Insights into the regulation of small RNA expression: SarA represses the expression of two sRNAs in *Staphylococcus aureus*

Tony Mauro[1,†], Astrid Rouillon[1,†,*], and Brice Felden[1,*]

[1]Inserm U835, Biochimie Pharmaceutique, University of Rennes 1, 35000 Rennes, France.

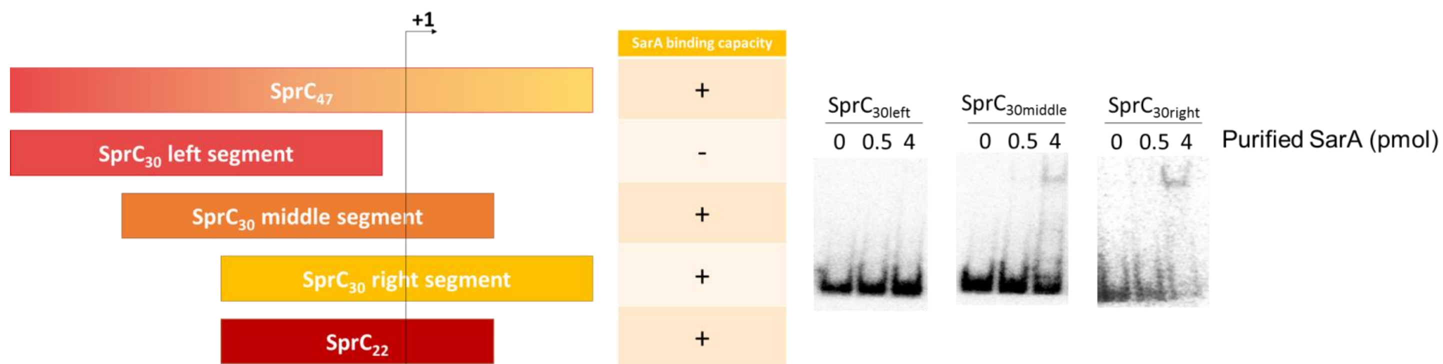
* To whom correspondence should be addressed: Tel: +33 2 2323 4851 Fax: +33 2 2323 4456; Email: astrid.rouillon@univ-rennes1.fr. Correspondence may also be addressed to brice.felden@univ-rennes1.fr.

† These authors contributed equally to this work.

SUPPORTING INFORMATIONS

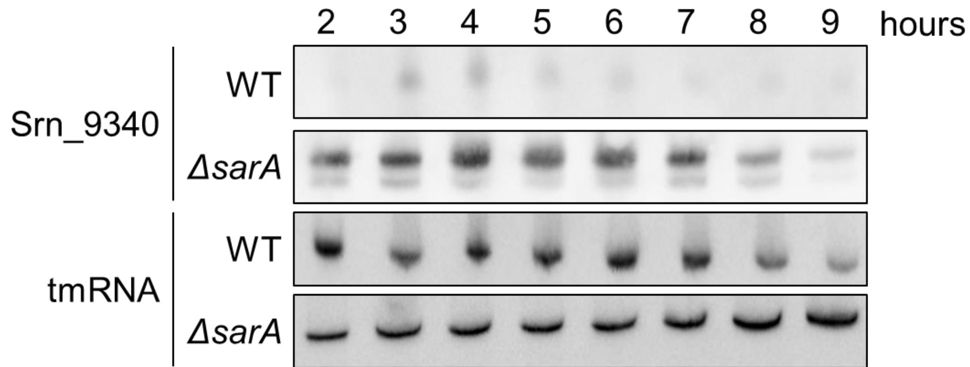
SUPPLEMENTARY FIGURE S1: A 30 nt overlapping sequence from *sprC₄₇* defines a 22 nt sequence necessary for SarA binding.

EMSA experiments were performed using 10 fmol radiolabeled 30 nt fragments from *sprC*$_{47}$ as probes, in the presence of an increasing amount (0.5 to 4 pmol) of 6His-tagged SarA.



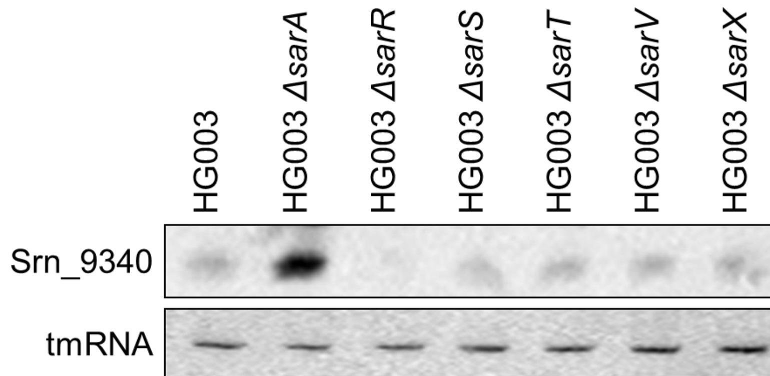
SUPPLEMENTARY FIGURE S2: *srn_9340* expression during bacterial growth of *S. aureus* HG003 and HG003Δ*sarA* strains.

Northern blot analysis of Srn_9340 in *S. aureus* HG003 strain (WT) and its isogenic HG003Δ*sarA* mutant. Samples were taken at the specified time points during bacterial growth, and tmRNA was used as a loading control.



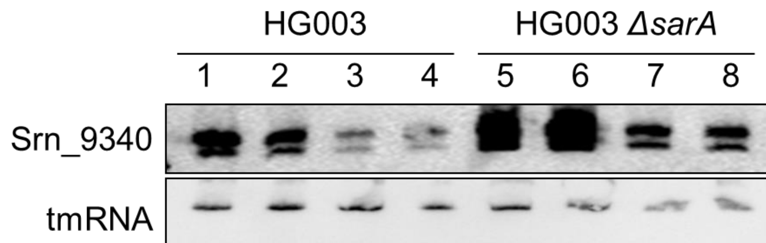
SUPPLEMENTARY FIGURE S3: *srn_9340* expression increases in the absence of SarA.

srn_9340 expression in the different *S. aureus* isogenic strains, each lacking a TF (SarA, SarR, SarS, SarT, SarV, and SarX). Northern blot analysis of Srn_9340 in the *S. aureus* HG003 strain and its isogenic mutants for SarA protein family members. All samples were taken after 5 hours of growth. 10 µg of total RNA were loaded and the Digoxigenin method (Roche) was used to reveal Srn_9340 and tmRNA (loading control).



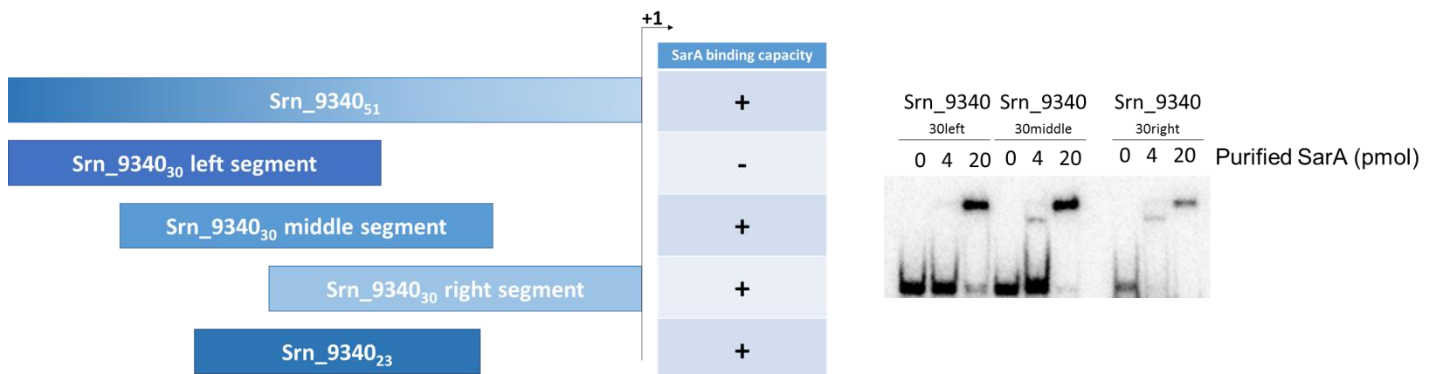
SUPPLEMENTARY FIGURE S4: Functional complementation studies.

Effect of SarA on the transcriptional activity of the *srn_9340* promoter. *S. aureus* HG003 or HG003Δ*sarA* strains were co-transformed with pCN36/pCN41c empty vectors (lanes 1 and 5); pCN36/pCN41c-P_{sprC} (lanes 2 and 6); pCN36-SarA /pCN41c (lanes 3 and 7); and pCN36-SarA/pCN41c-P_{sprC} (lanes 4 and 8). Srn_9340 and tmRNA (loading control) levels were assessed by Northern blot.



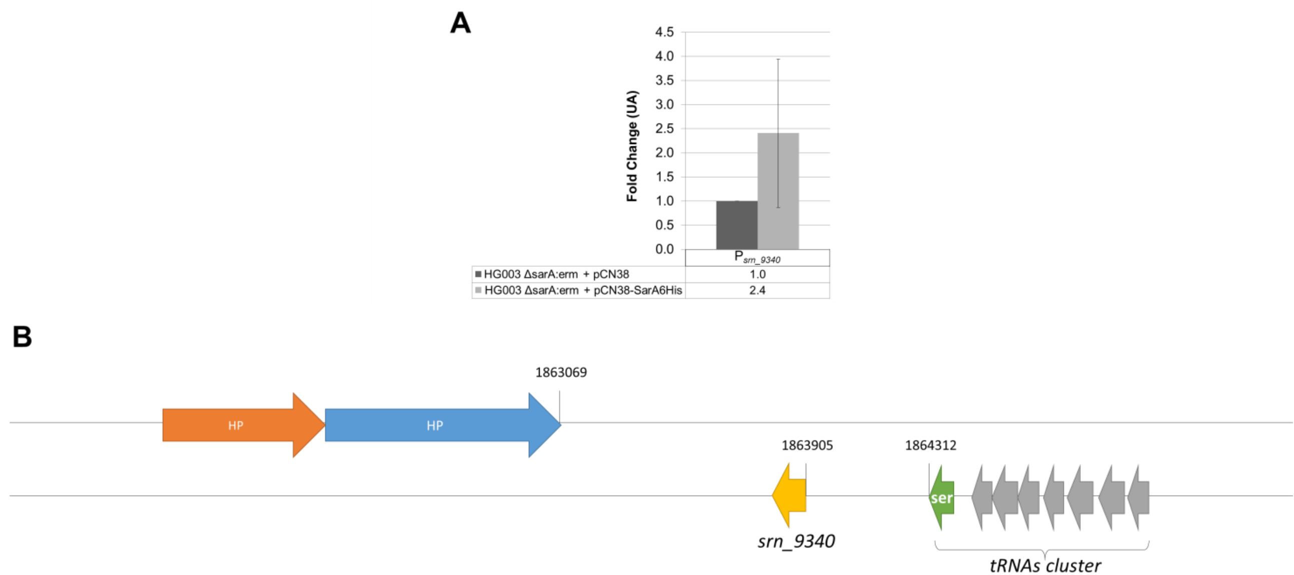
SUPPLEMENTARY FIGURE S5: Overlapping 30 nt sequences from srn_9340_{51} define a 23 nt sequence that is necessary for SarA binding.

EMSA experiments were performed using 10 fmol of radiolabeled 30 nt-long srn_9340_{51} fragments as probes in the presence of an increasing amount (4 to 20 pmol) of 6His-tagged SarA.



SUPPLEMENTARY FIGURE S6: tRNA gene clusters skewed ChIP analysis for RNA polymerase binding onto *srn_9340* promoter *in vivo*.

(A) The *S. aureus* HG003Δ*sarA* strain was transformed either with an empty pCN38 vector (dark gray) or with pCN38-SarA6His (light gray). ChIP experiments were conducted using antibodies specific for the σ70/σA RNA polymerase subunit. No statistically significant differences were observed in the *srn_9340* promoter fragment enrichments in the presence of SarA. Error bars show ± standard deviation. **(B)** Flawed ChIP analysis of the highly transcribed $tRNA^{Ser}$ gene, which is only 408 nt away from the *Srn_9340* promoter.



SUPPLEMENTARY TABLE S1 Strains used in this study

	Strains	Comments	References
***E. coli* strain**	XL1-Blue	Δ(ara-leu) 7697 araD139 fhuA ΔlacX74 galK16 galE15 e14-ϕ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL (StrR) rph spoT1 Δ(mrr-hsdRMS-mcrBC)	
***S. aureus* strains**	RN4220	Restriction-defective derivative of 8325-4	Kreiswirth et al., 1983 [47]
	HG003	rsbU and tcaR restored strain 8325	Herbert et al., 2010 [45]
	HG003 ΔsarA	HG003 strain deleted for sarA(erythromycin resistance)	This work
	HG003 ΔsarR	HG003 strain deleted for sarR (erythromycin resistance)	This work
	HG003 ΔsarS	HG003 strain deleted for sarS (erythromycin resistance)	This work
	HG003 ΔsarT	HG003 strain deleted for sarT (erythromycin resistance)	This work
	HG003 ΔsarV	HG003 strain deleted for sarV (erythromycin resistance)	This work
	HG003 ΔsarX	HG003 strain deleted for sarX(erythromycin resistance)	This work

SUPPLEMENTARY TABLE S2 DNA primers used in this study

	Name	Sequence	References
Cloning Primers	5'_BamHI_P*sprC*	GGCCGGATCCAAGTGTATAATATCAATTTACTAC	This work
		forward primer of *sprC* promoter flanked by BamHI restriction site	
	P*sprC*_EcoRI_3'	GGCCGAATTCATTTATATTATAATATAAATATT	This work
		reverse primer of *sprC* promoter flanked by EcoRI restriction site	
	5'_BamHI_P*sarA*	GGCCGGATCCCTATTGGTCTATTATGTATTTTG	This work
		forward primer of *sarA* promoter flanked by BamHI restriction site	
	*sarA*_KpnI_	GGCCGGTACCTATGTGATATATAAACCTAGG	This work

	3'	reverse primer of *sarA*gene flanked by KpnI restriction site	
	P*sarA*_BamHI_3'	GGCCGGATCCGTTTAAAACCTCCCTATTTG	This work
		reverse primer of *sarA* promoter flanked by BamHI restriction site	
	5'_PstI_P*sarA*	GGCCCTGCAGCTATTGGTCTATTATGTATTTTG	This work
		forward primer of *sarA* promoter flanked by PstI restriction site	
	T*sarA*_KpnI_3'	GGCCGGTACCGTTAATTAATGGGGAACATG	This work
		forward primer of *sarA* terminater flanked by KpnI restriction site	
	5'_KpnI_T*sarA*	GGCCGGTACCTAATTTTGTTTAGCGCAATTTGG	This work
		reverse primer of *sarA* terminater flanked by KpnI restriction site	
	5'_BamHI_*sarA*	GGCCGGATCCATGGCAATTACAAAAATCAATG	This work
		forward primer of *sarA* gene flanked by BamHI restriction site	
	*sarA6his*_KpnI_3'	GGCCGGTACCTTAGTGGTGGTGGTGGTGGTGTAGTTCAATTTCGTTGTTTGC	This work
		reverse primer of *sarA6His* gene flanked by KpnI restriction site	
Probe for Northern Blot	Srn_3610_SprC	CGGCTACTACATTCGCATGT	Le Pabic et *al,* 2015 [25]
		probe for Northern Blot analysis	
	SarA	AGCTAATTGAAAATTCCTTTTTAATTAAACTTTTTAATTTGTCAGCATAAGTGACCATTGATAACAACTCAAAGCAATCATTGATTTTTGTAATTGCCAT	Hallier M, laboratory
		probe for Northern Blot analysis	
	tmRNA	ACACGCTTAATGAGCTCGGG	This work
		probe for Northern Blot analysis	
	16S rRNA	TTCATCATATAACAAATGTAATGAGATATGATGTTGGTAGTAAAATTATTTTAACTTTTAATGATCAAACAGTTTATTTATGTGAAATTGCTGACATAAG	Hallier M, laboratory
		probe for Northern Blot analysis	
	Srn_9340	CGACTAACTCCTGGTTATGC	This work
		probe for Northern Blot analysis	

RACE primers	R1_srn_9340	CTGGTTATGCTACGCTTTAC	This work
	R2_srn_9340	CTTTACTTTATAATCCGCAC	This work
	F1_srn_9340	GTTAGTCGATTTTTAATAAATT	This work
	F2_srn_9340	CGACTAAATTATTAATAAAG	This work
EMSA,DNaseI Footprinting and *in vitro* transcription primers	5'_P*sprC*	AAGTGTATAATATCAATTTA	This work
		forward primer of *srn_3610_sprC* promoter	
	*sprC*_3'	ATCGCTTACTTCATCTAAAA	This work
		reverse primer of *srn_3610_sprC* gene	
	*srn_9340*_HindIII_3'	CCGGAAGCTTCGACTAACTCCTGGTTATGC	This work
		reverse primer of *srn_9340* gene flanked by HindIII restriction site	
	5'_P*srn_9340*	GTTTCATTATCTTCAAATTCTTTAATC	This work
		forward primer of *srn_9340* promoter	
	*srn_9340*_seq_3'	AGCTTCGACTAACTCCTGGTTATGC	This work
		reverse primer of *srn_9340* gene sequencing	
	*sprC*_seq_3'	AGCTTCTACTCTCATGGCAATTTATAC	This work
		reverse primer of *srn_3610_sprC* gene sequencing	
	5'_*16S*	CAAAAGTGAAAGACGGTCTTG	This work
		forward primer of *16S* promoter	
	267_*16S*_3'	CACATATGTTCTTCCCTAATAAC	This work
		reverse primer of *16S* gene	
	225_*16S*_3'	ACCTTCATCACTCACGCGGC	This work

		reverse primer of *16S* gene	
	P267Δ47*sprC_3'*	ATTCGCATGTACTTCGTTTTACTGTCCACGAATACTTAATTTTAAAAAATCC	This work
		reverse primer of *srn_3610_sprC*$_{P267\Delta 47}$	
	5'_P267Δ47 *sprC*	TTTCATAGGGGATTTTAAAATTAAGTATTCGTGGACAGTAAAACGAAGTAC	This work
		forward primer of *srn_3610_sprC*$_{P267\Delta 47}$	
	P225Δ51*sprC_3'*	ACTTAAAGAAAATCAAATCGGCATCTACGTGCGGA	This work
		reverse primer of *srn_9340*$_{P225\Delta 51}$	
	5'_P225Δ51 *sprC*	ACGTAGATGCCGATTTGATTTTCTTTAAGTAACAGC	This work
		forward primer of *srn_9340*$_{P225\Delta 51}$	
	5'_*sprC*_47	CATGGTCGTTGACTTATTTATATTATAATATAAATATTTTATTTTTC	This work
		forward 47-nt *srn_3610_sprC* oligonucleotides	
	*sprC*_47_3'	GAAAAATAAAATATTTATATTATAATATAAATAAGTCAACGACCATG	This work
		reverse 47-nt *srn_3610_sprC* oligonucleotides	
	5'_rdm_47	GAATTCTCAATAATATAAATTAATAAATTTCAAATTTTTTTGGATCC	This work
		forward 47-nt random oligonucleotides	
	rdm_47_*3'*	GGATCCAAAAAAATTTGAAATTTATTAATTTATATTATTGAGAATTC	This work
		reverse 47-nt random oligonucleotides	
	5'_srn_9340_51	AATATTATATATACGCTTGAAAAAATAAAATAATAATATTATAATATAATC	This work
		forward 51-nt *srn_9340*oligonucleotides	
	srn_9340_51_3'	GATTATATTATAATATTATTATTTTATTTTTTCAAGCGTATATATAATATT	This work
		reverse 51-nt *srn_9340*oligonucleotides	
	5'_rdm_*51*	ATATATTCATAATTAATAATGGATTTAATTTTATCAAAATATGAATATTTT	This work
		forward 51-nt random oligonucleotides	
	rdm_51_*3'*	AAAATATTCATATTTTGATAAAATTAAATCCATTATTAATTATGAATATAT	This work
		reverse 51-nt random oligonucleotides	

	P267Δ22*sprC_3'*	GTCCACGCATGGTCGTAAATATTTTATTTTTCAATAC	This work
		reverse primer of *srn_3610_sprC*$_{P267\Delta22}$	
	5'_P267Δ22 *sprC*	GAAAAATAAAATATTTACGACCATGCGTGGACAGTAAAACG	This work
		forward primer of *srn_3610_sprC*$_{P267\Delta22}$	
	5'_P225Δ23 *sprC*	CAAATATTATATATACGCTATAATATAATCAATCGGCATCTACG	This work
		forward primer of *srn_9340*$_{P225\Delta23}$	
	P225Δ23*sprC_3'*	GCCGATTGATTATATTATAGCGTATATATAATATTTGATTTTC	This work
		reverse primer of *srn_9340*$_{P225\Delta23}$	
	5'_*sprC*_30left	GAAAAATAAAATATTTATATTATAATATAA	This work
		forward 30-nt *srn_3610_sprC* oligonucleotides	
	*sprC*_30left_3'	TTATATTATAATATAAATATTTTATTTTTC	This work
		reverse 30-nt *srn_3610_sprC* oligonucleotides	
	5'_*sprC*_30 middle	TTGACTTATTTATATTATAATATAAATATT	This work
		forward 30-nt *srn_3610_sprC* oligonucleotides	
	*sprC*_30middle_3'	AATATTTATATTATAATATAAATAAGTCAA	This work
		reverse 30-nt *srn_3610_sprC* oligonucleotides	
	5'_*sprC*_30right	TATTATAATATAAATAAGTCAACGACCATG	This work
		forward 30-nt *srn_3610_sprC* oligonucleotides	
	*sprC*_30right_3'	CATGGTCGTTGACTTATTTATATTATAATA	This work
		reverse 30-nt *srn_3610_sprC* oligonucleotides	
	5'_*srn_9340*_30left	AATATTATATATACGCTTGAAAAAATAAA	This work
		forward 30-nt *srn_9340* oligonucleotides	
	*srn_9340*_30left_3'	TTTTATTTTTTCAAGCGTATATATAATATT	This work
		reverse 30-nt *srn_9340* oligonucleotides	
	5'_*srn_934*	ATACGCTTGAAAAAATAAAATAATAATATT	This work

	*0*_30middle	forward 30-nt *srn_9340* oligonucleotides	
	*srn_9340*_30middle_3'	AATATTATTATTTTATTTTTTCAAGCGTAT	This work
		reverse 30-nt *srn_9340* oligonucleotides	
	5'_*srn_9340*_30right	AAAATAAAATAATAATATTATAATATAATC	This work
		forward 30-nt *srn_9340* oligonucleotides	
	*srn_9340*_30right_3'	GATTATATTATAATATTATTATTTTATTTT	This work
		reverse 30-nt *srn_9340* oligonucleotides	
	3’_EcoRI_T*sprC*	GGCCGAATTCCGTATACTGTTATAACTGAA	This work
		reverse primer of *srn_3610_sprC* terminator by EcoRI restriction site	
	5'_BamHI_P*srn_9340*	GGCCGGATCCATCAAATGTGAATATGTTTTAAGCG	This work
		forward primer of *srn_9340* promoter flanking by BamHI restriction site	
	3’_EcoRI_T*srn_9340*	GGCCGAATTCTCAAATATCATTGAAACTTT	This work
		reverse primer of *srn_9340* promoter flanking by EcoRI restriction site	
qPCR primers	5'_*sprC*_qPCR	AAATAAGTCAACGACCATGCGTGGA	This work
		forward primer of *srn_3610_sprC* promoter	
	sprC_3'_qPCR	TCATAAACTGAAGCTTCTACTCTCA	This work
		reverse primer of *srn_3610_sprC* gene	
	5'_*Hla*_qPCR	AACACGTATAGTCAGCTCAGT	This work
		forward primer of *hla* promoter	
	*Hla*_3'_qPCR	GCATTAGCGACAGGATTCATTAA	This work
		reverse primer ofhla promoteur	
	5'_*srn_9340*_qPCR	ACTCCTGGTTATGCTACGCT	This work

		forward primer of *srn_9340* promoter	
	*srn_9340*_3'_qPCR	TCTGCATGACCTAATCGTTTTGA	This work
		reverse primer of *srn_9340* gene	

SUPPLEMENTARY TABLE S3 Plasmids used in this study

Plasmids	Comments	References
pCN36	Low-copy-number shuttle vector with ApR in *E. coli* and TetR in *S. aureus*	Charpentier et *al.*, 2004 [37]
pCN36-SarA	pCN36 with *sarA* under the control of its endogenous promoter	This study
pCN38	Low-copy-number shuttle vector with ApR in *E. coli* and EmR in *S. aureus*	Charpentier et *al.*, 2004 [37]
pCN38-SarA6His	pCN36 with 6Histidine tagged *sarA* under the control of its endogenous promoter	This study
pCN41	Low-copy-number shuttle vector with ApR in *E. coli*, EmR in *S. aureus* and *blaZ* gene	Charpentier et *al.*, 2004 [37]
pCN41c	pCN41 with CmR instead of EmR in S.aureus	This study
pCN41c-P*sprC*	pCN41c with *sprC* promoter	This study
pET42a	*E. coli* expression vector	Novagen
pET42a-SarA	pET42a with the intact *sarA* gene	Hallier M, laboratory

SUPPLEMENTARY TABLE S4 Recipes for buffers used in Chromatin immunoprecipitation assays

Dilution Buffer :	SDS	0.01 %
pH 8.1	Tris-HCl	16.7 mM
	Triton X-100	1.1 %
	EDTA	1.2 mM
	NaCl	167 mM

Low Salt Immune Wash Buffer :	SDS	0.1 %
pH 8.1	Tris-HCl	20 mM
	Triton X-100	1 %
	EDTA	2 mM
	NaCl	150 mM
High Salt Immune Wash Buffer :	SDS	0.1 %
pH 8.1	Triton X-100	1 %
	EDTA	2 mM
	Tris-HCl	20 mM
	NaCl	500 mM
LiCl Immune Complexe Wash Buffer :	LiCl	250 mM
pH 8.1	Tris-HCl	10 mM
	IGEPAL CA630	1 %
	Deoxycholic Acid (Sodium Salt)	1 %
	EDTA	1 mM
TE Buffer :	EDTA	1 mM
pH 8	Tris-HCl	10 mM
Elution Buffer :	SDS	1 %
	$NaHCO_3$	100 mM