Examination of cationic antimicrobial tolerance in *Escherichia coli* to identify phenotypic and genotypic adaptations

by

Nicola Cartwright

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Department of Medical Microbiology and Infectious Diseases
University of Manitoba
Winnipeg
Manitoba
CANADA

Abstract

Cationic antimicrobial (CA) agents describe a variety of positively charged antimicrobials that are widely used in many clinical, agricultural and industrial facilities to disinfect and prevent microbial growth. Increased tolerance to CAs by Gram-negative bacteria is a growing problem because CA tolerant bacteria frequently confer therapeutic antimicrobial cross-resistance. Previous studies have shown that CA tolerant bacteria frequently exhibit alterations in lipid modification pathways, up-regulation of efflux pumps and porins as a mechanism of tolerance but these changes have yet to be consistently identified in experiments containing the same species or strain exposed to different CAs. In this thesis, *E. coli* K12 was adapted to increasing concentrations of CAs, specifically, benzalkonium chloride, cetrimide bromide, chlorhexidine hydrochloride and colistin sulphate that belong to different antimicrobial classes to determine phenotypic and genotypic changes over 20-40 sub-cultures. It was revealed that CAs belonging to similar classes had similar growth phenotypes, antimicrobial cross-resistance and genotypic alterations. Experiments exploring the stability of CA-tolerant phenotypes when CA selection is removed over a 10-day period among revealed a dependence on previous CA exposure. Genotypic analysis involved identification of repeatedly identified single nucleotide variants (SNVs) in lipopolysaccharide biosynthesis pathways, antimicrobial transcriptional regulators, transposable elements, and to a lesser extent in efflux pump genes. This study suggests that CA adaptation may be dependent upon how each CA specifically disrupts the cell membrane, since each CA disrupts the membrane at potentially different outer membrane targets. It also reveals new insights and genetic markers associated with CA tolerance.

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List of Abbreviations (in alphabetical order)

ALX, alexidine dihydrochloride

AMK, amikacillin

AMP, ampicillin

AMOX, amoxicillin

AMR, antimicrobial resistance

AST, antimicrobial susceptibility testing

ATP, adenosine triphosphate

BG, bisbiguanide

Br, bromide

BZE, benzethonium

BZK, benzalkonium chloride

BZK-R, benzalkonium-resistant

Ca, calcium

CA, cationic antimicrobial

CAZ, ceftazidime

CDF, cationic diffuser facilitator

CDAB, cetyl dimethylammonium bromide

CET, cetrimide bromide

CET-A, cetrimide-adapted

CHG, chlorhexidine digluconate

CHL, chloramphenicol

CHX, chlorhexidine hydrochloride

CHX-A, chlorhexidine-adapted

CHX-R, chlorhexidine-resistant

CIP, ciprofloxacin

Cl. chlorine

CLSI, Clinical Laboratory Standards Institute

CPC, cetylpyridinium chloride

CPC-A, cetylpyridinium-adapted

COL, colistin sulphate

COL-A, colistin-adapted

CTAB, cetyltrimethylammonium bromide

CTX, cefotaxime

Da, Dalton

DDAB, didodecyldimethyl ammonium bromide

DDAC, didecyldimethyl ammonium chloride

DMAB, didecyldimethyl ammonium bromide

DG, davis glucose

DMSO, dimethyl sulphoxide

DNA, deoxyribonse nucleic acid

DOM, domiphen bromide

ECA, enterobacterial common antigen

EPS, extrapolymeric substance

ERY, erythromycin

ETC, electron transport chain

EUCAST, European Committee on Antimicrobial Susceptibility Testing

GENT, gentamycin

H_2SO_4, hydrogen sulphate

IMP, imipenem

K, potassium

KAN, kanamycin

LB, lysogeny broth

LPS, lipopolysaccharide

MATE, multidrug and toxin extruder

MDR, multidrug resistant

MFS, major facilitator superfamily

Mg, magnesium

Mg/L, milligram per litre

µg/ml, microgram per millilitre

MHB, mueller hinton broth

MIC, minimum inhibitory concentration

MV, methyl viologen

NA, nalidixic acid

Ng, nanogram

NOR, norfloxacin

OD, optical density

OMP, outer membrane protein

OMV, outer membrane vesicles

PACE, proteobacterial antimicrobial compound efflux

PEN, penicillin

PG, peptidoglycan

PHMB, polyhexamethylene biguanide

PIP, piperacillin

PMXB, polymyxin B

QAC, quaternary ammonium compound

rDNA, ribosomal deoxyribose nucleic acid

RND, resistance nodular division

SDS, sodium dodecyl sulphate

SFX, Sulfamethoxazole

SMR, small multidrug resistance

SNV, single nucleotide variant

Spp., species

STR, streptomycin

TAZ, tazobactam

TET, tetracycline

TLN, triclosan

TMP, trimethoprim

TOB, tobramycin

TSB, tryptic soy broth

WGS, whole genome sequencing

WHO, World Health Organisation

WT, wild type

CHAPTER 1. INTRODUCTION

1.1. Antimicrobials and their classifications

Antimicrobial is a general term used to describe substances including medicines that kill or slowly inhibit the growth of microorganisms when treating human or environmental surfaces[2]. This group can be classified into 2 main groups: biocides and antibiotics. Because biocides and antibiotics range in antimicrobial activity, other specific terms can be included to indicating how they inhibit growth or kill may be used: "-static," refers to agents which inhibit growth (e.g., bacteriostatic, fungistatic, and sporistatic) and "-cidal," refers to agents which kill the target organism (e.g., sporicidal, virucidal, and bactericidal)[2]. "Biocide" is an overarching term that describes a broad-spectrum chemical agent such as detergents, preservatives, antiseptics, and disinfectants, and possess either bacteriostatic (inhibit) or bacteriocidal (kill) properties towards the growth of microorganisms. Antiseptics are biocides that kill or inhibit the growth of microorganisms and are typically used on living tissues, whereas disinfectants are similar to antiseptics, but are used on inanimate objects or surfaces[2]. An antibiotic is a naturally occurring or synthetic medicine designed to kill or slow the growth of bacteria and some fungi, generally at low concentrations in or on living tissue and are therapeutically administered[2].

Biocides are now known to encompass a number of different types of compounds including detergents, quaternary ammonium compounds (QACs), bisbiguanides (BG), chlorine compounds, phenolics, iodine, alcohols, hydrogen peroxide, silver compounds and dyes[2]. Historically, biocides and other antimicrobial agents have been employed in a variety of forms for a number of centuries[3]. Historical empirical approaches included using vinegar and honey for cleansing wounds and copper and silver vessels for storing portable water in the 17th century. Later, reports stated the use

of iodine as a wound disinfectant, the use of chlorine water in obstetrics and phenol (carbolic acid) as a wound dressing in the antiseptic cleansing prior to surgery[4]. Over the course of the 20th century, other chlorine releasing agents and some QACs were introduced into use as reviewed by Russell (2002)[3]. By the mid 1940's, common biocides in use included phenolics, iodine, alcohols, hydrogen peroxide, silver compounds and dyes, with a lot of the agents remaining in use today[4]. The most important agents introduced within the last 60 years include amphoteric surfactants, bisphenols including triclosan (TLN) and biguanides including chlorhexidine (CHX) and alexidine (ALX)[3].

1.1.1. Cationic antimicrobials

Cationic antimicrobial agents (CAs) comprise a chemically diverse range of antimicrobial compounds that possess a positive charge at neutral pH[5] (Figure 1.1). CAs are commonly used to disinfect and sterilize in clinics[6], food preparation facilities[7], households[8], agriculture/ aquaculture[9], and industrial facilities[2,10]. In clinics, antiseptics and disinfectants are commonly used as part of infection control practices to prevent the spread of nosocomial infections by disinfecting surfaces[2]. The mechanism of action of CAs is their adsorption and penetration into the bacterial cell envelope; thereon follows a reaction with negatively charged outer membrane (lipids and proteins) followed by membrane disruption; this then causes cell leakage of cytoplasmic contents (including Magnesium (Mg^{2+}) and Calcium (Ca^{2+}) ions, degradation of nucleic acids and proteins and eventual cell death[5]. There are numerous biocide CAs in clinical use as reviewed by Maillard (2005)[6], however, due to the focus of this thesis, the following subsections will discuss three of the most frequently used CAs classes in antiseptic products and therapeutic medications: QACs, BGs, and polymyxins (PMX).

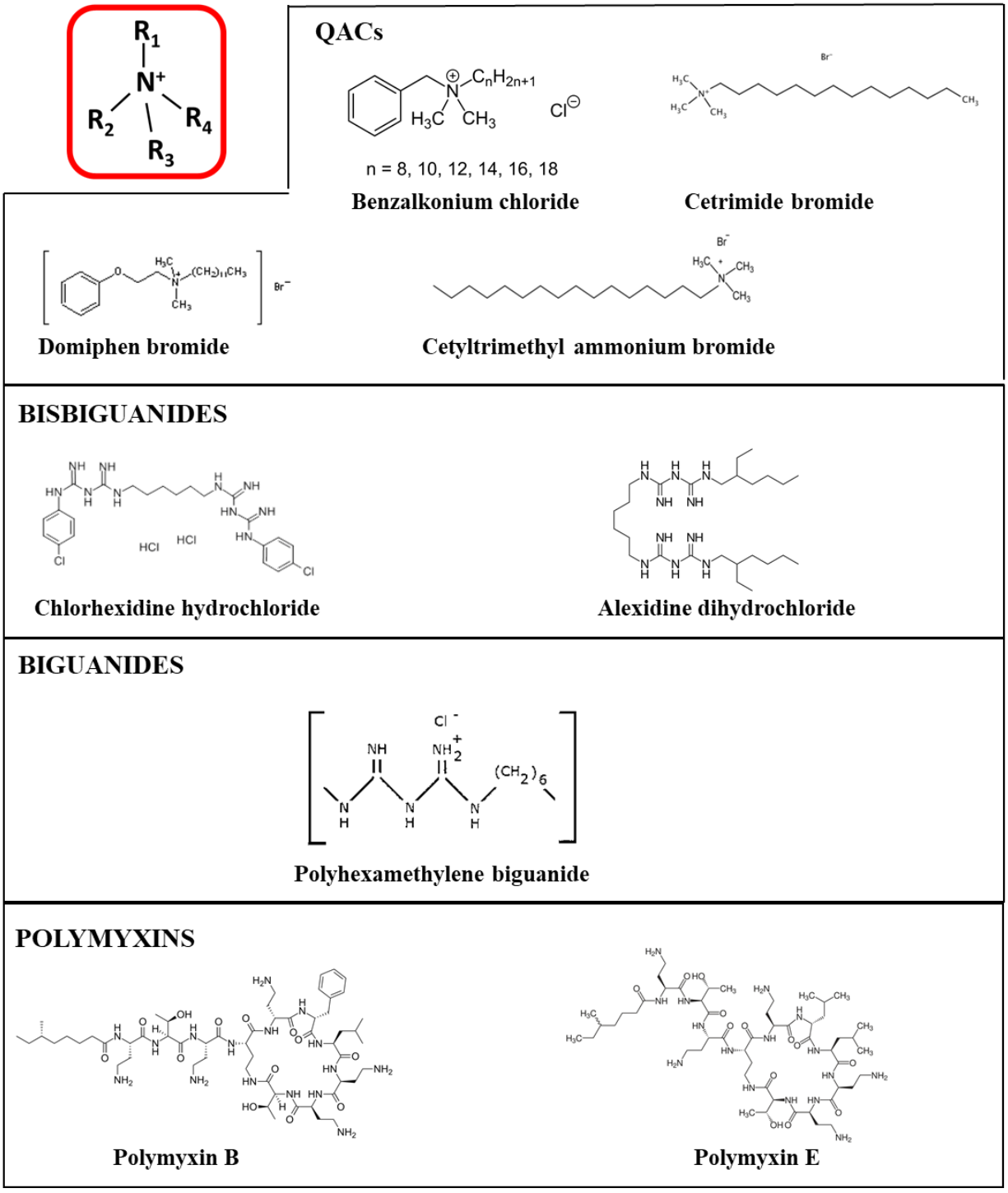

Figure 1.1 Examples of cationic antimicrobial chemical structures. Panels show QACs benzalkonium chloride; cetrimide bromide: domiphen bromide and cetyltrimethylammonium bromide: bisbiguanides chlorhexidine hydrochloride and alexidine dihydrochloride: biguanide polyhexamethylene biguanide and polymyxins polymyxin B and E.

1.1.2. Quaternary ammonium compounds (QACs)

QACs possess two distinct regions in their molecular structure, 3-4 hydrophobic acyl or aryl compound groups attached to one or more permanently charged nitrogen cations resulting in a quaternary bound ammonium atom[2,11] (Figure 1.1). Commonly used QACs such as benzalkonium chloride and cetrimide bromide are found in a number of products including disinfectants, surfactants, antiseptic creams and cosmetic products including shampoos, fabric softeners and detergents. QACs are relied upon for a variety of clinical disinfection purposes, including preoperative disinfection of skin, oral rinses, eye drops, and disinfection solutions and wipes[2].

QACs are membrane active agents, and therefore have a mechanism of action that targets the outer and cytoplasmic membranes of bacteria[2]. Specifically, QACs mechanism of action includes the progressive adsorption of the cationic nitrogen headgroup to acidic phospholipid headgroups situated on the outer or cytoplasmic membrane of the bacterium[11]. This leads to decreased fluidity of the cell membrane, creating voids within the membrane due to lipid vesicilization[11]. Reactive oxygen species (chemically reactive species containing oxygen) and reactive nitrogen species (chemically reactive species containing nitrogen) are generated which cause oxidative stress, thus denaturing proteins and DNA[12,13]. Eventual lysis of the bacterial cell is induced, and subsequent solubilization of phospholipids and proteins into QAC/phospholipid micelles occurs[11] (Figure 1.2B).

1.1.3. Bisbiguanides

Bisbiguanides (BG) are a class of chemically related compounds known for their bactericidal properties[2]. BGs, such as CHX (Figure 1.1), are widely used as antiseptics and can be found in items such as oral hygiene products, surgical handwashes, as well as topical

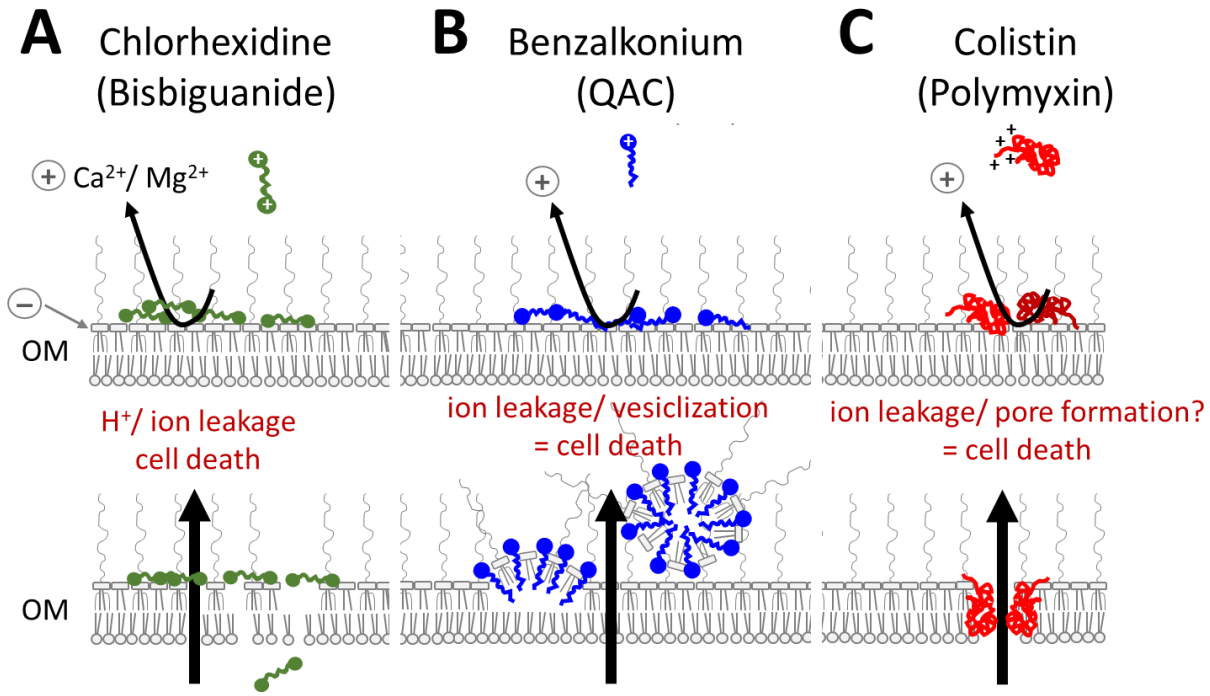

Figure 1.2. A cartoon diagram of known CA mechanisms of action at a Gram-negative bacterial outer membrane surface. The proposed mechanisms of action are compared between bisbiguanide (BG) (A), QACs (B) and polymyxin (PMX) (C).[11,14,15]

ointments/washes for wound infections[11]. BGs have similar chemical features to QACs in the sense that they are positively charged at nitrogen groups but at neutral pH and their main target site of bacterial entry is the membrane[11]. BGs have a similar mechanism of action to the QACs since BG cationic charges associate strongly with the anionic phospholipids and acidic protein sites in the cell membrane[16]. Like other CAs, BGs can subsequently induce displacement of membrane associated divalent cations (Mg^{2+}, Ca^{2+})[17]. Unlike QACs, the hydrophobic regions of membrane associated BGs do not become solubilized within the hydrophobic membrane core. The carbon chain length of BGs are shorter (6-8 C atoms) when compared to QAC aryl groups (12-18 C atoms). Since the shorter BG hydrophobic region is less flexible, it cannot penetrate deep enough into the bilayer to displace lipids like many QACs. Hence, BG specific mechanism of action creates gaps or bridges in between paired phospholipid headgroups and acidic proteins, displacing the associated divalent cations, causing eventual ion leakage that results in cell lysis and death[17] (Figure 1.2A).

Polyhexamethylene biguanide (PHMB) is a polymeric biguanide that is commonly used as a disinfectant in the food industry; it can also be found in cosmetics, leather preservatives, contact lens disinfectants, in treatment of hatching eggs, fibers and textiles and technical fluids like cutting oils and glues as well as a disinfectant in swimming pools[2]. PHMB, like CHX, is a membrane-acting agent that impairs the integrity of the outer membrane of Gram-negative bacteria[2]. PHMB is comprised of repeating basic biguanide units connected by hexamethylene hydrocarbon chains, which provides the compound with a cationic and amphipathic structure[18]. PHMB is different to other biguanides such as CHX and ALX in the sense that PHMB causes domain formation of the acidic phospholipids of the cytoplasmic membrane[18]. The sequence of events includes i) rapid attraction of PHMB toward the negatively charged bacterial outer membrane, with specific

adsorption to phosphate-containing compounds; ii) the integrity of the outer bacterial membrane is impaired, and subsequent attraction of PHMB to the cytoplasmic membrane ensues; iii) binding of PHMB to phospholipids occurs, with an increase in inner membrane permeability (K^+ loss) accompanied by bacteriostasis; and iv) complete loss of membrane function follows with precipitation of intracellular constituents and a bactericidal effect[2].

1.1.4. Polymyxins

Polymyxins (PMX) are therapeutic antimicrobial peptides that possess a polycationic charge at neutral pH[14]. PMXs were discovered over 6 decades ago and were derived from *Bacillus polymyxa*[19]. PMXs are amphiphilic, where they have net cationic charges and possess hydrophilic and hydrophobic/lipophilic regions; typically, a cyclic heptapeptide ring, attached to a tripeptide which in turn is attached to a hydrophobic fatty acid chain (Figure 1.1)[20]. Only PMXs B and E (colistin) are used clinically, frequently as last resort therapeutic antibiotics in the treatment of multidrug resistant Gram-negative bacterial infections[21]. PMXB is normally applied topically to treat eye, ear and skin infections, while colistin is used to treat diarrhea in children[21].

PMXs, like colistin, target the bacterial cell membrane, specifically, due to the polycationic peptide ring which interacts with the lipid A portion of negatively charged lipopolysaccharides (LPS), enabling colistin to penetrate through the outer membrane, potentially forming a pore and subsequently displacing Mg^{2+} and Ca^{2+}[22]. Insertion between the phospholipids of the cytoplasmic membrane leads to loss of membrane integrity and eventual bacterial cell death[23] (Figure 1.2C).

1.2. The problem of antimicrobial resistance (AMR)

Antimicrobial resistance (AMR) as determined by the World Health Organisation (WHO), is defined as:

> "the ability of a microorganism (like bacteria, viruses, and some parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it. As a result, standard treatments become ineffective, infections persist and may spread to others[24]"

AMR is a biological process that develops in bacteria over a period of time, due to selective pressure exerted by the antimicrobial[25]. As new AMR mechanisms are being discovered, it is becoming clear to healthcare professionals that AMR poses a global threat[24]. AMR mechanisms threaten our ability to treat infections, resulting in prolonged illness and even fatality[24]. According to the report by Jim O'Neill, it is predicted that by 2050, 10 million lives/annum and a 100 trillion USD worth of economic output are at risk due to the rise of AMR infections[26]. Although new antimicrobials are being developed, not nearly enough are in development and are expected to be completely ineffective against extremely multidrug resistant (MDR) bacteria. Therefore, it is critical to study AMR mechanisms to identify key new targets for therapeutic intervention and treatment. The report by Jim O' Neill in 2016 proposes that seven main interventions need to be implemented in order to attempt to tackle the growing crisis of AMR: i) introduce a global public awareness campaign; ii) improve sanitation and reduce the spread of infection; iii) reduce unnecessary use of antimicrobials; iv) improve global surveillance of drug resistant antimicrobials and their use; v) promote new and rapid diagnostics to reduce the unnecessary use of antimicrobials; vi) promote development and use of vaccines and other alternatives and vii) improve the number, pay and recognition of people working in infectious disease[26]. Implementation of these interventions in a timely manner will ideally tackle the global burden of AMR[26].

1.2.1. Critical priority AMR Gram-negative bacterial infections

According to the WHO, carbapenem resistant Gram-negative bacteria, in particular Enterobacteriaceae, are an emerging AMR problem and effectual cause of nosocomial acquired infections that pose a significant threat to public health[27]. In November 2017, the WHO published its first ever list of antibiotic-resistant "priority pathogens" and this was undertaken to guide and promote research and development of new antibiotics to try and address the growing issue of global resistance. The WHO list is divided into three categories according to the urgency of need for new antibiotics: critical, high and medium priority and consists of *Acinetobacter* spp., *Pseudomonas* spp. and various Enterobacteriaceae (including *Klebsiella* spp, and *Escherichia coli*) (Table 1.1). These opportunistic pathogens can cause severe and often fatal infections and have become resistant to many relevant antibiotics, including carbapenems and third generation cephalosporins, which are the first line treatment for treating MDR bacterial infections[1] . According to the WHO:

> "the second and third tiers in the list – the high and medium priority categories – contain other increasingly drug-resistant bacteria that cause more common diseases including gonorrhoea and food poisoning caused by *Salmonella* spp." (Table 1.1)[1].

Table 1.1. A summary of WHO priority AMR pathogens[1]

CRITICAL PRIORITIES	HIGH PRIORITIES	MEDIUM
Acinetobacter baumannii carbapenem-resistant	***Enterococcus faecium*** vancomycin-resistant	***Streptococcus pneumoniae*** penicillin-non-susceptible
Pseudomonas aeruginosa carbapenem-resistant	***Staphylococcus aureus*** methicillin-resistant vancomycin-intermediate & resistant	***Haemophilus influenzae*** ampicillin-resistant
Enterobacteriaceae carbapenem-resistant extended spectrum beta-lactamase producing	***Helicobacter pylori*** clairthormycin-resistant	***Shigella spp.*** fluoroquinolone-resistant
	Campylobacter spp. fluoroquinolone-resistant	
	Salmonellae fluoroquinolone-resistant	
	Neisseria gonorrhoeae cephalosporin-resistant fluoroquinolone-resistant	

1.2.2. The relevance of *E. coli* as a model organism to study AMR

Escherichia coli is a Gram-negative facultative anaerobic, rod shaped bacterium that is commonly found in the gut and lower intestines of most animals including humans[28]. The majority of *E. coli* strains are non-pathogenic and are part of the normal microbiota of the gut, however, some pathogenic serotypes can cause illness in humans, including diarrhea, vomiting, abdominal pain and fever[29]. Fecal–oral transmission is the major route of pathogenic infection that cause disease, and most cases of *E. coli* infection and illness are caused by improper food handling, cross-contamination of food utensils, consuming dairy products that have been left out too long or stored at the incorrect temperature, consuming foods that are not cooked to the correct temperature or duration of time, especially meats and poultry, consuming raw seafood products, drinking unpasteurized milk and consuming raw produce that has not been properly washed[30].

E. coli can be grown and cultured easily and inexpensively in a laboratory setting and has been intensively investigated for over 60 years[29]. *E. coli* is the most widely studied prokaryotic model organism, and an important species in the fields of biotechnology and microbiology, where it has served as the host organism for the majority of work with recombinant DNA[31]. The increased availability of genome sequences has provided the basis for comprehensive understanding of organisms at the molecular level. Besides sequence data, a large number of experimental and computational resources are required for genome-scale analyses. *E. coli* K-12 has been one of the best characterized organisms in molecular biology[29]. *E. coli* K-12 BW25113 is a common laboratory strain that was created in the laboratory of Barry L. Wanner and was utilized in a method using the bacteriophage lambda red recombination system to perform gene disruptions with double-stranded PCR products[32]. *E. coli* K-12 BW25113 later became the parental strain for the 'Keio collection', a major resource consisting of approximately 4,000 non-essential single-gene deletion mutants[33,34]. The 'Keio collection' provides a valuable resource not only for systematic

analyses of unknown gene functions and gene regulatory networks but also permits genome-wide testing of mutational effects in *E. coli* K-12 BW25113[33]. Additionally, a complete set of cloned genes corresponding to each essential and non-essential gene were cloned into the pCA24N expression vector with and without a green fluorescent protein C-terminal fusion tag and are known as the 'ASKA collection'[35]. The ASKA collection allows systematic gene complementation and functional analyses of the Keio mutants to examine phenotype[35]. Whole genome sequences are available for BW25113[36] and its two closely related K-12 strains, MG1655[37] and W3110[38]. Hence, *E. coli* K12 BW25113 and its derivatives are being used in countless laboratories for a variety of studies, including systematic phenotypic surveys[39] and synthetic biology efforts[40–42]. Despite the genetic characterization of *E. coli*, nearly 1/3 of the genes in the *E.coli* genome (designed as genes beginning with a 'y') have unknown function and/or are hypothetical functions, many of these are predicted to target the cell membrane, emphasizing the need to continue genotypic and phenotypic studies of *E. coli* [43,44].

1.3. CA resistance in Gram-negative species

CAs used as antiseptics and disinfectants can describe a wide variety of chemicals, each with their own working concentrations, properties, chemical modifications and counterion formulations (gluconate; acetate; Cl^-, Br^-, H_2SO_4-). In addition to chemical property variations, there is considerable confusion and controversy in determining what defines bacterial CA susceptibility, CA concentration thresholds or break points since there are no reference strains or agreed upon CA compounds for biocide testing thus far[45–48]. Standard biocide susceptibility testing methods are not available from the Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). There is also

considerable ambiguity in what defines minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) since most biocides are bactericidal. The problem is biocide 'resistance' and 'tolerance' are terms often used interchangeably in published articles leading to confusion when compared to antibiotics. The review article published by Cerf *et al* (2010)[49] attempts to define biocide 'resistance' and 'tolerance' as follows: 'resistance' should be used to describe bacterial killing (i.e. MBC values), while 'tolerance' should be used to describe bacterial adaptation to inhibitory biocide concentrations and used when describing MIC values of more than 2-fold change as compared to the WT strain[45,49].

Although most biocides are effective at killing Gram-negative pathogens at the manufacturer's recommended high concentrations (0.001-10% w/v)[6,8], many *Pseudomonas* and *Acinetobacter* spp. are intrinsically more tolerant to high concentrations of cationic biocides as compared to Enterobacteriaceae (see Table 1.2 for *E. coli* examples). Enterobacteriaceae species can also rapidly acquire tolerance to high concentrations of CA biocides when exposed to prolonged sub-inhibitory concentrations. CA resistance has been shown in *E. coli*, with QAC resistance demonstrating up to 4-fold increases in MIC[50,51] (Table 1.2). Reduced biocide susceptibility by opportunistic pathogens is associated with higher rates of hospital outbreaks (as reviewed by Weber *et al* (2007)[52], making biocide tolerance an important AMR aspect to consider with respect to environmental and clinical AMR stewardship initiatives.

Table 1.2. A summary of published MICs (mg/L) for biocide susceptible and biocide tolerant or biocide adapted *E. coli* strains.

Species	n	CHX	BZK	BZE	CET	DMAB	CPC	TLN	CAZ	CTX	IMP	MER	AMP
E. coli (ESBL producer)	174	0.5-4	4-32	16-32	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	2	5-10	25	ND	ND	ND	ND	ND	ND	ND	ND	ND	2-10
E. coli BZK-A	2	**60-240**	**150**	ND	ND	ND	ND	ND	ND	ND	ND	ND	**20-50**
E. coli K-12	1	ND	13	ND	ND	ND	ND	ND	ND	0.06	ND	ND	4
E. coli K-12 BZK-A	3	ND	**80-90**	ND	ND	ND	ND	ND	ND	**0.12-0.5**	ND	ND	4-8
E. coli	1	ND	10	ND	ND	ND	5	ND	ND	ND	ND	ND	5
E. coli CTAB-A	1	ND	**35**	ND	ND	ND	**20**	ND	ND	ND	ND	ND	**>1000**

Species	AMOX	PIP	PIP-TAZ	GEN	AMK	TOB	KAN	STR	NOR	CIP	TET	CHL	TMP
E. coli (ESBL producer)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
E. coli	ND	ND	ND	2	ND	ND	8-10	ND	0.1-0.4	ND	4	ND	ND
E. coli BZK-A	ND	ND	ND	**4**	ND	ND	10-16	ND	0.15	ND	**6-16**	ND	ND
E. coli K-12	ND	ND	ND	2-4	ND	ND	16	16-32	ND	0.06	2	8	ND
E. coli K-12 BZK-A	ND	ND	ND	1-2	ND	ND	2-8	4-16	ND	**0.25**	**4-8**	8-**128**	ND
E.coli	4	ND	ND	ND	ND	ND	5	ND	ND	ND	1.2	7	0.4
E. coli CTAB-A	**525**	ND	ND	ND	ND	ND	**15**	ND	ND	ND	**3.2**	**55**	**2**

Species	TMP-SFX[b]	SFX	ERY	NA	COL	PMXB	References
E. coli (ESBL producer)	ND	ND	ND	ND	ND	ND	Deus et al. 2017[53]
E. coli	ND	ND	100-140	4-8	ND	ND	Langsrud et al. 2004[54]
E .coli BZK-A	ND	ND	**160-180**	**30**	ND	ND	Langsrud et al. 2004[54]
E. coli K-12	ND	ND	ND	8	ND	ND	Bore et al. 2007[50]
E. coli K-12BZK-A	ND	ND	ND	**32-64**	ND	ND	Bore et al. 2007[50]
E. coli	ND	ND	250	25	ND	ND	Ishikawa et al. 2002[51]
E. coli CTAB-A	ND	ND	**800**	**250**	ND	ND	Ishikawa et al. 2002[51]

ND; not determined, BZK-A; benzalkonium-adapted species, CTAB-A; cetyltrimethylammonium bromide adapted species.
Biocide abbreviations: BZK; benzalkonium, CHX; chlorhexidine, BZE; benzethonium, CET; cetrimide, CTAB; cetyltrimethylammonium bromide, CPC; cetylpyridinium chloride, DMAB; didecyldimethylammonium bromide, TLN; triclosan
Antibiotic abbreviations: AMK; amikacillin, AMP; ampicillin, AMOX; amoxicillin, CAZ; ceftazidime, CHL; chloramphenicol, CIP; ciprofloxacin, COL; colistin, CTX; cefotaxime, ERY; erythromcyin, GENT; gentamycin, IMP; imipenem, KAN; kanamycin, NA; nalidixic acid NOR; norfloxacin, PEN; penicillin, PMXB; polymyxin B, PIP; piperacillin, SFX; sulfamethoxazole, STR; streptomycin, TAZ; tazobactam, TET; tetracycline, TOB; tobramycin, TMP; trimethoprim.

1.3.1. CA tolerance and adaptation in Enterobacteriaceae

There have been many *in vitro* laboratory experiments conducted to artificially 'adapt' various lab cultured Gram-negative species to study biocide CA tolerance. These biocide 'adaptation' (also known as 'exposure') experiments involve gradually exposing a pure or mixed bacterial culture to increasing concentrations of a CA (most frequently BZK and CHX) over multiple subcultures (5-40 sub-cultures) ultimately producing a culture that can tolerate higher biocide MICs (ranging from 2-100 fold) as compared to the initial 'un-adapted' strain (Table 1.2). In most studies, the 'stability' of the newly acquired biocide tolerant culture is verified by repeated sub-culturing in media lacking biocide followed by sub-culturing in media at the adapted biocide MIC value[55,56]. Many adaptation studies have also examined the fitness of the CA-adapted species, where the majority consensus suggests biocide adaptation came at significant cost (5-50% reduction) in their overall growth as compared to initial un-adapted strains[51,57,58]. However, some cationic biocide adapted strains showed no difference in fitness and even gained fitness as well as virulence as was the case for *Salmonella*[58,59]. It remains unclear if specific CAs have a greater fitness cost in certain species/ genera due to lack of studies examining more than 3 different classes of biocide CAs in a single study.

With respect to MIC values achieved by CA-adapted species, mostly modest (2-6 fold changes) and occasionally high (20-50 fold changes) MIC values were obtained for various Enterobacteriaceae as compared to their initial starting strains[50,60–62]. Factors influencing final CA-adapted MIC values may be related to the genetic background of starting strain/serotype used for adaptation[61,63] and variations in the total number of sub-cultures generated during the adaptation experiment[64]. Unfortunately, experimental variations in the number of sub-cultures (or lack of reported values) required to generate the final biocide adapted species making it difficult to determine if background species or final sub-culture numbers are more influential on final CA

adaptation thresholds. For example a study examining CHX adaptation of various *Klebsiella* spp. used a method that involved a fixed total of 10 sub-cultures for all experiments and reported differences in the final CA concentrations tolerated by each strain[64]. This suggests that different strains/serotypes were more adept than others to acquire higher CA tolerance supporting the importance of species/serotype background. The importance of CA adaptation order was also demonstrated in *Enterobacter* spp. experiments[61]. Adaptation of *Enterobacter* species to benzalkonium (BZK) demonstrated increased susceptibility to cetrimide (CET) and cetylpyridinium chloride (CPC), yet the same strains adapted first to CPC showed reduced susceptibility to BZK[61,63]. As a result, growing consensus from previous adaptation studies, in Enterobacteriaceae, suggests that different CA tolerance mechanisms may be responsible for adaptation to different antimicrobials (BZK versus CHX for example), rather than a single generic CA tolerant pathway or mechanism[50,51,64]. However, further evidence in support of this theory has not been shown to date. Hence, biocide adaptation to one CA does not necessarily confer cross-resistance to other CAs or biocides and highlights the importance of understanding the mechanisms of biocide action, cellular targets, and biocide synergies/antagonism, especially when many antiseptics/ disinfectants are formulated in commercial biocide solutions.

1.3.2. CA contributions to antimicrobial cross-resistance in *E. coli*

The consequences of increased CA tolerance by Enterobacteriaceae extend beyond increased tolerance to other biocides, as numerous studies surveying antibiotic cross-resistance of biocide adapted strains have demonstrated increased tolerance to particular antimicrobials (Table 1.2). CA-adapted strains have frequently demonstrated low level increases ($\geq$2 fold) in antibiotic MIC values to one or more antibiotics tested as compared to the un-adapted strain or MIC value

ranges reported for collections representing each species (Table 1.2). For example, CA-adapted *E. coli* strains have shown antibiotic cross-resistance to β-lactams: ampicillin, amoxicillin, penicillin and macrolide erythromycin[51,54], third generation cephalosporins ceftazidime, ceftiofur, cefotaxime[50,65,66], as well as naladixic acid and aminoglycoside tobramycin[67]. Additionally, CA-adapted strains also demonstrated reduced susceptibility to similar classes of CAs as well as antibiotic cross-resistance testing suggesting tolerance to one CA class may enhance tolerance to others. For example, the type of CA used for *Salmonella* spp. adaptation to BZK, CPC, chlorhexidine digluconate (CHG), and CET increases MIC value, CA tolerance and cross-resistance to various antimicrobials such as ampicillin, trimethoprim-sulfamethoxazole, nalidixic acid and tetracycline[57,61,63]. Furthermore, differences in MIC values to these four antimicrobials were also noted for CHX-adapted *S. enterica* serovars in two separate studies[57,63]; increased resistance was only demonstrated to tetracycline in both studies. This may suggest that species/ serovar/ serotype selection for biocide adaptation is an important factor for antimicrobial cross-resistance determination.

Regarding the impact of the final MIC values attained for a CA-adapted strains and its influence on antibiotic cross-resistance, CHX-adapted *K. pneumoniae* (4-8-fold increase in MIC from the un-adapted strain) demonstrated significant increases in MIC ranges towards many clinically relevant antibiotics tested[64]. Similar cross-resistance values were noted in *E. coli* strains adapted to DDAC that exhibited $\geq$3 fold MIC from the un-adapted starting strain[68]. These adaptation studies suggest that Enterobacteriaceae strains with higher final CA-adapted MIC values may concomitantly increase antibiotic resistance. It should be noted that other studies examining CA-tolerant strains tested for their antibiotic cross-resistance have also demonstrated

no significant differences in antibiotic cross-resistance[8,69,70]. These outcomes may be explained by many of the same factors discussed above as well as reduced culture fitness, and intrinsic differences in background CA tolerance due to variations between species/ strain/ serovar. Hence, bacterial CA tolerance, CA adaptation and cross-resistance experiments would benefit from further studies using known organisms, to address experimental irreproducibility, reference strain continuity, and more in-depth CA tolerance mechanism exploration.

Finally, CA adaptation has been reported to increase tolerance to other CAs, specifically aminoglycosides[51,54] and CA peptides such as PMXs[64,71] in various Enterobacteriaceae, including *E. coli* (Table 1.2). Based on the solubility, size, and net charge differences of these CA compounds at neutral pH, it would seem logical that tolerance to any CA would increase its tolerance of other CAs. However, antimicrobial cross-tolerance/resistance by Enterobacterial CA-adapted strains to both CA biocides or other antimicrobials has not been convincingly demonstrated in any species to date[50,51,54]. Some explanations for this may be due to the inherent differences between antimicrobial chemical properties such as size, hydrophobicity, aromaticity, net charge as well as mechanism(s) of drug action, and molecular mechanisms associated with its tolerance (Table 1.2). How the CA/drug disrupts the membrane, the concentrations at which it can do so, the target surface and/or membrane proteins/lipids it targets, whether or not the compound can pass through outer membrane porins or bypass porins in favour of self-promoted uptake/direct membrane penetration are all variables that may predict CA-antibiotic cross-resistance patterns but at the present time there are insufficient experiments to support this.

1.3.3. Mechanisms of CA tolerance

There are three main mechanisms of AMR and tolerance in bacteria; efflux pumps[72,73], lipid biosynthesis and transport[74] and outer membrane porin down regulation[75] (Figure 1.3). In addition, other AMR systems including transcriptional regulators such as marR[76], ramR[77], soxS[78] and PhoPQ[79] and lipid biosynthesis enzymes such as lpxL[80] and lpxM[81] have also been associated with CA tolerance mechanisms in bacteria[82,83]. Similar to some antibiotics (aminoglycosides), the mechanism of tolerance to CAs involves alterations of the bacterial membrane that reduce the entry of the antimicrobial into the bacterial cell. Previous studies have shown an association between mechanisms of CA tolerance and up-regulation of efflux pumps[50,84], as well as porin down-regulation[50,51,84,85] and altered lipid biosynthesis enzyme expression[86,87]. More importantly it should be noted that different genes/ proteins are often altered depending on the type of CA the cell was exposed to (ie. QAC versus BG) suggesting that although CAs disrupt the membranes, they may not share specific CA tolerance biomarkers[64]. CAs such as QACs are also known to denature proteins as well as disrupt the membrane, therefore, QAC-mediate disruption may influence electron transport chain activity as well and increase the level of reactive oxygen species radicals in the cell[50]. Therapeutic CAs such as COL are known to target the LPS on the surface of bacterial cells, therefore, the most commonly identified mechanism of tolerance can be observed via changes in LPS in a number of studies[51,88]. Since CA tolerance impacts many membrane-associated systems, including reducing drug permeability and membrane lipid composition alteration[11,51], each mechanism is discussed in further detail in the following sections below (as summary is provided in Figure 1.3).

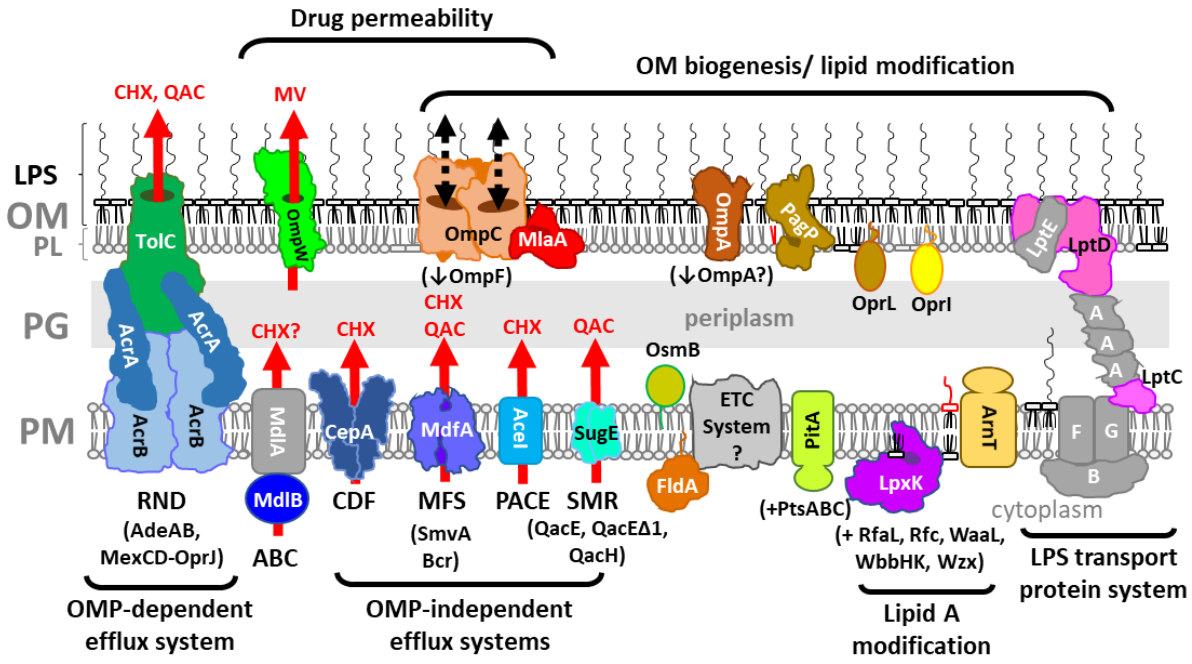

Figure 1.3. A cartoon summary of membrane proteins alterations caused by CA adaptation in various Enterobacteriaceae.

1.3.3.1. Outer membrane proteins (OMPs)

In Gram-negative bacteria, β-barrel forming proteins known as OMPs play numerous roles for the cell. Many OMPs (also known as porins) permit passive nutrient diffusion/osmoregulation (OmpC, OmpF) and participation in dedicated efflux and transport complexes (TolC). OMPs also help stabilize and maintain membrane integrity (OmpA) or possess enzymatic activity such as proteolysis (OmpT). Since porins regulate outer membrane permeability by limiting the entry of most compounds into the cell, porins are known to influence resistance to antimicrobials, such as β-lactams, tetracycline, chloramphenicol, and fluoroquinolones[76] when their expression and accumulation are altered; as reviewed by Fernandez and Hancock, 2012[75]. After surveying transcriptomic and proteomic experiments involving CA-adapted strains of Enterobacteriaceae, many OMPs previously associated with MDR are also linked to biocide adaptation.

OMP TolC is an archetypical member of the outer membrane efflux protein family and a component of numerous Resistance Nodular Division (RND) (AcrAB) and Major Facilitator Superfamily (MFS) (EmrAB) efflux systems in Enterobacteriaceae such as *E. coli*[89]. In transcriptomic studies of QAC-adapted *E. coli*, the outer membrane component of *acrAB* efflux system, *tolC* was shown to be significantly up-regulated in addition to *acrAB* as well as other *tolC*-dependent efflux systems[50]. TolC participates in a variety of multipartite membrane complexes responsible for iron siderophore release, toxin/ metabolite expulsion, and efflux pumps; hence, inactivation of TolC impairs cellular repair mechanisms and promotes metabolic shutdown as reviewed by Zgurskaya et al., 2011[89]. Hence, *tolC* up-regulation likely has beneficial pleotropic effects in QAC adapted strains beyond efflux-mediated tolerance mechanisms.

E. coli CA adaptation experiments have identified similar patterns of porin expression and accumulation by general diffusion porins, OmpC and OmpF, as observed for MDR Enterobacteriaceae[75]. In QAC tolerant and adapted *E. coli* strains, OmpC is up-regulated[50,51], while OmpF is down-regulated[50,51,85]. OmpF is slighter larger in pore size than OmpC and is down-regulated under conditions of high osmolarity and high oxidative stress (as reviewed by Falagas 2005[90]), similar to conditions that would be induced by QAC exposure. Under similar nutrient, oxidative, and stress culturing conditions, *E. coli ompC* expression increases, suggesting that biocide adaptation triggers similar stress responses regulating porin expression[91–93]. Due to the membrane disruptive actions of biocides, *in vitro* porin channel conductance experiments in artificial membranes cannot determine if biocides enter these pores, however, both pores can passively diffuse molecules ≤600 Da in size[90]. It is clear that OmpC and OmpF regulation is similar between cationic biocide adapted and antimicrobial resistant *E. coli* but its contributions in other CA tolerant genera remains hypothetical.

OMPs associated with membrane stability were identified among CA-adapted species. OmpA is one of the most abundant proteins in Gram-negative membranes. It has poor channel conductance as compared to OmpC and OmpF, but it confers an important pathogenic role with respect to host cell adhesion and invasion as reviewed by Confer and Alayew (2013)[94]. OmpA plays a stabilizing role for Gram-negative outer membranes due to its interactions with peptidoglycan (PG) and LPS[72,95,96]. Biocide adaptation is known to alter LPS[51] and PG[97] through direct contact, therefore, the loss of OmpA may either facilitate membrane alterations or is a consequence of altered LPS content in CA-tolerant bacterial membranes. Up-regulation of OMPs

responsible for membrane stability are an expected tolerance adaptation, especially under conditions where membranes must ward off the disruptive effects of CAs.

BZK, cetyltrimethylammonium bromide (CTAB), and CHX-tolerant species also demonstrated *ompW* up-regulation in *E. coli*[50,85]. Although it is unclear if BZK or CTAB can pass through OmpW pore as this has not been determined to date, OmpW has been shown to participate in QAC herbicide methyl viologen (MV) efflux as mediated by SMR efflux pump EmrE in *E. coli*[98]. QAC adapted *E. coli* showed down-regulation of *ompT*, a protease with specificity for cleaving paired basic residues[99], which is highly induced in response to heat shock and protein over-expression[100]. In *E. coli*, OmpT confers resistance to urinary CA peptides [101,102], regulates outer membrane vesicle biogenesis[103], and requires LPS for protease activity in *in vitro* experiments[104]. It remains unclear as to why *ompT* is down-regulated as a consequence of QAC adaptation. Based on OmpT's varied roles and regulation, it may be due to stress response signaling changes or perhaps a reduction in specifically modified LPS caused by CA adaptation.

1.3.3.2. Efflux pumps

In comparison to all other CA-resistant mechanisms, efflux pumps are the most well characterized and play an important part in reducing biocide susceptibility. Efflux pump activity expels drugs that enter cells and is fueled by primary active adenosine triphosphate (ATP) or secondary active proton motive force driven pumps. Efflux activity prevents drug action or targeting by expelling compounds that enter the cell. Gram-negative bacteria such as *E. coli* intrinsically possess a variety of distinct efflux pump systems, however, clinically relevant resistance to most antimicrobials including biocide CAs is conferred by an assortment of chromosomally encoded single or multi-component (OMP-dependent) efflux pump systems.

OMP-dependent efflux systems form a multipartite complex spanning both the plasma membrane (AcrB), the periplasm (AcrA) and outer membrane (TolC) of *E. coli* to completely remove the target compound, as reviewed by Li *et al* 2015[105]. In CA-adapted Enterobacterial studies reported so far, OMP-dependent efflux systems belonging to the resistance-nodulation division (RND) family play a large role in reducing CA susceptibility and are significantly upregulated in most CA-adapted species, including *E. coli*[50]. Biocide-selective *acrAB* orthologues are present in at least one or more copies within the genomes of Enterobacteriaceae and are often significantly upregulated in biocide-resistant and biocide-adapted species[50].

OMP-dependent efflux systems are augmented by the activities of single component/ OMP-independent efflux pumps that reside exclusively within the plasma membrane. As their name implies, these efflux pumps are not reliant on TolC or OMP in the outer membrane and frequently confer biocide tolerance when expressed as a single gene (as reviewed by Slipski *et al* 2017[106]). OMP-independent biocide selective efflux pumps include many transporter families: small multidrug resistance (SMR;[107]), proteobacterial antimicrobial compound efflux (PACE;[108]), cation diffusion facilitator (CDF;[109]), multidrug and toxin extruder (MATE) and MFS[110]. OMP-independent efflux pump systems enhance CA tolerance mechanisms in a variety of important ways. These pumps are conditionally expressed[111] and/or induced by oxidative and stress responses[83], such as MATE members NorM[112] and SMR *qac* genes[113]. Many OMP-independent efflux pumps are laterally inherited via integrons and multidrug-resistance plasmids[106] which expand a cells' ability to recognize and efflux a broader range of CAs not recognized by dominant OMP-dependent efflux systems[111,114]. Elevated tolerance to QACs and/or CHX have also been demonstrated in over-expression studies of MFS members *mdtM*[115], *mdfA* and *emrD*[83,114], but none

of the efflux pump has been directly identified in transcriptomic/proteomic analyses of QAC or CHX adapted *E. coli* strains. MATE member *mdtK* (*ydhE*) which has previously shown BZK (2-fold) tolerance when over expressed in *E. coli*[116], suggests that particular CAs may induce specific efflux pumps. By comparison to AcrAB pumps, most OMP-independent efflux pumps only modestly enhance CA tolerance (> 2-6 fold MIC values) based on overexpression studies as reviewed by Slipski *et al* 2017[106], emphasizing their supporting role in efflux-mediated AMR.

1.3.3.3. Transcriptional regulators, lipid modifiers, and other membrane proteins

When considering the multiple mechanisms of action induced by biocide CAs, the development of CA tolerance/adaptation may force cells to overcome multiple stressors at the membrane rather than a single target. Therefore, it is not surprising that many of the transcriptional regulators target a variety of membrane proteins. Analyses of CA tolerant and adapted Enterobacterial species have identified altered up-regulation of numerous transcriptional regulators (*marR, ramR, soxS, phoPQ*) in studies involving antimicrobial exposure, oxidative damage, and stress. Many of these systems control the expression of various efflux pump systems and antimicrobial-resistance genes[82,83] . QACs and CHX have been shown to up-regulate similar efflux pump regulators, *marRA* and *soxS*, in *E. coli;* both transcriptional regulators are known to positively regulate dominant pump *acrAB-tolC* [84,117].

In addition to efflux system regulation, transcriptional regulators that control LPS modifications have been implicated in CA-adapted Enterobacterial studies. In *E. coli* and *Salmonella enterica* serovar Typhimurium, *pmrD* regulates the expression of the *pmrHFIJKLM* (also known as *arnBCADTEF* operon), which enhances resistance to CA peptides including PMXB by 4-amino-4deoxy-α-L-arabinose modifications to lipid A[118,119]. In *E. coli*, PmrD acts as

a connector between the PmrAB and PhoPQ two component systems as reviewed by Dalebroux *et al* (2014)[120]. LPS alterations have demonstrated increased resistance to PMXs as well as other CA peptides as reviewed by Olaitan *et al* 2014[14]. Therefore, it makes sense that they may play an additional role in biocide CA tolerance. Other PMX resistance associated genes *rfaL, yefI, rfc, rfbX* have been identified from PHMB-adapted *E. coli*[88]. LPS O-antigen polysaccharide modifying enzymes *rfaL*(*waaL*), *yefI* (*wbbK*), *rfc* (*wbbH*), *rfbX (wzx*) were up-regulated in PHMB adapted *E. coli* strains[88], suggesting that modifications of LPS O-antigen sugars are increased in cationic biocide-adapted *E. coli*. QAC exposure in *E. coli* is known to alter LPS properties[51], suggesting an potential role for PMX-like LPS modifications in other CA tolerance mechanisms.

Outer membrane lipoproteins have been up-regulated in CA-adapted *E. coli*. The lipoprotein, *vacJ/ mlaA,* is an outer membrane component of the Mla pathway involved in phospholipid transport system[121]. MlaA directly interacts with OmpC and OmpF porins to maintain the phospholipid-LPS asymmetry of the outer membrane[122]. MlaA enhances outer membrane vesicle (OMV) formation and polymyxin resistance[123,124]. Up-regulation of *osmB*, a hyperosmotic stress inducible lipoprotein[125] in CA-adapted *E. coli* membranes may suggest that osmotic stress inducible proteins as well as OMV regulatory proteins are required to counteract the membrane disruptive actions of CAs. SoxRS-regulated electron transport chain (ETC) components were also up-regulated in BZK adapted *E. coli* and include flavoprotein FldA and cytosolic fumarate reductase FumC[50]. The increased oxidative stress inducible components as well as superoxide dismutase SodA up-regulation may help offset the chronic oxidative damage caused by CA exposure in these adapted cells. Additionally, CA-adapted *E. coli* and *S. Typhimurium* both demonstrated a variety of energy production, nucleotide metabolism, and carbohydrate

metabolism pathway components that were variably up and down-regulated; many which were biased towards MarAR and SoxSR inducible systems[50,126]. It is clear that many proteins responsible for membrane stability, maintenance, and lipid biosynthesis / modifications contribute towards CA tolerance mechanisms in addition to oxidative stress induced responses, however, due to the lack of experiments comparing alterations caused by different CA classes in the same experiment it is hard to pin point shared and unique mechanisms of CA tolerance and resistance attributed to CA adaptation. Future experiments will ideally clarify the cause and effect relationships linked to CA adaptation/tolerance to determine what phenotypic and genotypic alterations are essential for CA tolerance to develop.

1.4. Thesis objectives and hypotheses

The main aim of the study is to understand CA tolerance mechanisms by generating and characterizing *E. coli* strains adapted to various CAs. The main hypothesis for the project is as follows:

> *"**E. coli* K-12 strain BW25113 adapted to different CA classes (QACs, BGs, and PMXs) will result in similar phenotypic and genotypic alterations".**

To address the main hypothesis, the following sub-hypotheses for the project were examined:

1. Are CA tolerant phenotypes easily lost when CAs are removed from growth medium? The phenotypic stability of CA tolerance after the removal of CA selection over 10 days will gradually diminish CA tolerance phenotypes among CA-adapted *E. coli* strains.

2. Are CA-adapted *E. coli* more or less fit than un-adapted *E. coli* under similar growth conditions? The growth fitness of CA-adapted strains will be compromised as compared to un-adapted *E. coli* strains.
3. Does CA tolerance enhance cross-resistance to therapeutic antibiotics and other biocides? CA-adapted strains will enhance antimicrobial cross-resistance profiles as compared to the un-adapted *E. coli* strain.
4. Does CA adaptation result in genotypic alterations ie. single nucleotide variants (SNV)? If yes, are SNVs similar or different among similar CA-adapted strains? *E. coli* adapted to different CAs will confer similar genetic alterations (SNVs) in gene and pathways involving the outer membrane, lipid biosynthesis and trafficking, OMP/porins, and efflux pump systems.

CA-adapted *E. coli* K12 BW25113 strains were generated employing gradual exposure to four different CAs, BZK, CET, CHX, and COL involving the broth culture CA adaptation procedure described by Bore *et al.* 2007[50] in triplicate; resulting in a total of 3 independently adapted strains per drug tested. The antimicrobial susceptibility of these 12 strains was compared to un-adapted *E. coli* and experiments involving phenotypic stability and growth fitness was conducted by growing CA-adapted strains and un-adapted strains in various broth media. The genetic alterations present within each CA-adapted strain was determined via Illumina MiSeq whole genome sequencing (WGS) and bioinformatic analysis to map and characterize SNVs associated with each CA tolerance phenotype.

CHAPTER 2. MATERIALS AND METHODS

2.1. *E. coli* species, strains and chemicals used in study

CAs and chemicals used in this study were obtained from Tokyo Chemical Industry (TCI) America (OR, USA), Millipore Sigma (MA, USA), Fisher Scientific and VWR (Appendix i). *E.coli* K-12 BW25113[33] was obtained from the Coli Genetic Stock Centre (CGSC; http://cgsc2.biology.yale.edu)..

2.2. *E. coli* cryopreservation, stab culture methods, and growth conditions

CA-adapted *E. coli* sub-cultures were cryopreserved in Lysogeny Broth (LB) media (1% tryptone, 0.5% yeast extract, 0.5% sodium chloride), containing a final concentration of 16% (v/v) glycerol and stored at -80°C. LB+glycerol as a cryopreservant was selected based on prior CA adaptation experiments[50]. Samples were cryopreserved when ODs reached stationary phase from overnight incubation (LB media containing 16% v/v glycerol). Stab cultures of 1% LB agar were also prepared in line with standard protocol[127], incubated overnight at 37ºC in a non-shaking incubator and then stored at 4ºC. The viability of the cryopreserved stocks was tested by inoculating 5 ml of LB per sample and incubating overnight at 37ºC until turbidity was observed. All *E. coli* cultures were grown in a shaking incubator (New Brunswick Excella E25) at 37ºC in this study unless otherwise noted.

2.3. *E. coli* adaptation to CAs

E. coli BW25113 CA adaptation experiments were performed in LB broth medium using a repeated sub-culturing method described by Bore *et al.* 2007[50] with modifications listed below

(Figure 2.1A). Briefly, dimethylsulfoxide (DMSO) cryopreserved strains of *E. coli* K12 BW25113 were grown overnight (18 hours) in LB medium and subsequently diluted 10^{-2} into sterile LB containing one of four different CAs, BZK, CET, CHX and COL (Appendix i) at final concentrations ranging from 0.2-6µg/ml; these concentrations were equivalent to 20% of the respective MIC value for the CA based on the un-adapted WT strain. Triplicate cultures were inoculated for each CA to be tested and all test tubes incubated for 20–24 hours with shaking (150-170 rpm). The next day, cultures with the highest CA concentration and turbidity (growth) were selected and re-inoculated (10^{-2} dilution) into three new tubes of LB media, one at the same CA concentration and the remaining two at 2 and 4 µg/mL more CA than tube one, respectively. This sub-culturing cycle was repeated for each CA-adapted culture (three replicates total) until its gradual CA tolerance was unchanged after five consecutive subcultures and it reached a $\geq$ 2-fold higher MIC value than the original un-adapted *E. coli* BW25113 strain. COL adapted strains reached 32 subcultures both QAC adapted strains reached 40 subcultures (BZK and CET), and CHX adapted *E. coli* reach 20 subcultures; CHX adaptation experiments were extended to 30 subcultures to verify phenotypic enhancements were unaltered after 20 subcultures. *E. coli* BW25113 was also sub-cultured 40 times in LB only for use as un-adapted WT controls in all experiments. All sub-cultures were cryopreserved throughout the experiment as described above. *E. coli* BW25113 adapted to each of the four CAs were generated in biological triplicate resulting in a final total of 12 CA-adapted strains for analysis.

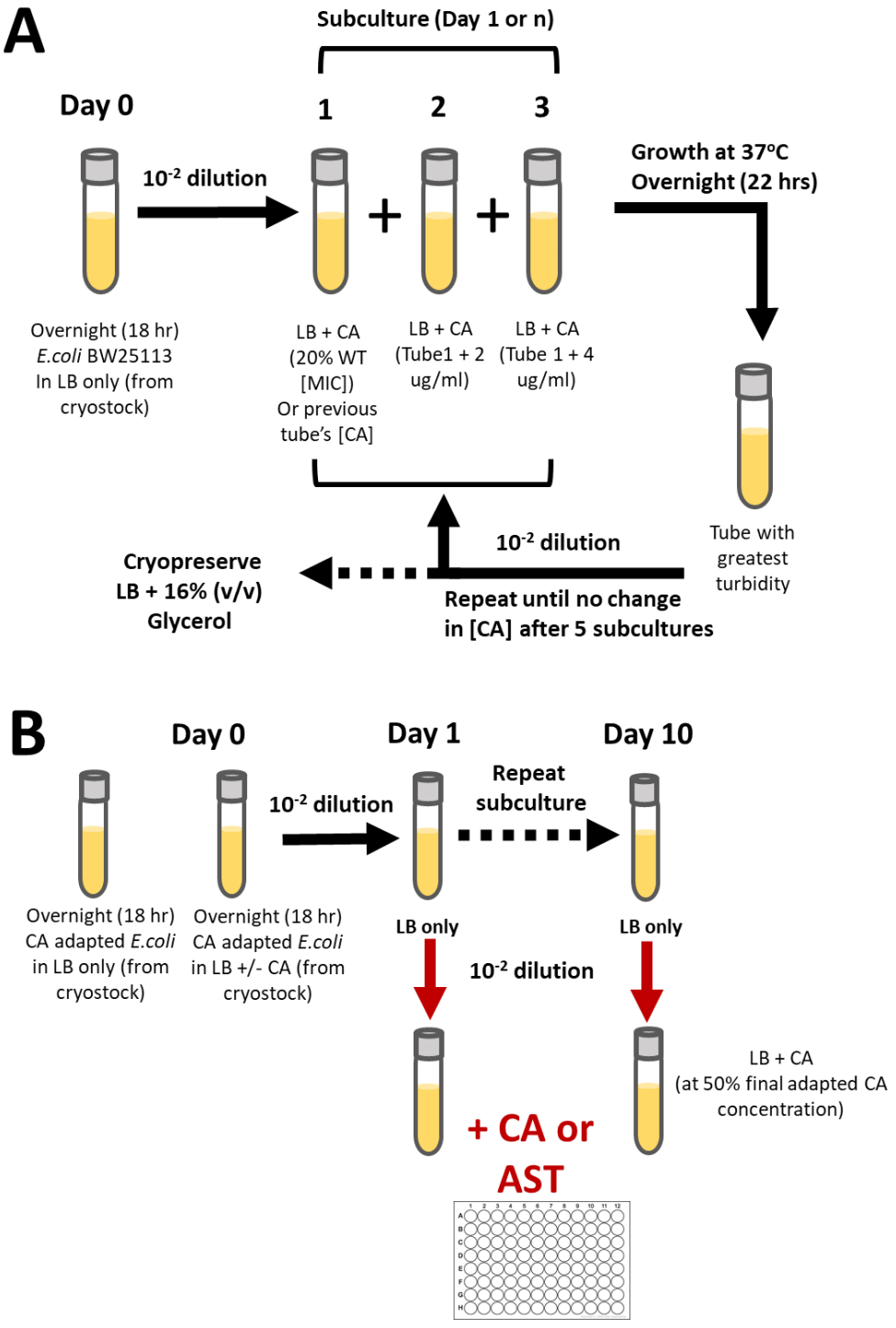

Figure 2.1. Diagram flow charts showing A) the CA adaptation process and B) the phenotypic stability testing experiment.

2.4 Antimicrobial Susceptibility Testing (AST) methods

A broth microdilution AST method was used to determine MIC values as described by Balouiri *et al.* 2016[128]. Briefly, final sub-culture cryopreserved stocks of each CA-adapted or un-adapted *E. coli* (three biological replicates per CA in technical triplicate) were inoculated into sterile LB medium and grown for 18 hours with shaking. The overnight cultures were sub-cultured into fresh tubes containing 50% of the final CA concentration they were adapted to (40 µg/mL BZK; 50 µg/mL CET; 1 µg/mL CHX; 55 µg/mL COL) and grown for 22-24 hours with shaking. Prior to inoculation for AST, overnight culture turbidity was adjusted spectrophotometrically to obtain an optical density at 600 nm (OD_{600nm}) of 1.0 units with LB. The standardized OD_{600nm} 1.0 adjusted cultures were diluted 10^{-2} into 96 well microtitre plates containing 2-fold serial dilutions of an antimicrobial stock solution (1-50 mg/mL) in LB broth medium to a final volume of 200 µL/ well. For antimicrobials stocks that required solubilization in ethanol or DMSO, control wells containing these adjuvants with and without inoculated culture were used as optical baselines. A total of 17 antimicrobial compounds were included for AST and are highlighted in bold in Appendix i. Triplicate CA-adapted/un-adapted *E. coli* strains were also repeated in technical triplicate (n=6) by AST and microplates were incubated for 18 hours with shaking (150 rpm) before OD_{600nm} measurement by a Multiskan Spectrum UV-Vis microplate reader (ThermoFisher, MA). MIC values were defined as the lowest concentration of CA tested per adapted strain that resulted in an OD_{600nm} value that was indistinguishable from the uninoculated control well (Student's t-test *p*-values $\geq$ 0.001). Significant changes in MIC were determined as a +/- 4-fold change as compared to the un-adapted WT control strain. Since AST involved a 2-fold serial

dilution of CAs the +/- 4-fold was deemed to be the minimum threshold and therefore, accounts for log 2 error.

2.5. Growth curve experiments for fitness determination

CA-adapted strain fitness was determined by comparing the growth of each strain in 96 well microplate rich and defined broth cultures to the un-adapted *E. coli* WT strain. 96 well microplate growth curves were set up as described for AST with the exception that only one specified CA concentration was selected for each CA to be tested. Final sub-inhibitory CA concentrations at 20% of the *E. coli* WT strain MIC was tested for each strain and compared to the growth of the un-adapted strain in media only. Growth curves were measured spectrophotometrically ($OD_{600\ nm}$) every 30 minutes over 24 hrs in Biotek Synergy Neo2 Hybrid Multimode reader. CA-adapted and un-adapted strains were grown in a variety of rich media: LB, LB + 0.2% (w/v) Glucose, Mueller Hinton broth (MHB), Tryptic Soy broth (TSB). Defined media was also tested: minimal nine salts (M9)[127] and Davis Glucose (DG)[127] minimal media to measure bacterial fitness in various growth conditions.

2.6. Phenotypic stability testing of CA-adapted strains when CA is removed

The stability of CA tolerance by CA-adapted strains grown in the absence of CA was determined using broth sub-culturing each CA-adapted *E. coli* strains in LB without CA for 10 days (summarized in Figure 2B). Each day of the 10-day sub-culturing stability experiment, the culture (-CA) was re-inoculated at 10^{-2} dilution into LB media containing CA at 50% of the drug concentration they were cryopreserved at (40µg/ml; CET 50µg/ml; CHX 1µg/ml; COL 55µg/ml).

All stability broth testing was grown for 22-24 hours with shaking and OD_{600nm} were measured for a minimum of 3 replicates/CA-adapted strains (n=3). To determine the stability of the MICs obtained following adaptation, CA-adapted strains were grown in the presence of CA and then sub-cultured in LB without CA for 10 days (Figure 2.1B). Each day of the 10-day sub-culturing stability experiment, AST was performed.

2.7. Whole genome sequencing analyses of CA-adapted strains

Genomic DNA was isolated from the final CA-adapted broth culture for each of the three biological replicates as well as the un-adapted *E. coli* WT strain using Invitrogen Purelink Microbiome DNA isolation kits in order to capture SNV changes within the population (ThermoFisher Scientific, MA). Genomic DNA (30-100 µl of 10-30 ng/µL) was extracted from 22-24 hour cultures grown in LB containing 50% its respective final adapted CA (BZK (40µg/ml; CET (50µg/ml); CHX (1µg/ml); COL (0.5µg/ml). Genome sequencing was performed by MicrobesNG (https://microbesng.uk/), which was supported by the BBRSC (grant number BB/L024209/1). An Illumina-MiSeq system (Illumina, Inc., CA) was used to sequence genome at a minimum of 30x coverage (Appendix ii), and all strains were verified to be *E. coli* BW25113 K-12 based on their 16S rDNA sequences and alignment to the BW25113 reference map (Genbank sequence CP009273.1). Genomic sequences for each CA-adapted strain replicate are available to download at the MicrobesNG project link (https://microbesng.uk/portal/projects/2626E4CE-05C7-45FC-A54C-EEF0DE0B8500/) and will be uploaded to NCBI in June 2019. Trimmed paired reads were generated and assembled using the MicrobesNG in-house pipeline where the *E. coli* BW25113 (CP009273.1) was used as the mapping reference.

SNV analysis was performed using Geneious® next generation sequencing bioinformatics software (v 11.1.5) to identify SNVs for each CA-adapted strain to the *E. coli* BW25133 reference genome. Briefly, the trimmed paired reads were assembled to provide contigs of the reads mapped to the reference genome. Contigs were reviewed to determine how the reads map against the reference genome and to ensure that the reads mapped to the expected distance apart, based upon the insert size specified when the reads were set up. A consensus sequence was generated to enable sequence base visualization (consensus of the reads only). Annotation and prediction of low and high coverage contig alignments was calculated so that low coverage regions could be excluded when SNVs were called. Gene annotation using Geneious® was performed to create an annotation track of SNVs added to the reference sequence. SNV annotations in coding and non coding regions was generated in separate tables. SNV annotations were then compared to the BW25113 reference genome to enable the filtering out of SNVs that were of low coverage. SNVs identified in the un-adapted WT strain grown for 40 subcultures (to account for genetic drift in LB media) were also identified and excluded from SNV analysis. SNV information was then extracted and exported into Microsoft Excel for further analyses.

2.8. Statistical analyses used

Data analysis was performed using Microsoft Excel 2016. Statistically significant differences in growth between CA-adapted and un-adapted strains were determined using a two-tailed Student's t-test using 2 -tailed paired heteroscedastic where p-values ≤ 0.001 were deemed significantly different.

CHAPTER 3. RESULTS

Adaptation, cross resistance to antimicrobials, stability testing, and growth curve experiments were conducted and led by N. Cartwright with experimental assistance from Kari Green. Whole genome sequencing was performed by Microbes NG and analysis was conducted by N. Cartwright using Geneious® software v 11.1.5.

3.1 *E. coli* adaptation to CA differs depending on the antimicrobial class it was adapted to

To remain consistent with previous experiments that 'adapted' lab cultured *E. coli* strains to biocides in broth media[50,51,54], *E.coli* BW25113 was adapted in LB at gradually increasing concentrations of one of four different CAs (BZK, CET, CHX, COL) over 20-40 sub-cultures (Figure 3.1) to produce a CA-adapted *E.coli* strain with an MIC $\geq$ 2 fold as compared to the initial 'un-adapted' BW25113 strain (Table 3.1). CA-adapted BW25113 strains were sub-cultured in triplicate and the adaptation experiment stopped when visible growth was not observed in the highest concentration after 5 sub-cultures; hence, a total of 12 CA-adapted bio-replicates (3 per each of the 4 CAs tested) were generated and we refer to them as strains based on their subtle difference in antimicrobial tolerance and fitness phenotypes as well as genotypes as discussed in further results sections in this chapter. Broth microdilution AST methods were selected as the primary method to determine MIC values of each CA-adapted strain. As compared to the un-adapted WT strain (WTG0), each CA-adapted strain showed a significant increase in MIC value ($\geq$ 4 fold) as compared to WTG0 towards its respective CA with the exception of CHX adapted strains (Table 3.1) For CHX, only one CHX adapted strain (CHXG20R1) showed an increase in MIC value (4-fold) compared to the WTG0, suggesting that the culture adapts to CHX at variable

rates. Surprisingly, COL adapted BW25113 demonstrated a 300-450-fold increase in MIC values (Table 3.1). When assessing the CA adaptation concentrations attained by each strain over the 20-40 days, as shown in Figure 3.1, the plotted outcome for QAC and COL adapted strain shows a linear increase in drug versus time; the only exception were the CHX adapted strains, which showed a stepwise increase due to the low concentrations of CHX and shorter time frame we examined. This suggests that BW25113 gradually and slowly adapted to increasing amounts of CAs for each subculture. This is important considering, parabolic and/or sigmoidal curves are often observed in antibiotic adaptative evolution experiments which imply that antimicrobial threshold must be reached prior to any gain in antimicrobial resistance[129,130]. Taken altogether the results of CA gradual adaptation demonstrate that BW25113 is capable of tolerating all four CAs but at significantly different concentrations as reflected by MIC values.

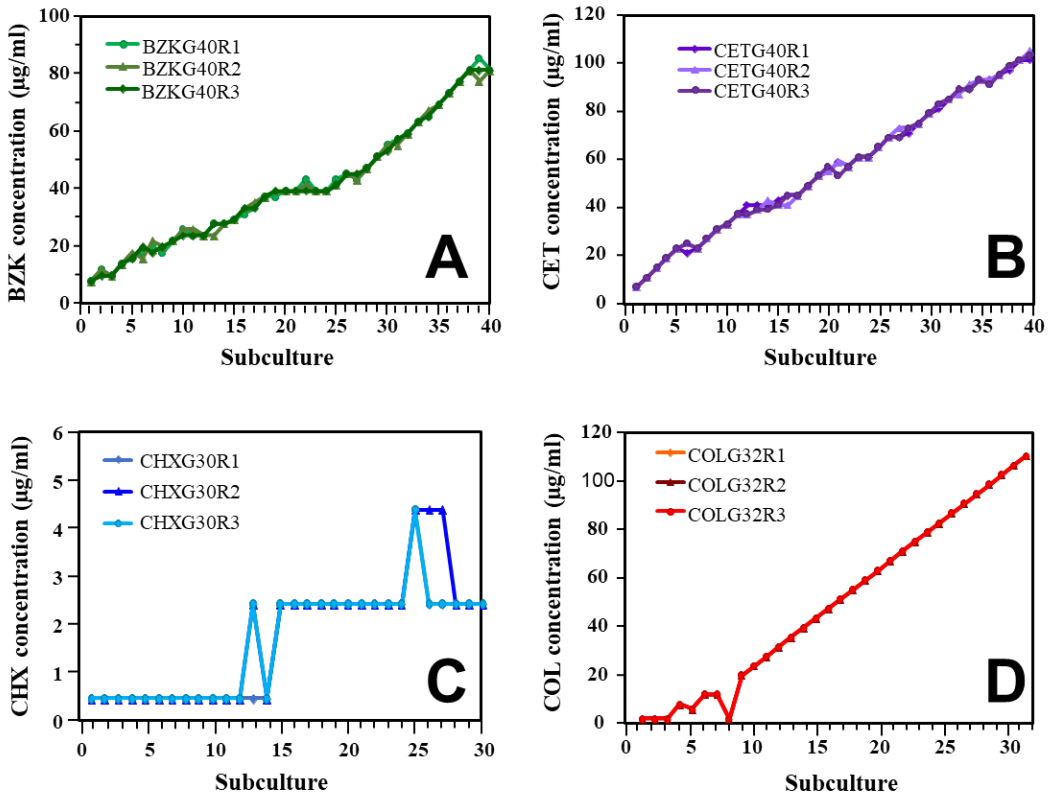

Figure 3.1. A summary of the gradually adapted maximum CA concentration achieved by each *E. coli* BW25113 subculture grown in LB broth in triplicate. CA concentrations (y-axis) are reported for each sub-cultured strain (x-axis) capable of turbid growth in LB media containing the specified concentration of CA after 22 hrs at 37°C. A summary of gradual adapted *E. coli* BW25113 strains (R1-R3 shown in panel legends) to increasing concentrations of **A)** BZK, **B)** CET, **C)** CHX and **D)** COL are shown.

Table 3.1 Breakdown of each adapted strain replicate and their final MIC as compared to un-adapted WT.

Experiment identifier	CA used for adaptation identifier	Adapted MIC (μg/ml)	WT MIC (μg/ml)
BZKG40R1	BZK	144	18
BZKG40R2	BZK	72	18
BZKG40R3	BZK	72	18
CETG40R1	CET	240	30
CETG40R2	CET	240	30
CETG40R3	CET	240	30
CHXG20R1	CHX	9.6	2.4
CHXG20R2	CHX	4.8	2.4
CHXG20R3	CHX	4.8	2.4
COLG32R1	COL	300	1
COLG32R2	COL	>450	1
COLG32R3	COL	>450	1

Experimental strains generated: R1; replicate strain 1, R2; replicate strain 2, R3; replicate strain 3 per CA used for adaptation of the WTG0 strain. G, refers to subculture generation.

3.2. CA-adapted *E. coli* show limited cross-tolerance to antimicrobials and antibiotics

Previous studies of CA-adapted strains have shown increased tolerance to various antimicrobials beyond their own CAs[51,131]. To determine if CA-adapted BW25113 strains generated herein have increased or decreased cross-tolerance as compared to WTG0, broth microdilution AST was repeated using a more extensive antimicrobial library. CAs including BZK and CET as well as additional QACs and BGs were included as well as therapeutic antibiotics representing PMXs, β-lactams, aminoglycosides, glycopeptides, quinolones, oxazolidinones, sulfonamides, tetracyclines, macrolides and ansamycins (Table 3.2). Overall, QAC adapted strains (BZK and CET) showed significantly higher MIC values (≥4 fold increase) to CPC and insignificant but higher (2-fold increases) to CDAB, CTAB, DOM as compared to WTG0. QAC adapted strain AST results for anionic disinfectant triclosan (TLN) demonstrated insignificant but higher MIC increases (2-fold) as compared to WTG0 suggesting that BZK and CET adaptation only marginally improved TLN tolerance. Previous studies have shown CA exposure (BZK) can increase cross-tolerance to TLN (5->100 fold increase in MIC as compared to the WT strain)[61]. Our AST results show low level increases in tolerance suggesting TLN mechanisms of action may have some overlap with CA mechanisms of tolerance. BZK and CET adapted strains exhibited greater susceptibility (2-16 fold) to a Gram-positive specific antibiotic vancomycin (VAN) as compared to the WT (Table 3.2). This finding suggests that QAC adaptations, particularly CET adapted strains, may alter the outer membrane and/or PG content to allow VAN easier access to the cell wall that is not possible in WT Gram-negative *E. coli*.

Table 3.2: Summary of MIC values of each adapted replicate strain when tested against other antimicrobial compounds. Values in bold indicate >2 fold change from the WTG0 value

Strain tested	Mean MIC (ug/ml) n= 3										
	BZK	**CET**	**CHX**	**COL**	**ERY**	**ALX**	**CEF**	**CDAB**	**CPC**	**CTAB**	**DDAB**
WTG0	18	30	2	1	512	2	2	16	8	16	8
BZKR1G40	**144**	**120**	2	1	256	4	1	>32	**>64**	>32	16
BZKR2G40	**72**	**240**	4	0.5	256	4	1	>32	**>64**	>32	16
BZKR3G40	**72**	**120**	4	0.5	256	4	1	>32	**>64**	>32	16
CETR1G40	**72**	**240**	2	0.5	512	4	1	>32	**>64**	>32	16
CETR2G40	**144**	**240**	2	0.5	256	4	1	>32	**>64**	>32	8
CETR3G40	**288**	**240**	2	0.5	256	4	1	>32	**>64**	>32	8
CHXR1G20	18	30	**9.6**	1	256	2	**0.5**	8	8	8	4
CHXR2G20	9	30	**4.8**	1	512	2	1	8	8	8	4
CHXR3G20	9	30	**4.8**	1	256	2	**0.5**	8	8	8	4
COLR1G32	9	15	2	**300**	**<32**	2	4	**4**	**2**	**4**	**2**
COLR2G32	**4.5**	30	2	**>450**	**<32**	2	4	**4**	**2**	**4**	**1**
COLR3G32	**4.5**	30	4	**>450**	256	2	4	**4**	**2**	**4**	**2**
Strain tested	**DOM**	**DOXY**	**KAN**	**LIN**	**MER**	**PMXB**	**RIF**	**SMX-TMP**	**TLN**	**VAN**	
WTG0	16	8	16	1024	0.03	1	512	16	0.25	256	
BZKR1G40	32	16	8	2048	0.015	NA	256	16	0.5	128	
BZKR2G40	32	16	8	2048	0.015	NA	256	16	0.5	128	
BZKR3G40	32	8	8	2048	0.03	NA	256	16	0.5	128	
CETR1G40	32	16	8	2048	0.015	NA	256	16	0.5	128	
CETR2G40	32	4	4	2048	0.015	NA	**128**	16	0.5	**16**	
CETR3G40	32	4	4	1024	0.015	NA	**128**	16	0.5	**16**	
CHXR1G20	8	4	16	1024	0.06	NA	512	16	0.25	512	
CHXR2G20	8	4	16	1024	0.03	NA	256	16	0.25	512	
CHXR3G20	8	**2**	16	**512**	0.03	NA	512	16	0.25	256	
COLR1G32	**4**	**0.5**	16	**32**	0.03	**128**	**0.125**	32	**0.0078**	**64**	
COLR2G32	**4**	**0.5**	8	**32**	0.03	**256**	**0.125**	32	**0.0078**	**32**	
COLR3G32	**4**	**0.5**	8	**32**	0.03	**256**	**0.125**	32	**0.0078**	**32**	

Abbreviation of antimicrobials: CET, BZK, CHX, COL, erythromucin (ERY), alexidine dihydrochloride (ALX), ceftazidime (CEF), cetyldiethyl ammonium bromide (CDAB), cetylpyridinium chloride (CPC), cetyl trimethyl ammonium bromide (CTAB), dimethyldidodecylammonium bromide (DDAB), domiphen bromide (DOM), doxycycline (DOXY) kanamycin (KAN), linezolid (LIN), meropenem (MER), polymyxin B (PMXB), rifamycin (Rif), trimethoprim-sulfamethoxazole (SMX-TMP), triclosan (TLN) and vancomycin hydrochloride (VAN).

CHX adapted strains showed no significant cross-tolerance to any of the antimicrobials we tested; all MIC values were either similar or insignificantly susceptible (2-fold reduction in MIC) as compared to WTG0 for the majority of antimicrobials tested, including alexidine (ALX) (Table 3.2). The results for ALX are striking when considering ALX chemically differs from CHX by the presence of two ethylhexyl moieties on each end of the molecule in contrast to the *p*-chlorophenyl groups of CHX. The ethylhexyl-end groups of ALX, are suggested to influence the ability of a biguanide to perturb LPS and lipid domains in the cytoplasmic membrane and this might, in turn, affect resistance patterns and cellular targets observed[132]. Previous studies using colourimetric biochemical *E. coli* membrane assays comparing ALX and CHX mechanisms of action show that both agents must saturate a number of envelope targets before penetration into the cytosol is possible and that ALX possessed a higher affinity towards these target sites than CHX[16]. Differences in the BG mechanism of action and target specificity between CHX and ALX may account for the lack of cross-tolerance in the CHX adapted strains expected. All three COL-adapted strains exhibited increased tolerance to PMXB as compared to WTG0 (Table 3.2), indicating that tolerance to one PMX confers cross-tolerance to other PMXs, similar to findings for QAC adapted strains. COL adapted strains demonstrated significant increases in susceptibility ($\leq$ 4-fold reduction in MIC as compared to WTG0) to the majority of antimicrobials tested, including QACs (CPC, CTAB, CDAB, DDAB), anionic disinfectant TLN, and therapeutic antibiotics ERY, DOX, DOM, LZD, RIF and VAN (Table 3.2). To explain the high susceptibility to most antimicrobials we observed by COL adapted *E.coli*, it is likely that adaptation has resulted in significant changes to the outer membrane, particularly targeting lipid A as observed in previous

studies (as reviewed by Biswas *et al* 2012 [133]). This would explain why COL adapted *E. coli* are more susceptible to most antimicrobials we tested, each with variable mechanisms of action.

In conclusion, these AST findings indicate that *E. coli* K-12 adaptation to QACs and PMXs only increases tolerance to its respective antimicrobial classes (i.e. QAC to QAC and PMX to PMX) and does not enhance cross-tolerance/resistance to other CAs or antimicrobials. *E. coli* CHX adaptation appears to be the exception to this trend, since it failed to enhance tolerance to ALX. This may highlight differences in BG mechanisms of action or highlight differences in cellular targets of each CHX and ALX during adaptation.

3.3 CA-adapted *E. coli* have similar growth phenotypes in rich media but reduced growth in minimal defined media

Since antimicrobial adaptation is known to come at significant growth fitness costs to some microorganisms[52,60,61], growth time-course (growth curve) experiments were performed for each CA-adapted strain in rich (LB +/ glucose, TSB, MHB) and minimal media (DG, M9). 24 hr growth curve experiments infer growth from $OD_{600\ nm}$ values over 30 minute time points in 96 well microtitre plates. These experiments were used to compare how CA adaptation altered the growth rate and cell titre (OD_{600nm}) with respect to WTG0. Growth curves of each CA adapted strain were compared to WTG0 in the presence and absence of sub-inhibitory CA concentrations as listed in Figures 3.2 – 3.4. Overall, CA-adapted strains demonstrated robust growth in all rich media tested with or without sub-inhibitory concentrations of CAs added as compared to WTG0. Some minor exceptions in rich media growth were noted; QAC adapted strains demonstrated a 2-4 hr extension of the lag-phase (Figures 3.2–3.3) and CHX adapted strains demonstrated slightly higher stationary

phase OD_{600nm} values (OD_{600nm}= 0.2 unit gain; Figure 3.2-3.3) as compared to WTG0. Although these findings were statistically non-significant (p>0.001), the findings were reproducible. This suggests that both QAC adapted strains have delayed growth fitness in rich medium, in contrast to CHX which appears to reach higher cell titres than WTG0 at stationary phase.

In minimal media, the growth curves of nearly all CA-adapted strains with and without sub-inhibitory CA concentrations added to the growth media demonstrated non-significant (*p*-value = $\geq$0.001) delays in lag phase growth at 2-6 hrs as well as reduced $OD_{600\ nm}$ values at stationary phase for all strains. Again, despite these findings being statistically non-significant (p>0.001), the findings were reproducible. The only exception was CHX adapted strains which maintained the same growth rate when compared to WTG0 (Figure 3.4). This suggests that CA addition in a more defined and lower osmolarity minimal growth medium has a significant impact on CA adapted strain fitness in contrast to rich media. An explanation for why minimal media is more detrimental to culture fitness is that CAs may adsorb to the diverse organic components in rich media reducing the antimicrobial concentration exposed to cells. In minimal medium, there are fewer components for CAs to adsorb onto upon increasing the exposure of cell and drug. Therefore, as observed in previous studies, *E. coli* CA adaptation comes at fitness cost when cultures are exposed to CAs in defined minimal medium.

3.4. QAC adapted *E. coli* have unstable tolerance phenotypes as compared to other CAs used for adaptation

Previous CA adaptation studies have examined the phenotypic ‘stability' of each CA-adapted strain when the selective pressure exerted by an antimicrobial compound is removed.

Typically, antimicrobial adaptation stability is determined from sub-culturing experiments, conducted over days to weeks, in growth medium lacking the antimicrobial compound (for examples refer to Méchin *et al*., 1999[134] and Gradel *et al*., 2005[135]). In this study, all 12 CA-adapted strains and the WTG0 were grown overnight without selection and repeatedly sub-cultured in medium lacking CA over a period of 10 days in LB broth. After each sub-culture day, the strains were re-exposed to media containing CAs at 50% of the final drug concentration they were cryopreserved at (refer to Table 3.1) and the results are summarized in Figure 3.5. *E. coli* strains adapted to COL and CHX maintained a stable CA tolerant phenotype to their respective CAs over the entire 10-day period. In contrast, BZK and CET-adapted strains lost tolerance to their respective CAs, quickly after the second day of sub-culturing in LB medium (Figure 3.5). These results indicate that antimicrobial tolerance phenotypes of CA-adapted *E. coli* BW25113 are stable for CHX and COL CAs, but not for QACs when selection is completely removed.

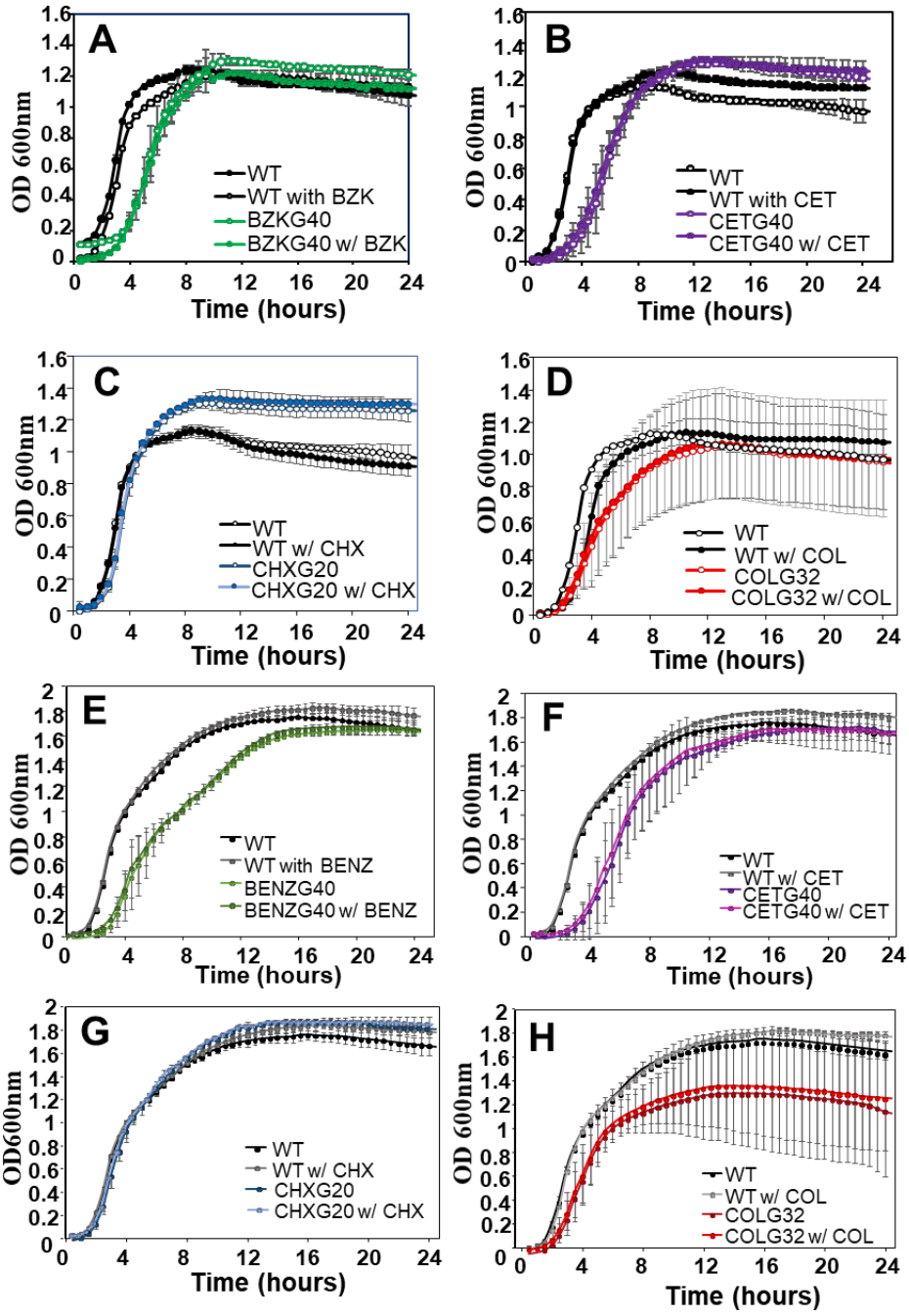

Figure 3.2. A summary of growth curves reached by each *E. coli* BW25113 subculture grown in LB broth (A-D) and MHB broth (E-H).Optical densities (y-axis) are reported as an average of each sub-cultured strain over the course of 24 hours (x-axis). A summary of gradual adapted *E. coli* BW25113 strains to sub-inhibitory concentrations of **A and E)** BZK, **B and F)** CET, **C and G)** CHX and **D and H)** COL are shown.

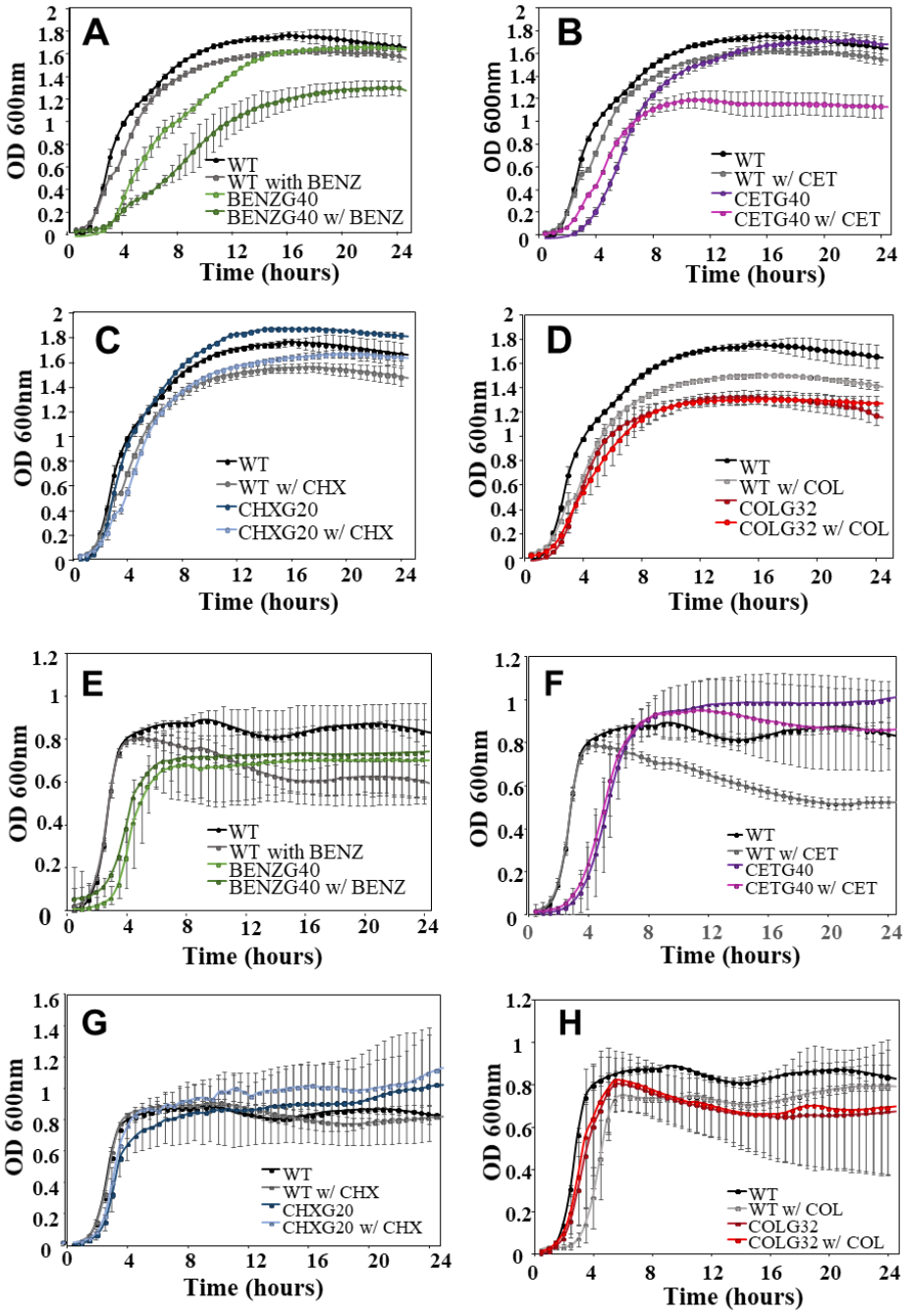

Figure 3.3. A summary of growth curves reached by each *E. coli* BW25113 subculture grown in TSB broth (A-D) and LB plus glucose broth (E-H). Optical densities (y-axis) are reported for each sub-cultured strain over the course of 24 hours (x-axis). A summary of gradual adapted *E. coli* BW25113 strains to sub-inhibitory concentrations of **A and E)** BZK, **B and F)** CET, **C and G)** CHX and **D and H)** COL are shown.

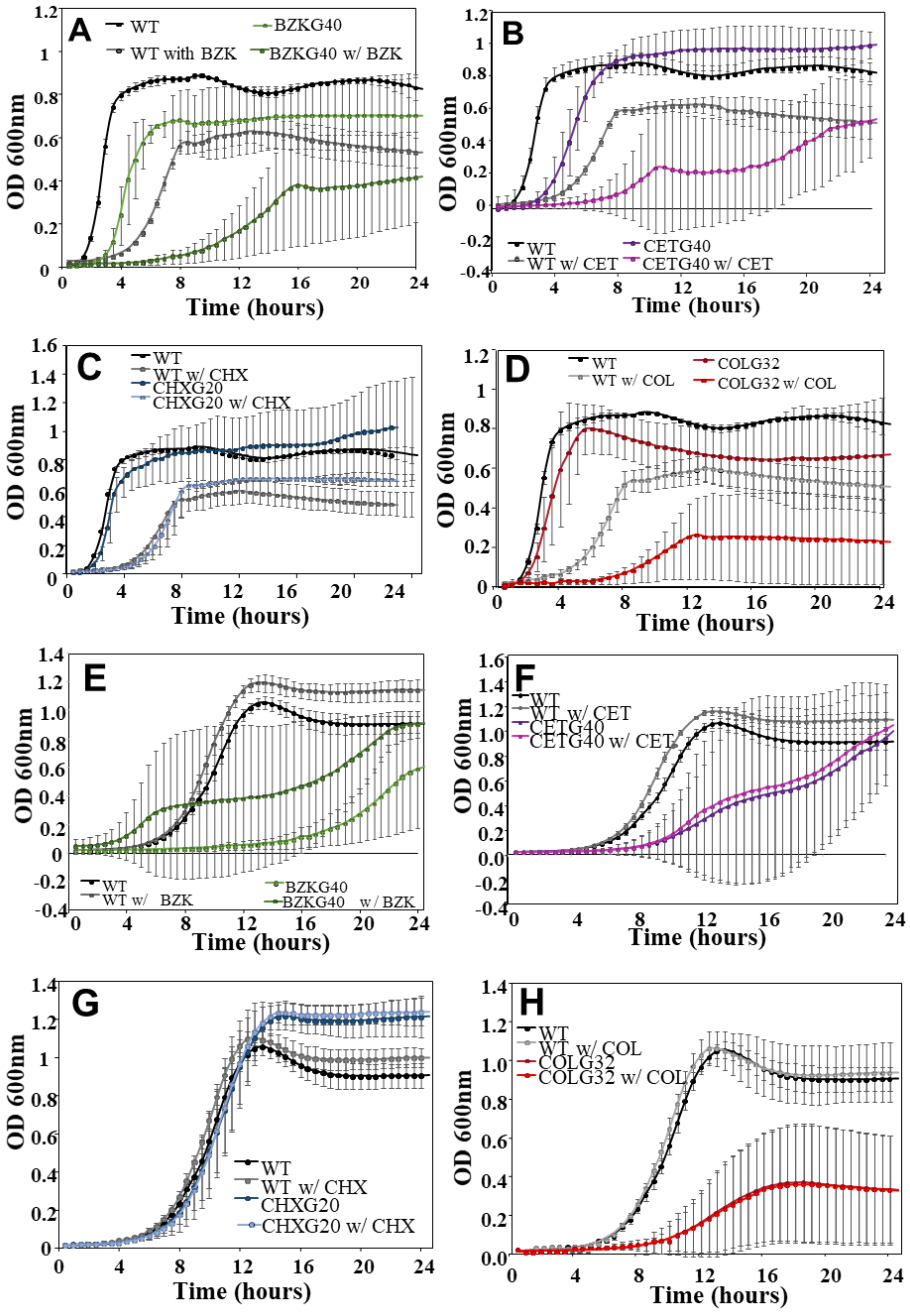

Figure 3.4. A summary of growth curves reached by each *E. coli* BW25113 subculture grown in DG broth (A-D) and M9 broth (E-H). Optical densities (y-axis) are reported as an average for each sub-cultured strain over the course of 24 hours (x-axis). A summary of gradual adapted *E. coli* BW25113 strains to sub-inhibitory concentrations of **A and E)** BZK, **B and F)** CET, **C and G)** CHX and **D and H)** COL are shown.

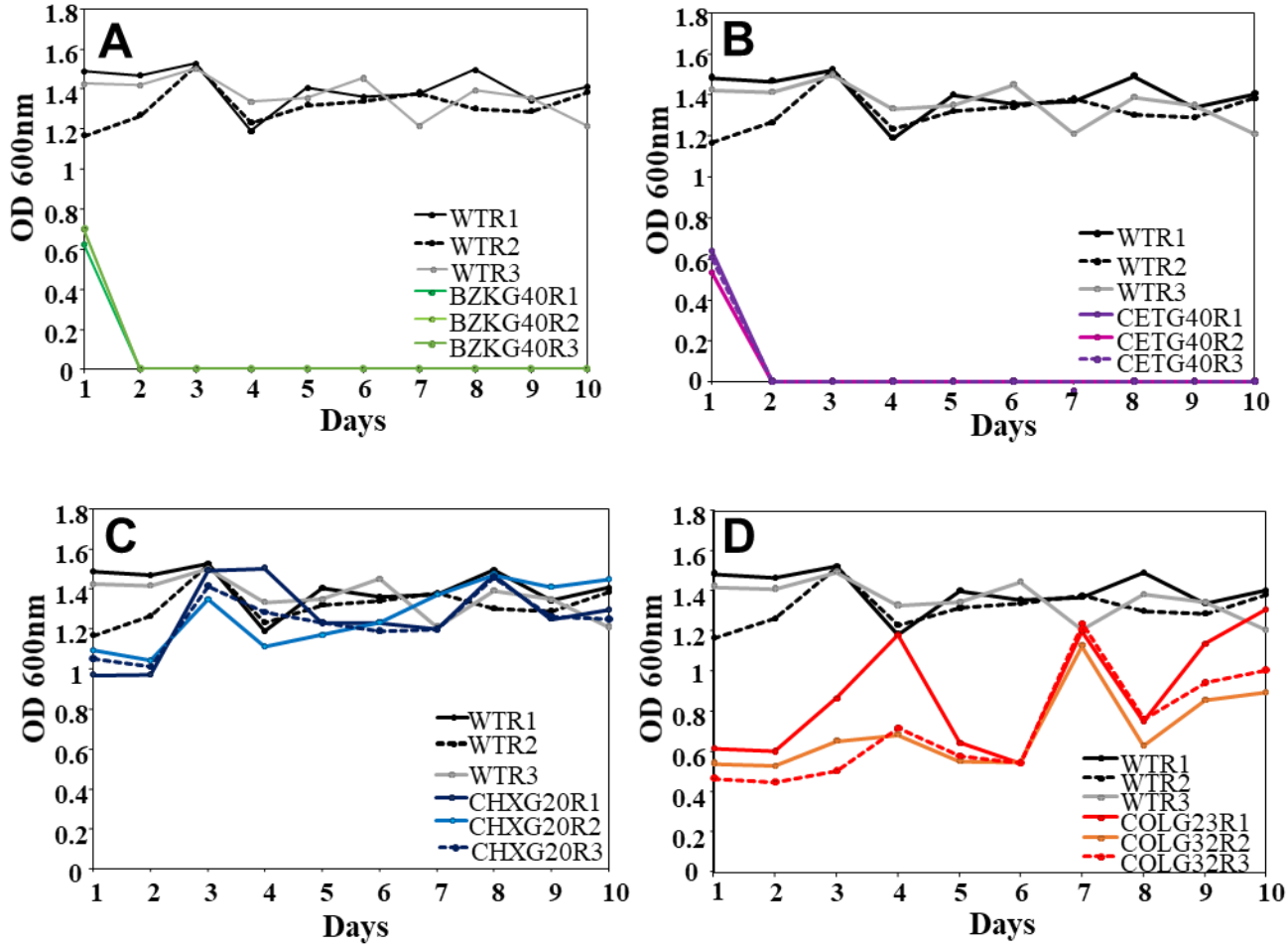

Figure 3.5 A summary of the initial stability experiment for each *E. coli* strain adapted to **A)** BZK, **B)** CET, **C)** CHX and **D)** COL are shown as compared to WTG0. The stability of each individual CA-adapted *E. coli* strain sub-cultured without selection in LB medium was measured over 10 days. On the x-axis, the growth outcome for each CA-adapted strain after each day when re-exposed to LB containing CA concentrations equal or greater than WTG0 MIC are plotted. All strains were compared to the WTG0 strain. In each panel, LB broth containing CAs at the following concentrations were measured: (BZK = 40µg/ml; CET = 50µg/ml; CHX = 1µg/ml and COL = 55µg/ml).

To follow up on these findings, the stability experiment was repeated, with one modification. At the very start of this experiment all CA-adapted strains were grown with CA selection to begin with (i.e. in media containing CA concentrations at 50% of the final concentration they were cryopreserved at) and then sub-cultured without selection in LB medium over a period of 10 days. At each daily time point, a complete broth microdilution AST panel was measured to precisely determine each strains respective CA tolerance (Figure 3.6). Interestingly, CA tolerant stability experiment AST results revealed that nearly all CA-adapted strains maintained a stable CA phenotype ($\geq$2-fold increase in MIC from WTG0) until day 10 of the experiment (Figure 3.6). On day 10, all CA-adapted strains began to show moderate reductions in MIC values that were still above the MIC of WTG0 with the exception of CHX adapted strains; CHX adapted strains demonstrated MIC value fluctuations of $\geq$ 8 µg/ml over the 10-day period that never decreased (Figure 3.6). This suggests that QAC and COL-adapted *E. coli* strains may be more prone to losing their CA-adapted phenotypes over time without CA selection in the medium as compared to CHX. This observation is consistent with other stability experiment findings (formaldehyde, glutaraldehyde/BZK compound, oxidizing compound, tar oil phenol, iodophor tested) that show changes in MIC values were only identified after a long period of drug removal and growth in media only[135]. Unfortunately, most CA tolerant stability studies have been performed with QACs; in QAC-adapted strains (BZK) resistance was maintained after several passages in broth without QACs[135]. Therefore, the CA tolerant stability experiments performed herein indicate that CA tolerance is quickly lost for QACs if CA tolerance is not added after cryopreservation. However, phenotypic CA tolerance is maintained over longer periods when CA tolerance of the strain is verified and then removed.

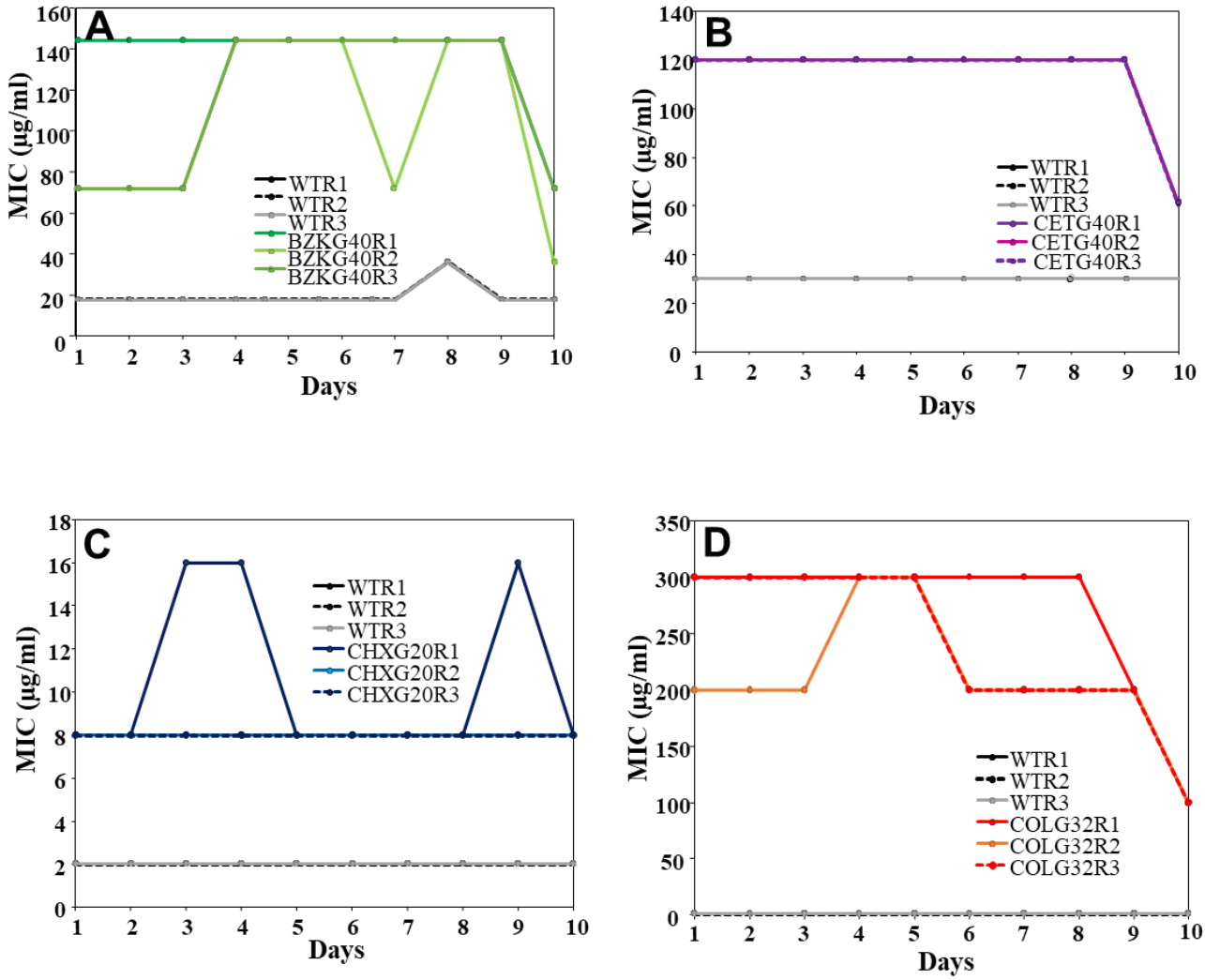

Figure 3.6 A summary of the stability experiment outcomes to determine MIC values for *E. coli* BW25113 strain adapted to **A)** BZK, **B)** CET, **C)** CHX and **D)** COL. Each CA-adapted *E. coli* subculture was grown in LB without CA selection for the indicated number of days plotted on the x-axis followed by AST to determine the MIC value of the culture to its respective CA (y-axis). The strains were cryopreserved at each daily time point in triplicate.

These stability experiments also indicate that QAC adapted strains have the least stable CA tolerance as compared to CHX and COL adapted strains suggesting that QAC adapted strains may have cellular alteration that are less permanent or less specific than those caused by CHX and COL due to their broad mechanism of action.

3.5. Whole genome sequencing (WGS) identifies different amounts of SNV to gene ratios within genes and non-coding regions of each CA-adapted *E. coli*

Previous studies of CA-adapted *E. coli* have demonstrated an association between down-regulated porin expression and increased efflux pump activity, specifically by QAC-adapted *E. coli*[51,52,88,136]. Lipid alterations have also been observed in other proteobacteria and summarized in a review by Tezel and Pavlostathis[137]. To examine what, if any, genetic alterations occurred in the genomes of CA-adapted *E. coli*, next generation WGS was performed on extracted genomic DNA isolated from each strain at 30X minimum sequencing coverage. 30X WGS coverage was deemed to be sufficient for SNV calling based on the size of the *E. coli* BW25113 genome (4631469 bp; 4.63 Mbp), N50 values (>100,000 bp) and >95% coverage as discussed by Chen et al 2015[138]. The N50, N75, and L75 values can also be found in Appendix ii. These values are an important measure of the quality of the assembled genome and based on fragmented contigs of different lengths. N50 values in this project exceeded 100MB ranging from 142,000-204,000 bps for all CA adapted strains, therefore, based on N50 and % coverage values the quality of our sequencing was acceptable for SNV calling[139]. SNVs were identified in each CA-adapted strain genome in both coding (gene) and non-coding (intergenic) regions after sequence alignment to the reference genome and comparison un-adapted BW25113 grown only in LB only for 40 subcultures

(WTG40). Based on the assembled genomes and using BW25113 sequence as reference template, the complete list of detailed SNVs found in each CA-adapted genome are provided in Appendix Table iii.

To address the final hypothesis of this study, which states:

> "*E. coli* adapted to different CAs will confer similar genetic alterations (SNVs) in gene and pathways involving the outer membrane, lipid biosynthesis and trafficking, OMP/porins, and efflux pump systems",

SNV analysis of each CA adapted strain (n=12) was performed using Geneious® software. SNV analysis was used to identify genes frequently targeted by SNVs in each CA adapted genome to identify if efflux, porin and lipid biosynthesis genes were specifically altered by CA exposure. This analysis focused on whether the same coding and non-coding SNVs occurred in *E. coli* strains adapted to the same CA and/or between different CAs used for adaptation. Tables 3.3 and 3.4 summarize total SNVs identified within more than one CA-adapted strains' genome, in either coding or non-coding regions. Since genetic alterations may be random, repeatedly identified SNVs that occurred between the same CA adapted strain genomes or amongst the genomes of different CAs were focused on for this study. This approach was taken in an effort to identify genetic regions/genes that may be linked to each CAs specific mechanism of action and tolerance.

Prior to gene analysis, SNV frequency of occurrence trends were compared between all 12 CA-adapted strains. Based on crude SNV totals per genome, the greatest number of SNVs occurred within BZK (53-101 SNVs) and CET (97-110 SNVs) adapted strain genomes (Tables 3.3 and 3.4). CHX and COL-adapted strains had slightly lower total SNVs; CHX-adapted had 59-74 SNVs and

Table 3.3. A summary of the number of coding SNVs repeatedly identified for CA-adapted *E. coli* BW25113 strains.

CA used	Strain name	Total number SNVs	Total number coding SNVs	Gene identified with SNV (total number of SNVs)
CET	CETG40R1	110	92	***marR* (37)***: *lon* (1): ***stfP* (4)**: *gfcA* (1): *sfmD* (1): ***msbA* (1)**: ***lpxL* (2)**: *ydjF* (3): *insA* (4): *fliR* (4): *bamD* (1): *garP* (6): *ispB* (9)*: ***yrhA* (5)**: *pitA* (5): *ydbA* (2): *CP4-6* (5): *rapA* (1)
	CETG40R2	97	72	*insA* (3): *CP4-6* (1): ***stfP (3)***: ***insX* (6)**: ***dmlA* (7)***: ***lpxM* (1)**: ***wbbL* (2)**: ***gyrA* (1)**: ***yghO* (10)**: ***yhcE* (11)***: *insH* (2): ***yhdP* (1)**: *yrhA* (22): ***rpoB* (1)**: ***rob* (1)**
	CETG40R3	96	66	***CP4-6* (*insX*) (7)**: ***stfP* (2)**: ***dmlA* (7)***: ***lpxM* (1)**: ***wbbL* (2)**: ***gyrA* (1)**: ***yhcE* (10)***: ***yghO* (7)**: *insH-1* (2): ***yhdP* (1)**: ***yrhA* (24)**: ***rpoB* (1)**: ***rob* (1)**
BZK	BZKG40R1	101	77	*dksA* (1): ***acrB* (1)**: *hokE* (1): *glnS* (1): ***rpsA* (1)**: ***msbA* (1)**: ***lpxL* (*waaM*) (1)**: ***stfP* (*ycfK*) (10)**: ***mipA* (1)**: *pdeA* (5): *ptsP* (31)*: *glcB* (1): ***yghO* (12)**: ***yhcE* (+ *insH*) (2)***: *kdsC* (2)
	BZKG40R2	53	33	*avtA* (1): *rpoC* (1): ***rob* (1)**: No gene in location (1)
				insH-1 (1): ***stfP* (*ycfK*) (1)**: ***wbbL* (4)**: ***mlaA* (7)**: *yghQ* (2): ***yhiS* (+ *insH-1*) (3)**: *hsdS* (15)*
	BZKG40R3	58	55	*rapA* (1): *ompX* (9): ***rpsA* (1)**: ***msbA* (1)**: *pqiB* (1): ***stfP* (*ycfK*) (6)**: ***mipA* (1)**: ***marR* (1)**: *truA* (1)**: *yhcE* (+*insH*) (8)***: *yrhA* (5): *kdsC* (2): *gyrB* (1): *gltU* (14): *rpoB* (1): *insH-1* (1): ***rob* (1)**
CHX	CHXG20R1	60	18	*cdaR* (1)*: ***mlaA* (2)**: *eutE* (1): ***yghQ* (14)***
	CHXG20R2	59	30	***marR* (18)***: ***mlaA* (7)**: ***yhiS* (+*insH*) (5)***
	CHXG20R3	74	45	*rsxC* (1): ***wbbL* (3)**: ***mlaA* (5)**: ***yghQ* (3)**: ***yhiS* (+ *insH*) (12)***: ***yhiS2* (+*insH*) (3)***: *hsdS* (18)*
COL	COLG32R1	18	18	*lpxC* (1): ***sbmA* (1)***: ***acrB* (1)**: *rpsA* (1): *mgrB* (1): ***rfaY* (*waaY*) (11)**: *cpxA* (1): ***basS* (*pmrB*) (1)**
	COLG32R2	56	50	***lpxC* (1)**: *pdhR* (1): ***acrB* (1)**: *cysS* (1): ***marR* (1)***: *yfdP* (2): *torI* (+*CPS-53*) (31): ***rfaY* (*waaY*) (10)***: ***pmrB* (*basS*) (1)**: *adiC* (1)
	COLG32R3	40	39	***lpxC* (1)**: *bamA* (1): ***sbmA (15)****: *acrB* (1): *ydbA* (1): *nuoC* (1)*: *eutE* (1): *pyrG* (1): *greA* (3): ***rfaY* (*waaY*) (11)***: *spoT* (1): ***pmrB* (*basS*) (1)**: *adiC* (1)

Bolded genes highlight genes with one or more SNVs identified in more than one CA adapted strain genome. Genes with asterisk highlight frameshift mutations.

Table 3.4. A summary of the number of SNVs identified in non-coding regions for adapted *E. coli* BW25113 replicate strains

CA used	Strain name	Total number SNVs	Total number non coding SNVs	Upstream genes (total number of non-coding SNVs per region)
CET	CETG40R1	110	18	***lacZ (10)***: *IS1* (1): *gfcA*; *insA* (6): *mlaA*; *ydfC* (1)
	CETG40R2	97	25	***insA* (3)**: ***ydiJ; pfkB* (13)**: ***IS5* (9)**
	CETG40R3	96	30	***insA* ((3)**: ***lacZ* (3)**: ***ydiJ; pfkB* (12)**: ***IS5* (9)**: *trmL* (3)
BZK	BZKG40R1	101	24	***lacZ* (1)**: ***lon* (11)**: *IS4* (6): ***murB* (2)**: ***fimA* (3)**: *rob*; *creA* (1)
	BZKG40R2	53	20	***lacZ* (2)**: tRNA (1): ***murB* (2)**: ***fimE* (1)**: *mdtM* (14)
	BZKG40R3	58	3	*ompX*; *rhtA* (3)
CHX	CHXG20R1	60	42	***lon*; (12)**: *mdfA* (9): ***mlaA*; *yfdC* (12)**: *trmL* (1): ***fimE* (8)**:
	CHXG20R2	59	29	***lacZ* (4)**: ***IS5* (6)**: *trmL* (1): ***fimE* (11)**: *fimA* (u) (7)
	CHXG20R3	74	29	***lacZ* (2)**: ***IS5* (9)**: *ynaJ* (2): ***fimE* (16)**
COL	COLG32R1	18	0	N/A (0)
	COLG32R2	56	6	***mlaA*; *yfdC* (6)**
	COLG32R3	40	1	*acrE* (1)

N/A; no genes identified or not applicable. Bolded non-coding regions highlight non-coding regions with one or more SNVs identified in more than one CA adapted strain genome
More details on SNV locations and the intergenic non-coding region are summarized in Appendix iii.

COL-adapted 18-56 SNVs in examined genome sequences. This outcome may suggest that QAC exposure exerts greater genetic alteration as compared to intermediate CHX and COL SNV totals.

It is noteworthy that the ratio of coding SNVs per total SNVs (SNV coding/ SNV total) was highest for COL-adapted strains (0.89 – 1). SNV coding/total ratios for QAC-adapted strains were intermediate (0.67 – 0.84 for CET, 0.63 – 0.94 for BZK) in value and SNV coding/total ratios for CHX-adapted (0.29 –0.61) were lowest among comparison of all CA adapted strain genomes. These SNV coding ratio values suggest that CHX-adapted strain genomes have fewer SNVs in genes as compared to SNVs in QAC or COL-adapted strains, which may indirectly relate to how each drug/compounds' mechanism of action exerts selective pressure to alter each genome. To elaborate, COL is believed to have the most focused mechanism of action, which primarily targets and alters lipid A biosynthesis and trafficking as reviewed by Olaitan *et al*. 2014[14]. In contrast, QACs (BZK and CET) and CHX have multiple mechanisms of action focused not only on outer membrane disruption but also include protein denaturation (CHX and QACs) as well as oxidative damage (QACs)[11]. Therefore, the observation that more non-specific and non-coding SNVs occur in antiseptic adapted strains may not be as surprising given the many multiple mechanisms of action by these biocides.

3.5.1 Repetitive SNVs were most frequently identified in various lipid biosynthesis, trafficking, and modifying genes or non-coding regulatory regions of each CA-adapted *E. coli* strain

To further examine SNV differences between each CA adapted genome, Figures 3.7 and 3.8 were generated and showing network diagrams of all coding and non-coding SNVs that may affect promoter/ enhancer regions of the genome; identified genes and potential regulatory non-coding regions in both figures are grouped based on their function and operon locations.

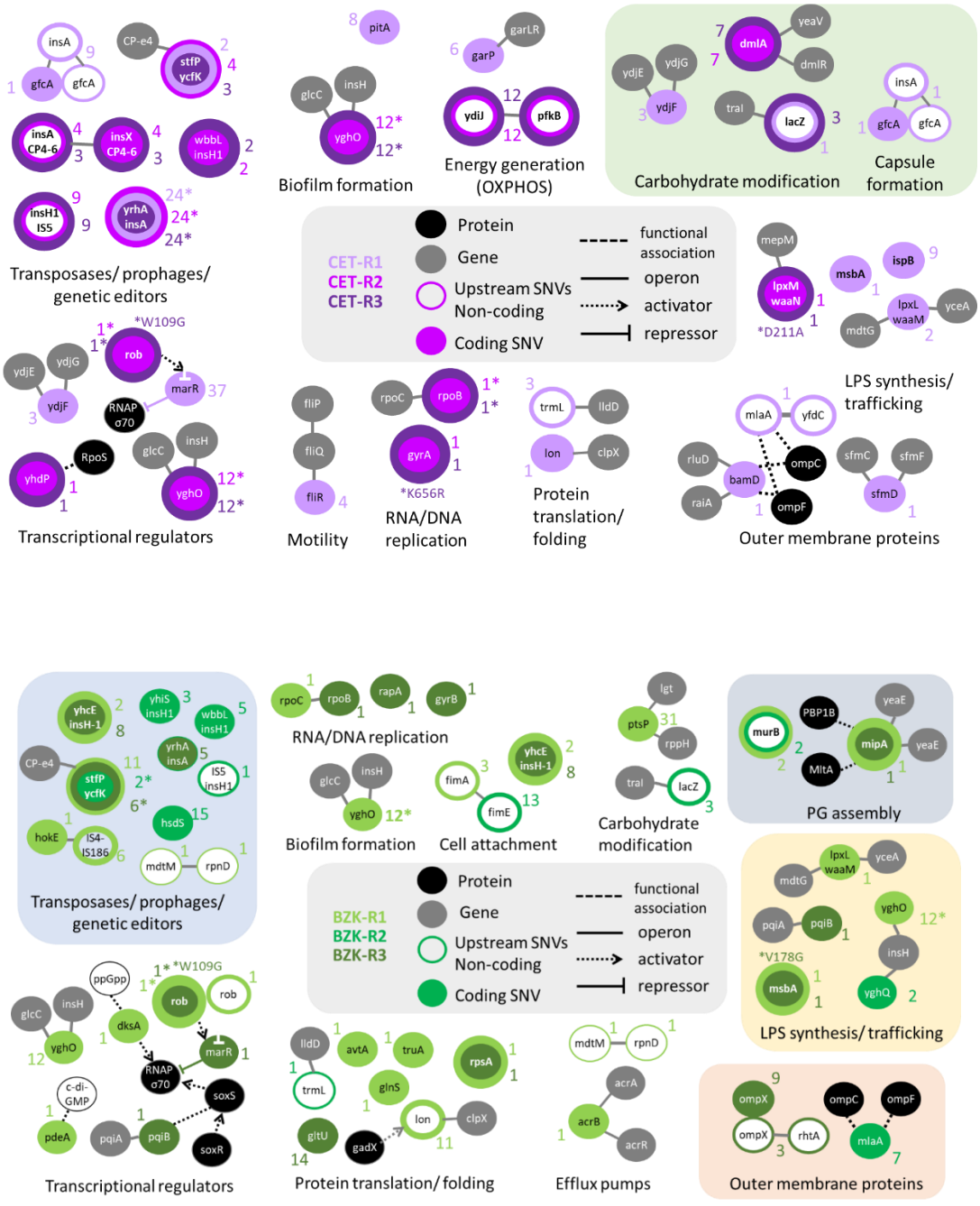

Figure 3.7 Network diagrams illustrating coding and non-coding SNVs identified from WGS of CET (purple) and BZK (green) adapted strains. Legends in the panels indicate the meaning of symbols and arrows shown in the diagrams.

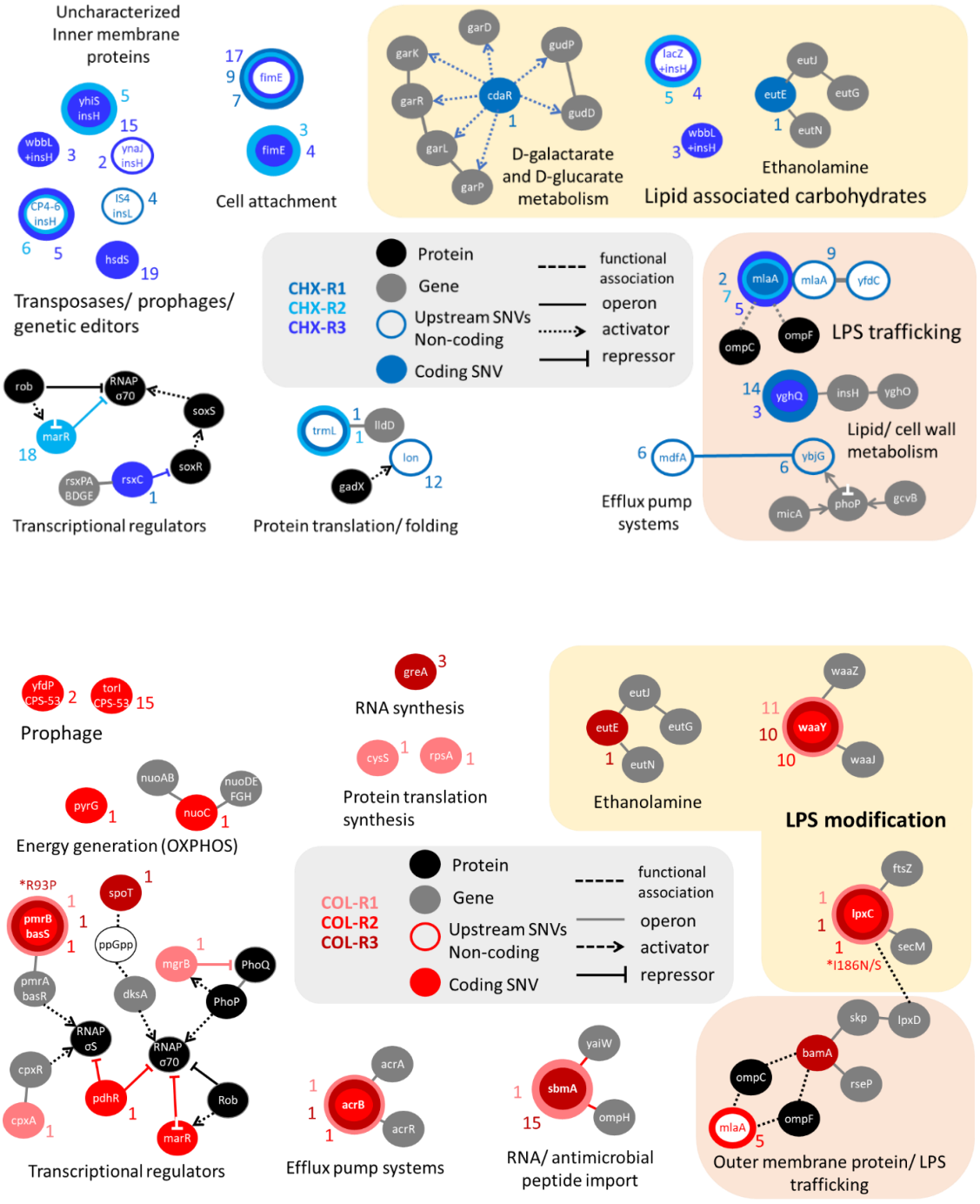

Figure 3.8 Network diagrams illustrating coding and non-coding SNVs identified from WGS of CHX (blue) and COL (red) adapted strains. Legends in the panels indicate the meaning of symbols and arrows shown in the diagram.

In Figures 3.7-3.8, SNVs repeatedly located in coding (filled circles) or non-coding (empty circles) regions within putative upstream regulatory/promoter regions of nearby genes are coloured and form 'bullseyes' that can easily be visualized as genetic regions with repetitive SNV targets for each genome. The more 'bullseyes' found on a network map, identify genes/ regulatory non-coding regions with SNVs which may be interpreted as less random SNV occurrence among independently CA adapted strains. Based on the hypothesis we expected that most repetitive SNV to gene/non-coding (or 'bullseyes') in Figures 3.7-3.8 would preferentially target efflux systems, porins, and lipid biosynthesis and/or lipid trafficking systems which were seen but were different for each CA used to adapt *E. coli*. More specifically, SNV 'bullseyes' were mainly observed in LPS biosynthesis or LPS trafficking pathways amongst all 4 CA-adapted strains and targeted genes located in the *lpx* genes (*lpxCLM*) *msbA*, *mlaA* or the *waa* operon (Figures 3.7-3.8).

Repetitive identification of deleterious SNVs (frame shifts, substitutions, indels) in *mlaA* in 2/3 CHX-adapted strains as well as BZK adapted strain (BKZG40R2) are important to note. MlaA is an outer membrane lipoprotein[140] implicated in a retrograde phospholipid trafficking pathway; MlaA is involved in maintaining outer membrane lipid asymmetry by removing mis-localized outer leaflet phospholipids and transporting them back to the inner membrane. Since BZK and CHX perturbs LPS molecules as part of their mechanisms of action[11], deleterious mutations to *mlaA* suggest inhibition of phospholipid recycling and may be a mechanism of tolerance toward CHX and BZK agents. SNVs were also noted in the upstream non-coding region of *mlaA* single CA adapted strains CETG40R1, CHXG20R1, and COLG40R2 highlighting the potential value of *mlaA* and its upstream non-coding regions as a biomarker for multiple CA adapted strains.

Repetitive deleterious SNVs found in *waaY* (*rfaY*) in all 3 COL-adapted strains (Figure 3.9) suggest core lipid A glycosylation is significantly affected by prolonged *E. coli* COL adaptation. WaaY is a heptose specific LPS core kinase which catalyses phosphorylation of the second heptose residue in the inner core of LPS[141]. Deletions mutants of *waaY* in *E. coli* have previously shown enhanced resistance towards human cathelicidin antimicrobial peptide (LL-37) and suggest COL adaptation also targets this gene.

Repetitive SNVs were identified in essential genes *lpxC* (COL-adapted), *lpxM* (CET-adapted), *lpxL* (CET and BZK adapted) which are all located in unrelated (*trans*) operons of the *E. coli* genome but all that function as lipid A biosynthesis enzymes to maintain outer membrane integrity[142]. Most SNVs identified in *lpx* genes were non-deleterious (in frame codon substitutions) and altered a specific codon. For example, SNVs in LpxM altered codons D211A in 2/3 BZK adapted strains and LpxC had I186N alterations in 3/3/ COL adapted strains (Figure 3.9). Hence, SNV mutations in *lpx* genes may serve as useful predictive genetic markers of CA adaptation and enhanced CA tolerance in *E. coli*. It is important to note, that repetitive non-deleterious (codon substitutions) SNVs were identified within *msbA*, an ATP-dependent lipid A-core flippase[143], in 2/3 BZK and 1/3 CET-adapted strain (CETG40R1) (Figures 3.7, 3.9). SNVs in BZK adapted genomes altered a single codon, V178G and in the single CETG40R1 altered A207V both were located in loop regions of the membrane transmembrane strand domain of the MsbA protein (Figure 3.9). *msbA* mutants have been previously reported to coincide with and rescue *lpxL* null mutations[144] (Figures 3.7, 3.9). *msbA* over-expression has been shown to rescue *lpxL* null mutants by increasing LPS translocation of tetra-acylated lipid A[145]. SNVs in both *msbA* and *lpxL* were noted in genomes of single CA adapted strains CETG40R1 and BZKG40R1, verifying the

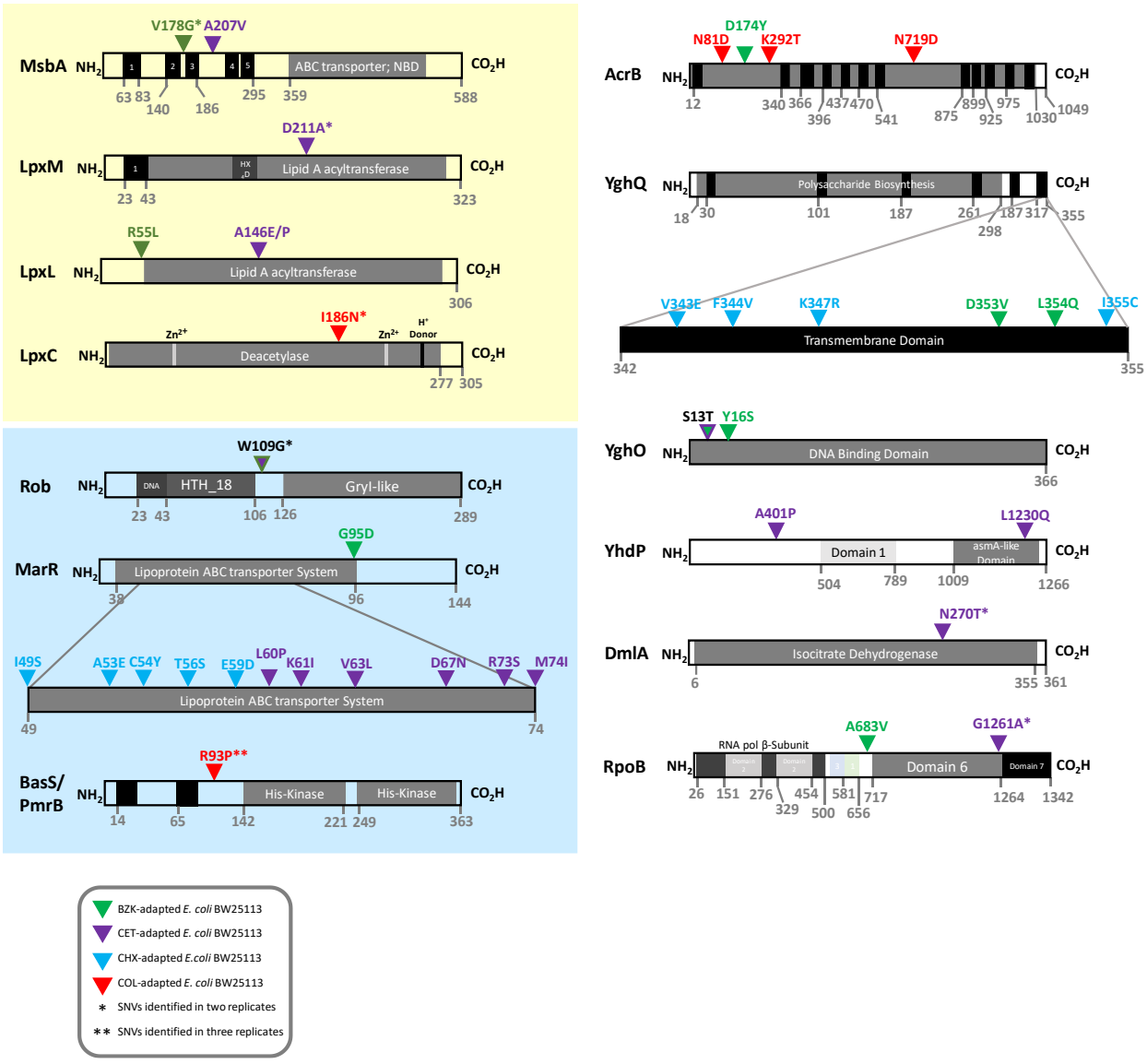

Figure.3.9 Repetitive SNVs identified within the coding regions of CA adapted strains shown as protein maps. Black and grey sections highlighted on each protein map indicate transmembrane strands and functional domains respectively. Coloured highlighting behind protein maps (shown as rectangles) indicate LPS biosynthesis and transport genes (yellow) and transcriptional regulation (blue) genes with SNVs shown as triangles (refer to in legend in the figure panel).

importance of these compensatory mutations in QAC adapted strains. The presence of *msbA-lpxL* SNVs may suggest that acylated lipid A modifications may one option to contribute to QAC adaptation and reaffirm the co-association between these lipid biosynthesis genes.

3.5.2 WGS of CA-adapted strains identifies SNVs few CA adapted strain efflux pump genes and outer membrane assembly proteins

SNVs were identified within efflux pump gene *acrB* and the upstream non-coding region of *mdfA* in BZK, CHX, and COL-adapted strain genomes (Figures 3.7 and 3.8). However, only COL-adapted strains identified repetitive SNVs in *acrB* (Figure 3.8). MdfA is a major facilitator superfamily transporter that recognizes a broad range of cation-selective efflux pump, particularly chloramphenicol[146]. AcrB is part of a dominant multipartite efflux pump system (AcrAB-TolC) in resistance nodulation and cell division (RND) superfamily[147,148] involved in the efflux of a wide variety of cationic and lipophilic molecules, including some QACs[149]. The lack of repetitive SNVs identification within efflux pump genes and regulatory regions of more than one CA-adapted strain is not surprising, since efflux pump regulation commonly occurs at the RNA transcript and protein translation level[150] in response to stressors, as noted in previous QAC adapted *E. coli* studies[50,51,54]. Therefore, it is surprising that repetitive SNVs were identified within *acrB* in COL-adapted strains that appear to alter many codons (Figure 3.9) but the functional outcome is unclear as these codon alterations have not been experimentally examined to date; further experimental validation and testing will ideally reveal the functional outcome of these codon variants with respect to enhanced COL tolerance.

SNVs identified in fimbrial associated OMPs, *ompX*[151] (BZKG40R3) and *sfmD*[152] (CETG40R1) genes and OMP lipoproteins *bamA* (COLG40R3) and *bamD* (CETG40R1) of the β-

barrel assembly machinery (BAM) complex[153] responsible for proper OMP folding and insertion were identified in only one CA-adapted strain genome but are worth mentioning (Figures 3.7-3.8). Previous mutational studies of *ompX,* a porin that associates with type 1 fimbriae, showed that *E. coli* strains had impaired motility, increased extrapolymeric substance (EPS) production (typically observed during biofilm formation), and increased tolerance to sodium dodecyl sulfate (SDS) and hydrophobic antibiotics[154] when *ompX* was mutated. The *sfmD* gene is a chaperone-usher protein that is typically cryptically expressed in *E. coli* but when expressed increases type fibrial formation and surface attachment to epithelial cells. Interestingly, in other the remaining 2 BZK adapted strains SNVs were noted in *yghO* (BZKG40R1) and *yghQ* (BZKG40R2) suggesting each BZK adapted strain may target alter different biofilm and/or surface attachment genes besides *ompX* (Figure 3.7). The identification of SNVs in only one CA adapted strain may still be worthwhile to explore in future studies, as these may include SNVs that act as compensatory mutations to maintain fimbriae formation and OMP secretion. Further experimental analyses of these genes should be undertaken to determine how these gene mutations affect CA tolerance and how they may compensate for other SNVs within specific CA adapted strains.

3.5.3 WGS of CA-adapted strains identify SNVs in previously identified antimicrobial resistance genes

Many repetitive SNVs were also identified in coding/non-coding regions in CA-adapted *E. coli* to different drugs that are known for their influence on antibiotic resistance genes. Repetitive SNVs in the antimicrobial resistance DNA-binding transcriptional regulator *rob*, part of the *marA/soxS/rob* regulon[155], was identified in 2/3 BZK and CET adapted strain genomes. Rob is a transcriptional activator known to bind the *mar-sox-rob* regulons upstream of ~50 genes[156]

involved in enhanced oxidative stress[157], solvent tolerance[158], antibiotic resistance[159] and heavy metal tolerance[160]. Interestingly, all *rob* SNVs identified from BZK and CET-adapted strain genomes altered the same codon W109G which is located in a protein region in between the helix-turn-helix (HTH_18) and GRYL-like domain of the transcription factor (Fig. 3.9). Rob is known to regulate antibiotic resistance, as well as superoxide resistance[157] and organic solvent tolerance[158], indicating that W109G may be an important variant and genetic marker for antimicrobials like QACs that have multiple mechanisms of action.

Repetitive SNVs were identified in 2/3 CET adapted strain *yhdP* genes at different codons A401P and L1230Q (Figure 3.9); YhdP is a regulatory polypeptide necessary for maintaining the outer membrane permeability barrier in *E. coli* by altering enterobacterial common antigen (ECA) levels and increases *E. coli* susceptibility to VAN resistance when deleted[161]. The association between ECA and QAC adaptation may be a useful direction to explore in future experiments.

An exciting and novel finding in 2 of 3 COL adapted strain genomes was the identification of deleterious (frame-shift causing) SNVs in *sbmA*, an antimicrobial peptide transport gene (Figure 3.8). Bacterial mutants and knockouts of *sbmA* have demonstrated resistance to antimicrobial peptides including microcin[162,163]; therefore, *sbmA* may be an intrinsically occurring *E. coli* biomarker to predict COL resistance.

Finally, as we expected from previous COL resistance studies[14], repetitive SNVs in *pmrA* (*basS*) in 3/3 COL adapted genomes both specifically altered codon R39P of this two-component sensor kinase protein (Figure 3.9). Activation of the PmrAB two component system leads to the upregulation of the *pmrCAB* and *arnBCADTEF-pmrE* (also called *pmrHFIJKLM-ugd*) operons that mediate the synthesis and transfer of phosphatidylethanolamine (PEtN) and 4-amino-4-deoxy-

L-arabinose (L-Ara4N), respectively, to lipid A[14]. PEtN and L-Ara4N modifications of lipid A in COL-resistant strains are well documented and considered to be a primary mechanism of COL resistance[14]. Their identification in 2/3 COL adapted strains suggest multiple pathways of COL adaptation and lipid A modification have occurred in this study, emphasizing its importance. SNVs were identified in DNA-binding transcriptional regulator *yghO* in 2/3 BZK (and BZKG40R1) adapted strain genomes (Figure 3.9). *YghO* influences antimicrobial resistance in bacteria grown as a biofilm, by an as yet unidentified functional mechanism[164] (Figure 3.7). Biofilm formation is known to be a multicellular mechanism of antimicrobial resistance employed by *E. coli* as well as many other Proteobacteria to resist CAs as well as antibiotics[165,166].

3.5.4 WGS of CA-adapted *E. coli* strains identified most SNVs within transposon and prophage regions

SNV network diagrams also highlighted a number of transposases/prophages in coding and non-coding regions by all CA-adapted strain genomes examined herein (Figures 3.7-3.8). Monitoring SNVs in transposable elements is important since, SNVs may alter the movement and regulation of transposons and prophages, ultimately changing the rate of recombination and multidrug resistance gene transfer in bacteria. Repetitive SNVs were identified in genes and non-coding regions of insertion sequence (IS) transposase *ins* genes (e.g. *insA, insH*) and IS5 transposase interrupted LPS rhamnosyltransferase *wbbL* gene in CET, BZK, and CHX-adapted strains (Fig. 3.7-3.8). Transposases such as InsH1 often facilitate the mobilization and transmission of antimicrobial resistance genes and factors[167]. Mutations and disruption of the *wbbL* gene interrupted by IS5 element have been shown to affect the synthesis of O-antigen of LPS[168], termed the *rfb*-50 mutation, which may contribute to antiseptic CA tolerance. Repetitive SNVs identified

in e14 prophage gene, *stfP* (*ycfK*) were also noted in CET and BZK-adapted strains (Figure 3.7). *StfP* has been shown to be upregulated in *E. coli* during antimicrobial drug exposure and associated with oxidative stress[169], which is known to be induced upon QAC exposure[12]. An increase in SNVs within transposons and prophage genes after prolonged exposure to stress(ors)[170–172] and antimicrobials[173] in *E. coli* and other bacteria. In experiments conducted herein, prolonged QAC and CHX exposure also appears to increase SNV occurrences in transposable elements, likely due to the variable mechanisms of action by QACs and CHX. SNVs were also identified in non-coding regions adjacent to insertion elements, *insA*, *insH1* in 2/ 3 CET-adapted strain genomes (Figure 3.7) and SNVs in cryptic prophage CP4-6 *insH* region of CHX-adapted strains (Figure 3.8). It is important to note that COL-adapted strain genomes had the lowest amount of repetitive SNVs in transposable element genes, which may suggest COL exerts less of generalized stress response as compared to antiseptics or exerts more focused selective pressure on lipid A as compared to cationic biocides.

3.5.5 WGS of CA-adapted strains identified SNVs within other essential genes and regulatory upstream non-coding regions

As discussed above, essential lipid biosynthesis genes, including *lpxL* and *lpxM* were also SNV targets, together with other outer membrane essential genes including *bamA* and *bamD*. However, essential genes associated with RNA/DNA replication, *rpoC*, *rpoB* and *gyrB* had repetitive SNVs in CET and BZK-adapted strains and essential 30S ribosomal subunit 1 gene *rpsA* required for protein translation had repetitive SNVs in CET-adapted strains. Mutations occurring in many of these essential genes we identified with SNVs after QAC/CHX adaptation, have also been noted in *rpoC*[174,175], *rpoB*[176], *gyrB*[177], and *rpsA*[178] genes from various antibiotic resistant

bacteria. Although we did not observe increased cross-resistance to antibiotics among our CA adapted strains in this study, our findings suggest that antiseptic exposure conditions could eventually select SNVs in essential genes that enhance antimicrobial cross-resistance to therapeutic drugs.

Non-coding regions were included in the gene network analysis if they occurred within upstream regions of a gene. Without performing a transcriptomic or proteomic analysis of each CA-adapted strain (a future project direction), we cannot confidently determine how non-coding SNVs alter gene expression. In light of this, only repetitive SNVs occurring in upstream genetic regions will be discussed here. BZK adapted strain genomes, identified repetitive SNVs within non-coding regions that potentially regulate *murB* as well as coding gene *mipA* (Figure 3.7), and both participate in peptidoglycan (PG) assembly and outer membrane integrity. Over expression of *mipA* can result in altered PBP1 binding and compromised membrane integrity[179], while *murB* is an essential gene[33] that catalyzes the second committed step of PG synthesis[180]. To date, PG assembly genes have been speculated to participate in antiseptic tolerance, but have yet to be directly identified[181] and highlighting the potential importance of these SNV findings.

As discussed above repetitive SNVs were also found upstream of *ydiJ* and *pfkB* in CET-adapted strains, both of which are associated energy generation as they encode for putative flavin adenine dinucleotide (FAD)-linked oxidoreductase[182] and 6-phosphofructokinase 2 enzyme, respectively. It has been noted that deletion of either *ydiJ* or *pfkB* have no apparent effect on cell growth but their individual deletions may impact growth on particular sugars[182,183]. It is worth noting here, that repetitive SNVs were located with in other energy generation pathway related genes, specifically in *dmlA* of CET adapted strain genomes (Figures 3.7, 3.9). DmlA is a D-malate

dehydrogenase that is essential for aerobic growth on D-malate[184]; SNVs identified in *dmlA* alter a single specific codon N270T, located quite close to the conserved isocitrate dehydrogenase region (244-263 aa) of the protein, which may alter the function of this enzyme. It is unclear how coding and non-coding SNVs may alter the regulation these energy generation proteins and further experimental analysis is needed. Since many energy generating proteins reside within the inner membrane, it is not surprising that CA adapted strains would impact these systems, and some SNVs may be compensate for membrane changes during CA adaptation.

As noted above, greater emphasis was placed on repetitively occurring SNVs in genes or non-coding regions, but most SNVs we identified only occurred in a single CA adapted strain genome (1/3 genomes). Despite their single occurrence in one CA-adapted strain, some of these SNVs also occurred in strains adapted to different CAs; therefore, some are worthy of noting. The Venn diagram shown in Figure 3.10, highlights all of the repetitive SNVs identified in coding and upstream non-coding gene regions. Of particular note within the centre of the Venn diagram, is multiple antibiotic resistance (MAR) transcriptional regulator, MarR and outer membrane lipoprotein MlaA (discussed in section 3.5.1 above; Figure 3.10) and were identified in at least one adapted strain per drug (MarR) or within ¾ of the CA adapted strain genomes (MlaA). *MarR* is part of the *marRAB* operon that that negatively regulates the expression of its own operon and represses expression of genes with a *mar-sox-rob* regulon as reviewed by Wales and Davis. 2015[7]. *MarR* regulates expression for a network of genes involved in antimicrobial resistance and includes outer membrane porin gene *ompF*, and efflux pump genes *acrAB-tolC*. Previous QAC and CHX-adapted studies have reported up-regulated efflux pump regulators linked to MarRA and

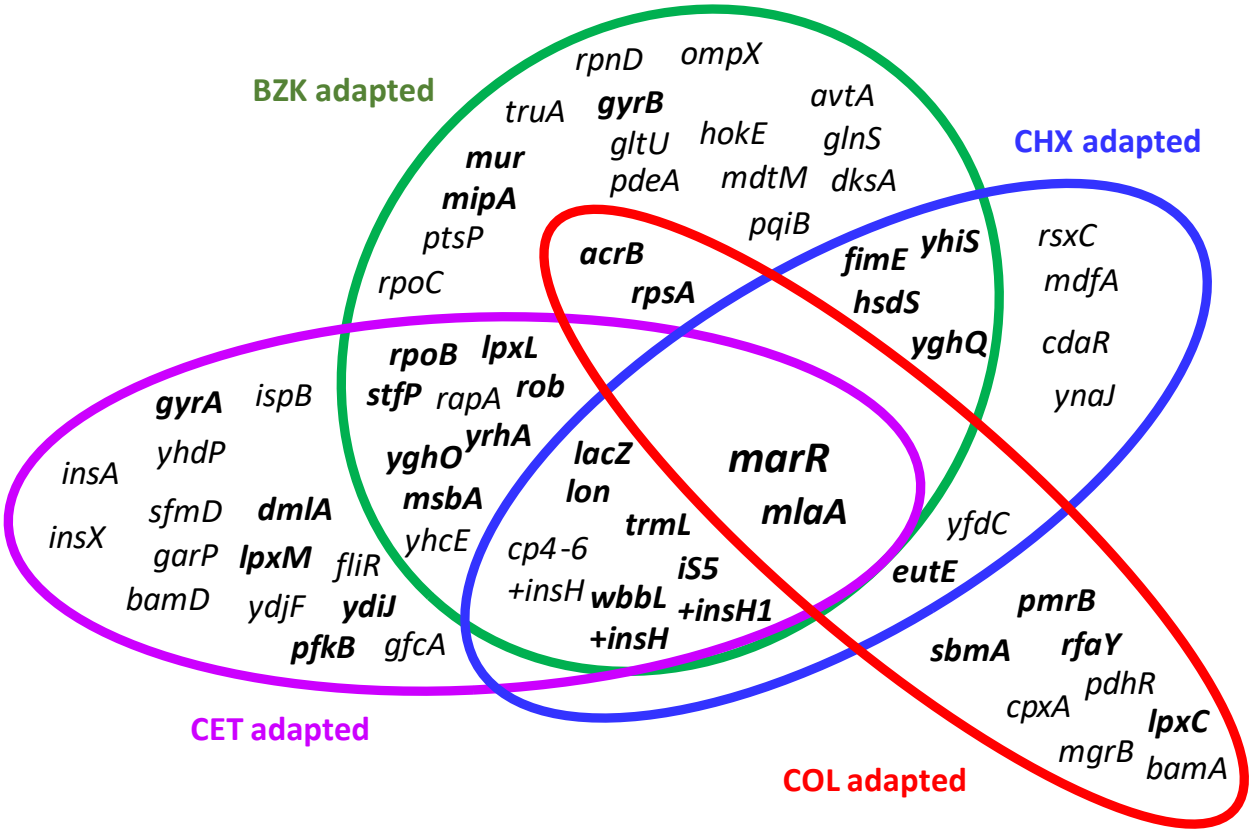

Figure 3.10. Venn diagram illustrating highly repetitive SNVs identified within one CA adapted strain genome and/or within multiple CAs. Bolded genes has SNVs in 2/3 or 3/3 strain genomes adapted to the same CA. Overlapping coloured circles denote genes that are shared between specific CAs. The diagram shows genes with identified SNVs for each CA adapted strain encircled by colour; BZK (Green), CET (purple), CHX (blue) and COL (red).

SoxS, which positively regulate dominant pump AcrAB-TolC in *E. coli*[84,117]. The *marRAB* operon is induced by antibiotics[7] (such as salicylate, tetracycline, and chloramphenicol) and was previously shown to be upregulated in BZK-adapted strain transcriptomic studies[50]. Most but not all *marR* SNVs were deleterious which suggest that the loss of this repressor should enhance *mar-sox-rob* regulon but future studies are needed to validate how *marR* mutants may act as a mechanism of CA tolerance. As previously noted at the start of this section, the majority of repetitive SNVs were found within the QAC-adapted strains as compared to CHX and COL-adapted strains. A number of SNVs were also identified as being shared across multiple CAs (CET, BZK and CHX) including *lon* (ATP-dependent protease responsible for degradation of misfolded proteins), *trmL* (methyltransferase) and a number of prophages/transposons (*cp4-6* and *insH*). The number of different types of genes identified between CET, BZK and CHX again supports the observation that multiple mechanisms of tolerance are developing as compared to COL-adapted strains. This would suggest that many random and compensatory genetic alterations are occurring specific for each CA we tested. Efflux pump *acrB* was also identified to SNVs within a single CA-adapted strain which also suggest a common tolerance mechanism triggered by BZK and COL.

In relation to the thesis hypothesis, this study showed that each CA used for *E. coli* K12 BW25113 adaptation resulted phenotypic as well as genotypic alterations that validated the hypothesis. The most highly repetitive SNVs were observed in transcriptional regulators associated with stress and antimicrobial tolerance, lipid A biosynthesis and LPS transport genes, a variety of outer membrane proteins, efflux pump *acrB*, and transposable elements with all three mechanisms together observed in some drug classes alone eg; BZK (*ompX*; *acrB* and *lpxL*). However, as stated earlier very few repetitive SNVs occurred at identical positions in the same

genes between strain genomes adapted to different CAs, where QACs BZK and CET has the closest relationship. This suggests that the types and number of highly repetitive SNVs may not be directly used to predict tolerance to a particular CA but with more experimental analyses may have use as a genetic antiseptic tolerance prediction tool.

CHAPTER 4. DISCUSSION

4.1. Summary of main findings

In summary, this project has identified that *E. coli* K12 BW25113, when exposed to increasing drug concentrations can produce CA tolerant strains that differ in antimicrobial tolerance, CA phenotypic stability and genotype depending on the type of CA used for initial drug adaptation. In terms of CA tolerant stability of each adapted strain, the loss of CA tolerance significantly differed depending upon the CA it was adapted to and if selection was initially verified in the starting culture at day 0 of the experiment. QAC adapted *E. coli* had the least stable CA-tolerant phenotypes after prolonged removal of drug and the addition of QAC at day 0 also had a big impact on the stability of the CA tolerant phenotype. Growth curve studies demonstrated that CA-adapted *E. coli* had similar growth phenotypes in rich media but reduced and delayed lag phase growth in minimal media. Furthermore, CA-adapted strains showed limited cross-resistance to antimicrobials and antibiotics unless they were closely chemically related, with the exception of CHX-adapted strains. Lastly, WGS identified few common SNVs within genes and non-coding regions of various CA-adapted *E. coli* but frequently identified genes with SNVs in operons and pathways related to outer membrane integrity and biosynthesis. These are important findings as the differences between each CA may be linked to its specific mechanism(s) of action. Hence, the findings of this study have provided new insights and genetic targets to explore related to CA adaptation and tolerance mechanisms.

4.2 Benefits and limitations of study

There are certain benefits to the approaches undertaken within this project. Laboratory based experiments for CA adaptation in a single antimicrobial susceptible reference *E. coli* K-12 strain allowed us to generate CA-tolerant reference strains and genomes free from other confounding antimicrobial cross-resistance mechanisms. This enabled the specific measurements of how exposing the same reference *E. coli* strain to various CAs could be compared. Undertaking this research in a laboratory based controlled environment also removed any external factors that may be found in the natural environment such a mobile genetic element acquisition that carry many antimicrobial, heavy metal and biocide resistance genes with them[7,185].

There were also limitations to conducting this type of project. The use of only one *E. coli* strain adapted to four drugs may make it difficult to compare to other *E. coli* strains as well as other bacterial species used in adaptation studies. However, our development of stable *E. coli* strains with a collection of single gene knockouts as part of the Keio[33] and ASKA[35] clone collections in Japan make this experiment extremely valuable for genetically validating other CAs and their identified SNV targets in future molecular experiments. Furthermore, only 4 CA drugs were included in the study and it would have been useful to include more CAs to expand CA classes and include other classes to further validate the CA patterns we observed. With regards to specific experiments, as lack of cross-resistance was observed with the CAs tested it would have been useful, time permitting to have tested more antibiotics in each respective class to ensure that the patterns being observed were consistent among other closely related drug types. Growth curve experiments could have also been improved by growing cells as biofilms, a more relevant *E. coli* growth physiology associated with antiseptic tolerance[186] and explored different media

formulations to explore other growth conditions. Lastly, SNV data provided useful information regarding alterations in coding and non-coding regions, however, this data cannot determine if RNA transcripts, translated proteins or synthesized lipids are affected, which is where the future work of this project will begin and is ongoing by other members of the Bay lab. SNVs that cause frame-shifts of significant DNA sequence insertions are the only alterations that can be easily interpreted directly from sequenced genomes without corresponding transcriptomic, proteomic and/or lipidomic analysis.

4.3 Findings in relation to hypotheses

4.3.1 Discussion of the first thesis sub-hypothesis

The first sub-hypothesis of this study that has been addressed is as follows:

> "The phenotypic stability of CA tolerance after the removal of CA selection over 10 days will gradually diminish CA tolerance phenotypes among CA-adapted *E. coli* strains".

The results of CA tolerance stability testing confirm that QACs but not CHX or COL adapted strains lose their tolerance to CAs faster over a 10 day period when grown in media lacking their respective CA (Figure 3.5). When this experiment was repeated, MICs for CA-adapted strains remained stable however stability diminished by day 10 (except CHX). Previous studies have demonstrated a loss of CA tolerant stability over time by various proteobacteria including *E. coli*, suggesting that CA stability is temporary and prone to rapid loss[134,135]. Considering the number of essential gene SNVs identified in QAC adapted strain genomes we examined this is likely due to the higher fitness costs exerted on *E. coli* to genetically adapt to phenotypically tolerate higher

QAC concentration exposures as compared to the un-adapted WT strain. Therefore, this hypothesis is only partially valid and is dependent on the type of antimicrobial used to initially adapt *E. coli*.

4.3.2 Discussion of the second thesis sub-hypothesis

The second thesis sub hypothesis stated:

> "The growth fitness of CA-adapted strains will be compromised as compared to un-adapted *E. coli* strains".

In order to characterize the dynamics of CA adaptation, it was important to quantify how the adapted strains compared to the original un-adapted WT strain by comparing planktonic cell growth. Previous adaptation studies have shown that CA adaptation came at a significant cost when the fitness of CA-adapted species (BZK[58], CHX[58] and CTAB[51]) was measured. However, some CA-adapted strains showed no difference in fitness and even gained fitness[58,59]. Despite these conflicting studies, this project indicated CA adapted *E. coli* growth/fitness was only compromised in minimal media, suggesting that more defined and lower osmotic minimal media may be an important factor for CA adaptation. If we were to repeat this CA adaptation experiment again, only this time in minimal media it is likely that the CA adaptation time and final CA tolerance would be much lower as compared to rich medium. Therefore, based on this study we can conclude that this sub-hypothesis is valid for CA-adapted *E. coli* K-12.

4.3.3 Discussion of the third thesis sub hypothesis.

The third thesis sub-hypothesis states that:

> "CA-adapted strains will enhance antimicrobial cross-resistance profiles as compared to the un-adapted *E. coli* strain".

Previous CA biocide cross-tolerance studies have reported low to moderate (2-6 fold) to high (20-50) increases in MIC values (2-6 fold) for CA adapted Enterobacteriaceae and *Pseudomonas* spp. strains when compared to their respective un-adapted strains[50,60,62]. In contrast, other studies have reported enhanced susceptibility to other biocides by CA-adapted Enterobacteriaceae strains[61,63,87]. Due to differences in experimental design and species/ strain differences it is difficult to determine any unifying theme or trend for any particular CA in the studies cited above, including *E. coli* studies. There are three main variables that make comparison between these studies difficult: 1) strain/ species differences, 2) drug concentration differences and exposure lengths, and 3) the chemical formulation of the CA used for strain adaptation. Although studies specifically exploring variables 1) to 3) have yet to be properly and independently conducted, the outcome of this study provides a framework to now test these experimental parameters. Regarding variable 1, my study shows that QACs adapted and COL- adapted strains conferred cross-tolerance to closely related chemicals expect CHX (Table 3.2). In this study, chlorhexidine hydrochloride (CHX) was used for CHX adaptation studies instead of chlorhexidine digluconate (CHG) which is more commonly used as a disinfectant and antiseptic in commercial formulations; in addition, CHX formulation is less soluble than CHG but releases very different counterions, e.g. HCl vs digluconate in solution. The counterion itself may also exert some selective pressure during adaptation as increased anionic charges may exert an adaptive osmotic regulatory pressure on the cells. Further experimental studies are needed to determine the validity of CA formulations. Hence, it can be suggested that biocide adaptation to one CA biocide does not necessarily confer cross-resistance to other CA biocides and highlights the importance of

understanding the mechanisms of biocide action, cellular targets, and biocide synergies/antagonism.

A number of studies analysing antibiotic cross-resistance of biocide adapted strains have demonstrated increased resistance to particular antimicrobials[50,51,54]. In this project, cross-resistance was observed in both the QAC strains for a few of the antibiotics tested, however what was interesting to note was that increased susceptibility of all 3 COL-adapted strains to nearly all of the antibiotics tested was observed suggesting a strain with a very permeant cell membrane, particularly due to COL adapted *E. coli* strain susceptibility to Gram-positive selective vancomycin. In previous studies, QAC and CHX biocide adapted *E. coli* strains have shown antibiotic cross-resistance to a number of antibiotics, including β-lactams and macrolides[51,54] and third generation cephalosporins[50,65,66] but my findings did not show increased resistance to these compounds (Table 3.2). It is possible that in some of these *E. coli* strains/ isolates, there may have already been some pre-existing antimicrobial resistance genes present to confer these phenotypes or perhaps the strains had prior antibiotic exposure before CA adaptation in these studies; the lack of any genotypic information for strains used in these experiments prevents any further speculation.

Altogether, the third sub-hypothesis appears to be valid for only QAC and COL-adapted strains but not for CHX-adapted strains based on our studies findings comparing antimicrobial cross-resistance/tolerance values collected under the same conditions. Again, understanding the mechanism of action is important here, particularly as variations in CA cross-tolerance profiles were observed in this study.

4.3.4. Discussion of the fourth sub-hypothesis of the thesis.

The fourth sub hypothesis of this thesis states:

> "*E. coli* adapted to different CAs will confer similar genetic alterations (SNVs) in gene and pathways involving the outer membrane, lipid biosynthesis and trafficking, OMP/porins, and efflux pump systems".

WGS performed on each CA-adapted strain in this study revealed that a number of SNVs were repetitively identified for each CA-adapted strain and between strains. The total amount of SNVs in coding and non-coding regions differed between each CA-adapted strain suggesting that CA mechanism of action influences different genetic alterations of *E. coli* K12. With respect to the fourth sub-hypothesis itself, repetitive SNVs were found in greatest frequency in transposable insertion element genes and non-coding regions as well as LPS modification/trafficking systems (*waaY, lpxCLM, mlaA, msbA*) of the CA-adapted strains. SNVs identified in genes and upstream non-coding regions in other pathways involving the membrane such as efflux pump systems (*acrB, mdfA*), outer membrane proteins (*ompX, bamAD, sfmD*), and peptidoglycan synthesis (*mipA, murB*) were also observed in some but not all CA- adapted partially supporting the fourth sub-hypothesis. CA adaptation also revealed repetitive SNV occurrence rates in genes/ non-coding regions associated with many transcriptional regulators (*marR, rob, pmrB, yghQ*) known for antimicrobial resistance gene regulation, biofilm formation (*yghQ*), DNA/RNA replication (*rpoBC, gyrA*) and protein translation/ folding (*rpsA, lon, trmL*) between various CA adapted strain genomes (Figures 3.7-3.8). However, these SNVs (including lipid biosynthesis/trafficking genes and transposases/prophages) were not consistently or repetitively identified in the same genes/ non-coding regions between all four sets of CA adapted strains which may interpreted in two ways.

The first interpretation is that therapeutic CA, COL exerts various amounts of selective pressure on *E. coli* K12 as compared to biocide CAs, CHX and QACs. Although all these compounds act to disrupt the cell membrane(s) and only COL is known to target lipid A specifically, where as QACs and CHX may target multiple anionic lipids and proteins forming a number of cellular targets for alteration. Greater amounts of SNVs located within transposable elements of QAC and CHX-adapted *E. coli* as compared to COL adapted strains may provide some evidence to support this. Transposable elements have been shown not only to regulate host gene expression but are often co-opted by the host to serve new cellular functions as reviewed by Navarro, 2017[187] and 'hop' within the genome potentially causing deleterious mutations and altered gene expression[187]. In addition, prophages can also contribute in rapid genome editing of the bacterial DNA chromosome or extrachromosomal plasmids[188], and also influencing rapid evolution of bacteria under stress[188]. A number of repetitive SNVs in similar regions of transposable elements were observed in CET, BZK and CHX-adapted strains, but very few were observed in COL-adapted strains. It is therefore entirely possible that transposable elements have been targeted in the biocide CA-adapted strains as a mechanism of stress response and creating a way to attempt to spread the possibility of antimicrobial resistance genes.

The second interpretation of the SNV data is that the mechanism of CA action and the stress it exerts on the cell plays a much bigger role in *E. coli* adaptation to CAs than assumed by the sub-hypothesis. As discussed in the results 3.5 section, QAC and CHX mechanisms of action[11] may place greater pressure on the cell to overcome a number of stressors beyond the sub-hypothesis pathways: lipid alterations, efflux pump enhancements and porin downregulation. In contrast, COL resistance is know to be associated with lipid A modifications specifically[14]. QACs

and CHX both disrupt membranes, denature proteins but so far, only QACs have experimental evidence showing they increase reactive oxygen/ nitrogen species and inducing DNA damage[189]. The identification of SNVs in genes associated with various transcriptional regulators, such as *rob*, *marR*, and *pmrB* (COL-adapted *E.coli* only) suggest that different stress pathways may be triggered and more transcriptional analysis such as RNA-seq or qPCR may shed more light on how these are regulated and perhaps give more insight into how mechanism of action or cellular targets influence CA genotypic adaptation.

Therefore, CA adapted *E. coli* strains do not share identical lipid, efflux and porin targets but do alter similar components related to these pathways that may be influenced by CA mechanism of action and/or the number of cellular targets they interact with to drive selective pressure. In relation to the overall hypothesis, *E. coli* K-12 strain BW25113 adapted to different CA classes (QACs, BGs, and PMX) has resulted in similar phenotypic and genotypic alterations (to a degree) and so therefore the project hypothesis can be concluded as being proven.

4.4 Future directions

Future work on this project and strains should focus on transcriptomic, proteomic and lipidomic approaches to determine whether specific genes, proteins, or lipids respectively have increased or decreased as a result of each CA adaptation. Furthermore, repeating CA adaptation experiments with different CA biocides eg;. ALX, CPC, DDAB, and different bacterial strains eg; *Pseudomonas* spp., *Acinetobacter. baumannii* could be performed to determine if phenotypic and genotypic alterations are consistently observed based on the findings for *E. coli* and the 4 CAs tested. It is also important that follow up work be undertaken further to the genetic targets

identified via WGS. One approach could be through site directed mutagenesis (SDM), where genes with specific SNV codon substitutions could be generated in clones and transformed into *E. coli* Keio collection library mutants of BW25113[33] (single gene deletion mutants) to determine if phenotypic alterations are caused by particular genes of interest identified from the WGS findings. Constructs from the ASKA collection[34], a plasmid library built in *E. coli* K-12 where each gene has been cloned in pCA24N (-) could be used for SDM experiments, to complement Keio mutants (assuming it is not an essential gene). Comparing the WT gene to the mutant gene (with SNVs we observed) by AST can determine if CA tolerance is altered and estimate an MIC value. This future work could provide further insight into the process of how CA adaptation occurs and identify phenotypic and genotypic targets in CA tolerance which are still uncertain.

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Appendix i A summary of chemicals and antimicrobials used in this study.

Chemical	Company	Stock concentration used (if applicable)	Antimicrobial or chemical compound designation
Alexidine dihydrochloride	Sigma Aldrich	1mg/ml	Bisbiguanide
Ammonium chloride	VWR	N/A	Medium
Ammonium sulphate	Thermo Fisher	N/A	Medium
Benzalkonium chloride	Sigma Aldrich	50mg/ml	Quaternary ammonium compound
Calcium chloride	Thermo Fisher	N/A	Medium
Ceftazidime	TCI America	50mg/ml	Cephalosporin antibiotic
Cetrimide bromide	Sigma Aldrich	50mg/ml	QAC
Cetyl dimethyl ammonium bromide (CDAB)	TCI America	30mg/ml	QAC
Cetyl pyridinium chloride (CPC)	TCI America	12.5mg/ml	QAC
Cetyl trimethyl ammonium bromide (CTAB)	VWR	30mg/ml	QAC
Chlorhexidine dihydrochloride	TCI America	180µg/ml	Bisbiguanide
Colistin sulphate	TCI America	30mg/ml	Polymyxin antibiotic
Dextrose	VWR	N/A	N/A
Dimethyldidodecylammonium bromide (DDAB)	TCI America	10mg/ml	QAC
Dimethylsulfoxide	VWR	N/A	Cryopreservation
Dipotassium sulphate	Thermo Fisher	N/A	Medium
Domiphen bromide	Sigma Aldrich	50mg/ml	Quaternary ammonium compound
Doxycycline	Biobasic	20mg/ml	Tetracycline antibiotic
Erythromycin	VWR	10mg/ml	Macrolide antibiotic
Ethanol	VWR	N/A	Alcohol
Glycerol	Thermo Fisher	N/A	Medium
Kanamycin	VWR	10mg/ml	Aminoglycoside antibiotic
Linezolid	Thermo Fisher	5mg/ml	Oxazolidinone antibiotic
Magnesium sulphate	Thermo Fisher	N/A	Medium
Meropenem trihydrate	TCI America	5 mg/ml	Carbapenem antibiotic
Monopotassium sulphate	TCI America	N/A	Medium
Mueller Hinton broth	VWR	N/A	Medium
Polymyxin B	Thermo Fisher	30mg/ml	Polymyxin antibiotic
Potassium hydrogen phosphate	Thermo Fisher	N/A	Medium
Rifamycin	Thermo Fisher	10mg/ml	Ansamycin antibiotic
Sodium citrate dihydrate	Thermo Fisher	N/A	Medium
Sodium chloride	VWR	N/A	Medium
Sodium hydrogen phosphate	Thermo Fisher	N/A	Medium
Thiamine	Thermo Fisher	N/A	Medium
Triclosan	TCI America	20mg/ml	Anionic biocide
Trimethoprim-sulfamethoxazole	Thermo Fisher	20mg/ml	Sulphonamide antibiotic
Tryptic Soy broth	Thermo Fisher	N/A	Medium
Tryptone	Thermo Fisher	N/A	Medium
Vancomycin hydrochloride	Biobasic	50mg/ml	Glycopeptide antibiotic
Yeast extract	Thermo Fisher	N/A	Medium

Appendix ii N50 and N75 values for WGS of CA-adapted strains

Strain sequenced	# contigs (>= 0 bp)	# contigs (>= 1000 bp)	Total length (>= 0 bp)	Total length (>= 1000 bp)	# contigs	Largest contig	Total length	GC (%)	N50	N75	L50	L75	# N's per 100 kbp
BZKG40R1	124	75	4586501	4565252	87	414105	4573381	50.74	142449	78770	10	20	0
BZKG40R2	116	64	4589328	4568537	70	414105	4573360	50.74	174915	76651	10	20	0
BZKG40R3	153	62	4607634	4571956	69	428761	4577188	50.75	178400	95180	9	17	0
CETG40R1	146	67	4600921	4569417	75	387978	4575491	50.74	148378	78770	11	21	0
CETG40R2	212	65	4634019	4570861	78	405507	4579924	50.73	176266	88592	10	19	0
CETG40R3	153	64	4604718	4570514	72	361542	4576954	50.74	150659	88592	11	20	0
CHXG20R1	116	72	4585779	4567624	79	428761	4573169	50.74	150852	69618	10	21	0
CHXG20R2	100	65	4584177	4569959	70	430631	4573860	50.74	178400	78770	9	18	0
CHXG20R3	96	67	4583674	4571496	71	414105	4574627	50.74	175283	78770	10	19	0
COLG32R1	105	64	4585650	4568226	71	414105	4573602	50.74	177179	95318	10	18	0
COLG32R2	119	64	4581092	4556860	75	414105	4564694	50.76	178400	95318	9	17	0
COLG32R3	116	74	4587651	4569640	82	430631	4575780	50.74	148613	78770	10	20	0
BW25113WT1	89	62	4579698	4569605	66	414105	4572571	50.74	204053	95180	8	17	0
BW25113WT2	95	62	4582030	4570231	65	414105	4572629	50.74	186809	112544	9	17	0

Appendix iii Complete list of SNVs for all adapted *E. coli* BW25113 adapted strains.

Biorep	Minimum	Maximum	Length	# Intervals	Amino Acid Change	Change	Codon Change	Coverage	Polymorphism Type	Protein Effect	Variant Frequency	Variant P-Value (	Max (with gap	Coding regions	Non-coding regions	Locus tag (if known)
BZK3	62726	62726	1	1	G -> V	C -> A	GGT -> GTT	126	SNP (transversion)	Substitution	0.992	0	62821	rapA		BW25113_0059
BZK1	156952	156952	1	1	W -> S	C -> G	TGG -> TCG	31	SNP (transversion)	Substitution	96.80%	3.10E-113	157051	dksA		BW25113_0145
BZK1	360327	360328	2	1		CT -> TC		33	Substitution		30.30%	1.50E-26	360573		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
BZK2	360295	360295	1	1		A -> G		63	SNP (transition)		0.254	8.2E-28	360547		lacZ (U); traI (D)	BW25113_0344, BW25113_0345
BZK2	360298	360298	1	1		C -> G		61	SNP (transversion)		0.262	4.9E-36	360550		lacZ (U); traI (D)	BW25113_0344, BW25113_0345
BZK1	454218	454218	1	1		T -> C		23	SNP (transition)		26.10%	2.50E-14	454532		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	454224	454227	4	1		ATTC -> CCCT		28 -> 30	Substitution		25.0% -> 30.0%	3.70E-19	454541		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	454229	454230	2	1		CG -> AA		32	Substitution		34.40%	8.00E-28	454544		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	454232	454238	7	1		CGTTGAA -> TTAGCGC		29 -> 32	Substitution		34.4% -> 40.0%	1.00E-26	454552		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	454240	454242	3	1		GTG -> TAT		30 -> 32	Substitution		43.3% -> 46.9%	1.50E-35	454556		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	454256	454258	3	1		CAT -> ATA		42	Substitution		38.10%	1.00E-48	454572		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	454260	454264	5	1		TACTG -> GCGCT		40 -> 43	Substitution		27.5% -> 32.6%	8.80E-32	454579		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	454266	454267	2	1		CG -> AC		39	Substitution		28.20%	8.30E-28	454582		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	454269	454270	2	1		AC -> TA		38	Substitution		26.30%	4.70E-28	454585		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	454272	454272	1	1		T -> G		40	SNP (transversion)		27.50%	5.80E-31	454587		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	454274	454274	1	1		T -> G		40	SNP (transversion)		25.00%	8.40E-28	454589		clpX (D): lon (U)	BW25113_0438; BW25113_0439
BZK1	479340	479340	1	1	D -> Y	C -> A	GAT -> TAT	30	SNP (transversion)	Substitution	100.00%	1.00E-111	479664	acrB		BW25113_0462
BZK1	603438	603438	1	1	K -> N	G -> C	AAG -> AAC	60	SNP (transversion)	Substitution	25.00%	1.70E-42	603856	hokE		BW25113_4415
BZK1	603445	603445	1	1		C -> T		66	SNP (transition)		28.80%	3.40E-47	603864		hokE (D); IS421 (U)	BW25113_4415; BW25113_0016
BZK1	603447	603448	2	1		GT -> AG		67 -> 69	Substitution		29.9% -> 30.4%	5.60E-48	603867		hokE (D); IS421 (U)	BW25113_4415; BW25113_0016
BZK1	603450	603450	1	1		G -> A		68	SNP (transition)		30.90%	1.40E-48	603869		hokE (D); IS421 (U)	BW25113_4415; BW25113_0016
BZK1	603452	603453	2	1		GC -> AT		67 -> 68	Substitution		30.9% -> 31.3%	1.40E-48	603872		hokE (D); IS421 (U)	BW25113_4415; BW25113_0016
BZK1	603459	603458	0	1		Unknown		66	Insertion		31.80%	6.80E-49	603878		hokE (D); IS421 (U)	BW25113_4415; BW25113_0016
BZK1	603461	603463	3	1		AAT -> TTC		63	Substitution		34.90%	8.00E-48	603883		hokE (D); IS421 (U)	BW25113_4415; BW25113_0016
BZK2	684507	684507	1	1		T -> A		74	SNP (transversion)		0.284	9.3E-50	684938		insH-1 (U)	BW25113_0259
BZK1	702329	702329	1	1	R -> S	C -> A	CGC -> AGC	53	SNP (transversion)	Substitution	100.00%	7.90E-197	702801	glnS		BW25113_0680
BZK3	845894	845897	4	1		TTTG -> CACT		58	Substitution		0.259	2.9E-38	846739		ompX (U): rhtA (U)	BW25113_0814; BW25113_0813
BZK3	845899	845900	2	1		GG -> AA		63	Substitution		31.7% -> 33.3%	8.6E-58	846742		ompX (U): rhtA (U)	BW25113_0814; BW25113_0813
BZK3	845902	845904	3	1		GGT -> CAG		58 -> 62	Substitution		36.2% -> 40.3%	6.6E-63	846746		ompX (U): rhtA (U)	BW25113_0814; BW25113_0813
BZK3	845907	845907	1	1	M -> K	T -> A	ATG -> AAG	62	SNP (transversion)	Start Codon Loss	0.403	1.5E-68	846749	ompX		BW25113_0814
BZK3	845909	845913	5	1	KK -> LA	AAAAA -> TTGGC	AAA,AAA -> TTG,GCA	60 -> 62	Substitution	Substitution	37.1% -> 38.3%	2.3E-70	846755	ompX		BW25113_0814
BZK3	845915	845918	4	1	IA -> AS	ATTG -> GCAT	ATT,GCA -> GCA,TCA	61	Substitution	Substitution	0.426	4.9E-78	846760	ompX		BW25113_0814
BZK3	845921	845922	2	1	C -> P	TG -> CC	TGT -> CCT	60 -> 61	Substitution	Substitution	44.3% -> 45.0%	9.9E-81	846764	ompX		BW25113_0814
BZK3	845933	845938	6	1	LA -> GN	CTGGCC -> GGTAAT	CTG,GCC -> GGT,AAT	69 -> 71	Substitution	Substitution	40.8% -> 42.0%	5.9E-80	846780	ompX		BW25113_0814
BZK3	845940	845950	11	1	AVLA -> DSNL	CAGTTCTGGCT -> ACTCCAACTTA	GCA,GTT,CTG,GCT -> GAC,TCC,AAC,TTA	71 -> 72	Substitution	Substitution	31.0% -> 38.0%	5.2E-66	846792	ompX		BW25113_0814
BZK3	845953	845953	1	1	F -> L	C -> G	TTC -> TTG	71	SNP (transversion)	Substitution	0.338	2E-56	846795	ompX		BW25113_0814
BZK3	845955	845956	2	1	T -> I	CC -> TA	ACC -> ATA	64 -> 71	Substitution	Substitution	26.6% -> 33.8%	2.1E-43	846798	ompX		BW25113_0814
BZK3	845961	845961	1	1	G -> V	G -> T	GGT -> GTT	63	SNP (transversion)	Substitution	0.254	3.5E-34	846803	ompX		BW25113_0814
BZK1	958217	958217	1	1	S -> C	C -> G	TCC -> TGC	34	SNP (transversion)	Substitution	100.00%	1.60E-126	958893	rpsA		BW25113_0911
BZK3	958245	958245	1	1	D -> E	T -> A	GAT -> GAA	109	SNP (transversion)	Substitution	1	0	959225	rpsA		BW25113_0911
BZK1	962609	962609	1	1	V -> G	T -> G	GTT -> GGT	35	SNP (transversion)	Substitution	100.00%	3.20E-123	963286	msbA		BW25113_0914
BZK3	962609	962609	1	1	V -> G	T -> G	GTT -> GGT	107	SNP (transversion)	Substitution	0.991	0	963594	msbA		BW25113_0914
BZK3	1009211	1009211	1	1	K -> T	A -> C	AAA -> ACA	80	SNP (transversion)	Substitution	0.988	3.2E-283	1010236	pqiB		BW25113_0951
BZK1	1111875	1111875	1	1	R -> L	C -> A	CGC -> CTC	34	SNP (transversion)	Substitution	100.00%	1.00E-119	1112661	lpxL; waaM		BW25113_1054
BZK1	1203196	1203197	2	1	PF -> PI	GT -> AA	CCG,TTT -> CCA,ATT	40	Substitution	Substitution	27.50%	5.40E-27	1204041	stfP (ycfk)		BW25113_1154
BZK1	1203201	1203202	2	1	G -> A	GC -> CA	GGC -> GCA	40	Substitution	Substitution	27.50%	7.20E-30	1204046	stfP (ycfk)		BW25113_1154
BZK1	1203204	1203205	2	1	D -> G	AT -> GA	GAT -> GGA	44	Substitution	Substitution	34.10%	2.30E-43	1204049	stfP (ycfk)		BW25113_1154
BZK1	1203208	1203208	1	1	I -> M	C -> G	ATC -> ATG	45	SNP (transversion)	Substitution	35.60%	2.50E-43	1204052	stfP (ycfk)		BW25113_1154
BZK1	1203210	1203216	6	2	KSD -> MPF	AATCGGA -> TGCCATT	AAA,TCG,GAT -> ATG,CCA,TTT	46 -> 48	Substitution	Substitution	34.8% -> 40.4%	6.20E-48	1204060	stfP (ycfk)		BW25113_1154
BZK1	1203219	1203222	4	1	GT -> AC	GCAC -> CTTG	GGC,ACT -> GCT,TGT	47 -> 49	Substitution	Substitution	40.4% -> 42.9%	3.50E-58	1204066	stfP (ycfk)		BW25113_1154
BZK1	1203224	1203234	11	1	VQTA -> YFFI	GTGCAAACGGC -> TATTTCTTCAT	GTG,CAA,ACG,GCT -> TAT,TTC,TTC,AT	42 -> 44	Substitution	Substitution	47.7% -> 50.0%	5.00E-64	1204078	stfP (ycfk)		BW25113_1154
BZK1	1203236	1203236	1	1	L -> F	C -> T	CTC -> TTC	42	SNP (transition)	Substitution	50.00%	8.50E-69	1204080	stfP (ycfk)		BW25113_1154
BZK1	1203239	1203240	2	1	E -> I	GA -> AT	GAA -> ATA	41 -> 42	Substitution	Substitution	51.2% -> 52.4%	4.30E-69	1204084	stfP (ycfk)		BW25113_1154
BZK1	1203245	1203245	1	1	L -> V	C -> G	CTT -> GTT	40	SNP (transversion)	Substitution	55.00%	7.10E-69	1204089	stfP (ycfk)		BW25113_1154
BZK2	1203262	1203263	2	1	GA -> GS	AG -> CT	GGA,GCA -> GGC,TCA	38	Substitution	Substitution	0.263	4.7E-28	1204021	stfP (ycfk)		BW25113_1154
BZK3	1203224	1203234	11	1	VQTA -> YFFI	GTGCAAACGGC -> TATTTCTTCAT	GTG,CAA,ACG,GCT -> TAT,TTC,TTC,AT	164 -> 68	Substitution	Substitution	25.0% -> 28.1%	8.6E-38	1204439	stfP (ycfk)		BW25113_1154
BZK3	1203236	1203236	1	1	L -> F	C -> T	CTC -> TTC	64	SNP (transition)	Substitution	0.297	1.3E-45	1204441	stfP (ycfk)		BW25113_1154
BZK3	1203239	1203240	2	1	E -> I	GA -> AT	GAA -> ATA	67 -> 68	Substitution	Substitution	34.3% -> 35.3%	3.3E-61	1204445	stfP (ycfk)		BW25113_1154
BZK3	1203245	1203245	1	1	L -> V	C -> G	CTT -> GTT	69	SNP (transversion)	Substitution	0.391	1.7E-73	1204450	stfP (ycfk)		BW25113_1154
BZK3	1203262	1203267	5	2	GAK -> GSA	AGCAAA -> CTCTGC	GGA,GCA,AAA -> GGC,TCT,GCA	72 -> 74	Substitution	Substitution	32.4% -> 33.3%	2.8E-70	1204472	stfP (ycfk)		BW25113_1154
BZK3	1203269	1203273	4	2	LN -> LP	CTCAA -> TTACC	CTC,AAT -> TTA,CCT	74 -> 76	Substitution	Substitution	27.0% -> 31.6%	5.8E-53	1204478	stfP (ycfk)		BW25113_1154
BZK1	1860644	1860644	1	1	G -> D	C -> T	GGC -> GAC	32	SNP (transition)	Substitution	96.90%	6.40E-114	1861949	mipA		BW25113_1782
BZK3	1860673	1860673	1	1		T -> A	GTA -> GTT	106	SNP (transversion)	None	0.981	0	1862551	mipA		BW25113_1782
BZK1	2027413	2027413	1	1		G -> A		23	SNP (transition)		100.00%	4.00E-88	2028832	No genes in location		
BZK2	2096425	2096425	1	1	K -> R	T -> C	AAG -> AGG	76	SNP (transition)	Substitution	0.316	4.4E-34	2097726	wbbL+ insH1		BW25113_4571
BZK2	2096430	2096432	2	2	G -> R	CCC -> GCG	GGG -> CGC	70 -> 78	Substitution	Substitution	30.0% -> 30.8%	9E-34	2097733	wbbL+ insH1		BW25113_4571
BZK2	2096435	2096436	2	1	ML -> IL	AC -> GT	ATG,TTA -> ATA,CTA	70	Substitution	Substitution	0.3	1.1E-35	2097737	wbbL+ insH1		BW25113_4571
BZK2	2096439	2096440	2	1	F -> S	AA -> TG	TTT -> TCA	68	Substitution	Substitution	0.279	5.3E-28	2097741	wbbL+ insH1		BW25113_4571
BZK2	2458376	2458381	6	1	NR -> LN	GCGGTT -> ATTTAA	AAC,CGC -> TTA,AAT	27	Substitution	Substitution	0.259	2.2E-21	2459891	mlaA		BW25113_2346
BZK2	2458383	2458387	5	1	GF -> YH	AACCC -> TGATA	GGG,TTC -> TAT,CAC	27 -> 29	Substitution	Substitution	25.9% -> 31.0%	2.2E-21	2459897	mlaA		BW25113_2346
BZK2	2458390	2458399	10	1	DPLE -> PDNQ	CTAACGGGTC -> GGTTGTCTGG	GAC,CGG,TTA,GAA -> CCA,GAC,AAC,CAA	28 -> 29	Substitution	Substitution	31.0% -> 34.5%	3.4E-27	2459909	mlaA		BW25113_2346
BZK2	2458403	2458410	8	1	QGR -> PPE	ACGCCCTT -> TTCAGGGG	CAA,GGG,CGT -> CCC,CCT,GAA	29 -> 32	Substitution	Substitution	40.6% -> 50.0%	4.3E-37	2459920	mlaA		BW25113_2346
BZK2	2458413	2458415	3	1	DQ -> DW	TGA -> CAG	GAT,CAG -> GAC,TGG	29 -> 32	Substitution	Substitution	50.0% -> 58.6%	6E-48	2459925	mlaA		BW25113_2346
BZK2	2458419	2458420	2	1		GT -> A		29	Deletion	Truncation	0.586	1.3E-40	2459930	mlaA		BW25113_2346

BZK2	2458426	2458433	8	1	CASS -> CKST	AACTCGCA -> TGGATTTG	TGT,GCG,AGT,TCC -> TGC,AAA,TCC,ACC	27 -> 28	Substitution	Substitution	25.0% -> 25.9%	1.9E-18	2459943	mlaA		BW25113_2346
BZK1	2509125	2509127	3	1	D -> L	ATC -> TAG	GAT -> CTA	46 -> 49	Substitution	Substitution	34.8% -> 38.8%	1.60E-49	2510888	pdeA		BW25113_2395
BZK1	2509130	2509131	2	1	LA -> FP	CT -> GA	TTA,GCT -> TTT,CCT	49	Substitution	Substitution	32.70%	8.30E-46	2510892	pdeA		BW25113_2395
BZK1	2509134	2509140	7	1	PQP -> PLI	TGGCTGC -> AATTAAA	CCG,CAG,CCA -> CCT,TTA,ATT	45 -> 48	Substitution	Substitution	31.3% -> 33.3%	1.10E-42	2510901	pdeA		BW25113_2395
BZK1	2509144	2509148	5	1	GR -> IS	CGACC -> GAGAT	GGT,CGC -> ATC,TCC	45 -> 47	Substitution	Substitution	29.8% -> 31.1%	2.60E-41	2510909	pdeA		BW25113_2395
BZK1	2509151	2509155	5	1		TCAAA -> AATCC		45 -> 47	Substitution	Truncation	26.7% -> 29.8%	4.50E-31	2510916	pdeA		BW25113_2395
BZK3	1613660	1613660	1	1	G -> D	G -> A	GGC -> GAC	117	SNP (transition)	Substitution	0.991	0	1615277	marR		BW25113_1530
BZK3	2428859	2428859	1	1	G -> D	C -> T	GGC -> GAC	116	SNP (transition)	Substitution	1	0	2431274	truA		BW25113_2318
BZK1	2961100	2961100	1	1	A -> T	C -> T	GCC -> ACC	18	SNP (transition)	Substitution	83.30%	2.60E-50	2963130	ptsP		BW25113_2829
BZK1	2961101	2961102	2	1		#NAME?		18	Deletion	Frame Shift	100.00%	6.30E-53	2963132	ptsP		BW25113_2829
BZK1	2961104	2961107	3	2		AAAG -> AGT		18	Deletion	Frame Shift	83.30%	1.00E-45	2963137	ptsP		BW25113_2829
BZK1	2961112	2961112	1	1	K -> E	T -> C	AAA -> GAA	16	SNP (transition)	Substitution	81.30%	1.80E-43	2963142	ptsP		BW25113_2829
BZK1	2961115	2961118	3	2	YS -> ER	TGTA -> GTTC	TAC,AGC -> GAA,CGC	17	Substitution	Substitution	70.60%	7.20E-36	2963148	ptsP		BW25113_2829
BZK1	2961119	2961120	2	1	R -> L	GC -> CA	CGC -> CTG	17	Substitution	Substitution	70.60%	3.90E-40	2963150	ptsP		BW25113_2829
BZK1	2961122	2961124	3	1	R -> A	GCG -> AGC	CGC -> GCT	16 -> 17	Substitution	Substitution	64.7% -> 68.8%	1.40E-35	2963154	ptsP		BW25113_2829
BZK1	2961122	2961124	3	1	R -> D	GCG -> ATC	CGC -> GAT	16 -> 17	Substitution	Substitution	25.0% -> 29.4%	1.10E-12	2963154	ptsP		BW25113_2829
BZK1	2961125	2961125	1	1		A -> G	TTT -> TTC	16	SNP (transition)	None	25.00%	1.10E-12	2963155	ptsP		BW25113_2829
BZK1	2961125	2961127	3	1	F -> P	AAA -> CGG	TTT -> CCG	16 -> 17	Substitution	Substitution	64.7% -> 68.8%	9.80E-31	2963157	ptsP		BW25113_2829
BZK1	2961128	2961128	1	1	E -> D	C -> A	GAG -> GAT	17	SNP (transversion)	Substitution	64.70%	4.00E-38	2963158	ptsP		BW25113_2829
BZK1	2961128	2961130	2	2	E -> Y	CTC -> ATA	GAG -> TAT	17	Substitution	Substitution	35.30%	4.90E-17	2963161	ptsP		BW25113_2829
BZK1	2961131	2961133	3	1		GTT -> CA		16 -> 17	Deletion	Frame Shift	47.1% -> 50.0%	2.40E-20	2963164	ptsP		BW25113_2829
BZK1	2961132	2961133	2	1	N -> G	TT -> CC	AAC -> GGC	16	Substitution	Substitution	37.50%	2.00E-18	2963164	ptsP		BW25113_2829
BZK1	2961134	2961134	1	1		(T)3 -> (T)2		16	Deletion (tandem repeat)	Frame Shift	100.00%	1.60E-45	2963165	ptsP		BW25113_2829
BZK1	2961137	2961139	3	1	A -> N	CGC -> GTT	GCG -> AAC	14 -> 16	Substitution	Substitution	50.0% -> 57.1%	5.10E-23	2963170	ptsP		BW25113_2829
BZK1	2961139	2961139	1	1	A -> T	C -> T	GCG -> ACG	14	SNP (transition)	Substitution	42.90%	4.00E-21	2963170	ptsP		BW25113_2829

BZK1	2961140	2961144	5	1	EE -> GI	TTCTT -> GATGC	GAA,GAA -> GGC,ATC	14	Substitution	Substitution	57.10%	1.20E-23	2963175	ptsP		BW25113_2829
BZK1	2961145	2961145	1	1	E -> K	C -> T	GAA -> AAA	14	SNP (transition)	Substitution	42.90%	1.90E-19	2963176	ptsP		BW25113_2829
BZK1	2961146	2961147	2	1	L -> Q	CA -> TT	CTG -> CAA	14	Substitution	Substitution	42.90%	1.20E-20	2963178	ptsP		BW25113_2829
BZK1	2961146	2961148	3	1	L -> S	CAG -> TGA	CTG -> TCA	14	Substitution	Substitution	57.10%	1.90E-24	2963179	ptsP		BW25113_2829
BZK1	2961149	2961149	1	1		C -> T	GCG -> GCA	14	SNP (transition)	None	42.90%	2.50E-22	2963180	ptsP		BW25113_2829
BZK1	2961149	2961150	2	1	A -> V	CG -> TA	GCG -> GTA	14	Substitution	Substitution	57.10%	7.50E-27	2963181	ptsP		BW25113_2829
BZK1	2961152	2961152	1	1		C -> A	GGG -> GGT	14	SNP (transversion)	None	100.00%	1.60E-52	2963183	ptsP		BW25113_2829
BZK1	2961155	2961155	1	1		G -> T	ACC -> ACA	14	SNP (transversion)	None	57.10%	3.00E-25	2963186	ptsP		BW25113_2829
BZK1	2961158	2961166	9	1	ERL -> FNG	CAGTCGTTC -> TCCATTAAA	GAA,CGA,CTG -> TTT,AAT,GGA	14 -> 15	Substitution	Substitution	53.3% -> 57.1%	7.50E-23	2963197	ptsP		BW25113_2829
BZK1	2961168	2961171	4	1	ER -> VT	CGTT -> GTAA	GAA,CGC -> GTT,ACC	14 -> 15	Substitution	Substitution	50.0% -> 53.3%	2.20E-22	2963202	ptsP		BW25113_2829
BZK1	2961173	2961174	2	1	L -> H	CA -> GT	CTG -> CAC	13 -> 14	Substitution	Substitution	42.9% -> 46.2%	1.20E-17	2963205	ptsP		BW25113_2829
BZK1	2961176	2961183	8	1	DPA -> AGC	AGCCGGAT -> GCATCCTG	GAT,CCG,GCT -> GCA,GGA,TGC	13 -> 14	Substitution	Substitution	30.8% -> 42.9%	1.10E-12	2963214	ptsP		BW25113_2829
BZK1	2961189	2961189	1	1	T -> R	G -> C	ACG -> AGG	12	SNP (transversion)	Substitution	25.00%	1.70E-09	2963220	ptsP		BW25113_2829
BZK1	2961192	2961194	3	1	AS -> AR	GAT -> CGG	GCA,TCA -> GCC,CGA	12	Substitution	Substitution	25.00%	6.90E-09	2963225	ptsP		BW25113_2829
BZK1	3116670	3116670	1	1		C -> T	CAG -> CAA	5	SNP (transition)	None	40.00%	1.60E-07	3118832	glcB		BW25113_2976
BZK1	3123453	3123454	2	1	P -> G	GG -> CC	CCC -> GGC	40	Substitution	Substitution	25.00%	3.50E-07	3125618	yghO		BW25113_2981
BZK1	3123456	3123456	1	1	Y -> S	T -> G	TAT -> TCT	41	SNP (transversion)	Substitution	26.80%	4.00E-09	3125620	yghO		BW25113_2981
BZK1	3123458	3123460	2	2	L -> I	AAG -> GAT	CTT -> ATC	44 -> 46	Substitution	Substitution	31.8% -> 32.6%	1.60E-10	3125624	yghO		BW25113_2981
BZK1	3123462	3123463	2	1	S -> I	GA -> AT	TCA -> ATA	46	Substitution	Substitution	32.60%	1.60E-10	3125627	yghO		BW25113_2981
BZK1	3123466	3123466	1	1	S -> T	A -> T	TCA -> ACA	46	SNP (transversion)	Substitution	32.60%	1.60E-10	3125630	yghO		BW25113_2981
BZK1	3123468	3123469	2	1	P -> K	GG -> TT	CCG -> AAG	47	Substitution	Substitution	31.90%	2.20E-10	3125633	yghO		BW25113_2981
BZK1	3123471	3123480	10	1	FIAF -> CQQN	AAAGCGATAA -> TTCTGTTGGC	TTT,ATC,GCT,TTT ->	48 -> 49	Substitution	Substitution	31.3% -> 32.7%	3.10E-10	3125644	yghO		BW25113_2981
BZK1	3123483	3123484	2	1	A -> I	GC -> AT	GCA -> ATA	48 -> 49	Substitution	Substitution	32.7% -> 33.3%	7.60E-10	3125648	yghO		BW25113_2981
BZK1	3123486	3123490	4	2	LK -> MP	TTAAG -> GGCAT	CTT,AAG -> ATG,CCG	48	Substitution	Substitution	35.40%	7.50E-11	3125654	yghO		BW25113_2981
BZK1	3123492	3123493	2	1	D -> P	TC -> GG	GAC -> CCC	46	Substitution	Substitution	37.00%	3.40E-11	3125657	yghO		BW25113_2981

BZK1	3123496	3123498	3	1	KN -> TH	TTT -> GCG	AAA,AAT -> ACG,CAT	46 -> 48	Substitution	Substitution	37.0% -> 39.6%	3.40E-11	3125662	yghO		BW25113_2981
BZK1	3123504	3123504	1	1	L -> P	A -> G	CTT -> CCT	47	SNP (transition)	Substitution	42.60%	1.80E-12	3125668	yghO		BW25113_2981
BZK2	3124707	3124707	1	1	L -> Q	A -> T	CTG -> CAG	42	SNP (transversion)	Substitution	0.262	3.2E-13	3126590	yghQ		BW25113_2983
BZK2	3124709	3124710	2	1	D -> V	GT -> TA	GAC -> GTA	42	Substitution	Substitution	0.262	3.2E-13	3126593	yghQ		BW25113_2983
BZK1	3358899	3358902	4	1	GT -> DQ	GAAC -> ATCA	GGA,ACA -> GAT,CAA	59 -> 60	Substitution	Substitution	26.7% -> 27.1%	1.10E-34	3361234	yhcE+ insH-1		BW25113_3217
BZK1	3358907	3358909	2	2		TCA -> GTTC		58 -> 59	Insertion	Frame Shift	25.4% -> 25.9%	1.20E-35	3361242	yhcE+ insH-1		BW25113_3217
BZK1	3358911	3358911	1	1	I -> T	T -> C	ATT -> ACT	58	SNP (transition)	Substitution	27.60%	4.90E-38	3361244	kdsC		BW25113_3198
BZK1	3358914	3358914	1	1	R -> K	G -> A	AGG -> AAG	62	SNP (transition)	Substitution	25.80%	6.60E-36	3361247	kdsC		BW25113_3198
BZK3	3358871	3358874	4	1		GGCA -> CATT		144 -> 145	Substitution	Truncation	26.9% -> 27.1%	1.1E-101	3362120	yhcE+ insH-1		BW25113_3217
BZK3	3358877	3358878	2	1	N -> G	AA -> GG	AAT -> GGT	150 -> 154	Substitution	Substitution	28.0% -> 29.9%	3E-110	3362124	yhcE+ insH-1		BW25113_3217
BZK3	3358880	3358884	4	2	DN -> QG	GACAA -> CAAGG	GAC,AAT -> CAA,GGT	156 -> 164	Substitution	Substitution	29.5% -> 32.3%	5.3E-131	3362130	yhcE+ insH-1		BW25113_3217
BZK3	3358888	3358888	1	1		G -> T	CCG -> CCT	171	SNP (transversion)	None	0.345	1.4E-160	3362134	yhcE+ insH-1		BW25113_3217
BZK3	3358890	3358891	2	1	G -> D	GC -> AT	GGC -> GAT	172 -> 173	Substitution	Substitution	35.5% -> 35.8%	1E-163	3362137	yhcE+ insH-1		BW25113_3217
BZK3	3358893	3358895	3	1	ST -> NA	GCA -> ATG	AGC,ACA -> AAT,GCA	182 -> 183	Substitution	Substitution	36.3% -> 36.8%	5.4E-168	3362141	yhcE+ insH-1		BW25113_3217
BZK3	3358899	3358902	4	1	GT -> DQ	GAAC -> ATCA	GGA,ACA -> GAT,CAA	194 -> 195	Substitution	Substitution	35.4% -> 35.6%	6.8E-171	3362149	yhcE+ insH-1		BW25113_3217
BZK3	3358907	3358909	2	2		TCA -> GTTC		189 -> 199	Insertion	Frame Shift	35.2% -> 37.0%	6.2E-177	3362158	yhcE+ insH-1		BW25113_3217
BZK3	3358911	3358911	1	1	I -> T	T -> C	ATT -> ACT	202	SNP (transition)	Substitution	0.347	2.2E-176	3362160	kdsC		BW25113_3198
BZK3	3358914	3358914	1	1	R -> K	G -> A	AGG -> AAG	200	SNP (transition)	Substitution	0.355	1.1E-172	3362163	kdsC		BW25113_3198
BZK3	3123504	3123504	1	1	L -> P	A -> G	CTT -> CCT	194	SNP (transition)	Substitution	0.258	5.6E-22	3126529	insH-1		BW25113_0259
BZK3	3576760	3576761	2	1	SI -> RF	CA -> AT	AGC,ATT -> AGA,TTT	132 -> 134	Substitution	Substitution	24.6% -> 25.0%	5.9E-49	3580212	yrhA		BW25113_3443
BZK3	3576767	3576768	2	1	I -> G	AT -> GG	ATA -> GGA	139 -> 140	Substitution	Substitution	25.7% -> 25.9%	7E-54	3580219	yrhA		BW25113_3443
BZK3	3576771	3576776	6	1		TTACTT -> AATGAG		136 -> 140	Substitution	Truncation	25.0% -> 25.7%	3.5E-52	3580227	yrhA		BW25113_3443
BZK3	3576780	3576781	2	1	D -> A	AC -> CA	GAC -> GCA	144 -> 148	Substitution	Substitution	28.4% -> 28.5%	3E-69	3580234	yrhA		BW25113_3443
BZK3	3576783	3576786	4	1	IK -> TS	TTAA -> CGTC	ATT,AAA -> ACG,TCA	146 -> 149	Substitution	Substitution	28.2% -> 28.8%	1.2E-66	3580239	yrhA		BW25113_3443
BZK2	3645364	3645365	2	1	LL -> LF	AC -> TT	CTA,CTT -> CTT,TTT	68	Substitution	Substitution	0.25	1.7E-39	3647541	yhiS + insH-1		BW25113_3504

BZK2	3645389	3645392	3	2	NL -> EL	AATT -> GAAC	AAT,TTA -> GAA,CTA	68	Substitution	Substitution	0.25	7E-43	3647570	yhiS + insH-1		BW25113_3504
BZK2	3646596	3646596	1	1		T -> A	GGT -> GGA	77	SNP (transversion)	None	0.299	2.4E-34	3648774	yhiS + insH-1		BW25113_3504
BZK1	3733088	3733088	1	1	D -> E	C -> A	GAC -> GAA	22	SNP (transversion)	Substitution	27.30%	9.50E-07	3735687	avtA		BW25113_3572
BZK2	3774516	3774516	1	1		G -> A		24	SNP (transition)		0.25	0.000000002	3776759		trmL (D): tRNA (U)	BW25113_3605; BW25113_3606
BZK3	3872347	3872346	0	1	A -> AAR	(CACGGG)2 -> (CACGGG)3	GCT -> GCT,GCC,CGT	79 -> 85	Insertion (tandem repeat)	Insertion	1	2E-261	3876076	gyrB		BW25113_3699
BZK3	3936821	3936822	2	1		AC -> CA		78	Substitution		0.282	2.3E-11	3940609	gltU		BW25113_3757
BZK3	3936824	3936825	2	1		GC -> AA		78 -> 81	Substitution		29.5% -> 29.6%	2.9E-12	3940612	gltU		BW25113_3757
BZK3	3936828	3936828	1	1		T -> C		79	SNP (transition)		0.316	5.5E-14	3940615	gltU		BW25113_3757
BZK3	3936831	3936831	1	1		C -> A		78	SNP (transversion)		0.333	4.2E-15	3940618	gltU		BW25113_3757
BZK3	3936833	3936836	4	1		CGGC -> GTTA		76 -> 80	Substitution		34.6% -> 38.8%	1.4E-18	3940623	gltU		BW25113_3757
BZK3	3936838	3936842	5	1		GTAAC -> CGCTT		83 -> 84	Substitution		41.0% -> 41.7%	3.6E-30	3940629	gltU		BW25113_3757
BZK3	3936844	3936844	1	1		G -> T		83	SNP (transversion)		0.422	9.4E-34	3940631	gltU		BW25113_3757
BZK3	3936858	3936858	1	1		C -> A		94	SNP (transversion)		0.415	2.4E-52	3940645	gltU		BW25113_3757
BZK3	3936861	3936861	1	1		G -> A		95	SNP (transition)		0.389	3.3E-41	3940648	gltU		BW25113_3757
BZK3	3936863	3936863	1	1		G -> C		95	SNP (transversion)		0.389	7.8E-45	3940650	gltU		BW25113_3757
BZK3	3936865	3936868	4	1		ACGC -> CTAA		95	Substitution		0.368	1.8E-41	3940655	gltU		BW25113_3757
BZK3	3936870	3936873	4	1		ACTT -> TTAA		93 -> 97	Substitution		33.3% -> 36.1%	2.6E-35	3940660	gltU		BW25113_3757
BZK3	3936875	3936886	12	1		CTGGTTTGTGAG -> GGTTGAACCATC		89 -> 93	Substitution		23.9% -> 31.2%	5E-25	3940673	gltU		BW25113_3757
BZK3	3936891	3936891	1	1		A -> G		94	SNP (transition)		0.255	3.2E-22	3940678	gltU		BW25113_3757
BZK1	4161853	4161853	1	1		C -> T		43	SNP (transition)		30.20%	9.30E-14	4164713		murB (U)	BW25113_3972
BZK1	4161893	4161897	5	1		CGTTT -> AAAGA		43 -> 44	Substitution		25.0% -> 25.6%	8.90E-09	4164757		murB (U)	BW25113_3972
BZK2	4161850	4161850	1	1		C -> A		55	SNP (transversion)		0.255	3.2E-12	4164305		murB (U)	BW25113_3972
BZK2	4161853	4161853	1	1		C -> T		55	SNP (transition)		0.255	3.2E-12	4164308		murB (U)	BW25113_3972
BZK3	4173220	4173220	1	1	A -> V	C -> T	GCG -> GTG	121	SNP (transition)	Substitution	1	0	4177220	rpoB		BW25113_3987
BZK1	4176791	4176791	1	1	D -> A	A -> C	GAC -> GCC	32	SNP (transversion)	Substitution	100.00%	1.60E-109	4179657	rpoC		BW25113_3988

BZK1	4199802	4199802	1	1		A -> G		40	SNP (transition)		40.00%	8.90E-16	4202680	No genes in location		
BZK1	4199805	4199806	2	1		TG -> GA		40	Substitution		40.00%	8.90E-16	4202684	No genes in location		
BZK2	4531775	4531775	1	1		T -> C		31	SNP (transition)		0.258	4.9E-21	4534443		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531777	4531779	3	1		TAT -> CTG		31 -> 33	Substitution		29.0% -> 33.3%	5E-24	4534447		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531781	4531784	4	1		GGGC -> TCTA		31 -> 33	Substitution		34.4% -> 36.4%	2.7E-31	4534452		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531786	4531788	3	1		AAT -> GGC		33 -> 34	Substitution		33.3% -> 35.3%	1.5E-26	4534456		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531790	4531795	6	1		TTGACC -> CCAGAT		34 -> 35	Substitution		41.2% -> 42.9%	1.4E-40	4534463		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531798	4531800	3	1		TTG -> CAA		35	Substitution		0.429	1E-43	4534468		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531802	4531803	2	1		GG -> CA		39	Substitution		0.487	3.4E-60	4534471		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531805	4531807	3	1		TTC -> GAT		39 -> 41	Substitution		48.7% -> 51.2%	2.7E-58	4534475		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531810	4531815	6	1		ATAGGT -> CATATC		42 -> 44	Substitution		53.5% -> 56.8%	1.5E-71	4534483		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531817	4531819	3	1		TTC -> GGG		41	Substitution		0.634	6.3E-81	4534487		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531821	4531821	1	1		T -> C		40	SNP (transition)		0.65	3.7E-89	4534489		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531823	4531829	7	1		CAAATAT -> TGTTCGC		40 -> 42	Substitution		70.0% -> 71.4%	1.4E-94	4534497		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531831	4531831	1	1		T -> C		42	SNP (transition)		0.714	1.1E-98	4534499		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK2	4531834	4531836	3	1		CAG -> TCC		55 -> 61	Substitution		78.2% -> 80.3%	1.4E-139	4534504		fimB (D); fimE (U)	BW25113_4312; BW25113_4313
BZK1	4532781	4532781	1	1		A -> C		23	SNP (transversion)		26.10%	6.40E-18	4535896		fimA (U)	BW25113_4314
BZK1	4532826	4532825	0	1		#NAME?		21	Insertion		28.60%	2.20E-19	4535943		fimA (U)	BW25113_4314
BZK1	4532828	4532834	7	1		GGGGAAA -> TTTTTTT		21	Substitution		28.60%	2.20E-19	4535952		fimA (U)	BW25113_4314
BZK2	4558528	4558528	1	1		C -> T		120	SNP (transition)		0.992	0	4561251		mdtM + rpnD (U)	BW25113_4337
BZK2	4570517	4570517	1	1	I -> L	T -> G	ATT -> CTT	41	SNP (transversion)	Substitution	0.707	7.8E-78	4573263	hsdS		BW25113_4348
BZK2	4570519	4570520	2	1	R -> F	CG -> AA	CGC -> TTC	34	Substitution	Substitution	0.324	1.4E-28	4573266	hsdS		BW25113_4348
BZK2	4570519	4570520	2	1		#NAME?		34	Deletion	Frame Shift	0.294	1.2E-15	4573266	hsdS		BW25113_4348
BZK2	4570521	4570523	2	2		TAG -> AA		34 -> 37	Deletion	Frame Shift	27.0% -> 29.4%	1.2E-15	4573269	hsdS		BW25113_4348
BZK2	4570521	4570522	2	1	L -> P	TA -> GG	CTA -> CCC	34	Substitution	Substitution	0.324	5.5E-22	4573268	hsdS		BW25113_4348

BZK2	4570524	4570525	2	1	I -> T	TA -> GG	ATA -> ACC	37	Substitution	Substitution	0.27	3.4E-25	4573271	hsdS		BW25113_4348
BZK2	4570527	4570528	2	1	P -> R	TG -> GC	CCA -> CGC	39	Substitution	Substitution	0.256	6.2E-23	4573274	hsdS		BW25113_4348
BZK2	4570531	4570532	2	1	H -> I	TG -> AT	CAT -> ATT	36 -> 39	Substitution	Substitution	25.6% -> 27.8%	1.9E-25	4573278	hsdS		BW25113_4348
BZK2	4570534	4570535	2	1	G -> L	CC -> AG	GGT -> CTT	39	Substitution	Substitution	0.256	1.9E-25	4573281	hsdS		BW25113_4348
BZK2	4570536	4570538	3	1	V -> P	AAC -> CGG	GTT -> CCG	38 -> 39	Substitution	Substitution	25.6% -> 26.3%	4.6E-23	4573284	hsdS		BW25113_4348
BZK2	4570540	4570541	2	1	G -> S	CC -> GA	GGT -> TCT	37 -> 38	Substitution	Substitution	26.3% -> 27.0%	3.4E-26	4573287	hsdS		BW25113_4348
BZK2	4570543	4570543	1	1	S -> I	C -> A	AGT -> ATT	37	SNP (transversion)	Substitution	0.27	3.4E-26	4573289	hsdS		BW25113_4348
BZK2	4570546	4570549	4	1	NE -> RR	TCAT -> CTTC	AAT,GAA -> AGA,AGA	37	Substitution	Substitution	0.27	3.5E-28	4573295	hsdS		BW25113_4348
BZK2	4570551	4570551	1	1		T -> C	CCA -> CCG	36	SNP (transition)	None	0.278	2.5E-28	4573297	hsdS		BW25113_4348
BZK2	4570556	4570560	5	1	SSK -> SSQ	TTGAT -> GACTC	TCA,TCA,AAG -> TCG,AGT,CAG	36	Substitution	Substitution	0.25	2.5E-25	4573306	hsdS		BW25113_4348
BZK3	4624803	4624803	1	1	W -> G	A -> C	TGG -> GGG	285	SNP (transversion)	Substitution	0.989	0	4629747	rob		BW25113_4396
BZK1	4624803	4624803	1	1	W -> G	A -> C	TGG -> GGG	44	SNP (transversion)	Substitution	100.00%	1.00E-154	4627996	rob		BW25113_4396
BZK1	4625210	4625210	1	1		G -> T		31	SNP (transversion)		100.00%	3.20E-109	4628403		rob (U): creA (U)	BW25113_4396; Unknown
CET2	20564	20564	1	1		T -> C		249	SNP (transition)		42.60%	1.10E-109	20727		insA (U); rpsT (D)	BW25113_0022; BW25113_0023
CET2	20566	20568	3	1		CCT -> GGC		243 -> 249	Substitution		27.7% -> 28.2%	1.50E-57	20731		insA (U); rpsT (D)	BW25113_0022; BW25113_0023
CET2	20572	20572	1	1		T -> G		220	SNP (transversion)		34.50%	7.90E-71	20735		insA (U); rpsT (D)	BW25113_0022; BW25113_0023
CET3	20564	20564	1	1		T -> C		195	SNP (transition)		0.39	3.1E-68	20669		insA (U); rpsT (D)	BW25113_0022; BW25113_0023
CET3	20566	20568	3	1		CCT -> GGC		187 -> 191	Substitution		29.3% -> 29.9%	3.2E-43	20673		insA (U); rpsT (D)	BW25113_0022; BW25113_0023
CET3	20571	20573	3	1		ATG -> TGA		166 -> 169	Substitution		26.0% -> 26.8%	1E-29	20678		insA (U); rpsT (D)	BW25113_0022; BW25113_0023
CET1	62322	62322	1	1	T -> P	T -> G	ACC -> CCC	125	SNP (transversion)	Substitution	100.00%	0	62402	rapA		BW25113_0059
CET1	274851	274853	3	1	LD -> HN	CGA -> TAT	CTC,GAT -> CAT,AAT	130 -> 132	Substitution	Substitution	25.0% -> 25.4%	6.40E-72	275140	afuB + insB1		BW25113_0263
CET1	274857	274857	1	1	S -> A	A -> C	TCG -> GCG	132	SNP (transversion)	Substitution	25.00%	6.40E-72	275144	afuB + insB1		BW25113_0263
CET1	274863	274871	7	3		CGATTTGGC -> GCACGGCTTCT		129 -> 133	Insertion	Frame Shift	25.6% -> 26.4%	2.00E-74	275160	afuB + insB1		BW25113_0263
CET1	274873	274873	1	1		C -> G	CCG -> CCC	127	SNP (transversion)	None	26.80%	1.30E-78	275162	afuB + insB1		BW25113_0263
CET1	274938	274938	1	1	S -> C	T -> A	AGC -> TGC	136	SNP (transversion)	Substitution	25.00%	3.10E-84	275227	insB1		Unknown

CET2	275515	275515	1	1		A -> G	ACT -> ACC	246	SNP (transition)	None	43.50%	1.80E-143	276016	tnpA + insA		Unknown
CET2	275642	275642	1	1	E -> Q	G -> C	GAG -> CAG	275	SNP (transversion)	Substitution	41.80%	1.50E-151	276143	IS30		BW25113_0256
CET2	275645	275646	2	1	N -> A	AA -> GC	AAC -> GCC	275 -> 277	Substitution	Substitution	40.0% -> 40.4%	2.70E-136	276147	insX		BW25113_4505
CET2	275649	275650	2	1	T -> M	CA -> TG	ACA -> ATG	271 -> 273	Substitution	Substitution	37.4% -> 37.6%	4.40E-120	276151	insX		BW25113_4505
CET2	275652	275655	4	1	NG -> MQ	ATGG -> TGCA	AAT,GGG -> ATG,CAG	262 -> 264	Substitution	Substitution	36.6% -> 37.4%	3.70E-133	276156	insX		BW25113_4505
CET2	275657	275660	4	1	LI -> AV	CTAA -> GCGG	CTA,ATT -> GCG,GTT	257 -> 261	Substitution	Substitution	36.0% -> 36.7%	3.60E-120	276161	insX		BW25113_4505
CET2	275663	275679	14	4	RQYFPK -> CYGATG	CGGCAGTACTTTCCTAA -> TGTTACGGGGCAACGGG	CGG,CAG,TAC,TTT,CCT,AAA -> TGT,TAC,GGG,GCA,ACG,GGA	219 -> 253	Substitution	Substitution	21.7% -> 25.1%	1.00E-69	276180	insX		BW25113_4505
CET2	275681	275681	1	1	K -> E	A -> G	AAG -> GAG	218	SNP (transition)	Substitution	27.10%	1.60E-88	276182	insX		BW25113_4505
CET3	275515	275515	1	1		A -> G	ACT -> ACC	185	SNP (transition)	None	0.346	1.2E-78	275926	tnpA + insA		Unknown
CET3	275642	275642	1	1	E -> Q	G -> C	GAG -> CAG	192	SNP (transversion)	Substitution	0.417	8.3E-106	276053	IS30		BW25113_0256
CET3	275645	275646	2	1	N -> A	AA -> GC	AAC -> GCC	196	Substitution	Substitution	0.398	3E-101	276057	insX		BW25113_4505
CET3	275649	275650	2	1	T -> M	CA -> TG	ACA -> ATG	191	Substitution	Substitution	0.366	5.8E-88	276061	insX		BW25113_4505
CET3	275652	275655	4	1	NG -> MQ	ATGG -> TGCA	AAT,GGG -> ATG,CAG	180 -> 184	Substitution	Substitution	35.3% -> 36.1%	9.7E-94	276066	insX		BW25113_4505
CET3	275657	275660	4	1	LI -> AV	CTAA -> GCGG	CTA,ATT -> GCG,GTT	178 -> 183	Substitution	Substitution	35.5% -> 36.0%	1.5E-92	276071	insX		BW25113_4505
CET3	275663	275674	10	3	RQYF -> CYGA	CGGCAGTACTTT -> TGTTACGGGGCA	CGG,CAG,TAC,TTT -> TGT,TAC,GGG,GCA	151 -> 175	Substitution	Substitution	24.5% -> 30.8%	1.7E-50	276086	insX		BW25113_4505
CET3	360294	360295	2	1		AA -> GG		137 -> 140	Substitution		26.3% -> 27.1%	1.9E-68	360813		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
CET3	360297	360297	1	1		T -> A		136	SNP (transversion)		0.257	3.5E-80	360815		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
CET3	360305	360305	1	1		A -> G		128	SNP (transition)		0.258	2.8E-59	360823		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
CET1	360294	360298	5	1		AATTC -> GGCAG		115 -> 121	Substitution		25.2% -> 27.3%	2.50E-56	360713		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
CET1	455017	455017	1	1	Q -> P	A -> C	CAG -> CCG	99	SNP (transversion)	Substitution	99.00%	2.5E-312	455532	lon		BW25113_0439
CET1	557425	557430	6	1	KNI -> TFL	AAAATA -> CCTTCC	AAA,AAT,ATT -> ACC,TTC,CTT	162 -> 171	Substitution	Substitution	24.1% -> 27.5%	1.70E-101	558048	sfmD		BW25113_0532
CET1	962696	962696	1	1	A -> V	C -> T	GCA -> GTA	129	SNP (transition)	Substitution	100.00%	0	963773	msbA		BW25113_0914
CET1	1045196	1045196	1	1	K -> I	T -> A	AAA -> ATA	155	SNP (transversion)	Substitution	25.80%	1.60E-71	1046342	gfcA;		BW25113_0987
CET1			1	1		A -> G		154	SNP (transition)		26.60%	3.70E-86	1046347		IS1 family transposase (	Unknown
CET1	1045208	1045208	1	1		C -> G		161	SNP (transversion)		26.10%	2.30E-100	1046354		gfcA (U): insA (U)	BW25113_0987; BW25113_0022

CET1	1045216	1045216	1	1		C -> A		165	SNP (transversion)		28.50%	8.10E-105	1046363		gfcA (U): insA (U)	BW25113_0987; BW25113_0022
CET1	1045221	1045221	1	1		C -> T		163	SNP (transition)		31.30%	2.50E-131	1046368		gfcA (U): insA (U)	BW25113_0987; BW25113_0022
CET1	1045226	1045226	1	1		A -> C		168	SNP (transversion)		29.20%	8.50E-110	1046373		gfcA (U): insA (U)	BW25113_0987; BW25113_0022
CET1	1045228	1045233	6	1		AATACG -> CCGCAA		168 -> 174	Substitution		29.9% -> 31.0%	3.50E-112	1046380		gfcA (U): insA (U)	BW25113_0987; BW25113_0022
CET1	1045283	1045283	1	1	F -> C	T -> G	TTC -> TGC	162	SNP (transversion)	Substitution	31.50%	1.40E-136	1046430		gfcA (U): insA (U)	BW25113_0987; BW25113_0022
CET1	1045360	1045360	1	1		T -> C	ACT -> ACC	168	SNP (transition)	None	31.00%	2.20E-128	1046507	tnpA + insA		Unknown
CET1	1111752	1111752	1	1	A -> E	G -> T	GCG -> GAG	100	SNP (transversion)	Substitution	34.00%	3.50E-86	1112972	lpxL		BW25113_1054
CET1	1111753	1111753	1	1	A -> P	C -> G	GCG -> CCG	101	SNP (transversion)	Substitution	65.30%	2.60E-191	1112973	lpxL		BW25113_1054
CET1	1203240	1203240	1	1	E -> V	A -> T	GAA -> GTA	84	SNP (transversion)	Substitution	26.20%	1.50E-55	1204544	stfP		BW25113_1154
CET1	1203245	1203245	1	1	L -> V	C -> G	CTT -> GTT	81	SNP (transversion)	Substitution	27.20%	5.80E-56	1204549	stfP		BW25113_1154
CET1	1203262	1203267	5	2	GAK -> GSA	AGCAAA -> CTCTGC	GGA,GCA,AAA -> GGC,TCT,GCA	78 -> 84	Substitution	Substitution	23.8% -> 25.6%	1.90E-50	1204571	stfP		BW25113_1154
CET1	1203269	1203269	1	1	L -> F	C -> T	CTC -> TTC	84	SNP (transition)	Substitution	25.00%	1.00E-54	1204573	stfP		BW25113_1154
CET2	1203262	1203267	5	2	GAK -> GSA	AGCAAA -> CTCTGC	GGA,GCA,AAA -> GGC,TCT,GCA	101 -> 104	Substitution	Substitution	29.8% -> 30.7%	6.10E-83	1204920	stfP		BW25113_1154
CET2	1203269	1203273	4	2	LN -> LP	CTCAA -> TTACC	CTC,AAT -> TTA,CCT	101 -> 104	Substitution	Substitution	27.7% -> 29.8%	1.10E-76	1204926	stfP		BW25113_1154
CET2	1203276	1203276	1	1	A -> V	C -> T	GCA -> GTA	98	SNP (transition)	Substitution	25.50%	4.20E-65	1204929	stfP		BW25113_1154
CET3	1203262	1203267	5	2	GAK -> GSA	AGCAAA -> CTCTGC	GGA,GCA,AAA -> GGC,TCT,GCA	77 -> 79	Substitution	Substitution	34.6% -> 36.4%	1.2E-77	1204667	stfP		BW25113_1154
CET3	1203269	1203273	4	2	LN -> LP	CTCAA -> TTACC	CTC,AAT -> TTA,CCT	75 -> 78	Substitution	Substitution	30.7% -> 33.3%	3.7E-62	1204673	stfP		BW25113_1154
CET1	1460458	1460458	1	1		T -> C	GGT -> GGC	152	SNP (transition)	None	27.00%	5.00E-66	1462017	ydbA		BW25113_1401
CET1	1460467	1460467	1	1		G -> A	GCG -> GCA	137	SNP (transition)	None	29.90%	2.10E-80	1462026	ydbA		BW25113_1401
CET1	1613537	1613541	5	1	CI -> YW	GTATT -> ACTGG	TGT,ATT -> TAC,TGG	46 -> 48	Substitution	Substitution	87.2% -> 89.6%	3.80E-133	1615266	marR		BW25113_1530
CET1	1613542	1613547	6	1		ACTCCG -> TAAAAC		44 -> 47	Substitution	Truncation	84.1% -> 89.4%	1.50E-111	1615272	marR		BW25113_1530
CET1	1613548	1613552	4	2	VE -> LP	GTTGA -> TTACC	GTT,GAA -> TTA,CCA	47	Substitution	Substitution	74.50%	1.60E-105	1615277	marR		BW25113_1530
CET1	1613554	1613556	3	1	L -> S	CTG -> TCC	CTG -> TCC	47	Substitution	Substitution	27.70%	2.20E-36	1615281	marR		BW25113_1530
CET1	1613555	1613555	1	1	L -> P	T -> C	CTG -> CCG	47	SNP (transition)	Substitution	72.30%	1.00E-119	1615280	marR		BW25113_1530
CET1	1613557	1613559	3	1	K -> A	AAA -> GCG	AAA -> GCG	47	Substitution	Substitution	70.20%	1.70E-91	1615284	marR		BW25113_1530

CET1	1613558	1613558	1	1	K -> I	A -> T	AAA -> ATA	47	SNP (transversion)	Substitution	29.80%	3.40E-38	1615283	marR		BW25113_1530
CET1	1613560	1613561	2	1	K -> A	AA -> GC	AAG -> GCG	50	Substitution	Substitution	66.00%	4.90E-90	1615286	marR		BW25113_1530
CET1	1613560	1613561	2	1	K -> W	AA -> TG	AAG -> TGG	50	Substitution	Substitution	34.00%	3.10E-47	1615286	marR		BW25113_1530
CET1	1613563	1613563	1	1	V -> L	G -> T	GTA -> TTA	50	SNP (transversion)	Substitution	34.00%	3.10E-47	1615288	marR		BW25113_1530
CET1	1613563	1613565	3	1	V -> R	GTA -> CGG	GTA -> CGG	48 -> 50	Substitution	Substitution	62.0% -> 63.3%	4.20E-81	1615290	marR		BW25113_1530
CET1	1613566	1613568	2	2	L -> I	TTG -> ATC	TTG -> ATC	47	Substitution	Substitution	36.20%	1.10E-46	1615293	marR		BW25113_1530
CET1	1613566	1613568	3	1	L -> Q	TTG -> CAA	TTG -> CAA	47	Substitution	Substitution	61.70%	1.10E-86	1615293	marR		BW25113_1530
CET1	1613569	1613571	3	1	S -> R	TCG -> AGA	TCG -> AGA	48 -> 50	Substitution	Substitution	37.5% -> 40.8%	1.80E-54	1615296	marR		BW25113_1530
CET1	1613570	1613571	2	1	S -> Y	CG -> AT	TCG -> TAT	49 -> 50	Substitution	Substitution	59.2% -> 60.0%	8.90E-89	1615296	marR		BW25113_1530
CET1	1613572	1613574	3	1	V -> R	GTC -> AGA	GTC -> AGA	47 -> 48	Substitution	Substitution	41.7% -> 42.6%	1.70E-59	1615299	marR		BW25113_1530
CET1	1613574	1613574	1	1		C -> G	GTC -> GTG	47	SNP (transversion)	None	57.40%	1.50E-79	1615299	marR		BW25113_1530
CET1	1613575	1613575	1	1	D -> N	G -> A	GAC -> AAC	49	SNP (transition)	Substitution	55.10%	3.20E-95	1615300	marR		BW25113_1530
CET1	1613575	1613577	2	2	D -> K	GAC -> AAG	GAC -> AAG	47 -> 49	Substitution	Substitution	44.9% -> 46.8%	5.90E-69	1615302	marR		BW25113_1530
CET1	1613578	1613580	2	2	L -> I	CTG -> ATC	CTG -> ATC	44 -> 45	Substitution	Substitution	48.9% -> 50.0%	1.30E-67	1615305	marR		BW25113_1530
CET1	1613578	1613580	3	1	L -> N	CTG -> AAT	CTG -> AAT	44 -> 45	Substitution	Substitution	50.0% -> 51.1%	3.30E-63	1615305	marR		BW25113_1530
CET1	1613582	1613583	2	1	G -> A	GA -> CC	GGA -> GCC	43 -> 44	Substitution	Substitution	100.00%	1.30E-142	1615308	marR		BW25113_1530
CET1	1613584	1613586	3	1	A -> I	GCA -> ATC	GCA -> ATC	44	Substitution	Substitution	40.9% -> 45.5%	5.60E-57	1615311	marR		BW25113_1530
CET1	1613584	1613586	3	1	A -> C	GCA -> TGC	GCA -> TGC	44	Substitution	Substitution	54.5% -> 59.1%	2.80E-77	1615311	marR		BW25113_1530
CET1	1613587	1613589	2	2		CTG -> TTA	CTG -> TTA	45 -> 46	Substitution	None	75.6% -> 76.1%	2.50E-106	1615316	marR		BW25113_1530
CET1	1613592	1613592	1	1		C -> G	ACC -> ACG	45	SNP (transversion)	None	84.40%	7.20E-130	1615319	marR		BW25113_1530
CET1	1613593	1613593	1	1	R -> S	C -> A	CGT -> AGT	44	SNP (transversion)	Substitution	68.20%	1.10E-97	1615320	marR		BW25113_1530
CET1	1613598	1613598	1	1	M -> I	G -> C	ATG -> ATC	44	SNP (transversion)	Substitution	84.10%	2.40E-126	1615325	marR		BW25113_1530
CET1	1613599	1613601	3	1	L -> C	CTG -> TGT	CTG -> TGT	43 -> 45	Substitution	Substitution	68.9% -> 72.1%	1.70E-96	1615328	marR		BW25113_1530
CET1	1613602	1613604	3	1	D -> L	GAT -> CTC	GAT -> CTC	44	Substitution	Substitution	75.00%	4.50E-109	1615331	marR		BW25113_1530
CET1	1613608	1613609	2	1	L -> W	CT -> TG	CTG -> TGG	48 -> 54	Substitution	Substitution	79.2% -> 81.5%	1.00E-127	1615336	marR		BW25113_1530

CET1	1613611	1613613	2	2	V -> I	GTC -> ATA	GTC -> ATA	52 -> 53	Substitution	Substitution	82.7% -> 83.0%	7.50E-146	1615340	marR		BW25113_1530
CET1	1613615	1613616	2	1	C -> L	GT -> TA	TGT -> TTA	53	Substitution	Substitution	98.10%	8.40E-176	1615343	marR		BW25113_1530
CET1	1613617	1613630	13	2	KGWVE -> PRHSL	AAAGGCTGGGTGGA -> CCGCGGCACAGTTT	AAA,GGC,TGG,GTG,GAA -> CCG,CGG,CAC,AGT,TTA	54 -> 63	Substitution	Substitution	73.0% -> 83.6%	1.90E-141	1615357	marR		BW25113_1530
CET1	1613636	1613643	7	2	LPN -> CSA	TGCCGAAC -> GCTCTGCT	TTG,CCG,AAC -> TGC,TCT,GCT	64 -> 71	Substitution	Substitution	95.3% -> 97.2%	7.80E-205	1615370	marR		BW25113_1530
CET1	1613645	1613647	3	1		CGA -> T		73	Deletion	Frame Shift	98.60%	7.90E-198	1615374	marR		BW25113_1530
CET1	1613650	1613654	5	1	DK -> PL	GACAA -> CCGCT	GAC,AAG -> CCG,CTG	76 -> 78	Substitution	Substitution	100.00%	4.00E-259	1615381	marR		BW25113_1530
CET2	1800527	1800527	1	1		T -> A		109	SNP (transversion)		25.70%	1.30E-75	1802954		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800529	1800530	2	1		TG -> CT		106 -> 107	Substitution		30.8% -> 31.1%	2.00E-75	1802957		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800536	1800540	5	1		TTTTA -> ACACC		115 -> 124	Substitution		35.3% -> 39.5%	1.10E-107	1802967		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800542	1800543	2	1		AT -> TC		141 -> 142	Substitution		47.5% -> 47.9%	4.90E-194	1802970		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800545	1800545	1	1		A -> T		147	SNP (transversion)		49.70%	3.60E-213	1802972		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800547	1800551	5	1		GCTCC -> CACTA		161 -> 170	Substitution		51.5% -> 52.7%	5.00E-250	1802978		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800555	1800555	1	1		A -> C		183	SNP (transversion)		55.20%	6.7E-311	1802982		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800557	1800557	1	1		A -> G		189	SNP (transition)		55.00%	0	1802984		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800559	1800559	1	1		C -> A		191	SNP (transversion)		56.00%	1.4E-319	1802986		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800561	1800562	2	1		TA -> GT		195	Substitution		56.90%	0	1802989		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800564	1800564	1	1		T -> G		194	SNP (transversion)		57.70%	0	1802991		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800566	1800570	5	1		TTAAT -> CAGCA		196 -> 205	Substitution		56.3% -> 57.4%	0	1802997		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET2	1800572	1800575	4	1		TCTT -> CACC		205 -> 208	Substitution		57.6% -> 58.7%	0	1803002		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800529	1800530	2	1		TG -> CT		85 -> 86	Substitution		25.9% -> 26.7%	7.6E-49	1802539		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800536	1800540	5	1		TTTTA -> ACACC		106 -> 113	Substitution		34.0% -> 38.1%	3.7E-94	1802549		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800542	1800543	2	1		AT -> TC		123 -> 124	Substitution		42.3% -> 42.7%	4.3E-137	1802552		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800545	1800545	1	1		A -> T		131	SNP (transversion)		0.443	1.9E-148	1802554		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800547	1800551	5	1		GCTCC -> CACTA		145 -> 153	Substitution		47.7% -> 48.6%	1.7E-191	1802560		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800555	1800555	1	1		A -> C		162	SNP (transversion)		0.512	2.1E-235	1802564		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800557	1800557	1	1		A -> G		165	SNP (transition)		0.515	2.6E-241	1802566		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800559	1800559	1	1		C -> A		164	SNP (transversion)		0.518	4E-250	1802568		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800561	1800562	2	1		TA -> GT		164	Substitution		0.518	4E-233	1802571		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800564	1800564	1	1		T -> G		163	SNP (transversion)		0.521	6E-225	1802573		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800566	1800570	5	1		TTAAT -> CAGCA		165 -> 176	Substitution		52.1% -> 54.5%	2.4E-253	1802579		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET3	1800572	1800575	4	1		TCTT -> CACC		177 -> 180	Substitution		54.8% -> 55.6%	3.9E-269	1802584		ydiJ (U): pfkB (U)	BW25113_1687; BW25113_1723
CET1	1848353	1848360	7	2		TTACCACA -> AACACTAT		155 -> 178	Substitution	Extension	23.9% -> 30.3%	1.10E-82	1850295	ydjF		BW25113_1770
CET1	1848362	1848366	4	2	QL -> LI	CAGCT -> AATAA	CAG,CTG -> CTT,ATT	179 -> 181	Substitution	Substitution	32.0% -> 32.4%	5.50E-140	1850301	ydjF		BW25113_1770
CET1	1848368	1848381	13	2		GATATTATTGTCTT -> TTGCAGTCATTACC		190 -> 195	Substitution	Truncation	33.3% -> 38.5%	4.20E-163	1850316	ydjF		BW25113_1770
CET2	1876968	1876969	2	1	A -> G	CC -> GT	GCC -> GGT	305	Substitution	Substitution	58.70%	0	1879840	dmlA		BW25113_1800
CET2	1876972	1876973	2	1		#NAME?		289	Deletion	Frame Shift	49.10%	5.10E-287	1879844	dmlA		BW25113_1800
CET2	1876977	1876977	1	1	N -> T	A -> C	AAT -> ACT	271	SNP (transversion)	Substitution	52.00%	0	1879848	dmlA		BW25113_1800
CET2	1876981	1876990	10	1		GGAACGCACT -> AACTTATTGA		240 -> 269	Substitution	Truncation	43.8% -> 50.6%	1.50E-290	1879861	dmlA		BW25113_1800
CET2	1876992	1876996	5	1		TCCCG -> AGTGT		214 -> 221	Substitution	Truncation	39.7% -> 41.2%	1.40E-211	1879867	dmlA		BW25113_1800
CET2	1876998	1877002	4	2	SL -> FM	CGCTC -> TTATG	TCG,CTC -> TTT,ATG	201 -> 207	Substitution	Substitution	31.8% -> 38.2%	4.60E-159	1879874	dmlA		BW25113_1800
CET3	1876968	1876969	2	1	A -> G	CC -> GT	GCC -> GGT	261 -> 262	Substitution	Substitution	52.1% -> 52.7%	0	1879304	dmlA		BW25113_1800
CET3	1876972	1876973	2	1		#NAME?		244 -> 247	Deletion	Frame Shift	46.2% -> 46.7%	1.6E-224	1879308	dmlA		BW25113_1800
CET3	1876977	1876977	1	1	N -> T	A -> C	AAT -> ACT	241	SNP (transversion)	Substitution	0.481	0	1879312	dmlA		BW25113_1800
CET3	1876981	1876990	10	1		GGAACGCACT -> AACTTATTGA		211 -> 236	Substitution	Truncation	43.6% -> 46.2%	1.2E-258	1879325	dmlA		BW25113_1800
CET3	1876992	1876996	5	1		TCCCG -> AGTGT		192 -> 198	Substitution	Truncation	38.3% -> 39.9%	3.3E-189	1879331	dmlA		BW25113_1800
CET3	1876998	1877002	4	2	SL -> FM	CGCTC -> TTATG	TCG,CTC -> TTT,ATG	173 -> 189	Substitution	Substitution	28.0% -> 30.6%	8.4E-132	1879337	dmlA		BW25113_1800
CET3	1877006	1877011	6	1		GAGCCT -> AGATAA		151 -> 162	Substitution	Truncation	25.8% -> 29.0%	7.1E-80	1879346	dmlA		BW25113_1800
CET2	1877006	1877011	6	1		GAGCCT -> AGATAA		176 -> 187	Substitution	Truncation	25.0% -> 27.8%	9.70E-100	1879883	dmlA		BW25113_1800
CET2	1933819	1933819	1	1	D -> A	T -> G	GAT -> GCT	155	SNP (transversion)	Substitution	99.40%	0	1936778	lpxM		BW25113_1855
CET3	1933819	1933819	1	1	D -> A	T -> G	GAT -> GCT	138	SNP (transversion)	Substitution	1	0	1936197	lpxM		BW25113_1855
CET1	2017004	2017011	8	1	LSI -> GDA	TTATCCAT -> GGTGATGC	TTA,TCC,ATT -> GGT,GAT,GCT	194 -> 209	Substitution	Substitution	26.8% -> 31.1%	5.00E-138	2019460	fliR		BW25113_1950
CET1	2017013	2017015	3	1	F -> A	TTT -> GCC	TTT -> GCC	188 -> 190	Substitution	Substitution	25.3% -> 25.5%	1.50E-123	2019464	fliR		BW25113_1950
CET1	2017016	2017017	2	1	V -> N	GT -> AA	GTT -> AAT	187 -> 188	Substitution	Substitution	25.0% -> 25.1%	3.10E-111	2019466	fliR		BW25113_1950
CET1	2017019	2017019	1	1	I -> F	A -> T	ATT -> TTT	180	SNP (transversion)	Substitution	27.20%	9.30E-123	2019468	fliR		BW25113_1950
CET2	2095224	2095224	1	1	K -> E	T -> C	AAA -> GAA	186	SNP (transition)	Substitution	31.70%	3.10E-47	2098373	wbbL +insH1		BW25113_2031
CET2	2095226	2095226	1	1	N -> S	T -> C	AAT -> AGT	207	SNP (transition)	Substitution	35.70%	2.70E-70	2098375	wbbL +insH1		BW25113_2031
CET3	2095224	2095224	1	1	K -> E	T -> C	AAA -> GAA	198	SNP (transition)	Substitution	0.278	7.6E-46	2097758	wbbL +insH1		BW25113_2031
CET3	2095226	2095226	1	1	N -> S	T -> C	AAT -> AGT	214	SNP (transition)	Substitution	0.304	6.6E-63	2097760	wbbL +insH1		BW25113_2031
CET2	2283593	2283593	1	1		A -> C		216	SNP (transversion)		32.40%	3.50E-104	2286991		insH-1 (U)	BW25113_0259
CET2	2283597	2283598	2	1		CG -> TA		220 -> 222	Substitution		31.5% -> 31.8%	3.90E-110	2286996		insH-1 (U)	BW25113_0259
CET2	2283600	2283604	5	1		TGTAG -> GCATC		216	Substitution		32.40%	3.50E-104	2287002		insH-1 (U)	BW25113_0259
CET2	2283608	2283608	1	1		C -> TG		218	Insertion		31.70%	6.90E-102	2287008		insH-1 (U)	BW25113_0259
CET2	2283610	2283610	1	1		A -> C		221	SNP (transversion)		31.70%	2.30E-103	2287010		insH-1 (U)	BW25113_0259
CET2	2283613	2283615	3	1		ACA -> TAT		222 -> 223	Substitution		31.4% -> 31.5%	4.00E-96	2287015		insH-1 (U)	BW25113_0259
CET2	2283617	2283618	2	1		AT -> CC		220 -> 222	Substitution		31.5% -> 31.8%	3.40E-103	2287018		insH-1 (U)	BW25113_0259
CET2	2283620	2283626	7	1		TTGCGGA -> ACAGAAT		214 -> 218	Substitution		30.7% -> 31.3%	4.20E-97	2287026		insH-1 (U)	BW25113_0259
CET2	2283628	2283628	1	1		C -> A		214	SNP (transversion)		33.20%	1.70E-99	2287028		insH-1 (U)	BW25113_0259
CET3	2283593	2283593	1	1		A -> C		216	SNP (transversion)		0.361	4.7E-128	2286339		insH-1 (U)	BW25113_0259
CET3	2283597	2283598	2	1		CG -> TA		217 -> 218	Substitution		33.9% -> 34.1%	5.3E-112	2286344		insH-1 (U)	BW25113_0259
CET3	2283600	2283604	5	1		TGTAG -> GCATC		214 -> 216	Substitution		34.3% -> 34.6%	3.5E-112	2286350		insH-1 (U)	BW25113_0259
CET3	2283608	2283608	1	1		C -> TG		215 -> 216	Insertion		34.7% -> 34.9%	2.2E-114	2286355		insH-1 (U)	BW25113_0259
CET3	2283610	2283610	1	1		A -> C		214	SNP (transversion)		0.35	1.4E-114	2286357		insH-1 (U)	BW25113_0259
CET3	2283613	2283615	3	1		ACA -> TAT		212 -> 215	Substitution		34.9% -> 35.4%	2.2E-114	2286362		insH-1 (U)	BW25113_0259
CET3	2283617	2283618	2	1		AT -> CC		212 -> 213	Substitution		35.2% -> 35.4%	9.4E-115	2286365		insH-1 (U)	BW25113_0259
CET3	2283620	2283626	7	1		TTGCGGA -> ACAGAAT		212 -> 215	Substitution		34.7% -> 35.4%	6.1E-115	2286373		insH-1 (U)	BW25113_0259

CET3	2283628	2283628	1	1		C -> A		213	SNP (transversion)		0.347	4.6E-120	2286375		insH-1 (U)	BW25113_0259
CET2	2330933	2330933	1	1	K -> R	T -> C	AAG -> AGG	137	SNP (transition)	Substitution	100.00%	0	2334395	gyrA		BW25113_2231
CET3	2330933	2330933	1	1	K -> R	T -> C	AAG -> AGG	126	SNP (transition)	Substitution	1	0	2333737	gyrA		BW25113_2231
CET1	2458523	2458523	1	1		G -> A		99	SNP (transition)	Truncation	99.00%	0	2461418		mlaA (U): yfdC (U)	BW25113_2346; BW25113_2347
CET1	2729684	2729684	1	1	N -> K	T -> G	AAT -> AAG	127	SNP (transversion)	Substitution	100.00%	0	2732870	bamD		BW25113_2595
CET2	3123458	3123460	2	2	L -> I	AAG -> GAT	CTT -> ATC	186 -> 188	Substitution	Substitution	26.9% -> 27.1%	9.00E-57	3127961	yghO		BW25113_2981
CET2	3123462	3123463	2	1	S -> I	GA -> AT	TCA -> ATA	182 -> 185	Substitution	Substitution	28.0% -> 28.1%	1.40E-57	3127964	yghO		BW25113_2981
CET2	3123466	3123466	1	1	S -> T	A -> T	TCA -> ACA	186	SNP (transversion)	Substitution	28.50%	3.20E-60	3127967	yghO		BW25113_2981
CET2	3123468	3123469	2	1	P -> K	GG -> TT	CCG -> AAG	187 -> 188	Substitution	Substitution	28.9% -> 29.3%	1.10E-61	3127970	yghO		BW25113_2981
CET2	3123471	3123480	10	1	FIAF -> CQQN	AAAGCGATAA -> TTCTGTTGGC	TTT,ATC,GCT,TTT -> TGC,CAA,CAG,AA	191 -> 226	Substitution	Substitution	27.1% -> 35.4%	1.90E-61	3127981	yghO		BW25113_2981
CET2	3123483	3123484	2	1	A -> I	GC -> AT	GCA -> ATA	232 -> 235	Substitution	Substitution	39.7% -> 40.4%	6.80E-101	3127985	yghO		BW25113_2981
CET2	3123486	3123490	4	2	LK -> MP	TTAAG -> GGCAT	CTT,AAG -> ATG,CCG	246 -> 257	Substitution	Substitution	42.8% -> 44.2%	4.40E-105	3127991	yghO		BW25113_2981
CET2	3123492	3123493	2	1	D -> P	TC -> GG	GAC -> CCC	265 -> 266	Substitution	Substitution	44.7% -> 45.1%	3.90E-103	3127996	yghO		BW25113_2981
CET2	3123496	3123498	3	1	KN -> TH	TTT -> GCG	AAA,AAT -> ACG,CAT	268 -> 289	Substitution	Substitution	45.5% -> 48.4%	1.10E-109	3128001	yghO		BW25113_2981
CET3	3123468	3123469	2	1	P -> K	GG -> TT	CCG -> AAG	188	Substitution	Substitution	0.25	1E-32	3126992	yghO		BW25113_2981
CET3	3123471	3123480	10	1	FIAF -> CQQN	AAAGCGATAA -> TTCTGTTGGC	TTT,ATC,GCT,TTT -> TGC,CAA,CAG,AA	188 -> 201	Substitution	Substitution	24.3% -> 29.4%	2.6E-29	3127003	yghO		BW25113_2981
CET3	3123483	3123484	2	1	A -> I	GC -> AT	GCA -> ATA	206 -> 214	Substitution	Substitution	30.6% -> 32.2%	1.5E-37	3127007	yghO		BW25113_2981
CET3	3123486	3123490	4	2	LK -> MP	TTAAG -> GGCAT	CTT,AAG -> ATG,CCG	219 -> 220	Substitution	Substitution	34.5% -> 34.7%	1.8E-50	3127013	yghO		BW25113_2981
CET3	3123492	3123493	2	1	D -> P	TC -> GG	GAC -> CCC	219 -> 220	Substitution	Substitution	36.8% -> 37.0%	6.2E-55	3127016	yghO		BW25113_2981
CET3	3123496	3123498	3	1	KN -> TH	TTT -> GCG	AAA,AAT -> ACG,CAT	225 -> 246	Substitution	Substitution	37.8% -> 42.3%	5.1E-58	3127021	yghO		BW25113_2981
CET3	3123504	3123504	1	1	L -> P	A -> G	CTT -> CCT	260	SNP (transition)	Substitution	0.458	6.4E-71	3127027	yghO		BW25113_2981
CET2	3123504	3123504	1	1	L -> P	A -> G	CTT -> CCT	308	SNP (transition)	Substitution	51.60%	1.20E-134	3128007	yghO		BW25113_2981
CET1	3267852	3267852	1	1	A -> T	C -> T	GCC -> ACC	137	SNP (transition)	Substitution	27.00%	5.90E-93	3271638	garP		BW25113_3127
CET1	3267861	3267864	3	2	RI -> CF	TTCG -> AACA	CGA,ATT -> TGT,TTT	151 -> 153	Substitution	Substitution	31.1% -> 32.0%	9.90E-126	3271650	garP		BW25113_3127
CET1	3267868	3267869	2	1	N -> I	GT -> TA	AAC -> ATA	153 -> 163	Substitution	Substitution	32.7% -> 36.8%	5.10E-133	3271655	garP		BW25113_3127

CET1	3267872	3267876	5	1	PA -> LL	GCCGG -> AATAA	CCG,GCG -> TTA,TTG	172 -> 178	Substitution	Substitution	40.7% -> 44.4%	1.00E-192	3271662	garP		BW25113_3127
CET1	3267878	3267880	3	1	SF -> SN	AAT -> TTG	TCA,TTC -> TCC,AAC	180	Substitution	Substitution	46.10%	1.30E-213	3271666	garP		BW25113_3127
CET1	3267883	3267890	8	1	EAP -> GND	TGGCGCTT -> GTCATTAC	GAA,GCG,CCA -> GGT,AAT,GAC	187 -> 190	Substitution	Substitution	47.3% -> 49.5%	2.70E-258	3271676	garP		BW25113_3127
CET1	3327886	3327887	2	1	E -> G	AA -> GT	GAA -> GGT	192 -> 193	Substitution	Substitution	45.6% -> 45.8%	7.00E-235	3331966	ispB		BW25113_3187
CET1	3327889	3327891	3	1		CAA -> ATGC		181 -> 182	Insertion	Frame Shift	27.5% -> 27.6%	1.40E-140	3331971	ispB		BW25113_3187
CET1	3327894	3327896	3	1	N -> P	AAC -> CCA	AAC -> CCA	181 -> 183	Substitution	Substitution	27.3% -> 27.6%	2.50E-120	3331976	ispB		BW25113_3187
CET1	3327897	3327897	1	1	A -> T	G -> A	GCT -> ACT	185	SNP (transition)	Substitution	41.60%	3.30E-209	3331977	ispB		BW25113_3187
CET1	3327901	3327903	3	1		GTG -> ACT		188 -> 189	Substitution	Truncation	27.0% -> 27.1%	1.40E-132	3331984	ispB		BW25113_3187
CET1	3327907	3327907	1	1	S -> F	C -> T	TCT -> TTT	182	SNP (transition)	Substitution	37.40%	6.30E-181	3331988	ispB		BW25113_3187
CET1	3327909	3327910	2	1	L -> S	CT -> AG	CTT -> AGT	169 -> 172	Substitution	Substitution	29.7% -> 30.2%	5.20E-135	3331991	ispB		BW25113_3187
CET1	3327913	3327913	1	1	E -> V	A -> T	GAA -> GTA	168	SNP (transversion)	Substitution	31.00%	5.70E-144	3331994	ispB		BW25113_3187
CET1	3327917	3327918	2	1		GA -> AT		156 -> 162	Substitution	Truncation	28.8% -> 30.2%	3.30E-105	3331999	ispB		BW25113_3187
CET2	3358856	3358861	6	1	LA -> YL	CTGGCG -> TATTTA	CTG,GCG -> TAT,TTA	199 -> 221	Substitution	Substitution	30.2% -> 35.3%	4.90E-161	3363652	yhcE + insH1		BW25113_3217
CET2	3358864	3358866	3	1	LA -> FY	AGC -> CTA	TTA,GCC -> TTC,TAC	221 -> 232	Substitution	Substitution	37.1% -> 39.7%	5.60E-229	3363657	yhcE + insH1		BW25113_3217
CET2	3358868	3358869	2	1	S -> L	AG -> CT	AGT -> CTT	229	Substitution	Substitution	42.40%	2.30E-254	3363660	yhcE + insH1		BW25113_3217
CET2	3358871	3358874	4	1		GGCA -> CATT		256 -> 259	Substitution	Truncation	48.4% -> 49.2%	0	3363665	yhcE + insH1		BW25113_3217
CET2	3358877	3358878	2	1	N -> G	AA -> GG	AAT -> GGT	277 -> 286	Substitution	Substitution	52.0% -> 53.5%	0	3363669	yhcE + insH1		BW25113_3217
CET2	3358880	3358884	4	2	DN -> QG	GACAA -> CAAGG	GAC,AAT -> CAA,GGT	293 -> 325	Substitution	Substitution	48.3% -> 53.6%	0	3363675	yhcE + insH1		BW25113_3217
CET2	3358888	3358888	1	1		G -> T	CCG -> CCT	338	SNP (transversion)	None	60.10%	0	3363680	yhcE + insH1		BW25113_3217
CET2	3358890	3358891	2	1	G -> D	GC -> AT	GGC -> GAT	348 -> 349	Substitution	Substitution	61.3% -> 61.5%	0	3363683	yhcE + insH1		BW25113_3217
CET2	3358893	3358895	3	1	ST -> NA	GCA -> ATG	AGC,ACA -> AAT,GCA	366 -> 372	Substitution	Substitution	60.9% -> 61.3%	0	3363687	yhcE + insH1		BW25113_3217
CET2	3358899	3358902	4	1	GT -> DQ	GAAC -> ATCA	GGA,ACA -> GAT,CAA	398 -> 399	Substitution	Substitution	64.7% -> 64.8%	0	3363694	yhcE + insH1		BW25113_3217
CET2	3358907	3358909	2	2		TCA -> GTTC		390 -> 391	Insertion	Frame Shift	66.0% -> 66.2%	0	3363702	yhcE + insH1		BW25113_3217
CET2	3358911	3358911	1	1	I -> T	T -> C	ATT -> ACT	391	SNP (transition)	Substitution	66.20%	0	3363705	yhcE + insH1		BW25113_3217
CET2	3358914	3358914	1	1	R -> K	G -> A	AGG -> AAG	387	SNP (transition)	Substitution	66.90%	0	3363708	yhcE + insH1		BW25113_3217

CET3	3358866	3358866	1	1	A -> D	C -> A	GCC -> GAC	224	SNP (transversion)	Substitution	0.259	2.5E-149	3362637	yhcE + insH1		BW25113_3217
CET3	3358868	3358869	2	1	S -> L	AG -> CT	AGT -> CTT	223	Substitution	Substitution	0.283	1.5E-151	3362640	yhcE + insH1		BW25113_3217
CET3	3358871	3358874	4	1		GGCA -> CATT		236 -> 242	Substitution	Truncation	33.1% -> 34.4%	3.6E-202	3362645	yhcE + insH1		BW25113_3217
CET3	3358877	3358878	2	1	N -> G	AA -> GG	AAT -> GGT	252 -> 255	Substitution	Substitution	36.9% -> 37.6%	2.4E-249	3362649	yhcE + insH1		BW25113_3217
CET3	3358880	3358884	4	2	DN -> QG	GACAA -> CAAGG	GAC,AAT -> CAA,GGT	253 -> 273	Substitution	Substitution	39.1% -> 43.6%	4.2E-265	3362655	yhcE + insH1		BW25113_3217
CET3	3358888	3358888	1	1		G -> T	CCG -> CCT	287	SNP (transversion)	None	0.456	0	3362659	yhcE + insH1		BW25113_3217
CET3	3358890	3358891	2	1	G -> D	GC -> AT	GGC -> GAT	296 -> 297	Substitution	Substitution	47.1% -> 47.6%	0	3362662	yhcE + insH1		BW25113_3217
CET3	3358893	3358895	3	1	ST -> NA	GCA -> ATG	AGC,ACA -> AAT,GCA	315 -> 318	Substitution	Substitution	47.5% -> 48.1%	0	3362666	yhcE + insH1		BW25113_3217
CET3	3358899	3358902	4	1	GT -> DQ	GAAC -> ATCA	GGA,ACA -> GAT,CAA	344 -> 345	Substitution	Substitution	49.9% -> 50.0%	0	3362673	yhcE + insH1		BW25113_3217
CET3	3358907	3358909	2	2		TCA -> GTTC		338 -> 345	Insertion	Frame Shift	49.3% -> 50.3%	0	3362682	yhcE + insH1		BW25113_3217
CET3	3358911	3358911	1	1	I -> T	T -> C	ATT -> ACT	344	SNP (transition)	Substitution	0.506	0	3362685	yhcE + insH1		BW25113_3217
CET3	3358914	3358914	1	1	R -> K	G -> A	AGG -> AAG	344	SNP (transition)	Substitution	0.5	0	3362688	yhcE + insH1		BW25113_3217
CET2	3385929	3385929	1	1	L -> Q	A -> T	CTG -> CAG	165	SNP (transversion)	Substitution	100.00%	0	3390754	yhdP		BW25113_4472
CET3	3388417	3388417	1	1	A -> P	C -> G	GCC -> CCC	86	SNP (transversion)	Substitution	0.977	2.3E-274	3392224	yhdP		BW25113_4472
CET2	3576729	3576730	2	1	L -> P	TA -> CG	CTA -> CCG	199 -> 215	Substitution	Substitution	25.1% -> 25.6%	1.90E-73	3581817	yrhA + insA		BW25113_3443
CET2	3576731	3576736	4	3	QE -> YH	CAAGAA -> TACCAT	CAA,GAA -> TAC,CAT	217 -> 226	Substitution	Substitution	25.8% -> 28.8%	6.60E-96	3581824	yrhA + insA		BW25113_3443
CET2	3576738	3576739	2	1	R -> H	GG -> AT	CGG -> CAT	229 -> 230	Substitution	Substitution	31.3% -> 31.4%	2.90E-127	3581827	yrhA + insA		BW25113_3443
CET2	3576742	3576742	1	1	L -> F	A -> C	TTA -> TTC	243	SNP (transversion)	Substitution	48.60%	7.60E-236	3581830	yrhA + insA		BW25113_3443
CET2	3576743	3576748	4	3	VI -> LL	GTGATC -> CTCCTA	GTG,ATC -> CTC,CTA	254 -> 288	Substitution	Substitution	35.8% -> 43.2%	1.90E-176	3581836	yrhA + insA		BW25113_3443
CET2	3576749	3576750	2	1		GG -> TA		288 -> 289	Substitution	Truncation	42.9% -> 43.1%	7.00E-289	3581838	yrhA + insA		BW25113_3443
CET2	3576753	3576753	1	1	D -> A	A -> C	GAT -> GCT	306	SNP (transversion)	Substitution	41.80%	0.00E+00	3581841	yrhA + insA		BW25113_3443
CET2	3576755	3576755	1	1	Y -> D	T -> G	TAT -> GAT	313	SNP (transversion)	Substitution	41.90%	9.80E-303	3581843	yrhA + insA		BW25113_3443
CET2	3576758	3576759	2	1	S -> F	AG -> TT	AGC -> TTC	315 -> 316	Substitution	Substitution	42.2% -> 42.7%	5.50E-308	3581847	yrhA + insA		BW25113_3443
CET2	3576762	3576762	1	1	I -> S	T -> G	ATT -> AGT	317	SNP (transversion)	Substitution	43.20%	5.4E-319	3581850	yrhA + insA		BW25113_3443
CET2	3576764	3576768	5	1	SI -> LA	TCAAT -> CTGGC	TCA,ATA -> CTG,GCA	316 -> 319	Substitution	Substitution	41.6% -> 41.8%	1.60E-307	3581856	yrhA + insA		BW25113_3443

CET2	3576770	3576772	3	1	F -> P	TTT -> CCG	TTT -> CCG	319	Substitution	Substitution	42.90%	5.6E-312	3581860	yrhA + insA		BW25113_3443
CET2	3576774	3576774	1	1	T -> N	C -> A	ACT -> AAT	321	SNP (transversion)	Substitution	42.70%	1.10E-290	3581862	yrhA + insA		BW25113_3443
CET2	3576776	3576778	2	2	Y -> Q	TAT -> CAG	TAT -> CAG	318 -> 320	Substitution	Substitution	42.8% -> 43.1%	2.5E-312	3581866	yrhA + insA		BW25113_3443
CET2	3576779	3576781	3	1	D -> R	GAC -> CGG	GAC -> CGG	320 -> 332	Substitution	Substitution	41.0% -> 42.5%	1.80E-299	3581869	yrhA + insA		BW25113_3443
CET2	3576783	3576787	5	1	IK -> KV	TTAAA -> AAGTG	ATT,AAA -> AAA,GTG	327 -> 341	Substitution	Substitution	39.9% -> 41.6%	1.40E-281	3581875	yrhA + insA		BW25113_3443
CET1	3576754	3576756	3	1	DY -> EG	TTA -> GGG	GAT,TAT -> GAG,GGT	171 -> 174	Substitution	Substitution	24.6% -> 25.3%	2.30E-49	3581074	yrhA + insA		BW25113_3443
CET1	3576759	3576759	1	1	S -> N	G -> A	AGC -> AAC	174	SNP (transition)	Substitution	25.30%	5.40E-52	3581077	yrhA + insA		BW25113_3443
CET1	3576761	3576762	2	1	I -> G	AT -> GG	ATT -> GGT	167 -> 170	Substitution	Substitution	27.5% -> 27.6%	1.00E-51	3581080	yrhA + insA		BW25113_3443
CET1	3576764	3576766	3	1	S -> R	TCA -> CGC	TCA -> CGC	169 -> 174	Substitution	Substitution	25.3% -> 26.0%	1.90E-43	3581084	yrhA + insA		BW25113_3443
CET1	3576767	3576769	3	1	I -> H	ATA -> CAT	ATA -> CAT	174 -> 175	Substitution	Substitution	25.1% -> 25.3%	2.30E-47	3581087	yrhA + insA		BW25113_3443
CET1	3576770	3576770	1	1	F -> L	T -> C	TTT -> CTT	175	SNP (transition)	Substitution	26.30%	1.20E-59	3581088	yrhA + insA		BW25113_3443
CET1	3576782	3576782	1	1	I -> V	A -> G	ATT -> GTT	182	SNP (transition)	Substitution	25.30%	8.60E-50	3581100	yrhA + insA		BW25113_3443
CET1	3576785	3576785	1	1	K -> Q	A -> C	AAA -> CAA	184	SNP (transversion)	Substitution	33.70%	2.10E-75	3581103	yrhA + insA		BW25113_3443
CET3	3576729	3576730	2	1	L -> P	TA -> CG	CTA -> CCG	143 -> 151	Substitution	Substitution	25.2% -> 26.5%	1.6E-60	3580736	yrhA + insA		BW25113_3443
CET3	3576731	3576736	4	3	QE -> YH	CAAGAA -> TACCAT	CAA,GAA -> TAC,CAT	154 -> 169	Substitution	Substitution	27.3% -> 28.4%	6.4E-84	3580743	yrhA + insA		BW25113_3443
CET3	3576738	3576739	2	1	R -> H	GG -> AT	CGG -> CAT	173 -> 176	Substitution	Substitution	28.9% -> 29.0%	8.8E-99	3580746	yrhA + insA		BW25113_3443
CET3	3576742	3576742	1	1	L -> F	A -> C	TTA -> TTC	188	SNP (transversion)	Substitution	0.426	2.3E-162	3580749	yrhA + insA		BW25113_3443
CET3	3576743	3576748	4	3	VI -> LL	GTGATC -> CTCCTA	GTG,ATC -> CTC,CTA	191 -> 219	Substitution	Substitution	33.5% -> 40.7%	2.1E-129	3580755	yrhA + insA		BW25113_3443
CET3	3576749	3576750	2	1		GG -> TA		219 -> 222	Substitution	Truncation	42.5% -> 42.8%	3.2E-226	3580757	yrhA + insA		BW25113_3443
CET3	3576753	3576753	1	1	D -> A	A -> C	GAT -> GCT	229	SNP (transversion)	Substitution	0.441	4.4E-267	3580760	yrhA + insA		BW25113_3443
CET3	3576755	3576755	1	1	Y -> D	T -> G	TAT -> GAT	229	SNP (transversion)	Substitution	0.441	5.4E-257	3580762	yrhA + insA		BW25113_3443
CET3	3576758	3576759	2	1	S -> F	AG -> TT	AGC -> TTC	232 -> 234	Substitution	Substitution	45.3% -> 45.7%	1.3E-250	3580766	yrhA + insA		BW25113_3443
CET3	3576762	3576762	1	1	I -> S	T -> G	ATT -> AGT	231	SNP (transversion)	Substitution	0.459	1.9E-261	3580769	yrhA + insA		BW25113_3443
CET3	3576764	3576768	5	1	SI -> LA	TCAAT -> CTGGC	TCA,ATA -> CTG,GCA	230 -> 234	Substitution	Substitution	45.3% -> 46.1%	4.5E-250	3580775	yrhA + insA		BW25113_3443
CET3	3576770	3576772	3	1	F -> P	TTT -> CCG	TTT -> CCG	231 -> 233	Substitution	Substitution	45.5% -> 45.9%	3.1E-231	3580779	yrhA + insA		BW25113_3443

CET3	3576774	3576774	1	1	T -> N	C -> A	ACT -> AAT	231	SNP (transversion)	Substitution	0.459	1.1E-229	3580781	yrhA + insA		BW25113_3443
CET3	3576776	3576778	2	2	Y -> Q	TAT -> CAG	TAT -> CAG	231 -> 233	Substitution	Substitution	0.459	3.5E-242	3580785	yrhA + insA		BW25113_3443
CET3	3576779	3576781	3	1	D -> R	GAC -> CGG	GAC -> CGG	233 -> 248	Substitution	Substitution	43.1% -> 45.9%	1.9E-249	3580788	yrhA + insA		BW25113_3443
CET3	3576783	3576787	5	1	IK -> KV	TTAAA -> AAGTG	ATT,AAA -> AAA,GTG	248 -> 252	Substitution	Substitution	42.1% -> 42.7%	5.9E-218	3580794	yrhA + insA		BW25113_3443
CET2	3577557	3577564	8	1		ACATTAAA -> GATGGGGC		240 -> 251	Substitution		46.1% -> 49.8%	1.10E-196	3582659	yrhA + insA		BW25113_3443
CET2	3577566	3577566	1	1		G -> A		241	SNP (transition)		46.10%	1.60E-218	3582661	yrhA + insA		BW25113_3443
CET2	3577568	3577569	2	1		AA -> GC		239 -> 240	Substitution		46.3% -> 46.4%	9.80E-208	3582664	yrhA + insA		BW25113_3443
CET2	3577571	3577572	2	1		TT -> AA		238	Substitution		46.20%	9.00E-228	3582667	yrhA + insA		BW25113_3443
CET2	3577574	3577575	2	1		TT -> GG		231	Substitution		44.60%	1.70E-203	3582670	yrhA + insA		BW25113_3443
CET2	3577578	3577581	4	1		AAAT -> CCGG		214 -> 219	Substitution		37.9% -> 40.2%	2.10E-177	3582676	yrhA + insA		BW25113_3443
CET2	3577583	3577584	2	1		AG -> GC		212	Substitution		36.8% -> 37.7%	4.80E-191	3582679	yrhA + insA		BW25113_3443
CET2	3577587	3577597	11	1		ATAATATTGGC -> GCCGGGCCAAG		181 -> 210	Substitution		25.4% -> 34.8%	2.20E-107	3582692	yrhA + insA		BW25113_3443
CET3	3577557	3577564	8	1		ACATTAAA -> GATGGGGC		174 -> 184	Substitution		36.9% -> 38.0%	2.4E-132	3581572	yrhA + insA		BW25113_3443
CET3	3577566	3577566	1	1		G -> A		173	SNP (transition)		0.382	1.9E-143	3581574	yrhA + insA		BW25113_3443
CET3	3577568	3577569	2	1		AA -> GC		170	Substitution		0.388	1.7E-137	3581577	yrhA + insA		BW25113_3443
CET3	3577571	3577572	2	1		TT -> AA		174	Substitution		0.374	4.8E-147	3581580	yrhA + insA		BW25113_3443
CET3	3577574	3577575	2	1		TT -> GG		168 -> 169	Substitution		35.7% -> 36.1%	2.1E-140	3581583	yrhA + insA		BW25113_3443
CET3	3577578	3577581	4	1		AAAT -> CCGG		164 -> 166	Substitution		33.3% -> 34.3%	2.4E-121	3581589	yrhA + insA		BW25113_3443
CET3	3577583	3577584	2	1		AG -> GC		163 -> 164	Substitution		31.7% -> 32.5%	4.9E-129	3581592	yrhA + insA		BW25113_3443
CET3	3577587	3577589	3	1		ATA -> GCC		160 -> 164	Substitution		25.6% -> 28.7%	4.6E-99	3581597	yrhA + insA		BW25113_3443
CET1	3631446	3631446	1	1	I -> F	A -> T	ATC -> TTC	74	SNP (transversion)	Substitution	25.70%	2.60E-42	3635824	pitA		BW25113_3493
CET1	3631452	3631452	1	1	K -> Q	A -> C	AAA -> CAA	73	SNP (transversion)	Substitution	30.10%	9.90E-53	3635830	pitA		BW25113_3493
CET1	3631463	3631463	1	1	S -> R	T -> G	AGT -> AGG	76	SNP (transversion)	Substitution	61.80%	2.60E-144	3635843	pitA		BW25113_3493
CET1	3631465	3631480	13	4	IFGSLI -> KVRTSP	TTTTCGGTTCTCTGAT -> AGGTGCGAACAAGTCC	ATT,TTC,GGT,TCT,CTG,ATC -> AAG,GTG,CGA,ACA,AGT,CCC	72 -> 80	Substitution	Substitution	48.8% -> 55.6%	1.30E-110	3635865	pitA		BW25113_3493
CET1	3631482	3631484	3	1		GTT -> TGA		79 -> 83	Substitution	Truncation	47.0% -> 49.4%	7.90E-113	3635869	pitA		BW25113_3493

CET1	3631486	3631490	5	1	SP -> YE	CCCCT -> ATGAG	TCC,CCT -> TAT,GAG	86 -> 88	Substitution	Substitution	39.8% -> 40.7%	1.40E-101	3635875	pitA		BW25113_3493
CET1	3631493	3631505	10	4	IVGLV -> IMFVI	TGTCGGCCTGGTG -> CATGTTTGTCATC	ATT,GTC,GGC,CTG,GTG -> ATC,ATG,TTT,GTC,ATC	78 -> 82	Substitution	Substitution	26.8% -> 30.9%	5.00E-58	3635890	pitA		BW25113_3493
CET1	3631507	3631510	4	1	FA -> WS	TTGC -> GGAG	TTT,GCT -> TGG,AGT	82 -> 83	Substitution	Substitution	25.3% -> 26.8%	1.20E-50	3635895	pitA		BW25113_3493
CET3	3774500	3774500	1	1		A -> G		55	SNP (transition)		0.273	9.4E-21	3778710		trmL (U): lldD (D)	BW25113_3606; BW25113_3605
CET2	4174954	4174954	1	1	G -> A	G -> C	GGT -> GCT	152	SNP (transversion)	Substitution	100.00%	0	4180811	rpoB		BW25113_3987
CET3	4174954	4174954	1	1	G -> A	G -> C	GGT -> GCT	136	SNP (transversion)	Substitution	0.993	0	4179613	rpoB		BW25113_3987
CET2	4624803	4624803	1	1	W -> G	A -> C	TGG -> GGG	929	SNP (transversion)	Substitution	99.60%	0	4631813	rob		BW25113_4396
CET3	4624803	4624803	1	1	W -> G	A -> C	TGG -> GGG	773	SNP (transversion)	Substitution	0.996	0	4630323	rob		BW25113_4396
CHX1	178959	178962	4	1		(TGGC)3 -> (TGGC)2		112	Deletion (tandem repeat)	Frame Shift	100.00%	2.5E-314	179112	cdaR		BW25113_0162
CHX2	360292	360292	1	1		T -> C		166	SNP (transition)		0.259	7.7E-77	360771		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
CHX2	360295	360295	1	1		A -> G		152	SNP (transition)		0.25	2E-67	360774		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
CHX2	360298	360298	1	1		C -> G		152	SNP (transversion)		0.263	7.1E-80	360777		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
CHX2	360303	360303	1	1		A -> T		153	SNP (transversion)		0.255	7.3E-93	360782		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
CHX3	360294	360296	3	1		AAT -> GGC		117 -> 119	Substitution		25.2% -> 25.6%	2.5E-65	360513		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
CHX3	360298	360298	1	1		C -> G		118	SNP (transversion)		0.254	9.1E-72	360515		lacZ (U); lacI (D)	BW25113_0344, BW25113_0345
CHX1	454232	454238	7	1		CGTTGAA -> TTAGCGC		73 -> 76	Substitution		25.0% -> 27.4%	2.80E-38	454666		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX1	454240	454242	3	1		GTG -> TAT		74	Substitution		29.70%	1.00E-57	454670		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX1	454256	454258	3	1		CAT -> ATA		77 -> 78	Substitution		55.8% -> 56.4%	5.10E-125	454686		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX1	454260	454264	5	1		TACTG -> GCGCT		74 -> 77	Substitution		49.4% -> 54.7%	1.30E-111	454692		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX1	454266	454267	2	1		CG -> AC		76 -> 77	Substitution		47.4% -> 48.1%	4.00E-108	454695		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX1	454269	454270	2	1		AC -> TA		75 -> 76	Substitution		46.1% -> 46.7%	5.40E-98	454698		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX1	454272	454272	1	1		T -> G		81	SNP (transversion)		40.70%	6.60E-87	454700		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX1	454274	454274	1	1		T -> G		84	SNP (transversion)		38.10%	3.80E-83	454702		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX1	454276	454277	2	1		AA -> TG		81	Substitution		35.80%	1.60E-74	454705		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX1	454279	454279	1	1		A -> G		78	SNP (transition)		33.30%	5.40E-66	454707		lon (U); clpX (D)	BW25113_0439; BW25113_0438

CHX1	454281	454283	3	1		ATG -> TAT		75 -> 76	Substitution		30.7% -> 31.6%	3.70E-62	454711		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX1	454286	454286	1	1		G -> C		74	SNP (transversion)		27.00%	5.70E-45	454717		lon (U); clpX (D)	BW25113_0439; BW25113_0438
CHX2	684507	684508	2	1		TA -> AGT		234	Insertion		0.269	7.8E-132	685376		insH-1 (U)	BW25113_0259
CHX2	684511	684513	3	1		ATT -> TAC		236 -> 238	Substitution		26.5% -> 26.7%	1.4E-137	685381		insH-1 (U)	BW25113_0259
CHX2	684516	684516	1	1		G -> T		239	SNP (transversion)		0.259	1.3E-128	685384		insH-1 (U)	BW25113_0259
CHX2	684518	684518	1	1		T -> G		237	SNP (transversion)		0.266	3.8E-125	685386		insH-1 (U)	BW25113_0259
CHX2	684521	684522	2	1		GC -> AG		230	Substitution		0.27	8.3E-130	685391		insH-1 (U)	BW25113_0259
CHX2	684527	684527	1	1		T -> C		233	SNP (transition)		0.266	1.4E-135	685396		insH-1 (U)	BW25113_0259
CHX3	684507	684508	2	1		TA -> AGT		145 -> 147	Insertion		25.2% -> 25.5%	1.5E-97	684950		insH-1 (U)	BW25113_0259
CHX3	684511	684513	3	1		ATT -> TAC		145	Substitution		0.262	7.6E-95	684955		insH-1 (U)	BW25113_0259
CHX3	684516	684516	1	1		G -> T		144	SNP (transversion)		0.264	1.4E-83	684958		insH-1 (U)	BW25113_0259
CHX3	684518	684518	1	1		T -> G		143	SNP (transversion)		0.266	1E-83	684960		insH-1 (U)	BW25113_0259
CHX3	684521	684522	2	1		GC -> AG		142	Substitution		0.268	1.9E-91	684964		insH-1 (U)	BW25113_0259
CHX3	684527	684527	1	1		T -> C		145	SNP (transition)		0.262	4.7E-91	684969		insH-1 (U)	BW25113_0259
CHX3	684530	684533	4	1		AAAG -> CGTT		144 -> 145	Substitution		25.5% -> 25.7%	3E-87	684975		insH-1 (U)	BW25113_0259
CHX3	684536	684536	1	1		A -> C		145	SNP (transversion)		0.255	6.7E-92	684978		insH-1 (U)	BW25113_0259
CHX3	684545	684545	1	1		T -> A		135	SNP (transversion)		0.252	2.1E-67	684987		insH-1 (U)	BW25113_0259
CHX1	878982	878982	1	1		A -> G		54	SNP (transition)		27.80%	2.70E-34	879819		ybjG (U); mdfA (U)	BW25113_0841; BW25113_0842
CHX1	879000	879000	1	1		A -> G		52	SNP (transition)		42.30%	1.70E-65	879838		ybjG (U); mdfA (U)	BW25113_0841; BW25113_0842
CHX1	879003	879005	3	1		GAA -> TCT		54	Substitution		44.40%	8.70E-65	879843		ybjG (U); mdfA (U)	BW25113_0841; BW25113_0842
CHX1	879007	879008	2	1		GC -> CA		57	Substitution		49.10%	5.90E-77	879846		ybjG (U); mdfA (U)	BW25113_0841; BW25113_0842
CHX1	879011	879011	1	1		A -> T		62	SNP (transversion)		43.50%	1.10E-69	879849		ybjG (U); mdfA (U)	BW25113_0841; BW25113_0842
CHX1	879013	879013	1	1		-C		63	Deletion		39.70%	6.90E-46	879851		ybjG (U); mdfA (U)	BW25113_0841; BW25113_0842
CHX1	879016	879016	1	1		T -> G		64	SNP (transversion)		39.10%	1.30E-70	879854		ybjG (U); mdfA (U)	BW25113_0841; BW25113_0842
CHX1	879018	879018	1	1		C -> T		64	SNP (transition)		39.10%	1.20E-60	879856		ybjG (U); mdfA (U)	BW25113_0841; BW25113_0842

CHX1	879021	879032	12	1		GTAATAATGTAA -> AATTGGTCAACG		64 -> 71	Substitution		23.9% -> 30.3%	2.10E-41	879874		ybjG (U); mdfA (U)	BW25113_0841; BW25113_0842
CHX3	1391504	1391504	1	1		T -> G		90	SNP (transversion)		0.289	1.2E-35	1392347		ynaJ (U); insH (D)	BW25113_1332; unknown
CHX3	1391516	1391516	1	1		T -> A		89	SNP (transversion)		0.281	1.9E-36	1392360		ynaJ (U); insH (D)	BW25113_1332; unknown
CHX2	1613498	1613502	5	1	AQ -> GI	CACAG -> GTATT	GCA,CAG -> GGT,ATT	52 -> 55	Substitution	Substitution	50.9% -> 53.8%	1.2E-87	1615300	marR		BW25113_1530
CHX2	1613503	1613511	8	2	FKV -> VGR	TTTAAGGTG -> GTCGGTCGA	TTT,AAG,GTG -> GTC,GGT,CGA	49 -> 55	Substitution	Substitution	40.7% -> 46.2%	4.9E-64	1615309	marR		BW25113_1530
CHX2	1613513	1613520	7	2	LCS -> PGS	TCTGCTCT -> CTGGGAGC	CTC,TGC,TCT -> CCT,GGG,AGC	49 -> 55	Substitution	Substitution	30.6% -> 39.6%	5E-44	1615318	marR		BW25113_1530
CHX2	1613522	1613522	1	1	I -> S	T -> G	ATC -> AGC	52	SNP (transversion)	Substitution	0.25	7.9E-32	1615320	marR		BW25113_1530
CHX2	1613522	1613525	4	1	IR -> TD	TCCG -> CTGA	ATC,CGC -> ACT,GAC	50 -> 52	Substitution	Substitution	26.0% -> 28.8%	1.1E-34	1615323	marR		BW25113_1530
CHX2	1613525	1613526	2	1	R -> L	GC -> TG	CGC -> CTG	49 -> 50	Substitution	Substitution	28.0% -> 28.6%	9.3E-38	1615324	marR		BW25113_1530
CHX2	1613527	1613528	2	1	C -> P	TG -> CC	TGC -> CCC	50	Substitution	Substitution	0.26	2.8E-37	1615326	marR		BW25113_1530
CHX2	1613527	1613529	3	1	C -> L	TGC -> CTT	TGC -> CTT	50	Substitution	Substitution	0.3	2.2E-39	1615327	marR		BW25113_1530
CHX2	1613530	1613532	3	1	A -> N	GCG -> AAC	GCG -> AAC	50 -> 51	Substitution	Substitution	30.0% -> 31.4%	7E-38	1615330	marR		BW25113_1530
CHX2	1613534	1613534	1	1	A -> E	C -> A	GCG -> GAG	49	SNP (transversion)	Substitution	0.327	2.1E-39	1615332	marR		BW25113_1530
CHX2	1613537	1613537	1	1	C -> Y	G -> A	TGT -> TAT	49	SNP (transition)	Substitution	0.347	2E-47	1615335	marR		BW25113_1530
CHX2	1613539	1613541	2	2	I -> L	ATT -> CTG	ATT -> CTG	50	Substitution	Substitution	0.38	9.5E-54	1615339	marR		BW25113_1530
CHX2	1613542	1613542	1	1	T -> S	A -> T	ACT -> TCT	50	SNP (transversion)	Substitution	0.4	4.7E-55	1615340	marR		BW25113_1530
CHX2	1613548	1613550	2	2	V -> L	GTT -> CTG	GTT -> CTG	55	Substitution	Substitution	0.418	2.5E-64	1615348	marR		BW25113_1530
CHX2	1613553	1613553	1	1	E -> D	A -> T	GAA -> GAT	58	SNP (transversion)	Substitution	0.362	6.6E-63	1615351	marR		BW25113_1530
CHX2	1613554	1613556	2	2	L -> I	CTG -> ATT	CTG -> ATT	57 -> 58	Substitution	Substitution	34.5% -> 35.1%	1.2E-51	1615354	marR		BW25113_1530
CHX2	1613558	1613561	4	1	KK -> TA	AAAA -> CCGC	AAA,AAG -> ACC,GCG	54 -> 55	Substitution	Substitution	34.5% -> 35.2%	1.2E-47	1615359	marR		BW25113_1530
CHX2	1613564	1613563	0	1		(G)2 -> (G)3		55	Insertion (tandem repeat)	Frame Shift	0.364	5E-54	1615362	marR		BW25113_1530
CHX3	1703187	1703187	1	1	P -> Q	C -> A	CCG -> CAG	72	SNP (transversion)	Substitution	0.264	2.1E-16	1704230	rsxC		BW25113_1629
CHX3	2096425	2096425	1	1	K -> R	T -> C	AAG -> AGG	110	SNP (transition)	Substitution	0.255	5E-42	2097696	wbbL + insH-7		BW25113_2031
CHX3	2096430	2096432	2	2	G -> R	CCC -> GCG	GGG -> CGC	104 -> 109	Substitution	Substitution	25.0% -> 25.7%	1.2E-46	2097703	wbbL + insH-7		BW25113_2031
CHX3	2096436	2096436	1	1	M -> I	C -> T	ATG -> ATA	104	SNP (transition)	Substitution	0.25	4.7E-44	2097707	wbbL + insH-7		BW25113_2031

CHX1	2458482	2458482	1	1	K -> T	T -> G	AAG -> ACG	76	SNP (transversion)	Substitution	25.00%	1.20E-49	2460632	mlaA		BW25113_2346
CHX1	2458484	2458484	1	1		C -> G	ATG -> ATC	78	SNP (transversion)	None	26.90%	1.70E-55	2460634	mlaA		BW25113_2346
CHX2	2458181	2458188	7	2		GCGGGTAA -> ACACTATC		71 -> 74	Substitution	Truncation	25.7% -> 28.8%	1.2E-46	2460871	mlaA		BW25113_2346
CHX2	2458190	2458201	11	2	GMVH -> TPTY	GTGGACCATCCC -> ATAAGTTGGAC	GGG,ATG,GTC,CAC -> ACT,CCA,ACT,TAT	71 -> 79	Substitution	Substitution	31.0% -> 41.8%	3.1E-55	2460884	mlaA		BW25113_2346
CHX2	2458203	2458208	6	1	PYQ -> PVM	TGATAA -> ATTACC	CCT,TAT,CAG -> CCG,GTA,ATG	79 -> 84	Substitution	Substitution	43.0% -> 47.0%	2.5E-97	2460891	mlaA		BW25113_2346
CHX2	2458218	2458218	1	1	Q -> P	T -> G	CAG -> CCG	85	SNP (transversion)	Substitution	0.318	8.5E-68	2460902	mlaA		BW25113_2346
CHX2	2458220	2458221	2	1	L -> S	CA -> TG	TTG -> TCA	85	Substitution	Substitution	0.318	1.7E-70	2460905	mlaA		BW25113_2346
CHX2	2458223	2458236	14	1	MVNYF -> SKLAA	GAAGTAGTTAACCA -> TGCTGCCAACTTAC	ATG,GTT,AAC,TAC,TTC -> AGT,AAG,TTG,GCA,GCA	84 -> 89	Substitution	Substitution	24.4% -> 30.6%	1.2E-57	2460920	mlaA		BW25113_2346
CHX2	2458238	2458240	2	2	V -> I	CAC -> GAT	GTG -> ATC	84 -> 85	Substitution	Substitution	25.0% -> 25.9%	2E-48	2460924	mlaA		BW25113_2346
CHX3	2458397	2458399	3	1	D -> P	GTC -> TGG	GAC -> CCA	31 -> 32	Substitution	Substitution	25.0% -> 25.8%	7.8E-22	2459882	mlaA		BW25113_2346
CHX3	2458403	2458410	8	1	QGR -> PPE	ACGCCCTT -> TTCAGGGG	CAA,GGG,CGT -> CCC,CCT,GAA	29 -> 32	Substitution	Substitution	28.1% -> 31.0%	2.8E-23	2459893	mlaA		BW25113_2346
CHX3	2458413	2458415	3	1	DQ -> DW	TGA -> CAG	GAT,CAG -> GAC,TGG	29 -> 35	Substitution	Substitution	32.3% -> 34.5%	2E-27	2459898	mlaA		BW25113_2346
CHX3	2458419	2458420	2	1		GT -> A		34 -> 35	Deletion	Truncation	37.1% -> 38.2%	2.9E-29	2459903	mlaA		BW25113_2346
CHX3	2458426	2458435	9	2	CASS -> GKST	AACTCGCACA -> TGGATTTGCC	TGT,GCG,AGT,TCC -> GGC,AAA,TCC,ACC	32 -> 41	Substitution	Substitution	29.3% -> 40.6%	1.8E-33	2459918	mlaA		BW25113_2346
CHX1	2458489	2458490	2	1		AT -> CA		77	Substitution		27.30%	9.90E-58	2460640		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458492	2458495	4	1		TCTC -> ATGT		76 -> 77	Substitution		26.3% -> 27.3%	3.80E-45	2460645		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458497	2458498	2	1		CT -> TG		77	Substitution		27.30%	1.60E-53	2460648		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458500	2458502	3	1		TTT -> AAA		75 -> 78	Substitution		29.3% -> 32.1%	2.00E-52	2460652		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458504	2458504	1	1		T -> A		76	SNP (transversion)		32.90%	2.40E-68	2460654		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458506	2458508	3	1		TAT -> AGG		75 -> 77	Substitution		32.9% -> 34.7%	6.20E-64	2460658		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458512	2458513	2	1		TT -> AA		74	Substitution	Extension	36.50%	1.80E-72	2460663		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458516	2458516	1	1	A -> G	G -> C	GCA -> GGA	75	SNP (transversion)	Substitution	37.30%	1.20E-72	2460666		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458524	2458543	18	3	VSFRHGW -> EIQGASL	CCATCCATGACGGAACGATA -> TAGACTGGCCCCCTGAATCT	GTA,TCG,TTC,CGT,CAT,GGA,TGG -> GAG,ATT,CAG,GGG,GCC,AGT,CTA	62 -> 70	Substitution	Substitution	39.7% -> 53.6%	9.50E-67	2460693		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458546	2458547	2	1	T -> L	GT -> AG	ACG -> CTG	70 -> 71	Substitution	Substitution	38.0% -> 38.6%	2.80E-73	2460697		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458549	2458552	4	1	AD -> GC	TCCG -> CAAC	GCG,GAT -> GGT,TGT	68 -> 69	Substitution	Substitution	33.8% -> 36.2%	3.90E-54	2460702		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2458554	2458559	5	2	KQ -> DI	CTGTTT -> AATATC	AAA,CAG -> GAT,ATT	68 -> 77	Substitution	Substitution	26.0% -> 33.8%	1.90E-42	2460709		MlaA (U): yfdC (U)	BW25113_2346, BW25113_2347
CHX1	2563835	2563835	1	1	L -> V	G -> C	CTG -> GTG	8	SNP (transversion)	Substitution	25.00%	4.40E-07	2566081	eutE		BW25113_2455
CHX1	3124704	3124704	1	1	I -> S	A -> C	ATT -> AGT	94	SNP (transversion)	Substitution	30.90%	3.70E-75	3127458	yghQ		BW25113_2983
CHX1	3124706	3124706	1	1		C -> G	CTG -> CTC	93	SNP (transversion)	None	31.20%	2.50E-75	3127460	yghQ		BW25113_2983
CHX1	3124709	3124711	2	2		GTC -> AT		89	Deletion	Frame Shift	31.50%	2.00E-59	3127465	yghQ		BW25113_2983
CHX1	3124715	3124723	9	1	LEA -> SFK	CGCTTCGAG -> TTTGAATGA	CTC,GAA,GCG -> TCA,TTC,AAA	88 -> 89	Substitution	Substitution	31.8% -> 32.6%	3.40E-70	3127477	yghQ		BW25113_2983
CHX1	3124728	3124728	1	1	K -> R	T -> C	AAG -> AGG	86	SNP (transition)	Substitution	32.60%	1.30E-70	3127482	yghQ		BW25113_2983
CHX1	3124731	3124732	2	1	V -> Y	AC -> TA	GTT -> TAT	84	Substitution	Substitution	33.30%	3.70E-68	3127486	yghQ		BW25113_2983
CHX1	3124734	3124735	2	1	G -> S	CC -> GA	GGC -> TCC	83	Substitution	Substitution	33.70%	1.50E-65	3127489	yghQ		BW25113_2983
CHX1	3124738	3124738	1	1	F -> V	A -> C	TTT -> GTT	83	SNP (transversion)	Substitution	33.70%	1.50E-65	3127495	yghQ		BW25113_2983
CHX1	3124740	3124740	1	1	V -> E	A -> T	GTG -> GAG	81	SNP (transversion)	Substitution	34.60%	2.70E-74	3127497	yghQ		BW25113_2983
CHX1	3124742	3124744	2	2	L -> I	CAG -> AAT	CTG -> ATT	81 -> 83	Substitution	Substitution	33.7% -> 34.6%	4.00E-71	3127501	yghQ		BW25113_2983
CHX1	3124747	3124746	0	1		#NAME?		82	Insertion	Frame Shift	30.50%	7.40E-65	3127507	yghQ		BW25113_2983
CHX1	3124751	3124751	1	1		G -> T	CTC -> CTA	74	SNP (transversion)	None	33.80%	3.40E-66	3127512	yghQ		BW25113_2983
CHX1	3124755	3124758	4	1	KP -> IG	GGCT -> CCAA	AAG,CCG -> ATT,GGG	74 -> 75	Substitution	Substitution	32.0% -> 32.4%	6.70E-56	3127519	yghQ		BW25113_2983
CHX1	3124760	3124769	8	3	LIVG -> FFLS	GCCGACGATC -> AGATAAAAAG	TTG,ATC,GTC,GGC -> TTC,TTT,TTA,TCT	74 -> 76	Substitution	Substitution	27.6% -> 33.3%	2.20E-47	3127530	yghQ		BW25113_2983
CHX3	3124738	3124738	1	1	F -> V	A -> C	TTT -> GTT	68	SNP (transversion)	Substitution	0.25	1.7E-39	3647579	yghQ		BW25113_2983
CHX3	3124740	3124740	1	1	V -> E	A -> T	GTG -> GAG	68	SNP (transversion)	Substitution	0.25	3.5E-41	3647584	yghQ		BW25113_2983
CHX3	3124742	3124742	1	1		C -> A	CTG -> CTT	67	SNP (transversion)	None	0.254	1.1E-44	3647590	yghQ		BW25113_2983
CHX3	3645349	3645354	5	2	FYF -> LET	TTATTT -> AGAAAC	TTT,TAT,TTC -> TTA,GAA,ACC	141 -> 146	Substitution	Substitution	24.8% -> 26.7%	3E-77	3647596	yhiS		BW25113_3504
CHX3	3645359	3645359	1	1	D -> Y	G -> T	GAT -> TAT	146	SNP (transversion)	Substitution	0.295	3E-118	3647599	yhiS		BW25113_3504
CHX3	3645363	3645365	3	1	LL -> RF	TAC -> GTT	CTA,CTT -> CGT,TTT	146 -> 148	Substitution	Substitution	28.4% -> 28.8%	3.8E-102	3647603	yhiS		BW25113_3504
CHX3	3645368	3645368	1	1	N -> D	A -> G	AAT -> GAT	148	SNP (transition)	Substitution	0.284	9.8E-115	3647608	yhiS		BW25113_3504
CHX3	3645371	3645371	1	1	I -> L	A -> C	ATA -> CTA	151	SNP (transversion)	Substitution	0.285	6.5E-109	3647613	yhiS		BW25113_3504
CHX3	3645374	3645374	1	1	N -> H	A -> C	AAT -> CAT	149	SNP (transversion)	Substitution	0.282	1.4E-114	3647619	yhiS		BW25113_3504

CHX3	3645377	3645378	2	1	F -> G	TT -> GG	TTC -> GGC	145 -> 149	Substitution	Substitution	28.2% -> 29.0%	5.3E-102	3647622	yhiS		BW25113_3504
CHX3	3645380	3645383	4	1	TD -> QH	ACTG -> CAGC	ACT,GAC -> CAG,CAC	144	Substitution	Substitution	0.285	9.8E-103	3648823	yhiS		BW25113_3504
CHX3	3645387	3645386	0	1		#NAME?		144	Insertion	Frame Shift	0.292	2.5E-115	3648826	yhiS		BW25113_3504
CHX3	3645389	3645392	3	2	NL -> EL	AATT -> GAAC	AAT,TTA -> GAA,CTA	147 -> 148	Substitution	Substitution	28.4% -> 28.6%	9.8E-115	3648831	yhiS		BW25113_3504
CHX3	3645395	3645395	1	1	G -> R	G -> A	GGG -> AGG	150	SNP (transition)	Substitution	0.28	3E-110	3648833	yhiS		BW25113_3504
CHX3	3646596	3646596	1	1		T -> A	GGT -> GGA	131	SNP (transversion)	None	0.534	1.2E-151	3648836	yhiS		BW25113_3504
CHX3	3646598	3646599	2	1		AC -> T		117	Deletion	Frame Shift	0.487	6.5E-81	3650682	yhiS		BW25113_3504
CHX3	3646604	3646604	1	1	I -> T	T -> C	ATT -> ACT	116	SNP (transition)	Substitution	0.491	2.8E-132	3650684	yhiS		BW25113_3504
CHX3	3646606	3646606	1	1	F -> I	T -> A	TTT -> ATT	116	SNP (transversion)	Substitution	0.483	2.1E-129	3650687	yhiS		BW25113_3504
CHX2	3646604	3646604	1	1	I -> T	T -> C	ATT -> ACT	197	SNP (transition)	Substitution	0.487	1.4E-211	3650674	yhiS		BW25113_3504
CHX2	3646606	3646606	1	1	F -> I	T -> A	TTT -> ATT	183	SNP (transversion)	Substitution	0.448	3.8E-185	3650677	yhiS		BW25113_3504
CHX2	3646609	3646609	1	1	L -> V	T -> G	TTA -> GTA	171	SNP (transversion)	Substitution	0.415	2.6E-143	3778739	yhiS		BW25113_3504
CHX2	3646596	3646596	1	1		T -> A	GGT -> GGA	207	SNP (transversion)	None	0.522	3.2E-242	3778748	yhiS		BW25113_3504
CHX2	3646598	3646599	2	1		AC -> T		194 -> 196	Deletion	Frame Shift	0.495	1.6E-145		yhiS		BW25113_3504
CHX2	3774505	3774505	1	1		C -> G		74	SNP (transversion)		0.27	3.5E-23	3777871		trmL (U); lldD (D)	BW25113_3606, BW25113_3605
CHX1	3774505	3774505	1	1		C -> G		66	SNP (transversion)		25.80%	6.10E-10			trmL (U); lldD (D)	BW25113_3606, BW25113_3605
CHX3	4487984	4487984	1	1		T -> G		81	SNP (transversion)		0.259	4.2E-55	4535838		fimE (U)	BW25113_4313
CHX1	4531814	4531815	2	1		GT -> TC		60 -> 64	Substitution		25.0% -> 26.7%	5.30E-40	4535850		fimE (U)	BW25113_4313
CHX1	4531817	4531819	3	1		TTC -> GGG		60 -> 61	Substitution		26.7% -> 27.9%	1.50E-42	4535855		fimE (U)	BW25113_4313
CHX1	4531821	4531821	1	1		T -> C		59	SNP (transition)		28.80%	4.30E-44	4535865		fimE (U)	BW25113_4313
CHX1	4531823	4531829	7	1		CAAATAT -> TGTTCGC		61 -> 63	Substitution		30.6% -> 32.8%	1.40E-53	4535867		fimE (U)	BW25113_4313
CHX1	4531831	4531831	1	1		T -> C		60	SNP (transition)		33.30%	4.10E-55	4535869		fimE (U)	BW25113_4313
CHX1	4531834	4531836	3	1		CAG -> TCC		62 -> 66	Substitution		38.7% -> 42.4%	9.60E-68	4535871		fimE (U)	BW25113_4313
CHX1	4531850	4531850	1	1		T -> G		73	SNP (transversion)		32.90%	4.50E-56	4534493		fimE (U)	BW25113_4313
CHX1	4531854	4531855	2	1	M -> K	GT -> AA	GTG -> AAG	70 -> 72	Substitution	Start Codon Loss	27.1% -> 29.2%	6.00E-41	4534499		fimE (U)	BW25113_4313

CHX3	4531764	4531764	1	1		T -> C		48	SNP (transition)		0.292	3E-35	4534502		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531766	4531770	5	1		ATCTT -> CATGA		48 -> 57	Substitution		31.3% -> 40.4%	1.1E-33	4534504		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531772	4531773	2	1		TT -> GA		57 -> 59	Substitution		38.6% -> 42.4%	5.5E-60	4534508		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531775	4531775	1	1		T -> C		62	SNP (transition)		0.516	2.8E-82	4534513		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531786	4531788	3	1		AAT -> GGC		72 -> 74	Substitution		58.3% -> 59.5%	2.8E-118	4534529		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531790	4531795	6	1		TTGACC -> CCAGAT		75 -> 79	Substitution		60.0% -> 62.0%	5E-118	4534532		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531805	4531807	3	1		TTC -> GAT		85 -> 89	Substitution		64.7% -> 67.4%	2.8E-157	4534548		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531810	4531815	6	1		ATAGGT -> CATATC		89 -> 93	Substitution		67.4% -> 73.3%	5.3E-179	4534550		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531817	4531819	3	1		TTC -> GGG		88 -> 89	Substitution		79.5% -> 79.8%	2.4E-199	4534558		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531821	4531821	1	1		T -> C		88	SNP (transition)		0.807	3E-217	4534560		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531823	4531829	7	1		CAAATAT -> TGTTCGC		90 -> 98	Substitution		76.7% -> 78.6%	1.2E-223	4534565		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531831	4531831	1	1		T -> C		101	SNP (transition)		0.832	5E-259	4536821		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX3	4531834	4531836	3	1		CAG -> TCC		115 -> 132	Substitution		85.2% -> 87.1%	5.5E-314	4536832		fimE (U); fimB (D)	BW25113_4313; BW25113_4312
CHX2	4531782	4531784	3	1		GGC -> CTA		75 -> 76	Substitution		26.7% -> 27.6%	7.8E-49	4536840		fimE (U)	BW25113_4313
CHX2	4531786	4531788	3	1		AAT -> GGC		78 -> 84	Substitution		28.2% -> 33.3%	4.9E-60	4536844		fimE (U)	BW25113_4313
CHX2	4531790	4531795	6	1		TTGACC -> CCAGAT		79 -> 82	Substitution		36.6% -> 43.9%	1.3E-65	4536852		fimE (U)	BW25113_4313
CHX2	4531798	4531800	3	1		TTG -> CAA		83 -> 84	Substitution		45.8% -> 46.4%	1.5E-98	4536856		fimE (U)	BW25113_4313
CHX2	4531802	4531803	2	1		GG -> CA		90	Substitution		0.5	9.7E-113	4536858		fimE (U)	BW25113_4313
CHX2	4531805	4531807	3	1		TTC -> GAT		91 -> 97	Substitution		47.3% -> 50.5%	6.3E-114	4536866		fimE (U)	BW25113_4313
CHX2	4531810	4531815	6	1		ATAGGT -> CATATC		105 -> 111	Substitution		54.2% -> 58.5%	1.7E-158	4536868		fimE (U)	BW25113_4313
CHX2	4531817	4531819	3	1		TTC -> GGG		110 -> 114	Substitution		62.7% -> 64.0%	1.6E-180	4536873		fimE (U)	BW25113_4313
CHX2	4531823	4531829	7	1		CAAATAT -> TGTTCGC		120 -> 128	Substitution		64.2% -> 65.6%	2.8E-236	4537514		fimE (U)	BW25113_4313
CHX2	4531831	4531831	1	1		T -> C		134	SNP (transition)		0.709	2.9E-261	4537518		fimE (U)	BW25113_4313
CHX2	4531834	4531836	3	1		CAG -> TCC		147 -> 165	Substitution		72.1% -> 75.2%	1.3E-317	4537532		fimE (U)	BW25113_4313
CHX2	4532476	4532476	1	1	F -> I	A -> T	TTT -> ATT	232	SNP (transversion)	Substitution	0.284	1.9E-146	4537548		fimE (D); fimA (U)	BW25113_4313; BW25113_4314

CHX2	4532480	4532480	1	1	C -> W	A -> C	TGT -> TGG	234	SNP (transversion)	Substitution	0.286	3.7E-122	4537551		fimE (D); fimA (U)	BW25113_4313; BW25113_4314
CHX2	4532489	4532494	4	3	F -> I	A -> T	TTT -> ATT	232	SNP (transversion)	Substitution	0.284	1.9E-146	4537548		fimE (D); fimA (U)	BW25113_4313; BW25113_4314
CHX2	4532498	4532498	1	1	F -> I	A -> T	TTT -> ATT	232	SNP (transversion)	Substitution	0.284	1.9E-146	4537548		fimE (D); fimA (U)	BW25113_4313; BW25113_4314
CHX2	4532508	4532510	3	1	F -> I	A -> T	TTT -> ATT	232	SNP (transversion)	Substitution	0.284	1.9E-146	4537548		fimE (D); fimA (U)	BW25113_4313; BW25113_4314
CHX2	4532513	4532513	1	1	F -> I	A -> T	TTT -> ATT	232	SNP (transversion)	Substitution	0.284	1.9E-146	4537548		fimE (D); fimA (U)	BW25113_4313; BW25113_4314
CHX2	4532771	4532772	2	1		TT -> AA		190	Substitution		0.253	1.7E-118	4537837		fimE (D); fimA (U)	BW25113_4313; BW25113_4314
CHX3	4558528	4558528	1	1		C -> T		294	SNP (transition)		0.997	0	4561308		fimE (U)	BW25113_4313
CHX3	4570507	4570507	1	1	V -> G	A -> C	GTA -> GGA	96	SNP (transversion)	Substitution	0.25	6.4E-60	4573315	hsdS		BW25113_4348
CHX3	4570511	4570512	2	1	SS -> RA	AA -> CC	AGT,TCT -> AGG,GCT	94	Substitution	Substitution	0.277	4.2E-66	4573320	hsdS		BW25113_4348
CHX3	4570517	4570517	1	1	I -> L	T -> G	ATT -> CTT	87	SNP (transversion)	Substitution	0.667	3.9E-140	4573325	hsdS		BW25113_4348
CHX3	4570519	4570520	2	1	R -> F	CG -> AA	CGC -> TTC	79	Substitution	Substitution	0.354	4.3E-55	4573328	hsdS		BW25113_4348
CHX3	4570519	4570520	2	1		#NAME?		79	Deletion	Frame Shift	0.253	1.3E-20	4573328	hsdS		BW25113_4348
CHX3	4570521	4570523	2	2		TAG -> AA		73 -> 75	Deletion	Frame Shift	26.7% -> 27.4%	2.3E-21	4573331	hsdS		BW25113_4348
CHX3	4570521	4570522	2	1	L -> P	TA -> GG	CTA -> CCC	73 -> 74	Substitution	Substitution	28.4% -> 28.8%	4.1E-35	4573330	hsdS		BW25113_4348
CHX3	4570524	4570525	2	1	I -> T	TA -> GG	ATA -> ACC	74 -> 75	Substitution	Substitution	26.7% -> 27.0%	5.7E-47	4573333	hsdS		BW25113_4348
CHX3	4570527	4570528	2	1	P -> R	TG -> GC	CCA -> CGC	73 -> 74	Substitution	Substitution	25.7% -> 26.0%	5.7E-45	4573336	hsdS		BW25113_4348
CHX3	4570531	4570532	2	1	H -> I	TG -> AT	CAT -> ATT	69 -> 70	Substitution	Substitution	30.0% -> 30.4%	2.4E-50	4573340	hsdS		BW25113_4348
CHX3	4570534	4570535	2	1	G -> L	CC -> AG	GGT -> CTT	67 -> 69	Substitution	Substitution	29.0% -> 29.9%	1.6E-45	4573343	hsdS		BW25113_4348
CHX3	4570536	4570541	6	1	GV -> SP	AACACC -> CGGGGA	GGT,GTT -> TCC,CCG	67 -> 69	Substitution	Substitution	27.5% -> 28.4%	5.1E-38	4573349	hsdS		BW25113_4348
CHX3	4570543	4570543	1	1	S -> I	C -> A	AGT -> ATT	68	SNP (transversion)	Substitution	0.294	7.6E-40	4573351	hsdS		BW25113_4348
CHX3	4570546	4570547	2	1	E -> R	TC -> CT	GAA -> AGA	67	Substitution	Substitution	0.358	9.3E-55	4573356	hsdS		BW25113_4348
CHX3	4570548	4570549	2	1	N -> R	AT -> TC	AAT -> AGA	67	Substitution	Substitution	0.299	5.6E-46	4573358	hsdS		BW25113_4348
CHX3	4570551	4570551	1	1		T -> C	CCA -> CCG	66	SNP (transition)	None	0.333	7.1E-54	4573360	hsdS		BW25113_4348
CHX3	4570556	4570559	4	1	SK -> SQ	TTGA -> GACT	TCA,AAG -> AGT,CAG	62 -> 64	Substitution	Substitution	24.2% -> 26.6%	2.9E-40	4573368	hsdS		BW25113_4348
CHX3	4570560	4570560	1	1		T -> C	TCA -> TCG	60	SNP (transition)	None	0.267	9.2E-38	4573369	hsdS		BW25113_4348

COL1	103600	103600	1	1	I -> N	T -> A	ATC -> AAC	112	SNP (transversion)	Substitution	100.00%	0	103743	lpxC		BW25113_0096
COL2	103600	103600	1	1	I -> S	T -> G	ATC -> AGC	147	SNP (transversion)	Substitution	0.993	0	103726	lpxC		BW25113_0096
COL3	103600	103600	1	1	I -> S	T -> G	ATC -> AGC	80	SNP (transversion)	Substitution	1	1E-280	103677	lpxC		BW25113_0096
COL2	118913	118913	1	1	A -> V	C -> T	GCT -> GTT	169	SNP (transition)	Substitution	0.994	0	119049	pdhR		BW25113_0113
COL3	196528	196528	1	1	A -> V	C -> T	GCT -> GTT	98	SNP (transition)	Substitution	0.286	4E-77	196688	bamA		BW25113_0177
COL3	392121	392121	1	1		G -> T	CCG -> CCT	35	SNP (transversion)	None	0.343	2.1E-31	392446	sbmA		BW25113_0377
COL3	392123	392124	2	1	G -> V	GA -> TT	GGA -> GTT	35 -> 36	Substitution	Substitution	25.0% -> 25.7%	1.1E-21	392449	sbmA		BW25113_0377
COL3	392125	392127	3	1	T -> G	ACG -> GGT	ACG -> GGT	36	Substitution	Substitution	0.278	2.5E-29	392452	sbmA		BW25113_0377
COL3	392129	392129	1	1	F -> C	T -> G	TTT -> TGT	36	SNP (transversion)	Substitution	0.417	5.5E-45	392454	sbmA		BW25113_0377
COL3	392131	392136	5	2	FL -> LE	TTTCTC -> CTGGAG	TTT,CTC -> CTG,GAG	36 -> 38	Substitution	Substitution	30.6% -> 34.2%	1.5E-31	392461	sbmA		BW25113_0377
COL3	392137	392145	9	1	SAF -> IQG	TCGGCCTTC -> ATTCAGGGG	TCG,GCC,TTC -> ATT,CAG,GGG	37 -> 38	Substitution	Substitution	34.2% -> 35.1%	5.3E-30	392470	sbmA		BW25113_0377
COL3	392147	392153	6	2	VWA -> ASL	TTTGGGC -> CCAGTCT	GTT,TGG,GCA -> GCC,AGT,CTA	37 -> 41	Substitution	Substitution	34.1% -> 37.8%	6E-37	392481	sbmA		BW25113_0377
COL3	392162	392164	3	1	AV -> GF	CCG -> GAT	GCC,GTT -> GGA,TTT	40	Substitution	Substitution	0.4	2.5E-44	392492	sbmA		BW25113_0377
COL3	392167	392168	2	1	I -> A	AT -> GC	ATC -> GCC	37	Substitution	Substitution	0.351	4.4E-34	392496	sbmA		BW25113_0377
COL3	392170	392181	11	2	FWQA -> PIFP	TTCTGGCAAGCC -> CCTATATTTCCA	TTC,TGG,CAA,GCC -> CCT,ATA,TTT,CC/	36 -> 39	Substitution	Substitution	25.6% -> 35.1%	6.3E-24	392509	sbmA		BW25113_0377
COL3	392183	392186	4	1	GG -> DM	GTGG -> ACAT	GGT,GGG -> GAC,ATG	39	Substitution	Substitution	0.256	6.3E-24	392514	sbmA		BW25113_0377
COL1	392895	392895	1	1		(G)4 -> (G)3		132	Deletion (tandem repeat)	Frame Shift	100.00%	0	393441	sbmA		BW25113_0377
COL3	393874	393875	2	1	F -> A	TT -> GC	TTT -> GCT	62 -> 63	Substitution	Substitution	25.4% -> 25.8%	1.1E-40	394203	sbmA		BW25113_0377
COL3	393878	393880	3	1		CCG -> GGT		66	Substitution	Truncation	0.288	4.3E-49	394208	sbmA		BW25113_0377
COL3	393883	393885	2	2	Q -> D	CAG -> GAT	CAG -> GAT	68 -> 69	Substitution	Substitution	27.5% -> 27.9%	1.3E-52	394213	sbmA		BW25113_0377
COL3	393888	393888	1	1		#NAME?		70	Deletion	Frame Shift	0.271	1.6E-48	394216	sbmA		BW25113_0377
COL2	477705	477705	1	1	N -> D	T -> C	AAC -> GAC	173	SNP (transition)	Substitution	1	0	478239	acrB		BW25113_0462
COL3	478985	478985	1	1	K -> T	T -> G	AAG -> ACG	106	SNP (transversion)	Substitution	0.368	4.8E-108	479396	acrB		BW25113_0462
COL1	479619	479619	1	1	N -> D	T -> C	AAC -> GAC	157	SNP (transition)	Substitution	100.00%	0	480297	acrB		BW25113_0462
COL2	550713	550713	1	1	Q -> P	A -> C	CAG -> CCG	196	SNP (transversion)	Substitution	0.985	0	551346	cysS		BW25113_0526

COL1	958245	958245	1	1	D -> E	T -> A	GAT -> GAA	121	SNP (transversion)	Substitution	100.00%	0	959513	rpsA		BW25113_0911
COL3	1460467	1460467	1	1		G -> A	GCG -> GCA	106	SNP (transition)	None	0.283	2E-55	1461663	ydbA		BW25113_4492
COL2	1613552	1613561	10	1				139	Deletion	Frame Shift	0.986	0	1615358	marR		BW25113_1530
COL1	1903022	1903022	1	1	M -> E	A -> T	GTG -> GAG	133	SNP (transversion)	Start Codon Loss	100.00%	0	1905378	mgrB		BW25113_1826
COL3	2396304	2396304	1	1		(T)4 -> (T)3		67	Deletion (tandem repeat)	Frame Shift	1	1.6E-161	2398269	nuoC		BW25113_2286
COL2	2459864	2459866	3	1		TTA -> CCT		19 -> 20	Substitution		89.5% -> 90.0%	1.4E-54	2462570		mlaA (U)	BW25113_2346
COL2	2459868	2459869	2	1		CA -> GT		17 -> 18	Substitution		82.4% -> 83.3%	2.6E-47	2462573		mlaA (U)	BW25113_2346
COL2	2459873	2459876	4	1		CGCT -> TATC		9 -> 10	Substitution		77.8% -> 80.0%	5.7E-23	2462580		mlaA (U)	BW25113_2346
COL2	2459879	2459879	1	1		C -> T		9	SNP (transition)		0.778	9E-26	2462583		mlaA (U)	BW25113_2346
COL2	2459881	2459885	5	1		TCCGT -> AAACC		7 -> 9	Substitution		57.1% -> 71.4%	2.1E-13	2462589		mlaA (U)	BW25113_2346
COL2	2459888	2459889	2	1		CA -> AT		4	Substitution		0.5	0.00000038	2462593		mlaA (U)	BW25113_2346
COL2	2467122	2467122	1	1	G -> R	G -> A	GGG -> AGG	2	SNP (transition)	Substitution	1	0.00000001	2469826	yfdP		BW25113_2359
COL2	2467256	2467256	1	1		T -> A	CGT -> CGA	2	SNP (transversion)	None	1	0.00000004	2469960	yfdP		BW25113_2359
COL2	2469909	2469909	1	1	V -> I	G -> A	GTT -> ATT	4	SNP (transition)	Substitution	1	0.000000004	2472614	torI		BW25113_4501
COL2	2469914	2469920	6	2	IHG -> IKT	CCACGGG -> AAAAACC	ATC,CAC,GGG -> ATA,AAA,ACC	6 -> 7	Substitution	Substitution	1	1.6E-11	2472625	torI		BW25113_4501
COL2	2469923	2469926	4	1	RA -> RH	AGCA -> GCAT	CGA,GCA -> CGG,CAT	7 -> 8	Substitution	Substitution	1	4E-16	2472631	torI		BW25113_4501
COL2	2469928	2469928	1	1	R -> K	G -> A	AGA -> AAA	9	SNP (transition)	Substitution	1	3.2E-23	2472633	torI		BW25113_4501
COL2	2469933	2469934	2	1	L -> R	TT -> CG	TTA -> CGA	10	Substitution	Substitution	1	1E-26	2472639	torI		BW25113_4501
COL2	2469936	2469940	5	1	YR -> GF	TATCG -> GGGTT	TAT,CGT -> GGG,TTT	11 -> 15	Substitution	Substitution	1	1E-22	2472645	torI		BW25113_4501
COL2	2469942	2469944	2	2	D -> K	GAC -> AAG	GAC -> AAG	15	Substitution	Substitution	1	3.2E-35	2472649	torI		BW25113_4501
COL2	2469947	2469947	1	1	H -> Q	T -> A	CAT -> CAA	17	SNP (transversion)	Substitution	1	7.9E-40	2472652	torI		BW25113_4501
COL2	2469949	2469955	7	1	CEF -> SSG	GTGAATT -> CGAGCGG	TGT,GAA,TTC -> TCG,AGC,GGC	17	Substitution	Substitution	1	2E-36	2472660	torI		BW25113_4501
COL2	2469958	2469967	10	1	KNKL -> SVLT	AAAATAAGCT -> GCGTACTTAC	AAA,AAT,AAG,CTC -> AGC,GTA,CTT,AC	18 -> 22	Substitution	Substitution	1	1E-45	2472672	torI		BW25113_4501
COL2	2469969	2469973	5	1	LS -> PH	TTAAG -> CCGCA	TTA,AGC -> CCG,CAC	22 -> 24	Substitution	Substitution	87.0% -> 90.9%	1E-55	2472678	torI		BW25113_4501
COL2	2469975	2469976	2	1	R -> S	CG -> TC	CGC -> TCC	27 -> 32	Substitution	Substitution	1	2.5E-76	2472681	torI		BW25113_4501

COL2	2469978	2469980	3	1	A -> I	GCC -> ATT	GCC -> ATT	32	Substitution	Substitution	1	1E-96	2472685	torI		BW25113_4501
COL2	2469982	2469983	2	1	N -> S	AT -> GC	AAT -> AGC	32 -> 33	Substitution	Substitution	1	1E-96	2472688	torI		BW25113_4501
COL2	2469989	2469989	1	1		A -> T		39	SNP (transversion)	Extension	1	1.6E-125	2472694	torI		BW25113_4501
COL2	2469991	2469991	1	1		A -> C		41	SNP (transversion)		1	5E-136	2472696	torI		BW25113_4501
COL2	2469993	2469995	3	1		AGC -> CAT		42 -> 43	Substitution		1	4E-135	2472700	torI		BW25113_4501
COL2	2469997	2470000	4	1		GGTA -> CCGC		44 -> 51	Substitution		1	1E-135	2472705	torI		BW25113_4501
COL2	2470002	2470007	6	1		AATATT -> TTGTCC		53 -> 59	Substitution		98.1% -> 98.3%	5E-165	2472712	torI		BW25113_4501
COL2	2470011	2470011	1	1		C -> T		59	SNP (transition)		1	2E-195	2472716	torI		BW25113_4501
COL2	2470013	2470014	2	1		CA -> GT		62 -> 63	Substitution		1	1.6E-211	2472719	torI		BW25113_4501
COL2	2470016	2470017	2	1		CT -> AA		65 -> 68	Substitution		1	1E-208	2472722	torI		BW25113_4501
COL2	2470019	2470021	3	1		AAA -> TGG		68 -> 78	Substitution		1	5E-227	2472726	torI		BW25113_4501
COL2	2470023	2470022	0	1		#NAME?		78	Insertion		1	6.3E-266	2472728	torI		BW25113_4501
COL2	2470024	2470024	1	1		C -> T		81	SNP (transition)		1	4E-276	2472730	torI		BW25113_4501
COL2	2470026	2470026	1	1		C -> A		81	SNP (transversion)		1	5E-268	2472732	torI		BW25113_4501
COL2	2470028	2470030	3	1		ATT -> GAG		81	Substitution		1	5E-268	2472736	torI		BW25113_4501
COL2	2470033	2470034	2	1		AA -> CC		84	Substitution		1	4E-261	2472740	torI		BW25113_4501
COL2	2470037	2470055	19	1		AATCCCCTGCTGCTTCAAG -> CTAAGGGCTAATTGCAGGT		90 -> 95	Substitution		97.8% -> 97.9%	4E-295	2472761	torI		BW25113_4501
COL2	2470057	2470057	1	1		A -> C		95	SNP (transversion)		1	0	2472763	torI		BW25113_4501
COL2	2470061	2470062	2	1		GT -> TC		98 -> 99	Substitution		0.99	0	2472768	torI		BW25113_4501
COL3	2563837	2563837	1	1	G -> A	C -> G	GGG -> GCG	10	SNP (transversion)	Substitution	0.3	7.6E-09	2565948	eutE		BW25113_2455
COL3	2902900	2902900	1	1	D -> E	A -> T	GAT -> GAA	96	SNP (transversion)	Substitution	1	0	2905264	pyrG		BW25113_2780
COL3	3321805	3321807	3	1		AGC -> TTA		42 -> 43	Substitution	Truncation	25.6% -> 26.2%	3.6E-26	3324499	greA		BW25113_3181
COL3	3321810	3321821	10	3	RVIFG -> LMPIS	CAAAAATAACGC -> TGATCGGCATT	CGC,GTT,ATT,TTT,GGT -> CTA,ATG,CCG,ATC,AGT	42 -> 46	Substitution	Substitution	35.7% -> 50.0%	3.1E-42	3324513	greA		BW25113_3181
COL3	3321833	3321833	1	1	P -> H	G -> T	CCC -> CAC	48	SNP (transversion)	Substitution	0.375	1.8E-54	3324525	greA		BW25113_3181
COL3	3407131	3407131	1	1	K -> R	T -> C	AAA -> AGA	36	SNP (transition)	Substitution	0.278	2.5E-25	3409889		acrE (U)	BW25113_3265

COL2	3793704	3793706	3	1	DL -> GL	GAT -> AGC	GAT,CTA -> GGC,TTA	79 -> 80	Substitution	Substitution	25.0% -> 25.3%	3.5E-52	3798058	waaY (rfaY)		BW25113_3625
COL2	3793708	3793710	3	1	R -> L	TCT -> GAG	AGA -> CTC	83 -> 87	Substitution	Substitution	27.7% -> 31.4%	2.9E-63	3798062	waaY (rfaY)		BW25113_3625
COL2	3793713	3793724	11	2	IKNEI -> SGEIL	TCTCATTTTTAA -> GAATTTCCCCGC	ATT,AAA,AAT,GAG,ATT -> AGC,GGG,GAA,ATT,CTT	88 -> 90	Substitution	Substitution	36.0% -> 40.0%	2.3E-80	3798076	waaY (rfaY)		BW25113_3625
COL2	3793726	3793737	12	1	RHYG -> KVRI	ACCGTAATGACG -> TATTCGCACCTT	CGT,CAT,TAC,GGT -> AAG,GTG,CGA,AT	95 -> 100	Substitution	Substitution	46.9% -> 54.5%	1.8E-110	3798089	waaY (rfaY)		BW25113_3625
COL2	3793739	3793739	1	1	E -> G	T -> C	GAG -> GGG	105	SNP (transition)	Substitution	0.629	1.5E-189	3798091	waaY (rfaY)		BW25113_3625
COL2	3793745	3793746	2	1	D -> S	TC -> GA	GAC -> TCC	106 -> 107	Substitution	Substitution	30.8% -> 33.0%	1E-78	3798098	waaY (rfaY)		BW25113_3625
COL2	3793748	3793748	1	1		A -> GG		105	Insertion	Frame Shift	0.276	3.6E-56	3798101	waaY (rfaY)		BW25113_3625
COL2	3793750	3793750	1	1		A -> G	CGT -> CGC	104	SNP (transition)	None	0.279	7.1E-68	3798103	waaY (rfaY)		BW25113_3625
COL2	3793754	3793754	1	1	D -> V	T -> A	GAT -> GTT	104	SNP (transversion)	Substitution	0.279	7.1E-68	3798108	waaY (rfaY)		BW25113_3625
COL2	3793756	3793758	3	1	K -> L	TTT -> AAG	AAA -> CTT	104 -> 105	Substitution	Substitution	25.0% -> 26.7%	8.7E-65	3798112	waaY (rfaY)		BW25113_3625
COL3	3794080	3794082	3	1	Y -> I	GTA -> TAT	TAC -> ATA	49 -> 50	Substitution	Substitution	24.0% -> 26.0%	2.2E-33	3797196	waaY (rfaY)		BW25113_3625
COL3	3794084	3794092	9	1	KGDY -> NKSL	TAATCACCT -> AGGGACTTG	AAA,GGT,GAT,TAT -> AAC,AAG,TCC,CT	46 -> 50	Substitution	Substitution	26.5% -> 30.4%	2.5E-28	3797206	waaY (rfaY)		BW25113_3625
COL3	3794095	3794103	9	1	SLL -> EGA	TAACAGAGA -> CGCACCTTC	TCT,CTG,TTA -> GAA,GGT,GCG	39 -> 45	Substitution	Substitution	31.1% -> 40.5%	2.1E-33	3797217	waaY (rfaY)		BW25113_3625
COL3	3794109	3794109	1	1	F -> L	A -> G	TTT -> CTT	42	SNP (transition)	Substitution	0.548	1.7E-53	3797223	waaY (rfaY)		BW25113_3625
COL3	3794113	3794114	2	1		AC -> G		36 -> 37	Deletion	Frame Shift	35.1% -> 36.1%	2.8E-17	3797228	waaY (rfaY)		BW25113_3625
COL3	3794117	3794117	1	1	E -> A	T -> G	GAA -> GCA	37	SNP (transversion)	Substitution	0.324	1.8E-27	3797231	waaY (rfaY)		BW25113_3625
COL3	3794120	3794120	1	1		T -> AA		36 -> 37	Insertion	Frame Shift	27.0% -> 27.8%	8.8E-19	3797235	waaY (rfaY)		BW25113_3625
COL3	3794123	3794123	1	1	R -> L	C -> A	CGT -> CTT	36	SNP (transversion)	Substitution	0.417	1.7E-37	3797238	waaY (rfaY)		BW25113_3625
COL3	3794126	3794132	7	1	KVK -> ISP	TTAACTT -> GGGGAAA	AAA,GTT,AAG -> ATT,TCC,CCG	34 -> 36	Substitution	Substitution	27.8% -> 31.4%	1.7E-18	3797250	waaY (rfaY)		BW25113_3625
COL3	3794134	3794147	14	1	KVFSP -> SQPRR	CGGAGAAAAAACCT -> TCTTCTCGGCTGAC	AAG,GTT,TTT,TCT,CCG -> AGT,CAG,CCG,AGA,AGA	35 -> 39	Substitution	Substitution	25.6% -> 28.6%	1.8E-20	3797265	waaY (rfaY)		BW25113_3625
COL3	3794149	3794149	1	1		A -> C	CTT -> CTG	39	SNP (transversion)	None	0.256	6.1E-20	3797267	waaY (rfaY)		BW25113_3625
COL1	3794182	3794187	5	2	VM -> LQ	CATAAC -> TTGGAG	GTT,ATG -> CTC,CAA	89 -> 92	Substitution	Substitution	25.6% -> 27.8%	3.20E-46	3798705	waaY (rfaY)		BW25113_3625
COL1	3794189	3794190	2	1		TT -> CA		84 -> 87	Substitution	Truncation	33.3% -> 34.5%	2.00E-73	3798708	waaY (rfaY)		BW25113_3625
COL1	3794192	3794195	4	1		GTAT -> TACC		83 -> 85	Substitution	Truncation	33.7% -> 36.5%	2.20E-64	3798713	waaY (rfaY)		BW25113_3625
COL1	3794205	3794207	3	1	RS -> HP	AAC -> GGT	CGT,TCT -> CAC,CCT	68 -> 73	Substitution	Substitution	50.0% -> 53.4%	1.10E-103	3798725	waaY (rfaY)		BW25113_3625

COL1	3794210	3794214	5	1	VF -> QH	AAAAC -> TGCTG	GTT,TTT -> CAG,CAT	63 -> 65	Substitution	Substitution	46.0% -> 47.7%	3.80E-90	3798732	waaY (rfaY)		BW25113_3625
COL1	3794216	3794219	4	1	IK -> SW	TTGA -> CAAC	ATC,AAG -> AGT,TGG	59 -> 64	Substitution	Substitution	40.7% -> 46.0%	7.30E-70	3798737	waaY (rfaY)		BW25113_3625
COL1	3794221	3794226	5	2	NI -> SV	GATATT -> TACTGA	AAT,ATC -> TCA,GTA	58 -> 61	Substitution	Substitution	33.9% -> 37.9%	2.80E-57	3798744	waaY (rfaY)		BW25113_3625
COL1	3794228	3794228	1	1	I -> K	A -> T	ATA -> AAA	59	SNP (transversion)	Substitution	33.90%	2.80E-61	3798746	waaY (rfaY)		BW25113_3625
COL1	3794231	3794231	1	1	N -> T	T -> G	AAT -> ACT	61	SNP (transversion)	Substitution	29.50%	8.10E-47	3798749	waaY (rfaY)		BW25113_3625
COL1	3794233	3794233	1	1		A -> G	TAT -> TAC	60	SNP (transition)	None	28.30%	6.00E-44	3798751	waaY (rfaY)		BW25113_3625
COL1	3794236	3794236	1	1		A -> T	TCT -> TCA	59	SNP (transversion)	None	27.10%	1.10E-42	3798754	waaY (rfaY)		BW25113_3625
COL3	3816888	3816888	1	1	E -> K	G -> A	GAG -> AAG	77	SNP (transition)	Substitution	1	1.6E-262	3820025	spoT;		BW25113_3650
COL1	4094750	4094750	1	1	H -> Y	G -> A	CAT -> TAT	70	SNP (transition)	Substitution	100.00%	1.00E-252	4099606	cpxA		BW25113_3911
COL1	4322812	4322812	1	1	R -> P	C -> G	CGC -> CCC	100	SNP (transversion)	Substitution	100.00%	0	4327897	pmrB (basS)		BW25113_4112
COL3	4322812	4322812	1	1	R -> P	C -> G	CGC -> CCC	96	SNP (transversion)	Substitution	1	0	4326329	pmrB (basS)		BW25113_4112
COL2	4322812	4322812	1	1	R -> P	C -> G	CGC -> CCC	168	SNP (transversion)	Substitution	1	0	4327782	pmrB (basS)		BW25113_4112
COL3	4325475	4325475	1	1		T -> A		89	SNP (transversion)		1	3.2E-312	4328997	adiC		BW25113_4115
COL2	4325475	4325475	1	1		T -> A		89	SNP (transversion)					adiC		BW25113_4115