

## Assembly of Lipopolysaccharide in *Escherichia coli* Requires the Essential LapB Heat Shock Protein\*

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\*Running title: *LapA- and LapB-dependent assembly of LPS in Escherichia coli*

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### Supplemental information

**Table S1.** Bacterial strains and plasmids used in this study.

Strains/Plasmids	Relevant characteristic	Reference or source
Strains		
W3110	$\lambda^-$ , <i>IN(rrnD-rrnE)1</i> , <i>rph-1</i>	<i>E. coli</i> Genetic Stock Center, Yale
BW25113	<i>lacI<sup>f</sup> rrnB<sub>T14</sub> ΔlacZ<sub>WJ16</sub> hsdR514 ΔaraBAD<sub>AH33</sub> ΔrhaBAD<sub>LD78</sub></i>	(19)
BW30207	MG1655 <i>rph<sup>+</sup></i>	<i>E. coli</i> Genetic Stock Center, Yale
BL21(DE3)	<i>F<sup>-</sup> ompT hsdSB(rB<sup>-</sup> mB<sup>-</sup>) gal dcm (DE3)</i>	Invitrogen
GK1942	BW25113 (pKD46)	This study
SR8035	W3110 <i>waaC&lt;&gt;aph</i>	(6)
SR8233	W3110 <i>waaC&lt;&gt;cat</i>	(6)
SR9521	BW25113 <i>lapA&lt;&gt;aph</i>	This study
SR9523	BW25113 <i>lapB&lt;&gt;aph</i>	This study
SR16087	BW25113 <i>lapA-lapB&lt;&gt;cat</i>	This study
SR17187	BW25113 <i>lapA-lapB&lt;&gt;aph</i>	This study
SR17420	BW25113 <i>lapA-lapB&lt;&gt;aph waaC&lt;&gt;cat</i>	This study
SR8392	W3110 <i>lapB&lt;&gt;aph waaC T187K</i>	This study
SR8431	BW30207 <i>lapB&lt;&gt;aph gmhA::IS1</i>	This study
SR8915	<i>rpoH::Kan</i>	(69)
BAS849	<i>lptD4213</i>	(54)
PK101	<i>(dnaK14 dnaJ14)::Kan</i>	(22)
GK3078	BW25113 <i>dnaK dnaJ::Kan</i>	This study
SR17161	GK3078 <i>lapA-lapB&lt;&gt;cat</i>	This study
SR17401	BW25113 $\phi$ ( <i>rpoHP3-lacZ</i> )	This study
SR17405	SR17401 <i>lapA-lapB&lt;&gt;aph</i>	This study
SR17432	BW25113 $\phi$ ( <i>rpoEP2-lacZ</i> )	This study
GK3481	SR17432 <i>lapA-lapB&lt;&gt;aph</i>	This study
SR7718	BW25113 $\phi$ ( <i>pcpxP-lacZ</i> )	(6)
SR16317	SR7718 <i>lapA-lapB&lt;&gt;cat</i>	This study
SR17662	BW25113 $\phi$ ( <i>lapABP2<sub>hs</sub>-lacZ</i> )	This study
SR6618	BW25113 $\phi$ ( <i>pgroESL-lacZ</i> )	This study
SR16389	BW25113 <i>lapA::3xFLAG&lt;&gt;frt</i>	This study
SR16430	BW25113 <i>lapA::3xFLAG&lt;&gt;frt-phs-lapB</i>	This study
SR16962	BW25113 <i>lapB::3xFLAG&lt;&gt;frt</i>	This study

GK2642	BW25113 <i>lapB</i> ::1xFLAG<> <i>frt</i>	This study
SR9609	BW25113 <i>lptD</i> ::3xFLAG<> <i>frt</i>	This study
SR17634	SR9609 <i>lapA-lapB</i> <> <i>cat</i>	This study
SR16215	BW25113 <i>waaC</i> ::3xFLAG<> <i>frt</i>	This study
SR16233	SR16215 <i>lapA-lapB</i> <> <i>aph</i>	This study
SR9237	BW25113 <i>waaO</i> ::3xFLAG<> <i>frt</i>	This study
SR17310	SR9237 <i>lapA-lapB</i> <> <i>aph</i>	This study
GK2626	BW25113 <i>lpxM</i> ::3xFLAG<> <i>aph</i>	(4)
GK2640	GK2626 <i>lpxM</i> ::3xFLAG <> <i>frt</i>	This study
GK3223	GK2640 <i>lapA-lapB</i> <> <i>aph</i>	This study
SR16143	SR16087 <i>lapA-lapB</i> <> <i>cat waaQ</i> ::Tn10	This study
SR16156	SR16087 <i>lapA-lapB</i> <> <i>cat waaG</i> <> <i>aph</i>	This study
SR16146	SR16087 <i>lapA-lapB</i> <> <i>cat lpp</i> ::Tn10	This study
SR16181	SR16087 <i>lapA-lapB</i> <> <i>cat lpxC186</i>	This study
SR17710	SR16087 <i>lapA-lapB</i> <> <i>cat lpxCA60</i>	This study
GK3193	BW25113 <i>lpp</i> <> <i>frt</i>	This study
GK3201	GK3193 <i>lapA-lapB</i> <> <i>cat</i>	This study
A8926	$\Delta$ <i>ftsH3</i> ::Kan <i>sfhC21 zad220</i> ::Tn10	(23)
GK3576	BW25113 <i>sfhC21 zad220</i> ::Tn10	This study
GK3584	GK3576 <i>lapA-lapB</i> <> <i>aph</i>	This study
GK3592	GK3576 $\Delta$ <i>ftsH3</i> ::Kan	This study
GK3606	GK3592 <i>lapA-lapB</i> <> <i>cat</i>	This study
SM101	<i>lpxA2</i> (ts)	<i>E. coli</i> Genetic Stock Center, Yale
SR17745	SR17187 <i>lpxA2</i>	This study
RL436	<i>lpxD36</i> (ts)	<i>E. coli</i> Genetic Stock Center, Yale
GK3558	SR17187 <i>lpxD36</i>	This study
RL25-1	<i>lpxD201</i> (ts)	<i>E. coli</i> Genetic Stock Center, Yale
SR17742	SR17187 <i>lpxD201</i>	This study
GK3097	BW25113 <i>yceK</i> <> <i>frt</i>	This study
SR17241	GK3097 <i>lapA-lapB</i> <> <i>cat</i>	This study
SR9423	Bw25113 <i>rseA</i> <> <i>ada</i>	This study
SR17458	SR17187 + <i>pslA</i> <sup>+</sup>	This study
SR17515	BW25113 $\phi$ ( <i>pslA-lacZ</i> )	This study
GK3539	SR17515 <i>rseA</i> <> <i>ada</i>	This study
GK2549	BW25113 <i>degP</i> <> <i>ada</i>	This study
SR17675	BW25113 <i>slrA</i> <sup>C</sup>	This study
SR17677	SR17675 <i>degP</i> <> <i>ada</i>	This study
GK3565	BW25113 <i>slrA</i> <> <i>frt</i>	This study
Plasmids		
pET28b	expression vector	Novagen
pCP20	ts replicon with inducible FLP recombinase	(19)
pKD3	<i>oriR6K</i> , <i>bla</i> (Amp <sup>R</sup> ), <i>kan</i> , <i>rgnB</i> (Ter), <i>cat</i>	(19)
pKD13	<i>oriR6K</i> , <i>bla</i> (Amp <sup>R</sup> ), <i>kan</i> , <i>rgnB</i> (Ter)	(19)
pKD46	<i>araBp-gam-bet-exo</i> , <i>bla</i> (Amp <sup>R</sup> ), <i>repA101</i> (ts)	(19)
pLC1921	pSC101-based low copy vectors spec <sup>R</sup>	(21)
pRS415	<i>lacZYA</i> transcriptional fusion vectors	(29)
pRS551	<i>lacZYA</i> transcriptional fusion vectors	(29)
pSUB312	1xFLAG	(30)
pSUB11	3xFLAG	(30)
pWSK29	pSC101-based low copy vectors amp <sup>R</sup>	(25)
pWSK30	pSC101-based low copy vectors amp <sup>R</sup>	(25)
pCA24N	<i>ptac</i> -based expression vector cm <sup>R</sup>	(26)

pSR16726	<i>lapAB</i> <sup>+</sup> in pWSK29 amp <sup>R</sup>	This study
pSR16730	<i>lapAB</i> <sup>+</sup> in pWSK30 amp <sup>R</sup>	This study
pSR16881	<i>lapB</i> <sup>+</sup> from pSR16726 amp <sup>R</sup>	This study
pGK3605	<i>lapA</i> <sup>+</sup> in pET28b kan <sup>R</sup>	This study
pSR7815	<i>lapB</i> <sup>+</sup> in pET28b kan <sup>R</sup>	This study
pSR15978	<i>lptC</i> <sup>+</sup> in pET28b kan <sup>R</sup>	This study
JW3596	<i>waaC</i> <sup>+</sup> in pCA24N cm <sup>R</sup>	(26)
JW0175	<i>fabZ</i> <sup>+</sup> in pCA24N cm <sup>R</sup>	(26)
pSR17452	<i>slrA</i> <sup>+</sup> in pRS415 amp <sup>R</sup>	This study
<i>plptBFG</i>	Spec <sup>R</sup> plasmid	(11)
pDM38	pWSK29 <i>dnaK</i> <sup>+</sup> <i>dnaJ</i> <sup>+</sup>	(70)

## References

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**Table S2. Primers**

**For gene disruptions:**

<b>lapA_for cm</b>	5'-TAA GTT GAC CAT AAT TTA TTC GCT CTA ACC ACA TAA CGG GAA GTA ATG TGC ATA TGA ATA TCC TCC TTA G-3'
<b>lapA_for kan</b>	5'-TAA GTT GAC CAT AAT TTA TTC GCT CTA ACC ACA TAA CGG GAA GTA ATG TGA TTC CGG GGA TCC GTC GAC C-3'
<b>lapB_for cm</b>	5'-ACG TGG CTG TAG TGC CGC ACT CGT CAG CGG CGA AGG AAT AAC TTT CTA TGC ATA TGA ATA TCC TCC TTA G-3'
<b>lapB_rev cm</b>	5'-TGT AAC TAA AGT CGG ATT TTT TTT AAA AAT TAC AGG CCA TCA AGA CCG CGG TGT AGG CTG GAG CTG CTT C-3'
<b>lapB_for kan</b>	5'-ACG TGG CTG TAG TGC CGC ACT CGT CAG CGG CGA AGG AAT AAC TTT CTA TGA TTC CGG GGA TCC GTC GAC C-3'
<b>lapB_rev kan</b>	5'-TGT AAC TAA AGT CGG ATT TTT TTT AAA AAT TAC AGG CCA TCA AGA CCG CGT GTA GGC TGG AGC TGC TTC G-3'
<b>slrA_for</b>	5'-ATC AAA AAT TAT GGA ACT TAT TGC CCA TCG TGA TGC TCC AAT CAT TAT GGA TTC CGG GGA TCC GTC GAC C-3'
<b>slrA_rev</b>	5'-CGG TTA TCG TTC GTT TTT TAT ACT GTT CAG GGA TAA AAA AAG GCC CCT GTT GTA GGC TGG AGC TGC TTC G-3'
<b>yceK_for</b>	5'-TTT TTT TAC GAA AGA GGT ATC AAA TGC GTT TAA TTG TGG TGA GCA ATC ATG GCA CCT TGC CGT AGA AGA AC-3'
<b>yceK_rev</b>	5'-CAG CAG CTG ATG AGT GGG ATG AAC GTT AAT CAC TCA TAC GGG CCA TGA ATG TAA CGC TTG AGT TAA GCC GC-3'
<b>lpp_for</b>	5'-ACT TGT AAC GCT ACA TGG AGA TTA ACT CAA TCT AGA GGG TAT TAA TAA TGA TTC CGG GGA TCC GTC GAC C-3'
<b>lpp_rev</b>	5'-GTG CGC CAT TTT TCA CTT CAC AGG TAC TAT TAC TTG CGG TAT TTA GTA GCT GTA GGC TGG AGC TGC TTC G-3'

**For cloning, sequencing and overexpression of specific genes:**

<b>lapA_for</b>	5'-ACG GGA AGT CAT ATG AAA TAT TTA CTC-3'
<b>lapA_rev</b>	5'-CCA GCA TAG ACT CGA GTT CCT TCG C-3'
<b>lapB_for</b>	5'-GAA TAA CTT TCC ATG GTG GAG TTG TTG-3'
<b>lapB_rev</b>	5'-TCG GAT TTT TTT TAA CTC GAG CAG GCC ATC AAG-3'
<b>waaC_for</b>	5'-TGA CGC TGC GGA GGG TTA TCA CCA-3'
<b>waaC_rev</b>	5'-CAT TGA TGA TTT CAG AGT GTA AGG-3'
<b>lptC_for</b>	5'- AAA GGG CAA TCC ATA TGA GTA AAG CCA GAC G -3'
<b>lptC_rev</b>	5'- TGG CAA GCA CAC TCG AGA GGC TGA GTT TG-3'
<b>slrA_for</b>	5'-CTG GCA GTG AGA ATT CAT CGC GCC TTC GA-3'
<b>slrA_rev</b>	5'-GCT CAA CGG ATC CCG GTT ATC GTT CG-3'
<b>lpxC_for</b>	5'-CCC AGC ATT CCT GCG TAA GCA AGC TGA-3'
<b>lpxC_rev</b>	5'-GAG AGA GTG CCA GAT TTG CCA GTC GA-3'

**For mutagenesis of *lapA-lapB* operon:**

<b>lapAB-1_for</b>	5'-TGG CGG TGG CAA CGT GGA TCC CGC AAC GAA TTT G-3'
<b>lapAB-2_rev</b>	5'-CAT TAA TAA TTA GAA GCT TGT AAC TAA AGT CGG-3'

**For promoter cloning:**

<b>p<sub>h</sub>s lapAB_rev</b>	5'-GTA CCA GGA TCC CGC CGT CTG CGA AA-3'
<b>plapAB_for</b>	5'-GTA GCT ACG TTG AAT TCG TGG GCG C-3'
<b>plapAB_rev</b>	5'-CCG TCG TTG CCC GGG CAC AAT GAA ACA TTA-3'

**pslrA\_for** 5'-CTGGCAGTGAGAATTCATCGCGCCTTCGA-3'  
**pslrA\_rev** 5'-GCTCAACGGATCCCGGTTATCGTTTCG-3'

**For promoter mapping:**

**Lap\_PE1** 5'-CAT TAC TTC CCG TTA TGT GGT TAG AGC GA-3'  
**Lap\_PE2** 5'-CGA AGA TCG CTA ACA CCA GTA AG-3'  
**slr\_RACE1** 5'-CGA GCA AGC ATC ATA TTG-3'  
**slr\_RACE2** 5'-GGC GAC ATG ATG CAA CGG TA-3'

**For epitope tagging:**

**lapA 3xFLAG\_for** 5'-TCA CCC GCG ACT GAC GTG GCT GTA GTG CCG CAC TCG TCA  
GCG GCG AAG GAA GAC TAC AAA GAC CAT GAC GG-3'  
**lapA 3xFLAG\_rev** 5'-CCA TAG GCA GCG GCT ACA GGC AAA AGC AGA AAC AAC AAC  
TCC AGC ATA GAC ATA TGA ATA TCC TCC TTA G-3'  
**lapB 3xFLAG\_for** 5'-CTT GTC GGG CCT GGT CAA CCA TTA AAC CGA TTC GCG GTC  
TTG ATG GCC TGG ACT ACA AAG ACC ATG ACG G-3'  
**lapB 3xFLAG\_rev** 5'-AAT GGA ACA TTA ATA ATT AGT ATG TTG TAA CTA AAG TCG  
GAT TTT TTT TAC ATA TGA ATA TCC TCC TTA G-3'  
**lapB 1xFLAG\_for** 5'-CTT GTC GGG CCT GGT CAA CCA TTA AAC CGA TTC GCG GTC  
TTG ATG GCC TGG ACT ACA AAG ATG ACG ACG A-3'  
**lapB 6xHis\_for** 5'-CTT GTC GGG CCT GGT CAA CCA TTA AAC CGA TTC GCG GTC  
TTG ATG GCC TGC ACC ACC ATC ATC ACC ATT AG-3'  
**lptD 3xFLAG\_for** 5'-GTA CGC AAG AGA TGC TGC GTT CGA ACA TTC TGC CGT ATC  
AAA ACA CTT TGG ACT ACA AAG ACC ATG ACG G-3'  
**lptD 3xFLAG\_rev** 5'-CAT ACT TTT TCC ATT TCA ATT AAC CGC ACT GCG GAT TAC  
GTG GTA AAT CAC ATA TGA ATA TCC TCC TTA G-3'  
**waaC 3xFLAG\_for** 5'-ATT TAA TTA ACC TCA ACA GTC AAG CAG TTT TGG AAA AGT  
TAT CAT CAT TAG ACT ACA AAG ACC ATG ACG G-3'  
**waaC 3xFLAG\_rev** 5'-AGT GTA AGG TTT CAA TGA ATG AAG TTT AAA GGA TGT TAG  
CAT GTT TTA CCC ATA TGA ATA TCC TCC TTA G-3'  
**waaO 3xFLAG\_for** 5'-TAA AAG GAT TTA GCA ACT ATC TTT TTT ATT TTA TTG AAA  
AGA TAA AGC ATG ACT ACA AAG ACC ATG ACG G-3'  
**waaO 3xFLAG\_rev** 5'-TAA AAG GAT TTA GCA ACT ATC TTT TTT ATT TTA TTG AAA  
AGA TAA AGC ATG ACT ACA AAG ACC ATG ACG G-3'