

1 **Supplementary information**

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3 **Enzyme IIA^{Ntr} regulates *Salmonella* invasion via 1,2-propanediol and propionate**
4 **catabolism**

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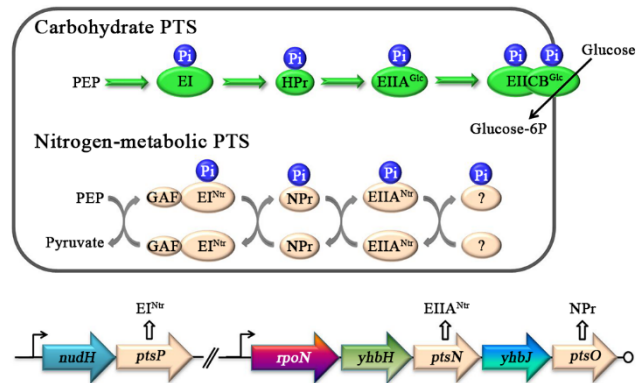
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20 **Figure S1. Organization of the nitrogen-PTS in *Salmonella Typhimurium*.** The nitrogen-

21 PTS catalyzes a cascade of phosphoryl-transfer reactions. First, EI^{Ntr} autophosphorylates with

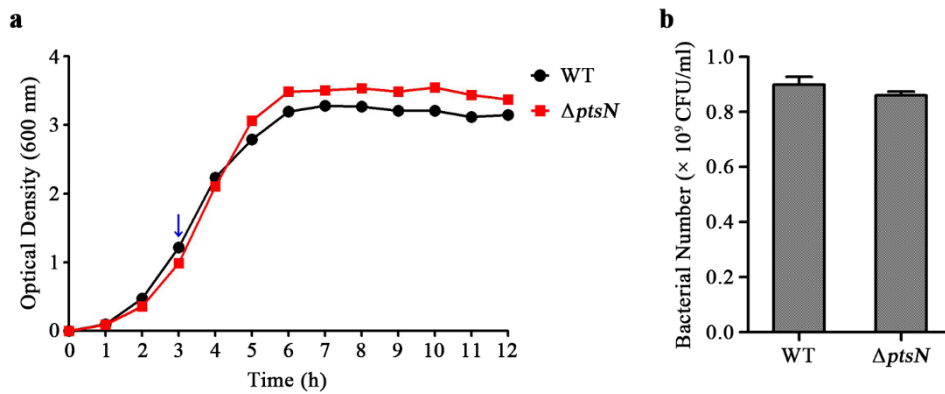
22 phosphoenolpyruvate and subsequently transfers the phosphoryl group to NPr, which then

23 phosphorylates EIIA^{Ntr}. The final acceptor for the phosphoryl groups of EIIA^{Ntr} remains

24 unknown. NPr and EIIA^{Ntr} are encoded in the *rpoN* operon. EI^{Ntr} is encoded elsewhere on the

25 chromosome in the *nudH-ptsP* operon.

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27

28 **Figure S2. No significant difference was found in bacterial growth rate or viability**

29 **between *Salmonella* Typhimurium SL1344 wild-type and the $\Delta ptsN$ mutant strain. (A)**

30 Overnight cultures of *Salmonella* Typhimurium SL1344 wild-type and $\Delta ptsN$ mutant strain

31 were harvested at $13,000 \times g$ for 2 min, and the cells were resuspended in fresh LB medium

32 and incubated at $37^\circ C$ with shaking. The OD₆₀₀ value was measured every 1 h to draw and

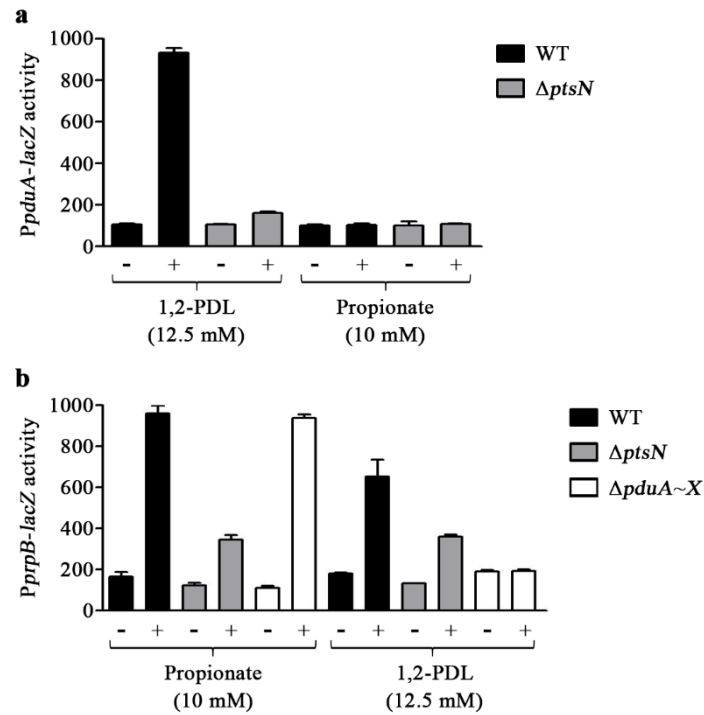
33 compare the growth curve. This experiment was performed in triplicate. (B) To compare the

34 bacterial cell viability between the wild-type and $\Delta ptsN$ mutant strains, cultures were collected

35 at 3 h (blue arrow, Fig. S3A) and then serially diluted in PBS. Dilutions of the suspension were

36 plated on LB agar medium to enumerate the CFU. This experiment was performed in triplicate.

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39 **Figure S3. The effects of *ptsN* on the expression of *pduA* and *prpB* genes in the presence**

40 **of 1,2-PDL or propionate. (A) The effects of *ptsN* mutation on the *pduA* gene measured by a**
 41 **β -galactosidase assay using a *PpduA-lacZ* reporter in the wild-type and $\Delta ptsN$ mutant strains.**

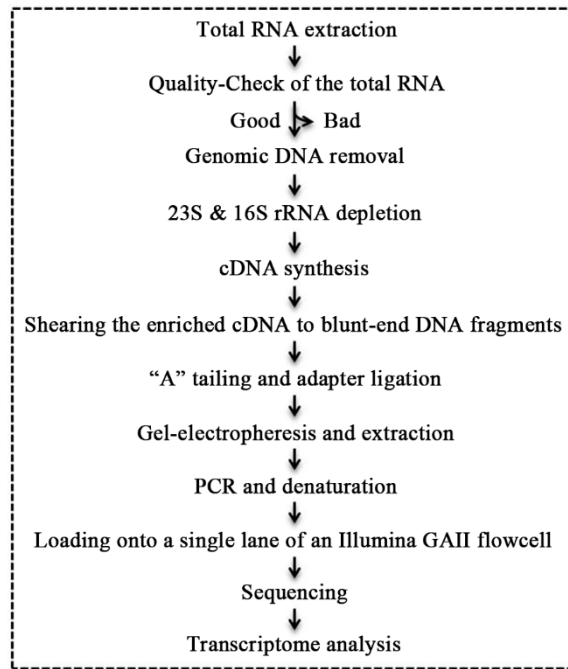
42 These strains were cultivated in LB medium in the presence or absence of either 1,2-PDL or

43 propionate. (B) Transcriptional expression of the *prpB* gene, also measured by a β -

44 galactosidase assay using a *PprpB-lacZ* reporter in the wild-type, $\Delta ptsN$, and $\Delta pduA\sim X$

45 strains. These were grown in LB medium in the presence or absence of either 1,2-PDL or

46 propionate.



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48 **Figure S4. The whole process of RNA sequencing.** After the quality-check of total RNA,

49 rRNA was depleted, and mRNA was converted into cDNA. The synthesized cDNA was

50 sheared to blunt-ends and ligated with adapter containing primer sites for sequencing. Adequate

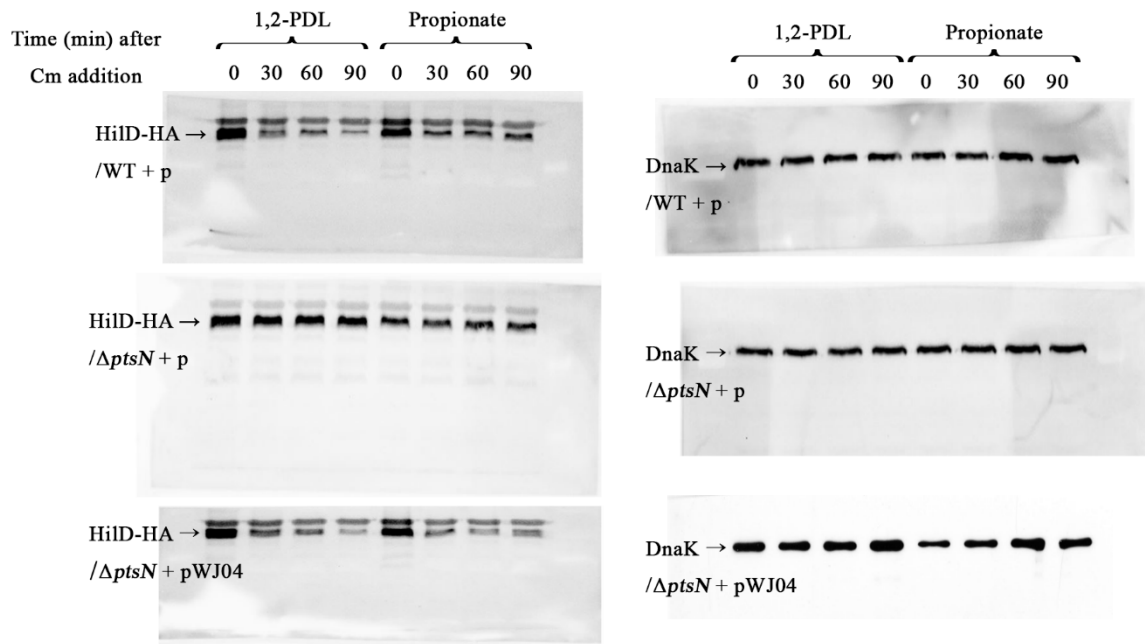
51 cDNA fragments were selected via gel-electrophoresis and then denatured to form a single

52 strand after PCR. The completed samples were loaded onto a single lane of an Illumina GAII

53 flow cell and sequenced. Finally, transcriptome analysis was conducted.

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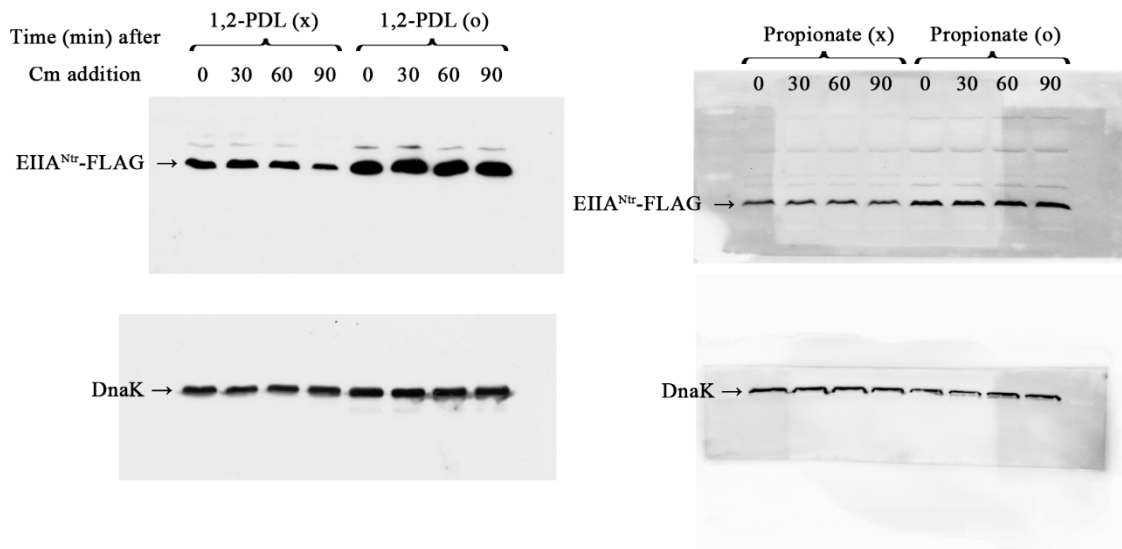
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57 **Figure S5. Full-length blots of Figure 3d and 3e.** Equivalent amounts of proteins were
 58 subjected to SDS-PAGE analysis and transferred to PVDF membranes. PVDF membranes
 59 were cut into two pieces to detect HiID-HA and DnaK, respectively. The blots of target
 60 proteins were indicated with an arrow.

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62

63 **Figure S6. Full-length blots of Figure 6c and 6d.** Equivalent amounts of proteins were
 64 subjected to SDS-PAGE analysis and transferred to PVDF membranes. PVDF membranes were
 65 cut into two pieces to detect EIIA^{Ntr}-FLAG and DnaK, respectively. The blots of target proteins
 66 were indicated with an arrow.

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TABLE S1 Summary of RNA-seq and data analysis results.				
	wild-type		<i>ΔptsN</i>	
	number	% of total	number	% of total
Total number of reads	22,388,642	100	14,255,406	100
Mapped reads	22,293,048	99.6	14,163,120	99.4
-uniquely mapped	21,285,343	95.1	13,820,149	97.0
-non-specifically mapped	1,007,705	4.5	342,971	2.4
Unmapped reads	95,594	0.4	92,286	0.6

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TABLE S2 Genes specifically regulated by *ptsN*.

Category	Gene	Fold expression	Function
Propionate catabolism	<i>prpR</i>	-1.95	propionate catabolism operon regulatory protein
	<i>prpB</i>	-1.86	carboxyvinyl-carboxyphosphonate phosphorylmutase
	<i>prpC</i>	-0.68	methylcitrate synthase
	<i>prpD</i>	-2.82	PrpD protein
	<i>prpE</i>	-1.14	PrpE protein
Vitamin B ₁₂ synthesis	<i>cobC</i>	0.18	alpha-ribazole-5'-phosphate phosphatase
	<i>cobD</i>	-0.80	putative aminotransferase CobD
	<i>cobB</i>	0.16	NAD-dependent deacetylase
	<i>cobT</i>	-1.37	nicotinate-nucleotide-dimethylbenzimidazole phosphoribosyltransferase
	<i>cobS</i>	-0.91	cobalamin (5'-phosphate) synthase
	<i>cobU</i>	-0.37	cobinamide kinase and guanylyltransferase
	<i>cbiP</i>	-1.18	putative cobyrinic acid synthase
	<i>cbiO</i>	-0.88	putative cobalt transport ATP-binding protein
	<i>cbiQ</i>	-0.79	putative cobalt transport protein
	<i>cbiN</i>	-0.34	putative cobalt transport protein CbiN
	<i>cbiM</i>	-0.43	cobalamin biosynthesis protein
	<i>cbiL</i>	-0.81	cobalt-precorrin-2 C(20)-methyltransferase

	<i>cbiK</i>	-0.41	sirohydrochlorin cobaltochelataase
	<i>cbiJ</i>	-0.78	cobalt-precorrin-6a reductase
	<i>cbiH</i>	-0.85	precorrin-3 C17-methyltransferase
	<i>cbiG</i>	-0.88	cobalamin biosynthesis protein
	<i>cbiF</i>	-0.84	precorrin-4 C(11)-methyltransferase
	<i>cbiT</i>	-0.68	precorrin-8W decarboxylase
	<i>cbiE</i>	-0.63	precorrin-6Y C5,15-methyltransferase
	<i>cbiD</i>	-1.04	cobalt-precorrin-6A synthase
	<i>cbiC</i>	-1.41	precorrin-8X methylmutase
	<i>cbiB</i>	-0.76	cobalamin biosynthesis protein
	<i>cbiA</i>	-0.62	cobyrinic acid A,C-diamide synthase
1,2-PDL utilization	<i>pocR</i>	-0.25	pdu/cob regulatory protein
	<i>pduF</i>	-1.97	putative propanediol diffusion facilitator
	<i>pduA</i>	-2.43	putative propanediol utilization protein
	<i>pduB</i>	-3.86	putative propanediol utilization protein
	<i>pduC</i>	-3.57	propanediol dehydratase large subunit
	<i>pduD</i>	-4.03	propanediol dehydratase medium subunit
	<i>pduE</i>	-3.86	propanediol dehydratase small subunit
	<i>pduG</i>	-4.34	putative propanediol utilization
	<i>pduH</i>	-4.58	putative propanediol utilization
	<i>pduJ</i>	-3.68	putative propanediol utilization
	<i>pduK</i>	-3.70	putative propanediol utilization

	<i>pduL</i>	-4.07	putative propanediol utilization
	<i>pduM</i>	-3.76	putative propanediol utilization
	<i>pduN</i>	-5.90	putative propanediol utilization
	<i>pduO</i>	-5.40	propanediol utilization protein
	<i>pduP</i>	-3.36	putative propionaldehyde dehydrogenase
	<i>pduQ</i>	-4.33	putative propanediol dehydrogenase
	<i>pduS</i>	-3.62	propanediol utilization ferredoxin
	<i>pduT</i>	-3.50	putative propanediol utilization protein
	<i>pduU</i>	-1.00	putative propanediol utilization protein PduU
	<i>pduV</i>	-31.40	putative propanediol utilization protein PduV
	<i>pduW</i>	-2.89	acetokinase
<i>Salmonella</i> pathogenicity island-1	<i>hilC</i>	0.89	AraC family transcriptional regulator
	<i>orgC</i>	1.88	hypothetical protein
	<i>orgB</i>	1.46	oxygen-regulated invasion protein
	<i>orgA</i>	1.77	oxygen-regulated invasion protein
	<i>prgK</i>	1.75	type III secretion system apparatus
	<i>prgJ</i>	1.53	type III secretion system apparatus
	<i>prgI</i>	1.67	type III secretion system apparatus
	<i>prgH</i>	1.65	type III secretion apparatus component
	<i>hilD</i>	1.43	AraC family transcriptional regulator
	<i>hilA</i>	1.04	invasion protein regulator

<i>iagB</i>	1.16	cell invasion protein
<i>sptP</i>	1.76	tyrosine phosphatase (associated with virulence)
<i>sicP</i>	2.11	chaperone (associated with virulence)
<i>iacP</i>	2.73	acyl carrier protein
<i>sipA</i>	1.92	pathogenicity island 1 effector protein
<i>sipD</i>	1.88	pathogenicity island 1 effector protein
<i>sipC</i>	2.11	pathogenicity island 1 effector protein
<i>sipB</i>	1.83	pathogenicity island 1 effector protein
<i>sicA</i>	1.95	type III secretion-associated chaperon
<i>spaS</i>	1.63	type III secretion system secretory apparatus
<i>spaR</i>	1.33	type III secretion system secretory apparatus
<i>spaQ</i>	0.83	type III secretion system secretory apparatus
<i>spaP</i>	1.74	type III secretion system secretory apparatus
<i>spaO</i>	1.06	surface presentation of antigens protein (associated with type III secretion and virulence)
<i>invJ</i>	0.93	surface presentation of antigens protein (associated with type III secretion and virulence)
<i>invI</i>	1.77	type III secretion system secretory apparatus
<i>invC</i>	1.14	secretory apparatus ATP synthase (associated with virulence)
<i>invB</i>	1.49	chaperone protein for type III secretion system effectors
<i>invA</i>	1.69	secretory apparatus of type III secretion system
<i>invE</i>	1.38	cell invasion protein
<i>invG</i>	1.27	type III secretion system secretory apparatus
<i>invF</i>	1.53	AraC family regulatory protein

	<i>invH</i>	1.80	outer membrane lipoprotein
<i>Salmonella</i>	<i>siiA</i>	2.29	type I secretion-related protein
pathogenicity	<i>siiB</i>	2.50	putative integral membrane protein
island-4	<i>siiC</i>	3.06	putative type I secretion protein
	<i>siiD</i>	3.19	putative type I secretion protein
	<i>siiE</i>	2.50	large repetitive protein
	<i>siiF</i>	3.16	putative type-1 secretion protein

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TABLE S3 Bacterial strains and plasmids used in this study.		
Strains	Description	Reference or source
<i>Salmonella enterica</i> serovar Typhimurium		
SL1344	Wild type, Sm ^R	1
SR7001	$\Delta ptsN$	2
SR7021	EIIA ^{Ntr} -FLAG	2
SR7024	HiID-HA	This study
SR7025	HiID-HA, $\Delta ptsN$	This study
SR7026	<i>PptsN-lacZ</i>	This study
SR7027	<i>PprpB-lacZ</i>	This study
SR7028	<i>PprpB-lacZ</i> , $\Delta ptsN$	This study
SR7029	<i>PprpB-lacZ</i> , $\Delta pduABCDEFGHIJKLMN O P Q S T U V W X$	This study
SR7030	<i>PpduA-lacZ</i>	This study
SR7031	<i>PpduA-lacZ</i> , $\Delta ptsN$	This study
<i>Escherichia coli</i>		
DH5 α	<i>gyrA96 recA1 relA1 endA1 thi-1 hsdR17 glnV44</i> <i>deoR</i> $\Delta(lacZYA-argF)U169$ [$\Phi 80d \Delta(lacZ)M15$]	3
Plasmids		
pKD46	Ap ^R P _{BAD} - <i>gam-beta-exo oriR101 repA101^{ts}</i>	4
pKD13	Ap ^R FRT Km ^R FRT PS1 PS4 <i>oriR6Kγ</i>	4
pCP20	Ap ^R Cm ^R <i>cI857 λP_Rflp oripSC101^{ts}</i>	4

pCE70	Km ^R FRT <i>tnpR lacZY⁺ oriR6Kγ</i>	5
pACYC184	Tet ^R Cm ^R p15A <i>ori</i>	6
pPtsN	pACYC184- <i>ptsN</i>	2
pPocR	pUHE21-2 <i>lacI^q-pocR</i>	This study
pPrpR	pUHE21-2 <i>lacI^q-prpR</i>	This study

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TABLE S4 Primers used for the construction of bacterial strains and plasmids.	
Primers	Sequences (5' to 3')
ptsN-del-F	CTG GCC ATC AAC CTG ACA GGA CAG GTT CTT AGG TGA AAT TTG TAG GCT GGA GCT GCT TCG
ptsN-del-R	CGT TTC GCC ACC AGC GAC AGC GTG TGC AGA TGC GTT TTA ATT CCG GGG CTC CGT CGA CC
ptsN-comple-F	AAA AAG CTT ACG CAC ATC TCG GAT GCG AC
ptsN-comple-R	AAA GCA TGC CCG CTG ACG ATC ATC AGT AC
hilD-HA-F	TAA AAC TAC GCC ATC GAC ATT CAT AAA AAT GGC GAA CCA TTA TCC GTA TGA TGT TCC TGA TTA TGC TAG CCT CTA ATG TAG GCT GGA GCT GCT TCG
hilD-HA-R	TTA ATA AAA ATC TTT ACT TAA GTG ACA GAT ACA AAA AAT GAT TCC GGG GAT CCG TCG ACC
ptsN-FLAG-F	TCA AAT CAT TAC TGA CAC CGA AGG TGA GCA GAA TGA GGC AGG CAG CGG CGA CTA CAA AGA CGA TGA CGA CAA GTA ATG TAG GCT GGA GCT GCT TCG
ptsN-FLAG-R	GTT TCT CCT CAC AAC GAC AGA AAT AAA TGC CAT TGA GTT GAT TCC GGG GAT CCG TCG ACC
ptsN-lacZ-F1	AAT CAT TAC TGA CAC CGA AGG TGA GCA GAA TGA GGC ATA ATG TAG GCT GGA GCT GCT TCG
ptsN-lacZ-R1	ACC ATG TAC CGT TTC TCC TCA CAA CGA CAG AAA TAA ATG CAT TCC GGG GAT CCG TCG ACC
pduA-lacZ-F1	CGA TGT AGA AAA AAT CTT ACC GAA GGG AAT TAG CCA ATG ATG TAG GCT GGA GCT GCT TCG

pduA-lacZ-R1	CAC ACG GGC AAT CAC CTG CGC CAT GAT CTG TTC CAC CAG CAT TCC GGG GAT CCG TCG ACC
prpB-lacZ-F1	CCC AAC GGG CCG GGT ATC AGG CTA TCT ATC TTT CTG GCG GGT GTA GGC TGG AGC TGC TTC
prpB-lacZ-R1	AAT CCC CAG ATC CGG CAG TCC GAG CGA GCC CGC CGC TAC GCT GTC AAA CAT GAG AAT TAA
pduA~X-del-F	AGC CGG ATA AGC CGC GTT CGG CAA CGG GTT AGA CTC AGC GGT GTA GGC TGG AGC TGC TTC
pduA~X-del-R	CGC GCT GCC GCC CTT TCC CGA CTT TAC ACC CCA CTA TTC AAT GGG AAT TAG CCA TGG TCC
pocR-over-F	AAA GGA TCC TTT CAC GCC GTT TTG TCA GT
pocR-over-R	AAA AAG CTT ACT ATC AAA AAT CGG CAA TAG C
prpR-over-F	AAA GGA TCC ACG ACT GCC CAC AGC GCT
prpR-over-R	AAA CTG CAG TTG TCT TAA TTA TCC GAC TGG TC

TABLE S5 Primers used in qRT-PCR analysis.	
Primers	Sequences (5' to 3')
qRT-copT-F1	TCT GAT CCC GTC GCA CTT TT
qRT-copT-R1	CCT CCG GCA GGA CAA TAT TAC T
qRT-cbiA-F1	CCC ACT ATC AGC TCC TCAA AA
qRT-cbiA-R1	CAC ATC CAC TGT TTG TTC CA
qRT-pocR-F1	TTT CAC GCC GTT TTG TCA GTT
qRT-pocR-R1	GAA CCT GCC CGC ATA AAA CA
qRT-pduF-F1	GCG GTA GCG TCG AAA GTT TG
qRT-pduF-R1	AAC GGC AAC CAG TAT CCC AAT
qRT-pduA-F1	GGA ATG GTA GAA ACC AAA GGC TTA
qRT-pduA-R1	GCC AAT CTT TTC ATA GCC CAC TA
qRT-pduC-F1	GTT CGC CCA GGA AAT CAT CA
qRT-pduC-R1	ATA ATG GCG GAG GTG TGC AA
qRT-pduP-F1	AAC CCG ACG GAA ACC ATC AT
qRT-pduP-R1	ATC TGC TGG GTC GCT TCG AA
qRT-pduW-F1	GGC TGA TTG AGC GTA TCG GTA T
qRT-pduW-R1	CGT TCG ATT TGC GCT AAC G
qRT-ptsN-F1	AGC GGC GTT CAT TGT CAG A
qRT-ptsN-R1	GGA GGT AAA CTG AGC TGT TTT GC
qRT-hilD-F1	CAA CGA CTT GGC GCT CTC TAT
qRT-hilD-R1	ACA GGA GAA CGC CGT TTT CA
qRT-hilA-F1	CTG CCG GTG ACC ATT ACG A

qRT-hilA-R1	GCG GGT TGG TGT TCT ATC AAC T
qRT-invF-F1	GGG TTT TGC TGA GTC CTG AGT T
qRT-invF-R1	TTC TCC CAG CAT TCT CAT CGT
qRT-spaS-F1	CCG CTA AAA AAG GCC AGT CA
qRT-spaS-R1	AAA TGA GCC ATA CGA CAC CAG AT
qRT-sopE2-F1	CCA CCC AGC ACT ACA GAA TCC
qRT-sopE2-R1	GAT GCA GGC TAA AAC GAT CTG A
qRT-sipA-F1	CCA ACG CTT CAT CCG AAA GT
qRT-sipA-R1	AAG CGG CTT CAC ATT CAC AAT
qRT-siiA-F1	ATG GCA GGC TGA GAA GCT TTT
qRT-siiA-R1	CCC GTT CAT ATC CCA GTC GTA
qRT-siiE-F1	TGG TTG CCA GCG TTG ATG T
qRT-siiE-R1	GCT CAA TCG TCG GCT TTT CT
qRT-gyrB-F1	ATA ACG CCA CGC AGA AAA TGA
qRT-gyrB-R1	TGG CTG ATA CAC CAG CTC TTT G
qRT-prpR-F1	CGG CTT TAC TTG CCT TTC AG
qRT-prpR-R1	TGT CAG ACG GGT CAT ATC CA
qRT-prpB-F1	GGG ATT TCT ACG CTG GAT GA
qRT-prpB-R1	CCA TCT CCT CTT TCG AGA C
qRT-prpC-F1	TTC AAC GCC TCG ACG TTT AC
qRT-prpC-R1	CCA AAG CCA ATC ACC ACC TC
qRT-prpD-F1	TCC GGT ACA GGC AGC TTT TA
qRT-prpD-R1	CGA TGC AGC CCT GAA TTT CA

qRT-prpE-F1	TTT ACA CCT CCG GCA CTA CC
qRT-prpE-R1	GGC GCG TAA ACG ATA TAG GA

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