

**Supplementary Materials for:**

**Title: An essential membrane protein modulates the proteolysis of LpxC to control lipopolysaccharide synthesis in *Escherichia coli***

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## MATERIALS AND METHODS

### *Construction of the $\Delta yejM$ strain EMF27 [ $\Delta yejM::kan^R/P_{lac}::yejM$ ]*

The  $kan^R$  cassette and flanking FRT sites was amplified from pKD4 using primers with homology domains flanking  $yejM$  coding sequence (forward primer:

AACGAAGACAAAGCGCACTAAGGGAAACAGATAACAGGTTGTGTAGGCTGGAGCT

GCTTC, reverse primer:

GATTGCAAGTAAGATATTTTCGCTAACTGATTTATAATTAACATATGAATATCCTCCTT

AG. This PCR product was then transformed into TB10 (8) harboring plasmid pEMF17

[ $P_{lac}::yejM$ ]. Transformants were selected on LB + Kan + Cm + IPTG (100  $\mu$ M). The

$\Delta yejM::kan^R$  allele was confirmed by PCR across the 5' junction using the primers

( $yejM\_kan\_seq\_F$ : CCCAACGCCAGGCAATTG, Kan-5' Out:

GCTTTCTACGTGTTCCGCTTCC) and 3' junction ( $yejM\_kan\_seq\_R$ :

TGCGCTACGTGCCGACTC, Kan-3' Out: TTCTATCGCCTTCTTGACGAGTTCTT) and

by testing for lack of growth in the absence of inducer. The  $\Delta yejM::kan^R$  allele was then

transduced from EMF25 into TB28/pEMF17 to generate strain EMF27. The genotype

was confirmed by PCR and lack of growth in the absence of inducer.

### *Construction of the $yejM_{(1-191)}$ strain EMF69 [ $yejM_{(1-191)}::kan^R/P_{lac}::yejM$ ]*

The  $yejM_{(1-191)}$  mutant (referred to as  $yejM-\Delta C$  in main text) was generated by first

amplifying the  $kan^R$  cassette and flanking FRT sites from pKD4 using primers with

homology domains designed to introduce a stop codon at residue 192 on the 5' end and

flanking the 3' end of the gene (forward primer:

ATGTGGTGTATATCTGGGCCTAAGCCAACCTTCTATCGCTTGTGTAGGCTGGAGCTG

CTTC, reverse primer:

GATTGCAAGTAAGATATTTTCGCTAACTGATTTATAATTAACATATGAATATCCTCCTT

AG) This PCR product was then transformed into TB10 (8) harboring plasmid pEMF17

[ $P_{lac}::yejM$ ], generating strain EMF68. Transformants were selected on LB + Kan + Cm

+ IPTG (100  $\mu$ M). The  $\Delta yejM::kan^R$  allele was confirmed by PCR across the 5' junction

using the primers (yejM\_kan\_seq\_F: CCCAACGCCAGGCAATTG, Kan-5' Out:

GCTTTCTACGTGTTCCGCTTCC) and 3' junction (yejM\_kan\_seq\_R:

TGCGCTACGTGCCGACTC, Kan-3' Out: TTCTATCGCCTTCTTGACGAGTTCTT). The

$\Delta yejM_{(1-191)}::kan^R$  allele was then transduced from EMF68 into TB28/pEMF17 to

generate strain EMF69.

### **Construction of the $\Delta yejM$ strain EMF30 [ $\Delta yejM::kan^R/P_{lac}::lpxC$ ]**

The  $\Delta yejM::kan^R$  allele was transduced into MG1655/pPR111 [WT/ $P_{lac}::lpxC$ ].

Transductants were selected on LB + Cam + Kan + 50  $\mu$ M IPTG. The transduction was confirmed via PCR (see above).

### *Molecular biology*

The polymerase chain reaction (PCR) was conducted using Q5 High fidelity polymerase (New England Biolabs) or GoTaq green master mix (Promega) following manufacturer's protocol. PCR products were purified using the PCR clean up kit from Qiagen or CWBiosciences. Plasmids were isolated using the Miniprep Kit from Qiagen or the plasmid purification kit from CWBiosciences. All plasmids were sequence verified by the DNA Resource Core of Dana-Farber/Harvard Cancer Center.

## *Plasmid construction*

### **pPR111**

LpxC was amplified from gDNA using primers LpxC\_nativeRBS\_XbaI5' (CCCCTCTAGATAATTTGGCGAGATAATACGATGATC) and lpxC\_R\_HindIII (TGATAAGCTTATTATGCCAGTACAGCTGAAGGCGC). The resulting insert (xbaI\_nativeRBS\_lpxC\_hindII) was cloned into vector pPR66 using restriction enzymes xbaI and hindIII.

### **pEMF15**

YejM was amplified from gDNA using primers pEMF15\_yejM\_xbaI\_F (TGGGTCTAGAGGGAAACAGATAACAGGTTATGGTAAC) and pEMF15\_yejM\_hindIII\_R (CGATAAGCTTAAGTAAGATATTTTCGCTAACTGATTTATAATTAATC). The resulting insert (xbaI\_nativeRBS\_yejM\_hindII) was cloned into vector pPR99 using restriction enzymes xbaI and hindIII.

### **pEMF17**

pEMF15 was digested with restriction enzymes xbaI and hindIII-HF and the xbaI\_nativeRBS\_yejM\_hindIII insert was gel-extracted (see Materials and Methods) and ligated into digested pPR66.

### **pEMF43**

acpT was amplified from gDNA using primers pEMF43\_F (CCCCTCTAGATTTAAGAAGGAGATATACATATGTATCGGATAGTTCTGGGGAAAG) and HindIII-acpT-3'R (TACCAAGCTTATCAGTTAACTGAATCGATCCATTGCAC). The insert (xbaI\_artificialRBS\_acpT\_hindIII) was ligated into pPR66 using restriction enzymes xbaI and hindIII.

### **pEMF20**

yejM was amplified from pEMF17 using primers yejM\_F\_gibson (GAAACAGCTATGACCATGATTACGAACTCCCGGGGATCTCGATCC) and yejM\_R\_gibson (TGGTACCGTGTCTGACTTACTCGAGAAGTAAGATATTTTCGCTAACTGATTTATAATTAATC) and inserted in pJLB11, which was digested with restriction enzymes smaI and xhoI, via isothermal assembly. This plasmid was constructed in strain JLB45 which expresses the *cI857*, in order to prevent zygotic induction.

### **pEMF33**

yejM was amplified from pEMF17 using primers xbaI\_phi10RBS\_yejM\_F (TGGGTCTAGATTTAAGAAGGAGATATACATATGGTAACTCATCGTCAGCGC) and pEMF31\_R\_hindIII (CGATAAGCTTCTGATTTATAATTAATCAGTTAGCGATAAAACGCTTCTC). The resulting insert (xbaI\_artificialRBS\_yejM\_hindII) was cloned into vector pPR66 using restriction enzymes xbaI and hindIII.

### **pEMF35**

*yejM* was amplified from pEMF17 using primers pEMF35\_F (CGGTGGATCCGTAACATCGTCAGCGCTAC) and pEMF35\_R (TGATAAGCTTATTTATAATTAATCAGTTAGCGATAAAACGC). The resulting product (BamHI\_ *yejM*\_hindIII) was cloned into vector pHCL149(7) using restriction enzymes BamHI and HindIII-HF.

### **pEMF36**

*lapB* was amplified from gDNA using primers pEMF36\_F (CCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGCTGGAGTTGTTGTTTCTG) and pEMF36\_R(CAGAGGATCCCAGGCCATCAAGACCGCG). The resulting PCR product (xbaI\_artificialRBS\_ *lapB*\_BamHI) was cloned into pHCL147(7) using restriction enzymes xbaI and BamHI. The ligation was then transformed into TB28/pTB102 Chung competent cells. The insert was then amplified and sequenced. Correct integration was confirmed via PCR (9). The integrated plasmid was then transduced into MG1655. The resulting strain was confirmed via PCR.

### **pEMF37**

*lapA* was amplified from gDNA using primers pEMF37\_F (TATACATATGAAATATTTACTCATTTTCTTACTGGTGTTAG) and pEM37\_R (CAGAGGATCCTTCCTTCGCCGCTGACGAG). The resulting product (ndel\_ *lapA*\_bamHI) was inserted into pHCL147(7) using restriction enzymes ndel and bamHI. The ligation was then transformed into TB28/pTB102 Chung competent cells. The insert was then amplified and sequenced. Correct integration was confirmed via PCR (9). The integrated plasmid was then transduced into MG1655. The resulting strain was confirmed via PCR.

### **pEMF38**

FtsH was amplified from gDNA using primers pEMF38\_F (CCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCGAAAAA CTAATACTCTGGC) and pEMF38\_R (GGACCCTCGAGACCAGAGGATCCCTTGTCGCCTAACTGCTCTG). The resulting product (xbaI\_artificialRBS\_ *ftsH*\_xhoI) was inserted into pHCL147(7) using restriction enzymes xbaI and xhoI. The ligation was then transformed into TB28/pTB102 Chung competent cells. The insert was then amplified and sequenced. Correct integration was confirmed via PCR(9). The integrated plasmid was then transduced into MG1655. The resulting strain was confirmed via PCR.

### **pEMF53**

artificialRBS\_ *lapB* was amplified from pEMF40 using primers pEMF36\_F (CCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGCTGGAGTTGTTGTTTCTG) and pEMF40\_R(TGATAAGCTTATTACAGGCCATCAAGACCGC). The resulting insert (xbaI\_artificialRBS\_ *lapB*\_hindIII) was digested using restriction enzymes xbaI and hindIII-HF and ligated into vector pMT116. The ligation was then transformed into TB28/pTB102 Chung competent cells. The insert was then amplified and

sequenced. Correct integration was confirmed via PCR(9). The integrated plasmid was then transduced into MG1655. The resulting strain was confirmed via PCR.

#### **pEMF54**

pEMF33 was digested with restriction enzymes *xba*I and *hind*III-HF. The artificialRBS\_ *yej*M insert was ligated into pMT13.

#### **pEMF55**

ArtificialRBS\_ *yej*M sequence was amplified from pEMF35 using primers pEMF55\_F(CGGCCTCGAGTAACTAGCAGGAGATACATATATGGTAACTCATCGTCA GCGC) and pEMF55\_R(TGATAAAGCTTAGTCGACTTATCAGTTAGCGATAAAACGCTTCTC). The resulting insert (*xho*I\_artificialRBS\_ *yej*M\_ *hind*III) was digested using restriction enzymes *xho*I and *hind*III-HF and ligated into vector pHCL149 directly downstream of the Para::popZ-H3H4-msfGFP-TM sequence.

#### **pEMF57**

pHC405 was digested with restriction enzymes *xba*I and *hind*III-HF. The *xba*I\_ *sf*- *gfp*\_ *hind*III insert was ligated into pMT13.

#### **pEMF65**

*yej*M was amplified from pEMF17 using primers pEMF35\_F (CGGTGATCCGTA ACTCATCGTCAGCGCTAC) and pEMF65\_R (TGATAAAGCTTATTTATAATTAATTAGGCCAGATATACACCACAT). The resulting product (*Bam*HI\_ *yej*M(1-191)STOP\_ *hind*III) was cloned into vector pHCL149(7) using restriction enzymes *Bam*HI and *Hind*III-HF.

#### **pEMF68**

The *yej*M<sub>(1-191)</sub> sequence was amplified from pEMF17 using primers *xba*I\_ *phi*10RBS\_ *yej*M\_F (TGGGTCTAGATTTAAGAAGGAGATACATATGGTAACTCATCGTCAGCGC) and pEMF65\_R (TGATAAAGCTTATTTATAATTAATTAGGCCAGATATACACCACAT). The resulting *xba*I\_ *yej*M<sub>(1-191)</sub>\_ *hind*III product was digested using restriction enzymes *Xba*I and *Hind*III-HF and ligated into pMT13.

## SUPPLEMENTAL REFERENCES

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