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Supplementary Information for

A high-throughput system to identify inhibitors of *Candidatus* Liberibacter asiaticus transcription regulators.

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Supplementary Information Text

Materials and Methods

Prediction of *Ca.* Liberibacter asiaticus (*C*Las) transcription regulators. Published *C*Las genome annotations (1, 2) were mined for proteins annotated as transcription regulators. To identify additional transcription regulators, we performed BLASTp sequence alignment analyses. To identify *C*Las and *Sme* orthologous transcriptional regulators, we performed bidirectional BLASTp analysis of the *C*Las Psy62 and *S. meliloti* (*Sme*) proteomes.

Strains and plasmids. All *Sme* strains (Table S2) used in this study are derived from CL150, the sequenced Rm1021 strain (3) with repaired *pstC* and *ecfR1* genes (4). *Sme* strains were grown in M9 sucrose (supplemented with 500 ng/mL biotin and 50 ng/mL $CoCl_2 \cdot 6H_2O$), LB (Luria Broth, 5 g/L NaCl) or TY medium at 30°C, as described (5). *E. coli* strains were grown in LB medium at 37°C. Antibiotics were used at the following concentrations: ampicillin (Ap), 50-100 μg mL^{-1}; chloramphenicol (Cm), 50 μg mL^{-1}; gentamicin (Gm), 5 μg mL^{-1} for *E. coli* and 50 μg mL^{-1} for *Sme*; and streptomycin (Sm), 500 μg mL^{-1}.

Standard techniques were used for cloning and PCR amplification. Oligonucleotide primers used in this study are listed in Table S7. *E. coli* strains DH5α and NEB5α (New England Biolabs; Ipswich, MA) were used as plasmid hosts. Triparental conjugations were conducted as described (5) to transfer plasmids (Table S2) from *E. coli* to *Sme*.

Regulator genes (Table 1) were cloned into the medium copy, Isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible vector, pSRKGm (6) as NdeI-KpnI DNA fragments. pSRKGm contains a *lacI-lac* promoter-operator complex and a *lacZ* ribosome binding site (RBS) sequence 6 nt upstream of the NdeI-site-ATG codon (AGGAAACAGCAT**ATG**). The *Ca*. L. asiaticus (*C*Las) genome is GC-poor (37%), while the *Sme* genome is GC-rich (67%) (3); therefore, we fully optimized the coding sequence of each *Ca*. L. asiaticus Psy62 (1) regulator for expression in *Sme* with the JCat Codon Adaptation Tool to a codon adaptation index of 1 compared to their pre-optimization values that ranged from 0.11 to 0.15 (7; http://www.jcat.de). We also used JCat options to remove internal Rho-independent terminators and RBSs from the regulator coding sequences. The *C*Las DNAs were synthesized by GeneArt Gene Synthesis service (Invitrogen, ThermoScientific), and their nucleotide sequences have been deposited in NCBI (GenBank accession numbers: MK359043-MK359048). Since *visN* and *visR* form an operon in *Sme*, optimized *C*Las *visN* and *visR* were synthesized as a single DNA and separated by the *Sme* 11-nt *visN-visR* intergenic sequence. *Sme ctrA*, *ldtR*, *rpoH1* and *visNR* coding sequences were each cloned into pSRKGm without changes to their DNA sequences. Genes cloned into pSRKGm were expressed by inducing with 0.5 mM IPTG.

Unmarked deletions of *Sme ctrA* (SMc00654), *ldtR* (SMc01768), *lsrB* (SMc01225), *phrR1* (SMc01110), *phrR2* (SMb21117), and the *visN-visR* operon (SMc03016-SMc03015), were constructed using the *sacB* vector, pJQ200 (8), and sucrose counterselection, as described previously (5), except that an antibiotic resistance cassette was not inserted. Construction of *ctrA*-deleted *Sme* was more complicated because CtrA is essential for viability in *Sme* and most other α-proteobacteria (9). A deletion of *Sme ctrA* was viable if *Sme ctrA* was carried in *trans* on pSRKGm (pMB913), and its expression induced with 0.5 mM IPTG. We failed to obtain a deletion when optimized *C*Las *ctrA* was similarly expressed (pMB876). Further tests with pMB913 and pMB876 in WT CL150, which retains a functional *ctrA* gene, revealed that expression of *C*Las *ctrA* conferred lethality on LB agar plates containing IPTG 0.2, 0.3, 0.4, 0.5 or 1 mM IPTG. Expression of *Sme ctrA* using these IPTG concentrations had no effect on viability. Since CL150 pMB876 was viable on LB with 0.1 mM IPTG and on M9 sucrose or M9 glycerol plates with 0.5 mM IPTG, we tried to obtain a *Sme* strain deleted for *ctrA* using these media with *C*Las *ctrA* in trans, but failed, suggesting that *C*Las *ctrA* cannot substitute for *Sme ctrA*.

We designed a modular enhanced green fluorescent protein (EGFP) expression cassette to clone into the KpnI-NheI sites of pSRKGm (Fig. S2). This cassette includes an EGFP coding sequence partially optimized for expression in *Sme*, a spacer sequence upstream of EGFP that can be replaced by promoter DNA, and flanking transcription terminators (Fig. S2). The core cassette feature is a GFP coding sequence with F64L and S65T enhancements, optimized for expression in *Sme* using Optimizer (10; http://genomes.urv.es/OPTIMIZER/) and manual editing. Two nucleotides in the EGFP coding region were changed to disrupt promoter-like sequences: 153 (T>C) and 432 (T>C). The final EGFP sequence has a *Sme* codon adaptation index of 0.80. Upstream of the EGFP ORF ATG is an optimal *Sme* RBS (GGAGGCTCTTCA**ATG**; 11), and a synthetic transcriptionally silent spacer, S1.2 (12), which can be excised with SpeI-XhoI and replaced by a promoter DNA sequence. A different transcriptionally silent random spacer, S1.1 (12), was inserted downstream of the KpnI site to create space between the bidirectional transcription terminator region (*rpoC* and *rnpB*) (12), and the upstream regulator gene. Two additional transcription terminators are located upstream of the promoter cloning site (M13 central T linker + *rrnD* terminator 1 and *rrnB* terminator 1) (12) to prevent extraneous expression of EGFP. The intrinsic terminator search tool ARNold (13, 14); http://rna.igmors.u-psud.fr/toolbox/arnold/) found only the four terminators used to construct the cassette. Synthesized by GeneArt Gene Synthesis service (Invitrogen), the cassette nucleotide sequence has been deposited in NCBI (Accession number MK387175). In the course of this work, pSRKGm plasmids containing the EGFP cassette with each *C*Las regulator gene, and candidate promoter sequences upstream of EGFP were constructed (Table S2).

Based on Affymetrix Gene Chip results for *Sme* strains ectopically expressing *C*Las regulators, candidate target promoters were amplified and cloned upstream of the EGFP cassette as SpeI-XhoI DNA fragments (Table S7). We focused primarily on genes showing a large increase in transcript

abundance in the *C*Las regulator strain compared to its control. We also considered other factors: consistency among three biological replicates in each Affymetrix GeneChip experiment; accuracy of the corresponding gene annotation; expression behavior of other genes in the operon (when applicable); availability of transcription and translation start site data (4); whether a given promoter was activated by the orthologous *Sme* regulator; and feasibility of cloning the promoter without including portions of nearby ORFs or overlapping promoters. Strains were tested for inducible fluorescence by streaking on agar plates ± 0.5 mM IPTG and imaging under blue epifluorescence, and by fluorometric assays of liquid cultures (emission=484 nm; excitation=507 nm). Promoter-EGFP plasmids are listed in Table S2 and include the following regulator (target promoter) pairs: CtrA (P*minC*); LdtR (P*tac*, P*smc04059*, P*ldtP*); LsrB (P*lrp3*, P*smc01834*); PhrR (P*ldtR*, P*smc00404*); RpoH (P*ibpA*, P*groES5*); and VisNR (P*rem*). With the exception of *tacA*, all promoters with strong increased expression in the Affymetrix GeneChip experiments showed enhanced EGFP fluorescence when expression of the regulator was induced with IPTG (*ibpA*, *groES5*, *rem*, *smc04059*). The lack of strong fluorescence with the *tacA* construct may be due to our inability to correctly identify the *tacA* promoter. *C*Las regulators that acted weakly at *Sme* target promoters, as shown by transcriptome analyses (e.g. CtrA, LsrB, PhrR), failed to significantly enhance EGFP fluorescence when their expression was induced with IPTG.

Phenotypic assays. Growth under heat stress was assayed on LB agar plates at the 37°C restrictive temperature. Swimming motility and deoxycholate sensitivity were assayed as previously described (15). Morphologies of cells ectopically expressing *Sme* LdtR (MB1101 pMB961) and optimized *C*Las LdtR (MB1101 pMB878) were examined using a Leica DM5000B microscope, with MB1101 pSRKGm as a control. Cells were grown in M9 sucrose medium with Gm, Sm, and 0.5 mM IPTG for 19 hours before imaging.

Affymetrix GeneChip analysis. For transcriptome analysis of *Sme*, we used a custom dual genome Affymetrix Symbiosis Chip (16). Each optimized *C*Las regulator was ectopically expressed as described above. Controls for comparison were *Sme* deletion strains carrying the empty pSRKGm plasmid (or in the case of CtrA, CL150 carrying pSRKGm). Three biological replicates were analyzed for each strain. For additional controls, in two instances (RpoH1 and CtrA) we also tested deletion strain behavior when the native *Sme* regulator gene was expressed from pSRKGm (Table S2). Overnight starter cultures were inoculated from single colonies in M9 sucrose medium with 50 μg mL^{-1} Gm and 500 μg mL^{-1} Sm. The Δ*rpoH1rpoH2* strain (RFF231) grows poorly when inoculated from single colonies into M9 sucrose medium, so these strains were grown overnight from a single colony in LB medium, then washed and the cells were resuspended in fresh M9 sucrose medium before inoculating the flask culture. For RNA purification, starter cultures were diluted to ~0.1 OD_{600} in 20 mL M9 sucrose medium at 50 μg mL^{-1} Gm and 500 μg mL^{-1} Sm, in 250 mL baffled flasks. Cultures were allowed to grow to mid-exponential phase (~0.5 to ~0.7 OD_{600}), with shaking (250 rpm) at 30°C. Replicates from a single experiment were grown to within ~0.1 OD_{600} units of each other. At mid-exponential phase, IPTG was added to a final

concentration of 0.5 mM and the cells were grown for one hour before harvest. All strains, including empty vector control strains, were induced with IPTG to account for effects of IPTG on global transcription. Cell harvest, RNA purification, cDNA synthesis and labeling, hybridization and scanning were performed as previously described (16), except for the Δ*phrR1R2* strain, which we discovered was resistant to lysozyme lysis. After troubleshooting, we obtained sufficient RNA amounts from the Δ*phrR1R2* strain by washing the cells with 1% Triton X-100 detergent before treatment with an enhanced activity lysozyme (Ready-Lyse, Epicentre Inc.). IPTG-induced expression of *C*Las PhrR in *Sme* Δ*phrR1phrR2* did not suppress its lysozyme-resistant phenotype and resulted in even poorer growth, suggesting that *C*Las *phrR* cannot substitute for *Sme phrR1* and that ectopic expression of *C*Las PhrR is deleterious to *Sme* growth.

Data were analyzed with Partek Genomics Suite 6.6 software with the following settings: RMA for background correction; quantile normalization; log base 2 transformation; median polish summarization; and ANOVA (with LS means used to calculate fold change, P-value $\leqslant 0.05$) for identification of differentially expressed genes. For experiments where *Sme* regulators were also tested (RpoH, CtrA), each pairwise comparison of the regulator strain to the pSRKGm control was performed separately. The Affymetrix GeneChip data have been deposited under Superseries accession number GSE124984 in the Gene Expression Omnibus (GEO) database.

High-throughput screening of compound libraries. Screening of strains carrying a *C*Las regulator and a target promoter-EGFP fusion was performed at the Stanford High-Throughput Bioscience Center (HTBC; http://med.stanford.edu/htbc.html). Three strains were used for screening: MB1101 pMB958 (LdtR); RFF231 pMB949 (RpoH); and MB1102 pMB956 (VisNR). Starter cultures were grown ~24 hours to saturation in M9 sucrose Gm Sm, then diluted to an OD_{600} = 0.15 in the same medium. Wells of Greiner 384-plates (polystyrene, black with clear bottom, tissue-culture treated [E&K Scientific #EK-30091]) were filled with: 40 μL M9 sucrose Gm Sm with 0.625 mM IPTG (0.5 mM final concentration after adding cells); 40 μL without IPTG (no inducer control); or 50 μL without IPTG (growth medium blank). Test compounds, diluted in DMSO, were transferred (100 nL) by using a V&P Scientific pin tool on a Caliper Life Sciences Staccato SciClone ALH3000 small molecule liquid handling system, after which 10 μL of 0.15 OD_{600} cells were added to wells with 40 μL growth medium. Plates were sealed with AeraSeal sterile adhesive microplate seals (Excel Scientific; Victorville, CA), lidded, and incubated at 30°C with shaking (250 rpm) for 17-19 hours. We used a Tecan Infinite M1000 Pro plate reader to read bottom fluorescence (emission=489 nm; excitation=507 nm) and absorbance (600 nm).

We screened a total of 10 compound libraries (Table S3) using the workflow shown in Fig. S3. Data were analyzed using MDL Assay Explorer software. All compounds in the Sigma LOPAC, Microsource Spectrum, Biomol ICCB and Biomol FDA libraries were screened at seven different concentrations (20, 10, 5, 2.5, 1.25, 0.625, 0.313 μM or for Biomol ICCB, 10, 5, 2.5, 1.25, 0.625, 0.313 0.156 μg/mL) (Table S3). Compounds in the NCIDTP library and NIH Clinical Collection

were screened at a single concentration in duplicate. Compounds in the ChemDiv, ChemBridge, and Specs libraries in the "Diverse" Collection (n=113,809) were screened at a single concentration, after which we devised a ranking system for rescreening the most promising compounds at multiple concentrations (Tables S4 and S5). 629 Diverse-Collection compounds were retested in duplicate, and at eight different concentrations (20, 10, 5, 2.5, 1.25, 0.625, 0.313 0.156 μM).

Testing purchased compounds. Retesting compounds obtained from sources other than the screening libraries is an essential follow-up for any high-throughput compound screen, because library compounds may not match their given structure or calculated concentration; this might occur due to degradation or errors in dispensing by the library supplier. To evaluate selected results from the high-throughput screen, we purchased 10 compounds that showed inhibitory activity for one or more of the three *C*Las regulators: six of these were from the Known Bioactive Collection and four from the Diverse Collection (Fig. 2, Fig. S4). Compounds and suppliers were: 4'-Demethylepipodophyllotoxin (Abcam #ab142634, Cambridge, MA); Orbifloxacin (Abcam #ab143425); Rosiglitazone maleate (Abcam #ab142461); Fisetin (Abcam #ab142429); Oxybenzone (Sigma-Aldrich #59647); Bortezomib (Sigma-Aldrich #5043140001); #5109513 (ChemBridge); #8013-5939 (ChemDiv); #C549-0604 (ChemDiv); and #D244-0326 (ChemDiv). Structures of the four compounds purchased from ChemBridge and ChemDiv (diluted in DMSO) were confirmed by 1D proton NMR at the NMR Facility in the Stanford University Chemistry Department. Stanford NMR spectra for all compounds matched the spectra provided by the suppliers. The three ChemDiv compounds appeared >95% pure, while ChemBridge compound #5109513 had other aromatic resonances that integrated to about 15% relative to the correct peaks.

The 10 purchased compounds were also tested on each of the three *Sme* deletion strains ectopically expressing the corresponding *Sme* regulator (LdtR, RpoH or VisNR) (Table S2). Testing was similar to the above high-throughput screening method, except that: 96-well plates (E&K Scientific, #EK-25090) contained 100 μL per well; compound solutions were pipetted into wells instead of being pinned; plates were shaken on a "Mix-EVR Invitroshaker" (Taitec; Koshigaya, Japan) at 1000 rpm for 18-19 hours; and top fluorescence and bottom absorbance were read with a Biotek Synergy H1 plate reader (emission=479 nm; excitation=520 nm). Each compound was tested twice at multiple concentrations.

Three candidate VisNR-inhibiting compounds (Bortezomib; ChemDiv #C549-0604; and ChemDiv #D244-0326) were assayed for effects on swim motility as before (15), except that 35x10 mm petri dishes were used (Falcon Corning #351008), each containing 4 mL swim agar medium (TY, 0.22% Bacto™agar, 50 μg mL^{-1}Gm, 500 μg mL^{-1} Sm, and 0.05 mM IPTG). One strain plus one treatment was assayed per plate. Each compound was tested at 20 μM, using DMSO as a control, with replicates (n=4) of four plasmid-containing strains: CL150 pSRKGm (WT, empty vector); MB1102 pSRKGm (Δ*visNR*, empty vector); MB1102 pMB877 (Δ*visNR*, *C*Las

VisNR); and MB1102 pMB877 (Δ*visNR*, *Sme* VisNR). In experiments using 0.5 mM IPTG, motility was enhanced compared to WT in strains ectopically expressing either *C*Las or *Sme* VisNR. Therefore, we lowered the IPTG concentration: using 0.05 mM IPTG, WT carrying the empty vector showed approximately the same motility as strains ectopically expressing VisNR. Swim colony diameters were measured at 4 days and statistical significance tested using a Student's T-test (one-tail distribution, two-samples, equal variance).

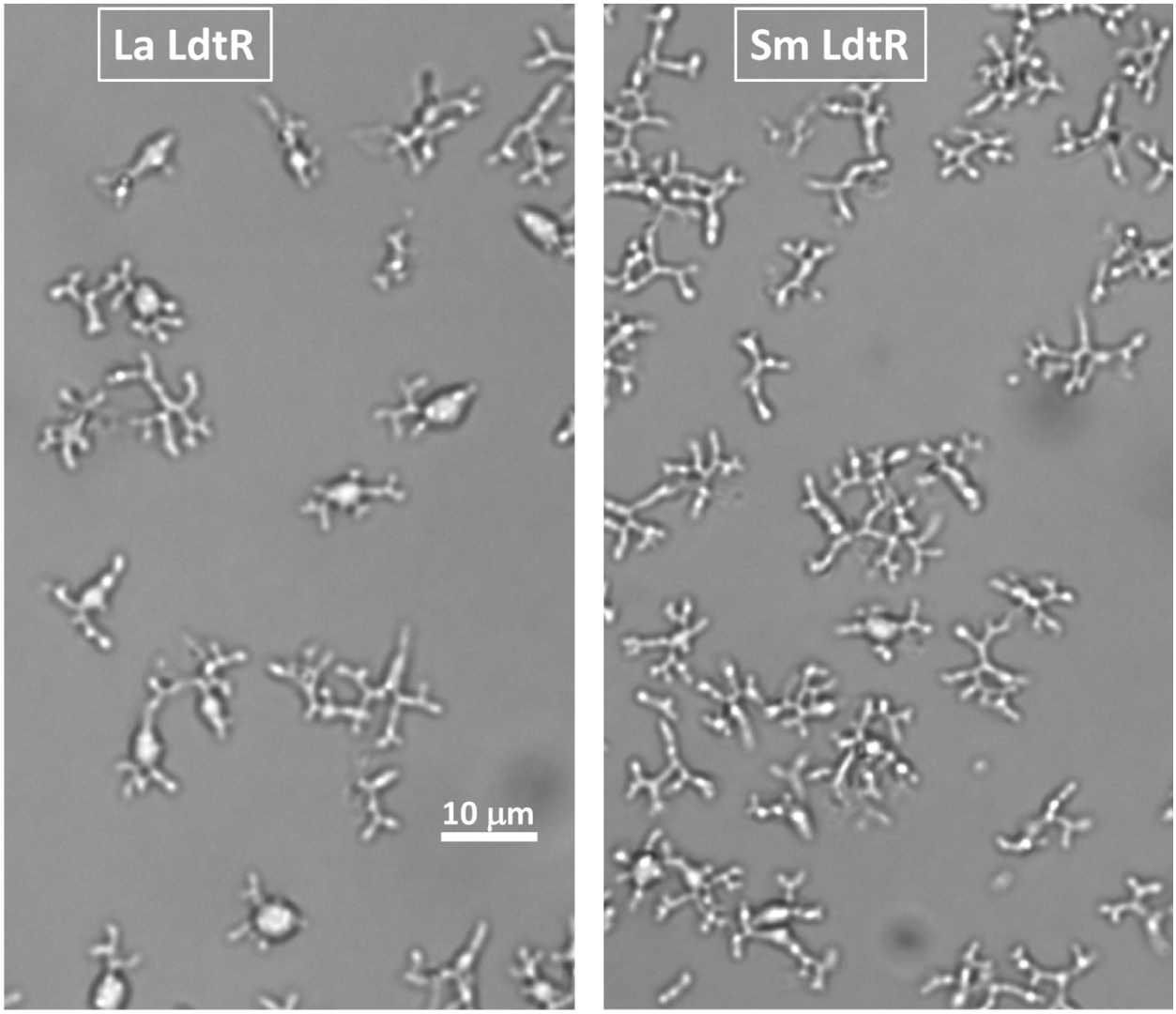

Fig. S1. *S. meliloti* Δ*ldtR* strain (MB1101) ectopically expressing *Ca*. L. asiaticus LdtR on pMB878 (left) or *S. meliloti* LdtR on pMB961 (right). Wild type *S. meliloti* cells are rod-shaped and about 2 µm long (17). Cultures were grown in M9 sucrose with 0.5 mM IPTG for 19 hours to exponential phase and imaged on a Leica DM5000B microscope using a 40X objective.

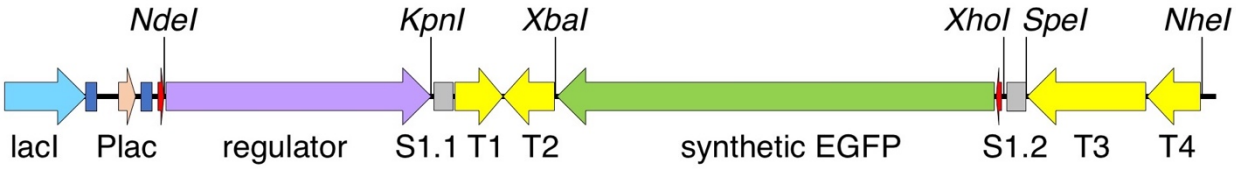

Fig. S2. Map of the modular transcription regulator–EGFP-expression cassette used in high-throughput compound screening. The cassette was cloned into pSRKGm (6) as described in Materials and Methods. The 3' end of *lacI* encoding the Lac repressor is shown in light blue, and its operator sequences, O3 (left) and O1, in dark blue. The *Ca*. L. asiaticus or *S. meliloti* transcription regulator gene (purple) is expressed from the *lac* operon promoter (pale orange) and RBS (red). A promoterless synthetic EGFP gene (green) with an optimal *S. meliloti* RBS (red) (11) can be expressed by replacing the synthetic transcriptionally silent random spacer (gray; S1.2 from Cambray et al. 2013 (12) with promoter DNA. A different random spacer is downstream of the regulator gene (gray, S1.1) (12). The EGFP ORF is flanked by transcription terminators (yellow) (12): T1, *rpoC*; T2, *rnpB*; T3, M13 central T linker + *rrnD* terminator 1; T4, *rrnB* terminator 1. KpnI, NdeI, NheI, SpeI, XbaI, XhoI restriction sites are unique.

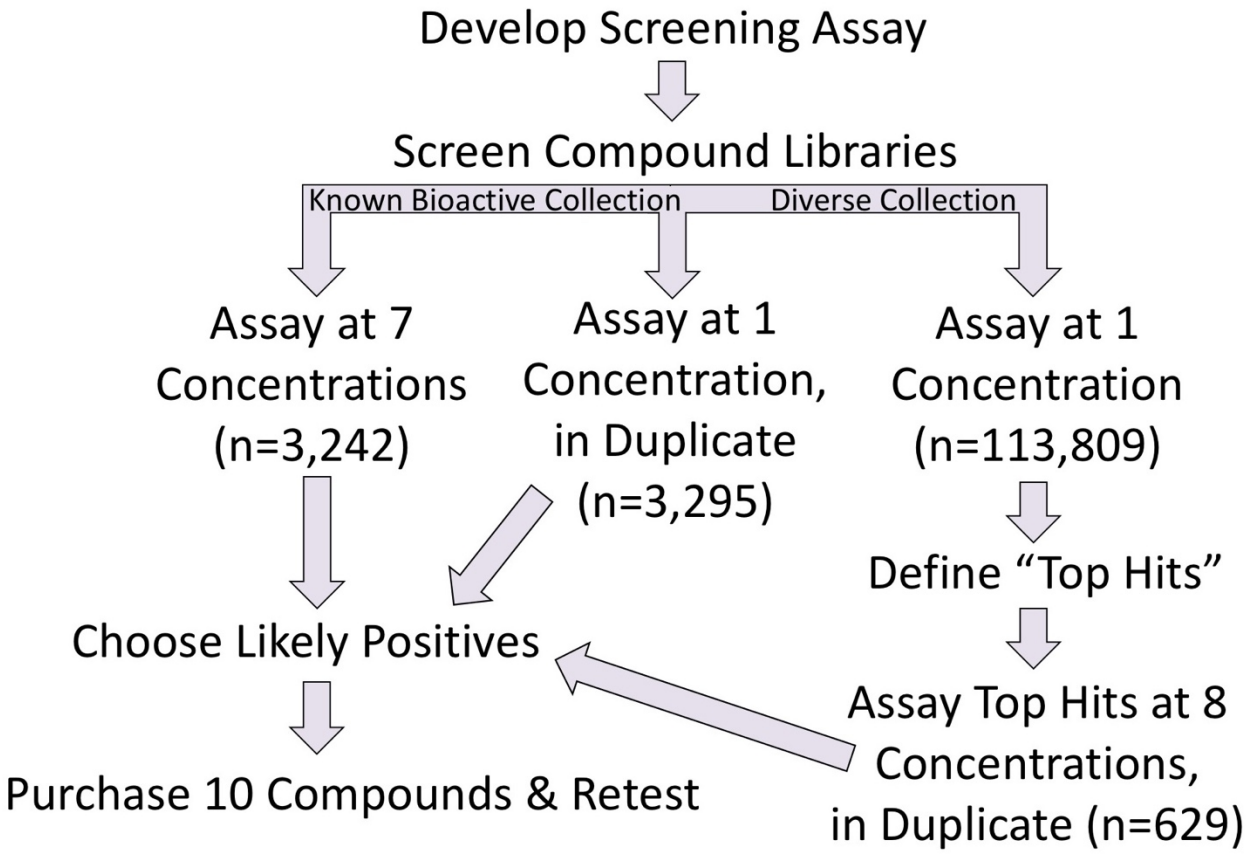

Fig. S3. Workflow for high-throughput compound screening. 120,346 compounds were screened; see Table S3 for libraries screened, compound concentrations, and number of compounds in each library.

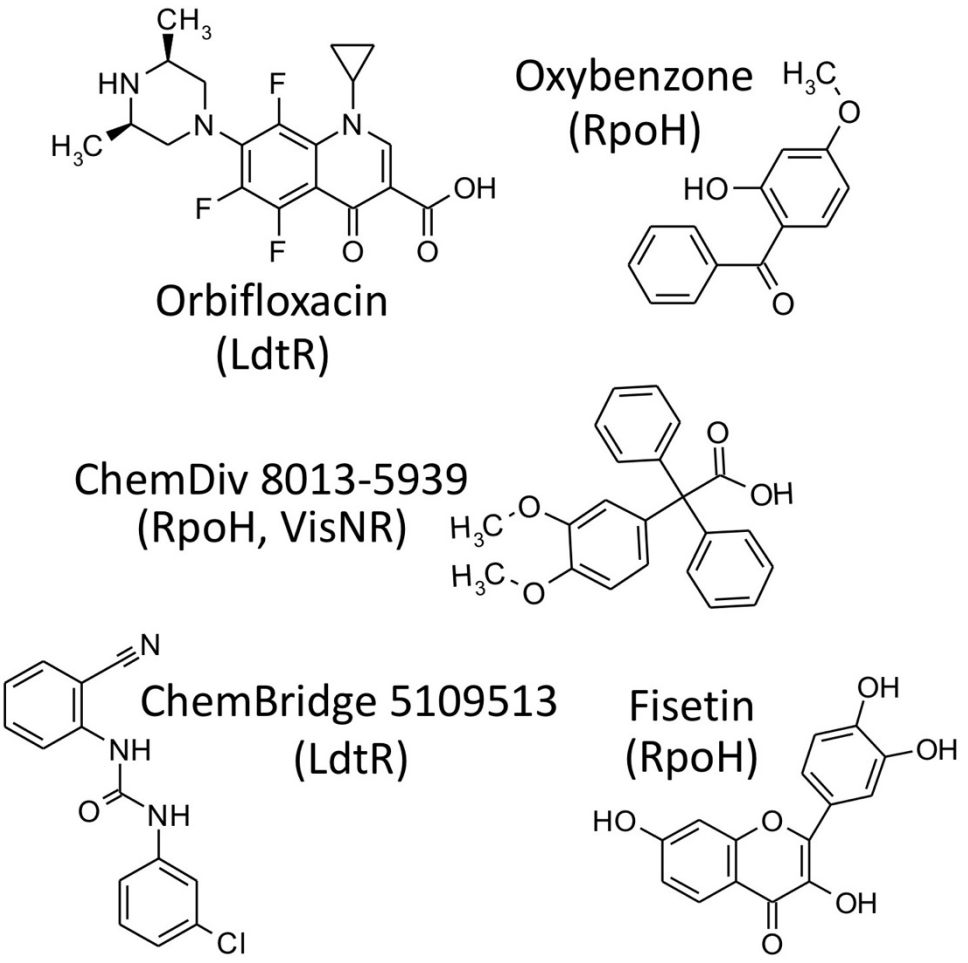

Fig. S4. Five compounds whose effects on the *Ca*. L. asiaticus and *S. meliloti* regulators (LdtR, RpoH/RpoH1, and VisNR) were not confirmed by retesting purchased compounds (Table S6). Regulator(s) identified via high-throughput screening as the putative target(s) of the inhibitory compound(s) are shown below the compound name (Bioactive Collection compounds) or supplier/catalog number (Diverse Collection compounds) in parentheses.

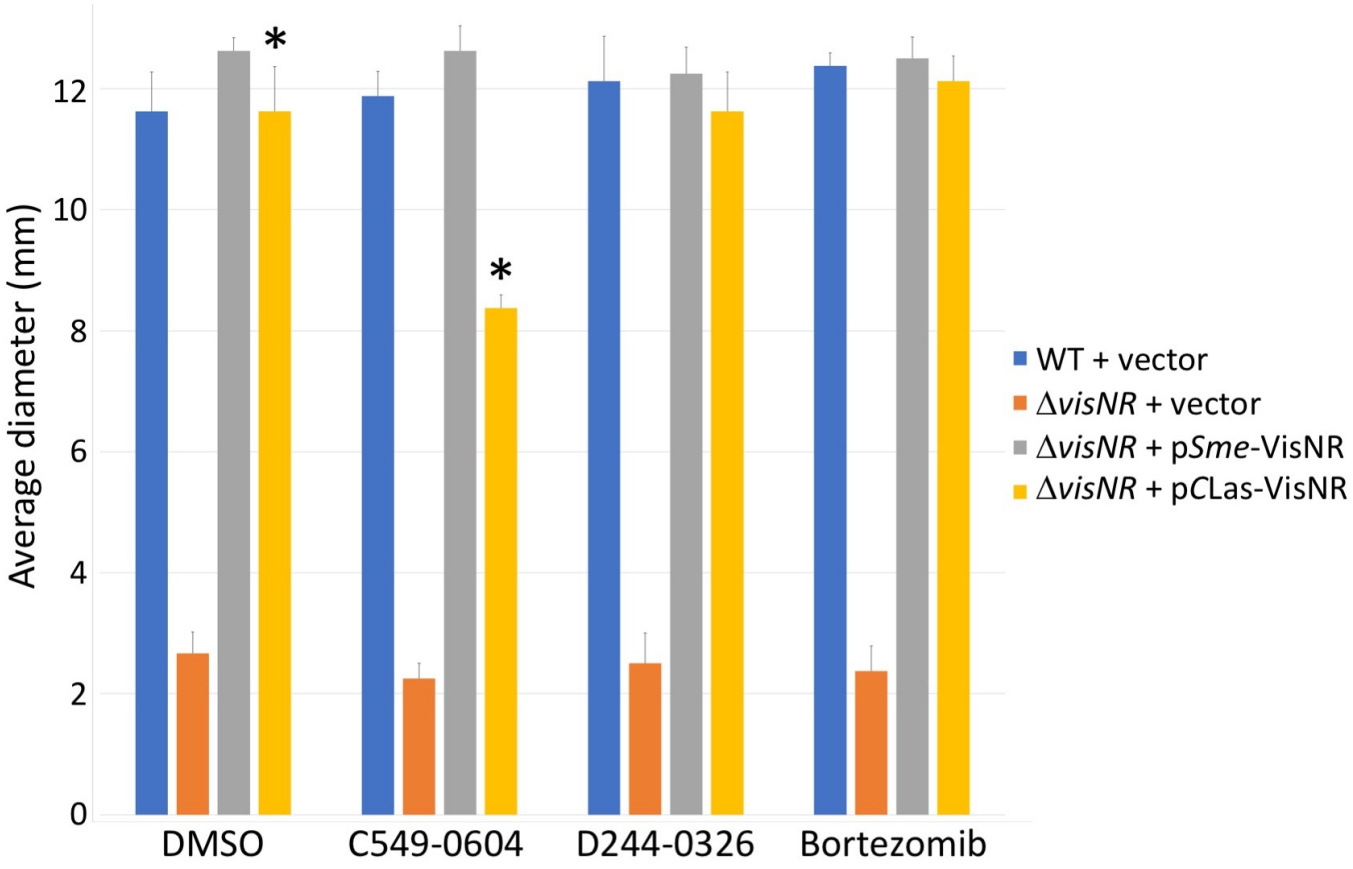

Fig. S5. Swim motility tests of three candidate VisNR-inhibiting compounds (ChemDiv #C549-0604; ChemDiv #D244-0326; and Bortezomib). Assays were performed as described in Supplementary Materials and Methods. Error bars show standard deviation for an average of four replicates. Each compound was tested at 20 μM. Swim motility mediated by ectopically expressed *C*Las VisNR was significantly inhibited by 20 μM ChemDiv #C549-0604 compared to DMSO controls (asterisks, P-value, 1.7×10^{-4}), but not by Bortezomib or ChemDiv #D244-0326.

Table S1. *Ca*. Liberibacter asiaticus (*C*Las) predicted transcription regulators, not listed in Table 1. Bioinformatic analyses predict up to 19 transcription regulators are encoded in the *C*Las genome.

*C*Las regulator, GenBank accession[1]	*Sinorhizobium meliloti* (*Sme*) ortholog(s)[2]	Percent identity between *C*Las and *Sme* proteins	Regulator type	Putative function of regulator in *Sme* (reference)	*C*Las fold change expression for plant vs. psyllid[3]
ACT56762	RpoD	72, 41	Sigma factor	Essential, primary sigma factor (18)	Not reported
ACT57093	RirA	63	IscR	Response to iron limitation (19)	2.1
ACT56890	PrbP (SMc03874)	73	CarD	Unknown	2.4
ACT57256	MucR	55	Ros/MucR	Regulation of exopolysaccharide production (20)	Not attempted
ACT56999	Rem	70	Response regulator	Regulation of motility (21)	Not reported
ACT57389	TacA	34	Enhancer binding protein	Possible involvement in polar development (22)	Not reported
ACT57691	None	n/a	HTH-XRE	n/a	Not reported
ACT57365	SMc00653	47	Response regulator	Unknown	7.9
ACT56949	DivK	78	Response regulator	Cell cycle control; essential gene (23)	2.1
ACT56904	GcrA (SMc02139)	45	GcrA	Cell cycle control; likely essential gene (24)	Not reported
ACT56785	CpdR1, CdpR2	87, 43	Response regulator	Cell cycle control; essential for symbiosis (25)	3.0
ACT56917	SMc02223	50	LexA-like C-terminal domain	Unknown	Not attempted

[1]Regulator accession numbers are for the '*Candidatus* Liberibacter asiaticus' Psy62 genome (1).

[2]If the gene name has not been annotated in GenBank, the *S. meliloti* 1021 unique locus tag is given in parentheses.

[3]Data published in Yan *et al.* 2013 (26). The fold change provided here was calculated from their reported $\log_2$ ratio values.
Not reported = the gene was listed in Yan *et al.* Supplementary Table 1 as "selected for qRT-PCR analysis" but a $\log_2$ ratio was not reported. Not attempted = the gene was not listed in Yan *et al.* Supplementary Table 1.

Table S2. Strains and plasmids used in this study.

Strain or plasmid	Description[1]	Reference
***Sinorhizobium meliloti* strains**		
CL150	Rm1021, *ecfR1, pstC* corrected; Sm^r	(4)
RFF231	CL150, Δ*rpoH1 rpoH2*; Sm^r	(27)
MB1101	CL150, Δ*ldtR*; Sm^r	This study
MB1102	CL150, Δ*visNR*; Sm^r	This study
MB1105	CL150, Δ*lsrB*; Sm^r	This study
MB1122 pMB913	CL150, Δ*ctrA*; pSRKGm with *Sme ctrA*; Gm^r Sm^r	This study
MB1138	CL150; Δ*phrR2*; Sm^r	This study
MB1139	CL150; Δ*phrR1*; Sm^r	This study
MB1142	CL150; Δ*phrR1 phrR2*; Sm^r	This study
Plasmids		
pSRKGm	Broad-host-range P*lac* expression vector; Gm^r	(6)
pMB861	pSRKGm; optimized *C*Las *rpoH*; Gm^r	This study
pMB865	pSRKGm; *Sme rpoH1*; Gm^r	This study
pMB874	pSRKGm; optimized *C*Las *lsrB*; Gm^r	This study
pMB875	pSRKGm; optimized *C*Las *phrR*; Gm^r	This study
pMB876	pSRKGm; optimized *C*Las *ctrA*; Gm^r	This study
pMB877	pSRKGm; optimized *C*Las *visNR*; Gm^r	This study
pMB878	pSRKGm; optimized *C*Las *ldtR*; Gm^r	This study
pMB876	pSRKGm; optimized *C*Las *ctrA*; Gm^r	This study
pMB913	pSRKGm; *Sme ctrA*; Gm^r	This study
pMB949	pSRKGm; optimized *C*Las *rpoH*; P*ibpA*; EGFP; Gm^r	This study
pMB950	pSRKGm; optimized *C*Las *rpoH*; P*groES5*; EGFP; Gm^r	This study
pMB951	pSRKGm; optimized *C*Las *lsrB*; P*smc01834*; EGFP; Gm^r	This study
pMB952	pSRKGm; optimized *C*Las *lsrB*; P*lrp3*; EGFP; Gm^r	This study
pMB953	pSRKGm; optimized *C*Las *phrR*; P*ldtR*; EGFP; Gm^r	This study
pMB954	pSRKGm; optimized *C*Las *phrR*; P*smc00404*; EGFP; Gm^r	This study
pMB955	pSRKGm; optimized *C*Las *ctrA*; P*minC*; EGFP; Gm^r	This study
pMB956	pSRKGm; optimized *C*Las *visNR*; P*rem*; EGFP; Gm^r	This study
pMB957	pSRKGm; optimized *C*Las *ldtR*; P*tacA*; EGFP; Gm^r	This study
pMB958	pSRKGm; optimized *C*Las *ldtR*; P*smc04059*; EGFP; Gm^r	This study
pMB959	pSRKGm; optimized *C*Las *ldtR*; P*ldtP*; EGFP; Gm^r	This study
pMB961	pSRKGm; *Sme ldtR*; Gm^r	This study
pMB962	pSRKGm; *Sme ldtR*; P*smc04059*; EGFP; Gm^r	This study
pMB973	pSRKGm; *Sme rpoH1*; P*ibpA*; EGFP; Gm^r	This study
pMB974	pSRKGm; *Sme visNR*; P*rem*; EGFP; Gm^r	This study
pMB975	pSRKGm; *Sme visNR*; Gm^r	This study

[1]*C*Las, *Ca*. Liberibacter asiaticus; *Sme*, *Sinorhizobium meliloti*.

Table S3. Compound libraries screened at the Stanford High-Throughput Biosciences Center.

Library description or vendor	Number of unique compounds[1]	Compound concentration(s)
Diverse Collection		
Specs	30,106	10 μM
ChemBridge	23,759	4 μg/ml
ChemDiv	49,946	10 μM
ChemDiv, Kinase-targeted	9,998	20 μM
Known-Bioactive Collection		
Sigma LOPAC	1,269	20, 10, 5, 2.5, 1.25, 0.625, 0.3125 μM
NIH Clinical Collection	377	10 μM, in duplicate
NCIDTP, Challenge, Natural Product, and Structural Diversity Sets	2,196	20 μM, in duplicate
NCIDTP, Mechanistic Diversity Set	722	2 μM, in duplicate
Microsource Spectrum	1,502	20, 10, 5, 2.5, 1.25, 0.625, 0.3125 μM
Biomol ICCB	296	10, 5, 2.5, 1.25, 0.625, 0.3125, 0156 μg/ml
Biomol FDA	175	20, 10, 5, 2.5, 1.25, 0.625, 0.3125 μM

[1]There is slight compound redundancy among the libraries, particularly in the Known-Bioactive Collection. Additional information about the libraries is available at: http://med.stanford.edu/htbc/compounds.html

Table S4. Criteria for ranking single concentration results obtained from screening the Diverse Collection libraries.

Rank[1]	% EGFP[2] Inhibition	Difference (%EGFP - %ABS[3] Inhibition)
1	> 40%	> 30%
2	> 40%	> 20%
2	> 30%	> 30%
3	> 40%	> 10%
3	> 30%	> 20%
4	> 30%	> 10%
4	> 20%	> 20%

[1]To choose compounds for retesting at multiple concentrations, the single concentration results for all three regulators (LdtR, RpoH, VisNR) were ranked from best to worst for their ability to inhibit EGFP fluorescence without significantly inhibiting growth. For example, each compound in Rank 1 showed both high EGFP inhibition and a large differential between % inhibition of EGFP fluorescence and % inhibition of growth.

[2]EGFP = fluorescence, indicative of inhibition of *Ca*. L. asiaticus regulator activity.

[3]ABS = absorbance, indicative of *S. meliloti* growth.

Table S5. Distribution by rank and *Ca*. L. asiaticus regulator of 629 Diverse-collection compounds chosen for retesting.

Selectivity[1]	Rank 1	Rank 2	Rank 3	Rank 4
LdtR	80	118	0	0
RpoH	1	55	41	9
VisNR	43	24	25	17
LdtR, RpoH	7	11	0	0
LdtR, VisNR	14	14	0	0
RpoH, VisNR	26	31	0	0
LdtR, RpoH, VisNR	46	67	0	0

[1]Selectivity denotes which *Ca*. L. asiaticus regulator(s) met the rank criteria (defined in Table S4) for single concentration compound results.

Table S6. Retesting summary for 10 purchased compounds.

	High-throughput screening		Retesting purchased compounds				
				*C*Las regulator		*Sme* regulator	
Compound	**Selectivity[1]**	**IC50[2]-EGFP**	**Selectivity**	**IC50-EGFP**	**IC50-ABS[3]**	**IC50-EGFP**	**IC50-ABS**
4'-Demethylepipodophyllotoxin	LdtR	10.3	LdtR	7.5	>>20	8.7	>>20
Bortezomib	VisNR	20.2	VisNR RpoH (*Sme*)	2.1 (VisNR)	>40 (VisNR)	2.4 (VisNR) <1.25 (RpoH)	>40 (VisNR) 13.0 (RpoH)
ChemDiv C549-0604	VisNR	2.6	VisNR	0.7	>>20	>20	>>20
ChemDiv D244-0326	VisNR	8.5	VisNR RpoH	5.3 (VisNR), 26.8 (RpoH)	>>20	18.5 (VisNR) 20.0 (RpoH)	>>20
Rosiglitazone maleate	RpoH	27.0	VisNR RpoH	15.7 (VisNR) 24.4 (RpoH)	>40	9.4 (VisNR) 3.5 (RpoH)	>40
ChemBridge 5109513	LdtR	4.8	Non-selective. Toxic[4], all strains				
ChemDiv 8013-5939	RpoH VisNR	11.2 (RpoH) 4.2 (VisNR)	Inactive[5]				
Fisetin	RpoH	19.1	Non-selective. Toxic, *C*Las and *Sme* RpoH strains				
Orbifloxacin	LdtR	<0.3125	Non-selective. Toxic, all strains				
Oxybenzone	RpoH	10.1	Non-selective. Toxic, all strains except *C*Las LdtR				

[1]Selectivity indicates which regulator strains showed inhibition of EGFP fluorescence (cutoff ≥ 30% inhibition). Non-selective: compound inhibited EGFP fluorescence because it affected growth. *C*Las, *Ca*. Liberibacter asiaticus. *Sme*, *Sinorhizobium meliloti.*

[2]IC50 is the micromolar concentration of an inhibitor where the response was reduced by half. IC50 values for inhibition are shown for only those compounds that specifically inhibited EGFP fluorescence of just one or two of the three regulators.

[3]ABS, absorbance. >, ABS inhibition at the highest concentration tested was ≥30% and <50%. >>, ABS inhibition at highest concentration tested < 30%.

[4]Toxic, ≥50% ABS inhibition at the highest concentration tested.

[5]Inactive, no EGFP or ABS inhibition.

Table S7. Oligonucleotide primers used in this study		
Description	**Primer 1**	**Primer 2**
Construct plasmids carrying Sinorhizobium meliloti regulators		
Amplify *S. meliloti rpoH1* as NdeI-KpnI DNA to create pMB865	TTCATATGGCCCGCAATACCTTGCCG	CAGGTACCTTAAGCGCCTTCAACCACGCG
Amplify *S. meliloti ctrA* as NdeI-KpnI DNA to create pMB913	TTCATATGCGGGTTCTACTGATCGA	CAGGTACCTCAGGCGGTTTCCAGGTAGT
Amplify *S. meliloti ldtR* as NdeI-KpnI DNA to create pMB961	TACATATGAACACCAAGATGAAGCC	CAGGTACCTAAACCCGCCCCGATTAGAG
Amplify *S. meliloti visNR* as NdeI-KpnI DNA to create pMB975	AACATATGGAAATGTCGCGGTCG	CAGGTACCCTAGCTGATATAGCCGAG
Construct genomic deletions of S. meliloti regulators		
Amplify DNA upstream of *visNR* as XbaI-NotI to create pMB891	AATCTAGAAGCTGGGCTTCGGCGGACAG	AAGCGGCCGCGTATTCTTGCCACCAAGCGA
Amplify DNA downstream of *visNR* as NotI-XhoI to create pMB891	AAGCGGCCGCGACGAACTTCGATTCTGCCA	AACTCGAGAGGCTCGATACGGCAACGGA
Amplify DNA upstream of *lsrB* as XbaI-NotI to create pMB887	AATCTAGATTCACGGCGCTTTCTCCGTG	AATCTAGATTCACGGCGCTTTCTCCGTG
Amplify DNA downstream of *lsrB* as NotI-XhoI to create pMB887	AAGCGGCCGCCGATCCGTTCAATTGCCCGA	AACTCGAGGATTGACGAGGTAGGTCTTC
Amplify DNA upstream of *phrR1* as XbaI-NotI to create pMB892	AATCTAGAATGCCGTTGATGATGCAGTC	AAGCGGCCGCTTGATGGCGAAATCCACTAT
Amplify DNA downstream of *phrR1* as NotI-XhoI to create pMB892	AAGCGGCCGCAAGTCGTCCGGCACGAAAGA	AACTCGAGGCCGATCGCGTAGGAAAGCT
Amplify DNA upstream of *phrR2* as XbaI-NotI to create pMB889	AATCTAGACGACATGCTCGCCCGAAATCA	AAGCGGCCGCTAAGTTTGCTTGAATGGACG
Amplify DNA downstream of *phrR2* as NotI-ApaI to create pMB889	AAGCGGCCGCTGTCCGTGACGACGATTGGC	AAGGGCCCCATGTCGAGGATCGGCTTGA
Amplify DNA upstream of *ctrA* as XbaI-NotI to create pMB890	AATCTAGAAGATCATGCTGCAGCTGCCG	AAGCGGCCGCGCCCAAGGTTGTCGTGGCAT
Amplify DNA downstream of *ctrA* as NotI-ApaI to create pMB890	AAGCGGCCGCCCGGCATTCCCGTAGGCGAC	AAGGGCCCCCAAGCTGGTGGAGCTCATC
Amplify DNA upstream of *ldtR* as XbaI-NotI to create pMB888	AATCTAGATAGGTCTTCTTGTCGCCCTT	AAGCGGCCGCTCGTGCCTCACTGTTTCGTT
Amplify DNA downstream of *ldtR* as NotI-XhoI to create pMB888	AAGCGGCCGCTCGGGGCGGGTTTACCTTCA	GGTTTGTCTCGAGCTGGGCGA
Check genomic *ldtR* deletion (strain=MB1101)	ACATCGAGTGCCGCATCGCG	AGGTCGCCCGACATCGACTG
Check genomic *visNR* deletion (strain=MB1102)	AATCTAGAATGGATTTCGTGGAGACACA	CAAACATGGCCGAGGCGCTG
Check genomic *ctrA* deletion (strain=MB1122)	AGATCATGCTGCAGCTGCCG	GGAGTTCGACGCGATATTCA
Check genomic *phrR1* deletion (strain=MB1139)	CTTGCGCTCGGCTTCGGCAT	CAGATCGACATAGATCGACA
Check genomic *phrR2* deletion (strain=MB1138)	CATCGAAACGACATCGGAGC	GCTCGCGCTGAAGCCGGCTG
Check genomic *lsrB* deletion (strain=MB1105)	GATCCGTCCGCACCAAGAGC	GCGATCTCCGCCGGTTCGAG

Clone promoters into EGFP expression vector		
Amplify *ibpA* promoter to clone as SpeI-XhoI	ATACTAGTCCAGAAGGAACATGCAAGT	AACTCGAGATAAGAGCAACGGTTGCGA
Amplify *rem* promoter to clone as SpeI-XhoI	ATACTAGTGCGACAATGCGGACAGATT	AACTCGAGTCCGAATCAGTATGGATGCC
Amplify *SMc04059* promoter to clone as SpeI-XhoI	ATACTAGTCACGTATGTCCCGCTTCTG	AACTCGAGATCGGCCTATGGTTGATCGT
Amplify *groES5* promoter to clone as SpeI-XhoI	ATACTAGTTGCTTCATCTCCGTTACAT	AACTCGAGTTGATGATCTTGTTCCCCA
Amplify *SMc01384* promoter to clone as SpeI-XhoI	ATACTAGTTGCCAGCAATATCATCAAGGC	AACTCGAGCAGTCTTGGGAGGACAGGC
Amplify *lrp3* promoter to clone as SpeI-XhoI	ATACTAGTATTTGCATCGGTTTTGACCC	AACTCGAGTTTATCCGCCGATCAACTGC
Amplify *ldtR* promoter to clone as SpeI-XhoI	ATACTAGTGTTGCTGATCGTGCTCCAC	AACTCGAGTGCCTCACTGTTTCGTTTGG
Amplify *SMc00404* promoter to clone as SpeI-XhoI	ATACTAGTACTTCGCCATTTCCACAACC	AACTCGAGGTTCCCTTTGTTGATCGAGCT
Amplify *minC* promoter to clone as SpeI-XhoI	ATACTAGTAAAGGAGAGAGGAACCGACA	AACTCGAGCTTCGTTAATTATTGGTTGCC
Amplify *tacA* promoter to clone as SpeI-XhoI	ATACTAGTTTAACCCTGAAGCGAGGCAA	AACTCGAGGACACGCGGGGTACTTTTG
Amplify *ldtP* promoter to clone as SpeI-XhoI	ATACTAGTTCCGACCTTTGCTGCTTTTC	AACTCGAGCGTCTCAATGCGCCAGAAG
Check promoters cloned as SpeI-XhoI (in *rrnB* terminator and EGFP)	TGAACACTCTCCCGACTAG	TTAAGGGTGAGCTTGCCGTA

Additional Data Set S1 (separate file)
Affymetrix GeneChip transcriptome data for expression of transcription regulators in *Sinorhizobium meliloti* regulator deletion strains.

Additional Data Set S2 (separate file)
Candidate inhibiting compounds identified by high-throughput screening of the Known Bioactive Collection.

Additional Data Set S3 (separate file)
Candidate inhibiting compounds identified by high-throughput screening of the Diverse Collection.

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