A role for the FtsQLB complex in cytokinetic ring activation revealed by an *ftsL* allele that accelerates division

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FIGURE LEGENDS

Fig. S1 FtsL* phenotype not due to protein stabilization. (**A**) Overnight cultures of TB28 [*WT*] or MT10 [*ftsL**] cells were diluted in fresh LB or M9 maltose medium and grown at 37°C until OD_{600} ~ 0.5-0.7 when the cells were harvested for whole-cell extract preparation. Proteins in the resulting extracts were separated by SDS-PAGE, transferred to PVDF, and FtsL was detected with anti-FtsL antisera. (**B**) TB28 or MT10 cells with expressing either the empty vector (pBAD33), FtsL(WT) (pMT27) or FtsL(E88K) (pMT28) were grown overnight in LB broth at 30°C. Following normalization for cell density (OD_{600} = 2), the resulting cultures were serially diluted (10^{-1} to 10^{-6}), and 5 μl of each dilution was spotted on the indicated medium. Plates were incubated overnight at the indicated temperature and photographed.

Fig. S2. Localization of GFP-FtsL*. Overnight cultures of MT10 [*ftsL**] containing the integrated GFP fusion constructs (**A, B**) *att*HKMT35 [P_{lac}*::gfp-ftsL(wt)*] or (**C, D**) *att*HKMT36 [P_{lac}*::gfp-ftsL**] were diluted in 0.5xLB-0N broth and grown to mid-log at 30°C. They were then diluted to a starting OD_{600} ~ 0.02 in fresh 0.5xLB-0N supplemented with 25 μM IPTG and grown at 42°C to an OD_{600} of 0.3 - 0.5 before they were visualized on 2% agarose pads with DIC (A, C) and GFP (B, D) optics. Bar = 4µm.

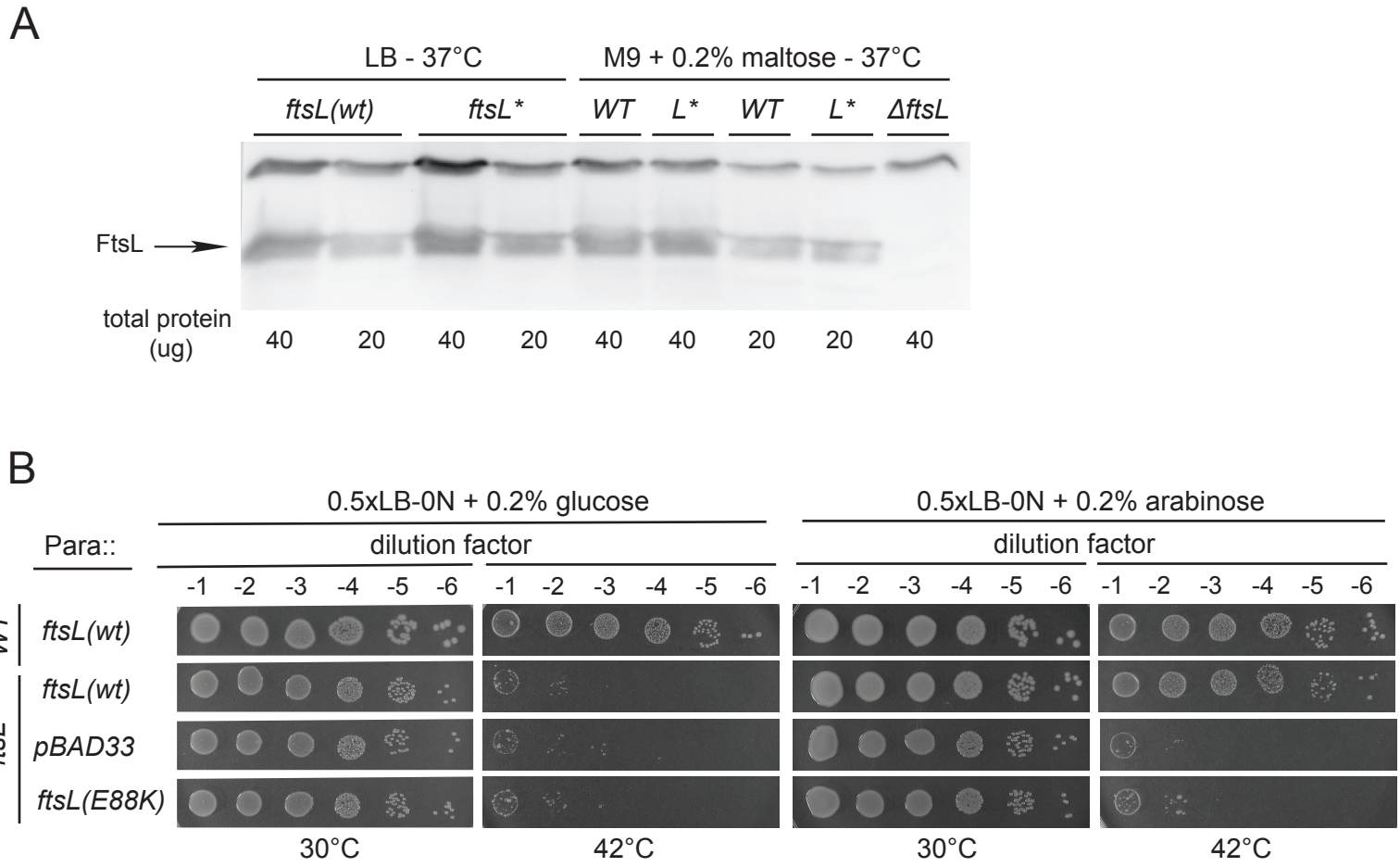
A
LB - 37°C
M9 + 0.2% maltose - 37°C
ftsL(wt)
ftsL*
WT
L*
WT
L*
ΔftsL
FtsL
total protein (ug)
40
20
40
20
40
40
20
20
40
B
0.5xLB-0N + 0.2% glucose
0.5xLB-0N + 0.2% arabinose
Para::
dilution factor
dilution factor
-1 -2 -3 -4 -5 -6 -1 -2 -3 -4 -5 -6
-1 -2 -3 -4 -5 -6 -1 -2 -3 -4 -5 -6
WT
ftsL*
ftsL(wt)
ftsL(wt)
pBAD33
ftsL(E88K)
30°C
42°C
30°C
42°C

Figure S1

MT10 [*ftsL**]

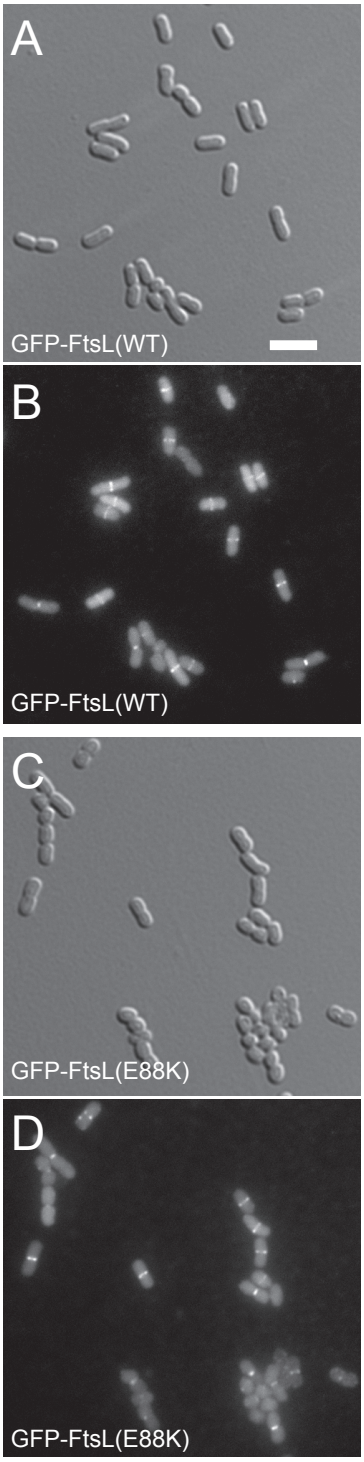

Figure S2

Plasmid Construction

Plasmids used in this study are listed below. PCR was performed using KOD polymerase (Novagen) for cloning purposes and *Taq* DNA polymerase (NEB) for diagnostic purposes, both according to the manufacturer's instructions. Unless otherwise indicated, MG1655 chromosomal DNA was used as the template. Restriction sites for use in plasmid constructions are bold, italicized and underlined in the primer sequences given below. Plasmid DNA and PCR fragments were purified using the Zyppy plasmid miniprep kit (Zymo Research) or the Qiaquick PCR purification kit (Qiagen), respectively.

pMM61

The plasmid pMM61 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::gfp-slmA*] was constructed as follows. The primers 5'-GCAT***AGATCT***GCACATTTCCCCGAAAAGTG-3' and 5'-GACGAAAGTGATTGCGCCTACC-3' were used to amplify *P$_{lac}$::gfp-slmA* from pTB99 [*att*HK022 *bla lacI*^{q} P$_{lac}$*::gfp-slmA*] (Bernhardt and de Boer, 2005) . The resulting PCR product was purified, digested with BglII and HindIII and ligated with similarly digested pDY75 [*att*HK022 *tetA tetR araC* P$_{ara}$*::*^{ss}*dsbA-amiB*] (Peters *et al.*, 2011).

pMT15

The plasmid pMT15 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::ftsL*] was constructed as follows. The primers 5'-GTCA***TCTAGA***CTGCGTATTGCAGAGAGGACGAATGCATG-3' and 5'-GTCA***AAGCTT***GCGTCGCGTTTATCCTTATTTTTGC-3' were used to amplify *ftsL* from genomic DNA. The resulting PCR produced was purified, digested with XbaI and HindIII

and ligated with similarly digested pNP20 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::nlpD-mCherry*] (Peters *et al.*, 2011).

pMT16

The plasmid pMT16 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::ftsL(E88K)*] was constructed as follows. The primers 5'-GTCA***TCTAGA***CTGCGTATTGCAGAGAGGACGAATGCATG-3'/ 5'-GAGCGCATT**CTT**TTCAAGGATCAGGTTGCGC-3' and 5'-GATCCTTGAA**AAG**AATGCGCTCGGCGACCATAG-3'/5'-GTCA***AAGCTT***GCGTCGCGTTTATCCTTATTTTTGC-3' were used in overlap extension PCR to amplify the *ftsL* gene from genomic DNA and to perform site directed mutagenesis to create the E88K mutation in the *ftsL* gene. The resulting PCR produce was purified, digested with XbaI and HindIII and ligated with similarly digested pNP20 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::nlpD-mCherry*].

pMT17

The plasmid pMT17 [*cat mobRP4 sacB mraZ-mraW-ftsL(E88K)-ftsI*] was constructed as follows. The primers 5'-GTCA***GAGCTC***GAGTCATTTTAAAACGGTGATGACGATGAGG-3'/5'-GAGCGCATT**CTT**TTCAAGGATCAGGTTGCGC-3' and 5'-GATCCTTGAA**AAG**AATGCGCTCGGCGACCATAG-3'/5'-GTCA***TCTAGA***GCAAATTACGATCTGCCACCTGTCC-3' were used in overlap extension PCR to amplify a 4kb region including the *ftsL* gene from genomic DNA and to perform site directed mutagenesis to create the E88K mutation in the *ftsL* gene. The

resulting PCR produce was purified, digested with SacI and XbaI and ligated with similarly digested pDS132 [*cat mobRP4 sacB*] (Philippe *et al.*, 2004).

pMT20

The plasmid pMT20 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::nlpD*] was constructed as follows. The *nlpD* containing XbaI/HindIII fragment of pTD23 [*att*HK022 *bla lacI*^{q} P$_{lac}$*::nlpD*] was used to replace the corresponding fragment of pNP20 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::nlpD-mCherry*].

pMT23

The plasmid pMT23 [t*etA tetR lacI*^{q} P$_{lac}$*::nlpD*] was constructed as follows. The primers 5'-GCT***GCGGCCGC***TTTAGATTGATTTAAAACTTC-3' and 5'-GCT***AAGCTT****AAAAAAACCCGCCCCGGCGGGTTTTTTTA*ATCACCGATACGCGAGCGAAC were used to amplify the ColE1 origin from pMLB1113 [*bla lacI*^{q} P$_{lac}$*::lacZ*] (de Boer *et al.*, 1989) and include the tL17 transcription termination sequence. The resulting PCR product was purified, digested with NotI and HindIII and ligated with similarly digested pMT20 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::nlpD*].

pMT27

The plasmid pMT27 [*cat araC* P$_{ara}$*::ftsL*) was constructed as follows. The *ftsL* containing XbaI/HindIII fragment of pMT15 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::ftsL*] was used to replace the corresponding fragment of pDY31 [*cat araC* P$_{ara}$*::amiB*](Yang *et al.*, 2012).

pMT28

The plasmid pMT28 [*cat araC* P_{ara}*::ftsL(E88K)*) was constructed as follows. The *ftsL(E88K)* containing XbaI/HindIII fragment of pMT16 [*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::ftsL(E88K)*] was used to replace the corresponding fragment of pDY31 [*cat araC* P_{ara}*::amiB*](Yang *et al.*, 2012).

pMT35

The plasmid pMT35 [*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::gfp-ftsL*] was constructed as follows. The primers 5'-GTCA***GGATCC***ATCAGCAGAGTGACAGAAGCTC-3' and 5'-GTCA***AAGCTT***GCGTCGCGTTTATCCTTATTTTTGC-3' were used to amplify *ftsL* from pMT15 [*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::ftsL*]. The resulting PCR produce was purified, digested with BamHI and HindIII and ligated with similarly digested pMM61 [*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::gfp-slmA*].

pMT36

The plasmid pMT36 [*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::gfp-ftsL(E88K)*] was constructed as follows. The primers 5'-GTCA***GGATCC***ATCAGCAGAGTGACAGAAGCTC-3' and 5'-GTCA***AAGCTT***GCGTCGCGTTTATCCTTATTTTTGC-3' were used to amplify *ftsL(E88K)* from pMT16 [*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::ftsL(E88K)*]. The resulting PCR produce was purified, digested with BamHI and HindIII and ligated with similarly digested pMM61 [*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::gfp-slmA*].

pMT74

The plasmid pMT74 [*tetA tetR lacI*^{q} P$_{lac}$*::sulA*] was constructed as follows. The primers 5'-CGTA***CATATG***TACACTTCAGGCTATGC-3' and 5'-CGTA***AAGCTT***AATGATACAAATTAGAGTG-3' were used to amplify *sulA* from genomic DNA. The resulting PCR product was purified, digested with NdeI and HindIII and ligated downstream of the *tac* promoter in a derivative of pTB85 (Bernhardt and de Boer, 2005) that also contained the *phi10* ribosome binding site, generating pHC705 [*bla lacI*^{q} P$_{tac}$*::sulA*]. The *sulA* containing XbaI/HindIII fragment of pHC705 was then used to replace the corresponding fragment of pMT23 [t*etA tetR lacI*^{q} P$_{lac}$*::nlpD*] to make pMT74.

pMT117

The plasmid pMT117 [*tetA tetR lacI*^{q} P$_{lac}$*::ftsK*] was constructed as follows. The primers 5'-GGGGAATTGTGAGCGGATAACAATTCCCC***TCTAGA***GAAAAGCCTGTAACCTGGAGAGCC-3' and 5'-GTCA***GTCGAC***TTAGTCAAACGGCGGTGGGG-3' were used to amplify *ftsK* from pTB66. The resulting PCR produce was purified, digested with XbaI and SalI and ligated with similarly digested pNP20 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::nlpD-mCherry*].

pNP102

The plasmid pNP102 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::ftsN*] was constructed as follows. The primers 5'-CAGC***TCTAGA***CATGGCGGGCTGACGA-3' and 5'-CAGC***AAGCTT***TCATCAACCCCCGGCGGCGAGCC-3' were used to amplify *ftsN* from genomic DNA. The resulting PCR produce was purified, digested with XbaI and HindIII

and ligated with similarly digested pMM60 [*att*HK022 *tetA tetR lacI*^{q} P$_{lac}$*::ycfM-sfgfp*] (Peters *et al.*, 2011).

pTB66

The plasmid pTB66 [*bla lacI*^{q} P$_{lac}$*::ftsK*] was constructed as follows. The *ftsK* gene was amplified from genomic DNA, digested with EcoRI and HindIII and ligated with similarly digested pRC7 (de Boer *et al.*, 1989).

pTD17

The plasmid pTD17 [*att*HK022 *bla lacI*^{q} *gfp*] was constructed as follows. The primers 5'-TTAGGCACCCCAGGCTTTACAC-3' and 5'-GTCA***GTCGAC***TTATTTGTATAGTTCATCCATGCCATGTGTAATC-3' were used to amplify *gfp* from pTB183 [*att*HK022 *bla lacI*^{q} P$_{lac}$*::gfp-zapA*] (Bendezú and de Boer, 2008). The resulting PCR product was purified, digested with XbaI and SalI and ligated into similarly digested pTD16 [*att*HK022 *bla lacI*^{q}] (Uehara *et al.*, 2009).

pTD19

The plasmid pTD19 [*att*HK022 *bla lacI*^{q} P$_{lac}$*::gfp*] was constructed as follows. The primers 5'-AATTCAATTGTGAGCGCTCACAATTCAAGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCAT-3' and 5'-CTAGATGAAATTGTTATCCGCTCACAATTCCACACATTATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGGGTGCTTGAATTGTGAGCGCTCACAATTG-3' were annealed

together to create a fragment containing the *Plac(UV5)* promoter, which was then ligated into pTD17 [*att*HK022 *bla lacI*^{q} *gfp*] digested with EcoRI and XbaI.

pTD22

The plasmid pTD22 [*att*HK022 *bla lacI*^{q} P$_{lac}$*::zipA-gfp*] was constructed as follows. The *zipA-gfp* containing XbaI/HindIII fragment of pTB98 [*att*HK022 *bla lacI*^{q} P$_{lac}$*::zipA-gfp*] (Bendezú *et al.*, 2009) was used to replace the corresponding fragment of pTD19 [*att*HK022 *bla lacI*^{q} P$_{lac}$*::gfp*].

pTD23

The plasmid pTD23 [*att*HK022 *bla lacI*^{q} P$_{lac}$*::nlpD*] was constructed as follows. The primers 5'-GTACCATATGAGCGCGGGAAGCCCA-3' and 5'-GTCAAAGCTTCCGCCGATTTATCGCTGC-3' were used to amplify *nlpD* from genomic DNA. The resulting PCR product was purified, digested with NdeI and HindIII and ligated into similarly digested pTD22 [*att*HK022 *bla lacI*^{q} P$_{lac}$*::zipA-gfp*].

Table S1. Strains used in this study.

Strain	Genotype[a]	Source/Reference[b]
DH5α	*F– hsdR17 deoR recA1 endA1 phoA supE44 thi-1 gyrA96 relA1 Δ(lacZYA-argF)U169 ϕ80dlacZΔM15*	Gibco BRL
MG1655	*rph-1 ilvG rfb-50*	(Guyer *et al.*, 1981)
TB28	MG1655 *ΔlacIZYA*::*frt*	(Bernhardt and de Boer, 2004)
CH2	*dadR trpE trpA tna ftsA(0) recA::Tn10*	(Hale and de Boer, 1999)
CH34	TB28 *ΔftsN::Kan^R*	(Gerding *et al.*, 2009)
CH43	TB28 *leu::Tn10*	from de Boer lab
HC261	TB28 *zapA*-GFP *Cm^R*	(Peters *et al.*, 2011)
MT10	TB28 *ftsL(E88K)*	Allelic replacement
MT13	TB28 *leu::Tn10 ftsL(E88K)*	P1(CH43) x MT10
MT23	TB28 *ftsL(E88K) proQ::MarTn*	This Study (Isolated Suppressor)
MT24	TB28 *ftsL(E88K) ftsP::MarTn*	This Study (Isolated Suppressor)
MT28	TB28 *ftsL(E88K) ftsN::Tn5-3*	This Study (Isolated Suppressor)
MT29	TB28 *ftsL(E88K) ddlB::Tn5-2*	This Study (Isolated Suppressor)
MT30	TB28 *ftsL(E88K) ftsK::Tn5-1*	This Study (Isolated Suppressor)
MT31	TB28 *ftsL(E88K) ftsN::Tn5-1*	This Study (Isolated Suppressor)
MT32	TB28 *ftsL(E88K) murD::Tn5*	This Study (Isolated Suppressor)
MT35	TB28 *ftsL(E88K) dedD::Tn5*	This Study (Isolated Suppressor)

Strain	Genotype[a]	Source/Reference[b]
MT38	TB28 *ftsK::Tn5-1*	P1(MT30) x TB28
MT39	TB28 *ftsN::Tn5-1*	P1(MT31) x TB28
MT70	TB28 *ΔftsN::Kan^R*	P1(CH34) x TB28
MT71	TB28 *ftsL(E88K) ΔftsN::Kan^R*	P1(CH34) x MT10
MT75	TB28 *ΔftsK::Kan^R*	λRed
MT76	TB28 *ftsL(E88K) ΔftsK::Kan^R*	P1(MT75) x MT10
MT78	TB28 *zapA*-GFP *frt leu::Tn10 ftsA(0)*	P1(MT13) x NP180
MT79	TB28 *zapA*-GFP *frt leu::Tn10 ftsL(E88K) ftsA(0)*	P1(MT13) x NP180
MT90	TB28 *ftsL(E88K) zapA*-GFP *Cm^R*	P1 (HC261) x MT10
MT102	TB28 *ftsL(E88K) zapA-mCherry Cm^R*	P1 (TU211) x MT10
NP1	TB28 *zapA*-GFP *frt*	(Peters *et al.*, 2011)
NP179	TB28 *zapA*-GFP *frt leu::Tn10*	P1(CH43) x NP1
NP180	TB28 *zapA*-GFP *frt ftsA(0)*	P1(CH2) x NP179
TU211	TB28 *zapA-mCherry Cm^R*	Peters et al. 2011

[a] The Kan^R and Cm^R cassettes are flanked by *frt* sites for removal by FLP recombinase. An *frt* scar remains following removal of the cassette using FLP expressed from pCP20.

[b] Strain constructions by P1 transduction are described using the shorthand: P1(donor) x recipient. Transductants were selected on Kan, Tet, Cm or minimal medium with no casamino acids plates where appropriate. Allelic replacement and λRec indicates strains were constructed by allelic exchange and recombineering respectively (see Experimental Procedures for details).

Table S2. Plasmids used in this study.

Plasmid	Genotype[a]	Origin	Source or Reference
pDB355	*aadA cI857(ts)* $P_{\lambda R}$*::ftsA*	pSC101	(Hale and de Boer, 1999)
pDS132	*cat mobRP4 sacB*	R6K	(Philippe *et al.*, 2004)
pDY31	*cat araC* P_{ara}*::amiB*	pACYC/p15A	(Yang *et al.*, 2012)
pKD46	*bla repA101(ts) araC* P_{ara}*::γ-β-exo*	pSC101	(Datsenko and Wanner, 2000)
pMG20	*cat araC* P_{ara}*::*^{ss}*torA-bfp-ftsN(71-105)*	pACYC/p15A	(Gerding *et al.*, 2009)
pMM61	*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::gfp-slmA*	R6K	This study
pMT15	*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::ftsL*	R6K	This study
pMT16	*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::ftsL(E88K)*	R6K	This study
pMT17	*cat mobRP4 sacB mraZ-mraW-ftsL(E88K)-ftsI*	R6K	This study
pMT23	*tetA tetR lacI*^{q} P_{lac}*::nlpD*	R6K	This study
pMT27	*cat araC* P_{ara}*::ftsL*	pACYC/p15A	This study
pMT28	*cat araC* P_{ara}*::ftsL(E88K)*	pACYC/p15A	This study
pMT35	*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::gfp-ftsL*	R6K	This study
pMT36	*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::gfp-ftsL(E88K)*	R6K	This study
pMT74	*tetA tetR lacI*^{q} P_{lac}*::sulA*	pBR/ColE1	This study
pMT117	*tetA tetR lacI*^{q} P_{lac}*::ftsK*	R6K	This study
pNP20	*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::nlpD-mCherry*	R6K	peters 2011
pNP102	*att*HK022 *tetA tetR lacI*^{q} P_{lac}*::ftsN*	R6K	This study
pTB102	*cat repA(ts) cI857(ts)* $P_{\lambda R}$*::int(HK022)*	pSC101	(Bernhardt and de Boer, 2005)

[a] $P_{\lambda R}$, P_{lac}, and P_{ara} indicate the phage λR, lactose, and arabinose promoters, respectively.

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