

Table S1: Bacterial strains and Plasmids

Strains/ Plasmids	Description	Source and Reference
Strains		
<i>E. coli</i>		
CC118	F ⁻ Δ(<i>ara-leu</i>) 7679 Δ(<i>lacZ</i>) 74Δ(<i>phoA</i>)20 <i>araD139 galE galK thi rpsE rpoB arfE^{am} recA1, λpir</i>	[1]
S17-1λpir	<i>recA1 thi pro hsdR</i> RP4-2Tc::Mu Km::Tn7 λpir	[2]
BL21λDE3	F ⁻ <i>ompT gal dcm lon hsdSB (rB2 mB2) gal</i> λDE3	[3]
<i>Y. pseudo-tuberculosis</i>		
YP111	+ pYV, wildtype	[4]
YP12	- pYV, wildtype strain cured of the virulence plasmid	[5]
YP52	YP111 Δ <i>csrC</i> , Kn ^R , Δ <i>csrB</i> , Ap ^R	[6]
YP53	YP111 Δ <i>csrA</i> , Kn ^R	[6]
YP66	YP111 Δ <i>lcrF</i> , Ap ^R	[7]
YP91	YP111 Δ <i>yopD</i>	this study
YP138	YP111 Δ <i>pnp</i>	this study
YP145	YP111 Δ <i>yopD</i> , Δ <i>csrA</i> , Kn ^R	this study
YP155	YP111 - pYV, <i>lcrF</i> ⁺ , Ap ^R	this study
Plasmids		
pACYC184	cloning vector, ori p15A, Tet ^R , Cm ^R	[8]
pAKH3	pGP704, <i>sacB</i> ⁺ , Ap ^R	[9]
pAKH56	pACYC184, P _{<i>csrA</i>} - <i>csrA</i> ⁺ , p15A, Cm ^R	[6]
pAKH172	pET28a, <i>csrA</i> ⁺ , Kn ^R	this study
pBAD18- <i>lacZ</i> (481)	translational <i>lacZ</i> fusion vector, Ap ^R , ori pBR, ori M13	[10]
pBAD33	expression vector, P _{<i>BAD</i>} , <i>araO2</i> , <i>araC</i> , ori p15A, Cm ^R	[11]
pBADmycC	expression vector, N-terminal His ₆ -tag, P _{<i>BAD</i>} , ori pBR322, Ap ^R	Invitrogen
pDM4	mutagenesis vector, ori R6K, <i>sacB</i> , Cm ^R	[12, 13]
pED07	pBAD18- <i>lacZ</i> (481), pBAD:: <i>lcrF-lacZ</i> (-124 to +74) ^a , mutation GUU-30/-28AAA), Ap ^R	[7]
pET28a	T7 overexpression vector, ori 3286, Kn ^R	Novagen
pFU31	<i>gfp_{mut3.1}</i> , ori ColE1, Ap ^R	[14]
pFU50	<i>dsRed2</i> , P _{<i>lac</i>} , ori 29807, Kn ^R	[14]
pFU51	<i>gfp</i> -RBS, pSC101*, Ap ^R	[14]
pFU72	<i>luxCDABE</i> , R6K mobRP4, Ap ^R	[14]
pFU98	promoterless <i>luxCDABE</i> operon, ori pSC101*, Cm ^R	[14]
pFU100	<i>luxCDABE</i> -RBS, R6Kmob, Cm ^R	this study
pFU166	<i>gapA-luxCDABE</i> , ori colE1, Ap ^R	[14]
pGFPmut3.1	<i>gfp_{mut3.1}</i> expression vector, ori pUC, Ap ^R	Clontech
pGP20	promoterless <i>lacZ</i> gene, ori pSC101, Tet ^R	[7]
pHSG576	cloning vector, ori pSC101, Cm ^R	[15]
pIV2mob	<i>Yersinia</i> cloning vector, ori 29807, Ap ^R	[13]
pIVO22	pT02, <i>rne-lacZ</i> (-646 to +44), ori pSC101, Ap ^R	this study
pIVO23	pT02, <i>pnp-lacZ</i> (-288 to +44), ori pSC101, Ap ^R	this study

pIVO25	pT03, <i>rne-lacZ</i> (-646 to -315), ori pSC101, Ap ^R	this study
pIVO26	pT03, <i>pnp-lacZ</i> (-288 to -87), ori pSC101, Ap ^R	this study
pJE2	pGFPmut3.1, <i>yadA-gfp</i> , ori pUC, Ap ^R	this study
pJE9	pJE2, <i>yadA-gfp</i> , ori p29807, Kn ^R	this study
pJH4	pKB63, <i>csrA'-lacZ</i> (-298 to +17) ^c , ori pSC101, Ap ^R	this study
pJH6	pKB63, <i>csrA'-lacZ</i> (-121 to +17) ^c , ori pSC101, Ap ^R	this study
pJH12	pET28a, <i>yopD⁺, lcrH⁺</i> , Kn ^R	this study
pKB12	pGP20, $\Delta yscW(\Delta+113$ to +298) ^b <i>lcrF'-lacZ</i> , ori pSC101, Tet ^R	this study
pKB14	pBAD18- <i>lacZ</i> (481), pBAD:: <i>lcrF-lacZ</i> (-124 to +74) ^a , ori pMB1, Ap ^R	[7]
pKB28	pACYC184, P _{<i>yscW</i>} :: <i>yscWlcrF</i> (-1076 to +893) ^a , ori p15A, Tet ^R	this study
pKB60	pHSG576, <i>csrA⁺</i> , ori pSC101, Cm ^R	this study
pKB63	pTS02, <i>csrA-lacZ</i> (-1071 to +17) ^c , ori pSC101, Ap ^R	[16]
pKB99	pED07- <i>kan</i> , pBAD:: <i>lcrF-lacZ</i> (-124 to +74) ^a , mutation GUU-30/-28AAA), ori pMB1, Kn ^R , Ap ^R	this study
pKD4	kanamycin cassette template, Kn ^R	[17]
pMP1	pBAD33-RBS _{<i>lcrF</i>} , 3'-UTR, ori M13, Cm ^R	this study
pRS1	pFU50, ori29807, Cm ^R	this study
pRS2	pRS1, <i>yopD⁺</i> , ori29807, Cm ^R	this study
pRS4	pRS1, <i>lcrQ⁺</i> , ori29807, Cm ^R	this study
pRS15	pRS1, ori p15A, Cm ^R	this study
pRS16	pRS2, <i>yopD⁺</i> , ori p15A, Cm ^R	this study
pRS18	pRS4, <i>lcrQ⁺</i> , ori p15A, Cm ^R	this study
pRS30	pDM4, $\Delta yscS$, Cm ^R	this study
pRS34	pDM4, $\Delta yopD$, Cm ^R	this study
pRS40	pBAD33, P _{BAD} :: <i>rne</i> (-15 to +1395) ^d , ori p15A, Cm ^R	this study
pRS50	pAKH3, Δpnp , Ap ^R , Kan ^R	this study
pSR1	pIV2mob, <i>gfp_{mut3.1}</i> , ori p29807, Kn ^R	this study
pTS02	pGP20, promoterless <i>lacZ</i> , ori pSC101, Ap ^R	[7]
pTS03	pGP20, <i>lacZ⁺</i> , ori pSC101, Ap ^R	[7]
pTS31	pFU98, <i>yadA-luxCDABE</i> +RBS, ori pSC101*, Cm ^R	[14]
pTS32	pFU98, <i>invA-luxCDABE</i> + RBS, ori pSC101*, Cm ^R	[14]
pTS34	pWO8, <i>lcrF-luxCDABE</i> + RBS, ori pSC101*, Cm ^R	this study
pWO3	pSR1 $\Delta yscW(\Delta+113$ to +298) ^b <i>lcrF-gfp</i> , ori p29807, Kn ^R	this study
pWO8	pFU51, P _{<i>yscW</i>} :: <i>yycsWlcrF</i> , ori SC101*, Ap ^R	this study
pWO13	pWO8, P _{<i>yscW</i>} :: <i>yycsWlcrF</i> , ori R6K mob, Ap ^R	this study
pWO14	pFU51 P _{<i>yscW</i>} :: <i>yycsWlcrF</i> , insertion sequence, ori R6Kmob, Amp ^R	this study
pWO41	pTS31 <i>yadA-luxCDABE</i> + RBS, SC101*, Amp ^R	this study
pWO42	pTS34 <i>lcrF-luxCDABE</i> +RBS, SC101*, Amp ^R	this study
pZA31luc	Expression vector, p15A, P _{<i>LtetO-1</i>} , Cm ^R	[18]

^a – relative to translational start codon of *lcrF*

^b – relative to transcriptional start site of *yscW*

^c – relative to translational start codon of *csrA*

^d – relative to transcriptional start site of *rne*

Table S1 References:

- X1. Manoil C, Beckwith J. A genetic approach to analyzing membrane protein topology. *Science*. 1986;233:1403-8.
2. Simon R, Priefer U, Puehler A. A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in gram negative bacteria. *Biotechnology*. 1983;1:784-91.
3. Studier FW, Moffatt BA. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression. *J Mol Biol*. 1986;189:113-30.
4. Bolin I, Norlander I, Wolf-Watz H. Temperature-inducible outer membrane protein of *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* is associated with the virulence plasmid. *Infect Immun*. 1982;37:506-12.
5. Dersch P, Isberg RR. A region of the *Yersinia pseudotuberculosis* invasin protein enhances integrin-mediated uptake into mammalian cells and promotes self-association. *EMBO J*. 1999;18(5):1199-213. Epub 1999/03/04. doi: 10.1093/emboj/18.5.1199. PubMed PMID: 10064587; PubMed Central PMCID: PMC1171211.
6. Heroven A, Bohme K, Rohde M, Dersch P. A Csr-type regulatory system, including small non-coding RNAs, regulates the global virulence regulator RovA of *Yersinia pseudotuberculosis* through RovM. *Mol Microbiol*. 2008;68(5):1179-95. Epub 2008/04/24. doi: MMI6218 [pii] 10.1111/j.1365-2958.2008.06218.x. PubMed PMID: 18430141.
7. Böhme K, Steinmann R, Kortmann J, Seekircher S, Heroven AK, Berger E, et al. Concerted actions of a thermo-labile regulator and a unique intergenic RNA thermosensor control *Yersinia* virulence. *PLoS Pathog*. 2012;8(2):e1002518. Epub 2012/02/24. doi: 10.1371/journal.ppat.1002518. PubMed PMID: 22359501; PubMed Central PMCID: PMC3280987.

8. Chang AC, Cohen SN. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. *J Bacteriol.* 1978;134(3):1141-56.
9. Bückler R, Heroven AK, Becker J, Dersch P, Wittmann C. The pyruvate-tricarboxylic acid cycle node: a focal point of virulence control in the enteric pathogen *Yersinia pseudotuberculosis*. *J Biol Chem.* 2014;289(43):30114-32. doi: 10.1074/jbc.M114.581348. PubMed PMID: 25164818; PubMed Central PMCID: PMC4208018.
10. Waldminghaus T, Fippinger A, Alfsmann J, Narberhaus F. RNA thermometers are common in alpha- and gamma-proteobacteria. *Biol Chem.* 2005;386(12):1279-86. Epub 2005/12/13. doi: 10.1515/BC.2005.145. PubMed PMID: 16336122.
11. Guzman LM, Belin D, Carson MJ, Beckwith J. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. *J Bacteriol.* 1995;177(14):4121-30.
12. Milton DL, O'Toole R, Horstedt P, Wolf-Watz H. Flagellin A is essential for the virulence of *Vibrio anguillarum*. *J Bacteriol.* 1996;178(5):1310-9.
13. Strauch E, Voigt I, Broll H, Appel B. Use of a plasmid of a *Yersinia enterocolitica* biogroup 1A strain for the construction of cloning vectors. *J Biotechnol.* 2000;79(1):63-72.
14. Uliczka F, Pisano F, Kochut A, Opitz W, Herbst K, Stolz T, et al. Monitoring of gene expression in bacteria during infections using an adaptable set of bioluminescent, fluorescent and colorigenic fusion vectors. *PLoS One.* 2011;6(6):e20425. doi: 10.1371/journal.pone.0020425. PubMed PMID: 21673990; PubMed Central PMCID: PMC3108616.

15. Takeshita S, Sato M, Tabo M, Masahashi W, Hashimoto-Gothoh T. High-copy-number and low-copy-number plasmid vectors for *lacZ* α -complementation and chloramphenicol- or kanamycin resistance selection. *Gene*. 1987;61:63-74.
16. Nuss AM, Schuster F, Kathrin Heroven A, Heine W, Pisano F, Dersch P. A direct link between the global regulator PhoP and the Csr regulon in *Y. pseudotuberculosis* through the small regulatory RNA CsrC. *RNA Biol*. 2014;11(5):580-93. PubMed PMID: 24786463.
17. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc Natl Acad Sci U S A*. 2000;97(12):6640-5. PubMed PMID: 10829079.
18. Lutz R, Bujard H. Independent and tight regulation of transcriptional units in *Escherichia coli* via the LacR/O, the TetR/O and AraC/I1-I2 regulatory elements. *Nucleic Acids Res*. 1997;25(6):1203-10. PubMed PMID: 9092630.

Table S2: Oligonucleotides

Name	Sequence (5'-3')	Restriction site(s)
90	CGCGGCGGATCCCCTTAACGTACGTTTTTCGTCCC	<i>Bam</i> HI
92	CGCGGCGGTACCCGCAGATATTAATGCCGCAGAGAC	<i>Bam</i> HI
555	CGGCGCGGATCCCTCTCACACCAGCTGTG	<i>Bam</i> HI
556	GGGGGCGTTCGACGGCAAACCTCAATATCCTG	<i>Sal</i> I
558	CGGCGCGGATCCGGACAATGGTCGATGAC	<i>Bam</i> HI
559	GGGGGCGTTCGACGTTACACGAGACGCTGC	<i>Sal</i> I
583	GGGCGCGGATCCGATTGGGCCGGAATCTAGC	<i>Bam</i> HI
168	CGGCGCCTCGAGGTAAGTCGTCGGTTGAGAC	<i>Xho</i> I
182	GCAATCAGCTAGTCAATTTG	
I214	GCGGCGTCTAGACCATTGAATCTTCACAATCTAATCCCG	<i>Xba</i> I
I222	GCGGCTGCAGCCATCTTGTGAATGCTCAACAACC	<i>Pst</i> I
I224	GCGGCCTGCAGGGCTGCAATGTAAGTACTAGGAATATGG	<i>Pst</i> I
I303	GCGCGCTGCAGGATTTTTAGGACAGTATAAC	<i>Pst</i> I
I404	TTTGAATTCGCCATCTTGTGAATGCTCAAC	<i>Eco</i> RI
I515	GCAGTTCATTTGGATCAATAC	
I661	GTGTAGGCTGGAGCTGCTTC	
I662	CATATGAATATCCTCCTTAGTTCC	
I746	CGTATGTCACGACCATTATCTCGCGTGGCTCAGCAAAAG	
I747	CTTTTGCTGAGCCACGCGAGATAATGGTCGTGACATACG	
I844	GCGGCGCATGCGGCTGCAATGTAAGTACTAGGAATATGG	<i>Sph</i> I
I964	GCACTGGATCCCTAATCTTGTGAATGCTCAACAACC	<i>Bam</i> HI
I972	GCATAGATCTTACGGGGTCTGACGCTCAGTG	<i>Bgl</i> II
I998	GCATCAGATCTTCTGTTGTTTGTTCGGTGAACG	<i>Bgl</i> II
II275	GGGGCCCTGCAGCGAGTCAGAATAAGCATTCTTTG	<i>Pst</i> I
II341	GCACGGAGCTCAGGCGAGTATCGTGATAG	<i>Sac</i> I
II342	GCACGGAGCTCGTCAGGTGGTCAACCTTCT	<i>Sac</i> I
II360	CGGCTGCAGGCGACAGGAGACTCGATG	<i>Pst</i> I
II361	CGGGCGGCCGCCAGTAGATATATATTATCTCAGC	<i>Not</i> I
II363	CGGACTAGTCAGGCAACACAAGTCGCCG	<i>Spe</i> I
II364	CGGGCATGCGCAAAATGCCGATCACAGCC	<i>Sph</i> I
II365	GGAATAACCATGACAATAAATATCGGTGTTGTCTGACCATTGATG	
II366	CATCAATGGTCAGACAACACCGATATTTATTGTCATGGTTAT TCC	
II496	GCGCGTTATAAGCTGTCAAACATGAGAATTACAAC	<i>Psi</i> I
II538	GCGCGGAGCTCCCGCCGGACATCAGCGC	<i>Sac</i> I
III233	GCGCGCTCTAGAAGGAGGTTGTTAAAGATGAAAAGAATGTTGATTAACGCG	<i>Xba</i> I
III234	GCGCGCGCATGCTTATTAACGCTTCTCAATAGCGTTTACTG	<i>Sph</i> I
III243	GCGGCGGAGCTCGTTGCTGACTTCGGTCGCGG	<i>Sac</i> I
III244	GAAGCAGCTCCAGCCTAC CGCAAAATTGTACCCTTATGCTTTGC	

III245	ACTAAGGAGGATATTCATATGGTAATTAAGTCATTCCA CAGCTCC	
III246	GCGGCGGAGCTCGCTCTAGTACAGAATCAAACG	<i>SacI</i>
III394	CCTCGTTCATAAGCACTCGTC	
III645	GGGCGC <u>ACTAGTTCAGACAACACCAAAAAGCG</u>	<i>SpeI</i>
III647	GGGCGCCTCGAGTCATCATGGGTTATCAACGCAC	<i>XhoI</i>
III731	GAATAAGCATTCTTTGCTCC	
III902	GGGCGCGCATGCGCTGACTCATACCAGGCC	<i>SphI</i>
III905	GGGCGCTCTAGAGCTCTGCCAGTGTTACAAC	<i>XbaI</i>
V066	GGGCGCGTAATACGACTCACTATAGGAATGTAATGGCT TACGTTTTTC	
V708	GGGCGCGTAATACGACTCACTATAGAACAGAGAGACC CGACTC	
VIII671	AAACCCCTCCGTTTAGAGAGGGGTTATGCTAGTTACC GGTTATTATTATTTTTGACACC	
IV436	GGGGCCGAATTCGCCAGCGGCATTAG	<i>EcoRI</i>
IV438	GGGGCCGAATTCGGACTCGACCAAGCTACTTAC	<i>EcoRI</i>
IV527	TTGCTGACTCCGATTATTCG	
IV528	GAAGACGACCGCGCCCAAC	
IV529	CGCGACTCAGCAAGAAGAG	
IV530	GCCGATGTCTGGGCGCAG	
IV706	GGGCGCCATATGATGACAATAAATATCAAGACAG	<i>NdeI</i>
IV708	GGGCGC <u>ACTAGTGTGATGCAACGCTGCTAGATG</u>	<i>SpeI</i>
IV783	GCGCCCCATGGATGCTTATTCTGACTCG	<i>NcoI</i>
V066	GGGCGCGTAATACGACTCACTATAGGAATGTAATGGCT TACGTTTTTC	
V631	GTTTCCCCGCCATTATCC	
V659	GCGGCGGAGCTCGTGATTTATTATATTGGTTTTGGTTG	<i>SacI</i>
V700	GGGCGCGTAATACGACTCACTATAGCAACAATACCGTG AAATGC	
V708	GGGCGCGTAATACGACTCACTATAGAACAGAGAGACC CGACTC	
V731	GGGCGCGTAATACGACTCACTATAGGGTGATTTATTAT ATTGGTTTTGGTTG	
V732	CTCTAGTGATGCCATAAATGTTATAC	
V830	GGGCGCGTAATACGACTCACTATAGCTTACATTTTATAT GAATGTAATGGC	
V831	CATACACCGTGAACCGTAAG	
V832	GGGCGCGTAATACGACTCACTATAGCACGGTGTATGAT GGATAATG	
VI085	CGGAGTCAGCAAATTGTACC	
VI386	GCGCGTAATACGACTCACTATAGGGCGGGAAACAGA GAG	
VI564	GGCTAATACGACTCACTATAGGGCTTACCGAAATAATG CG	
VI565	CATTCTTTTCATCTTTAACTTACTCG	
VI950	GCGCGTAATACGACTCACTATAGGTTTCGCGCGGCTAAT GAGAG	
VIII672	GCGCCGGATCCCGAACGTTTAGGTCTGCGTC	<i>BamHI</i>
VIII673	GCGCCGTGACCTAATGACAATCCTTAACTTTC	<i>SalI</i>
VIII674	GCGCCGTGACGTATGTTGACCATACTGGAAT	<i>SalI</i>

VIII675	<u>GCGCCGGATCCGTTATCGATCCGCTGACCG</u>	<i>Bam</i> HI
VIII676	<u>GCGCCGTCGACGTTTATTATTCCACAAGTCTCTTGG</u>	<i>Sal</i> I
VIII677	<u>GCGCCGTCGACCGCAACTCTTCTTGCTGAG</u>	<i>Sal</i> I
qRT-PCR Primer		
III393	CCGACGTAAAGCCGCGATAC	<i>sopB</i> (fw)
III394	CCTCGTTCATAAGCACTCGTC	<i>sopB</i> (rev)
IV529	CGCGACTCAGCAAGAAGAG	<i>rne</i> (fw)
IV530	GCCGATGTCTGGGCGCAG	<i>rne</i> (rev)
IV931	CGCCCAGACGCGCTTCGGCC	<i>pnp</i> (rev)
IV946	GTTGGGCGCGGTCGTCTTCGG	<i>pnp</i> (fw)
V89	CAGGGACTGCTTAGGACGAG	<i>csrB</i> (fw)
V90	CCTGCTCAATCCCTGAAAAC	<i>csrB</i> (rev)
VI98	GGAGCACAGTTACTCAGGATGAG	<i>csrC</i> (fw)
VI99	GGCGATTGCCCCGGCGCTC	<i>csrC</i> (rev)

Underlined – restriction sites

Dotted underlined – T7 promoter

Bold – sequences homologous to kanamycin resistance gene (pKD4)

Bold and underlined – terminator sequence