**Supplementary file 1a**

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| --- | --- | --- | --- |
|  | **HipBSTD233Q** | **HipBSTS57A** | **HipBSTS59A** |
| ***Data Collection*** Wavelength (Å)Resolution range (Å)Space groupUnit cell dimensions a, b, c, (Å) *α, β, γ* (o) | 1.5418049.9–2.9 (3.0–2.9)\*C121281.66,106.47, 57.7590, 90.75, 90 | 0.9762546.9–2.4 (2.5–2.4)\*C121281.68, 106.07, 57.5690, 90.65, 90 | 1.5418050.4–3.34 (3.5 – 3.4)\*C121285.26, 107.15, 58.4590, 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 solventNo. of reflectionsNo. of reflections (free)R-work (%)R-free (%)Number of protein (residues) solvent (atoms) ligand (atoms)rmsd (bonds, Å) rmsd (angles, degrees)Rotamer outliers (%)ClashscoreRamachandran statistics favoured (%) allowed (%) outliers (%)Rama-Z score whole helix sheet loop | 98.598.9-80.037,706 (3,719)1,914 (175)19.5 (31.8)22.8 (34.5)1,018203-0.0111.499.84.495.14.70.2-1.63-1.27-1.36-0.75 | 86.186.5166.776.766,112 (6,504)3,364 (326)21.0 (42.8)23.8 (46.2)1,017402200.013 1.756.02.796.03.60.4-0.44-0.400.21-0.16 | 119.4119.9118.678.322,570 (2,193)1086 (86)20.2 (29.2)24.3 (35.4)1,023110100.0131.6610.075.895.63.90.5-0.90-0.480.07-0.73 |

**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 |

|  |  |  |
| --- | --- | --- |
| 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 |