

Dataset S1. List of differentially expressed genes in the *hutC* deletion mutant background when compared with wild-type *P. fluorescens* SBW25. The differentially expressed genes were determined based on transcripts comparison normalized using the Median of Gene Expression Ratios methods, following the recommendations of Geneious 9.0.5.

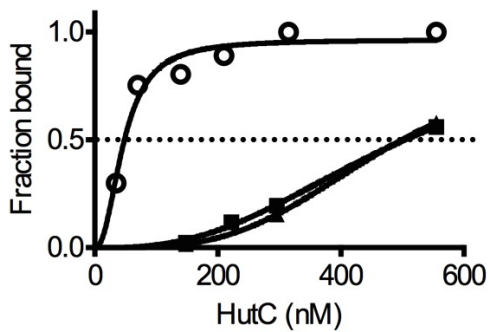


Figure S1. Determination of equilibrium dissociation constant (K_d) for HutC_{His6} binding with P_{hutU} probe DNAs.

The plots were made on EMSA data presented in Figure 1 for the wild-type probe PhutU-325 (open circles), and the EMSA data in Figure 3 for the two mutant probes PhutU-M1 (solid triangles) and PhutU-M2 (solid squares) carrying mutations of the Phut-I and Phut-II repeats respectively.

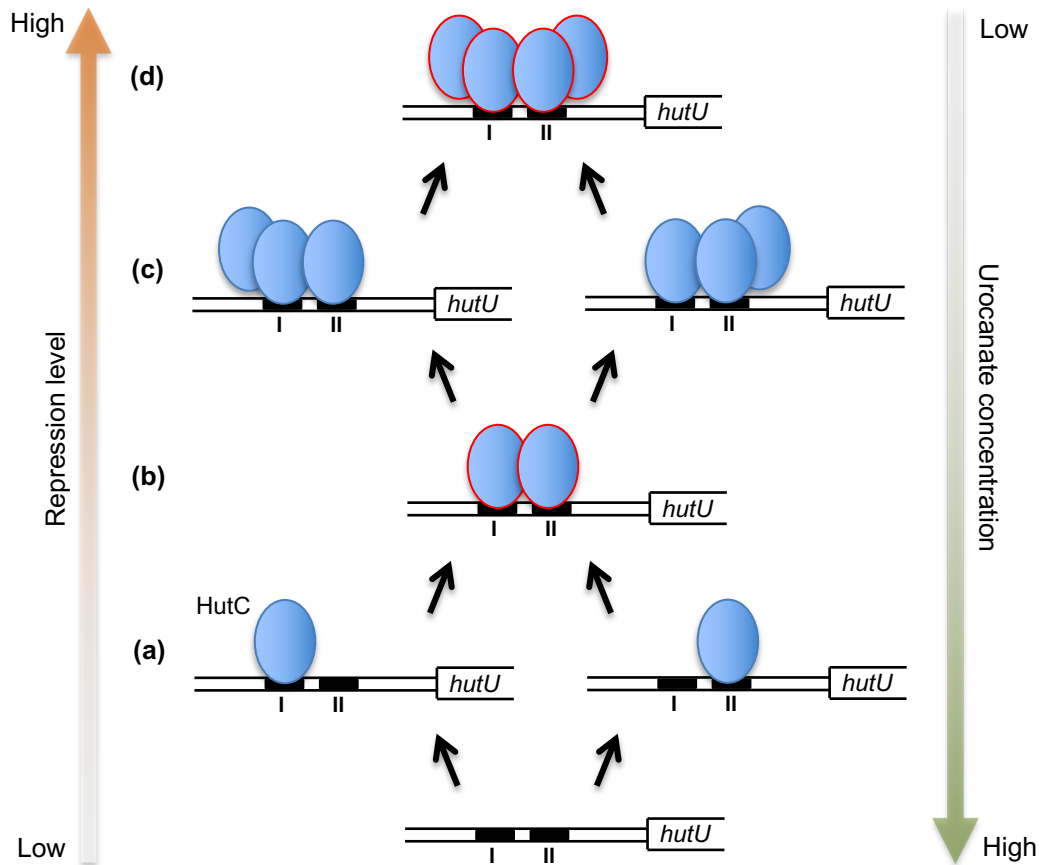


Figure S2. The proposed model of HutC action on the basis of data presented in this work.

When urocanate is present at high concentrations, HutC is dissociated from the P_{hutU} promoter region, and *hut* expression is de-repressed. On the decrease of urocanate, apo-HutC monomer binds to either Phut-I site or Phut-II site (a). However, efficient repression is achieved only when HutC forms a dimer and simultaneously binds to the two half-sites (b). As further decrease of urocanate, HutC forms a trimer (c), and eventually a tetramer, which tightly binds to the Phut site, resulting in stronger repression of the *hut* promoter (d). This dynamic process is reversed on the increase of urocanate.

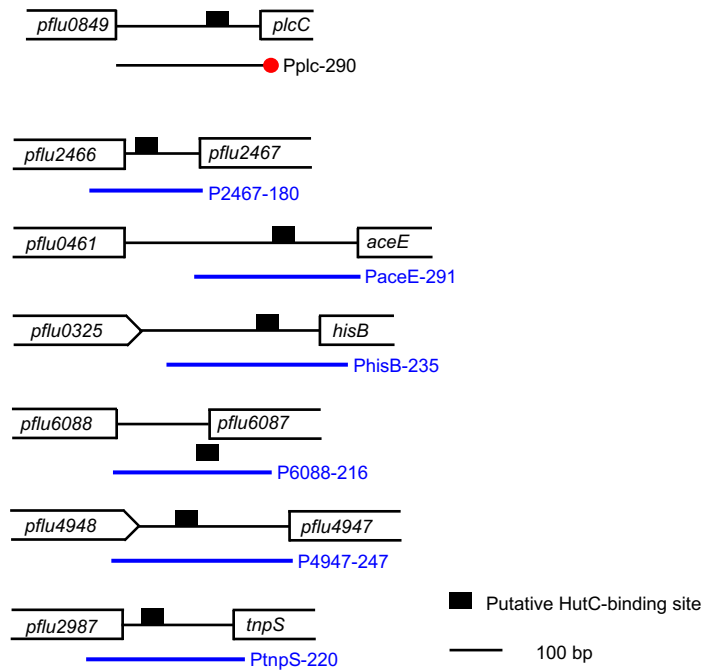


Figure S3. Probe DNAs used for EMSAs analysis of the predicted HutC binding sites. The Pplc-290 probe was labelled by biotin at 3'-end, whereas other probes were DIG-labelled at both ends.

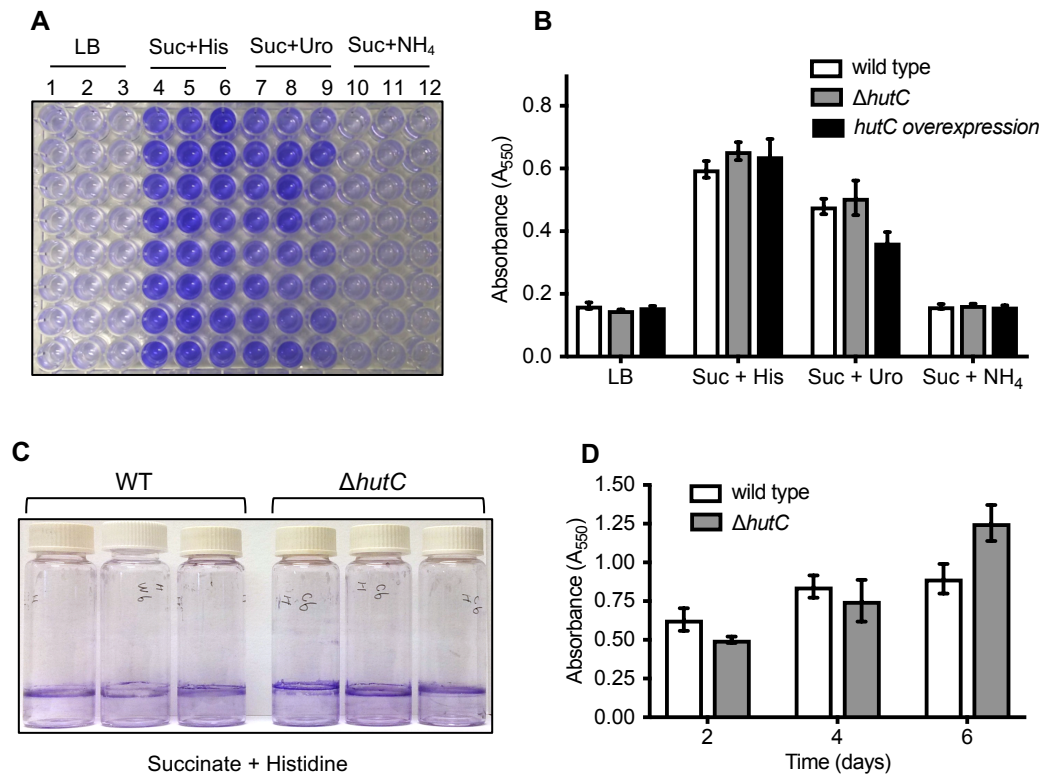


Figure S4. Assays for biofilm formation by wild-type SBW25 and its $\Delta hutC$ mutant.

(A) Bacteria were grown in a 96-well microtiter plate at 28°C for 3 days with eight replicates for each strain and each medium. Wild type: column 1, 4, 7 and 10; mutant $\Delta hutC$, column 2, 5, 8 and 11; mutant over-expressing *hutC*, column, 3, 6, 9 and 12. Biofilm was stained by crystal violet and solubilized for measuring absorbance at A_{550} nm. **(B)** Absorbance data are means and standard errors of eight repeats. **(C)** Biofilm formed when bacteria were grown in glass tubes under shaken conditions for 6 days at 28°C. **(D)** Data of absorbance (A_{550}) for biofilms formed by bacteria grown in minimal salt medium supplemented with succinate (20 mM) and histidine (10 mM) for 2, 4 and 6 days. Data are means and standard errors of three repeats.

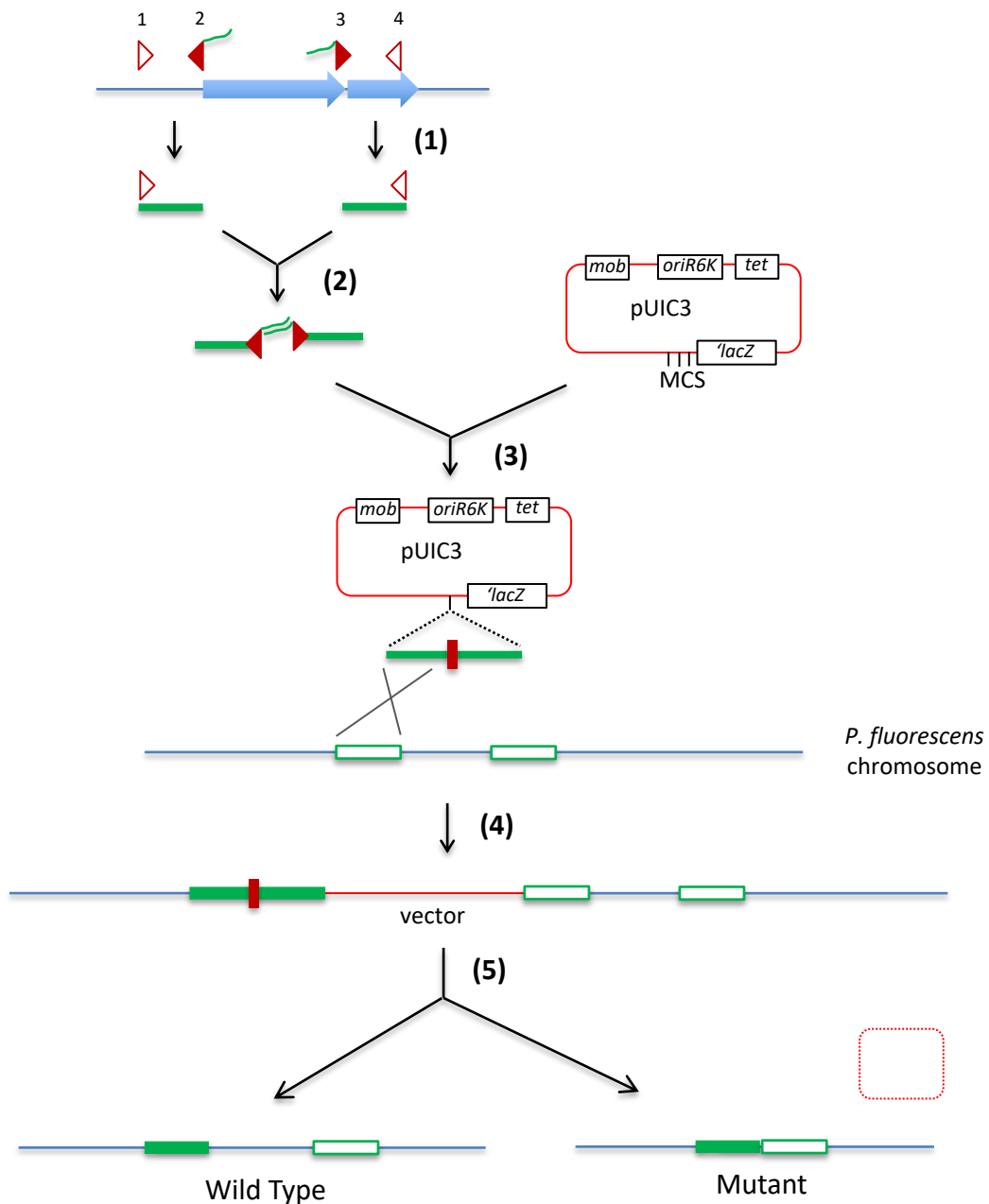


Figure S5. Gene deletion in *Pseudomonas* by SOE-PCR and a two-step allelic-exchange strategy.

(1) Two pairs of primers were designed to amplify the flanking DNA regions, and gene deletion or substitution mutation can be introduced into the overlapping sequence of the two outward facing primers. (2) The PCR products were then joined together in a second PCR using the two inward facing primers. (3) The resultant PCR product was then cloned into the plasmid vector pCR8/GW/TOPO using the TA Cloning Kit from Invitrogen Ltd. After sequence identity was confirmed by DNA sequencing, the insert DNA is then sub-cloned into the suicide-integration vector pUIC3 (Mob⁺). (4) The recombinant plasmid was then mobilized into *Pseudomonas* by a standard procedure of tri-parental conjugation on agar plate with the help of pRK2013 (Tra⁺). Integration by single homologous recombination was selected on LB agar supplemented with nitrofurantoin (100 µg/ml, to counter-select *E. coli*) and tetracycline (15 µg/ml). (5) To select for excision of the plasmid vector, purified single crossover mutants were subjected to cycloserine enrichment as previously described (Zhang and Rainey, 2007). The double crossover mutants were tet-sensitive and produced white colonies on LB agar containing X-gal (60 µg/ml). The desired mutants can be distinguished from wild-type revertants by PCR and DNA sequencing.

Table S1. Stoichiometric analysis of the HutC_{His6}-P_{hutU} complexes performed using the Hilmar Bading's method on the basis of EMSA gel image shown in Figure 1.

| | Migration Distance (cm) | HutC Molecular Weight (KDa) | Number of HutC |
|-----------|-------------------------|-----------------------------|----------------|
| Complex d | 4.75 | 119.74 | 4.19 |
| Complex c | 5.25 | 91.67 | 3.21 |
| Complex b | 5.80 | 66.38 | 2.32 |
| Complex a | 6.50 | 40.38 | 1.41 |
| Free DNA | 8.00 | -- | -- |

Table S2. Predicted HutC-binding sites in *P. fluorescens* SBW25

| p-value | Matched Sequence ^a | Motif Location | | | Putative or established function of candidate genes ^b |
|----------|-------------------------------|----------------|---------|---|--|
| | | Start | End | Distance to candidate genes | |
| 8.49E-09 | TATATGTATATACAAA | 396038 | 396053 | 27 bp | PFLU0358: HutF |
| 9.13E-09 | TGCTTGATGTACAAG | 397745 | 397760 | 71 bp | PFLU0361: HutU |
| 1.21E-06 | TCCATGTATAACAAG | 2682811 | 2682826 | 81bp to PFLU2467 | PFLU2466: hypothetical protein. PFLU2467: AraC family transcriptional regulator. |
| 2.49E-06 | TATTTGTATACAAAAG | 4800553 | 4800568 | 108 bp | PFLU4355: Xanthine/uracil permeases family protein. |
| 3.69E-06 | TATTTGTATGCACAGA | 6665739 | 6665754 | 120bp to PFLU6088 | PFLU6087: N-acetylmuramoyl-L-alanine amidase in peptidoglycan catabolism. PFLU6088: GTP cyclohydrolase |
| 4.59E-06 | GATATGTTTGTACAAG | 3934565 | 3934580 | | |
| 6.34E-06 | TGTATGTATATACAGC | 957430 | 957445 | 40bp to PFLU0848 | PFLU0848: Plc, phosphatidylcholine-hydrolyzing phospholipase C. PFLU0849: 5-dehydro-4-deoxyglucarate dehydratase, involved in D-glucarate degradation. |
| 6.47E-06 | TGTTTGAATATTCAAA | 5361498 | 5361513 | 130 bp | PFLU4885: hypothetical protein. A LTXXQ domain-containing protein, which shows similarity to a pilus assembly protein. |
| 7.22E-06 | TGTATGCAAATACAAA | 2875177 | 2875192 | Overlap PFLU2604 start codon | PFLU2604: hypothetical protein, which shows similarity to the GCN5 family acetyltransferase. |
| 8.74E-06 | TGCTTGTTTATATAAG | 3019426 | 3019441 | 123bp | PFLU2736: hypothetical protein. |
| 8.93E-06 | TGTTTGCATGTTCAAA | 1819996 | 1820011 | 386 bp | PFLU1658: Fnl2, NAD dependent epimerase/dehydratase. |
| 9.06E-06 | TGTTTCGTTTGTACAAG | 5428854 | 5428869 | 155 bp | PFLU4947: hypothetical protein. Its identified ortholog is pilus assembly protein PilZ. |
| 9.13E-06 | TGCATGTATGTGCATG | 5770150 | 5770165 | PFLU5255 coding region, 34bp from its start codon | PFLU5255: Hypothetical protein. Its ortholog is ribosome maturation protein RimP, which is important for maturation of the 30S ribosomal subunit. |
| 1.14E-05 | TATGTGGATATAAAAA | 3125190 | 3125205 | | |
| 1.15E-05 | TATATCAATTTACAAG | 1851392 | 1851407 | | |

| | | | | | |
|----------|------------------|---------|---------|--|---|
| 1.23E-05 | CATATATATATAAAAA | 192404 | 192419 | 447 bp | PFLU0172: hypothetical protein. |
| 1.56E-05 | TATTTATTTATATAAA | 817631 | 817646 | 95bp to PFLU0709 | PFLU0709: RspL, alternate sigma factor. PFLU0710: No information. |
| 1.57E-05 | TATTTGTATGTAGGAC | 4575786 | 4575801 | | |
| 1.79E-05 | GATTTTATATAGAAG | 4674453 | 4674468 | | |
| 2.36E-05 | TTTATGTTTATAAAAA | 5295164 | 5295179 | 210 bp | PFLU4815: putative glutathione S-transferase like protein. |
| 2.36E-05 | TTTTTGCATGTACAAC | 3711537 | 3711552 | 230 bp to PFLU3353, overlap PFLU3354 start codon | PFLU3353: putative amidase, its ortholog is acyl-homoserine lactone acylase subunit beta, which is involved in quorum sensing. PFLU3354: hypothetical protein. |
| 2.36E-05 | TACCTATATATACCAG | 5252378 | 5252393 | | |
| 2.62E-05 | TACTCGTATTTAGAAG | 3126232 | 3126247 | | |
| 2.97E-05 | TACTCGTATATACATT | 520016 | 520031 | 150bp to PFLU0460 | PFLU0460: AceE, pyruvate dehydrogenase subunit E1. PFLU0461: bifunctional glutamine-synthetase adenylyltransferase/deadenyltransferase. |
| 3.04E-05 | TATTTGATACACAAA | 4779233 | 4779248 | 199 bp | PFLU4327: putative sulfatase. |
| 3.17E-05 | TATTTGACTATAAAAA | 3433087 | 3433102 | PFLU3141 coding region, 7bp from its start codon | PFLU3141: LipB, lipase. |
| 3.19E-05 | CAGTTGTATGCACATG | 360518 | 360533 | 106 bp | PFLU0327: HisB, imidazoleglycerol-phosphate dehydratase. |
| 3.29E-05 | TATTTATATGAATAAA | 4501472 | 4501487 | 318 bp | PFLU4069: hypothetical protein. |
| 3.37E-05 | GATTTGTATAAATAAG | 2182043 | 2182058 | 247 bp | PFLU2013: CysA, D-serine/D-alanine/glycine transporter. |
| 3.43E-05 | TATATCGATATTCAAG | 4573285 | 4573300 | | |
| 3.43E-05 | TATTCGTACATAGAAG | 6418864 | 6418879 | 106 bp | PFLU5862: ArgA, N-acetylglutamate synthase involved in arginine biosynthesis pathway. |
| 3.54E-05 | TATTTTTTTGTAAAAG | 2045361 | 2045376 | 21 bp | PFLU1874: putative transporter-like acyltransferase protein. Its ortholog is glycerol acyltransferase. |
| 3.54E-05 | TATCTGTCTGTACGAA | 4678701 | 4678716 | | |
| 3.54E-05 | TATGTGTATGAGCAAG | 1584681 | 1584696 | | |
| 3.63E-05 | TAATTGGTTGTACAAG | 3192681 | 3192696 | | |
| 3.67E-05 | TAATTGTGAGTACAAG | 6160944 | 6160959 | 275 bp | PFLU5623: GlcB, malate synthase G. |
| 3.68E-05 | TTTTTGTAGGTAAAAG | 3761310 | 3761325 | | |
| 3.79E-05 | TATATGTCGATCCAAA | 1060899 | 1060914 | | |
| 3.79E-05 | TATATGTGAATACAGA | 3527700 | 3527715 | 91bp to PFLU3219 | PFLU3218: putative TonB-dependent outer membrane receptor. PFLU3219: SyrP-like protein. SyrP is a regulatory protein involved in syringomycin production and virulence in <i>P. syringae</i> . |
| 3.93E-05 | GATATGCATATTCAAA | 4101662 | 4101677 | Overlap PFLU3705 start codon | PFLU3705: hypothetical protein. |
| 4.04E-05 | TATATGAACATTCAAA | 4570067 | 4570082 | PFLU4126 B coding region, 27bp from its start | PFLU4126B: hypothetical protein. |

| | | | | | |
|----------|-------------------------|---------------|---------------|--|---|
| | | | | codon | |
| 4.14E-05 | TATAGGTATGTTAAAA | 1818852 | 1818867 | | |
| 4.18E-05 | TATATTTACGTGCAAA | 2720248 | 2720263 | | |
| 4.37E-05 | TATTTGGATGTAACAA | 3164038 | 3164053 | 2 bp | PFLU2900: hypothetical protein. |
| 4.45E-05 | CACTTTTATGTAAAAA | 3431252 | 3431267 | 79bp from PFLU3140 | PFLU3139: hypothetical protein. PFLU3140: putative lipoprotein. |
| 4.45E-05 | CCACTGTCCGAACAAC | 377028 | 377043 | 300 bp | PFLU0344: NtrB, nitrogen-specific signal transduction histidine kinase. |
| 4.45E-05 | TATATGGATGCAAAAA | 3468310 | 3468325 | 71bp to PFLU3176 | PFLU3176: aspartate aminotransferase, which catalyzes the interconversion of aspartate and α -ketoglutarate to oxaloacetate and glutamate. PFLU3177: hypothetical protein. Its ortholog is NAD-dependent dehydratase. |
| 4.45E-05 | TATATTTATGACCAAA | 4101101 | 4101116 | | |
| 4.56E-05 | TATATCTTTGTCCAAA | 5824380 | 5824395 | PFLU5305 coding region, 74bp from its start codon | PFLU5305: putative plasmid partitioning protein. |
| 4.56E-05 | TACATATTTGTAGAAA | 6297047 | 6297062 | | |
| 4.61E-05 | TATTTCCATGTACACA | 6639456 | 6639471 | 80bp to PFLU6064 | PFLU6064: GntR family transcriptional regulator. PFLU6065: putative regulatory protein. |
| 4.71E-05 | TACTTCTAGATGCAAG | 960179 | 960194 | Overlap PFLU0851 start codon | PFLU0851: putative sugar ABC transporter membrane protein. Its ortholog is glucarate transporter. |
| 4.81E-05 | TACTAGTATATAGAGG | 1612714 | 1612729 | 81bp to PFLU1465 | PFLU1465: NadB, L-aspartate oxidase, which participates in alanine and aspartate metabolism. PFLU1467: AlgU, a sigma factor, which regulates genes involved in alginate biosynthesis. |
| 4.95E-05 | TATTTGTACACACGAG | 2357140 | 2357155 | 287 bp | PFLU2175: hypothetical protein. |
| 5.15E-05 | TATTGGTCAATACAAG | 3188737 | 3188752 | | |
| 5.28E-05 | TATAGGTACAAACAAG | 4575654 | 4575669 | | |
| 5.47E-05 | TATTTCTGTGTACACG | 202548 | 202563 | | |
| 5.74E-05 | TATATGTACGTATACG | 2930280 | 2930295 | Overlap PFLU2657 start codon, 230bp upstream of PFLU2658 | PFLU2657: putative sulfite reductase. PFLU2658: hypothetical protein. |
| 5.89E-05 | TTCATGTCTAAACAAA | 2052141 | 2052156 | 305 bp | PFLU1879: hypothetical protein. |
| 5.89E-05 | TCTATGCATGTCCAAA | 2691737 | 2691752 | 370 bp | PFLU2477: hypothetical protein. |
| 5.99E-05 | CACATGTACATACAGA | 2409875 | 2409890 | 260 bp | PFLU2222: putative ABC transporter membrane protein. |
| 5.99E-05 | TACATGTAGATACTCA | 3192340 | 3192355 | 414 bp | PFLU2927: hypothetical protein. |
| 5.99E-05 | TACTTGGAGATACACA | 5654723 | 5654738 | 228bp to PFLU5155, 0bp to PFLU5156 | PFLU5155: putative MerR family transcriptional regulator. PFLU5156: hypothetical protein with very high similarity to antibiotic biosynthesis monooxygenase. |
| 5.99E-05 | AATACGTATGTTCAAA | 2065013 | 2065028 | Overlap PFLU1890 start codon | PFLU1890: hypothetical protein. |

| | | | | | |
|----------|------------------|---------|---------|------------------------------|--|
| 6.05E-05 | TATTTGGATGAACCAA | 2069374 | 2069389 | | |
| 6.05E-05 | TATTGGTATGTGCATA | 3168701 | 3168716 | | |
| 6.17E-05 | TACATGGAAATCCAAG | 2730579 | 2730594 | 433 bp | PFLU2516: hypothetical protein, a TetR family transcriptional regulator. |
| 6.17E-05 | TATTTGTTTGATCAAG | 1968004 | 1968019 | 55bp to PFLU1804 | PFLU1803: Gcl, glyoxylate carboligase, which participates in glycolate degradation. PFLU1804: hypothetical protein. Its ortholog is GlcG protein with unknown function. |
| 6.21E-05 | TATTCGTAAGTACACG | 2958127 | 2958142 | 31 bp | PFLU2681: PepN, aminopeptidase. |
| 6.24E-05 | AATTTGTAGGTTCAAG | 4649122 | 4649137 | 63 bp | PFLUt63: tRNA-Glu. |
| 6.25E-05 | TACTCGTATGAAGAAG | 5761130 | 5761145 | Overlap PFLU5248 start codon | PFLU5248: osmotically inducible protein Y. |
| 6.32E-05 | TGTTTTATATACAGT | 54429 | 54444 | 16 bp | PFLU0054: hypothetical protein with very high similarity to DNA repair photolyase. |
| 6.32E-05 | TGATCGTATCTACAAG | 4367523 | 4367538 | | |
| 6.37E-05 | TGCGTTTATCTACAAG | 5381873 | 5381888 | | |
| 6.37E-05 | TTCATGTATGTACTGG | 5604071 | 5604086 | 447 bp | PFLU5103: hypothetical protein. |
| 6.43E-05 | TGCATGGATTACAAT | 829311 | 829326 | 248 bp | PFLU0727: RspB, putative type III secretion protein. |
| 6.43E-05 | TGTATGTATTAATAAA | 2164052 | 2164067 | 22bp to PFLU1992 | PFLU1992: hypothetical protein, showing high similarity to amidase. PFLU1993: GntR family transcriptional regulator. |
| 6.49E-05 | CACATGGATGTACACA | 4109407 | 4109422 | | |
| 6.52E-05 | TGTTTTTTCTACAAG | 5256428 | 5256443 | | |
| 6.62E-05 | TACTCGTCTGTACGAG | 5539401 | 5539416 | 65bp to PFLU5040 | PFLU5040: PTS system sucrose-specific transporter subunit IIBC. PFLU5041: trehalose operon transcriptional repressor. |
| 6.68E-05 | TGTTTGTATAGACAGT | 1303654 | 1303669 | 93bp to PFLU1167 | PFLU1166: hypothetical protein. PFLU1167: putative regulatory protein, its ortholog is transcriptional repressor PrtR which regulates pyocin-producing genes. |
| 6.78E-05 | TGTATGCATATACAGT | 1304632 | 1304647 | 441 bp | PFLU1169: hypothetical protein. |
| 6.79E-05 | TGAATGTGTAAACAAA | 3336181 | 3336196 | | |
| 6.86E-05 | TGTACGTATTTACCAA | 3708943 | 3708958 | 134 bp | PFLUt50: tRNA-Cys. |
| 6.86E-05 | TGTCTGTATGCAAAA | 3777730 | 3777745 | | |
| 6.95E-05 | TGTCGGTATAAACAAG | 4334862 | 4334877 | 147 bp | PFLU3927: DNA-binding ATP-dependent protease La; heat shock K-protein. |
| 6.99E-05 | GGCATATATCTACAAG | 3832062 | 3832077 | | |
| 7.03E-05 | AGCATGGATATACAAC | 6590392 | 6590407 | 447 bp | PFLU6027: hypothetical protein. |
| 7.04E-05 | TGTTTATAAATGCAAA | 5351550 | 5351565 | 292bp to PFLU4872 | PFLU4870: aromatic amino acid transport protein. PFLU4872: hypothetical protein with high similarity to leucyl aminopeptidase (aminopeptidase T). |
| 7.04E-05 | TGTATGTTTGTACAGC | 1918052 | 1918067 | 25 bp | PFLU1750: hypothetical protein, a highly conserved transcriptional regulator in <i>Pseudomonas</i> . |
| 7.13E-05 | TGTATGGATGTACAGC | 2337963 | 2337978 | 69 bp | PFLU2157: TopB, DNA topoisomerase III. |

| | | | | | |
|----------|-------------------|---------|---------|--|--|
| 7.16E-05 | TGTATGTATGAACAGT | 3123864 | 3123879 | 20 bp | PFLU2842: hypothetical protein. Its ortholog is ATP-dependent hsl protease ATP-binding subunit hslU. |
| 7.18E-05 | TGTAATTGTATACAAG | 1967841 | 1967856 | 22bp to PFLU1803 | PFLU1803: Gcl, glyoxylate carboligase, which participates in glycolate degradation. PFLU1804: hypothetical protein. Its ortholog is GlcG protein with unknown function. |
| 7.24E-05 | TGCGTGAATATCCAAA | 5683522 | 5683537 | 33 bp | PFLU5187: putative amino acid transporter membrane protein. |
| 7.24E-05 | CGAATGTTTGTACAAA | 127586 | 127601 | | |
| 7.27E-05 | TGGTTGTCTGTTCAAA | 2366894 | 2366909 | | |
| 7.27E-05 | GGATTGCATGTACAAG | 4094071 | 4094086 | Overlap PFLU3698 start codon, 374bp upstream of PFLU3699 | PFLU3698: putative TonB-dependent outer membrane receptor protein. PFLU3699: putative GGDEF domain signaling protein. |
| 7.29E-05 | TGCTTGTAAGATAAA | 445895 | 445910 | 99bp to PFLU0402 | PFLU0402: PriA, primosome assembly protein. PFLU0403: RpmE, 50S ribosomal protein L31. |
| 7.39E-05 | TGCATTTATATCCACA | 2201527 | 2201542 | | |
| 7.44E-05 | TGCTTATCGATACAAA | 2574931 | 2574946 | 122 bp | PFLU2365: putative TonB-dependent siderophore receptor. |
| 7.52E-05 | GGTAGGGATATACAAA | 3018076 | 3018091 | 5 bp | PFLU2734: hypothetical protein. |
| 7.61E-05 | GGTATGTATATACGTA | 3255865 | 3255880 | 32bp from PFLU2987 | PFLU2987: putative phage cointegrase resolution protein. PFLU2988: TnpS, cointegrase. |
| 7.87E-05 | CGTAGGTATATACACA | 5503929 | 5503944 | 116 bp | PFLU5008: XerD, site-specific tyrosine recombinase. |
| 7.87E-05 | TGCATGAATAGATAAA | 5540677 | 5540692 | 21 bp | PFLU5042: putative regulatory protein. |
| 7.92E-05 | TGCAGGTCTGTACAAC | 2315111 | 2315126 | | |
| 7.98E-05 | TGCTGGTCTGTACAAT | 4750305 | 4750320 | 24 bp | PFLU4307: TenA family transcriptional regulator. |
| 7.98E-05 | TGTTTGTCTGAAAAAA | 2489624 | 2489639 | 351 bp | PFLU2290: putative acetyl-CoA synthetase. |
| 8.08E-05 | TGTTTGTCTGAAAAAA | 2490009 | 2490024 | | |
| 8.14E-05 | TGCATCTATGTAAATA | 3330114 | 3330129 | | |
| 8.25E-05 | TGTTTGTCTGTGAAAA | 3391717 | 3391732 | | |
| 8.32E-05 | TGCATCAATGTAAAAA | 792973 | 792988 | | |
| 8.35E-05 | TGCATGTATACCGAAG | 2443235 | 2443250 | | |
| 8.38E-05 | AGCTTTTATATTCAAG | 3752558 | 3752573 | | |
| 8.49E-05 | GGCTTCTATAAACAAAG | 4361503 | 4361518 | | |
| 8.57E-05 | TGCATGTATATAATGG | 5544003 | 5544018 | 34 bp | PFLU5044: inosine 5'-monophosphate dehydrogenase. |
| 8.57E-05 | CGTATCTATGCACAAG | 1513619 | 1513634 | | |
| 8.67E-05 | TGTTTCGGTGTACAAG | 4620202 | 4620217 | | |
| 8.67E-05 | TGCGTGTGTGTACACG | 2550391 | 2550406 | | |
| 8.87E-05 | TGCTGTAGAAACCAA | 5013760 | 5013775 | | |
| 8.99E-05 | TGCTTGCATAGCCAAA | 1627083 | 1627098 | | |
| 8.99E-05 | TGCATGTTTATCCACA | 5014262 | 5014277 | | |
| 9.14E-05 | TGCTTCTGTGTACATA | 1360685 | 1360700 | | |

| | | | | | |
|----------|----------------------------|---------|---------|--|--|
| 9.23E-05 | CGCAT GT TTGTAGAAA | 2011553 | 2011568 | | |
| 9.23E-05 | TGCATTTATGT CC CAA | 6232815 | 6232830 | Overlap PFLU5686 start codon | PFLU5686: BetA, choline dehydrogenase |
| 9.23E-05 | CGCAA AT ATGTACAAA | 935972 | 935987 | 157 bp | PFLU0826: DppA3, dipeptide ABC transporter substrate-binding protein. |
| 9.26E-05 | TGTTT GT CTGTACGTA | 3251963 | 3251978 | 80 bp | PFLU2983: putative lipoprotein. |
| 9.28E-05 | TGCTT GT AAGTTTAAA | 6251444 | 6251459 | 90 bp | PFLU5700: hypothetical protein. |
| 9.32E-05 | TGCAG GT AGAGACAAG | 3028964 | 3028979 | PFLU2745 coding region, 24bp from its start codon | PFLU2745: putative ABC transporter mannitol-binding protein. |
| 9.39E-05 | TGCTTTTT GT TTCAAG | 75295 | 75310 | 100 bp | PFLU0074: ZnuC, high-affinity zinc ABC transporter ATP-binding protein. |
| 9.39E-05 | TGTTT GC AGGTACACG | 709299 | 709314 | 401 bp | PFLU0626: SpeA, arginine decarboxylase. |
| 9.46E-05 | TGTTT GT GTGAACAGG | 2914511 | 2914526 | 286 bp | PFLU2640: putative substrate-binding transport protein. |
| 9.46E-05 | TGCTT GT AAGAAAAAG | 6506555 | 6506570 | | |
| 9.46E-05 | TGCATATATGAC CA AG | 6578846 | 6578861 | 77bp to PFLU6018 | PFLU6017: Homologue of a type VI secretion protein. PFLU6018: Homologue of the type VI secretion protein ImpA. |
| 9.48E-05 | TGCT GG GATGTACACA | 52910 | 52925 | 149 bp | PFLU0053: AdhB, alcohol dehydrogenase cytochrome C subunit. |
| 9.57E-05 | TGCTT GT CTGCACGAA | 875808 | 875823 | 7bp to PFLU0773 | PFLU0772: a type IV pilus-like protein. PFLU0773: hypothetical protein. Its ortholog is pilus assembly protein PilV. |
| 9.57E-05 | GGCA AG TAGGTACAAA | 1909814 | 1909829 | 73 bp | PFLU1740: putative two-component system response regulator, LuxR family transcriptional regulator. |
| 9.57E-05 | TGCAG GT ACGTGCAAA | 3122089 | 3122104 | 240 bp | PFLU2839: hypothetical protein. |
| 9.57E-05 | TGCAT GG ATGAACGAG | 486244 | 486259 | 62bp to PFLU0439 | PFLU0438: homologue of 3-oxoacyl-ACP synthase in fatty acid biosynthesis. PFLU0439: putative chromosome partitioning ParA-like protein. |
| 9.64E-05 | CGCAT GG ATGTGCAAG | 1174625 | 1174640 | 398 bp | PFLU1062: RecO, DNA repair protein. |
| 9.72E-05 | TGCTT GT TACGTCCAGG | 3067587 | 3067602 | 257 bp | PFLU2774: 3-phosphoshikimate 1-carboxyvinyltransferase involved in aromatic amino acid biosynthesis. |
| 9.75E-05 | TGCAT GC CAGTACAAG | 3799694 | 3799709 | 197 bp | PFLU3431: hypothetical protein. |
| 9.82E-05 | GGCTT GA ATGTGCAAG | 4276360 | 4276375 | | |
| 9.93E-05 | GGCAT GG ATGTACATG | 6436208 | 6436223 | 235 bp | PFLU5879: 2-octaprenyl-6-methoxyphenyl hydroxylase, which is involved in ubiquinone biosynthesis and oxidation-reduction process. |

^a Conserved palindromic half sites (Phut-I and Phut-II) are highlighted in bold font.

^b Genes shown here contain the related HutC-binding site in their regulatory region spanning from -500 bp to +100 bp relative to the first nucleotide of the start codon. Candidates in red font have been subjected to experimental verification by EMSA with purified HutC_{His6}.

Table S3. Oligonucleotides used in this work

| Primer | Sequence (5' - 3') ^a | Application |
|------------------------|--|---|
| HutC-ProF | aaatttaccatgggcatcatcatcatcatcatCCGACTCC GCCCCGCAAGTCTC | Expression of HutC protein from SBW25 |
| HutC-ProR | aaatttgaagccttGCGCCAGACGCTTATTGCACTC AT | |
| PhutU_D | aaatttactagtATTTGTTACCGAATGCCCCAGC | Amplifying PhutU-325 probe DNA |
| Bio-UR ^b | GCTTGTTACCGTGGGCGGCACGGAT | |
| PhutU_B | aaatttactagtGTCGGTACATCTATGACTGAAAC | Mutagenizing Phut-I site in P _{hutU} promoter |
| PhutU-mut1 | cttgatcacccggAGCATATGCAATCGAG | |
| PhutU-mut2 | catatgctccggTGTACAAGTAAAGATGTG | |
| hutU-T7R2 | TGTTTCATCAGCATGCGCAGC | |
| PhutU-mut3 | atctttacggccACATACAAGCATATGCAA | Mutagenizing Phut-II site in P _{hutU} promoter with primers PhutU_B and hutU-T7R2 |
| PhutU-mut4 | ttgatgtggccGTAAGATGTGTGCGTAA | |
| hutU-M1 | ttacggccacaccggAGCATATGCAATCGAGCGG CCA | Mutagenizing Phut-I and Phut-II site in P _{hutU} promoter with primers PhutU_B and hutU-T7R2 |
| hutU-M2 | atgctccgggtggccGTAAGATGTGTGCGTAAG AG | |
| PhutU_Rev | aaatttctgcagAGCTTGTTACCGTGGGCGGC | P _{hutU} transcriptional fusion to lacZ with primer PhutU_D |
| ntrB-Spel | gactaGTATTACCGGCAACACCCCGGTCGA | Amplifying P _{ntrBC} -154 probe DNA |
| Bio-ntrR3 ^b | ATGAAGGTTGTTTCAGGAAGTGG | |
| ntrB-HindIII | gaagccttGCTGATGGTCATTGGGACCTCTT | Mutagenizing Phut site in P _{ntrBC} promoter |
| P _{ntr} BM2-1 | TCATGGGATGGCACACCTTAC | |
| P _{ntr} BM2-2 | tgccaccacttaggtcACACTGATCCATCCCCACT | |
| P _{ntr} BM2-3 | agtgtgacctaagtGTGGCATGCCCGGACGGCC CGCGT | |
| P2467-F | GAAGGACTTGGGTTACCGT | Amplifying P2467 probe DNA |
| P2467-R | CATGGGCATGCACATCGATC | |
| PaceE-F | CATAGTTGTTGGCAGGGAAC | Amplifying P _{glnE} probe DNA |
| PaceE-R | TCTTGCATGGCTTGCTCCAG | |
| PhisB-F | AGCCACCTCGTTCTTTCAGT | Amplifying PhisB probe DNA |
| PhisB-R | TTACGTTTCGGCCATCACCAG | |
| P6088-F | TTGCGTATCTGTGTGGCGTG | Amplifying PamiA probe DNA |
| P6088-R | CATGAGCAGGTCCATCGTTG | |
| P4947-F | GCGTCCAGCTTCGTTATGATG | Amplifying P _{pilZ} probe DNA |
| P4947-R | CATGGTGAGATCGCCATCCA | |
| P2987-F | CATGGGAGGCCTTTGTGTAC | Amplifying P _{tnpS} probe DNA |
| P2987-R | AGGCGTGCCGTTTGA ACTAC | |
| Bio-plc ^b | CTGCGAGTTCAAGACCTGACAT | Amplifying P _{plcC} probe DNA |
| plc-R | ggactagtGGACTTCAGTTCTTGTGGATTC | |
| ntrB-Spel | gactaGIATTACCGGCAACACCCCGGTCGA | Transcriptional lacZ fusion to P _{ntrBC} |
| ntrB-HindIII | gaagccttGCTGATGGTCATTGGGACCTCTT | |
| plc-F | ggaagccttCTGCGAGTTCAAGACCTGACAT | P _{plcC} transcriptional fusion to lacZ with primer plc-R |
| hutC7 | gaagatCIGCAAGGATTCCCTGTGCC | hutC overexpression with primer HutC-ProR |
| hutF1 | gaagatCIGATCTGACGCGACAGTTC | hutC deletion |

| | | |
|-----------|-------------------------------|--|
| hutC-del2 | aagctatgacGCACAGGGAATCCTTGCAG | |
| hutC-del3 | attccctgtgCGTCATAGCTTGAAGGAC | |
| hutD1 | gaagatctTGGGTCAGTTCGATCAGGC | |

^a Artificial sequences integrated into the primers are shown in lowercase with restriction sites underlined.

^b Primers are labeled by biotin at the 5' end.