

# Natural Strain Variation Reveals Diverse Biofilm Regulation in Squid-Colonizing Vibrio fischeri

Ella R. Rotman,<sup>a</sup> Katherine M. Bultman,<sup>b</sup> John F. Brooks II,<sup>a\*</sup> Mattias C. Gyllborg,<sup>a</sup> Hector L. Burgos,<sup>b</sup> Michael S. Wollenberg,<sup>c</sup> Mark J. Mandel<sup>a,b</sup>

<sup>a</sup>Department of Microbiology-Immunology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA <sup>b</sup>Department of Medical Microbiology and Immunology, University of Wisconsin—Madison, Madison, Wisconsin, USA <sup>c</sup>Department of Biology, Kalamazoo College, Kalamazoo, Michigan, USA

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ABSTRACT The mutualistic symbiont Vibrio fischeri builds a symbiotic biofilm during colonization of squid hosts. Regulation of the exopolysaccharide component, termed Syp, has been examined in strain ES114, where production is controlled by a phosphorelay that includes the inner membrane hybrid histidine kinase RscS. Most strains that lack RscS or encode divergent RscS proteins cannot colonize a squid host unless RscS from a squid symbiont is heterologously expressed. In this study, we examine V. fischeri isolates worldwide to understand the landscape of biofilm regulation during beneficial colonization. We provide a detailed study of three distinct evolutionary groups of V. fischeri and find that while the RscS-Syp biofilm pathway is required in one of the groups, two other groups of squid symbionts require Syp independent of RscS. Mediterranean squid symbionts, including V. fischeri SR5, colonize without an RscS homolog encoded by their genome. Additionally, group A V. fischeri strains, which form a tightly related clade of Hawaii isolates, have a frameshift in rscS and do not require the gene for squid colonization or competitive fitness. These same strains have a frameshift in sypE, and we provide evidence that this group A sypE allele leads to an upregulation in biofilm activity. Thus, this work describes the central importance of Syp biofilm in colonization of diverse isolates and demonstrates that significant evolutionary transitions correspond to regulatory changes in the *syp* pathway.

**IMPORTANCE** Biofilms are surface-associated, matrix-encased bacterial aggregates that exhibit enhanced protection to antimicrobial agents. Previous work has established the importance of biofilm formation by a strain of luminous *Vibrio fischeri* bacteria as the bacteria colonize their host, the Hawaiian bobtail squid. In this study, expansion of this work to many natural isolates revealed that biofilm genes are universally required, yet there has been a shuffling of the regulators of those genes. This work provides evidence that even when bacterial behaviors are conserved, dynamic regulation of those behaviors can underlie evolution of the host colonization phenotype. Furthermore, this work emphasizes the importance of investigating natural diversity as we seek to understand molecular mechanisms in bacteria.

**KEYWORDS** Aliivibrio fischeri, BinK, RscS, Vibrio fischeri, biofilm, phosphorelay

A fundamental question in studying host-associated bacterial communities is understanding how specific microbial taxa assemble reproducibly in their host. Key insights into these processes were first obtained by studying plant-associated microbes, and the discovery and characterization of Nod factors in rhizobia were valuable to understanding how partner choice between microbe and host could be mediated at the molecular level (1, 2). There are complex communities in humans and other vertebrate animals, yet metagenomic and imaging analyses of these communities have **Citation** Rotman ER, Bultman KM, Brooks JF, II, Gyllborg MC, Burgos HL, Wollenberg MS, Mandel MJ. 2019. Natural strain variation reveals diverse biofilm regulation in squidcolonizing *Vibrio fischeri*. J Bacteriol 201:e00033-19. https://doi.org/10.1128/JB .00033-19.

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Address correspondence to Mark J. Mandel, mmandel@wisc.edu.

\* Present address: John F. Brooks II, Department of Immunology, The University of Texas Southwestern Medical Center, Dallas, Texas, USA.

E.R.R. and K.M.B. contributed equally to this work.

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revealed striking reproducibility in the taxa present and in the spatial arrangement of those taxa (3–5). Invertebrate animal microbiomes provide appealing systems in which to study microbiome assembly in an animal host: the number of taxa are relatively small, and examination and manipulation of these organisms have yielded abundant information about processes underlying host colonization (6). For this work, we focused on the binary symbiosis between Vibrio fischeri and bobtail squids, including the Hawaiian bobtail squid, Euprymna scolopes. Bobtail squid have an organ for the symbiont termed the light organ, and passage of specific molecules between the newly hatched host and the symbiont leads to light organ colonization specifically by planktonic V. fischeri and not by other bacteria (7-9). The colonization process involves initiation, accommodation, and persistence steps, resulting in light organ crypt colonization by V. fischeri. Upon colonization of the squid light organ, bacteria accumulate to high density and produce light. The bacterial light is modulated by the host to camouflage the moonlight shadow produced by the nighttime foraging squid in a cloaking process termed counterillumination (10, 11). A diel rhythm leads to a daily clearing of 90 to 95% of the bacteria from the crypts and regrowth of the remaining cells (12). However, the initial colonization process, including biofilm-based aggrega-

examines regulation of biofilm formation in diverse squid-colonizing *V. fischeri* strains. In the well-studied *V. fischeri* strain ES114, biofilm formation is required to gain entry into the squid host. RscS is a hybrid histidine kinase that regulates *V. fischeri* biofilm formation through a phosphorelay involving the hybrid histidine kinase SypF and the response regulator and  $\sigma^{54}$ -dependent activator SypG (13–15). This pathway regulates transcription of the <u>symbiosis polysaccharide</u> (Syp) locus, which encodes regulatory proteins (SypA, SypE, SypF, and SypG), glycosyltransferases, factors involved in polysaccharide export, and other biofilm-associated factors (14, 16). The products of the ES114 *syp* locus direct synthesis and export of a biofilm exopolysaccharide that is critical for colonization. Additional pathways have been identified to influence biofilm regulation in ES114, including the SypE-SypA pathway, and inhibition of biofilm formation by BinK and HahK (17–21).

tion on the host ciliated appendages, occurs only in newly hatched squid. This work

*V. fischeri* biofilm regulation is connected to host colonization specificity. In the Pacific Ocean, the presence of *rscS* DNA is strongly correlated to the ability to colonize squid (22). As one example, while the fish symbiont MJ11 carries a complete *syp* locus, it lacks RscS and does not robustly colonize squid. Heterologous expression of ES114 RscS in MJ11 activates the biofilm pathway and is sufficient to enable squid colonization (22). Similarly, addition of ES114 RscS to *mjapo*.8.1, a fish symbiont that encodes a divergent RscS that is not functional for squid colonization, allows the strain to colonize squid (22). RscS has also been shown to be necessary for squid colonization in certain strains. In addition to ES114, interruption of *rscS* in *V. fischeri* strains KB1A97 and MJ12 renders them unable to colonize squid. Previous phylogenetic analysis revealed that genomes of ancestral *V. fischeri* strains do not carry *rscS* and that it was acquired once during the organism's evolution, likely allowing for an expansion in host range. From this analysis, it was concluded that strains with *rscS* can colonize squid, with the only exception being the fish symbionts that harbor the divergent RscS, including *mjapo*.8.1 (22).

There are similar *Vibrio*-squid associations worldwide, yet only *V. fischeri* and the closely related *Vibrio logei* have been isolated from light organs (23–26). Our 2009 study revealed that although most symbionts have *rscS* DNA, there are Mediterranean *V. fischeri* strains (e.g., SR5) that do not have *rscS* yet can colonize squid (22, 24, 27). This unexpected finding prompted the current work to examine whether strains such as SR5 colonize with the known biofilm pathway or with a novel pathway. Here, we show that all *V. fischeri* strains tested require the *syp* locus to colonize a squid host, and we identify two groups of isolates that colonize with novel regulation. Given the exquisite specificity by which *V. fischeri* bacteria colonize squid hosts, this work reinforces the importance of biofilm formation and reveals different regulatory modes across the evolutionary tree.



**FIG 1** Bayesian phylogram (50% majority-rule consensus) inferred with a SYM+I+ $\Gamma$  model of evolution for the concatenated gene fragments *recA*, *mdh*, and *katA*. In this reconstruction, the root connected to a clade containing the four non-*V*. *fischeri* outgroup taxa. Statistical support is represented at nodes by the following three numbers: upper left, Bayesian posterior probability (of approximately 37,500 nondiscarded samples) multiplied by 100; upper right, percentage of 1,000 bootstrap maximum likelihood pseudoreplicates; bottom, percentage of 1,000 bootstrap maximum parsimony pseudoreplicates. Statistical support values are listed only at nodes where more than 2 methods generated support values of  $\geq$ 50%. Strains sharing identical sequences for a given locus fragment are listed next to a vertical bar at a leaf; because of a lack of space, some support values have been listed immediately to the right of their associated nodes and are marked with lowercase roman numerals in the phylogram. The isolation habitat and geography of each strain are indicated by symbol and color, respectively. The scale bar represents 0.01 substitution/site.

## RESULTS

**Most V. fischeri strains synthesize biofilm in response to RscS overexpression.** Biofilm formation is required for squid colonization, and overexpression of the biofilm regulator RscS in strain ES114 stimulates a colony biofilm on agar plates (15). Our previous work demonstrated that *V. fischeri* strain MJ11 synthesizes a colony biofilm under similar inducing conditions, which is notable because MJ11 does not encode RscS in its chromosome (22). While the ancestral strain MJ11 did not encode RscS, it had what seemed to be an intact *syp* locus, and overexpression of the heterologous RscS from ES114 was sufficient to enable robust squid colonization (22). We examined a phylogenetic tree of *V. fischeri* isolates (Fig. 1), and in this study we expand our analysis of RscS-Syp biofilm regulation in a wider group of *V. fischeri* strains.

Initially, we asked whether responsiveness to RscS overexpression would yield a similar colony biofilm in this diverse group of strains. We took the same approach as our previous study and introduced plasmid pKG11, which overexpressed ES114 RscS, into strains across the evolutionary tree (22, 28). We observed that almost all strains tested, including those that lack *rscS*, were responsive to overexpression of ES114 RscS (Fig. 2). The morphology of the colony biofilms differed across isolates, but in most cases colony



**FIG 2** Most *V. fischeri* strains tested form colony biofilm in response to RscS overexpression. Shown are spot assays of the indicated *V. fischeri* strains carrying pKV69 (vector) or pKG11 (*rscS1*; overexpressing ES114 *rscS*) after 24 and 48 h. Strains are MJM1268, MJM1269, MJM1246, MJM1247, MJM1266, MJM1267, MJM1219, MJM1221, MJM1238, MJM1239, MJM1104, MJM1266, MJM1277, MJM1270, MJM1271, MJM1258, MJM1259, MJM1254, MJM1255, MJM1242, MJM1243, MJM1240, MJM1241, MJM1272, MJM1273, MJM1274, MJM1275, MJM1275, MJM1278, MJM1279, MJM1279, MJM1111, MJM1280, MJM1281, MJM1260, MJM1261, MJM1244, MJM1245, MJM1255, MJM1255, MJM1255, MJM1279, MJM1270, MJM1270, MJM1274, MJM1274, MJM1274, MJM1276, MJM1277, MJM1270, MJM1274, MJM1274, MJM1275, MJM1278, MJM1279, MJM1279, MJM1270, MJM1274, MJM1274, MJM1274, MJM1276, MJM1277, MJM1270, MJM1274, MJM1274, MJM1274, MJM1276, MJM1277, MJM1270, MJM1274, MJM1274, MJM1276, MJM1277, MJM1270, MJM1274, MJM1274, MJM1276, MJM1276, MJM1279, MJM1279, MJM1274, MJM1274, MJM1276, MJM1276, MJM1277, MJM1276, MJM1274, MJM1274, MJM1274, MJM1276, MJM1277, MJM1279, MJM1274, MJM1274, MJM1274, MJM1276, MJM1277, MJM1279, MJM1274, MJM1274, MJM1274, MJM1276, MJM1276, MJM1277, MJM1276, MJM1274, MJM1274, MJM1274, MJM1274, MJM1276, MJM1276, MJM1277, MJM1276, MJM1274, MJM1274, MJM1274, MJM1276, MJM1276, MJM1276, MJM1276, MJM1276, MJM1276, MJM1274, MJM1276, MJM1277, MJM1276, MJM1276, MJM1276, MJM1276, MJM1276, MJM1276, MJM1276, MJM1276, MJM1277, MJM1276, MJM1276, MJM1276, MJM1276, MJM1277, MJM1276, MJM1276, MJM1276, MJM1276, MJM1276, MJM1277, MJM1276, MJM1277, MJM1276, MJM1276, MJM1276, MJM1276, MJM1276, MJM1277, MJM1276, MJM1276, MJM1276, MJM1276, MJM1277, MJM1276, MJM1276, MJM1276, MJM1276, MJM1277, MJM1276, MJ

biofilm was evident at 24 h and prominent at 48 h. All of the strains exhibited some wrinkled colony morphology at 48 h with the exception of CG101, which was isolated from the pineapplefish *Cleidopus gloriamaris* (25). These results demonstrated that most *V. fischeri* strains can produce biofilm in response to RscS overexpression, and this includes strains that presumably have not encountered *rscS* in their evolutionary history.

One unexpected observation was that there was a subset of *rscS*-encoding strains that were reproducibly delayed in their colony biofilm and had only a mild wrinkled colony phenotype at 48 h (strains MB11B1, ES213, and KB2B1) (Fig. 2). We considered whether this was due to differential growth of the strains, but resuspension of spots and dilution plating to determine CFU/spot demonstrated no significant growth difference between these strains and ES114 under these conditions. The strains are closely related (Fig. 1), and a previous study had noted that this group shared a number of phenotypic characteristics, e.g., reduced motility in soft agar (29). Those authors termed this tight clade "group A" *V. fischeri* (30). Our results shown in Fig. 2 argue that group A strains do not respond to RscS in the same manner as other *V. fischeri* strains, which prompted us to investigate the evolution of the RscS-Syp signaling pathway. We have maintained the group A nomenclature here, and furthermore we introduce the nomenclature of group B (a paraphyletic group of strains that contain *rscS*; this group includes the common ancestor of all *rscS*-containing strains) and group C (a paraphyletic



**FIG 3** Squid colonization in group C strain SR5, which does not encode RscS, is dependent on the *syp* polysaccharide locus. Single-strain colonization experiments were conducted, and circles represent individual animals. The limit of detection for this assay, represented by the dashed line, is 7 CFU/light organ (LO), and the horizontal bars represent the median for each set. Hatchling squid were inoculated with  $1.5 \times 10^3$  to  $3.2 \times 10^3$  CFU/ml bacteria, washed at 3 h and 24 h, and assayed at 48 h. Strains are MJM1100, MJM3010, MJM3062, MJM1125, and MJM3501. Statistical comparisons were done with the Mann-Whitney test. \*\*, P < 0.01; \*\*\*, P < 0.001; \*\*\*\*, P < 0.0001.

etic group of strains that contains the common ancestor of all *V. fischeri* strains; these strains do not contain *rscS*), as shown in Fig. 1.

**Colonization of** *E. scolopes* **by ancestral group C squid isolates is independent of RscS and dependent on Syp.** Group C strains generally cannot colonize squid, yet there are Mediterranean squid isolates that appear in this group (Fig. 1) (22). The best-studied of these strains, SR5, was isolated from *Sepiola robusta*, is highly luminous, and can colonize the Hawaiian bobtail squid *E. scolopes* (24). Nonetheless, this strain lacks *rscS* (27). We first asked whether the strain can colonize under our laboratory conditions, and we confirmed that it colonizes robustly, consistent with the result previously published by Fidopiastis et al. (24) (Fig. 3). We next asked whether it uses the Syp biofilm to colonize. To address this question, we deleted the 18-kb *syp* locus (i.e., *sypA* through *sypR*) in strains SR5 and ES114. Deletion of *rscS* or the *syp* locus in ES114 led to a substantial defect in colonization, consistent with a known role for these factors (Fig. 3). Similarly, deletion of the *syp* locus in SR5, a strain that does not contain *rscS*, led to a dramatic reduction in colonization (Fig. 3). Therefore, even though strain SR5 does not contain *rscS*, it can colonize squid, and it requires the *syp* locus to colonize normally.

RscS is dispensable for colonization in group A strains. We noted in the wrinkled colony biofilm assays shown in Fig. 2 that group A strains exhibited a more modest response to overexpression of RscS. Sequencing of the native rscS gene in these strains revealed a predicted -1 frameshift ( $\Delta$ A1141) between the PAS domain and the histidine kinase CA domain. Whereas ES114 and other group B strains have nine adenines at this position, the group A strains have eight, leading to a frameshift and then truncation at an amber stop codon, raising the possibility that group A strains have a divergent biofilm signaling pathway (Fig. 4A). Given the importance of RscS in the group B strains, including ES114, we considered the possibility that this apparent frameshift encoded a functional protein, either through ribosomal frameshifting or through the production of two polypeptides that together provided RscS function; there is precedent for both of these concepts in the literature (31, 32). We first introduced a comparable frameshift into a plasmid-borne overexpression allele of ES114 rscS, and this allele did not function with the deletion of the single adenine (Fig. 4B). This result suggested to us that the frameshift in the group A strains are not functional. Therefore, we proceeded to delete rscS in two group A strains (MB11B1 and ES213) and two group B strains (ES114 and MB15A4). The group B strains required RscS for squid colonization (Fig. 5A). However, the group A strains exhibited no deficit in the absence of rscS (Fig. 5A). We next attempted a more sensitive assay in which a group A strain was competed against MB15A4. Previous studies have demonstrated that in many cases group A strains outcompete group B strains (30, 33). We competed group



**FIG 4** Group A strains have a frameshift in *rscS*. (A) ES114 RscS protein domains. Nucleotides 1114 to 1173 in ES114 RscS (GenBank accession no. AF319618) and their homologous sequences in the other group B and group A strains are listed. The -1 frameshift is present in the group A *rscS* alleles. The ES114 reading frame is noted on the top of the alignment and the group A reading frame on the bottom, which is predicted to end at the amber stop codon. (B) Deletion of nucleotide A1141 in ES114 to mimic this frameshift in pKG11 renders it unable to induce a colony biofilm in a spot assay at 48 h. Strains are MJM1104, MJM1106, and MJM2226.

A strain MB11B1 against group B strain MB15A4 and observed a significant competitive advantage for the group A strain, as was observed previously (30). Deletion of *rscS* in the group A strain did not affect competitive fitness, demonstrating that MB11B1 can outcompete a group B strain even if MB11B1 lacks RscS (Fig. 5B).

The syp locus is broadly required for squid colonization. Given that group A strains seemed to represent a tight phylogenetic group in which RscS was not required for colonization or competitive fitness, we next asked whether this group requires the Syp biofilm for colonization. We proceeded to delete the entire *syp* locus in two group A and two group B strains and to conduct single-strain colonization analysis. In each strain assayed, the *syp* locus was required for full colonization, and we observed a 2- to 4-log-unit reduction in CFU per animal in the absence of the *syp* genes, pointing to a critical role for Syp biofilm in these strains (Fig. 6). In group A strains in particular, no colonization was detected in the absence of the *syp* locus.

**Group A strains carry an alternate allele of SypE.** It seemed curious to us that group A strains do not encode a functional RscS and do not require *rscS* for colonization, yet in many cases group A strains can outcompete group B strains (e.g., MB11B1 in Fig. 5B) (30, 33). We reasoned that if the Syp biofilm had a different regulatory architecture in group A strains, e.g., constitutively activated or activated by a different regulatory protein, then this could explain the Syp regulation independent of RscS. Genome sequencing of SR5 and MB11B1 did not identify a unique histidine kinase that was likely to directly substitute for RscS (27, 33). Given that the *syp* locus encodes biofilm regulatory proteins, we examined *syp* conservation. We used TBLASTN with the ES114 Syp proteins as queries to determine amino acid conservation in the other *V. fischeri* group A strain MB11B1, group C strain SR5, and the *Vibrio vulnificus* type strain



**FIG 5** Group A strains MB11B1 and ES213 do not require RscS for squid colonization. Wild-type (WT) and  $\Delta$ rscS derivatives of the indicated strains were assayed in a single-strain colonization assay (A) and competitive colonization against group B strain MB15A4 (B). Hatchling squid were inoculated at 3.5 × 10<sup>3</sup> to 14 × 10<sup>3</sup> CFU/ml bacteria, washed at 3 h and 24 h, and assayed at 48 h. Each dot represents an individual squid. (A) Strains are MJM1100, MJM3010, MJM2114, MJM3042, MJM1130, MJM3046, MJM1117, and MJM3017. The limit of detection is represented by the dashed line, and the horizontal bars represent the median for each set. In both panels, open dots are the wild type and filled dots are the  $\Delta$ rscS strain. (B) The competitive index (CI) is defined in Materials and Methods and is shown on a log<sub>10</sub> scale. Strains are MJM1130 and MJM3046, each competed against MJM2114. Values greater than 1 indicate more MB11B1. Statistical comparisons were made with the Mann-Whitney test. ns, not significant; \*\*\*\*\*, *P* < 0.0001.

ATCC 27562 (34, 35). As shown in Fig. 7, ES114 SypE, a response regulator and serine kinase/phosphatase that is a negative regulator of the Syp biofilm (17, 36), exhibited the lowest level of conservation among *syp* locus products. *V. vulnificus* does not encode a SypE ortholog (37), as the syntenic (but not homologous) RbdE gene encodes a predicted ABC transporter substrate-binding protein. The closest hit for SypE was AOT11\_RS12130 (9% identity), compared to 7% identity for RbdE. Due to the reduced conservation at both the strain and species levels, we analyzed *V. fischeri* MB11B1 SypE in greater detail. Examination of the *sypE* coding sequence revealed an apparent -1 frameshift mutation in which position 33 (guanine in ES114 and adenine in other group B and C strains examined) is absent from group A strains (Fig. 7B). We therefore considered the hypothesis that SypE is nonfunctional in group A, and that these strains can colonize because they lack a functional copy of this negative regulator that is itself regulated by RscS.



**FIG 6** Group B and group A strains require the *syp* locus for robust squid colonization. The wild type (WT) and  $\Delta syp$  derivatives of the indicated strains were assayed in a single-strain colonization assay. Hatchling squid were inoculated with 6.7 × 10<sup>2</sup> to 32 × 10<sup>2</sup> CFU/ml bacteria (ES114 and MB15A4 backgrounds) or 5.2 × 10<sup>2</sup> to 8.9 × 10<sup>2</sup> CFU/ml bacteria (MB11B1 and ES213 backgrounds), washed at 3 h and 24 h, and assayed at 48 h. Each dot represents an individual squid. The limit of detection is represented by the dashed line, and the bars represent the median for each set. Strains are MJM1100, MJM3062, MJM2114, MJM3071, MJM1130, MJM3065, MJM117, and MJM3068. Statistical comparisons were done with the Mann-Whitney test. \*\*\*\*, P < 0.0001.



**FIG 7** Group A strains have a frameshift in *sypE*. (A) Amino acid identity in the Syp locus. Results show the identity from TBLASTN query using the *V. fischeri* ES114 protein sequences as queries against genes in the homologous loci in *V. fischeri* strains or *V. vulnificus* ATCC 27562. The identity for SypE against *V. vulnificus* is plotted for the syntenous RbdE, although this is not the highest TBLASTN hit, as described in the text. (B) ES114 SypE protein domains. Nucleotides 1 to 60 in ES114 sypE and their homologous sequences in the other group C, B, and A strains are listed. A -1 frameshift is present in the group A sypE alleles. The ES114 reading frame is noted on the top of the alignment and the group A reading frame on the bottom, which is predicted to end at the amber stop codon. A possible GTG start codon for the resumption of translation in the ES114 reading frame is present at the position corresponding to the 18th codon in ES114 *sypE*.

To test this hypothesis, we relied on knowledge of the biofilm regulatory pathway from ES114, in which overexpression of SypG produces a wrinkled colony phenotype, but only in strains lacking SypE activity (38, 39). Therefore, we introduced the SypGoverexpressing plasmid pEAH73 into strains as a measure of whether the SypE pathway was intact. In the ES114 strain background, we observed cohesive wrinkled colony formation at 48 h in an ES114  $\Delta sypE$  strain but not in the wild-type parent (Fig. 8A). If the *sypE* frameshift observed in MB11B1 led to a loss of function, then introduction of that frameshift into ES114 would lead to a strain that is equivalent to the  $\Delta sypE$  strain. We constructed this strain, and upon SypG overexpression we observed wrinkled colony formation. Surprisingly, the biofilm phenotype was observed earlier (i.e., by 24 h) and leads to more defined colony biofilm architecture at 48 h. While the lack of SypE leads to increased and more rapid biofilm formation, in this assay we observed an even greater increase as a result of the frameshift in *sypE* (Fig. 8A).

We proceeded to conduct a similar assay in the MB11B1 strain background. The colony biofilm phenotypes were muted compared to that in the ES114 background, but the pattern observed is the same. Strains lacking the additional nucleotide at position 33 (i.e., the native MB11B1 allele) exhibited the strongest cohesion, whereas strains with the nucleotide to mimic ES114 *sypE* [i.e., added back in MB11B1 *sypE*(nt::33G)] were not cohesive (Fig. 8B). These results argue that a novel allele of *sypE* is found in group A strains, and this allele results in more substantial biofilm formation than it does in a  $\Delta sypE$  strain.

Our finding that the MB11B1 *sypE* allele promotes biofilm formation bolstered the model that this allele contributes to the ability of MB11B1 to colonize squid indepen-



**FIG 8** MB11B1 *sypE* frameshift leads to an enhanced biofilm phenotype upon SypG overexpression. Shown are spot assays of strains carrying the pKV69 vector or pEAH73 SypG overexpression plasmid. (A) ES114 strain background. Strains lacking SypE produce a wrinkled colony phenotype upon SypG overexpression. Deletion of nucleotide 33 in *sypE* to mimic the group A frameshift led to earlier wrinkling and a more pronounced colony biofilm at 48 h. Strains are MJM1104, MJM3455, MJM3418, MJM3419, MJM3364, and MJM3365. (B) Group A strain MB11B1, which naturally carries a -1 frameshift in *sypE*, exhibits a cohesive phenotype at 48 h with overexpression of SypG. Deletion of *sypE* reduces this phenotype, and repairing the frameshift by addition of a guanosine at nucleotide 33 further reduces the cohesiveness of the spot. Strains are MJM3370, MJM3371, MJM3411, MJM3412, MJM3398, and MJM3399.

dent of RscS. To test this model, we introduced the frameshift into ES114 or corrected the frameshift in MB11B1. We then conducted single-strain colonization assays, and in each case the *sypE* allele alone was not sufficient to alter the overall colonization behavior of the strain (Fig. 9). Therefore, these data suggest that the frameshift in the MB11B1 *sypE* is not sufficient to explain its ability to colonize independently of RscS, and therefore other regions of SypE and/or other loci in the MB11B1 genome contribute to its ability to colonize independently of RscS.

**BinK is active in group A, B, and C strains.** We recently described the histidine kinase BinK, which negatively regulates *syp* transcription and Syp biofilm formation (18). In ES114, overexpression of BinK impairs the ability of *V. fischeri* to colonize. We therefore reasoned that if BinK could function in group A strains and acted similarly to repress Syp biofilm, then overexpression of BinK would reduce colonization of these strains. We introduced the pBinK plasmid (i.e., ES114 *binK* [18]) and asked whether multicopy *binK* would affect colonization. In strain MB11B1, BinK overexpression led to a dramatic reduction in colonization of the group A strain MB11B1.

We attempted to ask the same question for group C strain SR5, but the pES213origin plasmids were not retained during squid colonization. Therefore, we instead asked whether deletion of BinK, a negative regulator of ES114 colonization, has a



**FIG 9** *sypE* –1 frameshift allele is not sufficient to affect colonization ability. The indicated strains were assayed in a single-strain colonization assay. Gray boxes denote alleles distinct from their wild-type background. Frameshift "fs" refers to alleles, relative to an ES114 reference, that lack *rscS* nucleotide A1141 or that lack *sypE* nucleotide G33. The wild-type MB11B1 strain contains natural frameshifts in these loci, and the ES114 nt33:: $\Delta$ G allele was constructed. Addition back of the nucleotide in MB11B1 syp*E* is denoted (+). Hatchling squid were inoculated with 6.8 × 10<sup>2</sup> to 8.4 × 10<sup>2</sup> CFU/ml bacteria (MB11B1 background) or 4.0 × 10<sup>3</sup> to 5.4 × 10<sup>3</sup> CFU/ml bacteria (ES114 background), washed at 3 h and 24 h, and assayed at 48 h. Each dot represents an individual squid. The limit of detection is represented by the dashed line, and the bars represent the median for each set. Strains are MJM1100, MJM33010, MJM3397. Statistical comparisons were done with the Mann-Whitney test. ns, not significant.

comparable effect in SR5 (18). We deleted *binK* and observed a 2.4-fold competitive advantage during squid competition (Fig. 10B), arguing that BinK in this group C strain is active and performs an inhibitory function similar to that in ES114.

We next examined the colony biofilm phenotype for strains lacking BinK. The MB11B1  $\Delta binK$  strain exhibited a mild colony biofilm phenotype at 48 h, as evidenced by the cohesiveness of the spot when disrupted with a toothpick (Fig. 10C). The colonies also exhibited an opaque phenotype. In a minority of experimental replicates, wrinkled colony morphology was evident at 48 h, but in all samples wrinkled colony morphology was evident at 48 h, but in all samples wrinkled colony morphology was visible at 7 days (data not shown). The SR5  $\Delta binK$  strain also exhibited slightly elevated biofilm morphology at 48 h, although the cells were not as cohesive as those of the MB11B1  $\Delta binK$  strain (Fig. 10C). Together, the results shown in Fig. 10 argue that BinK, a factor that has been characterized as a negative regulator of Syp biofilm, plays similar roles in group A and group C strains and has a widely conserved function across the *V. fischeri* evolutionary tree.

## DISCUSSION

This study examines regulation of a beneficial biofilm that is critical to host colonization specificity in *V. fischeri*. The Syp biofilm was discovered fourteen years ago and has been characterized extensively for its role in facilitating squid colonization by *V. fischeri*. This work establishes that the *syp* locus is required broadly across squid symbionts, and it uncovers three groups of *V. fischeri* that use different regulatory programs upstream of the *syp* locus. A simplified phylogenetic tree showing key features of squid symbionts in these three groups is shown in Fig. 11.

There are three nested evolutionary groups of *V. fischeri* that have been described separately in the literature, and here we formalize the nomenclature of groups A, B, and C. Group A is a monophyletic group, as are groups AB and ABC (Fig. 1). This work provides evidence that squid symbionts in each group have a distinct biofilm regulatory architecture. Most *V. fischeri* isolates that have been examined from the ancestral group C cannot colonize squid; however, those that can colonize do so without the canonical biofilm regulator RscS. We show that the known targets of RscS regulation, genes in the *syp* biofilm locus, are nonetheless required for squid colonization by this group. Group B strains include the well-characterized ES114 strain, which requires RscS



**FIG 10** BinK is active in groups A, B, and C. (A) Overexpression of pBinK inhibits colonization in group A strain MB11B1. Hatchling squid were inoculated with  $3.6 \times 10^3$  to  $6.8 \times 10^3$  CFU/ml bacteria, washed at 3 h and 24 h, and assayed at 48 h. Each dot represents an individual squid. The limit of detection is represented by the dashed line, and the bars represent the median of each set. The vector control is pVSV104. Strains are MJM1782, MJM2386, MJM2997, and MJM2998. (B) Deletion of *binK* confers a colonization defect in group C strain SR5. Strains are MJM1125 and MJM3571. The mean inoculum was 7.2 × 10<sup>3</sup> CFU/ml, and the median competitive index (Cl) was 0.38 (i.e., 2.4-fold advantage for the mutant). (C) Deletion of the native *binK* in MB11B1 yielded opaque and cohesive spots, which are stronger phenotypes than we observe in ES114. Strains are MJM1100, MJM2251, MJM1130, MJM3084, MJM2997, and MJM2998. Statistical comparisons were done with the Mann-Whitney test. \*\*\*\*, *P* < 0.0001.

and the *syp* locus to colonize squid. Group A strains differ phenotypically and behaviorally from the sister group B strains (30), and we demonstrate that these strains have altered biofilm regulation. Group A strains have a frameshift in *rscS* that renders it nonfunctional and a 1-bp deletion in *sypE*, and we provide evidence that the *sypE* allele promotes biofilm development in the absence of RscS. Additionally, we note that the



**FIG 11** Summary model of distinct modes of biofilm formation in squid-colonizing *V. fischeri*. The phylogenetic tree is simplified from Fig. 1 and illustrates key features of squid symbionts in the three groups. Shown are divergent aspects (RscS and SypE) and conserved regulation (BinK). In all groups, the *syp* exopolysaccharide locus is required for squid colonization.

*sypE* frameshift is not present in SR5, arguing for distinct modes of biofilm regulation in groups A, B, and C.

At the same time, this study provides evidence that some aspects of biofilm regulation are conserved in diverse squid symbionts, such as the effects of the strong biofilm negative regulator BinK. Published data indicate that evolved BinK alleles can alter colonization of H905 (group B) and MJ11 (group C), and that a deletion of MJ11 *binK* leads to enhanced colonization (20). Our experiments in Fig. 10 show a clear effect for BinK in all three phylogenetic groups. We also observed responsiveness to RscS overexpression in all squid symbionts examined (Fig. 2). CG101 was the only *V. fischeri* strain examined that did not exhibit a colony biofilm in response to RscS overexpression. CG101 was isolated from the Australian fish *Cleidopus gloriamaris*; based on these findings, we suspect that the strain does not have an intact *syp* locus or otherwise has divergent biofilm regulation.

It remains a formal possibility that the entire *syp* locus is not required in group A or group C but instead that only one or a subset of genes in the locus is needed. Aggregation in squid mucus has been observed for the group A strain MB13B2, and this aggregation is dependent on *sypQ* (40). In our data we note that group A strains were completely unable to colonize in the absence of the *syp* locus, unlike the tested group B and C strains that exhibited reduced colonization in their respective mutants (Fig. 3 and 6). Therefore, the simplest explanation is that the *syp* locus is required to function in divergent strains in a manner similar to how it is used in ES114. We think that the ability to completely delete the *syp* locus is an effective way to determine whether the locus is required for specific phenotypes, and our strains are likely to be useful tools in probing Syp protein function in diverse *V. fischeri* isolates.

It is intriguing to speculate as to how the two frameshifts in the group A strains arose and why the nonfunctional RscS is tolerated in this group. One possible scenario is that the group A strains acquired a new regulatory input into the Syp pathway, and that the presence of this new regulator bypassed the requirement for RscS. We note that comparative genomic analysis of Hawaiian D (dominant)-type strains, which largely overlap group A, revealed an additional 250 kb of genomic DNA not seen in other isolates, yielding a large cache of genes that could play a role in this pathway (33). A related possibility is that rscS-independent colonization results from altered regulation of the syp locus due to changes in regulators (e.g., SypF) or sites that are conserved with group B. An additional possibility is that the sypE frameshift enabled group A strains to colonize independently of rscS. Given that correction of this frameshift in MB11B1 does not significantly affect colonization ability (Fig. 9), this sequence of events seems less likely, and we expect that another regulator in MB11B1 is required for the RscSindependent colonization phenotype. There is evidence that under some conditions LuxU can regulate the syp biofilm (41), and as this protein is conserved in V. fischeri, it may play an important role in group A or group C.

Results from two experimental conditions suggest that the group A strains have an elevated baseline level of biofilm formation. Our data indicate that in the absence of BinK or upon SypG overexpression, MB11B1 colonies exhibit strong cohesion under conditions in which ES114 does not (Fig. 8 and 10). Furthermore, we note that the group A strain MB11B1, when lacking BinK, also exhibits a darker, or more opaque, colony phenotype (Fig. 10). This phenotype has been observed in some ES114 mutants (16) but not in the corresponding ES114  $\Delta binK$  strain (Fig. 10). The entire colonization lifecycle likely requires a balance between biofilm formation/cohesion and biofilm dispersal, and these data argue that group A strains are more strongly tilted toward the biofilm-producing state. There is evidence that strains lacking BinK exhibit a colonization advantage in the laboratory (18, 20), suggesting that this strategy of more readily forming biofilms provides a fitness advantage in nature. At the same time, the biofilm negative regulator BinK is conserved among *V. fischeri* strains examined (including MB11B1 [Fig. 10]), arguing that there is a benefit to reducing biofilm formation under some conditions.

Our study provides hints as to the role of SypE in MB11B1 and other group A strains.

In ES114, the C terminus is a PP2C serine kinase domain, whereas the N terminus of SypE is an RsbW serine phosphatase domain. SypE acts to phosphorylate and dephosphorylate SypA Ser-56, with the unphosphorylated SypA being the active form to promote biofilm development (17). The balance between SypE kinase and phosphatase is modulated by a central two-component receiver domain (17). Our data that the MB11B1 *sypE* allele promotes biofilm formation suggest that the protein is tilted toward the phosphatase activity. In MB11B1, the frameshift early in *sypE* suggests that there is a different (later) start codon. An alternate GTG start codon in MB11B1 occurs corresponding to codon 18 in ES114 *sypE* (Fig. 7), and this is likely the earliest start for the MB11B1 polypeptide. We attempted to directly identify the SypE N terminus by mass spectrometry, yet we could not identify the protein from either strain. Additional study is required to elucidate how MB11B1 SypE acts to promote biofilm formation.

*V. fischeri* strains are valuable symbionts in which to probe the molecular basis to host colonization specificity in animals (22, 25, 26). A paradigm has emerged in which biofilm formation through the RscS-Syp pathway is required for squid colonization but not for fish colonization. This study affirms a role of the Syp biofilm but at the same time points out divergent (RscS-independent) regulation in group C and group A isolates. In another well-studied example of symbiotic specificity, rhizobial Nod factors are key to generating specificity with the plant host, yet strains have been identified that do not use this canonical pathway (42, 43). Future work will elaborate on these RscS-independent pathways to determine how noncanonical squid colonization occurs in diverse natural isolates.

## **MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** *V. fischeri* and *Escherichia coli* strains used in this study can be found in Table 1. Plasmids are listed in Table 2. *E. coli* strains, used for cloning and conjugation, were grown in Luria-Bertani (LB) medium (25 g Difco LB broth [BD] per liter). *V. fischeri* strains were grown in Luria-Bertani salt (LBS) medium (25 g Difco LB broth [BD], 10 g NaCl, and 50 ml 1 M Tris buffer, pH 7.0, per liter). Growth media were solidified by adding 15 g Bacto agar (BD) per liter. When necessary, antibiotics (Gold Biotechnology) were added at the following concentrations: tetracycline, 5 µg/ml for *V. fischeri*; erythromycin, 5 µg/ml for *V. fischeri*; kanamycin, 50 µg/ml for *E. coli* and 100 µg/ml for *V. fischeri*; and chloramphenicol, 25 µg/ml for *E. coli*, 2.5 to 5 µg/ml for group B *V. fischeri*, and 1 to 2.5 µg/ml for group A *V. fischeri*. The two MB11B1/pKV69 strains listed reflect two separate constructions of this strain, although we have not identified any differences between them.

**Phylogenetic analysis.** Phylogenetic reconstructions assuming a tree-like topology were created with three methods—maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference (Bayes)—as previously described (22, 30). Briefly, MP reconstructions were performed by treating gaps as missing, searching heuristically using random addition, tree-bisection reconnection with a maximum of 8 for swaps, and swapping on best only with 1,000 repetitions. For ML and Bayesian analyses, likelihood scores of 1,500+ potential evolutionary models were evaluated using both the corrected and uncorrected Akaike information criterion, the Bayesian information criterion, and decision theory (performance-based selection) as implemented by jModelTest2.1 (44). For all information criteria, the most optimal evolutionary model was a symmetric model with a proportion of invariable sites and a gamma distribution of rate heterogeneity (SYM+1+ $\Gamma$ ).

ML reconstruction was implemented via PAUP\*4.0a163 (45) by treating gaps as missing, searching heuristically using random addition, tree-bisection reconnection for swaps, and swapping on best only with 1,000 repetitions. Bayesian inference was done by invoking the nst=6, rates=invgamma, and statefreqpr=fixed(equal) settings in the software package MrBayes3.2.6 (46). The Metropolis-coupled Markov chain Monte Carlo (MCMCMC) algorithm, used to estimate the posterior probability distribution for the sequences, was set up with temp=0.2 and one incrementally "heated" chain with three "cold" chains; these four chains were replicated two times per analysis to establish convergence of the Markov chains (i.e., "stationarity," as defined in reference 47 and interpreted previously in reference 30). For this work, stationarity was achieved after approximately 50,000 samples (5,000,000 generations) were collected, with 25% discarded. The  $\sim$ 37,500 samples included were used to construct a 50% majority-rule consensus tree from the sample distribution generated by MCMCMC and assess clades' posterior probabilities. For ML and MP analyses, the statistical confidence in the topology of each reconstruction was assessed using 1,000 bootstrap replicates. Phylogenetic trees were visualized with FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree); the final tree was edited for publication with Inkscape 0.91 (http://inkscape.org/) and GIMP 2.8.22 (http://www.gimp.org/).

DNA synthesis and sequencing. Each of the primers listed in Table 3 was synthesized by Integrated DNA Technologies (Coralville, IA). Full inserts from all cloned constructs were verified by Sanger DNA sequencing through ACGT, Inc., via the Northwestern University Feinberg School of Medicine NUSeq Core Facility or the University of Wisconsin—Madison Biotechnology Center. Sequence data were analyzed with SeqMan Pro (DNAStar software), SnapGene (GSL Biotech), and Benchling.

## TABLE 1 Bacterial strains

| Ctuain     | Canatuma   | Source or        |
|------------|--|------------------|
| V fischori | Genotype   | reference(s)     |
| MIM1059    | M111   | 25 53            |
| MJM1100    | F\$114   | 54               |
| MJM1104    | ES114 (MJM1100)/pKV69                            | This study       |
| MJM1106    | ES114 (MJM1100)/pKG11                            | This study       |
| MJM1109    | MJ11 (MJM1059)/pKV69                             | This study       |
| MJM1111    | MJ11 (MJM1059)/pKG11                             | This study       |
| MJM1114    | MJ12   | 53               |
| MJM1115    | CG101  | 25               |
| MJM1117    | ES213  | 55               |
| MJM1119    | EM18   | 25, 53           |
| MJM1120    | EM24   | 53, 56           |
|            |  | 53               |
| MIM1122    | SB2  | 57<br>74         |
| MIM1125    | SA1  | 24               |
| MJM1127    | KB1A97   | 29               |
| MJM1128    | KB2B1  | 29               |
| MJM1129    | KB5A1  | 29               |
| MJM1130    | MB11B1   | 29               |
| MJM1136    | EM17   | 56               |
| MJM1147    | тјаро.6.1  | 22               |
| MJM1149    | mjapo.7.1  | 22               |
| MJM1151    | mjapo.8.1  | 22               |
| MJM1153    | mjapo.9.1  | 22<br>This study |
|            | mjapo.8.1/pKV69                                  | This study       |
|            | MI12 (MIM1114)/pKV69                             | This study       |
| MIM1230    | MI12 (MIM1114)/pKG11                             | This study       |
| MJM1240    | SR5 (MJM1125)/pKV69                              | This study       |
| MJM1241    | SR5 (MJM1125)/pKG11                              | This study       |
| MJM1242    | SA1 (MJM1126)/pKV69                              | This study       |
| MJM1243    | SA1 (MJM1126)/pKG11                              | This study       |
| MJM1244    | MB11B1 (MJM1130)/pKV69                           | This study       |
| MJM1245    | MB11B1 (MJM1130)/pKG11                           | This study       |
| MJM1246    | EM17 (MJM1136)/pKV69                             | This study       |
| MJM1247    | EM17 (MJM1136)/pKG11<br>KB1407 (MJM1137)/=K/KG   | This study       |
| MIM1254    | KBTA97 (MJMTT27)/pKV69<br>KB1A07 (MJMTT27)/pKC11 | This study       |
| MIM1256    | KB2R1 (MIM1128)/pKV69                            | This study       |
| MJM1257    | KB2B1 (MJM1128)/pKG11                            | This study       |
| MJM1258    | KB5A1 (MJM1129)/pKV69                            | This study       |
| MJM1259    | KB5A1 (MJM1129)/pKG11                            | This study       |
| MJM1260    | ES213 (MJM1117)/pKV69                            | This study       |
| MJM1261    | ES213 (MJM1117)/pKG11                            | This study       |
| MJM1266    | EM18 (MJM1119)/pKV69                             | This study       |
| MJM1267    | EM18 (MJM1119)/pKG11                             | This study       |
| MJM1268    | EM24 (MJM1120)/pKV69                             | This study       |
| MJM1269    | EM24 (MJM1120)/pKG11                             | This study       |
|            | EM30 (MIM1121)/pKV09<br>EM30 (MIM1121)/pKC11     | This study       |
| MIM1277    | miano 6.1 (MIM1147)/pKV69                        | This study       |
| MJM1272    | miapo.6.1 (MIM1147)/pKG11                        | This study       |
| MJM1274    | mjapo.7.1 (MJM1149)/pKV69                        | This study       |
| MJM1275    | mjapo.7.1 (MJM1149)/pKG11                        | This study       |
| MJM1276    | <i>mjapo</i> .9.1 (MJM1151)/pKV69                | This study       |
| MJM1277    | mjapo.9.1 (MJM1151)/pKG11                        | This study       |
| MJM1278    | CG101 (MJM1115)/pKV69                            | This study       |
| MJM1279    | CG101 (MJM1115)/pKG11                            | This study       |
| MJM1280    | WH1 (MJM1122)/pKV69                              | This study       |
| MJM1281    | WH1 (MJM1122)/pKG11                              | This study       |
|            | EST14 (NUNTTOU) PVSV104                          | 10<br>20         |
| M IM2226   | FS114 (MIM1100)/nMIM33                           | 27<br>This study |
|            | ככואנואון/(סטדדאונאו) דרדכ                       | inis study       |

(Continued on next page)

|         |   | Source or        |
|---------|---|------------------|
| Strain  | Genotype  | reference(s)     |
| MJM2251 | ES114 (MJM1100) ΔbinK   | 18               |
| MJM2386 | ES114 (MJM1100)/pBinK   | This study       |
| MJM2997 | MB11B1 (MJM1130)/pVSV104  | This study       |
| MJM2998 | MB11B1 (MJM1130)/pBinK  | This study       |
| MJM2999 | ES213 (MJM1117)/pVSV104   | This study       |
| MJM3000 | ES213 (MJM1117)/pBinK   | This study       |
| MIM3010 | $FS114 (MIM1100) \Lambda rsc S$   | This study       |
| MJM3017 | FS213 (MJM1117) ArscS   | This study       |
| MJM3042 | $MB15A4 (MJM2114) \Delta rscS$  | This study       |
| MIM3046 | $MB11B1 (MJM1130) \Delta rscS$  | This study       |
| MIM3062 | FS114 (MIM1100) Asyn  | This study       |
| MIM3065 | MB11B1 (MJM1130) Asyn   | This study       |
| MIM3068 | FS213 (MIM1117) Asyn  | This study       |
| MJM3071 | $MB15A4 (MJM2114) \Lambda svp$  | This study       |
| MJM3084 | MB11B1 (MJM1130) <i>AbinK</i>   | This study       |
| MIM3354 | FS114 (MIM1100) svpE(ntG33A)  | This study       |
| MIM3364 | FS114 (MJM1100) sypE(ntG33A)/pKV69                                      | This study       |
| MIM3365 | FS114 (MIM1100) sypE(ntG33A)/pEAH73                                     | This study       |
| MIM3370 | MB11B1 (MJM1130)/pKV69  | This study       |
| MJM3371 | MB11B1 (MJM1130)/pEAH73   | This study       |
| MJM3394 | $FS114 (MJM1100) \Lambda rscS svpF(ntG33\Lambda)$                       | This study       |
| MIM3397 | MB11B1 (MIM1130) svpE(nt33G)  | This study       |
| MIM3398 | MB11B1 (MJM1130) sypE(nt33::G)/pKV69                                    | This study       |
| MJM3399 | MB11B1 (MJM1130) sypE(nt33::G)/pEAH73                                   | This study       |
| MIM3410 | $MB11B1 (MIM1130) \Delta svpF$  | This study       |
| MIM3411 | MB11B1 (MIM1130) $\Delta sypE$<br>MB11B1 (MIM1130) $\Delta sypE$ /pKV69 | This study       |
| MIM3412 | MB11B1 (MJM1130) $\Delta sypE/pEAH73$                                   | This study       |
| MIM3417 | FS114 (MIM1100) AsynF   | This study       |
| MIM3418 | $ES114$ (MJM1100) $\Delta sypE$<br>ES114 (MJM1100) $\Delta sypE$ /pKV69 | This study       |
| MJM3419 | $FS114$ (MJM1100) $\Delta sypE/pEAH73$                                  | This study       |
| MJM3423 | $FS114 (MJM1100) \Lambda rsc S \Lambda symF$                            | This study       |
| MJM3455 | ES114 (MJM1100)/pEAH73  | This study       |
| MJM3501 | SB5 (MJM1125) $\Lambda$ svp   | This study       |
| MJM3751 | SR5 (MJM1125) <i>\DeltabinK</i> ::erm                                   | This study       |
|         |   |                  |
| E. coli |   |                  |
| MJM534  | CC118 λpir/pEVS104  | 58               |
| MJM537  | DH5 $\alpha$ $\lambda$ pir  | Laboratory stock |
| MJM570  | DH5α/pEVS79   | 58               |
| MJM580  | DH5 $\alpha$ $\lambda$ pir/pVSV104                                      | 59               |
| MJM581  | DH5α/pKV69  | 60               |
| MJM583  | DH5a/pKG11  | 15               |
| MJM639  | XL1-Blue/pMJM33   | This study       |
| MJM658  | BW23474/pEVS107   | 61               |
| MJM2384 | DH5 $\alpha$ $\lambda$ pir/pBinK  | 18               |
| MJM2540 | KV5264/pEAH73   | 39               |
| MJM3008 | DH5 $\alpha$ /pEVS79- $\Delta$ rscS[MJM1100]                            | This study       |
| MJM3014 | DH5 $\alpha$ $\lambda$ pir/pEVS79- $\Delta$ rscS[MJM1117]               | This study       |
| MJM3039 | DH5 $\alpha$ $\lambda$ pir/pEVS79- $\Delta$ rscS[MJM2114]               | This study       |
| MJM3043 | DH5 $\alpha$ $\lambda$ pir/pEVS79- $\Delta$ rscS[MJM1130]               | This study       |
| MJM3060 | NEB5α/pEVS79-Δ <i>syp</i> [MJM1100]                                     | This study       |
| MJM3063 | NEB5α/pEVS79-Δ <i>syp</i> [MJM1130]                                     | This study       |
| MJM3066 | DH5 $\alpha$ $\lambda$ pir/pEVS79- $\Delta$ syp[MJM1117]                | This study       |
| MJM3069 | DH5α λpir/pEVS79-Δ <i>syp</i> [MJM2114]                                 | This study       |
| MJM3082 | NEB5α/pEVS79-Δ <i>binK</i> [MJM1130]                                    | This study       |
| MJM3287 | NEB5α/pHB1  | This study       |
| MJM3338 | DH5 $\alpha$ $\lambda$ pir/pEVS107- <i>sypE</i> [MJM1130](nt33::G)      | This study       |
| MJM3340 | DH5α λpir/pEVS107- <i>sypE</i> [MJM1100](ntG33Δ)                        | This study       |
| MJM3351 | NEB5α/pEVS79- <i>sypE</i> [MJM1130](nt33::G)                            | This study       |
| MJM3352 | NEB5α/pEVS79-sypE[MJM1100](ntG33Δ)                                      | This study       |
| MJM3409 | NEB5α/pEVS79-Δ <i>sypE</i> [MJM1130]                                    | This study       |
| MJM3416 | NEB5α/pEVS79-Δ <i>sypE</i> [MJM1100]                                    | This study       |

## TABLE 2 Plasmids

| Plasmid   | Relevant genotype <sup>a</sup>                                   | Source or reference |
|---|--|---------------------|
| pEVS79  | Vector backbone (Cam <sup>r</sup> ) for deletion construction    | 58                  |
| pKV69   | Vector backbone (Cam <sup>r</sup> /Tet <sup>r</sup> )            | 60                  |
| pKG11   | pKV69 carrying <i>rscS1</i>                                      | 15                  |
| pMJM33  | pKG11 <i>rscS1</i> (ntA1141::Δ)                                  | This study          |
| pEVS104   | Conjugation helper plasmid (Kan <sup>r</sup> )                   | 58                  |
| pEVS107   | Mini-Tn7 mobilizable vector (Erm' Kan')                          | 61                  |
| pEAH73  | pKV69 carrying sypG from ES114                                   | 39                  |
| pVSV104   | Complementation vector (Kan <sup>r</sup> )                       | 59                  |
| pBinK   | pVSV104 carrying <i>binK</i> from MJM1100                        | 18                  |
| pHB1  | pUC19 FRT-erm-FRT  | This study          |
| pEVS79-ΔrscS[MJM1100]                                     | pEVS79 carrying 1.6-kb US/1.6-kb DS of rscS from MJM1100         | This study          |
| pEVS79-Δ <i>rscS</i> [MJM1117]                            | pEVS79 carrying 1.6-kb US/1.6-kb DS of rscS from MJM1117         | This study          |
| pEVS79-ΔrscS[MJM2114]                                     | pEVS79 carrying 1.6-kb US/1.6-kb DS of rscS from MJM2114         | This study          |
| DH5 $\alpha$ $\lambda$ pir/pEVS79- $\Delta$ rscS[MJM1130] | pEVS79 carrying 1.6-kb US/1.6-kb DS of rscS from MJM1130         | This study          |
| pEVS79-Δ <i>syp</i> [MJM1100]                             | pEVS79 carrying 1.6-kb US of sypA/1.6-kb DS of sypR from MJM1100 | This study          |
| pEVS79-Δ <i>syp</i> [MJM1130]                             | pEVS79 carrying 1.6-kb US of sypA/1.6-kb DS of sypR from MJM1130 | This study          |
| pEVS79-Δ <i>syp</i> [MJM1117]                             | pEVS79 carrying 1.6-kb US of sypA/1.6-kb DS of sypR from MJM1117 | This study          |
| pEVS79-Δ <i>syp</i> [MJM2114]                             | pEVS79 carrying 1.6-kb US of sypA/1.6-kb DS of sypR from MJM2114 | This study          |
| pEVS79-Δ <i>binK</i> [MJM1130]                            | pEVS79 carrying 1.6-kb US/1.6-kb DS of <i>binK</i> from MJM1130  | This study          |
| pEVS107- <i>sypE</i> [MJM1130](nt33::G)                   | pEVS107 carrying the sypE(nt33::G) allele from MJM1130           | This study          |
| pEVS107- <i>sypE</i> [MJM1100](ntG33Δ)                    | pEVS107 carrying the <i>sypE</i> (ntG33Δ) allele from MJM1100    | This study          |
| pEVS79- <i>sypE</i> [MJM1130](nt33::G)                    | pEVS79 carrying the sypE(nt33::G) allele from MJM1130            | This study          |
| pEVS79- <i>sypE</i> [MJM1100](ntG33∆)                     | pEVS79 carrying the <i>sypE</i> (ntG33Δ) allele from MJM1100     | This study          |
| pEVS79-Δ <i>sypE</i> [MJM1130]                            | pEVS79 carrying 1.6-kb US/1.6-kb DS of sypE from MJM1130         | This study          |
| pEVS79-Δ <i>sypE</i> [MJM1100]                            | pEVS79 carrying 1.6-kb US/1.6-kb DS of <i>sypE</i> from MJM1100  | This study          |

<sup>a</sup>US, upstream; DS, downstream.

**Construction of gene deletions.** Deletions in *V. fischeri* strains ES114 and MB11B1 were made according to the laboratory's gene deletion protocol (https://doi.org/10.5281/zenodo.1470836). In brief, 1.6-kb upstream sequence and 1.6-kb downstream sequence of the targeted gene or locus were cloned into linearized plasmid pEVS79 (amplified with primers pEVS79\_rev\_690/pEVS79\_for\_691) using Gibson Assembly (NEBuilder HiFi DNA assembly cloning kit) with the primer combinations listed in Table S1 in the supplemental material. The Gibson mix, linking the upstream and downstream flanking regions, was transformed into *E. coli* on plates containing 5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactopyranoside (X-Gal) with several white colonies selected for further screening by PCR using primers flanking the upstream junction (Table 3 and Table S1). The resulting plasmid candidate was confirmed by sequencing and conjugated into the *V. fischeri* recipient by triparental mating with helper plasmid pEVS104, selecting for the chloramphenicol resistance of the plasmid backbone. *V. fischeri* colonies were first screened for single recombination into the chromosome by maintaining antibiotic resistance in the absence of selection and then screened for double recombination by the loss of both the antibiotic resistance cassette and the gene/locus of interest. Constructs were verified by PCR (Table 3) and sequencing.

Deletion of SR5 binK was conducted using splicing by overlap extension PCR (SOE-PCR) and natural transformation (method modified from reference 48). Oligonucleotides binK-F1 and binK-R1-LUH and oligonucleotides binK-F2-RUH and binK-R2 were used in a PCR with MJM1125 (SR5) genomic DNA as the template to amplify DNA fragments containing  $\sim$ 1 kb of sequence upstream and downstream, respectively, relative to binK. Using SOE-PCR, these fragments were fused on either side to a third DNA fragment containing an Erm<sup>r</sup> cassette, which was amplified using pHB1 as the template and oligonucleotides HB41 and HB42. We then used natural transformation with pLostfoX (49) to insert this mutagenic DNA into MJM1125, where the flanking sequences guide the Erm<sup>r</sup> cassette to replace *binK*, generating the desired gene deletion. Candidate SR5 AbinK mutants were selected after growth on LBS-Erm5 plates. Oligonucleotides binK-F1 and binK-R2 as well as HB8 and binK-FO were used to screen candidates for the correct deletion scar by PCR, and oligonucleotides KMB\_036 and KMB\_037 were used to confirm the absence of binK in the genome. The deletion was verified by Sanger sequencing with primers HB8, HB9, HB42, and HB146. The base plasmid pHB1 contains an erythromycin resistance cassette flanked by FLP recombination target sites and was constructed using oligonucleotides HB23 and HB39 with gBlock gHB1 (sequence in File S1) (Integrated DNA Technologies, Inc.) as the template to amplify the Erm<sup>r</sup> cassette flanked by HindIII and BamHI sites, which was then cloned into the corresponding site in pUC19.

For most constructs, the deleted genetic material was between the start codon and last six amino acids (50), with two exceptions:  $\Delta sypE$  in MJM1130 included the ATG that is two amino acids upstream of the predicted start codon but not the canonical start codon, and the  $\Delta binK$  alleles in MJM1117, MJM1130, and MJM2114, which were constructed to be equivalent to MJM2251 ( $\Delta binK$  in ES114) (18). The  $\Delta binK$  alleles in these strains include the start codon, the next six codons, two codons resulting from ATCGAT (Clal site), and the last three codons for a predicted 12-amino-acid peptide.

**Construction of sypE alleles.** To create  $sypE(ntG33\Delta)$  in MJM1100 and sypE(nt33:G) in MJM1130, the single point mutation was created by amplifying the gene in two halves, with the N-terminal portion

## TABLE 3 DNA oligonucleotides for PCR amplification and sequencing

| DAT_015F ACCAMAGAGCAGTACAGCAGTATAT<br>ES114_DRAFT GALARDAGCAGTACAGCAGTATAT<br>ES114_DRAFT GALARDAGCAGTACAGAGCAGTATAT<br>ES114_DRAFT GALARDAGCAGTACAGAGCAGGAGTATATATAGCAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTA  | Primer name            | Sequence <sup>a</sup> (5' to 3')                  |
|---|------------------------|---|
| ES14_DS_wr     GGATCTTAGATCACAGC       ES14_indel_rev     GTGTCTGATAGCCGGA       ES14_indel_rev     GTGTCTGATAGCGCGA       ES14_indel_rev     GTGTCTGATAGCGCGA       ES14_indel_rev     GTGTCTGATAGCGCGA       ES14_indel_rev     GTGTCTGATAGCGCTAA       ES14_indel_rev     GTGTCTGATAGCACATCGGA       ES14_indel_rev     GTGTCTGATAGCACATCATGCGA       ES14_indel_rev     gapgrapegaraganaGAGCCTTTAAATCATCGCGCACACACACACACACACACAC  | DAT_015F               | ACCAAGAAGCAGTACGACGATTAT                          |
| ESI-H_indel_for       TIACTITITICAGATACAMAGECE         ESI-H_indel_for       GTIGTEGTATAGTGEGTA         ESI-H_indel_for       GTIGTEGTATAGTGEGTA         ESI-H_indel_for       GEGETIAAATAATAGTGEGTA         Gib_ESI-H_indel_for       GEGETIAAATAATAGTGEGTA         Gib_ESI-H_indel_for       GEGAGTAAAATAGTGEGTA         Gib_ESI-H_indel_for       GeaggattegataGCGTATACATAGTCATATATAC         Gib_ESI-H_indel_for       GeaggattegataGCGTATACATATATAC         Gib_ESI-H_indel_for       GeaggattegataGCGTTATAATATAG         Gib_ESI-H_indel_for       geaggattegataGCGCTATAAATATAG         Gib_ESI-H_indel_for       geaggattegataGCGCTATAAATATAG         Gib_ESI-H_indel_for       geaggattegataGCGCTATAAATATAG         Gib_ESI-H_indel_for       geaggattegataGCGCTATAAATATAG         Gib_ESI-H_indel_for       geaggattegataGCCCAATAGTAGCCCAATATAG         Gib_ESI-H_indel_for       geaggattegataGCCCCAATAGTAGCGCCAATAGTAGG         Gib_ESI-H_indel_for       geaggattegataGCCCCCAATAGTAGCGCCAATAGTAGG         Gib_ESI-H_indel_for       geaggattegataGCCCCCAATAGTAGGTAGTAGTAGGTAGGTAGGTAGTAGTAGG         Gib_ESI-H_indel_for       geaggattegataGCCCCCAATAGTAGGCGCCAATAGTAGG         Gib_ESI-H_indel_for       geaggattegataGCCCCCAATAGTAGGGGGTATATTAG         Gib_ESI-H_indel_for       geaggattegataGCCCCAATGTCGGCGGTATTATTAG         Gib_ESI-H_indel_for   | ES114_DS_ver           | GGATGTTTTAGATGTTGCGG                              |
| ESI14_US_VP       CITCHICATAGTECOTOA         ESI14_US_VP       CICAACTAGAAACTECC         for_ver_syste       CCGGCTCAAACTATGCAG         Gib_BSI14_bhick_DS_for       galagtagattagatacagaCTTCAATACTGATTITAATCC         Gib_BSI14_bhick_DS_rev       galagtagattagatacagaCTCAAACTGTGACTAACAACTGACTITTAATCC         Gib_BSI14_bhick_US_rev       attacagattGGATGAATAATGATTTAATTCCTAAACTGACTITTAATCC         Gib_BSI14_bhick_US_rev       attacagattGGATGAACAATGACAATTAATTATTTATTACTAAAAAAC         Gib_BSI14_spit_US_S_rev       attacagattGGATGAAGAAGTGACAAAAACC         Gib_BSI14_spit_US_S_rev       galagtagattGGATGACAATTAAAAAC         Gib_BSI14_spit_US_S_rev       galagtagattGGATGAGAGAAAACCGTGACCAATTAAAAAC         Gib_BSI14_spit_US_rev       galagtagattGGATGACAACTCCACCCAATTAAAAAC         Gib_BSI14_spit_US_rev       galagtattagattGGTGAGATAAAAAAC         Gib_BSI14_spit_US_rev       galagtattagattGGTGAGATAAAAAAC         Gib_BSI14_spit_US_rev       galagtattagattGGTGAATAAAAAAC         Gib_BSI14_spit_US_rev       galagtattagattGGTGTAAGTAGCCACCAATTAAAAAAC         Gib_BSI14_spit_US_rev       galagtattagattCGGATAATTAAAAAC         Gib_BSI14_spit_US_rev       galagtattagattCGGATAATTAAAAAC         Gib_BSI14_spit_US_rev       galagtattagattCGGATTAATTACCCACTGTG         Gib_BSI14_spit_US_rev       galagtattagattCGGATTAAATAGCACCTGTG         Gib_BSI14_spit_US_rev   | ES114_indel_for        | TTACTTTTTCAGATACAAAGCCC                           |
| ESI14_US yer         ATCAACTCAAGAAATCTCCCC           Gib, ESI14_bink, DS, for         attatagatGCGTATACTATGCAG           Gib, ESI14_bink, DS, rev         geoggattagataGCGTATACTATGCAG           Gib, ESI14_bink, DS, rev         geoggattagataGCGTATACTATTGCAG           Gib, ESI14_bink, US, rev         geoggattagataGCGTATACTATTATTATCTATATATATATCA           Gib, ESI14_bink, US, rev         geoggattagataGCGTATATATGCATATTATTATCTATATATATATATATAT  | ES114_indel_rev        | GTTGTTCTGATAGTGCGTGA                              |
| for_ver_spiE         CCGCCTCAAACTATICGAG           ibiS114_binK_D5_for         antactogradicoGCTATACATAAATAATCATCATATATAC           ibiS114_binK_D5_for         gaggtactogradicagataCCCTTAAATAATCATCATATATAC           ibiS114_binK_U5_for         gaggtactogradicagataCCCTTAAATATCACATTATATCATAAAAAC           ibiS114_rot_D5_For         gaggtactogradicaGACACAATATATTCATAAAAAC           ibiS114_rot_D5_for         gaggtactogradicaGACCAATATATCACCATTGAC           ibiS114_rot_D5_for         gaggtactogradicaGACCAATATACCATTGACACATGAC           ibiS114_rot_D5_for         gaggtactogradicaGACAATATCACTTGCAGCCACAATACT           ibiS114_rot_D5_for         gaggtactogradicaGACATATAACCGCCAATATC           ibiS114_rot_D5_for         gaggtactogradicaGACATATAACCGCCAATATC           ibiS114_rot_D5_for         gaggtactogradicaGACCCAATATC           ibiS114_rot_D5_for         gaggtactogradicaGACCCAATATC           ibiS114_rot_D5_for         tigtactagCCTAATATACCCCCCAATATC           ibiS114_rot_D5_for         tigtactagCCTAATATAACCCCCCAATATC           ibiS114_rot_D5_for         tigtactagCCTAATATAACCCCCCAATATC           ibiS114_rot_D5_for         tigtactagCCTAATATAACCCCCCAATATCA           ibiS114_rot_D5_for         tigtactagCCTAATATAACCCCCCAATATCACAAAAAAAAAAAAAA   | ES114_US_ver           | ATCAACTCAAGAAACTCCCC                              |
| Gil, ESTi Juink, DS, for         attaskogatGCGTATACATIAATAATGATTCATTATAC           Gil, ESTI Juink, DS, for         geaggattagatasGATCATAATGATTTATTAGC           Gil, ESTI Juink, US, for         geaggattagatasGATCAATACTGGTTTTATAGC           Gil, ESTI Juink, US, for         geaggattagatasGATCAATACTGGATTATAGCAATGAATGATGATGATGATGAGT           Gil, ESTI Juink, US, for         geaggattagattasGAGAGATATGAATGAATGAATGAATGAATGAATGAATGA  | for_ver_sypE           | CCGGCTCAAACTATTGCAG                               |
| Gil-25114-binK_US_for geggpattcgajatcagagTTCA/TACTGTTTTATGC<br>Gib_25114-binK_US_for geggpattcgajatcagagACACTTTAAATCCCTAAC<br>Gib_25114-rsc5_DS_for tatgcagtGACAGATTAATGACCAATTAAAC<br>Gib_25114-rsc5_US_for geggpattcgajatcagagACAAAATCACTATTATTATCATAAAAAC<br>Gib_25114-rsc5_US_for geggpattcgajatcagagACAAAATCACCAATTAAAC<br>Gib_25114-rsc5_US_for geggpattcgajatcagagACAAAATCACCAATTAAAC<br>Gib_25114-rsc5_US_for geggpattcgajatcagagACAAAATCACCAATTAAAC<br>Gib_25114-rsc5_US_rev getattatgcATTAGCTCCTATAAATAGC<br>Gib_25114-rsc5_US_rev gegagaattcgajatcagagACGGTGAATGTAAGCTCAATGG<br>Gib_25114-rsc5_US_rev gegagaattcgajatcagagACGGTGAATGTAAGCTCCAATGG<br>Gib_25114-rsc5_US_rev gegagaattcgajatcagagCGGTGAATGTAAGCTCCAATGG<br>Gib_25114-rsc5_US_rev gegagaattcgajatcatCAAGCTCCAATGG<br>Gib_25114-rsc5_US_rev gegagaattcgajatcaCAAGCACCAATGG<br>Gib_25114-rsc5_US_rev gegagaattcgajatcatCAAGCTCCAATGGAATGCA<br>Gib_25114-rsc5_US_rev gegagaattcgajatcaCAAGCACCACCACTGACGAATGG<br>Gib_25114-rsc5_US_rev gegagaattcgajatCACAGCCACTGAATGTAAGCTCCAATGGA<br>Gib_25114-rsc5_US_rev gegagaattcgajatCACAGCACCACTGACTGACAGCAATGT<br>Gib_25114-rsc5_US_rev gegagagattcgajatCACAGCCACTGAATGTAAGC<br>Gib_25114-rsc5_US_rev gegagagattcgajatGCGAGCACACAATGTCAATGGA<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAGCACCACTGATAACG<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAGCACACTGATAACG<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAATGA<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAATGAAGCACTGATAACG<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAATAAGCCCACTGATAAAGC<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAATGAAGCAATAAGC<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAAAGCAATAAGC<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAAACAATAAGCACTGATGA<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAAAAGCAATAAGC<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAAAAGCAATAAGC<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAAAAGCAATAAGC<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAAAAGCAATAAGC<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAAAAGCAATAAGC<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAAAAGCAATAAGC<br>Gib_25114-rsc5_US_rev gegagaattcgajatGCAAATAATAGCCCAATAAAATG<br>Gib_25129_US_rev geg  | Gib_ES114_binK_DS_for  | attaatcgatGCGTATACATAAATAATGATTCATATAC            |
| Gib ES114 Junk US, for         gegicgarcggatcgataGAGCCTTTAAATCCCTAAC           Gib ES114 Junk US, rev         atgataGAGCATATTATATATCATATAAACAC           Gib ES114 Junk US, rev         gediggatcgataGAGAAGTAGAAAACCAATAAAC           Gib ES114 Junk US, rev         gediggatcgataGAGAAGTAGAAAACCAATAAAC           Gib ES114 Junk US, rev         gediggatcgataGAGAAGTAGAAACCAATAAAC           Gib ES114 Junk US, rev         gediggatcgataGAGAGTAGAAGTAGACGAATAAC           Gib ES114 Junk US, rev         gediggatcgataGAGGAGTAAAACGAATAAG           Gib ES114 Junk US, rev         gediggatCGAGAGTAGAAGTAGAGTAGACGAATAAGA           Gib ES114 Junk US, rev         gediggatCGAGAGTAGAAGTAGT  | Gib_ES114_binK_DS_rev  | gcaggaattcgatatcaagcTTTCAATACTGTGTTTTTATGC        |
| Gib ES114 Junk US, rev         atgrategoArCGATTAATGACATATTAATTACACAATTAAC           Gib ES114 Junk US, EoS, Jon         gaggtacgatacgatacGAGAACAATTAACACCATTAAC           Gib ES114 Junk Junk US, Jon         gaggtacgatacgatacGAGAACAATTAACTGGCATTGG           Gib ES114 Junk Junk US, Jon         gaggtacgatacgatacGAGAATGG           Gib ES114 Junk Junk US, Jon         gaggtacgatacgataCGGTGAATGTAAGCGCAATTAAACG           Gib ES114 Junk Junk US, Jon         gaggtacgatacgataCGGTGAATGTAAGCTCAATGG           Gib ES114 Junk Junk Junk Junk Junk Junk Junk Junk   | Gib_ES114_binK_US_for  | gaggtcgacggtatcgataaGAGCCTTTTAAATCCCCTAAC         |
| Gib _ES14 _ rsc, DS_ For<br>graggattragattagaAAAATAATAAATCATTGCACTG Gib _ES14 _ rsc, US_ rov<br>graggattragattagaAAAATAATACATGTGCACTG Gib _ES14 _ rsc, US_ rov<br>graggattragattagaAAAAATAAATGCATGGACATGAAAGA Gib _ES14 _ rsc, US_ rov<br>graggattragattagaTCGTGAATGTAGAACCAATAGA Gib _ES14 _ rsc, US_ rov<br>graggattaGAATGCTGAAAGAACCAATGCACACCACACGA Gib _ES14 _ rsc, US_ rov<br>graggattaGAATGTAGAAGCACCACACTGCAGAGCAATAGAA Gib _ES14 _ rsc, US_ rov<br>graggattaGAATGTAGACCGACACTCACAGCACCACACGAGCAGAATGT Gib _ES14 _ rsc, US_ rov<br>graggattaGAAGAGCGACACTCACTGCAGCACATAGT Gib _ES14 _ rsc, US_ rov<br>graggattaGAAGAGCGACACTCACTGCAGCACATAGT Gib _ES14 _ rsc, US_ rov<br>graggattaGAAGAGCGACACTCACTGCAGCGCAATAGT Gib _ES14 _ rsc, US_ rov<br>graggattaGAGAAGCGACACTCACTGCAGCGCAATAGT Gib _ES14 _ rsc, US_ rov<br>graggattaGGAGAAGGAGCGACACTCACTGCAGCGCAATAGT Gib _ES14 _ rsc, US_ rov<br>graggattaGGGAGAGGGGCGGTGTGCAGAAGTGCAATAGTGAGCGGCATAATTGG Gib _ES14 _ rsc, US_ rov<br>graggattaGGGAGGAGGCGCGTGTGCAGAAGTGGAAGTGGCTGAGAGGGGGGGG   | Gib_ES114_binK_US_rev  | atgtatacgcATCGATTAATGACATATTATTATTCATAAAAAAC      |
| Gib_ES141_rcs_DS_rev geagaattcgatactagacAAATACATTGTTGCACTTG Gib_ES141_rcs_US_For geaggattcgatactagacACGCTGTAAATGGCGCTGTAAATGGCGCGATAATGG Gib_ES14_SD_US_DS_for gcttattagATGTGTCGCGGCCGATAAAAC Gib_ES14_SD_US_DS_for geagaattcgatactagacAGGCGCGATAAATG Gib_ES14_SD_US_DS_rev geagaattcgatactagacAGGCGCGATAAATG Gib_ES14_SD_US_EC_rev ctagtggccaggatcgatactGGCAGCGGATAATG Gib_ES14_SD_US_DS_for gttattagATGGTGTAATGCGCGCGATAATG Gib_ES14_SD_US_DS_for gttattagATGGTGTAATGCGCGCGATAATG Gib_ES14_SD_US_DS_for gttattagATGGTGTAATGCGCGCGATAATG Gib_ES14_SD_US_US_For gaattcgataCCGACTCACTGCGCGCGATAATG Gib_ES14_SD_US_US_For gttattagATGGTGTATGGAAGCAATGCGCGCGCAATGG Gib_ES14_SD_US_US_FOR gaattcgataCCGACTCACTGCAGGCGAATGGCGCGCGGCGGCGGCGGCGGCGGGCG   | Gib_ES114_rscS_DS_for  | taatgcaatgGAGAAGTATGAAACACAATAAAC                 |
| Gib_ES14_rsc_US_fer gaggtcgagatagabaGACGTCAAAACTGAATCG Gib_ES14_rsc_US_fer gaggtcgagatagabaGACGTCAAAACTGAATCG Gib_ES14_syp_DS_for grttatagaTATTGCTCGAGGCAATAAAAC Gib_ES14_syp_DS_for gaggtcgagatcgabaCACCGTACGCCCAAATG Gib_ES14_syp_US_for gaggtcgagatcgabaCACCGTACGCCCAAATG Gib_ES14_syp_US_for gagtcgaatGATAATAGCCTCAGCGCAATG Gib_ES14_syp_EC_for cagatacaaCCAACTACTAGAGTCGC Gib_ES14_syp_ED_Fror tgtgatcgagatCGAATATAGCTTCACCGTCACCACTGCTGC Gib_ES14_syp_DE_for gagtcgagatCGAATATAGCTTCACACTGCATCACCG Gib_ES14_syp_LS_for gagtggatcgagatCGAATAAAAGCTAACTGAATGACGCACACTGCTGC Gib_ES14_syp_LS_for gagtggatcgagatCGAATAACGCGAACTGATAGCGCCAATGC Gib_ES14_syp_LS_for gagtggatcgagatCGAATAACGCACACTGCTGC Gib_ES14_syp_LS_for gagtggatcgagatCGAATAACGCGAACTGAATGC Gib_ES14_syp_LS_for gagtggatcgagatGCGAATAACGCGAACTGATACGC Gib_ES14_syp_LS_for gagtggatcgagatGCGAATAACGCGAACTGATGC Gib_ES14_syp_LS_for gagtggatcgagatGCGAATAACGCGAACTGCACTGCG Gib_ES14_syp_LS_for gagtggatcgatagaGCGCAASTGCGCACTGTG Gib_ES14_syp_LS_for gagtggatcgataGCGCACACTGTG Gib_ES14_syp_LS_for gagtggatcgataGCACCGCCATGTGC Gib_ES14_syp_LS_for gagtggatcgataGCACCGCCATGTGC Gib_ES14_syp_LS_for gagtggatcgataGCACCGCGGTAATAAAGG Gib_MB118_yp_LS_for gagtggatcgataGCACCGCGGTAATAACGCACGC Gib_MB118_yp_LS_for gagtggatcgataGCACCGCGGTAATAACGCACGC Gib_MB118_yp_LS_for gagtggatcgataGCACCGCGGTAATAACGCACGC Gib_MB118_yp_LS_for gagtggatcgataGCACCGCGGTAATAACGCACGC Gib_MB118_yp_LS_for gagtggatcgataGCACCGCGGTAATAACGCACGC Gib_MB118_yp_LS_for gagtggatcgataGCACCGCGGTAATAACGCACGC Gib_MB118_yp_LS_for gagtggatcgataGCACCGCGGCAATAACGCACGC Gib_MB118_yp_LS_for gagtggatcgataGCACCGCGGCGTTAATAGCGCCGCCGCCGCGCGCGCGCGC  | Gib_ES114_rscS_DS_rev  | gcaggaattcgatatcaagcAAAAATACATTGTTGCACTTG         |
| Gile_Estl.4_pcs_US_rev etactctcCATTGCATTAGCTCCTATAAATAG<br>Gile_Estl.4_pcs_US_rev gcagaattcgatactagaCAGGCCAATAAAAAC<br>Gile_Estl.4_pcs_US_rev gcaggattcgataCAGGCCAATAAAACC<br>Gile_Estl.4_pcs_US_rev gcaggattcgataCAGCACCAACGGCAATG<br>Gile_Estl.4_pcs_US_rev cagatccagaCCCAACTGCTAGGATCCAC<br>Gile_Estl.4_pcs_US_rev tgaggattcgataCCGAATCATAAAAAG<br>Gile_Estl.4_pcs_US_rev cagatccagaCCCAATGCACTACTGCAGCGCAATG<br>Gile_Estl.4_pcs_US_rev cagatccagaCCCAATGCACTACTAGAGTCG<br>Gile_Estl.4_pcs_US_rev cagatccagaCCCAATGCACTAAAAAG<br>Gile_Estl.4_pcs_US_rev cagatccagaCCCAATGCACTAATTAG<br>Gile_Estl.4_pcs_US_rev cagatccagaCCCAATGCACTAATTAGG<br>Gile_Estl.4_pcs_US_rev cagatccagaCCGACTGCAATGCAATGCAATGCAATGCAC<br>Gile_Estl.4_pcs_US_rev cagatccagaCCGCCTGCAATGCAATGCAATGCACGCCAGGCAATGCAG<br>Gile_Estl.4_pcs_US_US_rev cagatccagaGCGCCTGCAAGGCAATGCATGCATGCAGGCAATGCAATG   | Gib_ES114_rscS_US_for  | gaggtcgacggtatcgataaGACGTCTAAAACTGAATCG           |
| Gib _5114 _syp_D5_for gettattagATATTIGCTCGAGGCCANTAAAAAC<br>Gib _5114 _syp_U5_rev gaggatatcgataaGCTCCTAGGGAGTCAAC<br>Gib _5114 _syp_U5_rev gaggatatCTATATAGCTCCTCAGGGATATATAGC<br>Gib _5114 _syp_E_C.rev ctagtgcaggtatcgatATTAGGTCCACTCCATCATATATC<br>Gib _5114 _syp_E_D5_rev tgaggacggatgcaggatATTAGGTCCCACTCATCATATAGC<br>Gib _5114 _syp_E_D5_rev tgagggacggaggacgcagGTGTTTCACACTCAATAAAAG<br>Gib _5114 _syp_E_D5_rev tgagggacggaggacgcagGTGTTTCACACTCAATAAAAG<br>Gib _5114 _syp_E_D5_rev tgagggacggaggacGTGTTTCACACTCAATAAAAG<br>Gib _5114 _syp_E_D5_rev tgagggacggaggacGTGTTTCACACTCAATAAAAG<br>Gib _5114 _syp_E_U5_rev tgagggacggaggacGTGTTTCACACTCAATAGAAGTAG<br>Gib _5114 _syp_E_U5_rev tgagggacggaggacGTGTTTCACACTCAATACAG<br>Gib _5114 _syp_E_U5_rev tgagggacggacgCTGTTCACACTCAATAAAAG<br>Gib _5114 _syp_E_U5_rev tgagggacggacgCTGTTCACACTCAATAAAG<br>Gib _5114 _syp_E_U5_rev tgaggaggacggacGTGTTCACACTCAATACAG<br>Gib _5114 _syp_E_U5_rev tgaggaggacggacGCTGTAATAGAGAGAATGCAATTTAG<br>Gib _5113 _syp_E_U5_rev tgaggaggacgacGCTGTAATAAAG<br>Gib _MB118 _syp_E_C_rev tgaggacggacgCACTCCTAGGG<br>Gib _MB118 _syp_E_C_rev tgaggacggaggacGCTCTCAGACAATAAAG<br>Gib _MB118 _syp_E_C_rev tgaggaggacggaggacGCTCTCAGACAATAAGGTCCAAT<br>Gib _MB118 _syp_E_C_rev tgaggacggaggacgGCAGTTCACAATAGGTTCCATC<br>Gib _MB118 _syp_E_D_for tgaggagccgaggacgGCAGTTTCACAATCAAATAG<br>Gib _MB118 _syp_E_D_for tgaggagccgaggacgGCAGTTTCACAATCAAATAGC<br>Gib _MB118 _syp_E_D_for tgaggacggacggacgGCAGTTTCACAATCAAATAGC<br>Gib _MB118 _syp_E_U5_rev tgaggatgcgatgataGTGTTAATGCAAATAGAATAG<br>Gib _MB118 _syp_E_U5_rev tgaggatgcagtagataGTGTCAGAATAAAGT<br>Gib _SB53_syp_D_D_for gaggtgcaggatggatgatGTGTATTGCAACTCAAATAGC<br>Gib _SB53_syp_D_D_SF0 rev gaggatgcagtagataGTTTCACAATCAAATAGC<br>Gib _SB53_syp_D_D_SF0 rev gaggatgcagtagataGTTTCACAATCAAATAGC<br>Gib _SB53_syp_D_D_SF0 rev gaggatgcagtagataGTTCACAATCAAATAGC<br>Gib _SB53_syp_D_SF0 rev gaggatgcagtagataGTTTCACACTCAATACCCAATACCACTCAGTCACTCAG<br>Gib _SB53_syp_D_SF0 rev gaggatgcagtagatgatgatgatgatgTTCACACTCAAATAGC<br>Gib _SB53_syp_D_SF0 rev gaggatgcagatgatgatgatgatgatgatgatgatgatgatgatgat  | Gib_ES114_rscS_US_rev  | catacttctcCATTGCATTAGCTCCTATAAAATAG               |
| Gib _ 5114 _ sypD.S_rev         geaggaattegatatcaagcTGGTGATIGTAGGATCCAC           Gib _ 5114 _ sypU.S_rev         geaggaattegatatcaagcTACACCGTAGCCCACATG           Gib _ 5114 _ sypD.S_rev         cagattegatatcagatACACCGTACCCCACATG           Gib _ 5114 _ sypD.S_rev         ccagattegatatcACCGTACATCACAGCGG           Gib _ 5114 _ sypD.S_rev         ccactcittittegaaggtaTGACAACGGACACCGACACACACAACACAACAACAACAACA   | Gib_ES114_syp_DS_for   | gcttattatgATATTTGCTCGAGGCCAATAAAAAC               |
| Gib_S114_syp_US_rev     gagatcgagtatcgatacAACCGTAGCGCCAAATG       Gib_S114_sypE_C, for     cggataccaatCATATATAGCTCCATGCATATATAGC       Gib_S114_sypE_C, for     cggataccaatCATATATAGCTCCATGCATATATAGC       Gib_S114_sypE_DS_for     tipatactajqCTGTTATTGAGATCCAATTAAAAGG       Gib_S114_sypE_DS_for     tipatactajqCTGTTATTGAGATCCAATTAAAGTAG       Gib_S114_sypE_DS_for     tipatactajqCTGTTATTGAGATACCGACTAATTAGG       Gib_S114_sypE_DS_for     tipatactajqCTGTTATCGAAAAAGTAAAGTAG       Gib_S114_sypE_US_for     gagagtcgagtgacgCTGTTCCAACCACATGAAATGCA       Gib_S114_sypE_US_for     ggagtcgagtgacgCTGTTCCAACCACCAGTGAAGTGAAGTAGGAGGG       Gib_S114_sypE_US_for     ggagtcgagtgacgCGGCGGCGCTAAAAAGTAG       Gib_MD118_syp_US_for     ggagtcgagtacgCCGGGCGGGGGGGGGGGGG       Gib_MD118_sypE_C, for     cgagtaccaaGCCAACTCCCGGGGG       Gib_MD118_sypE_C, for     cgagtaccaaGCCAACTCCCGGGG       Gib_MD118_sypE_C, for     cgagtaccaaGCCAACATCACATAGCATCCCATGG       Gib_MD118_sypE_C, for     cgagtaccaaGCCAACATCACATAGCATCCATGG       Gib_MD118_sypE_D, for     cgagtaccaaGtggagtggggggggggggggggggggggg  | Gib_ES114_syp_DS_rev   | gcaggaattcgatatcaagcTGGTGAATGTAGGATCCAC           |
| Gib_S114_syp_LS_rev         gagaaataCATAATAAGCTCCTAGGGAATAATC           Gib_S114_sypE_C_rev         cagatacaCCAACATCATGAGATC           Gib_S114_sypE_DS_for         ttgatggacagatacctgaAATTAAGCTTCATCAC           Gib_S114_sypE_DS_for         ttgatggaccaggaccaggataCCGGATCAATAAAG           Gib_S114_sypE_DS_for         ttgatggaccaggatacctgaAAATAAGCTCCATCAAAAC           Gib_S114_sypE_N_rev         gagatggatgatGCCGGACAAAAAGAAAGTAAAGTAA           Gib_S114_sypE_US_rev         caattatacqCATACACAATCCAATCCAATCCAATCAC           Gib_S114_sypE_US_rev         caattatacqCATACACCACTCAAAAAGAAGAAGTAAAGTAAAGTAAAG  | Gib_ES114_syp_US_for   | gaggtcgacggtatcgataaCAACCGTAGCGCCAAATG            |
| Gib_Sil_sypE_C_for     cagatacaaaCCCACATCACTAGAGGCG       Gib_Sil_sypE_C_for     tigtaccagatcCCACATCACTAGAGGCG       Gib_Sil_sypE_DS, for     tigtaccagatcCCACATCACTACAAAAAG       Gib_Sil_sypE_DS, for     tigtaccagatcCCACATCACTACAAAAGG       Gib_Sil_sypE_DS, for     tigtagcaggtaccgagccGCATAATTAG       Gib_Sil_sypE_D, for     tigtagcaggtaccgagcagcaCCATAATTAG       Gib_Sil_sypE_DS, for     gaggtcgaggtaccgatarGAAAGTAC       Gib_Sil_sypE_US, for     gaggtcgaggtaccgatarGAAAGTAC       Gib_Sil_sypE_US, for     gaggtcgaggtaccgataGCACACTGAATAAAGG       Gib_Sil_sypE_US, for     gaggtcgacggtaccgataGCACACTGAATAAAAGG       Gib_MBilB_syp_US_for     gagtcgacggtaccgataGCACACTGAATAAACG       Gib_MBilB_syp_US_rev     gagtcgacggtaccgataGCACACTGATAACTAAATTAACGTCCATC       Gib_MBilB_sypE_C_for     cagtagcacggtaccgatCACACTGACTAAGCTAAACTAAATTAACGTCCATC       Gib_MBilB_sypE_DS     cagtagcacggtaccgataCCACTGATAACTAAACTAAATTAACGTCCATC       Gib_MBilB_sypE_DS     cagtagcacggataCGgataCCAGCACTGATAAATAACGTCCATC       Gib_MBilB_sypE_DS     cagtaggcccaggatcgataccgatCACTCAATACTAATACCATC       Gib_MBilB_sypE_DS     gaggtcgaggtactggataCGGATAATACACCATC       Gib_MBilB_sypE_US     gaggtcgaggtactggataCGGATAATACACCATC       Gib_MBilB_sypE_US     gaggtcgaggtcatcggaCGCGCGCACGCACTCCATAACTAACTACATTG       Gib_MBilB_sypE_US     gaggatcgatacgataccgatCATCAATACCATCCCATC       Gib_MBilB_sypE_US     gaggatcgatacgataccgatCCACATA  | Gib_ES114_syp_US_rev   | gagcaaatatCATAATAAGCTCCTAGGGAATAATC               |
| Gib_S114_sypE_C_rev     tdgtgccagglactcgaAATTAAACTTCAC       Gib_S114_sypE_DS_for     tgtgtgccagglactcgaAATTAAACTTCAC       Gib_S114_sypE_DS_rev     caacttttttcGAGATCAATAACCGGCATAATTAG       Gib_S114_sypE_N_rov     gtgatggggCtagggcacgcGCTGTTCACAACCC       Gib_S114_sypE_N_rov     gtgatggggCtagggcacgCCTGTTCACAAACCC       Gib_S114_sypE_N_rov     gtgatggggCtagggcacgCCTGTTCACAAACCC       Gib_S114_sypE_N_rov     gtgatggggCtagggatcgataGGCAAAATGCAAAGTGC       Gib_S114_sypE_N_rov     caatcacagCATGATTACACCACTGTTG       Gib_S131_sypE_N_rov     caatcacagCATGATTACACCACTGTTG       Gib_M1181_sypE_DS_for     gtgatggattcgataGCACCGGGG       Gib_M1181_sypE_C_rov     caatcacagCCAACCATCACTAAAAG       Gib_M1181_sypE_DS_rov     cagtgataggataGGCACACTCATACGGG       Gib_M1181_sypE_DS_rov     cagtggattgGCTGTTAATTGAAACCCATAGC       Gib_M1181_sypE_DS_rov     cagtggattgGCTGTTAATTGAAAATAGCACTCATAGC       Gib_M1181_sypE_DS_rov     cagtggattgGCTGTTAATTGAAAAACCAATAGC       Gib_M1181_sypE_DS_rov     cagtggattgGTGTAATTGAAATTAACACTCATAGC       Gib_M1181_sypE_DS_rov     cagtggtatgGTGTAATTGAAATTAACACTCATAGC       Gib_M1181_sypE_DS_rov     cagtggtatgGTGTAATTGAAAATAGCAAAATAGC       Gib_M1181_sypE_DS_rov     cagtggtatggattagataGGTCAAATTAAGCTCCACGC       Gib_M1181_sypE_US_rov     cgaggattggattagataGGTCAACAATTAAGCTCCACGC       Gib_M1181_sypE_US_rov     cgaggattggattagataGGTCAACAATAACTCAAATTAACACTCAATACCACGC  | Gib_ES114_sypE_C_for   | cagatacaaaCCCACATCACTAGAGTCG                      |
| Gib_Estil_sypE_D5_for     tigtaatcatgCTGTTAATTGAGAATCAATAAAAAG       Gib_Estil_sypE_D5_rev     caactutticcaagaigacgccTGTTTACATACCCGCATAATTAG       Gib_Estil_sypE_N_for     tiggaggcctaggaggcgcCGTTACACACCCAATTAG       Gib_Estil_sypE_U5_for     gagggcctaggatacgataTGGTCAAATGACCAATTAACC       Gib_Estil_sypE_U5_rev     cataagGATGGTTACACCACTGTG       Gib_Estil_sypE_U5_rev     cataagGATGGTTACACCACTGTG       Gib_Estil_sypE_U5_rev     cataaGCATGGTTACCCACTGAGGCAATTAACG       Gib_MB11B_syp_U5_for     gaggtcgacggatacgatagCATGGTACAAAAGC       Gib_MB11B_syp_U5_for     gaggtcgacggatacgataGCACTGATCAAATAACCTCCTGGG       Gib_MB11B_sypE_U5_rev     caggtcgacggatacgataGCACTCCTAGGG       Gib_MB11B_sypE_U5_rev     caggtcgacggatacgataGCCCCTAATACCTCCTGC       Gib_MB11B_sypE_U5_rev     caggggccatgggcgcGCGCCCCCCCCAATTACCACTCCACTC       Gib_MB11B_sypE_U5_rev     caggggccatgggccacGCCCCCCCCCCAATTGC       Gib_MB11B_sypE_U5_rev     caggggccatgggccacGCCCCCCCCCAATTGCCCTCCAATGC       Gib_MB11B_sypE_U5_rev     cagggaattcgatacgaaTGGTAAACACCCACTCCACTCCAATACTAATAATGC       Gib_PU579_PS_PS_SypE_rev     gcaggaattcgatatcgaaTGTCAAACACACTCAATACCAATTGC       Gib_PU579_VS_S_SypE_rev     gcaggaattcgatatcgaaTGTCAAACACACTCAATACAATACCAATCCACTCCAATACAAGCACACTCAATACAAGGG       Gib_SPS_Syp_D_S_F     gcaggaattcgatatcgaaCTCAAATTAACCCACTCAATACAAACACACCCAATACAAGGG       Gib_SPS_Syp_D_S_F     gcaggaattcgatatcgaaCTCACAAATTAACCCACACTCCAATACAAAGGGGGGGGGG  | Gib_ES114_sypE_C_rev   | ctagtggccaggtacctcgaAATTAAGCTTCCATCTTCAC          |
| Gib_Es114_sypE_DS_rev       caactettittecqaaggaTTGATACCGGCATAATTTAG         Gib_Es114_sypE_N.for       gtgatgtgggTTGTATCTGAAAATGACACTCAATCAC         Gib_Es114_sypE_US_for       gtgatgtgggTTGTATACCCCACTGTTG         Gib_Es114_sypE_US_for       gtgatgtgggTTGTATACCCCACTGTTG         Gib_MS1113_syp_US_for       gtgatgtgggttgataGCACACTGATAAATAGG         Gib_MS1113_syp_US_for       gatgtgacggatgataGCACACTGATAAATAGG         Gib_MS1113_syp_US_for       gatgtgacggataGCACACTGATAACAGG         Gib_MS1113_syp_US_for       gatgtgacggataGCAGACACTGATAACAGG         Gib_MS1113_sypE_C_for       cagtgacaataCTATAATAGGTCCATAGGAG         Gib_MS1113_sypE_DS_rev       cagtggatgGTGTTAATGGCACATAGAAT         Gib_MS1113_sypE_DS_rev       cagtggatggatcgataGCACACTGATAACAATAGC         Gib_MS1113_sypE_DS_rev       cagtggatgggtCGTGTTAATGCACAATAGGAATTGC         Gib_MS1113_sypE_N_rev       gagggccatgggtcggatGGAAATGGTCAATACTAATACTAATATATATC         Gib_MS1113_sypE_N_rev       gaggtcgacggtataGAATGGTCAGATAATGC         Gib_MS1113_sypE_US_rev       gaggtcgacggtataGAATGGTCAGATAATGC         Gib_MS1113_sypE_N_rev       gaggtcgacggtataGAATGGTCAGATATACTAATATATATATC         Gib_DS139_sypE_N_rev       gaggtcgacggtataGAATGGTCAGATATACTAATATATATATATC         Gib_DS139_sypE_US_rev       gaggtcgacggtataGAATGGTCAGATATACTAATACTAATATATATATC         Gib_DS139_sypE_US_rev       gaggtcgacggtataGAATGGTCAGATATACTCAA  | Gib_ES114_sypE_DS_for  | tgtaatcatgCTGTTAATTGAGAATCAATAAAAAG               |
| Glb_ES114_sypE_N_for       tqaqqqccctaqqccqcqcTGTTCACAATACC         Glb_ES114_sypE_N_rev       qqqtqqqtqqtqtatAAGGTCAAATGAAGTGA         Glb_ES114_sypE_US_rev       caataacqCATGATTACACCACTGTG         Glb_ES114_sypE_US_rev       caataacqCATGATTACACCACTGTGTG         Glb_ES114_sypE_US_rev       caataacqCATGATTACACCACTGTGTG         Glb_MB118_syp_DS_for       gqtqacqqtacqataaGCACACTGATACAAAG         Glb_MB118_syp_DS_rev       qqtqacqataGCACACTGATACAAAG         Glb_MB118_sypE_C, for       qqqtcacqataCACACTGATACAAAGC         Glb_MB118_sypE_C, rev       ctaqtqqcacqatcqqcaCACACTCATACAATAC         Glb_MB118_sypE_C, rev       ctaqtqqcacqqtcqqcqcATGTCCAACAATACACTACATACACATTACACATTG         Glb_MB118_sypE_D, rev       qqqqqatcqqqtatCqqTATAGAATACCACTGATGC         Glb_MB118_sypE_N, rev       tqqqqqqqtqqqtGTGTTTACAAATAGC         Glb_MB118_sypE_N, rev       tqqqqqqqqtqqqqtGTGTGTATGAATGACAATAGC         Glb_MB118_sypE_N, rev       tqqqqqqqqtqqqqqtqqqqtGCGTGTAGCACAGATAAATAG         Glb_MB118_sypE_N, rev       qqqqtqqqqqtqqqqqtqqqtGCGTGTAGCACAGATAAATAG         Glb_MB118_sypE_N, rev       qqqqtqqqqqtqqqtqGTGTGTATGAATACCACTGAATACCACGATAAATAGC         Glb_MB118_sypE_N, rev       qqqqtqqqqtqtqqtGTGTATAAAGTACCCAGATGAAGGTC         Glb_MB118_sypE_N, rev       qqqqtqqqqqtqtqqtGTGTGTAAATACCACAGTGTAAGTAGC         Glb_MB118_sypE_N, rev       qqqqqtqqqqqqtqtqqqtGTGTGTAAAAGTCCAAGTGAGGTCCA  | Gib_ES114_sypE_DS_rev  | caactctttttccgaaggtaTTGAGTAACCGGCATAATTTAG        |
| Glb_ES114_sypE_N_rev     gtqatgtggTTGTATCTGAAAAAAGGTAAAGTAG       Glb_ES114_sypE_US_for     gqatgtqggTTGTATCACCACTGTTG       Glb_ES114_sypE_US_rev     caattacagACTGATTACACCACTGTTG       Glb_ES113_syp_US_US_rev     caattacagACACACTGATAAATAG       Glb_MB118_syp_US_for     gqatgtcaggtatgataaGCACACTGATAAAATG       Glb_MB118_syp_US_for     gqatgtcaggtatgataaGCACACTGATAAAATG       Glb_MB118_syp_US_rev     cagtacaaBCCAACTGATTAAGCTCCTAGGG       Glb_MB118_sypE_C, for     cagtacaaBCCAACATGATTAAGCTCCTAGGG       Glb_MB118_sypE_DS_rev     ccagtagcaagtactGaTATAGGTCTTAGGG       Glb_MB118_sypE_N     for     cagtagcaagtactGaTGATAGGTTTTAACCACTCATG       Glb_MB118_sypE_N     for     tgagggccctggcgcgcgcGGTTTTAACACAATAGC       Glb_MB118_sypE_N     for     tgagggccctggcgcgcgcGGTTTAACACAATAACGTCATACTAATAATATTC       Glb_MB118_sypE_N     for     tgagggccctggcgcgcgcGAGTTTGAAATAACATAGC       Glb_MB118_sypE_N     for     tgagggccctggcgcgcgcGAGTTTGAAAATAG       Glb_MB118_sypE_N     for     tgagggccctggcgcgcgcGAGTTCAAATAACACTCAATACCAATAGC       Glb_DB118_sypE_N     for     tgagggccctggcgcgcgcGAGTTCCAATCAATAGCTCCATCC       Glb_DB118_sypE_NF     ggcgagattgatatGatAGGTCAAATAAAGC       Glb_DB118_sypE_NF     ggcgagattgatatGatAGGTCAATCAATAACTCAATACCAATATAATATTC       Glb_DB2NSPE     ggcgagattgatatGatCAATATAAGCTCCATACCAATCAATACCAATCAATACCATCATCACCAC  | Gib_ES114_sypE_N_for   | tagagggccctaggcgcgccTGTTTCACAACTCAATACC           |
| Gib_S114_sypE_US_for       gagtcgacggtatcgatadaTGGTCAGATGAAATGTCATTTTAG         Gib_S114_sypE_US_rev       catacttcCATTGTATTAGCCTCTTGTG         Gib_S114_sypE_US_rev       catacttcCATTGTATTAGCTCCTATAAAATGG         Gib_MB11B_syp_US_for       gagtcgacggtatcgataaGCACACTGATAAAAG         Gib_MB11B_syp_US_rev       gagcaataCATAATAAAGCTCCTAGAATCAAG         Gib_MB11B_syp_US_rev       gagcaataCATAATAAGCTCCTAGAGG         Gib_MB11B_sypE_C_rev       ctagtggccaggtatcgaTAAGCACACTGATCACATAGC         Gib_MB11B_sypE_DS_rev       gcaggattcgattcgaTCAACAATAAGCTCCATCG         Gib_MB11B_sypE_NP_Nop       tgagggtcgacggtccGATTTAGCAATAGC         Gib_MB11B_sypE_NP_Nop       tgagggtccaggtgcccCATTCACACATAGC         Gib_MB11B_sypE_NP_Nop       tgaggtcgacggtgcccCATTCACACAATAGC         Gib_MB11B_sypE_NP_Nop       tgaggtcgacggtgtcgataGCATTCACACATAGC         Gib_MB11B_sypE_NP_Nop       tgaggtcgacggtgtcgataGCATTCACACATTCATAATAATGTC         Gib_MB11B_sypE_NP_Nop       gaggtcgacggtgtcgataGCATTCACAACTCAATACC         Gib_DPV579_MB_sypE_rov       gcaggattcgatacagCAAATTAAGCTCCATTCACACCCATACC         Gib_PV579_MB_sypE_for       gaggtcgacggtgtatcgataCAGTTCACAACTCAATACC         Gib_SPS_syp_DS_rov       gaggtcgacggtgtatcgataCAGTATAAAAATG         Gib_SPS_syp_DS_rov       gaggtcgacggtgtatcgataCAGTTCACAACTCAATACC         Gib_SPS_syp_DS_rov       gaggtcgacggtgtatcgataCAGTTCACACCACTCAATACACCACTCT   | Gib_ES114_sypE_N_rev   | gtgatgtgggTTTGTATCTGAAAAAAGTAAAGTAG               |
| Gib_S114_sypE_US_rev       cattacacqCATGATTACACCACTGTTG         Gib_S231_srcs_US_rev       cqtattacqATGATTATAGCTCCATAAAATG         Gib_MB1181_syp_DS_for       gagacatatCATAATAAGGTCCATAAAAAG         Gib_MB1181_syp_US_rev       gagacatatCATAATAAGCTCCTAGGG         Gib_MB1181_syp_E_C_for       cagatacaaGCCAACATCATAAAAGC         Gib_MB1181_sypE_C_for       cagatacaaGCCAACATCACTAGAATC         Gib_MB1181_sypE_DS_rev       cagtagatagGCCAACATCAATAGCCCATCCATC         Gib_MB1181_sypE_DS_rev       cagtagatagGCCAACATCAATAGCCAATAGC         Gib_MB1181_sypE_DS_rev       cagtagatagGCCAACATCAATAGCCAATAGC         Gib_MB1181_sypE_NF_rev       tgagagtcqatacagATTAGGACAATATGC         Gib_MB1181_sypE_NF_rev       tgagagtcqatagataGCCAATGTCCAATCACATATATATC         Gib_MB1181_sypE_NF_rev       tgagagtcqatagatagataGCCAACATCAATACCACTGTCCACTCAATACTCAATACCC         Gib_DFV579_ES_sypE_rev       gagagtcqatagatagataGTTGCAAACTCAATACCC         Gib_DFV579_MB_sypE_rev       gagagtcqatagatagataGCGTGACGCAATAAAAG         Gib_SS_syp_DS_rev       gagagatagataCGGTGGGGGGGGGGGGGAATGAAGGG         Gib_SS_Syp_DS_rev       gagagatagataGCGAACATCAACACCATCAACACCATCAAGG         Gib_SS_syp_US_for       gagagtagaggatagataGGGGGGGGGGGGGGGGGGGGG  | Gib_ES114_sypE_US_for  | gaggtcgacggtatcgataaTGGTCAGATGAAATGTCATTTTTAG     |
| Gib. 25:13.rsC, US, rev       catattict.CATIGTATTAGCTCCTATAAAATAG         Gib. M811B1.syp, DS, for       gagtagaggtatgataaGCACACTGATAAAAG         Gib. M811B1.syp, DS, for       gagtagaataGCACACTGATAAAGGT         Gib. M811B1.syp, E, C       cagtacaaaGCCAACATCACTAGATAAG         Gib. M811B1.syp, E, C, rev       cagtagcaaggtatcgaattaGATCAACAATAAGCTCCATC         Gib. M811B1.syp, E, DS, for       cagtagcaaggtatcgaattaCATAAAAACCAATAGC         Gib. M811B1.syp, E, DS, for       cagtagcaaggtatcgaattaCaagcCAATTAGGACAATAGC         Gib. M811B1.syp, E, N, rev       gagagtagcacgtagcgcacGTTTCACAACAATCAACATAATAATATTG         Gib. M811B1.syp, E, N, rev       gagattacgaattacaagcAATTAAGCAAAAAGCAAAATAG         Gib. M811B1.syp, E, N, rev       gagattacgaattacaagcAATTAAGCAAAAAGCAAATAG         Gib. M811B1.syp, E, US, rev       gagattacgaattacaagcAATTAAGCACCAATTACACAATCAATAACATCAATAATATTG         Gib. M811B1.syp, E, US, rev       gagattacgaattacaagCAATTAAGCTTCCAATTCAATAATATTC         Gib. M811B1.syp, E, US, rev       gagagtagtagattagaattacagAATTAAGACTTCCAATTCAATAATATTC         Gib. pEV579.Sp. Syp, E, for       gagattagattacagCAATTAAGACTTCCAATTCAATAATATATTC         Gib. pEV579.M8.sypE, for       gagagtattagattacagCTAACAATTAAGCTTCCACTCAATAACAAT         Gib. SRS.syp, DS, for       gagattagattacagCTCAACAATTAAGCTTCCACTCAATAATATATTC         Gib. SRS.syp, US_ rev       gagacaatatCATAATATAGAGTCCCACTGAAGGAGGGGGGGGTGTAGAGAGTCCAATTCAAGAAGCTCCATTAAGAATTCACAAC  | Gib_ES114_sypE_US_rev  | caattaacagCATGATTACACCACTGTTG                     |
| Gib. JMB11B1_syp_LS_for       gettattajATATTIGCTCGAGGTCAATAAAAG         Gib. JMB11B1_syp_LS_rev       gagcaaatatCATAATAAGCTCGTAGGG         Gib. JMB11B1_syp_LC_rev       cagatacaaaGCCAACATCACTAGAATAC         Gib. JMB11B1_syp_LC_rev       cagatacaaaGCCAACATCACTAGAATAC         Gib. JMB11B1_syp_LC_rev       cagatacaaaGCCAACATCACTAGAATAGC         Gib. JMB11B1_syp_LD_S_for       cagatacaaaGCCAACATCACTAGAATAGC         Gib. JMB11B1_syp_LD_S_rev       cagagatcgatacaagACTTAGAAACCAATAGC         Gib. JMB11B1_syp_LD_S_rev       tagagtgcacggtaccgatacagAATTAGACTAAATAGC         Gib. JMB11B1_syp_LD_S_rev       tagatgttgcgTGTGTATCGAAAAGCGAAATGG         Gib. JMB11B1_syp_LU_S_rev       caattaacagCATACCACTGTTGATAATAACCAATACC         Gib. JMB11B1_syp_LU_S_rev       caattaacagCATACCACTGTTGATAAAAATG         Gib. JPS75_S_Syp_LFor       gaagtcgacgtatcgatatCagATCATACACTCAATACC         Gib. JPS75_MB_sypE_rev       gcaggattcgatatcaagCATGTCACAATCAATAATATC         Gib. JPS75_MB_sypE_rev       gcaggattcgatatcaagCTGTACACAATTAAGC         Gib. SPS. Syp_D_S_for       gaagtcgacgtatcgatatCaAGTCCAATCAATAAAAAG         Gib. SPS. Syp_LS_rev       gaagtactgatatcaagCTGAACGATAAAAAG         Gib. SPS. Syp_LS_rev       gaagtactgatatcaagCTGAACAATAAAAAACG         Gib. SPS. Syp_D_S_rev       gaagtatcgatatcaagCTGAACCAATAAAAAACGAATAACCATCAG         Gib. SPS. Syp_LS_rev       gaagtatcgatatcaagCTGAACCAACTGAG  | Gib_ES213_rscS_US_rev  | catacttctcCATTGTATTAGCTCCTATAAAATAG               |
| Gib_MB11B1_syp_LS_forgaggtaggatggataggataggataggataggatagg  | Gib_MB11B1_syp_DS_for  | gcttattatgATATTTGCTCGAGGTCAATAAAAG                |
| Gib_MB1181_syp_Lyp_C_(r)gagcaaatatCATAATAAGCTCCTAGGGGib_MB1181_sypE_C_revctagtagcaagGCCAAQCTACCTATCAGAATCGib_MB1181_sypE_C_revctagtagcaagGCCAAQCTACCTACTAGCATCGib_MB1181_sypE_D_S_forcagtagcaagCCAAQCTACATAGCATGCGib_MB1181_sypE_D_S_revgcaggaattcgaagCCAGTTTAAGAATCAATAGCGib_MB1181_sypE_N_rorttagagggccctaggcgcccAGTTTCACAACTCAATACTAATAATATTCGib_MB1181_sypE_US_forgaggtcgacggtatcgataCAACTGCAACTCAATACTAATAATATTCGib_MB1181_sypE_US_forgaggtcgacggtatcgataGAATGGTCACATGCAATGCAATGCACTGCAATGCGib_DB1181_sypE_US_revgagtgcacggtatcgataGCAACTACCACTCAATACCGib_DFV579_ES_sypE_forgagtgcacggtatcgataCATTACCACTCAATACCGib_DFV579_MB_sypE_revgcaggaattcgatactaagCTCAACAATTAAGCTTCCAATCCGib_SFS_syp_DS_forgagtgcacggtatcgataCAAGTAAAAAAGGib_SFS_syp_DS_forgagtcgacggtatcgataCAAGTAAAAAAGGib_SFS_syp_DS_forgagtcgacggtatcgataCAAGTAAAAAAGGib_SFS_syp_DS_forgagtcgacggtatcgataCAGTCAACAATTAAGCTCCATCGib_SFS_syp_DS_forgagtcgacgtatcgataCAGCGCCAAATAGCHB8ACAAAATTTAAGATCGCAGCGCCCAAATGCHB9GGGAGGAAATAATCTAGGAGCGGCGCCAAAGTCACACCCCTTAAGGAGCHB41CGATCTTGGGGTAGAGCAACTTCAAGCACCGCCCGCCTCTAGTTGGGAATCAAGGGCAGGAGGGCGGAGGAGGGCGTGAAGGHB42ACGAAGCGTACGCACCCCCGCCCCCCCCCCCCCCCCCCC   | Gib_MB11B1_syp_US_for  | gaggtcgacggtatcgataaGCACACTGATAACTAAATTATTAC      |
| Gib_MB1181_sypE_C_for       cagatacaaaGCCAACATCACTAGAATC         Gib_MB1181_sypE_DS_for       cagtggtatgCTGTTAATTGAAAACCAATTAGC         Gib_MB1181_sypE_DS_rev       gcaggaattcgatacaagcATTTAGGAATGTTTTAATAACAATTTG         Gib_MB1181_sypE_N_for       tagagggccagcATTTAGGATGTTTTAATAGAATGTC         Gib_MB1181_sypE_US_for       tagagggccaggcagcATTTAGGATGATTAATAATATTC         Gib_MB1181_sypE_US_for       tagagggccaggcaggcaTTTGCAACTCAATACATAATAATATTC         Gib_MB1181_sypE_US_for       gaggtcgagcggtatcgataGAATGGTCAATGCAATGCAATGC         Gib_MB1181_sypE_US_rev       caattaacagCATACCACTGTGATAAAAATC         Gib_DVS79_LS_sypE_for       gaggtcgacggtatcgataaGATTTCACAACTCAATACCC         Gib_PVS79_S_SypD_For       gcaggaattcgatacagaCATAACCACTCAATACCAATAATAATATTC         Gib_PVS79_MB_sypE_for       gaggtcgacgtatcgatacaagCCAACAATAAAAG         Gib_SR5_syp_DS_rev       gcaggaattcgatacagaCGTGAGCAATAAAAAG         Gib_SR5_syp_DS_rev       gcaggaattcgatacagaCGTGAGCAATAAAAAG         Gib_SR5_syp_US_rev       gaggtcgacgtatcgatacagCGTGAGCGTAGCAATAAAAGG         Gib_SR5_syp_US_rev       gaggtcgacgtatcgatacagCGTGAGCGTAGCAATAAAAGG         Gib_SR5_syp_US_rev       gaggtcgacgtatcgatacagaCGTAGCAATAAAAGG         Gib_SR5_syp_US_rev       gaggtcgacgtatcgatacagaCGTAGCAATAAAAGG         Gib_SR5_syp_US_rev       gaggtcgacgtatcgatacagaCGTAGCAGTAACACCATCAAGGG         Gib_SR5_syp_US_rev       gaggt  | Gib_MB11B1_syp_US_rev  | gagcaaatatCATAATAAGCTCCTAGGG                      |
| Glb_MB1181_sypE_D_S_fev     ctagtggtactggaftacttagaftCAACAATTAAGC       Glb_MB1181_sypE_D_S_rev     gcaggaattcgatatcaagcATTTAGGATGTTTTAACAAATTG       Glb_MB1181_sypE_N_for     tagaggtactgggcacAGTTTAGGACAAATCAATTGC       Glb_MB1181_sypE_N_for     tagaggtactgggcacAGTTCACACACTCAATACAATTGC       Glb_MB1181_sypE_N_SpE_N_v     tagattgggcTTGGATACTCAGACAAAATCA       Glb_MB1181_sypE_US_for     gaaggtcgacggtatcgataaGAATGGTCACAATGCACACTCAATACCC       Glb_MB1181_sypE_US_for     gaaggtcgacggtatcgataaGAATGGTCACAATCACC       Glb_PK579_ES_sypE_for     gaaggtcgacggtatcgataaGTTTCACAACTCAATACCC       Glb_PK579_MB_sypE_rev     gcaggaattcgataacgaCATTCAACCACTCAATACCC       Glb_PK579_MB_sypE_rev     gcaggaattcgataacagCTCAACAATTAAGCTTCCATC       Glb_SR5_syp_DS_rev     gcaggaattcgatatcaagCTGACAATTAAGCTTCCATC       Glb_SR5_syp_DS_for     gaaggtaggtgatagataACCGTAGGGCAAATAAAAG       Glb_SR5_syp_DS_rev     gcaggaattcgatatcaagCTGAGAGTATAATCC       Glb_SR5_syp_DS_rev     gcaggaattcgatatcaagCTGGAGTGTAGCACCAAAATG       Glb_SR5_syp_DS_rev     gaagcaatgtCTCATACACCTGTAGGGCAAAATACC       HB8     ACAAAATTTTAAGATACTGCACAACTACAACCACACCTCTAAG       HB9     GGGAGGAAATATCTCAGACACACACACACACACACACCACAGG       HB23     TTGGAAGCCTACCTTCAGAGACATACACCAGGCCCAGGCTCCATTCCTAGGAAAGTACAAGGGCAAGGTAAGTCGCAGGCAAGGTCCAAGTCCAGGCCCAGGCCCTAAGTGCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGCCAGGCCCAGGCCCTAAGTCCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGCAAGGGCAAGGGCAAGGCCAAGGCCAGGCCAGGCCAGGCCCAGGGCCAGGGCCAGGGCCAGGCCGCAGG   | Gib_MB11B1_sypE_C_for  | cagatacaaaGCCAACATCACTAGAATC                      |
| Glb_MB1181_sypE_DS_for     cagtggtatgCTGTTAATTGAAAACCAATAGC       Glb_MB1181_sypE_N_for     tagagggcctaggcggccAGTTTCACAAACAATTG       Gib_MB1181_sypE_N_for     tagagggcctaggcgccAGTTTCACAAACAAATAG       Gib_MB1181_sypE_N_rev     tgatgttggCTTGTATCGAAAAAAGCAAAATAG       Gib_MB1181_sypE_N_rev     tgatgttggCTGTGAATGGCAAATGGCAAATGGC       Gib_MB1181_sypE_N_rev     tgatgttggCtGAAATGGCAAATGGCAAATGCAAATGC       Gib_MB1181_sypE_N_rev     cagtgcaggtatcgataAGATGGCAAATGGCAAATGCA       Gib_pEV579_ES_sypE_for     gaggtcgacggtatcgataAGTTTCACAACTCAATACCAATGCAATGCA       Gib_pEV579_MB_sypE_rev     gcaggaattcgatacaagCAATTCAACACTCAATACATAATAATTC       Gib_pEV579_MB_sypE_ror     gcaggaattcgatacaagCAACACAATTAAAGCTTCCATCC       Gib_pEV579_MB_sypE_ror     gcaggaattcgatacaagCGGCAGAATAAAAAG       Gib_SR5_syp_DS_for     gcaggaattcgatacaagCGGCAGAATAATCC       Gib_SR5_syp_US_rov     gcaggaattggataCGGAAGCACATCAACATTAAGGCCAACTCAATGG       Gib_SR5_syp_US_rov     gaggtcgacggtatcgataCGAGCAATAATCC       H88     ACAAAATTTAAGATCCCACGCCCAATGG       Gib_SR5_syp_US_rov     gaggattggataGCAGCCAACCTCAATACGG       H839     TGGAAGGAAATAATCTAGAAGCAAACTTAAGGGTG       H841     CGATCTTGGGGTAGAGACACACCAGGCCCAAGGCCGCCCCCGCCCCTAGGGGGACAACAGGGAGGCCGCAAGGACGG       H844     CGATCTGTGGGGTAGAGAGCACC       H845     CGAACGAACAGCAAGCAAGCAGCAGC       bink-F1     GAAATTACCATGGGGCCAAAGGCAAGGC       bink-F2     <   | Gib_MB11B1_sypE_C_rev  | ctagtggccaggtacctcgaTCAACAATTAAGCTTCCATC          |
| Glb_MB11B1_sypE_DS_revgcaggaattcgatatcaagcATTTAGGATGTTTTAATAGCAATTGGlb_MB11B1_sypE_N_revtgatgttggcTTGTATCTGAAAAAAGCACTCCAATACTAATAATAATATTCGlb_MB11B1_sypE_US_forgaggtcgacggtattggataGAATGGTCAGATGAAATGCGlb_MB11B1_sypE_US_forgaggtcgacggtattggataGGTTGCACAGTGCAAATGCGlb_PK579_ES_sypE_revgcaggaattggatatcaagcAATTGACCAATCACTCAATACTAGlb_PK579_MB_sypE_rovgaggtcggtattggataGGTTCCACACTCAATACTCACTCACTGlb_PK579_MB_sypE_rovgcaggaattggatatcaagCAATCACACTCAATACTCACTCACTGlb_PK579_MB_sypE_rovgcaggaattggatatcaagCGAACTCACATCACTCAATATATATATTCGlb_SR5_syp_DS_forgagtcgacggtattggataCagataGAGTGTAGACACTCAATAAAGGTGlb_SR5_syp_DS_forgagttggattggatatcaagCTGACAATAAAGCTCCATCGlb_SR5_syp_DS_forgagtggacggttggatagataGGTGGGAGTGTAGAATCCATGCGlb_SR5_syp_US_forgagtggacggttggatagataGGTGGGGAGTGAGAATAAATGGGlb_SR5_syp_US_revgagaaatCGATAAATAAGCTCCAACACCACTCAAGGH88ACAAAATTTAAGATCGCACATCAAACACACACACTCAAGGH89GGGAGGAAATAATCTAGAAGGCAGCGCGCTGTGCTAAH839TAGGAAGCTACCAGAGCAGCGCGCTGCCAAAH841CGATCTGTGGGTAGAGACACCACGGCCCAGGCCCCGCCTCAGTTAGGAAATCAAGGCAGGAGCAGGTGCAAGGCCACGCGCCCCAGCCCCGCCTCAGTTAGAAGGCAAGGAGCACCACGGCCACAGCCCAGGCCCCGCCTAGTTAGAGAGAATCAAAGTGGH842ACGAGAGCGAGCGTCCTTGTATATATGCTTCGCCAGH844CGATCTGTGGGTAGAGACACCCH844CGAACTCGTGGGCAAAGCCCACGGCCACAGCCCGH844CGACTCTGTGGGTAGAGACACCCH842GGCACCACTCGCCAAGCCCACGGCCACGCGCCGGGGGAGAGTACAAGTGAGGGGCACACCCCGGGGGGGG  | Gib_MB11B1_sypE_DS_for | cagtggtatgCTGTTAATTGAAAACCAATAGC                  |
| Glb_MB11B1_sypE_N_fortagagggccctaggcgcgccqccAGTTCACAACTCAATAATAATATTCGlb_MB11B1_sypE_US_forgaggtgcaggtatcgataGAATGGTCAGAAAAACAAAATAGGlb_MB11B1_sypE_US_forgaggtcgacggtatcgataGAATGGTCAGATGAAAAACCAACTCAATACCGlb_PK579_ES_sypE_forgaggtcgacggtatcgataTGTTCACAACTCAATACCGlb_PK579_SE_sypE_revgcaggaattcgatatcaagcAATTAAGCTTCCATCTCACGlb_SPK579_MB_sypE_revgcaggaattcgatatcaagcTGACACAATTAAGCTTCCATCTCGlb_SPK579_NB_sypE_revgcaggaattcgatatcaagcTGAGGACAATAAAAGGlb_SPK579_NB_sypE_revgcaggaattcgatatcaagcTGAGGACAATAAAAGGlb_SPS_syp_DS_forgtagtgtagtatcaagcTGGGGAGTGAGAATCAAGCCCAATGGGlb_SPS_syp_US_forgaggtcgacggtatcgatacaagCGGGGAGTGAGAATCAATGGGlb_SPS_syp_US_forgaggtcgacggtatcgatacaagCGGGGAGTGAGCAATAAAAAGGlb_SPS_syp_US_revgagaattcgatatcaagCAGTGAGCGCAAATGGGlb_SPS_syp_US_revgagatcgattcGatATGCCCCTACGGAGTAGGHB8ACAAAATTTAAGATACGCACACCACACACACTCTAAGHB9GGGAGGAAATAATCTAGAAGCCGAGCGGCTCTATATATGCTCCGCCAGGAGAGTCCTATTCTGAGAAAGCTAHB41CGACTCTGTGGGTAGAGACACCCGGAGCCCGCAGCCCGCCTCAGTTGGGAATCAAGTGCAGGCCGAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG  | Gib_MB11B1_sypE_DS_rev | gcaggaattcgatatcaagcATTTAGGATGTTTTTAATAACAATTTG   |
| Gib_MB11B1_sypE_N_revtgatgtggCTTGTATCTGAAAAAAGCAAAATAGGib_MB11B1_sypE_US_forgagtgcaggtatcgataGAATGGTCAGATGAAATGCGib_MB11B1_sypE_US_revcaattaacgCATACCACTGTTGATAAAAATCGib_pEVS79_ES_sypE_forgagtgcaggtatcgataGATTGACTCCATCTCACGib_pEVS79_ES_sypE_revgcaggaattcgatacaagCAAATTAAGCTCCATCCATCACGib_pEVS79_MB_sypE_rovgcaggaattcgatacaagCTGAACAATTAAACTCCATCGib_pEVS79_MB_sypE_rovgcaggaattcgatacaagCTGAACAATTAAACTCCATCGib_SRS_syp_DS_forgcttgatatgataCGAACAATTAAACTCCATCGib_SRS_syp_DS_forgcttgatatgataCGAACAATAAAACAGGib_SRS_syp_DS_forgagtcgacggtatcgataaAACCGTGAGGGAATAAACGGib_SRS_syp_DS_forgagtgcacggtatcgataaAACCGTACGGAATAAACGGib_SRS_syp_US_forgagtgcacggtatcgataaAACCGTACGGAATAATGCH88ACAAAATTTTAAGATACTGCAACACTCTAAGGH89GGGAGAAATAATCTAGAATGGGAAAATGGAGGGGGAATAAGGH839TAGGAAGCTACGAGACGACGTGCTTCATATAGGTGCGCCAAGAGGAAGTCCTATTCTCTAGAAAGTAH841CGATCTTGTGGGTAGAGACATCCAGGCCAAGGGCGCCGCCGCTCTAGTTGGGAATCAAGTGCATGAGGCGCGGAAGAH842ACGAACGGAGCTCTTATATATGCTTCGCCAGGCCCCGCTCTAGTTGGGAATCAAGTGCATGAGCGCGGGAAGACTbink-F1GAAATTACCATGGGGTAGAGACATCbink-F2GGCATCTTGTGGGTAGAGACATCCCAGGCCCCGCCCTTATTTGTAGATATAATTATAACTATAATCGCbink-F2GGCATCATTATGGGGTAGAGACATCbink-F2GGCATCATTATGGGATACGAGCAACCAGCAGCAGCbink-F2GGCATCATTATGGCAACCAACAGCAGCAGCbink-F2GGCATCATTATGGCAACCAACTAAAAACCTAGCGCCTTATATTGAGAGTATGACGAGGCbink-F2GGCATCATTATGGCAACCAACTAGCGCbink-F2GGCATCATTATGGCAACCAACTACAAGGCbink-F2GGCATCATT  | Gib_MB11B1_sypE_N_for  | tagagggccctaggcgcgccAGTTTCACAACTCAATACTAATAATATTC |
| Gib_MB11B1_sypE_US_for gaggtcgacggtatcgataGAATGGTCAGATGAAATGTC<br>Gib_mB11B1_sypE_US_rev cattaacagCATACCACTGTGTAAAAAATC<br>Gib_pEVS79_ES_sypE_for gaggtcgacggtatcgataGATTAAGCTTCACAATACC<br>Gib_pEVS79_MB_sypE_rev gcaggaattcgatacagCAATAAAGCTCCAATCCAATACAATAATATTC<br>Gib_pEVS79_MB_sypE_rev gcaggaattcgatacagCAATAAAGCTCCAATCCAATACTAATAATATTC<br>Gib_pEVS79_MB_sypE_rev gcaggaattcgatacagCTGAACAATTAAGCTTCCATC<br>Gib_SR5_syp_DS_for gcttattagATATTGCTCGAGGACAATAAAAA<br>Gib_SR5_syp_DS_for gctgattgatacaagCTGAACAATAAAAAG<br>Gib_SR5_syp_US_for gaggtcgacggtatcgatacaagCTGAGGAGATAGAAAAG<br>Gib_SR5_syp_US_for gaggtcgacggtatcgatacaagCTGACGACATTAGGA<br>Gib_SR5_syp_US_rev gaggaattcGataCaagCTGAGGAGTAGGACAATACAAAG<br>Gib_SR5_syp_US_rev gagcaatatCATAATAAGCTCCTAGGGAATAATCC<br>HB8 ACAAAATTTTAAGATACTGGACTATCAACACACTCTAAG<br>HB9 GGGAGGAAATAATCTAGGACTATCAACACACTCTAAG<br>HB3 GGGAGGAAATAATCTAGGACTCATCAACACACTCTAAG<br>HB3 GGGAGGCAAATAATCTAGGACTCTAGGAGGAGTAGG<br>HB23 TTGGAGAGCCCACGTGCTCTTAGAAGCGAAGCTTCCTATGCAGGAGAGTTCCTATTCTCTAGAAAAGTA<br>TAGGAAGCTTCTTGGGGTGAGACACTCAAGGCCCACGGCCGCCGCCTCTAGTTTGGGAATCAAGTGCATGAGGCGCTGAAG<br>HB41 CGATCTTGTGGGTGAGACACTCCAGGTCAAGTCCAGGCCCCGCGCTCTAGTTTGGGAATCAAGTGCATGAGGCGCTGAAG<br>HB42 ACGAACTTCCTTGGGATGAGACCACCCAGGCCCAGG<br>HB44 CGATCTTGTGGGTAGAGACCATC<br>bink-F1 GAAATTACCATGGAGCCAACGCAAGCC<br>HB44 CGATCTTGTGGGTAGAGACCTC<br>bink-F2 GGCATCATTATGGCACACGCAGGCACAGCAAGGC<br>HB44 CGATCTTGTGGGTAGAGCCACCAGCAAGGC<br>HB44 CGATCTTGTGGGTAGAGCCACCAGCAAGGC<br>HB44 CGATCTTGTGGGTAGAGCCACCAGCAAGGC<br>HB44 CGATCTTGTGGGTAGAGCCACCAGCAAGGC<br>HB44 CGATCTTGTGGGTAGAGCCACCAGCAAGGC<br>HB44 CGATCTTGTGGGTAGAGCCACCAGCAAGGC<br>HB44 CGATCTTGTGGGTAGACCACTCAGGCC<br>HB46 CGATCTTGTGGGTAGACCACTCAGGCC<br>HB46 CGATCTTGTGGGTAGACCACTCAGGCC<br>HB46 CGATCTTGTGGGTAGACCACTGCCCAG<br>HB47 CCCGTTAATACTGGGATAGACCAGCCAGGC<br>HB47 CCCGTTAATACTGGGATACGACGGC<br>HB47 CCCGTTAATACTGGGATACGACGGC<br>HB47 CCCGTTAATACTGGGATACGAGGC<br>HB47 CCCGCTAATACTGGGATGCGCG<br>HB47 CTCAAATAGCAGTGGCGGG<br>HB47 CTCAAATGCAGGGGCCACGG<br>HB47 CTCAAATGCAGGAGGATTCAACGAGGC<br>HB47 CTCAAATGCAGGAGGCCCACGG<br>HB47 CTCAAATGCGACGAGCGCCAGG<br>HB47 CTCAAATGCGACGACGCCGCG<br>HB47 CTCAAATGCAGGGGCCACGG<br>HB47 CTCAAATGCAGGGGCCCACGG<br>HB47 CTC | Gib_MB11B1_sypE_N_rev  | tgatgttggcTTTGTATCTGAAAAAAGCAAAATAG               |
| Gib_MB11B1_sypE_US_revcaattaacagCATACCACTGTTGATAAAAATCGib_pEVS79_ES_sypE_forgagtgcagggttcgattaGataTGTTTCACAACTCAATCCAATCTAATAATATTCGib_pEVS79_MB_sypE_forgagtgcagggttcgataaaGTTTCACAATCAATCAATCAATAATATTCGib_pEVS79_MB_sypE_revgcaggaattcgattcaagcCAATTAAGCTTCCATCGib_pEVS79_MB_sypE_revgcaggaattcgattcaagCTGAACAATTAAAGCTTCCATCGib_SR5_syp_DS_revgcaggaattcgattcaagCTGACAATTAAAGAGGib_SR5_syp_US_revgaggtcgacggtatcgataaACCTAACAATCAATCGGGib_SR5_syp_US_revgaggtcgacggtatcgataaACCTACAACATCATGGGib_SR5_syp_US_revgaggtcgacggtatcgataaAACCTAGAGCGCCAAATGGGib_SR5_syp_US_revgaggtcgacggtatcgataaAACCGTACCAATCAACACTCTTAAGHB8ACAAAATTTTAAGATACTGCACTATCAACACACTCTTAAGHB9GGAGGCAAATAATCTAGAATCGCGGGAATAATCGHB23TTGGAGAGCCAGCTGCGTTCGCTAAHB39TAGGAAGCTTACGAGACGAACTAATCTAGAATGCAGGCCCAGGCAGCTCCTATTCTGAGAAAGTATAGGAACGCTTGCTGGGAAGAACATCCAGGCCAAGCTCCAGGCCAGGCAGCTGCAGTGCAGGCCGGAGGHB41CGATCTTGTGGGTAGAGACACTCCAGGCCAAGTCCAGGCCCGCGCTCTAGTTGGGAATCAAGTGCATGAGCGCTGAAGHB42ACGASACGAGCGCACCACGAGAGCCCCAGCCGCGCCGCG  | Gib_MB11B1_sypE_US_for | gaggtcgacggtatcgataaGAATGGTCAGATGAAATGTC          |
| Gib_pEVS79_ES_sypE_for gaggtcgacggtatcgatacgatacgatcATTAACC<br>Gib_pEVS79_ES_sypE_for gaggtcgacggtatcgatacagcATTAAGCTTCCATCAC<br>Gib_pEVS79_MB_sypE_for gaggtcgacggtatcgatacagcCATTAAGCTTCCATCCAC<br>Gib_SR5_syp_DS_for gcttatatcgatacagcTGCAGCAGATTAAGAG<br>Gib_SR5_syp_DS_for gcttatatcagCTGCTGCAGGGACAATTAAGAG<br>Gib_SR5_syp_US_for gaggaattcgatatcaagCTGCTGCAGGGCAAATGC<br>Gib_SR5_syp_US_for gaggaattcgatatcaagCTGCTGCAGGGCAAATGC<br>Gib_SR5_syp_US_for gaggaattcgatatCATATAAGCCGTAGGGCAAATGC<br>HB8 ACAAAATTTTAAGCTACTGCACTATCAACCACTCTTAAG<br>HB9 GGGAGGAAATAATCTGGACTATCAACACTCTTAAG<br>HB9 GGGAGGAAATAATCTGGACTATCAACACTCTTAAG<br>HB23 TTGGAGAGCCACGTGCGTTGGCTAA<br>HB39 ACGAAACTTCCTAGGAGCAGACGGG<br>HB41 CGATCTTGTGGGTAGAGCAACTCCAGGTCAGGCCCAGGGAAGTTCCTATTCTCAGAAAGTA<br>TAGGAACCTTCCTAGAAGCAACCTCCAGGTCAGGCCCGCGTCGCAG<br>HB42 ACGAGCGACGTCCTTATATATGCTCGCCAGGCAGTCGCATCAGGCAAGTGCATGAGGAATCAAGTGCATGAGGCGCTGAAG<br>HB46 CGATCTTGTGGGTAGAGACACTCCAGGTCAAGTCCAGGCCCAGGCAGTGGAATGAAGTGCATGAGGCATGAGGCGCTGAAG<br>HB46 CGATCTTGTGGGTAGAGACACTC<br>bink-F1 GAAATTACCATGGAGCCAACACCAGGCCCAGGCCTTAATTATGGATATAATTATAACTATAATGCAT<br>bink-F1 GAAATTACCATGGAGCCAACACCAGGCCAAGC<br>bink-F2 GGCATCATTATGGCAGCAACACGCCGCGCTTATTTTGTAGGAATGAAGTACGGGTAGAGTACGC<br>bink-F2 GGCATCATTATGGCAACCACTTAAAGACG<br>bink-F2 GGCATCATTATGGCAACCACTGCAAGGCCAACGC<br>KMB_037 CTCAAAATGGACGACGCAGGCC<br>JFB_287 MB11B1 ATGGAGTTCAAGGCCAAGG<br>JFB_288 TGTTATAACGACTGCAGG<br>JFB_288 TGTTATAACGACTGCCAGG<br>JFB_288 TGTTATAACGACGACGCAGGC  | Gib_MB11B1_sypE_US_rev | caattaacagCATACCACIGIIGATAAAAAIC                  |
| Gib_peVS79_ES_sypL_revgcaggaattcgatacaagcAATTAAGCTTCCATCGib_peVS79_MB_sypE_forgaggtacggattcgatacaagcTCAACAATTAAGCTTCCATCGib_pEVS79_MB_sypE_revgcaggaattcgatacaagcTCAACAATTAAGCTTCCATCGib_SR5_syp_DS_forgcttattatgATATTTGCTCGAGGACAATAAAAAGGib_SR5_syp_DS_forgcaggaattcgatacaagcTGGTGGTGTAGAATCCATTCGib_SR5_syp_US_revgaggtagcagtatggataaAACCGTAGCGCCAAATGGGib_SR5_syp_US_revgagcaaattCATAATAAGCTCCTAGGGAATAATCCHB8ACAAAATTTTAAGATACTGCACGAGAGCAATAAACCACTCTTAAGHB9GGGAGGAAATAATCAGAATGCGAGAGAGCAGGHB23TTGGACAGCCAGCTGCGTTCGCTAAHB39TAGGAAGCTCACGAGCAGCGCGCTCTTATATATGCTTCGCCAGGAAGTTCCTATTCTCTAGAAAGTAHB41CGATCTTGTGGGTAGAGCAACTCCAGGTCAGCCCGCCCGC  | Gib_pEVS79_ES_sypE_for | gaggtcgacggtatcgataalGTTLACAACICAAIACC            |
| Gib_pEVS/9_MB_sypE_for gaggtcgacggtatcgataaAGTTICACAACLCAATAACLAATAATATATC<br>Gib_SR5_syp_DS_for gcttattatgATATTTGCTCGAGGACAATTAAGCTTCCATC<br>Gib_SR5_syp_DS_for gcttattatgATATTTGCTCGAGGACAATTAAGCTTCCATC<br>Gib_SR5_syp_US_for gaggtatcgatacagctGGTGAGTGTAGAGATCAGATCAATTA<br>Gib_SR5_syp_US_rev gagcaatatCATAATAAGCTCCTAGGGAATAATCC<br>HB8 ACCAAAATTTTAAGATTCCTCAGGGAATAATCC<br>HB8 ACCAAAATTTTAAGATTCCCACGACACCACTCTTAAG<br>HB23 CGGAGGAAATAATCTGACACTGCACAATGG<br>HB39 TAGGAAGCTTACGAGAGCTGCGTCGCTAA<br>HB39 CGGAGGAAATAATCTGACACGACGCGCTCCTTATATATGCTTCGCCAGGAAGTTCCTATTCTCTAGAAAGTA<br>TAGGAAGCTTACCAGAGCCAGCGCGTCCGTCAA<br>HB39 CGGAGGAAATAATCTAGAAGCGAGCTCCTTATATATGCTTCGCCAGGAAGTTCCTATTCTCTAGAAAGTA<br>TAGGAAGCTTACGAGACCACGCGGCTCCGCGCA<br>HB42 ACCAGAGCCAGCGTCGTTCGCCAAGCCAGCCCCGCTCTAGTTTGGGAATCAAGTGCATGAGGCGCTGAAG<br>HB46 CGATCTTGTGGGTAGAGACATCCAGGCCAGCCCGCGCTCTAGTTTGGGAATCAAGTGCATGAGCGCTGAAG<br>HB146 CGATCTTGTGGGTAGAGACCATC<br>binK-F1 GAAATTACCATGGAGCCAACGCAAGCCAAGCCAAGCCAA  | GID_PEVS/9_ES_sypt_rev | gcaggaattcgatatcaagcAATAAGCTICCAICTICAC           |
| Gib_pbV5/9_MB_sypE_rev gcaggaattcgatatcaagcICAACAATIAAACGITCCAITC<br>gib_sbS_syp_DS_for gcttattatgATATTTGCTCGAGGACAATAAAAG<br>Gib_sbS_syp_DS_rev gcaggaattcgatacaagcTGGTGAGTGTAGGAATCAATGG<br>Gib_sbS_syp_US_rev gagcaaattCATAATAAGCTCCTAGGGAATAATCC<br>HB8 ACAAAATTTTAAGATACTGCACGGCACAATCGAAG<br>HB9 GGGAGGAAATAATCTAGAATGCGCACATCACACTCTAAG<br>HB9 GGGAGGAAATAATCTAGAATGCGAGAGGACTACTAAG<br>HB23 TTGGAGAGCCACGTGCGTTCGCTAA<br>HB39 CGGAGGAAATAATCTAGAAGCACACCTCTATATATAGCTTCCACGGAAGTTCCTATGCTAGAAAGTA<br>TAGGAAGCTTACCGAGACGAGCTGCTTAATATAGAGAGCCACGCGCCCGGTCTAGTTGGGAATCAAGTGCATGAGGAG<br>HB41 CGATCTTGTGGGTAGAGACAACCTAAGAGCCAGCCCCGCTCTAGTTTGGGAATCAAGTGCATGAGGCGCTGAAG<br>HB42 ACGAGACCGAGCTTCTTATATATGCTTCGCCAG<br>HB46 CGATCTTGTGGGTAGAGACATCCAGGTCAGTAGCCAGCCCGCGCTCTAGTTTGGGAATCAAGTGCATGAGGCGCTGAAG<br>HB42 ACGAGACCGAGCTTCTTATATATGCTTCGCCAG<br>HB46 CGATCTTGTGGGTAGAGACATC<br>bink-F1 GAAAATTACCATGGAGCCAACGCAAGCC<br>bink-F1 GAAATTACCATGGAGCCAACGCAAGCC<br>bink-F1 GAAATTACCATGGAGCCAACGCAAGCC<br>bink-F1 GAAATTACCATGGAGCCAACGCAAGCC<br>bink-F1 CGACATTAGGAGCCATC<br>bink-F2-RUH gacttgactggatgtcttacccacagaatgCGCTCATTGTATCTATGAGATATAATTATAACTATAATCGC<br>bink-F2<br>bink-F2 CGCATCATTATGCACAACCATTAAGAGC<br>bink-F2<br>KMB_036 CCACAATAGCAGATACAAATTCGCTTG<br>KMB_037 CTCAAAATGACAGTCAGAATCAGCA<br>JFB_287 ATGGAGTTCTACGTCAACCAGCA<br>JFB_288 TGTTATACGATTACGCGCAGCG<br>JFB_288 TGTTATAACGATTACAGTATAGC<br>JFB_288 TGTTATAACGAGTCAGCGCAGCG<br>JFB_288 TGTTATAACGAGTACAGTGAGCAGCG<br>JFB_288 TGTTATACGGCACCGGCAGCG<br>JFB_288 TGTTATACGGCACGGCGCGCGCCCGCCCGCCCGCCCGCCC  | GID_PEVS/9_MB_sypE_for | gaggtcgacggtatcgataaAGIIICACAACICAAIACIAAIAAIAIIC |
| Gib SHS_syp_DS_rorgcttattatqa1A111GC1CGAGGAGTA1AAAAAGGib_SHS_syp_DS_revgcaggaattcgatacaagcTGGTGAGTGTAGAAATCCATTCGib_SRS_syp_US_forgaggcaatatCATAATAAGCTCCTAGGGAGTGTAGAAATCCHB8ACAAAATTTTAAGATACTGCACTATCCAACACCACTCTAAGHB9GGGAGGAAATAATCTGACATCGACGACGAGGAGGAGHB23TTGGAGAGCCAGCGGCGGCGGCGGCGCGCGCGCGCGCGCG  | GID_pEVS/9_MB_sypE_rev | gcaggaattcgatatcaagcICAACAATTAAGCTICCATC          |
| Gib_SR5_syp_DS_rev gcaggattcgatacaagc1GGIGAGAATCCATTC<br>Gib_SR5_syp_US_for gaggtcgacggtatcgataaAACCGTAGGGCCAAATGG<br>Gib_SR5_syp_US_rev gagcaaatatCATAATAAGCTCCTAGGAATAATCC<br>HB8 ACAAAATTTTAAGATACTGCACTATCAACACACACTCTTAAG<br>HB9 GGGAGGAAATAATCTAGAATGCGAGGGGGGGG<br>HB23 TTGGAGAGCCAGCTGCGGTTCGCTAA<br>HB39 TGGAGAGCCTTACGAGAGCGAGCTTCGTAAGGAGAGTTCCTATTCTCTAGAAAGTA<br>TAGGAACTTCCTTAGAAGCAACGAGCTTCTTATATATGCTTCGCCAGGAAGTTCCTATTCTCTAGAAAGTA<br>TAGGAACTTCCTTAGAAGCAACCAGGTCCAGGTCAAGTGCAGGAGATCAAGTGCATGAAGGCCATGAAGCGACGCCGAGAGTTCGCAG<br>HB41 CGATCTTGTGGGTAGAGACATCCAGGTCAAGTCCAGCCCGCCTCTAGTTTGGGAATCAAGTGCATGAGCGCTGAAG<br>HB42 ACGAGACGTCTTATATATGCTTCGCCAG<br>HB44 CGATCTTGTGGGTAGAGACATC<br>binK-F1 GAAATTACCATGGAGCCAACAGCAAGC<br>binK-F1 GAAATTACCATGGAGCCAACAGCAAGAC<br>binK-F2-RUH ctggcgaagcatatataggagtctgtctgCATAAAAACCTAGCGCTTTATTTGTAGATATAATTATTAACTATAATCGC<br>binK-F2 GGCATCATTATGGCAACCACTA<br>binK-F0 CCGTTAATACGAGGACATCCATTAAAGACG<br>binK-F0 CCGTTAATATGGCAACCCATTAAAGACG<br>binK-F0 CCGTTAATACGATTATGGCAGCCATTGAAGTGACGGCGTTGATGTACTGAGGTACGGGTAGAG<br>KMB_036 CCACAATAGCAGAGTATTGGCTG<br>KMB_037 CTCAAAATGACAGTCGAAGTATCGCTG<br>KMB_037 CTCAAAATGACAGTCAAGCGAGGC<br>JFB_287 ATGGAGTTTCACGCAACCAGGA<br>JFB_288 TGTTATACGATTACAGGAGCG<br>JFB_288 TGTTATAACGATTACAGGGCGAGG<br>JFB_288 TGTTATACGATTACAGGGCCAGG<br>M13for   | GID_SR5_syp_DS_for     | gcttattatgAIAIIIGCICGAGGACAAIAAAAAG               |
| Gib_SR5_syp_US_rev gagcaacggtatcgatgatAACCG IAGCGCCAAAIGG<br>Gib_SR5_syp_US_rev gagcaaataCATAATAAGCTCCTAGGGAATAATCG<br>HB8 ACAAAATTTTAAGATACTGCACTATCAACACACTCTTAAG<br>HB9 GGGAGGAAATAATCTAGAATGCGACGATGG<br>HB23 TTGGAGAGCCAGCTGCGTTCGCTAA<br>HB39 TAGGAAGCTTACGAGAGCGAGCTTCTTATATATGCTTCGCCAGGAAGTTCCTATTCTCTAGAAAGTA<br>TAGGAACTTCCTTAGAAGCAAACTTAAGAGTGG<br>HB41 CGATCTTGTGGGTAGAGACATCCAGGTCAAGTCCAGCCCCGCTCTAGTTTGGGAATCAAGTGCATGAGCGCTGAAG<br>HB42 ACGAGACGACGACGTTCTTATATATGCTTCGCCAG<br>HB146 CGATCTTGTGGGTAGAGACATC<br>binK-F1 GAAATTACCATGGAGCCACACAGCAGC<br>binK-F1 GAAATTACCATGGAGCCAACAGCAAGC<br>binK-F2-RUH gacttgacatgaagactcgtctcgtCATAAAAACCTAGGGCTTATGTAGATATAATTATTAACTATAATCGC<br>binK-F2-RUH gacttgactggatgttcttacccacaagatcgCGCTCATTGTATGTAGATATGCTGAGGTTACG<br>binK-F0 CCGTTAATACTGGAATACCAGTGAAGTC<br>binK-F0 CCGTTAATACTGGAATACCAGTGAAGTC<br>MB_037 CTCAAAATGACAGAATACCAAATTCGCTG<br>KMB_037 CTCAAAATGACAGATACCAAATTCGAGC<br>JFB_287 MB11B1 ATGGAGTTTTACGTCAACCAGGA<br>JFB_288 TGTTATAACGATTGACTGAGGC   | GID_SR5_syp_DS_rev     | gcaggaattcgatatcaagc1GG1GAG1G1AGAA1CCA11C         |
| GID_SKS_SYP_05_FeV       gagcaaataCATAATAAGCTCCTAGGGAATAATCC         HB8       ACAAAATTTTAAGATACTGCACTATCAACACACTCTTAAG         HB9       GGGAGGAAATAATCTAGAATGCGAGAGTAGG         HB23       TTGGAGAGCCAGCTGCGTTCGCTAA         HB39       TAGGAAGCTTACGAGACGAGCGTCTTTATATATGCTTCGCCAGGAAGTTCCTATTCTCTAGAAAGTA         TAGGAACTTCCTTGGGGTAGAGACATCCAGGTCAGGCCAGCCGCCGCTCTAGTTTGGGAATCAAGTGCATGAGCGCTGAAG         HB41       CGATCTTGTGGGTAGAGACATCCAGGCCACAGC         HB42       ACGAGACGACCACCAGCAAGCA         bink-F1       GAAATTACCATGGAGCCAACAGCAAGCAAGC         bink-F2       bink-F1         cdgagaatataagaagctcgtctcgtCATAAAAAACCTAGCGCTTTATATTGAGATATAATTATTAACTATAATCGC         bink-F2       GGCATCATTATGGCAACCATTAAGAGCG         bink-F2       GGCATCATTATGGCAACCATTAAAGACG         bink-F2       GGCATCATTATGGCAACCATTAAAGACG         bink-F0       CCGTTAATACTGGATTATTCGCTTGAATTGAACG         KMB_037       CTCAAAATGACAGTACAGGATATCGAGGC         JFB_287       ATGGAGTTTTTACGTCAACCAGAA         JFB_288       TGTTATAACGAATTACCGCAGCAGG         JFB_288       TGTTATAACGAATTACCAGGAGA         JFB_365       GGAAAGAGAATGATTACAGGCGAGG   | GID_SR5_syp_US_for     |   |
| HB8       ACAAAATTTTAAGATACTGCACACACTACTAAGA         HB9       GGGAGGAAATAATCTAGAATGCGAGAGTAGG         HB23       TTGGAGGCCAGCTGCGTTCGCTTAA         HB39       TAGGAAGCTTACGAGACGAGCTTCTTATATATGCTTCGCCAGGAAGTTCCTATTCTCTAGAAAGTA         TAGGAACTTCCTTAGAAGCAAACTTCAGAGTGCG       TAGGAAGCTTCCTTGGGAGAGCAACTCCAGGTCCAGGTCCAGGTCCAGGTCCAGGTGGAATCAAGTGCATGAGGCGCTGAAG         HB41       CGATCTTGTGGGTAGAGACATCCAGGTCCAGGTCCAGCCCCGCCTCTAGTTTGGGAATCAAGTGCATGAGCGCTGAAG         HB42       ACGAGACGAGCTTCTTATATATGCTTCGCCAG         bink-F1       GAAATTACCATGGGGCCAACAGCAAGCA         bink-F1       GAAATTACCATGGGGCCAACAGCAAGAC         bink-F2-RUH       gacttgacctggatgctctaccacaagatcgCGCTCATTGTATCTATAGAGTATAATTATTAACTATAATCGC         bink-F2       GGCATCATTATGGCAACCATTAAGAACG         bink-F0       CCGTTAATACTGGATTATCGCTTGAATTGACG         KMB_036       CCCACAATAGCAGAATACAAATTCGCTG         KMB_037       CTCAAAATGACGACAGCAGCAGCA         JFB_287_MB1181       ATGGAGTTTTACGTCAACCAGAGA         JFB_288       TGTTATAACGATCAGCAGCG         JFB_288       TGTTATAACGATGCAACCAGGA         JFB_365       GGAAAGAATGATTAAG         M136or       GTAAAAGCGCCAGC  | GID_SR5_syp_US_rev     |   |
| HB9       GGGAGGAAATAATAATACTAAGGGAAGTAGG         HB23       TTGGAGAGCCAGCTGCGTTCGCTAA         HB39       TAGGAAGCTTACGAGAGCAGCTTCTTATATATGCTTCGCCAGGAAGTTCCTATTCTCTAGAAAGTA         TAGGAACTTCCTTAGAAGCAAACTTAAGAGTGG       TAGGAACTCCTTGTGGGTAGAGACATCCAGGTCCAGGTCCAGCTCCAGTTTGGGAATCAAGTGCATGAGCGCTGAAG         HB42       ACGAGACGAGCTTCTTATATATGCTTCGCCAG         HB146       CGATCTTGTGGGTAGAGACATC         bink-F1       GAAATTACCATGGAGCCAACAGCAACGC         bink-F1       GAAATTACCATGGAGCCAACAGCAACGC         bink-F2-RUH       gacttgactggagtctctcaccaagagtcgCGCTCATTGTATCTATAGAGTATGAGTATGAGTACG         bink-F2       GGCATCATTATGGCAACCATTAAAGACG         bink-F0       CCGTTAATATGGCAACCATTAAAGACG         KMB_036       CCACAATAGCAGAATACAAATTCGCTG         KMB_037       CTCAAAATGACAGTCAACGAGAGTATCGTAGGC         JFB_287       ATGGAGTTTTCACGTCAACCAGAA         JFB_288       TGTTATACGACGACGAGG         JFB_288       TGTTATACGAGCAGCG         JFB_365       GGAAAGAGAATGATTACATGCAGGC         JFB_365       GGAAAGAGAATGATTACATGCAGGC         JFB365       GGAAAGAGAATGATTACAAGGCAGC   |                        |   |
| HB23       HIGGAGAGCCAGCIGCICGCITATA         HB39       TAGGAAGCTTACGAGACGAGCGTCTTATATATGCTTCGCCAGGAAGTTCCTATTCTCTAGAAAGTA         HB41       CGATCTTGTGGGTAGAGACAACCCAGGTCAGGTCCAGCCCCGCTCTAGTTTGGGAATCAAGTGCATGAGCGCTGAAG         HB42       ACGAGACGAGCTTCTTATATATGCTTCGCCAG         HB146       CGATCTTGTGGGTAGAGACATC         binK-F1       GAAATTACCATGGAGCCAACAGCAAGAC         binK-R1-LUH       ctggcgaagcatatataagaagctcgtctgtCATAAAAAACCTAGCGCTTTATTGTAGATATAATTATTAACTATAATCGC         binK-R2       GGCATCATTATGGCAACCACTCAACAGCAACAGC         binK-R2       GGCATCATTATGGCAACCATTGAAGAGC         binK-R2       GGCATCATTATGGCAACCAATGCAGCGCCAACAGCGCTTTATTGTAGATATAATTATAACTATAATCGC         binK-R2       GGCATCATTATGGCAACCATTAAAGACG         binK-F0       CCGTTAATACTGGATTATTCGCTTGAACG         KMB_036       CCACAATAGCAGAATACAAATTCGCTG         KMB_037       CTCAAAATGACAGTCAGAGTATCGTAGGC         JFB_287       ATGGAGTTTCTACGTCAACCAGAG         JFB_287_MB11B1       ATGGAGTTTCTACGTCAACCAGAG         JFB_288       TGTTATAACGATTACAGCAGCG         JFB_288       TGTATAACGATTACAGGAGCG         JFB_365       GGAAAGAGAATGATTAAG         M13for       GTAAAACGACGGCCAG   | НВУ                    |   |
| HB39TAGGAAGCTTACGAAGCAGCTTCTTATATAGCTTCGCCAGGAAGTTCCTATCTCTAGAAGGTATAGGAACTTCCTTAGAAGCAGACGAGCTTCTTATAGGCTCGCAGGTCAGGGAAGTTCCAGCCCCGCTCTAGTTTGGGAATCAAGTGCATGAGCGCTGAAGHB41CGATCTTGTGGGTAGAGACATCCAGGTCCAGGCCAGGHB42ACGAGACGACGTTCTTTATATAGCTTCGCCAGbinK-F1GAAATTACCATGGAGCCAACAGCAACAGCbinK-R1-LUHctgcgaagcatatataagaagctcgtctgtCATAAAAAAACCTAGCGCTTTATTGTAGATATAATTATTAACTATAATCGCbinK-R2GGCATCATTATGGCAACCAATTAAAGACGbinK-F0CCGTTAATACTGGATTATTGGCTAGAGTATCGAGTKMB_036CCACAATAGCAGAATACAAATTCGCTGKMB_037CTCCAAAATGACAGTCAAGCAGAAJFB_287ATGGAGTTTCACGTCAACCAGAAJFB_288TGTTATAACGATTACGCAACCAGAAJFB_285GGAAAGAGATTACAAGTACAAGGJFB_365GGAAAGAGAATGATTAAGGM13forGTAAAACGACGCCCAGG  | HB23                   |   |
| HAGGAACTTCCTTAGAAGCAAACTTAGAAGCAAACTTAGAGGTGTGHB41CGATCTTGTGGGTAGAGACATCCAAGTCCAGCCCGCCCGC  | HB39                   |   |
| HB41CGATCHTGTGGGTAGAGACATCCAGGTCAAGTCCAGGTCCAGTCCAGGTCTAGTTGGGAATCAAGTGCAGGGCGAGAGHB42ACGAGACGAGGTTCTTATATATGCTTGGCCAGHB146CGATCTTGTGGGTAGAGACATCbinK-F1GAAATTACCATGGAGCCAACAGCAGCAbinK-R1-LUHctggcgaagcatatataagaagctcgtctgtCATAAAAAAACCTAGCGCTTTATTTGTAGATATAATTATTAACTATAATCGCbinK-F2-RUHgacttgacctggatgtctctacccacaagatgCGCTCATTGTATCTATAGAGTATGACTGAGTTACGbinK-F0CCGTTAATACTGGATTATTGGCAACCATTAAAGACGbinK-F0CCGTTAATACTGGATTATTCGCTTGAATTTGAACGKMB_036CCACAATAGCAGAATACAAATTCGCTGKMB_037CTCAAAATGACAGTCAGAGTATCGTAGGCJFB_287ATGGAGTTTCTACGTCAACCAGAAJFB_287_MB11B1ATGGAGTTTTACGTCAACCAGAAJFB_288TGTTATAACGATTACAGGCAGGGJFB_365GGAAAGAGAATGATTAAGM13forGTAAAACGACGGCCGG   | 110.41                 |   |
| HB42ACGAGACGAGCTTCTTATATAGCTTCGCCAGHB146CGATCTTGTGGGTAGAGACATCbinK-F1GAAATTACCATGGAGCCAACGCAAGCAAGCAbinK-R1-LUHctggcgaagcatatataagagactcgtccgtCATAAAAAAACCTAGCGCTTTATTTGTAGATATAATTATTAACTATAATCGCbinK-R2gacttgacctggatgtctctacccacaagatcgCGCTCATTGTATCTATAGAGTATGAACGAGTAGCGbinK-F0CCGTTAATACTGGATTATTGGAACCATTAAAGACGbinK-80CCACAATAGCAGAATACAAATTCGCTGAACTTGAACGKMB_036CCACAATAGCAGAATACAAATTCGCTGKMB_037CTCAAAATGACAGTCAGAGTATCGTAGGCJFB_287ATGGAGTTTCTACGTCAACCAGAAJFB_288TGTTATAACGATTACCGAGAGJFB_288GGAAAGAGAATGATTAAGJFB_365GGAAAGAGAATGATTAAGM13forGTAAAACGACGGCCAG  | HB41                   |   |
| HB146CGATCITIGIGGIAGAGACATCbink-F1GAAATTACCATGGAGCAACAGCAAGCAAGCAbink-R1-LUHctggcgaagcatatatagagagctcgtcgtCATAAAAAAACCTAGCGCTTTATTTGTAGATATAATTATTAACTATAATCGCbink-F2-RUHgacttgacctggatgtctctacccacaagatcgCGCTCATTGTATCTATAGAGTATGTACTGAGTTACGbink-R2GGCATCATTATGGCAACCATTAAAGACGbink-F0CCGTTAATACTGGATTATTCGAGATACAACTGCTTGAATTGAACGKMB_036CCACAATAGCAGAATACAAATTCGCTGKMB_037CTCAAAATGACAGTCAGAGTATCGTAGGCJFB_287ATGGAGTTTCTACGTCAACCAGAAJFB_287_MB11B1ATGGAGTTTTACGTCAACCAGAGJFB_288TGTTATAACGATTACTAGGCAGCGJFB_365GGAAAGAGAATGATTAAGM13forGTAAAACGACGGCCAG   | HB42                   |   |
| DINK-F1GAAATTACCATGGAGCCAACAGCAAGACbink-R1ctggcgaagcatatatagagagctcgtccgtCAACAGCAAGACbink-R1-LUHctggcgaagcatatatagagagctcgtcgtCAACAGCAAGAACCTAGCGCTTTATTTGTAGATATAATTATTAACTATAATCGCbink-F2-RUHgacttgacctggatgtctctacccacaagatcgCGCTCATTGTATCTATAGAGTATGTACTGAGTTACGbink-R2GGCATCATTATGGCAACCATTAAAGACGbink-F0CCGTTAATACTGGATTATTCGCTGAATTTGAACGKMB_036CCACAATAGCAGAATACAAATTCGCTGKMB_037CTCAAAATGACAGTCAGAGTATCGTAGGCJFB_287ATGGAGTTTCTACGTCAACCAGAAJFB_287_MB11B1ATGGAGTTTTACGTCAACCAGAGJFB_288TGTTATAACGATTACTGGCAGCGJFB_365GGAAAGAGAATGATTAAGM13forGTAAAACGACGGCCAG   | HB140                  |   |
| bink-R1-LOHctggcgaagcaalaaagaagccgictcgictGictGictGictGictGictGictGictGictGictG   |                        |   |
| bink-P2-ROH GGCATCATTATGGCAGCGG<br>bink-R2 GGCATCATTATGGCACCATTAAAGACG<br>bink-FO CCGTTAATACTGGATTATTCGCTGAATTGAACG<br>KMB_036 CCACAATAGCAGAATACAAATTCGCTG<br>KMB_037 CTCAAAATGACAGTCAGAGTATCGTAGGC<br>JFB_287 MB11B1 ATGGAGTTTCTACGTCAACCAGAA<br>JFB_288 TGTTATAACGATTACCAGCG<br>JFB_365 GGAAGAGAATGATTAAG   |                        |   |
| bink-n2 GGCATCATTATGGCACCATTATAGACG<br>bink-FO CCGTTAATACTGGATTATTGGCATCTTGAACTTGAACG<br>KMB_036 CCACAATAGCAGAATACAAATTGGCTG<br>KMB_037 CTCAAAATGACAGTATCAGAGTATCGTAGGC<br>JFB_287 ATGGAGTTTCTACGTCAACCAGAA<br>JFB_288 TGGTATAACGACTGACCAGAG<br>JFB_365 GGAAGAGAATGATTAAG<br>M13for GTAAAACGACGGCCAG  | bink P2                |   |
| blink-ro       CCGTAATACTGGATTATTGGATTGGATTGAACG         KMB_036       CCACAATAGCAGATACAAATTCGCTG         KMB_037       CTCAAAATGACAGTACCAGAGTATCGTAGGC         JFB_287       ATGGAGTTTCTACGTCAACCAGAG         JFB_287_MB11B1       ATGGAGTTTTACGTCAACCAGAG         JFB_288       TGTTATAACGACTACATGGCAGCG         JFB_365       GGAAAGAGAATGATTAAG         M13for       GTAAAACGACGGCCAG   | bink-rz                |   |
| KMB_035     CCCACAATGCCAGATACAAATGCGCG       KMB_037     CTCAAAATGACAGTACCAGAGTATCGTAGGC       JFB_287     ATGGAGTTTCTACGTCAGCCAGAG       JFB_287_MB11B1     ATGGAGTTTTTACGTCAACCAGAG       JFB_288     TGTTATAACGATTACATGGCAGCG       JFB_365     GGAAAGAGAATGATTAAG       M13for     GTAAAACGACGGCCAG   |                        |   |
| JFB_287 ATGGAGTTTCTACGTCAACCAGAA<br>JFB_287_MB11B1 ATGGAGTTTTACGTCAACCAGAG<br>JFB_288 TGTTATAACGATTACATGGCAGCG<br>JFB_365 GGAAGAGAATGATTAAG<br>M13for GTAAAACGACGGCCAG  | KMB 037                |   |
| JFB_287_MB11B1 ATGGAGTTTTACGTCAACCAGAG<br>JFB_288 TGTATAACGATTACATGGCAGCG<br>JFB_365 GGAAGAGAATGATTAAG<br>M13for GTAAAACGACGGCCAG   | IFR 287                |   |
| JFB_288 TGTTATAACGATTAACGAGGGGGGGG<br>JFB_365 GGAAGGAGAATGATTAAG<br>M13for GTAAAACGACGGCCAG   | IFR 287 MR11R1         |   |
| JFB_365 GGAAAGAGAATGATTAAG<br>M13for GTAAAACGACGGCCAG   | IFR 288                | TGTTATAACGATTACATGGCAGCG                          |
| M13for GTAAAACGACGGCCAG   | IFB 365                | GGAAAGAGAATGATTAAG                                |
|   | M13for                 | GTAAAACGACGACGAG                                  |

(Continued on next page)

## TABLE 3 (Continued)

| Primer name      | Sequence <sup>a</sup> (5' to 3')                |
|------------------|---|
| M13rev           | CAGGAAACAGCTATGAC                               |
| MB11B1_indel_for | GCTTTTTCAGATACAAAGCCA                           |
| MB11B1_indel_rev | ATACCTGATGGAAACGACCT                            |
| MJM-154F         | TAAAAAGGGAATTAATCCGC                            |
| MJM-306R         | AACTCTAACCAAGAAGCA                              |
| pEVS107_3837     | GGCGCGCCTAGGGCCCTC                              |
| pEVS107_3838     | TCGAGGTACCTGGCCACTAG                            |
| pEVS79_for_691   | GCTTGATATCGAATTCCTG                             |
| pEVS79_rev_690   | TTATCGATACCGTCGACC                              |
| rev_ver_sypE     | TTCACCATGAGTGCCAAATC                            |
| rscS_del1F       | CTTATCTTCTAGTTCTTTTTTTAGTGATGTCTCTTTCTACGGC     |
| rscS_del1R       | GCCGTAGAAAGAGACATCACTAAAAAAAAAAAGAACTAGAAGATAAG |
| rscS_ver_1       | GTAATTCAGTAATGCTACC                             |
| rscS_ver_2       | GTCGCACCGTCAGGTATA                              |
| rscS_ver_3       | AAGAAATTATTCGCTACC                              |
| rscS_ver_4       | AGTTAGTAGGCCATTACG                              |
| SR5_syp_ver_for  | TAGGCGTATCAAAAACCACCT                           |
| SR5_syp_ver_rev  | TCAGGAATGTCGATGGCAG                             |
| Syp_ver_DS_rev   | ATCGAGCATATTTTGCCAATC                           |
| Syp_ver_US_for   | ACCTATCAACTCTTAAGTCGATTC                        |
| syp4F            | TGAGGATCCCATCGTGCCATA                           |
| syp4R            | AGCTCCTTTGCAATGTTTGCTT                          |
| syp5F            | TATTAGGCCGTTTCCACCAGG                           |
| syp5F-B          | TATTAGGTCGTTTCCATCAGG                           |
| sypA_out         | AACAGGAATTGCGTTTTCAA                            |
| US_syp_flank_for | ACCACTGTGATAACTTGCAC                            |
| US_syp_flank_rev | ATGAGGCATAACCTGTTCCA                            |

<sup>&</sup>lt;sup>a</sup>For Gibson Assembly primers, capital letters indicate homology to the template. All primers were designed for this study, except MJM-154F and MJM-306R (22), JFB\_287, JFB\_288, and JFB\_365 (18), and M13 for and M13 rev.

consisting of approximately 300 bp upstream of *sypE* up through nucleotide 33 and the C-terminal portion consisting of nucleotide 33 and the remaining *sypE* gene. The overlap between the two halves contained the single-nucleotide polymorphism in the primers that connected them. The altered *sypE* alleles were initially cloned into plasmid pEVS107 (linearized with primers pEVS107\_3837/pEVS107\_3838) using Gibson Assembly, and then the entire altered *sypE* allele was subcloned into pEVS79 with Gibson Assembly (Table S1). After double recombination of the vector into *V. fischeri*, candidate colonies for the altered *sypE* in MJM1100 were screened with primers ES114\_indel\_for/ES114\_indel\_rev. The primer set anneals more strongly to the wild-type *sypE* sequence than to *sypE*(ntG33::Δ). Candidates in the MJM1100 background with a fainter PCR band were sequenced and confirmed to have the *sypE*(ntG33::Δ) allele. For MJM1130, the primer set MB11B1\_indel\_for/MB11B1\_indel\_rev anneals more strongly to the naturally occurring *sypE* allele, and candidates in MJM1130 that contained a more robust PCR band were selected for sequencing to be confirmed as being *sypE*(nt33::G).

**Construction of pKG11 rscS1(ntA1141::Δ).** Plasmid pKG11 encodes an overexpression allele of RscS, termed *rscS1* (15, 28). *rscS* nucleotide A1141 was deleted on the plasmid using the Stratagene QuikChange II site-directed mutagenesis kit with primers rscS\_del1F and rscS\_del1R. The resulting plasmid, pMJM33, was sequenced with primers MJM-154F and MJM-306R to confirm the single base pair deletion.

Squid colonization. Hatchling E. scolopes organisms were colonized by exposure to approximately  $3 \times 10^3$  CFU/ml (ranging from 5.2  $\times 10^2$  to  $1.4 \times 10^4$  CFU/ml, as specified in the figure legends) for each strain in a total volume of 40 ml of FSIO (filter-sterilized Instant Ocean) for 3 h. Squid were then transferred to 100 ml of FSIO to stop the inoculation and then transferred to 40 ml FSIO for an additional 45 h, with a water change at 24 h postinoculation. For Fig. 10A, kanamycin was added to the FSIO to keep selective pressure on the plasmid. After 48 h of colonization, the squid were euthanized and surface sterilized by storage at -80°C according to standard practices (51). For determination of CFU per light organ, hatchlings were thawed and homogenized, and 50  $\mu$ l of homogenate dilutions was plated onto LBS plates. Bacterial colonies from each plate were counted and recorded. Mock-treated, uncolonized hatchlings ("apo-symbiotic") were used to determine the limit of detection in the assay. The competitive index (CI) was calculated from the relative CFU of each sample in the output (light organ) versus the input (inoculum) as log10{[test strain (light organ)/control strain (light organ)]/[test strain (inoculum)/control strain (inoculum)]]. For competitions of natural isolates, the group A strain (or its  $\Delta rscS$  derivative) was the test strain and the group B strain was the control strain. Colony color was used to enumerate colonies from each, white for group A strains MB11B1 and ES213 and yellow for group B strains ES114 and MB15A4, along with PCR verification of selected colonies. For competition between SR5 and SR5 ΔbinK, 100 colonies per squid were patched onto LBS-Erm5 and LBS.

**Colony biofilm assays.** Bacterial strains were grown in LBS medium (Fig. 10C) or LBS-Cam2.5 medium (Fig. 2 and 8) for approximately 17 h, and then 10  $\mu$ l (Fig. 2) or 8  $\mu$ l (Fig. 8, 10C) was spotted

onto LBS plates (Fig. 10C) or LBS-Tet5 plates (Fig. 2 and 8). Spots were allowed to dry and the plates incubated at 25°C for 48 h. Images of the spots were taken at 24 and 48 h postspotting using a Leica M60 microscope and Leica DFC295 camera. After 48 h of growth, the spots were disrupted using a flat toothpick and imaged similarly.

**Analysis of DNA and protein sequences** *in silico*. Amino acid sequences for *V. fischeri* ES114 *syp* genes were obtained from RefSeq accession no. NC\_006841.2. Local TBLASTN queries were performed for each protein against nucleotide databases for the following strains, each of which were derived from the RefSeq cds\_from\_genomic.fna file: *V. fischeri* SR5 (GCA\_000241785.1), *V. fischeri* MB11B1 (GCA\_001640385.1), and *V. vulnificus* ATCC 27562 (GCA\_00224265.1). Percent amino acid identity was calculated as the identity in the BLAST query divided by the length of the amino acid sequence in ES114. Domain information is from the PFAM database (52).

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JB .00033-19.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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