**S2 Table. Primers used in this study**

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| --- | --- | --- |
| **Primers** | **Sequence (5'-3')** | **Application** |
| 313\_FBA1p\_F\_site1\_F | cgcggtggcggccgcataacaatactgacagtactaaataattgc | pRnKHK construction |
| 313\_FBA1p\_R\_site1 | TTCTTCCATTCTAGATTTGAATATGTATTACTTGGTTATGG |
| 313\_RnKHK\_frag\_F | tctagaatggaagaaaagcaaattttg |
| 313\_RnKHK\_frag\_R | GACATAACTACTAGTTTAGACGATACCATCGAAACC |
| 313\_CYC1t\_frag\_F | actagtagttatgtcacgcttacattcac |
| 313\_CYC1t\_Frag\_R | CAGCCCGGGGGATCCAGCTTGCAAATTAAAGCC |
| N-RnKHKtag\_1 | agtaatacatattcaaatctagaatgcatcaccatcacc | pNT/his-RnKHK construction |
| N-RnKHKtag\_2 | catcaccatcaccatcaccatcacgcgtctgcgtggtctcatccacaatttgaaaaagg |
| N-RnKHKtag\_3 | CAAAATTTGCTTTTCTTCTGGTCCTTGGAACAGGACTTCGAGACCACCTTTTTCAAATTG |
| TDH3p\_RnKHK\_F\_Frag | cagttcgagtttatcattatcaatactgc | p1  construction |
| TDH3p\_RnKHK\_R\_Frag | TTTGTTTGTTTATGTGTGTTTATTCGAAAC |
| TDH3p\_RnKHK\_F\_LV | acataaacaaacaaatctagaatggaagaaaagcaaattttgtg |
| TDH3p\_RnKHK\_R\_LV | GATAAACTCGAACTGCCGCGGTGGAGCTCC |
| 313\_site2\_FBA\_F | atcgataccgtcgacataacaatactgacagtactaaataattgc | pRnKHK-FBA1 construction |
| 313\_site2\_FBA\_R | CAAAAGCTGGGTACCGCTATCAAAAACGATAGATCGATTAG |
| 313\_site2\_LV\_F | ggtacccagcttttgttcc |
| 313\_site2\_LV\_R | GTCGACGGTATCGATAAGC |
| SpKHK frag for pFBA1\_F | taatacatattcaaaatgtcttcatacatcttgtttggtttgg | pSpKHK-FBA1 construction |
| SpKHK frag for pFBA1\_R | GACATAACTACTAGTTTAAGGTAAAGGGAACTGCTTTTTG |
| 313\_FBA1p\_R\_site1 | [same as above] |
| 313\_CYC1t\_frag\_F | [same as above] |
| NCU03742 frag for pFBA1\_F | taatacatattcaaaatggaaggaaggaaggaag | pNcKHK-FBA1 construction |
| NCU03742 frag for pFBA1\_R | GACATAACTACTAGTTTACCATCCCTTAACAATCC |
| 313\_FBA1p\_R\_site1 | [same as above] |
| 313\_CYC1t\_frag\_F | [same as above] |
| 315\_TEF1p\_frag\_F | agtggatcccccgggatagcttcaaaatgtttctactcc | pXI construction |
| 315\_CYC1t\_frag\_R | GTCGACGGTATCGATGGCCGCAAATTAAAGC |
| pRS315-LV\_F | atcgataccgtcgacctcg |
| pRS315-LV\_R | CCCGGGGGATCCACTAG |
| pRS316-CCW12p-frag\_F | agtggatcccccgggcaaagcaaaataaaagaaacttaatacg | p3 construction |
| pRS316-CCW12p-frag\_R | ACTAAGCTTCTGCAGTATTGATATAGTGTTTAAGCGAATGACAG |
| pRS316-CYC1t-frag\_F | ctgcagaagcttagttatgtcacgcttacattcac |
| pRS316-CYC1t-frag\_R | GTCGACGGTATCGATAGCTTGCAAATTAAAGCCTTC |
| 316\_CCW12p\_ADH1\_frag\_F | aacactatatcaataatgtctatcccagaaactcaaaaag | pADH1 construction |
| 316\_CYC1t\_ADH1\_frag\_F | AAGCGTGACATAACTTTATTTAGAAGTGTCAACAACGTATCTACC |
| 316\_LV\_CYC1t\_F | agttatgtcacgcttacattcacg |
| 316\_LV\_CCW12p\_R | TATTGATATAGTGTTTAAGCGAATGACAG |
| 316\_CCW12p\_GRE2\_frag\_F | aacactatatcaataatgtcagttttcgtttcaggtgc | pGRE2 construction |
| 316\_CCW12p\_GRE2\_frag\_R | AAGCGTGACATAACTTTATATTCTGCCCTCAAATTTTAAAATTTG |
| 316\_LV\_CYC1t\_F | [same as above] |
| 316\_LV\_CCW12p\_R | [same as above] |
| 316\_site2\_CCW12p\_frag\_F | agtggatcccccgggcaaagcaaaataaaagaaacttaatacg | pADH1-GRE2 construction |
| 316\_site2\_CYC1t\_frag\_R | ATCGAATTCCTGCAGAGCTTGCAAATTAAAGCCTTC |
| 316\_site2\_LV\_F | ctgcaggaattcgatatcaagc |
| 316\_site2\_LV\_R | CCCGGGGGATCCACTAG |
| xyIB\_F | gttctgttccagggtccgtatatcgggatagatcttgg | pET-xylB construction |
| xyIB\_R | CGGATCCGATTATACCTACGCCATTAATGGCAG |
| pETm\_F | taggtataatcggatccggctg |
| pETm\_R | CGGACCCTGGAACAGAAC |
| 423-his-PGM1-F | atgcatcatcatcatcatcatggtggtggttcacttctaatagattctgtaccaac | pRS423-NT/his-PGM1  Construction |
| 423-his-PGM1-R | TAATTACATGACTCGAGTTACTATGTGCGGACTGTTGG |
| 423-his-LV-F | taactcgagtcatgtaattag |
| 423-his-LV-R | TGATGATGATGATGATGCATTGGATCCACTAGTTCTAG |
| 423-his-PGM2-F | atgcatcatcatcatcatcatggtggtggttcatttcaaattgaaacgg | pRS423-NT/his-PGM2  Construction |
| 423-his-PGM2-R | TAATTACATGACTCGAGTTATTAAGTACGAACCGTTG |
| 423-his-LV-F | [same as above] |
| 423-his-LV-R | [same as above] |
| 423-his-PRM15-F | atgcatcatcatcatcatcatggtggtggtttgcaaggaattttagaaacc | pRS423-NT/his-PRM15  Construction |
| 423-his-PRM15-R | TAATTACATGACTCGAGTTATCAAAATTTTGTAACTATATTCATTTCATC |
| 423-his-LV-F | [same as above] |
| 423-his-LV-R | [same as above] |
| xks1del\_UP\_F | aaatgcgtttatatatatatattccagtg | S2 construction |
| xks1del\_UP\_R | TAAAGTACTAATCTCATCCTCCTTTTG |
| xks1del\_DOWN\_F | aatatgtttgagataatttatcatgc |
| xks1del\_DOWN\_R | GCCTCTTGGGGTAACTTTATAG |
| KanMX\_F | aaacaaaaggaggatgagattagtactttataggtctagagatctgtttagc |
| KanMX\_R | CAGGGCATGATAAATTATCTCAAACATATTATTAAGGGTTCTCGAGAG |
| pgm1\_up\_F | caggcatttatactttgg | S2 *pgm1Δ*::*nat*MX |
| pgm1\_up\_R | TACTATTCAATTGAGTTGTTAG |
| natMX\_F | tctctctctcatccactttcggttcaatagtgcaaaaataggtaactaacaactcaattgaatagtaaTAGGTCTAGAGATCTGTTTAGC |
| natMX\_R | CTCTACATATAAATTAAGATCAGTTAAAACATCTTTGGAAAATTAGTGCTTGTTCAAGACCAATAATTATTAAGGGTTCTCGAGAGC |
| pgm1\_down\_F | tggtcttgaacaagc |
| pgm1\_down\_R | GTTTTCTGAATTACCACC |
| pgm2\_up\_F | attaaaaaaggtctaacatcc | S2 *pgm2Δ*::*nat*MX |
| pgm2\_up\_R | ACTGAGAAAGATTGGTTG |
| natMX\_F | caataggataataagaagaagatcaaccaatctttctcagtaaaaaaagtaacaaaagttaacataacTAGGTCTAGAGATCTGTTTAGC |
| natMX\_R | TTTCTTCTTTACCGTTAATATTCATTGAAAAAGGTGAAAATCATTAAGCCATTAGTAAATCATTCGTTATTAAGGGTTCTCGAGAGC |
| pgm2\_down\_F | cgaatgatttactaatggc |
| pgm2\_down\_R | GGATTCTTGAAGGATGC |
| prm15\_up\_F | gacaagaatatctggagg | S2 *prm15Δ*::  *nat*MX |
| prm15\_up\_R | TTTTTGAAATTGCAGCAG |
| natMX\_F | attctttctcctttccccctaaatactcaacagtactctcgagccattagctgctgcaatttcaaaaaTAGGTCTAGAGATCTGTTTAGC |
| natMX\_R | AGGTACAAACAACAAATATAATGGCATTCTTTAAGTCGCTTGGTCTTAATGTATAGGTTAAAATAGTAATTAAGGGTTCTCGAGAGC |
| prm15\_down\_F | tattttaacctatacattaagacc |
| prm15\_down\_R | CATTCTATTATGTTTCCTTCC |