Table S1: Plasmids[a]

Plasmid	Genotype	
pDG268	*amyE::lacZ cat amp*	(Guérout-Fleury et al., 1996)
pDG1515	*tet amp*	(Guérout-Fleury et al., 1995)
pDP376	*ΩΔremsite34 mls amp*	
pDP377	*ΩΔremsite56 mls amp*	
pKB116	*P_{tac}-MBP-remA lacIq amp*	
pMAL-c2x	*P_{tac}-MBP-lacZ lacIq amp*	(New England Biolabs)
pMiniMAD2	*ori^{BsTs} amp mls*	(Patrick and Kearns, 2008)
pRLG770	transcription vector	(Ross et al., 1990)
pRLG1507	pSL6 circular permutation vector	(Gosink et al., 1993)
pRLG3422	pRLG770 containing *lacUV5 (-45/+1)*	(Gaal et al., 1997)
pRLG7340	pSL6 containing *rrnB* P1 *(-73/+50)*	
pRLG11810	pRLG770 containing P_{eps} *(-452/+35)*	
pRLG11811	pRLG770 containing P_{eps} *Δremsite34*	
pRLG11812	pRLG770 containing P_{eps} *Δremsite56*	
pRLG11825	pRLG770 containing $P_{eps}^{improved}$ *(-452/+35)*	
pRLG12757	pRLG770 containing P_{eps} *(-107/+35)*	
pRLG12759	pRLG770 containing P_{eps} *(-159/+35)*	
pRLG12760	pRLG770 containing P_{eps} *(-176/+35)*	
pRLG12764	pRLG770 containing P_{eps} *(-121/+35)*	
pRLG12766	pSL6 containing P_{eps} (-159/+35)	
pRLG12783	pSL6 containing P_{opuA} (-159/+35)	
pRLG12784	pRLG770 containing P_{opuA} *(-172/+27)*	
pRLG12785	pSL6 containing P_{tapA} (-168/+65)	
pRLG12786	pRLG770 containing P_{tapA} *(-168/+65)*	

[a]Numbers in parentheses indicate the boundaries of the promoter construct relative to the transcriptional start site.

Table S2: Primers

Primer	Sequence
1527	AGGAGGAATTCATGACGATTAAACTGATTAATATCGGA
1528	CTCCTGGATCCAACCCTCTTTCTTTCATGCGGCAA
1620	GCGCTACGGCGTTTCACTTC
2713	AGGAGGAATTCCTCCGCCATCAGCGCTTT
2714	CTCCTCTCGAGTCCTCTAATTTACTGCACTTC
2715	CTCCTCTCGAGCTTATTATTTTAAATTTTATAAAGAAC
2716	AGGAGCTCGAGTGTTCTCTAAAGAGAACTTATTG
2717	AGGAGCTCGAGGAGGAAAATCATGATTTTGTTC
2718	CTCCTGGATCCGATCGTGATCCTGAACGGCA
3250	ACGACTCACTATAGGGCGAATTG
3251	CTCACTAAGGGAACAAAAGCTGG
3318	TCCTGCCTAAACGCCGTCGT
3319	CAATTCGCCCTATAGTGAGTCGTCTGCCTTCTTTGTTTGTTTCC
3320	CCAGCTTTTGTTCCCTTTAGTGAGTGATATTGGCACCAGTATGAC
3321	GCATATGTATATCACAACTCGC
4633	AGGAGGAATTCAATGTTGCGCGGTCAGAAAATTA
4831	AGGAGGAATTCTGTACGGCTTGCACTAAATGT
5619	CTCCTAAGCTTCCCGCGGCTGGCTTCCCGC
5620	GCAGCGAATTCGGGAAGTGCAGTAAATTAGAGGAA
5892	GGCTTATTTTGCAATTTGTAAATAATAACGTTTTC
6097	GCGGCGGATCCCCCGCGGCTGGCTTCCCGC
6157	AGGAGGAATTCCCTCTATTCCTGTCGTTATTTC
6158	AGGAGGAATTCATTTCGTTCATTATAAGGAATTTTTCGT
6159	AGGAGGAATTCATTTAAAATAATAAGGGAAGTGCAGTAA
6463	AGGAGGAATTCGGATATGCATTTAAATTCTCACATAAC
6464	TCCTCAAGCTTCATATCTTACCTCCTGTAAAACACTG
6465	AGGAGGAATTCCTAAAGGTAGGAGAAAAATCACCCGT
6466	TCCTCAAGCTTCATATTTTCCCTCCATATTAAGCAAT

Table S3: β-galactosidase data

Genotype	*amyE::P_{eps}-lacZ*	*amyE::$P_{eps}^{improved}$-lacZ*
Wild type	12 ± 4 (DS1882)	25 ± 6 (DS9468)
sinR	480 ± 38 (DS2609)	1751 ± 320 (DS9467)
remA	1 ± 0 (DS2913)	14 ± 1 (DS9469)
remA sinR	1 ± 0 (DS2911)	7 ± 1 (DS9466)
sinI	1 ± 0 (DK444)	16 ± 2 (DK445)

[1]All cultures were grown to 1.5 OD_{600} in LB medium. All values are the average of three replicas (Miller units ± standard deviation). This table contains the raw data for Figure 1. Strains used to generate the data are indicated in parentheses.

Supplemental Figure Legends

Figure S1. RemA binds in an array upstream of target promoters. A) The sequences of the P_{eps}, P_{tapA}, and P_{opuA} promoters (Kearns et al., 2005; Chu et al., 2006; Hoffman et al., 2013). The -35 and -10 promoter elements and the +1 transcriptional start sites are underlined. RemA binding sites (BS) are indicated in red. B) A consensus binding site was predicted suing Weblogo assuming that each of the RemA binding sites is part of a series of direct repeats (Crooks et al., 2004).

Figure S2. RemA is highly conserved and is typically found upstream of the gene that encodes Gmk. A) Multiple sequence alignment and boxshade analysis of RemA sequences from the following bacteria: Bsu, *Bacillus subtilis*; Ban, *Bacillus anthracis*, Oih, *Oceanobacillus iheyensis*, Cbo, *Clostridium botulinum*, Tet, *Thermoanaerobacter ethanolicus*, Tma, *Thermotoga maritima*, Pmo, *Petrotoga mobilis*, Tye, *Thermodesulfovibrio yellowstonii*, Dvu, *Desulfovibrio vulgaris*, Det, *Dehalococcoides ethenogenes*, Npu, *Nostoc punciforme*, Ssp, *Synechococcus sp*. Carets indicate the sites of loss-of-function missense mutations *sor4* (R18W) and *sor31* (P29S) reported in Winkelman et al., 2009. B) Neighborhood analysis of genes surrounding *remA* in various genomes that correspond to the predicted RemA products aligned in Fig S2A. Neighborhood analysis was conducted using www.microbesonline.org. Named genes are color coded to indicate conservation in the various genomes. Gray genes are genes that are not highly conserved with other genomes in this neighborhood. Gene lengths and distances are not to scale.

Figure S1

A)

Peps

```
     BS6        BS5        BS4        BS3        BS2        BS1
ataAGGGAAGtgcAGTAAATtagAGGAAAAtcaTGATTTTgttCTCTAAAgagAACTTATtggcttattt
tgcaattttaaataataacgttttctttataatccaatca
       -35                        -10        +1
```

PtapA

```
     BS6        BS5        BS4        BS3        BS2        BS1
atgAGCAATActgAGCAAGActtTGTAATAtgaTGAAAACattCTTTTAAacgAACAAAAtgagcgattt
cggtgttttaaatctataaatcgttgattatactctatttg
       -35                        -10        +1
```

PopuA

```
                BS5        BS4        BS3        BS2        BS1
cccgtaaaatacaATCATATaggAGGATTAcagAGCATTTagaAGCATAAataAGATCATgtggtcacat
ggatgtttataaagaaatggtacagaataaaagagaatatgctgtttgtgtgggaagttacataaatgtt
acggtaataaag                                        -35
 -10       +1
```

B)

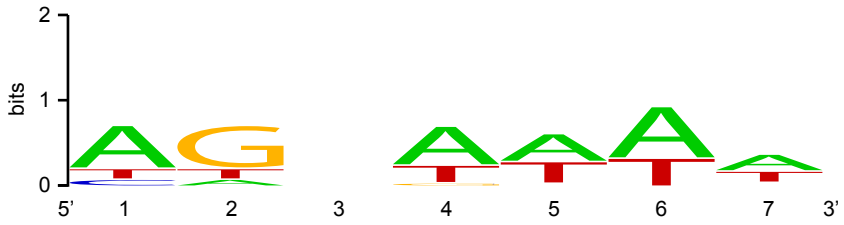
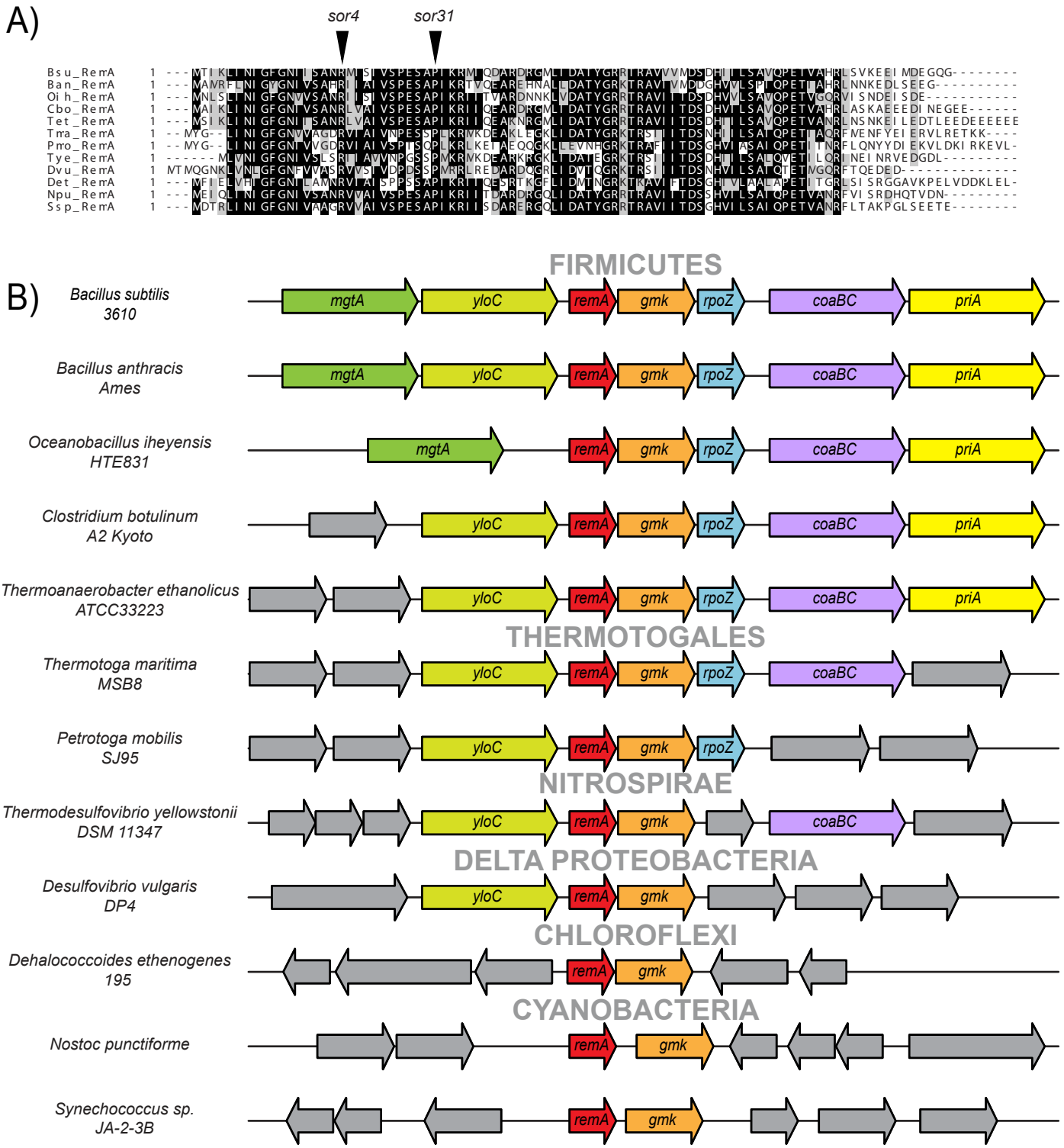


Figure S2


A)
sor4
sor31
Bsu_RemA
Ban_RemA
Oih_RemA
Cbo_RemA
Tet_RemA
Tma_RemA
Pro_RemA
Tye_RemA
Dvu_RemA
Det_RemA
Npu_RemA
Ssp_RemA
B)
FIRMICUTES
Bacillus subtilis 3610
Bacillus anthracis Ames
Oceanobacillus iheyensis HTE831
Clostridium botulinum A2 Kyoto
Thermoanaerobacter ethanolicus ATCC33223
THERMOTOGALES
Thermotoga maritima MSB8
Petrotoga mobilis SJ95
NITROSPIRAE
Thermodesulfovibrio yellowstonii DSM 11347
DELTA PROTEOBACTERIA
Desulfovibrio vulgaris DP4
CHLOROFLEXI
Dehalococcoides ethenogenes 195
CYANOBACTERIA
Nostoc punctiforme
Synechococcus sp. JA-2-3B
mgtA
yloC
remA
gmk
rpoZ
coaBC
priA