Supporting Information.

Roles for both FtsA and the FtsBLQ subcomplex in FtsN-stimulated cell constriction in *Escherichia coli*.

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Contents:

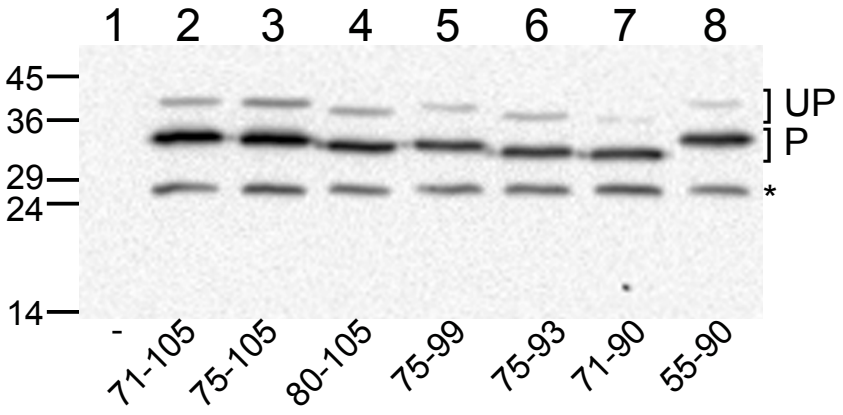

Figure S1. Immunoblot of periplasmic ^{TT}GFP fusions to various FtsN peptides.

Lanes contained whole cell extracts of strain TB28 [*wt*] harboring pMLB1113ΔH3 [vector] (1), pMG14 [P_{lac}::*torA*$^{1-43}$-*gfp*-*ftsN*$^{71-105}$] (2), pLP168 [P_{lac}::*torA*$^{1-43}$-*gfp*-*ftsN*$^{75-105}$] (3), pLP169 [P_{lac}::*torA*$^{1-43}$-*gfp*-*ftsN*$^{80-105}$] (4), pLP219 [P_{lac}::*torA*$^{1-43}$-*gfp*-*ftsN*$^{75-99}$] (5), pLP218 [P_{lac}::*torA*$^{1-43}$-*gfp*-*ftsN*$^{75-93}$] (6), pMG50 [P_{lac}::*torA*$^{1-43}$-*gfp*-*ftsN*$^{71-90}$] (7), or pLP221 [P_{lac}::*torA*$^{1-43}$-*gfp*-*ftsN*$^{55-90}$] (8). Overnight cultures were diluted 100-fold in M9-maltose medium with 50 μg/ml ampicillin and 100 μM IPTG and incubated at 30°C to OD_{600}=~0.5. Fusion proteins were detected using α-GFP polyclonal antibodies. Migration of molecular weight standards (kD) is indicated on the left. Unprocessed (UP) and processed (P, lacking the TorA signal sequence) forms of the fusions are indicated on the right. A degradation product is indicated with an asterisk (*). FtsN residues present in the various fusions are indicated at the bottom.

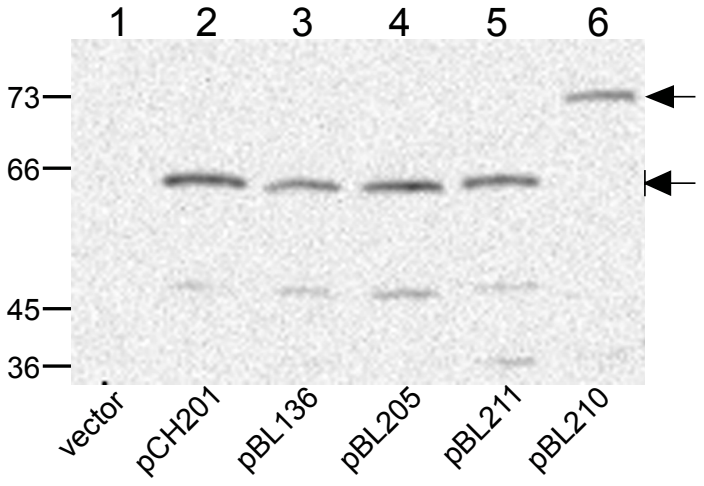

Figure S2. Immunoblot of GFP fusions to various FtsN deletion-substitution variants.

Lanes contained whole cell extracts of strain TB28 [*wt*] harboring pMLB1113ΔH3 [vector] (1), pCH201 [P_{lac}::*gfp-ftsN*] (2), pBL136 [P_{lac}::*gfp-ftsN*$^{\Delta(64\text{-}101)<>22}$] (3), pBL205 [$P_{lac}$::*gfp-ftsN*$^{\Delta(59\text{-}73)<>5}$] (4), pBL211 [$P_{lac}$::*gfp-ftsN*$^{\Delta(59\text{-}73)<>15}$] (5), or pBL210 [$P_{lac}$::*gfp-ftsN*$^{\Delta(59\text{-}73)<>95}$] (6). Overnight cultures were diluted 100-fold in LB medium with 50 μg/ml ampicillin and 5 μM IPTG and incubated at 30°C to OD_{600}=~0.5. Fusion proteins were detected using α-GFP polyclonal antibodies, and are marked by arrows on the right. Molecular weight standards (kD) are indicated on the left.

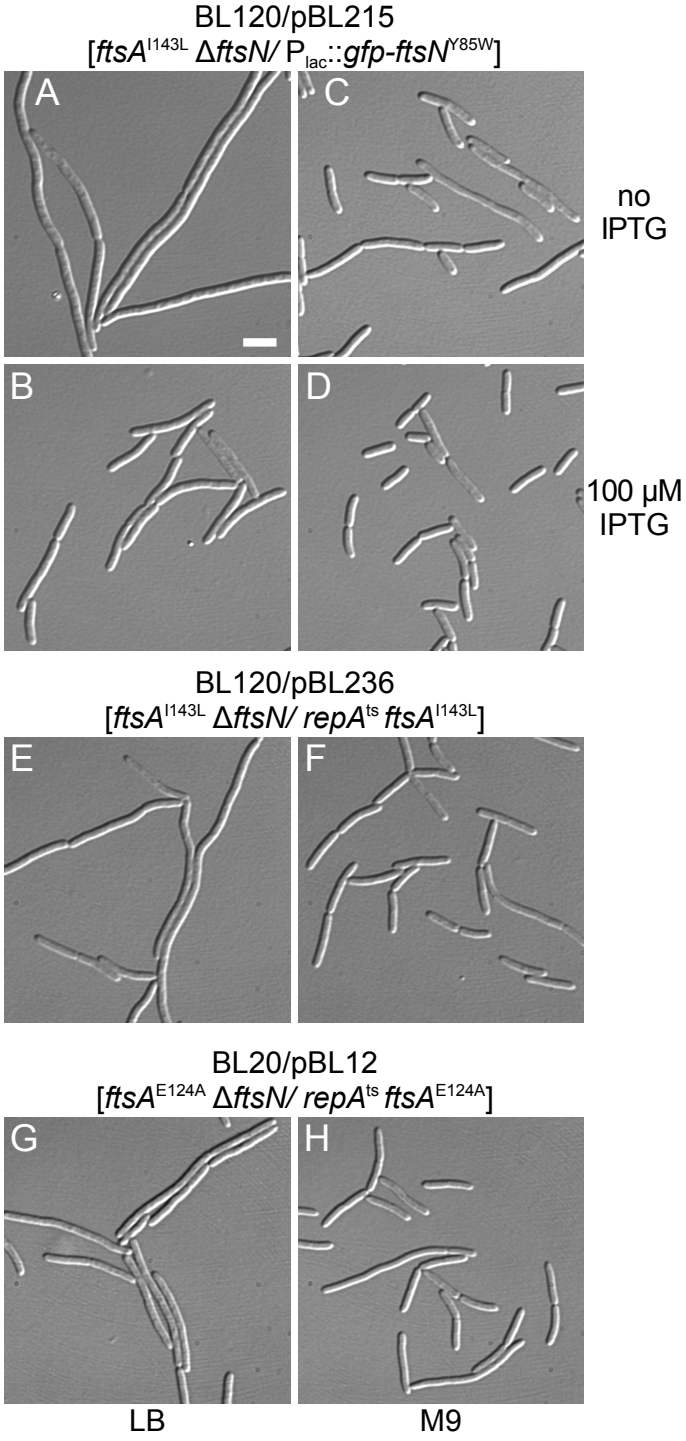

Figure S3. Cell fission in the absence of (functional) FtsN by (over)production of FtsA^{I143L} or FtsA^{E124A}.

Differential interference contrast images of live BL120 [*ftsA*^{I143L} Δ*ftsN*] cells carrying pBL215 [P_{lac}::*gfp-ftsN*^{Y85W}] (A-D) or pBL236 [*repA*^{ts} *ftsA*^{I143L}] (E and F), and of BL20 [*ftsA*^{E124A} Δ*ftsN*] cells carrying pBL12 [*repA*^{ts} *ftsA*^{E124A}] (G and H). Cells were grown to OD_{600}=~0.5 in LB (E, G), LB supplemented with 0.2% glucose (A) or 100 μM IPTG (B), M9-glucose (C, F, H), or M9-glucose with 100 μM IPTG (D). Bar equals 4 μm.

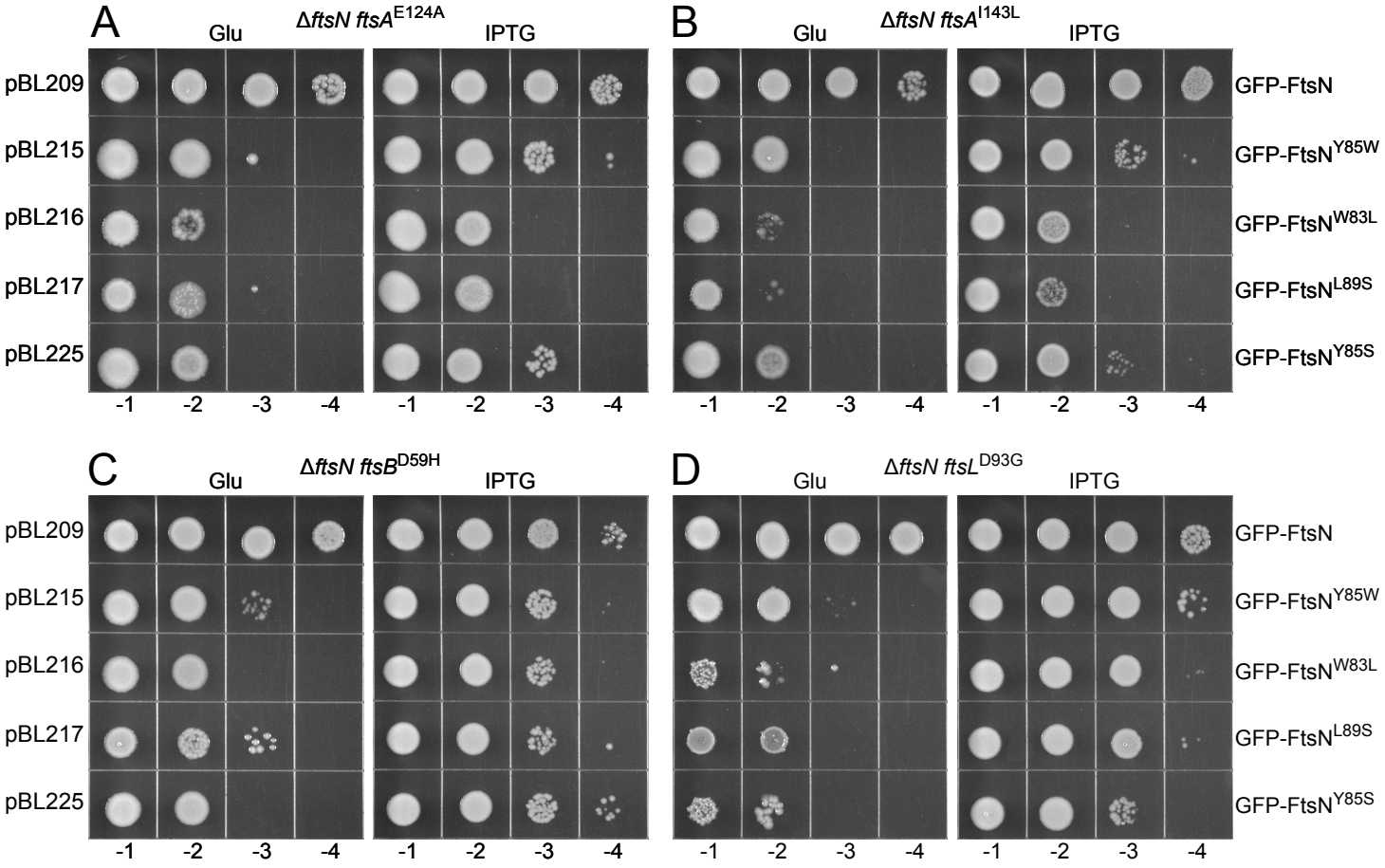

Figure S4. Little allele-specificity of ^{E}FtsN*-supressing mutations in *ftsA*, *ftsB*, or *ftsL*.

Spot titer analyses of strains BL20 [*ftsA*^{E124A} Δ*ftsN*] (A), BL120 [*ftsA*^{I143L} Δ*ftsN*] (B), BL141 [*ftsB*^{D59H} Δ*ftsN*] (C), and BL157 [*ftsL*^{D93G} Δ*ftsN*] (D), carrying mini-F plasmids encoding WT or mutant variants of GFP-FtsN under control of the *lac* regulatory region as indicated on the sides of each row. Cells were grown overnight at 30°C in LB with 100 μM IPTG, serially diluted to $OD_{600}=4\times10^{X}$, and 5 μl of each dilution was spotted on M9-maltose agar supplemented with 0.2% glucose or 100 μM IPTG, as indicated. Plates were incubated at 30°C for 42 hr.

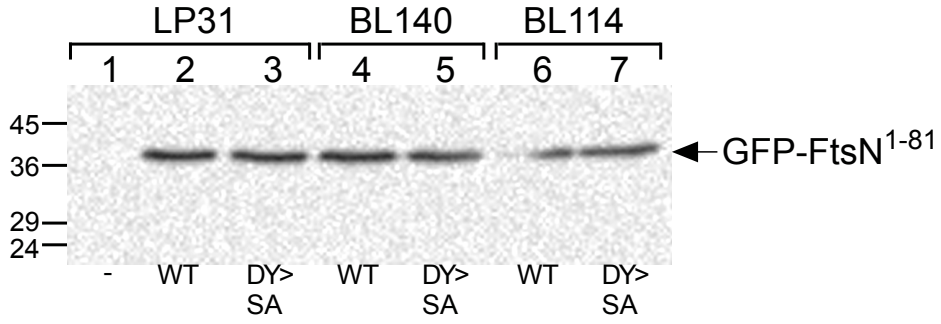

Figure S5. Immunoblot of GFP-FtsN$^{1-81}$ and GFP-FtsN$^{1-81,\ DY>SA}$ fusions.

Lanes contained whole cell extracts of strain LP31 [*recA*] harboring pMLB1113ΔH [vector] (1), pMG13 [P_{lac}::*gfp-ftsN*$^{1-81}$] (2), or pBL335 [P_{lac}::*gfp-ftsN*$^{1-81,\ DY>SA}$] (3), strain BL140 [*ftsB*^{D59H}] harboring pMG13 (4), or pBL335 (5), and strain BL114 [*ftsA*^{I143L}] harboring pMG13 (6), or pBL335 (7). Overnight cultures were diluted 100-fold in M9-maltose medium with 50 μg/ml ampicillin and 100 μM IPTG and incubated at 30°C to OD_{600}=~0.5. Fusion proteins were detected using α-GFP polyclonal antibodies. Molecular weight standards (kD) are indicated on the left.

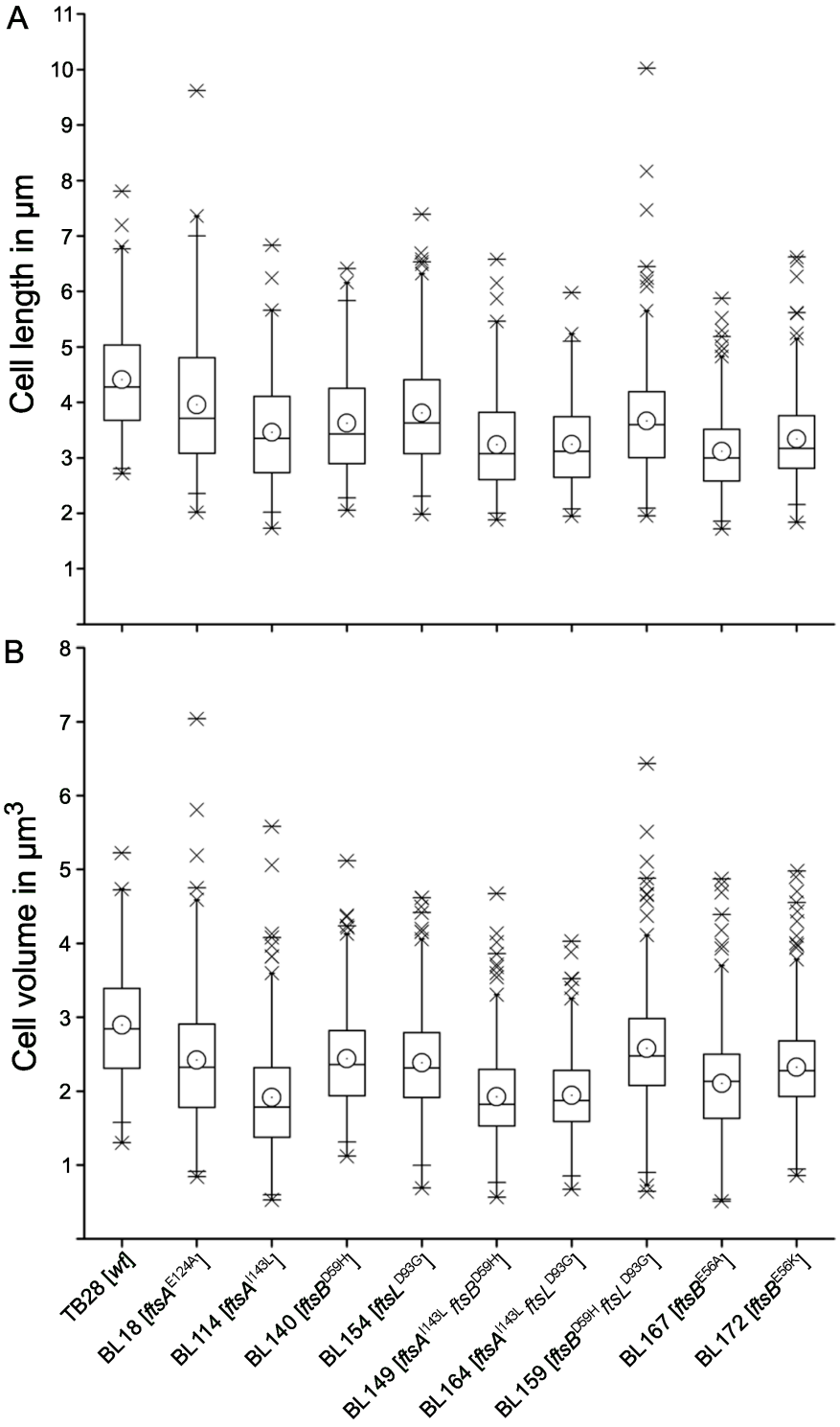

Figure S6. Cell size distributions of FtsN^{+} strains harboring ^{E}FtsN*-suppressing mutations.

Box plots showing cell length (A) and volume (B) distributions of the LB-grown strains listed in Table 5. In each plot, values within the 25th-50th and 50th-75th percentile ranges (boxed), minimum, maximum, 1st and 99th percentile values (horizontal cross-hatches), and average (⊙) and outlier (X) values are indicated. Values below Q1-1.5×IQR or above Q3+1.5×IQR (with Q1=first quartile, Q3=third quartile, and IQR=interquartile range) were considered outliers.

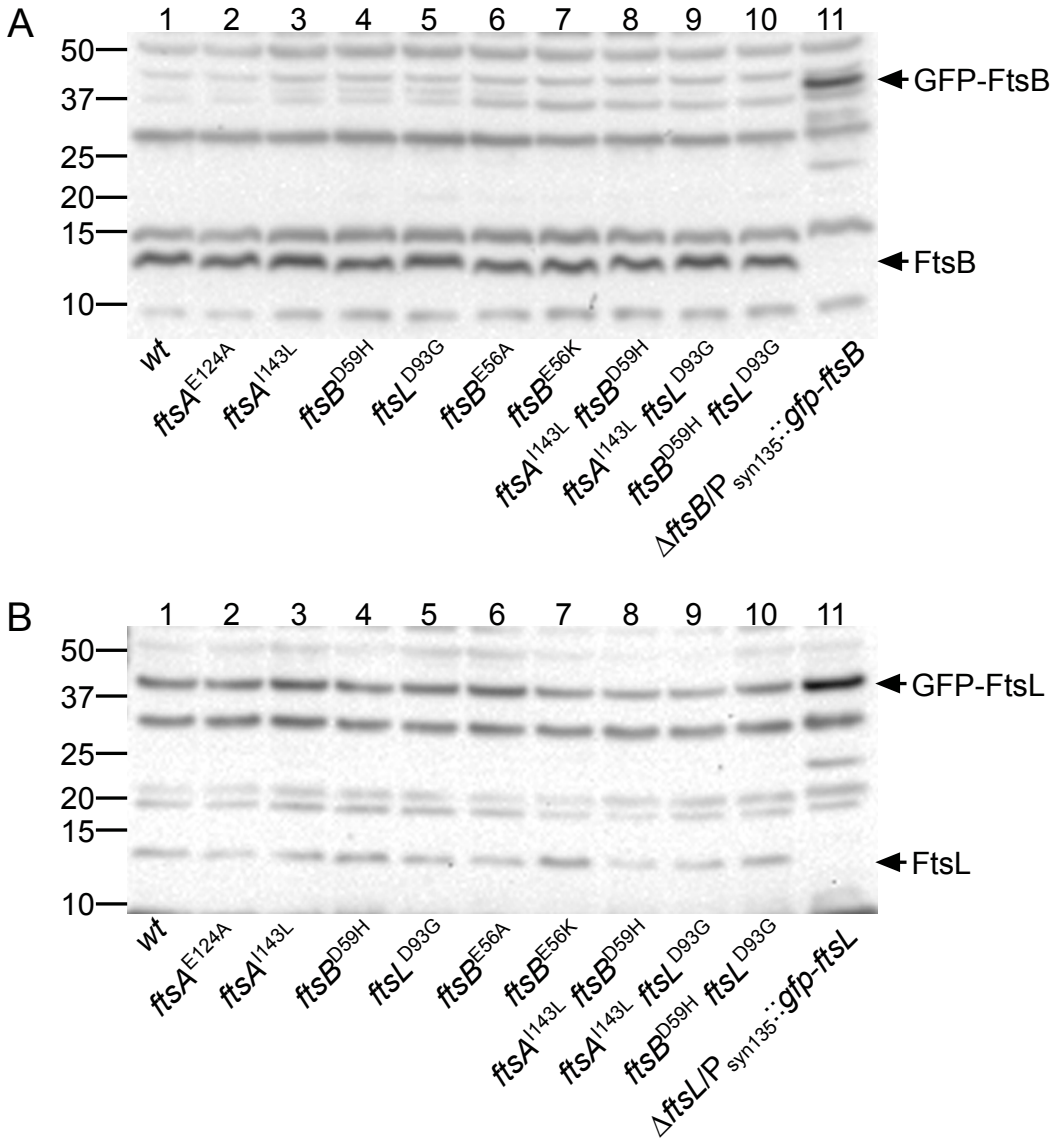

Figure S7. Immunoblot of FtsB, FtsL, and mutant variants.

(A, B) Lanes contained whole cell extracts of strain TB28 [*wt*] (1), BL18 [*ftsA*^{E124A}] (2), BL114 [*ftsA*^{I143L}] (3), BL140 [*ftsB*^{D59H}] (4), BL154 [*ftsL*^{D93G}] (5), BL167 [*ftsB*^{E56A}] (6), BL172 [*ftsB*^{E56K}] (7), BL149 [*ftsA*^{I143L} *ftsB*^{D59H}] (8), BL164 [*ftsA*^{I143L} *ftsL*^{D93G}] (9), BL159 [*ftsB*^{D59H} *ftsL*^{D93G}] (10), and BL155/pBL194 [Δ*ftsB* / P_{syn135}::*gfp-ftsB*] (11, panel A only), or BL156/pBL195 [Δ*ftsL* / P_{syn135}::*gfp-ftsL*] (11, panel B only). Cells were grown to OD_{600}=0.5-0.6 at 30°C in LB, whole cell extracts were prepared, and 40 µg of total protein was loaded in each lane of a 10-20% Tricine gradient gel (Invitrogen). Proteins were detected using α-FtsB (A) or α-FtsL (B) polyclonal antibodies. The positions of FtsB (A, lanes 1-10), GFP-FtsB (A, lane 11), FtsL (B, lanes 1-10), and GFP-FtsL (B, lane 11) are indicated. Most other bands represent non-specific cross-reactive species. Molecular weight standards (kD) are indicated on the left.

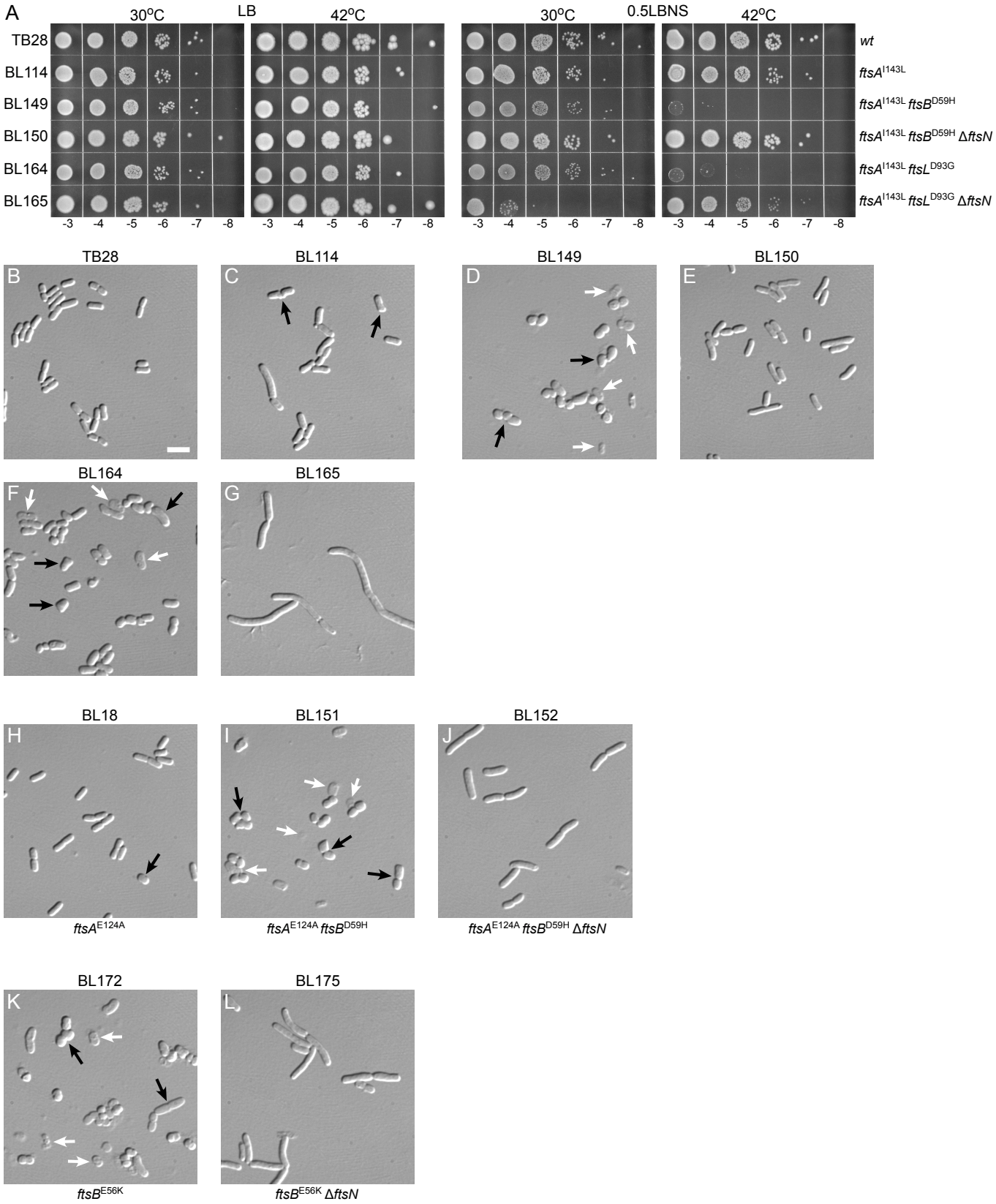
A
LB
30°C
42°C
0.5LBNS
30°C
42°C
TB28
BL114
BL149
BL150
BL164
BL165
wt
ftsA I143L
ftsA I143L ftsB D59H
ftsA I143L ftsB D59H ΔftsN
ftsA I143L ftsL D93G
ftsA I143L ftsL D93G ΔftsN
-3 -4 -5 -6 -7 -8 -3 -4 -5 -6 -7 -8 -3 -4 -5 -6 -7 -8 -3 -4 -5 -6 -7 -8
TB28
BL114
BL149
BL150
B
C
D
E
BL164
BL165
F
G
BL18
BL151
BL152
H
I
J
ftsA E124A
ftsA E124A ftsB D59H
ftsA E124A ftsB D59H ΔftsN
BL172
BL175
K
L
ftsB E56K
ftsB E56K ΔftsN

Figure S8. Conditional lethality of ^{E}FtsN*-suppressing mutations in FtsN^{+} cells.

(A) Cells of the indicated strains were grown overnight in LB at 30°C. Cultures were serially diluted in LB to $OD_{600}=4\times10^{X}$ as indicated, and 5 μl of each dilution was spotted on LB or 0.5LBNS agar. Plates were incubated for 16 hr at 42°C or 20 hr at 30°C. Growth phenotypes of the *ftsA*^{E124A} strains BL18, BL151, and BL152 (not shown) were similar to those of the *ftsA*^{I143L} strains BL114, BL149, and BL150, respectively.

(B-L) Cells of the indicated strains were grown overnight in LB at 30°C, diluted 400-fold in 0.5LBNS, grown for 3.5 hr at 42°C, and imaged live. Arrows point at examples of cells with shape defects (black) or of cell ghosts/debris (white).

Table S1. Single residue substitutions in the essential domain of FtsN obtained by site-directed and scanning mutagenesis.

Plasmid name	wt residue	wt codon	new codon	new residue	d/s[a]	> 1[b]	CH31 rescue[c]
pBL321	L75	CTA	GCT	A	d		+
pBL322	P76	CCA	GCA	A	d		+
pBL323	P77	CCA	GCA	A	d		+
pBL324	K78	AAA	GCA	A	d		+
pBL325	P79	CCA	GCA	A	d		+
pBL326	E80	GAA	GCA	A	d		+
pBL116(E81A)	E81	GAA	GCG	A	s		+
pBL93			AAG	K	d		+
pBL116(R82T)	R82	CGC	ACG	T	s		+
pBL116(W83A)	W83	TGG	GCG	A	s		-
pBL163			TTT	F	d		+
pBL116(W83G)			GGT	G	s		-
pBL116(W83L)			CTG	L	s		-
pBL116(W83M)			ATG	M	s		-
pBL116(W83Q)			CAG	Q	s		-
pBL180			ACC	T	d		-
pBL116(W83T)			ACG	T	s		-
pBL327	R84	CGC	GCC	A	d		+
pBL116(R84R)			CGT	R	s		+
pBL116(Y85A)	Y85	TAC	GCA	A	s	2	-
pBL116(Y85A2)			GCC	A	s		-
pBL116(Y85C)			TGT	C	s		-
pBL116(Y85E)			GAA	E	s	3	-
pBL165			TTC	F	d		+
pBL116(Y85G)			GGG	G	s		+
pBL116(Y85G2)			GGT	G	s		+
pBL116(Y85I)			ATT	I	s	2	-
pBL116(Y85K)			AAA	K	s	4	-
pBL116(Y85K2)			AAG	K	s	2	-

pBL116(Y85L)			CTC	L	s		-
pBL116(Y85L2)			CTG	L	s	2	-
pBL116(Y85P)			CCA	P	s	3	-
pBL116(Y85P2)			CCC	P	s	2	-
pBL116(Y85Q)			CAA	Q	s		-
pBL116(Y85R)			AGA	R	s		-
pBL116(Y85R2)			AGG	R	s		-
pBL116(Y85R3)			CGA	R	s		-
pBL94			CGC	R	d		-
pBL116(Y85R4)			CGC	R	s		-
pBL116(Y85R5)			CGG	R	s	2	-
pBL160			AGC	S	d		-
pBL116(Y85S)			AGC	S	s		-
pBL116(Y85T)			ACT	T	s	4	-
pBL116(Y85V)			GTA	V	s		-
pBL116(Y85W)			TGG	W	s		-
pBL116(Y85Y)			TAT	Y	s		+
pBL116(Y85*)			TAG	*	s		-
pBL116(I86R)	I86	ATT	CGC	R	s		+
pBL100	K87	AAA	GAA	E	d		+
pBL116(K87I)			ATT	I	s		+
pBL116(E88V)	E88	GAG	GTG	V	s		+
pBL116(L89A)	L89	CTG	GCA	A	s	2	-
pBL116(L89A2)			GCC	A	s	2	-
pBL328			GCG	A	d		-
pBL116(L89A3)			GCG	A	s		-
pBL116(L89C)			TGC	C	s		-
pBL116(L89E)			GAA	E	s		-
pBL116(L89F)			TTC	F	s	2	+
pBL116(L89F2)			TTT	F	s		+
pBL116(L89G)			GGG	G	s		-
pBL116(L89H)			CAC	H	s		-
pBL116(L89H2)			CAT	H	s	2	-
pBL116(L89I)			ATC	I	s		+

pBL116(L89K)			AAA	K	s		-
pBL116(L89L)			CTA	L	s		+
pBL116(L89L2)			TTA	L	s		+
pBL116(L89M)			ATG	M	s		+
pBL116(L89N)			AAC	N	s		-
pBL116(L89P)			CCA	P	s	2	-
pBL116(L89P2)			CCG	P	s		-
pBL116(L89P3)			CCT	P	s	3	-
pBL116(L89Q)			CAA	Q	s	2	-
pBL116(L89R)			CGA	R	s		-
pBL116(L89S)			TCA	S	s	2	-
pBL116(L89S2)			TCG	S	s		-
pBL116(L89T)			ACA	T	s		-
pBL116(L89T2)			ACC	T	s		-
pBL116(L89Y)			TAC	Y	s	3	+
pBL116(L89Y2)			TAT	Y	s	2	+
pBL116(L89*)			TAA	*	s		-
pBL329	E90	GAA	GCA	A	d		+
pBL116(E90*)			TAG	*	s		-
pBL116(S91H)	S91	AGT	CAC	H	s		+
pBL116(R92L)	R92	CGC	TTA	L	s		+
pBL116(Q93H)	Q93	CAG	CAC	H	s		+
pBL116(Q93K)			AAA	K	s		+

[a] Obtained by site-directed (d) or site-scanning (s) mutagenesis.

[b] Multiples of independent clones of an allele that were isolated and analyzed.

[c] Cells of strain CH31 [P_{BAD}::*ftsN*] harboring mutant derivatives of pCH201[*bla lacI*^{q} P_{lac}::*gfp-ftsN*] (d) or of pBL116 [*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G,\ S67G,\ +A102}$] (s) were colony purified on LB agar containing amplicillin and either no or 0.5% arabinose. +, good growth; -, no or very poor growth. Note that CH31 cells harboring unmutated pCH201 or pBL116 produce sufficient GFP-FtsN to allow normal growth, even in the absence of IPTG inducer.

Table S2. Isolation of plasmid-borne ^{E}FtsN*-suppressors in *ftsB* and *ftsL*.

Residue substitutions in FtsB				[a]Originating screen(s)			
wt residue	wt codon	new codon	new residue	mini-F plasmid	Protein under P_{lac} control	Selection temp.	[b]No. clones
A55	GCC	ACC	T	pBL215	GFP-FtsN^{Y85W}	37^{o}C	1
E56	GAA	GCA	A	pBL215	GFP-FtsN^{Y85W}	37^{o}C	1
E56	GAA	GGA	G	pBL215	GFP-FtsN^{Y85W}	42^{o}C	1
				pJH10	GFP-MalF$^{2-39}$-FtsN$^{55-320, Y85W}$	37^{o}C	2
				pJH11	GFP-MalF$^{2-39}$-FtsN$^{55-320, W83L}$	37^{o}C	1
E56	GAA	AAA	K	pBL216	GFP-FtsN^{W83L}	37^{o}C	1
				pJH11	GFP-MalF$^{2-39}$-FtsN$^{55-320, W83L}$	37^{o}C	2
E56	GAA	GTA	V	pBL215	GFP-FtsN^{Y85W}	37^{o}C	1
				pBL215	GFP-FtsN^{Y85W}	42^{o}C	2
				pBL216	GFP-FtsN^{W83L}	42^{o}C	2
D59	GAT	GTT	V	pJH10	GFP-MalF$^{2-39}$-FtsN$^{55-320, Y85W}$	37^{o}C	1*
No survivors				pBL217	GFP-FtsN^{Y85W}	37^{o}C	NA
				pBL217	GFP-FtsN^{Y85W}	42^{o}C	NA
Residue substitutions in FtsL							
E88	GAG	AAG	K	pBL215	GFP-FtsN^{Y85W}	42^{o}C	1
				pJH11	GFP-MalF$^{2-39}$-FtsN$^{55-320, W83L}$	37^{o}C	1*
E88	GAG	GTG	V	pBL215	GFP-FtsN^{Y85W}	37^{o}C	2
				pBL216	GFP-FtsN^{W83L}	42^{o}C	1
N89	AAT	AGT	S	pBL215	GFP-FtsN^{Y85W}	37^{o}C	1
				pBL215	GFP-FtsN^{Y85W}	42^{o}C	3
				pBL225	GFP-FtsN^{Y85S}	37^{o}C	1
D93	GAC	GGC	G	pBL215	GFP-FtsN^{Y85W}	42^{o}C	2
H94	CAT	TAT	Y	pBL215	GFP-FtsN^{Y85W}	37^{o}C	1
No survivors				pBL217	GFP-FtsN^{Y85W}	37^{o}C	NA
				pBL217	GFP-FtsN^{Y85W}	42^{o}C	NA
				pJH10	GFP-MalF$^{2-39}$-FtsN$^{55-320, Y85W}$	37^{o}C	NA

[a] Strain JH1/pBL200 [Δ*ftsN*<>*aph* Δ*recA*/ *aadA repA*^{ts} P_{syn135}::*ftsN I-SceI cI*857 $P_{\lambda R}$::*i-sceI*] harboring the indicated mini-F plasmid (*bla*) was transformed with a library of plasmid pBL336 [*tetA* P_{syn135}::*ftsB**] containing randomly mutated *ftsB* (upper rows), or with a library of plasmid pJH2 [*tetA*

P_{syn135}::*ftsL**] containing randomly mutated *ftsL* (lower rows). Cells were plated on LB agar with Kan, Amp, Tet, and 200 μM IPTG at 37°C or 42°C. Survivor colonies were purified, the absence of pBL200 was verified ($Spec^S$), and the plasmid-borne *ftsB* or *ftsL* allele was sequenced. pBL336 or pJH2 derivatives with multiple silent and/or missense mutations in *ftsB* or *ftsL*, respectively, were used for subcloning to unambiguously identify the ^{E}FtsN*-suppressing mutation as summarized in Table 4 and Figure 3.

[b] Number of independent clones isolated. Asterisk (*) indicates poor growth. NA, not applicable. Parallel transformation of JH1/pBL200 bearing pBL209 [P_{lac}::*gfp-ftsN*] with either mutant library under similar conditions yielded 700-1400 transformants per screen.

Table S3. $FtsB^{E56A}$ promotes early sPG synthesis and cell fission.

Strain [genotype]	TB28 [*wt*]	BL167 [$ftsB^{E56A}$]	
Number of cells measured	308	318	% WT
Average cell length in μm (SD)	4.35 (0.98)	3.36 (0.77)	77
Avgerage cell volume in μm^3 (SD)	3.96 (1.25)	3.06 (0.99)	77
% cells with 'HADA ring'	51	56	110
% cells with visible constriction	40	35	88
% 'HADA rings' without obvious constriction	23	40	174
% constrictions without obvious 'HADA ring'	2	2	100
Mass doubling time (min)	43	43	100

[a] Overnight cultures in LB were diluted to OD_{600}=0.02 in LB, and growth was continued at 30°C to OD_{600}=0.5-0.6. Cells were pulse-labeled with HADA for 1 min and immediately fixed in ethanol. SD, standard deviation of the mean. Average cell length and volume of BL167 was significantly smaller than those of TB28 by two-sample t-test ($p<0.005$). See also Fig.6.

Table S4. *E.coli* strains used in this study.

Strain	Relevant genotype	Source or Reference
BL17	TB28, *leu*::Tn10	This work
BL18	TB28, *ftsA*^{E124A}	(Gerding *et al.*, 2009)
BL19	TB28, *ftsA*^{E124A} *leu*::Tn10	This work
BL20*	TB28, *ftsA*^{E124A} *ftsN<>aph*	(Gerding et al., 2009)
BL23	TB10, *ponB<>cat*	This work
BL24	TB28, *ponB<>cat*	This work
BL71*	TB10, *ftsN<>cat*	This work
BL79	TB28, *ponA<>aph*	This work
BL84*	TB28, *ponB<>cat ftsN*^{slm117} [=*ftsN*::EZTnKan-2]	This work
BL85*	TB28, *leu*::Tn10 *ftsN<>aph*	This work
BL86*	TB28, *leu*::Tn10 *ftsN<>aph recA::cat*	This work
BL86-AK1*	TB28, *leu*::Tn10 *ftsN<>aph recA::cat ftsL*^{D93G}	This work
BL86-AK11*	TB28, *leu*::Tn10 *ftsN<>aph recA::cat ftsB*^{D59H}	This work
BL86-AK12*	TB28, *leu*::Tn10 *ftsN<>aph recA::cat ftsB*^{D59H}	This work
BL86-KK1*	TB28, *leu*::Tn10 *ftsN<>aph recA::cat ftsA*^{I143L}	This work
BL105	TB28, *ponA<>frt ftsN*^{slm117} [=*ftsN*::EZTnKan-2]	This work
BL114	TB28, *ftsA*^{I143L}	This work
BL120*	TB28, *ftsA*^{I143L} *ftsN<>aph*	This work
BL140	TB28, *ftsB*^{D59H}	This work
BL141*	TB28, *ftsB*^{D59H} *ftsN<>aph*	This work
BL148	TB28, *ftsA*^{I143L} *leu*::Tn10	This work
BL149	TB28, *ftsB*^{D59H} *ftsA*^{I143L} *leu*::Tn10	This work
BL150	TB28, *ftsB*^{D59H} *ftsA*^{I143L} *leu*::Tn10 *ftsN<>aph*	This work
BL151	TB28, *ftsB*^{D59H} *ftsA*^{E124A} *leu*::Tn10	This work
BL152	TB28, *ftsB*^{D59H} *ftsA*^{E124A} *leu*::Tn10 *ftsN<>aph*	This work
BL153*	TB28, *leu*::Tn10 *ftsL*^{D93G} *ftsN<>aph*	This work
BL154	TB28, *leu*::Tn10 *ftsL*^{D93G}	This work
BL155*	TB28, *ftsB<>aph*	This work
BL156*	TB28, *ftsL<>aph*	This work
BL157*	TB28, *leu*::Tn10 *ftsL*^{D93G} *ftsN<>aph*	This work
BL159	TB28, *leu*::Tn10 *ftsL*^{D93G} *ftsB*^{D59H}	This work
BL163	TB28, *leu*::Tn10 *ftsL*^{D93G} *ftsB*^{D59H} *ftsN<>aph*	This work
BL164	TB28, *leu*::Tn10 *ftsL*^{D93G} *ftsA*^{I143L}	This work

BL165	TB28, *leu*::Tn10 *ftsL*^{D93G} *ftsA*^{I143L} *ftsN*<>*aph*	This work
BL167	TB28, *ftsB*^{E56A}	This work
BL172	TB28, *ftsB*^{E56K}	This work
BL173	TB28, *ftsB*^{E56A} *ftsN*<>*aph*	This work
BL175	TB28, *ftsB*^{E56K} *ftsN*<>*aph*	This work
BTH101	*cya*-99 *araD*139 *galE*15 *galK*16 *rpsL*1 *hsdR*2 *mcrA*1 *mcrB*1	Karimova & Ladant
BW10724 (λ*recA*$^{+}$)	*recA*::*cat lac169 pho-510 thi*	(Wanner & Boline, 1990)
BW25113	Δ(*araD-araB*)567 Δ*lacZ*4787(::rrnB-3) λ^{-} *rph*-1 Δ(*rhaD-rhaB*)568 *hsdR*514	(Datsenko & Wanner, 2000)
CH31*	TB28, *aph araC* P_{BAD}::*ftsN*	(Gerding et al., 2009)
CH34*	TB28, *ftsN*<>*aph*	(Gerding et al., 2009)
CH82	TB28, *ponA*<>*frt*	This work
DH5α	*hsdR*17 *deoR recA*1 *endA*1 *phoA supE*44 *thi*-1 *gyrA*96 *relA*1 Δ(*lacZYA-argF*)U169 ϕ80d*lacZ*ΔM15	Gibco BRL
JH1*	TB28, *ftsN*<>*aph recA*::*cat*	This work
JW2669	BW25113, *recA*<>*aph*	(Baba *et al.*, 2006)
JW3359	BW25113, *ponA*<>*aph*	(Baba et al., 2006)
LP31	TB28, *recA*<>*aph*	This work
MDG277	Δ(*argF-lac*)U169 e14^{-} *flhD*5301 Δ(*fruK-yeiR*)725 *relA*1 *rpsL*150 *rbsR22* Δ(*fimB-fimE*)632 *deoC*1 *spoT1 ftsL*<>*aph* Δ(λ*attL-lom*)::*bla araC* P_{BAD}::*ftsL*	(Gonzalez & Beckwith, 2009)
MG1655	*ilvG rfb*50 *rph*1	(Guyer *et al.*, 1981)
NB946	Δ(*argF-lac*)U169 e14^{-} *flhD*5301 Δ(*fruK-yeiR*)725 *relA*1 *rpsL*150 *rbsR22* Δ(*fimB-fimE*)632 *deoC*1 *spoT1 ftsB*<>*aph* Δ(λ*attL-lom*)::*bla araC* P_{BAD}::*ftsB*	(Buddelmeijer *et al.*, 2002)
SG13109	*his rpsL sulA366 leu::*Tn10	(Gottesman *et al.*, 1981)
TB10	MG1655, *nadA*::Tn10 *gal*490 λ*cI*857 Δ(*cro-bioA*)	(Johnson *et al.*, 2004)
TB28	MG1655, *lacIZYA*<>*frt*	(Bernhardt & de Boer, 2003)
TB77	TB28, *ftsN*^{slm117} [=*ftsN*::EZTnKan-2]	(Gerding et al., 2009)

Note that strains marked with * required an appropriate plasmid, phage, and/or inducer for survival. The symbol <> denotes DNA replacement by recombineering, and *frt* a scar

sequence remaining after eviction of an *aph* or *cat* cassette by FLP recombinase (Datsenko & Wanner, 2000, Yu *et al.*, 2000).

Table S5. Plasmids used in this study.

Plasmid	Relevant genotype [a]	ori	Source or Reference
pAB12	*bla lacI*^{q} P_{T7}::*ftsL*	ColE1	This work
pACBSCE	SceI *cat araC* P_{BAD}::*i-sceI* γ β *exo*	pACYC	(Lee *et al.*, 2009)
pAH162	*att*ϕ80 *tetA*	R6K	(Haldimann & Wanner, 2001)
pBAD33	*cat araC* P_{BAD}::	pACYC	(Guzman *et al.*, 1995)
pBL3	*att*HK022 *bla lacI*^{q} P_{lac}::*zapC-le*	R6K	(Hale *et al.*, 2011)
pBL12	*cat repA*^{ts} *ftsA*^{E124A}	pSC101^{ts}	(Gerding et al., 2009)
pBL93	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{E81K}	ColE1	This work
pBL94	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{Y85R}	ColE1	This work
pBL100	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{K87E}	ColE1	This work
pBL114	*bla lacI*^{q} P_{T7}::*gfp-ftsN*$^{+A102}$	ColE1	This work
pBL115	*bla lacI*^{q} P_{T7}::*gfp-ftsN*$^{T64G, S67G, +A102}$	ColE1	This work
pBL116	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G, S67G, +A102}$	ColE1	This work
pBL116 (Y85W)	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G, S67G, Y85W, +A102}$	ColE1	This work
pBL116 (W83L)	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G, S67G, W83L, +A102}$	ColE1	This work
pBL116 (L89S)	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G, S67G, L89S, +A102}$	ColE1	This work
pBL116 (W83M)	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G, S67G, W83M, +A102}$	ColE1	This work
pBL116 (L89H)	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G, S67G, L89H, +A102}$	ColE1	This work
pBL120	*bla lacI*^{q} P_{lac}::*gfp-ftsI*	ColE1	This work
pBL136	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{\Delta(64-101)<>22}$	ColE1	This work
pBL138	*cat araC* P_{BAD}::*rfp-malF*$^{2-39}$*-ftsN*$^{55-123}$	pACYC	This work
pBL141	*cat araC* P_{BAD}::*rfp-ftsN*$^{1-105}$*-le*	pACYC	This work

pBL142	*cat araC* P_{BAD}::*rfp-ftsN*$^{1-90}$	pACYC	This work
pBL143	*bla lacI*^{q} P_{lac}::*gfp-ftsN* $^{\Delta(64-101)<>(SphI\_XhoI\text{-}stuffer)}$	ColE1	This work
pBL145	*aadA cI*857 $P_{\lambda R}$::*ftsN*$^{1-90}$	pSC101	This work
pBL147	*att*HK022 *bla lacI*^{q} P_{lac}::*gfp-ftsI*	R6K	This work
pBL153	*aadA* P_{syn135}::*ftsN*	pSC101	This work
pBL154	*aadA repA*^{ts} P_{syn135}::*ftsN*	pSC101^{ts}	This work
pBL160	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{Y85S}	ColE1	This work
pBL161	*att*HK022 *bla lacI*^{q} P_{lac}::*gfp-ftsN*^{Y85S}	R6K	This work
pBL163	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{W83F}	ColE1	This work
pBL165	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{Y85F}	ColE1	This work
pBL180	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{W83T}	ColE1	This work
pBL181	*att*HK022 *bla lacI*^{q} P_{lac}::*gfp-ftsN*^{W83T}	R6K	This work
pBL190	*aadA* SceI *cI*857 $P_{\lambda R}$::*ftsN*$^{1-90}$	pSC101	This work
pBL191	*aadA* SceI *cI*857 $P_{\lambda R}$::*sceI*	pSC101	This work
pBL193	*bla lacI*^{q} P_{lac}::*gfp-ftsB*	ColE1	This work
pBL194	*aadA repA*^{ts} P_{syn135}::*gfp-ftsB*	pSC101^{ts}	This work
pBL195	*aadA repA*^{ts} P_{syn135}::*gfp-ftsL*	pSC101^{ts}	This work
pBL200	*aadA repA*^{ts} P_{syn135}::*ftsN* SceI *cI*857 $P_{\lambda R}$::*i-sceI*	pSC101^{ts}	This work
pBL203	*bla lacI*^{q} P_{T7}::*gfp-ftsN*$^{\Delta(59-73)<>6}$	ColE1	This work
pBL205	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{\Delta(59-73)<>6}$	ColE1	This work
pBL206	*tetA repA*^{ts} *ftsA*^{E124A}	pSC101^{ts}	This work
pBL209	*bla lacI*^{q} P_{lac}::*gfp-ftsN*::^{G}*lacZYA*	F	This work
pBL210	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{\Delta(59-73)<>95}$	ColE1	This work
pBL211	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{\Delta(59-73)<>15}$	ColE1	This work
pBL215	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G,\ S67G,\ Y85W,\ +A102}$ ::^{G}*lacZYA*	F	This work
pBL216	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G,\ S67G,\ W83L,\ +A102}$ ::^{G}*lacZYA*	F	This work
pBL217	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G,\ S67G,\ L89S,\ +A102}$ ::^{G}*lacZYA*	F	This work
pBL218	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G,\ S67G,\ W83M,\ +A102}$ ::^{G}*lacZYA*	F	This work
pBL219	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{T64G,\ S67G,\ L89H,\ +A102}$ ::^{G}*lacZYA*	F	This work

pBL222	*cat araC* P_{BAD}::*ftsL*	pACYC	This work
pBL225	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{Y85S}::^{G}*lacZYA*	F	This work
pBL226	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{W83T}::^{G}*lacZYA*	F	This work
pBL236	*cat repA*^{ts} *ftsA*^{I143L}	pSC101^{ts}	This work
pBL289	*bla lacI*^{q} P_{T7}::*ftsB*	ColE1	This work
pBL294	*bla lacI*^{q} P_{lac}::*ftsB*	ColE1	This work
pBL295	*bla lacI*^{q} P_{lac}::*ftsL*	ColE1	This work
pBL300	*att*HK022 *bla lacI*^{q} P_{lac}::*ftsB*^{D59H}	R6K	This work
pBL301	*bla lacI*^{q} P_{lac}::*ftsL*^{D93G}	ColE1	This work
pBL303	*aadA* P_{syn135}::*ftsL*^{D93G}	pSC101	This work
pBL304	*aadA repA*^{ts} P_{syn135}::*ftsB*^{D59H}	pSC101^{ts}	This work
pBL305	*aadA repA*^{ts} P_{syn135}::*ftsL*^{D93G}	pSC101^{ts}	This work
pBL306 (E56A)	*aadA repA*^{ts} P_{syn135}::*ftsB*^{E56A} *ispD*$^{1-48}$	pSC101^{ts}	This work
pBL306 (E56K)	*aadA repA*^{ts} P_{syn135}::*ftsB*^{E56K} *ispD*$^{1-48}$	pSC101^{ts}	This work
pBL306 (D59H)	*aadA repA*^{ts} P_{syn135}::*ftsB*^{D59H} *ispD*$^{1-48}$	pSC101^{ts}	This work
pBL309	*aadA* P_{syn135}::*ftsL*	pSC101	This work
pBL312	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-14}$-*ftsN*$^{27-81}$-*le*	ColE1	This work
pBL313	*cat araC* P_{BAD}::*rfp-malF*$^{2-39}$-*ftsN*$^{55-105}$-*le*	pACYC	This work
pBL315	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-81}$-*le*	ColE1	This work
pBL321	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{L75A}	ColE1	This work
pBL322	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{P76A}	ColE1	This work
pBL323	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{P77A}	ColE1	This work
pBL324	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{K78A}	ColE1	This work
pBL325	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{P79A}	ColE1	This work
pBL326	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{E80A}	ColE1	This work
pBL327	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{R84A}	ColE1	This work
pBL328	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{L89A}	ColE1	This work
pBL329	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{E90A}	ColE1	This work
pBL330	*bla lacI*^{q} P_{lac}::*gfp-ftsN*^{D5S,Y6A}	ColE1	This work
pBL331	*tetA* P_{syn135}::*ftsL*^{H94Y}	pSC101	This work
pBL332	*tetA* P_{syn135}::*ftsL*^{E88V}	pSC101	This work
pBL333	*tetA* P_{syn135}::*ftsL*^{E88K}	pSC101	This work

pBL334	*tetA* P_{syn135}::*ftsL*^{N89S}	pSC101	This work
pBL335	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{1-81;\ D5S,Y6A}$*-le*	ColE1	This work
pBL336	*tetA* P_{syn135}::*ftsB*	pSC101	This work
pBL338	*tetA* P_{syn135}::*ftsB*^{E56V}	pSC101	This work
pBL339	*tetA* P_{syn135}::*ftsB*^{E56A}	pSC101	This work
pBL340	*tetA* P_{syn135}::*ftsB*^{A55T}	pSC101	This work
pBL341	*tetA* P_{syn135}::*ftsB*^{E56K}	pSC101	This work
pBL342	*tetA* P_{syn135}::*ftsB*^{E56G}	pSC101	This work
pBL343	*tetA* P_{syn135}::*ftsB*^{D59V}	pSC101	This work
pBL356	*bla* SceI P_{syn135}::*ftsB*^{E56K} *ispD*$^{1-48}$ SceI *sacB*	ColE1	This work
pBT3-N	*aph leu2* P_{CYC1}::*lexA-vp16-cub-*	ColE1, CEN	Dualsystems Biotech
pCH38	*bla lacI*^{q} P_{T7}::*zipA-h*	ColE1	(Hale *et al.*, 2000)
pCH181	*bla lacI*^{q} P_{lac}::*gfp-minD minE-le*	ColE1	(Bendezu & de Boer, 2008)
pCH195	*bla lacI*^{q} P_{lac}::*gfp-ftsL*	ColE1	(Hale & de Boer, 2002)
pCH198	*bla lacI*^{q} P_{T7}::*gfp-ftsN*	ColE1	(Hale & de Boer, 2002)
pCH201	*bla lacI*^{q} P_{lac}::*gfp-ftsN*	ColE1	(Hale & de Boer, 2002)
pCH276	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{1-123}$	ColE1	(Gerding et al., 2009)
pCH277	*bla lacI*^{q} P_{lac}::*amiC*$^{1-31}$*-ftsN*$^{52-123}$*-gfp*	ColE1	This work
pCH282	*bla lacI*^{q} P_{lac}::^{ss}*torA-gfp-ftsN*$^{55-319}$	ColE1	(Gerding et al., 2009)
pCH286	*bla lacI*^{q} P_{lac}::*amiC*$^{1-31}$*-ftsN*$^{52-123}$	ColE1	This work
pCH287	*aadA cI*857 $P_{\lambda R}$::*amiC*$^{1-31}$*-ftsN*$^{52-123}$	pSC101	This work
pCH288	*bla lacI*^{q} P_{lac}::^{ss}*torA-gfp-ftsN*$^{55-123}$	ColE1	(Gerding et al., 2009)
pCH309	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$*-mreC*$^{38-367}$*-le*	ColE1	This work
pCH310	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$*-ftsN*$^{55-123}$	ColE1	This work
pCH327	*aph leu2* P_{CYC1}::*lexA-vp16-cub-ftsN*$^{1-105}$	ColE1, CEN	This work
pCH354	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{1-243}$*-le*	ColE1	(Gerding et al., 2009)
pCH358	*aph* P_{lac}::*t25-rodZ*	pACYC	(Bendezu *et al.*, 2009)
pCH362	*aadA* P_{syn135}::*gfp-zapA*	pSC101	(Bendezu et al.,

			2009)
pCH371	*bla lacI*^{q} P_{lac}::*t18-rodZ*	ColE1	(Bendezu et al., 2009)
pCH391	*aph* P_{lac}::*t25-ftsN*$^{1-105}$	pACYC	This work
pCH391 (DY>SA)	*aph* P_{lac}::*t25-ftsN*$^{1-105;\ D5S,Y6A}$	pACYC	This work
pCH392	*bla lacI*^{q} P_{lac}::*t18-ftsN*$^{1-105}$	ColE1	This work
pCH392 (DY>SA)	*bla lacI*^{q} P_{lac}::*t18-ftsN*$^{1-105;\ D5S,Y6A}$	ColE1	This work
pCH399	*bla lacI*^{q} P_{lac}::*t18-ftsA*	ColE1	This work
pCH400	*bla lacI*^{q} P_{lac}::*t18-ftsQ*	ColE1	This work
pCH425	*bla lacI*^{q} P_{lac}::*t18-ftsB*	ColE1	This work
pCH433	*aph* P_{lac}::*t25-ftsI*	pACYC	This work
pCP20	*bla cat repA*^{ts} *cI*857 $P_{\lambda R}$::*flp*	pSC101^{ts}	(Cherepanov & Wackernagel, 1995)
pDB326	*aadA repA*^{ts}	pSC101^{ts}	(Hale & de Boer, 1997)
pDB344	*aadA cI*857 $P_{\lambda R}$::	pSC101	(Raskin & de Boer, 1997)
pDB357	*bla lacI*^{q} P_{T7}::*ftsN*	ColE1	This work
pDOC-C	*bla* <u>SceI</u> <u>SceI</u> *sacB*	ColE1	(Lee et al., 2009)
pET21A	*bla lacI*^{q} P_{T7}::	ColE1	Novagen
pFB243	*bla lacI*^{q} P_{lac}::*malF*$^{1-39}$-*mreC*$^{38-367}$	ColE1	(Bendezu et al., 2009)
pFB261	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-14}$-*rodZ*$^{111-319}$	ColE1	(Bendezu et al., 2009)
pJH1	*tetA repA*^{ts} P_{syn135}::*ftsL*	pSC101^{ts}	This work
pJH2	*tetA* P_{syn135}::*ftsL*	pSC101	This work
pJH3	*tetA* P_{syn135}::*ftsL*^{D93G}	pSC101	This work
pJH5	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-320;\ T64G,\ S67G,\ Y85W,\ +A102}$	ColE1	This work
pJH6	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-320;\ T64G,\ S67G,\ W83L,\ +A102}$	ColE1	This work
pJH10	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-320;\ T64G,\ S67G,\ Y85W,\ +A102}$::^{G}*lacZYA*	F	This work

pJH11	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-320;\ T64G,\ S67G,\ W83L,\ +A102}$::^{G}*lacZYA*	F	This work
pKD3	*bla frt-cat-frt*	R6K	(Datsenko & Wanner, 2000)
pKD46	*bla repA*^{ts} *araC* P_{BAD}::$\gamma\ \beta$ *exo*	pSC101^{ts}	(Datsenko & Wanner, 2000)
pKNT25	*aph* P_{lac}::*lacZ'-t25*	pACYC	(Karimova *et al.*, 2005)
pLP7	*bla lacI*^{q} P_{lac}::*t18-ponA*	ColE1	(van den Ent *et al.*, 2010)
pLP10	*aph* P_{lac}::*t25-ponA*	pACYC	This work
pLP13	*bla lacI*^{q} P_{lac}::*gfp-*	ColE1	(van den Ent et al., 2010)
pLP160	*cat araC* P_{BAD}::*rfp-malF*$^{2-39}$-*ftsN*$^{55-90}$	pACYC	This work
pLP163	*bla lacI*^{q} P_{lac}::*gfp-ftsN*	ColE1	This work
pLP164	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{75-105}$-*le*	ColE1	This work
pLP165	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{80-105}$-*le*	ColE1	This work
pLP168	*bla lacI*^{q} P_{lac}::^{ss}*torA-gfp-ftsN*$^{75-105}$-*le*	ColE1	This work
pLP169	*bla lacI*^{q} P_{lac}::^{ss}*torA-gfp-ftsN*$^{80-105}$-*le*	ColE1	This work
pLP170	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{1-31}$-*malF*$^{17-39}$-*ftsN*$^{55-81}$-*le*	ColE1	This work
pLP171	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{1-71}$-*le*	ColE1	This work
pLP218	*bla lacI*^{q} P_{lac}::^{ss}*torA-gfp-ftsN*$^{75-93}$-*le*	ColE1	This work
pLP219	*bla lacI*^{q} P_{lac}::^{ss}*torA-gfp-ftsN*$^{75-99}$-*le*	ColE1	This work
pLP221	*bla lacI*^{q} P_{lac}::^{ss}*torA-gfp-ftsN*$^{55-90}$	ColE1	This work
pMLB1113	*bla lacI*^{q} P_{lac}::*lacZ*	ColE1	(de Boer *et al.*, 1989)
pMLB1113ΔH	*bla lacI*^{q} P_{lac}::	ColE1	(Gerding *et al.*, 2007)
pMLB1115	*bla lacI*^{q} P_{lac}::*lacZ*	ColE1	(de Boer et al., 1989)
pMG12	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{1-105}$-*le*	ColE1	(Gerding et al., 2009)
pMG13	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{1-81}$-*le*	ColE1	(Gerding et al., 2009)
pMG14	*bla lacI*^{q} P_{lac}::^{ss}*torA-gfp-ftsN*$^{71-105}$-*le*	ColE1	(Gerding et al., 2009)
pMG21	*bla lacI*^{q} P_{lac}::*ftsN*	ColE1	This work
pMG47	*bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{1-90}$	ColE1	(Gerding et al., 2009)
pMG50	*bla lacI*^{q} P_{lac}::^{ss}*torA-gfp-ftsN*$^{71-90}$	ColE1	(Gerding et al., 2009)
pMG59	*att*HK022 *bla lacI*^{q} P_{lac}::*gfp-ftsN*	R6K	(Gerding et al., 2009)
pMG62	*att*HK022 *bla lacI*^{q} P_{lac}::*gfp-ftsN*$^{1-123}$	R6K	This work

pRC7	*bla lacI*^{q} P_{lac}::*galK*$^{1-54}$-*lacZ*$^{10-1024}$ *lacY lacA* [~P_{lac}::^{G}*lacZYA*]	F	(de Boer et al., 1989)
pTB8	*bla lacI*^{q} P_{lac}::*minCDE*::^{G}*lacZYA*	F	(Bernhardt & de Boer, 2004)
pTB37	*bla lacI*^{q} P_{lac}::*amiC*$^{1-31}$-*gfp*	ColE1	(Bernhardt & de Boer, 2003)
pTB51	*cat repA*^{ts} *araC* P_{BAD}::γ β *exo*	pSC101^{ts}	This work
pTB222	*att*HK022 *bla lacI*^{q} P_{lac}::*zipA-gfp*	R6K	(Bendezu et al., 2009)
pZAQ	*tetA ftsQ ftsA ftsZ*	ColE1	(Ward & Lutkenhaus, 1985)
pZC100	*aadA*	pSC101	(Wang *et al.*, 1991)

Genotypes indicate when constructs encode in-frame Gfpmut2 (*gfp*), mCherry (*rfp*), hexahistidine (*h*), Leu-Glu dipeptide (*le*), CyaA T18-domain (*t18*), CyaA T25-domain (*t25*), or the signal peptide of TorA (^{ss}*torA*). SceI indicates the presence of a substrate site for the I-SceI homing endonuclease. ^{G}*lacZ* refers to a *galK*$^{1-54}$-*lacZ*$^{10-1024}$ fusion in which the first 10 residues of LacZ are replaced with the first 54 residues of GalK (Koop *et al.*, 1987).

Strain construction:

Construction of strains involved P1-mediated transduction (transduction for short) (Miller, 1992), λRed-mediated recombineering (Datsenko & Wanner, 2000, Yu et al., 2000), Gene doctoring (Lee et al., 2009), Flp-mediated eviction of antibiotic cassettes (Cherepanov & Wackernagel, 1995), replacement of chromosomal genes using plasmids that are temperature sensitive for replication (Hamilton *et al.*, 1989), and other methods specified below or elsewhere in the text.

BL17 [*leu*::Tn10] was obtained by transduction of *leu*::Tn10 from SG13109 to TB28.

BL19 [*ftsA*^{E124A} *leu*::Tn10] was obtained by transduction of *leu*::Tn10 from SG13109 to BL18. Retention of *ftsA*^{E124A} was verified by the presence of a diagnostic FspI site on a fragment that was amplified from BL19 chromosomal DNA using primers 5'-CCGCTCTTCCGGTATGATCAAGGCGACGGACAG-3' and 5'-CCTCACTCGAGTTAAAACTCTTTTCGCAGC-3'.

For BL23 and BL24 [*ponB*<>*cat*], the *cat* cassette of pKD3 was amplified with primers 5'-AAATCGGGCTTTTGCGCCTGAATATTGCGGAGAAAAAGCCCATATGAATATCCTCCTTAG-3' and 5'-ATGGCAACTCGCCATCCGGTATTTCACGCTTAGATGTTAGTGTAGGCTGGAGCTGCTTCG-3', yielding a 1093 bp fragment with end sequences homologous to the chromosomal *ponB* locus (underlined). Recombination with the chromosome of TB10 yielded BL23 [*ponB*<>*cat*], in which 2538 bp of the *ponB* gene (from bp +1 to bp +2538) is replaced with *cat* and transcription of the latter is in the same direction as the replaced gene. Transduction of *ponB*<>*cat* from BL23 to TB28 resulted in BL24.

For BL71/pCH288 [*ftsN*<>*cat*/P_{lac}::^{ss}*torA-gfp-ftsN*$^{55-123}$], the *cat* cassette of pKD3 was amplified with primers 5'-CGGTTTCTCCCGCTATGGTCGCTATTGCTGCCGCCGTTCTTGTGCCATATGAATATCCTCCTTAG-3' and 5'- TCCAGCCATTGTTGGTGGTGATTTTCGAGTCAAAGCCTTCGTGTAGGCTGGAGCTGCTTCG -3', yielding a 1099 bp fragment with end sequences homologous to the chromosomal *ftsN* locus (underlined). Recombination with the chromosome of TB10/pCH288 in the presence of 250 μM IPTG yielded BL71/pCH288, in which 673 bp of the *ftsN* gene (from bp +136 to bp +809) is replaced with *cat* and transcription of the latter is in the same direction as the replaced gene.

BL79 [*ponA*<>*aph*] was obtained by transduction of *ponA*<>*aph* from JW3359 to TB28.

BL84(iMG62) [Δ*ponB ftsN*^{slm117}(P_{lac}::*gfp-ftsN*$^{1-123}$)] was obtained by transduction of *ponB*<>*cat* from BL23 to TB77(iMG62) in the presence of 500 μM IPTG.

BL85/pBL200 [*leu*::Tn10 *ftsN*<>*aph* / *aadA repA*^{ts} P_{syn135}::*ftsN* SceI *cI*857 $P_{λR}$::*i-sceI*] was obtained by transduction of *ftsN*<>*aph* from CH34/pCH201 to BL17/pBL200 at 30°C.

BL86/pBL200 [*leu*::Tn10 *ftsN*<>*aph recA::cat* / *aadA repA*^{ts} P_{syn135}::*ftsN* SceI *cI*857 $P_{λR}$::*i-sceI*] was obtained by transduction of *recA::cat* from BW10724 to BL85/pBL200 at 30°C.

BL86-AK1, BL86-AK11, BL86-AK12, and BL86-KK1 were obtained as described in the text.

BL105 [*ponA*<>*frt ftsN*^{slm117}] was obtained by transduction of *ftsN*::EZTnKan-2 [=*ftsN*^{slm117}] from TB77 to CH82.

For BL114 [*ftsA*^{I143L}], TB28 chromosomal *ftsA* was exchanged with the *ftsA*^{I143L} allele on pBL236 [*repA*^{ts} *ftsA*^{I143L}] by the method of Hamilton et al (Hamilton et al., 1989). To verify the presence and integrity of *ftsA*^{I143L} in BL114, chromosomal DNA was amplified using primers 5'-CCGCTCTTCCGGTATGATCAAGGCGACGGACAG-3' and 5'- CCTCACTCGAGTTAAAACTCTTTTCGCAGC-3', and the *ftsA*^{I143L} gene present on the resulting fragment was sequenced.

BL120/pX [*ftsA*^{I143L} *ftsN*<>*aph* /] strains were obtained by transduction of *ftsN*<>*aph* from CH34/pCH201 to BL114/pX in the presence of inducer if so required.

For BL140 [*ftsB*^{D59H}], TB28 chromosomal *ftsB* was exchanged with the *ftsB*^{D59H} allele on pBL306(D59H) [*repA*^{ts} P_{syn135}::*ftsB*^{D59H} *ispD*$^{1-48}$] by the method of Hamilton et al (Hamilton et al., 1989). To verify the presence and integrity of *ftsB*^{D59H} in BL140, chromosomal DNA was amplified using primers 5'- CGCATATGGGTAAACTAACGCTGCTGTTGC-3' and 5'-GCGTCGACTTAATGCGCCAGCAGCGCATGCACCG-3', and the *ftsB*^{D59H} gene present on the resulting fragment was sequenced.

BL141/pX [*ftsB*^{D59H} *ftsN*<>*aph* /] strains were obtained by transduction of *ftsN*<>*aph* from CH34/pCH201 to BL140/pX in the presence of inducer if so required.

BL148 [*ftsA*^{I143L} *leu*::Tn10] was obtained by transduction of *leu*::Tn10 from SG13109 to BL114. Retention of *ftsA*^{I143L} was verified by the presence of a diagnostic SmlI site on a fragment that was amplified from BL148 chromosomal DNA using primers 5'-CCGCTCTTCCGGTATGATCAAGGCGACGGACAG-3' and 5'- CCTCACTCGAGTTAAAACTCTTTTCGCAGC-3'.

BL149 [*ftsB*^{D59H} *ftsA*^{I143L} *leu*::Tn10] was obtained by co-transduction of *leu*::Tn10 and *ftsA*^{I143L} from BL148 to BL140. The presence of *ftsA*^{I143L} was verified as for BL148.

BL150 [*ftsB*^{D59H} *ftsA*^{I143L} *leu*::Tn10 *ftsN*<>*aph*] was obtained by transduction of *ftsN*<>*aph* from CH34/pCH201 to BL149.

BL151 [*ftsB*^{D59H} *ftsA*^{E124A} *leu*::Tn10] was obtained by co-transduction of *leu*::Tn10 and *ftsA*^{E124A} from BL19 to BL140. The presence of *ftsA*^{E124A} was verified as for BL19.

BL152 [*ftsB*^{D59H} *ftsA*^{E124A} *leu*::Tn10 *ftsN*<>*aph*] was obtained by transduction of *ftsN*<>*aph* from CH34/pCH201 to BL151.

For BL153/pBL215 [*leu*::Tn10 *ftsL*^{D93G} *ftsN*<>*aph* / *bla lacI*^{q} P_{lac}::*gfp-ftsN*^{Y85W}], a portion of pBL305 [P_{syn135}::*ftsL*^{D93G}] was amplified with primers 5'- CCGAATTCCATATGATCAGCAGAGTGACAG -3' and 5'- CGTGTCGACTTATTTTTGCACTACGAT -3', yielding a 386 bp *ftsL*^{D93G} fragment largely homologous to native chromosomal *ftsL* (underlined). Recombination with the chromosome of BL85/pBL200/pBL215/pTB51 and growth at 37°C, to cure both pBL200 [*aadA repA*^{ts} P_{syn135}::*ftsN* SceI *cI*857 $P_{\lambda R}$::*i-sceI*] and pTB51 [*repA*^{ts} *cat araC* P_{BAD}::γ β *exo*], in the presence of ampicillin and 100 μM IPTG yielded BL153/pBL215.

BL154 [*leu*::Tn10 *ftsL*^{D93G}] was obtained by co-transduction of *leu*::Tn10 and *ftsL*^{D93G} from BL153/pBL215 to TB28. To verify the presence and integrity of *ftsL*^{D93G} in BL154, chromosomal DNA was amplified using primers 5'- GATCGGCCATTACGGCCATGATCAGCAGAGTGACAGAAGC-3' and 5'- GCGTCGACTTAAACTTGTAACCACGCTACGCGTCC-3', and the *ftsL*^{D93G} gene present on the resulting fragment was sequenced.

BL155/pX [*ftsB*<>*aph* /] strains were obtained by transduction of *ftsB*<>*aph* from NB946 to TB28/pX in the presence of inducer if so required.

BL156/pX [*ftsL*<>*aph* /] strains were obtained by transduction of *ftsL*<>*aph* from MDG277 to TB28/pX in the presence of inducer if so required.

BL157/pX [*leu*::Tn10 *ftsL*^{D93G} *ftsN*<>*aph* /] strains were obtained by transduction of *ftsN*<>*aph* from CH34/pCH201 to BL154/pX in the presence of inducer if so required.

BL159 [*leu*::Tn10 *ftsL*^{D93G} *ftsB*^{D59H}] was obtained by co-transduction of *leu*::Tn10 and *ftsL*^{D93G} from BL154 to BL140. The presence of *ftsL*^{D93G} was verified by the presence of a diagnostic HaeIII site on a fragment that was amplified from BL159 chromosomal DNA using primers 5'- GATCGGCCATTACGGCCATGATCAGCAGAGTGACAGAAGC-3' and 5'- GCGTCGACTTAAACTTGTAACCACGCTACGCGTCC-3'.

BL163 [*leu*::Tn10 *ftsL*^{D93G} *ftsB*^{D59H} *ftsN*<>*aph*] was obtained by transduction of *ftsN*<>*aph* from CH34/pCH201 to BL159. The presence and integrity of native *ftsA* in this strain was verified by amplification of a 2153 bp fragment of BL163 chromosomal DNA using primers 5'-

GTAGTACGAATTCTGGAACTGGCGGAC-3' and 5'- ACTCGTCGACATCATCGTCGGCCTC-3', followed by nucleotide sequencing of the entire *ftsA* gene on the fragment.

BL164 [*leu*::Tn10 *ftsL*^{D93G} *ftsA*^{I143L}] was obtained by co-transduction of *leu*::Tn10 and *ftsL*^{D93G} from BL154 to BL114. The presence of both *ftsL*^{D93G} and *ftsA*^{I143L} was verified as for BL159 and BL148, respectively.

BL165 [*leu*::Tn10 *ftsL*^{D93G} *ftsA*^{I143L} *ftsN<>aph*] was obtained by transduction of *ftsN<>aph* from CH34/pCH201 to BL164.

For BL167 [*ftsB*^{E56A}], TB28 chromosomal *ftsB* was exchanged with the *ftsB*^{E56A} allele on pBL306(E56A) [*repA*^{ts} P_{syn135}::*ftsB*^{E56A} *ispD*$^{1-48}$] by the method of Hamilton et al (Hamilton et al., 1989). The presence and integrity of *ftsB*^{E56A} in BL167 was verified by sequencing, as described for BL140.

For BL172 [*ftsB*^{E56K}], the *ftsB*^{E56K} allele of pBL356 [*bla* SceI P_{syn135}::*ftsB*^{E56K} *ispD*$^{1-48}$ SceI *sacB*] was amplified with primers 5'-GTCGTCTTCGGATGCATGGGATGATGATGCCGTTTTTCAGGGGGCAGGATGGGTAAACTAACGCTGCTGTTGC -3' and 5'- GCGTCGACTTAATGCGCCAGCAGCGCATGCACCG-3', yielding a 533 bp *ftsB*^{E56K} fragment largely homologous to native chromosomal *ftsB* (underlined). This fragment was recombined with the chromosomal *ftsB<>aph* allele of BL155/pBL356/pACBSCE [*ftsB<>aph / bla* SceI P_{syn135}::*ftsB*^{E56K} *ispD*$^{1-48}$ SceI *sacB /* SceI *cat araC* P_{BAD}*::i-sceI* γ β *exo*] with selection for growth on LB containing 5% sucrose, to counterselect for pBL356, and for sensitivity to kanamycin, chloramphenicol and ampicillin. The presence and integrity of *ftsB*^{E56K} in BL172 was verified by sequencing, as described for BL140.

BL173 [*ftsB*^{E56A} *ftsN<>aph*] was obtained by transduction of *ftsN<>aph* from CH34/pCH201 to BL167. The integrity of native *ftsA* in this strain was verified by nucleotide sequencing as described for BL163. The integrity of native *ftsL* was verified by amplification of a 601 bp fragment of BL173 chromosomal DNA using primers 5'-GGTCTAGAGGTGGCTGAGAACCCTCGTGCC-3' and 5'-GCGTCGACTTAAACTTGTAACCACGCTACGCGTCC-3', followed by nucleotide sequencing of the *ftsL* gene on the fragment.

BL175 [*ftsB*^{E56K} *ftsN*<>*aph*] was obtained by transduction of *ftsN*<>*aph* from CH34/pCH201 to BL172. The integrity of native *ftsA* and *ftsL* in this strain was verified by nucleotide sequencing as described for BL163 and BL173, respectively.

CH34/pBL200 [*ftsN*<>*aph / aadA repA*^{ts} P_{syn135}::*ftsN* SceI *cI*857 $P_{\lambda R}$::*i-sceI*] was obtained by transduction of *ftsN*<>*aph* from CH34/pCH201 to TB28/pBL200.

CH82 [*ponA*<>*frt*] was obtained by eviction of *aph* from BL79, using pCP20.

JH1/pBL200 [*ftsN*<>*aph recA::cat / aadA repA*^{ts} P_{syn135}::*ftsN* SceI *cI*857 $P_{\lambda R}$::*i-sceI*] was obtained by transduction of *recA*<>*cat* from BW10724 to CH34/pBL200.

LP31 [*recA*<>*aph*] was obtained by transduction of *recA*<>*aph* from JW2669 to TB28.

Plasmid construction:

Unless indicated otherwise, MG1655 or TB28 chromosomal DNA was used as template in amplification reactions. Sites of interest (e.g. relevant restriction sites, those allowing for targeted recombination, or site-directed mutations) are underlined in primer sequences. When plasmids construction involved PCR amplification and/or mutagenesis, the nucleotide sequence of plasmid inserts was verified.

For pAB12 [P_{T7}::*ftsL*], *ftsL* was amplified with primers 5'-CCGAATTCCATATGATCAGCAGAGTGACAG-3' and 5'- CGTGTCGACTTATTTTTGCACTACGAT-3', and the 368 bp *Nde*I-*Sal*I fragment of the product was used to replace the 57 bp *Nde*I-*Sal*I fragment of pET21A [P_{T7}::].

For pBL93 [P_{lac}::*gfp-ftsN*^{E81K}], pMG59 [P_{lac}::*gfp-ftsN*] was mutagenized using 5'-GGACTACCACCAAAACCAGAAAAGCGCTGGCGCTACATTAAAGAG-3' and its reverse complement. The 973 bp *BamH*I-*Hind*III fragment of pMG59(E81K) was next used to replace the equivalent fragment of pCH201 [P_{lac}::*gfp-ftsN*].

For pBL94 [P_{lac}::*gfp-ftsN*^{Y85R}], pMG59 [P_{lac}::*gfp-ftsN*] was mutagenized using 5'-CCAGAAGAACGCTGGCGCCGCATTAAAGAGCTGGAAAG-3' and its reverse complement. The 973 bp

*BamH*I-*Hind*III fragment of pMG59(Y85R) was next used to replace the equivalent fragment of pCH201 [P_{lac}::*gfp-ftsN*].

For pBL100 [P_{lac}::*gfp-ftsN*^{K87E}], pMG59 [P_{lac}::*gfp-ftsN*] was mutagenized using 5'-GAACGCTGGCGCTACATTGAAGAGCTGGAAAGTCGCC-3' and its reverse complement. The 973 bp *BamH*I-*Hind*III fragment of pMG59(K87E) was next used to replace the equivalent fragment of pCH201 [P_{lac}::*gfp-ftsN*].

For pBL114 [P_{T7}::*gfp-ftsN*$^{+A102}$], one portion of pCH201 [P_{lac}::*gfp-ftsN*] was amplified with primers 5'- CAGCGAATTCCATATGGCACAACGAGATTATG-3' and 5'-CAGGCCGAAGGGGCCTCTGTGGGCGCACGCACTCCCGG-3' and digested with *EcoR*I and *Sfi*I, yielding a 318 bp fragment. A second portion was amplified with 5'-GTGAGGCCCCTTCGGCCGGTGGTGAAGTGAAAACGCCG-3' and 5'-TGAGAAGCTTAACCCCCGGCGGCGAG-3' and digested with *Sfi*I and *Hind*III, yielding a 652 bp fragment. The two fragments were then used in a three-way ligation with the 6119 bp *EcoR*I-*Hind*III fragment of pCH198 [P_{T7}::*gfp-ftsN*]. pBL114 encodes a fully functional version of GFP-FtsN in which an alanine residue is inserted between residues 101 and 102.

For pBL115 [P_{T7}::*gfp-ftsN* $^{T64G, S67G, +A102}$], one portion of pCH201[P_{lac}::*gfp-ftsN*] was amplified with primers 5'- CAGCGAATTCCATATGGCACAACGAGATTATG-3' and 5'-CTGGCCTTGCAGGCCCTCGGACTCTTCTTTCTTGTGATG-3' and digested with *EcoR*I and *Sfi*I, yielding a 205 bp fragment. A second portion was amplified with 5'-GTGGCCTGCAAGGCCAGAAAGTGACCGGAAACGG-3' and 5'-CAGGCCGAAGGGGCCTCTGTGGGCGCACGCACTCCCGG-3' and digested with *Sfi*I, yielding a 113 bp fragment. The two fragments were then used in a three-way ligation with the 6771 bp *EcoR*I-*Sfi*I fragment of pBL114 [P_{T7}::*gfp-ftsN*$^{+A102}$]. pBL115 encodes a fully functional version of GFP-FtsN in which an alanine residue is inserted between residues 101 and 102, and residues 64-67 (TLQS) have been replaced with GLQG. In addition, pBL115 contains two unique *Sfi*I sites flanking codons 68-101.

For pBL116 [P_{lac}::*gfp-ftsN* $^{T64G, S67G, +A102}$], the 976 bp *BamH*I-*Hind*III fragment of pBL115 [P_{T7}::*gfp-ftsN* $^{T64G, S67G, +A102}$] was used to replace the 973 bp *BamH*I-*Hind*III fragment of pCH201 [P_{lac}::*gfp-ftsN*]. pBL116 encodes a fully functional version of GFP-FtsN in which an alanine residue is inserted between residues 101 and 102, and residues 64-67 (TLQS) have been replaced with GLQG. In addition, pBL116 contains two unique *Sfi*I sites flanking codons 68-101.

Mutant derivatives of pBL116 were obtained by screening pBL116-based site-scanning libraries for derivatives that fail to rescue growth of strain CH31 [P_{BAD}::*ftsN*] in the absence of arabinose, as detailed in the Experimental Procedures.

For pBL120 [P_{lac}::*gfp-ftsI*], the 1780 bp *Sfi*I fragment of pCH433 [P_{lac}::*t25-ftsI*] was used to replace the 16 bp *Sfi*I fragment of pLP13 [P_{lac}::*gfp*-].

The construction of pBL131 [P_{BAD}::*rfp-dedD*$^{1-118}$-*le*] will be detailed elsewhere.

For pBL136 [P_{lac}::*gfp-ftsN*$^{\Delta(64-101)<>22}$], oligo 5'-CGAGGGCCTGCAAGGCCAGAAAGTGACCGGAAACGGACTCGAGCCGGGAGTGCGTGCGCCCACAGAGGCCCCTTCGGCCGGTG-3' and its reverse complement were annealed and the 62 bp *Sfi*I fragment of the product was used to replace the 113 bp *Sfi*I fragment of pBL116 [P_{lac}::*gfp-ftsN*$^{T64G,\ S67G,\ +A102}$]. In pBL136, *ftsN* codons 64-101 (TLQSQKVTGNGLPPKPEERWRYIKELESRQPGVRAPTE) are replaced by a *Sfi*I fragment containing a unique *Xho*I site, and encoding an in-frame arbitrary peptide of 22 residues (GLQGQKVTGNGLEPGVRAPTEA).

For pBL138 [P_{BAD}::*rfp-malF*$^{2-39}$-*ftsN*$^{55-123}$], a portion of pCH310 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-123}$] was amplified with primers 5'- GAGCTGTACAAAGCTAGCATGACTGGTG-3' and 5'-CTGGTCGACAAAACGACGGCCAGTGCCAAGC-3', and the 377 bp *BsrG*I-*Sal*I fragment was used to replace the 410 bp *BsrG*I-*Sal*I fragment of pBL131 [P_{BAD}::*rfp-dedD*$^{1-118}$-*le*].

For pBL141 [P_{BAD}::*ecrfp-ftsN*$^{1-105}$-*le*], a portion of pMG12 [P_{lac}::*gfp-ftsN*$^{1-105}$-*le*] was amplified with primers 5'- GAGCTGTACAAAGCTAGCATGACTGGTG-3' and 5'-CTGGTCGACAAAACGACGGCCAGTGCCAAGC-3', and the 371 bp *BsrG*I-*Xho*I fragment was used to replace the 401 bp *BsrG*I-*Xho*I fragment of pBL131 [P_{BAD}::*rfp-dedD*$^{1-118}$-*le*].

For pBL142 [P_{BAD}::*rfp-ftsN*$^{1-90}$], a portion of pMG47 [P_{lac}::*gfp-ftsN*$^{1-90}$] was amplified with primers 5'- GAGCTGTACAAAGCTAGCATGACTGGTG-3' and 5'-CTGGTCGACAAAACGACGGCCAGTGCCAAGC-3', and the 320 bp *BsrG*I-*Xho*I fragment was used to replace the 401 bp *BsrG*I-*Xho*I fragment of pBL131 [P_{BAD}::*rfp-dedD*$^{1-118}$-*le*].

For pBL143 [P_{lac}::*gfp-ftsN*$^{\Delta(64-101)<>(SphI\_XhoI\text{-}stuffer)}$], a portion of pCH201 [$P_{lac}$::*gfp-ftsN*] was amplified with primers 5'- CGAGGGCCTGCAAGGCCAGGCATGCAAAGTGACCGGAAACGGACTAC-3' and

5'- CACCGGCCGAAGGGGCCTCGAGTGTGGGCGCACGCACTCCCGGC-3', and the 122 bp *Sfi*I fragment of the 143 bp product was used to replace the 113 bp *Sfi*I fragment of pBL116 [P_{lac}::*gfp-ftsN*$^{T64G, S67G, +A102}$]. In pBL143, *ftsN* codons 64-101 are replaced by a non-coding *Sfi*I fragment containing unique *Sph*I and *Xho*I sites. The plasmid was used to facilitate directional cloning of 113 bp *Sfi*I mutant site scanning library fragments to generate pBL116 derivatives encoding GFP-FtsN variants with single amino acid substitutions within the FtsN$^{80-93}$ interval.

For pBL145 [*cI*857 $P_{\lambda R}$::*ftsN*$^{1-90}$], a portion of pDB357 [P_{T7}::*ftsN*] was amplified with primers 5'-GATCCCGCGAAATTAATACGACTCACTATAGGGG-3' and 5'-ATTTCTCGAGCTCTTTAATGTAGCGCCAGCGTTCTTCTGG-3', and the 306 bp *Xba*I-*Xho*I fragment was used to replace the 326 bp *Xba*I-*Xho*I fragment of pCH287 [*cI*857 $P_{\lambda R}$:: *amiC*$^{1-31}$-*ftsN*$^{52-123}$].

For pBL147 [P_{lac}::*gfp-ftsI*], the 605 bp *Xba*I-*Hind*III fragment of pBL3 [P_{lac}::*zapC-le*] was replaced with the 2578 bp *Xba*I-*Hind*III fragment of pBL120 [P_{lac}::*gfp-ftsI*].

For pBL153 [P_{syn135}::*ftsN*], the 1127 bp *Xba*I-*Hind*III fragment of pCH362 [P_{syn135}::*gfp-zapA*] was replaced with the 1000 bp *Xba*I-*Hind*III fragment of pDB357 [P_{T7}::*ftsN*].

To construct pBL154 [*repA*^{ts} P_{syn135}::*ftsN*], the 2128 bp *Nar*I-*Sph*I fragment of pBL153 [P_{syn135}::*ftsN*] was replaced with the 2128 bp *Nar*I-*Sph*I fragment of pDB326 [*repA*^{ts}].

pBL160 and pBL161 [P_{lac}::*gfp-ftsN*^{Y85S}] were obtained by site directed mutagenesis of pMG59 [P_{lac}::*gfp-ftsN*], using mutagenic primer 5'- CCAGAAGAACGCTGGCGCAGCATTAAAGAGCTGGAAAG-3' and its reverse complement. The 581 *BamH*I-*Age*I fragment of pMG59(Y85S) was next used to replace that of pCH201 [P_{lac}::*gfp-ftsN*], yielding pBL160, or that of pMG59, yielding pBL161.

pBL163 [P_{lac}::*gfp-ftsN*^{W83F}] was obtained by site directed mutagenesis of pMG59 [P_{lac}::*gfp-ftsN*], using mutagenic primer 5'- CCAAAACCAGAAGAACGCTTTCGCTACATTAAAGAGCTGG-3' and its reverse complement. The 581 *BamH*I-*Age*I fragment of pMG59(W83F) was next used to replace that of pCH201 [P_{lac}::*gfp-ftsN*].

pBL165 [P_{lac}::*gfp-ftsN*^{Y85F}] was obtained by site directed mutagenesis of pMG59 [P_{lac}::*gfp-ftsN*], using mutagenic primer 5'-ACCAGAAGAACGCTGGCGCTTCATTAAAGAGCTGGAAAGTC-3' and its reverse

complement. The 581 *BamH*I-*Age*I fragment of pMG59(Y85F) was next used to replace that of pCH201 [P_{lac}::*gfp-ftsN*].

pBL180 and pBL181 [P_{lac}::*gfp-ftsN*^{W83T}] were obtained by site directed mutagenesis of pMG59 [P_{lac}::*gfp-ftsN*], using mutagenic primer 5'-CCACCAAAACCAGAAGAACGCACCCGCTACATTAAAGAGCTGGAA-3' and its reverse complement. The 581 *BamH*I-*Age*I fragment of pMG59(W83T) was next used to replace that of pCH201 [P_{lac}::*gfp-ftsN*], yielding pBL180, or with that of pMG59, yielding pBL181.

For pBL190 [SceI *cI*857 $P_{\lambda R}$::*ftsN*$^{1-90}$], oligos 5'-AATTAGTTACGCTAGGGATAACAGGGTAATATA-3' and 5'- GATCTATATTACCCTGTTATCCCTAGCGTAACT-3' were annealed and the resulting fragment, with a unique I-*Sce*I site (SceI, underlined) and with *EcoR*I and *BamH*I compatible overhangs, was used to replace the 21 bp *EcoR*I-*BamH*I fragment of pBL145 [*cI*857 $P_{\lambda R}$::*ftsN*$^{1-90}$].

For pBL191 [SceI *cI*857 $P_{\lambda R}$::*I-sceI*], primers 5'- GCTCTAGACAGGAGGGTACCTATATGCATATG-3' and 5'- CAGGTCGACGCATGCGAATTCGACGTCGGGCCCTTATTTCAGGAAAGTTTCGGAGG-3' were used to amplify a portion of pACBSCE [P_{BAD}::*I-sce*I], and the 759 bp *Xba*I-*Sal*I fragment was used to replace the 315 bp *Xba*I-*Sal*I fragment of pBL190.

For pBL193 [P_{lac}::*gfp-ftsB*], the 1780 bp *Sfi*I fragment of pBL120 [P_{lac}::*gfp-ftsI*] was replaced with the 325 bp *Sfi*I fragment of pCH425 [P_{lac}::*t18-ftsB*].

For pBL194 [*repA*^{ts} P_{syn135}::*gfp-ftsB*], the 1000 bp *Xba*I-*Hind*III fragment of pBL154 [*repA*^{ts} P_{syn135}::*ftsN*] was replaced with the 1123 bp *Xba*I-*Hind*III fragment of pBL193 [P_{lac}::*gfp-ftsB*].

For pBL195 [*repA*^{ts} P_{syn135}::*gfp-ftsL*], the 1000 bp *Xba*I-*Hind*III fragment of pBL154 [*repA*^{ts} P_{syn135}::*ftsN*] was replaced with the 1142 bp *Xba*I-*Hind*III fragment of pCH195 [P_{lac}::*gfp-ftsL*].

For pBL200 [*repA*^{ts} P_{syn135}::*ftsN* SceI *cI*857 $P_{\lambda R}$::*I-sceI*], the 685 bp *Spe*I-*EcoR*I fragment of pBL154 [*repA*^{ts} P_{syn135}::*ftsN*] was replaced with the 2880 bp *Spe*I-*EcoR*I fragment of pBL191 [*I-SceI cI*857 $P_{\lambda R}$::*I-sceI*].

For pBL203 [P_{T7}::*gfp-ftsN*$^{\Delta(59-73)<>6}$], one portion of pCH201 [P_{lac}::*gfp-ftsN*] was amplified with primers 5'- CAGCGAATTCCATATGGCACAACGAGATTATG-3' and 5'-GCTAGGCCGGCCTCTTCCTGCGGCCGCTTGTGATGCGTAATGAAGTACAG-3' and digested with *EcoR*I

and *Fse*I, yielding a 202 bp fragment. A second portion was amplified with 5'-GCGGCCGGCCTACCACCAAAACCAGAAGAACGC-3' and 5'- TGAGAAGCTTAACCCCCGGCGGCGAG-3' and digested with *Fse*I and *Hind*III, yielding a 738 bp fragment. The two fragments were then used in a three-way ligation with the 6119 bp *EcoR*I-*Hind*III fragment of pCH198 [P_{T7}::*gfp-ftsN*]. pBL203 encodes a version of GFP-FtsN in which FtsN residues 59-73 (KEESETLQSQKVTGN) are replaced with the peptide RPQEEA.

For pBL205 [P_{lac}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>6}$], the 946 bp *BamH*I-*Hind*III fragment of pBL203 [P_{T7}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>6}$] was used to replace the 973 bp *BamH*I-*Hind*III fragment of pCH201 [P_{lac}::*gfp-ftsN*]. pBL205 encodes a version of GFP-FtsN in which FtsN residues 59-73 (KEESETLQSQKVTGN) are replaced with the peptide RPQEEA.

For pBL206 [*tetA repA*^{ts} *ftsA*^{E124A}], the 133 bp *Eag*I-*Cla*I fragment of pBL12 [*cat repA*^{ts} *ftsA*^{E124A}] was replaced with the 1325 bp *Eag*I-*Cla*I fragment of pAH162 [*tetA*], yielding pBL204 [*tetA cat repA*^{ts} *ftsA*^{E124A}]. Deletion of the 952 bp *BstB*I fragment of pBL204 then resulted in pBL206.

For pBL209 [P_{lac}::*gfp-ftsN*], the 2480 bp *Apa*I-*Hind*III fragment of pCH201 [P_{lac}::*gfp-ftsN*] was used to replace the 2503 bp *Apa*I-*Hind*III fragment of pTB8 [P_{lac}::*minCDE*].

For pBL210 [P_{lac}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>95}$], primers 5'- GTCAGCGGCCGCAACACCAGTACCAACCGCC-3' and 5'- GTCAGGCCGGCCTCCGCTACAGGCTCAGGCTGTGG-3' were used to amplify a portion of pCH38 [P_{T7}::*zipA-h*], and the 286 bp *Not*I-*Fse*I fragment was used to replace the 19 bp *Not*I-*Fse*I fragment of pBL205 [P_{lac}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>6}$]. pBL210 encodes a version of GFP-FtsN in which FtsN residues 59-73 (KEESETLQSQKVTGN) are replaced with a 95 residue P/Q-rich linker derived from ZipA$^{88\text{-}180}$ (RPQHQYQPPYASAQPRQPVQQPPEAQVPPQHAPHPAQPVQQPAYQPQPEQPLQQPVSPQVAPAPQPVHSAPQPAQQAFQPAEPVAAPQPEPVAEA).

For pBL211 [*gfp-ftsN*$^{\Delta(59\text{-}73)<>15}$], oligos 5'-GGCCGTCGCCGCAACACCAGTACCAACCGCCTTATGCGTCTGCCGG-3' and 5'-CAGACGCATAAGGCGGTTGGTACTGGTGTTGCGGCGAC-3' were annealed and the fragment with compatible overhangs was used to replace the 19 bp *Not*I-*Fse*I fragment of pBL205 [P_{lac}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>6}$]. pBL211 encodes a version of GFP-FtsN in which FtsN residues 59-73

(KEESETLQSQKVTGN) are replaced with a 15 residue P/Q-rich linker derived from ZipA$^{85-99}$ (RPSPQHQYQPPYASA).

Plasmids pBL215 [Y85W], pBL216 [W83L], pBL217 [L89S], pBL218 [W83M], and pBL219 [L89H] encode mutant versions of GFP-FtsN in which a single FtsN residue has been replaced, as indicated in brackets. They were obtained by replacing the 2503 bp *Apa*I-*Hind*III fragment of pTB8 [P_{lac}::*minCDE*] with the 2483 bp *Apa*I-*Hind*III fragment of mutant pBL116 derivatives carrying the corresponding allele.

For pBL222 [P_{BAD}::*ftsL*], the 24 bp *Xba*I-*Hind*III fragment of pBAD33 [P_{BAD}::] was replaced with the 414 bp *Xba*I-*Hind*III fragment of pAB12 [P_{T7}::*ftsL*].

For pBL225 [P_{lac}::*gfp-ftsN*^{Y85S}], the 2480 bp *Apa*I-*Hind*III fragment of pBL161 [P_{lac}::*gfp-ftsN*^{Y85S}] was used to replace the 2503 bp *Apa*I-*Hind*III fragment of pTB8 [P_{lac}::*minCDE*].

For pBL226 [P_{lac}::*gfp-ftsN*^{W83T}], the 2480 bp *Apa*I-*Hind*III fragment of pBL181 [P_{lac}::*gfp-ftsN*^{W83T}] was used to replace the 2503 bp *Apa*I-*Hind*III fragment of pTB8 [P_{lac}::*minCDE*].

For pBL236 [*ftsA*^{I143L}], primers 5'- GTAGTACGAATTCTGGAACTGGCGGAC-3' and 5'-GAGGCCGTAATCATCGTCGGCCTC-3' were used to amplify a chromosomal *ftsQA* fragment of strain BL86-KK1/pBL215 [*ftsA*^{I143L} Δ*ftsN* / P_{lac}::*gfp-ftsN*^{Y85W}]. The 852 bp *Bgl*II-*Asc*I fragment of the product was then used to replace that of pBL12 [*ftsA*^{E124A}].

For pBL289 [P_{T7}::*ftsB*], primers 5'- CGCATATGGGTAAACTAACGCTGCTGTTGC-3' and 5'-AAAGGGGGATGTGCTGCAAG-3' were used to amplify a portion of pBL193 [P_{lac}::*gfp-ftsB*], and the 334 bp *Nde*I-*Hind*III fragment of the product was used to replace the 63 bp *Nde*I-*Hind*III fragment of pET21A [P_{T7}::].

For pBL294 [P_{lac}::*ftsB*], the 1000 bp *Xba*I-*Hind*III fragment of pMG21 [P_{lac}::*ftsN*] was replaced with the 374 bp *Xba*I-*Hind*III fragment of pBL289 [P_{T7}::*ftsB*].

For pBL295 [P_{lac}::*ftsL*], the 1000 bp *Xba*I-*Hind*III fragment of pMG21 [P_{lac}::*ftsN*] was replaced with the 414 bp *Xba*I-*Hind*III fragment of pBL222 [P_{BAD}::*ftsL*].

For pBL300 [P_{lac}::*ftsB*^{D59H}], primers 5'-CGCATATGGGTAAACTAACGCTGCTGTTGC-3' and 5'-GTCAGGCCGAGGCGGCCTTATCGATTGTTTTGCCCCGC-3' were used to amplify chromosomal *ftsB*^{D59H} from strain BL86-AK11/pBL216 [*ftsB*^{D59H} Δ*ftsN* / P_{lac}::*gfp-ftsN*^{W83L}], and the 321 bp *Nde*I-*Sfi*I fragment was ligated to the 3521 *Nde*I-*Sfi*I fragment of pBL147 [P_{lac}::*gfp-ftsI*].

For pBL301 [P_{lac}::*ftsL*^{D93G}], primers 5'- CCGAATTCCATATGATCAGCAGAGTGACAG-3' and 5'-CGTGTCGACTTATTTTTGCACTACGAT-3' were used to amplify chromosomal *ftsL*^{D93G} from strain BL86-AK1/pBL215 [*ftsL*^{D93G} Δ*ftsN* / P_{lac}::*gfp-ftsN*^{Y85W}], and the 298 bp *Nsi*I-*Sal*I fragment was used to replace that of pBL295 [P_{lac}::*ftsL*].

For pBL303 [P_{syn135}::*ftsL*^{D93G}], the 1000 bp *Xba*I-*Hind*III fragment of pBL153 [P_{syn135}::*ftsN*] was replaced with the 414 bp *Xba*I-*Hind*III fragment of pBL301 [P_{lac}::*ftsL*^{D93G}].

For pBL304 [*repA*^{ts} P_{syn135}::*ftsB*^{D59H}], the 1000 bp *Xba*I-*Hind*III fragment of pBL154 [*repA*^{ts} P_{syn135}::*ftsN*] was replaced with the 358 bp *Xba*I-*Hind*III fragment of pBL300 [P_{lac}::*ftsB*^{D59H}].

For pBL305 [*repA*^{ts} P_{syn135}::*ftsL*^{D93G}], the 1000 bp *Xba*I-*Hind*III fragment of pBL154 [*repA*^{ts} P_{syn135}::*ftsN*] was replaced with the 414 bp *Xba*I-*Hind*III fragment of pBL301 [P_{lac}::*ftsL*^{D93G}].

For pBL306(D59H) [*repA*^{ts} P_{syn135}::*ftsB*^{D59H} *ispD*$^{1-48}$], primers 5'-CGCATATGGGTAAACTAACGCTGCTGTTGC-3' and 5'-GCGTCGACTTAATGCGCCAGCAGCGCATGCACCG-3' were used to amplify chromosomal *ftsB*^{D59H} and a portion of *ispD* from strain BL86-AK11/pBL216 [*ftsB*^{D59H} Δ*ftsN* / P_{lac}::*gfp-ftsN*^{W83L}], and the 209 bp *Mlu*I-*Sal*I fragment was used to replace the 58 bp *Mlu*I-*Sal*I fragment of pBL304 [*repA*^{ts} P_{syn135}::*ftsB*^{D59H}].

For pBL306(E56A) [*repA*^{ts} P_{syn135}::*ftsB*^{E56A} *ispD*$^{1-48}$], the 331 bp *Xba*I-*Cla*I fragment of pBL306(D59H) [*repA*^{ts} P_{syn135}::*ftsB*^{D59H} *ispD*$^{1-48}$] was replaced with the 347 bp *Xba*I-*Cla*I fragment of pBL339 [P_{syn135}::*ftsB*^{E56A}].

For pBL306(E56K) [*repA*^{ts} P_{syn135}::*ftsB*^{E56K} *ispD*$^{1-48}$], the 331 bp *Xba*I-*Cla*I fragment of pBL306(D59H) [*repA*^{ts} P_{syn135}::*ftsB*^{D59H} *ispD*$^{1-48}$] was replaced with the 347 bp *Xba*I-*Cla*I fragment of pBL341 [P_{syn135}::*ftsB*^{E56K}].

For pBL309 [P_{syn135}::*ftsL*], the 1000 bp *Xba*I-*Hind*III fragment of pBL153 [P_{syn135}::*ftsN*] was replaced with the 414 bp *Xba*I-*Hind*III fragment of pBL295 [P_{lac}::*ftsL*].

For pBL312 [P_{lac}::*gfp-malF*$^{2-14}$-*ftsN*$^{27-81}$-*le*], primers 5'-GTCGGCCGAAATGTGCCTGCGGTTTCTCCC-3' and 5'-AGCGCTCGAGTTCTTCTGGTTTTGGTGGTAGTCCGTTTCC-3' were used to amplify a portion of pMG13 [P_{lac}::*gfp-ftsN*$^{1-81}$], and the 169 bp *Eag*I-*Xho*I fragment was used to replace the 691 bp *Eag*I-*Xho*I fragment of pFB261 [P_{lac}::*gfp-malF*$^{2-14}$-*rodZ*$^{111-319}$].

For pBL313 [P_{BAD}::*rfp-malF*$^{2-39}$-*ftsN*$^{55-105}$-*le*], the 972 bp *Xba*I-*Xcm*I fragment of pBL138 [P_{BAD}::*rfp-malF*$^{2-39}$-*ftsN*$^{55-123}$] was used to replace the 1023 bp *Xba*I-*Xcm*I fragment of pBL141 [P_{BAD}::*rfp-ftsN*$^{1-105}$-*le*].

For pBL315 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-81}$-*le*], primers 5'-CTGTACGGATCCACGCATCACAAGAAAGAAGAGTCC-3' and 5'-AGCGCTCGAGTTCTTCTGGTTTTGGTGGTAGTCCGTTTCC-3' were used to amplify a portion of pCH201 [P_{lac}::*gfp-ftsN*], and the 87 bp *BamH*I-*Xho*I fragment was used to replace the 207 bp *BamH*I-*Xho*I fragment of pCH310 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-123}$].

For pBL321 [P_{lac}::*gfp-ftsN*^{L75A}], pMG59 [P_{lac}::*gfp-ftsN*] was mutagenized using 5'-GAAAGTGACCGGAAACGGAGCTCCACCAAAACCAGAAGAAC-3' and its reverse complement. The 581 bp *BamH*I-*Age*I fragment of pMG59(L75A) was next ligated to the 8804 bp *BamH*I-*Age*I fragment of pBL210 [P_{lac}::*gfp-ftsN*$^{\Delta(59-73)<>95}$].

For pBL322 [P_{lac}::*gfp-ftsN*^{P76A}], a mutagenized 732 bp fragment of pMG59 was obtained by asymmetric amplification and overlap extension (AAOE) according to Xiao and Pei (Xiao & Pei, 2011) using primer pairs 5'- GCTGCTGGGATTACACATGGC-3' with 5'-GTGACCGGAAACGGACTAGCACCAAAACCAGAAGAACGC-3', and 5'-GCGTTCTTCTGGTTTTGGTGCTAGTCCGTTTCCGGTCAC-3' with 5'- CGCAGGAGTTTGCAGCAGATC-3'. The 581 bp *BamH*I-*Age*I fragment encoding the P76A substitution was next ligated to the 8804 bp *BamH*I-*Age*I fragment of pBL210 [P_{lac}::*gfp-ftsN*$^{\Delta(59-73)<>95}$].

For pBL323 [P_{lac}::*gfp-ftsN*^{P77A}], pMG59 was mutagenized using 5'-CCGGAAACGGACTACCAGCAAAACCAGAAGAACGCTGG-3' and its reverse complement. The 581 bp

*BamH*I-*Age*I fragment of pMG59(P77A) was next ligated to the 8804 bp *BamH*I-*Age*I fragment of pBL210 [P_{lac}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>95}$].

For pBL324 [P_{lac}::*gfp-ftsN*^{K78A}], pMG59 was mutagenized using 5'-GGAAACGGACTACCACCAGCACCAGAAGAACGCTGGCGC-3' and its reverse complement. The 581 bp *BamH*I-*Age*I fragment of pMG59(K78A) was next ligated to the 8804 bp *BamH*I-*Age*I fragment of pBL210 [P_{lac}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>95}$].

For pBL325 [P_{lac}::*gfp-ftsN*^{P79A}], a mutagenized 732 bp fragment of pMG59 was obtained by asymmetric amplification and overlap extension using primer pairs 5'-GCTGCTGGGATTACACATGGC-3' with 5'- GGAAACGGACTACCACCAAAAGCAGAAGAACGCTGGCGCTAC-3', and 5'- GTAGCGCCAGCGTTCTTCTGCTTTTGGTGGTAGTCCGTTTCC-3' with 5'-CGCAGGAGTTTGCAGCAGATC-3'. The 581 bp *BamH*I-*Age*I fragment encoding the P79A substitution was next ligated to the 8804 bp *BamH*I-*Age*I fragment of pBL210 [P_{lac}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>95}$].

For pBL326 [P_{lac}::*gfp-ftsN*^{E80A}], a mutagenized 732 bp fragment of pMG59 was obtained by asymmetric amplification and overlap extension using primer pairs 5'-GCTGCTGGGATTACACATGGC-3' with 5'- CTACCACCAAAACCAGCAGAACGCTGGCGCTAC-3', and 5'-GTAGCGCCAGCGTTCTGCTGGTTTTGGTGGTAG-3' with 5'- CGCAGGAGTTTGCAGCAGATC-3'. The 581 bp *BamH*I-*Age*I fragment encoding the E80A substitution was next ligated to the 8804 bp *BamH*I-*Age*I fragment of pBL210 [P_{lac}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>95}$].

For pBL327 [P_{lac}::*gfp-ftsN*^{R84A}], a mutagenized 732 bp fragment of pMG59 was obtained by asymmetric amplification and overlap extension using primer pairs 5'-GCTGCTGGGATTACACATGGC-3' with 5'- CCAGAAGAACGCTGGGCCTACATTAAAGAGCTGGAAAG-3', and 5'- CTTTCCAGCTCTTTAATGTAGGCCCAGCGTTCTTCTGG-3' with 5'-CGCAGGAGTTTGCAGCAGATC-3'. The 581 bp *BamH*I-*Age*I fragment encoding the R84A substitution was next ligated to the 8804 bp *BamH*I-*Age*I fragment of pBL210 [P_{lac}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>95}$].

For pBL328 [P_{lac}::*gfp-ftsN*^{L89A}], pMG59 was mutagenized using 5'-GGCGCTACATTAAAGAGGCGGAAAGTCGCCAGCCGGG-3' and its reverse complement. The 581 bp *BamH*I-*Age*I fragment of pMG59(L89A) was next ligated to the 8804 bp *BamH*I-*Age*I fragment of pBL210 [P_{lac}::*gfp-ftsN*$^{\Delta(59\text{-}73)<>95}$].

For pBL329 [P_{lac}::*gfp-ftsN*^{E90A}], pMG59 was mutagenized using 5'-CGCTACATTAAAGAGCTGGCAAGTCGCCAGCCGGGAGTG-3' and its reverse complement. The 581 bp *BamH*I-*Age*I fragment of pMG59(E90A) was next ligated to the 8804 bp *BamH*I-*Age*I fragment of pBL210 [P_{lac}::*gfp-ftsN*$^{(1-58)-P/Q95-(74-319)}$].

For pBL330 [P_{lac}::*gfp-ftsN*^{D5S,Y6A}], a mutagenized 732 bp fragment of pMG59 [P_{lac}::*gfp-ftsN*] was obtained by asymmetric amplification and overlap extension using primer pairs 5'-GCTGCTGGGATTACACATGGC-3' with 5'-CCGAATTCCATATGGCACAACGATCTGCTGTACGCCGCAGCCAACCGGC-3', and 5'-GCCGGTTGGCTGCGGCGTACAGCAGATCGTTGTGCCATATGGAATTCGG-3' with 5'-CGCAGGAGTTTGCAGCAGATC-3'. The 581 bp *BamH*I-*Age*I fragment encoding the D5S and Y6A substitutions was next ligated to the 8804 bp *BamH*I-*Age*I fragment of pBL210 [P_{lac}::*gfp-ftsN*$^{\Delta(59-73)<>95}$].

For pBL331 [P_{syn135}::*ftsL*^{H94Y}], the 138 bp *Bpm*I-*Hind*III fragment of pJH2 [P_{syn135}::*ftsL*] was replaced with that of a mutant derivative [pJH2(L9V, V27A, H94Y)-pBL215-L6], selected from a library of mutant *ftsL* alleles that allow growth of strain JH1/pBL200/pBL215 at 37^{o}C.

For pBL332 [P_{syn135}::*ftsL*^{E88V}], the 138 bp *Bpm*I-*Hind*III fragment of pJH2 [P_{syn135}::*ftsL*] was replaced with that of a mutant derivative [pJH2(L77I, E88V)-pBL215-L7], selected from a library of mutant *ftsL* alleles that allow growth of strain JH1/pBL200/pBL215 at 37^{o}C.

Plasmid pBL333 [P_{syn135}::*ftsL*^{E88k}], was selected from a library of mutant *ftsL* alleles that allow growth of strain JH1/pBL200/pBL215 at 42^{o}C.

For pBL334 [P_{syn135}::*ftsL*^{N89S}], the 138 bp *Bpm*I-*Hind*III fragment of pJH2 [P_{syn135}::*ftsL*] was replaced with that of a mutant derivative [pJH2(K37R, N89S)-pBL215-L7], selected from a library of mutant *ftsL* alleles that allow growth of strain JH1/pBL200/pBL215 at 42^{o}C.

For pBL335 [P_{lac}::*gfp-ftsN*$^{1-81;D5S,Y6A}$-*le*], primers 5'- GCTGCTGGGATTACACATGGC-3' and 5'-AGCGCTCGAGTTCTTCTGGTTTTGGTGGTAGTCCGTTTCC-3' were used to amplify a portion of pBL330 [P_{lac}::*gfp-ftsN*^{D5S,Y6A}], and the 291 bp *Nhe*I-*Xho*I fragment was used to replace the 777 bp *Nhe*I-*Xho*I fragment of pCH354 [P_{lac}::*gfp-ftsN*$^{1-243}$-*le*].

For pBL336 [P_{syn135}::*ftsB*], the 414 bp *Xba*I-*Hind*III fragment of pJH2 [P_{syn135}::*ftsL*] was replaced with the 374 bp *Xba*I-*Hind*III fragment of pBL294 [P_{lac}::*ftsB*].

Plasmid pBL338 [P_{syn135}::*ftsB*^{E56V}], was selected from a library of mutant *ftsB* alleles that allow growth of strain JH1/pBL200/pBL215 at 42^{o}C.

For pBL339 [P_{syn135}::*ftsB*^{E56A}], the 121 bp *BstZ17*I-*Sap*I fragment of pBL336 [P_{syn135}::*ftsB*] was replaced with that of a mutant derivative [pBL336(K3R, E56A, S92L)-pBL215-B2], selected from a library of mutant *ftsB* alleles that allow growth of strain JH1/pBL200/pBL215 at 37^{o}C.

For pBL340 [P_{syn135}::*ftsB*^{A55T}], the 238 bp *Xba*I-*Sap*I fragment of pBL336 [P_{syn135}::*ftsB*] was replaced with that of a mutant derivative [pBL336(A55T, T83S)-pBL215-B3], selected from a library of mutant *ftsB* alleles that allow growth of strain JH1/pBL200/pBL215 at 37^{o}C.

Plasmid pBL341 [P_{syn135}::*ftsB*^{E56K}], was selected from a library of mutant *ftsB* alleles that allow growth of strain JH1/pBL200/pBL215 at 37^{o}C.

Plasmid pBL342 [P_{syn135}::*ftsB*^{E56G}], was selected from a library of mutant *ftsB* alleles that allow growth of strain JH1/pBL200/pBL215 at 37^{o}C.

Plasmid pBL343 [P_{syn135}::*ftsB*^{D59V}], was selected from a library of mutant *ftsB* alleles that allow growth of strain JH1/pBL200/pJH10 at 37^{o}C.

For pBL356 [SceI P_{syn135}::*ftsB*^{E56K} *ispD*$^{1-48}$ SceI *sacB*], the 58 bp *EcoR*I-*Sal*I fragment of pDOC-C [SceI SceI *sacB*] was replaced with the 573 bp *EcoR*I-*Sal*I fragment of pBL306(E56K) [P_{syn135}::*ftsB*^{E56K} *ispD*$^{1-48}$].

For pCH277 [P_{lac}::*amiC*$^{1-31}$-*ftsN*$^{52-123}$-*gfp*], primers 5'-GTCTCTGCTAGCTTACTTCATTACGCATCACAAGAAAGAAG-3' and 5'-CGAACTCGAGAAGCTGACGTTGTTCTGGTGTCAG-3' were used to amplify a portion of pCH276 [P_{lac}::*gfp-ftsN*$^{1-123}$], and the 217 bp *Nhe*I-*Xho*I fragment was used to replace the 19 bp *Nhe*I-*Xho*I fragment of pTB37 [P_{lac}::*amiC*$^{1-31}$-*gfp*].

For pCH286 [P_{lac}::*amiC*$^{1-31}$-*ftsN*$^{52-123}$], the 1859 bp *Xba*I-*Xho*I fragment of pCH181 [P_{lac}::*gfp-minD minE-le*] was replaced with the 326 bp *Xba*I-*Xho*I fragment of pCH277 [P_{lac}::*amiC*$^{1-31}$-*ftsN*$^{52-123}$-*gfp*].

For pCH287 [*cI*857 $P_{\lambda R}$::*amiC*$^{1-31}$-*ftsN*$^{52-123}$], the 6 bp *Xba*I-*Sal*I fragment of pDB344 [*cI*857 $P_{\lambda R}$::] was replaced with the 335 bp *Xba*I-*Sal*I fragment of pCH286 [P_{lac}::*amiC*$^{1-31}$-*ftsN*$^{52-123}$].

For pCH309 [P_{lac}::*gfp*-malF$^{2-39}$-*mreC*$^{38-367}$-*le*], primers 5'-GCAGAGATCTGATGTCATTAAAAAGAAACATTGGTGGC-3' and 5'-CGTTCTCGAGTTGCCCTCCCGGCGCACGCGCAGGC-3' were used to amplify a portion of pFB243 [P_{lac}::*malF*$^{1-39}$-*mreC*$^{38-367}$], and the 1116 bp *Bgl*II-*Xho*I fragment was used to replace the 330 bp *BamH*I-*Xho*I fragment of pMG12 [P_{lac}::*gfp-ftsN*$^{1-105}$-*le*].

For pCH310 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-123}$], the 1562 bp *Apa*I-*BamH*I fragment of pCH288 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{55-123}$] was replaced with the 1627 bp *Apa*I-*BamH*I fragment of pCH309 [P_{lac}::*gfp*-malF$^{2-39}$-*mreC*$^{38-367}$-*le*].

For pCH327 [P_{CYC1}::*lexA-vp16-cub-ftsN*$^{1-105}$], a portion of pMG12 [P_{lac}::*gfp-ftsN*$^{1-105}$-*le*]was amplified with primers 5'- CTGAGGCCATTACGGCCGTGGCACAACGAGATTATGTACG-3' and 5'-ATCGGGCCGAGGCGGCCTTAACCGGCAGAAGGTTCTGTGGG-3', and the 331 bp *Sfi*I fragment was used to replace the 27 bp *Sfi*I fragment of pBT3-N [P_{CYC1}::*lexA-vp16-cub*-].

For pCH391 [P_{lac}::*t25-ftsN*$^{1-105}$], the 1027 bp *Sfi*I fragment of pCH358 [P_{lac}::*t25-rodZ*] was replaced with the 331 bp *Sfi*I fragment of pCH327 [P_{CYC1}::*lexA-vp16-cub-ftsN*$^{1-105}$].

For pCH391(DY>SA) [P_{lac}::*t25-ftsN*$^{1-105;\ D5S,Y6A}$], the 2565 bp *Sfi*I fragment of pLP10 [P_{lac}::*t25-ponA*] was replaced with the 331 bp *Sfi*I fragment of pCH392(DY>SA) [P_{lac}::*t18-ftsN*$^{1-105;\ D5S,Y6A}$].

For pCH392 [P_{lac}::*t18-ftsN*$^{1-105}$], the 1027 bp *Sfi*I fragment of pCH371 [P_{lac}::*t18-rodZ*] was replaced with the 331 bp *Sfi*I fragment of pCH327 [P_{CYC1}::*lexA-vp16-cub-ftsN*$^{1-105}$].

For pCH392(DY>SA) [P_{lac}::*t18-ftsN*$^{1-105;\ D5S,Y6A}$], a portion of pBL330 [P_{lac}::*gfp-ftsN*^{D5S,Y6A}] was amplified with primers 5'- AAAAGGCCATTACGGCCGTGGCACAACGATCTGCTGTACGC-3' and 5'-ATCGGGCCGAGGCGGCCTTAACCGGCAGAAGGTTCTGTGGG-3', and the 331 bp *Sfi*I fragment was used to replace the 2565 bp *Sfi*I fragment of pLP7 [P_{lac}::*t18-ponA*].

For pCH399 [P_{lac}::*t18-ftsA*], a portion of pZAQ [*ftsQAZ*] was amplified with primers 5'-GGCCATTACGGCCATGATCAAGGCGACGGACAG-3' and 5'-GGCCGAGGCGGCCTTAAAACTCTTTTCGCAGCC-3', and the 1276 bp *Sfi*I fragment was used to replace the 1027 bp *Sfi*I fragment of pCH371 [P_{lac}::*t18-rodZ*].

For pCH400 [P_{lac}::*t18-ftsQ*], a portion of pZAQ [*ftsQAZ*] was amplified with primers 5'-GGCCATTACGGCCATGTCGCAGGCTGCTCTG-3' and 5'-GGCCGAGGCGGCCTCATTGTTGTTCTGCCTGTGCC-3', and the 844 bp *Sfi*I fragment was used to replace the 1027 bp *Sfi*I fragment of pCH371 [P_{lac}::*t18-rodZ*].

For pCH425 [P_{lac}::*t18-ftsB*], *ftsB* was amplified with primers 5'-GATCGGCCATTACGGCCATGGGTAAACTAACGCTGCTG-3' and 5'-GTCAGGCCGAGGCGGCCTTATCGATTGTTTTGCCCCGC-3', and the 325 bp *Sfi*I fragment was used to replace the 1027 bp *Sfi*I fragment of pCH371 [P_{lac}::*t18-rodZ*].

For pCH433 [P_{lac}::*t25-ftsI*], *ftsI* was amplified with primers 5'-GATCGGCCATTACGGCCATGAAAGCAGCGGCGAAAAC-3' and 5'-GATCGGCCGAGGCGGCCTTACGATCTGCCACCTGTC-3'. The product was digested with *Sfi*I, and the 1780 bp fragment was used to replace the 1027 bp *Sfi*I fragment of pCH358 [P_{lac}::*t25-rodZ*].

For pDB357 [P_{T7}::*ftsN*], *ftsN* was amplified with primers 5'-CAGCGAATTCCATATGGCACAACGAGATTATG-3' and 5'- TGAGAAGCTTAACCCCCGGCGGCGAG-3', and the 960 bp *Nde*I-*Hind*III fragment was used to replace the 63 bp *Nde*I-*Hind*III fragment of pET21A [P_{T7}::].

For pJH1 [*tetA repA*^{ts} P_{syn135}::*ftsL*], the 468 bp *EcoR*I-*Hind*III fragment of pBL309 [P_{syn135}::*ftsL*] was ligated to the 5779 bp *EcoR*I-*Hind*III fragment of pBL206 [*tetA repA*^{ts} *ftsA*^{E124A}].

For pJH2 [*tetA* P_{syn135}::*ftsL*], the 2674 bp *Afl*II-*ApaL*I fragment of pJH1 [*tetA repA*^{ts} P_{syn135}::*ftsL*] was replaced with the 2127 bp *Afl*II-*ApaL*I fragment of pBL153 [P_{syn135}::*ftsN*].

For pJH3 [*tetA* P_{syn135}::*ftsL*^{D93G}], the 414 bp *Xba*I-*Hind*III fragment of pJH2 [*tetA* P_{syn135}::*ftsL*] was replaced with that of pBL303 [*aadA* P_{syn135}::*ftsL*^{D93G}].

For pJH5 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-320;\ T64G,\ S67G,\ Y85W,\ +A102}$], a portion of pBL215 [$P_{lac}$::*gfp-ftsN*$^{T64G,\ S67G,\ Y85W,\ +A102}$::^{G}*lacZYA*] was amplified with primers 5'-CTGTACGGATCCACGCATCACAAGAAAGAAGAGTCC-3', and 5'- TGAGAAGCTTAACCCCCGGCGGCGAG-3', and the 794 bp *BamH*I-*Hind*III fragment was used to replace the 230 bp *BamH*I-*Hind*III fragment of pCH310 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-123}$].

For pJH6 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-320;\ T64G,\ S67G,\ W83L,\ +A102}$], a portion of pBL216 [$P_{lac}$::*gfp-ftsN*$^{T64G,\ S67G,\ W83L,\ +A102}$::^{G}*lacZYA*] was amplified with primers 5'-CTGTACGGATCCACGCATCACAAGAAAGAAGAGTCC-3', and 5'- TGAGAAGCTTAACCCCCGGCGGCGAG-3', and the 794 bp *BamH*I-*Hind*III fragment was used to replace the 230 bp *BamH*I-*Hind*III fragment of pCH310 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-123}$].

For pJH10 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-320;\ T64G,\ S67G,\ Y85W,\ +A102}$], the 30 bp *EcoR*I-*Hind*III fragment of pRC7 [P_{lac}::] was replaced with the 1774 bp *EcoR*I-*Hind*III fragment of pJH5.

For pJH11 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-320;\ T64G,\ S67G,\ W83L,\ +A102}$], the 30 bp *EcoR*I-*Hind*III fragment of pRC7 [P_{lac}::] was replaced with the 1774 bp *EcoR*I-*Hind*III fragment of pJH6.

For pLP10 [P_{lac}::*t25-ponA*], the 2566 bp *Sfi*I fragment of pLP7 [P_{lac}::*t18-ponA*] was used to replace the 1027 bp *Sfi*I fragment of pCH358 [P_{lac}::*t25-rodZ*].

For pLP160 [P_{BAD}::*rfp-malF*$^{2-39}$-*ftsN*$^{55-90}$], the 2232 bp *Xcm*I-*Nco*I fragment of pBL313 [P_{BAD}::*ecrfp-malF*$^{2-39}$-*ftsN*$^{55-105}$-*le*] was replaced with the 2181 bp *Xcm*I-*Nco*I fragment of pBL142 [P_{BAD}::*ecrfp-ftsN*$^{1-90}$].

For pLP163 [P_{lac}::*gfp-ftsN*], a portion of pCH201 [P_{lac}::*gfp-ftsN*] was amplified with primers 5'-GGTGAGATCTGTGGCACAACGAGATTATGTACGC-3' and 5'- TGAGAAGCTTAACCCCCGGCGGCGAG-3', and the 964 bp *Bgl*II-*Hind*III fragment was used to replace the 973 bp *BamH*I-*Hind*III fragment of pCH201. pLP163 is similar to pCH201, but 3 codons in the linker between *gfp* and *ftsN*, as well as three restriction sites (*BamH*I, *EcoR*I, and *Nde*I), have been eliminated.

For pLP164 [P_{lac}::*gfp-ftsN*$^{75-105}$-*le*], a portion of pMG14 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{71-105}$-*le*] was amplified with primers 5'- CATCGGATCCCTACCACCAAAACCAGAAGAACGC-3' and 5'-GCGATCGGCATAACCACCACGCTC-3', and the 955 bp *BamH*I-*Cla*I fragment of the product was used to replace the 1129 bp *BamH*I-*Cla*I fragment of pMG47 [P_{lac}::*gfp-ftsN*$^{1-90}$].

For pLP165 [P_{lac}::*gfp-ftsN*$^{80-105}$-*le*], a portion of pMG14 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{71-105}$-*le*] was amplified with primers 5'- GAAGGGATCCGAAGAACGCTGGCGCTACATTAAAGAG-3' and 5'-GCGATCGGCATAACCACCACGCTC-3', and the 940 bp *BamH*I-*Cla*I fragment of the product was used to replace the 1129 bp *BamH*I-*Cla*I fragment of pMG47 [P_{lac}::*gfp-ftsN*$^{1-90}$].

For pLP168 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{75-105}$-*le*], the 1629 bp *BamH*I-*Cla*I fragment of pCH282 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{55-319}$] was replaced with the 955 bp *BamH*I-*Cla*I fragment of pLP164 [P_{lac}::*gfp-ftsN*$^{75-105}$-*le*].

For pLP169 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{80-105}$-*le*], the 1629 bp *BamH*I-*Cla*I fragment of pCH282 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{55-319}$] was replaced with the 940 bp *BamH*I-*Cla*I fragment of pLP165 [P_{lac}::*gfp-ftsN*$^{75-105}$-*le*].

For pLP170 [P_{lac}::*gfp-ftsN*$^{1-31}$-*malF*$^{17-39}$-*ftsN*$^{55-81}$-*le*], a portion of pLP163 [P_{lac}::*gfp-ftsN*] was amplified with primers 5'- ATGACCATGATTACGAATTCCCG-3' and 5'-CCATCGGCCGGCAGGCAGATTTCGTTGCTTTTTCCG-3', and the 871 bp *Xba*I-*Eag*I fragment of the product was used to replace the 817 bp *Xba*I-*Eag*I fragment of pBL315 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-81}$-*le*].

For pLP171 [P_{lac}::*gfp-ftsN*$^{1-71}$-*le*], a portion of pCH201 [P_{lac}::*gfp-ftsN*] was amplified with primers 5'- GCTGCTGGGATTACACATGGC-3' and 5'- TAGGCTCGAGGGTCACTTTCTGGCTTTGCAG-3', and the 261 bp *Nhe*I-*Xho*I fragment was used to replace the 777 bp *Nhe*I-*Xho*I fragment of pCH354 [P_{lac}::*gfp-ftsN*$^{1-243}$-*le*].

For pLP218 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{75-93}$-*le*], a portion of pLP168 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{75-105}$-*le*] was amplified with primers 5'- GCTGCTGGGATTACACATGGC-3' and 5'-GGTCCTCGAGCTGGCGACTTTCCAGCTCTTTAATG-3', and the 96 bp *Nhe*I-*Xho*I fragment of the product was used to replace the 132 bp *Nhe*I-*Xho*I fragment of pLP168.

For pLP219 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{75-99}$-*le*], a portion of pLP168 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{75-105}$-*le*] was amplified with primers 5'- GCTGCTGGGATTACACATGGC-3' and 5'-GTGTCTCGAGGGGCGCACGCACTCCCGGCTG-3', and the 114 bp *Nhe*I-*Xho*I fragment of the product was used to replace the 132 bp *Nhe*I-*Xho*I fragment of pLP168.

For pLP221 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{55-90}$], the 72 bp *BamH*I-*Sal*I fragment of pLP218 [P_{lac}::^{ss}*torA-gfp-ftsN*$^{75-93}$-*le*] was replaced with the 117 bp *BamH*I-*Sal*I fragment of pLP160 [P_{BAD}::*ecrfp-malF*$^{2-39}$-*ftsN*$^{55-90}$].

For pMG21 [P_{lac}::*ftsN*], the 1746 bp *Xba*I-*Hind*III fragment of pCH201 [P_{lac}::*gfp-ftsN*] was replaced with the 1000 bp *Xba*I-*Hind*III fragment of pDB357 [P_{T7}::*ftsN*].

For pMG62 [P_{lac}::*gfp-ftsN*$^{1-123}$], the 1779 bp *Xba*I-*Hind*III fragment of pTB222 [P_{lac}::*zipA-gfp*] was replaced with the 1174 bp *Xba*I-*Hind*III fragment of pCH276 [P_{lac}::*gfp-ftsN*$^{1-123}$].

For pTB51 [*cat repA*^{ts} *araC* P_{BAD}::γ β *exo*], the *cat* cassette of pKD3 was amplified with primers 5'- ATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAATGAATATCCTCCTTAGTTCC-3' and 5'- TTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGGTGTAGGCTGGAGCTGCTTCG-3', yielding a 1091 bp fragment with end sequences homologous to those flanking the *bla* gene on pKD46 [*bla repA*^{ts} *araC* P_{BAD}::γ β *exo*] (underlined). Recombination of the fragment with the plasmid in strain TB28/pKD46, followed by selection for chloramphenicol resistance and ampicillin sensitivity, yielded TB28/pTB51. Plasmid pTB51 is similar to pKD46, except that an 888 bp *bla* fragment has been replaced with an 1010 bp *cat* fragment, which is transcribed in the same direction as the replaced gene.

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