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# Supplementary Information for

**Global control of bacterial nitrogen and carbon metabolism by a PTS$^{Ntr}$ regulated switch**

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**This PDF file includes:**

Supplementary Information Text
Figures S1 to S6
Tables S1 to S3
References for SI reference citations



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## Supplementary Information Text

## SI Materials and Methods.

**Cloning details.** PCR reactions for cloning were carried out according to manufacturer's instructions with Phusion High-Fidelity DNA Polymerase (ThermoFischer) and for mapping, with MangoTaq™ DNA Polymerase (Bioline). Restriction enzymes (New England Biolabs) were used according to manufacturer's instructions. Sanger sequencing was carried out by Eurofins Genomics.

The *manX* gene (RL0033, 402 bp) was amplified by PCR using primers pr1361-1362. A 3.7 kb was cloned into pJET1.2/blunt (ThermoFischer), resulting in plasmid pLMB661. Inverse PCR using primers pr1363-1364 with BamHI sites were used to delete *manX*. The PCR product was digested accordingly and ligated, rendering plasmid pLMB662. This plasmid was digested with BamHI and a BamHI ΩSpec cassette from pHP45 was inserted to give pLMB665. Plasmid pLMB665 was digested with NotI/XbaI and cloned into pJQ200SK (pLMB666), and this final plasmid was conjugated into Rlv3841 and selected for double-recombination using sucrose selection (1) to give LMB601. Strain LMB601 was mapped with mapping primers pr1367/pOTforward and pr1442/pr1443. An XbaI/PstI fragment from pLMB665 was cloned into pJQ200SK to make the *manX* in-frame deletion mutant, resulting in the markerless *manX* strain LMB692. Spectinomycin-sensitive, streptomycin-resistant colonies were mapped using primers pr1498-1499 to confirm the in-frame deletion.

The *hprK* gene (RL0034, 453 bp) was amplified by PCR using primers 'hprK for XbaI/hprK rev BamHI' containing the corresponding restriction sites and cloned into pJET1.2/blunt. The plasmid containing the deleted fragment was digested with Eco72I, inserting a SmaI ΩSpec cassette from pHP45 and ligated in its place. The resulting fragment was cloned XbaI/XhoI into the suicide vector pJQ200SK. This plasmid was conjugated into Rlv3841 and the transconjugants plated on TY agar supplemented with sucrose (5%), streptomycin and spectinomycin. Strain AA081 was mapped to confirm the cassette insertion.

Double- and triple-mutants were generated by transduction. *ptsN1*::ΩSpec mutation from LMB271 was transduced into Rlv3841 *manX* markerless mutant LMB691, generating the strain OPS0374, mapped with primers pOTforward/oxp0452 and pOTforward/oxp0453. *ptsN2*::ΩTet mutation from RU4391 was transduced into the same strain (LMB691), generating the strain OPS0849, and mapped with primers pOTforward/oxp0454 and pOTforward/oxp0455. LMB272 (*ptsN1*::ΩSpec; *ptsN2*::ΩTet) was converted to AA047

(*ptsN1*::ΩSpec; Δ*ptsN2*) to facilitate chromosomal replacement studies. Strain AA047 showed the same phenotype as its parental strain (LMB272).

Site-directed mutants of *ptsN1* (H66A and H66D) and *manX* (H10A and H10D) were constructed by overlap PCR using a Gibson Assembly Cloning Kit (New England Biolabs, NEBuilder Assembly Tool v1.11.0). Two internal primers complementary to each other carried the desired amino acid substitution, and two external primers, downstream and upstream of the gene, had the corresponding overlapping sequence for BD cloning into pJQ200SK (SI Appendix, Table S3). This vector was linearized by a XbaI/XhoI digestion in the case of *ptsN* and a XbaI/BamHI digestion in the case of *manX*. The plasmids generated (pOPS0373, pOPS0374, pOPS0375, pOPS0376) were introduced into the genomes of *ptsN1N2* (AA047) and *manX* (LMB601) mutants by conjugation. Subsequent plating on UMS agar supplemented with sucrose (10%) was followed by selection of the loss of antibiotic resistance. Strains OPS1102, OPS1104, OPS1012 and OPS1014 were confirmed to have the expected gene replacement by PCR-mapping and sequencing with primers oxp0452/oxp0453 (*ptsN1*) and oxp1626/oxp1627 (*manX*).

A deletion of 411 bp corresponding to the GAF domain of *ptsP* was carried out by fusion PCR, amplifying two different fragments before and after this domain, according to the pLMB180 plasmid used in previous work (2). The primer pairs were oxp0470/oxp0472 (fragment of 731 bp) and oxp0471/oxp0473 (fragment of 2,485 bp), where oxp0472 and oxp0473 had overlapping regions and oxp0470 and oxp0471 had the restriction sites BamHI and NotI, respectively. The resulting fused fragment *ptsP*-GAF was cloned into pJET1.2 following the manufacturer's instructions. A BamHI/XbaI fragment was cloned into pJQ200SK, generating plasmid pOPS0372. As before, the plasmid was conjugated into the genome of *ptsP* (PtsP107) mutant and plated on UMS agar supplemented with sucrose (10%), being selected for the loss of the antibiotic resistance. Strain OPS1010 (*ptsP*ΔGAF) was confirmed to have the expected gene replacement by PCR mapping and sequencing with primers oxp0456/oxp0457 (*ptsP*).

Complementing plasmids were made by amplifying the corresponding ORF with the primers listed on SI Appendix, Table S3 and cloned into pJET1.2 following the manufacturer's instructions. An XbaI/HindIII fragment was then cloned into pRK415 or KpnI/BamHI into pJP2.

For expression analysis using the *luxCDABE* cassette, the promoter region of each of the candidate genes was amplified using primers listed in SI Appendix, Table S3 including the restriction sites KpnI on the forward primer, and BamHI on the reverse one. Fragments

were purified and double-digested with both enzymes and cloned into pIJ11268 (3) digested with the same enzymes. Plasmids (SI Appendix, Table S2) were transferred into wildtype Rlv3841 and derived strains by tri-parental mating according to Figurski and Helinski, 1979 (4).

BACTH plasmids were constructed by amplifying the proteins tested with the corresponding primers listed on SI Appendix, Table S3. They were cloned in-frame into the BamHI/KpnI sites of pUT18C and pKNT25, rendering the different interacting plasmids on SI Appendix, Table S2. These plasmids were confirmed by PCR sequencing using primers M13/pKNT25 – 258R and pUT18C-711F/pUT18C-804R.

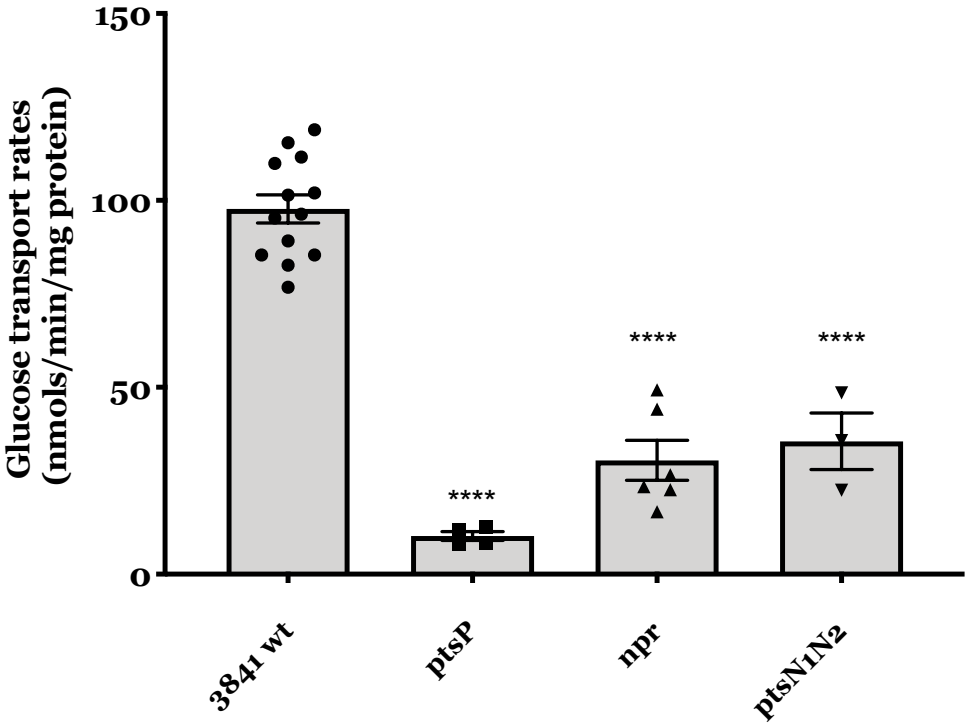


**Figure S1. Transport rates by PTS mutants.** Standard rates for glucose transport obtained from cultures grown on UMS with 10 mM glucose and 10 mM $NH_4Cl$. Rlv3841 wildtype, *ptsP* (PtsP107), *npr* (AA031) and *ptsN1N2* (AA047). All rates are expressed in nmol $min^{-1}$ mg $protein^{-1}$. Data are averages (±SEM) from at least three independent cultures analyzed by 1-way ANOVA with Dunnett's post-test for multiple comparisons (****) $P < 0.0001$ and n.s., not significant.

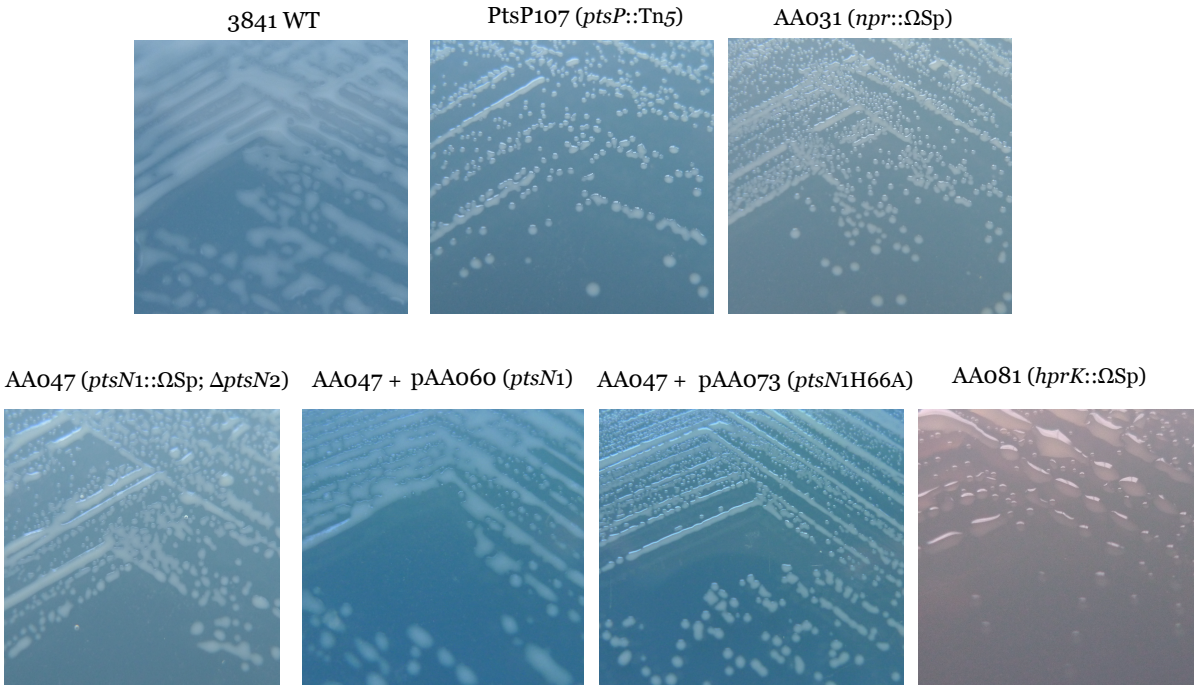


**Figure S2. Colony morphology of *R. leguminosarum* strains grown on TY agar plates.** The surface phenotype for PTS$^{Ntr}$ mutants is as follows: Rlv3841 wildtype, mucoid; PtsP107 (*ptsP*::Tn5), dry; AA031 (*npr*::ΩSpec), dry; AA047 (*ptsN1*::ΩSpec; Δ*ptsN2*), intermediate phenotype; AA047 + pAA060 (*ptsN1,* complemented mutant with the wild-type version of *ptsN1)*, mucoid; AA047 + pAA073 (*ptsN1* H66A, complemented mutant with the non-phosphorylatable version of *ptsN1)*, dry; AA081 (*hprK*::ΩSpec), hyper-mucoid phenotype.

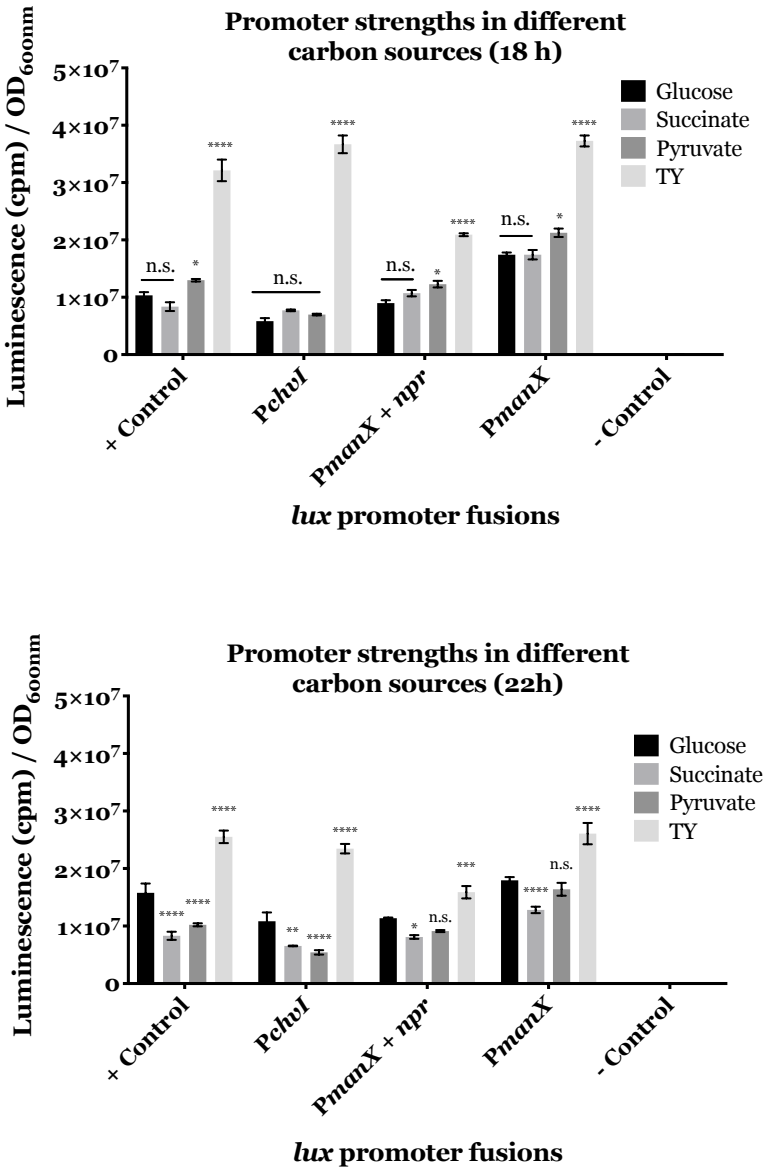


**Figure S3. Quantification of promoter activity from the promoter regions selected for testing at 18h and 22h under growth on different carbon sources**. The constitutive *nptII* promoter was used as a positive control on the plasmid pIJ11282 (+ Control) and the empty vector pJI11268 as a negative control (- Control). The promoter regions tested in the assay cloned upstream of *lux* were P*chvI* (in pOPS0298), P*manX* (in pOPS0606) and *PmanX+npr* (in pOPS0296). Cells were grown on TY as complex media or in UMS as minimal media, supplemented with 10 mM $NH_4Cl$ as nitrogen source and glucose (10 mM), succinate (20 mM) or pyruvate (30 mM) as carbon sources. All rates are expressed in counts per minute (cpm). Data are averages (±SEM) from two independent cultures with four replicates each analyzed by 1-way ANOVA with Dunnett's post-test for multiple comparisons (****) $P < 0.0001$, (***) $P < 0.001$, (**) $P < 0.01$, (*) $P < 0.1$ and n.s., not significant.

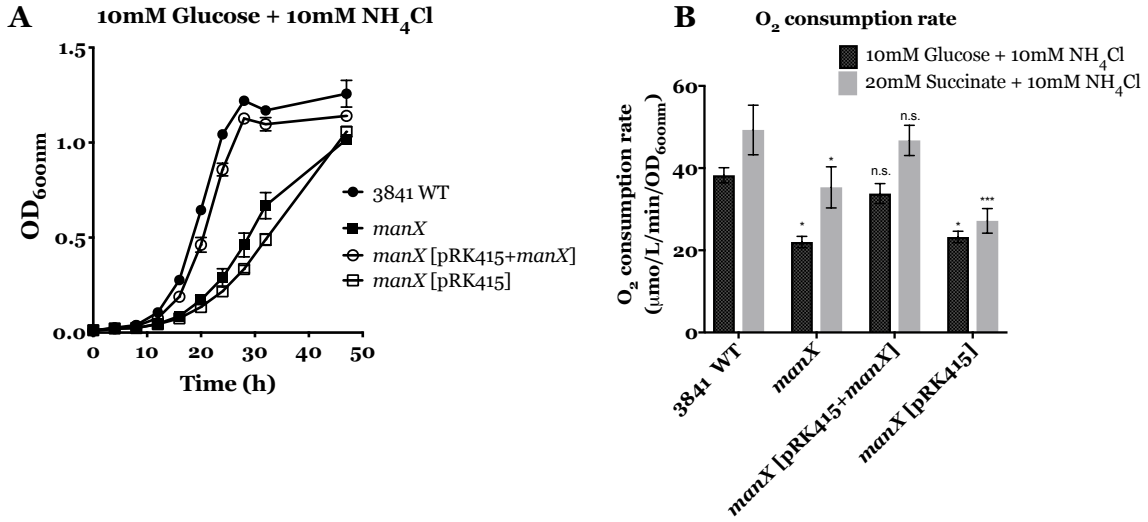


**Figure S4. Analysis of complemented *manX* mutant** (**A**) Growth of wildtype Rlv3841, *manX* mutant (LMB692), *manX* mutant with the complementing plasmid [pRK415 + *manX*], and *manX* mutant with the empty vector pRK415 (as a negative control). Cultures grown on UMS with 10 mM Glucose + 10 mM $NH_4Cl$. (**B**) Oxygen consumption of the same strains as in 5A grown in UMS supplemented with 10 mM glucose + 10 mM $NH_4Cl$ or 20 mM succinate + 10 mM $NH_4Cl$. Oxygen consumption rates are expressed in µmol $L^{-1}$ $min^{-1}$ $OD_{600nm}^{-1}$. Data are averages (±SEM) from three independent cultures; 2-way ANOVA with Dunnett's post-test for multiple comparisons (*) $P < 0.05$, (****) $P < 0.0001$ and n.s., not significant.

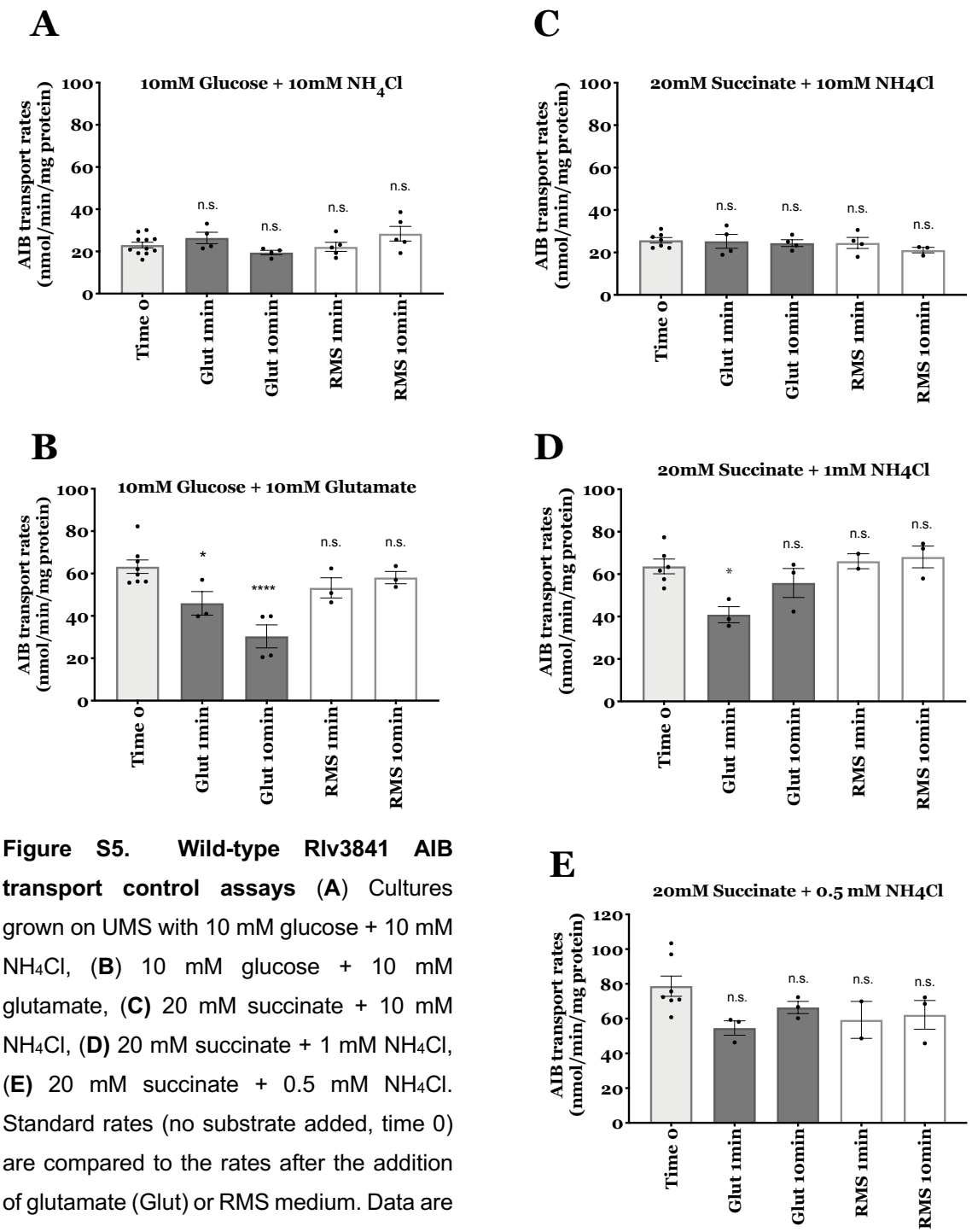


**Figure S5. Wild-type Rlv3841 AIB transport control assays** (**A**) Cultures grown on UMS with 10 mM glucose + 10 mM $NH_4Cl$, (**B**) 10 mM glucose + 10 mM glutamate, (**C)** 20 mM succinate + 10 mM $NH_4Cl$, (**D)** 20 mM succinate + 1 mM $NH_4Cl$, (**E)** 20 mM succinate + 0.5 mM $NH_4Cl$. Standard rates (no substrate added, time 0) are compared to the rates after the addition of glutamate (Glut) or RMS medium. Data are averages (±SEM) from at least three independent cultures, analyzed by 1-way ANOVA with Dunnett's post-test for multiple comparisons (*) $P < 0.01$, (****) $P < 0.0001$ and n.s., not significant.

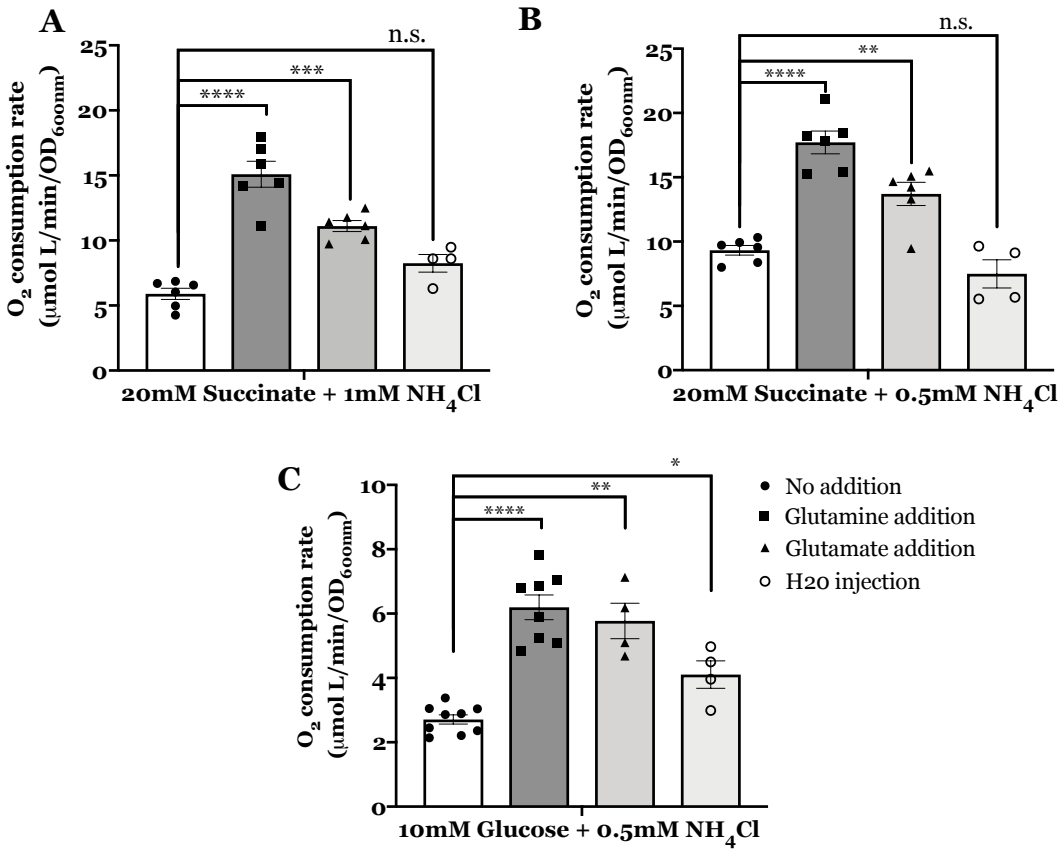


**Figure S6. Wild-type Rlv3841 oxygen consumption control assays** (**A**) Cultures grown on UMS with 20 mM succinate + 1 mM $NH_4Cl$, (**B**) 20 mM succinate + 0.5 mM $NH_4Cl$ and (**C)** 10 mM glucose + 0.5 mM $NH_4Cl$. Oxygen consumption rates are expressed in µmol $L^{-1}$ $min^{-1}$ $OD_{600nm}^{-1}$. Standard rates (no substrate added, represented by black dots) are compared to the rates after the addition of glutamine (black squares), glutamate (black triangles) or water (empty dots). Data are averages (±SEM) from at least three independent cultures, analyzed by 1-way ANOVA with Dunnett's post-test for multiple comparisons (*) P < 0.01, (**) P < 0.01, (***) P < 0.001, (****) P < 0.0001 and n.s., not significant.

**Table S1. Mean generation time for PTS mutants grown in UMS minimal medium supplemented with different carbon and nitrogen sources**.

| | | MEAN GENERATION TIME (hours) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Glucose 10 mM | Succinate 20 mM | Arabinose 10 mM | Pyruvate 30 mM | Glutamine 10 mM | Glutamate 10 mM | Aspartate 10 mM |
| Description | Strain | UMS Glc+$NH_4Cl$ | UMS Succ+$NH_4Cl$ | UMS Arab+$NH_4Cl$ | UMS Pyr+$NH_4Cl$ | UMS Glc+Gln | UMS Glc+Glut | UMS Glc+Asp |
| **wildtype** | **3841** | 3.42 ± 0.11 | 3.44 ± 0.28 | 3.25 ± 0.09 | 4.07 ± 0.19 | 3.73 ± 0.24 | 4.29 ± 0.08 | 11.41 ± 0.21 |
| ***ptsP*::Tn5** | **ptsP107** | 4.44 ± 0.16 | 5.65 ± 0.32 | 3.66 ± 0.12 | 7.63 ± 0.13 | 3.65 ± 0.13 | ∞ | 7.68 ± 0.21* |
| ***npr*::ΩSpec** | **AA031** | 4.43 ± 0.09 | 4.04 ± 0.43 | 3.39 ± 0.09 | 5.92 ± 0.41 | 3.45 ± 0.09 | ∞ | 11.91 ± 0.24 |
| ***ptsN1::ΩSpec; ΔptsN2*** | **AA047** | 3.33 ± 0.16 | 4.34 ± 0.35 | 3.41 ± 0.19 | 3.73 ± 0.03 | 3.51 ± 0.25 | 4.56 ± 0.30 | 12.51 ± 0.78 |
| ***ptsN1*::ΩSpec** | **LMB271** | 3.24 ± 0.16 | 4.76 ± 0.35 | 3.11 ± 0.05 | 3.99 ± 0.04 | 2.82 ± 0.10 | 4.79 ± 0.27 | 9.87 ± 0.38 |
| ***ptsN2*::ΩTet** | **RU4391** | 3.38 ± 0.14 | 4.63 ± 0.21 | 3.15 ± 0.08 | 3.54 ± 0.05 | 2.62 ± 0.22 | 4.38 ±0 .05 | 12.49 ± 0.66 |
| **Δ*manX*** | **LMB692** | 6.13 ± 0.12 | 6.15 ± 0.21 | 5.91 ± 0.25 | 5.24 ± 0.12 | 6.33 ± 0.18 | 7.64 ± 0.11 | 17.53 ± 0.28 |
| ***hprK*::ΩSpec** | **AA081** | 7.64 ± 0.17 | 5.48 ± 0.24 | 7.42 ± 0.25 | 8.25 ± 0.29 | 6.64 ± 0.51 | 9.11 ± 0.38 | 25.01 ± 0.67 |
| ***ptsN1::ΩSpec; ΔmanX*** | **OPS0374** | 5.46 ± 0.13 | 5.95 ± 0.34 | 5.87 ± 0.28 | 8.02 ± 0.37 | 6.09 ± 0.22 | 9.09 ± 0.35 | 14.02 ± 0.23 |
| ***ptsN2::ΩTet; ΔmanX*** | **OPS0849** | 5.98 ± 0.16 | 4.76 ± 0.08 | 6.54 ± 0.08 | 7.2 ± 0.15 | 6.45 ± 0.51 | 8.53 ± 0.36 | 16.88 ± 0.72 |
| ***ptsP*ΔGAF** | **OPS1010** | 4.30 ± 0.20 | 3.92 ± 0.18 | 4.37 ± 0.05 | 6.80 ± 0.32 | 3.01 ± 0.10 | 8.17 ± 0.39 | 10.44 ± 0.19 |
| ***ptsN1N2**H66A** | **OPS1102** | 3.42 ± 0.09 | 3.51 ± 0.11 | 3.20 ± 0.03 | 3.76 ± 0.19 | 3.53 ± 0.17 | 4.59 ± 0.15 | 13.63 ± 0.80 |
| ***ptsN1N2**H66D** | **OPS1104** | 4.48 ± 0.50 | 4.41 ± 0.08 | 3.47 ± 0.04 | 5.05 ± 0.34 | 3.87 ± 0.36 | 5.41 ± 0.61 | 16.33 ± 1.6 |
| ***manX**H9A** | **OPS1012** | 3.39 ± 0.11 | 3.46 ± 0.10 | 3.14 ± 0.02 | 4.00 ± 0.44 | 3.70 ± 0.13 | 4.38 ± 0.35 | 15.20 ± 0.80 |
| ***manX**H9D** | **OPS1014** | 3.92 ± 0.15 | 3.61 ± 0.12 | 3.46 ± 0.03 | 4.20 ± 0.07 | 3.83 ± 0.07 | 4.74 ± 0.25 | 14.31 ± 0.91 |

Glc: glucose, Succ: succinate, Arab: arabinose, Pyr: pyruvate, Gln: glutamine, Glut: Glutamate, Asp: aspartate. In white, strains with mean generation times (MGT) similar to Rlv3841 wildtype. In green, strains with an MGT value faster than Rlv3841 wildtype; in red, those strains with a slower MGT value compared to wild-type (light, 1h difference; medium, 2h difference; dark, more than 3h difference). Data are averages (±SEM) from at least 3 independent cultures. *Conditions leading to suppressor mutatations.

**Table S2. List of strains and plasmids.**

| Strains | Description | Reference |
|---|---|---|
| ***Escherichia coli*** | | |
| DH5α | *supE44, hsdR17, recA1 thi-1, ΔlacU169(ϕ801acZΔM15) endA1 gyrA96 relA1* | (5) |
| MAE01 | $F^-$ $\lambda^-$ *ilvG⁻ rfb-50 rph-1, ΔcyaA*::$Apra^R$ (host strain for BACTH assays) | (6) |
| ***Rhizobium leguminosarum*** | | |
| Rlv3841 | *R. leguminosarum* bv. viciae; $Str^r$ derivative of strain 300 | (7) |
| AA031 | Rlv3841 *npr*::ΩSpec | (2) |
| AA047 | Rlv3841 *ptsN2* (markerless mutation) in *ptsN1*::ΩSpec background (Rlv3841 *ptsN1/N2* double mutant) | This work |
| AA081 | Rlv3841 *hprK*::ΩSpec | This work |
| AA088 | AA081[pAA074] (Rlv3841 *hprK* mutant complemented with cloned *hprK*) | This work |
| AA089 | LMB601[pAA075] (Rlv3841 *manX* mutant complemented with cloned *manX*) | This work |
| AA090 | LMB601[pAA076] (Rlv3841 *manX* mutant complemented with cloned *manX* + *npr*) | This work |
| AA093 | LMB601[pAA038] (Rlv3841 *manX* mutant complemented with cloned *npr*) | This work |
| LMB271 | Rlv3841 *ptsN1*::ΩSpec | (8) |
| LMB272 | Rlv3841 *ptsN1*::ΩSpec *ptsN2*::ΩTet | (8) |
| LMB601 | Rlv3841 *manX*::ΩSpec | This work |
| LMB692 | Rlv3841 *manX* in-frame deletion | This work |
| OPS0374 | Transduction of *ptsN1*::ΩSpec into LMB692 (Rlv3841 *ptsN1/manX* double mutant) | This work |
| OPS0849 | Transduction of *ptsN2*::ΩTet into LMB692 (Rlv3841 *ptsN2/manX* double mutant) | This work |
| OPS1102 | AA047 with pOPS0373 used to replace *ptsN1*::ΩSpec with *ptsN1* H66A (Rlv3841 ptsN2 mutant with non-phosphorylatable PtsN1) | This work |
| OPS1104 | AA047 with pOPS0374 used to replace *ptsN1*::ΩSpec with *ptsN1* H66D (Rlv3841 *ptsN2* mutant with PtsN1 phosphomimic) | This work |
| OPS1010 | Rlv3841 *ptsP*ΔGAF (Rlv3841 with *ptsP* lacking GAF domain) | This work |
| OPS1012 | LMB601 with pOPS0375 used to replace *manX*::ΩSpec with *manX* H9A (Rlv3841 with non-phosphorylatable ManX) | This work |
| OPS1014 | LMB601 with pOPS0376 used to replace *manX*::ΩSpec with *manX* H9D (Rlv3841 with ManX phosphomimic) | This work |
| PtsP107 | Rlv3841 Tn5::*ptsP* | (8) |
| RU4391 | Rlv3841 *ptsN2*::ΩTet | (8) |

| **Plasmids** | | |
|---|---|---|
| pBBRMCS5 | Broad-host-range cloning vector; $Gm^R$ | (9) |
| pHP45ΩSpec | pBR322 derivative carrying ΩSpec interposon, pHP45 replicon; $Amp^R$, $Spec^R$ | (10) |
| pIJ11268 | Transcriptional bioreporter; *luxCDABE* cassette with upstream cloning site for driving Lux expression | (3) |
| pJET1.2/blunt | *E. coli* cloning vector | Thermo Fisher Scientific |
| pJP2 | Broad-host-range *gusA* transcriptional promoter probe vector; $Tet^R$ | (8) |
| pJP2neo | pJP2 derivative broad-host-range vector with *nptII promoter* cloned into pJP2 MCS, driving constitutive expression into the GUS reporter gene; $Tet^R$. | (11) |
| pJQ200SK | Suicide vector, pACYC derivative, P15A origin of replication; $Gm^R$, *lacZ sacB traJ* | (1) |
| pKNT25 | BACTH vector for fusions to the N-terminus of fragment T25; $Kana^R$ | Euromedex |
| pRK415 | IncP stable broad-host-range cloning vector; $Tet^R$ | Keen et al. (1988) |
| pUT18C | BACTH vector for fusions to the C-terminus of fragment T18; $Amp^R$ | Euromedex |
| pAA038 | *npr* for complementation cloned into pRK415 | (2) |
| pAA060 | *ptsN1* for complementation cloned into pRK415 | This work |
| pAA073 | *ptsN1 H66A* for complementation cloned into pRK415 | This work |
| pAA074 | *hprK* for complementation cloned into pJP2Neo | This work |
| pAA075 | *manX* for complementation cloned into pRK415 | This work |
| pIJ11282 | pIJ11268 with promoter region of *nptII* promoter cloned upstream of *luxCDABE, positive control for Lux expression studies* | (3) |
| pLMB661 | *manX* PCR-amplified from Rlv3841 with primers pr1361/pr1362 and cloned into pJET1.2 | This work |
| pLMB662 | Inverse PCR from pLMB661 to generate *manX* deletion with primers pr1363/pr1364 | This work |
| pLMB665 | pLMB662 with *manX*::ΩSpec | This work |
| pLMB666 | *manX*::ΩSpec disgested NotI/XbaI and cloned into pJQ200SK, used to generate *manX*::ΩSpec mutant | This work |
| pLMB720 | ΩSpec removed from pLMB666 by XbaI digestion, used to generate *manX* in-frame deletion mutant | This work |
| pOPS0172 | *ptsN1* PCR-amplified from Rlv3841 with primers oxp0516/0517 and cloned into BACTH vector pKNT25 with BamHI/KpnI | This work |
| pOPS0174 | C-terminus of *kdpD* PCR-amplified from Rlv3841 with primers oxp0520/0521 and cloned into BACTH vector pKNT25 with BamHI/KpnI | This work |
| pOPS0180 | *chvG* PCR amplified with oxp531/532 in BACTH vector cloned via BamHI/KpnI | This work |

| | | |
|---|---|---|
| pOPS0181 | *chvI* PCR-amplified from Rlv3841 with primers oxp0533/0534 and cloned into BACTH vector pKNT25 with BamHI/KpnI | This work |
| pOPS0186 | *ptsN1* PCR-amplified from Rlv3841 with primers oxp0516/0517 and cloned into BACTH vector pUT18C with BamHI/KpnI | This work |
| pOPS0188 | C-terminus of *kdpD* PCR-amplified from Rlv3841 with primers oxp0520/0521 and cloned into BACTH vector pUT18C with BamHI/KpnI | This work |
| pOPS0194 | *chvG* PCR-amplified from Rlv3841 with primers oxp0531/0532 and cloned into BACTH vector pUT18C with BamHI/KpnI | This work |
| pOPS0195 | *chvI* PCR-amplified from Rlv3841 with primers oxp0533/0534 and cloned into BACTH vector pUT18C with BamHI/KpnI | This work |
| pOPS0296 | P*npr* + *manX* PCR-amplified from Rlv3841 with primers oxp0624/0625 and cloned into pIJ11268 with BamHI/KpnI | This work |
| pOPS0297 | P*chvG* PCR-amplified from Rlv3841 with primers oxp0628/0629 and cloned into pIJ11268 with BamHI/KpnI | This work |
| pOPS0298 | P*chvI* PCR-amplified from Rlv3841 with primers oxp0630/0631and cloned into pIJ11268 with BamHI/KpnI | This work |
| pOPS0299 | P*chvI* + *chvG* PCR-amplified from Rlv3841 with primers oxp0630/0629 and cloned into pIJ11268 with BamHI/KpnI | This work |
| pOPS0313 | P*chvI* + *chvG* + *hprK* PCR-amplified from Rlv3841 with primers oxp0630/0627 and cloned into pIJ11268 with BamHI/KpnI | This work |
| pOPS0372 | *ptsP*ΔGAF generated by fusion PCR amplifying two fragments with primers oxp0470/oxp0472 and oxp0473/oxp0471 and cloned in pJQ200SK | This work |
| pOPS0373 | *ptsN1* H66A generated by PCR-amplification of two fragments with primers oxp0961/oxp0963 and oxp0964/oxp0962 and cloned in pJQ200SK by Gibson assembly | This work |
| pOPS0374 | *ptsN1* H66D generated by PCR-amplification of two fragments with primers oxp0961/oxp0965 and oxp0966/oxp0962 and cloned in pJQ200SK by Gibson assembly | This work |
| pOPS0375 | *manX* H9A generated by PCR-amplification of two fragments with primers oxp0967/oxp0969 and oxp0970/oxp0968 and cloned in pJQ200SK by Gibson assembly | This work |
| pOPS0376 | manX H9D generated by PCR-amplification of two fragments with primers oxp0967/oxp0971 and oxp0972/oxp0968 and cloned in pJQ200SK by Gibson assembly | This work |
| pOPS0603 | P*chvI* PCR-amplified from Rlv3841 with primers oxp0630/0631 and cloned into pIJ11268 with BamHI/KpnI | This work |
| pOPS0604 | P*chvI* + *chvG* + *hprK* PCR-amplified from Rlv3841with primers oxp0630/oxp0627 and cloned into pIJ11268 with BamHI/KpnI | This work |
| pOPS0605 | P*npr* PCR-amplified from Rlv3841with primers oxp0622/oxp0623 and cloned into pIJ11268 with BamHI/KpnI | This work |
| pOPS0606 | P*manX* PCR-amplified from Rlv3841 with primers oxp0624/oxp0625 and cloned into pIJ11268 with BamHI/KpnI | This work |
| pOPS1000 | P*pssA1* PCR-amplified from Rlv3841with primers oxp2396/oxp2397 and cloned into pIJ11268 with BamHI/KpnI | This work |

**Table S3. List of primers.**

| Primers | Description | Sequence |
|---|---|---|
| hprK comp XbaI | Forward for PCR of *hprK* with an XbaI site | CCCTCTAGATAAGGCAGATTGACCAATCC |
| hprK for XbaI | Forward for PCR to generate *hprK* mutant with an XbaI site | CCCTCTAGACGGGCGACATCGACAATATC |
| hprK rev BamHI | Reverse for PCR to generate *hprK* mutant with a BamHI site | TTTGGATCCTTGGCGCAGGCGCGAAGATG |
| oxp0452 | Mapping primer for *ptsN1* | GCCATCATCCTGGCGACCAT |
| oxp0453 | Mapping primer for *ptsN1* | AAGCAGGATTTCAACGCCGC |
| oxp0454 | Mapping primer for *ptsN2* | GCGATGACACTTGCGGATTCC |
| oxp0455 | Mapping primer for *ptsN2* | CTGGGAACACAATCACGCCG |
| oxp0456 | Mapping primer for *ptsP* | CTTCGTGCGCTCATCATTCG |
| oxp0457 | Mapping primer for *ptsP* | ATTTCGAGGATCGCGCTCTT |
| oxp0470 | Forward for PCR to generate *ptsP*ΔGAF mutant containing an XbaI site | TTTTTTCTAGACGTGCGCTCATCATTCGAAG |
| oxp0471 | Reverse for PCR to generate *ptsP*ΔGAF mutant containing a NotI site | TTTTTGCGGCCGCGGATGTCTTCAGCCCGGATC |
| oxp0472 | Overlapping primer to generate *ptsP*ΔGAF mutant | *GCCGGTGGCCAGCGGCTC*CGCCATCAACTCGCG |
| oxp0473 | Overlapping primer to generate *ptsP*ΔGAF mutant | *GAGCCGCTGGCCACCGGC*GAGCTCAAGAAGATC |
| oxp0516 | Forward for PCR of *ptsN1* and clone it into BATCH vectors with a BamHI site | TTTGGATCC**C**ATGGCATTGGCAGATTTGCT |
| oxp0517 | Reverse for PCR of *ptsN1* and clone it into BATCH vectors with a KpnI site | TTTGGTACC**CG**GGCAGCGTTCGACGTCTGCT |
| oxp0520 | Forward for PCR of *kdpD* and clone it into BATCH vectors with a BamHI site | TTTGGATCC**C**ACGACGGAAGACCTCTATCT |
| oxp0521 | Reverse for PCR of *kdpD* and clone it into BATCH vectors with a KpnI site | TTTGGTACC**CG**TCTGAGTTCATCCAGTTTCG |
| oxp0532 | Forward for PCR of *chvG* and clone it into BATCH vectors with a BamHI site | TTTGGATCC**C**ATGGCACAGTTGGTGCAGGA |
| oxp0533 | Reverse for PCR of *chvG* and clone it into BATCH vectors with a KpnI site | TTTGGTACC**CG**TGCTGCGGCGCCGATCGGTA |
| oxp0534 | Forward for PCR of *chvI* and clone it into BATCH vectors with a BamHI site | TTTGGATCC**C**ATGCCGACAATCGCGCTCGT |

| | | |
|---|---|---|
| oxp0535 | Reverse for PCR of *chvI* and clone it into BATCH vectors with a KpnI site | TTTGGTACC**CG**GGCTGCTTCGCGGAAGCGGT |
| oxp0622 | Forward for PCR of the promoter of *npr* with a KpnI site | TTTGGTACCTTGATCAAACTTGCCGGGGT |
| oxp0623 | Reverse for PCR of the promoter of *npr* with a BamHI site | TTTGGATCCCTGCACGAATTTGGCGGAAG |
| oxp0624 | Forward for PCR of the promoter of *manX* with a KpnI site | TTTGGTACCCACCCCTTGCGATCCTGATG |
| oxp0625 | Reverse for PCR of the promoter of *manX* with a BamHI site | TTTGGATCCCTGGTCCATGTCGTCTTCGG |
| oxp0626 | Forward for PCR of the promoter of *hprK* with a KpnI site | TTTGGTACCCTTCGGCCAGAATTCGGGG |
| oxp0627 | Reverse for PCR of the promoter of *hprK* with a BamHI site | TTTGGATCCCTGAACAACAGCCCCGTCTT |
| oxp0630 | Forward for PCR of the promoter of *chvI* with a KpnI site | TTTGGTACCGGCGGAAAAGTTGTAGCGAA |
| oxp0631 | Reverse for PCR of the promoter of *chvI* with a BamHI site | TTTGGATCCATATCCTTCGGCCTCCAGTG |
| oxp0961 | Forward for PCR of *ptsN1* | *GGCGAATTGGGTACCGGGCCCCCCCC*CCTGGATCGGACTCATCTGC |
| oxp0962 | Reverse for PCR of *ptsN1* | *GAGCTCCACCGCGGTGGCGGCCGCT*CGAATACGGCAAGCTGAAGC |
| oxp0963 | Reverse for PCR of *ptsN1* H66A | CAGCTTGCC**GGC**GGGAATGGCGATGCCGTT |
| oxp0964 | Forward for PCR of *ptsN1* H66A | GCCATTCCC**GCC**GGCAAGCTGGGAAATATC |
| oxp0965 | Reverse for PCR of *ptsN1* H66D | CAGCTTGCC**ATC**GGGAATGGCGATGCCGTT |
| oxp0966 | Forward for PCR of *ptsN1* H66D | GCCATTCCC**GAT**GGCAAGCTGGGAAATATC |
| oxp0967 | Forward for PCR of *manX* | *GATATCGAATTCCTGCAGCCCGGGG*CTACGCATCGGCCAGATC |
| oxp0968 | Reverse for PCR of *manX* | *GAGCTCCACCGCGGTGGCGGCCGCT*GCCGTCATCGAGGATCATG |
| oxp0969 | Reverse for PCR of *manX* H10A | CAGCTTGCC**GGC**CGTGACAAGCACAAGTCC |
| oxp0970 | Forward for PCR of *manX* H10A | CTTGTCACG**GCC**GGCAAGCTGGCTGAAGAG |
| oxp0971 | Reverse for PCR of *manX* H10D | CAGCTTGCC**ATC**CGTGACAAGCACAAGTCC |

| | | |
|---|---|---|
| oxp0972 | Forward for PCR of *manX* H10D | CTTGTCACG**GAT**GGCAAGCTGGCTGAAGAG |
| oxp1626 | *manX* mapping primer | TATTCTGGATCAGCTGGTCC |
| oxp1627 | *manX* mapping primer | GTGAAAACCGGATACCTCCC |
| oxp2396 | Forward for PCR of the promoter of *pssA* with a KpnI site | TTTGGTACCGGGACACCCGGCCG |
| oxp2396 | Reverse for PCR of the promoter of *pssA* with a BamHI site | TTTGGATCCGAAGGACTCTGTCGAAATGC |
| pKNT25 - 258R | Mapping primer for pKNT25 BATCH plasmid | GCGATTGCTGCATGGTCATT |
| pOTForward | Screening primer for gene replacement with Ω interposon | CGGTTTACAAGCATAAAGC |
| pr1361 | Forward for PCR to generate *manX* mutant containing an XbaI site | TTTTCTAGAGACCAGCGATTCCTTGCAGC |
| pr1362 | Reverse for PCR to generate *manX* mutant with a PstI site | TTTCTGCAGATATCTCCGACGCCTCCCGT |
| pr1363 | Inverse PCR of *manX*, with a BamHI site | TTTGGATCCTCCGATCATGATATTACTGC |
| pr1364 | Inverse PCR of *manX*, with a BamHI site | TTTGGATCCGGAAAATAACGGGCCTTCATG |
| pr1367 | Mapping primer for *manX* with Ω interposon | TCGGCTTCGGTCACCTTGAC |
| pr1442 | Mapping primer for *manX* with Ω interposon | TATTCTGGATCAGCTGGTCC |
| pr1443 | Mapping primer for *manX* with Ω interposon | GTGAAAACCGGATACCTCCC |
| pr1498 | Mapping primer for *manX* in-frame mutation | ATCATCAAGCCCATGATGGA |
| pr1499 | Mapping primer for *manX* in-frame mutation | CCTTCCGGATTGGTCAATCT |
| pUT18C - 711F | Mapping primer for pUT18C BACTH plasmid | GTCGCTGGGCGCAGTGGAAC |
| pUT18C - 804R | Mapping primer for pUT18C BACTH plasmid | GCAGATTGTACTGAGAGTGCAC |

*Restriction sites in primer sequences are underlined. Italics: overlapping sequences for BD cloning or Gibson assembly. Bold: extra bases needed for in-frame cloning of the amplified genes into BACTH plasmids or point mutations to generate the phosphomimic versions of *ptsN1* and *manX*.

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