Electronic Supplementary Material (ESI) for Metallomics.

SUPPORTING INFORMATION FOR

Zinc transporters YbtX and ZnuABC are required for the virulence of *Yersinia pestis* in bubonic and pneumonic plague in mice

by

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Supplemental Materials and Methods

The λ red recombinase method was used to inactivate *y3406* or *fadD (y2236)* in a *ybtS::kan* Δ*znuBC* mutant and *zntA (y0410)* in KIM6+. The PCR products for replacement of *fadD* and *y3406* with a *cam* cassette from pKD3 in the double *ybtS::kan* Δ*znuBC* mutant *(*KIM6-2070.3) carrying pWL204 were prepared using primer pairs Δfad5'/Δfad3', and y3406red-1/y3406red-2, respectively (Table S1). The presence of mutations in the resulting strains, KIM6-2070.5 and KIM6-2070.4, was verified by PCR using fadD C-3' and Cm-Alex-1, or y3406-R and Cm-Alex-1, respectively. These strains were cured of pWL204 by growth on TBAS plates.

Table S1. Bacterial strains, plasmids and primers used in this study.

Strains	**Relevant characteristics[b]**	**Reference or source**
***E. coli* strains**		
DH5□	Cloning strain	1
DH5□ *λpir*	Cloning strain for propagating plasmids with R6K origins; derived from DH5□	S.C. Straley
***Y. pestis* strains[a]**		
KIM5(pCD1Ap)+	Ap^r Hms^+ Ybt^+ Lcr^+; pMT1, pCD1Ap, pPCP1	2
KIM5-2067 (pCD1Ap)+	Ap^r Hms^+ Ybt^+ $YbtX^-$ (in frame *ΔybtX2067*) Lcr^+; pMT1, pPCP1; pCD1Ap electroporated into KIM6-2067	This study
KIM5-2070.1 (pCD1Ap)	Km^r Hms^+ Ybt^- (*ybtS::kan2070.1*) Lcr^+; pMT1, pPCP1; pCD1Ap electroporated into KIM6-2070.1	This study
KIM5-2070.3 (pCD1Ap)+	Ap^r Km^r Hms^+ Ybt^- $YbtS^-$(*ybtS::kan2070.1)* Znu^-	This study

	(*ΔznuBC2077*) Lcr$^+$; pMT1, pPCP1; pCD1Ap electroporated into KIM6-2070.3	
KIM5-2196.4 (pCD1Ap)+	Ap^r Km^r Hms$^+$ Ybt$^+$ ZntA$^-$ (*ΔzntA2196)* Zur$^-$ (*zur::kan2078*) Lcr$^+$; pMT1, pPCP1; pCD1Ap electroporated into KIM6-2196.4+	This study
KIM5-2197 (pCD1Ap)+	Ap^r Pgm$^+$ Znu$^-$ (in frame *ΔznuA2197*) Lcr$^+$; pMT1, pCD1Ap (*yadA::bla*), pPCP1; pCD1Ap electroporated into KIM6-2197+	3
KIM5-2197.2 (pCD1Ap)+	Ap^r Hms$^+$ Ybt$^+$ YbtX$^-$ (in frame *ΔybtX2067*) Znu$^-$ (*ΔznuA2197)* Lcr$^+$; pMT1, pPCP1; pCD1Ap electroporated into KIM6-2197.2	This study
KIM5-2197.4 (pCD1Ap)+	Ap^r Hms$^+$ Ybt$^+$ (*ybtX^{rp}*) Znu$^-$ (*ΔznuA2197*); in frame *ΔybtX2067* repaired in KIM5-2197.2 by replacement with *ybtX*$^+$; Lcr$^+$; pMT1, pPCP1; pSR47s-YbtX was electroporated into KIM5-2197.2 (pCD1Ap)+	This study
KIM6+	Hms$^+$ Ybt$^+$ Lcr$^-$; pMT1, pPCP1	4
KIM6-2046.1	Km^r Hms$^+$ Ybt$^-$(*irp2::kan2046.1*) Lcr$^-$; pMT1, pPCP1	5
KIM6-2046.7	Cm$^r{}_{15}$ Hms$^+$ Ybt$^-$ (*irp2ΔS52*) *Δy2360::cam2093* Lcr$^-$; pMT1, pPCP1	6
KIM6-2046.9	Cm$^r{}_{15}$ Hms$^+$ Ybt$^-$ (*irp2ΔS52*) Znu$^-$ (*ΔznuA2197)* *Δy2360::cam2093* Lcr$^-$; pMT1, pPCP1; pKNG-ΔZnuA electroporated into KIM6-2046.7	This study
KIM6-2056.1	Hms$^+$ Ybt$^-$ (in-frame *ΔybtE2056.1*) Lcr$^-$; pMT1, pPCP1	7
KIM6-2056.2	Hms$^+$ Ybt$^-$ (in-frame *ΔybtE2056.1*) Znu$^-$ (*ΔznuA2197)* Lcr$^-$; pMT1, pPCP1; pKNG-ΔZnuA electroporated into KIM6-2056.1	This study
KIM6-2067	Hms$^+$ Ybt$^+$ YbtX$^-$ (in frame *ΔybtX2067*) Lcr$^-$; pMT1, pPCP1	8
KIM6-2070.1	Km^r Hms$^+$ Ybt$^-$ (*ybtS::kan2070.1*) Lcr$^-$; pMT1, pPCP1	9
KIM6-2070.3	Km^r Hms$^+$ Ybt$^-$ (*ybtS::kan2070.1*) Znu$^-$ (*ΔznuBC2077*) Lcr$^-$; pMT1, pPCP1; pSucZnu3.5 electroporated into KIM6-2070.1	This study
KIM6-2070.4	Cm$^r{}_{15}$ Km^r Hms$^+$ Ybt$^-$ (*ybtS::kan2070.1)* Znu$^-$ (*ΔznuBC2077*) *y3406*$^-$ (*Δy3406::cam2201*) Lcr$^-$; pMT1, pPCP1; derived from KIM6-2070.3 (pWL204)	This study
KIM6-2070.5	Cm^r Km^r Hms$^+$ Ybt$^-$ (*ybtS::kan2070.1*) Znu$^-$ (*ΔznuBC2077*) FadD$^-$ (*Δy2236::cam2200)* Lcr$^-$; pMT1, pPCP1; derived from KIM6-2070.3 (pWL204)	This study
KIM6-2077+	Hms$^+$ Ybt$^+$ Znu$^-$ (*ΔznuBC2077*) Lcr$^-$; pMT1, pPCP1	10
KIM6-2077.7	Km^r Hms$^+$ Ybt$^-$ (*irp2::kan2046.3*) Znu$^-$ (*ΔznuBC2077*) Lcr$^-$; pMT1, pPCP1	3

KIM6-2077.8	Km^r Hms^+ Ybt^- (Δ*irp2-2046.3*) Znu^- (*ΔznuBC2077*) Lcr^-; pMT1, pPCP1	3
KIM6-2077.18	Km^r Hms^+ Ybt^- (*irp2::kan2046.1*) Psn^- (in-frame *Δpsn2045.1*) Znu^- (*ΔznuBC2077*) Lcr^-; pMT1, pPCP1	3
KIM6-2078+	Km^r Pgm^+ Zur^- (*zur::kan2078*) Lcr^-; pMT1, pPCP1; derived from KIM6+	11
KIM6-2196.1+	Hms^+ Ybt^+ $ZntA^-$ *(ΔzntA2196.1)* Lcr^-; pMT1, pPCP1; derived from KIM6+	This study
KIM6-2196.4+	Km^r Hms^+ Ybt^+ $ZntA^-$ (*ΔzntA2196.1)* Zur^- (*zur::kan2078*) Lcr^-; pMT1, pPCP1; pZur4 electroporated into KIM6-2196.1+ and second cross recovered	This study
KIM6-2197+	Hms^+ Ybt^+ Znu^- (in frame *ΔznuA2197*) Lcr-; pMT1, pPCP1	3
KIM6-2197.1	Hms^+ Ybt^- (in frame *Δirp2-2046.3*) Znu^- (in frame *ΔznuA2197*) Lcr-; pMT1, pPCP1	3
KIM6-2197.2	Hms^+ Ybt^+ $YbtX^-$ (in frame *ΔybtX2067*) Znu^- (*ΔznuA2197)* Lcr^-; pMT1, pPCP1	3
KIM6-2197.4+	Hms^+ Ybt^+ Znu^- (*ΔznuA2197*) $YbtX^+$ (in frame *ΔybtX2067* restored by introducing *ybtX*) Lcr^-; pMT1, pPCP1; pKNG-YbtX electroporated into KIM6-2197.2 and second cross recovered	This study
KIM6-2202.1+	Km^r Hms^+ Ybt^+ $Y3657^-$ (*Δy3657::kan2202*) Znu^- (*ΔznuBC2077*); *Δy3657::kan2202* introduced into KIM6-2077+	This study
KIM10+	Hms^+ Ybt^+ Lcr^-; pMT1; KIM6+ cured of pPCP1	12, 13
Plasmids	**Relevant characteristics**	**Reference or source**
pACYC184	4.24 kb, Cm^r, Tc^r, low copy cloning vector	1
pCD1Ap	71.7 kb, Ap^r, Lcr^+; pCD1 with *bla* cassette inserted into *'yadA* downstream of the frameshift mutation in this pseudogene (*'yadA::bla*)	2
pKD3	2.8 kb, Ap^r, Cm^r, template plasmid	14
pKD4	3.3 kb, Ap^r, Km^r, template plasmid	14
pKNG101	6.8 kb, Sm^r, *ori*R6K $SacB^+$ suicide vector	15
pKNG-ΔznuA	8.3 kb, Sm^r, *ori*R6K $SacB^+$, *ΔznuA2197*; suicide vector;	3
pKNG-YbtX-comp	9.24 kb, Sm^r; 2.67 kb ApaI-BamHI fragment was ligated into ApaI and BamHI sites of pKNG101 suicide vector.	This study
pQE30	3.5 kb, Ap^r, expression vector for IPTG-inducible His-tagged proteins	Qiagen
pQEYbtA	4.2 kb, Ap^r, expression vector for for IPTG-inducible YbtA-6xHis	16
pSkippy	5.4 kb, Ap^r, $SacB^+$; expresses FLP recombinase	17

pSR47s	6.58 kb, Km^r *oriT*RP4 *ori*R6K SacB$^+$; suicide vector	18
pSR-YbtX-comp	8.2 kb, Km^r; 2.66 kb XhoI-BamHI fragment was ligated into SalI and BamHI sites of pSR47s suicide vector.	This study
pSucZnu3.5	12.2 kb, Ap^r, *ΔznuBC2077* SacB$^+$, R6K ori; in suicide vector pSUC1	10
pYbtX	5.5 kb, Cm^r; *ybtX* expressed from native promoter in pACYC184	3
pYbtX-ZP	5.421 kb, Cm^r ,*ybtX*$^+$ with ZnuA promoter cloned into pACYC184	This study
pWL204	8.2 kb, Ap^r Sac^s λ-red recombinase helper plasmid containing *sacB*$^+$	19
pWSK29 5.4 Kb; Apr, low-copy-number cloning vector	5.4 Kb, Ap^r, low-copy-number cloning vector	20
pWSK-YbtX-comp	8.01 kb, Ap^r; 2.63 kb Y. pestis KIM6+ genomic region containing *ybtX* was ligated into HindIII and SmaI sites of pWSK29	This study
pZur4	7.5 kb, Ap^r, *zur::kan2078,* R6K *ori* suicide vector	11
Primer name	**Primer sequence (5'→3')**	**Purpose**
Δfad3'	TTTACCCACATTCGACTTCGGCAATTCGTCACGGAACTCCATATGAATATCCTCCTTAGT	construct *ΔfadD*
Δfad5'	CCCGCAGAGATTGATCCGGACCGCTATTCATCTTTGATAGTGTAGGCTGGAGCTGCTTC	construct *ΔfadD*
Cm-Alex-1	AATATCCAGCTGAACGGTCTG	confirm *Δy3406::cam ΔfadD::cam*
Cm-2	GAGATTTTCAGGAGCTAAGG	confirm *Δy0410::cam*
fadD-3'C	TTAAGCTGCGTCTGTCGTAC	confirm *ΔfadD::cam*
KM-1	ACTGGGCTATCTGGACAAGG	confirm *Δy3657::kan*
P27	TGCATGAGTGATGTTCAG	sequencing *ybtX* to confirm restoration of *ybtX*$^+$
P33	GCGAAATGGACTGGACAA	sequencing *ybtX* to confirm restoration of *ybtX*$^+$
pPQX-vector-2100	AGGAAGCAGCCCAGTAGTAG	sequencing *ybtX*

pPQX-vector-1300	CAGTTACCTCGGTTCAAAGAG	sequencing *ybtX*
Y0410red-1	CAACGTTAAGGGACATGGGCATATACCTGTTACATCC TGCATAGGGAGCGTGTAGGCTGGAGCTGCTTC	construct *Δy0410::cam*
Y0410red-2	CATTAGCCTATTCAAACATTGACCTAATCAAACATCTC GGACCTAATCACATATGAATATCCTCCTTAGT	construct *Δy0410::cam*
Y0410-pBADF	GGGCATATACCTGTTACATCCT	confirm *Δy0410::cam*
Y3406red-1	TCGTTGATTTATCAAAAGTGATCTATATGCAACTACAG GTAATGGTGTAGGCTGGAGCTGCTTC	construct *Δy3406::cam*
Y3406red-2	TCGTTCCGGTAAAGCTAAAAGCATTGATGGCGACAC GTTTGATTACATATGAATATCCTCCTTAGT	construct *Δy3406::cam*
Y3406-R	CCGTAGGCTTTCTCAACA	confirm *Δy3406::cam*
Y3657red-F	CATTGGCACAGTTCTCATGTCCTAAGAATCTCTAACC GCGAAATAGTGTAGGCTGGAGCTGCTTC	construct *Δy3657::kan*
Y3657red-R	CATTCCCCCGGATATTTTTTTGTCTGACGACTGCCCG CTACATTTTCATATGAATATCCTCCTTAGTTC	construct *Δy3657::kan*
Y3657 del	GGGGTTTGTCAGCAGTCTGA	confirm *Δy3657::kan*
YbtX-comp-Rev	GTGCGTTCTGCGTCGTTG	cloning *ybtX* into pWSK29
YbtX-comp-HindIII	GGAAAAGCTTGAGCGGTTTATGGCGATAG	cloning *ybtX* into pWSK29
ybtX-compl_R6K_R-SpeI	TATACTAGTCACTCTTCTTCTGCGAACTGG	cloning *ybtX* under *znuA* promoter
ZnuAprom-ybtX-BamHI	CAAAGGATCCATCATGATTATATTYGCAGAATCTAGT GAATGTTATAATATTACGCTTTACCCATCAAAACATTC TGGTGATGGAAGAGG	cloning *ybtX* under *znuA* promoter

[a] A plus sign (+) indicates an intact chromosomal 102-kb *pgm* locus. All other *Y. pestis* strains have a mutation within this locus or a deletion of the entire locus (*Δpgm*). Genes encoding for the synthesis and transport of Ybt as well as for transcriptional regulation of *ybt* genes are encoded within the *pgm* locus.

[b] Ap^r, Cm^r Km^r, $Sm^r$and Tc^r indicate resistance to ampicillin, chloramphenicol, kanamycin, streptomycin and tetracycline, respectively.

Supplemental Figures

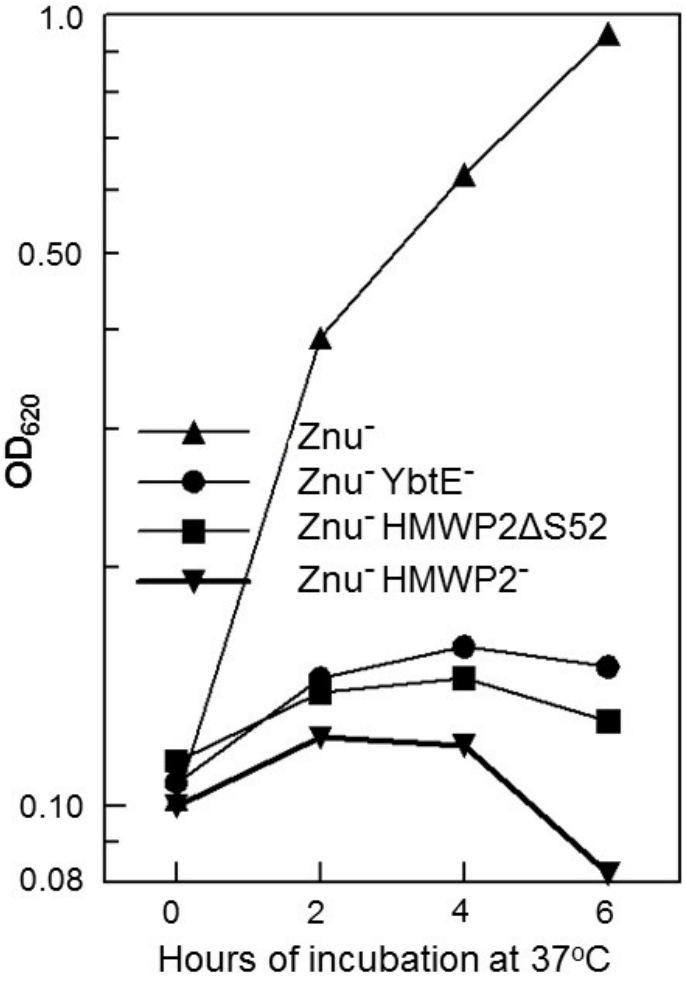

Fig. S1. Salicyl-AMP ligase YbtE and loading site for salicylate (Ser-52) onto the HMWP2 synthase are required for growth of the *znu* mutant under low Zn conditions. Growth rates of *Y. pestis* KIM6-2077+ (Znu$^-$; Δ*znuBC*), KIM6-2056.2 (YbtE$^-$ Znu$^-$; Δ*ybtE* Δ*znuBC*), KIM6-2046.9 (HMWP2ΔS52 Znu$^-$; *irp2*Δ*S52* Δ*znuA*) and KIM6-2077.7 (HMWP2$^-$ Znu$^-$; *irp2::kan* Δ*znuBC*). in **c**PMH2 supplemented with 0.6 μM $ZnCl_2$ and 1 μM $FeCl_3$. The growth curves shown are representative of two independent studies.

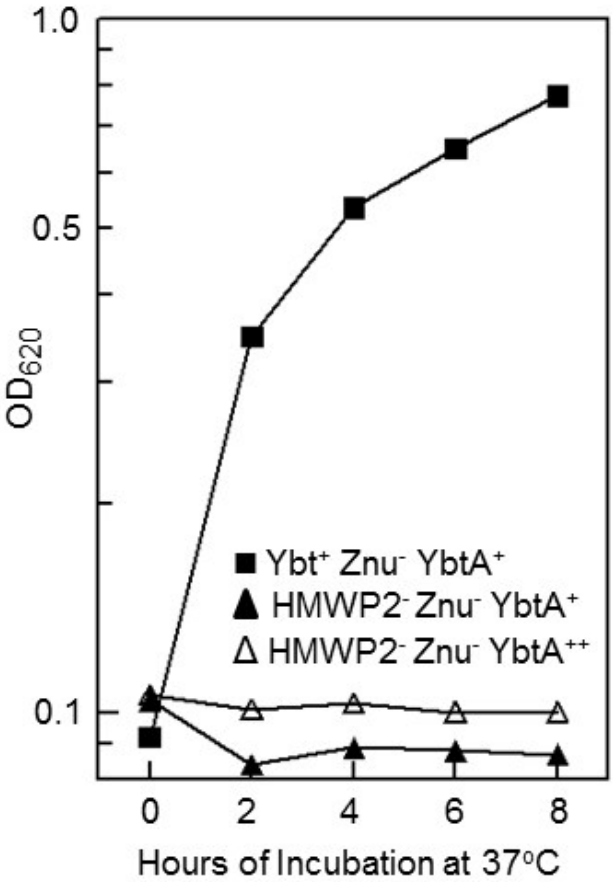

Fig. S2. Overexpression of the transcriptional regulator YbtA does not complement the severe growth defect of the *Y. pestis* Irp2$^-$ Znu$^-$ (*irp2 Δznu*) mutant under low Zn conditions. Growth rates of *Y. pestis* KIM6-2077(pQE30) (HMWP2$^+$ Znu$^-$ YbtA$^+$; Δ*znuBC*); KIM6-2077.8(pQE30) (HMWP2$^-$ Znu$^-$ YbtA$^+$; Δ*irp2* Δ*znuBC*); and KIM6-2077.8(pQEYbtA) (Irp2$^-$ Znu$^-$ YbtA^{++}Δ*irp2* Δ*znuBC ybtA*$^+$ overexpressed) in Chelex-100-treated PMH2 (cPMH2) supplemented with 0.6 μM $ZnCl_2$, 1.0 μM $FeCl_3$ and 1.0 mM IPTG. An Irp2- mutant cannot synthesis the Ybt siderophore and expresses reduced levels of *irp-psn-ybt* genes. Expression

plasmid pQEYbtA has *ybtA*$^{+}$ cloned behind the IPTG-inducible promoter in pQE30. The growth curves shown are representative of two independent studies.

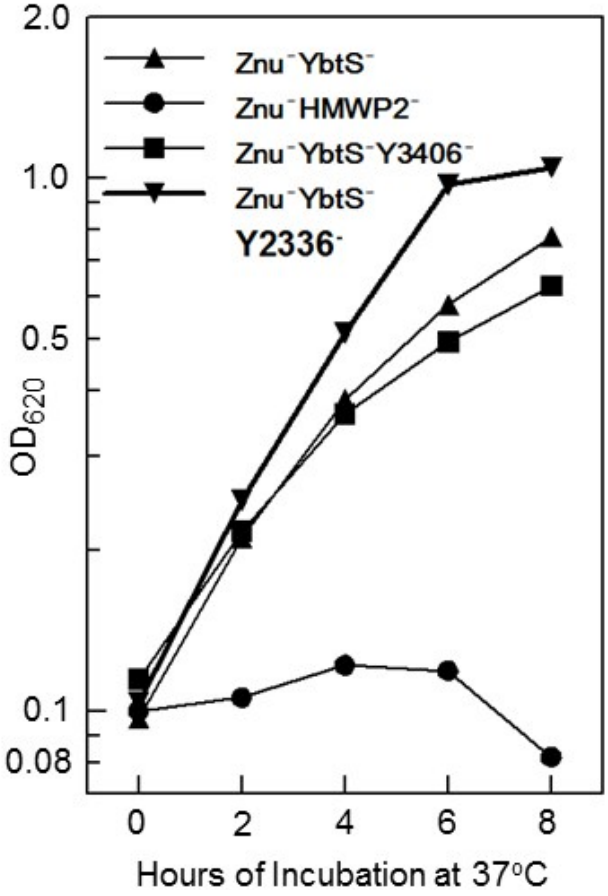

Fig. S3. Y3406 and Y2336 are not required for growth of the *znu YbtS* double mutant under low Zn conditions. Growth rates of *Y. pestis* KIM6-2070.3 (YbtS^{-} Znu^{-}; *ybtS::kan* Δ*znuBC*), KIM6-2077.7 (HMWP2^{-} Znu^{-}; *irp2::kan* Δ*znuBC*), KIM6-2070.4 (Y3406^{-} YbtS^{-} Znu^{-}; Δ*y3406::cam ybtS::kan* Δ*znuBC*) and KIM6-2070.5 (Y2236^{-} YbtS^{-} Znu^{-}; Δ*y2236::cam ybtS::kan* Δ*znuBC*). in **c**PMH2 supplemented with 0.6 μM $ZnCl_2$ and 1 μM $FeCl_3$. The growth curves shown are representative of two independent studies.

References

1. F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl, eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N. Y., 1987.
2. S. Gong, S. W. Bearden, V. A. Geoffroy, J. D. Fetherston and R. D. Perry, Characterization of the *Yersinia pestis* Yfu ABC iron transport system, *Infect. Immun.*, 2001, **67**, 2829-2837.
3. A. G. Bobrov, O. Kirillina, J. D. Fetherston, M. C. Miller, J. A. Burlison and R. D. Perry, The *Yersinia pestis* siderophore, yersiniabactin, and the ZnuABC system both contribute to Zinc acquisition and the development of lethal septicemic plague in mice, *Mol. Microbiol.*, 2014, **93**, 759-775.
4. J. D. Fetherston, P. Schuetze and R. D. Perry, Loss of the pigmentation phenotype in *Yersinia pestis* is due to the spontaneous deletion of 102 kb of chromosomal DNA which is flanked by a repetitive element, *Mol. Microbiol.*, 1992, **6**, 2693-2704.
5. J. D. Fetherston, J. W. Lillard, Jr. and R. D. Perry, Analysis of the pesticin receptor from *Yersinia pestis*: role in iron-deficient growth and possible regulation by its siderophore, *J. Bacteriol.*, 1995, **177**, 1824-1833.
6. M. C. Miller, J. D. Fetherston, C. L. Pickett, A. G. Bobrov, R. H. Weaver, E. DeMoll and R. D. Perry, Reduced synthesis of the Ybt siderophore or production of aberrant Ybt-like molecules activates transcription of yersiniabactin genes in *Yersinia* pestis *Microbiology*, 2010, **156**, 2226-2238.

7. S. W. Bearden, J. D. Fetherston and R. D. Perry, Genetic organization of the yersiniabactin biosynthetic region and construction of avirulent mutants in *Yersinia pestis*, *Infect. Immun.*, 1997, **65**, 1659-1668.
8. J. D. Fetherston, V. J. Bertolino and R. D. Perry, YbtP and YbtQ: two ABC transporters required for iron uptake in *Yersinia pestis*, *Mol. Microbiol.*, 1999, **32**, 289-299.
9. A. M. Gehring, E. DeMoll, J. D. Fetherston, I. Mori, G. F. Mayhew, F. R. Blattner, C. T. Walsh and R. D. Perry, Iron acquisition in plague: modular logic in enzymatic biogenesis of yersiniabactin by *Yersinia pestis*, *Chem. Biol.*, 1998, **5**, 573-586.
10. K. R. O. Hazlett, F. Rusnak, D. G. Kehres, S. W. Bearden, C. J. La Vake, M. E. La Vake, M. E. Maguire, R. D. Perry and J. D. Radolf, The *Treponema pallidum tro* operon encodes a multiple metal transporter, a zinc-dependent transcriptional repressor, and a semi-autonomously expressed phosphoglycerate mutase, *J. Biol. Chem.*, 2003, **278**, 20687-20694.
11. D. C. Desrosiers, S. W. Bearden, I. Mier, Jr, , J. Abney, J. T. Paulley, J. D. Fetherston, J. C. Salazar, J. D. Radolf and R. D. Perry, Znu is the predominant zinc importer in *Yersinia pestis* during *in vitro* growth but is not essential for virulence, *Infect. Immun.*, 2010, **78**, 5163-5177.
12. W. Deng, V. Burland, G. Plunkett, III, A. Boutin, G. F. Mayhew, P. Liss, N. T. Perna, D. J. Rose, B. Mau, S. Zhou, D. C. Schwartz, J. D. Fetherston, L. E. Lindler, R. R. Brubaker, G. V. Plano, S. C. Straley, K. A. McDonough, M. L. Nilles, J. S. Matson, F. R. Blattner and R. D. Perry, Genome sequence of *Yersinia pestis* KIM, *J. Bacteriol.*, 2002, **184**, 4601-4611.
13. R. D. Perry, M. L. Pendrak and P. Schuetze, Identification and cloning of a hemin storage locus involved in the pigmentation phenotype of *Yersinia pestis*, *J. Bacteriol.*, 1990, **172**, 5929-5937.
14. K. A. Datsenko and B. L. Wanner, One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 6640-6645.
15. K. Kaniga, I. Delor and G. R. Cornelis, A wide-host-range suicide vector for improving reverse genetics in Gram-negative bacteria: inactivation of the *blaA* gene of *Yersinia enterocolitica*, *Gene*, 1991, **109**, 137-141.
16. R. D. Perry, J. Abney, I. Mier, Jr., Y. Lee, S. W. Bearden and J. D. Fetherston, Regulation of the *Yersinia pestis* Yfe and Ybt iron transport systems, *Adv. Exp. Med. Biol.*, 2003, **529**, 275-283.
17. P. A. Price, J. Jin and W. E. Goldman, Pulmonary infection by *Yersinia pestis* rapidly establishes a permissive environment for microbial proliferation, *Proc. Natl. Acad. Sci USA*, 2012, **109**, 3083-3088.
18. H. L. Andrews, J. P. Vogel and R. R. Isberg, Identification of Linked *Legionella pneumophila* genes essential for intracellular growth and evasion of the endocytic pathway, *Infect. Immun.*, 1998, **66**, 950-958.
19. W. W. Lathem, P. A. Price, V. L. Miller and W. E. Goldman, A plasminogen-activating protease specifically controls the development of primary pneumonic plague, *Science*, 2007, **315**, 509-513.
20. R. F. Wang and S. R. Kushner, Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*, *Gene*, 1991, **100**, 195-199.