

Supplementary Materials

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Cross-recognition of the *ssgBp* promoter, which controls the expression of the sporulation-specific cell division gene *ssgB*, by nine SigB homologues in *Streptomyces coelicolor* A3(2)

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Table S1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotypes and relevant characteristics ^a	Reference or source
Strains		
<i>E. coli</i> DH5 α	F ⁻ <i>supE44</i> Δ <i>lacU169</i> (ϕ 80 <i>dlacZ</i> Δ M15) <i>hsdR17</i> <i>recA1</i> <i>endA1</i> <i>gyrA96</i> <i>thi-1</i> <i>relA1</i> Host strain for plasmid cloning and propagation	Invitrogen
<i>E. coli</i> XL1-Blue	<i>recA1</i> <i>endA1</i> <i>gyrA96</i> <i>thi-1</i> <i>hsdR17</i> <i>supE44</i> <i>relA1</i> <i>lac</i> [F' <i>proAB</i> <i>lacI</i> ^q Δ M15 <i>Tn10</i> (Tet ^r)]. Host strain for two-plasmid system	Stratagene
<i>E. coli</i> BW25113/pIJ790	Δ (<i>araD-araB</i>)567 Δ <i>lacZ</i> 4787(:: <i>rrnB-4</i>) <i>lacI</i> p-4000 (<i>lacI</i> ^Q) λ <i>rpoS</i> 369(Am) <i>rph-1</i> Δ (<i>rhaD-rhaB</i>) <i>hsdR514</i> . Host strain containing pIJ790 for REDIRECT strategy	[46]
<i>E. coli</i> ET12567/pUZ8002	<i>dam-13</i> :: <i>Tn9</i> <i>dcm-6</i> <i>hsdM</i> . A methylation-deficient host strain containing non-transmissible pUZ80023 for conjugation	[45]
<i>S. coelicolor</i> M145	wild-type, prototrophic SCP1 ⁻ SCP2 ⁻ Pgl ⁺	[45]
<i>S. coelicolor</i> Δ <i>sigB</i>	Apr ^R , deleted <i>sigB</i> gene in <i>S. coelicolor</i> M145	[30]
<i>S. coelicolor</i> Δ <i>sigF</i>	Tsr ^R , J1984, deleted <i>sigF</i> gene in <i>S. coelicolor</i> M145	[48]
<i>S. coelicolor</i> Δ <i>sigG</i>	Tsr ^R , deleted <i>sigG</i> gene in <i>S. coelicolor</i> M145	[18]
<i>S. coelicolor</i> Δ <i>sigH</i>	Apr ^R , deleted <i>sigH</i> gene in <i>S. coelicolor</i> M145	this study
<i>S. coelicolor</i> Δ <i>sigI</i>	Apr ^R , deleted <i>sigI</i> gene in <i>S. coelicolor</i> M145	[17]
<i>S. coelicolor</i> Δ <i>sigK</i>	Apr ^R , deleted <i>sigK</i> gene in <i>S. coelicolor</i> M145	this study
<i>S. coelicolor</i> Δ <i>sigL</i>	Apr ^R , deleted <i>sigL</i> gene in <i>S. coelicolor</i> M145	this study
<i>S. coelicolor</i> Δ <i>sigM</i>	Apr ^R , deleted <i>sigM</i> gene in <i>S. coelicolor</i> M145	this study
<i>S. coelicolor</i> Δ <i>sigN</i>	Apr ^R , deleted <i>sigN</i> gene in <i>S. coelicolor</i> M145	this study
Plasmids		
St5C7	Amp ^R , Kan ^R , SuperCos-1 derivative containing the <i>sigK</i> gene	[47]
St5H1	Amp ^R , Kan ^R , SuperCos-1 derivative containing the <i>sigL</i> gene	[47]
St55F8	Amp ^R , Kan ^R , SuperCos-1 derivative containing the <i>sigM</i> gene	[47]
2St5D60	Amp ^R , Kan ^R , SuperCos-1 derivative containing the <i>sigN</i> gene	[47]
pIJ773	Amp ^R , Apr ^R , <i>ori</i> colE1, <i>E. coli</i> plasmid vector	[46]
pIJ790	Clm ^R , <i>oriR101</i> , <i>E. coli</i> λ RED recombination plasmid	[46]
pBluescript II SK	Amp ^R , <i>ori</i> colE1, <i>E. coli</i> plasmid cloning vector	Stratagene
pSB40N	Amp ^R , promoter probe plasmid containing a promoterless <i>lacZ</i> α gene	[43]
pF81	Amp ^R , pSB40N containing a 750-bp <i>TaqI</i> DNA fragment with the <i>ssgBp</i> promoter	[31]

pSB40N-ssgBpA-J	Amp ^R , pSB40N containing 55-bp DNA fragments with mutant <i>ssgBp</i> promoters	this study
pMU1s*	Apr ^R , PhiBT1-based <i>Streptomyces</i> integration vector	[37]
pMU1s-kasOp1	Apr ^R , PhiBT1-based <i>Streptomyces</i> integration vector with the <i>kasOp</i> * promoter	[49]
pLux-kasOp	Kan ^R , PhiC31-based <i>Streptomyces</i> integration vector with the <i>kasOp</i> * promoter	this study
pLux-ssgBp	Kan ^R , pLux-kasOp containing a 750-bp DNA fragment with the <i>ssgBp</i> promoter	this study
pLux-ssgBpA-J	Kan ^R , pLux-kasOp containing 55-bp DNA fragments with <i>ssgBpA-J</i> promoters	this study
pAC5mut2	Clm ^R , expression plasmid vector with the <i>trc</i> promoter	[50]
pAC5-sigF1	Clm ^R , pAC5mut2 containing the <i>sigF</i> gene under the <i>trc</i> promoter	[43]
pAC5-sigG1	Clm ^R , pAC5mut2 containing the <i>sigF</i> gene under the <i>trc</i> promoter	[51]
pAC5-sigH1	Clm ^R , pAC5mut2 containing the <i>sigF</i> gene under the <i>trc</i> promoter	[52]
pAC5-sigB	Clm ^R , pAC5mut2 containing the <i>sigB</i> gene under the <i>trc</i> promoter	[30]
pAC5-sigI	Clm ^R , pAC5mut2 containing the <i>sigI</i> gene under the <i>trc</i> promoter	[30]
pAC5-sigK	Clm ^R , pAC5mut2 containing the <i>sigK</i> gene under the <i>trc</i> promoter	[30]
pAC5-sigL	Clm ^R , pAC5mut2 containing the <i>sigL</i> gene under the <i>trc</i> promoter	[30]
pAC5-sigM	Clm ^R , pAC5mut2 containing the <i>sigM</i> gene under the <i>trc</i> promoter	[30]
pAC5-sigN	Clm ^R , pAC5mut2 containing the <i>sigN</i> gene under the <i>trc</i> promoter	[30]
pAC5-sigF2	Clm ^R , pAC5mut2 containing the N-terminally truncated <i>sigF</i> gene under the <i>trc</i> promoter	[30]
pAC5-sigH2	Cm ^R , pAC5mut2 containing the <i>sigH</i> gene including longer N-terminal region under the <i>trc</i> promoter	[30]
pSB40BR5-phiC31int	Amp ^R , Kan ^R , PhiC31-based <i>Streptomyces</i> integration vector	[49]

^a Clm^R, chloramphenicol resistance; Amp^R, ampicillin resistance; Apr^R, apramycin resistance; Kan^R, kanamycin resistance; Str^R, streptomycin resistance; Tsr^R, thiostrepton resistance; Tet^R, tetracycline resistance

Table S2. Oligonucleotides used in this study. Cloning sites are underlined.

Oligonucleotide	Sequence (5' → 3')
ssgBpADir	GATCCACTCAGAGGGGTTTACAACGGCACCGTAGGGGGCATGTCGATTTGGTACC
ssgBpARev	TCGAGGTACCAAATCGACATGCCCCCTACGGTGCCGTTGTAAACCCCTCTGAGTG
ssgBpBDir	GATCCACTCAGAGGGGTTTACAACGGCACCGTAGGGGGTACGTCGATTTGGTACC
ssgBpBRev	TCGAGGTACCAAATCGACGTACCCCTACGGTGCCGTTGTAAACCCCTCTGAGTG
ssgBpCDir	GATCCACTCAGAGGGGTTTACAACGGCACCGTAGGGGGAAAGTCGATTTGGTACC
ssgBpCRev	TCGAGGTACCAAATCGACTTTCCCCCTACGGTGCCGTTGTAAACCCCTCTGAGTG
ssgBpDDir	GATCCACTCAGAGGGGTTTACAACGGCACCGTAGGGGGGACGTCGATTTGGTACC
ssgBpDRev	TCGAGGTACCAAATCGACGTCCCCCTACGGTGCCGTTGTAAACCCCTCTGAGTG
ssgBpEDir	GATCCACTCAGAGGGGTTTACAACGGCACCGTAGGGGGCACGTCGATTTGGTACC
ssgBpERev	TCGAGGTACCAAATCGACGTGCCCCCTACGGTGCCGTTGTAAACCCCTCTGAGTG
ssgBpFDir	GATCCACTCAGAGGGGTTTACAACGGCACCGTAGGGGGCAGGTCGATTTGGTACC
ssgBpFRev	TCGAGGTACCAAATCGACCTGCCCCCTACGGTGCCGTTGTAAACCCCTCTGAGTG
ssgBpGDir	GATCCACTCAGAGGGGTTTACAACGGCACCGTAGGGGGCAAGTCGATTTGGTACC
ssgBpGRev	TCGAGGTACCAAATCGACTTGCCCCCTACGGTGCCGTTGTAAACCCCTCTGAGTG
ssgBpHDir	GATCCACTCAGAGGGGTTTACAACGGCACCGTAGGGGGTATGTCGATTTGGTACC
ssgBpHRev	TCGAGGTACCAAATCGACATACCCCTACGGTGCCGTTGTAAACCCCTCTGAGTG
ssgBpIDir	GATCCACTCAGAGGGGTTTACAACGGCACCGTAGGGGGCTTGTCGATTTGGTACC
ssgBpIRev	TCGAGGTACCAAATCGACAAGCCCTACGGTGCCGTTGTAAACCCCTCTGAGTG
ssgBpJDir	GATCCACTCAGAGGGGTTTACAACGGCACCGTAGGGGGCGTGTCGATTTGGTACC
ssgBpJRev	TCGAGGTACCAAATCGACACGCCCTACGGTGCCGTTGTAAACCCCTCTGAGTG
-47	CGCCAGGGTTTTCCAGTCACGAC
luxCrev	GCTCTCGGGGAAGATCTCGACCTGG
SigKD1Dir	CCGGGCTGCCGCCCGGTGCGACGACGGGACCCCGCGCTCCGAGGGAGTTGCGACGCACC ATTCCGGGGATCCGTCGACC
SigKD1Rev	AACCCGGGACTCACCCGCCCGGACGCCGCTTCGCGCGAACGGAGCACGGTGACGGG CTGTAGGCTGGAGCTGCTTC
SigLD1Dir	CCCGGGTACCCGCAAGCGCGAAAAACCTAGGGAGAGCCGTGACTTTCGTGGGAGAGGC AATCCGGGGATCCGTCGACC
SigLD1Rev	GGGGCTCAGGCGCAGCCGAGTTCGCCAGCATGCCCTCGCGCAGCCGGGTGATGATCC TGTAGGCTGGAGCTGCTTC
SigMD1Dir	CAGCGCCGACGCCGTTCCCGTGGAAGTCCCTGAGACCACCGTTCAGGAGCGGATCCG

SigMD1Rev	CATTCCGGGGATCCGTCGACC GAGGAGTCTGCGCAGCACCGCGGGGTGCGGCATCAGCATCCGCCCCGTCCCCTCCCTG TGTAGGCTGGAGCTGCTTC
SigND1Dir	GCTGCGTTCCCGGCGTGAGCGTGTGCCGCGACCGTCCCGCGACCCAAAGGGGGTG GCATTCCGGGGATCCGTCGACC
SigND1Rev	CCCCGAGGTGCCACGGCGGTCGCCGTGCTGTCGTACGGGCTCTCCCGCGGCGCGGG CTGTAGGCTGGAGCTGCTTC
SigHD1Dir	GGCGCGGGACCCGGGCCGACGTGAGGGACGGGGACGGGCATTCCGGGGATCCGTCGACC
SigHD1Rev	CACCCGCCGACCGGCGGTTACTCCTCGACGAGCAGCTTCTGTAGGCTGGAGCTGCTTC



Figure S1. Cross-recognition of the *ssgBpA-J* promoters with all nine SigB homologous sigma factors from *S. coelicolor* A3(2) in the *E. coli* two-plasmid system. MacConkey agar plates (with Amp, Clm, IPTG) with transformation mixtures of all promoter-containing plasmids (pSB40N-*ssgBpA*, pSB40N-*ssgBpB*, pSB40N-*ssgBpC*, pSB40N-*ssgBpD*, pSB40N-*ssgBpE*, pSB40N-*ssgBpF*, pSB40N-*ssgBpG*, pSB40N-*ssgBpH*, pSB40N-*ssgBpI*, pSB40N-*ssgBpJ* and the previous pF81 [30] with combinations of the expression plasmid pAC5mut2 and the vector with cloned sigma factor genes under the control of the *trc* promoter (pAC-*sigB*, pAC-*sigF1*, pAC-*sigF2*, pAC-*sigG*, pAC-*sigH1*, pAC-*sigH2*, pAC-*sigI*, pAC-*sigK*, pAC-*sigL*, pAC-*sigM*, pAC-*sigN*) incubated for 72 h at 37 °C. Red colonies correspond to positive results and uncoloured colonies to negative results.

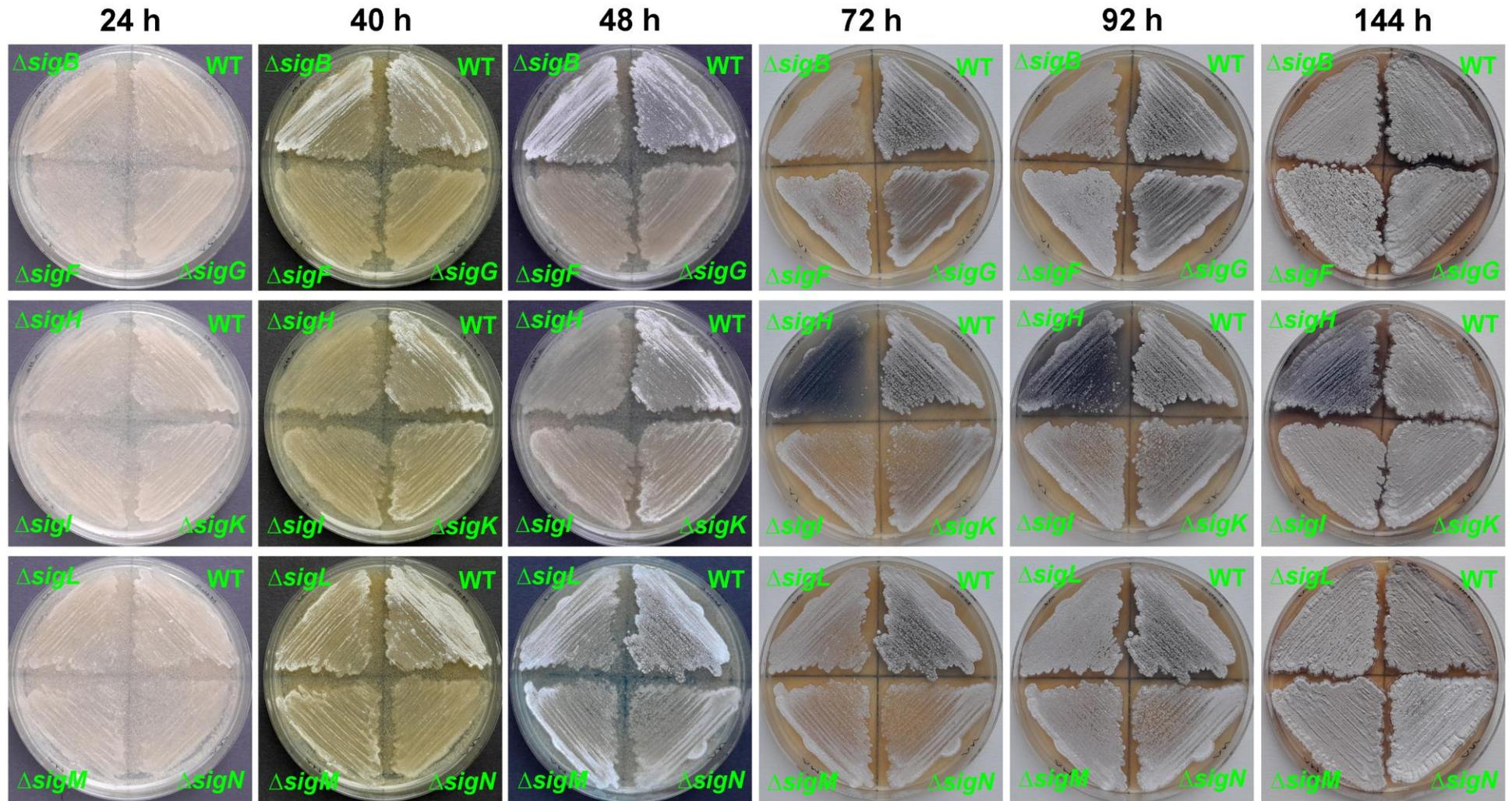


Figure S2. Phenotypic analysis of *S. coelicolor* mutant strains with deleted SigB-homologous gene. Spores of WT *S. coelicolor* M145, *S. coelicolor* $\Delta sigB$, *S. coelicolor* $\Delta sigF$, *S. coelicolor* $\Delta sigG$, *S. coelicolor* $\Delta sigH$, *S. coelicolor* $\Delta sigI$, *S. coelicolor* $\Delta sigK$, *S. coelicolor* $\Delta sigL$, *S. coelicolor* $\Delta sigM$ and *S. coelicolor* $\Delta sigN$ prepared in M145 genetic background were spread on the solid rich SFM medium and grown at the indicated time points at 28 °C.