**Supplementary file 1a**

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| --- | --- | --- | --- |
|  | **HipBSTD233Q** | **HipBSTS57A** | **HipBSTS59A** |
| ***Data Collection***  Wavelength (Å)  Resolution range (Å)  Space group  Unit cell dimensions  a, b, c, (Å)  *α, β, γ* (o) | 1.54180  49.9–2.9 (3.0–2.9)\*  C121  281.66,106.47, 57.75 90, 90.75, 90 | 0.97625  46.9–2.4 (2.5–2.4)\*  C121  281.68, 106.07, 57.56 90, 90.65, 90 | 1.54180  50.4–3.34 (3.5 – 3.4)\*  C121  285.26, 107.15, 58.45  90, 90.71, 90 |
| Total reflections | 74,587 (7,335) | 625,868 (25,597) | 41,777 (4,068) |
| Unique reflections | 37,716 (3,719) | 66,117 (6,503) | 22,580 (2,193) |
| Multiplicity | 2.0 (2.0) | 9.5 (3.9) | 1.9 (1.9) |
| Completeness (%) | 99.8 (99.3) | 99.9 (99.4) | 87.71 (84.84) |
| Rmeas (%) | 0.13 (1.105) | 0.27 (0.08) | 0.17 (1.01) |
| I/σ(I) | 6.62 (0.86) | 10.3 (0.72) | 7.10 (0.93) |
| CC1/2 | 0.99 (0.37) | 0.99 (0.37) | 0.97 (0.43) |
| ***Refinement***  Average B-factor (Å2)  protein  ligands  solvent  No. of reflections  No. of reflections (free)  R-work (%)  R-free (%)  Number of  protein (residues)  solvent (atoms)  ligand (atoms)  rmsd (bonds, Å)  rmsd (angles, degrees)  Rotamer outliers (%)  Clashscore  Ramachandran statistics  favoured (%)  allowed (%)  outliers (%)  Rama-Z score  whole  helix  sheet  loop | 98.5  98.9  -  80.0  37,706 (3,719)  1,914 (175)  19.5 (31.8)  22.8 (34.5)  1,018  203  -  0.011  1.49  9.8  4.4  95.1  4.7  0.2  -1.63  -1.27  -1.36  -0.75 | 86.1  86.5  166.7  76.7  66,112 (6,504)  3,364 (326)  21.0 (42.8)  23.8 (46.2)  1,017  402  20 0.013  1.75 6.0  2.7  96.0  3.6  0.4  -0.44  -0.40  0.21  -0.16 | 119.4  119.9  118.6  78.3  22,570 (2,193)  1086 (86)  20.2 (29.2)  24.3 (35.4)  1,023  110  10  0.013  1.66  10.07  5.8  95.6  3.9  0.5  -0.90  -0.48  0.07  -0.73 |

**Supplementary file 1b**

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| *E. coli* strains | Description | Reference or source |
| MG1655 | Wild-type K12 | (Blattner *et al.*, 1997) |
| TB28 | MG1655 *∆lacIZYA* | Laboratory collection |

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| --- | --- | --- |
| Plasmid | Description | Reference or source |
| pBAD33 | p15 *araC* PBAD, Cmr | (Guzman *et al.*, 1995) |
| pGH254 | Mini-R1, *lacZYA* transcriptional fusion vector, Kanr | Laboratory collection |
| pNDM220 | Mini-R1 *lacI*q PA1/04/03, Ampr | (Gotfredsen & Gerdes, 1998) |
| pET-15b | pBR322 *lacI* PT7, Ampr | Novagen |
| pKG127 | pUC57::*hipBSTO127* | (Vang Nielsen *et al.*, 2019) |
| pSVN1 | pBAD33::*hipT*, start codon GTG | (Vang Nielsen *et al.*, 2019) |
| pSVN68 | pUC57::*hipB*-*S*-*T*S57AHis6, optimized SDs for all genes | This work |
| pSVN78 | pET-15b::*hipB*-*S*-*T*S57AHis6, optimized SDs for all genes | This work |
| pSVN88 | pUC57::*hipB*-*S*-*T*D233QHis6, optimized SDs for all genes | This work |
| pSVN96 | pET-15b::*hipB*-*S*-*T*D233QHis6, optimized SDs for all genes | This work |
| pSVN109 | pNDM220::*hipS*, optimized SD | (Vang Nielsen *et al.*, 2019) |
| PSVN141 | pGH254::P*hipBST*-*hipB*’-*laZ*, transcriptional P*hipBST*-*hipB*’ *lacZ* fusion | This work |
| pSVN178 | pNDM220::*hipS*W65A, optimized SD | This work |
| pSVN181 | pBAD33::*hipB*-*S*-*T*D233Q, optimized SDs for all genes | This work |
| pSVN182 | pBAD33::*hipB*-*S*, optimized SDs for both genes | This work |
| pSVN185 | pBAD33::*hipB*-*T*D233Q, optimized SDs for both genes | This work |
| pSVN188 | pBAD33::*hipS*-*T*D233Q, optimized SDs for both genes | This work |
| pSVN189 | pBAD33::*hipB*, optimized SD | This work |
| pSVN190 | pBAD33::*hipS*, optimized SD | This work |
| pSVN193 | pBAD33::*hipT*D233Q, optimized SD | This work |
| pSVN194 | pBAD33::*hipT*S57D, start codon GTG | This work |
| pSVN195 | pBAD33::*hipT*S59D, start codon GTG | This work |
| pSVN199 | pBAD33::*hipT*S57A, start codon GTG | This work |
| pSVN201 | pBAD33::*hipT*S59A, start codon GTG | This work |
| pSNN1 | pET-15b::*hipT*S57AHis6, optimized SD | This work |
| pSNN2 | pET-15b::*hipT*S57A+D210AHis6, optimized SD | This work |
| pMME3 | pET-15b::*hipB-S-T*S59AHis6, optimized SDs for all genes | This work |
| pRBS1 | pET-15b::*hipB*-*S*-*T*D210AHis6, optimized SDs for all genes | This work |
| pRBS2 | pET-15b::*hipB*-*S*-*T*S57D,D210AHis6, optimized SDs for all genes | This work |
| pRBS3 | pET-15b::*hipB*-*S*-*T*S59D,D210AHis6, optimized SDs for all genes | This work |

**Supplementary file 1c**

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| Oligonucleotide | Sequence |
| FP1(GTG) | CCCCGTCGACGGATCCAAGGAGTTTTATAAGTGGCGAATTGTCGTATTCTG |
| FP21 | GGGGGGTACCGGATCCAAAATAAGGAGGAAAAAAAAATGATCTGCTCAGGACCAC |
| FP22 | CCCCCTCGAGGGATCCAAAATAAGGAGGAAAAAAAAATGCATCGGCGAGTGAAAG |
| FP43 | CCCCGAATTCCTCTCCCGATGAGATCAGC |
| FP46 | GGGGGTCGACCTGCAGAAAATAAGGAGGAAAAAAAAATGGCGAATTGTCGTATTCTG |
| FP47 | GGGGGGTACCGGATCCAAAATAAGGAGGAAAAAAAAATGGCGAATTGTCGTATTCTG |
| FP48 | GGGGGTCGACCTGCAGAAAATAAGGAGGAAAAAAAAATGGCGAATTGTCGTATTCTG |
| RP1 | CCCCCGCATGCGAATTCGCTCACAGCAGCCCCAGACG |
| RP11 | CCCCCTCGAGAAGCTTTCACAGCAGCCCCAGACG |
| RP14 | GGGGGAATTCAAGCTTTTATTCCTCCCAAGGTAAAATC |
| RP15 | GGGGGAATTCAAGCTTTCACTCGCCGATGCATAG |
| RP32 | CCCCGGATCCTCTGCAACTCCTGGAGTTG |
| RP42 | GGGGGTCGACCTGCAGTCACTCGCCGATGCATAG |
| HipT S57D Fw | GCGTCAACAAAAAGGGATGGATATTTCCGGTT |
| HipT S57D Rv | GGGCTGGTAACCGGAAATATCCATCCCTTTTT |
| HipT S59D Fw | GTCAACAAAAAGGGATGAGTATTGACGGTTAC |
| HipT S59D Rv | TTGGGCTGGTAACCGTCAATACTCATCCCTTT |
| HipT S59A Fw | GTCAACAAAAAGGGATGAGTATTGCCGGTTAC |
| HipT S59A Rv | TTGGGCTGGTAACCGGCAATACTCATCCCTTT |
| hipT D210A Fw | TAAATGCATCGCGTTATTACCCAGCAACAA |
| hipT D210A Rv | CTGGGTAATAACGCGATGCATTTACGAAACTTT |
| hipT S57S59A Fw | GGGATGAGTATTGCCGGTTACCAGCCCAAATTGCAA |
| hipT S57S59A Rv | GTAACCGGCAATACTCATCCCTTTTTGTTGACGCGG |
| hipS W65A Fw | CAGAAGGAGCTCTGCGTCAACGCTA |
| hipS W65A Rv | TGACGCAGAGCTCCTTCTGGCGC |
| hipX S57A Fw | AAGGGATGGCTATTTCCGGTTACCAGCC |
| hipX S57A Rv | CGGAAATAGCCATCCTTTTTGTTGACG |
| hipX D233Q Fw | CGGTGTATCAGTTTGTTTCTGTCGCTCCC |
| hipX D233Q Rv | GAAACAAACTGATACACCGGCGCTAACG |
| hipBS del Fw | ACGACAATTCGCCATTTTTTTTTCCTCCTTATTTTTCTAGAGGG |
| hipBS del Rv | TTCCCCTCTAGAAAAATAAGGAGGAAAAAAAAATGGCGAAT |
| Q5 HipT D210A Fw | GGTAATAACGctATGCATTTACGAAACTTTG |
| Q5 HipT D210A Rv | CAGCAACCAGGCGTAAAC |
| Q5 HipT S57D Fw | AAAGGGATGGaTATTTCCGGT |
| Q5 HipT S57D Rv | TTGTTGACGCGGAAGTTC |
| Q5 HipT S59D Fw | tagaCATCCCTTTTTGTTGACG |
| Q5 HipT S59D Rv | ttgatGGTTACCAGCCCAAATTG |