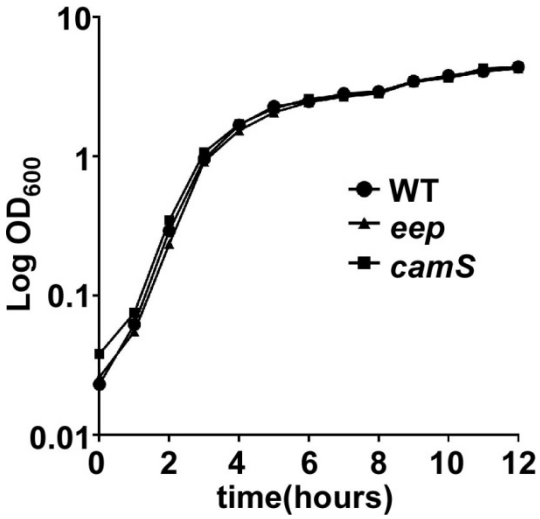

Fig. S1. The effect of the *eep*- and the *camS*-deletion on the growth of *S. aureus*.

OD_{600} in TSB was measured every hour for 12 h. WT, *S. aureus* USA300; *eep*, the *eep*-deletion mutant; *camS*, the *camS*-deletion mutant.

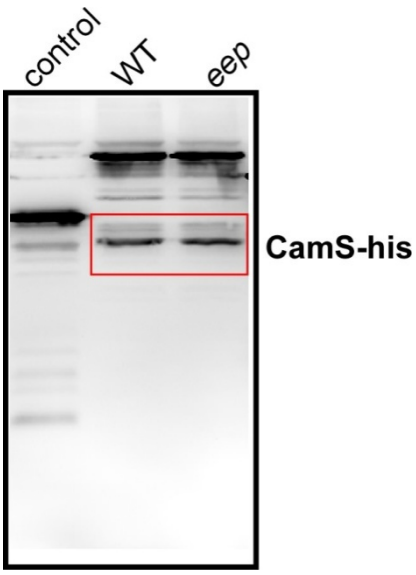

Fig. S2. The confirmation of the overexpression of CamS **by Western blot analysis**. An equal number of cell pellets was used for the analysis (see Materials and Methods). CamS protein was detected with anti-His-tag antibody.

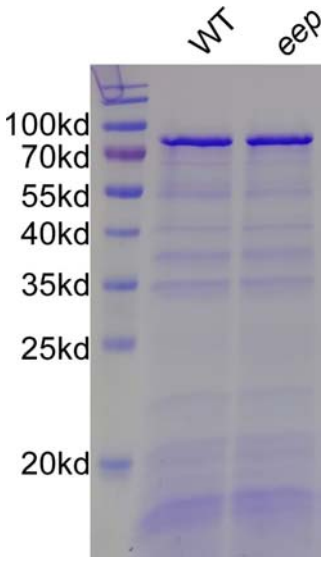

Fig. S3. Eep does not affect the gross protein-secretion profile. The supernatants of bacteria were collected by centrifugation, passed through 0.22 μm filter, and precipitated by TCA. The samples were loaded on 12% SDS-PAGE gel and stained with Coomassie brilliant blue. WT, *S. aureus* USA300; *eep*, the *eep*-deletion mutant.

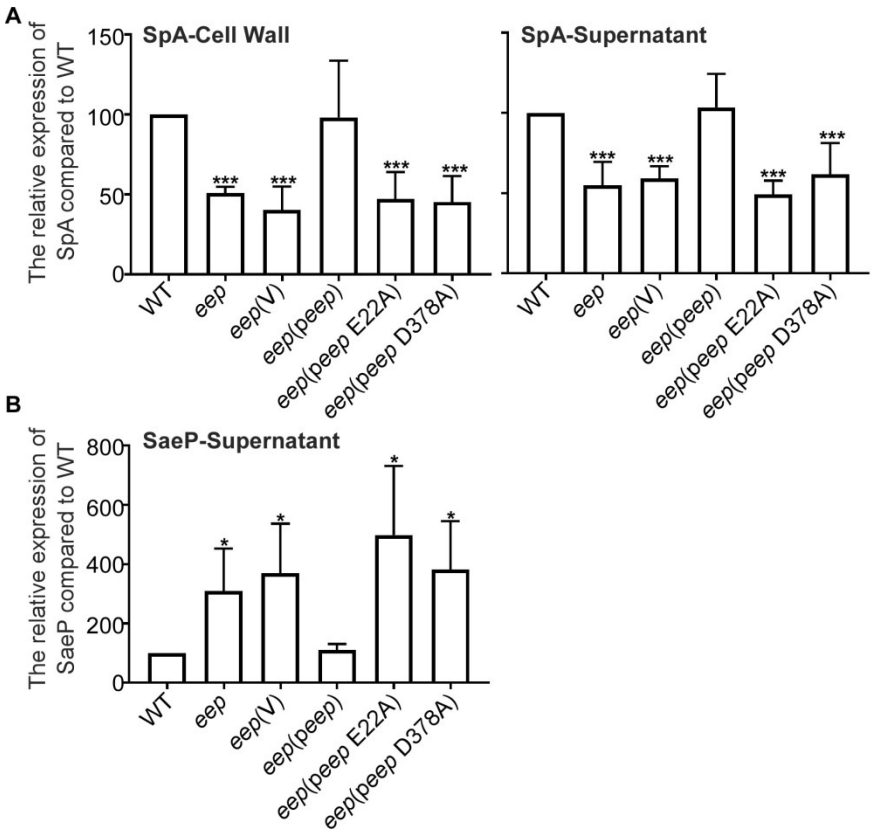

Fig. S4. The quantification of Western blot results for SpA (A) and SaeP (B).The protein bands were quantified by Image J.*, $p < 0.05$;***, $p < 0.001$ by unpaired, two-tailed Student's t-test. WT, *S. aureus* USA300; *eep*, the *eep*-deletion mutant; V, the vector pCL55; p*eep*, pCL55 containing the *eep* gene; p*eep* E22A, p*eep* containing the E22A mutation; p*eep* D378A, p*eep* containing the D378A mutation.

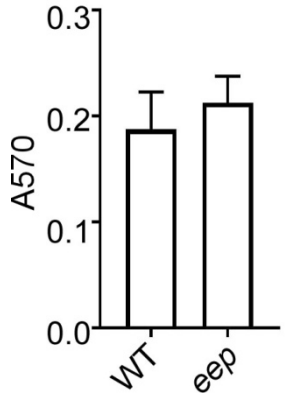

Fig. S5. Eep is not required for biofilm formation. The effect of the *eep*-deletion on the biofilm formation of *S. aureus*. The extent of biofilm formation was determined by crystal violet staining of the bacterial cells.

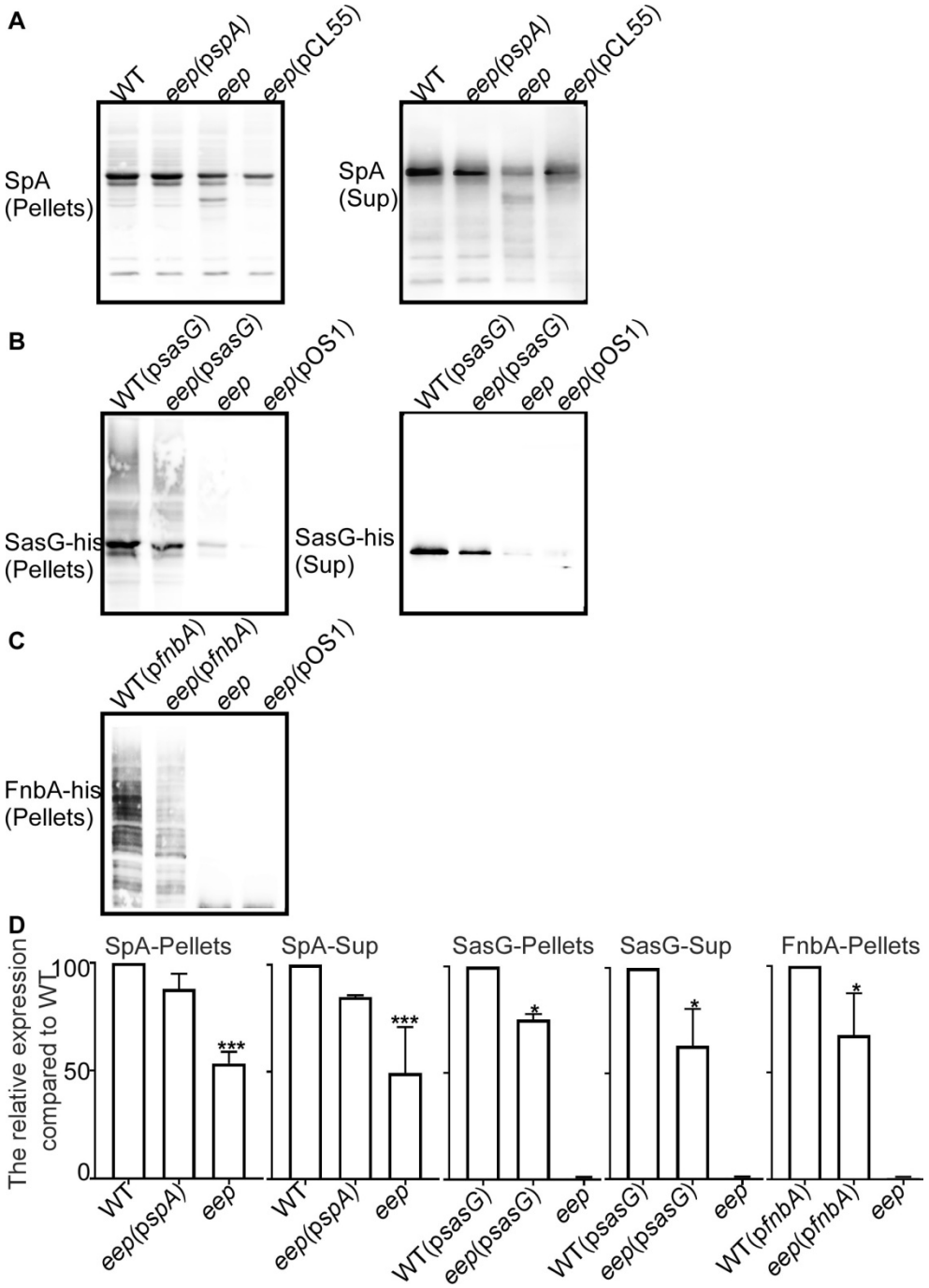

Fig. S6. The confirmation of the additional expression of the adhesins SpA，SasG, and FnbA. (A-C) Western blot analysis for SpA (A), SasG (B) and FnbA (C). An equal number of cells was used for the analysis (see Materials and Methods). SpA protein was detected with anti-SpA antibody, whereas SasG and FnbA proteins were detected with anti-His-tag antibody. Pellets, cell pellets; Sup, supernatant. Due to extensive degradation of FnbA, we could not compare the FnbA expression in the

culture supernatant. (D) The quantification of the Western blot results. The scanned images of the protein bands were quantified by Image J**.** *, $p < 0.05$; ***, $p < 0.001$ by unpaired, two-tailed Student's t-test. WT, *S. aureus* USA300; *eep*, *eep*-deletion mutant; p*spA*, pCL55-*spA*; p*sasG*, pOS1-*sasG*-His; p*fnbA*, pOS1-*fnbA*-His.

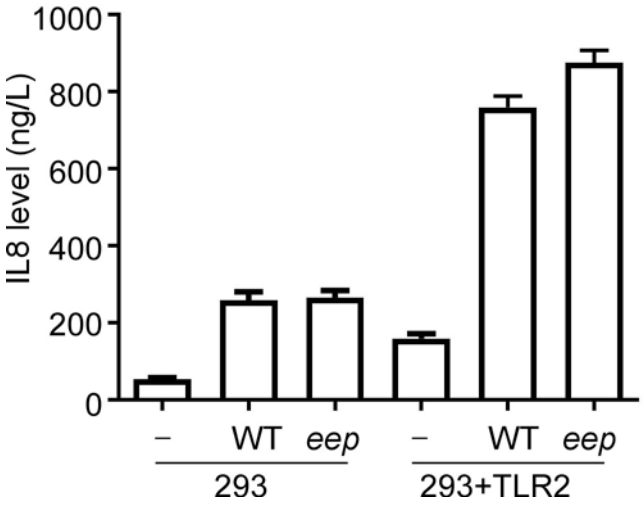

Fig. S7. The effect of the *eep*-deletion on the TLR2-mediated activation of IL8 secretion. The HEK293 cells were transfected with pcDNA3.0-TLR2 for 6 h. Then, the transfected cells were stimulated with either WT or the *eep*-deletion mutant for 24 h. Finally, the IL8 in the culture supernatant was measured by ELISA assay. -, no bacteria; WT, the wild-type USA300; *eep*, the *eep*-deletion mutant of USA300.

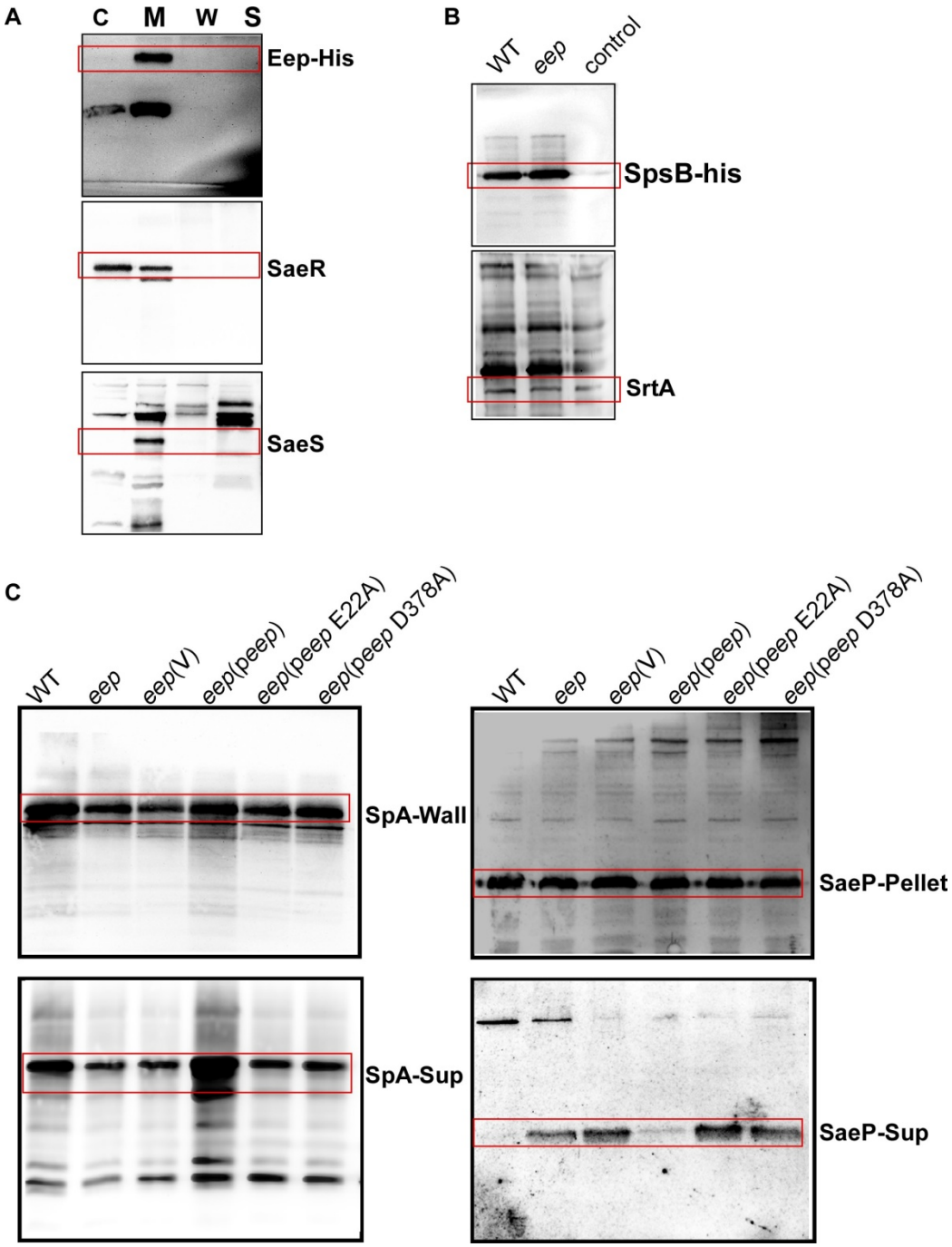

Fig. S8. Full-length Western blots.(A) Full-length blots for Fig 1D. (B) Full-length blots for Fig 3. (C) Full-length blots for Fig 4.

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

Strain or plasmid	Relevant characteristic	Origin or reference
E. coli		
DH5α	Plasmid free, restriction deficient	New England Biolabs
S. aureus		
RN4220	Restriction deficient, prophage cured	(1)
USA300-P23	USA300-0114 without plasmid 2 and 3	(2)
USA300Δ*eep*	USA300-P23with the deletion of *eep*	This study
USA300Δ*camS*	USA300-P23 with the deletion of*camS*	This study
NMΔ*phoB*	Newman strain with the deletion of *phoB*	(3)
Plasmid		
pKOR1	Allelic replacement plasmid	(4)
pIMAY	Allelic replacement plasmid	(5)
pKOR1Δ*camS*	pKOR1 containing *camS* deletion cassette	This study
pIMAYΔ*eep*	pIMAY containing *eep* deletion cassette	This study
pCL55	An integration vector for *S. aureus*	(6)
p*eep*	pCL55 carrying the *eep* gene with His-tag sequence at the C-terminus	This study
p*eep* E22A	p*eep* with the E22A mutation	This study
p*eep* D378A	p*eep* with the D378A mutation	This study
p*eep* S2-phoB	p*eep* where the *phoB* gene without signal	This study

	peptide sequence was inserted at S2 position	
p*eep* E38-phoB	p*eep* where the *phoB* gene without signal peptide sequence was inserted at E38 position	This study
p*eep* N91-phoB	p*eep* where the *phoB* gene without signal peptide sequence was inserted at N91 position	This study
p*eep* T250-phoB	p*eep* where the *phoB* gene without signal peptide sequence was inserted at T250 position	This study
p*eep* G339-phoB	p*eep* where the *phoB* gene without signal peptide sequence was inserted at G339 position	This study
p*eep* Y387-phoB	p*eep* where the *phoB* gene without signal peptide sequence was inserted at Y387 position	This study
p*eep* D422-phoB	p*eep* where the *phoB* gene without signal peptide sequence was inserted at Y387 position	This study
p*spA*	pCL55 carrying the *spa* gene with its own promoter	This study
pOS1	A multi-copy plasmid for *S. aureus*	(7)
pOS1-*camS*-his	pOS1 carrying the *camS* gene with His-tag at the C-terminus	This study
pOS1-*spsB*-His	pOS1 carrying the *spsB* gene with His-tag at the C-terminus	This study
pOS1-*fnbA*-His	pOS1 carrying the *fnbA* gene with His-tag at the C-terminus	This study

pOS1-*sasG*-His	pOS1 carrying the *sasG* gene with His-tag sequence at the C-terminus	This study

Table S2. Oligonucleotides used in this study.

Name	Sequence (5' → 3')	Target
P1986	ATTGGATTGGAAGTAC GCTTTGGCAGTTTATTCTTGACATGTA	LIC for pIMAY
P1987	ATTGGAAGTGGATAAC CGAAGTGATCTTCCGTCACAGGTATT	LIC for pIMAY
P2481	TACTTCCAATCCAATG GGCGGCCTTGTGTTAATGATA	For *eep* deletion
P2482	AATGCCTGCTCTTTTCGCAAAAAAC	For *eep* deletion
P2483	GTTTTTTGCGAAAAGAGCAGGCATT TTTAGGAGGATAAATAATTATG	For *eep* deletion
P2484	TTATCCACTTCCAATG CTAATTCAATATTATCTGTGCC	For *eep* deletion
P236	ATTGGAAGTGGATAACGGTACCGGTTCCGAGGCTC	LIC for pKOR1
P237	ATTGGATTGGAAGTACGGGCCCGAGCTTAAGACTGG	LIC for pKOR1
P474	TACTTCCAATCCAATG CGTTTCATGATGTAGCAGCTATG	For *cams* deletion
P475	TTGTAAATTAAAGATAATAAAAAGG	For *cams* deletion
P476	CCTTTTTATTATCTTTAATTTACAA GACATACCCCTCTAACTATTTA	For *cams* deletion
P477	TTATCCACTTCCAATG CAGGTGTAGTCACACCTACTGC	For *cams* deletion
P35	ATTGGAAGTGGATAAC CGGAGGAGGGATGTAAAATGTGG	pCL55
P80	ATTGGATTGGAAGTAC GAATTCTTGAAGACGAAAGGGCCTCG	pCL55
P249	TTATCCACTTCCAATG GCTAATATATTAGCATTGATTGC	For *eep* complement
P78	TACTTCCAATCCAATGctaatgatgatgatgatgatgTAAGAAATAT CGTCGAATATC	For *eep* complement
PL85	GTAACTGTTCAT gca TATGGCCATATG	For *eep* E22A
PL86	CATATGGCCATA TGC ATGAACAGTTAC	For *eep* E22A
PL87	CCTATTCCTGCACTA gca GGTGGTCGTATT	For *eep* D378A
PL88	AATACGACCACC TGC TAGTGCAGGAATAGG	For *eep* D378A
PL22	TATTTAGTTACAATAATTGCA	For p*eep* S2-phoB
PL23	GCTCACTCGCTACACCTCGATTG	For p*eep* S2-phoB
PL24	CAATCGAGGTGTAGCGAGTGAGC ATGCAATCCGATAAAAGTTCTAAAG	For p*eep* S2-phoB
PL25	TGCAATTATTGTAACTAAATA	For p*eep* S2-phoB

	CTTGAATATATCAAATATTATTTTTGC	
PL80	TTTGCGATCGGTATGGGGCCA	For p*eep* E38-phoB
PL81	TTCTGGACACATAATGCCTGCTC	For p*eep* E38-phoB
PL82	GAGCAGGCATTATGTGTCCAGAA ATGCAATCCGATAAAAGTTCTAAAG	For p*eep* E38-phoB
PL83	TGGCCCCATACCGATCGCAAA CTTGAATATATCAAATATTATTTTTGC	For p*eep* E38-phoB
PL26	GAAGAAAATGAAATAACACATATC	For p*eep* N91-phoB
PL27	ATTAAGTTTAATTTTAACGTTCATAC	For p*eep* N91-phoB
PL28	GTATGAACGTTAAAATTAAACTTAAT ATGCAATCCGATAAAAGTTCTAAAG	For p*eep* N91-phoB
PL29	GATATGTGTTATTTCATTTTCTTC CTTGAATATATCAAATATTATTTTTGC	For p*eep* N91-phoB
PL30	ACTGTTAAATTTGAACGTGATGG	For p*eep* T250-phoB
PL31	CGTCTTATTATCTTTAACTTTATC	For p*eep* T250-phoB
PL32	GATAAAGTTAAAGATAATAAGACG ATGCAATCCGATAAAAGTTCTAAAG	For p*eep* T250-phoB
PL33	CCATCACGTTCAAATTTAACAGT CTTGAATATATCAAATATTATTTTTGC	For p*eep* T250-phoB
PL34	ATTTATCATAACGTCGACTCAG	For p*eep* G339-phoB
PL35	ACCAACCGGACCATTTAACATATC	For p*eep* G339-phoB
PL36	GATATGTTAAATGGTCCGGTTGGT ATGCAATCCGATAAAAGTTCTAAAG	For p*eep* G339-phoB
PL37	CTGAGTCGACGTTATGATAAAT CTTGAATATATCAAATATTATTTTTGC	For p*eep* G339-phoB
PL38	GAAGCGATTTTCAGAAAACCAG	For p*eep* Y387-phoB
PL39	ATATATAACAAATAAAATACGAC	For p*eep* Y387-phoB
PL40	GTCGTATTTTATTTGTTATATAT ATGCAATCCGATAAAAGTTCTAAAG	For p*eep* Y387-phoB
PL41	CTGGTTTTCTGAAAATCGCTTC CTTGAATATATCAAATATTATTTTTGC	For p*eep* Y387-phoB
PL42	ATTCGACGATATTTCTTACATC	For p*eep* D422-phoB
PL43	ATCATTCCACGTTACTAATATC	For p*eep* D422-phoB
PL44	GATATTAGTAACGTGGAATGAT ATGCAATCCGATAAAAGTTCTAAAG	For p*eep* D422-phoB
PL45	GATGTAAGAAATATCGTCGAAT CTTGAATATATCAAATATTATTTTTGC	For p*eep* D422-phoB
PL428	GAG***CCCGGG*** CCAAACCTGGTCAATCAATGGCG	For SpsB-his expression
PL429	GCC***GTCGAC***ctaatgtgatgatgatgtgATTTTTAGTATTTTCAGG ATTGAAATTATG	For SpsB-his expression
PL351	GAGCCCGGG ATGAAGCGTACATTAGTATTATTG	For CamS-his expression
PL352	GAGGGATCC cta atg atg atg atg atg atg ATTACTGTAA ATATGAACTTGCGGTTC	For CamS-his expression
P221	GAG***CCCGGG***CGCAAGTGTGCTGT	For p*spA*

P222	GAGGGATCCTTATAGTTCGCGACGACGTC	For p*spA*
PL501	GAG***CCCGGG*** CTGATGACTTGAATACAATTTATAGG	For FnbA-his expression
PL502	GAGGGATCC ctaatgatgatgatgatgatg TGCTTTGTGATTCTT TTTATTTCTGCG	For FnbA-his expression
PL503	GAG***CCCGGG*** GAAATTCAATGCTATTGAGTTGATGGG	For SasG-his expression
PL504	GAGGGATCC ctaatgatgatgatgatgatg AATCCGGATTAAATT TACGTTCTTTCTTG	For SasG-his expression
PL464	cgc GGTACC G ATGCCACATACTTTGTGGATGG	For TLR2 overexpression
PL465	CGC TCTAGA CTAGGACTTTATCGCAGCTCTCAG	For TLR2 overexpression
P41	CAGCAAACCATGCAGATGCTA	Real-time PCR for *spA*
P42	ACCGATGAATGGATTTTCTTCAC	Real-time PCR for *spA*
PL152	CAACCATTGCGATTTCTTTACC	Real-time PCR for *saeP*
PL153	TTAGCTTTAGGTGCTTGTGG	Real-time PCR for *saeP*
P43	CAAATGATCACAGCATTTGGTACAG	Real-time PCR for *gyrB*
P44	CGGCATCAGTCATAATGACGAT	Real-time PCR for *gyrB*

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