Retraction ATPase motors from three orthologous type IVa pilus systems support promiscuous retraction of the *Vibrio cholerae* competence pilus

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 or jennichlebek@gmail.com

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 three orthologous T4aP can functionally complement *Vibrio cholerae* mutants that lack their endogenous motors. We found that the PilT and PilU retraction ATPases from the three T4aP systems tested are promiscuous and promote retraction of the *V. cholerae* competence T4aP despite a high degree of sequence divergence. In contrast, the orthologous extension ATPases from the same T4aP systems were not able to mediate extension of the *V. cholerae* competence T4aP despite exhibiting a similar degree of sequence divergence. Also, we show that one of the PilT orthologs characterized does not support PilU-dependent retraction and provide some data to indicate that the C-terminus of PilT is important for PilU-dependent retraction. Together, our data suggest that retraction ATPases may have maintained a high degree of promiscuity for promoting retraction of T4aP, while extension ATPases may have evolved to become 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 three T4aP are promiscuous, and capable of promoting retraction of a heterologous T4aP system. By contrast, the extension ATPase motors from these same T4aP systems are specific and cannot promote extension of a 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). The gene that encodes this motor is generally found in close proximity to other T4aP structural genes. 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 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). Unlike the extension motor, the genes encoding retraction motors are generally not located near other T4aP structural genes. These hexameric extension and retraction 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). A previous study demonstrated that the PilT retraction motor from *Pseudomonas aeruginosa* was able to complement a *V. cholerae pilT* mutant (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. For this reason, *A. baylyi* and *P. stutzeri* were selected for this study. 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 orthologous T4aP systems, extension ATPases display a high level of specificity for their cognate T4aP. Furthermore, our data identify a PilT ortholog that supports ATPase-dependent retraction, but not 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. Because PilU is an accessory retraction ATPase motor (4, 7), ectopic expression of $pilT_{Vc}$ alone is able to rescue 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**). Western blotting of functional N-terminally FLAG-tagged alleles of the PilT orthologs (4) confirmed that their expression was equal to or greater than native PilT in *V. cholerae* (**Fig. S1**).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**). Western blotting of functional N-terminally FLAG-tagged alleles of the PilU orthologs (4) demonstrated that their expression was equal to or greater than native PilU in *V. cholerae* (**Fig. S1)**. 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. S2**). Together, this suggests that while PilU is also promiscuous between these T4aP systems, 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 $tfpB_{Ab}$), *P. stutzeri* ($pilB_{Ps}$), and *E. coli* ($pilB_{Ec}$; also referred to as *hofB*) in a *V. cholerae* Δ*pilB* mutant and assessed natural transformation. While $pilB_{Vc}$ recovered natural transformation as expected, ectopic expression of $pilB_{Ab}$, $tfpB_{Ab}$, $pilB_{Ps,}$ and $pilB_{Ec}$ failed to complement the Δ*pilB* mutant (**Fig. 1C**). Importantly, Western blotting of N-terminally FLAG-tagged alleles of all PilB orthologs (14) indicated that expression of each was equal to or greater than native PilB in *V. cholerae* (**Fig. S1**). This suggests that extension ATPase orthologs from these species are not able to mediate extension of the *V. cholerae* competence pilus. These data indicate that there may be a high degree of specificity among extension ATPases, which is in stark contrast to the observed promiscuity among the retraction ATPases studied here.

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 PilT orthologs could interact with $PilC_{Vc}$ (**Fig. 1D**). Because PilU is a PilT-dependent retraction ATPase, it may need to interact with both PilT

143 and PilC to support pilus retraction. Indeed, all of the PilU orthologs could interact with $PilT_{Vc}$,
144 $PilT_{Vc}^{WA}$, and $PilC_{Vc}$, which is consistent with the ability of each PilU ortholog to support some
145 degree of natural transformation (**Fig. 1E**). For the PilB orthologs, we found that $PilB_{Vc}$ could
146 interact with $PilC_{Vc}$ as expected (**Fig. 1F**). Additionally, each of the PilB orthologs, apart from
147 $PilB_{Ec}$, could self-interact, which is consistent with the hexameric conformation that these
148 proteins likely adopt (**Fig. 1F**). Interestingly, $PilB_{Ab}$, $TfpB_{Ab}$, $PilB_{Ps}$, and $PilB_{Ec}$ varied in their
149 ability to interact with $PilC_{Vc}$. $PilB_{Ab,}$ $PilB_{Ps}$, could not interact with $PilC_{Vc}$ and $PilB_{Ec}$ only
150 interacted with $PilC_{Vc}$ in one direction (**Fig. 1F**), which could suggest that these proteins fail to
151 support extension because they cannot interact properly with the platform. However, $TfpB_{Ab}$
152 could interact with $PilC_{Vc}$ (**Fig. 1F**), but still failed to support natural transformation (**Fig. 1C**).
153 These data indicate that while interactions between PilB and PilC may be required for extension
154 of the competence pilus, these interactions are not sufficient to promote extension.

155 *The PilT ortholog from E. coli cannot support PilU-mediated retraction.*
156 Above, our results indicate that PilT orthologs from distinct T4aP can promote retraction of the
157 *V. cholerae* competence pilus in the absence of native $PilT_{Vc}$ and $PilU_{Vc}$. PilT, however, can also
158 support PilU-dependent retraction, in a manner that is not dependent on PilT ATPase activity
159 (4). So next, we wanted to test if the PilT orthologs among the studied T4aP could support PilU-
160 dependent retraction in conjunction with $PilU_{Vc}$. To test this, Walker A mutants of each *pilT* gene
161 ($pilT_{Vc}^{WA}$, $pilT_{Ab}^{WA}$, $pilT_{Ps}^{WA}$, $pilT_{Ec}^{WA}$) were ectopically expressed in a Δ*pilT* background (i.e.,
162 where $pilU_{Vc}$ is intact) and a Δ*pilTU* background. As expected, the ectopic expression of $pilT_{Vc}^{WA}$
163 fully restored the transformation efficiency of a Δ*pilT* background (since $pilU_{Vc}$ is intact) (**Fig.**
164 **2A**). Importantly, transformation was not restored by ectopic expression of any Walker A *pilT*
165 alleles in a Δ*pilTU* background (**Fig. 2A**), which indicates that these ATPase defective alleles of
166 PilT only support PilU-dependent retraction and cannot promote retraction on their own. When
167 $pilT_{Ab}^{WA}$ and $pilT_{Ps}^{WA}$ were ectopically expressed in the Δ*pilT* background, transformation was
168 rescued to parental levels (**Fig. 2A**). These data suggest that $PilU_{Vc}$ is able to mediate retraction
169 of the *V. cholerae* competence pilus in a $PilT_{Ab}$- and $PilT_{Ps}$-dependent manner, which further
170 demonstrates the promiscuity among these retraction ATPases. Ectopic expression of the
171 Walker A mutant allele of $pilT_{Ec}$, however, did not restore natural transformation in the Δ*pilT*
172 background (**Fig. 2A**). This suggests that $PilT_{Ec}^{WA}$ is incapable of promoting PilU-dependent
173 retraction in the presence of $PilU_{Vc}$.

174 The ability of PilU to interact with PilT is likely necessary for PilU-dependent retraction of the
175 competence pilus. As expected, $PilT_{Vc}$, $PilT_{Ab}$, $PilT_{Ps}$, and $PilT_{Ec}$ could self-interact (**Fig. 2B**) as
176 seen previously (**Fig. 1D**), and each allele could also interact with $PilU_{Vc}$ (**Fig. 2B**). Because
177 $PilU_{Vc}$-mediated retraction was possible when $pilT_{Vc}^{WA}$, $pilT_{Ab}^{WA}$, and $pilT_{Ps}^{WA}$ were ectopically
178 expressed, we hypothesized that each of these PilT alleles would be able to self-interact and
179 also interact with PilU. $PilT_{Vc}^{WA}$ and $PilT_{Ab}^{WA}$ were able to self-interact (**Fig. 2B**), which suggests
180 that the Walker A mutation does not inhibit the ability of these proteins to self-assemble into
181 hexamers. This is consistent with our previous results which demonstrate that purified $PilT_{Vc}^{WA}$
182 can still form hexameric rings (4). Unexpectedly, $PilT_{Ps}^{WA}$ and $PilT_{Ec}^{WA}$ did not exhibit self-
183 interactions in these BACTH assays (**Fig. 2B**). We also observed that $PilU_{Vc}$ could interact with
184 $PilT_{Vc}^{WA}$ and $PilT_{Ab}^{WA}$, however, it did not interact with $PilT_{Ec}^{WA}$ or $PilT_{Ps}^{WA}$ (**Fig. 2B**). The
185 absence of interactions between $PilU_{Vc}$ and $PilT_{Ec}^{WA}$ (**Fig. 2B**) is consistent with the inability of
186 $PilT_{Ec}^{WA}$ to promote PilU-dependent retraction (**Fig. 2A**). However, the absence of interactions
187 between $PilU_{Vc}$ and $PilT_{Ps}^{WA}$ was particularly surprising since this PilT allele can support PilU-
188 dependent retraction when expressed in *V. cholerae* (**Fig. 2A**). Because $PilT_{Ps}^{WA}$ supports PilU-
189 dependent retraction in *V. cholerae*, but does not exhibit self-interactions in BACTH assays, this
190 may suggest that self-assembly or hexamerization of PilT is not required for PilU-dependent
191 retraction. However, the inability of $PilT_{Ps}^{WA}$ to interact with $PilU_{Vc}$ in these same BACTH assays

192 (**Fig. 2B**), despite the ability of this allele to support PilU-dependent retraction (**Fig. 2A**) makes
193 this difficult to conclude. The observed inability of $PilT_{Ec}^{WA}$ and $PilT_{Ps}^{WA}$ to self-interact and/or
194 interact with $PilU_{Vc}$ in BACTH assays could either be due to an absence of interactions between
195 these proteins, or could be due to a poor affinity interaction between these proteins that is below
196 the detection limit for the assay.

197 The molecular mechanism underlying PilU-mediated retraction in a PilT-dependent manner
198 remains unclear. The ability of PilT to self-assemble and form a hexamer may be a prerequisite
199 for PilU-mediated retraction. However, other characteristics of PilT may also be responsible for
200 supporting PilU-mediated retraction. Because $pilT_{Ec}^{WA}$ lacked self-interactions, we constructed
201 an alternative mutant that also disrupted the ATPase activity of $PilT_{Ec}$ by mutating an invariantly
202 conserved glutamate in the Walker B domain of $pilT_{Ec}$ ($pilT_{Ec}^{E201A}$ = $pilT_{Ec}^{WB}$). Importantly,
203 $PilT_{Ec}^{WB}$ exhibited self-interactions in BACTH assays (**Fig. 2B**), suggesting that the Walker B
204 mutation allows for self-assembly and/or a higher affinity between subunits compared to the
205 Walker A mutant. When we ectopically expressed the $pilT_{Ec}^{WB}$ in a Δ*pilT* background (where
206 *pilU* is intact), however, we observed that this mutant did not restore transformation (**Fig. 2A**),
207 which is similar to the $pilT_{Ec}^{WA}$ allele. This indicates that self-assembly of an ATPase defective
208 $PilT_{Ec}$ is not sufficient to promote PilU-dependent retraction. Together, these data suggest that
209 $PilT_{Ec}$ may have unique features that prevents it from promoting PilU-dependent retraction. We
210 hypothesized that analyzing the differences between $PilT_{Ec}$ and the other PilT alleles ($PilT_{Vc}$,
211 $PilT_{Ab}$, and $PilT_{Ps}$) may help define the features of PilT that allow for PilU-dependent retraction.

212 *The C-terminus of $PilT_{Vc}$ contributes to PilU-mediated retraction*.
213 In order to narrow down the region of $PilT_{Vc}$ that is necessary and/or sufficient to support PilU-
214 dependent retraction, we constructed defined chimeras of $pilT_{Vc}$ and $pilT_{Ec}$. $PilT_{Vc}$ and $PilT_{Ec}$ are
215 345 residues and 326 residues long, respectively. The chimeras were constructed in intervals of
216 50 amino acids where the residues of one protein were replaced with the corresponding amino
217 acids of the other. These chimeras were first ectopically expressed in a Δ*pilTU* background to
218 test their ability to mediate retraction of the competence pilus in a manner that is dependent on
219 their ATPase activity – a property that both $PilT_{Vc}$ and $PilT_{Ec}$ shared (**Fig. 1A**). Surprisingly, only
220 three of the chimeras were able to restore parental levels of transformation in the Δ*pilTU*
221 background (**Fig. 3A**). Two of the functional chimeras consisted of portions of the N-terminus of
222 $PilT_{Vc}$ (100 or 200 amino acids) fused to the corresponding C-terminus of $PilT_{Ec}$. The remaining
223 functional chimera contained the first 300 amino acids of $PilT_{Ec}$ fused to the C-terminal 42 amino
224 acids of $PilT_{Vc}$. One potential explanation for this general lack of functionality is that the locations
225 of the junctions between the two proteins were not conducive to generating a functional ATPase
226 motor. PilT is composed of two domains, an N-terminal PAS-like domain and a C-terminal
227 RecA-like ATPase domain which are connected via a short linker (95-102 in $PilT_{Ec}$ and 98-105
228 in $PilT_{Vc}$). So, the chimeras at 100 residues should be within the linker that connects the two
229 domains of both proteins, and it was particularly surprising that the $PilT_{Ec}$(100) fusion was not
230 functional when the reciprocal $PilT_{Vc}$(100) fusion was functional. The three functional chimeras
231 of PilT obtained are the only alleles that we can be reasonably confident could potentially
232 support PilU-dependent retraction, thus, we only proceeded with characterizing these three
233 chimeras of PilT moving forward. To determine if these three chimeras could support PilU-
234 mediated retraction, we next introduced a Walker A mutation into each allele. None of the $pilT_{Vc}$
235 / $pilT_{Ec}$ chimeras with the Walker A mutation were able to rescue PilU-dependent transformation
236 efficiency in a Δ*pilT* background (**Fig. 3A**). Separately, a Walker B mutation was introduced into
237 these chimeras. Again, none of the 3 chimeras were able to restore PilU-dependent
238 transformation in a Δ*pilT* background (**Fig. 3A**). We reasoned that since $PilT_{Vc}$ is capable of
239 PilU-mediated retraction, that the chimera that is predominantly composed of $PilT_{Vc}$ might help
240 define a portion of the protein that plays an important role in PilU-mediated retraction (i.e. that

241 the part of $PilT_{Vc}$ missing in this chimera plays an important role). $PilT_{Vc}$(200) had the largest
242 portion of $PilT_{Vc}$ and still made a functional retraction motor when ATPase activity was intact
243 (**Fig. 3A**). This chimera had 200 residues of the $PilT_{Vc}$ N-terminus fused to the C-terminal 129
244 residues of $PilT_{Ec}$. Walker A and Walker B mutants of $PilT_{Vc}$(200) did not support PilU-
245 dependent retraction (**Fig. 3A**). So, we reasoned that the C-terminal 145 residues of $PilT_{Vc}$
246 (which were replaced with $PilT_{Ec}$ in these chimeras) must be playing an important role in PilU-
247 dependent retraction. However, this does not exclude the possibility that the N-terminus of $PilT_{Vc}$
248 also plays an important role in promoting PilU-dependent retraction.

249 In order to further investigate potential components of PilT that are required for supporting PilU-
250 mediated retraction, we constructed an alignment of the 4 PilT orthologs that were studied here.
251 The alignment revealed that the middle section of $PilT_{Ec}$ was conserved relative to all of the
252 other PilT orthologs (**Fig. S3**). However, the N-terminus and the C-terminus of $PilT_{Ec}$ were
253 poorly conserved relative to the other PilT orthologs. Because our data suggests that the C-
254 terminus of $PilT_{Vc}$ may be important for supporting PilU-mediated retraction, that is where we
255 focused our investigation. In particular, we observed that $pilT_{Ec}$ contains two substitutions in the
256 highly conserved AIRNLIRE domain of PilT (18). To determine if these substitutions were
257 responsible for the inability of $PilT_{Ec}$ to support PilU-mediated retraction (7), we mutated the two
258 residues in $pilT_{Ec}$ to the residues present in the other PilT orthologs ($PilT_{Ec}^{AVG\rightarrow AIR}$). Conversely,
259 to determine if these changes in $PilT_{Ec}$ contributed to its inability to support PilU-mediated
260 retraction, the residues in $pilT_{Vc}$ were mutated to the residues present in $pilT_{Ec}$ ($PilT_{Vc}^{AIR\rightarrow AVG}$).
261 These mutations were introduced into Walker A (WA) or Walker B (WB) mutant alleles of PilT so
262 that we could assess PilU-mediated retraction. The introduction of these mutations into $pilT_{Vc}$
263 did not result in the inability of $PilT_{Vc}$ to facilitate PilU-mediated retraction (**Fig. 3B**). Moreover,
264 the restoration of the two AIRNLIRE substitutions in $pilT_{Ec}$ did not rescue PilU-mediated
265 retraction (**Fig. 3B**). These data indicate that the substitutions in the AIRNLIRE domain of $pilT_{Ec}$
266 are not responsible for the inability of $PilT_{Ec}$ to support PilU-mediated retraction.

267 The alignment of the 4 PilT orthologs also revealed that 15 amino acids in the C-terminal
268 domain of $PilT_{Ec}$ were missing when compared to the other PilT orthologs that did support PilU-
269 mediated retraction ($PilT_{Vc}$, $PilT_{Ab}$, and $PilT_{Ps}$). We hypothesized that the deletion of the last 15
270 amino acids in $PilT_{Ec}$ could be responsible for the inability of this protein to support PilU-
271 mediated retraction. To test this, we deleted the last 15 amino acids from $PilT_{Vc}$ ($pilT_{Vc}^{\Delta 15aa}$).
272 Additionally, we fused the last 15 amino acids present at the C-terminus of $pilT_{Vc}$ to the end of
273 $PilT_{Ec}$ ($pilT_{Ec}^{+15aa}$). Again, these mutations were generated in Walker A and Walker B mutant
274 alleles of PilT so that we could assess PilU-mediated retraction. The $pilT_{Vc}^{\Delta 15aa,\ WA}$ mutant
275 displayed no reduction in transformation (**Fig. 3B**), suggesting that $PilT_{Vc}^{\Delta 15aa,\ WA}$ is still able to
276 facilitate PilU-mediated retraction. Conversely, the $pilT_{Ec}^{+15aa,\ WA}$ and $pilT_{Ec}^{+15aa,\ WB}$ mutations did
277 not restore transformation to parental levels (**Fig. 3B**), which suggests that $PilU_{Vc}$ cannot
278 mediate retraction in the presence of these $PilT_{Ec}$ alleles. Together, these data suggest that the
279 last 15 amino acids present at the C-terminus of $PilT_{Vc}$ is not necessary or sufficient to promote
280 PilU-mediated retraction.

281 Our results have suggested that, individually, the substitutions present in the conserved
282 AIRNLIRE domain of $PilT_{Ec}$ and the deletion of the last 15 amino acids in the C-terminus are not
283 responsible for the inability of this PilT allele to support PilU-mediated retraction. However, it is
284 possible that the combination of these two factors diminishes PilU-mediated retraction. To test
285 this, we constructed $pilT_{Vc}$ *alleles* that contained both the AIRNLIRE domain substitutions and
286 the C-terminal deletion of 15 amino acids ($pilT_{Vc}^{AIR\rightarrow AVG,\ \Delta 15aa}$). Also, we generated a $pilT_{Ec}$ allele
287 where we restored the AIRNLIRE domain and inserted the terminal 15 amino acids from $pilT_{Vc}$
288 onto the C-terminus ($pilT_{Ec}^{AVG\rightarrow AIR,\ +15aa}$). The combination of mutations in $pilT_{Vc}$ resulted in a loss
289 of function, even in a strain where the ATPase activity of the allele was intact (**Fig. 3B**).

290 Additionally, the *pilT*$_{Ec}^{AVG\rightarrow AIR,\ +15aa,\ WA}$ and *pilT*$_{Ec}^{AVG\rightarrow AIR,\ +15aa,\ WB}$ alleles did not restore
291 transformation to parental levels in the Δ*pilT* background, suggesting that PilU was unable to
292 mediate retraction in these backgrounds. This suggests that the combination of the 15 amino
293 acid deletion and the substitutions in the AIRNLIRE domain are not solely responsible for the
294 inability of PilT$_{Ec}$ to support PilU-mediated retraction.

295

296 **DISCUSSION**

297 This work demonstrates that the retraction ATPases PilT and PilU are promiscuous among the
298 subset of T4aP systems studied here, and that their functionality extends beyond species
299 boundaries. This contrasts with the fidelity displayed by the extension ATPases from the same
300 T4aP systems. Additionally, our data suggests that the C-terminus of PilT$_{Vc}$ plays a role in
301 supporting PilU-mediated retraction. However, further analysis determined that the genetic
302 differences between PilT$_{Vc}$ and PilT$_{Ec}$ in the AIRNLIRE domain and the extended C-terminus are
303 neither necessary nor sufficient to explain this discrepancy in activity. Because of this, the exact
304 residues in the PilT C-terminus that contribute to PilU-mediated retraction remain elusive. Also,
305 it is likely that the N-terminus of PilT$_{Vc}$ also contributes to PilU-dependent retraction, however,
306 the paucity of functional PilT$_{Vc}$ / PilT$_{Ec}$ chimeras made this difficult to narrow down.

307 Because the orthologous retraction motor ATPases were overexpressed in this study, we
308 cannot comment on the relative efficiency of the ectopically expressed retraction motors
309 compared to native PilT$_{Vc}$ *and* PilU$_{Vc}$. But it is important to note that similar overexpression of
310 the extension motor orthologs from the same T4aP did not promote dynamic competence pilus
311 activity, which indicates that the overabundance of a motor ATPase is not necessarily sufficient
312 for motor functionality. One possible explanation for the discrepancy in the promiscuity /
313 specificity among motor ATPases could be due to differences in sequence divergence; where
314 retraction motor orthologs are more similar to one another compared to the extension motor
315 orthologs. When we performed this analysis, however, we found that the PilT orthologs ranged
316 from ~62-80% similar (with PilT$_{Ec}$ being the most divergent), the PilU orthologs ranged from
317 ~70-74% similar, and the extension motor orthologs ranged from ~50-66% similar (**Fig. S4**).
318 These data indicate that overall sequence divergence among PilT and PilB orthologs is fairly
319 similar. Thus, suggesting that sequence divergence alone cannot account for the promiscuity /
320 specificity among these motor ATPases.

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

330 Some organisms, like *V. cholerae*, encode multiple T4P - competence pili, mannose sensitive
331 hemagglutinin (MSHA) pili, and toxin co-regulated pili (TCP) - and there are distinct
332 environmental conditions under which each pilus system is likely needed. Competence pili and
333 MSHA pili are likely critical for the survival of *V. cholerae* in its environmental reservoir, while
334 TCP are required for infection of its human host. In fact, it has previously been shown that the
335 inappropriate expression of MSHA pili during the context of an infection attenuates *V. cholerae*
336 (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 most 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), called N1D (6) that distinguishes them from the retraction ATPase motors (**Fig. S5**), and we found that much of the sequence divergence among extension ATPases was attributed to the N1D (**Fig. S4**). Thus, it is tempting to speculate that the N1D 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 among the T4aP studied here, 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). Strains were constructed by first inserting the ectopic expression construct of the pilus component into the genome. This was followed by the deletion of the native pilus component. All mutations and ectopic expression constructs were confirmed by PCR and/or sequencing.

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 (Δ*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 µM 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 µM IPTG, and arabinose (when appropriate; 0.05-0.2%) and grown to

383 late-log phase at 30 °C rolling. Next, ~10^8 cells were transferred to Instant Ocean medium (7
384 g/L; Aquarium Systems) containing 100 μM IPTG, arabinose (when appropriate; 0.05-0.2%),
385 and 200 ng of a transforming DNA (tDNA) PCR product. Reactions were then incubated
386 statically overnight at 30 °C. The tDNA product used throughout this study targeted a "neutral
387 gene" (the frame-shifted transposase, VC1807) for inactivation (ΔVC1807::Erm^R) as previously
388 described (27). A negative control, containing no tDNA, was included for each strain in all
389 transformation assays. After the overnight incubation with tDNA, reactions were outgrown by
390 adding 1 mL of LB to each reaction and incubating at 37 °C shaking for 2 hours. After the
391 outgrowth, reactions were plated for quantitative culture to distinguish between transformants
392 (plated on LB + erythromycin) and total viable counts (plated on plain LB). The transformation
393 frequency for each reaction was calculated by dividing the number of transformants by the total
394 viable counts.

395 *Bacterial Adenylate Cyclase Two-Hybrid Assay*
396 Using PCR, the desired motor ATPase genes were amplified and cloned into the BACTH
397 vectors pKT25 and pUT18C in order to produce N-terminal fusions between the motor ATPase
398 and the T25 and T18 fragments of adenylate cyclase, respectively. Miniprepped plasmids
399 (Qiagen) were then co-transformed into *E. coli* BTH101 cells. As a positive control, T25-zip and
400 T18-zip vectors were cotransformed into BTH101. And as a negative control, the pKT25 and
401 pUT18C empty vectors were cotransformed into BTH101. Transformations were plated onto LB
402 + kanamycin (50 μg/mL) + carbenicillin (100 μg/mL) plates in order to select for transformants
403 that received both plasmids. These transformants were then selected and grown overnight at 30
404 °C static in LB + kanamycin (50 μg/mL) + carbenicillin (100 μg/mL). Finally, 3 μL of the overnight
405 cultures were spotted onto LB agar plates supplemented with 500 μM IPTG, kanamycin (50
406 μg/mL), carbenicillin (100 μg/mL), and X-gal (40 μg/mL). These plates were allowed to incubate
407 statically at 30 °C for ~48 hours prior to imaging on a HP Scanjet G4010 flatbed scanner.

408 *Alignments*
409 The sequences of the genes that encode for the desired proteins were downloaded from
410 GenBank. Sequences were aligned using T-Coffee (28) and the resulting alignments were run
411 through the Sequence Manipulation Suite: Color Align Conservation tool (29) to generate the
412 final figures.

413 *Pairwise comparisons to assess similarity among ATPase motor orthologs*
414 Pairwise comparisons between ATPase motor orthologs were performed via global alignment
415 using the Needleman-Wunsch algorithm (30). The percent similarities generated by these
416 pairwise comparisons are reported in **Fig. S4**.

417 *Western Blotting*

418 Strains were grown to late-log phase in LB supplemented with 20 mM $MgCl_2$, 10 mM $CaCl_2$, 100
419 μM IPTG, and arabinose (when appropriate) exactly as described above for transformation
420 assays. Cells were washed and concentrated to an OD_{600} = 100 in 0.5X Instant Ocean medium,
421 and then lysed by adding Fastbreak lysis buffer (to 1X), lysozyme (80 μg/mL), and benzonase
422 (25U). Lysed samples were mixed 1:1 with 2X SDS sample buffer [220 mM Tris pH 6.8, 25%
423 glycerol, 1.8% SDS, 0.02% Bromophenol Blue, 5% β-mercaptoethanol] and 3 μL of each
424 sample was then separated on a 15% SDS PAGE gel. The proteins were transferred to a PVDF
425 membrane via electrophoresis, and the membrane was then incubated with the appropriate
426 primary antibody (α-FLAG polyclonal rabbit – for PilT and PilU; or α-FLAG M2 mouse
427 monoclonal – for PilB). A primary incubation with α-RpoA monoclonal mouse (BioLegend) was
428 also completed as a loading control. The blots were then washed and incubated with an
429 appropriate horseradish peroxidase (HRP) conjugated secondary antibody. And blots were

430 developed with Pierce™ ECL Western Blotting Substrate and imaged using a ProteinSimple
431 FluorChem R system.

432

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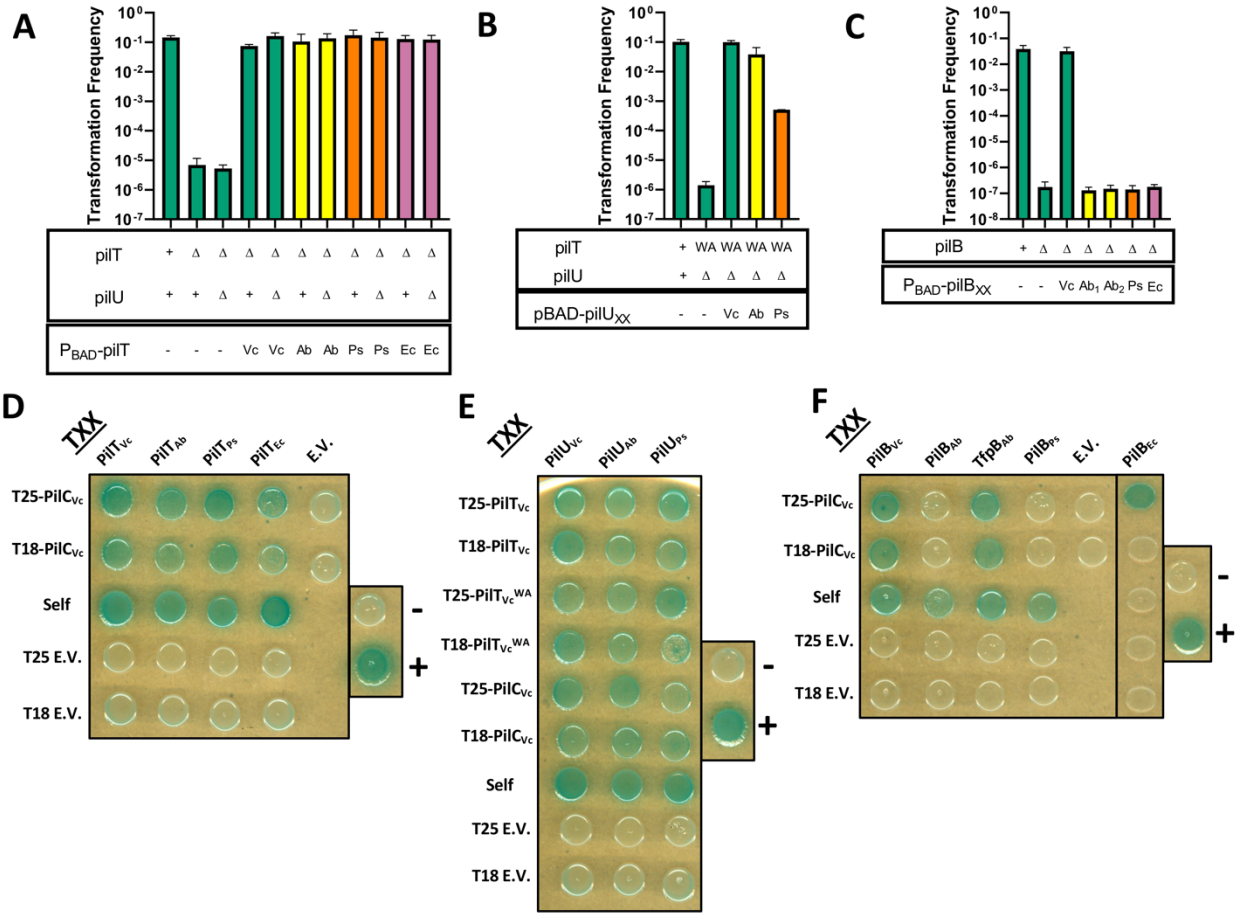
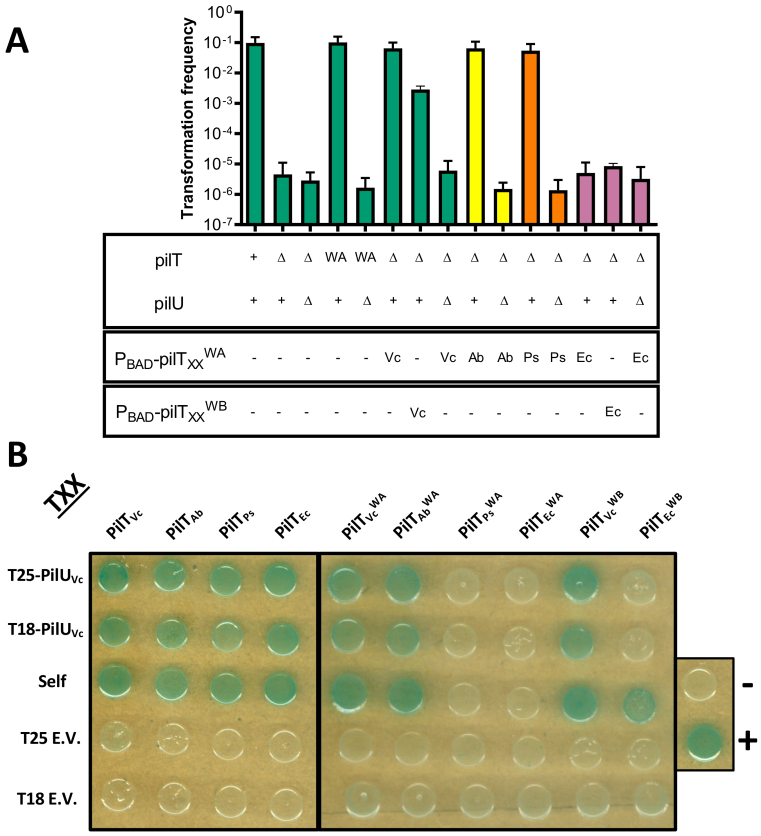


Fig. 1. ***Retraction ATPase orthologs are promiscuous among T4aP systems, while extension ATPase orthologs are not.*** Natural transformation assays were performed to test whether (**A**) PilT, (**B**) PilU, or (**C**) PilB/TfpB orthologs from 3 heterologous T4aP systems could complement *V. cholerae* 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 ± 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). For BACTH assays pilus components had an N-terminal fusion to the T25 or T18 fragment of adenylate cyclase as indicated. "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.



547

548 **Fig. 2. *The PilT homolog from E. coli cannot support PilU-mediated retraction*.** (**A**) Natural
549 transformation assays were performed to test whether retraction ATPase PilT mutants from 3
550 heterologous T4aP systems could complement *V. cholerae* mutants lacking their endogenous motor
551 ATPases. The relevant genotypes of strains are indicated below each bar, which includes the status of
552 the native *pilT*/*pilU* genes as well as the ectopically expressed motor ATPase allele (induced via P_{BAD}).
553 The color of the bar corresponds to the species from which the ectopically expressed *pilT* was derived
554 (green bars = *Vc pilT*, yellow bars = *Ab pilT*, orange bars = *Ps pilT*, and purple bars = *Ec pilT*). Data are
555 from at least 3 independent biological replicates and shown as the mean ± SD. (**B**) BACTH assays to test
556 whether the indicated PilT alleles can interact with themselves ("self") or $PilU_{Vc}$. For BACTH assays pilus
557 components had an N-terminal fusion to the T25 or T18 fragment of adenylate cyclase as indicated. "WA"
558 denotes a mutation in the Walker A domain of the tested allele, while "WB" denotes a mutation in the
559 Walker B domain of the tested allele. "+" and "–" denote positive and negative controls for the BACTH
560 assay. "E.V." denotes an empty vector. Data are representative of at least 2 independent experiments.

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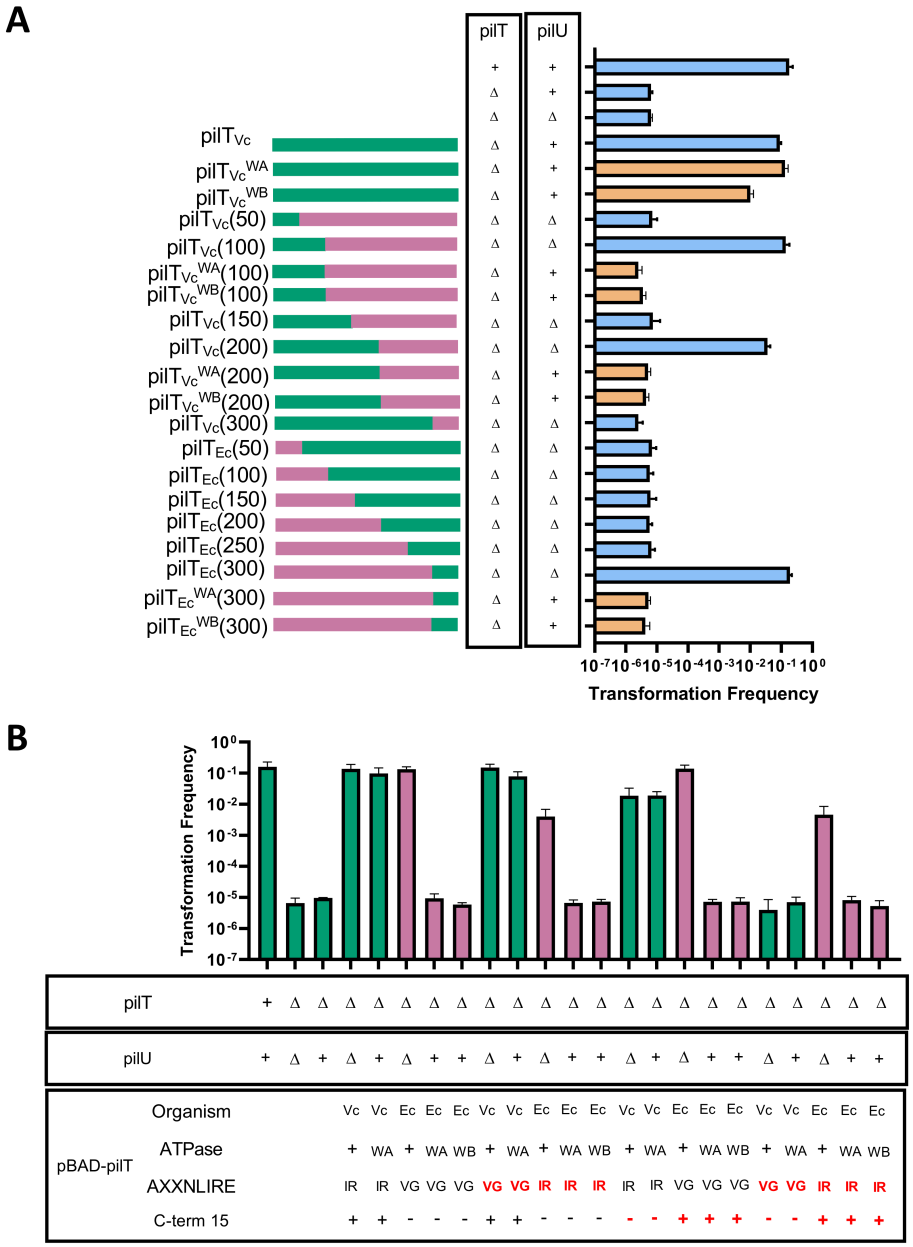

Fig. 3. *The C-terminus of PilT$_{Vc}$ contributes to PilU-mediated retraction*. Natural transformation assays were performed to test whether retraction ATPase chimeras between PilT$_{Vc}$ and PilT$_{Ec}$ can complement *V. cholerae* mutants lacking their endogenous motor ATPases. PilT chimeras either have (**A**) their ATPase activity intact to assess ATPase-dependent retraction, or 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. For strains that encode an ATPase active *pilT* allele, the bar is blue, while for strains that contain an ATPase inactive *pilT* allele (via a WA or WB mutation as indicated) the bar is tan. The number in parentheses indicates the number of N-terminal amino acids from the PilT allele indicated fused to its PilT ortholog. (**B**) 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

577 the native *pilT*/*pilU* genes as well as the ectopically expressed motor ATPase chimera (induced via P_{BAD}).
578 Four attributes of each ectopically expressed allele are indicated: the organism from which the PilT allele
579 is derived, the ATPase status of the allele ("+" denotes ATPase intact, "WA" denotes Walker A mutant,
580 and "WB" denotes Walker B mutant), the sequence of the AXXNLIRE domain (IR = AIRNLIRE, VG =
581 AVGNLIRE), and the presence / absence of the C-terminal 15 amino acids. If the attribute changes the
582 natural allele, it is displayed in red text. The color of the bar corresponds to the species from which the
583 ectopically expressed *pilT* was derived (green bars = *Vc pilT* and purple bars = *Ec pilT*). All data are from
584 at least 3 independent biological replicates and shown as the mean ± SD.

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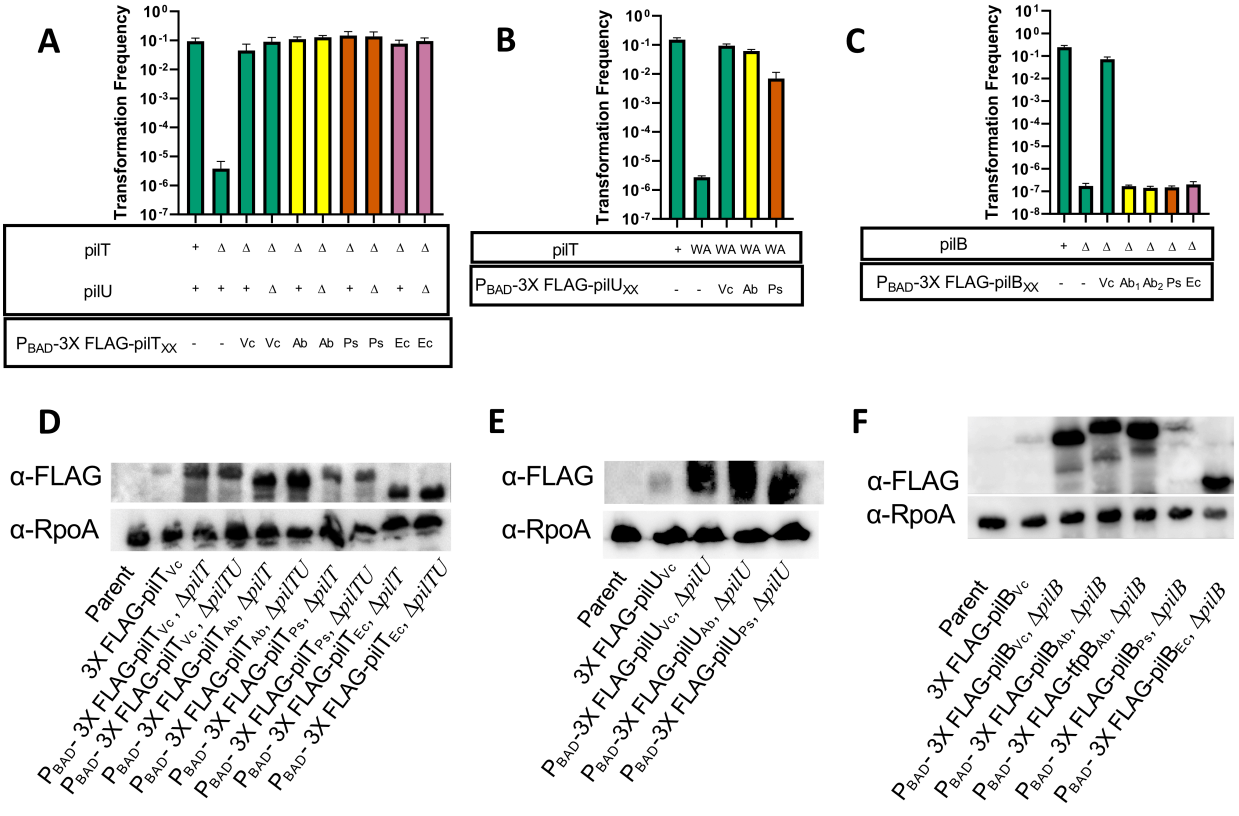


Fig. S1. ***Expression of ectopic constructs is equal to or greater than native levels of expression.*** Natural transformation assays were performed to confirm the functionality of the N-terminally FLAG-tagged alleles of **(A)** *pilT*, **(B)** *pilU*, and **(C)** *pilB*. The relevant genotypes of strains 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. Western blots to determine the levels of expression of the FLAG-tagged **(D)** *pilT*, **(E)** *pilU*, and **(F)** *pilB* alleles and RpoA as a loading control. The blots show that all ectopically expressed motors were expressed at or above the levels of the native *Vc* motor. Data is representative of at least two independent experiments.

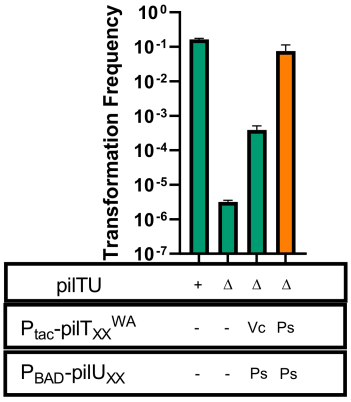


Fig. S2. *PilU$_{Ps}$ is a fully functional retraction ATPase motor.* Natural transformation assays were performed to assess whether PilU$_{Ps}$ can support pilus retraction in conjunction with its cognate PilT$_{Ps}^{WA}$ better than with the heterologous PilT$_{Vc}^{WA}$. The relevant genotypes of 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. The color of the bar corresponds to the species from which the ectopically expressed PilT motor ATPase was derived (green bars = *Vc* motors, orange bar = *Ps* motors). Data are from at least 3 independent biological replicates and shown as the mean ± SD.

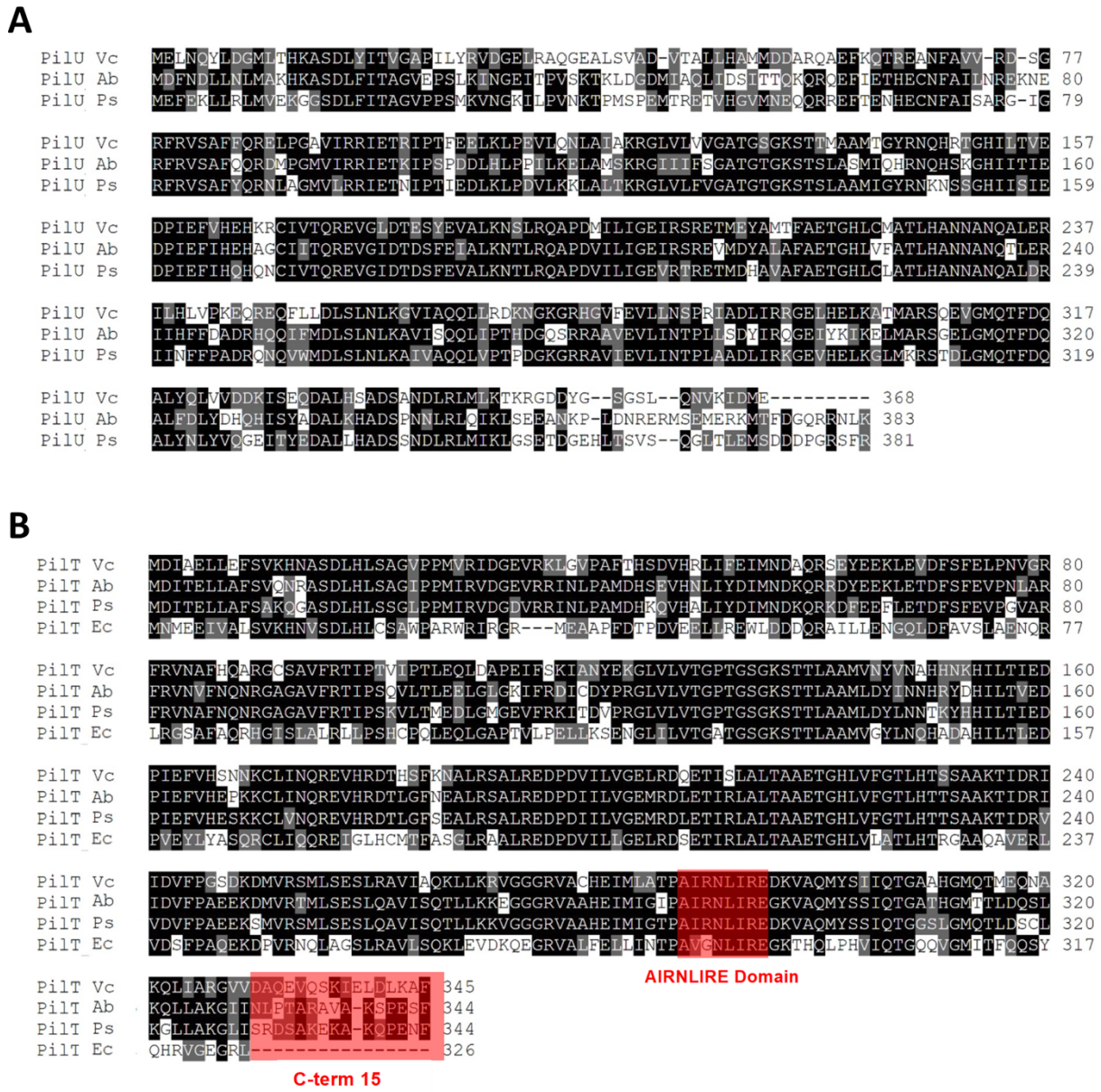


Fig. S3. ***Alignment of PilU and PilT sequences from the four T4aP studied.*** Alignments of (**A**) PilU orthologs and (**B**) PilT orthologs. Residues highlighted in black are identical, while those highlighted in gray are similar. The AIRNLIRE and C-terminal 15 residue domains experimentally tested 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%

full length

B

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

full length

C

	$PilB_{Vc}$	$PilB_{Ab}$	$TfpB_{Ab}$	$PilB_{Ps}$	$PilB_{Ec}$
$PilB_{Vc}$	100%	64%	53%	66%	50%
$PilB_{Ab}$	64%	100%	55%	75%	48%
$TfpB_{Ab}$	53%	55%	100%	54%	45%
$PilB_{Ps}$	66%	75%	54%	100%	49%
$PilB_{Ec}$	50%	48%	45%	49%	100%

full length

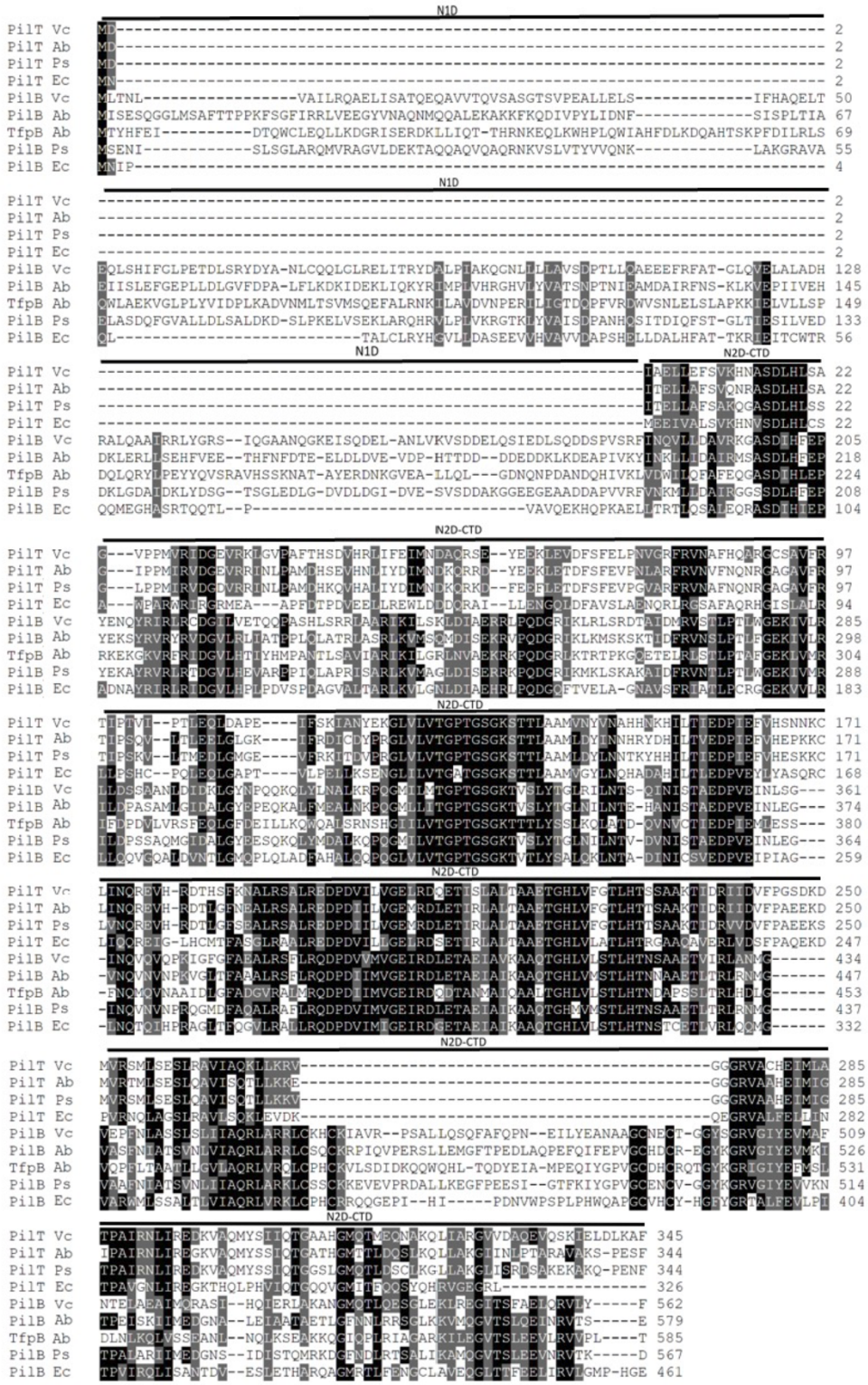
	$PilB_{Vc}$	$PilB_{Ab}$	$TfpB_{Ab}$	$PilB_{Ps}$	$PilB_{Ec}$
$PilB_{Vc}$	100%	75%	64%	75%	64%
$PilB_{Ab}$	75%	100%	64%	86%	62%
$TfpB_{Ab}$	64%	64%	100%	61%	60%
$PilB_{Ps}$	75%	86%	61%	100%	63%
$PilB_{Ec}$	64%	62%	60%	63%	100%

N2D-CTD

	$PilB_{Vc}$	$PilB_{Ab}$	$TfpB_{Ab}$	$PilB_{Ps}$	$PilB_{Ec}$
$PilB_{Vc}$	100%	41%	32%	49%	20%
$PilB_{Ab}$	41%	100%	38%	52%	22%
$TfpB_{Ab}$	32%	38%	100%	41%	16%
$PilB_{Ps}$	49%	52%	41%	100%	21%
$PilB_{Ec}$	20%	22%	16%	21%	100%

N1D

Fig. S4. ***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. The percent similarity is also denoted by the fill color of the box, where green represents high percent similarity and red represents lower percent similarity. PilB/TfpB orthologs were further analyzed for pairwise comparisons of the C-terminal domains that are shared between by extension and retraction motor ATPases (N2D and CTD) and the N-terminal domain (N1D) that is unique to extension motor ATPases (see **Fig. S5** for details on what portion of PilB represents the N1D vs N2D-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); Ec, *E. coli* PilB (AAC73218).



640

641 **Fig. S5.** ***Alignment of the PilB/TfpB and PilT sequences from the T4aP studied.*** Alignments
642 of PilB/TfpB orthologs and PilT orthologs. Residues highlighted in black are identical, while
643 those highlighted in gray are similar. The unique N-terminal domain in PilB orthologs is denoted
644 by the "N1D" bar above the sequence, while the two C-terminal domains shared by all motor

645 ATPases are denoted by “N2D-CTD”. The GenBank Accession numbers for the protein
646 sequences used are as follows: Vc, *Vibrio cholerae* PilT (AAF93635); Ab, *Acinetobacter baylyi*
647 PilT (CAG67808); Ps, *Pseudomonas stutzeri* PilT (AFN76400); Ec, *Escherichia coli* PilT
648 (NP_417425); Vc, *V. cholerae* PilB (AAF95567); Ab, *A. baylyi* PilB (CAG67312); Ab, *A. baylyi*
649 TfpB (CAG69034); Ps, *P. stutzeri* PilB (AFN76867); Ec, *E. coli* PilB (AAC73218).

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

Strain name in manuscript	Genotype and antibiotic resistance	Figure	Strain # / Reference
Parent	E7946 SmR, ΔluxO::miniFRT, lacZ::lacIq, pilA S56C, ΔVC1807::ZeoR, P_{tac}-*tfoX*	Fig. 1, 2, 3, S1, S2	TND0905 (SAD2468) / (4)
Δ*pilT*	E7946 SmR, ΔlacZ::lacIq, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔpilT::TmR	Fig. 1, 2, 3, S1	TND1035 (SAD2469) / (4)
Δ*pilTU*	E7946 SmR, ΔlacZ::lacIq, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔpilTU::TmR	Fig. 1, 2, 3, S2	JLC227 (SAD2470) / (4)
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) / (4)
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) / (4)
Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT	Fig. 1	TND2373 (SAD2787) / (13)

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) / (13)
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)
P_{BAD}-*pilB*$_{Ec}$, Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT, ΔlacZ:: P_{BAD}-pilB$_{Ec}$ CarbR	Fig. 1	EHC170 (SAD3283)
pilT^{WA}	E7946 SmR, ΔlacZ::lacIq, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C, pilT^{K136A}	Fig. 2	TND1077 (SAD2471) / (4)
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. S2	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. S2	EHC100 (SAD3256)
P_{BAD}-3X FLAG-*pilT*$_{Vc}$, Δ*pilT*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-3X FLAG-PilT$_{Vc}$ CarbR, ΔpilT::TmR	Fig. S1	EHC136 (SAD3285)
P_{BAD}-3X FLAG-*pilT*$_{Vc}$, Δ*pilTU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-3X FLAG-PilT$_{Vc}$ CarbR, ΔpilTU::TmR	Fig. S1	EHC137 (SAD3286)
P_{BAD}-3X FLAG-*pilT*$_{Ab}$, Δ*pilT*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-3X FLAG-PilT$_{Ab}$ CarbR, ΔpilT::TmR	Fig. S1	EHC138 (SAD3287)

P_{BAD}-3X FLAG-*pilT*$_{Ab}$, Δ*pilTU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-3X FLAG-PilT$_{Ab}$ CarbR, ΔpilTU::TmR	Fig. S1	EHC139 (SAD3288)
P_{BAD}-3X FLAG-*pilT*$_{Ps}$, Δ*pilT*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-3X FLAG-PilT$_{Ps}$ CarbR, ΔpilT::TmR	Fig. S1	EHC140 (SAD3289)
P_{BAD}-3X FLAG-*pilT*$_{Ps}$, Δ*pilTU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-3X FLAG-PilT$_{Ps}$ CarbR, ΔpilTU::TmR	Fig. S1	EHC141 (SAD3290)
P_{BAD}-3X FLAG-*pilT*$_{Ec}$, Δ*pilT*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-3X FLAG-PilT$_{Ec}$ CarbR, ΔpilT::TmR	Fig. S1	TND2978 (SAD3291)
P_{BAD}-3X FLAG-*pilT*$_{Ec}$, Δ*pilTU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::ZeoR, pilA S56C, ΔlacZ::P_{BAD}-3X FLAG-PilT$_{Ec}$ CarbR, ΔpilTU::TmR	Fig. S1	TND2982 (SAD3292)
P_{BAD}-3X FLAG-*pilU*$_{Vc}$, *pilT*^{WA}, Δ*pilU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C, pilT$_{Vc}^{K136A}$, ΔlacZ:: P_{BAD}-3X FLAG-PilU$_{Vc}$ CarbR, ΔpilU::TmR	Fig. S1	EHC161 (SAD3293)
P_{BAD}-3X FLAG-*pilU*$_{Ab}$, *pilT*^{WA}, Δ*pilU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C, pilT$_{Vc}^{K136A}$, ΔlacZ:: P_{BAD}-3X FLAG-PilU$_{Ab}$ CarbR, ΔpilU::TmR	Fig. S1	EHC152 (SAD3294)
P_{BAD}-3X FLAG-*pilU*$_{Ps}$, *pilT*^{WA}, Δ*pilU*	E7946 SmR, P_{tac}-tfoX, ΔluxO::miniFRT, ΔVC1807::KanR, pilA S56C, pilT$_{Vc}^{K136A}$, ΔlacZ:: P_{BAD}-3X FLAG-PilU$_{Ps}$ CarbR, ΔpilU::TmR	Fig. S1	EHC145 (SAD3295)
P_{BAD}-3X FLAG-*pilB*$_{Vc}$, Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT, ΔVCA0692:: P_{BAD}-3X FLAG-pilB$_{Vc}$ CarbR	Fig. S1	EHC166 (SAD3296)
P_{BAD}-3X FLAG-*pilB*$_{Ab}$, Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT, ΔVCA0692:: P_{BAD}-3X FLAG-pilB$_{Ab}$ CarbR	Fig. S1	EHC167 (SAD3297)
P_{BAD}-3X FLAG-*tfpB*$_{Ab}$, Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT, ΔVCA0692:: P_{BAD}-3X FLAG-tfpB$_{Ab}$ CarbR	Fig. S1	EHC168 (SAD3298)
P_{BAD}-3X FLAG-*pilB*$_{Ps}$, Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT, ΔVCA0692:: P_{BAD}-3X FLAG-pilB$_{Ps}$ CarbR	Fig. S1	EHC169 (SAD3299)
P_{BAD}-3X FLAG-*pilB*$_{Ec}$, Δ*pilB*	E7946 SmR, lacZ LPQEN:: KanR, P_{const}-tfoX, ΔluxO::miniFRT, pilA S56C, ΔVC1807::CmR, ΔpilB::miniFRT, ΔlacZ:: P_{BAD}-3X FLAG-pilB$_{Ec}$ CarbR	Fig. S1	EHC172 (SAD3300)
T25-PilT$_{Vc}$	TG1, pkT25-PilT$_{Vc}$ KanR	Fig. 1, 2	SAD2248 / (4)
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 / (4)
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 / (4)
T18-PilU$_{Vc}$	TG1, pUT18C-PilU$_{Vc}$ CarbR	Fig. 1, 2	SAD2251 / (4)
T25-PilC$_{Vc}$	TG1, pkT25-PilC$_{Vc}$ KanR	Fig. 1	SAD2260 / (4)
T18-PilC$_{Vc}$	TG1, pUT18C-PilC$_{Vc}$ CarbR	Fig. 1	SAD2261 / (4)
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) / (14)
T25-PilB$_{Ab}$	TG1, pkT25-PilB$_{Ab}$ KanR	Fig. 1	SAD2647 / (14)
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) / (14)
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 / (4)
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 / (4)
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-PilB$_{Ec}$	TG1, pkT25-PilB$_{Ec}$ KanR	Fig. 1	EHC142

			(SAD3301)
T18-PilB$_{Ec}$	TG1, pUT18C-PilB$_{Ec}$ CarbR	Fig. 1	EHC143 (SAD3302)
T25-Zip	TG1, pkT25-leucine zipper KanR	Fig. 1, 2	SAD2238 / (4)
T18-Zip	TG1, pUT18C-leucine zipper CarbR	Fig. 1, 2	SAD2241 / (4)
T25-vector	TG1, pkT25 vector KanR	Fig. 1, 2	SAD2236 / (4)
T18-vector	TG1, pUT18C vector CarbR	Fig. 1, 2	SAD2240 / (4)
Acinetobacter baylyi	ADP1	Purified gDNA	SAD631 / (23)
Pseudomonas stutzeri	JM300	Purified gDNA	SAD2178 / (24)
Escherichia coli	MG1655	Purified gDNA	SAD720 / (25)

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653 **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	caatttcacacaggatcccgggAGGAGGTAGTCCCATGGATATCACAGAAC	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	caatttcacacaggatcccgggAGGAGGTCTTAGTATGAATATGGAAGAAATTGTGG	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	caatttcacacaggatcccgggAGGAGGTAATACGATGGACTTTAAT GATTTACTC	Amplify pilU$_{Ab}$ for P$_{BAD}$-pilU F
BBC2810	tgtaggctggagctgcttcTTATTTGAGATTACGGCGTTGAC	Amplify pilU$_{Ab}$ for P$_{BAD}$-pilU R
JCP177	caatttcacacaggatcccgggAGGAGGTACGACGATGGAATTCGAG AAAC	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	caatttcacacaggatcccgggAGGAGGTTAACTAATGCTCACCAAC CTTGTTG	Amplify pilB$_{Vc}$ for P$_{BAD}$-pilB F
BBC1953	tgtaggctggagctgcttcTTAAAAGTAGAGCACACGCTG	Amplify pilB$_{Vc}$ for P$_{BAD}$-pilB R
BBC3320	caatttcacacaggatcccgggaggaggtATTATAATGATATCAGAATCA CAAGGCGG	Amplify pilB$_{Ab}$ for P$_{BAD}$-pilB F
BBC3310	tgtaggctggagctgcttcTTATTCACTGGTTACACGATTAATTTCC	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
ECP0040	caatttcacacaggatcccgggAGGAGGTGCGGCAATGAATATTCCACAG	Amplify pilB$_{Ec}$ for P$_{BAD}$-pilB$_{Ec}$ F
ECP0041	tgtaggctggagctgcttcTTACTCGCCATGCGGCATC	Amplify pilB$_{Ec}$ for P$_{BAD}$-pilB$_{Ec}$ 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	tccacctgctccacctgcCTCTAGAGTCGACCTGCAG	Amplify BACTH pUT18C to make T18-XX vectors R
BBC2518	gcaggtggagcaggtggaAAAGCGACCCAAACCTTACC	BACTH TXX-PilC$_{Vc}$ F
BBC2524	tgtaggctggagctgcttcTTAACCCAATACACTCATTAA GTTAAAG	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	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
ECP0043	gcaggtggagcaggtggaAATATTCCACAGCTCACTGC	BACTH TXX-PilB$_{Ec}$ F
ECP0044	tgtaggctggagctgcttcTTACTCGCCATGCGGCATC	BACTH TXX-PilB$_{Ec}$ 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
ECP0004	tccaccacttccacctgcCATGGGACTACCTCCTcccgggatcctg	3X FLAG-pilT$_{Ps}$ R1
ECP0005	gcaggtggagcaggtggaGATATCACAGAACTGCTGGCC	3X FLAG-pilT$_{Ps}$ F2
ECP0010	tccaccacttccacctgcCATATTTTCACCTCCTcccgggatcctg	3X FLAG-pilT$_{Ab}$ R1
ECP0011	gcaggtggagcaggtggaGATATAACAGAATTACTCGCGTTTTC	3X FLAG-pilT$_{Ab}$ F2

ECP0016	tccaccacttccacctgcCATCTGTCTACCTCCTcccgggatcctg	3X FLAG-pilU$_{Vc}$ R1
ECP0017	gcaggtggagcaggtggaGAGTTGAATCAATATCTGGATGGC	3X FLAG-pilU$_{Vc}$ F2
ECP0019	tccaccacttccacctgcCATTTAAATACCTCCTCCCGGGATCCTG	3X FLAG-pilT$_{Vc}$ R1
ECP0020	gcaggtggagcaggtggaGATATCGCTGAGTTACTGGAATTTAG	3X FLAG-pilT$_{Vc}$ F2
ECP0022	tccaccacttccacctgcCATCGTATTACCTCCTcccgggatcct	3X FLAG-pilU$_{Ab}$ R1
ECP0023	gcaggtggagcaggtggaGACTTTAATGATTTACTCAATTTGATGGC	3X FLAG-pilU$_{Ab}$ F2
ECP0025	tccaccacttccacctgcCATCGTCGTACCTCCTcccgggatcct	3X FLAG-pilU$_{Ps}$ R1
ECP0026	gcaggtggagcaggtggaGAATTCGAGAAACTGTTGCGC	3X FLAG-pilU$_{Ps}$ F2
ECP0028	tccaccacttccacctgcCATTAGTTAACCTCCTcccgggatcct	3X FLAG-pilB$_{Vc}$ R1
ECP0029	gcaggtggagcaggtggaCTCACCAACCTTGTTGCTATC	3X FLAG-pilB$_{Vc}$ F2
ECP0031	tccaccacttccacctgcCATTAGACCacctcctcccgggatcct	3X FLAG-pilB$_{Ab}$ R1
ECP0032	gcaggtggagcaggtggaTCAGCATTTACAACACCACCTAAGTTTTC	3X FLAG-pilB$_{Ab}$ F2
ECP0034	tccaccacttccacctgcCATTTATTTacctcctcccgggatcct	3X FLAG-tfpB$_{Ab}$ R1
ECP0035	gcaggtggagcaggtggaACATATCATTTTGAAATAGATACACAATGG	3X FLAG-tfpB$_{Ab}$ F2
ECP0037	tccaccacttccacctgcCATGAGGTTacctcctcccgggatcct	3X FLAG-pilB$_{Ps}$ R1
ECP0038	gcaggtggagcaggtggaAGCGAAAACATTTCCCTTTCC	3X FLAG-pilB$_{Ps}$ F2
ECP0045	tccaccacttccacctgcCATTGCCGCacctcctcccgggatcct	3X FLAG-pilB$_{Ec}$ R1
ECP0046	gcaggtggagcaggtggaAATATTCCACAGCTCACTGCCCTG	3X FLAG-pilB$_{Ec}$ F2

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