DNA transduction in *Sodalis* species: implications for the genetic modification of uncultured endosymbionts of insects

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Running title: Bacteriophage P1-mediated transduction in *Sodalis*

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.

Summary

A large number of economically important insects maintain intimate associations with maternally inherited endosymbiotic bacteria. Due to the inherit nature of these associations, insect endosymbionts cannot be usually isolated in pure culture nor 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.

49 **Introduction**

50 Bacteriophages (phages) are the most abundant and diverse biological entities on the
51 planet. With an estimated population size greater than $1x10^{31}$ (Hendrix *et al*. 1999),
52 these bacterial viruses play essential ecological and evolutionary functions. Phages
53 control the size of bacterial populations and shape the diversity of microbial
54 communities by modulating the abundance of bacterial lineages and promoting,
55 directly and indirectly, the exchange of genetic information among species (Touchon *et*
56 *al*., 2017; Soucy *et al*. 2015). Historically, phages have played a central role in the
57 development of molecular biology, enabling, for instance, the establishment of DNA as
58 the genetic material of living cells (Hershey and Chase, 1952). Today, phages are
59 widely used as tools in the study of bacteria. For instance, generalized transducing
60 phages such as P1 allow the rapid transfer of DNA among bacterial strains, greatly
61 facilitating genetic dissection of biological processes (Thomason *et al*. 2007).

62 P1 is a temperate bacteriophage capable of alternating between lytic and
63 lysogenic infection. P1 was initially described in studies involving lysogenic strains of
64 *Escherichia coli* (Bertani, 1951). This phage is capable of mediating generalized
65 transduction (Lennox, 1955), a property that has fostered its adoption as an important
66 experimental tool for the genetic analysis and manipulation of *E. coli* (Thomason *et al*.
67 2007, Yarmolinsky and Sternberg, 1988). Notably, in addition to its habitual *E. coli* host,
68 P1 can also infect a large number of Gram negative bacterial species (Yarmolinsky and
69 Sternberg, 1988; Goldberg *et al*., 1974; Ornellas and Stocker, 1974; Kaiser and Dworkin,
70 1975; Murooka and Harada, 1979). This broad host range, along with its well-
71 characterized molecular biology and established experimental procedures, has
72 prompted the use of this phage as an experimental tool for the delivery of DNA to a
73 large number of bacterial species (Streicher *et al*., 1971; O'Connor *et al*., 1983; Downard,

74 1988; Wolf-Watz *et al*., 1985; Westwater *et al*., 2002; Butela and Lawrence, 2012). Here
75 we establish that P1 is capable of infecting two members of the bacterial genus *Sodalis*,
76 including *Sodalis glossinidius* (Dale and Maudlin, 1999; Chari *et al*., 2015).
77 *Sodalis glossinidius* is a maternally inherited, Gram negative bacterial
78 endosymbiont of tsetse flies (*Glossina* spp.; Diptera: *Glossinidae*). Similar to other insect
79 endosymbionts, *S. glossinidius* exists in a stable, chronic association with its insect host,
80 and undergoes a predominantly maternal mode of transmission (Aksoy *et al*., 1997;
81 Cheng and Aksoy, 1999; De Vooght *et al*., 2015). Notably, like other insect
82 endosymbionts, this bacterium has undergone an extensive process of genome
83 degeneration as a result of a recent evolutionary transition from free-living existence to
84 permanent host association (McCutcheon *et al*., 2019; Moran *et al*., 2008). Because this
85 process is accompanied by the loss of metabolic capability and stress response
86 pathways (McCutcheon *et al*., 2019; Moran *et al*., 2008; Toh *et al*., 2006; Pontes *et al*. 2011;
87 Clayton *et al*., 2012; Pontes and Dale, 2006), *S. glossinidius* has proven refractory to harsh
88 artificial DNA transformation procedures that are commonly employed in model
89 organisms such as *Escherichia coli* (Pontes and Dale, 2006). Consequently, this bacterium
90 has remained genetically intractable (Kendra *et al.* 2020).
91 In this study, we demonstrate that the bacteriophage P1 is capable of infecting,
92 lysogenizing and promoting transduction in *Sodalis glossinidius*, and its free-living close
93 relative, the plant-associated and opportunistic pathogen *Sodalis praecaptivus* (Dale and
94 Maudlin, 1999; Chari *et al*., 2015). We demonstrate that P1 can be used to mediate
95 generalized transduction of chromosomal and extrachromosomal DNA in *S.*
96 *praecaptivus*. We use P1 to transduce autonomous replicating phagemids containing an
97 array of reporter genes and Tn*7* transposition systems harboring fluorescent proteins
98 for chromosomal tagging. Finally, we developed a suicide phagemid containing a

99 mariner transposase for random mutagenesis of bacterial strains susceptible to P1
100 infection. This study establishes a new efficient method for genetic manipulation of
101 *Sodalis* species (Fig. 1) that can be readily adapted to other Gram negative bacteria.
102 Furthermore, these results provide a potential means for the genetic modification of
103 bacterial endosymbionts, in their insect host, through the use of P1 as a DNA-delivery
104 system.
105

106 **Results**

107 **Bacteriophage P1 infects, lysogenizes and forms phage particles in *Sodalis***
108 ***glossinidius* and *Sodalis praecaptivus***

109 P1CM*clr*-100(ts) is a thermo-inducible P1 variant harboring a chloramphenicol resistant
110 marker. P1CM*clr*-100(ts) forms chloramphenicol resistant lysogens at low temperatures
111 (≤30°C) but produces phage particles at higher temperatures (≥37°C) (Rosner, 1972).
112 Consequently, infection of *E. coli* by P1CM*clr*-100(ts) yields chloramphenicol resistant
113 lysogens at 30°C. We took advantage of these P1CM*clr*-100(ts) properties to test if *S.*
114 *glossinidius* and *S. praecaptivus* were susceptible to P1 infection. We exposed cultures of
115 these bacteria to increasing concentrations of P1CM*clr*-100(ts) phage particles and
116 subsequently plated dilutions on solid medium containing chloramphenicol. We
117 established that, similar to the *E. coli* control (Fig. 2A), exposure to increasing
118 concentrations of P1CM*clr*-100(ts) particles yielded increasing numbers of
119 chloramphenicol resistant colonies in both *S. glossinidius* (Fig. 2B) and *S. praecaptivus*
120 (Fig. 2C). Importantly, no chloramphenicol resistant colonies were observed in cultures
121 that were not exposed to P1CM*clr*-100(ts) particles (Fig. 2B and C).

122 That these colonies were P1 lysogens, as opposed to recombinants harboring
123 only the P1-derived chloramphenicol resistant marker, was supported by several lines

124 of evidence. First, the presence of a P1 DNA fragment was detected by polymerase
125 chain reaction (PCR) in both chloramphenicol resistant *S. glossinidius* (Fig. 3A and B)
126 and *S. praecaptivus* clones (Fig. 3C and D), but not in the wild-type strains (Fig. 3A-D,
127 left side). This indicated that chloramphenicol resistant cells harbor at least part of the
128 P1CM*clr*-100(ts) genome. Second, lysates prepared from *S. glossinidius* and *S.*
129 *praecaptivus* chloramphenicol resistant clones, but not their wild-type counterparts,
130 formed plaques in soft agar cultures of *E. coli* grown at 37°C, a temperature that induces
131 P1CM*clr*-100(ts) lytic replication (Rosner, 1972) (Fig. 3E and F). This indicated that
132 chloramphenicol resistant *S. glossinidius* and *S. praecaptivus* clones can produce phage
133 particles that are lytic to *E. coli* grown at 37°C. Third, the lytic activity of lysates derived
134 from chloramphenicol resistant *S. praecaptivus* cultures propagated at 37°C was 10,000
135 times higher than those maintained at 30°C (Fig. 3G). This established that higher titers
136 of phage particles were being produced in *S. praecaptivus* chloramphenicol resistant
137 clones at a temperature where P1CM*clr*-100(ts) becomes lytic. Finally, lysates derived
138 from *S. glossinidius* and *S. praecaptivus* chloramphenicol resistant clones, but not their
139 wild-type isogenic counterparts, promoted the formation of chloramphenicol resistant
140 *E. coli* cells at 30°C (Fig. 3H). This indicated that the chloramphenicol resistant marker
141 can be transduced from *S. glossinidius* and *S. praecaptivus* back to *E. coli*.
142 In *S. glossinidius*, the frequency of chloramphenicol resistant colonies arising
143 following P1CM*clr*-100(ts) exposure was similar to those observed for the *E. coli* control
144 cells, indicating that P1 infection occurs efficiently in this bacterium. By contrast,
145 chloramphenicol resistant *S. praecaptivus* colonies emerged at a lower frequency, and
146 higher concentrations of bacterial cells were typically used in P1 infection experiments
147 (see Materials and Methods). Notably, in P1-resistant *Salmonella enterica*, the efficiency
148 of P1 infection can be drastically increased by mutations in either *galU* or *galE* (Ornellas

149 and Stocker, 1974). Because these mutations remove the O-antigen by truncating the
150 core region of the lipopolysaccharide (LPS) (Wilkinson and Stocker, 1968; Osborn,
151 1968), they presumably facilitate access of P1 to its host receptor—conserved structural
152 motifs within the LPS core (Yarmolinsky and Sternberg, 1988; Ornellas and Stocker,
153 1974). In particular, while the LPS of *S. praecaptivus* contains structural components
154 attached to its core region, *S. glossinidius* is devoid of such structures (Fig. S1A).
155 Nonetheless, the lower infectivity of P1 does not appear to be related to the physical
156 occlusion of the P1 receptor by components present in the outer portion of the *S.*
157 *praecaptivus* LPS. This is because a mutation in *galU* results in a truncated LPS in *S.*
158 *praecaptivus*, but does not affect P1 infectivity (Fig. S1). Hence, unlike *S. enterica*, this
159 phenotype is not due to the presence of P1-antagonizing structure(s) in the outer
160 portion of *S. praecaptivus* LPS. Taken together, these results indicate that phage P1 is
161 capable of infecting and lysogenize in *S. glossinidius* and *S. praecaptivus*.
162
163 **P1 generalized transduction in *Sodalis praecaptivus***
164 During the formation of P1 virions, approximately 0.05-0.5% of infective phage particles
165 package random DNA fragments derived from the bacterial host (Ikeda and Tomizawa,
166 1965). These particles can mediate the transfer of bacterial DNA across P1 susceptible
167 strains through generalized transduction. In the laboratory, generalized transduction of
168 DNA can be identified by virtue of genetic markers that are packaged in these phage
169 particles and transferred between bacterial strains. Accordingly, we sought to
170 determine if P1 could mediate generalized transduction in *S. praecaptivus*. First, we
171 exposed wild-type *S. praecaptivus* to phage lysates derived from a *S. praecaptivus*
172 P1CM*clr*-100(ts) lysogen harboring the ampicillin resistant (Amp^R) plasmid pSIM6
173 (Datta *et al*., 2006). Following lysate exposure, we were able to retrieve Amp^R *S.*

174 *praecaptivus* transductants. Importantly, Amp^R cells were absent from both phage
175 lysates alone and cultures of wild-type *S. praecaptivus* that were not exposed to phage
176 (data not shown). In agreement with the notion that these Amp^R clones were P1
177 transductants, diagnostic PCR revealed the presence of a pSIM6 fragment in these cells
178 (Fig. 4A).
179 Next, we attempted to transduce a chromosomal chloramphenicol resistant
180 marker (*rpoS-HA::Cm*) using the P1 lytic strain P1*vir*. [This P1 strain is widely used as a
181 transducing agent in *E. coli* due to its inability to lysogenize cells upon infection and the
182 ease with which transducing lysates can be generated (Thomason *et al*. 2007; Ikeda and
183 Tomizawa, 1965)]. We infected wild-type *S. praecaptivus* cells with P1*vir* lysates grown
184 in a *S. praecaptivus rpoS-HA::Cm* pSIM6 strain. Whereas chloramphenicol resistant
185 (Cm^R) cells emerged from wild-type *S. praecaptivus* exposed to phage, no Cm^R cells were
186 obtained from phage lysates or cultures of naïve wild-type *S. praecaptivus* alone (data
187 not shown). Notably, diagnostic PCR indicated that chloramphenicol resistant clones
188 were transductants harboring the *rpoS-HA::Cm* genetic modification (Fig. 4B).
189 Importantly, all of these *rpoS-HA::Cm* transductants were sensitive to ampicillin,
190 indicating that P1*vir* mediated the transduction of a discrete portion of the *S.*
191 *praecaptivus* genome. Taken together, these results indicate that bacteriophage P1 can be
192 used to mediate generalized transduction in *S. praecaptivus*.
193
194 **Introduction of exogenous DNA in *Sodalis glossinidius* and *Sodalis praecaptivus* by**
195 **P1-mediated guided transduction**
196 Whereas up to 0.5% of P1 particles can contain random fragments of bacterial host DNA
197 (Ikeda and Tomizawa, 1965), the vast majority of virions harbor P1 DNA. This is
198 because the packaging of P1 genome into phage particles is guided by elements

199 encoded within its DNA sequence (Sternberg and Coulby, 1987a and 1987b).
200 Particularly, this packaging element can be cloned into plasmids (to produce
201 phagemids) or incorporated into the bacterial chromosome to increase the frequency of
202 P1-mediated transduction of adjacent DNA (Westwater *et al*., 2002; Kittleson *et al*., 2012;
203 Huang and Masters, 2014). Indeed, the P1 packaging element can increase the
204 transduction of linked DNA by 1,600-fold above the levels obtained in generalized
205 transduction (Kittleson *et al*., 2012). Hence, this DNA element can be used to increase
206 the number of transducing particles and, consequently, the efficiency of DNA transfer
207 among bacterial strains that are susceptible to P1 infection.
208 The lack of genetic tools available for the manipulation of *Sodalis* species,
209 specifically *S. glossinidius*, prompted us to explore P1 as a plasmid DNA-delivery tool
210 for these bacteria. As a proof of principle, we used the aforementioned general
211 technique to transfer a number of P1 phagemids (Kittleson *et al*., 2012) (Fig. 5A) into *S.*
212 *glossinidius*. We were able to recover transductants expressing an array of phenotypic
213 traits encoded in the phagemids. These included light production (*luxCDABE* genes),
214 violacein pigment synthesis (*vioABCE*), β-galactosidase activity (*lacZ*) or green
215 fluorescence (*gfp*) (Fig. 5B and 5C). To expand the tool set available for the modification
216 of *Sodalis* species, we constructed (1) two phagemids for tagging bacterial chromosomes
217 with fluorescent genes at the Tn7 attachment site (Peters and Craig, 2011), and (2) a
218 suicide phagemid encoding a Himar1 transposition system for random mutagenesis
219 (Rubin *et al*., 1999) (Fig. S2). Following packaging into P1 virions in an *E. coli* P1CM*clr*-
220 100(ts) lysogen, these phagemids were efficiently delivered to *S. glossinidius* and *S.*
221 *praecaptivus* (Fig. 5D and 5E). Together, these results establish that bacteriophage P1 can
222 be used to efficiently deliver replication-competent and suicide vectors into *S.*
223 *glossinidius* and *S. praecaptivus* through a "guided transduction" strategy.

224

225 **Discussion**

226 In the current study, we demonstrate that bacteriophage P1 can infect, lysogenize and
227 promote transduction in two species of the genus *Sodalis*. We show that P1 can mediate
228 generalized transduction in *S. praecaptivus* (Fig. 4), and we establish that this
229 bacteriophage can be used for the delivery of plasmids and suicide vectors for the
230 genetic manipulation of *S. glossinidius* and *S. praecaptivus* (Fig. 5). While these results
231 constitute a significant advance in the development of genetic modification tools to
232 study these bacterial species, they also clear the way for the implementation of P1-based
233 DNA-delivery systems to uncultured *Sodalis* species (Heddi *et al*., 1998; Fukatsu *et al*.,
234 2007; Nováková and Hypsa, 2007; Chrudimský *et al*., 2012; Smith *et al*., 2013; Santos-
235 Garcia *et a*l., 2017; Šochová *et al*., 2017) and Gram negative insect endosymbionts
236 belonging to other genera.

237 Whereas *S. praecaptivus* can be genetically engineered with relative ease, the
238 ability of P1 to mediate generalized transduction provides a number of applications for
239 the manipulation of this bacterium. For instance, although *S. praecaptivus* can be readily
240 modified by recombineering functions of phage λ (λ-Red; Clayton *et al.* 2017; Enomoto
241 *et al*., 2017; Thomason *et al*., 2014), the use of this technique has two major drawbacks.
242 First, the expression of recombineering functions can be mutagenic (Murphy and
243 Campellone, 2003). This can potentially produce confounding results in subsequent
244 experiments as phenotypes associated with a particular engineered modification may
245 actually results from secondary mutation(s). Second, typical temperature-sensitive
246 plasmids (rep_{pSC101}^{ts} *ori*) harboring recombineering functions cannot be cured from *S.*
247 *praecaptivus* by propagating cells at non-permissive temperatures (≥37°C) in the absence
248 of plasmid selection (our unpublished results). The inability to cure these plasmids can

249 increase the chances of secondary mutations through leaky expression of
250 recombineering functions, and hinder the use of plasmids from the same
251 incompatibility group in downstream genetic analyses. Importantly, both of these
252 issues can be overcome by P1-mediated generalized transduction. That is, genomic
253 DNA fragments engineered using λ-Red can be transferred to naïve *S. praecaptivus* cells
254 that lack recombineering plasmids and, therefore, have not been exposed to potential
255 mutagenic events (Fig. 4B).
256 By contrast, the establishment of P1 mediated transduction provides a
257 considerable advancement in our ability to genetically manipulate *S. glossinidius*. This
258 is because *S. glossinidius* is recalcitrant to DNA transformation by standard techniques
259 such as heat-shock and electroporation (Pontes and Dale, 2006 and 2011; Kendra *et al*.,
260 2020). Whereas we have recently developed a method for DNA transfer to *S.*
261 *glossinidius* via conjugation (Kendra *et al*., 2020), P1-mediated transduction provides an
262 alternative, simpler method for the introduction of exogenous DNA into this bacterium.
263 Altered chromosomal fragments, replication competent plasmids carrying an array of
264 functions, and suicide vectors engineered for allelic replacement or containing
265 transposition systems can be quickly transduced into *S. glossinidius* in a simple protocol.
266 Given the large DNA packaging capability of P1 (up to 100 Kbp) (Thomason *et al*. 2007),
267 this bacteriophage can be efficiently used for a variety of applications, including the
268 delivery of bacterial artificial chromosomes (BAC vectors) or large plasmids encoding
269 multiple genome editing CRISPR systems (Adiego-Pérez *et al*., 2019) that are not easily
270 transferred by conjugation (Kendra *et al*., 2020). Additionally, the P1 packing sequence
271 can be incorporated into DNA fragments used in insertional mutagenesis (Huang and
272 Masters, 2014), enabling rapid and efficient combination of mutations via P1 "guided
273 transduction." This approach can greatly facilitate the implementation of several

274 analyses (e.g. complementation and epistasis) to identify and dissect genetic
275 components and pathways governing bacterial behaviors and interactions with
276 eukaryotic hosts.

277 Beyond the genus *Sodalis*, the results highlighted in this study have potential
278 broad implications for the genetic modification of uncultured Gram negative insect
279 endosymbionts. In Gram negative bacteria, the LPS is the major structural constituent
280 of the outer leaflet of the outer membrane. The LPS is composed of a highly conserved
281 lipid A "anchor," a conserved core polysaccharide and, sometimes, a hypervariable
282 outer component designated O-antigen (Fig. S1A; Silipo and Molinaro, 2010).
283 Bacteriophage P1 has a broad host range, in part, because it recognizes, as its host
284 receptor, structural features of the conserved LPS core (Yarmolinsky and Sternberg,
285 1988). In addition to *E. coli* and several species of the Gammaproteobacteria, P1 has
286 been shown to be capable of infecting various members within the Alpha, Beta- and
287 Deltaproteobacteria, and even bacterial species residing outside the proteobacteria
288 Phylum such as *Flavobacterium* sp. M64 (Yarmolinsky and Sternberg, 1988; Goldberg *et*
289 *al*., 1974; Ornellas and Stocker, 1974; Kaiser and Dworkin, 1975; Murooka and Harada,
290 1979).

291 Notably, a large number of economically important insect species—including
292 several disease vectors of animals and plants—harbor maternally inherited, Gram
293 negative bacterial endosymbionts (McCutcheon *et al*., 2019; Moran *et al*., 2008).
294 However, unlike *S. glossinidius* and a handful of other species, the vast majority of these
295 endosymbionts have not been isolated in pure culture (Dale and Maudlin, 1999;
296 Chrudimský *et al*., 2012; Hypsa and Dale, 1997; Sabri *et al*., 2011; Dale *et al*., 2006; Brandt
297 *et al*., 2017; Masson *et al*., 2018). This is because these bacteria undergo a process of
298 genome degeneration and size reduction during the course of long-term evolution and

299 specialization within their eukaryotic insect hosts. This process leads to the loss of
300 many physiological functions that are required for replication outside the host
301 (McCutcheon *et al*., 2019; Moran *et al*., 2008; Pontes *et al*., 2011; Pontes and Dale, 2006).
302 Notably, classical protocols of bacterial genetics require the manipulation of large
303 numbers of cells and the subsequent isolation of rare genetic events as bacterial colonies
304 on selective agar plates. Consequently, the implementation of genetics for the study of
305 insect endosymbionts has remained scarce and limited to species that can grow in
306 axenic culture, form colonies on agar plates and are receptive to exogenous DNA
307 (Pontes and Dale, 2006; Kendra *et al*., 2020; Masson *et al*., 2018).
308 The ability of bacteriophage P1 to deliver DNA to a large number of bacterial
309 species, suggests a clear method in which the requirement for culturing may be
310 bypassed. That is, similar to viral vectors that are commonly utilized in gene therapy in
311 mammalians (Kay *et al*., 2011), P1 could be used to deliver DNA to bacterial
312 endosymbionts inside their insect hosts. Specifically, insects could be microinjected (De
313 Vooght *et al*., 2015; Pontes *et al*., 2011; Boyle *et al*., 1993; Oliver *et al*., 2003; Doremus *et al*.,
314 2018) with P1 virions packaged with recombinant DNA. The establishment of
315 successful P1 infections and subsequent enrichment of transductant endosymbionts to
316 near-homogeneous or clonal populations could be attained by making use of
317 phenotypic markers (Fig. 5) and implementing antibiotic selection regiments in insects
318 (Wilkinson, 1998; Dale and Welburn, 2001; Dobson and Rattanadechakul, 2001; Koga *et*
319 *al*., 2007; Pais *et al*., 2008; McLean *et al*., 2011). Whereas this approach would
320 preferentially target recently acquired endosymbionts by virtue of their ability to exist
321 intra and extracellularly in various insect tissues (Aksoy *et al*., 1997 ; Cheng and Aksoy,
322 1999; De Vooght *et al*., 2015; Moran *et al*., 2008), ancient obligate intracellular

323 endosymbionts could also be subjected to infection and genetic modification via P1, if
324 they transiently exit host cells (Zaidman-Rémy *et al*., 2018).
325 The results presented in this study pave the way for the development of tractable
326 genetic systems for *S. glossinidius* and, potentially, a myriad of Gram negative bacterial
327 endosymbionts of insects. While this may empower the use of genetics to study these
328 obscure bacteria, it has also clear translational applications. P1-mediated DNA delivery
329 into insect endosymbionts may allow the engineering of bacterial traits aimed at
330 modifying aspects of insect ecology (Pontes and Dale, 2006; Kendra *et al*., 2020),
331 mitigating their burden on economic activities and human health.
332

333 **Material and Methods**

334 **Microbial strains, phages, plasmids and growth conditions**

335 Microbial strains, phages and plasmids used in this study are presented in Table S1.
336 Unless indicated, all *E. coli* strains were propagated at 30, 37 or 42˚C in Luria Bertani
337 (LB) broth or agar (1.5% w/v). *Sodalis glossinidius* was grown at 27˚C in brain-heart
338 infusion broth supplemented with 10 mM $MgCl_2$ (BHI) or on brain-heart infusion agar
339 (1.2% w/v) supplemented with 10 mM $MgCl_2$ (BHI agar). *Sodalis glossinidius* was also
340 propagated on BHI agar plates supplemented with 10% defibrinated horse blood
341 (BHIB). *Sodalis praecaptivus* was grown at 30, 39 or 42˚C in Luria Bertani (LB) broth or
342 agar (1.5% w/v) lacking sodium chloride. For experiments involving P1 infection or
343 generation of lysates, the growth media was supplemented with $CaCl_2$ and $MgCl_2$ to a
344 final concentration of 10 mM respectively. Growth of *Sodalis glossinidius* on BHIB agar
345 plates was carried out under microaerophilic conditions, which was achieved either
346 using BD GasPak EZ Campy Gas Generating sachets or a gas mixture (5% oxygen 95%
347 CO_2). For all strains, growth in liquid medium was carried out in shaking water bath

348 incubators with aeration (250 rpm). When required, medium was supplemented with
349 ampicillin (100 μg/mL), chloramphenicol (20 μg/mL for *E. coli* or *S. praecaptivus*, and 10
350 μg/mL for *S. glossinidius*), kanamycin (50 μg/mL for *E. coli* and 25 μg/mL for *S.*
351 *glossinidius* or *S. praecaptivus*). Arabinose was used at a concentration of 0.5 or 1%
352 (w/v); 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) was used at a
353 concentration of 100 μg/mL.
354
355 **Lipopolysaccharide extraction and detection**
356 Extraction of lipopolysaccharide (LPS) from *S. glossinidius* and *S. praecaptivus* cultures
357 were carried out as described (Davis and Goldberg, 2012). Extracted samples were
358 separated in a NuPAGE 10% Bis-Tris gel in NuPAGE MES SDS Running (ThermoFisher
359 Scientific). LPS in gels were stained with ProteoSilver Silver Stain Kit (Sigma Aldrich).
360
361 **Construction of phagemid pP1-Tn7-mCardinal**
362 Oligonucleotide sequences used in this study are presented in Table S2. Phusion®
363 High-Fidelity DNA Polymerase (New England BioLabs) was used in PCR reactions
364 with primers 469 and 470 and plasmid BBa_J72113-BBa_J72152 (Kittleson *et al*., 2012) as
365 template. The PCR product was ligated into pMRE-Tn7-163 (Schlechter *et al*., 2018),
366 previously digested with SbfI, using NEBuilder® HiFi DNA Assembly (New England
367 BioLabs). The integrity of the construct was verified by DNA sequencing and the
368 ability to be efficiently transduced by P1 particles.
369
370 **Construction of phagemid pP1-Tn7-mVenus**
371 Oligonucleotide sequences used in this study are presented in Table S2. Phusion®
372 High-Fidelity DNA Polymerase (New England BioLabs) was used in PCR reactions

373 with primers 469 and 470 and plasmid BBa_J72113-BBa_J72152 (Kittleson *et al*., 2012) as
374 template. The PCR product was ligated into pMRE-Tn7-166 (Schlechter *et al*., 2018),
375 previously digested with SbfI, using NEBuilder® HiFi DNA Assembly (New England
376 BioLabs). The integrity of the construct was verified by DNA sequencing and the
377 ability to be efficiently transduced by P1 particles.
378
379 **Construction of phagemid pP1-Himar**
380 Oligonucleotide sequences used in this study are presented in Table S2. Phusion®
381 High-Fidelity DNA Polymerase (New England BioLabs) was used in PCR reactions
382 with primers 475 and 476 and plasmid BBa_J72113-BBa_J72152 (Kittleson *et al*., 2012) as
383 template. The PCR product was ligated into pMarC9-R6k (Lee *et al*., 2019), previously
384 digested with EcoRI and HinDIII, using NEBuilder® HiFi DNA Assembly (New
385 England BioLabs). The integrity of the construct was verified by DNA sequencing and
386 the ability to be efficiently transduced by P1 particles.
387
388 **Recombineering procedure for *Sodalis praecaptivus***
389 Oligonucleotide sequences used in this study are presented in Table S2. A *S.*
390 *praecaptivus* strain harboring plasmid pSIM6 (Datta *et al*., 2006) was grown overnight in
391 LB broth supplemented with 100 μg/ml of ampicillin at 30˚C and 250 rpm. Cells were
392 diluted (1:100) in 30 ml of the same medium and grown to an OD_{600} between 0.45-0.5.
393 The culture flask was then grown in a water bath at 42˚C and 250 rpm for 25 min. Cells
394 were immediately transferred to a 50 ml conical tube, collected by centrifugation (7,000
395 rpm for 2.5 min at 4˚C), and resuspended in 40 ml of ice-cold dH_2O. Cells were
396 collected again by centrifugation, and this washing procedure was repeated a second
397 time. Finally, cells were resuspended in 150 μl of ice-cold dH_2O. Homologous

398 recombination was obtained by electroporating 70 µl of cell suspension with 10 µl of
399 purified PCR products generated with primers 251 and 252 (*galU::Kn*) or 84 and 85
400 (*rpoS-HA::Cm*) and plasmids pKD4 and pKD3 (Datsenko and Wanner, 2000) as
401 templates, respectively.
402
403 **Preparation of phage lysates derived from EMG16 P1 lysogens**
404 Lysates from *E. coli* EMG16 harboring selected phagemids were prepared following
405 arabinose induction as described (Kittleson *et al*., 2012).
406
407 **Preparation of stocks P1*vir* phage lysates**
408 Stocks of P1*vir* phage lysates were prepared by infecting *E. coli* MG1655 (Blattner *et al*.,
409 1997), as described (Thomason *et al*. 2007).
410
411 **Preparation of phage lysates derived from P1CM*clr*-100(ts) lysogens**
412 *Escherichia coli* P1CM*clr*-100(ts) lysogens were grown overnight at 30˚C. Cultures were
413 diluted (1:100, v/v) into fresh medium and grown to and OD_{600} value of 0.3-0.4.
414 Subsequently, cultures were shifted to 42˚C and propagated until extensive cell lysis (3-
415 4 h). At times, cultures were allowed to grow at 42˚C for 16 h prior to the preparation of
416 lysates. Partially lysed cells were disrupted by vortexing the cultures following the
417 addition of chloroform (1 volume of chloroform per 100 volumes of culture). Cell
418 debris was removed by centrifugation (12 min, 4,000 x rpms, room temperature), and
419 the supernatant was passed through a 0.22 µm polyethersulfone membrane filter.
420 *Sodalis praecaptivus* P1CM*clr*-100(ts) lysogens were prepared as described above, except
421 that cells were grown for 2 h at 42˚C and 16 h at 37˚C prior to lysate preparation. *Sodalis*
422 *glossinidius* P1CM*clr*-100(ts) lysogens were grown in BHI to an OD600 of 0.4. Cultures

423 were heat shocked at 37 ˚C for 2 h. The cultures were treated with chloroform (1
424 volume of chloroform per 100 volumes of culture) and processed as described for *E. coli*
425 and *S. praecaptivus*.
426
427 **Infection by P1*vir*, P1CM*clr*-100(ts) and P1 transducing particles**
428 Following overnight grow in LB, *E. coli* cultures were diluted in fresh LB supplemented
429 with 10 mM $CaCl_2$ and $MgCl_2$ to an OD_{600} of 1. 1 mL aliquots of these cell solutions
430 were incubated for 30 minutes at 30˚C in the absence or presence of various
431 concentrations of P1 lysate. Cells were subsequently collected by centrifugation (1 min,
432 13,000 x rpms, room temperature), and the supernatants were replaced by 1 ml of LB
433 containing 5 mM sodium citrate. Cells were grown for 1 h at 30˚C and 250 rpm prior to
434 plating. *Sodalis praecaptivus* cells grown overnight in LB were collected by
435 centrifugation (1 min, 13,000 x rpms, room temperature) and resuspended in fresh LB
436 supplemented with 10 mM $CaCl_2$ and $MgCl_2$. One ml of these resuspended solutions
437 was incubated for 30 minutes at 30˚C in the absence or presence of various
438 concentrations of P1 lysate. Cells were collected by centrifugation (1 min, 13,000 x
439 rpms, room temperature), and the supernatants were replaced by 1 ml of LB containing
440 5 mM sodium citrate. Cells were plated following 1 h of growth at 30˚C and 250 rpm.
441 *Sodalis glossinidius* cells were grown in BHI for 3-5 days, to an $OD_{600} \approx 0.5$. Cells were
442 collected by centrifugation and concentrated to an OD_{600} of 1. One ml of concentrated
443 cultures was incubated for 60 minutes at 30˚C in the absence or presence of various
444 concentrations of P1 lysate. Cells were collected by centrifugation (1 min, 13,000 x
445 rpms, room temperature), and the supernatants were replaced by 10 ml of BHI.
446 Cultures were incubated overnight at 27˚C overnight with shaking prior to plating.
447

448 **Curing of pP1-Tn7 phagemids**

449 Transduction of pP1-Tn7 phagemids into *S. glossinidius* and *S. praecaptivus* were initially
450 selected on plates containing ampicillin (Fig. 5D, Fig. S2). Because episomes harboring
451 rep_{pSC101}^{ts} origins of replication are not easily cured from *S. praecaptivus* (see discussion),
452 the curing of phagemids was only performed in *S. glossinidius*. The strategy used to
453 identify *S. glossinidius* clones lacking phagemids was similar to the one adopted
454 elsewhere (Pontes and Dale, 2001). Briefly, to identify *S. glossinidius* clones that
455 contained the chloramphenicol resistant marker at the Tn7 attachment site and had loss
456 the ampicillin resistant plasmid, cultures were propagated in BHI containing
457 chloramphenicol and 1% arabinose. After 4 passages, cells were diluted and plated.
458 Single colonies were screened for sensitivity to ampicillin. Transposon insertion at the
459 Tn7 attachment site was verified by PCR with primers 1018 and 1019.

460

461 **Image Acquisition, Analysis and Manipulation**

462 DNA agarose gel electrophoresis and bacterial colonies, with the exception of *S.*
463 *glossinidsius* macrocolonies (were detected using an Amersham Imager 680 (GE
464 Healthcare). *Sodalis glossinidsius* macrocolonies expressing GFP were detected using a
465 dark reader (Clare Chemical Research) and documented with an iPhone. When
466 oversaturated, the intensity of signals in images were adjusted across the entire images
467 using Preview (Apple).

468

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475
476 **Conflict of Interest**
477 The authors declare no conflict of interest.
478
479 **Supplemental Material**
480 Figure S1.
481 Figure S2.
482 Table S1.
483 Table S2.
484
485 **References**
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692 **Figure Legends**

693

694 **Fig. 1.** Cartoon representation depicting a workflow of the transduction procedure
695 developed for introduction of phagemids in *Sodalis* species. Following the direction of
696 the arrows: An *E. coli* P1 lysogen host is transformed with a P1 phagemid. The
697 phagemid is packaged following induction of the P1 prophage, and lysates derived
698 from culture supernatant are used to infect a *Sodalis* recipient strain. Cells receiving the
699 phagemid are subsequently isolated on plates containing a selective agent.

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701

702 **Fig. 2.** Infection of bacterial strains by phage P1. Lysates derived from an *E. coli*
703 P1CM*clr*-100(ts) lysogen (KL463) were used to infect *E. coli* MG1655 (A), *Sodalis*
704 *glossinidius* (B) and *Sodalis praecaptivus* (C). Plates depict the formation of
705 chloramphenicol resistant colonies as functions of the concentration of bacteria (vertical
706 axis) and the concentration of P1CM*clr*-100(ts) lysates (horizontal axis). Note that P1
707 infection conditions for the strains are different (see Materials and Methods), and
708 images do not reflect efficiency of P1 infection. Images show representative plates of at
709 least 3 routine experiments.

710

711

712 **Fig. 3.** Lysogenization and production of infective phage particles by *Sodalis glossinidius*
713 and *Sodalis praecaptivus* P1 lysogens. (A) Detection of P1 *pacB* gene by PCR and agarose
714 gel electrophoresis in *S. glossinidius* chloramphenicol resistant clones that emerged
715 following exposure to an *E. coli* P1CM*clr*-100(ts) lysogen (KL463). (B) Detection of a *S.*
716 *glossinidius* specific DNA fragment in clones depicted in (A) by PCR and agarose gel

717 electrophoresis. (C) Detection of P1 *pacB* gene by PCR and agarose gel electrophoresis
718 in *S. praecaptivus* chloramphenicol resistant clones that emerged following exposure to
719 an *E. coli* P1CM*clr*-100(ts) lysogen (KL463). (D) Detection of a *S. praecaptivus* specific
720 DNA fragment in clones depicted in (C) by PCR and agarose gel electrophoresis. (E-G)
721 Formation of phage plaques on soft agar embedded with *E. coli* MG1655. Soft agar
722 plates were spotted with dilutions of lysates derived from wild-type and *S. glossinidius*
723 chloramphenicol resistant (Cm^R) P1CM*clr*-100(ts) lysogen (MP1705) (E), wild-type and
724 *S. praecaptivus* Cm^R P1CM*clr*-100(ts) lysogen (MP1703) (F), and *S. praecaptivus* Cm^R
725 P1CM*clr*-100(ts) lysogen (MP1703) grown either at 37°C or 30°C. Plates are
726 representative of routine experiments. (H) Emergence of Cm^R *E. coli* MG1655 following
727 exposure to lysates derived from wild-type *S. glossinidius*, *S. glossinidius* Cm^R P1CM*clr*-
728 100(ts) lysogen (MP1705), wild-type *S. praecaptivus*, and *S. praecaptivus* Cm^R P1CM*clr*-
729 100(ts) lysogen (MP1703). Plate is representative of at least 3 experiments.
730
731
732 **Fig. 4.** Bacteriophage P1-mediated generalized transduction in *S. praecaptivus*. (A)
733 Detection of a pSIM6 DNA region by PCR and agarose gel electrophoresis in ampicillin
734 resistant (Amp^R) *S. praecaptivus* transductants following exposure to lysates derived
735 from *S. praecaptivus* Cm^R P1CM*clr*-100(ts) (MP1703) harboring Amp^R plasmid pSIM6.
736 (B) Detection of the *rpoS*-HA::Cm chromosomal insertion by PCR and agarose gel
737 electrophoresis in *S. praecaptivus* transductants following exposure to lysates of P1*vir*
738 grown in *S. praecaptivus rpoS*-HA::Cm pSIM6 (MP1522) strain.
739
740

741 **Fig. 5.** Transduction of P1 phagemids into *Sodalis* species. (A) Schematic representation
742 of replication competent P1 phagemids encoding a number of phenotypic markers
743 (Kittleson *et al*., 2012). (B) Comparison of wild-type (top quadrants) and *S. glossinidius*
744 transductants (lower quadrants) carrying P1 phagemid BBa_J72114-BBa_J72104
745 (Kittleson *et al*., 2012). Transductant colonies are purple due to the expression of
746 violacein biosynthetic genes (lower, left quadrant), and produce light due to the
747 expression of bioluminescence genes (lower, right quadrant). (C) Macrocolonies
748 derived from wild-type *S. glossinidius* (bottom row) and transductants carrying P1
749 phagemids BBa_J72114-BBa_J72104 (*luxCDABE*$^+$ and *vioABCE*$^+$), BBa_J72114-BBa_J72100
750 (*lacZ*$^+$) or BBa_J72113-BBa_J72152 (*gfp*$^+$) (upper row). (D) Transduction of pP1-Tn*7*-
751 mVenus into *S. praecaptivus* (left-hand side plate) and pP1-Tn*7*-mCardinal into *S.*
752 *glossinidius* (right-hand side plate). (E). Transduction of phagemid encoding a Himar1
753 transposition system (pP1-Himar) into *S. praecaptivus* (left-hand side plate) and *S.*
754 *glossinidius* (right-hand side plate).
755
756
757 **Fig. S1.** Effect of a *galU* mutation on LPS composition and P1 infectivity in *S.*
758 *praecaptivus*. (A) Schematic representation depicting the inner membrane, the
759 peptidoglycan and the outer membrane of a prototypical Gram negative bacterium.
760 Inlet shows a more detailed schematic of structural components of the LPS (left-hand
761 side). Silver-stained SDS-PAGE of LPS purified from *S. praecaptivus galU* (CMK36),
762 wild-type *S. praecaptivus* and wild-type *S. glossinidius* (right-hand side). (B) Plates
763 depicting the appearance of chloramphenicol resistant colonies as functions of the
764 concentration of bacteria (vertical axis) and the concentration of P1CM*clr*-100(ts) lysates
765 (horizontal axis). Cm^R colonies emerge at similar frequencies in wild-type *S.*

766 *praecaptivus* (left-hand side plate) and *S. praecaptivus galU* (CMK36) (right-hand side
767 plate) when cells are exposed to equal amounts of P1CM*clr*-100(ts) particles.
768
769 **Fig. S2.** Schematic representation of P1 phagemids used for tagging the bacterial
770 chromosome at the Tn7 attachment site(s) with genes encoding the fluorescent proteins
771 mCardinal (pP1-Tn7-mCardinal) or mVenus (pP1-Tn7-mVenus); and the suicide P1
772 phagemid for random mutagenesis with a Himar1 transposition system (pP1-Himar).

Figure 1

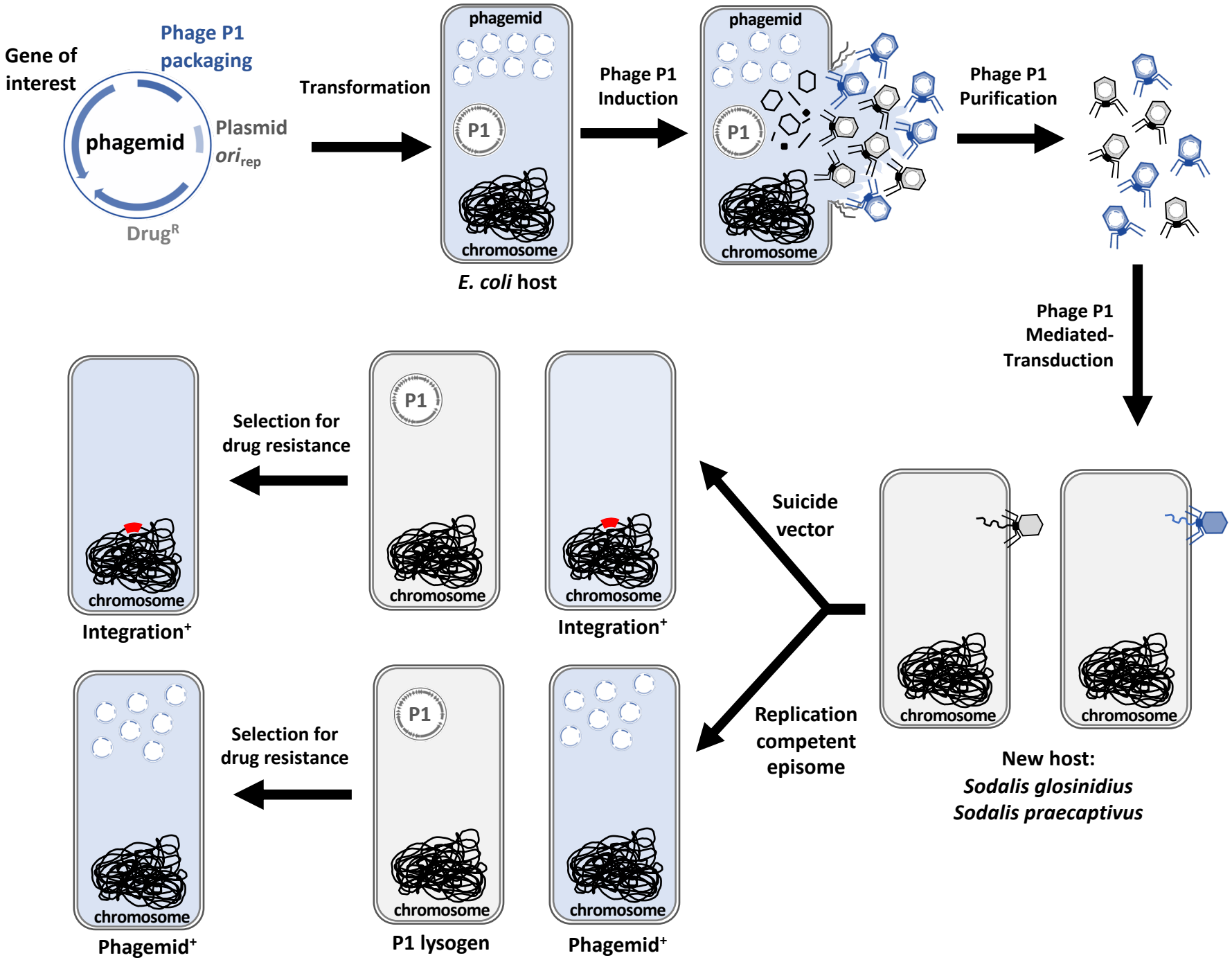

Gene of interest
Phage P1 packaging
phagemid
Plasmid
ori rep
Drug R
Transformation
phagemid
P1
chromosome
E. coli host
Phage P1 Induction
phagemid
P1
chromosome
Phage P1 Purification
Phage P1 Mediated-Transduction
Suicide vector
Replication competent episome
chromosome
chromosome
New host:
Sodalis glosinidius
Sodalis praecaptivus
P1
chromosome
Integration+
Selection for drug resistance
chromosome
Integration+
P1
chromosome
P1 lysogen
Selection for drug resistance
chromosome
Phagemid+
chromosome
Phagemid+

Figure 2

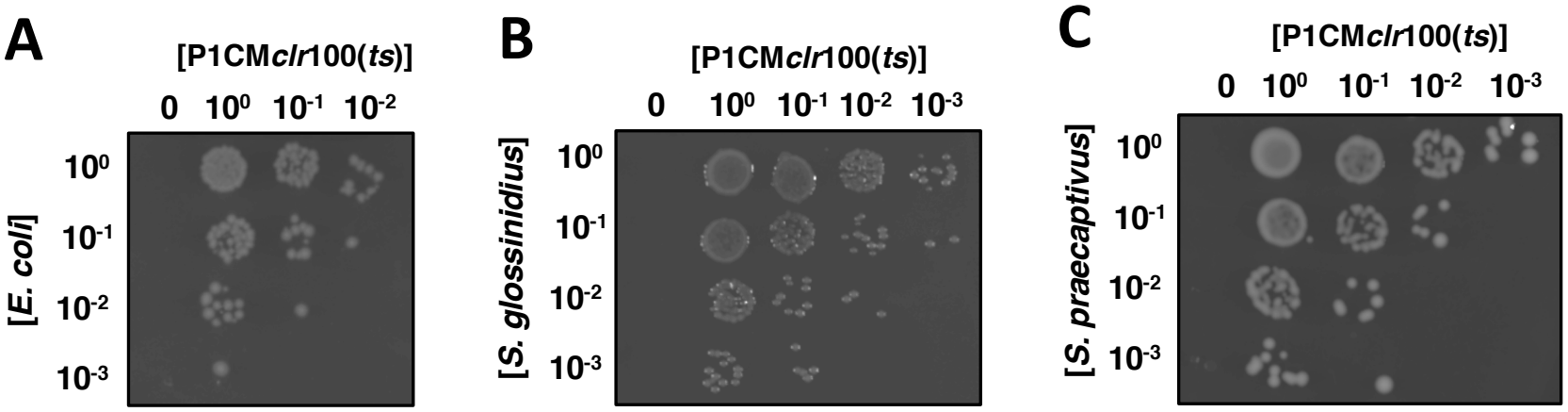

A
[P1CMclr100(ts)]
0 10^0 10^-1 10^-2
[E. coli]
10^0
10^-1
10^-2
10^-3
B
[P1CMclr100(ts)]
0 10^0 10^-1 10^-2 10^-3
[S. glossinidius]
10^0
10^-1
10^-2
10^-3
C
[P1CMclr100(ts)]
0 10^0 10^-1 10^-2 10^-3
[S. praecaptivus]
10^0
10^-1
10^-2
10^-3

Figure 3

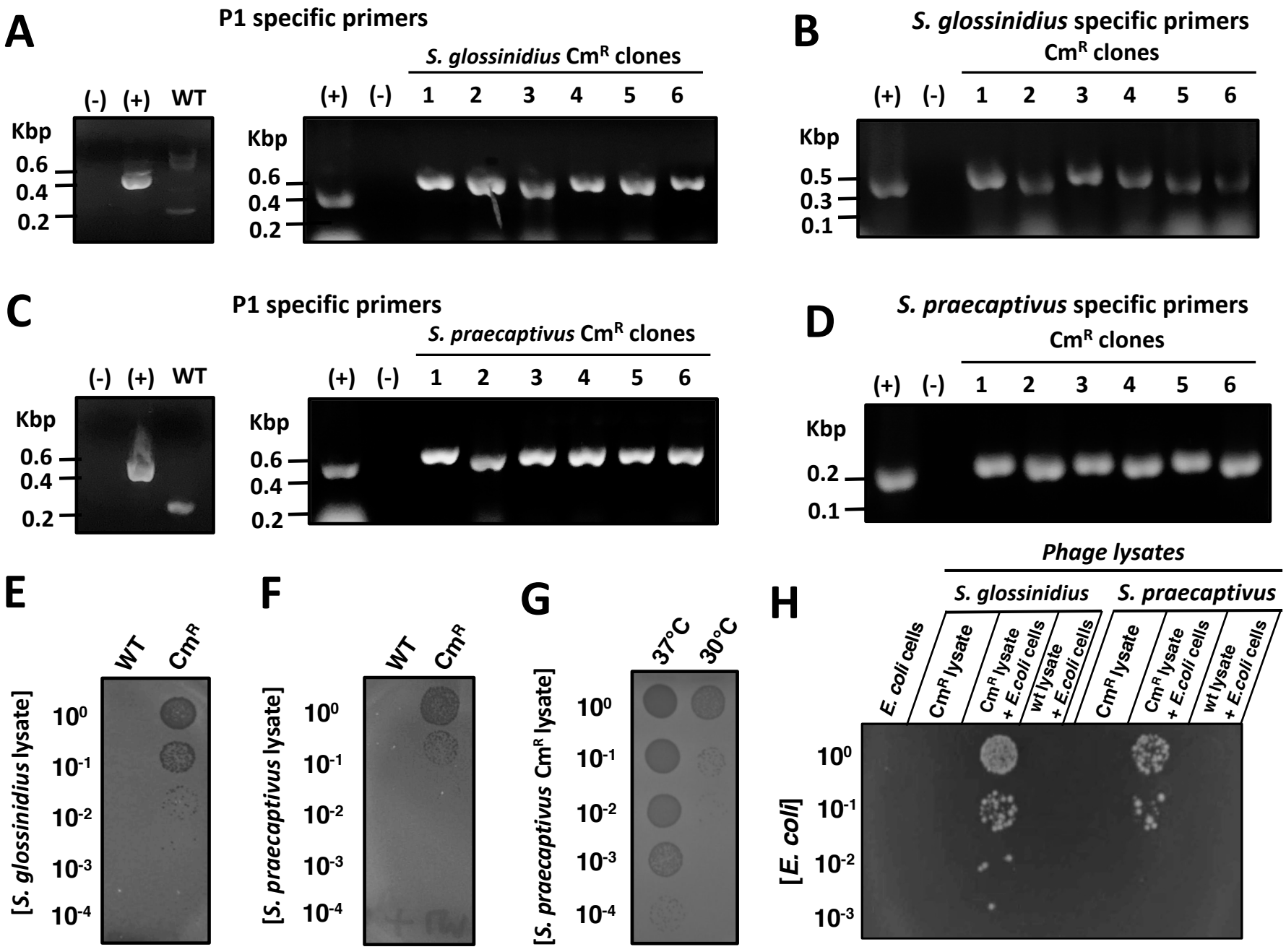

A
P1 specific primers
S. glossinidius CmR clones
(-) (+) WT
(+) (-) 1 2 3 4 5 6
Kbp
0.6
0.4
0.2
B
S. glossinidius specific primers
CmR clones
(+) (-) 1 2 3 4 5 6
Kbp
0.5
0.3
0.1
C
P1 specific primers
S. praecaptivus CmR clones
(-) (+) WT
(+) (-) 1 2 3 4 5 6
Kbp
0.6
0.4
0.2
D
S. praecaptivus specific primers
CmR clones
(+) (-) 1 2 3 4 5 6
Kbp
0.2
0.1
E
WT
CmR
[S. glossinidius lysate]
10^0
10^-1
10^-2
10^-3
10^-4
F
WT
CmR
[S. praecaptivus lysate]
10^0
10^-1
10^-2
10^-3
10^-4
G
37°C
30°C
[S. praecaptivus CmR lysate]
10^0
10^-1
10^-2
10^-3
10^-4
H
Phage lysates
S. glossinidius
S. praecaptivus
E. coli cells
CmR lysate
CmR lysate + E.coli cells
wt lysate + E.coli cells
CmR lysate
CmR lysate + E.coli cells
wt lysate + E.coli cells
[E. coli]
10^0
10^-1
10^-2
10^-3

Figure 4

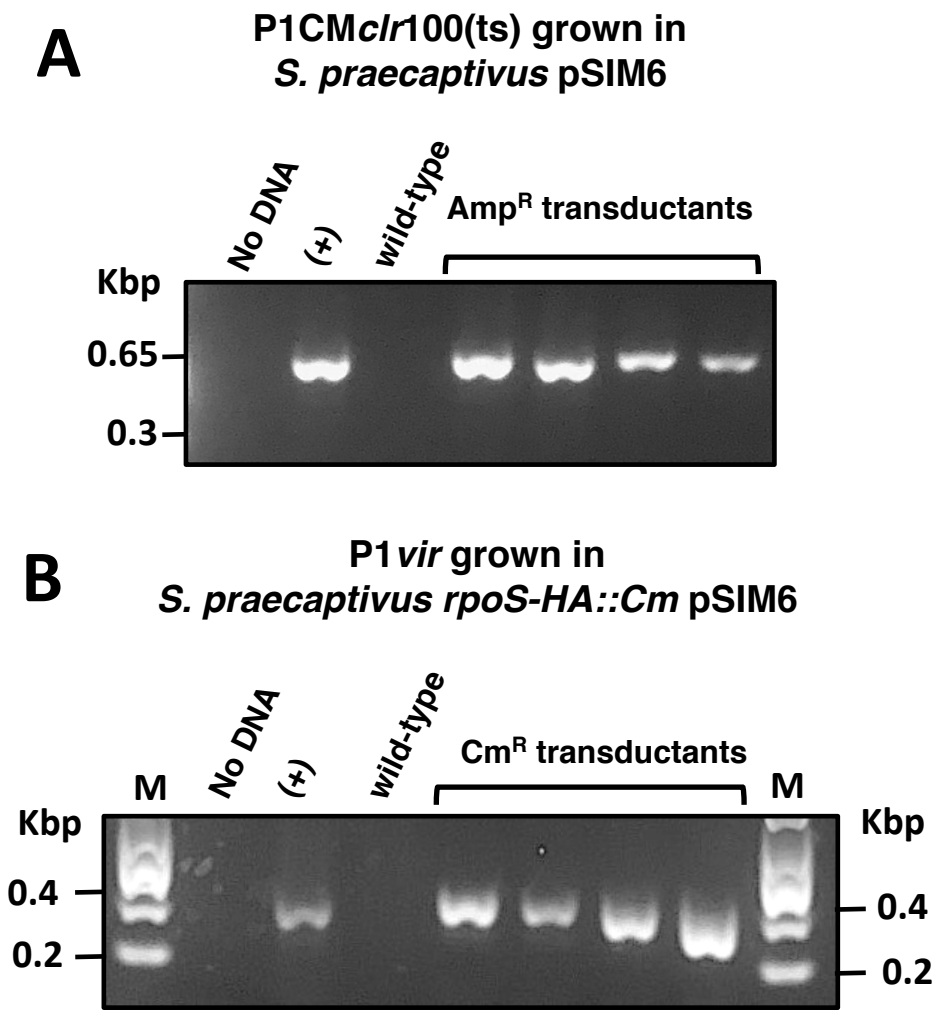

A
P1CM*clr*100(ts) grown in
S. praecaptivus pSIM6
No DNA
(+)
wild-type
Amp^R transductants
Kbp
0.65
0.3
B
P1*vir* grown in
S. praecaptivus rpoS-HA::Cm pSIM6
M
No DNA
(+)
wild-type
Cm^R transductants
M
Kbp
0.4
0.2
Kbp
0.4
0.2

Figure 5

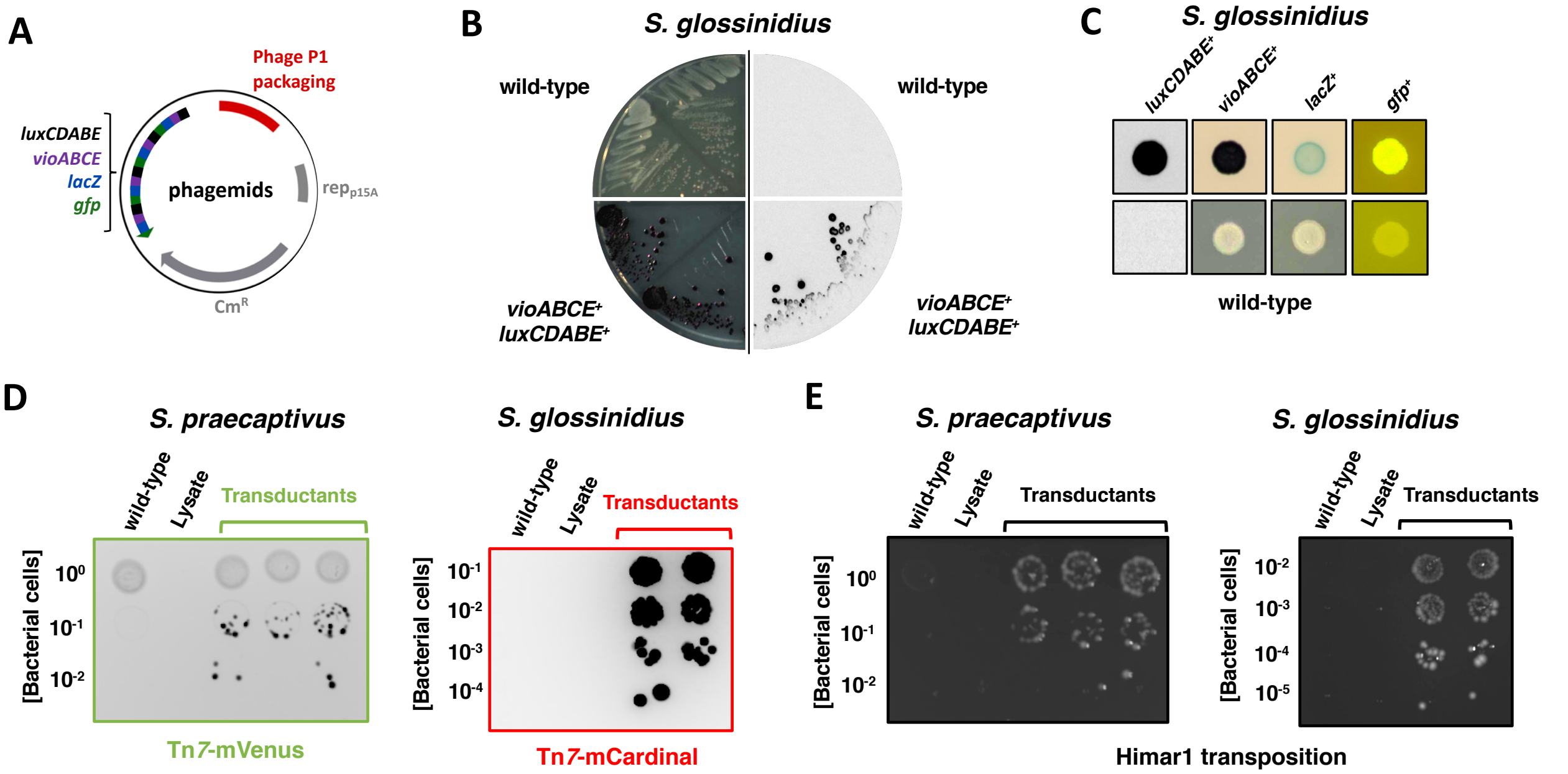

A
Phage P1 packaging
luxCDABE
vioABCE
lacZ
gfp
phagemids
rep$_{p15A}$
Cm^R
B
S. glossinidius
wild-type
wild-type
vioABCE+ luxCDABE+
vioABCE+ luxCDABE+
C
S. glossinidius
luxCDABE+
vioABCE+
lacZ+
gfp+
wild-type
D
S. praecaptivus
wild-type
Lysate
Transductants
[Bacterial cells]
10^0
10^{-1}
10^{-2}
Tn7-mVenus
S. glossinidius
wild-type
Lysate
Transductants
[Bacterial cells]
10^{-1}
10^{-2}
10^{-3}
10^{-4}
Tn7-mCardinal
E
S. praecaptivus
wild-type
Lysate
Transductants
[Bacterial cells]
10^0
10^{-1}
10^{-2}
S. glossinidius
wild-type
Lysate
Transductants
[Bacterial cells]
10^{-2}
10^{-3}
10^{-4}
10^{-5}
Himar1 transposition

Figure S1

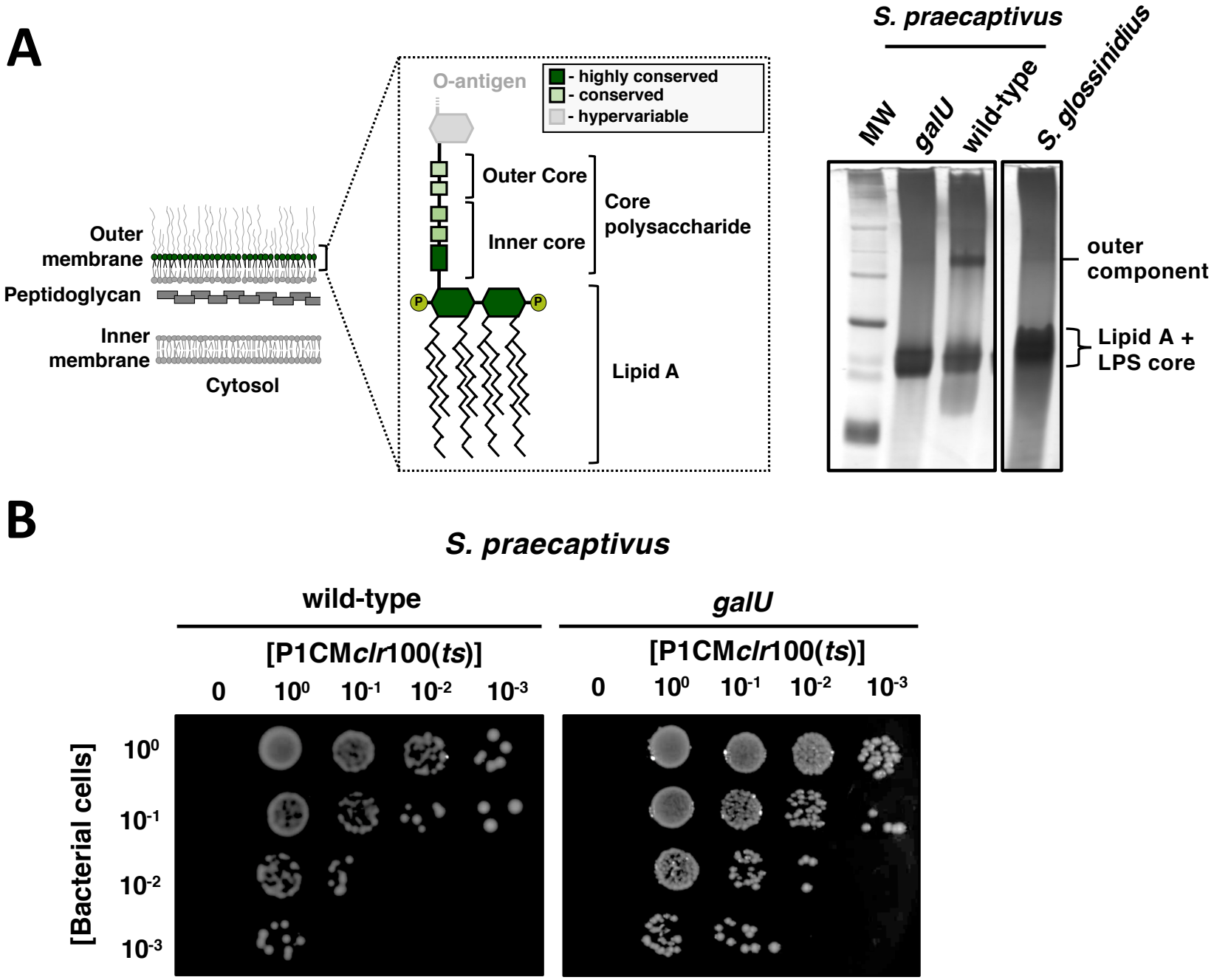
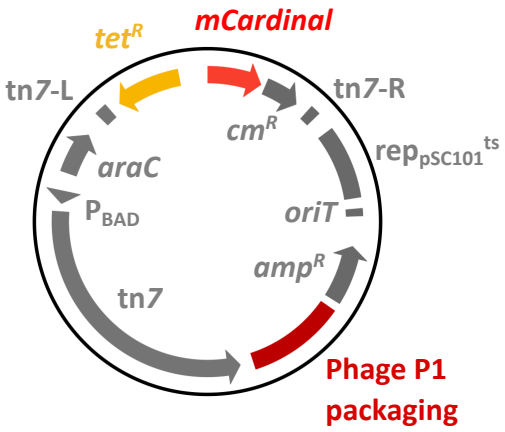
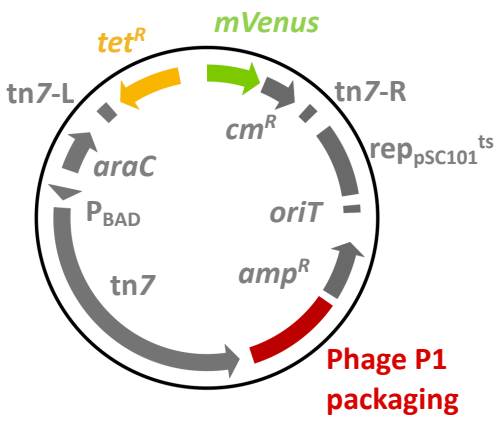
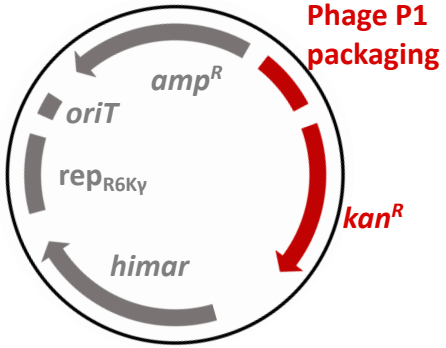

A
Outer membrane
Peptidoglycan
Inner membrane
Cytosol
O-antigen
- highly conserved
- conserved
- hypervariable
Outer Core
Inner core
Core polysaccharide
Lipid A
S. praecaptivus
MW
galU
wild-type
S. glossinidius
outer component
Lipid A + LPS core
B
S. praecaptivus
wild-type
galU
[P1CMclr100(ts)]
0
10^0
10^-1
10^-2
10^-3
[Bacterial cells]

Figure S2

pP1-Tn7-mCardinal



pP1-Tn7-mVenus



pP1-Himar