Temporal separation of distinct differentiation pathways by a dual specificity Rap-Phr system in *Bacillus subtilis*

Wiep Klaas Smits, Cristina Bongiorni, Jan-Willem Veening, Leendert W. Hamoen, Oscar P. Kuipers and Marta Perego

Legends to Supplementary Figures

Fig. S1: ComK and RghR can bind simultaneously to the promoter of *rapH*. **A**. Sequence of the upstream region of *rapH* and the start codon (white box, start) are derived from SubtiList (http://genolist.pasteur.fr/SubtiList/). Putative binding sites for RghR (RghR, in yellow), as well as the core promoter elements (-35 and -10, in gray) and transcriptional start site (+1), were determined by Hayashi and coworkers (Hayashi et al., 2006). The putative ComK binding site (K), composed of two AT boxes (AT1 and AT2), is postulated on the basis of the published consensus sequence (Hamoen *et al.*, 1998) and the position compared to the core promoter elements (Hamoen *et al.*, 2002). It has to be noted that another ComK binding-site can be identified that overlaps the promoter elements (Berka *et al.*, 2002;Hamoen *et al.*, 2002). **B**. Electrophoretic mobility shift assays of a [γ-^{32}P]-ATP labeled *rapH* promoter fragment in the presence of purified ComK and/or RghR. Grey bars indicate shifted complexes of DNA and protein, small triangles indicate super-shifted complexes. A black bar indicates free probe. X marks the lane to which no protein was added. ComK was added to a final concentration of 300 nM.

Fig. S2: Analysis of the genome-wide transcriptional effect of RapH/PhrH overexpression in strains isogenic with 168 (*trpC2*) (Kunst *et al.*, 1997). Genes significantly affected in a CyberT analysis (see Experimental procedures) were analyzed using FIVA software (Blom *et al.*, 2007) **A.** Effect of RapH/PhrH overproduction in Spizizen minimal medium. **B.** Effect of RapH/PhrH overproduction in Schaeffer's sporulation medium.

Fig. S3: Time course analysis of *abrB-gfp* reporter strains grown in MMF medium supplemented with 1% xylose as indicated in Experimental procedures. **A.** Wild type strain **B.** Strain XH, ectopically overexpressing RapH/PhrH from a xylose inducible promoter. Strains were grown in the presence (grey) or absence (red) of xylose. Colors are darker at later timepoints. Note the down-regulation of *abrB* transcription, as indicated by lower levels of fluorescence, in wild type or uninduced XH strains.

Fig. S4: ***RapH interacts with ComA and inhibits its DNA-binding activity.*** **A.** RapA does not interact with ComA in the native gel binding assay. Each protein was at 12µM final concentration. **B.** RapA does not inhibit the DNA binding activity of ComA. X: labeled probe only; RapA (lane 2: 5 µM, triangle: 5, 10 and 20 µM); ComA (5 µM). **C.** RapH does not interact with DegU in the native gel binding assay. H: RapH; U: DegU; each protein was at 10µM final concentration. Native gel analysis was carried out on 10% native Tris-Tricine gels as described in Bongiorni *et al.* (Bongiorni *et al.*, 2005).

Fig. S5: Time course of β-galactosidase activity of a *srfA-lacZ* (**A**) and a *rapA-lacZ* (**B**) reporter constructs in the following background strains: wild type (-■-); *spo0A* (-●-); *spo0AabrB* (-◆-). Strains were all isogenic to JH642 (*trpC*2, *phe*-1). Cells were grown in Schaeffer's sporulation medium. Samples were taken at hourly or half hourly intervals to represent the time of transition from exponential growth to stationary phase.

References

Berka,R.M., Hahn,J., Albano,M., Draskovic,I., Persuh,M., Cui,X. *et al.* (2002) Microarray analysis of the Bacillus subtilis K-state: genome-wide expression changes dependent on ComK. *Mol Microbiol* **43**: 1331-1345.

Blom, E.-J., Bosman, D.W.J., van Hijum, S.A.F.T., Breitling, R., Tijsma, L., Silvis, R. *et al.* (2007) FIVA: Functional Information Viewer and Analyzer - extracting biological knowledge from transcriptome data of prokaryotes. *Bioinformatics. Epub ahead of print.*

Bongiorni,C., Ishikawa,S., Stephenson,S., Ogasawara,N., and Perego,M. (2005) Synergistic regulation of competence development in Bacillus subtilis by two Rap-Phr systems. *J Bacteriol* **187**: 4353-4361.

Hamoen,L.W., Smits,W.K., de Jong,A., Holsappel,S., and Kuipers,O.P. (2002) Improving the predictive value of the competence transcription factor (ComK) binding site in *Bacillus subtilis* using a genomic approach. *Nucleic Acids Res* **30**: 5517-5528.

Hamoen,L.W., Van-Werkhoven,A.F., Bijlsma,J.J., Dubnau,D., and Venema,G. (1998) The competence transcription factor of *Bacillus subtilis* recognizes short A/T-rich sequences arranged in a unique, flexible pattern along the DNA helix. *Genes Dev* **12**: 1539-1550.

Kunst,F., Ogasawara,N., Moszer,I., Albertini,A.M., Alloni,G., Azevedo,V. *et al.* (1997) The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. *Nature* **390**: 249-256.

Figure S1 Smits *et al.*

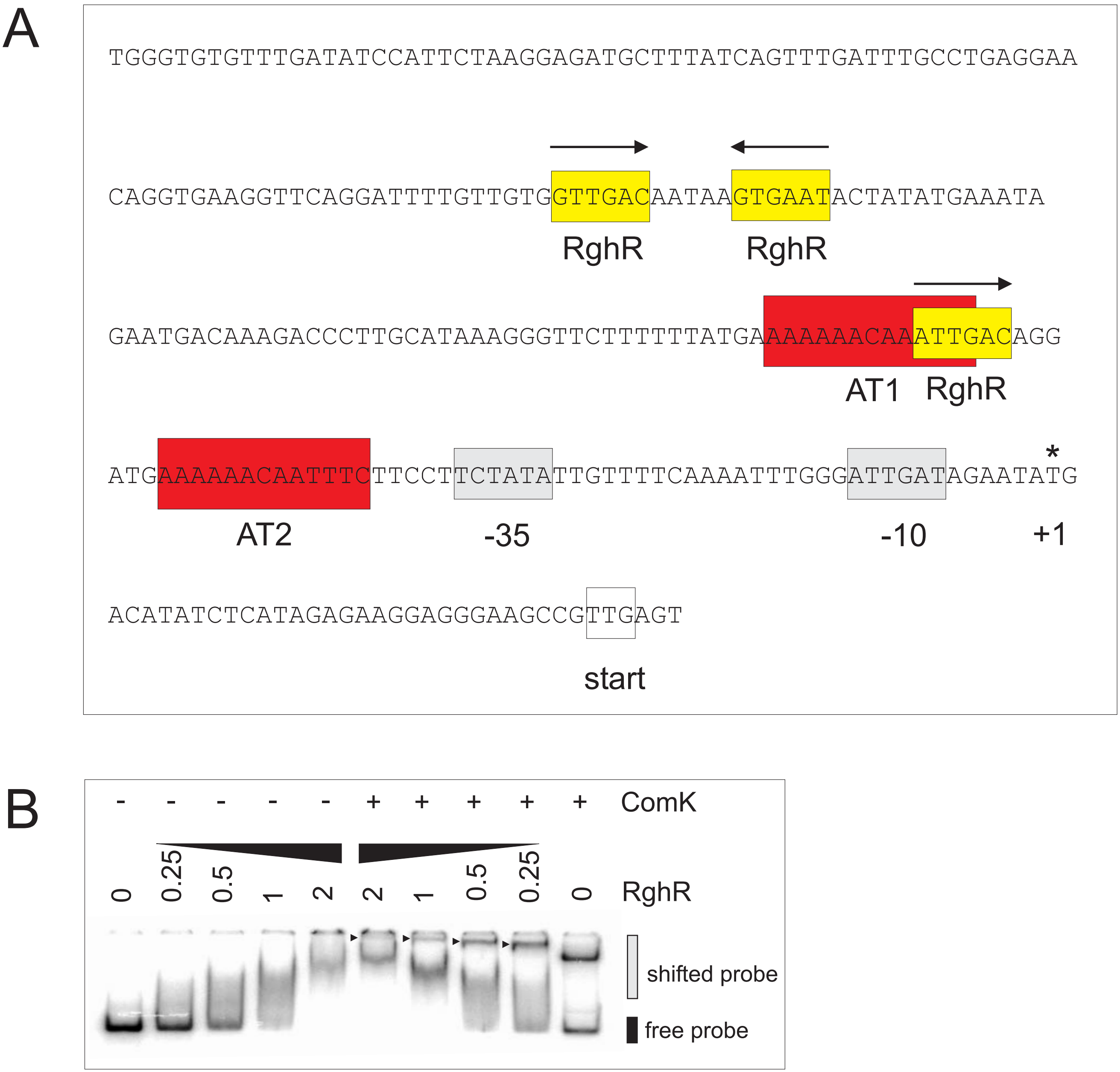
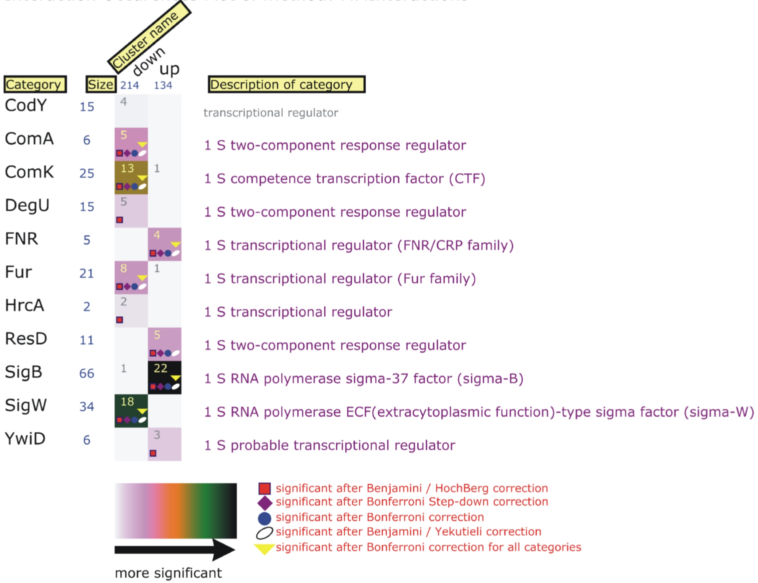


Figure S2 Smits *et al*

Interaction Occurences Plot of method: MMInteractions



Interaction Occurences Plot of method: SMInteractions

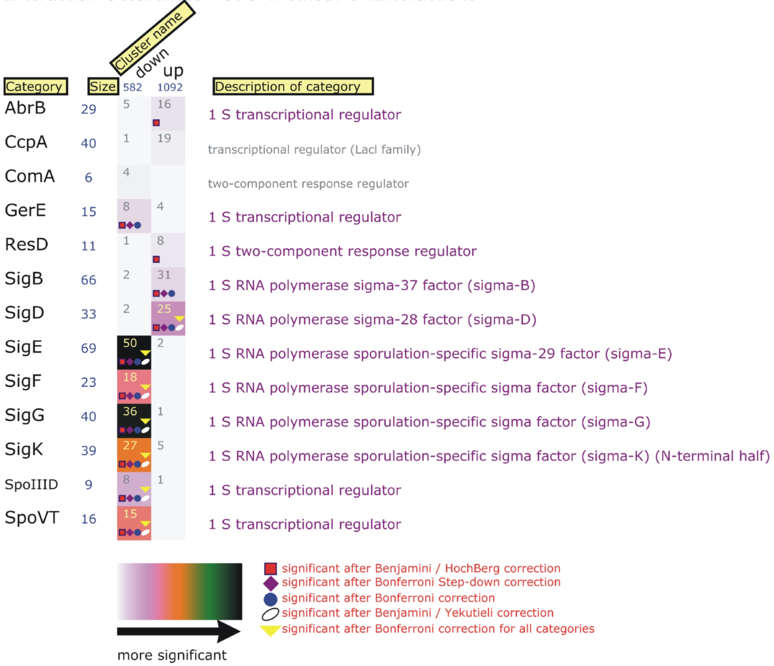


Figure S3 Smits *et al*

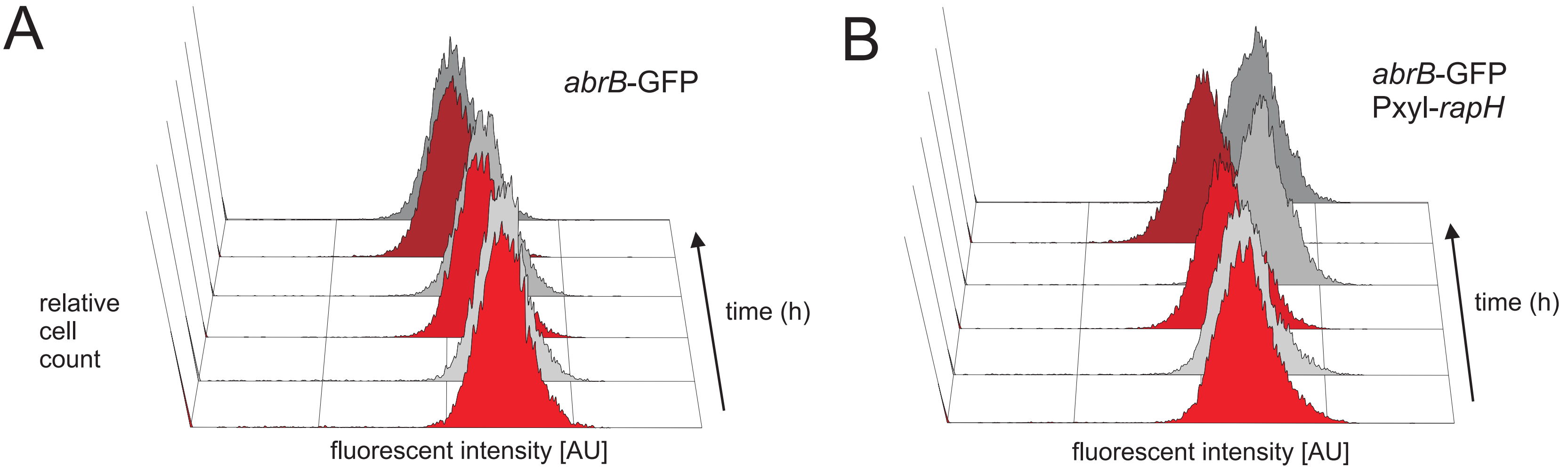


Fig. S4 Smits *et al.*

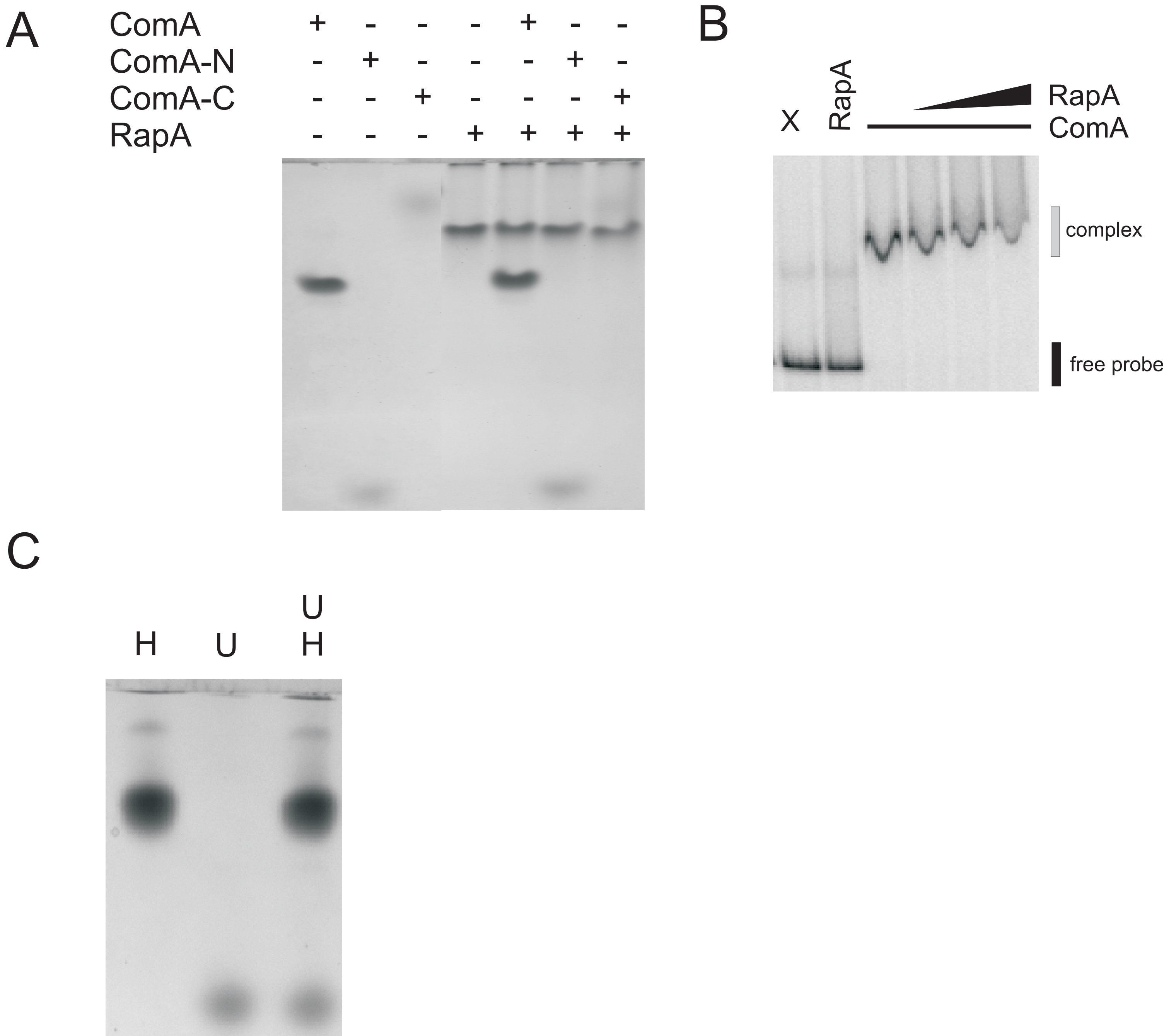


Figure S5 Smits *et al.*

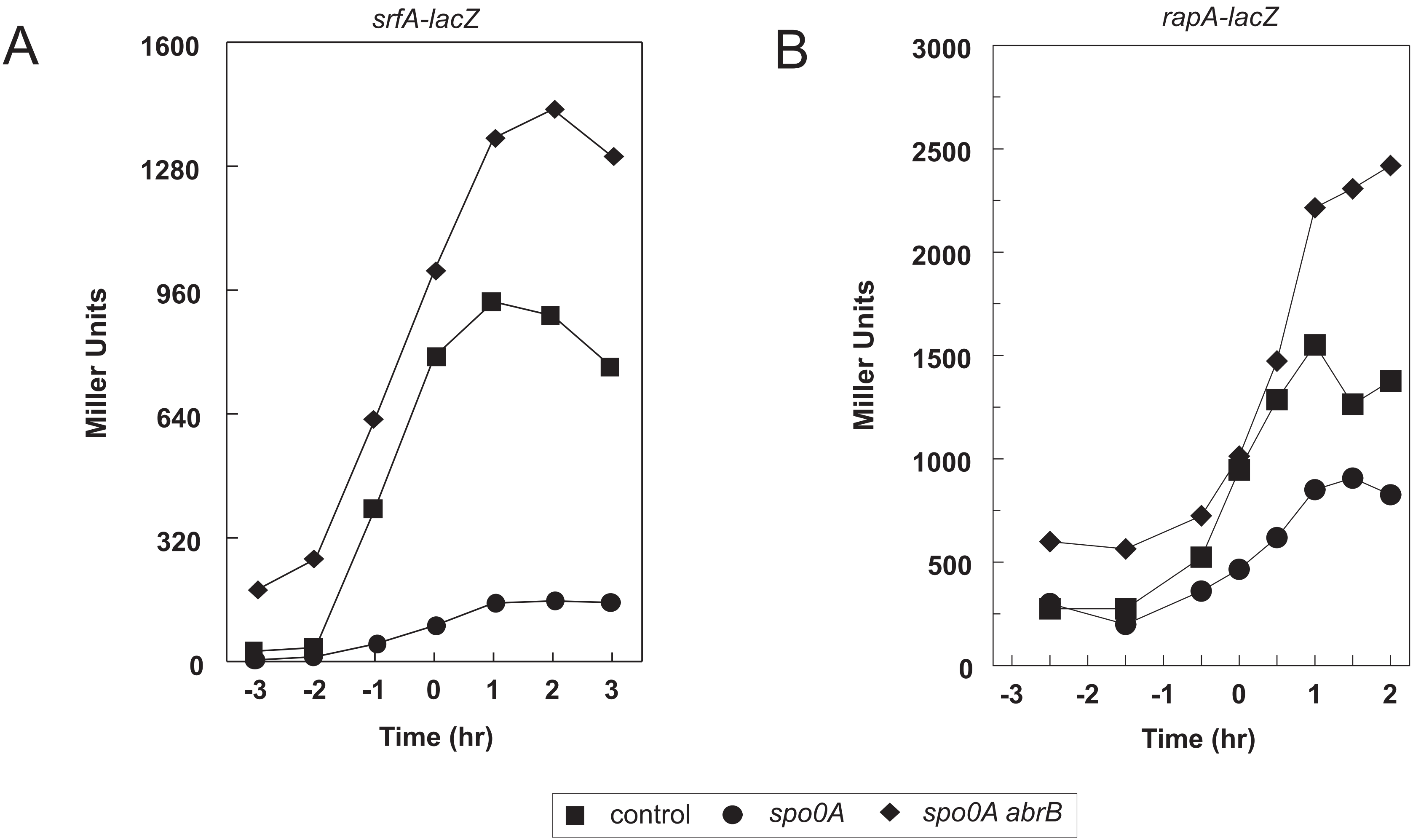


Table S1: *B. subtilis* strains used in this study

Strain	Description	Relevant Genotype[6]	Reference
168[1]	parental	*trpC2*	(Kunst *et al.*, 1997)
yvaN	deletion of *yvaN*	*yvaN::tet*	This study
Htet	deletion of *rapH* and *phrH*	*rapH-phrH::tet*	This study
HIC	pICFP-*rapH* →x[3] 168	*PrapH-icfp, cat*	This study
XH(Cm)	pX*rapHphrH* →xx[4] 168	*amyE, rapH*^{hi}[7]*-phrH*^{hi}*, cat*	This study
XH(Em)	pCm::Em →xx X*rapH*(Cm)	*amyE, rapH*^{hi}*-phrH*^{hi}*, erm*	This study
GAY(Cm)	pSG-comGAYFP →x 168	*PcomGA-yfp, cat*	This study
GAC(Cm)	pSG-comGACFP →x 168	*PcomGA-cfp, cat*	This study
GAY(Em)	pCm::Em →xx GAY(Cm)	*PcomGA-yfp, erm*	This study
GAC(Em)	pCm::Em →xx GAC(Cm)	*PcomGA-cfp, erm*	This study
abrB-gfp	pGFP-*abrB* →x 168	*PabrB-gfp, cat*	(Veening *et al.*, 2006a)
abrB-gfp XH	XH(Em) →[5] *abrB-gfp*	*PabrB-gfp, amyE, rapH*^{hi}*-phrH*^{hi}*, cat, erm*	This study
iyfp-IIA	pIYFP-*spoIIA* →x 168	*PspoIIA-iyfp, cat*	(Veening *et al.*, 2004)
IIA-IY(Sp)	pCm::Sp →xx iyfp-IIA	*PspoIIA-iyfp, spc*	This study
GAC IIA-IY	GAC → IIA-IY (Sp)	*PcomGA-cfp, PspoIIA-iyfp, cat, spc*	This study
GAC IIA-IY Htet	Htet → GAC IIA-IY	*PcomGA-cfp, PspoIIA-iyfp,rapH-phrH::tet, cat, spc*	This study
IIA-gfp	pGFP-*spoIIA* →x 168	*PspoIIA-gfp, cat*	(Veening *et al.*, 2005)
IIA-gfp XH	XH(Em) → IIA-*gfp*	*PspoIIA-gfp, amyE, rapH*^{hi}*-phrH*^{hi}*, cat, erm*	This study
srfA-gfp	pGFP-*srfA* → x168	*PsrfA-gfp, cat*	This study
srfA-gfp 0A	SWV215 → *srfA-gfp*	*PsrfA-gfp, spo0A::kan, cat*	This study; spo0A from SWV215 (Xu and Strauch, 1996)
srfA-gfp abrB	Δ*abrB* → *srfA-gfp*	*PsrfA-gfp, abrB::erm, cat*	This study; abrB (Smits *et al.*, 2005)
srfA-gfp Psp-abrB	Pspac-*abrB* →xx *srfA-gfp*	*PsrfA-gfp, Pspac-abrB, amyE, cat, spc*	This study; Pspac-abrB from BD2238 (Hahn *et al.*, 1995)
srfA-gfp XH	XH(Em) → *srfA-gfp*	*PsrfA-gfp, amyE, rapH*^{hi}*-phrH*^{hi}*, cat, erm*^{i}	This study
GA-gfp	pSG-*comGA* →x 168	*PcomGA-gfp, cat*	(Veening *et al.*, 2006b)
GA-gfp(Km)	pGA-GFP →x 168	*PcomGA-gfp, kan*	This study; pGA-GFP (Smits *et al.*, 2005)

GA-gfp comK	BV2004 → GA-*gfp*	*PcomGA-gfp, comK*::*spc, cat*	This study; comK from BV2004 {Hamoen, 2002 6 /id}
GA-gfp Htet	Htet → GA-*gfp*	*PcomGA-gfp, rapH-phrH*::*tet, cat*	This study
GA-gfp(Km) XH(Cm)	XH(Cm) → GA-*gfp*(Km)	*PcomGA-gfp, amyE, rapH*^{hi}*-phrH*^{hi}*, cat, kan*	This study
GA-gfp XH(Em)	XH(Em) → GA-*gfp*	*PcomGA-gfp, amyE, rapH*^{hi}*-phrH*^{hi}*, cat, erm*	This study
GAY HIC	HIC → GAY(Em)	*PcomGA-yfp, PrapH-icfp, erm, cat*	This study
GAY HIC Phs-comK	BD3836 → GAY HIC	*PcomGA-yfp, PrapH-icfp, amyE, Phyperspank-comK, erm, cat, spc*	This study; *Phs-comK* from BD3836 (Maamar and Dubnau, 2005)
GAY HIC comK	8G32 → GAY HIC	*PcomGA-yfp, PrapH-icfp, comK*::*kan, erm, cat*	This study
GAY HIC yvaN	*yvaN* → GAY HIC	*PcomGA-yfp, PrapH-icfp, yvaN*::*tet, erm, cat*	This study
GAY HIC yvaN comK	GAY HIC *comK* → GAY HIC *yvaN*	*PcomGA-yfp, PrapH-icfp, yvaN*::*tet, comK*::*kan, erm, cat*	This study
JH642[2]	parental	*trpC2, phe-1*	
JH11028	*rapA-lacZ* in *amyE* in *spo0A/abrB* double mutant	*rapA-lacZ, spo0A12, abrB*::*cat, kan*	(Perego *et al.*, 1988;Stephenson *et al.*, 2003)
JH12981	*rapA-lacZ* in *amyE*	*rapA-lacZ, amyE, kan*	(Stephenson *et al.*, 2003)
JH11432	*abrB-lacZ* in *amyE*	*abrB-lacZ, amyE, spc*	(Strauch *et al.*, 1989)
JH11205	*comG-lacZ* in *amyE*	*comG-lacZ, amyE, kan*	This study
JH11694	*srfA-lacZ* in *amyE*	*srfA-lacZ, amyE, kan*	This study
JH12546	*spo0A abrB* double mutant	*spo0A12, abrB*::Tn917*erm*	(Perego *et al.*, 1988)
JH27087	pBS19 → JH11205	*comG-lacZ, amyE, kan, cat*	This study
JH27088	pBS19-RapH2 → JH11205	*comG-lacZ, amyE, kan, cat, rapH*^{hi}[7]	This study
JH27089	pBS19-RapH3 → JH11205	*comG-lacZ, amyE, kan, cat, rapH*^{hi}*, phrH*^{hi}	This study
JH27090	pBS19 → JH12981	*rapA-lacZ, amyE, kan, cat*	This study
JH27091	pBS19-RapH2 → JH12981	*rapA-lacZ, amyE, kan, cat, rapH*^{hi}	This study
JH27092	pBS19-RapH3 → JH12981	*rapA-lacZ, amyE, kan,, cat, rapH*^{hi}*, phrH*^{hi}	This study
JH27093	pBS19 → JH11432	*abrB-lacZ, amyE, spc, cat*	This study
JH27094	pBS19-RapH2 → JH11432	*abrB-lacZ, amyE, spc, cat, rapH*^{hi}	This study
JH27095	pBS19-RapH3 → JH11432	*abrB-lacZ, amyE, spc, cat, rapH*^{hi}*, phrH*^{hi}	This study
JH19207	pHT315S → JH11028	*rapA-lacZ, amyE, kan, spo0A12, abrB*::*cat, erm*	This study

JH19208	pHT315S-RapH2 → JH11028	*rapA-lacZ, amyE, kan, spo0A12, abrB*::*cat, erm, rapH^{hi}*	This study
JH19209	pHT315S-RapH3 → JH11028	*rapA-lacZ, spo0A12, abrB*::*cat, erm,* amyE*, kan, rapH^{hi}-phrH^{hi}*	This study
JH27096	pJM115-RapHlac → JH642	*rapH-lacZ, amyE, kan*	This study
JH27097	QB4721 → JH27096	*rapH-lacZ*, *amyE, kan, comK*::*cat*	This study; QB4721 from Msadek *et al* (Msadek *et al.*, 1994)
JH19239	JH11694 → JH12546	*srfA-lacZ, spo0A12, abrB*::Tn917*erm, amyE, kan*	This study
JH27117	Htet → JH19239	*srfA-lacZ, spo0A12, abrB*::Tn917*erm, amyE, kan, rapH*::*tet*	This study

1) Following strains are all derivatives of strain 168 and therefore carry the *trpC2* auxotrophic marker.

2) Following strains are all derivatives of JH642 and therefore carry the *trpC2*, *phe-1* auxotrophic markers.

3) →x: indicates construction by transformation with plasmid DNA followed by single cross over homologous recombination.

4) →xx: indicates construction by transformation with plasmid DNA followed by double cross over integration.

5) →: indicates construction by transformation using chromosomal DNA or a replicative plasmid as donor.

6) Antibiotic resistance genes: *cat*=chloramphenicol; *erm*=erythromycin; *kan*=kanamycin; *spc*=spectinomycin, *tet*=tetracyclin.

7) ^{hi} indicates overproduction due to presence of multiple copies of the locus on a replicative plasmid (pBS19 derivatives), or overexpression by xylose induction (pX derivatives) or *spac* promoter constitutive transcription (pHT315S derivatives).

References

Bongiorni,C., Ishikawa,S., Stephenson,S., Ogasawara,N., and Perego,M. (2005) Synergistic regulation of competence development in *Bacillus subtilis* by two Rap-Phr systems. *J Bacteriol* **187**: 4353-4361.

Hahn,J., Roggiani,M., and Dubnau,D. (1995) The major role of Spo0A in genetic competence is to downregulate *abrB*, an essential competence gene. *J Bacteriol* **177**: 3601-3605.

Kunst,F., Ogasawara,N., Moszer,I., Albertini,A.M., Alloni,G., Azevedo,V. *et al.* (1997) The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* **390**: 249-256.

Maamar,H., and Dubnau,D. (2005) Bistability in the *Bacillus subtilis* K-state (competence) system requires a positive feedback loop. *Mol Microbiol* **56**: 615-624.

Msadek,T., Kunst,F., and Rapoport,G. (1994) MecB of *Bacillus subtilis*, a member of the ClpC ATPase family, is a pleiotropic regulator controlling competence gene expression and growth at high temperature. *Proc Natl Acad Sci USA* **91**: 5788-5792.

Perego,M., Spiegelman,G.B., and Hoch,J.A. (1988) Structure of the gene for the transition state regulator, *abrB*: regulator synthesis is controlled by the *spo0A* sporulation gene in *Bacillus subtilis*. *Mol Microbiol* **2**: 689-699.

Smits,W.K., Eschevins,C.C., Susanna,K.A., Bron,S., Kuipers,O.P., and Hamoen,L.W. (2005) Stripping *Bacillus*: ComK auto-stimulation is responsible for the bistable response in competence development. *Mol Microbiol* **56**: 604-614.

Stephenson,S., Mueller,C., Jiang,M., and Perego,M. (2003) Molecular analysis of Phr peptide processing in *Bacillus subtilis*. *J Bacteriol* **185**: 4861-4871.

Strauch,M.A., Perego,M., Burbulys,D., and Hoch,J.A. (1989) The transition state transcription regulator AbrB of *Bacillus subtilis* is autoregulated during vegetative growth. *Mol Microbiol* **3**: 1203-1209.

Veening,J.W., Hamoen,L.W., and Kuipers,O.P. (2005) Phosphatases modulate the bistable sporulation gene expression pattern in *Bacillus subtilis*. *Mol Microbiol* **56**: 1481-1494.

Veening,J.W., Kuipers,O.P., Brul,S., Hellingwerf,K.J., and Kort,R. (2006a) Effects of phosphorelay perturbations on architecture, sporulation, and spore resistance in biofilms of *Bacillus subtilis*. *J Bacteriol* **188**: 3099-3109.

Veening,J.W., Smits,W.K., Hamoen,L.W., Jongbloed,J.D., and Kuipers,O.P. (2004) Visualization of differential gene expression by improved cyan fluorescent protein and yellow fluorescent protein production in *Bacillus subtilis*. *Appl Environ Microbiol* **70**: 6809-6815.

Veening,J.W., Smits,W.K., Hamoen,L.W., and Kuipers,O.P. (2006b) Single cell analysis of gene expression patterns of competence development and initiation of sporulation in *Bacillus subtilis* grown on chemically defined media. *J Appl Microbiol* **101**: 531-541.

Xu,K., and Strauch,M.A. (1996) Identification, Sequence, and Expression of the Gene Encoding Gamma-Glutamyltranspeptidase in *Bacillus subtilis*. *J Bacteriol* **178**: 4319-4322.

Table S2: Plasmids used in this study		
Plasmid	**Description**	**Reference**
pGEM-T Easy	Cloning vector	Promega
pGT-*yvaN*	pGEM-T Easy derived vector containing a fragment of the *B. Subtilis* chromosome encompassing the *yvaN* gene	This study
pGT-*yvaN*-1514	pGT-*yvaN* derived vector where the *yvaN* ORF has been replaced with a tetracyclin resistance marker	This study
pICFP	Cloning vector for making C-terminal fusions with *cfp* variant	(Veening *et al.*, 2005)
pICFP-*rapH*	pICFP derived vector containing the promoter region and part of the ORF of *rapH*	This study
pSG1151	Cloning vector for GFP C-terminal fusions	(Lewis and Marston, 1999)
pSG1186	Cloning vector for CFP C-terminal fusions	(Feucht and Lewis, 2001)
pSG1187	Cloning vector for YFP C-terminal fusions	(Feucht and Lewis, 2001)
pX	Integration vector containing a xylose inducible promoter	(Kim *et al.*, 1996)
pGFP-srfA	pSG1151-derived vector containing the PsrfA promoter fused	This study
pSG-*comG*ACFP	pSG1186 derived vector containing PcomGA fused to CFP	This study
pSG-*comG*AYFP	pSG1186 derived vector containing PcomGA fused to YFP	This study
pX*rapHphrH*	pX derivative harboring the *rapHphrH* locus	This study
pCm::Em (pECE72)	Plasmid for the exchange of a chloramphenicol marker with a erythromycin marker	obtained from BGSC, (Steinmetz and Richter, 1994)
pCm::Sp (pECE74)	Plasmid for the exchange of a chloramphenicol marker with a spectinomycin marker	obtained from BGSC, (Steinmetz and Richter, 1994)
pGA-*gfp*	pUC-derived vector containing PcomGA fused to GFP	(Smits *et al.*, 2005)
pBS19	Replicative shuttle vector derivative of pBS42	(Band and Henner, 1984) and unpublished data
pHT315	Replicative shuttle vector	(Arantes and Lereclus, 1991)
pHT315S	Replicative shuttle vector containing the *spac* promoter	(Worner *et al.*, 2006)
pJM115	Transcriptional *lacZ* fusion vector Km^R derivative of pDH32	(Perego, 1993)
pET28a	Vector for protein expression	Novagen
pET28-RapH	pET28 carrying the *rapH* coding sequence as a BamHI fragment	This study
pBS19-RapH2	pBS19 carrying the *rapH* gene and its promoter, 1350bp	This study
pBS19-RapH3	pBS19 carrying the *rapH-phrH* genes and their promoter, 1560bp	This study
pHT315S-RapH2	pHT315S carrying the *rapH* gene and its promoter, 1350bp	This study
pHT315S-RapH3	pHT315S carrying the *rapH-phrH* genes and their promoter, 1560bp	This study
pJM115-RapHlac	*rapH-lacZ* transcriptional fusion	This study

References

Arantes,O., and Lereclus,D. (1991) Construction of cloning vectors for *Bacillus thuringiensis*. *Gene* **108**: 115-119.

Band,L., and Henner,D.J. (1984) *Bacillus subtilis* requires a "stringent" Shine-Delgarno region for gene expression. *DNA* **3**: 17-21.

Feucht,A., and Lewis,P.J. (2001) Improved plasmid vectors for the production of multiple fluorescent protein fusions in *Bacillus subtilis*. *Gene* **264**: 289-297.

Kim,L., Mogk,A., and Schumann,W. (1996) A xylose-inducible *Bacillus subtilis* integration vector and its application. *Gene* **181**: 71-76.

Lewis,P.J., and Marston,A.L. (1999) GFP vectors for controlled expression and dual labelling of protein fusions in *Bacillus subtilis*. *Gene* **227**: 101-110.

Perego,M. (1993) Integrational vectors for genetic manipulation in *Bacillus subtilis*. In *Bacillus subtilis* and Other Gram-Positive Bacteria: Biochemistry, Physiology, and Molecular Genetics. Sonenshein,A.L., Hoch,J.A., and Losick,R. (eds). Washington, D.C.: American Society for Microbiology, pp. 615-624.

Smits,W.K., Eschevins,C.C., Susanna,K.A., Bron,S., Kuipers,O.P., and Hamoen,L.W. (2005) Stripping *Bacillus*: ComK auto-stimulation is responsible for the bistable response in competence development. *Mol Microbiol* **56**: 604-614.

Steinmetz,M., and Richter,R. (1994) Plasmids designed to alter the antibiotic resistance expressed by insertion mutations in *Bacillus subtilis,* through *in vivo* recombination. *Gene* **142**: 79-83.

Veening,J.W., Hamoen,L.W., and Kuipers,O.P. (2005) Phosphatases modulate the bistable sporulation gene expression pattern in *Bacillus subtilis*. *Mol Microbiol* **56**: 1481-1494.

Worner,K., Szurmant,H., Chiang,C., and Hoch,J.A. (2006) Phosphorylation and functional analysis of the sporulation initiation factor Spo0A from *Clostridium botulinum*. *Mol Microbiol* **59**: 1000-1012.

Table S3: Oligonucleotide primers used in this study	
Oligonucleotide	**Sequence**
rapHup-F	GCGAACTTGCTGGAATATGG
rapHup-R	CGGGATCCACGGTATGGCTTGACTCAAC
rapHdown-F	GGGGTACCTTCCACATCGCGGCATTCCT
rapHdown-R	TCGGCACGCTGTAAGTCTTC
RNlacZfw	GGTTTTCCCAGTCACGACGTTGTAA
RNlacZrv	GTGAGCGGATAACAATTTCACACAGG
yvaNup-F	GGAAACTGCAGGGATTCGCTTGGCTACAACT
yvaNdown-R	GGAAACTGCAGGAACTCTGCCGCTTAGAT
yvaNdown-F	CGGGATCCCTGCTGATGACTGACTCTTG
yvaNup-R	CGGAATTCGCAATGCCCGTAATTGTTCG
rapHFP-F	GGGGTACCTAGTTGCCCAGGAAGAGCAT
rapHFP-R	AAAACTGCAGGCTAAGGGCTTTCTTCTGATC
PrapHFP-R	GGAATTCCGAAGACGGTATGGCTTGAC
pXrapH-F	GCTCTAGAGAAGGAGGGAAGCCG
pXrapH-R	CGGGATCCCTAGCTAAGGGCTTTCTTC
srfA-F	CCCAAGCTTGCTGAGAGAGCGTGAGCAGGATATG
srfA-R	CGGAATTCCATTTCCTCTCCTCCTCTAATCTTTATAAGCAGTGAACATGTGC
RapH5’Kpn	TTTGAGGTACCTGAGGAACAGGTGAAGGTTC
RapH3’Bam2	CATCAGGATCCTTCTTATATGGCATATAAACAC
RapH3’Bam3	GAAGGGATCCGCGATGTGGAAAATGGAAC
RapH5’Bam	GAAGGATCCTTGAGTCAAGCCATACC
RapHprom3’Bam	TTATAGGATCCATTAATCTTAACACCAAC
comG5’	CAGAAAGAATTCGTTTTTCAGCATATAACATC
comG3’	CGTAAGGGATCCGTTTTGCGGCTTTCGCCTTTC
DegU5’	GCGTGGCATATGACTAAAGTAAACATTGTTATTATC
DegU3’	CTATTCTCGAGTCTCATTTCTACCCAGCCATTTTTAATG
srfApromEco	TATGGAATTCATTGATATCGACAAAAATGTC
srfApromBam	CTTACGGATCCCCGCAAGATTTGAAATG