Retraction ATPase motors are promiscuous among type IVa pilus systems

Evan Couser[1], Jennifer L. Chlebek[1], and Ankur B. Dalia[1,*]

[1]Department of Biology, Indiana University, Bloomington, IN 47405.
*Correspondence to: ankdalia@indiana.edu.

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., Δ*pilT* and Δ*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 Δ*pilT* and Δ*pilTU* strains (**Fig. 1A**). Furthermore, we observed that ectopic expression of $pilT_{Ab}$, $pilT_{Ps}$, and $pilT_{Ec}$, all fully complemented natural transformation of Δ*pilT* and Δ*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

191 Walker A mutant. When we ectopically expressed the $pilT_{Ec}^{WB}$ in a Δ*pilT* background (where
192 *pilU* is intact), however, we observed that this mutant did not restore transformation (**Fig. 2A**),
193 which is similar to the $pilT_{Ec}^{WA}$ allele. This indicates that self-assembly of an ATPase defective
194 $PilT_{Ec}$ is not sufficient to promote PilU-dependent retraction. Together, these data suggest that
195 $PilT_{Ec}$ may have unique features that prevents it from promoting PilU-dependent retraction. We
196 hypothesized that analyzing the differences between $PilT_{Ec}$ and the other PilT alleles ($PilT_{Vc}$,
197 $PilT_{Ab}$, and $PilT_{Ps}$) may help define the features of PilT that allow for PilU-dependent retraction.

198 *The C-terminus of $PilT_{Vc}$ contributes to PilU-mediated retraction*.
199 In order to narrow down the region of $PilT_{Vc}$ that is necessary and/or sufficient to support PilU-
200 dependent retraction, we constructed defined chimeras of $pilT_{Vc}$ and $pilT_{Ec}$. These chimeras
201 were first ectopically expressed in a Δ*pilTU* background to test their ability to mediate retraction
202 of the competence pilus in a manner that is dependent on their ATPase activity – a property that
203 both $PilT_{Vc}$ and $PilT_{Ec}$ shared (**Fig. 1A**). Surprisingly, only three of the chimeras were able to
204 restore parental levels of transformation in the Δ*pilTU* background (**Fig. 3A**). These are the only
205 chimeras of PilT that we can be reasonably confident could potentially support PilU-dependent
206 retraction, thus, we only proceeded with characterizing these three chimeras of PilT moving
207 forward. Next, we introduced a Walker A mutation into these chimeras to determine if they were
208 able to support PilU-mediated retraction. Consistent with the idea that PilT self-assembly is
209 required for PilU-mediated retraction, none of the $pilT_{Vc}$ / $pilT_{Ec}$ chimeras with the Walker A
210 mutation were able to rescue transformation efficiency in a Δ*pilT* background (**Fig. 3B**).
211 Separately, a Walker B mutation was introduced into these chimeras. Again, none of the 3
212 chimeras were able to restore transformation in a Δ*pilT* background (**Fig. 3B**). Because the
213 chimera that contained the N-terminal ~200 amino acids of $PilT_{Vc}$ and C-terminal ~150 amino
214 acids of $PilT_{Ec}$ did not support PilU-dependent retraction, we reasoned that the C-terminus of
215 $PilT_{Vc}$ is important for PilU-dependent retraction.

216 In order to further investigate potential components of PilT that are required for supporting PilU-
217 mediated retraction, we constructed an alignment of the 4 PilT orthologs that were studied here.
218 The alignment revealed that the middle section of $PilT_{Ec}$ was conserved relative to all of the
219 other PilT orthologs (**Fig. S2**). However, the N-terminus and the C-terminus of $PilT_{Ec}$ were
220 poorly conserved relative to the other PilT orthologs. Because our data suggests that the C-
221 terminus of $PilT_{Vc}$ is required for supporting PilU-mediated retraction, that is where we focused
222 our investigation. In particular, we observed that $pilT_{Ec}$ contains two substitutions in the highly
223 conserved AIRNLIRE domain of PilT (18). To determine if these substitutions were responsible
224 for the inability of $PilT_{Ec}$ to support PilU-mediated retraction (7), we mutated the two residues in
225 $pilT_{Ec}$ to the residues present in the other PilT orthologs ($PilT_{Ec}^{AVG \rightarrow AIR}$). Conversely, to determine
226 if these changes in $PilT_{Ec}$ contributed to its inability to support PilU-mediated retraction, the
227 residues in $pilT_{Vc}$ were mutated to the residues present in $pilT_{Ec}$ ($PilT_{Vc}^{AIR \rightarrow AVG}$). These mutations
228 were introduced into Walker A (WA) or Walker B (WB) mutant alleles of PilT so that we could
229 assess PilU-mediated retraction. The introduction of these mutations into $pilT_{Vc}$ did not result in
230 the inability of $PilT_{Vc}$ to facilitate PilU-mediated retraction (**Fig. 3C**). Moreover, the restoration of
231 the two A<u>IR</u>NLIRE substitutions in $pilT_{Ec}$ did not rescue PilU-mediated retraction (**Fig. 3C**).
232 These data indicate that the substitutions in the AIRNLIRE domain of $pilT_{Ec}$ are not responsible
233 for the inability of $PilT_{Ec}$ to support PilU-mediated retraction.

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

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

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

262

263 **DISCUSSION**

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

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

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

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

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

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

314

315 **METHODS**

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

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

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

333 inactivating the quorum sensing negative regulator LuxO (Δ*luxO*) as previously described (8).
334 Chitin-independent natural transformation assays were performed exactly as previously
335 described (8, 14). Briefly, strains were grown overnight in 3 mL of LB broth containing 100 μM
336 IPTG at 30 °C rolling. Next, cells were subcultured into 3 mL of LB supplemented with 20 mM
337 $MgCl_2$, 10 mM $CaCl_2$, 100 μM IPTG, and arabinose (when appropriate; 0.05-0.2%) and grown to
338 late-log phase at 30 °C rolling. Next, ~10^8 cells were transferred to Instant Ocean medium (7
339 g/L; Aquarium Systems) containing 100 μM IPTG, arabinose (when appropriate; 0.05-0.2%),
340 and 200 ng of a transforming DNA (tDNA) PCR product. Reactions were then incubated
341 statically overnight at 30 °C. The tDNA product used throughout this study targeted a "neutral
342 gene" (the frame-shifted transposase, VC1807) for inactivation (ΔVC1807::Erm^R) as previously
343 described (27). A negative control, containing no tDNA, was included for each strain in all
344 transformation assays. After the overnight incubation with tDNA, reactions were outgrown by
345 adding 1 mL of LB to each reaction and incubating at 37 °C shaking for 2 hours. After the
346 outgrowth, reactions were plated for quantitative culture to distinguish between transformants
347 (plated on LB + erythromycin) and total viable counts (plated on plain LB). The transformation
348 frequency for each reaction was calculated by dividing the number of transformants by the total
349 viable counts.

350 *Bacterial Adenylate Cyclase Two-Hybrid Assay*
351 Using PCR, the desired motor ATPase genes were amplified and cloned into the BACTH
352 vectors pKT25 and pUT18C in order to produce N-terminal fusions between the motor ATPase
353 and the T25 and T18 fragments of adenylate cyclase, respectively. Miniprepped plasmids
354 (Qiagen) were then co-transformed into *E. coli* BTH101 cells. As a positive control, T25-zip and
355 T18-zip vectors were cotransformed into BTH101. And as a negative control, the pKT25 and
356 pUT18C empty vectors were cotransformed into BTH101. Transformations were plated onto LB
357 + kanamycin (50 μg/mL) + carbenicillin (100 μg/mL) plates in order to select for transformants
358 that received both plasmids. These transformants were then selected and grown overnight at 30
359 °C static in LB + kanamycin (50 μg/mL) + carbenicillin (100 μg/mL). Finally, 3 μL of the overnight
360 cultures were spotted onto LB agar plates supplemented with 500 μM IPTG, kanamycin (50
361 μg/mL), carbenicillin (100 μg/mL), and X-gal (40 μg/mL). These plates were allowed to incubate
362 statically at 30 °C for ~48 hours prior to imaging on a HP Scanjet G4010 flatbed scanner.

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

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

371

372 **ACKNOWLEDGEMENTS**

373 We would like to thank David Kysela for helpful discussions. This work was supported by grant
374 R35GM128674 from the National Institutes of Health (to ABD).

375 **References**

376 1. Craig L, Forest KT, Maier B. 2019. Type IV pili: dynamics, biophysics and functional
377 consequences. Nat Rev Microbiol 17:429-440.
378 2. Jakovljevic V, Leonardy S, Hoppert M, Sogaard-Andersen L. 2008. PilB and PilT are
379 ATPases acting antagonistically in type IV pilus function in Myxococcus xanthus. J
380 Bacteriol 190:2411-21.
381 3. Ellison CK, Dalia TN, Klancher CA, Shaevitz JW, Gitai Z, Dalia AB. 2021.
382 Acinetobacter baylyi regulates type IV pilus synthesis by employing two extension
383 motors and a motor protein inhibitor. Nat Commun 12:3744.
384 4. Chlebek JL, Hughes HQ, Ratkiewicz AS, Rayyan R, Wang JC, Herrin BE, Dalia TN,
385 Biais N, Dalia AB. 2019. PilT and PilU are homohexameric ATPases that coordinate to
386 retract type IVa pili. PLoS Genet 15:e1008448.
387 5. Whitchurch CB, Mattick JS. 1994. Characterization of a gene, pilU, required for
388 twitching motility but not phage sensitivity in Pseudomonas aeruginosa. Mol Microbiol
389 13:1079-91.
390 6. McCallum M, Tammam S, Khan A, Burrows LL, Howell PL. 2017. The molecular
391 mechanism of the type IVa pilus motors. Nat Commun 8:15091.
392 7. Adams DW, Pereira JM, Stoudmann C, Stutzmann S, Blokesch M. 2019. The type IV
393 pilus protein PilU functions as a PilT-dependent retraction ATPase. PLoS Genet
394 15:e1008393.
395 8. Ellison CK, Dalia TN, Vidal Ceballos A, Wang JC, Biais N, Brun YV, Dalia AB. 2018.
396 Retraction of DNA-bound type IV competence pili initiates DNA uptake during natural
397 transformation in Vibrio cholerae. Nat Microbiol 3:773-780.
398 9. Seitz P, Blokesch M. 2013. DNA-uptake machinery of naturally competent Vibrio
399 cholerae. Proc Natl Acad Sci U S A 110:17987-92.
400 10. Leong CG, Bloomfield RA, Boyd CA, Dornbusch AJ, Lieber L, Liu F, Owen A, Slay E,
401 Lang KM, Lostroh CP. 2017. The role of core and accessory type IV pilus genes in
402 natural transformation and twitching motility in the bacterium Acinetobacter baylyi.
403 PLoS One 12:e0182139.
404 11. Graupner S, Weger N, Sohni M, Wackernagel W. 2001. Requirement of novel
405 competence genes pilT and pilU of Pseudomonas stutzeri for natural transformation and
406 suppression of pilT deficiency by a hexahistidine tag on the type IV pilus protein PilAI. J
407 Bacteriol 183:4694-701.
408 12. Graupner S, Frey V, Hashemi R, Lorenz MG, Brandes G, Wackernagel W. 2000. Type
409 IV pilus genes pilA and pilC of Pseudomonas stutzeri are required for natural genetic
410 transformation, and pilA can be replaced by corresponding genes from nontransformable
411 species. J Bacteriol 182:2184-90.
412 13. Dalia TN, Chlebek JL, Dalia AB. 2020. A modular chromosomally integrated toolkit for
413 ectopic gene expression in Vibrio cholerae. Sci Rep 10:15398.
414 14. Chlebek JL, Dalia TN, Biais N, Dalia AB. 2021. Fresh Extension of Vibrio cholerae
415 Competence Type IV Pili Predisposes Them for Motor-Independent Retraction. Appl
416 Environ Microbiol 87:e0047821.
417 15. Chiang P, Sampaleanu LM, Ayers M, Pahuta M, Howell PL, Burrows LL. 2008.
418 Functional role of conserved residues in the characteristic secretion NTPase motifs of the
419 Pseudomonas aeruginosa type IV pilus motor proteins PilB, PilT and PilU. Microbiology
420 (Reading) 154:114-126.

421 16. Bischof LF, Friedrich C, Harms A, Sogaard-Andersen L, van der Does C. 2016. The
422 Type IV Pilus Assembly ATPase PilB of Myxococcus xanthus Interacts with the Inner
423 Membrane Platform Protein PilC and the Nucleotide-binding Protein PilM. J Biol Chem
424 291:6946-57.
425 17. Karimova G, Pidoux J, Ullmann A, Ladant D. 1998. A bacterial two-hybrid system based
426 on a reconstituted signal transduction pathway. Proc Natl Acad Sci U S A 95:5752-6.
427 18. Aukema KG, Kron EM, Herdendorf TJ, Forest KT. 2005. Functional dissection of a
428 conserved motif within the pilus retraction protein PilT. J Bacteriol 187:611-8.
429 19. Denise R, Abby SS, Rocha EPC. 2019. Diversification of the type IV filament
430 superfamily into machines for adhesion, protein secretion, DNA uptake, and motility.
431 PLoS Biol 17:e3000390.
432 20. Giltner CL, Nguyen Y, Burrows LL. 2012. Type IV pilin proteins: versatile molecular
433 modules. Microbiol Mol Biol Rev 76:740-72.
434 21. Hsiao A, Liu Z, Joelsson A, Zhu J. 2006. Vibrio cholerae virulence regulator-coordinated
435 evasion of host immunity. Proc Natl Acad Sci U S A 103:14542-7.
436 22. Floyd KA, Lee CK, Xian W, Nametalla M, Valentine A, Crair B, Zhu S, Hughes HQ,
437 Chlebek JL, Wu DC, Hwan Park J, Farhat AM, Lomba CJ, Ellison CK, Brun YV,
438 Campos-Gomez J, Dalia AB, Liu J, Biais N, Wong GCL, Yildiz FH. 2020. c-di-GMP
439 modulates type IV MSHA pilus retraction and surface attachment in Vibrio cholerae. Nat
440 Commun 11:1549.
441 23. Barbe V, Vallenet D, Fonknechten N, Kreimeyer A, Oztas S, Labarre L, Cruveiller S,
442 Robert C, Duprat S, Wincker P, Ornston LN, Weissenbach J, Marliere P, Cohen GN,
443 Medigue C. 2004. Unique features revealed by the genome sequence of Acinetobacter sp.
444 ADP1, a versatile and naturally transformation competent bacterium. Nucleic Acids Res
445 32:5766-79.
446 24. Busquets A, Pena A, Gomila M, Bosch R, Nogales B, Garcia-Valdes E, Lalucat J,
447 Bennasar A. 2012. Genome sequence of Pseudomonas stutzeri strain JM300 (DSM
448 10701), a soil isolate and model organism for natural transformation. J Bacteriol
449 194:5477-8.
450 25. Blattner FR, Plunkett G, 3rd, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J,
451 Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA,
452 Rose DJ, Mau B, Shao Y. 1997. The complete genome sequence of Escherichia coli K-
453 12. Science 277:1453-62.
454 26. Dalia AB. 2018. Natural Cotransformation and Multiplex Genome Editing by Natural
455 Transformation (MuGENT) of Vibrio cholerae. Methods Mol Biol 1839:53-64.
456 27. Dalia AB, McDonough E, Camilli A. 2014. Multiplex genome editing by natural
457 transformation. Proc Natl Acad Sci U S A 111:8937-42.
458 28. Notredame C, Higgins DG, Heringa J. 2000. T-Coffee: A novel method for fast and
459 accurate multiple sequence alignment. J Mol Biol 302:205-17.
460 29. Needleman SB, Wunsch CD. 1970. A general method applicable to the search for
461 similarities in the amino acid sequence of two proteins. J Mol Biol 48:443-53.

462

463

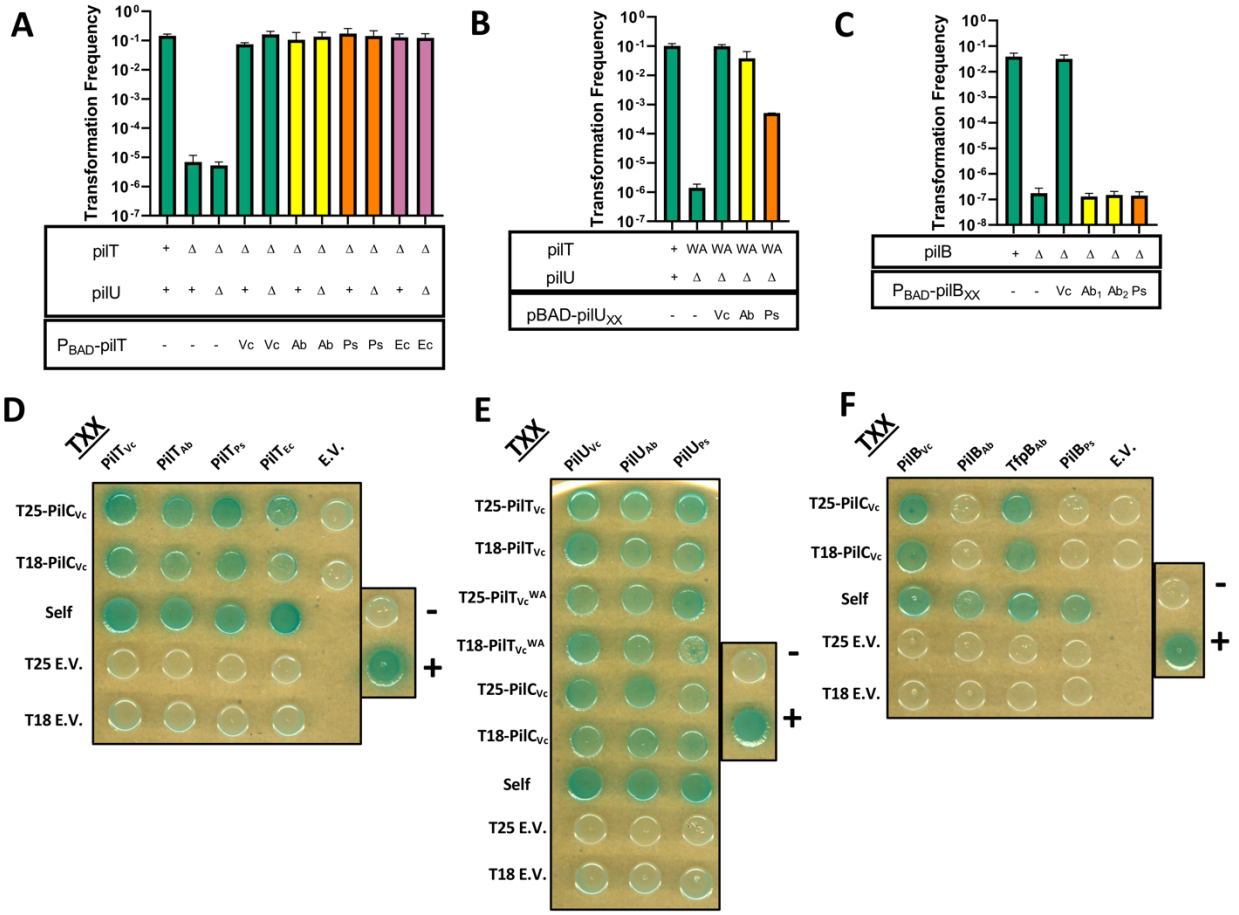

Fig. 1. ***Retraction ATPase orthologs are promiscuous among T4aP systems, while extension ATPase orthologs are not.*** Natural transformation assays were performed to test whether (**A**) PilT, (**B**) PilU, or (**C**) PilB/TfpB orthologs from 3 heterologous T4aP systems could complement *V. cholerae* 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 ± SD. The limit of detection for transformation frequency assays is ~10^{-7}. In **C**, Ab_1 denotes $pilB_{Ab}$ and Ab_2 denotes $tfpB_{Ab}$. In **C**, for all samples where the transformation frequency is ~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.

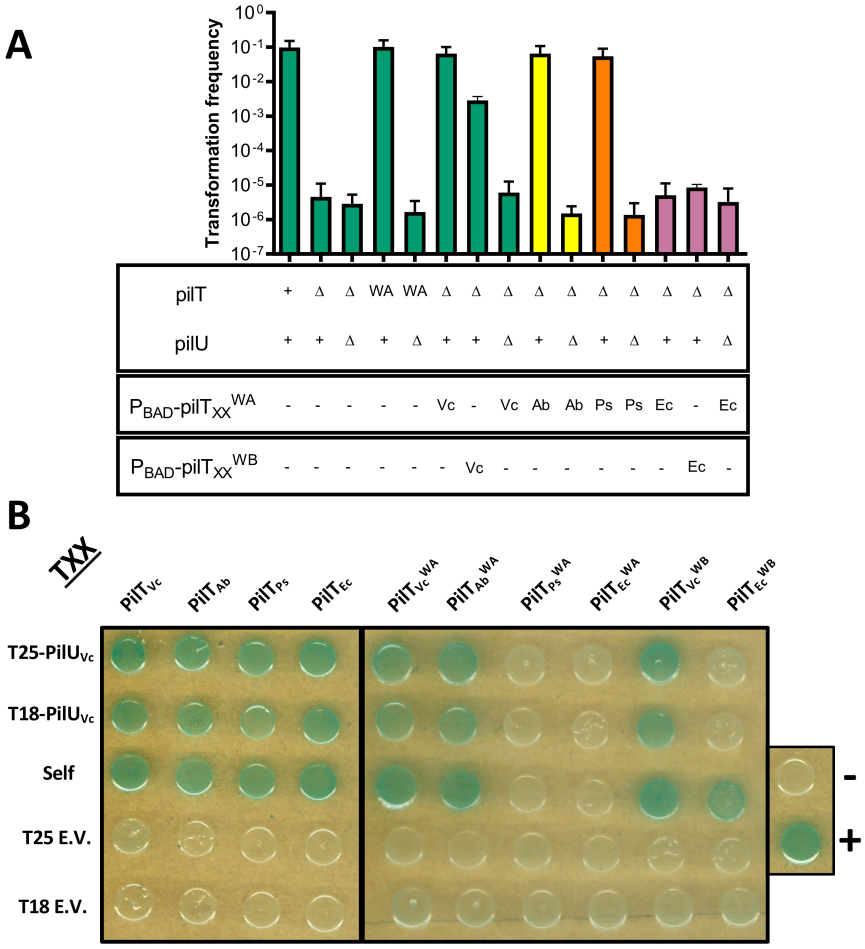

Fig. 2. *The PilT homolog from E. coli cannot support PilU-mediated retraction*. (**A**) Natural transformation assays were performed to test whether retraction ATPase PilT mutants from 3 heterologous T4aP systems could complement *V. cholerae* mutants lacking their endogenous motor ATPases. The relevant 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 ± 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.

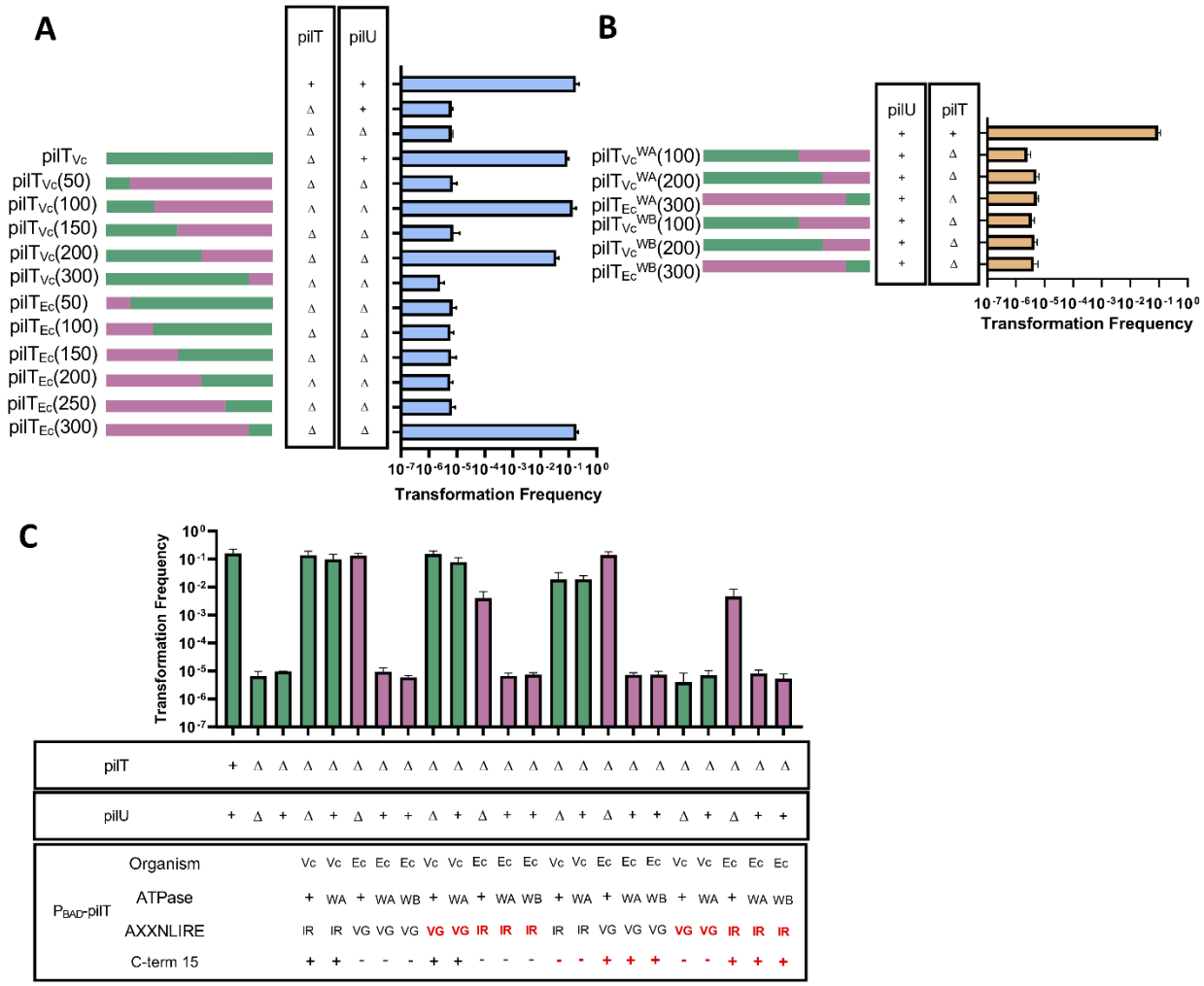

Fig. 3. *The C-terminus of PilT$_{Vc}$ contributes to PilU-mediated retraction*. Natural transformation assays were performed to test whether retraction ATPase chimeras between PilT$_{Vc}$ and PilT$_{Ec}$ can complement *V. cholerae* mutants lacking their endogenous motor ATPases. PilT chimeras either have (**A**) their ATPase activity intact to assess ATPase-dependent retraction, or (**B**) their ATPase activity inactivated via Walker A or Walker B mutations to assess PilU-mediated retraction. The relevant genotypes of strains are indicated to the left of each bar, which includes the status of the native *pilT*/*pilU* genes as well as the ectopically expressed motor ATPase chimera (induced via P$_{BAD}$). Diagrams to the left of bars denote the composition of each chimera with the fragments of PilT$_{Vc}$ denoted in green and PilT$_{Ec}$ denoted in pink. The number in parentheses indicates the number of N-terminal amino acids from the PilT allele indicated fused to its PilT ortholog. (**C**) Natural transformation assays were performed to test whether two elements at the C-terminus of PilT$_{Vc}$ (the AIRNLIRE domain and a 15-residue domain at the C-terminus) contribute to PilU-mediated retraction. 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 chimera (induced via P$_{BAD}$). Four attributes of each ectopically expressed allele are indicated: the organism from which the PilT allele is derived, the ATPase status of the allele ("+" denotes ATPase intact, "WA" denotes Walker A mutant, and "WB" denotes Walker B mutant), the sequence of the AXXNLIRE domain (IR = AIRNLIRE, VG = AVGNLIRE), and the presence / absence of the C-terminal 15 amino acids. If the attribute changes the natural allele, it is displayed in red text. The color of the bar corresponds to the species from which the ectopically expressed *pilT* was derived (green bars = *Vc pilT* and purple bars = *Ec pilT*). All data are from at least 3 independent biological replicates and shown as the mean ± SD.

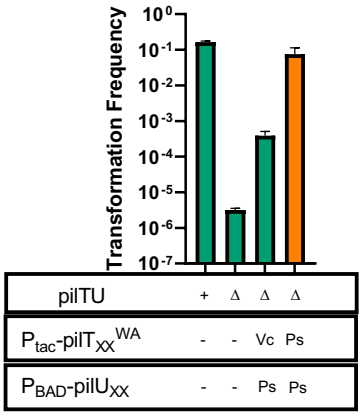

Fig. S1. *PilU$_{Ps}$ is a fully functional retraction ATPase motor.* Natural transformation assays were performed to assess whether PilU$_{Ps}$ can support pilus retraction in conjunction with its cognate PilT$_{Ps}^{WA}$ better than with the heterologous PilT$_{Vc}^{WA}$. The relevant genotypes of 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 ± SD.

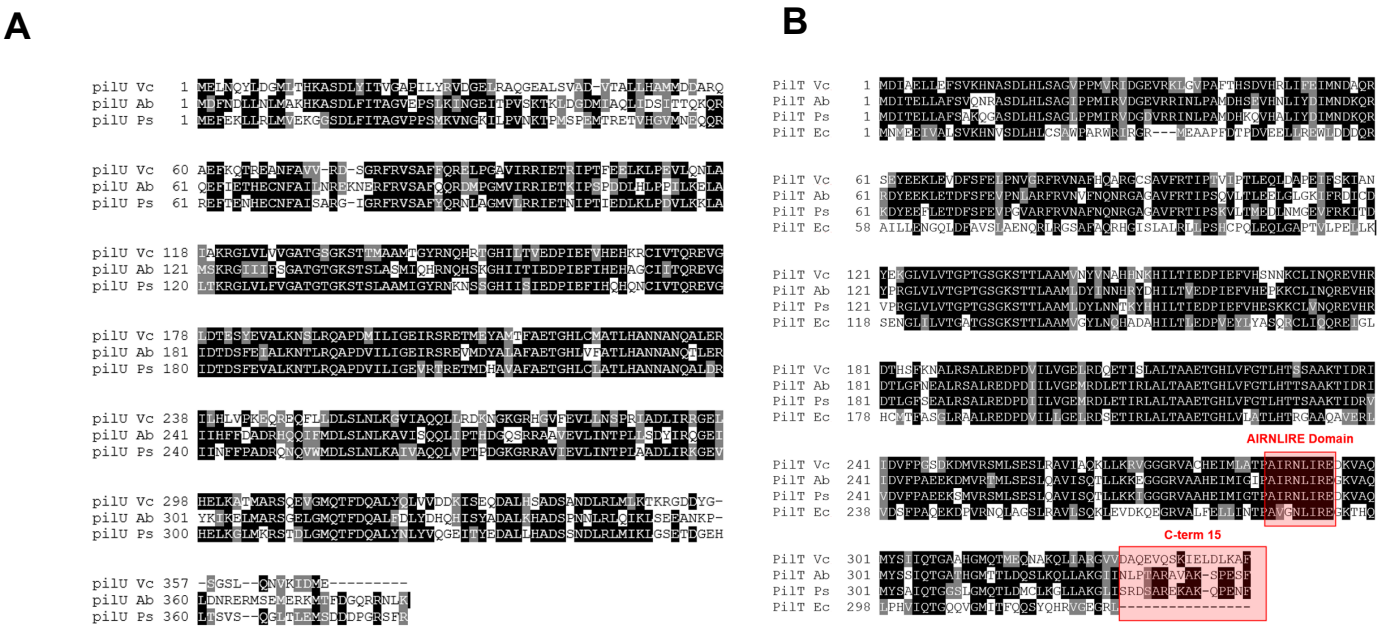

Fig. S2. *Alignment of PilU and PilT sequences from the four T4aP studied.* Alignments of (**A**) PilU orthologs and (**B**) PilT orthologs. Residues highlighted in black are identical, while those highlighted in gray are similar. The AIRNLIRE and C-terminal 15 residue domains experimentally tested in this study are boxed in red. The GenBank Accession numbers for the protein sequences used are as follows: Vc, *Vibrio cholerae* 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).

A

	$PilT_{Vc}$	$PilT_{Ab}$	$PilT_{Ps}$	$PilT_{Ec}$
$PilT_{Vc}$	100%	83%	80%	62%
$PilT_{Ab}$	83%	100%	91%	60%
$PilT_{Ps}$	80%	91%	100%	59%
$PilT_{Ec}$	62%	60%	59%	100%

B

	$PilU_{Vc}$	$PilU_{Ab}$	$PilU_{Ps}$
$PilU_{Vc}$	100%	70%	74%
$PilU_{Ab}$	70%	100%	78%
$PilU_{Ps}$	74%	78%	100%

C

full length

	$PilB_{Vc}$	$PilB_{Ab}$	$TfpB_{Ab}$	$PilB_{Ps}$
$PilB_{Vc}$	100%	64%	53%	66%
$PilB_{Ab}$	64%	100%	55%	75%
$TfpB_{Ab}$	53%	55%	100%	54%
$PilB_{Ps}$	66%	75%	54%	100%

CTD

	$PilB_{Vc}$	$PilB_{Ab}$	$TfpB_{Ab}$	$PilB_{Ps}$
$PilB_{Vc}$	100%	75%	64%	75%
$PilB_{Ab}$	75%	100%	64%	86%
$TfpB_{Ab}$	64%	64%	100%	61%
$PilB_{Ps}$	75%	86%	61%	100%

NTD

	$PilB_{Vc}$	$PilB_{Ab}$	$TfpB_{Ab}$	$PilB_{Ps}$
$PilB_{Vc}$	100%	41%	32%	49%
$PilB_{Ab}$	41%	100%	38%	52%
$TfpB_{Ab}$	32%	38%	100%	41%
$PilB_{Ps}$	49%	52%	41%	100%

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 MN------------------------------------------------------------
PilB Vc      1 MLTNL-----------------VAIDRQAELISATQEQAVVTQVSASGTSVPEALDEL--
PilB Ab      1 MISESQGGLMSAFTTPPKFSGFIRRLVEEGYVNAQNMQQALEKAKKFKQDIVPYLIDN--
TfpB Ab      1 MTYHFEI----------DTQWCLEQLLKDGRISERDKLLIQT-THRNKEQLKWHPLQWIA
PilB Ps      1 MSENI------------SLSGLARQMVRAGVLDEKTAQQAQVQAQRNKVSLVTYVVQN--

                                                NTD
PilT Vc      3 ------------------------------------------------------------
PilT Ab      3 ------------------------------------------------------------
PilT Ps      3 ------------------------------------------------------------
PilT Ec      3 ------------------------------------------------------------
PilB Vc     42 -----------SIFHAQELTEQLSHIFGLPETDISRYDYA-NLCQQLGLRELITRYDALP
PilB Ab     59 -----------FSISPLTIAEIISLEFGEPLLDIGVFDPA-LFLKDKIDEKLIQKYRIMP
TfpB Ab     50 HFDLKDQAHTSKPFDILRLSQWLAEKVGLPLYVIDPLKADVNMLTSVMSQEFALRNKILA
PilB Ps     47 -----------KLAKGRAVAELASDQFGVALLDISALDKD-SLPKELVSEKLARQHRVLP

                                                NTD
PilT Vc      3 ------------------------------------------------------------
PilT Ab      3 ------------------------------------------------------------
PilT Ps      3 ------------------------------------------------------------
PilT Ec      3 ------------------------------------------------------------
PilB Vc     90 IAKQGNLDLLLVSDPTLLQAEEEFRFATG-LQVPLAIADHRAIQAAIRRLYGRS--IQGA
PilB Ab    107 LVHRGHVDYVATSNPTNIEAMDAIRFNSK-LKVPPIIVEHDKIERLISEHPVEE--THFN
TfpB Ab    110 VDVNPERILIGTDQPFVRDWVSNLELSLAPKKIELVILSPDQMQRYIPEYMQVSRAVHSS
PilB Ps     95 LVKRGTKDYVAISDPANHQSITDIQFSTG-LTIPSIIVEDDKIGDAIDKLYDSG--TSGL

                                NTD                                CTD
PilT Vc      3 ----------------------------------------IAELIEFSVKHNASDLHLSA
PilT Ab      3 ----------------------------------------ITELIAFSVQNRASDLHLSA
PilT Ps      3 ----------------------------------------ITELIAFSAKQGASDLHLSA
PilT Ec      3 ----------------------------------------LEEIVALSVKHNVSDLHLCS
PilB Vc    147 ANQGKEISQDEL-ANLVKVSDDELQSIEDLSQDISPVSRFINQVILDAVRKGASDIHFEP
PilB Ab    164 FDTE-ELDLDVE-VDP-HTTDD--DDEDDKLKDDAPIVKYINKLLIDAIRMSASDLHFEP
TfpB Ab    170 KNAT-AYERDNKGVEA-LL-QL--GDNQNPDANDQHIVKLVDWILQFAFEQGASDIHLEP
PilB Ps    152 EDLG-DVDLDGI-DVE-SVSDDAKGGEEGEAADDAPVVRFVNKVILDAIRGGSSDLHFEP

                                                CTD
PilT Vc     23 G---VPPMVRIDGEVRKLG-VPAFTHS-DVHRLIFEIM---NDAQRSEYEEKLEVDFSFE
PilT Ab     23 G---IPPMIRVDGEVRRIN-LPAMDHS-EVHNLIYDIM---NDKQRFDYEEKLETDFSFE
PilT Ps     23 G---LPPMIRVDGDVRRIN-LPAMDHK-QVHALIYDIM---NDKQRKDYEEFLETDFSFE
PilT Ec     23 A---VPARVRIRGRM---E-AAPFDTP-DVEELLREWL---DDDQRAILLENGQLDFAVS
PilB Vc    206 YENQVRIRIRCDGILVETQ-QPASHLSRRLAA-RIKILSKLDIAERR-LPQDGRIKLRLS
PilB Ab    219 YEKSVRVRYRVDGVLRLIA-TPPLQLATRLAS-RIKVVSQLDISEKR-VPQDGRIKLKMS
TfpB Ab    225 RKEKGKVRFRIDGVLHTIYHMPANTLS-AVIA-RIKIIGRLNVAEKR-KPQDGRLKTRTP
PilB Ps    209 YEKAVRVRIRTDGILHEVA-RPPIQLAPRISA-RIKVVAGLDISERR-KPQDGRIKMKLS

                                                CTD
PilT Vc     75 LPNVGRFRVNAFHQARGCSAVFRTIPTVIP--TIEQLDAP----EIFSKLANYEKGLVLV
PilT Ab     75 VPNLARFRVNVFNQNRGAGAVFRTIPSQVL--TIEELGLG----KIFRDICDYPRGLVLV
PilT Ps     75 VPGVARFRVNAFNQNRGAGAVFRTIPSKVL--IMEDLNMG----EVFRKITDVPRGLVLV
PilT Ec     72 LAENQRLRGSAFAQRHGISLAIRLLPSHCP--QLEQLGAP----TVLPEILKSENGLILV
PilB Vc    263 RDTAIDMRVSVLPTLWGEKIVLRLLDSSAANLDIIKLGYNPQQKQLYLNALKRPQGMILM
PilB Ab    276 KSKTIDFRVNSLPTLFGEKIVLRILDPASAMLGIIALGYEPEQKALFMFALNKPQGMILI
TfpB Ab    282 KGQETELRLSILPTAFGEKIVMRIFDPDVLVRSFEQLGFDEILLKQMQALSRNSHGIILV
PilB Ps    266 KAKAIDFRVNILPTLWGEKIVMRILDPSSAQMGIIALGYEESQKQLYMIALKQPQGMILV

                                                CTD
PilT Vc    129 TGPTGSGKSTTLAAMVNYVNAHHNKHILTIEDPIEFVHSNNKCLINQREVH-RDTHSFKN
PilT Ab    129 TGPTGSGKSTTLAAMIDYINNHRYDHILTVEDPIEFVHEPKKCLINQREVH-RDTLGFNE
PilT Ps    129 TGPTGSGKSTTLAAMIDYLNNTKYHHILTIEDPIEFVHESKKCLINQREVH-RDTLGFSE
PilT Ec    126 TGATGSGKSTTLAAMVGYLNQHADAHILTIEDPVEYLYASQRCLIQQREIG-LHCMTFAS
PilB Vc    323 TGPTGSGKTVSLYTGLRILNTS-QINISTAEDPVEINLSG----INQVQVQPKIGFGFAE
PilB Ab    336 TGPTGSGKTVSLYTGLNILNTE-HANISTAEDPVEINLEG----VNQVNVNPKVGLTFAA
```


552

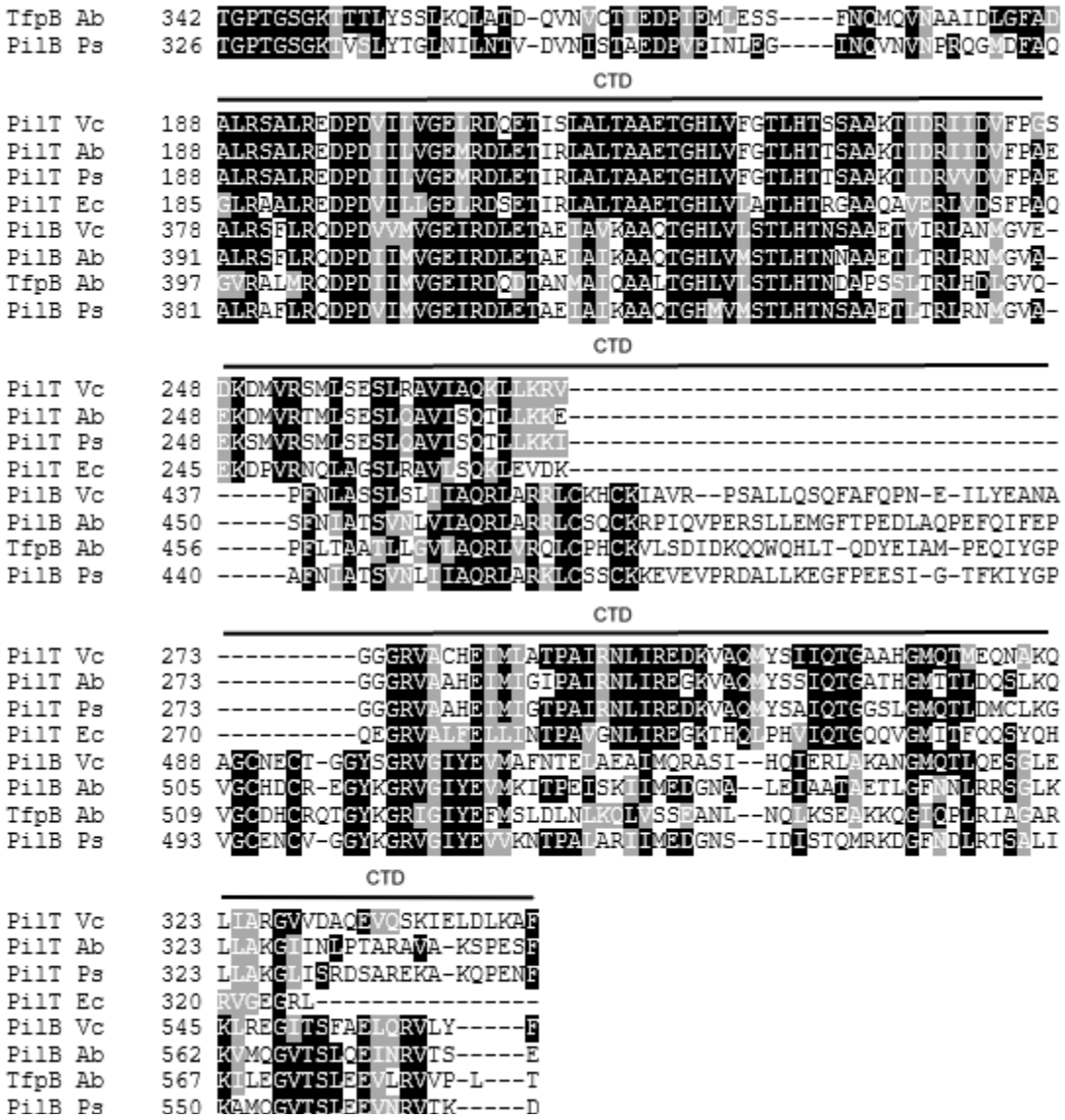

Fig. S4. ***Alignment of the PilB/TfpB and PilT sequences from the T4aP studied.*** Alignments of PilB/TfpB orthologs and PilT orthologs. Residues highlighted in black are identical, while those highlighted in gray are similar. The N-terminal domain and C-terminal domain of PilB are denoted by the “NTD” 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, ΔluxO::miniFRT, lacZ::lacIq, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*	Fig. 1, 2, 3, S1	TND0905 (SAD2468)
Δ*pilT*	E7946 SmR, ΔlacZ::lacIq, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔpilT::TmR	Fig. 1, 2, 3	TND1035 (SAD2469)
Δ*pilTU*	E7946 SmR, ΔlacZ::lacIq, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔpilTU::TmR	Fig. 1, 2, 3, S1	JLC227 (SAD2470)
P_{BAD}-*pilT*$_{Vc}$, Δ*pilT*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-PilT$_{Vc}$ CarbR, ΔpilT::TmR	Fig. 1, 3	TND1831 (SAD3199)
P_{BAD}-*pilT*$_{Vc}$, Δ*pilTU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-PilT$_{Vc}$ CarbR, ΔpilTU::TmR	Fig. 1, 3	JLC1155 (SAD3222)
P_{BAD}-*pilT*$_{Ab}$, Δ*pilT*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-PilT$_{Ab}$ CarbR, ΔpilT::TmR	Fig. 1	TND2148 (SAD3200)
P_{BAD}-*pilT*$_{Ab}$, Δ*pilTU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-PilT$_{Ab}$ CarbR, ΔpilTU::TmR	Fig. 1	JLC1153 (SAD3223)
P_{BAD}-*pilT*$_{Ps}$, Δ*pilT*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-PilT$_{Ps}$ CarbR, ΔpilT::TmR	Fig. 1	TND2125 (SAD3201)
P_{BAD}-*pilT*$_{Ps}$, Δ*pilTU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-PilT$_{Ps}$ CarbR, ΔpilTU::TmR	Fig. 1	JLC1157 (SAD3224)
P_{BAD}-*pilT*$_{Ec}$, Δ*pilT*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-PilT$_{Ec}$ CarbR, ΔpilT::TmR	Fig. 1	JLC1473 (SAD3225)
P_{BAD}-*pilT*$_{Ec}$, Δ*pilTU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-PilT$_{Ec}$ CarbR, ΔpilTU::TmR	Fig. 1	JLC1474 (SAD3226)
pilT^{WA}, Δ*pilU*	E7946 SmR, ΔlacZ::lacIq, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, pilT^{K136A}, ΔpilU::TmR	Fig. 1, 2	JLC444 (SAD2472)
P_{BAD}-*pilU*$_{Vc}$, *pilT*^{WA}, Δ*pilU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C, pilT$_{Vc}^{K136A}$, ΔlacZ:: P_{BAD}-PilU$_{Vc}$ CarbR, ΔpilU::TmR	Fig. 1	EHC062 (SAD3239)
P_{BAD}-*pilU*$_{Ab}$, *pilT*^{WA}, Δ*pilU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C, pilT$_{Vc}^{K136A}$, ΔlacZ:: P_{BAD}-PilU$_{Ab}$ CarbR, ΔpilU::TmR	Fig. 1	EHC060 (SAD3240)
P_{BAD}-*pilU*$_{Ps}$, *pilT*^{WA}, Δ*pilU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C, pilT$_{Vc}^{K136A}$, ΔlacZ:: P_{BAD}-PilU$_{Ps}$ CarbR, ΔpilU::TmR	Fig. 1	EHC061 (SAD3241)
Parent (P_{const}-*tfoX*)	E7946 SmR, ΔlacZ::lacIq, P_{const}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C	Fig. 1	TND1076 (SAD2513)
Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT	Fig. 1	TND2373 (SAD2787)

P_{BAD}-*pilB*$_{Vc}$, Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT, ΔVCA0692:: P_{BAD}-pilB$_{Vc}$ CarbR	Fig. 1	TND2403 (SAD2789)
P_{BAD}-*pilB*$_{Ab}$, Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT, ΔVCA0692:: P_{BAD}-pilB$_{Ab}$ CarbR	Fig. 1	TND2354 (SAD3202)
P_{BAD}-*tfpB*$_{Ab}$, Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT, ΔVCA0692:: P_{BAD}-tfpB$_{Ab}$ CarbR	Fig. 1	TND2372 (SAD3203)
P_{BAD}-*pilB*$_{Ps}$, Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT, ΔVCA0692:: P_{BAD}-pilB$_{Ps}$ CarbR	Fig. 1	TND2371 (SAD3204)
pilT^{WA}	E7946 SmR, ΔlacZ::lacIq, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C, pilT^{K136A}	Fig. 2	TND1077 (SAD2471)
P_{BAD}-*pilT*$_{Vc}^{WA}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ::P_{BAD}-pilT$_{Vc}^{K136A}$ CarbR, ΔpilT::TmR	Fig. 2, 3	JLC1609 (SAD3227)
P_{BAD}-*pilT*$_{Vc}^{WA}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ::P_{BAD}-pilT$_{Vc}^{K136A}$ CarbR, ΔpilTU::TmR	Fig. 2	EHC031 (SAD3242)
P_{BAD}-*pilT*$_{Vc}^{WB}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ::P_{BAD}-pilT$_{Vc}^{E204A}$ CarbR, ΔpilT::TmR	Fig. 2	EHC075 (SAD3243)
P_{BAD}-*pilT*$_{Ab}^{WA}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ::P_{BAD}-pilT$_{Ab}^{K136A}$ CarbR, ΔpilT::TmR	Fig. 2	EHC015 (SAD3244)
P_{BAD}-*pilT*$_{Ab}^{WA}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ::P_{BAD}-pilT$_{Ab}^{K136A}$ CarbR, ΔpilTU::TmR	Fig. 2	EHC019 (SAD3245)
P_{BAD}-*pilT*$_{Ps}^{WA}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ::P_{BAD}-pilT$_{Ps}^{K136A}$ CarbR, ΔpilT::TmR	Fig. 2	EHC016 (SAD3246)
P_{BAD}-*pilT*$_{Ps}^{WA}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ::P_{BAD}-pilT$_{Ps}^{K136A}$ CarbR, ΔpilTU::TmR	Fig. 2	EHC020 (SAD3247)
P_{BAD}-*pilT*$_{Ec}^{WA}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ::P_{BAD}-pilT$_{Ec}^{K133A}$ CarbR, ΔpilT::TmR	Fig. 2	JLC1499 (SAD3228)
P_{BAD}-*pilT*$_{Ec}^{WA}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ::P_{BAD}-pilT$_{Ec}^{K133A}$ CarbR, ΔpilTU::TmR	Fig. 2	JLC1498 (SAD3229)
P_{BAD}-*pilT*$_{Ec}^{WB}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ::P_{BAD}-pilT$_{Ec}^{E201A}$ CarbR, ΔpilT::TmR	Fig. 2, 3	EHC076 (SAD3248)
P_{BAD}-*pilT*$_{Vc}$(50)/*pilT*$_{Ec}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Vc}$(50)/pilT$_{Ec}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3069 (SAD3205)
P_{BAD}-*pilT*$_{Vc}$(100)/*pilT*$_{Ec}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Vc}$(100)/pilT$_{Ec}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3070 (SAD3206)
P_{BAD}-*pilT*$_{Vc}$(150)/*pilT*$_{Ec}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Vc}$(150)/pilT$_{Ec}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3071 (SAD3207)

P_{BAD}-*pilT*$_{Vc}$(200)/*pilT*$_{Ec}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Vc}$(200)/pilT$_{Ec}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3073 (SAD3208)
P_{BAD}-*pilT*$_{Vc}$(300)/*pilT*$_{Ec}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Vc}$(300)/pilT$_{Ec}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3072 (SAD3209)
P_{BAD}-*pilT*$_{Ec}$(50)/*pilT*$_{Vc}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Ec}$(50)/pilT$_{Vc}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3074 (SAD3210)
P_{BAD}-*pilT*$_{Ec}$(100)/*pilT*$_{Vc}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Ec}$(100)/pilT$_{Vc}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3075 (SAD3211)
P_{BAD}-*pilT*$_{Ec}$(150)/*pilT*$_{Vc}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Ec}$(150)/pilT$_{Vc}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3076 (SAD3212)
P_{BAD}-*pilT*$_{Ec}$(200)/*pilT*$_{Vc}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Ec}$(200)/pilT$_{Vc}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3077 (SAD3213)
P_{BAD}-*pilT*$_{Ec}$(250)/*pilT*$_{Vc}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Ec}$(250)/pilT$_{Vc}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3078 (SAD3214)
P_{BAD}-*pilT*$_{Ec}$(300)/*pilT*$_{Vc}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Ec}$(300)/pilT$_{Vc}$ CarbR, ΔpilTU::TmR	Fig. 3	TND3079 (SAD3215)
P_{BAD}-*pilT*$_{Vc}^{WA}$(100)/*pilT*$_{Ec}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Vc}^{K136A}$(100)/pilT$_{Ec}$ CarbR, ΔpilT::TmR	Fig. 3	TND3008 (SAD3216)
P_{BAD}-*pilT*$_{Vc}^{WA}$(200)/*pilT*$_{Ec}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Vc}^{K136A}$(200)/pilT$_{Ec}$ CarbR, ΔpilT::TmR	Fig. 3	TND3010 (SAD3217)
P_{BAD}-*pilT*$_{Ec}^{WA}$(300)/*pilT*$_{Vc}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Ec}^{K133A}$(300)/pilT$_{Vc}$ CarbR, ΔpilT::TmR	Fig. 3	TND3018 (SAD3218)
P_{BAD}-*pilT*$_{Vc}^{WB}$(100)/*pilT*$_{Ec}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Vc}^{E204A}$(100)/pilT$_{Ec}$ CarbR, ΔpilT::TmR	Fig. 3	EHC106 (SAD3249)
P_{BAD}-*pilT*$_{Vc}^{WB}$(200)/*pilT*$_{Ec}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Vc}^{E204A}$(200)/pilT$_{Ec}$ CarbR, ΔpilT::TmR	Fig. 3	EHC108 (SAD3250)
P_{BAD}-*pilT*$_{Ec}^{WB}$(300)/*pilT*$_{Vc}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-3X Flag-pilT$_{Ec}^{E201A}$(300)/pilT$_{Vc}$ CarbR, ΔpilT::TmR	Fig. 3	EHC115 (SAD3251)
P_{BAD}-*pilT*$_{Vc}^{AIR\rightarrow AVG}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Vc}$-$^{AIR\rightarrow AVG}$ CarbR, ΔpilTU::TmR	Fig. 3	JLC1622 (SAD3230)
P_{BAD}-*pilT*$_{Vc}^{WA,\ AIR\rightarrow AVG}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Vc}^{K136A,\ AIR\rightarrow AVG}$ CarbR, ΔpilT::TmR	Fig. 3	JLC1625 (SAD3231)
P_{BAD}-*pilT*$_{Vc}^{\Delta 15aa}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Vc}^{\Delta 15aa}$ CarbR, ΔpilTU::TmR	Fig. 3	TND2774 (SAD3219)
P_{BAD}-*pilT*$_{Vc}^{WA,\ \Delta 15aa}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Vc}^{K136A,\ \Delta 15aa}$ CarbR, ΔpilT::TmR	Fig. 3	JLC1611 (SAD3232)

P_{BAD}-*pilT*$_{Vc}^{AIR\rightarrow AVG, \Delta 15aa}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Vc-AIR\rightarrow AVG, \Delta 15aa}$ CarbR, ΔpilTU::TmR	Fig. 3	JLC1624 (SAD3233)
P_{BAD}-*pilT*$_{Vc}^{WA, AIR\rightarrow AVG, \Delta 15aa}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Vc}^{K136A, AIR\rightarrow AVG, \Delta 15aa}$ CarbR, ΔpilT::TmR	Fig. 3	JLC1627 (SAD3234)
P_{BAD}-*pilT*$_{Ec}^{AVG\rightarrow AIR}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Ec-AVG\rightarrow AIR}$ CarbR, ΔpilTU::TmR	Fig. 3	JLC1614 (SAD3235)
P_{BAD}-*pilT*$_{Ec}^{WA, AVG\rightarrow AIR}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Ec}^{K133A, AVG\rightarrow AIR}$ CarbR, ΔpilT::TmR	Fig. 3	JLC1615 (SAD3236)
P_{BAD}-*pilT*$_{Ec}^{WB, AVG\rightarrow AIR}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Ec}^{E201A, AVG\rightarrow AIR}$ CarbR, ΔpilT::TmR	Fig. 3	EHC095 (SAD3252)
P_{BAD}-*pilT*$_{Ec}^{+15aa}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Ec}^{+15aa}$ CarbR, ΔpilTU::TmR	Fig. 3	TND2730 (SAD3220)
P_{BAD}-*pilT*$_{Ec}^{WA, +15aa}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Ec}^{K133A, +15aa}$ CarbR, ΔpilT::TmR	Fig. 3	TND2727 (SAD3221)
P_{BAD}-*pilT*$_{Ec}^{WB, +15aa}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Ec}^{E201A, +15aa}$ CarbR, ΔpilT::TmR	Fig. 3	EHC093 (SAD3253)
P_{BAD}-*pilT*$_{Ec}^{AVG\rightarrow AIR, +15aa}$, Δ*pilTU*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Ec-AVG\rightarrow AIR, +15aa}$ CarbR, ΔpilTU::TmR	Fig. 3	JLC1620 (SAD3237)
P_{BAD}-*pilT*$_{Ec}^{WA, AVG\rightarrow AIR, +15aa}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Ec}^{K133A, AVG\rightarrow AIR, +15aa}$ CarbR, ΔpilT::TmR	Fig. 3	JLC1617 (SAD3238)
P_{BAD}-*pilT*$_{Ec}^{WB, AVG\rightarrow AIR, +15aa}$, Δ*pilT*	E7946 SmR, ΔluxO::miniFRT, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*, ΔlacZ:: P_{BAD}-pilT$_{Ec}^{E201A, AVG\rightarrow AIR, +15aa}$ CarbR, ΔpilT::TmR	Fig. 3	EHC097 (SAD3254)
P_{tac}-*pilT*$_{Vc}^{WA}$, P_{BAD}-*pilU*$_{Ps}$, Δ*pilTU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C, ΔlacZ::P_{BAD}-PilU$_{Ps}$ CarbR, ΔVC0692::P_{tac}-PilT$_{Vc}^{K136A}$ SpecR, ΔpilTU::TmR	Fig. S1	EHC099 (SAD3255)
P_{tac}-*pilT*$_{Ps}^{WA}$, P_{BAD}-*pilU*$_{Ps}$, Δ*pilTU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C, ΔlacZ::P_{BAD}-PilU$_{Ps}$ CarbR, ΔVC0692::P_{tac}-PilT$_{Ps}^{K136A}$ SpecR, ΔpilTU::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
Acinetobacter baylyi	ADP1	Purified gDNA	SAD631
Pseudomonas stutzeri	JM300	Purified gDNA	SAD2178
Escherichia coli	MG1655	Purified gDNA	SAD720

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

Primer name	Sequence (5' to 3')	Description
BBC374	TGGCAAAAAGCGAGAGAAGAAG	ΔluxO F1
BBC375	gtcgacggatccccggaatCATGAGGACATATTTTGTTTT CTGC	ΔluxO R1
BBC376	gaagcagctccagcctacaTAAGCGATGAGAGAATGGAT CAAC	ΔluxO F2
BBC377	TCACACCCGAATTTCCATCATGC	ΔluxO R2
ABD344	GATTAGCAACGATTCTAGCGCAGGAG	ΔVC1807 F1
ABD340	gtcgacggatccccggaatACGTTTCATTAGTCACCTCTA TTGT TAACTTGTTC	ΔVC1807 R1
ABD341	gaagcagctccagcctacaTAGTCGAAAATAAAAAAAAG AGG CTCGCCTC	ΔVC1807 F2
ABD345	CTTGCTAACCGTTGGTGTTACCAGC	ΔVC1807 R2
DOG0400	ACTTCTGGCTGAAGGTCAATTTTC	Amplify ΔpilT/U mutations F1
DOG0403	TCACGTGTTCGGCCAAAATC	Amplify ΔpilT/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	CACAATTTCTCGCTTAAAATGTCC	P_{tac}-XX @ VCA0692 R2
BBC1723	caatttcacacaggatcccgggAGGAGGTATTTAAATGGA 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	caatttcacacaggatcccgggAGGAGGTGAAAATATGGATATAACA GAATTACTCG	Amplify $pilT_{Ab}$ for P_{BAD}-pilT F
BBC2808	tgtaggctggagctgcttcTTAGAACGACTCTGGAGATTTTG	Amplify $pilT_{Ab}$ for P_{BAD}-pilT R
BBC3143	caatttcacacaggatcccgggAGGAGGTAGTCCCATGGATATCACA 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	caatttcacacaggatcccgggAGGAGGTCTTAGTATGAATATGGAA 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	CGCCAAGGTCGTCGAagcACCCGAGCCC	$pilT_{Vc}^{K136A}$ F2
BBC2403	GCTGCAAGGGTGGTTGAagcACCGGAACCAGTGG	$pilT_{Ab}^{K136A}$ R1
BBC2404	CCACTGGTTCCGGTgctTCAACCACCCTTGCAGC	$pilT_{Ab}^{K136A}$ F2
JCP135	CGCCAGAGTGGTGGAcgcGCCCGAACCGGTCGG	$pilT_{Ps}^{K136A}$ R1
JCP134	CCGACCGGTTCGGGCgcgTCCACCACTCTGGCG	$pilT_{Ps}^{K136A}$ F2
JCP132	CGCCAGCGTGGTAGAcgcGCCACTCCCCGTCGC	$pilT_{Ec}^{K133A}$ R1
JCP131	GCGACGGGGAGTGGCgcgTCTACCACGCTGGCG	$pilT_{Ec}^{K133A}$ F2
BBC2508	GTTTCTTGGTCACGCAGagCACCGACGAGGATCAC	$pilT_{Vc}^{E204A}$ R1

BBC2509	GTGATCCTCGTCGGTGctCTGCGTGACCAAGAAAC	pilT$_{Vc}^{E204A}$ F2
BBC4065	CTCGCTGTCACGCAGagCTCCGAGCAAAATCACATCAGGATCTTCCCG	pilT$_{Ec}^{E201A}$ R1
BBC4066	GCTCGGAGctCTGCGTGACAGCGAGACAATCCGTCTGGCACTGAC	pilT$_{Ec}^{E201A}$ F2
BBC3897	CAAGCGATGTACATCAGAATGGG	pilT$_{Vc}$(50)/pilT$_{Ec}$ R1
BBC3898	cccattctgatgtacatcgcttgCTGCGGGAGTGGCTGGATG	pilT$_{Vc}$(50)/pilT$_{Ec}$ F2
BBC3899	GGGAATGGTACGAAACACCGCC	pilT$_{Vc}$(100)/pilT$_{Ec}$ R1
BBC3900	ggcggtgtttcgtaccattcccTCGCACTGCCCGCAGCTCGAAC	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	gcgtgaagacccagatgtgatcTTGCTCGGAGAGCTGCGTG	pilT$_{Vc}$(200)/pilT$_{Ec}$ F2
BBC3907	GTCTTTGTCGCTGCCCGGAAAAAC	pilT$_{Vc}$(300)/pilT$_{Ec}$ R1
BBC3908	gtttttccgggcagcgacaaagacCCCGTGCGTAATCAACTGGC	pilT$_{Vc}$(300)/pilT$_{Ec}$ F2
BBC3909	CTGCGCCACTTTATCTTCACGG	pilT$_{Ec}$(50)/pilT$_{Vc}$ R1
BBC3910	ccgtgaagataaagtggcgcagTTACCGCATGTTATTCAAACC	pilT$_{Ec}$(50)/pilT$_{Vc}$ F2
BBC3911	CTCCCGCAGTAGCTCTTCG	pilT$_{Ec}$(100)/pilT$_{Vc}$ R1
BBC3912	cgaagagctactgcgggagATCATGAATGATGCCCAGCGC	pilT$_{Ec}$(100)/pilT$_{Vc}$ F2
BBC3913	GCAGTGCGAAGGTAACAGCCG	pilT$_{Ec}$(150)/pilT$_{Vc}$ R1
BBC3914	cggctgttaccttcgcactgcCCGACGCTAGAGCAACTGG	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	ggaaaaagacccagtgcgtTCCATGTTGTCGGAATCGC	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	tgtaggctggagctgcttcTTAAAAGTAGAGCACACGCTG	Amplify pilB$_{Vc}$ for P$_{BAD}$-pilB R
BBC3320	caatttcacacaggatcccgggaggaggtATTATAATGATATCAGAATCACAAGGCGG	Amplify pilB$_{Ab}$ for P$_{BAD}$-pilB F
BBC3310	tgtaggctggagctgcttcTTATTCACTGGTTACACGATTAATTTCC	Amplify pilB$_{Ab}$ for P$_{BAD}$-pilB R

BBC3327	caatttcacacaggatcccgggaggaggtAAATAAATGACATATCATTTTGAAATAGATA	Amplify $tfpB_{Ab}$ for P_{BAD}-tfpB F
BBC3328	tgtaggctggagctgcttcCTAAGTTAATGGGACCACCC	Amplify $tfpB_{Ab}$ for P_{BAD}-tfpB R
BBC3324	caatttcacacaggatcccgggaggaggtAACCTCATGAGCGAAAACATTTCCC	Amplify $pilB_{Ps}$ for P_{BAD}-pilB F
BBC3325	tgtaggctggagctgcttcTTAGTCCTTTGTCACCCGG	Amplify $pilB_{Ps}$ for P_{BAD}-pilB R
BBC2513	gaagcagctccagcctacaGATCCCCGGGTACCTAAGTAAC	Amplify BACTH pKT25-X to make T25-XX vectors F
BBC2512	tccacctgctccacctgcCTCTAGAGTCGACCCTGCAG	Amplify BACTH pKT25-X to make T25-XX vectors R
BBC2515	gaagcagctccagcctacaCCGAGCTCGAATTCATCGATATAAC	Amplify BACTH pUT18C to make T18-XX vectors F
BBC2514	tccacctgctccacctgcCTCTAGAGTCGACCTGCAG	Amplify BACTH pUT18C to make T18-XX vectors R
BBC2518	gcaggtggagcaggtggaAAAGCGACCCAAACCTTACC	BACTH TXX-$PilC_{Vc}$ F
BBC2524	tgtaggctggagctgcttcTTAACCCAATACACTCATTAAGTTAAAG	BACTH TXX-$PilC_{Vc}$ R
JCP027	gcaggtggagcaggtggaGATATCGCTGAGTTACTGGAATTTAG	BACTH TXX-$PilT_{Vc}$ F
BBC1724	tgtaggctggagctgcttcCTAAAATGCTTTTAAATCCAACTCG	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	tgtaggctggagctgcttcTTAAAAATTTTCCGGCTGCTTGG	BACTH TXX-$PilT_{Ps}$ R
BBC3923	gcaggtggagcaggtggaAATATGGAAGAAATTGTGGCCC	BACTH TXX-$PilT_{Ec}$ F
BBC3924	tgtaggctggagctgcttcTCACAAACGCCCTTCCCCCAC	BACTH TXX-$PilT_{Ec}$ R
BBC2638	gcaggtggagcaggtggaCTCACCAACCTTGTTGCTATC	BACTH TXX-$PilB_{Vc}$ F
BBC2639	tgtaggctggagctgcttcTTAAAAGTAGAGCACACGCTG	BACTH TXX-$PilB_{Vc}$ R
BBC3182	gcaggtggagcaggtggaTCAGCATTTACAACACCACC	BACTH TXX-$PilB_{Ab}$ F
BBC3183	tgtaggctggagctgcttcTTATTCACTGGTTACACGATTAAT	BACTH TXX-$PilB_{Ab}$ R
JCP188	gcaggtggagcaggtggaACATATCATTTTGAAATAGATACACAATG	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	gcaggtggagcaggtggaGAATTCGAGAAACTGTTGCG	BACTH TXX-PilU$_{Ps}$ F
JLC178	tgtaggctggagctgcttcGGCCTGTCAGCGGAAACTG	BACTH TXX-PilU$_{Ps}$ R
JCP318	GATTAACACACCCGCGaTtcGtAATTTGATTCGCGAAG	pilT$_{Ec}^{AVG\rightarrow AIR}$ F
JCP319	CTTCGCGAATCAAATTaCgaAtCGCGGGTGTGTTAATC	pilT$_{Ec}^{AVG\rightarrow AIR}$ R
JCP321	CTGGCGACGCCGGCAgTggGtAACTTGATCCGTGAAG	pilT$_{Vc}^{AIR\rightarrow AVG}$ F
JCP322	CTTCACGGATCAAGTTaCccAcTGCCGGCGTCGCCAG	pilT$_{Vc}^{AIR\rightarrow AVG}$ R
JCP264	tgtacttcttgcgcatcCAAACGCCCTTCCCCCAC	pilT$_{Ec}^{+15aa}$ R1
JCP259	ttggatttaaaagcatttTGAgaagcagctccagcctac	pilT$_{Ec}^{+15aa}$ F2
JCP262	gtaggctggagctgcttcCTACACCACACCACGCGCGATC	pilT$_{Vc}^{\Delta 15aa}$ R1
JCP263	gatcgcgcgtggtgtggtgTAGgaagcagctccagcctac	pilT$_{Vc}^{\Delta 15aa}$ F2

571