

Supplementary Information for

**Efficient septum formation is essential for chromosome segregation
in *Bacillus subtilis* when SMC function is impaired**

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Supplemental Materials and Methods

Strain construction.

B. subtilis strains were constructed by transforming plasmid DNA and genomic DNA isolated from existing strains using standard transformation assays in BMK competence medium (1).

BWX527 [*sacA::hbsu-mCherry (cat)*] was constructed by direct transformation of a three-way ligation into *B. subtilis* PY79. The ligation consisted of the pKM064 (D. Z. Rudner unpublished) backbone digested with EcoRI and BamHI, the *hbs* gene with its native promoter amplified from PY79 genomic DNA using primers oDR198 and oDR214 and digested with EcoRI and XhoI, and the *mCherry* gene amplified from pDR201 using primers oWX327 and oWX328 and digested with XhoI and BamHI. The ligation reaction was transformed into *B. subtilis* PY79 cells and plated on LB agar plates supplemented with 5 µg/mL chloramphenicol at 37°C. pKM064 is a cloning vector that contains *sacA (cat)*. pDR201 carries an *mCherry* gene codon-optimized for *B. subtilis* (D. Z. Rudner unpublished). The resulting strain was verified by PCR amplification of genomic DNA using primers oDR198 and oWX328, followed by Sanger sequencing using the same primers.

BWX2798 [PY79, *smc** (*spec*)] was generated by backcrossing the *smc** allele isolated from the mutant library described in the section “Construction and isolation of *smc* hypomorphic alleles” into a fresh WT background. The resulting strain contains a single point mutation V1119L and a spectinomycin resistance gene.

BWX2883 [Bs168, *trpC2*, *smc (kan)*] was constructed by direct transformation of an isothermal assembly product into Bs168 (2). The isothermal reaction contained three PCR fragments: 1) *smc* (amplified from the Bs168 genomic DNA using oWX821 and oWX849); 2) *loxP kan* (amplified from the pWX470 (3) plasmid using oWX438 and oWX823); and 3) downstream of *smc* (amplified from the Bs168 genomic DNA using oWX850 and oWX851). The ITA product was transformed into Bs168 cells and plated on LB agar plates supplemented with 10 µg/mL kanamycin at 37°C. The *smc* gene was sequenced using oWX523, oWX848, oWX1194, oWX1195, oWX1196 and oWX1624.

BWX2885 [Bs168, *trpC2*, *smc** (*kan*)] was constructed by direct transformation of an isothermal assembly product into Bs168 (2). The isothermal reaction contained three PCR fragments: 1) the 3.6-kb region containing *smc** (amplified from BWX2798 genomic DNA using oWX821 and

oWX849), 2) a kanamycin-resistance cassette (amplified from pWX470 (3) using oWX438 and oWX823), and 3) a 2.2-kb downstream region of *smc* (amplified from the Bs168 genomic DNA using oWX850 and oWX851). The ITA product was transformed into Bs168 cells and plated on LB agar plates supplemented with 10 µg/mL kanamycin at 37°C. The *smc** gene was sequenced using oWX523, oWX848, oWX1194, oWX1195, oWX1196 and oWX1624.

Plasmid construction.

pDR181 [*spoIIIE-gfp (kan)*] was built by ligating two DNA fragments: 1) pKL168 digested by EcoRI and XhoI, yielding a plasmid backbone containing *gfp* and a kanamycin-resistance cassette; 2) the C-terminus of *spoIIIE* amplified from PY79 genomic DNA using primers oDR238 and oDR239 and digested by EcoRI and XhoI. The resulting plasmid was integrated into the PY79 chromosome by single-crossover integration. The genomic DNA from the resulting strain was transformed into the Bs168 background by double backcrossing. pKL168 (Lemon and Grossman, unpublished) is a Campbell-type integration vector essentially identical to pKL147 (4), except that it carries kanamycin rather than spectinomycin resistance.

pWX209 [*pelB::tetO48 (cat)*] was built by a ligation reaction containing two DNA fragments: 1) pKM020 digested by EcoRI and HindIII to give *pelB::cat*; 2) pLAU29 (5) digested by EcoRI and HindIII to give *tetO48*. pKM020 is a cloning vector containing *pelB (cat)* (6).

pWX649 [*yvbJ::Pspank* (optRBS) spoIIIE (spec)*] was constructed by a ligation reaction containing two DNA fragments: 1) pMS051 digested by XmaI and SpeI to give *yvbJ::Pspank** (*spec*); 2) *spoIIIE* region amplified from PY79 using oWX1167 and oWX1168 and then digested by XmaI and SpeI. pMS051 is a cloning vector that contains *yvbJ::Pspank* (spec)* (D. Z. Rudner unpublished). The construct was sequenced using oWX486 and oWX487.

pWX659 [*yvbJ::Pspank* (optRBS) divIVA (spec)*] was constructed by a ligation reaction containing two DNA fragments: 1) pMS051 digested by XmaI and SpeI to give *yvbJ::Pspank** (*spec*); 2) *divIVA* region amplified from PY79 using oWX1188 and 1189 and then digested by XmaI and SpeI. pMS051 is a cloning vector that contains *yvbJ::Pspank* (spec)* (D. Z. Rudner unpublished). The construct was sequenced using oWX486 and oWX487.

pWX660 [*yvbJ::Pspank** (*optRBS*) *minJ* (*spec*)] was constructed by a ligation reaction containing two DNA fragments: 1) pMS051 digested by XmaI and SpeI to give *yvbJ::Pspank** (*spec*); 2) *minJ* region amplified from PY79 using oWX1190 and oWX1191 and then digested by XmaI and SpeI. pMS051 is a cloning vector that contains *yvbJ::Pspank** (*spec*) (D. Z. Rudner unpublished). The construct was sequenced using oWX486 and oWX487.

pWX723 [*yvbJ::Pspank* (*optRBS*) *parB* Δ *parS* *erm*] was constructed by a ligation reaction containing two DNA fragments: 1) pMS044 digested by XmaI and NheI to give *yvbJ::Pspank* (*erm*); 2) the (*optRBS*) *parB* Δ *parS* region was amplified from pWX589 (7) using oWX1668 and oWX999 and then digested by XmaI and NheI. pMS044 is a cloning vector that contains *yvbJ::Pspank* (*erm*) (D. Z. Rudner unpublished). The construct was sequenced using oWX486 and oDR829.

Table S1. Strains used in this study.

Strain	Genotype	Reference	Fig.
BWX2883	Bs168, <i>trpC2</i> , <i>smc</i> (<i>kan</i>)	This study	1A, 1B, 1D
BWX2885	Bs168, <i>trpC2</i> , <i>smc</i> [*] (<i>kan</i>)	This study	1A, 1B, 1D, 5C
BWX5786	Bs168, <i>trpC2</i> , <i>smc</i> (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>yycR</i> :: <i>tetO48</i> (<i>cat</i>)	This study	1C, 1E, 2A-D, 5B
BWX5790	Bs168, <i>trpC2</i> , <i>smc</i> [*] (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>yycR</i> :: <i>tetO48</i> (<i>cat</i>)	This study	1C, 1E, 2A-D, 5B
BWX5839	Bs168, <i>trpC2</i> , <i>smc</i> (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>yycR</i> :: <i>tetO48</i> (<i>cat</i>), Δ <i>parB</i> (<i>spec</i>)	This study	1E, 2A-D
BWX5831	Bs168, <i>trpC2</i> , <i>smc</i> (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>yycR</i> :: <i>tetO48</i> (<i>cat</i>), Δ <i>spolIIE</i> (<i>erm</i>)	This study	1E, 3A-D
BWX5837	Bs168, <i>trpC2</i> , <i>smc</i> (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>yycR</i> :: <i>tetO48</i> (<i>cat</i>), Δ <i>minJ</i> (<i>erm</i>)	This study	1E, 3A, 3E-G
BWX5835	Bs168, <i>trpC2</i> , <i>smc</i> (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>yycR</i> :: <i>tetO48</i> (<i>cat</i>), Δ <i>divIVA</i> (<i>erm</i>)	This study	1E, 3A, 3E-G
BWX5845	Bs168, <i>trpC2</i> , <i>smc</i> [*] (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>yycR</i> :: <i>tetO48</i> (<i>cat</i>), <i>yvbJ</i> :: <i>Pspank</i> (<i>optRBS</i>) <i>parB</i> Δ <i>parS</i> (<i>erm</i>), Δ <i>parB</i> (<i>spec</i>)	This study	1E, 2A-D
BWX5818	Bs168, <i>trpC2</i> , <i>smc</i> [*] (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>yycR</i> :: <i>tetO48</i> (<i>cat</i>), <i>yvbJ</i> :: <i>Pspank</i> [*] (<i>optRBS</i>) <i>spolIIE</i> (<i>spec</i>), Δ <i>spolIIE</i> (<i>erm</i>)	This study	1E, 3A-D
BWX5822	Bs168, <i>trpC2</i> , <i>smc</i> [*] (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>yycR</i> :: <i>tetO48</i> (<i>cat</i>), <i>yvbJ</i> :: <i>Pspank</i> [*] (<i>optRBS</i>) <i>minJ</i> (<i>spec</i>), Δ <i>minJ</i> (<i>erm</i>)	This study	1E, 3A, 3E-G, 5B
BWX5820	Bs168, <i>trpC2</i> , <i>smc</i> [*] (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>yycR</i> :: <i>tetO48</i> (<i>cat</i>), <i>yvbJ</i> :: <i>Pspank</i> [*] (<i>optRBS</i>) <i>divIVA</i> (<i>spec</i>), Δ <i>divIVA</i> (<i>erm</i>)	This study	1E, 3A, 3E-G, 5B
BWX6361	Bs168, <i>trpC2</i> , <i>smc</i> (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>pelB</i> :: <i>tetO48</i> (<i>cat</i>)	This study	4A, 4B
BWX6365	Bs168, <i>trpC2</i> , <i>smc</i> (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>pelB</i> :: <i>tetO48</i> (<i>cat</i>), Δ <i>spolIIE</i> (<i>erm</i>)	This study	4A, 4B
BWX6367	Bs168, <i>trpC2</i> , <i>smc</i> (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>pelB</i> :: <i>tetO48</i> (<i>cat</i>), Δ <i>minJ</i> (<i>erm</i>)	This study	4A, 4B
BWX6369	Bs168, <i>trpC2</i> , <i>smc</i> (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>pelB</i> :: <i>tetO48</i> (<i>cat</i>), Δ <i>divIVA</i> (<i>erm</i>)	This study	4A, 4B
BWX6363	Bs168, <i>trpC2</i> , <i>smc</i> [*] (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>pelB</i> :: <i>tetO48</i> (<i>cat</i>)	This study	4A, 4B
BWX6382	Bs168, <i>trpC2</i> , <i>smc</i> [*] (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>pelB</i> :: <i>tetO48</i> (<i>cat</i>), <i>yvbJ</i> :: <i>Pspank</i> [*] (<i>optRBS</i>) <i>spolIIE</i> (<i>spec</i>), Δ <i>spolIIE</i> (<i>erm</i>)	This study	4A, 4B
BWX6377	Bs168, <i>trpC2</i> , <i>smc</i> [*] (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>pelB</i> :: <i>tetO48</i> (<i>cat</i>), <i>yvbJ</i> :: <i>Pspank</i> [*] (<i>optRBS</i>) <i>minJ</i> (<i>spec</i>), Δ <i>minJ</i> (<i>erm</i>)	This study	4A, 4B
BWX6375	Bs168, <i>trpC2</i> , <i>smc</i> [*] (<i>kan</i>), <i>ycgO</i> :: <i>PftsW tetR-cfp</i> (<i>phleo</i>), <i>pelB</i> :: <i>tetO48</i> (<i>cat</i>), <i>yvbJ</i> :: <i>Pspank</i> [*] (<i>optRBS</i>) <i>divIVA</i> (<i>spec</i>), Δ <i>divIVA</i> (<i>erm</i>)	This study	4A, 4B

BWX6519	Bs168, <i>trpC2</i> , <i>smc*</i> (<i>kan</i>), <i>yvbJ::Pspank*</i> (<i>optRBS</i>) <i>minJ</i> (<i>spec</i>), Δ <i>minJ</i> (<i>erm</i>), Δ <i>minD</i> (<i>cat</i>)	This study	5A-C
BWX6517	Bs168, <i>trpC2</i> , <i>smc*</i> (<i>kan</i>), <i>yvbJ::Pspank*</i> (<i>optRBS</i>) <i>divIVA</i> (<i>spec</i>), Δ <i>divIVA</i> (<i>erm</i>), Δ <i>minD</i> (<i>cat</i>)	This study	5A-C
BWX6539	Bs168, <i>trpC2</i> , <i>smc*</i> (<i>kan</i>), Δ <i>minD</i> (<i>cat</i>)	This study	5C
BWX6514	Bs168, <i>trpC2</i> , <i>smc*</i> (<i>kan</i>), <i>yvbJ::Pspank*</i> (<i>optRBS</i>) <i>spolIIE</i> (<i>spec</i>), Δ <i>spolIIE</i> (<i>erm</i>), Δ <i>minD</i> (<i>cat</i>)	This study	5C
BWX5902	Bs168, <i>trpC2</i> , <i>sacA::hbsu-mCherry b.s.</i> (<i>cat</i>), <i>spolIIE-gfp</i> (<i>kan</i>), <i>smc loxP</i> (<i>spec</i>)	This study	6B, 6C, 6D
BWX5904	Bs168, <i>trpC2</i> , <i>sacA::hbsu-mCherry b.s.</i> (<i>cat</i>), <i>spolIIE-gfp</i> (<i>kan</i>), <i>smc*</i> (<i>spec</i>)	This study	6B, 6C, 6E
Strains that are used for strain building			
AG1468	Δ <i>parB</i> (<i>spec</i>)	(8)	
Bs168	<i>trpC2</i>	(2)	
BKO215	Bs168, <i>trpC2</i> , Δ <i>divIVA</i> (<i>erm</i>)	(1)	
BKO220	Bs168, <i>trpC2</i> , Δ <i>spolIIE</i> (<i>erm</i>)	(1)	
BKO3053	Bs168, <i>trpC2</i> , Δ <i>minJ</i> (<i>erm</i>)	(1)	
BWX527	<i>sacA::hbsu-mCherry</i> (<i>cat</i>)	This study	
BWX811	<i>ycgO::PftsW tetR-cfp</i> (<i>phleo</i>), <i>ycrR::tetO48</i> (<i>cat</i>)	(9)	
BWX2080	<i>smc</i> (<i>spec</i>)	(10)	
BWX2798	<i>smc*</i> (<i>spec</i>)	This study	
DK7279	Δ <i>minD</i> (<i>cat</i>)	(11)	

Table S2. Plasmids used in this study.

Plasmid	Description	Reference
pDR181	<i>spolIIE-gfp (kan)</i>	This study
pDR201	<i>mCherry b.s.</i>	D. Z. Rudner unpublished
pLAU29	<i>tetO48</i>	(5)
pKL168	<i>gfp (kan)</i>	Lemon and Grossman, unpublished
pKM020	<i>pelB::cat</i>	(6)
pKM064	<i>sacA::cat (amp)</i>	D. Z. Rudner unpublished
pMS051	<i>yvbJ::Pspank* (spec)</i>	D. Z. Rudner unpublished
pMS044	<i>yvbJ::Pspank (erm)</i>	D. Z. Rudner unpublished
pWX209	<i>pelB::tetO48 (cat)</i>	This study
pWX466	<i>loxP spec loxP</i>	(3)
pWX470	<i>loxP kan loxP</i>	(3)
pWX589	<i>yvbJ::Pspank-gfp-parB (ΔparS) (cat)</i>	(7)
pWX642	<i>pACYC TnKRM (spec) (amp) Mmel modified</i>	(12)
pWX649	<i>yvbJ::Pspank* (optRBS) spolIIE (spec)</i>	This study
pWX659	<i>yvbJ::Pspank* (optRBS) divIVA (spec)</i>	This study
pWX660	<i>yvbJ::Pspank* (optRBS) minJ (spec)</i>	This study
pWX723	<i>yvbJ::Pspank (optRBS) parB ΔparS (erm)</i>	This study

Table S3. Oligonucleotides used in this study.

Oligos	Sequence	Use
oDR198	gccctcgagtttccggcaactgcgtctttaagcgc	BWX527
oDR214	gccgaattcaaatcaccttaaatccttgacgagc	BWX527
oDR238	gccgaattctggcgctgagaagcttctggccg	pDR181
oDR239	cggctcgagagaagagagctcatcatatttctc	pDR181
oDR829	catcaaatcttacaatatgtag	sequencing
oWX327	accctcgagggatcaggacagggaccaatggcagcaagggagaggaag	BWX527
oWX328	tatggatccccgggctattattgtataattcgtccattccacc	BWX527
oWX438	gaccagggagcactgggtcaac	universal
oWX439	tccttctgctccctcgctcag	universal
oWX486	gccgctctagctaagcagaagggc	sequencing
oWX487	aacggtctgataagagacaccggc	sequencing
oWX523	cattcaggagtcgagattatcgctcag	sequencing
oWX821	taaaatcccccttatgactcaggggg	<i>smc</i> mutant library
oWX823	cagtaacgaggaaagagggtaaaagggatccttctgctccctcgctcag	<i>smc</i> mutant library
oWX847	ctttcagatacggcagagagctcttc	<i>smc</i> mutant library
oWX848	gaagagctctctgccgtatctgaaaag	<i>smc</i> mutant library
oWX849	ctgagcgagggagcagaaggatccctttaacctcttctcgttactg	<i>smc</i> mutant library
oWX850	gttgaccagtgtccctggctctaacgaggaaagagggtaaaagatgagc	<i>smc</i> mutant library
oWX851	cgtcagcctcaagcagcgcaagacgg	<i>smc</i> mutant library
oWX999	tttgctagccagagtggagggaagaacgccttaaccc	pWX723
oWX1167	aaaccgggacataaggaggaactactatggtggcaaagaaaaaacgaaa atc	pWX649
oWX1168	cgactagtttaagaagagagctcatcatatttctc	pWX649
oWX1188	aaaccgggacataaggaggaactactatgccattaacgccaatgatattca c	pWX659
oWX1189	cgactagtttattcctttctcaaatacagcgctcgac	pWX659
oWX1190	aaaccgggacataaggaggaactactatggtgtctgttcaatggggaattgaa c	pWX660
oWX1191	cgactagtttatgatcccgaagcgactgcttcgtc	pWX660
oWX1194	gggaaagtgaagagatcctgagc	sequencing
oWX1195	cttcacaatgaaaatgtcgaagag	sequencing
oWX1196	gcccggcattcatcatttctcggg	sequencing
oWX1624	ttcgatatcataggcagtcagcgc	sequencing
oWX1668	aaaccgggacataaggaggaactactatggctaaaggccttgaaaaggg	pWX723

Table S4. Tn-seq candidate genes after filtering by p value and count ratio.

Gene	p value	Count ratio (WT/mutant)	Visual inspection
<i>spoIIIE</i>	0.00000	14.1	candidate for further analysis
<i>yflE</i>	0.00006	30.3	low insertion in WT
<i>minJ</i>	0.00029	27.0	candidate for further analysis
<i>hprK</i>	0.00078	18.9	low insertion in WT
<i>parB</i>	0.00101	31.3	candidate for further analysis
<i>divIVA</i>	0.00443	1000.0	candidate for further analysis
<i>aroF</i>	0.00600	13.0	low insertion in WT
<i>yoqM</i>	0.00656	11.6	low insertion in WT
<i>atpF</i>	0.00831	20.4	low insertion in WT
<i>yabA</i>	0.01567	20.8	low insertion in WT; small gene
<i>yisI</i>	0.01771	23.8	low insertion in WT; small gene
<i>yomT</i>	0.01882	18.9	low insertion in WT; small gene
<i>sspL</i>	0.03740	37.0	low insertion in WT; small gene
<i>degQ</i>	0.03844	47.6	low insertion in WT; small gene
<i>yoqE</i>	0.03940	52.6	low insertion in WT; small gene
<i>yrzB</i>	0.03940	83.3	low insertion in WT; small gene
<i>nth</i>	0.04082	47.6	low insertion in WT; small gene
<i>yxxG</i>	0.04175	13.0	low insertion in WT

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