Supplementary Material

1 Supplementary Experimental Procedures

Construction of plasmids and strains.

Phusion DNA polymerase and restriction enzymes (Thermo Scientific) were used in plasmid constructions (primers are listed in Table S2). Plasmids were verified by sequencing.

To construct the *dnaA* allele that will encode either pseudo-phosphorylated or phospho-ablative protein, site-directed mutagenesis was conducted by using corresponding primers (Table S2) and pET28a-DnaA_{His6} as a template. Mutagenized sequence were obtained by PCR amplification and ligation into StuI and XhoI cut pET28-DnaA_{His6} plasmid.

For construction of $att\varphi C31$ attachment site integrative vector, harbouring different *dnaA* allele, pSET152 plasmid was digested with EcoRV and XbaI enzymes. The ca. 500 bp fragment of chromosomal DNA, localized upstream of the *dnaA* gene and partially within the ORF (reaching the first natural BamHI restriction site within *dnaA* gene), was amplified with primers flanked with XbaI and BamHI restriction site. The remaining *dnaA* coding sequence was obtained by digestion of pET28a-DnaA_{His6} WT/T486D vector with XhoI enzyme and filling in the 5' ends, followed by partial digestion with BamHI, resulting in 1900 bp fragment. Obtained DNA fragments were ligated, resulting in pSET-*dnaA_{His6}* WT/T486D/T486A integrative vector. An attempt to delete the native *dnaA* gene was made by generating apramycin-resistance cassette, derived from pIJ773, with primers dnaA_del_FW and dnaA_del_RV (Table S2). The *dnaA* gene on cosmid StH18 (John Innes Centre, Norwich) was replaced with obtained cassette. The modified cosmid was used for PCR targeting protocol as described elsewhere¹.

For construction of AK120 $\Delta afsK$ mutant, apramycin-resistance cassette, derived from pIJ773, was generated with primers afsK_del_FW and afsK_del_RV (Table S2). The *afsK* gene present on cosmid St6F11 (John Innes Centre, Norwich) was replaced with obtained cassette and PCR targeting protocol was used as described elsewhere¹ on AK120 strain.

2 Supplementary Figures and Tables

2.1 Supplementary Figures

sve:SVEN_3656272AAVAVAFAPARANNULFIYGESCLOKTHILLEAGUVARSLYPETKVRYSSEPTNEFI331mtu:FV0001188AALAIAEPARANNULFIYGESCLOKTHILLEAGUVARSLYPETKVRYSTEPTNEFI190ec:b3702152AALAIAEPARANNULFIYGEYGEKTHIMEAGUVALPSEKTVVISSEKTINEFI190ec:b3702152AALAIAEPARANNULFIYGEYGEKTHIMEAGUVALPSEKTVVISSEKTINEFI190sb:SAB0011136AASLAVAEPARANNULFIYGEYGEKTHIMEAGUVALPSEKTVVISSEKTINEFI195hpy:HP1529136AASLAVAEPARANNULFIYGEYGEKTHIMEAGUVALPSEKTVVISSEKTINEFI195sb:SAB0011136AASLAVAEPARANNULFIYGEYGEKTHIMEAGUANUNUVULNNEDAKUVISSEKTINEFI195hpy:HP1529136ETKKVAQSDTPPVNPVIYGETGEGKTHIMEAGUANUVULNNEDAKUVISSERTINEFI195sc:SC03879397NSIRDGKGDSFRKTKEMEMILLVDDIDFIADKESTCEFFHTFNTHENAKUVUSSERT187sc:SC03879397NSIRDGKGDSFRKTKEKEMILLVDDIDFIADKESTCEFFHTFNTHENAKUVUSSERT307bsuBSU00010191NSIRDBKAVDERNRYNDVULUDDIDFIADKESTCEFFHTFNTHENAKUVUSSERT307bsuBSU00010191NSIRDBKAVDERNRYNDVULUDDIGFIADKESTCEFFHTFNTHENAKUVISSERT250eco:SC03879457PKOLVTIEDERNRFENGITDVOPELETRIALEKAVGEOINAEPEVLEFTASRISK516sve:SVEN 3656392PKOLVTIEDERNRFENGITDVOPELETRIALEKAVGEOINAEPEVLEFTASRISK516sve:SVEN 3656392PKOLVTIEDERNRFENGITDVOPELETRIALEKAVGEOINAEPEVLEFTASRISK516sve:SVEN 3656392PKOLVTIEDERNRFENGITDVOPELETRIALEKAVGEOINAEPEVLEFTASRISK516sve:SVEN 3656392PKOLVTIEDERNRFENGITDVOPELETRIALEKAVGEOINAEPEVLEFTASRISK516sve:SVEN 3656392P	sco:SC03879	337	AAAVAVAEAPAKAYNPLFIYGESGLGKTHLLHAIGHYARSLYPGTRVRYVSSEEFTNEFI	396
bsu:SU00010131AASLAYAEAPARANNELFIYGGYGLGKTHLMHAIDHYYDDHNPSAKVYULSEKTINEFI190eco:b3702152AARROVADNPGGANNELFIYGGYGLGKTHLMHAIDHYYDNPSAKVYULSEKTINEFI191sab:SAB0001136AASLAYAEAPARANNELFIYGGYGLGKTHLMHAIDHNPSAKVYULSEKTINEFI195hpy:HP1529130EIAKKYAQSDTPPYNEVLFYGGTGLGKTHLMHAIDHNALDUNNDDAKVIYUSEDLTDFL187sco:SC03879397NSIRDGKGDSFRKRYKEMDILLVDDIOFIALKESTOEEFFHTENTIHNANKQIVISSDRP456sve:SVEN 3656332NSIRDGKGDAFRKRYKDVILLVDDIOFIALKESTOEEFFHTENTIHNANKQIVISSDRP391mtu:RV0001248NSIRDGKGDAFRKRYKDVILLVDDIOFIALKESTOEEFFHTENTIHNANKQIVISSDRP391bsu:BSU00010191NSIRDKKGDAFRKRYKDVILLVDDIOFIALKESTOEEFFHTENTIHNESKUISSDRP307bsu:BSU00010191NSIRDKKAGDAFRKRYKDVILLDDIOFIALKESTOEEFFHTENTIHNESKUISSDRP250bsu:BSU0011196KSIRDKVAFRKSYNDVVLUDIOFIALKESTOEEFFHTENTIHEEKSUISSDRP251py:HP1529186KNIENKAKVACFRSYNDVVLUDIOFIC/CKPRLEHFENTFIEHERANKOIVISSDRP255py:HP1529186KSIRDNEGEAFRRYNNIVULIDDIOFIC/KKYQTOEEFFHTENTIHASKISS247sco:SC03879457PKQLVTLEDRIRNERFGIITDVQPPELERRIATIKKAVGEONAPEVLEFIASRISR516sve:SVEN 3656392PKQLVTLEDRIRNERFGIITDVQPPELERRIATIKKAVGEONAPEVLEFIASRISR516sve:SUS00010251PKCIPTLEDRIRNERFGIITDVQPPELERRIATIKKAVGEONAPEVLEFIASRISR516sve:SUS00010251PKCIPTLEDRIRNERFGIITDVTPPULTRIATIKKAADERLIAKAPPOVLEIASSISR331sabiSAB0001256PKCIATLEDRIRNERFGIITDVTPPULTRIATIKKAADERLIAKATAPTPOVLES331sve:SUSN 365	sve:SVEN 3656	272	AAAVAVAEAPAKAYNELFIYGESGLGKTHLLHAIGHYARSLYPGTRVRYVSSEEFTNEFI	331
eco:b3702152AARQVADNPGAYNPLFLYGGVEGTEGKTHLLAVGNGIMARKPNAKVYMHSERVODVV211sab:SAB0001136AASLAVAFAPARANNELFYGGVEGTEGKTHLLAVGNGIMARKPNAKVYMHSERVODVV211hpy:HP1529130ELAKKAVASDTPYNVLFYGGTEGKTHLLAVGNGIMARKPNAKVYMHSERVODVV195sco:SC03879397NSIRDGKGDSFARRYKEMDILVDDIQFLAVKESTGEGEFHTENTILHNANKQTVLSSDRP456sve:SVEN 3656332NSIRDGKGDAFARRYKEMDILVDDIQFLAVKESTGEGEFHTENTILHNANKQTVLSSDRP307bsu:SU00010191NSIRDKAGDAFARRYKEMDILVDDIQFLAVKESTGEGEFHTENTILHNANKQTVLSSDRP307bsu:SSU00010191NSIRDNAVDFARRYKNAVULIDDIQFLAVKESTGEFFHTENTILHNANKQTVLSSDRP307bsu:SSU00010191NSIRDNAVDFARRYKNAVULIDDIQFLAVKESTGEFFHTENTILHANKQTVISSDRP250co:SC03879196KSIRDNEGKARKYHROVTLUDDIQFFAVKERSCEFFHTENTALEGNOTIISSDRP255hpy:HP1529188KHLDNKTMDSFARRYKNIDVLIDDIQFFAVKERSCEFFHTENALEGNOTUISSDRP255hpy:HP1529188KHLDNKTMDSFARRYKNIDVLIDDIQFFAVKERSCEFFHTENALEGNOTUISSDRP247soc:SC03879457FKQUVTLEDRINNFERGIITDUQPFELETRIAILRKAVQEQINA PEVLEFIASISR516sve:SVEN 3656392PKQULTLEDRINNFERGIITDUQPFELETRIAILRKAVQEQINA PEVLEFIASISR516sub:SAB0001251PKCUVTLEDRINNFERGIITDUQPFELETRIAILRKAVGEQINA PEVLEFIASISR516sub:SSU00010251PKCUVTLEDRINNFERGIITDUQPFELETRIAILRKAVQEQINA PEVLEFIASISR516sve:SVEN 3656392PKQUTLEDRINNFERGIITDUQPFELETRIAILRKAVQEQINA PEVLEFIASISR516sub:SSU00010251PKEINTLEDRINNFERGIITDUQPFELETRIAILRKAVQEQINA PEVLEFIASISS517	mtu:Rv0001	188	AAALAIAEAPARAYNPLFIWGESGLGKTHLLHAAGNYAORLFPGMRVKYVSTEEFTNDFI	247
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hpy:HP1529130EIAKKVAQSDTPPYNFVLFYGGTGIGKTHILNAIGNHALEKHKKVVLVTSDELTDFI i i*i137sco:SC03879 sve:SVEN 3656332NSIRDGKGDSFRKRYREMEDILVDDIOFLAXKESTOEEFFHTENT HNANKOTVLSDRP 456456sco:SC03879 sve:SVEN 3656332NSIRDGKGDAFKKYREMEDILVDDIOFLAXKESTOEEFFHTENT HNANKOTVLSDRP 456391bsu:BSU00010 eco:D3702 sab:SAB0001191NSIRDRKAVPENRYRNYDVLIDDIOFLAKESTOEEFFHTENT HNANKOTVLSDRP 250250hpy:HP1529188KH.DNKAVDENRYRNYDVLIDDIOFLAKESTOEEFFHTENT HENKOTVLSDRP 255250hpy:HP1529188KH.DNKTMDSKAKKYRKYRDIDVLIDDIOFLAKKSOEFFHTENT HENKOTVLSDRP 255257sco:SC03879 sve:SVEN 3656457PKQIVTLEDRIANKEKGITDVQPPLETRIAILRKKAVQEQINAPPEVLEFIASRISR 251516mu:Rv0071 subsub0010 eco:D3702251PKQIVTLEDRIANKEKGITDVQPPLETRIAILRKKAVQEQINAPPEVLEFIASRISR 251516sco:SC03879 sve:SVEN 3656 mu:Rv0071 sbusBSU00010 eco:D3702251PKQIVTLEDRIANKEKGITDVQPPLETRIAILRKKAVQEQINAPPEVLEFIASRISR 251516sco:SC03879 sve:SVEN 3656 hpy:HP1529517NIRELEGALIRVTAFASINRQPVDLGITEIVIKDITPPDLETRIAILRKKAVGEQINAPPEVLEFIASRISR 310310sco:SC03879 sve:SVEN 3656 hpy:HP1529517NIRELEGALIRVTAFASINRQPVDLGITEIVIKDITPEDLETRIAILRKKAKAEGIDIENTWLYIANOTOS 331330sco:SC03879 sve:SVEN 3656 hpy:HP1529517NIRELEGALIRVTAFASINRQPVDLGITEIVIKDIIPEGEDSAPEITSTAIMGATADYFG 3307576sco:SC03879 sve:SVEN 3656 hpy:HP1529517NIRELEGALIRVTAFASINRQPVDLGITEIVIKDIIPEGEDSAPEITAPAIMAATADYFG 3307576sco:SC03879 sve:SVEN 3656 h	eco:b3702	152	AAAROVADNPGGAYNFLFLYGGTGLGKTHLLHAVGNGIMARKPNAKVVYMHSERFVODMV	211
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eco:b3702212KALQNNAIEEEKRYYRSVALLIDDIOFANKERSOEEFFHTFNALLEGNOTIITSDRY271sab:SAB0001196KSIRDNEGEAFRERVRIDVLIDDIOFANKERSOEEFFHTFNALLEGNOTIITSDRY271hpy:HP1529188KHIDNKTMDSKAKVRHOFFILDDAOFLOGKPKLEEEFFHTFNELHONNKOTVISDRP255sco:SC03879457PKQLVTLEDRLRNRFEWGLITDVQPPELETRIAILRKKAVQEQLNAFPEVLEFIASRISR516sve:SVEN 3656392PKQLVTLEDRLRNRFEWGLITDVQPPELETRIAILRKKAVQEQLNAFPEVLEFIASRISR516mtu:Rv0001308PKQLATLEDRLRNRFEWGLITDVQPPELETRIAILRKKAVQEQLNAPPEVLEFIASRISR516swe:SVEN 3656392PKQLVTLEDRLRNRFEWGLITDVQPPELETRIAILRKKAVGEQLNAPPEVLEFIASRISR516swe:SVEN 3656271PKEIPTLEDRLRNRFEWGLITDVQPPELETRIAILRKKAVGEQLNAPPEVLEFIASRISR516swe:SVEN 3656292PKQLVTLEDRLRNRFEWGLITDVQPPELETRIAILRKKAVGEQLNAPPEVLEFIASRISR516swe:SVEN 3656291PKEIPTLEDRLRSRFEWGLITDVQPPELETRIAILRKKAVGEQLNAPPEVLEFIASRISR310sco:SC03879248PKIAQLEDRLKSRFEWGLITVATEPPETTRVAILMKKAPENDIRLFGEVAFFIAKRIRS307v::::::::::::::::::::::::::::::::::::			NSTRDNYAUNDONNYDYTTTDTOTTCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	
sab:SAB0001196KSTRDNEGEAFRERVENIDVLLIDDIOFIQNKVQTOEEFFYTFNE HQNNKQTVISSDRP255hpy:HP1529188KHLDNKTMDSFKARVRHCDFFLIDDAOFIQGEPFLEEFFHTFNE HANSKQTVLISDRP247sco:SC03879457FKQLVTLEDRLENRFENGLIDVQPELETRIAILRKKAVQEQLNAPPEVLEFIASRISR516mtu:Rv0001308FKQLATLEDRLENRFENGLIDVQPELETRIAILRKKAVQEQLNAPPEVLEFIASRISR516bsu:BSU00010251PKEIPTLEDRLENRFENGLIDVQPELETRIAILRKKAVGEQLNAPPEVLEFIASRISR310eco:b3702272PKEIPTLEDRLESRFENGLIDTPPDLETRIAILRKKAVGEQLNAPPEVLEFIASRISR311sab:SAB0001256PKEIQUDRLESRFENGLIDTPPDLETRIAILRKKAVGEQLNAPPEVLEFIASRISR310eco:b3702272PKEIPTLEDRLESRFENGLIDTPPDLETRIAILRKKAVGEQLNAPPEVLEFIASRISR310eco:b3702272PKEIPTLEDRLESRFENGLIDTPPDLETRIAILRKKAVERDINEFFARRIRS311hpy:HP1529248PKNIAGLEDRLESRFENGLIVDITPPDLETRIAILRKKAVERQUNATIONICOLS307**:::::**:::**:::**:::**:::**:::**:::				
hpy:HP1529188KHIDNKTMDSFKAKVRHCDFFILDDAOFLÖGKPRLÉEEFFHTENE HÄNSKÖIVIISDRS247sco:SC03879457PKQIVTLEDRINNFENGIITDVOPPELETRIAILRKKAVQEQLNAFPEVLEFIASRISR516sve:SVEN 3656392PKQIVTLEDRINNFENGIITDVOPPELETRIAILRKKAVQEQLNAFPEVLEFIASRISR451mtu:Rv0001308PKQIVTLEDRINNFENGIITDVOPPELETRIAILRKKAVQEQLNAFPEVLEFIASRISR451soc:SC03879251PKCIVTLEDRINNFENGIITDVOPPELETRIAILRKKAVQEQINAPPEVLEFIASRISR366ssu:BSU00010251PKEIPTLEDRINFENGIITDVOPPELETRIAILRKKAAREGLDIPNEVMLYIANQIDS310eco:b3702272PKEINGVEDRIKSRENGUITVATEPELETRVAILMKKADEDNDIRLIGEVAFFIAKRIRS331sab:SAB0001256PKEIAQLEDRIKSRENGUITVDITPPUTTNAILQKKIEEKKLDIPPELANYIANQIQS315hpy:HP1529248PKNIAGLEDRIKSRENGUITVATEPELETRVAILMKKADEQLNQITIPEEVMEYIAOHISD307scc:SC03879517NIRELEGALIRVTAFASINRQPVDLGITEIVIKDLIPGGEDSAPEITSTAIMGATADYFG576svc:SVEN 3656452NIRELEGALIRVTAFASINRQPVDLGITEIVIKDLIPGGEDSSPEITAPAIMAATADYFG511mtu:Rv0001311NIRELEGALIRVTAFASINRQPVDLGITEIVIKDLIPGGEDSAPEITSTAIMGATADYFG516subSU00010311NIRELEGALIRVTAFASINRQPVDLGITEIVIKDLIPGCDSSPEITAPAIMAATADYFG425sab:SAB0001316NIRELEGALIRVTAFASINRYPIKAALEIVIKDLIALQEKUTIIDNIQKTVAFYK388sab:SAB0001316NIRELEGALIRVVAYSLINKDINADLAAEAKDIIPSSKPKVITIKE QRVVGQOFN368sab:SAB0001316NIRELEGALIRVVAYSLINKDINADLAAEAKDIIQAFKSKKIIQDIQKIVAGYYN373sab:SAB0001316NIRELEGALIRVVAYSLINKNANNASIDLAKTVE DLQKHAEGSLENTLALAVASLINGA				
sco:SC03879457PKQLVTLEDRLRNRFEWGLITDVQPPELETRIAILRKKAVQEQLNAPPEVLEFIASRISR516sve:SVEN 3656392PKQLVTLEDRLRNRFEWGLITDVQPPELETRIAILRKKAVQEQLNAPPEVLEFIASRISR451mtu:Rv0001308PKQLATLEDRLRNRFEWGLITDVQPPELETRIAILRKKAVQEQLNAPPEVLEFIASRISR451bsu:BSU00010251PKEIPTLEDRLRNRFEWGLITDVQPPELETRIAILRKKAQMERLAVPDVLELIASSIER367bsu:BSU00010251PKEIPTLEDRLRSRFEWGLITDTTPPLETRIAILRKKAQMERLAVPDVLELIASSIER367bsu:BSU00010251PKEINGVEDRLSSRFEWGLIVDITPPLETRIAILRKKAQMERLAVPDVLEJASSIER367bsu:BSU00010256PKEIAQLEDRLSSRFEWGLIVDITPPLETRIAILRKKAQMERLAVPDVLEJASSIER331sab:SAB0001256PKEIAQLEDRLSSRFEWGLIVDITPPLETRIAILKKARAEGLDIPPELMYIANQIQS315hpy:HP1529248PKNIAGLEDRLSSRFEWGIIVALPPLETRIAILKKSIVKOCQLNQITLPEEVMEYIACHISD307sve:SVEN 3656452NIRELEGAIIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITSTAIMGATADYFG576sve:SVEN 3656452NIRELEGAIIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITAPAIMAATADYFG511mtu:Rv0001311NIRELEGAIIRVTAFASLNRTPIDKALAEIVLKDLIPGGEDSAPEITAPAIMAATAPYFD425bsu:BSU00010311NIRELEGAIIRVTAFASLNRTPIDKALAEIVLKDLIPGGEDSAPEITAPAIMAATAPYFD425sab:SAB0001316NIRELEGAIIRVTAFASLNKTPIDKALAEIVLKDLIPGGEDSAPEITAPAIMAATAPYFD425bsu:BSU00010311NIRELEGAIIRVTAFASLNKTPIDKALAEIVLKDLIPGGEDSAPEITAPAIMAATAPYFD425bsu:BSU00010311NIRELEGAIIRVAYSSINKDTNADLAAEALKDIIPSSK-PKVITIKE QRVVGQQFN368eco:b3702332NVKELEGAINRVIANANFTGRATIDFVREARDLLALQEKKVTIDUTQC			AND	
sve:SVEN 3656392PKQLVTLEDRLRNRFEWGLTTDVÖPPELETRIALRKAAVÖEÖLNAPPEVLEFTASRISR451mu:Rv0001308FKQLATLEDRLRNRFEWGLTDVÖPPELETRIALRKAAVMERLAVPDDVLELTASSIER367bsu:BSU00010251PKEIPTLEDRLRSRFEWGLIDDTPPLETRIALRKKAAKAGDEDIPNEVMLYTANQIDS310eco:b3702272PKEINGVEDRLKSRFEWGLIDDTPPLETRIALRKKAAKAGDEDIPNEVMLYTANQIDS311sab:SAB0001256PKEIAQLEDRLRSRFEWGLIVDITPPDYETRNALQKKKCQLNQITPEEVMEYTAQHISD307hpy:HP1529248PKNIAGLEDRLKSRFEWGITAKVMPPDLETKLSIVKOKCQLNQITPEEVMEYTAQHISD307sco:SC03879517NIRELEGALIRVTAFASLNRQPVDLGLTEIVLKDLIPGGEDSAPEITSTALMGATADYFG576sve:SVEN 3656452NIRELEGALIRVTAFASLNRQPVDLGLTEIVLKDLIPGGEDSAPEITAPAIMAATADYFG511mtu:Rv0001368NIRELEGALIRVTAFASLNRUPVDLGLTEIVLKDLIPGGEDSAPEITAPAIMAATAPYFD425bsu:BSU00010311NIRELEGALIRVTAFASLNRTPIDKALAEIVIPDLTALAGEKUVITIKE QRVGQQFN368eco:b3702332NVRELEGALIRVTAFASLNRTPIDKALAELVIIPSSKPKVITIKE QRVGQQFN368sab:SAB0001316NIRELEGALIRVTAFASLNRVAYSLINKDINADLAAEALKDIIPSSKPKVITIKE QRVGQQFN368sab:SAB0001316NIRELEGALIRVTAFASLNKTPIDKALAELVIIDUQAKAKKKITUDO CKVMAYK388sab:SAB0001316NIRELEGALIRVAYSLINKDINADLAAEALKDIIQAEKSKKITUDO CKVMAYK364	npy.ne1525	100		21/
mtu:Rv0001308PKQLATLEDRLRTRFENGLITDVÖPPELETRIATLRKKAQMERLAVPDDVLELIASSIER367bsu:BSU00010251PKEIPTLEDRLRSRFENGLITDITPPDLETRIATLRKKAQMERLAVPDDVLELIASSIER310eco:b3702272PKEINGVEDRLKSRFENGLITDITPPDLETRIATLRKKARAGIDIPNEVMLYIANOTDS310sab:SAB0001256PKEIAQLEDRLKSRFENGLITVATEPPELETRVATLMKKADENDIRLFGEVAFFIAKRIRS331hpy:HP1529248PKNIAGLEDRLKSRFENGLITATPPDLETRVATLMKKADENDIRLFGEVAFFIAKSIS307sco:SCO3879517NIRELEGALIRVTAFASLNROPVDLGITEIVIKDLIPGGEDSAPEITSTALMGATADYFG576sve:SVEN_3656452NIRELEGALIRVTAFASLNROPVDLGITEIVIKDLIPGGEDSAPEITAATMAATADYFG511mtu:Rv0001368NIRELEGALIRVTAFASLNROPVDLGITEIVIKDLIPGGEDSAPEITAATMAATAPYFG511su:BSU00010311NIRELEGALIRVTAFASLNROPVDLGITEIVIKDLIPGGEDSAPEITAATMAATAFYFD425bsu:BSU00010311NIRELEGALIRVTAFASLNKTPIDKALAEIVIRDLIAADEN-TMQISAATMAATAFYFD425sab:SAB0001316NIRELEGALIRVTAFASLNKTPIDKALAEIVIRDLIADCEKUVIINT CKTVAFYK388sab:SAB0001316NIRELEGALIRVIANATAFYFD VKALAFULALQEKUVIINT CKTVAFYK373hpy:HP1529308NIRQMEGAITKLAYSULMANLMASIDLNLAKTVEFDLQAKHAEGSLENTLLAVAQSLN374	sco:SC03879	457	PKOLVTLEDRIRNRFEWGLITDVOPPELETRIAIIRKKAVOEOINAPPEVLEFIASRISR	516
bsu:BSU00010251PKCIPTLEDRLENERSRENGLIIDITPPDLETRIATLRKKÄRAG.DIPNEVMLYTANQIDS310eco:b3702272PKEINGVEDRLKSRENGLIVDITPPDLETRIATLRKKÄRAG.DIPNEVMLYTANQIDS311sab:SAB001256PKEIAQLEDRLKSRENGLIVDITPPDVETRNATLOKKIEEKLDIPPEANYTANQIQS315hpy:HP1529248PKNIAGLEDRLKSRENGITAKVMPPDLETKLSIVKOKCQLNQITLFEEVMEYTAQHISD307sco:SCO3879517NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITSTAIMGATADYFG576svc:SVEN 3656452NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITSTAIMGATADYFG511mtu:Rv0001368NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITAATMAATADYFG511su:BSU00010311NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITAATMAATADYFG425sab:SAB0001316NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITAFATMAATADYFG516httl:Rv0051368S07328NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITAFATMAATADYFG511htt:Rv0051368NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITAFATMAATADYFG516bsu:BSU00010311NIRELEGALIRVTAFASLNKYFIDKAALEIVLRDLIALOFMKUTIKEIQRVKOQFN368eco:b3702332NVKELEGALNRVIANAFTGRATIDFVREALFDLALOFKKUTIDNIQKTVAFYKK388sab:SAB0001316NIRELEGALIRVLANAFTGRATIDFVREALFDLALOFSKKITIQDIQKIVGQYNN373hpy:HP1529308NIRQMEGATKLISVNANLMASIDLAKTVE DLQKAAEGSLENTLLAVAQSLN364	sve:SVEN 3656	392	PKOLVTLEDRERNRFEWGLTTDVOPPELETRIAILRKKAVOEQLNAPPEVLEFIASRISR	451
bsu:BSU00010251PKCIPTLEDRIRSRENGLIIDITPPDLETRIATLRKARAEGLDIPNEVMLYTANQIDS310eco:b3702272PKEINGVEDRIKSREGGIIVATEPPLETRVATLMKKADENDIRLEGVAFFTAKRLRS331sab:SAB0001256PKEIAQLEDRIRSREWGIIVDITPDUPTTRNATLOKKIEEKLDIPPEDANYTANQIQS315hpy:HP1529248PKNIAGLEDRIKSREWGITAKVMPPDLETKLSIVKOKCQLNQITLEEVMEYTAQHISD307***::::**::**::**::**::**::**::**::**:	mtu:Rv0001	308	PROLATIEDRIRTRFEWGLITDVOPPELETRIAILRKKAOMERLAVPDDVLELIASSIER	367
eco:b3702272PKEINGVEDRLKSREGNGITVATEPPELETRVATIMKADENDIRLEGEVAFFTAKRIRS331sab:SAB0001256PKETAQLEDRLRSREGNGITVATEPPELETRVATIQKKIEEEKLDIPPEALNYTANQIQS315hpy:HP1529248PKNIAGLEDRLKSREGNGITAKVMPPDLETKLSIVKQKCQLNQITLFEVMEYTAQHISD307**:::::::::::::::::::::::::::::::::::	bsu:BSU00010	251	PKEIPTLEDRERSREEWGLITDITPPDLETRIATLRKKAKAEGLDIPNEVMLYIANQIDS	310
hpy:HP1529248PKNIAĞLEDRLKSRFENGITAKVMPPDLETKLSIVÄQKCQLNQITLFEEVMEYTAQHTŠD307sco:SCO3879517NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITSTAIMGATADYFG576sve:SVEN 3656452NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSSPEITAPALMAATADYFG511mtu:Rv0001368NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITSTAIMGATADYFG512bsu:BSU00010311NIRELEGALIRVTAFASLNRQPVDLGITEIVLKDLIPGGEDSAPEITAPALMAATADYFG588eco:b3702332NVRELEGALIRVTAFASLNRVFTDKALTDLALQEKUVTIDN QKTVAEYYK388sab:SAB0001316NIRELEGALIRVLANANFTGRATIDEVREARDLALQEKUVTIDN QKTVAEYYK388hpy:HP1529308NIRQMEGATIRLIAYSQLEGRPTTELTAFALKDIQAPKSKKITQDTQKIVQYN373	eco:b3702	272	PKEINGVEDRLKSREGWGLTVAIEPPELETRVAILMKKADENDIRLEGEVAFFIAKRLRS	331
::::::::**:::**:::**:::**:::**:::	sab:SAB0001	256	PKEIAOLEDRIRSRFEWGLIVDITPPDYETRMATLOKKIEEEKLDIPPEALNYIANOTOS	315
::::::::**:::**:::**:::**:::**:::	hpy:HP1529	248	PKNIAGLEDRLKSRFEWGITAKVMPPDLETKLSIVKOKCOLNOITLPEEVMEYIAOHISD	307
sve:SVEN_3656452NIRELEGALIRVTAFASLNRÖPVDLGLTEIVLKDLIPGGEDSSPEITAPAIMAATADYFG511mtu:Rv0001368NIRELEGALIRVTAFASLNKTPIDKALAEIVLRDLIADANTMQISAATIMAATAPYFD425bsu:BSU00010311NIRELEGALIRVVAYSSLINKDINADLAAEAKDIIPSSKPKVITIKEIQRVVGQFN368eco:b3702332NVRELEGALNRVIANANFTGRATTIDFVREALRDLIADQEKLVTIDNIQKTVAEYYK383sab:SAB0001316NIRELEGALIRLAYSQLLGKPITTEITAEALKDIIQAPK-SKKITIQDIQKTVAEYYK383hpy:HP1529308NIRQMEGAIIKISVNANLMNASIDLNLAKTVEEDLQKDHAEGSSLENILLAVAQSLN364			**:: :****:.** **: . : **: **: :* :: * :. **. :	
mtu:Rv0001 368 NIRELEGALIRVTAFASLNKTPIDKALAEIVIRDLIADANTMQISAATIMAATAEYFD 425 bsu:BSU00010 311 NIRELEGALIRVTAFASLNKTPIDKALAELVDIPSKPKVITIKE QRVVGQQFN 368 eco:b3702 332 NVRELEGALIRVIANAFTGRATIDFVREALRDLIALQEKLVTIDNIQKTVAFQYK 388 sab:SAB0001 316 NIRELEGALIRLAYSQLLGKPITTEITAEALKDIIQAPK-SKKITQD QKVAFQYK 387 hpy:HP1529 308 NIRQMEGATIKISVNANLMNASIDLNLAKTVIFDLQKHAEGSSLENILLAVAQSLN 364	sco:SC03879	517	NIRELEGALIRVTAFASLNRQPVDLGLTEIVEKDLIPGGEDSAPEITSTAIMGATADYFG	576
bsu:BSU00010 311 NIRELEGALIRVVAYSSINKDINADLAAEALKDIPSSKPKVITIKEIQRVVGQQFN 368 eco:b3702 332 NVRELEGALNRVIANAFTGRATIDFVREARDLALQEKUVIDNIQKTVAEYYK 388 sab:SAB0001 316 NIRELEGALIRVLANAFTGRATIDEVREARDLALQESKUTIQDIQKVAEYYK 388 hpy:HP1529 308 NIRQMEGATIKISVNANLMNASIDLNLAKTVEDLQKDHAEGSSLENILLAVAQSLN 364	sve:SVEN 3656	452	NIRELEGALIRVTAFASLNRÖPVDLGLTEIVLKDLIPGGEDSSPEITAPAIMAATADYFG	511
eco:b3702 332 NVRELEGALNRVIANANFTGRAITIDFVREALRDLLALQEKLVTIDNTQKTVAFYYK 388 sab:SAB0001 316 NIRELEGALTRLLAYSQLLGKPITTELTAEALKDIIQAPKSKKITIQDTQKIVGQYYN 373 hpy:HP1529 308 NIRQMEGAIIKISVNANLMNASIDLNLAKTVLEDLQKDHAEGSSLENTLLAVAQSLN 364	mtu:Rv0001	368	NIRELEGALIRVTAFASLNKTPIDKALAEIVERDLIADANTMQISAATIMAATAEYFD	425
sab:SAB0001 316 NIRELEGALTRILAYSQLLGKPITTELTAEALKDIIQAPKSKKITQDIQKIVGQYYN 373 hpy:HP1529 308 NIRQMEGAIIKISVNANLMNASIDLNLAKTVLEDLQKDHAEGSSLENILLAVAQSLN 364	bsu:BSU00010		NIRELEGALIRVVAYSSLINKDINADLAAEALKDIIPSSKPKVITIKEIQRVVGQQFN	368
sab:SAB0001 316 NIRELEGALTRILAYSQLLGKPITTELTAEALKDIIQAPKSKKITQDIQKIVGQYYN 373 hpy:HP1529 308 NIRQMEGAIIKISVNANLMNASIDLNLAKTVLEDLQKDHAEGSSLENILLAVAQSLN 364	eco:b3702		NVRELEGALNRVIANANETGRATTIDEVREALRDLLALQEKLVTIDNIQKTVAEYYK	
	sab:SAB0001	316	NIRELEGALTRILAYSOLLGKPITTELTAEALKDIIQAPKSKKITIQDIQKIVGQYYN	373
:::***: :: . :.: : :*.*: : : *:	hpy:HP1529	308		364
	Non-Warding and Source of Con-		*:*::***: :: . :.: : :*.*: : : *:	

Fig. S1. High conservation of the DnaA domain III from various bacterial species.

Alignment of multiple DnaA domain III sequences from closely and distantly related bacterial species: *Streptomyces coelicolor, Streptomyces venezuelae, Mycobacterium tuberculosis, Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Helicobacter pylori* (indicated as sco:SCO3879, sve:SVEN_3656, mtu:Rv0001, bsu:BSU00010, eco:b3702, sab:SAB0001 and hpy:HP1529, respectively). Identical residues are marked by dark grey background and asterisk, similar residues are marked by light gray background and dot or colon. Numbers correspond to the position of given residue within protein sequence. *S. coelicolor* threonine 486 position and corresponding residues in other DnaA proteins are marked by red rectangle. Sequence alignment was made with Clustal Omega software². Protein sequence data was obtained from UniProt database.

2.2 Supplementary Tables

Table S1. Bacterial strains and plasmids.

Strain	Genotype	Source	
M600	Prototrophic, SCP1 ⁻ , SCP2 ⁻	laboratory stock	
M145	Prototrophic, SCP1 ⁻ , SCP2 ⁻		
M1101	M600 ∆afsK::aac(3)-IV	Hempel <i>et. al</i> , 2012; Prof. Mark Buttner	
M1102	M600 <i>∆ramC::aac(3)-IV</i>		
M1103	М600 <i>∆1468::aac(3)-IV</i>		
M1104	М600 <i>Д2244::aac(3)-IV</i>		
M1105	М600 <i>Д3102::aac(3)-IV</i>		
M1106	М600 Д3820-3821::aac(3)- IV		
M1107	М600 <i>Д4487-4488::aac(3)-</i> <i>IV</i>		
M1108	M600 ⊿4507::aac(3)-IV		
AK120	M145 <i>dnaN-EGFP</i> (in frame)	Prof. Dagmara Jakimowicz and dr Agnieszka Kois- Ostrowska, laboratory stock	
AK120 ∆afsK∷aac(3)-IV	M145 dnaN-FGFP (in		
AK120 tipAØ	M145 <i>dnaN-EGFP</i> (in frame); $attB_{\phi C31}$:: $tipA\emptyset$		
AK120 tipA afsK	M145 <i>dnaN-EGFP</i> (in frame); $attB_{\phi C31}$::tipA afsK		
M1101 pKF256	M600 $\Delta afsK::aac(3)-IV;$ $attB_{\varphi BTI}::afsK$	this work	
del-DnaA	M145 $attB_{\varphi C31}$:: $dnaA_{His6}$ WT; $\Delta dnaA$ (in frame)		
DnaA _{His6} WT	M145 $attB_{\varphi C31}$:: $dnaA_{His6}$ WT		
DnaA _{His6} T486D	$\begin{array}{c} M145 \\ T486D \end{array} attB_{\varphi C31} :: dnaA_{His6} \end{array}$		

S. coelicolor

Table S1 continued.

E. coli

Strain	Genotype	Source	
DH5a	F^- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20 $\varphi 80dlacZ\Delta M15 \Delta (lacZYA-argF)U169,$ hsdR17($r_K^-m_K^+$), λ^-		
ET pUZ8002	am-13::Tn9 dcm-6 hsdM, pUZ8002, Kan ^r	laboratory stock	
BL21(DE3)	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		
BL21	$B F^{-} ompT gal dcm lon hsdS_{B}(r_{B}^{-}m_{B}^{-})[malB^{+}]_{K-12}(\lambda^{S})$]	

Plasmids

Plasmid	General description	Source
pET28a-DnaA _{His6}	pET28a induced expression vector carrying <i>dnaA</i> _{His6} gene	laboratory stock
pIJ6902	vector integrating into <i>S. coelicolor</i> chromosomal φ C31 attachment site; carries thiostrepton-inducible <i>tipA</i> promoter	laboratory stock
pKF 592	92 pIJ6902 derivative, carries thiostrepton inducible <i>afsK</i> gene	
pSET152 vector integrating into <i>S. coelicolor</i> chromosomal φC31 attachment site		laboratory stock
pSET152-DnaA _{His6} vector integrating into S. coelicolor chromosomal φC31 attachment site; carries dnaA allele		this work
pIJ773 source of the <i>aac(3)IV</i> (apramycin) resistance cassette		laboratory stock

Primer	Sequence 5' - 3'	General description	
StuI_dnaA_FW	TCGCCGAGGCACCCG CCA		
XhoI_dnaA_RV	GTGGTGCTCGAGTGC GGC		
dnaA_T486D_UP	GCCGCCGGAGCTGGA GGACCGCATCGCGAT CCTGC	Primers used in site-directed mutagenesis of $dnaA_{His6}$ encoding pET28a plasmid.	
dnaA_T486D_DN	GCAGGATCGCGATGC GGTCCTCCAGCTCCG GCGGC		
dnaA_del_FW	TTGGAGCAACTTCTC GGAGAGGGGCCGCGG GCAGGGCGTCCATAT GATTCCGGGGGATCCG TCGACC	Primers used for generation of <i>dnaA</i> :	
dnaA_del_RV	GGGTCCCGGAGGCGC CTTCGTGACCGGTGG GGTCGGTCGCATATG TGTAGGCTGGAGCTG CTTC	<i>aac(3)-IV</i> disruption cassete.	
dnaA_pprom_FW	GGATCCAGTGCTCGT CCTTCGAC	Primers used for amplification of ~500 bp fragment of chromosomal DNA, localized	
dnaA_pprom_RV	TCTAGAGATGTCATG ACCCGACCGGGAT	upstream of the <i>dnaA</i> gene and partially within the ORF (see Construction of plasmid and strains in Supplementary procedures).	
afsK_del_FW	GTGGTGGATCAGCTG ACGCAGCACGATCCG CGGCGGATCATTCCG GGGATCCGTCGACC	Primers used for generation of <i>afsK</i> ::	
afsK_del_RV	TCACGTCGTACGGGC GGTCCCCGTGCCCTT CTCCGCGTCTGTAGG CTGGAGCTGCTTC	<i>aac(3)-IV</i> disruption cassete.	
afsK_FW	GGATCCGTGGTGGAT CAGCTGACGCA	Primers used for amplification of <i>afsK</i> gene	
afsK_RV	TCACGTCGTACGGGC GGT		
oriC_Bf2_IR700	CCTCGCACGTCCCCG CGTCTC	Primers used for generation of near-	
oriC_Br7_IR700	GGCATGTGGAGAAGC TGGTGATC	infrared labelled <i>oriC</i> fragment.	

References

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