Figure S1. Motility of Pto *fleQ* and its complemented strain.

Top panel. Swimming and surface motility assays were performed with the indicated strains and pictures were taken 48 hours and 24 hours after inoculation, respectively. Low panel. Transmission electron micrographs taken from negative staining preparations to visualize flagella. All experiments were performed at least three times.

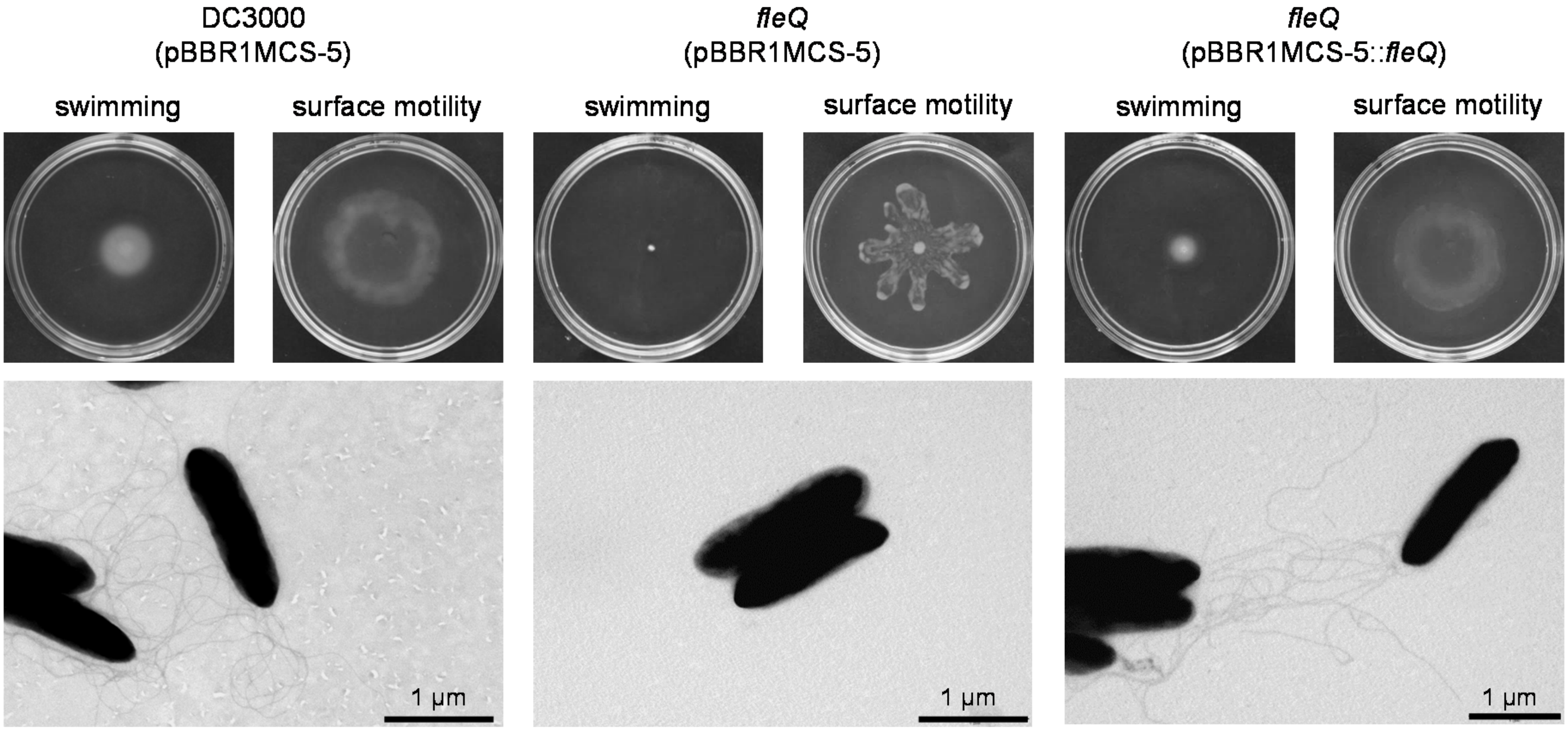

DC3000
(pBBR1MCS-5)
fleQ
(pBBR1MCS-5)
fleQ
(pBBR1MCS-5::*fleQ*)
swimming
surface motility
swimming
surface motility
swimming
surface motility
1 μm
1 μm
1 μm

Figure S2. Nucleotide sequence of the *flhB-flhA* intergenic region.

The -12 and -24 regions of P_{flhA} are shadowed, the +1 and the direction of transcription are indicated by an arrowhead, the stop codon of *flhB* and the coding sequence of *flhA* are shown in bold and the predicted site for FleQ binding (Baraquet *et al*., 2012; Jyot *et al*., 2002) is underlined.

flhB

TAGCCTTCAGTGGCTGTCATAAAGTTGCCTTAGCTCTCTTCCAACGCTCTGGCGTGCGCCTTTAAATGTACGGCGCGGCGCAG

ATCTGTGACGCGCAGCGTCACGACAGGCATTCCCATGCTGGAGTGGTAGGAACGATAGGTGTCCGGAAGAACACTATGGTGGC

CCATGCTCCGCGTGGGCATGCCGTTCTGGACGCTTTGCGTCCGCTCTTTAGTGTGCGCAGCGCGAGGTTCTCTGGCTTCACTG

ATCACCTCGTCTTGCGCAATTTCTACCTCTAAAGCCTTGCCGGCACTGGCTTTGTAAAAGTTGGACAGCTTTTTGCAATAAGG

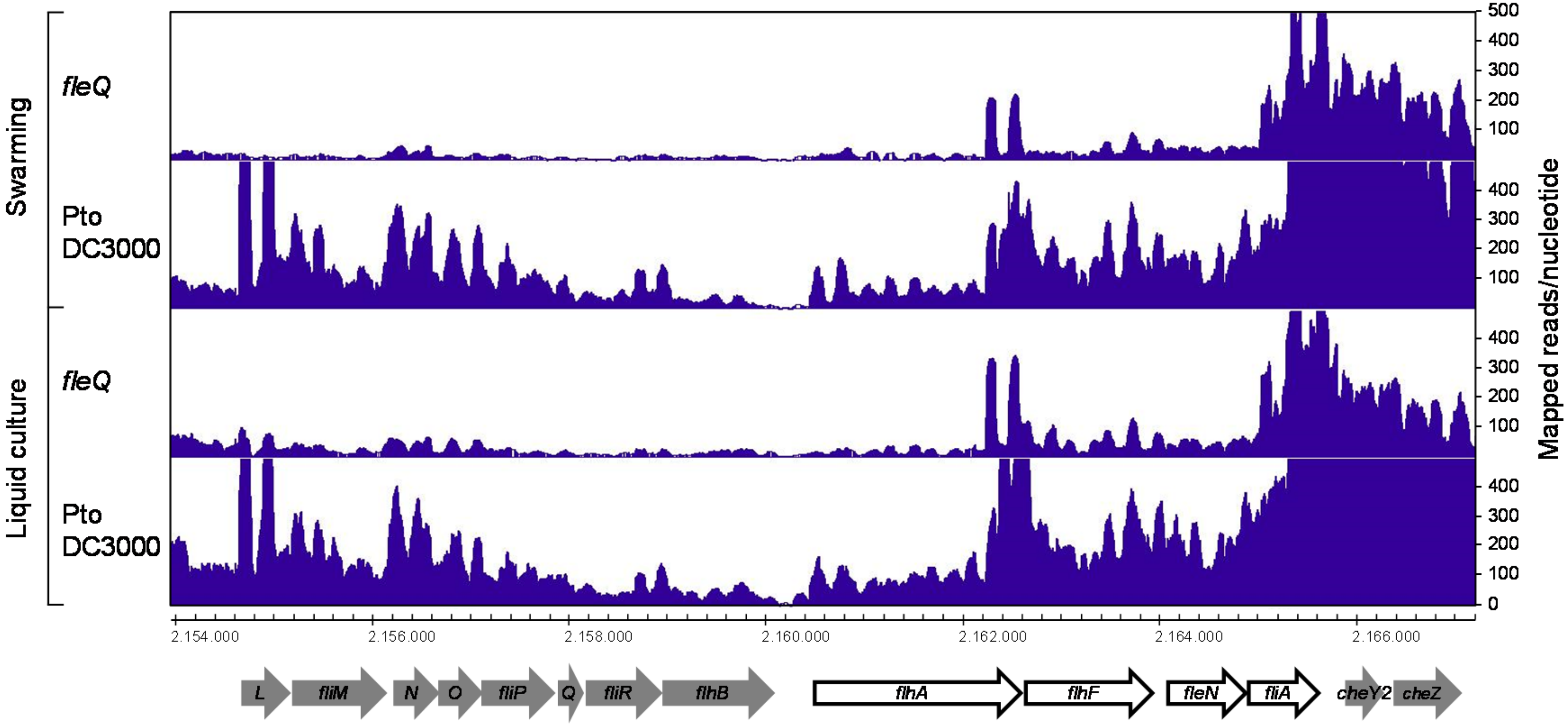
TCGAT*A*CAGGTCGCCATCGGCGTCAATGTTTTGATCTAGTGCGCAGGTAACGAATCA**GTGGATCGCTCTCAATTACTCAGT**

●----->

flhA

Figure S3. Mapped RNA reads in the *flhA*-*fliA* region.

RNA was obtained from Pto DC3000 and *fleQ* grown in swarming conditions and liquid cultures (MMR at 20ºC). Drawings are images from IGB software showing reads in several genes surrounding *flhA*. Genomic coordinates denote the position in kb of the Pto DC3000 genome and annotated ORFs are shown as arrows. The scale (counts) indicates the number of mapped reads per nucleotide position.


Swarming
Liquid culture
fleQ
Pto DC3000
fleQ
Pto DC3000
Mapped reads/nucleotide
500
400
300
200
100
0
2.154.000
2.156.000
2.158.000
2.160.000
2.162.000
2.164.000
2.166.000
L
fliM
N
O
fliP
Q
fliR
flhB
flhA
flhF
fleN
fliA
cheY2
cheZ

References

1. Baraquet, C., Murakami, K., Parsek, M.R., Harwood, C.S. 2012. The FleQ protein from *Pseudomonas aeruginosa* functions as both a repressor and an activator to control gene expression from the pel operon promoter in response to c-di-GMP. Nucleic Acids Res 40:7207-7218.
2. Jyot, J., Dasgupta, N., Ramphal, R. 2002. FleQ, the major flagellar gene regulator in *Pseudomonas aeruginosa*, binds to enhancer sites located either upstream or atypically downstream of the RpoN binding site. J Bacteriol 184:5251-5260.