Supplementary material to:

Recruitment of the TolA protein to cell constriction sites in *Escherichia coli* via three separate mechanisms, and a critical role for FtsWI activity in recruitment of both TolA and TolQ.

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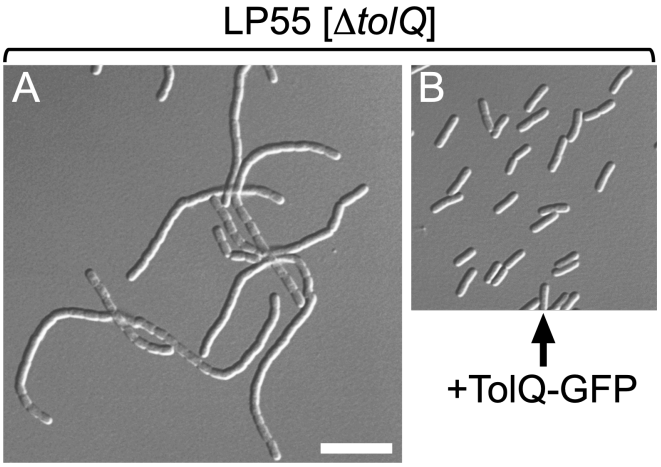

Figure S1. Correction of LP55 [Δ*tolQ*] cell chaining by TolQ-GFP fusion protein.

Differential interference contrast (DIC) images of chemically fixed cells of strain LP55 [Δ*tolQ*] carrying the vector control pMLB1113ΔH [P_{lac}::] (A), or pCH516 [P_{lac}::*tolQ-gfp*] (B). Cultures were grown to density overnight in regular LB (0.5% NaCl), diluted 200-fold in LBNS (no added NaCl) with 5 μM IPTG, and further incubated for ~ 5 mass doublings to OD_{600}=0.7. Bar equals 10 μm.

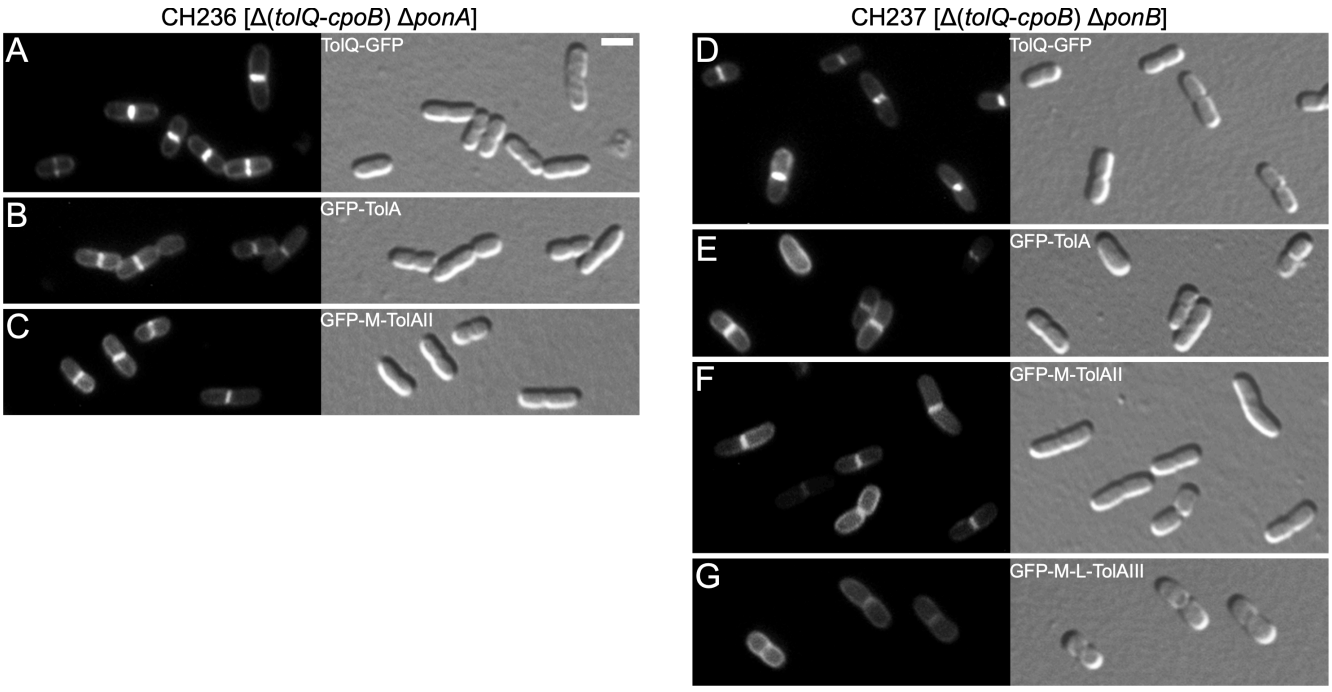

Figure S2. Septal localization of TolQ-GFP, GFP-TolA, or GFP-M-TolAII in the absence of the other Tol-Pal proteins and CpoB does not require PBP1A or PBP1B.

Shown are fluorescence (left) and DIC (right) images of live cells. Overnight cultures in LB with 1% NaCl were diluted in M9-based medium, and growth was continued for ~ 2 mass doublings to OD_{600}= 0.4-0.5. Bar equals 2 μm.

(A-C) CH236 [Δ(*tolQ-cpoB*) Δ*ponA*] cells carrying plasmid pCH516 [P_{lac}::*tolQ-gfp*] (A), or lysogenic for λNP4 [P_{lac}::*gfp-tolA*] (B) or λCH549 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-292}$] (C). Cells were grown in M9-maltose with 5 μM (A) or 37 μM (B and C) IPTG.

(D-G) CH237 [Δ(*tolQ-cpoB*) Δ*ponB*] cells carrying plasmid pCH633 [P_{BAD}::*tolQ-gfp*] (D), pCH634 [P_{BAD}::*gfp-tolA*] (E), pCH635 [P_{BAD}::*gfp-malF*$^{2-39}$-*tolA*$^{47-292}$] (F), or pCH636 [P_{BAD}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*tolA*$^{294-421}$] (G). Cells were grown in M9-maltose without arabinose (D) or in M9-glucose with 0.02 % arabinose (E-G). Note that, while CH236 cells grew about as well as TB28 [wt], CH237 cells grew poorly in both rich and minimal medium, and CH237 cultures contained a considerable amount of cell debris. Even so, the fusion proteins could readily be localized in the surviving cells. Also note that the GFP-M-L-TolAIII fusion in panel G did not accumulate at constriction sites, and served as a control.

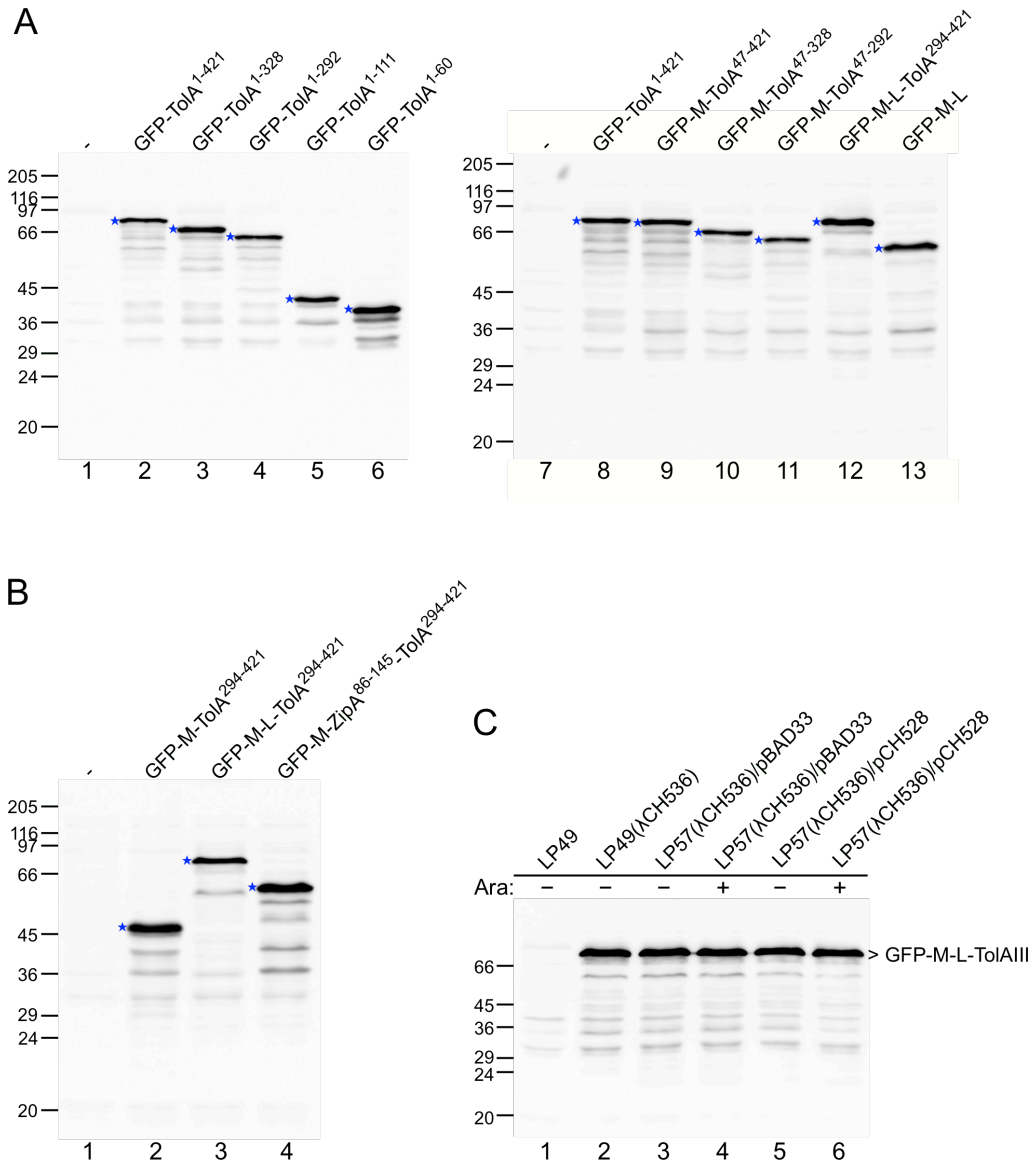

Figure S3. Immunodetection of GFP-TolA and its deletion/substitution variants.

Lanes contained equivalent amounts of whole cell extract, and fusion proteins were detected using α-GFP polyclonal antibodies. In panels A and B, the phage- (A) or plasmid-encoded (B) fusion protein is indicated above each lane (M corresponds to $MalF^{2-39}$, and L to $RodZ^{139-255}$), and a band corresponding to the intact fusion of interest is marked with a blue star. Other bands represent non-specific antigens or breakdown products. Migration of molecular weight standards (kD) is indicated on the left of each panel.

(A) Whole-cell extracts were prepared of strain LP57 [Δ*tolQ-cpoB*] that was either non-lysogenic (1, 7), or lysogenic for λNP4 [P_{lac}::*gfp-tolA*] (2, 8), λDE2 [P_{lac}::*gfp-tolA*$^{1-328}$] (3), λCH512 [P_{lac}::*gfp-tolA*$^{1-292}$] (4), λCH483 [P_{lac}::*gfp-tolA*$^{1-111}$] (5), λDE3 [P_{lac}::*gfp-tolA*$^{1-60}$] (6), λCH509 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-421}$] (9), λCH510 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-328}$] (10), λCH549 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-292}$] (11), λCH536 [P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*tolA*$^{294-421}$] (12), or λCH543 [P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$] (13). Cells were grown for ~ 3.5 mass doublings to OD_{600}=0.5-0.6 in M9-maltose with 37 μM IPTG.

(B) Whole-cell extracts were prepared of strain LP49 [Δ*tolA*] carrying either no plasmid (1), or carrying pCH535 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{294-421}$] (2), pCH536 [P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*tolA*$^{294-421}$] (3), or pCH538 [P_{lac}::*gfp-malF*$^{2-39}$-*zipA*$^{86-145}$-*tolA*$^{294-421}$] (4). Cells were grown for ~ 3.5 mass doublings to OD_{600}=0.5-0.6 in M9-glucose with 5 μM IPTG.

(C) Integrity of the GFP-M-L-TolAIII (GFP-MalF$^{2-39}$-RodZ$^{139-255}$-TolA$^{294-421}$) fusion protein in the presence or absence of Tol-Pal and CpoB proteins. Cells of strains LP49 [Δ*tolA*] (1), LP49(λCH536) [Δ*tolA* (P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*tolA*$^{294-421}$)] (2), or LP57(λCH536) [Δ*tolQ-cpoB* (P_{lac}:: *gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*tolA*$^{294-421}$)] (3-6) carrying either pBAD33 [P_{BAD}::] (3 and 4) or pCH528 [P_{BAD}::*tolB pal cpoB*] (5 and 6) were grown for ~ 3.5 mass doublings to OD_{600}=0.5-0.6 in M9-maltose with 37 μM IPTG and either no (1, 2, 3, 5) or 0.01% (4, 6) arabinose, as indicated.

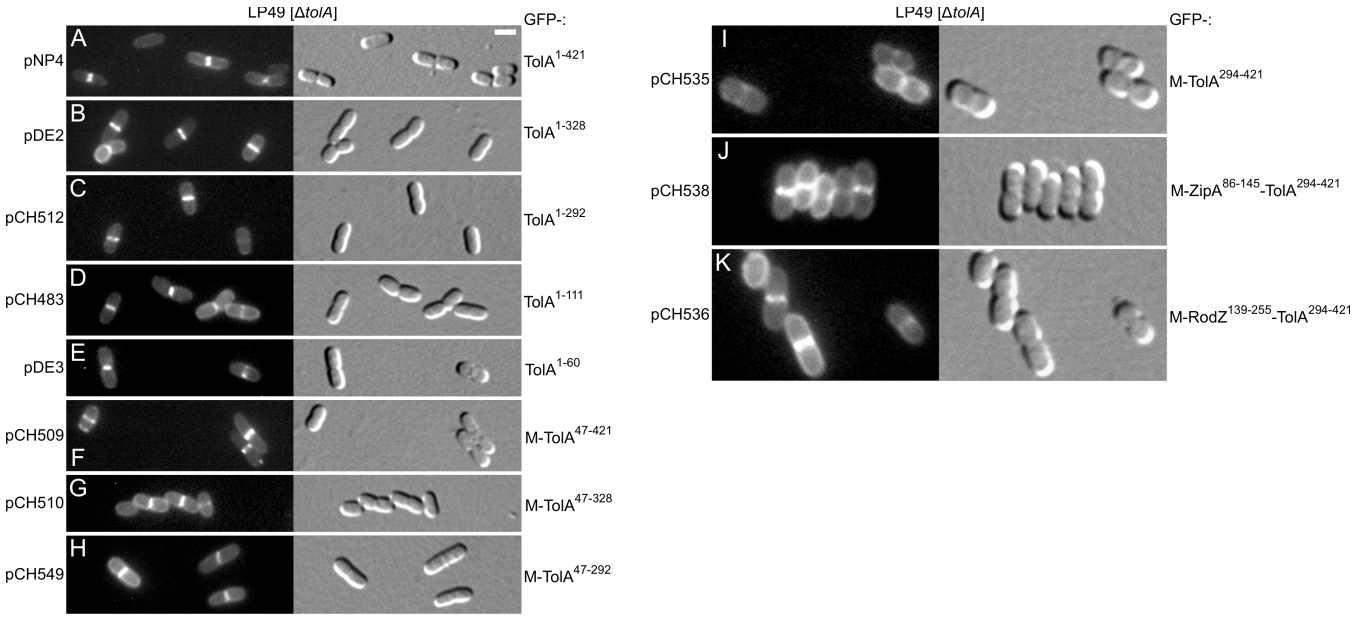

Figure S4. Localization of GFP-TolA and truncated derivatives in strain LP49 [Δ*tolA*].

Plasmids encoding GFP fusions to all (TolA$^{1-421}$) or part of TolA were introduced into strain LP49 [Δ*tolA*]. Cells were grown for ~ 3.5 mass doublings to OD_{600}= 0.5-0.6 in M9-glucose with 5 μM IPTG and imaged live using fluorescence and DIC optics. The name of the plasmid (left), and the fusion it encodes (right), are indicated on the sides of each panel. M (panels F-K) corresponds to MalF$^{2-39}$, which includes the first transmembrane helix of MalF (MalF$^{19-35}$). ZipA$^{86-145}$ (panel J) corresponds to a portion of the cytoplasmic linker that connects the TM and C-terminal domains of the ZipA protein. RodZ$^{139-255}$ (panel K) corresponds to the periplasmic linker that connects the TM and C-terminal domains of the RodZ protein. Bar equals 2.0 (A-H) or 1.2 (I-K) μm. Note that only plasmid pNP4 [P_{lac}::*gfp-tolA*] (panel A), encoding GFP fused to full-length TolA, conferred a Tol$^+$ phenotype to these cells. Save for GFP-MalF$^{2-39}$-TolA$^{294-421}$ (panel I), however, each fusion protein still accumulated at constriction sites to a significant degree.

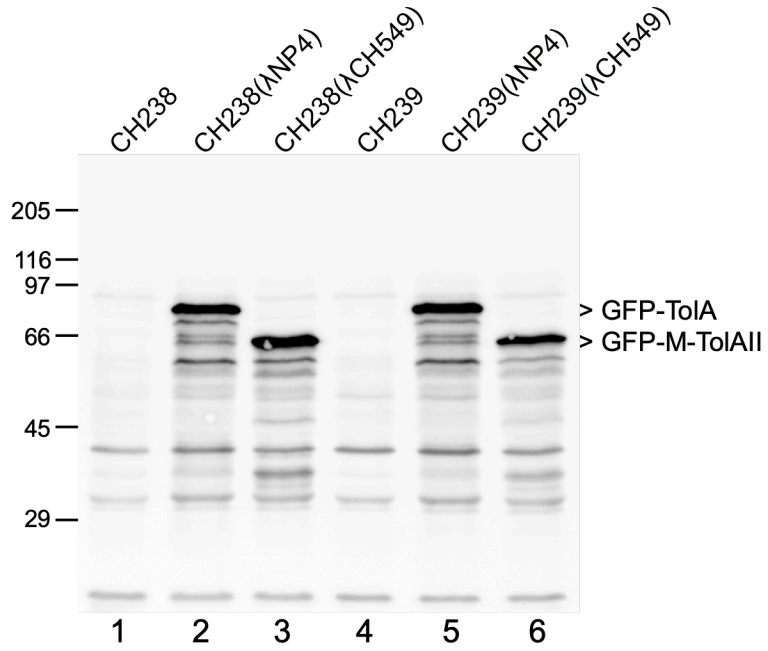

Figure S5. Integrity of GFP-TolA and GFP-M-TolAII fusion proteins in the presence and absence of FtsN.

Strains used were CH238 [Δ(*tolQ-cpoB*) *ftsB*^{E56A}] (1), CH238 lysogenic for λNP4 [P_{lac}::*gfp-tolA*] (2) or for λCH549 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-292}$] (3), strain CH239 [Δ(*tolQ-cpoB*) *ftsB*^{E56A} Δ*ftsN*] (4), and CH239 lysogenic for λNP4 [P_{lac}::*gfp-tolA*] (5) or for λCH549 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-292}$] (6). Cells were grown for ~ 3.5 mass doublings to OD_{600}=0.5-0.6 in M9-maltose with 37 μM IPTG, and whole cell extracts were prepared. Lanes contained equivalent amounts of extract, and fusion proteins were detected using α-GFP polyclonal antibodies. Bands corresponding to the intact fusion proteins are identified on the right. Other bands represent non-specific antigens (see lanes 1 and 4) or breakdown products. Migration of molecular weight standards (kD) is indicated on the left.

E. coli ZipA

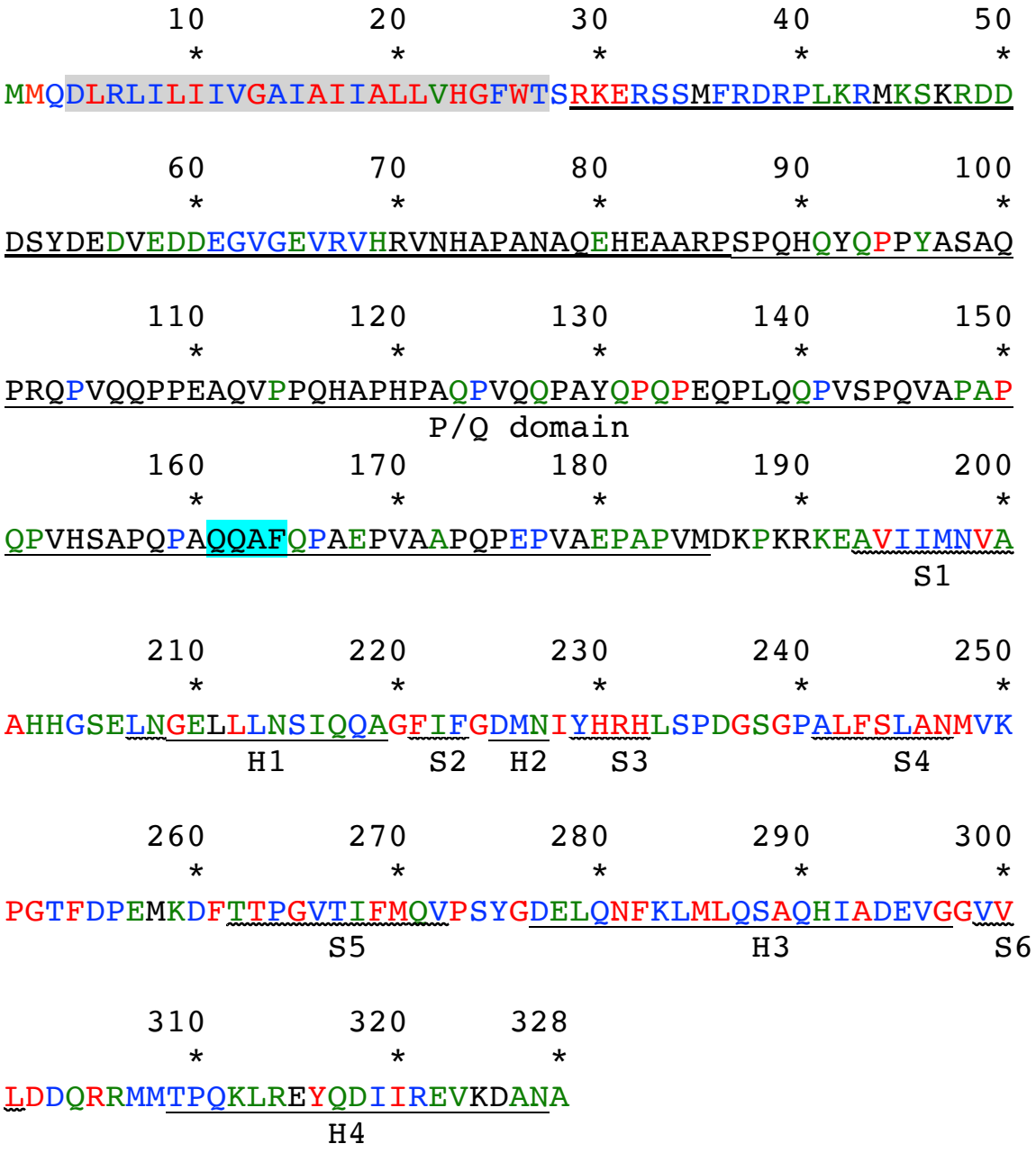

Figure S6. Primary structure of *E.coli* ZipA.

Residues are colored according to % identity in OMA group 1016422 (214 members) (1); red (100-80%), blue (80-60%), green (60-40%), and black (<40%). ZipA is a bitopic (N-out) inner membrane protein (2, 3) and transmembrane residues (ZipA$^{4-27}$, as predicted in the Mebranome database (4)) are highlighted in grey. The TM domain is followed by the highly charged domain

(ZipA$^{29-85}$, thick underline) the P/Q domain (ZipA$^{86-185}$, thin underline), and the globular domain that binds FtsZ (ZipA$^{186-328}$) (2, 3, 5). The tertiary structure of the latter has been determined (PDB:1F46) (6), and secondary structure elements (β-sheets S1-S6, and α-helices H1-H4) are indicated. In-frame replacement of poorly conserved residues 161-164 (highlighted in turquoise) within the P/Q domain with open reading frames of fluorescent proteins yielded functional ZipA-RFP^{SW} and ZipA-$_{SF}$GFP^{SW} sandwich fusions, as described in the text.

Table S1. Properties of TolA derivatives in the presence of other Tol-Pal proteins and CpoB.

[a]Host: LP49 [Δ*tolA*]				
[b]GFP-fusion			[c]at constr.	[d]corrects cell chaining
plasmid	residues	TolA domains		
pNP4	-TolA$^{1-421}$	I+II+III	+	+
pDE2	-TolA$^{1-328}$	I+II	+	-
pCH512	-TolA$^{1-292}$	I+II'	+	-
pCH483	-TolA$^{1-111}$	I	+	-
pDE3	-TolA$^{1-60}$	I	+	-
pCH509	-MalF$^{2-39}$-TolA$^{47-421}$	II+III	+	-
pCH510	-MalF$^{2-39}$-TolA$^{47-328}$	II	+	-
pCH549	-MalF$^{2-39}$-TolA$^{47-292}$	II'	+	-
pCH535	-MalF$^{2-39}$-TolA$^{294-421}$	III	-	-
pCH538	-MalF$^{2-39}$-ZipA$^{86-145}$-TolA$^{294-421}$	III	+	-
pCH536	-MalF$^{2-39}$-RodZ$^{139-255}$-TolA$^{294-421}$	III	+	-

[a] Strain LP49 [Δ*tolA*] was transformed with one of the listed plasmids.

[b] Indicated are the name of the plasmid encoding the fusion under control of the *lac* regulatory region, the TolA residues encoded, and the presence of intact TolAI (TolA$^{1-42}$), II (TolA$^{48-310}$), and/or III (TolA$^{314-421}$) domains in the fusion (7). II' indicates that the encoded TolAII domain is slightly truncated at its C-terminal end. GFP is N-terminal in all cases. MalF$^{2-39}$ includes the first transmembrane helix (MalF$^{19-35}$) of the MalF protein (8). ZipA$^{86-145}$ corresponds to a portion of the cytoplasmic linker that connects the TM and C-terminal domains of the ZipA protein (3, 5). RodZ$^{139-255}$ corresponds to the periplasmic linker that connects the TM and C-terminal domains of the RodZ protein (9).

[c] Cells were grown for ~ 3.5 mass doublings to OD_{600}= 0.5-0.6 in M9-glucose with 5 μM IPTG and imaged live by fluorescence and DIC microscopy. GFP-fusions appeared evenly distributed along the periphery of cells (-), or accumulated at sites of cell constriction (+).

[d] Cells were grown for ~ 5 mass doublings to OD_{600}= 0.9-1.1 in LBNS with 0, 5, or 25 μM of IPTG and examined by phase contrast microscopy. Cells displayed a normal (+) or chaining (-) morphology under all three conditions.

Table S2. Septal recruitment of TolAIII by TolB, Pal, and CpoB.

[a]Host: LP57(λCH536) [Δ(*tolQ-cpoB*) (P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*tolA*$^{294-421}$)]										
Plasmid	P_{BAD}::	Ara	all cells				constricted cells			
			[b]at midcell (% cells)			[d]N	[c]at midcell (% cells)			[d]N
			++	+-	--		++	+-	--	
pCH528	*tolB pal cpoB*	-	69	5	26	232	86	5	9	182
pCH518	*tolB pal*	-	0	2	98	178	0	3	97	143
pCH545	*pal cpoB*	-	0	0	100	312	0	0	100	238
pCH544	*tolB pal*^{0} *cpoB*	-	3	5	92	366	5	7	88	243
pBAD33	-	-	0	1	99	157	0	1	99	101
pCH518	*tolB pal*	+	1	1	98	587	1	2	97	449
pCH545	*pal cpoB*	+	2	2	96	123	2	2	96	82
pCH544	*tolB pal*^{0} *cpoB*	+	3	9	88	133	4	12	84	103
pBAD33	-	+	0	0	100	341	0	0	100	254

[a] LP57(λCH536) cells carrying the indicated plasmid were grown for ~ 3.5 mass doublings to OD_{600}= 0.5-0.6 in M9-maltose with 37 μM IPTG and either no (-) or 0.01% (+) arabinose.

[b,c] Percentage of all cells, or of those with a visible constriction, in which the GFP-fusion accumulated strongly (++) or weakly (+-) at the constriction site, or appeared evenly distributed along the cell periphery (--).

[d] Number of cells scored.

Table S3. Effects of low concentrations of Aztreonam on accumulation of TolQ-GFP or GFP-TolA at constriction sites of cells lacking the other Tol-Pal proteins and CpoB.

[a]Host: CH244 [Δ(*tolQ-cpoB*) *zipA-rfp*^{SW}]												
Row	GFP	Aztr. (ng/ml)	[b]Length (μm)		[c]Constrictions		[d]GFP Rings		[e](co)localization (%)			[f]N
			mean	total	total	L/C (μm)	total	L/R (μm)	RwC	CwR	Depl.	
1	TolQ-	0	3.4	97	23	4.2	23	4.2	100	100	0	28
2	"	20	4.8	124	31	4.0	32	3.9	94	97	0	26
3	"	50	8.3	307	36	8.5	38	8.1	95	97	0	37
4	-TolA	0	3.1	513	95	5.4	88	5.8	98	91	0	163
5	"	20	6.2	268	52	5.2	15	17.9	100	29	10	43
6	"	50	13.8	359	34	10.6	0	>359	-	0	44	26

[a]Three identical cultures each of strain CH244 carrying pCH633 [P_{BAD}::*tolQ-gfp*] (rows 1-3) or pCH634 [P_{BAD}::*gfp-tolA*] (rows 4-6) were inoculated to a starting density of OD_{600}=0.09 in M9-glucose with 0.005% (1-3) or 0.030 % (4-6) arabinose. After growth for 100 min, aztreonam was added as indicated, and growth was continued for another 210-240 min before live-cell imaging with DIC and fluorescence optics. Cellular parameters, including length (L) and the presence and location of visible constrictions (C) and of fluorescent rings (R) formed by TolQ-GFP (1-3) or GFP-TolA (4-6) were measured and analyzed using the ObjectJ plugin (10) in Fiji (11). See also figure 9.

[b-d] Total values represent the sums of lengths, or of the number of constrictions or fluorescent rings, of all cells analyzed. These values were also used to calculate the ratios of total cell length to total number of constrictions (L/C), or to total number of green-fluorescent rings (L/R).

[e] Percentages of fluorescent rings co-localized with a cell constriction (RwC), of cell constrictions co-localized with a fluorescent ring (CwR), and of cell constrictions at which green fluorescent signal appears depleted (Depl.) are given.

[f] Number of cells measured.

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

Strain	Relevant genotype	Source or Reference
BL78	TB28, *cpoB<>aph*	This work
BL130	TB28, *zipA-rfp*^{SW} *yfeN<>frt*	This work
BL167	TB28, *ftsB*^{E56A}	(12)
BL173	TB28, *ftsB*^{E56A} *ftsN<>aph*	(12)
BW25113	Δ(*araD-araB*)567 Δ *lacZ*4787(::rrnB-3) λ^{-} *rph*-1 Δ(*rhaD-rhaB*)568 *hsdR*514	(13)
CH82	TB28, *ponA<>frt*	(12)
CH119	TB28, *yfeN<>aph*	This work
CH120	TB28, Δ*galK* (λc1857 *cro-bioA<>tetA*) *yfeN<>aph*	This work
CH121*	TB28, Δ*galK* (λc1857 *cro-bioA<>tetA*) *zipA<>*P_{EM7}*::galK yfeN<>aph*	This work
CH123	TB28, Δ*galK* (λc1857 *cro-bioA<>tetA*) *zipA-rfp*^{SW} *yfeN<>aph*	This work
CH125	TB28, *zipA*$^{1-160}$*-rfp-zipA*$^{165-328}$ (*zipA-rfp*^{SW} *yfeN<>aph*	This work
CH127	TB28, Δ*galK* (λc1857 *cro-bioA<>tetA*) *zipA-*$_{sf}$*gfp*^{SW} *yfeN<>aph*	This work
CH128	TB28, *zipA-*$_{sf}$*gfp*^{SW} *yfeN<>aph*	This work
CH235	TB28, *ftsB*^{E56A} *ftsN<>frt*	This work
CH236	TB28, *ponA<>frt* (*tolQ-cpoB*)*<>aph*	This work
CH237	TB28, *ponB<>frt* (*tolQ-cpoB*)*<>aph*	This work
CH238	TB28, *ftsB*^{E56A} (*tolQ-cpoB*)*<>aph*	This work
CH239	TB28, *ftsB*^{E56A} *ftsN<>frt* (*tolQ-cpoB*)*<>aph*	This work
CH241	TB28, *zipA-rfp*^{SW} *yfeN<>frt tolQ<>aph*	This work
CH242	TB28, *zipA-rfp*^{SW} *yfeN<>frt tolA<>aph*	This work
JW0727-1	BW25113, *tolQ<>aph*	(14)
JW0728-1	BW25113, *tolR<>aph*	(14)
JW2399-2	BW25113, *yfeN<>aph*	(14)
JW5100-1	BW25113, *tolB<>aph*	(14)
KG11	TB28, Δ*galK* (λc1857 *cro-bioA<>tetA*)	This work
LP11	TB28, *ponB<>frt*	(15)
LP49	TB28, *tolA<>aph*	This work

LP50	TB28, (*tolA-pal*)<>*aph*	This work
LP51	TB28, (*tolR-pal*)<>*aph*	This work
LP53	TB28, *tolB*<>*aph*	This work
LP54	TB28, *tolR*<>*aph*	This work
LP55	TB28, *tolQ*<>*aph*	This work
LP56	TB28, (*tolQ-tolA*)<>*aph*	This work
LP57	TB28, (*tolQ-cpoB*)<>*aph*	This work
LP58	TB28, (*tolQ-tolR*)<>*aph*	This work
MG4	TB28, (*tolQ-pal*)<>*aph*	(16)
MG5	TB28, *pal*<>*aph*	(16)
MG24	TB28 (*tolB-pal*)<>*aph*	This work
MG28	TB10, *cpoB*<>*aph*	This work
MG31	TB28, *nadA*::Tn10 *gal*490 λ*cI*857 Δ(*cro-bioA*) *cpoB*<>*aph*	This work
MG1655	*ilvG rfb*50 *rph*1	(17)
SW102	*mcrA* Δ(*mrr-hsdRMS-mcrBC*) Δ*lac*X74 *deoR endA1* *araD*139 Δ(*ara*, *leu*)7697 *rpsL recA*1 *nupG* ϕ80*dlacZ*ΔM15 Δ*galK* (λc1857 *cro-bioA*<>*tetA*) *P1*^{R}	(18)
TB10	MG1655, *nadA*::Tn10 *gal*490 λ*cI*857 Δ(*cro-bioA*)	(19)
TB28	MG1655, *lacIZYA*<>*frt*	(20)

The symbol <> denotes DNA replacement by recombineering, and *frt* a scar sequence remaining after eviction of an *aph* or *cat* cassette by FLP recombinase (13, 21). *zipA-rfp*^{SW} is short notation for *zipA*$^{1-160}$-*mcherry-zipA*$^{165-328}$, and *zipA*-$_{sf}$*gfp*^{SW} for *zipA*$^{1-160}$-$_{sf}$*gfp-zipA*$^{165-328}$. (*) Note that strain CH121 required an appropriate plasmid for survival.

Table S5. Plasmids and phages used in this study.

Construct	Relevant genotype [a]	ori	Source or Reference
Plasmids:			
pBAD33	*cat araC* P_{BAD}::	pACYC	(22)
pBL34	*bla lacI*^{q} P_{lac}::*torA*$^{1-43}$-*gfp-cwlC*$^{181-255}$	ColE1	(23)
pBL74	*att*HK022 *bla lacI*^{q} P_{lac}::*dsbA*$^{1-24}$-*rfp-cwlC*$^{181-255}$	R6K	This work
pBL75	*bla lacI*^{q} P_{lac}::*dsbA*$^{1-24}$-*rfp-cwlC*$^{181-255}$	ColE1	This work
pBL126	*bla lacI*^{q} P_{lac}::*cpoB-rfp*	ColE1	This work
pBL134	*bla lacI*^{q} P_{lac}::*cpoB*	ColE1	This work
pBL169	*bla lacI*^{q} P_{lac}::*t18-dedD*$^{1-54}$-*le*	ColE1	(15)
pBSK-GS42810	*bla* $_{sf}$*gfp*	ColE1	Epoch Life Science
pCH151	*bla lacI*^{q} P_{lac}::*zipA-gfp*	ColE1	(20)
pCH181	*bla lacI*^{q} P_{lac}::*gfp-minD minE*^{K88E}	ColE1	(24)
pCH201	*bla lacI*^{q} P_{lac}::*gfp-ftsN*	ColE1	(25)
pCH279	*bla lacI*^{q} P_{lac}::*ftsA*^{R286W}	ColE1	This work
pCH310	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-123}$	ColE1	(12)
pCH311	*bla lacI*^{q} P_{lac}::*zipA-rfp*	ColE1	(9)
pCH364	*bla lacI*^{q} P_{lac}::*t18-'lacZ*	ColE1	(9)
pCH421	*bla lacI*^{q} P_{tac}::*gfp*-malF$^{2-39}$-*rodZ*$^{139-337}$	ColE1	This work
pCH455	*bla lacI*^{q} P_{lac}::*pal*$^{1-35}$-*rfp-*	ColE1	This work
pCH480	*bla lacI*^{q} P_{lac}::*t18- tolA*$^{1-111}$-*e*	ColE1	This work
pCH483	*bla lacI*^{q} P_{lac}::*gfp-tolA*$^{1-111}$-*e*	ColE1	This work
pCH491	*bla lacI*^{q} P_{T7}::*zipA*$^{1-160}$-*rfp-zipA*$^{165-328}$-*h*	ColE1	This work
pCH494	*bla lacI*^{q} P_{T7}::*zipA*$^{1-160}$-$_{sf}$*gfp-zipA*$^{165-328}$-*h*	ColE1	This work
pCH495	*bla lacI*^{q} P_{lac}::*t18-tolA*$^{294-421}$	ColE1	This work
pCH502	*bla lacI*^{q} P_{lac}::*t18-tolA*$^{47-421}$	ColE1	This work
pCH506	*bla lacI*^{q} P_{T7}::*h-sumo-kck-tolA*$^{47-421}$	ColE1	This work
pCH508	*bla lacI*^{q} P_{lac}::*gfp-t-malF*$^{2-39}$-	ColE1	This work
pCH509	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-421}$	ColE1	This work

pCH510	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-328}$-*le*	ColE1	This work
pCH511	*bla lacI*^{q} P_{T7}::*h-sumo-kck-tolA*$^{47-292}$-*h*	ColE1	This work
pCH512	*bla lacI*^{q} P_{lac}::*gfp-tolA*$^{1-292}$-*le*	ColE1	This work
pCH515	*bla lacI*^{q} P_{T7}::*tolQ-h*	ColE1	This work
pCH516	*bla lacI*^{q} P_{lac}::*tolQ-gfp*	ColE1	This work
pCH518	*cat araC* P_{BAD}::*tolB pal*	pACYC	This work
pCH519	*bla lacI*^{q} P_{lac}::*dsbA*$^{1-24}$-*rfp-tolA*$^{294-421}$	ColE1	This work
pCH520	*bla lacI*^{q} P_{lac}::*torA*$^{1-43}$-*gfp-tolA*$^{294-421}$	ColE1	This work
pCH522	*cat araC* P_{BAD}::*tolB pal*-$_{sf}$*gfp*	pACYC	This work
pCH525	*cat araC* P_{BAD}::*pal*	pACYC	This work
pCH528	*cat araC* P_{BAD}::*tolB pal cpoB*	pACYC	This work
pCH535	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{294-421}$	ColE1	This work
pCH536	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*tolA*$^{294-421}$	ColE1	This work
pCH537	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*zipA*$^{86-145}$-*pal*$^{63-173}$	ColE1	This work
pCH538	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*zipA*$^{86-145}$-*tolA*$^{294-421}$	ColE1	This work
pCH543	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*e*	ColE1	This work
pCH544	*cat araC* P_{BAD}::*tolB pal*^{0} *ybgF*	pACYC	This work
pCH545	*cat araC* P_{BAD}::*pal cpoB*	pACYC	This work
pCH546	*cat araC* P_{BAD}::*cpoB*	pACYC	This work
pCH549	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-292}$-*le*	ColE1	This work
pCH555	*cat araC* P_{BAD}::*tolA*	pACYC	This work
pCH633	*cat araC* P_{BAD}::*tolQ-gfp*	pACYC	This work
pCH634	*cat araC* P_{BAD}::*gfp-tolA*	pACYC	This work
pCP20	*bla cat repA*^{ts} *cI*857 $P_{\lambda R}$::*flp*	pSC101^{ts}	(26)
pCS7	*bla lacI*^{q} P_{T7}::*h-sumo-kck-*	ColE1	This work
pDB297	*bla lacI*^{q} P_{lac}::*ftsA*	ColE1	(27)
pDE2	*bla lacI*^{q} P_{lac}::*gfp-tolA*$^{1-328}$-*le*	ColE1	This work
pDE3	*bla lacI*^{q} P_{lac}::*gfp-tolA*$^{1-60}$-*le*	ColE1	This work
pET21	*bla lacI*^{q} P_{T7}::	ColE1	Novagen

pFB237	*bla lacI*^{q} P_{lac}::*gfp-rodZ*	ColE1	(9)
pFB259	*bla lacI*^{q} P_{lac}::*mreB'-linker-'mreB*	ColE1	(9)
pFB260	*bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-337}$	ColE1	This work
pFB283	*bla lacI*^{q} P_{lac}::*mreB'-*$_{sf}$*gfp-'mreB*	ColE1	This work
pFB291	*bla lacI*^{q} P_{tac}::*rodZ*	ColE1	(9)
pFB310	*aadA* $P_{\lambda R}$:: *mreB'-rfp-'mreB*	pSC101	(9)
pFB324	*bla lacI*^{q} P_{tac}::*gfp-rodZ*	ColE1	This work
p*galK*	*bla* P_{em7}::*galK*	ColE1	(18)
pJE102	*cat repA*^{ts} *ftsA*^{R286W}	pSC101^{ts}	(19)
pKD13	*bla aph*	R6K	(13)
pKD46	*bla repA*^{ts} *araC* P_{BAD}::$\gamma\ \beta$ *exo*	pSC101^{ts}	(13)
pKL4	*bla lacI*^{q} P_{lac}::*t18-tolA*	ColE1	(15)
pLP7	*bla lacI*^{q} P_{lac}::*t18-ponA*	ColE1	(28)
pLP14	*bla lacI*^{q} P_{lac}::*gfp-ponA*	ColE1	(28)
pLP15	*bla lacI*^{q} P_{lac}::*t18-malF*$^{2-39}$-*rodZ*$^{139-337}$	ColE1	This work
pLP25	*bla lacI*^{q} P_{lac}::*t18-malF*$^{2-39}$-*rfp*	ColE1	This work
pLP26	*bla lacI*^{q} P_{lac}::*t18-malF*$^{2-39}$-*rodZ*$^{139-255}$-*rfp*	ColE1	This work
pLP27	*bla lacI*^{q} P_{tac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*rfp*	ColE1	This work
pLP55	*bla lacI*^{q} P_{tac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*le*	ColE1	This work
pLP60	*loxP* P_{em7}::*galK aph*	R6K	This work
pLP95	*bla lacI*^{q} P_{lac}::*dsbA*$^{1-24}$-*rfp-pal*$^{63-173}$	ColE1	This work
pLP98	*bla lacI*^{q} P_{T7}::*h-sumo-kck-pal*$^{63-173}$	ColE1	This work
pLP102	*bla lacI*^{q} P_{lac}::*pal*$^{1-35}$-*rfp-pal*$^{63-173}$	ColE1	This work
pLP112	*bla lacI*^{q} P_{lac}::*pal*$^{1-35}$-*zipA*$^{86-145}$-*pal*$^{63-173}$	ColE1	This work
pLP146	*cat araC* P_{BAD}::*tolQ-le*	pACYC	This work
pLP147	*cat araC* P_{BAD}::*tolQR*	pACYC	This work
pLP148	*cat araC* P_{BAD}::*tolQRA*	pACYC	This work
pLP225	*cat araC* P_{BAD}::*tolR*	pACYC	This work
pMG4	*bla lacI*^{q} P_{lac}::*torA*$^{1-43}$-*gfp-ftsN*$^{241-319}$	ColE1	(23)

pMG20	*cat araC* P_{BAD}::*torA*$^{1-43}$*-bfp-ftsN*$^{71-105}$*-le*	pACYC	(23)
pMG36	*bla lacI*^{q} P_{lac}::*pal-rfp*	ColE1	(16)
pMG41	*bla lacI*^{q} P_{lac}::*pal*$^{1-35}$*-rfp*	ColE1	This work
pMG45	*cat araC* P_{BAD}::*tolB*	pACYC	This work
pNP2	*bla lacI*^{q} P_{lac}::*tolQ-gfp*	ColE1	(16)
pNP4	*bla lacI*^{q} P_{lac}::*gfp-tolA*	ColE1	(16)
pTB146	*bla lacI*^{q} P_{T7}::*h-sumo-*	ColE1	(9)
pTB223	*bla lacI*^{q} P_{lac}::*pal-*$_{sf}$*gfp*	ColE1	This work
pTB225	*att*HK022 *bla lacI*^{q} P_{lac}::*zipA-*$_{sf}$*gfp*	R6K	(29)
pTU136	*att*HK022 *bla lacI*^{q} P_{lac}::*dsbA*$^{1-24}$*-rfp-*	R6K	(29)
pTU148	*att*HK022 *bla lacI*^{q} P_{lac}::*dsbA*$^{1-23}$*-rfp*	R6K	(30)
pUNI10	*loxP aph*	R6K	(31)
pYT11	*bla lacI*^{q} P_{tac}::*relA*$^{1-455}$*-e*	ColE1	(24)
Phages:			
λDE2	*imm*^{21} *bla lacI*^{q} P_{lac}::*gfp-tolA*$^{1-328}$*-le*	λ	This work
λDE3	*imm*^{21} *bla lacI*^{q} P_{lac}::*gfp-tolA*$^{1-60}$*-le*	λ	This work
λCH483	*imm*^{21} *bla lacI*^{q} P_{lac}::*gfp-tolA*$^{1-111}$*-e*	λ	This work
λCH509	*imm*^{21} *bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$*-tolA*$^{47-421}$	λ	This work
λCH510	*imm*^{21} *bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$*-tolA*$^{47-328}$*-le*	λ	This work
λCH512	*imm*^{21} *bla lacI*^{q} P_{lac}::*gfp-tolA*$^{1-292}$*-le*	λ	This work
λCH536	*imm*^{21} *bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$*-rodZ*$^{139-255}$*-tolA*$^{294-421}$	λ	This work
λCH543	*imm*^{21} *bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$*-rodZ*$^{139-255}$*-e*	λ	This work
λCH549	*imm*^{21} *bla lacI*^{q} P_{lac}::*gfp-malF*$^{2-39}$*-tolA*$^{47-292}$*-le*	λ	This work
λNP4	*imm*^{21} *bla lacI*^{q} P_{lac}::*gfp-tolA*	λ	This work
λNT5	*imm*^{21} *'bla 'lacZ*	λ	Nancy Trun, (32)

Genotypes indicate when constructs encode in-frame GFPmut2 (*gfp*), super-folder GFP ($_{sf}$*gfp*), mCherry (*rfp*), CyaA T18-domain (*t18*), SUMO peptide (*sumo*), the peptide AEKCKEL (*kck*), hexahistidine (*h*), Leu-Glu dipeptide (*le*), and/or a single Glu residue (*e*).

Strain construction:

Construction of strains involved P1-mediated transduction (transduction for short) (33), λRed-mediated recombineering (13, 18, 21), Flp-mediated eviction of antibiotic cassettes (26), and other methods specified below or elsewhere in the text.

BL78 [*cpoB*<>*aph*] was obtained by transduction of *cpoB*<>*aph* from MG31 to TB28.

BL130 [*zipA-rfp*^{SW} *yfeN*<>*frt*] was obtained by eviction of *aph* from CH125.

CH119 [*yfeN*<>*aph*] was obtained by transduction of *yfeN*<>*aph* from JW2399-2 to TB28.

CH120 [Δ*galK* (λc1857 *cro-bioA*<>*tetA*) *yfeN*<>*aph*] was obtained by transduction of *yfeN*<>*aph* from JW2399-2 to KG11.

For CH121/pCH279 [Δ*galK* (λc1857 *cro-bioA*<>*tetA*) *zipA*<>P_{EM7}::*galK yfeN*<>*aph* / P_{lac}::*ftsA*^{R286W}], the P_{EM7}::*galK* portion of pLP60 [P_{em7}::*galK aph*] was amplified with primers 5'-GCGCCAGCGCCGCAGCCTGTGCATTCAGCACCGCAACCGGCATCCTGTTGACAATTAATCATCGGC-3' and 5'-TACAGGCTCAGGCTGTGGTGCCGCTACGGGTTCTGCAGGCTGTCAGCACTGTCCTGCTCCTTG-3', and the 1316 bp fragment was recombined with the chromosome of CH120/pCH279. Recombinants were selected on M9 agar with 0.2% galactose, 1 μgr/ml D-biotin, and 25 μM IPTG at 30°C. In CH121/pCH279, a 1231 bp P_{EM7}::*galK* fragment replaces codons 161-164 of chromosomal *zipA*, and production of FtsA^{R286W} encoded on the plasmid allows these cells to survive without ZipA (34).

For CH125 [*zipA-rfp*^{SW} *yfeN*<>*aph*], the *rfp* portion of pFB310 [$P_{\lambda R}$:: *mreB'-rfp-'mreB*] was amplified with primers 5'-GCGCCAGCGCCGCAGCCTGTGCATTCAGCACCGCAACCGGCATCTGGCTCGAGCATGGTTTCCAAGG -3' and 5'- TACAGGCTCAGGCTGTGGTGCCGCTACGGGTTCTGCAGGCTGGCCCGGCGCGCCAGATTTGTACAG -3', and the 819 bp fragment was recombined with the chromosome of CH121/pCH279. Recombinants were selected on M9 agar with 0.2% glycerol, 1 μgr/ml D-biotin, and 0.2% 2-deoxy-galactose (DOG) at 30°C, yielding CH123/pCH279 [Δ*galK* (λc1857 *cro-bioA*<>*tetA*) *zipA-rfp*^{SW} *yfeN*<>*aph* / P_{lac}::*ftsA*^{R286W}]. Co-transduction of *zipA-rfp*^{SW} with *yfeN*<>*aph* from CH123/pCH279 to TB28 then yielded CH125. Strain CH125 produces a functional sandwich fusion (ZipA-RFP^{SW}) in which codons

161-164 of chromosomal *zipA* have been replaced with a 735 bp fragment encoding in-frame RFP and linker peptides.

For CH128 [*zipA*-$_{sf}$*gfp*^{SW} *yfeN*<>*aph*], a portion of pCH494 [P_{T7}::*zipA*$^{1-160}$-$_{sf}$*gfp*-*zipA*$^{165-328}$-*h*] was amplified with primers 5'- GTCAGCGGCCGCAACACCAGTACCAACCGCC -3' and 5'- ATCGCTCGAGGTCAAGCACGACACCGC -3', and the 1389 bp fragment was recombined with the chromosome of CH121/pCH279. Recombinants were selected on M9 agar with 0.2% glycerol, 1 μgr/ml D-biotin, and 0.2% 2-deoxy-galactose (DOG) at 30°C, yielding CH127/pCH279 [Δ*galK* (λc1857 *cro-bioA*<>*tetA*) *zipA*-$_{sf}$*gfp*^{SW} *yfeN*<>*aph* / P_{lac}::*ftsA*^{R286W}]. Co-transduction of *zipA*-$_{sf}$*gfp*^{SW} with *yfeN*<>*aph* from CH127/pCH279 to TB28 then yielded CH128. Strain CH128 produces a functional sandwich fusion (ZipA-$_{sf}$GFP^{SW}) in which codons 161-164 of chromosomal *zipA* have been replaced with a 738 bp fragment encoding in-frame superfolder GFP and linker peptides.

CH235 [*ftsB*^{E56A} *ftsN*<>*frt*] was obtained by eviction of *aph* from BL173.

CH236 [*ponA*<>*frt* (*tolQ-cpoB*)<>*aph*] was obtained by transduction of *tolQ-cpoB*<>*aph* from LP57/pLP148 to CH82.

CH237 [*ponB*<>*frt* (*tolQ-cpoB*)<>*aph*] was obtained by transduction of *tolQ-cpoB*<>*aph* from LP57/pLP148 to LP11.

CH238 [*ftsB*^{E56A} *tolQ-cpoB*<>*aph*] was obtained by transduction of *tolQ-cpoB*<>*aph* from LP57/pLP148 to BL167.

CH239 [*ftsB*^{E56A} *ftsN*<>*frt tolQ-cpoB*<>*aph*] was obtained by transduction of *tolQ-cpoB*<>*aph* from LP57/pLP148 to CH235.

CH241 [*zipA-rfp*^{SW} *yfeN*<>*frt tolQ*<>*aph*] was obtained by transduction of *tolQ*<>*aph* from JW0727-1/pLP148 to BL130.

CH242 [*zipA-rfp*^{SW} *yfeN*<>*frt tolA*<>*aph*] was obtained by transduction of *tolA*<>*aph* from LP49/pNP4 to BL130.

Strain KG11 [Δ*galK* (λc1857 *cro-bioA*<>*tetA*)] is a P1-sensitive recombineering strain allowing the facile use of *galK* as a (counter)selectable marker during chromosome engineering. We obtained the similar strain SW102 [Δ*galK* (λc1857 *cro-bioA*<>*tetA*}] (18) from the NCI Biological Resources Branch, but found it to be a very poor host for phage P1. As the *galK* deletion lies close to the defective λ prophage in this strain, the whole relevant region was transferred to wt strain TB28 in a single recombineering step. Thus, SW102 chromosomal DNA was introduced in electrocompetent cells of strain TB28/pKD46 and Tet^R recombinants that were also Gal^- were selected at 30°C. This yielded strain KG9 bearing plasmid pKD46. As cells harboring this defective λ prophage display temperature-sensitive growth, plasmid pKD46 could not be cured from KG9/pKD46 by growth at elevated temperature. Unlike SW102, however, the strain readily allowed propagation of P1, and transduction of the Δ*galK* (λc1857 (*cro-bioA*)<>*tetA*] region from KG9/pKD46 to TB28, again with selection for Tet^R and Gal^- at 30°C, finally yielded strain KG11, which itself also proved a good host for phage P1, as desired.

For LP49 [*tolA*<>*aph*], a 1385 bp *tolA*<>*aph* fragment was generated by amplifying the *aph* cassette of pKD13 using primers 5'-AAAGAGAGCGGGTAACAGGCGAACAGTTTTTGGAAACCGAGAATTCCGGGGATCCGTCGACC-3' and 5'-CCCTGATGCGCCATTGTTTTTAGTATTACCACTCCCGGCGTGTAGGCTGGAGCTGCTTCG-3'. The fragment was recombined with the chromosome of TB28/pKD46, and a plasmid-free segregant was selected after growth at 37°C. In LP49, *aph* replaces bp 1190-2152 of the *ybgC-cpoB* operon, counting from the start of TolQ. The last 100 codons of *tolA*, containing putative promotors for downstream genes (35), are left intact.

For LP50 [*tolA-pal*<>*aph*], a 1387 bp *tolA-pal*<>*aph* fragment was generated by amplifying the *aph* cassette of pKD13 using primers 5'-AAAGAGAGCGGGTAACAGGCGAACAGTTTTTGGAAACCGAGAATTCCGGGGATCCGTCGACC-3' and 5'-CTGCTCATGCAATTCTCTTAGTAAACCAGTACCGCACGACGGTGTAGGCTGGAGCTGCTTCG-3'. The fragment was recombined with the chromosome of TB28/pKD46, and a plasmid-free segregant was selected after growth at 37°C. In LP50, *aph* replaces bp 1190-4410 of the *ybgC-cpoB* operon, counting from the start of TolQ.

For LP51 [*tolR-pal*<>*aph*], a 1385 bp *tolR-pal*<>*aph* fragment was generated by amplifying the *aph* cassette of pKD13 using primers 5'-CAGGCGTTTACCGTTAGCGAGAGCAACAAGGGGTAAGCCAATTCCGGGGATCCGTCGACC-3' and 5'-

CTGCTCATGCAATTCTCTTAGTAAACCAGTACCGCACGACGGTGTAGGCTGGAGCTGCTTCG-3'. The fragment was recombined with the chromosome of TB28/pKD46, and a plasmid-free segregant was selected after growth at 37°C. In LP51, *aph* replaces bp 698-4410 of the *ybgC-cpoB* operon, counting from the start of TolQ.

LP53 [*tolB*<>*aph*] was obtained by transduction of *tolB*<>*aph* from JW5100-1 to TB28. In LP53, *aph* replaces bp 2591-3859 of the *ybgC-cpoB* operon, counting from the start of TolQ.

LP54 [*tolR*<>*aph*] was obtained by transduction of *tolR*<>*aph* from JW0728-1 to TB28. In LP54, *aph* replaces bp 700-1104 of the *ybgC-cpoB* operon, counting from the start of TolQ.

LP55 [*tolQ*<>*aph*] was obtained by transduction of *tolQ*<>*aph* from JW0727-1/pLP148 to TB28. In LP55, *aph* replaces bp 3-673 of the *ybgC-cpoB* operon, counting from the start of TolQ.

For LP56 [*tolQ-tolA*<>*aph*], a 1384 bp *tolQ-tolA*<>*aph* fragment was generated by amplifying the *aph* cassette of pKD13 using primers 5'-CGTGCGCTTCCCAAGTCTATTGTCGCGGAGTTTAAGCAGTAATTCCGGGGATCCGTCGACC-3' and 5'-CCCTGATGCGCCATTGTTTTTAGTATTACCACTCCCGGCGTGTAGGCTGGAGCTGCTTCG-3'. The fragment was recombined with the chromosome of TB28/pKD46, and a plasmid-free segregant was selected after growth at 37°C. In LP56, *aph* replaces bp 3-2152 of the *ybgC-cpoB* operon, counting from the start of TolQ. The last 100 codons of *tolA*, containing putative promotors for downstream genes (35), are left intact.

For LP57 [*tolQ-cpoB*<>*aph*], a 1385 bp *tolQ-cpoB*<>*aph* fragment was generated by amplifying the *aph* cassette of pKD13 using primers 5'-CGTGCGCTTCCCAAGTCTATTGTCGCGGAGTTTAAGCAGTAATTCCGGGGATCCGTCGACC-3' and 5'-CACGACACGACCAGAAATAATGCGACTTCTGGTCGTGTGTTTGTAGGCTGGAGCTGCTTCG-3'. The fragment was recombined with the chromosome of TB28/pKD46, and a plasmid-free segregant was selected after growth at 37°C. In LP57, *aph* replaces bp 3-5242 of the *ybgC-cpoB* operon, counting from the start of TolQ.

For LP58 [*tolQ-tolR*<>*aph*], a 1394 bp *tolQ-tolR*<>*aph* fragment was generated by amplifying the *aph* cassette of pKD13 using primers 5'-CGTGCGCTTCCCAAGTCTATTGTCGCGGAGTTTAAGCAGTAATTCCGGGGATCCGTCGACC-3' and 5'-

TCTCTTTCAAGCAAGGGAAACGCAGATGTTTAGATAGGCTGCGTCATTAATGTAGGCTGGAGCTGCTTCG-3'. The fragment was recombined with the chromosome of TB28/pKD46, and a plasmid-free segregant was selected after growth at 37°C. In LP58, *aph* replaces bp 3-1104 of the *ybgC-ybgF* operon, counting from the start of TolQ.

For MG24 [*tolB-pal*<>*aph*], a 1385 bp *tolB-pal*<>*aph* fragment was generated by amplifying the *aph* cassette of pKD13 using primers 5'-ATTTTAGTTTGTTAACATTCTGCTAAATTATCGTGGGCCAATTCCGGGGATCCGTCGACC-3' and 5'-CTGCTCATGCAATTCTCTTAGTAAACCAGTACCGCACGACGGTGTAGGCTGGAGCTGCTTCG-3'. The fragment was recombined with the chromosome of TB28/pKD46, and a plasmid-free segregant was selected after growth at 37°C. In MG24, *aph* replaces bp 2565-4410 of the *ybgC-ybgF* operon, counting from the start of TolQ.

For MG28 [*nadA*::Tn10 *gal*490 λ*cI*857 Δ(*cro-bioA*) *cpoB*<>*aph*], a 1385 bp *cpoB*<>*aph* fragment was generated by amplifying the *aph* cassette of pKD13 using primers 5'-ATGAGCAGTAACTTCAGACATCAACTATTGAGTCTGTCGTAATTCCGGGGATCCGTCGACC-3' and 5'-CACGACACGACCAGAAATAATGCGACTTCTGGTCGTGTGTTTGTAGGCTGGAGCTGCTTCG-3', and the fragment was recombined with the chromosome of TB10. In MG28, *aph* replaces bp 4486-5242 of the *ybgC-cpoB* operon, counting from the start of TolQ.

MG31 [*nadA*::Tn10 *gal*490 λ*cI*857 Δ(*cro-bioA*) *cpoB*<>*aph*] was obtained by transduction of *cpoB*<>*aph* from MG28 to TB28. Further analyses revealed that the additional nearby markers [*nadA*::Tn10 *gal*490 λ*cI*857 Δ(*cro-bioA*)] had unintentionally co-transduced with *cpoB*<>*aph* in this strain.

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 plasmid construction involved PCR amplification and/or mutagenesis, the nucleotide sequence of the plasmid insert was verified.

For pBL74 [P_{lac}::*dsbA*$^{1-24}$-*rfp-cwlC*$^{181-255}$], the 229 bp *BamH*I-*Hind*III fragment of pBL34 [*torA*$^{1-43}$-*gfp-cwlC*$^{181-25}$] was used to replace the 18 bp *BamH*I-*Hind*III fragment of pTU136 [P_{lac}::*dsbA*$^{1-24}$-*rfp*-].

For pBL75 [P_{lac}::*dsbA*$^{1-24}$-*rfp-cwlC*$^{181-255}$], the 1075 bp *Xba*I-*Hind*III fragment of pBL74 [P_{lac}::*dsbA*$^{1-24}$-*rfp-cwlC*$^{181-255}$] was used to replace the 1746 bp *Xba*I-*Hind*III fragment of pCH201 [P_{lac}::*gfp-ftsN*].

For pBL126 [P_{lac}::*cpoB-rfp*], a *cpoB* fragment was amplified with primers 5'-GGTCTCTAGACTGGTTTACTAAGAGAATTGC -3' and 5'-CTACCTCGAGCATCGCGTTCAGACGTTTTTGTGC-3' and the 816 bp *Xba*I-*Xho*I fragment was used to replace the 584 bp *Xba*I-*Xho*I fragment of pMG36 [P_{lac}::*pal-rfp*].

For pBL134 [P_{lac}::*cpoB*], a portion of pBL126 [P_{lac}::*cpoB-rfp*] was amplified with primers 5'-GGTCTCTAGACTGGTTTACTAAGAGAATTGC-3' and 5'- AGTAAAGCTTATGATTCGCACGACACGACCAG-3', and the 873 bp *Xba*I-*Hind*III fragment was used to replace the 1557 bp *Xba*I-*Hind*III fragment of pBL126 itself.

Plasmid pBSK-GS42810 [$_{sf}$*gfp*] was custom ordered from Epoch Life Science Inc. and was produced by inserting a 762 bp synthetic fragment encoding a codon-optimized version of super-folder GFP into the *Sma*I site of pBluescript II SK(+) (Stratagene).

For pCH279 [P_{lac}::*ftsA*^{R286W}], the 728 bp *Bgl*II-*Hind*III fragment of pDB297 [P_{lac}::*ftsA*] was replaced with that of pJE102 [*'ftsQ ftsA*^{R286W} *ftsZ'*].

For pCH421 [P_{tac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-337}$], the 1282 bp *Xba*I-*Sal*I fragment of pFB324 [P_{tac}::*gfp-rodZ*] was replaced with the 988 bp *Xba*I-*Sal*I fragment of pFB260 [P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-337}$].

For pCH455 [P_{lac}::*pal*$^{1-35}$-*rfp*-], the 7809 bp *Xho*I-*Hind*III fragment of pMG41 [P_{lac}::*pal*$^{1-35}$-*rfp*] was ligated to the 750 bp *Xho*I-*Hind*III fragment of pTU136 [P_{lac}::*dsbA*$^{1-24}$-*rfp*-].

For pCH480 [P_{lac}::*t18-tolA*$^{1-111}$-*e*], a portion of pKL4 [P_{lac}::*t18-tolA*] was amplified with primers 5'-ACGTTCGAAGTTCTCGCC -3' and 5'- CGCCCTCGAGCCGCTCTTTCTCAAGTTGCTTCAG -3', and the 458 bp *Xba*I-*Xho*I fragment of the product was used to replace the 181 bp *Xba*I-*Xho*I fragment of pBL169 [P_{lac}::*t18-dedD*$^{1-54}$-*le*].

For pCH483 [P_{lac}::*gfp-t-tolA*$^{1-111}$-*e*], the 352 bp *Sfi*I fragment of pCH480 [P_{lac}::*t18-tolA*$^{1-111}$-*e*] was used to replace the 2566 bp *Sfi*I fragment of pLP14 [P_{lac}::*gfp-ponA*].

For pCH491 [P_{T7}::*zipA*$^{1-160}$-*rfp-zipA*$^{165-328}$-*h*], the chromosomal *zipA-rfp*^{SW} allele of strain CH125 [*zipA-rfp*^{SW} *yfeN*<>*aph*] was amplified with primers 5'- ACAGAGATCCATATGATGCAGGATTTGCGTCTG -3' and 5'- AAGTGTCGACGGCGTTGGCGTCTTTGAC -3', and treated with *Nde*I and *Sal*I. The resulting 1709 bp fragment was next used to replace the 77 bp *Nde*I-*Xho*I fragment of pET21b [P_{T7}::].

For pCH494 [P_{T7}::*zipA*$^{1-160}$-$_{sf}$*gfp-zipA*$^{165-328}$-*h*], the 722 bp *Xho*I-*Asc*I fragment of pFB283 [P_{lac}::*mreB'*-$_{sf}$*gfp-'mreB*] was used to replace the 719 bp *Xho*I-*Asc*I fragment of pCH491 [P_{T7}::*zipA*$^{1-160}$-*rfp-zipA*$^{165-328}$-*h*].

For pCH495 [P_{lac}::*t18-tolA*$^{294-421}$], a portion of pKL4 [P_{lac}::*t18-tolA*] was amplified with primers 5'- CCGGGGCCATTACGGCCGATGATATTTTCGGTGAGCTAAGC -3' and 5'- GTCAGGCCGAGGCGGCCTTACGGTTTGAAGTCCAATGG -3', and the 397 bp *Sfi*I fragment of the product was used to replace the 2565 bp *Sfi*I fragment of pLP7 [P_{lac}::*t18-ponA*].

For pCH502 [P_{lac}::*t18-tolA*$^{47-421}$], a portion of pKL4 [P_{lac}::*t18-tolA*] was amplified with primers 5'- TTTAGGCCATTACGGCCGGTTCGTCCATCGACGCTGTCATGG -3' and 5'- GTCAGGCCGAGGCGGCCTTACGGTTTGAAGTCCAATGG -3', and the 1141 bp *Sfi*I fragment of the product was used to replace the 2565 bp *Sfi*I fragment of pLP7 [P_{lac}::*t18-ponA*].

For pCH506 [P_{T7}::*h-sumo-kck-tolA*$^{47-421}$], the 349 bp *Sfi*I fragment of pLP98 [P_{T7}::*h-sumo-kck-pal*$^{63-173}$] was replaced with the 1141 bp *Sfi*I fragment of pCH502 [P_{lac}::*t18-tolA*$^{47-421}$].

To create pCH508 [P_{lac}::*gfp-malF*$^{2-39}$-], oligo's 5'- GATCGGCCATTACGGCCTAAGGCCGCCTCGGCCAG -3' and 5'- TCGACTGGCCGAGGCGGCCTTAGGCCGTAATGGCC -3' were annealed and the resulting fragment with *BamH*I and *Sal*I overhangs, and containing two distinct *Sfi*I sites, was used to replace the 216 bp *BamH*I-*Sal*I fragment of pCH310 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-123}$].

For pCH509 [P_{lac}::*gfp-t-malF*$^{2-39}$-*tolA*$^{47-421}$], the 16 bp *Sfi*I fragment of pCH508 [P_{lac}::*gfp-malF*$^{2-39}$-] was replaced with the 1141 bp *Sfi*I fragment of pCH502 [P_{lac}::*t18-tolA*$^{47-421}$].

For pCH510 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-328}$-*le*], the 973 bp *Xba*I-*Sph*I fragment of pCH509 [P_{lac}::*gfp-t-malF*$^{2-39}$-*tolA*$^{47-421}$] was used to replace the 979 bp *Xba*I-*Sph*I fragment of pDE2 [P_{lac}::*gfp-tolA*$^{1-328}$-*le*].

Plasmid pCH511 [P_{T7}::*h-sumo-kck-tolA*$^{47-292}$-*h*], was obtained by deletion of the 425 *Not*I fragment from pCH506 [P_{T7}::*h-sumo-kck-tolA*$^{47-421}$].

For pCH512 [P_{lac}::*gfp-tolA*$^{1-292}$-*le*], the 676 bp *Sph*I-*Xho*I fragment of pCH511 [P_{T7}::*h-sumo-kck-tolA*$^{47-292}$-*h*] was used to replace the 784 bp *Sph*I-*Xho*I fragment of pDE2 [P_{lac}::*gfp-tolA*$^{1-328}$-*le*].

For pCH515 [P_{T7}::*tolQ-h*], a portion of pNP2 [P_{lac}::*tolQ-gfp*] was amplified with primers 5'-TGCACATATGACTGACATGAATATCCTTG-3' and 5'- CCTGCTCGAGCCCCTTGTTGCTCTCGCTA-3', and the 692 bp *Nde*I-*Xho*I fragment of the product was used to replace the 77 bp *Nde*I-*Xho*I fragment of pET21b [P_{T7}::].

For pCH516 [P_{lac}::*tolQ-gfp*], the 1026 bp *Xba*I-*Xho*I fragment of pCH151 [P_{lac}::*zipA-gfp*] was replaced with the 732 bp *Xba*I-*Xho*I fragment of pCH515.

For pCH518 [P_{BAD}::*tolB pal*], the *tolB* and *pal* genes were amplified with primers 5'-TGTGTCTAGACCATCGGTCCAGATAAGGGAGATATG -3' and 5'-GCTGAAGCTTGTCGACTGGCCGAGGCGGCCTTAGTAAACCAGTACCGCACGACGG -3', and the 1130 bp *ApaL*I-*HinD*III fragment of the product was used to replace the 557 bp *ApaL*I-*HinD*III fragment of pMG45 [P_{BAD}::*tolB*].

For pCH519 [P_{lac}::*dsbA*$^{1-24}$-*rfp-tolA*$^{294-421}$], the 349 bp *Sfi*I fragment of pLP95 [P_{lac}::*dsbA*$^{1-24}$-*rfp-pal*$^{63-173}$] was replaced with the 397 bp *Sfi*I fragment of pCH495 [P_{lac}::*t18-tolA*$^{294-421}$].

For pCH520 [P_{lac}::*torA*$^{1-43}$-*gfp-tolA*$^{294-421}$], the 1580 bp *Apa*I-*BamH*I fragment of pCH519 [P_{lac}::*dsbA*$^{1-24}$-*rfp-tolA*$^{294-421}$] was replaced with the 1562 bp *Apa*I-*BamH*I fragment of pMG4 [P_{lac}::*torA*$^{1-43}$-*gfp-ftsN*$^{241-319}$].

For pCH522 [P_{BAD}::*tolB pal*-$_{sf}$*gfp*], the 869 bp *Sbf*I-*HinD*III fragment of pTB223 [P_{lac}::*pal*-$_{sf}$*gfp*] was used to replace the 142 bp *Sbf*I-*HinD*III fragment of pCH518 [P_{BAD}::*tolB pal*].

For pCH525 [P_{BAD}::*pal*], the 465 bp *Xba*I-*Sbf*I fragment of pTB223 [P_{lac}::*pal*-$_{sf}$*gfp*] was used to replace the 1759 bp *Xba*I-*Sbf*I fragment of pCH518 [P_{BAD}::*tolB pal*].

For pCH528 [P_{BAD}::*tolB pal cpoB*], the *pal* and *cpoB* genes were amplified with primers 5'-TACGGCTAGCGGCATGTTCTTCCAACAAGAACGCC -3' and 5'-AGTAAAGCTTATGATTCGCACGACACGACCAG -3', and the 977 bp *Sbf*I-*HinD*III fragment of the product was used to replace the 869 bp *Sbf*I-*HinD*III fragment of pCH522 [P_{BAD}::*tolB pal*-$_{sf}$*gfp*].

For pCH535 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{294-421}$], a portion of pCH520 [P_{lac}::*torA*$^{1-43}$-*gfp-tolA*$^{294-421}$] was amplified with primers 5'- ACTGGATCCCTCGAGGCCATTACGGCCGATGATATTTTCGG -3' and 5'-AAAGGGGGATGTGCTGCAAG -3', and the 428 bp *BamH*I-*HinD*III fragment of the product was used to replace the 230 bp *BamH*I-*HinD*III fragment of pCH310 [P_{lac}::*gfp-malF*$^{2-39}$-*ftsN*$^{55-123}$].

For pCH536 [P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*tolA*$^{294-421}$], the 1247 bp *Xba*I-*Xho*I fragment of pLP55 [P_{tac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*le*] was used to replace the 899 bp *Xba*I-*Xho*I fragment of pCH535 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{294-421}$].

For pCH537 [P_{lac}::*gfp-malF*$^{2-39}$-*zipA*$^{86-145}$-*pal*$^{63-173}$], the 563 bp *Xho*I-*HinD*III fragment of pLP112 [P_{lac}::*pal*$^{1-35}$-*zipA*$^{86-145}$-*pal*$^{63-173}$] was used to replace the 422 bp *Xho*I-*HinD*III fragment of pCH535 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{294-421}$].

For pCH538 [P_{lac}::*gfp-malF*$^{2-39}$-*zipA*$^{86-145}$-*tolA*$^{294-421}$], the 349 bp *Sfi*I fragment of pCH537 [P_{lac}::*gfp-malF*$^{2-39}$-*zipA*$^{86-145}$-*pal*$^{63-173}$] was replaced with the 396 bp *Sfi*I fragment of pCH520 [P_{lac}::*torA*$^{1-43}$-*gfp-tolA*$^{294-421}$].

For pCH543 [P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*e*], the 1655 bp *Xba*I-*Xho*I fragment of pCH512 [P_{lac}::*gfp-tolA*$^{1-292}$-*le*] was replaced with the 1247 bp *Xba*I-*Xho*I fragment of pCH536 [P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*tolA*$^{294-421}$].

To obtain pCH544 [P_{BAD}::*tolB pal*^{0} *cpoB*], pCH528 [P_{BAD}::*tolB pal cpoB*] was linearized with *Pci*I, treated with Klenow and dNTP's, and recircularized. This caused the insertion of an extra 4 nt (CATG), the creation of a new *NsiI* site, and a frameshift at codon 53 of the *pal* ORF. Hence, the *pal*^{0} allele on this plasmid encodes a peptide consisting of the first 52 residues of Pal (31 residues

after maturation), followed by the nonsense residues HVFRRAGSSANATAAAEQHRLLRSGQVRYPF.

For pCH545 [P_{BAD}::*pal cpoB*], the 1204 bp *Afl*II-*Sph*I fragment of pCH525 [P_{BAD}::*pal*] was used to replace the 2498 bp *Afl*II-*Sph*I fragment of pCH528 [P_{BAD}::*tolB pal cpoB*].

For pCH546 [P_{BAD}::*cpoB*], the 873 bp *Xba*I-*HinD*III fragment of pBL134 [P_{lac}::*cpoB*] was used to replace the 2736 bp *Xba*I-*HinD*III fragment of pCH528 [P_{BAD}::*tolB pal cpoB*].

For pCH549 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-292}$-*le*], the 979 bp *Xba*I-*Sph*I fragment of pCH512 [P_{lac}::*gfp-tolA*$^{1-292}$-*le*] was replaced with the 973 bp *Xba*I-*Sph*I fragment of pCH509 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-421}$].

For pCH555 [P_{BAD}::*tol*A], *tolA* was amplified with primers 5'-GGCGTCTAGAGCGGGTAACAGGCGAACAGTTTTTGG-3' and 5'-CGACGTCGACTTACGGTTTGAAGTCCAATGGCGCG -3', and the 1307 bp *Xba*I-*Sal*I fragment of the product was used to replace the 1026 bp *Xba*I-*Sal*I fragment of pMG20 [P_{BAD}::*torA*$^{1-43}$-*bfp-ftsN*$^{71-105}$-*le*].

For pCH633 [P_{BAD}::*tolQ-gfp*], the 24 bp *Xba*I-*Hind*III fragment of pBAD33 [P_{BAD}::] was replaced with the 1495 bp *Xba*I-*Hind*III fragment of pCH516 [P_{lac}::*tolQ-gfp*].

For pCH634 [P_{BAD}::*gfp-tolA*], the 6 bp *Xba*I-*Sal*I fragment of pBAD33 [P_{BAD}::] was replaced with the 2045 bp *Xba*I-*Sal*I fragment of pNP4 [P_{lac}::*gfp-tolA*].

For pCS7 [P_{T7}::*h-sumo-kck*-], a portion of pTB146 [P_{T7}::*h-sumo*-] was amplified with primers 5'-GATCCCGCGAAATTAATACGACTCACTATAGGGG-3' and 5'-CCGCAAGCTTGGAGCTCTTTGCATTTTTCCGCACCACCAATCTGTTCTCTG-3', and the 393 bp *Xba*I-*Sac*I fragment of the product was used to replace the 94 bp *Xba*I-*Sac*I fragment of pET21a [P_{T7}::]. The KCK-tag consists of seven residues (AEKCKEL) that immediately follow the SUMO-tag.

For pDE2 [P_{lac}::*gfp-tolA*$^{1-328}$-*le*], a portion of pNP4 [P_{lac}::*gfp-tolA*] was amplified with primers 5'-GCTGCTGGGATTACACATGGC-3' and 5'-ATTTCTCGAGTTTAGTATTACCACTCCCGGCAGG-3', and the

1023 bp *Nhe*I-*Xho*I fragment was used to replace the 1119 bp *Nhe*I-*Xho*I fragment of pCH181 [P_{lac}::*gfp-minD minE*^{K88E}].

For pDE3 [P_{lac}::*gfp-tolA*$^{1-60}$-*le*], a portion of pNP4 [P_{lac}::*gfp-tolA*] was amplified with primers 5'- GCTGCTGGGATTACACATGGC-3' and 5'- ACTGCTCGAGTACCGCACCTGAATCAACCATGAC-3', and the 219 bp *Nhe*I-*Xho*I fragment was used to replace the 1119 bp *Nhe*I-*Xho*I fragment of pCH181 [P_{lac}::*gfp-minD minE*^{K88E}].

For pFB260 [P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-337}$], a portion of pFB237 [P_{lac}::*gfp-rodZ*] was amplified with primers 5'- CGGGATCCCAGCAGGAAGAGATCACCACTATGGCCG-3' and 5'- CCGCTCGAGTTACTGCGCCGGTGATTGTTCGGC-3', and the 606 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 pFB283 [P_{lac}::*mreB'-*$_{sf}$*gfp-'mreB*], $_{sf}$*gfp* was amplified from pTB225 [P_{lac}::*zipA-*$_{sf}$*gfp*] with primers 5'- GCCGCTCGAGCATGTCTAAAGGTGAAGAACTGTTCACCGG -3' and 5'- CCCGGCGCGCCTTTGTAGAGCTCATCCATGCCG -3', and treated with *Xho*I and *Asc*I. The resulting 722 bp fragment was next used to replace the 17 bp *Xho*I-*Asc*I fragment of pFB259 [P_{lac}::*mreB'-linker-'mreB*].

For pFB324 [P_{tac}::*gfp-rodZ*], the 1076 bp *Xba*I-*Hind*III fragment of pFB291 [P_{tac}::*rodZ*] was replaced with the 1793 bp *Xba*I-*Hind*III fragment of pFB237 [P_{lac}::*gfp-rodZ*].

For pLP15 [P_{lac}::*t18-malF*$^{2-39}$-*rodZ*$^{139-337}$], a portion of pCH364 [P_{lac}::*t18-'lacZ*] was amplified with primers 5'- CCGTGAATTCTGCCGCCAGCGAGGCCACGGGC-3' and 5'- TGCGGCTAGCGCGTTCCACTGCGCCCAGCGACGG-3', and the 532 bp *EcoR*I-*Nhe*I fragment was used to replace the 816 bp *EcoR*I-*Nhe*I fragment of pFB260 [P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-337}$].

For pLP25 [P_{lac}::*t18-malF*$^{2-39}$-*rfp*], the 615 bp *BamH*I-*Sal*I fragment of pLP15 [P_{lac}::*t18-malF*$^{2-39}$-*rodZ*$^{139-337}$] was replaced with the 764 bp *BamH*I-*Sal*I fragment of pTU148 [P_{lac}::*dsbA*$^{1-23}$-*rfp*].

For pLP26 [P_{lac}::*t18-malF*$^{2-39}$-*rodZ*$^{139-255}$-*rfp*], a portion of pLP15 [P_{lac}::*t18-malF*$^{2-39}$-*rodZ*$^{139-337}$] was amplified with primers 5'- CGGGATCCCAGCAGGAAGAGATCACCACTATGGCCG-3' and 5'- GCACCTCGAGCGCATTCGGATCAGCCACCGGCGTGG-3', and the 353 bp *BamH*I-*Xho*I fragment was used to replace the 12 bp *BamH*I-*Xho*I fragment of pLP25 [P_{lac}::*t18-malF*$^{2-39}$-*rfp*].

For pLP27 [P_{tac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*rfp*], the 1121 bp *BamH*I-*Hind*III fragment of pLP26 [P_{lac}::*t18-malF*$^{2-39}$-*rodZ*$^{139-255}$-*rfp*] was used to replace the 606 bp *BamH*I-*Hind*III fragment of pCH421 [P_{tac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-337}$].

For pLP55 [P_{tac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*le*], the 1401 bp *Xba*I-*Xho*I fragment of pYT11 [P_{tac}::*relA*$^{1-455}$-*e*] was replaced with the 1247 bp *Xba*I-*Xho*I fragment of pLP27 [P_{tac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*rfp*].

For pLP60 [P_{em7}::*galK*], the 1248 bp *EcoR*I-*Xba*I fragment of p*galK* was used to replace the 298 bp *EcoR*I-*Xba*I fragment of pUNI10.

For pLP95 [P_{lac}::*dsbA*$^{1-24}$-*rfp-pal*$^{63-173}$], a portion of pMG36 [P_{lac}::*pal-rfp*] was amplified with primers 5'- GGCTGGATCCTCGGCCATTACGGCCCAACAGCTGCAGCAGAACAACATCG-3' and 5'-GCTGAAGCTTGTCGACTGGCCGAGGCGGCCTTAGTAAACCAGTACCGCACGACGG-3', and the 377 bp *BamH*I-*HinD*III fragment was used to replace the 229 bp *BamH*I-*HinD*III fragment of pBL75 [P_{lac}::*dsbA*$^{1-24}$-*rfp-cwlC*$^{181-255}$].

For pLP98 [P_{T7}::*h-sumo-kck-pal*$^{63-173}$], a portion of pLP95 [P_{lac}::*dsbA*$^{1-24}$-*rfp-pal*$^{63-173}$] was amplified with primers 5'- GGCTGAGCTCTCGGCCATTACGGCCCAACAG-3' and 5'-GCTGAAGCTTGTCGACTGGCCGAGGCGGCCTTAGTAAACCAGTACCGCACGACGG -3', and the 373 bp *Sac*I-*HinD*III fragment was used to replace the 9 bp *Sac*I-*HinD*III fragment of pCS7 [P_{T7}::*h-sumo-kck*-].

For pLP102 [P_{lac}::*pal*$^{1-35}$-*rfp-pal*$^{63-173}$], the 978 bp *EcoR*I-*BamH*I fragment of pCH455 [P_{lac}::*pal*$^{1-35}$-*rfp*-] was used to replace the 922 bp *EcoR*I-*BamH*I fragment of pLP95 [P_{lac}::*dsbA*$^{1-24}$-*rfp-pal*$^{63-173}$].

To create pLP112 [P_{lac}::*pal*$^{1-35}$-*zipA*$^{86-145}$-*pal*$^{63-173}$], a portion of pCH151 [P_{lac}::*zipA-gfp*] was amplified with primers 5'- CCGCTCGAGCCGTCGCCGCAACACCAG -3' and 5'-CGGGATCCCTGTGGCGAAACTGGCTGCTGC -3', and the 186 bp *Xho*I-*BamH*I fragment was used to replace the 732 bp *Xho*I-*BamH*I fragment of pLP102 [P_{lac}::*pal*$^{1-35}$-*rfp-pal*$^{63-173}$].

For pLP146 [P_{BAD}::*tolQ-le*], the 744 bp *Xba*I-*Xho*I fragment of pNP2 [P_{lac}::*tolQ-gfp*] was used to replace the 1017 bp *Xba*I-*Xho*I fragment of pMG20 [P_{BAD}::*torA*$^{1-43}$-*bfp-ftsN*$^{71-105}$-*le*].

For pLP147 [P_{BAD}::*tolQR*], a *tolQR* fragment was amplified with primers 5'-CCTGTCTAGAAATGAAGCCTCGTGCGCTTCC -3' and 5'-CGATGTCGACTTAGATAGGCTGCGTCATTAAACCAAC -3', and the 1178 bp *Xba*I-*Sal*I fragment was used to replace the 1026 bp *Xba*I-*Sal*I fragment of pMG20 [P_{BAD}::*torA*$^{1-43}$-*bfp-ftsN*$^{71-105}$-*le*].

For pLP148 [P_{BAD}::*tolQRA*], a *tolQRA* fragment was amplified with primers 5'-CCTGTCTAGAAATGAAGCCTCGTGCGCTTCC -3' and 5'-CGACGTCGACTTACGGTTTGAAGTCCAATGGCGCG -3', and the 2509 bp *Xba*I-*Sal*I fragment was used to replace the 1026 bp *Xba*I-*Sal*I fragment of pMG20 [P_{BAD}::*torA*$^{1-43}$-*bfp-ftsN*$^{71-105}$-*le*].

For pLP225 [P_{BAD}::*tolR*], a portion of pLP148 [P_{BAD}::*tolQRA*] was amplified with primers 5'-TTGTCTAGAGTTTACCGCGATTCTGCAC-3' and 5'- CGATGTCGACTTAGATAGGCTGCGTCATTAAACCAAC-3', and the 496 bp *Xba*I-*Sal*I fragment was used to replace the 1026 bp *Xba*I-*Sal*I fragment of pMG20 [P_{BAD}::*torA*$^{1-43}$-*bfp-ftsN*$^{71-105}$-*le*].

For pMG41 [P_{lac}::*pal*$^{1-35}$-*rfp*], a portion of pMG36 [P_{lac}::*pal-rfp*] was amplified with primers 5'-TCCCTCTAGACCCTGCCTGGTCGCCGTATCTGTG-3' and 5'-TCAGCTCGAGGCCTTCGCTGCCGTCATTGCTGGC-3', and the 170 bp *Xba*I-*Xho*I fragment was used to replace the 1026 bp *Xba*I-*Xho*I fragment of pCH311 [P_{lac}::*zipA-rfp*].

For pMG45 [P_{BAD}::*tolB*], *tolB* was amplified with primers 5'-TGTGTCTAGACCATCGGTCCAGATAAGGGAGATATG-3' and 5'-GTCGAAGCTTTTATCACAGATACGGCGACCAGG-3', and the 1328 bp *Xba*I-*Hind*III fragment was used to replace the 24 bp *Xba*I-*Hind*III fragment of pBAD33 [P_{BAD}::].

For pTB223 [P_{lac}::*pal*-$_{sf}$*gfp*], the 741 bp *Xho*I-HindIII fragment of pMG36 [P_{lac}::*pal-rfp*] was replaced with the 750 bp *Xho*I-HindIII fragment of pBSK-GS42810 [$_{sf}$*gfp*].

Phage construction:

Lysogenic phages λDE2 [P_{lac}::*gfp-tolA*$^{1-328}$-*le*], λDE3 [P_{lac}::*gfp-tolA*$^{1-60}$-*le*], λCH483 [P_{lac}::*gfp-tolA*$^{1-111}$-*e*], λCH509 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-421}$], λCH510 [P_{lac}::*gfp-malF*$^{2-39}$-*tolA*$^{47-328}$-*le*], λCH512 [P_{lac}::*gfp-tolA*$^{1-292}$-*le*], λCH536 [P_{lac}::*gfp-malF*$^{2-39}$-*rodZ*$^{139-255}$-*tolA*$^{294-421}$], λCH543 [P_{lac}::*gfp-malF*$^{2-39}$-

rodZ$^{139\text{-}255}$-*e*], λCH549 [P_{lac}::*gfp-malF*$^{2\text{-}39}$-*tolA*$^{47\text{-}292}$-*le*], and λNP4 [P_{lac}::*gfp-tolA*] were obtained by crossing the relevant inserts of pDE2, pDE3, pCH483, pCH509, pCH510, pCH512, pCH536, pCH543, pCH549, and pNP4, respectively, onto λNT5 [*imm*^{21} *'bla 'lacZ*] (32).

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