Plasmid	Description	Reference/Source
Bacterial two-hybrid constructs:		
pBRα	Encodes the full-length α subunit of RNAP under the control of tandem placUV5 and plpp promoters; confers resistance to Carb	[1]
ρΑCλCΙ	Encodes λ CI (residues 1-236) under the control of <i>lacUV5</i> promoter; confers resistance to Cm	[1]
pBRα -βflap (831–1057)	Encodes residues 1-248 of α fused by a three-alanine linker residues 831–1057 of the β subunit of <i>E. coli</i> RNAP under the control of tandem p <i>lacUV5</i> and p <i>lpp</i> promoters; confers resistance to Carb; used to generate Notl/BamHI-digested backbone to construct various α fusions.	[2]
pACλCI-βflap (831–1057)	Encodes λ CI (residues 1-236) fused via three alanines to residues 831–1057 of the β subunit of <i>E. coli</i> RNAP under the control of <i>lacUV5</i> promoter; confers resistance to Cm; used to generate Notl/BamHI-digested backbone to construct various λ CI fusions.	[2]
pBRα-FtsZ (pMT153)	Encodes residues 1-248 of α fused by a three-alanine linker to full-length <i>E. coli</i> FtsZ under the control of tandem placUV5 and plpp promoters; confers resistance to Carb	This study
pBRα-FtsZ∆66 (pDH198)	pBRα-FtsZ encoding <i>E. coli</i> FtsZ residues 2-317; the last 66 residues are deleted	This study
pBRa-FtsZ mutants isolated in two-hybrid screen: pBRa-FtsZ-L169P pBRa-FtsZ-L179R pBRa-FtsZ-S177P pBRa-FtsZ-D180N pBRa-FtsZ-F182S	pBRα-FtsZ encoding the indicated substitution in the FtsZ moiety of the fusion	This study
pBRα-FtsZ-D180K (pDH160)	pBRα-FtsZ encoding the D180K substitution in the FtsZ moiety of the fusion	This study
pACλCI-FtsZ (pMT152)	Encodes λ Cl fused by a three-alanine linker to full-length <i>E. coli</i> FtsZ under the control of <i>lacUV5</i> promoter; confers resistance to Cm	This study
pACλCI- <i>Bsu</i> FtsZ (pDH62)	Encodes λ CI fused by a three-alanine linker to full-length <i>B. subtilis</i> FtsZ under the control of <i>lacUV5</i> promoter; confers resistance to Cm	This study
pACλCI- <i>Bsu</i> FtsZ (loop ^{Eco}) (pDH69)	pAC λ CI- <i>Bsu</i> FtsZ with residues 169-183 (ILEIVDKNTPMLEAF) replaced with <i>Eco</i> FtsZ residues 168-182 (LLKVLGRGISLDAF) in the <i>Bsu</i> FtsZ moiety of the fusion.	This study

S3 Table. Plasmids used in this study.

pBRα-MreB (pMT151)	Encodes residues 1-248 of α fused by a three-alanine linker to <i>E. coli</i> MreB under the control of tandem p <i>lacUV5</i> and p <i>lpp</i> promoters; confers resistance to Carb	This study
pBRα-MreB mutants isolated in two-hybrid screen: pBRα-MreB-I126V (pDH257) pBRα-MreB-V173A (pDH256) pBRα-MreB-E196G (pDH259) pBRα-MreB-E262G (pDH258)	pBRα-MreB encoding the indicated substitution in the MreB moiety of the fusion	This study
Additional pBRα-MreB mutants: pBRα-MreB-K77D (pDH301) pBRα-MreB-D78K (pDH302) pBRα-MreB-F84A (pDH300) pBRα-MreB-F84A (pDH293) pBRα-MreB-R124D (pDH298) pBRα-MreB-R125D (pDH299) pBRα-MreB-R127D (pDH294) pBRα-MreB-R127D (pDH294) pBRα-MreB-R188D (pDH307) pBRα-MreB-R188D (pDH307) pBRα-MreB-E196K (pDH271) pBRα-MreB-E196K (pDH271) pBRα-MreB-R204D (pDH306) pBRα-MreB-R204D (pDH306) pBRα-MreB-S269F (pDH305) pBRα-MreB-S269F (pDH310) pBRα-MreB-S284D (pDH316)	pBRα-MreB encoding the indicated substitution in the MreB moiety of the fusion	This study
pACλCI-MreB (pMT150)	Encodes λ Cl fused by a three-alanine linker to full-length <i>E. coli</i> MreB under the control of <i>lacUV5</i> promoter; confers resistance to Cm	This study
pBRα-CbtA (pMT155)	Encodes residues 1-248 of α fused by a three alanine linker to CbtA under the control of tandem p <i>lacUV5</i> and p <i>lpp</i> promoters; confers resistance to Carb	This study
pBRα-YpjF (pMT174)	Encodes residues 1-248 of α fused by a three alanine linker to YpjF under the control of tandem p <i>lacUV5</i> and p <i>lpp</i> promoters; confers resistance to Carb	This study
pBRα-Ykfl (pMT173)	Encodes residues 1-248 of α fused by a three alanine linker to Ykfl under the control of tandem p <i>lacUV5</i> and p <i>lpp</i> promoters; confers resistance to Carb	This study
pACλCI-CbtA (pMT154)	Encodes λ Cl fused by a three-alanine linker to CbtA under the control of <i>lacUV5</i> promoter; confers resistance to Cm	This study
pACλCI-CbtA-F65S (pMT180)	pACλCI-CbtA encoding the F65S substitution in the CbtA moiety of the fusion	This study

pACλCI-CbtA-R15C (pDH246)	pACλCI-CbtA encoding the R15C substitution in the CbtA moiety of the fusion	This study
pACλCI-CbtA-V48E (pDH200)	pACλCI-CbtA encoding the V48E substitution in the CbtA moiety of the fusion	This study
рАСλСІ-ҮрјҒ (рМТ170)	Encodes λ Cl fused by a three-alanine linker to YpjF under the control of <i>lacUV5</i> promoter; confers resistance to Cm	This study
pACλCI-YpjF-F65S (pMT192)	pACλCI-YpjF encoding the F65S substitution in the YpjF moiety of the fusion	This study
pACλCI-YpjF-C15R (pDH267)	pACλCI-YpjF encoding the C15R substitution in the YpjF moiety of the fusion	This study
pACλCI-Ykfl (pMT169)	Encodes λ CI fused by a three-alanine linker to YkfI under the control of <i>lacUV5</i> promoter; confers resistance to Cm	This study
pACλCI-YkfI-F65S (pDH206)	pACλCI-Ykfl encoding the F65S substitution in the Ykfl moiety of the fusion	This study
pACλCI-YkfI-C15R (pDH266)	pACλCI-Ykfl encoding the C15R substitution in the Ykfl moiety of the fusion	This study
pACλCI-ZipA _{CTD} (pDH159)	Encodes λ CI fused by a three-alanine linker to residues 186-328 of ZipA under the control of <i>lacUV5</i> promoter; confers resistance to Cm	This study
pACλCI-RodZ _{NTD} (pDH238)	Encodes λ CI fused by a three-alanine linker to the NTD of RodZ (residues 2- 84) under the control of <i>lacUV5</i> promoter; confers resistance to Cm	This study
oxin expression constructs:		
p3-37	Derivative of pCA24N; Encodes full- length His ₆ -Ykfl-GFP under the control of <i>pT5-lac</i> promoter; pBR origin; confers resistance to Cm	[3]
pMT136	Derivative of pCA24N; Empty vector control plasmid encoding full-length His ₆ - GFP under the control of <i>pT5-lac</i> promoter; pBR origin; confers resistance to Cm	This study
pMT138	Derivative of pCA24N; Encodes full- length His ₆ -YpjF-GFP under the control of <i>pT5-lac</i> promoter; pBR origin; confers resistance to Cm	This study
pMT139	Derivative of pCA24N; Encodes full- length His ₆ -CbtA-GFP under the control of <i>pT5-lac</i> promoter; pBR origin; confers resistance to Cm	This study
pMT144	Derivative of pCA24N; Encodes full- length His ₆ -Ykfl-F65S-GFP under the control of <i>pT5-lac</i> promoter; pBR origin; confers resistance to Cm	This study

	Derivative of pCA24N; Encodes full-	This study
pMT146	length His ₆ -CbtA-F65S-GFP under the control of <i>pT5-lac</i> promoter; pBR origin; confers resistance to Cm	This study
	Derivative of pCA24N; Encodes full-	
pMT188	length His ₆ -YpjF-F65S-GFP under the control of <i>pT5-lac</i> promoter; pBR origin; confers resistance to Cm	This study
pDH253	Derivative of pCA24N; Encodes full- length His ₆ -CbtA-R15C-GFP under the control of <i>pT5-lac</i> promoter; pBR origin; confers resistance to Cm	This study
pDH262	Derivative of pCA24N; Encodes full- length His ₆ -CbtA-R15C/F65S-GFP under the control of <i>pT5-lac</i> promoter; pBR origin; confers resistance to Cm	This study
pBAD33	<i>araC</i> ; pACYC 184 origin; <i>pBAD</i> promoter with no insert; confers resistance to Cm	[4]
pDH212	<i>araC</i> ; pACYC 184 origin; <i>pBAD-cbtA-</i> <i>F65S</i> ; confers resistance to Cm	This study
pDH289	<i>araC</i> ; pACYC 184 origin; <i>pBAD-ypjF-</i> <i>F65S</i> ; confers resistance to Cm	This study
pSG360	Empty vector used for cloning genes downstream of a p <i>lacUV5</i> promoter with an additional <i>lacO</i> site; pCDF origin; confers resistance to Spec/Strep; <i>lacl^q</i>	S. Goldman
pDH325	pSG360 derivative encoding untagged CbtA; confers resistance to Spec/Strep	This study
pDH326	pSG360 derivative encoding untagged CbtA-F65S; confers resistance to Spec/Strep	This study
pDH327	pSG360 derivative encoding untagged CbtA-R15C; confers resistance to Spec/Strep	This study
pDH328	pSG360 derivative encoding untagged CbtA-R15C/F65S; confers resistance to Spec/Strep	This study
pSG369	Empty vector used for cloning genes downstream of a p <i>tet</i> promoter; pCDF origin; confers resistance to Spec/Strep; <i>tetR</i>	S. Goldman
pDH335	pSG369 derivative encoding untagged CbtA-F65S; confers resistance to Spec/Strep	This study
pDH337	pSG369 derivative encoding untagged CbtA-R15C/F65S; confers resistance to Spec/Strep	This study
Other constructs:		
pCX41	Bears a temperature-sensitive origin of replication and a HindIII/ClaI fragment containing wild-type <i>ftsZ</i> and flanking homology to <i>ftsA</i> and <i>lpxC</i> ; confers resistance to Cm (10 µg/mL)	[5]; T. Bernhardt
pCX41-F268C	pCX41 derivative bearing <i>ftsZ-F268C</i>	T. Bernhardt

pCX41-D180N (pDH33)	pCX41 derivative bearing ftsZ-D180N	This study
pCX41-S177P (pDH34)	pCX41 derivative bearing <i>ftsZ-S177P</i>	This study
pCX41-L169P (pDH35)	pCX41 derivative bearing ftsZ-L169P	This study
pDR3	Encodes full-length <i>E. coli</i> FtsZ under the control of <i>lac</i> promoter; ColE1 origin with <i>lacl</i> ^q ; confers resistance to Carb	[6,7]; T. Bernhardt
pDR3-D180N (pDH27)	pDR3 derivative encoding FtsZ-D180N	This study
pDR3-S177P (pDH28)	pDR3 derivative encoding FtsZ-S177P	This study
pDR3-F182S (pDH29)	pDR3 derivative encoding FtsZ-F182S	This study
pDR3-L169P (pDH30)	pDR3 derivative encoding FtsZ-L169P	This study
pDH84	Encodes His ₆ -GFP under the control of pHYPERSPANK; confers resistance to Carb in <i>E. coli</i> and to MLS in <i>B. subtilis</i>	This study
pDH85	Encodes His ₆ -CbtA-GFP under the control of <i>pHYPERSPANK;</i> confers resistance to Carb in <i>E. coli</i> and to MLS in <i>B. subtilis</i>	This study
pDH102	Encodes His ₆ -CbtA-F65S-GFP under the control of <i>pHYPERSPANK;</i> confers resistance to Carb in <i>E. coli</i> and to MLS in <i>B. subtilis</i>	This study
pDR111	Used for integration of sequences into <i>B.</i> subtilis amyE locus; confers resistance to Carb in <i>E. coli</i> and Spec in <i>B. subtilis</i>	D. Rudner
pMLB1113	Used as empty vector control for <i>mreB</i> complementation; ColE1 origin; Encodes LacZ under the control of the <i>lac</i> promoter; <i>lacl⁹</i> ; confers resistance to Carb	[8]; T. Bernhardt
pFB149	ColE1 origin; Encodes wild-type MreBCD under the control of the <i>lac</i> promoter. Residues LE are appended to the end of the MreD sequence; <i>lacl^q</i> ; confers resistance to Carb	[6]; T. Bernhardt
pDH278	pFB149 derivative encoding MreB- E262G and wild-type MreCD	This study
pDH332	pFB149 derivative encoding MreB- S269F and wild-type MreCD	This study

- 1. Dove SL, Joung JK, Hochschild A. Activation of prokaryotic transcription through arbitrary proteinprotein contacts. Nature. 1997 Apr 10;386(6625):627–30.
- 2. Deighan P, Diez CM, Leibman M, Hochschild A, Nickels BE. The bacteriophage λ Q antiterminator protein contacts the β -flap domain of RNA polymerase. Proc Natl Acad Sci U S A. National Acad Sciences; 2008 Oct 7;105(40):15305–10.
- Kitagawa M, Ara T, Arifuzzaman M, Ioka-Nakamichi T, Inamoto E, Toyonaga H, et al. Complete set of ORF clones of *Escherichia coli* ASKA library (A Complete Set of *E. coli* K-12 ORF Archive): Unique Resources for Biological Research. DNA Res. 2006 Jan 9;12(5):291–9.
- 4. Guzman LM, Belin D, Carson MJ, Beckwith J. Tight regulation, modulation, and high-level expression by vectors containing the arabinose P_{BAD} promoter. J Bacteriol. American Society for

Microbiology (ASM); 1995 Jul;177(14):4121–30.

- 5. Wang XD, de Boer PA, Rothfield LI. A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. EMBO J. 1991 Nov;10(11):3363–72.
- 6. Bendezu FO, de Boer PAJ. Conditional Lethality, Division Defects, Membrane Involution, and Endocytosis in *mre* and *mrd* Shape Mutants of *Escherichia coli*. J Bacteriol. 2008 Feb 20;190(5):1792–811.
- 7. Bernhardt TG, de Boer PAJ. SImA, a Nucleoid-Associated, FtsZ Binding Protein Required for Blocking Septal Ring Assembly over Chromosomes in *E. coli*. Mol Cell. 2005 May;18(5):555–64.
- 8. de Boer PA, Crossley RE, Rothfield LI. A division inhibitor and a topological specificity factor coded for by the minicell locus determine proper placement of the division septum in *E. coli*. Cell. 1989 Feb 24;56(4):641–9.