|  |  |  |
| --- | --- | --- |
| **Table S2**. Strains, plasmids, and primers used in this study. |  |  |
|  |  |  |  |
| Strains | notes | reference |  |
| Strain CT6 | isolated from Caltech soils | This study |  |
| Strain CT7 | isolated from Caltech soils | This study |  |
| Strain EM5 | isolated from soils near Echo Mountain (Altadena, CA) | This study |  |
| Strain DKN1213 | isolated from Caltech soils | This study |  |
| Strain CT9 | isolated from Caltech soils | This study |  |
| Strain CT12 | isolated from Caltech soils | This study |  |
| Mycobacterium fortuitum ATCC 6841 |  | ATCC strain |  |
| Mycobacterium smegmatis Mc2-155 |  | (1) |  |
| Rhodococcus sp. JVH1 |  | (2) |  |
| Rhodococcus sp. RHA1 |  | (3) |  |
| Rhodococcus sp. JVH1 pSD5-14347 |  | This study |  |
| Rhodococcus sp. JVH1 pSD5-14352-30529 |  | This study |  |
| Pseudomonas aeruginosa PA14 |  | (4) |  |
| Pseudomonas fluorescens 2-79 |  | (5) |  |
| Escherichia coli DH5alpha |  | D. Lies, Caltech |  |
| Shewanella oneidensis MR1 |  | (6) |  |
| Staphylococcus aureus MN8 |  | (7) |  |
| Agrobacterium tumefaciens NT1 | pDCI41E33 | (8) |  |
| M. fortuitum 6841 pJV53 | WT M. fortuitum containing the recombineering vector pJV53 | This study |  |
| MFORT\_16269::GmR | Allele replacement of MFORT\_16269 with a gentamicin resistance cassette from pMQ30 | This study |  |
| MFORT\_16319::GmR | Allele replacement of MFORT\_16319 with a gentamicin resistance cassette from pMQ30 | This study |  |
| MFORT\_16334::GmR | Allele replacement of MFORT\_16334 with a gentamicin resistance cassette from pMQ30 | This study |  |
| MFORT\_16349::GmR | Allele replacement of MFORT\_16349 with a gentamicin resistance cassette from pMQ30 | This study |  |
| MFORT\_14352::GmR | Allele replacement of MFORT\_14352 with a gentamicin resistance cassette from pMQ30 | This study |  |
| MFORT\_14347::GmR | Allele replacement of MFORT\_14347 with a gentamicin resistance cassette from pMQ30 | This study |  |
| MFORT\_30529::GmR | Allele replacement of MFORT\_30529 with a gentamicin resistance cassette from pMQ30 | This study |  |
| MFORT\_16224::GmR | Allele replacement of MFORT\_16224 with a gentamicin resistance cassette from pMQ30 | This study |  |
| MFORT\_16214-16204::GmR | Allele replacement of MFORT\_16214-16204 with a gentamicin resistance cassette from pMQ30 | This study |  |
| MFORT\_14352-30529::GmR | Allele replacement of MFORT\_14352-30529 with a gentamicin resistance cassette from pMQ30 | This study |  |
| MFORT-∆40kb::GmR | Allele replacement of ~40kb region in ATCC6841 from genes MFORT\_16204 – MFORT\_14352 with a gentamicin resistance cassette from pMQ30 | This study |  |
|  |  |  |  |
| plasmids | notes | reference |  |
| pMQ30 | plasmid containing gentamicin resistance cassette | (9) |  |
| pJV53 | Recombineering vector | (10) |  |
| pSD5 | Complementation vector | (11) |  |
| pSD5-16269-16264 | pSD5 with the genes encoding MFORT\_16269 and 16264 placed into the NdeI and PstI sites | This study |  |
| pSD5-16319 | pSD5 with the gene encoding MFORT\_16319 placed into the NdeI and PstI sites | This study |  |
| pSD5-16334-16339 | pSD5 with the genes encoding MFORT\_16334 and 16339 placed into the NdeI and PstI sites | This study |  |
| pSD5-16349 | pSD5 with the gene encoding MFORT\_16349 placed into the NdeI and PstI sites | This study |  |
| pSD5-14347 | pSD5 with the gene encoding MFORT\_16347 placed into the NdeI and PstI sites | This study |  |
| pSD5-14352-30529 | pSD5 with the genes encoding MFORT\_14352, 14347 and 30529 placed into the NdeI and PstI sites | This study |  |
|  |  |  |  |
| Primers | sequence | cut site | notes |
| Gent-F-Xba | AAAATCTAGAGGTTCGTAAACTGTAATGCAAGTAGCGT | XbaI | Only used for MFORT\_16334 mutant |
| Gent-F2-Xba | AAAATCTAGACTAAATACATTCAAATATGTATCCGCTCATGAGA | XbaI | Used for all other mutants |
| Gent-R-Not | TTTTGCGGCCGCTTAGGTGGCGGTACTTGGGTCGATATCAAAGT | NotI | Used for all mutants |
| 16269-us-F | TGACGCCGGCGAGCTCCGCCAAGGCCA |  |  |
| 16269-us-R-Xba | AAAATCTAGATGATGCGGGCCGAACCGCCGAATCGCCAT | XbaI |  |
| 16269-ds-F-Not | AAAAGCGGCCGCCCATCGATGCCCCGGTCGGCCTGACCCGCGA | NotI |  |
| 16269-ds-R | AACAAGGTGGTGTGGGCCAGGTGAACTGT |  |  |
| 16319-us-F | GTTCGAAGAATGCCAATCGCGACCGCCGGA |  |  |
| 16319-us-R-Xba | AAAATCTAGAGAATTTACCTTCCCGGCCCGCCCGCGTCAGT | XbaI |  |
| 16319-ds-F-Not | AAAAGCGGCCGCTGCACCGAAGCCGTCTTCGCCGACGGGA | NotI |  |
| 16319-ds-R | CAGCCCGCCCCCGAGGATCACCACGGCA |  |  |
| 16334-us-F | CCGACCCGGCCGTCGATCTGCGCTCGAT |  |  |
| 16334-us-R-Xba | TTTTTCTAGATTCCCTTCAGTTCACATTCGAGGACTGTT | XbaI |  |
| 16334-ds-F-Not | AAAAGCGGCCGCGCGTCGGAGAGATGGTGCAACCCACCGA | NotI |  |
| 16334-ds-R | GGCGATCTTGAAGCCCGTGTCCACCACCCGCA |  |  |
| 16349-us-F | GCTCTACCGGCAGCCCCTGGCGCTGCA |  |  |
| 16349-us-R-Xba | AAAATCTAGAGGATGATCCTTCGGGAGCGCTGTGGGCA | XbaI |  |
| 16349-ds-F-Not | AAAAGCGGCCGCGTACCACGATGGAATCCGCGACACAGCCGT | NotI |  |
| 16349-ds-R | TCGGGCTCCTCCCCGTACGAGATACGTGT |  |  |
| 14352-us-F | GGTGTTCGCCTCCAACAGGGCGATCTCGA |  |  |
| 14352-us-R-Xba | AAAATCTAGAAAGTGCCTCCCGCTACGGCAAATCA | XbaI |  |
| 14352-ds-F-Not | AAAAGCGGCCGCCGACGGCGGGCGCGACGTTCAGT | NotI |  |
| 14352-ds-R | AGCATGTACCCGGTGACGCCCACGAT |  |  |
| 14347-us-F | CGAAACCGGCGATTATCGGTGGCGTCGTA |  |  |
| 14347-us-R-Xba | AAAATCTAGAGAAGTTCCGCCGACTGAACGTCGCGCCCGCCGTCGT | XbaI |  |
| 14347-ds-F-Not | AAAAGCGGCCGCAATCCGTTGTTTCTGAGCACTATCCGCT | NotI |  |
| 14347-ds-R | TGAGCCTGGTGACGGCCTCGGCATCGGT |  |  |
| 30529-us-F | TCGTCGACGCGCTGCTCACCCAGTCGA |  |  |
| 30529-us-R-Xba | AAAATCTAGAGCGAACCTCCCGGAAACCTCGAT | XbaI |  |
| 30529-ds-F-Not | AAAAGCGGCCGCAGCGCGACAAAGCCGGTCCTGGGCGGGT | NotI |  |
| 30529-ds-R | GGCAGGGAGCTGAGCGGACCGGGGATCA |  |  |
| 16224-us-F | GCAGCGCGACACGGTGCCCATGAAGTTGGT |  |  |
| 16224-us-R-Xba | AAAATCTAGATCCGTCTCCTCCAATGGTGGTGAT | XbaI |  |
| 16224-ds-F-Not | AAAAGCGGCCGCGTCAACTATCTCGGCGCGATCCCGGGCA | NotI |  |
| 16224-ds-R | CGCATCGCCGACATCGCCGAAGCGGCCGGA |  |  |
| fwd.Gent.hom50.6841Mfor | AAGCTATCGCAAGTCAACTTGCGATAGAAGGTGGGAAGGTCTAAATACATTCAAATATGT | none | 16204-16214 mutant primers – PCR stitched  |
| rev.Gent.hom50.6841Mfor | GACGTCAAGTGTTGACGGTCGACCGGTTCCGCAAGCTGGTTTAGGTGGCGGTACTTGGGT | none | 16204-16214 mutant primers – PCR stitched |
| up.Gen.fusion.6841Mfor | ATGAGCGGATACATATTTGAATGTATTTAGACCTTCCCACCTTCTATCGCAAGTTGACTT | none | 16204-16214 mutant primers – PCR stitched |
| dn.Gen.fusion.6841Mfor | TATCGACCCAAGTACCGCCACCTAAACCAGCTTGCGGAACCGGTCGACCGTCAACACTTG | none | 16204-16214 mutant primers – PCR stitched |
| UpFwd2.Mf6841.16204-14Loss | CAGAATTCGCGCCGGGCGGCCTCGACTT | none | 16204-16214 mutant primers – PCR stitched |
| DnRev2.Mf6841.16204-14Loss | GGGTTCGCTCCAGTCCAGTGGGCACCGA | none | 16204-16214 mutant primers – PCR stitched |
| 40kb region-us-F | CCCGTGTGAGTTAGGTAACCCTTACT |  |  |
| 40kb region-us-R-Not | TTTTGCGGCCGCGACGACCGTATATCCCTGATCTCTAGA | NotI |  |
| 40kb region-ds-F-Xba | AAAATCTAGAAAGTGCCTCCCGCTACGGCAAATCATTCA | XbaI |  |
| 40kb region-ds-R | GCGCGGTGAAACTCCGCCAACATGTCGA |  |  |
| CT6RpoA-1 | CGTTCGTACAACTGCCTCAA |  | used for qRT-PCR |
| CT6RpoA-2 | GGGTGTCATCCAGGTCGTAG |  | used for qRT-PCR |
| RT16730-1 | CATCGTCAACTGGAACTGGA |  | used for qRT-PCR |
| RT16730-2 | GTACTGCCAGTCCGTGTCCT |  | used for qRT-PCR |
| RT16830-1 | AGGACGTGATCCGTTGTACC |  | used for qRT-PCR |
| RT16830-2 | GTGCGCGAAATGGACTTTAC |  | used for qRT-PCR |
| RT16860-1 | AACAGATTTCCGCATCGTTC |  | used for qRT-PCR |
| RT16860-2 | GGACTCGGTCGTCATGAACT |  | used for qRT-PCR |
| RT16890-1 | ACGACAAGCTGCTGTACGTG |  | used for qRT-PCR |
| RT16890-2 | ACGAGTTCCATCCTCACCTG |  | used for qRT-PCR |
| RT12670-1 | CTATCACCAGCTGACCACCA |  | used for qRT-PCR |
| RT12670-2 | GACCAGCTCGATGGTCTTGT |  | used for qRT-PCR |
| RT20730-1 | AGCGAAAGTACGTGGCTGAT |  | used for qRT-PCR |
| RT20730-2 | TTGAAGGTGAACGGGAAGTT |  | used for qRT-PCR |
| 16269-64-NdeI | AAAACATATGGACCCGATATCGGAGTTTCTCCGTACCGCCGCT | NdeI | for expression on pSD5 |
| 16269-63-PstI | TTTTCTGCAGTCACAAGAAGATCCCCAGGTTCGCCGTCAACA | PstI | for expression on pSD5 |
| 16319-F-NdeI | AAAACATATGAATGAAGTTGTCGCACTGGGCTATCT | NdeI | for expression on pSD5 |
| 16319-R-PstI | AAAACTGCAGTCAGTCACGGTGATGCCCCCACAAACT | PstI | for expression on pSD5 |
| 16334-39-NdeI | AAAACATATGGACCCGATATCGGAGTTTCTCCGTACCGCCGCT | NdeI | for expression on pSD5 |
| 16334-39-PstI | TTTTCTGCAGTCACAAGAAGATCCCCAGGTTCGCCGTCAACAAGGTGGT | PstI | for expression on pSD5 |
| 16349-F-NdeI | AAAACATATGGCTGTAGTCACCCAATTGAGTTATGT | NdeI | for expression on pSD5 |
| 16349-R-PstI | AAAACTGCAGTCACACCATCCTCATCAATCCGTCATGACCCCA | PstI | for expression on pSD5 |
| 14352-F-NdeI | AAAACATATGACAGGAAAAACGAAACCGGCGATTATCGGT | NdeI | for expression on pSD5 |
| 30529-R-PstI | AAAACTGCAGTCAGAATCCGGGTGGGCGCCGAAAACCACCGA | PstI | for expression on pSD5 |
| 14347-F-NdeI | AAAACATATGGTCGGTGGCGGGCTGGCTCTCGGCGCGGT | NdeI | for expression on pSD5 |
| 14347-R-PstI | AAAACTGCAGTCACTCCTGGGCGACCGGGTGGTTGCTCCGGA | PstI | for expression on pSD5 |

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