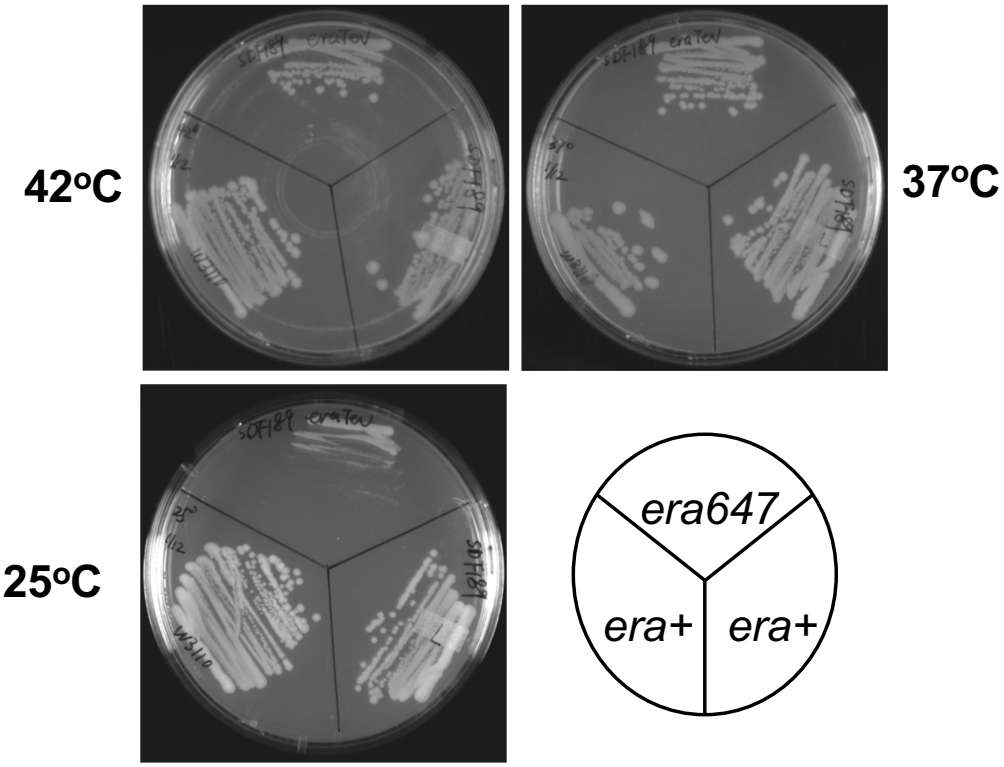

Fig. S1. Cold sensitivity of cells with *era647* at the native era locus. W3110 (*era*+), SDF189 (*era*+) or an *era647* derivative of SDF189 were streaked out on LB plates and incubated overnight at the temperatures indicated.

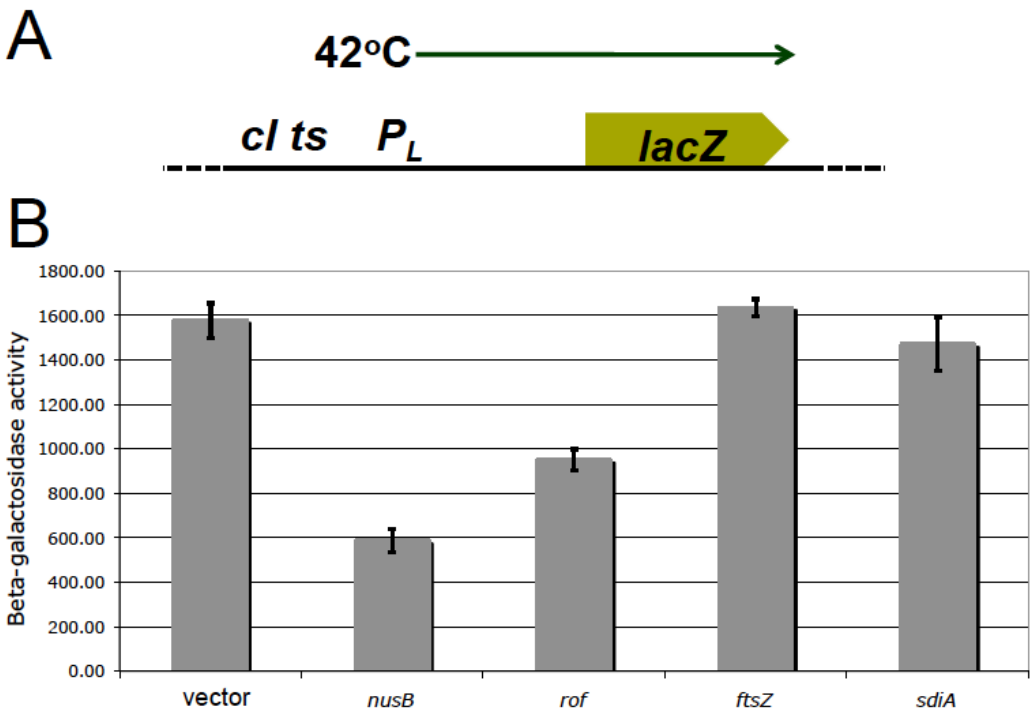

Fig. S2. Suppressor effects on expression from the *pL* operon.

The *lacZ* gene cloned to replace the *cIII* gene precisely at its ATG start codon (see Fig. S2) was used to measure β-galactosidase expression at 42°C in the presence of the different multicopy suppressor plasmids: The *nusB* gene on pXM14; the *rof* gene on pXM16; and the *ftsZ* gene on pXM15 the *sdiA* gene on pXM17. The pBR322 plasmid is the vector control. In the same assay without any plasmid, the *rho15 amp* mutant reduced expression to ~ 900 units of β-galactosidase. Units of activity were determined by the method of Miller (1).

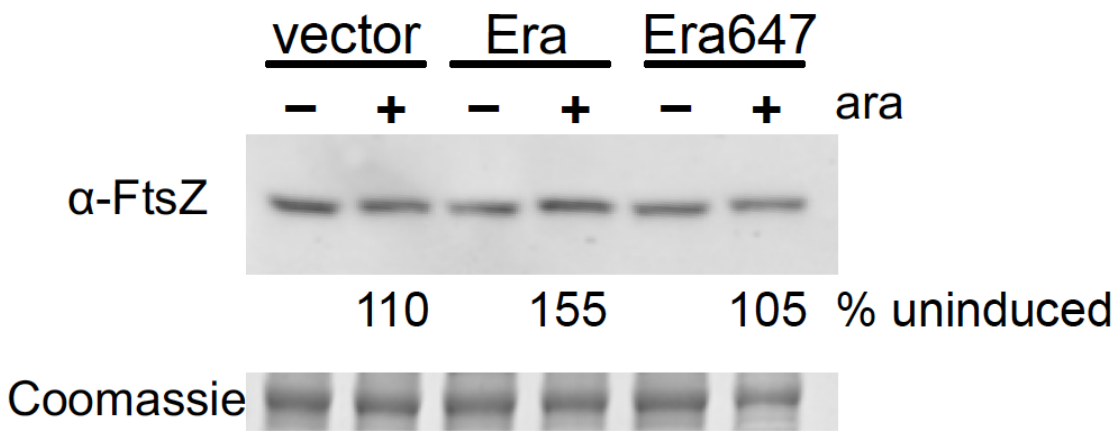

Fig. S3. Inhibition of cell division by Era647 is not due to a decrease in cellular FtsZ levels. Cultures of strains with pBAD30 vector, pBAD30-Era or pBAD30-Era647 were induced/not induced with 0.2% L-arabinose for 2 h at 30˚C, at equivalent optical densities. Two replicates of the 6 obtained samples were subjected to SDS-PAGE and Coomassie staining (bottom) or Western blotting with anti-FtsZ (top). Band intensities for each Coomassie-stained lane were quantified using Image Lab software (BioRad) and used to calculate a relative loading ratio for each lane. The same software was used to quantify FtsZ band intensities on the Western blot, which were then divided by the lane-specific relative loading ratio to obtain normalized values. The percent changes of FtsZ levels between induced and uninduced samples (shown) were obtained by dividing the mean values from the arabinose-induced replicates for each set by the uninduced average for each set.

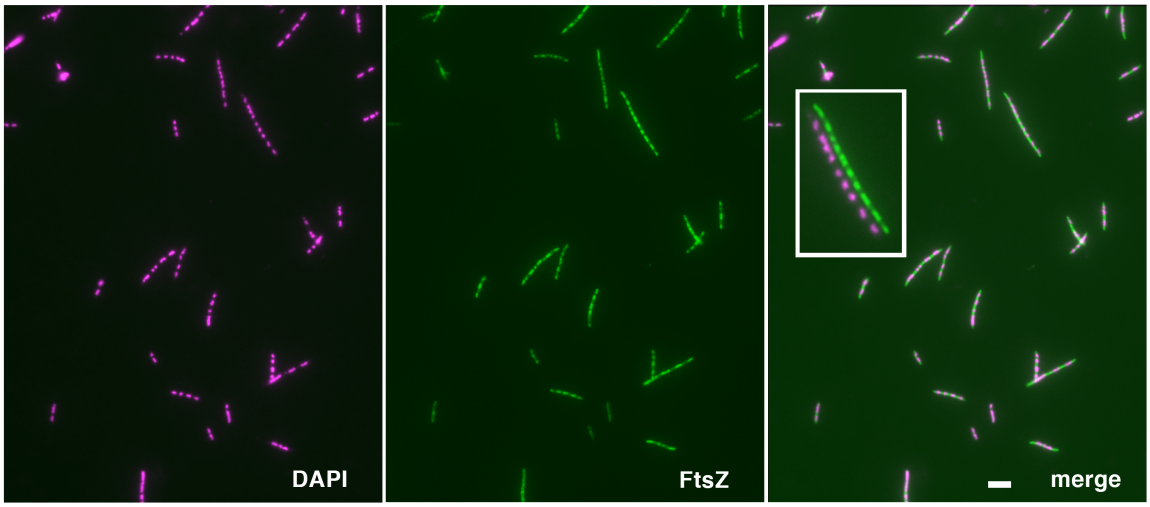

Fig. S4. FtsZ delocalizes to inter-nucleoid spaces in response to Era647 overproduction. TUC356 cells induced with L-arabinose for 2 h to overproduce Era647 were stained with DAPI to label nucleoids (magenta) or anti-FtsZ (green) as in Fig. 5, and the images were merged. The inset, magnified 2x, shows the DAPI and FtsZ channels from the longest visible filamentous cell in the field, in which one channel is spatially offset from the other to visualize the separate localization of FtsZ and nucleoids. Scale bar, 5 μm.

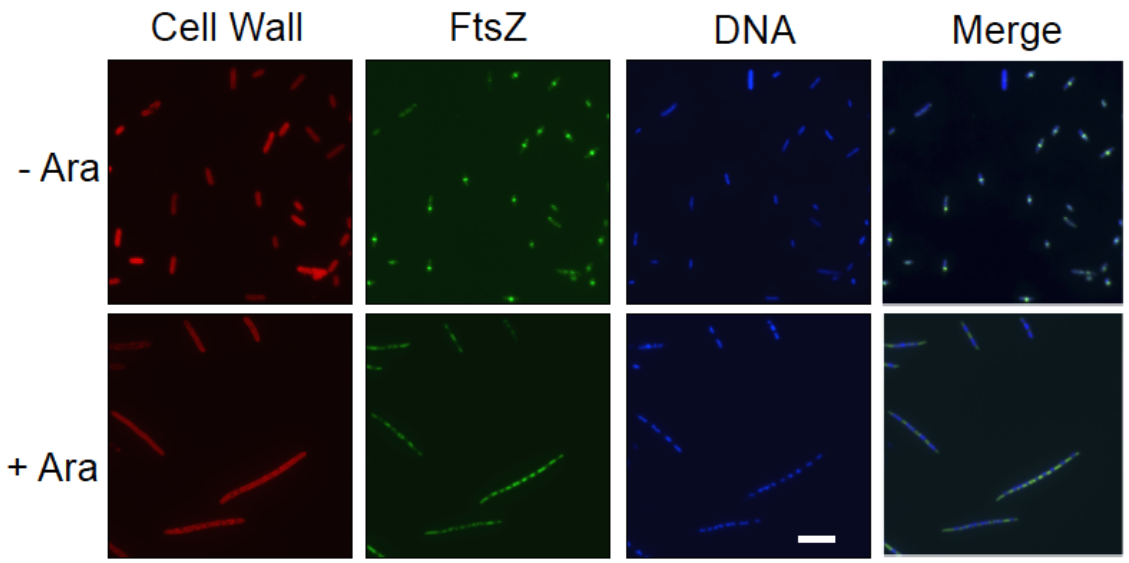

Fig. S5. Effect of Era647 overproduction on FtsZ rings in cells grown in minimal glycerol medium. Cells containing pBAD30 derivatives of Era or Era647 growing to early logarithmic phase in M63 glycerol were induced with L-arabinose (Ara) for 1 h, then fixed and stained with DAPI, fluorescent lectin, and immunostained with anti-FtsZ as described in Fig. 6. Scale bar, 5 μm.

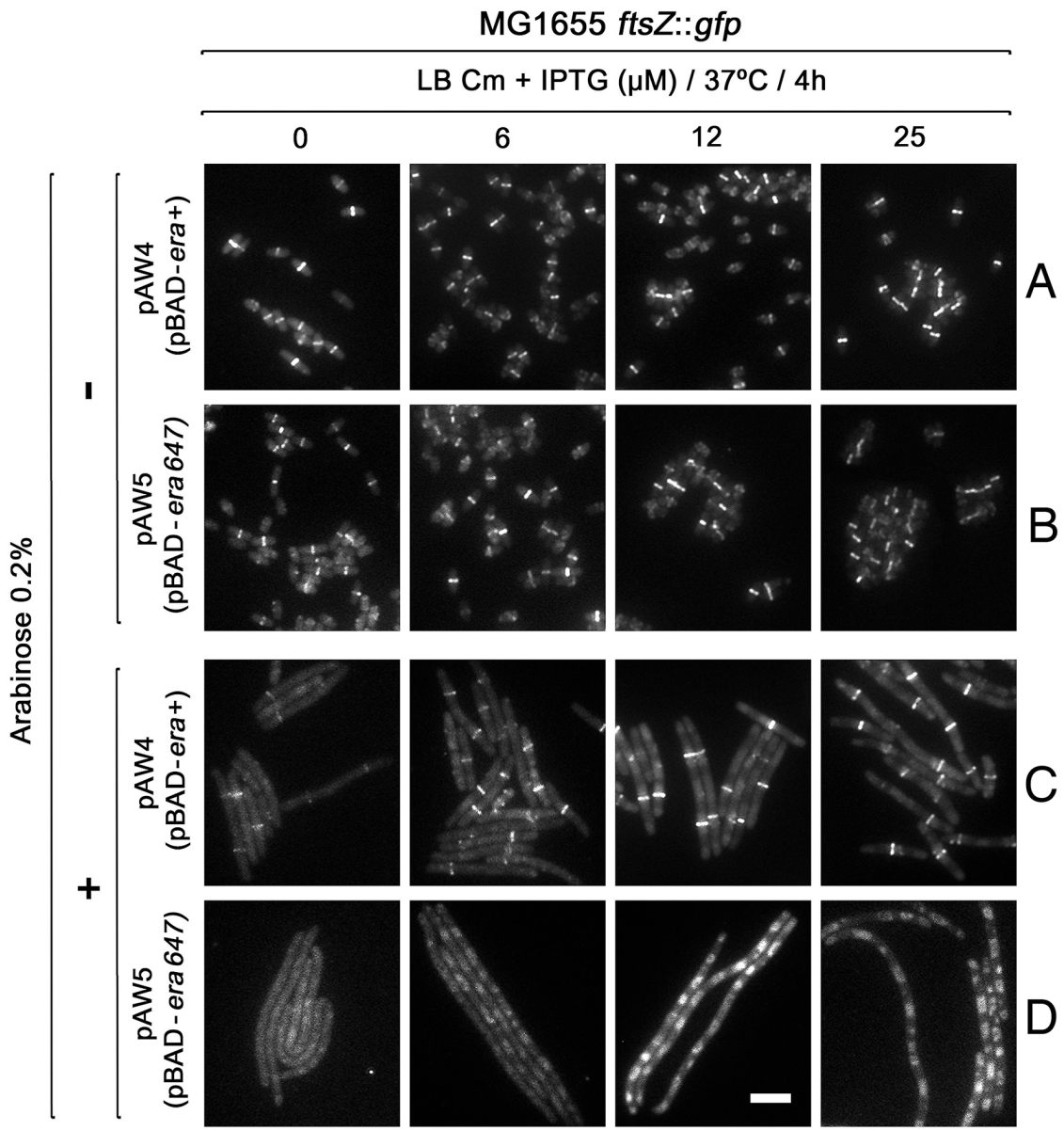

Fig. S6. Localization of FtsZ-GFP in response to Era or Era647 overproduction. MG1655 cells that express an ectopic *ftsZ-gfp* chromosomal fusion under IPTG control were transformed with either pAW4 (p*BAD-era*) or pAW5 (p*BAD-era647*) and grown in LB at 37˚C to early-logarithmic phase. Cells were then induced with (rows B, D) or without (rows A, C) arabinose in either 0, 6, 12 or 25 μM IPTG for 4 h, then placed on a slide with a thin layer of LB agarose and imaged by fluorescence microscopy. Scale bar, 5 μm.

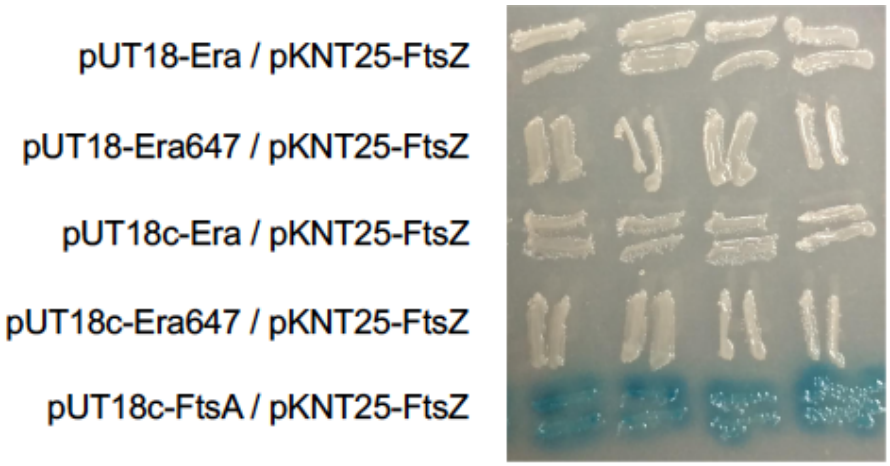

Fig. S7. Bacterial two-hybrid assay for Era-FtsZ interactions. Independent transformant colonies of BTH101 cells carrying the plasmid combinations shown were spotted onto LB supplemented with ampicillin, kanamycin, Xgal and 25 μM IPTG and incubated overnight at 30˚C, then photographed.

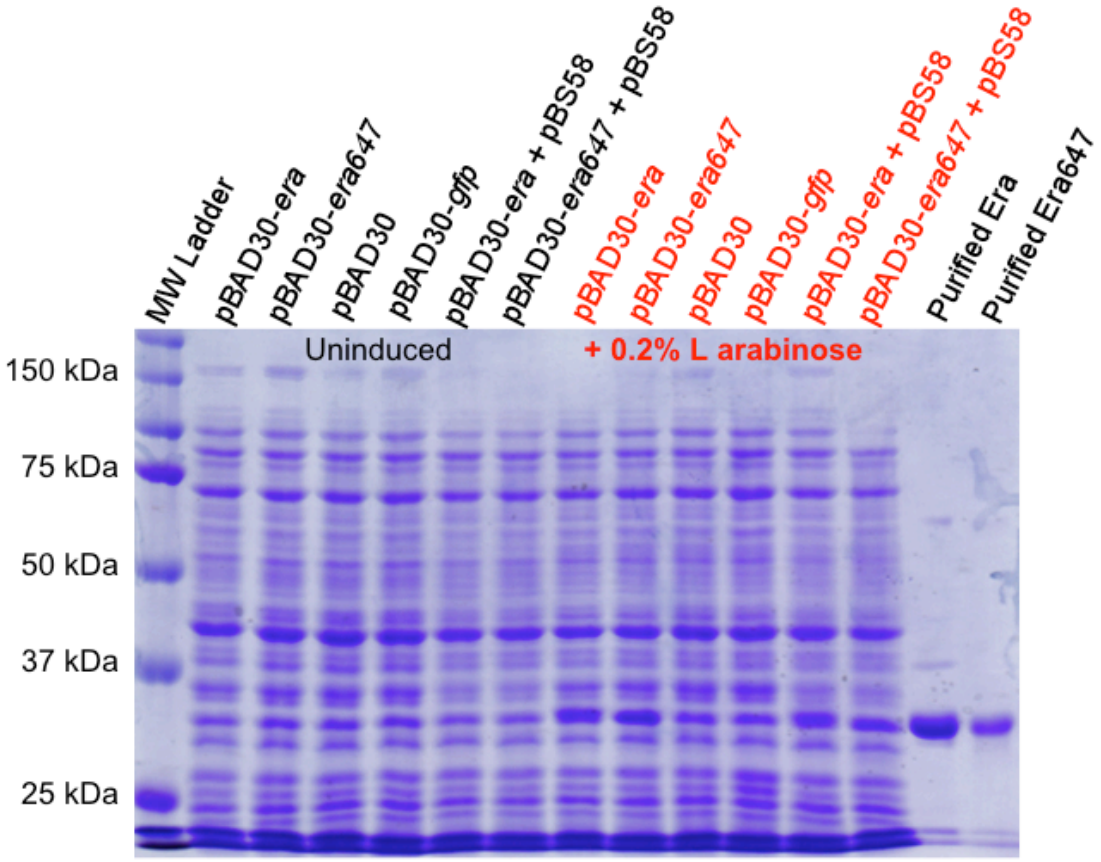

Fig. S8. Overproduction, purification, and standardization of Era or Era647 protein. Shown is a Coomassie-stained SDS polyacrylamide gel loaded with whole cell lysates of strains shown, normalized to OD_{600}. The two rightmost lanes of the gel were also loaded with purified Era or Era647 protein. Molecular weight standards in kDa are shown to the left.

Table S1. Bacterial strains and plasmids.

Strain	Description or relevant genotype †	Source/reference
W3110	*E. coli* K-12	Lab stock
HME45	W3110 *gal490 pgl*Δ8 λ *int red cIII cI857*Δ(*cro-bioA*)	(2)
SDF189	W3110 *yfhB*::Δ*Tn10*-A17	(3)
HT120	W3110 *rnc40*:: ΔTn*10*	(4)
MG1655	*E. coli* K-12	Lab stock
WM1074	MG1655 *lac-*	Lab stock
MT79	TB28 *leu*::Tn*10 ftsL** ($FtsL_{E88K}$)	(5)
TUC01	HME45 λ *int<>cat sacB red cIII cI857*Δ(*cro-bioA*)	This work
TUC03	HME45 λ (*cat sacB-cIII*)*<>era*$^{+}$ *cI857*Δ(*cro-bioA*)	This work
TUC05	HME45 λ (*cat sacB-cIII*)*<>era647 cI857*Δ(*cro-bioA*)	This work
TUC30	TUC05 /pSIM5	This work
TUC84	TUC05 λ *cI857 cro*$^{+}$ *lacZ* (Km^r)	This work
TUC85	TUC05 λ *cI857 cro27 lacZ* (Km^r)	This work
TUC93	SDF189 P_{rnc} *era67*	This work
TUC124	W3110 *ftsZ84* P_{rnc} *era67*	This work
TUC279	TUC05 /pACS1	This work

TUC303	TUC05 *sulA<>cat*	This work
TUC356	W3110 /pHKP67 (P_{BAD} *era647*)	This work
TUC357	TUC93 /pHKP67 (P_{BAD} *era647*)	This work
TUC358	W3110 /pHKP60 (P_{BAD} *era*$^{+}$)	This work
TUC359	TUC93 /pHKP60 (P_{BAD} *era*$^{+}$)	This work
TUC362	W3110 P_{rnc} *era647*	This work
TUC377	TUC124 P_{rnc} *era647*	This work
TUC382	W3110 /pBAD30	This work
TUC383	TUC93 /pBAD30	This work
TUC404	TUC05 /p*ftsQAZ*	This work
TUC412	TUC05 /pJPB71	This work
TUC413	TUC05 /pJPB223	This work
TUC414	TUC05 /pSEB25	This work
TUC415	TUC05 /pSEB162	This work
TUC421	TUC223 /p*ftsQAZ*	This work
TUC502	HME45 λ (*cat sacB-cIII*)*<>lacZ*	This work
TUC504	TUC502 /p*ftsQAZ*	This work
TUC540	HME45 λ (*cat sacB-cIII*)*<>era*$^{+}$	This work
TUC605	TUC05 /pBR322	This work

TUC606	TUC05 /pXM14	This work
TUC607	TUC05 /pXM15	This work
TUC609	TUC05 /pXM16	This work
TUC610	TUC05 /pXM17	This work
TUC612	TUC502 /pBR322	This work
TUC613	TUC502 /pXM14	This work
TUC614	TUC502 /pXM15	This work
TUC616	TUC502 /pXM16	This work
TUC617	TUC502 /pXM17	This work
TUC619	TUC124 /pBR322	This work
TUC620	TUC124 pXM14	This work
TUC621	TUC124 /pXM15	This work
TUC623	TUC124 /pXM16	This work
TUC624	TUC124 /pXM17	This work
TUC626	TUC05 *rho15<>amp*	This work
TUC630	TUC502 *rho15<>amp*	This work
TUC641	TUC05 *ftsZ84* (Tet^r)	This work
TUC708	MG1655 *lon*::Tn*10*	This work
TUC709	MG1655 *lon*::Tn*10 sulA<>cat*	This work

WM5166	MG1655 *Δ(λattL-lom)::bla lacI^q P$_{trc}$-ftsZ-gfp*	(6)
WM5570	WM5166 /pAW5	This work
WM6233	BW27783 *ftsZ*-mNG	(7)
WM6326	WM6233 *ftsL* leuO*::Tn*10* (P1 X MT79)	This work
WM6530	WM6326 /pAW5	This work
WM4915	WM1074 *ftsZ* leuO*::Tn*10*	(8)
WM6529	WM4915 /pAW5	This work

Plasmid	**Description**	**Source/reference**
pSIM5	pSC101 λ*cI857P$_L$*Δ(*kil-N*) (Cm^r)	(9)
pACS1	pBR322 *P$_{rnc}$ rnc$^+$ era$^+$* (Amp^r/Tet^r)	(4)
pBAD30	pACYC184 (Amp^r) with *P$_{araBAD}$*	(10)
pHKP60	pBAD30 *P$_{araBAD}$ era$^+$* (Amp^r)	This work
pHKP67	pBAD30 *P$_{araBAD}$ era647* (Amp^r)	This work
pGB2	pSC101 origin (Spc^r)	Lab stock
pJPB71	*ftsZ* cloned in pGB2 (Spc^r)	(11)
pJPB223	*ftsQAZ* cloned in pGB2 (Spc^r)	(12)

pSEB25	*ftsQZ* is pJPB223 with *ftsA* deleted (Spc^r)	(12)
pSEB162	*ftsQA* is pJPB223 with *ftsZ* deleted (Spc^r)	(12)
pBS58	*ftsQAZ* cloned in pGB2 (Spc^r)	(13)
pXM14	pBR322 *ribE*$^+$*nusB*$^+$ (Amp^r)	This work
pXM15	pBR322 *ftsZ* $^+$(Amp^r)	This work
pXM16	pBR322 *rof* $^+$*yaeP*$^+$ (Amp^r)	This work
pXM17	pBR322 *yecP*$^+$*sdiA*$^+$ (Amp^r)	This work
pBAD24Cm	pBAD24 with CAT (Cm^r) replacing *bla*	(10)
pAW4	pBAD24Cm-P_{araBAD} *era*$^+$ (Cm^r)	This work
pAW5	pBAD24Cm-P_{araBAD} *era647* (Cm^r)	This work
pKNT25-FtsZ	*E. coli* FtsZ fused to the N terminus of adenylate cyclase T25	(14)
pUT18C-FtsA	*E. coli* FtsA fused to the C terminus of adenylate cyclase T18	(15)
pUT18C-Era	*E. coli* Era fused to the C terminus of adenylate cyclase T18	This work
pUT18C-Era647	*E. coli* Era647 fused to the C terminus of adenylate cyclase T18	This work
pUT18-Era	*E. coli* Era fused to the N terminus of adenylate cyclase T18	This work
pUT18-Era647	*E. coli* Era647 fused to the N terminus of adenylate cyclase T18	This work

[†] The insertion site of *yfhB*::Δ*Tn10* A17 (4) has been determined here by Single Primer PCR analysis and sequencing (16).

Table S2. Frequency of cells with Z rings in strains with pBAD30-*era* or pAD30-*era647*, as measured by anti-FtsZ immunofluorescence.

Arabinose[1]	*era allele*	Cells with Z rings/total cells	% Cells with Z rings
0%	*era*	73/108	68%
	era647	54/65	83%
0.2%	*era*	100/135	74%
	era647	5/102	5%

[1] log-phase cells were grown with or without arabinose for 2 h

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