**Supplemental Table S1. Bacterial strains and plasmids**

**Bacterial strains used in this study**

| Name | Selection | Description | Reference |
|---|---|---|---|
| HI4320 | tet | Wild-type clinical isolate of *P. mirabilis* | 1 |
| Top 10 | | Routine *E. coli* host for cloning | Invitrogen |
| PMI0570::kan | kan | *sucB* targetron mutant | This study |
| PMI0641::kan | kan | *sanA* targetron mutant | This study |
| PMI0711::kan | kan | *serC* targetron mutant | This study |
| PMI1545::kan | kan | *guaA* targetron mutant | This study |
| PMI2870::kan | kan | PMI2870 targetron mutant | This study |
| PMI3210::kan | kan | *glpK* targetron mutant | This study |
| PMI3457::kan | kan | *argI* targetron mutant | This study |
| CFT073 | | Wild-type clinical isolate of *E. coli* | 2 |
| CFT073 *sucB*::Tn | kan | *sucB* transposon mutant from ordered library | 3 |
| CFT073 *sanA*::Tn | kan | *sanA* transposon mutant from ordered library | 3 |
| CFT073 *yicH*::Tn | kan | *PMI2870* transposon mutant from ordered library | 3 |
| CFT073 *glpK*::Tn | kan | *glpK* transposon mutant from ordered library | 3 |
| CFT073 *argI*::Tn | kan | *argI* transposon mutant from ordered library | 3 |

**Plasmids used in this study**

| Name | Selection | Description | Reference |
|---|---|---|---|
| pAR1219 | amp | T7 helper plasmid for targetron mutagenesis | 4 |
| pACD4K-CloxP-lacZ | chl | Targetron mutagenesis vector | Millipore Sigma |
| pSNS-2A1 | chl | pACD4K-CloxP with intron targeting PMI3457 *argI* | This study |
| pSNS-2A2 | chl | pACD4K-CloxP with intron targeting PMI0570 *sucB* | This study |
| pSNS-2A3 | chl | pACD4K-CloxP with intron targeting PMI2870 | This study |
| pSNS-2A4 | chl | pACD4K-CloxP with intron targeting PMI3210 *glpK* | This study |
| pSNS-2A5 | chl | pACD4K-CloxP with intron targeting PMI0641 *sanA* | This study |
| pSNS-2A7 | chl | pACD4K-CloxP with intron targeting PMI0711 *serC* | This study |
| pSNS-2A8 | chl | pACD4K-CloxP with intron targeting PMI1545 *guaA* | This study |
| pGEN-MCS | amp | Complementation plasmid; stably maintained without selection | 5 |
| guaA | amp | promoter | This study |
| pGEN-serC | amp | Complementation of *serC* under its native promoter | This study |
| pGEN-argI | amp | Complementation of *argI* under its native promoter | This study |

**References**


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3. Shea AE, Marzoa J, Himpsl SD, Smith SN, Zhao L, Tran L, Mobley HLT. *Escherichia coli* CFT073 fitness factors during urinary tract infection: identification using an ordered transposon library. Appl Environ Microbiol. 2020;86(13). Epub 2020/05/03. doi: 10.1128/AEM.00691-20. PubMed PMID: 32358013; PMCID: PMC7301846.

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5. Lane MC, Alteri CJ, Smith SN, Mobley HLT. Expression of flagella is coincident with uropathogenic *Escherichia coli* ascension to the upper urinary tract. Proc Natl Acad Sci U S A. 2007;104(42):16669-74.

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

| Name | Sequence | Purpose |
|---|---|---|
| CP-7 | CCAAGCAGAAGACGGCATACG | transposon outbound primer for confirmation of insertions |
| PMI0205 Rev | AGTTTGGTTGTCCCATCGTC | PCR confirmation of transposon insertion in PMI0205, paired with CP-7 |
| PMI0206 Rev | AGATGTGGGGATTGCTAACG | PCR confirmation of transposon insertion in PMI0206, paired with CP-7 |
| PMI0570 Rev | CGTCGTATCTTGTGCAGGTT | PCR confirmation of transposon insertion in PMI0570, paired with CP-7 |
| PMI0641 Rev | TCCATAACGATACGCGATGC | PCR confirmation of transposon insertion in PMI0641, paired with CP-7 |
| PMI0711 Rev | GCCACCATGACAGAACAGAA | PCR confirmation of transposon insertion in PMI0711, paired with CP-7 |
| PMI0765 Rev | TCAAAACGACCGAAACCAGT | PCR confirmation of transposon insertion in PMI0765, paired with CP-7 |
| PMI1151 Fwd | CCGCAATAAACCCTATCGGC | PCR confirmation of transposon insertion in PMI1151, paired with CP-7 |
| PMI1545 Fwd | GCCGGTATTAGGCATCTGCT | PCR confirmation of transposon insertion in PMI1545, paired with CP-7 |
| PMI1833 Fwd | ATTGCATGGCAGACCGAAGT | PCR confirmation of transposon insertion in PMI1833, paired with CP-7 |
| PMI2870 Rev | GTCTTTGTCTCGCTGTTCCA | PCR confirmation of transposon insertion in PMI2870, paired with CP-7 |
| PMI3180 Rev | TTTGTAAGCGGTTTTGCGTC | PCR confirmation of transposon insertion in PMI3180, paired with CP-7 |
| PMI3210 Rev | GGCACCACTTTAGGTAGCAT | PCR confirmation of transposon insertion in PMI3210, paired with CP-7 |
| PMI3301 Fwd | GTGAGCAGTCAACGACCTGT | PCR confirmation of transposon insertion in PMI3301, paired with CP-7 |
| PMI3316 Rev | CACCAAACGAAACGCAAGAA | PCR confirmation of transposon insertion in PMI3316, paired with CP-7 |
| PMI3457 Rev | AAGGATTTGCTAAGGTCGCC | PCR confirmation of transposon insertion in PMI3457, paired with CP-7 |
| PMI3528 Rev | GGTAGATGCTGGAACCCTTG | PCR confirmation of transposon insertion in PMI3528, paired with CP-7 |
| PMI0006 Fwd | AGCAGGTTGTGATCGCCTAA | PCR-confirmed lack of transposon insertion in PMI0006 |
| PMI0006 Rev | ATCGCAGGTAAAGAGAAGGC | PCR-confirmed lack of transposon insertion in PMI0006 |
| PMI3384 Fwd | CAAGACGGGAGGTTTACGCT | PCR-confirmed lack of transposon insertion in PMI3384 |
| PMI3384 Rev | ACCACGCTGTACTGAGCTAT | PCR-confirmed lack of transposon insertion in PMI3384 |
| PMI3431 Fwd | CGCGGAAAAGAACGGCTAATG | PCR-confirmed lack of transposon insertion in PMI3431 |
| PMI3431 Rev | GAGGGAATAGCGGTGTAAGC | PCR-confirmed lack of transposon insertion in PMI3431 |
| PMI3538 Fwd | CCATTTTTACCCGATGGGCG | PCR-confirmed lack of transposon insertion in PMI3538 |
| PMI3538 Rev | TCAACGTGTAACTCAGCCAC | PCR-confirmed lack of transposon insertion in PMI3538 |
| pACD4K-C-Gib-fwd | TAGGAGAACCTATGGGAAC | Amplification of targetron plasmid pACD4K-CloxP for Gibson cloning |
| pACD4K-C-Gib-rev | CTAGAGGGGAATTGTTATC | Amplification of targetron plasmid pACD4K-CloxP for Gibson cloning |
| intron-Gib-fwd | ggataacaattcccctctagAAAAAAGCTTATAATTATCCTTA | Amplification of targetron synthetic intron fragment for Gibson cloning |
| intron-Gib-rev | cgttcccataggttctcctaCAGATTGTACAAATGTGGTG | Amplification of targetron synthetic intron fragment for Gibson cloning |
| PMI0570-TTF | TTGTGTTAGAAGTACCGGCG | PCR confirmation of targetron insertion in PMI0570 *sucB* |
| PMI0570-TTR | GTCGGGCTAAGAGCATCATT | PCR confirmation of targetron insertion in PMI0570 *sucB* |
| PMI0641-TTF | GTTTGGGACAGATGGCTTTAC | PCR confirmation of targetron insertion in PMI0641 *sanA* |
| PMI0641-TTR | CTAGAAAACGCGGTTCACG | PCR confirmation of targetron insertion in PMI0641 *sanA* |
| PMI0711-TTF | TAGGTGAGAAAACCACCGC | PCR confirmation of targetron insertion in PMI0711 *serC* |

| | | |
|---|---|---|
| PMI0711-TTR | AGCAATCCCATCGATAGTCTC | PCR confirmation of targetron insertion in PMI0711 *serC* |
| PMI1545-TTF | CGAGAAAGGCTTGAAAACAGA | PCR confirmation of targetron insertion in PMI1545 *guaA* |
| PMI1545-TTR | TCTTCGGTAACATCCCATGC | PCR confirmation of targetron insertion in PMI1545 *guaA* |
| PMI2870-TTF | CAATGGGGTGCGCAAAAA | PCR confirmation of targetron insertion in PMI2870 |
| PMI2870-TTR | CAGATGAGACGCCGTAGTAA | PCR confirmation of targetron insertion in PMI2870 |
| PMI3210-TTF | TGAAACGACTATCGTCTGGG | PCR confirmation of targetron insertion in PMI3210 *glpK* |
| PMI3210-TTR | CGCCTTCAACATTATCGAGG | PCR confirmation of targetron insertion in PMI3210 *glpK* |
| PMI3457-TTF | AAAAGATTCCACCCGTACCC | PCR confirmation of targetron insertion in PMI3457 *argI* |
| PMI3457-TTR | TCAGCATATTTTGCCAGCAT | PCR confirmation of targetron insertion in PMI3457 *argI* |
| pGEN-GibsonF | AAGATCCCAACGAAAAGC | Amplification of complementation plasmid pGEN-MCS for Gibson cloning |
| pGEN-GibsonR | CAAGCTTCATATGCCCGG | Amplification of complementation plasmid pGEN-MCS for Gibson cloning |
| PguaBA-GibF | ccccgggcatatgaagcttgATCAATAAAAATTTTCAAAAAAAGCTAG | Amplification of *guaBA* promoter for Gibson cloning |
| PguaBA-GibR | ttgctgtcatagcaatatctcaccaagg | Amplification of *guaBA* promoter for Gibson cloning |
| guaA-GibF | agatattgctatgacagcaaatatccataatc | Amplification of *guaA* gene for Gibson cloning |
| guaA-GibR | acgcttttcgttgggatcttttaTTCCCACTCAATCGTTG | Amplification of *guaA* gene for Gibson cloning |
| serC_GibF | ccccgggcatatgaagcttgGTTTACAAAAGTCATTGCGCCAATTAG | Amplification of *serC* and promoter for Gibson cloning |
| serC_GibR | acgcttttcgttgggatcttttaAGCGTGGCGACGCTC | Amplification of *serC* and promoter for Gibson cloning |
| argI_GibF | ccccgggcatatgaagcttgCAAAAGTGACGTTTAAGG | Amplification of *argI* and promoter for Gibson cloning |
| argI_GibR | acgcttttcgttgggatcttttaAAAAGGATTTGCTAAGGTC | Amplification of *argI* and promoter for Gibson cloning |