# Improving Prodigiosin Production by Transcription factor Engineering and Promoter Engineering in *Serratia marcescens*

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Fig. S1

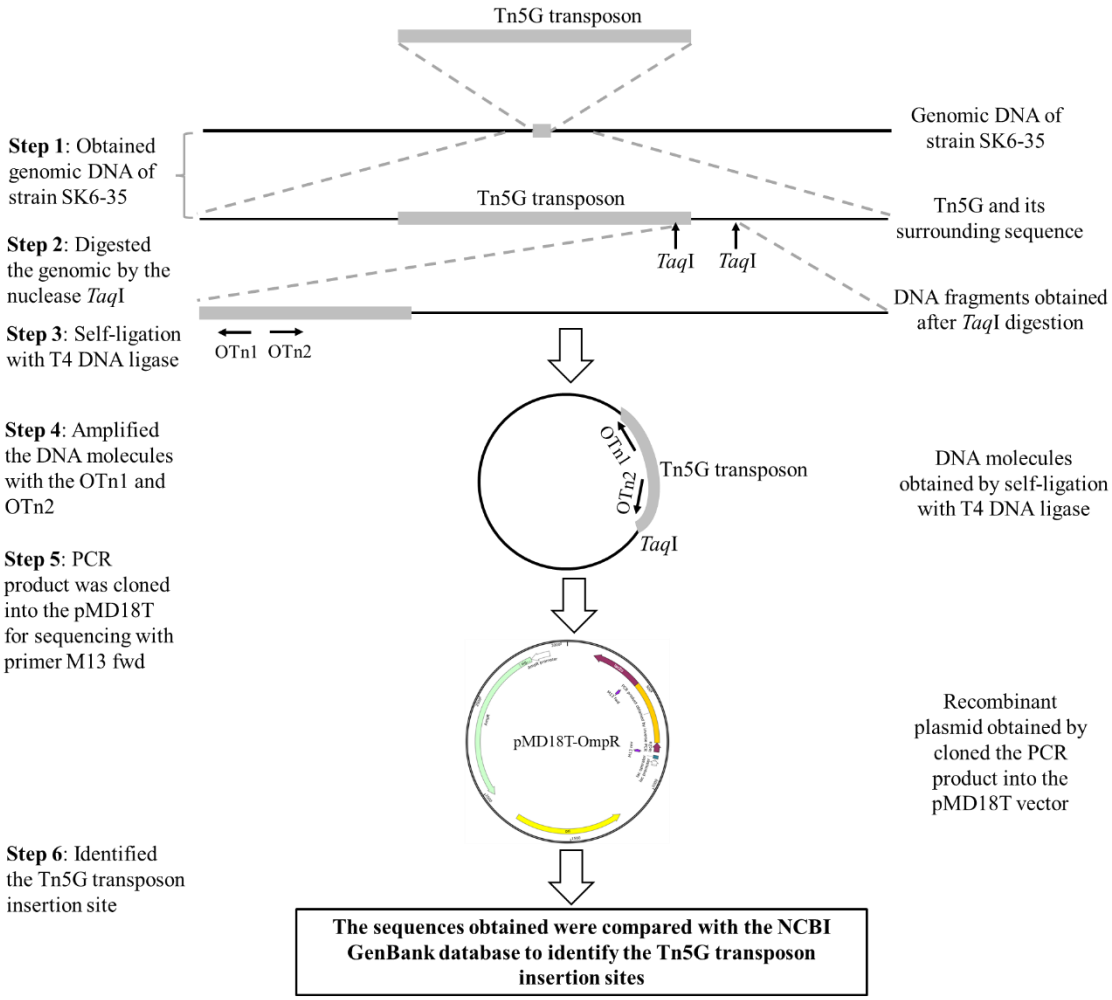


**Figure S1. Schematic diagram of Tn5G insertion site in mutant SK6-35 analyzed by inverse PCR**.

**Table S1. Primers used in this study**

| Primer | Primer Sequence (5'-3') | Function |
|---|---|---|
| OTn1 | GATCCTGGAAAACGGGAAAG | Identification of Tn5G in prodigiosin production |
| OTn2 | CCATCTCATCAGAGGGTAGT | mutants |
| OmpR-F1 | AAAACGACGGCCAGTGCCAAGCTTTCATGCCTTGCTGCCGTCC | Amplification of *ompR* gene to constructing of |
| OmpR-R1 | TTCGAGCTCGGTACCCGGGGATCCATGCAAGAGAATCATAAGATCCTGGTTGTC | plasmid pXW2010 |
| OmpR-arm-F1 | TAGGCCGAATTCGAGCTCGGTACCGATGCACGTTCATCATCAGTTCGC | Amplification of *ompR* gene upstream |
| OmpR-arm-R1 | AAACAAATAGGGGTTCCGCGATGAGGCGATTGCGCTTTTCAC | homologous arms |
| OmpR-arm-F2 | GAAAAGCGCAATCGCCTCATCGCGGAACCCCTATTTGTTTATTTTTCT | Amplification of *aacC3* gene |
| OmpR-arm-R2 | AAGCCTTTGGGAGTAATACCTCAGCCAATCGACTGGCGAG | |
| OmpR-arm-F3 | CTCGCCAGTCGATTGGCTGAGGTATTACTCCCAAAGGCTTTATTGCCT | Amplification of *ompR* gene downstream |
| OmpR-arm-R3 | ATTACAGCCGGATCCCCGGGTACCAACGTGAAAATCCCGGCCG | homologous arms |
| 18-egfp-F1 | AAAACGACGGCCAGTGCCAAGCTTCTATTTGTATAGTTCATCCATGCCATGTGTAATCC | Primers used to construct of plasmid pUCP18- |
| 18-egfp-R1 | TTCGAGCTCGGTACCCGGGGATCCATGAGTAAAGGAGAAGAACTTTTCACTGGAG | *egfp* |
| 18-egfp-F2 | GCCTGGGGTGCCTAATGAGT | Primers used to amplify the skeleton of plasmid |
| 18-egfp-R2 | ATGAGTAAAGGAGAAGAACTTTTCACTGGAGT | pUCP18-*egfp* |
| 18-ahpC-F1 | TTCTTCTCCTTTACTCATAATTACTCCTGTAATAGGTGTTAAAGGGTGGATG | Primers used to amplify the P1 ($P_{AhpC}$) promoter |

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|---|---|---|
| 18-ahpC-R1 | TCATTAGGCACCCCAGGCGGTCAAGCCCAAGCGTTTGAAAT | |
| 18-clpP-F1 | AGTTCTTCTCCTTTACTCATTACCGTCTCCTAATAGAAATTGCCTTGGC | Primers used to amplify the P2 ($P_{ClpP}$) promoter |
| 18-clpP-R1 | ACTCATTAGGCACCCCAGGCGAACCGGCGCACGATGAAAAAG | |
| 18-cpxP-F1 | AGTTCTTCTCCTTTACTCATTTCAACTCCTCAAGCTTTCTCACTTTGCG | Primers used to amplify the P3 ($P_{CpxP}$) promoter |
| 18-cpxP-R1 | ACTCATTAGGCACCCCAGGCTATGATTTACCTCCAGACGCAAAATACGTCATC | |
| 18-cspC-F1 | AGTTCTTCTCCTTTACTCATTTGTGTTTCCTTTACTTGATAAACCTGCCTTG | Primers used to amplify the P4 ($P_{CspC}$) promoter |
| 18-cspC-R1 | ACTCATTAGGCACCCCAGGCAGTTTCGTACCTGGATCGGTGAAAG | |
| 18-eno-F1 | AGTTCTTCTCCTTTACTCATTAGGTTTTCCTCAGTACAAGTTAAACTAAAACTCCAG | Primers used to amplify the P5 ($P_{Eno}$) promoter |
| 18-eno-R1 | ACTCATTAGGCACCCCAGGCCATATTTGGTAGGCGGCGCG | |
| 18-ftsZ-F1 | AGTTCTTCTCCTTTACTCATAGTTTCTCTCCGTTTTGTGCCTGTC | Primers used to amplify the P6 ($P_{FtsZ}$) promoter |
| 18-ftsZ-R1 | ACTCATTAGGCACCCCAGGCTGTTTCAACAAAGAGATCATGCTGAGTAATCTT | |
| 18-gltA-F1 | AGTTCTTCTCCTTTACTCATTTACTGTCTCCTTAGCGCCTTATTTTAAAAACC | Primers used to amplify the P7 ($P_{GltA}$) promoter |
| 18-gltA-R1 | ACTCATTAGGCACCCCAGGCACAGCTCTTATTATTTTCCTTCCTCCGG | |
| 18-lpp-F1 | AGTTCTTCTCCTTTACTCATTATAACACCCTCTAGATTGAGTTAATCTCCATGTAGC | Primers used to amplify the P8 ($P_{Lpp}$) promoter |
| 18-lpp-R1 | ACTCATTAGGCACCCCAGGCTATAAAACGTGACATAATTGTCTTGATCAAAACGCC | |
| 18-lpxC-F1 | AGTTCTTCTCCTTTACTCATCGTTTTATCTCGCAATGTTATCCATCCTACC | Primers used to amplify the P9 ($P_{LpxC}$) promoter |
| 18-lpxC-R1 | ACTCATTAGGCACCCCAGGCGAATATCCTGAGAATTTGGAATCTCCGCTCT | |

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| 18-nlpI-F1 | AGTTCTTCTCCTTTACTCATTTCCCACTCCCGAAGACAAACAT | Primers used to amplify the P10 ($P_{NlpI}$) promoter |
| 18-nlpI-R1 | ACTCATTAGGCACCCCAGGCCTGTATAGATAGTTTTACAGCTCCCCGC | |
| 18-ompA-F1 | AGTTCTTCTCCTTTACTCATTTTGCGCCTCGTTATCATCCAAAT | Primers used to amplify the P11 ($P_{OmpA}$) promoter |
| 18-ompA-R1 | ACTCATTAGGCACCCCAGGCGTAAATTTAGGATTTTTCCCATCATTTTCGCACC | |
| 18-ompC-F1 | AGTTCTTCTCCTTTACTCATCGTTATTATCCTCGTTAATTATGTCGAGCTACG | Primers used to amplify the P12 ($P_{OmpC}$) promoter |
| 18-ompC-R1 | ACTCATTAGGCACCCCAGGCGAATTTTACTCGCTGAAGGTGCGC | |
| 18-ompN-F1 | AGTTCTTCTCCTTTACTCATCATTATTACCCTCATTGGTGTTATTCGGACAC | Primers used to amplify the P13 ($P_{OmpN}$) promoter |
| 18-ompN-R1 | ACTCATTAGGCACCCCAGGCTTTATACGATCACACGTTTTTTAAACGATTTCGTTACAAA | |
| 18-ompW-F1 | AGTTCTTCTCCTTTACTCATTATCCATTCCATTTGTGTGGTTAATCGCT | Primers used to amplify the P14 ($P_{OmpW}$) promoter |
| 18-ompW-R1 | ACTCATTAGGCACCCCAGGCCTTGATTGCCCACCGACTCCT | |
| 18-ompX-F1 | AGTTCTTCTCCTTTACTCATAACCACCTCAAACGCGTTTTATTATTAAGTACA | Primers used to amplify the P15 ($P_{OmpX}$) promoter |
| 18-ompX-R1 | ACTCATTAGGCACCCCAGGCCCTTAACGGGCCTTTTTTGCGC | |
| 18-raiA-F1 | AGTTCTTCTCCTTTACTCATATAACTTACCTCTCTGTCTTTCCGTCTTGG | Primers used to amplify the P16 ($P_{RaiA}$) promoter |
| 18-raiA-R1 | ACTCATTAGGCACCCCAGGCTCGGCAACGGCAGAAAAACCA | |
| 18-rplJ-F1 | AGTTCTTCTCCTTTACTCATTAGCTTTTTGCTCCTGGATTAGCCG | Primers used to amplify the P17 ($P_{RplJ}$) promoter |
| 18-rplJ-R1 | ACTCATTAGGCACCCCAGGCTCGCACTTGCGATTATCGCTTTG | |
| 18-rpoB-F1 | AGTTCTTCTCCTTTACTCATAGGGTTCCTCAGCTCGCTGA | Primers used to amplify the P18 ($P_{RpoB}$) promoter |

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| 18-rpoB-R1 | ACTCATTAGGCACCCCAGGCGCCAACCTTTCCGGTTGCAG | |
| 18-rpoH-F1 | AGTTCTTCTCCTTTACTCATTCAAACCCTCTCTATGAGAATACAAAATCATGCAG | Primers used to amplify the P19 ($P_{RpoH}$) promoter |
| 18-rpoH-R1 | ACTCATTAGGCACCCCAGGCGATTTTTTTGGTATACTCTTCTCCTGCTGCT | |
| 18-rpsA-F1 | AGTTCTTCTCCTTTACTCATGTTGTTAATCTTCAGGGTTCTTTAGTTTAACGTCCA | Primers used to amplify the P20 ($P_{RpsA}$) promoter |
| 18-rpsA-R1 | ACTCATTAGGCACCCCAGGCGCGGCGCTATTGGCTTTTTGTC | |
| 18-rpsF-F1 | AGTTCTTCTCCTTTACTCATCGAATTGCTCCTTACGGATTATTCAGCC | Primers used to amplify the P21 ($P_{RpsF}$) promoter |
| 18-rpsF-R1 | ACTCATTAGGCACCCCAGGCAGTGCCTCTCTTACTTTTTGCCGT | |
| 18-rpsM-F1 | AGTTCTTCTCCTTTACTCATTATGCACTCCTACTATTTTATACAGCAACACCATTCT | Primers used to amplify the P22 ($P_{RpsM}$) promoter |
| 18-rpsM-R1 | ACTCATTAGGCACCCCAGGCTTATCTCGCATATTTTTCTTGCAAAGTTGGGTTGA | |
| 18-12140-F1 | AGTTCTTCTCCTTTACTCATGCGAACCTCCTTAAAAACGCCTG | Primers used to amplify the P23 ($P_{SMWW4\_v1c12140}$) |
| 18-12140-R1 | ACTCATTAGGCACCCCAGGCGGGACACCTCCAGAGGTGTTG | promoter |
| 18-29250-F1 | AGTTCTTCTCCTTTACTCATAGTGGCACCTTACAGTTTGTTTCACC | Primers used to amplify the P24 ($P_{SMWW4\_v1c29250}$) |
| 18-29250-R1 | ACTCATTAGGCACCCCAGGCAGCCGAAATGACGAGGCG | promoter |
| 18-sodB-F1 | AGTTCTTCTCCTTTACTCATTGCTTCCTCCTTTACAGCGCC | Primers used to amplify the P25 ($P_{SodB}$) promoter |
| 18-sodB-R1 | ACTCATTAGGCACCCCAGGCTTCCCTGTTCTGCGCGGT | |
| 18-tpiA-F1 | AGTTCTTCTCCTTTACTCATGTTTTTTCCTCCAACTAGGGAACGC | Primers used to amplify the P26 ($P_{TpiA}$) promoter |
| 18-tpiA-R1 | ACTCATTAGGCACCCCAGGCCGGCGCGGCATTTAATGAC | |

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|---|---|---|
| 18-trxA-F1 | AGTTCTTCTCCTTTACTCATGTTCTACTCCACAGGATTATGTCTACCTTGT | Primers used to amplify the P27 ($P_{TrxA}$) promoter |
| 18-trxA-R1 | ACTCATTAGGCACCCCAGGCAGTGTGGTAGAATATCAGCTAACTATTGCTTTACG | |
| 18-uspG-F1 | AGTTCTTCTCCTTTACTCATATCACGTCCTTTCTATGTAAACCGGG | Primers used to amplify the P28 ($P_{UspG}$) promoter |
| 18-uspG-R1 | ACTCATTAGGCACCCCAGGCTCTCGAGTAATAAATAATTAATCGCCTATCCGTTATCG | |
| 18-yabY-F1 | AGTTCTTCTCCTTTACTCATGGATCGGTCTCCTTTTTTATTATCAACATGTTGG | Primers used to amplify the P29 ($P_{YabY}$) promoter |
| 18-yabY-R1 | ACTCATTAGGCACCCCAGGCAAAGTTGTTACCTTTCTAATAATCATTTTGCGCAGT | |
| 18-yccA-F1 | AGTTCTTCTCCTTTACTCATAATGCTCTCTCTTATCAGGCCATCACAAAATAATC | Primers used to amplify the P30 ($P_{YccA}$) promoter |
| 18-yccA-R1 | ACTCATTAGGCACCCCAGGCGCAACGACGCGCACTATAAACAG | |
| 18-yfiD-F1 | AGTTCTTCTCCTTTACTCATTATGTTGCCTCCGTACAAGGGC | Primers used to amplify the P31 ($P_{YfiD}$) promoter |
| 18-yfiD-R1 | ACTCATTAGGCACCCCAGGCAAACGCTCTCCTTTGTTTTCTTTAGCAATTC | |
| 18-ygdI-F1 | AGTTCTTCTCCTTTACTCATTGAGGACTCCTTTGTAGTTTTAAATGCTGATAAATACC | Primers used to amplify the P32 ($P_{YgdI}$) promoter |
| 18-ygdI-R1 | ACTCATTAGGCACCCCAGGCACCCTGATTGTCACTGGCTTGAG | |
| 18-AmpR-F1 | AGTTCTTCTCCTTTACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGG | Primers used to amplify the AmpR promoter |
| 18-AmpR-R1 | ACTCATTAGGCACCCCAGGCCGCGGAACCCCTATTTGTTTATTTTTCTAAATAC | |
| eGFP-F1 | TGCCATGCCCGAAGGTTA | qPCR primers, coding region of gene *egfp* |
| eGFP-R1 | CGTGTCTTGTAGTTCCCGTCATC | |
| 16S rRNA-F1 | CACACCGCCCGTCACACCA | qPCR primers, coding region of gene 16S rRNA |

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| 16S rRNA-R1 | CGCAGGTTCCCCTACGGTTAC | |
| 18-PompR-ompR-F1 | AAAACGACGGCCAGTGCCAAGCTTTCATGCCTTGCTGCCGTCCG | Primers used to amplify the *ompR* gene under the |
| 18-PompR-ompR-R1 | TTCGAGCTCGGTACCCGGGGATCCCCCGACGGCTAAACGCCACC | control of its own promoter |
| 18-PpsrA-PsrA-F1 | GTAAAACGACGGCCAGTGCCAAGCTTTCAGGCAGAGTGATACTGTTCAGTAATCAG | Primers used to amplify the *psrA* gene under the |
| 18-PpsrA-PsrA-R1 | AATTCGAGCTCGGTACCCGGGGATCCGGGCCTTGGTGGTTCAACCAC | control of its own promoter |
| 18-PompRpsrA-ompRpsrA-F1 | GTAAAACGACGGCCAGTGCCAAGCTTTCATGCCTTGCTGCCGTCCG | Primers used to amplify the *psrA* and *ompR* genes |
| 18-PompRpsrA-ompRpsrA-R1 | AACAGTATCACTCTGCCTGACCCGACGGCTAAACGCCACC | under the control of their own promoters |
| 18-PompRpsrA-ompRpsrA-F2 | GGTGGCGTTTAGCCGTCGGGTCAGGCAGAGTGATACTGTTCAGTAATCAG | |
| 18-PompRpsrA-ompRpsrA-R2 | AATTCGAGCTCGGTACCCGGGGATCCGGGCCTTGGTGGTTCAACC | |
| 18-P17-ompR-F1 | GTAAAACGACGGCCAGTGCCAAGCTTTCATGCCTTGCTGCCGTCCG | Primers used to amplify the *ompR* gene under the |
| 18-P17-ompR-R1 | AATCCAGGAGCAAAAAGCTAATGCAAGAGAATCATAAGATCCTGGTTGTCGATG | control of the P17 promoter |
| 18-P17-ompR-F2 | ATCTTATGATTCTCTTGCATTAGCTTTTTGCTCCTGGATTAGCCGG | |
| 18-P17-ompR-R2 | AATTCGAGCTCGGTACCCGGGGATCCTCGCACTTGCGATTATCGCTTTG | |
| 18-P17-psrA-F1 | GTAAAACGACGGCCAGTGCCAAGCTTTCAGGCAGAGTGATACTGTTCAGTAATCAG | Primers used to amplify the *psrA* gene under the |
| 18-P17-psrA-R1 | AATCCAGGAGCAAAAAGCTAATGCCCGTAAATTTTGATCTCAACGATCT | control of the P17 promoter |
| 18-P17-psrA-F2 | AGATCAAAATTTACGGGCATTAGCTTTTTGCTCCTGGATTAGCCG | |
| 18-P17-psrA-R2 | AATTCGAGCTCGGTACCCGGGGATCCTCGCACTTGCGATTATCGCTTTG | |

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| 18-P17-ompRpsrA-F1 | GTAAAACGACGGCCAGTGCCAAGCTTTCAGGCAGAGTGATACTGTTCAGTAATCAG | Primers used to amplify the *ompR* and *psrA* gene |
| 18-P17-ompRpsrA-R1 | CGGACGGCAGCAAGGCATGAATGCCCGTAAATTTTGATCTCAACGATCT | under the control of the P17 promoter |
| 18-P17-ompRpsrA-F2 | AGATCAAAATTTACGGGCATTCATGCCTTGCTGCCGTCCG | |
| 18-P17-ompRpsrA-R2 | AATCCAGGAGCAAAAAGCTAATGCAAGAGAATCATAAGATCCTGGTTGTCGATGA | |
| 18-P17-ompRpsrA-F3 | ATCTTATGATTCTCTTGCATTAGCTTTTTGCTCCTGGATTAGCCG | |
| 18-P17-ompRpsrA-R3 | AATTCGAGCTCGGTACCCGGGGATCCTCGCACTTGCGATTATCGCTTTG | |

## Table S2. The 61 genes that highly expressed under all four conditions

| Gene | Average FPKM | Product |
|---|---|---|
| *ompA* | 21640.7025 | Outer membrane protein A |
| *hupA* | 9812.94 | DNA-binding transcriptional regulator, alpha subunit |
| *ompW* | 5126.42 | Outer membrane protein W |
| *ompN* | 10778.6525 | Outer membrane pore protein |
| *ybaY* | 8141.1775 | Outer membrane lipoprotein |
| *raiA* | 9965.2425 | Cold shock protein associated with 30S ribosomal subunit |
| *ompX* | 6679.55 | Outer membrane protein X |
| *ompC* | 4732.635 | Outer membrane porin protein C |
| *gapA* | 5062.2025 | Glyceraldehyde-3-phosphate dehydrogenase A |
| *cspC* | 8650.905 | Stress protein, member of the CspA-family |
| *SMWW4_v1c29250* | 2818.635 | Hypothetical protein |
| *ahpC* | 3612.45 | Alkyl hydroperoxide reductase, C22 subunit |
| *sodB* | 2468.105 | Superoxide dismutase |
| *lpp* | 8164.8725 | Murein lipoprotein |
| *gltA* | 2364.4775 | Citrate synthase |
| *pgk* | 2444.4925 | Phosphoglycerate kinase |
| *rplJ* | 5684.93 | 50S ribosomal subunit protein L10 |
| *rpsF* | 5404.0175 | 30S ribosomal subunit protein S6 |
| *rseA* | 2895.525 | Sigma-E factor negative regulatory protein RseA |
| *uspG* | 1753.1375 | Universal stress protein UP12 |
| *yfiD* | 3249.205 | Autonomous glycyl radical cofactor |
| *uspA* | 2927.7325 | Universal stress global response regulator |
| *SMWW4_v1c12140* | 1975.2125 | Hypothetical protein |
| *lpxC* | 2541.38 | UDP-3-O-acyl N-acetylglucosamine deacetylase |
| *rpsD* | 4388.445 | 30S ribosomal subunit protein S4 |
| *ftsZ* | 1703.47 | Cell division protein FtsZ |

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|---|---|---|
| *rpsM* | 5304.5475 | 30S ribosomal subunit protein S13 |
| *tpiA* | 2013.625 | Triosephosphate isomerase |
| *SMWW4_v1c16980* | 4148.5875 | CspA family cold shock transcriptional regulator |
| *rpoS* | 2251.38 | RNA polymerase, sigma S (sigma 38) factor |
| *rplF* | 3818.43 | 50S ribosomal subunit protein L6 |
| *rpsE* | 4551.18 | 30S ribosomal subunit protein S5 |
| *rpoA* | 2810.17 | RNA polymerase, alpha subunit |
| *rpsR* | 3253.605 | 30S ribosomal subunit protein S18 |
| *rpsA* | 2618.3375 | 30S ribosomal subunit protein S1 |
| *ygdI* | 2606.52 | Putative lipoprotein |
| *eno* | 1959.8025 | Enolase |
| *rplE* | 3278.4675 | 50S ribosomal subunit protein L5 |
| *fusA2* | 2736.9575 | Elongation factor G |
| *hupB* | 3855.9675 | DNA-binding protein HU-beta |
| *rplM* | 2622.89 | 50S ribosomal subunit protein L13 |
| *rplO* | 2843.77 | 50S ribosomal subunit protein L15 |
| *secY* | 2546.31 | Preprotein translocase membrane subunit |
| *ihfB* | 1780 | Integration host factor (IHF) |
| *rplC* | 2428.4275 | 50S ribosomal subunit protein L3 |
| *rpoH* | 1774.61 | RNA polymerase, sigma 32 (sigma H) factor |
| *pal* | 1621.7925 | peptidoglycan-associated outer membrane lipoprotein |
| *rpoB* | 1454.715 | RNA polymerase, beta subunit |
| *clpA* | 1163.19 | ATP-dependent serine protease |
| *rpsH* | 2558.6 | 30S ribosomal subunit protein S8 |
| *clpP* | 1144.5125 | ATP-dependent Clp protease |
| *skp* | 1289.2625 | periplasmic chaperone |
| *rpsJ* | 2430.1975 | 30S ribosomal subunit protein S10 |
| *trxA* | 1508.045 | thioredoxin 1 |
| *rpsG* | 1823.7575 | 30S ribosomal subunit protein S7 |

| | | |
|---|---|---|
| *hfq* | 1926.645 | Host factor for RNA phage Q beta replication |
| *arcA* | 1205.485 | DNA-binding response regulator |
| *yccA* | 1318.955 | HflBKC-binding inner membrane protein |
| *cspE* | 4084.85 | DNA-binding transcriptional repressor |
| *nlpI* | 1242.08 | Lipoprotein |
| *cpxP* | 1919.7225 | Cpx response inhibitor |