SUPPLEMENTARY FIGURE LEGENDS:

Supplemental Figure 1. Alignment of the amino acid sequence of DnaA Domains III and IV from six different species. Blue colouring represents the conservation of a particular amino acid between this species. Domains, important structural features, and the hypermorphic and suppressor substitutions are highlighted.

Supplemental Figure 2. The *oriC*-to-terminus ratios of the independently cloned DnaA suppressor mutants were determined using marker frequency analysis (MFA) in the presence and absence of Soj^{G12V} over expression (1% Xylose). Westerns blots showing the intracellular DnaA and DivIVA levels are shown below. Wild-type (HM524), $DnaA^{L294R}$ (HM527), $DnaA^{V323D}$ (HM528), $DnaA^{L337P}$ (HM529), $DnaA^{A341V}$ (HM530).

Supplemental Figure 3. Soj forms a complex with DnaA bound to DNA. (A) In vitro crosslinking assay using formaldehyde in the presence of DNA (pBSoriC4; 3 nM), Soj^{G12V} (12 uM) and ATP (2 mM). Protein complexes were separated by SDS-PAGE and the DnaA protein was visualized by western blotting. The identity of the DnaA proteins (3 μM) are indicated below. Triangles above the lanes represent an increasing concentration of formaldehyde (0.05, 0.1, 0.5 and 1%). (B) qPCR analysis of the amount of pBsoriC4 found in the complex Soj:DnaA:DNA complex captured with 0.5% formaldehyde.

Supplemental Figure 4. A cartoon representation of monomeric DnaA from the *A. aeolicus* crystal structure (PDB ID: 1L8Q) bound to ADP (ball and stick). Domains IIIa, IIIb (green) and IV (cyan) as well as the Soj^{G12V} hypermorph (black) and suppressor (red) substitutions are indicated. Large amino acid references indicate the mutations isolated in *B. subtilis* DnaA and the smaller residues in parenthesis are the corresponding residue found in *A. aeolicus.*

Supplemental Figure 5. EMSA of DnaA proteins with DNA. Wild-type and mutant DnaA proteins were two-fold serially diluted from 12 uM to 0.047 uM in oligomer formation buffer with NaCl (400 mM) and supplemented with ATP (2 mM). Either pBsoriC4 or pUC18 (120 fmol) were added to the reaction. Nucleoprotein complexes were separated on a 1% agarose gel and visualized by staining with ethidium bromide.Vertical arrows indicated DnaA concentrations used in the oligomer formation assays.

Supplemental Figure 6. (A) In vitro helix formation assay using a cysteine specific cross linker (BMOE). Crosses located above a lane indicate the presence of BMOE, nucleotide and/or DNA (120 fmol of pBsoriC4). The DnaA protein (3 μM) is indicated below. DnaA proteins were separated by SDS-PAGE and visualized by western blotting. (B) In vitro helix formation assay using a cysteine specific cross linker (BMOE) in the presence of ATP (2 mM). DnaA proteins were separated by SDS-PAGE and visualized by western blotting. Triangles across lanes indicate the presence of Soj^{G12V} protein (12, 24, 32 μM). The DnaA protein (3 μM) and DNA substrates (120 fmol) are indicated below. ssDNA is oGJS159.

Supplemental Figure 7. (A) In vitro crosslinking assay using the primary amine specific cross linker (BS^3) in the presence of DNA (pBSoriC4; 3 nM) and ATP (2 mM). Protein complexes were separated by SDS-PAGE and the DnaA protein was visualized by western blotting. Pluses located above each lane indicate the presence of BS^3 and/or Soj proteins (32 μM). The identity of the DnaA proteins (3 μM) are indicated below. The identity of the Soj proteins is indicated above the respective gel. (B) In vitro helix formation assay using a cysteine specific cross linker (BMOE) in the presence of pBSoriC4 (3 nM) and ATP (2 mM). DnaA proteins (3 μM) were separated by SDS-PAGE and visualized by

western blotting. Triangles across lanes indicate the presence of Soj^{G12V} protein (12, 24, 32 μM).

Supplemental Figure 8. (A) Soj proteins do not affect the ATPase activity of DnaA. A malachite green phosphate assaywas used to measure the ATPase activity of DnaA in the presence of monomeric and dimeric Soj proteins. The observed decrease in ATPase activity caused by both Soj proteins is likely non-specific becasuse an equivalent molar addition of either IgG or BSA produced the same response. (B) In vitro helix formation assay using a cysteine specific cross linker (BMOE) in the absence of DNA. Protein complexes were separated by SDS-PAGE and the DnaA protein visualized by western blotting. The DnaA proteins (3 μM) are indicated below and triangles above the lanes represent an increasing concentration of Soj^{G12V} protein (12, 24 and 36 μM). The panel below the full length gel shows the same image with increased contrast to better visualize the high molecular weight DnaA oligomers. The bar chart at the bottem shows the quantification of the gel, where DnaA was defined as being in a helix if found in a dimer or higher molecular weight complex.

Supplemental Figure 9. (A) Marker frequency analysis was used to determine the replication initiation frequency of wild-type DnaA and DnaA^{CC} strains. Wild-type DnaA (HM557), DnaA^{CC} (sGJS006). (B) Western blot analysis of Soj protein overexpression in strains harbouring DnaA^{CC}. Soj expression was induced with 1% xylose as described in Experimental Procedures. DivIVA was used to normalize the samples. DnaA^{CC} Soj^{G12V} (sGJS006), DnaA^{CC} Soj^{R189A} (sGJS033).

Supplemental Table 1. Table of strains used in this study.

Supplemental Table 2. Table of plasmids used in this study.

Supplemental Table 3. Table of oligonucleotides used in this study.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES:

Marker frequency analysis. Starter cultures were grown in LB medium at 30˚C overnight and then diluted 1:100 into fresh medium (with inducer where indicated). Sodium azide (0.5%; Sigma) was added to exponentially growing cells (A_{600} = 0.2-0.4) to prevent further growth. Chromosomal DNA was isolated using a DNeasy Blood and Tissue Kit (Qiagen). Rotor-Gene SYBR Green PCR Kit was used for PCR reactions (Qiagen). Q-PCR was performed in a Rotor-Gene Q Instrument (Qiagen). By use of crossing points (C_T) and PCR efficiency a relative quantification analysis ($\Delta\Delta C_T$) was performed using Rotor-Gene Software version 2.0.2 (Qiagen) to determine the *ori/ter* ratio of each sample. These results were normalized to the *ori/ter* ratio of a DNA sample from *B. subtilis* spores which only have one chromosome and thus an in *ori/ter* ratio of 1.

Surface plasmon resonance. SPR experiments were conducted on the Proteon XPR36 system using a GLC sensor chip. Protein solutions were prepared at room temperature and held within the Proteon at 4°C. The interaction analysis was performed at 25°C. Amongst other possibilities this system allows the simultaneous measurement of real-time interactions between five ligands (in five of the six available channels) and a single analyte at six different concentrations, the final channel was used as a reference channel. The system was primed with chip preparation buffer (25 mM HEPES pH 7.6, 200 mM NaCl, 10 mM MgCl, 1 mM EDTA and 0.005% P20) and the chip surface was pre-prepared with concurrent 60 second injections of 0.5% SDS, 50 mM NaOH and 100 mM HCL. The surface was activated with a 210 second injection of a 50:50 mix of 0.2 M EDC and 50 mM NHS (BioRAD). Reactive disulphide groups were introduced by a 300 second injection of

80 mM PDEA (BioRAD) in 0.1 M sodium borate pH 8.5. Unreacted esters were then blocked with a 300 second injection of 1 M ethanolamine. Five different DnaA ligands harbouring a cysteine at the end of the C-terminal histidine tag (mutation H485C) were diluted into binding buffer (25 mM sodium acetate pH 4.5, 500 mM NaCl and 0.005% P20) and injected over five channels until ~1000 RU had been immobilized. BSA was also diluted in the same buffer and immobilized to ~1000 RU in the remaining channel to act as a reference. The remaining reactive disulphide groups were deactivated with a 300 second injection of 50 mM cysteine and 1 M NaCl in 0.1 M sodium acetate pH 4. The system was then primed with oligomer formation buffer (25 mM HEPES pH 7.6, 200 mM NaCl, 100 mM potassium glutamate and 10 mM MgCl) supplemented with 5 mM ATP and 0.005% P20. Soj was exchanged (PD-10 column, GE Healthcare) into interaction buffer and supplemented with 5 mM nucleotide. The Soj analytes were then systemically injected over the DnaA surface for a contact time of 300 seconds. The surface was regenerated between each injection with a 8 second injection of regeneration buffer (50 mM NaOH, 200 mM NaCl, 1 mM EDTA and 0.005% P20). The response reported is the response observed from the channel harbouring the BSA subtracted from the channel harbouring the DnaA ligand.

Screen for DnaA mutants that suppress growth inhibition of Soj^{G12V}. Error-prone PCR was performed using Phire DNA polymerase (Finnzymes) on chromosomal DNA from strain HM387 with primers oHM56 and oQPCR33. Twenty 50 µL reactions were independently run to increase the diversity of mutations. Reactions were denatured at 98℃ for 30 seconds, followed by 20 cycles of 98℃ for 30 seconds, 60℃ for 30 seconds, and 72℃ for 8 minutes. The reactions were purified using a PCR Purification Kit (Qiagen) and pooled. Mutagenized DNA was transformed into HM240 and plated onto nutrient agar plates at 37℃ in the presence of chloramphenicol and 1% xylose (to induce expression of *soj*^{G12V}).

Large colonies were selected (200 from a starting pool of 20,000 clones) and streaked onto nutrient agar plates at 37℃ in the presence or absence of 1% xylose. Chromosomal DNA from 14 candidates that grew similarly in the presence and absence of xylose was isolated and backcrossed into HM240 to confirm that the suppressor mutation was linked to the chloramphenicol marker. The *oriC* region of these suppressors was amplified using primers oHM254 and oHM255, followed by sequencing to identify the mutations. The four amino acid substitutions in DnaA that specifically suppressed Soj^{G12V} ($DnaA^{L294R}$, $DnaA^{V323D}$, $DnaA^{L337P}$, $DnaA^{A341V}$) were introduced into the *dnaA* gene on pHM304 using quick-change mutagenesis, subcloned back into the pHM304 vector (*Bgl*II-*Sal*I), and integrated into HM240 by double cross-over to demonstrate that these mutations caused suppression of Soj^{G12V}.

Purification of in vivo protein-protein complexes. DnaA-His_{12} protein complexes were purified from *B. subtilis* as described (Ishikawa et al., 2006, 2007) with the following modifications. Strains were grown at 30˚C in 40ml of LB medium until the A_{600} reached 0.4–0.5. After crosslinking cells were washed with phosphate-buffered saline prior to brief storage in liquid nitrogen. Cell pellets were resuspended in buffer UT and disrupted by sonication (20 min at level 6 using a Sonics Vibracell). The eluate was passed through a Microcon-10 filter (Millipore) to concentrate the sample. Crosslinks were dissociated by heating at 90˚C for 60 min and half of the sample was used for SDS-PAGE (4%–12% NuPAGE Novex Bis-Tris Gel; Invitrogen) followed by western blot analysis using α-Soj polyclonal antibodies.

Soj-His_6 purification. Soj-his_6 was purified as previously described (Scholefield et al., 2011).

DnaA-His$_6$ purification. (note that DnaA^{L337P} was omitted from all the in vitro assays as it was found to be inactive) BL21 (DES) pLysS (B F- dcm ompT hsdS gal λ(DE3); Stratagene) harbouring the required DnaA expression plasmid (see Table S1) was inoculated into LB containing ampicillin at 75 mg/ml and chloramphenicol at 10 mg/ml and incubated overnight at 37°c. The culture was diluted 1:100 into LB containing 75 mg/ml ampicillin and incubated at 37°c until the A_{600} ~0.6. Cultures were then induced with 1 mM IPTG and moved to 30°c for 3 hours. The cells were collected by centrifugation at 5000 g and 4°c for 10 minutes. Cells were resuspended in DnaA lysis buffer (25 mM HEPES pH 7.6, 500 mM potassium glutamate and 1 mM DTT) such that they were concentrated 25-fold. One complete EDTA-free protease inhibitor (Roche) tablet and 32 mg/ml lysozyme (Biochemika) were added. Cells were incubated on ice for 1 hour with gentle agitation. Cells were lysed by sonication at 4°c (5 min at level 8). Cell debris was removed by centrifugation at 31000 g and 4°c for 45 minutes and the supernatant transferred to a clean tube. Proteins were precipitated by the addition of 0.34 g/ml ammonium sulphate. Precipitated proteins were collected by centrifugation at 27000 g and 4°C for 30 minutes. Proteins were resuspended in DnaA resuspension buffer (25 mM HEPES pH 7.6, 200 mM potassium glutamate, 10 mM magnesium acetate, 30 mM imidazole and 20% sucrose) such that they were concentrated 4-fold. Remaining ammonium sulphate was removed by overnight dialysis against DnaA resuspension buffer. The protein solution was applied to a 1 ml HisTrap FF column (GE Healthcare), washed with 20 ml DnaA resuspension buffer and then eluted with 5 ml DnaA elution buffer (DnaA binding buffer supplemented with 500 mM imidazole). The eluate was diluted to 50 ml with Mono Q binding buffer (30 mM TRIS pH 7.6, 100 mM potassium glutamate, 10 mM magnesium acetate, 1 mM DTT and 20% sucrose) and applied to a 1ml HiTrap Q HP column (GE Healthcare), washed with 20 ml Mono Q binding buffer and eluted with a linear gradient, 0 – 1000 mM of potassium glutamate (Mono Q binding buffer supplemented with 900 mM potassium glutamate).

Fractions were then analyzed by SDS-PAGE, pooled and dialyzed against DnaA final dialysis buffer (40 mM HEPES pH 7.6, 150 mM potassium glutamate, 1 mM DTT, 20% sucrose and 20% polyethylene glycol 12000 MW) overnight using a 3 kDa cut off dialysis membrane. All the mutant DnaA proteins were purified in an identical manner with the exception of $DnaA^{CC,\ I190A}$. This protein was found not to bind to a Mono Q column; therefore it was purified using a Mono SP column. In addition, the Tris in the Mono Q binding buffer was replaced with HEPES at the some concentration and pH.

$DnaA^{nat}$ purification. *E.coli* strain AQ3519 (argH, deo, dnaA850::Tn10, his-29, metB, metD88, pro, rnh::cat, thyA, trpA9605; Krause, *et al.* (1997)) harbouring pBsdna1 was grown as above except the media was 2x LB supplemented with 10 mg/ml thiamine and 20 mg/ml thymine. Native DnaA lacking a histidine tag was purified as above except the protein was loaded directly onto a Mono Q column after removal of the ammonium sulphate by dialysis.

In vitro formaldehyde cross-linking assay. DnaA was diluted into oligomer formation buffer supplemented with ATP (2 mM), imidazole (40 mM), Soj^{G12V} (12 uM) and pBsoriC4 (3 nM) and incubated at 37°C for 15 minutes. Complexes were cross-linked with 0.5% formaldehyde for 2 minutes before the reaction was quench by the addition of Tris (pH 8; 100 mM final). Complexes were bound to 5 ul of nickel beads (Promega) via the histidine tag on Soj and incubated for 15 minutes at room temperature with gentle agitation. Complexes were then washed 7 times with 1 ml of oligomer formation buffer supplemented with ATP (2 mM) and imidazole (40 mM) and resuspended in 100 ul of the same buffer. Crosslinks were then reversed by incubating samples at 90°C for 2 hours. Tubes were then cooled to room temperature, beads removed, and DNA purified using a PCR purification kit (QIAgen). DNA was then quantified by qPCR using oligonucleotides

oHM03/oHM04. A standard curve was generated using a 10-fold serial dilution of pBsoriC4.

In vivo helix formation assay. We found that the two cysteines in a wild-type DnaA background were quite unstable (cysteines in the $DnaA^{Hyp}$ background were stable in the presence of Soj^{G12V} overexpression). Therefore, fresh transformants were immediately subjected to in vivo cross-linking with a sample of the overnight cultures being frozen at -80°C. It should be noted that strains producing $DnaA^{Hyp}$ (sGJS036, sGJS037 and sGJS038) require 1% xylose to grow effectively. Therefore, for these strains 1% xylose was always present in the media used for overnight growth and dilution and at an A_{600} ~0.1 xylose was removed by washing the cells twice in an equal volume of cross-linking media.

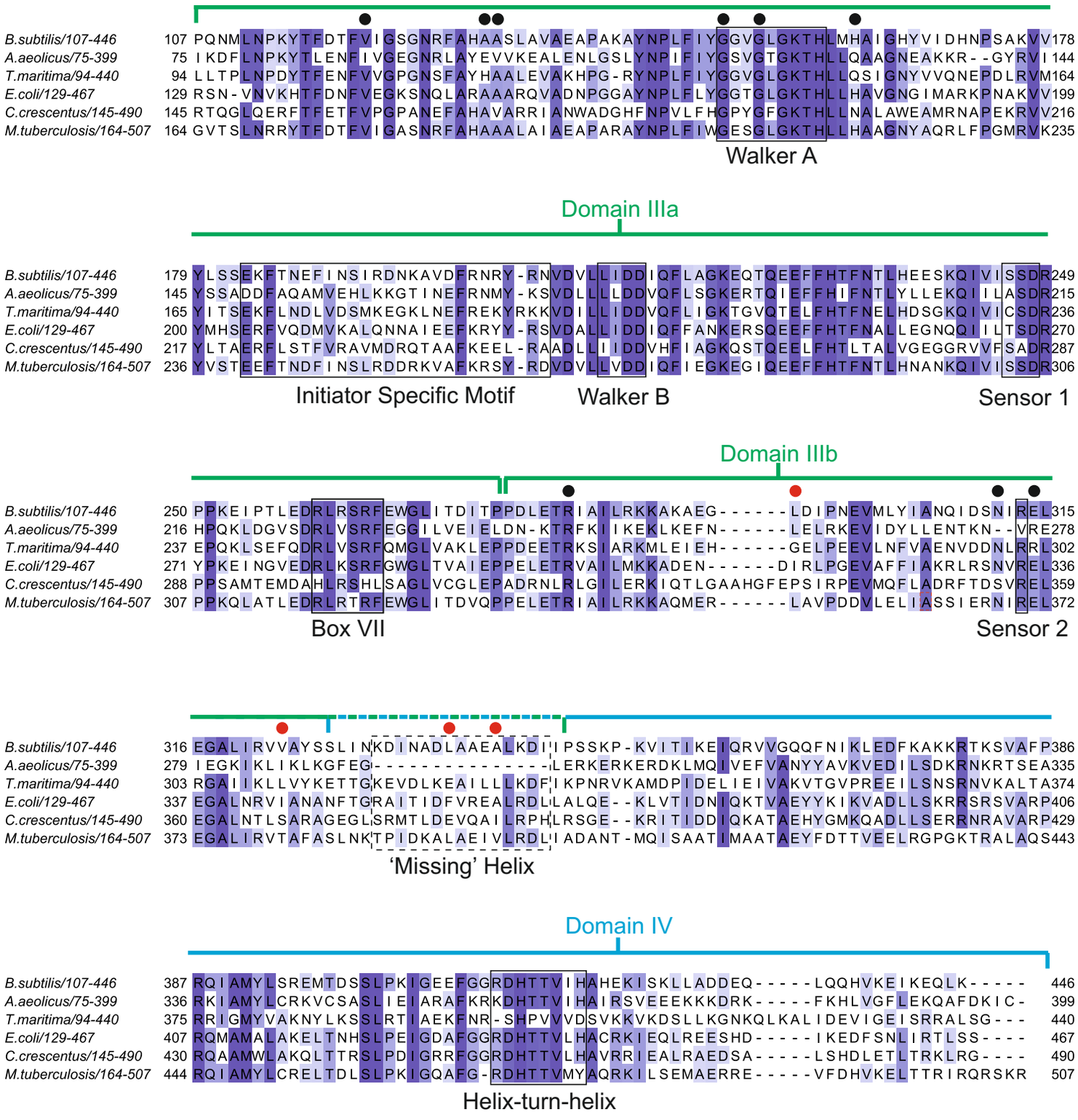
Electrophoretic mobility shift assay (EMSA). DnaA was serially diluted into oligomer formation buffer and incubated at 25°C for 3 minutes. Plasmid DNA (120 fmol) was then added and the reaction was left to proceed for 15 minutes at 37°C. Nucleoprotein complexes were separated on a 1% agarose gel (both the running buffer and the gel contained 0.5X Tris borate and 5 mM $MgCl_2$). To visualize the DNA the gel was post-stained in running buffer containing ethidium bromide (1 ug/ml) for 30 minutes and then de-stained for 10 minutes in water before being imaged on a Typhoon 8600 fluoroimager (GE Healthcare).

Malachite Green ATPase assay. The malachite green assay solution was created by mixing 0.0812% (w/v) malachite green, 2.23% (w/v) polyvinyl alcohol, 5.72% (w/v) ammonium molybdate in HCl (6 M) and H2O in a ratio of 2:1:1:2. The solution was incubated for 2 h with gentle agitation (during this time the solution turned from a muddy brown colour to a golden yellow). Reactions with and without Soj (36 μM) were diluted into

oligomer formation buffer supplemented with ATP (5 mM), pBSoriC4 (3 nM). DnaA (3 μM) was then added and the reaction incubated for a total of 6 hours. 10 μl samples were taken every 2 hours (including time zero) and mixed with 160 μl of malachite green assay solution, followed by the addition of 20 μl sodium citrate (34%). The colour was allowed to stabilize for 10 min before the absorbance was detected at 620 nm. A standard curve was created using a serial dilution of sodium phosphate (3–500 mM). The A_{620} was converted into mole Pi produced per mole of protein using a phosphate standard.

ATP binding assay. Samples were prepared and treated in an identical manner to the oligomer formation assay up until the addition of BMOE. At this point 5 μl of magnetic nickel beads (Promega) were added and incubated at room temperature for 2 minutes. Proteins were then collected using a magnet and washed twice with 1 ml of helix formation buffer supplemented with imidazole (10 mM). After the final wash the beads were resuspended in 10 μl methanol to denature the proteins and release the bound nucleotide. Denatured proteins collected using a magnet and 1 μl of the supernatant was then spotted onto PEI cellulose paper (Merck) and subjected to thin layer chromatography (TLC) using a solvent composed of 5% formic acid and 0.5 M LiCl. TLC plates were exposed onto phospoimager sceens and nucleotide was detected using a Typhoon imager.

Sup Figure 1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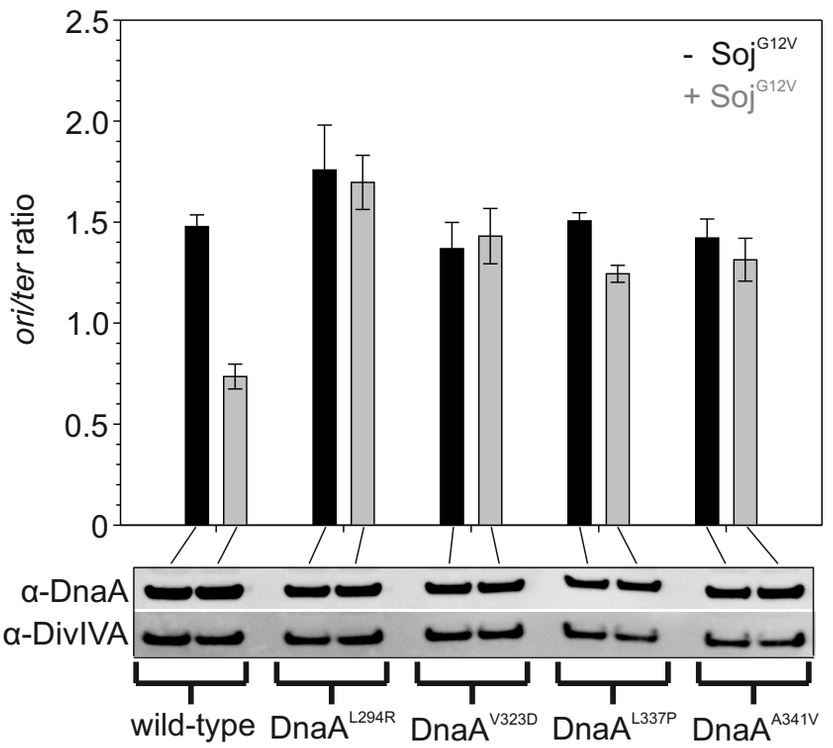
DnaA hypermorphic substitutions
DnaA supressor substitutions

Sup Figure 2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Sup Figure 3

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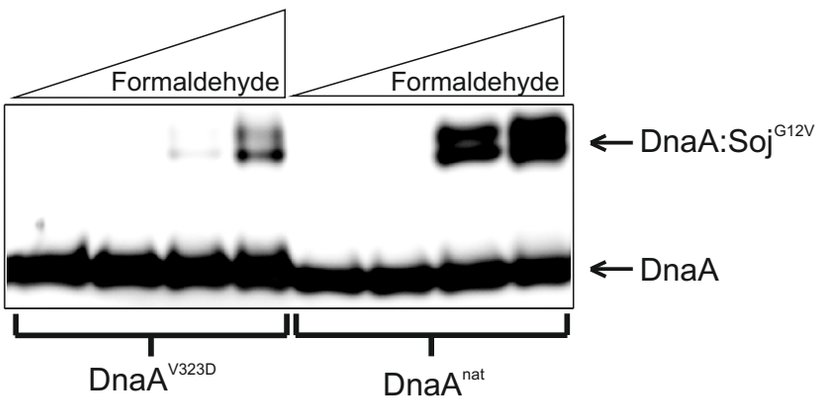

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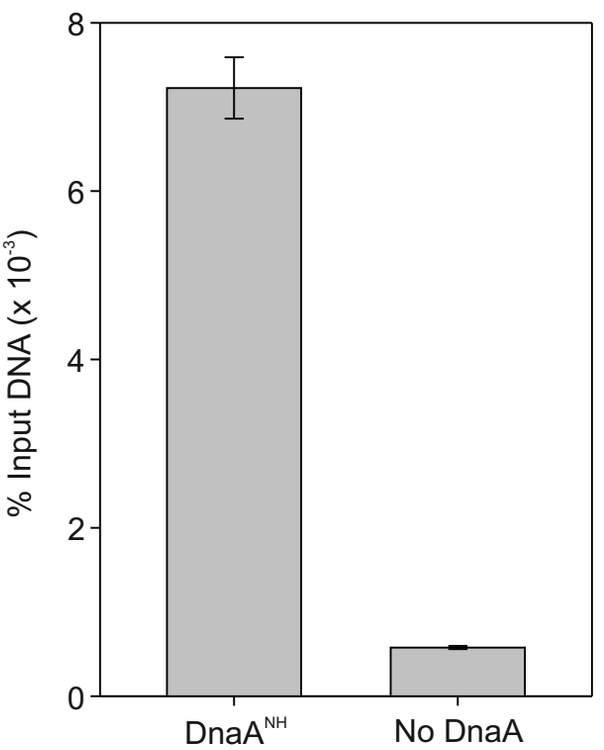

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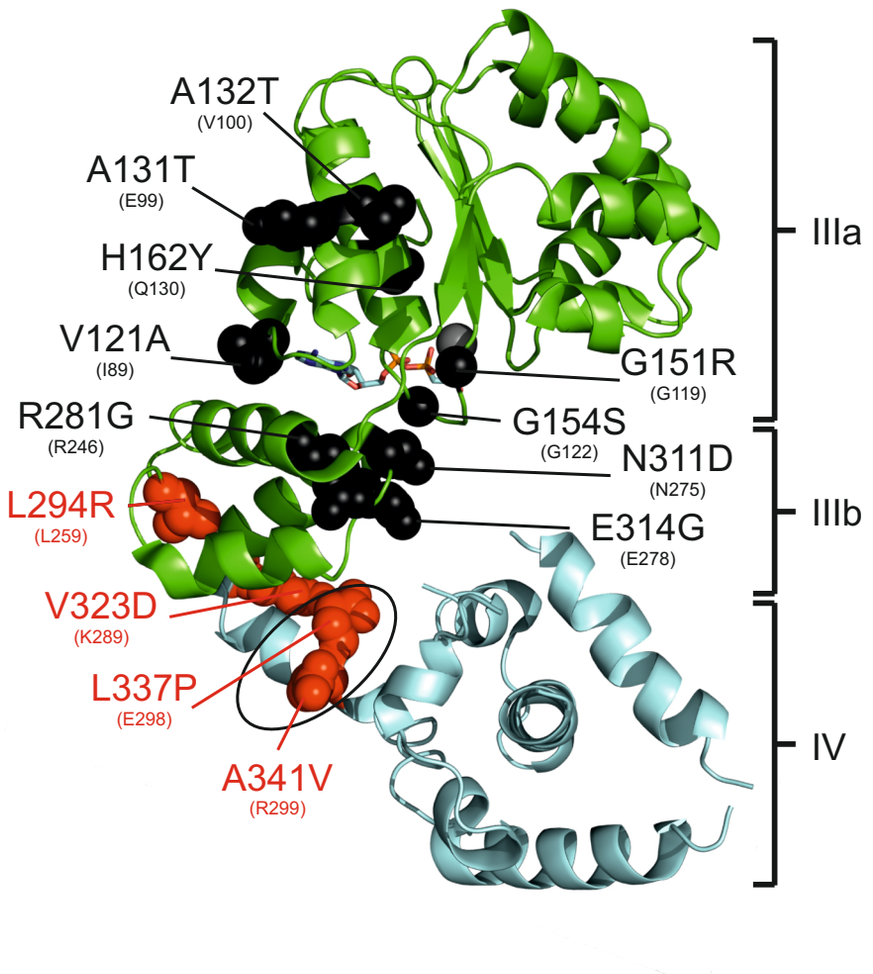
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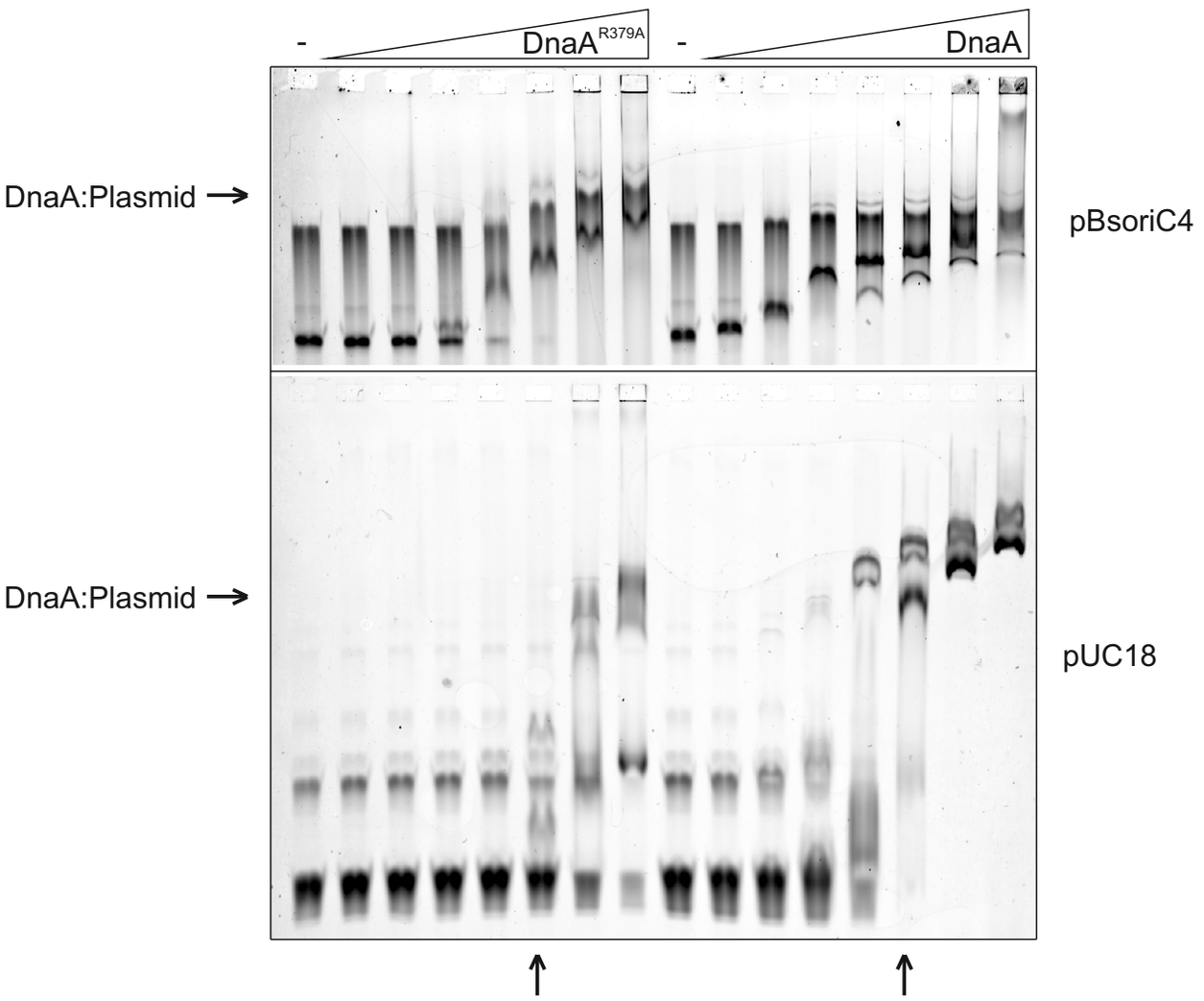
IV

Sup Figure 5



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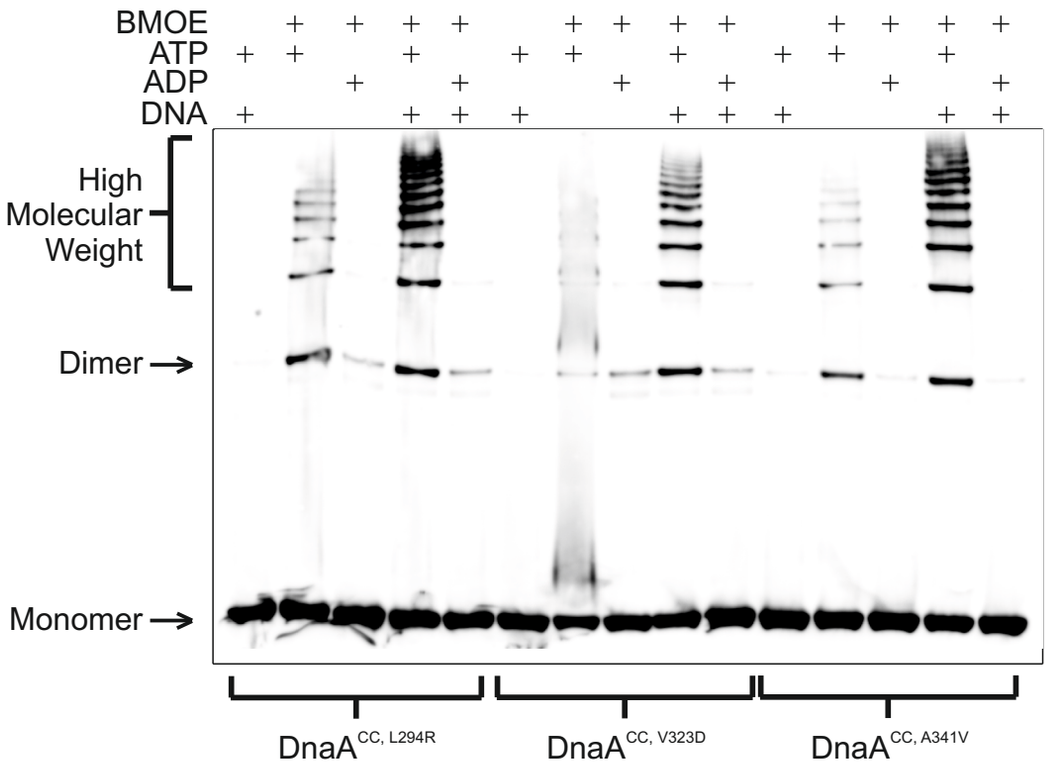

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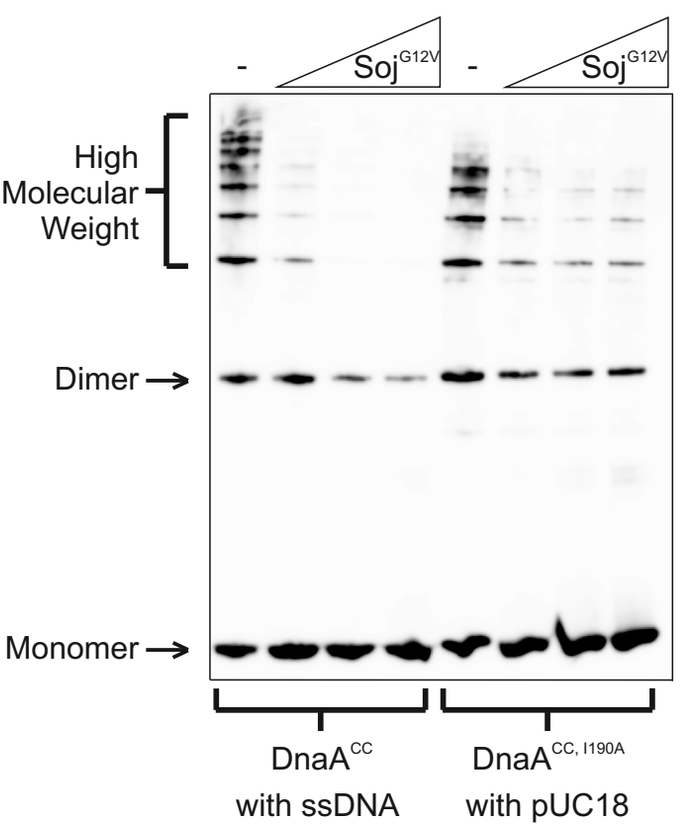

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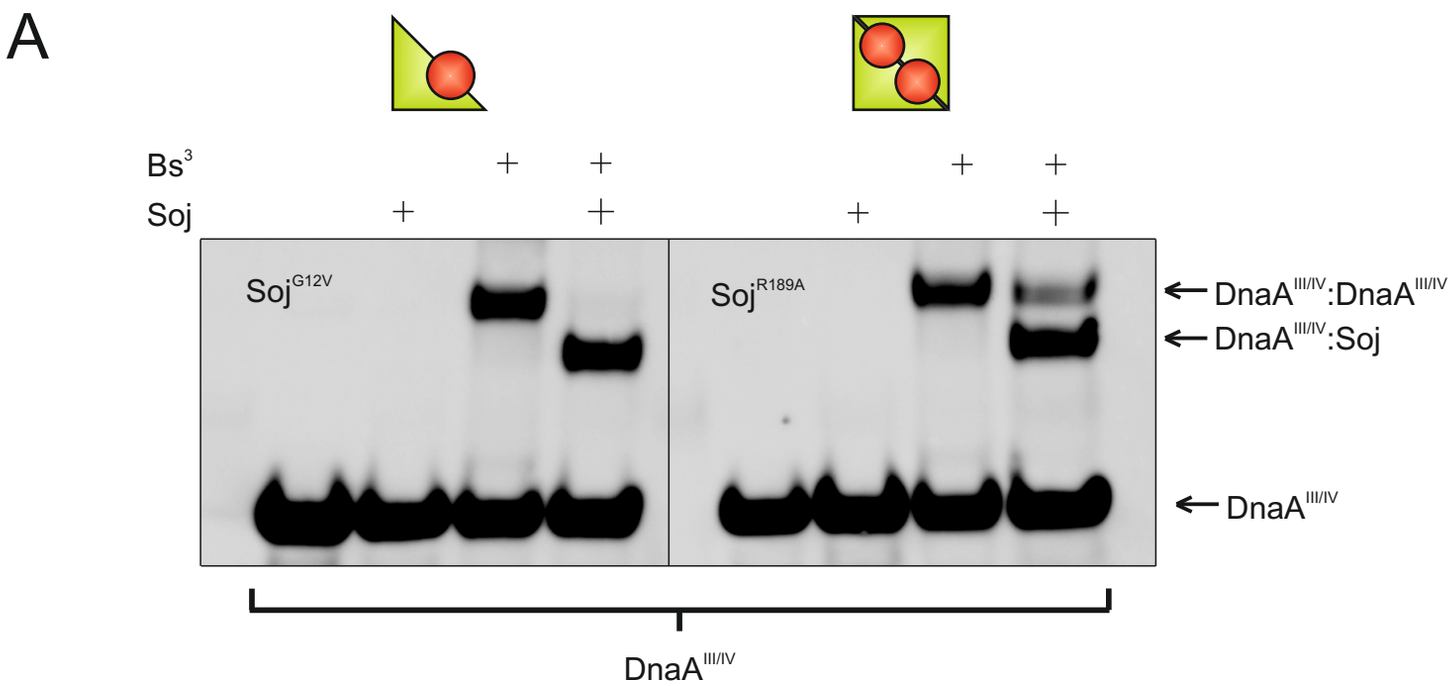
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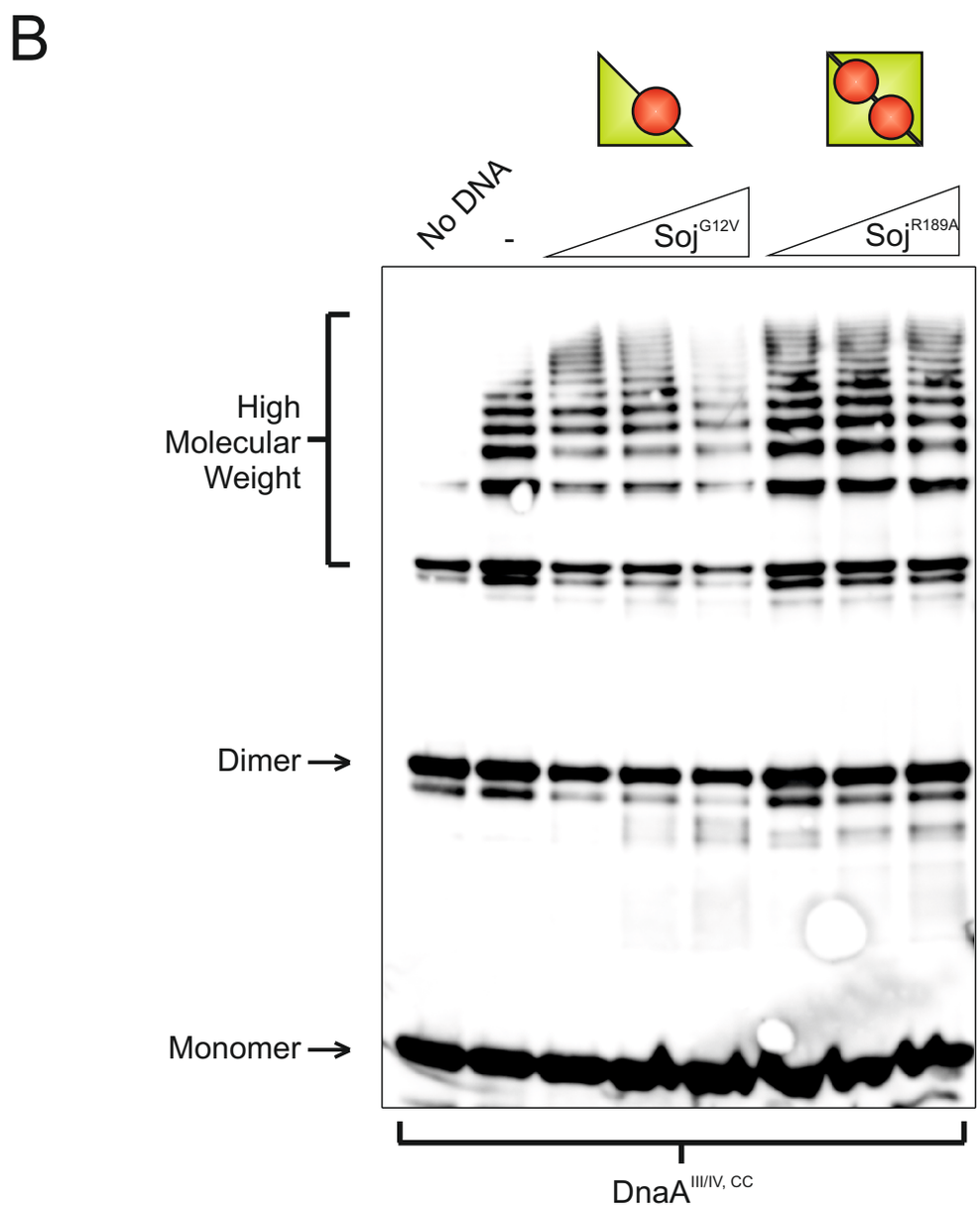
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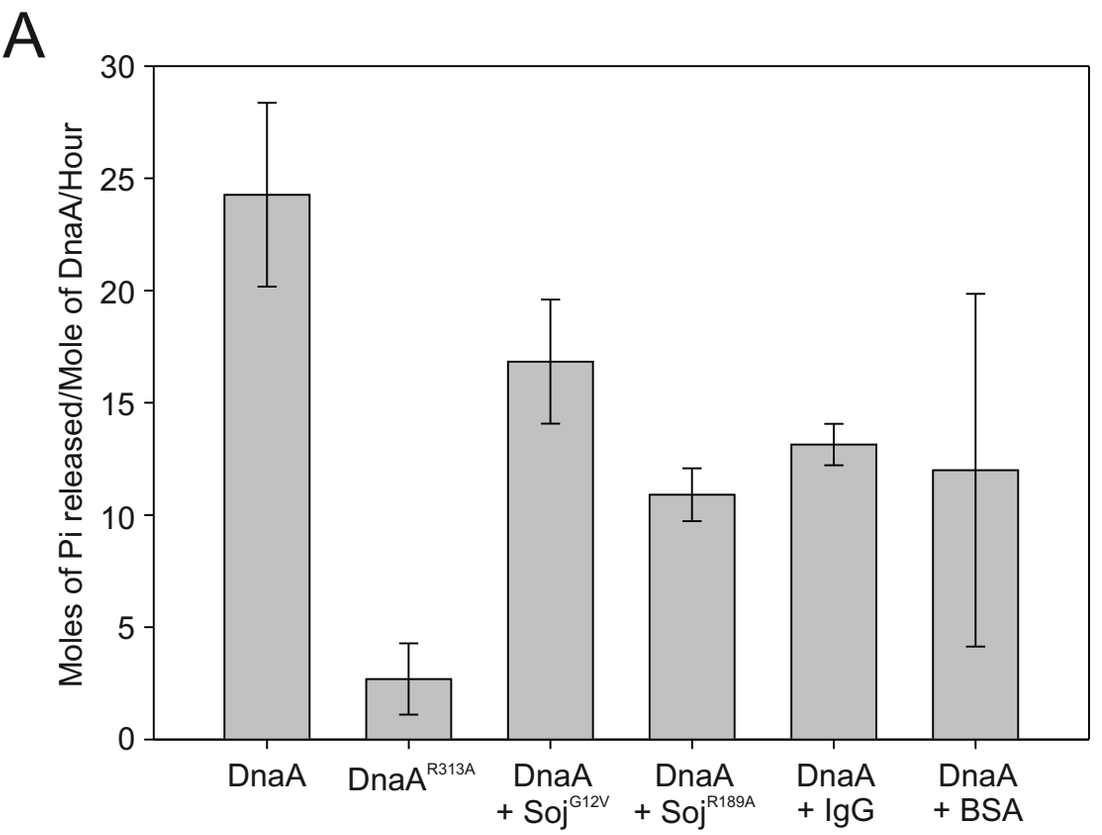
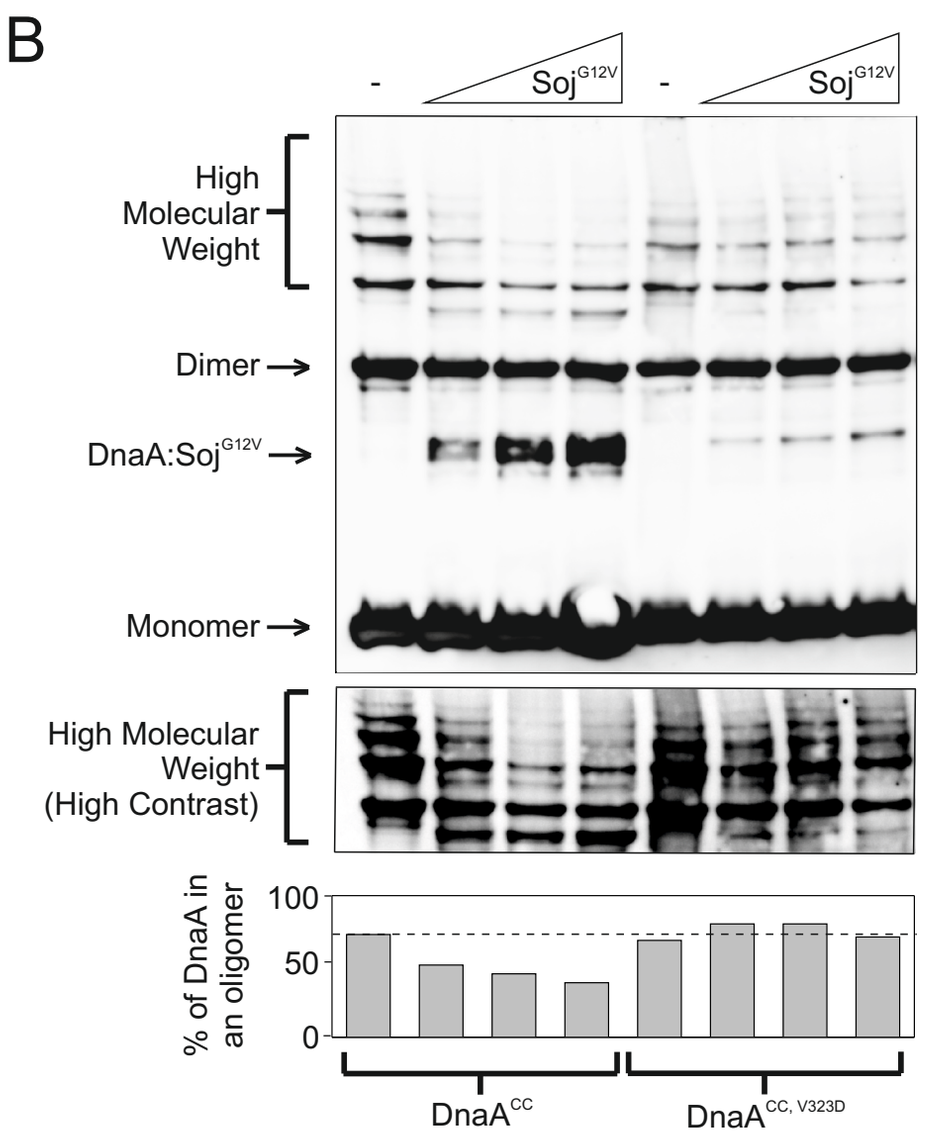
Dimer
Monomer
DnaAIII/IV, CC

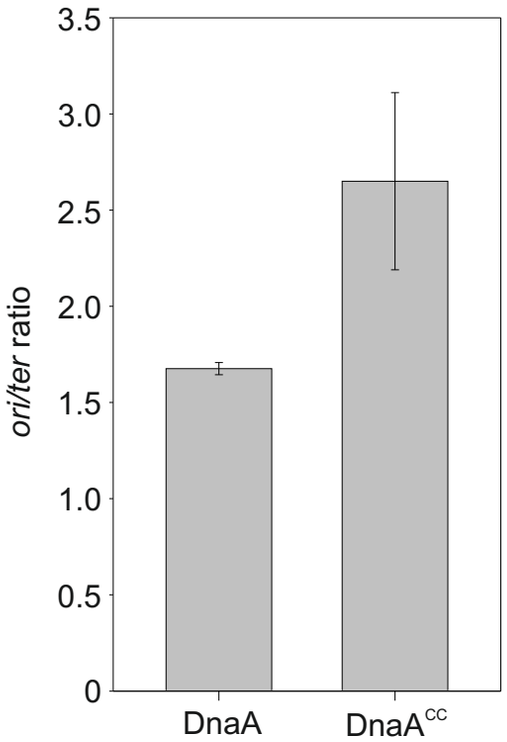
Sup Figure 8


A
Moles of Pi released/Mole of DnaA/Hour
30
25
20
15
10
5
0
DnaA
DnaA^R313A
DnaA + Soj^G12V
DnaA + Soj^R189A
DnaA + IgG
DnaA + BSA

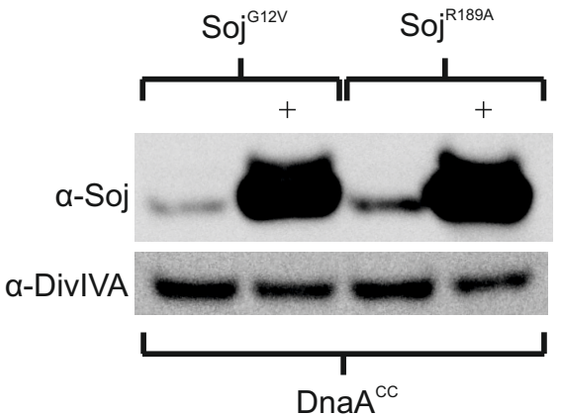

B
-
Soj^G12V
-
Soj^G12V
High Molecular Weight
Dimer
DnaA:Soj^G12V
Monomer
High Molecular Weight (High Contrast)
% of DnaA in an oligomer
100
50
0
DnaA^CC
DnaA^CC, V323D

Sup Figure 9

A



B



Sup Table 1

Plasmid	Genotype	Method of construction or Reference
pBsoriC4	*B. subtilis oriC* (position -211 to 2314)	Krause, M *et al.* (1997)
pBsdnaA1	$P_{A1-03/04}$-*dnaA amp*^{r}	Krause, M *et al.* (1997)
pMS103	see reference	Su'etsugu and Errington (2011)
pET21-d	P_{T7}-*mcs-his*$_6$ *amp*^{r}	Novagen
pGJS001	P_{T7}-*soj-his*$_6$ *amp*^{r}	Scholefield, G *et al.* (2011)
pGJS003	P_{T7}-*soj*^{K16A}-*his*$_6$ *amp*^{r}	Scholefield, G *et al.* (2011)
pGJS004	P_{T7}-*soj*^{G12V}-*his*$_6$ *amp*^{r}	Scholefield, G *et al.* (2011)
pGJS005	P_{T7}-*soj*^{R189A}-*his*$_6$ *amp*^{r}	Scholefield, G *et al.* (2011)
pGJS012	P_{T7}-*dnaA*^{V323D}-*his*$_6$ *amp*^{r}	Quickchange of pHM239 using oGJS067/068
pGJS016	P_{T7}-*dnaA*^{V323D,H485C}-*his*$_6$ *amp*^{r}	Quickchange of pGJS012 using oGJS083/084
pGJS028	P_{T7}-*dnaA*^{H485C}-*his*$_6$ *amp*^{r}	Quickchange of pHM239 using oGJS083/084
pGJS029	P_{T7}-*dnaA*^{R313A}-*his*$_6$ *amp*^{r}	Quickchange of pHM239 using oGJS035/036
pGJS031	P_{T7}-*dnaA*^{L294R,H485C}-*his*$_6$ *amp*^{r}	Quickchange of pGJS028 using oGJS097/098
pGJS033	P_{T7}-*dnaA*^{A341V,H485C}-*his*$_6$ *amp*^{r}	Quickchange of pGJS028 using oGJS101/102
pGJS035	P_{T7}-*dnaA*$^{III/IV}$-*his*$_6$ *amp*^{r}	PCR (oGJS070/071) from 168ed --> pET21-d (NcoI/XhoI)
pGJS042	P_{T7}-*dnaA*^{A198C}-*his*$_6$ *amp*^{r}	Quickchange of pHM239 using oGJS144/145
pGJS043	P_{T7}-*dnaA*^{N191C,A198C}-*his*$_6$ *amp*^{r}	Quickchange of pGJS042 using oGJS153/154
pGJS045	P_{T7}-*dnaA*^{N191C,A198C,R264A}-*his*$_6$ *amp*^{r}	Quickchange of pGJS043 using oGJS081/082
pGJS046	P_{T7}-*dnaA*^{N191C,A198C,V323D}-*his*$_6$ *amp*^{r}	Quickchange of pGJS043 using oGJS067/068
pGJS047	P_{T7}-*dnaA*^{N191C,A198C,A341V}-*his*$_6$ *amp*^{r}	Quickchange of pGJS043 using oGJS101/102
pGJS048	P_{T7}-*dnaA*^{N191C,A198C,L294R}-*his*$_6$ *amp*^{r}	Quickchange of pGJS043 using oGJS184/185
pGJS049	P_{T7}-*dnaA*^{L294R}-*his*$_6$ *amp*^{r}	Quickchange of pHM239 using oGJS097/098
pGJS050	P_{T7}-*dnaA*^{A341V}-*his*$_6$ *amp*^{r}	Quickchange of pHM239 using oGJS101/102
pGJS058	P_{T7}-*dnaA*^{N191C,A198C,R313A}-*his*$_6$ *amp*^{r}	Quickchange of pGJS043 using oGJS097/098
pGJS061	P_{T7}-*dnaA*^{N191C,A198C,I190A}-*his*$_6$ *amp*^{r}	Quickchange of pGJS042 using oGJS234/235
pGJS063	P_{T7}-*dnaA*^{N191C,A198C,R379A}-*his*$_6$ *amp*^{r}	Quickchange of pGJS042 using oGJS238/239
pGJS064	P_{T7}-*dnaA*$^{III/IV,\ A198C\ N191C}$-*his*$_6$ *amp*^{r}	Quickchange of pGJS035 using oGJS144/145 then oGJS153/154
pGJS101	*dnaA*^{N191C,A198C}*::cat amp*^{r}	Quickchange of pGJS100 using oGJS153/154
pGJS104	*dnaA*^{N191C,A198C,V323D}*::cat amp*^{r}	Quickchange of pGJS101 using oGJS067/068
pGJS106	*dnaA*^{A198C}*::cat amp*^{r}	Quickchange of pHM327 using oGJS144/145
pGJS107	*dnaA*^{N191C}*::cat amp*^{r}	Quickchange of pHM327 using oGJS142/143
pGJS113	*dnaA*^{N191C,A198C,A132T}*::cat amp*^{r}	Quickchange of pGJS101 using oGJS178/179
pGJS114	*dnaA*^{N191C,A198C,G154S}*::cat amp*^{r}	Quickchange of pGJS101 using oGJS180/181
pGJS115	*dnaA*^{N191C,A198C,R281G}*::cat amp*^{r}	Quickchange of pGJS101 using oGJS182/183
pGJS120	*dnaA*$^{A198C\ N191C\ R264A}$*::cat amp*^{r}	Quickchange of pGJS101 using oGJS081/082
pHM239	P_{T7}-*dnaA-his*$_6$ *amp*^{r}	PCR (oHM224/225) from 168ed --> pET21-d (NcoI/NotI)
pHM327	*dnaA::cat amp*^{r}	PCR (oQPCR40/oHM272) from 168ed --> pMS103 (AatII/SalI)

Sup Table 2

Strain	Genotype	Reference
168ed	*trpC2*	Kobayashi *et al.* (2003)
HM527	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{L294R} ::cat*	This work
HM528	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{V323D} ::cat*	This work
HM529	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{L337P} ::cat*	This work
HM530	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{A341V} ::cat*	This work
HM555	*trpC2 dnaA ^{V323D} -his $_{12}$ ::cat*	This work
HM557	*trpC2 dnaA ::cat*	This work
HM657	*trpC2 dnaA-his $_{12}$ ::cat*	This work
HM658	trpC2 *dnaA ^{L337P} -his $_{12}$*::cat	This work
HM705	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{G151R} ::cat*	This work
HM706	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{G154S} ::cat*	This work
HM707	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{H162Y} ::cat*	This work
HM708	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{R281G} ::cat*	This work
HM709	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{N311T} ::cat*	This work
HM710	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{A132T} ::cat*	This work
HM711	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{E314G} ::cat*	This work
HM712	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{N311D} ::cat*	This work
HM713	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{V121A} ::cat*	This work
HM714	*trpC2 Δsoj ::neo amyE ::spc(P $_{xyl}$ -soj ^{G12V}) dnaA ^{A131T} ::cat*	This work
HM716	*trpC2 dnaA ^{L294R} -his $_{12}$ ::cat*	This work
HM725	*trpC2 dnaA ^{A341V} -his $_{12}$ ::cat*	This work
sGJS006	*trpC2 dnaA $^{A198C\ N191C}$ ::cat Δsoj::neo amyE::spc(P $_{xyl}$ -soj ^{G12V})*	This work
sGJS008	*trpC2 dnaA $^{A198C\ N191C\ V323D}$ ::cat Δsoj::neo amyE::spc(P $_{xyl}$ -soj ^{G12V})*	This work
sGJS021	*trpC2 dnaA ^{N198C} ::cat Δsoj::neo amyE::spc(P $_{xyl}$ -soj ^{G12V})*	This work
sGJS022	*trpC2 dnaA ^{A191C} ::cat Δsoj::neo amyE::spc(P $_{xyl}$ -soj ^{G12V})*	This work
sGJS033	*trpC2 dnaA $^{A198C\ N191C}$ ::cat Δsoj::neo amyE::spc(P $_{xyl}$ -soj ^{R189A})*	This work
sGJS036	*trpC2 dnaA $^{A198C\ N191C\ A132T}$ ::cat Δsoj::neo amyE::spc(P $_{xyl}$ -soj ^{G12V})*	This work
sGJS037	*trpC2 dnaA $^{A198C\ N191C\ G154S}$ ::cat Δsoj::neo amyE::spc(P $_{xyl}$ -soj ^{G12V})*	This work
sGJS038	*trpC2 dnaA $^{A198C\ N191C\ R281G}$ ::cat Δsoj::neo amyE::spc(P $_{xyl}$ -soj ^{G12V})*	This work
sGJS050	*trpC2 spoIIIJ(359°)::kan tet(oriN) amyE::spc(P $_{xyl}$ -soj ^{G12V})*	This work
sGJS051	*trpC2 dnaA $^{A198C\ N191C}$ ::cat spoIIIJ(359°)::kan tet(oriN) amyE::spc(P $_{xyl}$ -soj ^{G12V})*	This work
sGJS052	*trpC2 dnaA $^{A198C\ N191C\ R264A}$ ::cat spoIIIJ(359°)::kan tet(oriN) amyE::spc(P $_{xyl}$ -soj ^{G12V})*	This work

Sup Table 3

Oligonucleotide	Sequence (5' -> 3')
oGJS035	CGCGAATCAAATCGACAGCAATATTGCGGAACTCGAAGGA
oGJS036	TCCTTCGAGTTCCGCAATATTGCTGTCGATTTGATTCGCG
oGJS067	CGAAGGAGCATTAATCAGAGTTGACGCTTATTCATCTTTAATTAATA
oGJS068	TATTAATTAAAGATGAATAAGCGTCAACTCTGATTAATGCTCCTTCG
oGJS081	CTTGAAGACAGATTGCGCTCAGCTTTTGAATGGGGACTTATTAC
oGJS082	GTAATAAGTCCCCATTCAAAAGCTGAGCGCAATCTGTCTTCAAG
oGJS083	CCACCACCACCACCACTGCTGACCGGCTGCTAACAAAGCC
oGJS084	GGCTTTGTTAGCAGCCGGTCAGCAGTGGTGGTGGTGGTGG
oGJS097	CCAAAGCAGAGGGCCGCGATATTCCGAACGA
oGJS098	TCGTTCGGAATATCGCGGCCCTCTGCTTTGG
oGJS101	TGATCTGGCCGCTGAGGTGTTGAAAGATATTATTCC
oGJS102	GGAATAATATCTTTCAACACCTCAGCGGCCAGATCA
oGJS142	TGAGAAATTTACAAACGAATTCATCTGCTCTATCCGAGATAATAAAGCCGTC
oGJS143	GACGGCTTTATTATCTCGGATAGAGCAGATGAATTCGTTTGTAAATTTCTCA
oGJS153	TGAGAAATTTACAAACGAATTCATCTGCTCTATCCGAGATAATAAATGCGTC
oGJS154	GACGCATTTATTATCTCGGATAGAGCAGATGAATTCGTTTGTAAATTTCTCA
oGJS159	CACAAGCCGAACATAATAAACAGAGCAAAATGAAAAAATAGTTAAGCATGTTTTGGGAATAGAT
oGJS178	CCGATTTGCACATGCTACTTCCCTCGCAGTAGC
oGJS179	GCTACTGCGAGGGAAGTAGCATGTGCAAATCGG
oGJS180	TTATCTATGGGGGCGTCAGCTTAGGGAAAACACAC
oGJS181	GTGTGTTTTCCCTAAGCTGACGCCCCCATAGATAA
oGJS182	CACACCGCCTGATCTAGAAACGGGAATTGCAATTTTAAGAAAAAAG
oGJS183	CTTTTTTCTTAAAATTGCAATTCCCGTTTCTAGATCAGGCGGTGTG
oGJS184	CGCGAATCAAATCGACAGCAATATTGCGGAACTCGAAGGA
oGJS185	TCCTTCGAGTTCCGCAATATTGCTGTCGATTTGATTCGCG
oGJS234	TTCTGAGAAATTTACAAACGAATTCGCCTGCTCTATCCGAGATAATAAATGC
oGJS235	GCATTTATTATCTCGGATAGAGCAGGCGAATTCGTTTGTAAATTTCTCAGAA
oGJS238	ATATTAAACTCGAGGATTTCAAAGCAAAAAAAGCGACAAAGTCAGTAGCTT
oGJS239	AAGCTACTGACTTTGTCGCTTTTTTTGCTTTGAAATCCTCGAGTTTAATAT
oHM056	CCAAAGCACTCTTACGGCGTGTTAGTTC
oHM189	ATGTGCTTTTGATAGATGATATTC
oHM224	TTTAAATCCATGGAAAATATATTAGACCTGTGG
oHM225	AAATTTGGTTGCGGCCGCCTAGTGATGGTGATGGTGATGCGATCCTC
oHM254	GCTGCGTTTACGGTTATTCG
oHM255	CGTGGTTCTGGATGAAACTG
oHM272	GGGGGGACGTCTAAGAAAATATATTAGACCTGTGGAAC
oQPCR33	GATCCGATTTCGCATCACAG
oQPCR40	TTCCTGGGTTTGTTCTTTCC
oQPCR06	GATTTCTGGCGAATTGGAAG