

# Regulation of the First Committed Step in Lipopolysaccharide Biosynthesis Catalyzed by LpxC Requires the Essential Protein LapC (YejM) and HslVU Protease

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## Supplementary Materials

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

Strains/Plasmids	Relevant characteristic	Reference or source
<b>Strains</b>		
BW25113	<i>lacI<sup>q</sup> rrrnB<sub>T14</sub> ΔlacZ<sub>WJ16</sub> hsdR514 ΔaraBAD<sub>AH33</sub> ΔrhaBAD<sub>LD78</sub></i>	[65]
T7 Express	<i>lysY/I<sup>q</sup> MiniF lysY fhuA2 lacZ::T7 gene1 [lon] ompT</i>	NEB
GK1942	BW25113 (pKD46)	[17]
SR7753	BW25113 ( <i>lapB</i> ) $\langle$ <i>aph</i>	[17]
SR9523	BW25113 ( <i>lapB</i> ) $\langle$ <i>frt</i>	[17]
SR8348	SR7753 <i>lapC</i> 377fs	This study
SR16087	BW25113 <i>lapA-lapB</i> $\langle$ <i>cat</i>	[17]
SR17187	BW25113 <i>lapA-lapB</i> $\langle$ <i>aph</i>	[17]
SR19796	SR17187+ <i>phslV</i> <sup>+</sup>	This study
SR17432	BW25113 $\phi$ ( <i>rpoEP6-lacZ</i> )	[17]
SR18868	BW25113 $\phi$ ( <i>rpoEP2/P3-lacZ</i> )	[18]
SR18987	BW25113 $\phi$ ( <i>rpoEP2*-lacZ</i> -12 and -24 mut)	[18]
SR19041	SR18987 <i>lapC</i> 190 stop	This study
SR19750	SR18987 <i>lapC</i> 377fs	This study
SR22861	SR18987 <i>lapC</i> 377fs	This study
SR22862	SR18987 <i>lapC</i> F349S	This study
GK6075	BW25113 $\Delta$ <i>lapC</i> 190	This study
GK6078	GK6075 <i>lpxC</i> K270T	This study
GK6093	GK6075 <i>lpxC</i> V37G	This study
SR22727	GK6075 <i>lpxC</i> R230C	This study
SR22728	GK6075 <i>lpxC</i> R230C	This study
SR22729	GK6075 <i>lpxC</i> R230C	This study
SR22731	GK6075 <i>lpxC</i> V37G	This study
SR22732	GK6075 <i>lpxC</i> V37G	This study
SR22738	GK6075 <i>lpxC</i> V37L	This study
SR22739	GK6075 <i>lpxC</i> V37G	This study
GK6094	GK6075 <i>lpxC</i> fs306 stop codon	This study
GK6085	GK6075 <i>lapA</i> IS after 34 nt	This study

GK6089	GK6075 <i>lapA</i> fs 137 nt	This study
GK6090	GK6075 <i>lapA</i> IS after 103 nt	This study
GK6097	GK6075 <i>lapA</i> L8 to TGA stop codon	This study
SR22734	GK6075 <i>lapA</i> IS after 34 nt	This study
SR22736	GK6075 <i>lapA</i> FS after 69 nt	This study
SR22737	GK6075 IS at -106 in <i>lapAB</i> promoter region	This study
GK6084	GK6075 <i>lapB</i> D124Y	This study
GK6087	GK6075 <i>lapB</i> R125L	This study
SR22724	GK6075 <i>lapB</i> H325P	This study
SR22725	GK6075 <i>lapB</i> H325L	This study
SR22726	GK6075 <i>lapB</i> A88V	This study
SR22733	GK6075 <i>lapB</i> R115H	This study
GK6095	GK6705 <i>ftsH</i> A296V	This study
SR22766	BW25113 $\Delta$ <i>hslUV</i> $\rightarrow$ <i>aph</i>	This study
SR22776	SR18987 <i>lapC190</i>	This study
SR22777	SR17432 <i>lapC190</i>	This study
SR22433	SR18987 <i>lapC377fs</i>	This study

### Plasmids

pET24b	expression vector	Our collection
pET28b	expression vector	Our collection
pCP20	ts replicon with inducible FLP recombinase	[65]
pKD3	<i>oriR6K<sub>s</sub></i> , <i>bla</i> (Amp <sup>R</sup> ), <i>kan</i> , <i>rgnB</i> (Ter), <i>cat</i>	[65]
pKD13	<i>oriR6K<sub>s</sub></i> , <i>bla</i> (Amp <sup>R</sup> ), <i>kan</i> , <i>rgnB</i> (Ter)	[65]
pKD46	<i>araBp-gam-bet-exo</i> , <i>bla</i> (Amp <sup>R</sup> ), <i>repA101</i> (ts)	[65]
pCA24N	IPTG-inducible expression vector cm <sup>R</sup>	[40]
pSR19788	<i>hslV</i> <sup>+</sup> in pCA24N	This study
pSR19796	<i>hslVU</i> <sup>+</sup> in pCA24N	This study
JW2176	<i>lapC</i> <sup>+</sup> in pCA24N	[40]
pSR22821	<i>hslVU</i> <sup>+</sup> in pET24b	This study
pSR22901	<i>lapC</i> <sup>+</sup> in pET28b	This study

**Table S2. Primers**

**For gene disruption:**

*lapC* For 5'-CTC TAT CAA CGA AGA CAA AGC GCA CTA AGG GAA ACA GAT AAC AGG  
TTA TGA TTC CGG GGA TCC GTC GAC CC-3'

*lapC* Rev 5'-AGA TAT TTC GCT AAC TGA TTT ATA ATT AAT CAG TTA GCG ATA AAA  
CGC TTT GTA GGC TGG AGC TGC TTC G-3'

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

*lapC* For 5'-CCG CAT CCC ATG GCT AAA ATT AAA CAT CAC CAT CAC CAT CAC CAC  
CAT CAC CAT ATG GTA ACT CAT CGT CAG C-3'

*lapC* Rev 5'-GCA AGT AAG AGA ATT CGC TAA CTG-3'

*hslV* For 5'-AGG GGT CAG CAT ATG ACA ACT ATA GTA AG-3'

*hslU* Rev 5'-GAT TGA ACG CTC GAG TCA TTA ATG ATG ATG GTG ATG ATG TAG GAT  
AAA ACG GCT CAG-3'

**For sequencing and PCR amplification:**

*lapC* For 5'-GCG CCT TAC AGT CCT CTA TCA AC-3'

*lapC* Rev 5'-CGT TTT CCA CAC CGA TTG CAA G-3'

*lapA* For 5'-GTT GAT TTC GTG GGC GCT GGT G-3'

*lapB* Rev 5'-GTC GTT GCC GGA GCA CAA TGG-3'

*ftsH* For 5'-GGA TAT AGA GTA TCC TGA CGC-3'

*ftsH* Rev 5'-CGG TAC AAA TAC AGT CAT CTG-3'

**For q-RT-PCR:**

*qlapC* For 5'-ATT CGC AAG GTC AGG ATT TG-3'

*qlapC* Rev 5'-GCG ATA AAA CGC TTC TCG TC-3'