**Supplementary File 5. Primers used in this study for generating complementation plasmids and empty vector.**

|  |  |  |  |
| --- | --- | --- | --- |
| Deletion | PCR Fragment Generated | Primer Name | Sequence (5’ to 3’) |
| SagA | homology arm 1 | oSK025 | TTTTGTTTAACTTTAAGAAGGAGATATACAAACTCCATGCATTAGTGGTGAC |
| oSK026 | ATCTATCTTGAGTTTCAATCTATTTTTTTTTCTTCATTCCTCCGACTGGC |
| cmR cassette | oSK008 | TAAATTTAACGATCACTCAAAAAATTATAAAAGCCAGTC |
| oSK009 | AAAAAAAATAGATTGAAACTCAAGATAGATATGTTATTG |
| homology arm 2 | oSK027 | TTTATAATTTTTTGAGTGATCGTTAAATTTAGAATGATTAATCAAAAAGAGTTAGTCCAG |
| oSK028 | GCCGGATCTCAGTGGTGGTGGTGGTGGTGCGTAAGGAAAAAATGGAATGGCAG |
| complete deletion construct | oSK041 | AACTCCATGCATTAGTGGTGAC |
| oSK042 | GTAAGGAAAAAATGGAATGGCAG |
| verification of deletion | oSK368 | CTTTCTAACTATGAACTTCATGGC |
| oSK369 | TCCAGTACGGGTATCTTC |

|  |  |  |  |
| --- | --- | --- | --- |
| Purpose | Fragment | Primer Name | Sequence (5’ to 3’) |
| generating pSK060 by swapping cmR for ermR in p*sagA* using two-fragment Gibson assembly | p*sagA* backbone | oSK406 | TTACTTCCAAAACCTAAATTCACGTTG |
| oSK407 | TAAATTATTTAATAAGTAATTTTTAGATTTTGAAAGTGAATTTAATTTTATACACGTAAG |
| ermR cassette | oSK408 | GATTTTTGGCAACGTGAATTTAGGTTTTGGAAGTAACATGTTCATATTTATCAGAGCTCG |
| oSK409 | TTCAAAATCTAAAAATTACTTATTAAATAATTTATAGCTATTGAAAAGAGATAAGAATTG |
| generating pSK069 by removing *sagA* from p*sagA* using Q5-SDM (NEB) | linearized pSK069 plasmid | oSK475 | GTCGACCGATGCCCTTGAGAGC |
| oSK476 | AGGATCCACAGGACGGGTGTG |
| generating pSK104 using two-fragment Gibson assembly | pSK060 backbone | oSK562 | TATGTTTGGGGCGGTAAAGATC |
| oSK563 | AGTGTAGGCCCTAAGAACCTTG |
| alternate *sagA* sequence | oSK564 | TTTTATTACAAGGTTCTTAGGGCCTACACT |
| oSK565 | TCCACTTGGATCTTTACCGCCCCAAACATA |

Mutagenesis of the *sagA* gene in p*sagA* was accomplished using the Q5 Site-Directed Mutagenesis kit (NEB), according to the manufacturer’s instructions. The primers used for mutagenesis are reported in **Supplementary File 6**.