antibiotics

Molecular Characterization of the *Burkholderia cenocepacia dcw* Operon and FtsZ Interactors as New Targets for Novel Antimicrobial Design

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Supplementary figures

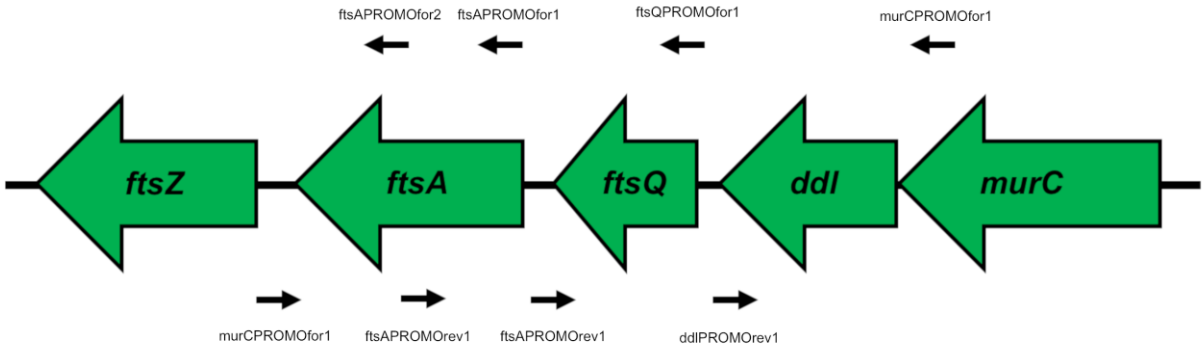

Figure S1. Representation of the distal genes of the *dcw* cluster. The black arrows represent the primers used for the amplification of the fragments tested for their promoter activity.

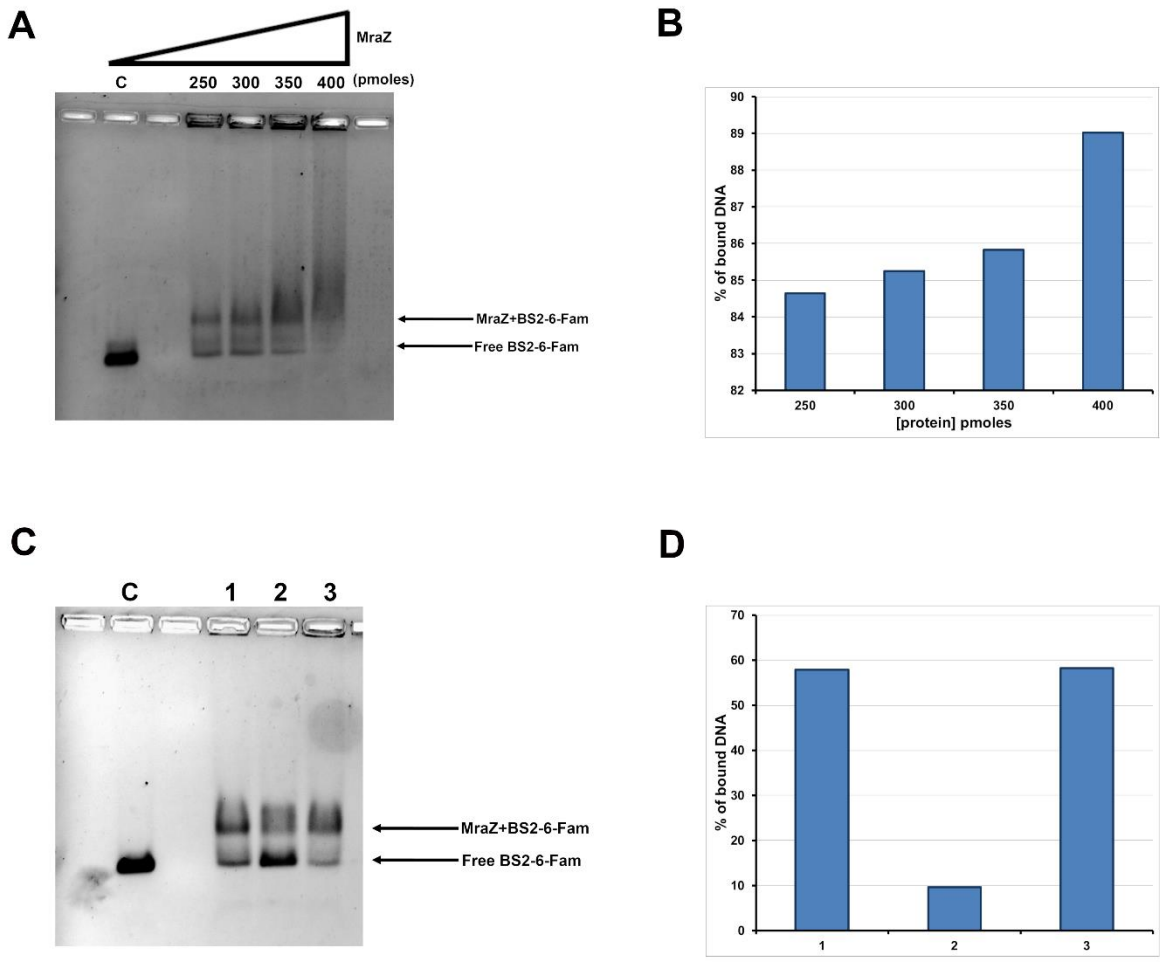

Figure S2. Electrophoretic mobility shift assay of the MraZ protein. (A) The gel represents the band shift using a constant BS2-6-Fam DNA amount (0.34 pmoles) and increasing quantities of MraZ. (B) The bars indicate the relative quantification of the percentage of the bound DNA obtained by densitometry analysis in function of the protein concentration. (C) Demonstration of the specificity of the binding of MraZ to the fragment BS2, using a fixed amount of protein (300 pmoles) and BS2-6-Fam (0.34 pmoles) (lane 1), a 20X excess of non-labeled BS2 fragment which competes for the binding of MraZ (lane 2) , or a 20X concentration of non-specific, non-labeled competitor (a 250 bp *cepI* fragment) (lane 3). (D) The bars indicate the relative quantification of the percentage of the bound DNA of the corresponding lanes obtained by densitometry analysis.

Supplementary tables

Table S1

Primer	Sequence 5′- 3′	Expected fragment
mraZRTfor1	GTGTTCCAAGGGGCGTCGGC	661bp

mraWRTrev1	GCATCCTTGATGCCCTGCGCCGTCT	
mraWRTfor1	AAGTCGTACTAGACGCGGCA	503bp
ftsLRTrev1	TCGAGATCGGCTGCATTTTCAGCGA	
ftsLRTfor1	GCCTCAATATCTTCCTGCTG	509bp
ftsIRTrev1	CCTGCTTCTGATAGAACGCG	
ftsIRTfor1	GGGTAGCGGCGCAAAGCATA	514bp
murERTrev1	CGGGCATCCCGGTGCCGAGCGT	
murERTfor1	GGCTCGAGTCGGTCAATGGC	561bp
murFRTrev1	ATCAGGCGGGCGGCTTCGCCG	
murFRTfor1	AAGATGGAGCGCGTGGTCGA	512bp
mraYRTrev1	ACAGCCCGATCACCGATTGC	
mraYRTfor1	ACCTGCTGTTCCCGCACATC	571bp
murDRTrev1	CCTCGCGGGTATCGGCAATA	
murDRTfor1	TCGACATGTTCACGAACTAC	529bp
ftsWRTrev1	GTTCACGCCCTTGCCGACAT	
ftsWRTfor1	ACGATGCTCGTGTGGCTGTC	581bp
murGRTrev1	GGTGCCGCCTGCCATCACCA	
murGRTfor1	AGGCTACCGACGAAGTCGCG	568bp
murCRTrev1	GAACGACGCATCCGACTCGT	
murCRTfor1	CTGGCGCTGGCGGAGTTCAA	544bp
ddlRTrev1	GAACAACACTGCCACCTTGC	
ddlRTfor1	CGCGACGCACGACAAGATCG	512bp
ftsQRTrev1	CGCCAACACGAGCAGCAGCA	
ftsQRTfor1	GGCAGGCATGCGGTTCCTGAC	557bp
ftsARTrev1	CCCGTCACGATGTGCACCTTC	
ftsARTfor1	AAGACATCAAGGTCGGCTACGGGAT	616bp
ftsZRTrev1	TGGTCTCGGTTTCCAGCATT	
ftsZRTfor1	GAAGAAGCAGCAGTCGGCAC	581bp
BCAL3456RTrev1	AATCCGAAGATCACCACCCG	
BCAL3456RTfor1	CAAATTCGAAGTGAGCGATG	573bp

lpxCRTrev1	TCGACATACAGGTTGTCGAT

Table S1: List of primers used for the transcription analysis of the *dcw* operon, and expected length of the PCR fragments.

Table S2

Primer	Sequence 5′- 3′	Restriction site
dcwPROMOfor1_2	GGTATCGATAAGCTTACGCCCGTCTGGGCCGTCT T	*Hind*III
dcwPROMOrev2	TCTAGCTAGAAGCTTTTCCGCTCTCCCGTTCAGG	*Hind*III
dcwPROMOfor2_2	GGTATCGATAAGCTTATTCGTCAAAAAAGCGGG CC	*Hind*III
murCPROMOfor1	GGTATCGATAAGCTTCATTCAACAGAAGGCATG AC	*Hind*III
ddlPROMOrev1	TCTAGCTAGAAGCTTACGTCGTTCCGTTCCTGCG T	*Hind*III
ftsQPROMOfor1	GGTATCGATAAGCTTATGTGGAACAACGTTCGCC A	*Hind*III
ftsQPROMOrev1	TCTAGCTAGAAGCTTAAAGTGCTCTTGCGTGTGA T	*Hind*III
ftsAPROMOfor1	GGTATCGATAAGCTTATGAGCAAAGACTACAAG GA	*Hind*III
ftsAPROMOrev1	TCTAGCTAGAAGCTTGATGTCGACCAGCACCAC GC	*Hind*III
ftsAPROMOfor2	GGTATCGATAAGCTTGGCGGCGGCACGACGGAC AT	*Hind*III
ftsAPROMOrev2	TCTAGCTAGAAGCTTTGTTGCCTCCGTCAAGAGA A	*Hind*III
mraZBS1for	GATTGGCGCCGGGGTGGCGT	
mraZBS1rev	GGAGCGGCCCGCTTTTTTGA	
mraZBS2for	AAGTTGCACTAGCTCATTCA	
mraZBS2rev	TTTCCGCTCTCCCGTTCAGG	
mraZpET28aFOR	ATGGGTCGCGGATCCCTGGAAGTTCTGTTCCAGG GGCCCaTGTTCCAAGGGGCGTCGGC	*Bam*HI
mraZpET28aREV	TGCGGCCGCAAGCTTTCAGAACGTGAAATTCTTC A	*Hind*III
sulApBADM41for	TTCAGGGCGCCATGGggCTGGAAGTTCTGTTCCA GGGGCCCCACCCCGCCCTCGCC	*Nco*I
sulApBADM41rev	ACGGAGCTCGAATTCTCAGGCGACGGCGCC	*Eco*RI
ftsASUMOfor	GAGAACAGATTGGTGGTATGAGCAAAGACTACA AGGA	
ftsASUMOrev	ATACCTAAGCTTGTCTTCAGAAGTTGCTCAGGAA CC	
ZipASUMOFor	GAGAACAGATTGGTGGTTGGCAGGGCGCGAAAG TGCGGCGC	

ZipASUMORev	ATACCTAAGCTTGTCTTTACTGGCTGAAGAGGCG GCGCGTGAC
mraZBSNSfor	ACACGAACTCGCGGCGGATC
mraZBSNSrev	TTGCGGCAGCGGCATGTCCT
mraZRACErev1	CATTCCCAACAACATGACTT
RA1	GACCACGCGTATCGATGTCGAC$(T)_{16}$
mraZRACErev2	CGGAAACAGCAACAGGCAGC
RA2	GACCACGCGTATCGATGTCGAC
mraZRACErev3	GTCACAGTCACCCGTCCTTC

Table S2: List of primers used for gene and promoter sequences cloning into plasmids, EMSA and 5'-RACE. The restriction site is underlined and the prescission protease sequence is in blue.

Table S3

Plasmid	Description	Source
pSU11	*E. coli-Burkholderia* shuttle vector containing *lacZ* reporter gene downstream of the MCS, Gm^r	Jenul *et al.*, 2018
pSU11-161	pSU11 containing the *dcw* predicted promoter sequence in a 161 bp fragment	This study
pSU11-289	pSU11 containing the *dcw* predicted promoter sequence in a 289 bp fragment	This study
pSU11-ftsQp	pSU11 containing the DNA sequence of the gene *ddl*	This study
pSU11-ftsAp	pSU11 containing the DNA sequence of the gene *ftsQ*	This study
pSU11-ftsZp1	pSU11 containing the first half of the *ftsA* gene sequence	This study
pSU11-ftsZp2	pSU11 containing the second half of the *ftsA* gene sequence	This study
pET28a	Expression vector IPTG inducible, Kan^r	Novagen
pET28a-MraZ	pET28a containing MraZ coding sequence	This study
pBADM-41	Expression vector for toxic or unstable proteins, containing MBP fusion, controlled by *araBAD* promoter, Amp^r	Laboratory collection
pBADM-41-SulA	pBADM-41 containing SulA coding sequence	This study
pETSUMO	Expression system incorporating a SUMO fusion, IPTG inducible, Kan^r	Invitrogen
pETSUMO-FtsA	pETSUMO containing FtsA coding sequence	This study
pETSUMO-ZipA	pETSUMO containing ZipA coding sequence	This study
pUT18	Derivative of pUC19. A MCS allows construction of in-frame fusions at the N-terminal end of the T18 polypeptide under the control of a lac promoter.	Karimova *et al.*, 1998

pUT18C	Derivative of pUC19. A MCS allows construction of in-frame fusions at the C-terminal end of the T18 polypeptide under the control of a lac promoter.	Karimova *et al.*, 1998
pKT25	Derivative of pSU40. A multicloning site sequence (MCS) allows construction of in-frame fusions at the N-terminal end of the T25 polypeptide under the control of a lac promoter.	Karimova *et al.*, 1998
pKNT25	Derivative of pSU40. A MCS allows construction of in-frame fusions at the C-terminal end of the T25 polypeptide under the control of a lac promoter.	Karimova *et al.*, 1998
pUT18FtsZ	pUT18 containing the T18 fused to the C-terminal end of FtsZ	This study
pUT18CFtsZ	pUT18C containing the T18 fused to the N-terminal end of FtsZ	This study
pKT25FtsZ	pKT25 containing the T25 fused to the N-terminal end of FtsZ	This study
pKNT25FtsZ	pKNT25 containing the T25 fused to the C-terminal end of FtsZ	This study
pUT18FtsA	pUT18 containing the T18 fused to the C-terminal end of FtsA	This study
pUT18CFtsA	pUT18C containing the T18 fused to the N-terminal end of FtsA	This study
pKT25FtsA	pKT25 containing the T25 fused to the N-terminal end of FtsA	This study
pKNT25FtsA	pKNT25 containing the T25 fused to the C-terminal end of FtsA	This study
pUT18FtsE	pUT18 containing the T18 fused to the C-terminal end of FtsE	This study
pKNT25FtsE	pKNT25 containing the T25 fused to the C-terminal end of FtsE	This study
pUT18FtsQ	pUT18 containing the T18 fused to the C-terminal end of FtsQ	This study
pKNT25FtsQ	pKNT25 containing the T25 fused to the C-terminal end of FtsQ	This study
pUT18FtsI	pUT18 containing the T18 fused to the C-terminal end of FtsI	This study
pUT18CFtsI	pUT18C containing the T18 fused to the N-terminal end of FtsI	This study
pKT25FtsI	pKT25 containing the T25 fused to the N-terminal end of FtsI	This study
pKNT25FtsI	pKNT25 containing the T25 fused to the C-terminal end of FtsI	This study
pUT18FtsN	pUT18 containing the T18 fused to the C-terminal end of FtsN	This study
pUT18CFtsN	pUT18C containing the T18 fused to the N-terminal end of FtsN	This study
pKT25FtsN	pKT25 containing the T25 fused to the N-terminal end of FtsN	This study
pKNT25FtsN	pKNT25 containing the T25 fused to the C-terminal end of FtsN	This study

pUT18ZipA	pUT18 containing the T18 fused to the C-terminal end of ZipA	This study
pUT18CZipA	pUT18C containing the T18 fused to the N-terminal end of ZipA	This study
pKT25ZipA	pKT25 containing the T25 fused to the N-terminal end of ZipA	This study
pKNT25ZipA	pKNT25 containing the T25 fused to the C-terminal end of ZipA	This study
pUT18SulA	pUT18 containing the T18 fused to the C-terminal end of SulA	This study
pKNT25SulA	pKNT25 containing the T25 fused to the C-terminal end of SulA	This study
pUT18ZapA	pUT18 containing the T18 fused to the C-terminal end of ZapA	This study
pUT18CZapA	pUT18C containing the T18 fused to the N-terminal end of ZapA	This study
pKT25ZapA	pKT25 containing the T25 fused to the N-terminal end of ZapA	This study
pKNT25ZapA	pKNT25 containing the T25 fused to the C-terminal end of ZapA	This study

Table S3: List of plasmids used in this work.

TableS4

Strain	Genotype	Source
Escherichia coli		
DH5α	F⁻, ϕ80d*lacZ*ΔM15 Δ(*lacZYA-argF*)*U169*, *endA1*, *recA1*, *hsdR17*($r_K^-m_K^+$), *supE44*, *thi-1*, Δ*gyrA96*, *relA1*	Laboratory stock
BL21(DE3)	F⁻, *ompT*, *hsdS*$_B$($r_B^-m_B^-$), *gal*, *dcm* (DE3)	Laboratory stock
TOP10	F⁻, *mcr*A, Δ(*mrr-hsd*RMS-*mcr*BC), φ80*lacZ*ΔM15, Δ*lacX74*, *rec*A1, *ara*D139, Δ(*ara-leu*)7697, *gal*U, *gal*K λ⁻ *rps*L(Str^r), *end*A1, *nup*G	Laboratory stock
XL1Blue	*recA1*, *endA1*, *gyrA96*, *thi-1*, *hsdR17*, *supE44*, *relA1*, *lac* [*F´ proAB lacI*^{q}*ZΔM15 Tn10* (Tet^r)].	Laboratory stock
BTH101	F⁻, *cya-99*, *araD139*, *galE15*, *galK16*, *rpsL1* (Str^r), *hsdR2*, *mcrA1*, *mcrB1*	Euromedex
Burkholderia cenocepacia		
J2315	Wild type strain	Laboratory stock
K56-2	Wild type strain	Laboratory stock

Table S4: List of strains used in this work.

Table S5

Primer	Sequence 5′- 3′	Restriction site

FtsZpUT18pKNT25For	GATTACGCCAAGCTTGATGGAATTCGAAATGCTGGA	*HindIII*
FtsZpUT18pKNT25Rev	GGTACCCGGGGATCCTCGTCAGCCTGCTTGCGCAGGAAAG	*BamHI*
FtsZpUT18CFor	GACTCTAGAGGATCCCATGGAATTCGAAATGCTGGA	*BamHI*
FtsZpUT18CRev	GAATTCGAGCTCGGTACCCGGTCAGCCTGCTTGCGCAGGAAAG	*KpnI*
FtsZpKT25For	GACTCTAGAGGATCCCATGGAATTCGAAATGCTGGA	*BamHI*
FtsZpkT25Rev	CTTAGTTACTTAGGTACCCGGTCAGCCTGCTTGCGCAGGA	*KpnI*
SulApUT18pKNT25For	GATTACGCCAAGCTTGATGCACCCCGCCCTCGCCCATCCTG	*HindIII*
SulApUT18Rev	CGGTACCCGGGGATCCTCGGCGACGGCGCCGGCGATCGTGGCC	*BamHI*
SulApKNT25Rev	GTACCCGGGGATCCTCGGCGACGGCGCCGGCGATCGTGGCC	*BamHI*
SulApUT18CpKT25For	GACTCTAGAGGATCCCATGCACCCCGCCCTCGCCCATCCT	*BamHI*
SulApUC18CRev	GTTATATCGATGAATTCGAGGCGACGGCGCCGGCGATCGTGGCC	*EcoRI*
SulApKT25Rev	GTTACTTAGGTACCCGGGCGACGGCGCCGGCGATCGTGGCC	*KpnI*
FtsApUT18pKNT25For	GATTACGCCAAGCTTGATGAGCAAAGACTACAAGGATC	*HindIII*
FtsApUT18pKNT2Rev	TTCGAGCTCGGTACCCGGAAGTTGCTCAGGAACC	*KpnI*
FtsApUT18CpKT25For	GACTCTAGAGGATCCCATGAGCAAAGACTACAAGGATC	*BamHI*
FtsApUT18CRev	GAATTCGAGCTCGGTACCCGGAAGTTGCTCAGGAACCATTCC	*KpnI*
FtsApKT25Rev	GTTACTTAGGTACCCGGAAGTTGCTCAGGAACCATTCC	*KpnI*
ZipApUT18pKNT25For	GATTACGCCAAGCTTGATGGACGAGTTGACACTCGGTTTG	*HindIII*
ZipApUT18pKNT25Rev	CGGGGATCCTCTAGAGTCTGGCTGAAGAGGCGGCGCGTGA	*XbaI*
ZipApUT18CpKT25For	GACTCTAGAGGATCCCATGGACGAGTTGACACTCGGTTTG	*BamHI*
ZipApUT18CRev	GAATTCGAGCTCGGTACCCGCTGGCTGAAGAGGCGGCGCGTGAC	*KpnI*
ZipApKT25Rev	GTTACTTAGGTACCCGCTGGCTGAAGAGGCGGCGCGTGAC	*KpnI*
ZapApUT18pKNT25For	GATTACGCCAAGCTTGATGAGCACCAAGCAGATCGAAGTCT	*HindIII*
ZapApUT18pKNT25rev	GTACCCGGGGATCCTCCTGCGTCTCGTGCTGTGCGAGC	*BamHI*
ZapApUT18CpKT25For	GACTCTAGAGGATCCCATGAGCACCAAGCAGATCGAAGTCT	*BamHI*

ZapApUT18CRev	CGAGCTCGGTACCCGCTGCGTCTCGTGCTGTGCGAGC	*KpnI*
ZapApKT25Rev	GTTACTTAGGTACCCGCTGCGTCTCGTGCTGTGCGAGC	*KpnI*
FtsEpUT18pKNT25For	GATTACGCCAAGCTTGATGATCCGCCTCGAACGCATCGAC	*HindIII*
FtsEpUT18pKNT25Rev	GTACCCGGGGATCCTCGAACGCCGGCACGCCTTGCGCGAG	*BamHI*
FtsEpUT18CpKT25For	GACTCTAGAGGATCCCATGATCCGCCTCGAACGCATCGAC	*BamHI*
FtsEpUT18CRev	CGAGCTCGGTACCCGGAACGCCGGCACGCCTTGCGCGAG	*KpnI*
FtsEpKT25Rev	GTTACTTAGGTACCCGGAACGCCGGCACGCCTTGCGCGAG	*KpnI*
FtsQpUT18pKNT25For	GATTACGCCAAGCTTGATGTGGAACAACGTTCGCCAAC	*HindIII*
FtsQpUT18pKNT25Rev	GTACCCGGGGATCCTCCTTCTTGCGCTTGTCGGTATCG	*BamHI*
FtsQpUT18CpKT25For	GACTCTAGAGGATCCCATGTGGAACAACGTTCGCCAAC	*BamHI*
FtsQpUT18CRev	CGAGCTCGGTACCCGCTTCTTGCGCTTGTCGGTATCG	*KpnI*
FtsQpKT25Rev	GTTACTTAGGTACCCGCTTCTTGCGCTTGTCGGTATCG	*KpnI*
FtsIpUT18pKNT25for	GATTACGCCAAGCTTGATGAAGCCGTCCCAGAAGCGC	*HindIII*
FtsIpUT18pKNT25rev	GTACCCGGGGATCCTCTCGAACTACTCCTGGTGAATTAC	*BamHI*
FtsIpUT18CFor	CAGGTCGACTCTAGAGATGAAGCCGTCCCAGAAGCGC	*XbaI*
FtsIpUT18CRev	GTTATATCGATGAATTCGATCGAACTACTCCTGGTGAATTAC	*EcoRI*
FtsIpKT25For	GGGTCGACTCTAGAGATGAAGCCGTCCCAGAAGCGC	*XbaI*
FtsIpKT25Rev	AACGACGGCCGAATTCTCATCGAACTACTCCTGGTGAATTAC	*EcoRI*
FtsNpUT18pKNT25For	GATTACGCCAAGCTTGGTGCTGGGCCTGATCGTCGGCCTCG	*HindIII*
FtsNpUT18pKNT25Rev	GTACCCGGGGATCCTCCTGCTTCGTGAAGCGGATCACC	*BamHI*
FtsNpUT18CpKT25For	GACTCTAGAGGATCCCGTGCTGGGCCTGATCGTCGGCCTCG	*BamHI*
FtsNpUT18CRev	CGAGCTCGGTACCCGCTGCTTCGTGAAGCGGATCACC	*KpnI*
FtsNpKT25Rev	GTTACTTAGGTACCCGCTGCTTCGTGAAGCGGATCACC	*KpnI*
pUT18pKNT25CheckFor	CTTTATGCTTCCGGCTCG	
pUT18CheckRev	GTTCGCGATCCAGGCCGC	
pKNT25CheckRev	GCGTAACCAGCCTGATGCG	

pUT18CCheckFor	GTCACCCGGATTGCGGCG	
pUT18CCheckRev	GTGTCGGGGCTGGCTTAAC	
pKT25CheckFor	GCAGTTCGGTGACCAGCGG	
pKT25CheckRev	GCAAGGCGATTAAGTTGGG	

Table S5: List of primers used for cloning the divisome genes into the BACTH plasmids. The restriction site is underlined.