Supporting Material

Chiral Introduction of Positive Charges to PNA for Double-duplex Invasion to Versatile Sequences

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DNA1 (used for invasion into G-C rich sequence; Figure 4)

TTGAGAGCCTTCAACCCAGTCAGCTCCTTCCGGTGGGCGCGGGGCATGAC

TATCGTCGCCGCACTTATGACTGTCTTCTTTATCATGCAACTCGTA*GGAC*

target site 3

*AGGTG**C**C**GGCAGCGC**TC*TGGGTCATTTTCGGCGAGGACCGCTTTCGCTGG

target site 1 target site 2

AGCGCGACGATGATCGGCCTGTCGCTTGCGGTATTCGGAATCTTGCACGC

CCTCGCTCAAGCCTTCGTCACTGGTC

DNA2 (used for invasion into A-T rich sequence; Figures 5, 6 and 7)

TGCACCATTATGTTCCGGATCTGCATCGCAGGATGCTGCTGGCTACCCTG

TGGAACACCTACATCTGTATTAACGAAGCGCTGGCATTGACCCTGAGTGA

TTTTTCTCTGGTCCCGCCGCATCCATACCGCCAGTTGTTTACCCTCACAA

CGTTCCAGTAACCGGGCATGTTCA***TCATCAGTAA***CCCGTATCGTGAGCAT

target site 4

CCTCTCTCGTTTCATCGGTATCATTACCCCCATGAACAGAAATCCCCCTT

ACACGGAGGCATCAGTGACCAAACAGGAAAAAACCGCCCTTAACATGGCC

CGCTTTATCAGAAGCCAGACATTAACGCTTCTGGAGAAACTCAACGAGCT

GGACGCGGATGAACAGGCAGACATCTGTGAATCGCTTCACGACCACGCTG

ATGAGCTT

DNAs used for T_m measurements

DNA_{11c} 5′-TCATCAGTAA-3′

DNA_{12c} 5′-TTACTGATGA-3′

Supplemental Figure 1. DNA sequences used in this study.

DNA1 and DNA2 were prepared by PCR from pBR322 plasmid DNA, and used for invasion assay. DNA1 and DNA2 correspond to T665-C890 and T1651-T2058 in pBR322, respectively. The sequences in italic are the target site of PNAs; target site 1 (red) for PNA1-4, target site 2 (blue) for PNA5-8, target site 3 (bold) for PNA17, 18 and target site 4 (black bold) for PNA9-16. DNA_{11c} and DNA_{12c} are used for T_m measurements.

Supplemental Table 1. PNA sequences used in this study

	sequence[a)]	D and U_s	G and C	chiral unit	cation
PNA1	H_2N-(Lys)CCU_sGU_sCCDCG(Lys)-H	3	7	0	3
PNA2	H-(Lys)GGDCDGGU_sGC(Lys)-NH_2	3	7	0	3
PNA3	H_2N-(Lys)CCU_sGU_sCCDCG(Lys)-H	3	7	2 (D)	5
PNA4	H-(Lys)GGDCDGGU_sGC(Lys)-NH_2	3	7	2 (D)	5
PNA5	H_2N-(Lys)CCGU_sCGCGDG(Lys)-H	2	8	0	3
PNA6	H-(Lys)GGCDGCGCU_sC(Lys)-NH_2	2	8	0	3
PNA7	H_2N-(Lys)CCGU_sCGCGDG(Lys)-H	2	8	2 (D)	5
PNA8	H-(Lys)GGCDGCGCU_sC(Lys)-NH_2	2	8	2 (D)	5
PNA9	H_2N-(Lys)DGTDGU_sCATU_s(Lys)-H	4	3	0	3
PNA10	H-(Lys)U_sCAU_sCDGTAD(Lys)-NH_2	4	3	0	3
PNA11	H_2N-(Lys)DGTDGU_sCATU$_s$(Lys)-H	4	3	2 (D)	5
PNA12	H-(Lys)U_sCAU_sCDGTAD(Lys)-NH_2	4	3	2 (D)	5
PNA13	H_2N-(Lys)DGTDGU_sCATU$_s$(Lys)-H	4	3	2 (L)	5
PNA14	H-(Lys)U_sCAU_sCDGTAD(Lys)-NH_2	4	3	2 (L)	5
PNA15	H_2N-$(Lys)_2$DGTDGU_sCAT$U_s$$(Lys)_2$-H	4	3	0	5
PNA16	H-$(Lys)_2$$U_sCAU_s$CDGTAD$(Lys)_2$-$NH_2$	4	3	0	5
PNA17	H_2N-(Lys)GGCCGU_sCGCG(Lys)-H	1	9	1	4
PNA18	H-(Lys)CCGGCDGCGC(Lys)-NH_2	1	9	1	4

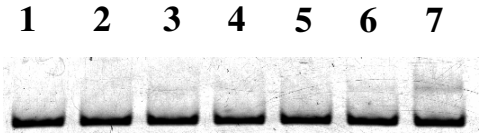
a) D and U_s bear 2,6-diaminiopurine and 2-thiouracil in place of conventional bases, respectively. Chiral units are underlined (e.g. A, T).

Supplemental Table 2. Molecular mass of PNAs measured by MALDI-TOF Mass Spectrometry

	sequence[a)]	theoretical *m/z*	observed *m/z*
PNA1	H_2N–(Lys)CCU$_s$GU$_s$CCDCG(Lys)–H	2937.2	2937.4
PNA2	H–(Lys)GGDCDGGU$_s$GC(Lys)–NH_2	3079.3	3079.1
PNA3	H_2N–(Lys)CCU$_s$GU$_s$CCDCG(Lys)–H	3081.0	3079.2
PNA4	H–(Lys)GGDCDGGU$_s$GC(Lys)–NH_2	3223.4	3220.9
PNA5	H_2N–(Lys)CCGU$_s$CGCGDG(Lys)–H	3000.2	3000.2
PNA6	H–(Lys)GGCDGCGCU$_s$C(Lys)–NH_2	3000.2	3000.1
PNA7	H_2N–(Lys)CCGU$_s$CGCGDG(Lys)–H	3142.4	3142.0
PNA8	H–(Lys)GGCDGCGCU$_s$C(Lys)–NH_2	3142.4	3141.5
PNA9	H_2N–(Lys)DGTDGU$_s$CATU$_s$(Lys)–H	3030.2	3030.7
PNA10	H–(Lys)U$_s$CAU$_s$CDGTAD(Lys)–NH_2	2999.2	2999.0
PNA11	H_2N–(Lys)DGTDGU$_s$CATU$_s$(Lys)–H	3172.3	3172.4
PNA12	H–(Lys)U$_s$CAU$_s$CDGTAD(Lys)–NH_2	3141.3	3141.4
PNA13	H_2N–(Lys)DGTDGU$_s$CATU$_s$(Lys)–H	3172.3	3172.1
PNA14	H–(Lys)U$_s$CAU$_s$CDGTAD(Lys)–NH_2	3141.3	3140.8
PNA15	H_2N–$(Lys)_2$DGTDGU$_s$CATU$_s$$(Lys)_2$–H	3286.4	3286.5
PNA16	H–$(Lys)_2$U$_s$CAU$_s$CDGTAD$(Lys)_2$–NH_2	3255.4	3255.3
PNA17	H_2N–(Lys)GGCCGU$_s$CGCG(Lys)–H	3072.3	3072.1
PNA18	H–(Lys)CCGGCDGCGC(Lys)–NH_2	3054.4	3054.2

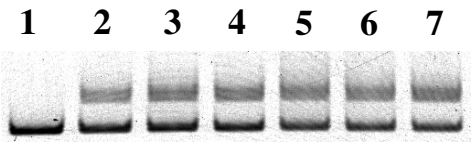
a) D and U_s bear 2,6-diaminiopurine and 2-thiouracil in place of conventional bases, respectively. Chiral units are underlined (e.g. A, T).

(a) PNA5/PNA6 (without chiral PNA monomers)



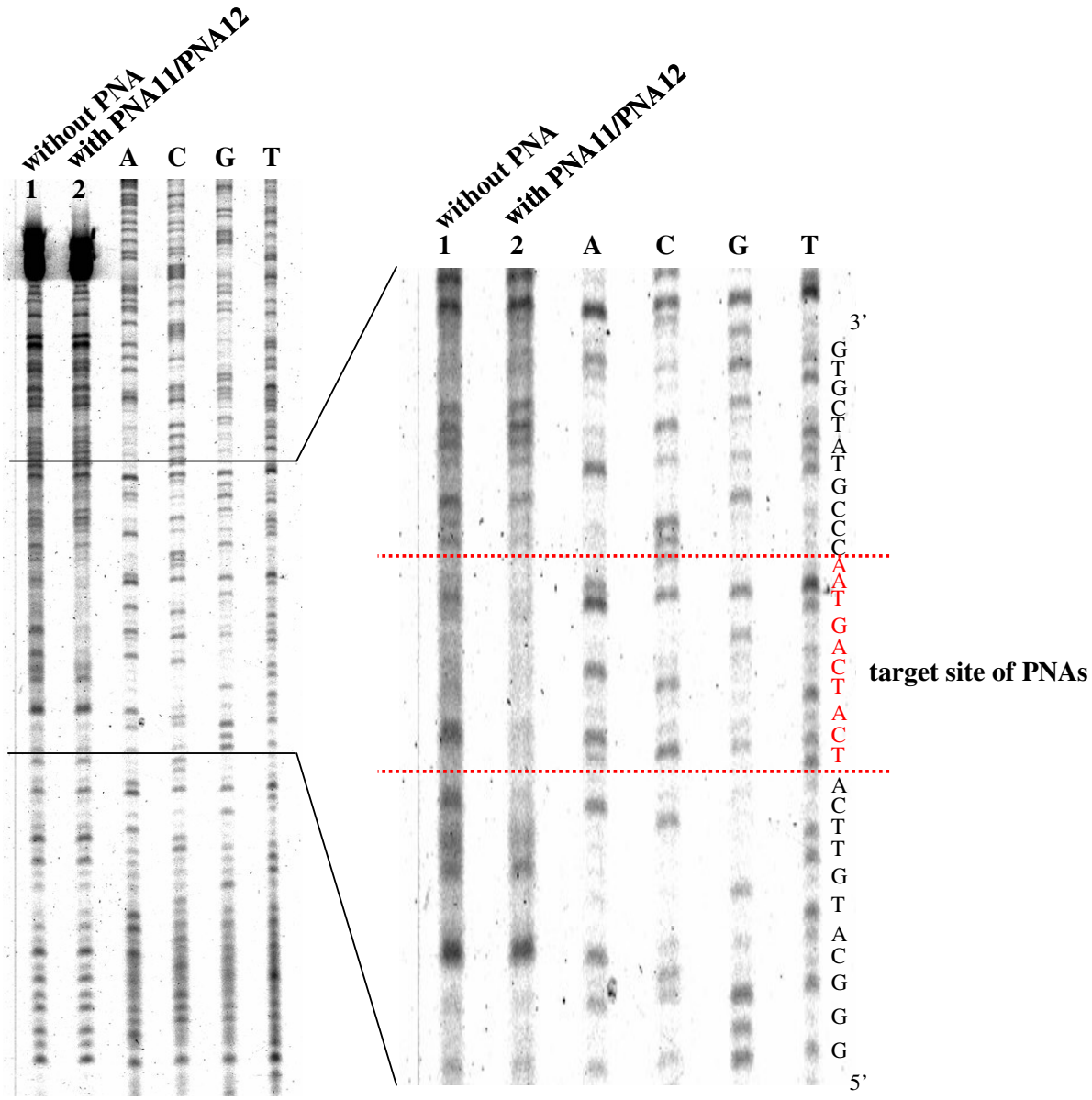
[PNA] = 0 5 10 20 30 40 50 nM
(each strand)

(b) PNA7/PNA8 (with chiral PNA monomers)



[PNA] = 0 5 10 20 30 40 50 nM
(each strand)

Supplemental Figure 2. Effect of PNA concentration on the formation of invasion complex.
(a) PNA5/PNA6 (without chiral PNA monomers), (b) PNA7/PNA8 (with chiral PNA monomers). Invasion conditions: [DNA1] = 5 nM at pH 7.0 (Hepes buffer) and 50 °C, 1.5 h. The gel-shift assay was performed at 20 °C.



Supplemental Figure 3. DNase I foot printing assay for the invasion complex composed of PNA11 and PNA12. A 150-mer double stranded DNA labeled at 5'-end of one strand with FAM was used as the substrate. Lane 1, without PNA; lane 2, with PNA11 and PNA12; A, C, G, T, the Sanger standard lanes. Invasion conditions: [FAM-labeled DNA] = 1 μM, [PNA11] = [PNA12] = 3 μM, [Hepes] = 5 mM, pH 7.0, 50 °C, 1 h. After the invasion complex was formed in 30 μl solution, 3 μl of 0.2 U/μl DNase I and 3 μl of DNase I buffer (both from Takara) were added, and then incubated for 15 minutes at 16 °C. The resultant digests were subjected to 10% denaturing PAGE.