**Supplementary File 3. List of Plasmids and Primers used in the study**

|  |  |  |  |
| --- | --- | --- | --- |
| **Construct Plasmid** | **Relevant genotype** | **Ori** | **Source of Reference** |
| pBOMBL | *bla* P*tet*::*mCherry* P*Nm*::*gfp* | pUC19 | (1, 2) |
| pBOMBL-spcr | *aadA Ptet::mCherry* P*Nm*::*gfp* | pUC19 | This study |
| pBOMBL-*dacA*\_6xH | *bla* P*tet*::Ctr\_*dacA \_6xH* P*Nm*::*gfp* | pUC19 | This study |
| pBOMBL- *dacA* | *bla* P*tet*::Ctr\_*dacA* P*Nm*::*gfp* | pUC19 | This study |
| pBOMBL- *dacA*(D164N) | *bla* P*tet*::Ctr\_*dacA*(D164N)P*Nm*::*gfp* | pUC19 | This study |
| pBOMBL- *ybbR*\_6xH | *bla* P*tet*::Ctr\_ *ybbR*\_6xH P*Nm*::*gfp* | pUC19 | This study |
| pBOMBL-*dacA-ybbR*\_6xH | *bla* P*tet*::Ctr\_*dacA-ybbR*\_6xH P*Nm*::*gfp* | pUC19 | This study |
| pBOMBL-spcr-*dacA-ybbR*\_6xH | *aadA Ptet::*Ctr*\_dacA-ybbR\_*6xHP*Nm*::*gfp* | pUC19 | This study |
| pBOMBL-*dacA(D164N)-ybbR*\_6xH | *bla* P*tet*::Ctr\_*dacA*(D164N)*-ybbR\_6xH* P*Nm*::*gfp* | pUC19 | This study |
| pL12CRia(*dacA*-IGR) | *bla* P*tet*::*ddAsCpf1*vaa P*dnaKmut*::As\_crRNA (*dacA*)P*Nm*::*gfp* | pUC19 | This study |
| pL12CRia(*dacA*-IGR)-*dacA-ybbR*\_6xH | *bla* P*tet*::*ddAsCpf1vaa*-*dacA*-*ybbR*\_6xH P*dnaKmut*::As\_crRNA (*dacA*) P*Nm*::*gfp* | pUC19 | This study |
| pBOMBLv2-ΔTM*dacA* | *aadA Ptet::*Ctr*\_*ΔTM*dacA* P*Nm*::*gfp* | pUC19 | This study |
| pBOMBLv2-ΔTM*dacA*(D164N) | *aadA Ptet::*Ctr*\_*ΔTM*dacA*(D164N)P*Nm*::*gfp* | pUC19 | This study |

|  |  |  |  |
| --- | --- | --- | --- |
| **Primer name** | **Sequence** | **Features** | **Usage** |
| dacA/(pBOMBL)/F | aaagatcttcacacaggacatctgcATGTTCGTAGGTATAACGTATTACAC | lower case for plasmid overlap construction | for amplification of *dacA+/-6xH and dacA-ybbR\_6xH* |
| dacA\_6xH/(pBOMB)/R | acatatttgaatggtcgaccggtacttaatggtgatggtgatggtgTTTTTTACGCATCCAGGAG | lower case for plasmid overlap construction and lower case with underline for 6xHis tag | for amplification of *dacA\_6xH* |
| dacA/pBOMBL/R | tttgaatggtcgaccggtacTCATTTTTTACGCATCCAGG | lower case for plasmid overlap construction | for amplification of *dacA* without a tag |
| ybbR/(pBOMBL)/F | aaagatcttcacacaggacatctgcATGATCAATTTCGTCTCTTG | lower case for plasmid overlap construction | for amplification of *ybbR\_6xH* |
| ybbR\_6xH/(pBOMB)/R | acatatttgaatggtcgaccggtacttaatggtgatggtgatggtgGGAAGATTTTTTCTTTAGAGAGG | lower case for plasmid overlap construction and 6xHis tag | for amplification of *ybbR\_6xH and dacA-ybbR\_6xH* |
| dacA/(dCas12)/F | cgtagctgcttaagtaccgg*aggagaatctgc*ATGTTCGTAGGTATAACGTATTAC | lower case for plasmid overlap construction, lower case and underlined with italics for RBS site | For amplification of *dacA-ybbR\_6xH* to complement *dacA*-KD |
| dacA87/(pBOMBL)/F | tcttcacacaggacatctgcATGTCTAGGATACGCTTGC | lower case for plasmid overlap construction | for amplification of ΔTM*dacA* |
| ct012\_dacA qPCR\_F | CGCTTGCGTAGAGGGAAAT | qPCR primer | Amplification of *dacA* |
| ct012\_dacA qPCR\_R | AGCTCCGATTTGTCGTTCAG | qPCR primer | Amplification of *dacA* |
| ct011\_ybbR qPCR\_F | GCTTCGCCCTTCCAATCT | qPCR primer | Amplification of *ybbR* |
| ct011\_ybbR qPCR\_R | GATGTCTGTGTCCAAGCATACTA | qPCR primer | Amplification of *ybbR* |
| hctA qPCR F | AAGCTAAAGCTGCTGCTAAGA | qPCR primer | Amplification of *hctA* |
| hctA qPCR R | GTTGGTTTGACCTTTGCTTTAGT | qPCR primer | Amplification of *hctA* |
| omcB qPCR F | CGGTAGGATCTCCCTATCCTATT | qPCR primer | Amplification of *omcB359-457nt* |
| omcB qPCR R | CGAACTCTGCTTCACATGGTA | qPCR primer | Amplification of *omcB359-457nt* |
| euo qPCR F | CGAAGACTACTCGTTGGGAAATA | qPCR primer | Amplification of *euo179-278nt* |
| euo qPCR R | AACAGAAGCTCTCCTTGATAAGT | qPCR primer | Amplification of *euo179-278nt* |

|  |  |  |  |
| --- | --- | --- | --- |
| **gBlock name** | **Sequence** | **Features** | **Usage** |
| *dacA* crRNA  | tgtgaaagtgggtcttaagacgtcggtactgcatgtgacgcacgtagatcatgca*TTCACCGGTGGAGACGGTTTTCTTATAATGACACC*TAATTTCTACTCTTGTAGAT**CTGAAAATAATGAAAACACTT**CAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATcaacagcggtctactgaatctgagctagtgcgtgatataattaaaattatattca | Lower case for plasmid overlap and spacer, *italicized* for PdnaKmut promoter sequence, underlined for crRNA scaffold, **bold** for *dacA* targeting sequence, Upper case for rrnB1 terminator | For CRISPRi knockdown of *dacA* operon |

1. Bauler LD, Hackstadt T.2014. Expression and targeting of secreted proteins from Chlamydia trachomatis. J Bacteriol 196:1325-34.

2. Ouellette SP, Blay EA, Hatch ND, Fisher-Marvin LA.2021. CRISPR Interference To Inducibly Repress Gene Expression in Chlamydia trachomatis. Infect Immun 89:e0010821.