH</i>	217.49	0.43	0.11056	<i>ptsG</i>	21717.47	1.95	4.96E-12
<i>potI</i>	232.34	0.95	0.00049	<i>ptsH</i>	4126.03	-1.22	1.92E-05
<i>poxB</i>	384.53	1.56	4.78E-07	<i>ptsl</i>	30095.50	-0.12	0.66056
<i>ppa</i>	9336.79	0.29	0.36476	<i>ptsJ</i>	268.96	-1.41	7.45E-05
<i>ppc</i>	4940.09	-1.10	2.25E-06	<i>ptsN</i>	3436.20	-1.09	0.00020
<i>pphA</i>	216.45	-0.58	0.07217	<i>ptsO</i>	252.61	-0.99	3.67E-05
<i>pphB</i>	812.95	-0.50	0.20657	<i>ptsP</i>	4115.32	-0.02	0.93439
<i>ppiA</i>	1713.40	0.41	0.13649	<i>ptxA</i>	41.63	1.78	1.94E-05
<i>ppiB</i>	3796.94	-1.04	0.00118	<i>purA</i>	9152.56	0.92	4.55E-05
<i>ppiC</i>	3329.94	-1.41	4.05E-06	<i>purB</i>	3086.23	0.44	0.06584
<i>ppk</i>	2776.22	-1.41	2.31E-08	<i>purC</i>	1356.38	0.12	0.66385
<i>ppnK</i>	1479.24	-1.98	2.17E-13	<i>purD</i>	378.12	0.79	0.00068
<i>pps</i>	2761.83	-0.50	0.20347	<i>purE</i>	115.43	0.70	0.01378
<i>ppx</i>	1143.14	0.37	0.11246	<i>purF</i>	1265.04	0.66	0.00893
<i>pqaA</i>	393.80	-0.46	0.22092	<i>purG</i>	1875.03	0.67	0.01478
<i>pqiA</i>	1415.58	-0.64	0.00979	<i>purH</i>	801.52	0.15	0.55505
<i>pqiB</i>	3472.92	-0.12	0.62768	<i>purK</i>	452.18	1.15	6.74E-05
<i>prc</i>	10388.61	0.70	0.01021	<i>purM</i>	661.44	1.25	0.00014
<i>prfA</i>	3494.10	1.58	1.62E-07	<i>purN</i>	391.88	-0.26	0.33847
<i>prfB</i>	14348.44	0.48	0.03325	<i>purR</i>	2054.49	0.63	0.00504
<i>prfC</i>	7358.41	1.34	2.23E-08	<i>purT</i>	518.25	0.44	0.15123
<i>prfH</i>	356.54	0.20	0.42340	<i>purU</i>	2446.61	-0.04	0.87967
<i>prgH</i>	13662.40	1.06	0.01895	<i>putA</i>	1899.86	-1.68	0.00035

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>putP</i>	2095.70	-1.08	0.00510	<i>rfaA</i>	4914.31	-0.79	0.00122
<i>pykA</i>	2388.20	-0.15	0.65905	<i>rfaB</i>	5876.92	0.21	0.39209
<i>pykF</i>	24284.86	1.57	2.14E-10	<i>rfaC</i>	4834.83	-0.07	0.80417
<i>pyrB</i>	420.73	0.47	0.05653	<i>rfaD</i>	4181.41	0.37	0.17081
<i>pyrC</i>	450.19	-0.04	0.87761	<i>rfaF</i>	11084.22	0.57	0.05927
<i>pyrD</i>	952.32	0.46	0.08680	<i>rfaG</i>	12071.16	-0.33	0.24854
<i>pyrE</i>	1050.36	-0.18	0.40628	<i>rfaH</i>	26828.78	0.51	0.05479
<i>pyrF</i>	911.87	-0.45	0.10088	<i>rfaI</i>	8180.68	0.17	0.61966
<i>pyrG</i>	21562.47	-0.34	0.20133	<i>rfaJ</i>	8527.51	0.04	0.89484
<i>pyrH</i>	8361.91	0.20	0.46598	<i>rfaK</i>	12364.14	0.14	0.65137
<i>pyrI</i>	272.69	-0.35	0.18373	<i>rfaN</i>	3131.53	-0.67	0.05503
<i>pyrL</i>	20.04	0.13	0.76800	<i>rfaP</i>	12074.98	-0.23	0.52458
<i>qor</i>	655.50	-2.37	7.42E-13	<i>rfaU</i>	5240.15	-0.90	0.02404
<i>queA</i>	1447.26	0.24	0.27309	<i>rfaV</i>	5778.91	-0.34	0.32501
<i>queF</i>	917.80	-0.88	7.69E-05	<i>rfaX</i>	5980.78	0.23	0.55081
<i>radA</i>	589.57	-0.15	0.52952	<i>rfaY</i>	1259.25	1.12	0.00120
<i>radC</i>	350.25	-0.65	0.01958	<i>rfaZ</i>	4561.39	-1.07	6.81E-05
<i>ramA</i>	247.03	-0.53	0.16696	<i>rffA</i>	2434.18	0.35	0.18812
<i>ramR</i>	319.69	-0.77	0.00178	<i>rffC</i>	948.14	0.06	0.84269
<i>rarD</i>	684.24	0.01	0.97244	<i>rffD</i>	1990.09	0.26	0.30834
<i>ratA</i>	877.14	0.20	0.39546	<i>rffE</i>	3206.70	0.31	0.19104
<i>rbfA</i>	6492.80	-0.15	0.65414	<i>rffG</i>	2290.77	0.27	0.31627
<i>rbn</i>	552.43	-1.25	5.00E-05	<i>rffH</i>	187.87	0.34	0.24257
<i>rbsA</i>	916.23	-1.90	7.42E-10	<i>rffM</i>	1500.83	0.18	0.41162
<i>rbsB</i>	17576.22	0.59	0.08174	<i>rffT</i>	1972.19	0.07	0.74798
<i>rbsC</i>	561.37	-1.93	4.50E-08	<i>rhaA</i>	163.77	1.27	1.17E-05
<i>rbsD</i>	5319.04	-5.27	2.38E-77	<i>rhaB</i>	250.99	0.73	0.03237
<i>rbsK</i>	778.27	-1.60	1.19E-07	<i>rhaD</i>	449.91	-0.32	0.24515
<i>rbsR</i>	570.99	-1.18	8.63E-06	<i>rhaR</i>	209.84	-0.30	0.40003
<i>rcaA</i>	1474.64	-0.09	0.73949	<i>rhaS</i>	258.41	-0.74	0.00573
<i>rcaB</i>	5556.83	0.22	0.41018	<i>rhaT</i>	173.38	1.58	2.35E-06
<i>rcaC</i>	3909.44	1.02	1.07E-05	<i>rhlB</i>	12045.38	-0.03	0.91801
<i>rcaF</i>	714.41	-0.71	0.01594	<i>rhlE</i>	4890.87	0.98	0.00045
<i>rdgC</i>	1427.18	-0.05	0.82793	<i>rho</i>	40425.10	-0.27	0.30406
<i>rec2</i>	488.55	0.61	0.03092	<i>rhtB</i>	336.57	-0.74	0.01223
<i>recA</i>	11877.04	-1.79	3.15E-15	<i>rhtC</i>	324.79	-0.27	0.23877
<i>recB</i>	4354.83	-0.20	0.38957	<i>rhuM</i>	1747.38	-1.70	4.02E-10
<i>recC</i>	2397.97	-0.58	0.00791	<i>ribA</i>	3261.57	0.16	0.50940
<i>recD</i>	676.59	0.44	0.06845	<i>ribB</i>	11159.87	0.67	0.00629
<i>recEa</i>	596.17	1.52	9.82E-10	<i>ribD</i>	1027.37	0.11	0.62674
<i>recEb</i>	999.46	1.28	4.54E-06	<i>ribE</i>	1806.07	-1.31	4.48E-08
<i>recF</i>	2335.09	-0.06	0.79049	<i>ribF</i>	3451.92	-0.27	0.32310
<i>recG</i>	2758.60	-0.31	0.20218	<i>ribH</i>	2169.83	0.71	0.01270
<i>recJ</i>	3308.16	-0.04	0.84705	<i>rihA</i>	347.78	-1.33	0.00266
<i>recN</i>	1330.24	-0.87	0.00148	<i>rihC</i>	1009.15	-0.18	0.42448
<i>recO</i>	2299.80	1.02	1.85E-05	<i>rimI</i>	665.02	-0.89	0.00031
<i>recQ</i>	2414.32	-0.37	0.08723	<i>rimJ</i>	2765.39	-2.89	5.24E-19
<i>recR</i>	567.09	0.86	0.00011	<i>rimK</i>	682.92	-0.23	0.29492
<i>relA</i>	5263.77	-1.00	1.81E-05	<i>rimL</i>	272.55	-1.36	2.13E-07
<i>relB</i>	214.90	-1.69	2.85E-09	<i>rimM</i>	18143.74	-0.78	0.00414
<i>relE</i>	58.81	-0.35	0.35688	<i>rimO</i>	2906.23	1.01	0.00036
<i>rep</i>	3299.55	-1.06	2.21E-06	<i>rl18</i>	26807.01	-0.29	0.28470
<i>res</i>	4828.49	-0.02	0.92161	<i>rlmL</i>	5429.61	0.48	0.05752
<i>rfaB</i>	2224.76	-0.31	0.24205	<i>rlpA</i>	1477.01	0.18	0.42904
<i>rfaC</i>	1490.41	-0.12	0.58245	<i>rlpB</i>	1741.42	0.71	0.01027
<i>rfaD</i>	4114.19	-1.27	8.87E-06	<i>rluA</i>	1854.10	0.08	0.74018
<i>rfaE</i>	1754.53	0.85	0.00116	<i>rluB</i>	490.70	0.62	0.00594
<i>rfaF</i>	1605.01	-0.36	0.13198	<i>rluC</i>	1671.82	0.40	0.11950
<i>rfaG</i>	1590.78	-0.33	0.24269	<i>rluD</i>	3983.19	-0.25	0.23683
<i>rfaH</i>	1230.84	-1.62	1.23E-08	<i>rmbA</i>	87.83	0.83	0.01461
<i>rfaI</i>	2863.64	0.62	0.01931	<i>rmf</i>	1296.89	1.11	8.15E-07
<i>rfaJ</i>	2006.83	0.44	0.11882	<i>rna</i>	1344.62	-1.15	4.45E-06
<i>rfaK</i>	3496.98	-0.70	0.06598	<i>rnb</i>	5542.51	0.18	0.46037
<i>rfaL</i>	11707.12	0.90	0.01235	<i>rnc</i>	5517.99	1.28	4.27E-07
<i>rfaP</i>	943.66	-0.94	0.00022	<i>rnd</i>	1139.17	1.04	9.72E-05
<i>rfaQ</i>	3279.04	-1.16	2.99E-06	<i>rne</i>	16755.07	0.87	0.00790
<i>rfaY</i>	2193.35	0.34	0.24516	<i>rnfA</i>	632.77	-0.31	0.28086
<i>rfaZ</i>	1530.37	-0.45	0.17638	<i>rnfB</i>	441.69	-0.44	0.13524

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>rnfC</i>	2489.52	0.25	0.30156	<i>rpsH</i>	53975.46	-1.16	0.00011
<i>rnfD</i>	733.65	0.51	0.06831	<i>rpsI</i>	4428.05	0.82	0.01199
<i>rnfE</i>	1628.75	0.38	0.17197	<i>rpsJ</i>	63445.16	-0.19	0.55201
<i>rnfG</i>	808.46	0.08	0.77216	<i>rpsK</i>	14725.47	-0.71	0.00833
<i>rnfH</i>	388.59	-0.55	0.05729	<i>rpsL</i>	10697.58	-2.32	6.85E-12
<i>rnhA</i>	1337.73	-0.43	0.15380	<i>rpsM</i>	58931.60	0.06	0.83821
<i>rnhB</i>	2154.68	0.86	0.00311	<i>rpsN</i>	21252.58	0.16	0.57660
<i>rnk</i>	281.64	0.29	0.40345	<i>rpsO</i>	10129.29	-0.20	0.71799
<i>rnpA</i>	289.21	-0.29	0.41341	<i>rpsQ</i>	11082.66	0.02	0.94336
<i>rnr</i>	52823.25	-1.10	0.00683	<i>rpsR</i>	1254.06	0.03	0.93357
<i>rnt</i>	912.24	1.12	1.64E-05	<i>rpsS</i>	22864.97	-0.32	0.26500
<i>rob</i>	3951.59	-0.89	0.00052	<i>rpsT</i>	24124.15	1.55	0.00140
<i>rodA</i>	6739.17	-0.97	0.00043	<i>rpsU</i>	1826.30	1.19	0.01417
<i>rof</i>	222.09	-2.33	6.05E-10	<i>rpsV</i>	2054.45	-0.28	0.44988
<i>rpe</i>	1034.39	-0.02	0.92854	<i>rrmA</i>	1419.15	1.08	6.97E-05
<i>rph</i>	2056.76	0.31	0.29240	<i>rsd</i>	2283.05	-3.44	3.02E-17
<i>rpiA</i>	1870.21	-1.33	9.25E-07	<i>rseA</i>	2924.79	-0.33	0.33406
<i>rpl19</i>	38487.92	0.21	0.39575	<i>rseB</i>	1047.75	0.43	0.12738
<i>rpl20</i>	44675.84	-0.52	0.11744	<i>rseC</i>	273.48	0.79	0.02164
<i>rpl32</i>	5120.59	-0.37	0.24235	<i>RseX</i>	55.17	-1.69	0.00191
<i>rpl35</i>	140846.29	-1.01	0.00161	<i>rsmC</i>	1108.23	1.08	9.97E-07
<i>rplA</i>	46746.58	1.77	5.67E-12	<i>rsmD</i>	462.14	-0.74	0.00129
<i>rplB</i>	26957.60	0.32	0.19635	<i>rspA</i>	159.52	1.26	0.00026
<i>rplC</i>	58907.40	0.31	0.27146	<i>rspB</i>	64.68	1.36	0.00017
<i>rplD</i>	40740.57	-0.04	0.87639	<i>rstA</i>	1286.37	-1.06	0.00014
<i>rplE</i>	50255.77	0.32	0.28259	<i>rstB</i>	251.33	0.46	0.08774
<i>rplF</i>	46394.13	-0.42	0.11729	<i>rsuA</i>	1103.28	0.73	0.01445
<i>rplI</i>	16407.91	2.81	1.34E-16	<i>rtcA</i>	113.09	0.58	0.03801
<i>rplJ</i>	18152.86	-0.40	0.16315	<i>rtcB</i>	694.94	-0.60	0.02301
<i>rplK</i>	9449.12	0.57	0.06019	<i>rtcR</i>	1912.49	0.12	0.62671
<i>rplL</i>	68171.48	1.06	0.00420	<i>rtn</i>	945.65	-1.36	8.73E-10
<i>rplM</i>	54287.74	-0.54	0.05807	<i>rtsA</i>	1322.76	0.43	0.25648
<i>rplN</i>	28725.22	-2.20	1.21E-11	<i>rtsB</i>	316.40	-1.21	0.00218
<i>rplO</i>	32546.92	-0.39	0.15847	<i>rumA</i>	1832.03	-1.82	5.96E-17
<i>rplP</i>	55980.59	-0.91	0.00399	<i>rumB</i>	870.70	0.53	0.02780
<i>rplQ</i>	4097.83	0.23	0.49444	<i>ruvA</i>	1811.27	-0.15	0.58201
<i>rplU</i>	25965.27	-0.18	0.65643	<i>ruvB</i>	1037.32	-0.09	0.77201
<i>rplV</i>	39398.37	0.73	0.01522	<i>ruvC</i>	315.12	1.48	2.85E-10
<i>rplW</i>	28830.72	-0.25	0.39879	<i>RybA</i>	7729.89	-3.86	1.06E-35
<i>rplX</i>	44731.39	-0.45	0.09775	<i>RybB</i>	2.40	-0.55	0.54448
<i>rplY</i>	5234.04	0.69	0.06848	<i>RybD</i>	3.46	2.21	0.01461
<i>rpmA</i>	8692.11	1.06	0.00747	<i>RydB</i>	88.11	1.38	8.65E-06
<i>rpmB</i>	5992.40	-0.18	0.70694	<i>RyDC</i>	211.97	0.07	0.83004
<i>rpmC</i>	5557.52	-0.29	0.44755	<i>RyeA</i>	19.08	-1.59	0.00157
<i>rpmD</i>	4529.78	-1.54	3.66E-05	<i>RyeC</i>	125.88	0.46	0.11383
<i>rpmE</i>	3339.45	-0.09	0.81689	<i>RyeF</i>	14.26	1.55	0.00447
<i>rpmE2</i>	888.41	-3.70	6.05E-19	<i>RyfA</i>	1781.45	2.68	2.25E-20
<i>rpmG</i>	2517.09	1.76	8.06E-05	<i>RygC</i>	187.65	-0.97	0.00520
<i>rpmH</i>	5562.17	-0.72	0.09935	<i>RygD</i>	29.90	1.28	0.00101
<i>rpmJa</i>	883.79	-1.57	9.18E-06	<i>RyhB1</i>	93.57	1.26	0.00017
<i>rpmJb</i>	21226.81	0.14	0.63548	<i>RyhB2</i>	12.83	-2.12	0.00044
<i>rpoB</i>	83480.52	0.90	4.28E-05	<i>rzpR</i>	46.62	1.04	0.01217
<i>rpoC</i>	73377.10	0.61	0.00422	<i>sadA</i>	1521.38	0.05	0.87985
<i>rpoD</i>	24626.89	0.54	0.02402	<i>safA</i>	643.70	-1.24	0.00064
<i>rpoE</i>	6686.93	-0.53	0.09228	<i>safB</i>	141.55	1.17	4.84E-05
<i>rpoH</i>	18231.94	-0.70	0.02029	<i>safC</i>	1318.17	0.15	0.53004
<i>rpoN</i>	10044.66	-1.23	5.14E-05	<i>safD</i>	556.57	-0.08	0.78442
<i>rpoS</i>	18250.62	0.82	0.00688	<i>sanA</i>	596.43	-0.68	0.01124
<i>rpoZ</i>	3565.39	-0.59	0.06209	<i>sapA</i>	1001.36	0.08	0.72760
<i>RprA</i>	120.32	-1.65	2.88E-05	<i>sapB</i>	305.60	0.38	0.09565
<i>rps16</i>	15465.79	-0.64	0.07625	<i>sapC</i>	549.98	0.52	0.02415
<i>rpsA</i>	116236.35	0.03	0.91702	<i>sapD</i>	1127.71	0.03	0.90637
<i>rpsB</i>	74994.91	-0.54	0.02245	<i>sapF</i>	554.95	0.14	0.54420
<i>rpsC</i>	63897.93	-0.24	0.31832	<i>sapG</i>	7015.63	3.07	3.18E-32
<i>rpsD</i>	103788.22	-0.34	0.18444	<i>sbcB</i>	2299.24	-1.07	7.62E-05
<i>rpsE</i>	9514.53	-0.46	0.11878	<i>sbcC</i>	2192.25	-0.21	0.30152
<i>rpsF</i>	15329.93	0.90	0.00203	<i>sbcD</i>	734.58	-0.46	0.03796
<i>rpsG</i>	35643.62	-0.33	0.27857	<i>sbmA</i>	2810.41	0.12	0.62705

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>sbp</i>	1383.62	-1.56	0.00113	<i>shdA</i>	815.49	0.90	0.00023
<i>SC4B5.11c</i>	749.97	0.61	0.00691	<i>sicA</i>	5795.11	-0.86	0.08661
<i>sciA</i>	1843.71	-1.48	2.87E-07	<i>sicP</i>	4470.74	-2.44	1.03E-09
<i>sciB</i>	163.58	-0.47	0.16128	<i>sifA</i>	771.30	-1.80	2.05E-07
<i>sciC</i>	275.32	0.75	0.01801	<i>sifB</i>	179.04	0.29	0.48214
<i>sciD</i>	64.60	-0.17	0.61903	<i>siiA</i>	2700.22	-4.09	8.78E-17
<i>sciE</i>	110.12	0.48	0.12297	<i>siiB</i>	2862.07	-2.78	2.46E-08
<i>sciF</i>	194.93	1.35	4.21E-06	<i>siiC</i>	1665.78	-3.05	3.46E-11
<i>sciG</i>	341.17	1.26	2.72E-05	<i>siiD</i>	4188.21	-4.47	1.66E-19
<i>sciH</i>	74.75	1.31	0.00021	<i>siiE</i>	34220.04	-2.13	4.29E-07
<i>sciI</i>	268.05	0.96	0.00161	<i>siiF</i>	3713.61	-2.10	1.39E-06
<i>sciJ</i>	93.97	1.16	0.00080	<i>sinH</i>	235.88	1.77	6.74E-07
<i>sciK</i>	92.76	-0.03	0.92600	<i>sinI</i>	96.85	1.28	0.00035
<i>sciK1</i>	106.22	0.80	0.00877	<i>sinR</i>	746.16	-0.68	0.04895
<i>sciN</i>	64.62	2.21	3.78E-09	<i>sipA</i>	17033.53	-0.51	0.31341
<i>sciO</i>	140.26	2.20	6.46E-12	<i>sipB</i>	17770.39	-0.28	0.58917
<i>sciP</i>	132.71	1.48	3.74E-06	<i>sipC</i>	17973.45	-1.15	0.01667
<i>sciQ</i>	136.68	1.43	1.54E-05	<i>sipD</i>	6146.10	-0.07	0.88985
<i>sciR</i>	107.71	2.16	3.12E-10	<i>sirA</i>	2872.48	-0.82	0.02469
<i>sciS</i>	505.26	1.44	3.48E-08	<i>sirC</i>	397.53	1.05	3.20E-05
<i>sciT</i>	115.58	1.74	7.86E-07	<i>sitA</i>	3578.66	0.57	0.15962
<i>sciU</i>	75.45	0.60	0.09666	<i>sitB</i>	2670.22	1.61	8.14E-06
<i>sciV</i>	148.67	0.79	0.01725	<i>sitC</i>	748.24	2.23	3.64E-09
<i>sciW</i>	88.55	0.79	0.01618	<i>sitD</i>	3592.93	2.36	3.87E-14
<i>sciX</i>	330.85	-1.47	6.47E-06	<i>sixA</i>	1631.61	0.13	0.64297
<i>sciY</i>	213.83	-2.17	1.60E-08	<i>SL1344_0014</i>	455.19	-1.06	0.00029
<i>sciZ</i>	2248.45	-0.47	0.09966	<i>SL1344_0015</i>	87.41	0.92	0.03818
<i>scsA</i>	120.07	0.60	0.03376	<i>SL1344_0016</i>	64.55	1.66	3.59E-05
<i>scsB</i>	456.35	1.38	1.07E-07	<i>SL1344_0017</i>	78.67	0.09	0.80845
<i>scsC</i>	259.00	1.27	3.60E-05	<i>SL1344_0019</i>	463.18	0.82	0.00313
<i>scsD</i>	74.37	1.39	2.27E-05	<i>SL1344_0020</i>	135.44	1.95	5.12E-08
<i>sdaA</i>	5797.54	2.14	3.79E-22	<i>SL1344_0029</i>	137.27	0.22	0.44450
<i>sdaB</i>	939.97	-0.21	0.58423	<i>SL1344_0030</i>	71.60	1.56	0.00020
<i>sdaC</i>	3179.52	-1.76	7.76E-08	<i>SL1344_0031</i>	124.89	1.62	5.91E-06
<i>sdhA</i>	3052.38	-0.84	0.09810	<i>SL1344_0032</i>	62.21	2.43	3.52E-08
<i>sdhB</i>	4059.70	-1.37	0.01265	<i>SL1344_0033</i>	336.30	0.56	0.09925
<i>sdhC</i>	9215.21	-3.42	1.80E-26	<i>SL1344_0034</i>	410.25	-0.51	0.07325
<i>sdhD</i>	871.07	-1.32	0.00044	<i>SL1344_0035</i>	125.21	1.56	3.56E-05
<i>sdiA</i>	1838.11	-2.07	2.05E-11	<i>SL1344_0036</i>	419.06	0.86	0.00103
<i>secA</i>	19593.95	1.31	2.72E-07	<i>SL1344_0037</i>	274.77	-0.67	0.01864
<i>secB</i>	4018.72	-2.01	2.65E-08	<i>SL1344_0038</i>	130.93	1.03	0.00280
<i>secD</i>	11773.20	0.79	0.00060	<i>SL1344_0039</i>	714.45	-0.53	0.08566
<i>secE</i>	5108.92	-0.01	0.97907	<i>SL1344_0042</i>	451.51	0.82	0.00313
<i>secF</i>	5530.75	1.27	4.41E-07	<i>SL1344_0043</i>	227.04	1.63	1.47E-08
<i>secG</i>	29451.56	0.72	0.06532	<i>SL1344_0051</i>	1995.42	-1.43	5.37E-11
<i>selA</i>	808.09	-0.71	0.00149	<i>SL1344_0055</i>	179.64	2.94	2.57E-17
<i>selB</i>	1101.51	-0.27	0.18789	<i>SL1344_0056</i>	162.65	3.60	1.20E-19
<i>selD</i>	2139.50	-0.62	0.05154	<i>SL1344_0058</i>	186.28	2.80	2.87E-15
<i>seqA</i>	4052.56	-1.11	0.00049	<i>SL1344_0081</i>	975.36	2.57	4.17E-09
<i>serA</i>	2076.86	-1.32	2.36E-06	<i>SL1344_0082</i>	1145.22	-2.50	1.87E-09
<i>serB</i>	1427.49	-0.35	0.14380	<i>SL1344_0083</i>	210.81	-0.91	0.00895
<i>serC</i>	3516.42	-0.85	0.00160	<i>SL1344_0083A</i>	152.72	-1.48	0.00012
<i>serS</i>	12210.13	-0.01	0.95079	<i>SL1344_0084</i>	482.91	-0.25	0.39947
<i>setB</i>	294.69	0.65	0.02277	<i>SL1344_0085</i>	31.94	1.19	0.00424
<i>sfbA</i>	1135.96	-2.71	2.70E-14	<i>SL1344_0099</i>	142.00	-0.41	0.19357
<i>sfbB</i>	306.09	-0.60	0.05040	<i>SL1344_0099A</i>	7.47	0.45	0.47616
<i>sfbC</i>	128.40	-0.20	0.48647	<i>SL1344_0100</i>	321.42	-2.86	8.43E-18
<i>sfcA</i>	2358.35	0.26	0.27341	<i>SL1344_0148</i>	175.71	-0.37	0.15656
<i>sfsA</i>	484.89	-1.76	1.36E-12	<i>SL1344_0149</i>	383.38	0.21	0.55323
<i>sgaB</i>	29.58	0.73	0.06016	<i>SL1344_0155</i>	845.45	2.00	8.24E-13
<i>sgaE</i>	225.80	1.03	2.06E-05	<i>SL1344_0156</i>	160.72	0.77	0.01802
<i>sgaH</i>	118.21	1.89	1.21E-08	<i>SL1344_0160</i>	9446.82	-0.24	0.52209
<i>sgaT</i>	281.93	1.50	3.01E-07	<i>SL1344_0163</i>	250.58	1.80	8.27E-10
<i>sgaU</i>	118.92	1.36	2.61E-05	<i>SL1344_0165</i>	715.04	-0.54	0.04803
<i>sgbE</i>	84.21	1.39	1.33E-05	<i>SL1344_0202</i>	306.62	-1.21	0.00015
<i>sgbH</i>	38.64	2.45	5.88E-09	<i>SL1344_0213</i>	1114.31	-0.82	0.00046
<i>sgbU</i>	88.66	2.65	6.97E-13	<i>SL1344_0234</i>	1084.36	-0.03	0.88643
<i>SgrS</i>	18.05	0.90	0.04705	<i>SL1344_0252</i>	525.28	-0.66	0.02366

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
SL1344_0272	88.05	0.86	0.03874	SL1344_0639	1269.84	-0.37	0.40662
SL1344_0273	47.38	-0.84	0.02704	SL1344_0640	1075.54	-1.29	1.11E-07
SL1344_0286	1076.03	-0.10	0.72185	SL1344_0649	444.11	-0.72	0.08208
SL1344_0286A	102.35	-0.39	0.35718	SL1344_0661	349.85	-1.67	1.52E-08
SL1344_0286B	384.49	-0.26	0.32502	SL1344_0671	413.85	0.12	0.63920
SL1344_0291	127.71	0.64	0.09550	SL1344_0672	212.11	1.27	1.61E-06
SL1344_0293	23.29	2.85	3.12E-08	SL1344_0673	178.84	-0.37	0.18233
SL1344_0301	55.55	-0.50	0.23776	SL1344_0674	741.50	0.03	0.91590
SL1344_0319	569.39	-1.70	7.74E-09	SL1344_0681	4409.87	5.61	7.23E-46
SL1344_0322	37154.31	-4.88	5.48E-32	SL1344_0696	123.77	-1.45	4.19E-05
SL1344_0323	656.63	-0.39	0.29385	SL1344_0698	95.90	0.01	0.97275
SL1344_0326	191.85	1.04	0.00051	SL1344_0699	65.32	2.24	2.80E-10
SL1344_0327	228.73	0.51	0.04230	SL1344_0700	111.10	1.64	2.31E-05
SL1344_0328	405.14	-0.65	0.00695	SL1344_0702	163.66	2.89	1.67E-14
SL1344_0329	319.07	-1.78	5.98E-07	SL1344_0703	177.92	1.64	6.47E-06
SL1344_0330	153.21	-1.67	1.94E-06	SL1344_0704	111.80	2.46	4.26E-15
SL1344_0336	506.49	0.21	0.45170	SL1344_0705	121.33	2.43	7.00E-13
SL1344_0337	220.48	1.26	7.76E-05	SL1344_0706	176.81	1.15	0.00024
SL1344_0338	775.95	0.27	0.29542	SL1344_0707	92.83	1.28	1.63E-05
SL1344_0339	1034.64	-1.49	9.91E-10	SL1344_0708	451.61	1.13	4.68E-05
SL1344_0340	266.69	0.00	0.98877	SL1344_0709	32.77	-0.10	0.77980
SL1344_0341	170.63	0.23	0.51498	SL1344_0713	21919.35	-2.94	6.58E-19
SL1344_0342	140.47	-0.12	0.75355	SL1344_0738	462.50	1.95	2.90E-13
SL1344_0343	38.76	0.90	0.04722	SL1344_0739	392.95	1.02	8.78E-05
SL1344_0344	212.70	-0.75	0.02582	SL1344_0740	1268.94	-2.93	2.64E-11
SL1344_0345	124.55	0.70	0.02803	SL1344_0741	177.59	1.21	0.00070
SL1344_0346	297.90	1.79	4.17E-08	SL1344_0742	143.71	3.13	3.61E-14
SL1344_0347	63.26	2.20	3.88E-08	SL1344_0746	67.49	1.92	6.48E-08
SL1344_0348	363.74	1.64	1.91E-06	SL1344_0747	275.89	1.75	5.58E-08
SL1344_0349	86.31	-0.57	0.10945	SL1344_0748	230.08	-0.48	0.04504
SL1344_0350	60.42	-0.61	0.07078	SL1344_0754	155.84	-0.05	0.88848
SL1344_0351	641.26	0.48	0.08178	SL1344_0785	98.45	1.24	0.00035
SL1344_0354	271.98	1.41	3.14E-06	SL1344_0786	145.27	1.31	3.15E-05
SL1344_0355	380.74	2.12	5.34E-14	SL1344_0794	458.17	-0.96	0.00177
SL1344_0356	208.23	1.72	5.37E-10	SL1344_0810	1660.67	-0.38	0.11058
SL1344_0357	100.28	-1.19	0.00082	SL1344_0814	535.63	-0.78	0.01331
SL1344_0358	952.11	-0.62	0.02672	SL1344_0830	62.17	-0.02	0.96714
SL1344_0376	224.95	-0.72	0.01900	SL1344_0831	208.92	0.36	0.27183
SL1344_0377	365.86	1.33	5.12E-08	SL1344_0832	299.95	-0.01	0.98367
SL1344_0404	520.52	-0.40	0.19280	SL1344_0833	477.92	0.00	0.98883
SL1344_0405	475.89	0.60	0.00993	SL1344_0834	440.67	0.00	0.99314
SL1344_0430A	241.00	-0.97	0.00658	SL1344_0835	449.36	-0.36	0.18280
SL1344_0431	714.33	-0.48	0.10453	SL1344_0836	1365.37	-2.02	5.17E-09
SL1344_0432	1443.40	-1.41	3.08E-05	SL1344_0843	621.43	0.94	0.00231
SL1344_0452	345.23	-0.52	0.04893	SL1344_0844	820.65	-0.81	0.00166
SL1344_0472	2227.11	-1.90	4.08E-15	SL1344_0845	323.95	-1.48	7.42E-06
SL1344_0490	496.55	-0.28	0.28045	SL1344_0846	2428.21	0.39	0.21941
SL1344_0502	582.96	-0.63	0.03786	SL1344_0860	255.05	0.65	0.07080
SL1344_0513	135.53	2.99	1.56E-15	SL1344_0861	110.17	0.45	0.17434
SL1344_0531	422.66	-0.76	0.00143	SL1344_0862	387.03	-0.19	0.50842
SL1344_0532	328.99	-0.64	0.01869	SL1344_0869	923.25	-2.24	2.45E-15
SL1344_0544	75.90	0.24	0.53579	SL1344_0871	782.23	-0.01	0.96063
SL1344_0546	473.11	-1.98	1.95E-11	SL1344_0886	533.84	-1.71	1.68E-11
SL1344_0547	820.92	-0.46	0.17697	SL1344_0887	408.04	-1.75	2.01E-10
SL1344_0550	750.75	1.53	6.76E-08	SL1344_0888	139.85	0.56	0.09315
SL1344_0553	186.07	4.14	1.46E-17	SL1344_0889	227.38	-1.14	0.00039
SL1344_0555	1993.41	-1.79	1.69E-12	SL1344_0890	814.48	-1.24	2.11E-06
SL1344_0559	628.55	-0.14	0.59582	SL1344_0892	1688.69	-0.72	0.06878
SL1344_0560	100.77	1.18	0.00037	SL1344_0908	385.57	-1.24	1.16E-06
SL1344_0561	82.83	1.79	1.18E-07	SL1344_0926	430.27	-0.60	0.01474
SL1344_0565	184.47	-1.72	2.46E-08	SL1344_0938	266.41	-1.00	0.00057
SL1344_0598	346.00	5.09	7.59E-43	SL1344_0940	228.48	0.23	0.54346
SL1344_0599	1255.67	3.65	2.49E-41	SL1344_0942	2508.93	-0.82	0.00175
SL1344_0600	167.96	3.14	2.69E-20	SL1344_0943	130.56	0.60	0.06881
SL1344_0601	387.16	0.87	0.00067	SL1344_0944	48.93	0.63	0.06836
SL1344_0619	414.70	-0.13	0.66340	SL1344_0945	335.05	1.67	2.85E-08
SL1344_0637	410.23	4.62	5.92E-32	SL1344_0947	63.89	0.48	0.28250
SL1344_0638	477.53	3.01	1.39E-19	SL1344_0948	14.21	0.67	0.17297

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
SL1344_0949	26.93	-0.22	0.56546	SL1344_1193	165.58	-0.65	0.01993
SL1344_0950	2699.21	-0.42	0.13141	SL1344_1194	134.87	0.71	0.01081
SL1344_0952	234.38	-0.42	0.24874	SL1344_1195	95.86	1.15	5.80E-05
SL1344_0953	96.75	1.66	3.12E-06	SL1344_1196	407.77	0.07	0.79874
SL1344_0954	44.84	1.26	0.00203	SL1344_1197	347.16	1.07	0.00013
SL1344_0955	65.48	-0.85	0.00486	SL1344_1198	636.49	-1.70	6.49E-07
SL1344_0956	3264.23	-0.80	0.01838	SL1344_1200	63.15	0.67	0.04200
SL1344_0957	1019.61	-3.31	1.79E-28	SL1344_1201	3727.24	-2.56	4.02E-12
SL1344_0958	133.28	-0.38	0.16616	SL1344_1202	122.55	-0.51	0.07519
SL1344_0960	1.75	1.16	0.23449	SL1344_1203	68.67	-2.12	1.33E-05
SL1344_0961	294.96	0.27	0.42354	SL1344_1208	425.94	-1.10	5.27E-05
SL1344_0962	2120.45	-2.39	3.44E-11	SL1344_1209	0.19	0.33	0.74250
SL1344_0963	333.68	1.27	2.33E-07	SL1344_1212	382.71	-2.22	3.43E-16
SL1344_0964	3945.53	-1.20	3.64E-08	SL1344_1222	493.75	0.71	0.00565
SL1344_0965A	45.66	0.53	0.12761	SL1344_1223	575.54	-0.48	0.06524
SL1344_0967	66.18	1.34	0.00053	SL1344_1224A	118.87	-0.41	0.17284
SL1344_0968	220.04	0.79	0.01380	SL1344_1235	471.11	-2.18	2.60E-12
SL1344_0969	337.49	1.33	4.02E-07	SL1344_1236	424.57	1.16	4.71E-05
SL1344_0971	429.25	1.70	1.05E-12	SL1344_1243	6788.57	-0.09	0.85853
SL1344_0972	589.77	1.09	0.00020	SL1344_1244	1956.87	1.83	1.23E-08
SL1344_0973	48.03	1.17	0.00101	SL1344_1259	1731.99	-1.87	1.16E-12
SL1344_0974	40.36	-0.08	0.83744	SL1344_1263	4048.13	-1.85	0.00047
SL1344_0975	98.47	0.56	0.08761	SL1344_1264	149.65	-2.80	3.73E-10
SL1344_0976	78.69	0.63	0.03747	SL1344_1265	312.70	-0.80	0.01758
SL1344_0977	143.79	0.74	0.01865	SL1344_1300	413.14	0.86	0.02657
SL1344_0978	74.38	0.94	0.00467	SL1344_1302	981.67	1.14	8.88E-06
SL1344_0979	129.20	1.06	4.64E-05	SL1344_1330A	192.23	-2.43	5.97E-12
SL1344_0980	1336.23	0.48	0.07530	SL1344_1344	84.28	1.99	1.72E-05
SL1344_0981	79.86	0.91	0.00224	SL1344_1373	618.19	-0.46	0.07154
SL1344_0982	688.99	-1.12	5.31E-07	SL1344_1403	171.33	1.16	9.93E-05
SL1344_0984	287.96	-0.55	0.07477	SL1344_1414	1003.48	-0.03	0.90080
SL1344_0985	116.32	0.49	0.10935	SL1344_1421	1662.80	1.57	0.00012
SL1344_0986	73.69	2.00	6.10E-10	SL1344_1422	464.51	1.05	0.02420
SL1344_0987	553.29	0.33	0.21548	SL1344_1423	1006.82	0.89	0.26141
SL1344_0988	341.10	0.85	0.00383	SL1344_1424	498.66	0.03	0.94555
SL1344_0989	79.17	1.42	0.00028	SL1344_1426	465.18	0.68	0.01653
SL1344_0990	19.45	1.16	0.00902	SL1344_1427	280.93	4.14	5.17E-37
SL1344_0993	211.17	1.39	7.40E-07	SL1344_1443	262.79	0.70	0.02478
SL1344_0994	39.17	0.53	0.12546	SL1344_1457	2855.48	0.19	0.55746
SL1344_1000	322.86	0.30	0.22212	SL1344_1458	192.67	-0.15	0.55919
SL1344_1018	192.02	-1.35	1.25E-06	SL1344_1459	330.90	0.02	0.94788
SL1344_1021	231.42	-0.13	0.60909	SL1344_1461	108.76	1.17	0.00014
SL1344_1022	253.90	-0.40	0.11091	SL1344_1464	47.00	1.27	0.00011
SL1344_1028	215.72	-2.41	4.64E-08	SL1344_1469	557.76	0.45	0.09480
SL1344_1032	97.31	-1.77	2.65E-05	SL1344_1470	1928.49	-1.67	1.41E-06
SL1344_1036	915.74	-2.14	8.89E-17	SL1344_1471	271.51	1.20	0.00051
SL1344_1048	119.78	0.81	0.01096	SL1344_1472	227.69	1.52	2.04E-06
SL1344_1049	152.00	-0.52	0.08206	SL1344_1474	234.20	0.56	0.18345
SL1344_1061	307.85	-0.95	0.00025	SL1344_1475	345.09	0.01	0.97386
SL1344_1065	1254.61	0.06	0.81927	SL1344_1476	164.76	-1.90	2.67E-11
SL1344_1066	1191.16	-0.88	0.04915	SL1344_1477	545.76	-1.72	4.66E-11
SL1344_1067	371.76	-1.26	0.11883	SL1344_1478	461.29	-1.89	5.57E-12
SL1344_1068	3695.21	0.46	0.67538	SL1344_1479	306.48	-1.41	4.20E-06
SL1344_1070	1358.32	1.56	0.09431	SL1344_1480	221.57	-2.33	1.77E-13
SL1344_1084	228.72	-0.73	0.01610	SL1344_1481	217.32	-0.96	0.01245
SL1344_1124	1524.31	-1.40	1.27E-08	SL1344_1482	308.96	-0.06	0.86396
SL1344_1125	323.29	-1.21	4.23E-07	SL1344_1483	80.46	1.33	0.00050
SL1344_1165	450.33	-0.55	0.02807	SL1344_1484	3960.07	0.88	0.00526
SL1344_1166	1828.37	0.55	0.08295	SL1344_1485	150.33	0.33	0.25066
SL1344_1174	147.94	2.05	6.07E-10	SL1344_1486	307.65	0.39	0.16419
SL1344_1177	1197.02	-0.20	0.53079	SL1344_1487	511.41	0.30	0.22130
SL1344_1186	970.92	0.39	0.26123	SL1344_1488	1632.97	1.99	1.62E-07
SL1344_1187	4078.67	-0.27	0.57317	SL1344_1489	497.09	1.91	6.02E-09
SL1344_1188	418.64	-0.11	0.71473	SL1344_1490	451.35	2.19	2.84E-10
SL1344_1189	1344.17	1.41	0.00045	SL1344_1491	363.17	1.74	6.22E-08
SL1344_1190	9562.74	2.32	3.02E-09	SL1344_1504	954.02	0.47	0.18289
SL1344_1191	633.05	0.33	0.24354	SL1344_1506	578.70	-0.65	0.02200
SL1344_1192	131.59	0.90	0.00582	SL1344_1516	504.97	-0.71	0.00338

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
SL1344_1517	2556.73	1.56	1.33E-09	SL1344_1786	460.68	-0.56	0.03231
SL1344_1530A	130.51	-0.17	0.62509	SL1344_1787	499.63	-0.58	0.03312
SL1344_1536	408.27	-0.87	0.00107	SL1344_1788	377.15	-0.45	0.08158
SL1344_1537	1734.77	-0.51	0.04581	SL1344_1789	847.17	-1.77	1.07E-09
SL1344_1542	162.47	0.35	0.18703	SL1344_1791	32.43	0.93	0.01683
SL1344_1543	44.07	0.66	0.09097	SL1344_1792	54.64	-0.19	0.60687
SL1344_1544	156.30	0.64	0.03511	SL1344_1794	25.16	0.29	0.45239
SL1344_1545	45.76	-0.26	0.45702	SL1344_1795	256.81	0.58	0.03613
SL1344_1546	46.55	-0.28	0.39550	SL1344_1796	16.50	1.34	0.00861
SL1344_1547	63.27	1.01	0.00326	SL1344_1798	32.93	1.20	0.00169
SL1344_1548	151.11	-0.34	0.27581	SL1344_1801	67.68	0.35	0.30920
SL1344_1550	195.39	0.35	0.30034	SL1344_1802	328.72	-1.49	2.28E-07
SL1344_1551	147.83	0.16	0.60368	SL1344_1803	114.93	0.77	0.03103
SL1344_1553	337.40	0.73	0.00125	SL1344_1804	37.95	3.01	2.66E-09
SL1344_1554	345.57	-0.13	0.59940	SL1344_1805	141.61	2.93	4.64E-15
SL1344_1558	505.11	1.47	3.90E-07	SL1344_1806	205.31	-0.38	0.14295
SL1344_1560	174.88	1.68	0.00020	SL1344_1807	417.02	-0.54	0.11722
SL1344_1562	99.42	0.37	0.31953	SL1344_1808	983.27	-0.05	0.85778
SL1344_1563	247.23	-0.29	0.30793	SL1344_1811	218.89	-0.30	0.29965
SL1344_1564	36.68	1.74	0.00010	SL1344_1845	429.69	0.11	0.66994
SL1344_1565	36.00	0.58	0.16492	SL1344_1846	1395.90	-0.43	0.15011
SL1344_1566	87.00	1.47	0.00028	SL1344_1866	108.15	0.44	0.12734
SL1344_1567	254.85	-0.07	0.84433	SL1344_1867	701.35	0.97	0.00103
SL1344_1568	1945.70	-0.98	0.00251	SL1344_1872	3177.55	-1.77	2.65E-09
SL1344_1573	479.21	0.99	0.00011	SL1344_1873	1902.52	-2.67	7.95E-15
SL1344_1579	37.35	1.80	5.27E-06	SL1344_1874	617.22	-3.18	5.89E-31
SL1344_1580	81.36	0.71	0.02732	SL1344_1896	530.08	-2.69	1.60E-22
SL1344_1583	365.99	-2.04	7.24E-15	SL1344_1916	528.67	1.60	9.58E-13
SL1344_1584	147.32	-1.46	0.00019	SL1344_1922	165.06	-1.47	0.00018
SL1344_1588	572.49	-0.99	0.00077	SL1344_1927	264.86	-2.27	1.98E-14
SL1344_1595	185.75	-0.56	0.03938	SL1344_1927A	347.32	-0.86	0.00098
SL1344_1596	264.02	-1.61	3.64E-07	SL1344_1927B	709.59	-1.09	0.00016
SL1344_1597	74.94	0.12	0.76668	SL1344_1929	196.92	0.21	0.44978
SL1344_1598	71.04	0.53	0.10634	SL1344_1930	200.91	0.57	0.05651
SL1344_1599	175.77	1.90	5.95E-08	SL1344_1931	50.41	2.03	1.45E-06
SL1344_1600	82.85	2.03	1.01E-06	SL1344_1932	75.03	0.40	0.24150
SL1344_1602	1626.81	-2.10	1.74E-17	SL1344_1933	111.39	0.89	0.00325
SL1344_1603	801.43	-1.22	6.49E-07	SL1344_1934	18.46	0.75	0.09226
SL1344_1604	244.07	1.12	0.00180	SL1344_1935	93.40	0.03	0.92641
SL1344_1605	591.58	2.30	1.93E-10	SL1344_1936	133.22	0.58	0.02691
SL1344_1606	622.02	-0.73	0.00783	SL1344_1937	199.23	1.04	0.00020
SL1344_1607	244.73	-0.92	0.00027	SL1344_1938	427.20	0.77	0.00543
SL1344_1608	351.37	-0.20	0.38762	SL1344_1939	69.48	0.35	0.28075
SL1344_1627	775.74	-1.56	2.08E-09	SL1344_1940	49.60	-1.08	0.00084
SL1344_1629	1.11	-1.25	0.26733	SL1344_1941	217.19	0.21	0.45786
SL1344_1635	51.98	-1.05	0.00113	SL1344_1942	139.92	0.38	0.30178
SL1344_1664	122.28	-0.76	0.03584	SL1344_1943	13.06	0.89	0.06519
SL1344_1672	403.93	-0.04	0.89514	SL1344_1944	29.33	0.90	0.03577
SL1344_1678	1759.51	-2.89	1.70E-16	SL1344_1945	16.82	0.47	0.34304
SL1344_1688	831.77	-0.50	0.05103	SL1344_1946	39.20	0.11	0.76484
SL1344_1713	1479.28	-1.28	4.20E-07	SL1344_1947	153.18	0.81	0.01052
SL1344_1715	174.43	1.85	3.81E-07	SL1344_1948	79.38	1.08	0.00270
SL1344_1722	24.40	-1.63	0.00013	SL1344_1949	145.96	1.05	0.00177
SL1344_1723	3081.64	-1.26	0.00046	SL1344_1950	321.97	0.54	0.08866
SL1344_1737	2844.99	-1.62	4.36E-09	SL1344_1951	143.15	0.29	0.43649
SL1344_1738	590.07	-1.45	2.79E-05	SL1344_1952	52.36	-0.54	0.12385
SL1344_1756	530.89	0.48	0.02969	SL1344_1953	214.96	-1.51	3.42E-10
SL1344_1759	6219.42	1.31	1.90E-05	SL1344_1954	154.51	-0.46	0.22553
SL1344_1760	1689.52	3.13	3.50E-15	SL1344_1956	85.64	0.69	0.04591
SL1344_1761	6274.18	4.16	2.18E-28	SL1344_1957	99.48	-0.20	0.51680
SL1344_1762	172.65	1.84	1.11E-09	SL1344_1958	477.96	-0.30	0.24997
SL1344_1768	9472.63	-0.41	0.18316	SL1344_1959	166.60	-0.06	0.83363
SL1344_1770	1548.93	0.98	0.00381	SL1344_1960	287.50	-0.03	0.89755
SL1344_1772	416.47	0.65	0.00749	SL1344_1961	368.13	-0.68	0.02741
SL1344_1778	2315.38	1.05	3.85E-06	SL1344_1962	311.00	-0.59	0.03292
SL1344_1780	273.14	1.10	1.64E-05	SL1344_1963	232.33	-0.19	0.55894
SL1344_1783	1738.78	-0.79	0.01232	SL1344_1964	322.59	-0.61	0.01260
SL1344_1785	119.25	-0.60	0.12683	SL1344_1965	536.97	-1.83	2.95E-12

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
SL1344_1966	2053.32	-3.16	2.24E-24	SL1344_2283	6150.39	-2.20	3.01E-07
SL1344_1967	9400.94	-0.53	0.06299	SL1344_2298	138.21	-0.31	0.33465
SL1344_1968	778.58	-1.22	0.00103	SL1344_2301	1170.80	-1.13	4.64E-06
SL1344_1969	439.58	0.23	0.56437	SL1344_2305	2418.12	-0.96	7.30E-05
SL1344_1970	266.19	-0.23	0.56801	SL1344_2309	810.87	2.42	4.19E-17
SL1344_1971	238.40	0.18	0.63322	SL1344_2310	303.90	2.54	4.54E-16
SL1344_1972	281.77	0.18	0.58790	SL1344_2311	290.27	2.28	6.54E-18
SL1344_1973	425.56	0.49	0.08213	SL1344_2312	115.83	0.98	0.00296
SL1344_1974	157.82	-0.16	0.62008	SL1344_2313	120.52	-1.04	0.00194
SL1344_1975	49.02	1.41	0.00013	SL1344_2314	479.66	-1.04	3.50E-05
SL1344_1976	5016.46	-2.86	2.56E-23	SL1344_2315	1609.07	-0.35	0.16720
SL1344_1978	783.29	-3.19	1.08E-18	SL1344_2326	1242.59	-0.94	1.87E-05
SL1344_1979	798.85	-2.00	2.56E-11	SL1344_2327	209.51	0.83	0.00093
SL1344_1980	452.97	-1.67	9.39E-08	SL1344_2328	249.12	0.86	0.00968
SL1344_1981	390.84	-0.52	0.07038	SL1344_2329	235.00	0.45	0.20127
SL1344_1982	650.44	-0.33	0.29677	SL1344_2330	1767.10	-2.99	2.19E-15
SL1344_1983	358.40	0.04	0.87347	SL1344_2341	637.19	-0.19	0.41024
SL1344_1985	113.45	0.44	0.14104	SL1344_2342	120.01	-0.06	0.85338
SL1344_1987	926.82	-3.17	1.18E-14	SL1344_2343	36.51	1.13	0.00592
SL1344_1988	1319.11	-2.07	4.24E-10	SL1344_2344	62.86	-0.01	0.98732
SL1344_1989	582.94	-1.96	8.61E-11	SL1344_2345	153.92	-0.07	0.80148
SL1344_2042	14.07	0.30	0.53760	SL1344_2346	1267.28	0.41	0.13289
SL1344_2103	465.17	0.02	0.93590	SL1344_2368	1967.20	-2.06	4.19E-11
SL1344_2109	91.18	1.49	8.29E-06	SL1344_2372	230.26	0.67	0.01686
SL1344_2110	43.33	1.72	5.50E-06	SL1344_2373	253.83	0.37	0.15039
SL1344_2111	475.05	0.60	0.02485	SL1344_2374	567.05	-0.80	0.00248
SL1344_2115	138.45	0.24	0.43850	SL1344_2383	33.71	-0.34	0.50181
SL1344_2116	40.14	0.88	0.01890	SL1344_2389	29.33	-0.49	0.23821
SL1344_2125	2610.87	-2.04	1.36E-08	SL1344_2397	127.22	0.09	0.77437
SL1344_2134	341.92	-0.78	0.01471	SL1344_2409	1344.77	0.27	0.32437
SL1344_2152	250.65	0.49	0.14830	SL1344_2412	485.83	-0.79	0.00373
SL1344_2153	104.34	-0.80	0.02747	SL1344_2415	173.75	0.15	0.55855
SL1344_2154	92.18	0.25	0.65054	SL1344_2416	446.00	0.07	0.76474
SL1344_2155	350.01	-0.91	0.09998	SL1344_2438	2443.20	-2.96	9.08E-18
SL1344_2156	235.48	0.43	0.31806	SL1344_2441	631.71	-1.17	6.00E-05
SL1344_2157	1099.91	-1.90	2.25E-14	SL1344_2447	1246.57	-0.44	0.05946
SL1344_2163	411.67	-0.56	0.09376	SL1344_2449	852.22	-1.18	1.33E-06
SL1344_2172	154.45	-0.12	0.66814	SL1344_2455	125.57	-0.37	0.28926
SL1344_2173	270.59	0.22	0.39088	SL1344_2457	1452.83	-0.40	0.09005
SL1344_2174	67.33	1.90	2.11E-06	SL1344_2466	435.27	0.53	0.10777
SL1344_2175	307.66	1.06	0.00010	SL1344_2467	33.46	-1.10	0.00369
SL1344_2187	290.56	-0.83	0.00270	SL1344_2468	451.04	0.40	0.40920
SL1344_2202	1167.85	-0.51	0.10356	SL1344_2469	285.99	-1.65	3.30E-05
SL1344_2206	423.57	-2.20	1.69E-13	SL1344_2470	171.23	-0.28	0.39762
SL1344_2207	95.82	-0.88	0.06078	SL1344_2489	178.56	0.56	0.05041
SL1344_2209	215.36	-0.96	0.00887	SL1344_2490	111.36	3.26	6.97E-25
SL1344_2210	44.41	0.64	0.08058	SL1344_2491	57.73	1.44	8.16E-05
SL1344_2211	191.27	1.31	3.91E-05	SL1344_2492	256.09	1.62	7.87E-08
SL1344_2212	42.53	2.18	8.73E-09	SL1344_2494	1909.82	-0.01	0.97696
SL1344_2213	88.01	2.14	1.81E-11	SL1344_2495	626.65	-1.55	9.50E-09
SL1344_2214	4068.62	-1.48	9.20E-06	SL1344_2496	426.85	-0.80	0.01714
SL1344_2215	137.79	1.28	0.00011	SL1344_2507	4700.59	-0.61	0.05892
SL1344_2216	309.91	0.84	0.00216	SL1344_2509	563.32	-0.21	0.37711
SL1344_2218	36.60	1.58	0.00041	SL1344_2513	246.36	0.53	0.06060
SL1344_2219	78.04	1.34	0.00030	SL1344_2514	416.13	-1.07	1.47E-06
SL1344_2220	24.84	0.33	0.53976	SL1344_2532	455.15	-0.84	0.01497
SL1344_2221	167.41	-0.07	0.86172	SL1344_2535	489.14	-0.25	0.29546
SL1344_2242	317.97	0.04	0.90335	SL1344_2536	193.87	1.37	0.00026
SL1344_2243	174.19	2.40	1.45E-09	SL1344_2537	280.62	-0.28	0.42467
SL1344_2244	424.49	-2.00	6.50E-09	SL1344_2547	686.32	-0.32	0.20636
SL1344_2249	692.25	-0.68	0.00388	SL1344_2548	19.63	0.45	0.33697
SL1344_2250	3151.98	-3.59	1.32E-41	SL1344_2549	86.10	0.02	0.94381
SL1344_2257	60.34	0.71	0.04232	SL1344_2550	49.33	2.73	1.30E-08
SL1344_2258	248.34	1.23	1.52E-07	SL1344_2551	44.47	2.91	1.37E-11
SL1344_2262	1359.04	-0.35	0.16434	SL1344_2552	493.14	0.06	0.83615
SL1344_2269	1420.85	2.43	6.96E-23	SL1344_2552A	15.22	1.63	0.00091
SL1344_2271	213.87	0.86	0.00410	SL1344_2553	411.07	1.73	9.56E-10
SL1344_2272	448.59	-1.10	0.00154	SL1344_2554	85.08	2.05	4.17E-09

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
SL1344_2555	138.90	0.60	0.04585	SL1344_2682	12.21	1.05	0.06006
SL1344_2556	259.86	-0.48	0.07210	SL1344_2683	0.57	1.47	0.20124
SL1344_2557	55.42	1.19	0.00138	SL1344_2684	19.51	3.09	1.59E-08
SL1344_2558	981.14	-0.06	0.81804	SL1344_2685	165.03	1.47	3.45E-06
SL1344_2559	27.85	1.93	7.38E-06	SL1344_2688	1.65	2.87	0.00875
SL1344_2560	107.53	2.10	6.53E-10	SL1344_2689	30.40	2.06	1.08E-05
SL1344_2561	316.79	0.37	0.14300	SL1344_2690	40.40	4.08	1.34E-14
SL1344_2562	179.48	0.44	0.10627	SL1344_2691	92.97	2.25	1.89E-11
SL1344_2564	321.97	-0.38	0.11826	SL1344_2692	82.66	3.68	1.74E-15
SL1344_2565	58.28	0.68	0.06120	SL1344_2693	170.33	2.43	1.09E-14
SL1344_2566	87.30	0.66	0.04795	SL1344_2694	177.23	1.41	5.70E-06
SL1344_2567	293.27	0.81	0.00263	SL1344_2695	1473.61	-0.92	0.00157
SL1344_2568	62.20	1.93	3.95E-07	SL1344_2696	2502.91	-2.57	1.55E-12
SL1344_2569	227.72	0.93	0.00116	SL1344_2697	2944.75	-0.67	0.10280
SL1344_2570	445.01	0.59	0.02166	SL1344_2698	2429.19	-2.03	2.83E-09
SL1344_2571	12.85	2.20	9.76E-05	SL1344_2699	562.66	-3.07	1.15E-22
SL1344_2572	391.83	1.29	1.42E-05	SL1344_2700	195.41	-2.33	8.82E-11
SL1344_2573	49.80	0.86	0.02721	SL1344_2701	294.06	1.00	0.00119
SL1344_2574	633.13	-2.53	2.03E-08	SL1344_2702	93.38	0.25	0.50193
SL1344_2575	89.70	1.16	5.97E-05	SL1344_2703	47.51	-0.07	0.88943
SL1344_2576	104.37	0.13	0.68481	SL1344_2704	41.09	0.08	0.87468
SL1344_2577	837.23	-1.21	2.55E-07	SL1344_2705	751.70	-0.59	0.05844
SL1344_2578	393.51	-2.27	7.45E-10	SL1344_2709	374.70	0.03	0.91788
SL1344_2579	120.93	0.64	0.05698	SL1344_2710	852.45	-2.20	4.72E-11
SL1344_2580	0.93	0.57	0.61292	SL1344_2712	250.10	-0.45	0.21777
SL1344_2581	141.81	0.03	0.93886	SL1344_2713	1904.88	-0.89	0.03111
SL1344_2582	153.45	-0.63	0.03398	SL1344_2714	113.91	-1.19	0.00158
SL1344_2583	697.80	-2.24	3.73E-16	SL1344_2715	952.79	-2.26	2.86E-08
SL1344_2584	953.06	-3.41	3.11E-32	SL1344_2716	191.33	0.17	0.52235
SL1344_2585	1326.58	-0.70	0.04318	SL1344_2717	38.06	-0.25	0.48786
SL1344_2586	57.54	0.05	0.87377	SL1344_2718	80.74	1.70	2.61E-06
SL1344_2587	128.75	0.67	0.03136	SL1344_2719	122.96	0.18	0.51353
SL1344_2588	72.75	0.44	0.15577	SL1344_2720	5.29	3.26	0.00021
SL1344_2589	95.04	0.36	0.25493	SL1344_2721	16.37	2.31	6.85E-06
SL1344_2593	13114.03	-1.90	9.92E-07	SL1344_2722	522.59	0.14	0.63303
SL1344_2594	18884.54	-1.57	3.26E-05	SL1344_2723	426.63	-0.30	0.35817
SL1344_2595	155.92	-0.41	0.14701	SL1344_2725	134.75	2.27	8.08E-11
SL1344_2596	179.10	-0.91	0.00440	SL1344_2726	3046.29	0.02	0.95210
SL1344_2597A	340.63	1.66	2.64E-09	SL1344_2727	1571.69	-2.18	6.95E-12
SL1344_2598	47.31	0.50	0.15300	SL1344_2728	26.85	3.26	2.63E-09
SL1344_2599	24.85	0.71	0.09048	SL1344_2729	202.97	1.92	5.09E-07
SL1344_2600	666.12	-1.28	4.86E-07	SL1344_2730	3805.41	-0.40	0.23822
SL1344_2619	370.00	-1.47	1.27E-05	SL1344_2731	2183.90	0.01	0.98131
SL1344_2626	412.14	-0.20	0.41397	SL1344_2732	645.27	-1.69	1.00E-11
SL1344_2632	2867.04	-0.92	0.00155	SL1344_2733	77.07	2.28	2.50E-11
SL1344_2633	1587.21	-1.33	3.97E-08	SL1344_2734	46.84	2.94	1.76E-11
SL1344_2634	45.01	1.73	0.00033	SL1344_2735	4.55	1.32	0.07183
SL1344_2636	1.42	-0.46	0.65082	SL1344_2736	127.29	2.37	2.48E-10
SL1344_2637	19.33	1.55	0.00239	SL1344_2737	358.36	-0.56	0.04134
SL1344_2638	29.93	2.61	3.93E-06	SL1344_2738	1299.98	-0.57	0.05481
SL1344_2639	8.23	-0.09	0.89097	SL1344_2741	85.08	2.63	8.29E-11
SL1344_2640	161.64	-1.47	0.00016	SL1344_2742	256.05	1.21	0.00134
SL1344_2641	243.40	1.22	4.51E-05	SL1344_2743	254.71	0.85	0.01256
SL1344_2644	7042.92	-2.98	2.10E-19	SL1344_2744	129.97	1.23	0.00014
SL1344_2653	1548.80	-1.39	7.53E-07	SL1344_2745	315.99	0.30	0.23589
SL1344_2662	106.67	0.85	0.00387	SL1344_2746	391.16	-0.67	0.02591
SL1344_2663	308.76	2.40	5.12E-14	SL1344_2750	86.42	0.54	0.15008
SL1344_2664	175.01	0.08	0.81458	SL1344_2751	958.92	0.59	0.07932
SL1344_2665	163.42	-1.86	9.72E-09	SL1344_2752	9715.29	-2.20	3.01E-14
SL1344_2666	205.93	0.11	0.71991	SL1344_2753	365.73	-1.66	2.83E-08
SL1344_2668	325.98	2.42	1.42E-14	SL1344_2754	596.40	-1.80	3.79E-13
SL1344_2671	75.88	1.86	6.48E-09	SL1344_2770	220.53	0.00	0.99800
SL1344_2672	173.01	1.40	6.58E-06	SL1344_2771	24.27	2.06	6.55E-06
SL1344_2673	46.49	1.59	0.00022	SL1344_2772	378.94	-0.67	0.00758
SL1344_2675	120.77	0.45	0.15310	SL1344_2781	2435.34	-2.65	8.09E-13
SL1344_2676	259.70	1.37	1.72E-05	SL1344_2784	545.04	0.79	0.00980
SL1344_2680	67.03	2.96	9.07E-14	SL1344_2787	308.27	-0.11	0.66754
SL1344_2681	9.85	2.29	0.00044	SL1344_2788	106.56	-0.55	0.07667

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
SL1344_2789	195.88	1.52	0.00012	SL1344_3105	135.50	-1.06	0.00081
SL1344_2797	651.77	0.45	0.05294	SL1344_3106	285.86	0.61	0.06162
SL1344_2801	351.77	-0.25	0.34069	SL1344_3107	234.14	0.23	0.56075
SL1344_2824	152.15	1.01	0.00107	SL1344_3112	9241.46	1.03	7.62E-05
SL1344_2834	996.51	-2.03	4.98E-06	SL1344_3115	167.61	0.30	0.32290
SL1344_2880	1363.18	-0.88	0.00995	SL1344_3116	307.88	1.03	0.00112
SL1344_2881	170.00	-0.60	0.07451	SL1344_3126	1773.43	0.09	0.75475
SL1344_2882	147.79	-0.65	0.01288	SL1344_3128	2455.76	-1.89	3.80E-08
SL1344_2883	124.21	0.52	0.16753	SL1344_3129	819.13	-1.81	1.49E-05
SL1344_2884	305.28	-2.72	1.78E-23	SL1344_3130	511.46	-0.80	0.03112
SL1344_2885	517.46	-1.32	1.06E-06	SL1344_3134	282.44	-2.15	3.86E-14
SL1344_2887	326.82	-0.50	0.19021	SL1344_3140	226.40	0.53	0.07174
SL1344_2889	99.02	1.54	3.71E-05	SL1344_3141	1673.92	-2.50	4.75E-24
SL1344_2890	185.51	0.47	0.17716	SL1344_3143	417.92	-0.95	0.00325
SL1344_2891	300.01	-0.36	0.27837	SL1344_3144	48.09	0.09	0.79562
SL1344_2892	285.50	1.16	2.85E-05	SL1344_3149	447.06	0.85	0.00022
SL1344_2893	100.64	2.09	1.37E-09	SL1344_3166	164.60	0.23	0.36182
SL1344_2899	439.23	-2.29	2.63E-16	SL1344_3167	442.19	0.26	0.25755
SL1344_2900	65.90	1.84	2.66E-07	SL1344_3171	151.71	-0.27	0.35992
SL1344_2901	158.02	0.75	0.00866	SL1344_3189	13275.75	-0.18	0.54223
SL1344_2902	141.36	0.46	0.16032	SL1344_3224	384.10	-1.08	0.00121
SL1344_2917	394.77	1.57	6.35E-11	SL1344_3227	59.99	2.92	1.36E-14
SL1344_2919	175.47	2.39	3.59E-17	SL1344_3228	400.19	1.44	1.83E-08
SL1344_2921	111.67	0.92	0.00194	SL1344_3229	150.73	2.28	2.41E-12
SL1344_2922	338.69	0.81	0.00052	SL1344_3230	378.32	0.34	0.22136
SL1344_2929	566.16	-1.16	0.00041	SL1344_3250	121.24	0.76	0.04762
SL1344_2934	35.15	-1.94	1.13E-05	SL1344_3251	117.01	0.38	0.28798
SL1344_2935	57.13	-1.75	8.37E-06	SL1344_3263	1682.60	-1.88	7.62E-14
SL1344_2936	922.85	-2.12	9.15E-11	SL1344_3315	3161.29	-0.51	0.09326
SL1344_2940	444.87	-0.59	0.01590	SL1344_3322	121.54	1.35	8.91E-05
SL1344_2944	2452.74	-1.62	1.87E-08	SL1344_3325	15.87	0.75	0.09818
SL1344_2966	433.76	-0.48	0.16565	SL1344_3328	172.71	2.36	1.34E-12
SL1344_2990	456.53	-1.83	5.72E-09	SL1344_3329	651.38	-0.02	0.94717
SL1344_2998	611.49	-0.69	0.00419	SL1344_3330	1004.83	-0.66	0.00514
SL1344_2999	226.79	-0.42	0.15942	SL1344_3334	826.64	-0.06	0.86580
SL1344_3000	1103.54	0.49	0.04549	SL1344_3350	763.53	-1.43	0.00013
SL1344_3009	1084.57	-1.41	2.00E-07	SL1344_3351	473.41	-1.01	8.72E-05
SL1344_3010	124.06	0.49	0.15176	SL1344_3361	725.03	-0.18	0.47541
SL1344_3011	888.36	-2.16	4.68E-06	SL1344_3368	1201.91	-0.58	0.02053
SL1344_3012	3790.17	-3.18	1.51E-15	SL1344_3378	678.68	-1.55	7.65E-06
SL1344_3013	1821.14	-3.89	4.77E-36	SL1344_3427	58.44	-1.23	0.00042
SL1344_3014	1091.11	-0.92	0.00012	SL1344_3428	3482.70	-2.55	4.26E-18
SL1344_3028	266.07	0.36	0.24039	SL1344_3475	640.45	-1.30	1.49E-07
SL1344_3047	40.96	2.13	1.45E-08	SL1344_3483	400.05	-2.55	2.71E-15
SL1344_3048	30.73	1.60	0.00039	SL1344_3484	697.49	-3.51	3.99E-43
SL1344_3049	58.52	2.11	4.63E-08	SL1344_3487	332.23	-0.57	0.02565
SL1344_3050	128.47	-0.31	0.31616	SL1344_3494	2336.35	-1.82	1.96E-10
SL1344_3051	66.20	0.64	0.03341	SL1344_3495	1516.12	-1.25	1.58E-07
SL1344_3055	284.00	-0.31	0.30549	SL1344_3496	299.14	0.35	0.23023
SL1344_3056	61.57	1.00	0.00231	SL1344_3497	223.70	1.52	0.00015
SL1344_3057	168.45	0.68	0.03947	SL1344_3498	337.45	1.53	1.20E-06
SL1344_3058	140.13	0.11	0.73285	SL1344_3499	332.60	0.54	0.05250
SL1344_3059	544.10	-1.76	1.82E-05	SL1344_3500	1993.21	-1.72	1.87E-13
SL1344_3060	7742.16	-2.03	9.15E-18	SL1344_3513	272.46	0.13	0.71728
SL1344_3061	267.91	0.35	0.18286	SL1344_3514	197.94	2.43	7.63E-14
SL1344_3073	336.46	-0.38	0.11356	SL1344_3515	106.03	2.40	7.35E-10
SL1344_3091	2953.44	-4.51	4.48E-29	SL1344_3516	228.93	1.34	8.27E-06
SL1344_3092	1540.39	-2.85	3.07E-14	SL1344_3545	3076.18	-0.22	0.43334
SL1344_3093	121.37	-0.89	0.01033	SL1344_3560	656.99	-1.56	2.26E-08
SL1344_3094	734.71	-2.47	2.73E-13	SL1344_3563	270.44	0.25	0.42228
SL1344_3095	359.73	-1.30	8.90E-05	SL1344_3564	226.79	1.45	0.00069
SL1344_3096	1060.65	-1.01	0.00021	SL1344_3565	179.64	1.25	0.00015
SL1344_3097	186.89	0.75	0.02392	SL1344_3566	703.07	-0.79	0.00691
SL1344_3098	464.53	-1.82	5.16E-08	SL1344_3567	1310.76	-1.00	0.00040
SL1344_3099	199.61	0.19	0.50363	SL1344_3569	1191.96	-1.82	3.07E-08
SL1344_3100	411.84	0.98	0.00033	SL1344_3570	44.91	1.41	0.00073
SL1344_3101	247.15	0.24	0.33624	SL1344_3597	414.72	-0.73	0.01413
SL1344_3103	459.72	0.01	0.97971	SL1344_3598	239.75	-0.64	0.07315

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
SL1344_3599	617.66	-0.85	0.00121	SL1344_3956	414.59	-0.49	0.09571
SL1344_3616	2282.86	-1.35	7.97E-07	SL1344_3957	247.40	0.34	0.26871
SL1344_3617	424.35	-2.00	9.67E-11	SL1344_3958	173.96	0.85	0.00514
SL1344_3618	207.65	-1.40	1.93E-06	SL1344_3959	134.89	0.31	0.52909
SL1344_3622	708.20	-0.94	0.00342	SL1344_3960	121.48	1.07	0.00561
SL1344_3635	100.07	2.51	7.51E-09	SL1344_3961	235.83	1.58	5.52E-06
SL1344_3643	200.78	0.09	0.76034	SL1344_3976	595.01	-1.32	1.57E-08
SL1344_3644	306.31	1.16	0.00018	SL1344_3977	183.19	-1.69	1.81E-08
SL1344_3646	277.76	0.39	0.16904	SL1344_3978	533.36	-1.15	0.00011
SL1344_3653	836.58	-1.82	4.26E-08	SL1344_3979	504.96	-2.29	3.58E-12
SL1344_3655	143.48	0.96	0.01684	SL1344_3980	193.22	-1.00	0.00020
SL1344_3661	604.23	0.54	0.02770	SL1344_3987	998.71	-1.79	1.21E-05
SL1344_3662	327.74	0.21	0.46801	SL1344_3989	261.49	-2.30	3.89E-18
SL1344_3663	625.82	-0.94	0.00020	SL1344_3990	495.59	-2.14	2.60E-10
SL1344_3664	135.43	-0.21	0.44066	SL1344_3991	1277.89	-1.86	2.08E-13
SL1344_3673A	706.37	2.57	1.41E-16	SL1344_3993	482.77	-0.82	0.00452
SL1344_3702	590.47	-0.79	0.00063	SL1344_4000	87.96	0.87	0.02307
SL1344_3703	520.47	-0.36	0.10825	SL1344_4001	230.90	1.05	0.00035
SL1344_3704	752.14	1.05	0.00011	SL1344_4002	90.90	0.83	0.03667
SL1344_3711	900.10	0.04	0.85866	SL1344_4003	244.86	0.58	0.06346
SL1344_3717	424.97	-1.00	0.00080	SL1344_4006	347.92	-1.15	0.00055
SL1344_3731	704.40	-1.80	4.08E-09	SL1344_4014	265.30	0.60	0.08683
SL1344_3732	91.13	1.32	2.04E-06	SL1344_4015	183.79	1.44	1.71E-05
SL1344_3733	185.35	1.13	0.00017	SL1344_4016	530.18	-0.06	0.76765
SL1344_3734	240.04	0.77	0.01175	SL1344_4017	831.86	-0.62	0.00825
SL1344_3735	110.73	1.23	2.17E-05	SL1344_4018	205.11	-2.10	1.72E-12
SL1344_3736	83.66	0.00	0.98881	SL1344_4020	143.97	-1.09	0.00071
SL1344_3737	55.88	-0.36	0.31079	SL1344_4029	833.73	-0.03	0.89199
SL1344_3738	2673.11	-1.43	9.85E-09	SL1344_4046	718.07	-0.73	0.00382
SL1344_3739	145.53	0.53	0.14152	SL1344_4047	1282.00	-2.05	6.68E-11
SL1344_3740	1421.46	-2.41	1.53E-08	SL1344_4051	326.91	-0.83	0.00520
SL1344_3743	1354.72	-4.39	4.35E-53	SL1344_4052	383.59	-0.40	0.25977
SL1344_3744	114.57	-1.83	1.97E-08	SL1344_4053	521.13	-1.71	1.06E-07
SL1344_3745	131.97	0.69	0.02841	SL1344_4054	459.53	-0.43	0.11665
SL1344_3746	154.58	1.55	2.64E-09	SL1344_4084	204.19	-1.55	7.78E-06
SL1344_3747	537.26	1.39	7.89E-09	SL1344_4094	664.39	-2.19	2.07E-07
SL1344_3748	535.11	1.54	1.39E-10	SL1344_4095	459.00	-4.39	2.25E-21
SL1344_3749	517.52	0.74	0.02155	SL1344_4097	251.78	-2.66	3.89E-11
SL1344_3750	619.77	-2.43	5.83E-10	SL1344_4121	112.69	-0.81	0.00322
SL1344_3751	4200.58	-4.70	4.40E-48	SL1344_4126	22.63	-0.74	0.08692
SL1344_3757	244.88	-0.74	0.00352	SL1344_4127	755.49	-1.85	3.35E-08
SL1344_3758	298.63	0.05	0.85514	SL1344_4130	776.03	-0.50	0.02773
SL1344_3759	868.54	-2.80	1.13E-17	SL1344_4131	174.56	1.48	3.17E-08
SL1344_3760	540.03	-1.93	1.23E-13	SL1344_4132	49.84	0.27	0.53134
SL1344_3765	416.28	-0.81	0.01798	SL1344_4133	51.92	0.72	0.07377
SL1344_3767	377.32	-0.88	0.00109	SL1344_4134	76.05	1.49	1.42E-06
SL1344_3799	174.99	1.91	4.64E-07	SL1344_4135	415.88	2.07	2.02E-11
SL1344_3800	403.87	0.52	0.06698	SL1344_4136	82.62	0.19	0.54451
SL1344_3801	1457.89	-2.11	1.20E-18	SL1344_4137	142.52	1.12	0.00058
SL1344_3808	277.83	-0.77	0.00296	SL1344_4138	115.66	1.20	0.00057
SL1344_3812	2249.50	-2.41	4.74E-12	SL1344_4139	2043.91	-0.31	0.34005
SL1344_3813	1460.46	-2.29	5.38E-09	SL1344_4142	155.52	0.47	0.09380
SL1344_3825	1183.00	-1.41	1.03E-05	SL1344_4143	340.17	0.83	0.00056
SL1344_3826	130.32	1.36	6.28E-05	SL1344_4144	5.08	1.16	0.11417
SL1344_3827	631.73	-0.76	0.01381	SL1344_4145	188.17	0.89	0.00266
SL1344_3830	752.10	-0.22	0.38273	SL1344_4146	327.84	1.75	4.64E-09
SL1344_3866	890.34	-3.25	1.34E-18	SL1344_4147	117.26	0.75	0.02154
SL1344_3867	226.44	-1.87	4.73E-09	SL1344_4148	95.82	-0.12	0.70956
SL1344_3870A	117.15	-1.66	2.19E-05	SL1344_4149	159.46	1.09	0.00087
SL1344_3871	19.26	0.40	0.39931	SL1344_4150	6.15	1.15	0.08869
SL1344_3886	862.33	0.38	0.14329	SL1344_4151	42.92	2.06	2.17E-06
SL1344_3895	179.97	-0.42	0.10911	SL1344_4152	21.83	0.34	0.48257
SL1344_3896	148.24	-1.17	0.00152	SL1344_4153	40.87	2.71	8.05E-11
SL1344_3897	107.19	-0.96	0.01343	SL1344_4154	28.88	3.69	1.38E-11
SL1344_3899	121.79	-2.75	2.24E-18	SL1344_4155	416.01	-0.97	0.00300
SL1344_3899A	155.61	-0.18	0.57418	SL1344_4178	932.60	-0.38	0.17924
SL1344_3920	497.71	-0.41	0.15349	SL1344_4188	20.54	0.14	0.75930
SL1344_3934	1438.62	-1.29	6.56E-06	SL1344_4189	387.81	-0.62	0.01939

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
SL1344_4191	28.54	-0.09	0.82952	SL1344_4449	441.83	-0.85	0.00303
SL1344_4203	321.81	-0.59	0.02831	SL1344_4450	477.49	1.69	7.61E-09
SL1344_4206	608.59	-1.20	0.00019	SL1344_4451	8.58	0.84	0.16889
SL1344_4207	375.23	-0.52	0.05443	SL1344_4453	364.00	-1.33	0.00139
SL1344_4208	123.57	0.90	0.00471	SL1344_4459	736.85	-2.73	2.83E-27
SL1344_4212	451.69	-3.07	2.38E-21	SL1344_4460	291.59	-1.36	1.76E-08
SL1344_4239	178.52	0.21	0.55652	SL1344_4465	1405.44	-1.23	4.21E-08
SL1344_4242	739.19	2.95	1.97E-21	SL1344_4466	80.81	0.18	0.63594
SL1344_4243	226.80	2.47	1.09E-19	SL1344_4467	41.46	2.41	5.75E-08
SL1344_4244	243.16	1.59	2.52E-06	SL1344_4468	79.66	2.62	2.59E-13
SL1344_4245	245.51	2.09	2.93E-15	SL1344_4469	128.09	1.63	1.54E-09
SL1344_4246	333.62	1.45	9.00E-06	SL1344_4470	223.39	2.61	9.50E-17
SL1344_4247	895.61	2.17	8.81E-10	SL1344_4471	1486.90	1.61	3.87E-12
SL1344_4248	177.57	-0.59	0.13577	SL1344_4476	172.61	-0.21	0.49848
SL1344_4249	97.90	-0.72	0.03802	SL1344_4480	1214.96	-2.57	2.57E-28
SL1344_4252	318.68	-0.43	0.15411	SL1344_4482	1260.52	-1.11	0.00135
SL1344_4253	384.52	-1.81	6.86E-12	SL1344_4483	2667.55	-1.22	0.00067
SL1344_4254	259.09	-0.01	0.97301	SL1344_4501	379.40	0.15	0.57605
SL1344_4256	49.72	1.26	0.00512	SL1344_4502	403.33	-0.10	0.66792
SL1344_4257	641.57	-2.34	2.69E-09	SL1344_4522	126.71	0.47	0.17842
SL1344_4258	1425.37	-1.92	3.58E-08	SL1344_4523	335.42	-1.18	4.15E-05
SL1344_4288	4403.12	-2.65	2.79E-14	slmA	888.89	-0.20	0.43491
SL1344_4305	234.76	1.23	3.48E-06	slrB	72.35	3.12	1.40E-14
SL1344_4323	60.11	-0.69	0.06005	slrP	6199.00	-0.49	0.08630
SL1344_4345	691.65	-1.00	4.31E-05	sIsA	393.92	-0.63	0.05077
SL1344_4346	182.86	0.49	0.13315	sIt	2155.32	-0.01	0.96969
SL1344_4354A	65.75	1.77	5.99E-05	slyA	7346.89	-0.10	0.73690
SL1344_4355	161.99	0.66	0.05633	slyB	9195.69	-1.48	8.35E-10
SL1344_4360	116.25	2.42	1.41E-15	slyD	19696.36	-0.88	0.00133
SL1344_4364	322.69	1.97	1.10E-09	slyX	2050.41	-2.74	9.79E-15
SL1344_4369A	54.68	-0.76	0.05290	smg	8576.54	-2.63	6.82E-12
SL1344_4370	157.80	-1.90	1.54E-09	smp	326.12	-0.92	0.00039
SL1344_4371	152.98	-1.59	5.85E-06	smpA	3013.05	-0.98	0.00110
SL1344_4372	30.56	-0.65	0.10454	smpB	2981.25	-1.43	0.00047
SL1344_4373	192.29	-0.05	0.88264	smtA	1726.89	-0.92	0.00029
SL1344_4374	177.20	0.63	0.15586	smvA	3939.08	-0.29	0.33735
SL1344_4375	153.72	1.01	0.00142	sodA	20986.43	-1.92	1.08E-12
SL1344_4376	243.01	1.14	3.91E-05	sodB	19664.88	0.65	0.02013
SL1344_4377	165.42	1.32	2.33E-06	sodCa	4064.38	-0.08	0.80072
SL1344_4378	880.40	-1.00	0.00263	sodCb	433.29	0.92	0.00033
SL1344_4383	65.04	0.01	0.96442	sohB	1754.04	-0.21	0.32700
SL1344_4388	1600.40	-1.41	6.93E-08	solA	1453.17	-1.16	9.55E-05
SL1344_4393	398.72	1.92	1.63E-05	sopA	2067.80	-1.40	0.00012
SL1344_4394	963.03	1.12	0.00042	sopB	6337.34	-2.85	6.53E-09
SL1344_4395	1291.32	4.64	1.38E-18	sopD	2681.22	-2.43	7.79E-09
SL1344_4396	225.21	1.80	0.02386	sopD2	843.62	-0.44	0.07886
SL1344_4397	837.09	0.82	0.31389	sopE	20805.20	-1.47	0.00285
SL1344_4408	401.47	-1.39	5.13E-06	sopE2	1626.34	-2.10	8.54E-05
SL1344_4417	353.53	-1.72	1.10E-08	sotB	589.87	1.24	2.17E-06
SL1344_4418	4282.25	-1.32	1.63E-09	soxR	936.71	-2.47	8.92E-08
SL1344_4419	1881.40	-2.22	7.71E-15	soxS	2159.45	1.48	4.57E-05
SL1344_4420	5025.52	0.54	0.03416	spaO	2748.36	1.93	8.24E-05
SL1344_4421	4824.58	-0.41	0.10828	spaP	5899.54	2.11	5.87E-05
SL1344_4422	1356.98	-0.32	0.21788	spaQ	358.58	2.68	1.40E-08
SL1344_4423	1896.05	-0.10	0.70829	spaR	1404.73	2.58	1.06E-07
SL1344_4424	12214.05	0.22	0.47514	spaS	1788.76	0.80	0.09330
SL1344_4425	6449.93	0.01	0.94790	speA	3948.98	0.17	0.48216
SL1344_4426	634.19	-0.55	0.02805	speB	2990.18	1.27	1.82E-08
SL1344_4427	919.38	-0.30	0.22289	speC	912.19	-0.08	0.73437
SL1344_4431	122.36	-1.32	3.23E-05	speD	10946.98	-0.92	0.00013
SL1344_4432	109.79	1.50	0.00011	speE	10959.95	-1.56	5.08E-13
SL1344_4433	9770.37	-0.39	0.30178	speF	8752.76	4.70	5.64E-35
SL1344_4434	393.23	-0.80	0.01265	speG	1165.22	0.24	0.34030
SL1344_4435	290.76	-1.03	0.00100	Spf	1148.12	-0.33	0.39866
SL1344_4436	316.89	-1.29	3.42E-06	spoT	8124.21	-0.01	0.95991
SL1344_4439	24.59	-1.47	0.00123	spoU	1250.06	-0.01	0.96110
SL1344_4440	1407.30	-1.60	1.68E-06	sppA	2084.14	0.26	0.24068
SL1344_4441	230.82	0.63	0.06209	spr	7135.34	-1.69	6.72E-06

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>sprB</i>	9987.96	2.77	1.32E-10	<i>stbE</i>	115.94	0.51	0.09159
<i>sprT</i>	299.05	-0.33	0.18052	<i>stcA</i>	422.67	3.01	3.12E-14
<i>sptP</i>	21602.33	-2.48	3.24E-09	<i>stcB</i>	127.37	3.21	2.42E-17
<i>SraA</i>	75.33	-0.11	0.69858	<i>stcC</i>	1087.25	2.61	3.85E-18
<i>SraB</i>	270.68	-0.92	0.00719	<i>stcD</i>	569.99	2.92	1.33E-17
<i>SraF</i>	18.69	-0.36	0.45348	<i>stdA</i>	152.25	1.31	0.00017
<i>SraL</i>	188.77	0.94	0.00928	<i>stdB</i>	199.73	2.44	3.92E-17
<i>srcA</i>	65.71	-0.56	0.06325	<i>stdC</i>	48.36	2.51	2.50E-11
<i>srfA</i>	1290.80	-0.25	0.28654	<i>steA</i>	8165.07	-2.94	3.40E-20
<i>srfB</i>	2954.38	-0.56	0.06611	<i>steB</i>	372.18	-1.49	4.58E-05
<i>srfC</i>	1717.35	-0.09	0.79825	<i>steC</i>	426.44	-0.11	0.70977
<i>srfJ</i>	303.12	0.95	0.00154	<i>stfA</i>	159.95	-0.08	0.81898
<i>srlA</i>	306.06	0.07	0.83770	<i>stfC</i>	297.49	1.00	0.00115
<i>srlE</i>	283.64	2.29	1.91E-17	<i>stfD</i>	105.15	1.41	4.61E-05
<i>srlR</i>	1151.70	-1.70	4.30E-10	<i>stfE</i>	80.78	2.78	3.58E-10
<i>srnB</i>	5630.36	0.77	0.00084	<i>stfF</i>	23.92	0.86	0.07025
<i>sRNA1</i>	37.98	1.39	0.00052	<i>stfG</i>	40.87	2.54	8.28E-06
<i>sRNA10</i>	27.69	-0.15	0.69179	<i>sthA</i>	99.16	0.89	0.00483
<i>sRNA3</i>	100.20	-2.39	3.76E-11	<i>sthB</i>	464.19	0.47	0.16364
<i>SroA</i>	24.24	-0.74	0.06276	<i>sthD</i>	82.35	0.61	0.08090
<i>SroC</i>	119.97	-1.02	0.02608	<i>sthE</i>	241.29	0.16	0.52848
<i>ssaB</i>	40.14	1.59	0.00078	<i>stiA</i>	80.74	0.98	0.00809
<i>ssaC</i>	110.45	1.92	1.09E-06	<i>stiB</i>	68.56	1.65	3.29E-05
<i>ssaD</i>	218.09	1.29	0.00017	<i>stiC</i>	188.68	1.52	6.51E-07
<i>ssaE</i>	61.93	-0.13	0.75111	<i>stiH</i>	725.88	0.73	0.00247
<i>ssaG</i>	19.09	1.50	0.00338	<i>stjA</i>	1011.40	-1.06	0.00021
<i>ssaH</i>	4.19	1.36	0.09300	<i>stjB</i>	727.01	-0.43	0.07592
<i>ssal</i>	23.32	2.87	3.00E-06	<i>stjC</i>	173.09	-0.48	0.12804
<i>ssaJ</i>	119.71	1.84	1.48E-05	<i>STM2208</i>	183.30	-0.76	0.05286
<i>ssaK</i>	17.26	1.58	0.00448	<i>STM2209</i>	22.14	-0.59	0.25283
<i>ssaL</i>	297.11	0.25	0.40612	<i>STM3025</i>	217.70	-0.28	0.35927
<i>ssaM</i>	28.25	1.89	1.17E-05	<i>STM3025.1N</i>	81.90	0.87	0.01105
<i>ssaN</i>	284.34	0.29	0.21799	<i>STM3026</i>	270.01	0.93	0.01304
<i>ssaO</i>	40.93	-0.12	0.77562	<i>STnc1010</i>	0.64	-0.65	0.56705
<i>ssaP</i>	66.99	0.70	0.03404	<i>STnc1060</i>	3187.00	2.12	6.81E-13
<i>ssaQ</i>	522.64	-0.23	0.39602	<i>STnc1080</i>	1.09	-1.93	0.09177
<i>ssaR</i>	192.24	0.26	0.44345	<i>STnc1110</i>	392.94	0.75	0.00786
<i>ssaS</i>	30.50	0.76	0.07587	<i>STnc1120</i>	1.27	0.51	0.63782
<i>ssaT</i>	222.71	-0.28	0.48045	<i>STnc1150</i>	53.49	0.20	0.59209
<i>ssaU</i>	1252.45	-2.98	2.91E-18	<i>STnc1160</i>	3.39	-2.24	0.02312
<i>ssaV</i>	288.16	1.64	4.23E-06	<i>STnc1210</i>	26.74	-0.83	0.04836
<i>ssb</i>	7214.90	-1.41	4.75E-11	<i>STnc1270</i>	343.74	-0.04	0.88777
<i>sscA</i>	75.47	-0.47	0.15833	<i>STnc1340</i>	1977.64	-1.99	5.68E-07
<i>sscB</i>	26.79	0.54	0.22030	<i>STnc1380</i>	72.00	-0.67	0.03806
<i>sseA</i>	132.92	-1.75	1.60E-07	<i>STnc1390</i>	0.30	-0.59	0.59639
<i>sseBa</i>	249.11	-1.18	0.00019	<i>STnc1400</i>	50.90	1.16	0.00083
<i>sseBb</i>	2276.67	1.19	1.45E-06	<i>STnc1460</i>	3034.05	-0.19	0.55142
<i>sseC</i>	547.67	0.09	0.73928	<i>STnc1470</i>	15.70	-0.10	0.84167
<i>sseD</i>	72.67	1.66	7.15E-06	<i>STnc150</i>	140.08	-1.35	9.96E-05
<i>sseE</i>	28.17	0.99	0.01953	<i>STnc1590</i>	10.83	0.52	0.33848
<i>sseF</i>	99.29	1.36	6.67E-05	<i>STnc1600</i>	89.37	-1.51	1.77E-05
<i>sseG</i>	104.12	0.79	0.00713	<i>STnc1640</i>	0.69	0.11	0.92284
<i>sseI</i>	918.49	0.46	0.13908	<i>STnc1650</i>	3.20	-2.81	0.00460
<i>sseJ</i>	175.46	0.28	0.46105	<i>STnc2040</i>	0.19	0.33	0.74250
<i>sseK1</i>	1389.05	-2.89	2.53E-16	<i>STnc2090</i>	0.16	-0.15	0.87517
<i>sseK2</i>	261.63	-1.58	7.43E-06	<i>STnc2130</i>	9.37	1.86	0.00518
<i>sseK3</i>	513.45	-2.03	1.65E-08	<i>STnc2160</i>	3.02	0.04	0.96625
<i>sseL</i>	269.08	0.60	0.03634	<i>STnc290</i>	717.73	-1.37	0.00849
<i>sspA</i>	8854.44	0.19	0.52034	<i>STnc400</i>	23.45	-0.67	0.19589
<i>sspB</i>	2302.40	0.84	0.00447	<i>STnc440</i>	28.01	0.65	0.18854
<i>sspH2</i>	1326.95	0.12	0.56426	<i>STnc470</i>	18.19	-0.50	0.35156
<i>ssrA</i>	1335.52	-1.61	5.89E-07	<i>STnc490</i>	4.40	1.23	0.10005
<i>ssrB</i>	266.30	-1.89	4.85E-11	<i>STnc500</i>	16.29	0.91	0.09123
<i>SsrS</i>	267547.28	2.34	1.85E-14	<i>STnc520</i>	846.39	-1.43	2.65E-09
<i>stbA</i>	45.81	3.47	3.73E-15	<i>STnc540</i>	43.50	-0.90	0.01693
<i>stbB</i>	69.01	1.97	9.75E-07	<i>STnc560</i>	496.28	-0.62	0.10502
<i>stbC</i>	511.08	0.19	0.47367	<i>STnc580</i>	173.47	-0.22	0.60123
<i>stbD</i>	126.75	0.88	0.00280	<i>STnc700</i>	129.88	-0.99	0.00312

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
STnc710	2390.70	1.91	1.74E-12	thiQ	227.59	0.48	0.09135
STnc730	219.35	1.50	1.40E-06	thiS	26.54	-0.18	0.66503
STnc740	57.72	-0.32	0.40520	thrA	1676.00	-1.32	2.51E-07
STnc750	34.12	0.83	0.02106	thrB	1088.76	-1.75	5.19E-08
STnc770	429.39	-2.42	8.58E-22	thrC	3289.41	0.41	0.11624
STnc780	277.78	-1.68	3.89E-06	thrL	1768.70	-2.93	1.15E-14
STnc840	35.11	-0.78	0.03815	thrS	42630.95	-0.90	0.00682
STnc850	0.15	-0.15	0.87517	thyA	1748.34	0.85	0.00077
STnc870	104.75	-1.86	1.23E-06	tig	28076.93	0.59	0.05995
STnc880	3.29	0.09	0.91148	tktA	12578.77	0.24	0.38198
stpA	3362.99	0.28	0.32891	tktB	1840.77	2.13	2.37E-10
sucA	10815.88	0.51	0.28137	tldD	3286.48	-0.70	0.01905
sucB	9948.19	2.12	1.35E-10	tmk	1215.85	0.90	0.00015
sucC	6193.15	2.25	1.57E-13	tnp	1784.94	0.55	0.07862
sucD	3286.73	2.72	1.74E-21	tnpA_1a	2587.27	0.38	0.24398
sufA	1385.75	3.17	6.72E-27	tnpA_1b	1822.93	0.49	0.11600
sufB	5450.27	3.79	3.79E-46	tnpA_1c	2417.85	0.60	0.05260
sufC	2221.41	3.86	2.73E-47	tnpA_1d	1775.20	0.45	0.15839
sufD	8609.66	4.61	2.80E-86	tnpA_2b	1847.31	0.51	0.09415
sufI	1328.19	-0.19	0.39568	tolA	2985.13	1.04	6.39E-05
sufS	4624.56	4.40	2.29E-86	tolB	7182.97	-0.12	0.59571
sugE	1047.62	-2.53	9.71E-15	tolC	7753.24	-0.99	0.00114
suhB	13640.02	1.93	4.17E-06	tolQ	1486.58	1.63	9.20E-12
sulA	3007.80	-3.49	6.12E-25	tolR	694.93	1.27	3.36E-07
surA	6013.83	0.65	0.00745	tonB	1622.68	1.07	1.97E-05
surE	761.53	-0.26	0.23881	topA	8195.42	0.88	0.00034
sxy	471.55	-1.46	4.42E-08	topB	3222.48	0.53	0.01312
syd	916.48	-1.06	0.00145	torA	403.71	1.40	4.29E-05
symE	62.42	-0.94	0.01371	torC	275.99	0.33	0.32250
tag	711.18	-1.83	3.08E-15	torD	81.15	1.33	1.05E-05
tal	680.33	1.89	9.10E-09	torR	476.33	-2.04	2.45E-15
talB	6912.15	-0.30	0.26549	torS	777.92	-0.46	0.05258
talC	599.98	0.99	0.00018	torT	291.95	0.11	0.64195
tas	2123.85	-1.04	0.00809	tpiA	9351.09	0.46	0.05763
tatA	18292.90	-0.24	0.46466	tppB	7797.14	0.09	0.69686
tatB	1721.74	0.02	0.93332	tpx	8210.86	-0.99	0.00629
tatC	2416.05	0.09	0.76385	tqsA	1139.25	-0.19	0.47750
tatD	529.11	-0.59	0.01333	treA	916.96	0.64	0.04282
tatE	995.62	0.69	0.11213	treC	360.63	1.63	1.82E-09
tbpA	1130.33	-0.81	0.00119	treF	1399.14	0.88	0.00016
tcp	5291.21	-0.02	0.95010	treR	410.58	0.22	0.36938
tctD	1512.03	-2.73	6.02E-11	trg	1273.16	-0.81	0.03488
tctE	521.78	-0.97	0.00666	trkH	1252.68	-0.54	0.01397
tdcA	221.75	-0.13	0.60338	trmA	1641.05	-0.23	0.32734
tdcC	2098.02	2.25	1.77E-06	trmB	1683.31	-1.62	5.84E-08
tdcD	1994.03	4.73	1.31E-17	trmD	29396.20	-1.78	4.74E-17
tdcE	7455.93	5.63	3.25E-38	trpA	245.14	0.18	0.55082
tdcG	5148.18	5.33	6.87E-51	trpB	853.99	-0.03	0.92065
tdh	3285.52	0.38	0.10107	trpC	408.17	-0.73	0.01352
tdk	2038.63	-0.09	0.73832	trpD	207.94	-0.32	0.28020
tehA	591.86	-0.79	0.00119	trpE	338.80	-0.90	0.00146
tehB	1279.40	-0.10	0.71585	trpH	1767.32	-1.32	8.57E-08
tesA	953.36	-0.57	0.02944	trpR	318.73	-1.57	5.29E-08
tesB	1063.90	-1.39	5.85E-07	trpS	5723.86	0.63	0.00473
tgt	9790.40	0.91	1.89E-05	trpS2	425.24	-0.61	0.05758
thdF	1517.19	1.00	8.88E-06	truA	1305.34	0.90	8.00E-05
thiC	1287.24	-2.06	8.84E-14	truB	6043.58	0.82	0.00719
thiD	492.24	-0.38	0.11359	truD	2041.82	-0.13	0.57587
thiE	163.47	-0.34	0.35645	trxA	7332.14	-1.32	2.21E-05
thiF	168.97	0.15	0.63085	trxB	29613.28	2.84	1.07E-24
thiG	181.30	0.49	0.11641	trxC	14677.72	4.34	3.10E-59
thiH	424.91	0.10	0.71415	tsaA	29559.52	3.42	3.48E-28
thiI	4865.07	0.27	0.30788	tsf	92910.27	0.40	0.13982
thiJ	218.19	0.35	0.14049	tsr	13720.72	0.05	0.86017
thiK	884.93	-0.65	0.01100	tsx	2424.82	-0.84	0.01171
thiL	1435.19	0.22	0.27518	ttdA	168.16	2.22	1.61E-11
thiM	553.66	-1.07	0.00036	ttdB	146.80	1.50	1.24E-06
thiP	337.81	-0.01	0.97571	ttrA	301.82	1.71	1.77E-10

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
ttrB	83.08	2.06	3.32E-11	wcaK	120.87	1.87	3.38E-08
ttrC	76.67	1.28	8.81E-05	wcaL	97.38	2.48	1.05E-11
ttrR	160.87	-0.30	0.31411	wcaM	210.26	1.80	2.22E-10
ttrS	873.83	-1.19	0.00034	wrbA	2158.98	1.07	9.25E-06
tufA	203763.07	-0.74	0.01081	wza	172.78	2.55	3.68E-17
tufB	104896.54	0.33	0.20057	wzb	34.48	3.16	5.78E-12
tus	372.34	-1.15	5.12E-06	wzc	280.60	2.34	1.87E-12
typA	16400.74	0.76	0.00148	wzx	199.87	2.63	7.44E-12
tyrA	1016.49	-1.84	6.47E-13	wzxE	1305.12	0.26	0.28573
tyrB	1719.94	-1.24	0.00016	wzzB	13416.58	1.49	8.72E-10
tyrP	854.91	-0.43	0.04082	wzzE	4731.30	-0.34	0.16115
tyrR	2494.70	-1.31	2.28E-08	xapB	316.56	1.06	0.00469
tyrS	5690.78	0.02	0.94582	xapR	701.34	-0.82	0.00246
ubiA	1574.54	-1.04	1.73E-05	xerC	823.90	-0.10	0.67640
ubiC	3993.36	-3.31	5.86E-45	xerD	2926.57	-1.25	5.86E-07
ubiE	3208.51	-1.58	1.71E-08	xseA	1038.74	-0.09	0.70749
ubiF	827.47	-1.30	5.43E-08	xseB	663.33	-2.26	9.82E-15
ubiG	1926.21	-0.41	0.06331	xthA	1865.91	1.32	2.34E-06
ubiH	766.07	0.59	0.01597	xylA	441.98	0.09	0.74864
ubiX	386.14	0.01	0.95143	xylB	422.14	0.65	0.01272
ucpA	8162.54	-0.61	0.07156	xylR	1266.42	0.54	0.01741
udg	2368.78	1.86	3.60E-12	yaaA	4689.39	2.29	3.64E-14
udhA	6006.35	-0.53	0.11034	yaaH	1619.81	-2.55	7.17E-25
udk	730.31	-0.30	0.44448	yaaI	225.81	-0.49	0.13613
udp	1909.48	-2.44	3.35E-20	yaaJ	806.72	-1.69	3.74E-14
ugpA	109.78	1.68	1.37E-08	yaaU	271.18	0.34	0.27410
ugpB	565.75	-0.62	0.04964	yaaY	966.25	-1.02	0.21336
ugpC	280.99	0.56	0.03323	yabF	111.91	1.28	7.50E-05
ugpE	78.80	1.40	6.73E-05	yabI	756.16	0.99	0.00036
ugpQ	654.86	-0.34	0.16131	yabN	782.93	-1.46	4.96E-08
ugtL	396.80	-2.73	7.14E-13	yacA	1697.88	0.73	0.00873
uhpA	145.21	1.42	0.00028	yacC	2474.78	-2.98	8.69E-16
uhpB	295.43	0.82	0.00663	yacF	1133.36	-0.42	0.04863
uhpC	304.08	0.23	0.40372	yacG	506.28	-1.11	2.04E-06
uhpT	332.56	0.66	0.05947	yacH	232.05	0.76	0.00272
umuC	567.35	-1.05	2.65E-05	yacL	6022.40	-2.78	4.80E-17
umuD	581.77	-3.00	1.15E-27	yadB	961.17	1.69	2.73E-10
ung	1215.38	-0.29	0.30888	yadE	831.33	0.32	0.18593
upp	3382.27	0.21	0.58210	yadF	5003.63	-1.40	1.17E-05
uppS	4352.12	-0.11	0.69591	yadG	2629.81	0.12	0.62618
uraA	709.39	0.82	0.00086	yadH	629.75	1.50	4.58E-10
usg	2656.78	1.05	2.77E-05	yadI	97.77	0.17	0.59195
ushA	1264.54	-0.22	0.33823	yadR	2251.27	-0.67	0.03673
uspA	21345.03	-3.96	1.93E-22	yadS	1074.26	-0.91	0.00058
uspB	529.52	-1.43	6.07E-10	yaeB	300.45	-0.84	0.00032
uup	5859.03	0.36	0.09998	yaeD	1539.75	-0.55	0.07838
uvrA	2810.69	0.08	0.74093	yaeE	800.38	-1.24	2.64E-06
uvrB	5165.15	-1.35	2.82E-10	yaeH	2353.23	-2.96	2.19E-15
uvrC	2168.00	0.67	0.00387	yaeJ	553.31	-1.56	3.73E-08
uvrD	5013.91	-0.45	0.04437	yaeL	9479.89	0.13	0.57307
uxaC	1196.27	0.20	0.50661	yaeP	5684.89	-3.26	4.63E-28
uxuA	3569.70	-2.04	3.57E-13	yaeQ	546.75	0.28	0.25678
uxuB	674.04	-0.10	0.82465	yaeR	1088.06	-1.07	0.00361
uxuR	3271.06	-2.92	6.36E-19	yaeT	21799.39	-0.01	0.96598
vacJ	1920.79	0.50	0.03010	yafC	853.82	-2.09	5.21E-12
valS	7155.91	0.63	0.01473	yafD	969.68	-1.80	6.91E-08
vgrS	342.48	0.61	0.02078	yafE	604.99	-0.34	0.21650
virK	3652.74	-2.62	1.17E-10	yafJ	1387.46	-1.46	8.47E-05
visC	2497.91	0.36	0.13322	yafK	1385.01	-2.15	7.74E-12
vsr	110.22	0.95	0.00045	yafP	384.39	-0.64	0.01739
wcaA	182.18	0.75	0.01241	yafS	454.43	-1.23	0.00010
wcaB	68.07	2.12	2.73E-07	yafV	1328.47	-2.17	6.53E-17
wcaC	77.06	1.90	4.29E-07	yahN	352.62	-1.20	1.11E-05
wcaD	291.90	1.79	6.13E-10	yahO	254.18	-0.70	0.02024
wcaE	89.24	2.36	3.23E-13	yaiA	3315.03	-1.82	3.10E-09
wcaF	30.92	2.20	2.56E-07	yaiB	4013.86	-2.40	1.09E-13
wcaI	165.80	2.56	1.62E-13	yaiE	529.51	-2.26	1.15E-17
wcaJ	127.37	1.23	0.00037	yaiI	625.70	-1.20	3.08E-07

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
yaiU	990.12	0.12	0.64905	ybfA	891.83	-3.47	4.80E-30
yaiV	156.62	-0.31	0.42811	ybfE	345.63	-1.16	0.00117
yaiW	1942.93	0.84	0.00160	ybfF	426.53	-0.69	0.00689
yaiY	1664.41	-1.43	0.00243	ybfM	1220.08	-1.22	0.00094
yaiZ	552.98	-2.03	3.06E-11	ybfN	106.70	0.00	0.99315
yajB	727.83	-0.48	0.03666	ybgC	2610.77	-0.67	0.02218
yajC	5056.25	0.39	0.13530	ybgE	1068.87	2.47	1.19E-18
yajD	4681.03	-2.57	9.76E-15	ybgF	4333.39	2.29	2.63E-16
yajF	406.02	-0.82	0.00107	ybgH	779.05	1.36	2.55E-07
yajG	918.25	0.16	0.45633	ybgI	1066.83	-2.16	2.03E-09
yajI	567.23	0.44	0.30046	ybgJ	513.50	-0.39	0.15273
yajO	1426.61	-1.68	3.01E-14	ybgK	326.94	-0.80	0.00767
yajQ	3730.04	-1.58	8.36E-07	ybgL	548.91	0.32	0.21381
yajR	711.48	-0.23	0.33300	ybgR	383.29	0.48	0.07309
yaoF	442.54	-1.27	4.10E-05	ybgS	4367.01	4.21	7.34E-27
ybaB	4600.22	1.03	6.41E-06	ybgT	751.53	1.51	3.28E-07
ybaE	677.73	-1.43	6.75E-05	ybhA	1835.38	-0.66	0.00540
ybaJ	21948.53	-3.34	4.91E-14	ybhB	154.21	-0.58	0.08066
ybaK	383.98	-0.41	0.10808	ybhC	1601.24	0.45	0.05093
ybaL	2153.53	-0.13	0.59232	ybhE	2064.66	-0.58	0.07215
ybaM	124.53	-0.82	0.00277	ybhF	921.84	-0.34	0.19513
ybaO	3053.92	-3.10	1.08E-15	ybhK	553.30	0.74	0.00214
ybaP	403.18	-0.31	0.22073	ybhL	4189.48	-0.76	0.00663
ybaW	471.31	-1.73	7.49E-10	ybhM	73.58	1.28	0.00075
ybaX	450.46	0.18	0.49794	ybhN	333.10	1.14	2.38E-05
ybaY	3103.59	1.17	0.00034	ybhO	212.86	1.68	2.48E-08
ybaZ	461.35	-1.08	1.88E-05	ybhP	150.36	1.92	1.42E-10
ybbA	275.54	0.42	0.15008	ybhQ	1286.47	-2.10	6.24E-17
ybbB	1406.08	-0.98	7.40E-05	ybhR	672.41	-0.52	0.01562
ybbJ	242.29	0.33	0.26100	ybhS	883.74	-0.08	0.76294
ybbK	1051.68	-0.91	0.00245	ybhT	476.55	-2.75	1.54E-15
ybbL	420.89	-0.88	0.00039	ybiB	555.56	-0.49	0.11074
ybbM	357.83	-0.31	0.19638	ybiF	180.53	0.95	0.00020
ybbN	1410.83	0.60	0.01622	ybiH	1030.90	-1.82	2.50E-11
ybbO	842.70	-0.58	0.01149	ybiI	228.20	-0.84	0.00145
ybbP	1363.53	1.54	2.68E-12	ybiJ	1687.74	2.72	3.57E-20
ybbS	156.11	-0.35	0.40267	ybiK	945.57	-0.49	0.10754
ybbY	177.33	0.44	0.12701	ybiN	496.40	0.47	0.04465
ybcI	216.99	-0.50	0.03391	ybiO	935.86	-0.48	0.03044
ybcJ	147.00	1.32	4.09E-05	ybiP	4168.04	-2.74	1.06E-14
ybdA	1476.64	1.53	3.26E-06	ybiR	598.73	0.15	0.53918
ybdB	3781.32	3.77	3.34E-67	ybiS	4136.99	1.95	1.39E-15
ybdD	331.75	-1.47	0.00284	ybiT	8648.18	1.45	4.82E-07
ybdF	182.70	-1.13	1.30E-05	ybiU	536.72	-0.94	7.05E-05
ybdG	1034.81	0.23	0.32297	ybiV(1)	1495.35	-0.88	0.00700
ybdH	434.18	-1.15	1.01E-05	ybiV(2)	58.46	-0.12	0.78323
ybdJ	88.59	-0.06	0.82921	ybjC	291.89	-1.60	1.20E-09
ybdK	543.81	1.46	1.36E-10	ybjD	2083.99	-0.03	0.90386
ybdL	747.88	-2.53	1.00E-13	ybjE	475.52	-0.43	0.12875
ybdM	344.11	-0.27	0.30241	ybjG	943.20	1.27	2.22E-07
ybdN	574.14	-0.05	0.82743	ybjM	12821.47	4.72	1.04E-90
ybdO	1189.04	-1.18	0.00067	ybjN	586.15	-0.01	0.98115
ybdQ	5172.42	-3.10	8.33E-19	ybjO	1046.99	0.62	0.01351
ybdR	265.70	0.59	0.02934	ybjP	2016.61	-0.25	0.32144
ybdZ	18.87	1.22	0.00833	ybjR	313.18	-0.46	0.06592
ybeA	1715.41	0.60	0.00651	ybjT	324.13	0.07	0.79498
ybeB	2138.17	-0.20	0.52782	ybjX	18256.00	-0.74	0.00716
ybeD	2333.67	0.51	0.09670	ybjY	4315.14	-1.78	2.54E-08
ybeF	867.79	0.18	0.44216	ybjZ	2062.15	0.31	0.16738
ybeJ	419.58	0.19	0.47692	ycaD	587.98	1.65	2.65E-08
ybeL	7149.37	-4.92	1.23E-71	ycaJ	3798.76	1.21	9.05E-08
ybeQ	478.86	-0.54	0.07069	ycaL	393.15	-0.47	0.05324
ybeR	172.65	1.57	1.94E-07	ycaM	386.29	0.66	0.03315
ybeS	163.40	2.21	1.52E-10	ycaO	4849.94	2.18	2.35E-18
ybeU	126.76	1.79	2.83E-09	ycaP	274.13	1.23	5.11E-05
ybeV	303.78	0.73	0.01397	ycaQ	648.51	-0.45	0.07727
ybeX	2695.29	0.33	0.32501	ycaR	234.59	-0.76	0.00879
ybeY	1774.74	-0.94	0.00058	ycbB	1980.38	-0.83	0.00077

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>ycbC</i>	313.32	-0.06	0.80316	<i>yciM</i>	2400.73	-0.10	0.63542
<i>ycbG</i>	2286.43	-3.07	6.03E-25	<i>yciN</i>	385.28	-0.72	0.01990
<i>ycbK</i>	2791.68	-0.41	0.10049	<i>yciO</i>	586.56	-0.17	0.54825
<i>ycbL</i>	1331.53	0.67	0.00224	<i>yciR</i>	941.36	-0.80	0.00048
<i>ycbW</i>	1711.57	0.64	0.02133	<i>yciS</i>	1279.74	-0.95	0.00278
<i>yccA</i>	3620.73	1.43	7.83E-07	<i>yciT</i>	1415.19	-0.07	0.79717
<i>yccD</i>	123.89	-0.46	0.12176	<i>yciU</i>	1270.10	-1.41	3.75E-05
<i>yccF</i>	213.26	0.07	0.83511	<i>yciW</i>	424.83	-0.69	0.01959
<i>yccJ</i>	1972.04	1.96	3.31E-09	<i>ycjE</i>	194.21	-0.33	0.28793
<i>yccK</i>	1192.61	-0.20	0.63060	<i>ycjF</i>	393.46	0.04	0.90511
<i>yccS</i>	1240.33	0.19	0.37871	<i>ycjG</i>	535.06	-1.11	1.92E-06
<i>yccT</i>	216.09	-0.54	0.09629	<i>ycjI</i>	484.45	-0.84	0.00053
<i>yccV</i>	497.87	-1.06	0.00044	<i>ycjX</i>	470.62	-0.61	0.01110
<i>yccW</i>	837.72	-0.10	0.68506	<i>ydaA</i>	6626.52	-2.70	1.89E-19
<i>yccX</i>	98.11	-0.62	0.04774	<i>ydaL</i>	818.47	-1.94	2.21E-15
<i>ycdC</i>	376.45	-0.36	0.25930	<i>ydaO</i>	1953.84	0.49	0.01832
<i>ycdW</i>	2273.64	-0.27	0.31890	<i>ydbH</i>	1113.73	-0.26	0.27915
<i>ycdX</i>	1462.28	-0.67	0.01315	<i>ydbL</i>	514.75	-0.06	0.84359
<i>ycdY</i>	2478.05	0.26	0.28960	<i>ydcF</i>	723.43	0.44	0.12338
<i>ycdZ</i>	1423.28	-0.35	0.23862	<i>ydcG</i>	3071.49	1.05	7.62E-05
<i>yceA</i>	977.23	-0.05	0.87404	<i>ydcI</i>	1424.24	-2.57	5.27E-13
<i>yceB</i>	6952.43	-1.63	6.25E-06	<i>ydcK</i>	203.08	-0.15	0.63682
<i>yceD</i>	38759.79	-0.82	0.01118	<i>ydcN</i>	932.78	-2.73	1.52E-31
<i>yceE</i>	512.94	-0.15	0.53883	<i>ydcP</i>	1595.76	0.82	0.00085
<i>yceG</i>	1921.07	0.85	7.06E-05	<i>ydcR</i>	597.56	-1.11	5.56E-05
<i>yceH</i>	638.29	-0.39	0.24115	<i>ydcW</i>	732.60	-0.62	0.01525
<i>yceI</i>	599.34	-0.22	0.50452	<i>ydcX</i>	1801.09	-0.60	0.05353
<i>yceJ</i>	256.08	-0.19	0.46769	<i>ydcY</i>	857.76	-2.16	1.92E-18
<i>yceK</i>	152.70	1.45	4.94E-07	<i>ydcZ</i>	111.75	-0.90	0.00095
<i>yceL</i>	283.42	-0.09	0.74519	<i>yddE</i>	372.29	-0.40	0.15668
<i>yceO</i>	55.27	-1.69	3.51E-05	<i>yddG</i>	77.15	0.87	0.01882
<i>ycfC</i>	833.00	0.22	0.35447	<i>yddX</i>	488.00	-1.80	1.96E-05
<i>ycfD</i>	1579.73	0.08	0.71592	<i>ydeD</i>	658.87	-1.15	1.89E-05
<i>ycfF</i>	1721.99	-1.77	2.13E-08	<i>ydeE</i>	1958.78	-3.84	7.48E-20
<i>ycfH</i>	2086.76	0.50	0.04578	<i>ydeI</i>	173.34	0.26	0.35748
<i>ycfJ</i>	4151.28	0.15	0.74214	<i>ydeJ</i>	194.30	1.07	0.00016
<i>ycfL</i>	323.13	0.03	0.91537	<i>ydeV</i>	609.39	-0.75	0.01195
<i>ycfM</i>	495.74	0.15	0.54963	<i>ydeW</i>	1612.20	-1.93	2.07E-10
<i>ycfP</i>	1411.29	-1.14	0.00027	<i>ydeY</i>	236.84	0.28	0.47700
<i>ycfQ</i>	1002.62	-0.79	0.00092	<i>ydeZ</i>	246.85	0.65	0.13332
<i>ycfR</i>	8141.75	0.36	0.41994	<i>ydfG</i>	1591.38	-1.85	1.85E-11
<i>ycfS</i>	1604.63	0.99	0.00215	<i>ydfH</i>	699.52	-0.38	0.14133
<i>ycfU</i>	1012.03	0.92	0.00014	<i>ydfI</i>	266.87	0.22	0.40350
<i>ycfW</i>	1336.03	0.81	0.00032	<i>ydfJ</i>	151.81	1.83	1.07E-08
<i>ycfX</i>	864.74	0.44	0.06656	<i>ydfZ</i>	111.65	-1.04	0.00175
<i>ycgB</i>	1307.95	-1.37	3.20E-07	<i>ydgA</i>	4251.49	0.96	0.00011
<i>ycgL</i>	793.33	-1.84	1.33E-09	<i>ydgC</i>	346.17	-0.36	0.17237
<i>ycgM</i>	1186.44	0.13	0.70324	<i>ydgE</i>	217.81	0.81	0.01511
<i>ycgN</i>	776.87	0.33	0.31501	<i>ydgF</i>	2493.31	-1.16	0.00451
<i>ycgR</i>	1380.87	-1.31	1.93E-09	<i>ydgH</i>	3145.14	-0.25	0.33405
<i>ychA</i>	5359.14	1.88	4.03E-13	<i>ydgl</i>	1573.02	1.25	5.11E-08
<i>ychE</i>	238.57	-0.70	0.00713	<i>ydgJ</i>	2728.58	-0.60	0.02492
<i>ychF</i>	5256.17	1.24	7.70E-07	<i>ydgK</i>	1790.84	-0.96	0.00379
<i>ychH</i>	1275.32	-4.39	3.13E-43	<i>ydgT</i>	4414.38	-0.95	0.02737
<i>ychJ</i>	2102.44	-0.44	0.05301	<i>ydgU</i>	196.96	-1.54	9.70E-07
<i>ychK</i>	2249.80	-1.49	2.13E-08	<i>ydhB</i>	239.01	0.18	0.53103
<i>ychM</i>	1021.48	-0.21	0.38401	<i>ydhC</i>	3667.56	3.99	3.85E-53
<i>ychN</i>	967.46	0.15	0.61675	<i>ydhD</i>	5063.69	-0.16	0.60608
<i>ychP</i>	2745.90	0.36	0.16595	<i>ydhF</i>	1013.26	-0.69	0.01087
<i>yciA</i>	844.88	0.40	0.18807	<i>ydhl</i>	105.65	-0.60	0.03585
<i>yciC</i>	2501.14	0.50	0.02850	<i>ydhJ</i>	678.57	0.08	0.73362
<i>yciE</i>	467.51	3.08	6.14E-17	<i>ydhl</i>	62.84	-0.58	0.09380
<i>yciF</i>	145.21	1.30	0.00059	<i>ydhM</i>	836.96	0.53	0.09375
<i>yciG</i>	137.39	1.90	1.69E-05	<i>ydhO</i>	1005.22	-0.13	0.68916
<i>yciH</i>	344.58	-0.87	0.00761	<i>ydiA</i>	471.33	-0.99	0.00623
<i>ycil</i>	564.88	-0.41	0.16878	<i>ydiD</i>	383.69	0.56	0.03675
<i>yciK</i>	3436.56	-0.15	0.70892	<i>ydiE</i>	777.75	-0.01	0.96883
<i>yciL</i>	5330.34	2.30	3.26E-25	<i>ydiF</i>	194.08	0.81	0.01102

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
ydiH	573.73	-0.11	0.77473	yeeX	5248.21	-1.45	0.00064
ydiJ	1746.54	0.15	0.67051	yeeY	497.58	-0.44	0.08985
ydiK	826.77	0.54	0.02629	yeeZ	1821.58	-1.78	9.76E-13
ydiL	166.31	-0.14	0.64809	yegB	327.69	0.50	0.09923
ydiM	225.35	2.60	6.15E-14	yegD	636.30	-0.10	0.74362
ydiO	258.86	0.17	0.50559	yegE	918.70	0.18	0.48613
ydiP	96.61	0.25	0.51961	yegH	1885.22	0.76	0.00063
ydiQ	112.95	0.88	0.00390	yegN	668.15	1.54	6.72E-13
ydiR	150.61	0.61	0.02536	yegO	616.84	1.10	6.12E-06
ydiS	216.57	0.66	0.01725	yegQ	3309.11	1.85	6.68E-11
ydiT	30.81	2.28	3.80E-07	yegS	1058.68	2.58	1.16E-13
ydiU	1730.10	-1.56	1.03E-09	yegT	471.44	1.05	7.87E-06
ydiV	287.80	0.80	0.00549	yegU	256.88	0.52	0.02356
ydiY	782.53	-1.13	0.00021	yegV	149.00	0.39	0.14881
ydiZ	1148.22	-0.35	0.20517	yegW	329.11	-0.61	0.01233
ydjA	2316.77	-1.91	1.52E-09	yehE	5428.57	5.79	8.59E-52
ydjM	222.79	-0.71	0.00634	yehR	530.28	-1.45	3.41E-05
ydjN	8428.37	2.69	2.84E-11	yehS	1453.79	-0.33	0.31195
yeaA	3337.94	-3.37	3.53E-28	yehT	321.97	-1.35	7.57E-06
yeaB	149.12	-0.17	0.50161	yehU	809.10	-1.57	3.86E-09
yeaC	1312.32	-1.49	4.57E-05	yehV	158.85	-0.88	0.00054
yeaD	751.73	0.39	0.08761	yehW	1103.23	-1.87	1.53E-15
yeaG	1558.21	0.75	0.00762	yehX	214.60	0.65	0.03704
yeaH	1042.36	-0.65	0.01355	yehY	462.24	1.21	5.17E-07
yeaJ	655.09	0.08	0.78653	yehZ	910.41	0.59	0.00684
yeaK	324.67	-0.21	0.44677	yeiA	854.37	1.63	1.31E-10
yeaL	130.07	-0.72	0.01464	yeiB	2332.65	3.06	4.16E-28
yeaM	210.84	-0.44	0.08373	yeiE	1220.86	-1.37	5.50E-08
yeaN	519.27	0.86	0.00981	yeiG	970.57	-1.15	6.32E-05
yeaO	402.85	-0.97	0.00035	yeiH	435.91	0.60	0.00814
yeaQ	909.39	0.33	0.18452	yeiP	3172.33	-0.88	4.54E-05
yeaS	1314.09	-0.82	0.00165	yeiR	490.11	-1.09	2.72E-05
yeaY	1220.04	0.81	0.00108	yeiU	3253.10	-0.30	0.21380
yeaZ	1812.83	-0.16	0.47098	yejA	861.30	0.18	0.39939
yebA	2915.71	1.05	0.00014	yejB	540.86	-0.54	0.01219
yebB	442.16	-0.91	0.00082	yejE	292.36	-0.82	0.00312
yebC	5276.73	0.50	0.04000	yejF	1718.06	-0.43	0.06809
yebE	2970.82	-0.02	0.94447	yejG	4078.19	-0.94	0.00369
yebF	862.76	-0.41	0.17009	yejH	753.12	0.04	0.85772
yebG	2764.71	-2.61	3.36E-16	yejK	2003.57	0.03	0.91193
yebN	352.51	-0.49	0.19033	yejL	1778.45	-1.28	0.00122
yebR	1787.80	-1.49	4.69E-09	yejM	2253.14	0.31	0.15498
yebS	694.59	0.13	0.60221	yfaE	281.70	2.46	1.05E-19
yebU	1156.43	1.33	6.16E-09	yfaO	962.71	-0.56	0.04991
yebW	56.43	0.20	0.53249	yfaV	412.15	1.46	4.99E-10
yecA	4116.45	-2.53	1.03E-19	yfaW	766.69	1.47	1.45E-10
yecC	566.04	1.04	6.94E-05	yfaX	173.06	-0.72	0.01075
yecD	794.85	-1.69	5.59E-09	yfaZ	2144.14	0.10	0.73450
yecE	214.31	-0.42	0.09947	yfbB	407.90	1.67	1.57E-12
yecF	1958.14	-2.04	2.94E-05	yfbE	3751.82	2.85	5.42E-25
yecG	2662.05	-5.01	1.81E-53	yfbG	7056.94	2.94	4.27E-37
yecH	86.32	-0.85	0.00641	yfbK	585.91	0.20	0.43757
yecM	389.31	-0.60	0.00919	yfbQ	1506.61	-0.66	0.00802
yecN	237.36	0.14	0.59018	yfbS	782.45	0.99	1.06E-05
yecO	428.47	0.83	0.00114	yfbT	1168.89	0.67	0.01617
yecP	1097.85	0.60	0.02222	yfbU	2686.06	-1.21	0.00049
yecS	510.44	0.35	0.14589	yfcA	577.83	-0.41	0.06458
yedA	238.41	0.77	0.00209	yfcB	2221.52	-0.13	0.58175
yedD	572.53	-1.19	1.38E-06	yfcC	480.00	0.39	0.10388
yedE	270.71	-0.52	0.05162	yfcE	1012.12	0.29	0.28903
yedF	153.15	-1.53	3.73E-08	yfcF	625.20	-1.43	8.87E-07
yedI	543.69	0.32	0.22745	yfcG	294.89	-1.08	0.01182
yedJ	166.29	-0.36	0.15385	yfcH	446.28	-1.19	7.09E-08
yeeA	795.78	-0.78	0.00024	yfcL	611.47	0.68	0.08210
yeeF	9507.71	0.98	0.00068	yfcM	799.30	0.06	0.81784
yeeI	2187.17	-3.50	4.64E-48	yfcN	576.60	-1.10	1.44E-06
yeeN	7744.88	-1.39	0.00029	yfcZ	2635.08	-3.45	4.00E-15
yeeO	728.57	1.01	2.58E-05	yfdC	317.86	0.57	0.05195

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
yfdZ	2088.44	-2.26	1.21E-25	ygdL	2244.84	-0.32	0.13560
yfeA	967.40	0.74	0.00687	ygdQ	1479.16	1.10	6.35E-07
yfeC	784.38	-3.41	3.50E-30	ygdR	2437.88	-2.20	2.33E-08
yfeD	367.35	-2.35	3.39E-13	ygeA	668.84	-1.42	1.88E-05
yfeH	607.94	-0.29	0.22939	ygeD	770.72	0.38	0.09444
yfeJ	321.27	-1.38	1.05E-07	ygfA	2451.67	-3.92	7.70E-41
yfeK	78.77	0.10	0.75609	ygfB	4476.48	-2.17	2.11E-21
yfeL	262.32	0.33	0.22846	ygfE	848.59	-3.70	8.29E-25
yfeN	257.95	-0.29	0.39093	ygfX	2134.08	-1.28	3.81E-09
yfeR	214.54	0.64	0.06809	ygfY	3239.57	-2.32	6.51E-16
yfeY	489.52	0.93	0.00250	ygfZ	2774.32	-1.12	0.00015
yfeZ	245.74	-0.36	0.15193	yggA	521.71	-0.91	0.01797
yffB	1106.99	-1.05	0.00349	yggB	557.09	0.53	0.02650
yffH	1043.46	-0.85	0.00221	yggE	6784.42	1.66	4.00E-05
yfgA	10437.17	-1.22	2.64E-05	yggG	2782.22	0.94	0.00469
yfgB	4641.59	1.31	1.48E-06	yggJ	596.21	-0.06	0.79020
yfgD	302.79	0.22	0.42674	yggL	2493.81	-1.39	6.67E-07
yfgE	1608.28	0.79	0.00185	yggM	386.89	-0.82	0.01256
yfgJ	167.33	1.25	8.57E-06	yggN	4163.44	-0.49	0.04575
yfgL	6954.93	1.63	5.87E-08	yggS	1600.22	-1.17	4.37E-05
yfgM	2650.07	1.62	6.26E-10	yggT	785.46	-0.14	0.57663
yfhA	2227.67	0.67	0.01166	yggU	294.54	-0.07	0.78589
yfhB	259.92	-0.84	0.00142	yggV	803.58	0.67	0.00731
yfhC	288.65	0.38	0.26008	yggW	1378.73	-0.44	0.04807
yfhD	1305.32	0.15	0.50487	yggX	2592.94	0.00	0.99960
yfhG	801.63	0.31	0.21815	yghA	2070.60	1.86	1.03E-07
yfhH	348.31	-1.44	2.46E-06	yghB	376.49	-1.35	1.04E-06
yfhJ	945.57	0.30	0.28347	yghJ	488.55	2.22	2.22E-18
yfhK	1731.30	-0.23	0.27305	yghU	1149.35	-0.64	0.01418
yfhL	416.21	-0.11	0.79072	yghW	666.01	-2.08	0.00016
yfhP	9288.84	1.27	5.47E-05	ygiB	2073.25	0.05	0.86937
yfiA	147598.13	-6.09	7.02E-37	ygiC	5337.38	1.59	1.87E-07
yfiC	666.45	-0.37	0.07923	ygiD	1933.22	-1.35	1.61E-08
yfiD	1784.55	-0.42	0.42378	ygiE	1482.10	-1.62	1.13E-12
yfiE	3419.86	-1.66	1.70E-09	ygiF	1009.72	-0.15	0.51021
yfiF	2270.45	1.44	2.74E-09	ygiH	923.02	-0.90	1.80E-05
yfiH	3916.97	-0.37	0.10616	ygiK	732.38	-0.75	0.00057
yfiK	235.20	1.18	0.00132	ygiM	3511.92	0.24	0.46307
yfiM	103.77	0.93	0.00433	ygiN	422.95	-1.23	1.67E-05
yfiN	773.83	-0.28	0.27865	ygiR	1753.41	0.81	0.00170
yfiO	4378.72	0.27	0.37357	ygiW	2066.06	-0.32	0.25463
yfiP	865.89	1.88	3.85E-16	ygiX	162.71	-0.95	0.00184
yfiQ	2598.89	-0.12	0.68436	ygiY	293.39	-0.15	0.53295
yfiR	243.88	-0.79	0.01038	ygiO	2761.82	-0.34	0.15130
yfiG	854.48	-1.03	2.34E-05	ygiP	342.91	-1.82	2.23E-11
ygaC	3631.92	-0.21	0.64502	ygiQ	275.19	-0.56	0.04808
ygaD	861.41	-1.47	1.83E-10	ygiR	962.79	-2.09	3.61E-09
ygaE	206.93	0.88	0.00330	ygiT	2356.63	-0.14	0.58210
ygaF	164.54	1.21	0.00032	ygiU	3642.98	-3.37	1.73E-30
ygaM	762.39	-0.75	0.04480	yhaH	200.97	-0.29	0.24050
ygaP	2231.98	-1.57	1.58E-08	yhaJ	467.37	-0.55	0.06297
ygaU	2230.68	1.40	2.40E-08	yhaK	580.29	2.23	2.36E-07
ygbA	1913.03	-4.72	9.97E-36	yhaL	185.30	-1.26	0.00204
ygbE	212.45	-0.23	0.36334	yhaN	3240.40	3.19	2.73E-22
ygbF	894.61	0.80	0.00171	yhaO	825.30	2.23	3.47E-21
ygbI	609.79	-1.74	4.50E-08	yhbC	8769.72	-1.05	0.00234
ygbJ	108.60	0.96	0.02641	yhbE	2144.68	0.46	0.06229
ygbK	106.93	1.52	0.00016	yhbG	1373.71	0.21	0.41143
ygbL	107.34	1.34	0.00036	yhbH	4756.42	-1.23	0.00035
ygcB	556.47	0.43	0.14778	yhbJ	1337.48	0.12	0.70044
ygcF	4187.38	-0.80	0.00653	yhbL	984.35	-0.96	0.00031
ygcH	275.35	3.00	4.02E-28	yhbN	2467.70	0.07	0.79680
ygcY	628.28	-0.22	0.57132	yhbO	326.64	1.09	0.00046
ygdD	123.30	0.28	0.31216	yhbP	257.12	-1.51	4.15E-08
ygdE	4867.55	0.07	0.76395	yhbQ	394.18	-1.98	1.30E-10
ygdH	6512.51	-0.71	0.03986	yhbS	792.05	-1.29	0.00078
ygdI	1797.68	0.36	0.22635	yhbT	2336.83	-2.38	7.24E-14
ygdK	440.63	1.26	8.02E-07	yhbU	121.40	2.27	2.66E-11

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
yhbV	218.92	1.89	2.40E-09	yhjJ	3030.34	-0.15	0.58682
yhbW	282.36	-0.58	0.17634	yhjK	1657.86	0.12	0.61219
yhbY	4734.71	-0.06	0.88300	yhjL	2959.46	0.14	0.55989
yhcB	11951.98	-2.55	8.85E-15	yhjN	1399.96	0.40	0.16159
yhcC	232.88	-0.12	0.73798	yhjQ	279.63	-0.24	0.29697
yhcG	479.90	-0.66	0.00549	yhjR	322.14	-1.35	4.62E-06
yhcH	1111.68	0.42	0.60332	yhjS	1169.65	-1.07	1.59E-06
yhcK	1118.03	-0.85	0.00080	yhjT	9.40	-0.07	0.90155
yhcM	1890.20	-0.69	0.00652	yhjU	789.97	0.27	0.24267
yhcN	2611.23	-1.18	0.02809	yhjV	1329.67	-0.52	0.06281
yhcO	511.88	-1.24	1.29E-05	yhjW	2818.09	-0.46	0.11430
yhcP	820.33	-0.37	0.11940	yhjY	622.15	-2.04	7.81E-14
yhcQ	524.39	-0.86	0.01032	yiaB	87.63	0.28	0.44878
yhcR	403.16	-1.30	0.00033	yiaC	208.31	-0.87	0.00890
yhcS	645.81	-1.29	3.77E-07	yiaD	2765.78	1.13	0.00089
yhdA	2827.59	-0.50	0.01492	yiaE	1351.88	-0.72	0.01201
yhdG	18498.23	-0.35	0.22172	yiaF	6495.86	-0.97	0.00010
yhdH	1131.09	-1.57	1.24E-11	yiaG	1604.82	-1.03	0.00170
yhdJ	724.72	-0.41	0.10547	yiaH	222.09	0.12	0.69169
yhdN	335.12	-0.66	0.01426	yiaJ	439.13	-0.35	0.18051
yhdP	3182.22	0.16	0.58032	yiaK	99.87	1.13	0.00333
yhdT	236.94	-0.02	0.95038	yiaL	37.44	0.83	0.05029
yhdU	604.65	-1.74	3.25E-09	yiaM	69.08	3.80	1.34E-17
yhdV	2336.82	-1.15	0.00320	yiaN	79.55	2.05	2.48E-08
yheL	335.45	-1.15	2.44E-05	yiaO	127.56	4.26	7.62E-24
yheM	319.74	0.21	0.43595	yibD	694.60	2.02	1.32E-15
yheN	296.89	0.41	0.07614	yibF	1003.27	-1.89	6.25E-18
yheO	2053.85	-0.89	0.00184	yibK	137.50	-0.04	0.87235
yheR	1394.16	-2.84	5.04E-21	yibL	2158.16	-2.17	1.31E-06
yheS	1954.03	-0.40	0.08275	yibN	4603.27	-1.23	0.00035
yheT	1199.61	-1.49	6.10E-11	yibP	2262.92	-0.32	0.16411
yheU	113.47	-1.32	1.84E-06	yibR	84.60	0.04	0.89276
yheV	751.12	-2.10	2.16E-07	yicC	4360.58	-0.62	0.00963
yhfA	2308.61	-2.43	2.17E-21	yicE	610.16	-0.62	0.01177
yhfC	1417.63	0.35	0.14892	yicH	1069.47	-1.42	1.30E-05
yhfG	162.34	0.46	0.09181	yicI	539.70	0.61	0.02578
yhfK	1386.20	-0.68	0.00111	yicJ	303.81	0.11	0.69288
yhfL	1017.14	-1.94	5.84E-07	yicL	934.32	-0.76	0.00373
yhgE	1005.25	-0.22	0.35311	yicN	557.20	-1.40	3.44E-07
yhgF	4868.10	0.55	0.02858	yidA	2426.06	-0.57	0.05930
yhgG	993.82	2.68	1.24E-22	yidC	11208.09	1.62	1.51E-11
yhgI	10544.13	0.22	0.35422	yidE	752.32	-0.39	0.14886
yhhA	1326.14	-2.65	1.18E-24	yidF	1387.77	-3.27	1.14E-17
yhhJ	482.28	0.26	0.27808	yidG	348.50	-1.37	6.20E-08
yhhK	777.00	-1.17	8.51E-06	yidH	466.48	-2.48	1.68E-12
yhhL	215.07	-0.97	0.00083	yidQ	1492.88	-3.26	3.10E-40
yhhM	152.36	-1.33	3.51E-06	yidR	623.68	-0.29	0.25302
yhhN	395.31	-1.82	2.47E-09	yidY	678.19	-1.06	3.01E-05
yhhP	1114.15	-0.38	0.29725	yidZ	640.51	-1.54	1.19E-10
yhhQ	698.30	-0.49	0.02336	yieE	800.19	-1.14	0.00039
yhhS	649.62	-0.06	0.82426	yieF	854.61	-0.05	0.87168
yhhT	444.57	1.26	5.76E-05	yieG	1520.66	1.18	3.05E-07
yhhW	575.23	0.43	0.32531	yieH	1427.30	-0.36	0.14627
yhhX	1163.45	-0.78	0.02702	yieM	1080.18	-1.01	0.00048
yhhY	182.57	-0.94	0.00036	yieN	1738.90	-2.05	8.88E-16
yhiH	796.91	0.15	0.55567	yieP	5305.60	-3.43	4.89E-36
yhiI	566.48	-1.36	4.32E-07	yifA	3007.83	-3.01	4.74E-35
yhiN	1048.77	-0.93	6.55E-05	yifE	7196.75	-1.62	2.57E-09
yhiP	3155.11	-2.32	3.47E-10	yifK	2303.06	-0.83	0.00030
yhiQ	511.48	1.90	1.30E-14	yifL	336.29	-2.08	3.95E-14
yhiR	2542.22	-1.25	8.23E-08	yifZ	596.97	-0.12	0.59260
yhjA	218.63	3.22	2.37E-30	yigA	597.80	0.07	0.79827
yhjB	163.96	0.05	0.87466	yigB	550.57	-0.23	0.29448
yhjC	655.19	-1.91	2.92E-08	yigC	8099.45	-1.00	5.04E-05
yhjD	483.53	-0.74	0.01311	yigF	75.63	-0.83	0.00711
yhjE	323.66	0.11	0.69302	yigG	152.32	-0.56	0.16433
yhjG	1149.47	-0.96	0.00090	yigI	463.66	-2.37	6.56E-10
yhjH	2526.49	-2.31	7.10E-11	yigL	676.10	-0.15	0.52542

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
yigM	243.82	-0.22	0.46816	yjfC	231.05	0.89	0.00152
yigN	2926.93	-2.40	4.07E-19	yjfH	2521.94	0.53	0.18018
yigP	2532.56	-0.85	7.78E-05	yjfl	157.84	-0.88	0.02872
yigQ	857.08	0.83	0.00031	yjfJ	119.58	0.24	0.54833
yigZ	1487.21	-0.41	0.08442	yjfK	107.01	0.54	0.06716
yihD	4930.44	-1.88	4.68E-08	yjfl	71.76	-0.01	0.95918
yihG	3184.01	-1.19	0.00023	yjfm	43.90	0.60	0.07989
yihI	2336.21	-0.16	0.60355	yjfn	3299.91	-4.40	3.38E-21
yihO	226.51	1.51	2.60E-06	yjfo	1198.33	-4.43	6.84E-47
yihP	329.01	1.09	0.00011	yjfp	611.94	-2.05	5.74E-07
yihQ	329.47	0.88	0.00087	yjfq	381.75	-0.94	0.00139
yihR	239.09	-0.77	0.01265	yjfr	268.15	0.44	0.09659
yihS	241.19	1.15	0.00193	yjfy	265.17	-1.16	2.90E-05
yihT	113.17	1.85	3.07E-07	yjgA	3954.58	-1.18	0.00165
yihU	85.60	2.18	5.75E-08	yjgB	595.08	1.14	2.62E-05
yihV	198.34	0.63	0.11550	yjgD	8449.78	-3.08	8.89E-18
yihW	2178.19	-3.02	1.10E-40	yjgF	1657.02	-0.77	0.01610
yihX	2027.94	-2.54	9.76E-15	yjgK	331.80	-1.17	1.79E-05
yihZ	223.08	-1.38	1.04E-07	yjgM	399.63	-1.26	1.34E-06
yiiD	1084.32	-1.21	4.60E-06	yjgN	882.10	-0.98	0.00041
yiiG	188.07	0.44	0.14505	yjgP	3762.60	-0.16	0.48508
yiiL	177.98	0.16	0.55555	yjgQ	3581.80	0.91	0.00024
yiiM	412.12	-1.54	5.16E-06	yjhP	662.70	0.40	0.11140
yiiQ	796.95	-1.73	6.83E-09	yjiA	772.69	-0.37	0.19237
yiiR	1665.62	-2.08	5.83E-17	yjiE	313.68	-0.23	0.37590
yiiU	13888.08	-2.47	1.30E-14	yjiG	60.75	-1.46	0.00047
yijC	2470.45	-1.91	1.01E-14	yjiH	134.17	1.13	0.00130
yijD	404.45	-0.49	0.05643	yjiJ	477.49	-0.84	0.00236
yijF	3322.32	5.22	7.93E-70	yjiN	439.85	0.11	0.69269
yijO	526.91	-0.67	0.00734	yjiO	309.34	1.35	1.57E-07
yijP	3470.38	-0.05	0.83588	yjiS	47.61	-0.76	0.02433
yjaB	2948.26	-1.83	4.30E-10	yjiX	217.07	-1.88	1.00E-10
yjaG	3275.42	-1.02	0.00022	yjiA	485.10	-0.55	0.05235
yjaH	1044.05	-0.64	0.02082	yjiG	1252.98	-0.01	0.97645
yjbA	483.84	-1.18	1.14E-06	yjil	409.60	-0.44	0.14097
yjbB	2608.72	-0.59	0.00923	yjiK	4229.71	0.16	0.48446
yjbC	1811.19	-0.02	0.92032	yjiP	235.26	-0.38	0.16363
yjbD	1569.11	-2.18	1.82E-13	yjiQ	90.37	1.03	0.00734
yjbE	498.50	1.88	9.74E-10	yjiU	597.25	0.60	0.03312
yjbF	228.76	2.56	2.53E-22	yjiV	208.78	1.11	4.78E-06
yjbG	243.55	3.12	3.91E-25	yjiW	137.14	-0.31	0.33373
yjbH	1588.84	1.13	2.59E-06	yjiX	336.76	-0.02	0.92688
yjbJ	4615.38	0.05	0.83750	yjiY	3798.85	-2.09	1.70E-10
yjbN	1534.29	0.25	0.29029	ykgB	148.69	2.21	3.99E-07
yjbQ	1008.14	-1.91	4.51E-09	ykgC	1034.08	1.66	6.52E-06
yjbR	310.06	-1.03	0.00075	ykgD	278.24	-0.31	0.26834
yjbB	469.35	-0.26	0.45897	ylaB	2928.83	-2.26	1.47E-12
yjbC	1505.31	-1.79	1.02E-06	ylaC	570.79	-1.02	0.00031
yjbD	956.55	1.20	0.00019	ylbA	555.12	0.63	0.02652
yjbE	1953.88	-0.38	0.24890	ylbE	112.87	3.19	1.25E-17
yjbH	61.64	0.76	0.07212	ylbF	79.74	2.91	9.40E-13
yjbO	926.27	-0.51	0.01907	yliA	798.37	-0.41	0.06319
yjbB	4600.59	1.02	0.00029	yliB	834.66	-0.03	0.87896
yjbC	1021.92	-0.58	0.03417	yliC	307.69	0.83	0.00283
yjbL	4144.82	3.67	1.33E-39	yliD	807.62	1.37	1.16E-07
yjeA	1948.45	-0.46	0.04552	yliI	240.36	-1.34	4.83E-06
yjeE	792.68	-1.36	1.77E-07	yliJ	1017.72	-1.34	1.27E-05
yjeF	1412.29	-1.63	2.75E-06	ymbA	1000.01	-0.19	0.56312
yjeH	967.47	-1.23	0.00024	ymdA	351.03	0.49	0.10076
yjeI	3892.17	-2.06	3.46E-13	ymdC	2587.15	0.03	0.92723
yjeJ	665.65	-1.16	0.00047	ymdF	565.81	0.04	0.87593
yjeK	3549.06	-1.49	6.48E-08	ymfB	167.09	1.02	5.17E-05
yjeM	660.70	0.41	0.07709	ymgE	80.74	0.97	0.00311
yjeO	399.28	-1.11	0.00060	ymiA	442.72	-1.55	8.58E-07
yjeP	3010.88	0.01	0.96494	ynaF	4548.65	-3.51	9.39E-22
yjeQ	1207.08	-0.76	0.00144	ynaI	676.00	-1.01	0.00119
yjeS	3513.42	-2.22	1.03E-09	ynaJ	1713.73	-0.72	0.01222
yjeV	203.77	-3.76	8.08E-20	ynbE	28.43	-0.11	0.77164

Gene	baseMean	log2FC	p value	Gene	baseMean	log2FC	p value
<i>yncA</i>	279.16	-1.53	4.81E-09	<i>yqhA</i>	1106.15	-0.48	0.02997
<i>yncB</i>	1500.95	-0.15	0.57765	<i>yqhC</i>	1039.18	-1.31	4.68E-07
<i>yncC</i>	428.90	1.03	0.00074	<i>yqhD</i>	974.27	-1.16	4.09E-05
<i>yncD</i>	631.79	1.15	0.00056	<i>yqiA</i>	775.97	-0.94	5.02E-05
<i>yncJ</i>	178.12	-1.49	4.99E-06	<i>yqiB</i>	977.59	-1.89	1.56E-16
<i>yneA</i>	239.54	0.64	0.09456	<i>yqiC</i>	3227.37	-2.39	1.72E-19
<i>yneB</i>	343.38	0.42	0.18396	<i>yqiK</i>	963.90	-0.19	0.40875
<i>yneC</i>	114.04	-1.34	3.23E-06	<i>yqjA</i>	1786.80	-1.26	4.02E-08
<i>yneG</i>	174.18	-1.09	2.06E-05	<i>yqjB</i>	733.50	-1.13	0.00019
<i>yneH</i>	639.34	0.21	0.38665	<i>yqjC</i>	3667.98	-0.49	0.03223
<i>yneI</i>	299.45	0.49	0.12794	<i>yqjE</i>	1447.12	-0.64	0.00580
<i>yneJ</i>	125.57	-0.01	0.96772	<i>yqjF</i>	228.28	1.11	0.00562
<i>ynfA</i>	93.74	1.85	3.30E-07	<i>yqjG</i>	467.24	0.26	0.37778
<i>ynfB</i>	2817.67	-1.93	5.30E-14	<i>yqjH</i>	774.53	-0.02	0.92354
<i>ynfC</i>	1026.23	-2.24	7.19E-24	<i>yqjI</i>	2117.14	-2.21	1.94E-19
<i>ynfD</i>	835.50	-1.71	9.56E-12	<i>yqjK</i>	165.50	-1.89	2.12E-11
<i>ynfI</i>	1420.14	1.26	1.95E-05	<i>yraL</i>	470.96	-0.23	0.35814
<i>ynfL</i>	244.83	0.11	0.71123	<i>yraM</i>	1948.10	0.45	0.03382
<i>ynfM</i>	685.43	0.43	0.05723	<i>yraN</i>	122.98	0.17	0.51742
<i>ynhA</i>	1587.45	3.46	2.39E-43	<i>yraO</i>	2335.18	-0.23	0.51344
<i>ynhF</i>	887.50	0.11	0.67906	<i>yraP</i>	596.89	-0.34	0.29593
<i>ynhG</i>	2426.61	2.19	2.84E-19	<i>yraR</i>	397.83	-0.64	0.00917
<i>yniB</i>	752.15	-0.32	0.17175	<i>yrbA</i>	1223.45	-0.97	0.00078
<i>yniC</i>	1536.08	0.76	0.00743	<i>yrbB</i>	3410.49	1.26	4.12E-06
<i>yoaA</i>	1335.53	-0.48	0.03622	<i>yrbC</i>	3648.92	0.01	0.98190
<i>yoaB</i>	1216.70	-0.31	0.41535	<i>yrbD</i>	2786.65	-0.76	0.00270
<i>yoaE</i>	993.80	1.65	4.73E-15	<i>yrbE</i>	498.46	-0.11	0.61760
<i>yoaG</i>	316.06	-2.94	2.85E-18	<i>yrbF</i>	2190.97	-0.29	0.29411
<i>yoaH</i>	373.29	-1.91	1.92E-07	<i>yrbG</i>	920.37	-0.37	0.08762
<i>yobA</i>	944.09	-2.03	5.16E-09	<i>yrbH</i>	1629.44	-0.42	0.05042
<i>yobF</i>	2652.51	0.14	0.74524	<i>yrbI</i>	2022.73	-0.56	0.01304
<i>yobG</i>	952.99	-0.50	0.02208	<i>yrbK</i>	2257.56	-0.35	0.21798
<i>yodD</i>	100.49	-0.55	0.06832	<i>yrbL</i>	495.41	-1.29	2.18E-05
<i>yoel</i>	326.66	0.65	0.10011	<i>yrbN</i>	1055.67	1.15	0.00019
<i>yohC</i>	237.46	-0.96	0.00184	<i>yrdA</i>	1596.54	-1.73	3.96E-14
<i>yohD</i>	207.67	-1.08	8.94E-05	<i>yrdB</i>	428.29	0.68	0.01598
<i>yohF</i>	135.19	0.85	0.00544	<i>yrdC</i>	279.99	-0.08	0.77584
<i>yohG</i>	227.48	0.72	0.01359	<i>yrdD</i>	568.88	-0.68	0.01927
<i>yohI</i>	358.57	0.78	0.00573	<i>yrfG</i>	805.09	-0.68	0.00156
<i>yohJ</i>	224.13	-0.44	0.09323	<i>ysaA</i>	304.18	-0.13	0.61062
<i>yohK</i>	196.06	0.17	0.55993	<i>yshB</i>	241.41	-0.12	0.75669
<i>yohL</i>	3796.44	-2.27	8.62E-13	<i>ytfB</i>	1735.10	-1.92	3.29E-12
<i>yohM</i>	736.74	-0.02	0.95920	<i>ytfE</i>	965.35	-1.12	0.00206
<i>yohO</i>	12.56	-2.17	0.00025	<i>ytfF</i>	432.47	-1.59	1.34E-06
<i>yojI</i>	2021.79	0.95	2.17E-05	<i>ytfG</i>	129.48	0.61	0.03114
<i>yojN</i>	3686.04	-0.34	0.13270	<i>ytfH</i>	219.23	-0.40	0.17438
<i>ypdK</i>	204.87	1.27	0.00174	<i>ytfJ</i>	586.63	-2.27	2.65E-11
<i>ypeC</i>	5223.49	-0.41	0.30531	<i>ytfK</i>	19475.52	-0.96	0.00275
<i>ypfG</i>	703.15	1.81	1.49E-12	<i>ytfL</i>	5301.24	-0.25	0.43537
<i>ypfI</i>	645.53	0.35	0.12803	<i>ytfM</i>	1543.81	-1.46	6.74E-09
<i>ypfM</i>	28171.15	-6.41	8.60E-50	<i>ytfN</i>	3554.21	-0.62	0.00474
<i>yqaA</i>	4147.01	-1.78	1.99E-10	<i>ytfP</i>	402.59	-0.98	0.00130
<i>yqaB</i>	2618.87	-2.03	1.59E-12	<i>ytgA</i>	241.04	-1.35	8.58E-05
<i>yqaE</i>	681.30	0.02	0.94040	<i>zipA</i>	4111.69	-1.88	5.68E-13
<i>yqcB</i>	670.74	0.87	0.00019	<i>zntA</i>	3066.24	-3.24	1.17E-19
<i>yqcC</i>	49.15	0.47	0.21453	<i>zntB</i>	1237.20	0.00	0.99327
<i>yqeF</i>	971.82	-2.10	4.85E-07	<i>zntR</i>	862.61	-1.43	2.56E-08
<i>yqfA</i>	5612.58	-0.29	0.26756	<i>znuA</i>	1771.83	-0.72	0.01304
<i>yqfB</i>	1415.92	-2.83	5.87E-16	<i>znuB</i>	514.55	0.23	0.33289
<i>yqgA</i>	557.36	0.20	0.48445	<i>znuC</i>	476.44	-1.04	0.00091
<i>yqgB</i>	578.07	0.56	0.04725	<i>zraP</i>	4258.37	-4.77	1.45E-29
<i>yqgD</i>	149.86	-0.51	0.08066	<i>zur</i>	2054.29	-1.59	8.20E-10
<i>yqgE</i>	765.66	-0.82	0.00214	<i>zwf</i>	15335.94	1.72	1.32E-08
<i>yqgF</i>	138.88	0.26	0.32854				