RESEARCH PAPER

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# Sequence specificity at targeting double-stranded DNA with a γ-PNA oligomer modified with guanidinium G-clamp nucleobases

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**Key words:** strand-invasion, gamma-PNA, duplex DNA recognition, duplex DNA capture, plasmid DNA purification

**Abbreviations:** bp, base pair; C, strand-invasion complex; lin, linear; MB, magnetic beads eluate; NT, no target DNA; PNA, peptide nucleic acid; PM, perfect match; SM, single mismatch; sc, supercoiled; SN, supernatant; WC, watson-crick

γ-PNA, a new class of peptide nucleic acids, promises to overcome previous sequence limitations of double-stranded DNA (dsDNA) targeting with PNA. To check the potential of γ-PNA, we have synthesized a biotinylated, pentadecameric γ-PNA of mixed sequence carrying three guanidinium G-clamp nucleobases. We have found that strand invasion reactions of the γ-PNA oligomer to its fully complementary target within dsDNA occurs with significantly higher binding rates than to targets containing single mismatches. Association of the PNA oligomer to mismatched targets does not go to completion but instead reaches a stationary level at or below 60%, even at conditions of very low ionic strength. Initial binding rates to both matched and mismatched targets experience a steep decrease with increasing salt concentration. We demonstrate that a linear DNA target fragment with the correct target sequence can be purified from DNA mixtures containing mismatched target or unrelated genomic DNA by affinity capture with streptavidin-coated magnetic beads. Similarly, supercoiled plasmid DNA is obtained with high purity from an initial sample mixture that included a linear DNA fragment with the fully complementary sequence. Based on the results obtained in this study we believe that γ-PNA has a great potential for specific targeting of chosen duplex DNA sites in a sequence-unrestricted fashion.

## Supplementary Material

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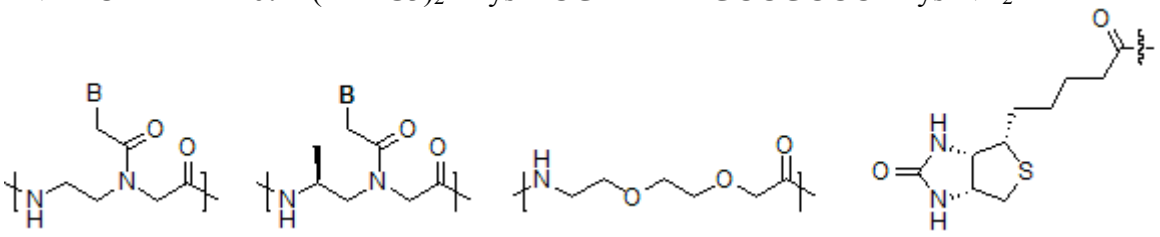
# SUPPLEMENTARY INFORMATION

A

aegPNA1 Biotin-(mPEG3)$_2$-$^{L}$Lys-TXGTAAATGXCGCXC-$^{L}$Lys-NH$_2$
aegPNA1-U Biotin-(mPEG3)$_2$-$^{L}$Lys-TCGTAAATGCCGCCC-$^{L}$Lys-NH$_2$
PNA1 Biotin-(mPEG3)$_2$-$^{L}$Lys-**TXGTAAATGXCGCXC**-$^{L}$Lys-NH$_2$
PNA1-U Biotin-(mPEG3)$_2$-$^{L}$Lys-**TCGTAAATGCCGCCC**-$^{L}$Lys-NH$_2$



aegPNAs PNA1; PNA1-U (γ-PNAs) mPEG3 Biotin

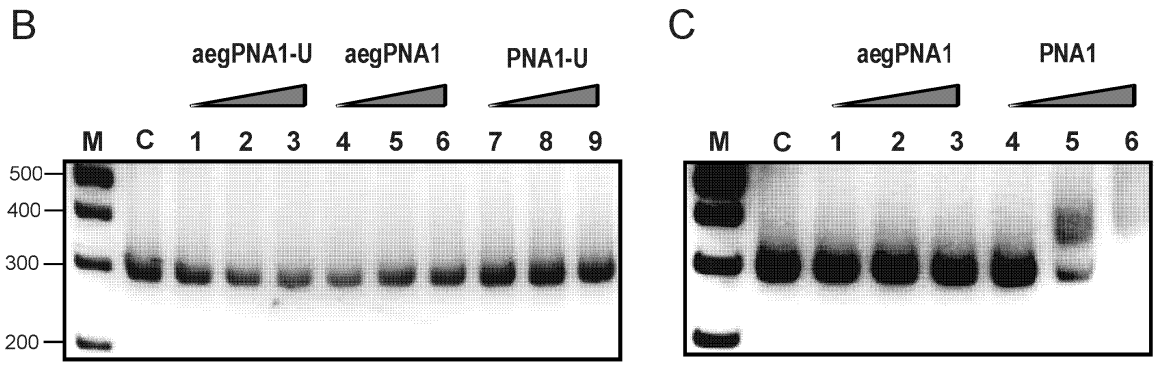



**Figure S1.** Strand-invasion capability of four PNA oligomers that are complementary to the same sequence in dsDNA. (a) Sequence and chemical structure of the synthesized PNA oligomers. PNAs carry cytosine or G-clamp nucleobases (X) at three positions and are either comprised of the regular or a γ-modified N-(2-aminoethyl) glycine backbone. (B, C) Electrophoretic mobility-shift assay. A 291-bp-long dsDNA fragment (0.1 M) containing the perfectly matched (PM) target sequence for the PNA oligomers was incubated for 16 h at 37$^{o}$C with various concentrations of a PNA oligomer in 10 mM sodium phosphate buffer (pH 7.4). Samples were subsequently separated on a 10% nondenaturing gel and stained with SYBR-Gold for visualization. PNA/DNA ratios employed were 1:1 (lanes 1, 4, and 7), 3:1 (lanes 2, 5, and 8), and 5:1 (lanes 3, 6, and 9). Lanes C are controls without PNA. M: DNA ladder.

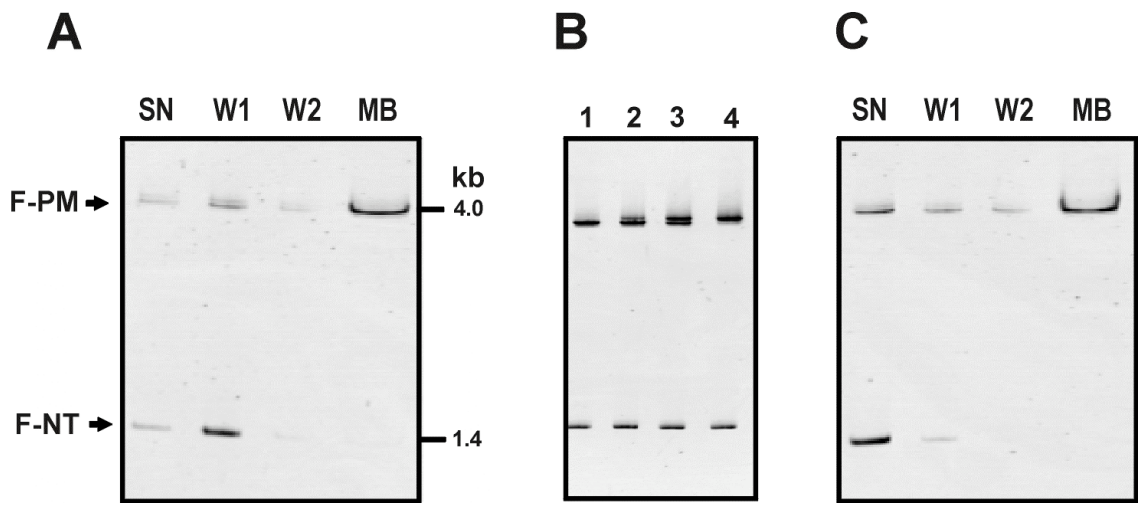



**Figure S2.** (A, C) Affinity capture with linearized pPM containing two fragments F-PM and F-NT. Following incubation with PNA1, samples were purified by gel filtration through Sephadex G-50 mini-columns pre-equilibrated with TE buffer (A) or TE buffer containing 20 mM NaCl (C). Samples were subsequently added to dry, pre-washed streptavidin-coated magnetic beads. Lane SN, supernatant; lane W1, wash with TE buffer containing 50 mM NaCl; lane W2, wash with TE buffer containing 100 mM NaCl; MB, eluate from magnetic beads following incubation for 30 at 60$^{o}$C in TE buffer with 500 mM NaCl. (B) Gel-shift analysis at incubation of F-PM and F-NT with PNA1. Incubations were performed at PNA1 concentrations of 0 M (control, lane 1), 1 M (lane 2), 1.5 M (lane 3), and 2 M (lane 4). For F-PM, two bands can be observed at partial formation of strand-invasion complex (lanes 2-3).

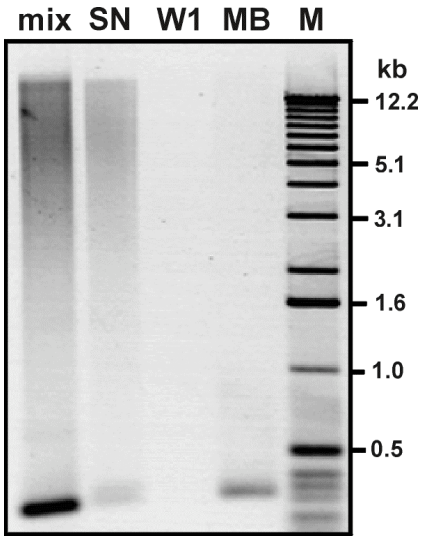



**Figure S3.** Capture of the short, perfectly matched target in the presence of human genomic DNA. The capture experiment was carried out analogous to the one described in Figure 4D. However, in this experiment, samples were analyzed on a 1 % agarose gel.