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Ákos T. Kovács, Wiep Klaas Smits, Aleksandra M. Mirończuk, Oscar P. Kuipers

Legends to Supplementary Figures

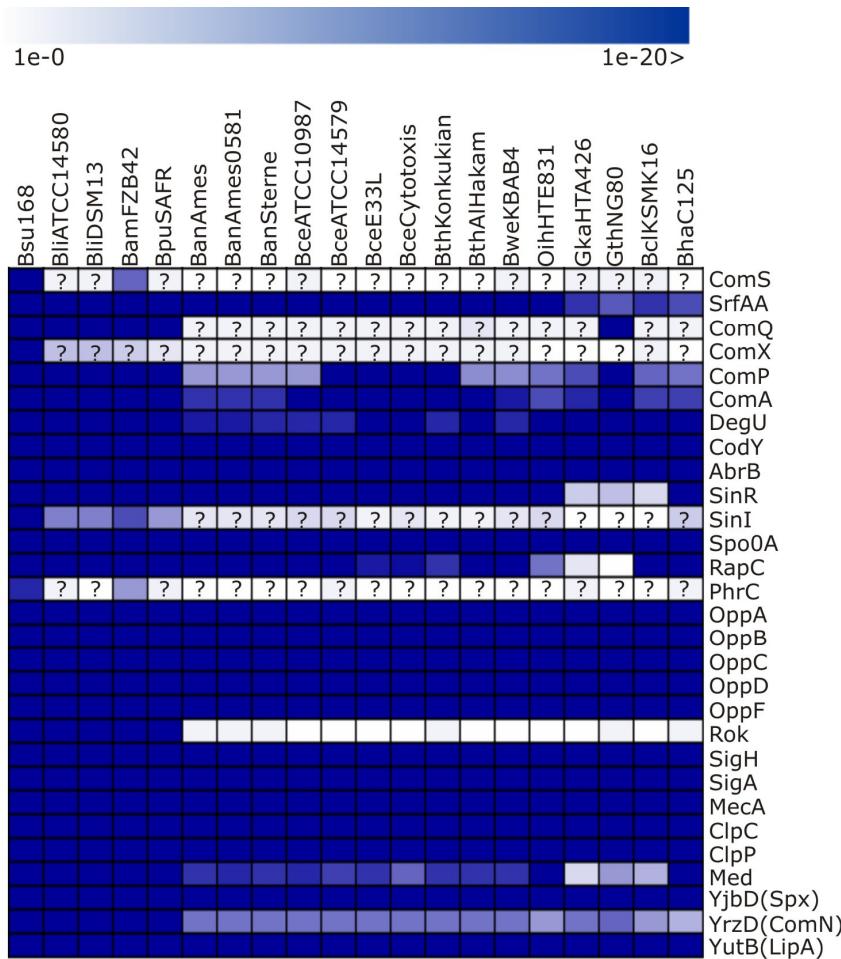
Supplementary Figure 1

Presence of homologs of proteins involved in the regulation of competence in *Bacillus* and closely related species. Results of BLAST queries were visualized with Genesis 1.6 software: white is absent (with e-value of E-0), dark blue is present (e-value <E-20). BLAST analysis was performed with *B. subtilis* protein sequences against the translated protein database of a given genome. Question marks denote small ORFs where identification is uncertain using the available bioinformatic tools that can miss homologues. Where the search yielded no hit (or with an e-value below E-05) an additional tblastn was performed. Protein names are indicated on the right. Bs_u (*B. subtilis*), Bam (*B. amyloliquefaciens*), Bl_i (*B. licheniformis*), Bpu (*B. pumilus*), Ban (*B. anthracis*), Bce (*B. cereus*), Bth (*B. thuringiensis*), Bwe (*B. weihenstephanensis*), Oih (*O. iheyensis*), Gka (*G. kaustophilus*), Gth (*G. thermodenitrificans*), Bcl (*B. clausii*), Bha (*B. halodurans*).

Supplementary Figure 2

Multiple alignments of ComG_{A-G} homologues. Black background represents conserved amino acids and grey background represents similar amino acids. Alignment was performed using Clustal W (Thompson et al., 1994), and presented using the Boxshade 3.21 program. For abbreviations of species names see Supplementary Fig. 1. Conserved domains of *B. subtilis* ComG proteins are indicated with arrows above the alignment. AAA+, ATPase domain (Smart accession number: SM00382); GSPII-F, general secretion pathway domain (PFAM accession number: PF00482); TMS, transmembrane segment; N-met, N-methyl domain often found at N-terminus of pilins and proteins involved in secretion (PFAM accession number: PF07963); SSeq, signal peptide sequence.

Supplementary Figure 1



Supplementary Figure 2

ComGA

Bsu168 1 -LDSIEKVSKNLLIEAYLTASDIHIVPRERDAITFPRUDHALKQRDVKKEBCVRLSHFKFLSAMDIGERRKPONGSLILKKEGN-VELRSTLPTI
 BamFZB42 1 -VKNIETYSRNLNEAYTARASDIHIVPRERDAITFPRIGALVKKRVLKKEECVRLSHFKFLSAMDIGERRKPONGSLILPPTET-VELRSTLPTI
 Bls 1 -LYTIESLSGRLLDEAARMRASDIHIVPGEKEAVVRFRIDDELQFDGTTENECRSRLESHFKFLSSMDIGERRLPQSALIYINREP-VELRSTLPTI
 BpuSAFR 1 -MNGIEESFAIMIKKEACRVQASDLHIVPRKDVAVOLRIGKDLMTYQCEKEKEFGEKLVSHFKFLASMDIGERRKPONGSLILQIDGE-E-VLRLSTLPTI
 Ban 1 -MNGIEESFAIMIKKEACRVQASDLHIVPRKDVAVOLRIGKDLMTYQCEKEKEFGEKLVSHFKFLASMDIGERRKPONGSLILQIDGE-E-VLRLSTLPTI
 Bth 1 -MNGIEESFAIMIKKEACRVQASDLHIVPRKDVAVOLRIGKDLMTYQCEKEKEFGEKLVSHFKFLASMDIGERRKPONGSLILQIDGE-E-VLRLSTLPTI
 Bce 1 -MNGIEESFAIMIKKEACRVQASDLHIVPRKDVAVOLRIGKDLMTYQCEKEKEFGEKLVSHFKFLASMDIGERRKPONGSLILQIDGE-E-VLRLSTLPTI
 Bwe 1 -MNSVELFLAMIMIKKEACRVQASDLHIVPRKDVAVOLRIGKDLMTYQCEKEKEFGEKLVSHFKFLASMDIGERRKPONGSLILQIDGE-E-VLRLSTLPTI
 Gth 1 VLDDEIEQVANRLLAEAVQRKRNASLHLVPRHDAILRIGDMLIDVGTISKETTAERVIVHFKFLAGMDIGERRRPOSKAMEVNESGET-VLRLSTLPTI
 Oih 1 -MNEVKKLSLSDSLLHSAWSLQSSDIHFYIYPTOTEIYFRIHGRRTLHRKISSNQYRLLITYMKFIISGMDIGESENIPQHTIIWTEKSNT-YSLRSTLPHH
 BhaC125 1 -LTIIHNNQSKRSLLQALRQACASDIHSGTREDIIIFRNGRAFPQRPLQTAERLNSHFKFSQGIDIGERRKPONGSLILQIDGE-E-VLRLSTLPTI
 BclKSMK16 1 -MADIEGKSTAIIYEAFQSBASDIHFTSKYGLVYRALGEIHAGOTIKAEDGRINSYFKYACGMIDIGERRKPQSKAIISYDWQGNG-YILRSTLPAK

AAA+

Bsu168 99 NEESLVIRLMPQYNPSPIDLSSLFPKICATLILS-FLKHSHGLMLIFTGPTGSKTTTLYSLQYAKHFNRLRIVTLEDPVEFRDEDVLOVO-NEKAGITYS
 BamFZB42 99 NDESLSVIRLPEKROIPPLDQLSSLFRGACAAILS-FLKHSHGLMLFTGPTGSKTTTLYSLVNYAKRHNRRNTRIVTLEDPVEFRDEDVLOVO-NEKAGITYS
 Bls 99 HDESLSVIRLPKNSKPLTLSSLFPSPATRKLLS-FLKHSHGLMLFTGPTGSKTTTLYSLIDYAKRHFKNRINTTLEDPVEFRNEDVLOQ-NEKAGITYS
 BpuSAFR 99 NDESLSVIRLPPDHVKLKLSSLFPKASTKLLS-FLNHSHGLMLFTGPTGSKTTTLYSLQOFAKKNFQAKRNRNTRIVTLEDPVEFRNEDVLOQ-NEKAGITYS
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 Bth 99 YQESLSVIRLHLQASDOPSLSSLFPSTAALKLLS-FLYRSHGLLVTFTGPTGSKTTTMYALLEVIAKKKURRIVTLEDPVEFRNDDVLOIQINEKAGITYE
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 Bwe 99 YQESLSVIRLHLQASDOPSLSSLFPSTAALKLLS-FLYRSHGLLVTFTGPTGSKTTTMYALLEVIAKKKURRIVTLEDPVEFRNDDVLOIQINEKAGITYE
 Gth 100 YDESLSVIRLPEQRFSIPLRELSSLFSHTAALFSLFQOQPQGILLLFTGPTGSKTTTLYLLOVCQAERCRNLIITLEDEPIERNRERFLQ-
 Oih 99 LLESTAIRHPQDQHPIKEKFLFFNQIRHKMEWSNQAGIILLTGPTGSKTTTLYAOKALHMONLIVTLEDPIEQLNPAFTI-
 BhaC125 100 LFEAVVARLHPQDQHPIFALESHPIKRQKQOLLS-LWTRQGILLTGAFTGSKTTTLYAOKALHMONLIVTLEDPIEQLNPAFTI-
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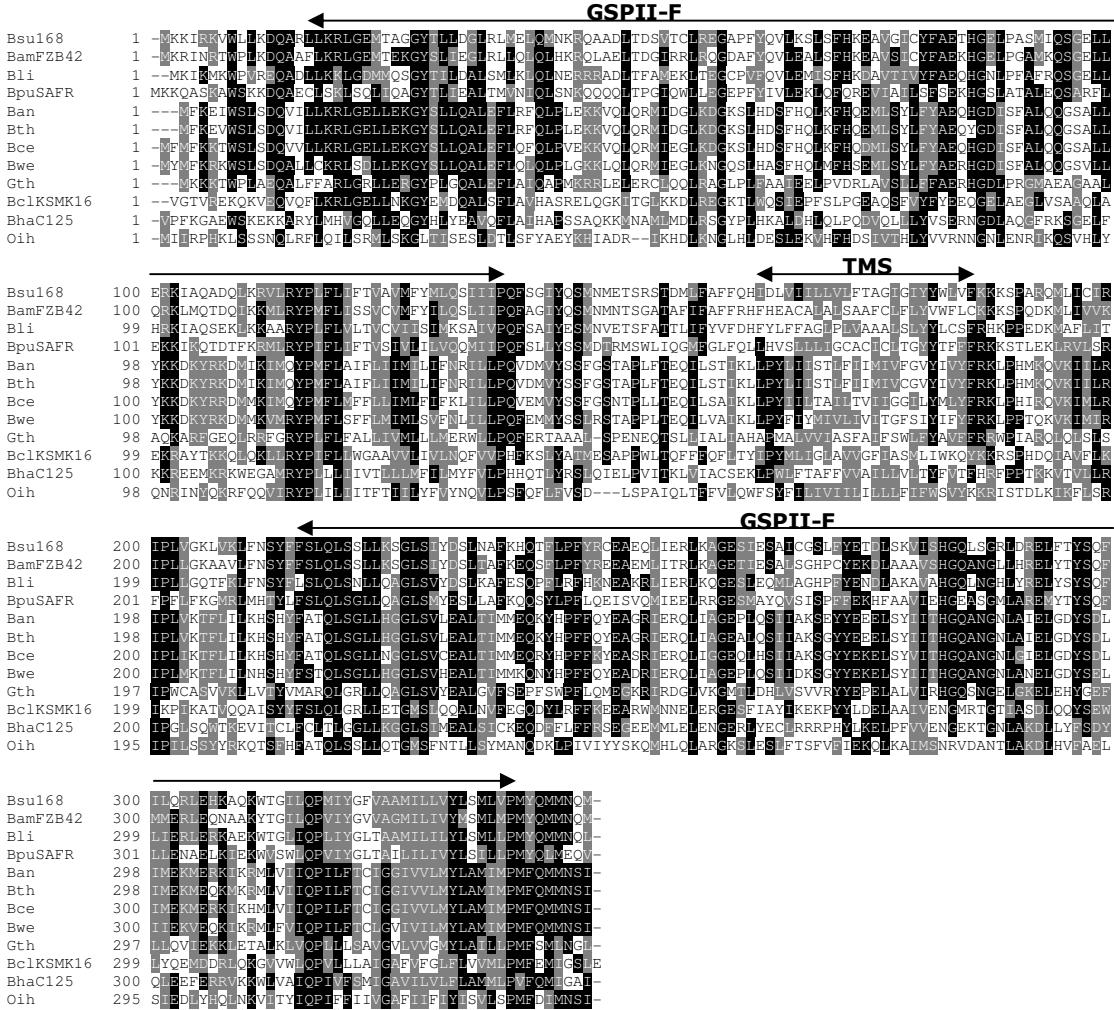
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 Bls 198 AGLKAILRHDPDMIIIGEIRDAETAIAVRAALTGHLVLTSLHAKNAKGAIYRLLELGVDIEIEQTILVSAQRVLVIDCPFCG-DSCYLYCRLSKFVR
 BpuSAFR 198 AGLKAILRHDPDMIVLGEIRDAETAIAVRAALTGHLVLTSLHAKNAKGALYRMLIEFGVTTIEIECTMVAIAAQRLLIDTCPCFCG-ETCQLYCRLNRFVR
 Ban 198 AGLKAILRHDPDMIVLGEIRDEETAKTAIRASLTGHLMVMTLHTNDARGAIYRFMDFCITRQEIEQSLLAIAAQRVLVBLKCPFCR-RKCSFLCKSVMRVR
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 Bce 198 TGLKAILRHDPDVILVGEIRDEETAKTAIRASLTGHLMVMTLHTNDARGAIYRFMDYGLTRQEIEQSLLAIAAQRVLVBLKCPFCR-GKCATLCKSVMRVR
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 Oih 199 PSLKAAALRHDPDVLMVGEIRDHUTAPIAVESALTGLHVVSIAADAVGAVVRLRFFGVSPCDIAETLLAISAOQLVBLRCPICG-DNCHPACERIIRR
 BhaC125 199 VGLKAAALRHDPDVIMVGEIRDHUTAPIAVESALTGLHVVSIAADAVGAVVRLRFFGVSPCDIAETLLAISAOQLVBLRCPICG-DNCHPACERIIRR
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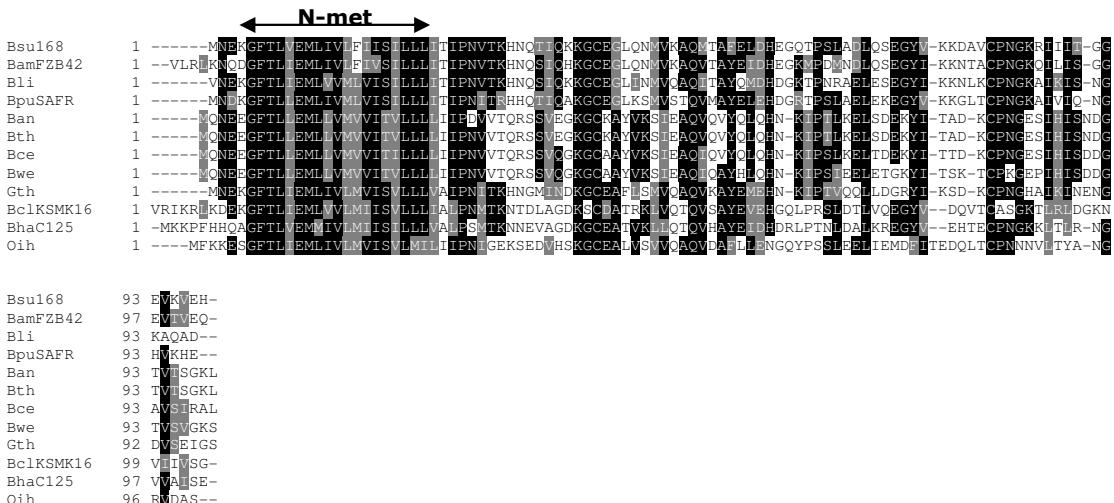
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 Bls 297 RASFYELLYCKNSLQEAEKRCGG-IKTDLKLMIKGHALGYLPSKIERWIGHED-----
 BpuSAFR 297 RTNVEELLGKCECIEKEAKGBYAH--SSYETLQRIRKGVALGYLSKNTHRWVYEEASL-----
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 Bth 297 QASIYELLYCYEIKQAIKEANGECVT--IKHETLQSSIRKGYALGELBEDDVW-----
 Bce 297 QASIYELLYCYEIKQAIKEANGECVT--IKHETLQSSIRKGYALGELBEDDVW-----
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 Oih 285 RAAYHELLYGSALFDVRESLSGEGERQLRPHFLARLIRKGHALGYLPTRALELVGGER-----
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ComG_B



ComG_C



ComG_D

← N-met →

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Bli	1 -----MNKGFTLLESLLVLSLASELAVETTIDPPAYNTAWROKTEOLQKDIDIAEETAAEHKRTKIDPLPKHHYKQS----GGIIVERSDS-
BpuSAFR	1 MNKLIFDEKGFTTIDPMLLVLVSLMIIIGVKUPNVTEGAEAKROVNIMKDFOLAAAYTAEVKTRVVVPEHLPEVSYQIDQKDG---EVIAFOHOK
Ban	1 -----VKQKGFTLLEMILLVIFASVLSMVTFVNVSILYEKQKDFQLRQFSNDILYMQQLAINRQKHYTLRWHDRHMYYIGES--SDLIIIRENDSD
Bth	1 -----VKQKGFTLLEMILLVIFASVLSMVTFVNVSILYEKQKDFQLRQFSNDILYMQQLAINRQKHYTLRWHDRHMYYIGES--SDLIIIRENDSD
Bwe	1 -----VKQKGFTLLEMILLVIFASVLSMVTFVNVSILYEKQKDFQLRQFSNDILYMQQLAINRQKHYTLRWHDRHMYYIGES--EDLIIIRENDSD
Bce	1 -----VKQQGFTTIDMLLVIFASVLSMVTFVNVSILYEKQKDFQLRQFSNDILYMQQLAINRQKHYTLRWHDRHMYYIGES--GKYYIVRREPED
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Oih	1 -----MPPSKNGFTTIDMLLVIFASVLSMVTFVNVSILYEKQKDFQLRQFSNDILYMQQLAINRQKHYTLRWHDRHMYYIGES--MNPQIRPFPKG
BhaC125	1 -----VSNSNGFTLMEITLIVIFASVLSMVTFVNVSILYEKQKDFQLRQFSNDILYMQQLAINRQKHYTLRWHDRHMYYIGES--SRSYLSRAPEM
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BamFZB42	95 ---DHTLVLWLPESLEENERGHPNAGGKHRRKGH-ITPLVYLGSGRNVAARK-----
Bli	95 ---DAIKKSSLKPILPENPQGKIVDTQHASEIITIYLGSGRHVK-----
BpuSAFR	98 ---GSIAKASHEPDGIYENVNGHSSSGGSLLIQPGSCKYKUITYLGSGRHVK-----
Ban	94 ---IQDLDNLHEPENPMTNPGNINRGGTTILSYRSYKYSIVEQLGRGRGRTYEMSKRVSDBG
Bth	94 ---IQDLDNLHEPENPMTNPGNINRGGTTILSYRSYKYSIVEQLGRGRGRTYEMSKRVSDBG
Bwe	94 ---VQMDLHHEPENPMTNPGNINRGGTTILSYRGYKYSIVEQLGRGRGRTYEVSKRISNG
Bce	94 ---IQDIDHHEPENPMTNPGNINRGGTTILSYRNKYKYSIVEQLGRGRGRTYEMSKRIHNG
Gth	95 ---WRFQIGLRLNEVETDNGNIEQQGGIVWKSSSENSYKVITFLGGRGRYVQKM-----
Oih	93 ---ISVTTFGRSE-IYENNSGTFIQPRTIHYGASNYSRITPELGRGRGYIPEI-----
BhaC125	94 GMYFEETGLGNGEDEELPNCGRFRGCSISLYAGDROYRITIMIGGKVYERL-----
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ComG_E

← SSeq →

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Bth	1 ---VCKQKGSVIAEMIVAIStLMMVVSLLPLQTVLVMGER-----KNIQLERKAAVLLKKEAAIYMYENE--AKQQKEKINGNVVITYNGGN
Bce	1 ---VCKQKGSVIAEMIVAIStLMMVVSLLPLQTVLVMGER-----KNIQLERKAAVLLKKEAAIYMYENE--DKRIIEKNINGVYYTYNGGN
Bwe	1 ---VCKQKGSVIAEMIVAIStLMMVVSLLPLQTVLVMGER-----KNIQLERKAAVLLKKEAAIYMYENE--EKRVEQVIKGVVITYPGD
Gth	1 ---MYKKCSGGFLLVEALFAIAALWTAALVLLPLYQHITER-----NNIVLEEKHLRLLTIALYEEPVGE--TAVADG---RTVFLRQARNEG
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BhaC125	1 ---MNDCKGXTLIEVLVLAIVCWSINTAAFPFWNMES-----ADLK-RVKVAQFPLEDAYHEYLIH--ELRTETIVGETIYQONLKESE
Oih	1 ---LRSNGFSFFEIIMARLFFSMIILHPLFSLSLMEEQ-----VVIQEERTIAYTIDKIQSSLWSNRPLEISTFNLENTOEVITIBETN
BclKSMK16	1 VYTRILKEEGGFTLIEHIIIGLVLSVVFCPSLQAGYSDPGEFDAQFPLNHTKEAKEELTWKDALLTKDGSASVYEWRPQKRORRDGKGV

Bsu168	85 GEYNQVIIKAAAYKE-----SFCLSLILQTLWILHAS-----
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Bli	85 GDDYEVCTSIKDRS-----QRHCFTILRACKYIYPS-----
Ban	84 ---EVCMWRKDVK-----EMMECCFTAGKGIN-----
Bth	84 ---EVCMWRKDVK-----EMMECCFTAGKGIN-----
Bce	84 ---EVTWVWDVK-----EMMECCFTAGKGIN-----
Bwe	84 ---EVCTWVWDVK-----EMMECCFTAGKGIN-----
Gth	83 GQMMKVCVRNDVYV-----RPERCFCGKGR-----
BpuSAFR	84 ---KGCVSILNLHK-----OTICFHQADEF-----
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ComG_F

← N-met →

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Bli	1 MIMKYMASLLKKETAKGTVSPFCGQCFITLINVIFSMVHMFAGTASFHLIFLSPE--RNGSDHPREWITABQILKEERSNS--IDIRSAGDHQSIA
Ban	1 -----MLLCRKRKNKEAGFTLIMEVCFCLFLSSTF-VLFPLRHPFQGEN--KQLKGNDNEWDFLICQVQLEPREVSFVEKIVPENRESMLR
Bth	1 -----MLLCRKRKNKEAGFTLIMEVCFCLFLSSTF-VLFPLRHPFQGEN--KQLKGNDNEWDFLICQVQLEPREVSFVEKIVPENRESMLR
Bce	1 -----MLLCRKRKNKEAGFTLIMEVCFCLFLSSTF-LLFPLRHPFQGEN--KQSKGNDNEWDVYHQMQLEPREVSFGEQIAFENGESMLR
Bwe	1 -----MQRKNNKEAGFTLIMEVCFCLFLSSTF-LLFPLRHPFQGEN--THSKGNDNEWDFLICQVQLEPREVSFVEKIVPENRESMLR
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Gth	1 -----VRVRAOAVAGGGSFTLIFAPJAAATAAAITPAALSFRPAGAD-VSDVGETHLEWRWFLOQLODELHET---ERWSTDGATLY
BhaC125	1 -----MRVQLQNCKGFTLIEVLLALIMLTVPLPLPLPS---SSIYFNFIAAEPQS--VRIEFDSPSRRL
Oih	1 -----MVNIKDNHGFSLHLLMIAIIITIPFISFLQLOSEIFPS-TKEEISVYQCFIFRDVIRSINA---VTTNDKLAITI
BclKSMK16	1 -----MWLSIFIKNFRP-----AIPITLTFIVLGGYFSYQFHYER-----PRLFVEQQLOLELEN-----ALQLAID

Bsu168	83 CTNLSGODIREDIVHSM-IRKRVIDGKGVFETIDHITAMKADLENGVVLKIDESEDOKVYOTAFPVYSYMIGGG--
BamFZB42	96 CRKTCGSEVREEDVHKM-IRKRVNCKGVFETIDQNAASLADKNGHLIIESSVQQNKAVTPVYSSFKGD--
Bli	96 MTNRGIGTIVYRYEYOTV-IRKRVNCKGVFETIDQNAASLADKNGHLIIESSVQQNKAVTPVYSSFKGD--
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Bth	85 FRLRTGDEVIYEKLNNH-LIRKVNMGREVLQNLVERVSYEVTPHILFNVKDRSGIIYEGVAIIFYSEMEINI
Bce	85 FRSRNGSBEVYKEKENSRLIRKVNMRGREGVLQNLITGTVSYKTPHVLIIINVDPGSKYIYQGVYMRYSEIGINV-
Bwe	82 FQLRNGDQVYKEKLNSN-LIRKVNMRGREGVLQNLITGTVSYKTPHILYVVDISGERYEGVAVRYSEIEIET-
BpuSAFR	78 YVSDGCRVNTIPYQEM-IRKRNDOAGHLPLQKVKECRVRTNQYRAIIKVDFESGKIVYEAFFYTKGVPTL-
Gth	82 LQKWNKGKVSFSLVKSN-PIRODGTGNTALRHRAVSYSTGICLTFQVTAIDGMVYEFVARAF-----
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BclKSMK16	63 ILASEGDETEDKHYPMTGTVSIVQAKEDKSYELVATLNGAIPKARIAVNADWEMVSYEG-----

ComG_G

