Supplementary Materials

Cell-type-specific activation of two nitrogenase gene clusters by CnfR1 or CnfR2 in the cyanobacterium Anabaena variabilis

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Fig. S1. Phylogenetic tree of CnfR proteins. For strains that have two nif systems and thus two CnfR proteins, each copy is labeled on the tree. Blue box = CnfR in unicellular strains, Purple box = other CnfR genes, Grey box = CnfR2 family, Green box = CnfR1 genes in heterocystous cyanobacteria. Accession numbers for the genes are provided in Table S1. This BioNJ distance phylogeny tree was constructed using the program SeaView (1) with 100-replicate bootstrap values.
Table S1: Strains included in Fig. S1.

| IMG Gene ID | Genome Name | IMG Locus Tag |
|-------------|-------------|---------------|
| 637232894   | Nostoc sp. PCC 7120 | all2512 |
| 640625288   | Crocosphaera chwakensis CCY 0110 | CY0110_23386 |
| 642599653   | Nostoc punctiforme PCC 73102 | Npun_R0334 |
| 643475300   | Rippkaea orientalis PCC 8801 | PCC8801_2251 |
| 643480389   | Gloeothecae citriformis PCC 7424 | PCC7424_2132 |
| 643588188   | Cyanothecae sp. PCC 7425 | Cyan7425_5231 |
| 646565923   | Anabaena variabilis ATCC 29413 – cnfR1 | Ava_0444 |
| 646569759   | Anabaena variabilis ATCC 29413 – cnfR2 | Ava_4260 |
| 648188610   | Gloeotheca verrucosa PCC 7822 | Cyan7822_3694 |
| 2503614847  | Chroococcidiopsis thermalis PCC 7203 | Chro_4567 |
| 250379381   | Nostoc sp. PCC 7107 | Nos7107_0753 |
| 2504129697  | Anabaena cylindrica PCC 7122 | Anacy_0077 |
| 2509812238  | Nostoc sp. PCC 7524 | Nos7524_4670 |
| 2550718290  | Fischeraea thermalis PCC 7521 | UYKDRAFT_03084 |
| 2631162521  | Nostoc sp. Moss2 | Ga0080672_123114 |
| 2631163828  | Nostoc sp. Moss5 – cnfR2 | Ga0080674_104223 |
| 2631167682  | Nostoc sp. Moss5 – cnfR1 | Ga0080674_104408 |
| 2631177118  | Nostoc sp. Moss4 | Ga0080676_1211926 |
| 2634115732  | Nostoc sp. Moss3 | Ga0080673_0878 |
| 2634127375  | Nostoc sp. Moss6 – cnfR1 | Ga0080675_0504 |
| 2634128697  | Nostoc sp. Moss6 – cnfR2 | Ga0080675_1826 |
| 2774718376  | Leptolyngbya boryana dg5 | Ga0226171_122313 |
| 2776140273  | Tolypothrix tenuis PCC 7101 NIES-37 – cnfR2 | Ga0263545_16524 |
| 2776145131  | Tolypothrix tenuis PCC 7101 NIES-37 – cnfR1 | Ga0263545_165389 |
| 2833621481  | Nostoc linckia NIES-25 | Ga0263765_836 |
| 2843035298  | Nostoc linckia z1 | Ga0336783_054_19484_21073 |
| 2845924250  | Nostoc sp. ATCC 53789 | Ga0336842_155_87608_89203 |
| 2883181653  | Trichormus variabilis 0441 – cnfR2 | Ga0443053_01_273221_274816 |
| 2883182992  | Trichormus variabilis 0441 – cnfR1 | Ga0443053_01_1861810_1863441 |
| 2883410175  | Anabaena sp. YBS01 – cnfR2 | Ga0439566_01_365100_366695 |
| 2883411055  | Anabaena sp. YBS01 – cnfR1 | Ga0439566_01_1343754_1345343 |
| 2886593859  | Cyanothecae sp. BG0011 | Ga0452603_049_1738_3336 |
| 2909711544  | Nostoc sp. 2RC – cnfR1 | Ga0478412_158_79026_80615 |
| 2909716834  | Nostoc sp. 2RC – cnfR2 | Ga0478412_853_43_1659 |

[IMG: https://img.jgi.doe.gov/]
Fig. S2. Expression of cnfR2 under the control of the cnfR1 promoter or cnfR1 under the control of the cnfR2 promoter. Expression from P_{cnfR1::cnfR2} (BP870) and P_{cnfR2::cnfR1} (BP871) was determined by RT-qPCR in aerobic cells grown 24 h -N +O2, leading to formation of heterocysts that activate the cnfR1 promoter, or anaerobic cells grown 6 h -N -O2, leading to vegetative cells that activate the cnfR2 promoter. Expression of cnfR1 and cnfR2 was normalized to rnpB and the values on the y-axis represents the relative log2 fold differences in expression. Statistical analysis: $P < 0.001$ (**). The horizontal bars below the $P$-values provide statistical comparisons of the means for the two values immediately below the ends of the bar and do not include values between these ends.
| Strains          | Relevant characteristics                                                                 | Source or reference |
|-----------------|-------------------------------------------------------------------------------------------|---------------------|
| *Anabaena variabilis* FD | A derivative of *A. variabilis* 29413                                                     | (2)                 |
| *Nostoc* sp. PCC 7120    | A filamentous nitrogen-fixing cyanobacterium that differentiates heterocysts, but lacks the Nif2 system |                     |
| *Synechocystis* sp. PCC 6803 | non-filamentous, non-nitrogen fixing microorganism and source of the Co^{2+}-inducible promoter (PcoaT). |                     |
| BP291            | *Nostoc* sp. strain PCC 7120 derivative that expresses frtRABC.                          | (3)                 |
| BP870            | pBP870 (*PcnfR1:cnfR2* fusion) recombed into the *cnfR1* promoter of *A. variabilis* BP894 (*ΔcnfR1 ΔcnfR2* strain). | This study          |
| BP871            | pBP871 (*PcnfR2:cnfR1* fusion) recombed into the *cnfR2* promoter of *A. variabilis* BP894 (*ΔcnfR1 ΔcnfR2* strain). | This study          |
| BP873            | pBP873 (*PcnfR2:cnfR2:cnfR1HTH* fusion) recombed into the *cnfR2* promoter of *A. variabilis* BP894 (*ΔcnfR1 ΔcnfR2* strain). | This study          |
| BP893            | pBP890 (*nif2* operon with Sp' cassette) in BP291 (*Nostoc* sp. strain PCC 7120 containing the frtRABC operon) | This study          |
| BP894            | *A. variabilis* FD containing a deletion mutation in both *cnfR1* and *cnfR2* (Δ*cnfR1 ΔcnfR2*). | (4)                 |
| BP907            | pBP907 (*PcnfR2:cnfR1:cnfR2HTH* fusion) recombed into the *cnfR2* promoter of *A. variabilis* BP894 (*ΔcnfR1 ΔcnfR2* strain). | This study          |
| BP910            | pBP910 (*PcnfR1:cnfR2:cnfR1HTH* fusion) recombed into the *cnfR1* promoter of *A. variabilis* BP894 (*ΔcnfR1 ΔcnfR2* strain). | This study          |
| BP920            | pBP920 (*PcnfR1:cnfR1* fusion) recombed into the *cnfR1* promoter of *A. variabilis* BP894 (*ΔcnfR1 ΔcnfR2* strain). | This study          |
| Code | Description |
|------|-------------|
| BP921 | pBP921 (*PcnfR2*:*cnfR2* fusion) recombined into the *cnfR2* promoter of *A. variabilis* BP894 (*ΔcnfR1 ΔcnfR2* strain). | This study |
| BP950 | pBP950 (*PcnfR1:*cnfR1:*cnfR2:*cnfR1HTH* fusion) recombined into the *cnfR1* promoter of *A. variabilis* BP894 (*ΔcnfR1 ΔcnfR2* strain). | This study |
| BP951 | pBP951 (*PcnfR1:*cnfR1:*cnfR2:*cnfR1HTH* fusion) recombined into the *cnfR1* promoter of *A. variabilis* BP894 (*ΔcnfR1 ΔcnfR2* strain). | This study |
| BP952 | pBP952 (*PcnfR1:*cnfR1:*cnfR2:*cnfR1HTH* fusion) recombined into the *cnfR1* promoter of *A. variabilis* BP894 (*ΔcnfR1 ΔcnfR2* strain). | This study |
| BP1101 | *PnifB2:B1:B2* promoter singly recombined to drive the *nifB2* operon in JE9 (a *xisA-nifE1* deletion strain of *A. variabilis*). | This study |
| BP1107 | pBP1107 doubly recombined both upstream and downstream of *hetR* in *A. variabilis* FD to create a *hetR* deletion. | This study |
| BP1108 | pBP1108 doubly recombined both upstream and downstream of *nrrA* in *A. variabilis* FD to create a *nrrA* deletion. | This study |
| BP1141 | pBP1141 (*PcoaT:*lacZ* fusion*) in *A. variabilis* FD | This study |
| BP1142 | pBP1142 (*PcoaT:*cnfR1* fusion*) in *A. variabilis* BP894 (*ΔcnfR1ΔcnfR2* strain) | This study |
| BP1143 | pBP1143 (*PcoaT:*cnfR2* fusion*) in *A. variabilis* BP894 (*ΔcnfR1ΔcnfR2* strain) | This study |
| BP2197 | pBP1197 (*PnifB2:B1:B2:*lacZ* fusion*) in *A. variabilis* BP1142 (*PcoaT:*cnfR1* in *ΔcnfR1ΔcnfR2* strain) | This study |
| BP3197 | pBP1197 (*PnifB2:B1:B2:*lacZ* fusion*) in *A. variabilis* BP1143 (*PcoaT:*cnfR2* in *ΔcnfR1ΔcnfR2* strain) | This study |
| BP7142 | pBP1142 (*PcoaT:*cnfR1* fusion*) in *A. variabilis* MM3 (*ntca* strain) | This study |
| BP7143 | pBP1143 (*PcoaT:*cnfR2* fusion*) in *A. variabilis* MM3 (*ntca* strain) | This study |
| JE9       | *xisA-nifEl* region of the *nif1I* genes interrupted with an Nm<sup>+</sup> gene | (5) |
|-----------|-----------------------------------------------------------------------------|-----|
| MM3       | *A. variabilis* FD containing a mutation in *ntcA*.                            | (6) |
| Plasmids             | Description                                                                                   | Source          |
|---------------------|-----------------------------------------------------------------------------------------------|-----------------|
| pAAWY22574         | Plasmid from JGI containing the *nrrA* region                                                 | JGI             |
| pAAWY8706          | Plasmid from JGI containing the *hetR* region                                                  | JGI             |
| pAAWZ1787          | Fosmid from JGI containing the *nif2* cluster from *ava_4241-ava_4266.*                       | JGI             |
| pBP639             | Vector with a promoterless *lacZ* for promoter fusions and a Tc' cassette for selection of promoter fragments; allows for recombination into a gene promoter region of the chromosome; Km'Tc' | (7)             |
| pBP716             | 1.9-kb Smal fragment containing the *aadA* cassette (Sp'Sm') from pRL5801 into the Smal site of pRL2948a, containing *oriT* site required for conjugation into cyanobacteria. | This study      |
| pBP744             | Vector with a promoterless *lacZ* for promoter fusions and a Tc' cassette for selection of promoter fragments; allows for recombination into the *frtBC* region of the chromosome; Km'Tc' | (4)             |
| pBP870             | *PcnfR1*:cnfR2 fusion was inserted between the BglIII and SacI sites of pBP639.               | (4)             |
| pBP871             | *PcnfR2*:cnfR1 fusion was inserted between the BglIII and SacI sites of pBP639.               | (4)             |
| pBP872             | *PcnfR1*:cnfR1cnfR2HTH fusion was created using fusion PCR; *PcnfR1*:cnfR1HTH- and cnfR2HTH fragments were amplified using primers sets patB1L102 + patB1HTHB2-R and patB1HTHB2-L + patB2-R(Fus), respectively, fused into a single fragment, and then inserted between the BglIII and SacI sites of pBP639. | This study      |
| pBP873             | *PcnfR2*:cnfR2cnfR1HTH fusion was created using fusion PCR; *PcnfR2*:cnfR2HTH- and cnfR1HTH fragments were amplified using primer sets patB2L201 + patB2HTHB1-R and patB2HTHB1-L + patB1-R(Fus), respectively, fused into a single fragment, and then inserted between the BglIII and SacI sites of pBP639. | This study      |
| pBP890 | 2.1-kb PCR fragment, containing the oriT site and aada (Sp'Sm') cassette, was generated from pBP716 using primers FosOriTSp-L2 + FosOriTSp-R2 and recombineered into pAAWZ1787 at the Cmr cassette. This study |
| pBP907 | *PcnfR2:cnfR1cnfR2HTH* fusion was created using fusion PCR; *PcnfR2* and *cnfR1:cnfR2HTH* were amplified from pBP873 using primer set patB2-L201 + P2patB1-R and from pBP872 using primer set P2patB1-L + patB2-R(Fus), respectively, fused into a single fragment, and then inserted between the BglII and SacI sites of pBP639. This study |
| pBP910 | *PcnfR1:cnfR2cnfR1HTH* fusion was created using fusion PCR; *PcnfR1* and *cnfR2:cnfR1HTH* were amplified from pBP872 using primer set patB1L102 + P1patB2-R and from pBP873 using primer set P1patB2-L + patB1-R(Fus), respectively, fused into a single fragment, and then inserted between the BglII and SacI sites of pBP639. This study |
| pBP920 | *PcnfR1:cnfR1* (wild-type) was amplified by PCR using primers patB1L102 and patB1-R(Fus) and then inserted between the BglII and SacI sites of pBP639. This study |
| pBP921 | *PcnfR2:cnfR2* (wild-type) was amplified by PCR using primers patB2L-201 and patB2-R(Fus) and then inserted between the BglII and SacI sites of pBP639. This study |
| pBP950 | *PcnfR1:cnfR1*1-179 cnfR2174-1425 cnfR1HTH1420-1590 fusion was created using fusion PCR; *pcnfR1:cnfR1*1-179 and *cnfR2*174-1425 *cnfR1HTH1420-1590* fragments were amplified from pBP872 using primer set patB1L102 + P1cnfR2fusion1-R and from pBP873 using primer set P1cnfR2fusion1-L + patB1-R (fus), respectively, fused into a single fragment, digested with BglII and SacI, and then inserted between the BglII and SacI sites of pBP639. This study |
| pBP951 | *PcnfR1:cnfR1*1-476 cnfR2486-1425 cnfR1HTH1420-1590 fusion was created using fusion PCR; *pcnfR1:cnfR1*1-476 and *cnfR2*486-1425 *cnfR1HTH1420-1590* fragments were amplified from pBP872 using primer set patB1L102+ P1cnfR2fusion2-R and from pBP873 This study |
| **pBP952** | **PcnfR1:cnfR1**$^{1-992}$**cnfR2**$^{1002-1425}$**cnfR1HTH**$^{1420-1590}$ fusion was created using fusion PCR; **PcnfR1:cnfR1**$^{1-992}$ and **cnfR2**$^{1002-1425}$**cnfR1HTH**$^{1420-1590}$ fragments were amplified from pBP902 using primer set **patB1L102+ P1cnfR2fusion3-R** and from pBP903 using primer set **P1cnfR2fusion3-L + patB1-R (fus)**, respectively, fused into a single fragment, digested with BglII and Sacl, and then inserted between the BglII and Sacl sites of pBP639. |
| **pBP1101** | **PnifB2:nifB1:nifB2** promoter was amplified by PCR from pSM54 using primers **pnifB2-L1(JJ) & pnifB2-BsaR1** and an additional 870-bp region at the 5’ end of the nifB2 gene was amplified from FD by PCR using primers **pnifB2-BsaL1 & nifB2-SmaR2** to create a larger region for recombination for the hybrid promoter. These fragments were digested with BglII & BsaI and BsaI & SmaI, respectively, and inserted between the BglII and SmaI sites of pBP639. |
| **pBP1104** | A **hetR** deletion was created using fusion PCR; fragments upstream and downstream of **hetR** were amplified from pAAWY8706 using primer sets **hetRmut-L1(BamHI) & hetRmut-R1** and **hetRmut-L2 & hetRmut-R2 (PstI)**, respectively, fused into a single fragment, digested with BamHI and PstI, and then inserted between the BamHI and PstI sites of pUC18. |
| **pBP1105** | A **nrrA** deletion was created using fusion PCR; fragments upstream and downstream of **nrrA** were amplified from pAAWY22574 using primer sets **nrrAmut-L1(BamHI) & nrrAmut-R1** and **nrrAmut-L2 & nrrAmut-R2 (PstI)**, respectively, fused into a single fragment, digested with BamHI and PstI, and then inserted between the BamHI and PstI sites of pUC18. |
| **pBP1107** | 6-kb Sacl fragment of pRL2948a cloned into the Sacl site of pBP1104. |
| Vector | Description | Source |
|--------|-------------|--------|
| pBP1108 | 6-kb SacI fragment of pRL2948a cloned into the SacI site of pBP1105 | This study |
| pBP1193 | Inserted 2-kb HindIII fragment from pRL2949a containing the Sp' cassette into the HindIII site of pMH5, a modA integration vector. | This study |
| pBP1195 | Inserted 1.7-kb BglII-Smal fragment from pBP639 containing the Tc' cassette between the BglII and Smal sites of pBP1193. | This study |
| pBP1197 | Inserted 1.1-kb BglII-Sacl fragment containing PnifB2nifB1nifB2 from pSM54 into the 12.2-kb BglII-Sacl fragment of pBP1195. | This study |
| pBP1141 | PcoaT: lacZ fusion was created using fusion PCR; PcoaT and lacZ fragments were amplified from Synechocystis sp. PCC 6803 using primer set pcoaR-L(BglII) + pcoaT: lacZ-R and from pBP639 using primer set pcoaT: lacZ-L + lacZ-R(Sacl) respectively, fused into a single fragment, digested with BglII and Sacl, and then inserted between the BglII and Sacl sites of pBP744. | This study |
| pBP1142 | PcoaT: cnfR1 fusion was created using fusion PCR; PcoaT and cnfR1 fragments were amplified from Synechocystis sp. PCC 6803 using primer set pcoaR-L(BglII) + pcoaT: cnfR1-R and from pBP920 using primer set pcoaT: cnfR1-L + patB1-R(Fus), respectively, fused into a single fragment, digested with BglII and Sacl, and then inserted between the BglII and Sacl sites of pBP744. | This study |
| pBP1143 | PcoaT: cnfR2 fusion was created using fusion PCR; PcoaT and cnfR2 fragments were amplified from Synechocystis sp. PCC 6803 using primer set pcoaR-L(BglII) + pcoaT: cnfR2-R and from pBP921 using primer set pcoaT: cnfR2-L + patB2-R(Fus), respectively, fused into a single fragment, digested with BglII and Sacl, and then inserted between the BglII and Sacl sites of pBP744. | This study |
| pJU410 | PnifH1: lacZ fusion vector; Km' | (3) |
| pKA6 | Source of 2.6-kb orf-modA region | (8) |
| pKM208 | Plasmid containing all the genes for recombineering under the Ptac promoter; red and gam genes are | (9) |
turned off using $lacI$; and has a temperature-sensitive origin of replication which allows it to be removed from the strain by growing at 37°C.

| pMH2    | A 1.4-kb XbaI fragment containing the $modAE$ from pKA6 was blunted and inserted into the Scal site of pJU410 to create a $lacZ$ vector that could integrate into the $modAE$ region in cyanobacteria. | This study |
|---------|-------------------------------------------------------------------------------------------------|------------|
| pMH5    | 1.1-kb PCR product, containing the Em′ cassette was amplified from pRL2948a using primers Em(BglII)-L + Em(HindIII)-R, digested with BglII and HindIII, and inserted between the BglII and HindIII sites of pMH2. | This study |
| pRL2948a| Source of mobilization site ($oriT$) and $sacB$ gene which confers sucrose sensitivity; Cm′Em′ | C.P. Wolk |
| pRL2949a| Source of mobilization site, $oriT$, and $sacB$ gene which confers sucrose sensitivity; Sp′ | C.P. Wolk |
| pRL5801 | Source of $aadA$ cassette (Sp′Sm′) | C.P. Wolk |
| pSM54   | Source of hybrid $nifB2:nifB1:nifB2$ promoter fused to $lacZ$ | (10) |
| pUC18   | Plasmid cloning vector; Ap′ | (11) |
| Oligonucleotide for cloning | Purpose | Sequence (5′→3′) DNA |
|----------------------------|---------|---------------------|
| Em(BglII)-L | Amplifies from the 5' end of the Em' cassette | ATTAAGATCTCTCTAGCTCCTGAAAATCTCG |
| Em(HindIII)-R | Amplifies from the 3' end of the Em' cassette | TATAAAGCTTGCACCTGCTCCTTTAATTACTTA |
| FosOriTSp-L2 | Amplifies the oriT and Sp' cassette | GGCGTATTTTTTGAGTTATCGAGATTTTCAGGAGCTAAGG AAGCTAAAAATAGTAACGGCAGGTATATGTGATG |
| FosOriTSp-R2 | Amplifies the oriT and Sp' cassette | TTATCAGTTATCCAGCGTACCAAGCAGCGTCTTAAGGGC ACCAAATACTTCTGAACGAAATTGAGACATTAGT |
| hetRmutchk-L | Spans the hetR deletion | TACGGGAGGAATGAGCATCTCG |
| hetRmutchk-R | Spans the hetR deletion | GTCGCGTTGGCTTAATTCTTG |
| HetRmut-L1(BamHI) | Amplifies 5' region in hetR deletion | ATTAGGATCCCAGCTATTATTAGTGCAAGCGTACC |
| HetRmut-L2 | Amplifies 3' region in hetR deletion | TTGTAATATGAGTAACGACATCGGTCTGTGTTGGTGA AAAGAAGATTAAGC |
| HetRmut-R1 | Amplifies 5' region in hetR deletion | CTTTACAGTTCTCTCTGTTACCAACACCAGATCGATGCTGTT ACTCATATTACAA |
| HetRmut-R2(PstI) | Amplifies 3' region in hetR deletion | TATACTGAGCTCATTTACCTTTGGCATTGC |
| lacZ-R(Sacl) | Amplifies the lacZ portion of the PcoAT::lacZ fusion | CAGTGCAGAGCTCGTTATCG |
| nifB2-SmaR2 | Extends the nifB2 region of pnifB2::nifB1:nifB2 | ATTACCCGGGCGATTATCTCTTTGATTTTGATCTGATTTTATG |
| nrrAmutchk-L | Spans the nrrA deletion | TATCACTGCGCTCTGGACAA |
| nrrAmutchk-R | Spans the nrrA deletion | AAACCAAGCCGATGAAGATAAG |
| Primer Name          | Function                                      | Amplified Region                                      | Sequences                                      |
|---------------------|-----------------------------------------------|-------------------------------------------------------|------------------------------------------------|
| nrrAmut-L1(BamHI)   | Amplifies 5’ region in nrrA deletion          | TAATGGATCCGGTTTCTATATTCTGAATTTTGACAATC                |
| nrrAmut-L2          | Amplifies 3’ region in nrrA deletion          | GTGGGTTCGGTTTGTATTGAAGTGAGAGGGAGTTAGGAG              |
| nrrAmut-R1          | Amplifies 5’ region in nrrA deletion          | CTCTAATCCCCTACCTCTCCTACCTCAATAACACCCGAAAC            |
| nrrAmut-R2(PstI)    | Amplifies 3’ region in nrrA deletion          | TAATCTGCAGCAAGGAATACAGTTAAATATCCATATCCACTG          |
| P1cnfR2fusion1-L    | Amplifies cnfR2<sup>174-1425</sup>-cnfR1HTH<sup>1420-1590</sup> portion of the hybrid fusion in BP950 | TGTGATAGCCTGTCAGTAAACATCCCCATCCCCCTCTACACAGCCAAAAAG |
| P1cnfR2fusion1-R    | Amplifies PcnfR1:cnfR1<sup>1-179</sup> portion of the hybrid fusion in BP950 | CTTTTTTCGGGAGGAGGATTGGGATTTTACTGGACAAAGTCATCACAC  |
| P1cnfR2fusion2-L    | Amplifies cnfR2<sup>486-1425</sup>-cnfR1HTH<sup>1420-1590</sup> portion of the hybrid fusion in BP951 | ATGATAGCAAGCCAGTTGAGTATGGGATAGGAGCTGCTTCGAGC     |
| P1cnfR2fusion2-R    | Amplifies PcnfR1:cnfR1<sup>1-476</sup> portion of the hybrid fusion in BP951 | GCAGGTGCATACAAACAGTGCTCTAACATATTACCTACATTGGCTGCTACAT |
| P1cnfR2fusion3-L    | Amplifies cnfR2<sup>1002-1425</sup>-cnfR1HTH<sup>1420-1590</sup> portion of the hybrid fusion in BP952 | GTTATTATTAAACAAAAATGGGAAGGAGACATGCTAGTCTTCTGTGAAAATCTGCTATGCTGTACAT |
| P1cnfR2fusion3-R    | Amplifies PcnfR1:cnfR1<sup>1-992</sup> portion of the hybrid fusion in BP952 | AGCATAGGAATTGTAATGCACGTGTTCCATTTTGGTTTAAATAAAC    |
| P1patB2-L           | Amplifies cnfR2:cnfR1HTH portion in the PcnfR1:cnfR2:cnfR1HTH fusion, BP910 | ACGATTAGGGTGGGAAGAAGTTATATGCCCTACGGCATTACTATCG |
| Primer | Description | Sequence | Primer | Description | Sequence |
|--------|-------------|----------|----|-------------|----------|
| P1patB2-R | Amplifies *PcnfR1* portion in the *PcnfR1:cnfR2cnfR1HTH* fusion, BP910 | CGATTAGTAATGGCGTAGGGCATATAACTTTCTTCCCCACCC TAATCGT | P2patB1-L | Amplifies *PcnfR2* portion in the *PcnfR2:cnfR1cnfR2HTH* fusion, BP907 | CTACCTGAAAATCAAGTTGCAAAAAACTAGCTTTATACACAT TCCTAAACACAGTTG |
| P2patB1-R | Amplifies *cnfR1:cnfR2HTH* portion in the *PcnfR2:cnfR1cnfR2HTH* fusion, BP907 | CAACGTTGGAAGATTGTAATAGCGATTTTGTGCAACTGATTTTCAAGTAG | patB1HTHB2-L | Amplifies *cnfR2HTH* portion in the *PcnfR1:cnfR1:cnfR2HTH* fusion; BP872 | CAAAAAAAACCCCGAGGCTTCTCTCTACTAGAATTTA TTATTGAGGTAGAA |
| patB1HTHB2-R | Amplifies *cnfR1:cnfR1HTH* portion in the *PcnfR1:cnfR1:cnfR2HTH* fusion; BP872 | TTCTAGCCTCATAAATAATTCTCTAGAAAGATCACCTGCGGTTTTTATTTTTT |
| patB1L102 | Amplifies promoter region in *cnfR1* fusion constructs | TTAGATCTTTTTATCCGGTGAGAAACTTACTA | patB1-R (Fus) | Amplifies the C-terminal end of *cnfR1* fusion constructs | ATTAGAGCTCAGGTTTAACCTTAGAAATTCTGCTTAT |
| patB2HTHB1-L | Amplifies *cnfR1HTH* portion in the *PcnfR2:cnfR2:cnfR1HTH* fusion; BP873 | AGACAAACTTAAAACCGCAATAACTTATGGTGAGCA AATTTTGGAAAG | patB2HTHB1-R | Amplifies *PcnfR2:cnfR2HTH* portion in the *PcnfR2:cnfR2:cnfR1HTH* fusion; BP873 | CTTGCAAATTTGTGCTACCAACTAAAGTTATGTCGCGTTTT AAGTTTTGTCT |
| Fusion       | Description                                                                 | Sequence                                                                 |
|-------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------|
| patB2-L201  | Amplifies promoter region in *cnfR2* fusion constructs                      | TATTAGATCTGCACTCATCAAGAAACCCTATTTTAGTTT                                     |
| patB2-R(Fus)| Amplifies the C-terminal end of *cnfR2* fusion constructs                   | ATTAGAGCTCAGGGTGAGGTTTTGAATAGTTAATTCTT                                      |
| pcoaR-L (BglII) | Amplifies *PcoaT* region in the *PcoaT*:fusions                          | ATTAAGATCTCTAAAGACAAAGTGAGATAGCAGTGCC                                       |
| pcoaT:cnfR1-L | Amplifies *cnfR1* gene in the *PcoaT*:cnfR1 fusion, BP1142               | AGGTTAAAATCCCAAAGTTAAAAGCATGCTTATAACATTCCTAAACACAGTT                        |
| pcoaT:cnfR1-R | Amplifies *PcoaT* region in the *PcoaT*:cnfR1 fusion, BP1142             | CAACTGTTGTAGGAAATTGTATAAGGCGATGCTTTTTAAACTTGATTTTACCT                       |
| pcoaT:cnfR2-L | Amplifies *cnfR2* gene in the *PcoaT*:cnfR2 fusion, BP1143              | AGGTTAAAATCCCAAAGTTAAAAGCATGCCCTACCGCATTATACTCG                             |
| pcoaT:cnfR2-R | Amplifies *PcoaT* region in the *PcoaT*:cnfR2 fusion, BP1143             | CGATTAGTAATGGCGTAGGGCATGCTTTTTAAACTXGATTTTTACCT                              |
| pcoaT:lacZ-L | Amplifies *lacZ* gene in the *PcoaT*:lacZ fusion, BP1141                | AGGTTAAAATCCCAAAGTTAAAAGCATGACCAGATTACGCGATCTG                              |
| pcoaT:lacZ-R | Amplifies *PcoaT* region in the *PcoaT*:lacZ fusion, BP1141             | CAGTGAATCCCGTAATCATGGTCATGCTTTTTAACTTGGATTTTTACCT                           |
| pnifB2-BsaL1 | Amplifies the *pnifB2:nifB1:nifB2* promoter to extend the *nifB2* region in BP1101 | ATGGTCTCATTTCAACATCGCCACTCCACAA                                              |
| pnifB2-BsaR1 | Amplifies and extends the *nifB2* gene of *pnifB2:nifB1:nifB2* in BP1101 | ATGGTCTCAAGAAGTCTGAGGTGAGGTGGTGGTGC                                      |
| pnifB2-L1 (JJ) | Amplifies the *pnifB2:nifB1:nifB2* promoter to extend                  | AGATCTGCTACAAAGACGCTTTAATCTATTACC                                           |
| Oligonucleotide for qPCR | Purpose | Sequence (5'→3') DNA |
|-------------------------|---------|----------------------|
| qcnfR2-mutL            | qPCR primer that amplifies the deleted region of cnfR2; Fig. 2C | CCCTTGGGAAAECTGATGAGTCG |
| qcnfR2-mutR            | qPCR primer that amplifies the deleted region of cnfR2; Fig. 2C | TGATGGCTCTGATTGTACGCT |
| qcnfRg1-R2             | qPCR primer that specifically amplifies pcnfR2:cnfR1 fusion; Fig. S2 | GATTTTGATTGCCACCGTAG |
| qcnfRg2-R1             | qPCR primer that specifically amplifies pcnfR1:cnfR2 fusion; Fig. S2 | CCGTCTATAACTTTGATCGCA |
| qcnfRp1-L1             | qPCR primer that specifically amplifies pcnfR1:cnfR2 fusion; Fig. S2 | CATCTCAACGATTAGGGTG |
| qcnfRp2-L2             | qPCR primer that specifically amplifies pcnfR2:cnfR1 fusion; Fig. S2 | ACGGCTGAATGTTCCAATA |
| qhesA2-L               | qPCR primer that amplifies the hesA2 gene; Fig. 6 | TTCAATCCGCGATGAGCCT |
| qhesA2-R               | qPCR primer that amplifies the hesA2 gene; Fig. 6 | AAGCATCCATCCATCCATT |
| qnifB1-L               | qPCR primer that amplifies the nifB1 gene; Fig. 1, Fig. 3A&C | TTGCAGACAAAGCCCGAGATA |
| Primer Name | qPCR primer that amplifies the nif gene; | Sequence |
|-------------|----------------------------------------|----------|
| qnifB1-R    | qnifB1 gene; Fig. 1, Fig. 3A&C | CGCCTCGCACCTCTATAAC |
| qnifB2 -L   | qnifB2 gene; Fig. 1, Fig. 3B&D, Fig. 5, Fig. 7B | ACACCACCACGTACGGATG |
| qnifB2 -R   | qnifB2 gene; Fig. 1, Fig. 3B&D, Fig. 5, Fig. 7B | CCGGACGGCTTTCAATTTCAC |
| qnifEN2 -L  | qnifEN2 gene; Fig. 6 | GTCCCTGATCATTCCACCTCCC |
| qnifEN2 -R  | qnifEN2 gene; Fig. 6 | ACCCGAACTACCATCTGTCG |
| qnifH2 -L   | qnifH2 gene; Fig. 7B | CAAAATGGACATCCACCCACC |
| qnifH2 -R   | qnifH2 gene; Fig. 7B | ACTACTGCTTTGCCCTGCTT |
| qnifZ2 -L   | qnifZ2 gene; Fig. 6 | TGAAACATAGCCAACCTTCCT |
| qnifZ2 -R   | qnifZ2 gene; Fig. 6 | ACCAGCATTTGAAATTGCCGCA |
| qpatB1-L    | qPCR primer that amplifies the deleted region of cnfR1; Fig. 2C | GAGAAACGCAAAGACCTCAGC |
| qpatB1-R    | qPCR primer that amplifies the deleted region of cnfR1; Fig. 2C | AGCGGCTATTGCGACTGAAA |
| qpatB25' -L | qPCR primer that amplifies the 5’ end of the cnfR2 gene; Fig. 7B | TTGACAAAACTATGCCCTACG |
| qpatB25' -R | qPCR primer that amplifies the 5’ end of the cnfR2 gene; Fig. 7B | ACACAAAACTAGGGTCAATCCAGTAATC |

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