SUPPORTING INFORMATION FOR:

Cyclopentane-Peptide Nucleic Acids for Qualitative, Quantitative, and Repetitive Detection of Nucleic Acids

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Abbreviations:
(ACN) acetonitrile; (BLB) 2% BSA, 25 mM Tris, 150 mM NaCl, 0.05% Tween 20, 0.1 mM EDTA, pH 7.4; (BLBs) BLB with 0.1 μg/mL single stranded salmon sperm DNA; (BSA) bovine serum albumin; (CAP) 25 mM Lys, 10 mM NaH_2PO_4, 100 mM NaCl, 0.1 mM EDTA, pH 8.0; (DCM) dichloromethane; (DIEA) N,N-diisopropylethylamine; (DMF) N,N-dimethylformamide; (DTDP) 2,2'-dithiodipyridine; (DTT) dithiothreitol; (Et_2O) diethyl ether; (HATU) *O* -(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethylammoniumhexafluorophosphate; (HBTU) *O*-benzatriazole-N,N,N',N'-tetramethyluroniumhexafluorophosphate; (IB) immoblization buffer (100 mM Na_2CO_3, pH 9.6); (MBHA) methyl-benzhydrylamine; (pHRP) poly-horseradish peroxidase; (EDTA) sodium salt of ethylene-diaminetetraacetic acid; (NMP) N-methyl-2-pyrolidinone; (1 × PBS) phosphate buffered saline solution, 9.0 g/L NaCl, 144 mg/L KH_2PO_4, 795 mg/L Na_2HPO_4, pH 7.4; (PBST) 1 × PBS with 0.05% Tween 20; (mPEG) 8-amino-3,6-dioctanoic acid; (NHS) N-hydroxysuccinimide; (PNA) peptide nucleic acid; (RT) room temperature; (SA) streptavidin; (SM-PEG_4-Mal) succinimidyl-([N-maleimidopropionamido]-tetraeythyleneglycol) ester ; (SPDP) 3-(2-pyridyldithio)-propionic acid; (SPPS) solid-phased peptide synthesis; (TEMED) Tetra-methylethylenediamine; (TFA) trifluoroacetic acid; (TFMSA) trifluoromethanesulfonic acid; (TIPS) triisopropylsilane; (TMB) 3,3'5,5'-tetramethylbenzdine; (Tris) tris(hydroxy-methyl)aminomethane; (Tween 20) polysorbate 20.

Reagent Sources:
All Boc- and Fmoc- peptide nucleic acid monomers were purchased from Applied Biosystems (Carlsbad, CA) or PolyOrg, Inc. (Leominster, MA); cyclopentane T PNA monomer was made following previously published procedures,[1] ACN, BSA, DCM, DIEA, DMF, DTDP, EDTA, Et_2O, 1 M DTT (in water), Kaiser[2] test reagents, m-cresol, MeOH, NaCl, Na_2CO_3, NMP, NaH_2PO_4, piperidine, sealing tape for multiwell plates, ssDNA, TFA, TFMSA, TIPS, thioanisole, Tween 20 were purchased from Sigma-Aldrich (St. Louis, MO). 1-Step™ Ultra-TMB, 96-well Nunc Immobilizer Amino plates, NHS ester of PEG_4 maleimide, pHRP linked SA, and TMB Stop Solution were obtained from Thermo Scientific (Fairlawn, NJ); PBS buffer was purchased from Quality Biological (Gaithersburg, MD); High purity water (18 MΩ) was generated from a Millipore (Billerica, MA) MilliQ water system. SPDP was purchased from Bachem (Switzerland). HATU was purchased from Applied Biosystems; Boc- and Fmoc-mPEG-OH (t-butyloxycarbonyl- or 9-fluorenylmethoxycarbonyl-8-amino-3,6-dioxaoctanoic acid and Boc-$mPEG_3$-OH (t-butyloxycarbonyl-11-amino-3,6,9-trioxaundecanoic acid) were purchased from Peptides International (Louisville, KY); HBTU, Boc-Lys(Fmoc)-OH, Fmoc-Lys(Boc)-OH, H-Lys-OH•HCl, MBHA resin were purchased from Advanced Chemtech (Louisville, KY). H_2SO_4 was purchased from EMD Chemicals (Gibbstown, NJ). Synthetic control and target DNA's were purchased from Integrated DNA Technologies (Coralville, IA).

General Method for PNA Synthesis:

PNA's were prepared on 5 or 25 μmol scale using either Boc- or Fmoc- SPPS on an Applied Biosystems 433a automated peptide synthesizer using HBTU activation. All PNA's were synthesized on Lys downloaded MBHA resin or modified resin (see below).

Resin Downloading Protocol:

MBHA resin was downloaded from 0.3 mmol/g to 0.1 mmol/g using Boc-Lys(2-Cl-Z)-OH. 1.0 g of resin was swelled with DCM for 1 h in a peptide synthesis vessel. The following solutions were prepared: (A) 0.4 M Boc-Lys(2-Cl-Z)-OH in NMP, (B) 0.2 M HATU in DMF, and (C) 0.5 M DIEA in NMP. 450 μL (A), 460 μL (C) and 1.59 mL NMP were combined and mixed (Solution 1) and 550 μL (B) were diluted with 1.95mL NMP (Solution 2). Solutions 1 and 2 were combined and pre-mixed for ~30 s before adding to the drained, swelled resin. The resin/coupling mixture was agitated for 1 h before draining and washing with DMF (4x), DCM (4x), 5% DIEA in DCM (1x-30 s) and finally DCM (4x). Any remaining active sites were capped using capping cocktail (1:25:25 Ac_2O:NMP:pyridine) for 20 min. The reaction was drained and rinsed with DMF (3x) and DCM (3x). The progress of the capping was followed by qualitative Kaiser[2] test. If the test was positive, the resin was resubmitted to capping. After a negative test for primary amines, the resin was washed with DCM (3x) and dried under vacuum for 30-60 min and then stored in a dessicator.

Coupling N-terminus of resin-bound RP3 to SM-PEG_4-Mal:

Coupling of maleimide was performed manually in a glass peptide reaction vessel at RT. To 50 mg (5 μmol) of preswelled resin, containing the RP3 sequence with a free amine at the N-terminus, in a minimal volume of DMF was added SM-PEG_4-Mal (7.7 mg, 3.0 eq.) and DIEA (1.7 μL, 2.0 eq.). The coupling was shaken for 2 h, drained and rinsed with DMF (3x) and DCM (3x). The progress of the coupling was followed by Kaiser[2] test. If it was positive, it was resubmitted to coupling. After a negative test for primary amine, the resin was rinsed with DMF (2x), then ~1.5 mL of capping cocktail was added and shaken for 4 min. Afterward, the resin was drained and washed with DMF (3x) and DCM (3x).

Manual Synthesis of C-terminus of SPDP-containing SP3:

The resin downloading procedure is listed earlier in the general methods section of this paper and was used to prepare Boc-Lys(Fmoc) downloaded MBHA resin. The swelled downloaded resin was then Fmoc- deprotected by rinsing (2x) with 20% piperidine in DMF and then adding 2 mL of the piperidine solution to the resin, agitating for 20 min, draining and rinsing with DMF (3x) and DCM (3x). Deprotection was followed by Kaiser[2] test for amine. Then Fmoc-mPEG-OH (56 mg, 5.0 eq.) was dissolved in a minimal volume of NMP in a glass vial. DIEA (87 μL, 20.0 eq.) was added to the solution. Also, in a separate glass vial, HATU (38 mg, 4.0 eq.) was dissolved in a minimal volume of DMF. The two solutions were then premixed for 30 s before adding to the resin. Minimal NMP was added to give proper agitation of the resin. The resin was agitated for 1 h before draining, rinsing with DMF (3x) and DCM (3x) and

monitoring the coupling progress by Kaiser[2] test. After sufficient coupling, the resin was capped using capping cocktail.

SPDP NHS-ester (41 mg, 5.0 eq.) was added dry to the drained resin. A minimal volume of NMP was added to dissolve the SPDP, before adding DIEA (87 µL, 20.0 eq.) The mixture was agitated for 2 h before draining and washing with DMF (3x) and DCM (3x) and monitoring by Kaiser[2] test for complete coupling. After sufficient coupling, the resin was capped with capping cocktail.

The initial two thymine residues were then coupled using the following procedure: the resin was rinsed with DCM (2x) before rinsing with 5% m-cresol in TFA (2x). Deprotection of the Boc- group of Lys commenced by adding 5% m-cresol in TFA and agitating with N_2 (3x - 4 min) followed by rinsing with DCM (3x) and monitoring by Kaiser[2] test. Once deprotected, Boc-T-OH (48 mg, 5.0 eq.) was dissolved in a minimal volume of NMP in a glass vial. DIEA (87 µL, 20.0 eq.) was added to the solution. Also, in a separate glass vial, HATU (38 mg, 4.0 eq.) was dissolved in a minimal volume of DMF. The two solutions were then premixed for 30 s before adding to the resin. Minimal NMP was added to give proper agitation of the resin. The resin was agitated for 1 h before draining, rinsing with DMF (3x) and DCM (3x) and monitoring coupling by Kaiser[2] test. After sufficient coupling, the resin was capped with capping cocktail. The process was repeated for the second Boc-T-OH and then the sequence was completed by automated SPPS.

Cleavage and recovery of crude PNA from resin:

The resin, in a peptide synthesis vessel, was first washed with TFA (2x for 4 min). To the drained resin, cleavage cocktail (1.5 mL, 60:20:10:10 TFA/TFMSA/thioanisole/m-cresol), cooled over ice, was added and reacted for 1 h. The cleavage mixture was collected in a glass vial using N_2 pressure to drain the vessel. The resin was resubmitted to fresh cleavage cocktail and cleaved for 1 h, and was drained into the first cleavage fraction. The volatiles were removed by flowing dry N_2 over the solution to produce a yellow-brown oil.

Approximately 10 mL of Et_2O was added to the cleavage oil to create a suspended white precipitate. The suspension was partitioned into five 2 mL microcentrifuge tubes and chilled over dry ice for 10 min. The tubes were centrifuged at 12,000 rpm for 40 s to produce a white pellet. Et_2O was carefully decanted, leaving the white crude PNA solid. Further washing was performed by adding ~1.6 mL of Et_2O to each tube, mixing to resuspend the precipitate, then chilling on dry ice for 5 min. Following centrifugation and decanting, the washes were repeated twice without dry ice. After the final wash, the white precipitate was dried by carefully passing a stream of dry N_2 over the crude PNA.

Reprotection of SP3 following cleavage:

In some instances, it was necessary to reprotect the thiol of SP3 following cleavage from the resin. In order to reprotect 2-pyridylthiol protecting group of SP3[3], 400 µL MeOH and 90 µL of 1 M Tris•HCl (pH 7.5) were added to one microcentrifuge tube of crude SP3. 44 mg DTDP (~ 40 eq.) was added and the mixture was shaken for 2.5 h before purifying by RP-HPLC.

Purification of crude PNA and Characterization:

Purification was performed on an Agilent (Santa Clara, CA) 1100 Series RP-HPLC with automatic (SP1, SP2, RP1 & RP2) or manual fraction collection (SP3 & RP3) using UV detection at 260 nm. Waters (Milford, MA) XBridge C18 (10 x 250 mm, 5 µm) columns were used in conjunction with Solvents A and B. Solvent A was 0.05% TFA in water and Solvent B consisted of 90% ACN in water. PNA's were purified using the following elution gradients: SP1 and RP1– (4.0 mL/min) 10% B over 5 min, 25% over 25 min and elution at 17 and 27 min, respectively; SP2 – (4.5 mL/min) 0% B for 2 min, 8% over 3 min, 14% over 22 min with elution at 21 min; SP3 – (4.0 mL/min) 0% B for 2 min, 15.5% over 3 min, 10.5% over 22 min with elution at 18 min; RP2 – (4.0 mL/min) 0 % B for 2 min, 12% for 3 min, 10% for 22 min and elution at 18 min. RP3 – (5.0 mL/min) 0% B for 2 min, 28% for 3 min, 16% for 21 min and elution at 11 min. PNA HPLC isolates were characterized using ESI-MS on a Waters/Micromass LCT Premier™ time-of-flight mass spectrometer. The instrument was operated in W-mode at a nominal resolution of 10,000. The electrospray capillary voltage was 2 kV and the sample cone voltage was 60 V. The desolvation temperature was 275 °C and the desolvation gas was N_2 with a flow of 300 L/h. Accurate masses were obtained using the internal reference standard method. The sample was introduced into the mass spectrometer via the direct loop injection method. Deconvolution of multiply charged ions was performed with MaxEnt I. All PNA oligomers gave molecular ions consistent with the calculated theoretical product values.

Table S1 – PNA Molecular Weight Characterization

Probe	Sequence	Theoretical MW	Observed MW
SP1	H_2N-(mPEG)$_5$-ATCCTTAT$_{cyp}$CAATATT-$CONH_2$	4776.7	4776.0
SP2	Ac-(mPEG)$_2$-ATCCTTATCAATATT-Lys(mPEG-Cys-NH_2)-$CONH_2$	4719.7	4719.5
SP3	H_2N-(mPEG)$_2$-ATCCTTAT$_{cyp}$CAATATT-Lys(mPEG-SPDP)-$CONH_2$	4666.7	4665.8
RP1	Ac-TAACAATAATCC-mPEG-{Lys[(mPEG-3)$_2$-BT]}$_2$-[Lys(mPEG-3-BT)]$_2$-[(Lys(mPEG-BT)]$_2$-Lys-$CONH_2$	7088.9	7089.0
RP2	Mal-mPEG-TAACAATAATCC-Lys(mPEG-Ac)-$CONH_2$	4080.1	4080.1
RP3	Mal-mPEG-TAACAATAATCC-mPEG-{Lys[(mPEG-3)$_2$-BT]}$_2$-[Lys(mPEG-3-BT)]$_2$-[(Lys(mPEG-BT)]$_2$-Lys-$CONH_2$	7590.4	7590.0
XP1	SP2-S-Mal-RP2	8799.7	8799.3

Table Notes: mPEG: 8-amino-3,6-dioxaoctanoic acid; mPEG-3: 11-amino-3,6,9-trioxaundecanoic acid; Mal: (N-maleimidopropionamido)-tetraeythyleneglycol; SPDP: 3-(2-pyridyldithio)-propionic acid; T_{cyp} is S,S-transcyclopentane modified thymine[1]

Table S2 – Nucleic Acid Sequences

Name	Nucleic Acid Type	Sequence	# of bases
TS1	DNA	5' - GGA-TTA-TTG-TTA-AAT-ATT-GAT-AAG-GAT - 3'	27
TS2	DNA (ds)	5' - GGA-TTA-TTG-TTA-AAT-ATT-GAT-AAG-GAT - 3' 3' - CCT-AAT-AAC-AAT-TTA-TAA-CTA-TTC-CTA - 5'	27
TS3	DNA (ds)	5' - GCTGAAATATAGGATTATTGTTAAATATTGATAAGGATGTAATGATAATA - 3' 3' - CGACT TTATATCCTAATAACAAT TTATAACTAT TCCT ACAT TACTAT TA - 5'	50
SS1	DNA	5' - TGC-AGT-CTG-TTA-CAA-TGA-CCT-ACT - 3'	24
SS4	DNA (ds)	5' - GCTGAAATATAAAGATAAATAGGTGTCAATTGTAGAACGTAATGATAATA - 3' 3' - CGACT TTATATT TCTATTTATCCACAGT TAACATCT TGCATTACTATTAT - 5'	50
TC mismatch	DNA	5'- GGA TTC TTG TTA AAT ATT GAT AAG GAT-3'	27
TG mismatch	DNA	5'- GGA TTG TTG TTA AAT ATT GAT AAG GAT-3'	27
TT mismatch	DNA	5'- GGA TTT TTG TTA AAT ATT GAT AAG GAT-3'	27

Thermal Melting Analysis:

UV concentration determination was determined by adding 1 µL of nucleic acid solution to 175 µL milliQ water, unless the concentration was too intense and consequently the 1 µL was diluted with 351 µL of water. Water was blanked against the background at 80 °C on an Agilent 8453 UV/Vis spectrometer equipped with an Agilent 89090A peltier temperature controller and a computer interface. Then the unknown solution was added to the quartz cell (Helma) and vigorously shaken, replaced in the spectrophotometer and the absorbance was read at 260 nm. The mixing and reading was repeated 3 more times. Values were converted to concentration, based on average absorbance. After initial measurement by UV, the concentration was determined based on appropriate ε_{260} (calculated on nearest neighbor approximation for PNA or provided by IDT or Thermo

Scientific for oligonucleotides) and then used from that point forward for additional experiments.

Thermal melting experiments were performed by preparing 0.5 – 5.0 μM oligonucleotide solution in 1 × PBS. Experiments traversed from 90 °C to 20 °C and back to 90 °C at 1 °C intervals while monitoring at 260 nm. An equilibration of 60 s at each temperature measurement step before readings was employed. Cooling and heating profiles were generated for each run with duplicates for each. The T_m (melting temperature) for duplexes was determined using the maximum derivative of the cooling and heating curves, then taken as an average of both runs.

Table S3 – Oligonucleotide Thermal Melting Temperatures

Oligonucleotide Set	# of bp's	T_m	Concentration[i]
TS1 (ss)	27	-	1.0 µM
TS2 (ds)	27	54.6 °C	1.0 µM
TS3 (ds)	50	64.6 °C	0.5 µM
SP1 / TS1	15	52.9 °C	1.0 µM
SP2 / TS1	15	43.8 °C	1.0 µM
SP3 / TS1	15	51.6 °C	2.5 µM
RP1 / TS1	12	50.3 °C	1.0 µM
RP2 / TS1	12	46.7 °C	1.0 µM
RP3 / TS1	12	48.3 °C	2.5 µM
SP1/RP1/TS1	27	53.2 °C	1.0 µM
SP2/RP2/TS1	27	48.5 °C	1.0 µM
SP3/RP3/TS1	27	52.0 °C	1.0 µM
SS1 / SP1	15	-	1.0 µM
SS1 / RP1	15	-	1.0 µM
SS1 / SP3	12	-	1.0 µM
SS1 / RP3	12	-	1.0 µM

[i] Concentration of individual sequence(s).

1:1 SP2-RP2 Coupling Protocol:
No complementary DNA template:
6.4 µL SP2 [2.5 mM, 200 µmol] were combined with 20 µL RP2 [800 µM, 200 µmol] in 53.6 µL of 1X PBS buffer with 0.1 mM EDTA at pH 6.6 in an Eppendorf tube and mixed the contents. The reaction was monitored by HPLC at 1 h, 4 h and 16 h. The yield of the cross coupling product reached about 10% after 16 h (see Figure S1 (A)), ESI-MS samples also confirmed the cross-coupled XPL1 product formation (see Table S1). Yield was calculated based on the estimated extinction coefficient at 260 nm of XPL1.

With complementary DNA template:
122 µL SP2 [41 µM, 10 µmol] and 15 µL RP2 [331 µM, 10 µmol] were combined with 33 µL TS1 [150 µM, 10 µmol] in 330 µL of 1X PBS buffer with 0.1 mM EDTA at pH 7.2 in a glass vial and mixed the contents. The reaction was monitored by HPLC at initial, 1 h, 2 h, 4 h, 7 h and 24 h. Results (Figure S1 (B)) showed that the coupling reaction was nearly complete after 7 h, and basically all of the RP2 is consumed, while a small amount of SP2 remains. Yield was calculated based on the estimated extinction coefficient at 260 nm of XPL1.

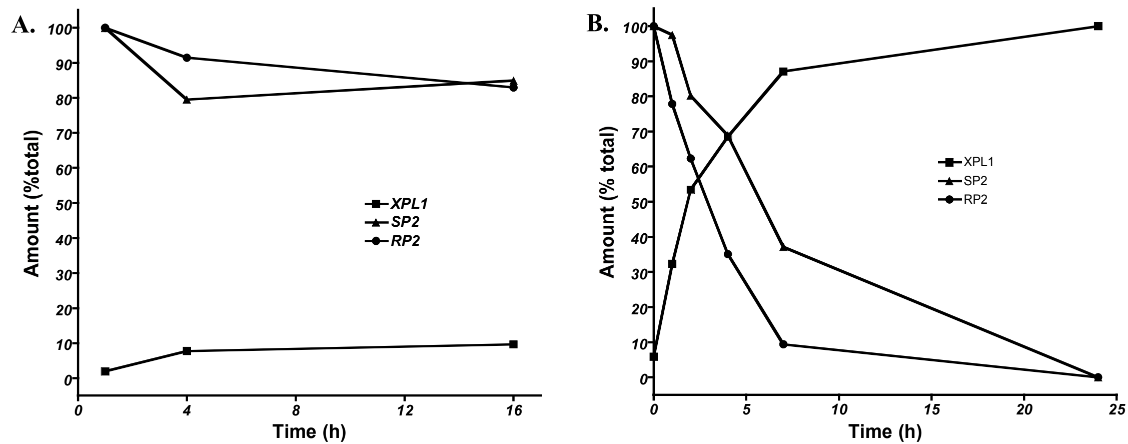

Figure S1. Amount changes of SP2, RP2 and XPL1 of the SP2-RP2 coupling reaction vs. reaction time. **A.** no DNA; **B.** in the presence of ssDNA TS1.

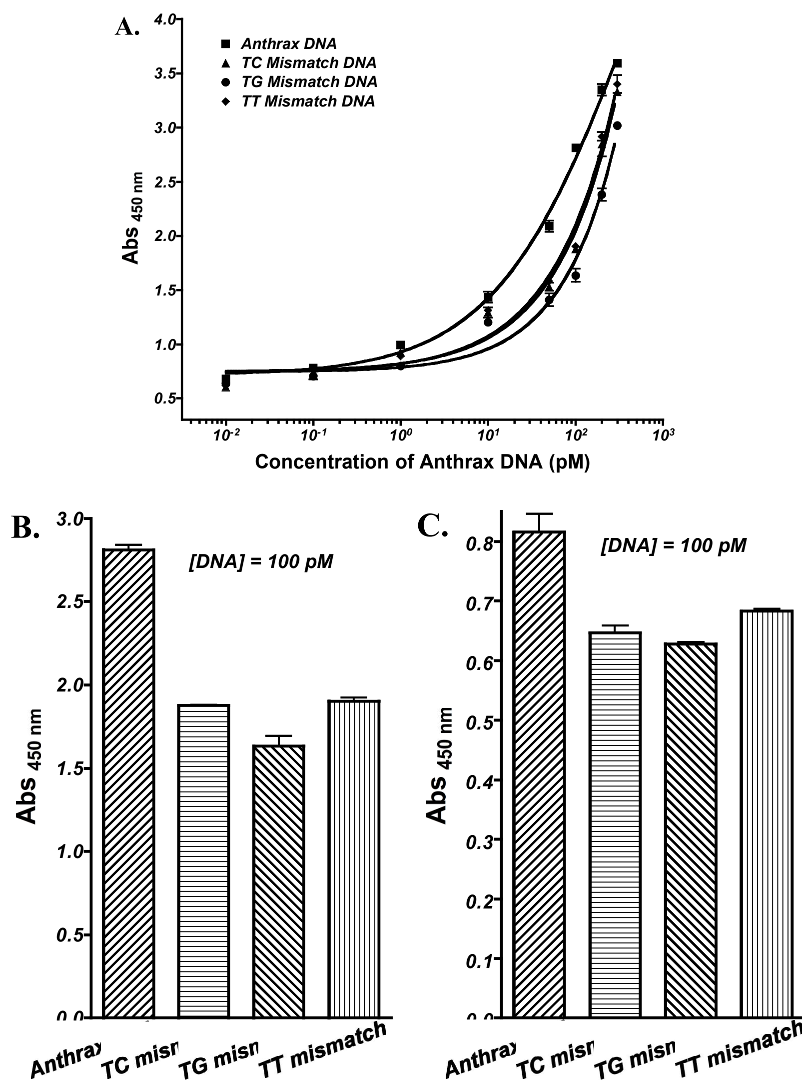

Figure S2. Detection of mismatches ssDNA using PNA probes SP1 & RP1, and SP3 & RP3. **A.** Signal response plot (Absorbance at 450 nm vs. DNA concentration) obtained after quenching of enzymatic oxidation with H_2SO_4 using SP1 and RP1 PNA probes. **B.** Comparison of the absorbance at 450 nm of 100 pM mismatch and native DNAs with SP1 & RP1 PNA probes. C. Comparison of the absorbance at 450 nm of 100 pM mismatch and native DNAs with SP3 & RP3 PNA probes.

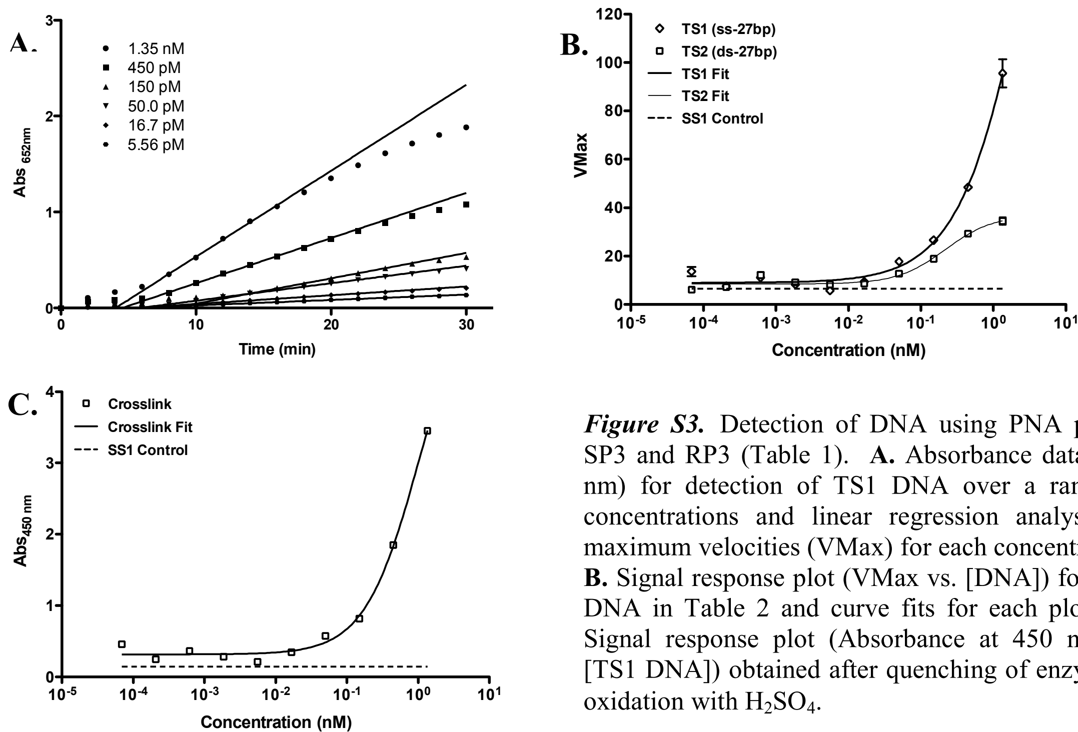

Figure S3. Detection of DNA using PNA probes SP3 and RP3 (Table 1). **A.** Absorbance data (652 nm) for detection of TS1 DNA over a range of concentrations and linear regression analyses of maximum velocities (VMax) for each concentration. **B.** Signal response plot (VMax vs. [DNA]) for each DNA in Table 2 and curve fits for each plot. **C.** Signal response plot (Absorbance at 450 nm vs. [TS1 DNA]) obtained after quenching of enzymatic oxidation with H_2SO_4.

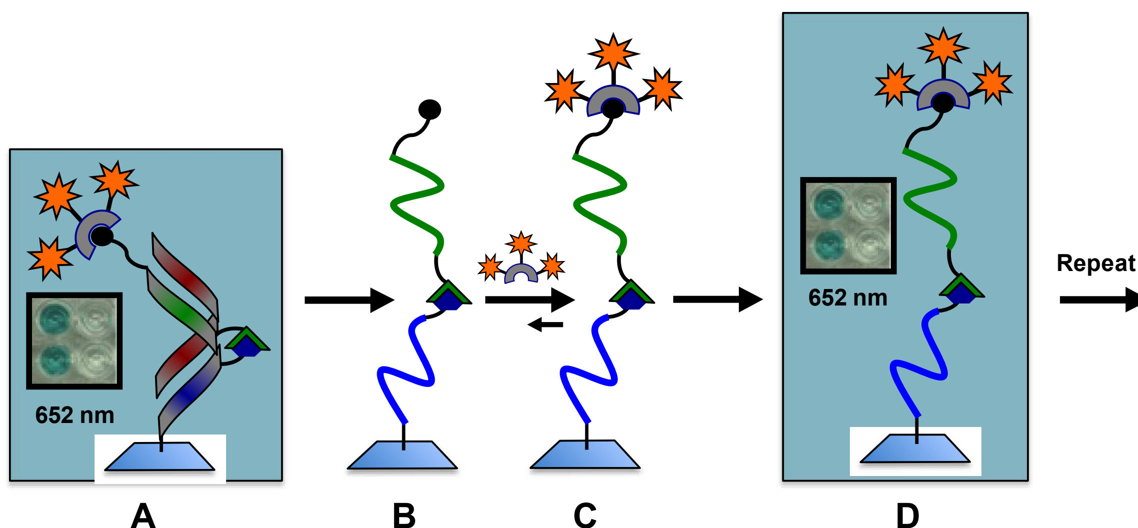

Figure S4. Scheme for repetition of detection using covalent sandwich. After the initial assay is performed (A), the plate is extensively washed and the DNA target is presumably removed along with the pHRP-SA. The crosslink between the two PNAs preserves the original information about the presence of the target DNA (B). After various time points, the pHRP-SA is introduced again (C), and the detection signal is re-determined (D).

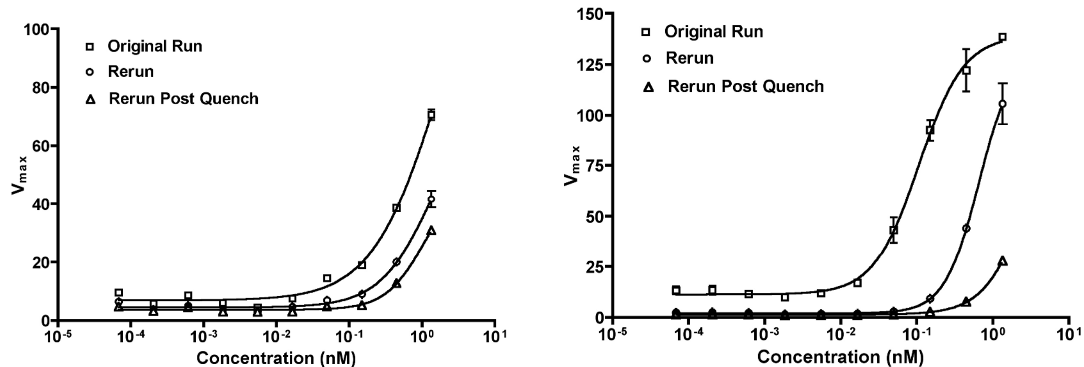

Figure S5. Detection of signal after DNA target has been washed away. **A**. Signal response plot using probes SP3 and RP3 where the crosslink preserves the signal for subsequent analyses. **B**. Signal response plot using probes SP1 and RP1 where the detection signal rapidly decreases as the non-covalent sandwich complex is washed away along with target DNA.

Table S4 – 4-PL Model Analysis of Concentration-dependent VMax$_{652nm}$ Results

Name	Nucleic Acid Type	4-PL Model Equation	Fit
Template Assay			
TS1	ssDNA	$y = 12.75 + ((247.3 - 12.75) / (1+10^{(\text{Log } 0.2041 - x) * 0.9217}))$	0.979
TS2	dsDNA (27-bp)	$y = 11.27 + ((172.1 - 11.27) / (1+10^{(\text{Log } 0.1713 - x) * 1.487}))$	0.994
TS3	dsDNA (50-bp)	$y = 18.39 + ((134.5 - 18.39) / (1+10^{(\text{Log } 0.1740 - x) * 1.512}))$	0.972
RS1	ssRNA	$y = 7.776 + ((126.4 - 7.776) / (1+10^{(\text{Log } 0.1737 - x) * 1.927}))$	0.972
Crosslink Assay			
TS1	ssDNA	$y = 8.971 + ((318.3 - 8.971) / (1+10^{(\text{Log } 4.009 - x) * 0.8699}))$	0.986
TS2	dsDNA (27-bp)	$y = 8.470 + ((37.02 - 8.470) / (1+10^{(\text{Log } 0.2191 - x) * 1.311}))$	0.967
RS1	ssRNA	$y = 6.993 + ((161.7 - 6.993) / (1+10^{(\text{Log } 0.7883 - x) * 1.279}))$	0.996

4-PL model equation: $y = A + ((D - A) / (1 + 10^{(\text{Log } C - x) * B}))$, where A is the response at a concentration of zero (baseline); B is the slope factor; C is the inflection point (IC_{50}); D is the response at infinite concentration; Y is the response; X is the analyte concentration.

Table S5 – 4-PL Model Analysis of Concentration-dependent Abs_{450nm} Results

Name	Nucleic Acid Type	4-PL Model Equation	Fit
Template Assay			
TS1	ssDNA	$y = 0.635 + ((3.65 - 0.635) / (1+10^{(\text{Log } 0.0193 - x) * 1.80}))$	0.999
TS2	dsDNA (27-bp)	$y = 1.14 + ((3.75 - 1.14) / (1+10^{(\text{Log } 0.0107 - x) * 1.52}))$	0.994
RS1	ssRNA	$y = 1.06 + ((3.68 - 1.06) / (1+10^{(\text{Log } 0.0226 - x) * 2.70}))$	0.972
Crosslink Assay[£]			
TS1	ssDNA	$y = 0.314 + ((5.76 - 0.314) / (1+10^{(\text{Log } 1.03 - x) * 1.14}))$	0.994
TS2	dsDNA (27-bp)	$y = 0.267 + ((0.660 - 0.267) / (1+10^{(\text{Log } 0.0960 - x) * 1.72}))$	0.940
RS1	ssRNA	$y = 0.264 + ((4.08 - 0.264) / (1+10^{(\text{Log } 0.378 - x) * 1.56}))$	0.995

4-PL model equation: $y = A + ((D - A) / (1 + 10^{(\text{Log } C - x) * B}))$, where A is the response at a concentration of zero (baseline); B is the slope factor; C is the inflection point (IC_{50}); D is the response at infinite concentration; Y is the response; X is the analyte concentration. [£]Data were analyzed the day after the initial kinetic assay following washing, relabeling and a second kinetic assay, as listed under "Plate Reruns" section of SI.

Limit of Quantification (LoQ) Determination:

The limits of quantitation[4] for the nucleic acid types investigated were determined using the 4-PL models with Prism 5.0 software by calculating the upper limit of the zero analyte concentration parameter error using a 90% confidence interval. These values provide a limit that is distinguishable from background noise with 96% certainty (Minimal distinguishable differential concentration – MDDC).

References:

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