**Supplementary File 4: Primers, plasmids and strains**

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| gene expressions from pSEVA  |
| plasmid in strain TOP10 | name | primers used for gene amplification |
| M027 | pSEVA238 | MCSSEVA238-5 GCAAGAAGCGGATACAGGAGMCSSEVA238-3 GGTTTTCCCAGTCACGACGC |
| R591 | pSEVA-tgt | 5tgtEcoRI CGCGGAATTCGTGAAATTAAAATTTGAACTG and 3tgtXbaI CGCGTCTAGATCAGGCTTTGTCTTTTTGTAGTGG |
| Q298 | pSEVA-rsxA | ZIP753 GCCCGAATTCCATTTGCCACTTATTGCG and ZIP754 GCCGCCGAATTCTTACAGTTTCACCAATCCGGTAAAGCC |
| Q294 | pSEVA-soxR | ZIP757 GGGGCCCCCCCCGAATTCGTAAAGCGTTTTTTTAATAAAACGGG and ZIP758 CCGAATTCTTAACGGCTCCACTCTTCTGGATGCGATAAGCG |
| Q291 | pSEVA-katG | ZIP755 GCGCGCCCCGAATTCATTTCCCATTTAGTGAAAAGGG and ZIP756 GAATTCTTACATCGCGGCCAGTTTTGCCACC |
| tRNA overexpression fragments cloned in pTOPO-blunt-kanamycin R (Ptrc promoter and VCt002 terminator are underlined, anticodon in red) |
| plasmid in TOP10 | name | sequence |
| L961 | Ptrc-eco-tRNA-Tyr wtGUA | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGGGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACTGTAAATCTGCCGTCACAGACTTCGAAGGTTCGAATCCTTCCCCCACCACCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| M660 | Ptrc-eco-tRNA-Tyr mutAUA | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGGGTGGGGTTCCCGAGCGGCCAAAGGGAGCAGACTATAAATCTGCCGTCACAGACTTCGAAGGTTCGAATCCTTCCCCCACCACCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| L957 | Ptrc-eco-tRNA-Asp wt GUC | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGGGAGCGGTAGTTCAGTCGGTTAGAATACCTGCCTGTCACGCAGGGGGTCGCGGGTTCGAGTCCCGTCCGTTCCGCCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| L960 | Ptrc-vch-tRNA-Tyr wtGUA | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGGGAGGGGTTCCCGAGTGGCCAAAGGGAGCAGACTGTAAATCTGCCGGCTCCGCCTTCGATGGTTCGAATCCGTCCCCCTCCACCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| L959 | Ptrc-vch-tRNA-Tyr mutAUA | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGGGAGGGGTTCCCGAGTGGCCAAAGGGAGCAGACTATAAATCTGCCGGCTCCGCCTTCGATGGTTCGAATCCGTCCCCCTCCACCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| L956 | Ptrc-vch-tRNA-Asp wtGUC | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGGGAGCGGTAGTTCAGTCGGTTAGAATACCGGCCTGTCACGCCGGGGGTCGCGGGTTCGAGTCCCGTCCGCTCCGCCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| L955 | Ptrc-vch-tRNA- mutAspAUC | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGGGAGCGGTAGTTCAGTCGGTTAGAATACCGGCCTATCACGCCGGGGGTCGCGGGTTCGAGTCCCGTCCGCTCCGCCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| M651 | Ptrc-vch-tRNA-Asn wtGUU  | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGTCCTCCTTAGCTCAGTCGGTAGAGCGACGGACTGTTAATCCGCAGGTCGCTGGTTCAAGTCCAGCAGGAGGAGCCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| L962 | Ptrc-vch-tRNA-Asn mutAUU | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGTCCTCCTTAGCTCAGTCGGTAGAGCGACGGACTATTAATCCGCAGGTCGCTGGTTCAAGTCCAGCAGGAGGAGCCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| M646 | Ptrc-vch-tRNA-HIS wtGUG | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGGTGGCTATAGCTCAGTTGGTAGAGCCCCGGATTGTGATTCCGGTTGTCGTGGGTTCGAGCCCCATTAGCCACCCCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| L997 | Ptrc-vch-tRNA-HIS mutAUG | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGGTGGCTATAGCTCAGTTGGTAGAGCCCCGGATTATGATTCCGGTTGTCGTGGGTTCGAGCCCCATTAGCCACCCCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| M653 | Ptrc-vch-tRNA-Phe wtGAA | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGGCCCGGATAGCTCAGTCGGTAGAGCAGAGGATTGAAAATCCTCGTGTCGGTGGTTCGATTCCGCCTCCGGGCACCACTTATTCGAGCTTAAGCTCAAAAAACTACA |
| translational fusions ordered in pUC\_IDT (carbenicillin R) |
| plasmid in strain DH5α | name | sequence |
| R973 | Ptrc-rsxATAC-gfp | TTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCGCCGCATGACCGAATACCTTTTGTTGTTAATCGGCACCGTGCTGGTCAATAACTTTGTACTGGTGAAGTTTTTGGGCTTATGTCCTTTTATGGGCGTATCAAAAAAACTAGAGACCGCCATTGGCATGGGGTTGGCGACGACATTCGTCCTCACCTTAGCTTCGGTGTGCGCTTACCTGGTGGAAAGTTACGTGTTACGTCCGCTCGGCATTGAGTACCTGCGCACCATGAGCTTTATTTTGGTGATCGCTGTCGTAGTACAGTTCACCGAAATGGTGGTGCACAAAACCAGTCCGACACTCTACCGCCTGCTGGGCATTTTCCTGCCACTCATCACCACCAACTGTGCGGTATTAGGGGTTGCGCTGCTCAACATCAACGAAAATCACAACTTTATTCAATCGATCATTTACGGTTTTGGCGCTGCTGTTGGCTTCTCGCTGGTGCTCATCTTGTTCGCTTCAATGCGTGAGCGAATCCATGTAGCCGATGTCCCCGCTCCCTTTAAGGGCGCATCCATTGCGATGATCACCGCAGGTTTAATGTCTTTGGCCTTTATGGGCTTTACCGGATTGGTGAAACTGGCTAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCTACATACGGAAAGCTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTGACCTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCATATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGAACTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACTCGAGTACAACTATAACTCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTCGCCACAACATTGAAGATGGAGGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAATAA |
| R972 | Ptrc-rsxATATWT-gfp | TTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCGCCGCATGACCGAATATCTTTTGTTGTTAATCGGCACCGTGCTGGTCAATAACTTTGTACTGGTGAAGTTTTTGGGCTTATGTCCTTTTATGGGCGTATCAAAAAAACTAGAGACCGCCATTGGCATGGGGTTGGCGACGACATTCGTCCTCACCTTAGCTTCGGTGTGCGCTTATCTGGTGGAAAGTTACGTGTTACGTCCGCTCGGCATTGAGTATCTGCGCACCATGAGCTTTATTTTGGTGATCGCTGTCGTAGTACAGTTCACCGAAATGGTGGTGCACAAAACCAGTCCGACACTCTATCGCCTGCTGGGCATTTTCCTGCCACTCATCACCACCAACTGTGCGGTATTAGGGGTTGCGCTGCTCAACATCAACGAAAATCACAACTTTATTCAATCGATCATTTATGGTTTTGGCGCTGCTGTTGGCTTCTCGCTGGTGCTCATCTTGTTCGCTTCAATGCGTGAGCGAATCCATGTAGCCGATGTCCCCGCTCCCTTTAAGGGCGCATCCATTGCGATGATCACCGCAGGTTTAATGTCTTTGGCCTTTATGGGCTTTACCGGATTGGTGAAACTGGCTAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCTACATACGGAAAGCTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTGACCTATGGTGTTCAATGCTTTTCCCGTTATCCGGATCATATGAAACGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAACGCACTATATCTTTCAAAGATGACGGGAACTACAAGACGCGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACTCGAGTACAACTATAACTCACACAATGTATACATCACGGCAGACAAACAAAAGAATGGAATCAAAGCTAACTTCAAAATTCGCCACAACATTGAAGATGGAGGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCGACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCTTGAGTTTGTAACTGCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAATAA |
| R975 | Ptrc-gfpTAC | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACACATATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTACGGTGTTCAATGCTTTGCGAGATACCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTACGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTACAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATACAACTACAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTACCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTACCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATACAAATAA |
| R974 | Ptrc-gfpTAT | GAGCTGTTGACAATTAATCATCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACACATATGCGTAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATTCTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATATGGAAAACTTACCCTTAAATTTATTTGCACTACTGGAAAACTACCTGTTCCATGGCCAACACTTGTCACTACTTTCGGTTATGGTGTTCAATGCTTTGCGAGATATCCAGATCATATGAAACAGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAGGAAAGAACTATATTTTTCAAAGATGACGGGAACTATAAGACACGTGCTGAAGTCAAGTTTGAAGGTGATACCCTTGTTAATAGAATCGAGTTAAAAGGTATTGATTTTAAAGAAGATGGAAACATTCTTGGACACAAATTGGAATATAACTATAACTCACACAATGTATATATCATGGCAGACAAACAAAAGAATGGAATCAAAGTTAACTTCAAAATTAGACACAACATTGAAGATGGAAGCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGATGGCCCTGTCCTTTTACCAGACAACCATTATCTGTCCACACAATCTGCCCTTTCGAAAGATCCCAACGAAAAGAGAGACCACATGGTCCTTCTTGAGTTTGTAACAGCTGCTGGGATTACACATGGCATGGATGAACTATATAAATAA |
| plasmid in strain (WT/*∆tgt*) | codon replacement | introduction of point mutation in circular pTOPO-TA (kanamycin R) by PCR using primers : |
| N509/N511 | bla wt  |  Tyr103TAC Asp129GAT |
| N512/N514 | bla Tyr103TAC> Tyr TAT synonymous | ZIP555 GACTTGGTTGAGTATTCACCAGTCACAGAAAAGC ZIP556 TCTGTGACTGGTGAATACTCAACCAAGTCATTCTGAGAATAG |
| plasmid in strain TOP10 | primers for transcriptional fusion PrsxA-gfp |
| Q282 | ZIP796ZIP812ZIP813ZIP200 | GGATACAAAAAAGTAAACCCCTCCTTTACGCATAGTTATATAAATGTTTGCTTCCGATCCCGGCATTATCCTGCAGGATAATGCCGGGATCGGAAGCAAACATTTATATAACTATGCGTAAAGGAGTATCAAGCTTATTTGTATAGTTCATCCATGCC |
| target for amplification | primers for q-RT-PCR tRNAs  |
| tRNATyr | ZIP719ZIP720 | GGAGGGGTTCCCGAGTGGGGTGGAGGGGGACGGATT |
| tRNAAsp | ZIP721ZIP722 | GGAGCGGTAGTTCAGTCGTGGCGGAGCGGACGGGAC |
| tRNAHis | ZIP723ZIP724 | GTGGCTATAGCTCAGTTGTGGGGTGGCTAATGG |
| tRNAAsn | ZIP725 ZIP726 | TCCTCCTTAGCTCAGTCGGTGGCTCCTCCTGCTGG |
| gyrA | gyrA\_FgyrA\_R | AAT GTG CTG GGC AAC GAC TGGAG CCA AAG TTA CCT TGG CC |
| target for amplification | primers for digital-RT-PCRfluorescent probes |
| *tgt* | fwd CAA CAC CAC TGG ATC CTC ATTrev GGT AGT AAC GCA GGT TAT GG5’ - [FAM] A CCT GCA TCA TCT GGA TCG CTG TAA - 3’ [BHQ2] |
| *rsxA* | fwd TCA CGC ATT GAA GCG AACrev CAC CAA CTG TGC GGT ATT AG5’ - [FAM] A GCG CCA AAA CCA TAA ATG ATC GAT - 3’ [BHQ2] |
| *gyrA* | fwd AAT GTG CTG GGC AAC GAC TGrev GAG CCA AAG TTA CCT TGG CC5’ - [CY5] - CAC CCT CAT GGT GAC AGT GCG GTT T - 3’ [BHQ2] |
| Strains |  |
| *V. cholerae* | strain # | reference |
| *N16961 hapR+* | F606 | laboratory collection |
| *N16961 hapR+ ∆lacZ* | K329 | Babosan et al, 2022 |
| *∆tgt::spec* | J420 | Babosan et al, 2022 |
| *∆tolA::kan* | J983 | Negro et al, 2019 |
| *∆crp::kan* | Q081 | Deletion of *crp* in F606 as described in Lang et al, 2021 |
| *∆rluF::kan* | M567 | Babosan et al, 2022 |
| *∆rluF::kan ∆tgt::spec* | M569 | Deletion of *rluF* in J420, as described for deletion of *rluF* in WT in Babosan et al, 2022 |
| *E. coli* | strain # | construction |
| *MG1655* |  C349 | laboratory collection |
| *∆tgt::kan* |  J233 | P1 transduction of tgt::kan from Keio collection into MG1655 |
| *∆tgt* | R181 | Kanamycin resistance cassette was removed using the FLP/FRT system (Zhu et al, 1995)  |
| *∆rsxA::kan* |  R207 | P1 transduction of tgt::kan from Keio collection into MG1655 |
| *∆tgt ∆rsxA::kan* |  R223 | P1 transduction of rsxA::kan from Keio collection into R181 *∆tgt* |