Retraction ATPase motors are promiscuous among type IVa pilus systems

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Running title: T4aP retraction ATPase motors are promiscuous

ABSTRACT

Bacterial surface appendages called type IVa pili (T4aP) promote diverse activities including DNA uptake, twitching motility, and virulence. These activities rely on the ability of T4aP to dynamically extend and retract from the cell surface. Dynamic extension relies on a motor ATPase commonly called PilB. Most T4aP also rely on specific motor ATPases, commonly called PilT and PilU, to dynamically and forcefully retract. Here, we systematically assess whether motor ATPases from 4 distinct T4aP could functionally complement *Vibrio cholerae* mutants that lacked their endogenous motors. We found that the retraction ATPases PilT and PilU are highly promiscuous and promote retraction of the *V. cholerae* competence T4aP despite a high degree of sequence divergence. In contrast, orthologous extension ATPases were not able to mediate extension of the *V. cholerae* competence T4aP despite a similar degree of sequence divergence. Also, we show that one of the PilT orthologs characterized does not support PilU-dependent retraction and we attributed this loss of activity to the 3' end of the gene, which suggests that the C-terminus of PilT plays an important role in promoting PilU-dependent retraction. Together, our data suggest that retraction ATPases have maintained a high degree of promiscuity for promoting retraction of diverse T4aP, while extension ATPases have evolved to become highly specific for their cognate systems.

IMPORTANCE

One way that bacteria interact with their environments is via hair-like appendages called type IVa pili (T4aP). These appendages dynamically extend and retract from the cell surface via the action of distinct ATPase motors. T4aP are present in diverse bacterial species. Here, we demonstrate that retraction motors from T4aP are highly promiscuous, and capable of promoting retraction of heterologous T4aP systems. By contrast, extension ATPase motors are highly specific and cannot promote extension of heterologous T4aP. Thus, these results suggest that T4aP extension may be more tightly regulated compared to T4aP retraction.

INTRODUCTION

Type IVa pilus systems (T4aP) are encoded by diverse bacterial species. These systems promote the dynamic extension and retraction of filamentous appendages called pili from the bacterial surface. Through this dynamic activity, T4aP carry out a variety of functions including twitching motility, surface attachment, biofilm formation, virulence, and DNA uptake (1). Dynamic extension of all T4aP relies on the activity of a dedicated motor ATPase, which is commonly called PilB (2). Some systems also have an additional accessory extension ATPase motor called TfpB (3). Dynamic retraction of T4aP canonically relies on a dedicated retraction ATPase called PilT. Additionally, many T4aP systems also contain an accessory retraction

ATPase called PilU. PilU is a PilT-dependent retraction ATPase that is required for forceful retraction of the competence T4aP in *V. cholerae* (4) and is required for twitching motility in other bacterial species (5). These hexameric motors are thought to function via a mechanical interaction with the pilus platform, which together take pilin subunits from the inner membrane and polymerize them into a filament during pilus extension or depolymerize these subunits and return them to the inner membrane during pilus retraction. Conformational changes in the ATPase motors due to ATP binding and hydrolysis are thought to power these processes (6). Previous studies have demonstrated that retraction motors can be interchangeable between some T4aP systems (7). However, a systematic analysis to assess motor promiscuity among T4aP is lacking.

Vibrio cholerae is a facultative pathogen that encodes a competence T4aP, which promotes DNA uptake for natural transformation (8, 9). *Acinetobacter baylyi* and *Pseudomonas stutzeri* encode functionally analogous competence T4aP that also promote horizontal gene transfer (3, 10-12). All of these T4aP systems encode canonical PilB, PilT, and PilU motor ATPases. The T4aP of *A. baylyi* also encodes an additional extension motor called TfpB that is required for initiating pilus extension (3). Some strains of *Escherichia coli* also encode an ortholog of PilT (also referred to as *yggR*) though these strains are not predicted to encode a PilU ortholog.

Here, we sought to formally test promiscuity among T4aP extension and retraction ATPases by using the motors from the systems discussed above. We and others have demonstrated that the competence pilus of *Vibrio cholerae* is a highly genetically tractable system for studying T4aP dynamic activity (4, 7, 8, 13). Thus, we assessed promiscuity among T4aP motors by determining if ectopic expression of heterologous motors could functionally complement *V. cholerae* mutants that lack their endogenous motor ATPases. Our data suggests that while the retraction ATPases PilT and PilU may be promiscuous in their ability to mediate retraction of T4aP systems, extension ATPases display a high level of specificity for their cognate T4aP. Furthermore, our data suggest that the C-terminus of PilT may play an important role in supporting PilU-dependent retraction.

RESULTS

Retraction ATPase orthologs are promiscuous among T4aP systems, while extension ATPase orthologs are not.

Because previous studies have shown that retraction ATPases from T4aP systems may be promiscuous (7), we first wanted to determine if this characteristic is conserved among other bacterial species. To test this, we ectopically expressed *pilT* from *A. baylyi* (*pilT_{Ab}*), *P. stutzeri* (*pilT_{Ps}*), and *E. coli* (*pilT_{Ec}*) in *V. cholerae* strains where the native *pilT* or *pilTU* genes were inactivated (i.e., $\Delta pilT$ and $\Delta pilTU$ backgrounds). As a control, we also ectopically expressed *V. cholerae pilT* (*pilT_{Vc}*). Competence pili in *V. cholerae* promote DNA uptake during natural transformation, a function that requires both pilus extension and retraction (8, 14). Thus, we functionally assessed competence pilus dynamic activity by performing natural transformation assays. As expected, ectopic expression of *pilT_{Vc}* rescued the transformation defect of the $\Delta pilT$ and $\Delta pilTU$ strains (**Fig. 1A**). Furthermore, we observed that ectopic expression of *pilT_{Ab}*, *pilT_{Ps}*, and *pilT_{Ec}*, all fully complemented natural transformation of $\Delta pilT$ and $\Delta pilTU$ *V. cholerae* (**Fig. 1A**). These data indicate that all of these retraction ATPases are capable of mediating retraction of the *V. cholerae* competence T4aP, suggesting that the promiscuity of PilT retraction ATPases is conserved across these species.

In addition to the dominant retraction ATPase PilT, many T4aP also encode an accessory retraction ATPase called PilU that can promote pilus retraction in a PilT-dependent manner (4,

7). So next, we wanted to test whether PilU from *A. baylyi* and *P. stutzeri* could complement a *V. cholerae* *pilU* mutant (the *E. coli* strain does not have a *pilU* ortholog). To test this, we ectopically expressed *pilU_{Ab}* and *pilU_{Ps}* in a *V. cholerae* strain where native *pilT* contained a Walker A mutation (*pilT^{K136A}* = *pilT^{WA}*) and the native *pilU* gene was inactivated (i.e., Δ *pilU*). Mutation of the invariantly conserved lysine in the Walker A domain (K136A) inhibits the ATPase activity of *pilT* (15), thus, efficient pilus retraction in this background (and correspondingly a high frequency of natural transformation) is reliant on PilU ATPase activity (4, 7). As expected, ectopic expression of *pilU_{Vc}* recovered the transformation defect of the *pilT^{WA}* Δ *pilU* strain to parental levels (**Fig. 1B**). The expression of *pilU_{Ab}* also showed almost full recovery of transformation when compared to the parent strain (**Fig. 1B**). Interestingly, ectopic expression of *pilU_{Ps}* only partially complemented the transformation defect of this strain (**Fig. 1B**). This suggested that PilU_{Ps} was either (1) generally a less functional PilU motor, or perhaps (2) specifically less active at promoting pilus retraction in conjunction with PilT_{Vc}. To test this further, we ectopically expressed *pilU_{Ps}* in a background that also ectopically expressed a Walker A mutant of its cognate PilT (*pilT_{Ps}^{WA}*). We found that in the presence of PilT_{Ps}^{WA}, PilU_{Ps} was able to completely rescue natural transformation (**Fig. S1**). Together, this suggests that while PilU is also promiscuous between T4aP, it is perhaps less promiscuous than PilT.

Because we found that retraction ATPases are, indeed, promiscuous between these T4aP, we next wanted to test whether the extension ATPases from these same organisms were similarly promiscuous. To test this, we ectopically expressed *pilB* orthologs from *A. baylyi* (*pilB_{Ab}* and *tfpB_{Ab}*) and *P. stutzeri* (*pilB_{Ps}*) in a *V. cholerae* Δ *pilB* mutant and assessed natural transformation (the *E. coli* PilB ortholog, HofB, was not tested). While *pilB_{Vc}* recovered natural transformation as expected, ectopic expression of *pilB_{Ab}*, *tfpB_{Ab}*, and *pilB_{Ps}* failed to complement the Δ *pilB* mutant (**Fig. 1C**). This suggests that extension ATPase orthologs from these species are not able to mediate extension of the *V. cholerae* competence pilus. These data suggest that there may be a high degree of specificity among extension ATPases, which is in stark contrast to the observed promiscuity among retraction ATPases.

Extension and retraction ATPases likely interact with the pilus machinery platform protein PilC to promote dynamic pilus activity (16). Thus, the observed promiscuity and/or specificity among these motor ATPases may be attributed to their ability and/or inability to interact with PilC. To test this, we assessed protein-protein interactions genetically using Bacterial Adenylate Cyclase Two-Hybrid (BACTH) assays (17). First, looking at PilT, we found that each of the PilT orthologs could self-interact, which is consistent with the hexameric conformation that likely all pilus motors adopt (**Fig. 1D**). Also, consistent with the observation that all of the PilT orthologs tested support natural transformation, we found that all of them could interact with PilC_{Vc} (**Fig. 1D**). Because PilU is a PilT-dependent retraction ATPase, it may need to interact with both PilT and PilC to support pilus retraction. Indeed, all of the PilU orthologs could interact with PilT_{Vc}, PilT_{Vc}^{WA}, and PilC_{Vc}, which is consistent with the ability of each PilU ortholog to support some degree of natural transformation (**Fig. 1E**). For the PilB orthologs, we found that PilB_{Vc} could interact with PilC_{Vc} as expected (**Fig. 1F**). Additionally, each of the PilB orthologs could self-interact, which is consistent with the hexameric conformation that these proteins likely adopt (**Fig. 1F**). Interestingly, PilB_{Ab}, TfpB_{Ab}, and PilB_{Ps} varied in their ability to interact with PilC_{Vc}. PilB_{Ab} and PilB_{Ps} could not interact with PilC_{Vc} (**Fig. 1F**), which could suggest that this interaction is required for extension of the competence pilus. However, TfpB_{Ab} could interact with PilC (**Fig. 1F**), but it could not support natural transformation (**Fig. 1C**). These data indicate that while interactions between PilB and PilC may be required for extension of the competence pilus, these interactions are not sufficient to promote extension.

The PilT ortholog from E. coli cannot support PilU-mediated retraction.

Above, our results indicate that PilT orthologs from distinct T4aP can promote retraction of the *V. cholerae* competence pilus in the absence of native PilT_{Vc} and PilU_{Vc}. PilT, however, can also support PilU-dependent retraction, in a manner that is not dependent on PilT ATPase activity (4). So next, we wanted to test if the PilT orthologs among the studied T4aP could support PilU-dependent retraction in conjunction with PilU_{Vc}. To test this, Walker A mutants of each *pilT* gene (*pilT*_{Vc}^{WA}, *pilT*_{Ab}^{WA}, *pilT*_{Ps}^{WA}, *pilT*_{Ec}^{WA}) were ectopically expressed in a Δ *pilT* background (i.e., where *pilU*_{Vc} is intact) and a Δ *pilTU* background. As expected, the ectopic expression of *pilT*_{Vc}^{WA} fully restored the transformation efficiency of a Δ *pilT* background (since *pilU*_{Vc} is intact) (**Fig. 2A**). Importantly, transformation was not restored by ectopic expression of any Walker A *pilT* alleles in a Δ *pilTU* background (**Fig. 2A**), which indicates that these ATPase defective alleles of PilT only support PilU-dependent retraction and cannot promote retraction on their own. When *pilT*_{Ab}^{WA} and *pilT*_{Ps}^{WA} were ectopically expressed in the Δ *pilT* background, transformation was rescued to parental levels (**Fig. 2A**). These data suggest that PilU_{Vc} is able to mediate retraction of the *V. cholerae* competence pilus in a PilT_{Ab}- and PilT_{Ps}-dependent manner, which further demonstrates the promiscuity among these retraction ATPases. Ectopic expression of the Walker A mutant allele of *pilT*_{Ec}, however, did not restore natural transformation in the Δ *pilT* background (**Fig. 2A**). This suggests that PilT_{Ec}^{WA} is incapable of promoting PilU-dependent retraction in the presence of PilU_{Vc}.

The ability of PilU to interact with PilT is likely necessary for PilU-dependent retraction of the competence pilus. As expected, PilT_{Vc}, PilT_{Ab}, PilT_{Ps}, and PilT_{Ec} could self-interact (**Fig. 2B**) as seen previously (**Fig. 1D**), and each allele could also interact with PilU_{Vc} (**Fig. 2B**). Because PilU_{Vc}-mediated retraction was possible when *pilT*_{Vc}^{WA}, *pilT*_{Ab}^{WA}, and *pilT*_{Ps}^{WA} were ectopically expressed, we hypothesized that each of these PilT alleles would be able to self-interact and also interact with PilU. PilT_{Vc}^{WA} and PilT_{Ab}^{WA} were able to self-interact (**Fig. 2B**), which suggests that the Walker A mutation does not inhibit the ability of these proteins to self-assemble into hexamers. This is consistent with our previous results which demonstrate that purified PilT_{Vc}^{WA} can still form hexameric rings (4). Unexpectedly, PilT_{Ps}^{WA} and PilT_{Ec}^{WA} did not exhibit self-interactions in these BACTH assays (**Fig. 2B**). We also observed that PilU_{Vc} could interact with PilT_{Vc}^{WA} and PilT_{Ab}^{WA}, however, it did not interact with PilT_{Ec}^{WA} or PilT_{Ps}^{WA} (**Fig. 2B**). The absence of interactions between PilU_{Vc} and PilT_{Ec}^{WA} (**Fig. 2B**) is consistent with the inability of PilT_{Ec}^{WA} to promote PilU-dependent retraction (**Fig. 2A**). However, the absence of interactions between PilU_{Vc} and PilT_{Ps}^{WA} was particularly surprising since this PilT allele can support PilU-dependent retraction when expressed in *V. cholerae* (**Fig. 2A**). Because PilT_{Ps}^{WA} supports PilU-dependent retraction in *V. cholerae*, but does not exhibit self-interactions in BACTH assays, this may suggest that self-assembly or hexamerization of PilT is not required for PilU-dependent retraction. However, the inability of PilT_{Ps}^{WA} to interact with PilU_{Vc} in these same BACTH assays (**Fig. 2B**), despite the ability of this allele to support PilU-dependent retraction (**Fig. 2A**) makes this difficult to conclude. The observed inability of PilT_{Ec}^{WA} and PilT_{Ps}^{WA} to self-interact and/or interact with PilU_{Vc} in BACTH assays could either be due to an absence of interactions between these proteins, or could be due to a poor affinity interaction between these proteins that are below the detection limit for the assay.

The molecular mechanism underlying PilU-mediated retraction in a PilT-dependent manner remains unclear. The ability of PilT to self-assemble and form a hexamer may be a prerequisite for PilU-mediated retraction. However, other characteristics of PilT may also be responsible for supporting PilU-mediated retraction. Because *pilT*_{Ec}^{WA} lacked self-interactions, we constructed an alternative mutant that also disrupted the ATPase activity of PilT_{Ec} by mutating an invariantly conserved glutamate in the Walker B domain of *pilT*_{Ec} (*pilT*_{Ec}^{E201A} = *pilT*_{Ec}^{WB}). Importantly, PilT_{Ec}^{WB} exhibited self-interactions in BACTH assays (**Fig. 2B**), suggesting that the Walker B mutation allows for self-assembly and/or a higher affinity between subunits compared to the

Walker A mutant. When we ectopically expressed the *pilT_{Ec}^{WB}* in a Δ *pilT* background (where *pilU* is intact), however, we observed that this mutant did not restore transformation (**Fig. 2A**), which is similar to the *pilT_{Ec}^{WA}* allele. This indicates that self-assembly of an ATPase defective PilT_{Ec} is not sufficient to promote PilU-dependent retraction. Together, these data suggest that PilT_{Ec} may have unique features that prevents it from promoting PilU-dependent retraction. We hypothesized that analyzing the differences between PilT_{Ec} and the other PilT alleles (PilT_{Vc}, PilT_{Ab}, and PilT_{Ps}) may help define the features of PilT that allow for PilU-dependent retraction.

The C-terminus of PilT_{Vc} contributes to PilU-mediated retraction.

In order to narrow down the region of PilT_{Vc} that is necessary and/or sufficient to support PilU-dependent retraction, we constructed defined chimeras of *pilT_{Vc}* and *pilT_{Ec}*. These chimeras were first ectopically expressed in a Δ *pilTU* background to test their ability to mediate retraction of the competence pilus in a manner that is dependent on their ATPase activity – a property that both PilT_{Vc} and PilT_{Ec} shared (**Fig. 1A**). Surprisingly, only three of the chimeras were able to restore parental levels of transformation in the Δ *pilTU* background (**Fig. 3A**). These are the only chimeras of PilT that we can be reasonably confident could potentially support PilU-dependent retraction, thus, we only proceeded with characterizing these three chimeras of PilT moving forward. Next, we introduced a Walker A mutation into these chimeras to determine if they were able to support PilU-mediated retraction. Consistent with the idea that PilT self-assembly is required for PilU-mediated retraction, none of the *pilT_{Vc} / pilT_{Ec}* chimeras with the Walker A mutation were able to rescue transformation efficiency in a Δ *pilT* background (**Fig. 3B**). Separately, a Walker B mutation was introduced into these chimeras. Again, none of the 3 chimeras were able to restore transformation in a Δ *pilT* background (**Fig. 3B**). Because the chimera that contained the N-terminal ~200 amino acids of PilT_{Vc} and C-terminal ~150 amino acids of PilT_{Ec} did not support PilU-dependent retraction, we reasoned that the C-terminus of PilT_{Vc} is important for PilU-dependent retraction.

In order to further investigate potential components of PilT that are required for supporting PilU-mediated retraction, we constructed an alignment of the 4 PilT orthologs that were studied here. The alignment revealed that the middle section of PilT_{Ec} was conserved relative to all of the other PilT orthologs (**Fig. S2**). However, the N-terminus and the C-terminus of PilT_{Ec} were poorly conserved relative to the other PilT orthologs. Because our data suggests that the C-terminus of PilT_{Vc} is required for supporting PilU-mediated retraction, that is where we focused our investigation. In particular, we observed that *pilT_{Ec}* contains two substitutions in the highly conserved AIRNLI_{RE} domain of PilT (18). To determine if these substitutions were responsible for the inability of PilT_{Ec} to support PilU-mediated retraction (7), we mutated the two residues in *pilT_{Ec}* to the residues present in the other PilT orthologs (PilT_{Ec}^{AVG→AIR}). Conversely, to determine if these changes in PilT_{Ec} contributed to its inability to support PilU-mediated retraction, the residues in *pilT_{Vc}* were mutated to the residues present in *pilT_{Ec}* (PilT_{Vc}^{AIR→AVG}). These mutations were introduced into Walker A (WA) or Walker B (WB) mutant alleles of PilT so that we could assess PilU-mediated retraction. The introduction of these mutations into *pilT_{Vc}* did not result in the inability of PilT_{Vc} to facilitate PilU-mediated retraction (**Fig. 3C**). Moreover, the restoration of the two AIRNLI_{RE} substitutions in *pilT_{Ec}* did not rescue PilU-mediated retraction (**Fig. 3C**). These data indicate that the substitutions in the AIRNLI_{RE} domain of *pilT_{Ec}* are not responsible for the inability of PilT_{Ec} to support PilU-mediated retraction.

The alignment of the 4 PilT orthologs also revealed that 15 amino acids in the C-terminal domain of PilT_{Ec} were missing when compared to the other PilT orthologs that did support PilU-mediated retraction (PilT_{Vc}, PilT_{Ab}, and PilT_{Ps}). We hypothesized that the deletion of the last 15 amino acids in PilT_{Ec} could be responsible for the inability of this protein to support PilU-mediated retraction. To test this, we deleted the last 15 amino acids from PilT_{Vc} (*pilT_{Vc}^{Δ15aa}*). Additionally, we fused the last 15 amino acids present at the C-terminus of *pilT_{Vc}* to the end of

PilT_{Ec} (*pilT*_{Ec}^{+15aa}). Again, these mutations were generated in Walker A and Walker B mutant alleles of PilT so that we could assess PilU-mediated retraction. The *pilT*_{Vc}^{Δ15aa, WA} mutant displayed no reduction in transformation (**Fig. 3C**), suggesting that PilT_{Vc}^{Δ15aa, WA} is still able to facilitate PilU-mediated retraction. Conversely, the *pilT*_{Ec}^{+15aa, WA} and *pilT*_{Ec}^{+15aa, WB} mutations did not restore transformation to parental levels (**Fig. 3C**), which suggests that PilU_{Vc} cannot mediate retraction in the presence of these PilT_{Ec} alleles. Together, these data suggest that the last 15 amino acids present at the C-terminus of PilT_{Vc} is not necessary or sufficient to promote PilU-mediated retraction.

Our results have suggested that, individually, the substitutions present in the conserved AIRNLIRE domain of PilT_{Ec} and the deletion of the last 15 amino acids in the C-terminus are not responsible for the inability of this PilT allele to support PilU-mediated retraction. However, it is possible that the combination of these two factors diminishes PilU-mediated retraction. To test this, we constructed *pilT*_{Vc} alleles that contained both the AIRNLIRE domain substitutions and the C-terminal deletion of 15 amino acids (*pilT*_{Vc}^{AIR→AVG, Δ15aa}). Also, we generated a *pilT*_{Ec} allele where we restored the AIRNLIRE domain and inserted the terminal 15 amino acids from *pilT*_{Vc} onto the C-terminus (*pilT*_{Ec}^{AVG→AIR, +15aa}). The combination of mutations in *pilT*_{Vc} resulted in a loss of function, even in a strain where the ATPase activity of the allele was intact (**Fig. 3C**). Additionally, the *pilT*_{Ec}^{AVG→AIR, +15aa, WA} and *pilT*_{Ec}^{AVG→AIR, +15aa, WB} alleles did not restore transformation to parental levels in the Δ*pilT* background, suggesting that PilU was unable to mediate retraction in these backgrounds. This suggests that the combination of the 15 amino acid deletion and the substitutions in the AIRNLIRE domain are not solely responsible for the inability of PilT_{Ec} to support PilU-mediated retraction.

DISCUSSION

This work demonstrates that the retraction ATPases PilT and PilU are highly promiscuous among T4aP systems, which contrasts with the fidelity displayed by the extension ATPases from the same systems. Additionally, our data suggests that the C-terminus of PilT_{Vc} likely plays an important role in supporting PilU-mediated retraction. However, the exact residues in the PilT C-terminus that contribute to PilU-mediated retraction remain elusive.

One possible explanation for the discrepancy in the promiscuity / specificity among motor ATPases could be due to differences in sequence divergence; where retraction motor orthologs are more similar to one another compared to the extension motor orthologs. When we performed this analysis, however, we found that the PilT orthologs ranged from ~62-80% similar (with PilT_{Ec} being the most divergent), the PilU orthologs ranged from ~70-74% similar, and the PilB orthologs ranged from ~64-66% similar (and the TfpB ortholog was ~53% similar to PilB_{Vc}) (**Fig. S3**). These data indicate that overall sequence divergence among PilT and PilB orthologs is fairly similar. Thus, suggesting that sequence divergence alone cannot account for the promiscuity / specificity among these motor ATPases.

Why are retraction ATPases promiscuous and extension ATPases specific? One possibility is that it is advantageous for T4aP systems to maintain promiscuous retraction ATPases. T4aP are highly diverse and retraction ATPases are generally expressed at distinct loci from the other T4aP components. Some T4aP systems are likely transferred between organisms through horizontal gene transfer (19). The inability to retract T4aP is likely disadvantageous in many circumstances since these surface appendages are highly immunogenic and can also be receptors for diverse phages (20). Thus, it is tempting to speculate that one potential advantage to retraction ATPase promiscuity is that this would allow cells that acquire a new T4aP the ability to retract pili from the surface.

Some organisms, like *V. cholerae*, encode multiple T4P - competence pili, mannose sensitive hemagglutinin (MSHA) pili, and toxin co-regulated pili (TCP) - and there are distinct environmental conditions under which each pilus system is likely needed. Competence pili and MSHA pili are likely critical for the survival of *V. cholerae* in its environmental reservoir, while TCP are required for infection of its human host. In fact, it has previously been shown that the inappropriate expression of MSHA pili during the context of an infection attenuates *V. cholerae* (21). A high degree of specificity among the extension ATPase motors for these distinct T4P may represent one mechanism by which cells can exhibit tight control over when these distinct pili are made. In contrast, the retraction of these pili may not require the same level of regulation because pilus extension is so tightly regulated. Indeed, in *V. cholerae*, PilT and PilU promote retraction of both competence pili and MSHA pili (8, 22).

The molecular mechanism underlying the specificity of extension ATPase motors remains unclear. One possibility is that the N-terminal domain of PilB provides specificity. It is thought that the N-terminal domain of PilB is responsible for interactions with PilM, which is likely required for proper pilus extension (6). An alignment of the extension and retraction motor orthologs showed that the extension motors contain an extended N-terminal domain (~184 residues) that distinguishes them from the retraction ATPase motors (**Fig. S4**), and we found that much of the sequence divergence among extension ATPases was attributed to this N-terminal domain (**Fig. S3**). Thus, it is tempting to speculate that the N-terminal domain of extension ATPases plays a critical role in defining the specificity of these motors for their cognate T4aP system.

While our results indicate that retraction motors are promiscuous and extension motors are specific, it remains unclear which of these phenotypes is the exception vs the rule. To distinguish between these possibilities, it would be interesting to test the degree of specificity / promiscuity among other T4aP components. Addressing this question may continue to shed light on the evolutionary pressures that have helped shaped these nearly ubiquitous molecular machines, and will be the focus of future research.

METHODS

Bacterial Strains and Growth Conditions

Vibrio cholerae strains were grown in LB Miller broth and LB Miller agar plates that contained erythromycin (10 µg/mL), carbenicillin (20 µg/mL), and/or kanamycin (50 µg/mL) when necessary. A list of all strains used in this study can be found in **Table S1**.

Construction of Mutant Strains and Constructs

All of the *V. cholerae* strains used in this study are derivatives of the El tor isolate E7946. The motor ATPase orthologs from other species were amplified from genomic DNA of the following strains: *Acinetobacter baylyi* ADP1 (23), *Pseudomonas stutzeri* JM300 (24), and *Escherichia coli* MG1655 (25). All mutant constructs were made via splicing-by-overlap extension (SOE) PCR, exactly as previously described (13, 26). A detailed list of all primers used to generate mutant constructs can be found in **Table S2**. Mutant constructs were introduced onto the *V. cholerae* genome by natural transformation and natural cotransformation exactly as previously described (26).

Natural Transformation Assays

In this study, we aimed to address the role of distinct T4aP components on competence pilus function. Thus, we opted to bypass the natural mechanism of competence induction in *V. cholerae* by ectopically expressing the master competence regulator TfoX (*P_{tac}-tfoX*) and

inactivating the quorum sensing negative regulator LuxO ($\Delta luxO$) as previously described (8). Chitin-independent natural transformation assays were performed exactly as previously described (8, 14). Briefly, strains were grown overnight in 3 mL of LB broth containing 100 μ M IPTG at 30 °C rolling. Next, cells were subcultured into 3 mL of LB supplemented with 20 mM $MgCl_2$, 10 mM $CaCl_2$, 100 μ M IPTG, and arabinose (when appropriate; 0.05-0.2%) and grown to late-log phase at 30 °C rolling. Next, $\sim 10^8$ cells were transferred to Instant Ocean medium (7 g/L; Aquarium Systems) containing 100 μ M IPTG, arabinose (when appropriate; 0.05-0.2%), and 200 ng of a transforming DNA (tDNA) PCR product. Reactions were then incubated statically overnight at 30 °C. The tDNA product used throughout this study targeted a “neutral gene” (the frame-shifted transposase, VC1807) for inactivation ($\Delta VC1807::Erm^R$) as previously described (27). A negative control, containing no tDNA, was included for each strain in all transformation assays. After the overnight incubation with tDNA, reactions were outgrown by adding 1 mL of LB to each reaction and incubating at 37 °C shaking for 2 hours. After the outgrowth, reactions were plated for quantitative culture to distinguish between transformants (plated on LB + erythromycin) and total viable counts (plated on plain LB). The transformation frequency for each reaction was calculated by dividing the number of transformants by the total viable counts.

Bacterial Adenylate Cyclase Two-Hybrid Assay

Using PCR, the desired motor ATPase genes were amplified and cloned into the BACTH vectors pKT25 and pUT18C in order to produce N-terminal fusions between the motor ATPase and the T25 and T18 fragments of adenylate cyclase, respectively. Miniprep plasmids (Qiagen) were then co-transformed into *E. coli* BTH101 cells. As a positive control, T25-zip and T18-zip vectors were cotransformed into BTH101. And as a negative control, the pKT25 and pUT18C empty vectors were cotransformed into BTH101. Transformations were plated onto LB + kanamycin (50 μ g/mL) + carbenicillin (100 μ g/mL) plates in order to select for transformants that received both plasmids. These transformants were then selected and grown overnight at 30 °C static in LB + kanamycin (50 μ g/mL) + carbenicillin (100 μ g/mL). Finally, 3 μ L of the overnight cultures were spotted onto LB agar plates supplemented with 500 μ M IPTG, kanamycin (50 μ g/mL), carbenicillin (100 μ g/mL), and X-gal (40 μ g/mL). These plates were allowed to incubate statically at 30 °C for ~48 hours prior to imaging on a HP Scanjet G4010 flatbed scanner.

Alignments

The sequences of the genes that encode for the desired proteins were downloaded from GenBank. Sequences were aligned using T-Coffee (28) and the resulting alignments were run through Boxshade to generate the final figures.

Pairwise comparisons to assess similarity among ATPase motor orthologs

Pairwise comparisons between ATPase motor orthologs were performed via global alignment using the Needleman-Wunsch algorithm (29). The percent similarities generated by these pairwise comparisons are reported in **Fig. S3**.

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Fig. 1. Retraction ATPase orthologs are promiscuous among T4aP systems, while extension ATPase orthologs are not. Natural transformation assays were performed to test whether (A) PilT, (B) PilU, or (C) PilB/TfpB orthologs from 3 heterologous T4aP systems could complement *V. cholerae* mutants lacking their endogenous motor ATPases. The relevant genotypes of strains are indicated below each bar, which includes the status of the native *V. cholerae* pilT/pilU/pilB genes (top) as well as the ectopically expressed motor ATPase allele (induced via P_{BAD}) (bottom). The color of the bar corresponds to the species from which the ectopically expressed motor ATPase was derived (green bars = *Vc* motors, yellow bars = *Ab* motors, orange bars = *Ps* motors, and purple bars = *Ec* motors). Data are from at least 3 independent biological replicates and shown as the mean $\pm$ SD. The limit of detection for transformation frequency assays is $\sim 10^{-7}$. In C, Ab₁ denotes pilB_{Ab} and Ab₂ denotes tfpB_{Ab}. In C, for all samples where the transformation frequency is $\sim 10^{-7}$, at least 1 replicate contained zero transformants and the limit of detection for that sample is displayed. BACTH assays to test whether the indicated (D) PilT, (E) PilU, or (F) PilB/TfpB alleles can interact with themselves ("self") or the indicated T4P components (PilC or PilT). "WA" denotes a Walker A mutation in the tested allele. "+" and "-" denote positive and negative controls for the BACTH assay. "E.V." denotes an empty vector. Data are representative of at least 2 independent experiments.

Fig. 2. The *PilT* homolog from *E. coli* cannot support *PilU*-mediated retraction. (A) Natural transformation assays were performed to test whether retraction ATPase *PilT* mutants from 3 heterologous T4aP systems could complement *V. cholerae* mutants lacking their endogenous motor ATPases. The relevant genotypes of strains are indicated below each bar, which includes the status of the native *pilT/pilU* genes as well as the ectopically expressed motor ATPase allele (induced via P_{BAD}). The color of the bar corresponds to the species from which the ectopically expressed *pilT* was derived (green bars = *Vc pilT*, yellow bars = *Ab pilT*, orange bars = *Ps pilT*, and purple bars = *Ec pilT*). Data are from at least 3 independent biological replicates and shown as the mean $\pm$ SD. (B) BACTH assays to test whether the indicated *PilT* alleles can interact with themselves ("self") or *PilU*_{vc}. "WA" denotes a mutation in the Walker A domain of the tested allele, while "WB" denotes a mutation in the Walker B domain of the tested allele. "+" and "-" denote positive and negative controls for the BACTH assay. "E.V." denotes an empty vector. Data are representative of at least 2 independent experiments.

Fig. S1. *PilU_{Ps}* is a fully functional retraction ATPase motor. Natural transformation assays were performed to assess whether *PilU_{Ps}* can support pilus retraction in conjunction with its cognate *PilT_{Ps}^{WA}* better than with the heterologous *PilT_{Vc}^{WA}*. The relevant genotypes of each strain are indicated below each bar, which includes the status of the native *pilT/pilU* genes as well as the ectopically expressed retraction motor ATPases. Data are from at least 3 independent biological replicates and shown as the mean $\pm$ SD.

A

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piIU Vc 1 MFLNOYIDGMI THKASDLITVCAFLYRVVGGELRAQGEALSVALVTALHAMMDLARO
piIU Ab 1 MFLNDLNIIMAKHKASDLFITAGVPSLRKNGEIEPVSKTKIDGDMIAQLIDSHITQKOR
piIU Ps 1 MFEFKLIRIMYKEGSDLFITAGVPPSMKVNGKILPVNKTIPSEMTRETVHGVANEQOR

piIU Vc 60 AEFKQTEFNFAVV-RT-SGRFRVSAPFQRELPGAVIRRIETRIPTFEELKLPBVLONLA
piIU Ab 61 QEFTEHECNFAILNBEKNERFRVSAPFQRMPPGMVIRRIETRIPTFPDDILHPEILKELA
piIU Ps 61 REFEENHECNFAISARG-IGRRFRVSAPFQRLNAGMVRRIETRIPTFEELKLPDVLKELA

piIU Vc 118 LAKRGLVLVVGATGSGKSTMAAMGYRNQRFQGHIIITVEDPIEFVHEHKEICIVTOREVG
piIU Ab 121 MKKRGIIIPSGATGTGKSTSLASMIQRNQHSGKHIIITIEDPIEFIEHAGCIITOREVG
piIU Ps 120 LAKRGLVLVVGATGSGKSTSLAAMIGYRNKNSGKHIIITIEDPIEFIEHAGCIIVTOREVG

piIU Vc 178 IDTDSFEVALKNSLRQAPDIIIGEIRSRRETMAYMTFAETGHLCHNATHANNANQALER
piIU Ab 181 IDTDSFEVALKNSLRQAPDIIIGEIRSRREMDYALAFATGHLVATLHANNANQALER
piIU Ps 180 IDTDSFEVALKNSLRQAPDIIIGEIRSRRETMHAFATGHLCHNATHANNANQALER

piIU Vc 238 ITHLVPRQRQCFLLDLSNLNKGVIQAQQLRDNGKGRHVEEVLINSEIADLRIRGEL
piIU Ab 241 ITHFFDADRHQQIFMDLSNLNKAIVISQQLIPTHDGSRRAAVEVLINTEPLISVIROGEL
piIU Ps 240 ITHFFPADRQNOVWMDLSNLNKAIVQAQQLVPTDGGKRRAVEVLINTEPLADLRIRGEL

piIU Vc 298 HELKNTMARSCEVGMQTFDQALYQLVDDKISECDALHSADSNLRLMIRTKRGDYG-
piIU Ab 301 YKRELMARSCEVGMQTFDQALYQLVDDKHISADALHADSNNLRQIKLSEBANKP-
piIU Ps 300 HELKGLMKRSIDLGMQTFDQALYNLYVQGEIITVEDALHADSNDLRLMIKLGSEITGEH

piIU Vc 357 -GSL--QNVKIDME-----
piIU Ab 360 LQNRERMSEVERKVFIDQQRNLK
piIU Ps 360 LQSVS--QSLTIDMSDDPCRSFR

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B

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PiIT Vc 1 MDIAELLFSSVKHNASDLHLSAGPPMIRIDGVRRLGVPATHSVDVHLLITIMNDQOR
PiIT Ab 1 MDITELLAFSVNRRASDLHLSAGPPMIRIDGVRRLINLPAMDHSVHLLIYIMNDKOR
PiIT Ps 1 MDITELLAFSPCCASDLHLSAGPPMIRIDGVRRLINLPAMDHGVHLLIYIMNDKOR
PiIT Ec 1 MNTEELVATSVKHNSDLHLCSMIFARWRIRGR---EAAPHTFDVDELRWIMDDQOR

PiIT Vc 61 SEYEKLELDFSEFELPNVGRFRVNAHRCARGCSAVFRITIPVITPLEOIDAPETESKIAN
PiIT Ab 61 KDYEKLELDFSEFELPNARFRVNMNQNRGASAVFRITIPSVLITLEDGLGRIFRKHED
PiIT Ps 61 KDYEKLELDFSEFELVAFRFRVNAHRCARGCSAVFRITIPSVLITLEDNNMGVFRKHED
PiIT Ec 58 AILLINGQIDFAVSAENQDILGSAFACRGTISLATILPSPCPGLEQLGAPTVLPDLK

PiIT Vc 121 VGRGLVLVTGPTGSGKSTTLAAMNYNNHNRHILITIEDPIEFVHNNKCLINQREVHR
PiIT Ab 121 VPRGLVLVTGPTGSGKSTTLAAMDYNNHNRHILITIEDPIEFVHNNKCLINQREVHR
PiIT Ps 121 VPRGLVLVTGPTGSGKSTTLAAMDYNNHNRHILITIEDPIEFVHNNKCLINQREVHR
PiIT Ec 118 SENGILLVTGPTGSGKSTTLAAMGYLNQADAHILITIEDPIEFVHNNKCLINQREVHGL

PiIT Vc 181 DTLSKRNALRSALREDPDMILVGLRLDETISLALTAAGHLVFGILLTSSAAKTIDRI
PiIT Ab 181 DTLGSEALRSALREDPDMILVGMRDLETIRLALTAAGHLVFGILLTSSAAKTIDRI
PiIT Ps 181 DTLGSEALRSALREDPDMILVGMRDLETIRLALTAAGHLVFGILLTSSAAKTIDRI
PiIT Ec 178 HCTEASLRSALREDPDMILVGLRLDETIRLALTAAGHLVFGILLTNGAAQAVRLK

AIRNLIRES Domain

PiIT Vc 241 IDVFPEKDKMVRSMLESISRAVIAKLLKLLKGGGRVAHEIMLATFAIRNLIREKVAQ
PiIT Ab 241 IDVFPAEEKDMVRSMLESISRAVISCHLLKKKGGGRVAHEIMIGTFAIRNLIREKVAQ
PiIT Ps 241 IDVFPAEEKDMVRSMLESISRAVISCHLLKKKGGGRVAHEIMIGTFAIRNLIREKVAQ
PiIT Ec 238 IDVFPAEEKDMVRSMLESISRAVISCHLLKLLKGGGRVAHEIMIGTFAIRNLIREKVAQ

C-term 15

PiIT Vc 301 MYSHTOTGAIHGMCTHECNKOLANGVDAQEVOSFIELDKAE
PiIT Ab 301 MYSHTOTGAIHGMCTHLDQSLKOLLAKGILNLPARAVAK-SPTSE
PiIT Ps 301 MYSHTOTGSSGMCTHLEKLLAKGLSRDSAREKAK-QPNE
PiIT Ec 298 MPRHTOTGQVGRHIFVPSYQHRVPSER

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Fig. S2. Alignment of PiIU and PiIT sequences from the four T4aP studied. Alignments of (A) PiIU orthologs and (B) PiIT orthologs. Residues highlighted in black are identical, while those highlighted in gray are similar. The AIRNLIRES and C-terminal 15 residue domains experimentally tested in this study are boxed in red. The GenBank Accession numbers for the protein sequences used are as follows: Vc, *Vibrio cholerae* PiIT (AAF93635); Ab, *Acinetobacter baylyi* PiIT (CAG67808); Ps, *Pseudomonas stutzeri* PiIT (AFN76400); Ec, *Escherichia coli* PiIT (NP_417425); Vc, *V. cholerae* PiIU (AAF93636); Ab, *A. baylyi* PiIU (CAG67807); Ps, *P. stutzeri* PiIU (AFN76401).

Fig. S3. Pairwise comparisons of the indicated motor ATPases. Percent similarities are reported for pairwise comparisons of the indicated (A) PilT, (B) PilU, and (C) PilB/TfpB orthologs. PilB/TfpB orthologs were further analyzed for pairwise comparisons of the C-terminal domains (CTD) and N-terminal domains (NTD) (see Fig. S4 for details on what portion of PilB represents the NTD vs CTD). The GenBank Accession numbers for the protein sequences used in these comparisons are as follows: Vc, *Vibrio cholerae* PilT (AAF93635); Ab, *Acinetobacter baylyi* PilT (CAG67808); Ps, *Pseudomonas stutzeri* PilT (AFN76400); Ec, *Escherichia coli* PilT (NP_417425); Vc, *V. cholerae* PilU (AAF93636); Ab, *A. baylyi* PilU (CAG67807); Ps, *P. stutzeri* PilU (AFN76401); Vc, *V. cholerae* PilB (AAF95567); Ab, *A. baylyi* PilB (CAG67312); Ab, *A. baylyi* TfpB (CAG69034); Ps, *P. stutzeri* PilB (AFN76867).

		NTD	
PilT Vc	1	MD-----	-----
PilT Ab	1	MD-----	-----
PilT Ps	1	MD-----	-----
PilT Ec	1	MD-----	-----
PilB Vc	1	MLTNL-----VAIRQAELISATQEQAVVTQVSASGTSPEALTEL--	-----
PilB Ab	1	MISESQGGLMSAFTTPPKFSGFIRRVVEEGYNAQNMQQALEKAKKFKQDIVPYLIDN--	-----
TfpB Ab	1	MTYHFEI-----DTQWCLEQLKDGRISERDKLLIQT-THRNKEQKWHPLQWIA	-----
PilB Ps	1	MSENI-----SLSGLARQVRAGVDEKTAQQAQVQAQRNKVSIVTYVQON--	-----
		NTD	
PilT Vc	3	-----	-----
PilT Ab	3	-----	-----
PilT Ps	3	-----	-----
PilT Ec	3	-----	-----
PilB Vc	42	-----SIFHAQELTEQLSHIFGLPETDISRYDYA-NLCQQLGLRELITRYDAIP	-----
PilB Ab	59	-----FSISPLTAEIISLEFGEPLLDIGVFDPA-LFLKDKIDEKLIQKYRIIP	-----
TfpB Ab	50	HFDLKDQAHTSKPFDILRSQWLAEKVGPLYVIDPLKADVNMLTSVMSQEFALRNKIIP	-----
PilB Ps	47	-----KLAKGRAIAELASDQFVALLDISALDKD-SLPKELVSEKLARQHRVIP	-----
		NTD	
PilT Vc	3	-----	-----
PilT Ab	3	-----	-----
PilT Ps	3	-----	-----
PilT Ec	3	-----	-----
PilB Vc	90	IAKQGNLILAVSDETLTQAEEEFRAFATG-LOVELAADHRAIAAARRIRGRS--IQGA	-----
PilB Ab	107	IVHRGHVLYVATSNPTNIEAMDARFNSK-LKVEPIIVVEHDKLERLISEHVEE--THFN	-----
TfpB Ab	110	VDVNPERILIGTDQPFVRDWSNLELSLAPKKDELVLSPDQLQRYPEYQVSRVAVHSS	-----
PilB Ps	95	IVKRGTKLYVAISDPANHQSIIDIQFSTG-LTIESILVEDDKIGDAIDKIDSG--TSGL	-----
		NTD CTD	
PilT Vc	3	-----	ITELLIEFSVKNASDLHLISA
PilT Ab	3	-----	ITELLAFSVQNRASDLHLISA
PilT Ps	3	-----	ITELLAFSAQCGASDLHLISA
PilT Ec	3	-----	IEEIVALSVMHNVSDDLHLCS
PilB Vc	147	ANQKKEISQDEL-ANLVKVSDELQSIEDLSQDQSPVSRFINQVILLDAVVKGASDIHFEP	-----
PilB Ab	164	FDTE-ELDLIVE-VDP-HTTDD--DEDDDKLKDCAPIVYINKLLIDATMSASDLHFEP	-----
TfpB Ab	170	KNAT-AYERDNKGVEA-LL-QL--GDNQNPDANDQHIVLVDWILQFAFEQASDIHLEP	-----
PilB Ps	152	EDLG-DVDLDGI-DVE-SVSDDAKGGEEGAADAPVVFVFNKAILLDAIRGSSDDLHFEP	-----
		CTD	
PilT Vc	23	G---VPPMIRNDGEVRKIG-VPAFTHS-IVHRILIFEIM---NDAQRSEYEEKLEVDFSFE	-----
PilT Ab	23	G---IPPMIRNDGEVRRIN-IPAMDHS-EVHNLIYDIM---NDKQRDDYEEKLETDFSFE	-----
PilT Ps	23	G---LPPMIRNDGDVRRIN-IPAMDHK-CVHEALIYDIM---NDKQRDDYEEFLETDFSFE	-----
PilT Ec	23	E---IPARRIRGRM---E-AAPFDTP-IVEELLREW---DDORAILLNGOLDFAVS	-----
PilB Vc	206	YENQYRIRIRCDGILVETQ-QPASHLSRR-AM-RIKILSKIDIAERR-IPQDGRIKIRIS	-----
PilB Ab	219	YEKSYRVRIRNDGVLRLIA-TBELQLATRIAS-RIKVMSONDISERR-VPQDGRIKLKMS	-----
TfpB Ab	225	RKEKGVRRIRNDGVLHTIYH-PANTLS-AVIA-RIKILGRNVAEKR-KPQDGRILKTRTP	-----
PilB Ps	209	YEKAYRVRIRNDGVLHEVA-RBELQLAPRISA-RIKVMAGDISERR-KPQDGRILKMKIS	-----
		CTD	
PilT Vc	75	LPNVGRFRVNAFHQARGCSAVERTIPTVTP--TLEQLDAP----EIEFSKIANYEKGLVILV	-----
PilT Ab	75	VPNLARFRVNVFNQNRGAGAVERTIPSKQL--TLEELGLG----KIFRDIQDYPRGLVILV	-----
PilT Ps	75	VPGVARFRVNAFNQNRGAGAVERTIPSKQL--TMEDLNMG----EVFRKIDVPRGLVILV	-----
PilT Ec	72	LAENQRLRGSFAAQRHGISAIRLLPSHCP--QLEQLGAP----TVLPETILKSENGLILV	-----
PilB Vc	263	RDTAIDMRVSLPTLWGEKIVRILDPSSAANLDIILKLGNNPOQKQLYLNALRPOGMILV	-----
PilB Ab	276	KSKTIDFRVNSLPTLFGKEIVRILDPASAMLGIDALGKEPEQKAFIMQALNKPQGMILV	-----
TfpB Ab	282	KGOETELRSLPTAFGEKIVRILDPDVLVRSFEQLGDEILLKQQAQSENHGIILV	-----
PilB Ps	266	KAKAIDFRVNSLPTLWGEKIVRILDPSSAQMGIIDALGVEESQKQLYMIALKQPOGMILV	-----
		CTD	
PilT Vc	129	TGPTGSGKSTTLAAMNYNAHHNKHILITIEDPIEFVHSNNKCLINQREVEH-RDTHSEKN	-----
PilT Ab	129	TGPTGSGKSTTLAAMLDYINMHRYDHILITIEDPIEFVHEPKKCLINQREVEH-RDILGFNE	-----
PilT Ps	129	TGPTGSGKSTTLAAMLDYLNNTKYHHILITIEDPIEFVHESKKCLINQREVEH-RDILGFSE	-----
PilT Ec	126	TGATGSGKSTTLAAMGYLNQHADAHILITIEDPVEYLYASQCLIQOREIG-LHCMTFAS	-----
PilB Vc	323	TGPTGSGKIVSLYTGIRILNLS-QINISTAEEDPVEINLSG----INQVQVPRIGFGFAE	-----
PilB Ab	336	TGPTGSGKIVSLYTGILNLS-QINISTAEEDPVEINLSG----INQVQVPRVGLTFFAE	-----

TfpB Ab	342	TGPTGSGKTTTLYSSIKQLATD-QVNVCTIEDPTMDESS----FNQMQVAAIDLGFAQ
PilB Ps	326	TGPTGSGKIVSLYTGLNINLNTV-DVNIISTAEDPVEINLEG----INQVNVNFRQGGDFAQ
		CTD
PilT Vc	188	ALRSALREDPDVILVGEIRDQETISIALTAAETGHLVFGTLHTSSAAKTIDRIIDVFPES
PilT Ab	188	ALRSALREDPDVILVGEIRDLETIRLALTAETGHLVFGTLHTSSAAKTIDRIIDVFPPE
PilT Ps	188	ALRSALREDPDVILVGEIRDLETIRLALTAETGHLVFGTLHTSSAAKTIDRIIDVFPPE
PilT Ec	185	ELRALREDPDVILVGEIRDSEITIRLALTAETGHLVLTATLHTRGAAQAVERLNDSPFAQ
PilB Vc	378	ALRSELRODPDVVIVGEIRDLETAETAVKAACTGHLVLTSTLHTNSAAETVIRIANMGVE-
PilB Ab	391	ALRSELRODPDVILVGEIRDLETAETAVKAACTGHLVMTSTLHTNMAAETLIRIRNMGVA-
TfpB Ab	397	GVRALRODPDVILVGEIRDQETANMAAALGHLVLTSTLHTNDAPSSLTIRHDLGVQ-
PilB Ps	381	ALRALRODPDVILVGEIRDLETAETAVKAACTGHLVMTSTLHTNSAAETLIRIRNMGVA-
		CTD
PilT Vc	248	EKDMVRSMLESLSRAVIAQKLLKRV-----
PilT Ab	248	EKDMVRIMLESLSRAVISQTLKKK-----
PilT Ps	248	EKDMVRSMLESLSRAVISQTLKKK-----
PilT Ec	245	EKDFVRNQLAGSLRAVLSQKLEVDK-----
PilB Vc	437	-----PFLNASSLSLIAQRIARLCKHCKIAVR--PSALLQSQFAFQPN-E-ILYEANA
PilB Ab	450	-----SFNIAATSVNLVIAQRIARLCSQCKRPIQVPERSLLEMGFTPEDLAQPEFQIFEP
TfpB Ab	456	-----PFLTAATLLGVIAQRIARLCPHCKVLSIDIDKQWQHLT-QDYEIAM-PEQIYGP
PilB Ps	440	-----AFNIAATSVNLVIAQRIARLCSCKKEVEVPRDALLKEGFPEESI-G-TFKIYGP
		CTD
PilT Vc	273	-----GGGRVACHEIMATPAIRNLIREDKVAQMYSSIQTGAAGHGMOTMEQNAKO
PilT Ab	273	-----GGGRVAHEIMIGTPAIRNLIREDKVAQMYSSIQTGATHGMTILDQSLKQ
PilT Ps	273	-----GGGRVAHEIMIGTPAIRNLIREDKVAQMYSAIQTGSSLGMOITLDMCLKG
PilT Ec	270	-----QEGRVAFELLINTPAVGNLIREGTHOLPHVIQTGQQVGMHIFQCSYQH
PilB Vc	488	AGQNECT-GGYSGRVGIYEYVAFNTEAEAIMQASII--HQLERLAKANGMOTLQESGLE
PilB Ab	505	VGCHDCR-EGYKGRVGIYEYVKITPETSKITMEDGNA--LEIAATPETHLGFNLRSGLK
TfpB Ab	509	VGCHDCRQIGYKGRIGIYEYFMSLDLNLKLVSSGANL--NQKSEAKKQGTCELRIAGAR
PilB Ps	493	VGCENCV-GGYKGRVGIYEYVKNTPALARIIMEDGNS--IDISTQMRKDGFDLRTSALI
		CTD
PilT Vc	323	LLRGGVWDACEVQSKIELDLKAF
PilT Ab	323	LLRGGVINTPTARAVA-KSPESF
PilT Ps	323	LLRGGVISRDSAREKA-KQPENF
PilT Ec	320	RVGGGRL-----
PilB Vc	545	KLRGGVTSFAELQRVLY-----E
PilB Ab	562	KVMCGVTSLSQETNRVTS-----E
TfpB Ab	567	KILEGVTSLEEVLRVVP-L---T
PilB Ps	550	KAMCGVTSLEEVNRVTK-----D

Fig. S4. Alignment of the PilB/TfpB and PilT sequences from the T4aP studied. Alignments of PilB/TfpB orthologs and PilT orthologs. Residues highlighted in black are identical, while those highlighted in gray are similar. The N-terminal domain and C-terminal domain of PilB are denoted by the “NTD” and “CTD” bars above the sequence. The GenBank Accession numbers for the protein sequences used are as follows: Vc, *Vibrio cholerae* PilT (AAF93635); Ab, *Acinetobacter baylyi* PilT (CAG67808); Ps, *Pseudomonas stutzeri* PilT (AFN76400); Ec, *Escherichia coli* PilT (NP_417425); Vc, *V. cholerae* PilB (AAF95567); Ab, *A. baylyi* PilB (CAG67312); Ab, *A. baylyi* TfpB (CAG69034); Ps, *P. stutzeri* PilB (AFN76867).

567 **Table S1. Strains used in this study**

Strain name in manuscript	Genotype and antibiotic resistance	Figure	Strain #
Parent	E7946 SmR, Δ luxO::miniFRT, lacZ::lacIq, pilA S56C, Δ VVC1807::ZeoR, P _{tac} -tfoX	Fig. 1, 2, 3, S1	TND0905 (SAD2468)
Δ pilT	E7946 SmR, Δ lacZ::lacIq, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, Δ pilT::TmR	Fig. 1, 2, 3	TND1035 (SAD2469)
Δ pilTU	E7946 SmR, Δ lacZ::lacIq, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, Δ pilTU::TmR	Fig. 1, 2, 3, S1	JLC227 (SAD2470)
P _{BAD} -pilT _{Vc} , Δ pilT	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, Δ lacZ::P _{BAD} -PilT _{Vc} CarbR, Δ pilT::TmR	Fig. 1, 3	TND1831 (SAD3199)
P _{BAD} -pilT _{Vc} , Δ pilTU	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, Δ lacZ::P _{BAD} -PilT _{Vc} CarbR, Δ pilTU::TmR	Fig. 1, 3	JLC1155 (SAD3222)
P _{BAD} -pilT _{Ab} , Δ pilT	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, Δ lacZ::P _{BAD} -PilT _{Ab} CarbR, Δ pilT::TmR	Fig. 1	TND2148 (SAD3200)
P _{BAD} -pilT _{Ab} , Δ pilTU	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, Δ lacZ::P _{BAD} -PilT _{Ab} CarbR, Δ pilTU::TmR	Fig. 1	JLC1153 (SAD3223)
P _{BAD} -pilT _{Ps} , Δ pilT	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, Δ lacZ::P _{BAD} -PilT _{Ps} CarbR, Δ pilT::TmR	Fig. 1	TND2125 (SAD3201)
P _{BAD} -pilT _{Ps} , Δ pilTU	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, Δ lacZ::P _{BAD} -PilT _{Ps} CarbR, Δ pilTU::TmR	Fig. 1	JLC1157 (SAD3224)
P _{BAD} -pilT _{Ec} , Δ pilT	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, Δ lacZ::P _{BAD} -PilT _{Ec} CarbR, Δ pilT::TmR	Fig. 1	JLC1473 (SAD3225)
P _{BAD} -pilT _{Ec} , Δ pilTU	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, Δ lacZ::P _{BAD} -PilT _{Ec} CarbR, Δ pilTU::TmR	Fig. 1	JLC1474 (SAD3226)
pilT ^{WA} , Δ pilU	E7946 SmR, Δ lacZ::lacIq, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::ZeoR, pilA S56C, pilT ^{K136A} , Δ pilU::TmR	Fig. 1, 2	JLC444 (SAD2472)
P _{BAD} -pilU _{Vc} , pilT ^{WA} , Δ pilU	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::KanR, pilA S56C, pilT ^{K136A} , Δ lacZ::P _{BAD} -PilU _{Vc} CarbR, Δ pilU::TmR	Fig. 1	EHC062 (SAD3239)
P _{BAD} -pilU _{Ab} , pilT ^{WA} , Δ pilU	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::KanR, pilA S56C, pilT ^{K136A} , Δ lacZ::P _{BAD} -PilU _{Ab} CarbR, Δ pilU::TmR	Fig. 1	EHC060 (SAD3240)
P _{BAD} -pilU _{Ps} , pilT ^{WA} , Δ pilU	E7946 SmR, P _{tac} -tfoX, Δ luxO::miniFRT, Δ VVC1807::KanR, pilA S56C, pilT ^{K136A} , Δ lacZ::P _{BAD} -PilU _{Ps} CarbR, Δ pilU::TmR	Fig. 1	EHC061 (SAD3241)
Parent (P _{const} -tfoX)	E7946 SmR, Δ lacZ::lacIq, P _{const} -tfoX, Δ luxO::miniFRT, Δ VVC1807::KanR, pilA S56C	Fig. 1	TND1076 (SAD2513)
Δ pilB	E7946 SmR, lacZ LPQEN:: KanR, P _{const} -tfoX, Δ luxO::miniFRT, pilA S56C, Δ VVC1807::CmR, Δ pilB::miniFRT	Fig. 1	TND2373 (SAD2787)

$P_{BAD-pilB_{Vc}}, \Delta pilB$	E7946 SmR, lacZ LPQEN:: KanR, $P_{const-tfoX}$, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::CmR$, $\Delta pilB::miniFRT$, $\Delta VCA0692::P_{BAD-pilB_{Vc}}$ CarbR	Fig. 1	TND2403 (SAD2789)
$P_{BAD-pilB_{Ab}}, \Delta pilB$	E7946 SmR, lacZ LPQEN:: KanR, $P_{const-tfoX}$, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::CmR$, $\Delta pilB::miniFRT$, $\Delta VCA0692::P_{BAD-pilB_{Ab}}$ CarbR	Fig. 1	TND2354 (SAD3202)
$P_{BAD-tfpB_{Ab}}, \Delta pilB$	E7946 SmR, lacZ LPQEN:: KanR, $P_{const-tfoX}$, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::CmR$, $\Delta pilB::miniFRT$, $\Delta VCA0692::P_{BAD-tfpB_{Ab}}$ CarbR	Fig. 1	TND2372 (SAD3203)
$P_{BAD-pilB_{Ps}}, \Delta pilB$	E7946 SmR, lacZ LPQEN:: KanR, $P_{const-tfoX}$, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::CmR$, $\Delta pilB::miniFRT$, $\Delta VCA0692::P_{BAD-pilB_{Ps}}$ CarbR	Fig. 1	TND2371 (SAD3204)
$pilT^{WA}$	E7946 SmR, $\Delta lacZ::lacIq$, $P_{tac-tfoX}$, $\Delta luxO::miniFRT$, $\Delta VC1807::KanR$, pilA S56C, $pilT^{K136A}$	Fig. 2	TND1077 (SAD2471)
$P_{BAD-pilT_{Vc}^{WA}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Vc}^{K136A}}$ CarbR, $\Delta pilT::TmR$	Fig. 2, 3	JLC1609 (SAD3227)
$P_{BAD-pilT_{Vc}^{WA}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Vc}^{K136A}}$ CarbR, $\Delta pilTU::TmR$	Fig. 2	EHC031 (SAD3242)
$P_{BAD-pilT_{Vc}^{WB}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Vc}^{E204A}}$ CarbR, $\Delta pilT::TmR$	Fig. 2	EHC075 (SAD3243)
$P_{BAD-pilT_{Ab}^{WA}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ab}^{K136A}}$ CarbR, $\Delta pilT::TmR$	Fig. 2	EHC015 (SAD3244)
$P_{BAD-pilT_{Ab}^{WA}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ab}^{K136A}}$ CarbR, $\Delta pilTU::TmR$	Fig. 2	EHC019 (SAD3245)
$P_{BAD-pilT_{Ps}^{WA}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ps}^{K136A}}$ CarbR, $\Delta pilT::TmR$	Fig. 2	EHC016 (SAD3246)
$P_{BAD-pilT_{Ps}^{WA}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ps}^{K136A}}$ CarbR, $\Delta pilTU::TmR$	Fig. 2	EHC020 (SAD3247)
$P_{BAD-pilT_{Ec}^{WA}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{K133A}}$ CarbR, $\Delta pilT::TmR$	Fig. 2	JLC1499 (SAD3228)
$P_{BAD-pilT_{Ec}^{WA}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{K133A}}$ CarbR, $\Delta pilTU::TmR$	Fig. 2	JLC1498 (SAD3229)
$P_{BAD-pilT_{Ec}^{WB}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{E201A}}$ CarbR, $\Delta pilT::TmR$	Fig. 2, 3	EHC076 (SAD3248)
$P_{BAD-pilT_{Vc}(50)/pilT_{Ec}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X Flag-pilT_{Vc}(50)/pilT_{Ec}}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3069 (SAD3205)
$P_{BAD-pilT_{Vc}(100)/pilT_{Ec}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X Flag-pilT_{Vc}(100)/pilT_{Ec}}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3070 (SAD3206)
$P_{BAD-pilT_{Vc}(150)/pilT_{Ec}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, pilA S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X Flag-pilT_{Vc}(150)/pilT_{Ec}}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3071 (SAD3207)

$P_{BAD-pilT_{Vc}(200)/pilT_{Ec}, \Delta pilTU}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Vc}(200)/pilT_{Ec}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3073 (SAD3208)
$P_{BAD-pilT_{Vc}(300)/pilT_{Ec}, \Delta pilTU}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Vc}(300)/pilT_{Ec}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3072 (SAD3209)
$P_{BAD-pilT_{Ec}(50)/pilT_{Vc}, \Delta pilTU}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Ec}(50)/pilT_{Vc}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3074 (SAD3210)
$P_{BAD-pilT_{Ec}(100)/pilT_{Vc}, \Delta pilTU}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Ec}(100)/pilT_{Vc}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3075 (SAD3211)
$P_{BAD-pilT_{Ec}(150)/pilT_{Vc}, \Delta pilTU}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Ec}(150)/pilT_{Vc}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3076 (SAD3212)
$P_{BAD-pilT_{Ec}(200)/pilT_{Vc}, \Delta pilTU}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Ec}(200)/pilT_{Vc}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3077 (SAD3213)
$P_{BAD-pilT_{Ec}(250)/pilT_{Vc}, \Delta pilTU}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Ec}(250)/pilT_{Vc}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3078 (SAD3214)
$P_{BAD-pilT_{Ec}(300)/pilT_{Vc}, \Delta pilTU}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Ec}(300)/pilT_{Vc}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND3079 (SAD3215)
$P_{BAD-pilT_{Vc}^{WA}(100)/pilT_{Ec}, \Delta pilT}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Vc}^{K136A}(100)/pilT_{Ec}$ CarbR, $\Delta pilT::TmR$	Fig. 3	TND3008 (SAD3216)
$P_{BAD-pilT_{Vc}^{WA}(200)/pilT_{Ec}, \Delta pilT}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Vc}^{K136A}(200)/pilT_{Ec}$ CarbR, $\Delta pilT::TmR$	Fig. 3	TND3010 (SAD3217)
$P_{BAD-pilT_{Ec}^{WA}(300)/pilT_{Vc}, \Delta pilT}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Ec}^{K133A}(300)/pilT_{Vc}$ CarbR, $\Delta pilT::TmR$	Fig. 3	TND3018 (SAD3218)
$P_{BAD-pilT_{Vc}^{WB}(100)/pilT_{Ec}, \Delta pilT}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Vc}^{E204A}(100)/pilT_{Ec}$ CarbR, $\Delta pilT::TmR$	Fig. 3	EHC106 (SAD3249)
$P_{BAD-pilT_{Vc}^{WB}(200)/pilT_{Ec}, \Delta pilT}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Vc}^{E204A}(200)/pilT_{Ec}$ CarbR, $\Delta pilT::TmR$	Fig. 3	EHC108 (SAD3250)
$P_{BAD-pilT_{Ec}^{WB}(300)/pilT_{Vc}, \Delta pilT}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-3X}$ Flag- $pilT_{Ec}^{E201A}(300)/pilT_{Vc}$ CarbR, $\Delta pilT::TmR$	Fig. 3	EHC115 (SAD3251)
$P_{BAD-pilT_{Vc}^{AIR \rightarrow AVG}, \Delta pilTU}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Vc}^{AIR \rightarrow AVG}}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	JLC1622 (SAD3230)
$P_{BAD-pilT_{Vc}^{WA, AIR \rightarrow AVG}, \Delta pilT}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Vc}^{K136A, AIR \rightarrow AVG}}$ CarbR, $\Delta pilT::TmR$	Fig. 3	JLC1625 (SAD3231)
$P_{BAD-pilT_{Vc}^{\Delta 15aa}, \Delta pilTU}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Vc}^{\Delta 15aa}}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND2774 (SAD3219)
$P_{BAD-pilT_{Vc}^{WA, \Delta 15aa}, \Delta pilT}$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Vc}^{K136A, \Delta 15aa}}$ CarbR, $\Delta pilT::TmR$	Fig. 3	JLC1611 (SAD3232)

$P_{BAD-pilT_{Vc}^{AIR \rightarrow AVG, \Delta 15aa}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Vc}^{AIR \rightarrow AVG, \Delta 15aa}}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	JLC1624 (SAD3233)
$P_{BAD-pilT_{Vc}^{WA, AIR \rightarrow AVG, \Delta 15aa}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Vc}^{K136A, AIR \rightarrow AVG, \Delta 15aa}}$ CarbR, $\Delta pilT::TmR$	Fig. 3	JLC1627 (SAD3234)
$P_{BAD-pilT_{Ec}^{AVG \rightarrow AIR}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{AVG \rightarrow AIR}}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	JLC1614 (SAD3235)
$P_{BAD-pilT_{Ec}^{WA, AVG \rightarrow AIR}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{K133A, AVG \rightarrow AIR}}$ CarbR, $\Delta pilT::TmR$	Fig. 3	JLC1615 (SAD3236)
$P_{BAD-pilT_{Ec}^{WB, AVG \rightarrow AIR}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{E201A, AVG \rightarrow AIR}}$ CarbR, $\Delta pilT::TmR$	Fig. 3	EHC095 (SAD3252)
$P_{BAD-pilT_{Ec}^{+15aa}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{+15aa}}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	TND2730 (SAD3220)
$P_{BAD-pilT_{Ec}^{WA, +15aa}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{K133A, +15aa}}$ CarbR, $\Delta pilT::TmR$	Fig. 3	TND2727 (SAD3221)
$P_{BAD-pilT_{Ec}^{WB, +15aa}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{E201A, +15aa}}$ CarbR, $\Delta pilT::TmR$	Fig. 3	EHC093 (SAD3253)
$P_{BAD-pilT_{Ec}^{AVG \rightarrow AIR, +15aa}}, \Delta pilTU$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{AVG \rightarrow AIR, +15aa}}$ CarbR, $\Delta pilTU::TmR$	Fig. 3	JLC1620 (SAD3237)
$P_{BAD-pilT_{Ec}^{WA, AVG \rightarrow AIR, +15aa}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{K133A, AVG \rightarrow AIR, +15aa}}$ CarbR, $\Delta pilT::TmR$	Fig. 3	JLC1617 (SAD3238)
$P_{BAD-pilT_{Ec}^{WB, AVG \rightarrow AIR, +15aa}}, \Delta pilT$	E7946 SmR, $\Delta luxO::miniFRT$, $pilA$ S56C, $\Delta VC1807::ZeoR$, $P_{tac-tfoX}$, $\Delta lacZ::P_{BAD-pilT_{Ec}^{E201A, AVG \rightarrow AIR, +15aa}}$ CarbR, $\Delta pilT::TmR$	Fig. 3	EHC097 (SAD3254)
$P_{tac-pilT_{Vc}^{WA}}, P_{BAD-pilU_{Ps}}, \Delta pilTU$	E7946 SmR, $P_{tac-tfoX}$, $\Delta luxO::miniFRT$, $\Delta VC1807::KanR$, $pilA$ S56C, $\Delta lacZ::P_{BAD-pilU_{Ps}}$ CarbR, $\Delta VC0692::P_{tac-pilT_{Vc}^{K136A}}$ SpecR, $\Delta pilTU::TmR$	Fig. S1	EHC099 (SAD3255)
$P_{tac-pilT_{Ps}^{WA}}, P_{BAD-pilU_{Ps}}, \Delta pilTU$	E7946 SmR, $P_{tac-tfoX}$, $\Delta luxO::miniFRT$, $\Delta VC1807::KanR$, $pilA$ S56C, $\Delta lacZ::P_{BAD-pilU_{Ps}}$ CarbR, $\Delta VC0692::P_{tac-pilT_{Ps}^{K136A}}$ SpecR, $\Delta pilTU::TmR$	Fig. S1	EHC100 (SAD3256)
T25-PilT _{Vc}	TG1, pkT25-PilT _{Vc} KanR	Fig. 1, 2	SAD2248
T25-PilT _{Ab}	TG1, pkT25-PilT _{Ab} KanR	Fig. 1, 2	SAD2659
T25-PilT _{Ps}	TG1, pkT25-PilT _{Ps} KanR	Fig. 1, 2	EHC043 (SAD3257)
T25-PilT _{Ec}	TG1, pkT25-PilT _{Ec} KanR	Fig. 1, 2	EHC047 (SAD3258)
T18-PilT _{Vc}	TG1, pUT18C-PilT _{Vc} CarbR	Fig. 1, 2	SAD2249
T18-PilT _{Ab}	TG1, pUT18C-PilT _{Ab} CarbR	Fig. 1, 2	SAD2660
T18-PilT _{Ps}	TG1, pUT18C-PilT _{Ps} CarbR	Fig. 1, 2	EHC044 (SAD3259)
T18-PilT _{Ec}	TG1, pUT18C-PilT _{Ec} CarbR	Fig. 1, 2	EHC042 (SAD3260)
T25-PilU _{Vc}	TG1, pkT25-PilU _{Vc} KanR	Fig. 1, 2	SAD2250

T18-PilU _{Vc}	TG1, pUT18C-PilU _{Vc} CarbR	Fig. 1, 2	SAD2251
T25-PilC _{Vc}	TG1, pkT25-PilC _{Vc} KanR	Fig. 1	SAD2260
T18-PilC _{Vc}	TG1, pUT18C-PilC _{Vc} CarbR	Fig. 1	SAD2261
T25-PilU _{Ab}	TG1, pkT25-PilU _{Ab} KanR	Fig. 1	EHC053 (SAD3261)
T25-PilU _{Ps}	TG1, pkT25-PilU _{Ps} KanR	Fig. 1	EHC048 (SAD3262)
T18-PilU _{Ab}	TG1, pUT18C-PilU _{Ab} CarbR	Fig. 1	SAD2664
T18-PilU _{Ps}	TG1, pUT18C-PilU _{Ps} CarbR	Fig. 1	EHC064 (SAD3263)
T25-PilB _{Vc}	TG1, pkT25-PilB _{Vc} KanR	Fig. 1	EHC052 (SAD3264)
T25-PilB _{Ab}	TG1, pkT25-PilB _{Ab} KanR	Fig. 1	SAD2647
T25-TfpB _{Ab}	TG1, pkT25-TfpB _{Ab} KanR	Fig. 1	SAD2878
T25-PilB _{Ps}	TG1, pkT25-PilB _{Ps} KanR	Fig. 1	SAD2959
T18-PilB _{Vc}	TG1, pUT18C-PilB _{Vc} CarbR	Fig. 1	EHC050 (SAD3265)
T18-PilB _{Ab}	TG1, pUT18C-PilB _{Ab} CarbR	Fig. 1	SAD2648
T18-TfpB _{Ab}	TG1, pUT18C-TfpB _{Ab} CarbR	Fig. 1	SAD2879
T18-PilB _{Ps}	TG1, pUT18C-PilB _{Ps} CarbR	Fig. 1	SAD2960
T25-PilT _{Vc} ^{WA}	TG1, pkT25-PilT _{Vc} ^{K136A} KanR	Fig. 1, 2	SAD2285
T25-PilT _{Ab} ^{WA}	TG1, pkT25-PilT _{Ab} ^{K136A} KanR	Fig. 2	EHC054 (SAD3266)
T25-PilT _{Ps} ^{WA}	TG1, pkT25-PilT _{Ps} ^{K136A} KanR	Fig. 2	EHC056 (SAD3267)
T25-PilT _{Ec} ^{WA}	TG1, pkT25-PilT _{Ec} ^{K133A} KanR	Fig. 2	EHC057 (SAD3268)
T18-PilT _{Vc} ^{WA}	TG1, pUT18C-PilT _{Vc} ^{K136A} CarbR	Fig. 1, 2	SAD2286
T18-PilT _{Ab} ^{WA}	TG1, pUT18C-PilT _{Ab} ^{K136A} CarbR	Fig. 2	EHC055 (SAD3269)
T18-PilT _{Ps} ^{WA}	TG1, pUT18C-PilT _{Ps} ^{K136A} CarbR	Fig. 2	EHC059 (SAD3270)
T18-PilT _{Ec} ^{WA}	TG1, pUT18C-PilT _{Ec} ^{K133A} CarbR	Fig. 2	EHC058 (SAD3271)
T25-PilT _{Vc} ^{WB}	TG1, pkT25-PilT _{Vc} ^{E204A} KanR	Fig. 2	SAD2287
T25-PilT _{Ec} ^{WB}	TG1, pkT25-PilT _{Ec} ^{E201A} KanR	Fig. 2	EHC078 (SAD3272)
T18-PilT _{Vc} ^{WB}	TG1, pUT18C-PilT _{Vc} ^{E204A} CarbR	Fig. 2	SAD2288
T18-PilT _{Ec} ^{WB}	TG1, pUT18C-PilT _{Ec} ^{E201A} CarbR	Fig. 2	EHC079 (SAD3273)
T25-Zip	TG1, pkT25-leucine zipper KanR	Fig. 1, 2	SAD2238
T18-Zip	TG1, pUT18C-leucine zipper CarbR	Fig. 1, 2	SAD2241
T25-vector	TG1, pkT25 vector KanR	Fig. 1, 2	SAD2236
T18-vector	TG1, pUT18C vector CarbR	Fig. 1, 2	SAD2240
<i>Acinetobacter baylyi</i>	ADP1	Purified gDNA	SAD631
<i>Pseudomonas stutzeri</i>	JM300	Purified gDNA	SAD2178
<i>Escherichia coli</i>	MG1655	Purified gDNA	SAD720

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570 **Table S2. Primers used in this study**

Primer name	Sequence (5' to 3')	Description
BBC374	TGGCAAAAAGCGAGAGAAGAAG	Δ luxO F1
BBC375	gtcgacggatccccggaatCATGAGGACATATTTTGTTC CTGC	Δ luxO R1
BBC376	gaagcagctccagcctacaTAAGCGATGAGAGAATGGAT CAAC	Δ luxO F2
BBC377	TCACACCCGAATTTCCATCATGC	Δ luxO R2
ABD344	GATTAGCAACGATTCTAGCGCAGGAG	Δ VC1807 F1
ABD340	gtcgacggatccccggaatACGTTTCATTAGTCACCTCTA TTGT TAACTTGTTT	Δ VC1807 R1
ABD341	gaagcagctccagcctacaTAGTCGAAAATAAAAAAAG AGG CTCGCCTC	Δ VC1807 F2
ABD345	CTTGCTAACCGTTGGTGTACCAGC	Δ VC1807 R2
DOG0400	ACTTCTGGCTGAAGGTCAATTTTC	Amplify Δ pilT/U mutations F1
DOG0403	TCACGTGTTTCGGCCAAAATC	Amplify Δ pilT/U mutations R2
ABD332	GGCTGAACGTGGTTGTCGAAAATGAC	P _{BAD} -XX @ lacZ F1
ABD256	CCCAAATACGGCAACTTGGCG	P _{BAD} -XX @ lacZ R2
BBC832	GCTTTTTGCTACAACGACCG	P _{tac} -XX @ VCA0692 F1
BBC834	CACAATTTCTCGCTTAAATGTCC	P _{tac} -XX @ VCA0692 R2
BBC1723	caatttcacacaggatcccggaAGGAGGTATTTAAATGGA TATCGCTGAGTTAC	Amplify pilT _{Vc} for P _{BAD} -pilT or P _{tac} -pilT F
BBC1724	tgtaggctggagctgcttcCTAAAATGCTTTTAAATCCAAC TCG	Amplify pilT _{Vc} for P _{BAD} -pilT or P _{tac} -pilT R
BBC2807	caatttcacacaggatcccggaAGGAGGTGAAATATGGATATAACA GAATTACTCG	Amplify pilT _{Ab} for P _{BAD} -pilT F
BBC2808	tgtaggctggagctgcttcTTAGAACGACTCTGGAGATTTTG	Amplify pilT _{Ab} for P _{BAD} -pilT R
BBC3143	caatttcacacaggatcccggaAGGAGGTAGTCCCATGGATATCACA GAAC	Amplify pilT _{Ps} for P _{BAD} -pilT or P _{tac} -pilT F
BBC3144	tgtaggctggagctgcttcTTATCGGTTGTTAAAAATTTTCCGG	Amplify pilT _{Ps} for P _{BAD} -pilT or P _{tac} -pilT R
BBC3140	caatttcacacaggatcccggaAGGAGGTCTTAGTATGAATATGGAA GAAATTGTGG	Amplify pilT _{Ec} for P _{BAD} -pilT F
BBC3141	tgtaggctggagctgcttcCATGGCAACGTCACAAACG	Amplify pilT _{Ec} for P _{BAD} -pilT R
HPQ0001	GGGCTCGGGTgctTCGACGACCTTGGCG	pilT _{Vc} ^{K136A} R1
HPQ0002	CGCCAAGGTCGTCAgacACCCGAGCCC	pilT _{Vc} ^{K136A} F2
BBC2403	GCTGCAAGGGTGGTTGAagcACCGGAACCAAGTGG	pilT _{Ab} ^{K136A} R1
BBC2404	CCACTGGTTCCGGTgctTCAACCACCTTGCAGC	pilT _{Ab} ^{K136A} F2
JCP135	CGCCAGAGTGGTGGACgcGCCCGAACCGGTCGG	pilT _{Ps} ^{K136A} R1
JCP134	CCGACCGGTTCCGGGcgcTCCACCACTCTGGCG	pilT _{Ps} ^{K136A} F2
JCP132	CGCCAGCGTGGTAGAcgcGCCACTCCCCGTCGC	pilT _{Ec} ^{K133A} R1
JCP131	GCGACGGGGAGTGGCgcgcTCTACCACGCTGGCG	pilT _{Ec} ^{K133A} F2
BBC2508	GTTTCTTGGTCACGCAGagCACCGACGAGGATCAC	pilT _{Vc} ^{E204A} R1

BBC2509	GTGATCCTCGTCGGTGctCTGCGTGACCAAGAAAC	pilT _{Vc} ^{E204A} F2
BBC4065	CTCGCTGTACGCAGagCTCCGAGCAAAATCACATCAGGATCTTCCCG	pilT _{Ec} ^{E201A} R1
BBC4066	GCTCGGAGctCTGCGTGACAGCGAGACAATCCGTCTGGCAC TGAC	pilT _{Ec} ^{E201A} F2
BBC3897	CAAGCGATGTACATCAGAATGGG	pilT _{Vc} (50)/pilT _{Ec} R1
BBC3898	cccattctgatgtacatcgcttgCTGCGGGAGTGGCTGGATG	pilT _{Vc} (50)/pilT _{Ec} F2
BBC3899	GGAATGGTACGAAACACCGCC	pilT _{Vc} (100)/pilT _{Ec} R1
BBC3900	ggcgggtgttcgtaccattcccTCGCACTGCCCGCAGCTCGAAC	pilT _{Vc} (100)/pilT _{Ec} F2
BBC3901	ATGAGCATTGACATAGTTCACC	pilT _{Vc} (150)/pilT _{Ec} R1
BBC3902	ggtgaactatgtcaatgctcatGCCGATGCGCATATTCTGACG	pilT _{Vc} (150)/pilT _{Ec} F2
BBC3903	GATCACATCTGGGTCTTCACGC	pilT _{Vc} (200)/pilT _{Ec} R1
BBC3904	gcgtaagaccagatgtgatcTTGCTCGGAGAGCTGCGTG	pilT _{Vc} (200)/pilT _{Ec} F2
BBC3907	GTCTTTGTCGCTGCCCGGAAAAAC	pilT _{Vc} (300)/pilT _{Ec} R1
BBC3908	gttttccgggagcagacaaagacCCCGTGCGTAATCAACTGGC	pilT _{Vc} (300)/pilT _{Ec} F2
BBC3909	CTGCGCCACTTTATCTTCACGG	pilT _{Ec} (50)/pilT _{Vc} R1
BBC3910	ccgtaagataaagtggcgcagTTACCGCATGTTATTCAAACC	pilT _{Ec} (50)/pilT _{Vc} F2
BBC3911	CTCCCGCAGTAGCTCTTCG	pilT _{Ec} (100)/pilT _{Vc} R1
BBC3912	cgaagagctactgcgggagATCATGAATGATGCCAGCGC	pilT _{Ec} (100)/pilT _{Vc} F2
BBC3913	GCAGTGCGAAGGTAACAGCCG	pilT _{Ec} (150)/pilT _{Vc} R1
BBC3914	cggctgttaccttcgactgcCCGACGCTAGAGCAACTGG	pilT _{Ec} (150)/pilT _{Vc} F2
BBC3915	CGCATCGGCATGTTGATTGAG	pilT _{Ec} (200)/pilT _{Vc} R1
BBC3916	ctcaatcaacatgccgatgcgCATATTTTGACCATTGAAGATCCG	pilT _{Ec} (200)/pilT _{Vc} F2
BBC3917	TCCGAGCAAAATCACATCAGG	pilT _{Ec} (250)/pilT _{Vc} R1
BBC3918	cctgatgtgattttgctcggaGAGCTGCGTGACCAAGAAACG	pilT _{Ec} (250)/pilT _{Vc} F2
BBC3919	ACGCACtGGGTCTTTTTCC	pilT _{Ec} (300)/pilT _{Vc} R1
BBC3920	ggaaaaagaccagtgctTCCATGTTGTCGGAATCGC	pilT _{Ec} (300)/pilT _{Vc} F2
DOG0565	AGATTGGTCTATTTTATGGCTCAAG	ΔpilB F1
DOG0570	gtcgacggatccccggaatCATTAGTTAATTGTTGCACCTGC	ΔpilB R1
DOG0571	gaagcagctccagcctacaCAGCGTGTGCTCTACTTTTAACTG	ΔpilB F2
DOG0572	AAAGAGTTGATGTTGAATCCCATC	ΔpilB R2
BBC1725	caatttcacacaggatcccgggAGGAGGTAGACAGATGGAGTTGAATCAATATC	Amplify pilU _{Vc} for P _{BAD} -pilU F
BBC1726	tgtaggctggagctgcttcCTACTCCATATCAATCTTCACATTC	Amplify pilU _{Vc} for P _{BAD} -pilU R
BBC2809	caatttcacacaggatcccgggAGGAGGTAATACGATGGACTTTAATGATTTACTC	Amplify pilU _{Ab} for P _{BAD} -pilU F
BBC2810	tgtaggctggagctgcttcTTATTTGAGATTACGGCGTTGAC	Amplify pilU _{Ab} for P _{BAD} -pilU R
JCP177	caatttcacacaggatcccgggAGGAGGTACGACGATGGAATTCGAGAAAC	Amplify pilU _{Ps} for P _{BAD} -pilU F
JCP178	tgtaggctggagctgcttcGGCCTGTCAGCGGAAACTG	Amplify pilU _{Ps} for P _{BAD} -pilU R
ABD334	AGTGCTCCGGACTCTTTGCTCTG	P _{BAD} -pilU @ lacZ F1
BBC1747	AACATCCGTTAGACAATGACC	P _{BAD} -pilU @ lacZ R2
BBC1952	caatttcacacaggatcccgggAGGAGGTTAACTAATGCTCACCAACCTTGTTG	Amplify pilB _{Vc} for P _{BAD} -pilB F
BBC1953	tgtaggctggagctgcttcTAAAAAGTAGAGCACACGCTG	Amplify pilB _{Vc} for P _{BAD} -pilB R
BBC3320	caatttcacacaggatcccgggaggaggtATTATAATGATATCAGAATCA CAAGGCGG	Amplify pilB _{Ab} for P _{BAD} -pilB F
BBC3310	tgtaggctggagctgcttcTTATTTACTGGTTACACGATTAATTTCC	Amplify pilB _{Ab} for P _{BAD} -pilB R

BBC3327	caatttcacacaggatcccgggaggaggtAAATAAATGACATATCATTTT GAAATAGATA	Amplify tfpB _{Ab} for P _{BAD} -tfpB F
BBC3328	tgtaggctggagctgcttcCTAAGTTAATGGGACCACCC	Amplify tfpB _{Ab} for P _{BAD} -tfpB R
BBC3324	caatttcacacaggatcccgggaggaggtAACCTCATGAGCGAAAACAT TTCCC	Amplify pilB _{Ps} for P _{BAD} -pilB F
BBC3325	tgtaggctggagctgcttcTTAGTCCTTTGTCACCCGG	Amplify pilB _{Ps} for P _{BAD} -pilB R
BBC2513	gaagcagctccagcctacaGATCCCCGGGTACCTAAGT AAC	Amplify BACTH pKT25-X to make T25-XX vectors F
BBC2512	tccacctgctccacctgcCTCTAGAGTCGACCCTGCAG	Amplify BACTH pKT25-X to make T25-XX vectors R
BBC2515	gaagcagctccagcctacaCCGAGCTCGAATTCATCGAT ATAAC	Amplify BACTH pUT18C to make T18-XX vectors F
BBC2514	tccacctgctccacctgcCTCTAGAGTCGACCCTGCAG	Amplify BACTH pUT18C to make T18-XX vectors R
BBC2518	gcaggtggagcaggtggaAAAGCGACCCAAACCTTACC	BACTH TXX-PilC _{Vc} F
BBC2524	tgtaggctggagctgcttcTTAACCCAATACACTCATTAA GTAAAG	BACTH TXX-PilC _{Vc} R
JCP027	gcaggtggagcaggtggaGATATCGCTGAGTTACTGGAATTTAG	BACTH TXX-PilT _{Vc} F
BBC1724	tgtaggctggagctgcttcCTAAAATGCTTTTAAATCCAAC TCG	BACTH TXX-PilT _{Vc} R
BBC3194	gcaggtggagcaggtggaGATATAACAGAATTACTCGCGTT	BACTH TXX-PilT _{Ab} F
BBC3195	tgtaggctggagctgcttcTTAGAACGACTCTGGAGATTTTG	BACTH TXX-PilT _{Ab} R
BBC3921	gcaggtggagcaggtggaGATATCACAGAACTGCTGGCC	BACTH TXX-PilT _{Ps} F
BBC3922	tgtaggctggagctgcttcTAAAAATTTCCGGCTGCTTGG	BACTH TXX-PilT _{Ps} R
BBC3923	gcaggtggagcaggtggaAATATGGAAGAAATTGTGGCCC	BACTH TXX-PilT _{Ec} F
BBC3924	tgtaggctggagctgcttcTCACAAACGCCCTTCCCCAC	BACTH TXX-PilT _{Ec} R
BBC2638	gcaggtggagcaggtggaCTCACCAACCTTGTGCTATC	BACTH TXX-PilB _{Vc} F
BBC2639	tgtaggctggagctgcttcTAAAAGTAGAGCACACGCTG	BACTH TXX-PilB _{Vc} R
BBC3182	gcaggtggagcaggtggaTCAGCATTTACAACACCACC	BACTH TXX-PilB _{Ab} F
BBC3183	tgtaggctggagctgcttcTTATTCAGTGGTTACACGATTAAT	BACTH TXX-PilB _{Ab} R
JCP188	gcaggtggagcaggtggaACATATCATTTTGAATAGATACACAAT G	BACTH TXX-tfpB _{Ab} F
BBC3328	tgtaggctggagctgcttcCTAAGTTAATGGGACCACCC	BACTH TXX-tfpB _{Ab} R
BBC3890	gcaggtggagcaggtggaAGCGAAAACATTTCCCTTTCC	BACTH TXX-PilB _{Ps} F

BBC3891	tgtaggctggagctgcttcTTAGTCCTTTGTCACCCGGTTG	BACTH TXX-PilB _{Ps} R
BBC2434	gcaggtggagcaggtggaGAGTTGAATCAATATCTGGATGG	BACTH TXX-PilU _{Vc} F
BBC1726	tgtaggctggagctgcttcCTACTCCATATCAATCTTCACATTC	BACTH TXX-PilU _{Vc} R
BBC3197	gcaggtggagcaggtggaGACTTTAATGATTTACTCAATTTGATGG	BACTH TXX-PilU _{Ab} F
BBC3198	tgtaggctggagctgcttcTTATTTGAGATTACGGCGTTGACC	BACTH TXX-PilU _{Ab} R
BBC3981	gcaggtggagcaggtggaGAATTCGAGAACTGTTGCG	BACTH TXX-PilU _{Ps} F
JLC178	tgtaggctggagctgcttcGGCCTGTCAGCGGAACTG	BACTH TXX-PilU _{Ps} R
JCP318	GATTAACACACCCGCGaTtcGtAATTTGATTCGCGAAG	pilT _{Ec} ^{AVG→AIR} F
JCP319	CTTCGCGAATCAAATTaCgaAtCGCGGGTGTGTTAATC	pilT _{Ec} ^{AVG→AIR} R
JCP321	CTGGCGACGCCGGCAGTggGtAACTTGATCCGTGAAG	pilT _{Vc} ^{AIR→AVG} F
JCP322	CTTCACGGATCAAGTTaCccAcTGCCGGCGTCGCCAG	pilT _{Vc} ^{AIR→AVG} R
JCP264	tgtacttctgcgcatcCAAACGCCCTTCCCCAC	pilT _{Ec} ^{+15aa} R1
JCP259	ttggatttaaagcatttTGAgagcagctccagcctac	pilT _{Ec} ^{+15aa} F2
JCP262	gtaggctggagctgcttcCTACACCACACCACGCGCGATC	pilT _{Vc} ^{Δ15aa} R1
JCP263	gatcgcgcgtggtgtggtgTAGgaagcagctccagcctac	pilT _{Vc} ^{Δ15aa} F2

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