**Supplementary file 1**

***Nostoc* and *E. coli* strains**

|  |  |  |
| --- | --- | --- |
| **Strains** | **Description/genotype** | **Source/reference** |
| ***Nostoc* strains** |  |  |
| *Nostoc* PCC 7120 | Wild type strain (WT) | Pasteur Institute Collection |
| WT/P*petE-patS* | WT strain containing the pRL1272-P*petE-patS* plasmid | This study |
| WT/P*petE-patS* P*petE-hetL* | WT strain containing the pRL1272-P*petE-patS* plasmid and pRL25T-P*petE-hetL* plasmid | This study |
| WT/P*petE-patS* P*petE-hetL*D151A | WT strain containing the pRL1272-P*petE-patS* plasmid and pRL25T-P*petE-hetL*D151Aplasmid derivative where the *hetL* gene has been mutated to encode for a D151A substitution | This study |
| WT/P*petE-hetL* | WT strain containing the pRL25T-P*petE-hetL* plasmid | This study |
| WT/P*petE-hetL*D151A | WT strain containing the pRL25T-P*petE-hetL*D151Aplasmid | This study |
| WT/P*patS-hetL* | WT strain containing the pRL25T-P*patS-hetL* plasmid | This study |
| WT/P*rbcL-hetL* | WT strain containing the pRL25T-P*rbcL-hetL* plasmid | This study |
| Δ*hetR* | *Nostoc* deletion mutant of the *hetR* gene | (Borthakur, Orozco, Young-Robbins, Haselkorn, & Callahan, 2005) |
|  |  |  |
| ***E. coli* strains** |  |  |
| TG1 | *supE thi*-1 ∆(*lac-proAB*) ∆(*mcrB-hsdSM*)5 (rK– mK–) [F´ *traD36 proAB lacIqZ∆M15*] | Stratagene |
| DH5α | *fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17* | (Taylor, Walker, & McInnes, 1993) |
| StellarTM | *F–, endA1, supE44, thi-1, recA1, relA1, gyrA96, phoA, Φ80d lacZΔ M15, Δ (lacZYA - argF) U169, Δ (mrr - hsdRMS - mcrBC), ΔmcrA, λ–* | Takara |
| BL21DE3 | *fhuA2 [lon] ompT gal (λ DE3) [dcm] ∆hsdS λDE3 = λsBamHIo ∆EcoRI-int::(lacI::PlacUV5::T7 gene1) i21∆nin5* | NEB |
| BTH101 | F−, cya-99, araD139, galE15, galK16, rpsL1, hsdR2, mcrA1, mcrB1 | (Karimova et al., 1998) |
| eXX1 | TG1 containing the plasmid pKT25-*hetR* | This study |
| eXX2 | TG1 containing the plasmid pKT25-*hetL* | This study |
| eXX3 | TG1 containing the plasmid pKT25-*hetL*D151A | This study |
| eXX4 | TG1 containing the plasmid pUT18C-*hetR* | This study |
| eXX5 | TG1 containing the plasmid pUT18C-*hetR*R223W | This study |
| eXX6 | TG1 containing the plasmid pUT18C-*hetL* | This study |
| eXX7 | TG1 containing the plasmid pUT18C-*hetL* RBS*-patS* | This study |
| eXX8 | TG1 containing the plasmid *hetR*hood-pUT18 | This study |
| eXX9 | TG1 containing the plasmid pUT18C-all3256 | This study |
| eXX10 | TG1 containing the plasmid pUT18C-all4303 | This study |
| eBR1 | TG1 containing the plasmid pET28a-*his-hetR* | (Roumezi et al., 2019) |
| eXX11 | StellarTM containing the plasmid pRL1272-P*petE-patS* | This study |
| eXX12 | StellarTM containing the plasmid pRL25T-P*petE-hetL* | This study |
| eXX13 | StellarTM containing the plasmid pRL25T-P*petE-hetL*D151A | This study |
| eSC1 | StellarTM containing the plasmid pRL25T-P*patS-hetL* | This study |
| eSC2 | StellarTM containing the plasmid pRL25T-P*rbcL-hetL* | This study |
| eXX14 | BTH101 containing plasmids pKT25-zip and pUT18C-zip | This study |
| eXX15 | BTH101 containing plasmids pKT25 and pUT18C | This study |
| eXX16 | BTH101 containing plasmids pKT25-*hetL* and pUT18C | This study |
| eXX17 | BTH101 containing plasmids pKT25 and pUT18C-*hetR* | This study |
| eXX18 | BTH101 containing plasmids pKT25-*hetR* and pUT18C-*hetR* | This study |
| eXX19 | BTH101 containing plasmids pKT25-*hetL* and pUT18C-*hetR* | This study |
| eXX20 | BTH101 containing plasmids pKT25-*hetL* and pUT18C-*hetL* | This study |
| eXX21 | BTH101 containing plasmids pKT25-*hetL* and *hetR*hood-pUT18 | This study |
| eXX22 | BTH101 containing plasmids pKT25-*hetL*D151A and pUT18C-*hetL* | This study |
| eXX23 | BTH101 containing plasmids pKT25-*hetR* and pUT18C-*hetR*R223W | This study |
| eXX24 | BTH101 containing plasmids pKT25-*hetL*D151A and pUT18C-*hetR* | This study |
| eXX25 | BTH101 containing plasmids pKT25-*hetL* and pUT18C-*hetR*R223W | This study |
| eXX26 | BTH101 containing plasmids pKT25-*hetR* and pUT18C-*hetL* | This study |
| eXX27 | BTH101 containing plasmids pKT25-*hetR* and pUT18C-*hetL-*RBS*-patS* | This study |
| eXX28 | BTH101 containing plasmids pKT25-*hetR* and pUT18C-*hetL-*RBS*-patS6* | This study |
| eXX29 | BTH101 containing plasmids pKT25-*hetR* and pUT18C-all3256 | This study |
| eXX30 | BTH101 containing plasmids pKT25-*hetR* and pUT18C-all4303 | This study |

**Plasmids**

|  |  |  |
| --- | --- | --- |
| **Plasmids** | **Description** | **Source/reference** |
| pKT25-zip | Two hybrid plasmid KanR | (Karimova et al., 1998) |
| pUT18C-zip | Two hybrid plasmid AmpR | (Karimova et al., 1998) |
| pKT25 | Two hybrid plasmid. T25 at the N terminus KanR | (Karimova et al., 1998) |
| pUT18C | Two hybrid plasmid. T18 at the N terminus AmpR | (Karimova et al., 1998) |
| pKNT25 | Two hybrid plasmid. T25 at the C terminus KanR | (Karimova et al., 1998) |
| pUT18 | Two hybrid plasmid. T18 at the C terminus. AmpR | (Karimova et al., 1998) |
| pET28a | His-tagged protein expression plasmid in *E. Coli* KanR | Novagen |
| pRL1272 | Replicative in *Nostoc* EryR | (Wolk et al., 1988) |
| pRL25T | Replicative in *Nostoc* NeoR | (Yang et al., 2013) |
| pXX1 | pKT25-*hetR* | This study |
| pXX2 | pKT25-*hetL* | This study |
| pXX3 | pKT25-*hetL*D151A | This study |
| pXX4 | pUT18C-*hetR* | This study |
| pXX5 | pUT18C-*hetR*R223W | This study |
| pXX6 | pUT18C-*hetL* | This study |
| pXX7 | pUT18C-*hetL-*RBS*-patS* | This study |
| pXX8 | pUT18C-*hetL-*RBS*-patS6* | This study |
| pXX9 | *hetR*hood-pUT18 | This study |
| pXX10 | pUT18C-all3256 | This study |
| pXX11 | pUT18C-all4303 | This study |
| pBR1 | pET28a-*his-hetR* | (Roumezi et al., 2019) |
| pXX12 | pET28a-*hetL-his* | This study |
| pCSB270 | pRL1272-P*petE* | Lab collection |
| pXX13 | pRL1272-P*petE-patS* | This study |
| pCSB265 | pRL25T-P*petE* | Lab collection |
| pXX14 | pRL25T-P*petE-hetL* | This study |
| pXX15 | pRL25T-P*petE-hetL*D151A | This study |
| pSC1 | pRL25T-P*patS-hetL* | This study |
| pSC2 | pRL25T-P*rbcL-hetL* | This study |

**Primers**

|  |  |  |  |
| --- | --- | --- | --- |
| Name | Sequence (5’-3’) | Experiment | |
| 16S rRNA rt fw | TCCTGGTGTAGCGGTGAAAT | Quantitative RT-PCR analysis | |
| 16S rRNA rt rv | AGCCACGCCTAGTATCCATC |  |
| *hetP* rt fw | TGGCTGGTAAATACTCTTGGG |  |
| *hetP* rt rv | ACCTACTACTTCCAGATAGGC |  |
| *hetL* rt fw | GACATTATGCTGCTGGCAAA |  |
| *hetL* rt rv | CAAGTCGCGTCTGACGTAAA |  |
| *hetR* rt fw | GCGTCGTCTGCTTTACTCTG |  |
| *hetR* rt rv | CCCAGTCTTTCATCATGCGG |  |
| *hetR* dh fw T25 | TTTTCTGCAGGGATGAGTAACGACATCGATCT |  |
| *hetR* dh fw T18 | TTTTCTGCAGGATGAGTAACGACATCGATCTGA |  |
| *hetR* dh rv | TTTTTGAATTCTTAATCTTCTTTTCTACCAAACACCATTTG | Two hybrid assays |
| Mut *hetR*R223W fw | CCAGCAGACGACCAAGAGTGGACTTATATTATGGTGGAA |  |
| Mut *hetR*R223W rv | TTCCACCATAATATAAGTCCACTCTTGGTCGTCTGCTGG |  |
| *hetL* dh fw T25 | TTTTCTGCAGGGATGAATGTGGGTGAAAT |  |
| *hetL* dh fw T18 | TTTTCTGCAGGATGAATGTGGGTGAAAT |  |
| *hetL* dh rv | TTTTTGAATTCTCAATCATGAATTGAACCATCAGG |  |
| RBS*-patS dh* fw T18 | TTTTTGTCGACAGGTTAGGAGAACCATATG |  |
| RBS*-patS dh* rv T18 | TTTTTCTCGAGGATTGAGTGGTCGGAACGA |  |
| RBS*-patS6 dh* fw T18 | AAGCTTATCGATACCGTCGACAGGTTAGGAGAACCATATGGAGCGCGGTAGTGGTAGATAGAACG |  |
| RBS*-patS6 dh* rv T18 | GATGAATTGCTCGAGGTCGACGATTGAGTGGTCGGAACGAATGC |  |
| *hetR*hood dh fw | TTTTCTGCAGGTATGCCCCAGCAGAC |  |
| *hetR*hood dh rv | TTTTTGAATTCGATCTACCAAACACCATTTGTAAAATCATGG |  |
| all3256dh fw T18 | TTTTCTGCAGGATGGCAAATCTAGAGCAT |  |
| all3256dh rv | TTTTTGAATTCCTAATCATGCCTTGAAGAGTCA |  |
| all4303dh fw T18 | TTTTCTGCAGGATGAATATTGACGCTATT |  |
| all4303dh rv | TTTTTGAATTCTTACCCATTACCAATTTCTAATATTGTCCCT |  |
| Mut *hetL*D151A fw | GCAGATTTAAGCTACGCTGCCCTGAGAGCGGCTTCTCTA |  |
| Mut *hetL*D151A rv | TAGAGAAGCCGCTCTCAGGGCAGCGTAGCTTAAATCTGC |  |
| *hetR* pET28 fw | CATATGAGTAACGACATCGATCTG |  |
| *hetR* pET28 rv | GGATCCTTAATCTTCTTTTCTACC |  |
| *hetL* pET28 fw | AGGAGATATACCATGGGCAATGTGGGTGAAATTCTGAGACA | Protein production for BLI assays |
| *hetL* pET28 rv | GGTGGTGGTGCTCGAGACCTTGAAAATAAAGATTTTCATCATGAATTGAACCATCA |  |
| *patS* pRL fw | GCCCATCGATGGATCCATGAAGGCAATTATGTTAGTG |  |
| *patS* pRL rv | CGTCGACCCGGGATCCATGACTATTGACCAAATGACTATTG |  |
| *hetL* pRL fw | GAGCTCGTCGACCCGGGATCCTCAATCATGAATTGAACCATCAGGC | Construction of recombinant plasmids for *Nostoc* |
| *hetL* pRL rv | ATGGGGCCCATCGATGGATCCATGAATGTGGGTGAAATTCTGAGAC |  |
| P*patS* fw | TGAGATTATCAAAAAGGATCCAGATCC  TGAATTTGTTTTGGGAAC |  |
| P*patS* rv | CCACATTCATAATCTTAACCTCCCTGAATTACTTTTCAACAGAACATTT |  |
| *hetL* P*patS* fw | TAAGATTATGAATGTGGGTGAAATTCT  GAGAC |  |
| *hetL* P*patS* rv | GAGTAGAATTCCCGGGGATCCTCAATC  ATGAATTGAACCATCAGGC |  |
| P*rbcL* fw | TGAGATTATCAAAAAGGATCCGCAGGGGAAGTAAAGAAGAATGAC |  |
| P*rbcL* rv | CACCCACATTCATATCTATCCTTCCAAGATGTCAC |  |
| *hetL* P*rbcL* fw | GATAGATATGAATGTGGGTGAAATTCTGAGAC |  |
| *hetL* P*rbcL* rv | GAGTAGAATTCCCGGGGATCCTCAATCATGAATTGAACCATCAGGC |  |
| P*hetP* fw | [6FAM]ATTTAGTGGTAAATTCTCTT | EMSA assay |
| P*hetP* rv | TGAGTTATACGCTATATCAA |  |

**Supplementary Table 1: PRPs encoding genes in the genome of *Nostoc***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene ID/name** | **Num**  **of**  **AA** | **Num**  **of PRs domains** | **Additional**  **Domain(s)** | **Cellular**  **location** | **Gene expression (number of reads)**  **0h 6h 12h 21h** |
| **Genes whose expression changes in response to combined nitrogen starvation** | | | | | |
| alr4610 | 164 | 2 | - | P/L | 33 30 88 92 |
| alr1298 | 167 | 1 | - | C | 79 65 151 119 |
| all0186 | 168 | 2 | - | M | 13 6 74 30 |
| alr1746 | 182 | 3 | - | C | 843  519 134 159 |
| all4303 | 213 | 4 | - | C | 39 51 33 80 |
| all3048 | 217 | 2 | DnaJ | C | 96 84 131 195 |
| all2395 (FraF) | 222 | 3 | - | C | 15 11 105 80 |
| alr1579 | 222 | 3 | - | P/L | 4 5 67 8 |
| all3740 (HetL) | 237 | 4 | - | C | 16 18 50 78 |
| all4152 | 450 | 1 | - | M | 9 21 28 27 |
| all3305(PatL) | 496 | 5 | - | M | 76 94 163 137 |
| alr3268 | 524 | 2 | Kinase | C | 6 8 17 23 |
| all3114 | 576 | 5 | - | C | 19 17 56 68 |
| alr9014\* | 679 | 3 | - | M | 40 40 87 73 |
| alr0704 | 693 | 3 | - | M | 21 28 39 42 |
| all0813 (HglK) | 727 | 4 | RDD domain | M | 65 52 316 222 |
| **Genes whose expression is not impacted by combined nitrogen starvation** | | | | | |
| all1812 | 125 | 1 | - | C |  |
| alr5209 | 129 | 2 | - | C |  |
| alr0433 | 143 | 2 | - | P/L |  |
| all4220 | 152 | 1 | - | P/L |  |
| alr2741 | 182 | 2 | - | M |  |
| all3332 | 206 | 1 |  | M |  |
| all3306 | 252 | 2 | - | C |  |
| alr2768 | 256 | 1 | - | L/P |  |
| all3256 | 268 | 4 | - | C |  |
| alr7125\* | 369 | 2 | - | C |  |
| all3869 | 376 | 4 | Endoribonuclease L-PSP | C |  |
| all0958 | 475 | 2 | - | C |  |
| alr1142 | 521 | 3 | Pentapeptide 4 (9PRs) | C |  |
| alr7124\* | 586 | 2 | - | M |  |
| alr3131 | 953 | 2 | - | C |  |
| all8023 | 1010 | 3 | - | C |  |

**Supplementary table 2**

**List of active residues used as input for HetRhood:HetL docking simulations**

|  |  |
| --- | --- |
| ***Protein*** | ***Active residues number*** |
| HetL | 0,1,2,3,5,7,8,9,10,11,12,13,14,15,16,18,19,20,21,23,24,26,28,29,31,33,36,39,43,44,46,49,51,53,54,58,59,61,64,66,68,71,74,78,79,81,83,84,88,89,91,93,94,96,98,99,103,104,106,108,111,113,114,116,119,121,123,124,126,128,129,130,131,132,133,134,136,139,141,143,144,146,149,151,153,158,159,161,163,164,166,169,171,174,175,176,177,178,179,180,181,184,186,187,189,191,192,194,197,199,201,204,206,207,209,211,212,214,215,216,217,218,219,220,221,222,224,225,226,227,228,230,231,232,233,234,235,236 |
| HetR hood | 222,223,224,225,227,228,229,231,233,235,236,239,240,241,243,244,245,246,247,249,250,252,253,254,256,259,260,261,262,263,264,266,267,268,269,271,272,273,274,276,279,280,281,282,283,285,287,296,297,298 |