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Supplementary Information for

Optimizing *Rhizobium*-legume symbioses by simultaneous measurement of rhizobial competitiveness and N_2 fixation in nodules

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Supplementary Information Text

SI Materials and Methods.

Designing Ps*nifH* consensus promoter.

The sequence of *nifH* (pRL100162) and its upstream Inter Genic Region (IGR) from *R. leguminosarum* biovar *viciae* 3841 (Rlv3841), Rlv3841*nifH*+IGR, was used as a template to find other genomes of the genus *Rhizobium* in the Integrated Microbial Genomes (IMG) system (1). As a result, 118 genomes (some of them as draft genomes) were collected. *BLASTn* (DNA vs. DNA E-value of $1e^{-5}$) was run on the 118 genomes. Scaffolds were generated of each strain and those with an *E-value* of 0 were extracted. Each extraction was confirmed in the *National Centre for Biotechnology Information* (*NCBI)* (2) to verify they contained *nifH* (although some strains contained more than one copy of *nifH*). In these cases, only the copies with the complete *nifHDK* operons were considered for the design of the consensus *nifH* promoter. Following initial analysis, a new extraction was performed, this time only of the IGR-*nifH* and then aligned by ClustalW in the bioinformatics software Geneious® (Steps in Fig. S1). The final alignment of the consensus *nifH* IGR from 48 strains of the genus *Rhizobium* is 227 b (Rlv3841*nifH* IGR is 717 bp long (Fig. S2*a*)). The features are: BsaI sites with GGAC and AATG overhangs for Golden Gate assembly as a PU module (nt 6 through 11 and nt 247 through 242), a UAS (nt 42 through 57), the RpoN-binding site (nt 144 through 157), and the RBS (nt 225 through 231), with a start codon ATG at nt 238 (Fig. S2*b*).

Plasmid ID as a Golden Gate T module

A Universal Primer binding site (5'-CGTTTTACAACGTCGTGACTGGG-3') was added between the 12-nt Golay barcode and T0. *In silico* analysis (with up to 5 mismatches) of the Universal Primer was performed on the full plasmid maps to ensure that it binds only to this specific region. The final construction was completed with addition of type IIS restriction enzyme recognition sites and the specific overhangs at either end of a Golden Gate T module. Plasmid IDs were designed as sense and antisense oligonucleotides (Table S3), so that they can be used directly in a Golden Gate reaction after annealing, without requiring BsaI digestion.

Plasmid construction by Golden Gate Cloning

Golden Gate Level 1 cloning reactions were performed with T4 DNA ligase and BsaI in a one-tube one-step reaction (3). Final plasmids were confirmed by EcoRI digestion and sequenced to confirm the vector/insert junctions (using primers inTable S2).

Plasmid ID library

A total of 96 individual and unique reporter plasmids were built by Golden Gate Level 1 one-tube reaction (BsaI digestion and ligation) (3) with the following modules utilising a 'master-mix' method for 100 reactions: vector; pOGG026, PU module; P*nifH* from pOGG043, SC module; sfGFP from pOGG037. The mix was distributed into a 96-well plate and the product from annealing the oligonucleotide Plasmid IDs was added (T module) (Fig. S5) according to the position designated in Table S7 to make individual clones (Fig. S6 and Fig. S7). Chemically competent *E. coli* ST18 cells were used for transformations with 5-aminolevulinic acid (ALA) added to all media (final concentration 50 µg mL^{-1}).

High-throughput conjugation from *E. coli* into rhizobial strains in 96-well microtiter plates

Rhizobial strains to be conjugated (recipients) were inoculated into a 96-deep-well plate with 1.2 ml of media and relevant antibiotics, then covered with a sterile hydrophobic porous sealing film to reduce contamination. Strains were grown in a shaking incubator, 150 rpm at 28°C for 3 d. This plate was used for re-inoculation of 5 plates to allow concentration of a high number of cells. The day before the conjugation, *E. coli* ST18 (donor strain) was inoculated into 96-deep-well plate with 1.2 ml of media, relevant antibiotics and ALA, and covered with film. Strains were grown in a shaking incubator, 150 rpm at 37°C. On the day of the conjugation experiment, all plates were centrifuged at 4000 rpm for 15 min. The five rhizobial pellets were combined into a single plate to concentrate the bacterial cells. The final plate with rhizobial strains and that with *E. coli* ST18 were washed with fresh media three times to remove any traces of antibiotics. The final pellets were resuspended in 1.2 ml of TY media. In a PCR plate, 100 µl donor was mixed with 100 µl recipient and spun down at 4,000 rpm for 15 min. The supernatant was discarded, and pellet re-suspended in 30 µl of TY-ALA media. The re-suspended mix was transferred to a microtiter plate with 150 µl of TY-ALA agar and and covered with film. The plate was incubated for 48 h at 28°C. 100 µl TY was added to the bacteria microfilm growing on top of the agar and transferred to a 96-deep-well plate with 1.5 ml of TY media and relevant antibiotics. To have better aeration which results in an increase of bacterial growth, we prepared each 96-deep-well plate with a TY agar slope in each of the well. The 96-deep-well plate was incubated in a shaking incubator, 150 rpm at 28°C for 3 d. Serial dilutions were done and 100µl of each conjugation was plated onto TY agar (with relevant antibiotics) to get single colonies. Single colonies were re-grown in a 96-deep-well plate with TY liquid media with relevant antibiotics in a shaking incubator, 150 rpm at 28 °C for 3 d. OD_{600} of a 200 µl sample from each well was measured to confirm bacterial growth (where OD_{600} 1.0 = $1x10^9$ cfu) using a FLUOStar Omega Microplate Reader (BMG). To eliminate possible spontaneous resistance to relevant antibiotics, positive plasmid acquisition was verified by colony PCR. Final conjugations shown in Fig. S13.

Primer design for multiplex Ion Torrent sequencing strategy

We designed primers (Table S4) for a two-step polymerase chain reaction (PCR) (4) taking into account the following restrictions: 1) First-step PCR primers needed to add specific barcodes to our amplicon sequence with the aim of identifying column and row samples in a 96-well plate, 2) should insert 'landing pads' for the second-step PCR preventing hairpin formation or binding to our amplicon sequence, 3) Second-step forward primers should add Ion Xpress™ Barcodes for library identification, 4) Second-step reverse primer should introduce the trP1 sequencing adapter and 5) the final sequencing template should not exceed 200bp in length. The size of the First-step PCR forward primers was minimized to include only 18 nt from the original 29 nt of the 'landing pad' compatible with Second-step PCR Forward primers. A landing pad for synthetic sequences was designed based on the Illumina Nextera transposase sequence (5). All primers were analyzed *in silico* as individual units and as a complete sequence, and no hairpin formations were found with up to five mismatches.

Ion Torrent sequencing and analysis

Sequencing results were analysed following the workflow in Fig. S8**.** Pre-processing steps were carried out with Trimmomatic-0.36 (6). We developed a Python script including the Biopython (7) and Pandas (8) libraries (Fig. S9) to separate and count the reads by amplicon (Plasmid ID) and sample-specific (primer barcodes). Script available in: script available in: https://github.com/marcelamendoza/Plasmid-ID.

Data manipulation was carried out in Microsoft Excel using PivotTables. After the high-quality filtering, where no mismatches were allowed, all samples identified with reads below 2% of the total number of unique sequences were removed to reduce false positive reads (9).

Extraction of DNA from nodules

Protocol used was modified from that of (10). Nodules were crushed in Eppendorf tubes and alkaline PEG 200 added at 10× sample volume. Samples were incubated for 15 min at 60°C and vortexed. Plant tissue was precipitated at 1,000 rpm for 15 min. The supernatant was transfered to sterile PCR-tubes and used in a 20 - 50 µL PCR reaction.

General plant growth

P. sativum cv. Aveola (Thompson and Morgan, UK), common bean (Tendergreen) (Thompson and Morgan, UK) white clover and soybean seeds were surface-sterilized by immersing in 95% ethanol for 30 sec, followed by washing with sterile water. Seeds were then immersed in 2% sodium hypochlorite for 5 min. After washing the seeds 5× with sterile water, they were placed onto 1% DWA plates and incubated in the dark at room temperature for 4-5 d. Alfalfa seeds were sterilized with 75% ethanol for 45 min with

continuous mixing, followed by washing with sterile water, and 2% sodium hypochlorite for 15 min with continuous mixing. After washing the seeds several times with sterile water, these were placed onto DWA plates and placed in the dark at room temperature for 2 d. Seedlings were placed in sterile 1 L pots with medium vermiculite and supplied with 400 ml nitrogen-free rooting solution (11). Common bean and soybean were grown in fine vermiculite and supplied with 400 ml of bean nitrogen-free rooting solution. The inoculation was approximately $1x10^7$ cfu of rhizobial strain per pot. Plants without inoculation were grown as a negative control (water control - WC). Plants were grown (random positioning of pots) in a controlled growth chamber at 21°C, 16-h/8-h day/night cycle. Alfalfa, white clover and pea plants were harvested at 21 dpi. Common bean and soybean plants were harvested at 42 dpi.

Competition assay: co-inoculation with two strains

Rhizobium cultures to be used as inoculum were grown to early stationary phase (OD_{600} < 0.6 - 0.8). Cultures were adjusted to 1000 cfu/ ml. An inoculum mix was prepared with approx. 1000 cfu in total of three different inoculation ratios: 10:1, 1:1 and 1:10 strain A vs strain B, This mix was added to 75 ml 2.6× concentrated nitrogen-free rooting solution (11) and distributed homogenously into 500 ml sterile pots with a mix of 50% silver sand/fine vermiculite. Similar sized 5-d old pea seedlings were transferred to pre-inoculated pots. Plants without inoculum were grown as a negative control (Water Control or WC). Plants were harvested at 21 dpi and were sequentially stained first with Magenta-glcA and then with X-gal after thermal treatment of 1 h at 70°C (12).

Assessment of Effectiveness at N_2-fixation

Full methodology described in (13). For measurement of shoot DW, pea plants were grown for 6 wk in 2-L pots. Shoots were cut, placed in paper envelopes and dried at 70 °C for 48 h. Dried shoots were weighed using an Acculab Sartorius Group scale model ALC-80-40.

Analysis of agricultural soil for competition assays

Soil was dried it at room temperature for 2 wk, then mixed thoroughly and removed stones with a 5 mm pore-diameter sieve. Soil samples were sent to James Hutton Limited (Aberdeen, Scotland) where they were stored at 4°C on receipt. A sub-sample was obtained and air dried (30°C) prior to sieving to pass a 2mm aperture. Determination of soil pH and elemental concentrations extracted with 0.43M acetic acid were obtained from the air-dry soil. Elemental concentrations were determined using inductively coupled plasma–optical emission spectroscopy (ICP-OES). Nitrate and ammonium concentrations were obtained using colourimetry after extraction of the field moist soil with 1 M KCl. The moisture content of both the air-dried (30°C) and field moist soil were determined at 105°C (Table S8).

Most probable number (MPN) of indigenous rhizobia from Yatesbury soil

Full methodology described in (13). Surface-sterilized pea (Avolar) seeds were placed in boiling tubes filled with sterile fine vermiculite and 20 ml nitrogen-free rooting solution (4 biological replicates) (11). WC for each dilution was placed randomly amongst the inoculated plants. Pea plants were harvested at 21 dpi and assessed for the presence (nodulation-positive) or absence (nodulation-negative) of nodules.

Image acquisition for green and red fluorescence expression

Samples were exposed for 1 s with filters for GFP (excitation 475/20 nm and emission 520/10 nm) and mCherry (excitation 550/10 nm and emission 620/10 nm). All images were analyzed with the software IndiGO (Berthold Technologies).

Image acquisition for a full root system (peas)

For a full root system, a blue-light transilluminator (VWR) together with a Kodak Wratten Gelatine Filter no 49 were used. All images were analysed with the software IndiGO (Berthold Technologies). To assess root systems that had nodules formed by indigenous rhizobia and nodules formed by tagged-strains expressing GFP, roots were exposed to a blue-light transilluminator. A mobile phone camera with an orange filter was used to photograph fluorescent nodules. Nodules were counted using open-source image analysis Fiji (14).

Confocal microscopy for bacteroids analysis

Pea nodules (42 dpi) were crushed and resuspended in 200 µl of water. 20 µl was placed on a microscope slide to analyse individual bacteroids. A GFP TIF image stack was loaded into MorphoGraphX (15), filtered using a Gaussian Blur Filter with a radius of 0.1 µm, and converted into a binary stack (threshold: 800). Meshes were generated using the Marching Cubes Algorithm at a cube spacing of 0.1 µm (3x smoothing) to obtain the volume of individual bacteroids. Individual bacteroids were color-identified according to volume.

SUPPORTING INFORMATION

Table S1. Strains and plasmids used in this study.

Strain	Details	Source
***E. coli* strains**		
α-Select Gold	Competent cells. Genotype: F - *deoR endA1 recA1 relA1 gyrA96 hsdR17(rk -, mk +) supE44 thi-1 phoA Δ(lacZYA argF)U169 Φ80lacZΔM15*λ -	Bioline
DH5α	Competent cells. Genotype: F- *Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17(rk-, mk+) phoA supE44 thi-1 gyrA96 relA1* λ-	Invitrogen
ST18	S17 *Δpir ΔhemA*	(16)
Rhizobial strains		
Rlv3841	*Rhizobium leguminosarum* bv. *viciae,* derivative of strain 300, Str^r	(17)
RlvA34	*R. leguminosarum* bv. *viciae*, Str^r	(18)
CFN42	*Rhizobium etli*	(19)
CIAT899	*Rhizobium tropici*, Rf^r	(20)
4292	*R. leguminosarum* bv. *phaseoli*, Rf^r	(18)
WSM1325	*R. leguminosarum* bv. *trifolii*	(21)
WSM419	*Sinorhizobium medicae*	(22)
HH103	*Sinorhizobium fredii*	(23)
WSM1521	Host of origin: *Lathyrus*. Effectiveness class in pea: high, Nit^r	John Howieson
54 n4	Host of origin: *P. sativum*. Effectiveness class in pea: high, Nit^r	John Howieson
60 n1	Host of origin: *P. sativum*. Effectiveness class in pea: medium, Nit^r	John Howieson
71 n4	Host of origin: *P. sativum*. Effectiveness class in pea: medium, Nit^r	John Howieson
SARDI 962	Host of origin: *P. sativum*. Effectiveness class in pea: low, Nit^r	John Howieson
SU303	Host of origin: unknown. Effectiveness class in pea: high, Nit^r	John Howieson
WSM1455	Host of origin: *V. faba*. Effectiveness class in pea: medium, Nit^r	John Howieson
WSM1475	Host of origin: *V. faba*. Effectiveness class in pea: medium, Nit^r	John Howieson
WSM1480	Host of origin: *V. faba*. Effectiveness class in pea: medium, Nit^r	John Howieson
WSM1481	Host of origin: *Vicia* spp. Effectiveness class in pea: low, Nit^r	John Howieson
WSM1488	Host of origin: *V. faba*. Effectiveness class in pea: low, Nit^r	John Howieson
WSM1529	Host of origin: *V. faba*. Effectiveness class in pea: high, Nit^r	John Howieson
WSM4458	Host of origin: *P. sativum*. Effectiveness class in pea: low, Nit^r	John Howieson
WSM4459	Host of origin: *P. sativum*. Effectiveness class in pea: low, Nit^r	John Howieson

WSM4460	Host of origin: *P. sativum*. Effectiveness class in pea: medium, Nit^r	John Howieson
WSM4461	Host of origin: *P. sativum*. Effectiveness class in pea: high, Nit^r	John Howieson
WSM4462	Host of origin: *P. sativum*. Effectiveness class in pea: low, Nit^r	John Howieson
WSM4645	Host of origin: *P. sativum*. Effectiveness class in pea: high, Nit^r	John Howieson
WSM937	Effectiveness class in pea: high, Nit^r	John Howieson
3841*celB*	*R. leguminosarum* bv. *viciae*, Str^r	(24)
UPM791*gusA*	*R. leguminosarum* bv. *viciae*, Str^r	(24)
UPM791	*R. leguminosarum* bv. *viciae*, Str^r	(24)
VSX1	*R. leguminosarum* bv. *viciae*, Nit^r	(25)
VXS7	*R. leguminosarum* bv. *viciae*, Nit^r	(25)
VSX10	*R. leguminosarum* bv. *viciae*, Nit^r	(25)
VSX11	*R. leguminosarum* bv. *viciae*, Nit^r	(25)
VSX16	*R. leguminosarum* bv. *viciae*, Nit^r	(25)
VSX28	*R. leguminosarum* bv. *viciae*, Nit^r	(25)
VSX32	*R. leguminosarum* bv. *viciae*, Nit^r	(25)
13	*Rhizobium* sp., Nit^r	Euan James
23_1	*Rhizobium* sp., Nit^r	Euan James
24	*Rhizobium* sp., Nit^r	Euan James
263	*Rhizobium* sp., Nit^r	Euan James
2B-1	*Rhizobium* sp., Nit^r	Euan James
364	*Rhizobium* sp., Nit^r	Euan James
367	*Rhizobium* sp., Nit^r	Euan James
370	*Rhizobium* sp., Nit^r	Euan James
387	*Rhizobium* sp., Nit^r	Euan James
388	*Rhizobium* sp., Nit^r	Euan James
42	*Rhizobium* sp., Nit^r	Euan James
535	*Rhizobium* sp., Nit^r	Euan James
536	*Rhizobium* sp., Nit^r	Euan James
585	*Rhizobium* sp., Nit^r	Euan James
CellTech	*Rhizobium* sp., Nit^r	Euan James
Corus 1+2 1-1	*Rhizobium* sp., Nit^r	Euan James
Corus 16 2-2	*Rhizobium* sp., Nit^r	Euan James
E2-1A	*Rhizobium* sp., Nit^r	Euan James
E2-1B	*Rhizobium* sp., Nit^r	Euan James
JED2	*Rhizobium* sp., Nit^r	Euan James
LA-2A	*Rhizobium* sp., Nit^r	Euan James
LegTech	*Rhizobium* sp., Nit^r	Euan James
Magnus 1+2 (2)	*Rhizobium* sp., Nit^r	Euan James
Magnus 16 (2)	*Rhizobium* sp., Nit^r	Euan James
PB3-3	*Rhizobium* sp., Nit^r	Euan James
PB6-3B	*Rhizobium* sp., Nit^r	Euan James
PEA NW	*Rhizobium* sp., Nit^r	Euan James

VF2	*Rhizobium* sp., Nit^r	Euan James
VF5	*Rhizobium* sp., Nit^r	Euan James
G004	Isolated using legume host: *P. sativum*, Nit^r	(26)
G007	Isolated using legume host: *P. sativum*, Nit^r	(26)
G008	Isolated using legume host: *P. sativum*, Nit^r	(26)
G011	Isolated using legume host: *P. sativum*, Nit^r	(26)
G016	Isolated using legume host: *P. sativum*, Nit^r	(26)
G028	Isolated using legume host: *P. sativum*, Nit^r	(26)
G051	Isolated using legume host: *P. sativum*, Nit^r	(26)
G067	Isolated using legume host: *P. sativum*, Nit^r	(26)
G073	Isolated using legume host: *P. sativum*, Nit^r	(26)
G077	Isolated using legume host: *P. sativum*, Nit^r	(26)
G083	Isolated using legume host: *P. sativum*, Nit^r	(26)
G088	Isolated using legume host: *P. sativum*, Nit^r	(26)
G093	Isolated using legume host: *P. sativum*, Nit^r	(26)
G094	Isolated using legume host: *P. sativum*, Nit^r	(26)
G099	Isolated using legume host: *P. sativum*, Nit^r	(26)
G108	Isolated using legume host: *P. sativum*, Nit^r	(26)
G109	Isolated using legume host: *P. sativum*, Nit^r	(26)
H005	Isolated using legume host: *V. faba*, Nit^r	(26)
H009	Isolated using legume host: *V. faba*, Nit^r	(26)
H011	Isolated using legume host: *V. faba*, Nit^r	(26)
H012	Isolated using legume host: *V. faba*, Nit^r	(26)
H031	Isolated using legume host: *V. faba*, Nit^r	(26)
H082	Isolated using legume host: *V. faba*, Nit^r	(26)
H127	Isolated using legume host: *V. faba*, Nit^r	(26)
H130	Isolated using legume host: *V. faba*, Nit^r	(26)
H174	Isolated using legume host: *V. faba*, Nit^r	(26)
H178	Isolated using legume host: *V. faba*, Nit^r	(26)
L002	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L008	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L010	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L018	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L019	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L070	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L074	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L079	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L082	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L102	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L104	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L111	Isolated using legume host: *L. culinaris*, Nit^r	(26)
L117	Isolated using legume host: *L. culinaris*, Nit^r	(26)
V002	Isolated using legume host: *V. sativa*, Nit^r	(26)
V004	Isolated using legume host: *V. sativa*, Nit^r	(26)
V006	Isolated using legume host: *V. sativa*, Nit^r	(26)

V008	Isolated using legume host: *V. sativa*, Nit^r	(26)
V010	Isolated using legume host: *V. sativa*, Nit^r	(26)
V014	Isolated using legume host: *V. sativa*, Nit^r	(26)
V030	Isolated using legume host: *V. sativa*, Nit^r	(26)
V043	Isolated using legume host: *V. sativa*, Nit^r	(26)
V050	Isolated using legume host: *V. sativa*, Nit^r	(26)
V057	Isolated using legume host: *V. sativa*, Nit^r	(26)
V060	Isolated using legume host: *V. sativa*, Nit^r	(26)
V067	Isolated using legume host: *V. sativa*, Nit^r	(26)
V068	Isolated using legume host: *V. sativa*, Nit^r	(26)
V069	Isolated using legume host: *V. sativa*, Nit^r	(26)
V074	Isolated using legume host: *V. sativa*, Nit^r	(26)
V100	Isolated using legume host: *V. sativa*, Nit^r	(26)
V116	Isolated using legume host: *V. sativa*, Nit^r	(26)
OPS0657	Rlv3841[pOPS0253], Kan^r/Nm^r, Str^r ^{r}	This work
OPS0658	Rlv3841[pOPS0254], Kan^r/Nm^r, Str^r	This work
OPS0660	Rlv3841[pOPS0263], Kan^r/Nm^r, Str^r	This work
OPS0717	HH103[pOPS0262], Kan^r/Nm^r, Nit^r	This work
OPS0718	HH103[pOPS0263], Kan^r/Nm^r, Nit^r	This work
OPS0719	CFN42[pOPS0262], Kan^r/Nm^r, Nit^r	This work
OPS0720	CFN42[pOPS0263], Kan^r/Nm^r, Nit^r	This work
OPS0721	WSM419[pOPS0262], Kan^r/Nm^r, Chl^r	This work
OPS0722	WSM419[pOPS0263], Kan^r/Nm^r, Chl^r	This work
OPS0723	Sm1022[pOPS0262], Kan^r/Nm^r, Chl^r	This work
OPS0724	Sm1022[pOPS0263], Kan^r/Nm^r, Chl^r	This work
OPS0725	Sm1021[pOPS0262], Kan^r/Nm^r, Str^r	This work
OPS0726	Sm1021[pOPS0263], Kan^r/Nm^r, Str^r	This work
OPS0729	RlvA34[pOPS0262], Kan^r/Nm^r, Str^r	This work
OPS0730	RlvA34[pOPS0263], Kan^r/Nm^r, Str^r	This work
OPS0731	Rl bv. *phaseoli* 4292[pOPS0262], Kan^r/Nm^r, Rf^r	This work
OPS0732	Rl bv. *phaseoli* 4292[pOPS0263], Kan^r/Nm^r, Rf^r	This work
OPS0733	CIAT899[pOPS0262], Kan^r/Nm^r, Rf^r	This work
OPS0734	CIAT899[pOPS0263], Kan^r/Nm^r, Rf^r	This work
OPS0736	WSM1325[pOPS0262], Kan^r/Nm^r, Nit^r	This work
OPS0737	WSM1325[pOPS0263], Kan^r/Nm^r, Nit^r	This work
OPS0738	Rlv3841[pOPS0262], Kan^r/Nm^r, Str^r	This work
OPS0816	Rlv3841[pOPS0314], Kan^r/Nm^r, Str^r	This work
OPS0842	WSM1488[pLMB640], Tet^r, Nit^r	This work
OPS0845	WSM1481[pLMB640], Tet^r, Nit^r	This work
OPS0852	UPM791[pLMB640], Tet^r, Str^r	This work
OPS0898	Rlv3841[pOPS0384], Kan^r/Nm^r, Str^r	This work
OPS0899	Rlv3841[pOPS0378], Kan^r/Nm^r, Str^r	This work
OPS0900	Rlv3841[pOPS0380], Kan^r/Nm^r, Str^r	This work
OPS0901	Rlv3841[pOPS0382], Kan^r/Nm^r, Str^r	This work
OPS0902	Rlv3841[pOPS0383], Kan^r/Nm^r, Str^r	This work

OPS0903	Rlv3841[pOPS0377], Kan^r/Nm^r, Str^r	This work
OPS0904	Rlv3841[pOPS0379], Kan^r/Nm^r, Str^r	This work
OPS0905	Rlv3841[pOPS0381], Kan^r/Nm^r, Str^r	This work
OPS1086	Rlv3841[pOPS0750], Kan^r/Nm^r, Str^r	This work
OPS1087	UPM791[pOPS0750], Kan^r/Nm^r, Str^r	This work
OPS1511	Rlv3841[pOPS0385], Kan^r/Nm^r, Str^r	This work
OPS1511	Rlv3841[pOPS0385], Kan^r/Nm^r, Str^r	This work
OPS1512	Rlv3841[pOPS0387], Kan^r/Nm^r, Str^r	This work
OPS1513	Rlv3841[pOPS0386], Kan^r/Nm^r, Str^r	This work
OPS1514	UPM791gusA[pOPS0548], Kan^r/Nm^r, Str^r	This work
OPS1515	Rlv3841celB[pOPS0564], Kan^r/Nm^r, Str^r	This work
OPS1516	Rlv3841[pOPS0491], Kan^r/Nm^r, Str^r	This work
OPS1517	Rlv3841[pOPS0503], Kan^r/Nm^r, Str^r	This work
OPS1518	UPM791[pOPS0491], Kan^r/Nm^r, Str^r	This work
OPS1519	UPM791[pOPS0503], Kan^r/Nm^r, Str^r	This work
OPS1520	WSM1521[pOPS0383], Kan^r/Nm^r, Str^r	This work
OPS1521	WSM1481[pOPS0383], Kan^r/Nm^r, Str^r	This work
OPS1522	WSM1475[pOPS0515], Kan^r/Nm^r, Str^r	This work
OPS1523	WSM1529[pOPS0539], Kan^r/Nm^r, Str^r	This work
OPS1524	WSM1521[pOPS0504], Kan^r/Nm^r, Str^r	This work
OPS1525	WSM1481[pOPS0527], Kan^r/Nm^r, Str^r	This work
OPS1526	Rlv SU303[pOPS0383], Kan^r/Nm^r, Str^r	This work
13 [pOPS0515]	LB-ID 13[pOPS0515], Kan^r/Nm^r, Nit^r	This work
23--1 [pOPS0522]	LB-ID 23--1[pOPS0522], Kan^r/Nm^r, Nit^r	This work
24 [pOPS0539]	LB-ID 24[pOPS0539], Kan^r/Nm^r, Nit^r	This work
2B-1 [pOPS0570]	LB-ID 2B-1[pOPS0570], Kan^r/Nm^r, Nit^r	This work
364 [pOPS0505]	LB-ID 364[pOPS0505], Kan^r/Nm^r, Nit^r	This work
367 [pOPS0545]	LB-ID 367[pOPS0545], Kan^r/Nm^r, Nit^r	This work
387 [pOPS0534]	LB-ID 387[pOPS0534], Kan^r/Nm^r, Nit^r	This work
536 [pOPS0542]	LB-ID 536[pOPS0542], Kan^r/Nm^r, Nit^r	This work
CellTech [pOPS0503]	LB-ID CellTech[pOPS0503], Kan^r/Nm^r, Nit^r	This work
CORUS 1+2 [pOPS0509]	LB-ID CORUS 1+2[pOPS0509], Kan^r/Nm^r, Nit^r	This work
CORUS 16 [pOPS0508]	LB-ID CORUS 16[pOPS0508], Kan^r/Nm^r, Nit^r	This work
E2-1A [pOPS0582]	LB-ID E2-1A[pOPS0582], Kan^r/Nm^r, Nit^r	This work
E2-1B [pOPS0540]	LB-ID E2-1B[pOPS0540], Kan^r/Nm^r, Nit^r	This work
G004 [pOPS0519]	LB-ID G004 pOPS0519], Kan^r/Nm^r, Nit^r	This work
G007 [pOPS0529]	LB-ID G007[pOPS0529], Kan^r/Nm^r, Nit^r	This work

G008 [pOPS0532]	LB-ID G008[pOPS0532], Kan^r/Nm^r, Nit^r	This work
G011 [pOPS0501]	LB-ID G011[pOPS0501], Kan^r/Nm^r, Nit^r	This work
G016 [pOPS0571]	LB-ID G016[pOPS0571], Kan^r/Nm^r, Nit^r	This work
G028 [pOPS0566]	LB-ID G028[pOPS0566], Kan^r/Nm^r, Nit^r	This work
G051 [pOPS0530]	LB-ID G051[pOPS0530], Kan^r/Nm^r, Nit^r	This work
G067 [pOPS0567]	LB-ID G067[pOPS0567], Kan^r/Nm^r, Nit^r	This work
G073 [pOPS0521]	LB-ID G073[pOPS0521], Kan^r/Nm^r, Nit^r	This work
G077 [pOPS0520]	LB-ID G077[pOPS0520], Kan^r/Nm^r, Nit^r	This work
G083 [pOPS0553]	LB-ID G083[pOPS0553], Kan^r/Nm^r, Nit^r	This work
G088 [pOPS0568]	LB-ID G088[pOPS0568], Kan^r/Nm^r, Nit^r	This work
G093 [pOPS0551]	LB-ID G093[pOPS0551], Kan^r/Nm^r, Nit^r	This work
G094 [pOPS0556]	LB-ID G094[pOPS0556], Kan^r/Nm^r, Nit^r	This work
G099 [pOPS0552]	LB-ID G099[pOPS0552], Kan^r/Nm^r, Nit^r	This work
G108 [pOPS0555]	LB-ID G108[pOPS0555], Kan^r/Nm^r, Nit^r	This work
G109 [pOPS0554]	LB-ID G109[pOPS0554], Kan^r/Nm^r, Nit^r	This work
H009 [pOPS0536]	LB-ID H009[pOPS0536], Kan^r/Nm^r, Nit^r	This work
H012 [pOPS0524]	LB-ID H012[pOPS0524], Kan^r/Nm^r, Nit^r	This work
H031 [pOPS0584]	LB-ID H031[pOPS0584], Kan^r/Nm^r, Nit^r	This work
H082 [pOPS0564]	LB-ID H082[pOPS0564], Kan^r/Nm^r, Nit^r	This work
H127 [pOPS0548]	LB-ID H127[pOPS0548], Kan^r/Nm^r, Nit^r	This work
H130 [pOPS0560]	LB-ID H130[pOPS0560], Kan^r/Nm^r, Nit^r	This work
H174 [pOPS0565]	LB-ID H174[pOPS0565], Kan^r/Nm^r, Nit^r	This work
H178 [pOPS0572]	LB-ID H178[pOPS0572], Kan^r/Nm^r, Nit^r	This work
JED2 [pOPS0504]	LB-ID JED2[pOPS0504], Kan^r/Nm^r, Nit^r	This work
L008 [pOPS0557]	LB-ID L008[pOPS0557], Kan^r/Nm^r, Nit^r	This work
L010 [pOPS0575]	LB-ID L010[pOPS0575], Kan^r/Nm^r, Nit^r	This work
L018 [pOPS0576]	LB-ID L018[pOPS0576], Kan^r/Nm^r, Nit^r	This work
L019 [pOPS0558]	LB-ID L019[pOPS0558], Kan^r/Nm^r, Nit^r	This work

L070 [pOPS0577]	LB-ID L070[pOPS0577], Kan^r/Nm^r, Nit^r	This work
L074 [pOPS0578]	LB-ID L074[pOPS0578], Kan^r/Nm^r, Nit^r	This work
L079 [pOPS0579]	LB-ID L079[pOPS0579], Kan^r/Nm^r, Nit^r	This work
L082 [pOPS0580]	LB-ID L082[pOPS0580], Kan^r/Nm^r, Nit^r	This work
L102 [pOPS0527]	LB-ID L102[pOPS0527], Kan^r/Nm^r, Nit^r	This work
L104 [pOPS0528]	LB-ID L104[pOPS0528], Kan^r/Nm^r, Nit^r	This work
LegTech [pOPS0518]	LB-ID LegTech[pOPS0518], Kan^r/Nm^r, Nit^r	This work
PB3-3 [pOPS0541]	LB-ID PB3-3[pOPS0541], Kan^r/Nm^r, Nit^r	This work
PB6-3B [pOPS0506]	LB-ID PB6-3B[pOPS0506], Kan^r/Nm^r, Nit^r	This work
PeanNW [pOPS0544]	LB-ID PeanNW[pOPS0544], Kan^r/Nm^r, Nit^r	This work
Rlv3841 [pOPS0492]	LB-ID Rlv3841[pOPS0492], Kan^r/Nm^r, Nit^r	This work
SU303 [pOPS0517]	LB-ID SU303[pOPS0517], Kan^r/Nm^r, Nit^r	This work
UPM791 [pOPS0491]	LB-ID UPM791[pOPS0491], Kan^r/Nm^r, Str^r	This work
V002 [pOPS0513]	LB-ID V002[pOPS0513], Kan^r/Nm^r, Nit^r	This work
V004 [pOPS0525]	LB-ID V004[pOPS0525], Kan^r/Nm^r, Nit^r	This work
V006 [pOPS0533]	LB-ID V006[pOPS0533], Kan^r/Nm^r, Nit^r	This work
V008 [pOPS0537]	LB-ID V008[pOPS0537], Kan^r/Nm^r, Nit^r	This work
V010 [pOPS0549]	LB-ID V010[pOPS0549], Kan^r/Nm^r, Nit^r	This work
V014 [pOPS0500]	LB-ID V014[pOPS0500], Kan^r/Nm^r, Nit^r	This work
V030 [pOPS0561]	LB-ID V030[pOPS0561], Kan^r/Nm^r, Nit^r	This work
V050 [pOPS0573]	LB-ID V050[pOPS0573], Kan^r/Nm^r, Nit^r	This work
V057 [pOPS0585]	LB-ID V057[pOPS0585], Kan^r/Nm^r, Nit^r	This work
V060 [pOPS0499]	LB-ID V060[pOPS0499], Kan^r/Nm^r, Nit^r	This work
V067 [pOPS0523]	LB-ID V067[pOPS0523], Kan^r/Nm^r, Nit^r	This work
V068 [pOPS0511]	LB-ID V068[pOPS0511], Kan^r/Nm^r, Nit^r	This work
V069 [pOPS0535]	LB-ID V069[pOPS0535], Kan^r/Nm^r, Nit^r	This work
V074 [pOPS0547]	LB-ID V074[pOPS0547], Kan^r/Nm^r, Nit^r	This work
V100 [pOPS0559]	LB-ID V100[pOPS0559], Kan^r/Nm^r, Nit^r	This work

V116 [pOPS0531]	LB-ID V116[pOPS0531], Kan^r/Nm^r, Nit^r	This work
VF2 [pOPS0507]	LB-ID VF2[pOPS0507], Kan^r/Nm^r, Nit^r	This work
VF5 [pOPS0516]	LB-ID VF5[pOPS0516], Kan^r/Nm^r, Nit^r	This work
VSX1 [pOPS0502]	LB-ID VSX1[pOPS0502], Kan^r/Nm^r, Nit^r	This work
VSX10 [pOPS0526]	LB-ID VSX10[pOPS0526], Kan^r/Nm^r, Nit^r	This work
VSX11 [pOPS0538]	LB-ID VSX11[pOPS0538], Kan^r/Nm^r, Nit^r	This work
VSX16 [pOPS0550]	LB-ID VSX16[pOPS0550], Kan^r/Nm^r, Nit^r	This work
VSX28 [pOPS0574]	LB-ID VSX28[pOPS0574], Kan^r/Nm^r, Nit^r	This work
VSX32 [pOPS0586]	LB-ID VSX32[pOPS0586], Kan^r/Nm^r, Nit^r	This work
VSX7 [pOPS0514]	LB-ID VSX7[pOPS0514], Kan^r/Nm^r, Nit^r	This work
WSM1475 [pOPS0493]	LB-ID WSM1475[pOPS0493], Kan^r/Nm^r, Nit^r	This work
WSM1481 [pOPS0494]	LB-ID WSM1481[pOPS0494], Kan^r/Nm^r, Nit^r	This work
WSM1488 [pOPS0496]	LB-ID WSM1488[pOPS0496], Kan^r/Nm^r, Nit^r	This work

Plasmid		Source	Addgene ID
EC15071	pL0M-SC-mCherry. Level 0 SC module cloned in pMS, Sp^r	ENSA	
pOGG003	pL0M-T-pharma. Level 0 T module cloned in pMS, Sp^r	Invitrogen	
pOGG004	pLVC-P1-Lv1 (Golden Gate Level 1 cloning site with cloned *lacZ*) position 1 module for vector construction cloned in pMS, Sp^r	(27)	113979
pOGG008	pLVC-P2-neo, neomycin-resistance gene, position 2 module for vector construction cloned in pMS, Sp^r, Nm^r/Kan^r	(27)	113982
pOGG010	pLVC-P3-RK2, RK2 origin of replication and *oriT* from *E. coli*, position 3 module for vector construction cloned in pMS, Sp^r	(27)	113984
pOGG012	pLVC-P4-par, partition genes (*parABCDE* from pMS) for plasmid stability, position 4 module for vector construction cloned in pMS, Sp^r	(27)	113986
pOGG014	pLVC-ELT-4, connecting position 4 to position 1 to circularise vector, endlinker module for vector construction cloned in pMS, Sp^r	(27)	113988
pOGG026	pL1V-Lv1-neo-RK2-par-ELT4, 6.6 kb, low copy, environmentally-stable, broad-host range Level 1 cloning vector, Nm^r/Kan^r	(27)	113992
pOGG037	pL0M-SC-sfGFP, pMS Level 0 SC module cloned in pMS, Sp^r	(27)	113995
pOGG043	pL0M-PU-psNifH. Synthetic consensus promoter to drive nodule-specific expression in biovars and strains of *R.*	This work	133123

	leguminosarum. Level 0 PU module synthesised as a fragment, Sp^r		
pOGG050	pL0M-SC-celB. Level 0 SC module cloned in pMS, Sp^r, Spc^r	(27)	113999
pOGG054	Destination vector for pL1V-F2, Amp^r	(28)	
pOGG068	Destination vector for pL0V-PU, Sp^r	(28)	
pOGG072	Destination vector for pL0V-SC, Sp^r	(28)	
pOGG082	pL0M-PU-pNifH, promoter 714 bp upstream of the ATG of *nifH* for nodule-specific gene expression in *R. leguminosarum*. Made by PCR using oxp0474 and oxp0475 primers and cloned in destination vector pOGG068, Sp^r, Spc^r	(27)	114000
pOGG083	pL0M-SC-gusA, *gusA* from pJP2. Made by PCR using oxp0376 and oxp0377 primers and cloned in destination vector pOGG072, Sp^r, Spc^r	(27)	114001
pOPS0253	Reporter plasmid for *R. leguminosarum* constructed with Rlv3841pNifH (pOGG082), *gusA* (pOGG083) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	(27)	115505
pOPS0254	Reporter plasmid for *R. leguminosarum* constructed with Rlv3841pNifH (pOGG082), *celB* (pOGG050) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	(27)	115506
pOPS0262	Reporter plasmid for *R. leguminosarum* constructed with psNifH (pOGG043), *celB* (pOGG050) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133227
pOPS0263	Reporter plasmid for *R. leguminosarum* constructed with psNifH (pOGG043), *gusA* (pOGG083) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133228
pOPS0314	Reporter plasmid for *R. leguminosarum* constructed with constitutive promoter pNeo (pOGG001), *celB* (pOGG050) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	(27)	115507
pOPS0377	Reporter plasmid for *R. leguminosarum* constructed with constitutive promoter pNeo (pOGG001), sfGFP (pOGG037) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	(27)	115509
pOPS0378	Reporter plasmid for *R. leguminosarum* constructed with constitutive promoter pNeo (pOGG001), mCherry (EC15071) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133229
pOPS0379	Reporter plasmid for *R. leguminosarum* constructed with constitutive promoter Rlv3841pNifH (pOGG082), sfGFP (pOGG037) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	(27)	115510
pOPS0380	Reporter plasmid for *R. leguminosarum* constructed with constitutive promoter Rlv3841pnifH (pOGG082), mCherry (EC15071) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133230
pOPS0381	Reporter plasmid for *R. leguminosarum* constructed with constitutive promoter psNifH (pOGG043), sfGFP (pOGG037) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133231
pOPS0382	Reporter plasmid for *R. leguminosarum* constructed with constitutive promoter psNifH (pOGG043), mCherry (EC15071) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133232

pOPS0383	Reporter plasmid for *R. leguminosarum* constructed with NoP (pOGG113), sfGFP (pOGG037) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133233
pOPS0384	Reporter plasmid for *R. leguminosarum* constructed with NoP (pOGG113), mCherry (EC15071) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133234
pOPS0385	Reporter plasmid for *R. leguminosarum* constructed with NoP (pOGG113), *gusA* (pOGG083) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133235
pOPS0386	Reporter plasmid for *R. leguminosarum* constructed with NoP (pOGG113), *celB* (pOGG050) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133236
pOPS0491	psNifH-sfGFP-A1 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TCCCTTGTCTCC Golay reference name:806rcbc0. Nm^r/Kan^r	This work	133132
pOPS0492	psNifH-sfGFP-A2 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ACGAGACTGATT Golay reference name:806rcbc1. Nm^r/Kan^r	This work	133133
pOPS0493	psNifH-sfGFP-A3 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GCTGTACGGATT Golay reference name:806rcbc2. Nm^r/Kan^r	This work	133134
pOPS0494	psNifH-sfGFP-A4 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ATCACCAGGTGT Golay reference name:806rcbc3. Nm^r/Kan^r	This work	133135
pOPS0495	psNifH-sfGFP-A5 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TGGTCAACGATA Golay reference name:806rcbc4. Nm^r/Kan^r	This work	133136
pOPS0496	psNifH-sfGFP-A6 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ATCGCACAGTAA Golay reference name:806rcbc5. Nm^r/Kan^r	This work	133137
pOPS0497	psNifH-sfGFP-A7 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GTCGTGTAGCCT Golay reference name:806rcbc6. Nm^r/Kan^r	This work	133138
pOPS0498	psNifH-sfGFP-A8 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AGCGGAGGTTAG Golay reference name:806rcbc7. Nm^r/Kan^r	This work	133139
pOPS0499	psNifH-sfGFP-A9 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ATCCTTTGGTTC Golay reference name:806rcbc8. Nm^r/Kan^r	This work	133140
pOPS0500	psNifH-sfGFP-A10 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TACAGCGCATAC Golay reference name:806rcbc9. Nm^r/Kan^r	This work	133141
pOPS0501	psNifH-sfGFP-A11 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ACCGGTATGTAC Golay reference name:806rcbc10. Nm^r/Kan^r	This work	133142

pOPS0502	psNifH-sfGFP-A12 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AATTGTGTCGGA Golay reference name:806rcbc11. Nm^r/Kan^r	This work	133143
pOPS0503	psNifH-sfGFP-B1 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TGCATACACTGG Golay reference name:806rcbc12. Nm^r/Kan^r	This work	133144
pOPS0504	psNifH-sfGFP-B2 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AGTCGAACGAGG Golay reference name:806rcbc13. Nm^r/Kan^r	This work	133145
pOPS0505	psNifH-sfGFP-B3 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ACCAGTGACTCA Golay reference name:806rcbc14. Nm^r/Kan^r	This work	133146
pOPS0506	psNifH-sfGFP-B4 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GAATACCAAGTC Golay reference name:806rcbc15. Nm^r/Kan^r	This work	133147
pOPS0507	psNifH-sfGFP-B5 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GTAGATCGTGTA Golay reference name:806rcbc16. Nm^r/Kan^r	This work	133148
pOPS0508	psNifH-sfGFP-B6 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TAACGTGTGTGC Golay reference name:806rcbc17. Nm^r/Kan^r	This work	133149
pOPS0509	psNifH-sfGFP-B7 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CATTATGGCGTG Golay reference name:806rcbc18. Nm^r/Kan^r	This work	133150
pOPS0510	psNifH-sfGFP-B8 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CCAATACGCCTG Golay reference name:806rcbc19. Nm^r/Kan^r	This work	133151
pOPS0511	psNifH-sfGFP-B9 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GATCTGCGATCC Golay reference name:806rcbc20. Nm^r/Kan^r	This work	133152
pOPS0512	psNifH-sfGFP-B10 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CAGCTCATCAGC Golay reference name:806rcbc21. Nm^r/Kan^r	This work	133153
pOPS0513	psNifH-sfGFP-B11 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CAAACAACAGCT Golay reference name:806rcbc22. Nm^r/Kan^r	This work	133154
pOPS0514	psNifH-sfGFP-B12 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GCAACACCATCC Golay reference name:806rcbc23. Nm^r/Kan^r	This work	133155
pOPS0515	psNifH-sfGFP-C1 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GCGATATATCGC Golay reference name:806rcbc24. Nm^r/Kan^r	This work	133156

pOPS0516	psNifH-sfGFP-C2 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CGAGCAATCCTA Golay reference name:806rcbc25. Nm^r/Kan^r	This work	133157
pOPS0517	psNifH-sfGFP-C3 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AGTCGTGCACAT Golay reference name:806rcbc26. Nm^r/Kan^r	This work	133158
pOPS0518	psNifH-sfGFP-C4 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GTATCTGCGCGT Golay reference name:806rcbc27. Nm^r/Kan^r	This work	133159
pOPS0519	psNifH-sfGFP-C5 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CGAGGGAAAGTC Golay reference name:806rcbc28. Nm^r/Kan^r	This work	133160
pOPS0520	psNifH-sfGFP-C6 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CAAATTCGGGAT Golay reference name:806rcbc29. Nm^r/Kan^r	This work	133161
pOPS0521	psNifH-sfGFP-C7 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AGATTGACCAAC Golay reference name:806rcbc30. Nm^r/Kan^r	This work	133162
pOPS0522	psNifH-sfGFP-C8 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AGTTACGAGCTA Golay reference name:806rcbc31. Nm^r/Kan^r	This work	133163
pOPS0523	psNifH-sfGFP-C9 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GCATATGCACTG Golay reference name:806rcbc32. Nm^r/Kan^r	This work	133164
pOPS0524	psNifH-sfGFP-C10 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CAACTCCCGTGA Golay reference name:806rcbc33. Nm^r/Kan^r	This work	133165
pOPS0525	psNifH-sfGFP-C11 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TTGCGTTAGCAG Golay reference name:806rcbc34. Nm^r/Kan^r	This work	133166
pOPS0526	psNifH-sfGFP-C12 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TACGAGCCCTAA Golay reference name:806rcbc35. Nm^r/Kan^r	This work	133167
pOPS0527	psNifH-sfGFP-D1 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CACTACGCTAGA Golay reference name:806rcbc36, Nm^r/Kan^r	This work	133168
pOPS0528	psNifH-sfGFP-D2 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TGCAGTCCTCGA Golay reference name:806rcbc37, Nm^r/Kan^r	This work	133169
pOPS0529	psNifH-sfGFP-D3 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ACCATAGCTCCG Golay reference name:806rcbc38. Nm^r/Kan^r	This work	133170

pOPS0530	psNifH-sfGFP-D4 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TCGACATCTCTT Golay reference name:806rcbc39. Nm^r/Kan^r	This work	133171
pOPS0531	psNifH-sfGFP-D5 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GAACACTTTGGA Golay reference name:806rcbc40. Nm^r/Kan^r	This work	133172
pOPS0532	psNifH-sfGFP-D6 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GAGCCATCTGTA Golay reference name:806rcbc41. Nm^r/Kan^r	This work	133173
pOPS0533	psNifH-sfGFP-D7 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TTGGGTACACGT Golay reference name:806rcbc42. Nm^r/Kan^r	This work	133174
pOPS0534	psNifH-sfGFP-D8 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AAGGCGCTCCTT Golay reference name:806rcbc43. Nm^r/Kan^r	This work	133175
pOPS0535	psNifH-sfGFP-D9 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TAATACGGATCG Golay reference name:806rcbc44. Nm^r/Kan^r	This work	133176
pOPS0536	psNifH-sfGFP-D10 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TCGGAATTAGAC Golay reference name:806rcbc45. Nm^r/Kan^r	This work	133177
pOPS0537	psNifH-sfGFP-D11 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TGTGAATTCGGA Golay reference name:806rcbc46. Nm^r/Kan^r	This work	133178
pOPS0538	psNifH-sfGFP-D12 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CATTCGTGGCGT Golay reference name:806rcbc47. Nm^r/Kan^r	This work	133179
pOPS0539	psNifH-sfGFP-E1 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TACTACGTGGCC Golay reference name:806rcbc48. Nm^r/Kan^r	This work	133180
pOPS0540	psNifH-sfGFP-E2 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GGCCAGTTCCTA Golay reference name:806rcbc49. Nm^r/Kan^r	This work	133181
pOPS0541	psNifH-sfGFP-E3 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GATGTTCGCTAG Golay reference name:806rcbc50. Nm^r/Kan^r	This work	133182
pOPS0542	psNifH-sfGFP-E4 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CTATCTCCTGTC Golay reference name:806rcbc51. Nm^r/Kan^r	This work	133183
pOPS0543	psNifH-sfGFP-E5 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ACTCACAGGAAT Golay reference name:806rcbc52. Nm^r/Kan^r	This work	133184

pOPS0544	psNifH-sfGFP-E6 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ATGATGAGCCTC Golay reference name:806rcbc53. Nm^r/Kan^r	This work	133185
pOPS0545	psNifH-sfGFP-E7 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GTCGACAGAGGA Golay reference name:806rcbc54. Nm^r/Kan^r	This work	133186
pOPS0546	psNifH-sfGFP-E8 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TGTCGCAAATAG Golay reference name:806rcbc55. Nm^r/Kan^r	This work	133187
pOPS0547	psNifH-sfGFP-E9 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CATCCCTCTACT Golay reference name:806rcbc56. Nm^r/Kan^r	This work	133188
pOPS0548	psNifH-sfGFP-E10 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TATACCGCTGCG Golay reference name:806rcbc57. Nm^r/Kan^r	This work	133189
pOPS0549	psNifH-sfGFP-E11 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AGTTGAGGCATT Golay reference name:806rcbc58. Nm^r/Kan^r	This work	133190
pOPS0550	psNifH-sfGFP-E12 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ACAATAGACACC Golay reference name:806rcbc59. Nm^r/Kan^r	This work	133191
pOPS0551	psNifH-sfGFP-F1 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CGGTCAATTGAC Golay reference name:806rcbc60. Nm^r/Kan^r	This work	133192
pOPS0552	psNifH-sfGFP-F2 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TGGAGTAGGTGG Golay reference name:806rcbc61. Nm^r/Kan^r	This work	133193
pOPS0553	psNifH-sfGFP-F3 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GCTCGAAGATTC Golay reference name:806rcbc62. Nm^r/Kan^r	This work	133194
pOPS0554	psNifH-sfGFP-F4 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AGGCTTACGTGT Golay reference name:806rcbc63. Nm^r/Kan^r	This work	133195
pOPS0555	psNifH-sfGFP-F5 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TCTCTACCACTC Golay reference name:806rcbc64. Nmr/Kanr	This work	133196
pOPS0556	psNifH-sfGFP-F6 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ACTTCCAACTTC Golay reference name:806rcbc65. Nm^r/Kan^r	This work	133197
pOPS0557	psNifH-sfGFP-F7 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CTCACCTAGGAA Golay reference name:806rcbc66. Nm^r/Kan^r	This work	133198

pOPS0558	psNifH-sfGFP-F8 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GTGTTGTCGTGC Golay reference name:806rcbc67. Nm^r/Kan^r	This work	133199
pOPS0559	psNifH-sfGFP-F9 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CCACAGATCGAT Golay reference name:806rcbc68. Nm^r/Kan^r	This work	133200
pOPS0560	psNifH-sfGFP-F10 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TATCGACACAAG Golay reference name:806rcbc69. Nm^r/Kan^r	This work	133201
pOPS0561	psNifH-sfGFP-F11 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GATTCCGGCTCA Golay reference name:806rcbc70. Nm^r/Kan^r	This work	133202
pOPS0562	psNifH-sfGFP-F12 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CGTAATTGCCGC Golay reference name:806rcbc71. Nm^r/Kan^r	This work	133203
pOPS0563	psNifH-sfGFP-G1 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GGTGACTAGTTC Golay reference name:806rcbc72. Nm^r/Kan^r	This work	133204
pOPS0564	psNifH-sfGFP-G2 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GTGGAGTCTCAT Golay reference name:806rcbc73. Nm^r/Kan^r	This work	133205
pOPS0565	psNifH-sfGFP-G3 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TAGGCATGCTTG Golay reference name:806rcbc74. Nm^r/Kan^r	This work	133206
pOPS0566	psNifH-sfGFP-G4 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AACTAGTTCAGG Golay reference name:806rcbc75. Nm^r/Kan^r	This work	133207
pOPS0567	psNifH-sfGFP-G5 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ATTCTGCCGAAG Golay reference name:806rcbc76. Nm^r/Kan^r	This work	133208
pOPS0568	psNifH-sfGFP-G6 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: AGCATGTCCCGT Golay reference name:806rcbc77. Nm^r/Kan^r	This work	133209
pOPS0569	psNifH-sfGFP-G7 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GTACGATATGAC Golay reference name:806rcbc78. Nm^r/Kan^r	This work	133210
pOPS0570	psNifH-sfGFP-G8 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GTGGTGGTTTCC Golay reference name:806rcbc79. Nm^r/Kan^r	This work	133211
pOPS0571	psNifH-sfGFP-G9 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TAGTATGCGCAA Golay reference name:806rcbc80. Nm^r/Kan^r	This work	133212

pOPS0572	psNifH-sfGFP-G10 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TGCGCTGAATGT Golay reference name:806rcbc81. Nm^r/Kan^r	This work	133213
pOPS0573	psNifH-sfGFP-G11 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ATGGCTGTCAGT Golay reference name:806rcbc82. Nm^r/Kan^r	This work	133214
pOPS0574	psNifH-sfGFP-G12 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GTTCTCTTCTCG Golay reference name:806rcbc83. Nm^r/Kan^r	This work	133215
pOPS0575	psNifH-sfGFP-H1 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CGTAAGATGCCT Golay reference name:806rcbc84. Nm^r/Kan^r	This work	133216
pOPS0576	psNifH-sfGFP-H2 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GCGTTCTAGCTG Golay reference name:806rcbc85. Nm^r/Kan^r	This work	133217
pOPS0577	psNifH-sfGFP-H3 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GTTGTTCTGGGA Golay reference name:806rcbc86. Nm^r/Kan^r	This work	133218
pOPS0578	psNifH-sfGFP-H4 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GGACTTCCAGCT Golay reference name:806rcbc87. Nm^r/Kan^r	This work	133219
pOPS0579	psNifH-sfGFP-H5 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CTCACAACCGTG Golay reference name:806rcbc88. Nm^r/Kan^r	This work	133220
pOPS0580	psNifH-sfGFP-H6 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: CTGCTATTCCTC Golay reference name:806rcbc89. Nm^r/Kan^r	This work	133221
pOPS0581	psNifH-sfGFP-H7 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ATGTCACCGCTG Golay reference name:806rcbc90. Nm^r/Kan^r	This work	133222
pOPS0582	psNifH-sfGFP-H8 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TGTAACGCCGAT Golay reference name:806rcbc91. Nm^r/Kan^r	This work	133223
pOPS0584	psNifH-sfGFP-H10 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: ATGGGTTCCGTC Golay reference name:806rcbc93. Nm^r/Kan^r	This work	133224
pOPS0585	psNifH-sfGFP-H11 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: TTGGCTCTATTC Golay reference name:806rcbc94. Nm^r/Kan^r	This work	133225
pOPS0586	psNifH-sfGFP-H12 in pOGG026 Plasmid constructed by GG with universal primer binding site. Confirmed BC sequence: GATCCCACGTAC Golay reference name:806rcbc95. Nm^r/Kan^r	This work	133226

pOSP0750	Reporter Plasmid constructed with constitutive promoter pNeo (pOGG001), *gusA* (pOGG083) and T-pharma (pOGG003) assembled in pOGG026, Nm^r/Kan^r	This work	133237

GG, Golden Gate Cloning; BC, Barcode. Amp^r, ampicillin resistance; Gm^r, gentamicin resistance; Kan^r, kanamycin resistance; Nm^r, neomycin resistance; Nit^r, nitrofurantoin resistance; Rf^r, rifampicin resistance, Tet^r, tetracycline resistance.

Table S2. Primers used in Golden Gate constructions.

Primer	Sequence (5'-3')	Description	Source
oxp0282	CCTACCTAGATCCTCGC	Forward primer. Used for screening plasmid constructions in pOGG026 backbone vector	This work
oxp0283	AGCGTTCTGAACAAATCC	Reverse primer. Used for screening plasmid constructions in pOGG026 backbone vector	This work
oxp1334	CACTCTGTGGTCTCAGGAGCATCCTCCCTAATGCGCC	Forward primer for amplification of promoterless region from pIJJ11268 for PU module	This work
oxp1335	CACTTCGTGGTCTCACATTCGTATCCTCCAAGCCTGAA	Reverse primer for amplification of promoterless region from pIJJ11268 for PU module	This work

Table S3. Plasmid IDs as sense and antisense oligonucleotides to be assembled as Golden Gate T modules.

Oligo name	Full Sequence (5' to 3')
806rcbc0	GCTTTCCCTTGTCTCCCGTTTTACAACGTCGTGACTGGG
806rcbc0_anti	AGCGCCCAGTCACGACGTTGTAAAACGGGAGACAAGGGA
806rcbc1	GCTTACGAGACTGATTCGTTTTACAACGTCGTGACTGGG
806rcbc1_anti	AGCGCCCAGTCACGACGTTGTAAAACGAATCAGTCTCGT
806rcbc2	GCTTGCTGTACGGATTCGTTTTACAACGTCGTGACTGGG
806rcbc2_anti	AGCGCCCAGTCACGACGTTGTAAAACGAATCCGTACAGC
806rcbc3	GCTTATCACCAGGTGTCGTTTTACAACGTCGTGACTGGG
806rcbc3_anti	AGCGCCCAGTCACGACGTTGTAAAACGACACCTGGTGAT
806rcbc4	GCTTTGGTCAACGATACGTTTTACAACGTCGTGACTGGG
806rcbc4_anti	AGCGCCCAGTCACGACGTTGTAAAACGTATCGTTGACCA
806rcbc5	GCTTATCGCACAGTAACGTTTTACAACGTCGTGACTGGG
806rcbc5_anti	AGCGCCCAGTCACGACGTTGTAAAACGTTACTGTGCGAT
806rcbc6	GCTTGTCGTGTAGCCTCGTTTTACAACGTCGTGACTGGG
806rcbc6_anti	AGCGCCCAGTCACGACGTTGTAAAACGAGGCTACACGAC
806rcbc7	GCTTAGCGGAGGTTAGCGTTTTACAACGTCGTGACTGGG
806rcbc7_anti	AGCGCCCAGTCACGACGTTGTAAAACGCTAACCTCCGCT
806rcbc8	GCTTATCCTTTGGTTCCGTTTTACAACGTCGTGACTGGG
806rcbc8_anti	AGCGCCCAGTCACGACGTTGTAAAACGGAACCAAAGGAT
806rcbc9	GCTTTACAGCGCATACCGTTTTACAACGTCGTGACTGGG
806rcbc9_anti	AGCGCCCAGTCACGACGTTGTAAAACGGTATGCGCTGTA
806rcbc10	GCTTACCGGTATGTACCGTTTTACAACGTCGTGACTGGG
806rcbc10_anti	AGCGCCCAGTCACGACGTTGTAAAACGGTACATACCGGT
806rcbc11	GCTTAATTGTGTCGGACGTTTTACAACGTCGTGACTGGG
806rcbc11_anti	AGCGCCCAGTCACGACGTTGTAAAACGTCCGACACAATT
806rcbc12	GCTTTGCATACACTGGCGTTTTACAACGTCGTGACTGGG
806rcbc12_anti	AGCGCCCAGTCACGACGTTGTAAAACGCCAGTGTATGCA
806rcbc13	GCTTAGTCGAACGAGGCGTTTTACAACGTCGTGACTGGG
806rcbc13_anti	AGCGCCCAGTCACGACGTTGTAAAACGCCTCGTTCGACT
806rcbc14	GCTTACCAGTGACTCACGTTTTACAACGTCGTGACTGGG
806rcbc14_anti	AGCGCCCAGTCACGACGTTGTAAAACGTGAGTCACTGGT
806rcbc15	GCTTGAATACCAAGTCCGTTTTACAACGTCGTGACTGGG
806rcbc15_anti	AGCGCCCAGTCACGACGTTGTAAAACGGACTTGGTATTC
806rcbc16	GCTTGTAGATCGTGTACGTTTTACAACGTCGTGACTGGG
806rcbc16_anti	AGCGCCCAGTCACGACGTTGTAAAACGTACACGATCTAC
806rcbc17	GCTTTAACGTGTGTGCCGTTTTACAACGTCGTGACTGGG
806rcbc17_anti	AGCGCCCAGTCACGACGTTGTAAAACGGCACACACGTTA
806rcbc18	GCTTCATTATGGCGTGCGTTTTACAACGTCGTGACTGGG

806rcbc18_anti	AGCGCCCAGTCACGACGTTGTAAAACGCACGCCATAATG
806rcbc19	GCTTCCAATACGCCTGCGTTTTACAACGTCGTGACTGGG
806rcbc19_anti	AGCGCCCAGTCACGACGTTGTAAAACGCAGGCGTATTGG
806rcbc20	GCTTGATCTGCGATCCCGTTTTACAACGTCGTGACTGGG
806rcbc20_anti	AGCGCCCAGTCACGACGTTGTAAAACGGGATCGCAGATC
806rcbc21	GCTTCAGCTCATCAGCCGTTTTACAACGTCGTGACTGGG
806rcbc21_anti	AGCGCCCAGTCACGACGTTGTAAAACGGCTGATGAGCTG
806rcbc22	GCTTCAAACAACAGCTCGTTTTACAACGTCGTGACTGGG
806rcbc22_anti	AGCGCCCAGTCACGACGTTGTAAAACGAGCTGTTGTTTG
806rcbc23	GCTTGCAACACCATCCCGTTTTACAACGTCGTGACTGGG
806rcbc23_anti	AGCGCCCAGTCACGACGTTGTAAAACGGGATGGTGTTGC
806rcbc24	GCTTGCGATATATCGCCGTTTTACAACGTCGTGACTGGG
806rcbc24_anti	AGCGCCCAGTCACGACGTTGTAAAACGGCGATATATCGC
806rcbc25	GCTTCGAGCAATCCTACGTTTTACAACGTCGTGACTGGG
806rcbc25_anti	AGCGCCCAGTCACGACGTTGTAAAACGTAGGATTGCTCG
806rcbc26	GCTTAGTCGTGCACATCGTTTTACAACGTCGTGACTGGG
806rcbc26_anti	AGCGCCCAGTCACGACGTTGTAAAACGATGTGCACGACT
806rcbc27	GCTTGTATCTGCGCGTCGTTTTACAACGTCGTGACTGGG
806rcbc27_anti	AGCGCCCAGTCACGACGTTGTAAAACGACGCGCAGATAC
806rcbc28	GCTTCGAGGGAAAGTCCGTTTTACAACGTCGTGACTGGG
806rcbc28_anti	AGCGCCCAGTCACGACGTTGTAAAACGGACTTTCCCTCG
806rcbc29	GCTTCAAATTCGGGATCGTTTTACAACGTCGTGACTGGG
806rcbc29_anti	AGCGCCCAGTCACGACGTTGTAAAACGATCCCGAATTTG
806rcbc30	GCTTAGATTGACCAACCGTTTTACAACGTCGTGACTGGG
806rcbc30_anti	AGCGCCCAGTCACGACGTTGTAAAACGGTTGGTCAATCT
806rcbc31	GCTTAGTTACGAGCTACGTTTTACAACGTCGTGACTGGG
806rcbc31_anti	AGCGCCCAGTCACGACGTTGTAAAACGTAGCTCGTAACT
806rcbc32	GCTTGCATATGCACTGCGTTTTACAACGTCGTGACTGGG
806rcbc32_anti	AGCGCCCAGTCACGACGTTGTAAAACGCAGTGCATATGC
806rcbc33	GCTTCAACTCCCGTGACGTTTTACAACGTCGTGACTGGG
806rcbc33_anti	AGCGCCCAGTCACGACGTTGTAAAACGTCACGGGAGTTG
806rcbc34	GCTTTTGCGTTAGCAGCGTTTTACAACGTCGTGACTGGG
806rcbc34_anti	AGCGCCCAGTCACGACGTTGTAAAACGCTGCTAACGCAA
806rcbc35	GCTTTACGAGCCCTAACGTTTTACAACGTCGTGACTGGG
806rcbc35_anti	AGCGCCCAGTCACGACGTTGTAAAACGTTAGGGCTCGTA
806rcbc36	GCTTCACTACGCTAGACGTTTTACAACGTCGTGACTGGG
806rcbc36_anti	AGCGCCCAGTCACGACGTTGTAAAACGTCTAGCGTAGTG
806rcbc37	GCTTTGCAGTCCTCGACGTTTTACAACGTCGTGACTGGG
806rcbc37_anti	AGCGCCCAGTCACGACGTTGTAAAACGTCGAGGACTGCA
806rcbc38	GCTTACCATAGCTCCGCGTTTTACAACGTCGTGACTGGG

806rcbc38_anti	AGCGCCCAGTCACGACGTTGTAAAACGCGGAGCTATGGT
806rcbc39	GCTTTCGACATCTCTTCGTTTTACAACGTCGTGACTGGG
806rcbc39_anti	AGCGCCCAGTCACGACGTTGTAAAACGAAGAGATGTCGA
806rcbc40	GCTTGAACACTTTGGACGTTTTACAACGTCGTGACTGGG
806rcbc40_anti	AGCGCCCAGTCACGACGTTGTAAAACGTCCAAAGTGTTC
806rcbc41	GCTTGAGCCATCTGTACGTTTTACAACGTCGTGACTGGG
806rcbc41_anti	AGCGCCCAGTCACGACGTTGTAAAACGTACAGATGGCTC
806rcbc42	GCTTTTGGGTACACGTCGTTTTACAACGTCGTGACTGGG
806rcbc42_anti	AGCGCCCAGTCACGACGTTGTAAAACGACGTGTACCCAA
806rcbc43	GCTTAAGGCGCTCCTTCGTTTTACAACGTCGTGACTGGG
806rcbc43_anti	AGCGCCCAGTCACGACGTTGTAAAACGAAGGAGCGCCTT
806rcbc44	GCTTTAATACGGATCGCGTTTTACAACGTCGTGACTGGG
806rcbc44_anti	AGCGCCCAGTCACGACGTTGTAAAACGCGATCCGTATTA
806rcbc45	GCTTTCGGAATTAGACCGTTTTACAACGTCGTGACTGGG
806rcbc45_anti	AGCGCCCAGTCACGACGTTGTAAAACGGTCTAATTCCGA
806rcbc46	GCTTTGTGAATTCGGACGTTTTACAACGTCGTGACTGGG
806rcbc46_anti	AGCGCCCAGTCACGACGTTGTAAAACGTCCGAATTCACA
806rcbc47	GCTTCATTCGTGGCGTCGTTTTACAACGTCGTGACTGGG
806rcbc47_anti	AGCGCCCAGTCACGACGTTGTAAAACGACGCCACGAATG
806rcbc48	GCTTTACTACGTGGCCCGTTTTACAACGTCGTGACTGGG
806rcbc48_anti	AGCGCCCAGTCACGACGTTGTAAAACGGGCCACGTAGTA
806rcbc49	GCTTGGCCAGTTCCTACGTTTTACAACGTCGTGACTGGG
806rcbc49_anti	AGCGCCCAGTCACGACGTTGTAAAACGTAGGAACTGGCC
806rcbc50	GCTTGATGTTCGCTAGCGTTTTACAACGTCGTGACTGGG
806rcbc50_anti	AGCGCCCAGTCACGACGTTGTAAAACGCTAGCGAACATC
806rcbc51	GCTTCTATCTCCTGTCCGTTTTACAACGTCGTGACTGGG
806rcbc51_anti	AGCGCCCAGTCACGACGTTGTAAAACGGACAGGAGATAG
806rcbc52	GCTTACTCACAGGAATCGTTTTACAACGTCGTGACTGGG
806rcbc52_anti	AGCGCCCAGTCACGACGTTGTAAAACGATTCCTGTGAGT
806rcbc53	GCTTATGATGAGCCTCCGTTTTACAACGTCGTGACTGGG
806rcbc53_anti	AGCGCCCAGTCACGACGTTGTAAAACGGAGGCTCATCAT
806rcbc54	GCTTGTCGACAGAGGACGTTTTACAACGTCGTGACTGGG
806rcbc54_anti	AGCGCCCAGTCACGACGTTGTAAAACGTCCTCTGTCGAC
806rcbc55	GCTTTGTCGCAAATAGCGTTTTACAACGTCGTGACTGGG
806rcbc55_anti	AGCGCCCAGTCACGACGTTGTAAAACGCTATTTGCGACA
806rcbc56	GCTTCATCCCTCTACTCGTTTTACAACGTCGTGACTGGG
806rcbc56_anti	AGCGCCCAGTCACGACGTTGTAAAACGAGTAGAGGGATG
806rcbc57	GCTTTATACCGCTGCGCGTTTTACAACGTCGTGACTGGG
806rcbc57_anti	AGCGCCCAGTCACGACGTTGTAAAACGCGCAGCGGTATA
806rcbc58	GCTTAGTTGAGGCATTCGTTTTACAACGTCGTGACTGGG

806rcbc58_anti	AGCGCCCAGTCACGACGTTGTAAAACGAATGCCTCAACT
806rcbc59	GCTTACAATAGACACCCGTTTTACAACGTCGTGACTGGG
806rcbc59_anti	AGCGCCCAGTCACGACGTTGTAAAACGGGTGTCTATTGT
806rcbc60	GCTTCGGTCAATTGACCGTTTTACAACGTCGTGACTGGG
806rcbc60_anti	AGCGCCCAGTCACGACGTTGTAAAACGGTCAATTGACCG
806rcbc61	GCTTGTGGAGTCTCATCGTTTTACAACGTCGTGACTGGG
806rcbc61_anti	AGCGCCCAGTCACGACGTTGTAAAACGATGAGACTCCAC
806rcbc62	GCTTGCTCGAAGATTCCGTTTTACAACGTCGTGACTGGG
806rcbc62_anti	AGCGCCCAGTCACGACGTTGTAAAACGGAATCTTCGAGC
806rcbc63	GCTTAGGCTTACGTGTCGTTTTACAACGTCGTGACTGGG
806rcbc63_anti	AGCGCCCAGTCACGACGTTGTAAAACGACACGTAAGCCT
806rcbc64	GCTTTCTCTACCACTCCGTTTTACAACGTCGTGACTGGG
806rcbc64_anti	AGCGCCCAGTCACGACGTTGTAAAACGGAGTGGTAGAGA
806rcbc65	GCTTACTTCCAACTTCCGTTTTACAACGTCGTGACTGGG
806rcbc65_anti	AGCGCCCAGTCACGACGTTGTAAAACGGAAGTTGGAAGT
806rcbc66	GCTTCTCACCTAGGAACGTTTTACAACGTCGTGACTGGG
806rcbc66_anti	AGCGCCCAGTCACGACGTTGTAAAACGTTCCTAGGTGAG
806rcbc67	GCTTGTGTTGTCGTGCCGTTTTACAACGTCGTGACTGGG
806rcbc67_anti	AGCGCCCAGTCACGACGTTGTAAAACGGCACGACAACAC
806rcbc68	GCTTCCACAGATCGATCGTTTTACAACGTCGTGACTGGG
806rcbc68_anti	AGCGCCCAGTCACGACGTTGTAAAACGATCGATCTGTGG
806rcbc69	GCTTTATCGACACAAGCGTTTTACAACGTCGTGACTGGG
806rcbc69_anti	AGCGCCCAGTCACGACGTTGTAAAACGCTTGTGTCGATA
806rcbc70	GCTTGATTCCGGCTCACGTTTTACAACGTCGTGACTGGG
806rcbc70_anti	AGCGCCCAGTCACGACGTTGTAAAACGTGAGCCGGAATC
806rcbc71	GCTTCGTAATTGCCGCCGTTTTACAACGTCGTGACTGGG
806rcbc71_anti	AGCGCCCAGTCACGACGTTGTAAAACGGCGGCAATTACG
806rcbc72	GCTTGGTGACTAGTTCCGTTTTACAACGTCGTGACTGGG
806rcbc72_anti	AGCGCCCAGTCACGACGTTGTAAAACGGAACTAGTCACC
806rcbc73	GCTTATGGGTTCCGTCCGTTTTACAACGTCGTGACTGGG
806rcbc73_anti	AGCGCCCAGTCACGACGTTGTAAAACGGACGGAACCCAT
806rcbc74	GCTTTAGGCATGCTTGCGTTTTACAACGTCGTGACTGGG
806rcbc74_anti	AGCGCCCAGTCACGACGTTGTAAAACGCAAGCATGCCTA
806rcbc75	GCTTAACTAGTTCAGGCGTTTTACAACGTCGTGACTGGG
806rcbc75_anti	AGCGCCCAGTCACGACGTTGTAAAACGCCTGAACTAGTT
806rcbc76	GCTTATTCTGCCGAAGCGTTTTACAACGTCGTGACTGGG
806rcbc76_anti	AGCGCCCAGTCACGACGTTGTAAAACGCTTCGGCAGAAT
806rcbc77	GCTTAGCATGTCCCGTCGTTTTACAACGTCGTGACTGGG
806rcbc77_anti	AGCGCCCAGTCACGACGTTGTAAAACGACGGGACATGCT
806rcbc78	GCTTGTACGATATGACCGTTTTACAACGTCGTGACTGGG

806rcbc78_anti	AGCGCCCAGTCACGACGTTGTAAAACGGTCATATCGTAC
806rcbc79	GCTTGTGGTGGTTTCCCGTTTTACAACGTCGTGACTGGG
806rcbc79_anti	AGCGCCCAGTCACGACGTTGTAAAACGGGAAACCACCAC
806rcbc80	GCTTTAGTATGCGCAACGTTTTACAACGTCGTGACTGGG
806rcbc80_anti	AGCGCCCAGTCACGACGTTGTAAAACGTTGCGCATACTA
806rcbc81	GCTTTGCGCTGAATGTCGTTTTACAACGTCGTGACTGGG
806rcbc81_anti	AGCGCCCAGTCACGACGTTGTAAAACGACATTCAGCGCA
806rcbc82	GCTTATGGCTGTCAGTCGTTTTACAACGTCGTGACTGGG
806rcbc82_anti	AGCGCCCAGTCACGACGTTGTAAAACGACTGACAGCCAT
806rcbc83	GCTTGTTCTCTTCTCGCGTTTTACAACGTCGTGACTGGG
806rcbc83_anti	AGCGCCCAGTCACGACGTTGTAAAACGCGAGAAGAGAAC
806rcbc84	GCTTCGTAAGATGCCTCGTTTTACAACGTCGTGACTGGG
806rcbc84_anti	AGCGCCCAGTCACGACGTTGTAAAACGAGGCATCTTACG
806rcbc85	GCTTGCGTTCTAGCTGCGTTTTACAACGTCGTGACTGGG
806rcbc85_anti	AGCGCCCAGTCACGACGTTGTAAAACGCAGCTAGAACGC
806rcbc86	GCTTGTTGTTCTGGGACGTTTTACAACGTCGTGACTGGG
806rcbc86_anti	AGCGCCCAGTCACGACGTTGTAAAACGTCCCAGAACAAC
806rcbc87	GCTTGGACTTCCAGCTCGTTTTACAACGTCGTGACTGGG
806rcbc87_anti	AGCGCCCAGTCACGACGTTGTAAAACGAGCTGGAAGTCC
806rcbc88	GCTTCTCACAACCGTGCGTTTTACAACGTCGTGACTGGG
806rcbc88_anti	AGCGCCCAGTCACGACGTTGTAAAACGCACGGTTGTGAG
806rcbc89	GCTTCTGCTATTCCTCCGTTTTACAACGTCGTGACTGGG
806rcbc89_anti	AGCGCCCAGTCACGACGTTGTAAAACGGAGGAATAGCAG
806rcbc90	GCTTATGTCACCGCTGCGTTTTACAACGTCGTGACTGGG
806rcbc90_anti	AGCGCCCAGTCACGACGTTGTAAAACGCAGCGGTGACAT
806rcbc91	GCTTTGTAACGCCGATCGTTTTACAACGTCGTGACTGGG
806rcbc91_anti	AGCGCCCAGTCACGACGTTGTAAAACGATCGGCGTTACA
806rcbc92	GCTTAGCAGAACATCTCGTTTTACAACGTCGTGACTGGG
806rcbc92_anti	AGCGCCCAGTCACGACGTTGTAAAACGAGATGTTCTGCT
806rcbc93	GCTTTGGAGTAGGTGGCGTTTTACAACGTCGTGACTGGG
806rcbc93_anti	AGCGCCCAGTCACGACGTTGTAAAACGCCACCTACTCCA
806rcbc94	GCTTTTGGCTCTATTCCGTTTTACAACGTCGTGACTGGG
806rcbc94_anti	AGCGCCCAGTCACGACGTTGTAAAACGGAATAGAGCCAA
806rcbc95	GCTTGATCCCACGTACCGTTTTACAACGTCGTGACTGGG
806rcbc95_anti	AGCGCCCAGTCACGACGTTGTAAAACGGTACGTGGGATC

Table S4. PCR primers for multiplex Ion Torrent sequencing strategy.

First- step PCR primers for Ion Torrent sequencing for well tagging Forward primers (column tagging). Sequence (5'-3')			
Name	**Forward landing pad***	**Barcode**	**Primer**
1_IT	GCCCAGTCTACTCGAGGG	GCTA	CATCACGCATGGTATGGA
2_IT	GCCCAGTCTACTCGAGGG	TGTGT	CATCACGCATGGTATGGA
3_IT	GCCCAGTCTACTCGAGGG	AGTCTG	CATCACGCATGGTATGGA
4_IT	GCCCAGTCTACTCGAGGG	ATCA	CATCACGCATGGTATGGA
5_IT	GCCCAGTCTACTCGAGGG	GACGA	CATCACGCATGGTATGGA
6_IT	GCCCAGTCTACTCGAGGG	TCGTCG	CATCACGCATGGTATGGA
7_IT	GCCCAGTCTACTCGAGGG	TGCT	CATCACGCATGGTATGGA
8_IT	GCCCAGTCTACTCGAGGG	CAGTT	CATCACGCATGGTATGGA
9_IT	GCCCAGTCTACTCGAGGG	ACATGT	CATCACGCATGGTATGGA
10_IT	GCCCAGTCTACTCGAGGG	GCGG	CATCACGCATGGTATGGA
11_IT	GCCCAGTCTACTCGAGGG	GTTGA	CATCACGCATGGTATGGA
12_IT	GCCCAGTCTACTCGAGGG	GTGGCT	CATCACGCATGGTATGGA

Reverse primers (row tagging). Sequence (5'-3')			
Name	**Reverse landing pad†**	**Barcode**	**Primer**
A_IT	ATCTCGGTGGTCGCCGTA	GCTC	CCCAGTCACGACGTTGTAAAACG
B_IT	ATCTCGGTGGTCGCCGTA	CTAGT	CCCAGTCACGACGTTGTAAAACG
C_IT	ATCTCGGTGGTCGCCGTA	TAGATC	CCCAGTCACGACGTTGTAAAACG
D_IT	ATCTCGGTGGTCGCCGTA	TCGC	CCCAGTCACGACGTTGTAAAACG
E_IT	ATCTCGGTGGTCGCCGTA	CCTTA	CCCAGTCACGACGTTGTAAAACG
F_IT	ATCTCGGTGGTCGCCGTA	CATAAC	CCCAGTCACGACGTTGTAAAACG
G_IT	ATCTCGGTGGTCGCCGTA	CAGA	CCCAGTCACGACGTTGTAAAACG
H_IT	ATCTCGGTGGTCGCCGTA	TGTTC	CCCAGTCACGACGTTGTAAAACG

Second-step PCR primers for Ion Torrent sequencing

Plate tagging Forward primers (Library tagging). Sequence (5'-3')*			
Name	**IT adapter**	**Ion Xpress™ Barcode**	**Primer for Forward landing pad**
IT_A_FP_1	CCATCTCATCCCTGCGTGTCT CCGACTCAG	CTAAGGT AAC	GATATAAAACCGCCCAGTCTA CTCGAGGG
IT_A_FP_2	CCATCTCATCCCTGCGTGTCT CCGACTCAG	TAAGGAG AAC	GATATAAAACCGCCCAGTCTA CTCGAGGG
IT_A_FP_3	CCATCTCATCCCTGCGTGTCT CCGACTCAG	AAGAGGA TTC	GATATAAAACCGCCCAGTCTA CTCGAGGG

IT_A_FP_4	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TACCAAGATC	GATATAAAACCGCCCAGTCTACTCGAGGG
IT_A_FP_5	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CAGAAGGAAC	GATATAAAACCGCCCAGTCTACTCGAGGG
IT_A_FP_6	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTGCAAGTTC	GATATAAAACCGCCCAGTCTACTCGAGGG
IT_A_FP_7	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TTCGTGATTC	GATATAAAACCGCCCAGTCTACTCGAGGG
IT_A_FP_8	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TTCCGATAAC	GATATAAAACCGCCCAGTCTACTCGAGGG
IT_A_FP_9	CCATCTCATCCCTGCGTGTCTCCGACTCAG	TGAGCGGAAC	GATATAAAACCGCCCAGTCTACTCGAGGG
IT_A_FP_10	CCATCTCATCCCTGCGTGTCTCCGACTCAG	CTGACCGAAC	GATATAAAACCGCCCAGTCTACTCGAGGG

Reverse primer compatible with synthetic amplicons (IT adaptor). Sequence (5'-3')		
Name	**trP1 sequencing adapter**	**Primer for reverse landing pad**
IT_trP1_FP_Synthetic	CCTCTCTATGGGCAGTCGGTGAT	ATCTCGGTGGTCGCCGTA

[*] **Modified from** (29)

[†] **Modified from Illumina Nextera transposase sequence**

Table S5. P*snifH* evaluation in a competition assay of RlvUPM791[pOPS0263] vs Rlv3841[pOPS0262].

Inoculation % of UPM791	Replicate	Blue nodules (Rlv3841)	Pink nodules (UPM791)	Mixed nodules	% Blue nodules (Rlv3841)	% Pink nodules (UPM791)	% Mixed nodules
50	1	NA	NA	NA	NA	NA	NA
50	2	60	94	2	38.46	60.26	1.28
50	3	71	86	2	44.65	54.09	1.26
50	4	39	138	3	21.67	76.67	1.67
50	5	62	98	2	38.27	60.49	1.23
50	6	69	81	1	45.70	53.64	0.66
50	7	52	108	3	31.90	66.26	1.84
50	8	63	141	5	30.14	67.46	2.39
50	9	72	101	4	40.68	57.06	2.26
50	10	32	122	2	20.51	78.21	1.28
50	11	58	121	3	31.87	66.48	1.65
50	12	67	144	4	31.16	66.98	1.86
50	13	62	100	2	37.80	60.98	1.22
50	14	59	127	3	31.22	67.20	1.59
90	1	7	172	1	3.89	95.56	0.56
90	2	8	185	2	4.10	94.87	1.03
90	3	15	184	2	7.46	91.54	1.00
90	4	7	188	2	3.55	95.43	1.02
90	5	8	141	1	5.33	94.00	0.67
90	6	18	119	0	13.14	86.86	0.00
90	7	10	168	1	5.59	93.85	0.56
90	8	30	189	3	13.51	85.14	1.35
90	9	26	146	2	14.94	83.91	1.15
90	10	3	114	1	2.54	96.61	0.85

90	11	8	168	2	4.49	94.38	1.12
90	12	4	205	3	1.89	96.70	1.42
90	13	1	80	0	1.23	98.77	0.00
90	14	0	160	1	0.00	99.38	0.62
10	1	181	61	2	74.18	25.00	0.82
10	2	NA	NA	NA	NA	NA	NA
10	3	115	20	0	85.19	14.81	0.00
10	4	221	4	2	97.36	1.76	0.88
10	5	NA	NA	NA	NA	NA	NA
10	6	154	95	1	61.60	38.00	0.40
10	7	151	98	1	60.40	39.20	0.40
10	8	192	81	1	70.07	29.56	0.36
10	9	122	44	1	73.05	26.35	0.60
10	10	123	115	2	51.25	47.92	0.83
10	11	NA	NA	NA	NA	NA	NA
10	12	72	36	0	66.67	33.33	0.00
10	13	152	43	2	77.16	21.83	1.02
10	14	135	52	1	71.81	27.66	0.53

*NA: Dead plant or lost sample

Table S6. Fluorescence from expression of sfGFP, acetylene reduced and shoot DW from nodules of pea plants inoculated with different rhizobial strains, harvested at 28, 35 and 42 dpi.

Strain	Nodule fluorescence *	ARA †	Shoot DW ‡	No. of nodules	Nodule area §
28dpi					
WSM1475	6.19E+06 ± 1.42E+06^{b}	1189.35 ± 74.78^{b}	ND	141.33 ± 24.92	1.70 ± 0.06 ^{a}
Rlv3841	2.92E+08 ± 1.28E+07 ^{a}	2758.50 ± 181.65^{b}	ND	182.67 ± 24.88	2.23 ± 0.24
UPM791	3.78E+08 ± 5.35E+07 ^{a}	4613.00 ± 562.91 ^{a}	ND	276.00 ± 37.75	1.88 ± 0.27
WSM1521	5.02E+08 ± 8.67E+07	9600.97 ± 1984.58	ND	176.00 ± 35.12	2.89 ± 0.50
35dpi					
WSM1475	1.47E+07 ± 1.57E+06 ^{a}	2849.84 ± 385.46	333.33 ± 66.67*	266.67 ± 23.47	1.44 ± 0.09 ^{a}
Rlv3841	1.43E+08 ± 2.67E+07	4531.97 ± 932.35	776.00 ± 167.29 ^{a}	219.33 ± 44.36	2.16 ± 0.38
UPM791	1.55E+08 ± 1.22E+07	5481.30 ± 621.64	916.67 ± 101.38	293.67 ± 23.90	1.93 ± 0.08
WSM1521	1.51E+08 ± 5.88E+07	3305.21 ± 525.85	1033.33 ± 185.59	138.00 ± 15.52	3.50 ± 0.74
42dpi					
WSM1475	2.01E+07 ± 2.12E+06	ND	800.00 ± 100^{b}	269.67 ± 18.85	1.67 ± 0.21^{a}
Rlv3841	3.67E+08 ± 5.34E+07	ND	850.00 ± 28.87^{b}	295.67 ± 32.63	2.21 ± 0.28
UPM791	1.79E+08 ± 7.04E+07	ND	1233.33 ± 185.59 ^{a}	289.00 ± 41.58	2.58 ± 0.34
WSM1521	7.42E+07 ± 3.29E+07	ND	1750.00 ± 132.29	152.00 ± 13.58	2.96 ± 0.26

***[cps][plant]$^{-1}$, †Acetylene reduced in nanomoles of [C_2H_2 reduced] [hour]$^{-1}$[plant]$^{-1}$, ‡[mg][plant]$^{-1}$, § mm^2[nodule]$^{-1}$. ND, not determined.**

Values are the mean from at least three biological replicates ± SEM. In the case of ARA values were obtained from three biological and three technical replicates.

Statistical analysis for each strain within the same time point were compared by One-way ANOVA (Dunnett's multiple comparisons test). Those that are significantly different to WSM1521 are indicated with ^{a} (P<0.05) and with ^{b} (P<0.005).

Table S7. Golay barcode sequences and assigned position in a 96-well pate to be used as plasmid IDs.

Golay barcode	Sequence	Assigned position	Golay barcode	Sequence	Assigned position
806rcbc0	TCCCTTGTCTCC	A1	**806rcbc48**	TACTACGTGGCC	E1
806rcbc1	ACGAGACTGATT	A2	**806rcbc49**	GGCCAGTTCCTA	E2
806rcbc2	GCTGTACGGATT	A3	**806rcbc50**	GATGTTCGCTAG	E3
806rcbc3	ATCACCAGGTGT	A4	**806rcbc51**	CTATCTCCTGTC	E4
806rcbc4	TGGTCAACGATA	A5	**806rcbc52**	ACTCACAGGAAT	E5
806rcbc5	ATCGCACAGTAA	A6	**806rcbc53**	ATGATGAGCCTC	E6
806rcbc6	GTCGTGTAGCCT	A7	**806rcbc54**	GTCGACAGAGGA	E7
806rcbc7	AGCGGAGGTTAG	A8	**806rcbc55**	TGTCGCAAATAG	E8
806rcbc8	ATCCTTTGGTTC	A9	**806rcbc56**	CATCCCTCTACT	E9
806rcbc9	TACAGCGCATAC	A10	**806rcbc57**	TATACCGCTGCG	E10
806rcbc10	ACCGGTATGTAC	A11	**806rcbc58**	AGTTGAGGCATT	E11
806rcbc11	AATTGTGTCGGA	A12	**806rcbc59**	ACAATAGACACC	E12
806rcbc12	TGCATACACTGG	B1	**806rcbc60**	CGGTCAATTGAC	F1
806rcbc13	AGTCGAACGAGG	B2	**806rcbc61**	GTGGAGTCTCAT	F2
806rcbc14	ACCAGTGACTCA	B3	**806rcbc62**	GCTCGAAGATTC	F3
806rcbc15	GAATACCAAGTC	B4	**806rcbc63**	AGGCTTACGTGT	F4
806rcbc16	GTAGATCGTGTA	B5	**806rcbc64**	TCTCTACCACTC	F5
806rcbc17	TAACGTGTGTGC	B6	**806rcbc65**	ACTTCCAACTTC	F6
806rcbc18	CATTATGGCGTG	B7	**806rcbc66**	CTCACCTAGGAA	F7
806rcbc19	CCAATACGCCTG	B8	**806rcbc67**	GTGTTGTCGTGC	F8
806rcbc20	GATCTGCGATCC	B9	**806rcbc68**	CCACAGATCGAT	F9
806rcbc21	CAGCTCATCAGC	B10	**806rcbc69**	TATCGACACAAG	F10
806rcbc22	CAAACAACAGCT	B11	**806rcbc70**	GATTCCGGCTCA	F11
806rcbc23	GCAACACCATCC	B12	**806rcbc71**	CGTAATTGCCGC	F12
806rcbc24	GCGATATATCGC	C1	**806rcbc72**	GGTGACTAGTTC	G1
806rcbc25	CGAGCAATCCTA	C2	**806rcbc73**	ATGGGTTCCGTC	G2
806rcbc26	AGTCGTGCACAT	C3	**806rcbc74**	TAGGCATGCTTG	G3
806rcbc27	GTATCTGCGCGT	C4	**806rcbc75**	AACTAGTTCAGG	G4
806rcbc28	CGAGGGAAAGTC	C5	**806rcbc76**	ATTCTGCCGAAG	G5
806rcbc29	CAAATTCGGGAT	C6	**806rcbc77**	AGCATGTCCCGT	G6
806rcbc30	AGATTGACCAAC	C7	**806rcbc78**	GTACGATATGAC	G7

806rcbc31	AGTTACGAGCTA	C8	**806rcbc79**	GTGGTGGTTTCC	G8
806rcbc32	GCATATGCACTG	C9	**806rcbc80**	TAGTATGCGCAA	G9
806rcbc33	CAACTCCCGTGA	C10	**806rcbc81**	TGCGCTGAATGT	G10
806rcbc34	TTGCGTTAGCAG	C11	**806rcbc82**	ATGGCTGTCAGT	G11
806rcbc35	TACGAGCCCTAA	C12	**806rcbc83**	GTTCTCTTCTCG	G12
806rcbc36	CACTACGCTAGA	D1	**806rcbc84**	CGTAAGATGCCT	H1
806rcbc37	TGCAGTCCTCGA	D2	**806rcbc85**	GCGTTCTAGCTG	H2
806rcbc38	ACCATAGCTCCG	D3	**806rcbc86**	GTTGTTCTGGGA	H3
806rcbc39	TCGACATCTCTT	D4	**806rcbc87**	GGACTTCCAGCT	H4
806rcbc40	GAACACTTTGGA	D5	**806rcbc88**	CTCACAACCGTG	H5
806rcbc41	GAGCCATCTGTA	D6	**806rcbc89**	CTGCTATTCCTC	H6
806rcbc42	TTGGGTACACGT	D7	**806rcbc90**	ATGTCACCGCTG	H7
806rcbc43	AAGGCGCTCCTT	D8	**806rcbc91**	TGTAACGCCGAT	H8
806rcbc44	TAATACGGATCG	D9	**806rcbc92**	AGCAGAACATCT	H9
806rcbc45	TCGGAATTAGAC	D10	**806rcbc93**	TGGAGTAGGTGG	H10
806rcbc46	TGTGAATTCGGA	D11	**806rcbc94**	TTGGCTCTATTC	H11
806rcbc47	CATTCGTGGCGT	D12	**806rcbc95**	GATCCCACGTAC	H12

Table S8. Methods performed to obtain chemical composition.

Methods	Accreditation*
pH Determination	DM007
Soil Extraction – 0.43M Acetic Acid – Ca, Mg, K & P	DM005
Inductively coupled plasma–Optical Emission Spectroscopy (ICP-OES)	BM005
Soil Extraction – 1M KCl – NO_3 & NH_4	N/A
Analysis 1M KCl Extractions	N/A
Moisture Determination	DM007

***United Kingdom Accreditation Service (UKAS) approved methods**

Table S9. Chemical composition of Yatesbury House Farm soil.

Sample	pH	Ca*	K*	Mg*	P†	NO_3†	NH_4†
Yatesbury soil	7.75	458	0.58	3.25	115.6	64.02	13.05

Values are expressed in *[cmol] $[kg]^{-1}$ or †[mg]$[kg]^{-1}$

Table S10. Full list of the strain occurrence.

Strain or combination	Occurrence	Strain or combination	Occurrence	Strain or combination	Occurrence
Rlv3841-WSM1475-G093-H082-H031	1	**24-H082**	1	**G0077-L111**	2
Rlv3841-WSM1475-V050	1	**WSM1475-G093-H082-H031**	1	**G093-H082-H031**	2
Rlv3841-WSM1488	1	**Rlv3841-WSM1475-V116-V006**	1	**SU303-G083**	2
BeanLA_2A	1	**WSM1475-G093-L111**	1	**G093-H031**	2
Rlv3841-WSM1488-G0077-G093-VSX28	1	**G051-VSX28-H031**	1	**V006-H031**	2
G004	1	**WSM1475-H031**	1	**WSM1475-13**	2
SU303-G0077-G083-G109	1	**G083-L010**	1	**V006-H082**	2
536-H174	1	**WSM1475-SU303**	1	**Rlv3841-G093-H082-E2_1A**	2
V006-L018-E2_1A	1	**G093-G083**	1	**Rlv3841-WSM1475-G093**	2
G007-G109	1	**WSM1475-SU303-G083**	1	**WSM1475-G051**	2
G051-G083-G067-VSX28-H031	1	**G093-H174**	1	**WSM1475-V006**	2
G0077-G093-H082-H031	1	**WSM1475-V043**	1	**WSM1529**	2
G067-L111	1	**G109-H174**	1	**SU303-G109**	2
SU303-G067	1	**WSM1475-V116**	1	**Rlv3841-H082-E2_1A-H031**	2
G083-H178	1	**H174-VSX28-H031**	1	**G109-L111**	2
SU303-G083-G067	1	**WSM1475-VSX28-L111**	1	**G109-G067-L111**	2
G088	1	**L102-V006**	1	**SU303-L111**	2
SU303-H174-G067-L111	1	**WSM1475-WSM1488-G0077**	1	**Rlv3841-G051-VSX28-H031**	2
G093-G067	1	**Rlv3841-H031**	1	**L082**	2
SU303-V116	1	**WSM1488-13-G0077-L111**	1	**H082-E2_1A**	2
G093-G083-H082	1	**Rlv3841-PB3-3-G109-G067**	1	**G094**	2
UPM791-G067	1	**WSM1488-G051**	1	**24**	3
G093-H082-G067-H031	1	**Rlv3841-V006-G093-H082-H031**	1	**V057**	3
UPM791-V006	1	**WSM1488-V006-H082**	1	**Rlv3841-G067**	3
G093-L018	1	**Rlv3841-WSM1475-13-VSX28**	1	**V116-V006**	3
V006-24-H082	1	**Rlv3841-G093-H082-E2_1A-H031**	1	**VF2**	3
G109-H082-VSX28-H031	1	**13-G0077-V006-G109-L111**	1	**G093-H082-E2_1A-H031**	3
V006-G067	1	**Rlv3841-H082-E2_1A**	1	**H178**	4
H082-G067	1	**G0077-G109**	1	**L018-E2_1A**	4
V006-G083-L008	1	**H082-E2_1A-H031**	1	**VSX28**	5
H174-G067	1	**G088-L111**	1	**L102**	5
V006-G094	1	**V006-H082-E2_1A**	1	**L111**	6
L008-H082	1	**G109-H082**	1	**UPM791**	6
V006-G109-H082	1	**E2_1A-H031**	1	**L079**	6
L102-G083	1	**L008-L018-H031**	1	**L018**	6

V006-H082-H031	1	**Rlv3841-E2_1A**	1	**V050**	7
PB3-3-G093	1	**Rlv3841-H174**	1	**536**	7
V006-L018	1	**Rlv3841-536-E2_1A**	1	**WSM1488**	7
Rlv3841-G067-VSX28	1	**Rlv3841-V116-L079**	1	**G051**	8
V010	1	**Rlv3841-SU303-L079-E2_1A**	1	**L010**	9
Rlv3841-H082	1	**Rlv3841-WSM1475-V116-H174-VSX28-H031**	1	**E2_1A**	9
V030	1	**VSX28-L018-E2_1A**	1	**PB3-3**	13
Rlv3841-L008-H031	1	**G093-G109**	1	**H174**	15
V116-H082	1	**WSM1475-L018-E2_1A**	1	**L008**	16
Rlv3841-SU303	1	**PB3-3-L008**	1	**H082**	19
V116-H174-L111	1	**WSM1475-G0077-L018-E2_1A**	1	**G093**	20
Rlv3841-V006-G067-VSX28-H031	1	**Rlv3841-WSM1475-G0077-G093**	1	**SU303**	22
V116-VSX28-H031	1	**PB3-3-E2_1A**	1	**H031**	24
Rlv3841-V006-H082-H031	1	**H082-VSX28**	1	**V006**	30
VSX28-H031	1	**Rlv3841-G093-E2_1A**	1	**WSM1475**	30
Rlv3841-WSM1475-13-V006-H031	1	**G067-VSX28**	1	**Rlv3841**	33
VSX28-L111-H031	1	**Rlv3841-536-L008-E2_1A**	1	**G0077**	34
Rlv3841-WSM1475-G0077-G083	1	**Rlv3841-V006**	1	**G109**	39
WSM1475-G0077-V006-G093-H031	1	**L008-E2_1A**	1	**G067**	40
Rlv3841-WSM1475-G0077-V006	1	**G0077-G083**	2	**V116**	54
WSM1475-G093-G109	1	**V006-G093-H082-H031**	2	**G083**	117

Table S11. Full statistical analysis of Competitiveness Index (CI)

ANOVA summary	
F	16.02
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.3269

Brown-Forsythe test	
F (DFn, DFd)	1.599 (9, 297)
P value	0.1147
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

Bartlett's test	
Bartlett's statistic (corrected)	26.68
P value	0.0016
P value summary	**
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	4136	9	459.5	F (9, 297) = 16.02	P<0.0001
Residual (within columns)	8517	297	28.68		
Total	12652	306			

Data summary	
Number of treatments (columns)	10
Number of values (total)	307

Number of families	**1**
Number of comparisons per family	45
Alpha	0.05

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value	
SU303 vs. H031	0.1387	-4.197 to 4.475	ns	>0.9999	A-B
SU303 vs. V006	-0.1681	-4.504 to 4.168	ns	>0.9999	A-C
SU303 vs. WSM1475	-0.3748	-4.711 to 3.961	ns	>0.9999	A-D
SU303 vs. Rlv3841	-0.9531	-5.325 to 3.419	ns	0.9995	A-E
SU303 vs. G0077	-1.46	-5.796 to 2.876	ns	0.9869	A-F
SU303 vs. G109	-1.092	-5.464 to 3.280	ns	0.9986	A-G
SU303 vs. G067	-1.633	-5.969 to 2.703	ns	0.9719	A-H

SU303 vs. V116	-3.597	-7.933 to 0.7390	ns	0.2019	A-I
SU303 vs. G083	-12.87	-17.24 to -8.496	****	<0.0001	A-J
H031 vs. V006	-0.3068	-4.643 to 4.029	ns	>0.9999	B-C
H031 vs. WSM1475	-0.5135	-4.850 to 3.823	ns	>0.9999	B-D
H031 vs. Rlv3841	-1.092	-5.464 to 3.280	ns	0.9986	B-E
H031 vs. G0077	-1.599	-5.935 to 2.737	ns	0.9755	B-F
H031 vs. G109	-1.23	-5.603 to 3.142	ns	0.9965	B-G
H031 vs. G067	-1.772	-6.108 to 2.565	ns	0.9525	B-H
H031 vs. V116	-3.736	-8.072 to 0.6003	ns	0.1603	B-I
H031 vs. G083	-13.01	-17.38 to -8.634	****	<0.0001	B-J
V006 vs. WSM1475	-0.2068	-4.543 to 4.129	ns	>0.9999	C-D
V006 vs. Rlv3841	-0.785	-5.157 to 3.587	ns	>0.9999	C-E
V006 vs. G0077	-1.292	-5.628 to 3.044	ns	0.9946	C-F
V006 vs. G109	-0.9237	-5.296 to 3.448	ns	0.9996	C-G
V006 vs. G067	-1.465	-5.801 to 2.871	ns	0.9866	C-H
V006 vs. V116	-3.429	-7.765 to 0.9071	ns	0.2616	C-I
V006 vs. G083	-12.7	-17.07 to -8.328	****	<0.0001	C-J
WSM1475 vs. Rlv3841	-0.5782	-4.950 to 3.794	ns	>0.9999	D-E
WSM1475 vs. G0077	-1.085	-5.422 to 3.251	ns	0.9986	D-F
WSM1475 vs. G109	-0.7169	-5.089 to 3.655	ns	>0.9999	D-G
WSM1475 vs. G067	-1.258	-5.594 to 3.078	ns	0.9956	D-H
WSM1475 vs. V116	-3.222	-7.558 to 1.114	ns	0.3484	D-I
WSM1475 vs. G083	-12.49	-16.86 to -8.121	****	<0.0001	D-J
Rlv3841 vs. G0077	-0.5073	-4.879 to 3.865	ns	>0.9999	E-F
Rlv3841 vs. G109	-0.1387	-4.546 to 4.269	ns	>0.9999	E-G
Rlv3841 vs. G067	-0.6798	-5.052 to 3.692	ns	>0.9999	E-H
Rlv3841 vs. V116	-2.644	-7.016 to 1.728	ns	0.65	E-I
Rlv3841 vs. G083	-11.91	-16.32 to -7.507	****	<0.0001	E-J
G0077 vs. G109	0.3686	-4.003 to 4.741	ns	>0.9999	F-G
G0077 vs. G067	-0.1726	-4.509 to 4.164	ns	>0.9999	F-H
G0077 vs. V116	-2.137	-6.473 to 2.199	ns	0.8611	F-I
G0077 vs. G083	-11.41	-15.78 to -7.035	****	<0.0001	F-J
G109 vs. G067	-0.5412	-4.913 to 3.831	ns	>0.9999	G-H
G109 vs. V116	-2.505	-6.877 to 1.867	ns	0.7177	G-I
G109 vs. G083	-11.78	-16.18 to -7.368	****	<0.0001	G-J
G067 vs. V116	-1.964	-6.300 to 2.372	ns	0.9122	H-I
G067 vs. G083	-11.23	-15.61 to -6.863	****	<0.0001	H-J
V116 vs. G083	-9.271	-13.64 to -4.899	****	<0.0001	I-J

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
SU303 vs. H031	3.272	3.133	0.1387	1.36	31	31	0.1442	297
SU303 vs. V006	3.272	3.44	-0.1681	1.36	31	31	0.1747	297
SU303 vs. WSM1475	3.272	3.646	-0.3748	1.36	31	31	0.3897	297
SU303 vs. Rlv3841	3.272	4.225	-0.9531	1.371	31	30	0.9828	297
SU303 vs. G0077	3.272	4.732	-1.46	1.36	31	31	1.518	297
SU303 vs. G109	3.272	4.363	-1.092	1.371	31	30	1.126	297
SU303 vs. G067	3.272	4.905	-1.633	1.36	31	31	1.698	297
SU303 vs. V116	3.272	6.869	-3.597	1.36	31	31	3.74	297
SU303 vs. G083	3.272	16.14	-12.87	1.371	31	30	13.27	297
H031 vs. V006	3.133	3.44	-0.3068	1.36	31	31	0.319	297
H031 vs. WSM1475	3.133	3.646	-0.5135	1.36	31	31	0.534	297

H031 vs. Rlv3841	3.133	4.225	-1.092	1.371	31	30	1.126	297
H031 vs. G0077	3.133	4.732	-1.599	1.36	31	31	1.663	297
H031 vs. G109	3.133	4.363	-1.23	1.371	31	30	1.269	297
H031 vs. G067	3.133	4.905	-1.772	1.36	31	31	1.842	297
H031 vs. V116	3.133	6.869	-3.736	1.36	31	31	3.884	297
H031 vs. G083	3.133	16.14	-13.01	1.371	31	30	13.41	297
V006 vs. WSM1475	3.44	3.646	-0.2068	1.36	31	31	0.215	297
V006 vs. Rlv3841	3.44	4.225	-0.785	1.371	31	30	0.8095	297
V006 vs. G0077	3.44	4.732	-1.292	1.36	31	31	1.344	297
V006 vs. G109	3.44	4.363	-0.9237	1.371	31	30	0.9525	297
V006 vs. G067	3.44	4.905	-1.465	1.36	31	31	1.523	297
V006 vs. V116	3.44	6.869	-3.429	1.36	31	31	3.565	297
V006 vs. G083	3.44	16.14	-12.7	1.371	31	30	13.1	297
WSM1475 vs. Rlv3841	3.646	4.225	-0.5782	1.371	31	30	0.5963	297
WSM1475 vs. G0077	3.646	4.732	-1.085	1.36	31	31	1.129	297
WSM1475 vs. G109	3.646	4.363	-0.7169	1.371	31	30	0.7392	297
WSM1475 vs. G067	3.646	4.905	-1.258	1.36	31	31	1.308	297
WSM1475 vs. V116	3.646	6.869	-3.222	1.36	31	31	3.35	297
WSM1475 vs. G083	3.646	16.14	-12.49	1.371	31	30	12.88	297
Rlv3841 vs. G0077	4.225	4.732	-0.5073	1.371	30	31	0.5231	297
Rlv3841 vs. G109	4.225	4.363	-0.1387	1.383	30	30	0.1418	297
Rlv3841 vs. G067	4.225	4.905	-0.6798	1.371	30	31	0.7011	297
Rlv3841 vs. V116	4.225	6.869	-2.644	1.371	30	31	2.727	297
Rlv3841 vs. G083	4.225	16.14	-11.91	1.383	30	30	12.19	297
G0077 vs. G109	4.732	4.363	0.3686	1.371	31	30	0.3801	297
G0077 vs. G067	4.732	4.905	-0.1726	1.36	31	31	0.1794	297
G0077 vs. V116	4.732	6.869	-2.137	1.36	31	31	2.222	297
G0077 vs. G083	4.732	16.14	-11.41	1.371	31	30	11.76	297
G109 vs. G067	4.363	4.905	-0.5412	1.371	30	31	0.5581	297
G109 vs. V116	4.363	6.869	-2.505	1.371	30	31	2.584	297
G109 vs. G083	4.363	16.14	-11.78	1.383	30	30	12.04	297
G067 vs. V116	4.905	6.869	-1.964	1.36	31	31	2.042	297
G067 vs. G083	4.905	16.14	-11.23	1.371	31	30	11.59	297
V116 vs. G083	6.869	16.14	-9.271	1.371	31	30	9.56	297

Table S12. Full statistical analysis of effectiveness by GFP detection.

ANOVA summary	
F	19.83
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.608

Brown-Forsythe test	
F (DFn, DFd)	3.538 (14, 179)
P value	<0.0001
P value summary	****
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	2.187E+14	14	1.56E+13	F (14, 179) = 19.83	P<0.0001
Residual (within columns)	1.41E+14	179	7.88E+11		
Total	3.596E+14	193			

Data summary	
Number of treatments (columns)	15
Number of values (total)	194

Number of families	**1**
Number of comparisons per family	105
Alpha	0.05

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value	
G083 vs. V116	1779988	1081663 to 2478313	****	<0.0001	A-B
G083 vs. G067	1918909	1006968 to 2830850	****	<0.0001	A-C
G083 vs. G109	2187275	1243159 to 3131391	****	<0.0001	A-D
G083 vs. G0077	2122117	1099071 to 3145162	****	<0.0001	A-E
G083 vs. Rlv3841	2662809	1531648 to 3793970	****	<0.0001	A-F
G083 vs. H031	2359658	1228497 to 3490819	****	<0.0001	A-G
G083 vs. L008	1189810	-12639 to 2392259	ns	0.0557	A-H
G083 vs. WSM1475	2117194	825801 to 3408586	****	<0.0001	A-I
G083 vs. H082	2489873	1083377 to 3896370	****	<0.0001	A-J

G083 vs. PB3-3	2314184	907688 to 3720681	****	<0.0001	A-K
G083 vs. H174	1714805	151463 to 3278146	*	0.0173	A-L
G083 vs. G093	2089565	526223 to 3652906	***	0.0008	A-M
G083 vs. SU303	2847993	1053452 to 4642534	****	<0.0001	A-N
G083 vs. WSM1488	2715069	920528 to 4509610	****	<0.0001	A-O
V116 vs. G067	138921	-904758 to 1182600	ns	>0.9999	B-C
V116 vs. G109	407287	-664620 to 1479194	ns	0.9929	B-D
V116 vs. G0077	342129	-799910 to 1484168	ns	0.9994	B-E
V116 vs. Rlv3841	882821	-356999 to 2122642	ns	0.4824	B-F
V116 vs. H031	579670	-660151 to 1819490	ns	0.9549	B-G
V116 vs. L008	-590178	-1895365 to 715009	ns	0.9657	B-H
V116 vs. WSM1475	337206	-1050356 to 1724767	ns	>0.9999	B-I
V116 vs. H082	709885	-785395 to 2205165	ns	0.9491	B-J
V116 vs. PB3-3	534196	-961084 to 2029476	ns	0.9962	B-K
V116 vs. H174	-65183	-1708858 to 1578492	ns	>0.9999	B-L
V116 vs. G093	309577	-1334098 to 1953252	ns	>0.9999	B-M
V116 vs. SU303	1068005	-796937 to 2932946	ns	0.8131	B-N
V116 vs. WSM1488	935081	-929860 to 2800023	ns	0.9228	B-O
G067 vs. G109	268366	-953503 to 1490235	ns	>0.9999	C-D
G067 vs. G0077	203208	-1080627 to 1487042	ns	>0.9999	C-E
G067 vs. Rlv3841	743900	-627644 to 2115445	ns	0.8668	C-F
G067 vs. H031	440749	-930796 to 1812293	ns	0.9987	C-G
G067 vs. L008	-729099	-2160005 to 701807	ns	0.9132	C-H
G067 vs. WSM1475	198285	-1308137 to 1704706	ns	>0.9999	C-I
G067 vs. H082	570964	-1035224 to 2177152	ns	0.9964	C-J
G067 vs. PB3-3	395275	-1210913 to 2001463	ns	>0.9999	C-K
G067 vs. H174	-204104	-1949282 to 1541074	ns	>0.9999	C-L
G067 vs. G093	170656	-1574522 to 1915834	ns	>0.9999	C-M
G067 vs. SU303	929084	-1025907 to 2884074	ns	0.9486	C-N
G067 vs. WSM1488	796160	-1158830 to 2751151	ns	0.9863	C-O
G109 vs. G0077	-65158	-1372044 to 1241727	ns	>0.9999	D-E
G109 vs. Rlv3841	475534	-917610 to 1868679	ns	0.9976	D-F
G109 vs. H031	172383	-1220762 to 1565528	ns	>0.9999	D-G
G109 vs. L008	-997465	-2449088 to 454159	ns	0.5452	D-H
G109 vs. WSM1475	-70081	-1596195 to 1456032	ns	>0.9999	D-I
G109 vs. H082	302598	-1322074 to 1927270	ns	>0.9999	D-J
G109 vs. PB3-3	126909	-1497763 to 1751581	ns	>0.9999	D-K
G109 vs. H174	-472470	-2234675 to 1289734	ns	0.9998	D-L
G109 vs. G093	-97710	-1859915 to 1664494	ns	>0.9999	D-M

G109 vs. SU303	660718	-1309487 to 2630922	ns	0.998	D-N
G109 vs. WSM1488	527795	-1442410 to 2497999	ns	0.9998	D-O
G0077 vs. Rlv3841	540693	-907106 to 1988491	ns	0.994	E-F
G0077 vs. H031	237541	-1210258 to 1685340	ns	>0.9999	E-G
G0077 vs. L008	-932306	-2436461 to 571848	ns	0.7118	E-H
G0077 vs. WSM1475	-4923	-1581087 to 1571240	ns	>0.9999	E-I
G0077 vs. H082	367757	-1304017 to 2039530	ns	>0.9999	E-J
G0077 vs. PB3-3	192068	-1479706 to 1863841	ns	>0.9999	E-K
G0077 vs. H174	-407312	-2213034 to 1398410	ns	>0.9999	E-L
G0077 vs. G093	-32552	-1838274 to 1773170	ns	>0.9999	E-M
G0077 vs. SU303	725876	-1283346 to 2735098	ns	0.9957	E-N
G0077 vs. WSM1488	592953	-1416269 to 2602175	ns	0.9995	E-O
Rlv3841 vs. H031	-303152	-1829265 to 1222962	ns	>0.9999	F-G
Rlv3841 vs. L008	-1472999	-3052677 to 106679	ns	0.0968	F-H
Rlv3841 vs. WSM1475	-545616	-2194007 to 1102775	ns	0.9983	F-I
Rlv3841 vs. H082	-172936	-1912974 to 1567101	ns	>0.9999	F-J
Rlv3841 vs. PB3-3	-348625	-2088663 to 1391412	ns	>0.9999	F-K
Rlv3841 vs. H174	-948005	-2817105 to 921095	ns	0.916	F-L
Rlv3841 vs. G093	-573245	-2442345 to 1295855	ns	0.9993	F-M
Rlv3841 vs. SU303	185183	-1881184 to 2251551	ns	>0.9999	F-N
Rlv3841 vs. WSM1488	52260	-2014108 to 2118628	ns	>0.9999	F-O
H031 vs. L008	-1169847	-2749525 to 409830	ns	0.4129	G-H
H031 vs. WSM1475	-242464	-1890855 to 1405927	ns	>0.9999	G-I
H031 vs. H082	130216	-1609822 to 1870253	ns	>0.9999	G-J
H031 vs. PB3-3	-45473	-1785511 to 1694564	ns	>0.9999	G-K
H031 vs. H174	-644853	-2513953 to 1224247	ns	0.9973	G-L
H031 vs. G093	-270093	-2139193 to 1599007	ns	>0.9999	G-M
H031 vs. SU303	488335	-1578033 to 2554703	ns	>0.9999	G-N
H031 vs. WSM1488	355412	-1710956 to 2421780	ns	>0.9999	G-O
L008 vs. WSM1475	927383	-770719 to 2625486	ns	0.8607	H-I
L008 vs. H082	1300063	-487138 to 3087264	ns	0.4445	H-J
L008 vs. PB3-3	1124374	-662827 to 2911575	ns	0.6898	H-K
L008 vs. H174	524995	-1388090 to 2438079	ns	0.9998	H-L
L008 vs. G093	899755	-1013330 to 2812839	ns	0.9527	H-M
L008 vs. SU303	1658183	-448054 to 3764420	ns	0.309	H-N
L008 vs. WSM1488	1525259	-580978 to 3631496	ns	0.4525	H-O
WSM1475 vs. H082	372680	-1475536 to 2220895	ns	>0.9999	I-J
WSM1475 vs. PB3-3	196991	-1651225 to 2045206	ns	>0.9999	I-K
WSM1475 vs. H174	-402389	-2372593 to 1567816	ns	>0.9999	I-L

WSM1475 vs. G093	-27629	-1997833 to 1942576	ns	>0.9999	I-M
WSM1475 vs. SU303	730799	-1427452 to 2889050	ns	0.9978	I-N
WSM1475 vs. WSM1488	597876	-1560375 to 2756127	ns	0.9998	I-O
H082 vs. PB3-3	-175689	-2106087 to 1754709	ns	>0.9999	J-K
H082 vs. H174	-775068	-2822565 to 1272428	ns	0.9932	J-L
H082 vs. G093	-400308	-2447805 to 1647188	ns	>0.9999	J-M
H082 vs. SU303	358120	-1870912 to 2587151	ns	>0.9999	J-N
H082 vs. WSM1488	225196	-2003836 to 2454228	ns	>0.9999	J-O
PB3-3 vs. H174	-599379	-2646876 to 1448117	ns	0.9996	K-L
PB3-3 vs. G093	-224619	-2272116 to 1822877	ns	>0.9999	K-M
PB3-3 vs. SU303	533809	-1695223 to 2762840	ns	>0.9999	K-N
PB3-3 vs. WSM1488	400885	-1828147 to 2629917	ns	>0.9999	K-O
H174 vs. G093	374760	-1783491 to 2533011	ns	>0.9999	L-M
H174 vs. SU303	1133188	-1197989 to 3464365	ns	0.9388	L-N
H174 vs. WSM1488	1000265	-1330913 to 3331442	ns	0.9781	L-O
G093 vs. SU303	758428	-1572749 to 3089605	ns	0.9986	M-N
G093 vs. WSM1488	625505	-1705673 to 2956682	ns	0.9998	M-O
SU303 vs. WSM1488	-132923	-2625057 to 2359210	ns	>0.9999	N-O

Test details	**Mean 1**	**Mean 2**	**Mean Diff.**	**SE of diff.**	**n1**	**n2**	**q**	**DF**
G083 vs. V116	3088426	1308438	1779988	203033	81	25	12.4	179
G083 vs. G067	3088426	1169517	1918909	265140	81	13	10.24	179
G083 vs. G109	3088426	901151	2187275	274495	81	12	11.27	179
G083 vs. G0077	3088426	966309	2122117	297443	81	10	10.09	179
G083 vs. Rlv3841	3088426	425616	2662809	328877	81	8	11.45	179
G083 vs. H031	3088426	728768	2359658	328877	81	8	10.15	179
G083 vs. L008	3088426	1898616	1189810	349603	81	7	4.813	179
G083 vs. WSM1475	3088426	971232	2117194	375463	81	6	7.975	179
G083 vs. H082	3088426	598553	2489873	408928	81	5	8.611	179
G083 vs. PB3-3	3088426	774242	2314184	408928	81	5	8.003	179
G083 vs. H174	3088426	1373621	1714805	454530	81	4	5.335	179
G083 vs. G093	3088426	998861	2089565	454530	81	4	6.501	179
G083 vs. SU303	3088426	240433	2847993	521750	81	3	7.72	179
G083 vs. WSM1488	3088426	373356	2715069	521750	81	3	7.359	179
V116 vs. G067	1308438	1169517	138921	303442	25	13	0.6475	179
V116 vs. G109	1308438	901151	407287	311649	25	12	1.848	179
V116 vs. G0077	1308438	966309	342129	332039	25	10	1.457	179
V116 vs. Rlv3841	1308438	425616	882821	360469	25	8	3.464	179

V116 vs. H031	1308438	728768	579670	360469	25	8	2.274	179
V116 vs. L008	1308438	1898616	-590178	379474	25	7	2.199	179
V116 vs. WSM1475	1308438	971232	337206	403423	25	6	1.182	179
V116 vs. H082	1308438	598553	709885	434742	25	5	2.309	179
V116 vs. PB3-3	1308438	774242	534196	434742	25	5	1.738	179
V116 vs. H174	1308438	1373621	-65183	477886	25	4	0.1929	179
V116 vs. G093	1308438	998861	309577	477886	25	4	0.9161	179
V116 vs. SU303	1308438	240433	1068005	542218	25	3	2.786	179
V116 vs. WSM1488	1308438	373356	935081	542218	25	3	2.439	179
G067 vs. G109	1169517	901151	268366	355249	13	12	1.068	179
G067 vs. G0077	1169517	966309	203208	373265	13	10	0.7699	179
G067 vs. Rlv3841	1169517	425616	743900	398766	13	8	2.638	179
G067 vs. H031	1169517	728768	440749	398766	13	8	1.563	179
G067 vs. L008	1169517	1898616	-729099	416025	13	7	2.478	179
G067 vs. WSM1475	1169517	971232	198285	437981	13	6	0.6402	179
G067 vs. H082	1169517	598553	570964	466987	13	5	1.729	179
G067 vs. PB3-3	1169517	774242	395275	466987	13	5	1.197	179
G067 vs. H174	1169517	1373621	-204104	507398	13	4	0.5689	179
G067 vs. G093	1169517	998861	170656	507398	13	4	0.4756	179
G067 vs. SU303	1169517	240433	929084	568399	13	3	2.312	179
G067 vs. WSM1488	1169517	373356	796160	568399	13	3	1.981	179
G109 vs. G0077	901151	966309	-65158	379967	12	10	0.2425	179
G109 vs. Rlv3841	901151	425616	475534	405047	12	8	1.66	179
G109 vs. H031	901151	728768	172383	405047	12	8	0.6019	179
G109 vs. L008	901151	1898616	-997465	422049	12	7	3.342	179
G109 vs. WSM1475	901151	971232	-70081	443706	12	6	0.2234	179
G109 vs. H082	901151	598553	302598	472361	12	5	0.906	179
G109 vs. PB3-3	901151	774242	126909	472361	12	5	0.38	179
G109 vs. H174	901151	1373621	-472470	512348	12	4	1.304	179
G109 vs. G093	901151	998861	-97710	512348	12	4	0.2697	179
G109 vs. SU303	901151	240433	660718	572822	12	3	1.631	179
G109 vs. WSM1488	901151	373356	527795	572822	12	3	1.303	179
G0077 vs. Rlv3841	966309	425616	540693	420937	10	8	1.817	179
G0077 vs. H031	966309	728768	237541	420937	10	8	0.7981	179
G0077 vs. L008	966309	1898616	-932306	437322	10	7	3.015	179
G0077 vs. WSM1475	966309	971232	-4923	458258	10	6	0.01519	179
G0077 vs. H082	966309	598553	367757	486056	10	5	1.07	179
G0077 vs. PB3-3	966309	774242	192068	486056	10	5	0.5588	179
G0077 vs. H174	966309	1373621	-407312	525000	10	4	1.097	179

G0077 vs. G093	966309	998861	-32552	525000	10	4	0.08769	179
G0077 vs. SU303	966309	240433	725876	584166	10	3	1.757	179
G0077 vs. WSM1488	966309	373356	592953	584166	10	3	1.435	179
Rlv3841 vs. H031	425616	728768	-303152	443706	8	8	0.9662	179
Rlv3841 vs. L008	425616	1898616	-1472999	459280	8	7	4.536	179
Rlv3841 vs. WSM1475	425616	971232	-545616	479258	8	6	1.61	179
Rlv3841 vs. H082	425616	598553	-172936	505903	8	5	0.4834	179
Rlv3841 vs. PB3-3	425616	774242	-348625	505903	8	5	0.9746	179
Rlv3841 vs. H174	425616	1373621	-948005	543427	8	4	2.467	179
Rlv3841 vs. G093	425616	998861	-573245	543427	8	4	1.492	179
Rlv3841 vs. SU303	425616	240433	185183	600781	8	3	0.4359	179
Rlv3841 vs. WSM1488	425616	373356	52260	600781	8	3	0.123	179
H031 vs. L008	728768	1898616	-1169847	459280	8	7	3.602	179
H031 vs. WSM1475	728768	971232	-242464	479258	8	6	0.7155	179
H031 vs. H082	728768	598553	130216	505903	8	5	0.364	179
H031 vs. PB3-3	728768	774242	-45473	505903	8	5	0.1271	179
H031 vs. H174	728768	1373621	-644853	543427	8	4	1.678	179
H031 vs. G093	728768	998861	-270093	543427	8	4	0.7029	179
H031 vs. SU303	728768	240433	488335	600781	8	3	1.15	179
H031 vs. WSM1488	728768	373356	355412	600781	8	3	0.8366	179
L008 vs. WSM1475	1898616	971232	927383	493711	7	6	2.656	179
L008 vs. H082	1898616	598553	1300063	519616	7	5	3.538	179
L008 vs. PB3-3	1898616	774242	1124374	519616	7	5	3.06	179
L008 vs. H174	1898616	1373621	524995	556215	7	4	1.335	179
L008 vs. G093	1898616	998861	899755	556215	7	4	2.288	179
L008 vs. SU303	1898616	240433	1658183	612373	7	3	3.829	179
L008 vs. WSM1488	1898616	373356	1525259	612373	7	3	3.522	179
WSM1475 vs. H082	971232	598553	372680	537355	6	5	0.9808	179
WSM1475 vs. PB3-3	971232	774242	196991	537355	6	5	0.5184	179
WSM1475 vs. H174	971232	1373621	-402389	572822	6	4	0.9934	179
WSM1475 vs. G093	971232	998861	-27629	572822	6	4	0.06821	179
WSM1475 vs. SU303	971232	240433	730799	627495	6	3	1.647	179
WSM1475 vs. WSM1488	971232	373356	597876	627495	6	3	1.347	179
H082 vs. PB3-3	598553	774242	-175689	561249	5	5	0.4427	179
H082 vs. H174	598553	1373621	-775068	595294	5	4	1.841	179
H082 vs. G093	598553	998861	-400308	595294	5	4	0.951	179
H082 vs. SU303	598553	240433	358120	648075	5	3	0.7815	179
H082 vs. WSM1488	598553	373356	225196	648075	5	3	0.4914	179
PB3-3 vs. H174	774242	1373621	-599379	595294	5	4	1.424	179

PB3-3 vs. G093	774242	998861	-224619	595294	5	4	0.5336	179
PB3-3 vs. SU303	774242	240433	533809	648075	5	3	1.165	179
PB3-3 vs. WSM1488	774242	373356	400885	648075	5	3	0.8748	179
H174 vs. G093	1373621	998861	374760	627495	4	4	0.8446	179
H174 vs. SU303	1373621	240433	1133188	677773	4	3	2.364	179
H174 vs. WSM1488	1373621	373356	1000265	677773	4	3	2.087	179
G093 vs. SU303	998861	240433	758428	677773	4	3	1.583	179
G093 vs. WSM1488	998861	373356	625505	677773	4	3	1.305	179
SU303 vs. WSM1488	240433	373356	-132923	724569	3	3	0.2594	179

Table S13. Results of effectiveness assessment of selected strains by ARA at 21 dpi

	No. of nodules	Total nodule fresh weight *	Average nodule fresh weight †	ARA ‡
WC	0	0	0	0.10 ± 0.03
Rlv3841	142.75± 13.24	0.38 ± 0.03	2.75 ± 0.22	4.72 ± 0.55
V116	165.75± 15.14	0.37 ± 0.03	2.23 ± 0.15	4.26 ± 0.39
G083	112.75± 23.10	0.34 ± 0.02	3.32 ± 0.59	7.53 ± 1.17

***[gr] [plant]$^{-1}$, † [mg] [nodule]$^{-1}$[plant]$^{-1}$, ‡ Acetylene reduced in nanomoles of [µmoles of C_2H_2 reduced][hr]$^{-1}$[plant]$^{-1}$. Values are the mean from at least four biological replicates ± SEM. In the case of ARA values were obtained from four biological and three technical replicates.**

Table S14. Full statistical analysis of effectiveness assessment of selected strains by ARA[plant]$^{-1}$

Table Analyzed	ARA/plant
Data sets analyzed	A-D
ANOVA summary	
F	20.70
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.8381
Brown-Forsythe test	
F (DFn, DFd)	17.81 (3, 12)
P value	0.0001
P value summary	***
Are SDs significantly different (P < 0.05)?	Yes
Bartlett's test	
Bartlett's statistic (corrected)	17.77
P value	0.0005
P value summary	***
Are SDs significantly different (P < 0.05)?	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	112.8	3	37.61	F (3, 12) = 20.70	P<0.0001
Residual (within columns)	21.80	12	1.817		
Total	134.6	15			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	16				

Number of families	1
Number of comparisons per family	3
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value	D-?	
G083 vs. WC	7.436	4.879 to 9.993	****	<0.0001	A	WC
G083 vs. Rlv3841	2.813	0.2555 to 5.370	*	0.0310	B	Rlv3841
G083 vs. V116	3.270	0.7130 to 5.827	*	0.0130	C	V116

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
G083 vs. WC	7.532	0.09592	7.436	0.9531	4	4	7.802	12
G083 vs. Rlv3841	7.532	4.719	2.813	0.9531	4	4	2.951	12
G083 vs. V116	7.532	4.262	3.270	0.9531	4	4	3.431	12

Table S15. Results of effectiveness assessment of selected strains by DW at 42dpi.

	No. of nodules [plant]$^{-1}$	Total nodule fresh weight *	Average nodule fresh weight †	No. of pods [plant]$^{-1}$	Pods DW ‡	Total shoot DW ‡
WC	0	0	0	1 ± 0	172.87 ± 33.03	300 ± 81.65
Rlv3841	139.5 ± 18.80	0.68 ± 0.05	4.87 ± 1.10	3 ± 0	1232.67 ± 54.26	2275 ± 94.65
V116	198.75 ± 23.48	1.06 ± 0.13	5.34 ± 0.03	3 ± 1.08	1001.12 ± 334.87	2575 ± 165.20
G083	118.75 ± 10.21	0.75 ± 0.06	6.27 ± 0.75	4.25 ± 0.25	1883 ± 138.30	3475 ± 893.84

***[gr] [plant]$^{-1}$, † [mg] [nodule]$^{-1}$[plant]$^{-1}$, ‡ [mg] [plant]$^{-1}$. Values are the mean from at least four biological replicates ± SEM.**

Table S16. Full statistical analysis of effectiveness assessment of selected strains by DW[plant]$^{-1}$

Table Analyzed	DW/plant
Data sets analyzed	A-D
ANOVA summary	
F	119.0
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.9675
Brown-Forsythe test	
F (DFn, DFd)	0.2632 (3, 12)
P value	0.8506
P value summary	ns
Are SDs significantly different (P < 0.05)?	No
Bartlett's test	
Bartlett's statistic (corrected)	1.259
P value	0.7390
P value summary	ns
Are SDs significantly different (P < 0.05)?	No

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	21496875	3	7165625	F (3, 12) = 119.0	P<0.0001
Residual (within columns)	722500	12	60208		
Total	22219375	15			
Data summary					
Number of treatments (columns)	4				
Number of values (total)	16				

Number of families	1
Number of comparisons per family	3
Alpha	0.05

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value	B-?			
G083 vs. Rlv3841	1200	734.5 to 1666	****	<0.0001	A	Rlv3841		
G083 vs. V116	900.0	434.5 to 1366	***	0.0006	C	V116		
G083 vs. WC	3175	2709 to 3641	****	<0.0001	D	WC		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
G083 vs. Rlv3841	3475	2275	1200	173.5	4	4	6.916	12
G083 vs. V116	3475	2575	900.0	173.5	4	4	5.187	12
G083 vs. WC	3475	300.0	3175	173.5	4	4	18.30	12

SI Figures

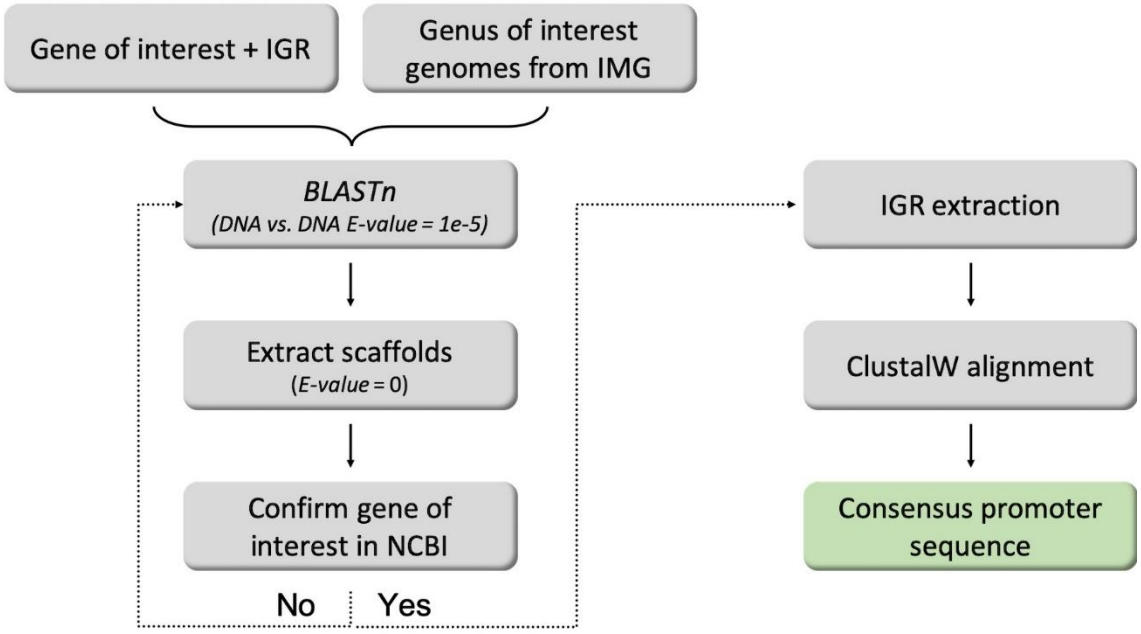
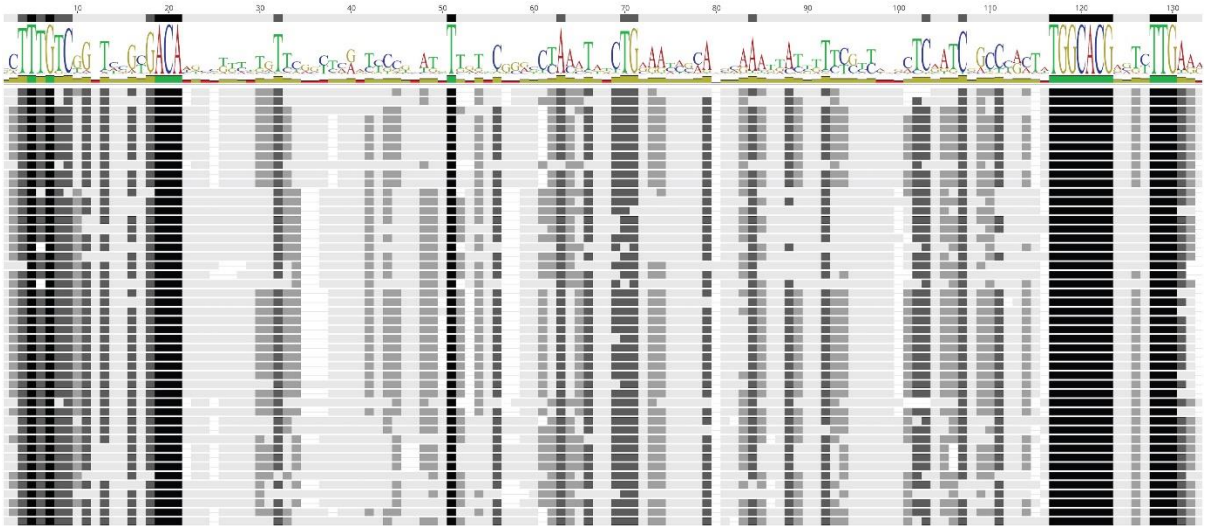


Fig. S1. Process followed to determine a consensus sequence for a nodule-specific promoter expressed in a wide range of biovars and strains of *R. leguminosarum.*

a



b

```
1          10         20         30         40         50         60         70         80         90         100
TCTGTGGTCTCAGGAGCTCG ACACGACATTGTCGTCACCT TTGTCGGGTCCGCGACAAGC TTTTTGTTCCTCGATCCCGT ATGTTTTCGCCTAAATATCT
110        120        130        140        150        160        170        180        190        200
GAAACAGCAGCAAATTATTT TTCGTTAGCTCAATCGGCCC ACTTGGCACGAGTCTTGAAA TTTATTGCGAGGCGGCGGAG CTGCCGGCCGCATCCGTGCG
210        220        230        240        250        258
GCATAACCACGGTTGTGAAC AAATGAAGGAAGGCCAAATG TGAGACCACGAAGTGAAG
```

Fig. S2. Design of Ps*nifH*, a consensus promoter for *nifH* in *Rhizobium*. a) Alignment of the IGR upstream of *nifH* from 48 rhizobial strains, b) Ps*nifH* synthesized as a fragment with terminal BsaI restriction sites for Golden Gate assembly as a PU module. The following features are indicated; UAS (orange), RpoN-binding site (blue) and RBS (yellow), with a start codon ATG (red).

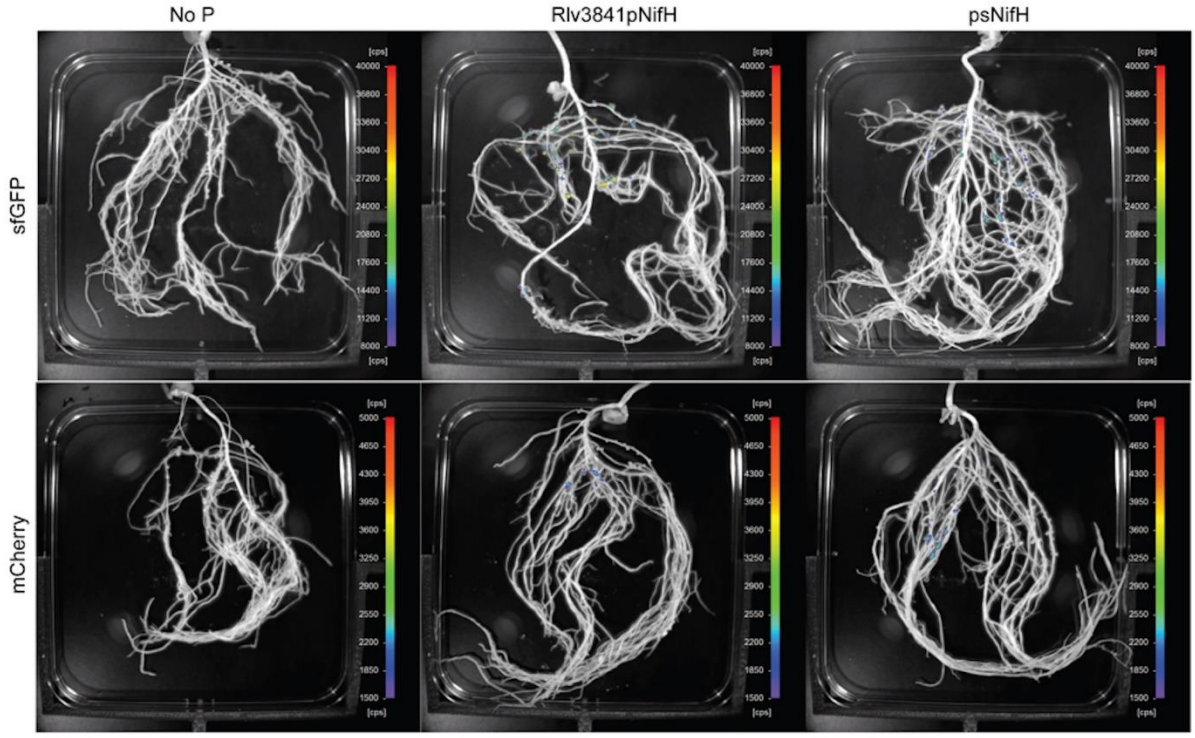


Fig. S3. Pea root system from plants harvested at 21 dpi. Fluorescence measurement from Rlv3841 expressing GFP under the control of i) No promoter, Rlv3841[pOPS0383] (negative control), ii) Rlv3841[pOPS0379] (positive control) and iii) Rlv3841[pOPS0381] (P*snifH*) (scale, 8,000-40,000 cps). Rlv3841 expressing mCherry under the control of i) No promoter, Rlv3841[pOPS0384] (negative control), ii) Rlv3841[pOPS0380] (positive control) and iii) Rlv3841[pOPS0382] (P*snifH*) (scale, 1,500-5,000 cps). Images shown are representative of four biological replicates.

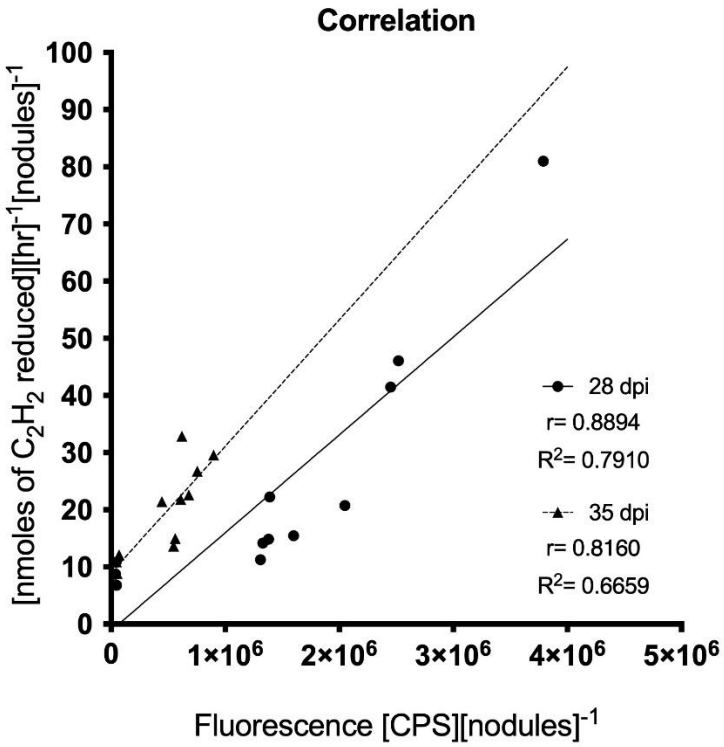


Fig. S3. Correlation of nodule fluorescence and nitrogenase activity. Pea plants at 28 and 35 dpi. GFP fluorescence and nitrogenase activity are normalized by number of nodules per plant. Each dot represents a single plant.

Fig. S4. Plasmid ID Golden Gate T module. Terminal BsaI restriction sites and overhangs (grey, sequence 5' to 3'). Plasmid ID is due to 12-nt Golay barcode (orange) and the universal primer binding site (blue).

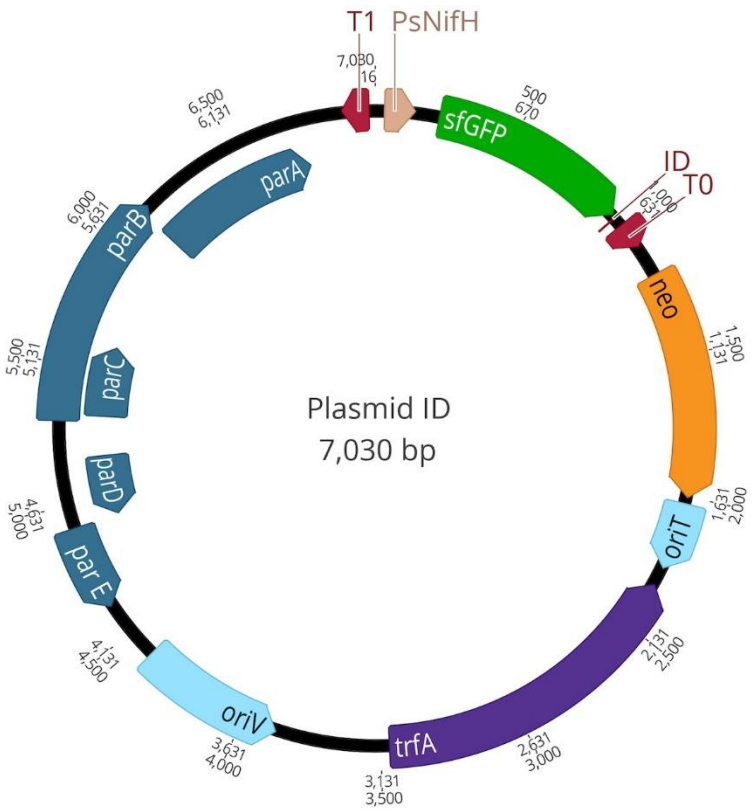


Fig.S5. Schematic map of 95 plasmids constructed with unique plasmid IDs. Plasmid ID (in red) is in T module position. Components for construction of all plasmids were identical, except the Plasmid ID T module, which was different in each case.

	1	2	3	4	5	6	7	8	9	10	11	12
A	pOPS0491	pOPS0492	pOPS0493	pOPS0494	pOPS0495	pOPS0496	pOPS0497	pOPS0498	pOPS0499	pOPS0500	pOPS0501	pOPS0502
B	pOPS0503	pOPS0504	pOPS0505	pOPS0506	pOPS0507	pOPS0508	pOPS0509	pOPS0510	pOPS0511	pOPS0512	pOPS0513	pOPS0514
C	pOPS0515	pOPS0516	pOPS0517	pOPS0518	pOPS0519	pOPS0520	pOPS0521	pOPS0522	pOPS0523	pOPS0524	pOPS0525	pOPS0526
D	pOPS0527	pOPS0528	pOPS0529	pOPS0530	pOPS0531	pOPS0532	pOPS0533	pOPS0534	pOPS0535	pOPS0536	pOPS0537	pOPS0538
E	pOPS0539	pOPS0540	pOPS0541	pOPS0542	pOPS0543	pOPS0544	pOPS0545	pOPS0546	pOPS0547	pOPS0548	pOPS0549	pOPS0550
F	pOPS0551	pOPS0552	pOPS0553	pOPS0554	pOPS0555	pOPS0556	pOPS0557	pOPS0558	pOPS0559	pOPS0560	pOPS0561	pOPS0562
G	pOPS0563	pOPS0564	pOPS0565	pOPS0566	pOPS0567	pOPS0568	pOPS0569	pOPS0570	pOPS0571	pOPS0572	pOPS0573	pOPS0574
H	pOPS0575	pOPS0576	pOPS0577	pOPS0578	pOPS0579	pOPS0580	pOPS0581	pOPS0582	Control	pOPS0584	pOPS0585	pOPS0586

Fig. S6. Plasmid IDs library, position in a 96-well plate. All plasmids are identical, apart from the plasmid ID which is different in each case.

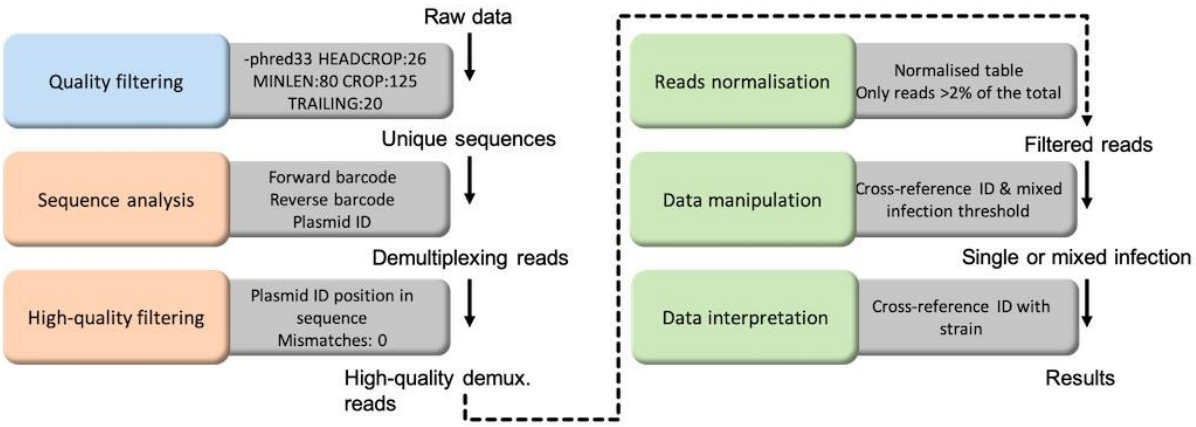


Fig. S7 Workflow for data analysis from Ion Torrent reads. Pre-processing steps in blue rectangles, sequence analysis in orange rectangles and data manipulation in green rectangles**.**

```
import sys
import getopt
import csv
import gzip
import itertools
import pandas as pd
import numpy as np
from Bio import SeqIO
from Bio.Seq import Seq


def fasta_to_dict(path, reverse):
	ret_dict = SeqIO.to_dict(SeqIO.parse(path, "fasta"))
	ret_dict = {k:v.seq for k, v in ret_dict.items()}
	if(reverse):
		ret_dict = {k:v.reverse_complement() for k, v in ret_dict.items()}
	ret_dict = {k:str(v) for k, v in ret_dict.items()}
	#print(ret_dict)
	return(ret_dict)

def read_plasmids_table(path):
	plasmids = {}
	with open(path, 'r') as csvfile:
		table_reader = csv.DictReader(csvfile, delimiter=',', quotechar='"')
		for row in table_reader:
			plasmids[row["Barcode name"]]= row["ID plasmid sequence"]
	return(plasmids)

def find_hash_position(sequence, tags):
	for key, value in tags.items():
		position = sequence.find(value)
		if position != -1:
			return (key, position)
	return ("none", -1)

def find_plasmid_positions(sequence, forward_dict, reverse_dict, plasmids,
orientation):
	fw_name, fw_pos = find_hash_position(sequence, forward_dict)
	rv_name, rv_pos = find_hash_position(sequence, reverse_dict)
	pl_name, pl_pos = find_hash_position(sequence, plasmids)

	if(fw_pos + rv_pos + pl_pos == -3 ):
		orientation = "."

	return ([fw_name, str(fw_pos), rv_name, str(rv_pos), pl_name,str(pl_pos),
orientation])


def write_raw_counts(fq, output_prefix, forward_dict, reverse_dict, plasmids):
	fw_f = fq
```

```
	distances_f = output_prefix + "_distances_merge.txt"
	with gzip.open(fw_f, "rt") as r1,open(distances_f,"w") as f3:

f3.write("fw_name,fw_pos,rev_name,rev_pos,pl_name,pl_pos,orientation\n")
		for fw in SeqIO.parse(r1, "fastq") :
			str_seq = str(fw.seq)
			values = find_plasmid_positions(str_seq, forward_dict, reverse_dict,
plasmids, "+")

			if values[6] == ".":
				str_seq = str(fw.seq.reverse_complement())
				values = find_plasmid_positions(str_seq, forward_dict,
reverse_dict, plasmids, "-")

			f3.write(",".join(values))
			f3.write("\n")

def write_summary(output_prefix):
	distances_f = output_prefix + "_distances_merge.txt"
	df = pd.read_csv(distances_f)
	summ=df[['fw_name', 'rev_name', 'pl_name', 'pl_pos']].groupby(['fw_name',
'rev_name', 'pl_name']).agg( ['count','mean'])
	summ.to_csv(output_prefix + "summary_merge.csv", sep=',')

def usage():
	print ("Usage: " + sys.argv[0]  + " --plasmid=<plasmids.csv> --
forward=<forward_ids.fa> --reverse=<reverse_ids.fa> --
sequences=<sequences.fq.gz> --output_prefix=<file_prefix>")


def main(argv):
	try:
		opts, args = getopt.getopt(argv,'p:f:r:s:o:h',
['plasmid=','forward=','reverse=','sequences=', 'output_prefix=', 'help'])
	except getopt.GetOptError:
		usage()
		sys.exit(2)

	if not opts:
			print ('No options supplied')
			usage()

	for opt, arg in opts:
		if opt in ('h', '--help'):
			usage()
			sys.exit(2)
		elif opt in ('p', '--plasmid'):
			plasmid_path = arg
			plamids_dict = read_plasmids_tables_table(plasmid_path)
		elif opt in ('f', '--forward'):
```

```
            forward_path = arg
            forward_dict = fasta_to_dict(forward_path, False)
        elif opt in ('r', '--reverse'):
            reverse_path = arg
            reverse_dict = fasta_to_dict(reverse_path, True)
        elif opt in ('s', '--sequences'):
            sequences_path = arg
            print(arg)
        elif opt in ('o', '--output_prefix'):
            output_prefix = arg

    write_raw_counts(sequences_path, output_prefix, forward_dict, reverse_dict,
plamids_dict)
    write_summary(output_prefix)

if __name__ == '__main__':
    main(sys.argv[1:])
```

Fig. S8 Biopython script to identify reads by amplicon (plasmid ID) and sample-specific barcodes.
Full script available in: https://github.com/marcelamendoza/Plasmid-ID

		Column Barcode																			
Row		1		2		3		4		5		6		7		8		9		10		11		12	
Barcode	ID	E10	G2	E10	G2	E10	G2	E10	G2	E10	G2	E10	G2	E10	G2	E10	G2	E10	G2	E10	G2	E10	G2	E10	G2
A		3E+04	2E+03	5E+04		6E+04		3E+04			1E+05		2E+05		3E+05										
B		4E+04	2E+05	2E+05	5E+03	2E+05	4E+03	1E+05			4E+05		4E+05		4E+05										
C		1E+05	2E+04	2E+05	2E+03	1E+05	3E+03				4E+05		5E+05												
D		1E+04	1E+05	2E+05	3E+03	1E+05	2E+03				3E+05		4E+05												
E				1E+05		1E+05					2E+05		4E+05												
F				2E+05		1E+05					3E+05	4E+03	4E+05												
G				2E+05		1E+05					3E+05		4E+05												
H				1E+05		9E+04					3E+05		5E+05												

Fig. S9. Total counts of plasmid ID E10 and G2 assigned to column and row barcodes. Results are classified in graded coloured scale where green is the highest value, yellow is the midpoint percentile and red is the minimum value, cells in white do not show reads.

	1	2	3	4	5	6	7	8	9	10	11	12
A	UPM791	Rlv3841	WSM1475	WSM1481	WSM1529	WSM1488	WSM1521	370	V060	V014	G011	VSX1
B	CellTech	JED2	364	PB6-3B	VF2	CORUS 16	CORUS 1	388	V068	V043	V002	VSX7
C	13	VF5	SU303	LegTech	G004	G077	G073	23--1	V067	H012	V004	VSX10
D	L102	L104	G007	G051	V116	G008	V006	387	V069	H009	V008	VSX11
E	24	E2-1B	PB3-3	536	42	PeanNW	367	BeanLA	V074	H127	V010	VSX16
F	G093	G099	G083	G109	G108	G094	L008	L019	V100	H130	V030	VSX25
G	H011	H082	H174	G028	G067	G088	L002	2B-1	G016	H178	V050	VSX28
H	L010	L018	L070	L074	L079	L082	L111	E2-1A	Control	H031	V057	VSX32

Fig. S11. Final selected Rlv strains (Rlv-library) arranged in a 96-well plate. Strains in yellow were studied in competition assays. Strains in blue were studied for effectiveness at fixing nitrogen in laboratory conditions, strains in grey were studied for effectiveness at fixing nitrogen in field conditions, strains in green have been phylogenetically analyzed and strains in orange came from the same soil but were selected by different hosts.

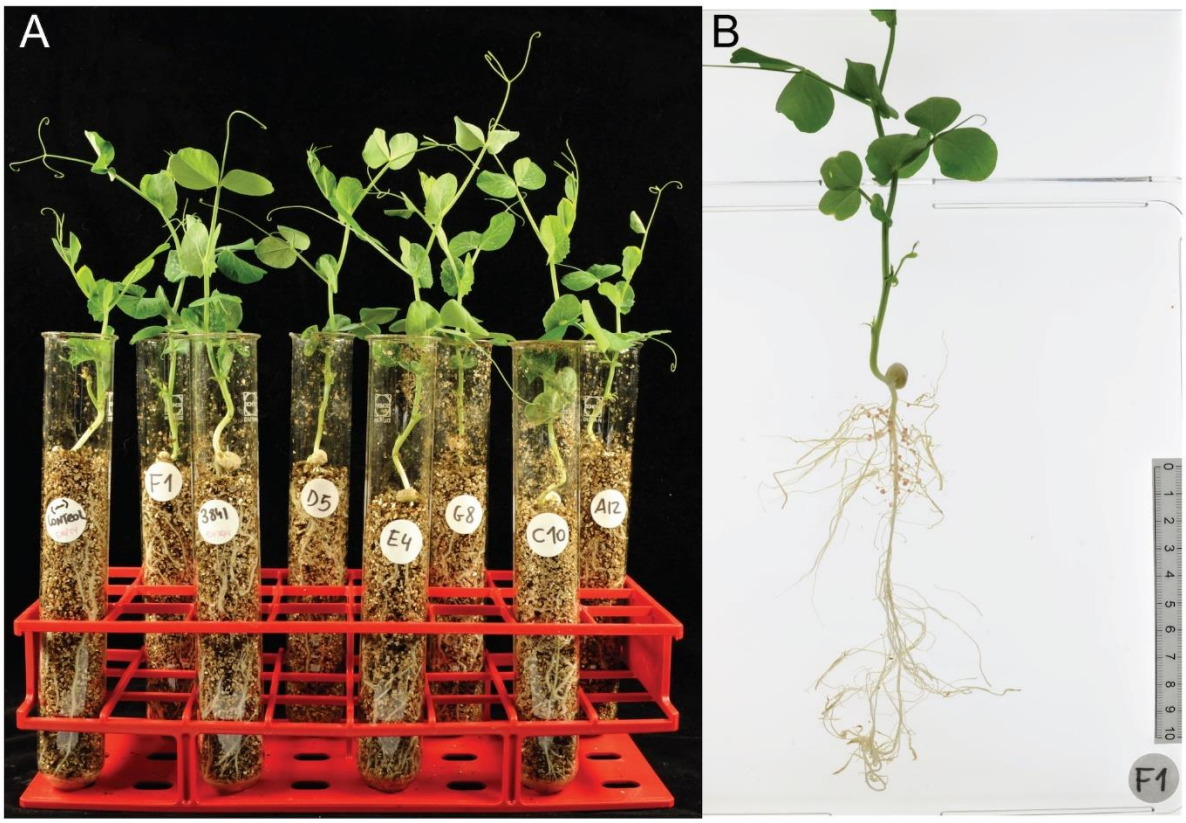


Fig. S10. Nodulation of pea plant seedlings by rhizobial strains. A) Nodulation test in pea plants using the 95 selected rhizobial strains from Fig. S11. B) Example of a pea plant showing root nodules indicative of an effective symbiosis.

	1	2	3	4	5	6	7	8	9	10	11	12
A	UPM791 [pOPS0491]	Rlv3841 [pOPS0492]	WSM1475 [pOPS0493]	WSM1481 [pOPS0494]		WSM1488 [pOPS0496]			V060 [pOPS0499]	V014 [pOPS0500]	G011 [pOPS0501]	VSX1 [pOPS0502]
B	CellTech [pOPS0503]	JED2 [pOPS0504]	364 [pOPS0505]	PB6-3B [pOPS0506]	VF2 [pOPS0507]	CORUS 16 [pOPS0508]	CORUS 1+2 [pOPS0509]		V068 [pOPS0511]		V002 [pOPS0513]	VSX7 [pOPS0514]
C	13 [pOPS0515]	VF5 [pOPS0516]	SU303 [pOPS0517]	LegTech [pOPS0518]	G004 [pOPS0519]	G077 [pOPS0520]	G073 [pOPS0521]	23--1 [pOPS0522]	V067 [pOPS0523]	H012 [pOPS0524]	V004 [pOPS0525]	VSX10 [pOPS0526]
D	L102 [pOPS0527]	L104 [pOPS0528]	G007 [pOPS0529]	G051 [pOPS0530]	V116 [pOPS0531]	G008 [pOPS0532]	V006 [pOPS0533]	387 [pOPS0534]	V069 [pOPS0535]	H009 [pOPS0536]	V008 [pOPS0537]	VSX11 [pOPS0538]
E	24 [pOPS0539]	E2-1B [pOPS0540]	PB3-3 [pOPS0541]	536 [pOPS0542]		PeanNW [pOPS0544]	367 [pOPS0545]		V074 [pOPS0547]	H127 [pOPS0548]	V010 [pOPS0549]	VSX16 [pOPS0550]
F	G093 [pOPS0551]	G099 [pOPS0552]	G083 [pOPS0553]	G109 [pOPS0554]	G108 [pOPS0555]	G094 [pOPS0556]	L008 [pOPS0557]	L019 [pOPS0558]	V100 [pOPS0559]	H130 [pOPS0560]	V030 [pOPS0561]	
G		H082 [pOPS0564]	H174 [pOPS0565]	G028 [pOPS0566]	G067 [pOPS0567]	G088 [pOPS0568]		2B-1 [pOPS0570]	G016 [pOPS0571]	H178 [pOPS0572]	V050 [pOPS0573]	VSX28 [pOPS0574]
H	L010 [pOPS0575]	L018 [pOPS0576]	L070 [pOPS0577]	L074 [pOPS0578]	L079 [pOPS0579]	L082 [pOPS0580]		E2-1A [pOPS0582]		H031 [pOPS0584]	V057 [pOPS0585]	VSX32 [pOPS0586]

Fig. S11. Final library of tagged Rlv strains (tagged-strains) with plasmid IDs. Empty cells do not contain any bacteria.

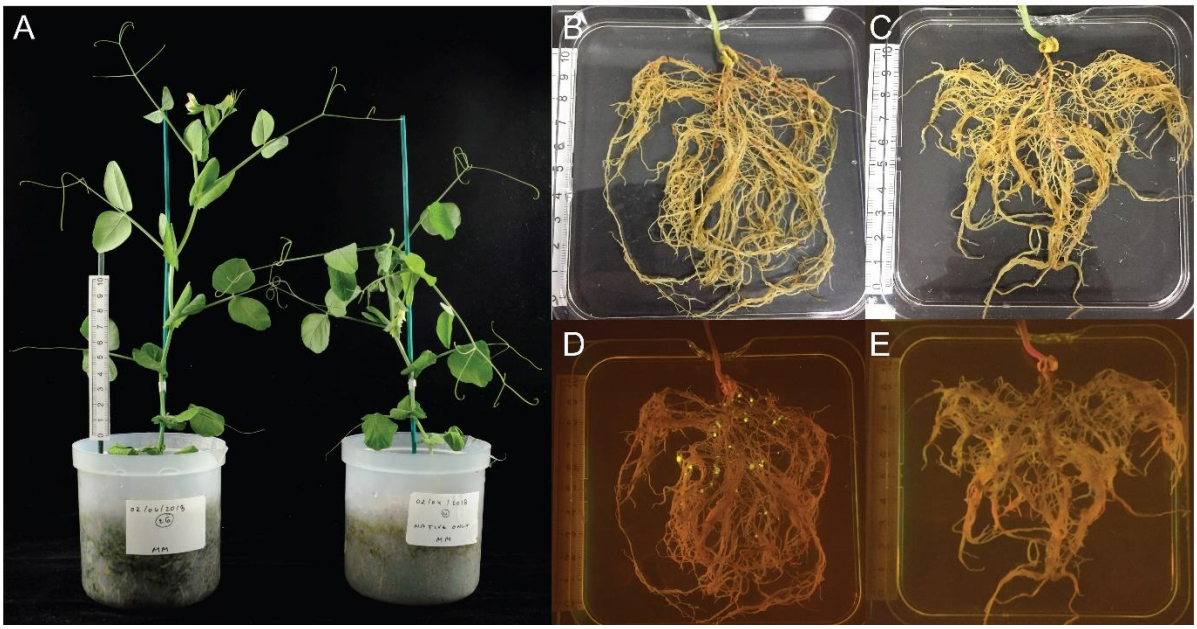


Fig. S12. Pea plants grown in Yatesbury soil. A) Plant at 21 dpi with tagged-strain inoculum on the left and -ve control on the right. (B) Root system of inoculated and (C) -ve control plants showing typical symbiotically-effective root nodules exposed to a blue-light transilluminator examining fluorescence from nodules formed by tagged-strains expressing GFP under Ps*nifH* control. Photographs taken with a camera fitted with a blue light filter. D) Plant inoculated with tagged-strains showing nodules with green fluorescence. E) Nodules from -ve controls show no green fluorescence.

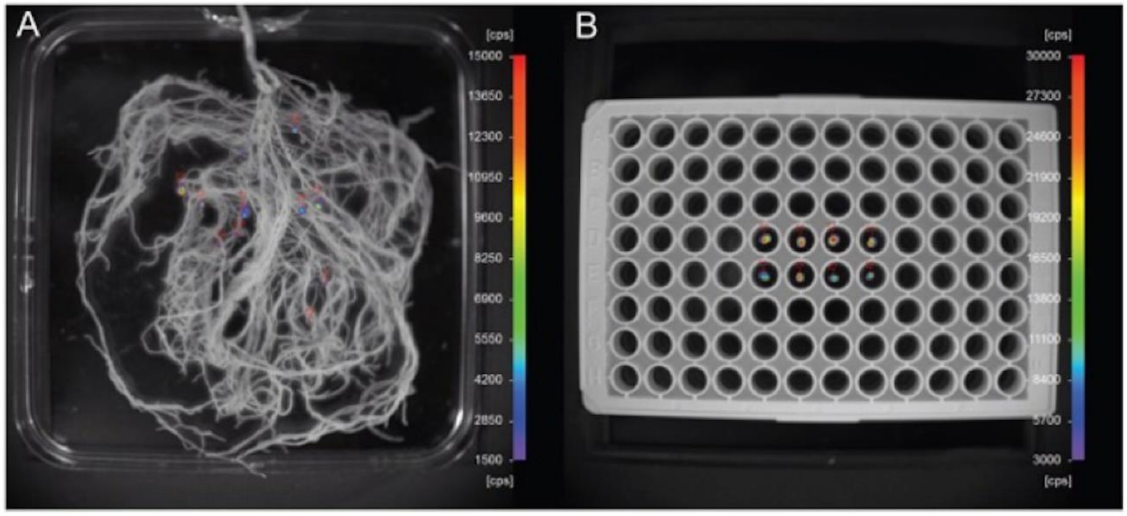


Fig. S13. Analysis of fluorescence from nodules. A) Quantification of green fluorescence with a NightOWL CCD camera of whole root systems to identify the brightest nodules. B) Fluorescence of eight selected individual nodules was quantified in a plate reader.

SI VIDEO

Video S1. 3D structures of individual bacteroids. Bacteroids formed by Rlv3841[pOPS0381] (GFP under the control pf Ps*nifH*) from crushed pea root nodules. 3D segmentation using a GFP TIF image stack. Meshes were generated to obtain the volume of individual bacteroids. Individual bacteroids were color-identified according to volume (scale from 0 to 60 μm^3).

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