

SUPPLEMENTAL FIGURE LEGENDS

FIGURE S1. Clustal W alignment of BLM orthologs was performed using default parameters (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>). Note that highlighting identifies similar residues. Sequence names include the aa residues used in the alignment: *Hs*, human; *Dr*, *Danio rerio*; *Ol*, *Oryzias latipes*; *Xl*, *Xenopus laevis*; *Gg*, *Gallus gallus*; *Dm*, *Drosophila melanogaster*; *Kl*, *Kluvermyces lactis*; *Sc*, *Saccharomyces cerevisiae*. (A) RecQ domains. (B) N-terminal domains. The BDHCT domain is underlined.

FIGURE S2. The indicated GST fusion proteins were subjected to EMSA but the reaction products were incubated for 30 min on ice with or without α -GST prior to gel electrophoresis.

FIGURE S3. (A) SDS-PAGE analysis of purified recombinant proteins. Proteins were expressed and purified from bacteria with either an N-terminal GST-tag (GST) or a C-terminal V5-His6 tag (V5). Approximately 1.5 μ g of each recombinant protein was resolved by 15% SDS-PAGE and the gel was stained with Coomassie blue. Molecular weight standards are shown in kDa. (B) V5-His6-tagged N-terminal domains of BLM orthologs bind d(T)174. The indicated V5-His6-tagged proteins were assayed by EMSA as described in Fig.1D.

FIGURE S4. The SE domain lacks meltase and nuclease activity. (A) A SE reaction (1) was carried out using 1 nM of a 32 P-labeled donor DNA with flush ends plus 5 nM of recipient oligo (lanes 1-9, 14). In reaction (2) the substrate consisted of 1 nM 32 P-labeled oligo 6 alone (lanes 10-13). Both reactions were performed as described in the standard SE assay except that oligo 11 (25 nM final concentration) was added with the stop buffer in lanes 7-14 and incubated at 37°C for 30 min (3). (B) One nM of 32 P-labeled oligo 6 or 1 nM of 32 P-labeled dsDNA (oligo 6 plus 14) was incubated with the indicated amount of protein at 37°C for 30 min and analyzed as described in SE reaction.

FIGURE S5. (A) A SE reaction was carried out in the presence of Sgs1₁₀₃₋₃₂₂ and the indicated concentrations of Mg²⁺. (B) A SE reaction containing two potential recipient ssDNAs was carried out in the presence or absence of Mg²⁺. (C) Sgs1₁₀₃₋₃₂₂ was titrated into a SE reaction employing 10 nM flush-ended duplex as donor and 50 nM ssDNA as recipient. The products of the indicated reactions were quantified and its efficiency is presented as the percent of total signal present in the slower migrating band. Shown below these values is the ratio of molecules of ssDNA (in nucleotides) to molecules of protein used in the reaction.

Table S1. Oligonucleotides used in this study.

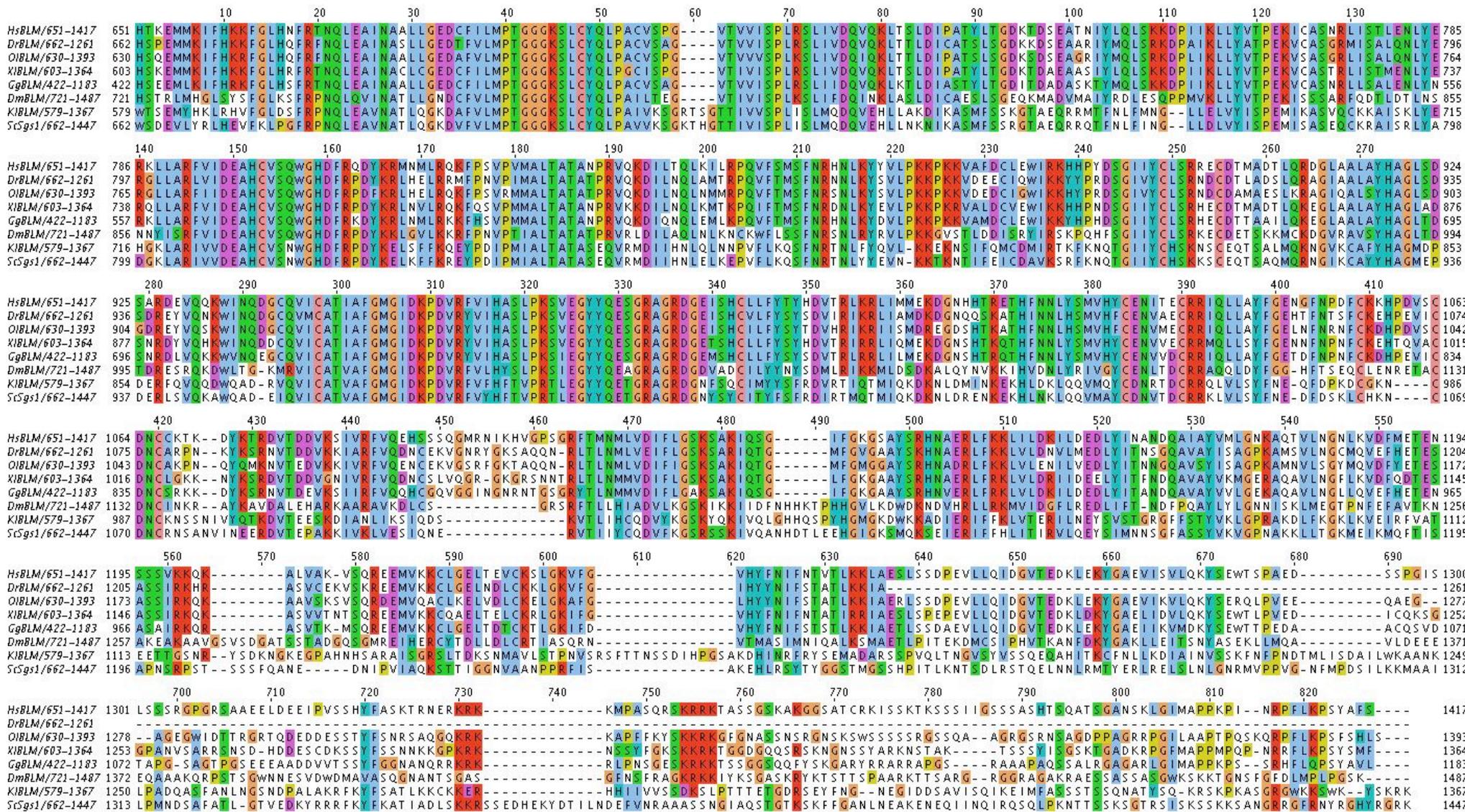


Figure S1a

HsBlm/1-651 1 --MAAVPQNNLQEQLERHSARTLNNKLSLSPKPKFGFTFKKNTSSDNNVSVTVNSVAKTAVLNRNKDVTEDFS-----FSEPLPNTINQQRVKDFFFKNAFAGQETQRGGSKSLDFLQTLQT 114
DrBlm/1-662 1 --MSSLPQNNLKEHLERHNNAAQN-KLSLLKPKPGGFCFKKSSGISKVEVPQKVTGSSVLANRSVNI PSNFVETKNPVTFSQKPERVAP-KVN-FFTAPSK-PKNTVNPANPLFALNKT 116
OIBLM/1-630 1 --MSSLPQNNLEQLARHNNANQS-KLSLAKPKTGAFLLKKSSTSGTINVEYPAKVTASSVLANRNVNPKNSLLTKSSVTFSNKLERQKSKINSFFTVNSK-CQSDSVIPACR-----NP 113
XIBLM/1-603 1 --MAALPQNNLQKQLLELPAKGTSNKLSLQKTKSSVFTFKKCSFNVS-ASTGFIFPQQHVLKDKNVNPKQD-----GTHTALPKATERNKINCFPTVYVYV-KSGQPPQVVAL-----K 105
GgBlm/1-659 1MEEARAATGGSGESQKLSNGEKSSQLLEPGDVGNELLADILEEDDYLDVVPPSPEEELPSFSSVRNVSNIFK----ESPDTGRSAVHGTESPEELMAPKQPAEQDSSAEHADKG----L 114
DmBlm/1-704 1--MSKKPVAQRKQLTLSFIGLDGSQKPKSRAASVRSKPPAVYNIIFLDASSDDETTETISSQSNGTIATKSSRDPRTAKLKKHTYLDLSSVPLAELLSAKKYARDSPPKPTSLDLSVLS 120
KIBLM/1-621 1--MVKPSHNLRRREHKWLKDT EAYLPDKSYLGLLQKHVTAKSSATLNSGVENDLARRTGSTLDQVPRNLTSSMPVSS-GAVTTAQTAVAGTWDEGSYNTTITAVATVLDSDGDSLELILADR 118
ScSgs1/1-674 1--MVTKPSHNLRRREHKWLKETATLQEDKDFVFOAIQKHIANRPRKTNSPPTTSPKDECGPGTNTNFITSLIPASGPTNTATKQHEVMQTLSDNTEWLSYATASNQYADVPMVDIPASTSVVSNP 120

130 140 150 160 170 180 190 200 210 220 230 240
HsBlm/1-651 115 PKEVVCTTQ-----NTPTVKKSRDTALKKLEFSSPDLSLSTINDWDDMDDFDTSETS-----S FVTPPQSHFVRVSTAQK--SKKGRNFFKAQLYTNTVKTDLPPSSSES EQDITLIEQK 225
DrBlm/1-662 117 PLVQSTIKA-----LPKSAALPKDSTNDQTVLKHDIQLSDFNEWDDLDDFETPVKSR--VAPVAGTSTKKPSVSD-----QNTSSSCSSKCEETKVNEESQATETITAPKDVLSAANG 224
OIBLM/1-630 114 PAVSGNNGA-----ITQTKT--DNHSINATNAPSFDPSQFPLDDWDDDFDFTPVKTK--NDSFSGKVTKPSDEEFPFTKQSSDTSHTPELIIEDDQPCMEIKEPEHIVSNNST 224
XIBLM/1-603 106 DHVHGNDISA-----NKPPST--EDAASKTGINTSFGSVTSLLEEWDDLDDFDTSWSP--KSHAGKGGKTPQKCKNT-----SPVASFKIQSISPEGPTTEKHDCAKLLYDNNEVAS EPR 211
GgBlm/1-659 115 HLEQKLYSM-----MEDICKLVDAIPLHELTSISCAKELLQQLRRKLLADSGALNT--NSVNGPRNWKACVQKQDSSRRPGTLLCSGPRGVS SVGSTPKSTNLPVLSVRTVNS SFS 227
DmBlm/1-704 121 PLAELPAKK-----SDRDPPPKPVQVENSYYRGLSESPVENKSGDTRLRKPPPKERK--TSIVWLSDSPEKKVTQNERKILDSLQRFSEDFPNKENGNRHLLTLDSPPPPPQPVKK 233
KIBLM/1-621 119 PSANNSNVT-----SSITAQITQKTEINTNGKTSDDQLHRLVLSLLWQQ--SELQEK--CEILESTLSLQDNKRSKIGSFINPTLMRIKQQISAMDNLLPSLPQARAVPDLOSFPVSR 229
ScSgs1/1-674 121 RTPNGSKT HNFNTFRPHMASSLVENDSSRNLSGRNNKSVINDNSIGKQLENDIKLEVIRLQGSILMALKEQSKLLQKCSIIESTSLSEDAKRLQLSRDIRQLSNMSIRIDSLKEIKKA 242

250 260 270 280 290 300 310 320 330 340 350 360
HsBlm/1-651 226 DDE---WLSGDVLCIDDGPIAEVHINEDAESDLSKTHLEDE-----RDNSEKKNLEEAELHSTEKVPCIEFDDDDYD-----DFVPPSPEEIISSASSSKCLSTLKDLDTSR 330
DrBlm/1-662 225 VS-----TETAEREPEDSPIKSKRPKKSVQHTALLSDTEDEEIIHCVSPDTNOKNFKSVAVAEKDKWGEFNVISDDCEENNHYEGFEDFIPPSPIPEEISLSVSDKEKSSSEVTPANK 340
OIBLM/1-630 225 VSPGGPILQEQVQFEVDSVVRGTRR--RPFHKSVLSDSED-----DTNVVEKIETKSDGKKWIDPKVIELQDNS E-----EVDFDICIPSPDETSTYSALQTRSKTGKTQSRF 332
XIBLM/1-603 212 KN-----LHAKTAESPQ-SLVCLASVEPTNLERDMCR-----NTDYLGTDLLEHQDETLSQVLI EEDDC E-----DFIPSPSDESLSPPVLLKVISQQRKHKVSSL 305
GgBlm/1-659 228 IR-----NQTLDKLDTSYSSKETDQEVICLEPAALPSPKVNKGK-----GSTLSRPSSEASFNQSWCEKPTGRDSSGNWRVPERPTASTALKAQHTAPAGNPASGCWVDVNDTDFDLDFDI 336
DmBlm/1-704 234 PEKT-MWQNEKTIQDKDSPANPLVSNLASISTLLDSSRAPN-----TYKGSRNLFESPEKSGSGEQGNLGSAKENIETKPTATASLERNVSTSSPSAAPLKPRTSVAFDNLADY 348
KIBLM/1-621 230 NAS-----ASISRRNSPTPHATESVNPISSEIDIDIPSE-----SQTLTQDQHNSSPKIQPSRRLRHRDPNQ-----YIPIPTLSQMDENPHLLQESLLSKRRTSTIN-- 323
ScSgs1/1-674 243 KKG---MSKDGKGRSQVSSQDDNIISILPSPLEYNTSSRN-----SNLSTTATTVTKALAITGAKQNTNNTGKNSNDSNNDLIIQVLDDEDIDICDPPVILKEGAPHSAPFPH 353

370 380 390 400 410 420 430 440 450 460 470 480
HsBlm/1-651 331 KEDVLTSTSKDLLSKPEKMSMQELNPESTDCDARQISLQQQLIHVMEH-ICKLIDTIPDD-KLKLDDCGNELLQQRNIRKLLTEVDFNKSDAS-----LGLSLWRYRPDSLDGPMED 442
DrBlm/1-662 341 KE-----SSRSASGLPALDQSAKG--LKGADDALFSVMES-ICCLVDTIPEH-ELIALTCGTELLLQRAHRKRILAKGGSRTSHSDSVSTPGFLNRPRTFGVTPSNLTSLTPTV 446
OIBLM/1-630 333 FS-----AQSASSAPTRPEFSLCLLS--KNSTREQLYSIMES-ICALVDSIPEH-ELISLSCGNELLLRRAHRKRILATGDFSRMQQPDST--VISDASFKETSS-CCVLTSS 435
XIBLM/1-603 306 TD-----VNDCENTTDHLOGQSVSTSLDSKVPSQLTLMLE-ICDLVKIIPIS-ELHVLSGLDLKKKRDMRKRLLSNDSVFRSSPADSSTVS--LTSCTSTQNRD FNVNAPKG 411
GgBlm/1-659 337 DDFDG--WEEAVAEAAPEAPPQWQPLREGSASLRCLLAAAGS-APGPHPTAPKSGCGISAKSS EPLVHNPAAHERFRGMKFSHEEMLKI FHRKFG LHSFRTNQLEA INAALLGED 455
DmBlm/1-704 349 LKDLAQ-----NDNFSIDPNKQNTETLTKSTLGFRRNTVELMEK-YCSLIDQIPAMHFNEIAGFQPNFLKLVVRQKFKKARTLVQNSLDKKSLEKAEQEALEKEEIQEQARQ 460
KIBLM/1-621 324-----NNIHHNDNDSASAEAESEDSHFLTMEE-DQIYDNELEHESDKDFVVDGNYLDYDEGPEADAEYHEEDDSNVPEKERSNQI I IDSSPGSSPRHNLNPPSQPP 425
ScSgs1/1-674 354 LHMTSE-----EQDELTRRRNMRSEPVNYRIPDRDDPFDYVMGKSLRDDYPDVEREDELTEMEADAHSSYMTTRDEEKEENELLNQSDDFVVDLDDPTQDQTDYHDNDV SANIQ 467

490 500 510 520 530 540 550 560 570 580 590 600
HsBlm/1-651 443 SCPTGNSMKELNFSHLPSNSVSPGDCLLTTTLG-----KTGFSATRKNLFRPLFN-----THLQKSFVSSNWA-----ETPRLGKKNSSYFPGNVLSTAVKDNKHTASINDL 543
DrBlm/1-662 447 SGRREGVKTFGRKSIASVMSVGDSEVDFDCIINGVETPGTWNPNSSSTKISARDTFNGSIQTLKSKPESKTKDCYSR--LSFNESNQDPLFYSPKRWDSGSRNDSVLEINIA GSS 565
OIBLM/1-630 436 SAVLDSRKSQQPRRP--SVISLDYDSHSDR-----TAFNPLSSKSSRAICMEDESIC--DSLSTPRLLKQPFCFS--ESTSRLEDDEDAHFFSPKPKVSEQNKSKTPTCTADNT 539
XIBLM/1-603 412 AESLSGSSSVKFKFNKLAVHDI GTKEENSAN-----SAPNFMKIKNKTSS--FRAGDLSIMENSFN--FHSVLSNSRFNTPQNEKPISSSTCTRPYSQPIDDM 510
GgBlm/1-659 456 CFILMPTGGGKSLCYQLPACVSAQTVVVISPLR-----SLIIDQVQKLLTLDIASTYLTGDTIDADASKTY--MQLSKDDPIKLLYVPEKVCASNRLLSALENLYNRKL 559
DmBlm/1-704 461 TVLSSSSPEKCRPIMLPLPKVQEI KDEKIPNRNQLIPDLGCEPDNFSPPSSPRDTQLIPKRQQLINDLCEGDDPFGPPSKQNDPHLLRCKEELVHDLCEEPDDYLAQSMMLDGLLEEQLNGP 582
KIBLM/1-621 426 LQQQTQNTSQEFQPTQLRLRLDDDDVFELEDPIS-----ESLPQDHTVAVHSEEFEP LSTDL EEFDAEREDQ-----TQFDEIKELDDDLKILSEAKLDAEDVLSFAIKKE 528
ScSgs1/1-674 468 ESSQEGDTRSTILSLQKNVQVILSSPTAQSVP-----SNGQNLGVEHIDLLEDDLEKDAI LDDSMSFSFGRQHMPMSHSDLELIDS EKENEDFEEDNNNGI EYLS DSD 573

620 630 640 650 660 670 680 690 700 710 720 730
HsBlm/1-651 544 ER-----ETQPSYDINDFIDDFDDDDWED-----IMHNLAASSSTAAYQPIKEGRPIKSVS ERLSSAKTDCLPVSSTAQNI TFS ES IQNYTDKSAQNLASRLNKHERRFGLSFPH 651
DrBlm/1-662 566 SLRT--GAEPVDDFLLDDFIDDFDEND-----IPDYEEPPSSVLESRNNS--GVKTPVQEGGSSKS-FERK-TFPPAPKSIKTPNPEPLRNPKAHRRFRGSLFPH 662
OIBLM/1-630 540-----ETDDFYDDFDIDDFDES-----IPEYEEAPTSSVPRQNS-----STAT-AVKEGSSKSSWDKPKSSSVSTPKPKKLSCEPTFRNPAHRRFRGFNPH 630
XIBLM/1-603 511 D-----NPDLDFIDNFDIEDLDDIH-----CLDSPAAPSVS SKNVFQ-----YPTIREAQLDSRNKEKNTRNNTGDTTNSLLSDLLKPIENPAHERFRGFNPH 603
GgBlm/1-659 560 LAR----FVIDEAEHCVSQWGHDFRKYKRLNM-----LRKKFHSVPMALTATAN-----PRVQKDIQNQLEMLKPVFTMSFNRRHLKYDVLKPKPKKVAMDCLWKY-HPH 659
DmBlm/1-704 583 TQGTTS GMDGDDLEGLLAEI EDEHQMQARRSEFNGYSYKEL EAVKVKKHKHPIINISLDDDGFP EYDEAMF EQMHSQAANKSRVSSAGPSTSKSVVPTKQTSALHSQKLSGNFAN 704
KIBLM/1-621 529 PK-----SLDTQLQTELVVPEVIDED-----DFSMADLEPYMEETEKT-----RENSVQPYAWTSEMYHKLRLVFG LDFRPNQLEAVNATLQKDAFVLMPTGG 621
ScSgs1/1-674 574 LER----FDEERENRTQVADIQLEDNDLKIITER-----KLTGDNEHPPPSWSPKIK-----REKSSVQKAEDEDDFDDFSLSDIVSKNSLSKNTGPTVPSDEVLRYLHEVF 674

Figure 1b

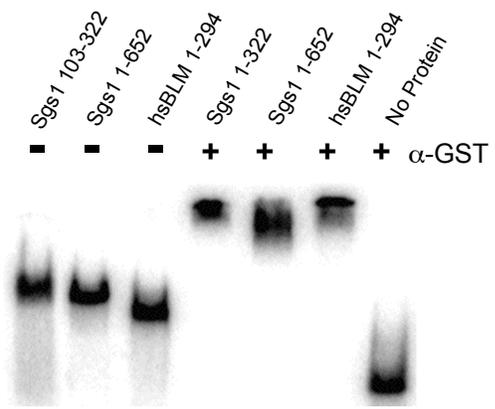


Figure S2

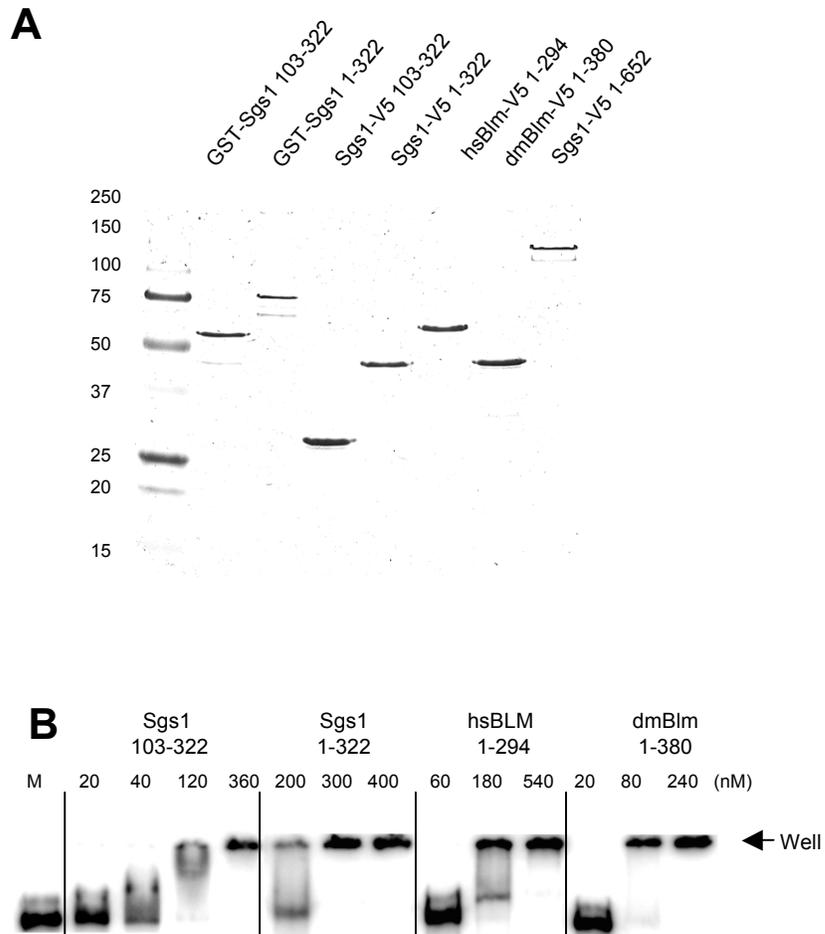


Figure S3

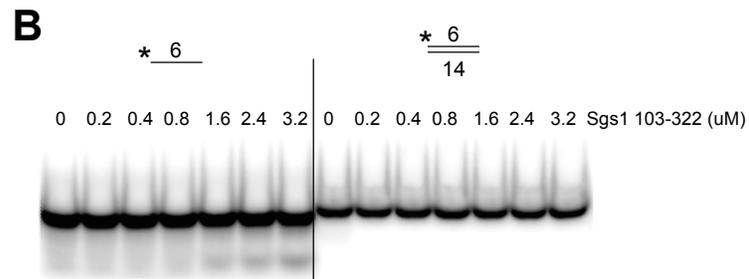
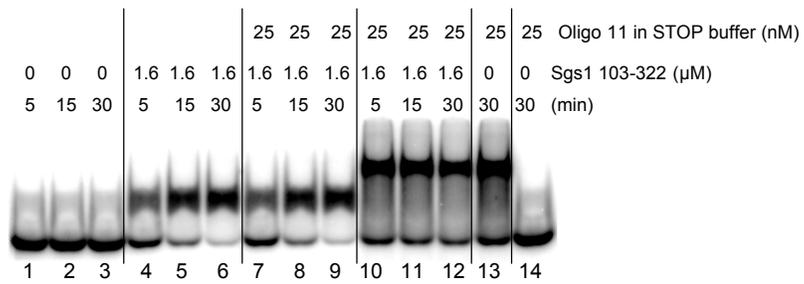
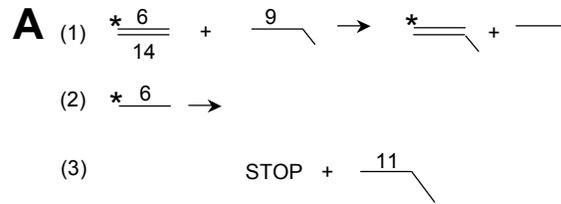


Figure S4

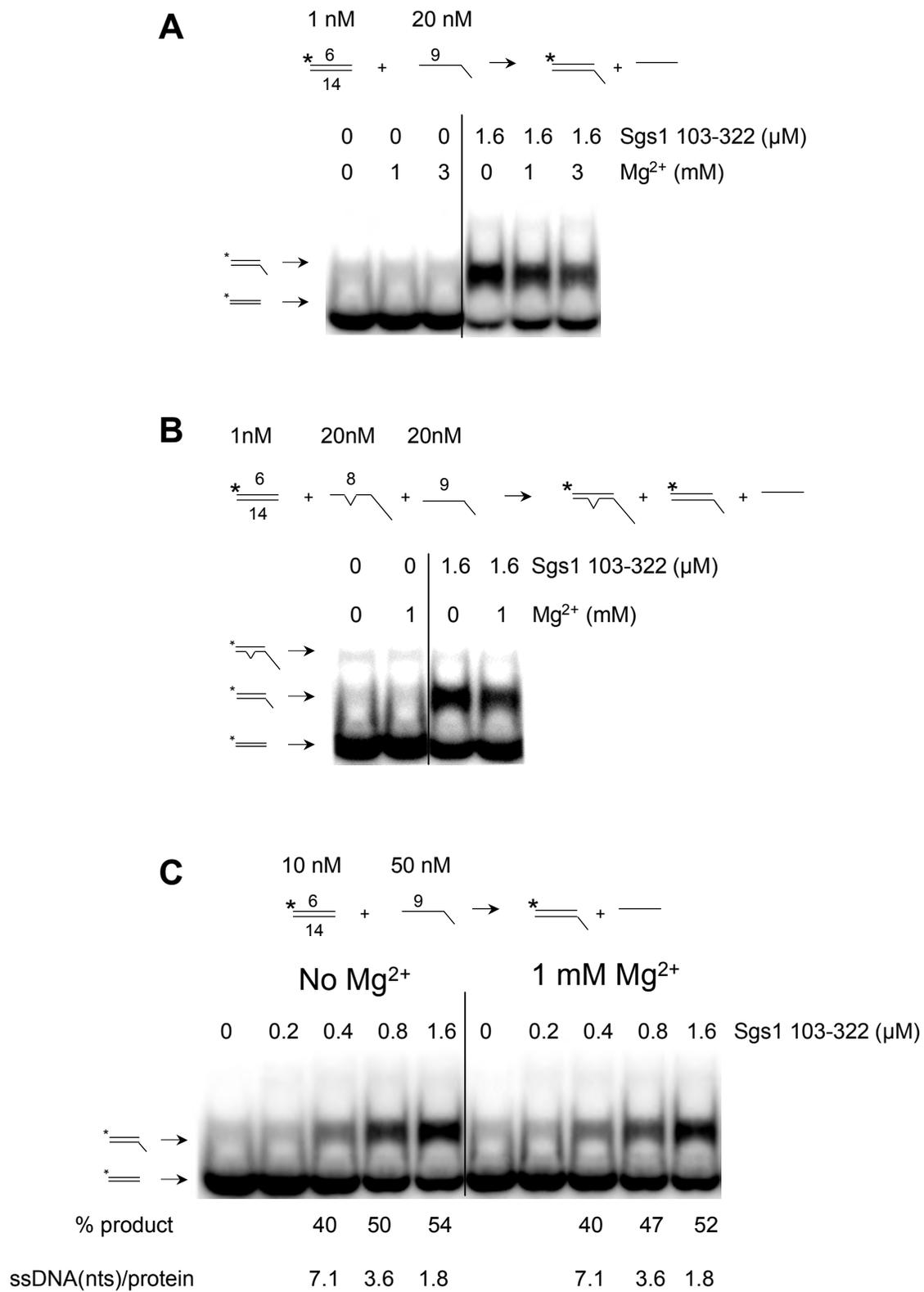


Figure S5

Supplementary Table S1

Oligonucleotides used in this study

Oligo Name	Original name	Size	DNA sequence (5'-3')
1	1253	50	TGGGTCAACGTGGGCAAAGATGTCCTAGCAATGTAATCGTCTATGACGTT
2	1254	50	TGCCGAATTCTACCAGTGCCAGTGATGGACATCTTTGCCACGTTGACCC
3	2558	25	TGGGTCAACGTGGGCAAAGATGTCC
4	2559	25	GGACATCTTTGCCACGTTGACCCA
5	2600	25	TCACTGGCACTGGTAGAATTCGGCA
6	2625	32	TCCTTTTGATAAGAGGTCATTTTTGCGGATGG
7	2640	32	TCCTTTTGATAAGAGCTCATTTTTGCGGATGG
8	2651	94	CTTTAGCTGCATATTTACAACATGTTGACCTACAGCACCAGATT CAGCAATTAAGCTCTAAGCCATCCGCAAAAATGAGCTCTTATCA AAAGGA
9	2655	57	CCAGATTCAGCAATTAAGCTCTATCCCATCCGCAAAAATGACCT CTTATCAAAGGA
10	2624	57	CCATCCGCAAAAATGACCTCTTATCAAAGGATGGACATCTTTG CCCACGTTGACCC
11	2626	94	CTTTAGCTGCATATTTACAACATGTTGACCTACAGCACCAGATT CAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCA AAAGGA
12	2653	34	TCTCCTTTTGATAAGAGGTCATTTTTGCGGATGG
13	2654	34	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCT
14	2639	32	CCATCCGCAAAAATGACCTCTTATCAAAGGA
15	2641	32	CCATCCGCAAAAATGAGCTCTTATCAAAGGA
16	2129 (Phi-X174)	50	AAAGGTCGCAAAGTAAGAGCTTCTCGAGCTGCGCAAGGATAGG TCGAATT
17	2044 (D-loop)	50	CGTTCTTCGGGGCGAAAACCTCAAGGATCTTACCGCTGTTGA GATCCAG
18	1317 (DNA binding)	90	GATCCGAATTCTGGCTTGCTAGGACATCTTTGCCACGTTGACC CGGGTTGGCGTTAGGAGATAGTCAGTTATAGCTGCGGCTGCTA AGG