Regulatable vectors for environmental gene expression in *Alphaproteobacteria*

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Supplementary material

Bacterial strains and growth conditions

The bacterial strains used in this study are described in table S1.

Construction of pLMB51. The *tauA* promoter and *tauR* gene of *S. meliloti* 1021 were PCR amplified from genomic DNA using primers pr0079 and pr0080 (Table S2) using previously described conditions (3). A KpnI restriction site was included at the 5' end of each primer to enable cloning. The PCR product was blunt ligated into vector pJET1.2/blunt (Fermentas). The insert was excised by KpnI restriction digestion and cloned into KpnI digested vector, pJP2 forming pLMB51.

Construction pLMB509. pLMB509 was formed utilising BD In-Fusion™ technology (Clontech) to combine three linear fragments (two amplified regions derived from pLMB51 and pRLU1357 and a linearized vector, pRU1097). pRU1097 was linearized by a ClaI and SacI restriction digest. The *tauAp TauR* region of pLMB51 and the *gfpmut3.1* region of pRU1357 were amplified from purified plasmid preparations using primers pr1266, pr1267 and pr1268, pr1269 respectively (Table S2). PCR amplifications were performed in 50 μl volume using 1 unit Phusion polymerase (Thermo scientific), 1 μM primer and 0.2 mM dNTPs. Cycling conditions were: One cycle of 98^{o}C for 30 s, 30 cycles of 98^{o}C for 10 s, 67^{o}C for 30 s,

72°C for 40 s with a final extension for 7 minutes at 72°C. The 5' ends of primers pr1267 and pr1268 were complementary to the linearized ends of pRU1097, primers pr1266 and pr1269 were complementary to a region of pRU1357 and pLMB51 respectively. These fragments were combined using the BD method in a 3:3:1 ratio (insert:insert:backbone) using manufactures reaction conditions. Additional features incorporated into the primers are given in table S2.

References supplementary material

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5. **Meade, H. M., S. R. Long, G. B. Ruvkun, S. E. Brown, and F. M. Ausubel.** 1982. Physical and genetic-characterization of symbiotic and auxotrophic

mutants of *Rhizobium meliloti* induced by transposon Tn5 mutagenesis. J. Bacteriol. **149:**114-122.

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Strain or plasmid	Description	Source/reference
Escherichia. coli		
E. coli α- select	F$^-$ *deo*R *end*A1 *rec*A1 *rel*A1 *gyr*A96 *hsd*R17(r_k^-, m_k^+) *sup*E44 *thi*-1 *pho*A Δ(*lac*ZYA *arg*F)U169 Φ80*lac*ZΔM15λ	Bioline
Rhizobium leguminosarum		
Rlv3841	Str^r derivative of *R. leguminosarum* bv viciae 300	(2)
LMB150	Rlv3841 Containing pLMB51	This work
LMB434	Rlv3841 containing pLMB509	This work
RU4125	Rlv3841 Containing pRU1357	(4)
LMB461	Rlv3841 Containing pRU548	This work
Rhizobium. etli		
CE3	Str^r derivative of *R. etli* CFN42	(6)
LMB470	Rlv3841 Containing pLMB51	This work
Rhizobium sp		
NGR234		(9)
LMB469	NG234 Containing pLMB51	This work
Sinorhizobium meliloti		
1021		(5)
RU2041	1021 Containing pRU1357	(4)
LMB152	1021 Containing pLMB51	This work
Rhodobacter sphaeriodes		
WS8N	Nal^r derivative of *R. sphaeriodes* WS8	(8)
LMB422	WS8N Containing pLMB51	This work
Plasmids		
pRK2013	Km^r, ColE1 replicon with RK2 *tra* genes. Used to conjugate mob$^+$ plasmids from *E.coli* to *Alphaproteobacteria*	(1)
pLMB51	Tc^r , pJP2 backbone containing *tauAp* and *tauR* from 1021	This work
pLMB509	Gm^r , pRU1097 backbone with *tauAp, tauR* from 1021 and a His-tagged protein (*gfpmut3.1*)	This work

pRU1357	Gm^r, pRU1097 backbone with *tauAp* upstream of *gfpmut3.1* but without *tauR* regulator	(4)
pRU1097	Gm^r promoter probe vector with a promoter-less *gfpmut3.1*	(3)
pJP2	Tc^r , stable broad host range cloning vector	(7)
pLMB548	Gm^r, Solute binding protein SMc02774 in vector pLMB509	This work

TABLE S1. Bacterial strains and plasmids used in this study (Abbreviations Gm, Gentamycin, Km, Kanamycin, Nal, Nalidixic acid, Str, Streptomycin, Tc, Tetracycline)

Primer	**Sequence**	**Gene/comment**
pr1266	GTTCTTCTCCTTTACGCATATGTTCTTCCTCCTGTC GTTACCCTCTTTGGTT	*tauAp* and *tauR* reverse. 5' pRU1357 complementary region
pr1267	CGGATCTAGATATCGATACCCCGCTCCGCGATCC GCG	*tauAp* and *tauR* forward. 5' pRU1097 complementary region
pr1268	ATTCATTATTTGTAGAGCTCTTAGTGATGATGGT GATGATGCATATGTTTGTATAGTTCATCCATGC	*gfpmut3.1* reverse. Contains NdeI RE site, 6 His-tag and stop codon. 5' pRU1097 complementary region
pr1269	AACCAAAGAGGGTAACGACAGGAGGAAGAACA TATGCGTAAAGGAGAAGAAC	*gfpmut3.1* forward. 5' pLMB51 complementary region
pr0079	GGTACCTCTGCAAGGCTCATTCTAACC	*tauAp* and *tauR* forward 5' KpnI RE site
pr0080	GGTACCCTACATGTCGTTACCCTCTTTGGTTA	*tauAp* and *tauR* reverse 5' KpnI RE site

TABLE S2. Primers used in this study