

Supplementary Information

Strand invasion of conventional PNA to arbitrary sequence in DNA assisted by single-stranded DNA binding protein

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Characterization of PNA strands

All PNAs were purified and characterized by MALDI-TOF mass spectrometry (Bruker Autoflex) using a 3,5-dimethoxy-4-hydroxycinnamic acid (matrix; #85429, Aldrich) dissolved in a 3:7 mixture of ACN and water containing 0.1% TFA.

Table S1. MALDI-TOF characterization of PNAs used in this study

	Sequence ^{a)}	Calcd.	Found.
PNA10 ^D	H- (Lys) TC D T C D G T D D (Lys) -NH ₂	3025.30	3026.37
PNA12	H- (Lys) C A T C A T C A G T A A (Lys) -NH ₂	3491.47	3492.68
PNA12 ^D	H- (Lys) C D T C D T C D G T D D (Lys) -NH ₂	3566.52	3566.25
PNA13	H- (Lys) T C A T C A T C A G T A A (Lys) -NH ₂	3757.57	3759.37
PNA13 ^D	H- (Lys) T C D T C D T C D G T D D (Lys) -NH ₂	3832.62	3834.70
PNA15	H- (Lys) G T T C A T C A T C A G T A A (Lys) -NH ₂	4314.78	4316.30
PNA15 ^D	H- (Lys) G T T C D T C D T C D G T D D (Lys) -NH ₂	4389.83	4390.54
PNA17	H- (Lys) A T G T T C A T C A T C A G T A A (Lys) -NH ₂	4855.99	4856.84
PNA20	H- (Lys) G G C A T G T T C A T C A T C A G T A A (Lys) -NH ₂	5689.31	5690.86

a) All PNA strands bearing L-lysine residue at its C- and N-terminus. The signs D show that D (2,6-diaminopurine) was used in place of A (adenine) bases.

(a) MALD-TOF-MS spectra of conventional PNA-strands

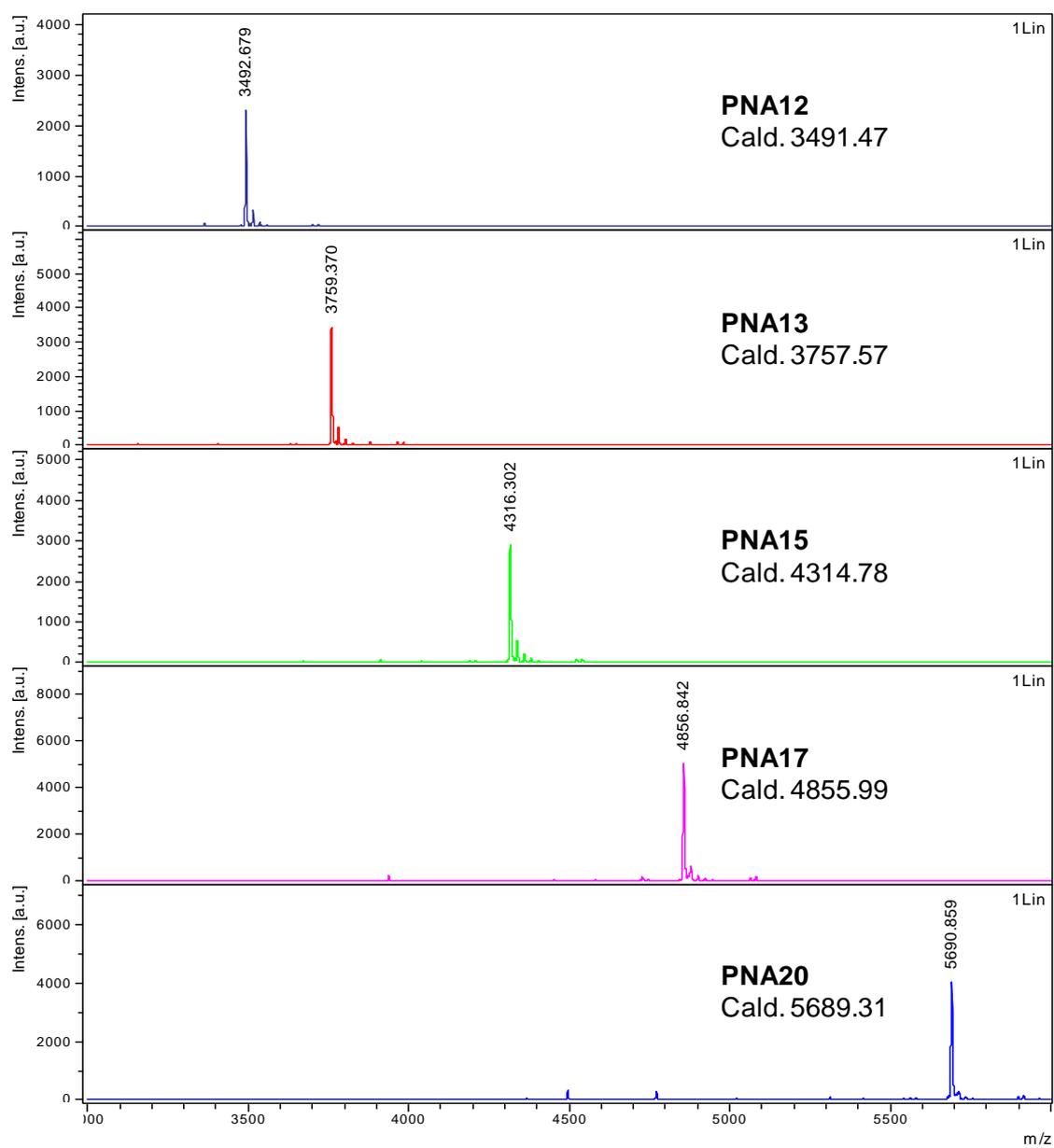


Figure S1. Continued

(b) MALDI-TOF-MS spectra of PNA strands involving 2,6-diaminopurine (D) in place of adenine (A)

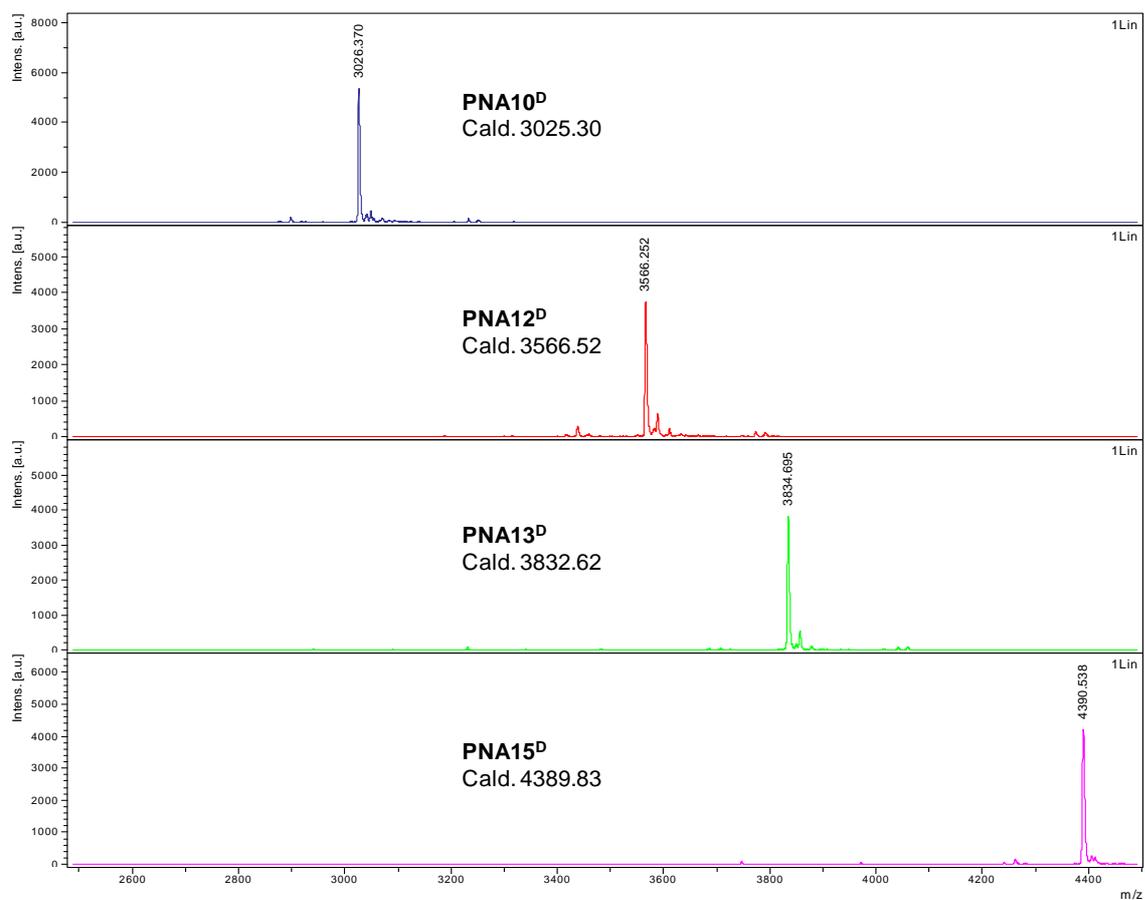


Figure S1. MALDI-TOF-MS spectra of PNA strands used in this study. All PNA strands bearing L-lysine residue at its C- and N-terminus.

Preparation of substrate DNAs

The parent fully-matched 203-mer dsDNA substrate (203 bp; C1724–G1926) was synthesized from pBR322 by PCR using primer-for and primer-rev. Mutated 203-mer dsDNA was first prepared from pBR322 by overlapping PCR using the corresponding primers, and was inserted into pBR322 by a conventional method. After cloning in JM109 (Toyobo), the plasmid was purified by QIAprep Spin Miniprep Kit (from QIAGEN). The resultant mutated plasmid was amplified with the two primers (primer1-for and primer2-rev). Non-complementary 226-mer dsDNA (226 bp; T665–C890) was prepared from pBR322 by PCR using primer2-for and primer2-rev. The DNA sequences (203-mer dsDNA and 226-mer dsDNA) and the sequencing analyses of the mutated 203-mer DNAs are summarized in Figure S3, respectively.

(a) The 203-mer PCR fragment which was used as the target for the invasion

(Only one of the strands is shown and the target site of PNA20 is underlined)

5'-CGAAGCGCTGGCATTGACCCTGAGTGATTTTTCTCTGGTCCCGCCGCATCCATAACCGCCAGTT
GTTTACCCTCACAACGTTCCAGTAACCGGGCATGTTTCATCATCAGTAACCCGTATCGTGAGCATC
CTCTCTCGTTTCATCGGTATCATTACCCCATGAACAGAAATCCCCCTTACACGGAGGCATCAGT
GACCAAACAG-3'

Primer1-for: 5'-CGAAGCGCTGGCATTGAC-3' (18 mer)

Primer2-rev: 5'-CTGTTTGGTCACTGATG-3' (17 mer)

(b) The 226-mer PCR fragment which has no target site

5'-TTGAGAGCCTTCAACCCAGTCAGCTCCTTCCGGTGGGCGCGGGGCATGACTATCGTCGCCGC
ACTTATGACTGTCTTCTTTATCATGCAACTCGTAGGACAGGTGCCGGCAGCGCTCTGGGTCATTT
TCGGCGAGGACCGCTTTCGCTGGAGCGCGACGATGATCGGCCTGTCGCTTGCGGTATTCGGAAT
CTTGACGCCCTCGCTCAAGCCTTCGTCACCTGGTC-3'

Primer2-for: 5'-TTGAGAGCCTTCAACCC-3' (17 mer)

Primer2-rev: 5'-GACCAGTGACGAAGGC-3' (16 mer)

Figure S2. DNA sequences used in this study. 203-mer dsDNA and 226-mer dsDNA were prepared by PCR from pBR322 plasmid DNA and purified by QIAquick PCR Purification Kit (#28106, QIAGEN). They correspond to C1724–G1926 and T665–C890 in pBR322, respectively.

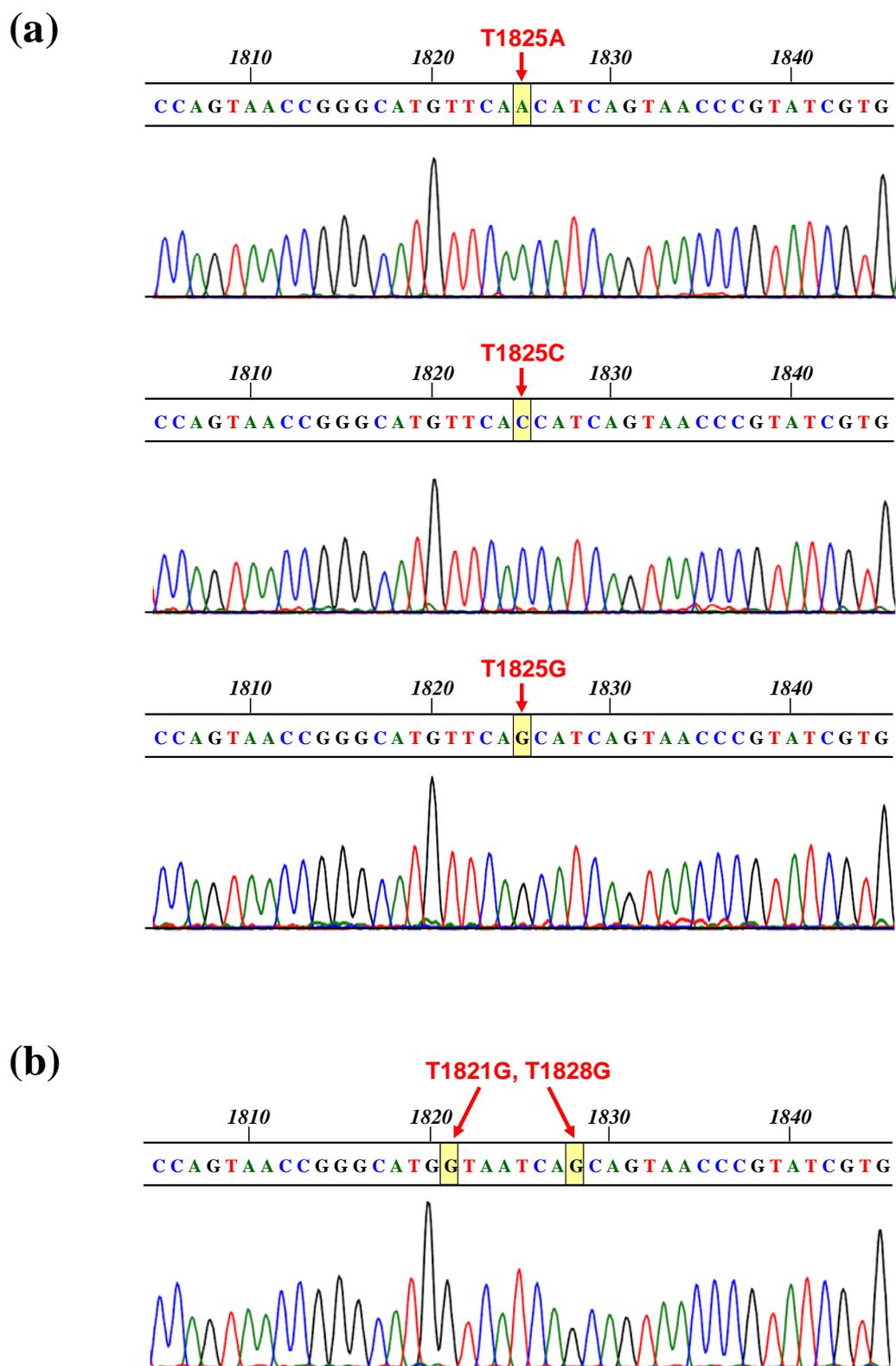


Figure S3. Sequencing of all mutated DNAs. (a) Mutated DNA for one base mismatch system. (b) Mutated DNA for two bases mismatches system. These DNAs were identified by genetic analyzer (Applied Biosystems).

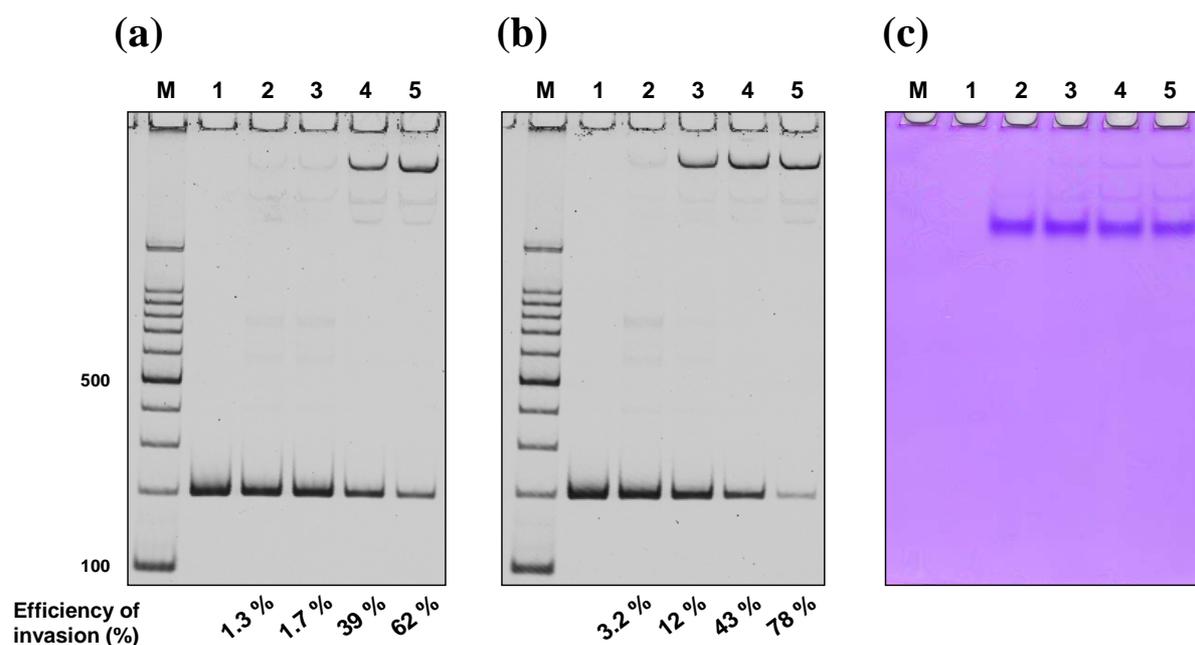


Figure S5. Quantitative analysis of the invasion efficiency by conventional PNA (a) and modified PNA (b). The gels (a) and (b) are the same as Figures 4a and 4b in text, except for the fact that the efficiencies of the invasion, determined by a FUJIFILM FLA-3000G imaging analyzer, are shown in the bottom of the gels. In (c), the results of CBB-staining of the gel in Figure S5b are presented. Note that the bands for the dsDNA/PNA^D/SSB are clearly stained with CBB (Quick-CBB, #299-50101, Wako).

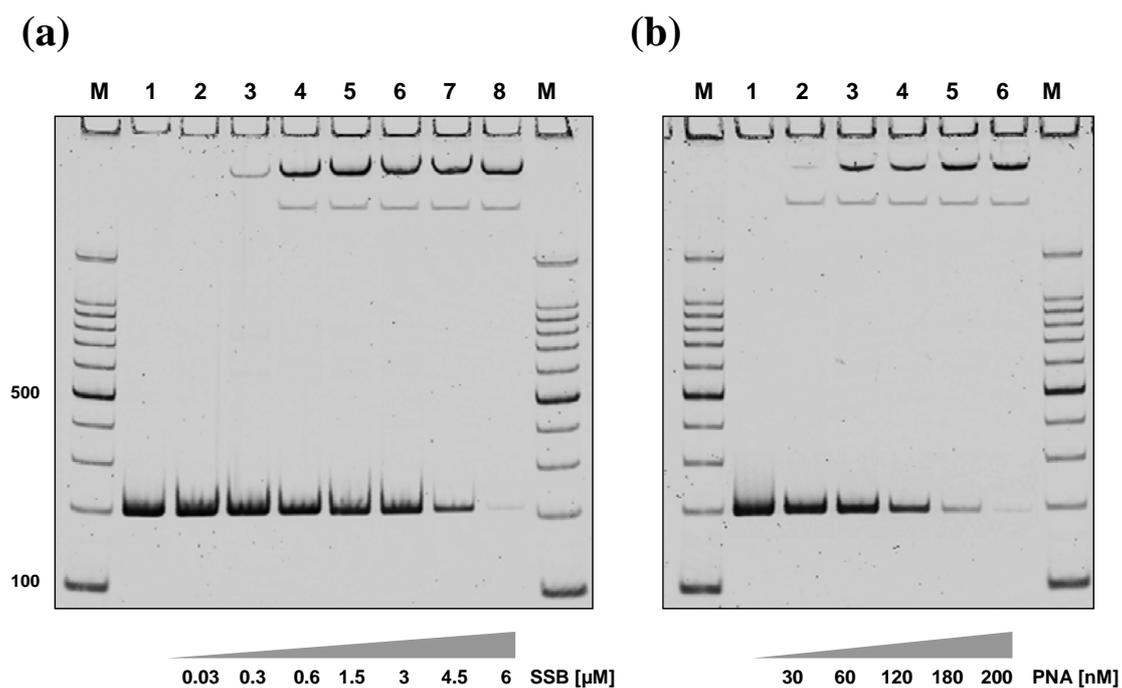


Figure S6. (a) SSB concentration-dependence of strand invasion by PNA20. The concentrations of PNA20 and DNA were kept constant at 200 nM and 30 nM, respectively. Lanes 1-8; [SSB as tetramer] = 0, 0.03, 0.3, 0.6, 1.5, 3, 4.5 and 6 μM . (b) PNA concentration-dependence of strand invasion by PNA20 ([SSB] = 6 μM). Lanes 1-6; [PNA] = 0, 30, 60, 120, 180, and 200 nM. Lane M, 100 bp ladder marker. Other invasion conditions are the same as described in Figure 2.

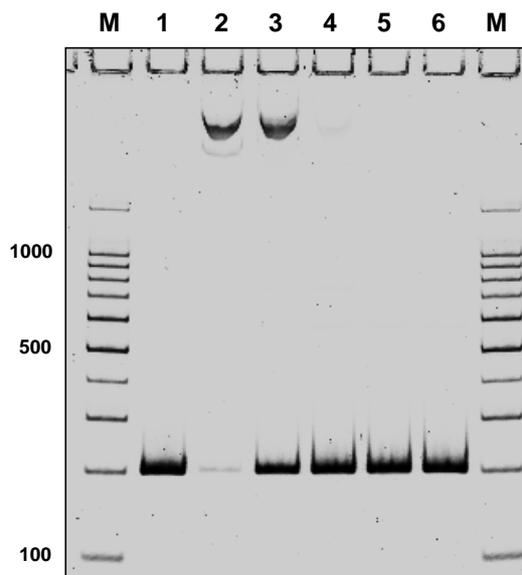


Figure S7. Effect of NaCl concentration on SSB-induced strand invasion by PNA20. The concentrations of PNA20, DNA, and SSB as tetramer were kept constant at 200 nM, 30 nM, and 6 μ M, respectively. Lane 1, DNA only; lanes 2-6, [NaCl] = 20, 25, 30, 35, and 40 mM. Other invasion conditions are the same as described in Figure 2.