SUPPLEMENTARY MATERIAL

A search for small noncoding RNAs in *Staphylococcus aureus* reveals a conserved sequence motif for regulation.

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Content:

MATERIALS AND METHODS

Cell culture

One colony of *S. aureus* (RN6390, COL) was grown overnight at 37°C in 2 to 3 ml of medium, either in brain-heart infusion (BHI), or in NZM medium (10 g/l Casein Tryptone digested; 5 g/l NaCl; 2 g/l $MgSO_4$-$7H_2O$; pH 7.0). From this pre-culture, 200 μl was placed in 10 to 20 ml of medium (BHI or NZM) and incubated at 37°C for 2 h (OD 600 nm 0.5 in BHI, and 0.2 in NZM, exponential phase) and for 6 h (OD 600 nm 5 in BHI, and 2.5 in NZM, late-exponential phase).

Preparation of the genomic DNA

DNA was prepared from 2 ml of RN6390 or COL strains grown in BHI medium for 4 h at 37°C. The cells were centrifuged (3273 g, 4°C, 10 min) and the pellet was dissolved gently in 200 μl of lysis buffer (20 mM Tris-HCl pH 8; 2 mM EDTA; 1.2% Triton X-100). Cell lysis was performed in the presence of 2 μl of lysostaphin 2.5 mg/ml at 37°C for 30 min. The genomic DNA was purified using the DNeasy Tissue kit following the protocol of the company. The genomic DNA was kept at -20°C in a commercial buffer.

Preparation of total RNAs and 3' end-labeling

Total RNAs were prepared from 20 ml of *S. aureus* grown in BHI or NZM medium as described above. After centrifugation of the cells (3273 g, 4°C, 10 min), total RNAs were extracted using the fastRNA Pro Blue kit (Qbiogen, France), and kept at -20°C at a concentration of 1 μg/μl in sterile water. The RNAs (20 μg) were purified by electrophoresis on a 6% polyacrylamide- 8 M urea gel to remove the highly abundant ribosomal RNAs. Bands corresponding to 50-600 nt long RNAs were cut into pieces and the RNAs were eluted at 4°C overnight in 1.5 ml of elution buffer (0.5 M ammonium acetate; 1 mM EDTA; 0.1% SDS). The RNAs were precipitated with 2.5 vol of ethanol and washed in 80% ethanol. The purified RNAs were kept in sterile water at -20°C at a concentration of 5 μg/μl.

The RNAs were labeled at their 3' end using T4 RNA ligase and [α-^{32}P]pCp. The reaction was done at 4°C overnight in 20 μl containing 10 μg total RNAs, 2 μl of commercial buffer 10x, 5 U of T4 RNA ligase (Ambion) and 5 μl [α-^{32}P]pCp (3000 Ci/mmol, 10 mCi/ml, Amersham). Labeled RNAs were purified on a gel filtration column (Micro-Bio Spin P-6, Bio-Rad) to remove the excess of pCp. The quality of the RNAs was controlled by electrophoresis on a 8% polyacrylamide- 8 M urea gel. Labeled RNAs were kept at -20°C.
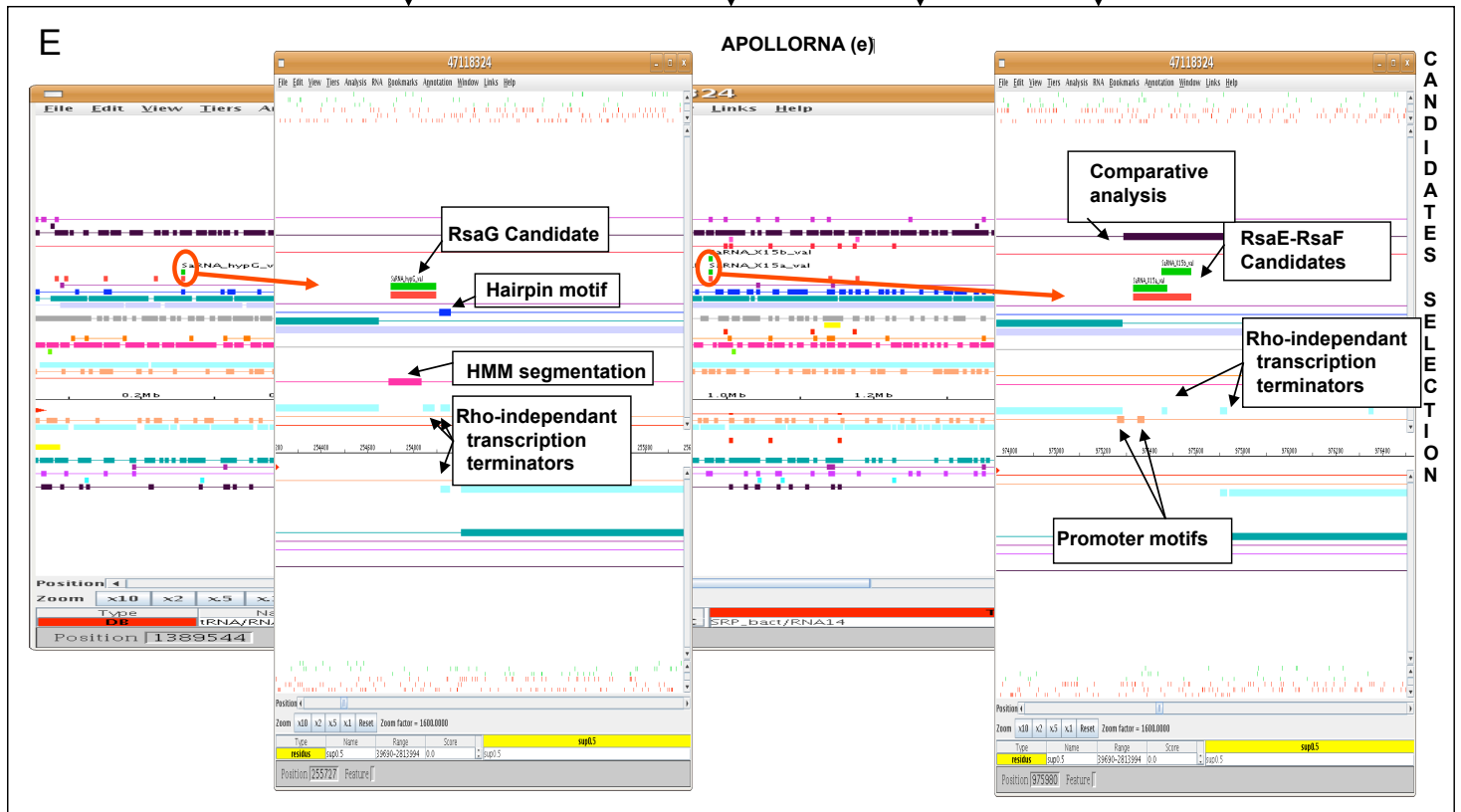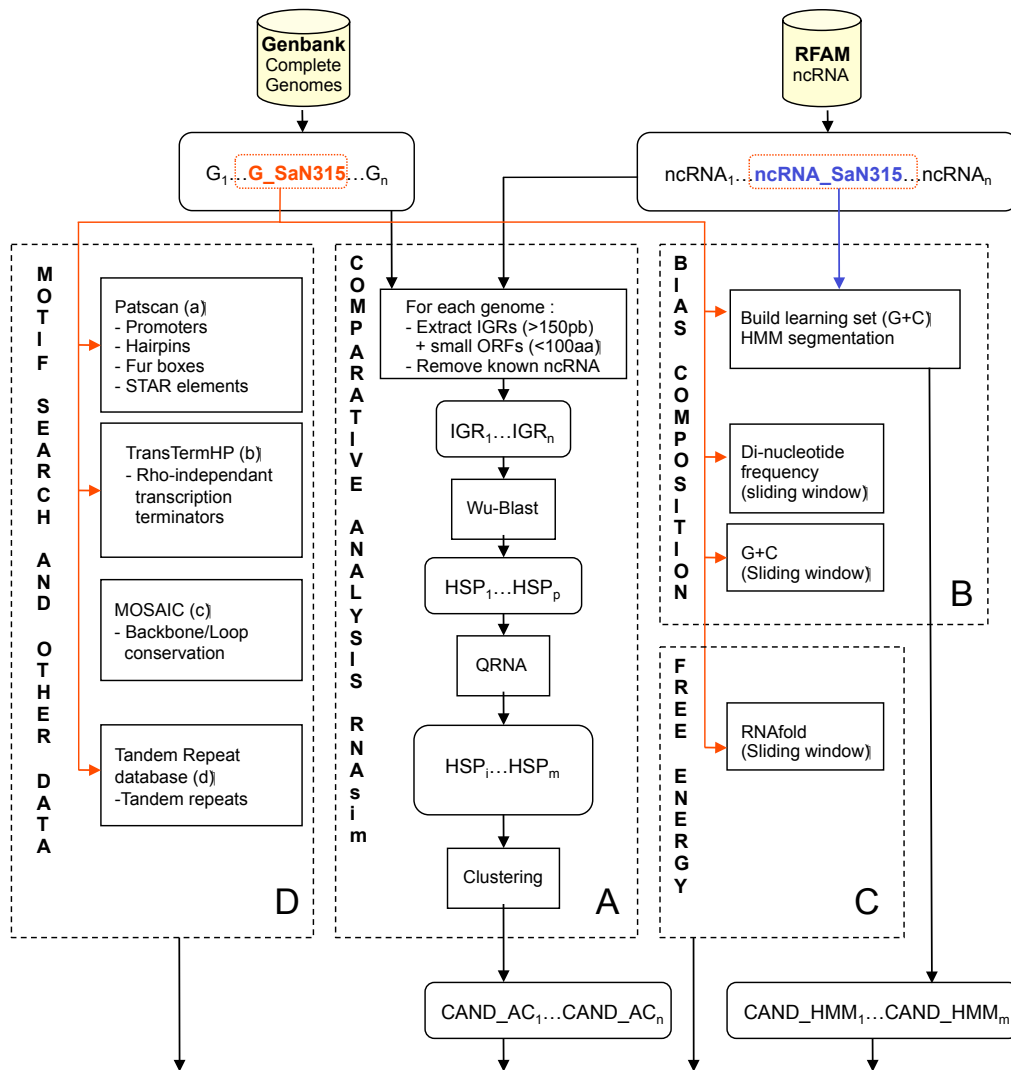
Home-made macroarrays

Macroarrays were done with dsDNAs corresponding to the 36 selected IGRs. The IGRs were PCR amplified from *S. aureus* RN6390 genomic DNA using a set of oligonucleotides (Table S4). The PCR reaction was carried out in a total volume of 50 µl containing 5 µl of commercial buffer 10x, 100 µM dNTP mix, 20 ng genomic DNA, 1 U of Taq DNA polymerase, and 50 pmol of each oligonucleotide. The hybridization temperature varies from 45 to 55°C depending on the stability of the duplexes. After 35 cycles of 30 sec denaturation at 94°C, 30 sec hybridization at 45-55°C, and 1 min elongation at 72°C, the quality of the amplified dsDNA fragments was determined by electrophoresis on a 1% agarose gel. Then, 5 µl of a PCR reaction were mixed in 5 µl of a solution containing 95 % formamide, 18 mM EDTA, and 0.025% SDS, and the dsDNA was denatured 3 min at 90°C followed by cooling on ice. The denatured DNA (2 µl) was then spotted in duplicate on a nitrocellulose membrane and dried. The membrane was incubated 1 h at 42°C in the hybridization buffer (50 mM sodium phosphate pH 7.2, 7 % SDS, 1 mM EDTA). The hybridization buffer was changed and the 3' end-labeled total RNAs, which were previously denatured, were added to the membrane. Hybridization was performed at 42°C overnight. The membrane was washed twice in buffer containing 40 mM sodium phosphate, 5 % SDS, and 1 mM EDTA for 15 min at 42°C. The radioactive spots were visualized by autoradiography of the hybridized membrane, which was previously exposed overnight.

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Figure S1. Strategy used to identify the functional intergenic regions of the *Staphylococcus aureus* N315 genome. Our strategy integrates data generated from complementary approaches (A-C) and data obtained from different sources (D) with the goal to select the most promising candidates. (A) Description of the comparative analysis implemented in a pipeline called RNAsim. Only clusters containing at least one IGR of *S. aureus* N315 genome longer than 150 nt were kept for further analysis. (B) The biased composition analysis relies on three approaches (HMM, di-nucleotide and G+C frequencies). A simple Hidden Markov Model (HMM) was used as an automatic segmentation approach, and was computed by considering known ncRNAs and riboswitches available in RFAM and Genbank databases. Di-nucleotides, G+C frequencies (B) and free energy values (C) were computed on a 90 nt sliding window along the genome. (D) Other data (promoters, STAR, different hairpin motifs) were computed using PatScan. Rho-independent terminators were obtained using TransTermHP. MOSAIC database was used to identify conserved and strain-specific regions among available *S. aureus* genomes. Tandem repeats were extracted from the Tandem Repeat database. (D) The data and the quantitative variables (free energy, G+C content) were integrated into ApolloRNA to select the IGR for functional analysis. Two examples are illustrated: RsaG was selected based on the HMM segmentation, the presence of a promotor and a Rho-independent terminator, and the orientation of the flanking genes; RsaE and RsaF were selected based on RNAsim, the presence of promotors and Rho-independent terminators, and the orientation of the flanking genes. (a-e) web sites from which softwares and/or data were obtained : (a) PatScan (http://www-new.mcs.anl.gov/compbio/PatScan/HTML/scanner.html), (b) TransTermHP (http://transterm.cbcb.umd.edu/index.php), (c) MOSAIC (http://genome.jouy.inra.fr/mosaic), (d) the tandem Repeat Database (http://minisatellites.u-psud.fr), (e) ApolloRNA (http://carlit.toulouse.inra.fr/ApolloRNA/index.html).

Genbank Complete Genomes

RFAM ncRNA

G_1...**G_SaN315**...G_n

$ncRNA_1$...**ncRNA_SaN315**...$ncRNA_n$

M O T I F   S E A R C H   A N D   O T H E R   D A T A

Patscan (a)
- Promoters
- Hairpins
- Fur boxes
- STAR elements

TransTermHP (b)
- Rho-independant transcription terminators

MOSAIC (c)
- Backbone/Loop conservation

Tandem Repeat database (d)
- Tandem repeats

D

C O M P A R A T I V E   A N A L Y S I S   R N A s i m

For each genome :
- Extract IGRs (>150pb) + small ORFs (<100aa)
- Remove known ncRNA

IGR_1…IGR_n

Wu-Blast

HSP_1…HSP_p

QRNA

HSP_i…HSP_m

Clustering

A

B I A S   C O M P O S I T I O N

Build learning set (G+C) HMM segmentation

Di-nucleotide frequency (sliding window)

G+C (Sliding window)

B

F R E E   E N E R G Y

RNAfold (Sliding window)

C

$CAND\_AC_1$…$CAND\_AC_n$

$CAND\_HMM_1$…$CAND\_HMM_m$

E

APOLLORNA (e)

RsaG Candidate

Hairpin motif

HMM segmentation

Rho-independant transcription terminators

Comparative analysis

RsaE-RsaF Candidates

Rho-independant transcription terminators

Promoter motifs

C A N D I D A T E S   S E L E C T I O N

Figure S2. Strategy implemented in RNAsim. (A) Wu-blast comparisons are realized between intergenic regions of genomes A, B and C (including *S. aureus* strains, Staphylocococci or firmicutes). (B) QRNA is used to filter pairwise alignments in order to retain only putative structured RNAs. In this example, the alignment between A4 and C2 is removed from the set of pairwise alignments. (C) Pairwise alignments are clustered when at least one sequence in an alignment overlaps one of the two sequences in another alignment. In this example, A2 aligns with B1 and B1 aligns with C3. A2, B1 and C3 are thus clustered as well as A3, B2 and C1.

Wu-blast comparisons between A, B and C
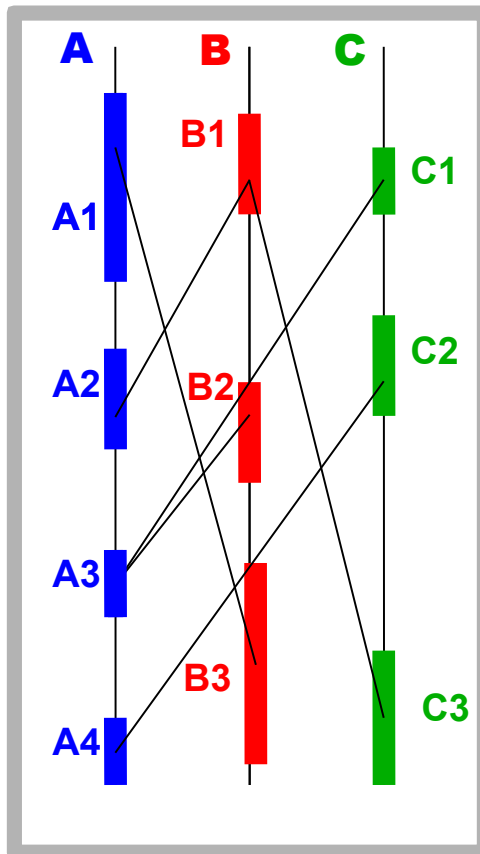
QRNA filtering : retain structured putative RNA
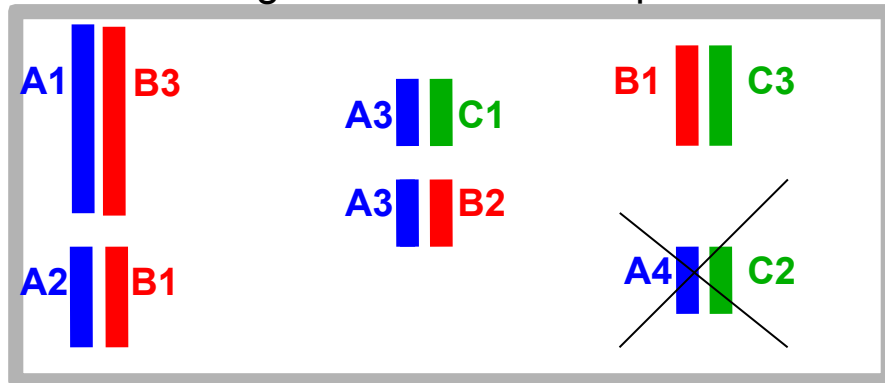
Clustering of sequences covering same loci
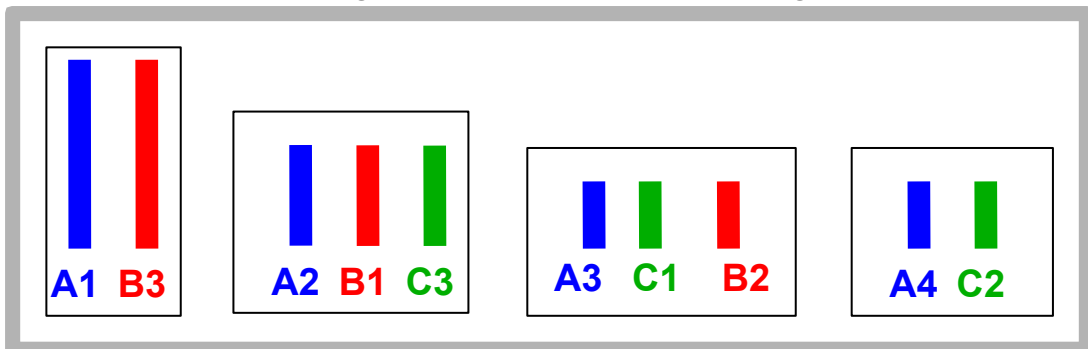
Figure S2- Geissmann et al.

Figure S3. Analysis of Rsa RNA expression. (B) Northern blot analysis of RsaA, RsaC, RsaE, RsaF, and RsaH in various *S. aureus* strains (RN6390, COL, and Newman), LUG774 (RN6390-Δ*rnc*), and LUG911 (RN6390-Δ*hfq*)) grown in BHI (rich medium) or NZM (stringent medium) at the exponential (E) and stationary (S) phases of growth. COL strain was grown under various stress conditions: osmotic stress (NaCl), oxidative stress (H_2O_2, paraquat), iron chelating agent (dipyridyl), acidic pH, cold shock (25°C), and heat shock (42°C). (B) Determination of the half-lives of RsaA, RsaD, RsaE, RsaG, and RsaI. The cells were treated with rifampicin and total RNAs were extracted after 1, 2, 5, 10, 20, 30, and 60 min at 37°C in BHI medium. 5S rRNA was probed to quantify the yield of RNA in each lane. (C) Northern blot analysis of RsaF in *S. aureus* strains: RN6390, RN6911 (Δ*agr*), LUG1160 (RN6390-Δ*rot*, *rot* encodes the repressor of toxins), LUG911 (RN6390-Δ*hfq*), SH1000, COL, and Newman. Total RNAs were prepared from cells grown in BHI medium at the exponential (E) and stationary (S) phases of growth.
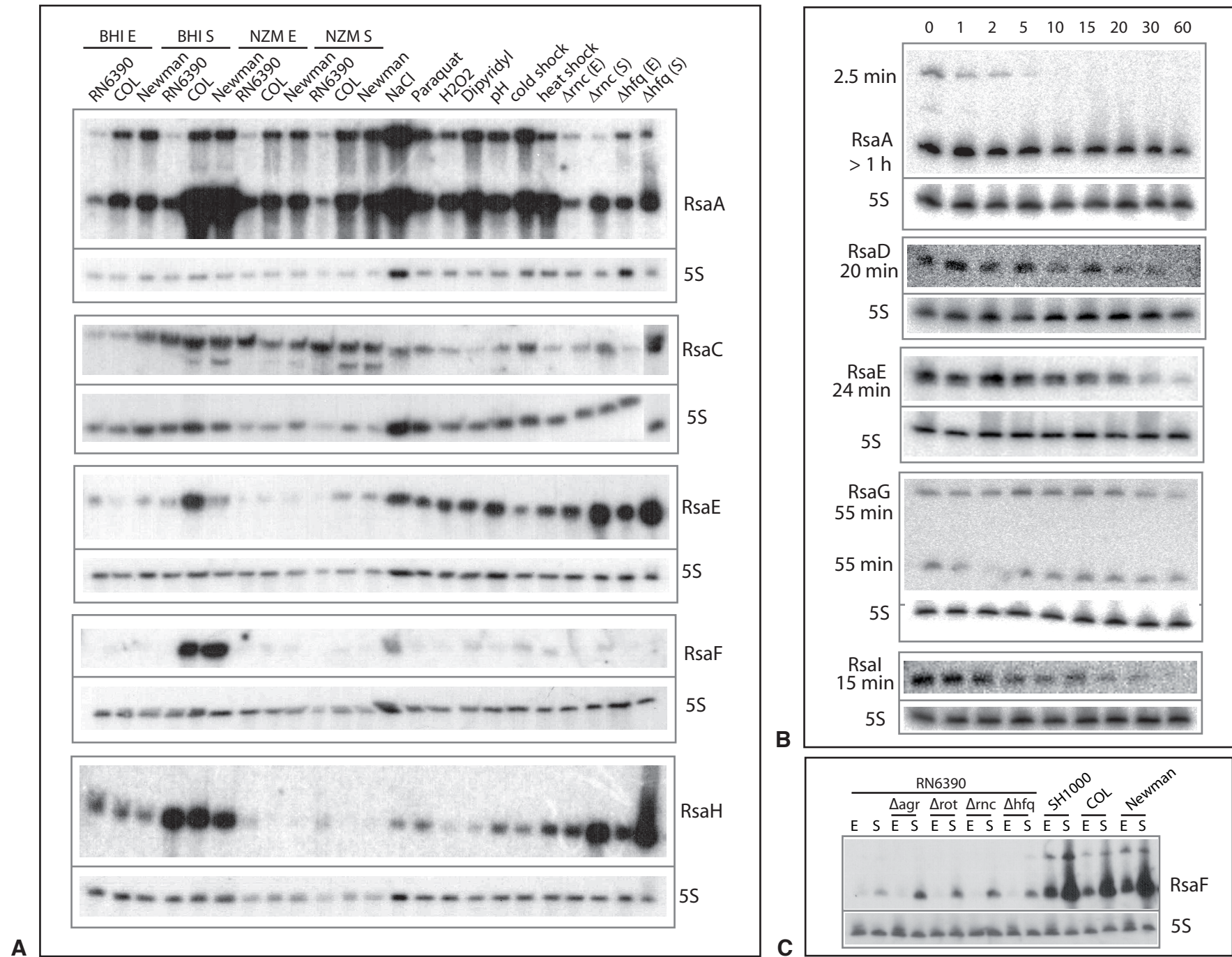
Figure S3-Geissmann et al.

Figure S4. Examples of conserved *cis*-regulatory leader regions of mRNAs in *S. aureus*. (A) Northern blot analysis of RsaK in *S. aureus* strains (RN6390, COL, and Newman). Total RNAs were prepared from cells grown in BHI at the exponential (2 h) or stationary (6 h) phases of growth. Lane L: RNA ladder (Ambion). (B) Sequence of *S. aureus* RsaK corresponding to the leader region of *glcA* mRNA. The leader contains a conserved RAT (ribonucleic antiterminator) motif which is recognized by the glucose sensing regulatory protein GlcT. The stabilization of this structure allows the RNA polymerase to proceed transcription of *glcA* mRNA. (C) The regulatory leader regions of *S. aureus pyr*R and *pyr*P mRNAs. The two regions contain a AGAGAG hairpin loop which was proposed to be the main recognition element for the regulatory protein PyrR in *B. subtilis* (1). In the presence of UMP, PyrR binds to the target mRNAs, and favors the formation of a terminator to induce premature termination of transcription. Alternative pairings leading to the formation of a terminator or of an anti-terminator structure are schematized by encircled grey and black residues.

Figure S4 -Geissmann et al.
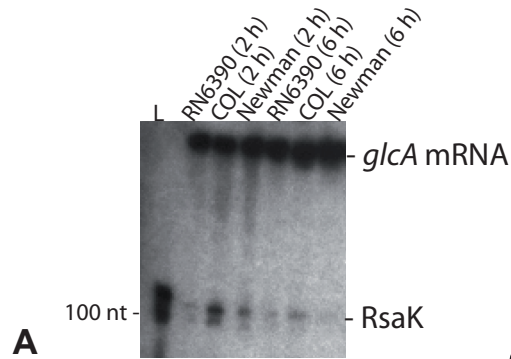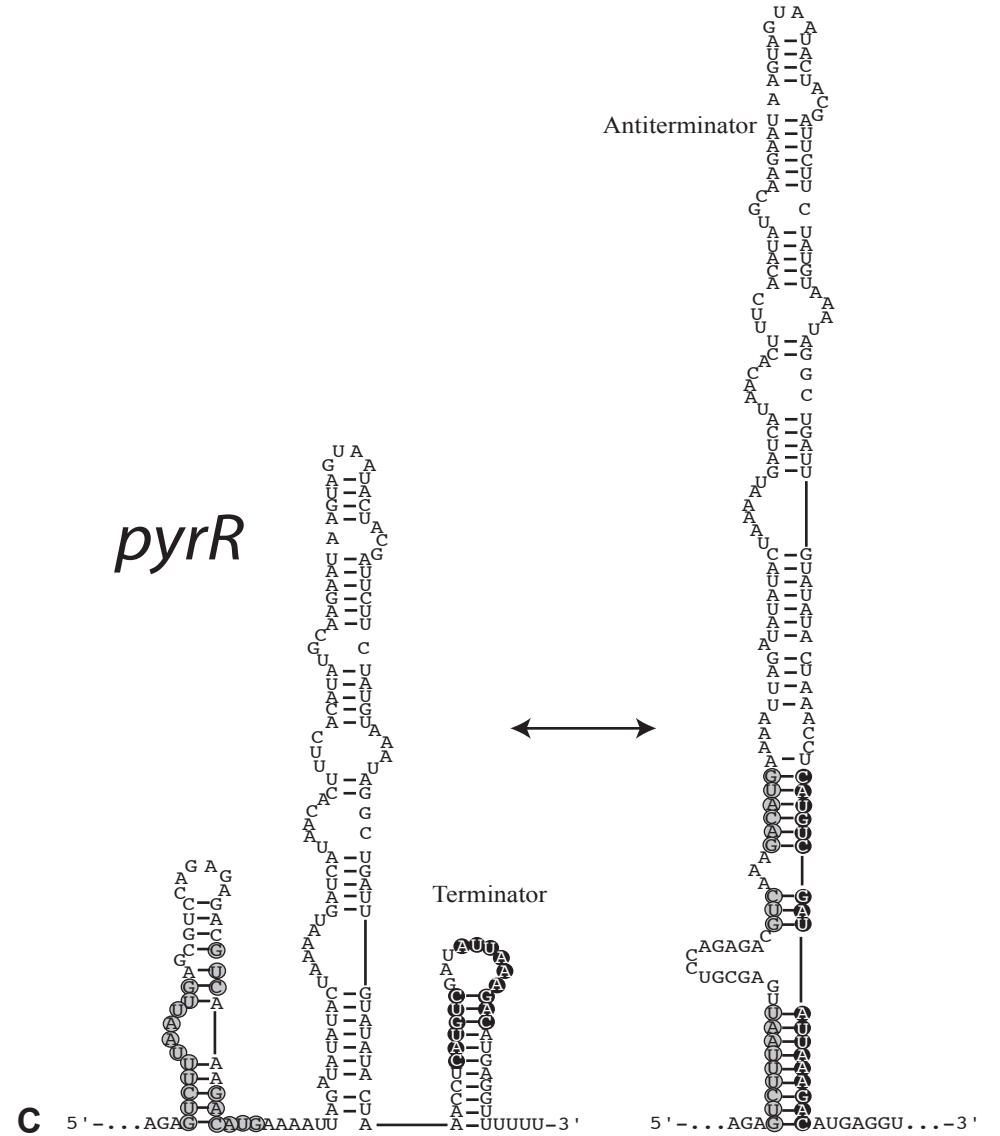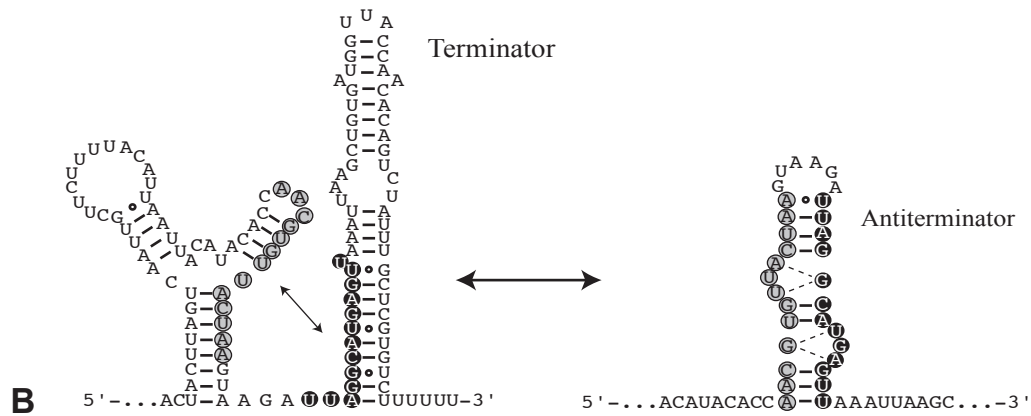
Figure S5. Genomic organization and sequence conservation of RsaE among *Staphylococcaceae* and *Bacillaceae*. (A) The *rsa*E-*rsa*F locus. RsaE is made from an autonomous IGR which contains a σ^A-consensus binding site located at the appropriate distance from the start site of *rsa*E, and a typical Rho-independent terminator of transcription (T1). In contrast to RsaE, RsaF is only present at high levels in strains expressing a fully active σ^B factor. A divergent σ^B-consensus binding site was predicting overlapping the σ^A-promoter. The size of RsaF (104 nts) was evaluated from Northern blots and the 5' start was determined by RACE. The presence of a longer RNA species on Northern blots suggests that RsaF may be the result of an endoribonuclease processing event. It remains to be determined whether the *S. aureus*-specific RsaF is functional, and the significance of the differential expression pattern of the two Rsa RNAs. (B) A schematic view of the sequence alignments of the intergenic region containing *rsa*E and *rsa*F from various *Staphylococcaceae* and *Bacillaceae*: 1-13, *S. aureus* Mu3, JH1, JH9, Mu50, MSSA476, MW2, USA300_TCH1516, Newman, NCTC8325, USA300_FPR3757, COL, MRSA252, and RF122, respectively; 14-15, *S. epidermidis* ATCC12228 and RP62A, respectively; 16, *S. haemolyticus* JCSC1435; 17, *S. saprophyticus* ATCC 15305; 18, *S. carnosus* TM300; 19, *Macrococcus caseolyticus* JCSC5402; 20, *Bacillus pumilus* SAFR-032; 21-22, *B. licheniformis* ATCC 14580 and DSM 13, respectively; 23, *B. subtilis* 168; 24, *B. amyloliquefaciens* FZB42; 25, *Anoxybacillus flavithermus* WK1; 26, *Lysinibacillus sphaericus* C3-41; 27-28, *B. anthracis* A0248 and CDC 684; 29-34, *B. cereus* 03BB102, Q1, G9842, AH820, B4264, and AH187, respectively; 35, *B. weihenstephanensis* KBAB4; 36-37 and 41-42, *B. cereus* cytotoxis NVH, ATCC 14579, ATCC 10987 and E33L, respectively; 38 and 40, *B. thuringiensis* Al Hakam and serovar konkukian str. 97-27, respectively; 43-44, *B. anthracis* Ames Ancestor and Ames, respectively; 45, *Geobacillus* sp. WCH70; 46, *G. thermodenitrificans* NG80-2; 47, *G. kaustophilus* HTA426. The alignment was performed using BlastN (http://www.ncbi.nlm.nih.gov/blast/). The five colors represent the alignment scores. The red line below the color code corresponds to the whole IGR comprises *rsa*E-*rsa*F of *S. aureus* N315 used as the reference sequence. The numbering of the nucleotides is given and the black arrows correspond to *rsa*E and *rsa*F genes. Only *rsa*E (except the terminator) shows a high degree of conservation in *Stapholococcaceae* and *Bacillaceae*, and the C-rich sequence motifs are strictly conserved in all strains (Fig. 3).
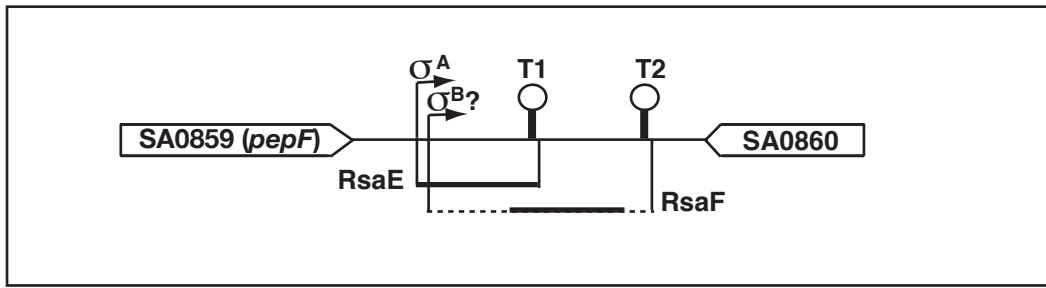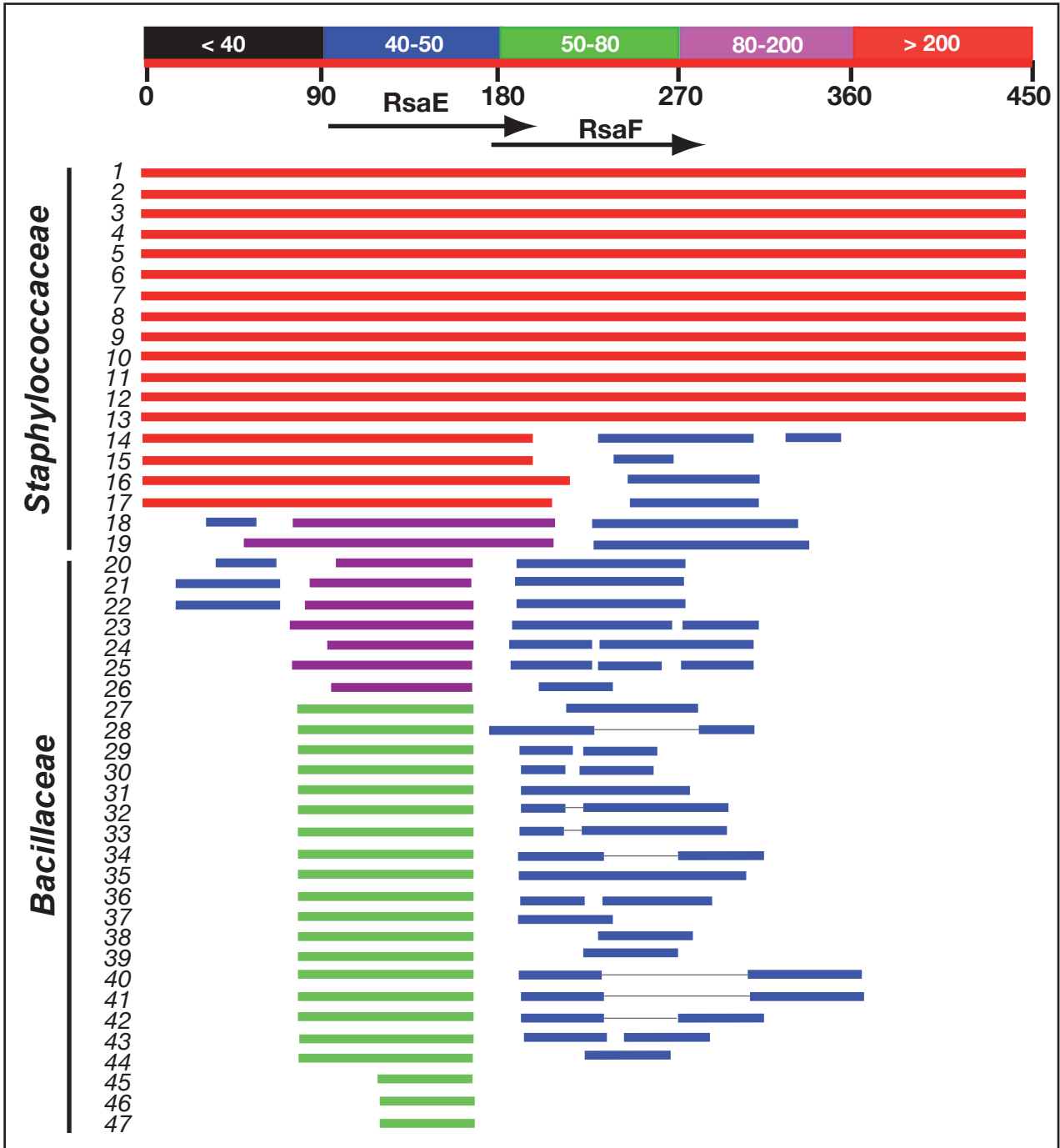
Figure S5- Geissmann et al.

Figure S6. Secondary structure of several Rsa RNAs. The secondary structures were predicted using RNAFold from the Vienna package (2). Strictly conserved nucleotides of RsaD, RsaH, and RsaI in four staphylococcal species (*S. aureus* N315, *S. epidermidis*, *S. saphrolyticus,* and *S. haemolyticus*) are boxed. The conserved C-rich boxes are shown in red characters in RsaB, RsaC, RsaD, RsaH, and RsaJ.

Fig. S6- Geissmann et al.

Figure S7. *S. aureus* RsaA and its potential target mRNAs. (A) Secondary structure of RsaA. The nucleotides in red are strictly conserved in *S. aureus*, *S. epidermidis*, *S. saprophyticus*, and *S. haemolyticus*. (B) RsaA potentially forms stable complexes with the ribosome binding sites of several mRNAs which are repressed by σ^B (3,4). The minimum free energy was calculated with the cofold program for each duplex (Vienna Package, http://rna.tbi.univie.ac.at/cgi-bin/RNAcofold.cgi). The Shine-Dalgarno (SD) sequence is given in red characters.

A

G A G C
G C
A G
A G—C A
A—U
U • G
A—U
U—A
20 G • U — 40
U—A
U—A
A—U
A—U
A C
A U
C A U G
U A
A C—G
C—G
A—U
U—A 60
U—A
A G—C G U U U A U A U G U A G U G A U

C C A
C U—G
C U—A
U—G
A G U—A
A A U • G
U—A
G—C
U—A
G—C

A U G
U C—G
G—C
U—A
U—A
C—G
A—U
A—U
C—G
A—G
G—C
G • U
70 A—U C—G—U U

B

-29.9 kcal/mol *set11* mRNA
exotoxin 11
UUG**GGAGA**AU---AG-UACUAUGAAAUUAAA-AACGUUAGCU
||||||||| || |||||| || | |||||||||
AACCCUCUUGAAAUUGAUGAUAGUGAUGUAUAUUGCAAUUGG

-26.12 kcal/mol *citM* mRNA
Mg-citrate transporter
GGGAGAAAA-UAAU**AUG**AAUAGUGAUAAUAUGUG---GUUAAC
|||||| |||||| | ||| || || ||||| |||||
CCCUCUUGAAAUUA-A--UGAUAGUGAUGUAUAUUUGCAAUUG

-25 kcal/mol SA2226 mRNA
APC aminoacid permease
UCCUG**AGGAGG**AA---AAUU**AUG**GCAAGAAAAUUGCAUAGAGAGUUGA
||||| ||||||| || ||||| |||||| || ||||||
AGGAACCCUCUUGAAAUUGAUGAUAG-----UGAUGUAUAUUGCAAUU

-21 kcal/mol SA0977 mRNA
29kd cell surface protein
UCCUU**AGGAGG**AAAACAAC**AUG**ACAAAACAUUAUUUA-AAC
||||| ||||||| ||| || || |||||| ||| |||
AGGAACCCUCUUGAAAUUGA--UGAUA-GUGAUGUAUAUUG

-21 kcal/mol SA0089 mRNA
DNA helicase-hypothetical protein
GG**UGGGA**-UUUC**AUG**AGU-CAAUUGCUAA--AUGAUACGUUA-UC
|| ||||| || || || | || |||| |||| || |
CC-CUCUUGAA--AUUGAUGAUAGUGAUGUAUAUUG--CAAUUGG

-24 kcal/mol *pycA* mRNA
Pyruvate carboxylase
UAGUUU-A**GAGGAG**AGUGU-AUC-GCUA**UUG**AAACAAAUAAAAAAGUUA-CUU
|||||| || |||| || |||||| |||| || || |||| ||||
AUCAGA AC-CCUCUUGAAAUUGAUGAUAGUGAUGUAUAUUG---CAAUUGGA
      A A
      G G

-22 kcal/mol SA0022 mRNA
5' nucleotidase
AGUU---AAUGG**AGGGA**ACGAA**AUG**AAAGCU-UUAUUACUUAAAAC
|||| ||| ||||| || ||| |||||| |||||||
UCAGAAGGAACC-CUCUUGAA--AUUGAUGAUAGUGAUGUAUAUUG

Figure S7-Geissmann et al.

Table S1. List of predicted conserved IGRs selected by RNAsim.

Start	End	strand	Conservation	Comments
114	510	+	Staphylococcus	Leader *dnaA*
1956	2155	+	Firmicutes	Leader *dnaN*
3417	3585	+	*S. aureus*	
10609	10859	+	Staphylococcus	
12441	12792	+	Firmicutes	T-box upstream of *serS*
14417	14692	+	Staphylococcus	
15776	16092	+	Firmicutes	S-box-SAM riboswitch
22218	22447	+	Staphylococcus	
24336	24625	+	*S. aureus*	Livny et al. (5)
30822	30996	+	*S. aureus*	
35750	36434	+	Staphylococcus	Includes small ORF
39893	40055	-	Firmicutes	Upstream of *pre* +Term, HMM, C-Box
42881	43180	+	Staphylococcus	HMM, RsaX01
43481	43716	+	Staphylococcus	RsaX01
49266	49722	+	Staphylococcus	
53428	53638	+	Firmicutes	Term upstream of SA0045
54468	54805	+	*S. aureus*	Livny et al. (5)
55552	56001	+	*S. aureus*	Cis-acting leader of *ermA*
62881	63210	-	Staphylococcus	Livny et al. (5)
66426	66629	-	Staphylococcus	Livny et al. (5)
68424	68723	-	Staphylococcus	
71351	71650	-	Firmicutes	Upstream of *tnp*
72895	73149	-	Firmicutes	Long IGR
76849	77115	+	Staphylococcus	Includes small ORF
81407	81949	+	Staphylococcus	RsaX02, Livny et al. (5)
87180	87668	+	Staphylococcus	
89956	90119	-	Staphylococcus	STAR
91349	91520	+	Staphylococcus	Term upstream of SA0081
95179	95634	+	Staphylococcus	RsaX03, Marchais et al. (8)
96951	97164	+	*S. aureus*	
97632	97851	+	*S. aureus*	
98489	98727	+	*S. aureus*	
102443	102644	+	*S. aureus*	
103633	103851	+	Staphylococcus	
115157	115385	-	*S. aureus*	
120468	120672	+	*S. aureus*	
122442	122644	+	*S. aureus*	Leader of *spa*, Roberts et al. (7)
124209	124406	-	*S. aureus*	
125182	125542	-	Staphylococcus	
141953	142143	+	*S. aureus*	
143771	143961	+	*S. aureus*	
147787	148049	+	Staphylococcus	
148698	148998	+	*S. aureus*	
149745	149927	+	Staphylococcus	RsaX04
150689	150922	+	*S. aureus*	
158609	158807	+	Staphylococcus	
161970	162120	+	*S. aureus*	
164074	164428	+	*S. aureus*	
167221	167408	+	*S. aureus*	
184882	185158	+	*S. aureus*	
186732	186949	+	Staphylococcus	
188293	188619	+	Staphylococcus	STAR
189594	189781	+	*S. aureus*	Term upstream of SA0166

193338	193534	+	*S. aureus*	
195127	195484	+	*S. aureus*	
196748	197105	+	*S. aureus*	
210408	210644	-	*S. aureus*	
212086	212272	-	*S. aureus*	
217068	217417	+	Firmicutes	RsaK, leader of *glc*A
222171	222426	+	*S. aureus*	
229090	229339	+	*S. aureus*	
232633	232800	+	*S. aureus*	
232830	233059	+	*S. aureus*	
243288	243477	+	*S. aureus*	
244236	244446	+	*S. aureus*	
252268	252525	+	*S. aureus*	
253038	253267	+	Staphylococcus	
258499	258846	+	*S. aureus*	
264490	264674	+	*S. aureus*	
275317	275537	+	*S. aureus*	
276925	277225	+	*S. aureus*	
278704	278900	-	*S. aureus*	
279310	279599	-	*S. aureus*	Small ORF (SAS007)
280772	281015	+	*S. aureus*	
281088	281255	+	*S. aureus*	Roberts et al. (7)
284212	284494	+	*S. aureus*	
285503	285741	+	*S. aureus*	
292549	292777	+	*S. aureus*	
296522	296764	+	*S. aureus*	
297966	298168	+	*S. aureus*	
304334	304490	+	*S. aureus*	
311529	311757	+	Staphylococcus	STAR, HMM
312788	312948	-	*S. aureus*	
315234	315409	-	*S. aureus*	
316427	316782	+	*S. aureus*	
325672	325930	-	*S. aureus*	
326827	327089	+	*S. aureus*	
341093	341259	+	*S. aureus*	
345753	346269	+	*S. aureus*	
348343	348530	-	*S. aureus*	
349847	350002	+	*S. aureus*	
353312	353489	+	*S. aureus*	
354601	354868	+	*S. aureus*	
360696	360850	+	Staphylococcus	
365096	365455	+	Staphylococcus	
374306	374590	+	*S. aureus*	
379765	379926	+	Staphylococcus	
383854	384128	+	*S. aureus*	
387801	388115	+	Staphylococcus	STAR, HMM
388699	388870	+	*S. aureus*	
394432	394587	+	*S. aureus*	
407471	407816	+	Staphylococcus	T-box upstream of SA0347, Marchais et al. (8)
407899	408121	+	Staphylococcus	
412795	413226	+	Firmicutes	End of *rpsR*+Term and downstream of IGR+Term
417756	417939	+	*S. aureus*	
418043	418608	+	Firmicutes	Includes SA0360 and downstream of IGR
423119	423530	+	Staphylococcus	
428000	428312	+	*S. aureus*	

430626	430930	+	Firmicutes	Purine riboswitch upstream of *xprT*
438863	439249	-	*S. aureus*	
440633	440824	+	*S. aureus*	
442870	443053	+	*S. aureus*	
445513	445727	+	*S. aureus*	
453907	454123	+	*S. aureus*	
456769	457081	+	*S. aureus*	
464508	464684	+	*S. aureus*	
476027	476316	+	*S. aureus*	
484610	484907	+	*S. aureus*	
490504	490655	+	Staphylococcus	
496737	496910	+	*S. aureus*	Upstream of tRNA
497009	497168	+	*S. aureus*	Downstream of tRNA
501512	501994	+	Firmicutes	4.5S RNA (SRP)
505754	506160	+	Staphylococcus	Upstream of rRNA
507976	508186	+	Staphylococcus	Between tRNA and 5.8S rRNA
511304	511472	+	Staphylococcus	Downstream of tRNA+term, Livny et al. (5)
511510	511749	+	Staphylococcus	IGR+Term
511895	512130	+	Staphylococcus	Term upstream of SA0439
526716	526951	+	*S. aureus*	
530335	530625	+	*S. aureus*	
538614	538786	+	*S. aureus*	
542978	543170	+	*S. aureus*	
544110	544263	+	Staphylococcus	
545202	545410	+	*S. aureus*	
547095	547406	+	Staphylococcus	Term upstream of *lysS*, T-box
555815	556094	+	Staphylococcus	Upstream of tRNA, IGR+Term
560474	560625	+	Staphylococcus	
565362	565614	+	*S. aureus*	
568157	568636	+	*S. aureus*	
570146	570317	+	Staphylococcus	Term upstream of *cys*E, T-box
576540	576690	+	*S. aureus*	Leader of *rplA*, Roberts et al. (7)
577440	577710	+	Staphylococcus	L10 leader+Term, upstream of *rplJ*
578633	578795	+	Staphylococcus	Term upstream of SA0499
579409	579564	+	Staphylococcus	
586955	587112	+	Staphylococcus	Small ORF
587214	587416	+	Firmicutes	Leader of *rpsL*, Roberts et al. (7)
590574	590789	+	Staphylococcus	Term upstream of *tufA*
591975	592251	+	Staphylococcus	
594790	595053	+	*S. aureus*	
597734	597896	+	Staphylococcus	
604788	605214	+	*S. aureus*	
608160	608441	+	*S. aureus*	
612630	612992	+	*S. aureus*	
619632	619904	-	*S. aureus*	
621878	622099	+	*S. aureus*	
625046	625392	+	Staphylococcus	
630855	631008	+	*S. aureus*	
631050	631324	+	*S. aureus*	
632189	632341	+	*S. aureus*	
636758	637304	+/-	Staphylococcus	RsaA (+ strand)
640216	640541	+	Staphylococcus	
646069	646253	+	*S. aureus*	
646366	646552	+	*S. aureus*	
650163	650446	+	*S. aureus*	

651569	651954	+	Staphylococcus	STAR, HMM, Livny et al. (5)
652533	652685	+	*S. aureus*	
655059	655507	+	*S. aureus*	
656598	656828	+	*S. aureus*	Upstream of SA0563-*argS*, T-box ?
659828	660066	+	*S. aureus*	RsaX06
665140	665294	+	*S. aureus*	
666098	666298	+	Staphylococcus	RsaX07
666842	667117	-	Staphylococcus	
667125	667630	-	Staphylococcus	
669192	669369	+	*S. aureus*	
675825	676113	+	*S. aureus*	
679723	680024	-	*S. aureus*	RsaC
680509	680738	-	*S. aureus*	RsaC
686710	687007	+	*S. aureus*	
692039	692383	+	*S. aureus*	
694133	694433	+	*S. aureus*	
695728	696143	+	Staphylococcus	RsaD, Livny et al. (5)
697192	697355	+	Staphylococcus	
703029	703495	+	*S. aureus*	
712969	713456	+	Staphylococcus	
715250	715686	+	*S. aureus*	
716497	716842	+	Staphylococcus	
717493	718174	+	*S. aureus*	
720870	721025	+	*S. aureus*	
722073	722559	+	Staphylococcus	
728170	728344	+	Staphylococcus	
731774	731963	+	Staphylococcus	
735892	736086	+	Staphylococcus	
738044	738220	+	Staphylococcus	
741648	741935	+	Staphylococcus	
742699	742879	-	*S. aureus*	
744682	744959	+	Staphylococcus	
746121	746316	+	Staphylococcus	
749975	750264	+	*S. aureus*	
751463	751682	+	Staphylococcus	
753034	753240	+	*S. aureus*	
757175	757551	-	Staphylococcus	
758259	758527	-	*S. aureus*	
761087	761229	+	Staphylococcus	RsaX08, pre-Q1 riboswitch upstream of SA0668 Marchais et al. (8)
765821	766226	+	Staphylococcus	
768200	768422	+	Staphylococcus	
772138	772335	+	*S. aureus*	
776137	776453	+	Staphylococcus	STAR, HMM, Livny et al. (5)
777120	777557	+	Staphylococcus	STAR, HMM, Livny et al. (5)
780748	780920	-	*S. aureus*	
782415	782637	+	*S. aureus*	
782659	783086	+	Staphylococcus	Purine riboswitch upstream of *nrdl*
790883	791038	+	Staphylococcus	STAR
794592	794959	+	*S. aureus*	
798801	798968	-	Staphylococcus	
800069	800355	+	Staphylococcus	
803253	803584	+	*S. aureus*	
805998	806284	+	*S. aureus*	
808967	809331	+	Staphylococcus	Includes small ORF
810429	810772	+	*S. aureus*	Includes small ORF

812626	812888	+	*S. aureus*	
817939	818247	+	Staphylococcus	STAR, HMM
823225	824090	+	Staphylococcus	STAR, HMM
827069	827237	+	*S. aureus*	
827447	827597	+	Staphylococcus	
828251	828448	+	*S. aureus*	
829368	829873	+	Staphylococcus	RsaH, Livny et al. (5)
830837	831322	+	Staphylococcus	STAR, HMM
838883	839113	+	*S. aureus*	
839881	840052	+	Staphylococcus	
844742	845141	+	Staphylococcus	Downstream of tmRNA
846434	847030	+	*S. aureus*	
848235	848481	+	*S. aureus*	
851455	851614	+	*S. aureus*	
853205	853501	+	*S. aureus*	
854562	854863	+	*S. aureus*	
855441	855747	+	Firmicutes	Upstream of SA0746 (staphylococcal nuclease)
856473	856765	+	Staphylococcus	
857190	857552	+	Staphylococcus	Term far upstream of SA0748
859310	859768	+	*S. aureus*	
865573	865726	+	Staphylococcus	Roberts et al. (7)
872157	872606	+	*S. aureus*	
873353	873699	+	*S. aureus*	Livny et al. (5)
873882	874206	+	Staphylococcus	STAR, HMM
874260	874502	+	Staphylococcus	STAR, HMM
875429	875597	+	Firmicutes	SAM riboswitch upstream of SA0769
878278	878529	+	*S. aureus*	
879854	880109	+	Staphylococcus	Includes small ORF
886286	886588	+	*S. aureus*	
893715	893905	+	Staphylococcus	
897895	898263	+	*S. aureus*	Includes small ORF
904608	904758	+	*S. aureus*	
912118	912341	-	*S. aureus*	
920248	920429	+	*S. aureus*	
921586	921871	+	*S. aureus*	
924454	924635	+	Staphylococcus	STAR, HMM
928516	928811	+	Staphylococcus	
930204	930359	+	*S. aureus*	
939508	939672	+	Staphylococcus	
941308	941464	+	*S. aureus*	
944084	944612	+	Staphylococcus	STAR
953726	953966	+	*S. aureus*	
954414	954644	+	*S. aureus*	
954859	955148	+	*S. aureus*	Term upstream of *fabh*, riboswitch ?
957808	957990	+	Staphylococcus	
963726	963926	+	*S. aureus*	
970542	970834	+	Staphylococcus	
971369	971600	+	Staphylococcus	
975288	975460	+	Firmicutes	RsaE, Livny et al. (5)
975460	975740	+	*S. aureus*	RsaF
977641	977812	+	*S. aureus*	
983797	984032	+	*S. aureus*	
986113	986457	+	*S. aureus*	
988928	989089	+	Staphylococcus	
992059	992486	+	Staphylococcus	

996902	997115	+	*S. aureus*	
1002460	1002628	+	*S. aureus*	upstream of tRNA gene
1003508	1003661	-	*S. aureus*	
1003804	1004015	-	Staphylococcus	In antisense orientation to SA0883
1006559	1006849	+	*S. aureus*	
1010523	1010773	+	*S. aureus*	Long IGR
1011366	1011547	+	*S. aureus*	
1013265	1013422	+	*S. aureus*	
1019781	1019947	+	*S. aureus*	Downstream of *memb,* Potential sRNA with Term
1022764	1022962	-	*S. aureus*	
1023012	1023200	-	*S. aureus*	
1032642	1033043	+	Staphylococcus	
1039067	1039361	-	*S. aureus*	
1039680	1040504	-	Staphylococcus	STAR, HMM
1041406	1041601	+	Staphylococcus	
1052839	1053096	+	Staphylococcus	
1055907	1056798	+	Firmicutes	Antisense orientation of TPP, repeat elements
1058320	1058707	+	*S. aureus*	
1062659	1063092	+	Staphylococcus	
1066487	1066727	+	Staphylococcus	Livny et al. (5)
1068543	1069063	-	Firmicutes	
1069685	1069846	+	*S. aureus*	
1083867	1084046	-	Staphylococcus	
1085788	1085953	+	Staphylococcus	
1087945	1088099	+	*S. aureus*	
1089128	1089413	+	*S. aureus*	
1090887	1091225	+	Staphylococcus	
1095856	1096173	+	Staphylococcus	
1097586	1097756	+	*S. aureus*	
1106608	1106790	-	*S. aureus*	
1107856	1108058	+	*S. aureus*	
1112944	1113182	+	*S. aureus*	
1113949	1114289	+	Firmicutes	T-box upstream of *pheS*
1119148	1119545	+	*S. aureus*	
1127040	1127317	+	Staphylococcus	
1130625	1130822	+	*S. aureus*	
1132777	1133220	+	*S. aureus*	Includes small ORF
1134351	1134993	+	*S. aureus*	Term upstream of SA1002
1135503	1135759	+	*S. aureus*	
1136760	1137488	+	Staphylococcus	STAR, HMM, Livny et al. (5)
1137644	1137875	+	*S. aureus*	Long IGR
1137937	1138593	+	*S. aureus*	Long IGR
1138630	1139083	+	Staphylococcus	RsaX10
1141537	1142098	-	*S. aureus*	
1142166	1143006	-	Staphylococcus	STAR, HMM
1145459	1145817	+	Staphylococcus	
1149556	1149966	+	Staphylococcus	Includes small ORF
1151613	1151839	-	Staphylococcus	STAR
1151978	1152191	-	*S. aureus*	Upstream of tRNA gene
1152270	1152713	+	Staphylococcus	Upstream of small ORF
1154325	1154496	+	*S. aureus*	
1166960	1167207	+	*S. aureus*	
1171092	1171307	+	Staphylococcus	T-box upstream of *ileS*
1175179	1175939	+	Staphylococcus	STAR, HMM, RsaX11
1177413	1177766	+	Staphylococcus	Cis-regulatory leader *pyrR*

1178313	1178505	+	Staphylococcus	Cis-regulatory leader *pyrP* (PyrR)
1187782	1188280	+	*S. aureus*	
1188815	1188998	+	*S. aureus*	
1191840	1192077	+	*S. aureus*	
1195742	1196199	+	*S. aureus*	
1197660	1197816	+	Staphylococcus	
1204379	1204587	+	*S. aureus*	
1207056	1207252	+	Firmicutes	Long IGR, antisense orientation to *rpmB*
1207368	1207623	+	Staphylococcus	Long IGR
1209822	1210010	+	*S. aureus*	
1212081	1212259	+	Staphylococcus	
1215618	1215904	+	Staphylococcus	Repeats, Term 80 nt upstream of *hmrB*
1223881	1224165	+	Firmicutes	Leader of *rpsP*
1229115	1229512	+	*S. aureus*	
1235976	1236140	+	*S. aureus*	
1237018	1237171	+	Staphylococcus	
1240749	1241012	+	*S. aureus*	
1245038	1245198	+	Staphylococcus	Leader of *rpsB*
1245984	1246147	+	Firmicutes	Small ORF, leader of EF-TS
1248617	1248800	+	Staphylococcus	Term upstream of *uppS*, Livny et al. (5)
1250407	1250591	+	*S. aureus*	
1253679	1253869	+	*S. aureus*	
1263619	1263776	+	*S. aureus*	Leader of *truB-ribC-rpsO*
1265989	1266427	+	Staphylococcus	Leader of *pnpA*
1270493	1270665	+	*S. aureus*	
1279039	1279226	+	*S. aureus*	
1280406	1280564	+	Staphylococcus	
1281617	1281966	+	Staphylococcus	
1283591	1283812	+	*S. aureus*	
1283831	1284194	+	Staphylococcus	
1290733	1291002	+	*S. aureus*	
1296413	1296565	+	*S. aureus*	
1306539	1306761	+	*S. aureus*	
1309256	1309601	+	*S. aureus*	
1309685	1310025	+	Staphylococcus	
1310031	1310492	+	*S. aureus*	Includes small ORF
1310499	1311089	+	*S. aureus*	Includes small ORF
1311163	1311668	+	*S. aureus*	
1311817	1312415	+	*S. aureus*	Includes small ORF
1312805	1313249	+	Staphylococcus	Includes small ORF
1313887	1314054	+	Staphylococcus	Includes small ORF
1314277	1314447	+	*S. aureus*	
1315518	1315773	+	*S. aureus*	
1317511	1317692	+	*S. aureus*	
1322977	1323131	+	*S. aureus*	
1325311	1325491	+	Staphylococcus	
1329618	1329858	+	*S. aureus*	
1331898	1332087	+	Staphylococcus	
1336837	1337115	+	*S. aureus*	Includes small ORF
1337755	1337964	+	Staphylococcus	Includes small ORF
1338420	1338619	+	Staphylococcus	Includes small ORF
1340640	1340865	+	*S. aureus*	
1346496	1346695	+	Staphylococcus	
1348594	1349059	+	Staphylococcus	STAR, HMM
1352000	1352173	+	*S. aureus*	

1352697	1352869	+	*S. aureus*	
1360589	1360804	+	*S. aureus*	
1363256	1363611	+	*S. aureus*	
1366136	1366335	+	*S. aureus*	
1368171	1368382	+	Firmicutes	
1378888	1379152	+	*S. aureus*	
1381748	1382022	+	*S. aureus*	
1387702	1387901	-	*S. aureus*	
1388353	1388520	+	*S. aureus*	
1395079	1395520	-	Staphylococcus	STAR, HMM
1395695	1395951	-	Firmicutes	Includes small ORF
1398843	1399123	+	Staphylococcus	RsaX12 upstream of lysine riboswitch
1399339	1399740	+	Staphylococcus	Lysine riboswitch upstream of *lysC*
1408636	1408787	-	*S. aureus*	
1408937	1409164	-	Firmicutes	Leader of *cspA*
1409542	1409751	+	Staphylococcus	
1413176	1413388	-	*S. aureus*	
1417320	1417878	-	Staphylococcus	
1424188	1424971	-	Staphylococcus	Upstream of truncated *arlR*
1427366	1427562	-	*S. aureus*	
1432388	1432583	-	*S. aureus*	
1433547	1433788	-	*S. aureus*	
1436007	1436171	+	*S. aureus*	
1437004	1437246	+	Staphylococcus	STAR, HMM, Livny et al. (5)
1469803	1470083	-	*S. aureus*	
1475356	1475822	-	*S. aureus*	
1480194	1480447	+	*S. aureus*	
1483726	1484114	-	Firmicutes	RNase P, HMM
1485561	1485911	+	*S. aureus*	
1491105	1491401	-	*S. aureus*	
1492707	1492871	-	Staphylococcus	
1499069	1499225	-	*S. aureus*	
1499634	1499839	-	*S. aureus*	
1506684	1507446	-	*S. aureus*	
1510729	1511138	-	Firmicutes	Leader of *hu*
1514878	1515160	-	Staphylococcus	
1515273	1515551	-	*S. aureus*	
1523885	1524137	-	Firmicutes	FMN upstream of SA1316, HMM
1530598	1530818	-	*S. aureus*	
1544160	1544364	+	Staphylococcus	
1550247	1550438	-	*S. aureus*	
1553370	1553696	-	*S. aureus*	
1562522	1562927	-	*S. aureus*	
1568050	1568228	-	Staphylococcus	
1571964	1572117	+	*S. aureus*	
1577475	1577690	-	*S. aureus*	
1583172	1583325	-	Staphylococcus	
1585629	1585811	-	Firmicutes	Leader of *rpmG*
1588657	1588896	-	*S. aureus*	
1600077	1600363	+	Staphylococcus	T-Box upstream of *glyS*, Marchais et al. (8)
1605769	1606026	-	*S. aureus*	
1608584	1609165	-	Firmicutes	Leader of *rpsU*
1618277	1618638	-	Staphylococcus	STAR, HMM
1620964	1621273	-	Firmicutes	In antisense orientation to *rps*T
1631726	1631958	-	*S. aureus*	

1632072	1632346	-	*S. aureus*	Includes part of SA1429
1632385	1632595	-	*S. aureus*	Includes part of SA1429
1633477	1633790	-	*S. aureus*	Upstream of STAR, HMM, Livny et al. (5)
1640858	1641060	-	*S. aureus*	
1645154	1645434	-	Staphylococcus	
1649154	1649422	-	Firmicutes	T-box upstream of *alaS*
1652768	1653061	-	Staphylococcus	STAR, HMM, Livny et al. (5)
1660490	1660646	-	Staphylococcus	Roberts et al. (7)
1663947	1664110	-	Staphylococcus	T-Box upstream of *hisS*, HMM, Livny et al. (5)
1667827	1668128	-	*S. aureus*	
1671068	1671269	-	*S. aureus*	
1682744	1682923	-	Staphylococcus	
1683462	1683761	-	Staphylococcus	
1685414	1685863	+	Firmicutes	Cis-regulatory leader of *ermA*
1696574	1696752	+	Firmicutes	T-box upstream of *valS*
1705181	1705379	-	Staphylococcus	
1711867	1712034	-	Firmicutes	L20 leader upstream of *infC*
1713606	1713993	-	Firmicutes	Lysine riboswitch upstream of *lysP*
1715961	1716354	-	Firmicutes	T-box upstream of *thrS*, HMM
1720401	1720569	-	Staphylococcus	
1724751	1725005	-	*S. aureus*	
1726510	1726993	-	*S. aureus*	
1732201	1732547	+	Staphylococcus	
1734098	1734256	+	*S. aureus*	
1736967	1737251	-	Staphylococcus	Livny et al. (5)
1740661	1740878	-	*S. aureus*	
1745150	1745402	-	Staphylococcus	STAR, HMM, Term upstream of SA1526
1746749	1746903	+	*S. aureus*	
1758029	1758239	-	*S. aureus*	
1761531	1761713	+	Staphylococcus	
1771001	1771159	+	Staphylococcus	
1773791	1774166	+	Staphylococcus	T-box upstream of *tyrS*
1775089	1775274	+	Staphylococcus	
1777967	1778142	+/-	*S. aureus*	RsaB (+ strand)
1780001	1780272	-	*S. aureus*	
1785343	1785657	-	*S. aureus*	
1787090	1787505	-	*S. aureus*	
1799158	1799538	-	*S. aureus*	
1801010	1801172	-	Staphylococcus	RsaX14
1801193	1801556	-	Staphylococcus	RsaX14
1803836	1804170	-	Staphylococcus	STAR, HMM
1804196	1804363	-	Staphylococcus	STAR, HMM
1807199	1807350	+	*S. aureus*	
1815565	1815759	-	*S. aureus*	
1822306	1822720	-	*S. aureus*	
1823612	1823809	+	Staphylococcus	
1828395	1828720	-	Firmicutes	FMN upstream of *ribD*, HMM
1830320	1830823	+	*S. aureus*	Term upstream of SA1591
1833394	1833678	+	Staphylococcus	
1833892	1834060	+	Staphylococcus	
1835663	1835820	+	*S. aureus*	
1839186	1839704	+	Staphylococcus	RsaX15, HMM
1839706	1839931	+	Firmicutes	Truncated transposase
1844412	1844781	+	Firmicutes	Leader of *pckA,* in antisense orientation to SAM riboswitch
1850782	1850936	-	*S. aureus*	

1851426	1851728	+	*S. aureus*	
1856369	1856798	+	Staphylococcus	sprA, Pichon and Felden (6), HMM
1857482	1857823	-	Staphylococcus	
1860738	1861018	-	*S. aureus*	
1865118	1865490	+	*S. aureus*	Term upstream of *splA*
1865836	1866079	+	*S. aureus*	
1866672	1867028	+	*S. aureus*	sprB, Pichon and Felden (6)
1867248	1867528	+	*S. aureus*	Downstream of sprB
1871172	1871931	+	Staphylococcus	sprC, Pichon and Felden (6)
1873386	1873642	+	Staphylococcus	
1873858	1874112	+	Staphylococcus	
1877123	1877283	-	*S. aureus*	
1881673	1881834	-	*S. aureus*	Downstream of tRNA cluster
1882658	1882992	-	*S. aureus*	Upstream of tRNA cluster
1883713	1883866	-	*S. aureus*	Term upstream of SA1649, riboswitch ?
1887313	1887560	+	Staphylococcus	
1899115	1899440	-	*S. aureus*	
1899478	1899762	-	*S. aureus*	HMM, Term
1901329	1901482	-	*S. aureus*	
1901976	1902324	-	Staphylococcus	
1907967	1908121	-	Staphylococcus	Upstream of STAR, HMM
1914230	1914658	+	Staphylococcus	RsaX16
1916129	1916289	+	*S. aureus*	3' UTR of *tnp* and upstream of tRNA cluster
1921856	1922066	-	Staphylococcus	Upstream of rRNA
1923904	1924623	-	Staphylococcus	Upstream of rRNA
1929336	1929486	+	Staphylococcus	
1931363	1931530	-	*S. aureus*	
1938347	1938528	-	*S. aureus*	
1939381	1939606	-	*S. aureus*	
1941930	1942087	+	*S. aureus*	
1944228	1944386	+	Staphylococcus	
1945105	1945410	+	*S. aureus*	
1950480	1950858	+	Staphylococcus	STAR, HMM
1951996	1952301	+	Staphylococcus	STAR, HMM, Livny et al. (5)
1952892	1953043	-	*S. aureus*	
1955115	1955395	+	Staphylococcus	
1955903	1956286	+	*S. aureus*	
1961321	1962071	-	Staphylococcus	STAR, HMM, Livny et al. (5)
1965286	1965636	+	Staphylococcus	
1975263	1975577	+	*S. aureus*	
1975655	1976120	+	*S. aureus*	
1977802	1978014	+	*S. aureus*	
1978693	1978946	-	Staphylococcus	
1991902	1993014	+	Staphylococcus	HMM, RsaX17 Detection of a stable RNA in antisense, Roberts et al. (7), Livny et al. (5)
2000792	2001169	+	Staphylococcus	HMM
2002762	2003025	+	*S. aureus*	
2004766	2004935	-	*S. aureus*	
2005435	2006027	-	*S. aureus*	
2006124	2006498	-	*S. aureus*	
2006879	2007560	+	*S. aureus*	sprD, Pichon and Felden (6)
2009578	2009756	-	*S. aureus*	
2010950	2011172	-	Staphylococcus	sprG, antisense to sprF, Pichon and Felden (6) ; Roberts et al. (7), C-rich motif
2012392	2012550	-	*S. aureus*	

2023371	2023533	-	Staphylococcus	
2044276	2044498	+	*S. aureus*	
2044531	2044689	+	*S. aureus*	
2046589	2046936	+	*S. aureus*	
2050407	2050637	+	*S. aureus*	
2052735	2053160	+	*S. aureus*	
2054442	2054834	+	Staphylococcus	STAR, HMM
2056712	2056982	+	*S. aureus*	
2057885	2058037	+	*S. aureus*	
2058852	2059635	+	*S. aureus*	
2060672	2061075	+	Staphylococcus	
2066059	2066357	-	*S. aureus*	
2074274	2074451	+	Firmicutes	
2076511	2076703	+	*S. aureus*	
2077355	2077666	+	Staphylococcus	STAR and Term upstream of SA1841
2078721	2078937	+	Staphylococcus	RNAIII-C-rich motif
2079188	2079445	+	Staphylococcus	RNAIII-C-rich motif
2082286	2082571	+	*S. aureus*	
2086113	2086282	-	*S. aureus*	
2089116	2089363	-	*S. aureus*	
2090087	2090243	+	Staphylococcus	
2092236	2092595	+	Staphylococcus	
2097090	2097527	+	Staphylococcus	Term upstream of *ilvD*, riboswitch ?
2112153	2112350	-	Staphylococcus	Upstream of rRNA
2112351	2112621	-	Staphylococcus	Upstream of rRNA
2114673	2115087	-	*S. aureus*	Upstream of tRNA
2117717	2118136	-	*S. aureus*	
2120826	2121111	-	*S. aureus*	
2125946	2126096	-	*S. aureus*	
2140430	2140694	+	*S. aureus*	
2142896	2143098	+	*S. aureus*	
2145816	2145986	+	Staphylococcus	
2150134	2150353	-	*S. aureus*	THI riboswitch upstream of *tenA*
2151848	2152017	+	*S. aureus*	
2154589	2154831	-	Staphylococcus	
2170438	2170674	-	Firmicutes	Leader of *rpmE*
2172033	2172272	-	Staphylococcus	
2173713	2173918	-	*S. aureus*	
2175623	2175838	-	*S. aureus*	
2176973	2177125	+	Staphylococcus	
2179408	2179559	-	*S. aureus*	
2183309	2183669	-	*S. aureus*	
2186103	2186358	+	Staphylococcus	
2191191	2191375	-	Staphylococcus	
2193769	2194327	-	*S. aureus*	
2195501	2195682	-	*S. aureus*	
2198126	2198299	+	Staphylococcus	
2198782	2198996	+	*S. aureus*	Livny et al. (5)
2199743	2200192	+	*S. aureus*	Cis-regulatory leader of *ermA*
2211959	2212517	+	Staphylococcus	Antisense to GlmS riboswitch
2217927	2218109	+	*S. aureus*	
2228994	2229181	-	Staphylococcus	
2233852	2234049	-	Staphylococcus	
2234050	2234320	-	Staphylococcus	Upstream of rRNA
2235891	2236330	-	Staphylococcus	Upstream of rRNA

2241225	2241396	-	*S. aureus*	
2244132	2244347	-	Staphylococcus	
2245730	2245955	-	Staphylococcus	
2256957	2257130	-	Staphylococcus	
2258694	2258999	-	*S. aureus*	
2269141	2269392	-	*S. aureus*	
2270166	2270385	+	*S. aureus*	
2271593	2271847	+	*S. aureus*	
2273257	2273473	+	*S. aureus*	
2278693	2279037	+	*S. aureus*	
2282469	2282662	-	Staphylococcus	
2283771	2283931	-	*S. aureus*	
2285904	2286114	-	*S. aureus*	
2287009	2287178	-	*S. aureus*	
2288114	2288407	+	*S. aureus*	
2290679	2290831	-	Staphylococcus	L13 leader upstream of *rplM*
2294193	2294358	-	*S. aureus*	End of rplQ, Roberts et al. (7)
2297295	2297786	-	Firmicutes	Cis-regulatory leader of *infA*
2315523	2315785	+	Staphylococcus	STAR, HMM
2317119	2317270	+	*S. aureus*	
2322654	2322811	-	*S. aureus*	
2326152	2326412	-	Staphylococcus	STAR, HMM
2326455	2326612	-	Staphylococcus	STAR, HMM
2335098	2335370	+	*S. aureus*	
2336178	2336334	+	Staphylococcus	
2338812	2339179	-	*S. aureus*	
2342611	2342890	+	Staphylococcus	
2347937	2348096	+	*S. aureus*	
2348445	2348807	-	Staphylococcus	
2351998	2352576	+	Staphylococcus	
2353589	2353825	+	Staphylococcus	
2356433	2356657	+	*S. aureus*	
2357081	2357300	+	Staphylococcus	
2365373	2365625	-	*S. aureus*	
2367831	2368208	+	Staphylococcus	RsaI, Marchais et al. (8)
2370043	2370414	+	*S. aureus*	
2371369	2371738	+	*S. aureus*	
2373129	2373403	+	Staphylococcus	
2375948	2376119	+	Staphylococcus	
2378340	2378664	+	Staphylococcus	
2384868	2385044	-	*S. aureus*	
2389095	2389322	+	*S. aureus*	
2389754	2389995	+	*S. aureus*	
2390997	2391225	+	Staphylococcus	
2393252	2393404	+	*S. aureus*	
2398087	2398249	-	*S. aureus*	
2399072	2399253	+	*S. aureus*	
2399983	2400209	+	*S. aureus*	
2405538	2405697	+	*S. aureus*	
2406372	2406582	+	*S. aureus*	
2409885	2410108	+	*S. aureus*	
2411321	2411574	-	*S. aureus*	
2412971	2413192	-	*S. aureus*	
2414829	2415053	+	*S. aureus*	
2425527	2425678	+	*S. aureus*	

2426297	2426582	-	*S. aureus*	
2429796	2430015	-	*S. aureus*	
2430501	2430698	-	*S. aureus*	
2431985	2432135	+	Staphylococcus	
2435998	2436269	+	*S. aureus*	RsaX18, stable RNA in antisense orientation, Roberts et al. (7)
2437028	2437317	+	Staphylococcus	Downstream of RsaX18
2439937	2440194	+	*S. aureus*	
2440561	2440869	+	Staphylococcus	HMM, repeats, RsaX19
2442167	2442330	-	*S. aureus*	
2442698	2443032	+	*S. aureus*	
2443583	2443744	+	*S. aureus*	
2445484	2445745	+	Staphylococcus	
2447564	2447719	+	*S. aureus*	
2456483	2456710	-	*S. aureus*	
2461228	2461426	-	*S. aureus*	
2461971	2462198	-	*S. aureus*	
2463308	2463504	-	*S. aureus*	
2466457	2466755	-	*S. aureus*	
2467832	2468006	+	*S. aureus*	
2473362	2473553	-	Staphylococcus	RsaX20
2474277	2474452	-	*S. aureus*	
2475454	2475778	+	*S. aureus*	
2475795	2476058	+	*S. aureus*	
2477641	2477908	+	*S. aureus*	
2478860	2479149	+	*S. aureus*	
2479216	2479421	+	*S. aureus*	
2486709	2486881	-	*S. aureus*	RsaJ
2490706	2491116	-	Staphylococcus	STAR, HMM
2497788	2498049	+	Staphylococcus	
2503698	2503855	+	*S. aureus*	
2509313	2509478	-	*S. aureus*	
2514267	2514440	+	Staphylococcus	
2516120	2516273	+	*S. aureus*	
2519098	2519353	-	*S. aureus*	
2525577	2525767	+	*S. aureus*	
2526558	2526857	+	Staphylococcus	
2536141	2536452	-	*S. aureus*	
2536625	2536820	-	*S. aureus*	
2544138	2544290	-	Staphylococcus	
2546465	2546691	-	Staphylococcus	
2549419	2549630	-	*S. aureus*	
2551315	2551599	-	*S. aureus*	
2556804	2557057	+	*S. aureus*	
2557826	2558087	+	*S. aureus*	
2562110	2562398	-	*S. aureus*	HMM
2563833	2564100	-	*S. aureus*	
2564499	2564800	+	*S. aureus*	
2568115	2568322	-	*S. aureus*	
2571236	2571482	-	*S. aureus*	
2571601	2571886	-	*S. aureus*	
2575158	2575471	-	*S. aureus*	
2580982	2581163	-	*S. aureus*	
2583795	2583980	-	*S. aureus*	
2585432	2585672	+	*S. aureus*	
2586700	2586952	+	*S. aureus*	RsaX22

2590828	2591139	+	Staphylococcus	RsaX23, sprA3, Pichon and Felden (6), HMM
2596738	2596915	+	*S. aureus*	
2599037	2599326	+	*S. aureus*	
2600725	2600877	+	*S. aureus*	
2601516	2601689	+	*S. aureus*	Livny et al. (5)
2605324	2605790	+	*S. aureus*	
2609352	2609550	-	*S. aureus*	
2611619	2611889	-	*S. aureus*	RAT motif in leader of *ptsG*
2614788	2614993	-	Staphylococcus	
2616463	2616619	+	Staphylococcus	
2618504	2618750	+	*S. aureus*	
2619921	2620083	+	Staphylococcus	
2620690	2621010	+	*S. aureus*	
2629104	2629317	+	*S. aureus*	
2635052	2635382	+	Staphylococcus	Includes part of repeats, Livny et al. (5)
2644680	2644981	-	*S. aureus*	
2646952	2647361	-	*S. aureus*	
2647851	2648018	-	*S. aureus*	
2648968	2649154	-	*S. aureus*	
2649183	2649430	-	*S. aureus*	RsaX24
2650531	2650761	+	*S. aureus*	
2651470	2651647	+	Staphylococcus	
2651696	2651846	+	*S. aureus*	
2652667	2652901	+	Staphylococcus	STAR, HMM
2661386	2661635	-	*S. aureus*	
2663861	2664068	+	*S. aureus*	
2666489	2666751	+	*S. aureus*	
2671087	2671338	-	Staphylococcus	Livny et al. (5)
2672085	2672534	+	*S. aureus*	Cis-regulatory leader of *ermA*
2678310	2678645	-	*S. aureus*	
2683676	2683834	+	*S. aureus*	
2684016	2684324	+	Staphylococcus	
2687272	2687440	+	Staphylococcus	
2688908	2689084	+	*S. aureus*	
2690843	2691222	-	*S. aureus*	
2697740	2697970	+	Staphylococcus	
2698571	2698723	+	Staphylococcus	
2700573	2700856	-	*S. aureus*	
2708048	2708199	-	*S. aureus*	
2708791	2709614	-	*S. aureus*	
2716436	2716702	+	Staphylococcus	
2721001	2721275	-	*S. aureus*	
2726839	2727174	-	*S. aureus*	
2727805	2727992	-	*S. aureus*	
2729572	2729953	-	*S. aureus*	
2730482	2730708	+	*S. aureus*	
2731227	2731379	+	*S. aureus*	
2739394	2739588	+	Staphylococcus	
2762069	2762602	-	*S. aureus*	
2763342	2763605	+	*S. aureus*	
2766556	2766758	-	*S. aureus*	
2768925	2769664	-	*S. aureus*	In antisense orientation to RsaX25
2773960	2774226	+	Staphylococcus	STAR
2776631	2777268	-	*S. aureus*	
2784309	2784518	-	*S. aureus*	

2792452	2792677	+	*S. aureus*	
2795645	2795809	+	*S. aureus*	
2796286	2796475	+	*S. aureus*	
2798095	2798255	+	*S. aureus*	
2800056	2800210	+	*S. aureus*	
2806131	2806408	+	Staphylococcus	Leader of *cspB*
2808484	2808774	-	Staphylococcus	
2814540	2814811	-	Firmicutes	Leader of *rpmH*

The boundaries of the conserved regions and their locations on the DNA strand are given. Comments include the known regulatory regions as defined by RFAM, the Rsa RNAs, the IGRs which have been predicted (5), and stable RNAs which were previously identified (6-8). Interesting features of conserved regions are mentioned. Term is for terminator of transcription.

Table S2. List of predicted IGRs found by a Hidden Markov Model (HMM).

HMM	Start-End	Flanking genes	Comments
1	24159-24341	*purA/vicR*	tRNA
2	39699-40058	*Pre-bleO*	RNAsim
3	42881-43180	SA0035-SA0036	RsaX01, RNAsim
4	233122-233343	SA0197-*oppF*	
5	254692-254833	*uhpT*-SA0215	RsaG
6	311527-311657	*bglA*- SA0257	RNAsim
7	387896-388065	SA0329-*appF*	RNAsim
8	407757-407873	SA0347-SA0348	T-Box, RNAsim, Marchais et al. (8)
9	469644-469972	SA0410-*ndhF*	Long IGR with small ORF
10	496930-497008	*gltD-treP*	tRNA
11	501419-501690	SA0434-SA0435	SRP
12	506179-507975	recR-rRNA	rRNA
13	508203-511102	rRNA-rRNA	rRNA
14	511185-511878	rRNA-SA0439	5S rRNA
15	549617-550468	*lysS*-16S rRNA	tRNA
16	550607-552312	tRNA-23S rRNA	rRNA
17	555476-555860	23S rRNA-SA0476	rRNA
18	651668-651853	SA0557-SA0558	RNAsim, Livny et al. (5)
19	659030-659146	*argS*- SA0565	
20	777116-777256	SA0680- SA0681	RNAsim, Livny et al. (5)
21	777392-777519	SA0680- SA0681	RNAsim, Livny et al. (5)
22	818082-818207	*uvrA- hprK*	RNAsim
23	830889-831241	SA0725-*gapR*	RNAsim
24	843796-844174	*ssrP*-SA0738	tmRNA
25	873970-874214	truncated-SA*-SA0768	RNAsim
26	874300-874421	truncated-SA*- SA0768	RNAsim
27	875413-875457	SAS023-SA0769	RNAsim, SAM riboswitch
28	924504-924632	gudb*-glpQ	RNAsim
29	1002623-1002696	SA0881*-SA0882	tRNA
30	1040000-1040140	SA0914-folD	RNAsim
31	1056349-1056701	SA0929-SAS030	Repeats downstream of a TPP riboswitch
32	1137073-1137371	SA1004-SAS035	RNAsim, Livny et al. (5)
33	1142669-1142793	SA1008-SA1009	RNAsim
34	1152072-1152271	SA1016-SA1018	tRNA
35	1175550-1175624	SA1037-*lsp*	RNAsim, RsaX11
36	1348702-1348948	*opuD-citB*	RNAsim
37	1353221-1351302	SA1186-SA1187	STAR
38	1395440-1395567	SA1221-truncated SA	RNAsim
39	1399134-1399210	SA1224-*lysC*	RsaX12, RNAsim, upstream of Lysine riboswitch
40	1437057-1437239	SA1265-SA1266	RNAsim, Livny et al. (5)
41	1483795-1483974	SA1277-SA1279	RNase P, RNAsim
42	1523747-1523924	SA1316-SA1317	FMN riboswitch, RNAsim
43	1618576-1618787	*hemN-lepA*	RNAsim
44	1633168-1633298	SA1430- SA1431	RNAsim, Livny et al. (5)
45	1649265-1649307	*alaS*- SA1447	T-Box
46	1652906-1653242	SA1448- SA1449	Livny et al. (5)
47	1660504-1660682	SA1455- *aspS*	6S RNA
48	1663953-1664007	*hisS-lytH*	RNAsim, Livny et al. (5)
49	1716110-1716151	*thrS-dnaI*	RNAsim
50	1745253-1745539	SA1526- SA1527	RNAsim
51	1804057-1804207	SA1572- SA1573	RNAsim
52	1828454-1828572	*ribD*- SA1590	FMN riboswitch, RNAsim
53	1839422-1839492	SA1602- truncated-SA	RsaX15, RNAsim
54	1856483-1856685	truncated-SA-*tnp*	sprA, Pichon and Felden (6), RNAsim
55	1881838-1882594	*seo*-SA1649	tRNA
56	1899470-1899757	SA1662-SA1663	Long IGR + Term, RNAsim
57	1907748-1907903	SA1670- SAS053	RNAsim

58	1916292-1918962	*tnp*-23S	rRNA
59	1918961-1921962	tRNA- tRNA	tRNA
60	1923883-1925438	23S- SA1678	rRNA
61	1950616-1950792	*map*- SA1705	Leader of *map*, Marchais et al. (8), RNAsim
62	1952064-1952292	SA1705- SA1706	RNAsim, Livny et al. (5)
63	1961413-1961597	SA1714- SA1715	RNAsim, Livny et al. (5)
64	1992295-1992365	SA1738- SAS056	RsaX17, RNAsim
65	2001219-2001316	SAS057- SA1749	RNAsim
66	2011128-2011190	SA1760-*sep*	downstream of sprG, Pichon and Felden (6)
67	2054610-2054739	SA1814- SA1815	RNAsim
68	2094306-2094381	SA1853- SA1854	
69	2108489-2109159	*ilvA*-23S	rRNA
70	2109258-2112139	5S-16S	rRNA
71	2112585-2114171	23S-2tRNA	rRNA-tRNA
72	2114295-2114494	16S- SA1867	rRNA
73	2145394-2145685	SA1892- SA1893	Long IGR + Term
74	2212146-2212213	*glmS*- *mtlF*	glmS riboswitch
75	2230198-2230858	Arg-5S	rRNA
76	2230957-2232838	5S-16S	rRNA
77	2234884-2237870	23S- SA1969	rRNA
78	2282200-2282274	*alsS*- SA2010	long IGR
79	2315574-2315703	SA2053- SA2054	RNAsim
80	2326436-2326506	SA2062- *moaA*	RNAsim
81	2440668-2440861	SA2171- *gltT*	RNAsim, RsaX19
82	2490989-2491119	SA2217- SA2219	RNAsim
83	2491406-2491543	SA2217- SA2219	RNAsim, sprA2, Pichon and Felden (6)
84	2513401-2513603	*opuCA*- SA2238	long IGR + Term
85	2544500-2544573	SA2267- SA2268	Upstream of RsaX28
86	2560653-2560793	SA2283-SA2285	Long IGR
87	2561037-2561177	SA2283-SA2285	
88	2561421-2561521	SA2283-SA2285	
89	2561805-2561945	SA2283-SA2285	
90	2562189-2562329	SA2283-SA2285	Long IGR, RNAsim
91	2590865-2590957	*Fbp*-SA2305	sprA3, Pichon and Felden (6), RNAsim
92	2652623-2652866	SA2360SA2361	RNAsim
93	2769241-2769328	SA2457-*icaR*	RsaX25

This analysis exploits the composition bias between known RNAs (tRNAs, rRNAs, tmRNA) and the rest of the genome. Known RNAs (underlined in violin), annotated in RFAM database, include stable and universally conserved bacterial RNAs (rRNAs, tRNAs, SRP, 6S RNA, tmRNA, RNase P) and several *cis*-acting leader of mRNAs regulated by metabolites (riboswitches: TPP, thiamine pyrophosphate; SAM, S-adenosyl methionine, FMN, flavin mononucleotide; Gln6P, glucosamine 6-phosphate). STAR and sequence repeats are underlined in orange. This HMM analysis predicts 28 new ncRNAs (no color). Other comments include the Rsa RNAs studied in this work, the IGRs which were predicted by RNAsim, and previously identified or predicted ncRNAs.

Table S3. Genes showing differential expression between RN6390 and Δ*rsaE* mutant strains analyzed by microarray.

Gene name	Function	Fold change	COG category
Genes upregulated by RsaE			
arcA	Arginine deiminase	3,87	E
arcB	Ornithine carbamoyltransferase	2,02	E
arcC	Carbamate kinase	2,08	E
arcD	Arginine/ornithine antiporter	2,51	E
arcR	Transcriptional regulator, Crp/Fnr family	1,96	
atl	Bifunctional autolysin precursor	2,63	MG
beta	Choline dehydrogenase	6,14	E
betB	Betaine aldehyde dehydrogenase	33,97	C
carB	Carbamoyl phosphate synthase large subunit	1,95	E
comK	Competence transcription factor	3,76	
cudT	Osmoprotectant transporter, BCCT family	8,14	M
fdh	Formate dehydrogenase	2,01	C
fmtA	Autolysis and methicillin resistant-related protein	2,29	V
fnbA	Fibronectin-binding protein precursor	1,88	M
glpQ	Glycerophosphoryl diester phosphodiesterase	1,92	M
hlgC	Gamma-hemolysin component C	2,19	V
isdC	Iron transport associated domain protein	1,92	M
SAOUHSC_02608	LysR family regulatory protein	1,8	K
Plc	1-phosphatidylinositol phosphodiesterase	2,17	I
pnbA	Para-nitrobenzyl esterase	1,85	I
purK	Phosphoribosylaminoimidazole carboxylase, ATPase subunit	1,97	F
pyrC	Dihydroorotase	3,93	F
pyrR	Pyrimidine regulatory protein	10,05	F
ribB	Riboflavin synthase subunit alpha	2,77	H
ribD	Riboflavin biosynthesis protein	3,67	H
rnc	Ribonuclease III	1,98	K
SA0423	Hypothetical protein	3,28	S
SA1598	Hypothetical protein	2,79	S
SA2309	Hypothetical protein	1,93	S
SAOUHSC_00025	Putative 5'-nucleotidase	2,16	F
SAOUHSC_00367	Putative sodium:dicarboxylate symporter protein	2,9	R
SAOUHSC_00484	MesJ/Ycf62 family protein	1,83	D
SAOUHSC_00765	Putative comf operon protein 1	2,40	L
SAOUHSC_01036	Hypothetical protein	2,57	S
SAOUHSC_02723	Putative glycerate kinase	2,32	S
SAOUHSC_02770	Similar to diaminopimelate epimerase	3,15	E
SAOUHSC_02794	Hypothetical protein	2,40	S
SAOUHSC_02842	Hypothetical protein	2,63	S
SAOUHSC_02855	LysM domain protein	3,44	R
SAR1150	Antibacterial protein	2,92	V
SAOUHSC_02129	Hypothetical protein	2,17	S
SAV0884	Hypothetical protein	2,46	S
ssaA	Secretory antigen precursor SsaA	5,66	R
sspA	Serine protease; V8 protease; glutamyl endopeptidase	2,65	V
Genes downregulated by RsaE			
bioA	Adenosylmethionine-8-amino-7-oxononanoate	0,33	H
bioB	Biotin synthase	0,18	H
bioD	Dethiobiotin synthase	0,18	H
capI	Capsular polysaccharide synthesis protein CapI	0,48	M
capJ	Capsular polysaccharide synthesis protein CapJ	0,46	M
capL	Capsular polysaccharide synthesis protein CapL	0,55	M
capM	Capsular polysaccharide synthesis protein CapM	0,51	M
capN	Capsular polysaccharide synthesis protein CapN	0,49	M
capO	Capsular polysaccharide synthesis protein CapO	0,53	M
fadA	Acyl-CoA acetyltransferase	0,27	I
fadB	3-hydroxyacyl-CoA dehydrogenase	0,32	I

Gene	Description	Fold change	COG
fadE	Acyl-CoA synthetase	0,46	I
ilvB	Acetolactate synthase small subunit	0,19	E
ilvC	Ketol-acid reductoisomerase	0,18	E
ilvD	Dihydro-acid dehydratase	0,16	E
ilvN	Acetolactate synthase small subunit	0,18	E
leuA	2-isopropylmalate synthase	0,36	E
leuB	3-hydroxyacyl-CoA dehydrogenase	0,3	C
lldp2	L-lactate permease	0,5	C
SAS1649	Putative soluble hydrogenase subunit	0,34	S
oppA	Oligopeptide transport system ATP-binding protein OppA	0,39	E
oppB	Oligopeptide transport system ATP-binding protein OppB*	0,48	E
oppC	Oligopeptide transport system ATP-binding protein OppC	0,43	E
oppD	Oligopeptide transport system ATP-binding protein OppD	0,48	E
oppF	Oligopeptide transport system ATP-binding protein OppF	0,45	E
pgk	Phosphoglycerate kinase	0,53	G
purF	Phosphoribosylpyrophosphate amidotransferase	0,54	F
SACOL2620/SA2393	4-aminobutyrate aminotransferase*	0,17	F
SA0769	ABC transporter ATP-binding protein	0,41	P
SA2491	Hypothetical protein	0,55	S
SACOL1020/SA0873	Hypothetical protein*	0,45	S
Sak	Staphylokinase precursor	0,53	V
SAOUHSC_00401	Hypothetical protein	0,43	S
SAOUHSC_00949	Sodium:alanine symporter family protein	0,51	E
SAOUHSC_01138	Acetyltransferase (GNAT) family protein	0,51	
SAOUHSC_01319	Aspartate kinase	0,35	E
SAOUHSC_02713	8-amino-7-oxononanoate synthase	0,2	H
SAOUHSC_03019	ABC transporter ATP-binding protein	0,55	P
SAOUHSC_03020	Similar to integral membrane protein	0,49	S
SAOUHSC_00842	ABC transporter ATP-binding protein	0,42	P
sucC	Succinyl-CoA synthetase subunit alpha*	0,62	G
sucD	Succinyl-CoA synthetase subunit beta*	0,65	G
thrB	Homoserine kinase	0,42	E
thrC	Threonine synthase	0,23	E

COG category	Number of genes	Functional groups
C	4	Energy production and conversion
D	1	Cell cycle, cell division, chromosome partitioning
E	21	Amino acid transport and metabolism
F	6	Nucleotide transport and metabolism
G	4	Carbohydrate transport and metabolism
H	6	Coenzyme transport and metabolism
I	5	Lipid transport and metabolism
K	2	Transcription
L	1	Replication, recombination and repair
M	12	Cell wall/membrane/envelope biogenesis
P	3	Inorganic ion transport and metabolism
R	3	General function prediction only
S	14	Function unknown
V	3	Defense mechanisms

(Top) Fold change corresponds to RN6390 versus Δ*rsaE* mutant data: values > 1 correspond to genes that are overexpressed in RN6390 strain whereas values < 1 correspond to genes that are overexpressed in the Δ*rsaE* mutant strain. *Predicted RsaE-mRNA base pairings. (Bottom) The number of RsaE-dependent regulated genes corresponding to a defined group of function (COG) is given. The complete microarray data set has been posted on the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/) under accession number GPL7137 for the platform design and GSE17135 for the original data set.

Table S4. List of oligonucleotides.

PCR intergenic regions, northern blot[1]			
rsaX01f	CATGCTTGCCTAGGGGTATG	rsaX01r	ATGTCCCAAGCTCCATTTTG
rsaX02f	TTTTTCATCATTTACGATACATCATTC	rsaX02r	GCCAAGTTACATTGCTTAATCTCC
rsaX03f	TGATCACGATGAAGTCATTCAA	rsaX03r	GGTGCAAAATTAAAAATACATCAGG
rsaX04f	GAAAAATCGTGTGATTATCTGAGC	rsaX04r	TCAGTCAACTACTGCCAATATAACAT
rsaKf	TTGGTAACCATCACAGCTTAATTT	rsaKr	TTGAAAACGCATACTTCACCA
rsaX05f	GGTCTCGTCTAGGCAAAGCA	rsaX05r	AGACACTGCATCACGGTACG
rsaAf	CCATTACAAAAATTGTATAGAGTAGC	rsaAr	ACCCGAGTAGTCTTCCTTGG
rsaX06f	CGCTGTGAAGTTAGATAGATGAGTTT	rsaX06r	GCAGTTGCTGCACAAACAAA
rsaX07f	AGGCAATGTGAAAAAGCTGA	rsaX07r	AGGAAGATAGCCGCATAACG
rsaCf	CATCGGATGAGTGTGGCTTA	rsaCr	ACGCCATTCCCTACACACTC
rsaDf	CTTGGCTTTTATGGGAAATGA	rsaDr	TCTGCTATAATGTTTTTAACTTCTTCA
rsaX08f	ACGAAAGAAATGGGTTGCTC	rsaX08r	TTCCTAGCTGAAACCCTCTATAAAAA
rsaHf	TCATTATTGTTGTTCAAAGGTTATACA	rsaHr	ACGATTAACGTACGGGTCCA
rsaX09f	TGATCGTGATAGCGGATACATTT	rsaX09r	GCTTTTATGTTATATTTTCAAGCAAGA
rsaEf	CCCCTTTGTTTGAAGTGAAAA	rsaEr	AATCGTTAATGTATAAAAAGTGCAT
rsaE-RT	TTTTCACTTCAAACAAAGGGG	rsaE-Cmotif	AAAGGGGATGGGAGAAA
rsaX10f	TCTAGAAATCTTTTTCAATTATATG	rsaX10r	TTCCATGTTGTCAAACCCTTT
rsaX11f	GTATTAATGGCGAGACTCCTGA	rsaX11r	CTTCGTATTGAATGGCTTCG
rsaX12f	ATACTTCGTATTGAATGGCTTCG	rsaX12r	GGACACGAGGCTGGGATATT
rsaX13f	GCTAAAAGAAGAATCAACAAAGC	rsaX13r	AAACAGTGGTTCTTTATCATTTCTG
rsaX14f	AATCCTCAACCAATGCCTACA	rsaX14r	CCATGAAAATGGGATAGCAA
rsaX15f	GGCTGGGACATAAATCCCTAA	rsaX15r	AGACTCCTGAGGGAGCAGTG
rsaX16f	TTAACTTTAACAATGATTAGTTGGCTA	rsaX16r	CATGACATTGATTAGTGAGTAGATTG
rsaX17f	GAAGGGACCCAACACAGAAA	rsaX17r	CTCAGCCTTGGTCTTCGACT
rsaIf	CACGTGCTAGCCGACAAATA	rsaIr	TAACAGGGGGAGCGATTAAA
rsaX18f	AGTGCGATTTCAAGATGCTG	rsaX18r	TCAAATCAACAACAATAACCATCA
rsaX19f	GCATGCATAAAAGCCCCTAA	rsaX19r	GCCCCTTACAACCTTTGGT
rsaX20f	TTTCGAGTATCCCTAGCTCGTT	rsaX20r	GCTGCATGTACACAAGGAGTG
rsaJf	GGGCAGATATAAACAGCGATTC	rsaJr	GGGCAATAGTGAGGGGATTT
rsaX21f	TTCCAAATAAATAAAGGGATTTGTGA	rsaX21r	ATGCGTTCATTCATGCCATT
rsaX22f	GCTGAAAGTCACCTAAAATCCTTT	rsaX22r	TGTGTGGAAGACAGGGTAAA
rsaX23f	TTGCGAGTAGTGAGGGGATT	rsaX23r	TTAGTTGATTTTGCATTTTT
rsaX24f	CGCAATTAAAAAGCAATCAACA	rsaX24r	AATGTTATTGTGAATTGCGAGAA
rsaX25f	TACCTTGCGTAACCCGTCAC	rsaX25r	TGACAATACAAATCGATATTGAGAA
rsaGf	GTGCGTGAAGAGATGAAAGATAC	rsaGr	CTTCATAACAGGGAATTATG
rsaBf	GTGTTAATTGTAGCGCATCCC	rsaBr	GGTTGCAATATTTAATTGGG
RT-RNAIII5	AATACATAGCACTGAGTCCAAGG	RT-RNAIII6	CCTAGATCACAGAGATGTGATGG
6Sf	ATGGGTTTTCTTGCAGCGTA	6Sr	CGTGTACGCTGCAAGAAACCC
Anti-rsaA	GTTAAAGTTCTCCCAAGGAAG		
Anti-rsaC	GGTTTCCTCCCCCCATAGATC		
Anti-rsaH	TTGACTCCCTTTAGTAGTGG		
Anti-rsaE	ATTTCTCCCATCCCCTTTGT		
Anti-rsaG	GATACTGCTTCTACCCTTGC		
5SrRNAr	AACAGGTGTGACCTCCTTGC		

In vitro transcription/toeprint

rsaG-5'-*Stu*I	TATAGGCCTCTGCCCCGACGGCATGTGCG	rsaG-3'-*Bam*HI	TATGGATCCACAATACTCCCTACAATATTG
rsaA-*Bam*HI-T7	AAAGGATCCTAATACGACTCACTATAGGTTAACCATTACAAAAATTGTATAG	rsaA-*Dra*I-*Eco*RI	TTGAATTCTTTAAAGTACACTTTGCTCATAGCAAAGTGTACCC
rsaH-	AAAGGATCCTAATACGACTCACTATA	rsaH-*Dra*I-	TTGAATTCTTTAAAAACGACCCGCAC

BamHI-T7	GGTACCTTCGATAACGAATAAACATC TC	EcoRI	GATTAACGTACGGG
rsaE-BamHI-T7	AAAGGATCCTAATACGACTCACTATA GGAAATTAATCACATAACAAACATAC CCCTT	rsaE-DraI-EcoRI	TTGAATTCTTTAAAAAAACGTCGTGTC TGAATACACG
oppB-BamHI-T7	AAAGGATCCTAATACGACTCACTATA GGAATGATTTTTTAATTTCTTCAGC	oppB-EcoRI	AAAGAATTCTGCGTAGCTACAGGATC
opptoe1	GGCGAACCTGGCATTAATTTC		
sa0873-BamHI-T7	AAAAAAGGATCCGATCCCGCGAAAT TAATACGACTCACTATAGGTTAAACA AAGGAGGAATTCAGA	sa0873-sacI	AAAAAAGAGCTCAATTTCAAATGGCG CTTTAATTGT

Deletion/replacement ΔrsaE/aphA-3 mutant of *S. aureus* RN6390

rsaE-140-smaI	TTTCCCGGGAGGT TTACTACATA TG
rsaE-2041r-EcoRV	TAAGATATCG TTCATAATAT AACATGCTAT C
rsaE-2163-SalI	CGTGTCGACA TGTTCTTTTT TAATAAGAGA G
rsaE-3214r-XhoI	TGACTCGAGA TGGCTGGAGA ATTAC
rsaE-70-CT	GTTCAGATAG AGGTAATGAC
rsaE-3283r-CT	GATTAACTGC TAATCATATG GTG

[1]Oligonucleotides used for the PCR reactions: r is for reverse and f for forward; RT oligonucleotide used for primer extension.