List of primers used in this study

|  |  |  |
| --- | --- | --- |
| **Primer number** | **Primer name** | **Sequence** |
| 6395 | ponA\_check\_FP | CAAGACCTCTTTCCCCCTGC |
| 6396 | ponA\_check\_RP | CTGCACGGAATTACAAGGCG |
| 6389 | pbpD\_check\_FP | CGGTTTGCTGCCATGCATTA |
| 6390 | pbpD\_check\_RP | AGGGGAGGGGAGCATACATT |
| 6391 | pbpF\_check\_FP | CCGTTTCCCACAAGGTCAGA |
| 6392 | pbpF\_check\_RP | AGGATTGCAGCATCGACTCC |
| 6393 | pbpG\_check\_FP | TGCGCCACCATTTCGTGATA |
| 6394 | pbpG\_check\_RP | AAAACGAGCCTGCTTCCAGA |
| 7289 | ecsA\_check\_FP | TGATGTCCAGAACCCTGTCTC |
| 7290 | ecsA\_check\_RP | CCATCACCCAAAAGGACGGA |
| 7291 | ytxG\_check\_FP | AGCCGTTCTCATCTTCATGGG |
| 7292 | ytxG\_check\_RP | CACCGATGATTCCCCCGATT |
| 7692 | rasP\_check\_FP | AGTAGCTGTCGCTGCCTTTT |
| 7693 | rasP\_check\_RP | CGGAGCGTAGGAATAAGCGT |
| 7545 | sigW\_check\_FP | ATCGTCTAGAACTTTGACTCCGTCATGCGT |
| 8670 | sigW\_check\_RP | AAGATTCGGCTGCTTGGACA |
| 8671 | sigV\_check\_FP | TATTTTTCAGCCTGCCGCGT |
| 8672 | sigV\_check\_RP | TCGCTTTGACGATTTTGCCG |
| 6954 | sigI\_check\_FP | ATCTCTAGAAAGAGACGAAGCAAAGCGGT |
| 6957 | sigI\_check\_RP | ATCGGTACCCGGATGGTTTGCTGGAGTCA |
| 8664 | ftsL\_check\_FP | ACGGCCAACGAGGCCAGGCGTTACAGAAGTAGACGGCA |
| 8669 | ftsL\_check\_RP | AAGGCCTTATTGGCCTGCGCCACTTTTGTCATGCT |
| 8666 | ftsL\_seq\_check | AAAAGGAGGTCATCAGCCTATG |
| 7074 | rsgI\_check\_FP | AGCTTCCGGTCAAACAGCTT |
| 7075 | rsgI\_check\_RP | GTTCTGATACAGTCGCACGC |
| VP136 | rsiW\_check\_FP | TAAACATTCCTGTGGGGACT |
| VP137 | rsiW\_check\_RP | CATTTCGATTTAGCAAGTGA |
| 6588 | sigM\_check\_FP | CCATTGTGCCACTCCTTCAC |
| 6589 | sigM\_check\_RP | TGCAGTCATTTCCTGGTCGC |
| 9126 | mreBH\_check\_FP | AGAAATGCTCATGCCTCCAAGA |
| 9127 | mreBH\_check\_RP | TCATCTACTTTTCTCACAACACCT |
| 8495 | lytE\_check\_FP | CGATATATGAGCCGACCCCG |
| 8496 | lytE\_check\_RP | TAGGAGCAACCAAAACGCCT |
| 6976 | gsiB\_check\_FP | TCAAACAGGCGGACAGTACC |
| 6979 | gsiB\_check\_RP | ATGCCTTCAGAACCGTCCAG |
| 6851 | fabI\_check\_FP | CAACTTTGTCCGTGAAGCGG |
| 6852 | fabI\_check\_RP | CTCCTGCTGCTTCTCATGGT |
| 6048 | bcrC\_check\_FP | ACTTAACGATGCACGGGGAA |
| 6051 | bcrC\_check\_RP | AGTGAAGACAGCGGAAACCA |
| 8802 | walK\_check\_FP | AAGGCCAACGAGGCCATGCCGCGAAGTCAGAAAGA |
| 8803 | walK\_check\_RP | AAGGCCTTATTGGCCCTTTCACATCCCAATGCGGC |
| 8810 | walK\_seq\_check | AGGCGATGGAGCTTGCGAAA |
| 7088 | qRT\_mreBH\_FP | ATGGCAGGAAACAACGCCAC |
| 7089 | qRT\_mreBH\_RP | GCCGGTTGCCAATGTGGTAG |
| 7086 | qRT\_lytE\_FP | CATTGCCAGCCCGTCTGTTG |
| 7087 | qRT\_lytE\_RP | TGTACGCCGTCAGATCCTGC |
| 8726 | qRT\_gyrA\_FP | GGCGGCCATGCGTTATACAG |
| 8727 | qRT\_gyrA\_RP | GCCATACCTACCGCAATGCC |
| 7848 | ecsA-HindIII-F  | ATCGAAGCTTACACTATAAGGGGAGAAACTATGTC |
| 7849 | ecsA-XbaI-R  | ATCGTCTAGACCGCGACTGCCAAATATCAAG |
| 7850 | ecsAB-XbaI-R  | ATCGTCTAGAGCAGCTGATTATCTGTCATTTGAT |
| 7851 | ytxG-HindIII-F  |  ATCGAAGCTTATCCTGCAGCAAGGAGGTTT |
| 7852 | ytxG-XbaI-R  |  ATCGTCTAGATGCTCATCATTATTCCTCCTCTT |
| 8499 | RasP-XmaI-RBS-F   | ATCGCCCGGGTAAGGAGGCAAAATCATGTTCGTGAATACAGTTATAGCGTT |
| 8500 | RasP-SphI-R   | ATCGGCATGCGGAGCGTAGGAATAAGCGT |
| 8662 | ftsL-gRNA-F   | TACGTTGACAATCACTTTTTTCTC |
| 8663 | ftsL-gRNA-R   | AAACGAGAAAAAAGTGATTGTCAA |
| 8664 | CRISPR-ftsL-up-F   | ACGGCCAACGAGGCCAGGCGTTACAGAAGTAGACGGCA |
| 8665 | CRISPR-ftsL-up-R   | CATAGGCTGATGACCTCCTTTT |
| 8666 | ftsL-mid-F   | AAAAGGAGGTCATCAGCCTATG |
| 8667 | ftsL-mid-R   | ATTTATTCTTTTTTGGCATTTGAATCATGCATAATCAGGAACATCATAAGGATA |
| 8668 | CRISPR-ftsL-down-F   | TATCCTTATGATGTTCCTGATTATGCATGATTCAAATGCCAAAAAAGAATAAAT |
| 8669 | CRISPR-ftsL-downR   | AAGGCCTTATTGGCCTGCGCCACTTTTGTCATGCT |
| 8519 | gBlock-ftsL-delta25-HA  | TTAAAAGGAGGTCATCAGCCTATGAAAAGGGCTTCCATTACTCTCGGAGAAAAAGTGCTTCTTGTCCTCTTTGCTGCGGCGGTGCTCAGCGTATCGCTTTTGATCGTATCGAAGGCGTATGCGGCATATCAAACCAATATTGAGGTGCAAAAGCTTGAGGAGCAAATTTCATCCGAAAATAAGCAAATTGGTGACCTCGAAAAAAGCGTTGCTGATTTAAGCAAACCGCAGCGCATTATGGACATTGCGAAAAAGAACGGCTTGAACCTTAAAGATAAGAAAGTGAAAAACATACAGGAAGGATCAGGCCAAGGACCGGGAAGCGGACAAGGCCCTTATCCTTATGATGTTCCTGATTATGCTTGATAA |
| 9115 | sigI-HindIII-FP | CCCAAGCTTTCCCTATCAAATTAGCTATCA |
| 9116 | sigI-Xba1-RP | CTAGTCTAGATCTACTATAATCCCTCTTCTCA |
| 6461 | PM-up-for(HindIII) | GCGAAGCTTTGAGCCTTCGTCCTTTAGTGAC |
| 6457 | PM-up-rev | TTTTGCCAACCATTG**A**AC**A**TTTTCTTCTGGTGAAAAGTTTCGATT |
| 6458 | PM-do-for | AAATGTTCAATGGTTGGCAAAA |
| 6462 | PM-do-rev(BamHI) | GCGGGATCCTCATTGTTTTTGATATAGGAAG |
| 6485 | maf-for(SalI) | GCGGTCGACAGTATCTTTTTTTCTTAAGCAA |
| 6486 | maf-rev(AsiSI) | GCGGCGATCGCTCTTTCCCTTCCCGTTTATAGA |
| 7426 | Delta-Pm-rodA-up-F  | AAGGCCAACGAGGCCTCTGCTGAACACAGTCACTT |
| 7427 | Delta-Pm-rodA-up-R | CGCTTTTTCAGCTACACGAAATGCGATAATGTGTTATGTTCCC |
| 7428 | Delta-Pm-rodA-down-F | GGGAACATAACACATTATCGCATTTCGTGTAGCTGAAAAAGCG |
| 7429 | Delta-Pm-rodA-down-R | AAGGCCTTATTGGCCCTCATTTGAAGCAGACACCC |
| 7430 | ProdA-deltaPm-gRNA-F | TACGCGTTTTTTAACAAATTCTAT |
| 7431 | ProdA-deltaPm-gRNA-R | AAACATAGAATTTGTTAAAAAACG |
| VP475 | SfiI PM ponA up for  | AAGGCCAACGAGGCCGTCTCTCCCGGTTAACATTCTTTGCTCT |
| VP476 | SfiI pm ponA down rev  | AAGGCCTTATTGGCCCGCCCTTTGTAAATCCCATTGTAGTCT |
| VP477 | Int repair frag F1  | CCAATCTG TTTTCGTTCC**A**TCTAATGAGTGG |
| VP478 | Int repair R1  | CCACTCATTAGATGGAACGAAAACAGATTGG |
| VP479 | Int repair F2  | ACGAAGGATTTTGTATAATAGGAGTCTGAATAGTGTTTTGTTCA |
| VP480 | Int repair R2 | TGAACAAAACACTATTCAGACTCCTATTATACAAAATCCTTCGT |
| VP481 | Int repair F3  | ACTGCCTTTTAAGTCTGTCATTGACCAGCC |
| VP482 | Int repair R3  | GGCTGGTCAATGACAGACTTAAAAGGCAGT |
| 9113 | MreBH\_FP\_HindIII | CCCAAGCTTtAAGGAGGTGAACATGTTTCAATCAACTG |
| 9114 | MreBH\_RP\_XbaI | CTAGTCTAGAAGAAGCAAGAAATTTGCC |
| 9140 | lytE-SmaI-FP | TCCCCCGGGAGGAGGAAAATATGAAAAAGCAAA |
| 9141 | lytE-XbaI-RP | CTAGTCTAGACAATGAACGGGTTTTCTCTAAA |
| 9130 | lacZ-check-FP | AGTCGTTTGCCGTCTGAATTTG |
| 9131 | lacZ-check-RP | TTTTGATGGACCATTTCGGCAC |
| 9132 | lacI-check-FP | TGACGGCGGGATATAACATGAG |
| 9133 | lacI-check-RP | ATGCCGGTGTCTCTTATCAGAC |
| 7082 | F-pSpachy-check | GCAACGCCAATCAGCAACGA |
| 7083 | R-pSpachy-check | AGACCTCACTAGGCACCTTA |
| 9144 | mreBH-EcorI-FP | CCGGAATTCCGGAAGGAGGTGAACATGTTTCAATCAACTG |
| 9145 | mreBH-SpeI-RP  | GGACTAGTCCAAGAAGCAAGAAATTTGCC |
| 9231 | xylR-CDS-check-FP  | TTCCAAATGGCGGTATTGAT |
| 9230 | mreBH-middle-check-RP  | CATGGCCAATTTCCATTTTT  |
| 8802 | WalK-Up-F  | AAGGCCAACGAGGCCATGCCGCGAAGTCAGAAAGA |
| 8803 | WalK-Down-R  | AAGGCCTTATTGGCCCTTTCACATCCCAATGCGGC |
| 8806 | WalK-Asp274Ala-up-R  | TAATCGCTCCGTTTCGGTTTGTAGCGATAACGCCAGCTGTCATATAAGCAATGACAGAGG |
| 8807 | WalK-Asp274Ala-down-F  | CCTCTGTCATTGCTTATATGACAGCTGGCGTTATCGCTACAAACCGAAACGGAGCGATTA |
| 8814 | sgRNA-walK-Asp274Ala-F  | TACGAATCGCTCCGTTTCGGTTTG |
| 8815 | sgRNA-walK-Asp274Ala-R  | AAACCAAACCGAAACGGAGCGATT |
| **Primers used for TnSeq** |   |   |
|   | Adapterbarcode501A | TTCCCTACACGACGCTCTTCCGATCTTATAGCCTNN |
|   | Adapterbarcode501B | AGGCTATAAGATCGGAAGAGCGTCGTGTAGGGAAAGAG |
|   | Adapterbarcode502A | TTCCCTACACGACGCTCTTCCGATCTATAGAGGCNN |
|   | Adapterbarcode502B | GCCTCTATAGATCGGAAGAGCGTCGTGTAGGGAAAGAG |
|   | Adapterbarcode503A | TTCCCTACACGACGCTCTTCCGATCTCCTATCCTNN |
|   | Adapterbarcode503B | AGGATAGGAGATCGGAAGAGCGTCGTGTAGGGAAAGAG |
|   | Adapterbarcode504A | TTCCCTACACGACGCTCTTCCGATCTGGCTCTGANN |
|   | Adapterbarcode504B | TCAGAGCCAGATCGGAAGAGCGTCGTGTAGGGAAAGAG |
|   | Adapterbarcode505A | TTCCCTACACGACGCTCTTCCGATCTAGGCGAAGNN |
|   | Adapterbarcode505B | CTTCGCCTAGATCGGAAGAGCGTCGTGTAGGGAAAGAG |
|   | Adapterbarcode506A | TTCCCTACACGACGCTCTTCCGATCTTAATCTTANN |
|   | Adapterbarcode506B | TAAGATTAAGATCGGAAGAGCGTCGTGTAGGGAAAGAG |
|   | Adapterbarcode507A | TTCCCTACACGACGCTCTTCCGATCTCAGGACGTNN |
|   | Adapterbarcode507B | ACGTCCTGAGATCGGAAGAGCGTCGTGTAGGGAAAGAG |
|   | Adapterbarcode508A | TTCCCTACACGACGCTCTTCCGATCTGTACTGACNN |
|   | Adapterbarcode508B | GTCAGTACAGATCGGAAGAGCGTCGTGTAGGGAAAGAG |
|   | oKrmit-Tnseq2 | CAAGCAGAAGACGGCATACGAAGCGCCTACGAGGAATTTGTATCG |
|   | oAdapterPCR | AATGATACGGCGACCACCGAGATCACACTCTTTCCCTACACGACGCTCTTCC |