Bacteriophages (phages) are ubiquitous in nature. These viruses play a number of central roles in microbial ecology and evolution by, for instance, promoting horizontal gene transfer (HGT) among bacterial species. The ability of phages to mediate HGT through transduction has been widely exploited as an experimental tool for the genetic study of bacteria. As such, bacteriophage P1 represents a prototypical generalized transducing phage with a broad host range that has been extensively employed in the genetic manipulation of *Escherichia coli* and a number of other model bacterial species. Here we demonstrate that P1 is capable of infecting, lysogenizing, and promoting transduction in members of the bacterial genus *Sodalis*, including the maternally inherited insect endosymbiont *Sodalis glossinidius*. While establishing new tools for the genetic study of these bacterial species, our results suggest that P1 may be used to deliver DNA to many Gram-negative endosymbionts in their insect host, thereby circumventing a culturing requirement to genetically manipulate these organisms.

**ABSTRACT**  Bacteriophages (phages) are ubiquitous in nature. These viruses play a number of central roles in microbial ecology and evolution by, for instance, promoting horizontal gene transfer (HGT) among bacterial species. The ability of phages to mediate HGT through transduction has been widely exploited as an experimental tool for the genetic study of bacteria. As such, bacteriophage P1 represents a prototypical generalized transducing phage with a broad host range that has been extensively employed in the genetic manipulation of *Escherichia coli* and a number of other model bacterial species. Here we demonstrate that P1 is capable of infecting, lysogenizing, and promoting transduction in members of the bacterial genus *Sodalis*, including the maternally inherited insect endosymbiont *Sodalis glossinidius*. While establishing new tools for the genetic study of these bacterial species, our results suggest that P1 may be used to deliver DNA to many Gram-negative endosymbionts in their insect host, thereby circumventing a culturing requirement to genetically manipulate these organisms.

**IMPACT** A large number of economically important insects maintain intimate associations with maternally inherited endosymbiotic bacteria. Due to the inherent nature of these associations, insect endosymbionts cannot be usually isolated in pure culture or genetically manipulated. Here we use a broad-host-range bacteriophage to deliver exogenous DNA to an insect endosymbiont and a closely related free-living species. Our results suggest that broad-host-range bacteriophages can be used to genetically alter insect endosymbionts in their insect host and, as a result, bypass a culturing requirement to genetically alter these bacteria.

**KEYWORDS** *Sodalis praecaptivus*, *Sodalis glossinidius*, insect endosymbiont, symbiont, transformation, transduction, genetic modification, plasmid transfer, transposition, bacteriophage P1, gene disruption, mutation, paratransgenesis, bacteriophage transduction, symbiosis

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**Using phage to genetically modify a bacterial insect endosymbiont:** @PontesLab @ChrisKendra5 @RobertoEBruna1

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that has fostered its adoption as an important experimental tool for the genetic analysis and manipulation of *E. coli* (5, 8). Notably, in addition to its habitual *E. coli* host, P1 can also infect a large number of Gram-negative bacterial species (8–12). This broad host range, along with its well-characterized molecular biology and established experimental procedures, has prompted the use of this phage as an experimental tool for the delivery of DNA to a large number of bacterial species (13–18). Here we establish that P1 is capable of infecting two members of the bacterial genus *Sodalis*, including *Sodalis glossinidius* (19, 20).

*Sodalis glossinidius* is a maternally inherited, Gram-negative bacterial endosymbiont of tsetse flies (*Glossina* spp.; Diptera: *Glossinidae*). Similar to other insect endosymbionts, *S. glossinidius* exists in a stable, chronic association with its insect host and undergoes a predominantly maternal mode of transmission (21–23). Notably, like other insect endosymbionts, this bacterium has undergone an extensive process of genome degeneration as a result of a recent ecological transition from free-living existence to permanent host association (24, 25). Because this process is accompanied by the loss of metabolic capability and stress response pathways (24–29), *S. glossinidius* has proven refractory to harsh artificial DNA transformation procedures that are commonly employed in model organisms such as *Escherichia coli* (29). Consequently, this bacterium has remained genetically intractable (30).

In this study, we demonstrate that the bacteriophage P1 is capable of infecting, lysogenizing, and promoting transduction in *Sodalis glossinidius*, and its free-living close relative, the plant-associated and opportunistic pathogen *Sodalis praecaptivus* (19, 20). We demonstrate that P1 can be used to mediate generalized transduction of chromosomal and extrachromosomal DNA in *S. praecaptivus*. We use P1 to transduce autonomous replicating phagemids containing an array of reporter genes and Tn7 transposition systems harboring fluorescent proteins for chromosomal tagging. Finally, we developed a suicide phagemid containing a mariner transposase for random mutagenesis of bacterial strains susceptible to P1 infection. This study establishes a new efficient method for genetic manipulation of *Sodalis* species (Fig. 1) that can be readily adapted to other Gram-negative bacteria. Furthermore, these results provide a potential means for the genetic modification of bacterial endosymbionts, in their insect host, through the use of P1 as a DNA delivery system.

**RESULTS**

**Bacteriophage P1 infects, lysogenizes, and forms phage particles in *Sodalis glossinidius* and *Sodalis praecaptivus*.** P1CMclr-100(ts) is a thermo-inducible P1 variant harboring a chloramphenicol-resistant marker. P1CMclr-100(ts) forms chloramphenicol-resistant lysogens at low temperatures (≤30°C) but produces phage particles at higher temperatures (≥37°C) (31). Consequently, infection of *E. coli* by P1CMclr-100(ts) yields chloramphenicol-resistant lysogens at 30°C. We took advantage of these P1CMclr-100(ts) properties to test whether *S. glossinidius* and *S. praecaptivus* were susceptible to P1 infection. We exposed cultures of these bacteria to increasing concentrations of P1CMclr-100(ts) phage particles and subsequently plated dilutions on solid medium containing chloramphenicol. We established that, similar to the *E. coli* control (Fig. 2A), exposure to increasing concentrations of P1CMclr-100(ts) particles yielded increasing numbers of chloramphenicol-resistant colonies in both *S. glossinidius* (Fig. 2B) and *S. praecaptivus* (Fig. 2C). Importantly, no chloramphenicol-resistant colonies were observed in cultures that were not exposed to P1CMclr-100(ts) particles (Fig. 2B and C).

That these colonies were P1 lysogens, as opposed to recombinants harboring only the P1-derived chloramphenicol-resistant marker, was supported by several lines of evidence. First, the presence of a P1 DNA fragment was detected by polymerase chain reaction (PCR) in both chloramphenicol-resistant *S. glossinidius* (Fig. 3A and B) and *S. praecaptivus* clones (Fig. 3C and D), but not in the wild-type strains (Fig. 3A to D, left side). This indicated that chloramphenicol-resistant cells harbor at least part of the P1CMclr-100(ts) genome. Second, lysates prepared from *S. glossinidius* and *S.
praecaptivus} chloramphenicol-resistant clones, but not their wild-type counterparts, formed plaques in soft agar cultures of \textit{E. coli} grown at 37°C, a temperature that induces P1CM\textsuperscript{clr}-100(ts) lytic replication (31) (Fig. 3E and F). This indicated that chloramphenicol-resistant \textit{S. glossinidius} and \textit{S. praecaptivus} clones can produce phage particles that are lytic to \textit{E. coli} grown at 37°C. Third, the lytic activity of lysates derived from chloramphenicol-resistant \textit{S. praecaptivus} cultures propagated at 37°C was 10,000 times higher than those maintained at 30°C (Fig. 3G). This established that higher titers of phage particles were being produced in \textit{S. praecaptivus} chloramphenicol-resistant clones at a temperature where P1CM\textsuperscript{clr}-100(ts) becomes lytic. Finally, lysates derived from \textit{S. glossinidius} and \textit{S. praecaptivus} chloramphenicol-resistant clones, but not their

**FIG 1** Cartoon representation depicting a workflow of the transduction procedure developed for introduction of phagemids in \textit{Sodalis} species. Following the direction of the arrows, an \textit{E. coli} P1 lysogen host is transformed with a P1 phagemid. The phagemid is packaged following induction of the P1 prophage, and lysates derived from culture supernatant are used to infect a \textit{Sodalis} recipient strain. Cells receiving the phagemid are subsequently isolated on plates containing a selective agent.

**FIG 2** Infection of bacterial strains by phage P1. Lysates derived from an \textit{E. coli} P1CM\textsuperscript{clr}-100(ts) lysogen (K0463) were used to infect \textit{E. coli} MG1655 (A), \textit{Sodalis glossinidius} (B), and \textit{Sodalis praecaptivus} (C). Plates depict the formation of chloramphenicol-resistant colonies as functions of the concentration of bacteria (vertical axis) and the concentration of P1CM\textsuperscript{clr}-100(ts) lysates (horizontal axis). Note that P1 infection conditions for the strains are different (see Materials and Methods), and images do not reflect efficiency of P1 infection. Images show representative plates of at least three routine experiments.
The wild-type isogenic counterparts, promoted the formation of chloramphenicol-resistant E. coli cells at 30°C (Fig. 3H). This indicated that the chloramphenicol-resistant marker can be transduced from S. glossinidius and S. praecaptivus back to E. coli.

In S. glossinidius, the frequency of chloramphenicol-resistant colonies arising following P1CMclr-100(ts) exposure was similar to those observed for the E. coli control cells, indicating that P1 infection occurs efficiently in this bacterium. In contrast, chloramphenicol-resistant S. praecaptivus colonies emerged at a lower frequency, and higher concentrations of bacterial cells were typically used in P1 infection experiments (see Materials and Methods). Notably, in P1-resistant Salmonella enterica, the efficiency of P1 infection can be drastically increased by mutations in either galU or galE (10).

Because these mutations remove the O antigen by truncating the core region of the lipopolysaccharide (LPS) (32, 33), they presumably facilitate access of P1 to its host receptor—conserved structural motifs within the LPS core (8, 10). In particular, while the LPS of S. praecaptivus contains structural components attached to its core region, S. glossinidius is devoid of such structures (see Fig. S1A in the supplemental material).

Nonetheless, the lower infectivity of P1 does not appear to be related to the physical occlusion of the P1 receptor by components present in the outer portion of the S. praecaptivus LPS. This is because a mutation in galU results in a truncated LPS in S. praecaptivus but does not affect P1 infectivity (Fig. S1). Hence, unlike S. enterica, this phenotype is not due to the presence of a P1-antagonizing structure(s) in the outer portion.
of S. praecaptivus LPS. Taken together, these results indicate that phage P1 is capable of infecting and lysogenize in S. glossinidius and S. praecaptivus.

**P1 generalized transduction in S. praecaptivus.** During the formation of P1 virions, approximately 0.05 to 0.5% of infective phage particles package random DNA fragments derived from the bacterial host (34). These particles can mediate the transfer of bacterial DNA across P1-susceptible strains through generalized transduction. In the laboratory, generalized transduction of DNA can be identified by virtue of genetic markers that are packaged in these phage particles and transferred between bacterial strains. Accordingly, we sought to determine whether P1 could mediate generalized transduction in S. praecaptivus. First, we exposed wild-type S. praecaptivus to phage lysates derived from an S. praecaptivus P1CMclr-100(ts) lysogen harboring Amp<sup>r</sup> plasmid pSIM6 (35). Following lysate exposure, we were able to retrieve Amp<sup>r</sup> S. praecaptivus transductants. Importantly, Amp<sup>r</sup> cells were absent from both phage lysates alone and cultures of wild-type S. praecaptivus that were not exposed to phage (data not shown). In agreement with the notion that these Amp<sup>r</sup> clones were P1 transductants, diagnostic PCR revealed the presence of a pSIM6 fragment in these cells (Fig. 4A).

Next, we attempted to transduce a chromosomal chloramphenicol-resistant marker (rpoS-HA::Cm) using the P1 lytic strain P1<sup>vir</sup>. (This P1 strain is widely used as a transducing agent in E. coli due to its inability to lysogenize cells upon infection and the ease with which transducing lysates can be generated [5, 34].) We infected wild-type S. praecaptivus cells with P1<sup>vir</sup> lysates grown in an S. praecaptivus P1CMclr-100(ts) lysogen harboring the ampicillin-resistant (Amp<sup>r</sup>) plasmid pSIM6 (35). Following lysate exposure, we were able to retrieve Amp<sup>r</sup> S. praecaptivus transductants. Importantly, Amp<sup>r</sup> cells were absent from both phage lysates alone and cultures of wild-type S. praecaptivus that were not exposed to phage (data not shown). In agreement with the notion that these Amp<sup>r</sup> clones were P1 transductants, diagnostic PCR revealed the presence of a pSIM6 fragment in these cells (Fig. 4A).

![Image of Figure 4](image-url)

**FIG 4** Bacteriophage P1-mediated generalized transduction in S. praecaptivus. (A) Detection of a pSIM6 DNA region by PCR and agarose gel electrophoresis in ampicillin-resistant (Amp<sup>r</sup>) S. praecaptivus transductants following exposure to lysates derived from S. praecaptivus Cm<sup>r</sup> P1CMclr-100(ts) (MP1703) harboring Amp<sup>r</sup> plasmid pSIM6. (B) Detection of the rpoS-HA::Cm chromosomal insertion by PCR and agarose gel electrophoresis in S. praecaptivus transductants following exposure to lysates of P1<sup>vir</sup> grown in S. praecaptivus rpoS-HA::Cm pSIM6 (MP1522) strain.

Taken together, these results indicate that P1<sup>vir</sup> can be used to mediate generalized transduction in S. praecaptivus.
Introduction of exogenous DNA in *S. glossinidius* and *S. praecaptivus* by P1-mediated guided transduction. Whereas up to 0.5% of P1 particles can contain random fragments of bacterial host DNA (34), the vast majority of virions harbor P1 DNA. This is because the packaging of P1 genome into phage particles is guided by elements encoded within its DNA sequence (36, 37). Particularly, this packaging element can be cloned into plasmids (to produce phagemids) or incorporated into the bacterial chromosome to increase the frequency of P1-mediated transduction of adjacent DNA (17, 38, 39). Indeed, the P1 packaging element can increase the transduction of linked DNA by 1,600-fold above the levels obtained in generalized transduction (38). Hence, this DNA element can be used to increase the number of transducing particles and, consequently, the efficiency of DNA transfer among bacterial strains that are susceptible to P1 infection.

The lack of genetic tools available for the manipulation of *Sodalis* species, specifically *S. glossinidius*, prompted us to explore P1 as a plasmid DNA delivery tool for these bacteria. As a proof of principle, we used the aforementioned general technique to transfer a number of P1 phagemids (38) (Fig. 5A) into *S. glossinidius*. We were able to recover transductants expressing an array of phenotypic markers encoded in the phagemids. These traits included light production (*luxCDABE* genes), violacein pigment synthesis (*vioABCE*), β-galactosidase activity (*lacZ*), or green fluorescence (*gfp*) (Fig. 5B and C). To expand the tool set available for the modification of *Sodalis* species, we constructed two phagemids for tagging bacterial chromosomes with fluorescent genes at the Tn7 attachment site (40) and a suicide phagemid encoding a Himar1 transposition system for random mutagenesis (41) (Fig. S2). Following packaging into P1 virions in an *E. coli* P1CMclr-100(ts) lysogen, these phagemids were efficiently delivered to *S. glossinidius* and *S. praecaptivus* (Fig. 5D and E). Together, these results establish that bacteriophage P1 can be used to efficiently deliver replication-competent and suicide vectors into *S. glossinidius* and *S. praecaptivus* through a “guided transduction” strategy.
DISCUSSION

In the current study, we demonstrate that bacteriophage P1 can infect, lysogenize, and promote transduction in two species of the genus Sodalis. We show that P1 can mediate generalized transduction in S. praecaptivus (Fig. 4), and we establish that this bacteriophage can be used for the delivery of plasmids and suicide vectors for the genetic manipulation of S. glossinidius and S. praecaptivus (Fig. 5). While these results constitute a significant advance in the development of genetic modification tools to study these bacterial species, they also clear the way for the implementation of P1-based DNA delivery systems to uncultured Sodalis species (42–48) and Gram-negative insect endosymbionts belonging to other genera.

Whereas S. praecaptivus can be genetically engineered with relative ease, the ability of P1 to mediate generalized transduction provides a number of applications for the manipulation of this bacterium. For instance, although S. praecaptivus can be readily modified by recombineering functions of phage λ (λ-Red) (49–51), the use of this technique has two major drawbacks. First, the expression of recombineering functions can be mutagenic (52). This can potentially produce confounding results in subsequent experiments as phenotypes associated with a particular engineered modification may actually result from secondary mutation(s). Second, typical temperature-sensitive plasmids (Rep_{PSI1} ori) harboring recombineering functions cannot be cured from S. praecaptivus by propagating cells at nonpermissive temperatures (\(\geq 37^\circ C\)) in the absence of plasmid selection (our unpublished results). The inability to cure these plasmids can increase the chances of secondary mutations through leaky expression of recombineering functions and hinder the use of plasmids from the same incompatibility group in downstream genetic analyses. Importantly, both of these issues can be overcome by P1-mediated generalized transduction. That is, genomic DNA fragments engineered using λ-Red can be transferred to naive S. praecaptivus cells that lack recombineering plasmids and, therefore, have not been exposed to potential mutagenic events (Fig. 4B).

In contrast, the establishment of P1-mediated transduction provides a considerable advancement in our ability to genetically manipulate S. glossinidius. This is because S. glossinidius is recalcitrant to DNA transformation by standard techniques such as heat shock and electroporation (29, 30, 53). Whereas we have recently developed a method for DNA transfer to S. glossinidius via conjugation (30), P1-mediated transduction provides an alternative, simpler method for the introduction of exogenous DNA into this bacterium. Altered chromosomal fragments, replication-competent plasmids carrying an array of functions, and suicide vectors engineered for allelic replacement or containing transposition systems can be quickly transduced into S. glossinidius in a simple protocol. Given the large DNA packaging capability of P1 (up to 100 kbp) (5), this bacteriophage can be efficiently used for a variety of applications, including the delivery of bacterial artificial chromosomes (bacterial artificial chromosome [BAC] vectors) or large plasmids encoding multiple genome editing CRISPR systems (54) that are not easily transferred by conjugation (30). Additionally, the P1 packing sequence can be incorporated into DNA fragments used in insertional mutagenesis (39), enabling rapid and efficient combination of mutations via P1 “guided transduction.” This approach can greatly facilitate the implementation of several analyses (e.g., complementation and epistasis) to identify and dissect genetic components and pathways governing bacterial behaviors and interactions with eukaryotic hosts.

Beyond the genus Sodalis, the results highlighted in this study have potential broad implications for the genetic modification of uncultured Gram-negative insect endosymbionts. In Gram-negative bacteria, the LPS is the major structural constituent of the outer leaflet of the outer membrane. The LPS is composed of a highly conserved lipid A “anchor,” a conserved core polysaccharide and, sometimes, a hypervariable outer component designated O antigen (see Fig. S1A in the supplemental material) (76). Bacteriophage P1 has a broad host range, in part, because it recognizes, as its host receptor, structural features of the conserved LPS core (8). In addition to E. coli and
several species of the Gammaproteobacteria, P1 has been shown to be capable of infecting various members within the Alpha, Beta-, and Deltaproteobacteria, and even bacterial species residing outside the Proteobacteria phylum such as Flavobacterium sp. strain M64 (8–12).

Notably, a large number of economically important insect species—including several disease vectors of animals and plants—harbor maternally inherited, Gram-negative bacterial endosymbionts (24, 25). However, unlike S. glossinidius and a handful of other species, the vast majority of these endosymbionts have not been isolated in pure culture (14, 19, 55–59). This is because these bacteria undergo a process of genome degeneration and size reduction during the course of long-term evolution and specialization within their eukaryotic insect hosts. This process leads to the loss of many physiological functions that are required for replication outside the host (24, 25, 27, 29). Notably, classical protocols of bacterial genetics require the manipulation of large numbers of cells and the subsequent isolation of rare genetic events as bacterial colonies on selective agar plates. Consequently, the implementation of genetics for the study of insect endosymbionts has remained scarce and limited to species that can grow in axenic culture, form colonies on agar plates, and are receptive to exogenous DNA (29, 30).

The ability of bacteriophage P1 to deliver DNA to a large number of bacterial species suggests a clear method in which the requirement for culturing may be bypassed. That is, similar to viral vectors that are commonly utilized in gene therapy in mammals (60), P1 could be used to deliver DNA to bacterial endosymbionts inside their insect hosts. Specifically, insects could be microinjected (23, 27, 61–63) with P1 virions packaged with recombinant DNA. The establishment of successful P1 infections and subsequent enrichment of transductant endosymbionts to near-homogeneous or clonal populations could be attained by making use of phenotypic markers (Fig. 5) and implementing antibiotic selection regiments in insects (64–69). Whereas this approach would preferentially target recently acquired endosymbionts by virtue of their ability to exist intracellularly and extracellularly in various insect tissues (21–23, 25), ancient obligate intracellular endosymbionts could also be subjected to infection and genetic modification via P1, if they transiently exit host cells (70).

The results presented in this study pave the way for the development of tractable genetic systems for S. glossinidius and, potentially, a myriad of Gram-negative bacterial endosymbionts of insects. While this may empower the use of genetics to study these obscure bacteria, it also has clear translational applications. P1-mediated DNA delivery into insect endosymbionts may allow the engineering of bacterial traits aimed at modifying aspects of insect ecology (29, 30), mitigating their burden on economic activities and human health.

MATERIALS AND METHODS

Microbial strains, phages, plasmids, and growth conditions. Microbial strains, phages, and plasmids used in this study are presented in Table S1 in the supplemental material. Unless indicated, all Escherichia coli strains were propagated at 30, 37, or 42°C in Luria-Bertani (LB) broth or agar (1.5% [wt/vol]). Sodalis glossinidius was grown at 27°C in brain heart infusion broth supplemented with 10 mM MgCl₂ (BHI) or on brain heart infusion agar (1.2% [wt/vol]) supplemented with 10 mM MgCl₂ (BHI agar). S. praecaptivus was also propagated on BHI agar plates supplemented with 10% defibrinated horse blood (BHB). Sodalis praecaptivus was grown at 30, 39, or 42°C in Luria-Bertani broth or agar (1.5% [wt/vol]) lacking sodium chloride. For experiments involving P1 infection or generation of lysates, the growth medium was supplemented with CaCl₂ and MgCl₂ to a final concentration of 10 mM, respectively. Growth of S. glossinidius on BHB agar plates was carried out under microaerophilic conditions, which was achieved either using BD GasPak EZ Campy Gas Generating sachets or a gas mixture (5% oxygen and 95% CO₂). For all strains, growth in liquid medium was carried out in shaking water incubators with aeration (250 rpm). When required, medium was supplemented with ampicillin (100 µg/ml), chloramphenicol (30 µg/ml) for E. col i or S. praecaptiv us and 10 µg/ml for S. glossinidius), kanamycin (50 µg/ml) for E. coli and 25 µg/ml for S. glossinidius or S. praecaptiv us). Arabinose was used at a concentration of 0.5 or 1% (wt/vol); S-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was used at a concentration of 100 µg/ml.

Lipopolysaccharide extraction and detection. Extraction of lipopolysaccharide (LPS) from S. glossinidius and S. praecaptivus cultures was carried out as described previously (71). Extracted samples were
infecting E. coli presented in Table S2. An were collected by centrifugation and concentrated to an OD600 of 1. One milliliter of concentrated cul-
al material). Because episomes harboring repSC101 was initially selected on plates containing ampicillin (Fig. 5D; see also Fig. S2 in the supplemen-
gens were grown overnight at 30°C. Cultures were diluted (1:100 [vol/vol]) into fresh medium and grown
Cm harboring selected phagemids were prepared following arabinose induction as described previously (38).

Preparation of lysates. Partially lysed cells were disrupted by vortexing the cultures following the addition of chloro-
and grown to an optical density at 600 nm (OD600) between 0.45 and 0.5. The
sulfone membrane (Sigma-Aldrich). LPS in
the presence of various concentrations of P1 lysate. Cells were subsequently collected by centrifugation (1 min, 13,000 rpm, room temperature), and the supernatants were replaced by 1 ml of LB containing 5 mM so-

Preparation of stocks of P1vir phage lysates. Stocks of P1vir phage lysates were prepared by infecting E. coli MG1655 (75), as described previously (5).

Preparation of phage lysates derived from P1CMcr-100(ts) lysogens. E. coli P1CMcr-100(ts) lyso-
gens were grown overnight at 30°C. Cultures were diluted (1:100 [vol/vol]) into fresh medium and grown to an OD600 value of 0.3 to 0.4. Subsequently, cultures were shifted to 42°C and propagated until exten-
sive cell lysis (3 to 4 h). At times, cultures were allowed to grow at 42°C for 16 h prior to the preparation of lysates. Partially lysed cells were disrupted by vortexing the cultures following the addition of chloro-
form (1 volume of chloroform per 100 volumes of culture). Cell debris was removed by centrifugation
(12 min, 4,000 rpm, room temperature), and the supernatant was passed through a 0.45-μm polyethy-
sulfone membrane filter. S. praecaptivus P1CMcr-100(ts) lysogens were prepared as described above, except that cells were grown for 2 h at 42°C and 16 h at 37°C prior to lysis preparation. S. glossinidius P1CMcr-100(ts) lysogens were grown to an OD600 of 0.4. Cultures were heat shocked at 37°C for 2 h. The cultures were treated with chloroform (1 volume of chloroform per 100 volumes of culture) and processed as described for E. coli and S. praecaptivus.

Infection by P1vir, P1CMcr-100(ts), and P1 transducing particles. Following overnight growth in LB, E. coli cultures were diluted in fresh LB supplemented with 10 mM CaCl2 and MgCl2 to an OD600 of 1. One-milliliter aliquots of these cell solutions were incubated for 30 min at 30°C in the presence or absence of various concentrations of P1 lysi. Cells were subsequently collected by centrifugation (1 min, 13,000 rpm, room temperature), and the supernatants were replaced by 1 ml of LB containing 5 mM so-
dium citrate. Cells were grown for 1 h at 30°C and 250 rpm prior to plating. S. praecaptivus cells grown
in fresh LB supplemented with 10 mM CaCl2 and MgCl2. One milliliter of these resuspended solu-
tions was incubated for 30 min at 30°C in the presence or absence of various concentrations of P1 lysi. Cells were collected by centrifugation (1 min, 13,000 rpm, room temperature), and the supernatants were replaced by 1 ml of LB containing 5 mM sodium citrate. Cells were plated following 1 h of growth at 30°C and 250 rpm. S. glossinidius cells were grown in BHI for 3 to 5 days to an OD600 of ≈0.5. Cells were collected by centrifugation and concentrated to an OD600 of 1. One milliliter of concentrated cultures
was incubated for 60 min at 30°C in the presence or absence of various concentrations of P1 lysi. Cells were collected by centrifugation (1 min, 13,000 rpm, room temperature), and the supernatants were replaced by 10 ml of BHI. Cultures were incubated overnight at 27°C overnight with shaking prior to plating.

Curing of pP1-Tn7 phagemids. Transduction of pP1-Tn7 phagemids into S. glossinidius and S. praecaptivus was initially selected on plates containing ampicillin (Fig. 5D; see also Fig. S2 in the supplemen-
tal material). Because episomes harboring repSC101 origins of replication are not easily cured from S. praecaptivus (see Discussion), the curing of phagemids was performed only in S. glossinidius. The strategy used to identify S. glossinidius clones lacking phagemids was similar to the one adopted elsewhere (53). Briefly, to identify S. glossinidius clones that contained the chloramphenicol-resistant marker at the
Tn7 attachment site and had lost the ampicillin-resistant plasmids, cultures were propagated in BHI containing chloramphenicol and 1% arabinose. After four passages, cells were diluted and plated. Single colonies were screened for sensitivity to ampicillin. Transposon insertion at the Tn7 attachment site was verified by PCR with primers 1018 and 1019.

**Image acquisition, analysis, and manipulation.** DNA agarose gel electrophoresis and bacterial colonies, with the exception of S. glossinidius macrocolonies, were detected using an Amersham ImageMaster 680 (GE Healthcare). S. glossinidius macrocolonies expressing green fluorescent protein (GFP) were detected using a dark reader (Clare Chemical Research) and documented with an iPhone. When oversaturated, the intensity of signals in images were adjusted across the entire images using Preview (Apple).

**SUPPLEMENTAL MATERIAL**

Supplemental material is available online only.

**FIG S1** TIF file, 2.4 MB.

**FIG S2** TIF file, 2.6 MB.

**TABLE S1** XLSX file, 0.01 MB.

**TABLE S2** XLSX file, 0.01 MB.

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We declare that we have no conflicts of interest.

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