Expression of *kinA* and *kinB of Bacillus subtilis*, Necessary for Sporulation Initiation, Is under Positive Stringent Transcription Control

Supplemental material

Files in this Data Supplement:

*Supplemental file 1 – Table S1, Oligonucleotide primers used in this study. Fig. S1, *In situ* replacement of adenine with guanine at nucleotide +1 of the *kinA* promoter.

Table S1. Oligonucleotide primers used in this study

Oligo-nucleotide	Sequence[a]
ABu-F	gtgaaaacaccgtctgatccgaa
ABu-R	gcactatcaacacactcttaagaaacattctcctcccaagacatt
ABd-F	ggagctaaagaggtccctagcttaaataatcatttcttgtacaaaa
ABd-R	cgcaagacatgaaatccactgca
EM-F	cttaagagtgtgttgatagtgc
EM-R	ctagggacctctttagctcc
KA-F1	gtgtctagattgacgttcaccataagaata
KA-R1	gtgggatccacttttaccTagtatgattcg
KA-R1g	gtgggatccacttttaccCagtatgattcg
KA-R1c	gtgggatccacttttaccGagtatgattcg
KB-F1	gtgtctagatcttaataaaggaattttatat
KB-R1	gtgggatcctataaaataTgaatctattataa
KB-R1g	gtgggatcctataaaataCgaatctattataa
KB-R1c	gtgggatcctataaaataGgaatctattataa
MF-F	cgacagcggaattgactcaagc
MF-R	cgcggatcctacccaatcagtacgttaattttg
KA-F2	ctcctcgcaaagaccaaaaaat
KA-R2g	ctgattgggtaggatccgcattgacttttaccCagtatgattcgc
KA-F3g	gagtcaattccgctgtcgggcgaatcatactGggtaaaagtcaat
KA-R3	ggttttgatccccgtttatataa
KB-F2	gatcggcagcgtttgttcaaaa
KB-R2g	ctgattgggtaggatccgcttcgtataaaataCgaatctattataacactaa
KB-F3g	gagtcaattccgctgtcggttataatagattcGtattttatacgaa

KB-R3	cacatccgcctgttttggatga
KA-R2c	ctgattgggtaggatccgcattgacttttaccGagtatgattcgc
KA-F3c	gagtcaattccgctgtcgggcgaatcatactCggtaaaagtcaat
KB-R2c	ctgattgggtaggatccgcttcgtataaaataGgaatctattataacactaa
KB-F3c	gagtcaattccgctgtcggttataatagattcCtattttatacgaa

[a] The underlined 26- and 27 base-sequences of the *kinA* and *kinB* short repeated regions have the respective transcription initiation bases where the replacement of adenine with guanine or cytosine was performed. Bases corresponding to nucleotide +1 are in capitals.

Fig. S1.

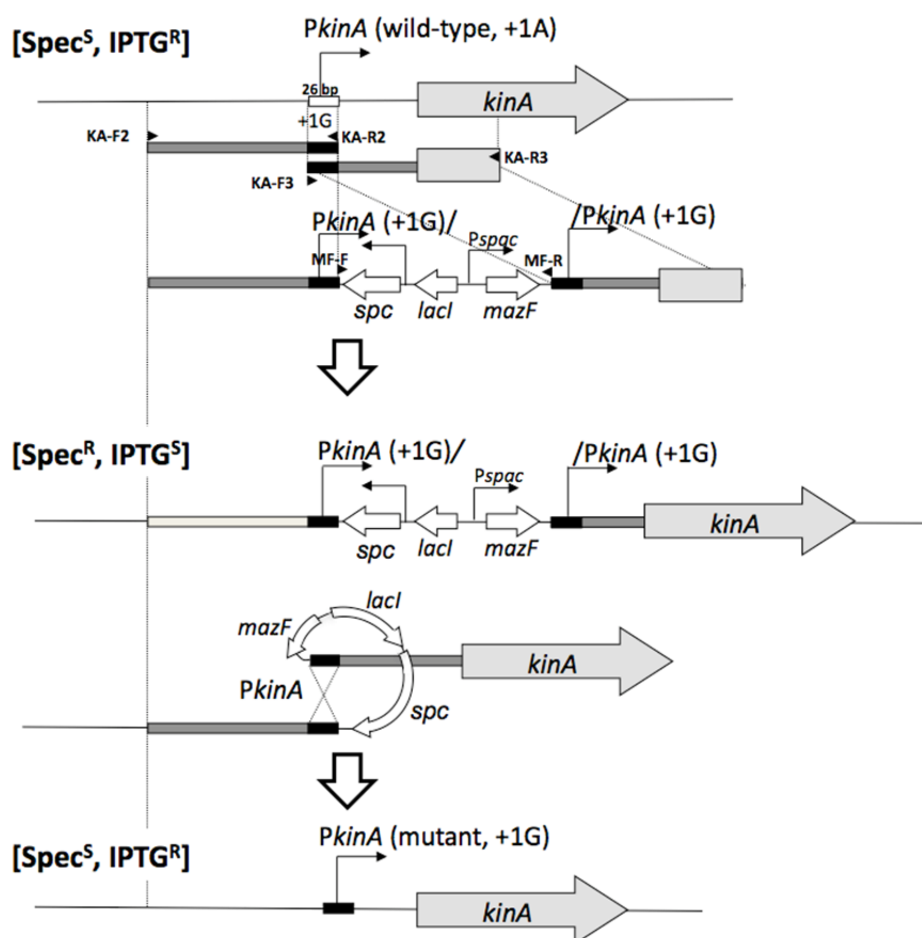

Fig. S1. *In situ* replacement of adenine with guanine at nucleotide +1 of the *kinA* promoter. The guanine replacement of adenine at nucleotide +1 of the *kinA* promoter was performed in three steps. First, the *kinA* promoter regions upstream and downstream of the transcription initiation site, both having the guanine replacement of adenine at nucleotide +1, and the *mazF* cassette possessing the spectinomycine-resistance gene (*spc*), *lacI*, and *mazF* under the control of the P_{spac} promoter (1) were separately amplified by PCR, and were combined by the subsequent PCR using the most outside primer pair. Secondly, strain 168 [spectinomycin-sensitive ($Spec^{s}$), IPTG-resistant ($IPTG^{r}$)] was transformed with the above combined PCR product to yield cells [Spec-resistant ($Spec^{r}$), IPTG-sensitive ($IPTG^{s}$)]. Thirdly, one colony

of the cells (Spec^s, IPTG^r) resulting from a single-crossover to pinch off the *mazF* cassette was designated as the *kinA* (A+1G) strain FU1102. Details of the isolation of this strain are given in Materials and Methods. The *kinB* (A+1G) strain FU1113 and the *kinA* (A+1C) strain FU1156 were similarly constructed by the above three-step procedure using the *mazF* gene.

Reference

1. Morimoto, T., K. Ara, K. Ozaki, and N. Ogasawara. 2009. A new simple method to introduce marker-free deletions in the *Bacillus subtilis* genome. Genes Genet. Syst. **84**: 315-318.