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Genetic Manipulation of Pathogenicity Loci in Non-Typhimurium *Salmonella*

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Abstract

The traditional genetic tools used in *Salmonella enterica* serovar Typhimurium rely heavily on a high-transducing mutant of bacteriophage P22. P22 recognizes its hosts by the structure of their O-antigens, which vary among serovars of *Salmonella*; therefore, it cannot be used in most non-Typhimurium *Salmonella*, including the majority of those causing food-borne illnesses in both humans and livestock. Bacteriophage P1 infects a variety of enteric bacteria, including *galE* mutants of serovar Typhimurium; however, the degree to which the presence of coimmune prophages, the lack of required attachment sites or the lack of host factors act as barriers to using phage P1 in natural isolates of *Salmonella* is unknown. Here, we show that recombineering can be used to make virtually any serovar of *Salmonella* susceptible to P1 infection; as a result, P1 can be utilized for facile genetic manipulation of non-Typhimurium *Salmonella*, including movement of very large pathogenicity islands. A toolkit for easy manipulation of non-Typhimurium serovars of *Salmonella* is described.

Keywords

Salmonella enterica; Recombineering; Non-Typhimurium *Salmonella*; Bacteriophage P1; Transduction

1. Introduction

Salmonella enterica is responsible for millions of food-borne illnesses per year; its high prevalence and significant financial impact led Voetsch *et al.* (Voetsch *et al.*, 2004) to conclude that *Salmonella* “presents a major ongoing burden to public health.” Commercially important animal species are also infected by serovars of *S. enterica enterica* (Rabsch *et al.*, 2002), where virulence factors differ among strains (Ochman and Groisman, 1996). While molecular recombineering enables direct manipulation of bacterial chromosomes, it can be time-consuming and labor intensive to move these systems out of model organisms and into natural isolates. In contrast, traditional bacteriophage-mediated transduction allows easy movement of well-characterized mutations into natural isolates or introduction of chromosome regions from natural isolates into the test-bed of a model organism. Use of *Salmonella* serovar Typhimurium as a model organism is greatly facilitated by the use of bacteriophage P22 (Schmieger, 1972). P22 uses the *rfb*-encoded O-antigen polysaccharide

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as an attachment site, and loss of the O-antigen renders cells P22 resistant. While temporary disabling of restriction endonucleases via heat-shock renders other serovars of the Typhimurium subgroup bearing the 1,4,[5],12 O-antigen (e.g., Saintpaul, Heidelberg, Paratyphi B) sensitive to P22 (Edwards et al., 1999), infection does not normally occur in strains carrying structurally distinct O-antigens. The closely related bacterium *Escherichia coli* can be rendered P22 sensitive by the introduction of the Typhimurium *rfb* operon (Neal et al., 1993), resulting in expression of an O-antigen permissive for infection.

Bacteriophage P1 has been used extensively for the genetic manipulation of laboratory strains of *Escherichia coli* and *Klebsiella aerogenes* (Lennox, 1955; Streicher et al., 1971). Despite its close relatedness to these species, *Salmonella* strains are resistant to P1. However, mutations in the *galE* gene confer sensitivity to phage P1 in serovar Typhimurium LT2 (Ornellas and Stocker, 1974); these mutants are unable to epimerize UDP-glucose to UDP-galactose, a required building block for the lipopolysaccharide (LPS) core antigen that forms the foundation for anchoring the O-antigen to the outer membrane (Figure S1). UDP-galactose is also required by many *Salmonella* serovars to initiate O-antigen biosynthesis (Samuel and Reeves, 2003). As a result, *galE* mutants cannot present O-antigens on the outside of the cell, thus exposing the membrane-proximal portion of the LPS core antigen. Given that the *galE* gene is conserved among the *Salmonellae*, use of this mutation to render non-Typhimurium serovars P1 sensitive would be more straightforward than methods attempting to confer P22 sensitivity. In addition, the large P1 capsid size allows the packaging of more than 100 kb of DNA in a transducing particle (Hanks et al., 1988), whereas phage P22 only encapsulates ~44 kb of DNA in transducing particles (Jackson et al., 1978). Thus, phage P1 is the better choice for the manipulation of large genomic regions such as pathogenicity islands. The advantage of P1 in DNA packaging size is offset by the lower rate of production of transducing particles; P1-*vir* lysates contain ~0.1% transducing particles, compared to ~40% for the commonly-used terminase mutant of P22 (Schmieger, 1972).

Although P1 has the potential to permit the rapid, reliable transfer of large genomic regions among strains, its utility has not been examined in other, non-Typhimurium serovars of *Salmonella*, likely for two reasons. First, receptors for bacteriophage attachment are notoriously hypervariable and experience strong selection for variants conferring phage resistance. For example, variation in the LamB maltose receptor among strains of *E. coli* results in strain-specific resistance to phage λ (Clement et al., 1983). Second, coimmune prophages often leave cells resistant to phages of interest, even if cells present the appropriate receptors for phage attachment. For these reasons, it is unlikely that any one bacteriophage would infect diverse members of any bacterial species (Hyman and Abedon, 2010). Serovar Typhimurium LT2 must possess the correct receptor for phage P1 attachment because its *galE* mutants gain sensitivity to phage P1; it is possible that non-Typhimurium *Salmonella* also possess a P1 receptor. Moreover, coimmune prophages that confer resistance to phage P1 are not evident in the genomes of *Salmonella* sequenced to date, suggesting that coimmunity to phage P1 may not be an issue in non-Typhimurium strains. Here, we discuss the development of genetic manipulation of non-Typhimurium *Salmonella* using bacteriophage P1.

2. Materials and Methods

2.1. Media and growth conditions

Bacterial strains were propagated at 37°C in LB medium (Difco), with plates using 1.2% agar (EMB). E medium (Vogel and Bonner, 1956) was supplemented with 0.2% glucose and NCE (No Carbon E, lacking the citrate chelator) was supplemented with 10 mM MgSO₄ and 0.2% galactose. Biotin and nicotinic acid were added to a final concentration of 0.1 mM

each. P1 Dilution Buffer was prepared as 0.9% NaCl supplemented with 5 mM CaCl₂ and 10 mM MgSO₄. P1 Top Agar was prepared as LB medium supplemented with 0.7% agar, 5 mM CaCl₂, and 10 mM MgSO₄. Antibiotic concentrations used include: kanamycin at 20 µg/mL for routine propagation and at 30 µg/mL for selection of directed gene knockouts; tetracycline at 20 µg/mL for selection of plasmid-borne markers and 10 µg/mL for selection of chromosomal markers; chloramphenicol at 20 µg/mL; spectinomycin at 100 µg/mL; hygromycin at 100 µg/mL; gentamycin at 30 µg/mL and ampicillin at 100 µg/mL. Non-Typhimurium strains used in these experiments were obtained from the *Salmonella* Reference Collection B (SARB) (Boyd et al., 1993); SARB strains 1, 3, 8, and 36 were found to be defective for purine biosynthesis and required the supplementation of 0.3 mM guanosine to minimal media to support growth.

2.2. Bacteriophage propagation and transduction

Lysates of bacteriophages P22 and ES18 were prepared using the protocols of Davis *et al.* (1980) and Schmieger and Schicklmaier (1999); transductions with these phages were performed by mixing 100 µl of an overnight bacterial culture with 100 µL of phage lysate diluted to a titer of 10⁸ PFU/mL, incubating with shaking at 37°C for 1 hour and plating the transduction mixture directly to selective media. For phage P22 transductions, selective media was supplemented with 10 mM EGTA as a magnesium chelator. For use of P22 with strains containing *galE* mutations, LB broth was supplemented with 0.2% glucose and 0.02% galactose, and all other growth media was supplemented with 0.02% galactose. Lysates of bacteriophage P1-*vir* were prepared and used with an alteration of the protocol of Kolko *et al.* (2001), where growth media was supplemented with 0.2% glucose, and cells were propagated for 30 minutes after phage addition to reduce clumping.

2.3. Bacteriophage P1-*vir* plaque assay

Strains were assessed for sensitivity to phage P1-*vir* using two methods: lysate preparation in liquid media and plaque assay on solid media. Plaque assays were conducted by diluting 500 µL of cells grown overnight at 37°C into 5 mL LB and incubating with shaking for 60 minutes at 37°C. Cells were pelleted with centrifugation and resuspended in 2.5 mL P1 Dilution Buffer; a 100 µL aliquot of cells was mixed with 2.5 mL P1 Top Agar and plated to a room-temperature LB plate. P1-*vir* lysate was serially diluted to 10⁻⁸ in P1 Dilution Buffer and spotted onto plates in 10 µL aliquots; plates were allowed to dry at room temperature. Plaque formation was assessed after overnight incubation at 37°C.

2.4. Directed gene replacements

Gene replacements followed the procedure of Poteete and Fenton (2000) and Datsenko and Wanner (2000). Primers were designed so that their 3' ends were complementary to sequences flanking the antibiotic resistance cassettes *cat* (chloramphenicol), *aph* (kanamycin), *tet* (tetracycline) or *hph* (hygromycin) and 45 bases at their 5' ends were complementary to sequences within the *S. enterica* gene targeted for replacement. Replacement of chromosomal genes with antibiotic resistance cassettes was confirmed by PCR amplification of the target locus, associated phenotypes, and DNA sequencing when possible. All constructs were backcrossed into the LT2 laboratory strain of *S. enterica* via P1 or P22 bacteriophage transduction. Sequences used to target gene replacements are listed in Table 1.

3. Results and Discussion

3.1. The presence of O-antigens confers P1 resistance in serovar Typhimurium LT2

Although the identity of the P1 receptor is unclear, the terminal glucose of the LPS core antigen has been implicated in P1 adsorption to *E. coli* K12 (Sandulache et al., 1984).

Mutations in either the *galU* or *galE* genes, which result in the formation of truncated, galactose-depleted LPS core antigens, both confer P1 sensitivity in *Salmonella enterica* Typhimurium LT2 (Ornellas and Stocker, 1974) and prevent display of O-antigens (Figure S1A). The sensitivity of *galE* mutants to phage P1 may reflect the removal of the O-antigen's steric inhibition on a polysaccharide receptor within the LPS core antigen in *Salmonella* (Yarmolinski and Bonner, 1988). If so, then it is unclear why retention of the O-antigen permits P1 sensitivity in *E. coli* K12, considering that the polysaccharide structures of the *E. coli* and *Salmonella* LPS core antigens are very similar (Kajimura et al., 2005). Although these genetic data link the LPS core antigen to phage P1 infection, it is unknown if these polysaccharides are actual P1 attachment sites. Alteration of the LPS core antigen could result in the formation of a structure that mimics the genuine P1 receptor present on the *E. coli* K12 O-antigen.

Because the nature of the P1 receptor is ambiguous for *Salmonella*, we investigated the role of O-antigen chain length in P1 sensitivity. A less severe truncation of the O-antigen could confer P1 sensitivity while still permitting P22 sensitivity via partial presentation of the O-antigen. To test this, we constructed a series of strains in the serovar Typhimurium LT2 background bearing O-antigens of different lengths and tested the sensitivity of these strains to phage P1. Mutant derivatives were created by directed gene knockout (Datsenko and Wanner, 2000; Poteete and Fenton, 2000), replacing the coding sequences of the *galE*, *wzzB*, *fepE*, *rfc* or *rfb* loci with genes encoding antibiotic resistance determinants (Fig. 1A). Strains with mutations at either the *galE* or *rfb* locus completely lack an O-antigen, with *galE* strains also producing a slightly truncated form of the LPS core antigen. Chain length in cells displaying O-antigens was controlled experimentally via the introduction of mutations in the O-antigen chain length determining genes *rfc*, *wzzB*, and *fepE* (Figure S1, Table 2). O-antigen chain length distribution is trimodal in serovars Typhimurium and Enteritidis (Murray et al., 2003). Alone, the Rfc polymerase generates O-antigens with 1-16 repeats in length (Collins and Hackett, 1991), whereas interaction with the WzzB or FepE chain length determinants add additional monomers to the O-antigen, creating antigens of 16-35 or 100+ repeats in length, respectively (Bastin et al., 1993; Murray et al., 2003). Because both donor and recipient cells are genetically identical in terms of host restriction systems, differences in transduction efficiency can be attributed to differences in the ability of phages P1 or P22 to infect each cell type.

The relationship of O-antigen chain length to sensitivity to phages P1 and P22 was examined by transducing the *galE*-2842::*aadA* marker into the O-antigen chain length mutant strain collection (Table 2, Figure S1), where recovery of transductants indicates sensitivity to infection by bacteriophage P1. Transductants were only obtained in the recipient strains bearing mutations in the *galE* or *rfb* locus. Thus, complete absence of the O-antigen is required for P1 attachment in *Salmonella*, as the one O-antigen monomer present in KAB704 (*rfc*-3303::*cat*) is sufficient to block phage P1 infection. These data would argue against the P1 sensitivity of wild-type strains of *E. coli* arising from the lack of steric hindrance of the P1 attachment site within the LPS core; rather, it seems likely that some polysaccharide portion of the *E. coli* K12 O-antigen itself is recognizable by P1.

While the single O-antigen subunit borne by *rfc* mutants was sufficient to block P1 attachment to *Salmonella*, it was insufficient to promote P22 attachment, as *rfc* mutants were nearly insensitive to both phages (Table 2). Although all strains having an O-antigen chain length greater than 1 unit were sensitive to phage P22, there was a general trend towards increased P22 sensitivity with increasing O-antigen chain lengths. The slightly reduced sensitivity of the *galE* mutant strain KAB007 can be attributed to the dependence of O-antigen production on the amount of galactose in the growth medium.

Although both *galE* and *rfb* mutations confer P1 sensitivity, use of the *galE* mutation is a better strategy for the manipulation of non-Typhimurium strains. Unlike the hypervariable *rfb* locus, the *galE* gene is well-conserved among *Salmonella* (Figure S2), which permits the design of molecular-based directed gene replacement strategies for a large array of non-Typhimurium strains (Fig. 1A). To identify appropriate sequences to direct *galE* deletions in natural isolates, the complete genome sequences for 16 strains of *Salmonella* were downloaded from NCBI (Pruitt et al., 2009) and the regions surrounding the *gal* operon were aligned (Figure S2). The *galE* gene was well conserved in these genomes with the exception of serovar Arizonae, allowing for the identification of two 40 bp regions that were nearly identical in all 16 strains; these regions are contained within the PCR primers used to generate the *galE*-2841::aph gene replacement cassette (Table 1). Moreover, *galE* mutations can be easily repaired after completion of other genetic manipulations using *rfb* strains as transduction donors by selecting for the ability to utilize galactose as a sole carbon source. Repair of the *rfb* locus is impractical, especially for non-Typhimurium strains.

3.2. Reversal of the *galE* mutation

All serovars of *Salmonella* must acquire a mutation in either the *galE* or *rfb* locus to permit sensitivity to phage P1. Once genetic manipulations have been completed, these mutations must be reverted in order to permit proper display of the O-antigen. To address this issue, a serovar Typhimurium LT2 donor strain (KAB371) lacking an intact *rfb* locus ($\Delta rfbP$ -*rfbB*-3302::hph) and wherein the intact *galE* gene was flanked by mutations in the *bio* operon ($\Delta bioABCDF$ -631::cat) and *nadA* gene (*nadA*-1151::aph) was engineered. Using this strain as a donor, *galE* mutants can be transduced to Gal⁺ on minimal media lacking biotin and nicotinamide and containing galactose as a sole carbon source (Fig. 1B). Use of the flanking markers $\Delta bioABCDF$ -631::cat and *nadA*-1151::aph restricts the size of the incoming DNA fragment when counterselecting these markers, thereby limiting the region of DNA transduced among strains; typically ~25% of Gal⁺ transductants would be Bio⁺ Nad⁺. Experimental limitation of the amount of transduced DNA among strains is crucial for use with phage P1 due to the relatively large amount of DNA transduced by this phage.

3.3. The *galE* mutation confers sensitivity to phage P1 in non-Typhimurium *Salmonella*

Allelic replacement (Datsenko and Wanner, 2000) was used to introduce the *galE*-2841::aph construct into non-Typhimurium strains from the SARB collection (Boyd et al., 1993); this collection represented the breadth of genetic diversity within subspecies *Salmonella enterica enterica*, which includes pathogenic *Salmonellae*. The resulting collection of 13 *galE*-2841::aph derived SARB strains (Table 3) was tested for P1 sensitivity using plaque assays on solid media and for the ability to amplify P1 in liquid media. In all cases, strains displayed varying degrees of P1 sensitivity in both assays only when carrying the *galE* mutation; Gal⁺ strains were uniformly P1 resistant.

3.4. Transduction from Typhimurium LT2 donors into non-Typhimurium recipients

The ability for *galE* derivatives of non-Typhimurium serovars to act as recipients for P1 transduction was tested by transducing a variety of markers from serovar Typhimurium LT2. Donor markers included mutations at the *galE*, *fljBA*, *flhCD*, *rfb* and *org/prg* loci. While some transductions were limited by natural antibiotic resistance in the recipient strain, all others were successful (Table 3). The widespread success of transduction into a diverse collection of SARB strains suggests that the host restriction systems in non-Typhimurium serovars do not significantly impact transfer of LT2-derived DNA.

3.5. Transduction from non-Typhimurium donors into Typhimurium LT2 recipients

Next, the ability of non-Typhimurium serovars to act as donors for P1 transduction using serovar Typhimurium LT2 as a recipient was tested (Table 4). Here, P1 must replicate successfully in natural isolates of *Salmonella*, which may not permit phage replication. The *rfb* operon was selected as the target locus for transduction as it requires large incoming DNA fragments to escape cleavage by serovar Typhimurium LT2's restriction endonucleases. The region between the Typhimurium *gnd* and *galF* genes was replaced with a chloramphenicol or hygromycin resistance cassette and a linked *hisD9953::MudJ* mutation was introduced. Transductions moving the region encompassing *his* and *rfb* from SARB donors were selected for histidine prototrophy and screened for chloramphenicol or hygromycin sensitivity to validate co-transduction of the linked *rfb* operon. This transduction was successful for 12 of 13 SARB donors (Table 4), replicated with *rfb::cat* and *rfb::hph* insertion mutations; transfer of novel *rfb* operons was verified by antibody agglutination tests. While serovar Stanley could act as a P1 recipient, it failed as a P1 donor; because Stanley is His⁺, this likely reflects the failure of P1 to amplify on this strain.

Different serovars of *Salmonella* harbor different *hsd*-encoded restriction modification systems. These differences contributed to very low transduction frequencies using P22 (Edwards et al., 1999), suggesting that P1 transduction might be equally hampered by differences in restriction modification systems. However, linkage between the *hisD9953::MudJ* and $\Delta(rfbP-rfbB)$ -3301::*cat* markers fell into the range of 60% - 90% for most strains (Table 4), corresponding to a physical distance of 3-10 kb which is consistent with the size of the inter-operonic region in complete genome sequences (McClelland et al., 2001). If restriction enzymes were cleaving incoming DNA with high frequency, one would expect far lower cotransduction frequencies. Therefore, restriction endonucleases do not represent a general barrier to using transduction as a genetic tool among natural isolates of *Salmonella*.

3.6. Transduction among non-Typhimurium donors and recipients

To demonstrate that effective P1 transduction in *Salmonella* does not require Typhimurium LT2 as either a donor or a recipient, genetic material was moved between natural isolates, here transducing the *rfb* locus between non-Typhimurium serovars (Table 4). A diverse set of recipient strains was created by introducing the *hisD* and $\Delta(rfbP-rfbB)$ mutations from serovar Typhimurium LT2 into *galE* derivatives of SARB strains. The region encompassing the *his* and *rfb* operons was transduced between donor and recipient strains, again selecting for histidine prototrophy and screening for chloramphenicol sensitivity. Several *rfb* regions were successfully moved among non-Typhimurium strains (Table 4); these regions conferred changes in O-antigen serotype as determined by antibody agglutination. Therefore, *galE* derivatives of non-Typhimurium serovars of *Salmonella* can be readily manipulated by P1 using diverse donor strains. Critically, it is not impeded by restriction endonucleases and easily moves more than 50 kb of DNA.

3.7. Differential P1 sensitivity in *galE* and *rfb* derivatives

While *rfb* mutants do not synthesize an O-antigen at all and produce fully intact LPS core antigens (Schnaitman and Klena, 1993), *galE* mutants are able to synthesize O-antigens but are unable to present them on the outside of the cell, as they produce a truncated core antigen. As previously reported (Ornellas and Stocker, 1974), P1 behaves differently when amplified on these two mutants of serovar Typhimurium.

Bacteriophage P1 has two different infection phases determined by expression of alternative tail fiber genes; lysates of one phase will contain approximately 0.1% of particles with the alternative tail fiber phase created by site-specific inversion (Iida, 1984). The low relative

titer of the alternative phase necessitates several rounds of amplification when moving between hosts that are recognized by the alternate phase. To examine differences in P1 infectivity in *galE* and *rfb* mutant strains, lysates of *galE* mutants were used to seed lysates of *rfb* mutants and *vice versa*. While *galE* mutants were equally cleared by lysates produced by either *galE* or *rfb* mutants, *rfb* mutants were only effectively cleared by lysates made from another *rfb* mutant. Several rounds of amplification were required to produce a high-titer lysate on *rfb* mutant strains using P1 initially raised on *galE* mutants. These data suggest that *rfb* strains are infected by only a single phase of P1, whereas *galE* strains are infected by both phases; these results were replicated three times. Differences in infection cannot be attributed to host restriction systems, as all strains were derived from the same serovar Typhimurium LT2 background. The infectivity differences between *galE* and *rfb* mutant strains further highlight the advantage of choosing the *galE* mutation to confer P1 sensitivity in non-Typhimurium *Salmonella*. A *galE* mutant is easily repaired by P1 grown on Gal⁺ *rfb* mutants; however, the reverse is not true.

4. Conclusions

This toolkit enables rapid and reversible use of bacteriophage P1 for genetic manipulation of natural isolates of *Salmonella*. Conserved oligonucleotide sequences allow for high-efficiency introduction of either a *galE* gene or *rfb* deletion to confer P1 sensitivity in non-Typhimurium strains. The *galE*-2841::*aph* mutation confers phage P1 sensitivity in a group of SARB strains representing diversity within Subspecies 1 of *Salmonella* (Boyd et al., 1993), indicating its potential for broad applicability within *Salmonella*. One of the P1 tail fiber phases appears to recognize the LPS core antigen, ensuring widespread distribution of the attachment site. The ease, widespread distribution of the readily-accessed receptor, robustness to variability in restriction endonucleases, apparent lack of coimmune prophages, and ability to transfer large amounts of DNA make this toolkit valuable to those interested in performing classical genetics in natural isolates of *Salmonella*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Mutations in *galE* confer sensitivity to phage P1 in non-Typhimurium *Salmonella*
- P1 can transfer large regions of DNA among serovars
- Transduction is not hindered by coimmune prophages or endonucleases
- Mutations in *galE* are easily repaired using a Δrfb donor strain
- Phage P1 can be used for genetic manipulation of natural isolates of *Salmonella*

A. Making strains P1^S

Electroporate pKD46 selecting Ap^R

Electroporate *aadA* fragment selecting Sp^R

Screen for Gal⁻

B. Restoring strains to Gal⁺ P1^R

Transduce selecting Gal⁺,

Screen for Bio⁺ NAD⁺ Kn^S Cm^S Hg^S

Figure 1.

A. Protocol for creating a directed knock-out of the *galE* gene in natural isolates of *Salmonella enterica*. After introduction of a plasmid bearing the λ Red recombination system, a gene cassette is introduced to replace the *galE* gene with an antibiotic resistance cassette. **B.** Protocol for reverting *galE* derivatives of *Salmonella* natural isolates to Gal⁺. A P1 lysate is prepared on a P1^S Gal⁺ Δrfb strain of serovar Typhimurium wherein the *galE* locus is flanked by additional mutations. This fragment is transduced into P1^S natural isolates and Gal⁺ revertants are selected; screens for Bio⁺ Nad⁺ transductants minimize cotransduction of flanking markers.

Table 1

Primers used to target gene knock-outs.

Locus	Primer	Sequence homologous to target genes
<i>bioABCD</i>	BioF	5' TTTGACTCACCAGAAAGTGTAGCCAGAAGCAGCATCGCTTACCGCT
<i>bioABCD</i>	BioR	5' ATTATTGGCTAACGAGAAGGGAAAAATTGAGGCGTAGACGCTCACTTT
<i>galE</i>	GalF	5' GGTCATGACGTCGTCATCCTCGATAACCTCTGCAACAGCAAGCGC
<i>galE</i>	GalR	5' CTCGCGATCGGCTTTGCTGGCATCCGCCCAATACGCCGGGAGATC
<i>nadA</i>	NadF	5' ACGCTTTCATTCGGTTGTTTCGTAAAGTCAGTAAACGAGAAGCC
<i>nadA</i>	NadR	5' TGTGCGTACTAAAAAATCCATTCTCTCCCCAAAGATTTCCGTC
<i>rfbP-rfbB</i>	RfbF	5' TTTATTGGCAAATTAATACCACATTAAATACGCCTTATGGAATAGAAAA
<i>rfbP-rfbB</i>	RfbR	5' TTTACGCAGGCTAATTTATACAATTATTATTAGTACTTCTCGGTAAGCT

Table 2

Sensitivity of *Salmonella* serovar Typhimurium LT2 O-antigen chain length derivatives to bacteriophages P22 and P1 as determined by transduction.

Strain	Genotype	O-antigen Chain Length	Sensitivity to Phage ^a	
			P22	P1
KAB002	Wild-type	16-35; 100+	++++	-
KAB701	<i>wzzB</i> -101:: <i>aph</i>	1-16; 100+	++++	-
KAB702	<i>fepE</i> -221:: <i>tet</i>	16-35	++++	-
KAB703	<i>wzzB</i> -101:: <i>aph</i> <i>fepE</i> -221:: <i>tet</i>	1-16	+++	-
KAB704	<i>rfc</i> -3301:: <i>cat</i>	1	-/+	-/+
KAB007	<i>galE</i> -2841:: <i>aph</i>	None; truncated core antigen	-/+	++++
KAB007 ^b	<i>galE</i> -2841:: <i>aph</i>	16-35; 100+	+++	-
KAB030	$\Delta(rfbP-rfbB)$ -3301:: <i>cat</i>	None	-	++++

^a Amount of countable transductants (30-300 colony forming units per plate) observed are indicated on a (+/-) scale in relation to the dilution of transducing bacteriophage added to cells. +++++, 10^{-2} (P22) or 10^{-1} (P1) phage dilution; +++, 10^{-1} P22 phage dilution; -/+, less than 30 colonies per plate observed for undiluted P22 or P1 phage. Data represent observations across at least two replicates.

^b Grown in the presence of 0.02% galactose

Table 3
Constructs transduced into *galE2841::aph* derivatives of non-Typhimurium *Salmonella* using bacteriophage P1.

Serovar ^a	O-antigen Serotype	Donor Marker from Serovar Typhimurium LT2							
		<i>galE-2842::aadA</i>	<i>fliBA-8062::tet</i>	<i>fliHDC-8061::cat</i>	<i>Δ(rfbP-rfbB)-3301::cat</i>	<i>Δ(rfbP-rfbB)-3302::lph</i>	<i>Δ(orgC-prgH)-4301::cat</i>		
Agona (1)	1,4,12	Y ^b	Y	Y	Y	Y	Y	Y	Y
Anatum (2)	3,10	Y	Y	Y	Y	Y	Y	Y	ND
Brandenburg (3)	1,4,12	Y	Y	Y	Y	Y	Y	Y	Y
Decatur (8)	6,7	Y	Y	Y	Y	Y	Y	Y	ND
Emek (20)	8,20	Y	Y	Y	Y	Y	Y	Y	Y
Montevideo (30)	6,7	Y	Y	Y	Y	Y	Y	Y	Y
Muenchen (34)	6,8	NA	ND	Y	Y	ND	ND	ND	ND
Newport (36)	6,8	Y	Y	Y	Y	Y	Y	Y	Y
Pullorum (52)	1,9,12	Y	Y	Y	Y	Y	Y	Y	ND
Rubislaw (54)	11	Y	Y	Y	Y	Y	Y	Y	Y
Saintpaul (55)	1,4,[5],12	Y	Y	Y	Y	Y	Y	Y	ND
Senftenberg (59)	1,3,19	Y	Y	Y	Y	Y	Y	Y	Y
Stanley (60)	1,4,[5],12,27	NA	ND	ND	ND	Y	Y	Y	ND

^aNumber in parentheses indicates SARB strain number

^bY: attempted transduction; successful. ND: transduction not attempted. NA: not attempted due to natural antibiotic resistance. Data represent at least three replicates.

Table 4

The *rfb* operon was transduced among Typhimurium and non-Typhimurium *Salmonella* via bacteriophage P1.

Donor Serovar ^a	Recipient Serovar ^b	O-serotype conversion	Linkage ^c
Agona	Typhimurium	(<u>1</u> ,4,[5],12) to (<u>1</u> ,4,12)	97%
Anatum	Typhimurium	(1,4,[5],12) to (3,10)	69%
Brandenburg	Typhimurium	(1,4,[5],12) to (<u>1</u> ,4,12)	73%
Decatur	Typhimurium	(1,4,[5],12) to (6,7)	ND
Emek	Typhimurium	(1,4,[5],12) to (8, <u>20</u>)	64%
Montevideo	Typhimurium	(1,4,[5],12) to (6,7)	55%
Muenchen	Typhimurium	(1,4,[5],12) to (6,8)	93%
Newport	Typhimurium	(1,4,[5],12) to (6,8)	54%
Pullorum	Typhimurium	(1,4,[5],12) to (<u>1</u> ,9,12)	ND
Rubislaw	Typhimurium	(1,4,[5],12) to (11)	87%
Saintpaul	Typhimurium	(1,4,[5],12) to (<u>1</u> ,4,[5],12)	100%
Senftenberg	Typhimurium	(1,4,[5],12) to (<u>1</u> ,3,19)	ND
Stanley	Typhimurium	Failed	
Senftenberg	Emek	(8, <u>20</u>) to (<u>1</u> ,3,19)	ND
Brandenburg	Emek	(8, <u>20</u>) to (<u>1</u> ,4,12)	ND
Anatum	Montevideo	(6,7) to (3,10)	ND
Decatur	Senftenberg	(<u>1</u> ,3,19) to (6,7)	ND

^a All strains are *galE*-2841::*aph* derivatives

^b All strains are *hisD*9953::MudJ Δ (*rfbP-rfbB*)-3301::*cat* derivatives

^c Linkage between *hisD* and the *rfb* operon. ND: transduction not attempted.