Escherichia coli genes that are required for the Ff phage lifecycle

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Abstract

Ff phages have remarkably few genes, yet are able to perform the complex task of infecting *Escherichia coli* via a process involving F pilus binding and retraction at the start of its life cycle, and releasing progeny from a host cell by a secretion-like process without killing it. The Ff phage-encoded assembly-secretion (egress) machinery operates across all layers of E. coli envelope – inner membrane, periplasm, peptidoglycan and outer membrane. Nevertheless, phage encodes only three proteins that build a functional assembly-secretion machine. In contrast, analogous trans-envelope systems in bacteria, that mediate pilus assembly, conjugation, DNA transfer and protein secretion require tens of proteins. To date, only a few host proteins required for Ff phages infection and DNA replication have been identified, of which only one was found to be involved in assembly, and only in one of three known Ff phages. Because the Ff phages are able to be assembled and secreted using a strategy similar to those that complex transenvelope secretion-assembly systems do, yet only use 3 phage-encoded proteins for this, a hypothesis can be made that the Ff phage is using the host (*E. coli)*-encoded proteins to complete its lifecycle.

The hypothesis that many host proteins are involved in the Ff lifecycle was tested by systematic screening of 212 *E. coli* single knockout mutants, which were mated to become F positive, in a Ff phage plaque and phagemid transduction assays. The screen found 66 *E. coli* proteins which were required for the phage lifecycle, most of which had impaired the infection step, which is dependent on the F-pilus as the primary receptor. Of the mutants that failed to be infected by Ff phage, there is some overlap with previously identified mutants that were defective in F pilus conjugation donor function, which indicates that there is an overlap between the F-pilus Ff receptor and conjugation function. Some mutants identified in this thesis were not functional as donors in conjugation, hence they added to the tally of *E. coli* chromosomal genes that cannot conjugate using the F pilus.

Majority of the chromosomal mutants required for the Ff lifecycle found in this screen encode proteins that play a role in stress response pathways, or control the envelope morphology. Many proteins encoded by mutated genes that did not fall into these two broad categories were also found. Due to the large number of mutants found that are implicated in the Ff phage infection and conjugation, from just a small systematic screen in this study, a

further genetic screen is warranted to find the true extent of *E. coli* genes that are required for filamentous phage infection and conjugation.

Phage infection and conjugation play major roles in horizontal gene transfer between bacteria. Thus finding *E. coli* genes that are vital for these processes is crucial to fully understand the mechanisms of inter-bacterial horizontal gene transfer. Having a complete list of genes involved in gene transfer mediated by filamentous phage infection and conjugation would give more targets to control the spread of antibiotic resistance during antibiotic treatment, or to develop other biotechnology applications.

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Abbreviations

Amp:	Ampicillin
Amp^R:	Ampicillin resistant
bp:	Base pair(s)
CDS:	Coding DNA Sequence
Cm:	Chloramphenicol
Cm^R	Chloramphenicol resistant
DNA:	Deoxyribonucleic acid
dsDNA:	Double stranded DNA
EDTA:	Ethylenediaminetetraacetic acid
E. coli:	*Escherichia coli*
Ff:	F-pilus specific filamentous phage(s) of *E. coli*
F':	F fertility factor plasmid containing additional genes derived from *E. coli* chromosome or other plasmids
g:	gram
IM:	Inner Membrane
kb:	kilobase
Km:	Kanamycin
Km^R:	Kanamycin resistant
LPS:	LipoPolySaccharide
M:	Molar
mL:	Millilitre
μM:	Micromolar
μL:	Microlitre
NEB:	New England Biolabs
OM:	Outer Membrane
ori:	Origin of replication
PCR:	Polymerase Chain Reaction
PP:	Phagemid Particle
RF:	Replicative Form
RNA:	RiboNucleic Acid

ssDNA: Single stranded DNA

Tet: Tetracycline

Tet^R: Tetracycline resistant

T2SS: Type II secretion system

T4PS: Type IV pilus-assembly system

º C: Degree Celsius

(-): Negative

(+): Positive

2xYT: 2x Yeast Extract Tryptone medium

f2, MS2, R17, Qβ: small F-pilus-specific RNA bacteriophages

1. LITERATURE REVIEW

1.1 Ff bacteriophages

1.1.1 Overview

Within the bacteriophage phylogeny lies the *Inoviridae* family, more commonly known as the filamentous bacteriophage. They are one of the simplest known viruses, having short single-stranded circular DNA genomes that encode only a small number of proteins (Knezevic *et al.*, 2021). The Ff phages, which make up the *Inovirus* genus, are filamentous bacteriophages that are so named because they infect bacteria with the F fertility factor. The three most studied filamentous phages are Ff phages, f1, fd, and M13. They were the first three filamentous phages to be discovered, having been identified independently by three separate laboratories in the 1960s (Hoffman-Berling *et al.*, 1963; Hofschneider, 1963; Loeb, 1960). The genomes of all three are 98% identical to each other and as such, they are considered interchangeable for the purposes of study and application (Beck & Zink, 1981; Smeal *et al.*, 2017a). For brevity, the rest of this literature review will use Ff phages to refer to f1, fd and M13.

Ff phages infect *Escherichia coli* by binding to the tip of the F pilus. The pilus then retracts to pull the virion to its secondary receptor in the TolQRA complex (Lin *et al.*, 2011; Lubkowski *et al.*, 1999). After Ff phage entry, its DNA is then released into the host cytoplasm and replicated using a rolling circle mechanism (Dotto *et al.*, 1984a). The DNA is also transcribed and translated to make Ff phage proteins (Smeal *et al.*, 2017a). When high amounts of phage proteins have built up in the cell, it acts as a signal to start the packaging of the phage DNA to start virion exit from the cell.

Phage exit is when one of the most unique characteristic of filamentous bacteriophages is observed. Unlike the majority of other viruses, filamentous bacteriophages do not lyse their host cell to release their progeny. Instead, the phage virions are secreted through the host membrane without killing the cell (Marciano *et al.*, 2001).

There are two main reasons why studying the Ff phage continues to be of great importance. Firstly, the Ff phage is used as a model organism to guide research on the other filamentous bacteriophage species. This is especially salient as in a recent meta analysis of prokaryotic sequences in public databases has revealed that filamentous bacteriophage genome sequences are present in a majority of bacterial and some archaeal phyla, with the pervasiveness of this virus across two domains suggesting that this family has been present in, and evolved alongside their microbial hosts since ancient times (Roux *et al.*, 2019). As such, increased

understanding of Ff phages will further increase knowledge of all filamentous bacteriophages and give more insight into the unique evolutionary relationship between themselves and their hosts (Knezevic *et al.*, 2021). Secondly, there is an ever increasing use of filamentous phages, especially the Ff, in many biotechnological applications which would benefit from increased understanding of these phages' biology and structure (Branston *et al.*, 2011; Chung *et al.*, 2010; Gillespie *et al.*, 2015; Sattar *et al.*, 2015).

1.1.2 Structure

The Ff phage is remarkably simple, with a genome that only encodes 11 proteins (Smeal *et al.*, 2017a). Yet with only those small number of proteins it is able to undergo the complex task of completing a full viral lifecycle while keeping the cell viable. These proteins follow the naming convention of the letter 'p' followed by either a Roman or Arabic numeral from I to XI. The numbers from both styles do refer to the same proteins (for example, p1 and p4 are pI and pIV). For consistency, all proteins will be named by the roman numeral form in this literature review.

Only five proteins make up the structure of the Ff phage (Bennett *et al.*, 2011). The arrangement of these proteins around the backbone viral DNA gives the virions their characteristic shape. As their name would imply, filamentous phages are long and thin, like a filament. The Ff phage virion has a diameter of 6 nm and length of 680 nm. The length of the virion is determined by the length of its genome (Sattar *et al.*, 2015). This is because the majority of the virion length consists of just its circular single stranded DNA (ssDNA) surrounded by the major coat protein pVIII. To fit within the narrow space, the single-stranded circular DNA is stretched out so the sides of the circle are brought close together, though forming a quasi-Watson-Crick double-helix (Marvin *et al.*, 2006). Two minor coat proteins flank each side of the virion, pIII and pVI are on one end while pVII and pIX are on the other (Ploss & Kuhn, 2011). There are five copies of each of these minor code proteins while thousands of copies of pVIII make up the structure (Conners *et al.*, 2023; Papavoine *et al.*, 1998).

Three other proteins, pII, pV, and pX are involved in the DNA replication of the Ff phage genome (Smeal *et al.*, 2017b). The final three proteins encoded by the Ff phage are involved in the phage egress process. Inserted into the *E. coli* outer membrane is the pIV protein, which is a barrel-shaped channel of the bacterial secretin family with a gated pore that allows the Ff phage virion to egress (Conners *et al.*, 2021). pI and pXI are found in the inner membrane and assemble the virion during egress (Feng *et al.*, 1997; Haigh & Webster, 1999).

1.1.3 Ff phage lifecycle

1.1.3.1 Infection

Ff phage infection begins with the N2 domain of the pIII protein binding to its primary receptor on *E. coli*, the tip of the F pilus (Fig 1.1) (Lin *et al.*, 2011). The F pilus then retracts which brings the pIII (N1 domain) to its secondary receptor, the periplasmic domain of the protein TolA (Deng & Perham, 2002). It was initially proposed that the binding of the Ff phage triggers the F pilus retraction, however, live cell imaging studies show that the retraction is part of normal F pilus movement (Clarke *et al.*, 2008). The mechanism in which the F pilus retraction brings the virion through the outer membrane to interact with TolA has still not yet been resolved (Bennett *et al.*, 2011). Likewise, little is known about the steps that occur after pIII binds to TolA. It is only known that binding to TolA causes the virion to disassemble in a way that its coat proteins integrate into the inner membrane and its DNA is released into the cytoplasm (Fig. 1.1) (Bennett & Rakonjac, 2006). Despite only its interaction with TolA having been resolved, it has been long understood that all three proteins in the TolQRA complex (TolQ, TolR, and TolA) are necessary for successful Ff phage infection (Click & Webster, 1998). Though it has been demonstrated phage infection can still occur in a non-functional TolQRA complex and a TolQR deletion if the homologous proteins, ExbB and ExbD, are present (Samire *et al.*, 2020). Despite being the primary receptor, the F pilus is nevertheless not absolutely required for phage infection, though the infectivity rate of Ff phage in F-negative (F^-) *E. coli* is extremely low, 10^{-6} compared to F^+ *E. coli* (Russel *et al.*, 1988).

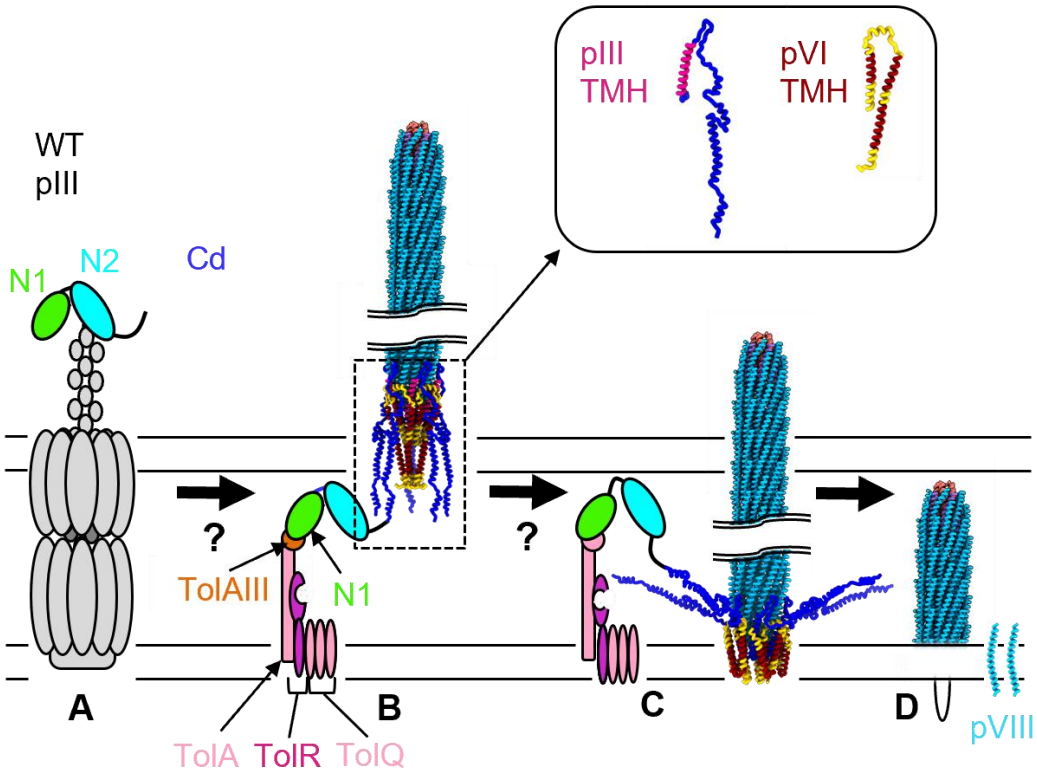

Figure 1.1: Diagram of Ff phage entry

To the left of the image in part A, the first step of Ff phage infection, binding of the N2 Domain of pIII to the F pilus, is depicted. Though a yet unresolved mechanism, retraction of the F pilus brings the Ff phage through the *E. coli* outer membrane so the N1 domain can bind to TolA of the TolQRA complex shown in part B. Parts C and D show the viral proteins disassembling into the inner membrane after binding to TolA, which causes the release of the Ff phage genome into the cytoplasm of the infected cell. Image used with permission (Rakonjac *et al.*, 2024).

1.1.3.2 DNA replication

The single stranded DNA that is encapsulated in the virion and released into the host cytoplasm during infection step of the lifecycle corresponds to the (+) strand of the DNA (Fig. 1.2). Replication of Ff phage DNA begins with synthesizing the complementary (-) strand. The synthesis starts at the (-) origin of replication on the (+) strand when the *E. coli* RNA polymerase binds to the origin and forms a short RNA primer (Higashitani *et al.*, 1996). The recognition of the (-) origin of replication by RNA polymerase is possible because the ssDNA of this site forms a secondary structure that mimics the features of the transcriptional promoter sequence in *E. coli*. The affinity of the (-) origin of replication to RNA polymerase

even exceeds the affinity of RNA polymerase to some promoters within the *E. coli* genome (Higashitani *et al.*, 1997). DNA Polymerase III from *E. coli* then extends the (-) strand from the free 3'-OH end of the RNA primer synthetized by the RNA polymerase to create double stranded Ff phage DNA, also called the replicative form DNA (RF DNA). Transcription of Ff phage proteins then immediately begins from all five promoters on the RF DNA for the synthesis of all 11 proteins (Smeal *et al.*, 2017a). The synthesis of pII is required for replication of the Ff phage (+) strand DNA. pII nicks the RF DNA at the (+) origin of replication. The free 3'-OH created by the nick is then recognised by DNA Polymerase III which will then synthesize the new (+) strand by the rolling-circle mechanism (Dotto *et al.*, 1984a).

The (+) DNA strand that was formed can be used in one of two ways. Early in infection, the (+) strand is once again used as a template for the (-) strand synthesis, to produce RF DNA. In late infection, however, the (+) strand is marked for encapsulation into the virion particle (Fig 1.2). The regulation of these processes is carried out by pV which has dual functions, both of which are triggered by the accumulation of pV in the cell. The first function is that it prevents further synthesis of pII and pX by binding to their mRNA as an operator (Michel & Zinder, 1989). The other role is to sequester the (+) single stranded DNA for packaging into virions. It does the latter by binding to the DNA to form a filament protein-DNA complex that compresses the circular ssDNA into its long quasi-double-helix forn required for it to fit into the filamentous virion (Guan *et al.*, 1995).

Along with pII and pV, pX also functions to regulate the DNA synthesis of Ff phage. The gene encoding pX is entirely nestled within the pII gene and is translated from the same mRNA from an internal ribosome binding site. Though the role of pX has not yet been fully studied, it is believed to bind to pII to prevent it from nicking the RF DNA for replication (Smeal *et al.*, 2017b).

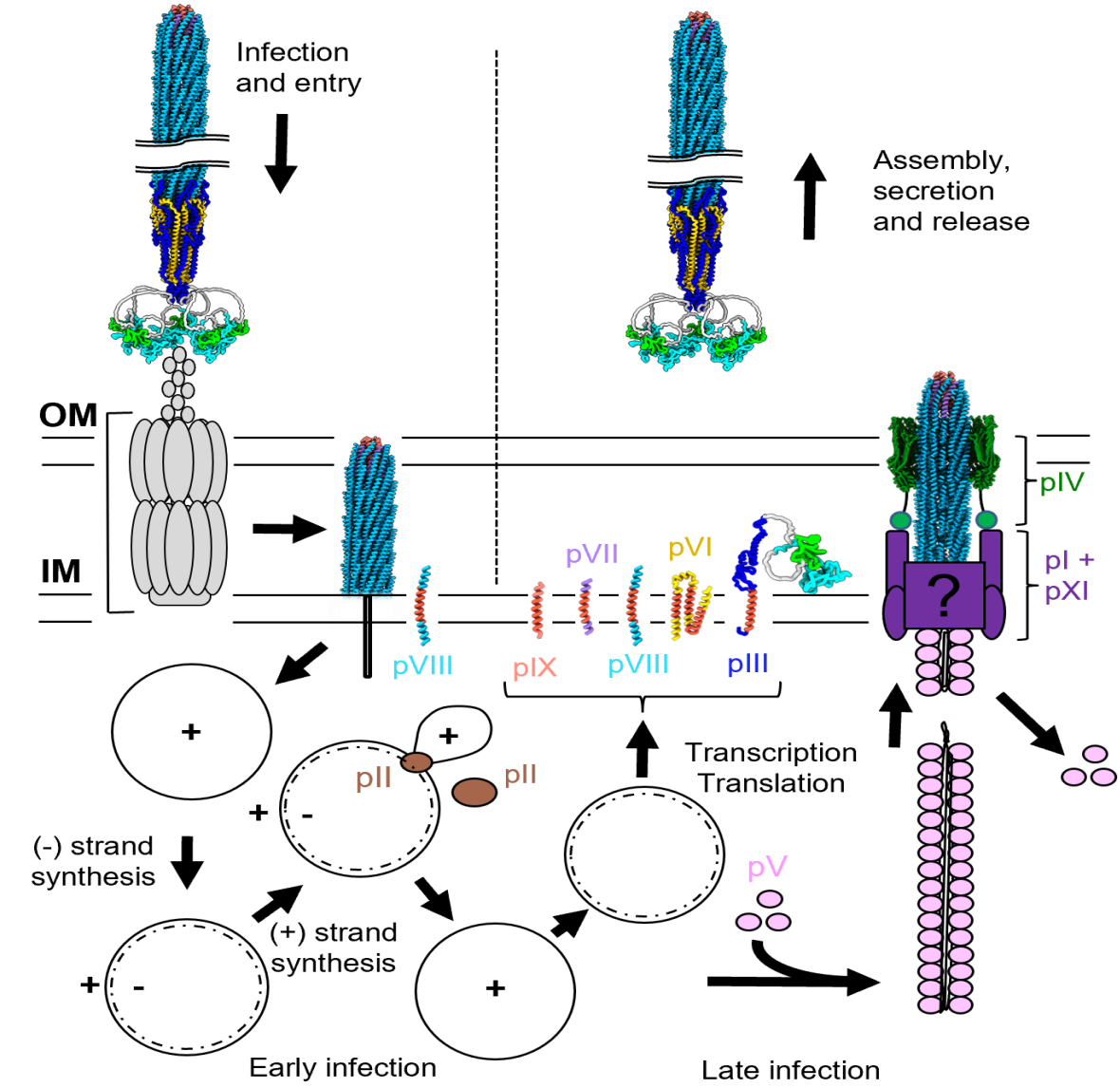

Figure 1.2: Diagram of the Ff phage lifecycle

On the top left, Ff phage which is bound to the F-pilus is shown. The F-pilus will then retract to pull the phage to the TolQRA complex through which the Ff phage will enter the cell. The TolQRA-mediated entry is depicted immediately to the right. On the bottom half of the image, the fate of the phage genome released into the host cell is depicted. The genome is used for complementary negative strand synthesis which would then allow for the synthesis of more positive strand phage DNA or transcription and translation of the phage protein. Later in the infection cycle, pV binds to the positive strand phage genome to prevent more negative strand synthesis and to transport the DNA to the pI/pXI complex as depicted on the right of the image. There the DNA will be packaged by the Ff phage structural proteins as the virion is being secreted through pIV (Rakonjac *et al.*, 2024) adopted with permission.

1.1.3.3 Assembly and egress

As phage DNA replication occurs, Ff phage structural and egress genes are also being transcribed from the RF DNA and the resulting mRNA translated into proteins. The coat proteins of Ff phage are targeted to and become integral membrane proteins of the inner membrane of the *E. coli* in preparation for phage assembly (Endemann & Model, 1995; Park *et al.*, 2010) (Fig 2). Integration of large amounts of major coat protein pVIII into the inner bacterial membrane, especially the amount that would be required for phage production, would usually be prevented by the limited amount of the SecYEG protein translocation complex. However, Ff phage overcomes this by the major coat protein pVIII using the Sec-independent YidC translocon complex (Chen *et al.*, 2002; Ploss & Kuhn, 2011).

Along with the phage coat proteins, the three proteins that make up the phage secretion complex are also targeted to the *E. coli* membranes. Specifically, pIV is targeted to the outer membrane of the cell, while pI and pXI are targeted to the inner membrane (Spagnuolo *et al.*, 2010). The pIV is a bacterial secretin family member that has a double-barrel-like shape with a gated channel in the centre that allows for the virion to egress through it (Marciano *et al.*, 2001). The pI and pXI proteins are both essential for the assembly of the virion with the pI containing an ATP binding domain which carries out the ATP hydrolysis necessary for phage assembly (Feng *et al.*, 1997; Haigh & Webster, 1999).

Ff phage assembly begins when the packaging signal, a hairpin structure in the Ff phage genome, associates with minor coat proteins, pVII and pIX. Mechanistic and structural details of assembly have not been determined, however genetic data show that these two proteins interact with pI, an ATPase which plays the role of inner membrane assembly machine. The pV-DNA complex likely threads through pI (which is known to be embedded in the inner membrane), a process that leads to pV disassociation from the DNA and replacement by pVIII. (Fig. 1.2) (Russel & Model, 1989). When pVIII has covered all the DNA, the distal end of the virion is capped by pIII and pVI, and the fully formed virion is released from the cell (Rakonjac *et al.*, 1999).

1.2 *E. coli* components required for the Ff lifecycle

1.2.1 F pilus

The F pilus, which serves as the Ff primary receptor, is the first example of conjugative plasmid transfer system to be discovered when it was found in 1946 (Lederberg & Tatum, 1946).

It mainly acts by attaching to the recipient cell and retracting, bringing it closer to the donor cell to form and stabilise a mating pair where the F plasmid DNA is then transferred to the recipient directly through the host cell membranes (Panicker & Minkley, 1985). There is, however, some evidence that direct contact is not always necessary and transfer of DNA through the F pilus itself is theoretically possible (Harrington & Rogerson, 1990).

There are two groups of *E. coli* bacteriophages that infect the host *via* the F pilus. Ff filamentous phages (f1, M13 and fd) and small RNA phages [(f2, MS2, R17 and Qβ (Schoulaker & Engelberg-Kulka, 1978)]. Ff relies on the retraction ability of the F pilus to bring the virus to the host cell membranes and to the secondary receptor, specifically the inner membrane-periplasm-located TolQRA complex, for infection to occur (Bennett *et al.*, 2011).

F pilus assembly-retraction machinery is a large and complex structure, and there are a couple dozen host genes required for the F pilus donor function in conjugation that have recently been identified (Alalam *et al.*, 2020). Nevertheless, it is not known if any *E. coli* genes outside of the F pilus are involved in supporting the Ff phage infection process.

1.2.2 *E. coli* proteins required for Ff phage lifecycle

As can be seen from its description above, the Ff phage is a remarkably simple system. Using a genome that only encodes 11 proteins, it somehow balances the delicate task of being a very productive virus yet still keeping the host *E. coli* alive (Lerner & Model, 1981). However, there is some evidence that the system may not be as simple as it appears.

One area in which there are potential gaps in the knowledge, is the phage assembly-secretion system. Biologically the assembly-secretion system of the Ff phage most closely resembles that of the Type II and III secretion and the Type IV pilus assembly system (Spagnuolo *et al.*, 2010). However all those secretion systems have complex multiple-protein structures with many components (Abrusci *et al.*, 2013; Gold *et al.*, 2015; Yan *et al.*, 2017; Zilkenat *et al.*, 2016). Meanwhile, Ff phage only uses three proteins for its assembly-secretion process.

The structure of the Ff phage outer membrane channel, secretin family protein pIV, has been solved and it is known that it is a double-walled-barrel multimeric channel protein with 15 identical radially arranged subunits in the complex. High-resolution structure identified, in the outer wall of the barrel, not only the expected hydrophobic ring corresponding to the interface with outer membrane lipid bilayer, but also a second hydrophobic ring within the part of the pIV that is located within the aqueous environment of the periplasm and formed by the C-terminal residues of each subunit. Interestingly, this is an evolutionarily conserved sequence amongst the filamentous phage secretins, and also in some type 4 pilus assembly secretins (PilQ) which is a binding surface for an accessory protein, named pilotin, which helps target the secretin to the outer membrane (Conners *et al.*, 2021). An unresolved question behind the pIV protein is how its subunits would be targeted to and assemble at the outer membrane. Similar secretins usually have a pilotin protein that guide monomers to initiate the assembly and target the secretin protein complex to the outer membrane. The presence of the hydrophobic ring strongly suggests that pIV may have a pilotin as well, however its identity is not known (Conners *et al.*, 2021).

A strong indicator that a pilotin may be necessary for pIV targeting is that when pIV chimeras are created which have their hydrophobic girdle disrupted by fusing an extra domain to the C-terminus and pIV was rendered nonfunctional. This extra domain (referred to as ”S”) is present in the Type II and III secretins, and is required for targeting to the outer membrane *via* interaction with a cognate protein referred to as a “pilotin”. Pilotins are typically outer membrane lipoproteins. Interestingly, the function of those pIV-domain S chimeras can be restored if a cognate pilotin is co-expressed in the same cell (Daefler *et al.*, 1997; Daefler & Russel, 1998). Given that the Ff phage genome does not encode a pilotin, if the pIV requires one for its function, such protein must be hijacked from the *E. coli* to serve as a pilotin.

The idea that Ff phage has hijacked *E. coli* proteins for its own use is not just conjecture. It is a virus after all and, as shown above, it is already known that it uses the host proteins for cell entry and DNA replication, namely the F pilus, TolQRA complex, RNA polymerase, and DNA Polymerase III. There is another known host protein that is used by Ff phage for its assembly and egress, thioredoxin (Russel & Model, 1986). Thioredoxin functions as an oxidoreductase in *E. coli* but its reducing ability is not used by Ff phage. The thioredoxin interacts with pI during the Ff phage virion assembly. So, it is likely that Ff phage may be using other *E. coli* proteins to assist its secretion process.

The Ff phage and *E. coli* have a very unique symbiotic relationship. As a virus, Ff phage is using host resources for its own reproduction, and there is strong evidence that it may be using more *E. coli* proteins beyond what is already known. So, more research needs to be done on the *E. coli* genes required to support the Ff phage system. While a few *E. coli* genes have already been identified as being essential for the Ff phage replication and assembly, the complexity of the infection and assembly indicates that there is more to be found. The identification of putative *E. coli* genes that are necessary for filamentous phage infection, replication and assembly is important because it will provide insight into the coevolution of Ff phage and *E. coli* and, by extension, the evolution of filamentous phages and their hosts. Beyond furthering evolutionary knowledge there exists many benefits to practical applications that can result from further research into this area. Filamentous phages are increasingly being used for many biotechnological applications. A greater understanding of how filamentous phages are formed can be used to increase the efficiency of their production and possibly allow for greater manipulation of these phages to further expand their uses.

1.2.3 Effects of Ff phage infection on *E. coli*

One unique characteristic of Ff phage infection is that the viral progeny are released via secretion from the host cell membrane, instead of lysing the cell. Thus, the infected *E. coli* remains viable (Marciano *et al.*, 2001). Despite the host survival, the high production rate of Ff phage (1000 per cell per generation) places tremendous stress on the host cell, so infected *E. coli* grows and divides slower than uninfected, having twice the doubling time compared to uninfected cells. The slowed growth rate is the most observable trait of infected cells, with the infected *E. coli* forming small transparent colonies or thinner bacterial lawns compared to uninfected *E. coli* (Rakonjac *et al.*, 2017). This decrease in *E. coli* growth rate is a result of Ff phage hijacking its host cell resources to sustain its production. The virus uses the DNA replication and transcription machinery of the host to form the many copies of the viral genome and proteins required to sustain its high progeny production rate (Dotto *et al.*, 1984b; Higashitani *et al.*, 1997). The assembly of Ff phage also requires the use of host ATP (Loh *et al.*, 2017). The host resources allocated to Ff phage production decreases in subsequent generations of *E. coli* as each generation produces fewer Ff phage per cell (Smeal *et al.*, 2017a, 2017b).

Another characteristic of Ff phage production is the high levels of membrane stress that it causes to *E. coli*. One known cause of this stress is Ff-encoded outer membrane assembly channel pIV, which is sometimes mistargeted to the inner membrane of *E. coli* instead of the

outer membrane. The large diameter of pIV appears to cause the induction of Phage Shock Protein (PSP), an inner membrane stress response (Brissette *et al.*, 1990; Mai-Prochnow *et al.*, 2015). Another cause of membrane stress is the large number (million per cell) of the major coat protein copies that accumulate in the inner membrane (Rakonjac *et al.*, 2017). Like other Ff virion proteins, the pVIII major coat protein has first integrate into the inner membrane before they are fed into the Ff assembly machinery (Park *et al.*, 2010). The amount of protein that can be integrated into the inner membrane is limited by the SecYEG translocation complex, however, pVIII bypasses this limit by using an alternative translocon protein, YidC (Chen *et al.*, 2002; Ploss & Kuhn, 2011).

On top of targeting its coat proteins to the inner membrane, Ff phage infection also changes the composition of phospholipids in the *E. coli* inner membrane. *E. coli* has two groups of phospholipids in its inner membrane, zwitterionic and acidic phospholipids, which are both kept at a constant ratio to each other. Its zwitterionic phospholipid is phosphatidylethanolamine, and its two acidic phospholipids are phosphatidylglycerol and cardiolipin (Rowlett *et al.*, 2017). However, Ff phage infection causes an increase in the amount of cardiolipin in the cell, without the corresponding expected decrease in phosphatidylglycerol or increase in phosphatidylethanolamine. Conversely, the levels of phosphatidylethanolamine are decreased and phosphatidylglycerol levels are increased (Woolford *et al.*, 1974). In wild type Ff phage infection, the change in membrane composition is temporary and reverts to normal levels after the phage progeny start being secreted. However, in Ff phage mutants which cannot assemble or secrete phage, the changes in membrane composition remain and alterations in the membrane morphology can be observed. It appears that infection with these mutants causes large and numerous folds in the inner membrane, that resemble mitochondrial cristae. These structures are not normally observed in cells infected with wild type Ff phage, unless the *E. coli* is grown at 42°C (Schwartz & Zinder, 1968; Woolford *et al.*, 1974).

Ff phage infection also results in modifications to the physiology of the *E. coli* outer membrane. Infection with Ff phage results in the continuous release of lipopolysaccharide (LPS) from the outer membrane into the surrounding media. The release rate of LPS remains constant even in late infection when secretion of Ff phage is reduced (Roy & Mitra, 1970). This LPS release is believed to cause some changes in the morphology of the outer membrane of the infected cell. Their outer membrane becomes less rough than that of the

uninfected cells and decreases in rigidity. The infected cells also form a more hydrophobic bacterial lawn (Chen *et al.*, 2009).

Whether by the result of the morphological effects of Ff phage infection mentioned above or as a result of other yet unknown reasons, there are a few effects on the survivability of *E. coli* under certain conditions as a result of Ff phage infection. One impact is that infected *E. coli* has a reduced temperature range it can survive in. When grown at 42°C the infected cells die after some time despite *E. coli* normally being able to grow at that temperature (Schwartz & Zinder, 1968). Infected cells also have a reduced survival rate when subjected to freeze-thaw cycles (Roy & Mitra, 1970). Osmotic shock is another condition that infected *E. coli* are largely susceptible to, whereas uninfected *E. coli* are not. There are also detergents that will inactivate Ff-infected, but not uninfected *E. coli*, specifically, Sarkosyl and sodium dodecyl sulphate. Despite Ff phage infection causing the shedding of LPS from the outer membrane, [which is also brought on by ethylenediamine tetraacetate (EDTA) treatment to make *E. coli* susceptible to lysozyme], there was no difference in effect of lysozyme treatment between infected and uninfected *E. coli* (Roy & Mitra, 1970). Despite most effects of Ff phage infection largely being negative for the survivability of *E. coli* there is one known effect of infection that does confer increased selective advantage to the *E. coli*. The Ff phage protein pIII causes retraction of the F pilus and also obstructs the TolQRA complex, protecting *E. coli* from being infected by lytic phage that use either of those as receptors (Rakonjac *et al.*, 2017) and also protecting it from colicins that use TolQRA as a receptor (Cascales *et al.*, 2007; Sun & Webster, 1986).

1.3 Thesis aims

I hypothesize that there are yet unknown *E. coli* genes that are necessary for the Ff phage lifecycle. To identify these genes, Ff phage production and infection will be tested on curated set of *E. coli* knockout mutants from a genome-wide knockout collection. Analysis of genes required for filamentous phage infection and assembly will increase our understanding of the mechanism of these processes and identify ways to increase production for biotechnological applications. It will further identify targets for preventing conjugational transfer of antibiotic resistance genes.

2. MATERIALS AND METHODS

2.1 *E. coli* strains and growth conditions

All strains used were *E. coli* K12, derived from the Keio knockout collection (Baba *et al.*, 2006) unless otherwise specified. All strains and their genotypes are listed in Appendix I.

The *E. coli* strains were grown at 37°C on 2xYT 1.5% agar plates for solid media (25 mL per plate), and 2xYT for liquid media. For the phage and phagemid infection assays and titration, *E. coli* was grown in thin 2xYT 0.5% (soft) agar layer (3 mL) which was overlayed on top of the 1.5% Agar plates at the time of plating.

The Agar plates used for initial growth of *E. coli* taken from the Keio knockout stocks contained Kanamycin at a concentration of 50 μg/mL.

The agar plates used for F' plasmid conjugation and phage infection assays of *E. coli* contained Kanamycin at a concentration of 50 μg/mL and Tetracycline at a concentration of 15 μg/mL.

The agar plates used for f1 phage growth, titration and phage infection assays of control strain K2245 contained no antibiotic.

The agar plates used for phagemid infection assays of *E. coli* contained either a single layer of 1.5% agar (25 mL per plate) with Ampicillin at a concentration of 60 μg/mL or had two layers of 1.5% agar with the bottom layer of agar (25 mL) containing 30 μg/mL chloramphenicol and the top layer (9 mL) without antibiotic.

2.2 Phages and plasmids

The f1 phage strains are described in Table 2.1 and plasmids in Table 2.2.

Table 2.1 Phage strains

Name	Genotype	Reference
f1	Wild-type	(Loeb, 1960)
R408	f1, Δ*PS, IX T30A, IR1 gtrxA2*	(Russel *et al.*, 1986)

Table 2.2 Plasmids

Name	Description	Resistance	Reference
pUC118	High copy number phagemid vector	Amp	(Vieira & Messing, 1987)
pNJB51	Phagemid vector expressing phage protein pIII C-terminal domain under the control of the *psp* promoter	Cm	(Bennett, 2010)

Phage stocks were made on strain K2245 as a host using standard protocols (Sambrook & Russell, 2001). Phagemid particle stocks were created by infecting K2245 transformed with pUC118 or pNJB51 with the helper phage R408 using standard protocols (Sambrook & Russell, 2001). The supernatant from the cultures where phage or phagemid particles were produced were filtered through 0.22 μm syringe filters to eliminate the host cells.

Titration of the phage and phagemid particle stocks was performed as described in the phage and phagemid infection assays below. The titre of the f1 and R408 phage stocks used in the experiments was approximately 1-4x10^{10} pfu/mL. As large amounts of phage stock were required for the number of phage assays done, new phage stock was constantly made over the course of a year, thus titres would vary between batches of plates. As with the phage titre, the phagemid titre varied over the course of the study because of the amount of batches needed to be made. The approximate phagemid titre was 2-4 x10^{9} for the phagemid particles produced by pUC118 and 6-8 x10^{9} for phagemid particles produced by pNJB51.

2.3 Conjugation

Three colonies of each of the *E. coli* strains revived from the frozen Keio collection stocks (Km^R; Table 2.1.1) were crossed with the donor K2245 (Tet^R) on two-antibiotics (Kanamycin, Tetracycline) plates. Colonies that grew were then picked and clonally purified by passaging onto a fresh Kanamycin Tetracycline plate to ensure there is no contamination by the parent strains.

2.4 Ff phage infection assay

From an undiluted stock of Ff phage, serial hundred-fold dilutions were made in the sterile 2xYT medium (10^{-2}, 10^{-4}, 10^{-6}, 10^{-8} and 10^{-10}).

A single colony from an F-positive clonally-purified transconjugant *E. coli* strain obtained as described in the section above was then picked and dissolved in 100 μL of 2xYT solution, which was then mixed into 3 mL of 0.5% 2xYT (soft) agar. The soft agar was spread out on either a Kanamycin Tetracycline plate for the strains of interest, or a plate without antibiotics for the control strain K2245, and left to solidify.

A 10 μL drop of each Ff phage dilution, including undiluted, was then placed on the soft agar of each plate. The plates were left to allow the phage/phagemid stocks to dry before being moved to the 37°C warm room for overnight incubation. Assay results were scored positive when individual plaques were detected at 10^{-6} dilution (Fig. 2.1), or negative if the plaques were absent at the same dilution. Confluent clearance by undiluted stock was observed with mutants that do not support assembly, but allow infection, and these were also scored negative (not shown). Complete lack of clearance indicates mutants that do not allow infection or replication.

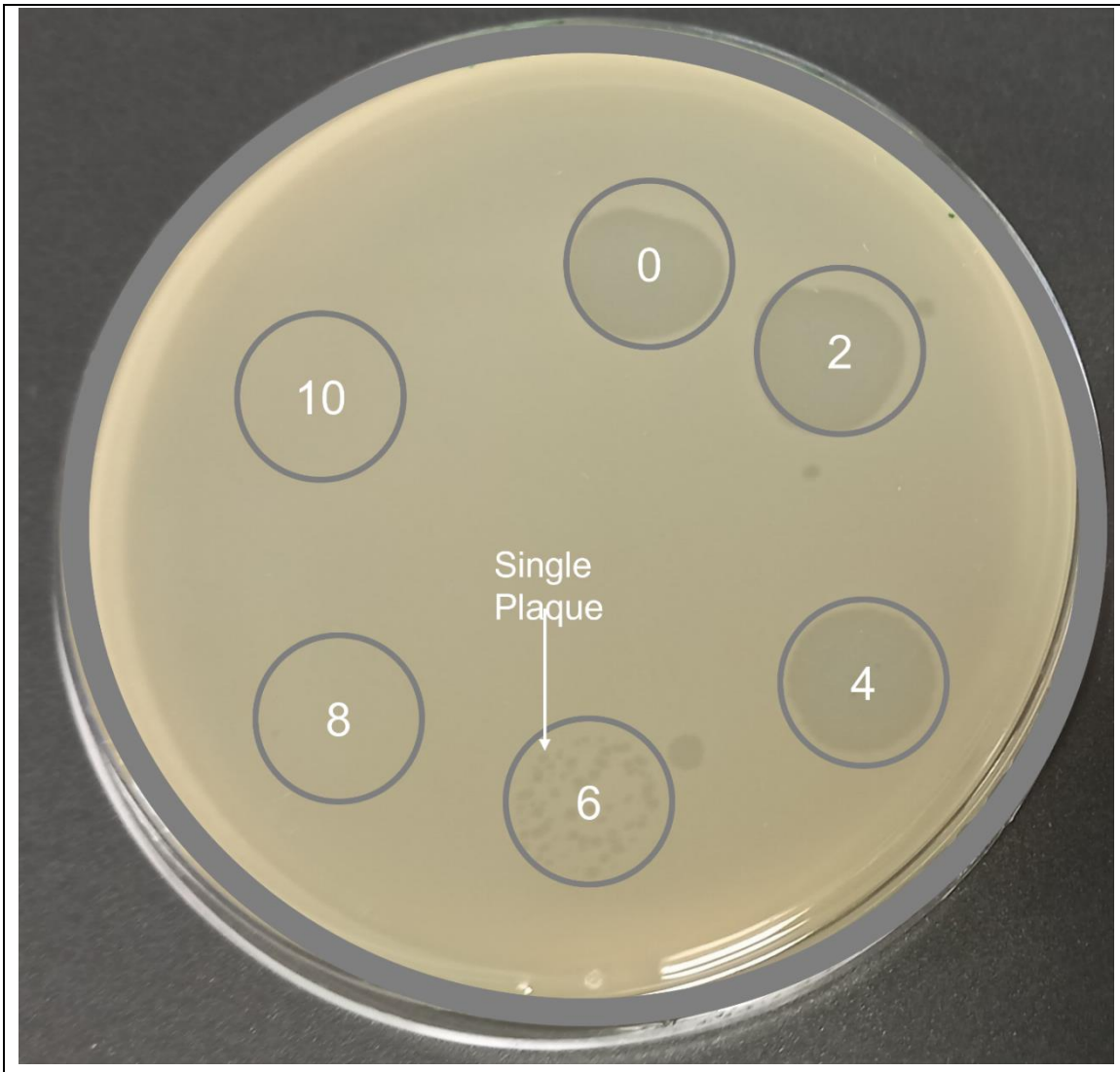

Figure 2.1: Ff Phage infection assay, positive control strain, plaques from phage infection

Dilutions of Ff phage f1 stock were spotted onto positive control strain K2245 which supports the phage lifecycle. Soft agar was inoculated with K2245 *E. coli* and was spread onto the plate to create the *E. coli* lawn. The dilutions of f1 phage stocks (10 µL each) were then spotted onto the plate, as indicated by the small grey circles in the image. The number within those circles corresponds to the dilution factor of the f1 phage stock pipetted onto the agar surface in those spots, with 0 corresponding to undiluted stock, 2 for the 10^{-2} dilution, 4 for the 10^{-4} dilution, and so on until the final dilution of 10^{-10} in the circle marked 10. The darker surfaces within the *E. coli* lawn indicate the area of the confluent phage infection (terme confluent "lysis" in case of lytic phages), where the undiluted to 10^{-4} drops were placed represent confluent areas of f1 infection causing the *E. coli* corresponding to the whole drop surface to grow more slowly due to effects of the Ff infection of all cells in the area covered by the phage stock dilution drop. As the dilution factor increases, single plaques can be seen in the lawn under the 10^{-6} drop. Single plaques correspond to the zone of the *E. coli* lawn where a single cell was infected initially with a single phage. One of these plaques have been indicated by a white arrow in the image for clarity.

2.5 Phagemid infection (transduction) assay

Strains that produced notable differences in comparison to the positive control (K2245) in the phage infection assay were further tested in a phagemid infection assay to differentiate between infection and replication- or assembly-interfering mutants..

The dilution and drop titration part of the protocol for the phagemid infection assay were carried out the same way as was described in the phage infection assay. The main change in the protocol being that phagemid stocks are used instead of Ff phage stocks, and the plates used contained antibiotics to select for host cells transduced by the phagemid which acquired the phagemid-encoded antibiotic resistance marker. Ampicillin plates were used to assay the transduction by pUC118 encoding β-lactamase (Amp^R) and two-layer chloramphenicol plates were used to check transduction with phagemid pNJB51 encoding chloramphenicol acetyl transferase (Cat; Cm^R marker). Use of chloramphenicol-resistance-encoding phagemid avoids the known phenomenon of cross-protection related to the Amp^R selection. This is due to the leakage of β-lactamase from transduced colonies into the surrounding medium which results in dense satellite colonies, impeding the counting of colonies derived from the true transductants.

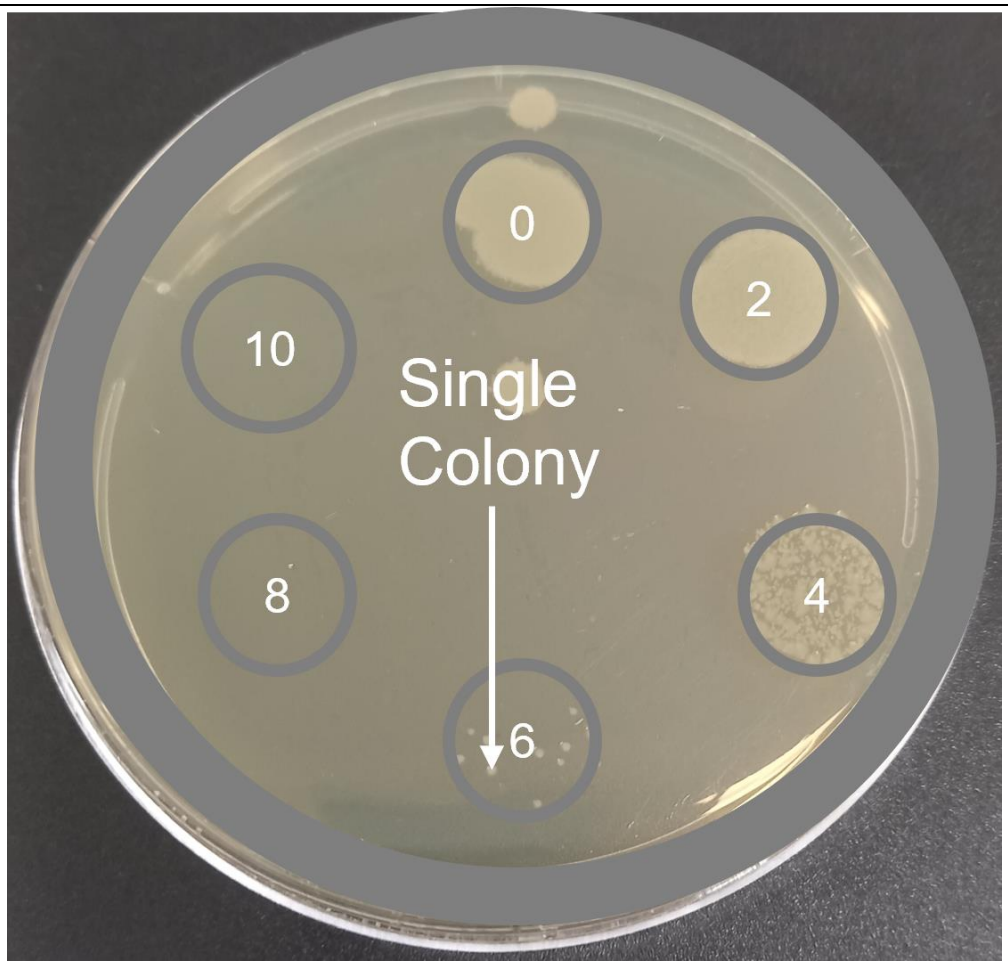

Figure 2.2 Phagemid plating assay, positive control strain, colony growth from phage transduction

Dilutions of phagemid pNJB51 stock were spotted onto lawn of K2245 on chloramphenicol plate. Successfully transduced *E. coli* acquire the chloramphenicol resistance gene of the phagemid and form a colony. The pNJB51 phagemid stock dilutions (10 μL each) were then spotted (same method as phage stocks in the plaque assay), indicated as the small grey circles in the image. The number within those circles corresponds to the dilution factor of the phagemid stocks in those spots, with 0 being where the undiluted phagemid stock was placed, 2 for the 10^{-2} dilution, 4 for the 10^{-4} dilution, and so on until the final dilution of 10^{-10} in the circle marked 10. Single bacterial colonies can be seen in the lawn under the 10^{-6} spot which would correspond to a single phagemid particle. One of these colonies is indicated by a white arrow.

2.6 Secondary cross to test mutant conjugation ability

Selected F^+ transconjugant mutant strains (Km^R, Tet^R) that could not be transfected by phagemid were subjected to a secondary cross to test their functionality as donors for F pilus conjugation. A Cm^R F^- (F-negative) strain as a recipient and selection for double resistant transconjugants on Tet Cm plates. These strains were grown on kanamycin tetracycline plates to ensure that they still retained Tet^R marker from the F plasmid received in the initial cross. Single colonies of the three clones from previous tests were then picked and each inoculated into 100 µL of 2xYT media, of which 3 µL were pipetted and spread into separate small rectangular sections of a tetracycline chloramphenicol (Tet and Cm-containing) plate. A sterilised metal loop was then used to pick up a colony of the F^- recipient strain K2607, which carries the Cm^R gene. The recipient was initially streaked onto a section of the plate that did not have the Tet^R carrying mutants, as a control to show that the K2607 could not survive on the plate without conjugation. The loop was then streaked through the section containing the F^+ mutants being tested. If the mutant strain could still produce a functioning F pilus, Tet^R Cm^R transconjugant colonies would grow in the area where the K2607 and the mutant have been crossed and conjugation has occurred.

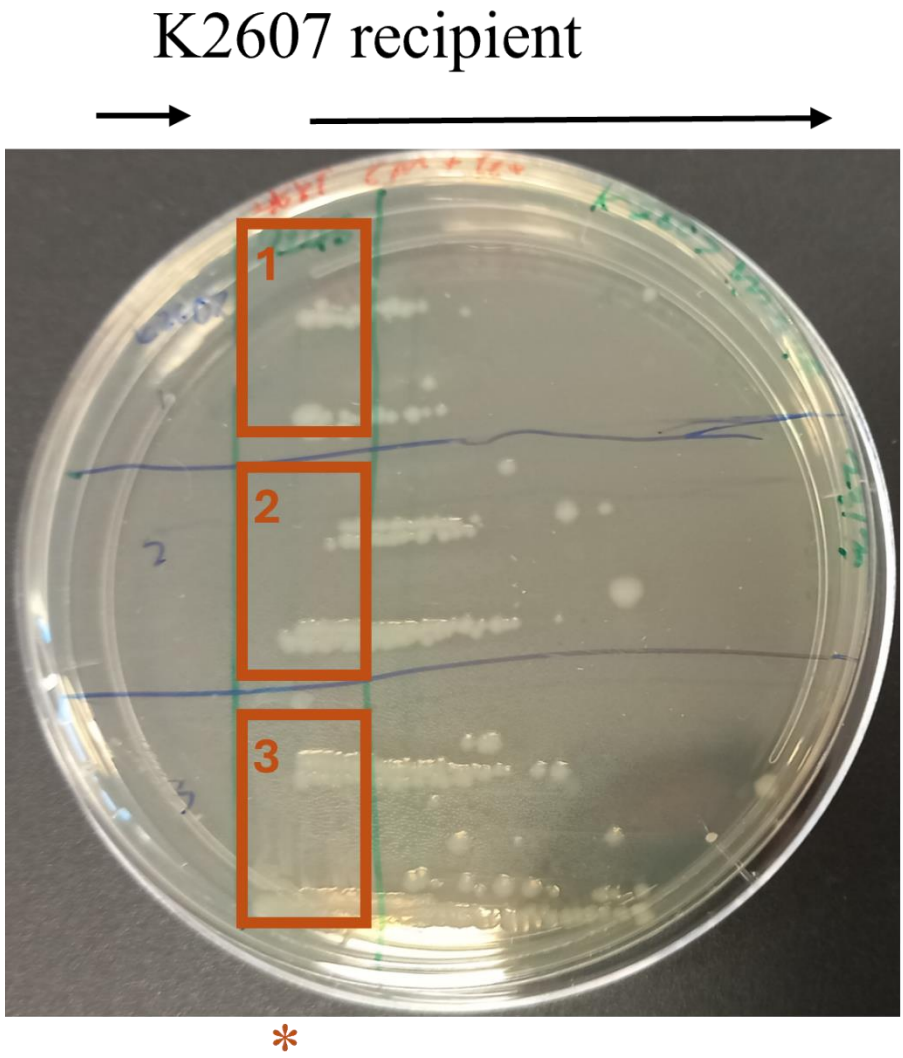

Figure 2.3 Assay of the Keio collection F' strains for conjugation donor function

Materials of colonies from donor (F' Tet^R) and recipient (Cm^R) were crossed on a selection plate for transconjugants containing both Tet and Cm. In this case, donor was control strain K2245 and recipient was K2607. As expected transconjugants only grow where the two strains overlapped. *Boxes 1, 2 and 3 represent three independent colonies of K2245.

2.7 PCR confirmation of gene knockouts

Colony PCR was used to confirm the gene knockouts that are specified by the Keio Collection. A bacterial colony was suspended in 20 µL of the autoclaved MilliQ water, of which 2 µL of the solution was used for the PCR reaction. The remainder of the PCR reaction consisted of 4 µL of NEB Taq 5× Master Mix, 0.4 µL of both the forward and reverse primer for a concentration of 2 µM, and water to make up a total reaction volume of 20 µL.

The PCR conditions were a 95°C initial denaturation step for 5 minutes, then 35 cycles of denaturation steps at 95°C for 15 seconds, annealing steps at 54°C for 30 seconds, and extension steps at 68°C for 2 minutes, followed by a single final extension step of 68°C for 5 minutes.

3. RESULTS

3.1 Introduction

The aim of this study is to identify chromosomally-encoded *E. coli* genes required for filamentous phage lifecycle. The strategy for identifying such genes was to test *E. coli* mutants in known genes for susceptibility to Ff infection by plaque assay. Furthermore, we determined whether mutation prevents the initial step of the phage cycle, infection, by susceptibility to transduction of antibiotic resistance marker by phagemid particles.

Mutants were initially selected for screening based on (i) either known physiological changes to *E. coli* caused by Ff phage infection, or (ii) the proteins within type II secretion system (T2SS) or type IV pilus-assembly systems (T4PS) which occasionally "lend" their outer membrane channels to filamentous phages (in Pseudomonades and Vibrio species).

(i) Mutations of three *clsABC* genes, *degP*, and *dnaK* mutants were selected based on known physiological changes due to the Ff infection. ClsABC are three enzymes catalysing synthesis of cardiolipin, a phospholipid that accumulates in the *E. coli* inner membrane when translation of Ff phage proteins initially begins (Rakonjac et al., 2024). It was therefore possible that inhibition of cardiolipin synthesis would prevent Ff production. The degP gene was selected as Ff-infected *E. coli* were unable to survive at 42°C, and as DegP is a heat shock protein involved in folding and quality control of periplasmic and outer membrane proteins whose mutation is lethal to *E. coli* at 42°C I hypothesised that Ff phage life cycle may directly or indirectly prevent DegP function. A dnaK mutant was also chosen as there is evidence of other phages being unable to undergo DNA replication phase of their cycle in *E. coli* without DnaK (Osipiuk *et al.*, 1993), thus it is likely that replication of Ff phage DNA might also require it.

(ii) *E. coli* chromosome contains genes encoding a complete type II secretion system (*gspCDEFGHIJKLMO*, *gspAB* and *gspS*; (Francetic *et al.*, 2000) The *gsp* cluster in *E. coli* genome include several genes encoding components that are typical in the Type II secretion systems but are missing from the Ff phage-encoded own assembly-secretion system. I hypothesised that the Ff phage might be using these other proteins from the *gsp* gene cluster on *E. coli* chromosome to build the assembly machinery that is more complex than just three phage-encoded proteins, pI, pXI and pIV. Another candidate for a protein that might be required by the Ff phage secretion system could be a lipoprotein, as the secretin channels homologous to the Ff-encoded pIV have lipoproteins which act as pilotins, whose role is to

target the secretin subunits to the outer membrane of gram-negative bacteria and assist with their assembly.

During the testing of these candidate mutants, heterogeneous results were observed from three supposedly identical clones retrieved from the Keio collection stock. Specifically, two of the candidate mutants, *gspG* and *gspJ*, were retrieved from the Keio library plate 47, and a suspicion was raised of poor handling of the clone library by the vendor (Thermo Fisher) from whom the library was purchased. As they were the first mutants discovered from that collection that had one clone from the same library well-being negative for phage infection while the other two were positive, it was thought that the two negative results might be caused by contamination from another well on that plate. Thus, the whole 96-well plate (#47) was also subsequently systematically screened to examine potential cross-contamination patterns between Keio mutants stocked in the wells of the same plate.

3.2 Initial F^+ cross and analyses of known mutants to confirm the validity of assay

Three mutants of known phenotype related to Ff infection and assembly were selected to confirm the validity of the Ff filamentous phage susceptibility assays used in this thesis. A *recO* mutant was selected as the negative control (susceptible host) as deletion of the gene has no known effect on the production of Ff phage. RecO mutation has been used for growth of Ff filamentous phage ssDNA due to a lower level of episomal and ssDNA recombination (Deng & Perham, 2002). The use of a Keio mutant instead of the parent strain BW25113 was also decided in order to demonstrate that the effect of the Keio knock-out library mutants on phage production is a result of the specified gene deletion and lack of the encoded protein, rather than due to the Km^R marker or gene deletion methods used to create the Keio collection.

Two mutations that are known to make *E. coli* resistant to Ff phage were used as positive controls. First mutation was deletion of *tolA*, the chromosome-encoded receptor for Ff. It was used as a positive control for disruption of the phage infection and entry into *E. coli*. The second mutation was in *trxA*, gene encoding Thioredoxin, a small redox protein that is required for the f1 phage assembly (Russel & Model, 1986) and was therefore chosen as a positive control for disruption of phage assembly.

All three strains (*recO, tolA* and *trxA* deletion mutants) were from the Keio collection, where Km^R cassette replaces the CDS (Baba *et al.*, 2006). In order to allow infection with Ff phage, each strain was made F' by conjugation with XL1-Blue. The F' plasmid in XL1-Blue contains the Tet^R marker, and the chromosomal marker is *rpsL* (Str^R). The transconjugants were hence selected as Km^R Tet^R, as described in Chapter 2. The recO mutant was further subjected to the protocol to remove the Km^R cassette as described (Baba *et al.*, 2006). Three independent clones revived from the frozen mutant Keio stock collection were each conjugated to construct three independent F-positive transconjugants, which were in turn each tested in the plaque and phagemid infection assays. The reason for these precautions was in that cases of strain cross-contamination were reported in the Keio collection (Baba *et al.*, 2006). Furthermore, our collection was purchased from Thermo as a series of 96-well plates containing each 96 different mutant stocks, covered with poorly adherent metallic film that likely facilitates dispersion of small volumes of aerosol derived from stock microdrops attached to the cover across the plate as it is being uncovered to reach a specific designated well.

Two sets of experiments were done to test susceptibility of mutants to Ff infection: i) plaque-forming assay by wild-type Ff phage f1 (or phage titration assay; Table 3.1) to examine ability of a mutant to support a complete cascade of replication cycles resulting in a plaque formation from initial infection with a single phage (Fig. 2.1); ii) transduction by phagemid particles (phagemid titration assay; Table 3.2) to test only for infection and entry (Fig. 2.2). Phagemid particles (PPs) are Ff-derived virions/particles containing encapsulated ssDNA corresponding to phagemid vectors. These are plasmids containing the Ff origin of replication and packaging signal. Phagemids were derived from pUC118 (Amp^R) or pNJB51 (Cm^R); phagemid titration assay) by infection of the transformed cells with the helper phage R408 which does not carry any antibiotic resistance genes. In the presence of a helper Ff phage, replication protein pII encoded by the helper mediates ssDNA replication from the f1 positive-strand origin of replication [*(+) ori*] and all proteins of the Ff virion and assembly machinery. As a result, phagemid ssDNA (that contains the Ff packaging signal) is assembled into a phage-like phagemid particles or PPs.

Table 3.1 Plaque assay – known mutants

Keio Strain	Mutation	Results			Comments
		Plate 1	Plate 2	Plate 3	
JW2549 F'	*recO (no expected effect on Ff infection)*	✓[a]	✓	✓	Positive Control
JW0729 F'	*tolA* (required for Ff infection and entry)	× [b]	×	×	
JW5856 F'	*trxA* (required for f1 assembly)	×	×	×	A noticeably thinner lawn was observed under the area where the undiluted through to 10^{-4} dilutions of phage were placed; this is typical for assembly mutants, which infect the host cells but do not assemble and therefore cannot form plaques.

[a]A ✓ mark indicates that plaque formation was observed on the plate under the 10^{-6} and 10^{-8} stock dilution drops (Fig. 2.1). [b]An × mark indicates no plaques were observed. Plate 1, 2 and 3 refer to three independent Keio collection clones that were tested for each mutant.

For the *recO* mutant, there was thinning of the bacterial lawn where the 10 µL drops of undiluted to 10^{-4} dilutions of phage were placed. Where the 10^{-6} and 10^{-8} drops were placed (Fig. 2.1), individual separate plaques could be observed which corresponded with the number of phage expected in 10 µL of those dilutions, based on the known titre of the particular phage stock on other susceptible *E. coli* strains of roughly 1-4x10^{10}pfu/mL.

For the *tolA* mutant (Russel & Model, 1986), there were no observable differences in the bacterial lawn between the areas of the plates that had been spotted with phage and those that had not. This is the expected result if Ff phage infection was prevented. However, this result would also be observed if Ff phage was able to infect *E. coli*, but viral DNA replication could not occur. Because of previous studies, it is already known that *tolA* mutations would have this result as the mutation prevents Ff phage infection due to the lack of the secondary receptor that mediates the entry of DNA encapsulated in phage or phage-like particles into the cell.

For the *trxA* mutant (Russel & Model, 1986), there was a thinner bacterial lawn under the undiluted to the 10^{-4} drops of phage, but no individual plaques were observed where the 10^{-6} and 10^{-8} phage dilutions were placed. This phenomenon is sometimes referred to as "clearance" or "growth suppression" in literature about Ff phage. This result is expected when there is a high multiplicity of infection, with all host cells in the lawn are being infected with the phage, despite the absence of phage assembly for which the TrxA protein is required. The infected *trxA* mutant *E. coli* still has its ATP and resources hijacked by the Ff phage to replicate DNA and make phage proteins, thus still exhibits slower growth. However, since this mutant cannot secrete the phage progeny, there are no phages produced by the infected cells hence there is no infection observed in the surrounding of the initial infected *E. coli* cell and its daughter cells, which is why there is an absence of plaque formation at lower phage dilutions.

Table 3.2 Phagemid transduction assay with control mutants

Keio Strain	Mutation	Results		
		Plate 1	Plate 2	Plate 3
JW2549	*recO*	✓[a]	✓	✓
JW0729	*tolA*	×[b]	×	×
JW5856	*trxA*	✓	✓	✓

[a]A ✓ mark indicates that single colonies grew on the antibiotic plate under the 10^{-4} and 10^{-6} stock dilution drops (Fig. 2.2). [a]An × mark indicates no single-colony growth was observed. Plate 1, 2 and 3 refer to three independent Keio collection clones that were tested for each mutant.

To further discern between mutations impairing the initial infection steps from those affecting f1 replication and assembly, only the infection step was assessed by susceptibility of mutants to be transduced with Phagemid Particles (PPs). These particles are composed of exactly the same proteins as are Ff phages, but instead of the Ff phage ssDNA, phagemid ssDNA forms the backbone of the virion (Vieira & Messing, 1987).

Because the PPs contain an antibiotic resistance marker (Amp^R in the case of pUC118) and a plasmid origin of replication, once the ssDNA is transduced into a host, they behave like cells transformed with any plasmid, i.e. they replicate from the plasmid (pUC) origin and the pUC118 transductants are selected for as colonies growing on plates containing ampicillin. Therefore, the only requirement for acquiring Amp^R by transduction with pUC118 PPs is

successful receptor binding and ssDNA entry mediated by the Ff phage proteins pIII, pVI and pVIII of the PPs. No other phage function is required for transduction, hence the phagemid titration only examines susceptibility of *E. coli* mutants to infection and entry of the ssDNA into the cytoplasm, and the synthesis of the (-) strand of phagemid DNA required for conversion to the double-stranded circular DNA. This form of DNA is required for commencement of replication from the pUC/pMB1 plasmid origin and eventual formation of a colony on the selective (Amp-containing) plates.

The ampicillin plate containing the mutant *recO* in the soft agar layer showed a confluent Amp^R transductant lawn patch, in the areas where the undiluted and 10^{-2} phagemid stock drops were placed. Individual colonies were observed under the 10^{-4} and 10^{-6} stock dilution drops (Fig. 2.1), corresponding to a phagemid stocks with titres 1-4x10^9/ml. This shows that the phagemid was successfully able to transduce antibiotic resistance into an Ff-susceptible *E. coli* host, allowing it to grow on an antibiotic plate, hence there was no impairment of the phagemid infection mechanism.

The *tolA* (Ff secondary receptor) mutant had no visible colonies on the selective Amp-containing plates when exposed to the pUC118 phagemid particles, which implies that the infection and entry of the phagemid ssDNA was disrupted, and by extension would be for the Ff phage).

The *trxA* mutant plate resembles that of the positive control, *recO*, demonstrating that there was no impairment of phagemid ssDNA infection and entry due to a *trxA* mutation. This result demonstrates that comparison of PPs transfection assay and the plaque assay clearly differentiates between defect in host susceptibility between the infection-entry *vs.* the rest of the phage lifecycle.

3.3 Testing candidate mutants – *degP*, *dnaK*, *clsABC*, *gspAC-O*

Having confirmed the validity of Ff susceptibility assays, several host mutants of unknown susceptibility were initially chosen for further analysis. The choice was based on the knowledge about the effect of the phage on physiology of *E. coli* and involvement of specific candidate membrane and periplasmic proteins in Ff assembly.

3.3.1 Cardiolipin synthesis genes

As stated in the introduction, after Ff infection the ratio of cardiolipins to phosphoethanolamines in the inner membrane of *E. coli* increases dramatically, with cardiolipins constituting up to 30% of inner membrane lipids, in comparison to 5% in uninfected cells (Woolford *et al.*, 1974). There are three cardiolipin synthetases in *E. coli*, encoded by three genes, *clsA, clsB* and *clsC*. To determine whether either of these genes are required for Ff susceptibility, *E. coli* single mutants of each of the individual *cls* genes were subjected to the plaque assay (Table 3.3). All three mutants were positive in the plaque assay, in agreement with reported redundancy in the enzymes required for cardiolipin synthesis (Carranza *et al.*, 2017). In order to determine the role of cardiolipins in the Ff replication, it will be necessary to construct a triple-knock-out mutant of *clsA, clsB* and *clsC* genes.

3.3.2 DegP protein

Protein DegP is a major periplasmic chaperone involved in control of folding of periplasmic and outer membrane proteins and could therefore be required for infection and assembly of machinery like F pilus and pIV channel required for secretion-assembly (Spagnuolo *et al.*, 2010).

DegP is also a heat shock protein, and *degP* mutants of *E. coli* are not viable at 42ºC. Ff phage infection kills *E. coli* at 42ºC, hence I hypothesised that the DegP protein could be sequestered by phage assembly, resulting in cell death at that temperature. From this, *degP* could also be required at other temperatures for phage assembly.

The plaque (Table 3.3) and phagemid transduction (Table 3.4) assays were negative for one out of 3 tested *degP* F' strains. One of 3 *degP* clones, therefore, did not support receptor binding and entry of ssDNA into the host cell. From this experiment it is not discernible whether other steps of Ff life cycle is affected by *degP* mutation.

3.3.3 DnaK protein

Protein DnaK encodes chaperone Hsp70 (heat shock protein 70 kDa) (Schonfeld *et al.*, 1995). It has been reported to be required for rearrangement of DNA replication complexes and for DNA replication of λ phage (Osipiuk *et al.*, 1993), thus it might also play a role in the Ff DNA replication. DnaK is also required for replication of F plasmid from the double-

stranded-DNA origin and therefore for maintenance of this plasmid in the host cell as an episome (Nakamura *et al.*, 2007).

The Keio collection *dnaK* mutants were made F+ by conjugation with XL1-Blue as described in material and methods. Successful selection of Tet^R Kan^R transconjugants of *dnaK* mutants is likely a result of the F' integration into the genome, forming an Hfr strain. The dnaK mutant clones failed to support f1 plaque formation, and were not tranducible in the phagemid assay (Tables 3.3 and 3.4). DnaK is not required for pUC plasmid replication (Giraldo-Suarez *et al.*, 1993) hence it is most likely that DnaK is required for replication of the negative strand of the phagemid after entry of the (+) strand (ssDNA) and/or processes required for single stranded DNA entry.

3.3.4 Gsp (General secretory pathway) or type II secretion system

Gsp mutants were tested because the Ff phage assembly machinery is remarkably simple, consisting of only 3 proteins, compared to a related, but much more complex type II secretion system. Another related system, for type IV pilus assembly, is also complex, as is less related to type III secretion system. All three related systems have dozens of components. The most conserved protein in these three systems and Ff assembly system are outer membrane "secretin" family channels through which phage, pilus or secretion substrates are secreted. There are examples of *Vibrio cholerae* and Pseudomonas filamentous phages (CTXϕ and Pf4, respectively) that do not encode their own secretins, but rather use the secretin channels, respectively, of the type II secretion system or type IV pilus assembly systems encoded by their host for phage assembly and secretion. Type II secretion system or type IV pilus assembly systems are closely related to each other. Another conserved component is an inner membrane hexameric ATPase which powers assembly and secretion of substrates. The Ff assembly system, however, does not encode additional inner membrane and periplasmic components that are typical for the type II secretion and type IV pilus assembly systems (Rakonjac *et al.*, 2024).

E. coli K12 genome contains 14 genes annotated as *gsp* which is typical for the type II secretion system nomenclature, but are also homologous to known type IV pilus assembly systems (Francetic *et al.*, 2000) Here I hypothesised that some of the inner membrane components encoded by *gsp*, for example, could be "highjacked" by the Ff assembly system and therefore required for assembly of this phage. Mutants of all 14 *gsp* genes encoded by the

E. coli chromosome identified above were tested for Ff infection and replication. Three F' transconjugants derived ultimately from three independent clones recovered from the same frozen stock (well) of the Keio collection were tested for each mutant, due to a reported cross-contamination in the Keio collection, as stated above (Baba *et al.*, 2006). Only two of the 14 *gsp* mutants, encoding minor pilins GspG and GspJ, had clones that failed to support plaque formation and could not be transduced by a phagemid, suggesting that they are involved, at a minimum, in the Ff infection step. None of the other 12 *gsp* genes' mutations impaired susceptibility to f1 phage infection (Tables 3.3 and 3.4).

Table 3.3 Plaque assay of the candidate mutants

Keio Strain	Mutation	Results		
		Plate 1	Plate 2	Plate 3
JW1241F'	*clsA*	✓[a]	✓	✓
JW0772F'	*clsB*	✓	✓	✓
JW5150F'	*clsC*	✓	✓	✓
JW0157F'	*degP*	×[b]	✓	✓
JW0013F'	*dnaK*	×	×	×
JW3285F'	*gspA*	✓	✓	✓
JW3297F'	*gspO*	✓	✓	✓
JW3286F'	*gspC*	✓	✓	✓
JW3288F'	*gspE*	✓	✓	✓
JW3289F'	*gspF*	✓	✓	✓
JW3290F'	*gspG*	×	✓	✓
JW3291F'	*gspH*	✓	✓	✓
JW3293F'	*gspJ*	✓	✓	×
JW3294F'	*gspK*	✓	✓	✓
JW5704F'	*gspM*	✓	✓	✓
JW5705F'	*gspL*	✓	✓	✓
JW5706F'	*gspI*	✓	✓	✓
JW5707F'	*gspD*	✓	✓	✓
JW2938F'	*yghG*	✓	✓	✓

[a]A ✓ mark indicates that plaque formation was observed on the plate. [b]An × mark indicates no plaques were observed. Plate 1, 2 and 3 refer to three independent Keio collection clones that were tested for each mutant.

Table 3.4 Phagemid transduction assay of candidate mutants negative in plaque assay

Keio Strain	Mutation	Results		
		Plate 1	Plate 2	Plate 3
JW0157F'	*degP*	×	✓[a]	✓
JW0013F'	*dnaK*	×[b]	×	×
JW3290F'	*gspG*	×	✓	✓
JW3293F'	*gspJ*	✓	✓	×

[a]A ✓ mark indicates that colonies grew on the antibiotic plate. [b]An × mark indicates no colony growth was observed. Plate 1, 2 and 3 refer to three independent Keio collection clones that were tested for each mutant.

The unsusceptible clones of *gspG* and *gspJ* mutant demonstrated the most unexpected phenotype observed so far. Given that these genes are homologues of the minor type II pilin mutants involved in the initiation of pseudopilus assembly in type II secretion system and of the type IV pilus which are functionally analogous to initiation of Ff phage assembly, they were expected to be involved in assembly rather than infection as proposed in the sections 3.1 and 3.3.4. The type II secretion system of Klebsiella, however, has been shown to be involved in uptake of DNA, hence it is also possible that the two minor pilins of the *E. coli* K12 type II secretion system play a role in the ssDNA uptake during Ff infection, in conjunction with *tolQRA* or F-pilus. The involvement in the infection does not exclude a role in phage assembly, hence it is plausible that the two minor pilins have a role in both infection and assembly of the Ff phages.

In summary, of all the candidate mutants selected, all but four, *degP*, *dnaK*, *gspG*, *gspJ*, were able to replicate phage successfully as indicated by the presence of plaques for all three clones (all the plates), each corresponding to independent clones from the Keio collection. In one of those four mutants, *dnaK*, all three colonies showed the same phenotype; i.e. they all failed to form plaques. At high phage to bacteria ratio, there was no clearance of the bacteria lawn indicating failure to infect or replicate rather than failure of phage assembly. This is in agreement with *dnaK* being required for phage DNA replication.

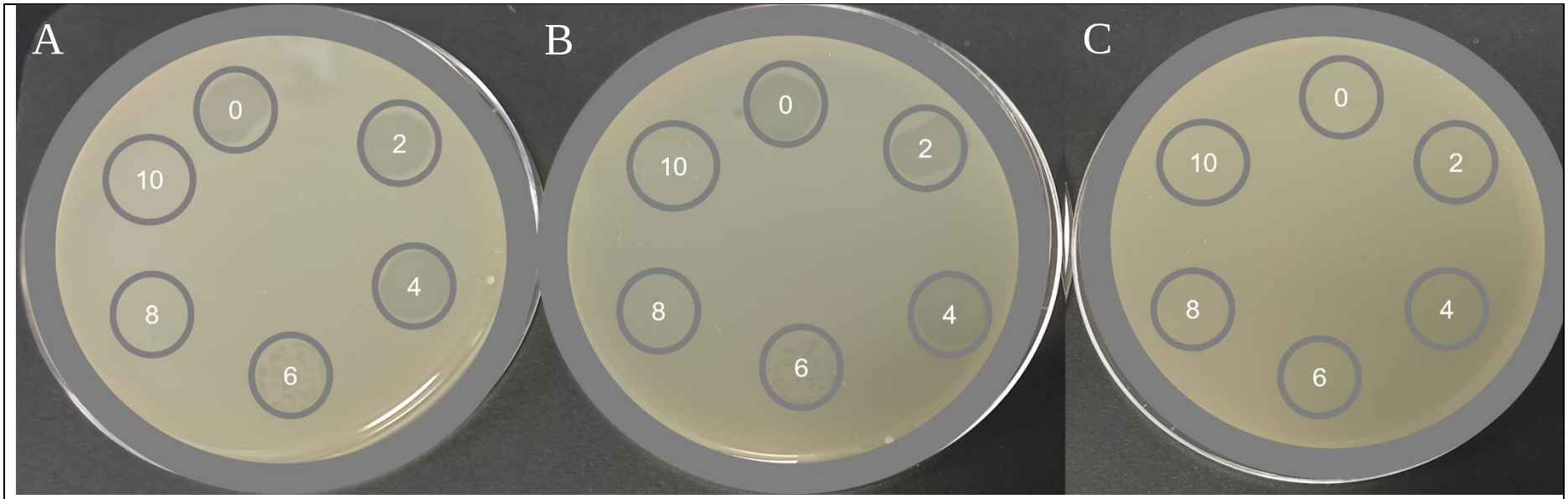

Figure 3.1 Ff phage infection assay, gspG mutant clones, with one of the three clones not having visible plaques

Dilutions of Ff phage f1 were spotted onto three independent F' *gspG* clones. A F' *gspG1*, B and C correspond to three different clones of F' *gspG*. In detail, soft agar was inoculated with a clone the mutant *E. coli* and was spread onto a plate to create the bacterial lawn. The dilutions of a f1 phage stock (10ul each) were then spotted onto the plate, as indicated by the small grey circles in the image. The numeral within those circles corresponds to the dilution factor of the f1 phage stock in those spots, with 0 being where a drop of the undiluted stock was placed, 2 for the 10^{-2} dilution drop, 4 for the 10^{-4} dilution, and so on until the final dilution of 10^{-10} in the circle marked 10. In plates A and B, the darker surfaces within the *E. coli* lawn where the undiluted to 10^{-4} drops were placed represent confluent areas of f1 infection causing the *E. coli* corresponding to the whole drop surface to grow more slowly due to effects of the Ff infection, and single plaques can be seen in the lawn under the 10^{-6} drop correspond to the *E. coli* lawn of cells infected initially with a single phage particle. In plate C however, no thinner lawn or single plaques can be observed on the plate.

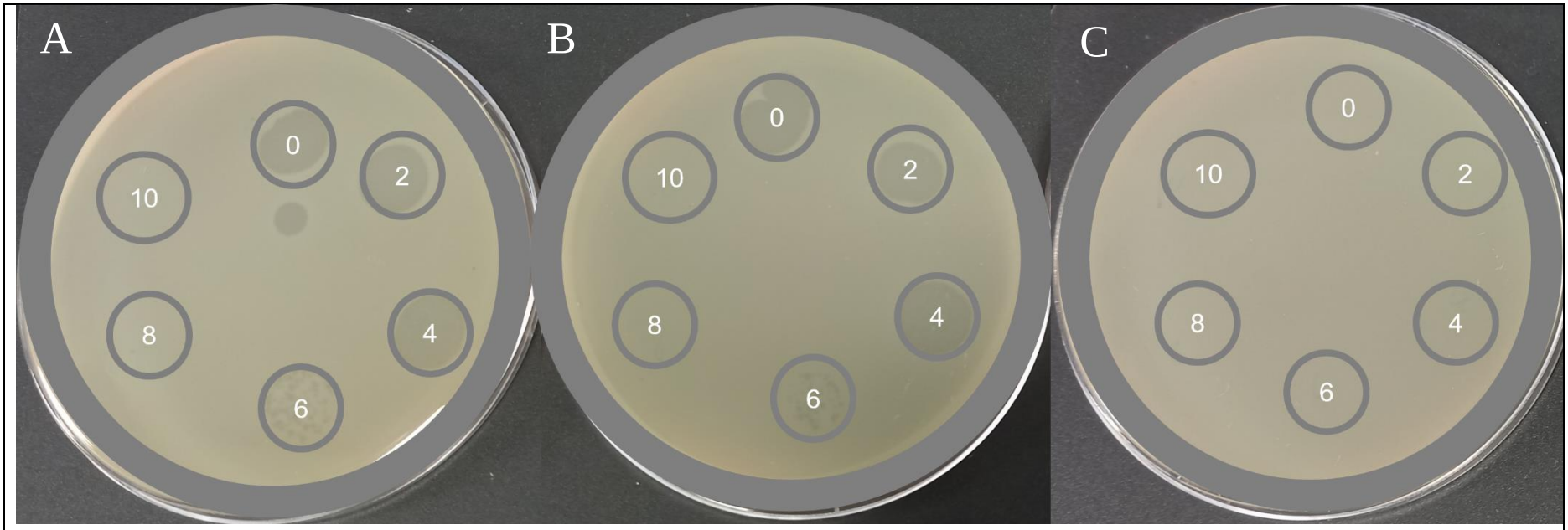

Figure 3.2 Ff phage infection assay, gspJ mutant clones, with one of the three clones not having visible plaques

Dilutions of Ff phage f1 were spotted onto three independent F' *gspJ* mutants. Soft agar was inoculated with the mutant *E. coli* and was spread onto the plate to create the *E. coli* lawn. The dilutions of f1 phage stocks (10 μL each) were then spotted onto the plate, as indicated by the small grey circles in the image. The number within those circles corresponds to the dilution factor of the f1 phage in those spots, with 0 being where a drop of the undiluted phage stock was placed, 2 for the 10^{-2} dilution drop, 4 for the 10^{-4} dilution, and so on until the final dilution of 10^{-10} in the circle marked 10. In plates A and B, the darker surfaces within the *E. coli* lawn where the undiluted to 10^{-4} drops were placed represent confluent areas of f1 infection causing the *E. coli* corresponding to the whole drop surface to grow more slowly due to effects of the Ff infection, and single plaques can be seen in the lawn under the 10^{-6} drop correspond to the *E. coli* lawn of cells infected initially with a single phage particle. In plate C however, no thinner lawn or single plaques can be observed on the plate.

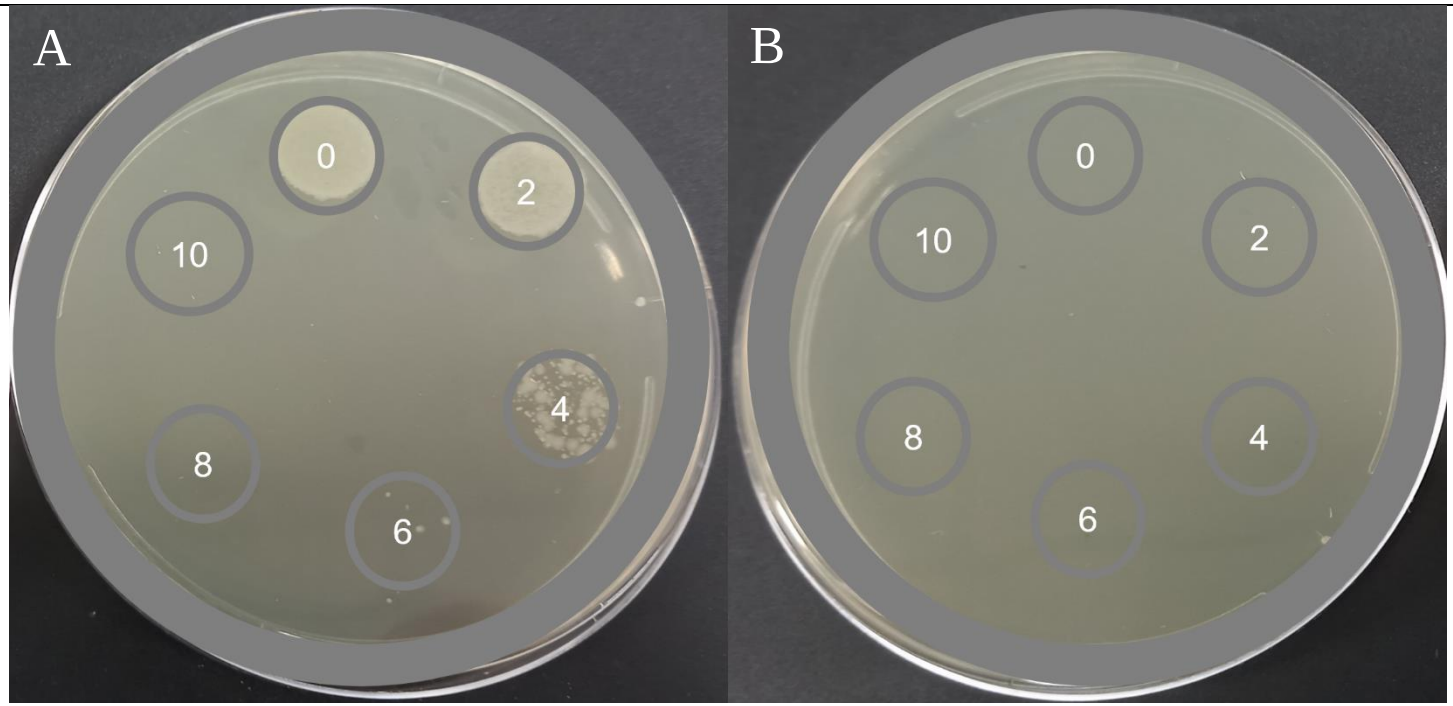

Figure 3.3 Phagemid transduction assay, gspG mutant clones; one of the clones fails to form colonies

Dilutions of phagemid pNJB51 stock were spotted onto a lawn of two F' *gspG* mutants on Chloramphenicol plates. Successfully transfected *E. coli* will acquire the chloramphenicol resistance gene of the phagemid and form a colony. The pNJB51 phagemid stock dilutions were then spotted (same as plaques) into the small grey circles in the image. The number within those circles corresponds to the dilution factor of the phagemid in those spots, with 0 being where the undiluted phagemid drop was placed, 2 for the 10^{-2} dilution drop, 4 for the 10^{-4} dilution, and so on until the final dilution of 10^{-10} in the circle marked 10. In plate A, which corresponds to a clone from the Ff phage infection assay that did form plaques, a lawn of *E. coli* can be observed where the drops of undiluted stock and 10^{-2} dilution was placed, and colonies can be observed where the 10^{-4} and 10^{-6} dilutions were. In plate B, which corresponds to the plate in the Ff phage dilution assay that did not form plaques, no growth of any *E. coli* is observed.

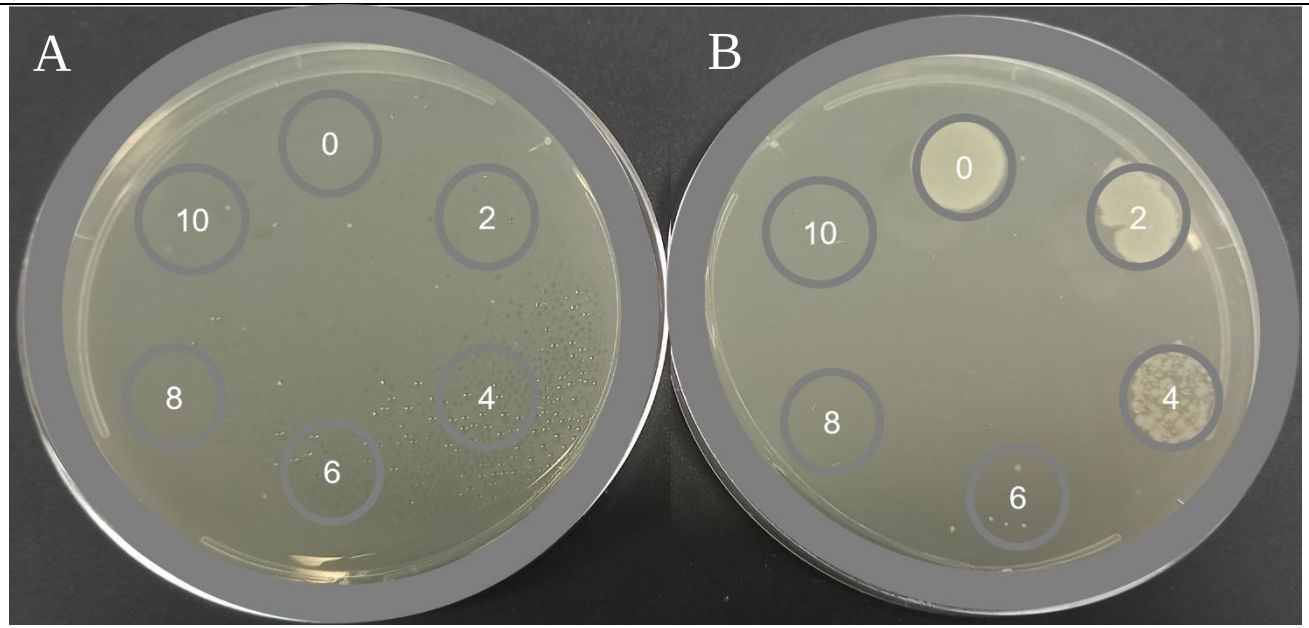

Figure 3.4 Phagemid transduction assay, gspJ mutant clones; one of the clones fails to form colonies

Dilutions of phagemid pNJB51 stock were spotted onto a lawn of two F' *gspJ* mutants on Chloramphenicol plates. Successfully transfected *E. coli* will acquire the chloramphenicol resistance gene of the phagemid and form a colony. The pNJB51 phagemid stock dilutions were then spotted (same as plaques) into the small grey circles in the image. The number within those circles corresponds to the dilution factor of the phagemid in those spots, with 0 being where the undiluted phagemid drop was placed, 2 for the 10^{-2} dilution drop, 4 for the 10^{-4} dilution, and so on until the final dilution of 10^{-10} in the circle marked 10. In plate A, which corresponds to the plate in the Ff phage dilution assay that did not form plaques, no growth of any *E. coli* is observed. In plate B, which corresponds to a clone from the Ff phage infection assay that did form plaques, a lawn of *E. coli* can be observed where the drops of undiluted stock and 10^{-2} dilution was placed, and colonies can be observed where the 10^{-4} and 10^{-6} dilutions were.

3.3.5 Confirming the mutant clones' identities

The same clones of *degP*, *gspG*, and *gspJ* mutants that did not form plaques in the plaque assay also were not transduced in the phagemid infection assay (Tables 3.3 - 3.4; Fig 3.1 – 3.4). This would suggest that these mutants are not able to facilitate the infection process for Ff phage or transfection by phagemid. The other two clones of those mutants that supported plaque formation after phage infection, likewise were susceptible to the phagemid transduction.

The one in three clones derived from the same supposed mutant stock having different phenotype could have been caused by the reported cross-contamination of the Keio collection, where many mutants contained varying amounts of contamination in the wells (Baba *et al.*, 2006) which was the reason in the first place for testing three independent clones of each mutant was used for each test. Given this, different results in triplicates could have arisen because of cross-contamination with cells from neighbouring stocks of the Keio library stored in the 96-well plates. For mutants where individual clones produced different results, all three were tested either phenotypically if the mutation has an easily tested phenotype, or by PCR if the mutation does not have an easily testable phenotype, to check for the presence of the expected mutations based on their Keio collection assignment.

The presence of *degP* mutation was tested by growth at 42ºC because *degP* mutants do not grow at that temperature. Of the three individual clones, the one that was unable to grow at 42ºC, was also unable to be infected by Ff phage or transduced by phagemid pUC118. From this, it can be concluded that DegP protein is required for the infection step of the degP filamentous phage lifecycle.

GspG and GspJ do not have any detectable phenotypes so they were confirmed by PCR testing (Fig 3.5; 3.6).

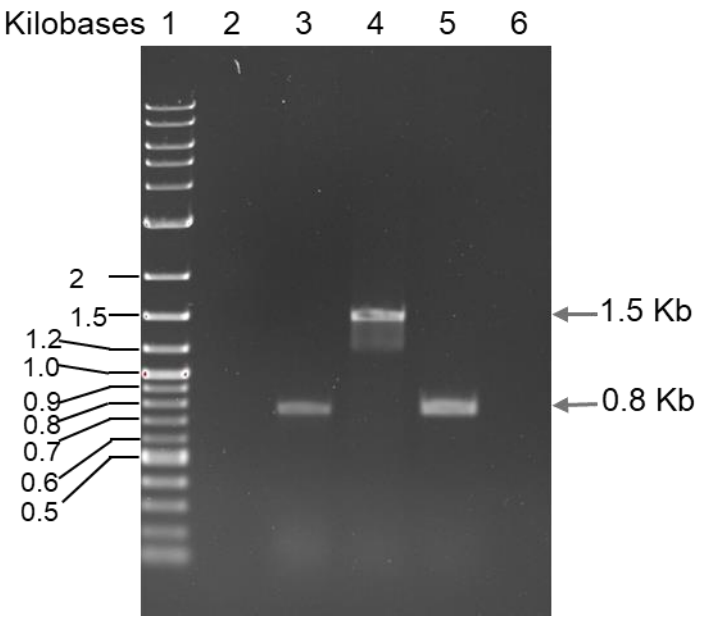

Figure 3.5 Confirming gspJ::Km^R mutants using PCR

Agarose gel electrophoresis of the PCR products using flanking primers and chromosomal DNA as a template. Lanes: 1, 1 Kb plus dsDNA linear standard ladder; 2, No-template control; 3, K2245 (wild-type *gspJ* control); 4, *gspJ3* mutant candidate (clone) that did not produce plaques; 5 and 6, mutant candidates (clones) *gspJ1* and *gspJ2* that produced plaques.

To test for the presence of the Km^R cassette replacing *gspG* and *gspJ ORFs*, a PCR was carried out on each tested clone. Primers that flanked the gene of interest were used to differentiate between the wild-type and the corresponding Km^R-cassette-replaced locus.

Confirmation of the *gspJ*::Km^R and *gspG*::Km^R is shown in Figures 3.5 And 3.6, respectively. Chromosomal DNA was purified from the cultures of individual clones and used as a template in PCR reactions. Strain K2245 was used as a wild-type control.

The PCR reactions on *gspJ* candidate clones showed that the *gspJ3* clone gives a PCR product of the size expected for Km^R cassette replacement hence is a true mutant. The *gspJ1* PCR product size corresponds to the wild-type locus and is therefore not a true gspJ1 mutant. The PCR using gspJ2 DNA did not give a product, hence the reaction failed, and the status of this clone remains undetermined. The PCR based on one phage resistant and one susceptible clone confirms the phenotype correlates with the *gspJ* mutation.

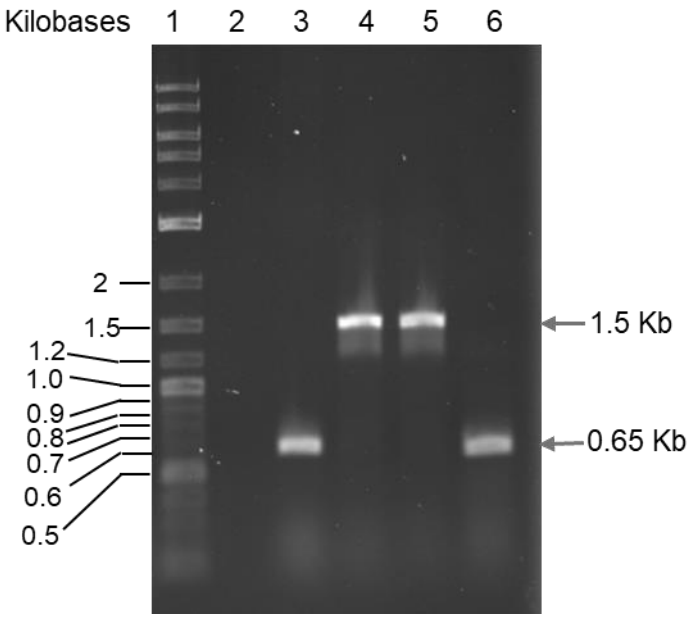

Figure 3.6 Confirming gspG::Km^R mutants using PCR

Agarose gel electrophoresis of the PCR products using flanking primers and chromosomal DNA as a template. Lanes: 1, 1 Kb plus dsDNA linear standard ladder; 2, No-template control; 3, K2245 (wild-type *gspG* control); 4, F' *gspG1* mutant candidate that did not produce plaques; 5 and 6, mutant candidates, *gspG2* and *gspG3*, that produced plaques.

The PCR reactions on *gspG* candidate clones showed that the *gspG1* and *gspG2* candidate clones gives a PCR product of the size expected for the Km^R cassette replacement in this locus, hence are true mutants. The *gspG3* candidate PCR product size corresponds to the wild-type locus and is therefore not a *gspG* mutant. While the *gspG1* and *gspG3* (actual wild-type for the *gspG* locus) correlate with the resistant *vs.* susceptible phenotype, gspG2 is a mutant, yet supports plaque formation by Ff. This result suggests that in case of *gspG*, there may be heterogeneity of phenotypes, possibly related to the F pilus maintenance or function in the Ff infection.

3.3.6 Discerning the roles of mutated proteins between F pilus function and phage infection independent of F pilus

gspG, *gspJ*, *dnaK*, and *degP* were shown to be required for infection of the host cells by filamentous phage. The failure to infect could be caused either by a direct role in the infection, DNA entry or (-) strand synthesis, or indirect role via the effect on the F pilus function or maintenance. Reasoning that the function in Ff infection, which depends on the

pilus retraction, partially overlaps with the function as the F plasmid donor in conjugation, we tested whether these mutants are functional as donors of the F plasmid to an F-negative recipient in a secondary cross (Fig. 3.7 and 3.8). In this assay failure to conjugate means that the reason for the failure of the phagemid infection is the loss of the F-pilus function. None of the *gspG*, *gspJ*, *dnaK*, and *degP*, clones that were confirmed to be true mutants and failed to be infected in the phagemid transduction assay could serve as donors in F' crosses, showing that a defect in the F pilus structure or function correlates with resistance to infection.

Interestingly, there was difference in phenotype between two clones of the *gspG* mutant stock that were both confirmed to be mutants (i.e. both had identical *gspG*::Km^R allele). One clone could and another could not plate phage, be transduced by the pUC118 phagemid particles or serve as a donor in conjugation, whereas the other one could. Given that phage plating phenotype between these two clones correlates to the F pilus conjugation donor ability, potential explanation for this is that *gspG* gene mutation has incomplete penetration when it comes to the F pilus replication or function, resulting in clone-specific deletions within the F-plasmid and/or integration of incomplete F-plasmid into the chromosome. If the latter is the case, in some F-positive clones the F pilus functionality could be lost and in some retained. This could, for example, be a result of partial integration of the F plasmid fragment containing the selective marker (Tet^R) due to propagation of the strain in the Tet-containing media, but loss of F-plasmid genes required for function as a conjugation donor and receptor in Ff infection. This remains to be resolved in the future.

From this, it can be concluded that the proteins encoded by *gspG*, *gspJ*, *dnaK*, and *degP* are required for the F pilus structure or function and their effect on the Ff infection is through the F pilus functionality. This, however, does not exclude a direct effect as part of pleiotropic functions, especially in the case of *dnaK*, and *degP* that control folding of many proteins involved in, respectively, replication and periplasmic protein folding and assembly.

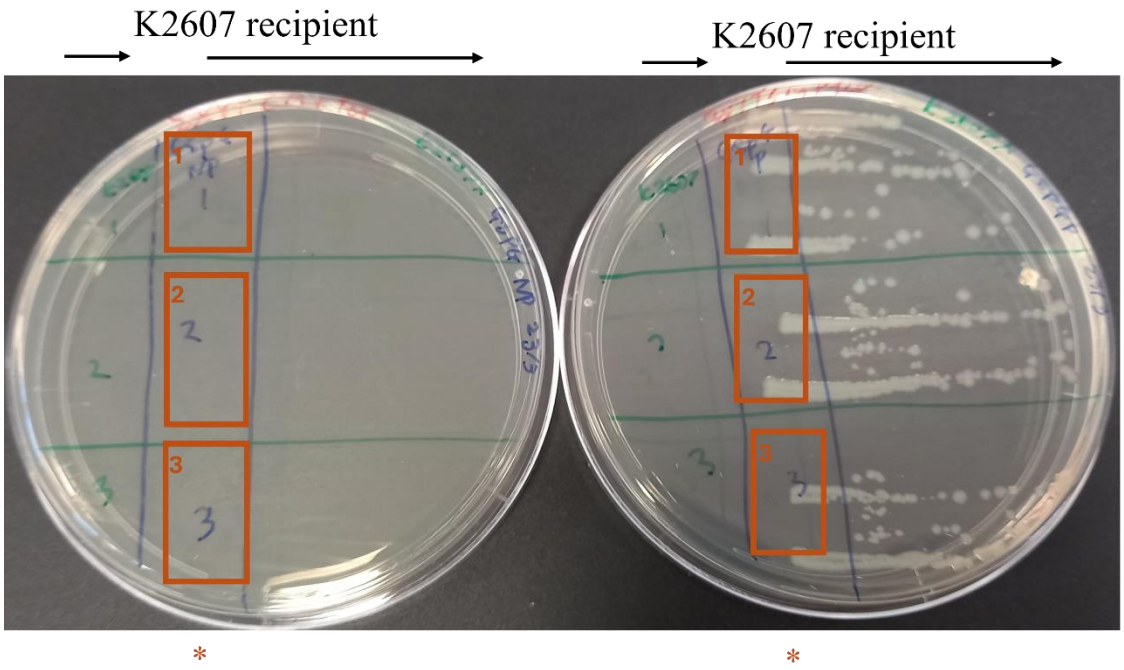

Figure 3.7 Assay of gspG mutant vs. wild-type clone for the F' donor function, with no transconjugants detected on the plate containing Ff-resistant gspJ clone as a donor

Materials of colonies from donor (F' Tet^R) and a recipient (Cm^R) were crossed on a selection plate for transconjugants containing both Tet^R and Cm^R marker. In this case, the donors were two different *gspG* mutant clones and recipient was K2607. On the left plate, the *gspG* clone used was the clone that failed to replicate phage or be transfected by phagemid in the respective phage and phagemid assays. No colonies grew on this plate indicating a failure of the *gspG* clone to donate the F plasmid to K2607. The right plate had a *gspG* clone that was able to replicate phage and be transfected by phagemid. As expected transconjugants grew where the two strains overlapped. *Boxes 1,2 and 3 represent three independent colonies of each *gspG* clone.

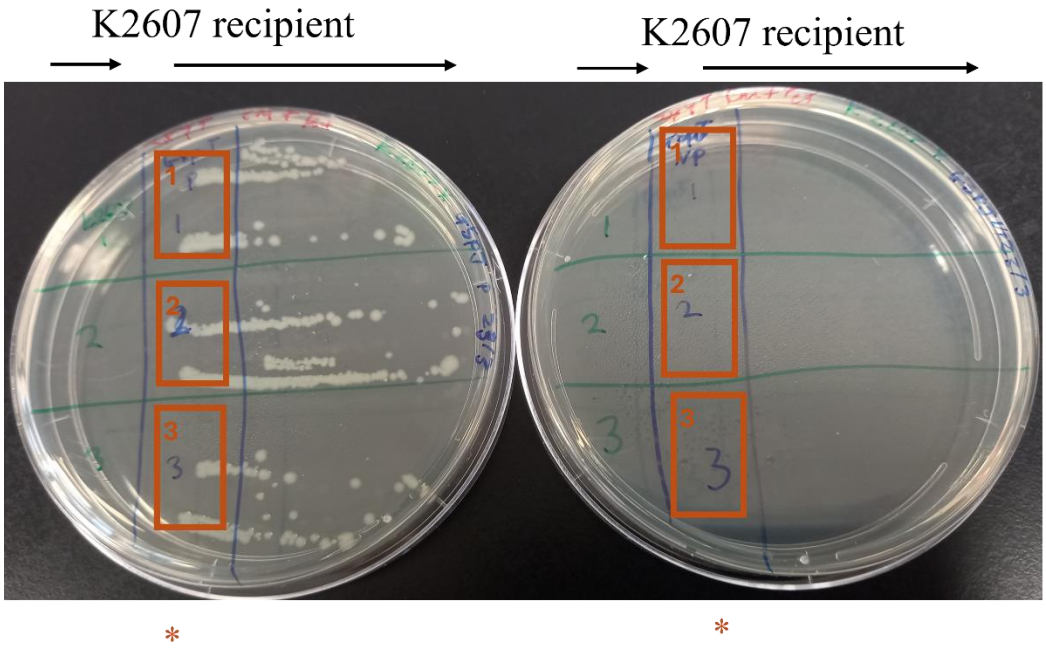

Figure 3.8 Assay of gspJ mutant vs wild-type clone as F' donors, with no transconjugants detected on the plate containing the Ff-resistant gspJ clone as a donor

Materials of colonies from donor (F' Tet^R) and a recipient (Cm^R) were crossed on a selection plate for transconjugants containing both Tet and Cm. In this case, the donors were two different *gspJ* candidate clones and recipient was K2607. The left plate had a clone (determined by PCR to be the wild-type for the *gspJ* locus) that was able to replicate phage and be transfected by phagemid in the respective phage and phagemid assays. On the right plate, the *gspJ* clone used was the true mutant as determined by PCR, and had failed to replicate phage or be transduced by a phagemid. No colonies grew on this plate indicating a failure of the true *gspJ* mutant to serve as an F plasmid donor in conjugation. * Boxes 1,2 and 3 represent three independent colonies of each *gspJ* clone.

3.4 Systematic testing of lipoprotein mutants

As lipoproteins were the most likely candidates for a putative Ff phage pilotin that binds to pIV secretin during assembly (Conners *et al.*, 2021), a list of *E. coli* lipoproteins-encoding genes needed to be made for testing. Two online databases were used to create the list of lipoproteins, EcoCyc and UniProt. (Keseler *et al.*, 2021).

A search was first carried out on EcoCyc, an online resource with all the known genes of *E. coli* K-12 (the main laboratory strain used as a model Gram-negative bacterium). The search term "lipoprotein" returned 94 results for genes. Knock-out mutants of 80 of these genes were available in the Keio Collection. All the available lipoprotein-encoding genes' mutants were tested.

A later search on UniProt, an online protein database, for "lipoprotein" was carried out, which yielded 70 additional results that were not found on EcoCyc because of the differences in the way the two search engines worked. Knock-out mutants of additional 61 of the UniProt-annotated *E. coli* K12 lipoprotein-encoding genes were available in the Keio Collection; 23 of those were tested due to the time constraints. In total, 97 annotated lipoprotein gene deletion mutants were tested for susceptibility to Ff phage infection. Negative strains were then further tested for infection step of the Ff using phagemid transduction assay.

3.4.1 Plaque assay of lipoprotein-encoding mutants

Of the 97 mutants tested in the phage plating assay, 21 did not support plaque formation (Table 3.5). All of these produced heterogeneous phenotypes amongst the three clones isolated from each mutant stock. Three of these mutants, *slp*, *osmB* and *mltB*, had two clones that failed to plate phage while the third clone plated phage normally. The remaining 18 mutants had one out of three clones that could not plate phage. The knock-out mutans with one strain not plating Ff were *yaiW, yajG, lpoA, yhfL, yiaD, yjbF, yghJ, yfiL, ygeQ, acrE, acrA, chiQ, rzoD, yidX, cyoA, cpxA, mltA,* and *ompA*. For all 21 mutants, the clones that failed to plate phage (negative plaque assay) were likewise unable to be transduced by phagemid, while the clones that plated phage normally were also transduced by phagemid (Table 3.6).

Table 3.5 Plaque assay on lipoprotein-encoding genes' knock-out mutants

Keio strain	Mutation	1	2	3	Keio Strain	Mutation	1	2	3	Keio strain	Mutation	1	2	3
JW2373F'	*ypdl*	✓[a]	✓	✓	JW1667F'	*lpp*	✓	✓	✓	JW5351F'	*yehR*	✓	✓	✓
JW2462F'	*bamC*	✓	✓	✓	JW3132F'	*nlpI*	✓	✓	✓	JW5412F'	*yfiL*	✓	✓	×
JW0755F'	*ybhC*	✓	✓	✓	JW2938F'	*yghG*	✓	✓	✓	JW5461F'	*ygeQ*	×	✓	✓
JW0731F'	*pal*	✓	✓	✓	JW3119F'	*dolP (yraP)*	✓	✓	✓	JW0240F'	*yafY*	✓	✓	✓
JW0188F'	*nlpE*	✓	✓	✓	JW3116F'	*lpoA (yraM)*	✓	×	✓	JW3635F'	*nlpA*	✓	✓	✓
JW1795F'	*yeaY*	✓	✓	✓	JW3235F'	*yhdV*	✓	✓	✓	JW5921F'	*yfjS*	✓	✓	✓
JW3474F'	*slp*	×[b]	×	✓	JW3332F'	*yhfL*	✓	×	✓	JW2343F'	*mlaA*	✓	✓	✓
JW2425F'	*yfeY*	✓	✓	✓	JW5657F'	*yiaD*	✓	✓	×	JW3233F'	*acrE*	×	✓	✓
JW1892F'	*yecR*	✓	✓	✓	JW3532F'	*ysaB*	✓	✓	✓	JW0452F'	*acrA*	✓	✓	×
JW1913F'	*yedD*	✓	✓	✓	JW3867F'	*yiiG*	✓	✓	✓	JW5448F'	*ygdl*	✓	✓	✓
JW1782F'	*yoaF*	✓	✓	✓	JW3989F'	*yjbH*	✓	✓	✓	JW0668F'	*chiQ*	✓	×	✓
JW1631F'	*mliC*	×	✓	✓	JW0628F'	*rlpA*	✓	✓	✓	JW5080F'	*rzoD*	✓	×	✓
JW2539F'	*qseG*	✓	✓	✓	JW0192F'	*rcsF*	✓	✓	✓	JW5258F'	*ynfC*	✓	✓	✓
JW2504F'	*yfhM*	✓	✓	✓	JW2982F'	*yqhH*	✓	✓	✓	JW5858F'	*yidX*	×	✓	✓
JW1377F'	*ynbE*	✓	✓	✓	JW1698F'	*nlpC*	✓	✓	✓	JW0422F'	*cyoA*	✓	✓	×
JW1427F'	*ydcL*	✓	✓	✓	JW1275F'	*osmB*	×	×	✓	JW3882F'	*cpxA*	✓	✓	×
JW1050F'	*yceB*	✓	✓	✓	JW1728F'	*osmE*	✓	✓	✓	JW2373F'	*ypdI*	✓	✓	✓
JW1545F'	*rzpQ**	✓	✓	✓	JW1633F'	*slyB*	✓	✓	✓	JW2784F'	*mltA*	×	✓	✓
JW0369F'	*yaiW*	✓	×	✓	JW0546F'	*borD*	✓	✓	✓	JW2671F'	*mltB*	✓	×	×
JW0206F'	*yafT*	✓	✓	✓	JW4108F'	*ecnB*	✓	✓	✓	JW0940F'	*ompA*	✓	✓	×
JW0118F'	*yacC*	✓	✓	✓	JW5412F'	*yifL*	✓	✓	✓	JW2047F'	*wza*	✓	✓	✓
JW0126F'	*yadE*	✓	✓	✓	JW5711F'	*yjbF*	×	✓	✓	JW0561F'	*cusC*	✓	✓	✓
JW2586F'	*yfiB*	✓	✓	✓	JW5633F'	*YidQ*	✓	✓	✓	JW2712F'	*nlpD*	✓	✓	✓
JW0443F'	*ybaY*	✓	✓	✓	JW5736F'	*yjel*	✓	✓	✓	JW2903F'	*loiP*	✓	✓	✓
JW0424F'	*yajG*	✓	×	✓	JW5737F'	*ecnA*	✓	✓	✓	JW2265F'	*yfbK*	✓	✓	✓

JW0969F’	*gfcB*	✓	✓	✓	JW5743F’	*bsmA*	✓	✓	✓	JW0732F’	*cpoB*	✓	✓	✓
JW1374F’	*hslJ*	✓	✓	✓	JW5925F’	*yghJ*	✓	✓	×	JW0908F’	*ldtD*	✓	✓	✓
JW0849F’	*ybjP*	✓	✓	✓	JW5213F’	*rzoR*	✓	✓	✓	JW2670F’	*pncC*	✓	✓	✓
JW4110F’	*blc*	✓	✓	✓	JW5151F’	*yceK*	✓	✓	✓	JW1486F’	*digH*	✓	✓	✓
JW0966F’	*gfcE*	✓	✓	✓	JW5127F’	*pqiC*	✓	✓	✓	JW2833F’	*actS*	✓	✓	✓
JW0967F’	*gfcD*	✓	✓	✓	JW5157F’	*lpoB*	✓	✓	✓	JW0193F’	*metQ*	✓	✓	✓
JW0224F’	*yafP*	✓	✓	✓	JW5056F’	*yajI*	✓	✓	✓					
JW2801F’	*ygdR*	✓	✓	✓	JW5400F’	*yfgH*	✓	✓	✓					

[a]A ✓ mark indicates that plaque formation was observed on the plate. [b]An × mark indicates no plaques were observed. Plate 1, 2 and 3 refer to three independent Keio collection clones that were tested for each mutant. **rzpQ* was found to encode a lipoprotein during the database search, however the Keio collection does not contain an *rzpQ* deletion mutant. *rzpQ* is a deletion mutant of an annotated membrane protein that was used in its stead as it contained *rzpQ* as a nested gene (Moore *et al.*, 2024).

Table 3.6 Phagemid transduction assay of the lipoprotein gene knock-out mutants negative in the plaque assay

Keio Strain	Mutation	1	2	3	Keio Strain	Mutation	1	2	3	Keio Strain	Mutation	1	2	3
JW3474F’	*slp*	×[b]	×	✓	JW5711F’	*yjbF*	×	✓	✓	JW5080F’	*rzoD*	✓	×	✓
JW0369F’	*yaiW*	✓[a]	×	✓	JW5925F’	*yghJ*	✓	✓	×	JW5858F’	*yidX*	×	✓	✓
JW0424F’	*yajG*	✓	×	✓	JW5412F’	*yfiL*	✓	✓	×	JW0422F’	*cyoA*	✓	✓	×
JW3116F’	*lpoA*	✓	×	✓	JW5461F’	*ygeQ*	×	✓	✓	JW3882F’	*cpxA*	✓	✓	×
JW3332F’	*yhfL*	✓	×	✓	JW3233F’	*acrE*	×	✓	✓	JW2784F’	*mltA*	×	✓	✓
JW5657F’	*yiaD*	✓	✓	×	JW0452F’	*acrA*	✓	✓	×	JW2671F’	*mltB*	✓	×	×
JW1275F’	*osmB*	×	×	✓	JW0668F’	*chiQ*	✓	×	✓	JW0940F’	*ompA*	✓	✓	×

[a]A ✓ mark indicates that colonies grew on the antibiotic plate. [b]An × mark indicates no colony growth was observed. Plate 1, 2 and 3 refer to three independent Keio collection clones that were tested for each mutant.

One common trait that links a large proportion of the lipoprotein mutants that did not support Ff phage infection is that they are involved in membrane stress response pathways or play a key role in the morphology of the membrane.

Of the stress response genes, there is *slp*, which encodes a starvation lipoprotein that is upregulated when *E. coli* is grown in acidic or low-carbon conditions (Alexander & St John, 1994; Tucker *et al.*, 2002). Expression of another lipoprotein-encoding gene, *yfiL*, is linked to general stress response and regulation of this gene is controlled by the sigma S factor (Erickson *et al.*, 2017). Gene *cpxA* encodes an inner membrane stress response sensor kinase (Fleischer *et al.*, 2007); *ompA* is required for the *cpx* response (Cho *et al.*, 2023). OsmB is predicted to be an inner-membrane-anchored lipoprotein with the rest of the protein located in the periplasm. This location is in agreement with findings that OsmB is implicated in membrane stress response to hypoosmotic conditions (Jung *et al.*, 1990). Furthermore, while the function of OsmB is still unknown, the expression of *osmB* gene is upregulated during *E. coli* growth in hypoosmotic media and is induced by envelope stress (Boulanger *et al.*, 2005). The *yjbF* gene is part of the *yjbEFGH* operon that mediates the production and secretion of an extracellular polysaccharide (EPS) that is different from colanic acid, and plays a role in the response to osmotic stress (Ferrieres *et al.*, 2007; Ferrieres & Clarke, 2003).

YhfL, YgeQ, and YidX are still-uncharacterised inner membrane lipoproteins. Genes encoding these proteins are upregulated as part of the response to multiple stressors, indicating that they may have a stress response role (Abdelwahed *et al.*, 2022).

Among identified genes, whose deletion prevents infection, some are required for maintenance of the cell envelope structure, cell morphology and physiology. For example, *mltA* and *mltB* encode lytic murein transglycosylases, which catalyse reactions required for cell wall formation and remodelling (Kraft *et al.*, 1999; Lommatzsch *et al.*, 1997). Another gene, *yaiW*, seems to have a role in outer membrane stability, as its deletion mutants have defective outer membranes (Natarajan *et al.*, 2017). OmpA belongs to the porin family of outer membrane proteins of which YiaD is also a member. The lack of major Omp proteins including OmpA destabilises the outer membrane (Manning *et al.*, 1977). OmpA is implicated in F-pilus mediated conjugation and is shown to be a receptor for some bacteriophages (Datta *et al.*, 1977; Van Alphen *et al.*, 1977). It forms complexes in the outer membranes with RcsF and Bam complex. OmpA and NlpE are required to activate the cpx response (Dekoninck *et al.*, 2020). Cells of the *ompA* deletion mutants have reduced outer

membrane stiffness which results in lower tolerance of mechanical perturbations to the membrane (Rojas *et al.*, 2018).

YiaD is an outer membrane lipoprotein, a member of the OmpA-OmpF porin family. YiaD seems to be involved in the function of BamD, an essential protein and a component of a protein complex encoded by *bamABCDE* (Selkrig *et al.*, 2014) that mediates assembly of beta-barrel outer membrane proteins, such as porins.

RzoD is a lipoprotein encoded as part of a lysis cassette from the cryptic DLP12 prophage. It plays a role in *E. coli* peptidoglycan metabolism and curli pili production; its absence alters *E. coli* cell wall structure (Toba *et al.*, 2011). Regulation of the lysis cassette gene expression is regulated by the stress response factor RpoE (Rueggeberg *et al.*, 2015).

LpoA is required for the activation of Penicillin-binding Protein 1a (PBP1a) to enable its peptidoglycan synthesis function, thus deletion of *lpoA* may have an impact on cell wall synthesis (Paradis-Bleau *et al.*, 2010).

There are a few genes encoding proteins that seemingly do not fit into either of these categories. Gene *yajG* encodes a protein of unknown function, however it is known that expression of this gene is repressed by the global regulator protein, Fnr, under anaerobic growth conditions (Salmon *et al.*, 2003). ChiQ likewise is a lipoprotein of unknown function. Interestingly, the *chiQ* mutation has been shown to cause increased stop codon readthrough in comparison to the wild-type parent, indicating interaction with ribosome (Gagarinova *et al.*, 2016). Gene *yghJ* encodes a potentially amyloidogenic lipoprotein (Antonets *et al.*, 2016), i.e. it contains a domain that forms aggregates with a fibrillar morphology. In pathogenic *E. coli*, YghJ protein is secreted by a type II secretion system. In *E. coli* K12 laboratory strains it cannot be detected in the secreted protein fraction, but rather in the cell lysates, indicating that it is cell-attached or located in the periplasm (Tan *et al.*, 2016). As a potential Type II secretion system substrate, it may be functionally connected to the role of GspJ and GspG, the type II secretion system minor pilins in the Ff infection, observed in the previous section.

CyoA is subunit II of the cytochrome b_o complex which is required for generating the proton motive force in the electron transport chain in *E. coli* (Choi *et al.*, 2017). AcrA and AcrE are the periplasmic components of the AcrAB-TolC and AcrEF-TolC multidrug efflux pumps, respectively; they share 65% amino acid sequence identity (Kobayashi *et al.*, 2001; Ma *et al.*, 1993; Wang *et al.*, 2017). Increased expression of either of these efflux pump complexes leads to greater resistance of *E. coli* to various antibiotics (Nishino *et al.*, 2003).

3.5 Systematic testing of the Keio library plate 47

Two of the earliest mutants tested, *gspG* and *gspJ*, that failed to replicate phage in only 1 out of 3 of clones taken from the same stock, were located on plate 47. The library was supplied in 96-well plates covered with sticky foil which snapped on removal from the plate and sprayed the frozen liquid containing bacteria. Because of this it was concluded that most likely heterogeneity of clones could have been caused by cross-contamination between stocks. To test this hypothesis, a systematic analysis of one library plate was performed to monitor the patterns of strains and clones that cannot be infected with Ff among 96 mutants (i.e. how frequent would the mutations be and how they will be arranged on the plate).

There was also the question of how high the frequency of proteins required for the Ff phage lifecycle amongst the uncurated *E. coli* mutants. Having no leads for required proteins outside of lipoproteins and the short list of candidate mutants, it was decided that a systematic probe of one of the 96-well Keio plates would be carried out.

As there was suspicion that plate 47 would already have contained a required mutant because of the suspected contamination of *gspG* and *gspJ*, this plate was a prime candidate for a systematic probe to identify the host genes required for Ff phage lifecycle. Plate 47 also contained a good spread of gene deletions that would have implications on a wide range of processes in *E. coli* which would allow for the testing of how modifications of various biological pathways in *E. coli* would affect its production of Ff Phage. All 96 mutants from this plate were retrieved, made F' by conjugation and analysed for ability to plate Ff phage by analysing plaque formation (Table 3.7). The clones that did not give plaques were tested for phagemid infection to test if the infection step of the phage lifecycle had been compromised (Table 3.8).

All strains that showed failure to form plaques when infected with wild type Ff phage were next tested for susceptibility to transduction for antibiotic resistance by phagemid pUC118, the determine if the block is at the infection stage. All negative mutants, with the exception of clone 2 of the *agp* mutant, also had defects in the infection/entry step of the lifecycle.

Table 3.7 Plaque assay – plate 47 mutants

Keio Strain	Mutation	Plate			Keio Strain	Mutation	Plate			Keio Strain	Mutation	Plate		
		1	2	3			1	2	3			1	2	3
JW3290F’	*gspG*	×[b]	✓	✓	JW1595F’	*pntA*	✓	✓	✓	JW2924F’	*ansB*	✓	✓	✓
JW3291F’	*gspH*	✓[a]	✓	✓	JW1611F’	*hdhA*	×	✓	✓	JW2946F’	*glcD*	✓	✓	✓
JW3293F’	*gspJ*	✓	✓	×	JW1643F’	*gloA*	✓	✓	✓	JW2947F’	*glcC*	×	×	✓
JW3294F’	*gspK*	✓	✓	✓	JW1756F’	*ansA*	✓	✓	✓	JW2956F’	*gss*	✓	×	✓
JW3309F’	*fkpA*	✓	✓	✓	JW1803F’	*sdaA*	×	✓	✓	JW2972F’	*yghA*	✓	×	×
JW3324F’	*fic*	✓	✓	✓	JW2010F’	*ugd*	✓	✓	✓	JW3143F’	*glmM*	✓	✓	✓
JW3513F’	*dppA*	✓	✓	✓	JW2023F’	*rfbC*	✓	✓	✓	JW3181F’	*gltF*	✓	✓	✓
JW3584F’	*secB*	✓	✓	✓	JW2024F’	*rfbA*	×	✓	✓	JW3192F’	*nanE*	✓	✓	×
JW3684F’	*trmE*	×	✓	✓	JW2025F’	*rfbD*	✓	✓	✓	JW3300F’	*chiA*	✓	✓	✓
JW0116F’	*speD*	✓	×	✓	JW2026F’	*rfbB*	✓	✓	×	JW3414F’	*ugpQ*	✓	✓	✓
JW0181F’	*ldcC*	✓	✓	✓	JW2027F’	*galF*	✓	✓	✓	JW3485F’	*gadA*	✓	×	✓
JW0202F’	*gloB*	✓	✓	✓	JW2033F’	*cpsG*	✓	×	✓	JW3553F’	*sgbH*	✓	✓	✓
JW0315F’	*yahI*	✓	✓	✓	JW2037F’	*fcl*	✓	×	✓	JW3591F’	*tdh*	✓	✓	✓
JW0330F’	*cynT*	✓	✓	×	JW2038F’	*gmd*	×	✓	✓	JW3592F’	*kbl*	✓	✓	×
JW0331F’	*cynS*	✓	✓	✓	JW2233F’	*glpQ*	✓	✓	✓	JW5594F’	*aslB*	✓	✓	✓
JW0469F’	*ushA*	✓	✓	✓	JW2363F’	*dsdA*	✓	✓	✓	JW3773F’	*aslA*	×	✓	×
JW0495F’	*gcl*	✓	✓	✓	JW2487F’	*ppx*	✓	×	✓	JW3794F’	*pldA*	✓	✓	✓
JW0510F’	*ybcF*	✓	✓	✓	JW2525F’	*hcaB*	✓	✓	×	JW5584F’	*pldB*	×	✓	✓
JW0661F’	*nagD*	✓	×	✓	JW2636F’	*gabD*	✓	✓	✓	JW3913F’	*metF*	✓	✓	✓
JW0663F’	*nagA*	✓	×	✓	JW2720F’	*cysC*	✓	✓	✓	JW4053F’	*phnP*	✓	✓	✓
JW0664F’	*nagB*	✓	✓	×	JW2721F’	*cysN*	✓	✓	×	JW3866F’	*fdhD*	✓	×	✓
JW0680F′	*speF*	✓	×	✓	JW2722F’	*cysD*	✓	✓	✓	JW3895F’	*fpr*	✓	✓	✓
JW0808F’	*ybiY*	✓	×	✓	JW2732F’	*cysH*	✓	×	×	JW4031F’	*nrfA*	✓	✓	✓

JW0963F’	*appA*	×	✓	✓	JW2733F’	*cysI*	✓	✓	×	JW4033F’	*nrfC*	✓	×	✓
JW0987F’	*agp*	×	×[c]	×	JW2734F’	*cysJ*	✓	✓	✓	JW4034F’	*nrfD*	✓	✓	✓
JW0999F’	*putA*	✓	✓	✓	JW2759F’	*gudX*	✓	×	✓	JW4036F’	*nrfF*	✓	✓	✓
JW1005F’	*phoH*	✓	✓	×	JW2768F’	*sdaB*	×	✓	✓	JW4037F’	*nrfG*	×	✓	✓
JW1178F’	*dadA*	✓	✓	✓	JW2842F’	*yqeA*	✓	✓	✓	JW4040F’	*fdhF*	✓	✓	✓
JW1179F’	*dadX*	✓	✓	✓	JW2871F’	*gcvP*	×	✓	✓	JW4198F’	*treC*	✓	✓	✓
JW1310F’	*ycjU*	✓	✓	✓	JW2872F’	*gcvH*	✓	✓	✓	JW4222F’	*idnT*	✓	✓	✓
JW1488F’	*gadB*	✓	✓	✓	JW2873F’	*gcvT*	✓	✓	×	JW4223F’	*idnO*	×	×	×
JW1594F’	*pntB*	✓	✓	×	JW2905F’	*speA*	✓	✓	✓	JW4224F’	*idnD*	✓	✓	✓

[a]A ✓ mark indicates that plaque formation at a similar plaque number and appearance as positive control was observed on the plate. [b]An × mark indicates no plaques were observed. [c]This second agp mutant clone formed highly mucoid colonies, whereas those of the other two clones were not mucoid. Plate 1, 2 and 3 refer to three independent Keio collection clones that were tested for each mutant.

Table 3.8 Phagemid transduction assay – plate 47 mutants

Keio Strain	Mutation	Plate			Keio Strain	Mutation	Plate			Keio Strain	Mutation	Plate		
		1	2	3			1	2	3			1	2	3
JW3290F′	*gspG*	×	✓	✓	JW1803F′	*sdaA*	×	✓	✓	JW2947F′	glcC	×	×	✓
JW3293F′	*gspJ*	✓	✓	×	JW2024F′	*rfbA*	×	✓	✓	JW2956F′	*gss*	✓	×	✓
JW3684F′	*trmE*	×	✓	✓	JW2026F′	*rfbB*	✓	✓	×	JW2972F′	*yghA*	✓	×	×
JW0116F′	*speD*	✓	×	✓	JW2033F′	*cpsG*	✓	×	✓	JW3192F′	*nanE*	✓	✓	×
JW0330F′	*cynT*	✓	✓	×	JW2037F′	*fcl*	✓	×	✓	JW3485F′	*gadA*	✓	×	✓
JW0661F′	*nagD*	✓	×	✓	JW2038F′	*gmd*	×	✓	✓	JW3592F′	*kbl*	✓	✓	×
JW0663F′	*nagA*	✓	×	✓	JW2487F′	*ppx*	✓	×	✓	JW3773F′	*aslA*	×	✓	×
JW0664F′	*nagB*	✓	✓	×	JW2525F′	*hcaB*	✓	✓	×	JW5584F′	*pldB*	×	✓	✓
JW0680F′	*speF*	✓	×	✓	JW2721F′	*cysN*	✓	✓	×	JW3866F′	*fdhD*	✓	×	✓
JW0808F′	*ybiY*	✓	×	✓	JW2732F′	*cysH*	✓	×	×	JW4033F′	*nrfC*	✓	×	✓
JW0963F′	*appA*	×	✓	✓	JW2733F′	*cysI*	✓	✓	×	JW4037F′	*nrfG*	×	✓	✓
JW0987F′	*agp*	×	✓	×	JW2759F′	*gudX*	✓	×	✓	JW4222F′	*idnT*	✓	✓	✓
JW1005F′	*phoH*	✓	✓	×	JW2768F′	*sdaB*	×	✓	✓	JW4223F′	*idnO*	×	×	×
JW1594F′	*pntB*	✓	✓	×	JW2871F′	*gcvP*	×	✓	✓					
JW1611F′	*hdhA*	×	✓	✓	JW2873F′	*gcvT*	✓	✓	×					

[a]A ✓ mark indicates that colonies grew on the antibiotic plate. [b]An × mark indicates no colony growth was observed. Plate 1, 2 and 3 refer to three independent Keio collection clones that were tested for each mutant.

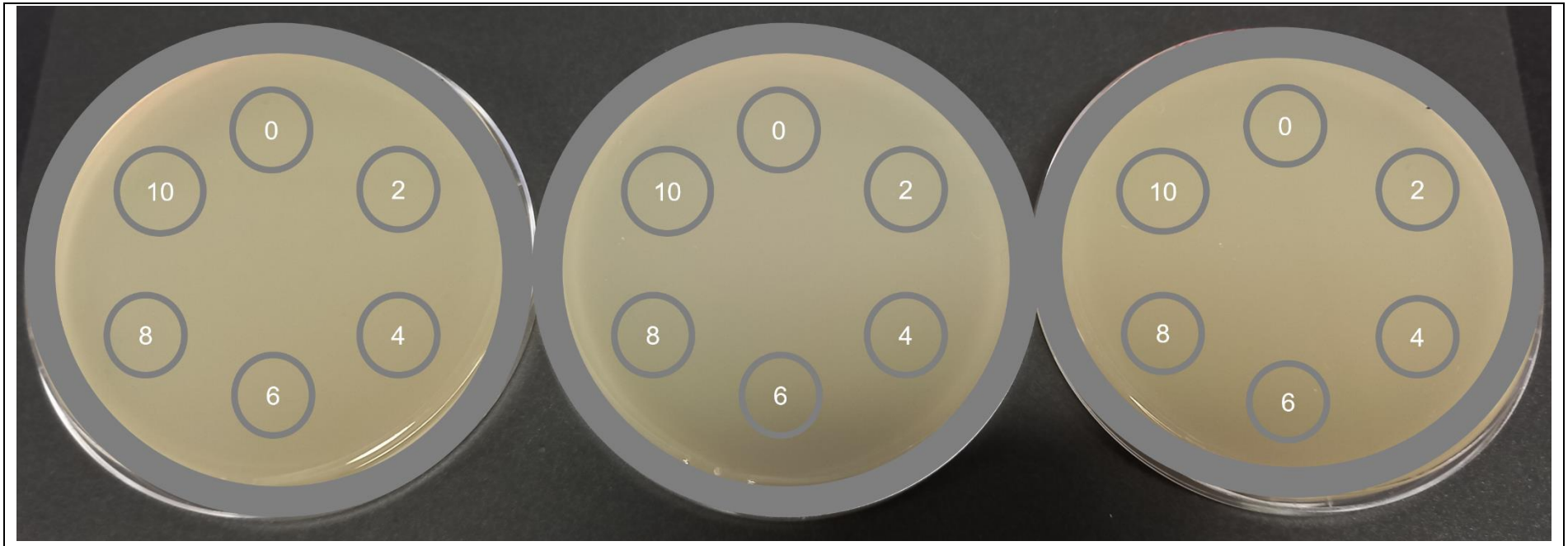

Figure 3.9 Phage plating assay, idnO mutant, all three clones lacking visible plaques

Dilutions of Ff phage f1 were spotted onto three independent F' *idnO* clones. A, B and C correspond to three different clones of F' *idnO.* In detail, soft agar was inoculated with a clone the mutant *E. coli* and was spread onto a plate to create the bacterial lawn. The dilutions of a f1 phage stock (10ul each) were then spotted onto the plate, as indicated by the small grey circles in the image. The numeral within those circles corresponds to the dilution factor of the f1 phage stock in those spots, with 0 being where a drop of the undiluted stock was placed, 2 for the 10^{-2} dilution drop, 4 for the 10^{-4} dilution, and so on until the final dilution of 10^{-10} in the circle marked 10. In all three plates of the *idnO* mutant, no thinner lawn or single plaques can be observed.

Among the mutants that did not plate phage, *gspG* and *gspJ* were discussed in previous section where PCR analysis confirmed that there was contamination from other mutants (section 3.3.5)

In *idnO* all three clones showed the same phenotype, the mutant did not form plaques at all. This indicates that *idnO* cannot support phage lifecycle. The mutant was further tested for susceptibility to phagemid transduction to see if the infection step of the lifecycle is compromised. The *idnO* mutant infection was blocked. The F' *idnO* mutant clones were tested in a secondary F' conjugation and were shown not to be functional as a donors (Fig. 3.10), hence the effect of this mutation on the F pilus was a reason for the lack of susceptibility to phagemid infection .

The identities of *idnO* mutant clones were confirmed by PCR testing (Fig. 3.12). When primers specific for *idnO*-flanking sequences were used, a PCR product of 1.5 Kb was detected using DNA isolated from the three *idnO* (::Km^R) F' clones and 0.65 Kb for the wild-type *idnO* control strain K2245, as expected. These PCRs therefore confirmed *idnO*::Km^R mutations in all three clones that failed to plate the Ff phage.

Among the mutant clones failing to plate Ff phage, all but one also failed to support phagemid transduction. The exception is one of the three clones of the *agp* mutant which showed a unique pattern of phenotypes. None of the three clones formed any plaques, however the phagemid infection essay showed only 2 out of 3 failed to be transduced in the phagemid assay. Interestingly, the colony phenotype of the clone that allowed infection was different than that of the other two that could not be infected, with the former showing a mucoid growth pattern whereas the two that were resistant in the phagemid assay did not show mucoidy. Furthermore, the mucoid clone which had functional infection step, also showed some clearance under higher titres in the phage infection assay similar to *trx* and other assembly mutants, indicating a defect in the assembly, rather than infection step of the phage lifecycle. This in turn shows that among the *agp* candidate clones there are two different mutations, one resistant to the phage infection-entry step, and one unable to support assembly, which also results in overproduction of extracellular polysaccharides.

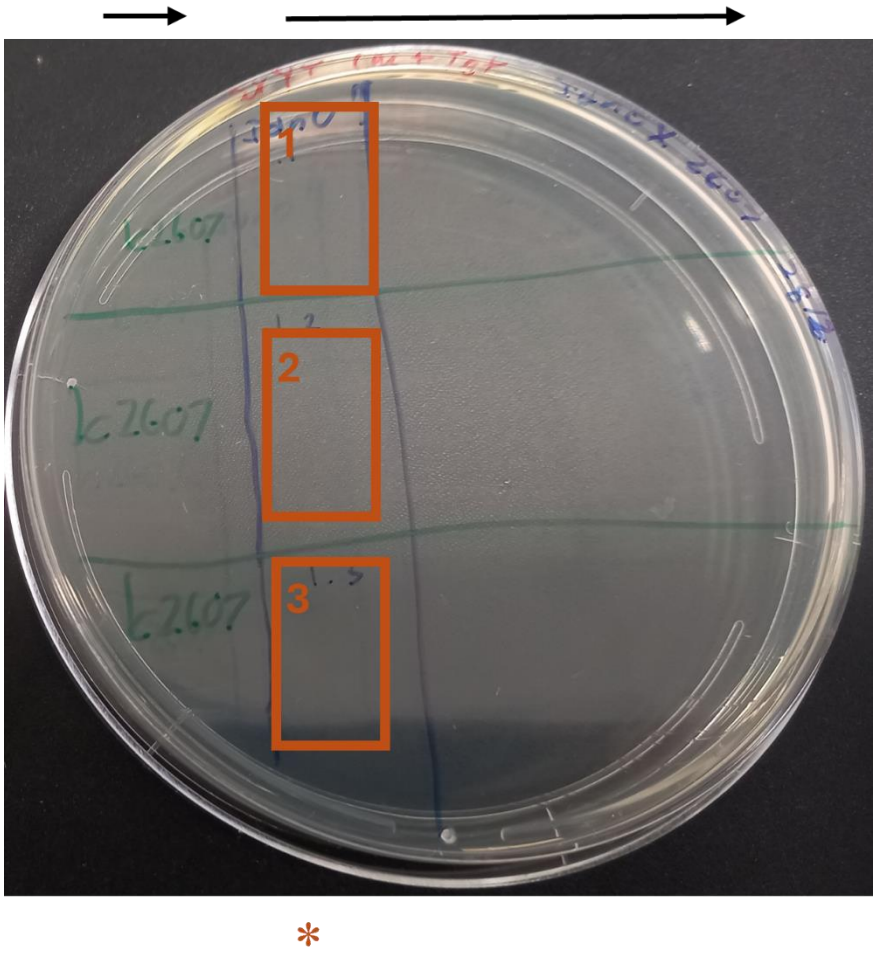

Figure 3.10 Assay of idnO mutant F' strains donor function with no transconjugant growth observed

Materials of colonies from donor (F' Tet^R) and recipient (Cm^R) were crossed on a selection plate for transconjugants containing both Tet and Cm markers. In this case, the donor was i*dnO F'* and recipient was K2607. No colonies grew on this plate indicating a failure of the tested *idnO F'* clone to conjugate the F plasmid to K2607. 1,2 and 3 represent three independent colonies of *idnO F'*. *Donor *idnO* Mutant, three independent colonies.

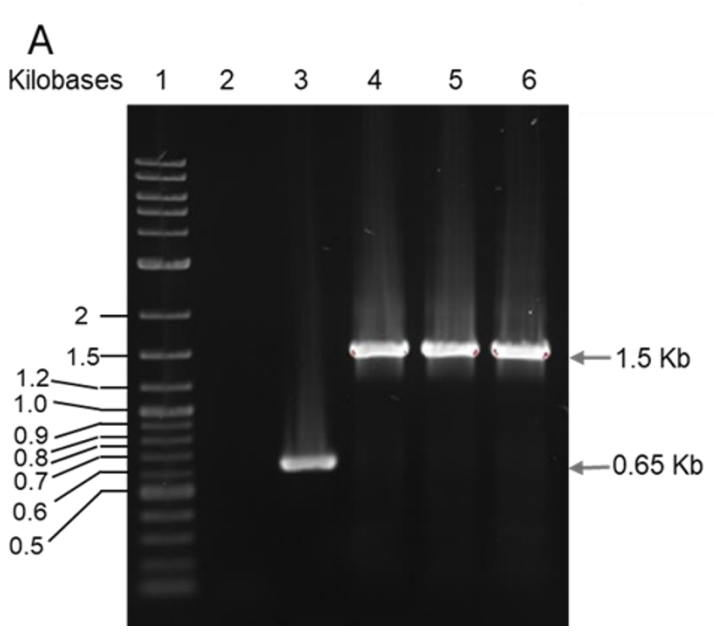

Figure 3.11 Confirming idnO mutant clones using PCR

Agarose gel electrophoresis of the PCR products using flanking primers and chromosomal DNA as a template. Lanes: 1 1KB+ DNA standard ladder; 2-6, PCR reactions; 2, no-template control; 3 K2245 (wild type *idnO*), 4-6 *idnO*::Km^R mutant clones 1-3, respectively.

As seen in Table 3.7, the number of heterogenous clones in the terms of plating phage was quite high with 41 out of 96 mutants having clones with at least one clone that was not susceptible to Ff infection. In 36 of those tested mutants, only one out of three clones showed a failure to form plaques. This heterogeneity between clones indicates likely high level of cross-contamination with another strain from the plate. In addition, there were four mutant strains where two out of three clones showed a failure to form plaques. Furthermore, there was also a single mutant, *agp*, which had the same phenotype under the phage assay, as all three clones had failed to form plaques, but in the phagemid assay one of those three clones could be transduced in the phagemid assay. This high level of heterogeneity in phenotypes within clones from what should be the same mutant, indicates that there might also be some contamination within the stocks used in the preparation of the library plates, rather than cross-contamination at the time of retrieving of the clones.

3.5.1 Pattern of clones with non-uniform phenotype

It was unknown if the contamination came from within the same plate or an outside source. To analyse if there was any pattern to the contamination in plate 47, which would increase the likelihood that some mutants on the plate were the contamination source for the surrounding wells, a chart was generated of the plate with the strains that had heterogeneous phenotypes highlighted (Fig. 3.12).

	1	2	3	4	5	6	7	8	9	10	11	12
A	gspG	trmE	gcl	agp	pntA	rfbD	ppx	cysJ	ansB	chiA	pldA	nrfD
B	gspH	speD	ybcF	putA	hdhA	rfbB	hcaB	gudX	glcD	ugpQ	pldB	nrfF
C	gspJ	ldcC	nagD	phoH	gloA	galF	gabD	sdaB	glcC	gadA	metF	nrfG
D	gspK	gloB	nagA	dadA	ansA	cpsG	cysC	yqeA	gss	sgbH	phnP	fdhF
E	fkpA	yahI	nagB	dadX	sdaA	fcl	cysN	gcvP	yghA	tdh	fdhD	treC
F	fic	cynT	speF	ycjU	ugd	gmd	cysD	gcvH	glmM	kbl	fpr	idnT
G	dppA	cynS	ybiY	gadB	rfbC	glpQ	cysH	gcvT	gltF	aslB	nrfA	**idnO**
H	secB	ushA	appA	pntB	rfbA	dsdA	cysI	speA	nanE	aslA	nrfC	idnD

Figure 3.12 Pattern of wells containing Ff-resistant mutant clones on plate 47

The orange highlighted genes are those where one out three clones failed to plate phage and be transduced by phagemid. These include *gspG, gspJ, trmE, speD, cynT, nagD, nagA, nagB, speF, ybiY, appA, phoH, pntB, hdhA, sdaA, rfbA, rfbB, cpsG, fcl, gmd, ppx, hcaB, cysN, cysI, gudX, sdaB, gss, gcvP, gcvT, nanE, gadA, kbl, pldB, fdhD, nrfC, nrfG*, The blue highlighted genes are those where two out three clones failed to plate phage and be transduced by phagemid. These include *cysH, glcC, AslA, yghA*. The *agp* mutant was highlighted yellow to distinguish it, as it had two clones that could not plate phage or be transduced by phagemid, and one clone that cloud not plate phage but could be transduced by phagemid. The *idnO* text is in red as all three clones could not plate phage or be transduced by phagemid, and the identity of all three clones has been confirmed to be *idnO* by PCR (Fig. 3.12).

The spread of stocks containing Ff-resistant clones is widely distributed throughout the plate except for the leftmost and rightmost columns of plate 47, with the clones in those areas expressing the same phenotype in terms of supporting phage lifecycle. The distribution of clones with different phenotypes also appears to have a mosaic pattern instead of being clustered around any specific genes, which implies a high likelihood that there may be

multiple sources of contamination, rather than just a few wells contaminating their surroundings, so therefore it appears that there is a high frequency of mutants that prevent infection step of the phage lifecycle. A notable example that points to other sources of contamination is the *agp* mutant. One of the clones sourced from the *agp* well showed a mucoid colony phenotype, which is not a known phenotype of *agp* mutants as Glucose-1-phosphatase does not contribute to the formation of a polysaccharide wall (Lee *et al.*, 2003), nor was that phenotype observed from any other mutants grown from the plate. This particular clone could be a contaminant from a different plate or a laboratory where the mutant library was handled. The analysis of *gspG* and *gspJ* clones in the Section 3.3 the clones that plated phage were contaminants with the Keio clones containing wild-type *gspG* and *gspJ*, which indicates a high frequency of cross-contamination of stocks in the 96-well plates containing the library. Therefore, it is likely that the rest of the stocks from the 96-well plate that have heterogenous phenotype are likely contaminated with different library clones. The library was purchased from Thermo, and it was unknown how often the library was amplified by them, and what measures were taken to prevent cross-contamination.

4. DISCUSSION

4.1 General discussion

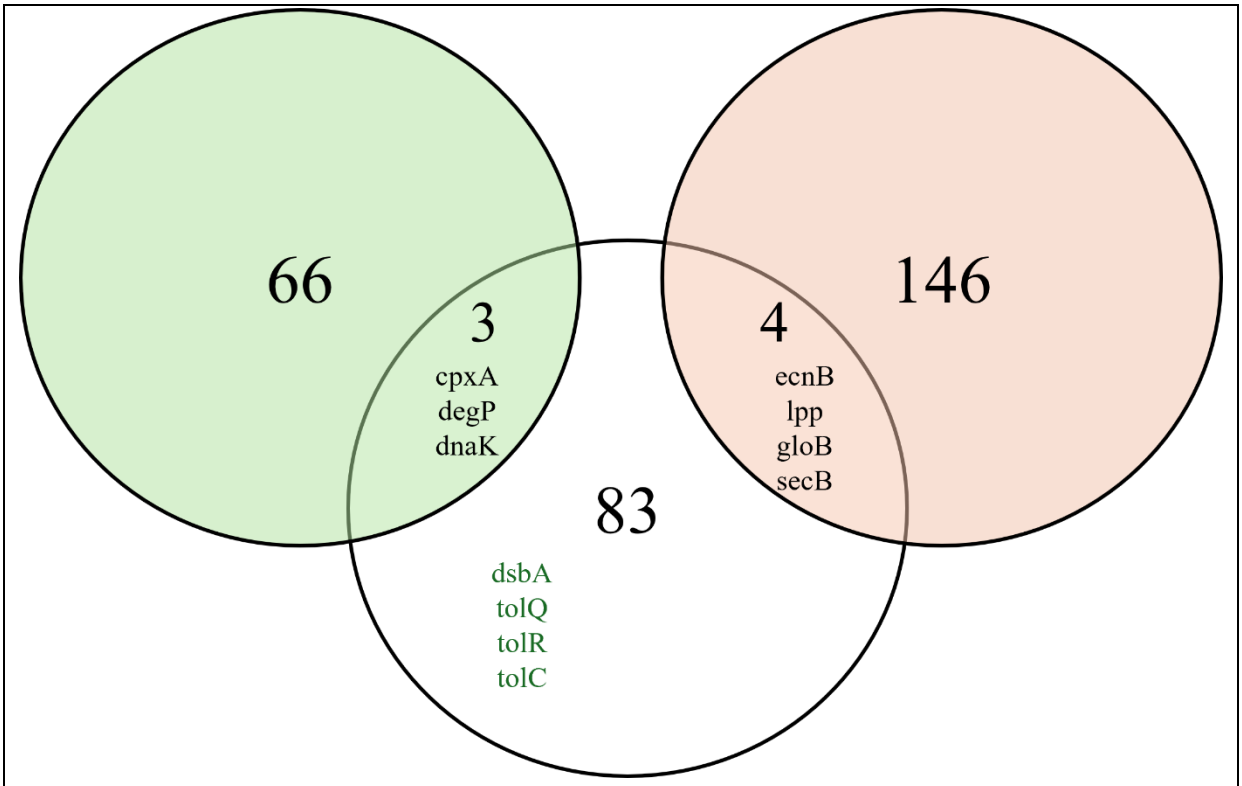

Figure 4.1 Venn diagram of genes required for conjugation and Ff phage infection

The Venn diagram above depicts how the genes found to impact Ff phage infection from this thesis overlap with the genes found to be required for F pilus conjugation from a published study (Alalam *et al.*, 2020). The green circle represents the 66 genes found to impair Ff phage infection, and the red circle is for the 146 genes that had no impact from this thesis work, while the white circle is for the 83 genes that Alalam *et al*. (2020) found to impair conjugation. Between the green and white circles, the three genes that directly overlap, thus have impacts on both Ff phage infection and conjugation, *cpxA*, *degP*, and *dnaK* are listed. The overlap of the red and white circle lists the four genes that hinder conjugation but have no impact on Ff phage infection, *ecnB, lpp, gloB,* and *secB.* Also shown in green text within the white circle are four genes that are already known to have an impact on Ff phage infection from other studies, and were found to have an impact on F pilus conjugation as well.

This thesis describes investigation of *E. coli* chromosomal genes required for Filamentous phage lifecycle (reproduction). It identified 66 genes by screening 212 mutants of the Keio

collection, where in each mutant most of the coding sequence was replaced by a Km^R cassette (leaving only 6 codons on each end of the CDS).

Interestingly, most of the genes identified in this screen that have an impact on the Ff phage lifecycle seemed to hinder it at the infection stage of the lifecycle. Two steps have so far been resolved for Ff phage infection to occur, first the phage binds to the F pilus, which retracts to bring the pIII protein of the virion to the secondary receptor, periplasmic domain of an inner-membrane-anchored trans-periplasmic protein, TolA, a component of TolQRA complex. This stage is followed by the genome (circular ssDNA) entry into the host cytoplasm, accompanied by insertion of major coat protein pVIII into the inner membrane (Bennett & Rakonjac, 2006).

The mechanism by which pilus retraction allows the virion to bypass the outer membrane to reach the inner-membrane-bound TolA is still unknown so there may also be proteins required to facilitate that process. To try and differentiate whether the proteins encoded by the identified genes were impacting the F pilus functions or later steps of infection (e.g. entry), a literature search was performed to see if any of them have been previously implicated in the F pilus function. A published study which screened for *E. coli* mutants that were required for F pilus function showed some overlaps with the mutants identified in this thesis (Alalam *et al.*, 2020). These were *dnaK*, *cpxA*, and *degP* genes (Table 3.3 – 3.6).

However, there were many genes that did not overlap between the two. Of those, some genes that were found are different between the two screens, but they encode proteins that belong to the same functional groups. One group are components of efflux pumps; *acrB* in the conjugation study and *acrA*, and *acrE* in this thesis work. Efflux pumps together with outer membrane component TolC, could be involved in the uptake of nutrients and aid intensive energy-requiring processes such as F pilus conjugation and Ff phage infection.

Conjugation screen found *cysE* as required for donor function in conjugation, whereas in this thesis *cysN*, *cysH*, and *cysI* were required for infection. All four genes are in the pathway of sulphate reduction and cysteine biosynthesis and could be involved in assembly and maintenance of the Fe-S-containing redox proteins of the electron-transport chain or proteins involved in other redox reactions in the cell dependent on sulphur- or cysteine- containing oxidoreductases., which are internally linked to generation of protonmotive force or other essential redox reactions.

Another overlap between Ff infection and conjugation is TolQ, component of the TolQRA complex required for phage entry, which was also found to be required for conjugation.

There were also some genes that were reported to impact F pilus conjugation (Alalam *et al.*, 2020), but in this thesis they showed no observable impact on the Ff phage infection; e.g. *ecnB*, *lpp* and *gloB*. Some of these genes encode proteins that may play a role in conjugational transfer of DNA from donor to recipient, but do not have a significant impact on the structure of the F pilus where Ff phage binding occurs, allowing infection to proceed as normal. This hypothesis is corroborated by a study which shows that a *traC* mutant which could not form the full F pilus structure and had greatly diminished conjugation ability, could still be infected by the Ff phages with only a slight decrease in efficiency (Schandel *et al.*, 1987).

Though the published conjugation study screened the entire Keio collection, they did not colony-purify individual mutants they revived from the stocks (Alalam *et al.*, 2020). Seeding their assay cultures with unpurified stocks from the 96-well Keio library plates would include a number of contaminating conjugation-positive clones that masked conjugation-negative clones. This may be why the published study did not identify several key mutants that the much smaller screen performed in this thesis found to interfere with donor function in conjugation, such as *gspG*, *gspJ*, and *idnO*.

4.2 Future directions

Further study should be done on the mutants that were identified in this study to be deficient for Ff phage infection, to further test if their deficiency is caused by a defect in the F pilus function or the later steps of infection i.e. DNA entry. This can be done by a series of the follow-up conjugation assays with the individual mutants that have been identified as not susceptible to phagemid transduction in this study.

The mutants identified in this thesis should also be further tested to see if they might also have impact on the phage DNA replication or assembly stages of the Ff phage lifecycle as well, given that some might play multiple roles. For example, DnaK plays a role in Ff phage infection via the F pilus, but it may also be required for Ff phage (-) strand DNA replication that is necessary for the phagemid ssDNA to be converted to dsDNA so that it can express antibiotic resistance marker and be maintained by replication from a plasmid origin.

Moreover, other phages have been reported to require DnaK for their DNA replication (Nakamura *et al.*, 2007).

A way to bypass infection and test solely for phage assembly is to use phagemid-helper plasmid system to assay replication and assembly. In this assay the phagemid production uses the same processes as Ff phages for DNA replication and assembly, but have advantage that their DNA can be introduced by transformation, into cells that contain helper plasmid expressing all other phage proteins (Quezada, 2021). Phagemid production assay would then be used to determine whether the replication from the Ff origin and assembly are functional.

Further work should be done to confirm the identity of mutations in clones that do not support phage life cycle by PCR analyses, to confirm that the results are attributed to the correct mutations.

In the future, this experiment can be repeated, but instead of testing clones individually, the strains from the Keio library can be pooled together to reduce workload. Normally Ff phage infection is not lethal to *E. coli*, however there are some mutant Ff phage strains (e.g. gI, gIV or gVIII phage mutants) that will kill infected cells (Pratt *et al.*, 1969). So, these "killer" mutants, which result in death of all infected cells, will allow for the non-infected cells to be positively selected. The identities of these surviving clones can be determined in the pool of selected clones by deep sequencing that detects the location of the kanamycin resistance cassettes that have been engineered for replacements of the mutated genes in the Keio collection.

These series of experiments will give a complete picture of the host genes required for the Ff lifecycle, and will pinpoint at which stage(s) of the cycle they are required. A comprehensive selection approach can also be used to identify the full complement of genes required for the Ff conjugation. The two sets of genes will provide new targets for adjuvanting molecules that could be used in the future with antibiotics, to decrease horizontal transfer of antibiotic resistance in the gut during the oral antibiotic therapy.

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6. APPENDIX I

Table A1.1. *Escherichia coli* K12 strains used in this thesis

Row	Strain	Mutation	Genotype	Reference
1	K2245	*recO*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrecO737, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	(Conners et al., 2023)
2	JW0729	*tolA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔtolA788::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
3	JW0729F'	*tolA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔtolA788::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
4	JW5856	*trxA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔtrxA732::kan, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
5	JW5856F'	*trxA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔtrxA732::kan, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
6	JW1241	*clsA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔclsA756::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
7	JW1241F'	*clsA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔclsA756::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
8	JW0772	*clsB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔclsB761::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
9	JW0772F'	*clsB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔclsB761::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
10	JW5150	*clsC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔclsC788::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
11	JW5150F'	*clsC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔclsC788::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
12	JW0157	*degP*	*F-, Δ(araD-araB)567, ΔdegP775::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
13	JW0157F'	*degP*	*Δ(araD-araB)567, ΔdegP775::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
14	JW0013	*dnaK*	*F-, ΔdnaK734::kan, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
15	JW0013F'	*dnaK*	*ΔdnaK734::kan, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
16	JW3285	*gspA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspA733::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
17	JW3285F'	*gspA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspA733::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
18	JW3297	*gspO*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspO745::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
19	JW3297F'	*gspO*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspO745::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
20	JW3286	*gspC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspC734::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
21	JW3286F'	*gspC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspC734::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
22	JW3288	*gspE*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspE736::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
23	JW3288F'	*gspE*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspE736::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
24	JW3289	*gspF*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspF737::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
25	JW3289F'	*gspF*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspF737::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

26	JW3290	*gspG*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspG738::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
27	JW3290F'	*gspG*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspG738::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
28	JW3291	*gspH*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspH739::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
29	JW3291F'	*gspH*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspH739::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
30	JW3293	*gspJ*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspJ741::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
31	JW3293F'	*gspJ*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspJ741::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
32	JW3294	*gspK*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspK742::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
33	JW3294F'	*gspK*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspK742::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
34	JW5704	*gspM*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspM744::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
35	JW5704F'	*gspM*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspM744::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
36	JW5705	*gspL*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspL743::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
37	JW5705F'	*gspL*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspL743::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
38	JW5706	*gspI*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspI740::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
39	JW5706F'	*gspI*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspI740::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
40	JW5707	*gspD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspD735::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
41	JW5707F'	*gspD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgspD735::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
42	JW2938	*yghG*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyghG745::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
43	JW2938F'	*yghG*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyghG745::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
44	JW2373	*ypdl*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔypdI785::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
45	JW2373F'	*ypdl*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔypdI785::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
46	JW2462	*bamC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔbamC726::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
47	JW2462F'	*bamC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔbamC726::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
48	JW0755	*ybhC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔybhC744::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
49	JW0755F'	*ybhC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔybhC744::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
50	JW0731	*pal*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), Δpal-790::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
51	JW0731F'	*pal*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), Δpal-790::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
52	JW0188	*nlpE*	*F-, Δ(araD-araB)567, ΔnlpE789::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
53	JW0188F'	*nlpE*	*Δ(araD-araB)567, ΔnlpE789::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

54	JW1795	*yeaY*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyeaY731::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
55	JW1795F'	*yeaY*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyeaY731::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
56	JW3474	*slp*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δslp-761::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
57	JW3474F'	*slp*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δslp-761::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
58	JW2425	*yfeY*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfeY761::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
59	JW2425F'	*yfeY*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfeY761::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
60	JW1892	*yecR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyecR754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
61	JW1892F'	*yecR*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyecR754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
62	JW1913	*yedD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyedD774::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
63	JW1913F'	*yedD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyedD774::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
64	JW1782	*yoaF*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyoaF789::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
65	JW1782F'	*yoaF*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyoaF789::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
66	JW1631	*mliC (ydhA)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔydhA788::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
67	JW1631F'	*mliC (ydhA)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔydhA788::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
68	JW2539	*qseG (yfhG)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfhG729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
69	JW2539F'	*qseG (yfhG)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfhG729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
70	JW2504	*yfhM*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfhM766::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
71	JW2504F'	*yfhM*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfhM766::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
72	JW1377	*YnbE*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔynbE746::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
73	JW1377F'	*YnbE*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔynbE746::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
74	JW1427	*ydcL*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔydcL720::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
75	JW1427F'	*ydcL*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔydcL720::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
76	JW1050	*yceB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyceB733::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
77	JW1050F'	*yceB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyceB733::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
78	JW1545	*Q* (within rzp*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrzpQ775::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
79	JW1545F'	*Q* (within rzp*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrzpQ775::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
80	JW0369	*yaiW*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔyaiW743::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
81	JW0369F'	*yaiW*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔyaiW743::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

82	JW0206	*yafT*	*F-, Δ(araD-araB)567, ΔyafT735::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
83	JW0206F'	*yafT*	*Δ(araD-araB)567, ΔyafT735::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
84	JW0118	*yacC*	*F-, Δ(araD-araB)567, ΔyacC740::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
85	JW0118F'	*yacC*	*Δ(araD-araB)567, ΔyacC740::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
86	JW0126	*yadE*	*F-, Δ(araD-araB)567, ΔyadE747::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
87	JW0126F'	*yadE*	*Δ(araD-araB)567, ΔyadE747::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
88	JW2586	*yfiB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfiB768::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
89	JW2586F'	*yfiB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfiB768::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
90	JW0443	*ybaY*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔybaY739::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
91	JW0443F'	*ybaY*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔybaY739::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
92	JW0424	*yajG*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔyajG720::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
93	JW0424F'	*yajG*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔyajG720::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
94	JW0969	*gfcB (ymcC)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgfcB730::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
95	JW0969F'	*gfcB (ymcC)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgfcB730::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
96	JW1374	*hslJ*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔhslJ743::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
97	JW1374F'	*hslJ*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔhslJ743::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
98	JW0849	*ybjP*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔybjP766::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
99	JW0849F'	*ybjP*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔybjP766::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
100	JW4110	*blc*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, Δblc-776::kan, hsdR514*	(Baba *et al.* , 2006)
101	JW4110F'	*blc*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, Δblc-776::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
102	JW0966	*gfcE (yccZ)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgfcE727::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
103	JW0966F'	*gfcE (yccZ)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgfcE727::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
104	JW0967	*gfcD (ymcA)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgfcD728::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
105	JW0967F'	*gfcD (ymcA)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgfcD728::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
106	JW0224	*yafP*	*F-, Δ(araD-araB)567, ΔyafP752::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
107	JW0224F'	*yafP*	*Δ(araD-araB)567, ΔyafP752::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
108	JW2801	*ygdR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔygdR758::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
109	JW2801F'	*ygdR*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔygdR758::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

110	JW1667	*lpp*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δlpp-752::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
111	JW1667F'	*lpp*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δlpp-752::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
112	JW3132	*nlpI*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔnlpI775::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
113	JW3132F'	*nlpI*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔnlpI775::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
114	JW2938	*yghG*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyghG745::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
115	JW2938F'	*yghG*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyghG745::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
116	JW3119	*dolP (yraP)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyraP763::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
117	JW3119F'	*dolP (yraP)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyraP763::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
118	JW3116	*lpoA (yraM)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyraM760::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
119	JW3116F'	*lpoA (yraM)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyraM760::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
120	JW3235	*yhdV*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyhdV785::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
121	JW3235F'	*yhdV*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyhdV785::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
122	JW3332	*yhfL*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyhfL777::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
123	JW3332F'	*yhfL*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyhfL777::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
124	JW5657	*yiaD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyiaD736::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
125	JW5657F'	*yiaD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyiaD736::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
126	JW3532	*ysaB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔysaB743::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
127	JW3532F'	*ysaB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔysaB743::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
128	JW3867	*yiiG*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔyiiG759::kan, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
129	JW3867F'	*yiiG*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔyiiG759::kan, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
130	JW3989	*yjbH*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔyjbH725::kan, hsdR514*	(Baba et al., 2006)
131	JW3989F'	*yjbH*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔyjbH725::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
132	JW0628	*rlpA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔrlpA772::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
133	JW0628F'	*rlpA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔrlpA772::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
134	JW0192	*rcsF*	*F-, Δ(araD-araB)567, ΔrcsF721::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
135	JW0192F'	*rcsF*	*Δ(araD-araB)567, ΔrcsF721::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
136	JW2982	*yqhH*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyqhH786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
137	JW2982F'	*yqhH*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyqhH786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

138	JW1698	*NlpC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔnlpC782::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
139	JW1698F'	*NlpC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔnlpC782::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
140	JW1275	*osmB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔosmB720::kan, rph-1, Δ(rhaD-rhaB)568,*	(Baba *et al.* , 2006)
141	JW1275F'	*osmB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔosmB720::kan, rph-1, Δ(rhaD-rhaB)568,; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
142	JW1728	*osmE*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔosmE738::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
143	JW1728F'	*osmE*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔosmE738::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
144	JW1633	*slyB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔslyB790::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
145	JW1633F'	*slyB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔslyB790::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
146	JW0546	*borD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔborD766::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
147	JW0546F'	*borD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔborD766::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
148	JW4108	*ecnB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔecnB774::kan, hsdR514*	(Baba *et al.* , 2006)
149	JW4108F'	*ecnB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔecnB774::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
150	JW5412	*yifL*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfiL765::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
151	JW5412F'	*yifL*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfiL765::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
152	JW5711	*yjbF*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔyjbF723::kan, hsdR514*	(Baba *et al.* , 2006)
153	JW5711F'	*yjbF*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔyjbF723::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
154	JW5633	*YidQ*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔyidQ724::kan, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
155	JW5633F'	*YidQ*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔyidQ724::kan, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
156	JW5736	*yjeI*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔyjeI769::kan, hsdR514*	(Baba *et al.* , 2006)
157	JW5736F'	*yjeI*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔyjeI769::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
158	JW5737	*ecnA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔecnA773::kan, hsdR514*	(Baba et al., 2006)
159	JW5737F'	*ecnA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔecnA773::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
160	JW5743	*bsmA (yjfO)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔyjfO739::kan, hsdR514*	(Baba *et al.* , 2006)
161	JW5743F'	*bsmA (yjfO)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔyjfO739::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
162	JW5925	*yghJ*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyghJ747::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
163	JW5925F'	*yghJ*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyghJ747::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
164	JW5213	*rzoR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrzoR729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
165	JW5213F'	*rzoR*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrzoR729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

166	JW5151	*yceK*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyceK721::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
167	JW5151F'	*yceK*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyceK721::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
168	JW5127	*pqiC (ymbA)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔymbA768::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
169	JW5127F'	*pqiC (ymbA)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔymbA768::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
170	JW5157	*lpoB (ycfM)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔycfM767::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
171	JW5157F'	*lpoB (ycfM)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔycfM767::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
172	JW5056	*yajI*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔyajI774::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
173	JW5056F'	*yajI*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔyajI774::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
174	JW5400	*yfgH*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfgH754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
175	JW5400F'	*yfgH*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfgH754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
176	JW5351	*yehR*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyehR748::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
177	JW5351F'	*yehR*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyehR748::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
178	JW5412	*yfiL*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfiL765::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
179	JW5412F'	*yfiL*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfiL765::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
180	JW5461	*ygeQ*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔygeQ786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
181	JW5461F'	*ygeQ*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔygeQ786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
182	JW0240	*yafY*	*F-, Δ(araD-araB)567, ΔyafY769::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
183	JW0240F'	*yafY*	*Δ(araD-araB)567, ΔyafY769::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
184	JW3635	*nlpA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔnlpA764::kan, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
185	JW3635F'	*nlpA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔnlpA764::kan, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
186	JW5921	*yfjS*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfjS720::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
187	JW5921F'	*yfjS*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfjS720::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
188	JW2343	*mlaA (vacJ)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔmlaA754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
189	JW2343F'	*mlaA (vacJ)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔmlaA754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
190	JW3233	*acrE*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔacrE783::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
191	JW3233F'	*acrE*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔacrE783::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
192	JW0452	*acrA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔacrA748::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
193	JW0452F'	*acrA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔacrA748::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

194	JW5448	*ygdI*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔygdI737::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
195	JW5448F'	*ygdI*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔygdI737::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
196	JW0668	*chiQ (ybfN)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔybfN730::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
197	JW0668F'	*chiQ (ybfN)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔybfN730::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
198	JW5080	*rzoD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔrzoD765::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
199	JW5080F'	*rzoD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔrzoD765::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
200	JW5258	*ynfC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔynfC733::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
201	JW5258F'	*ynfC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔynfC733::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
202	JW5858	*yidX*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔyidX732::kan, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
203	JW5858F'	*yidX*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔyidX732::kan, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
204	JW0422	*CyoA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔcyoA789::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
205	JW0422F'	*CyoA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔcyoA789::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
206	JW3882	*CpxA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔcpxA771::kan, hsdR514*	(Baba et al., 2006)
207	JW3882F'	*CpxA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔcpxA771::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
208	JW2373	*ypdI*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔypdI785::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
209	JW2373F'	*ypdI*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔypdI785::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
210	JW2784	*MltA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔmltA741::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
211	JW2784F'	*MltA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔmltA741::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
212	JW2671	*MltB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔmltB776::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
213	JW2671F'	*MltB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔmltB776::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
214	JW0940	*OmpA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔompA772::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
215	JW0940F'	*OmpA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔompA772::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
216	JW2047	*Wza*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δwza-760::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
217	JW2047F'	*Wza*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δwza-760::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
218	JW0561	*CusC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔcusC781::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
219	JW0561F'	*CusC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔcusC781::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
220	JW2712	*NlpD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔnlpD747::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
221	JW2712F'	*NlpD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔnlpD747::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

222	JW2903	*LoiP (yggG)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyggG784::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
223	JW2903F'	*LoiP (yggG)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyggG784::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
224	JW2265	*YfbK*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfbK752::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
225	JW2265F'	*YfbK*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyfbK752::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
226	JW0732	*CpoB (ybgF)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔybgF720::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
227	JW0732F'	*CpoB (ybgF)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔybgF720::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
228	JW0908	*LdtD (ycbB)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔycbB742::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
229	JW0908F'	*LdtD (ycbB)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔycbB742::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
230	JW2670	*PncC (ygaD)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔygaD775::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
231	JW2670F'	*PncC (ygaD)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔygaD775::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
232	JW1486	*DigH (yddW)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyddW784::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
233	JW1486F'	*DigH (yddW)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyddW784::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
234	JW2833	*actS (ygeR)*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔygeR787::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
235	JW2833F'	*actS (ygeR)*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔygeR787::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
236	JW0193	*MetQ*	*F-, Δ(araD-araB)567, ΔmetQ722::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
237	JW0193F'	*MetQ*	*Δ(araD-araB)567, ΔmetQ722::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
238	JW3309	*fkpA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔfkpA754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
239	JW3309F'	*fkpA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔfkpA754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
240	JW3324	*fic*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δfic-769::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
241	JW3324F'	*fic*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δfic-769::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
242	JW3513	*dppA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔdppA728::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
243	JW3513F'	*dppA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔdppA728::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
244	JW3584	*secB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔsecB721::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
245	JW3584F'	*secB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔsecB721::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
246	JW3684	*trmE*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔtrmE737::kan, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
247	JW3684F'	*trmE*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔtrmE737::kan, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
248	JW0116	*speD*	*F-, Δ(araD-araB)567, ΔspeD738::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
249	JW0116F'	*speD*	*Δ(araD-araB)567, ΔspeD738::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

250	JW0181	*ldcC*	*F-, Δ(araD-araB)567, ΔldcC783::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
251	JW0181F'	*ldcC*	*Δ(araD-araB)567, ΔldcC783::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
252	JW0202	*gloB*	*F-, Δ(araD-araB)567, ΔgloB731::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
253	JW0202F'	*gloB*	*Δ(araD-araB)567, ΔgloB731::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
254	JW0315	*yahI*	*F-, Δ(araD-araB)567, ΔgloB731::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
255	JW0315F'	*yahI*	*Δ(araD-araB)567, ΔgloB731::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
256	JW0330	*cynT*	*F-, Δ(araD-araB)567, ΔcynT780::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
257	JW0330F'	*cynT*	*Δ(araD-araB)567, ΔcynT780::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
258	JW0331	*cynS*	*F-, Δ(araD-araB)567, ΔcynS781::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
259	JW0331F'	*cynS*	*Δ(araD-araB)567, ΔcynS781::kan, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
260	JW0469	*ushA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔushA763::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
261	JW0469F'	*ushA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔushA763::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
262	JW0495	*gcl*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), Δgcl-790::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
263	JW0495F'	*gcl*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), Δgcl-790::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
264	JW0510	*ybcF*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔybcF734::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
265	JW0510F'	*ybcF*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔybcF734::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
266	JW0661	*nagD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔnagD724::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
267	JW0661F'	*nagD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔnagD724::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
268	JW0663	*nagA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔnagA726::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
269	JW0663F'	*nagA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔnagA726::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
270	JW0664	*nagB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔnagB727::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
271	JW0664F'	*nagB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔnagB727::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
272	JW0680	*speF*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔspeF741::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
273	JW0680F'	*speF*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), ΔspeF741::kan, λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
274	JW0808	*ybiY*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔybiY724::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
275	JW0808F'	*ybiY*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔybiY724::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
276	JW0963	*appA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔappA724::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
277	JW0963F'	*appA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔappA724::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

278	JW0987	*agp*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δagp-746::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
279	JW0987F'	*agp*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δagp-746::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
280	JW0999	*putA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔputA758::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
281	JW0999F'	*putA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔputA758::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
282	JW1005	*phoH*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔphoH766::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
283	JW1005F'	*phoH*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔphoH766::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
284	JW1178	*dadA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔdadA778::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
285	JW1178F'	*dadA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔdadA778::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
286	JW1179	*dadX*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔdadX779::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
287	JW1179F'	*dadX*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔdadX779::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
288	JW1310	*ycjU*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔycjU753::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
289	JW1310F'	*ycjU*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔycjU753::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
290	JW1488	*gadB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgadB786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
291	JW1488F'	*gadB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgadB786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
292	JW1594	*pntB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔpntB750::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
293	JW1594F'	*pntB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔpntB750::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
294	JW1595	*pntA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔpntA751::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
295	JW1595F'	*pntA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔpntA751::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
296	JW1611	*hdhA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔhdhA767::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
297	JW1611F'	*hdhA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔhdhA767::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
298	JW1643	*gloA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgloA729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
299	JW1643F'	*gloA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgloA729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
300	JW1756	*ansA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔansA765::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
301	JW1756F'	*ansA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔansA765::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
302	JW1803	*sdaA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔsdaA738::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
303	JW1803F'	*sdaA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔsdaA738::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
304	JW2010	*ugd*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δugd-726::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
305	JW2010F'	*ugd*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δugd-726::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

306	JW2023	*rfbC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrfbC736::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
307	JW2023F'	*rfbC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrfbC736::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
308	JW2024	*rfbA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrfbA737::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
309	JW2024F'	*rfbA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrfbA737::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
310	JW2025	*rfbD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrfbD738::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
311	JW2025F'	*rfbD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrfbD738::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
312	JW2026	*rfbB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrfbB739::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
313	JW2026F'	*rfbB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrfbB739::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
314	JW2027	*galF*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgalF731::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
315	JW2027F'	*galF*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgalF731::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
316	JW2033	*cpsG*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcpsG746::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
317	JW2033F'	*cpsG*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcpsG746::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
318	JW2037	*fcl*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δfcl-750::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
319	JW2037F'	*fcl*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δfcl-750::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
320	JW2038	*gmd*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δgmd-751::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
321	JW2038F'	*gmd*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δgmd-751::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
322	JW2233	*glpQ*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔglpQ790::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
323	JW2233F'	*glpQ*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔglpQ790::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
324	JW2363	*dsdA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δrbn-750::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
325	JW2363F'	*dsdA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δrbn-750::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
326	JW2487	*ppx*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δppx-750::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
327	JW2487F'	*ppx*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δppx-750::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
328	JW2525	*hcaB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔhcaB786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
329	JW2525F'	*hcaB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔhcaB786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
330	JW2636	*gabD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgabD742::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
331	JW2636F'	*gabD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgabD742::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
332	JW2720	*cysC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysC752::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
333	JW2720F'	*cysC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysC752::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

334	JW2721	*cysN*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysN753::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
335	JW2721F'	*cysN*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysN753::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
336	JW2722	*cysD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysD754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
337	JW2722F'	*cysD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysD754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
338	JW2732	*cysH*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysH764::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
339	JW2732F'	*cysH*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysH764::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
340	JW2733	*cysI*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysI765::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
341	JW2733F'	*cysI*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysI765::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
342	JW2734	*cysJ*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysJ766::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
343	JW2734F'	*cysJ*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔcysJ766::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
344	JW2759	*gudX*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgudX786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
345	JW2759F'	*gudX*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgudX786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
346	JW2768	*sdaB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔsdaB724::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
347	JW2768F'	*sdaB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔsdaB724::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
348	JW2842	*yqeA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyqeA725::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
349	JW2842F'	*yqeA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔyqeA725::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
350	JW2871	*gcvP*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgcvP754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
351	JW2871F'	*gcvP*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgcvP754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
352	JW2872	*gcvH*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgcvH755::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
353	JW2872F'	*gcvH*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgcvH755::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
354	JW2873	*gcvT*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgcvT756::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
355	JW2873F'	*gcvT*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgcvT756::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
356	JW2905	*speA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔspeA786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
357	JW2905F'	*speA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔspeA786::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
358	JW2924	*ansB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔansB731::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
359	JW2924F'	*ansB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔansB731::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
360	JW2946	*glcD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔglcD753::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
361	JW2946F'	*glcD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔglcD753::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

362	JW2947	*glcC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔglcC754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
363	JW2947F'	*glcC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔglcC754::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
364	JW2956	*gss*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δgss-729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
365	JW2956F'	*gss*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δgss-729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
366	JW2972	*yghA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δgss-729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
367	JW2972F'	*yghA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δgss-729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
368	JW3143	*glmM*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔglmM784::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
369	JW3143F'	*glmM*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔglmM784::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
370	JW3181	*gltF*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgltF742::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
371	JW3181F'	*gltF*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgltF742::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
372	JW3192	*nanE*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔnanE751::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
373	JW3192F'	*nanE*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔnanE751::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
374	JW3300	*chiA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔchiA748::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
375	JW3300F'	*chiA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔchiA748::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
376	JW3414	*ugpQ*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔugpQ779::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
377	JW3414F'	*ugpQ*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔugpQ779::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
378	JW3485	*gadA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgadA772::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
379	JW3485F'	*gadA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔgadA772::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
380	JW3553	*sgbH*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔsgbH764::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
381	JW3553F'	*sgbH*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔsgbH764::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
382	JW3591	*tdh*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δtdh-728::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
383	JW3591F'	*tdh*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δtdh-728::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
384	JW3592	*kbl*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δkbl-729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
385	JW3592F'	*kbl*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, Δkbl-729::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
386	JW5594	*aslB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrfaD731::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
387	JW5594F'	*aslB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, ΔrfaD731::kan, rph-1, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
388	JW3773	*aslA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔaslA748::kan, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
389	JW3773F'	*aslA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔaslA748::kan, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

390	JW3794	*pldA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔpldA766::kan, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
391	JW3794F'	*pldA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔpldA766::kan, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
392	JW5584	*pldB*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔpldB770::kan, Δ(rhaD-rhaB)568, hsdR514*	(Baba *et al.* , 2006)
393	JW5584F'	*pldB*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔpldB770::kan, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
394	JW3913	*metF*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔmetF728::kan, hsdR514*	(Baba et al., 2006)
395	JW3913F'	*metF*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔmetF728::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
396	JW4053	*phnP*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔphnP788::kan, hsdR514*	(Baba *et al.* , 2006)
397	JW4053F'	*phnP*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔphnP788::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
398	JW3866	*fdhD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔfdhD758::kan, Δ(rhaD-rhaB)568, hsdR514*	(Baba et al., 2006)
399	JW3866F'	*fdhD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, ΔfdhD758::kan, Δ(rhaD-rhaB)568, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
400	JW3895	*fpr*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, Δfpr-783::kan, hsdR514*	(Baba *et al.* , 2006)
401	JW3895F'	*fpr*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, Δfpr-783::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
402	JW4031	*nrfA*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔnrfA765::kan, hsdR514*	(Baba et al., 2006)
403	JW4031F'	*nrfA*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔnrfA765::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
404	JW4033	*nrfC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔnrfC767::kan, hsdR514*	(Baba *et al.* , 2006)
405	JW4033F'	*nrfC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔnrfC767::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
406	JW4034	*nrfD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔnrfD768::kan, hsdR514*	(Baba et al., 2006)
407	JW4034F'	*nrfD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔnrfD768::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
408	JW4036	*nrfF*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔnrfF770::kan, hsdR514*	(Baba *et al.* , 2006)
409	JW4036F'	*nrfF*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔnrfF770::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
410	JW4037	*nrfG*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔnrfG771::kan, hsdR514*	(Baba et al., 2006)
411	JW4037F'	*nrfG*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔnrfG771::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
412	JW4040	*fdhF*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔfdhF774::kan, hsdR514*	(Baba *et al.* , 2006)
413	JW4040F'	*fdhF*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔfdhF774::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
414	JW4198	*treC*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔtreC786::kan, hsdR514*	(Baba et al., 2006)
415	JW4198F'	*treC*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔtreC786::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
416	JW4222	*idnT*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔidnT736::kan, hsdR514*	(Baba *et al.* , 2006)
417	JW4222F'	*idnT*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔidnT736::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

418	JW4223	*idnO*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔidnO737::kan, hsdR514*	(Baba et al., 2006)
419	JW4223F'	*idnO*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔidnO737::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study
420	JW4224	*idnD*	*F-, Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔidnD738::kan, hsdR514*	(Baba *et al.* , 2006)
421	JW4224F'	*idnD*	*Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, ΔidnD738::kan, hsdR514; F'[::Tn10 proAB+ lacIq Δ(lacZ)M15]*	This study

Rows 1-5, control mutants of known phenotype related to the Ff lifecycle

Rows 5-43, candidate mutants based on the known functions of encoded genes

Rows 44-237, mutants of genes encoding lipoproteins

Rows 238-421, mutants from the library plate 47