Electronic Supplementary Material (ESI) for Chemical Science

Bioorthogonal Reactions Challenged: DNA Templated Native Chemical Ligation during PCR

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1 Materials and methods

1.1 Chemicals

Boc/Cbz-protected PNA-monomers were purchased from Applied Biosystems (Darmstadt, Germany) and ASM Research Chemicals (Burgwedel, Germany). Fmoc-protected amino acids and MBHA-resin were supplied by Novabiochem (Schwalbach, Germany). Boc-protected amino acids were purchased from NeoMPS (Strasbourg, France). PyBOP was acquired from Chemcube (Bochum, Germany). *6*-carboxyfluorescein (FAM) and *6*-carboxytetramethylrhodamine (TMR) were purchased from ChemPep (Wellington, FL, USA). Synthetic DNA-templates and primers were purchased from BioTez Berlin-Buch-GmbH (Berlin, Germany) in HPLC-purification grade. PCR-reagents, namely 10× reaction buffer high specificity S, 5× enhancer solution P, magnesium chloride, dNTP-mix long range and peqGOLD Taq-DNA-polymerase as well as 6× loading dye for PAGE-experiments were purchased from peqLab Biotechnologie GmbH (Erlangen, Germany). Human genomic wildtype DNA (WT-HGD) from human blood (buffy coat) was acquired from Roche Diagnostics Deutschland GmbH (Mannheim, Germany) and human genomic mutant DNA (MT-MEL) derived from the human melanoma cell line SK-MEL28 was purchased from LGC standards (Wesel, Germany) in partnership with the American Type Culture Collection (ATCC, product code: HTB-72D). Acrylamide-buffer for PAGE-analysis was supplied by Carl Roth GmbH + Co. KG (Karlsruhe, Germany). Gene Ruler™ Ultra Low Range DNA-ladder (ready-to-use) was obtained from Fermentas GmbH (St. Leon-Rot, Germany) and 10000× SYBR-Gold for gel-staining was purchased from Invitrogen (Molecular Probes). All other chemicals were purchased from Acros, Sigma-Aldrich and Fluka and used without further purification. Water was used after purification with an Astacus Milli-Q Ultra Pure Water Purification System from membraPure GmbH (Bodenheim, Germany).

1.2 Instruments and Equipment

Nuclear magnetic resonance (NMR) spectroscopy was performed on an Avance II 500 instrument from Bruker in deuterated DMSO at room temperature. Chemical shifts δ are given in ppm. Spectra were calibrated to the characteristic signals of the residual non-deuterated solvent (^{1}H: quintuplet, 2.50 ppm, ^{13}C: septuplet, 39.52 ppm). Coupling constants J are given in Herz (Hz). Abbreviations for signal multiplicities are s (singlet), d (doublet), t (triplet) and m (multiplet).

High resolution mass spectrometry was performed on a Finnigan LTQ-FT machine from ThermoElectron. Samples were dissolved in acetonitrile and ionization was achieved using electro spray (ESI). Values are given in mass-to-charge ratios (m/z).

Solid phase syntheses were performed in 2 mL polyethylene syringe reactors (Braun) equipped with a frit.

Analytical UPLC measurements were performed on a Waters Acquity UPLC system and either a Waters-X-Bridge-C18-BEH130 column (1.7µ, 50×2.1mm, pore size 130Å) or a Waters-X-Bridge-C18-CSH130 column (1.7µ, 50×2.1mm, pore size 130Å) temperated to 50 °C. Absorbance was measured using a photo diode array detector at 260 nm. Samples were eluted using solvents A (98.9% water, 1% acetonitrile, 0.1% TFA) and B (98.9% acetonitrile, 1% water, 0.1% TFA) in linear gradients (gradient I: 3% B → 30% B in 2 min, gradient II: 3% B → 15% B in 8 min) at flow rates of 0.6 - 1 $mL \cdot min^{-1}$.

Semi-preparative HPLC purification of crude PNA-conjugates was achieved by using an Agilent 1100 series instrument equipped with a Varian Polaris C18 A column (5µ, PN A 2000-250×10mm, pore size 220Å) at room temperature. Absorbance was measured using a photo diode array detector at 260 nm. Samples were eluted using solvents A (98.9% water, 1% acetonitrile, 0.1% TFA) and B (98.9% acetonitrile, 1% water, 0.1% TFA) in linear gradients (gradient III: 3% B → 30% B in 30 min) at a flow rate of 6 $mL \cdot min^{-1}$.

Purification of small amounts was carried out via analytical HPLC on a Merck-Hitachi Elite LaChrom instrument equipped with a Varian Polaris C18 A column (5µ, 250×4.6mm, pore size 220Å) at 55 °C. Absorbance was measured using a photo diode array detector at 260 nm. Samples were eluted using solvents A (98.9% water, 1% acetonitrile, 0.1% TFA) and B (98.9% acetonitrile, 1% water, 0.1% TFA) in linear gradients (gradient IV: 3% B → 40% B in 30 min) at a flow rate of 1 $mL \cdot min^{-1}$.

ESI-MS was performed on an Agilent 1100 series LC/MS quadrupol mass spectrometer. Samples were measured in 50% water, 50% acetonitrile and 0.1% FA. Values are given in mass-to-charge ratios (m/z).

MALDI-TOF mass spectra were recorded using a PerSeptive Biosystems Voyager-DE™ Pro Biospectrometry Workstation. Sinapinic acid (10 $mg \cdot ml^{-1}$ in 70% water, 30% acetonitrile, 0.1% TFA, saturated solution) was used as matrix. Values are given in mass-to-charge ratios (m/z).

UV-vis-spectroscopy for concentration determination of DNA and PNA stock solutions was performed by using a Varian Cary 100 Bio instrument using quartz cuvettes with a path length of d = 1 cm. An aliquot of the stock solution was diluted to 100 µL with buffer (10 mM NaH_2PO_4, 100 mM NaCl, pH 7.0 for DNA, TMR-labeled PNAs and competitor-PNA **8** or 100 mM NaH_2PO_4, pH 9.0 for FAM-labeled PNAs). A baseline of the used buffer was determined and measurements of the extinction E at 260 nm (for DNA), 565 nm (for TMR-labeled PNAs) or 492 nm (for FAM-labeled PNAs) yielded concentrations using Lambert-Beer-Law ($E = \varepsilon_\lambda \cdot c \cdot d$) with molar extinction coefficients ε_{260nm} given by the supplier (DNA), calculated using the nearest-neighbor model (competitor-PNA **8**),[1] or $\varepsilon_{565nm} = 91000\ L \cdot mol^{-1} \cdot cm^{-1}$ for TMR and $\varepsilon_{492nm} = 78000\ L \cdot mol^{-1} \cdot cm^{-1}$ for FAM.

Denaturation experiments for melting temperature (T_M) analyses were performed on a Varian Cary 100 Bio instrument using quartz cuvettes with a path length of d = 1 cm. PNA conjugates and DNA templates were diluted in 1 mL buffer (10 mM NaH_2PO_4, 150 mM NaCl, pH 7.4) to a final concentration of 1 µM each. For Cys-PNAs 4 mM TCEP were included to prevent oxidation during heating of the solutions (MESNa could not be used due to its immanent temperature dependent absorption at 260 nm). The solutions were heated to 95 °C (10 °C·min^{-1}), equilibrated for 5 min, cooled to 25 °C (1 °C·min^{-1}) and equilibrated again for 5 min. Absorption at 260 nm was monitored three times during heating solutions from 25 °C to 95 °C (0.5 °C·min^{-1}) with a data interval of 0.1 °C ·min^{-1}. First derivatives of melting curves were calculated using the machine software and the average of their maxima yielded the T_M.

Fluorescence spectroscopy was performed by using a Varian Cary Eclipse instrument in 1 mL quartz fluorescence cuvettes with a path length of 1 cm. For all measurements, excitation wavelength was adjusted to 470 nm and fluorescence intensities were measured at 523 nm (FAM) and 585 nm (TMR) for kinetic studies. Each data point was averaged over 1.5 sec measuring time. Fluorescence spectra were recorded from 480 to 700 nm with a scan rate of 600 nm·min^{-1}. Filter slit widths were ± 5 nm each. The photo multiplier detector voltage was set to “medium”.

Polymerase chain reactions were performed by using the iQ™5 Multicolor Real-Time Detection System from BioRad Laboratories GmbH (München, Germany) in 0.2 mL semi-skirted 96 well plates sealed with heat resistant transparent plastic foils from peqLab. Master mixes were prepared in 1.7 mL SafeSeal microcentrifuge tubes (low binding polymer

technology) from Sorenson BioScience, Inc (Salt Lake City, UT, USA). For FAM-fluorescence detection an excitation filter with a wavelength of 490 ± 20 nm and an emission filter with a wavelength of 530 ± 30 nm was used. To monitor the FRET-induced TMR-emission an excitation filter with a wavelength of 485 ± 30 nm and an emission filter with a wavelength of 585 ± 20 nm was used.

Polyacrylamide gel electrophoreses were carried out with a PS 9009 TX voltage supply from Apelex (Lisses, France). Buffer-stocks were 0.5 M EDTA-buffer (73 g EDTA filled up to 500 mL with water, pH 8 (NaOH)), 10× TBE-buffer (108 g TRIS, 55 g boric acid, 40 mL 0.5 M EDTA-buffer filled up to 1 L with water), 0.5× TBE-buffer (50 mL 10× TBE-buffer filled up to 1 L with water), 20% acrylamide-buffer (100 mL Rotiphorese® Gel 40 (19:1 acrylamide:bisacrylamide) and 10 mL 10× TBE-buffer filled up to 200 mL with water). Gels were produced as follows: For a separating gel (8% acrylamide) of 15 cm length 12 mL 20% acrylamide-buffer were filled up to 30 mL with 0.5× TBE-buffer and centrifuged for 5 min at 4300 rpm. 150 µL ammonium persulfate in water (1:9, wt:v, APS) and 30 µL tetramethylethylenediamine (TMED) were added and the solution was spilled between glass plates. After polymerization finished, the separation gel was topped with a 1 cm collecting gel layer (5% acrylamide) prepared from 2 mL 20% acrylamide-buffer filled up to 8 mL with 0.5× TBE-buffer, 40 µL APS and 8 µL TMED. Samples from PCR (5 µL) were spiked with 1 µL 6× loading dye and deposited on the gel pockets. Gel electrophoreses were performed by using 0.5× TBE-buffer at 150 - 300 Volts for 1.5 - 2 h in gel chambers at 4 °C. Afterwards, gels were isolated and stained with 20 mL 1× SYBR-Gold in 0.5× TBE-buffer. Imaging was realized on an Image Station 4000 MM PRO from Kodak ($\lambda_{ex.}$ = 470 nm, $\lambda_{em.}$ = 535 nm).

1.3 Modified PNA-monomers

The lysine-derived, Alloc-protected PNA-monomer **9** was prepared as previously described.[2] A modified procedure allowed synthesis of the lysine-derived, Fmoc-protected PNA-monomer **10** and will be published elsewhere.

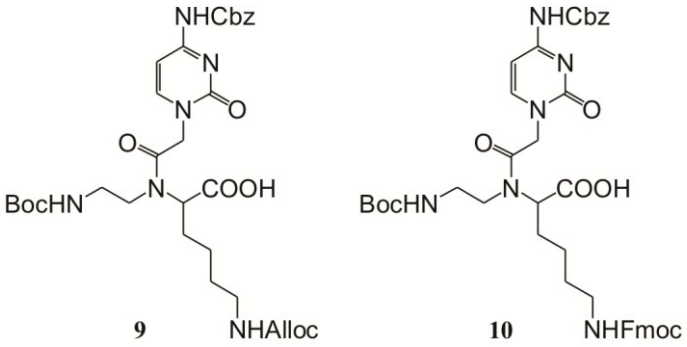


Figure S1. Structures of modified orthogonal protected PNA-monomers **9** and **10**.

Analytical data for **10**: TLC: R_f = 0.66 (ethyl acetate/methanol/formic acid 94:5:1, v:v:v); HR-FTMS (ESI): $[M+H]^+$ = 797.3505 (calculated: = 797.3505); M = 796.86 g·mol^{-1}; $C_{42}H_{48}N_6O_{10}$.

^{1}H-NMR (500 MHz, DMSO-d$_6$, both rotamers): δ = 1.23-1.32 (m, 2H, C**H$_2$**(side chain)), 1.33-1.56 (m, 11H, 3× C**H$_3$**(Boc), C**H$_2$**(side chain)), 1.67-1.91 (m, 2H, C**H$_2$**(side chain)), 2.95-3.48 (m, 6H, 2× C**H$_2$**(backbone), C**H$_2$**(side chain)), 4.20 (t, J = 6.8, 1H, C**H**(Fmoc)), 4.29 (d, J = 6.9, 2H, O-C**H$_2$**(Fmoc)), 4.37 (m, 1H, C**H**(backbone)), 4.72 and 4.82 (2× s, 2H, C**H$_2$**CO(methylene-linker)), 5.19 (s, 2H, O-C**H$_2$**(Cbz)), 6.85-6.91 (m, 1H, N**H**), 7.00 and 7.02 (2× d, J = 7.3 and 7.3, 1H, C**H**(cytosine)), 7.26-7.36 (m, 4H, 4× C**H$_{arom.}$**(Fmoc)), 7.37-7.42 (m, 5H, 5× C**H$_{arom.}$**(Cbz)), 7.68 (d, J = 7.3, 2H, 2× C**H$_{arom.}$**(Fmoc)), 7.88 (d, J = 7.5, 2H, 2× C**H$_{arom.}$**(Fmoc)), 7.93 and 7.96 (d, J = 7.4 and 7.3, 1H, C**H**(cytosine)).

APT-NMR (125 MHz, DMSO-d$_6$, both rotamers): δ = 23.04 (**C**H$_2$(side chain)), 28.05 (3× **C**H$_3$(Boc)), 28.20 (**C**H$_2$(side chain)), 29.00 (**C**H$_2$(side chain)), 38.93 (**C**H$_2$(backbone)), 39.93 (**C**H$_2$(side chain)), 45.62 (**C**H$_2$(backbone)), 46.67 (**C**H (Fmoc)), 49.80 and 50.11 (**C**H$_2$CO(methylene-linker)), 58.73 (**C**H(backbone)), 65.09 (O-**C**H$_2$(Fmoc)), 66.35 (O-**C**H$_2$(Cbz)), 77.91 (**C$_q$**(Boc)), 93.69 (**C**H(cytosine)), 119.95 (2× **C**H$_{arom.}$(Fmoc)), 125.03 (2× **C**H$_{arom.}$(Fmoc)), 126.91 (2× **C**H$_{arom.}$(Fmoc)), 127.44 (2× **C**H$_{arom.}$(Fmoc)), 127.76 (2× **C**H$_{arom.}$(Cbz)), 127.99 (**C**H$_{arom.}$(Cbz)), 128.34 (2× **C**H$_{arom.}$(Cbz)), 135.86 (**C$_q$**(Cbz)), 140.61 (2× **C$_q$**(Fmoc)), 143.83 (2× **C$_q$**(Fmoc)), 150.84 (**C**H(cytosine)), 153.09

(**C**O(Cbz)), 154.87 (**C**O(Cytosin)), 155.60 (**C**O(Boc)), 155.96 (**C**O(Fmoc)), 163.07 ($\mathbf{C}_q$(cytosine)), 167.15 (CH_2**C**O(methylene-linker)), 172.10 (**C**OOH).

1.4 Manual Solid Phase Synthesis of PNA conjugates according to Boc/Cbz-strategy

Syntheses were performed on a methylbenzhydrylamine hydrochloride (MBHA) resin, loaded with Fmoc-Gly-OH according to standard loading protocols (ca. 0.1 mmol·g^{-1}) (see: Novabiochem catalog 2010/2011). The resin was swollen in DMF (peptide synthesis grade) prior to each synthesis for 0.5 h.

Initial Fmoc-cleavage: The resin was treated with piperidine/DMF (1:4, v:v, 1 mL, 4× 5 min) and washed (15× DMF, 1 mL).

Coupling of PNA-monomers or amino acids: The resin was treated with a solution of 4 eq. protected PNA-monomer or amino acid (corresponding to resin loading), 4 eq. PyBOP and 8 eq. NMM in DMF (concentration of the monomers: ca. 0.1 M) for 2× 30 min and washed (5× DMF, 1 mL).

Capping: A mixture of DMF/*2,6*-lutidine/acetic anhydride (89:6:5, v:v:v) was added to the resin (1 mL, 1× 3 min), which was subsequently washed (5× DMF, 10× CH_2Cl_2, 1 mL).

Boc-cleavage: The resin was treated with TFA/*m*-cresol (19:1, v:v, 1 mL, 1× 5 min) and washed (10× CH_2Cl_2, 5× DMF, 1 mL).

Coupling of fluorophores:

Method a: The resin was washed (5× degassed CH_2Cl_2, 1 mL). For cleavage of the Alloc-group from modified PNA-monomer **9**, a scavenger solution containing 10 eq. dimethyl barbituric acid in 250 µL degassed CH_2Cl_2 and a cleavage solution containing 1 eq. $Pd(PPh_3)_4$ in 300 µL degassed CH_2Cl_2 were prepared and subsequently added to the resin. After 30 min, resin was washed (5× CH_2Cl_2, 5× DMF, 5× degassed CH_2Cl_2, 1 mL) and the former steps were repeated once. The resin was washed (5× CH_2Cl_2, 5× DMF, 2× dioxane/water (9:1, v:v), 2× methanol, 15× DMF, 1 mL) and treated with a solution of 4 eq. 6-carboxyfluorescein, 4 eq. PyBOP and 10 eq. NMM in DMF (concentration of the dye: ca. 0.1 M) for 2× 1 h and washed (10× DMF, 10× CH_2Cl_2).

Method b: The resin was washed (5× DMF, 1 mL). For cleavage of the Fmoc-group of the modified PNA-monomer **10**, the resin was treated with piperidine/DMF (1:4, v:v, 1 mL, 2×

5 min) and washed (15× DMF, 1 mL). A solution of 4 eq. *6*-carboxytetramethylrhodamine (TMR), 4 eq. PyBOP and 10 eq. NMM in DMF (concentration of the dye: ca. 0.1 M) was added to the resin (2× 1 h), which was subsequently washed (10× DMF, 10× CH_2Cl_2).

Cleavage from the solid support and cleavage of permanent protecting groups:

Method A: The resin was washed (5× DMF, 10× CH_2Cl_2, 1 mL), treated with a mixture of TFA/TFMSA/*m*-cresol (16:3:1, v:v:v, 700 µL, 3 h) and washed with TFA (3× 300 µL).

Method B: The resin was washed (5× DMF, 10× CH_2Cl_2, 1 mL), treated with a mixture of TFA/*m*-cresol (19:1, v:v, 1 mL, 3× 5 min), washed (10× CH_2Cl_2, 1 mL), treated with a mixture of TFA/TFMSA/*m*-cresol (16:3:1, v:v:v, 700 µL, 3 h) and washed with TFA (3× 300 µL).

Work up: The combined cleavage and washing solutions were concentrated to ca. 100 µL under reduced pressure followed by precipitation of the crude product with cold diethyl ether (1.5 mL). After centrifugation the supernatant was discarded and the precipitate was washed once with cold diethyl ether (1.5 mL). The crude product was dissolved in water/acetonitrile mixtures not exceeding 50% acetonitrile and purified by semi-preparative HPLC (gradient III). The fractions containing the desired products were combined, solvent was removed under reduced pressure and products were dissolved in degassed water. Product identity and purity was verified by UPLC (gradient I) and ESI-MS.

2 Experimental details

2.1 Synthesis and characterization of PNA conjugates

FAM-labeled PNA-thioesters were synthesized using the modified, Alloc-protected PNA-monomer **9** at the desired position, whereas for the TMR-labeled Cys-PNAs the modified, Fmoc-protected PNA-monomer **10** was used.

Ac-ccactc((CH$_2$)$_4$NHFAM)catcgaga-Gly-SR (3)

The FAM-labeled PNA-Gly-thioester **3** was synthesized in a 5 µmol scale on a MBHA-resin loaded with Fmoc-Gly-OH (0.1 mmol·g^{-1}). After the initial Fmoc-cleavage, the resin was treated with a solution of 10 eq. S-trityl-protected 3-mercaptopropionic acid, 10 eq. PyBOP and 12 eq. NMM in DMF (concentration of monomer: ca. 0.2 M) for 2× 30 min and washed (5× DMF, 1 mL). After capping, the trityl group was cleaved by treatment with TFA/(*i*Pr)$_3$SiH (19:1, v:v, 1 mL, 2× 15 min) and the resin was washed (10× CH_2Cl_2, 5× DMF, 1 mL). Coupling of Boc-Gly-OH and the PNA-sequence as well as cleavage of the Alloc-group from the internal PNA-monomer **9** and coupling of FAM (method a) was performed following the described protocol in 1.4. Cleavage from the solid support was accomplished using method A. Yield: OD_{492nm} = 8.00, 102.6 nmol, 2.1%; ESI-MS (m/z): $[M+3H]^{3+}$: 1471.8 (calculated: 1472.4), $[M+4H]^{4+}$: 1104.2 (calculated: 1104.6); UPLC: t_R = 1.02 min (gradient I); $C_{182}H_{218}N_{84}O_{50}S_1$.

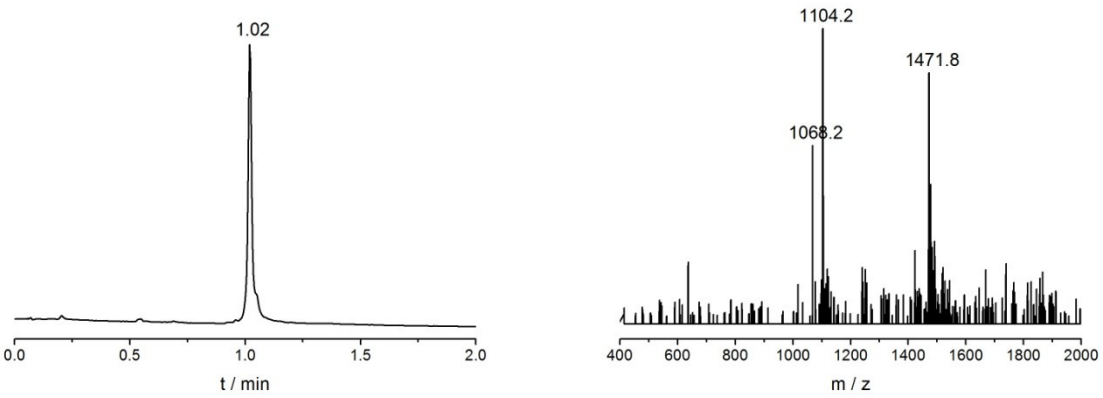


Figure S2. UPLC-trace (left) and ESI-MS spectrum (right) of purified **3** (mercaptopropionic acid thioester).

The mercaptopropionic acid thioester was converted to the corresponding MESA-thioester by shaking in MESNA-buffer (100 mM NaH_2PO_4, 100 mM MESNA, 20% acetonitrile, pH 7.4) at room temperature for 8 h and subsequent purification by semi-preparative HPLC (gradient III). ESI-MS (m/z): $[M+4H]^{4+}$: 1099.4 (calculated: 1099.3), $[M+5H]^{5+}$: 879.8 (calculated:

879.7), $[M+6H]^{6+}$: 733.3 (calculated: 733.2), $[M+7H]^{7+}$: 628.7 (calculated: 628.6); UPLC: t_R = 1.10 min (gradient I); $C_{179}H_{213}N_{82}O_{51}S_2$.

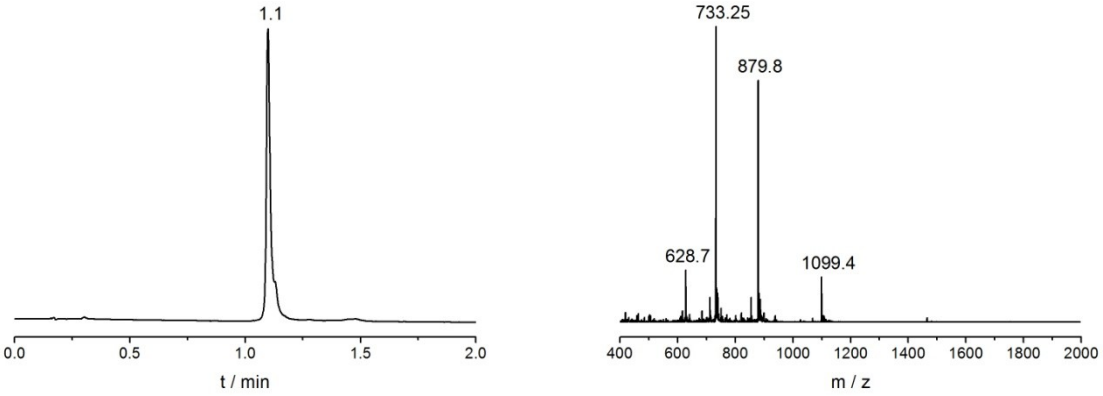


Figure S3. UPLC-trace (left) and ESI-MS spectrum (right) of purified **3** (MESA thioester).

Ac-ccactc((CH$_2$)$_4$NHFAM)catcgaga-βAla-SR (4)

The FAM-labeled PNA-βAla-thioester **4** was synthesized in a 10 µmol scale on a MBHA-resin loaded with Fmoc-Gly-OH (0.1 mmol·g^{-1}) according to the synthesis of **3**. Instead of Boc-Gly-OH, Boc-βAla-OH was coupled to the mercaptopropionic acid moiety. Yield: OD_{492nm} = 12.56, 161.0 nmol, 1.6%; ESI-MS (m/z): $[M+3H]^{3+}$: 1476.4 (calculated: 1477.1), $[M+4H]^{4+}$: 1107.6 (calculated: 1108.1); UPLC: t_R = 1.03 min (gradient I); $C_{183}H_{220}N_{84}O_{50}S_1$.

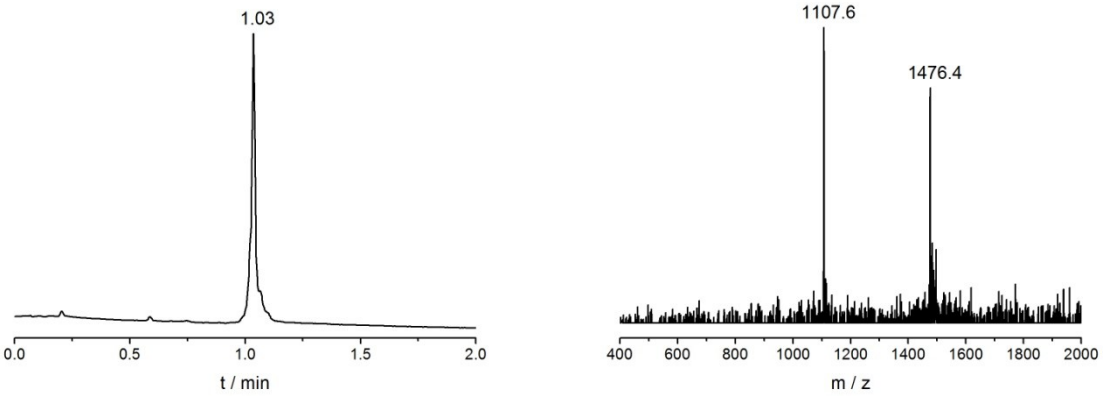


Figure S4. UPLC-trace (left) and ESI-MS spectrum (right) of purified **4** (mercaptopropionic acid thioester).

The mercaptopropionic acid thioester was converted to the corresponding MESA-thioester by shaking in MESNA-buffer (100 mM NaH_2PO_4, 100 mM MESNA, 20% acetonitrile, pH 7.4) at room temperature for 8 h and subsequent purification by semi-preparative HPLC (gradient III). ESI-MS (m/z): $[M+3H]^{3+}$: 1470.2 (calculated: 1470.1), $[M+4H]^{4+}$: 1102.9 (calculated: 1102.8), $[M+5H]^{5+}$: 882.5 (calculated: 882.5), $[M+6H]^{6+}$: 735.7 (calculated: 735.6), $[M+7H]^{7+}$: 630.8 (calculated: 630.6); UPLC: t_R = 1.19 min (gradient I); $C_{180}H_{215}N_{82}O_{51}S_2$.

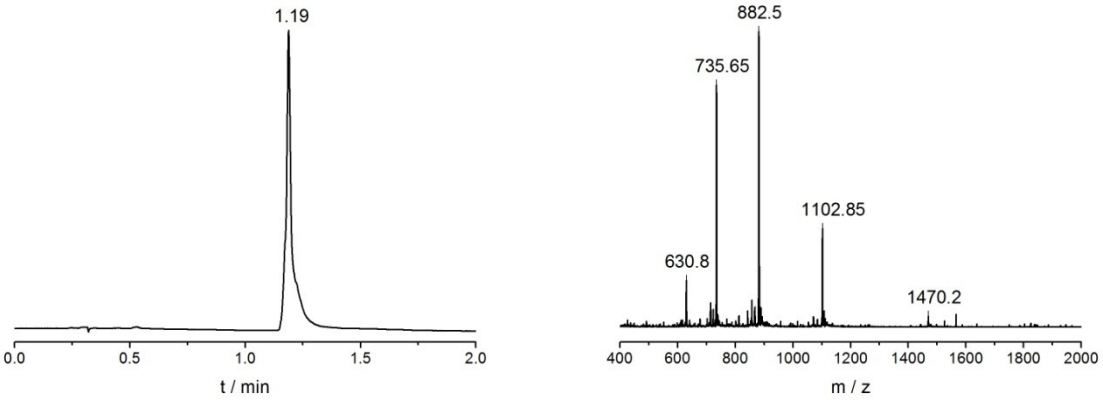


Figure S5. UPLC-trace (left) and ESI-MS spectrum (right) of purified **4** (MESA thioester).

Ac-ccactc((CH$_2$)$_4$NHFAM)catcgaga-βAla-OH (5)

20 nmol **4** were dissolved in 100 µL 0.1 M NaOH. After 2 h at room temperature the reaction was quenched by adding 100 µL TFA and the solvent was evaporated under reduced pressure. The residue was dissolved in 150 µL water/acetonitrile/TFA (98:1:1, v:v:v) and purified by analytical HPLC (gradient IV). Yield: quantitative; ESI-MS (m/z): $[M+4H]^{4+}$: 1072.0 (calculated: 1072.0), $[M+5H]^{5+}$: 857.8 (calculated: 857.8), $[M+6H]^{6+}$: 715.1 (calculated: 715.0), $[M+7H]^{7+}$: 613.1 (calculated: 613.0); UPLC: t_R = 1.03 min (gradient I); $C_{178}H_{212}N_{82}O_{49}$.

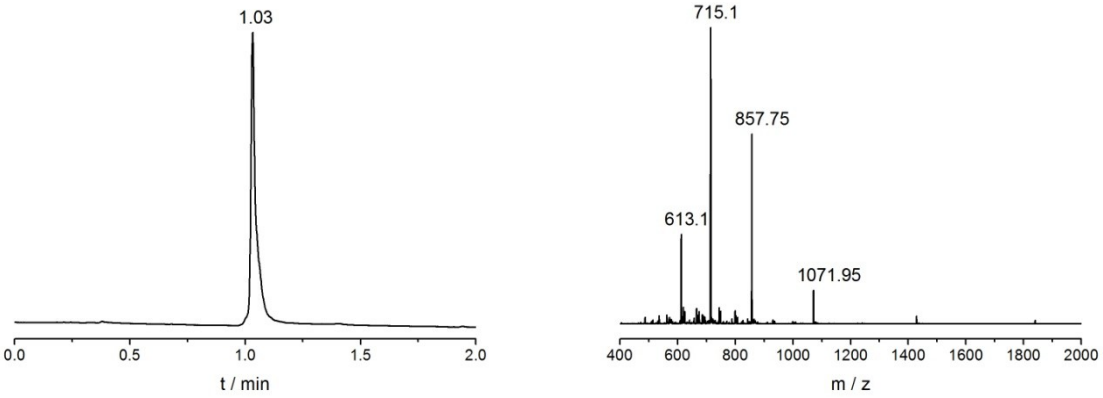


Figure S6. UPLC-trace (left) and ESI-MS spectrum (right) of purified **5**.

H-Cys-tttc((CH$_2$)$_4$NHTMR)tctgtag-Lys-Lys-Lys-Gly-NH$_2$ (6)

The TMR-labeled Cys-PNA **6** was synthesized in a 5 µmol scale on a MBHA-resin loaded with Fmoc-Gly-OH (0.1 mmol·g^{-1}). Sequence assembly, cleavage of the Fmoc-group from the internal PNA-monomer **10** and coupling of TMR (method b) was performed following the described protocol in 1.4. Cleavage from the solid support was accomplished by using method B. Yield: OD_{565nm} = 60.60, 665.9 nmol, 13.3%; ESI-MS (m/z): $[M+4H]^{4+}$: 1001.6 (calculated:

1001.8), $[M+5H]^{5+}$: 801.5 (calculated: 801.6), $[M+6H]^{6+}$: 668.2 (calculated: 668.2), $[M+7H]^{7+}$: 572.9 (calculated: 572.9); UPLC: t_R = 1.17 min (gradient I); $C_{171}H_{225}N_{67}O_{47}S_1$.

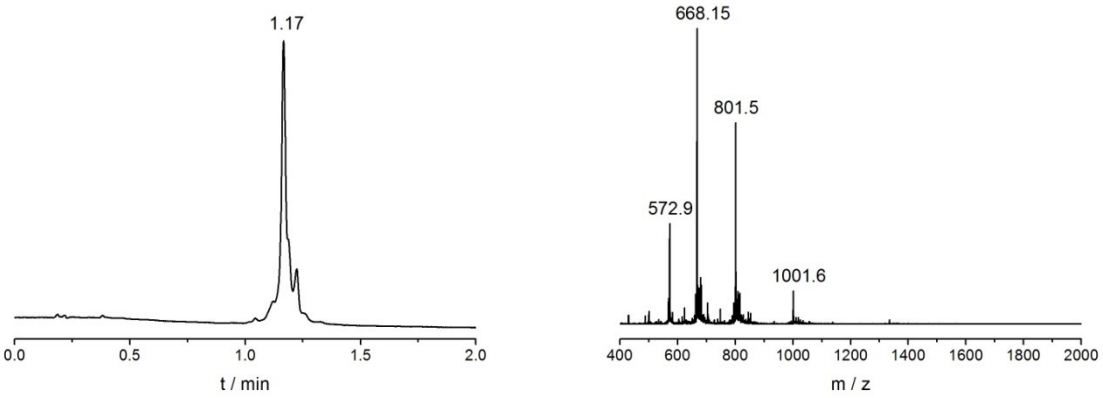


Figure S7. UPLC-trace (left) and ESI-MS spectrum (right) of purified **6**.

H-Cys-tttc((CH₂)₄NHTMR)actgtag-Lys-Lys-Lys-Gly-NH₂ (7)

The TMR-labeled Cys-PNA **7** was synthesized in a 5 µmol scale on a MBHA-resin loaded with Fmoc-Gly-OH (0.1 mmol·g^{-1}) according to the synthesis of **6**. Before Boc-Cys^{Trt}-OH and TMR were coupled, half of the batch was kept for the synthesis of **8**. Yield: OD_{565nm} = 18.00, 197.8 nmol, 7.9%; ESI-MS (m/z): $[M+4H]^{4+}$: 1003.9 (calculated: 1004.0), $[M+5H]^{5+}$: 803.3 (calculated: 803.4), $[M+6H]^{6+}$: 669.7 (calculated: 669.7), $[M+7H]^{7+}$: 574.3 (calculated: 574.2); UPLC: t_R = 1.08 min (gradient I); $C_{171}H_{224}N_{70}O_{45}S_1$.

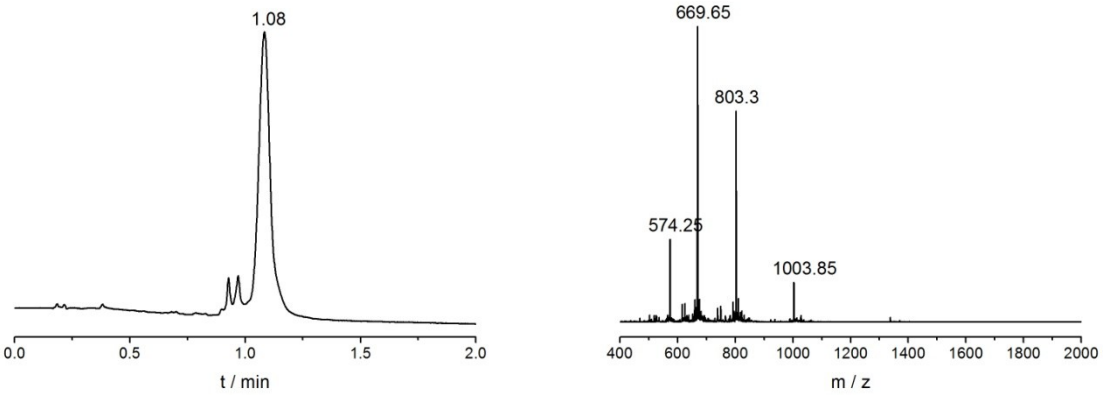


Figure S8. UPLC-trace (left) and ESI-MS spectrum (right) of purified **7**.

H-tttc((CH₂)₄NH₂)actgtag-Lys-Lys-Lys-Gly-NH₂ (8)

The unlabeled competitor-PNA **8** was synthesized from the resin kept from the synthesis of **7**. After cleavage of the Fmoc-group from the internal PNA-monomer **10** by treatment with piperidine/DMF (1:4, v:v, 1 mL, 2× 5 min) the product was cleaved from the solid support using method B. Yield: OD_{260nm} = 54.75 (ε_{260nm} = 102900 L·mol^{-1}·cm^{-1}), 532.1 nmol, 21.3%; ESI-MS (m/z): $[M+2H]^{2+}$: 1748.2 (calculated: 1749.3), $[M+3H]^{3+}$: 1166.0 (calculated:

1166.5), $[M+4H]^{4+}$: 875.0 (calculated: 875.1); UPLC: t_R = 0.63 min (gradient I); $C_{143}H_{199}N_{67}O_{40}$.

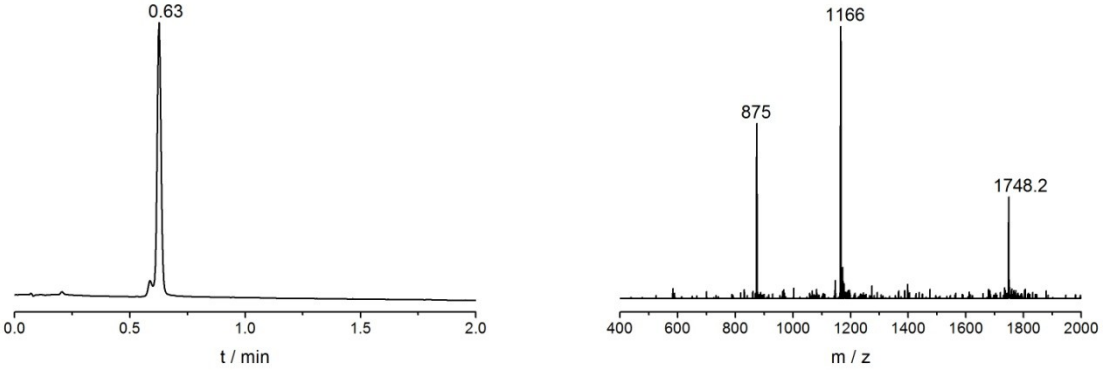


Figure S9. UPLC-trace (left) and ESI-MS spectrum (right) of purified **8**.

2.2 Experimental details for hydrolysis of PNA-thioesters

FAM-labeled PNA-Gly-thioester **3** or PNA-βAla-thioester **4** was dissolved in 200 µL hydrolysis buffer (100 mM NaH_2PO_4, pH 8.5) to concentrations of 2 µM. The mixture was agitated at 50 °C. Aliquots of 15 µL were retrieved after certain reaction times, quenched with 1 µL TFA and analyzed by UPLC (10 µL injection volume, gradient II). MALDI-TOF spectra were recorded from aliquots after 0 min, 2 h and 8 h (Figure S10).

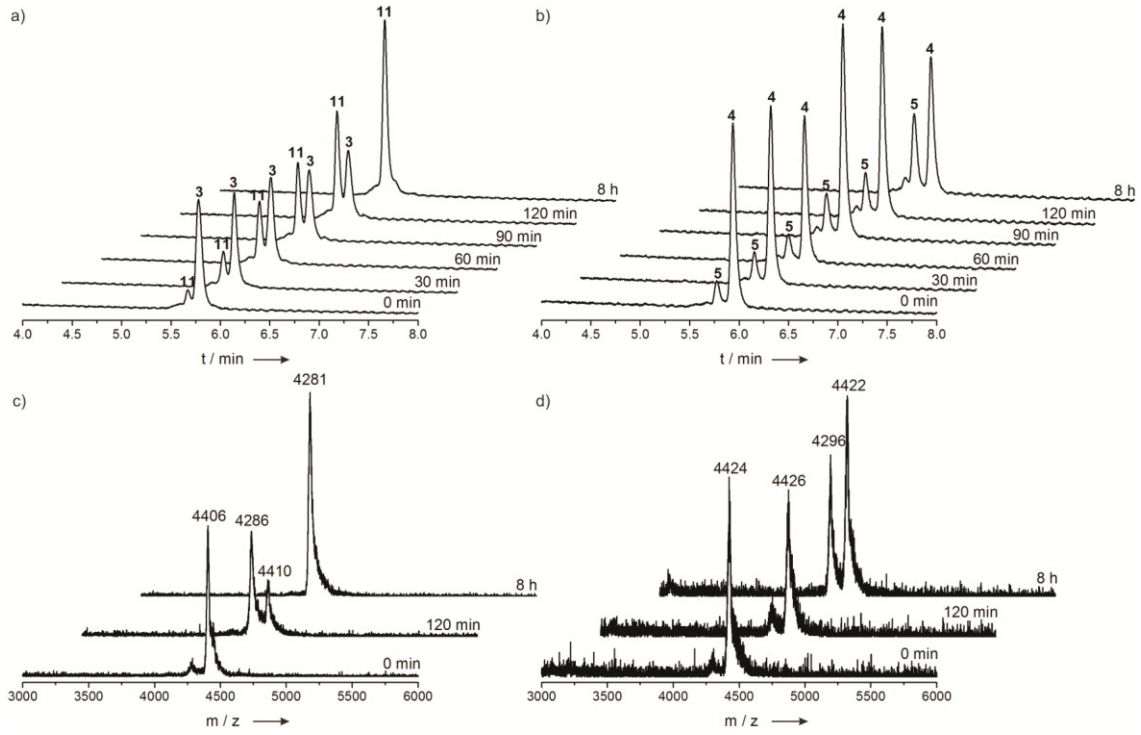


Figure S10. UPLC-traces for the hydrolysis of a) PNA-Gly-thioester **3** and b) PNA-βAla-thioester **4** and MALDI-TOF spectra of c) PNA-Gly-thioester **3** and d) PNA-βAla-thioester **4** after indicated reaction times at pH 8.5 and 50 °C (2 µM thioester, 100 mM NaH_2PO_4).

Fractions of intact thioesters were calculated from the relative peak areas of the thioesters to their corresponding hydrolysis products **11** and **5**. The impurities of hydrolyzed products at 0 min arose from thioester syntheses. Deviations in MALDI-TOF spectra from theoretical masses are probably due to the high salt content of samples, but mass differences between thioesters and carboxylic acids agree well with the expected value (ΔM = 124 g·mol^{-1}).

2.3 General procedure for the conversion of PNA conjugates with fluorescence read-out on the fluorescence spectrometer

In order to avoid oxidation of the Cys-PNAs, all manipulations were carried out with minimal exposure to oxygen under argon atmosphere. FAM-labeled PNA-thioesters (**3** or **4**) or PNA-carbonic acid (**5**) and TMR-labeled Cys-PNAs (**6** or **7**) were dissolved separately in degassed

(1 h, 140 mbar) ligation buffer (10 mM NaH_2PO_4, 150 mM NaCl, 10 mM MESNA, pH 7.4) to concentrations of 2 µM and incubated for 1 h at room temperature. Equal volumes of both solutions were combined (final concentration of PNA conjugates: 1 µM) and the resulting mixture was partitioned to fluorescence quartz cuvettes (1 mL each). For reactions containing competitor-PNA **8**, this conjugate was added to the cuvettes (5 nmol each, final concentration of competitor-PNA: 5 µM). After equilibrating the mixtures at the reaction temperature (50 °C), fluorescence spectra (0 min) were recorded. Next, kinetic measurements were started ($\lambda_{ex.}$ = 470 nm, $\lambda_{em.}$(FAM) = 523 nm, $\lambda_{em.}$(TMR) = 585 nm) and paused after 2 min. DNA-templates were added (1 nmol each, final concentration 1 µM) and the reactions were monitored for 60 min. Finally, fluorescence spectra (1 h) were recorded. To obtain the relative change of the fluorescence intensity ratio the quotient $(F_{TMR}/F_{FAM})_t/(F_{TMR}/F_{FAM})_{t=0}$ was calculated for each data point, in which the denominator was taken from the ratio of the first measured data point in the 2 min initial reaction phase in absence of the DNA-template. Signal increases in early reaction phases were obtained from the slopes of linear regressions within the quasi-linear ranges of ligations. Accelerations of signal increases due to DNA-template addition and sequence specifities were determined by dividing the slopes of the match-templated reactions by that of the no template containing controls or the mismatch-templated reactions, respectively (see below).

2.4 Experimental details for the conversion of PNA conjugates with fluorescence read-out on the fluorescence spectrometer

Figure S11 shows the early reaction phases of the conversion of FAM-labeled PNA-Gly-thioester **3** and PNA-βAla-thioester **4** with TMR-labeled Cys-PNA **6** (as shown in Figure 1c), linear regressions and their calculated slopes together with the corresponding R-squared (R^2) values.

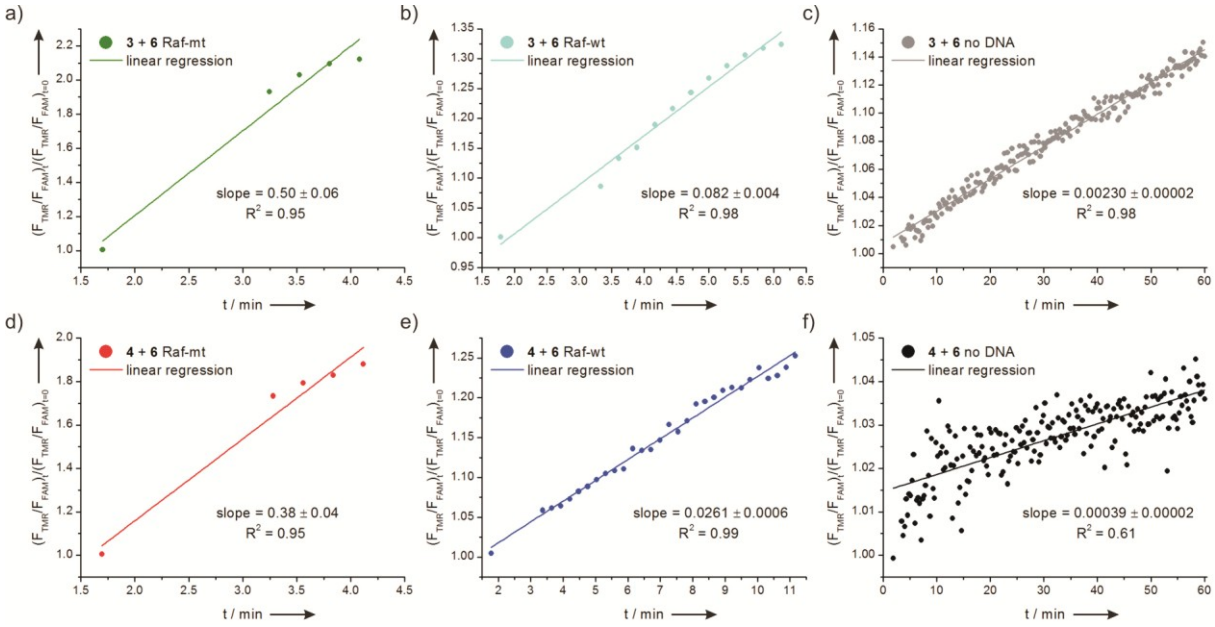


Figure S11. Relative increase of fluorescence ratio 585 nm (F_{TMR}) / 523 nm (F_{FAM}) for NCL of FAM-labeled PNA-thioester **3** or **4** with TMR-labeled Cys-PNA **6** at 50 °C ($\lambda_{ex.}$ = 470 nm) in early reaction phases with corresponding linear regressions. a) Gly-thioester **3** with Cys-PNA **6** in presence of matched DNA Raf-mt. b) Gly-thioester **3** with Cys-PNA **6** in presence of mismatched DNA Raf-wt. c) Gly-thioester **3** with Cys-PNA **6** in absence of DNA. d) βAla-thioester **4** with Cys-PNA **6** in presence of matched DNA Raf-mt. e) βAla-thioester **4** with Cys-PNA **6** in presence of mismatched DNA Raf-wt. f) βAla-thioester **4** with Cys-PNA **6** in absence of DNA (1 µM PNA conjugates, 1 µM DNA template when added, 10 mM NaH_2PO_4, 150 mM NaCl, 10 mM MESNA, pH 7.4).

Figure S12 shows the emission spectra of the above mentioned conversions before the template was added and after 1 h reaction time. Excitation was carried out at 470 nm to minimize direct excitation of the TMR-fluorophore.

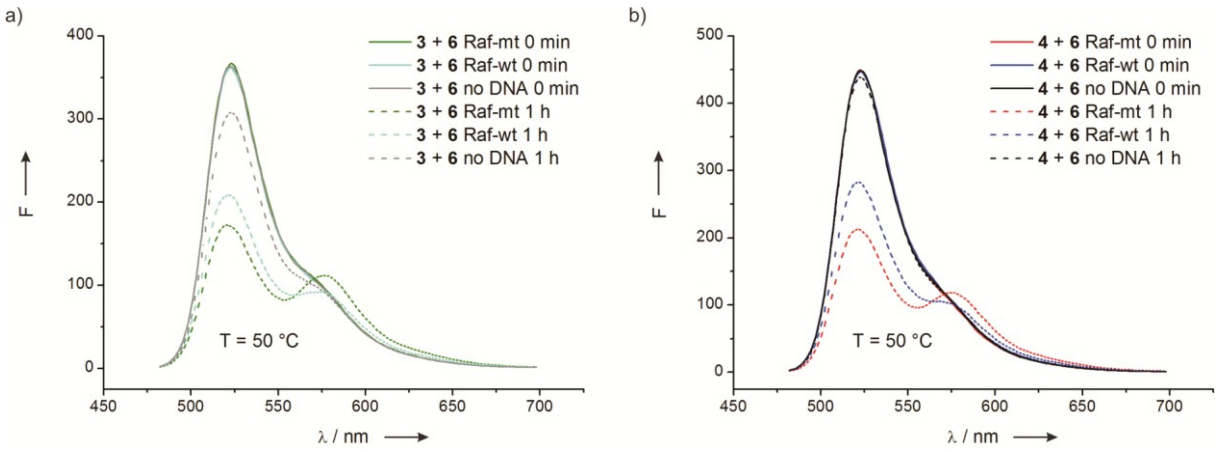


Figure S12. Emission-spectra of FAM-labeled PNA-thioester **3** or **4** with TMR-labeled Cys-PNA **6** at 50 °C before DNA-addition and after 1 h ($\lambda_{ex.}$ = 470 nm). a) Gly-thioester **3** with Cys-PNA **6**. b) βAla-thioester **4** with Cys-PNA **6** (1 µM PNA conjugates, 1 µM DNA template when added, 10 mM NaH_2PO_4, 150 mM NaCl, 10 mM MESNA, pH 7.4).

Figure S13 shows the early reaction phases of the conversion of FAM-labeled PNA-βAla-thioester **4** with wildtype-specific TMR-labeled Cys-PNA **7** (as shown in Figure 1e), linear regressions and their calculated slopes together with the corresponding R-squared (R^2) values.

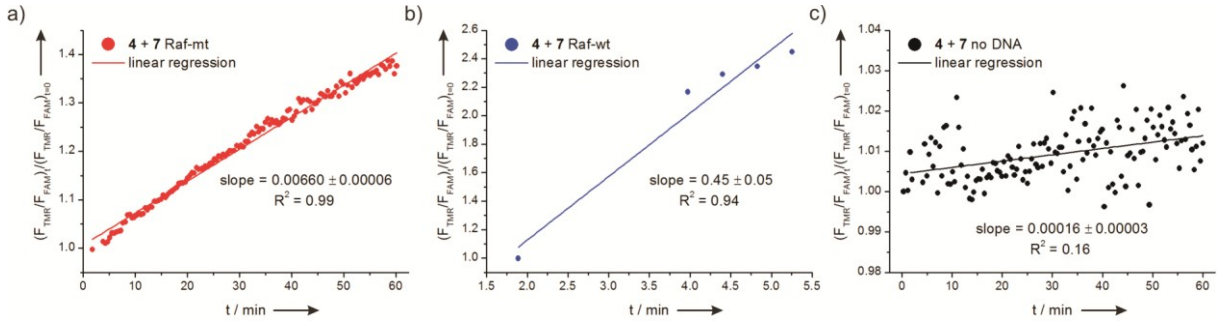


Figure S13. Relative increase of fluorescence ratio 585 nm (F_{TMR}) / 523 nm (F_{FAM}) for NCL of FAM-labeled PNA-thioester **4** with wt-specific TMR-labeled Cys-PNA **7** at 50 °C ($\lambda_{ex.}$ = 470 nm) in early reaction phases with corresponding linear regressions. a) **4** + **7** in presence of mismatched DNA Raf-mt. b) **4** + **7** in presence of matched DNA Raf-wt. c) **4** + **7** in absence of DNA (1 µM PNA conjugates, 1 µM DNA template when added, 10 mM NaH_2PO_4, 150 mM NaCl, 10 mM MESNA, pH 7.4).

Figure S14 shows the early reaction phases of the conversion of FAM-labeled PNA-βAla-thioester **4** with TMR-labeled Cys-PNA **6** in absence and in presence of 5 eq. competitor-PNA **8** (as shown in Figure 1f), linear regressions and their calculated slopes together with the corresponding R-squared (R^2) values.

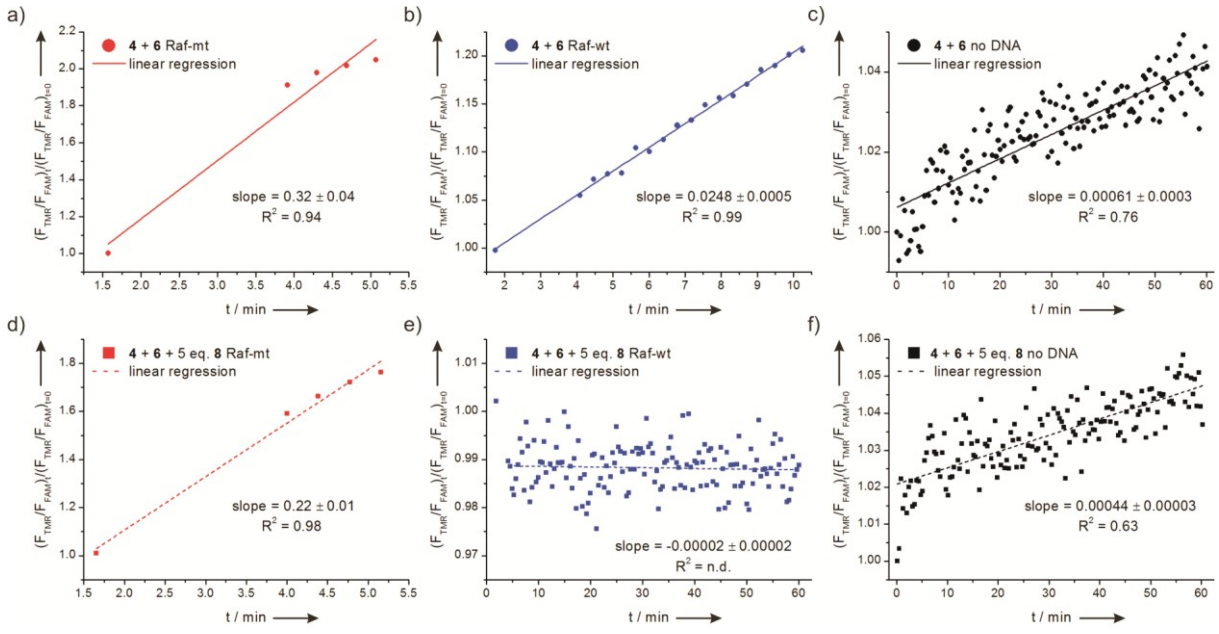


Figure S14. Relative increase of fluorescence ratio 585 nm (F_{TMR}) / 523 nm (F_{FAM}) for NCL of FAM-labeled PNA-thioester **4** with TMR-labeled Cys-PNA **6** in absence or presence of 5 eq. competitor-PNA **8** at 50 °C ($\lambda_{ex.}$ = 470 nm) in early reaction phases with corresponding linear regressions. a) **4** + **6** without **8** in presence of matched DNA Raf-mt. b) **4** + **6** without **8** in presence of mismatched DNA Raf-wt. c) **4** + **6** without **8** in absence of DNA. d) **4** + **6** + 5 eq. **8** in presence of matched DNA Raf-mt. e) **4** + **6**+ 5 eq. **8** in presence of mismatched DNA Raf-wt. f) **4** + **6** + 5 eq. **8** in absence of DNA (1 µM PNA conjugates, 5 µM competitor-PNA **8** when added, 1 µM DNA template when added, 10 mM NaH_2PO_4, 150 mM NaCl, 10 mM MESNA, pH 7.4).

2.5 General procedure for the conversion of PNA conjugates with fluorescence read-out during PCR

FAM-labeled PNA-βAla-thioester (**4**) or PNA-carbonic acid (**5**) and TMR-labeled Cys-PNAs (**6** or **7**) were dissolved separately under argon atmosphere in degassed (1h, 140 mbar) buffer (100 mM TRIS, 10 mM MESNA, pH 8.5) to a twentyfold (4 - 6 µM) of the final concentration in the PCR-mix and incubated for 1 h at room temperature. PCR master mixes, containing argon saturated water, reaction buffer high specificity S (final concentration: 1×), enhancer solution P (final concentration: 1×), magnesium chloride (final concentration: 2.5 mM), dNTP-mix long range (final concentration: 200 µM each dNTP), forward-primer (final concentration: 400 nM), reverse-primer (final concentration: 400 nM for symmetric PCR, 50 nM for asymmetric PCR), Taq-DNA-polymerase (final concentration: 1 u) and the denoted amount of human genomic DNA template WT-HGD or MT-MEL (100 - 0.01 ng, in the no template controls (NTCs) DNA was replaced by water) were prepared on ice. PNA conjugates were subsequently spiked to the master mixes intermitted by vortexing, to avoid a template independent background reaction at higher concentrations. This gave final concentrations of 200 - 300 nM PNA conjugates, 10 mM TRIS and 1 mM MESNA. 3× 20 µL (triple experiments) of each reaction mixture were transferred to a well-plate, sealed with a plastic foil and centrifuged for 20 sec at 1300 rpm to destroy any air bubbles. Temperature protocol of PCR included initial denaturation at 95 °C (20 sec.), followed by 60 - 70 cycles of denaturation (95 °C, 10 sec.), primer-annealing (50 °C, 30 sec.) and elongation (72 °C, 20 sec.) and finally cooling to 4 °C until analysis by gel electrophoresis. Fluorescence detection was carried out during the 30 sec. annealing step by exciting the FAM-fluorophore at $\lambda_{ex.} = 490$ nm (FAM-detection) and 485 nm (TMR-detection) and measuring FAM-fluorescence at $\lambda_{em.}$(FAM) = 530 nm and TMR-fluorescence at $\lambda_{em.}$(TMR) = 585 nm. To obtain the normalized FRET signal the quotient $(F_{TMR}/F_{FAM})_t/(F_{TMR}/F_{FAM})_{cycle20}$ was calculated for each data point (the denominator was taken from the ratio of the measured data point in cycle 20 of each PCR experiment, where the amount of PCR-product is still small) and normalized to the maximal value (set to 1) and the value in cycle 20 (set to 0). In all figures, one representative data curve for each experiment is shown. PAGE-analysis of PCR-products was accomplished as described above.

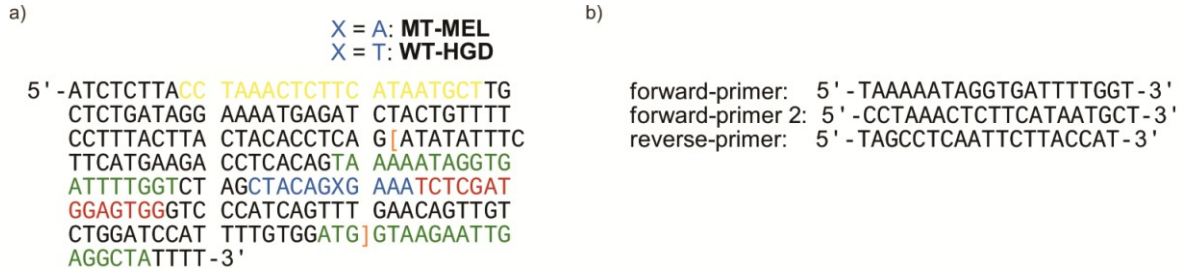


Figure S15. a) Section of the human BRaf-gene containing the V600E (T1799A) point mutation site (X) in the exon 15 (embedded in orange brackets). The binding site for the TMR-labeled Cys-PNA (**6** or **7**) is marked blue, for the FAM-labeled PNA-thioester (**4**) or -carbonic acid (**5**) red and primer annealing sites are marked green and yellow (forward-primer 2). The mutant DNA (derived from the human melanoma cell line SK-MEL28) harbors the mutation (X = A, MT-MEL) whereas the wildtype DNA lacks it (X = T, WT-HGD). b) Sequences of used forward- and reverse-primers.

2.6 Experimental details for the conversion of PNA conjugates with fluorescence read-out during PCR

2.6.1 PCR efficiency in presence of sodium 2-mercaptoethane sulfonate (MESNA)

A PCR containing 10 ng of wildtype human genomic DNA (WT-HGD) template or water (NTC) and the intercalator dye SYBR-Gold was spiked with different amounts of MESNA (0, 1 or 10 mM final concentration) and fluorescence intensity of the dye was followed over the course of the PCR (Figure S16a). Fluorescence increases as well as threshold cycle numbers were not affected by MESNA-concentrations of 1 or 10 mM in the template containing reactions, compared to the no MESNA containing control. After later cycle numbers SYBR-Gold emission also increased for the NTCs. This was caused by the formation of primer dimers. PAGE-analysis revealed that the desired 208 bp PCR-products were formed in all template containing reactions, whereas the bands corresponding to the NTCs are in agreement with the assumption that primer dimers have the approximate doubled length of a single primer (Figure S16b).

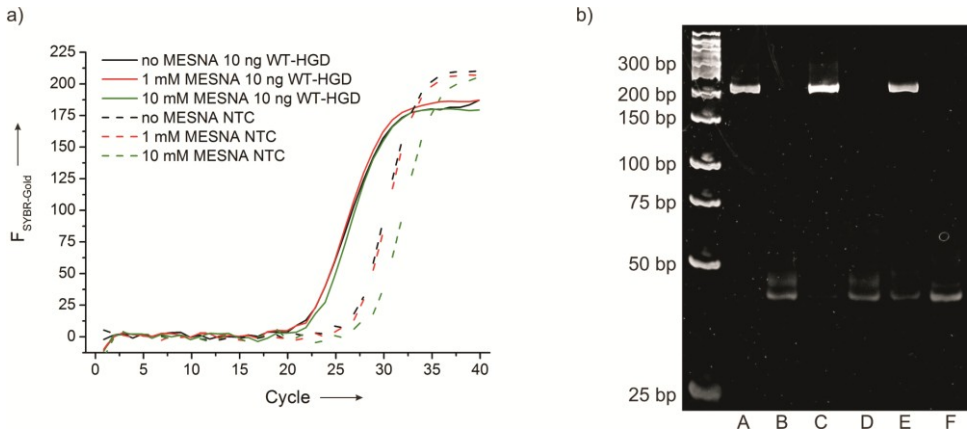


Figure S16. a) SYBR-Gold fluorescence during PCR containing different concentrations of MESNA (100 nM forward primer 2, 100 nM reverse primer, 200 µM dNTPs, 2.5 mM $MgCl_2$, 0 - 10 mM MESNA, 10 mM TRIS, pH 8.5, 1 u Taq-Pol, 10 ng template or NTC, PCR protocol: 10 sec. 95 °C, 20 sec. 50 °C (detection), 20 sec. 72 °C, $\lambda_{ex.} = 490$ nm, $\lambda_{em.} = 530$ nm). b) PAGE-analysis of PCR-products. Lane A: no MESNA, 10 ng WT-HGD; lane B: no MESNA, NTC; lane C: 1 mM MESNA, 10 ng WT-HGD; lane D: 1 mM MESNA, NTC; lane E: 10 mM MESNA, 10 ng WT-HGD; lane F: 10 mM MESNA, NTC (native gel, 8% polyacrylamide, staining: SYBR-Gold).

2.6.2 PCR efficiency in presence of reactive PNA conjugates

A PCR containing 10 ng of wildtype human genomic DNA (WT-HGD) template or water (NTC) and 10 mM MESNA was spiked with different amounts of FAM-labeled PNA-thioester **4** and TMR-labeled Cys-PNA **7** (1000, 500 or 100 nM final concentration). PAGE-analysis revealed that the desired 208 bp PCR-products were formed in all template containing reactions (Figure S17).

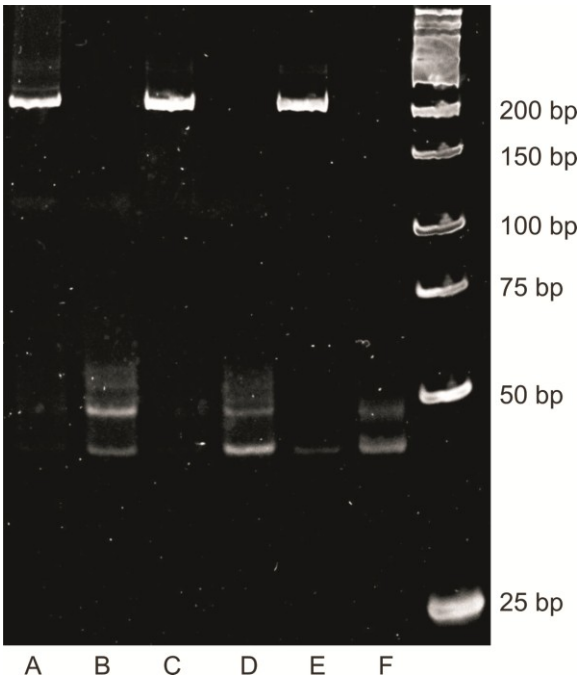


Figure S17. PAGE-analysis of PCR-products (PCR conditions: 100 nM forward primer 2, 100 nM reverse primer, 200 µM dNTPs, 2.5 mM $MgCl_2$, 1000 – 100 nM PNA conjugates, 10 mM MESNA, 10 mM TRIS, pH 8.5, 1 u Taq-Pol, 10 ng template or NTC, PCR protocol: 10 sec. 95 °C, 20 sec. 50 °C (detection), 20 sec. 72 °C). Lane A: 1000 nM PNA conjugates, 10 ng WT-HGD; lane B: 1000 nM PNA conjugates, NTC; lane C: 500 nM PNA conjugates, 10 ng WT-HGD; lane D: 500 nM PNA conjugates, NTC; lane E: 100 nM PNA conjugates, 10 ng WT-HGD; lane F: 100 nM PNA conjugates, NTC (native gel, 8% polyacrylamide, staining: SYBR-Gold).

2.6.3 Comparison of PNA ligation and adjacent hybridization of fluorophore-labeled PNA conjugates during PCR

A PCR containing either the reactive pair **4** + **7** (PNA-thioester + Cys-PNA) or the unreactive pair **5** + **7** (PNA-carbonic acid + Cys-PNA) was carried out. Signal increase was significantly lower with unreactive PNA conjugates (Figure S18a). Polyacrylamide gel electrophoresis of the amplification products of PCRs are shown in Figure S18b. The expected 108 bp PCR-products were generated in both template containing reactions (note that a different forward primer was used than for the MESNA and PNA conjugate concentration screenings in Figure S16 and S17).

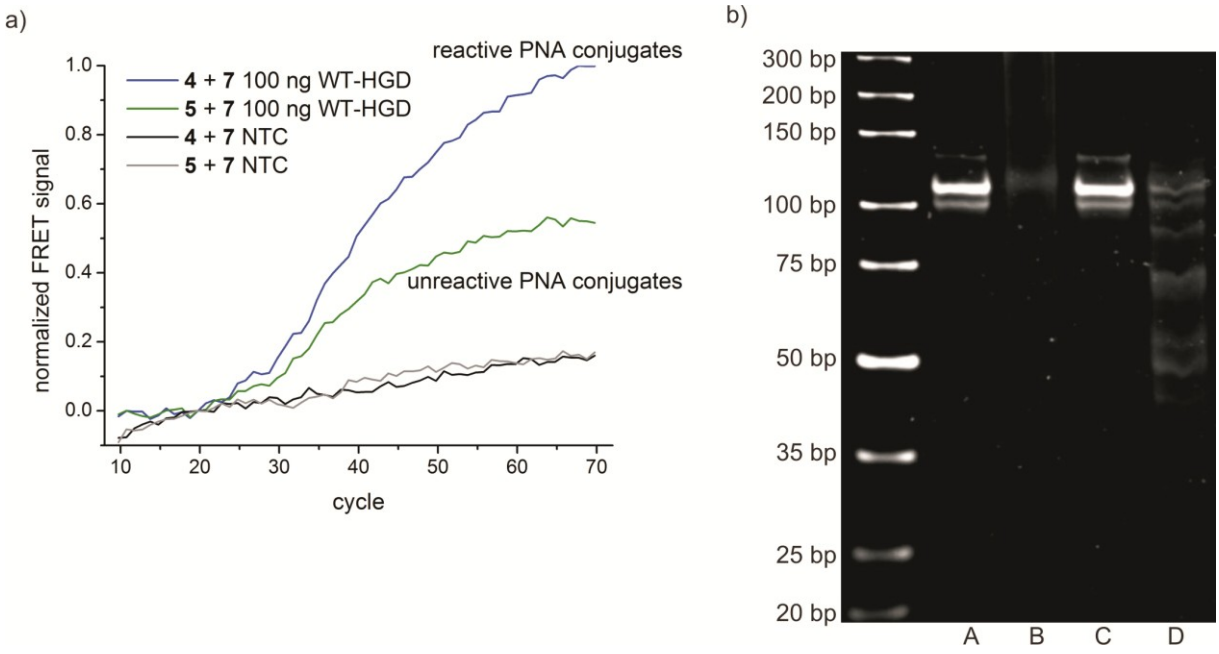


Figure S18. a) Normalized FRET signal 585 nm (F_{TMR}) / 530 nm (F_{FAM}) for the conversion of fluorophore-labeled reactive (**4** + **7**) or unreactive (**5** + **7**) pair in presence or absence of matched-template WT-HGD (400 nM forward primer, 50 nM reverse primer, 200 µM dNTPs, 2.5 mM $MgCl_2$, 300 nM PNA conjugates, 1 mM MESNA, 10 mM TRIS, pH 8.5, 1 u Taq-Pol, 100 ng template (when added), PCR protocol: 10 sec. 95 °C, 30 sec. 50 °C (detection), 20 sec. 72 °C, $\lambda_{ex.}$ = 490 nm (FAM), 485 nm (TMR), $\lambda_{em.}$ = 530 nm (FAM), 585 nm (TMR)). b) PAGE-analysis of PCR-products. Lane A: reactive pair **4** + **7**, 100 ng WT-HGD; lane B: reactive pair **4** + **7**, NTC; lane C: unreactive pair **5** + **7**, 100 ng WT-HGD; lane D: unreactive pair **5** + **7**, NTC (native gel, 8% polyacrylamide, staining: SYBR-Gold).

2.6.4 Scope of the PCR-based PNA ligation system

Polyacrylamide gel electrophoresis of the amplification products of PCRs containing the WT-specific pair **4** + **7** and different amounts of match-template WT-HGD or mismatch template MT-MEL are shown in Figure S19. The expected 108 bp PCR-products were generated in all template containing reactions.

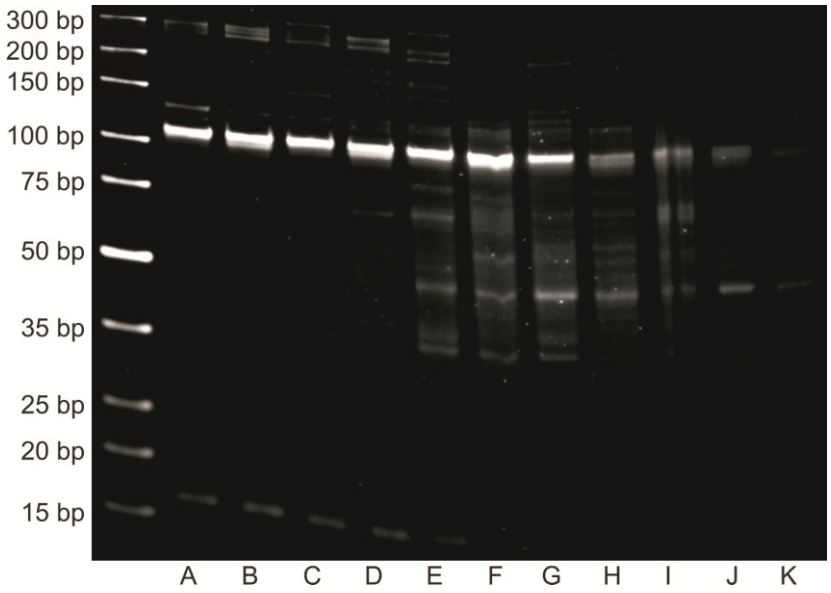


Figure S19. PAGE-analysis of PCR-products from experiments shown in Figure 2b. Lane A: 100 ng matched DNA WT-HGD; lane B: 100 ng mismatched DNA MT-MEL; lane C: 10 ng matched DNA WT-HGD; lane D: 10 ng mismatched DNA MT-MEL; lane E: 1 ng matched DNA WT-HGD; lane F: 1 ng mismatched DNA MT-MEL; lane G: 0.1 ng matched DNA WT-HGD; lane H: 0.1 ng mismatched DNA MT-MEL; lane I: 0.01 ng matched DNA WT-HGD; lane J: 0.01 ng mismatched DNA MT-MEL; lane K: NTC (native gel, 8% polyacrylamide, staining: SYBR-Gold).

Polyacrylamide gel electrophoresis of the amplification products of PCRs containing the MT-specific pair **4** + **6** and different amounts of match-template MT-MEL or mismatch template WT-HGD are shown in Figure S20. The expected 108 bp PCR-products were generated in all template containing reactions.

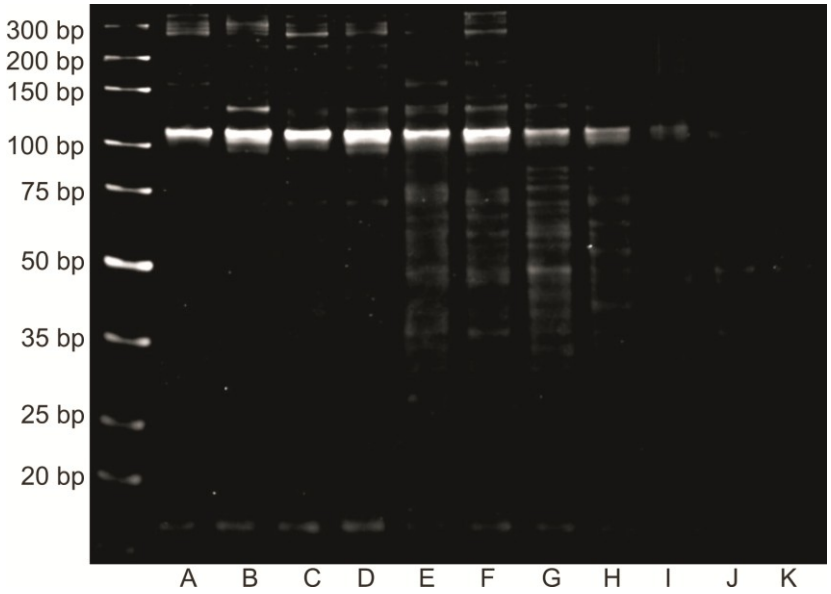


Figure S20. PAGE-analysis of PCR-products from experiments shown in Figure 2c. Lane A: 100 ng matched DNA MT-MEL; lane B: 100 ng mismatched DNA WT-HGD; lane C: 10 ng matched DNA MT-MEL; lane D: 10 ng mismatched DNA WT-HGD; lane E: 1 ng matched DNA MT-MEL; lane F: 1 ng mismatched DNA WT-HGD; lane G: 0.1 ng matched DNA MT-MEL; lane H: 0.1 ng mismatched DNA WT-HGD; lane I: 0.01 ng matched DNA MT-MEL; lane J: 0.01 ng mismatched DNA WT-HGD; lane K: NTC (native gel, 8% polyacrylamide, staining: SYBR-Gold).

Assuming that the average molecular weight of a base pair is 650 $g \cdot mol^{-1}$ and the human genome consists of approximately $3 \cdot 10^9$ base pairs, the copy number can be calculated using Avogadro's constant. The lowest template load that triggered a signal gain (0.1 ng) corresponds to ca. 30 template molecules and in a volume of 20 µL this corresponds to a concentration of ca. 2.5 attomolar. Lower amounts of template probably did not lead to a signal increase because of insufficient template amplification (Lanes I and J in Figures S19 and S20).

2.7 Abbreviations

Ac	acetyl
Alloc	allyloxycarbonyl
APS	ammonium persulfate
a. u.	arbitrary units
Boc	*tert*-butyloxycarbonyl
Cbz	benzyloxycarbonyl
DMF	*N*, *N*-dimethyl formamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
eq.	equivalent(s)
ESI	electrospray ionization
et al.	et alii
FA	formic acid
FAM	*6*-carboxyfluorescein
Fmoc	9-fluorenylmethyloxycarbonyl
FRET	Förster resonance energy transfer
HPLC	high performance liquid chromatography
HR-FTMS	high resolution Fourier-transform mass spectrometry
MALDI	matrix assisted laser desorption ionization
MBHA	*para*-methylbenzhydrylamine
MESA	2-mercaptoethanesulfonic acid
MESNA	sodium-2-mercaptoethanesulfonate

MS	mass spectrometry
NCL	native chemical ligation
NMM	*N*-methyl morpholine
NMR	nuclear magnetic resonance
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PNA	peptide nucleic acid
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
Raf	fibroblastic rat sarcoma
R_f	retention factor
RFU	relative fluorescence units
rpm	rounds per minute
TFA	trifluoroacetic acid
TFMSA	trifluoromethanesulfonic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMED	tetramethylethylenediamine
TMR	*6*-carboxytetramethylrhodamine
TOF	time of flight
t_R	retention time
Trt	trityl
UV	ultraviolet
vis	visible

2.8 References

[1] http://biophysics.idtdna.com/UVSpectrum.html; also see : A. V. Tataurov, Y. You, R. Owczarzy, *Biophys. Chem.* **2008**, *133*, 66-70.

[2] C. Dose, O. Seitz, *Bioorg. Med. Chem.* **2008**, *16*, 65-77.

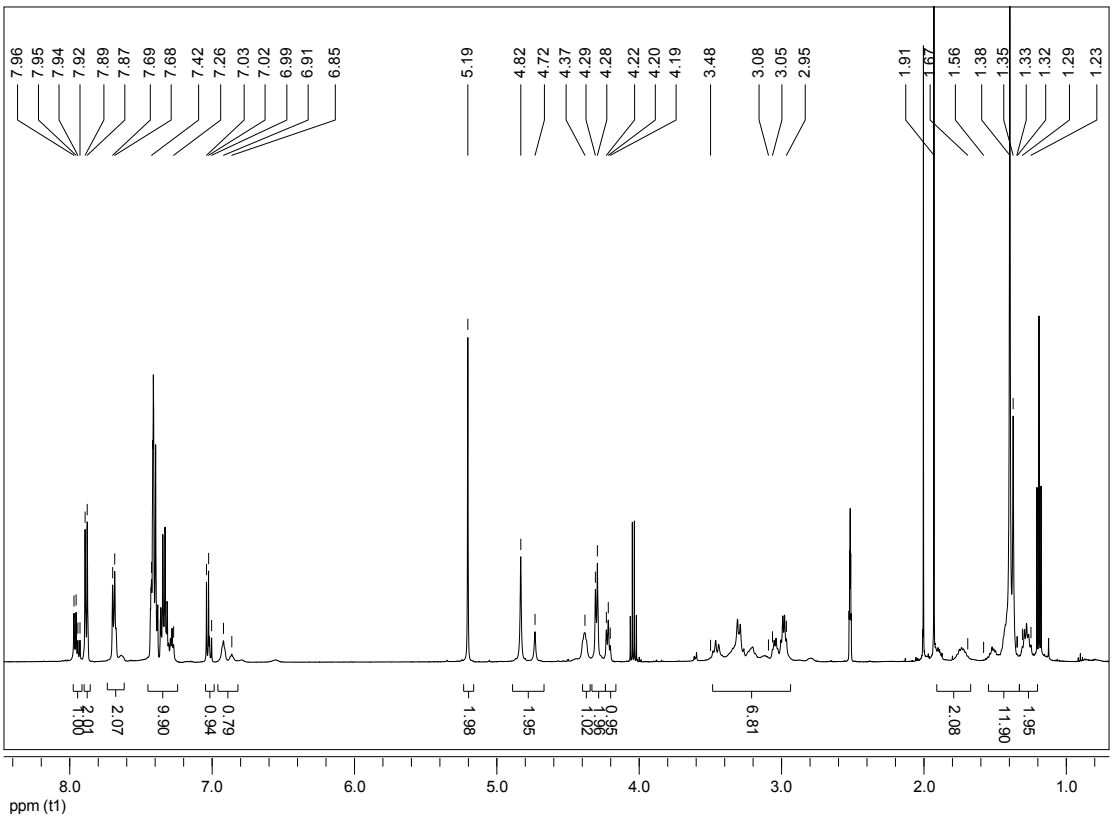
Complete citation of reference 16:

a) H. Davies, G. R. Bignell, C. Cox, P. Stephens, S. Edkins, S. Clegg, J. Teague, H. Woffendin, M. J. Garnett, W. Bottomley, N. Davis, E. Dicks, R. Ewing, Y. Floyd, K. Gray, S. Hall, R. Hawes, J. Hughes, V. Kosmidou, A. Menzies, C. Mould, A. Parker, C. Stevens, S. Watt, S. Hooper, R. Wilson, H. Jayatilake, B. A. Gusterson, C. Cooper, J. Shipley, D. Hargrave, K. Pritchard-Jones, N. Maitland, G. Chenevix-Trench, G. J. Riggins, D. D. Bigner, G. Palmieri, A. Cossu, A. Flanagan, A. Nicholson, J. W. C. Ho, S. Y. Leung, S. T. Yuen, B. L. Weber, H. F. Seigler, T. L. Darrow, H. Paterson, R. Marais, C. J. Marshall, R. Wooster, M. R. Stratton, P. A. Futreal, *Nature* **2002**, *417*, 949-954;

b) P. M. Pollock, U. L. Harper, K. S. Hansen, L. M. Yudt, M. Stark, C. M. Robbins, T. Y. Moses, G. Hostetter, U. Wagner, J. Kakareka, G. Salem, T. Pohida, P. Heenan, P. Duray, O. Kallioniemi, N. K. Hayward, J. M. Trent, P. S. Meltzer, *Nat. Genet.* **2003**, *33*, 19-20.

2.9 NMR-spectra of modified PNA-monomer 10

^{1}H-NMR (500 MHz, DMSO-d_6):



APT-NMR (125 MHz, DMSO-d_6):