

## FIGURE LEGENDS

Table S1. tblastn analysis suggests that  $\sigma^{\text{AntA}}$  is only encoded by antimycin-producing organisms taxa.

Table S2. Oligonucleotide primers used in this study.

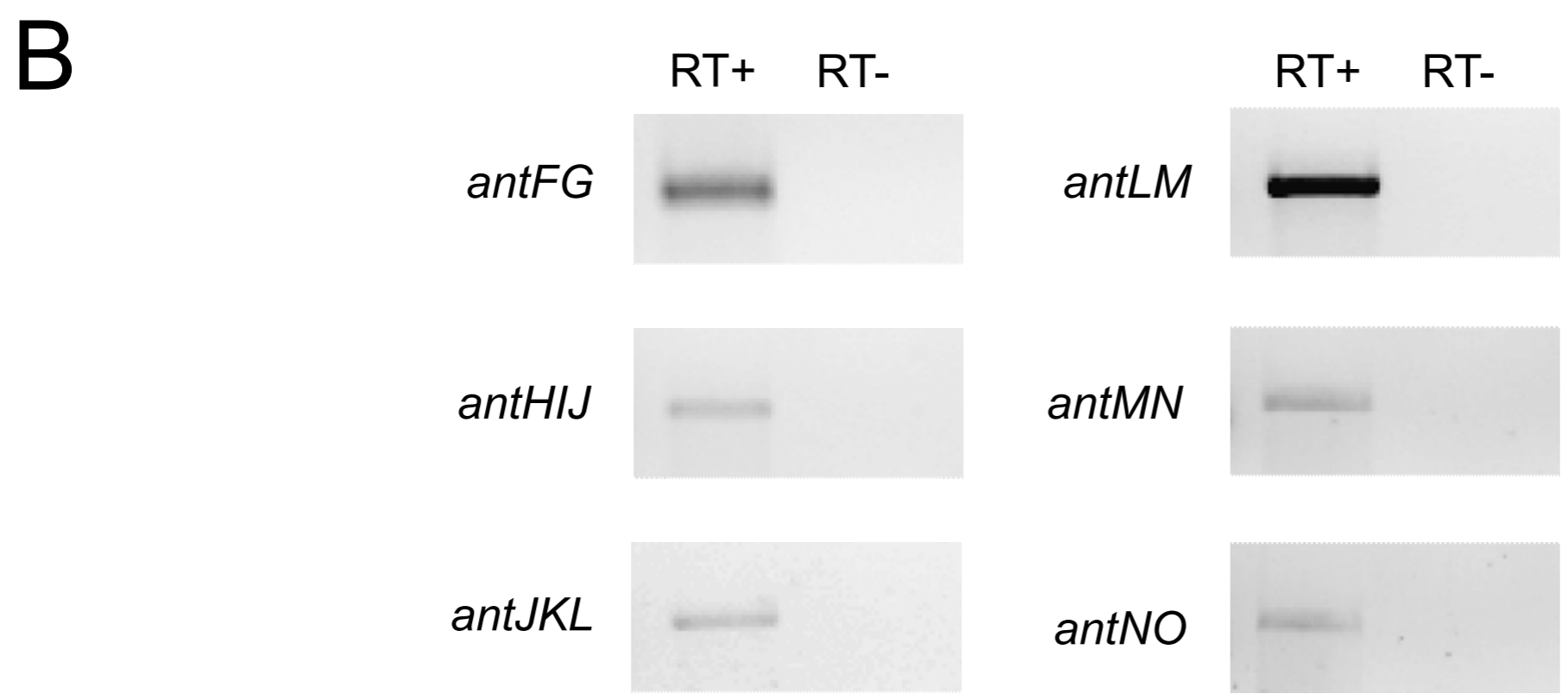
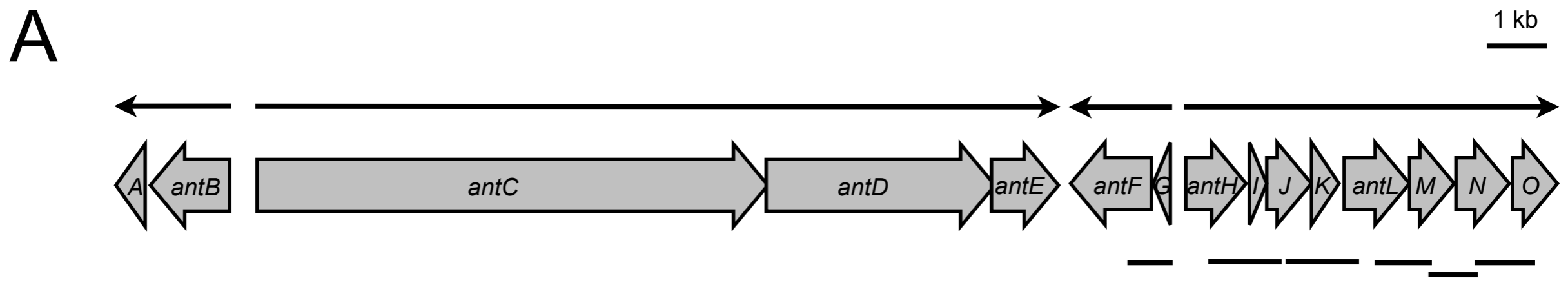
Fig. S1. *antFG* and *antHIJKLMNO* comprise two divergently co-transcribed operons. (A) Schematic representation of the antimycin gene cluster. Narrow black arrows represent predicted (*antAB*, *antCDE*) operons and experimentally proven (*antFG*, *antHIJKLMNO*) operons and the direction of transcription. Horizontal bars denote the location of DNA targeted for RT-PCR analysis. (B) Agarose gel electrophoresis of RT-PCR products. RT+, cDNA reverse transcribed  $\Delta antA/pIJ10257-antA$  RNA, RT-reverse transcriptase reaction mix with enzyme omitted. PCR products originating from cDNA template were cloned into pCRII-TOPO or pGEM-T Easy and sequenced.

Fig. S2. Clustal  $\Omega$  alignment of  $\sigma^{\text{AntA}}$  orthologues identified a conserved Ala-Ala. Predicted  $\sigma_2$  (green) and  $\sigma_4$  domains are indicated by shading. The terminal Ala-Ala motif conserved among  $\sigma^{\text{AntA}}$  orthologues is bolded. Clustal  $\Omega$  program available at: <http://www.ebi.ac.uk/Tools/msa/clustalo/> was developed by Sievers *et al.*, 2011.

Fig. S3.  $\sigma^{\text{AntA}}$  comprises a new ECF RNA polymerase  $\sigma$  factor subfamily.  $\sigma^{\text{AntA}}$  proteins were aligned to random proteins from each ECF RNA polymerase  $\sigma$  factor subfamily reported by (Staro *et al.*, 2009) using Clustal $\Omega$  (Sievers *et al.*, 2011) and PhyML (Guindon *et al.*, 2010) used to generate the phylogenetic tree, which was visualised using FigTree v1.4. All  $\sigma^{\text{AntA}}$  cluster together and possess a distinct lineage from all known ECF RNA polymerase  $\sigma$  factor subfamilies.

Fig. S4. Bacterial two-hybrid analysis did not detect an interaction between  $\sigma^{\text{AntA}}$  and the putative transmembrane protein STRS4\_02195.  $\beta$ -galactosidase activity measured for DHM1 cells harbouring empty pKT25 plasmid (black bars), pKT25-*antA* (dark grey bars), pKT25-STRS4\_02195 (light grey bars), pKT25-*sigB* (white bar), pKT25-*zip* (hashed bar) and the indicated pUT18C plasmid are shown. Each bar represents the mean value of enzyme activities (represented as Miller units) from three technical replicates and the standard error is also shown. The plasmid pair pKT25-*zip* + pUT18C-*zip* is a positive procedural control.

Figure S1. The antimycin gene cluster is organised into four transcriptional units



# Figure S2. Clustal $\Omega$ alignment of AntA orthologues

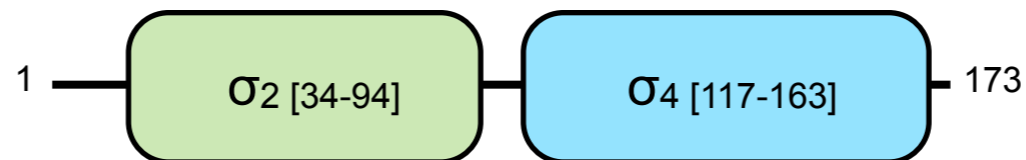
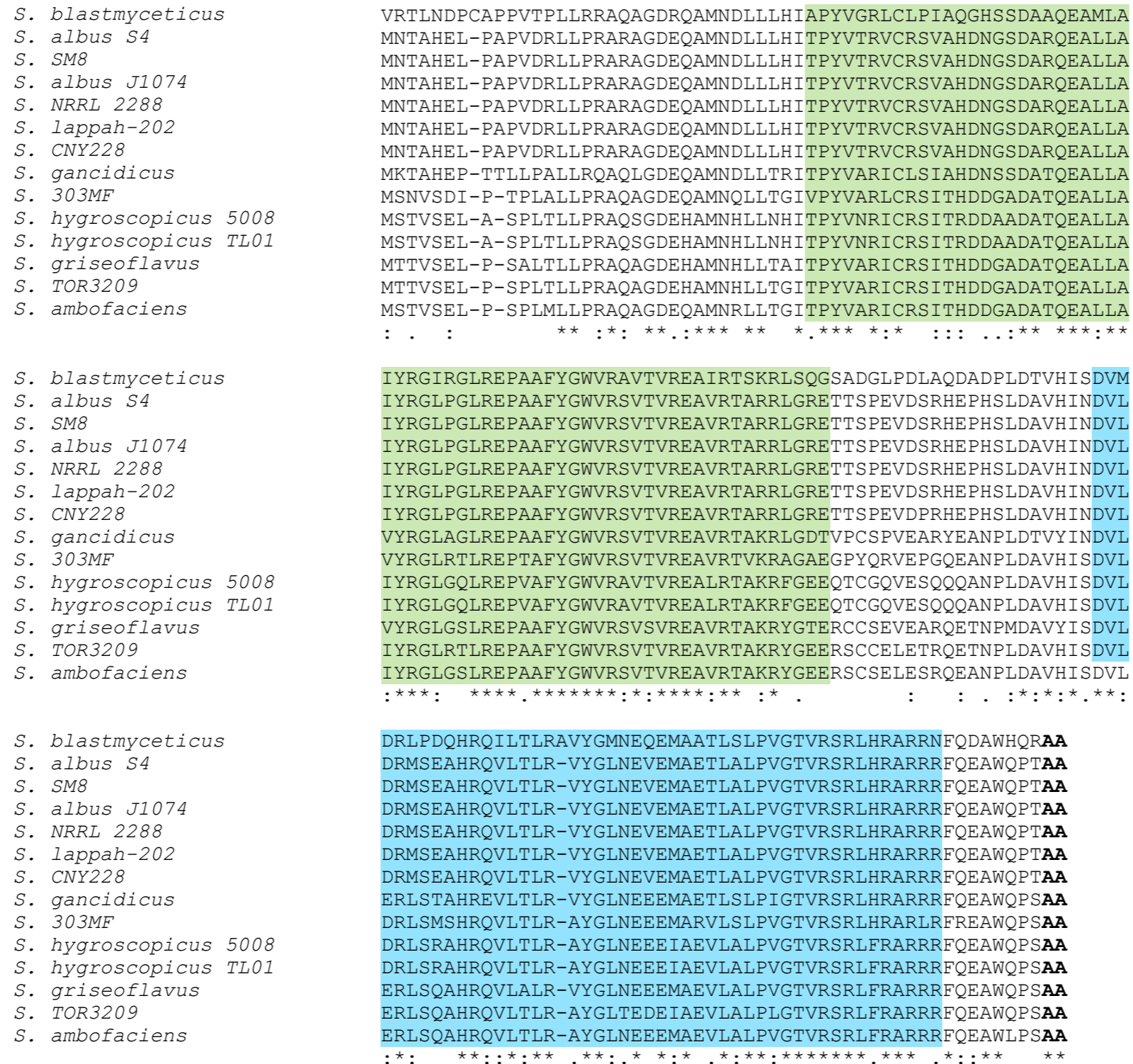


Figure S3.  $\sigma^{\text{AntA}}$  comprises a new subfamily of ECF RNA polymerase  $\sigma$  factors

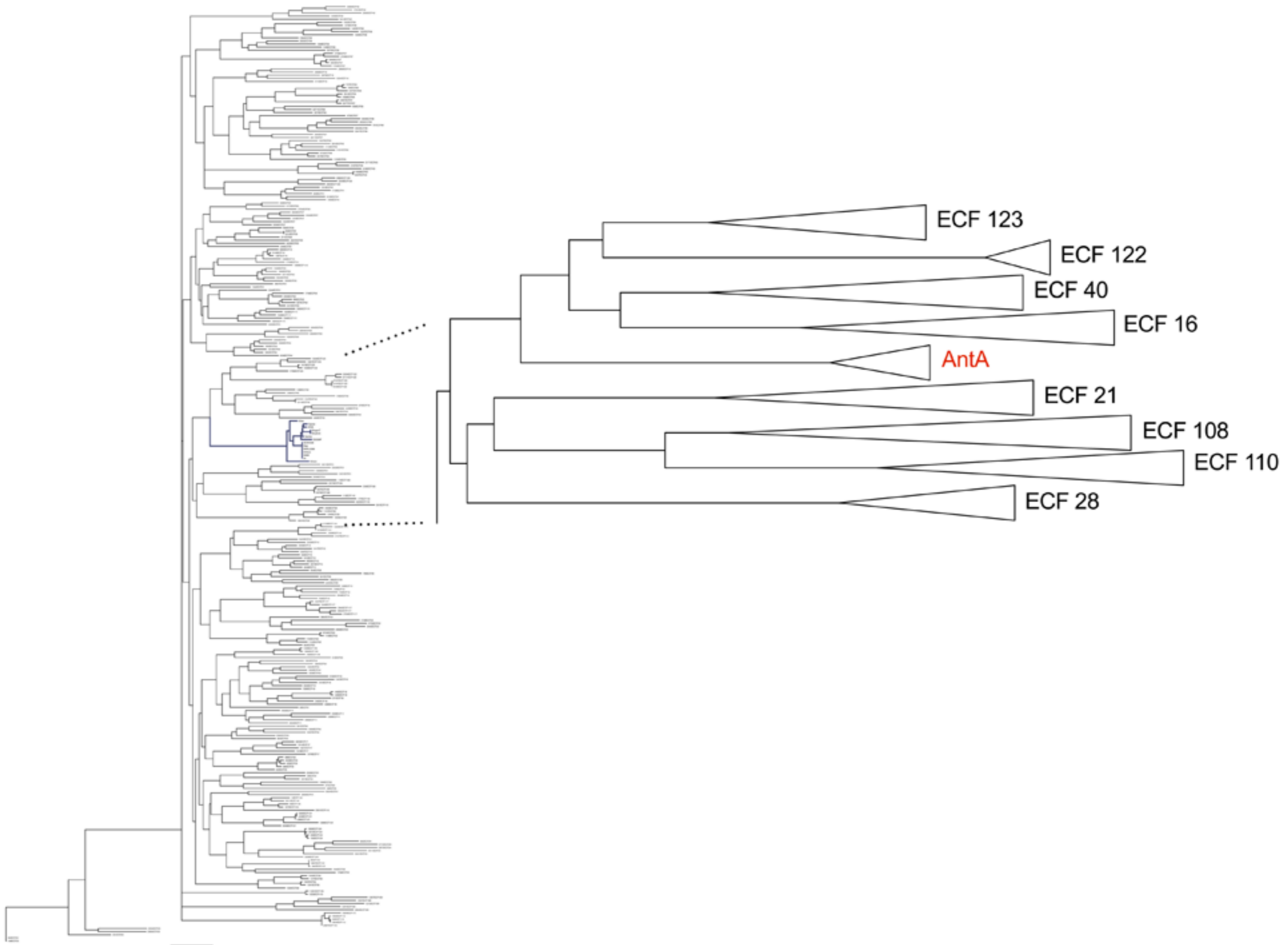


Figure S4.  $\sigma^{\text{AntA}}$  does not interact with STRS4\_02195

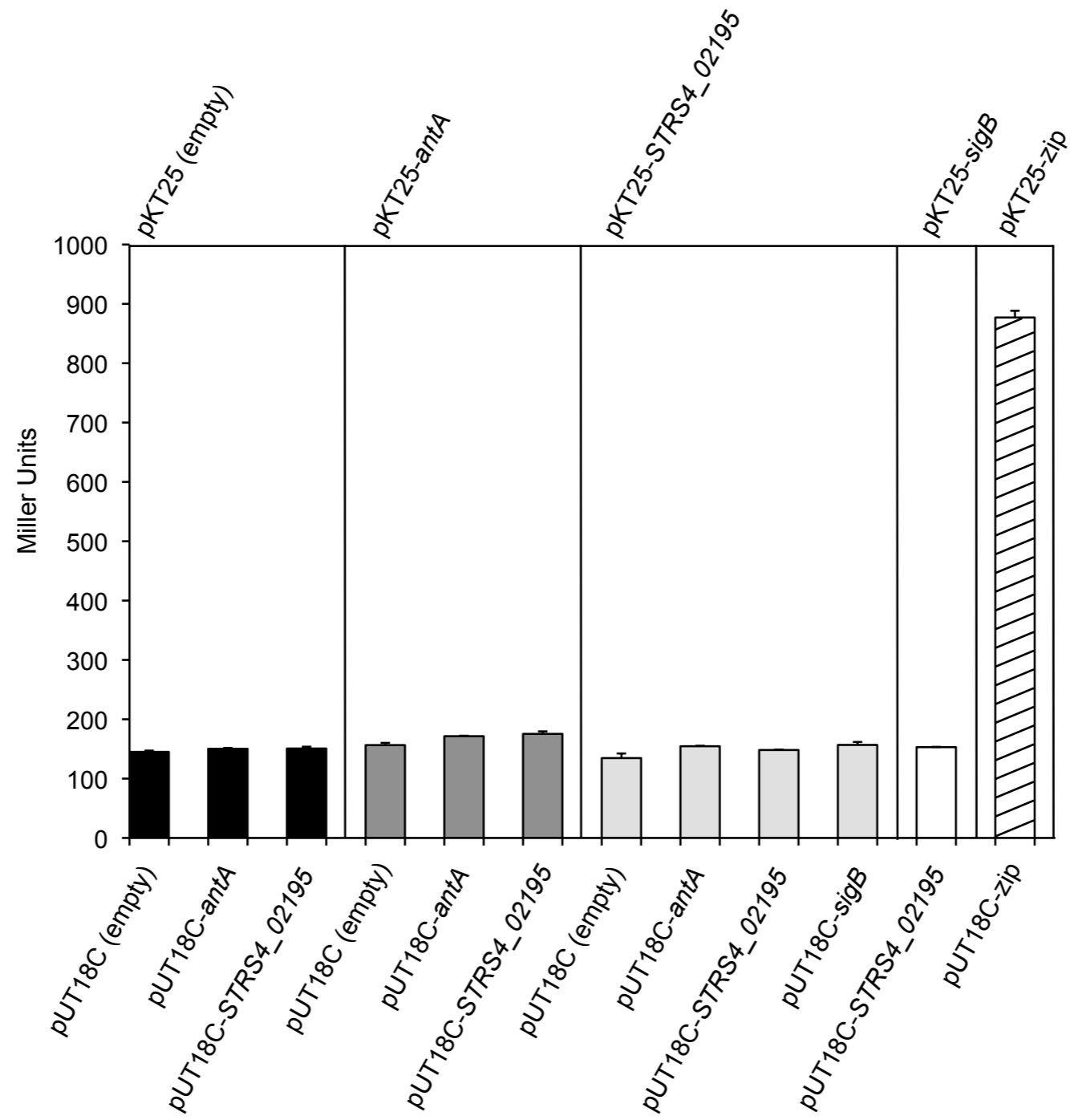


Table S1 tblastn analysis suggests that  $\sigma^{\text{AntA}}$  is only encoded by antimycin-producers

Species	Identity to $\sigma^{\text{AntA}}$	AA motif ?	Predicted targets	Source	E-value
<i>Streptomyces albus</i> J1074	100%	Yes	Antimycin biosynthesis	tblastn	3.00E-100
<i>Streptomyces</i> sp. SM8	100%	Yes	Antimycin biosynthesis	tblastn	3.00E-100
<i>Streptomyces</i> sp. NRRL 2288	100%	Yes	Antimycin biosynthesis	Yan et al 2012	3.00E-100
<i>Streptomyces</i> sp. LaPpaH-202	100%	Yes	Antimycin biosynthesis	tblastn	3.00E-100
<i>Streptomyces</i> sp. CNY228	99%	Yes	Antimycin biosynthesis	tblastn	2.00E-99
<i>Streptomyces ambofaciens</i> ATCC 23877	76%	Yes	Antimycin biosynthesis	Choulet et al 2006	1.00E-68
<i>Streptomyces</i> sp. 303MFC015.2	69%	Yes	Antimycin biosynthesis	tblastn	8.00E-67
<i>Streptomyces gancidicus</i> BKS 13-15	74%	Yes	Antimycin biosynthesis	Kumar et al 2013	2.00E-66
<i>Streptomyces</i> sp. TOR3209	73%	Yes	Antimycin biosynthesis	Hu et al 2012	4.00E-66
<i>Streptomyces griseoflavus</i> Tu4000	73%	Yes	Antimycin biosynthesis	tblastn	3.00E-66
<i>Streptomyces hygrosopicus</i> subsp. jinggangensis TL01	72%	Yes	Antimycin biosynthesis	tblastn	9.00E-65
<i>Streptomyces hygrosopicus</i> subsp. jinggangensis 5008	72%	Yes	Antimycin biosynthesis	tblastn	9.00E-65
<i>Streptomyces blastmyceticus</i> NBRC 12747	66%	Yes	Antimycin biosynthesis	Yan et al 2012	1.00E-59

Table 2. Oligonucleotides used in this study

Primer alias	Sequence (5'-3')*	Description
RFS172	<b>aagctt</b> acgatctggtcctcgggctc	PCR: screening cosmid library for <i>antC</i> -containing cosmids
RFS173	<b>ggatcc</b> cagccggtggaaggtgac	PCR: screening cosmid library for <i>antC</i> -containing cosmids
RFS184	ccattattatcatgacattaa	End sequencing of Supercos1 cosmids
RFS185	gtccgtggaatgaacaatgg	End sequencing of Supercos1 cosmids
RFS186	<u>cacgcgcccgcgtgtctcaccgccatggtggcc</u> <u>gtcatgtaggctggagctgctc</u>	PCR: <i>antA</i> REDIRECT knockout cassette
RFS187	<u>cgcccgcaccgtcctccccgcaaggagagcacc</u> <u>ccatgattccggggatccgtcgacc</u>	PCR: <i>antA</i> REDIRECT knockout cassette
RFS188	cttgcggggaggacggtgcggcgggcggtccgc ggtcatgtaggctggagctgctc	PCR: <i>antB</i> REDIRECT knockout cassette
RFS189	tgccaagatcggggccttctcgtctcccctcgccg tgattccgggatccgtcgacc	PCR: <i>antB</i> REDIRECT knockout cassette
RFS222	gtacccccgcccttcccccgattgggtacctcca tgattccgggatccgtcgacc	PCR: <i>STRS4_0213-17</i> REDIRECT knockout cassette
RFS223	cacgtgtgcgcgggcaccctggccccgtccggcgt ctcatgtaggctggagctgctc	PCR: <i>STRS4_0213-17</i> REDIRECT knockout cassette
RFS226	<u>ctccgtcgcgcaccacggatcagaaggggacac</u> <u>acatgattccg gggatccgtcgacc</u>	PCR: <i>STRS4_02194</i> REDIRECT knockout cassette
RFS227	<u>gcgaggccgcggccgggaagacctgaggccc</u> <u>gtgatcatgtaggctggagctgctc</u>	PCR: <i>STRS4_02194</i> REDIRECT knockout cassette
RFS228	acggctggaccgagatcgtcta	PCR: $\Delta$ <i>STRS4_02194</i> knockout confirmation
RFS229	tgttccgcgcaccgtcctca	PCR: $\Delta$ <i>STRS4_02194</i> knockout confirmation
RFS230	ata <b>catatg</b> aacaccgcgcacgaact	PCR: AntA coding sequence for complementation plasmid
RFS231	ata <b>aagctt</b> caggcggcggtgggctgcc	PCR: AntA coding sequence for complementation plasmid
RFS232	cccttgccgtcagagagatc	PCR: confirmation of $\Delta$ <i>antA</i> mutant strain
RFS233	gcttccccgcagtagatg	PCR: confirmation of $\Delta$ <i>antA</i> mutant strain
RFS258	acagcctgaccgcatggaa	PCR: confirmation of $\Delta$ <i>STRS4_0213-17</i> mutant strain
RFS259	actgaccgaggacgtcgtacgtgag	PCR: confirmation of $\Delta$ <i>STRS4_0213-17</i> mutant strain
RFS260	cctcgttcaggccgtaca	RT-PCR: <i>antA</i>
RFS261	acgaaccccactcactcg	RT-PCR: <i>antA</i>
RFS262	atcgtcacggtcaggaggt	RT-PCR: <i>antB</i>
RFS263	gcgtacatcgaggaactgaac	RT-PCR: <i>antB</i>
RFS264	gtcaacatcgtcccctcag	RT-PCR: <i>antC</i>
RFS265	accgagatctggaggtcgt	RT-PCR: <i>antC</i>
RFS266	agtacatgtgcccccgaag	RT-PCR: <i>antE</i>
RFS267	gccgaagagctgacggta	RT-PCR: <i>antE</i>
RFS268	ggacctgcgtagtctc	RT-PCR: <i>antF</i>
RFS269	aacgtctacccctcgagat	RT-PCR: <i>antF</i>
RFS270	ctcgaccaggaggaccag	RT-PCR: <i>antG</i>
RFS271	ctcaccggtaccaccgagta	RT-PCR: <i>antG</i>
RFS272	gctgtgccacacctgta	RT-PCR: <i>antH</i>
RFS273	agagcgccttcatcaggctc	RT-PCR: <i>antH</i>
RFS274	caccgcaccagggtctac	RT-PCR: <i>antO</i>
RFS275	cagatgcatccggtcgtgta	RT-PCR: <i>antO</i>
RFS276	<u>tacctggcgtccgcggggagacggcatgatcggc</u> <u>ccatgattccg gggatccgtcgacc</u>	PCR: <i>STRS4_02195</i> REDIRECT knockout cassette
RFS277	<u>tttccggtgccgcctcccgtcctgcccgcgagaac</u> <u>tcatgtaggctggagctgctc</u>	PCR: <i>STRS4_02195</i> REDIRECT knockout cassette
RFS278	ggtagcgcctcccgcac	PCR: confirmation of $\Delta$ <i>STRS4_02195</i> mutant strain
RFS279	ctttccggtgccgcctc	PCR: confirmation of $\Delta$ <i>STRS4_02195</i> mutant strain

RFS280	<u>ataggatcc</u> <u>aatgcccaggg</u> aacgcgtact	PCR: STRS4_02195 coding sequence for bacterial two-hybrid plasmids
RFS281	<u>ataggatcc</u> ttcgcggtccgccggagccgg	PCR: STRS4_02195 coding sequence for bacterial two-hybrid plasmids
RFS282	<u>ataggatcc</u> <u>aatgaacaccg</u> cgcacgaac	PCR: AntA coding sequence for bacterial two-hybrid plasmids
RFS283	<u>ataggatcc</u> ttggcggtgggctgccatg	PCR: AntA coding sequence for bacterial two-hybrid plasmids
RFS284	<u>ataggatcc</u> <u>aatgtccgcacag</u> actgaagc	PCR: STRS4_04339 (SigB orthologue) coding sequence for bacterial two-hybrid plasmids
RFS285	<u>ataggatcc</u> ttctctcggcgagcatccccg	PCR: STRS4_04339 (SigB orthologue) coding sequence for bacterial two-hybrid plasmids
RFS286	cgtcgctgggcgagtggaacgcc	PCR: sequencing pUT18C inserts
RFS287	cttaactatgcgcatcagagc	PCR: sequencing pUT18C inserts
RFS288	gttcgccattatgccgcatc	PCR: sequencing pKT25 inserts
RFS289	ggatgtgctgcaaggcgatt	PCR: sequencing pKT25 inserts
RFS298	gtcgacggtgacgcccttg	RT-PCR: <i>antFG</i> co-transcription
RFS299	atgagcaccatcagggagttgct	RT-PCR: <i>antFG</i> co-transcription
RFS302	tcaccaacgcgctgctcgtc	RT-PCR: <i>antJKL</i> co-transcription
RFS303	ggccgctccggaacctccag	RT-PCR: <i>antJKL</i> co-transcription
RFS304	gctgaaggacgcgtacgtcg	RT-PCR: <i>antLM</i> co-transcription
RFS306	gctgtcggggacgggttcc	RT-PCR: <i>antMN</i> co-transcription
RFS307	ggagtcggtgagcgcggaga	RT-PCR: <i>antMN</i> co-transcription
RFS308	gcgtgaggcggttgtaagg	RT-PCR: <i>antNO</i> co-transcription
RFS309	acgtcgtcgaaggctcggg	RT-PCR: <i>antNO</i> co-transcription
RFS329	gctctggattctcaacaatgc	PCR: confirmation of $\Delta$ STRS4_02212 mutant strain
RFS330	tgaggcggcgattgatt	PCR: confirmation of $\Delta$ STRS4_02212 mutant strain
RFS317	agcgcgttcatgaaggatg	RT-PCR: <i>antAB</i> operon 5'RLM-RACE
RFS318	cccgccctcctgcggatca	RT-PCR: <i>antAB</i> operon 5'RLM-RACE
RFS323	agctctccaccacctcgat	RT-PCR: <i>antGF</i> operon 5'RLM-RACE
RFS324	caactgggtcagccacatca	RT-PCR: <i>antGF</i> operon 5'RLM-RACE
RFS340	tcaagcggcacaccaacgac	RT-PCR: <i>antHIJ</i> co-transcription
RFS341	atggtcggcgccctggtgat	RT-PCR: <i>antHIJ</i> co-transcription
RFS342	tggtcgtggccgtagtga	RT-PCR: <i>antLM</i> co-transcription
RFS345	cgccgagtacaggtaca	RT-PCR: <i>antHIJKLMNO</i> operon 5'RLM-RACE
RFS346	ctgcatcccgatgatct	RT-PCR: <i>antHIJKLMNO</i> operon 5'RLM-RACE
RFS351	ataga <u>attc</u> ggcgggagaaaaga	PCR: <i>antA</i> native promoter complementation plasmids
RFS352	ata <b>ggatcct</b> cagtcgctggtgggctgc	PCR: <i>antA</i> native promoter complementation plasmid and introduces A172D A173D point mutations

\* non-homologous sequences are underlined and engineered restriction endonuclease sites are bolded