Supplemental Information

Centromere interactions promote the maintenance of the multipartite genome in *Agrobacterium tumefaciens*

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Inventory of Supplemental information

Six Supplemental Figures and Figure Legends

Supplemental Tables
• Table S1A: Strains used in this study.
• Table S1B: Plasmids used in this study.
• Table S1C: Oligonucleotides used in this study.
• Table S1D: Next-Generation-Sequencing samples used in this study.

Supplemental Materials and Methods: This provides a detailed description of the methods applied in this study.
Ren Figure S1

ParB1 depletion 0h 6h 12h 18h
ori1
ori2
oAt
Figure S1. ParB1 is important for genome maintenance.

Visualization of origin localization when ParB1 was depleted for the indicated durations in AtWX496 (top) and AtWX498 (bottom). The origins are labeled using *mcherry-parB^{P1}-parS^{P1}* at 50 kb away from ori1 (green, top panel), or *ygfp-parB^{pMT1}-parS^{pMT1}* at 57 kb away from ori2 (red, top panel) or 11 kb away from oAt (green, bottom panel). Pseudo-colors were assigned as indicated. Protein levels can be found in Figure 1B. Scale bar represents 2 μm. ParB1 depletion was performed by washing away the inducers (1 μM AHL and 2 mM theophylline).
Ren Figure S2

A

Cell length distribution

H

Cell width distribution

I

Strains

WT

ΔpodJ

ΔpopZ

ΔZ ΔJ

Δgpr

- number of cells

- anucleated cells

- cells with 1 ori1 and 1 ori2

- cells with 2 ori1 and 2 ori2

- total number of ori1 foci

- total number of ori2 foci

- ori1 colocalized with ori2

- ori2 colocalized with ori1
Figure S2. The polar organizers are required for polar localization of the origins. 

(A) 10-fold serial dilutions of the indicated strains spotted on ATGN plate (left) or LB plate (right).

(B-F) Plots of the localization of ori1 (green) and ori2 (red) in (C) WT (AtWX263), (C) ΔpodJ (AtWX307), (D) ΔpopZ (AtWX303), (E) ΔpopZ ΔpodJ (AtWX305), (F) Δgpr (AtW309) for cells containing a single ori1 and ori2 focus (top), or two ori1 and ori2 foci (bottom). The percentage of cells in these subpopulations can be found in (H).

(G-H) Distribution of cells length (G) and cell width (H) in the indicated strains.

(I) Image analysis. Colocalization was defined as a pair of green and red foci that are with an inter-focal distance of less than 6 pixels. Images were analyzed using Oufti software, see Materials and Methods.
Figure S3. PopZ is enriched at ori1 and ori2, which requires ParB1 and RepBCh2 respectively.

(A-C) ChIP enrichment (ChIP/input) of (A) GFP-PopZ (AtWX234), (B) GFP-PodJ (AtWX263) and (C) GFP-GPR (AtWX236). Whole-genome profiles are shown on the left in 1-kb bins and high-resolution plots of ori1 and ori2 regions are shown on the right in 100-bp bins. Black asterisks indicate an enrichment peak present in all of our anti-GFP ChIP-seq experiments regardless of the fusion protein. Blue and gray dotted lines indicate the parS1 and parS2 sites, respectively.

(D) High-resolution ChIP enrichment (ChIP/input) of GFP-PopZ at ori1 (top) and ori2 (bottom) in ParB1⁺ (AtWX289 with inducers 1 μM AHL and 2 mM theophylline), ParB1⁻ (AtWX289 without inducers), and ∆repBCh2 (AtWX291). The enrichment of GFP-PopZ at the ori1 and ori2 regions depends on the presence of ParB1 and RepBCh2, respectively.
Ren Figure S4
**Figure S4. Quantification of inter-replicon interactions.**

(A-D) Quantifications of ori1-ori2 interactions (orange region) and Ch1-Ch2 alignment (blue regions) in different strains. Regions used for quantification are shown in (A-B). Details can be found in Materials and Methods. Interactions in $\Delta\text{repB}_{\text{Ch2}}$ is set as the background (0%, black dotted lines). After subtracting background, the percentage of interactions relative to the WT (100%) is shown.

(E-G) *A. tumefaciens* 15955 strain showed similar phenotype. Normalized Hi-C contact maps for (E) 15955 WT (11) (F) 15955 $\Delta\text{popZ}$ (IB173), (G) 15955 $\Delta\text{podJ}$ (IB172) grown in ATGN.
Figure S5. ChIP-seq enrichment of ParB1 and RepB\textsuperscript{Ch2} at cognate sites and reciprocal sites.

(A) ParB1 enrichment in wild-type cells (11). Sequencing reads from ChIP and input samples were normalized to the total number of reads and plotted in 1-kb bins. x-axis shows genome positions.

(B) RepB\textsuperscript{Ch2} enrichment in wild-type cells (11).

(C) High-resolution plots of ParB1 enrichment from (A) at 50-kb regions in encompassing ori1 (left panel) and ori2 (right panel). parS1 and parS2 sites are indicated by blue and gray dotted lines, respectively. Data are plotted in 100-bp bins.

(D) High-resolution plots of RepB\textsuperscript{Ch2} enrichment from (B) at 50-kb regions in encompassing ori1 (left panel) and ori2 (right panel).
Ren Figure S6

A

B

C

D
Figure S6. ParB1-RepB<sub>Ch2</sub> interactions could not be detected in BACTH or in vitro pulldown assays.

(A) BACTH interactions between ParB1 and RepB<sub>Ch2</sub>. E. coli strain BTH101 (56) expressing protein fusions to different domains (T25 and T18) of an adenylate cyclase. T25 and T18 fused to the same leucine zipper domain (“zip”) from yeast GCN4 serve as both positive and negative controls (38). This experiment detected interactions between ParB1 and ParB1, and between RepB<sub>Ch2</sub> and RepB<sub>Ch2</sub>, but not between ParB1 and RepB<sub>Ch2</sub>.

(B) An SDS-PAGE gel of an in vitro pulldown experiment. Affinity-purified ParB1 polyclonal antibodies were crosslinked to magnetic ProteinA Sepharose beads, which were then incubated with 50 μg of ParB1 protein and 50 μg of RepB<sub>Ch2</sub> proteins in 1 ml 1xPBS solution, singly or doubly. Similarly, beads crosslinked with purified RepB<sub>Ch2</sub> antibodies were incubated with proteins. Proteins were eluted in sample loading buffer at 65°C and separated by stain-free precast 4-20% polyacrylamide gradient gels (Bio-Rad 4561096). L is for protein ladder (BioRad 1610363). The gels were imaged using ProteinSimple Fluorchem R gel documentation system.

(C) An SDS-PAGE gel of an in vitro pulldown experiment using ParB1 beads similar to that in (B). The beads were incubated with ParB1, RepB<sub>Ch2</sub>, 1 mM CTP, 3 μM parS1 and parS2 DNA fragments. ParB1-RepB<sub>Ch2</sub> interactions were not detected.

(D) The SDS-PAGE gel in (C) was immunoblotted using RepB<sub>Ch2</sub> antibodies. A low amount of RepB<sub>Ch2</sub> protein could be detected when incubated with ParB1 beads alone, but this level did not increase in the presence of the ParB1 protein.
| Strain     | Genotype                                                                 | Reference | Figure |
|------------|---------------------------------------------------------------------------|-----------|--------|
| **A. tumefaciens used in main figures**                                                                                      |
| AtWX063    | C58, wild type                                                            | (1)       | 1B, 3A, S2A, S4ACD, S5ABCD |
| AtWX089    | C58, ΔrepB<sub>C</sub>(Atu3923/ATU_RS18280)::amp                         | (2)       | 1B, 3B, S2A, S4BCD |
| AtWX192    | C58, ΔtraI, tetRA::gen Ptra-riboswitch-parB1(Atu2828/ATU_RS13770) traR    | (2)       | 1B     |
| AtWX356    | C58, mcherry-parB<sub>PI</sub>-parS<sub>PI</sub> inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from ori1, ygfp-parB<sub>PMT1</sub>-par<sub>PMT1</sub> inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from ori2 | This study | 1CF |
| AtWX359    | C58, ygfp-parB<sub>PMT1</sub>-par<sub>PMT1</sub> inserted between Atu5336/ATU_RS25500 and Atu5337/ATU_RS25505, 11 kb from oAt | (2)       | 1CF |
| AtWX402    | C58, ΔrepB<sub>C</sub>(Atu3923/ATU_RS18280)::amp, mcherry-parB<sub>PI</sub>-parS<sub>PI</sub> inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from ori1, ygfp-parB<sub>PMT1</sub>-par<sub>PMT1</sub> inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from ori2 | This study | 1DF |
| AtWX500    | C58, ΔrepB<sub>C</sub>(Atu3923/ATU_RS18280)::amp, ygfp-parB<sub>PMT1</sub>-par<sub>PMT1</sub> inserted between Atu5336/ATU_RS25500 and Atu5337/ATU_RS25505, 11 kb from oAt | This study | 1DF |
| AtWX496    | C58, ΔtraI, tetRA::gen Ptra-riboswitch-parB1(Atu2828/ATU_RS13770) traR, mcherry-parB<sub>PI</sub>-parS<sub>PI</sub> inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from ori1, ygfp-parB<sub>PMT1</sub>-par<sub>PMT1</sub> inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from ori2 | This study | 1EF, S1 |
| AtWX498    | C58, ΔtraI, tetRA::gen Ptra-riboswitch-parB1(Atu2828/ATU_RS13770) traR, ygfp-parB<sub>PMT1</sub>-parS<sub>PMT1</sub> inserted between Atu5336/ATU_RS25500 and Atu5337/ATU_RS25505, 11 kb from oAt | This study | 1EF, S1 |
| AtWX277    | C58, ygfp-parB<sub>PMT1</sub>-par<sub>PMT1</sub> inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from ori1 | (2)       | 1F     |
| AtWX295    | C58, ygfp-parB<sub>PMT1</sub>-par<sub>PMT1</sub> inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from ori2 | (2)       | 1F     |
| Strain | Description | Notes |
|--------|-------------|-------|
| AtWX351 | C58, ygfp-parB<sup>PM1</sup>-parS<sup>PM1</sup> inserted between Atu0647/ATU_RS23235 and Atu0648/ATU_RS23240, 4 kb from oTi | (2) 1F |
| AtWX263 | C58, carrying pWX970, pSRKKm Plac rfp-repB<sup>Ch2</sup> (Atu3923/ATU_RS18280) terminator Plac egfp-parB1 (Atu2828/ATU_RS13770) terminators | (2) 2A, S2B |
| AtWX307 | C58, ΔpodJ (Atu0499/ATU_RS02460), containing pWX970 | This study 2B, S2C |
| AtWX303 | C58, ΔpopZ (Atu1720/ATU_RS08420), containing pWX970 | This study 2C, S2D |
| AtWX305 | C58, ΔpopZ (Atu1720/ATU_RS08420) ΔpodJ (Atu0499/ATU_RS02460), containing pWX970 | This study 2D, S2E |
| AtWX309 | C58, Δgpr (Atu1348/ATU_RS06650), containing pWX970 | This study 2E, S2F |
| AtWX283 | C58, ΔpodJ (Atu0499/ATU_RS02460) | This study 3C, S2A, S4CD |
| AtWX110 | C58, ΔpopZ (Atu1720/ATU_RS08420) | This study 3D, S2A, S4CD |
| AtWX121 | C58, ΔpopZ (Atu1720/ATU_RS08420) ΔpodJ (Atu0499/ATU_RS02460) | This study 3E, S2A, S4CD |
| AtWX286 | C58, Δgpr (Atu1348/ATU_RS06650) | This study 3F, S2A, S4CD |

**A. tumefaciens used for strain building and in supplemental figures**

| Strain | Description | Notes |
|--------|-------------|-------|
| AtWX234 | C58, containing pWX822, pSRKKm msfgfp-popZ (Atu1720/ATU_RS08420) | This study S3A |
| AtWX265 | C58, containing pMAT3, pSRKKm msfgfp-popJ (Atu0499/ATU_RS02460) | This study S3B |
| AtWX236 | C58, containing pJZ253, pSRKGm gfp-gpr (Atu1348/ATU_RS06650) | This study S3C |
| AtWX289 | C58, ΔtraI, tetRA::gen PtraI-riboswitch-parB1 (Atu2828/ATU_RS13770) traR, pSRKKm msfgfp-popZ (Atu1720/ATU_RS08420) | This study S3D |
| AtWX291 | C58, ΔrepB<sup>Ch2</sup>, pSRKKm msfgfp-popZ (Atu1720/ATU_RS08420) | This study S3D |
| AtWX486 | C58, ΔtraI, tetRA::gen PtraI-riboswitch-parB1 (Atu2828/ATU_RS13770) traR, ygfp-parB<sup>p1</sup>-parS<sup>p1</sup> inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from ori1 | This study |
| AtWX050 | 15955, wild type | (3) S4E |
| IB172 | 15955, ΔpodJ (ISGA_411) | This study S4F |
| IB173 | 15955, ΔpopZ (ISGA_1749) | This study S4G |

**B. subtilis strains used in main figures**

| Strain | Description | Notes |
|--------|-------------|-------|
| BWX5333 | pelB::Psoj mcherry-parB1<sub>Ai</sub> tet, parS<sub>2Ai</sub> cluster at -91° kan, ΔparB<sub>Bs</sub> spec | This study 4BCD |
| BWX5359 | ycgO::Phyperspank-optRBS-mgfpmut3-repB<sup>Ch2</sup>cat, parS<sub>2Ai</sub> cluster at -91° kan, ΔparB<sub>Bs</sub> spec | This study 4BCD |
| BWX5341 | pelB::Psoj mCherry-parB1<sub>Ai</sub> tet, ycgO::Phyperspank-optRBS-mgfpmut3-repB<sup>Ch2</sup>cat, ΔparB<sub>Bs</sub> spec | This study 4C |
| Strain Code | Genotype Description | References |
|------------|----------------------|------------|
| BWX5353   | pelB::Pssoj mCherry-parB1At tet, ycgO::Phyperspank-optRBS-mgfpmut3-repBCh2Al cat, parS2Al cluster at -91° kan, parSΔ9, ΔparB Bs (ΔparS) spec | This study 4D |
| AG1468    | Δspo0J::spec, trpC2, pheA1 | (4) |
| BWX2423   | ΔparB (ΔparS) spec | (5) |
| BWX3212   | parSΔ9 no a.b. | (6) |
| BWX3379   | parSΔ9 no a.b., parS at -91° ytuf kan | (7) |
| BWX5258   | pelB::Pssoj mcherry-parB1At tet | This study |
| BWX5260   | ycgO::Pssoj mgfpmut3-repBCh2Al cat | This study |
| BWX5265   | parS2Al cluster at -91° kan | This study |
| BWX5309   | parSΔ9 no a.b., ycgO::Phyperspank-optRBS-mgfpmut3-repBCh2Al cat | This study |
| BWX5329   | ycgO::Phyperspank mgfpmut3-repBCh2Al cat, parSΔ9 | This study |
| BWX5349   | pelB::Pssoj mCherry-parB1At tet, ycgO::Phyperspank-optRBS-mgfpmut3-repBCh2Al cat, parS2Al cluster at -91° kan, ΔparB Bs spec | This study |

**B. subtilis** strains used for strain building and in supplemental figures

1. **BWX5353**
   - pelB::Pssoj mCherry-parB1At tet, ycgO::Phyperspank-optRBS-mgfpmut3-repBCh2Al cat, parS2Al cluster at -91° kan, parSΔ9, ΔparB Bs (ΔparS) spec

2. **AG1468**
   - Δspo0J::spec, trpC2, pheA1

3. **BWX2423**
   - ΔparB (ΔparS) spec

4. **BWX3212**
   - parSΔ9 no a.b.

5. **BWX3379**
   - parSΔ9 no a.b., parS at -91° ytuf kan

6. **BWX5258**
   - pelB::Pssoj mcherry-parB1At tet

7. **BWX5260**
   - ycgO::Pssoj mgfpmut3-repBCh2Al cat

8. **BWX5265**
   - parS2Al cluster at -91° kan

9. **BWX5309**
   - parSΔ9 no a.b., ycgO::Phyperspank-optRBS-mgfpmut3-repBCh2Al cat

10. **BWX5329**
    - ycgO::Phyperspank mgfpmut3-repBCh2Al cat, parSΔ9

11. **BWX5349**
    - pelB::Pssoj mCherry-parB1At tet, ycgO::Phyperspank-optRBS-mgfpmut3-repBCh2Al cat, parS2Al cluster at -91° kan, ΔparB Bs spec
| Plasmid       | Description                                                                 | Reference                      |
|--------------|-----------------------------------------------------------------------------|--------------------------------|
| pFHC2973     | The plasmid carries $\text{cfp-parB}^{\text{PT}}$ and $\text{ygfparB}^{\text{PTT}}$   | (8)                            |
| pGM9         | pNPTS138 $\Delta$podJ (Atu0499/ATU_RS02460) (kan)                           | Fuqua Lab, unpublished         |
| pIB315       | pNPTS138 15955 $\Delta$popZ (ISGA_1749) (kan)                              | This study                     |
| pIB316       | pNPTS138 15955 $\Delta$podJ (ISGA_411) (kan)                               | This study                     |
| pJW005       | yhdG::Phyerspank-opt.rbs-sirA (phleo)                                       | (9)                            |
| pJZ25        | pSRK6m Plac gfpgpr (Atu1348/ATU_RS06650) (kan)                              | (10)                           |
| pJZ298       | pBSKII+ plasmid with sacB carb carrying 2kb sequencing homologous to gpr (Atu1348/ATU_RS06650) | (10)                           |
| pKNT18       | BACTH plasmid contains MCS t18 (amp)                                        | (11)                           |
| pKNT25       | BACTH plasmid contains MCS t25 (kan)                                        | (11)                           |
| pKT18        | BACTH plasmid contains t18 MCS (amp)                                        | (11)                           |
| pKT25        | BACTH plasmid contains t25 MCS (kan)                                        | (11)                           |
| pKT25zip     | BACTH Plasmid was used to express t25-zip (kan)                             | (11)                           |
| pUT18Czip    | BACTH Plasmid was used to express t18-zip (amp)                             | (11)                           |
| pMAT3        | pSRKKm Plac msfgfp-podJ (Atu0499/ATU_RS02460) (kan)                         | Fuqua Lab, unpublished         |
| pNPTS138     | onT sacB                                                     | (12)                           |
| pSRKKm       | Broad host-range, Plac (kan)                                                 | (13)                           |
| pSRKKm msfGFP| pSRKKm Plac msfgfp (kan)                                                     | (14)                           |
| mini-Tn7     | pUC18-mini-Tn7T gen Plac ha                                                | (14)                           |
| pWX294       | pACYC origin with MCS (amp)                                                  | This study                     |
| pWX563       | pelB::Psoj-mgfpmut3-spo0J (parS*) (tet)                                      | (5)                            |
| pWX564       | pelB::Psoj-mcherry-spo0J (parS*) (tet)                                       | (15)                           |
| pWX588       | ycgO::Pspank* (optRBS) gfsp-spo0J (parS*) cat                             | This study                     |
| pWX822       | pSRKKm Plac msfgfp-popZ (Atu1720/ATU_RS08420) (kan)                         | (13)                           |
| pWX839       | pNPTS138 $\Delta$popZ (Atu1720/ATU_RS08420) (kan)                         | This study                     |
| pWX845       | BACTH Plasmid was used to express t25-parB1 (kan)                          | This study                     |
| pWX846       | BACTH Plasmid was used to express t25-repB$^{\text{CH2}}$ (kan)            | This study                     |
| pWX847       | BACTH Plasmid was used to express parB1-t25 (kan)                          | This study                     |
| pWX848       | BACTH Plasmid was used to express repB$^{\text{CH2}}$-t25 (kan)            | This study                     |
| pWX849       | BACTH Plasmid was used to express t18-parB1 (amp)                          | This study                     |
| pWX850       | BACTH Plasmid was used to express t18-repB$^{\text{CH2}}$ (amp)            | This study                     |
| pWX851       | BACTH Plasmid was used to express parB1-t18 (amp)                          | This study                     |
| pWX852       | BACTH Plasmid was used to express repB$^{\text{CH2}}$-t18 (amp)            | This study                     |
| pWX854       | pNPTS138 repB$^{\text{CH2}}$ (Atu3923/ATU_RS18280)::ampR (kan)             | This study                     |
| pWX915       | pACYC terminator Ppen (amp)                                                  | This study                     |
| pWX916       | pACYC terminator Ppen cfp-parB$^{\text{PT}}$-parS$^{\text{PT}}$ (amp)       | This study                     |
| pWX930       | pNPTS138 Ppen cfp-parB$^{\text{PT}}$-parS$^{\text{PT}}$ kan at Atu3054/ATU_RS14060 | This study     |
| pWX936       | pNPTS138 PT7strong cfp-parB$^{\text{PT}}$-parS$^{\text{PT}}$ at Atu3054/ATU_RS14060 | This study     |
| pWX962  | pNPTS138 PT7strong cfp-parB^{P1}-parS^{P1} at Atu0048/ATU_RS00235 | This study |
| pWX967  | pNPTS138 PT7strong yGFP-parB^{MT1}-parS^{MT1} at Atu3973/ATU_RS18530 | This study |
| pWX970  | pSRKKm Plac rfp-repB^{Chz} (Atu3923/ATU_RS18280) terminator (Atu2828/ATU_RS13770) parB1-egfp Plac terminators | (2) |
| pWX995  | pNPTS138 terminators PT7strong mcherry-parB^{P1}-parS^{P1} at Atu0048/ATU_RS00235 | This study |
| pWX1005 | pNPTS138 yGFP-parB^{MT1}-parS^{MT1} Atu5337/ATU_RS25505 | (2) |
Table S1C. Oligonucleotides used in this study.

| Oligo   | Sequence                                                                 | Use          |
|---------|--------------------------------------------------------------------------|--------------|
| oML83   | CCTCATCCTCTTCATCCTC                                                     | sequencing   |
| oML85   | AATAGCGTCCTTGGCTCCTCGT                                                  | sequencing   |
| IBE140  | GGATCCAGAGCTCGATCATGTGCCGGG                                             | IB172        |
| IBE141  | CATCCGTTGCAAACGTTGATCATCTTTCGCTGCTTCG                                 | IB172        |
| IBE142  | GCGAAGCGAGCGAAAGGTAGTACCCGTTGGCAGAGTGATA                              | IB172        |
| IBE143  | GCTAGCAGCCAGCTTTCCGCGCCGGAAA                                          | IB172        |
| IBE144  | TTAGCGGGGAAAAGGGCCTCC                                                  | IB172        |
| IBE145  | CGTACGGCCCGAGAGGCGCC                                                   | IB172        |
| IBE146  | GGATCCACTGCGTGTGGCGTGGTGCGATA                                          | IB173        |
| IBE147  | ATGCGAGCAGCAGAGCTCATATCATCAATCCCCGCTTTCC                               | IB173        |
| IBE148  | GGGAAAGGCGGGATGGATATGGATGGTACCCGTTGGCAGAGTGATA                         | IB173        |
| IBE149  | GCTAGCAGCTGTGTTCCACTCACGCTTCGTG                                        | IB173        |
| IBE150  | CAGACCTTGTCACGGAGGC                                                    | IB173        |
| IBE151  | TCGAAGATTGGCCGGGCGCA                                                    | IB173        |
| oWX439  | TCCTTCTGCTCCCTCCTGCTCAG                                                 | BWX5265      |
| oWX776  | ATGGGCTGGAAGCCAGCGGAGG                                                 | sequencing   |
| oWX998  | AAACCCCGGACATAAGGAGGAACCTACTATGAGTAAAGG                                | pWX588       |
| oWX999  | TTTGCTAGCCAGAGTGGAAGAACAGCGCCTTAAACC                                  | pWX588       |
| oWX1279 | CTAATCCGACAGCTACCTCGAGCC                                              | BWX5265      |
| oWX1282 | CGATAAAGCGGACGGAGGATGCGGAGTC                                      | BWX5265      |
| oWX1283 | TCCTATTTGCACTCGGCCTCC                                                   | sequencing   |
| oWX1782 | TGAGTTCAGCTCCTACCGGAT                                                | sequencing   |
| oWX1783 | ACCAGCGGAGACTCAATGCTG                                                  | sequencing   |
| oWX1789 | CATCTTGCCAACCTCGCCGAG                                                  | sequencing   |
| oWX1790 | CCTCTTCTGCTATAGCGCCAGC                                                | sequencing   |
| oWX1835 | GCCAGGGTTTCACCGGCA                                                    | sequencing   |
| oWX1854 | CGCCAGGGTTTTGCCGACGAGGC                                               | sequencing   |
| oWX1855 | TCACACGAGAAACAGGTAGCC                                                 | sequencing   |
| oWX2044 | CAATTTCACACAGGGAAACAGCATATGAGTAAAGGTCAGCCACTGCTGTTCC                  | pWX822       |
| oWX2046 | GACGTCTCGACATCCTGTTAGTATGCTATCGGCCGT                                    | pWX822       |
| oWX2051 | TATAAAAGGCTGGAAGACCTCCAGCAGGCGTGCCAGGAGTCAGTGTAGTGGCCGTGAAAGGC         | pWX822       |
| oWX2052 | CTCGAGGTGTAAGTGGGAGGACCTCGGTAGGATGAAAGGTCAGGCGGAGGTGGCCGTGAAAGGC       | pWX822       |
| oWX2060 | GGAAGCGGCGAGCGGTGAGGC                                                  | sequencing   |
| oWX2061 | GAGGTTATGATGAGGAGGAGAGC                                                | sequencing   |
| oWX2076 | TGCGCGCAGGGCTATTCTGAGGATAGCAGCAGAGTGGCAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG | sequencing   |
| oWX2077 | GCTAGCGAATCTGCTGGATCCAGATCTAACCAGGCGCAGACCAGACCAGACCAGACCAGACCAGAC    | sequencing   |
| oWX2160   | CTGGCGCAAGCTTCTCTGCAGGATTGCCAAGGCAACTGTCTATCG | pWX839, sequencing |
| oWX2161   | GGCAGCGATTGAGCCCTGCGGAATATCAATCCCGGTTTCTACTC | pWX839 |
| oWX2162   | GAGTAGAAAAACGGGGATTGTGATATCCCGCAGCTACAATCGCTGCC | pWX839, sequencing |
| oWX2163   | AGCTAGCGAATTCGTTGCCAGATAGCTGGTGGTTTCATCAGGTTGC | pWX839, sequencing |
| oWX2190   | CGCTCTAGAGTAACACACAGGAAGACGTATGAGTGATCTTTCG | pWX847, pWX851 |
| oWX2191   | ATGCCCGGGCCGCAACCGGCTACCTTCTGTCCAGCAGCC | pWX847, pWX851 |
| oWX2193   | ATGCCCGGGTTTATTTTCTGCTCCAGCAGCC | pWX845, pWX849 |
| oWX2194   | CGCTCTAGAGTAACACACAGGAAGACGTATGAGCGGAAACAGATAATTCG | pWX848, pWX852 |
| oWX2195   | ATGCCCGGGCCGCAACCGGCTACCTTCTGCTCCAGGCTTTCG | pWX848, pWX852 |
| oWX2197   | ATGCCCGGGTTTACTCGTTGAGCCGTTTATTCG | pWX846, pWX850 |
| oWX2202   | CGCTCTAGAGGGCAGCGGCTACCTTCTGCTCCAGGCTTTCG | pWX845, pWX849 |
| oWX2203   | CGCTCTAGAGGGCAGCGGCTACCTTCTGCTCCAGGCTTTCG | pWX846, pWX850 |
| oWX2291   | GGCTGATTTGCGCATGACAATATTGCAGTGTCG | sequencing |
| oWX2292   | GTTCTCGGATCGGACAGTAGAAGTCAGGG | sequencing |
| oWX2377   | GGCTTCTTTGTTATCAAGGCGCCAG | sequencing |
| oWX2385   | GCTGAATTCCCGCGGAAGCGGGGTTTTTTTTTCGCTTGGGAAACGAGGTCATCATTTC | pWX915 |
| oWX2386   | TTTAAGCTTGAATTTTGATTGATGCGTACAGGATGAAG | pWX915 |
| oWX2387   | TCAATAATTTAAGCTTGAAGGAGGAGGTGGAACATGAGTAAAGGGAAGAAGAATTTTC | pWX916 |
| oWX2388   | TCTTAAATGACTCGCGAGAACTCGAGTTAATAGGAAATTTGATGGCGAAG | pWX916 |
| oWX2389   | CTGCCGATTTCAAATTCTACTTGAATCGTCTCAGGATGTTTACTCGAGTGCCGACTTTT | pWX916 |
| oWX2390   | GCCGATACTGAGTCGACATGGGCAGACGATCTCCGTGAATCAATCGTGCCGAATT | pWX916 |
| oWX2395   | TCTTTCGATTACGGCAGACATCC | sequencing |
| oWX2396   | CCGTCAATTGTGTCATTTACGTCGGAATT | sequencing |
| oWX2397   | GATGACGGTAAACTCAAAACCC | sequencing |
| oWX2407   | CTCTAGATAGCAGCATGCTGATTC | pWX930, pWX962 |
| oWX2408   | GGTATGCTAGTTATGGCTGAGC | pWX930, pWX962 |
| oWX2420   | GGCAGCGAAGTCTCTGCAGGATCCAGATACGATCC | sequencing |

14
| oWX2590   | GGTGGAATGGACGAATTATACAAAGAATTTCGAGCTCATGGTCGAGCAG | pWX995 |
| oWX2597   | CGCCAAGCTTCTCTGAGGATATCCCAGGGATGGCATTAAGGTCC      | sequencing |
| oWX2600   | AGCGAATTCGATCCAGATATCCTTCCGACAACGTCGTGGATGCC      | sequencing |
| oWX2649   | AAGCTTACATAAGGAGGAACTACTATGAGTAAAGGAGAAGAACTTTTCAC | BWX5309 |
| oWX2650   | CAGCTATGACAAACAAATGAAACAGC                        | BWX5309, BWX5329 |
| oWX2651   | GGATGCCGATACGGCTGAAGCG                           | BWX5309, BWX2651 |
| oWX2655   | ATAGTAGTTCCTCCTTTATGAAGCTTAATTGTTATCCGCTCAC      | BWX5309 |
| oWX2668   | TGCCCTCAAGCTAGAGTGCGATGTTCCAGACGTCCATTGCAGAG    | BWX5309 |
| oWX2669   | GAAGCTGAGCGTCTGAACATCGACTTCTCTAGCTTGAGGCATC      | BWX5309 |
| oWX2674   | CCGAATTAGCTTGCATGCAGTCAGCTTGGAGGACGTTCCATTCCGAG | BWX5329 |
| oWX2675   | AATACCGGTCAAAGCCATGTGCAATGCAAGCTATTGCGCTGG       | BWX5329 |
Table S1D. Next generation sequencing samples used in this study.

| Sample name | Figure | Reference | Identifier |
|-------------|--------|-----------|------------|
| 401_Wang_HiC_AtWX063_ATGN | 3A, S4ACD | (2) | GSM5542437 |
| 408_Wang_HiC_AtWX089_ATGN | 3B, S4BCD | (2) | GSM5542444 |
| 443_Wang_HiC_AtWX283_ATGN | 3C, S4CD | This study | GSM5870438 |
| 444_Wang_HiC_AtWX110_ATGN | 3D, S4CD | This study | GSM5870439 |
| 445_Wang_HiC_AtWX121_ATGN | 3E, S4CD | This study | GSM5870440 |
| 446_Wang_HiC_AtWX286_ATGN | 3F, S4CD | This study | GSM5870441 |
| 447_Wang_ChIP_anti_mCherry_BWX5333_CH | 4CD | This study | GSM5870442 |
| 448_Wang_input_BWX5333_CH | 4CD | This study | GSM5870443 |
| 449_Wang_ChIP_anti_GFP_BWX5359_CH_20uMIPTG1h | 4CD | This study | GSM5870444 |
| 450_Wang_input_BWX5359_CH_20uMIPTG1h | 4CD | This study | GSM5870445 |
| 451_Wang_ChIP_anti_GFP_BWX5341_CH_20uMIPTG1h | 4C | This study | GSM5870446 |
| 452_Wang_input_BWX5341_CH_20uMIPTG1h | 4C | This study | GSM5870447 |
| 453_Wang_ChIP_anti_mCherry_BWX5353_CH_20uMIPTG1h | 4D | This study | GSM5870448 |
| 454_Wang_input_BWX5353_CH_20uMIPTG1h | 4D | This study | GSM5870449 |
| 458_Wang_ChIP_anti_GFP_AtWX234_ATGN_halfmMIPTG4h | S3A | This study | GSM5870453 |
| 459_Wang_input_AtWX234_ATGN_halfmMIPTG4h | S3A | This study | GSM5870454 |
| 460_Wang_ChIP_anti_GFP_AtWX236_ATGN_halfmMIPTG4h | S3C | This study | GSM5870455 |
| 461_Wang_input_AtWX236_ATGN_halfmMIPTG4h | S3C | This study | GSM5870456 |
| 462_Wang_ChIP_anti_GFP_AtWX265_ATGN_halfmMIPTG4h | S3B | This study | GSM5870457 |
| 463_Wang_input_AtWX265_ATGN_halfmMIPTG4h | S3B | This study | GSM5870458 |
| 464_Wang_ChIP_anti_GFP_AtWX289_LB_2mMTheo_1uMAHL_halfmMIPTG4h | S3D | This study | GSM5870459 |
| 465_Wang_input_AtWX289_LB_2mMTheo_1uMAHL_halfmMIPTG4h | S3D | This study | GSM5870460 |
| 466_Wang_ChIP_anti_GFP_AtWX289_LB_halfmMIPTG4h_4h | S3D | This study | GSM5870461 |
| 467_Wang_input_AtWX289_LB_halfmMIPTG4h_4h | S3D | This study | GSM5870462 |
| 468_Wang_ChIP_anti_GFP_AtWX291_ATGN_halfmMIPTG4h | S3D | This study | GSM5870463 |
| 469_Wang_input_AtWX291_ATGN_halfmMIPTG4h | S3D | This study | GSM5870464 |
| 415_Wang_HiC_AtWX050_LB | S4E | (2) | GSM5542451 |
| 470_Wang_HiC_IB173_ATGN | S4F | This study | GSM5870465 |
| 471_Wang_HiC_IB172_ATGN | S4G | This study | GSM5870466 |
| 403_Wang_input_AtWX063_ATGN_rep2 | S5A-D | (2) | GSM5542439 |
| 404_Wang_ChIP_anti_AtParB_AtWX063_ATGN | S5AC | (2) | GSM5542440 |
| 405_Wang_ChIP_anti_AtRepBCh2_AtWX063_ATGN | S5BD | (2) | GSM5542441 |
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SUPPLEMENTAL MATERIALS AND METHODS

Plasmid construction

**pWX588** [ycgO::Pspank* (optRBS) gfp-spo0J (parS*) (cat)] was constructed by a ligation reaction containing two DNA fragments: 1) pAM12 (1) was digested by Xmal and NheI to give ycgO::Pspank* cat; 2) (optRBS) gfp-spo0J (parS*) was amplified using oWX998 and oWX999 from pKM256 (2).

**pWX822** [pSRKKm msfgfp-popZ (Atu1720/ATU_RS08420) (kan)] was constructed by an isothermal assembly reaction containing three DNA fragments: 1) pSRKKm digested by Ndel and HindIII; 2) msfgfp amplified using oWX2044 and oWX2046 from pSRKKm msfgfp; 3) At popZ amplified using oWX2051 and oWX2052 from C58 genomic DNA. The construct was sequenced using oWX1835, oWX2060 and oWX2061.

**pWX839** [pNPTS138 ΔpopZ (Atu1720/ATU_RS08420) (kan)] was constructed by an isothermal assembly reaction containing three gel-purified fragments: 1) pNPTS138 digested by EcoRV; 2) At popZ upstream region amplified using oWX2160 and oWX2161 from C58 genomic DNA; 3) At popZ downstream region amplified using oWX2162 and oWX2163 from C58 genomic DNA. The construct was sequenced using oWX1854 and oWX1855.

**pWX845** [pKT25 t25-parB1 (Atu2828/ATU_RS13770) (kan)] was constructed by ligating two DNA fragments: 1) pKT25 digested by Xbal and Xmal; 2) At parB1 amplified using oWX2202 and oWX2193 from C58 gDNA and then digested by Xbal and Xmal. The construct was sequenced using oWX1789 and oWX1790.

**pWX846** [pKT25 t25-repBCh2 (Atu3923/ATU_RS18280) (kan)] was constructed by ligating two DNA fragments: 1) pKT25 digested by Xbal and Xmal; 2) At repBCh2 amplified using oWX2203 and oWX2197 from C58 gDNA and then digested by Xbal and Xmal. The construct was sequenced using oWX1789 and oWX1790.
pWX847 [pKNT25 parB1-t25 (Atu2828/ATU_RS13770) (kan)] was constructed by ligating two DNA fragments: 1) pKNT25 digested by XbaI and XmaI; 2) At parB1 amplified using oWX2190 and oWX2191 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1782 and oWX1783.

pWX848 [pKNT25 repBCh2-t25 (Atu3923/ATU_RS18280) (kan)] was constructed by ligating two DNA fragments: 1) pKNT25 digested by XbaI and XmaI; 2) At repBCh2 amplified using oWX2194 and oWX2195 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1782 and oWX1783.

pWX849 [pKT18 t18-parB1 (Atu2828/ATU_RS13770) (amp)] was constructed by ligating two DNA fragments: 1) pKT18 digested by XbaI and XmaI; 2) At parB1 amplified using oWX2202 and oWX2193 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1789 and oWX1790.

pWX850 [pKT18 t18-repBCh2 (Atu3923/ATU_RS18280) (amp)] was constructed by ligating two DNA fragments: 1) pKT18 digested by XbaI and XmaI; 2) At repBCh2 amplified using oWX2203 and oWX2197 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1789 and oWX1790.

pWX851 [pKNT18 parB1-t18 (Atu2828/ATU_RS13770) (amp)] was constructed by ligating two DNA fragments: 1) pKNT18 digested by XbaI and XmaI; 2) At parB1 amplified using oWX2190 and oWX2191 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1782 and oWX1783.

pWX852 [pKNT18 repBCh2-t18 (Atu3923/ATU_RS18280) (amp)] was constructed by ligating two DNA fragments: 1) pKNT18 digested by XbaI and XmaI; 2) At repBCh2 amplified using oWX2194 and oWX2195 from C58 gDNA and then digested by XbaI and XmaI. The construct was sequenced using oWX1782 and oWX1783.
pWX915 [pACYC terminator Ppen] was constructed by ligating two DNA fragments: 1) pWX294 digested by EcoRI and HindIII; 2) Ppen amplified using oWX2385 and oWX2386 from gWX46. pWX294 is an empty cloning vector with pACYC origin. Ppen is a constitutive promoter the penicillinase gene from B. licheniformis. The construct was sequenced using oWX2395.

pWX916 [pACYC terminator Ppen cfp-parB^P1-parS^P1] was constructed by an isothermal assembly reaction containing three gel-purified fragments: 1) pWX915 digested by HindIII and BamHI; 2) rbs-cfp-parB^P1 amplified using oWX2387 and oWX2388 from pFHC2973 (3); 3) parS^P1 amplified using oWX2389 and oWX2390 from gDNA of TND1379 (4). The construct was sequenced using oWX2395, oWX2396, oWX2397 and 2377.

pWX930 [pNPTS138 Ppen cfp-parB^P1-parS^P1 kan at Atu3054/ATU_RS14060] was constructed by an isothermal assembly reaction containing four gel-purified fragments: 1) pNPTS138 digested by EcoRV; 2) a part of Atu3054/ATU_RS14060 amplified using oWX2420 and oWX2421 from C58 gDNA; 3) cfp-parB^P1-parS^P1 amplified using oWX2407 and oWX2408 from pWX916 4) a part of Atu3055/ATU_RS14065 amplified using oWX2422 and oWX2423 from C58 gDNA. The construct was sequenced using oWX2424, oWX2426, oWX2377 and oWX2425.

pWX936 [pNPTS138 PT7strong cfp-parB^P1-parS^P1 at Atu3054/ATU_RS14060] was constructed by an isothermal assembly reaction containing one gel-purified fragments: pWX930 backbone amplified using oWX2431 and oWX2432. The construct was sequenced using oWX2424, oWX2426, oWX2377 and oWX2425.

pWX962 [pNPTS138 PT7strong cfp-parB^P1-parS^P1 at Atu0048/ATU_RS00235] was constructed by an isothermal assembly reaction containing four gel-purified fragments: 1) pNPTS138 digested by EcoRV; 2) PT7strong cfp-parB^P1-parS^P1 amplified using oWX2407 and oWX2408 from pWX936; 3) a part of Atu0047/ATU_RS00230 amplified using oWX2502 and oWX2503 from C58 gDNA; 4) a part of Atu0048/ATU_RS00235
amplified using oWX2504 and oWX2505 from C58 gDNA. The construct was sequenced using oWX2506, oWX2377, oWX2426, oWX2507.

**pWX995** [pNPTS138 terminators PT7strong mcherry-parB\(^{P1}\)-par\(^{S1}\) at Atu0048/ATU_RS00235] was constructed by an isothermal assembly reaction containing two gel-purified fragments: 1) pWX962 backbone amplified using oWX2589 and oWX2590 on pWX962; 2) mcherry amplified using oWX2584 and oWX2585 from gDNA of BWX2208 (5). The construct was sequenced using oWX2506, oWX2377, oWX2426 and oWX2507.

**pIB315** [pNPTS138 15955 ∆popZ (ISGA_1749) (kan)] was constructed in two steps. First, At 15955 popZ upstream amplified using IPB140 and IBP141 and At 15955 popZ downstream amplified using IPB142 and IBP143 from 15955 gDNA were stitched together by PCR and then ligated into pGEM T-easy (Promega), confirmed by sequencing (6). Next the stitched fragment digested using BamH1 and Nhe1 and pNPTS138 digested with the same enzymes were ligated together.

**pIB316** [pNPTS138 15955 ∆podJ (ISGA_411) (kan)] was constructed in two steps. First, At 15955 podJ upstream amplified using IPB146 and IBP147 and At 15955 podJ downstream amplified using IPB148 and IBP149 from 15955 gDNA were stitched together by PCR and then ligated into pGEM T-easy (Promega), confirmed by sequencing (6). Next the stitched fragment digested using BamH1 and Nhe1 and pNPTS138 digested with the same enzymes were ligated together.

**A. tumefaciens** Strain construction
In general, in-frame deletions of C58 A. tumefaciens strains were constructed using a previously described allelic replacement method (6). Briefly, regions flanking the gene to be deleted were PCR amplified using Phusion (NEB M0530) or Q5 polymerase (NEB M0491) and cloned into pNPTS138 (7), a ColE1 suicide plasmid that confers kanamycin resistance and sucrose sensitivity, by isothermal assembly reactions. See Plasmid construction for details. pNPTS138 deletion constructs were then introduced into A.
tumefaciens C58 via mating with E. coli S17-1/λpir (8) carrying the appropriate construct. Screening for plasmid integration and target gene deletion was performed as previously described (6, 9). Colony PCR was used to amplify the region to confirm the deletion mutants. Specifically,

C58, mcherry-parB<sup>P1</sup>-parS<sup>P1</sup> inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from ori1, ygfp-parB<sup>MT1</sup>-parS<sup>MT1</sup> inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from ori2 (AtWX356) was generated in two steps. First, pWX967 was used to insert ygfp-parB<sup>MT1</sup>-parS<sup>MT1</sup> between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from ori2, generating AtWX295. This strain was confirmed using oWX2508 and oWX2511. Next, pWX995 was used to insert mcherry-parB<sup>P1</sup>-parS<sup>P1</sup> between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from ori1, generating AtWX356. This strain was confirmed using oWX2502 and oWX2505.

C58, ∆repB<sup>Ch2</sup>, mcherry-parB<sup>P1</sup>-parS<sup>P1</sup> inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from ori1, ygfp-parB<sup>MT1</sup>-parS<sup>MT1</sup> inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from ori2 (AtWX402) was generating using pWX854 on AtWX356 (see above), and conformed using oWX2076 and oWX2077.

C58, ∆traI, tetRA::gen PtraI-riboswitch-parB1(Atu2828/ATU_RS13770) traR, mcherry-parB<sup>P1</sup>-parS<sup>P1</sup> inserted between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from ori1, ygfp-parB<sup>MT1</sup>-parS<sup>MT1</sup> inserted between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from ori2 (AtWX496) was generated in two steps. First, pWX967 was used to insert ygfp-parB<sup>MT1</sup>-parS<sup>MT1</sup> between Atu3973/ATU_RS18530 and Atu3974/ATU_RS18535, 57 kb from ori2 on AtWX192 (10), generating AtWX486. This strain was confirmed using oWX2508 and oWX2511. Next, pWX995 was used to insert mcherry-parB<sup>P1</sup>-parS<sup>P1</sup> between Atu0047/ATU_RS00230 and Atu0048/ATU_RS00235, 50 kb from ori1 on AtWX486, generating AtWX496. This strain was confirmed using oWX2502 and oWX2505.
AtWX192 contains ΔtraI, tetRA::gen Ptra-riboswitch-parB1(Atu2828/ATU_RS13770) traR (10).

C58, ΔtraI, tetRA::gen Ptra-riboswitch-parB1(Atu2828/ATU_RS13770) traR, ygfp-parB<sub>pMT1</sub>-parS<sub>pMT1</sub> inserted between Atu5336/ATU_RS25500 and Atu5337/ATU_RS25505, 11 kb from oAt (AtWX498) was generated using pWX1005 on AtWX192 (10), and confirmed using oWX2597 and oWX2600.

C58, ΔrepB<sup>Ch2</sup>, ygfp-parB<sub>pMT1</sub>-parS<sub>pMT1</sub> inserted between Atu5336/ATU_RS25500 and Atu5337/ATU_RS25505, 11 kb from oAt (AtWX500) was generated using pWX1005 on AtWX089 (10), and confirmed using oWX2597 and oWX2600. AtWX089 contains ΔrepB<sup>Ch2</sup> (10).

C58, ΔpodJ (Atu0499/ATU_RS02460) (AtWX283) was generated using pGM9, and confirmed using oWX2291 and oWX2292.

C58, ΔpopZ (Atu1720/ATU_RS08420) (AtWX110) was generated using pWX839, and confirmed using oWX2160 and oWX2163.

C58, ΔpopZ (Atu1720/ATU_RS08420) ΔpodJ (Atu0499/ATU_RS02460) (AtWX121) was generated using pGM9 on AtWX110, and confirmed using oWX2291 and oWX2292.

C58, Δgpr (Atu1348/ATU_RS06650) (AtWX286) was generated using pJZ298 (11), and confirmed using oWX2530 and oWX2531.

15955, ΔpodJ (ISGA_411) (IB172) was generated using plB316, and confirmed using IBP144 and IBP145.

15955, ΔpopZ (ISGA_1749) (IB173) was generated using plB315, and confirmed using IBP150 and IBP151.
Replicative plasmids were introduced to *A. tumefaciens* by electroporation as previously described (6). pWX822, pJZ253, pMAT3 were electroporated into C58 WT, generating AtWX234, AtWX236, AtWX265. pWX970 was electroporated into AtWX110, AtWX121, AtWX283 and AtWX286 to generate AtWX303, AtWX305, AtWX307 and AtWX309. pWX822 was electroporated into AtWX089 (10) and AtWX192 (10), generating AtWX291 and AtWX289, respectively.

**B. subtilis** Strain construction

*pelB::Psoj mCherry-parB1At tet* (BWX5258) A ligation reaction containing the following two DNA fragments was directly transformed to PY79: 1) pWX564 [*pelB::Psoj-mcherry-spo0J (parS*) (tet)*] (12) cut with BamHI and Xhol to remove *spo0J (parS*)*; 2) *parB1At* (amplified from C58 genomic DNA using oWX2563 and oWX2564, and then cut with BamHI and Xhol). The transformants were amplified using oWX776 and oML85 and sequenced using oWX776 and oML85.

*ycgO::Psoj mgfpmut3-RepB^{Ch2}_At cat* (BWX5260) A ligation reaction containing the following three DNA fragments was directly transformed to PY79: 1) an empty cloning vector pKM077 [*ycgO::cat*] cut with EcoRI and BamHI; 2) *repB^{Ch2}_At* (amplified from C58 genomic DNA using oWX2566 and oWX2567, and then cut with BamHI and Xhol); 3) *Psoj mgfpmut3* liborated from pWX563 using EcoRI and Xhol. The transformants were amplified and sequenced using oWX2497 and oWX2568. pWX563 (13) contains *pelB::Psoj-mgfpmut3-spo0J (parS*) tet*.

*parS2At cluster at -91°* kan (BWX5265) An isothermal assembly reaction containing the following three PCR products was directly transformed to PY79: 1) the region containing *ytuf* upstream region (amplified from PY79 genomic DNA using oWX1279 and oWX2569); 2) the *parS2At* region (amplified from C58 genomic DNA using oWX2570 and oWX2571); 3) the region containing *kan*, *ytuf* and *ytuf* downstream (amplified from BWX3379 genomic DNA (14) using primers oWX439 and oWX1282).
The transformants were amplified using oWX1283 and oML83 and sequenced using oWX1283 and oML83.

**parS\(\Delta9\) no a.b., ycgO::Phyperspank-optRBS-mgfpmut3-repB\(Ch_2\)\(_{At}\) cat (BWX5309)**

An isothermal assembly reaction containing the following three PCR products was directly transformed to BWX3212 (15): 1) the region containing ycgO downstream (amplified from PY79 genomic DNA using oWX2668 and oWX2650); 2) the *Phyperspank* promotor amplified from pJW005 (16) using oWX2655 and oWX2669); 3) the region containing *mgfpmut3-repB*\(Ch_2\)\(_{At}\), cat, ycgO downstream (amplified from genomic DNA of BWX5260 using primers oWX2649 and oWX2651). The transformants were amplified using oWX2568 and oWX2560 and sequenced using oWX2568 and oWX2497.

**ycgO::Phyperspank mgfpmut3-repB\(Ch_2\)\(_{At}\) cat, parS\(\Delta9\) (BWX5329)**

An isothermal assembly reaction containing the following three PCR products was directly transformed to BWX3212 (15): 1) the region containing ycgO downstream and *Phyperspank mgfpmut3-repB*\(Ch_2\)\(_{At}\) (amplified from genomic DNA of BWX5309 (see above) using oWX2674 and oWX2650); 2) the *lacl-cat* and ycgO upstream (amplified from pWX588 using oWX2675 and oWX2651). pWX588 contains ycgO::Pspank\(^*\) (optRBS) gfp-spo0J (parS\(^*\)) cat. The transformants were amplified using oWX2568 and oWX2560 and sequenced using oWX2568 and oWX2497.

After individual *B. subtilis* constructs were built as above, their genomic DNA was extracted and used in successive transformations to build BWX5333, BWX5341, BWX5349, BWX5353, BWX5359.
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