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(54) **CONTROL OF NITROGEN FIXATION IN RHIZOBIA THAT ASSOCIATE WITH CEREALS**

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(60) Provisional application No. 62/820,765, filed on Mar. 19, 2019.

Publication Classification

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C12N 15/87 (2006.01)
(52) **U.S. Cl.**
CPC *C12N 1/20* (2013.01); *C05F 11/08* (2013.01); *C12N 15/87* (2013.01); *C12N 2510/00* (2013.01)

(57) ABSTRACT

Disclosed herein are engineered rhizobia having nif clusters that enable the fixation of nitrogen under free-living conditions, as well as ammonium and oxygen tolerant nitrogen fixation under free-living conditions. Also provided are methods for producing nitrogen for consumption by a cereal crop using these engineered rhizobia.

Specification includes a Sequence Listing.

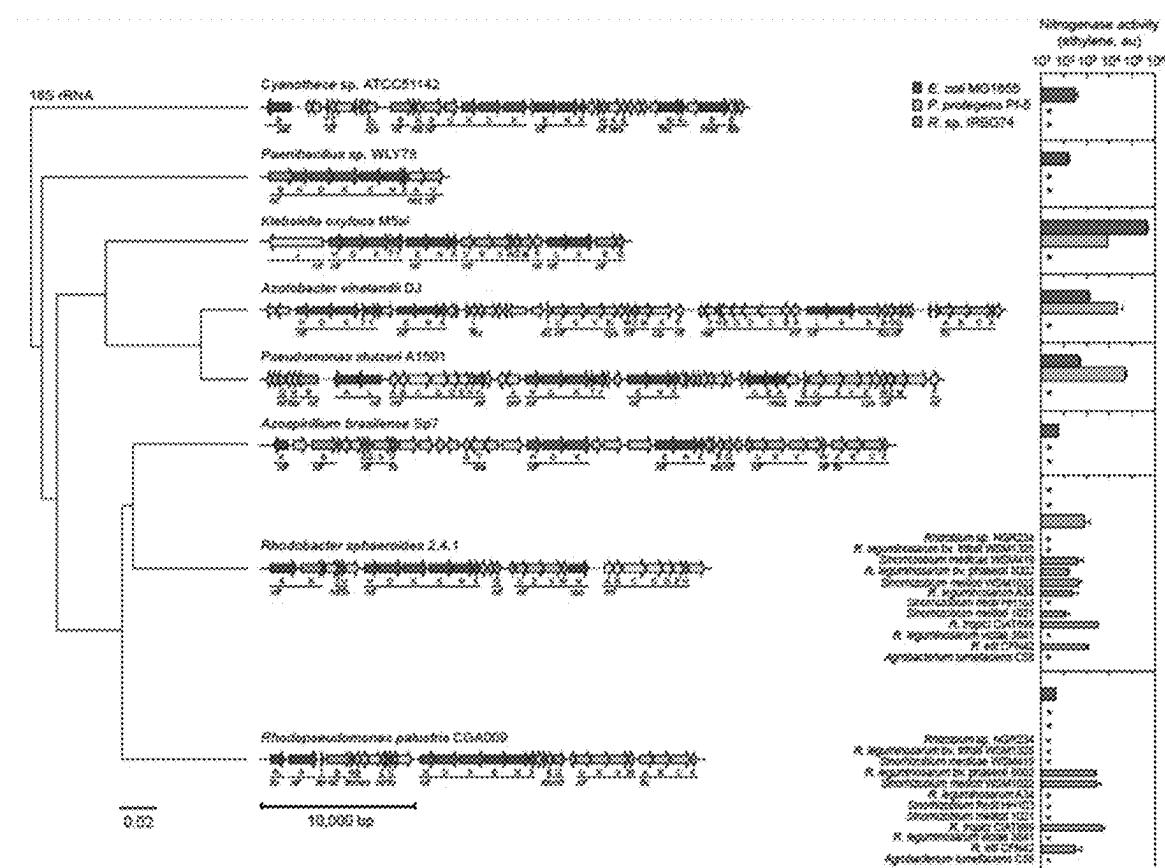


FIG. 1A

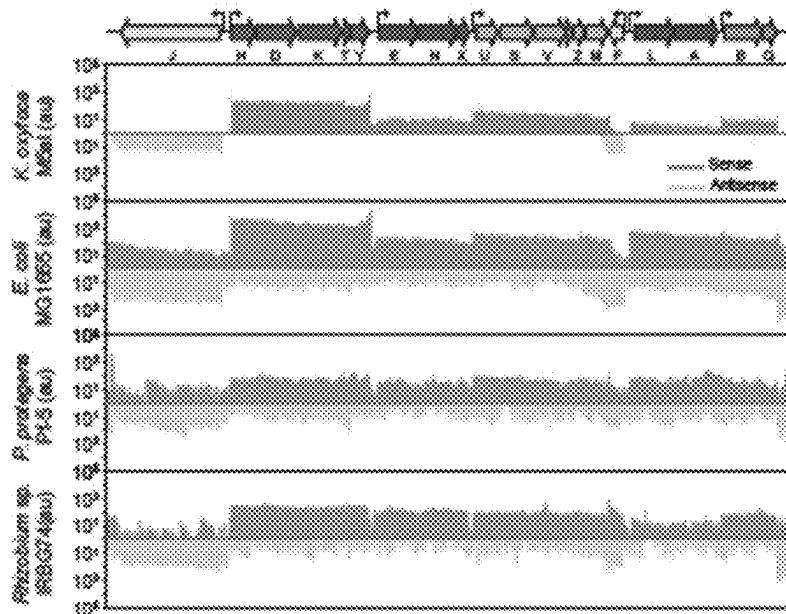


FIG. 1B

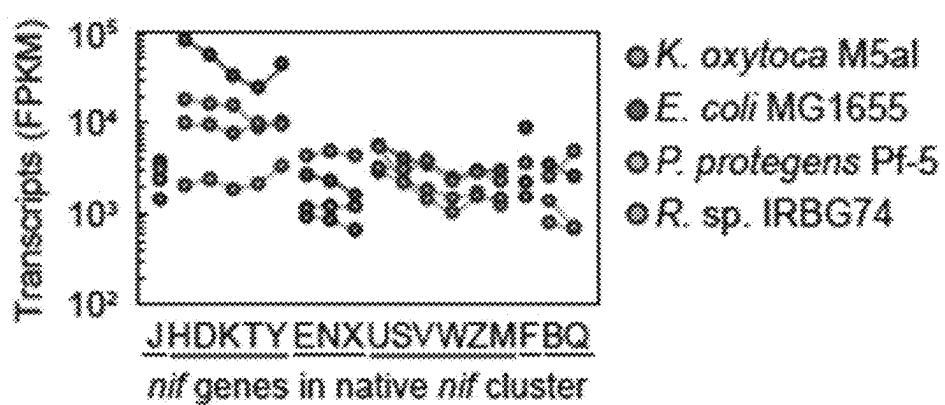


FIG. 1C

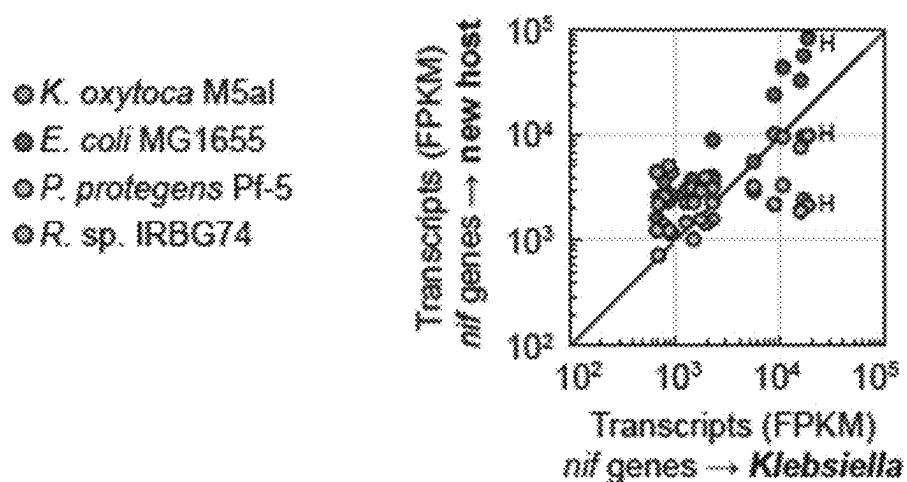


FIG. 1D

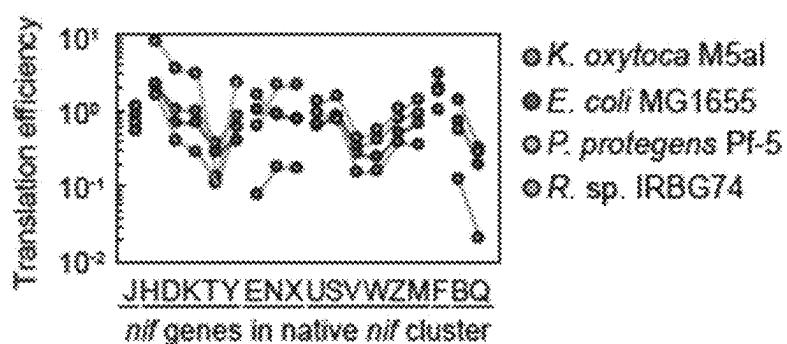


FIG. 1E

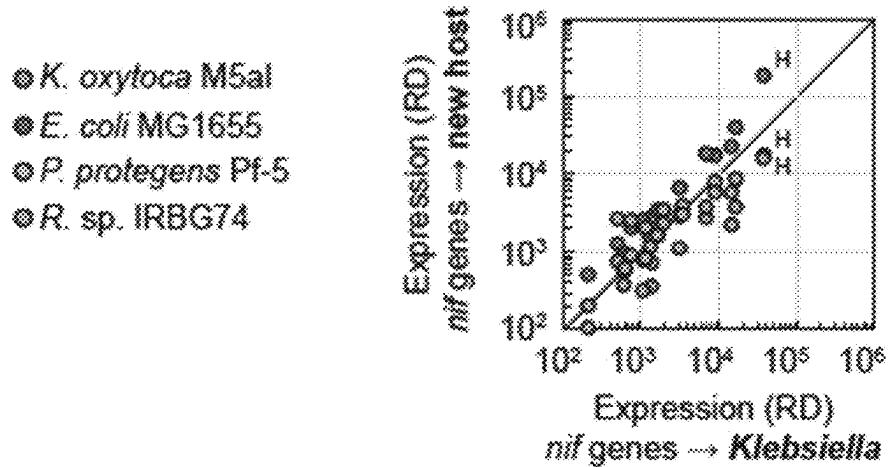


FIG. 1F

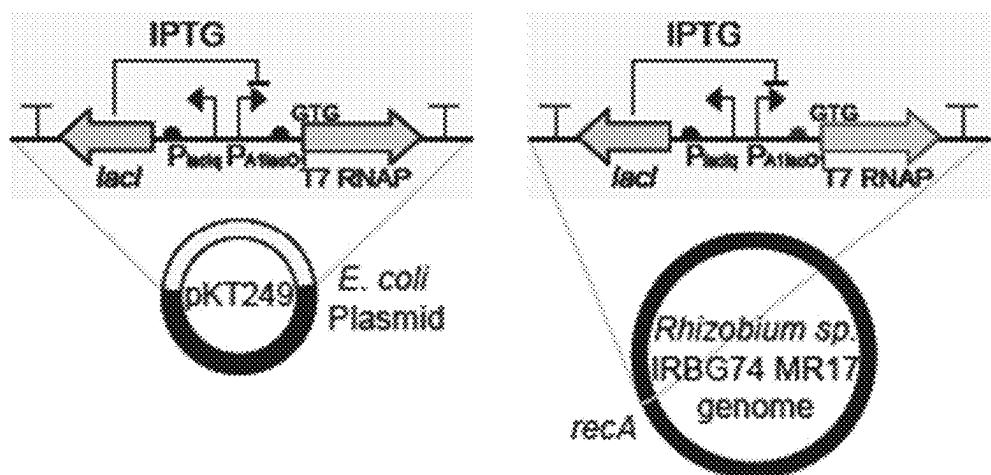


FIG. 2A

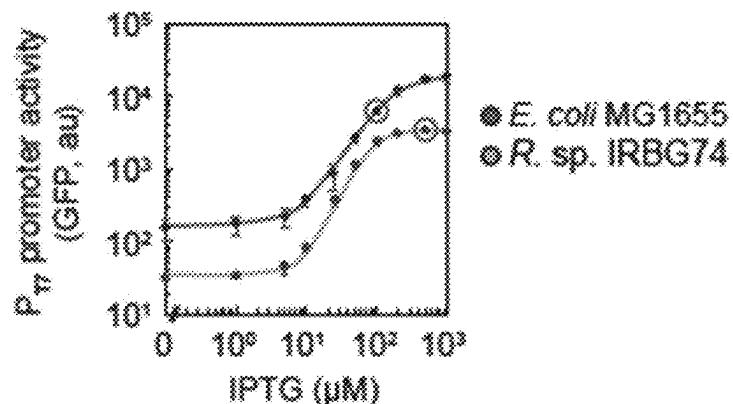


FIG. 2B

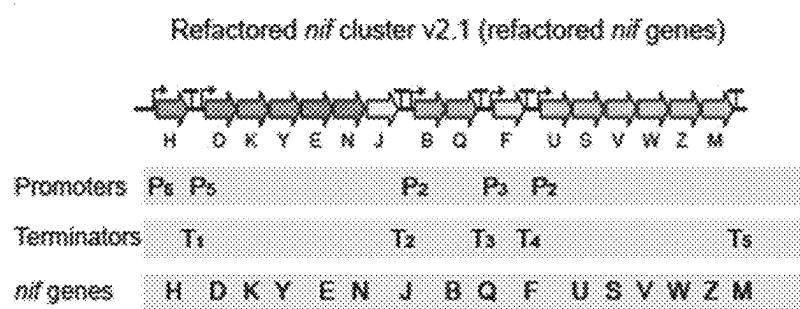


FIG. 2C

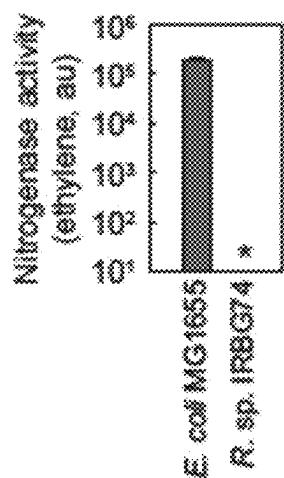


FIG. 2D

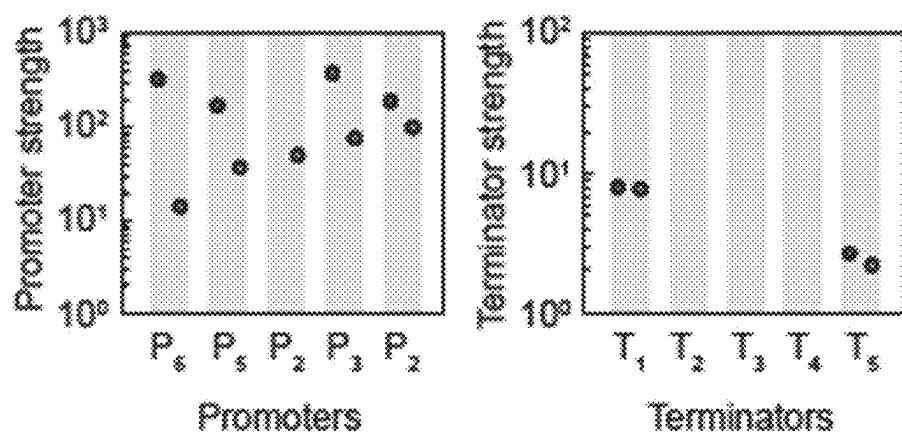


FIG. 2E

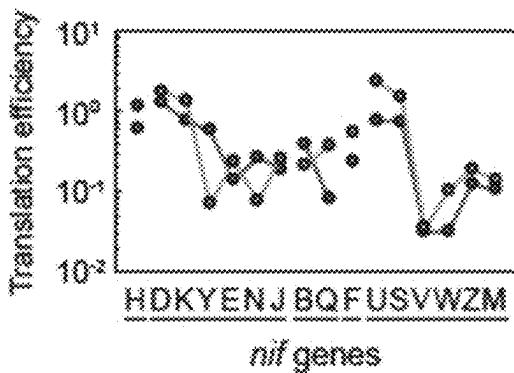


FIG. 2F

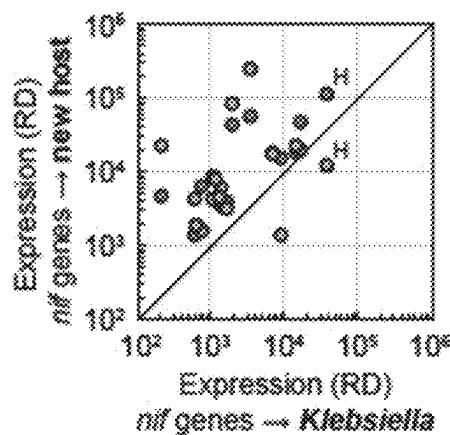


FIG. 2G

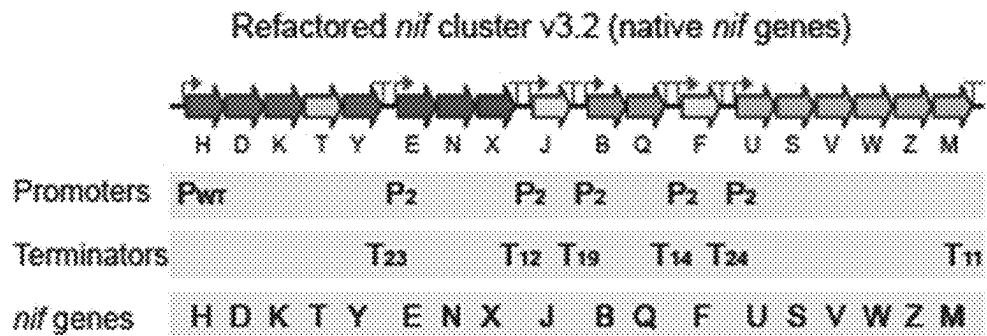


FIG. 2H

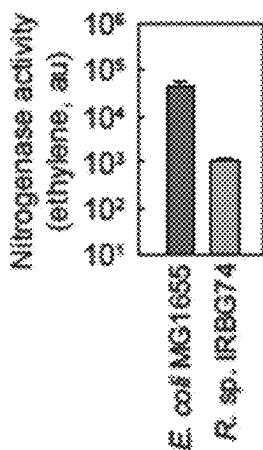


FIG. 2I

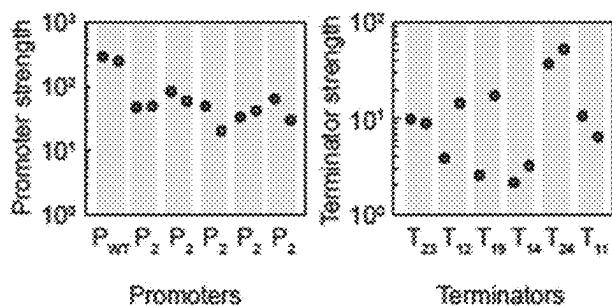


FIG. 2J

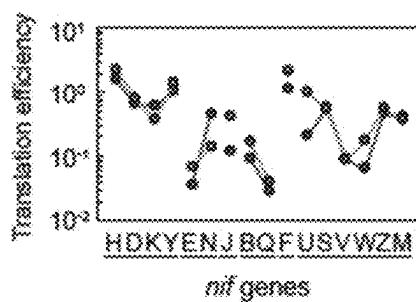


FIG. 2K

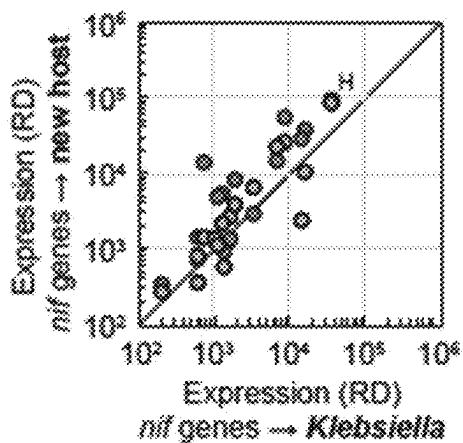


FIG. 2L

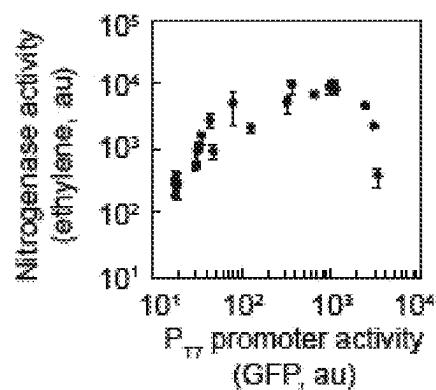


FIG. 2M

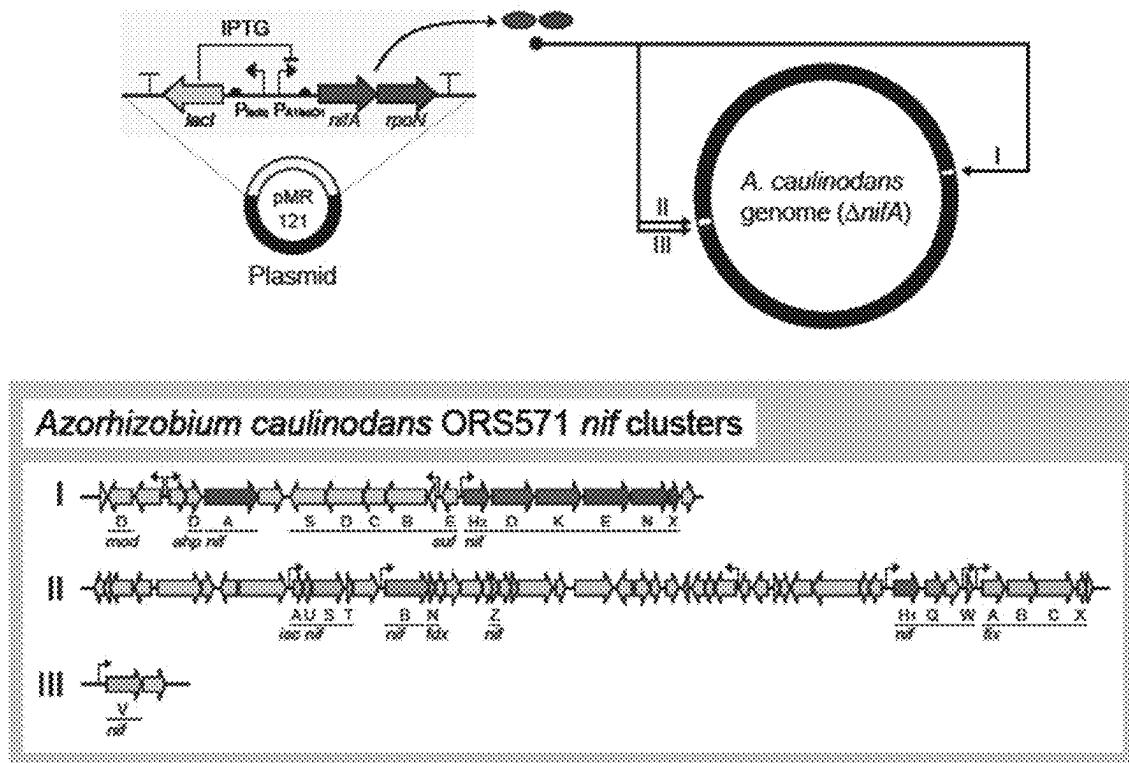


FIG. 3A

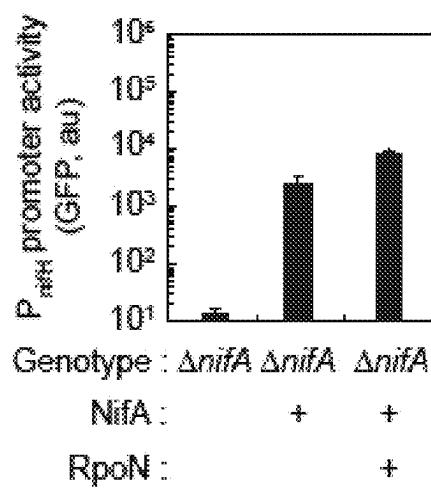


FIG. 3B

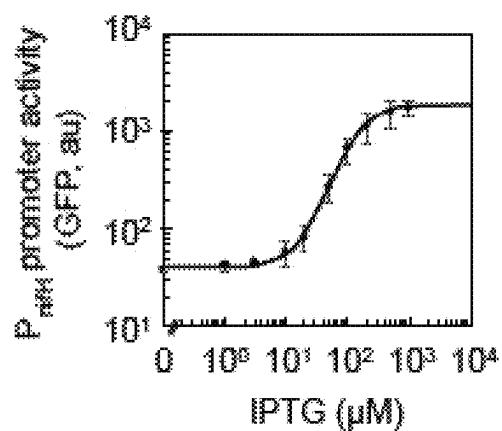


FIG. 3C

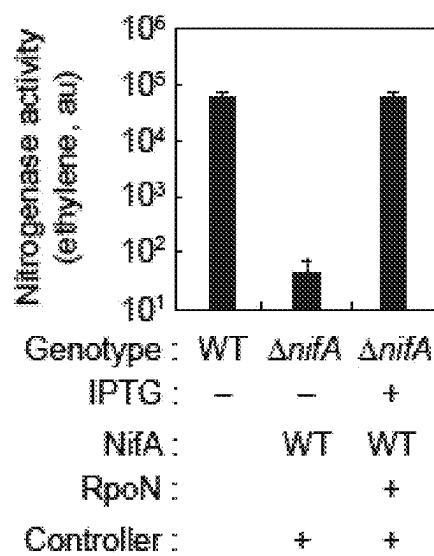


FIG. 3D

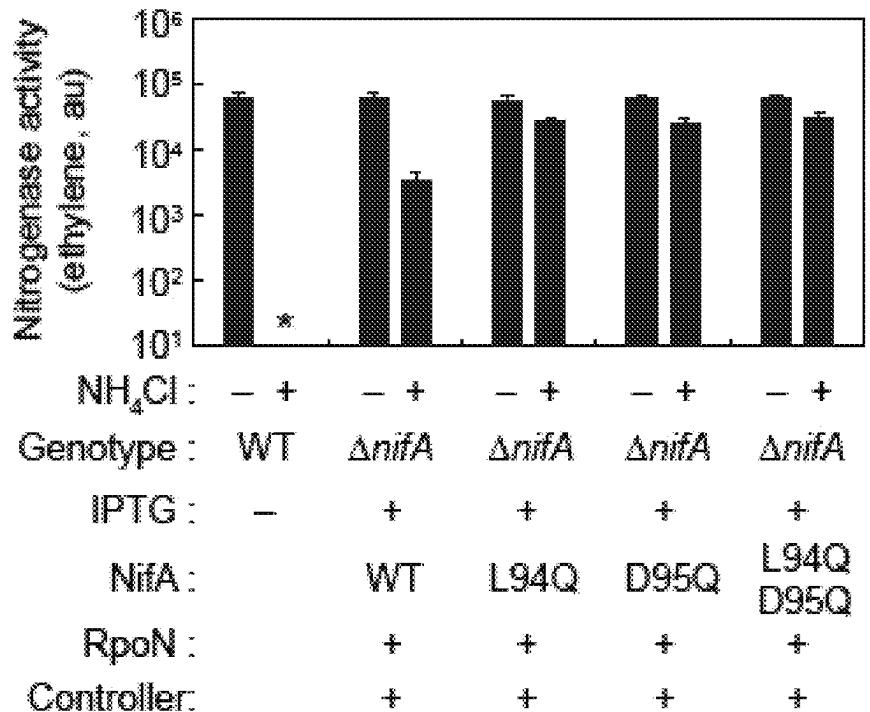


FIG. 3E

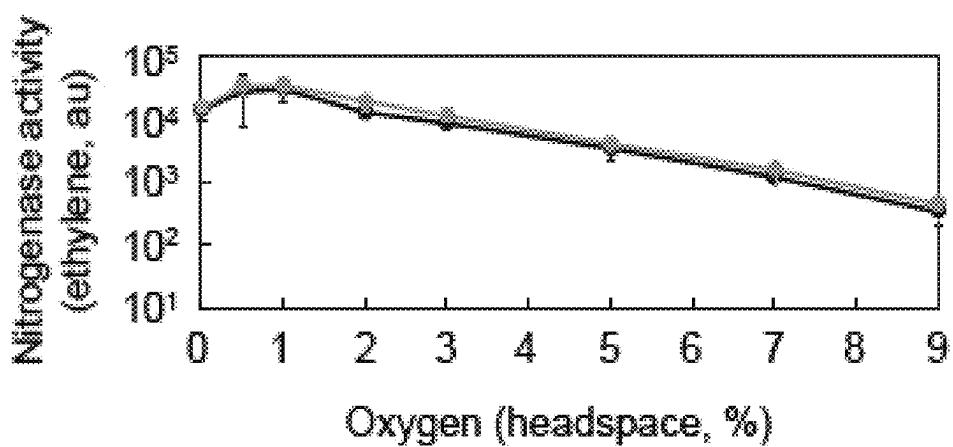


FIG. 3F

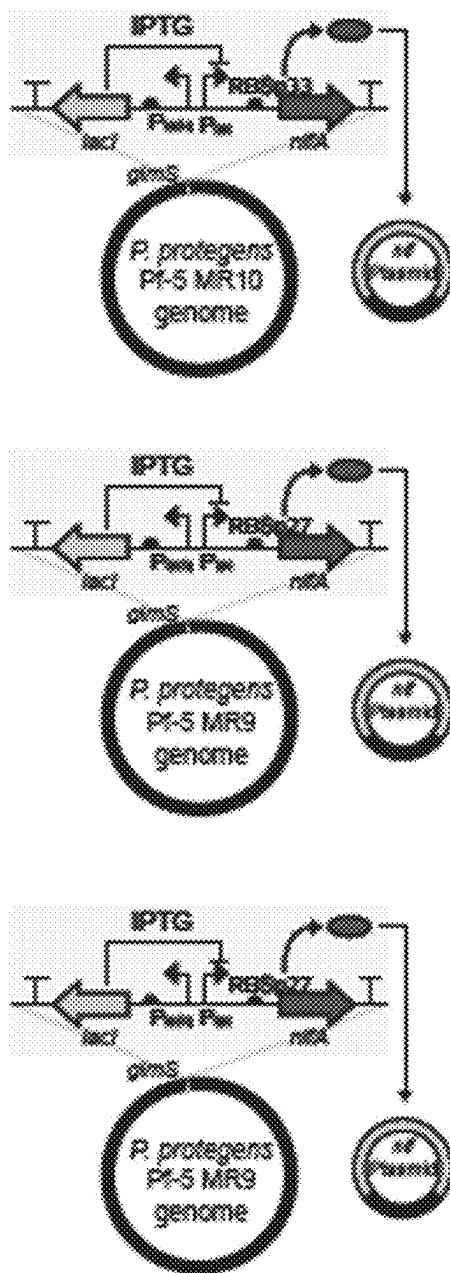


FIG. 4A

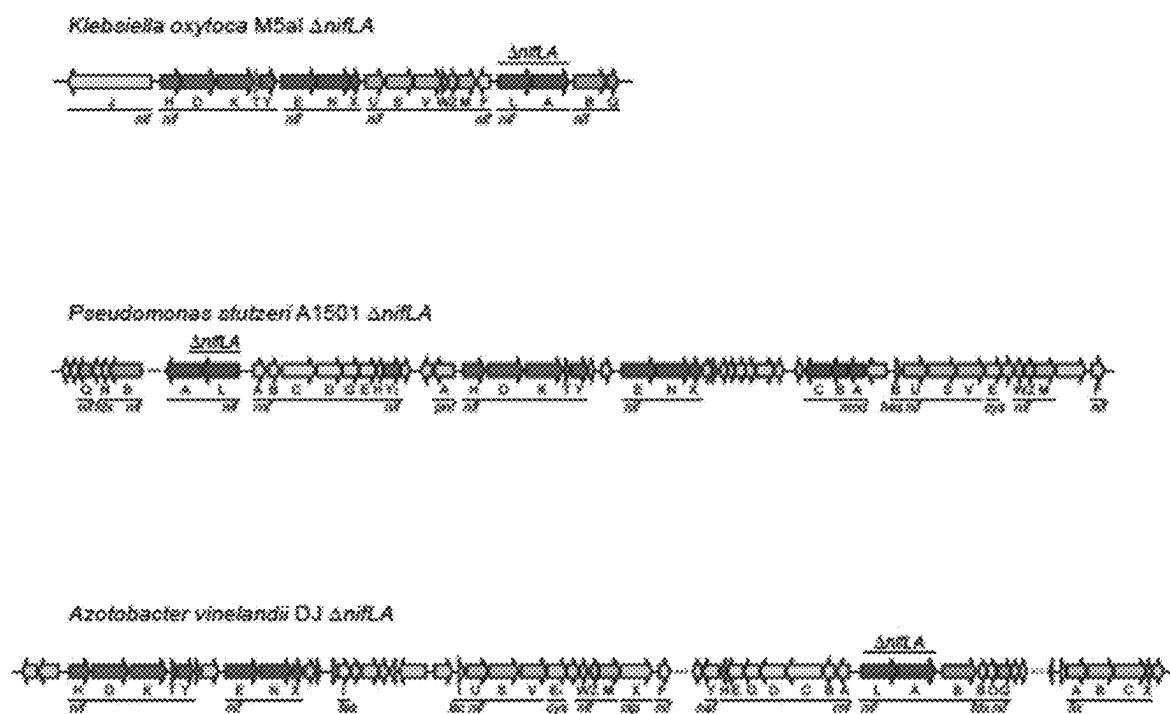


FIG. 4B

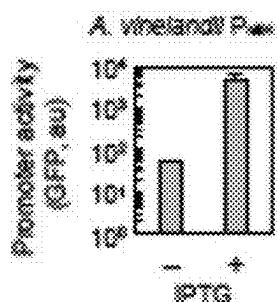
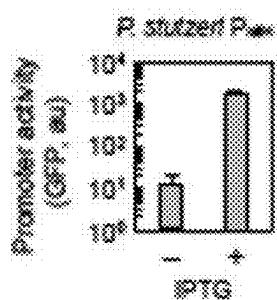
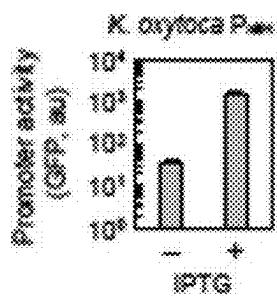


FIG. 4C

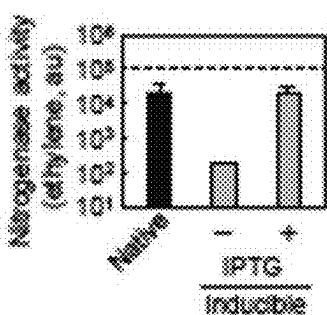
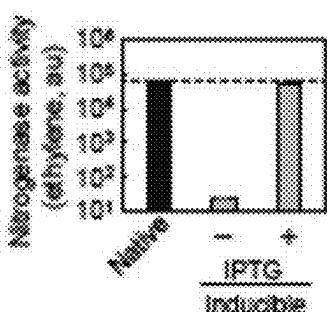
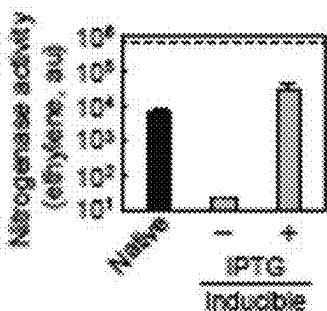


FIG. 4D

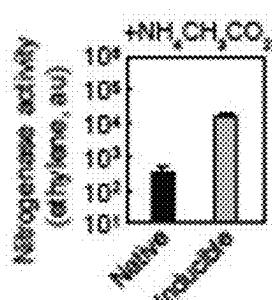
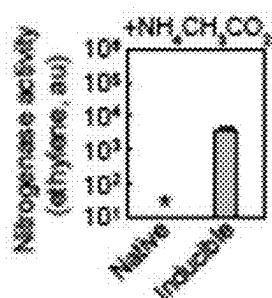
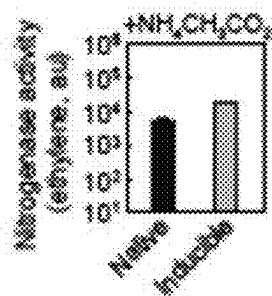


FIG. 4E

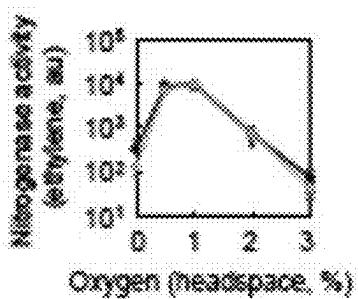
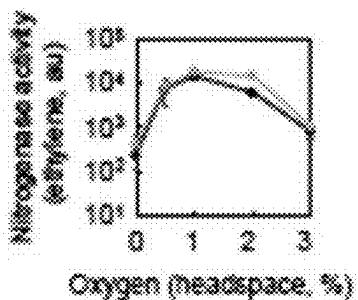
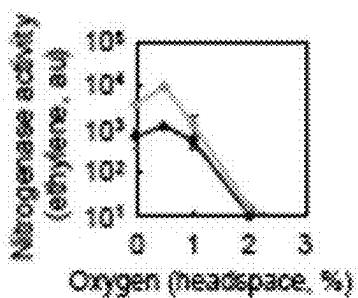


FIG. 4F

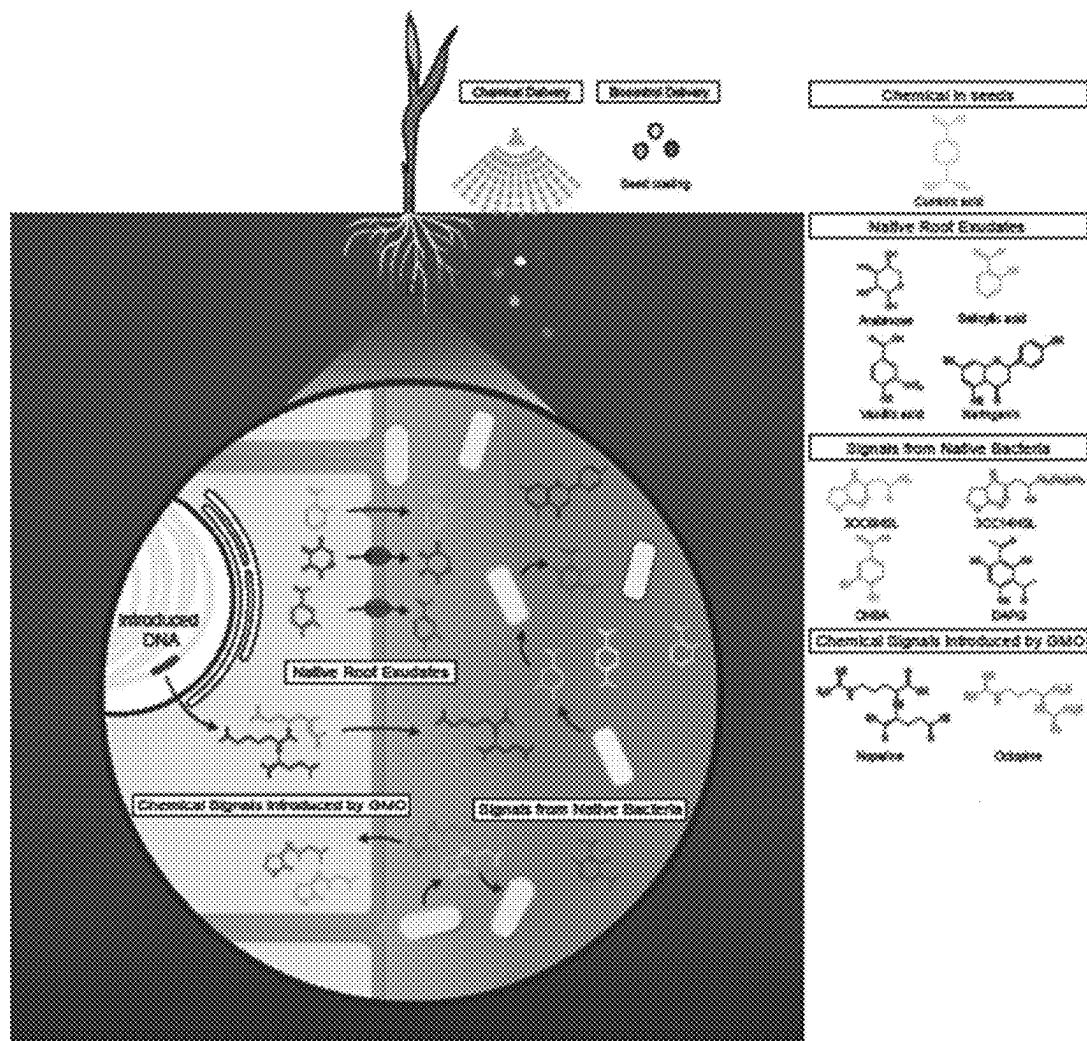


FIG. 5A

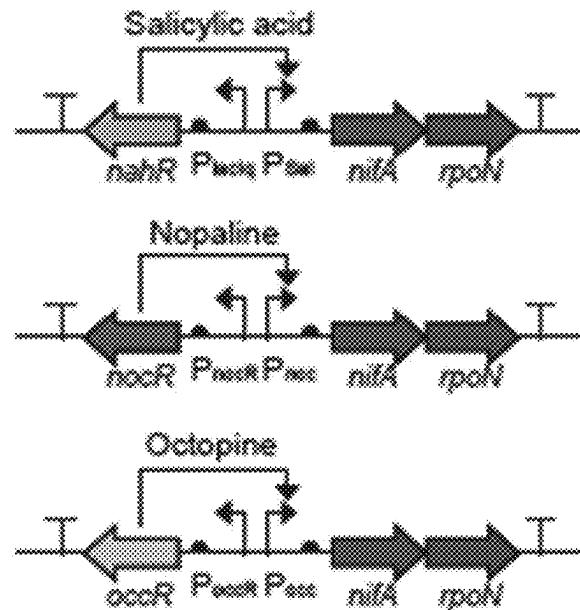


FIG. 5B

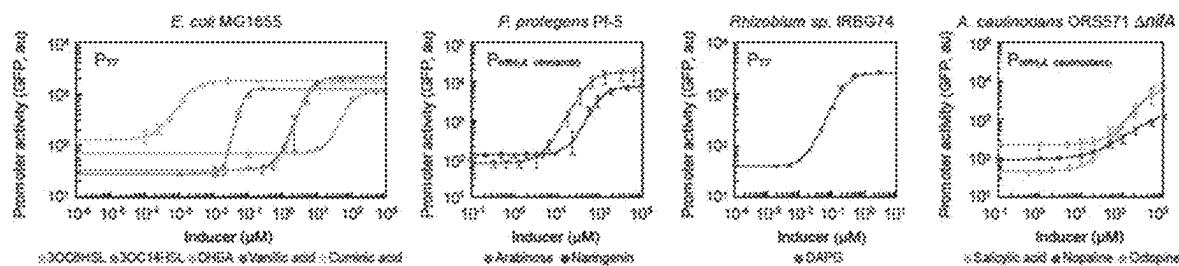


FIG. 5C

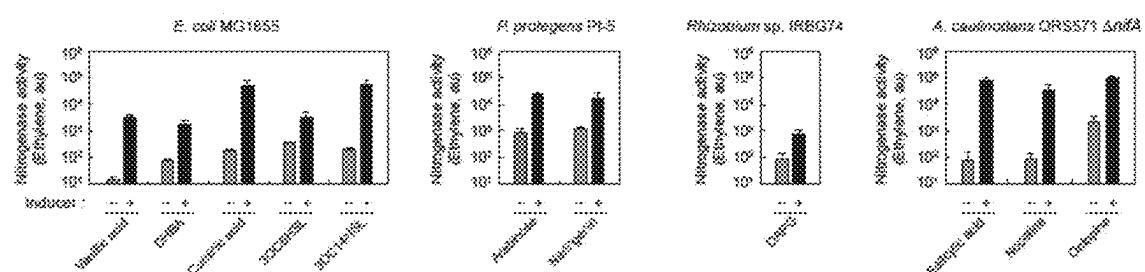


FIG. 5D

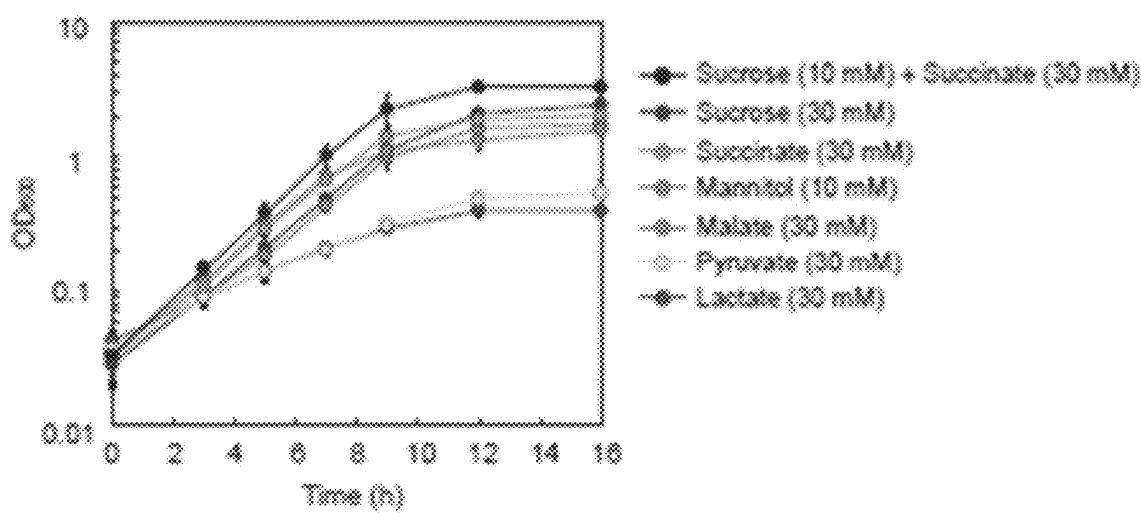


FIG. 6

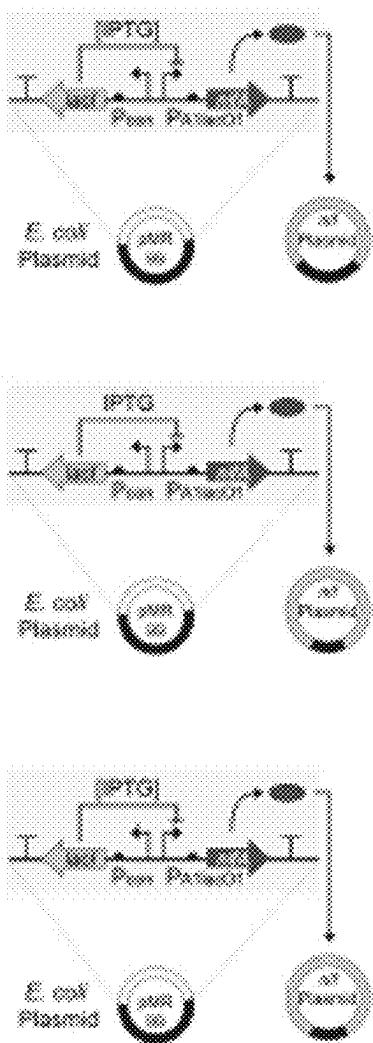


FIG. 7A

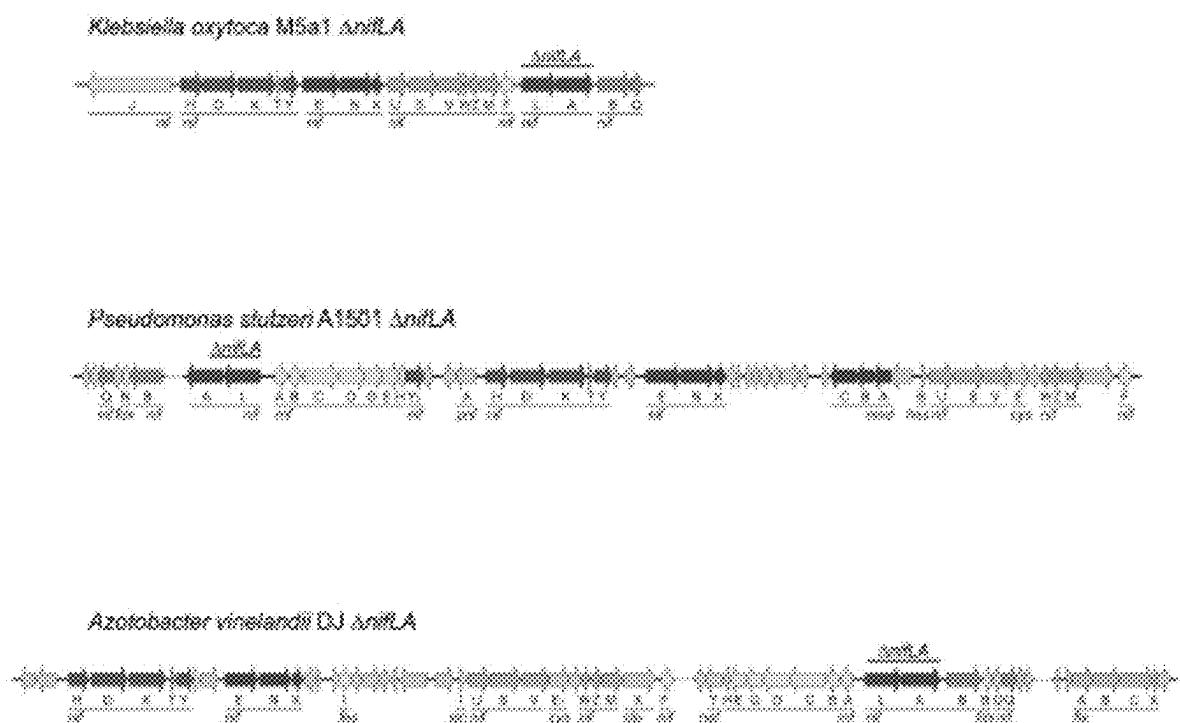


FIG. 7B

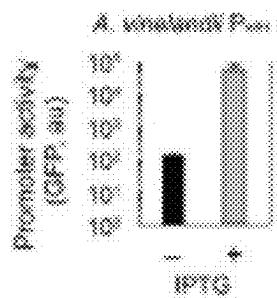
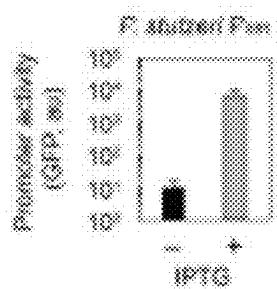
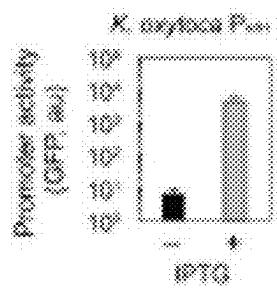


FIG. 7C

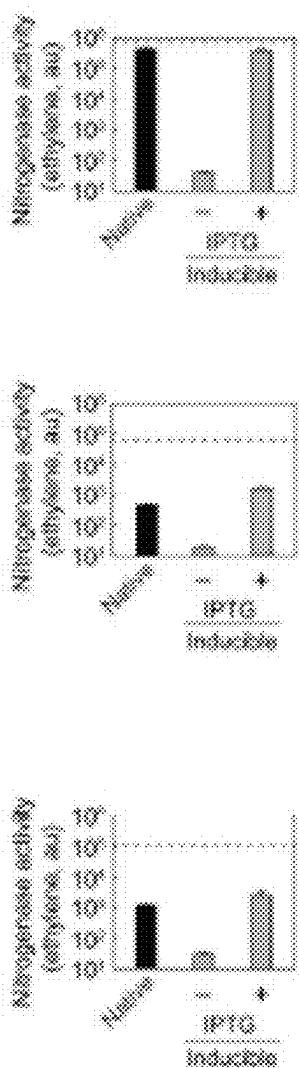


FIG. 7D

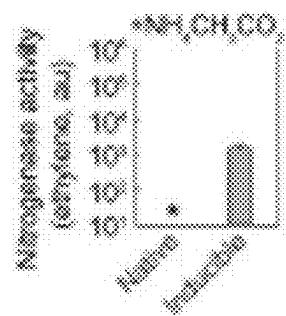
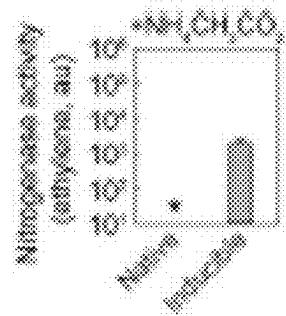
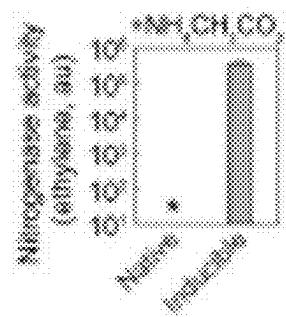


FIG. 7E

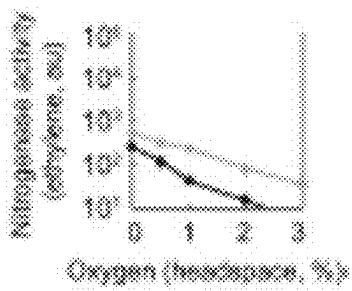
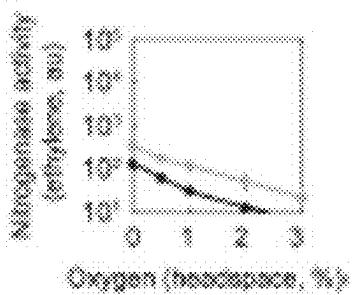
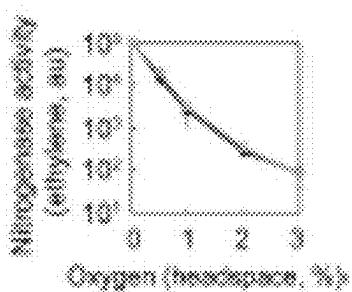


FIG. 7F

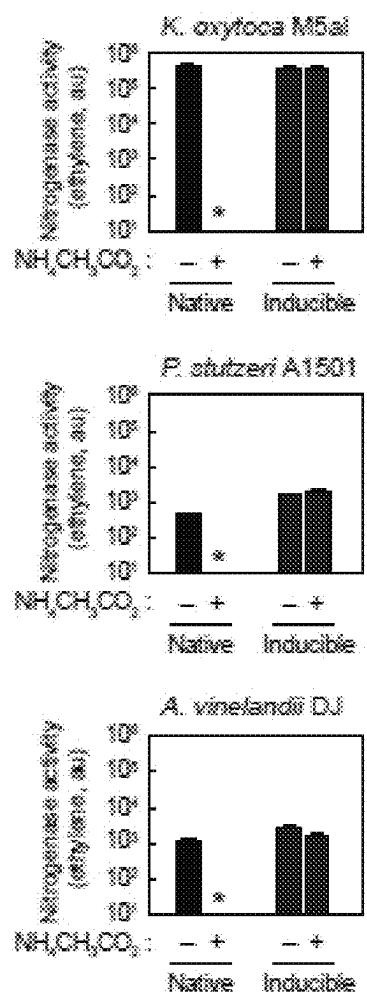


FIG. 8A

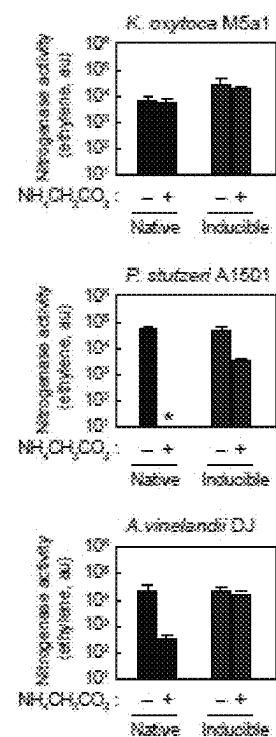


FIG. 8B

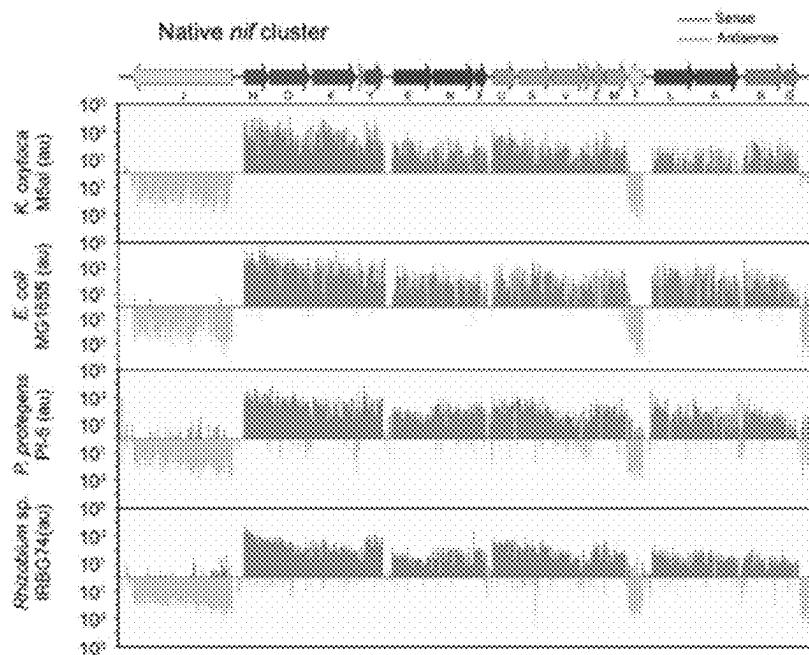


FIG. 9

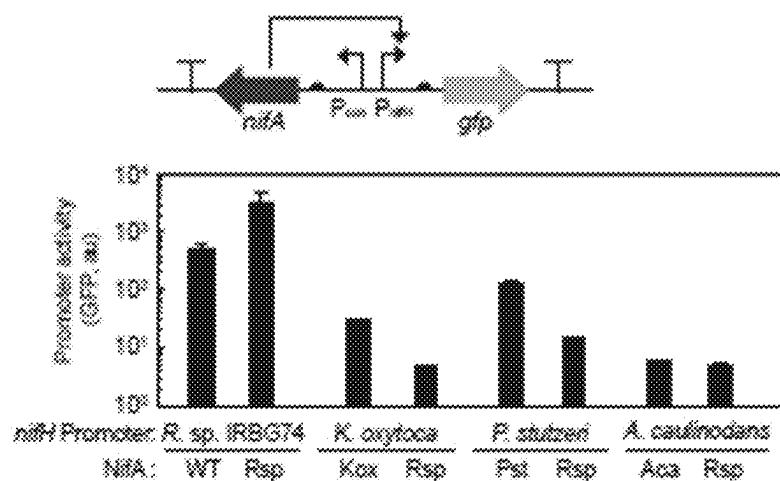


FIG. 10A

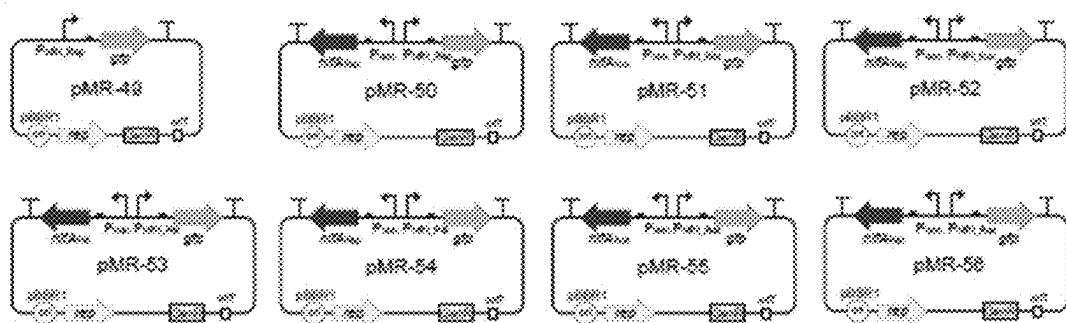


FIG. 10B

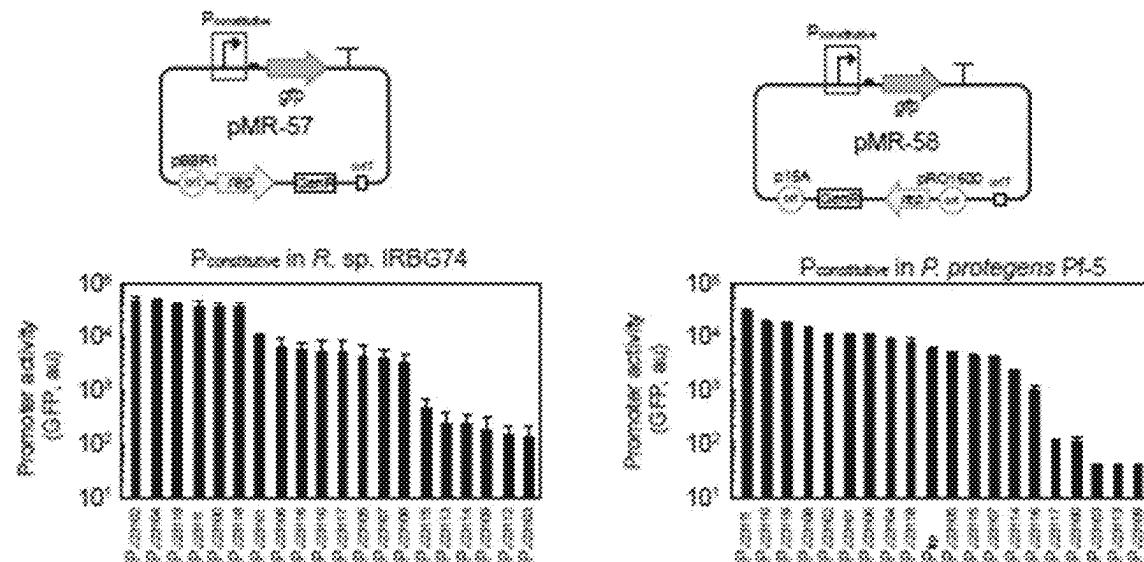


FIG. 11A

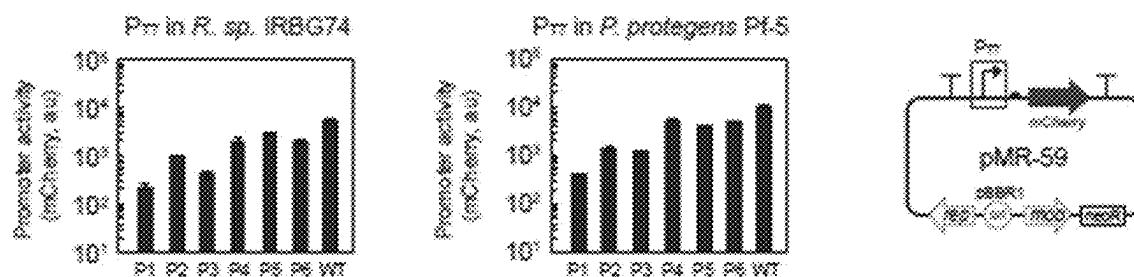


FIG. 11B

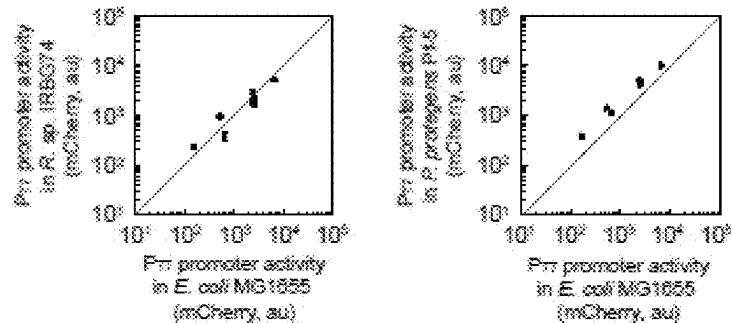


FIG. 11C

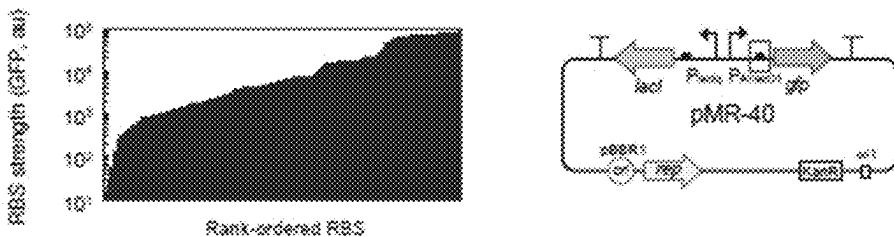


FIG. 12A

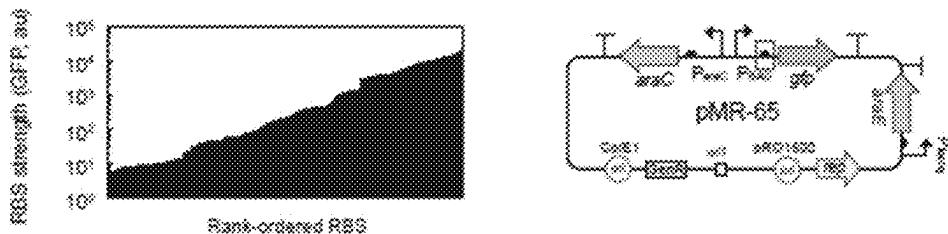


FIG. 12B

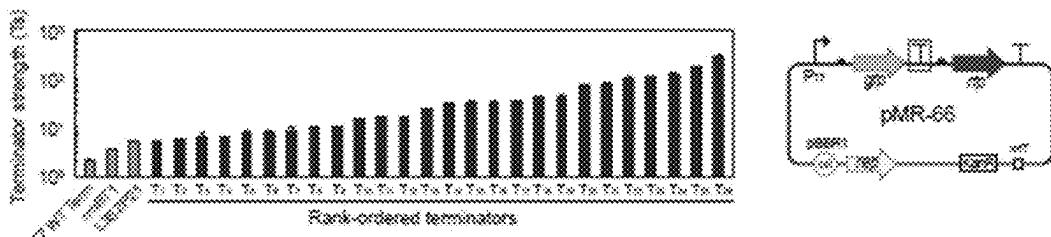


FIG. 13A

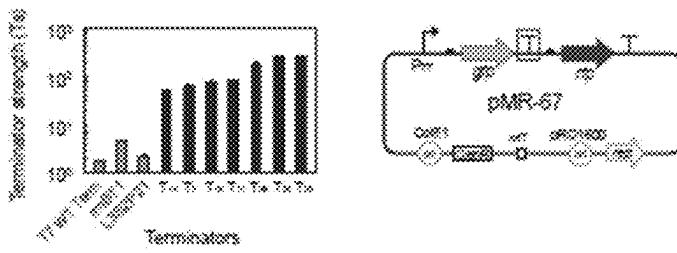


FIG. 13B

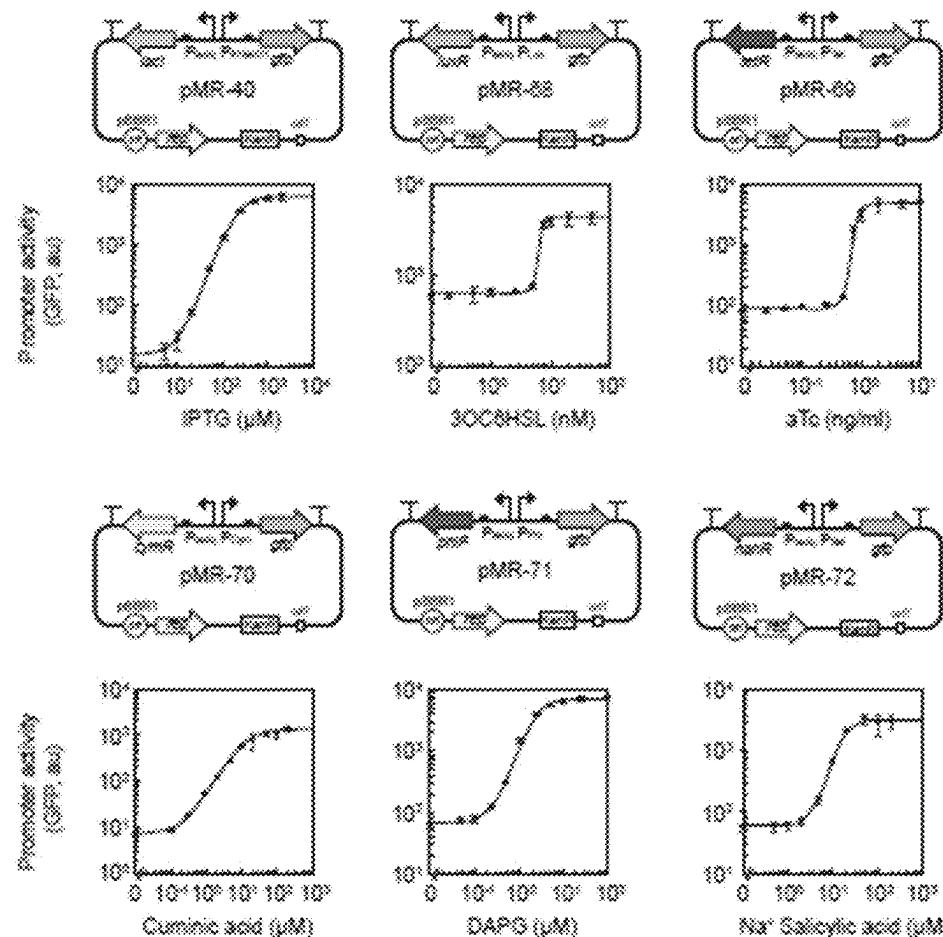


FIG. 14

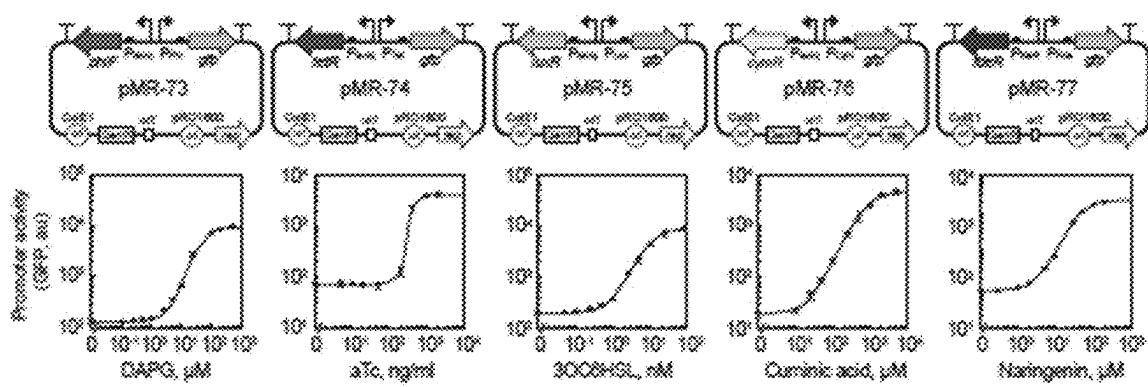


FIG. 15A

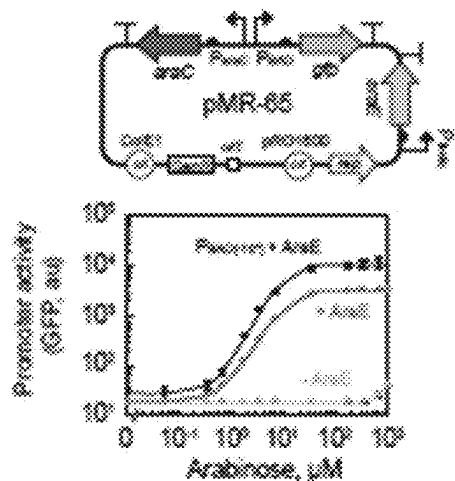


FIG. 15B

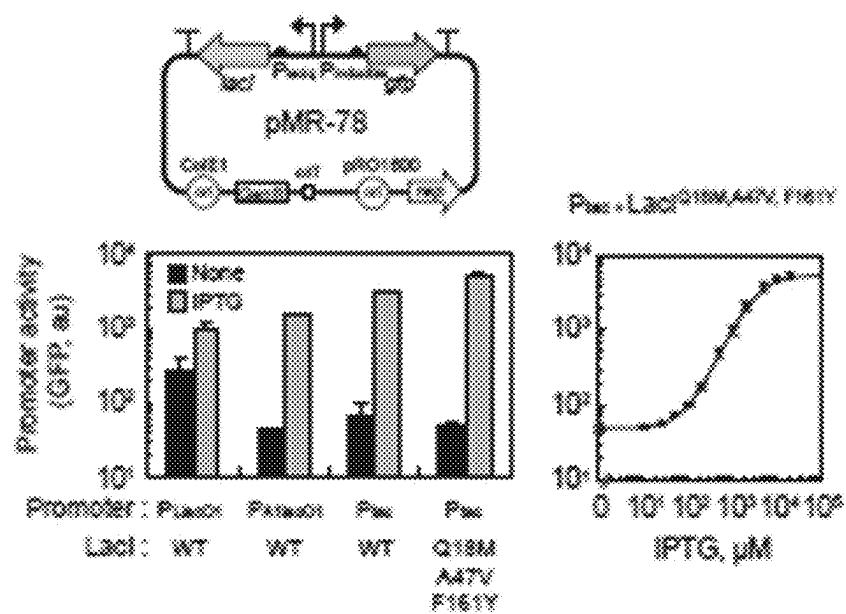


FIG. 15C

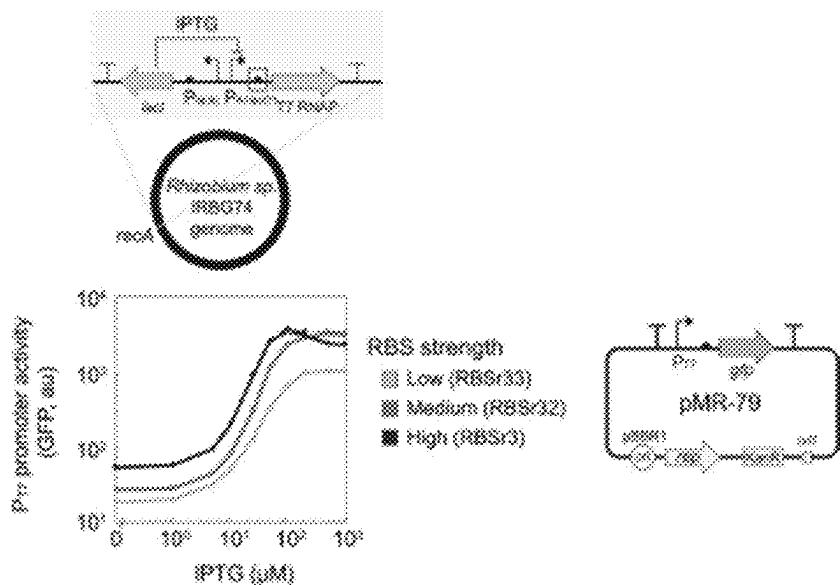


FIG. 16

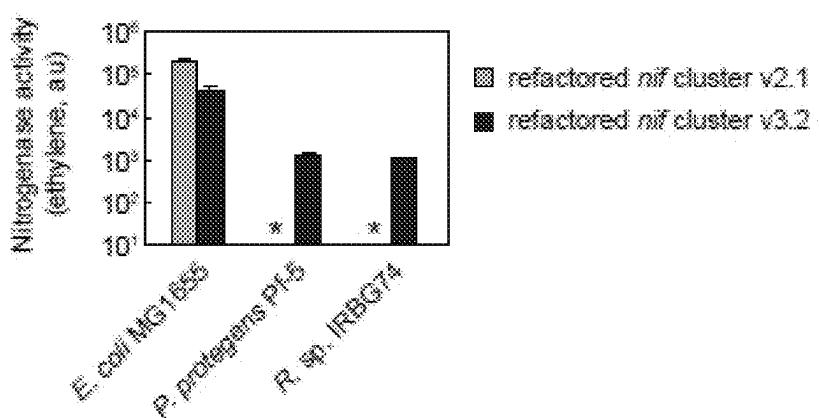


FIG. 17

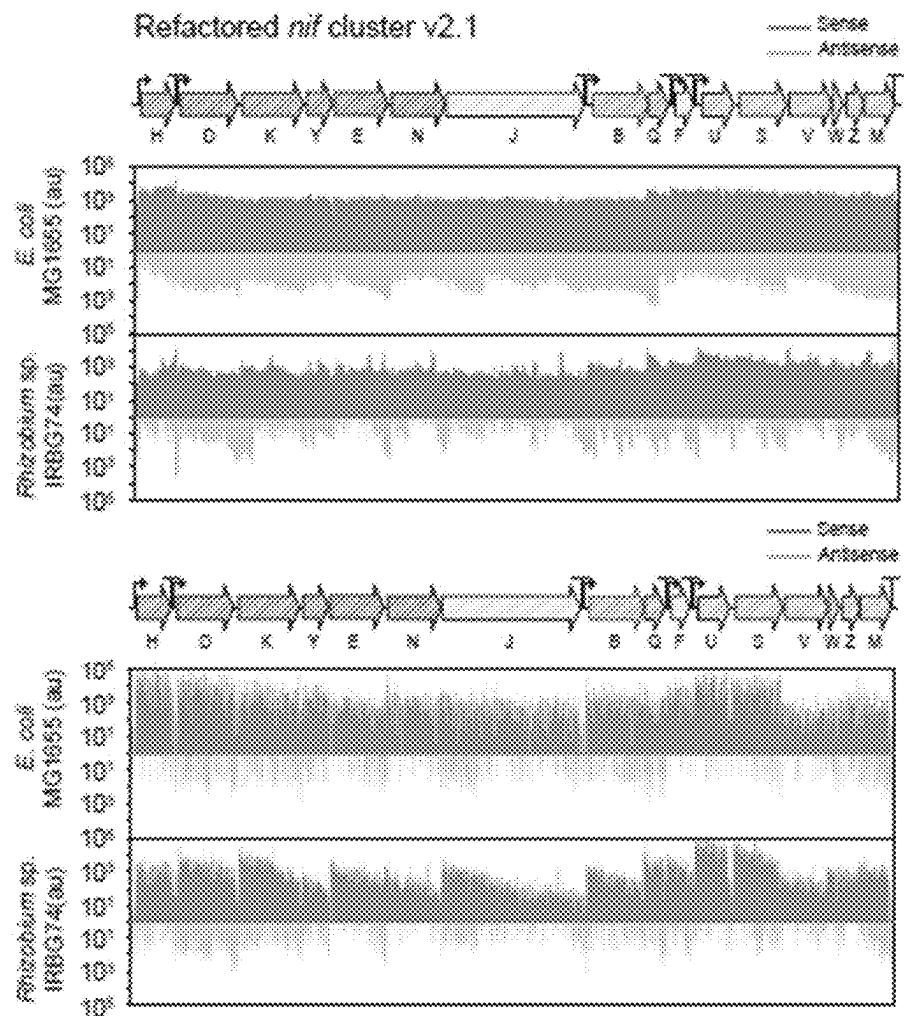


FIG. 18

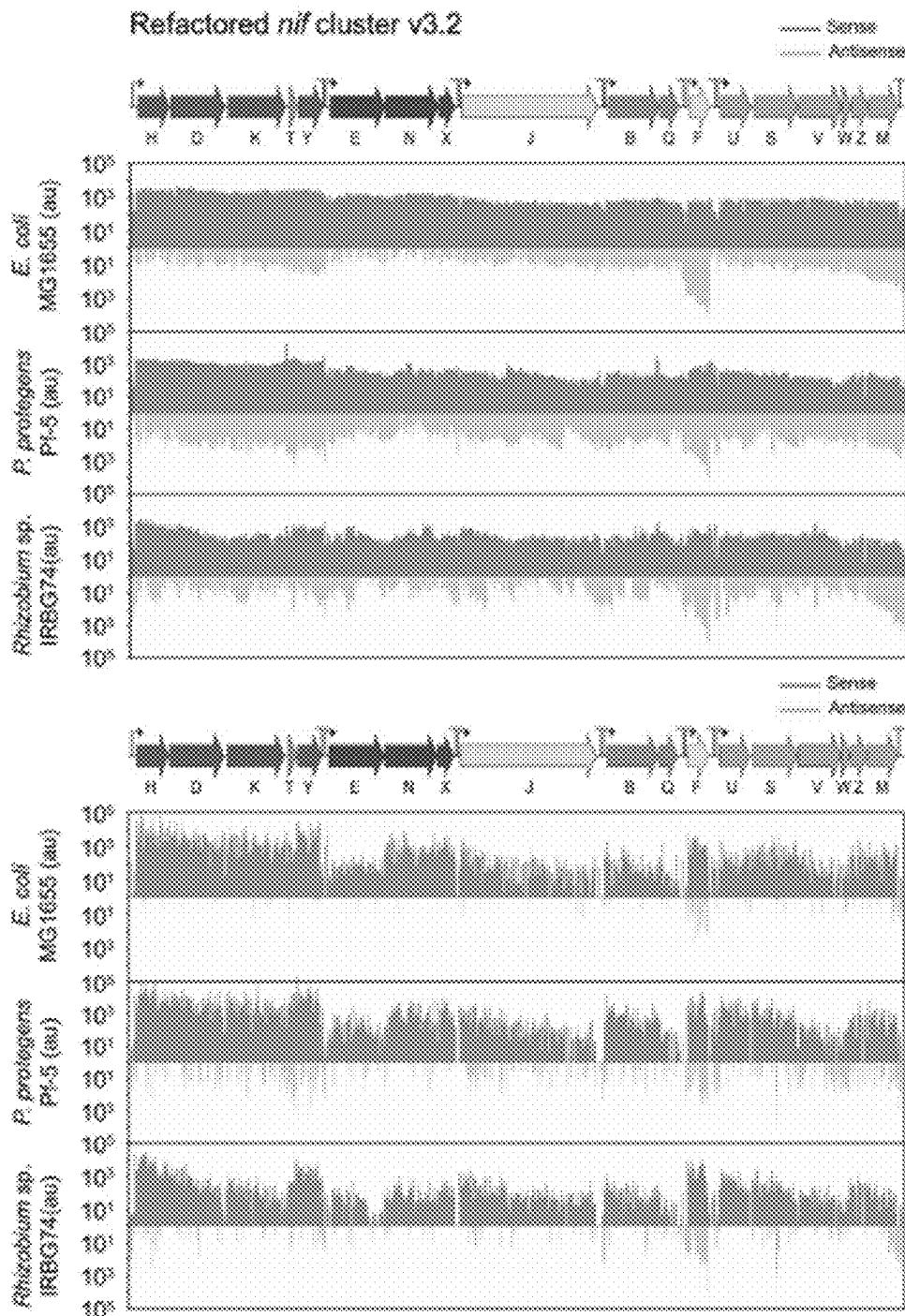


FIG. 19

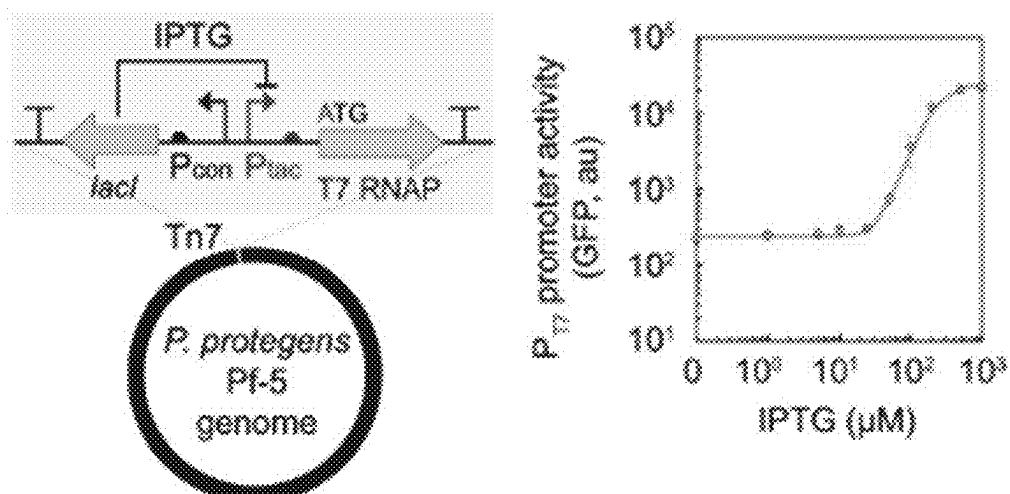


FIG. 20A

Refactored *nif* cluster v3.2 (native *nif* genes)

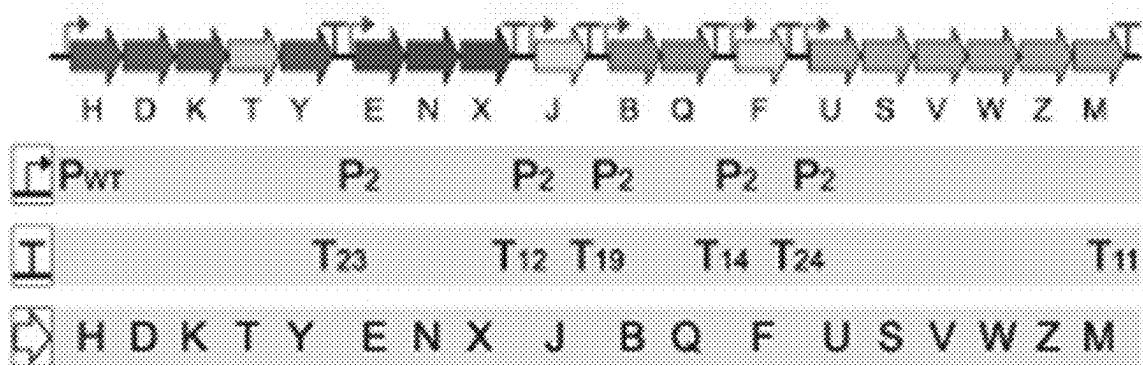


FIG. 20B

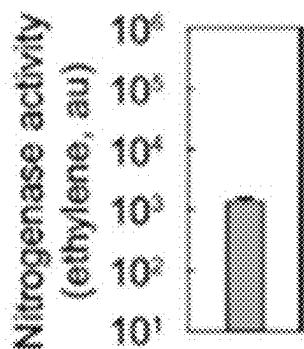


FIG. 20C

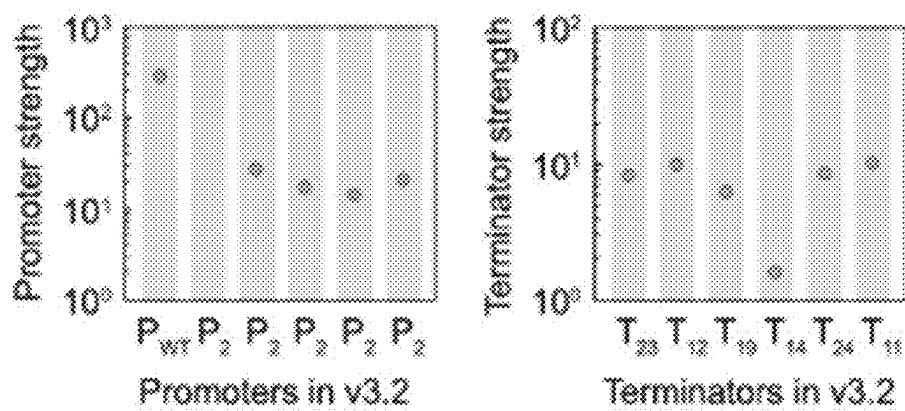


FIG. 20D

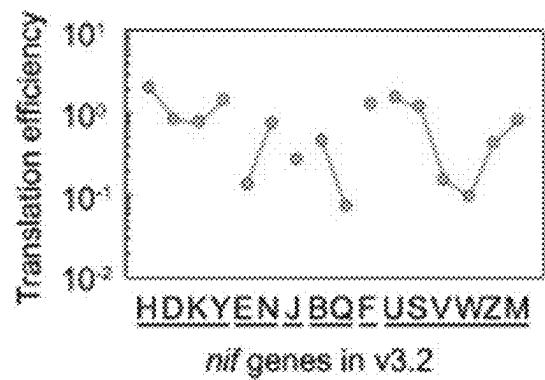


FIG. 20E

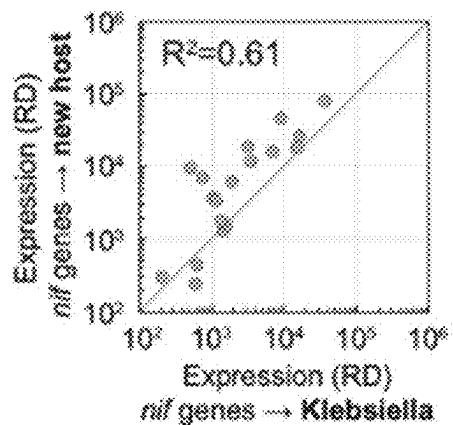


FIG. 20F

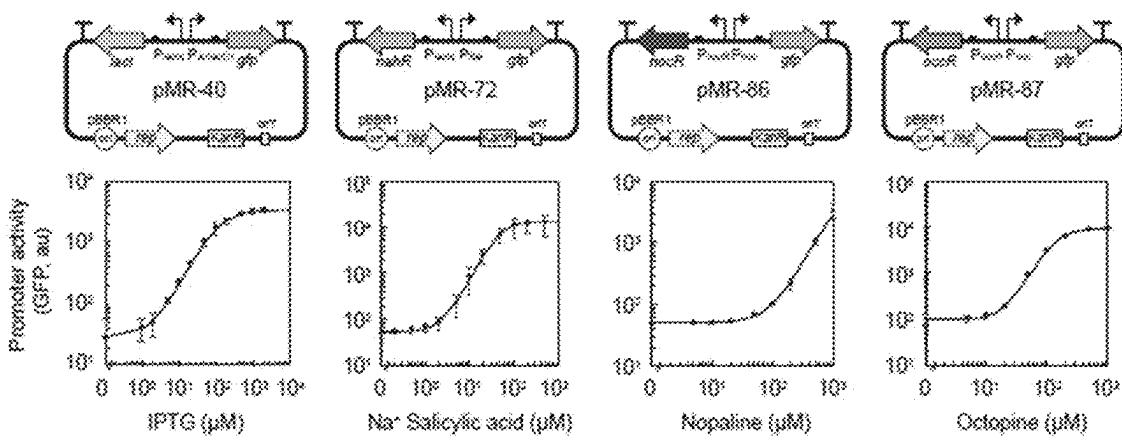


FIG. 21

R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]
R. sphaeroides	[REDACTED]	[REDACTED]	[REDACTED]
A. caulinodans	[REDACTED]	[REDACTED]	[REDACTED]

FIG. 22

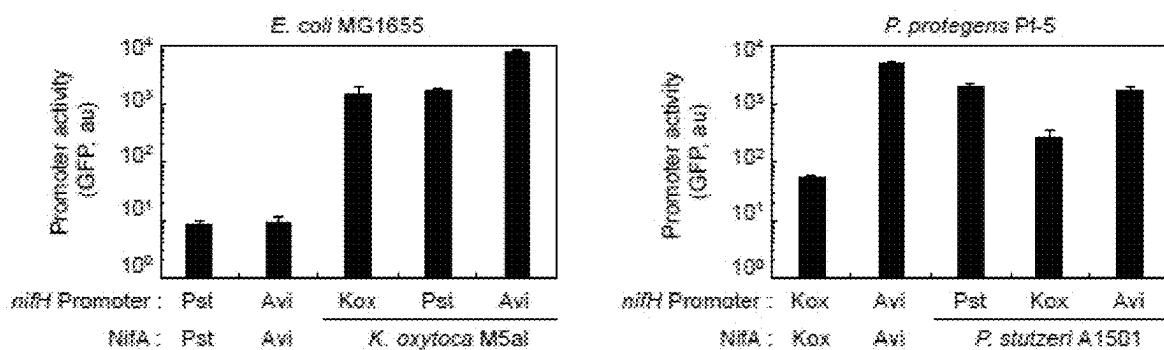


FIG. 23A

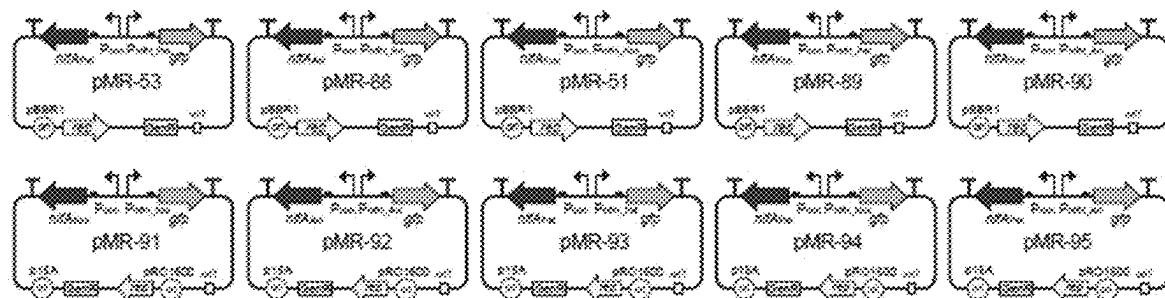


FIG. 23B

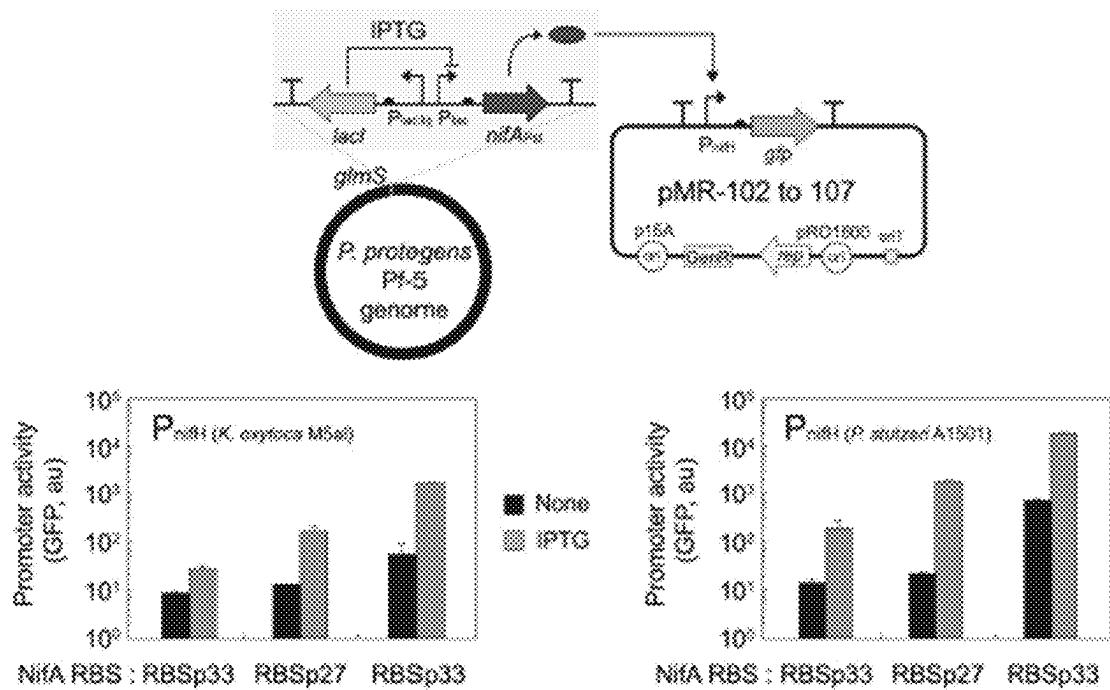


FIG. 24A

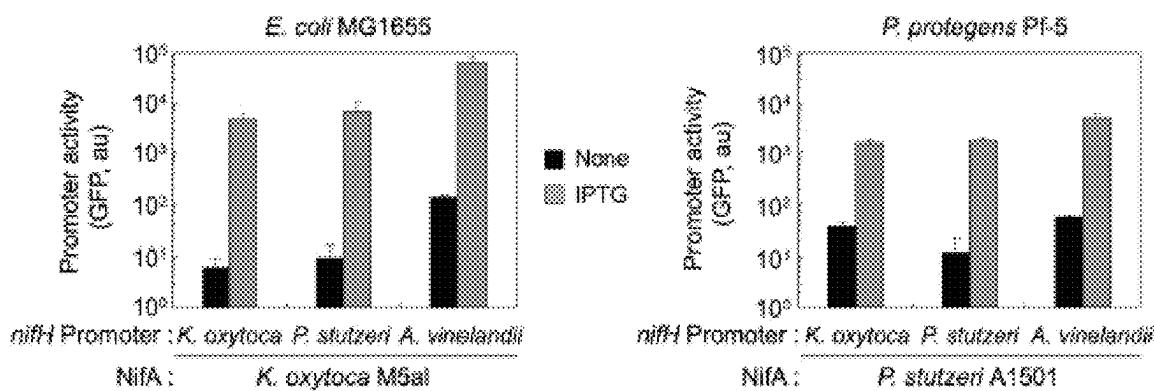


FIG. 24B

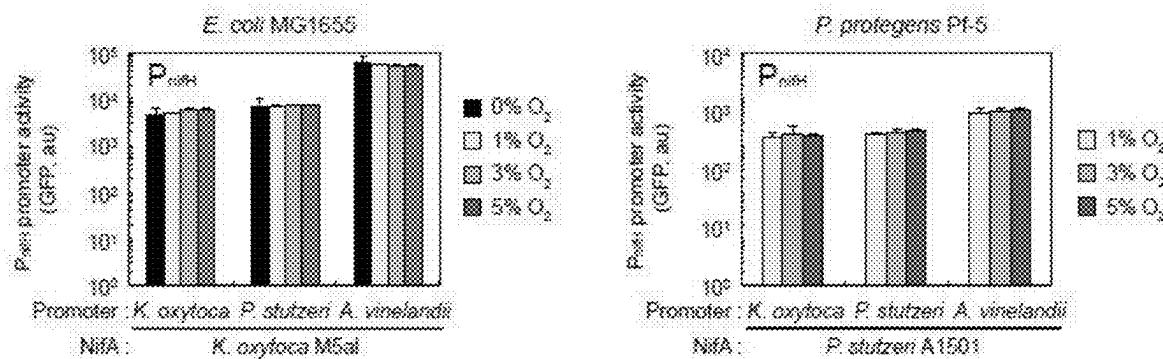


FIG. 25

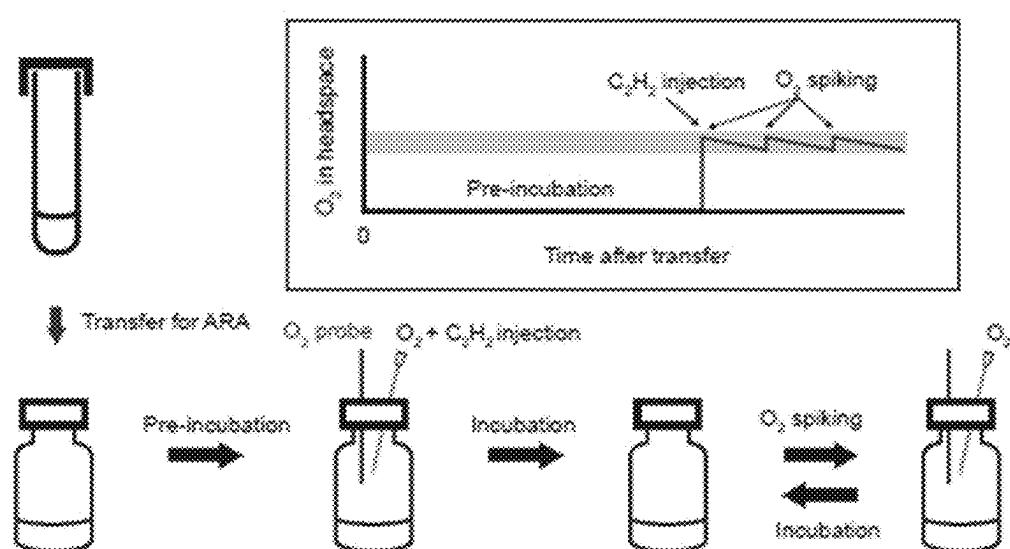


FIG. 26A

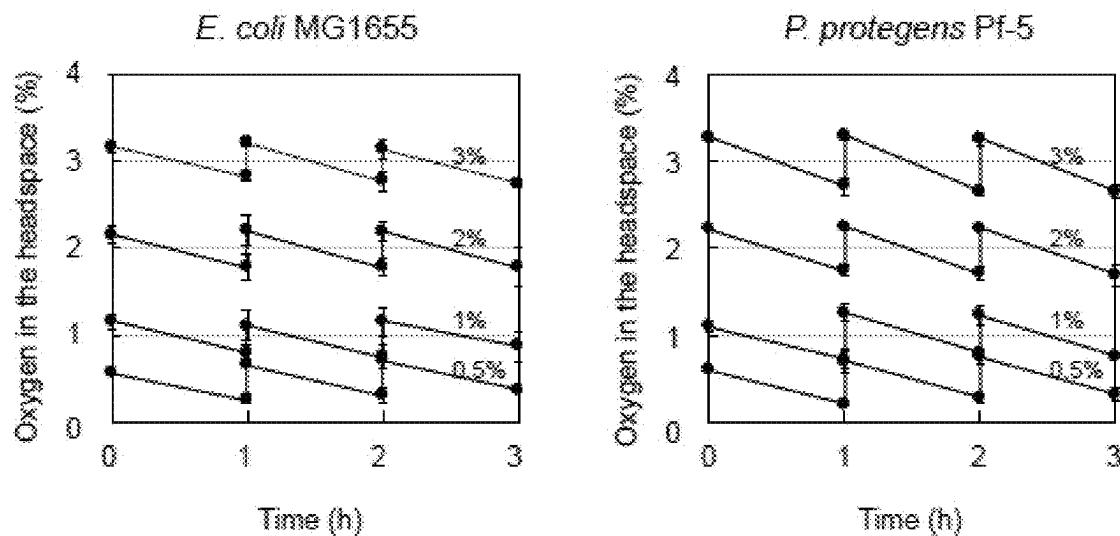


FIG. 26B

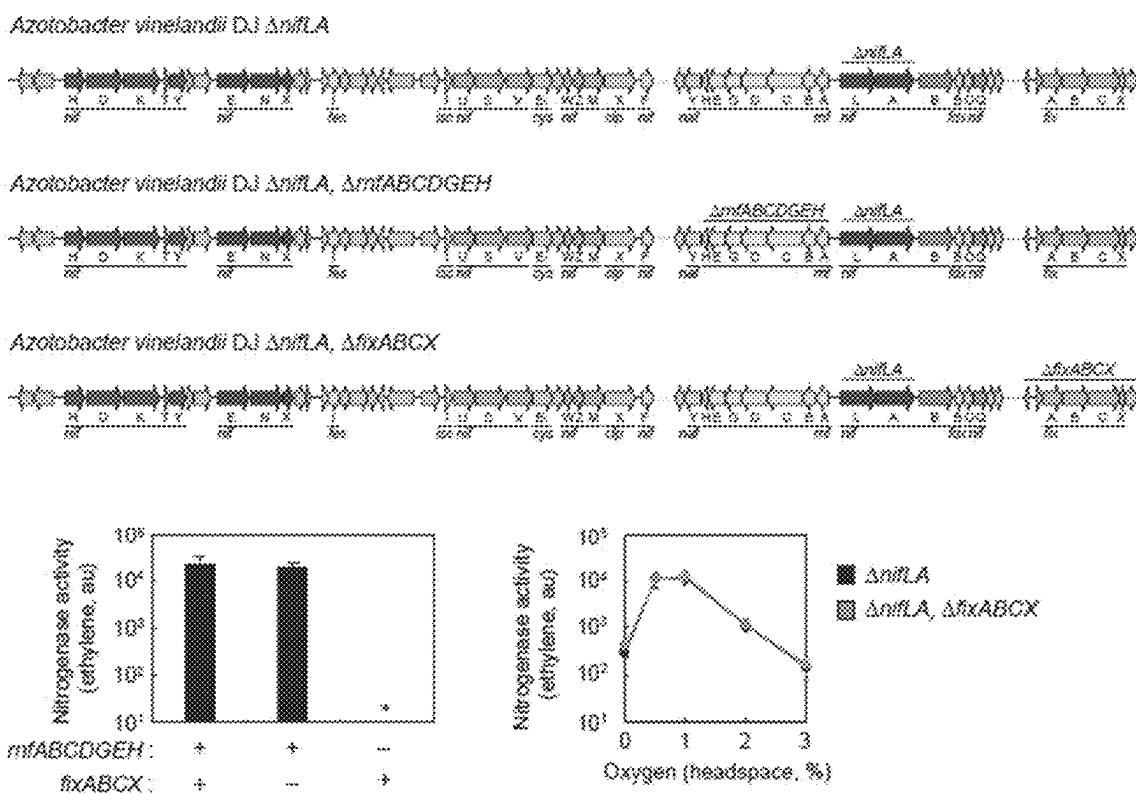


FIG. 27

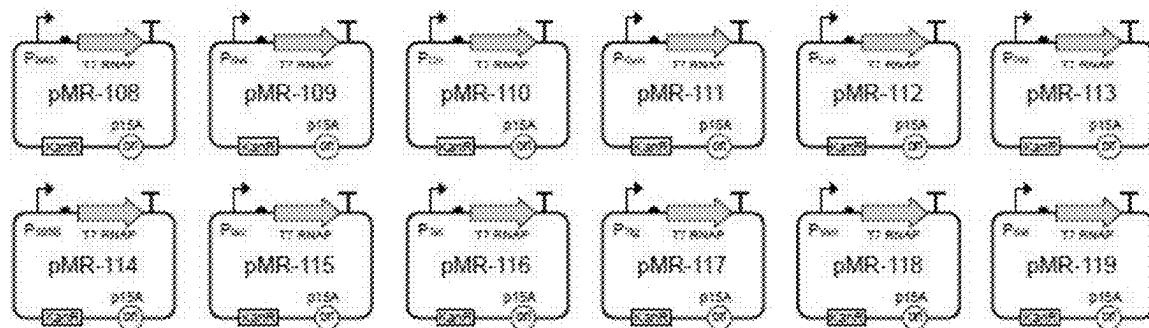


FIG. 28A

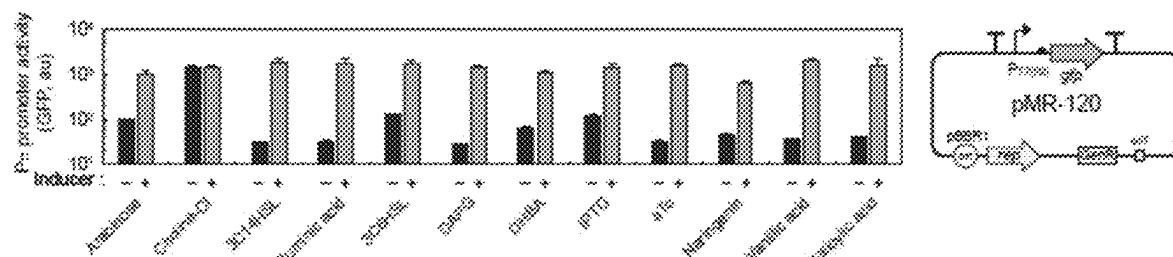


FIG. 28B

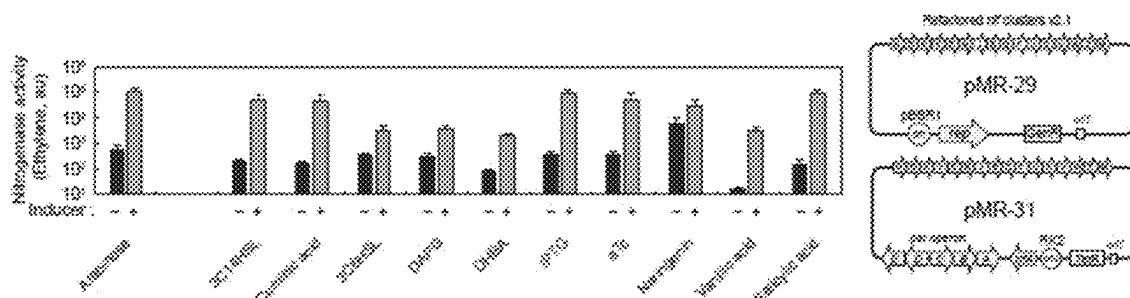


FIG. 28C

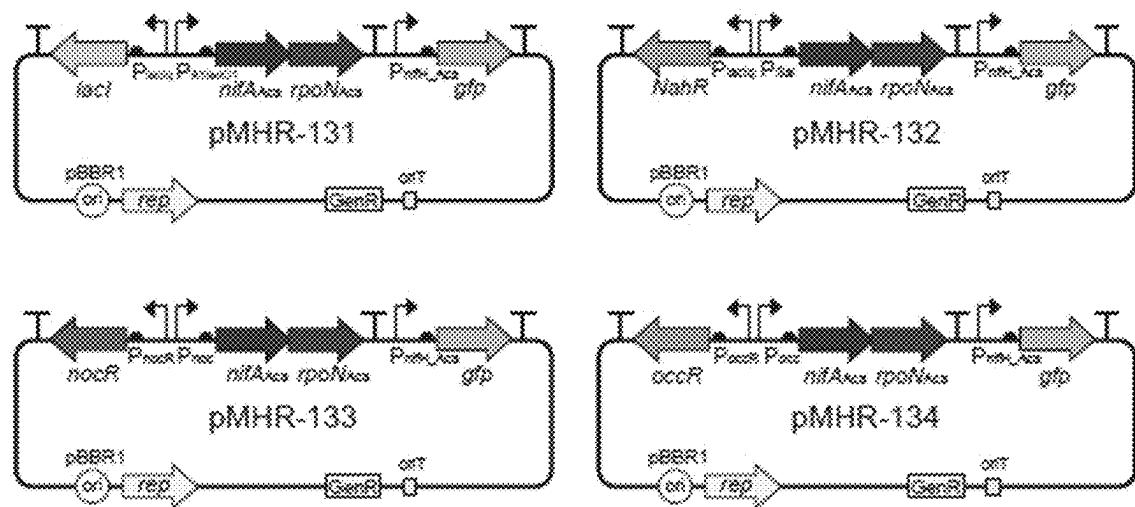


FIG. 29

CONTROL OF NITROGEN FIXATION IN RHIZOBIA THAT ASSOCIATE WITH CEREALS

RELATED APPLICATION

[0001] This application claims priority under 35 U.S.C. § 119 (e) to U.S. Provisional Application Ser. No. 62/820,765, filed Mar. 19, 2019, the entire contents of which are incorporated by reference herein.

GOVERNMENT SUPPORT

[0002] This invention was made with government support under Grant No. IOS1331098, awarded by the National Science Foundation (NSF). The government has certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] In agriculture, nitrogen is a limiting nutrient that needs to be added as fertilizer to those crops that cannot produce it on their own, including the cereals rice, corn, and wheat. In contrast, legumes are able to obtain nitrogen from the atmosphere using nitrogen-fixing bacteria that reside in root nodules. However, the majority of the world's calories are from cereals; thus, it has been a longstanding problem in genetic engineering to transfer this ability to these crops. This would reduce the need for nitrogenous fertilizer and the economic, environmental, and energy burdens that it brings.

SUMMARY OF THE INVENTION

[0004] The present disclosure is based, at least in part, on rhizobia and methods for making rhizobia that can fix nitrogen under aerobic free-living conditions. The present disclosure also provides refactored nif-clusters that confer the ability to fix nitrogen under aerobic free-living conditions.

[0005] Accordingly, one aspect of the present disclosure provides a *rhizobium* that can fix nitrogen under aerobic free-living conditions, comprising a symbiotic *rhizobium* having an exogenous nif cluster, wherein the exogenous nif cluster confers nitrogen fixation capability on the symbiotic *rhizobium* under aerobic free-living conditions, and wherein the *rhizobium* is not *Azorhizobium caulinodans*. In some embodiments, the exogenous nif cluster is from a free-living diazotroph. In some embodiments, the exogenous nif cluster is from a symbiotic diazotroph. In some embodiments, the exogenous nif cluster is from a photosynthetic Alphaproteobacteria. In some embodiments, the exogenous nif cluster is from a Gammaproteobacteria. In some embodiments, the exogenous nif cluster is from a cyanobacteria. In some embodiments, the exogenous nif cluster is from a firmicutes. In some embodiments, the exogenous nif cluster is from *Rhodobacter sphaeroides*. In some embodiments, the exogenous nif cluster is from *Rhodopseudomonas palustris*. In some embodiments, the exogenous nif cluster is an inducible refactored nif cluster. In some embodiments, the inducible refactored nif cluster is an inducible refactored *Klebsiella* nif cluster. In some embodiments, the *rhizobium* is IRBG74. In some embodiments, the exogenous nif cluster comprises 6 nif genes. In some embodiments, the 6 nif genes are nifHDK (T)Y, nifEN(X), nifJ, nifBQ, nifF, and nifUSVWZM. In some embodiments, each nif gene of the exogenous nif cluster is preceded by a T7 promoter. In some embodiments, the T7 promoter is a wild-type promoter. In some embodiments,

the *rhizobium* further comprises an endogenous nif cluster. In some embodiments, the nif cluster has a nifV gene. In some embodiments, the nifV gene is endogenous. In some embodiments, the exogenous nif cluster further comprises a terminator. In some embodiments, the T7 promoter has a terminator and the terminator is downstream from the T7 promoter. In some embodiments, the exogenous nif cluster is a refactored v3.2 nif cluster as shown in FIG. 2H.

[0006] Another aspect of the present disclosure provides a plant growth promoting bacterium that can fix nitrogen under aerobic free-living conditions, comprising a bacterium having an exogenous nif cluster having at least one inducible promoter, wherein the exogenous nif cluster confers nitrogen fixation capability on the bacterium, under aerobic free-living conditions, and wherein the bacterium is not *Azorhizobium caulinodans*. In some embodiments, the bacterium is a symbiotic bacterium. In some embodiments, the bacterium is an endophyte. In some embodiments, the endophyte is *rhizobium* IRBG74. In some embodiments, the bacterium is an epiphyte. In some embodiments, the epiphyte is *pseudomonas* protiens PF-5. In some embodiments, the plant growth promoting bacterium is associated with a genetically modified cereal plant. In some embodiments, the genetically modified cereal plant includes an exogenous gene encoding a chemical signal. In some embodiments, the nitrogen fixation is under the control of the chemical signal. In some embodiments, the chemical signal is opine, phloroglucinol or rhizopene. In some embodiments, the exogenous nif cluster comprises 6 nif genes. In some embodiments, the 6 nif genes are nifHDK (T)Y, nifEN(X), nifJ, nifBQ, nifF, and nifUSVWZM. In some embodiments, the inducible promoter is a T7 promoter. In some embodiments, the inducible promoter is P_{A1lacO1} promoter. In some embodiments, the inducible promoter is activated by an agent selected from a group that includes IPTG, sodium salicylate, octapine, nopaline, the quorum signal 3OC6HSL, aTc, cuminic acid, DAPG, and salicylic acid. In some embodiments, the exogenous nif cluster further comprises a terminator. In some embodiments, the inducible promoter has a terminator and the terminator is downstream from the inducible promoter.

[0007] Another aspect of the present disclosure provides an *Azorhizobium caulinodans* capable of inducible ammonium-independent nitrogen fixation in a cereal crop, comprising:

[0008] (i) a modified nif cluster, wherein an endogenous nifA gene is deleted or altered; and

[0009] (ii) at least one operon comprising nifA and RNA polymerase sigma factor (RpoN), wherein the operon comprises a regulatory element including an inducible promoter. In some embodiments, the inducible promoter is P_{A1lacO1} promotor. In some embodiments, the inducible promoter is activated by an agent selected from the group consisting of IPTG, sodium salicylate, octapine, nopaline, the quorum signal 3OC6HSL, aTc, cuminic acid, DAPG, and salicylic acid. In some embodiments, the endogenous nifA gene is altered with at least one of the following substitutions: (i) L94Q, (ii) D95Q, and (iii) both L94Q and D95Q.

[0010] Another aspect of the present disclosure provides a method of engineering a *rhizobium* that can fix nitrogen under aerobic free-living conditions, comprising transferring an exogenous nif cluster to a symbiotic *rhizobium*,

wherein the exogenous nif cluster confers nitrogen fixation capability on the symbiotic *rhizobium*, under aerobic free-living conditions, and wherein the *rhizobium* is not *Azorhizobium caulinodans*. In some embodiments, the exogenous nif cluster comprises 6 nif genes. In some embodiments, the 6 nif genes are nifHDK(T)Y, nifEN(X), nifI, nifBQ, nifF and nifUSVWZM. In some embodiments, each of the nif genes is preceded by a wild-type T7 promoter. In some embodiments, the exogenous nif cluster is transferred to the *rhizobium* in a plasmid. In some embodiments, the exogenous nif cluster further comprises a terminator. In some embodiments, the wild-type T7 promoter has a terminator, and the terminator is downstream from the wild-type T7 promoter. In some embodiments, the endogenous NifL gene is deleted.

[0011] Another aspect of the present disclosure provides a method of producing nitrogen for consumption by a cereal plant, comprising providing a plant growth promoting bacterium that can fix nitrogen under aerobic free-living conditions in proximity of the cereal plant, wherein the plant growth promoting bacterium is a symbiotic bacterium having an exogenous nif cluster, wherein the exogenous nif cluster confers nitrogen fixation capability on the symbiotic bacterium, enabling nitrogen fixation under aerobic free-living conditions. In some embodiments, the plant growth promoting bacterium is a *rhizobium*. In some embodiments, the plant growth bacterium is a bacterium as described in the present disclosure. In some embodiments, the cereal plant is a genetically modified cereal plant. In some embodiments, the genetically modified cereal plant includes an exogenous gene encoding a chemical signal. In some embodiments, the nitrogen fixation is under the control of the chemical signal. In some embodiments, the chemical signal is opine, phloroglucinol or rhizopene. In some embodiments, the nitrogen fixation is under the control of a chemical signal. In some embodiments, the chemical signal is a root exudate, biocontrol agent or phytohormone. In some embodiments, the root exudate is selected from the group consisting of sugars, hormones, flavonoids, and antimicrobials. In some embodiments, the chemical signal is vanillate. In some embodiments, the chemical signal is IPTG, aTe, cuminic acid, DAPG, and salicylic acid, 3,4-dihydroxybenzoic acid, 3OC6HSL or 3OC14HSL.

[0012] The details of one or more embodiments of the invention are set forth in the description below. Other features or advantages of the present invention will be apparent from the following drawings and detailed description of several embodiments, and also from the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present disclosure, which can be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein. For purposes of clarity, not every component may be labeled in every drawing. It is to be understood that the data illustrated in the drawings in no way limit the scope of the disclosure. In the drawings:

[0014] FIGS. 1A-1F include diagrams showing transfer of nif clusters across species. (FIG. 1A) Eight nif clusters from free-living nitrogen fixing bacteria are aligned based on phylogenetic relationships of 16S rRNA sequences. The

genes and operons are based on *K. oxytoca* M5al. Dots in the DNA line indicate where multiple regions were cloned from genomic DNA and combined to form one large plasmid-borne nif cluster. A complete list of strain genotypes is provided in Table 8. Nitrogenase activity from transfer of the native nif clusters was measured in three species. The activities of the *R. palustris* and *R. sphaeroides* nif clusters were also measured in 12 Rhizobia strains. Asterisks indicate ethylene production below the detection limit (<10 a.u.). Error bars represent s.d. from three independent experiments. (FIG. 1B) Transcriptomic profile of the native *K. oxytoca* nif cluster in *K. oxytoca*, compared with those obtained from its transfer to the indicated species. (FIG. 1C) Transcription levels (FPKM) of the native *K. oxytoca* nif cluster across species. Transcriptional units are underlined. (FIG. 1D) Transcription levels (FPKM) of the *K. oxytoca* nif genes in *K. oxytoca* (\rightarrow *Klebsiella*) compared to that obtained when transferred to a new host. (FIG. 1E) Same as in (FIG. 1C), except the translational efficiency is compared, as calculated using ribosome profiling. (FIG. 1F) Same as in (FIG. 1D), except the ribosome densities (RD) are compared, as calculated using ribosome profiling. R2 in log-log plots was calculated from the line ($y=x+b$), where b is an expression variable between hosts.

[0015] FIGS. 2A-2M include diagrams showing the transfer of the refactored *K. oxytoca* nif clusters to *R. sp.* IRBG74. (FIG. 2A) The genetic systems for the controller for *E. coli* MG1655 (left) and *R. sp.* IRBG74 (right) are shown. A variant of T7 RNAP (R6232S, N-terminal Ion tag, GTG start codon) is used for the *E. coli* controller. Several genetic parts were substituted to build the *R. sp.* IRBG74 controller (red) (FIG. 16). The sequences for the genetic parts are provided in Table 10. (FIG. 2B) The response functions for the controllers with the reporter plasmid pMR-79 (Table 9 and Table 10). The IPTG concentrations used to induce nitrogenase were circled in red. (FIG. 2C) The genetic parts used to build the refactored v2.1 nif gene cluster are shown (Table 10). (FIG. 2D) The activity of the refactored nif gene cluster v2.1 in different hosts is shown. Asterisks indicate ethylene production below the detection limit (<10 a.u.). (FIG. 2E) The activities of the v2.1 promoters and terminators in *E. coli* MG1655 and *R. sp.* IRBG74 as calculated from RNA-seq data (see Materials and Methods). (FIG. 2F) The translation efficiency of the v2.1 nif genes in *E. coli* MG1655 and *R. sp.* IRBG74, as calculated using ribosome profiling and RNA-seq. Lines connect points that occur in the same operon. (FIG. 2G) The ribosome density (RD) is compared for the refactored v2.1 nif genes in a new host (*E. coli* MG155; *R. sp.* IRBG74) versus that measured for the nif genes from the native *K. oxytoca* cluster in *K. oxytoca* (\rightarrow *Klebsiella*). The points corresponding to nifH is marked H. (FIGS. 2H-2L) The same as (FIGS. 2C-2G) but with the refactored nif cluster v3.2. Genetic parts are provided in Table 10. (FIG. 2M) Nitrogenase activity is shown as a function of T7 promoter strength. The refactored nif cluster v3.2 was expressed from three controller strains with varying strengths (FIG. 16). Error bars represent s.d. from three independent experiments.

[0016] FIGS. 3A-3F include diagrams showing the control of nitrogen fixation in *A. caulinodans* ORS571. (FIG. 3A) The controller is shown, carried on a pBBR1 origin plasmid (genetic parts are provided in Table 10). NifA and RpoN co-induce the expression of three sites in the genome

(identified by consensus NifA binding sequences). (FIG. 3B) Expression from the nifH promoter was evaluated using a fluorescent reporter (see Materials and Methods). NifA and RpoN were complemented (+) individually or in combination in the *A. caulinodans* ΔnifA strain where the genomic rpoN remains intact. (FIG. 3C) The response function for the induction of the nifH promoter by the controller is shown. (FIG. 3D) The nitrogenase activity is shown for wild-type *A. caulinodans* ORS571 compared to the ΔnifA complemented with the controller plasmid (+) and the addition of 1 mM IPTG (+). (FIG. 3E) The effect of the absence or presence of 10 mM ammonium chloride is shown. The WT NifA from *A. caulinodans* ORS571 is compared to different combinations of amino acid substitutions with additional RpoN expression. NifA/RpoN expression is induced by 1 mM IPTG (+) for the ΔnifA strain containing the controller plasmid pMR-121, 122, 123, and 124 (+). Asterisks indicate ethylene production below the detection limit (<10 au). (FIG. 3F) The nitrogenase activity is shown as a function of the oxygen concentration in the headspace (see Materials and Methods). The native nif cluster (wild-type *A. caulinodans* ORS571) is compared to the inducible version including the controller plasmid and 1 mM IPTG. Error bars represent s.d. from three independent experiments.

[0017] FIGS. 4A-4F include diagrams showing Nitrogenase activity of the inducible nif clusters in *Pseudomonas protegens* Pf-5. (FIG. 4A) The controllers, based on *P. stutzeri* NifA, were used for all three clusters. Plasmids and genetic parts are provided in Table 9 and Table 10. (FIG. 4B) The nif clusters from *K. oxytoca*, *P. stutzeri*, and *A. vinelandii* are shown. The deleted regions corresponding the NifLA regulators are marked. The dotted lines indicate that multiple regions from the genome were cloned and combined for form the nif cluster. The clusters were carried the plasmids pMR-4, 6, 8 (Table 9). (FIG. 4C) The induction of the nifH promoters from each species by the controller are shown (0.5 mM IPTG) (see Materials and Methods). (FIG. 4D) The nitrogenase activities of the native cluster (intact nifLA) is compared to the inducible clusters in the presence and absence of 0.5 mM IPTG. The dashed lines indicate the activity of the native clusters in the wild-type context (top to bottom, *K. oxytoca* M5al, *P. stutzeri* A1501 and *A. vinelandii* DJ). (FIG. 4E) The sensitivity of the native and inducible (+0.5 mM IPTG) nif clusters to 17.1 mM ammonium acetate are compared. Asterisks indicate ethylene production below the detection limit (<10 au). (FIG. 4F) The nitrogenase activity is shown as a function of the oxygen concentration in the headspace (see Materials and Methods). The native nif cluster is compared to the inducible version including the controller plasmid and 0.5 mM IPTG. Error bars represent s.d. from three independent experiments.

[0018] FIGS. 5A-5D include diagrams showing the control of nitrogenase activity with sensors that respond to diverse chemicals in the rhizosphere. (FIG. 5A) Schematic showing the origins of the chemicals. "Introduced DNA" refers to the genetic modification of the plant to produce nopaline and octopine. (FIG. 5B) The genetic sensors built for *A. caulinodans* are shown. Sequences for the genetic parts are provided in Table 10. (FIG. 5C) The response functions for the sensors are shown. Either the sensor expresses T7 RNAP, which then activates PT7, or it expresses NifA (*P. protegens* Pf-5) or NifA/RpoN (*A. caulinodans*) and activates the nifH promoter (species origin in parentheses). (FIG. 5D) The nitrogenase activity is mea-

sured in the presence or absence of inducer (see Materials and Methods). The refactored *Klebsiella* nif clusters v2.1 and v3.2 were used in *E. coli* MG1655 and R. sp. IRBG74, respectively. The inducible *A. vinelandii* nif cluster was used in *P. protegens* Pf-5. The controller containing nifA/rpoN was used in *A. caulinodans* ΔnifA. The inducer concentrations are: 50 μM vanillic acid, 500 μM DHBA, 50 μM cuminic acid, 25 nM 3OC6HSL, 500 nM 3OC14HSL, 33 μM arabinose, 100 μM naringenin, 100 nM DAPG, 200 μM salicylic acid, 1 mM nopaline and 1 mM octopine. Error bars represent s.d. from three independent experiments.

[0019] FIG. 6 includes a plot of the growth curve of R. sp. IRBG74 in UMS minimal medium with varying carbon sources. Cultures grown overnight in 2 mL TY medium in 15 mL culture tubes at 30° C. and 250 rpm were diluted to an OD₆₀₀ of 0.02 into 1 mL of UMS minimal medium plus varying carbon sources in 96-deepwell plates and incubated for 16 hours at 30° C. and 900 rpm. Bacterial growth was spectrophotometrically monitored at OD₆₀₀ nm. Error bars represent s.d. from three independent experiments.

[0020] FIGS. 7A-7F include diagrams showing the nitrogenase activity when different inducible nif clusters are transferred to *E. coli* MG1655. (FIG. 7A) The same controller system based on *K. oxytoca* NifA was used for all three clusters. The controller plasmid pMR-99 and genetic parts are provided in Table 9 and Table 10. (FIG. 7B) The nif clusters from *K. oxytoca*, *P. stutzeri*, and *A. vinelandii* are shown. The deleted regions corresponding the NifLA regulators are marked. The dotted lines indicate that multiple regions from the genome were cloned and combined for form the nif cluster. The clusters were carried the plasmids pMR-3, 5, 7 (Table 9). (FIG. 7C) The induction of the nifH promoters from each species by the controller is shown (50 μM IPTG) (see Materials and Methods) (FIG. 7D) The nitrogenase activities of the native cluster (intact nifLA) is compared to the inducible clusters in the presence and absence of 50 μM IPTG. The dashed lines indicate the activity of the native clusters in the wild-type context (top to bottom, *K. oxytoca* M5al, *P. stutzeri* A1501 and *A. vinelandii* DJ). (FIG. 7E) Regulation of nitrogenase activity by ammonia. Ammonium tolerance of nitrogenase from the native (black bar) and inducible (gray bar) systems was tested in the presence of 17.1 mM ammonium acetate. Asterisks indicate ethylene production below the detection limit (<10 au). (FIG. 7F) Regulation of nitrogenase activity by oxygen. The native nif cluster is compared to the inducible version including the controller plasmid and 50 μM IPTG. Nitrogenase activities were measured after 3 h of incubation at constant oxygen concentrations (0 to 3%) in the headspace (see Materials and Methods). Error bars represent s.d. from three independent experiments.

[0021] FIGS. 8A-8B include plots showing ammonium repression of the transferred nif clusters. Nitrogenase sensitivity to ammonium was measured by nitrogenase assay in the absence (-) or presence (+) of 17.1 mM ammonium acetate. The sensitivity of the native and inducible nif clusters in *E. coli* MG1655 (FIG. 8A) and *P. protegens* Pf-5 (FIG. 8B). Note that the data are from FIGS. 4A-4F and FIGS. 7A-7F. The nif clusters were induced by 50 μM and 0.5 mM IPTG in *E. coli* MG1655 and *P. protegens* Pf-5, respectively. Asterisks indicate ethylene production below the detection limit (<10 au). Error bars represent s.d. from three independent experiments.

[0022] FIG. 9 includes a diagram showing the ribosome profiling data for the *K. oxytoca* native nif cluster in *K. oxytoca* M5al, *E. coli* MG1655, *P. protegens* Pf-5 and R. sp. IRBG74 (see Materials and Methods).

[0023] FIGS. 10A-10B include diagrams showing the effect of NifA overexpression on the nifH promoter activity in R. sp. IRBG74. (FIG. 10A) The reporter construct used to measure nifH promoter activity is shown. The nifH promoter activity was analyzed in the R. sp. IRBG74 wild-type background using flow cytometry. Additional copies of NifA of R. sp. IRBG74 increased activity of the R. sp. IRBG74 nifH promoter but failed to complement or enhance activity of the other nifH promoters including *K. oxytoca*, *P. stutzeri* and *A. caulinodans*. Error bars represent s.d. from three independent experiments. (FIG. 10B) Plasmid maps used to assess the effect of NifA overexpression in R. sp. IRBG74. WT, wild-type; Rsp, R. sp. IRBG74; Kox, *K. oxytoca* M5al; Pst, *P. stutzeri* A1501; Aca, *A. caulinodans* ORS571

[0024] FIGS. 11A-11C include diagrams showing Promoter characterization in R. sp. IRBG74 and *P. protegens* Pf-5. (FIG. 11A) Constitutive promoters are rank-ordered by their strength. Plasmids used to measure promoter activity are depicted on the top. (FIG. 11B) The strength of the T7 promoter wild-type and its variants was analyzed in the controller strains containing the IPTG-inducible T7 RNAP on the genome of R. sp. IRBG74 and *P. protegens* Pf-5 with 1 mM IPTG induction. A reporter plasmid used to measure T7 promoter activity is shown on the right. (FIG. 11C) Correlation of T7 promoter strength between species. Error bars represent s.d. from three independent experiments.

[0025] FIGS. 12A-12B include diagrams showing RBS characterization in R. sp. IRBG74 and *P. protegens* Pf-5. RBS library for GFP was designed using the RBS library calculator at the highest-resolution mode. (FIG. 12A) The strengths of the synthetic RBSs in R. sp. IRBG74 were analyzed in the plasmid pMR-40 containing the IPTG-inducible system with 1 mM IPTG induction. 33 of the RBSs spanning a range of 5,684-fold expression were selected and their sequences are provided in Table 11. (FIG. 12B) The strengths of the synthetic RBSs in *P. protegens* Pf-5 was analyzed in the plasmid pMR-65 containing the arabinose-inducible system with 7 μ M arabinose induction. 33 of the RBSs spanning a range of 1,075-fold expression were selected and their sequences are provided in Table 11.

[0026] FIGS. 13A-13B include diagrams showing the characterization of terminators for T7 RNAP in R. sp. IRBG74 (FIG. 13A) and *P. protegens* Pf-5 (FIG. 13B). (FIG. 13A) The strength of terminators was analyzed in the controller R. sp. IRBG74 strains MR16 containing the IPTG-inducible T7 RNAP on the genome with 1 mM IPTG induction. (FIG. 13B) Plasmids used to measure terminator strength are shown on right. Genetic parts are provided in Table 10. Error bars represent s.d. from three independent experiments.

[0027] FIG. 14 includes diagrams showing the response functions for the sensors in R. sp. IRBG74. Plasmids used to characterize the sensors are shown on top of each panel and provided in Table 9. Genetic parts are provided in Table 10. Error bars represent s.d. from three independent experiments. Experimental details are provided in Methods.

[0028] FIGS. 15A-15C include diagrams showing the response functions for the sensors in *P. protegens* Pf-5. The output changes as a function of input inducer concentrations. Plasmids used to characterize the sensors are shown on top

of each panel. (FIG. 15A) Inducible promoter characterization in *P. protegens* Pf-5. (FIG. 15B) Optimization of the arabinose-inducible systems. Constitutive expression of a plasmid-borne AraE transporter decreased a dissociation constant of arabinose (dark gray). A mutation in the -10 region (TACTGT to TATATT) of the P_{BAD} promoter increased promoter strength (black). (FIG. 15C) Optimization of IPTG-inducible systems. The IPTG-inducible promoters were induced by 1 mM IPTG. The combination of the P_{lacZ} promoter and the LacI (Q18M/A47V/F161Y) protein yielded an expression range of 110-fold. Plasmids and genetic parts are provided in Table 9 and Table 10. Error bars represent s.d. from three independent experiments.

[0029] FIG. 16 includes diagrams showing the tuning controller strength in R. sp. IRBG74. The controller containing the IPTG-inducible T7 RNAP is integrated into the genome of R. sp. IRBG74 (top). Controller strengths were adjusted by modulating the RBS of T7 RNAP in the plasmids pMR-81, 82, and 83. Response functions of the T7 promoter were measured with the reporter plasmid pMR-79 (right) in the R. sp. IRBG74 controller strains MR16, MR17, and MR18. Genetic parts and RBS sequences are provided in Table 10 and Table 5. Error bars represent s.d. from three independent experiments.

[0030] FIG. 17 includes a plot showing the nitrogenase activity of the refactored nif clusters across species. Error bars represent s.d. from three independent experiments.

[0031] FIG. 18 includes diagrams showing RNA-seq (top) and Ribosome profiling (bottom) data, respectively in *E. coli* MG1655 and R. sp. IRBG74. The nif genes were induced by 1 mM IPTG for 6 hours (see Materials and Methods).

[0032] FIG. 19 includes diagrams showing RNA-seq (top) and ribosome profiling (bottom) data, respectively, in *E. coli* MG1655 and *P. protegens* Pf-5 and R. sp. IRBG74. The nif genes were induced by 1 mM, 0.1 mM, and 0.5 mM IPTG for 6 h in *E. coli* MG1655, *P. protegens* Pf-5 and R. sp. IRBG74, respectively (see Materials and Methods).

[0033] FIGS. 20A-20F include diagrams showing the transfer of the refactored nif cluster v3.2 in *P. protegens* Pf-5. (FIG. 20A) Controllers whose output is T7 RNAP from the genome of *P. protegens* Pf-5 are described. Substituted genetic parts including a new RBS and IPTG-inducible promoter for the controller optimization compared to the controller module pKT249 in *E. coli* MG1655 highlighted in red. The response functions for the controllers with the reporter plasmid pMR-80 was measured in the *P. protegens* Pf-5 controller strain MR7. Controllers driving the expression of GFP by the T7 promoter achieved large dynamic to 96-fold activation by IPTG. Error bars represent s.d. from three independent experiments. (FIG. 20B) The genetic parts used to build the refactored v3.2 nif gene cluster are shown (Table 10). (FIG. 20C) The activity of the refactored nif cluster v3.2. Nitrogenase expression was induced by 1 mM IPTG. (FIG. 20D) Function of the transcriptional parts of the cluster v3.2 was analyzed by RNA-seq (FIG. 19). The performance of the promoters (left) and terminators (right) was calculated (see Materials and Methods). (FIG. 20E) The translation efficiency of the nif genes v3.2 as calculated using ribosome profiling and RNA-seq. Lines connect points that occur in the same operon. (FIG. 20F) The ribosome density (RD) is compared for the refactored v3.2 nif genes in *P. protegens* Pf-5 versus that measured for the nif genes from the native *K. oxytoca* cluster in *K. oxytoca* (\rightarrow *Klebsiella*).

[0034] FIG. 21 includes diagrams showing the response function of inducible promoters in *A. caulinodans* ORS571. Plasmids used to characterize inducible promoters are shown on top of each panel and provided in Table 9. Genetic parts are provided in Table 10. Error bars represent s.d. from three independent experiments.

[0035] FIG. 22 includes a diagram showing the multiple sequence alignment of NifA of *A. caulinodans* ORS571 with *R. sphaeroides* 2.4.1 was generated using MUSCLE2. The corresponding residues for ammonium tolerance in *R. sphaeroides* are outlined in red. The *A. caulinodans* strand corresponds to SEQ ID NO: 293, and the *R. sphaeroides* strand corresponds to SEQ ID NO: 292.

[0036] FIGS. 23A-23B include diagrams showing functional testing of the NifA homologues that activate the nifH promoters. (FIG. 23A) The ability of the various NifA to activate the nifH promoters was tested with pairwise combinations of the nifH promoters and the NifA in *E. coli* MG1655 and *P. protegens* Pf-5. Error bars represent s.d. from three independent experiments. (FIG. 23B) Plasmids used to measure nifH promoter activity by NifA overexpression are shown and provided in Table 9. Genetic parts are provided in Table 10. Pst, *P. stutzeri* A1501; Avi, *A. vinelandii* DJ; Kox, *K. oxytoca* M5al

[0037] FIGS. 24A-24B include diagrams showing optimization of the controllers in *P. protegens* Pf-5 and *E. coli* MG1655 that induce the nifH promoters. (FIG. 24A) The controllers with different strengths were designed by RBS replacement and tested with the reporter plasmids (pMR 103-105) in which each of the three nifH promoter is fused to sfgfp (Methods). The nifH promoters were induced with 0.5 mM IPTG. Genetic parts and RBS sequences are provided in Table 10 and 11, respectively. (FIG. 24B) Activation of the nifH promoters in the *E. coli* MG1655 containing the controller plasmid pMR102 was tested with the reporter plasmids pMR106-108. The *P. protegens* Pf-5 controller strain MR10 was used to drive expression of the nifH promoter of *K. oxytoca* and the controller strain MR9 was used to drive expression of the nifH promoters of *P. stutzeri* and *A. vinelandii*. The nifH promoters were induced with 0.05 mM IPTG and 0.5 mM IPTG in *E. coli* MG1655 and *P. protegens* Pf-5, respectively. Error bars represent s.d. from three independent experiments.

[0038] FIG. 25 includes diagrams showing the effect of oxygen on the activity of the nifH promoters. Expression from the nifH promoters was analyzed in *E. coli* MG1655 containing the controller plasmid pMR 102, *P. protegens* Pf-5 MR 10 (for *K. oxytoca*) and MR9 (for *P. stutzeri* and *A. vinelandii*) at varying initial oxygen levels in the headspace. The three nifH promoters were induced with 0.05 mM IPTG and 0.5 mM IPTG in *E. coli* MG1655 and *P. protegens* Pf-5, respectively, and incubated at varying initial oxygen concentrations. Oxygen has no effects on nifH expression in both strains. Error bars represent s.d. from three independent experiments.

[0039] FIGS. 26A-26B include diagrams describing the nitrogenase activity assay. (FIG. 26A) Nitrogenase activity assay at constant oxygen levels in the headspace. Experimental setup used in this study to analyze oxygen tolerance of nitrogenase. Following the expression induction of nitrogenase with preincubation under low oxygen conditions, targeted oxygen concentrations in the headspace is maintained by oxygen spiking while monitoring with oxygen

monitoring system (Methods). (FIG. 26B) Nitrogenase activity in *E. coli* MG1655 and *P. protegens* Pf-5 over a course of three hours.

[0040] FIG. 27 includes diagrams showing the effect of the rnf and fix complex on nitrogenase activity. The modified nif clusters of *A. vinelandii* on the plasmids pMR25-28 were analyzed in the controller strain *P. protegens* Pf-5 MR9. The deleted regions from the clusters were provided in Table 9. Nitrogenase was induced with 0.5 mM IPTG. Removing the mf complex from the cluster abrogated activity. The cluster without the fixABCX complex showed identical oxygen tolerance to the cluster with the complex. Error bars represent s.d. from three independent experiments.

[0041] FIGS. 28A-28C include diagrams showing regulation of nitrogenase activity in *E. coli* MG1655 "Marionette" strain5. (FIG. 28A) Controller plasmids used to drive expression of T7 promoters. (FIG. 28B) Inducibility of the T7 promoter by the controller plasmids encoding T7 RNAP under the regulation of the 12 sensors was tested with a reporter plasmid pMR121 (right). (FIG. 28C) Inducible control of nitrogenase activity in response to 12 inducers was with the plasmid pMR 136 (right) carrying the refactored nif cluster v2.1 on pBBR1 origin. The choline-C1 inducible system was omitted for activity assay as the system was not inducible. For the DAPG-, DHBA-, and vanillic acid-inducible system, the refactored cluster was carried on a lower copy number plasmid pMR31 (right) as transformation of the plasmid pMR29 gave rise to no colony formation. The inducers concentrations are: 400 μ M arabinose, 1 mM choline-C1, 500 nM 3OC14HSL, 50 μ M cuminic acid, 25 nM 3OC6HSL, 25 μ M DAPG, 500 μ M DHBA, 1 mM IPTG, 100 nM aTc, 250 μ M naringenin, 50 μ M vanillic acid, and 250 M salicylic acid. Plasmid and genetic parts are provided in Table 9 and 10. Error bars represent s.d. from three independent experiments.

[0042] FIG. 29 includes schematic plasmid maps used to assess the effect of inducible expression of NifA/RpoN on the activity of the nifH promoter in *A. caulinodans* ORS571.

DETAILED DESCRIPTION OF THE INVENTION

[0043] Nitrogen fixation in the root nodules of leguminous plants is a major contributor to world food production and therefore, the practical applications of this field are of major interest. Legumes obtain nitrogen from air through bacteria residing in root nodules, some species of which also associate with cereals but do not fix nitrogen under these conditions. Disabling native regulation can turn on expression, even in the presence of nitrogenous fertilizer and low O₂, but continuous nitrogenase production confers an energetic burden.

[0044] The present disclosure in some aspects describes the surprising discovery that bacteria can be genetically altered in a manner that will enable the bacteria to deliver fixed nitrogen to cereal crops. Several strategies to implement control over nitrogen fixation in bacteria that live on or inside the roots of cereals are described. At least two approaches can be taken. In one embodiment, the native regulation is replaced. In alternative embodiments, a nif cluster is transferred from another species and placed under inducible control. The Examples section below includes a description of the achievement of these two approaches in multiple species with multiple constructs. For example, *A. caulinodans*, ammonium-independent control was achieved

using a sensor to drive the co-expression of a NifA mutant and RpoN in a Δ nifA strain. *Rhizobium* sp. IRBG74 can be engineered to express functional nitrogenase under free living conditions either by transferring a native nif cluster from *Rhodobacter* or a refactored cluster from *Klebsiella*. Multiple approaches enable *P. protegens* Pf-5 to express functional nitrogenase, of which the transfer of the nif cluster from *Azotobacter vinelandii* DJ yields the highest activity and O₂ tolerance.

[0045] To date, it has not been shown that a *Rhizobium* strain can be engineered to fix nitrogen under free-living conditions when it does not do so naturally. Some Rhizobia isolated from legume root nodules are also cereal endophytes, however most are unable to fix nitrogen under free-living conditions (outside of the nodule) (Ramachandran, V. K., East, A. K., Karunakaran, R., Downie, J. A. & Poole, P. S. Adaptation of *Rhizobium leguminosarum* to pea, alfalfa and sugar beet rhizospheres investigated by comparative transcriptomics. *Genome biology* 12, R106 (2011); Frans, J. et al. in Nitrogen Fixation 33-44 (Springer, 1990)). There have been reports of cereal yield improvements due to these bacteria, including a 20% increase for rice by *Rhizobium* sp. IRBG74, but this is likely due to other growth-promoting mechanisms, such as improved nutrient uptake or root formation (Ramachandran, V. K., East, A. K., Karunakaran, R., Downie, J. A. & Poole, P. S. Adaptation of *Rhizobium leguminosarum* to pea, alfalfa and sugar beet rhizospheres investigated by comparative transcriptomics. *Genome biology* 12, R106 (2011); Delmotte, N. et al. An integrated proteomics and transcriptomics reference data set provides new insights into the *Bradyrhizobium japonicum* bacteroid metabolism in soybean root nodules. *Proteomics* 10, 1391-1400 (2010); Hoover, T. R., Imperial, J., Ludden, P. W. & Shah, V. K. Homocitrate is a component of the iron-molybdenum cofactor of nitrogenase. *Biochemistry* 28, 2768-2771 (1989)). *Azorhizobium caulinodans* ORS571 is exceptional because it is able to fix nitrogen in both aerobic free-living and symbiotic states, has been shown to be a rice and wheat endophyte, and does not rely on plant metabolites to produce functional nitrogenase. However, when Rhizobia or *Azorhizobium* are living in cereal roots, there is low nitrogenase expression and 15N₂ transfer rates suggest any reported uptake is due to bacterial death.

Cereal Crops, Nitrogen Fixation, and Bacteria

[0046] Cereal crops are broadly defined as any grass cultivated for the edible components of its grain (also referred to as caryopsis), composed of the endosperm, germ, and bran. Cereal crops are considered staple crops in many parts of the world. They are grown in greater quantities and provide more food energy worldwide than any other type of crop. Non-limiting examples of cereal crops include maize, rye, barley, wheat, sorghum, oats, millet and rice. As used herein, the terms “cereal crop” and “cereal plant” are used interchangeably.

[0047] Nitrogen fixation is the process by which atmospheric nitrogen is assimilated into organic compounds as part of the nitrogen cycle. The fixation of atmospheric nitrogen associated with specific legumes is the result of a highly specific symbiotic relationship with rhizobial bacteria. These indigenous bacteria dwell in the soil and are responsible for the formation of nodules in the roots of leguminous plants as sites for the nitrogen fixation. Most *Rhizobium* symbioses are confined to leguminous plants.

Furthermore, *Rhizobium* strains which fix nitrogen in association with the agriculturally-important temperate legumes are usually restricted in their host range to a single legume genus.

[0048] The nif genes are genes encoding enzymes involved in the fixation of atmospheric nitrogen into a form of nitrogen available to living organisms. The primary enzyme encoded by the nif genes is the nitrogenase complex which converts atmospheric nitrogen (N₂) to other nitrogen forms (e.g. ammonia) which the organism can process. As used herein, the term “nif cluster” refers to a gene cluster comprising nif genes. As used herein, the term “refactored” refers to an engineered gene clusture, i.e. its genes have reordered, deleted or altered in some way.

[0049] Rhizobia are diazotrophic bacteria. In general, they are gram negative, motile, non-sporulating rods. In terms of taxonomy, they fall into two classes: alphaproteobacteria and betaproteobacteria. Non-limiting examples of rhizobia include *Azorhizobium caulinodans*, *Rhizobium* (R.) sp. IRBG74, *R. radiobacter*, *R. rhizogenes*, *R. rubi*, *R. vallis*, Alfalfa Rhizobia (*R. meliloti*), Chickpea Rhizobia (*Rhizobium* sp.), Soybean Rhizobia (*Bradyrhizobium japonicum*), Leucaena Rhizobia (*Rhizobium* sp.), *R. leguminosarum* by *trifoli*, *R. leguminosarum* by *phaseoli*, and *Rhizobium leguminosarum* by *viciae* (see for example U.S. Pat. No. 7,888,552, herein incorporated by reference). In some embodiments, the rhizobia of the present invention are *Azorhizobium caulinodans*. In some embodiments, the rhizobia of the present invention are not *Azorhizobium caulinodans*.

[0050] As used herein, the term “free-living conditions” refers to a bacterium (e.g. *rhizobium*) that is not within a leguminous root nodule. It generally refers to something that has not formed a parasitic (or dependent) relationship with another organism or is not on a substrate. As used herein, the term “symbiotic” refers to the interaction between two organisms living in close proximity. Close proximity can be about 0.2 μ m, 0.4 μ m, 0.6 μ m, 0.8 μ m, 1 μ m, 5 μ m, 10 μ m, 20 μ m, 50 μ m, 100 μ m, 500 μ m, 1 mm, 1 cm, 5 cm, 10 cm. Close proximity can also be less than 0.2 μ m. In many cases, a symbiotic relationship refers to a mutually beneficial interaction.

[0051] As used herein, “aerobic free-living conditions” refer to conditions under which a bacterium is not within a leguminous root nodule and the bacterium is in the presence of oxygen. Aerobic free-living conditions can also be referred to as nonsymbiotic or non-parasitic conditions in the presence of oxygen. The bacterium can be in close proximity to a crop, as defined above.

[0052] As used herein, the term “endophyte” refers to a group of organisms, often fungi and bacteria, that live within living plant cells for at least part of its life cycle without having an apparent detrimental effect on the plant cell. This is contrasting with an epiphyte, which is a plant that grows on another plant, without being parasitic.

[0053] As used herein, the term “diazotroph” refers to microorganisms that are able to grow without external sources of fixed nitrogen. The group includes some bacteria and some archaea. There are free-living and symbiotic diazotrophs. An example of a free-living diazotroph is *Klebsiella pneumoniae*. *K. pneumoniae* is a facultative anaerobes—these species can grow either with or without oxygen, but they only fix nitrogen anaerobically.

[0054] As used herein, the term “Alphaproteobacteria” refers to a diverse class of bacteria falling under the phylum Proteobacteria. Non-limiting examples of Alphaproteobacteria include species *Rhodobacter sphaeroides* and *Rhodopseudomonas palustris*. As used herein, the term “Gammaproteobacteria” refers to another class of bacteria falling under the phylum of Proteobacteria. All proteobacteria are gram negative. As used herein, the term “Cyanobacteria” refers to a phylum of bacteria that obtain their energy through photosynthesis. They are also referred to as Cyanophyta. They have characteristic internal membranes and thylakoids, the latter being for photosynthetic purposes. As used herein, the term “Firmicutes” refer to a phylum of bacteria. This phylum includes the classes Bacilli, Clostridia, and Thermolithobacteria.

Nif Genes

[0055] Typically, the genes necessary for nitrogen fixation occur together in a gene cluster, including the nitrogenase subunits, the biosynthesis of metalloclusters cluster and, e-transport, and regulator proteins. Nif genes are genes that encode the enzyme involved in nitrogen fixation. In most cases nif genes occur as an operon. Some of these genes encode the subunits for the nitrogenase complex, which is the primary enzyme imparting the ability to convert atmospheric nitrogen (N_2) to forms of nitrogen accessible to living organisms. In most genes, the regulation of the nif gene transcription is conducted by NifA protein, which is responsive to nitrogen levels. When there are nitrogen deficits, NtrC activates NifA expression, which in turn leads to the activation of the remaining nif genes. When nitrogen levels are adequate or in excess, NifL protein, encoded by NifL. NifL inhibits NifA activity.

[0056] Nif gene pathways are generally sensitive to small changes in expression. The genes that form nitrogenase. Important genes include nifHDK, which form the subunits for nitrogenase. The chaperone NifY is required to achieve full activity and broadens the tolerance to changes in expression level. NifJ and nif regulate electron transport. The nifUSVWZM operon encodes proteins for early Fe—S cluster formation (NifUS) and proteins for component maturation (NifVWZ for Component I and NifM for Component II), whereas nifBQ encodes proteins for FeMo-co core synthesis (NifB) and molybdenum integration (NifQ). NifEN is tolerant to varied expression levels.

[0057] Exemplary sequences for various nif genes are provided in Table 10. Non-limiting examples of nif genes include nifH, nifD, nifK, nifE, nifN, nifU, nifS, nifV, nifW, nifX, nifB, nifQ, nifY, nifT, nifJ, nifF, nifX, nifU, and nifS Nitrogen fixation and Regulatory Elements

[0058] The nitrogen fixation (nif) genes are organized as genomic clusters, ranging from a 10.5 kb single operon in *Paenibacillus* to 64 kb divided amongst three genomic locations in *A. caulinodans*. Conserved genes include those encoding the nitrogenase enzyme (nifHDK), FeMoCo biosynthesis, and chaperones. Species that can fix nitrogen under more conditions tend to have larger gene clusters that include environment-specific paralogues, alternative electron transport routes, and oxygen protective mechanisms. Often, the functions of many genes in the larger clusters are unknown.

[0059] There is evolutionary evidence for the lateral transfer of nif clusters between species (Pascuan, C., Fox, A. R., Soto, G. & Ayub. N. D. Exploring the ancestral mechanisms

of regulation of horizontally acquired nitrogenases. Journal of molecular evolution 81, 84-89 (2015); Kechris, K. J., Lin, J. C., Bickel, P. J. & Glazer, A. N. Quantitative exploration of the occurrence of lateral gene transfer by using nitrogen fixation genes as a case study. Proceedings of the National Academy of Sciences 103, 9584-9589 (2006)). However, achieving such a transfer via genetic engineering poses a challenge as many things can go awry, including differences in regulation, missing genes, and the intracellular environment (Frans, J. et al. in Nitrogen Fixation 33-44 (Springer, 1990); Poudel, S. et al. Electron transfer to nitrogenase in different genomic and metabolic backgrounds. Journal of bacteriology 200, e00757-00717 (2018); Thony, B., Anthamatten, D. & Hennecke, H. Dual control of the *Bradyrhizobium japonicum* symbiotic nitrogen fixation regulatory operon fixR nifA: analysis of cis- and trans-acting elements. Journal of bacteriology 171, 4162-4169 (1989); Han, Y. et al. Interspecies Transfer and Regulation of *Pseudomonas stutzeri* A1501 Nitrogen Fixation Island in *Escherichia coli*. Journal of microbiology and biotechnology 25, 1339-1348 (2015)). Nitrogenase is under stringent control because it is oxygen sensitive and energetically expensive: it can make up 20% of the cell mass and each NH₃ requires ~40 ATP. It is also irreversibly deactivated by oxygen. Across species, transcription of nif genes is strongly repressed by fixed nitrogen (ammonia) and oxygen with these signals converging on the NifA regulatory protein that works in concert with the sigma factor RpoN. Diverse, species-specific, and often poorly understood signals control these regulators, including plant-produced chemicals, ATP, reducing power, temperature, and carbon sources. Those bacteria that can fix nitrogen in a wider range of environmental conditions tend to be controlled by more complex regulatory networks.

[0060] When a nif cluster is transferred from one species to another, it either preserves its regulation by environmental stimuli or has an unregulated constitutive phenotype. Maintaining the native regulation, notably ammonium repression, limits their use in agriculture because such levels are likely to fluctuate according to soil types, irrigation, and fertilization. Nitrogen-fixing diazotrophs have been engineered to reduce ammonia sensitivity by disrupting NifL or mutating NifA and placing the entire cluster under the control of T7 RNA polymerase (RNAP). Constitutive expression of nitrogenase is also undesirable as it imparts a fitness burden on the cells. For example, when the nif cluster from *P. stutzeri* A1501 was transferred to *P. protegens* Pf-5, this was reported to result in sufficient ammonia production to support maize and wheat growth, but the bacteria quickly declined after a month when competing with other species in soil. Constitutive activity is detrimental even before the bacteria are introduced to the soil, impacting production, formulation, and long-term storage. Therefore, uncontrolled nitrogenase production could lead to more expensive production, shorter shelf life, and more in-field variability.

[0061] An important aspect of the nif clusters or nif genes the present disclosure is that they can each be under the control of a regulatory element. In some embodiments 2 or more genes are under the control of a regulatory element. In some embodiments, all the genes are under the control of a regulatory element. The regulatory elements may also be activation elements or inhibitory elements. An activation element is a nucleic acid sequence that when presented in context with a nucleic acid to be expressed will cause

expression of the nucleic acid in the presence of an activation signal. An inhibitory signal is a nucleic acid sequence that when presented in context with a nucleic acid to be expressed will cause expression of the nucleic acid unless an inhibitory signal is present. Each of the activation and inhibitory elements may be a promoter, such as a bacteriophage T7 promoter, sigma 70 promoter, sigma 54 promoter, lac promoter, etc. As used herein, the term "promoter" is intended to refer to those regulatory sequences which are sufficient to enable the transcription of an operably linked DNA molecule. Promoters may be constitutive or inducible. As used herein, the term "constitutive promoter" refers to a promoter that is always on (i.e. causing transcription at a constant level). Examples of constitutive promoters include, without limitation, sigma 70 promoter, bla promoter, lacL promoter, etc. Non-limiting examples of inducible promoters are shown in Table 6. The $P_{A1lacO1}$ promoter is another example of an inducible promoter that can be used in the present invention.

TABLE 6

Examples of regulatory elements (e.g. inducible promoters, repressors).		
Name	Chemical inducer and/or repressor	Essential regulatory gene(s)
ParaBAD ("PBAD")	L-arabinose (ON) & glucose (OFF)	araC
PphaBAD	L-rhamnose (ON) & glucose (OFF)	rhaR & rhaS
Plac	lactose or IPTG (ON) & glucose (OFF)	lacI
Ptac	lactose or IPTG (ON)	lacI
Plux	acyl-homoserine lactone (ON)	luxR
Ptet	tetracycline or aTc (ON)	tetR
Psal	salicylate (ON)	nahR
Ptrp	tryptophan (OFF)	(NONE)
Ppho	phosphate (OFF)	phoB & phoR

[0062] Inducible promoters allow regulation of gene expression and can be regulated by exogenously supplied compounds, environmental factors such as temperature, or the presence of a specific physiological state, e.g., acute phase, a particular differentiation state of the cell, or in replicating cells only. Inducible promoters and inducible systems are available from a variety of commercial sources, including, without limitation, Invitrogen, Clontech and Ariad. Many other systems have been described and can be readily selected by one of skill in the art. Examples of inducible promoters regulated by exogenously supplied promoters include the zinc-inducible sheep metallothionein (MT) promoter, the dexamethasone (Dex)-inducible mouse mammary tumor virus (MMTV) promoter, the T7 polymerase promoter system [WO 98/10088]; the ecdysone insect promoter [No et al, Proc. Natl. Acad. Sci. USA, 93:3346-3351 (1996)], the tetracycline-repressible system [Gossen et al, Proc. Natl. Acad. Sci. USA, 89:5547-5551 (1992)], the tetracycline-inducible system [Gossen et al, Science, 268:1766-1769 (1995), see also Harvey et al, Curr. Opin. Chem. Biol., 2:512-518 (1998)], the RU486-inducible system [Wang et al, Nat. Biotech., 15:239-243 (1997) and Wang et al, Gene Ther., 4:432-441 (1997)] and the rapamycin-inducible system [Magari et al, J. Clin. Invest., 100: 2865-2872 (1997)]. Still other types of inducible promoters which may be useful in this context are those which are

regulated by a specific physiological state, e.g., temperature, acute phase, a particular differentiation state of the cell, or in replicating cells only.

[0063] As used herein, the term "terminator" (as referred to as a transcription terminator) is a section of nucleic acid sequence that marks the end of a gene or operon in genomic DNA during transcription. They stop transcription of a polymerase. Terminators can be classified into several groups. At the first group of termination signals the core enzyme can terminate in vitro at certain sites in the absence of any other factors (as tested in vitro). These sites of termination are called intrinsic terminators or also class I terminators. Intrinsic terminators usually share one common structural feature, the so called hairpin or stem-loop structure. On the one hand the hairpin comprises a stem structure, encoded by a dG-dC rich sequence of dyad symmetrical structure. On the other hand the terminator also exhibits a dA-dT rich region at the 3'-end directly following the stem structure. The uridine rich region at the 3' end is thought to facilitate transcript release when RNA polymerase pauses at hairpin structures. Two or more terminators can be operatively linked if they are positioned to each other to provide concerted termination of a preceding coding sequence. Particularly preferred, the terminator sequences are downstream of coding sequences, i.e. on the 3' position of the coding sequence. The terminator can e.g. be at least 1, at least 10, at least 30, at least 50, at least 100, at least 150, at least 200, at least 250, at least 300, at least 400, at least 500 nucleotides downstream of the coding sequence or directly adjacent. Examples of terminators include, but are not limited to, T7 terminator, rrnBT1, L3S2P21, tonB, rrnA, rrnB, rrnD, RNAl, crp, his, ilv lambda, M13, rpoC, and up (see for example U.S. Pat. No. 9,745,588, incorporated herein by reference).

RpoN

[0064] As used herein "RpoN" refers to a gene that encodes the sigma factor sigma-54 (σ 54, sigma N, or RpoN), a protein in *Escherichia coli* and other species of bacteria. Sigma factors are initiation factors that promote attachment of RNA polymerase to specific initiation sites and are then released. Bacteria normally only have one functional copy of the alternative sigma factor, σ 54 or RpoN, which regulates a complex genetic network that extends into various facets of bacterial physiology, including metabolism, survival in strenuous environments, production of virulence factors, and formation of biofilms. RpoN is one of seven RNA polymerase sigma subunits in *E. coli* required for promoter-initiated transcription and RpoN plays a major role in the response of *E. coli* to nitrogen-limiting conditions. Under such conditions, RpoN directs the transcription of at least 14 *E. coli* operons/regulators in the nitrogen regulatory (Ntr) response. RpoN also plays an important role in stress resistance (e.g. resistance to osmotic stress) and virulence of bacteria. RpoN is structurally and functionally distinct from the other *E. coli* σ factors. It is able to bind promoter DNA in the absence of core RNA polymerase and it recognizes promoter sequences with conserved GG and GC elements located -24 to -12 nucleotides upstream of the transcription start site. Additionally, Regulatory proteins like NtrB and NtrC can activate σ 54 holoenzyme.

[0065] Without being bound by theory or mechanism, it is believed that RpoN works in concert with NifA to turn on

the transcription of nif clusters. An exemplary sequence for RpoN is provided in Table 10.

Gene Cluster Nucleic Acids

[0066] In some embodiments of the present disclosure a genetic cluster includes a nucleotide sequence that is at least about 85% or more homologous or identical to the entire length of a naturally occurring genetic cluster sequence, e.g., at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50% or more of the full length naturally occurring genetic cluster sequence). In some embodiments, the nucleotide sequence is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% homologous or identical to a naturally occurring genetic cluster sequence. In some embodiments, the nucleotide sequence is at least about 85%, e.g., is at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% homologous or identical to a genetic cluster sequence, in a fragment thereof or a region that is much more conserved, such as an essential, but has lower sequence identity outside that region.

[0067] Calculations of homology or sequence identity between sequences (the terms are used interchangeably herein) are performed as follows. To determine the percent identity of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The length of a reference sequence aligned for comparison purposes is at least 80% of the length of the reference sequence, and in some embodiments is at least 90% or 100%. The nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein nucleic acid "identity" is equivalent to nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0068] In some embodiments the gene clusters are native gene clusters. In some embodiments, the gene clusters are refactored gene clusters. In some instances, the nucleic acids may include non-naturally occurring nucleotides and/or substitutions, i.e. Sugar or base substitutions or modifications.

[0069] One or more substituted sugar moieties include, e.g., one of the following at the 2' position: OH, SH, SCH₃, F, OCN, OCH₃OCH₃, OCH₃O(CH₂)_nCH₃, O(CH₂)_nNH₂ or O(CH₂)_nCH₃ where n is from 1 to about 10; Ci to C₁₀ lower alkyl, alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF₃; OCF₃; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; SOCH₃; SO₂CH₃; ONO₂; NO₂; N₃; NH₂; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of a nucleic acid; or a group for improving the pharmacodynamic properties of a nucleic acid and other substituents having similar properties. Similar modifications may also be made at other positions on the nucleic acid, particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of

5' terminal nucleotide. Nucleic acids may also have sugar mimetics such as cyclobutyls in place of the pentofuranosyl group.

[0070] Nucleic acids can also include, additionally or alternatively, nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include adenine (A), guanine (G), thymine (T), cytosine (C) and uracil (U). Modified nucleobases include nucleobases found only infrequently or transiently in natural nucleic acids, e.g., hypoxanthine, 6-methyladenine, 5-Me pyrimidines, particularly 5-methylcytosine (also referred to as 5-methyl-2' deoxycytosine and often referred to in the art as 5-Me-C), 5-hydroxymethylcytosine (HMC), glycosyl HMC and gentobiosyl HMC, isocytosine, pseudouracil, as well as synthetic nucleobases, e.g., 2-aminoadenine, 2-(methylamino) adenine, 2-(imidazolylalkyl) adenine, 2-(aminoalkylamino) adenine or other heterosubstituted alkyladenines, 2-thiouracil, 2-thiothymine, 5-bromouracil, 5-hydroxymethyluracil, 5-propynyluracil, 8-azaguanine, 7-deazaguanine, N6 (6-aminohexyl) adenine, 6-aminopurine, 2-aminopurine, 2-chloro-6-aminopurine and 2,6-diaminopurine or other diaminopurines. See, e.g., Kornberg, "DNA Replication," W. H. Freeman & Co., San Francisco, 1980, pp 75-'7 7; and Gebeyehu, G., et al. Nucl. Acids Res., 15:4513 (1987)). A "universal" base known in the art, e.g., inosine, can also be included.

[0071] As used herein, the equivalent terms "expression" or "gene expression" are intended to refer to the transcription of a DNA molecule into RNA, and the translation of such RNA into a polypeptide.

[0072] As used herein, a "gene cluster" refers to a set of two or more genes that encode gene products. As used herein, a "nif gene cluster" refers to a set of two or more genes that encode nitrogen fixation genes.

[0073] "Exogenous" with respect to genes indicates that the nucleic acid or gene is not in its natural (native) environment. For example, an exogenous gene can refer to a gene that is from a different species. In contrast, "endogenous" with respect to genes indicates that the gene is in its native environment. As used herein, the terms "endogenous" and "native" are used interchangeably.

[0074] As used herein, the term "delete" or "deleted" refers to the removal of a gene (e.g. endogenous gene) from a sequence or cluster. As used herein, the term "alter" or "altered" refers to the modification of one or more nucleotides in a gene or the deletion of one or more base pairs in a gene. This alteration may render the gene dysfunctional. Herein, "ΔnifA" refers to a strain or cluster within which NifA was deleted or altered. Method of deletion and alteration, in the context of genes, are known in the art.

[0075] As used herein, the term "chemical signals" refers to chemical compounds. Any substance consisting of two or more different types of atoms (chemical elements) in a fixed stoichiometric proportion can be termed a chemical compound. Chemical signals can be synthetic or natural chemical compounds. In some embodiments of the present invention, a bacterium of the present disclosure or a sensor of the present disclosure is under the control of a chemical signal. In some embodiments, the signal is a native biological signal (e.g. root exudate, biological control agent, etc.). In some embodiments, the chemical signal is a quorum sensing signal from the bacterium. Non-limiting examples of chemical signals include root exudates (as defined below), bio-

control agents (as defined below), phytohormones, vanillate, IPTG, aTc, cuminic acid, DAPG, and salicylic acid, 3,4-dihydroxybenzoic acid, 3OC6HSL and 3OC14HSL.

[0076] As used herein, the term "root exudate" refers to chemicals secreted or emitted by plant roots in response to their environment. These allow plant to manipulate or alter their immediate environment, specifically their rhizosphere. Root exudates are a complex mixture of soluble organic substances, which may contain sugars, amino acids, organic acids, enzymes, and other substances. Root exudates include, but are not limited to, ions, carbon-based compounds, amino acids, sterols, sugars, hormones (phytohormones), flavonoids, antimicrobials, and many other chemical compounds. The exudates can serve as either positive regulators or negative regulators.

[0077] As used herein, the term "phytohormone" refers plant hormones and they are any of various hormones produced by plants that influence process such as germination, growth, and metabolism in the plant.

[0078] As used herein, the term "vanillate" refers to a methoxybenzoate that is the conjugate base of vanillic acid. It is a plant metabolite.

[0079] Biological control or biocontrol is a method of controlling pests such as insects, mites, weeds and plant diseases using other organisms. Natural enemies of insect pests, also known as biological control agents, include predators, parasitoids, pathogens, and competitors. Biological control agents of plant diseases are most often referred to as antagonists. Biological control agents of weeds include seed predators, herbivores and plant pathogens. The inducible clusters or promoters of the present invention may be modulated by a secretion of (or chemical otherwise associated with) a biological control agent. Herein, that is referred to as a "biocontrol agent".

[0080] Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present invention to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

EXAMPLES

[0081] Herein, inducible nitrogenase activity is engineered in two cereal endophytes (*Azorhizobium caulinodans* ORS571 and *Rhizobium* sp. IRBG74) and the epiphyte *Pseudomonas protegens* Pf-5, a maize seed inoculant. For each organism, different strategies are taken to eliminate ammonium repression and place nitrogenase expression under the control of agriculturally-relevant signals, including root exudates, biocontrol agents, and phytohormones. The present disclosure demonstrates that *Rhizobium* sp. (e.g., IRBG74) can be engineered to fix nitrogen under free living conditions, inter alia, by transferring either a nif cluster from *Rhodobacter* or *Klebsiella*. For *P. protegens* Pf-5, the transfer of an inducible cluster from *Azotobacter vinelandii* yields the highest ammonia and oxygen tolerance. Collectively, data from the transfer of 12 nif gene clusters between diverse species (including *E. coli* and 12 additional Rhizobia) help identify the barriers that must be overcome to engineer a bacterium to deliver a high nitrogen flux to a cereal crop and provide a solution such that *Rhizobium* can be engineered to fix nitrogen under free living conditions.

Materials and Methods

[0082] Bacterial strains and growth media. All bacterial strains and their derivatives used in this study are listed in Table 7. *E. coli* DH10-beta (New England Biolabs, MA, Cat #C3019) was used for cloning. *E. coli* K-12 MG1655 was used for the nitrogenase assay. *P. protegens* Pf-5 was obtained from the ATCC (BAA-477). Strains used in this study are listed in Table 8. For rich media, LB medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl), LB-Lennox medium (10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl), and TY medium (5 g/L tryptone, 3 g/L yeast extract, 0.87 g/L CaCl₂·2H₂O) were used. For minimal media, BB medium (0.25 g/L MgSO₄·7H₂O, 1 g/L NaCl, 0.1 g/L CaCl₂·2H₂O, 2.9 mg/L FeCl₃, 0.25 mg/L Na₂MoO₄·2H₂O, 1.32 g/L NH₄CH₃CO₂, 25 g/L Na₂HPO₄, 3 g/L KH₂PO₄ pH [7.4]), UMS medium (0.5 g/L MgSO₄·7H₂O, 0.2 g/L NaCl, 0.375 mg/L EDTA-Na₂, 0.16 ZnSO₄·7H₂O, 0.2 mg/L Na: MoO₄·2H₂O, 0.25 mg/L H₃BO₃, 0.2 mg/L MnSO₄·H₂O, 0.02 mg/L CuSO₄·5H₂O, 1 mg/L CoCl₂·6H₂O, 75 mg/L CaCl₂·2H₂O, 12 mg/L FeSO₄·7H₂O, 1 mg/L thiamine hydrochloride 2 mg/L D-pantothenic acid hemicalcium salt, 0.1 mg/L biotin, 87.4 mg/L K₂HPO₄, 4.19 g/L MOPS pH [7.0]), and Burk medium (0.2 g/L MgSO₄·7H₂O, 73 mg/L CaCl₂·H₂O, 5.4 mg/L FeCl₃·6H₂O, 4.2 mg/L Na₂MoO₄·2H₂O, 0.2 g/L KH₂PO₄, 0.8 g/L K₂HPO₄ pH [7.4]) were used. Antibiotics were used at the following concentrations (μg/mL): *E. coli* (kanamycin, 50; spectinomycin, 100; tetracycline, 15; gentamicin, 15). *P. protegens* Pf-5 (kanamycin, 30; tetracycline, 50; gentamicin, 15; carbenicillin, 50). R. sp. IRBG74 (neomycin, 150; gentamicin, 150; tetracycline, 10; nitrofurantoin, 10). *A. caulinodans* (kanamycin, 30; gentamicin, 15; tetracycline, 10; nitrofurantoin, 10). Chemicals including inducers used in this study are listed in Table 12.

[0083] Strain construction. In order to increase transformation efficiency in R. sp. IRBG74, a type-I restriction modification system was inactivated by deleting hsdR, which encodes a restriction enzyme for foreign DNA (this strain was the basis for all experiments) (Ferri, L., Gori, A., Biondi, E. G., Mengoni, A. & Bazzicalupo, M. J. P. Plasmid electroporation of *Sinorhizobium* strains: The role of the restriction gene hsdR in type strain Rm1021. 63, 128-135 (2010)). A sacB markerless insertion method was utilized to allow replacements of a native locus with synthetic parts by homologous recombination. Two homology arms of ~500 bp flanking the hsdR gene were amplified by PCR, cloned and yielded a suicide plasmid pMR-44. The suicide plasmid was mobilized into R. sp. IRBG74 by triparental mating. Single-crossover recombinants were selected for resistance to gentamicin and subsequently grown and plated on LB plates supplemented with 15% sucrose to induce deletion of the vector DNA part containing the counter selective marker sacB which converts sucrose into a toxic product (levan). Two native nif gene clusters encompassing nifHDKENX (genomic location 219,579-227,127) and nifSW-fixABCX-nifAB-fdxN-nifTZ (genomic location 234,635-234,802) of R. sp. IRBG74 were sequentially deleted using pMR45-46. To increase genetic stability recA gene was deleted using the plasmid pMR47. The R. sp. IRBG74 Δnif, hsdR, recA strain was the basis for all experiments unless indicated otherwise. Two homology arms of ~900 bp flanking the nifA gene were amplified by PCR, cloned and yielded a suicide plasmid pMR-47 to generate nifA deletion in *A. caulinodans* ORS571, The suicide plasmid pMR47 in *E. coli* was mobi-

lized into *A. caulinodans* by triparental mating. Single-crossover recombinants were selected for resistance to gentamicin and subsequently grown and plated on plain TY plates supplemented with 15% sucrose to induce deletion of the vector DNA part. All markerless deletions were confirmed by gentamicin sensitivity and diagnostic PCR. A list of the mutant strains is provided in Table 8.

[0084] Plasmid system. Plasmids with the pBBR1 origin were derived from pMQ131 and pMQ132. Plasmids with the pRO1600 origin were derived from pMQ80. Plasmids with the RK2 origin were derived from pJP2. Plasmids with the RSF1010 origin were derived from pSEVA651. Plasmids with the IncW origin were derived from pKT249. Plasmids used in this study are provided in Table 9.

[0085] Phylogenetic analysis of nif clusters. Phylogenetic analysis was performed based on the full-length 16S rRNA gene sequences (*K. oxytoca*, BWI76_05380; *A. vinelandii*, Avin_55000; *R. sphaeroides*, DQL45_00005; Cyanobacte ATCC51142, cce_RNA045; *A. brasiliense*, AMK58_25190; *R. palustris*, RNA_55; *P. protegens*, PST_0759; *Paenibacillus* sp. WLY78, JQ003557). A multiple sequence alignment was generated using MUSCLE (Edgar, R. C. J. N. a. r. MUSCLE: multiple sequence alignment with high accuracy and high throughput. 32, 1792-1797 (2004)). A phylogenetic tree was constructed using the Geneious software (R9.0.5) with the Jukes-Cantor distance model and UPGMA as a tree build method, with bootstrap values from 1,000 replicates.

[0086] nif cluster construction. To obtain large nif clusters on mobilizable plasmids that carry origin of transfer (oriT) for conjugative transfer of the plasmids, the genomic DNAs from *K. oxytoca*, *P. stutzeri*, *A. vinelandii*, *A. caulinodans* and *R. sphaeroides* were purified using Wizard genomic DNA purification kit, following the isolation protocol for gram negative bacteria (Promega, Cat #A1120). The genomic DNAs of Cyanobacte ATCC51142, *A. brasiliense* ATCC29729, *R. palustris* ATCC BAA-98, and *G. diazotrophicus* ATCC49037 were obtained from ATCC. Each nif cluster was amplified into several fragments (4-10 kb) with upstream and downstream 45 bp linkers at the 5' and 3' most end of the cluster by PCR with primer sets (Table 7) and assembled onto linearized *E. coli*-yeast shuttle vectors pMR-1 for *E. coli* and Rhizobia, and pMR-2 for *P. protegens* Pf-5 using yeast recombineering. For the nif cluster of *Paenibacillus* sp. WLY78, the DNA sequence information were gleaned from contig ALJV01 and the DNA of the nif cluster was synthesized by GeneArt gene synthesis (Thermo Fisher Scientific, MA) into four fragments that were used as templates for PCR amplification and assembly. Amplified fragments from two to eight (Table 7) were assembled with a linearized vector into a single large plasmid by one-pot yeast assembly procedure (Shanks, R. M. et al. *Saccharomyces cerevisiae*-based molecular tool kit for manipulation of genes from gram-negative bacteria. 72, 5027-5036 (2006)). Once assembled, the nif cluster-plasmids were isolated from yeast using Zymoprep Yeast Miniprep kit (Zymo Research Cat #D2004) and transformed into *E. coli*. The purified plasmid was isolated from *E. coli* and sequenced to verify the correct assembly and sequence (MGH CCIB DNA Core facility, Cambridge, MA). *E. coli* containing a mutation-free plasmid were stored for further experiments. Plasmids containing nif clusters are provided in Table 9.

[0087] Construction of refactored nif v3.2. The six transcriptional units (nifHDKTY, nifENX, nifJ, nifBQ, nifF, nifUSVWZM) were amplified from the plasmid pMR-3 that harbors the native *Klebsiella* nif cluster. Each unit was divided onto six level-1 module plasmids where the nif genes are preceded by a terminator. T7 promoter wild-type or T7 promoter variant PT7.P2 was placed between a terminator and the first gene of the transcriptional unit. Assembly linkers (~45 bp) were placed at both ends of the units. The level-1 plasmids (pMR32-37) were provided in Table 9 and 10. Each of the six plasmids was linearized by digestion with restriction enzymes and assembled with a linearized pMR-1 or pMR-2 vector into a single large plasmid by one-pot yeast assembly procedure, yielding pMR38 and pMR39.

[0088] Transformation. Electroporation was used to transfer plasmids into *P. protegens* Pf-5. A single colony was inoculated in 4 mL of LB and grown for 16 h at 30° C. with shaking at 250 rpm. The cell pellets were washed twice with 2 mL of 300 mM sucrose and dissolved in 100 μ L of 300 mM sucrose at RT. A total of 50-100 ng DNA was electroporated and recovered in 1 mL of LB media for 1 h before plating on selective LB plates. Triparental mating was used to transfer DNA from *E. coli* to Rhizobia. An aliquot of 40 μ L of late-log phase (OD_{600} ~0.6) donor cells and 40 μ L of late-log phage helper cells containing pRK7013 were mixed with 200 μ L of late-log phase (OD_{600} ~0.8) recipient Rhizobia cells and washed in 200 μ L of TY medium. Mating was initiated by spotting 20 μ L of the mixed cells on TY plates and incubated at 30° C. for 6 h. The mating mixtures were plated on TY medium supplemented with nitrofurantoin to isolate Rhizobia transconjugants.

[0089] Construction and characterization genetic parts for Rhizobia. Genetic part libraries were built on a pBBR1-ori plasmid pMR-1 using Gibson assembly (New England Biolabs, Cat #E2611). The fluorescence proteins, GFPmut3b and mRFP1 were used as reporters. The Anderson promoter library (Anderson, J. et al. BglBricks: A flexible standard for biological part assembly. 4, 1 (2010)) on the BioBricks Registry were utilized for the characterization of constitutive promoters (FIGS. 11A-11C). To characterize inducible promoters, a regulator protein is constitutively expressed by the *PlacIq* promoter, and GFP expression is driven by a cognate inducible promoter from the opposite direction, facilitating replacement of the reporter with gene of interest (e.g., T7 RNAP and nifA) and transfer of the controller unit across different plasmid backbones for diverse microbes. The following combinations of cognate regulators and inducible promoters were characterized. IPTG inducible *LacI*-A1lacO1, DAPG inducible *PhIF-PPhI*, aTc inducible *TetR*-PTet, 3OC6HSL inducible *LuxR-P_{Lux}*, salicylic acid inducible *Nahr-R-P_{Sal}*, and cuminic acid inducible *CymR-P_{Cym}* systems were optimized for *R. sp.* IRBG74 (FIG. 14). Opine inducible *OccR-P_{occ}*, and nopaline inducible *NocR-Pnoc* systems were optimized for *A. caulinodans* (FIGS. 20A-20F and Tables 9 and 10). For RBS characterization, an IPTG-inducible GFP expression plasmid pMR-40 was used and GFP was expressed to the highest levels with 1 mM IPTG (FIGS. 12A-12B). RBS library for GFP was designed using the RBS library calculator at the highest-resolution mode, and the 3' end of the 16S rRNA sequences were adjusted according to the species (3'-ACCTCCCTTC-5' for *R. sp.* IRBG74). Terminators for T7 RNAP were characterized by placing a terminator between two fluorescence reporters

expressed from a single T7 wild-type promoter located upstream of the first fluorescence protein GFP. The expression of the two fluorescence proteins is enabled by the controller strain MR18 encoding the IPTG-inducible T7 RNAP system by 1 mM IPTG (FIGS. 13A-13B). The terminator strength (T_s) was determined by normalizing fluorescence levels of a terminator construct by a reference construct pMR-66 where a 40 bp spacer was placed between the reporters. All genetic parts for Rhizobia were characterized as follows. Single colonies were inoculated into 0.5 ml TY supplemented with antibiotics in 96-deepwell plates (USA Scientific, Cat #18962110) and grown overnight at 30° C., 900 rpm in a Multitron incubator (INFORS HT, MD). 1.5 μ l of overnight cultures was diluted into 200 μ l of TY with antibiotics and appropriate inducers in 96-well plates (Thermo Scientific, Cat #12565215) and incubated for 7 h at 30° C., 1,000 rpm in an ELMI DTS-4 shaker (ELMI, CA). After growth, 8 μ l of culture sample was diluted into 150 μ l PBS with 2 mg/mL kanamycin for flow cytometry analysis. Plasmids and genetic parts are listed in Table 9 and 10.

[0090] Construction and characterization genetic parts for *P. protegens*. Genetic part libraries were built on a pRO1600-ori plasmid pMR-2 using Gibson assembly (New England Biolabs, Cat #E2611). The fluorescence proteins, GFPmut3b and mRFP1 were used as reporters. The Anderson promoter library on the BioBricks Registry were utilized for the characterization of constitutive promoters (FIGS. 11A-11C). The following combinations of cognate regulators and inducible promoters were characterized. IPTG inducible LacI-P_{tac}, DAPG inducible PhIF-P_{phb}, aTc inducible TetR-P_{ter}, 3OC6HSL inducible LuxR-P_{lux}, arabinose inducible AraC-P_{BAD}, cuminic acid inducible CymR-P_{Cym}, and naringenin inducible FdeR-P_{Fde} were optimized (FIGS. 15A-15C). For RBS characterization, an arabinose-inducible GFP expression plasmid pMR-65 was used and GFP was expressed with 1 mM IPTG (FIGS. 12A-12B). RBS library for GFP was designed using the RBS library calculator at the highest-resolution mode, and the 3' end of the 16S rRNA sequences were adjusted according to the species (3'-ACCTCCTTA-5' for *P. protegens* Pf-5). Terminators for T7 RNAP were characterized by placing a terminator between two fluorescence reporters expressed from a single T7 wild-type promoter located upstream of the first fluorescence protein GFP. The expression of the two fluorescence proteins is enabled by an IPTG-inducible T7 RNAP expression system of the controller strain MR7 (FIGS. 13A-13B). All genetic parts for *P. protegens* Pf-5 were characterized as follows. Single colonies were inoculated into 1 ml LB supplemented with antibiotics in 96-deepwell plates (USA Scientific, Cat #18962110) and grown overnight at 30° C., 900 rpm in a Multitron incubator (INFORS HT, MD). 0.5 μ l of overnight cultures was diluted into 200 μ l of LB with antibiotics and appropriate inducers in 96-well plates (Thermo Scientific, Cat #12565215) and incubated for 7 h at 30° C., 1,000 rpm in an ELMI DTS-4 shaker (ELMI, CA). After growth, 10 μ l of culture sample was diluted into 150 μ l PBS with 2 mg/mL kanamycin for flow cytometry analysis. Plasmids and genetic parts are listed in Tables 9 and 10.

[0091] Genomic integration and characterization of controllers. The mini-Tn7 insertion system was used to introduce a controller into the genome of *P. protegens* Pf-5. The IPTG-inducible T7 RNAP expression system and a tetracycline resistant marker tetA was placed between two Tn7 ends

(Tn7L and Tn7R). The controller plasmid pMR-85 was introduced into *P. protegens* Pf-5 by double transformation with pTNS3 encoding the TasABCD transposase. A genetically-integrated controller located 25 bp downstream of the stop codon of glmS was confirmed by PCR and sequencing. A markerless insertion method using homologous recombination was employed in R. sp. IRBG74. A controller encoding inducible T7 RNAP system flanked by two homology fragments that enables the replacement of recA was cloned into a suicide plasmid. These controller plasmids (IPTG-inducible, pMR82-84; DAPG-inducible, pMR85) in *E. coli* was mobilized into R. sp. IRBG74 MR 18 (AhsdR. Δnif) by triparental mating, generating the controller strains (MR 19, 20, 21 and 22, respectively). The controller integration in the genome was confirmed by gentamicin sensitivity and diagnostic PCR. All controllers were characterized in a manner identical to that described in genetic part characterization.

[0092] Construction and characterization of Marionette-based controllers. To regulate nitrogenase expression in the *E. coli* Marionette MG1655, the yfp in the 12 reporter plasmids was replaced with T7 RNAP while keeping other genetic parts (e.g., promoters and RBSs) unchanged (FIGS. 28A-28C). The reporter plasmid pMR-120 in which gfpmut3b is fused to the PT7 (P2) promoter (FIGS. 28A-28C) was co-transformed to analyze the response functions of each of the 12 T7 RNAP controller plasmids. To characterize controllers, single colonies were inoculated into 1 ml LB supplemented with antibiotics in 96-deepwell plates (USA Scientific, Cat #18962110) and grown overnight at 30° C., 900 rpm in a Multitron incubator (INFORS HT, MD). 0.5 μ l of overnight cultures was diluted into 200 μ l of LB with antibiotics and appropriate inducers in 96-well plates (Thermo Scientific, Cat #12565215) and incubated for 6 h at 30° C., 1,000 rpm in an ELMI DTS-4 shaker (ELMI, CA). After growth, 4 μ l of culture sample was diluted into 150 μ l PBS with 2 mg/mL kanamycin for flow cytometry analysis.

[0093] Flow cytometry. Cultures with fluorescence proteins were analyzed by flow cytometry using a BD Biosciences LSRII Fortessa analyzer with a 488 nm laser and 510/20-nm band pass filter for GFP and a 561 nm laser and 610/20 nm band pass filter for mCherry and mRFP1. Cells were diluted into 96-well plates containing phosphate buffered saline solution (PBS) supplemented with 2 mg/mL kanamycin after incubation. Cells were collected over 20,000 events which were gated using forward and side scatter to remove background events using FlowJo (TreeStar Inc., Ashland, OR). The median fluorescence from cytometry histograms was calculated for all samples. The median autofluorescence was subtracted from the median fluorescence and reported as the fluorescence value in arbitrary unit (au).

[0094] Nitrogenase assay (*E. coli* and *K. oxytoca*). Cultures were initiated by inoculating a single colony into 1 mL of LB supplemented with appropriate antibiotics in 96-deepwell plates (USA Scientific, Cat #18962110) and grown overnight at 30° C., 900 rpm in a Multitron incubator. 5 μ l of overnight cultures was diluted into 500 μ l of BB medium with 17.1 mM NH4CH3CO2 and appropriate antibiotics in 96-deepwell and incubated for 24 h at 30° C., 900 rpm in a Multitron incubator. Cultures were diluted to an OD600 of 0.4 into 2 mL of BB medium supplemented with appropriate antibiotics, 1.43 mM serine to facilitate nitrogenase depression, and an inducer (if necessary) in 10 mL glass vials with

PTFE-silicone septa screw caps (Supelco Analytical, Cat #SU860103). Headspace in the vials was replaced with 100% argon gas using a vacuum manifold. Acetylene freshly generated from CaC₂ in a Burris bottle was injected to 10% (vol/vol) into each culture vial to begin the reaction. The acetylene reduction was carried out for 20 h at 30° C. with shaking at 250 rpm in an Innova 44 shaking incubator (New Brunswick) to prevent cell aggregations, followed by quenching via the addition of 0.5 mL of 4 M NaOH to each vial.

[0095] Nitrogenase assay (*P. protegens* Pf-5). Cultures were initiated by inoculating a single colony into 1 mL of LB supplemented with appropriate antibiotics in 96-deepwell plates (USA Scientific, Cat #18962110) and grown overnight at 30° C., 900 rpm in a Multitron incubator. 5 µl of overnight cultures was diluted into 500 µl of BB medium with 17.1 mM NH₄CH₃CO₂ and appropriate antibiotics in 96-deepwell and incubated for 24 h at 30° C., 900 rpm in a Multitron incubator. Cultures were diluted to an OD₆₀₀ of 0.4 into 2 mL of BB medium supplemented with appropriate antibiotics, 1.43 mM serine and an inducer (if necessary) in 10 mL glass vials with PTFE-silicone septa screw caps. Headspace in the vials was replaced with 99% argon and 1% oxygen gas (Airgas, MA USA) using a vacuum manifold. Acetylene was injected to 10% (vol/vol) into each culture vial to begin the reaction. The acetylene reduction was carried out for 20 h at 30° C. with shaking at 250 rpm, followed by quenching via the addition of 0.5 mL of 4 M NaOH to each vial.

[0096] Nitrogenase assays (Rhizobia strains). Cultures were initiated by inoculating a single colony into 0.5 mL of TY medium supplemented with appropriate antibiotics in 96-deepwell plates (USA Scientific, Cat #18962110) and grown overnight at 30° C., 900 rpm in a Multitron incubator. 5 µl of overnight cultures was diluted into 500 µl of UMS medium with 30 mM succinate, 10 mM sucrose, and 10 mM NH₄Cl and appropriate antibiotics in 96-deepwell and incubated for 24 h at 30° C., 900 rpm in a Multitron incubator. Cultures were diluted to an OD₆₀₀ of 0.4 into 2 mL of UMS medium plus 30 mM succinate and 10 mM sucrose supplemented with appropriate antibiotics, 1.43 mM serine and an inducer (if necessary) in 10 mL glass vials with PTFE-silicone septa screw caps. Headspace in the vials was replaced with 99% argon and 1% oxygen gas using a vacuum manifold. Acetylene was injected to 10% (vol/vol) into each culture vial to begin the reaction. The acetylene reduction was carried out for 20 h at 30° C. with shaking at 250 rpm, followed by quenching via the addition of 0.5 mL of 4 M NaOH to each vial.

[0097] Nitrogenase assays (*A. caulinodans* and *P. stutzeri*). Cultures were initiated by inoculating a single colony into 0.2 mL of TY medium supplemented with appropriate antibiotics in 96-deepwell plates and grown overnight at 37° C. and 30° C. for *A. caulinodans* and *P. stutzeri*, respectively. 900 rpm in a Multitron incubator. 5 µl of overnight cultures was diluted into 500 µl of UMS medium with 30 mM lactate and 10 mM NH₄Cl and appropriate antibiotics in 96-deepwell and incubated for 24 h at 37° C. and 30° C. for *A. caulinodans* and *P. stutzeri*, respectively, 900 rpm in a Multitron incubator. Cultures were diluted to an OD₆₀₀ of 0.4 into 2 mL of UMS medium plus 30 mM lactate supplemented with appropriate antibiotics and an inducer (if necessary) in 10 mL glass vials with PTFE-silicone septa screw caps. Headspace in the vials was

replaced with 99% argon plus 1% oxygen gas using a vacuum manifold. Acetylene was injected to 10% (vol/vol) into each culture vial to begin the reaction. The acetylene reduction was carried out for 20 h at 30° C. with shaking at 250 rpm, followed by quenching via the addition of 0.5 mL of 4 M NaOH to each vial.

[0098] Nitrogenase assays (*A. vinelandii*). Cultures were initiated by inoculating a single colony into 0.5 mL of Burk medium supplemented with appropriate antibiotics in 96-deepwell plates (USA Scientific, Cat #18962110) and grown overnight at 30° C., 900 rpm in a Multitron incubator. 5 µl of overnight cultures was diluted into 500 µl of Burk medium with 17.1 mM NH₄CH₃CO₂ and appropriate antibiotics in 96-deepwell and incubated for 24 h at 30° C., 900 rpm in a Multitron incubator. Headspace in the vials was replaced with 97% argon and 3% oxygen gas (Airgas, MA USA) using a vacuum manifold. Acetylene was injected to 10% (vol/vol) into each culture vial to begin the reaction. The acetylene reduction was carried out for 20 h at 30° C. with shaking at 250 rpm, followed by quenching via the addition of 0.5 mL of 4 M NaOH to each vial.

[0099] Nitrogenase activity assay in the presence of ammonium. Following overnight incubation in minimal medium with a nitrogen source (described above), cultures were diluted to an OD₆₀₀ of 0.4 in 2 mL of nitrogen-free minimal medium, 1.43 mM serine (for *E. coli* and *P. protegens* Pf-5) and an inducer (for inducible systems) in 10 mL glass vials with PTFE-silicone septa screw caps. Ammonium (17.1 mM NH₄CH₃CO₂ for *E. coli* and *P. protegens* Pf-5 and 10 mM NH₄Cl for Rhizobia) was added to a nitrogen-free minimal medium when testing ammonium tolerance of nitrogenase activity. Headspace in the vials was replaced with either 100% argon gas for *E. coli*, 99% argon plus 1% oxygen for *Pseudomonas* and Rhizobia using a vacuum manifold. Acetylene was injected to 10% (vol/vol) into each culture vial to begin the reaction. The acetylene reduction was carried out for 20 h at 30° C. with shaking at 250 rpm followed by quenching via the addition of 0.5 mL of 4 M NaOH to each vial.

[0100] Nitrogenase activity assay at varying oxygen levels. Following overnight incubation in minimal medium with a nitrogen source (described above), cultures were diluted to an OD₆₀₀ of 0.4 in 2 mL of minimal medium, 1.43 mM serine (for *E. coli* and *P. protegens* Pf-5), and an inducer (for inducible systems) in 10 mL glass vials with PTFE-silicone septa screw caps. The vial headspace was replaced with either 100% nitrogen gas for *E. coli* or 99% nitrogen plus 1% oxygen for *P. protegens* Pf-5 and *A. caulinodans* using a vacuum manifold. Cultures were incubated with shaking at 250 rpm at 30° C. for 6 h and 9 h for *P. protegens* Pf-5 and *A. caulinodans*, respectively, after which oxygen concentrations in the headspace were recorded with the optical oxygen meter FireStingO2 equipped with a needle-type sensor OXF500PT (Pyro Science, Germany). After the induction period, no oxygen remained in the headspace for all species as confirmed by the oxygen meter. The initial oxygen levels in the headspace were adjusted by injecting pure oxygen via syringe into the headspace of the vials and stabilized with shaking at 250 rpm at 30° C. for 15 m followed by the injection of acetylene to 10% (vol/vol) into each culture vial to begin the reaction and initial oxygen concentrations in the headspace were recorded concomitantly. The oxygen levels in the headspace were maintained around the setting points (<±0.25% (2) while incubating at

250 rpm and 30° C. by injecting oxygen every hour for 3 h with oxygen monitoring before and after oxygen spiking (FIGS. 26A-26B). The reactions were quenched after 3 h of incubation by the injection of 0.5 mL of 4 M NaOH to each vial using a syringe.

[0101] Ethylene quantification. Ethylene production was analyzed by gas chromatography using an Agilent 7890A GC system (Agilent Technologies, Inc., CA USA) equipped with a PAL headspace autosampler and flame ionization detector as follows. An aliquot of 0.5 mL headspace preincubated to 35° C. for 30 s was injected and separated for 4 min on a GS-CarbonPLOT column (0.32 mm×30 m, 3 microns; Agilent) at 60° C. and a He flow rate of 1.8 mL/min. Detection occurred in a FID heated to 300° C. with a gas flow of 35 mL/min H₂ and 400 mL/min air. Acetylene and ethylene were detected at 3.0 min and 3.7 min after injection, respectively. Ethylene production was quantified by integrating the 3.7 min peak using Agilent GC/MSD ChemStation Software.

[0102] Sample preparation for RNA-seq and Ribosome profiling. Cultures of *K. oxytoca*, *E. coli*, *P. protegens* Pf-5 or R. sp. IRBG74 were grown following the same protocol as used for nitrogenase activity assay (described above) with a few changes. Following overnight incubation in minimal medium with a nitrogen source, cultures were diluted to an OD₆₀₀=0.4 in 25 mL of minimal medium (with an inducer, if needed) and antibiotics in 125 mL Wheaton serum vials (DWK Life Sciences, Cat #223748) with septum stoppers (Fisher Scientific, Cat #FB57873). The vial headspace was replaced with either 100% nitrogen gas for *E. coli* and *K. oxytoca* or 99% nitrogen plus 1% oxygen for *P. protegens* Pf-5 and R. sp. IRBG74 using a vacuum manifold. Cultures grown 6 h at 30° C., 250 rpm were filtered onto a nitrocellulose filter 0.45 μM pore size (Fisher Scientific, Cat #GVS1215305). Cell pellets were combined from three vials using a stainless-steel scoopula, followed by flash-frozen in liquid nitrogen. The frozen pellets were added to 650 μL of frozen droplets of lysis buffer (20 mM Tris (pH 8.0), 100 mM NH₄Cl, 10 mM MgCl₂, 0.4% Triton X-100, 0.1% NP-40, 1 mM chloramphenicol and 100 U/mL DNase I) in prechilled 25 mL canister (Retsch, Germany, Cat #014620213) in liquid nitrogen and pulverized using TissueLyser II (Qiagen USA) with a setting at 15 Hz for 3 min for 5 times with intermittent cooling between cycles. The pellet was removed by centrifugation at 20,000 rcf at 4° C. for 10 min and the lysate was recovered in the supernatant.

[0103] RNA-seq experiments. RNA-seq and Ribosome footprint profiling was carried out according to the method described earlier with a few modifications (Li, G.-W., Oh, E. & Weissman, J. S. J. N. The anti-Shine-Dalgarno sequence drives translational pausing and codon choice in bacteria. 484, 538 (2012); Li, G.-W., Burkhardt, D., Gross, C. & Weissman, J. S. Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. Cell 157, 624-635 (2014)). The total RNA was isolated using the hot phenol-SDS extraction method. The rRNA fractions were determined and subtracted from the total using the MICROBExpress kit (Thermo Fisher Scientific, Cat #AM1905). The remaining mRNAs and tRNAs were fragmented by RNA fragmentation reagents (Thermo Fisher Scientific, Cat #AM8740) at 95° C. for 1 m 45 s. RNA fragments (10-45 bp) were isolated from a 15% TBE-Urea polyacrylamide gel (Thermo Fisher Scientific, Cat #EC6885). The 3' ends of the RNA fragments were dephos-

phorylated using T4 polynucleotide kinase (1 U/μL, New England Biolabs, Cat #M0201S) in a 20 μL reaction volume supplemented with 1 μL of 20 U SUPERase. In at 37° C. for 1 h, after which the denatured fragments (5 pmoles) were incubated at 80° C. for 2 min and ligated to 1 μg of the oligo (/5rApp/CTGTAGGCACCATCAAT/3ddc/, Integrated DNA technologies) (SEQ ID NO: 1) in a 20 μL reaction volume supplemented with 8 μL of 50% PEG 8000, 2 μL of 10x T4 RNA ligase 2 buffer, 1 μL of 200 U/μL truncated K277Q T4 ligase 2 (New England Biolabs, Cat #M0351) and 1 μL of 20 U/μL of SUPERase. In at 25° C. for 3 h. The ligated fragments (35-65 bp) were isolated from a 10% TBE-Urea polyacrylamide gel (Invitrogen, Cat #EC6875). cDNA libraries from the purified mRNA products were reverse-transcribed using Superscript III

[0104] (Thermo Fisher Scientific, Cat #18080044) with oCJ485 primer (/5Phos/AGATCG-GAAGAGCGTCGTGTAGGGAAAGAGTGT/iSp18/CAAGCAGAAGA CGGCATACGAGATATT-GATGGTGCCTACAG (SEQ ID NO: 2, SEQ ID NO: 3)) at 50° C. for 30 min and RNA products subsequently were hydrolyzed by the addition of NaOH at a final concentration of 0.1 M, followed by incubation at 95° C. for 15 min. The cDNA libraries (125-150 bp) were isolated from on a 10% TBE-Urea polyacrylamide gel (Invitrogen, Cat #EC6875). The cDNA products were circularized in a 20 μL reaction volume supplemented with 2 μL of 10x CircLigase buffer, 1 μL of 1 mM ATP, 1 μL of 50 mM MnCl₂ and 1 μL of CircLigase (Epicenter, Cat #CL4115K) at 60° C. for 2 h and heat-inactivated at 80° C. for 10 min. 5 μL of circularized DNA was amplified using Phusion HF DNA polymerase (New England Biolabs, Cat #M0530) with o231 primer (CAAGCAGAAGACGGCATACGA (SEQ ID NO: 4)) and indexing primers (AATGATACTGGCGACCACCGAGATCTACACGATCGGAAGAGCACACGTCTGAACCTCAGTCACNNNNNNACACTCTTCCTACAC (SEQ ID NO: 5)) for 7 to 10 cycles. The amplified products (125-150 bp) were recovered from an 8% TBE-Urea polyacrylamide gel (Invitrogen, Cat #EC62152). The purified products were analyzed by BioAnalyzer (Agilent, CA USA) and sequenced with a sequencing primer (CGACAGGTTACAGAGTTCTACAGTCCGACGATC (SEQ ID NO: 6)) using an Illumina HiSeq 2500 with a rapid run mode. To generate the RNA-seq read profile for each nif cluster, the raw trace profiles are multiplied by 107 and normalized by respective total reads from coding sequences of each species (*K. oxytoca* M5a1, CP020657.1; *E. coli* MG1655, NC_000913.3; *P. protegens* Pf-5, CP000076; R. sp. IRBG74 HG518322, HG518323, HG518324 and an appropriate plasmid carrying a nif cluster). The mRNA expression level of each gene was estimated using total sequencing reads mapped onto the gene, representing fragments per kilobase of transcript per million fragments mapped units (FPKM).

[0105] Ribo-seq experiments. 0.5 mg of RNA was diluted into 195 μL of the lysis buffer including 0.5 U RNase inhibitor SUPERase. In (Invitrogen, Cat #AM2694), 5 mM CaCl₂ and were treated with 5 μL of 750 U of micrococcal nuclease (Sigma Aldrich, Cat #10107921001) at 25° C. for 1 h to obtain ribosome-protected monosomes. The digestions were quenched by the addition of EGTA to a final concentration of 6 mM and then kept on ice before the isolation of monosomes. Subsequently, the monosome fraction was collected by sucrose density gradient (10-55% w/v)

ultracentrifugation at 35,000 rpm for 3 h, followed by a hot phenol-SDS extraction to isolate ribosome-protected mRNA fragments. The mRNA fragments (15–45 bp) were isolated from a 15% TBE-Urea polyacrylamide gel. The 3' ends of the purified fragments were dephosphorylated and ligated to the modified oligo. cDNA libraries generated by Superscript III were circularized by CircLigase as described above. rRNA products were depleted by a respective biotinylated oligo mix for *E. coli* and *P. protegens* Pf-5. 5 µl of circularized DNA was amplified using Phusion HF DNA polymerase with 0231 primer and indexing primers for 7 to 10 cycles. The amplified products (125–150 bp) were recovered from an 8% TBE-Urea polyacrylamide gel. The purified products were analyzed by BioAnalyzer and sequenced with a sequencing primer (CGACAGGTTCAGAGTTCTA-CAGTCGACGATC (SEQ ID NO: 7)) using an Illumina HiSeq 2500 with a rapid run mode. Sequences were aligned to reference sequences using Bowtie 1.1.2 with the parameters-k1-m2-v1. A center-weighting approach was used to map the aligned footprint reads ranging from 22 to 42 nucleotides in length. To map P-site of ribosome from footprint reads, 11 nucleotides from the both ends were trimmed, and the remaining nucleotide were given the same score, normalized by the length of the center region. Aligned reads (10–45 nucleotides) were mapped to the reference with equal weight of each nucleotide. A Python 3.4 script was used to perform the mapping. To generate the Ribo-seq read profile for each nif cluster, the raw trace profiles are multiplied by 10⁸ and normalized by respective total reads from coding sequences of each species. To calculate the ribosome density of each gene, read densities were first normalized in the following ways: (i) The first and last 5 codons of the gene are excluded for the calculation to remove the effects of translation initiation and termination. (ii) A genome-wide read density profile was fitted to an exponential function and the density at each nucleotide on a given gene was corrected using this function. (iii) If the average read density on a gene is higher than 1, a 90% winsorization was applied to reduce the effect of outliers. The sum of normalized reads on a gene was normalized by the gene length and the total read densities on coding sequences to yield the ribosome density.

[0106] Calculation of genetic part strengths based on-seq data. The activity of a promoter is defined as the change in RNAP flux 8/around a transcription start site X_{iss} (Gorochowski, T. E. et al. Genetic circuit characterization and debugging using RNA-seq. 13, 952 (2017)). The promoter strength is calculated by

$$\delta \textcircled{2} = \frac{\textcircled{2}}{\textcircled{2}} [\textcircled{2} m(i) - \textcircled{2} m(i)] \quad (1)$$

② indicates text missing or illegible when filed

where m (i) is the number of transcripts at each position I from FPKM-normalized transcriptomic profiles, y=0.0067 s" is the degradation rate of mRNA, n is the window length before and after x_{tss}. The window length is set to 10. The terminator strength T_s is defined as the fold-decrease in transcription before and after a terminator, which can be quantified from FPKM-normalized transcriptomic profiles as

$$\textcircled{2} = \frac{\textcircled{2} m(i)}{\textcircled{2} m(i)} \quad (2)$$

② indicates text missing or illegible when filed

where x₀ and x₁ are the beginning and end positions of the terminator part, respectively. Translation efficiency was calculated by dividing the ribosome density by the FPKM.

[0107] nifH expression analysis. Complementation of NifA was tested using plasmid pMR-128 to 130 that contains the sfgfp fused to the nifH promoter in the *A. caulinodans* ΔnifA mutant. The inducible NifA/RpoN expression was provided by the plasmid pMR-121 into which sfgfp driven by the nifH promoter was added to analyze nifH promoter activity, yielding pMR-131 (FIG. 29). The IPTG-inducible system in the plasmid pMR-124 was substituted with other inducible systems including the salicylic acid-inducible, nopaline-inducible and octopine-inducible systems, yielding pMR-125, 126, and 127, respectively. Each of the plasmids was mobilized into the *A. caulinodans* ΔnifA mutant, which was grown following the same protocol as used for nitrogenase activity (described herein). Following overnight incubation in minimal medium with a nitrogen source, cultures were diluted to an OD₆₀₀=0.4 in 2 mL of UMS medium plus 30 mM lactate, antibiotics and an inducer (for inducible systems) in 10 mL glass vials with PTFE-silicone septa screw caps. Headspace in the vials was replaced with 99% argon plus 1% oxygen using a vacuum manifold. The vials were incubated with shaking at 250 rpm at 30° C. for 9 h, after which 10 µl of cultures was diluted into 150 µl PBS with 2 mg/mL kanamycin for flow cytometry analysis. To test activation of the nifH promoters by diverse NifA proteins, the plasmids pMR-51, 53, 88, 89 and 90 were introduced into *E. coli* MG1655 and the plasmids pMR-91, 92, 93, 94 and 95 to *P. protegens* Pf-5. The plasmid pMR-101 was used to provide inducible NifA expression by IPTG in *E. coli*. The controller encoding the IPTG-inducible NifA was inserted into the genome of *P. protegens* Pf-5 using the plasmids pMR-96, 97 and 98. The IPTG-inducible system of the NifA controller plasmid pMR-96 was replaced with the arabinose-inducible and the naringenin-inducible system, yielding pMR-99 and 100, respectively. The inducibility of nifH expression was assessed by the reporter plasmids pMR-105 to 107 and pMR102 to 104 for *E. coli* and *P. protegens* Pf-5, respectively. The controller plasmids were transformed into *E. coli* or *P. protegens* Pf-5 with the reporter plasmids. Following overnight incubation in minimal medium with a nitrogen source, cultures were diluted to an OD_{600=0.4} in 2 mL of BB medium, antibiotics and an inducer (for inducible systems) in 10 mL glass vials with PTFE-silicone septa screw caps. Headspace in the vials was replaced with either 100% argon for *E. coli* or 99% argon plus 1% oxygen for *P. protegens* Pf-5 using a vacuum manifold. The vials were incubated with shaking at 250 rpm at 30° C. for 9 h, after which 10 µl of cultures was diluted into 150 µl PBS with 2 mg/mL kanamycin for flow cytometry analysis.

[0108] Sequence alignment. NifA sequences of *R. sphaeroides* 2.4.1 (RSP_0547) and *A. caulinodans* ORS571 (AZC_1049) were obtained from NCBI. NifA protein sequences were aligned with MUSCLE (<https://www.ebi.ac.uk/Tools/msa/muscle/>) with a default settings (FIG. 22).

Results

Performance of Native Nif Clusters in *E. coli*, *P. Protegens* Pf-5, and Symbiotic Rhizobia

[0109] A set of diverse native nif clusters were cloned in order to determine their relative performance in different strains and the associated species barriers (FIG. 1A). Previously-defined boundaries for the well-studied nif cluster from *K. oxytoca* (Arnold, W., Rump, A., Klipp, W., Priefer, U. B. & Pühler, A. J. J. o. m. b. Nucleotide sequence of a 24.206-base-pair DNA fragment carrying the entire nitrogen fixation gene cluster of *Klebsiella pneumoniae*. 203, 715-738 (1988)) and the small (10 kb) cluster from *Paenibacillus polymyxa* WLY7870 were used. Similarly, the published boundaries (43.7 kb) of the *P. stutzeri* A1501 (Yan, Y. et al. Nitrogen fixation island and rhizosphere competence traits in the genome of root-associated *Pseudomonas stutzeri* A1501. Proceedings of the National Academy of Sciences (2008).) and *A. vinelandii* DJ clusters were used (Hamilton, T. L. et al. Transcriptional profiling of nitrogen fixation in *Azotobacter vinelandii*. *J Bacteriol* 193, 4477-4486, doi:10.1128/JB.05099-11 (2011)). A region of the *P. stutzeri* A1501 nif cluster (Pst1307-Pst1312) was excluded as these genes are predicted to have no effect on nitrogenase. *A. vinelandii* DJ contains three putative electron transport systems (the Rnf1 and Rnf2 complexes and the Fix complex) located in other regions of the genome. RNA-seq data shows that Rnf2 is not co-expressed with the nif genes, so only the Rnf1 and Fix complexes were included by fusing their DNA to create a single 46.9 kb construct. The nif cluster (40.1 kb) from Azospirillum brasiliense Sp7 was selected because this species is a cereal endophyte and fixes nitrogen in free-living conditions. Several less-studied gene clusters were also cloned in order to probe species barriers. As a representative of cyanobacteria, the gene cluster from *Cyanothece* sp. ATCC51142 was cloned following published boundaries. Its transcriptional activator PatB occurs outside of the nif cluster, which was cloned along with its native promoter and fused to nif cluster to form a single construct (31.7 kb). Several gene clusters were selected from photosynthetic purple bacteria (*Rhodopseudomonas palustris* CGA009 (Oda, Y. et al. Functional genomic analysis of three nitrogenase isozymes in the photosynthetic bacterium *Rhodopseudomonas palustris*. 187, 7784-7794 (2005)) and *Rhodobacter sphaeroides* 2.4.1 (Haselkorn, R. & Kapatal, V. in Genomes and genomics of nitrogen-fixing organisms, 71-82 (Springer, 2005))) as these are members of the same alphaproteobacteria class as Rhizobia. The rnf cluster, encoded on a separate chromosome of *R. sphaeroides* 2.4.1, was added to the nif cluster to provide electrons to nitrogenase. Finally, the gene clusters from the sugarcane and rice endosymbiant *Gluconacetobacter diazotrophicus* PA15 (28.9 kb) as well as the three nif clusters from *A. caulinodans* ORS571 (64 kb)³⁷ were cloned together with an upstream regulator fixLJK, but these were found to be inactive in all species tested, so they are not shown in FIGS. 1A-1F. The precise genomic locations for all the nif clusters are provided in Table 7 and the plasmids containing nif clusters are provided in Table 8.

[0110] Each cluster was amplified from genomic DNA as multiple fragments by PCR and assembled with the plasmid backbone using yeast assembly (see Methods and Materials Section). The *P. polymyxa* WLY78 cluster was de novo synthesized based on the DNA sequence on contig ALJV01 (Shanks, R. M. et al. *Saccharomyces cerevisiae*-based

molecular tool kit for manipulation of genes from gram-negative bacteria. 72, 5027-5036 (2006)). The clusters were cloned into different plasmid systems to facilitate transfer. For transfer to *E. coli* and R. sp. IRBG74, the broad-host range plasmid based on a pBBR1 origin was used (a second compatible RK2-origin plasmid was used for the nif cluster from *A. caulinodans* ORS571). These plasmids contain the RK2 oriT to enable the conjugative transfer of large DNA (see Materials and Methods). For transfer to *P. protegens* Pf-5, this plasmid system was found to be unstable and produce a mixed population. To transfer into this strain, the *Pseudomonas*-specific plasmid pRO1600 with the oriT was used. After construction, all of the plasmids were verified using next-generation sequencing (see Methods and Materials Section).

[0111] The set of 10 nif clusters were transferred into *E. coli* MG1655, the cereal epiphyte *P. protegens* Pf-5, and the cereal endophyte R. sp. IRBG74 to create 30 strains (FIG. 1A). *E. coli* was selected as a control as most of the published successful transfers have been to this recipient. Native *P. protegens* Pf-5 does not fix nitrogen. R. sp. IRBG74 contains two nif clusters in different genomic locations, which were left intact, but does not have nitrogenase activity under free living conditions. The genomic cluster does not have the required NifV enzyme as it obtains homocitrate from the plant. All of the clusters in the set have nifV, except the one from *P. polymyxa* WLY78. A test was run to determine whether the expression of recombinant nifV from *A. caulinodans* ORS571 in R. sp. IRBG74 would result in active nitrogenase, but no activity was detected.

[0112] The bacteria were grown in appropriate media, including antibiotics, and then evaluated for nitrogenase activity using an acetylene reduction assay (see Methods and Materials Section). *E. coli* and *Pseudomonas* were grown at 30° C. in BB minimal media, as described previously⁷¹. However, no growth was observed for R. sp. IRBG74 under these conditions. Different media and carbon sources were tested and it was found that UMS media with dicarboxylic acids (malate or succinate), the major carbon source from plants¹⁴⁷, with 10 mM sucrose yielded the highest growth rates (FIG. 6). After overnight growth, cells were transferred to stoppered test tubes in ammonium-free minimal media to a final OD₆₀₀ of 0.4. For *E. coli*, the headspace air is completely replaced with argon gas. For *P. protegens* Pf-5 and R. sp. IRBG74, the initial headspace concentration of oxygen was maintained at 1% because these bacteria require oxygen for their metabolism. The cells are incubated at 30° for 20 hours in the presence of excess acetylene and the conversion to ethylene was quantified by GC-MS (see Methods and Materials Section). There was no significant growth for any of the strains under these conditions, so the nitrogenase activities reported correspond to the same cell densities.

[0113] A surprising 6 out of 10 clusters were functional in *E. coli* MG1655, with the *K. oxytoca* cluster producing the highest activity (FIG. 1A). The *K. oxytoca* cluster is also functional in *P. protegens* Pf-5, albeit with 60-fold less activity as compared to that in *E. coli* MG1655. Interestingly, the clusters from *P. stutzeri* and *A. vinelandii*—both obligate aerobes—are able to achieve high activities in *P. protegens* Pf-5. The resulting nitrogenase activities are 3- to 7-fold higher than that achieved from *K. oxytoca*, which only fixes nitrogen under strict anaerobic conditions. These

clusters have common organizational features and similar electron transport chains, such as the Rnf complex.

[0114] A single gene cluster, from *R. sphaeroides*, yielded nitrogenase activity in *R. sp.* IRBG74 (FIG. 1A). Notably, both *Rhizobium* and *Rhodobacter* are alphaproteobacter and their nif clusters may contain interchangeable genes. When the native nif clusters are knocked out of *R. sp.* IRBG74, introducing the *R. sphaeroides* cluster alone does not yield active nitrogenase. These data point to a complex complementation between the endogenous and introduced gene clusters. To determine whether this approach could be generalized to other symbiotic Rhizobia, the *Rhodobacter* and *Rhodopseudomonas* gene clusters were transferred to a panel of 12 species isolated from diverse legumes (FIG. 1A). Remarkably, the transfer of these clusters was able to produce detectable nitrogenase activity in 7 of the strains.

[0115] Hereafter, studies were conducted to better characterize the extent to which changes in transcription and translation impacted the differences in activity observed when a native cluster is transferred between species. Differences in promoter activity, ribosome binding sites, and codon usage could change the expression levels of nif genes in detrimental ways. To quantify this effect, RNA-seq and ribosome profiling experiments were performed to evaluate the expression *K. oxytoca* nif cluster in *K. oxytoca* as well as *E. coli* MG1655, *P. protegens* Pf-5, and *R. sp.* IRBG74. RNA-seq experiments provide mRNA levels of genes (calculated as FPKM) and can be used to measure the performance of promoters and terminators. Ribosome profiling can be used to quantify protein synthesis rates, ribosome binding site (RBS) strength and ribosome pausing internal to genes. The ribosome density (RD) has been shown to correlate with protein expression rates. The translation efficiency is calculated by normalizing the RD by the number of transcripts (FPKM from Ribo-seq). Ribosome profiling has been applied to determine the relative levels of proteins expressed in multi-subunit complexes.

[0116] The RNA-seq profiles in both the sense and anti-sense direction are very close when compared between *K. oxytoca* and *E. coli* (FIGS. 1B-1C) and the ratios between mRNAs is preserved ($R^2=0.89$) (FIG. 1D). This is consistent with the observation that this cluster yields a similar activity in both hosts. In contrast, the RNA-seq profiles differ more significantly for *P. protegens* Pf-5 and *R. sp.* IRBG74 (FIGS. 1B-1C), and there was no correlation between mRNA transcripts (FIG. 1D).

[0117] The ratios between protein expression rates were measured using ribosome profiling (FIG. 1E and FIG. 9). It is noteworthy that the ratios measured in *K. oxytoca* almost perfectly correlate with immunoblotting assays of *A. vinelandii* and the stoichiometry of H:D:K reflects the known 2:1:1 ratio. Interestingly, unlike mRNA levels, the ratios in expression rates are strongly correlated when the cluster is transferred between species: *E. coli* ($R^2=0.94$), *P. protegens* Pf-5 ($R^2=0.61$), and *R. sp.* IRBG74 ($R^2=0.71$) (FIGS. 1E-1F). The production of NifH is significantly lower in *R. sp.* IRBG as compared to other strains. In an attempt to increase the induction of the cluster in this host, NifA was overexpressed, but this proved unsuccessful in producing high levels of active nitrogenase (FIGS. 10A-10B).

[0118] The following summarizes the results of the transfer of native nif clusters to new species. The most successful recipient is *E. coli*. However, this is not a viable agricultural

strain and activity is eliminated in the presence of 17.1 mM ammonium, consistent with previous results (FIGS. 7A-7E, and FIGS. 8A-8B). Moderately high activity can be obtained in *P. protegens* Pf-5, but this yields a constitutively-on response (the *K. oxytoca* cluster) or is strongly repressed by ammonium (the *A. vinelandii* cluster). It was also found that the *P. stutzeri* cluster in *P. protegens* Pf-5 is inactive in the presence of ammonium, in disagreement with previously published results (Setten, L. et al. Engineering *Pseudomonas protegens* Pf-5 for nitrogen fixation and its application to improve plant growth under nitrogen-deficient conditions. PLOS One 8, e63666 (2013)). In previous studies, the published strain is not made available by the authors nor is its sequence, thus it is impossible to replicate the strain perfectly and differences in the cluster boundary or mutations to the regulation during construction could explain the discrepancy in results. Only low levels of activity could be obtained by transferring clusters to Rhizobia. To address these issues, different approaches were applied to engineer the clusters to generate higher activity, exhibit less repression by ammonium, and be inducible.

Transfer of Refactored *Klebsiella* Nif Clusters to *R. Sp.* IRBG74

[0119] The process of refactoring a gene cluster involves the complete reconstruction of the genetic system from the bottom-up, using only well-characterized genetic parts. An exhaustive approach is to recode the genes (to eliminate internal regulation), reorganize into operons, control expression with synthetic ribosome binding sites (RBSs), and use T7 RNAP promoters and terminators. A separate “controller,” carried in a genetically distinct location, links synthetic sensors and circuits to the expression of T7 RNAP. For various applications, this approach has proven useful for transferring multi-gene systems between species, simplifies optimization through part replacement and enzyme mining, and enables the replacement of environmental signals that naturally control the cluster with the stimuli that induce the synthetic sensors (Smanski, M. J. et al. Synthetic biology to access and expand nature’s chemical diversity. *Nature Reviews Microbiology* 14, 135 (2016); Song, M. et al. Control of type III protein secretion using a minimal genetic system. 8, 14737 (2017); Guo, C.-J. et al. Discovery of reactive microbiota-derived metabolites that inhibit host proteases. 168, 517-526. e518 (2017); Ren, H., Hu, P., Zhao, H. J. B. & bioengineering. A plug-and-play pathway refactoring workflow for natural product research in *Escherichia coli* and *Saccharomyces cerevisiae*. 114, 1847-1854 (2017)). In previous studies, the *Klebsiella* nif cluster was refactored, which was subsequently used as a platform to optimize activity by changing the genetic organization and the parts controlling expression. The top variant (v2.1) fully recovered activity in a *K. oxytoca* nif knockout and is functional in *E. coli*. An interesting observation during optimization is that the genetic organization of the native cluster, including the existence of operons, was not correlated with activity.

[0120] The present disclosure sought to study the performance of the refactored v2.1 cluster in *R. sp.* IRBG74. An advantage of using T7 RNAP is that it is functional in essentially all prokaryotes, so the refactored cluster can be transferred as-is and transcription induced by expressing T7 RNAP in the new host. However, a new controller needs to be built for each host based on regulation and regulatory parts that work in that species. Previously, a controller for *E. coli* was designed based on the IPTG-inducible T7 RNAP

carried on a plasmid (pKT249) (FIG. 2A). To transfer the refactored cluster to R. sp. IRBG74, first a controller was constructed that functions in this species and produces an equivalent range of T7 RNAP expression.

[0121] While a handful of inducible systems and sets of genetic parts have been previously described for Rhizobia, a new part collection needed to be built and characterized in order to have those needed to create a controller with sufficient dynamic range. First, a set of 20 constitutive promoters (Anderson, J. et al. BglBricks: A flexible standard for biological part assembly. 4, 1 (2010)) and seven T7 RNAP-dependent promoters (emme, K., Zhao, D. & Voigt, C. A. Refactoring the nitrogen fixation gene cluster from *Klebsiella oxytoca*. *Proceedings of the National Academy of Sciences* 109, 7085-7090 (2012) that were found to span a range of 382-fold and 23-fold expression, respectively, were characterized (FIGS. 11A-11C). Second, a library of 285 ribosome binding sites (RBSs) were screened using the RBS Library Calculator, representing an expression range of 5,600-fold (FIGS. 12A-12B). Finally, a set of 29 terminators was characterized, of which 17 were found to have a terminator strength >10 (FIGS. 13A-13B). Using these part libraries, six inducible systems for R. sp. IRBG74 were then constructed that respond to IPTG, the quorum signal 3OC6HSL, aTe, cuminic acid, DAPG, and salicylic acid (FIG. 14). After optimization, these systems generate between 7- to 400-fold induction.

[0122] A controller was then constructed by using the optimized IPTG-inducible system to drive the expression of a variant of T7 RNAP (R6232S, N-terminal lon tag, GTG start codon) (FIG. 2A). RBS variants controlling T7 RNAP expression were tested and an intermediate strength was selected to maximize induction while limiting toxicity (FIG. 16). The controller was carried on the genome by replacing recA (see Methods and Materials). The response function of the final controller is compared to that obtained for pKT249 in *E. coli*, showing that they sweep through the same range of expression at intermediate levels of induction (FIG. 2B). To achieve the same level of induction in the two species, 0.1 mM IPTG is selected for *E. coli* and 0.5 mM for R. sp. IRBG74 (circled points in FIG. 2B).

[0123] The refactored v2.1 cluster was then transferred to R. sp. IRBG74, but no activity was observed (FIGS. 2C-2D). Activity was also not observed when the v2.1 cluster was transferred to *P. protegens* Pf-5 (FIG. 17). To determine if the genetic parts that make up the refactored cluster were functioning as designed, RNA-seq and ribosome profiling experiments were performed (FIG. 18). From these data, the strengths of promoters/terminators and the transcription level and translation rates of genes could be calculated (see Methods and Materials). The performance of the promoters in R. sp. IRBG74 was systematically lower than *E. coli*, particularly the first promoter controlling nifH (FIG. 2E). The terminators were functioning the same in the two species, albeit weakly, and no termination could be detected from the three terminators in the center of the cluster (FIG. 2E). The translation of the genes differed significantly between organisms (FIG. 2F). When the expression rates of the nif genes from the refactored cluster are compared with their levels in their native context in *K. oxytoca*, there is almost no correlation (FIG. 2F). Importantly, there is 9-fold less NifH expressed from the refactored cluster in R. sp. IRBG74 as compared to the same cluster in *E. coli*. Thus, the refactored cluster produces wildly different expression lev-

els of the component genes when transferred between organisms, even when transcription is matched between them using different controllers.

[0124] Based on these results, a new refactored cluster (v3.2) (FIG. 2G) was designed. A very strong promoter was chosen for nifH. The transcription was broken up by adding promoters to divide nifENX and nif and selecting stronger terminators. Noting that the expression ratios between nif genes are better preserved when the native cluster is transferred to a new host (FIG. 1D) but not the refactored cluster (FIG. 2F), it was hypothesized that this could be due to the disruption of the operon structures and the associated translational coupling between genes. The *K. oxytoca* operons were cloned intact, including native RBSs and replaced these regions of the refactored cluster (FIG. 2G). Note that this also preserves nifT and nifX, which were not included in first versions because they were either inessential (Simon, H. M., Homer, M. J. & Roberts, G. P. J. J. o. b. Perturbation of nifT expression in *Klebsiella pneumoniae* has limited effect on nitrogen fixation. 178, 2975-2977 (1996)) or inhibitory (Gosink, M. M., Franklin, N. M. & Roberts, G. P. J. J. o. b. The product of the *Klebsiella pneumoniae* nifX gene is a negative regulator of the nitrogen fixation (nif) regulon. 172, 1441-1447 (1990)).

[0125] Compared to v2.1, the v3.2 cluster is less active in *E. coli* but is active in R. sp. IRBG74 (FIG. 2H) and *P. protegens* Pf-5 (FIG. 17). This experiment was performed in the double nif knockout strain in R. sp. IRBG74, thus indicating that the refactored cluster is self-contained in producing nitrogenase activity. RNA-seq and ribosome profiling was applied to evaluate the performance of v3.2 in all three species (FIG. 2I, FIG. 19, and FIGS. 20A-20F). The promoters perform similarly in the different hosts, but there was significant diversity in terminator function. Despite this, the translation rates (RD) of the genes were remarkably consistent and NifH expression is nearly identical (FIG. 2J). The higher expression of NifH and the preserved ratios between proteins is the likely reason that the refactored cluster is functional in R. sp. IRBG74. The next attempt was to increase expression level of the nif genes in R. sp. IRBG74 by increasing the concentration of inducer used, but a clear optimum beyond which increased expression caused a rapid decline in activity was found (FIG. 2M). This indicates a potential upper limit in obtaining activity in R. sp. IRBG74 under free living conditions using only the genes from *K. oxytoca*.

Replacement of *A. caulinodans* Nif Regulation with Synthetic Control

[0126] The *A. caulinodans* nif genes are distributed across three clusters in different genomic locations. The regulatory signals converge on the NifA activator that, in concert with the RpoN sigma factor, turns on transcription of the genomic nif clusters. Numerous and not fully characterized environmental signals are integrated upstream of this node, including NtrBC (Kaminski, P. A. & Elmerich, C. J. M. m. The control of *Azorhizobium caulinodans* nifA expression by oxygen, ammonia and by the HF-I-like protein, NrfA. 28, 603-613 (1998)), NtrXY (Pawlowski, K., Klosse, U., De Bruijn, F. J. M. & MGG, G. G. Characterization of a novel *Azorhizobium caulinodans* ORS571 two-component regulatory system, NtrY/NtrX, involved in nitrogen fixation and metabolism. 231, 124-138 (1991), FixLJK (Kaminski, P. & Elmerich, C. J. M. m. Involvement of fixLJ in the regulation of nitrogen fixation in *Azorhizobium caulinodans*. 5, 665-

673 (1991); Kaminski, P., Mandon, K., Arigoni, F., Desnoes, N. & Elmerich, C. J. M. m. Regulation of nitrogen fixation in *Azorhizobium caulinodans*: identification of a fixK-like gene, a positive regulator of nifA. 5, 1983-1991 (1991)), NrfA (Kaminski, P. A. & Elmerich, C. J. M. m. The control of *Azorhizobium caulinodans* nifA expression by oxygen, ammonia and by the HF-I-like protein, NrfA. 28, 603-613 (1998)), and PII proteins (e.g., GlnB and GlnK (Michel-Reydellet, N. & Kaminski. P. A. J. J. o. b. *Azorhizobium caulinodans* Pland GlnK proteins control nitrogen fixation and ammonia assimilation. 181, 2655-2658 (1999))). The clusters (64 kb total, containing 76 genes) were cloned into the plasmid systems described above and transferred into R. sp. IRBG74 and *P. protegens* Pf-5, but no activity was found in either strain. Overexpression of *A. caulinodans* NifA and RpoN did not lead to activity and, upon further investigation, these regulators were found to be inactive in these strains. The size of the clusters and the lack of genetic and gene function information would complicate fully refactoring the system. For these reasons, it was decided to modify the regulation controlling nif such that it can be placed under the control of synthetic sensors.

[0127] The primary goal herein was to eliminate ammonium repression of nitrogenase activity, which converges on the regulation of NifA. The native nifA gene was knocked out of the genome using the sacB markerless deletion method (see Methods and Materials), with the intent of placing NifA under inducible control (FIG. 3A). There is only basal activity from the nifH promoter in the ΔnifA strain (FIG. 3B). When NifA is overexpressed, the promoter turns on and its activity is further enhanced by the co-expression of RpoN in an operon (note that the genomic rpoN gene is left intact for these experiments). The IPTG-inducible system designed for *Rhizobium* (previous section) was tested in *A. caulinodans* carried on a pBBR1-ori plasmid. Using GFP, this was found to induce expression over several orders of magnitude (FIG. 21). Then, the *A. caulinodans* nifA and rpoN gene was placed under IPTG control and the fluorescent reporter fused to the *A. caulinodans* nifH promoter (encompassing 281 nt upstream of the ATG), carried on the same plasmid (see Materials and Methods). The response function from the nifH promoter was analyzed at the condition used for nitrogen fixation, exhibiting a wide dynamic range to 45-fold (FIG. 3C).

[0128] The controller was designed to co-express NifA and RpoN and tested for its ability to induce nitrogenase (FIG. 3D). When fully induced, there is a complete recovery of activity as compared to the wild-type strain. The repression of nitrogenase activity by ammonium was then evaluated. The presence of 10 mM ammonium chloride leads to no detectable activity by the wild-type strain (FIG. 3E). Even when both NifA and RpoN are under inducible control, there is strong repression with only 5% of the nitrogenase activity of the wild-type. This suggests that the post-transcriptional control of NifA activity by ammonium remains intact.

[0129] In related alphaproteobacteria, mutations have been identified in NifA that abrogate ammonium repression (Paschen, A., Drepper, T., Masepohl, B. & Klipp, W. *Rhodobacter capsulatus* nifA mutants mediating nif gene expression in the presence of ammonium. FEMS microbiology letters 200, 207-213 (2001); Rey, F. E., Heiniger, E. K. & Harwood, C. S. Redirection of metabolism for biological hydrogen production. *Applied and environmental microbiology* 73, 1665-1671 (2007)). These mutations occur in the

N-terminal GAF domain. Using a multiple sequence alignment, two equivalent residues were identified to mutate in *A. caulinodans* (L94Q and D95Q) (FIG. 22). These mutations were made and then tested individually and in combination (FIG. 3D). When the double mutant of NifA is co-expressed with RpoN, the presence of ammonium only results in a slight decrease in activity.

[0130] Oxygen irreversibly inhibits nitrogenase and represses nif clusters. The inducible nif clusters were tested for oxygen sensitivity, noting that *A. caulinodans* is an obligate aerobe and fixes nitrogen under micro-aerobic conditions. The tolerance of nitrogenase to oxygen was then assessed as a function of the concentration of oxygen in the headspace, held constant by injecting oxygen while monitoring its level (Methods and FIG. 26A). The native and inducible gene clusters responded nearly identically to oxygen (FIG. 3F). The optimum activity occurs between 0.5% to 1% with a wide tolerance (30% activity at 3% oxygen).

Introduction of Controllable Nif Activity in *P. protegens* Pf-5

[0131] The native *K. oxytoca*, *P. stutzeri*, and *A. vinelandii* nif clusters are all functional in *P. protegens* Pf-5 (FIG. 1A). However, when the native *P. stutzeri* and *A. vinelandii* clusters are transferred, nitrogenase is strongly repressed. In contrast, transferring the native *K. oxytoca* cluster produces uncontrolled (constitutively on) nitrogenase activity (FIG. 4E). For these three clusters in *P. protegens* Pf-5, it was sought to gain regulatory control by removing the nifA master regulators from the clusters and expressing them from a controller (FIG. A).

[0132] As with Rhizobia, it was found that first, part libraries for *P. protegens* Pf-5 had to be built before building controllers with sufficient dynamic range. A range of 20 constitutive promoters and seven T7 promoters that span a range of 778-fold and 24-fold expression, respectively, was characterized (FIGS. 11A-11C). A library of 192 RBSs was screened, representing an expression range of 4,079-fold (FIGS. 12A-12B). A set of seven terminators that share no sequence homology between each other and have a terminator strength >10 in R. sp. IRBG74 was selected and characterized together with the three well-used terminators (e.g., T7 terminator, rrmBT1, and L3S2P21). These seven terminators showed a terminator strength >50 (FIGS. 13A-13B).

[0133] The inducible systems designed for *Rhizobium* were transferred as-is to a *Pseudomas*-specific pRO1600 plasmid (see Methods and Materials). The 3OC6HSL-, aTe-, cuminic acid-, and DAPG-inducible systems were all found to be functional (FIG. 15A). In addition, a naringenin-inducible system based on the *P_{fde}* promoter was constructed and found to be functional. The strength of arabinose inducible system was increased by substituting the -10 box in *P_{BAD}* promoter and arabinose import was improved by constitutive expression of the arabinose transporter AraE (FIG. 15B). Finally, the IPTG-inducible system was optimized for *P. protegens* Pf-5 by replacing the *P_{41lacO1}* promoter with the *P_{tac}* promoter and making three amino acid substitutions to LacI (Meyer, A. J., Segall-Shapiro, T. H., Glassey, E., Zhang, J. & Voigt, C. A. J. N. c. b. *Escherichia coli* "Marionette" strains with 12 highly optimized small-molecule sensors. 1 (2018).). This effort resulted in seven new inducible systems that produce 41- to 554-fold induction in *P. protegens* Pf-5 (FIG. 15C).

[0134] To simplify the comparison between clusters, it was sought to build a single, universal controller that could induce all three. Each has a different NifA sequence, so the ability to cross induce the gene clusters was tested. To do this, the nifH promoters from each nif cluster were cloned and fused to gfp to build plasmid-based reporters (see Methods and Materials). The ability of the various NifA homologues to activate the nifH promoters was evaluated in *E. coli* and *P. protegens* Pf-5 (FIG. 23A-23B). The results suggest that it is more important to express a NifA variant from a similar species as the host, as opposed to expressing the NifA variant that is cognate to the transferred cluster. This may be due to the need for NifA to recruit host transcriptional machinery, whereas the NifA binding sites in the promoters are well conserved across species. Based on these data, the controller was constructed using the *P. stutzeri* NifA, placed under the control of the optimized IPTG-inducible system, described above. The RBSs of NifA were synthetically designed to span a wide range of expression of nif genes (FIG. 24A). The controller was inserted into the genome 25 bp downstream of the stop codon of glmS using the mini-Tn7 system. The ability for this controller to induce the nifH promoter from each cluster using a fluorescent reporter is shown in FIG. 4C and FIG. 24B.

[0135] The nitrogenase activity for each of the gene clusters in *P. protegens* Pf-5 was then assessed (FIG. 4D). The three *P. protegens* Pf-5 strains containing the transferred clusters were modified to insert the controller and delete the native nifLA genes from each cluster (FIG. 4B). All three are inducible, with nitrogenase activity showing dynamic ranges of 1,200-fold, 2,300-fold, and 130-fold for the *K. oxytoca*, *P. stutzeri*, and *A. vinelandii* nif clusters, respectively. When induced, these systems all produce similar or even higher nitrogenase activities than can be achieved by the transfer of the unmodified native clusters (FIG. 4D). For reference, the nitrogenase activities produced by *K. oxytoca*, *P. stutzeri*, and *A. vinelandii* are shown as dashed lines in FIG. 4D (top to bottom) (see Methods and Materials). All three inducible clusters produce similar levels of activity that approach those measured from wild-type *P. stutzeri* and *A. vinelandii*.

[0136] The native *P. stutzeri* and *A. vinelandii* clusters are strongly repressed by ammonium: the presence of 17.1 mM eliminates activity or reduces it 7-fold, respectively (FIG. 4E and FIGS. 8A-8B). The inducible clusters show little reduction in activity and the inducible *A. vinelandii* cluster exhibits almost no ammonia repression. While the native *K. oxytoca* cluster in *P. protegens* Pf-5 generates a constitutive response, there is still some repression, which is reduced by the inducible version.

[0137] The inducible nif clusters were tested for oxygen sensitivity. Note that wild-type *A. vinelandii* is able to fix nitrogen under ambient conditions due to genetic factors internal and external to the cluster. First, it was established that the controller in *P. protegens* Pf-5 could induce transcription from the three nifH promoters in the presence of oxygen (FIGS. 26A-26B). The tolerance of nitrogenase to oxygen was then assessed as a function of the concentration of oxygen in the headspace, as described for *A. caulinodans* (previous section). The native and inducible clusters exhibited the same oxygen response (FIG. 4F). The nif cluster from *K. oxytoca* was the most sensitive, generating the highest activity under anaerobic conditions, but this is quickly abolished in the presence of O₂. In contrast, the nif clusters from *P. stutzeri* and *A. vinelandii* showed wider

tolerance with optima at 1% and 0.5%, respectively. However, both clusters lose activity at lower oxygen concentrations than *A. caulinodans*.

[0138] To explore the impact of the electron transport chains, several mutants to the *A. vinelandii* cluster were made (FIG. 27). The *A. vinelandii* cluster contains two potential electron transport systems to nitrogenase and the redundant system may help maintain redox status for nitrogenase at various oxygen levels. The dependence of nitrogenase activity on the oxygen concentration in various mutant backgrounds was re-measured. No effect was seen by adding the rnf2 operon or deleting the fix operon, however deleting rnf1 eliminated activity. This suggests that the rnf operon is the sole source of electrons in *P. protegens* Pf-5 under these conditions and the Fix complex cannot compensate the Rnf complex unlike the case of *A. vinelandii*. Control of Nitrogen Fixation with Agriculturally-Relevant Sensors

[0139] The careful design and characterization of the controller has the benefit of simplifying the process by which different synthetic sensors are used to induce nitrogenase expression. By knowing the dynamic range required to go from inactive to active nitrogenase, one can quantitatively select sensors that have the produce a compatible response. This allows different environmental signals—or combinations of signals using genetic logic circuits—to be used to control expression. To demonstrate this, 11 synthetic sensors were selected that respond to a variety of chemical signals of relevance to the rhizosphere and demonstrate that these can be used to create inducible nitrogenase in our engineered strains of *E. coli* (carrying the refactored v2.1 nif), R. sp. IRBG74 (carrying the refactored v3.2 nif), *P. protegens* Pf-5 (carrying the inducible *A. vinelandii* nif), and *A. caulinodans* (inducible nifA/rpoN) (FIGS. 5A-5D).

[0140] The roles of the chemical signals in the rhizosphere are shown in FIG. 5A. Cuminic acid is present in plant seeds and functions as a fungicide. Natural root exudates may include sugars, amino acids, organic acids, phenolic compounds, phytohormones, and flavonoids. These represent potential signals to control nitrogenase production close to the root surface. Cereals have been shown to release arabinoose, vanillic acid, and salicylic acid. In addition, salicylic acid regulates the plant innate immune response and the impact of its exogenous addition to cereals has been studied. Naringenin is a common precursor for many flavonoids and improves endophytic root colonization when applied to rice and wheat. Genistein, a product from naringenin catalyzed by the isoflavone synthase, is released from maize roots. A quorum sensing mimic released by rice can regulate the 3OC6HSL receptor protein LuxR, which has been visualized using *E. coli* biosensor strains.

[0141] Bacteria either native to the rhizome or added as biocontrol agents introduced as a spray inoculant or seed coating produce chemical signatures. Inoculation of cereals with root colonizing *Pseudomonas* strains that produce DAPG elicits protection against fungal pathogens. Many bacteria produce quorum molecules, such as N-acyl homoserine lactones, as a means of communication and plants can respond to these signals2. The bacterium *Sinorhizobium meliloti* produces 3OC14HSL, which enhances *Medicago* nodulation and has been shown to induce systemic resistance in cereals. DHBA can be produced by root colonizing bacteria to increase iron solubility and play a role as a chemoattractant for *Agrobacterium* and *Rhizobium*.

[0142] Sensors for these chemicals were constructed based on the controllers for each species. For *E. coli* MG1655, a strain that contains 12 optimized sensors, carried in the genome, that respond to various small molecules ("Marionette") had been previously constructed (Meyer, A. J., Segall-Shapiro, T. H., Glassey, E., Zhang, J. & Voigt, C. A. J. N. c. b. *Escherichia coli* "Marionette" strains with 12 highly optimized small-molecule sensors. 1 (2018).). The response functions of these sensors were characterized in standard units, making it simple to identify those that can be connected to nitrogenase expression without further tuning. Marionette contains sensors for vanilllic acid, DHBA, cuminic acid, 3OC6HSL, and 3OC14HSL. For each sensor, the output promoter was transcriptionally fused to T7 RNAP and the response of the responsive promoter (PT7) was measured as a function of inducer concentration (FIG. 5B and FIG. 28B). Then, the v2.1 refactored nif cluster was introduced and nitrogenase activity was measured in the presence and absence of inducer (FIG. 5C and FIG. 28C). The inducible systems constructed for *P. protegens* Pf-5 that respond to arabinose and naringenin were used to drive NifA expression for the control of the *A. vinelandii* nif cluster (FIG. 4A). The induction of the nifH promoter by these sensors was first confirmed using a reporter (FIG. 5B). When this is replaced with the nif gene cluster, it results in an inducible response of nitrogenase activity (FIG. 5C). The best nitrogenase activity in R. sp. IRBG74 is low; however, herein it was demonstrated that it could be placed under inducible control. The DAPG-inducible system developed for R. sp. IRBG74 was connected to the control of T7 RNAP and this produces a strong response from PT7 (FIG. 5B). However, when used to drive the expression of the v3.2 refactored pathway, only a 9-fold induction is observed, consistent with the low nitrogenase activity observed in this strain (FIG. 5C). Finally, the salicylic acid sensor designed for *Rhizobium* was used to control NifA (L94Q/D95Q)/RpoN expression in *A. caulinodans* (FIG. 3A and FIG. 5B). This yielded a 1000-fold dynamic range of nitrogenase activity (FIG. 5C).

[0143] Plants could be engineered to release an orthogonal chemical signal that could then be sensed by a corresponding engineered bacterium. This would have the benefit of only inducing nitrogenase in the presence of the engineered crop. Further, if the molecule is metabolizable by the engineered bacterium, it could serve as a mechanism around which a synthetic symbiosis could be designed, where the plant provides the carbon and the bacterium fixed nitrogen in an engineered relationship. To this end, legumes and *Arabidopsis* have been engineered to produce opines, including nopaline and octopine. Sensors were constructed for these two opines for *A. caulinodans* based on the LysR-type transcriptional activators OccR (octopine) and NocR (nopaline) and their corresponding P_{occ} and P_{noc} promoters (FIG. 5D and FIG. 21). These sensors were connected to the expression of NifA (L94Q/D95Q)/RpoN and the response from P_{nifH} was measured using a fluorescent reporter. Both response functions had a large dynamic range (FIG. 5B) and produced highly-inducible nitrogenase activity (FIG. 5C). The nopaline sensor yielded a 412-fold dynamic range and the octopine sensor led to 40% higher nitrogenase activity than the wild-type.

DISCUSSION

[0144] Towards designing a bacterium that can deliver fixed nitrogen to a cereal crop, this work provides a side-

by-side comparison of diverse species, natural nif clusters, and engineering strategies. The goal was to obtain inducible nitrogenase activity in a strain that can associate with cereals as an endophyte or epiphyte. To this end, ~100 strains involving the transfer of 10 natural nif clusters ranging in size from 10 kb to 64 kb to 16 diverse species of Rhizobia, *Azorhizobium*, *Pseudomas*, and *E. coli* were constructed. Different approaches were taken to make these nif clusters inducible, from bioinformatics and protein engineering to complete genetic reconstruction from the ground-up (refactoring). In addition to the highest activity, it is important that nitrogen fixation be robust to the addition of nitrogenous fertilizer (ammonia) and microaerobic environments. Two lead candidates have emerged from this effort. The most promising endophyte is a variant of *Azorhizobium* where nifA is knocked out of the genome and a nifA mutant and rpoN are complemented on a plasmid. For the epiphyte *P. protegens* Pf-5, the most versatile strain is based on the transfer of the *A. vinelandii* nif cluster and placement of nifA of *P. stutzeri* under inducible control. In both cases, nitrogenase activities were obtained that are nearly identical to wild-type *A. caulinodans* and *P. stutzeri*, respectively. Neither showed significant repression by ammonia and optimal activity was obtained in 1% oxygen. Based on these strains, it was demonstrated that nitrogenase can be placed under inducible control in response to cereal root exudates (arabinose, salicylic acid), phytohormones (naringenin) and putative signaling molecules that could be released by genetically modified plants (nopaline and octopine).

[0145] Because R. sp. IRBG74 can fix nitrogen in a legume nodule and also associates with rice, significant effort was directed to engineering this strain to fix nitrogen when cereal-associated. The first attempt was simply complementing nifV, as this is absent in R. sp. IRBG74 and produces a metabolite provided by the plant, but this attempt was unsuccessful. Then, it was found that all of the initial nif clusters transferred, some of which have high activity in *P. protegens* Pf-5 and *E. coli*, are non-functional in R. sp. IRBG74, which led to trying clusters from alphaproteobacteria, one of which produced a very low level of activity that was dependent on the nif genes native to R. sp. IRBG74. The previously-published refactored gene clusters based on *Klebsiella* nif were attempted in R. sp. IRBG74 but these showed no activity. It was only after the construction of a new refactored cluster (v3.2) that activity was obtained under free-living conditions that was not dependent on the native nif genes. This allowed an increase in the expression levels, and an optimum was discovered beyond which activity was lost. This is the first time that nif activity has been engineered in a *Rhizobium* under free-living conditions that could otherwise not perform this function. This sets the foundation for further development and optimization of this strain.

[0146] The present disclosure encompasses different degrees of nif pathway re-engineering to promote heterologous transfer. The most ambitious is the complete refactoring of all the nif genes and regulation, where all regulatory genetic parts are replaced, genes are recoded, operons are reorganized, and transcription is performed by the orthogonal T7 RNAP. When this project was initiated, DNA synthesis was a novelty and a lack of DNA assembly methods made it difficult to make alternative designs. Further, the evaluation of performance relied on the overall nitrogenase activity, rather than an understanding of the underlying

parts. As such, the first refactored pathway performed poorly. In subsequent studies, better part libraries and DNA assembly and automation platforms enabled the synthesis of many variants. Further, as the cost of RNA-seq declined, it was used to evaluate the performance of internal parts, such as promoters and terminators. This revealed that the first designs were effectively large single operons with little differential control over the transcription levels of individual genes. With these techniques allowed the optimization of the function of the refactored nif pathway and the discovery that many of the underlying genetic structure were not needed to achieve high activities.

[0147] In the present disclosure, ribosome profiling, a new technique that enables the measurement of translational parts (e.g., ribosome binding sites), was applied and expression levels were inferred. Further, nitrogenase activity and the function of underlying parts were assessed as the clusters were moved between species. Interestingly, the native *Klebsiella* nif cluster could be transferred and it performed similarly but the refactored cluster yielded widely varying expression levels in the different hosts, sometimes leading to a total loss in activity. This could be recovered by maintaining the native operon structure in the refactored cluster, implying that it was not due to the synthetic sensors, T7 RNAP, or promoters/terminators. This is one of the hypothesized functions of operons. Achieving this required maintenance of the codon usage and translational coupling of the native cluster. However, this does not mean that it will not be possible to also encode this function synthetically. There have been computational advances that enable the calculation of RBSs internal to upstream genes when encoded on an operon. If coupled with codon optimization algorithms, this would allow the design of de novo genetic parts that achieve a desired degree of translational coupling and expression level.

[0148] This work herein is the first step of a larger effort to build strains that can efficiently deliver fixed nitrogen to cereals. The present disclosure demonstrates the deregulation of nif clusters in *A. caulinodans* and *P. protegens* Pf-5, enabling them to be placed under the control of cereal root exudates. This derepresses the pathway in the presence of exogenous nitrogenous fertilizer—critical for the use of the bacterium as part of an integrated agricultural solution. Further, these organisms retain the ability to fix nitrogen in microaerobic environments, thus avoiding the need for a root nodule that enforces strict anaerobiosis. The complete deregulation of the nif pathway makes the bacterium non-competitive in the soil and lost quickly, thus limiting its impact to particular phases of the growth cycle. Thus, it is demonstrated that nitrogenase can be placed under the control of chemical root exudates. Fully realizing the goal of engineering microbial delivery to a cereal will require significant additional genetic engineering to maximize their ability to catabolize carbon sources from the plant and increase the flux of fixed nitrogen delivery by redirecting metabolism, introducing transporters, and the optimization of electron transfer. An intriguing possibility is to also genetically engineer the plant to produce orthogonal carbon sources, such as opines or less common sugars, and then placing the corresponding catabolism pathways into the bacterium.

EMBODIMENTS

- [0149] 1. A *rhizobium* that can fix nitrogen under aerobic free-living conditions, comprising a symbiotic *rhizobium* having an exogenous nif cluster, wherein the exogenous nif cluster confers nitrogen fixation capability on the symbiotic *rhizobium* under aerobic free-living conditions, and wherein the *rhizobium* is not *Azorhizobium caulinodans*.
- [0150] 2. The *rhizobium* of paragraph 1, wherein the exogenous nif cluster is from a free-living diazotroph.
- [0151] 3. The *rhizobium* of paragraph 1, wherein the exogenous nif cluster is from a symbiotic diazotroph.
- [0152] 4. The *rhizobium* of paragraph 1, wherein the exogenous nif cluster is from a photosynthetic Alphaproteobacteria.
- [0153] 5. The *rhizobium* of paragraph 1, wherein the exogenous nif cluster is from a Gammaproteobacteria.
- [0154] 6. The *rhizobium* of paragraph 1, wherein the exogenous nif cluster is from a cyanobacteria.
- [0155] 7. The *rhizobium* of paragraph 1, wherein the exogenous nif cluster is from a firmicutes.
- [0156] 8. The *rhizobium* of paragraph 1, wherein the exogenous nif cluster is from *Rhodobacter sphaeroides*.
- [0157] 9. The *rhizobium* of paragraph 1, wherein the exogenous nif cluster is from *Rhodopseudomonas palustris*.
- [0158] 10. The *rhizobium* of paragraph 1, wherein the exogenous nif cluster is an inducible refactored nif cluster.
- [0159] 11. The *rhizobium* of paragraph 10, wherein the inducible refactored nif cluster is an inducible refactored *Klebsiella* nif cluster.
- [0160] 12. The *rhizobium* of any one of the preceding paragraphs, wherein the *rhizobium* is IRBG74.
- [0161] 13. The *rhizobium* of any one of the preceding paragraphs, wherein the exogenous nif cluster comprises 6 nif genes.
- [0162] 14. The *rhizobium* of paragraph 13, wherein the 6 nif genes are nifHDK(T)Y, nifEN(X), nifF, nifBQ, nifF, and nifUSVWZM.
- [0163] 15. The *rhizobium* of paragraphs 13 or 14, wherein each nif gene of the exogenous nif cluster is preceded by a T7 promoter.
- [0164] 16. The *rhizobium* of paragraph 15, wherein the T7 promoter is a wild-type promoter.
- [0165] 17. The *rhizobium* of any one of the preceding paragraphs, further comprising an endogenous nif cluster.
- [0166] 18. The *rhizobium* of any one of the preceding paragraphs, wherein the nif cluster has a nifV gene.
- [0167] 19. The *rhizobium* of paragraph 18, wherein the nifV gene is endogenous.
- [0168] 20. The *rhizobium* of any one of the preceding paragraphs, wherein the exogenous nif cluster further comprises a terminator.
- [0169] 21. The *rhizobium* of any one of paragraphs 15-20, wherein the T7 promoter has a terminator and wherein the terminator is downstream from the T7 promoter.
- [0170] 22. The *rhizobium* of paragraph 12, wherein the exogenous nif cluster is a refactored *rhizobium* IRBG74 nif cluster.
- [0171] 23. A plant growth promoting bacterium that can fix nitrogen under aerobic free-living conditions, comprising a bacterium having an exogenous nif cluster having at least one inducible promoter, wherein the exogenous nif cluster confers nitrogen fixation capability on the bacterium, under aerobic free-living conditions, and wherein the bacterium is not *Azorhizobium caulinodans*.

- [0172] 24. The plant growth promoting bacterium of paragraph 23, wherein the bacterium is a symbiotic bacterium.
- [0173] 25. The plant growth promoting bacterium of paragraph 23, wherein the bacterium is an endophyte.
- [0174] 26. The plant growth promoting bacterium of paragraph 25, wherein the endophyte is *rhizobium* IRBG74.
- [0175] 27. The plant growth promoting bacterium of paragraph 23, wherein the bacterium is an epiphyte.
- [0176] 28. The plant growth promoting bacterium of paragraph 27, wherein the epiphyte is *pseudomonas* protogens PF-5.
- [0177] 29. The plant growth promoting bacterium of any one of paragraphs 23-28, wherein the plant growth promoting bacterium is associated with a genetically modified cereal plant.
- [0178] 30. The plant growth promoting bacterium of paragraph 29, wherein the genetically modified cereal plant includes an exogenous gene encoding a chemical signal.
- [0179] 31. The plant growth promoting bacterium of paragraph 29, wherein the nitrogen fixation is under the control of the chemical signal.
- [0180] 32. The plant growth promoting bacterium of paragraphs 30 or 31, wherein the chemical signal is opine, phloroglucinol or rhizopene.
- [0181] 33. The *rhizobium* of any one of paragraphs 23-32, wherein the exogenous nif cluster comprises 6 nif genes.
- [0182] 34. The *rhizobium* of paragraph 33, wherein the 6 nif genes are nifHDK(T)Y, nifEN(X), nifJ, nifBQ, nifF, and nifUSVWZM.
- [0183] 35. The *rhizobium* of any one of paragraphs 23-34, wherein the inducible promoter is a T7 promoter.
- [0184] 36. The *rhizobium* of any one of paragraphs 23-34, wherein the inducible promoter is $P_{A1lacO1}$ promoter.
- [0185] 37. The *rhizobium* of any one of paragraphs 23-36, wherein the inducible promoter is activated by an agent selected from a group that includes IPTG, sodium salicylate, octapine, nopaline, the quorum signal 3OC6HSL, aTe, cuminic acid, DAPG, and salicylic acid.
- [0186] 38. The *rhizobium* of any one of paragraphs 23-37, wherein the exogenous nif cluster further comprises a terminator.
- [0187] 39. The *rhizobium* of any one of paragraphs 23-37, wherein the inducible promoter has a terminator and wherein the terminator is downstream from the inducible promoter.
- [0188] 40. An *Azorhizobium caulinodans* capable of inducible ammonium-independent nitrogen fixation in a cereal crop, comprising:
- [0189] (i) a modified nif cluster, wherein an endogenous nifA gene is deleted or altered; and
 - [0190] (ii) at least one operon comprising nifA and RNA polymerase sigma factor (RpoN), wherein the operon comprises a regulatory element including an inducible promoter.
- [0191] 41. The *Azorhizobium caulinodans* of claim 40, wherein the inducible promoter is $P_{A1lacO1}$ promotor.
- [0192] 42. The *Azorhizobium caulinodans* of paragraphs 40 or 41, wherein the inducible promoter is activated by an agent selected from IPTG, sodium salicylate, octapine, nopaline, the quorum signal 3OC6HSL, aTe, cuminic acid, DAPG, and salicylic acid.
- [0193] 43. The *Azorhizobium caulinodans* of any one of paragraphs 40-42, wherein the endogenous nifA gene is altered with at least one of the following substitutions:

- [0194] (i) L94Q;
- [0195] (ii) D95Q; and
- [0196] (iii) both L94Q and D95Q.
- [0197] 44. A method of engineering a *rhizobium* that can fix nitrogen under aerobic free-living conditions, comprising transferring an exogenous nif cluster to a symbiotic *rhizobium*, wherein the exogenous nif cluster confers nitrogen fixation capability on the symbiotic *rhizobium*, under aerobic free-living conditions, and wherein the *rhizobium* is not *Azorhizobium caulinodans*.
- [0198] 45. The method of paragraph 44, wherein the exogenous nif cluster comprises 6 nif genes.
- [0199] 46. The method of paragraph 45, wherein the 6 nif genes are nifHDK(T)Y, nifEN(X), nifJ, nifBQ, nifF and nifUSVWZM.
- [0200] 47. The method of paragraph 45 or 46, wherein each of the nif genes is preceded by a wild-type T7 promoter.
- [0201] 48. The method of any one of paragraphs 44-47, wherein the exogenous nif cluster is transferred to the *rhizobium* in a plasmid.
- [0202] 49. The method of any one of paragraphs 44-48, wherein the exogenous nif cluster further comprises a terminator.
- [0203] 50. The method of any one of paragraphs 47-49, wherein the wild-type T7 promoter has a terminator, and wherein the terminator is downstream from the wild-type T7 promoter.
- [0204] 51. The method of any one of paragraphs 44-50, wherein the endogenous NifL gene is deleted.
- [0205] 52. A method of producing nitrogen for consumption by a cereal plant, comprising providing a plant growth promoting bacterium that can fix nitrogen under aerobic free-living conditions in proximity of the cereal plant, wherein the plant growth promoting bacterium is a symbiotic bacterium having an exogenous nif cluster, wherein the exogenous nif cluster confers nitrogen fixation capability on the symbiotic bacterium, enabling nitrogen fixation under aerobic free-living conditions.
- [0206] 53. The method of paragraph 52, wherein the plant growth promoting bacterium is a *rhizobium*.
- [0207] 54. The method of paragraph 52, wherein the plant growth bacterium is the bacterium of any one of paragraphs 1-22 and 23-39.
- [0208] 55. The method of any one of paragraphs 52-54, wherein the cereal plant is a genetically modified cereal plant.
- [0209] 56. The method of paragraph 55, wherein the genetically modified cereal plant includes an exogenous gene encoding a chemical signal.
- [0210] 57. The method of paragraph 56, wherein the nitrogen fixation is under the control of the chemical signal.
- [0211] 58. The method of paragraph 56 or 57, wherein the chemical signal is opine, phloroglucinol or rhizopene.
- [0212] 59. The method of any one of paragraphs 52-55, wherein the nitrogen fixation is under the control of a chemical signal.
- [0213] 60. The method of paragraph 57 or 59, wherein the chemical signal is a root exudate, biocontrol agent or phytohormone.
- [0214] 61. The method of paragraph 60, wherein the root exudate is selected from the group consisting of sugars, hormones, flavonoids, and antimicrobials.
- [0215] 62. The method of paragraph 57 or 59, wherein the chemical signal is vanillate.

[0216] 63. The method of paragraph 57 or 59, wherein the chemical signal is IPTG, aTc, cuminic acid, DAPG, and salicylic acid, 3,4-dihydroxybenzoic acid, 3OC6HSL or 3OC14HSL.

[0217] All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

[0218] From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

EQUIVALENTS

[0219] While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters, dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

[0220] All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

[0221] All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

[0222] The indefinite articles "a" and "an," as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean "at least one."

[0223] The phrase "and/or," as used herein in the specification and in the claims, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B," when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

[0224] As used herein in the specification and in the claims, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the claims, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the claims, shall have its ordinary meaning as used in the field of patent law.

[0225] As used herein in the specification and in the claims, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or, equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

[0226] It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

TABLE 7

Primers used for nif cluster cloning.

Nif cluster	Forward primer (SEQ ID Nos.: 8-64)	Reverse Primer (SEQ ID Nos.: 65-121)	Genomic location	GenBank accession No.
<i>Klebsiella oxytoca</i> M5a1	CCTAGGGCGATAATCAGCTGGCACGAAAGTGAATTCTAGCTGGTAAAGTC	GTGAGCTCGCTGCGTATCAGGTTTG ATCAGGCGATATTGAAATGAT TTRACTGAGCGGCCGCTCTAG AGTGACCAAAGCTTCGGAAACCC	3, 897, 443-3, 909, 294 3, 909, 255-3, 920, 878	CP020657.1 CP020657.1
<i>Pseudomonas stutzeri</i> A501	GCCGGGAGGCAAGCCGTAGGGCGCATTAATGCAGCTGG CACGAACTGGTTAGTTGCGCTGAAATTGCGTGT GCCTCACTTCGATTTCGTCGGTGGCTGCTGCTGAGT GATGCCGA CGCTGTATTGCGCTGATGAAACAGG TGACGCTGTGTTGACCAACGGC ATGGAACCTGTGGCACCGCTGA CGAACCGTTGGGTAGGTGG GACGTCCATCGCTGGCTGCCTICA CTGCATGACGACSTGTGAGCATCATGGTTCA	ACTACGCACTCACTAGCAGGGCAG CACCGCGACGAAATCGAGTGG TTGTCGACATCCCGGGCTGAC GGCTTAACGGCATGTTCCGGGT GTAATCGCTGTTGTCGGCGAAACT AAACCATACATCTGGTGGCGC CGTAGGGCACAACGCTCGA CTATGAGCTGGACTGAACGGCGATG GAAAATACGGCATAGCGCCATTG TGATGTATTACTGAGC GCCCTGGCGGAATCTCTTCCTCGGTGCG	1, 410, 207-1, 414, 229 1, 419, 757-1, 424, 637	NC_009434 NC_009434
<i>Azotobacter vinelandii</i> DJ	ATCCATCTCAGCTGTCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT GATCCAGGGCAAGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT GGGGCAGCCAGTGGAAAGG CTACGGGAAGCTGGCTGTTGCA GTCGGAAAGCTGGCTGAAAC AAATCAGAACATTCTACGCCACGG TCTACCATGGCTGACTCTCGG ACTCGTCT CGC CTTGGATAGGAGGAGCAGC GCCGGCTCCCTGAACTGAAAGGGCGGAGGATGATG	GCCTTCGAAACATGTTGTCAG TCGAGTTGAGGAGCTTCTCCAGC AGCAACATACCTGTCGG TGGGCTTCCCCTGTTCTCCA GGCGGTGTTAGCTTCCGGAGT TTAACGGCAGAGAACGAGTCTGGGG TTGTCAGGGTGGGGTGG CATCATCTGGCCCTTCAGGTGCA GGACCCGGCTTG GCAAGCCTACCTCACTGAGAA ACAGGTTCGGCAGTTCAAGC GCTGATTTGATGTCACAAATTCTGG GAAAGCCTACACGAGCAAGG CTTGAGGAATCTGCGGGCGCT ATCCACAAATCAAACACCTGCG AAAGCGTTCCAGTCAGGTAC	134, 732-144, 115 144, 076-148, 534 148, 500-152, 895 152, 861-157, 152 157, 101-162, 181 5, 161, 399-5, 168, 611 5, 168, 561-5, 175, 635 995, 860-1, 000, 698 23, 686-26, 413 26, 364-27, 763 27, 714-29, 113 29, 064-30, 463 30, 414-31, 813 31, 764-34, 402 NC_012560 NC_012560 NC_012560 NC_012560 NC_012560 NC_012560 NC_012560 ALUV01.1 ALUV01.1 ALUV01.1 ALUV01.1 ALUV01.1 contig00089 contig00089 contig00089 contig00089 contig00089 contig00089	NC_012560 NC_012560 NC_012560 NC_012560 NC_012560 NC_012560 NC_012560 ALUV01.1 ALUV01.1 ALUV01.1 ALUV01.1 ALUV01.1 contig00089 contig00089 contig00089 contig00089 contig00089 contig00089
<i>Paenibacillus polymyxa</i> WLY7 ⁸	GAATTGGATAATGTCAGGATTCTAG CCAAGCATTTCAGATGGGATG CGGAGGTGCGGGTATGAGCGA GAAGTTGAGGAAAGGGCG GGGATGATGAGAATACTCCCG GGTGACCTGGATGATGGAGGAGAG	GCTGATTTGATGTCACAAATTCTGG GAAAGCCTACACGAGCAAGG CTTGAGGAATCTGCGGGCGCT ATCCACAAATCAAACACCTGCG AAAGCGTTCCAGTCAGGTAC	26, 364-27, 763 27, 714-29, 113 29, 064-30, 463 30, 414-31, 813 31, 764-34, 402	contig00089 contig00089 contig00089 contig00089 contig00089

TABLE 7-continued

Primers used for nif cluster cloning.					
Nif cluster	Forward primer (SEQ ID Nos.: 8-64)	Reverse Primer (SEQ ID Nos.: 65-121)	Genomic location		GenBank accession No.
Cyanothece ATCC51142	GGCCCGGTTAGGTGGCTGAATTGGTGTATCCCC GGAGATACTTAACGCCCTGTAGGGCG GGTTTITTTTGATAGTCAGCTATCAGAACGGATC TAATTCCCATACATCTGATCATATAAGTGGGAA CTCTCAGC AATGTAATTCTCATGCATGGGAGC GTTATCTGGCTGATGGTTGTGTT GTCAAAACTGTCTGGTTAAAGCG TTAAGGTCATGGCAGGAGAACTAAGGCCCGCGTTAGG TGGTATAAAAAGGCCCGGAATGATCTCGGGGC CTGCGAAATACAACTCGAGATC GAGACTGAATAGGAGCGGGAAATG TATGTCZACGGCCGACAAGCG GATTGTCGGPATCAGCACAGAG CGAAGGAGTGGCCCAGTCATTTC AATAGCATGGCATGTCCTAGGTAATACGAC GAGAGTAACTGTTGGGATTGTGAT CCAAAGZAAAGGACACCCCT ACCTCCATATGCGACCTAACCGA TTGTCATGTCGGACCTAACCGA CAATACGATGGCATGTCCTAGGTAATACGACTCACTATAG GGAGATGCAATTACGCTCGGATC CCGCCCTACCGAGACAC ATCGAGAAGTTCTACATGCCGT GCAAAAAAAAACCCGCCCTGACAGGGGGTTTTTT TITCAATTGGACTCTGGATGGAGCAAG CTCGCATCCATTCTAGGTGTCCTCTCTAG AGTCGGAGCTTGGGGCTTAAGGGTTTGAGGGT TITTTGGTGTCTCGAGCGAGCT ATAGGCATACCATCGATGCGTTAACTGATAAGGA GGCCACTGGCTGG	GAGACTTCGCCACCTTATTAT GCATGAGATGTTATGGGATTAA ACCTGACATATTACAGCG CAAATAATGATGACATTTGACAC CGTTAACATTGTCGCAAAACTTCS ACCAGGCGAATCTCTTCCTCGTGTG CGATCACGGTACTTCGC CAAYAAAGGCCGGATGATGATCTC CGGGCCAGATCAGGTAACTGCTCAG TGGCTCTCTCGGGCATCGTCA AGAAATTCATTGGGACAGCG TTCATAATGTTAGGAGATCGGCTCG CGGTGTTAGAAATAATTTCAGATAGAC GTCACAAAGGGCCTTAATTGTTATCGTTA TCACCTTGTTT GTCGCCGGTCTCGATACAC GGTGTGGGATCATCACTTTC GACGGTAGGTGTCGAC TCACCTTGTTG TCACATAAGGGTCTTGGGATATCACTTTC ACGACAATGGAGGGATAG TCCGATGGCATGTCCTTGGC GTGGCTTCTCCAGGGAGGC AATGGCCCGCCCTGGGGGGGGTTTT TGCGAGGCCATTCCGTC GTCACAAAGGGCCTTAATTGTTATCGTTA AGCTTGCTTGGGAAAGCTGGGGCTAG GCCCGGGAGGGATCTCGGGGCTTCTCAT GCGTTGA CAGCTTGATGATGAGTCAGTGC CTGATCAGGCCCTCATCG 1, 931, 343-1, 929, 132 555, 364-562, 941 562, 897-570, 603 570, 558-577, 494 577, 449-584, 687 1, 043, 795-1, 035, 563 5, 215, 514-5, 207, 699 5, 207, 743-5, 201, 639 5, 201, 687-5, 196, 113 5, 196, 162-5, 187, 847 2, 285, 634-2, 279, 216 2, 279, 260-2, 271, 404 2, 271, 450-2, 264, 419 245, 956-252, 936 5, 290, 244-5, 293, 483 1, 183, 854-1, 175, 614 NC_010546.1 NC_010546.1 NC_010546.1 NC_010546.1 NC_010546.1 CP012914 CP012914 CP012914 CP012914 CP012914 NC_005296.1 NC_005296.1 NC_005296.1 NC_005296.1 NC_007493 NC_007493 NC_007493 NC007494 NC_009937 NC_009937 NC_009937	NC_010546.1 NC_010546.1 NC_010546.1 NC_010546.1 NC_010546.1 CP012914 CP012914 CP012914 CP012914 CP012914 NC_005296.1 NC_005296.1 NC_005296.1 NC_005296.1 NC_007493 NC_007493 NC_007493 NC007494 NC_009937 NC_009937 NC_009937		
Rhodopseudomonas palustris CGA009	2, 4, 1. GGAGATGCAATTACGCTCGGATC CCGCCCTACCGAGACAC ATCGAGAAGTTCTACATGCCGT GCAAAAAAAAACCCGCCCTGACAGGGGGTTTTTT TITCAATTGGACTCTGGATGGAGCAAG CTCGCATCCATTCTAGGTGTCCTCTAG AGTCGGAGCTTGGGGCTTAAGGGTTTGAGGGT TITTTGGTGTCTCGAGCGAGCT ATAGGCATACCATCGATGCGTTAACTGATAAGGA GGCCACTGGCTGG	GGAGATGCAATTACGCTCGGATC CCGCCCTACCGAGACAC ATCGAGAAGTTCTACATGCCGT GCACAAAGGGCCTTAATTGTTATCGTTA AGCTTGCTTGGGAAAGCTGGGGCTAG TCCGATGGCATGTCCTTGGC GTGGCTTCTCCAGGGAGGC AATGGCCCGCCCTGGGGGGGGTTTT TGCGAGGCCATTCCGTC GTCACAAAGGGCCTTAATTGTTATCGTTA AGCTTGCTTGGGAAAGCTGGGGCTAG GCCCGGGAGGGATCTCGGGGCTTCTCAT GCGTTGA CAGCTTGATGATGAGTCAGTGC CTGATCAGGCCCTCATCG 1, 183, 854-1, 175, 614	NC_007493 NC_007493 NC_007493 NC_007493 NC_007493 NC_009937 NC_009937 NC_009937		
Rhodobacter shaaroides					
Azorhizobium caulinodans ORS571					

TABLE 7-continued

Primers used for nif cluster cloning.					
Nif cluster	Forward primer (SEQ ID Nos: 8-64)	Reverse Primer (SEQ ID Nos: 65-121)	Genomic location		GenBank accession No.
CGATGCGTCCAGCACCTC CTGCCACGGTCCAAAGTTC	GACATGTCGGTCTCCCTGGAAC TTCTGGAAATTGGTACCGAGTAGTAACTGGCACA GCCCTCG ATCAAGGCATATTGAAATGTATTACTGAG CGGGCCPACGTACTTGCGGGT CAGTTCCGGCTGGGGTTAGCAGGCCACC TGCAGTTAATAAGGGCTCTTCTGATTTCG GCTGCTGTGGAGAGATCG	1, 175, 653-1, 170, 712 1, 179, 751-1, 162, 529 3, 922, 323-3, 919, 341			NC_009937 NC_009937 NC_009937
TAAAAAGCGGTAACCAAGCGGCTTTTACGTCGAA GTGTTGCGAAGCTTATGCGC	CGCTGCTTAAGGTCACTCAGCAGGAGAACTTAAGGCCGCC TCTGCGAAAGGAATAGCGTC CTATCGCCGACCTGACCC CGTCAGAACGGCTCTGACGCATCAGGAGA AGTAATATTGGCGATCGC2ACCGAGGAAAG GGTGGTCAATTGGCAACGGTTGGAAAG TCCCAGAGCCCCAACCGTTCGGAGCGAA	GTCGGTGAGATTGATCATGGCC TGCATGTCGGTTCTCGCTG ACATGTCIGAATTCCTTGAAACC TGCATTGGCTGGTTGCTCC TGTCAAGGGCAGGGCAGGGCC	3, 934, 220-3, 937, 923 3, 937, 871-3, 941, 205 3, 941, 164-3, 959, 444 3, 959, 405-3, 962, 598 3, 962, 559-3, 966, 562		NC_009937 NC_009937 NC_009937 NC_009937 NC_009937
Gluconacetobacter diazotrophicus PA1 5	TIAAGGTCATGAGCAGGAGAACTAAAGGCCGGAAATATCTTCGGGGGC GATCGAGAAATCGACCTG ATATTCGGAAATCGTAATGCCTATAAC CGCCACETCGTAATGCCTATAAC TGACCAACCGTCAAGACATCC	TCACAGCGGTATCGGAAATATCGCAGGATCAT GAATCC ACGATTCCATGCCAAGGTC CCTCAGAACCTCTCGATG GTCGAAGGAGCCTTAATTGTTATCGTTT ATCAAGTTSC TTGGGCAATAACCTGAGACGTTCA	1, 759, 465-1, 754, 718		CP001189

TABLE 8

Strains used in this study			
Name	Strain	Source	Description
MR1	<i>E. coli</i> DH10-beta	NEB	Cat# C3019
MR2	<i>E. coli</i> K-12 MG1655	Voigt lab	
MR3	<i>Klebsiella oxytoca</i> M5al	Voigt lab	
MR4	<i>Pseudomonas stutzeri</i> A1501	Poole lab	
MR5	<i>Azotobacter vinelandii</i> DJ	Peters lab	
MR6	<i>Pseudomonas protegens</i> Pf-5	ATCC	BAA-477
MR7	<i>P. protegens</i> Pf-5 controller (P_{lacZ} -T7RNAP)	This study	generated by pMR86
MR8	<i>P. protegens</i> Pf-5 controller v1 (P_{lacZ} -nifA)	This study	generated by pMR97
MR9	<i>P. protegens</i> Pf-5 controller v2 (P_{lacZ} -nifA v2)	This study	generated by pMR98
MR10	<i>P. protegens</i> Pf-5 controller v3 (P_{lacZ} -nifA v3)	This study	generated by pMR99
MR11	<i>P. protegens</i> Pf-5 controller v4 ($P_{BAD,10}$ -nifA)	This study	generated by pMR100
MR12	<i>P. protegens</i> Pf-5 controller v5 (P_{Fde} -nifA)	This study	generated by pMR101
MR13	<i>Rhizobium</i> sp. IRBG74	Ané lab	
MR14	R. sp. IRBG74 Δ hsdR	This study	generated by pMR44
MR15	R. sp. IRBG74 Δ recA	This study	generated by pMR47
MR16	R. sp. IRBG74 Δ nif	This study	generated by pMR45-46. Two nif clusters (227,127-219,579 and 234,635-234,802) were removed.
MR17	R. sp. IRBG74 Δ hsdR, recA	This study	
MR18	R. sp. IRBG74 Δ hsdR, Anif	This study	
MR19	R. sp. IRBG74 Δ hsdR, recA Anif	This study	
MR20	R. sp. IRBG74 Δ hsdR Δ nif Δ recA:: $P_{A1lacO1}$ -T7RNAP v1	This study	generated by pMR82
MR21	R. sp. IRBG74 Δ hsdR Δ nif Δ recA:: $P_{A1lacO1}$ -T7RNAP v2	This study	generated by pMR83
MR22	R. sp. IRBG74 Δ hsdR Δ nif Δ recA:: $P_{A1lacO1}$ -T7RNAP v3	This study	generated by pMR84
MR23	R. sp. IRBG74 Δ hsdR Δ nif Δ recA:: P_{phr} -T7RNAP	This study	generated by pMR85
MR24	<i>Azorhizobium caulinodans</i> ORS571	Poole lab	
MR25	<i>Azorhizobium caulinodans</i> ORS571 Δ nifA	This study	generated by pMR48
MR26	R. spp NGR234	Poole lab	
MR27	<i>R. leguminosarum</i> bv. <i>Trifoli</i> WSM1325	Poole lab	
MR28	<i>Sinorhizobium medicae</i> WSM419	Poole lab	
MR29	<i>R. leguminosarum</i> 8002	Poole lab	
MR30	<i>Sinorhizobium meliloti</i> WSM1022	Poole lab	
MR31	<i>R. leguminosarum</i> A34	Poole lab	
MR32	<i>Sinorhizobium fredii</i> HH103	Poole lab	
MR33	<i>Sinorhizobium meliloti</i> 1021	Poole lab	
MR34	<i>R. tropici</i> CIAT899	Poole lab	
MR35	<i>R. leguminosarum</i> <i>viciae</i> 3841	Poole lab	
MR36	<i>R. etli</i> CFN42	Poole lab	
MR37	<i>Agrobacterium tumefaciens</i> C58	Poole lab	

TABLE 9

Plasmids used in this study			
Name	Origin of replication	Marker	Description
pMR1	pBBR1	Kanamycin	Plasmid for nif cluster cloning
pMR2	pRO1600,	Gentamicin	Plasmid for nif cluster cloning
	p15A		
pMR3	pBBR1	Kanamycin	Native nif cluster of <i>K. oxytoca</i> M5al
pMR4	pRO1600,	Gentamicin	Native nif cluster of <i>K. oxytoca</i> M5al
	p15A		
pMR5	pBBR1	Kanamycin	Native nif cluster of <i>P. stutzeri</i> A1501
pMR6	pRO1600,	Gentamicin	Native nif cluster of <i>P. stutzeri</i> A1501
	p15A		
pMR7	pBBR1	Kanamycin	Native nif cluster of <i>A. vinelandii</i> DJ
pMR8	pRO1600,	Gentamicin	Native nif cluster of <i>A. vinelandii</i> DJ
	p15A		
pMR9	pBBR1	Gentamicin	Native nif cluster of <i>Cyanothece</i> ATCC51142
pMR10	pRO1600,	Gentamicin	Native nif cluster of <i>Cyanothece</i> ATCC51142
	p15A		
pMR11	pBBR1	Kanamycin	Native nif cluster of <i>P. polymyxa</i> WLY78
pMR12	pRO1600,	Gentamicin	Native nif cluster of <i>P. polymyxa</i> WLY78
	ColE1		
pMR13	pBBR1	Kanamycin	Native nif cluster of <i>A. brasiliense</i> Sp7
pMR14	pRO1600,	Gentamicin	Native nif cluster of <i>A. brasiliense</i> Sp7
	ColE1		
pMR15	pBBR1	Kanamycin	Native nif cluster of <i>R. sphaeroides</i> 2.4.1
pMR16	pRO1600,	Gentamicin	Native nif cluster of <i>R. sphaeroides</i> 2.4.1
	ColE1		

TABLE 9-continued

Plasmids used in this study			
Name	Origin of replication	Marker	Description
pMR17	pBBR1	Kanamycin	Native nif cluster of <i>R. palustris</i> CGA009
pMR18	pRO1600, ColE1	Gentamicin	Native nif cluster of <i>R. palustris</i> CGA009
pMR19	pBBR1	Kanamycin	Native nif cluster of <i>A. caulinodans</i> ORS571 (Part1 of 2)
pMR20	RK2	Tetracycline	Native nif cluster of <i>A. caulinodans</i> ORS571 (Part2 of 2)
pMR21	pBBR1	Kanamycin	Native nif cluster of <i>G. diazotrophicus</i> PA1 5
pMR22	pRO1600, ColE1	Gentamicin	Native nif cluster of <i>G. diazotrophicus</i> PA1 5
pMR23	pRO1600, p15A	Gentamicin	nifLA (3,915,521-3,918,529) deletion in the nif cluster of <i>K. oxytoca</i> M5al
pMR24	pRO1600, p15A	Gentamicin	nifLA (1,420,874-1,423,084) deletion in the nif cluster of <i>P. stutzeri</i> A1501
pMR25	pRO1600, p15A	Gentamicin	nifLA (5,168,709-5,171,731) deletion in the nif cluster of <i>A. vinelandii</i> DJ
pMR26	pRO1600, p15A	Gentamicin	Native nif cluster of <i>A. vinelandii</i> DJ with the mf2 operon
pMR27	pRO1600, p15A	Gentamicin	mf1 (5,168,156-5,162,716) operon deletion in the nif cluster of <i>A. vinelandii</i> DJ
pMR28	pRO1600, p15A	Gentamicin	fix operon (995,860-1,000,698) deletion in the nif cluster of <i>A. vinelandii</i> DJ
pMR29	pBBR1	Kanamycin	Refactored nif cluster v2.1
pMR30	pRO1600, p15A	Gentamicin	Refactored nif cluster v2.1
pMR31	RK2	Tetracycline	Refactored nif cluster v2.1
pMR32	ColE1	Gentamicin	P _{nifT} -nifHDKTY
pMR33	ColE1	Gentamicin	P ₂ -nifeNX
pMR34	ColE1	Gentamicin	P ₂ -nifJ
pMR35	ColE1	Gentamicin	P ₂ -nifBQ
pMR36	ColE1	Gentamicin	P ₂ -nifF
pMR37	ColE1	Gentamicin	P ₂ -nifUUSVWZM
pMR38	pBBR1	Kanamycin	Refactored nif cluster v3.2
pMR39	pRO1600, p15A	Gentamicin	Refactored nif cluster v3.2
pMR40	pBBR1	Kanamycin	LacI, P _{A1lacO1} -gfpmut3b
pMR41	RSF1010	Gentamicin	LacI, P _{A1lacO1} -gfpmut3b
pMR42	RK2	Tetracycline	LacI, P _{lac} -gfpmut3b
pMR43	pRO1600, ColE1	Gentamicin	LacI, P _{A1lacO1} -gfpmut3b
pMR44	p15A	Gentamicin	Suicide plasmid for hsdR deletion in <i>R. sp.</i> IRBG74
pMR45	p15A	Gentamicin	Suicide plasmid for the nif cluster I (219,579-227,127) deletion in <i>R. sp.</i> IRBG74
pMR46	p15A	Gentamicin	Suicide plasmid for the nif cluster II (234,635-234,802) deletion in <i>R. sp.</i> IRBG74
pMR47	p15A	Gentamicin	Suicide plasmid for recA deletion in <i>R. sp.</i> IRBG74
pMR48	p15A	Gentamicin	Suicide plasmid for nifA deletion in <i>A. caulinodans</i> ORS571
pMR49	pBBR1	Gentamicin	LacI, P _{A1lacO1} -nifV (<i>A. caulinodans</i> ORS571)
pMR50	pBBR1	Gentamicin	P _{nifH} (<i>R. sp.</i> IRBG74)-sf GFP
pMR51	pBBR1	Gentamicin	NifA(<i>R. sp.</i> IRBG74), P _{nifH} (<i>R. sp.</i> IRBG74)-sf GFP
pMR52	pBBR1	Gentamicin	NifA(<i>K. oxytoca</i>), P _{nifH} (<i>K. oxytoca</i>)-sf GFP
pMR53	pBBR1	Gentamicin	NifA(<i>R. sp.</i> IRBG74), P _{nifH} (<i>K. oxytoca</i>)-sf GFP
pMR54	pBBR1	Gentamicin	NifA(<i>P. stutzeri</i>), P _{nifH} (<i>P. stutzeri</i>)-sf GFP
pMR55	pBBR1	Gentamicin	NifA(<i>R. sp.</i> IRBG74), P _{nifH} (<i>P. stutzeri</i>)-sf GFP
pMR56	pBBR1	Gentamicin	NifA(<i>A. caulinodans</i>), P _{nifH} (<i>A. caulinodans</i>)-sf GFP
pMR57	pBBR1	Gentamicin	NifA(<i>R. sp.</i> IRBG74), P _{nifH} (<i>A. caulinodans</i>)-sf GFP
pMR58	pBBR1	Kanamycin	Plasmid for constitutive promoter characterization. P _{constitutive} -gfpmut3b
pMR59	pRO1600, p15A	Gentamicin	Plasmid for constitutive promoter characterization. P _{constitutive} -gfpmut3b
pMR60	pBBR1	Kanamycin	PT7(WT)-mCherry
pMR61	pBBR1	Kanamycin	PT7(P1)-mCherry
pMR62	pBBR1	Kanamycin	PT7(P2)-mCherry
pMR63	pBBR1	Kanamycin	PT7(P3)-mCherry
pMR64	pBBR1	Kanamycin	PT7(P4)-mCherry
pMR65	pBBR1	Kanamycin	PT7(P5)-mCherry
pMR66	pRO1600, ColE1	Gentamicin	AraE, AraC, P _{BAD} .10-gfpmut3b
pMR67	pBBR1	Kanamycin	Plasmid for terminator characterization. P _{T7} -gfpmut3b-mrfp1
pMR68	pRO1600, ColE1	Gentamicin	Plasmid for terminator characterization. P _{T7} -gfpmut3b-mrfp1
pMR69	pBBR1	Kanamycin	LuxR, P _{Lux} -gfpmut3b
pMR70	pBBR1	Kanamycin	TetR, P _{Tet} -gfpmut3b
pMR71	pBBR1	Kanamycin	CymR, P _{Cym} -gfpmut3b
pMR72	pBBR1	Kanamycin	PhlF, P _{Phl} -gfpmut3b
pMR73	pBBR1	Kanamycin	NahR, P _{Sar} -gfpmut3b
pMR74	pRO1600, ColE1	Gentamicin	PhlF, P _{Phl} -gfpmut3b

TABLE 9-continued

Plasmids used in this study			
Name	Origin of replication	Marker	Description
pMR75	pRO1600, ColE1	Gentamicin	TetR, P_{Ter} -gfpmut3b
pMR76	pRO1600, ColE1	Gentamicin	LuxR, P_{Lux} -gfpmut3b
pMR77	pRO1600, ColE1	Gentamicin	CymR, P_{Cym} -gfpmut3b
pMR78	pRO1600, ColE1	Gentamicin	FdeR, P_{Fde} -gfpmut3b
pMR79	pRO1600, ColE1	Gentamicin	LacI(Q18M/A47V/F161Y), P_{lac} -gfpmut3b
pMR80	pBBR1	Kanamycin	P_{T7} -gfpmut3b
pMR81	pRO1600, p15A	Gentamicin	P_{T7} -gfpmut3b
pMR82	p15A	Gentamicin	Controller for R. sp. IRBG74, LacI, $P_{A1lacO1}$ -T7RNAP (RBSr33 for T7RNAP)
pMR83	p15A	Gentamicin	Controller for R. sp. IRBG74, LacI, $P_{A1lacO1}$ -T7RNAP (RBSr32 for T7RNAP)
pMR84	p15A	Gentamicin	Controller for R. sp. IRBG74, LacI, $P_{A1lacO1}$ -T7RNAP (RBSr3 for T7RNAP)
pMR85	p15A	Gentamicin	Controller for R. sp. IRBG74, PhIF, P_{PhIF} -T7RNAP (RBSf33 for T7RNAP)
pMR86	ColE1	Tetracycline	Controller for <i>P. protegens</i> Pf-5, LacI(Q18M/A47V/F161Y), P_{lac} -T7RNAP
pMR87	pBBR1	Kanamycin	NocR, P_{noc} -gfpmut3b
pMR88	pBBR1	Kanamycin	OccR, P_{occ} -gfpmut3b
pMR89	pBBR1	Gentamicin	NifA(<i>A. vinelandii</i>), P_{nifH} (<i>A. vinelandii</i>)-sgfp
pMR90	pBBR1	Gentamicin	NifA(<i>K. oxytoca</i>), P_{nifH} (<i>P. stutzeri</i>)-sgfp
pMR91	pBBR1	Gentamicin	NifA(<i>K. oxytoca</i>), P_{nifH} (<i>A. vinelandii</i>)-sgfp
pMR92	pRO1600, p15A	Gentamicin	NifA(<i>K. oxytoca</i>), P_{nifH} (<i>K. oxytoca</i>)-sgfp
pMR93	pRO1600, p15A	Gentamicin	NifA(<i>A. vinelandii</i>), P_{nifH} (<i>A. vinelandii</i>)-sgfp
pMR94	pRO1600, p15A	Gentamicin	NifA(<i>P. stutzeri</i>), P_{nifH} (<i>P. stutzeri</i>)-sgfp
pMR95	pRO1600, p15A	Gentamicin	NifA(<i>P. stutzeri</i>), P_{nifH} (<i>K. oxytoca</i>)-sgfp
pMR96	pRO1600, p15A	Gentamicin	NifA(<i>P. stutzeri</i>), P_{nifH} (<i>A. vinelandii</i>)-sgfp
pMR97	ColE1	Tetracycline	NifA controller for <i>P. protegens</i> Pf-5, LacI(Q18M/A47V/F161Y), P_{lac} -nifA(<i>P. stutzeri</i>) (RBSp32 for NifA)
pMR98	ColE1	Tetracycline	NifA controller for <i>P. protegens</i> Pf-5, LacI(Q18M/A47V/F161Y), P_{lac} -nifA(<i>P. stutzeri</i>) (RBSp27 RBS for NifA)
pMR99	ColE1	Tetracycline	NifA controller for <i>P. protegens</i> Pf-5, LacI(Q18M/A47V/F161Y), P_{lac} -nifA(<i>P. stutzeri</i>) (RBSp33 for NifA)
pMR100	ColE1	Tetracycline	NifA controller for <i>P. protegens</i> Pf-5, AraE, AraC, $P_{BAD,10}$ -nifA
pMR101	ColE1	Tetracycline	NifA controller for <i>P. protegens</i> Pf-5, FdeR, P_{Fde} -nifA
pMR102	IncW	Spectinomycin	NifA controller plasmid for <i>E. coli</i> , LacI, $P_{A1lacO1}$ -nifA(<i>K. oxytoca</i>)
pMR103	pRO1600, p15A	Gentamicin	P_{nifH} (<i>K. oxytoca</i>)-sgfp
pMR104	pRO1600, p15A	Gentamicin	P_{nifH} (<i>P. stutzeri</i>)-sgfp
pMR105	pRO1600, p15A	Gentamicin	P_{nifH} (<i>A. vinelandii</i>)-sgfp
pMR106	pBBR1	Gentamicin	P_{nifH} (<i>K. oxytoca</i>)-sgfp
pMR107	pBBR1	Gentamicin	P_{nifH} (<i>P. stutzeri</i>)-sgfp
pMR108	pBBR1	Gentamicin	P_{nifH} (<i>A. vinelandii</i>)-sgfp
pMR109	p15A	Kanamycin	P_{BAD} -T7RNAP
pMR110	p15A	Kanamycin	P_{Bet} -T7RNAP
pMR111	p15A	Kanamycin	P_{Cin} -T7RNAP
pMR112	p15A	Kanamycin	P_{Cm} -T7RNAP
pMR113	p15A	Kanamycin	P_{Lux} -T7RNAP
pMR114	p15A	Kanamycin	P_{Phf} -T7RNAP
pMR115	p15A	Kanamycin	P_{SBSB} -T7RNAP
pMR116	p15A	Kanamycin	P_{lac} -T7RNAP
pMR117	p15A	Kanamycin	P_{Ter} -T7RNAP
pMR118	p15A	Kanamycin	P_{Tig} -T7RNAP
pMR119	p15A	Kanamycin	P_{Van} -T7RNAP
pMR120	p15A	Kanamycin	P_{Sal} -T7RNAP
pMR121	pBBR1	Gentamicin	$P_{T7}(P2)$ -gfpmut3b
pMR122	pBBR1	Gentamicin	NifA controller for <i>A. caulinodans</i> , LacI, $P_{A1lacO1}$ -nifA-rpoN
pMR123	pBBR1	Gentamicin	NifA controller for <i>A. caulinodans</i> , LacI, $P_{A1lacO1}$ -nifA(L94Q)-rpoN
pMR124	pBBR1	Gentamicin	NifA controller for <i>A. caulinodans</i> , LacI, $P_{A1lacO1}$ -nifA(D95Q)-rpoN(<i>A. caulinodans</i>)
pMR125	pBBR1	Gentamicin	NifA controller for <i>A. caulinodans</i> , LacI, $P_{A1lacO1}$ -nifA(L94Q/D95Q)-rpoN
pMR126	pBBR1	Gentamicin	NifA controller for <i>A. caulinodans</i> , NahR, P_{Sal} -nifA(L94Q/D95Q)-rpoN
pMR127	pBBR1	Gentamicin	NifA controller for <i>A. caulinodans</i> , NocR, P_{noc} -nifA(L94Q/D95Q)-rpoN
pMR128	pBBR1	Gentamicin	NifA controller for <i>A. caulinodans</i> , OccR, P_{occ} -nifA(L94Q/D95Q)-rpoN

TABLE 9-continued

Plasmids used in this study			
Name	Origin of replication	Marker	Description
pMR129 pBBR1	Gentamicin	$P_{nifH}(A. caulinodans)$ -sfgfp	
pMR130 pBBR1	Gentamicin	NifA, $P_{nifH}(A. caulinodans)$ -sfgfp	
pMR131 pBBR1	Gentamicin	NifA, RpoN, $P_{nifH}(A. caulinodans)$ -sfgfp	
pMR132 pBBR1	Gentamicin	LacI, $P_{41lacO1}$ -nifA(L94Q/D95Q)-rpoN, P_{nifH} -sfgfp	
pMR133 pBBR1	Gentamicin	NahR, PSal-nifA(L94Q/D95Q)-rpoN, P_{nifH} -sfgfp	
pMR134 pBBR1	Gentamicin	NocR, Pnoc-nifA(L94Q/D95Q)-rpoN, P_{nifH} -sfgfp	
pMR135 pBBR1	Gentamicin	OccR, Pocc-nifA(L94Q/D95Q)-rpoN, P_{nifH} -sfgfp	
pMR136 pBBR1	Gentamicin	Refactored nif cluster v2.1	

TABLE 10-continued

Name	Genetic part DNA sequence (SEQ ID Nos: 122-225)	Genetic part sequences used in this study			
P_{WT}	CATCTAAAGTGGAAACTGTGCGGGACCAATTCCCTCCACGGGCCGTTAATCGAGGAAGCAGTATGGCTGGGCTTCCTGAAACGACACAGCTGGCG GGATGTAACCCGGAGGGCCCTGAAAGGGAGATACTAGCAGGGCTAAGTGAAGGCTAACAACTGACATAGTGGACATCACCTCCAAACAGGAACTACACAT				
P_1	Promoter ⁸	TAATACGACTCACTAACGGAGA			
P_2	Promoter ⁸	TAATACGACTCACTAACGGAGA			
P_3	Promoter ⁸	TAATACGACTCACTAACGGAGA			
P_4	Promoter ⁸	TAATACGACTCACTAACGGAGA			
P_5	Promoter ⁸	TAATACGACTCACTAACGGAGA			
P_6	Promoter ⁸	TAATACGACTCACTAACGGAGA			
nifY of <i>A. caulinodans</i>	Gene	GTGTTCCGGGGAGGCCTGCATGCTGCGCAAAGAACCCGCCAAACCCGCCATGGCCGCTTCCTGAAACGACACAGCTGGCG CGACGGGGAGAGGGCGCGGGCTGGTCCCTGCTGGCCAAAGGAGATACTCCCGCAAGAACATCCATGCCATGCTGCAATGTTCCATGCTG ACCGCAACGCCCGCCGCAATGGCCGCAAGG CCATGAGGGAGG CAAGCTGGGGAGGGAGGATGCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGG GCGAGGATTCCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGG CGGCAACTGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGG GCGGAACTGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGG TGTCTCGGCTGCTGCTGAAGGCTGGCCGCACTATGAAAGCTGAACTGCTGAATGGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCG CCGGCTGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCG TTGGCTGCTGCAACCAAGGAGAATGTTCCGGGAGAACGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCG GCCGCGCTGGCCGAGGGCAATCTGA			
P_{nifH} of <i>K. oxytoca</i>	Promoter	TGTGCTGCAACGCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCG CCAAGAGATGCTGGCTGAATAGACAGGGGGCAAGGCTGCTTATGCTGGCCGCTTATGCTGGCCGCTTATGCTGGCCGCTTATGCTGGCC GTTTCCCACATTGCTGGCCCTTATGCTGGCCGCTTATGCTGGCCGCTTATGCTGGCCGCTTATGCTGGCCGCTTATGCTGGCC AMACAGCGACCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCC TGTCTGGCTGCAACGCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCC TTGTTAACTATGCAACCGCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCC AAGGCTGCTATGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCC P. _{nifH} of <i>P. stutzeri</i>	TGTCTGGCTGCAACGCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCC TTGTTAACTATGCAACCGCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCC AAGGCTGCTATGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCC P. _{nifH} of <i>A. vinelandii</i>	TGTCTGGCTGCAACGCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCCGCTGGCC AGTAGGGGAGTGTAGGGGATTGTT ATGTT TTGACAAAGCTCCGAGAAAGGGGGCCCTAACCCCTCTAACGGCTGATGGCAAGTATACTGTTGCTGATGGCTAACGTTGATAGGGAC GCTATACGACAACGCTGGTACAATTGTCGTTGCTGCGGCAAGGATTTCCTCTGCTGGGGGCTCTTCTGCTGGGGCTCTTCTGCTGGGG R. sp.	

TABLE 10-continued

Name	Genetic part DNA sequence (SEQ ID Nos: 122-225)	Genetic part sequences used in this study	
IRBG74	AATCAAGAAAATAACTTATTTCGTCTAACCGAAGGGTCTCCGGTACGGGTTGTAACCATTCCTTGGAAGCCACCGGAGAT		
<i>P_{nifH}</i> of <i>A. caulinodans</i>	TGTGGCGTTGAAAACAGGGGCTTITGGAACCGTTGATTTCTGCATGGCAATGGCCTGTTGGTGTGGTACGGGTTCCGGTACCGGTTCCGGCAC		
<i>nifA</i> of <i>K. oxytoca</i>	ATGATCCATAAAATCCGATTGGACACCACCGTCAGACGGTTCGATGCTCTCCAGGAGTTACCGCCATGGAGGGATAAGGGTGGCTCTGGAG TGCGTCACCGAGGAAACCTGGTAAACCTGGTACGGGGTCTGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGG ACAGCCAGGAGGAGATCCTGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGG GAAGGAACTGGGTTCCGGTGTGATGGGCCCTGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGG GTACGGACTATGACCTGGCCCTTATGCCGTTCCGGTGTGATGGGCCCTGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGG AGGAAGGGGGGTGGCGCTGCAACCGGTTCTGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGGTACGGGG GGCGCGCGAGGGGGGGAGGG AAGCCCGCGGATGCGGGGAGGG AAGAGACTCATGGCCAACGCAATCCACCATTAATTCTGGGGGCGGG CTTGGAGGGGAGCTGGTTGGGGTACGG CTTATACCTCTCATGAGGATCGGGAAAGGG GGCGAGGAAACCTGGGGTCAAGTGCGGAACTTACCGGGGACCAACCGGGCATCTGGGCGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG CTATACTACGGCTGACCTATGGCTATGCGTACGCTATGGCTATGGCTATGGCTATGGCTATGGCTATGGCTATGGCTATGGCTATGGCTATGG AAAAATTCGCCCCAACAGGGGGAACGGGGGCTTGG AACTGCGAAACATGCGCTGG CCGAAAAGCGCTGG AGGGGGGGCTGG CGCGACTGGTGA	ATGAAACGCCACATTCCGAAACGCCAACGCCAACGCCAACGCCAACGCCAACGCCAACGCCAACGCCAACGCCAACGCCAACGCCAACGCC CCTTAAACGCCGCGGG TCTGCAACCCGAGGAGGG ATGGGCGCAAGGG GGCGCTGTACGACATGACCTCTGGCCCTTATGCCGCTGGCCATGACCTCTGGCCCTTATGCCGCTGGCCATGACCTCTGGCCCTTATGCC GCGGGCGCGGAGGGGCTGACCCGGGACGG GAGGAAGGG GGCTCTCATGGCCGG AGCTGATGCGGAGGG GAAATCGGAAACTTCTGG GTTCCCTCGAGAGATGG GGAGGG TATATATATAATTATTTTTAA	
<i>nifA</i> of <i>P. stutzeri</i>	GATCGCGCGCAGAGGG TGGAAAACCTGG GGTCCCGGG GA	ATGAAATGCCAACATCCCTAACGGGCTGGGCAACAAACGAACCCGGTGAACCTCTATGACCTCTGCAATGCGGGCCCTGGGGAACCTCTA GCTCGCGCGGAAACACAGATGCAAACTCTCGAACAGCTCTCGAACAGCTCTCGAACAGCTCTCGAACAGCTCTCGAACAGCTCTCGAACAG TTCCGACGCCGAAACRGGGGCCCTGAGATGGGECATCCACAGATGGGECATCCACAGATGGGECATCCACAGATGGGECATCCACAGATGG AGCGCGCTGG GGTCCCGGG GA	
<i>nifA</i> of <i>A. vinelandii</i>		ATGAAATGCCAACATCCCTAACGGGCTGGGCAACAAACGAACCCGGTGAACCTCTATGACCTCTGCAATGCGGGCCCTGGGGAACCTCTA GCTCGCGCGGAAACACAGATGCAAACTCTCGAACAGCTCTCGAACAGCTCTCGAACAGCTCTCGAACAGCTCTCGAACAGCTCTCGAACAG TTCCGACGCCGAAACRGGGGCCCTGAGATGGGECATCCACAGATGGGECATCCACAGATGGGECATCCACAGATGGGECATCCACAGATGG AGCGCGCTGG GGTCCCGGG GA	

TABLE 10-continued

Name	Genetic part DNA sequence (SEQ ID Nos: 122-225)	Genetic part sequences used in this study
P _{D3100}	Promoter ⁹	TAGGTGTTGACGGCTAGCTCAGTCCTAGGTAAGTGCTAGTCAGTCTAGA
P _{D3101}	Promoter ⁹	TAGGTGTTAACGCTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3102}	Promoter ⁹	TAGGTGTTGAGCTAGCTCAGTCCTAGGTAAGTGCTAGTCAGTCTAGA
P _{D3103}	Promoter ⁹	TAGGTGTTGATAGCTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3104}	Promoter ⁹	TAGGTGTTGACGGCTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3105}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGTAAGTGCTAGTCAGTCTAGA
P _{D3106}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGTAAGTGCTAGTCAGTCTAGA
P _{D3107}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3108}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3109}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3110}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3111}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3112}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3113}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3114}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3115}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3116}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3117}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3118}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{D3119}	Promoter ⁹	TAGGTGTTAACGGTAGCTCAGTCCTAGGATTATGCTAGTCAGTCTAGA
P _{Ap}	Promoter ¹⁰	TAGGTGTTAACGGATTATTCCTACGAACTTAATGAGTACGAAAGTT

TABLE 10-continued

Name	Genetic part DNA sequence (SEQ ID Nos: 122-225)	Genetic part sequences used in this study
T ₇₇	Terminator ¹¹	TAGCATAACCCCTTGGGCCTTAACGGGCTTGTAGGGGTTTTGT
T _{77.2}	Terminator ¹¹	TACTCGAACCCCTAGCCGCTCTTATCGGGGGCTAGGGGTTTTGT
T _{F7.3}	Terminator ¹¹	TACATATCGGGGGTAGGGGTTTTGT
T _{mBT1}	Terminator ¹²	CCAGGCATCAAATAAACGAAAGGCTCAGTGAAGACTGGCCTTCGTTATCTGTTGTTGCTGGTGAACGCTCTC
T _{L3SP21}	Terminator ¹³	CTCGGTACCAAAATTCCAGAAAAAGGGCCTCCGAAAGGGGGCTTTTGTTGGT
T ₁	Terminator ¹³	CTCGGTAACCAAAATTCCAGAAAAAGGGCTCAGTGAAGACTGGCCTTCGTTATCTGTTGTTGCTGGCCTCCATCCTAGATGGCAGTAAAAAA ATCCITPAGCTTCGCTAAGGATGATTCTCATAGCAAAATAGATGCACTGCC
T ₂	Terminator ¹⁴	CCAGGCATCAAATAAACGAAAGGCTCAGTGAAGACTGGCCTTCGTTATCTGTTGTTGCTGGTGAACGCTCTCTACTAGAGTAC ACTGGCTCACCTCGGGGCCCTTCGCGTTATA
T ₃	Terminator ¹³	CCAGGCATCAAATAAACGAAAGGCTCAGTGAAGACTGGCCTTCGTTATCTGTTGTTGCTGGCCTTCGCTACTAGAGTAC ACTGGCTCACCTCGGGGCCCTTCGCGTTATA
T ₄	Terminator ¹³	GTCCTGTCACTACCTCGAGTAATGCGGGAGGATCGGCCTTCTCTCAATGACTGCACTAAATGAACTACATAAATA
T ₅	Terminator ¹³	CAGATAAAAAAAATCCTTAGCTTGCCTAAGGATGATTCTTCGCTCCATCCTTAGATAGCTCGTACCAAAATCCAGAAAAGACA CCCGAAAGGGCTGGTTTCTGGTCCATAGCAAAATAGATGCACTAAATGAACTACATAAATA
T ₆	Terminator ¹³	CCAGGCATCAAATAAACGAAAGGCTCAGTGAAGACTGGCCTTCGTTATCTGTTGTTGCTGGCCTCCATCTAGCATAACCC CTGGGGCCCTAAACGGTTTGAAGGGTTTTG
T ₇	Terminator ¹³	CTCGGTACCAAAATTCCAGAAAAAGGACGCTTTCGAGCGTTTTCTGTTGCTCCATCTAGCAATAAGCTGCACTGCC AAAGGGGGATTATCTCCCTTAATTCTCATAGCAATAAGCTGCACTGCC
T ₈	Terminator ¹³	CGCAGATAGCAAAAAGGCCCTTAAAGCGCTTCTACATGGGCTTCTGATAGCTAGAGCTGACGAAACCTCG CTCCGGGGGGTTTCTGATAGCTAGCACTGCCATGCTGCC
T ₉	Terminator ¹⁴	TCGGTCAGTTCACCTGATTACGTTAAACCCGCTTGGGGTTTCTCTGGAGGGCAGAAAGATGAACTGTC
T ₁₀	Terminator ¹⁴	GCCCCGGAAATACCTCGGGGCTTCTATGGCCTGGCGTGAATAGGGCCGGCTGATAGCTGGGCTACCTGAGGG CCATTTGTCT
T ₁₁	Terminator ¹⁴	GTAATCGTTAAATCGGAAATAACGTTAAACCGCTTCGGGGTTTTTATGGGGGAGTTAGGGAAAGGAGTTGCA AAAAAAACCCGCCCTGACAGGGGGTTTTT
T ₁₂	Terminator ¹⁴	TCGGCAATTAAGGGCTAACACGGCGCTTCTGATAGCTGAACTGAACTTAACGGATGAAAGCCC CGGAAATGCACTTCGGGGCTTCTGCTCTGGCCCTCATCTAGATAG
T ₁₃	Terminator ¹³	GGAGACCATACTGGAAACAGAAAAAGGCCAACCTGACAGTGGCTCATGGCAATACGATGCT ATTGGAGATAGCAAAAGGGCTTAAAGGGCTTACATGGCTCATGGCAATACGATGCT
T ₁₄	Terminator ¹³	

TABLE 10-continued

Name	Genetic part DNA sequence (SEQ ID Nos: 122-225)	Genetic part sequences used in this study
T ₁₅	Terminator ¹³ TCGGGCAATTAAGGGCTAACCAACGGCGTTTACGTCTGCANCCCTGCCATCCTAGATACTCGTACCAAAATTCCAG AAAAGGGCCTCCGAAAGGGGGCTTTTCGTTTGCCATAGGAATAAGATGCGATGTCATGATGCAATGTC	
T ₁₆	Terminator ¹³ TTCAGGCCAAACTAAGACGCCGTCCTGCAACTACTTGAGATGCGTGAAGGATCGGGTTTCCTGCTTGGCCCATAGCCA CATAGAAGATAAGAAATTACTCGGTACAAATTCAGAAAAGGGCTTCCGAAAGGGGGCTTTTCGTTTGCCATAGCCA	
T ₁₇	Terminator ¹³ TTCAGGCCAAACTAAGACGCCGTCCTGCAACTACTTGAGATGCGTGAAGGATCGGGTTTCCTGCTTGGCCCATAGCCA CTGGGCCCTCCATCCTAGATACTGGCAATTAAAAAGGGCTAACCCAGCGCTTTCAGCTAGATGCGATAGTC	
T ₁₈	Terminator ¹³ CTCGGTACCAAATTCCGAAAGAGGGCCCTCCGAAAGGGGGCTTTTCGTTTGCTGACTGAAATAAGAAAGGAAACATTAACCGA TGAGAAAGCCCCGGAAAGATACCTTCGGGGCTTTTATTGGCTCTTGGCCCTCATCCTTAGATG	
T ₁₉	Terminator ¹³ CTCGGTACCAAATTCCGAAAGAGGCCCTCCGAAAGGGGGCTTTTCGTTTGCTCCTGGCCCTCATCCTTAGATGTCGGCA ATTAAGAAAGGGCTAACCGCCGCTTTTACGTCTGCACTAGGAATAAGATGCGATGTCATGTCATGTCATGTC	
T ₂₀	Terminator ¹³ CTCGGTACCAAAGAGCAACATAAGCGTAAAAGCTTTTCGTTGGCTCTAAATAGAAAGAACATAGAAATATTTCAGGCAA AAACTTAAGAGCCCGGTCTGCACTACTTGCACTGAGTAATGCGTGGCAAGGATCGGGTTTCCTCTCATCCTGGCCCTC CATCCTAGATG	
T ₂₁	Terminator ¹⁴ GGAACTGCAAGACATCAAATAAAAGCTAGTCGAAGACTGGCCCTTTGTTATCTGTGTTATCTGTGTTGTTGCGTGAACACTCTCCGA CTAGTAGCGCGCTGCAAGAGGAGGA	
T ₂₂	Terminator ¹³ AACGGCATGAAAGCCCCGAAAGATCACCTCCGGGGCTTTTATGGCTATAGCAATGCGATGTCATGTCCTCGGCAATTAAA AAGCGGCTAACACGGCGCTTTTACGTCTGCACTCCCTGGCCCATCTAGAG	
T ₂₃	Terminator ¹⁴ GGGAACCTGCAAGACATCAAATAAAAGCTAGTCGAAGACTGGCCCTTTGTTATCTGTGTTATCTGTGTTGCGTGAACACTCTCCG	
T ₂₄	Terminator ¹⁴ AAAGCAAGCTGATAACCGATAAAAGCTCTTGGAGCTTCTGGAGCTTTGGAGATTTCACATGAAAGGGCTCATGGAAAGCTGGCCCTTGTGTTATCTGTGTTGCGTGA CAGATAGCGCGGGRACCTCGAGACATCAAATAACAAAGGTCTAGTCATGGAAAGCTGGCCCTTGTGTTATCTGTGTTGCGTGA ACACTCTCCCG	
T ₂₅	Terminator ¹³ AAATAAAAAAATTATTAZATT CCAGAAAGGGCCCTCCGAAAGGGGGCTTTTCGTTGCTCATAGGCAATAACGATCGCATGTC AAATAAAAAAATTATTAZATTAA	
T ₂₆	Terminator ¹⁴ TTAAGACCGGGCTCTGTCCTACTCTGGAGATACTGGTGTGTTATATGAGGACTCTAGA CGCATGTC	
P _{Lox}	Promoter ¹⁵ CCTAGGACCTGTAGGATCGTACAGGTTACCCAAAGAAATGGTTGTTACTTCGAATAATCTAGA	
P _{fet}	Promoter ¹⁶ CGGTGGATCCCTATAGTGTAGATAATTGATCCTCATAGTGTGTTATATGAGGACTCTAGA	
P _{Cm}	Promoter ⁵ AACAAACAGAAATCTGGTGTGTTATATGAGGAAATTTCGTTGTTATATGAGGACTCTAGA	
P _{Phl}	Promoter ¹⁷ AAAAAGAGTTCACATGATAAGAAAGCTACGGTATGTTAGGTTACTAGTGTAGA	

TABLE 10 -continued

Name	Genetic part	DNA sequence (SEQ ID Nos: 122-225)	Genetic part sequences used in this study
P_{Fde}	Promoter ¹⁸	TCATGTGTTATGAGCGCTCATATCATGAATCAAACAAATCATTGATTAATCAAGGTCACCTCTAAGCTTCACTATCCCTGTGAT	
fdeR	Gene	ATGCGGTTCAAAAGETCGAACCTCAATCTTGGTGCCCTGGATGCACTGCTCACGGGATGACCATGCGGCGCATGCCAAAGATCCA TCTGAGSCAEGCGCATGAGCAATGCCCTGGCGCGCTGGCGGCTGGCGGAGTATTTCGAGTATGATTGGTGTGATCAAGGTCGGTCAAGGGC CCAGCGGCGGCCGGCGAGGTTGGCGAGTTGGCGAGTCGGTGGCGGAGTGGCGCTGGCGGCTGGCGGCTGGCGGCGCATGGCGCG GGCGAGAATCCAGGGCGGGAGTTGGCGAGTCGGTGGCGGAGTGGCGGCTGGCGGCTGGCGGCGCATGGCGGCGCGCGCGCGCG AATTCCGCAAGCCGCAATCCTCGCGAAGAGGTGCGTCTCCCGCGAAGCGCACTGCGTGGCGCCTGGCGCCTGGCGCGCGCGCG CTGACGGTGGACGCTAAATGGCGTCAAGGCGTGGCGAGTGGCGTGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCG GCTGGGGCGTTGGCG ATGCCCGCGTGGCG TGGCGATGCTTACCGCAGCAATTGGCATCTCTAGTGGCTCGGGTTTCTGGCTGGTGTGATCAAGGAGACTGGCGAGGAGATGGCG CATGGCTGA	
$P_{BAD.10}$	Promoter	CAGACATATGGACAATTGGTTTCTCTGAATGGGGAGATGAAGATGGCTGAAGCGCAAATGATCCCCGTTTATAAAGCAATTCTGA CCAAAAGCCATGACAAAAAGCGTAACAAAAGTGTCTATATCAGGGAAAGTCACZTTGATTATTGCAAGGCTACACTTCTCATACCGGTT GCCATACCATTTATCATATAAGTAGCGGATACCTACGCTTACGGTACCTGCGAACTCTATATTCTCATACCGGTTTTCGCG TAGCGGATT	
araC	Gene	ATGCAATATGGACAATTGGTTTCTCTGAATGGGGAGATGAAGATGGCTGAAGCGCAAATGATCCCCGTTTATAAAGCAATTCTGA GTTTAATGCCATTCTCGGGGTTAACCGCGATTGAGCGGCTTATCGGATTTTATCTGACGCGGCGGAAATGAAAGTT ATATTTCANISTCACCATCGCGGCTCATCGGGAGCTTCGAATGAGGTTGATGGTCAACAGTGGTTTACTTGCGACGCGGCGGTTTGC CGAGGAGAGATTCATCACTAACTGCTCATCGGGAGCTTCGAATGAGGTTGATGGTCAACAGTGGTTTACTTGCGACGCGGCGGTT ATGCTTAATGGCCGCTCATATTGGCCATACGCTTACCGGGTTCGCGGCTTACGGGATAATTCTGCTGATGGGATAATCTGCTG TCATTAACGCCGGGAGGGAGGGCTGGCGTATTCTGGAGCTTCTGGGATAATTCTGCTGATGGGATAATCTGCTG AACAGATTCCTCATACACCGATGATACTACCGGATGATACTACCGGATGATACTACCGGATGATACTACCGGATGATACTACCGG CAGCGTGCAGCAGCAGCTTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGG ACCAAGCTATAGCCGGGAGGCTTGGACACACCCTGGCTATGCCACCTGGCTTCGGCTCCGGTGGTGGTGGAGAAACTC TATTTCGCGGGTATTAAAAATCACCAGGGGAGGCCAGAGCTGGAGTTGGTGGAGAAATGGTGAATGTAAGTGA GTGTCATATAA	
araE	Gene	ATGGTACTATCAATACGGAACTCTGTTAACGCCACGTTCTTGGGGTACGGGGTATGATAATGGTTTCGTTGGTAGCTGGCG CCAGGGATTGGTATTGGCTGTGATATGGGGTAACTGGGTTGCTTAACTACGGTAACTTGTGCTGGCTGGCTGGCGCTGG ATATGG ATGGTGGTGGTCAACCTGGCATGCGCTGGCTGGGTTTATGGGTTTATGGGTTTATGGGTTTATGGGTTTATGGGTTTATGG AGTGGTGGTCAACCTGGCATGCGCTGGCTGGGTTTATGGGTTTATGGGTTTATGGGTTTATGGGTTTATGGGTTTATGG GTTTACAGGAGATCTGGTGGTGGGTTTATGGGTTTATGGGTTTATGGGTTTATGGGTTTATGGGTTTATGGGTTTATGG AGAAGPATTGCTGATTCGGCGATAGCTGGAAAGCGCGAGAGAACTCAAGGAATTGCGAAAGCTGAGTTAAACGGGGGG GGGCACTGTTAGATACGTAAGTCTCGTCTCTGCTCTGCTCTGCTCTGCTCTGCTCTGCTCTGCTCTGCTCTGCTCT ATCATEFACTAGCGCCGCGCATCTCAAAATGGGGGGCTTACGACCAAGAAACACAGATGTTGGTGTGTTGGTGTGTTGGTGT TATGGTGGTCAACCTTATGGGGTTTACGGGGTTTACGGGGTTTACGGGGTTTACGGGGTTTACGGGGTTTACGGGGTTTACGG CTCTGGTGGTGGCTATTGGCTGATGGCCAGGCGCAACTGCTGGTGTGTTGGTGTGTTGGTGTGTTGGTGTGTTGGTGT ATTGGCGGTATTGGATGAGGCCGCGCAACTGGGGGGTGTGATATGGCTCTGCTCTGCTCTGCTCTGCTCTGCTCTGCTCT TTCGACCAACCGCAACTGGGGGGTGTGATATGGCTCTGCTCTGCTCTGCTCTGCTCTGCTCTGCTCTGCTCTGCTCT TCTACACTCGCGTGAACATGGGTTGGGCAATTRACTTCTGGTCTCATTCGCGAACCCAAAATGTCAGCTGGAAACAAAT CTGATGGCAGGGAGGAGTGGAAATAATGGCGCTGA	

TABLE 10-continued

Name	Genetic part DNA sequence (SEQ ID Nos: 122-225)	Genetic part sequences used in this study
P _{lac}	Promoter ¹⁰ CTCGAGTGTTGACAATTAAATATCGGCCCTGGATAATGTGTTGAATTGTGCTTCACATTACACAVCTAGA	
P _{nos}	Promoter AACAAATACAAATGGGGCAATGCCATTATGCCATTGGCATAATGGAGGAAGTATTGATAAACATAGAAAATGGGCTTACTCTCGAA GAATGCACTGCGCCTCACCTCAACAGCTCTGCACTGCTGCACTGAGATGTGATGGCAGATTGCCAGGGGTTGCTCCGGTGT	
nocR	Gene ATGACGTCAGCAGCAGTCAGGATACGGAGATACGCCGGATACGCCGGTTACGCGGTGAACTTGATCAGGGATCTCGGAAGAGGAATGGATCAGCCCTTC CGAAAGAACGGCAACCGTTAACGCTTAAGCGGGGCTTAATCTTCAAGGAGGGCGGTATCTCTTCAAGGAGTCGGACATTTCACGGGTTACGGCAATCTCG ACAAAGTCGGCCTGAGCTGAGAAGCTCACTATGGGTCATGGGTCATGGGGCTAACCTGACAGTTCTCCAGACGGGCTTCAAGGTTGCTCCAGCA ATTAGCAGCTCATGCCGATGCCGATGCCGCGCCTGGGGCTAACCTGACAGTTCTCCAGACGGGCTTCAAGGTTGCTCCAGCA CTACGAACTGGGAAATGGGAAATGATCCTGGGACTTAACTGCAAGGAAACTGTTCAAGGAACTCTGGCAGGAACTGGGACTTAACTGCAAGGAACTGGG CGGGGATCNCCTCGGAGAACGAGAAACGGGAAACTGTTCAAGGAACTCTGGCAGGAACTTGAAGGAGGTCAATGATTGTCAGGCCTTCAGCA ATGCAAACGGAGCCGGCACTGGGAACTGGGTTGGGTAGTCAGGAACTTCACTGGCAGGAACTGGGACTTAACTGCAAGGAACTGGGACTTAACTG CAGCAGGAAATGGGTTGGGTGGGTAGTCAGGAACTGGGTTGGGTAGTCAGGAACTGGGACTTAACTGCAAGGAACTGGGACTTAACTG CCTACCAATTTCCTTCTATAGTTCCTTCCGACGAGCCCCTTCACTGGCAGGAACTGGGACTTAACTGCAAGGAACTGGGACTTAACTGCAAGGAA TTGCCCCCTTAAACCATTGGA	
P _{occ}	Promoter AAACGACCAATACACATCTGCTTATICCTGGCCGGTCATTATGAAATTGCAATGAAATTGAAATGTAAGCTAACCCATTATAACACAC TCTTAACTACAGCT CTTTATACAGAGCT	
occR	Gene ATGAATCTCAGGAGGTGAGGGCTTCCGGGCACTGATGGTGCACGGGAAATGAGGGGGCTGGAACTAATCTGGGACTCTAGCCGGC CATCATGGCCTTAATPAGGCCTTCAACCGCCACAACTGCGGCTTCAAGGAGGAGCTGGCAGCCTGGGAACTGAGGCA AGACGCTGTGGAAGGGTCAATCGGGGTTCATGGCTTCTGGCCTTAATCATATGGCAACTCTGGGCACTGGGACTCTGGGACT CTTCCGGATTGCTGCAATTCGCTCTGGCAAAAGGGCTCTGGCCTCTGGGAACTGGGACTCTGGGACTCTGGGACT CCTAATGGGAATCTGGCTCCCAAGTGETCATGGCAAGGGCTTCTGGCCTGGCTGCTGGCAGGCACTGGGACT GTTTCTTAATGCAAACTGGGTTCGGCTGGCTGGGTGGGGGGGACTGGGACTCTGGGACTCTGGGACT ATTGGAGTGAACGGCTCTGGCAACTTGCACTGGGCAACTGGGACTTGCACTGGGCAACTGGGACT CGGACAGGATCTACTGGCACTGGGCAACTTGCACTGGGCAACTGGGACT GATGGTTTCACACGAAATTGGGACTTCAATGAGGTTTCAATGAGGAATGGGACTTAACTGAGGAACTGGCTTAATGGAGTAA AGGGGGATTGGTGAAGGGATTGTTAACATGACAATTGTTGGAGCTTCATATGCTAGC	
P _{Bcr}	Promoter ¹⁷ ACGGGGGTAGAGGGATTGGTACGGGAAATTGAGATACATGTTGGAGCTTCATATGCTAGC	
P _{Cln}	Promoter CCCTTGTGCGTCACAGGACGCCAGGGCTTAAGCCGGTGCACATTGCTCCGGCCTTCAGCTTGTGCTGC ATGTCGTTATGCAAAACCGCTGCAACTTITGCGGACTGTCTGATCCCCTCATCTGGGGGCTATCTGAGGGAAATTGCGATCG	
P _{AB5B}	Promoter ⁵ TTTTGGTGTGAGTATGGAAATTGAGATACATGTTGGAGCTTCATATGCTAGC A	
P _{TTg}	Promoter ¹⁹ CACCCAGCAGTATTACAAACCATGAGCTAGCTGGCTTAGCTTCTAGGAA	
P _{Van}	Promoter ²⁰ ATTGGATCCAATTGAGAGTGTCTGGCTTAGTACCATGGATCCAAT	

TABLE 11

RBS sequences used in this study			Strength (GFP, au)
Name	Strain	RBS sequence ^a (SEQ ID NOS: 226-291)	
RBSr1	R. sp. IRBG74	ATTCACACATCTAGAGCTAATCATCTCGTACTAAAGAGGAGAAATTAAACC <u>ATG</u>	8242
RBSr2	R. sp. IRBG74	ATTCACACATCTAGAGCTAATCATCGCGTACTCAGGAGGAAG <u>TAATG</u>	7181.5
RBSr3	R. sp. IRBG74	ATTCACACATCTAGAATTAAAGAGGAGAAATTAAACC <u>ATG</u>	6238.5
RBSr4	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCTCGTACTAAAGAGGCAAG <u>TAATG</u>	3618
RBSr5	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCGCGTACTAAGGAGGCAAG <u>TAATG</u>	3560
RBSr6	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCGCGTACTCAAGAGGCAAG <u>TAATG</u>	2614.5
RBSr7	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCGCGTACTAAAGAGGCAAG <u>TAATG</u>	2418.5
RBSr8	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCTCGTACTCAGGAGGCAAG <u>TAATG</u>	1882.5
RBSr9	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCTCGTACTAATGAGGCAAG <u>TAATG</u>	1593.5
RBSr10	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCGCGTACTAATGAGGCAAG <u>TAATG</u>	1590
RBSr11	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCGCGTACTCACGAGGCAAG <u>TAATG</u>	1554
RBSr12	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCGCGTACTAAAAGGCAAG <u>TAATG</u>	1138
RBSr13	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCGCGTACTAAAAGGCAAG <u>TAATG</u>	895.5
RBSr14	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCGCGTACTAAGAAGGCAAG <u>TAATG</u>	632.5
RBSr15	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCTCGTACTAAATAGGCAAG <u>TAATG</u>	648.5
RBSr16	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCTCGTACTAATAAGGCAAG <u>TAATG</u>	532
RBSr17	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCTCGTACTAAAGAGGCAAG <u>TAATG</u>	488
RBSr18	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCGCGTACTCAATAGGCG <u>AGTAATG</u>	305.5
RBSr19	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCGCGTACTAAAGTAGGCAAG <u>TAATG</u>	242
RBSr20	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCTCGTACTAACAGGCAAG <u>TAATG</u>	248
RBSr21	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCGCGTACTCAGCAGGCAAG <u>TAATG</u>	183
RBSr22	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCGCGTACTAAAGTAGGCAAG <u>TAATG</u>	130
RBSr23	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCGCGTACTAATTAGGCAAG <u>TAATG</u>	84.4
RBSr24	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCTCGTACTAACAGGCAAG <u>TAATG</u>	75.15
RBSr25	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCTCGTACTCAATAGGCAAG <u>TAATG</u>	45.45
RBSr26	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCTCGTACTAACAGCAG <u>TAATG</u>	36
RBSr27	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCATCGCGTACTAACTACGCAAG <u>TAATG</u>	12.2
RBSr28	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCGCGTACTAAAGAACG <u>CAAGTAATG</u>	13
RBSr29	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCGCGTACTAAAACG <u>CAAGTAATG</u>	4.6
RBSr30	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCGCGTACTAACAAACG <u>CAAGTAATG</u>	2.95
RBSr31	R. sp. IRBG74	TAACAATTCACACATCTAGAGCTAATCTCTCGTACTCATGACG <u>CAAGTAATG</u>	1.45
RBSr32	R. sp. IRBG74	ATTCACACATCTAGAATTAAAGAGAAGAAATTAAACC <u>ATG</u>	N/A ^b
RBSr33	R. sp. IRBG74	CTAGTGC <u>G</u> AAC <u>T</u> AGCTCATACCG <u>CAGA</u> <u>TG</u>	N/A ^b
RBSp1	P. <i>protegens</i> Pf-5	CTAGCGCAGGTCCAACGTTTTCTAAGCAAGGAGGT <u>CATATG</u>	25090
RBSp2	P. <i>protegens</i> Pf-5	CTAGCGAAGGTCCAACGTTTTCTAAGCAAGGAGGT <u>CATATG</u>	21590
RBSp3	P. <i>protegens</i> Pf-5	CTAGCGAAGGTCCAACGTTTTCTAAGCCAGGAGGT <u>CATATG</u>	19690

TABLE 11-continued

RBS sequences used in this study			
Name	Strain	RBS sequence ^a (SEQ ID NOS: 226-291)	Strength (GFP, au)
RBSp4	<i>P. protegens</i> Pf-5	CTAGCGCAGGTCCAACGTTTCTAAGCCAGGAGGT <u>CATATG</u>	19490
RBSp5	<i>P. protegens</i> Pf-5	CTAGCGAAGCTCCAACGTTTCTAAGCAAGGAGGT <u>CATATG</u>	17990
RBSp6	<i>P. protegens</i> Pf-5	GAATTCTACACTAACGGACAGGAGGGT <u>CCGATG</u>	14490
RBSp7	<i>P. protegens</i> Pf-5	GAATTCTAAACTAACGGACAGGAGGGT <u>CCGATG</u>	13390
RBSp8	<i>P. protegens</i> Pf-5	GAATTCTAACGCTAACGGACAGGAGGGT <u>CCGATG</u>	12790
RBSp9	<i>P. protegens</i> Pf-5	GAATTCTAACGCTAACGGACAGGAGGGT <u>CCGATG</u>	11490
RBSp10	<i>P. protegens</i> Pf-5	GAATTCTACACTAACGGACAGGAGGGT <u>CGGATG</u>	11090
RBSp11	<i>P. protegens</i> Pf-5	GAATTCTACGCTAACGGACAGGAGGGT <u>CCGATG</u>	10390
RBSp12	<i>P. protegens</i> Pf-5	GAATTCTAACGCTAACGGACAGGAGGGT <u>CCGATG</u>	9590
RBSp13	<i>P. protegens</i> Pf-5	GAATTCTAACGCTAACGGACAGGAGGGT <u>CGGATG</u>	8918
RBSp14	<i>P. protegens</i> Pf-5	GAATTCTCAGCTAACGGACAGGAGGGT <u>CCGATG</u>	8766
RBSp15	<i>P. protegens</i> Pf-5	GAATTCTCAACTAACGGACAGGAGGGT <u>CCGATG</u>	7596
RBSp16	<i>P. protegens</i> Pf-5	GAATTCTACGCTAACGGACAGGAGGGT <u>CGGATG</u>	6055
RBSp17	<i>P. protegens</i> Pf-5	GAATTCTCAACTAACGGACAGGAGGAT <u>TACATATG</u>	5939
RBSp18	<i>P. protegens</i> Pf-5	GAATTCTCAGCTAACGGACAGGAGGGT <u>CGGATG</u>	5915
RBSp19	<i>P. protegens</i> Pf-5	GAATTCTAAACTAACGGACAGGAGGGT <u>CGGATG</u>	4867
RBSp20	<i>P. protegens</i> Pf-5	GAATTCTCAGCTAACGGACAGGAGGGT <u>CGGATG</u>	4426
RBSp21	<i>P. protegens</i> Pf-5	GAATTCTCAACTAACGGACAGGAGGGT <u>CGGATG</u>	4110
RBSp22	<i>P. protegens</i> Pf-5	GAATTCTACACTAACGGACAGGAGGGT <u>CGGATG</u>	3977
RBSp23	<i>P. protegens</i> Pf-5	GAATTCTAACGCTAACGGACAGGAGGGT <u>CGGATG</u>	3829
RBSp24	<i>P. protegens</i> Pf-5	GAATTCTCAACTAACGGACAGGAGGGT <u>CGGATG</u>	3661
RBSp25	<i>P. protegens</i> Pf-5	GAATTCTCACACTAACGGACAGCAGGGT <u>CGGATG</u>	3542
RBSp26	<i>P. protegens</i> Pf-5	CTAGCGCAGGTCCAACCTT+32CTAACGCAAGTAGGT <u>CATATG</u>	2139
RBSp27	<i>P. protegens</i> Pf-5	GAATTCTCAGCTAACGGACAGCAGGGT <u>CGGATG</u>	1265
RBSp28	<i>P. protegens</i> Pf-5	CTAGCGCAGGTCCAACCTTCTAACGCAACTAGGT <u>CATATG</u>	389
RBSp29	<i>P. protegens</i> Pf-5	CTAGCGAAGGTCCAACCTTCTAACGCCAGTAGGT <u>CATATG</u>	377
RBSp30	<i>P. protegens</i> Pf-5	GAATTCTACGCTAACGGACAGCAGGGT <u>CGGATG</u>	221
RBSp31	<i>P. protegens</i> Pf-5	GAATTCTCCGCTAACGGACAGGAGGGT <u>CCGATG</u>	23.3
RBSp32	<i>P. protegens</i> Pf-5	CTTCTCGGCCAGCTGACAGGGGAAGCT <u>CGCATG</u>	N/A ^b
RBSp33	<i>P. protegens</i> Pf-5	CTTCTCGGCCAGCTGACAGGGAGGAAGCT <u>CGCATG</u>	N/A ^b

^aThe start codon is underlined.^bRBSs are rationally designed for the controllers by the RBS Calculator²

TABLE 12

Chemicals used in this study		
Chemicals	Source	Identifier
Tryptone Yeast extract	Fisher Scientific BD Bacto	Cat# BP1421 Cat# DF0127

TABLE 12-continued

Chemicals used in this study		
Chemicals	Source	Identifier
NaCl	Fisher Scientific	Cat# S271
CaCl ₂ •2H ₂ O	Sigma-Aldrich	Cat# C3306
MgSO ₄ •7H ₂ O	Fisher Scientific	Cat# M80
FeCl ₃	Alfa Aesar	Cat# AA1235709
Ni ₂ MoO ₄ •2H ₂ O	Sigma-Aldrich	Cat# 331058
NH ₄ CH ₃ CO ₂	Sigma-Aldrich	Cat# A1542
Ni ₂ HPO ₄	Fisher Scientific	Cat# S375
KH ₂ PO ₄	Sigma-Aldrich	Cat# P9791
EDTA—Na ₂	Sigma-Aldrich	Cat# E5134
ZnSO ₄ •7H ₂ O	ACROS Organics	Cat# AC424605000
H ₃ BO ₃	Fisher Scientific	Cat# A73
MnSO ₄ •H ₂ O	MP Biomedicals	Cat# ICN225099
CuSO ₄ •5H ₂ O	Aldon Corp	Cat# CC0535
CoCl ₂ •6H ₂ O	Sigma-Aldrich	Cat# C8661
FeSO ₄ •7H ₂ O	Sigma-Aldrich	Cat# 215422
Thiamine hydrochloride	ACROS Organics	Cat#148990100
D-pantothenic acid hemicalcium salt	Sigma-Aldrich	Cat# P5155
Biotin	Sigma-Aldrich	Cat# B4501
Nicotinic acid	Sigma-Aldrich	Cat# 72309
MOPS	Fisher Scientific	Cat# BP308
Isopropyl-beta-D-thiogalactoside (IPTG)	GoldBio	Cat# 12481
L-arabinose	Sigma	Cat# A3256
Anhydrotetracycline hydrochloride (aTc)	Sigma	Cat# 37919
N-(3-Oxohexanoyl)-L-homoserine lactone (3OC6HSL)	Sigma	Cat# K3007
N-(3-Hydroxytetradecanoyl)-DL-homoserine lactone (3OC14HSL)	Sigma	Cat# 51481
Naringenin	Sigma	Cat# N5893
2,4-Diacetylphloroglucinol (DAPG)	Santa Cruz	Cat# sc-206518
Salicylic acid sodium salt	Sigma	Cat# S3007
3,4-Dihydroxybenzoic acid (DHBA)	Sigma	Cat# 37580
Vanillic acid	Sigma	Cat# 94770
Cuminic acid	Sigma	Cat# 268402
Nopaline	Toronto Research Chemicals	Cat# N650600
Octopine	Toronto Research Chemicals	Cat# 0239850
Choline chloride	Sigma	Cat# C7017
Tris (1M), pH 8.0	Invitrogen	Cat# AM9855
Triton X-100	Sigma-Aldrich	Cat# T8787
Tergitol solution	Sigma-Aldrich	Cat# NP40S
DNase I	Sigma-Aldrich	Cat# 4716728001
RNA Fragmentation Reagents	Invitrogen	Cat# AM8740
T4 Polynucleotide kinase	New England Biolabs	Cat# M0201
SUPERase•In	Invitrogen	Cat# AM2694
PEG 8000	Sigma-Aldrich	Cat# 1546605
T4 RNA ligase 2, truncated K277Q	New England Biolabs	Cat# M0351
Superscript III reverse transcriptase	Invitrogen	Cat# 18080044
CircLigase ssDNA ligase	Epicentre	Cat# CL4115K
Phusion High-Fidelity DNA polymerase	New England Biolabs	Cat# M0530
Micrococcal nuclease	Roche	10107921001

SEQUENCE LISTING

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Sequence total quantity: 293
SEQ ID NO: 1      moltype = DNA  length = 17
FEATURE          Location/Qualifiers
misc_feature     1..17
                  note = Synthetic polynucleotide
misc_feature     1
                  note = linked to /5rApp/
misc_feature     17
                  note = linked to /3ddc/
source           1..17
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 1
ctgttaggcac catcaat

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17

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SEQ ID NO: 2      moltype = DNA  length = 33

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FEATURE          Location/Qualifiers
misc_feature    1..33
note = Synthetic polynucleotide
misc_feature    1
note = linked to /5Phos/
misc_feature    33
note = linked to /iSp18/
source          1..33
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 2
agatcgaaag acgcgtcgtgt agggaaagag tgt                         33

SEQ ID NO: 3          moltype = DNA length = 41
FEATURE          Location/Qualifiers
misc_feature    1..41
note = Synthetic polynucleotide
misc_feature    1
note = linked to /iSp18/
source          1..41
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 3
caagcagaag acggcatacg agatattgtat ggtgcctaca g                         41

SEQ ID NO: 4          moltype = DNA length = 21
FEATURE          Location/Qualifiers
misc_feature    1..21
note = Synthetic polynucleotide
source          1..21
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 4
caagcagaag acggcatacg a                         21

SEQ ID NO: 5          moltype = DNA length = 85
FEATURE          Location/Qualifiers
misc_feature    1..85
note = Synthetic polynucleotide
source          1..85
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 5
aatgatacgg cgaccaccga gatctacacg atcggaaagag cacacgtctg aactccagtc 60
acnnnnnnnac acttttccc tacac                         85

SEQ ID NO: 6          moltype = DNA length = 32
FEATURE          Location/Qualifiers
misc_feature    1..32
note = Synthetic polynucleotide
source          1..32
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 6
cgacaggttc agagttctac agtccgacga tc                         32

SEQ ID NO: 7          moltype = DNA length = 32
FEATURE          Location/Qualifiers
misc_feature    1..32
note = Synthetic polynucleotide
source          1..32
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 7
cgacaggttc agagttctac agtccgacga tc                         32

SEQ ID NO: 8          moltype = DNA length = 68
FEATURE          Location/Qualifiers
source          1..68
mol_type = genomic DNA
organism = Klebsiella oxytoca

SEQUENCE: 8
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ttaaggtc                         68

SEQ ID NO: 9          moltype = DNA length = 24

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-continued

FEATURE	Location/Qualifiers
source	1..24
	mol_type = genomic DNA
	organism = Klebsiella oxytoca
SEQUENCE: 9	
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SEQ ID NO: 10	moltype = DNA length = 74
FEATURE	Location/Qualifiers
source	1..74
	mol_type = genomic DNA
	organism = Pseudomonas stutzeri
SEQUENCE: 10	
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cctgaattcg gtgt	74
SEQ ID NO: 11	moltype = DNA length = 48
FEATURE	Location/Qualifiers
source	1..48
	mol_type = genomic DNA
	organism = Pseudomonas stutzeri
SEQUENCE: 11	
ggctcaacttc gatttcgtcc gcggtgcgta ccctgctagt gatgcgtta	48
SEQ ID NO: 12	moltype = DNA length = 25
FEATURE	Location/Qualifiers
source	1..25
	mol_type = genomic DNA
	organism = Pseudomonas stutzeri
SEQUENCE: 12	
cgccctgattt cgcctgtatga acagg	25
SEQ ID NO: 13	moltype = DNA length = 20
FEATURE	Location/Qualifiers
source	1..20
	mol_type = genomic DNA
	organism = Pseudomonas stutzeri
SEQUENCE: 13	
tgacgctgtt gaccaccggc	20
SEQ ID NO: 14	moltype = DNA length = 23
FEATURE	Location/Qualifiers
source	1..23
	mol_type = genomic DNA
	organism = Pseudomonas stutzeri
SEQUENCE: 14	
atggaagtgg tcggcacccgg cta	23
SEQ ID NO: 15	moltype = DNA length = 22
FEATURE	Location/Qualifiers
source	1..22
	mol_type = genomic DNA
	organism = Pseudomonas stutzeri
SEQUENCE: 15	
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SEQ ID NO: 16	moltype = DNA length = 23
FEATURE	Location/Qualifiers
source	1..23
	mol_type = genomic DNA
	organism = Pseudomonas stutzeri
SEQUENCE: 16	
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SEQ ID NO: 17	moltype = DNA length = 37
FEATURE	Location/Qualifiers
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	mol_type = genomic DNA
	organism = Pseudomonas stutzeri
SEQUENCE: 17	
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FEATURE	Location/Qualifiers
source	1..101
	mol_type = genomic DNA

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SEQUENCE: 18          organism = Azotobacter vinelandii
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tactgcgta cggggttgcg gggcagcca gtggaaaaag g               101

SEQ ID NO: 19          moltype = DNA length = 21
FEATURE
source
1..21
mol_type = genomic DNA
organism = Azotobacter vinelandii

SEQUENCE: 19
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SEQ ID NO: 20          moltype = DNA length = 22
FEATURE
source
1..22
mol_type = genomic DNA
organism = Azotobacter vinelandii

SEQUENCE: 20
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SEQ ID NO: 21          moltype = DNA length = 24
FEATURE
source
1..24
mol_type = genomic DNA
organism = Azotobacter vinelandii

SEQUENCE: 21
aaatcagaca ttcatggcca cagg                                         24

SEQ ID NO: 22          moltype = DNA length = 22
FEATURE
source
1..22
mol_type = genomic DNA
organism = Azotobacter vinelandii

SEQUENCE: 22
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SEQ ID NO: 23          moltype = DNA length = 43
FEATURE
source
1..43
mol_type = genomic DNA
organism = Azotobacter vinelandii

SEQUENCE: 23
actcgtcttc tgtccgttta aactcccgga actctaccac cgc             43

SEQ ID NO: 24          moltype = DNA length = 21
FEATURE
source
1..21
mol_type = genomic DNA
organism = Azotobacter vinelandii

SEQUENCE: 24
cttggataga cgaggcacag c                                         21

SEQ ID NO: 25          moltype = DNA length = 36
FEATURE
source
1..36
mol_type = genomic DNA
organism = Azotobacter vinelandii

SEQUENCE: 25
gccggctctt gcaacctgaa ggggcccggagg atgatg                      36

SEQ ID NO: 26          moltype = DNA length = 30
FEATURE
source
1..30
mol_type = genomic DNA
organism = Paenibacillus polymyxa

SEQUENCE: 26
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source
1..24
mol_type = genomic DNA
organism = Paenibacillus polymyxa

SEQUENCE: 27
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FEATURE
source
1..21
mol_type = genomic DNA
organism = Paenibacillus polymyxa
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SEQ ID NO: 29      moltype = DNA  length = 22
FEATURE
source
1..22
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organism = Paenibacillus polymyxa
SEQUENCE: 29
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FEATURE
source
1..23
mol_type = genomic DNA
organism = Paenibacillus polymyxa
SEQUENCE: 30
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SEQ ID NO: 31      moltype = DNA  length = 26
FEATURE
source
1..26
mol_type = genomic DNA
organism = Paenibacillus polymyxa
SEQUENCE: 31
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1..118
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source
1..118
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 32
ggcccgccgtt aggttggcctt gaattcggtt tttatccccc ggagataacgt aaaaaaaaaaa 60
accccgccctt gtcaggggcg gggttttttt ttgtataagtc aagctatcag aaccgatc 118

SEQ ID NO: 33      moltype = DNA  length = 48
FEATURE
misc_feature
1..48
note = Cyanothece
source
1..48
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 33
taattcccat aacatctgca tgcataataa ggtggggaaa gtctcagc                                48

SEQ ID NO: 34      moltype = DNA  length = 24
FEATURE
misc_feature
1..24
note = Cyanothece
source
1..24
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 34
aatgttatttc tgatcgatgc gacg                                24

SEQ ID NO: 35      moltype = DNA  length = 24
FEATURE
misc_feature
1..24
note = Cyanothece
source
1..24
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 35
gttatctggc tgatgttgtt ggtg                                24

SEQ ID NO: 36      moltype = DNA  length = 24
FEATURE

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misc_feature          1..24
                      note = Cyanothece
source               1..24
                      mol_type = genomic DNA
                      organism = unidentified
SEQUENCE: 36
gtcaaaactgt cttgtttaaa gccg                                24

SEQ ID NO: 37          moltype = DNA  length = 104
FEATURE              Location/Qualifiers
source               1..104
                      mol_type = genomic DNA
                      organism = Azospirillum brasiliense
SEQUENCE: 37
ttaaggatcat gcagcaggag aactaaaaggc ccgcgttagg ttggtaataa aaaagccccc 60
ggaatgatct tccggggggcc ctgcgc当地 acaacatcg aatc                                104

SEQ ID NO: 38          moltype = DNA  length = 26
FEATURE              Location/Qualifiers
source               1..26
                      mol_type = genomic DNA
                      organism = Azospirillum brasiliense
SEQUENCE: 38
gacgactgaa taaggatcgc ggaatg                                26

SEQ ID NO: 39          moltype = DNA  length = 23
FEATURE              Location/Qualifiers
source               1..23
                      mol_type = genomic DNA
                      organism = Azospirillum brasiliense
SEQUENCE: 39
tatgtcacag gccccgacaaa gcg                                23

SEQ ID NO: 40          moltype = DNA  length = 23
FEATURE              Location/Qualifiers
source               1..23
                      mol_type = genomic DNA
                      organism = Azospirillum brasiliense
SEQUENCE: 40
gattgtcggg tatcgacacac gag                                23

SEQ ID NO: 41          moltype = DNA  length = 25
FEATURE              Location/Qualifiers
source               1..25
                      mol_type = genomic DNA
                      organism = Azospirillum brasiliense
SEQUENCE: 41
cgaaggagtt cgccccagtc tattc                                25

SEQ ID NO: 42          moltype = DNA  length = 68
FEATURE              Location/Qualifiers
source               1..68
                      mol_type = genomic DNA
                      organism = Rhodopseudomonas palustris
SEQUENCE: 42
aatacgtatcg catgttcttag gtaatacgtac tcactatagg gagaggtaat cagtggtaga 60
tttgatgt                                         68

SEQ ID NO: 43          moltype = DNA  length = 20
FEATURE              Location/Qualifiers
source               1..20
                      mol_type = genomic DNA
                      organism = Rhodopseudomonas palustris
SEQUENCE: 43
ccaagcaaag gaccaccctc                                20

SEQ ID NO: 44          moltype = DNA  length = 22
FEATURE              Location/Qualifiers
source               1..22
                      mol_type = genomic DNA
                      organism = Rhodopseudomonas palustris
SEQUENCE: 44
agtttcgata tcatccgctg at                                22

SEQ ID NO: 45          moltype = DNA  length = 23
FEATURE              Location/Qualifiers

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source          1..23
               mol_type = genomic DNA
               organism = Rhodopseudomonas palustris
SEQUENCE: 45
ttgttcatgt cggacctaac cga                                23
SEQ ID NO: 46      moltype = DNA  length = 67
FEATURE
source          1..67
               mol_type = genomic DNA
               organism = Rhodobacter sphaeroides
SEQUENCE: 46
caatacgcgc gcatgtccta ggtaatacga ctcactatacg ggagatgcat ttcacgcctc 60
gcgattc                                67
SEQ ID NO: 47      moltype = DNA  length = 20
FEATURE
source          1..20
               mol_type = genomic DNA
               organism = Rhodobacter sphaeroides
SEQUENCE: 47
ccgccttac cagagacacc                                20
SEQ ID NO: 48      moltype = DNA  length = 23
FEATURE
source          1..23
               mol_type = genomic DNA
               organism = Rhodobacter sphaeroides
SEQUENCE: 48
atcgagaagt tctacgatgc cgt                                23
SEQ ID NO: 49      moltype = DNA  length = 68
FEATURE
source          1..68
               mol_type = genomic DNA
               organism = Rhodobacter sphaeroides
SEQUENCE: 49
gaaaaaaaaa accccgcccc tgacaggcg gggttttttt tttcaattgg acctggatgg 60
gcagcaag                                68
SEQ ID NO: 50      moltype = DNA  length = 106
FEATURE
source          1..106
               mol_type = genomic DNA
               organism = Azorhizobium caulinodans
SEQUENCE: 50
ctcgcatcca ttctcaggct gtctcgctc gtctctctag agtcggagct ctggggcct 60
ctaaacgggt cttaggggt ttttgttgc ttgcacgcg aagtc                106
SEQ ID NO: 51      moltype = DNA  length = 53
FEATURE
source          1..53
               mol_type = genomic DNA
               organism = Azorhizobium caulinodans
SEQUENCE: 51
ataggcaata cgatcgcatg tccgtttaaa ctgataagga cggcactggc tgg      53
SEQ ID NO: 52      moltype = DNA  length = 19
FEATURE
source          1..19
               mol_type = genomic DNA
               organism = Azorhizobium caulinodans
SEQUENCE: 52
cgatgccgtc cagcacctc                                19
SEQ ID NO: 53      moltype = DNA  length = 21
FEATURE
source          1..21
               mol_type = genomic DNA
               organism = Azorhizobium caulinodans
SEQUENCE: 53
ctgcccacggt tcccaagggtt c                                21
SEQ ID NO: 54      moltype = DNA  length = 62
FEATURE
source          1..62

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mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 54
taaaaaagcg gctaaccacg ccgttttt tacgtctgca gtgttgcga agcttgatgc 60
gc                                         62

SEQ ID NO: 55      moltype = DNA length = 60
FEATURE
source
1..60
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 55
cgctgcttaa ggtcatgcag caggagaact aaaggcccg tctgcgaaag gaatagcgtc 60

SEQ ID NO: 56      moltype = DNA length = 19
FEATURE
source
1..19
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 56
ctatcgccgc cacctgacc                                         19

SEQ ID NO: 57      moltype = DNA length = 30
FEATURE
source
1..30
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 57
cgtcagaacg gctctgacgc atcagggaga                                         30

SEQ ID NO: 58      moltype = DNA length = 32
FEATURE
source
1..32
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 58
agtaatatgg cgatcgccg acgacgagg aa                                         32

SEQ ID NO: 59      moltype = DNA length = 25
FEATURE
source
1..25
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 59
ggtgttatt ggcaacgggt cgaag                                         25

SEQ ID NO: 60      moltype = DNA length = 31
FEATURE
source
1..31
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 60
tcccccaag cccaaccgtt cgggagcga a                                         31

SEQ ID NO: 61      moltype = DNA length = 99
FEATURE
source
1..99
mol_type = genomic DNA
organism = Gluconacetobacter diazotrophicus
SEQUENCE: 61
ttaaggcat gcagcaggag aactaaaggc ccgcgtttagg ttggtaataa aaaagccccc 60
ggaatgtatct tccggggggcc gatcgaggaa atcgacgtg 99

SEQ ID NO: 62      moltype = DNA length = 28
FEATURE
source
1..28
mol_type = genomic DNA
organism = Gluconacetobacter diazotrophicus
SEQUENCE: 62
atattccgga tacggctggt gaggtgga                                         28

SEQ ID NO: 63      moltype = DNA length = 24
FEATURE
source
1..24
mol_type = genomic DNA
organism = Gluconacetobacter diazotrophicus

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SEQUENCE: 63
cgccacgtcg tcaatgccta taac                                24

SEQ ID NO: 64      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
source           1..21
mol_type = genomic DNA
organism = Gluconacetobacter diazotrophicus

SEQUENCE: 64
tgaccaccgt gcagaagatc c                                21

SEQ ID NO: 65      moltype = DNA  length = 23
FEATURE          Location/Qualifiers
source           1..23
mol_type = genomic DNA
organism = Klebsiella oxytoca

SEQUENCE: 65
tgacgctcg cgtatcaggc ttg                                23

SEQ ID NO: 66      moltype = DNA  length = 69
FEATURE          Location/Qualifiers
source           1..69
mol_type = genomic DNA
organism = Klebsiella oxytoca

SEQUENCE: 66
atcaggcgca tatttgaatg tatttactgc agcggccgct tctagagtga cccaaagctt 60
ccgcaaccc                                69

SEQ ID NO: 67      moltype = DNA  length = 48
FEATURE          Location/Qualifiers
source           1..48
mol_type = genomic DNA
organism = Pseudomonas stutzeri

SEQUENCE: 67
actacgcata actagcaggc cacgcaccgc ggacgaaatc gaagttag                                48

SEQ ID NO: 68      moltype = DNA  length = 22
FEATURE          Location/Qualifiers
source           1..22
mol_type = genomic DNA
organism = Pseudomonas stutzeri

SEQUENCE: 68
tttgtcgactc ccggggtctg ac                                22

SEQ ID NO: 69      moltype = DNA  length = 23
FEATURE          Location/Qualifiers
source           1..23
mol_type = genomic DNA
organism = Pseudomonas stutzeri

SEQUENCE: 69
ggcttaacg gcatgttccg ggt                                23

SEQ ID NO: 70      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
source           1..24
mol_type = genomic DNA
organism = Pseudomonas stutzeri

SEQUENCE: 70
gtatcgatcg ttgtggccga actc                                24

SEQ ID NO: 71      moltype = DNA  length = 22
FEATURE          Location/Qualifiers
source           1..22
mol_type = genomic DNA
organism = Pseudomonas stutzeri

SEQUENCE: 71
aaagcatcat ctgggtcgg gc                                22

SEQ ID NO: 72      moltype = DNA  length = 21
FEATURE          Location/Qualifiers
source           1..21
mol_type = genomic DNA
organism = Pseudomonas stutzeri

SEQUENCE: 72
cgtcgagcga caacgcctcg a                                21

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SEQ ID NO: 73      moltype = DNA length = 25
FEATURE          Location/Qualifiers
source           1..25
mol_type = genomic DNA
organism = Pseudomonas stutzeri
SEQUENCE: 73
ctatgagctg gactgaacctc cgatg                                25

SEQ ID NO: 74      moltype = DNA length = 74
FEATURE          Location/Qualifiers
source           1..74
mol_type = genomic DNA
organism = Pseudomonas stutzeri
SEQUENCE: 74
gaaaataccg catcaggcgcatatttgaat gtatttactgcagcggccgc tggcaatct 60
ccttcctcggttcg                                         74

SEQ ID NO: 75      moltype = DNA length = 22
FEATURE          Location/Qualifiers
source           1..22
mol_type = genomic DNA
organism = Azotobacter vinelandii
SEQUENCE: 75
gccttcgaac atgttgtccc ag                                22

SEQ ID NO: 76      moltype = DNA length = 24
FEATURE          Location/Qualifiers
source           1..24
mol_type = genomic DNA
organism = Azotobacter vinelandii
SEQUENCE: 76
tcgagttcga gcagtttctc cagc                                24

SEQ ID NO: 77      moltype = DNA length = 20
FEATURE          Location/Qualifiers
source           1..20
mol_type = genomic DNA
organism = Azotobacter vinelandii
SEQUENCE: 77
agcgaacaat acctgtggcc                                     20

SEQ ID NO: 78      moltype = DNA length = 21
FEATURE          Location/Qualifiers
source           1..21
mol_type = genomic DNA
organism = Azotobacter vinelandii
SEQUENCE: 78
tggcgcttgc ccttggttcca a                                21

SEQ ID NO: 79      moltype = DNA length = 54
FEATURE          Location/Qualifiers
source           1..54
mol_type = genomic DNA
organism = Azotobacter vinelandii
SEQUENCE: 79
gcgcggtggtaagttccgg gagtttaaac ggacagaaga cgagtcgtgc gggc      54

SEQ ID NO: 80      moltype = DNA length = 20
FEATURE          Location/Qualifiers
source           1..20
mol_type = genomic DNA
organism = Azotobacter vinelandii
SEQUENCE: 80
ttgctcaggc tcgggttggc                                     20

SEQ ID NO: 81      moltype = DNA length = 39
FEATURE          Location/Qualifiers
source           1..39
mol_type = genomic DNA
organism = Azotobacter vinelandii
SEQUENCE: 81
catcatcctc ggccccttca gggttgcggaa gcccggcttgc            39

SEQ ID NO: 82      moltype = DNA length = 21
FEATURE          Location/Qualifiers
source           1..21

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SEQUENCE: 82	mol_type = genomic DNA organism = Azotobacter vinelandii	
gcaaggccact ccactgacga a		21
SEQ ID NO: 83	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
source	1..22	
	mol_type = genomic DNA	
	organism = Paenibacillus polymyxa	
SEQUENCE: 83		
acaggttccg cagttcacaa gc		22
SEQ ID NO: 84	moltype = DNA length = 25	
FEATURE	Location/Qualifiers	
source	1..25	
	mol_type = genomic DNA	
	organism = Paenibacillus polymyxa	
SEQUENCE: 84		
gctgatttgt atcgacaata ttgcgg		25
SEQ ID NO: 85	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
source	1..22	
	mol_type = genomic DNA	
	organism = Paenibacillus polymyxa	
SEQUENCE: 85		
gaaaaggctac acgaaggaaa gg		22
SEQ ID NO: 86	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
source	1..22	
	mol_type = genomic DNA	
	organism = Paenibacillus polymyxa	
SEQUENCE: 86		
cttgagaatc tgccgggcgc ct		22
SEQ ID NO: 87	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
source	1..22	
	mol_type = genomic DNA	
	organism = Paenibacillus polymyxa	
SEQUENCE: 87		
atccacaaat caacaccctg cg		22
SEQ ID NO: 88	moltype = DNA length = 22	
FEATURE	Location/Qualifiers	
source	1..22	
	mol_type = genomic DNA	
	organism = Paenibacillus polymyxa	
SEQUENCE: 88		
aaagcgttcc agtcacggtc ac		22
SEQ ID NO: 89	moltype = DNA length = 48	
FEATURE	Location/Qualifiers	
misc_feature	1..48	
	note = Cyanothece	
source	1..48	
	mol_type = genomic DNA	
	organism = unidentified	
SEQUENCE: 89		
gagactttcc ccacacctt atgcatgcag atgttatggg aattaacg		48
SEQ ID NO: 90	moltype = DNA length = 23	
FEATURE	Location/Qualifiers	
misc_feature	1..23	
	note = Cyanothece	
source	1..23	
	mol_type = genomic DNA	
	organism = unidentified	
SEQUENCE: 90		
accttgacaa tcattacaca gcg		23
SEQ ID NO: 91	moltype = DNA length = 28	
FEATURE	Location/Qualifiers	
misc_feature	1..28	

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source          note = Cyanothece
               1..28
               mol_type = genomic DNA
               organism = unidentified
SEQUENCE: 91
caaatataat gatcgacatt ttcaccac                                28

SEQ ID NO: 92      moltype = DNA  length = 24
FEATURE          Location/Qualifiers
misc_feature     1..24
note = Cyanothece
source          1..24
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 92
cgtaacttt gtcgcaaaac ttctg                                24

SEQ ID NO: 93      moltype = DNA  length = 102
FEATURE          Location/Qualifiers
misc_feature     1..102
note = Cyanothece
source          1..102
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 93
accaaggcga atctccttcc tcgggtcgcg atcacgtac tcggccaata aaaaagcccc 60
cggatgtatc ttccgggggc cagattcagg taactgctca ag                  102

SEQ ID NO: 94      moltype = DNA  length = 23
FEATURE          Location/Qualifiers
source          1..23
mol_type = genomic DNA
organism = Azospirillum brasiliense
SEQUENCE: 94
tggtgttttc tcgggcatcg tca                                23

SEQ ID NO: 95      moltype = DNA  length = 23
FEATURE          Location/Qualifiers
source          1..23
mol_type = genomic DNA
organism = Azospirillum brasiliense
SEQUENCE: 95
agaaaattga ttgcggacga gcg                                23

SEQ ID NO: 96      moltype = DNA  length = 27
FEATURE          Location/Qualifiers
source          1..27
mol_type = genomic DNA
organism = Azospirillum brasiliense
SEQUENCE: 96
ttcaataagt taagcagatc ggccctcg                                27

SEQ ID NO: 97      moltype = DNA  length = 33
FEATURE          Location/Qualifiers
source          1..33
mol_type = genomic DNA
organism = Azospirillum brasiliense
SEQUENCE: 97
cggtgttacg aataaatatt tctacgaata gac                                33

SEQ ID NO: 98      moltype = DNA  length = 68
FEATURE          Location/Qualifiers
source          1..68
mol_type = genomic DNA
organism = Azospirillum brasiliense
SEQUENCE: 98
gctccaaaag gagccttaa ttgtatcggt ttatcgtt gttttttcc gcgggtctcg 60
ataacaacg                                              68

SEQ ID NO: 99      moltype = DNA  length = 22
FEATURE          Location/Qualifiers
source          1..22
mol_type = genomic DNA
organism = Rhodopseudomonas palustris
SEQUENCE: 99
ggtcttgccg atcatcaatt tc                                22

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SEQ ID NO: 100      moltype = DNA  length = 20
FEATURE
source
1..20
mol_type = genomic DNA
organism = Rhodopseudomonas palustris
SEQUENCE: 100
gacggtcagg tggtccgaac                                         20

SEQ ID NO: 101      moltype = DNA  length = 23
FEATURE
source
1..23
mol_type = genomic DNA
organism = Rhodopseudomonas palustris
SEQUENCE: 101
ggtgagaatg atcatgtatcg gcc                                         23

SEQ ID NO: 102      moltype = DNA  length = 67
FEATURE
source
1..67
mol_type = genomic DNA
organism = Rhodopseudomonas palustris
SEQUENCE: 102
ctcggaaaagg agccttaat tttatcggtt ttttagcttg ctttgacgac aagtggagaa 60
ggatag                                         67

SEQ ID NO: 103      moltype = DNA  length = 22
FEATURE
source
1..22
mol_type = genomic DNA
organism = Rhodobacter sphaeroides
SEQUENCE: 103
tccccatggtc atgtcctttg cg                                         22

SEQ ID NO: 104      moltype = DNA  length = 21
FEATURE
source
1..21
mol_type = genomic DNA
organism = Rhodobacter sphaeroides
SEQUENCE: 104
gtgcgcgtt ccacgaggagc c                                         21

SEQ ID NO: 105      moltype = DNA  length = 66
FEATURE
source
1..66
mol_type = genomic DNA
organism = Rhodobacter sphaeroides
SEQUENCE: 105
aattgaaaaa aaaaaccccg ccctgtcagg ggccggggttt tttttgcag cgcccatcc 60
gtcttc                                         66

SEQ ID NO: 106      moltype = DNA  length = 66
FEATURE
source
1..66
mol_type = genomic DNA
organism = Rhodobacter sphaeroides
SEQUENCE: 106
gctccaaag gaggccttaa ttgtatcggt ttatcagtt gttttggaga aagcctgcgc 60
ggtag                                         66

SEQ ID NO: 107      moltype = DNA  length = 65
FEATURE
source
1..65
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 107
gccccccggaa ggtgatctc cggggggctt ctcatgcgtt gacagcgtt agatagatca 60
agtgc                                         65

SEQ ID NO: 108      moltype = DNA  length = 20
FEATURE
source
1..20
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 108
ctgatccagg ctttcatcggt                                         20

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SEQ ID NO: 109      moltype = DNA  length = 23
FEATURE
source
1..23
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 109
gacatgtctg gtctccttgg aac                                23

SEQ ID NO: 110      moltype = DNA  length = 42
FEATURE
source
1..42
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 110
ttctggaatt tggtaaccgag tcagtaacgt gccacagcct cg          42

SEQ ID NO: 111      moltype = DNA  length = 117
FEATURE
source
1..117
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 111
atcaggcgca tatttgaatg tatttactgc agcggccgc acgtacttgtt ggggtcagtt 60
cccgctgggg gttcagcagc cacctgcagt taattaaggc gtcctttcc tgattcg 117

SEQ ID NO: 112      moltype = DNA  length = 20
FEATURE
source
1..20
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 112
gctgctgtgt ggagagatcg                                20

SEQ ID NO: 113      moltype = DNA  length = 22
FEATURE
source
1..22
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 113
gtcggtgaga ttgatcatgg cc                                22

SEQ ID NO: 114      moltype = DNA  length = 20
FEATURE
source
1..20
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 114
tgcatgtccg ttcctcgctg                                20

SEQ ID NO: 115      moltype = DNA  length = 24
FEATURE
source
1..24
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 115
acatgtcttg aattccttcg aacc                                24

SEQ ID NO: 116      moltype = DNA  length = 18
FEATURE
source
1..18
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 116
tgcattgcgt tcgctccc                                18

SEQ ID NO: 117      moltype = DNA  length = 19
FEATURE
source
1..19
mol_type = genomic DNA
organism = Azorhizobium caulinodans
SEQUENCE: 117
tgtcaggggca ggcaggggcc                                19

SEQ ID NO: 118      moltype = DNA  length = 41
FEATURE

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source          1..41
               mol_type = genomic DNA
               organism = Gluconacetobacter diazotrophicus
SEQUENCE: 118
tcaccagccg tatacgaaat atgtcaggat catgacatcc c           41

SEQ ID NO: 119      moltype = DNA  length = 20
FEATURE
source          1..20
               mol_type = genomic DNA
               organism = Gluconacetobacter diazotrophicus
SEQUENCE: 119
acgatttcca tgcccaggc           20

SEQ ID NO: 120      moltype = DNA  length = 20
FEATURE
source          1..20
               mol_type = genomic DNA
               organism = Gluconacetobacter diazotrophicus
SEQUENCE: 120
cctccagcac ctttcgatg           20

SEQ ID NO: 121      moltype = DNA  length = 67
FEATURE
source          1..67
               mol_type = genomic DNA
               organism = Gluconacetobacter diazotrophicus
SEQUENCE: 121
gctccaaagg gagccttaa ttgtatcggt ttatcagtt gctttggca atacctgaga 60
cgtttca           67

SEQ ID NO: 122      moltype = DNA  length = 70
FEATURE
misc_feature    1..70
               note = Synthetic polynucleotide
source          1..70
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 122
agagtgttga ctttgagcg gataacaatg atacttagat tcaattgtga gcggataaca 60
atttcacaca           70

SEQ ID NO: 123      moltype = DNA  length = 2652
FEATURE
misc_feature    1..2652
               note = Synthetic polynucleotide
source          1..2652
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 123
atgaacaccta ttaacatcgc taagaacgc ttctctgaca tcgaactggc tgctatccg 60
ttcaacactc tggctgacca ttacggtag cgtttagctc gcgaacagg ggcccttgag 120
catgagtctt acggatggg tgaacgcgc ttccgcaaga tggttggccg tcaacttaaa 180
gctggtaggg ttgcggataa cgctgcgc aagcctctca tcactacct actccctaag 240
atgattgcac gcatcaacag ctgggtttcgag gaagtgaaa ctaagcgggg caagcgcgg 300
acagecctcc agttcctgc aaaaaatcaag ccggaaaggccg tagcgtacat caccattaag 360
accactctgg cttgcctaac cagtgctgaa aataacaaccc ttccaggctgt agcaagcgc 420
atcggctggg ccattgagga cgaggctgc ttccggctgta tccgtgaccc tgaagctaa 480
cacttcaaga aaaaacgttgc ggaacaactc aacaagcgc tagggcacgt ctacaaggaa 540
gcatttatgc aagtgttgcg ggctgacatg ctctctaagg gtctacttgg tggcgaggcg 600
tggtcttcgtt ggcataaagg agactctt catgttaggg tacgctgcat cgagatgctc 660
attgagtcac ccggaaatgg tagtttacac cgccaaaatg ctggcgtagt aggtcaagac 720
tctgagacta tcaactcgca acctgtaaatc gctgaggctc tccgcaacccg tgcaggcgc 780
ctggctggca tctctccgtat gttccaaacct tgctgttgc ctccataaggc gtggacttgc 840
attactgttgc tggtgttattt ggcttacacggt cgtcgcttgc tggcgctgtt gctgtactc 900
agtaagaaatg cactgtgcg ctacgaaac gtttacatgc ctggaggctt caaaggcgatt 960
aacattgcgc aaaacaccgc atggaaaatc aacaagaaatg tcccttagggt cgccaaacgt 1020
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ccgatgaac ccggaaagacat cgacatgtat cctggggctc tcacccgcgt gaaacgtgt 1140
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gactggcgcc gtcgtgttgc cgtgtgtca atgttcaacc cgcaaggtaa cgatgtgacc 1320
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aaaatccacg gtgcacactg tgccgggtgtc gacaagggtt cgttccctga ggcgtatcgc 1440
ttcatttgagg aaaaccacga gaacatcatg gcttgcgtca agtctccact ggagaacact 1500
tggtggctgtt gcaagatcc tccgttgc ttccttgcgt tctgttttgc gtcacgtgg 1560

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gtacagcacc	acggcctgag	ctataactgc	tcccttcgc	tgccgttga	cgggtcttc	1620
tctggcatcc	agacttctc	cgcgatgctc	cgagatgagg	taggtgtcgc	cgccgttaac	1680
ttgttctca	tgaaaacctgt	tcaggacatc	tacgggattt	ttgtctaa	aggtaacagg	1740
atttacaag	cagacgcaat	caatgggacc	gataacaa	tagttacccgt	gaccgatgag	1800
aacactgggt	aatatctctga	gaaagtcaag	ctgggcaacta	aggcactggc	tggtaatgg	1860
ctggcttacg	gtgttactcg	cagtgtgact	aaggcggtcg	tcatgacgt	ggcttacggg	1920
tccaaagagt	tcgggttccg	tcgaaactgt	ctggaagata	ccatccagcc	agctattgtat	1980
tccggcaagg	gtctgtatgtt	cactcagccg	aatcaggctgt	ctggatacat	ggctaactgt	2040
atttggaaat	ctgtgagcgt	gacgggtggta	gctgcgggtt	aagcaatgaa	ctggcttaag	2100
tctgtctcta	actgtctggc	tgctgaggatc	aaagataa	agactggaga	gattttcgc	2160
aagcgttgcg	tggtgtcg	ggtacttct	gatggtttcc	tggtgtggc	ggaataacaag	2220
aaggcttattc	agacgcgtt	gaaactgtat	ttctctggtc	agttccgc	acagcctacc	2280
attaacacca	acaaagatag	cgagattgtat	gcacacaac	aggagtctgg	tatcgctct	2340
aactttgtac	acagcacaaga	cggtagccac	cttcgtaa	ctgtgtgt	ggcacacgag	2400
aagtacggaa	tcgaatcttt	tgcactgatt	cacgacttct	teggtaatgt	tccggctgac	2460
gtctgcgaaacc	tgttcaa	agtgccgaa	actatgggtt	acatcatatg	gtcttgcgtat	2520
gtactggctg	atttctacga	ccagttcgct	gaccgttgc	acgagtctca	attggacaaa	2580
atgccagcac	ttccggctaa	aggtaacttg	aacctccgt	acatctttaga	gtcggacttc	2640
gcgttgcgt	aa					2652

SEQ ID NO: 124 moltype = DNA length = 51
 FEATURE Location/Qualifiers
 source 1..51
 mol_type = genomic DNA
 organism = Azorhizobium caulinodans

SEQUENCE: 124
 cgaatggtgc aaaacctttc gcggtatggc atgatagcgc ccggaaagaga g 51

SEQ ID NO: 125 moltype = DNA length = 1545
 FEATURE Location/Qualifiers
 misc_feature 1..1545
 note = Synthetic polynucleotide
 source 1..1545
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 125
 atggcgatga gccaaagat ggagttccgc cagagccagt ctctggatg gacggccag 60
 ctgatgcagg ccatcaagct gctcgagtc tccaaatctcg aactggtcgc ctatgtggag 120
 gcccggatcc aacgcaatcc gctgtggag cggcgagacg agccggaaag ccccgagcac 180
 gatccggccgaa agggccggcc accccgccgt acatggcgcg gccgggtgtcc 240
 ggcgactgtgaa tgaaaaggca catggctcg agccggcagg ccatcgagac ccggctggac 300
 accgacccgtc gcaatgtctt tcccgatgat ggcggggccg agccgtatcg gccggggcagc 360
 ggccggccgtc cgccatcgat atggggctcg ggcggccgagg gggggccggaa ctacaatccg 420
 gaageccttcc tcgctggcga gacgacgtcg gccgaccatc tggaaggccca getctccgt 480
 gccggccgcg atccggccgcg ccgccttcac ggccctaaac tcatecgcc catcgacgag 540
 acgggttatt ttcggccgca cctcgatcg gttggccgagg aactggccgc caccacgat 600
 caagggtggccg acgtgtcgcc cgctcgatcc agcttcgacg cgtccggcgt cgccgcacgg 660
 tcgctcgtcg atgcgttgcg cctcgatcc agccgtccatcg tgccgtccatg 720
 caggcgctgcg tcgacaatct ggaactccgc gcccggccacg accgcacgcg gctgaacgc 780
 atctcgccggg tggacgcccc agacctcgccg gacatgtatcg gcgagatccg ccgcctcgat 840
 cccaaagccgcg gctctggcata tgccggccgc gtcgtccacc ccgtgttgcg ggaactgtt 900
 gtgcgcgagg gtcggcaccg cagctggatc tgccggacta atcccgagac gtcgcgcgc 960
 gtgtgttgcg aggacacatc tcacggcgcg tgccggccacg cggccgcgc ggcggaggaa 1020
 aagaccttc tcggccactg cctccagago gcttcctggc ttacccgcgc gctcgaccag 1080
 cgggctcgca ccatactcaa ggtggccgcg gagatcgccg gccagccaga cgccttcctc 1140
 gtgcacccgcg tgccggccatc ggcggccctgt aacctcgccca cggccggccgc tgccatcgcc 1200
 atgcacaaat ccacccgttc gccgggtacc tcgaaactgt acatctccac cccgcgggg 1260
 gtgtgttgcg tgaaggatcc ttctcttcac tcacatcgatcc cctcggttgc tgccggaggcc 1320
 catgcggccgg aggccgtcgcc ccacccgcata aagacccgtca tcgaggccgc gactgtccgc 1380
 gacgtgtcgat cccggccacac gtcgtgtccg acgtgtccgcg acgacccgcgat cgtatcgcc 1440
 cccggccggcc tcgcaataa tcgccggccgcg atgaaatcc cgtccctcggt ccacgcgcgc 1500
 cccggaaaaggc aggccctcgcc cagcgacgc gccggccggcc gctgca 1545

SEQ ID NO: 126 moltype = DNA length = 1083
 FEATURE Location/Qualifiers
 misc_feature 1..1083
 note = Synthetic polynucleotide
 source 1..1083
 mol_type = other DNA
 organism = synthetic construct

SEQUENCE: 126
 gtgaaaccatg taacgttata cgatgtcgca gagtatggccg gtgtcttcttca ctagaccgtt 60
 tcccgcttgg tgaaccaggc cagccacgtt tctcgaaaaa cgcggggaaaaa agtggaaagcg 120
 gcgatggccgg agctgaatca cattccaaac cgcgtggccac aacaactggc gggcaaacag 180
 tcgttgtatcgatggccgttgc cacctccatcg tggccctgc acgcggccgtc gcaaattgtc 240
 cccggccgatataatctcgcc cgtatcaactg ggtggcccg gttgtgtgtc gatggtagaa 300

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cgaaagcggcg tcgaagcctg taaagcggcg gtgcacaatc ttctcgccca acgcgtcagt 360
gggctgatcca ttaactatcc gctggatgac caggatgcca ttgtctgga agctgcctgc 420
actaatgttc cggcggttatt ttttgcgtc tctgaccaga caccatcaa cagtattatt 480
tttcccattt aagacggtagc gcgactgggc gtggagcatc tggtcgatt gggcacccag 540
caaatacgcc tggtagcggg cccattaagt tctgtctcg cgctgtcgcc tctggctggc 600
tggcataaat atctcaactcg caatcaaatt cagccgatag cggaaacggga aggccactgg 660
atgtccatgt cgggtttca acaaaccatg caaatgtcta atgaggcat ctgtttccact 720
gegatgtgg ttgccaacgt tcagatggcg ctggcgca tgcgcocat taccgagtc 780
gggctgecg ttgggtcgga tatctcggtt gtgggatagc acgataccga agacagctca 840
tgttatatcc cggccgttaac caccatcaa caggattttc gctgtctggg gcaaaccagg 900
gtggaccgc tggcgtcaact ctctcaaggcg cggccgttga agggcaatca gctgttggcc 960
gttctactgg tggaaaagaaa aaccacctgg ggcggccaaatc cgccaaaccgc ctctccccgc 1020
gcgttgccg attcatatcc gcagctggca cgacagggtt cccgactggaa aagcgggcag 1080
tga 1083
SEQ_ID NO: 127 moltype = DNA length = 717
FEATURE Location/Qualifiers
misc_feature 1..717
note = Synthetic polynucleotide
source 1..717
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 127
atgagtaaag gagaagaact ttcaactgga gttgtcccaa ttcttgttga attagatgg 60
gatgttaatg ggcacaattt ttctgttagt ggagagggtg aagggtatgc aacatacgg 120
aaacttaccc ttaattttt ttgcactact gaaaaactac ctgttccatg gccaacact 180
gtcaactactt tgggtttatgg ttttgcgttgc ttggcgatg accccagatca tatgaaacag 240
catgactttt tcaagagtgc catgcccggaa ggttatgtac agggaaagaaat tatattttc 300
aaagatgacg ggaactacaa gacacgtgtc gaagtcgttga ttgaagggttga tacccttgg 360
aatagaatcg agttaaaagg tattgattt aaagaagatg gaaacattct tggacacaaa 420
ttggaataaca actataactc acacatgtt tacatcatgg gagacaaaca aaagaatgg 480
atcaaaggta acttcaaaaat tagacacaaatc attgaagatg gaagcggttca actacggcag 540
cattatcaac aaaactactcc aatttgcgttgc ggcctgttcc ttttaccaga caaccattac 600
ctgtccacac aatctgcctt ttcaaaatg cccaaacgaa agagagacca catggcctt 660
tttgagtttgc taacagctgc tgggattaca catggcatgg atgaactata caaatag 717
SEQ_ID NO: 128 moltype = DNA length = 717
FEATURE Location/Qualifiers
misc_feature 1..717
note = Synthetic polynucleotide
source 1..717
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 128
atcgtaaag gogaagagct ttcaactggt gtctgtccctt ttctgggttga actggatgg 60
gatgtcaacg gtcataatgtt ttccgtgcgtt ggcgagggtg aagggtacgc aactaatgg 120
aaactgtacgc tgaagttcat ctgtactact ggttaactctgc cggtacccctg gccgactctg 180
gttaacgacgc tgactttatgg ttgtcaatgtt ttggctgttgc atccggacca tatgaaacag 240
catgactttt tcaagtcgc catgcccggaa ggctatgtgc aggaacggcac gattttttt 300
aggatgacg gcacgtacaa aacgcgtgtc gaagtgaaaat ttgaagggttga tacccttgg 360
aaccgcattt acgttggaaagg ctttgactttt aaagaagatc gcaatattctt gggccataa 420
ctgttataaca atttttacacg ccacatgtt tacatcaccc cgcataaaca aaaaaatggc 480
attaaaggcga attttttaaaatc tggccacaaatc gtggaggatg gcaacgttca gctggctgtt 540
cactaccgc aaaacactcc aatcggtat ggtccctgttgc tgctggccaga caatcactat 600
ctggacacgc aagecgcttgc gtctaaatg cccaaacgaga aacgcgtatca tatggttctg 660
ctggagtttgc taacccgcgc gggcatcaccg catggatggt atgaactgtt caaatag 717
SEQ_ID NO: 129 moltype = DNA length = 678
FEATURE Location/Qualifiers
misc_feature 1..678
note = Synthetic polynucleotide
source 1..678
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 129
atggcttcctt ccgaagacgt tatcaaagat ttcatgcgtt tcaaagttcg tatggaaagg 60
tcggtaacg gtcacgatgtt cggaaatcgaa ggttgggttgc aagggtcgcc gtacgaaatgtt 120
acccagacgc tcaaactgaa agttacccaaat ggtggtccgc tgccgttcgc ttgggacatc 180
ctgtccccgc agttccatgtt cgggtccatgtt aacacccggc tgacatcccg 240
gactacctgtt aactgtccctt cccggaaagggtt ttcacatgggg aacgtgttat gaaacttcgaa 300
gacgggtgtt ttgttaccgt taccatggac tccctccatgc aagacgttgc gttcatctac 360
aaagtttacac tgcgtgttgc caacttcccg tccgacgggtt cgggttatgca gaaaaaaacc 420
atgggttggg aagcttccac cgaacgtatg taccatggaa acgggtgttgc gaaagggttgc 480
atcaaaaatgc gtctgaaact gaaagacgggtt ggttactacg acgttgcgtt gaaaccacc 540
tacatggcttca aaaaaccgggtt tcacatggcc ggttgcgttaca aaaccgacat caaatgttgc 600
atcacccccc acaacgaaatc ctacaccatc gtttgcgttgc tgaagggttgc 660

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cactccaccg gtgcttaa	678
SEQ ID NO: 130	moltype = DNA length = 711
FEATURE	Location/Qualifiers
misc_feature	1..711
	note = Synthetic polynucleotide
source	1..711
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 130	
atggtttgcg aggccgagga ggataacatg gccatcatca aggagttcat gcgttcaag 60	
gtgcacatgg agggctccgt gaacggccac gagttcgaga tcgaggccga gggcgagggc 120	
cgccttacg agggcaccca gaccccaag ctgaagggtc ccaagggtgg cccctgccc 180	
ttcgccctgg acatccgtc ccctcagtgc atgtacggct ccaaggctca cgtaaagcac 240	
cccgccgaca tccccgacta ctgtaaagctc tccttccccg agggttcaa gtgggagcgc 300	
gtgatgaaact tccgaggacgg cgccgttggt accgtgaccg aggactcctc cttgcaggac 360	
ggcgaggtca tctacaagggt gaagctgcgc ggcaccaact tccctccga cggcccccgt 420	
atgcagaaga agacgatggg ctgggaggcc tcctccgagc ggatgtaccc cgaggacggc 480	
gcccgttggg gcgagatggc gcagaggtc aagctgaagg acggccgcctt ctacgacgct 540	
gagggtcaagg ccacccatcaa gccaagaag cccgtgcgc tgccggccgc ctacaacgtc 600	
aacatcaagt tggacatcac ctcccacaac gaggactaca ccatgtgga acagtacgaa 660	
cgccgcgagg gccgccactc caccggccgc atggacgacg tgtacaagta a 711	
SEQ ID NO: 131	moltype = DNA length = 23
FEATURE	Location/Qualifiers
misc_feature	1..23
	note = Synthetic polynucleotide
source	1..23
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 131	
taatacgact cactataggg aga	23
SEQ ID NO: 132	moltype = DNA length = 23
FEATURE	Location/Qualifiers
misc_feature	1..23
	note = Synthetic polynucleotide
source	1..23
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 132	
taatacgact cactacaggc aga	23
SEQ ID NO: 133	moltype = DNA length = 23
FEATURE	Location/Qualifiers
misc_feature	1..23
	note = Synthetic polynucleotide
source	1..23
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 133	
taatacgact cactagagag aga	23
SEQ ID NO: 134	moltype = DNA length = 23
FEATURE	Location/Qualifiers
misc_feature	1..23
	note = Synthetic polynucleotide
source	1..23
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 134	
taatacgact cactaatggg aga	23
SEQ ID NO: 135	moltype = DNA length = 23
FEATURE	Location/Qualifiers
misc_feature	1..23
	note = Synthetic polynucleotide
source	1..23
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 135	
taatacgact cactaaaggc aga	23
SEQ ID NO: 136	moltype = DNA length = 23
FEATURE	Location/Qualifiers
misc_feature	1..23

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source          note = Synthetic polynucleotide
                1..23
                mol_type = other DNA
                organism = synthetic construct

SEQUENCE: 136
taatacact cactataagg aga                                23

SEQ ID NO: 137      moltype = DNA  length = 23
FEATURE          Location/Qualifiers
source           1..23
                mol_type = genomic DNA
                organism = Azorhizobium caulinodans

SEQUENCE: 137
taatacact cactattggg aga                                23

SEQ ID NO: 138      moltype = DNA  length = 1221
FEATURE          Location/Qualifiers
source           1..1221
                mol_type = genomic DNA
                organism = Klebsiella oxytoca

SEQUENCE: 138
gtgttcctgc ggaggccctgc catgtcgcc aagacacccgc caaaccccgcc gcccgttcag 60
cggacggcgt tcctgaacgt caccacgtgc cggacggcgtc agcaggccgc gggtgtcgcc 120
ttcacccgcga aggagaagat ccggatcgcc gcccgccttg cggccgcgg tgcccgag 180
atcgaggccg gaacgcgcgc catggcgac gaagagggtgg aaaccatccg ctccatcgtc 240
tcgctgaacc tccccacgcgc cgtcatggcc tggtgccgcga tgagcgaggaa cgacccgtatg 300
ggccgcgtcg cggccggcgat gaagatcgtd aatgtctcca ttcccaacctc cgacccggcaa 360
ctggccggca agctcgccaa ggatcgccgc tggccgtcgcc ggggtgtggc ggagggtgtg 420
acactcgccg gtccgtcgcc ttgtgagggtg gccgttagggg gcgaggattc ctgcggggcc 480
gatcccgatt ttctctgcgc tctcgccgg acggcgaagg cggccggccgc ctttcgctg 540
cggtcgatgg acacgcgtgg cgtgttgcgc cccatgcatt ggtgcgcgg 600
gtggccgcga ccacccgatc cgagatgtgg tttccacgcgc atgacgatct cggccgttgcc 660
acccgcaata cgctggccgc ggtgtatggc ggagcgccgt acggccagcgt caccgtcgcc 720
gggctcgccg agcgcgcggg caatgccgcg ctggaggaaatggccatcgc cctgcgcac 780
acggcgcggg cggagacccgc categctcgcc gccgcgtcgca agccgtcgcc cgaactagt 840
tgccgcgcgcg ccgcggccgc ggtgcgcgcg ggcaggccca tgcgtccgcg ggtatgtttc 900
acccacaggt cgggcattcca tgcgtccgcg ctgtcaagg accggggcacatctgaagat 960
ctgaatccgg aactgttcgg gctgtggccac acgggtggtc tcggaaagca ttccggtctt 1020
ggccgcgtgg agaaggccgt ggcgcacgag ggcacatccac tggatgcgtt ggcgcggccg 1080
gccattctcg acccgggtcgcc ggcattttgcgttgcgcacca aggagaatgt ttcccgccgag 1140
acgctgtcgc gtttataca ggcacgttc accgagtcgg cgtctgcgtt gggccggcc 1200
ggcggtggaaag ggcacatctg a                                1221

SEQ ID NO: 139      moltype = DNA  length = 323
FEATURE          Location/Qualifiers
source           1..323
                mol_type = genomic DNA
                organism = Pseudomonas stutzeri

SEQUENCE: 139
tgttgcctca agcacagcct gtgcacagtc gcggatgaca gaagagtttg cgcgaattca 60
acccgttatg aagagatgc cccgcgcgcg cgccaaaggaa ttgcgttgaa taagacacag 120
ggggcgacaa gctgttgaac aggccacaaa ggcgcacccat ggcccgccca ggcgaatttgc 180
tttcgtttcc cacatgttgc cgccttatttgcgttttgcgttcc tgcgcggccg 240
caaataacta acttcataaaa aatcataaga atacataaac aggcacggctt ggtatgttcc 300
ctgcacttct ctgtggccaa aca                                323

SEQ ID NO: 140      moltype = DNA  length = 233
FEATURE          Location/Qualifiers
source           1..233
                mol_type = genomic DNA
                organism = Azotobacter vinelandii

SEQUENCE: 140
tgtcatgttc gcaacagttt ccggaaagggtt ggaaaacccgg cgcttggccc ggccgatctt 60
tttgtcgccaa ttgcacacgt caggcgttc ggttggtaatc tgcgttgcg ccggatggatg 120
ttgtctagtaa ttaattttt ctaattaaaaa caatgtcttta gatttttta gaaacgttgg 180
caaaaagggtt gctatggcc tggccgcgc gcttgcgttgcgttcc cac 233

SEQ ID NO: 141      moltype = DNA  length = 256
FEATURE          Location/Qualifiers
misc_feature    1..256
                note = R. sp. IRBG74
source           1..256
                mol_type = genomic DNA
                organism = unidentified

SEQUENCE: 141
tgtcagttt gtcacagggg gccggaccag gatggtggac gtcgtatggg gatgtcgcc 60

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SEQ ID NO: 142	moltype = DNA length = 294
FEATURE	Location/Qualifiers
source	1..294
	mol_type = genomic DNA
	organism = Azorhizobium caulinodans
SEQUENCE: 142	
ttgacaaaacg ctcggagaag	agcgccttcc aacccctcct cagccctgat cggcagtatc 60
atcttgtca atcctaacgt	ctgataggca acgtatacg acaaacgtg gttacaattg 120
tcgggtccgc gacaagaatt	tgcgttgcgtt ggccgggtgt ctattttag ctaagttagt 180
gagaaatcatg gaaaaacaaaa	ctctatccgg tctaccggc gagttggcac gggctttgt 240
accatccctg cgcaggccgc	gaaagccacc ggcgatattc atgtcgccc caac 294
SEQ ID NO: 143	moltype = DNA length = 215
FEATURE	Location/Qualifiers
source	1..215
	mol_type = genomic DNA
	organism = Klebsiella oxytoca
SEQUENCE: 143	
tgtcgcggtt gaaacacggg	gctttggaa ccgttcgatt ctgcaatgca ctgattttac 60
ttgattaatt cgaccacacg	accactggca caccctgtgc aaaacccctt ggtgcaggcg 120
acgggttgcg ggtctgggtt	gccccatctcc tcgatccccc gctaccgacc cgcctccgaa 180
aagtccggc cogatccagt	tcgggggggg cacac 215
SEQ ID NO: 144	moltype = DNA length = 1575
FEATURE	Location/Qualifiers
source	1..1575
	mol_type = genomic DNA
	organism = Pseudomonas stutzeri
SEQUENCE: 144	
atgatccata aatccgattc	ggacaccacc gtcaagacgt tcgatcttc ccagcagtt 60
accggccatgc agcggataag	cgtggctctg agtgcgcgcc cggaaacggg caaaacctg 120
caaggagggtc tgagcgtgtc	acataacat gccttatgc agcacggat gatttgctg 180
tacgacagcc agcaggagat	cctgagcata gaagcgtgc acggaaacggg agatcagacg 240
ctggccggca gtacgcaaat	tgcgttcccg ccggggggaa gattagtcgg taccgtgtg 300
gcccggggcc agtcgtgggt	gtgcgcgcgc accagcggtt ttcgcgtcgt 360
ctgagcgtgt acgactatga	cctgcgtt atgcgcgtt cgctgtatggg ccccccactc 420
cgcccccattcg	cggtactggc ggcgcagccg atggcgcgtc aggaagagcg gtcggccgc 480
tgcacggcgt ttctcgaaat	cgtggccaaat ctgatcgccc agacgattcg cctgtatgatc 540
ctgccaacat cgcggccgca	ccggccggccgca gagagccccca gaatagagcc cccggccgc 600
tgtacccctt cgcggcggtt	cggctggaa aatattgtcg gtaaaaagccc ggcgtatgcgg 660
cagattatgg atattattcg	tcaggttcc cgctggata ccacgggtgtt ggtacggcc 720
gagagcggca cccggaaaga	gctcatcgcc aacggccatcc accataatc tccggccgc 780
gcccggggcgt tgcgtcaatt	taactggcgcg cgcgtccggg acaacctgtt ggagagccgag 840
ctgtttggtc atgagaagg	cgcgtttacc ggccgggtgc ggcageggaa aggccgcattt 900
gagctggccg acggccgac	cttattctc gatgagatcg gcgaaacgg cgcctcggtt 960
caggcttaacg tactcgat	tctgtcaaggg ggggagatgg agcgcgtccg cggccgcggaa 1020
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cggctgggtt attcccgca	ggatcttac taccgcgtt acgtatgcc tatecgctg 1140
ccggccgtgc	cgagccgcca ggaggatata gccgagctgg cgcacttctt ggtgcggaaaa 1200
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gagtcacgtt	ggccggggaa cgtgcgcgcgat cggaaacact gtctcgaaacg ttcggcggtg 1320
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ccgaaagcgc	tcggccagccg ccggccggccg gaggacggcgt ggctcgataa cagccgtcgc 1440
gacgcggcgc	ggctgtatcgc cgccctggaa aaacggggcgt gggtgccggc caaagccgg 1500
cgccgtgtcg	gcatgacccc ggcgcagggt gctatcgca ttcaagattat ggatatcacc 1560
atgcgcgcac	tgtga 1575
SEQ ID NO: 145	moltype = DNA length = 1566
FEATURE	Location/Qualifiers
source	1..1566
	mol_type = genomic DNA
	organism = Azotobacter vinelandii
SEQUENCE: 145	
atgaacgcaca cattcgccga	acgccccaga ggcggccaaacc gcaacgaaact gctggatgcc 60
caactcgagg cgctggcgca	atccttaacc gtcggccgcg catcgaggaa 120
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atcctcaacg atggcaacag	cgtgggtgcgc gggcgatccg acggccaaacc ggcgtttctc 360
gaccgactgg cgctgtacga	catggacccgt cccttcatcg cctgtgcgcgat caaggccgtc 420
gacggccacca	ccatcgccgt gctggatgcc cagcccgacc gccggccgcgatcgatcgt 480

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cccgaaacgca	cccggttgat	ggaaatcgtc	gcccgcctac	tggcgcagac	cgtgcgcctg	540
gttgtgtgaacc	ttcgaggacgg	ccaggaagtg	gtcgacgacg	gcgacgact	acgcgcgcaa	600
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cagccgcgtgg	taacgttgaa	ctggccgcgg	ctaccggaaa	ccctgtctca	atcggaactg	840
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caaggcagaat	tccggaaaga	cctctaact	cgcttcac	tatggccat	ccgcgtcccg	1140
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gcccgcacgc	agggtcgca	actcaagctg	accgacagcg	ccctgtgtct	gctgtatgagc	1260
caccgcgtgc	cgggcaacgt	gcccgtact	gaaaaactgc	tggAACGCTC	ggccatcatcg	1320
agegaggatcg	tcacatcg	ccgcgtact	ccgcgtact	ccgcgtact	ccacgcacgc	1380
acgcgcgtgg	cgccgtcc	cgaaatcgac	ctcgccgacg	acagcttcga	cgaccgcgag	1440
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atctga						1566

SEQ ID NO: 146	moltype = DNA	length = 1569				
FEATURE	Location/Qualifiers					
misc_feature	1..1569					
	note = R. sp. IRBG74					
source	1..1569					
	mol_type = genomic DNA					
	organism = unidentified					
SEQUENCE: 146						
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caattgcagg	ccctggcgag	catgcggccg	acgctcggcc	gcgaacaaca	gatcgacgaa	120
ctgctcgaac	agggtcgcc	cgtactcga	aatgacccctg	gcctgtctca	tggctgtgt	180
accatttcgg	accggaaaca	cgggccctg	catagatcgcc	ccatccacac	cgactcgaa	240
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gtgctcaagc	acggcaacag	cgtgtgtc	ggggcgtatc	ccggcgcaccc	gcccgttctc	360
gaccgcgtcc	cgctgtacga	cctggaaatg	ccgttcatcg	ccgtgcggat	caagaacccc	420
gagggcacaca	ccatcggt	gctggggcc	cagccggact	gccgcgcga	cgagcacatg	480
cccgccgcga	ccgcgttct	ggagatcgac	gccaacctgc	tggcgacagac	cgtgcgtctg	540
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gtcgccgcga	agatcggtt	cgagacatcg	gtggtggggc	acacccccc	catgcgcgg	660
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aaggcgaaat	tccggggagg	cctctactac	cgatcgac	tatggccat	ccgcattccg	1140
ccgcgtcgcc	acgcgtcgcc	cgacatcccc	gaacttggccg	aattctctgt	cggaagatc	1200
ggccgcgcac	aggggccccc	gctyacgtc	acggacacgc	ccatccgcct	gctgtatgagc	1260
caccgcgtgc	ggggcaacgt	gcccgtact	gagaactgc	tggagcgctc	ggcgtatcg	1320
agcgaggacg	gcacccatcac	ccgcgtact	gtctcgatcg	ccgggtcgca	caacgagacg	1380
ccgcgcgtcg	ccgcgcgtcg	gcccgtact	aaactggccg	acgagaccc	ggacgcacgc	1440
gaacccggatcg	tccggccct	cgacacggcc	gggtgggtgc	aggccaaaggc	ccgcgtgt	1500
ctggggatcg	ccgcgcggca	gatcgctac	cgcatccaga	ccctcaacat	ccacatgcgc	1560
aagatctga						1569

SEQ ID NO: 147	moltype = DNA	length = 1695				
FEATURE	Location/Qualifiers					
source	1..1695					
	mol_type = genomic DNA					
	organism = Azorhizobium caulinodans					
SEQUENCE: 147						
atgctgcaca	atgggctaa	tgagggtatg	actgaacgt	ccgctaaac	catccacaaa	60
ccggatttc	ggggcagccg	tatctatcg	atatcgaaag	ttttgttgg	tccacacgt	120
ctcgagacga	agtttgcata	tgttcatca	gcctctcc	taatttctcc	aatgcggcgc	180
ggcgcaatcg	tctgttctaa	tgtttaagg	gagccccaga	tgggttcaat	gctgggccta	240
gagcaagcat	ctcaaggcgc	ccgctccatt	ccggcggagg	ctgcgtatga	tagaatcg	300
gccaaaggcg	ccgcgtgtt	cgttccggac	atttgcgt	cggacgtt	ccaggcggag	360
cttcaccaacca	actcgacgc	cacaggccca	gccacgttcc	ttggcgcc	gatgaaggc	420
aaaaaaaat	cgcttggaa	actatggatc	gaccgcgc	aaatggcg	cactaggatc	480
caatttgcgg	aaagggtcg	cttctctcc	atgtcgcca	acctttcgcc	ccgggcatt	540
tggctggatc	gccaccagag	ccgcgtatgt	cagccaaatcg	tggcgagga	aggaactcg	600
aagacttagt	caggcgacaa	ggaactgccc	aatctgccc	gacaaaggcc	cacaaaatc	660
gattggatc	tcggggaaaag	ccctgcctc	aaacgggtgg	ttgaaagcgt	caaagtctt	720
gcaacaacca	attctgcgt	gttctcagg	ggcgaaaggc	gcacggccaa	ggatgtctt	780
gaaaaggcc	tccacgagct	ttcataccgg	aaaagaagac	ccttcgtgaa	tttgaactgc	840

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gcgcgcgtgt	ctgcaggcgt	tttggaatcg	gaattgtttg	gacatgaaaaa	gggcgccttc	900
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ctcgatgaga	tccggcgcacat	tccgggggc	ttccaagcga	aactgttgcg	cgttgcag	1020
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tattaccgga	tcatatgtggt	gcgccttattt	ctggcgccct	tccgggagcg	aaatggggat	1200
attccacgcc	tttcgcgagat	tttcctcgcc	cgattcaaca	gggaaaacaa	tcgcgatctc	1260
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gagcttggaa	actcgctcg	caggaccgc	actctcgccg	gttggagac	gatcgttcca	1380
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cgtccacttg	gggatcatgt	caatgggtt	gcatacgatg	agatgttgc	ggtgcatacg	1500
cgcatacgcc	tcgggtactc	caatggaccg	gccggctta	cggtggacc	acatctaag	1560
gaccgcgagc	tgctaatcag	tgcgatggag	aaggccggtt	gggttcaggc	aaaggcagct	1620
cgatctcg	gcctcacacc	gacacaggtc	ggctatgtt	tacgttaggc	tcgtatacag	1680
gtgaagaaaa	tctaa					1695

SEQ ID NO: 148	moltype = DNA length = 1848					
FEATURE	Location/Qualifiers					
misc_feature	1..1848					
	note = Synthetic polynucleotide					
source	1..1848					
	mol_type = other DNA					
	organism = synthetic construct					
SEQUENCE: 148						
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gacatcgcgc	cgtcatccat	caccacgcgg	ggcgcgcgtgc	cgccgcgggg	agggatgcct	120
gtgtccatgt	cgcgggggac	ctcgcccgac	gttgcactca	tccgggtcta	tgagatate	180
aagatccatg	cgccgcggcc	gcgcctcgaa	gtcacgcgtc	ccaaatgtgt	gaacgtgc	240
tccctccatgc	tgcagatgcg	gtatggcgt	atctgcattc	tccagacgcg	gggcgatccc	300
gacatgttgtt	ccaccacccg	ctggacgcct	gagatggcg	gccagatccg	cgccgtatgt	360
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accccccgtt	tccaggccaa	gctgtgcgg	gtgtgcagg	aggcgcgtt	cgaggggtc	1140
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gacgtgtgtc	ggcgctgtca	tcccccggg	aacgtgcgcg	agttggagaa	ctgtatccg	1440
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ggccagtcgc	tctcgccat	gtcttggaa	gtctcgcc	cgaaacctgt	gatggcgac	1560
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ccccgtcgcc	cgacggccggc	gcccggcc	gacacgcgtc	cggtactcg	ccccggccacc	1680
gaggccgtgc	ccgggtgtcc	ccccggccag	agcgaaaagg	agcgtgttgc	ccaggccatc	1740
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gtgggttatg	cgctgcgca	atatgacatc	gacatcaac	gtttctgc		1848

SEQ ID NO: 149	moltype = DNA length = 47				
FEATURE	Location/Qualifiers				
misc_feature	1..47				
	note = Synthetic polynucleotide				
source	1..47				
	mol_type = other DNA				
	organism = synthetic construct				
SEQUENCE: 149					
taggtgttga	cggtctagtc	agtccctagg	acagtgtcg	ctctaga	47

SEQ ID NO: 150	moltype = DNA length = 47				
FEATURE	Location/Qualifiers				
misc_feature	1..47				
	note = Synthetic polynucleotide				
source	1..47				
	mol_type = other DNA				
	organism = synthetic construct				
SEQUENCE: 150					
taggtgttta	cagcttagtc	agtccctagg	attatgtcg	ctctaga	47

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SEQ ID NO: 151      moltype = DNA length = 47
FEATURE           Location/Qualifiers
misc_feature      1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 151
taggtgttga cagctagctc agtcctaggt actgtgctag ctctaga        47

SEQ ID NO: 152      moltype = DNA length = 47
FEATURE           Location/Qualifiers
misc_feature      1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 152
taggtgctga tagctagctc agtcctaggg attatgctag ctctaga        47

SEQ ID NO: 153      moltype = DNA length = 47
FEATURE           Location/Qualifiers
misc_feature      1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 153
taggtgttga cagctagctc agtcctaggt atttgtgctag ctctaga        47

SEQ ID NO: 154      moltype = DNA length = 47
FEATURE           Location/Qualifiers
misc_feature      1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 154
taggtgttta cggctagctc agtcctaggt actatgctag ctctaga        47

SEQ ID NO: 155      moltype = DNA length = 47
FEATURE           Location/Qualifiers
misc_feature      1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 155
taggtgttta cggctagctc agtcctaggt atatgtgctag ctctaga        47

SEQ ID NO: 156      moltype = DNA length = 47
FEATURE           Location/Qualifiers
misc_feature      1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 156
taggtgttta cggctagctc agccctaggt attatgctag ctctaga        47

SEQ ID NO: 157      moltype = DNA length = 47
FEATURE           Location/Qualifiers
misc_feature      1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 157
taggtgtga cagctagctc agtcctaggt ataatgctag ctctaga        47

SEQ ID NO: 158      moltype = DNA length = 47
FEATURE           Location/Qualifiers
misc_feature      1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA

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SEQUENCE: 158      organism = synthetic construct
taggtgttta cagctagctc agtcctaggg actgtgctag ctctaga          47

SEQ ID NO: 159      moltype = DNA  length = 47
FEATURE           Location/Qualifiers
misc_feature       1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 159      organism = synthetic construct
taggtgttta cggctagctc agtcctaggt acaatgctag ctctaga          47

SEQ ID NO: 160      moltype = DNA  length = 47
FEATURE           Location/Qualifiers
misc_feature       1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 160      organism = synthetic construct
taggtgttga cggctagctc agtcctaggt atatgtctag ctctaga          47

SEQ ID NO: 161      moltype = DNA  length = 47
FEATURE           Location/Qualifiers
misc_feature       1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 161      organism = synthetic construct
taggtgtgta tagctagctc agtcctaggg attatgtctag ctctaga          47

SEQ ID NO: 162      moltype = DNA  length = 47
FEATURE           Location/Qualifiers
misc_feature       1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 162      organism = synthetic construct
taggtgtgta tggctagctc agtcctaggg attatgtctag ctctaga          47

SEQ ID NO: 163      moltype = DNA  length = 47
FEATURE           Location/Qualifiers
misc_feature       1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 163      organism = synthetic construct
taggtgttta tggctagctc agtcctaggt acaatgctag ctctaga          47

SEQ ID NO: 164      moltype = DNA  length = 47
FEATURE           Location/Qualifiers
misc_feature       1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 164      organism = synthetic construct
taggtgttta tagctagctc agcccttggt acaatgctag ctctaga          47

SEQ ID NO: 165      moltype = DNA  length = 47
FEATURE           Location/Qualifiers
misc_feature       1..47
note = Synthetic polynucleotide
source            1..47
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 165      organism = synthetic construct
taggtgtgta cagctagctc agtcctaggg actatgtctag ctctaga          47

SEQ ID NO: 166      moltype = DNA  length = 47
FEATURE           Location/Qualifiers

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misc_feature	1..47	
	note = Synthetic polynucleotide	
source	1..47	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 166		
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SEQ ID NO: 167	moltype = DNA length = 47	
FEATURE	Location/Qualifiers	
misc_feature	1..47	
	note = Synthetic polynucleotide	
source	1..47	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 167		
taggttgtga cggcttagctc agtccctaggtt atttgtgctag ctctaga		47
SEQ ID NO: 168	moltype = DNA length = 47	
FEATURE	Location/Qualifiers	
misc_feature	1..47	
	note = Synthetic polynucleotide	
source	1..47	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 168		
taggttgtga cagcttagctc agtccctaggtt ataatgctag ctctaga		47
SEQ ID NO: 169	moltype = DNA length = 47	
FEATURE	Location/Qualifiers	
misc_feature	1..47	
	note = Synthetic polynucleotide	
source	1..47	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 169		
taggttgtga cattattcca tcgaaactagt taacttagtac gaaagtt		47
SEQ ID NO: 170	moltype = DNA length = 48	
FEATURE	Location/Qualifiers	
misc_feature	1..48	
	note = Synthetic polynucleotide	
source	1..48	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 170		
tagcataacc ctttggggcc tctaaacggg tcttgagggg tttttgt		48
SEQ ID NO: 171	moltype = DNA length = 48	
FEATURE	Location/Qualifiers	
misc_feature	1..48	
	note = Synthetic polynucleotide	
source	1..48	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 171		
tactcgaacc cctagccgc tcttatcggg cggttagggg tttttgt		48
SEQ ID NO: 172	moltype = DNA length = 29	
FEATURE	Location/Qualifiers	
misc_feature	1..29	
	note = Synthetic polynucleotide	
source	1..29	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 172		
tacatatcgg ggggttaggg gttttttgt		29
SEQ ID NO: 173	moltype = DNA length = 80	
FEATURE	Location/Qualifiers	
misc_feature	1..80	
	note = Synthetic polynucleotide	
source	1..80	
	mol_type = other DNA	
	organism = synthetic construct	
SEQUENCE: 173		

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ccaggcatca aataaaacga aaggctcagt cgaaagactg ggccttcgt tttatctgtt 60
gttgcggtaa gaacgctctc                                80

SEQ ID NO: 174      moltype = DNA  length = 61
FEATURE           Location/Qualifiers
misc_feature      1..61
note = Synthetic polynucleotide
source            1..61
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 174
ctcggtacca aattccagaa aagaggcctc ccgaaagggg ggcctttt cgtttggtc 60
c                                61

SEQ ID NO: 175      moltype = DNA  length = 146
FEATURE           Location/Qualifiers
misc_feature      1..146
note = Synthetic polynucleotide
source            1..146
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 175
ctcggtacca aattccagaa aagacaccgg aaagggtgtt tttcggttt ggtcctcctt 60
ggccctccat ctttagatag cagataaaaa aaatccttag cttcgctaa ggatgattc 120
ttcataggca atacgatcgc atgtcc                                146

SEQ ID NO: 176      moltype = DNA  length = 129
FEATURE           Location/Qualifiers
misc_feature      1..129
note = Synthetic polynucleotide
source            1..129
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 176
ccaggcatca aataaaacga aaggctcagt cgaaagactg ggccttcgt tttatctgtt 60
gttgcggtaa gaacgctctc tactagagtc acactggctc accttcgggt gggccttct 120
gcgtttata                                129

SEQ ID NO: 177      moltype = DNA  length = 129
FEATURE           Location/Qualifiers
misc_feature      1..129
note = Synthetic polynucleotide
source            1..129
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 177
ccaggcatca aataaaacga aaggctcagt cgaaagactg ggccttcgt tttatctgtt 60
gttgcggtaa gaacgctctc tactagagtc acactggctc accttcgggt gggccttct 120
gcgtttata                                129

SEQ ID NO: 178      moltype = DNA  length = 168
FEATURE           Location/Qualifiers
misc_feature      1..168
note = Synthetic polynucleotide
source            1..168
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 178
ggcttgcggc actaccttgc agtaatgcgg tggacaggat cggcggttt ctttcttt 60
ctcaatgact gaatagaaaa gacgaacatt aacgcgttag aagcccccg gaagatcacc 120
ttccgggggc ttttttattt cgctacaaat gaaagtacat agaaatta                                168

SEQ ID NO: 179      moltype = DNA  length = 146
FEATURE           Location/Qualifiers
misc_feature      1..146
note = Synthetic polynucleotide
source            1..146
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 179
caataaaaa aaatccttag ctttcgtaa ggatgatttc ttccctggcc ctccatcctt 60
agatagctcg gtaccaaatt ccagaaaaga cacccgaaag ggtttttt cgtttggtc 120
ctcataggca atacgatcgc atgtcc                                146

SEQ ID NO: 180      moltype = DNA  length = 128
FEATURE           Location/Qualifiers

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misc_feature          1..128
                      note = Synthetic polynucleotide
source               1..128
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 180
ccaggcatca aataaaacga aaggctcagt cgaaagactg ggccttcgt tttatctgtt 60
gtttgtcggt gaacgctctc ctagcataac cccttgggg ctctaaacgg gtcttgaggg 120
gttttttg                                         128

SEQ ID NO: 181      moltype = DNA length = 152
FEATURE
misc_feature          1..152
                      note = Synthetic polynucleotide
source               1..152
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 181
ctcggtagcca aattccagaa aagagacgct ttcgagcgctc ttttttcgtt ttggccctcc 60
tttggccctcc atccttagat agatctaacc aaaaaggggg gattttatct ccccttaat 120
ttttccttca taggcaatac gatcgcatgt cc                                         152

SEQ ID NO: 182      moltype = DNA length = 144
FEATURE
misc_feature          1..144
                      note = Synthetic polynucleotide
source               1..144
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 182
cgagatagc aaaaaagcgc cttagggcg ctttttaca ttgggtggcc ttggccctcc 60
atccttagat agaggcgact gacaaacct cgctccggcg gggtttttg ttatctgcat 120
cataggcaat acgatcgcat gtcc                                         144

SEQ ID NO: 183      moltype = DNA length = 82
FEATURE
misc_feature          1..82
                      note = Synthetic polynucleotide
source               1..82
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 183
tcggtcagtt tacacctgatt tacgtaaaaa cccgcttcgg cgggttttg ctttggagg 60
ggcagaaaaga tgaatgactg tc                                         82

SEQ ID NO: 184      moltype = DNA length = 103
FEATURE
misc_feature          1..103
                      note = Synthetic polynucleotide
source               1..103
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 184
gcccccggaa gatcacctc cgggggcttt ttattggcg gccggctgat tgatcaggcg 60
ggccggctgat tggcgcgtta cctggtagcg cgcattttg ttt                                         103

SEQ ID NO: 185      moltype = DNA length = 83
FEATURE
misc_feature          1..83
                      note = Synthetic polynucleotide
source               1..83
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 185
gtaatcgtta atccgcaaat aacgtaaaaa cccgcttcgg cgggttttt tatgggggga 60
gtttagggaa agagcatttgc                                         83

SEQ ID NO: 186      moltype = DNA length = 41
FEATURE
misc_feature          1..41
                      note = Synthetic polynucleotide
source               1..41
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 186
aaaaaaaaaac cccgccccctg acagggcggg gtttttttt t                                         41

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SEQ ID NO: 187      moltype = DNA  length = 152
FEATURE
misc_feature        Location/Qualifiers
1..152
note = Synthetic polynucleotide
source              1..152
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 187
tccggcaatt aaaaaagcg  ctaaccacgc cgctttttt acgtctgcat gactgaatag 60
aaaagacgaa cattaacgc tgagaagcc cccggaatg caccttcgg gggcttttt 120
attgcgtcc ttggccctcc atccttagat ag                                152

SEQ ID NO: 188      moltype = DNA  length = 167
FEATURE
misc_feature        Location/Qualifiers
1..167
note = Synthetic polynucleotide
source              1..167
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 188
ggaagaccat actgaaaca  cagaaaaaa cccgcacctg acagtgcggg ctttttttt 60
cgacccaaagg tgactgaata gaaaagacga acatcgcag atagaaaaa agcgcctta 120
gggcgcctttt ttacatttgtt ggtcatggc aatacgtcg catgtcc                                167

SEQ ID NO: 189      moltype = DNA  length = 160
FEATURE
misc_feature        Location/Qualifiers
1..160
note = Synthetic polynucleotide
source              1..160
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 189
tccggcaatt aaaaaagcg  ctaaccacgc cgctttttt acgtctgcat ctttggccct 60
ccatcccttag atagctcggt accaaattcc agaaaagagg cttccggaaa ggggggcctt 120
tttcgtttt ggtcctcata ggcaatacga tcgcatgtcc                                160

SEQ ID NO: 190      moltype = DNA  length = 199
FEATURE
misc_feature        Location/Qualifiers
1..199
note = Synthetic polynucleotide
source              1..199
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 190
ttcagccaaa aaacttaaga  ccgcgggtct tgcactac ctgcgttgc 60
aggatcggcg gttttttttt ctcttctcaa tacatgaaag tacatagaaa ttactcggt 120
ccaatttca gaaaagaggc ctccggaaaag gggggcctt tttcggtttt gtcctcatag 180
gcaatacgtt cgcgttcc                                199

SEQ ID NO: 191      moltype = DNA  length = 189
FEATURE
misc_feature        Location/Qualifiers
1..189
note = Synthetic polynucleotide
source              1..189
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 191
ttcagccaaa aaacttaaga  ccgcgggtct tgcactac ctgcgttgc 60
aggatcggcg gttttttttt ctcttctcaa tccttggccc tccatccta gatagtccgg 120
caatttcaaa agcggctaac cacggccgtt ttttacgtc tgcatacatag gcaatacgtt 180
cgcatgtcc                                189

SEQ ID NO: 192      moltype = DNA  length = 164
FEATURE
misc_feature        Location/Qualifiers
1..164
note = Synthetic polynucleotide
source              1..164
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 192
ctcgggtacca aattccagaa aagaggccctc ccgaaaggggg ggcctttttt cgtttggtc 60
ctgactgaat agaaaagacg aacattaacg catgagaaag ccccccggaaatcacccttcc 120
ggggggcctttt ttattgcgtt ccttggccctt ccatttcgtt atag                                164

SEQ ID NO: 193      moltype = DNA  length = 159

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FEATURE          Location/Qualifiers
misc_feature    1..1159
                  note = Synthetic polynucleotide
source          1..1159
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 193
ctcggatcacca aattccagaa aagaggcctc ccgaaagggg ggcctttt cgtttggtc 60
ctccttgccc ctccatcctt agatgtccgg caattaaaaa agcggttaac cacgcccgtt 120
tttttacgtc tgcatcatag gcaatacgtat cgcgtatgtcc 159

SEQ ID NO: 194      moltype = DNA length = 197
FEATURE          Location/Qualifiers
misc_feature    1..1197
                  note = Synthetic polynucleotide
source          1..1197
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 194
ctcggatcacca aagacgaaca ataagacgt gaaaagcgctc tttttcggtt ttgggtccatc 60
aaatgaaagt acatagaaat tattcagcca aaaaacttaa gaccgcgggtt cttgtccact 120
accttgcagt aatgcgggtgg acaggatcggtt cggttttctt ttcttcttc aatccttggc 180
cctccatcctt tagatag 197

SEQ ID NO: 195      moltype = DNA length = 121
FEATURE          Location/Qualifiers
misc_feature    1..1121
                  note = Synthetic polynucleotide
source          1..1121
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 195
ggaaactgcc agacatcaaataaaacaa ggctcagtcg gaagactggg cctttgttt 60
tatctgttgtt ttgtcggtga acactctccc gacttagtcg ggccgcgtca gaaagaggag 120
a 121

SEQ ID NO: 196      moltype = DNA length = 152
FEATURE          Location/Qualifiers
misc_feature    1..1152
                  note = Synthetic polynucleotide
source          1..1152
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 196
aacgcgtatgg aaagccccccg gaagatcacc ttccgggggg tttttatgtt cgctcatagg 60
caatacgtatc gcatgtcttc cggcaattaa aaaagcggtt aaccacgcgtt cttttttac 120
gttgcgtatcc ttggccctcc atccttagat ag 152

SEQ ID NO: 197      moltype = DNA length = 91
FEATURE          Location/Qualifiers
misc_feature    1..91
                  note = Synthetic polynucleotide
source          1..91
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 197
ggaaactgcc agacatcaaataaaacaa ggctcagtcg gaagactggg cctttgttt 60
tatctgttgtt ttgtcggtga acactctccc g 91

SEQ ID NO: 198      moltype = DNA length = 195
FEATURE          Location/Qualifiers
misc_feature    1..195
                  note = Synthetic polynucleotide
source          1..195
                  mol_type = other DNA
                  organism = synthetic construct
SEQUENCE: 198
aaagcaagct gataaaccgatacaattaaa ggctcctttt ggagcctttt tttttggaga 60
tttcaacat gaaaaaaaaatatttgcgtt atcagatgcg ggcggggaaac tgccagacat 120
caaataaaaaac aaaagggtca gtccggaaatc tggccctttt gttttatctgtt tggtttgtcg 180
gtgaacactc tcccg 195

SEQ ID NO: 199      moltype = DNA length = 164
FEATURE          Location/Qualifiers
misc_feature    1..164
                  note = Synthetic polynucleotide

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source          1..164
               mol_type = other DNA
               organism = synthetic construct
SEQUENCE: 199
aacgcatgag aaagcccccg gaagatcacc ttccgggggc ttttttatg cgctccttgg 60
ccctccatcc ttagatagct cggtaccaaa ttccagaaaa gaggcctccc gaaagggggg 120
ccttttttcg ttttgtctt cataggcaat acgatcgcat gtcc                164

SEQ ID NO: 200      moltype = DNA  length = 193
FEATURE           Location/Qualifiers
misc_feature      1..193
note = Synthetic polynucleotide
source            1..193
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 200
aacgcatgag aaagcccccg gaagatcacc ttccgggggc ttttttatg cgctccttgg 60
ccctccatcc ttagatagtt cagccaaaaa acttaagacc gccgggtttt tccactacct 120
tgcagtaatg cggtgacag gatcgccgt tttttttctt ctctcaatc ataggcaata 180
cgatcgcatg tcc                                         193

SEQ ID NO: 201      moltype = DNA  length = 67
FEATURE           Location/Qualifiers
misc_feature      1..67
note = Synthetic polynucleotide
source            1..67
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 201
cttaggacct gtaggatcgt acaggtttac gcaagaaaat ggtttgttac tttcgaataa 60
atctaga                                         67

SEQ ID NO: 202      moltype = DNA  length = 68
FEATURE           Location/Qualifiers
misc_feature      1..68
note = Synthetic polynucleotide
source            1..68
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 202
cggtgaaatc cctatcagtg atagagattt acatccat ttttgttata tataatgagc 60
actctaga                                         68

SEQ ID NO: 203      moltype = DNA  length = 90
FEATURE           Location/Qualifiers
misc_feature      1..90
note = Synthetic polynucleotide
source            1..90
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 203
aacaaacacaga caatctggtc tgtttgtatt atggaaaatt ttttgtata atagattcaa 60
caaacacacaca atctggtctg ttttgtattat 90

SEQ ID NO: 204      moltype = DNA  length = 58
FEATURE           Location/Qualifiers
misc_feature      1..58
note = Synthetic polynucleotide
source            1..58
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 204
aaaaagagtt tgacatgata cgaaacgtac cgtatcgta aggttactag agtctaga     58

SEQ ID NO: 205      moltype = DNA  length = 116
FEATURE           Location/Qualifiers
misc_feature      1..116
note = Synthetic polynucleotide
source            1..116
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 205
ggggcctcgc ttgggttatt gctggtgccc ggccgggcgc aatattcatg ttgtatgattt 60
attatatatac gagtggtgtt tttatattata ttgtttgttc cgttaccgtt attaac    116

SEQ ID NO: 206      moltype = DNA  length = 753

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FEATURE	Location/Qualifiers
misc_feature	1..753
	note = Synthetic polynucleotide
source	1..753
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 206	
atgaaaaaac taaatgccga cgacacatac agaataatta ataaaattaa agcttgtaga 60	
agcaataatg atattaatca atgttatct gatatgacta aaatggtaca ttgtgaatat 120	
tatttactcg cgcattatcc tcctatttc atgttaaat ctgatatttc aatcttagat 180	
aattacccca aaaaatggag gcaatataatc gatgacgtca atttaataaa atatgatcc 240	
atagtagatt attctaactc caatcattca ccaattaatc ggaatataatt tgaaaacaat 300	
gctgttaata aaaaatctcc aatgttaatt aaagaagcga aaacatcagg tcttgcact 360	
gggttttagt tccctattca tacggctaa aatggcttc gaatgtttag ttttgcacat 420	
tcagaaaaag aacaactata agatgtttt ttttacatg cgtgtatgaa cattaccata 480	
atttgttccct ctcttagttgaa taattatcga aaaataataa tagcaaaatg taaatcaaac 540	
aacgattttaa cccaaagaga aaaagaatgt ttacgtggg catgcgaagg aaaaagctct 600	
tgggatattt caaaaatattt agggttgcgt gaggctactg tcactttca tttaaccaat 660	
gcgc当地atgaa aactcaatac aacaaccgc tgccaaatgt ttctaaagc aattttaaaca 720	
ggagcaattt attgcccata cttaaaaaat taa 753	
SEQ ID NO: 207	moltype = DNA length = 624
FEATURE	Location/Qualifiers
misc_feature	1..624
	note = Synthetic polynucleotide
source	1..624
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 207	
atgtccagat tagataaaaag taaatgttta acacagcgcatt tagagctgtc taatgaggc 60	
ggaatcgaag gtttacaaac ccgttaactc gcccagaagc taggtgtaga gcgcctaca 120	
ttgttattggc atgttaaaaaaa taaggggctt ttgcgtcagc ctttagccat tgagatgtta 180	
gataggcacc atactcactt ttgccttta gaagggaaa gctggcaaga tttttacgt 240	
aataacgcta aaggttttag atgtgttta ctaagtcatc gcgtggagc aaaagtacat 300	
tttaggttacgc ggcacatcaga aaaaactgtat taaaatcattt acccttttta 360	
tgccacaacaa gtttttactt agagaatgcata ttatatgcac tcagcgtgtt gggccatTTT 420	
acttttaggtt ggttatttttttta agatcaagag catcaagtcg cttaaaagaag aaggaaaca 480	
cctactactg atagatgttca gccatttta cgacaagctt tgcattttttt ttttttttta 540	
gggtgcagacgc cagcccttattt attcggccattt gattttatca tatgcgtttt agaaaaacaa 600	
cttaaatgtt aagatgggtt cttaa 624	
SEQ ID NO: 208	moltype = DNA length = 612
FEATURE	Location/Qualifiers
misc_feature	1..612
	note = Synthetic polynucleotide
source	1..612
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 208	
atgagccccga aacgtcgta ccaggcagaa cgtcaatggg aaacccaggaa taaactgtt 60	
gcacgcac gtttttttttgcgtgtat ggttatgtat tgcacatgtt 120	
ccgggttgcag cgggttttag ccgtgtcga cagagccat attttccac caaactggaa 180	
ctgtgttgcg caacccatgaa atgggttgcat gacggatcca ccggaaatgtt ccgtgcacgt 240	
ctggcaaaaac tggaaacccggaa agatgttgcattt attcagcggaa tgctggatgc tgcacatgtt 300	
tttttttgcgg atgtatgttgcattt tagcatcggc ctggatgttgcatggccat tgcacatgtt 360	
ccggcaactgc gttggatgttgcattt ctggatgttgcattt gtttttttttgcgg tggaaatgtt 420	
atgtgttgcg gttgtgttgcgtt gacccgtgtt ctggatgttgcattt gtttttttttgcgg tggaaatgtt 480	
tgggtgttgcgtt ttaacacgttgcattt ttttttttttgcgg ttttttttttgcgg ttttttttttgcgg 540	
gaacgttttgcgtt aacgtgttgcgtt taatagcacc ctggaaatggccat ttttttttttgcgg ttttttttttgcgg 600	
ttcaaacgttgcattt ttttttttttgcgg ttttttttttgcgg ttttttttttgcgg ttttttttttgcgg 612	
SEQ ID NO: 209	moltype = DNA length = 603
FEATURE	Location/Qualifiers
misc_feature	1..603
	note = Synthetic polynucleotide
source	1..603
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 209	
atggcacgtt ccccgagccg tagcagcattt ggttagccgtc ttagtccgca taccataaaa 60	
gcaattctga ccagcattat tggaaatcttttgcgtt aaaaatgtt gttatgttgc tctgacatt 120	
gaaaggcggtt cgcgtgtgc cgggtcaatc aaaccggatc tttatgttgc tggccaaat 180	
aaaggcgttc acgtgtatgttgcattt gtttttttttgcgg ttttttttttgcgg ttttttttttgcgg 240	
gatctgggtt gtttttttttgcgg ttttttttttgcgg ttttttttttgcgg ttttttttttgcgg 300	
cgtgttgcgtt ttttttttttgcgg ttttttttttgcgg ttttttttttgcgg ttttttttttgcgg 360	
gcaacccttgcgtt gtttttttttgcgg ttttttttttgcgg ttttttttttgcgg ttttttttttgcgg 420	

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ctgggttggaaa atgccattag caatgggtgaa ctggccgaaag ataccaatcg tgaactgctg	480
cttgatgtg ttttttgtt ttgttgatcg cgcctgtcg ccgaacagct gaccgttgaa	540
caggatattg aagaatttac cttctgtcg attaatggtg ttgtccggg tacacagcgt	600
taa	603
SEQ ID NO: 210	moltype = DNA length = 906
FEATURE	Location/Qualifiers
misc_feature	1..906
	note = Synthetic polynucleotide
source	1..906
	mol_type = other DNA
	organism = synthetic construct
 SEQUENCE: 210	
atggaaactgc gtgacctgga tttaaacctg ctgggttggt tcaaccagg tgcggcgc	60
agacgcgtct ctgtcactgc ggagaacctg ggccgtgacc agcctgcgt gagcaatgcg	120
ctgaaacccc tgcgcaccc tgcataaggac ccacttcgc tgccgcacaca tcaggaaat	180
gaaccccac acctatggcc gcatactggcc gggccgtca ctccggccat gcacgcactg	240
cggaaacgccc tacagcacca tggaaacctc gatccgtcg ccagegacgc tacccatcc	300
ctggccatcg cggacattgg cgagatctac ttcatgcgc ggctgtatgg tgcgttggct	360
caccaggccc ccaatttgcgt gatcatcg tggccgcaca gttcgatgag cctgtatgcag	420
gccttgcaga acggaaacctg ggacttggcc gtgggcctgc ttccaaatct gcaaaactggc	480
tttttcagec gcccgtgtcg ccagaatctc tacgtgtgc tatgtcgccaa ggaccatcca	540
gtcaccggcg aaccggctgc tctggagcgc ttctgttccct acggccacgt gctgttcatc	600
ggccgtggca cggccacgg cgagggtggac acgtatcgca cacgggtccg catccggcgc	660
gacatccgtc tggaaagtggc gcacttcgc gccgttggcc acatccatca ggcacccat	720
ctgtctcgcca ctgtggcgat atgttagcc gactgtcgcc tagagccctt cggccctaagc	780
gccttgcgc accccagtcgt ctgtgcgtt atagccatca acatgttctg gcatgcgaaag	840
taccacaagg accttagccaa tattttgggtt cggcaactga tgtttgaccc gtttacggat	900
tgataa	906
SEQ ID NO: 211	moltype = DNA length = 90
FEATURE	Location/Qualifiers
misc_feature	1..90
	note = Synthetic polynucleotide
source	1..90
	mol_type = other DNA
	organism = synthetic construct
 SEQUENCE: 211	
tcaatgtatt gatggcgcc atatcatgaa tcaaaaacaat ccatttgcgc aatatcaagc	60
tcactcttaa gcttactca tccgtgcgt	90
SEQ ID NO: 212	moltype = DNA length = 930
FEATURE	Location/Qualifiers
misc_feature	1..930
	note = Synthetic polynucleotide
source	1..930
	mol_type = other DNA
	organism = synthetic construct
 SEQUENCE: 212	
atgcgttca acaagctcgatccctcaatctt ctggtcgcccc tggatgcact gctcacggag	60
atggatcgatca ggcggcgccgc cgaaaaatgc cattctgacgc agtccggccat gagaatgcgc	120
ctggcgccgc tgcgcgagta ttccatgtatcg gatgtcgatc tccagggtgg ccggcgcatg	180
gagcccaacgc cggcgccgcga ggtgtcaag gatgcgggtc atgtatgtct gccggcgatc	240
gatggcttca tgcggcgctt gccggccctt gtggccggccg agtccacgcg cgagggttgc	300
atctcggtttt cggatcttac gtcgttccgtt ctcatcccccc ggggtgtggc gcggcgccac	360
ggccggggca agcacatccg ctttgcgttgc atgcgcgggg tgcagacacc gacccggctcg	420
ctggatcgccg cggagggtggc cctgtcgatcg ttggccgggg aattctgcac gcccgatcat	480
cctggccaaag aggtttccg cgaacggcat gtgtcgatcg ttcggcgccgca cagtcgcgt	540
ggcccaaggccg agctgacgcgtt ggaacgcgtt atggccctcg gccatgtggt gatgttgcgc	600
cctggggccat atgcgtcgatc ggtggggccg tggatggccca ggaaggtggg ctttgcgcgc	660
cggggtggaaat tgaccatcgat cgttgcgtcg ccgtgttaca gggggacggac	720
cgcatcgccca cgggtgcgttc cggcgccggc cagtcgtcgatcg ctccgcata gccgggtgg	780
atcaaggaga gtccgcgttc gctggggccgag atgcggcagaat tgatgcgtt gcatcgatc	840
cggcggccatc atccctggccat ccgtggccgtt cgtccgggtt ttctggagat gtcgcaggag	900
atggatcgccg cgttgcgttc catctgtcgat	930
SEQ ID NO: 213	moltype = DNA length = 286
FEATURE	Location/Qualifiers
misc_feature	1..286
	note = Synthetic polynucleotide
source	1..286
	mol_type = other DNA
	organism = synthetic construct
 SEQUENCE: 213	
cagacattgc cgtcactgcg tcttttactg gtcgttctcg ctaaccaaacc cggtaacccc	60
gcttattaa agcattctgtt aacaaaggcc gaccaaaacc cgcgtaaacaa	120

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aagtgtctat aatcacggca gaaaagtcca cattgattat ttgcacggcg tcacacttg	180
ctatgcata gaatttttat ccataagatt agcggatcc acctgaacct ttttatcgca	240
actctctata ttttctccat acccgaaaa ttgggctage gaattc	286

SEQ ID NO: 214	moltype = DNA length = 930
FEATURE	Location/Qualifiers
misc_feature	1..930
	note = Synthetic polynucleotide
source	1..930
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 214	
atgcaatatg gacaatttgtt ttcttcctgt aatggcgaaa gtatgaaaag tatggctgaa	60
ggccaaaaatg atccccctgtc gcggggatad tcgtttaatg cccatctgtt ggcgggttta	120
acggccgatgg aggccaaacgg tttttatcgat cccggccgtt ggaaatgaaa 180	
gtttagatattc tcaatctcaccat cttcgccgtt cagggggttgg taaaaatca gggacgaaaa	240
tttttttgcg gacgggggtga tattttgtgtt ttcccgccag gagagattca tcactacgg	300
cgtcatccgg aggctcgccg atggatcac tttttatcgat cccggccgtt ggaaatgaaa	360
tggcataatg gctttaaactt gccgtcaata ttttcccaata cgggggtttt tgcccccggat	420
gaaggcgcacc acccgccattt cttggccaaat ttttttttttgg cggggcaagg	480
gaaggggcgat attccggatgt gctggcgata aatctgttgg agcaattgtt actggggcg	540
atggaaagcga ttaacggatc gtcctatcca ccgtatggata atccggatcg cgaggctgt	600
cagtacatca gcatcaccat ggcagacago aattttgtata tggccatcgat cgcacagcat	660
ttttgtttgtt cggccgtcgccg tttttcccgat cttttcccgat cggccatcgat	720
ttaagctggc gggaggacca acgtatcago cttggccaaat ttttttttttgg cggggcaagg	780
atgcctatcg ccaccgtcgcc tggcaatgtt gtttttgcgtt atcaactcta tttttcccgat	840
gtatataaaa aatgcacccgg gggccggccg agcgaggatcc gtttttgcgtt tgaagaaaaa	900
gttgaatgtatg tagccgtcaa gttgtccat	930

SEQ ID NO: 215	moltype = DNA length = 1419
FEATURE	Location/Qualifiers
misc_feature	1..1419
	note = Synthetic polynucleotide
source	1..1419
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 215	
atgggttacta tcaatacgga atctgttttta acggccacgtt ctttgcggga tacggccgtt	60
atgaatatgtt ttttttttttgcg agctgtcggtt gtcggcaggat ttttttttttgcg ttttttttttgcg	120
ggcgtaatcg ccggggatgtt gccgttattt accggatcatc ttttttttttgcg ctttttttttgcg	180
cagaatgggg ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	240
tggctgtcgat ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	300
cttggccgtttt ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	360
gttgcgtttttt ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	420
gcaatgtttttt ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	480
atcgatgtttttt ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	540
tttgggggtttt ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	600
agccggcgat ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	660
cttcggccat ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	720
ttaaaacagg gggccggccg ttttttttttgcg ttttttttttgcg ttttttttttgcg	780
cttcggatgtt ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	840
gcccggccat ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	900
cttcggccat ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	960
gcaaggccgtt ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	1020
cttcggccat ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	1080
tcttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	1140
tggatcttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	1200
accaccacgg ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	1260
agcatggccg ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	1320
attacttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	1380
ctgtatggccg ttttttttttgcg ttttttttttgcg ttttttttttgcg ttttttttttgcg	1419

SEQ ID NO: 216	moltype = DNA length = 75
FEATURE	Location/Qualifiers
misc_feature	1..75
	note = Synthetic polynucleotide
source	1..75
	mol_type = other DNA
	organism = synthetic construct
SEQUENCE: 216	
ctcgaggatgtt gacaatataat catcgccatcg tataatgtgtt ggaattgtgtt ggcgtcacaa	60
tttcacacat cttaga	75

SEQ ID NO: 217	moltype = DNA length = 221
FEATURE	Location/Qualifiers
misc_feature	1..221

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source          note = Synthetic polynucleotide
                1..221
                mol_type = other DNA
                organism = synthetic construct

SEQUENCE: 217
aacaaataca catggcgca tgccttac tgcccttgcg atatggaaagg caagttta  60
gtaacaatag aaaactgggt cctacttcg aagaatgcac tgcggcggtc acgtcaacac 120
gtgctgacc gttgagaatg aatgtgggc agattgcac cgccgtcatt ttccggctgc 180
ccgtcctcac gttttgcgc tgcacatcgaa gagattggaa a 221

SEQ ID NO: 218      moltype = DNA length = 849
FEATURE
misc_feature        Location/Qualifiers
1..849
note = Synthetic polynucleotide
source             1..849
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 218
atgacgtcag cagcgaatct ggtgaggata acgcagcccc cgatcagccg gctgtatcagg 60
gatctcgaag agggaaattgg gatagccctc ttccaaagaa cggcaacccg gttacgtcct 120
acgcgggagg ccgttattct gttcaaggaa gtgtcgccgc atttcaacgg gattcagcac 180
atcgacaaag tccggctga actaagaagaa ttcataatggg ggtcccttaag ggtcgccctgt 240
tatacagcgc cagcttcgtatggg ttttatgtcc gggtgcatttcc agacgttcat ccggcgatccgg 300
cccgacgtgtt cgggttacactt ccgacatggg ctttcccaaa cgggttccgc atttgcgtc 360
ctccagcact acgatctgg aatatcgata ttggctggc actatctgg ttcacccacc 420
gaacctgtcc ttcccttcg tgcggctgc ctgtcgccg cggggcatcg ttcgtaaagac 480
aaggaaacttgc ttcatcgccg ggacatttggaa ggagagtcat tgatttgctt ctctccatgt 540
agccttcac ggatgcacaaac ggacgcccgc ctggacacgtt cggcgctcca ctgtaaatcgc 600
aggatagaaa gtatgttgc gctgaatctc tgcgtatggg taaggcaggaa aatgggggtt 660
ggatctgtcg acccccttcac tgccgactac tacagtgcata atccggatcc tcagcgctcc 720
tttgcatttttgc ttgttgccttccatgttgc atatgttgc ctggacacag cccaccggcg 780
cggttgcgtt gggatgttgc ggcacgttgc ctgtatgttgc tgaaaggctt gcccattatgaa 840
accatttga 849

SEQ ID NO: 219      moltype = DNA length = 119
FEATURE
misc_feature        Location/Qualifiers
1..119
note = Synthetic polynucleotide
source             1..119
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 219
aaacgcacca taacatctgc ttattcttc cccgttattt tgaatttgc cgaatgcata 60
tcgaatgtaa agctcacccctt ataaatcaca actttccggg gccaacccggg atcagacgt 119

SEQ ID NO: 220      moltype = DNA length = 897
FEATURE
misc_feature        Location/Qualifiers
1..897
note = Synthetic polynucleotide
source             1..897
mol_type = other DNA
organism = synthetic construct

SEQUENCE: 220
atgaatctca ggcagggtcga ggcgttccgg gcagtcatgc tgacggggca aatgacggcg 60
gcggctgaac taatgtctgg tgcgttccgg gccatcagtc gcctaataaa ggactttgaa 120
caggcgacaa aactgcgtgtt ctgcgtatggg cgtggaaacc atattatccc gacacaggag 180
gcaagacgc tggtaaaaaaa ggtcgatcggtt ggttgcgtcg ggcttaatca tataggcaac 240
ctggctgcgc acatcgccag gcaggcagecg gggacgttccgc gatgtgtcgc aatgcgtct 300
ctggcaaaac ggcgttccgg ggcgttccgg tgcgtatggc accaaatctc 360
cagggttccca taatggggactt ggcctcaagc atgggtatggg aagccgttgc gtcggcgagg 420
ggccacatcg gttatgcgc tggccacacag gggcccaaaatggggatccatcgatccgg 480
tgcgttcccg ctgttgcgc tggccatggc gggacatcgatccatcgatccgg 540
acgcccacagg accttgcggg tgagcgatattt ataaaacagg agactggcac tctttcgcc 600
atgcgggttag aggtggcgat tggatgttgc caacggccgc cgtcaatggc agtggacgttgc 660
tcgcataatcg ctgtatgttgc cgtccggcaaa ggcgcggggatccatcgatccgg 720
ggccgcgtatcg agttcacccgcg caggatcgatccatcgatccgg 780
ggatccctcg aagtccggc agcaatttgc gttccctcaaa ccatcgatccatcgatccgg 840
accgaatttca tgatgttgc atgaaggcaga acggccatcgatccgg 897

SEQ ID NO: 221      moltype = DNA length = 63
FEATURE
misc_feature        Location/Qualifiers
1..63
note = Synthetic polynucleotide
source             1..63
mol_type = other DNA
organism = synthetic construct

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SEQUENCE: 221
agcgcgggtg agagggattc gttaccaata gacaatttat tggacgttca atataatgtc 60
    agc                      63

SEQ ID NO: 222      moltype = DNA  length = 226
FEATURE           Location/Qualifiers
misc_feature      1..226
                    note = Synthetic polynucleotide
source            1..226
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 222
ccctttgtgc gtccaaacgg acgcacggcg ctctaaaggcg ggtcgcgatc ttttagattc 60
gtccctcgcg ctttcgtct ttgtttggc gcatgtcgat atcgcaaaac cgctgcacac 120
tttgcgcga catgtctga cttccctcat ctgggggggc ctatctgagg gaatttcgga 180
tccggctgcg ctgaaccatt ctgtttcca cgaacttgaa aacgtc 226

SEQ ID NO: 223      moltype = DNA  length = 93
FEATURE           Location/Qualifiers
misc_feature      1..93
                    note = Synthetic polynucleotide
source            1..93
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 223
tttgcgttgc ttatcgaaca aattattgaa atatcgaaca aaacctctaa actactgtgg 60
cactgaatca aaaaattata aacctgtatc aga                      93

SEQ ID NO: 224      moltype = DNA  length = 51
FEATURE           Location/Qualifiers
misc_feature      1..51
                    note = Synthetic polynucleotide
source            1..51
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 224
caccagcag tatttacaaa caaccatgaa tgtaagtata ttccttagca a 51

SEQ ID NO: 225      moltype = DNA  length = 50
FEATURE           Location/Qualifiers
misc_feature      1..50
                    note = Synthetic polynucleotide
source            1..50
                    mol_type = other DNA
                    organism = synthetic construct

SEQUENCE: 225
atggatcca attgacagct agctcagtc taggtaccat tggatccaat 50

SEQ ID NO: 226      moltype = DNA  length = 54
FEATURE           Location/Qualifiers
misc_feature      1..54
                    note = R. sp. IRBG74
source            1..54
                    mol_type = genomic DNA
                    organism = unidentified

SEQUENCE: 226
atttcacaca tcttagagcta atcatctcgat actaaagagg agaaattaac catg 54

SEQ ID NO: 227      moltype = DNA  length = 49
FEATURE           Location/Qualifiers
misc_feature      1..49
                    note = R. sp. IRBG74
source            1..49
                    mol_type = genomic DNA
                    organism = unidentified

SEQUENCE: 227
atttcacaca tcttagagcta atcatcgatc actcaggagg caagtaatg 49

SEQ ID NO: 228      moltype = DNA  length = 40
FEATURE           Location/Qualifiers
misc_feature      1..40
                    note = R. sp. IRBG74
source            1..40
                    mol_type = genomic DNA
                    organism = unidentified

SEQUENCE: 228

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atttcacaca tctagaatta aagaggagaa attaaccatg	40
SEQ ID NO: 229 moltype = DNA length = 54	
FEATURE Location/Qualifiers	
misc_feature 1..54	
source note = R. sp. IRBG74	
SEQUENCE: 229 1..54	
taacaatttc acacatctag agctaatacat ctcgtactaa agaggcaagt aatg	54
SEQ ID NO: 230 moltype = DNA length = 54	
FEATURE Location/Qualifiers	
misc_feature 1..54	
source note = R. sp. IRBG74	
SEQUENCE: 230 1..54	
taacaatttc acacatctag agctaatacat cgcgtaactaa ggaggcaagt aatg	54
SEQ ID NO: 231 moltype = DNA length = 54	
FEATURE Location/Qualifiers	
misc_feature 1..54	
source note = R. sp. IRBG74	
SEQUENCE: 231 1..54	
taacaatttc acacatctag agctaatacat cgcgtaactca agaggcaagt aatg	54
SEQ ID NO: 232 moltype = DNA length = 54	
FEATURE Location/Qualifiers	
misc_feature 1..54	
source note = R. sp. IRBG74	
SEQUENCE: 232 1..54	
taacaatttc acacatctag agctaatactt cgcgtaactaa agaggcaagt aatg	54
SEQ ID NO: 233 moltype = DNA length = 54	
FEATURE Location/Qualifiers	
misc_feature 1..54	
source note = R. sp. IRBG74	
SEQUENCE: 233 1..54	
taacaatttc acacatctag agctaatacat ctcgtactca ggaggcaagt aatg	54
SEQ ID NO: 234 moltype = DNA length = 54	
FEATURE Location/Qualifiers	
misc_feature 1..54	
source note = R. sp. IRBG74	
SEQUENCE: 234 1..54	
taacaatttc acacatctag agctaatacat ctcgtactaa tgaggcaagt aatg	54
SEQ ID NO: 235 moltype = DNA length = 54	
FEATURE Location/Qualifiers	
misc_feature 1..54	
source note = R. sp. IRBG74	
SEQUENCE: 235 1..54	
taacaatttc acacatctag agctaatacat cgcgtaactaa tgaggcaagt aatg	54
SEQ ID NO: 236 moltype = DNA length = 54	
FEATURE Location/Qualifiers	
misc_feature 1..54	
source note = R. sp. IRBG74	

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source          1..54
               mol_type = genomic DNA
               organism = unidentified
SEQUENCE: 236
taacaatttc acacatctag agctaattcat cgcgtaactca cgaggcaagt aatg      54

SEQ ID NO: 237      moltype = DNA  length = 54
FEATURE
misc_feature        Location/Qualifiers
1..54
note = R. sp. IRBG74
source              1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 237
taacaatttc acacatctag agctaattcat cgcgtaactaa aaaggcaagt aatg      54

SEQ ID NO: 238      moltype = DNA  length = 54
FEATURE
misc_feature        Location/Qualifiers
1..54
note = R. sp. IRBG74
source              1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 238
taacaatttc acacatctag agctaattctt cgcgtaactaa aaaggcaagt aatg      54

SEQ ID NO: 239      moltype = DNA  length = 54
FEATURE
misc_feature        Location/Qualifiers
1..54
note = R. sp. IRBG74
source              1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 239
taacaatttc acacatctag agctaattctt cgcgtaactaa gaaggcaagt aatg      54

SEQ ID NO: 240      moltype = DNA  length = 54
FEATURE
misc_feature        Location/Qualifiers
1..54
note = R. sp. IRBG74
source              1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 240
taacaatttc acacatctag agctaattcat ctgcgtactaa ataggcaagt aatg      54

SEQ ID NO: 241      moltype = DNA  length = 54
FEATURE
misc_feature        Location/Qualifiers
1..54
note = R. sp. IRBG74
source              1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 241
taacaatttc acacatctag agctaattcat ctgcgtactaa taaggcaagt aatg      54

SEQ ID NO: 242      moltype = DNA  length = 54
FEATURE
misc_feature        Location/Qualifiers
1..54
note = R. sp. IRBG74
source              1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 242
taacaatttc acacatcttag agctaattctt ctgcgtactaa agaggcaagt aatg      54

SEQ ID NO: 243      moltype = DNA  length = 54
FEATURE
misc_feature        Location/Qualifiers
1..54
note = R. sp. IRBG74
source              1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 243
taacaatttc acacatctag agctaattcat cgcgtaactca ataggccagt aatg      54

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SEQ ID NO: 244	moltype = DNA length = 54
FEATURE	Location/Qualifiers
misc_feature	1..54
	note = R. sp. IRBG74
source	1..54
	mol_type = genomic DNA
	organism = unidentified
SEQUENCE: 244	
taacaatttc acacatctag	agctaatacat cgcgtaactaa gtaggcaagt aatg
	54
SEQ ID NO: 245	moltype = DNA length = 54
FEATURE	Location/Qualifiers
misc_feature	1..54
	note = R. sp. IRBG74
source	1..54
	mol_type = genomic DNA
	organism = unidentified
SEQUENCE: 245	
taacaatttc acacatctag	agctaatacat ctcgtactaa cgaggcaagt aatg
	54
SEQ ID NO: 246	moltype = DNA length = 54
FEATURE	Location/Qualifiers
misc_feature	1..54
	note = R. sp. IRBG74
source	1..54
	mol_type = genomic DNA
	organism = unidentified
SEQUENCE: 246	
taacaatttc acacatctag	agctaatacat cgcgtaactca gcaggcaagt aatg
	54
SEQ ID NO: 247	moltype = DNA length = 54
FEATURE	Location/Qualifiers
misc_feature	1..54
	note = R. sp. IRBG74
source	1..54
	mol_type = genomic DNA
	organism = unidentified
SEQUENCE: 247	
taacaatttc acacatctag	agctaatactt cgcgtaactaa gtaggcaagt aatg
	54
SEQ ID NO: 248	moltype = DNA length = 54
FEATURE	Location/Qualifiers
misc_feature	1..54
	note = R. sp. IRBG74
source	1..54
	mol_type = genomic DNA
	organism = unidentified
SEQUENCE: 248	
taacaatttc acacatctag	agctaatactt cgcgtaactaa ttaggcaagt aatg
	54
SEQ ID NO: 249	moltype = DNA length = 54
FEATURE	Location/Qualifiers
misc_feature	1..54
	note = R. sp. IRBG74
source	1..54
	mol_type = genomic DNA
	organism = unidentified
SEQUENCE: 249	
taacaatttc acacatctag	agctaatactt ctcgtactaa caaggcaagt aatg
	54
SEQ ID NO: 250	moltype = DNA length = 54
FEATURE	Location/Qualifiers
misc_feature	1..54
	note = R. sp. IRBG74
source	1..54
	mol_type = genomic DNA
	organism = unidentified
SEQUENCE: 250	
taacaatttc acacatctag	agctaatacat ctcgtactca ataggcaagt aatg
	54
SEQ ID NO: 251	moltype = DNA length = 54
FEATURE	Location/Qualifiers
misc_feature	1..54
	note = R. sp. IRBG74
source	1..54
	mol_type = genomic DNA

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SEQUENCE: 251          organism = unidentified
taacaatttc acacatctag agctaatcat ctcgtactaa gcacgcaagt aatg      54

SEQ ID NO: 252          moltype = DNA  length = 54
FEATURE
misc_feature
1..54
note = R. sp. IRBG74
source
1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 252          taacaatttc acacatctag agctaatcat cgcgtactaa ctacgcaagt aatg      54

SEQ ID NO: 253          moltype = DNA  length = 54
FEATURE
misc_feature
1..54
note = R. sp. IRBG74
source
1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 253          taacaatttc acacatctag agctaatttt cgcgtactaa gaacgcaagt aatg      54

SEQ ID NO: 254          moltype = DNA  length = 54
FEATURE
misc_feature
1..54
note = R. sp. IRBG74
source
1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 254          taacaatttc acacatctag agctaatttt cgcgtactaa aaacgcaagt aatg      54

SEQ ID NO: 255          moltype = DNA  length = 54
FEATURE
misc_feature
1..54
note = R. sp. IRBG74
source
1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 255          taacaatttc acacatctag agctaatttt cgcgtactaa caacgcaagt aatg      54

SEQ ID NO: 256          moltype = DNA  length = 54
FEATURE
misc_feature
1..54
note = R. sp. IRBG74
source
1..54
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 256          taacaatttc acacatctag agctaatttt ctcgtactca tgacgcaagt aatg      54

SEQ ID NO: 257          moltype = DNA  length = 40
FEATURE
misc_feature
1..40
note = R. sp. IRBG74
source
1..40
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 257          atttcacaca tctagaatta aagagaagaa attaaccatg      40

SEQ ID NO: 258          moltype = DNA  length = 29
FEATURE
misc_feature
1..29
note = R. sp. IRBG74
source
1..29
mol_type = genomic DNA
organism = unidentified
SEQUENCE: 258          ctatgtgcgaa ctagctcata ccgcagatg      29

SEQ ID NO: 259          moltype = DNA  length = 42
FEATURE

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source          1..42
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 259
ctagcgcagg tccaacgttt ttctaaagcaa ggaggtcata tg        42

SEQ ID NO: 260      moltype = DNA  length = 42
FEATURE
source          1..42
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 260
ctagcgaagg tccaacgttt ttctaaagcaa ggaggtcata tg        42

SEQ ID NO: 261      moltype = DNA  length = 42
FEATURE
source          1..42
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 261
ctagcgaagg tccaacgttt ttctaaagcca ggaggtcata tg        42

SEQ ID NO: 262      moltype = DNA  length = 42
FEATURE
source          1..42
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 262
ctagcgcagg tccaacgttt ttctaaagcca ggaggtcata tg        42

SEQ ID NO: 263      moltype = DNA  length = 42
FEATURE
source          1..42
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 263
ctagcgaagc tccaacgttt ttctaaagcaa ggaggtcata tg        42

SEQ ID NO: 264      moltype = DNA  length = 33
FEATURE
source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 264
gaattctaca ctaacggaca ggagggtccg atg                  33

SEQ ID NO: 265      moltype = DNA  length = 33
FEATURE
source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 265
gaattctaaa ctaacggaca ggagggtccg atg                  33

SEQ ID NO: 266      moltype = DNA  length = 33
FEATURE
source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 266
gaattctaag ctaacggaca ggagggtccg atg                  33

SEQ ID NO: 267      moltype = DNA  length = 33
FEATURE
source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 267
gaattctaa ctaacggaca ggagggtccg atg                  33

SEQ ID NO: 268      moltype = DNA  length = 33
FEATURE
source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 268

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-continued

gaattctaca ctaacggaca ggagggtcgg atg	33
SEQ ID NO: 269 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 269	
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SEQ ID NO: 270 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 270	
gaattctcaa ctaacggaca ggagggtcgg atg	33
SEQ ID NO: 271 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 271	
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SEQ ID NO: 272 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 272	
gaattctcag ctaacggaca ggagggtcgg atg	33
SEQ ID NO: 273 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 273	
gaattctcaa ctaacggaca ggagggtcgg atg	33
SEQ ID NO: 274 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 274	
gaattctacg ctaacggaca ggagggtcgg atg	33
SEQ ID NO: 275 moltype = DNA length = 35	
FEATURE Location/Qualifiers	
source 1..35	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 275	
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SEQ ID NO: 276 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 276	
gaattctcag ctaacggaca ggagggtcgg atg	33
SEQ ID NO: 277 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 277	
gaattctaaa ctaacggaca ggagggtcgg atg	33
SEQ ID NO: 278 moltype = DNA length = 33	
FEATURE Location/Qualifiers	

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source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 278
gaattctcg ctcacggaca ggagggtcgg atg                         33

SEQ ID NO: 279      moltype = DNA  length = 34
FEATURE
source          1..34
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 279
gaattctca a ctaacggaca ggagggtcgg gatg                        34

SEQ ID NO: 280      moltype = DNA  length = 33
FEATURE
source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 280
gaattctaca ctcacggaca ggagggtcgg atg                         33

SEQ ID NO: 281      moltype = DNA  length = 33
FEATURE
source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 281
gaattctaag ctcacggaca ggagggtcgg atg                         33

SEQ ID NO: 282      moltype = DNA  length = 33
FEATURE
source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 282
gaattctcaa ctcacggaca ggagggtcgg atg                         33

SEQ ID NO: 283      moltype = DNA  length = 33
FEATURE
source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 283
gaattctaca ctaacggaca gcagggtcgg atg                         33

SEQ ID NO: 284      moltype = DNA  length = 42
FEATURE
source          1..42
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 284
ctagcgcagg tccaaacctt ttctaaagcaa gtaggtcata tg                  42

SEQ ID NO: 285      moltype = DNA  length = 33
FEATURE
source          1..33
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 285
gaattctcg ctaacggaca gcagggtcgg atg                         33

SEQ ID NO: 286      moltype = DNA  length = 42
FEATURE
source          1..42
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 286
ctagcgcagg tccaaacctt ttctaaagcaa cttagtcata tg                  42

SEQ ID NO: 287      moltype = DNA  length = 42
FEATURE
source          1..42
               mol_type = genomic DNA
               organism = Pseudomonas protegens
SEQUENCE: 287

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ctagcgaagg tccaaacctt ttctaagcca gtaggtcata tg	42
SEQ ID NO: 288 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 288	
gaattctacg ctcacggaca gcagggtcgg atg	33
SEQ ID NO: 289 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 289	
gaattctccg ctcacggaca ggagggtcgg atg	33
SEQ ID NO: 290 moltype = DNA length = 33	
FEATURE Location/Qualifiers	
source 1..33	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 290	
cttctcgccc agctgacagg ggaagctcg atg	33
SEQ ID NO: 291 moltype = DNA length = 34	
FEATURE Location/Qualifiers	
source 1..34	
mol_type = genomic DNA	
organism = Pseudomonas protegens	
SEQUENCE: 291	
cttctcgccc agctgacagg aggaagctcg catg	34
SEQ ID NO: 292 moltype = AA length = 582	
FEATURE Location/Qualifiers	
source 1..582	
mol_type = protein	
organism = Rhodobacter sphaeroides	
SEQUENCE: 292	
MDTSAARSGA VAERGEEYLT LDALCEIAKL LTGASDPIAC MPAVFGVLGA FMGLRHGALA 60	
ILQEGQAET QRNARHVNPy VIAATASGVP PAGAEARAIp AQVARHVFNR GVSLVSCDIL 120	
EERFGAEALPP GLGDSRQALV AVPFDLQDANS PVFLGVLCAY RSLKDNGARY LDTDLRVLN 180	
VAAVLEQSIR PRRLLVARDRD RIVQEAREAI RVAEEATAGP PVEAPAEAL EGVIIGSSPAI 240	
QRVIGGQIRKV AGTHTPVLL GESGTGKEVF ARHALHSER RDKAFLIKVNC AALSQSLIES 300	
ELFGHEKGSF TGAVQQKKGR PEMAEGGTLF LDEIGEISLE FQAKLLRILQ EGEFEVRGGT 360	
RTLRVDRLTV TATNKLDERA VANGTFRADL YFRICVVPIV LPPLRDRKED IGLLAQGLLE 420	
RFNKRNGMVK KLHPSAVAAL AQCNPPGNVR ELENCIARVA ALSPETVIHA DDLACHHDHC 480	
LSADLWRLQT GSASPVGGLA QGPLLELPVLG SRPPAAAPSA PPPPPPPTVPS APLDGEAAER 540	
ELIEAMERA GWVQAKAARL RGMTPRQIGY ALKKYNIRVE KF 582	
SEQ ID NO: 293 moltype = AA length = 615	
FEATURE Location/Qualifiers	
source 1..615	
mol_type = protein	
organism = Azorhizobium caulinodans	
SEQUENCE: 293	
MPMTDAFQVR VPRVSSSTAG DIAASSITTR GALPRPGGM VSMSRGTSPE VALIGVYEIS 60	
KILTAPRRL E VTLANVNVNL SSMLQMRHGM ICILDSEGDPM DMVATTGWTP EMAGOIRAHV 120	
PQKAIDQIVA TQMPLVQDV TADPLFAGHE DLFGPPEEAT DSFIGVPIKA DHVMGTLISI 180	
DRIWDGDTARF RFDEDVRFLL MVANLVQGTV RLHKLVASDR DRLIAQTHR EKALREEKSG 240	
AEPEVAAEAAN GSAMGIVGDS PLVKRLIATA QVVARNSNSTV LLRGESGTGK ELFARAIHEL 300	
SPRKKGPKFK VNCAALPESV LESELFQHEK GAFTGALNMR QGRFELAHGG TLFLDEIDEI 360	
TPAFQAKLLR VLQEGERFV GGNRTLKVDV RLVCATNKNL EA AVSKGEFR ADLYYRIHV 420	
PLILPPLRER PGDIPKLAKN FLDRFNKENK LHMLSLAPAI DVLRRCYFPG NVRELENCIR 480	
RTATLAHDAV ITPHDFACDS GQCLSAMLWK GSAPKPVMPH VPPAPTPLTP LSPAPLATAA 540	
PAASAPAPAA DSLPVTCPGT EACPAVPPRQ SEKEQLLQAM ERSGWVQAKA ARLLNLTPRQ 600	
VGYALRKYDI DIKRF 615	

What is claimed is:

1. A *rhizobium* that can fix nitrogen under aerobic free-living conditions, comprising a symbiotic *rhizobium* having an exogenous nif cluster, wherein the exogenous nif cluster confers nitrogen fixation capability on the symbiotic *rhizo-*

bium under aerobic free-living conditions, and wherein the *rhizobium* is not *Azorhizobium caulinodans*.

2. The *rhizobium* of claim 1, wherein the exogenous nif cluster is selected from a group consisting of a free-living diazotroph, a symbiotic diazotroph, a photosynthetic Alp-

haproteobacteria, a Gammaproteobacteria, a cyanobacteria, a firmicutes, a *Rhodobacter sphaeroides*, and a *Rhodopseudomonas palustris*.

3. The *rhizobium* of claim **1**, wherein the exogenous nif cluster is an inducible refactored nif cluster.

4. The *rhizobium* of claim **3**, wherein the inducible refactored nif cluster is an inducible refactored *Klebsiella* nif cluster.

5. The *rhizobium* of any one of claims **1-4**, wherein the *rhizobium* is IRBG74.

6. The *rhizobium* of any one of claims **1-5**, wherein the exogenous nif cluster comprises 6 nif genes.

7. The *rhizobium* of claim **6**, wherein the 6 nif genes are nifHDK(T)Y, nifEN(X), nifJ, nifBQ, nifF, and nifUSVWZM.

8. The *rhizobium* of claim **6** or **7**, wherein each nif gene of the exogenous nif cluster is preceded by a T7 promoter.

9. The *rhizobium* of any one of claims **1-8**, further comprising an endogenous nif cluster.

10. The *rhizobium* of any one of claims **1-9**, wherein the exogenous nif cluster further comprises a terminator.

11. The *rhizobium* of any one of claims **8-10**, wherein the T7 promoter has a terminator and wherein the terminator is downstream from the T7 promoter.

12. The *rhizobium* of claim **12**, wherein the exogenous nif cluster is a refactored *rhizobium* IRBG74 nif cluster.

13. A plant growth promoting bacterium that can fix nitrogen under aerobic free-living conditions, comprising a bacterium having an exogenous nif cluster having at least one inducible promoter, wherein the exogenous nif cluster confers nitrogen fixation capability on the bacterium, under aerobic free-living conditions, and wherein the bacterium is not *Azorhizobium caulinodans*.

14. The plant growth promoting bacterium of claim **13**, wherein the bacterium is a symbiotic bacterium.

15. The plant growth promoting bacterium of claim **13**, wherein the bacterium is an endophyte.

16. The plant growth promoting bacterium of claim **15**, wherein the endophyte is *rhizobium* IRBG74.

17. The plant growth promoting bacterium of claim **13**, wherein the bacterium is an epiphyte.

18. The plant growth promoting bacterium of claim **17**, wherein the epiphyte is *pseudomonas* protogens PF-5.

19. The plant growth promoting bacterium of any one of claims **13-18**, wherein the plant growth promoting bacterium is associated with a genetically modified cereal plant.

20. The plant growth promoting bacterium of claim **19**, wherein the genetically modified cereal plant includes an exogenous gene encoding a chemical signal.

21. The plant growth promoting bacterium of claim **19**, wherein the nitrogen fixation is under the control of the chemical signal.

22. The plant growth promoting bacterium of claim **20** or **21**, wherein the chemical signal is opine, phloroglucinol or rhizopene.

23. The *rhizobium* of any one of claims **13-22**, wherein the inducible promoter is a T7 promoter, and optionally wherein the inducible promoter is $P_{A1lacO1}$ promoter.

24. The *rhizobium* of any one of claims **13-23**, wherein the inducible promoter is activated by an agent selected from a group that includes IPTG, sodium salicylate, octapine, nopaline, the quorum signal 3OC6HSL, aTe, cuminic acid, DAPG, and salicylic acid.

25. The *rhizobium* of any one of claims **13-24**, wherein the inducible promoter has a terminator and wherein the terminator is downstream from the inducible promoter.

26. An *Azorhizobium caulinodans* capable of inducible ammonium-independent nitrogen fixation in a cereal crop, comprising:

(i) a modified nif cluster, wherein an endogenous nifA gene is deleted or altered; and

(ii) at least one operon comprising nifA and RNA polymerase sigma factor (RpoN), wherein the operon comprises a regulatory element including an inducible promoter.

27. The *Azorhizobium caulinodans* of claim **26**, wherein the endogenous nifA gene is altered with at least one of the following substitutions:

(i) L94Q;

(ii) D95Q; and

(iii) both L94Q and D95Q.

28. A method of engineering a *rhizobium* that can fix nitrogen under aerobic free-living conditions, comprising transferring an exogenous nif cluster to a symbiotic *rhizobium*, wherein the exogenous nif cluster confers nitrogen fixation capability on the symbiotic *rhizobium*, under aerobic free-living conditions, and wherein the *rhizobium* is not *Azorhizobium caulinodans*.

29. The method of any one of claims **26-28**, wherein the exogenous nif cluster is transferred to the *rhizobium* in a plasmid.

30. The method of any one of claim **28** or **29**, wherein the endogenous NifL gene is deleted.

31. A method of producing nitrogen for consumption by a cereal plant, comprising providing a plant growth promoting bacterium that can fix nitrogen under aerobic free-living conditions in proximity of the cereal plant, wherein the plant growth promoting bacterium is a symbiotic bacterium having an exogenous nif cluster, wherein the exogenous nif cluster confers nitrogen fixation capability on the symbiotic bacterium, enabling nitrogen fixation under aerobic free-living conditions.

32. The method of claim **31**, wherein the plant growth bacterium is the bacterium of any one of claims **1-19** and **23-39**.

33. The method of any one of claim **31** or **32**, wherein the cereal plant is a genetically modified cereal plant.

* * * * *