

SI EXPERIMENTAL PROCEDURES

Table S1. Strains used in this study

Table S2. Plasmids used in this study

Table S3. Oligonucleotides used in this study

Table S4. Strains used in each figure

Regulation of DNA replication initiation by ParA is independent of *parS* location in *Bacillus subtilis*

Supplementary material

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Supplementary Figures

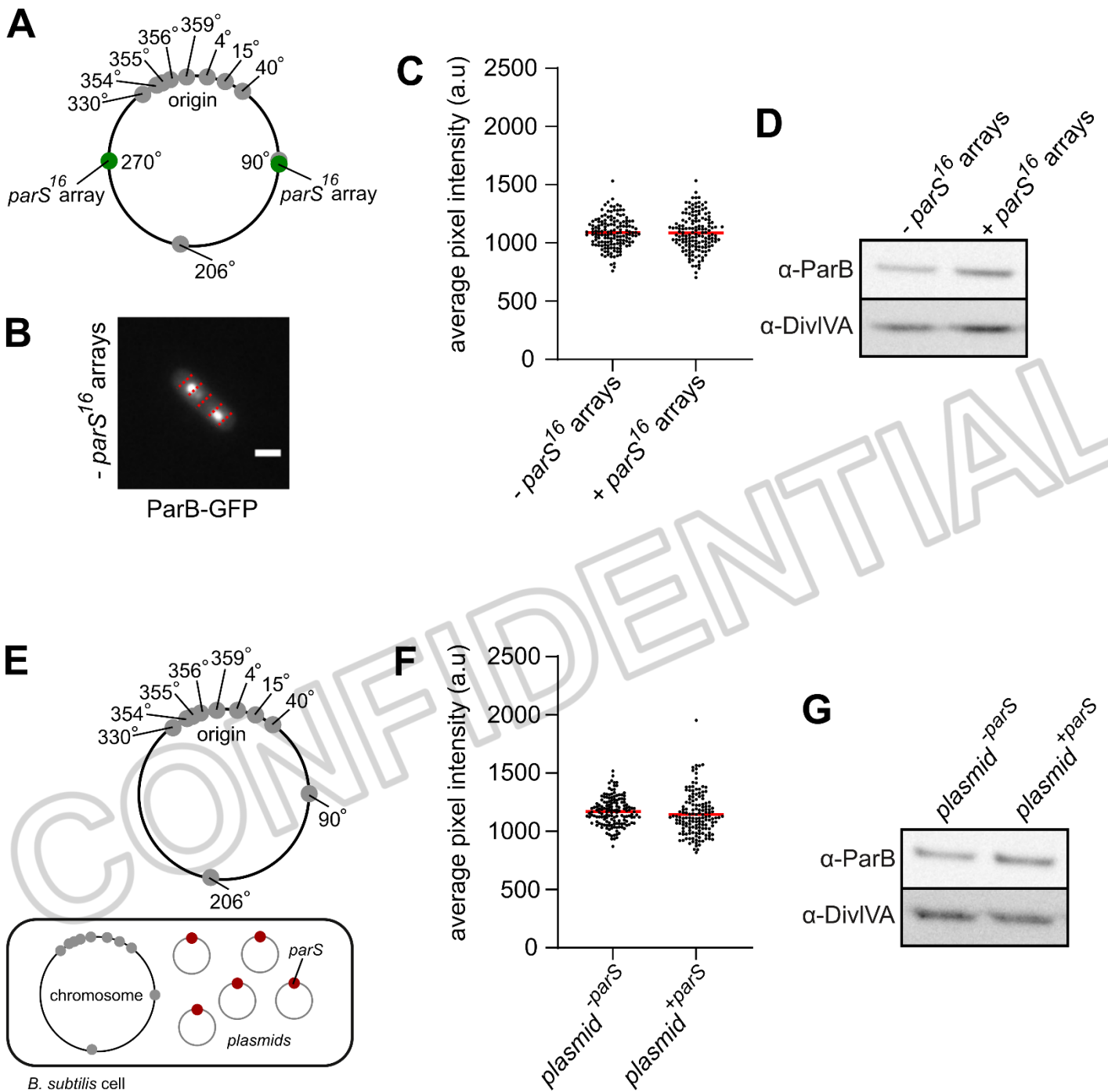


Figure S1: Intracellular level of ParB remains unchanged in cells harbouring extra *parS* sites. **(A)** Schematic diagram showing the location of *B. subtilis* endogenous chromosomal *parS* (grey circles) and the *parS*¹⁶ arrays (green circles). **(B)** Fluorescent image of a representative cell used for the intensity profile plot. Dotted lines represent the area measured to generate the intensity profile of individual cells. Scale bar, 1 μm . **(C)** The average fluorescent intensity for ParB-

GFP in strains containing chromosomal *parS*¹⁶ arrays was measured for 150 individual cells. P-value of 0.9462. **(D)** Intracellular ParB level in strains containing chromosomal *parS*¹⁶ arrays was detected by immunoblot using an α -ParB antibody (top panel) and α -DivIVA antibody as a loading control (bottom panel). **(E)** Schematic diagram showing the location of *B. subtilis* endogenous chromosomal *parS* (grey circles) and plasmid containing a single *parS* (red circles). **(F)** The average fluorescent intensity for ParB-GFP in strains containing extrachromosomal *parS* was measured for 150 individual cells. P-value of 0.1377. **(G)** Intracellular ParB level in strains containing extrachromosomal *parS* was detected by Western blot using an α -ParB antibody (top panel) and α -DivIVA antibody as a loading control (bottom panel).

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parS mutants

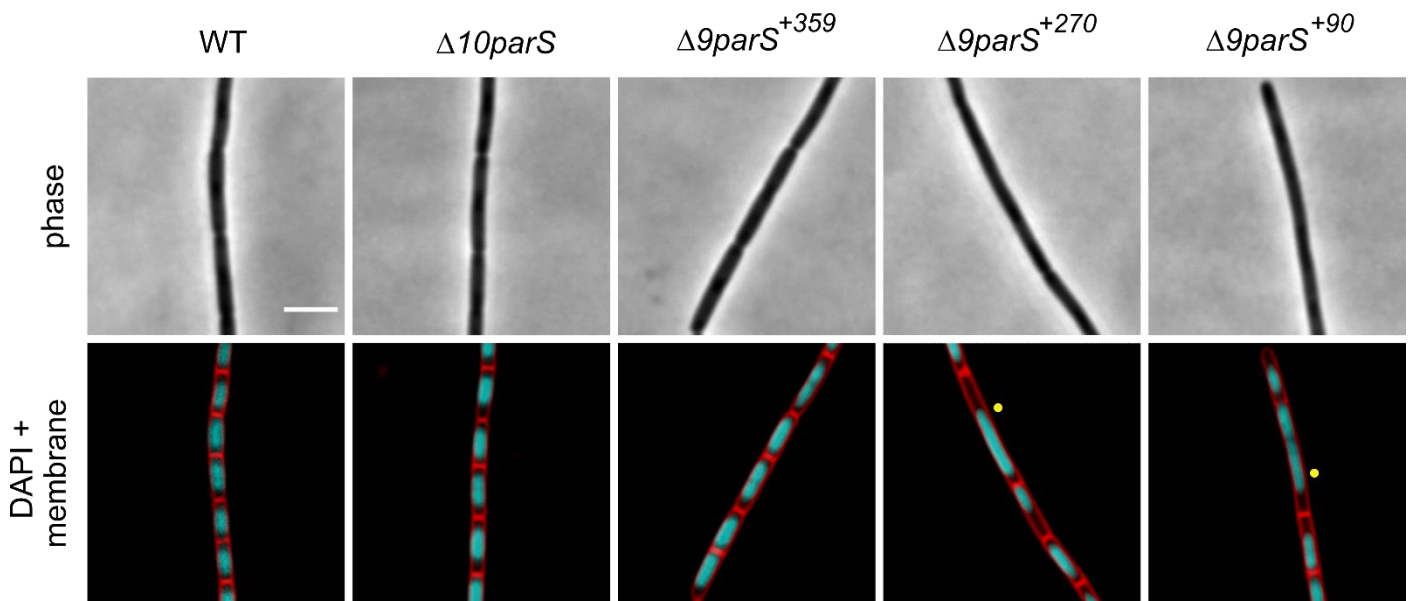


Figure S2: Chromosome organization was disrupted when *parS* was located away from the origin. DAPI staining of chromosomal DNA in strains that differ in the location of *parS*. Phase

contrast (top panel) and membrane dye FM 5-95 combined with DNA stain DAPI (bottom panel).

Scale bar, 3 μ m.

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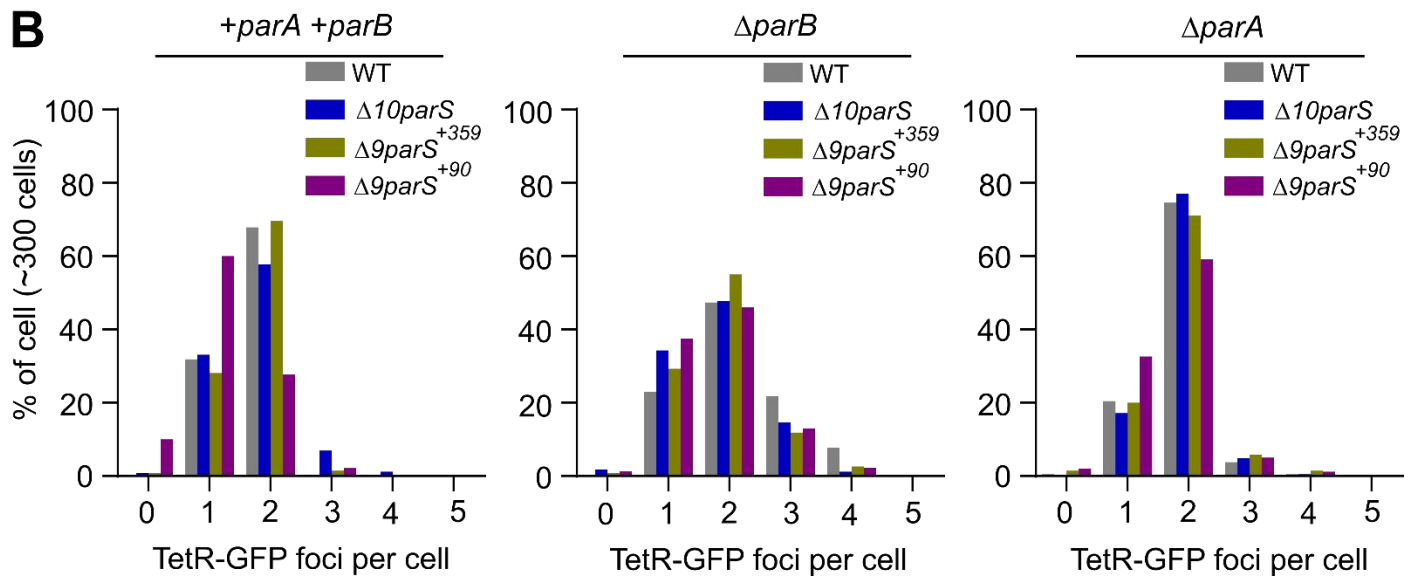
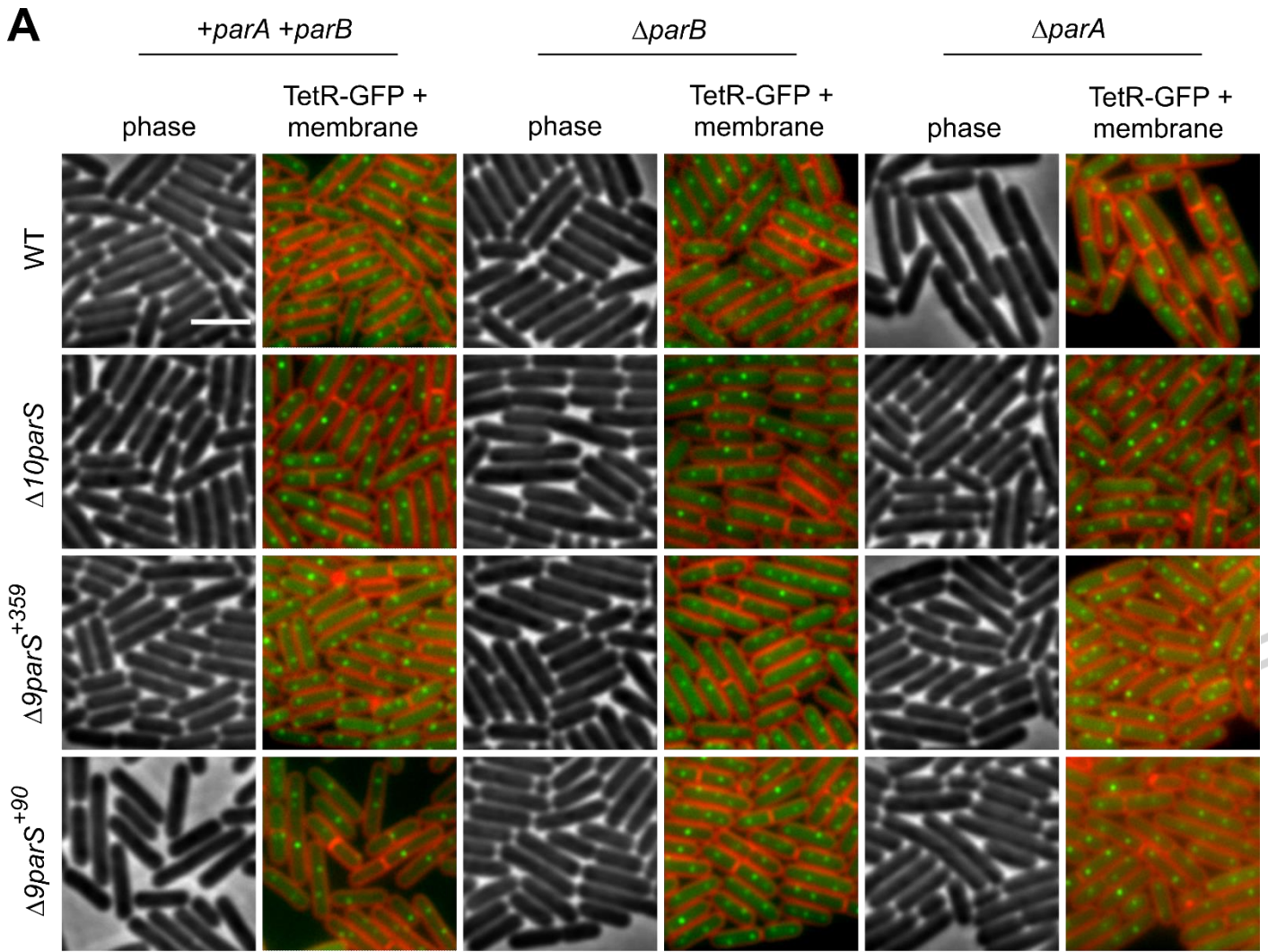


Figure S3: A single *parS* redistribute ParB to disrupt chromosome origin segregation. (A)

Origin localization in a wild-type strain and strains that differ in the location of *parS*. The *oriC*

region was labelled using an array of *tetO* operators bound by TetR-GFP. Phase contrast (left panel), and membrane dye FM 5-95 combined with TetR-GFP (right panel). **(B)** ParB dependent decrease when *parS* was located away from the origin. Scale bar, 3 μ m.

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Supplementary Table 1: Strains used in this study

Strains	Genotype ^a	Construction ^b	Source
<i>B. subtilis</i> 168CA	<i>trpC2</i> wild type	-	[1]
<i>B. subtilis</i> 168CA AK47	<i>trpC2 tetR-gfp(spc)/tetO⁻⁷(ery)</i>	-	[2]
<i>B. subtilis</i> 168CA AK83	<i>trpC2 cotS::parS¹⁶ array cat</i>	PSL23 → 168CA	this work
<i>B. subtilis</i> 168CA AK87	<i>trpC2 parA-parB::neo tetR-gfp/tetO⁻⁷ parS¹⁶ arrays</i>	AK83 → AK99	this work
<i>B. subtilis</i> 168CA AK89	<i>trpC2 ΔparA::neo tetR-gfp/tetO⁻⁷ parS¹⁶ arrays</i>	HM766 → AK97	this work
<i>B. subtilis</i> 168CA AK91	<i>trpC2 yheH::parS¹⁶ array cat</i>	PSL25 → 168CA	this work
<i>B. subtilis</i> 168CA AK93	<i>trpC2 yheH::parS¹⁶ array tet</i>	pcat::tet → AK91	this work
<i>B. subtilis</i> 168CA AK95	<i>trpC2 tetR-gfp/tetO⁻⁷ yheH::parS¹⁶ array tet</i>	AK93 → AK47	this work
<i>B. subtilis</i> 168CA AK97	<i>trpC2 tetR-gfp/tetO⁻⁷ parS¹⁶ arrays</i>	AK83 → AK95	this work
<i>B. subtilis</i> 168CA AK99	<i>trpC2 parA-parB::neo tetR-gfp/tetO⁻⁷ yheH::parS¹⁶ array tet</i>	HM765 → AK95	this work
<i>B. subtilis</i> 168CA AK123	<i>trpC2 ΔparB::neo tetR-gfp/tetO⁻⁷ parS¹⁶ arrays</i>	HM907 → AK97	this work
<i>B. subtilis</i> 168CA AK150	<i>trpC2 parS¹⁶ arrays</i>	AK83 → AK93	this work
<i>B. subtilis</i> 168CA AK153	<i>trpC2 parB-gfp::neo parS¹⁶ arrays</i>	HM756 → AK150	this work
<i>B. subtilis</i> PY79 AK169	<i>tetR-gfp/tetO⁻⁷</i>	BKM865 → BKM918	this work
<i>B. subtilis</i> PY79 AK171	<i>Δ8parS tetR-gfp/tetO⁻⁷</i>	AK47 ^c → BNS1657	this work
<i>B. subtilis</i> PY79 AK177	<i>Δ8parS parA-parB::neo tetR-gfp/tetO⁻⁷</i>	pHM142 → AK171	this work
<i>B. subtilis</i> PY79 AK179	<i>Δ8parS ΔparB::neo tetR-gfp/tetO⁻⁷</i>	pHM456 → AK171	this work
<i>B. subtilis</i> PY79 AK181	<i>parA-parB::neo tetR-gfp/tetO⁻⁷</i>	pHM48 → AK169	this work
<i>B. subtilis</i> PY79 AK183	<i>ΔparB::neo tetR-gfp/tetO⁻⁷</i>	HM907 → AK169	this work
<i>B. subtilis</i> PY79 AK187	<i>ΔparA::neo tetR-gfp/tetO⁻⁷</i>	pAK98 → AK169	this work
<i>B. subtilis</i> 168CA AK199	<i>trpC2 gfp-parA::neo parS¹⁶ arrays</i>	HM740 → AK150	this work
<i>B. subtilis</i> PY79 AK205	<i>Δ9parS (native parS⁹⁰)</i>	-	[3]
<i>B. subtilis</i> PY79 AK207	<i>Δ9parS parA-parB::neo tetR-gfp/tetO⁻⁷</i>	pAK09 → AK177	this work
<i>B. subtilis</i> PY79 AK209	<i>Δ9parS ΔparB::neo tetR-gfp/tetO⁻⁷</i>	pAK09 → AK179	this work
<i>B. subtilis</i> PY79 AK213	<i>Δ9parS (native parS²⁰⁶)</i>	pAK23 → BNS1657	this work
<i>B. subtilis</i> PY79 AK215	<i>Δ10parS</i>	-	[3]
<i>B. subtilis</i> PY79 AK225	<i>Δ10parS parA-parB::neo tetR-gfp/tetO⁻⁷</i>	pAK23 → AK207	this work
<i>B. subtilis</i> PY79 AK227	<i>Δ10parS ΔparB::neo tetR-gfp/tetO⁻⁷</i>	pAK23 → AK209	this work
<i>B. subtilis</i> PY79 AK239	<i>parA-parB::neo</i>	pAK26 → PY79	this work
<i>B. subtilis</i> PY79 AK241	<i>ΔparB::neo</i>	-	[3]
<i>B. subtilis</i> PY79 AK243	<i>Δ10parS parA-parB::neo</i>	-	[3]
<i>B. subtilis</i> PY79 AK245	<i>Δ10parS ΔparB::neo</i>	pAK30 → AK215	this work
<i>B. subtilis</i> PY79 AK259	<i>Δ9parS⁺³⁵⁹ parA-parB::neo</i>	pAK26 → AK215	this work
<i>B. subtilis</i> PY79 AK261	<i>Δ9parS⁺³⁵⁹ ΔparB::neo</i>	pAK28 → AK215	this work
<i>B. subtilis</i> PY79 AK277	<i>gfp-parA::neo</i>	pHM23 → PY79	this work
<i>B. subtilis</i> PY79 AK281	<i>Δ10parS gfp-parA::neo</i>	pAK48 → AK215	this work

<i>B. subtilis</i> PY79 AK305	$\Delta 9parS^{+359} gfp-parA::neo$	pHM23 → AK215	this work
<i>B. subtilis</i> PY79 AK315	$\Delta 9parS^{+90}$	pAK56 → AK205	this work
<i>B. subtilis</i> PY79 AK323	$\Delta 9parS^{+90} parA-parB::neo$	pAK32 → AK315	this work
<i>B. subtilis</i> PY79 AK325	$\Delta 9parS^{+90} \Delta parB::neo$	pAK30 → AK315	this work
<i>B. subtilis</i> PY79 AK367	<i>parB-gfp::neo</i>	pAK82 → PY79	this work
<i>B. subtilis</i> PY79 AK369	$\Delta 10parS parB-gfp::neo$	pAK83 → AK215	this work
<i>B. subtilis</i> PY79 AK373	$\Delta 9parS^{+90} parB-gfp::neo$	pAK83 → AK315	this work
<i>B. subtilis</i> PY79 AK399	$\Delta 9parS^{+90} gfp-parA::neo$	pAK48 → AK315	this work
<i>B. subtilis</i> PY79 AK405	$\Delta 9parS^{+90} tetR-gfp/tetO^{-7}$	pAK56 → AK207	this work
<i>B. subtilis</i> PY79 AK519	$\Delta 9parS tetR-gfp/tetO^{-7}$	pAK23 → AK171	this work
<i>B. subtilis</i> PY79 AK521	$\Delta 10parS tetR-gfp/tetO^{-7}$	pAK09 → AK519	this work
<i>B. subtilis</i> 168CA AK547	<i>trpC2 Δspo0J gfp-parA::neo</i>	HM13 → 168CA	this work
<i>B. subtilis</i> PY79 AK553	$\Delta 9parS^{+90} \Delta (parA-parB)::neo$	pAK100 → AK315	this work
<i>B. subtilis</i> PY79 AK555	$\Delta 9parS^{+90} \Delta parA::neo$	pAK101 → AK315	this work
<i>B. subtilis</i> PY79 AK557	<i>ΔparA::neo</i>	pAK98 → PY79	this work
<i>B. subtilis</i> PY79 AK559	$\Delta (parA-parB)::neo$	pAK99 → PY79	this work
<i>B. subtilis</i> PY79 AK561	$\Delta 9parS^{+359} \Delta (parA-parB)::neo$	pAK99 → AK215	this work
<i>B. subtilis</i> PY79 AK563	$\Delta 10parS \Delta (parA-parB)::neo$	pAK100 → AK215	this work
<i>B. subtilis</i> 168CA AK569	<i>trpC2 Δspo0J gfp-soj::neo plasmid^{+parS}</i>	pHM430 → AK547	this work
<i>B. subtilis</i> 168CA AK571	<i>trpC2 Δspo0J gfp-soj::neo plasmid^{+parS}</i>	pHM432 → AK547	this work
<i>B. subtilis</i> 168CA AK573	<i>trpC2 Δ(parA-parB) tetR-gfp/tetO⁻⁷</i>	HM749 → AK47	this work
<i>B. subtilis</i> 168CA AK575	<i>trpC2 Δ(parA-parB)::neo tetR-gfp/tetO⁻⁷ parS¹⁶ arrays</i>	HM749 → AK97	this work
<i>B. subtilis</i> 168CA AK577	<i>trpC2 ΔparB gfp-parA::neo parS¹⁶ arrays</i>	AK547 → AK150	this work
<i>B. subtilis</i> 168CA AK585	<i>trpC2 Δsoj Δspo0J tetR-yfp/tetO⁻¹⁵⁰</i>	HM194 → 168CA	this work
<i>B. subtilis</i> 168CA AK587	<i>trpC2 Δsoj Δspo0J tetR-yfp/tetO⁻¹⁵⁰ plasmid^{+parS}</i>	pHM430 → AK585	this work
<i>B. subtilis</i> 168CA AK589	<i>trpC2 Δsoj Δspo0J tetR-yfp/tetO⁻¹⁵⁰ plasmid^{+parS}</i>	pHM432 → AK585	this work
<i>B. subtilis</i> PY79 AK591	$\Delta 9parS^{+359} tetR-gfp/tetO^{-7}$	pAK26 → AK521	this work
<i>B. subtilis</i> PY79 AK593	$\Delta 9parS^{+359} parB-gfp::neo$	pAK82 → AK215	this work
<i>B. subtilis</i> PY79 AK597	$\Delta 9parS^{+270} parB-gfp::neo$	pAK180 → AK369	this work
<i>B. subtilis</i> PY79 AK599	$\Delta 9parS^{+270} gfp-parA::neo$	pAK180 → AK281	this work
<i>B. subtilis</i> PY79 AK603	$\Delta 9parS^{+270} soj-parB::neo$	pAK180 → AK243	this work
<i>B. subtilis</i> PY79 AK605	$\Delta 9parS^{+270} \Delta parB::neo$	pAK180 → AK245	this work
<i>B. subtilis</i> PY79 AK607	$\Delta 9parS^{+270} \Delta (parA-parB)::neo$	pAK180 → AK563	this work
<i>B. subtilis</i> PY79 AK615	$\Delta 9parS^{+90} \Delta parB::neo tetR-gfp/tetO^{-7}$	pAK56 → AK209	this work
<i>B. subtilis</i> PY79 AK617	$\Delta 10parS \Delta parA::neo$	pAK101 → AK215	this work
<i>B. subtilis</i> PY79 AK639	$\Delta 9parS^{+359} \Delta parB::neo tetR-gfp/tetO^{-7}$	pAK28 → AK521	this work
<i>B. subtilis</i> PY79 AK649	$\Delta 9parS^{+270} \Delta parA::neo$	pAK180 → AK617	this work
<i>B. subtilis</i> PY79 AK667	$\Delta 8parS^{+359} \Delta parA::neo$	pHM160 → AK205	this work
<i>B. subtilis</i> PY79 AK669	$\Delta 9parS^{+359} \Delta parA::neo$	AK213 → AK667	this work

<i>B. subtilis</i> PY79 AK673	$\Delta 9parS^{+90} \Delta parA::neo tetR-gfp$	AK171 → AK555	this work
<i>B. subtilis</i> PY79 AK675	$\Delta 10parS \Delta parA::neo tetR-gfp$	AK171 → AK617	this work
<i>B. subtilis</i> PY79 AK677	$\Delta 9parS^{+359} \Delta parA::neo tetR-gfp$	AK171 → AK669	this work
<i>B. subtilis</i> PY79 AK683	$\Delta 9parS^{+90} \Delta parA::neo tetR-gfp/tetO^{-7}$	AK171 → AK673	this work
<i>B. subtilis</i> PY79 AK685	$\Delta 10parS \Delta parA::neo tetR-gfp/tetO^{-7}$	AK171 → AK675	this work
<i>B. subtilis</i> PY79 AK687	$\Delta 9parS^{+359} \Delta parA::neo tetR-gfp/tetO^{-7}$	AK171 → AK677	this work
<i>B. subtilis</i> PY79 BKM865	$tetR-gfp/tetO^{-7}::cat$	-	[4]
<i>B. subtilis</i> PY79 BKM918	$tetO^{-7}::ery$	-	[4]
<i>B. subtilis</i> PY79 BNS1657	$\Delta 8parS$	-	[5]
<i>B. subtilis</i> 168ED HM4	$trpC2 gfp-parA::neo$	-	[6]
<i>B. subtilis</i> 168ED HM13	$trpC2 \Delta parB gfp-parA::neo$	-	[6]
<i>B. subtilis</i> 168ED HM34	$trpC2 parA-parB::neo$	-	[6]
<i>B. subtilis</i> 168ED HM61	$trpC2 parB-gfp::neo$	-	[7]
<i>B. subtilis</i> 168ED HM130	$trpC2 (spo0J tetO^{-150})::neo cgeD::(P_{pen-tetR-yfp}) tet$	-	[6]
<i>B. subtilis</i> 168ED HM168	$trpC2 (\Delta spo0J tetO^{-150})::neo cgeD::(P_{pen-tetR-yfp}) tet$	-	[6]
<i>B. subtilis</i> 168ED HM171	$trpC2 (\Delta soj tetO^{-150})::neo cgeD::(P_{pen-tetR-yfp}) tet$	-	[6]
<i>B. subtilis</i> 168ED HM183	$trpC2 \Delta (parA-parB)::neo$	-	[6]
<i>B. subtilis</i> 168ED HM194	$trpC2 (\Delta soj \Delta spo0J tetO^{-150})::neo cgeD::(P_{pen-tetR-yfp}) tet$	-	[6]
<i>B. subtilis</i> 168ED HM226	$trpC2 pheA \Delta parB::neo$	-	[6]
<i>B. subtilis</i> 168ED HM227	$trpC2 pheA \Delta parA::neo$	-	[6]
<i>B. subtilis</i> 168CA HM740	$trpC2 gfp-parA::neo$	HM4 → 168CA	this work
<i>B. subtilis</i> 168CA HM747	$trpC2 parA-parB::neo$	HM34 → 168CA	this work
<i>B. subtilis</i> 168CA HM748	$trpC2 \Delta parA::neo$	HM227 → 168CA	this work
<i>B. subtilis</i> 168CA HM749	$trpC2 \Delta (parA-parB)::neo$	HM183 → 168CA	this work
<i>B. subtilis</i> 168CA HM754	$trpC2 \Delta parB::neo$	HM226 → 168CA	this work
<i>B. subtilis</i> 168CA HM756	$trpC2 parB-gfp::neo$	HM61 → 168CA	this work
<i>B. subtilis</i> 168CA HM765	$trpC2 parA-parB::neo tetR-gfp/tetO^{-7}$	HM747 → AK47	this work
<i>B. subtilis</i> 168CA HM766	$trpC2 \Delta parA::neo tetR-gfp/tetO^{-7}$	HM748 → AK47	this work
<i>B. subtilis</i> 168CA HM821	$trpC2 spo0J-gfp::neo plasmid^{parS}$	pHM430 → HM756	this work
<i>B. subtilis</i> 168CA HM823	$trpC2 spo0J-gfp::neo plasmid^{+parS}$	pHM432 → HM756	this work
<i>B. subtilis</i> 168CA HM831	$trpC2 \Delta spo0J tetR-yfp/tetO^{-150}$	HM168 → 168CA	this work
<i>B. subtilis</i> 168CA HM832	$trpC2 \Delta soj tetR-yfp/tetO^{-150}$	HM171 → 168CA	this work
<i>B. subtilis</i> 168CA HM834	$trpC2 \Delta spo0J tetR-yfp/tetO^{-150} plasmid^{parS}$	pHM430 → HM831	this work
<i>B. subtilis</i> 168CA HM835	$trpC2 \Delta soj tetR-yfp/tetO^{-150} plasmid^{parS}$	pHM430 → HM832	this work
<i>B. subtilis</i> 168CA HM837	$trpC2 \Delta spo0J tetR-yfp/tetO^{-150} plasmid^{+parS}$	pHM432 → HM831	this work
<i>B. subtilis</i> 168CA HM838	$trpC2 \Delta soj tetR-yfp/tetO^{-150} plasmid^{+parS}$	pHM432 → HM832	this work
<i>B. subtilis</i> 168CA HM853	$trpC2 tetR-yfp/tetO^{-150}$	HM130 → 168CA	this work
<i>B. subtilis</i> 168CA HM855	$trpC2 tetR-yfp/tetO^{-150} plasmid^{parS}$	pHM430 → HM853	this work

<i>B. subtilis</i> 168CA HM856	<i>trpC2 tetR-yfp/tetO⁻¹⁵⁰ plasmid^{+parS}</i>	pHM432 → HM853	this work
<i>B. subtilis</i> 168CA HM870	<i>trpC2 gfp-soj::neo plasmid^{+parS}</i>	pHM430 → HM740	this work
<i>B. subtilis</i> 168CA HM871	<i>trpC2 gfp-soj::neo plasmid^{+parS}</i>	pHM432 → HM740	this work
<i>B. subtilis</i> 168CA HM907	<i>trpC2 ΔparB::neo tetR-gfp/tetO⁻⁷</i>	HM754 → AK47	this work
<i>B. subtilis</i> JH642 PSL23	<i>trpC2 Δ6parS parB-gfp cotS::parS¹⁶ array cat</i>	-	[8]
<i>B. subtilis</i> JH642 PSL25	<i>trpC2 Δ6parS parB-gfp yheH::parS¹⁶ array cat</i>	-	[8]
<i>B. subtilis</i> PY79	wild type prototroph	-	[9]

^a *B. subtilis* antibiotic resistance markers are listed respectively. *neo*, kanamycin resistance; *ery*, erythromycin resistance; *cat*, chloramphenicol resistance; *spc*, spectinomycin resistance; *tet*, tetracycline resistance.

^b Plasmids or genomic DNA of the indicated strain transformed into the parent strain used in the genetic construction.

^c DNA fragments amplified from AK47 gDNA using primer pairs oAK5/oAK6 and oAK7/oAK8.

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Supplementary Table 2: Plasmids used in this study

Plasmid	Genotype ^a	Construction ^b	Source
pAK09	<i>bla cat 'sigX ΔparS²⁰⁶::zeo resE'</i>	-	[3]
pAK23	<i>bla cat 'yhaX ΔparS⁹⁰::tet hemZ'</i>	-	[3]
pAK26	<i>bla cat parB::neo</i>	-	[3]
pAK28	<i>bla cat ΔparB::neo</i>	-	[3]
pAK30	<i>bla cat ΔparB ΔparS³⁵⁹::neo</i>	pHM456 (oAK55/oGJS431) → pHM23	this work
pAK32	<i>bla cat parB ΔparS³⁵⁹::neo</i>	-	[3]
pAK48	<i>bla cat gfp-parA ΔparS³⁵⁹::neo</i>	pHM142 (oAK52/oAK53) → pHM23	this work
pAK56	<i>bla cat 'yhaX parS⁹⁰::tet hemZ'</i>	pHM48 (oAK61/oAK62) → pAK23	this work
pAK82 ^c	<i>bla cat parB-gfp::neo</i>	pHM110 → pAK26	this work
pAK83 ^c	<i>bla cat parB-gfp ΔparS³⁵⁹::neo</i>	pHM110 → pAK32	this work
pAK98	<i>bla cat ΔparA::neo</i>	pHM160 (oAK58/oHM20) → pAK26	this work
pAK99	<i>bla cat Δ(parA-parB)::neo</i>	pHM160 (oAK58/oHM20) → pAK28	this work
pAK100	<i>bla cat Δ(parA-parB ΔparS³⁵⁹)::neo</i>	pHM160 (oAK58/oHM20) → pAK30	this work
pAK101	<i>bla cat ΔparA ΔparS³⁵⁹::neo</i>	pHM160 (oAK58/oHM20) → pAK32	this work
pAK128	<i>bla cat 'ytcC::zeo</i>	160CA (oAK191/oAK192) → pHM457	this work
pAK130	<i>bla cat zeo ytxO'</i>	160CA (oAK189/oAK190) → pHM457	this work
pAK136 ^c	<i>bla cat 'ytcC::zeo ytxO'</i>	pAK130 → pAK128	this work
pAK178	<i>bla cat 'ytcC::erm ytxO'</i>	pHM432 (oAK227/oAK257) → pAK136	this work
pAK180	<i>bla cat 'ytcC::erm parS²⁷⁰ ytxO'</i>	pHM48 (oAK216/oAK217) → pAK178	this work
pcat::tet	<i>bla cat::tet</i>	-	[10]
pHM23	<i>bla cat gfp-parA::neo</i>	-	[6]
pHM48	<i>bla cat parB::neo</i>	-	[6]
pHM52	<i>bla cat ΔparB::neo</i>	-	[6]
pHM59	<i>bla cat Δ(parA-parB)::tet</i>	-	[6]
pHM110	<i>bla cat parB-gfp::neo</i>	-	[7]
pHM142	<i>bla cat parB ΔparS³⁵⁹::neo</i>	-	[3]
pHM160	<i>bla cat ΔparA::neo</i>	-	[6]
pHM430	<i>ery</i>	pNZ8907	[11]
pHM432	<i>ery 'spo0J-parS-spo0J'</i>	168CA (oAK61/oAK62) → pHM430	this work
pHM456 ^c	<i>bla cat ΔparB ΔparS³⁵⁹::neo</i>	pHM142 → pHM52	this work
pHM457	<i>bla cat zeo</i>	-	[3]

^a *E. coli* and *B. subtilis* antibiotic resistance markers are listed first and second respectively. *bla*, ampicillin resistance; *kan*, kanamycin resistance; *ery*, erythromycin resistance; *cat*, chloramphenicol resistance; *spc*, spectinomycin resistance; *tet*, tetracycline resistance; *phl*, phleomycin resistance; zeomycin resistance; *zeo*.

^b DNA fragments amplified with primer pair indicated in parentheses using template from either plasmids or genomic DNA indicated in parentheses before the amplified products are cloned into the parent plasmid used in the genetic construction.

^c DNA fragments are digested by restriction enzymes and cloned into the parent plasmid used in the genetic construction.

Supplementary Table 3: Oligonucleotides used in this study

Primer	Sequence (5'→3')
oAK52	AAGGGAAGGGCCGCGGTCAAGAGGTGCGGAAGTATATTTAG
oAK53	AAGGGAAGGGTCTAGAAGTGTGGATCCCCCGGGCTGC
oAK55	AATTTAATTTCTAGGTAAGGAAAATCATAGCAATTACGAAC
oAK58	TTTATGCTTCCGGCTCGTATG
oAK61	AATTTAATTTGCGGCCGCGACTGCTGACACTGCCAG
oAK62	AATTTAATTTGCGGCCGCCATTTATGATTCTCGTTCAGAC
oAK91	AATTTAATTTGAGCTCGCGTTCTCTAATTTACAAGAGG
oAK92	GATTGGAATACGGATTCTG
oAK189	AAGGGAATTCTCGAGGTGACTTTATGCCTTGCGCTTTAC
oAK190	AAGGGAATTGGTACCGCCTCTATTTCTAAACTGGATCTCGAC
oAK216	AAGGGAATTTCTAGAGACTGCTGACACTGCC
oAK217	AAGGGAATTTCTAGACATTTATGATTCTCGTTCAGAC
oAK227	AAGGGAATTGGATCCGACTCATAGAATTATTTCTCCCG
oAK257	AATTTAATTTCTCGAGTATCACGAGGCCCTTTCGTCTTCAAGAATTGATCC
oGJS431	GGTGGTCCCGCGGCATCTTCGTTTGAAAGATGC GGC
oHM20	TTTTCTTGGCTGATAAGGATTAGGGCGTAAATCG

Supplementary Table 4: Strains used in each figures

- Fig.1 HM756 (*parB-gfp*), AK153 (*parB-gfp parS¹⁶* arrays)
- Fig. 2 HM821 (*parB-gfp plasmid^{parS}*), HM823 (*parB-gfp plasmid^{+parS}*)
- Fig. 3 HM765 (*tetR-gfp/tetO⁻⁷*), AK87 (*tetR-gfp/tetO⁻⁷ parS¹⁶* arrays), HM907 (*tetR-gfp/tetO⁻⁷ ΔparB*), AK123 (*tetR-gfp/tetO⁻⁷ parS¹⁶* arrays Δ *parB*), HM766 (*tetR-gfp/tetO⁻⁷ ΔparA*), AK89 (*tetR-gfp/tetO⁻⁷ parS¹⁶* arrays Δ *parA*)
- Fig. 4 HM855 (*tetR-yfp/tetO⁻⁷ plasmid^{parS}*), HM856 (*tetR-yfp/tetO⁻⁷ plasmid^{+parS}*), HM834 (Δ *parB tetR-yfp/tetO⁻⁷ plasmid^{parS}*), HM837 (Δ *parB tetR-yfp/tetO⁻⁷ plasmid^{+parS}*), HM835 (Δ *parA tetR-yfp/tetO⁻⁷ plasmid^{parS}*), HM838 (Δ *parA tetR-yfp/tetO⁻⁷ plasmid^{+parS}*)
- Fig. 5 HM765 (*tetR-gfp/tetO⁻⁷*), AK87 (*tetR-gfp/tetO⁻⁷ parS¹⁶* arrays), HM907 (*tetR-gfp/tetO⁻⁷ ΔparB*), AK123 (*tetR-gfp/tetO⁻⁷ parS¹⁶* arrays Δ *parB*), AK573 (*tetR-gfp/tetO⁻⁷ ΔparA ΔparB*), AK575 (*tetR-gfp/tetO⁻⁷ parS¹⁶* arrays Δ *parA ΔparB*), HM766 (*tetR-gfp/tetO⁻⁷ ΔparA*), AK89 (*tetR-gfp/tetO⁻⁷ parS¹⁶* arrays Δ *parA*), HM740 (*gfp-parA*), AK199 (*gfp-parA parS¹⁶* arrays), AK547 (*gfp-parA ΔparB*), AK577 (*gfp-parA ΔparB parS¹⁶* arrays), HM855 (*tetR-yfp/tetO⁻⁷ plasmid^{parS}*), HM856 (*tetR-yfp/tetO⁻⁷ plasmid^{+parS}*), HM834 (Δ *spo0J tetR-yfp/tetO⁻⁷ plasmid^{parS}*), HM837 (Δ *spo0J tetR-yfp/tetO⁻⁷ plasmid^{+parS}*), AK587 (Δ *soj Δspo0J tetR-yfp/tetO⁻⁷ plasmid^{parS}*), AK589 (Δ *soj Δspo0J tetR-yfp/tetO⁻⁷ plasmid^{+parS}*), HM835 (Δ *soj tetR-yfp/tetO⁻⁷ plasmid^{parS}*), HM838 (Δ *soj tetR-yfp/tetO⁻⁷ plasmid^{+parS}*), HM870 (*gfp-soj plasmid^{parS}*), HM871 (*gfp-soj plasmid^{+parS}*), AK569 (Δ *spo0J gfp-soj plasmid^{parS}*), AK571 (Δ *spo0J gfp-soj plasmid^{+parS}*).
- Fig. 6 AK367 (*parB-gfp*), AK369 (*parB-gfp Δ10parS*), AK593 (*parB-gfp Δ9parS⁺³⁵⁹*), AK597 (*parB-gfp Δ9parS⁺²⁷⁰*), AK373 (*parB-gfp Δ9parS⁺⁹⁰*), AK239 (wild type), AK243 (Δ *10parS*), AK259 (Δ *9parS⁺³⁵⁹*), AK603 (Δ *9parS⁺²⁷⁰*), AK323 (Δ *9parS⁺⁹⁰*), AK241 (Δ *parB*), AK245 (Δ *parB Δ10parS*), AK261 (Δ *parB Δ9parS⁺³⁵⁹*), AK605 (Δ *parB Δ9parS⁺²⁷⁰*), AK325 (Δ *parB Δ9parS⁺⁹⁰*), AK559 (Δ *parA ΔparB*), AK563 (Δ *parA ΔparB Δ10parS*), AK561 (Δ *parA ΔparB Δ9parS⁺³⁵⁹*), AK607 (Δ *parA ΔparB Δ9parS⁺²⁷⁰*), AK553 (Δ *parA ΔparB Δ9parS⁺⁹⁰*), AK557 (Δ *parA*), AK617 (Δ *parA Δ10parS*), AK669 (Δ *parA Δ9parS⁺³⁵⁹*), AK649 (Δ *parA Δ9parS⁺²⁷⁰*), AK555 (Δ *parA Δ9parS⁺⁹⁰*), AK277 (*gfp-parA*), AK281 (*gfp-parA Δ10parS*), AK305 (*gfp-parA Δ9parS⁺³⁵⁹*), AK599 (*gfp-parA Δ9parS⁺²⁷⁰*), AK399 (*gfp-parA Δ9parS⁺⁹⁰*)

Fig. S1 HM756 (*parB-gfp*), AK153 (*parB-gfp parS¹⁶* arrays), HM821 (*parB-gfp plasmid^{parS}*), HM823 (*parB-gfp plasmid^{+parS}*), HM765 (*tetR-gfp/tetO⁻⁷*), AK87 (*tetR-gfp/tetO⁻⁷ parS¹⁶* arrays), HM855 (*tetR-yfp/tetO⁻⁷ plasmid^{parS}*), HM856 (*tetR-yfp/tetO⁻⁷ plasmid^{+parS}*)

Fig. S2 AK239 (wild type), AK243 ($\Delta 10parS$), AK259 ($\Delta 9parS^{359+}$), AK603 ($\Delta 9parS^{270+}$), AK323 ($\Delta 9parS^{90+}$)

Fig. S3 AK181 (*tetR-gfp/tetO⁻⁷*), AK225 (*tetR-gfp/tetO⁻⁷ $\Delta 10parS$*), AK591 (*tetR-gfp/tetO⁻⁷ $\Delta 9parS^{359+}$*), AK405 (*tetR-gfp/tetO⁻⁷ $\Delta 9parS^{90+}$*), AK183 (*tetR-gfp/tetO⁻⁷ $\Delta parB$*), AK227 (*tetR-gfp/tetO⁻⁷ $\Delta parB$ $\Delta 10parS$*), AK639 (*tetR-gfp/tetO⁻⁷ $\Delta parB$ $\Delta 9parS^{359+}$*), AK615 (*tetR-gfp/tetO⁻⁷ $\Delta parB$ $\Delta 9parS^{90+}$*), AK187 (*tetR-gfp/tetO⁻⁷ $\Delta parA$*), AK685 (*tetR-gfp/tetO⁻⁷ $\Delta parA$ $\Delta 10parS$*), AK687 (*tetR-gfp/tetO⁻⁷ $\Delta parA$ $\Delta 9parS^{359+}$*), AK683 (*tetR-gfp/tetO⁻⁷ $\Delta parA$ $\Delta 9parS^{90+}$*)

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