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 µg 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 37ºC 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 Sgs1$_{103\text{-}322}$ and the indicated concentrations of Mg^{2+}. (**B**) A SE reaction containing two potential recipient ssDNAs was carried out in the presence or absence of Mg^{2+}. (**C**) Sgs1$_{103\text{-}322}$ 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.

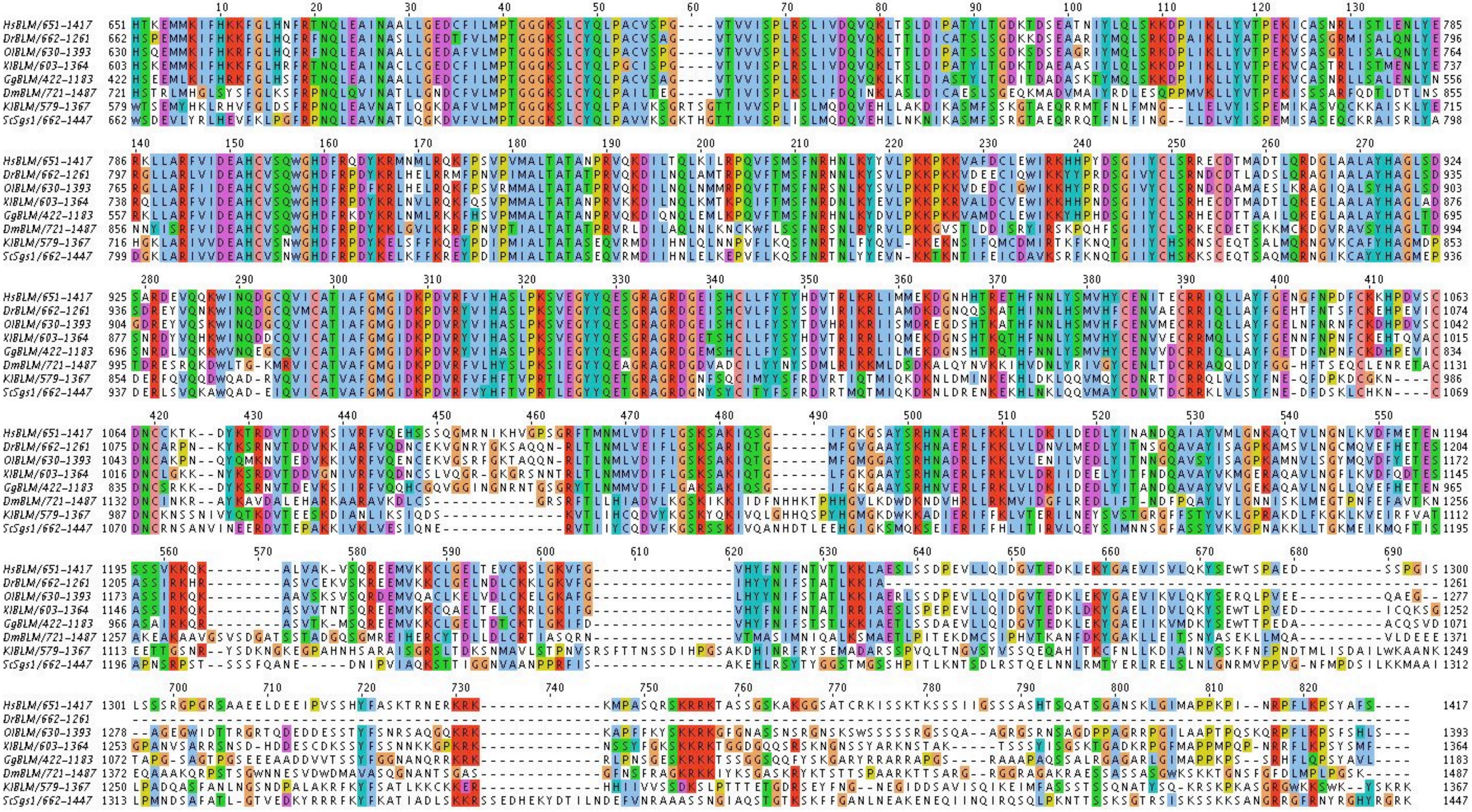
HsBLM/651-1417
DrBLM/662-1261
OlBLM/630-1393
XlBLM/603-1364
GgBLM/422-1183
DmBLM/721-1487
KlBLM/579-1367
ScSgs1/662-1447

Figure S1a

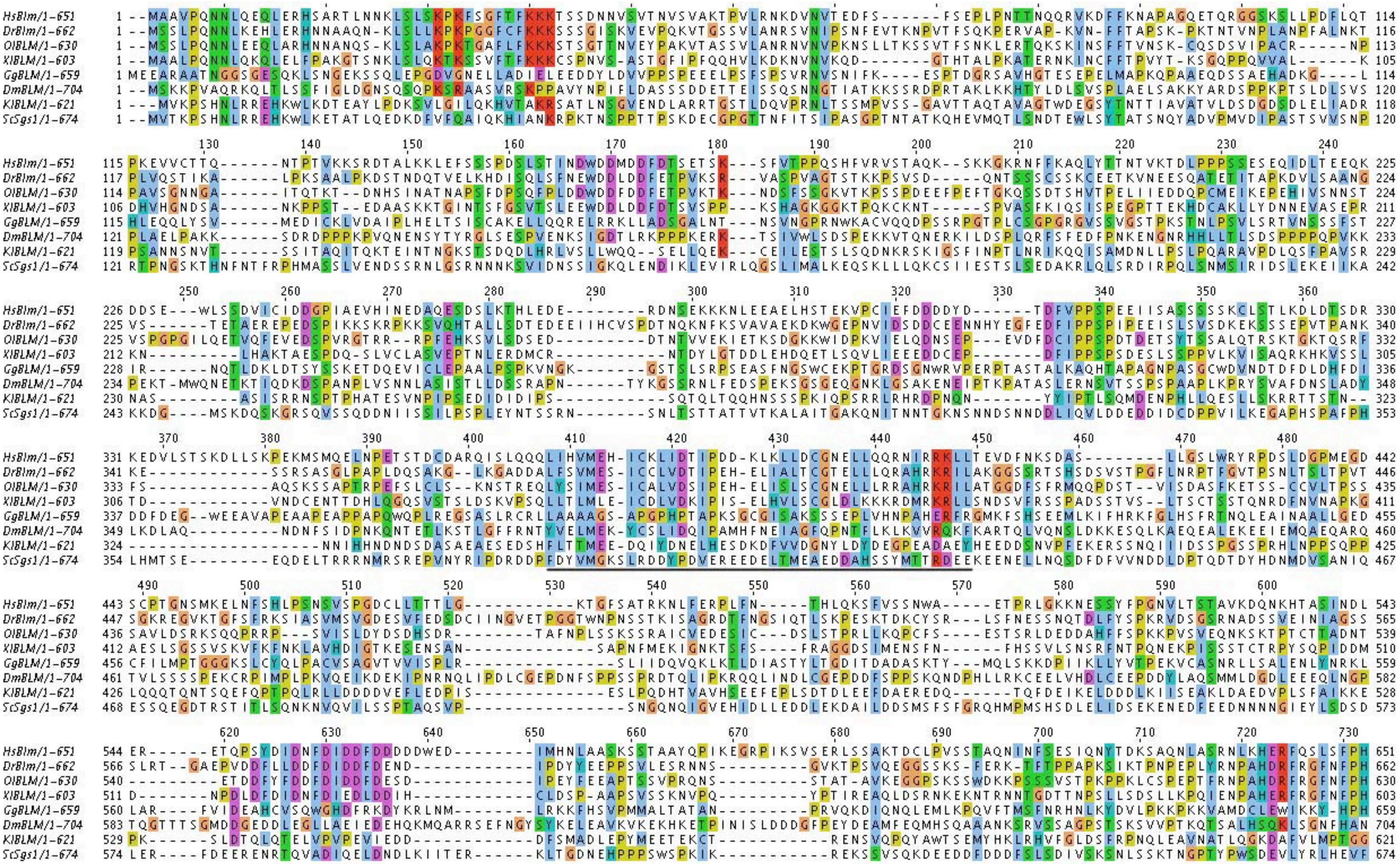
HsBlm/1-651
DrBlm/1-662
OlBLM/1-630
XlBLM/1-603
GgBLM/1-659
DmBLM/1-704
KlBLM/1-621
ScSgs1/1-674

Figure S1b

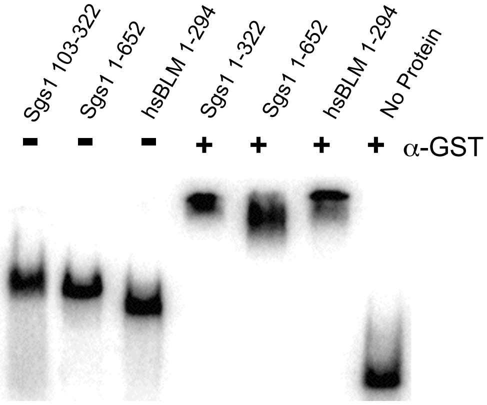
Sgs1 103-322
Sgs1 1-652
hsBLM 1-294
Sgs1 1-322
Sgs1 1-652
hsBLM 1-294
No Protein
-
-
-
+
+
+
+
α-GST

Figure S2

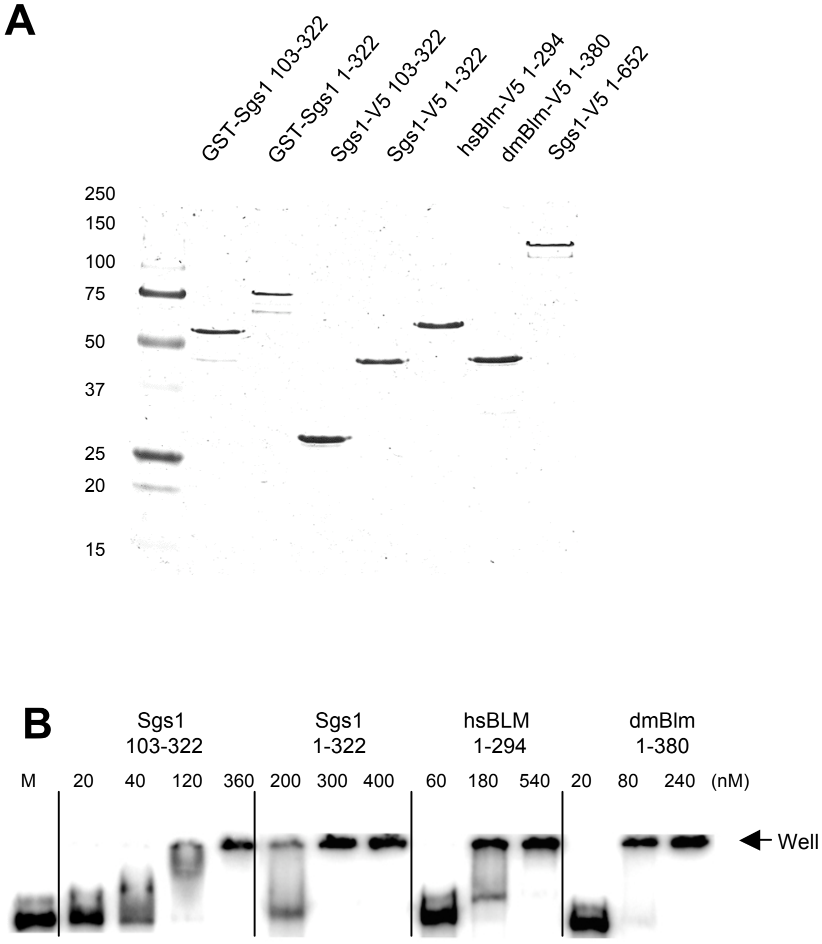
A
GST-Sgs1 103-322
GST-Sgs1 1-322
Sgs1-V5 103-322
Sgs1-V5 1-322
hsBlm-V5 1-294
dmBlm-V5 1-380
Sgs1-V5 1-652
250
150
100
75
50
37
25
20
15
B
Sgs1
103-322
Sgs1
1-322
hsBLM
1-294
dmBlm
1-380
M
20
40
120
360
200
300
400
60
180
540
20
80
240
(nM)
Well

Figure S3

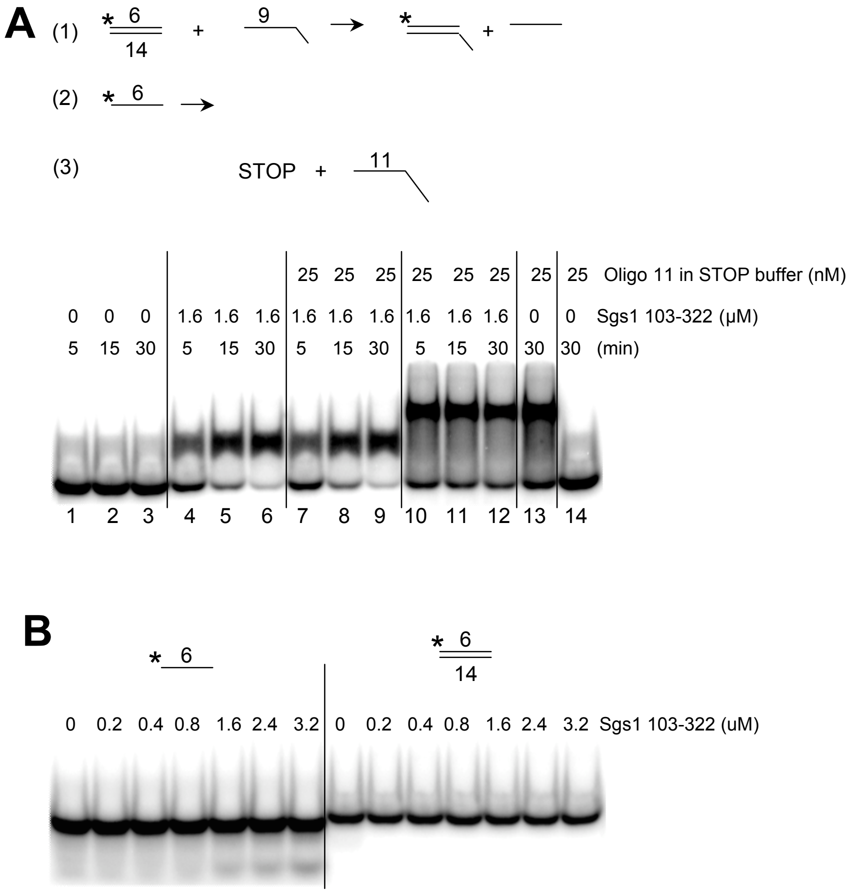
A
(1) * 6 14 + 9 → * +
(2) * 6 →
(3) STOP + 11
25 25 25 25 25 25 25 25 Oligo 11 in STOP buffer (nM)
0 0 0 1.6 1.6 1.6 1.6 1.6 1.6 1.6 1.6 1.6 0 0 Sgs1 103-322 (μM)
5 15 30 5 15 30 5 15 30 5 15 30 30 30 (min)
1 2 3 4 5 6 7 8 9 10 11 12 13 14
B
* 6
* 6 14
0 0.2 0.4 0.8 1.6 2.4 3.2 0 0.2 0.4 0.8 1.6 2.4 3.2 Sgs1 103-322 (uM)

Figure S4

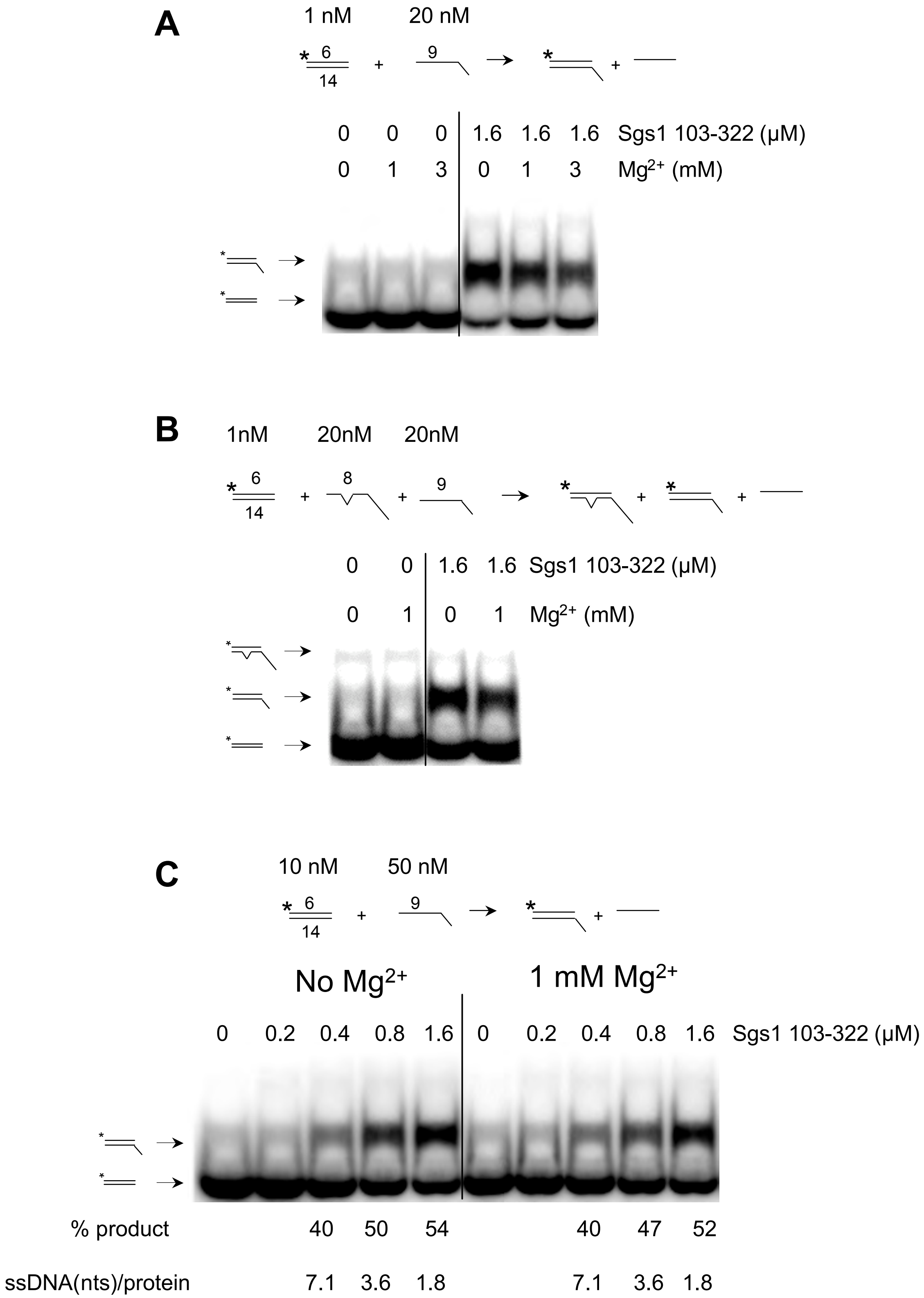
A
1 nM
20 nM
6
14
9
Sgs1 103-322 (μM)
0 0 0 1.6 1.6 1.6
Mg2+ (mM)
0 1 3 0 1 3
B
1nM
20nM
20nM
6
14
8
9
Sgs1 103-322 (μM)
0 0 1.6 1.6
Mg2+ (mM)
0 1 0 1
C
10 nM
50 nM
6
14
9
No Mg2+
1 mM Mg2+
Sgs1 103-322 (μM)
0 0.2 0.4 0.8 1.6 0 0.2 0.4 0.8 1.6
% product
40 50 54 40 47 52
ssDNA(nts)/protein
7.1 3.6 1.8 7.1 3.6 1.8

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	TGCCGAATTCTACCAGTGCCAGTGATGGACATCTTTGCCCACGTTGACCC
3	2558	25	TGGGTCAACGTGGGCAAAGATGTCC
4	2559	25	GGACATCTTTGCCCACGTTGACCCA
5	2600	25	TCACTGGCACTGGTAGAATTCGGCA
6	2625	32	TCCTTTTGATAAGAGGTCATTTTTGCGGATGG
7	2640	32	TCCTTTTGATAAGAGCTCATTTTTGCGGATGG
8	2651	94	CTTTAGCTGCATATTTACAACATGTTGACCTACAGCACCAGATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGAGCTCTTATCAAAAGGA
9	2655	57	CCAGATTCAGCAATTAAGCTCTATCCCATCCGCAAAAATGACCTCTTATCAAAAGGA
10	2624	57	CCATCCGCAAAAATGACCTCTTATCAAAAGGATGGACATCTTTGCCCACGTTGACCC
11	2626	94	CTTTAGCTGCATATTTACAACATGTTGACCTACAGCACCAGATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAAAGGA
12	2653	34	TCTCCTTTTGATAAGAGGTCATTTTTGCGGATGG
13	2654	34	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCT
14	2639	32	CCATCCGCAAAAATGACCTCTTATCAAAAGGA
15	2641	32	CCATCCGCAAAAATGAGCTCTTATCAAAAGGA
16	2129 (Phi-X174)	50	AAAGGTCGCAAAGTAAGAGCTTCTCGAGCTGCGCAAGGATAGGTCGAATT
17	2044 (D-loop)	50	CGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAG
18	1317 (DNA binding)	90	GATCCGAATTCTGGCTTGCTAGGACATCTTTGCCCACGTTGACCCGGGTTGGCGTTAGGAGATAGTCAGTTATAGCTGCGGCTGCTAAGG