Supporting information:

Fluorescence-based Detection of Single Nucleotide Permutation in DNA via Catalytically Templated Reaction

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1. General experimental procedures

Reactions for the synthesis of PNAs and their monomers were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions. Anhydrous solvents were obtained by passing them through commercially available alumina column (Innovative technology, Inc., MA). PNA monomers were prepared as previously described. DNA oligomers were purchased from Sigma Proligo. Substituted polystyrene resins (100-200 mesh, 1% DVB) were purchased from Novabiochem. Deionized water was obtained by purification with a Mili-Q Ultra Pure Water system. Reversed-phase chromatography was performed using pre-packed columns Supelco Discovery DSC-18. NMR spectra were recorded on Bruker Advance-400 instrument and calibrated using residual undeuterated solvent as an internal reference. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad. IR spectra were measured using a FT-IR Nicolet Magna 200 spectrophotometer, in reflective mode (ATR) directly from solid or liquid pure samples; wave numbers are given in cm$^{-1}$. The mass spectra were measured on a Surveyor MSQ spectrometers (ESI) using Hewlett Packard Series 1100 HPLC, column Interchrom C18, US0550, HS Strategy 3 RP, 50x2 (mm), particle size 3.0μm 0-1 min with water and acetonitrile containing 0.01% of TFA: 0% MeCN, 1-5 min: 0-100% MeCN, 5-6 min: 100% MeCN, 6-7 min 100%-0% MeCN, 7-8 min 0% MeCN. The MALDI spectra were measured using Brucker Daltonics AutoflexII TOF/TOF spectrometer. Automated PNA syntheses were performed on an Applied biosystems Expdite 9000. The fluorescence measurements were made using microplate fluorometer GeminiXS (Molecular Devices).

Preparation of the (O-TBS)-serine-modified PNA monomer 4-S:

![Scheme S-1. Synthesis of 2-(O-TBDMS-hydroxymethyl)-PNA monomer.](image)

Supplementary information 2
Fmoc-Ser-OH (17): (S)-serine 10 (1.05 g, 10.0 mmol, 1.0 equiv) was dissolved in 50 mL of sat. aq. NaHCO₃. Fmoc-OSu (3.37 g, 10.0 mmol, 1.0 equiv) was dissolved in acetonitrile (50 mL). The solutions were mixed and stirred for 12 h after which the acetonitrile was evaporated and the resulting mixture was extracted with EtOAc (3 x 50 mL). The organic layers were combined and washed 3x100 mL 0.5% aqueous HCl, 2x100 mL brine, dried with Na₂SO₄ and concentrated in vacuo to afford 3.02 g of 17 as a white solid (92% yield).

**1H NMR (CDCl₃) δ (ppm):**

7.69 (2H, d, J = 7.5 Hz), 7.55 (2H, t, J = 7.5 Hz), 7.43 (1H, br s), 7.33 (2H, t, J = 7.5 Hz), 7.24 (2H, t, J = 7.5 Hz), 6.75 (1H, br s), 6.34 (1H, d, J = 7.5 Hz), 4.46 (1H, m), 4.32 (2H, m), 4.11 (1H, m), 4.10-3.80 (2H, br m).

**13C NMR δ (ppm):**

173.8, 156.8, 143.6, 141.2, 127.7, 127.1, 125.1, 119.9, 67.4, 62.8, 55.8, 46.9.

**MS ESI [M+H]+:** 328.15, RT = 5.18 min.

**HRMS MALDI (m/z):** C₁₈H₁₇NO₅Na [M+Na]+ = calc. 350.1005, measured 350.1004.

**IR ν (cm⁻¹):** 3310, 2964, 1694, 1539, 1449, 1337, 1254, 1050.

Fmoc-Ser-OBn (11): Fmoc-Ser-OBn 17 (2.75 g, 8.4 mmol, 1.0 equiv) was dissolved in CH₂Cl₂ (50 mL) and benzyl alcohol (4.35 mL, 42 mmol, 5.0 eq) was added prior to cooling the reaction mixture to 0 °C. Thionyl chloride (0.92 mL, 12.6 mmol, 1.5 equiv) was added dropwise and the solution was stirred for 3 h at 0°C. Another portion of SOCl₂ (0.92 mL, 12.6 mmol, 1.5 equiv) was then added and reaction was stirred at 23 °C for 6 h before quenching with sat. aq. NaHCO₃ (100 mL). The mixture was extracted EtOAc (2 x 100 mL) and the combined organic layers were washed brine (3 x 100 mL), dried over Na₂SO₄ and evaporated. The crude product was purified with flash chromatography (hexanes:EtOAc 3:1) affording 2.55 g (73% yield) of the desired ester 11 as white solid.

**1H NMR (CDCl₃) δ (ppm):**

7.79 (2H, d, J = 7.5 Hz), 7.62 (2H, d, J = 7.5 Hz), 7.44 (2H, t, J = 7.5 Hz), 7.38 (5H, s), 7.34 (2H, t, J = 7.5 Hz), 5.77 (1H, d, J = 6.4 Hz), 5.26 (2H, s), 4.52 (1H, m), 4.45 (2H, d, J = 3.8 Hz), 4.25 (1H, ddd, J = 6.4 Hz, 1.6 Hz, 1.5 Hz), 4.06 (1H, dd, J = 10.5 Hz, 1.6 Hz), 3.97 (1H, dd, J = 10.5 Hz, 1.5 Hz), 2.47 (1H, br s).

**13C NMR δ (ppm):** 143.6, 141.3, 135.1, 128.7, 128.6, 128.2, 127.7, 127.1, 127.0, 125.0, 120.0, 67.6, 67.3, 63.3, 56.1, 47.1 (two quaternary carbons missing)

**MS ESI [M+H]+:** 418.21, RT = 4.98 min.

**HRMS MALDI (m/z):** C₂₅H₂₃NO₅Na [M+Na]+ = calc. 440.1474, measured 440.1485.

**IR ν (cm⁻¹):** 3065, 2948, 1717, 1519, 1450, 1338, 1195, 1059, 737.
**Fmoc-Ser(TBS)-OBn (18):** Fmoc-Ser-OBn (11) (2.33 g, 5.58 mmol, 1.0 equiv) was dissolved in DMF (10 mL) together with imidazole (1.52 g, 22.32 mmol, 4.0 equiv) and tert-butyldimethylsilyl chloride (1.68 g, 11.16 mmol, 2.0 eq) was added. The reaction was stirred for 5 h at 23°C then quenched with brine (100 mL) and extracted with EtOAc (3 x 50 mL). The organic layers were combined and washed brine (3 x 100 mL), dried with Na₂SO₄ and concentrated in vacuo affording 2.62 g of a crude product which was engaged directly in the subsequent step. **¹H NMR (CDCl₃)** δ(ppm): 7.85 (2H, d, J = 7.3 Hz), 7.68 (2H, t, J = 6.8 Hz), 7.47-7.34 (9H, m), 5.76 (1H, d, J = 8.8 Hz), 5.26 (1H, d, J = 3.9 Hz), 5.26 (1H, d, J = 3.9 Hz) 4.56 (1H, dt, J = 2.5 Hz, 8.8 Hz), 4.46-4.41 (2H, m), 4.30 (1H, ddd, J = 8.8 Hz, 3.0 Hz, 2.6 Hz), 4.18 (1H, dd, J = 10.0 Hz, 2.6 Hz), 3.94 (1H, dd, J = 10.0 Hz, 3.0 Hz), 0.94 (9H, s), 0.07 (3H, s), 0.06 (3H, s). **¹³C NMR δ(ppm):** 170.4, 156.0, 143.8, 141.3, 135.3, 128.7, 128.6, 128.4, 128.3, 127.8, 127.7, 127.2, 127.1, 125.2, 125.1, 125.0, 120.0, 67.3, 63.7, 56.1, 47.1, 25.7, 18.1, -3.5, -5.5. **MS ESI [M+H]⁺:** 532.44, RT = 6.19 min. **HRMS MALDI (m/z):** C₃₁H₃₈NO₅Si [M+H]⁺ = calc. 532.2520, measured 532.2539. **IR ν(cm⁻¹):** 3035, 2955, 2883, 2856, 1741, 1721, 1499, 1451, 1343, 1251, 1191, 1058, 740.

**H-Ser(TBS)-OBn (12):** The crude Fmoc-Ser(TBS)-OBn 18 (2.62 g) was dissolved in DMF (40 mL) and piperidine (10 mL) was added. After 30 min. at 23°C, the solvents were evaporated under reduced pressure and the resulting crude was purified by flash chromatography (hexanes:EtOAc 2:1 to hexanes:EtOAc 1:3) to afford 1.36 g (78% after 2 steps) of the H-Ser(TBS)-OBn 12 as a white crystalline solid. **¹H NMR (CDCl₃)** δ(ppm): 7.35 (5H), 5.18 (1H, d, J = 1.5 Hz), 5.18 (1H, d, J = 1.5 Hz), 3.98 (1H, dd, J = 9.8 Hz, 4.4 Hz), 3.83 (1H, dd, J = 9.8 Hz, 3.9 Hz), 3.57 (1H, dd, J = 4.4 Hz,
N-(Fmoc-aminoethyl)-Ser(TBS)-OBn (14): H-Ser(TBS)-OBn 12 (317 mg, 1.024 mmol, 1.0 equiv) dissolved in MeOH (3 mL) at 0°C with Fmoc glycinal 13 were stirred at that temperature for 30 min prior to the addition of sodium cyanoborohydride (35.4 mg, 0.563 mmol, 0.55 equiv) and acetic acid (32.2 µL, 0.563 mmol, 0.55 equiv). After 1 h, TLC analysis indicated complete consumption of the substrates and the solvents were evaporated. The crude product was then stirred with sat. aq. NaHCO₃ (50 mL) and extracted EtOAc (3 x with 50 mL). The organic layers were combined and washed 2x with sat. aq. NaHCO₃, once with brine, dried with anhydrous Na₂SO₄ and concentrated in vacuo. The crude product was purified with flash chromatography (hexanes:EtOAc 2:1) affording 372 mg (63% yield) of a translucid oil which crystalized upon drying in vacuo. 

\[ ^1H \text{ NMR (CDCl}_3 \] \( \delta(\text{ppm}): \] 7.80 (2H, d, \( J = 7.5 \) Hz), 7.63 (2H, d, \( J = 7.5 \) Hz), 7.40 (2H, t, \( J = 7.5 \) Hz), 7.34 (2H, t, \( J = 7.5 \) Hz), 5.38 (1H, m), 5.20 (2H, s), 4.41 (2H, d, \( J = 7.0 \) Hz), 4.27 (1H, t, \( J = 7.0 \) Hz), 3.93 (1H, dd, \( J = 4.3 \) Hz, 9.7 Hz), 3.86 (1H, m), 3.42 (1H, m), 3.36-3.26 (2H, m), 2.90-2.69 (2H, m), 0.90 (9H, s), 0.07 (3H, s), 0.05 (3H, s). 

\[ ^13C \text{ NMR } \delta(\text{ppm}): \] 173.0, 156.5, 144.0, 141.3, 135.7, 128.6(CH), 128.3(CH), 128.2(CH), 127.6(CH), 127.0(CH), 125.1(CH), 119.9(CH), 66.7(CH₂), 66.6(CH₂), 64.5(CH₂), 62.8(CH), 47.3(CH₂), 47.2(CH), 40.9(CH₂), 25.8(CH₃), 18.2, -5.5(CH₃), -5.6(CH₃). 

MS ESI [M+H]⁺: 575.45 RT = 5.41 min. HRMS MALDI (m/z): \( \text{C}_{33}\text{H}_{43}\text{N}_{2}\text{O}_{5}\text{Si} \ [\text{M+H}]^+ = \text{calc. 575.2942, measured 575.2889.} \] IR γ(\text{cm}⁻¹): 3063, 2952, 2929, 2883, 2856, 1716, 1667, 1450, 1333, 1253, 1102, 734.
**N-(Fmoc-aminoethyl)-Ser(TBS)-OH (15):** Benzyl ester 14 (913 mg, 1.59 mmol, 1.0 equiv) dissolved in 35 mL of methanol was mixed with 5% Pd/C (1 wt.% Pd, 183 mg) and after deoxygenation (triple flush of the 500 mL reaction flask with N₂/vacuum) was stirred vigorously for 1h under hydrogen at atmospheric pressure. TLC analysis indicated complete consumption of the starting material (EtOAc 1:2, Rₜ starting material 0.6, the product on the start line) and the reaction flask was then degassed, flushed 3 times with N₂ and the suspension was filtered through a celite pad, which was subsequently washed with additional 100 mL of methanol. After evaporation and drying overnight in vacuo, the expected product was obtained pure (690 mg, 90% yield) as white solid. 

**1H NMR (CDCl₃) δ(ppm):**
7.76 (2H, d, J = 7.3 Hz), 7.63 (2H, d, J = 7.3 Hz), 7.49 (1H, s), 7.39 (2H, t, J = 7.3 Hz), 7.29 (2H, t, J = 7.3 Hz), 4.47 (1H, br s), 4.32 (2H, m), 4.20 (2H, m), 4.16 (1H, m), 3.65 (2H, br s), 3.60 (1H, m), 3.29 (2H, br m), 0.87 (9H, s), 0.07 (6H, s).

**1H NMR (DMSO) δ(ppm):**
7.90 (2H, d, J = 7.3 Hz), 7.68 (2H, d, J = 7.3 Hz), 7.48 (1H, s), 7.39 (2H, t, J = 7.3 Hz), 7.32 (2H, t, J = 7.3 Hz), 4.31 (2H, d, J = 6.72 Hz), 4.22 (1H, t, J = 6.44 Hz), 3.93-3.86 (2H, m), 3.30 (1H, s), 3.24 (2H, d, J = 5.36 Hz), 2.88 (2H, d, J = 6.2 Hz), 0.84 (9H, s), 0.03 (6H, d).

**13C NMR (CDCl₃) δ(ppm):** 170.5, 157.0, 143.9, 141.2, 127.7, 127.1, 125.3, 119.9, 67.1, 63.2, 61.0, 49.6, 47.1, 46.7, 25.7, 18.1, -5.6.

**MS ESI [M+H]+:** 485.41, RT = 5.05 min.

**HRMS MALDI (m/z):** C₂₆H₃₇N₂O₅Si [M+H]+ = calc. 485.2472, measured 485.2440.

**IR ν(cm⁻¹):** 3067, 2952, 2928, 2884, 2856, 1702, 1634, 1539, 1407, 1252, 1087, 838, 739.

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**Modified PNA-monomer 4-S:** N⁷-Boc-cytosine-2-acetic acid 16 (263 mg, 0.98 mmol, 1.1 eq, preparation described in [1]) was dissolved in DMF and HBTU (337 mg, 0.89 mmol, 1.0 eq) followed by Et₂Pr₂N (191 μL, 1.16 mmol, 1.3 equiv) were added. After stirring 20 min. at 23 °C, Et₂Pr₂N (191 μL, 1.16 mmol, 1.3 equiv) and backbone 15 (430 mg, 0.89 mmol, 1.0 equiv) were added subsequently and the reaction was quenched after 10 min with 0.5% HCl (200 mL). The precipitate thus obtained was filtered, washed with water (50 mL), dissolved in 100 mL EtOAc, dried with anhydrous Na₂SO₄ concentrated in vacuo. The crude product was purified with flash chromatography (acetone 100%, then acetone:MeOH 19:1, 9:1, 3:1) to afford 220 mg (33% yield) of the pure product 4. 

**1H NMR (DMSO) δ(ppm):** 10.26 (1H, s), 7.87 (2H, m), 7.66 (2H+1H, m), 7.39 (2H, m), 7.30 (2H, m), 6.96 (1H, m), 4.75 (1H, m), 4.19 (2H, m), 4.12 (2H, m), 3.93 (1H, m), 3.70-3.10 (6H, m), 1.44 (9H, s), 0.83
7-azidocoumarin-4-acetic acid (3): 7-aminocoumarin-4-acetic acid ([1] (3.0 g, 13.69 mmol, 1.0 equiv) was suspended in water (65 mL) and cooled to 0°C. Concentrated sulfuric acid (16 mL) was added slowly to maintain the reaction at 0°C and the solution became homogeneous. A solution of sodium nitrite (1.13 g, 16.42 mmol, 1.2 equiv) in water (20 mL) was added drop wise over 30 min and the mixture was stirred 1h at 0°C. A solution of sodium azide (1.47 g, 22.58 mmol, 1.65 equiv) in water (12 mL) was added drop wise to the reaction mixture and it was stirred overnight while warming up to room temperature. The product precipitated as dark-yellow solid. It was filtered on glass fritted funnel, washed extensively with water and dried in vacuo to remove traces of water thus affording 2.66 g (79% yield) of pure azidocoumarin 2. As chromatography resulted in partial decomposition and the product was pure by LCMS and NMR techniques, the compound was used for coupling with PNA oligomers after careful drying without further purification. 

**1H NMR (DMSO)** δ (ppm): 7.71 (1H, d, J = 8.2 Hz), 7.15 (1H, s), 7.11 (1H, d, J = 8.8 Hz), 6.44 (1H, s), 3.90 (2H, s).

**13C NMR (DMSO)** δ (ppm): 171.0, 160.0, 154.6, 150.0, 143.8, 127.6, 116.5, 116.1, 115.5, 107.4, 37.6.

**MS ESI [M+H]^+:** 246.05, RT = 4.34 min. **HRMS MALDI (m/z):** C_{11}H_{8}N_{3}O_{4} [M+H]^+ = calc. 246.0515, measured 246.0508. **IR ν (cm⁻¹):** 2124, 2103, 1729, 1689, 1614, 1393, 1311, 1169.

**Synthesis of the PNA oligomers:**

**preparation of the lysine-functionalized solid support:** Rink resin (500 mg, 0.7 mmol/g, 0.35 mmol, 3.5 equiv 100-200 mesh, Novabiochem) was swollen in CH₂Cl₂ (5 mL) for 20 min. The Fmoc group was removed (20% piperidine/DMF, 20 min.) and the resin washed extensively with DMF and CH₂Cl₂. Fmoc-Lys(Boc)-OH (46.5 mg, 0.1 mmol, 1.0 equiv) was dissolved in anhydrous N-methylpyrrolidone (NMP, 5 mL) and HOBt (101 mg, 0.75 mmol, 7.5 equiv) followed by diisopropylcarbodiimide (DIC, 77.4 μL, 0.5 mmol, 5.0 equiv) were added. The mixture was stirred for 15 min prior to addition to the resin and then 3 hr with the resin. The unreacted amino groups were capped (30 min) with solution of acetic anhydride (0.92 mL) and 2,6-lutidine (1.3 mL) in DMF (20 min.) and filtered on glass fritted funnel, washed with DMF and dried in vacuo to afford 3.45 mg (79% yield) of pure ester.

**1H NMR (CD₂OD)** δ (ppm): 168.2, 163.9, 162.7, 157.6, 149.9, 148.9, 143.8, 141.1, 140.3, 128.8, 126.6, 123.7, 119.3, 95.5, 82.1, 66.7, 60.1, 54.4, 53.1, 46.9, 44.3, 42.1, 24.8, 24.7, 17.4, -4.9. **MS ESI [M+H]^+:** 736.84, [M+H-Boc]^+: 636.63. **HRMS MALDI (m/z):** C_{37}H_{49}N_{5}O_{9}SiNa [M+Na]^+ = calc. 758.3198, measured 758.3292. **IR ν (cm⁻¹):** 2928, 1748, 1653, 1558, 1498, 1450, 1369, 1240, 1149.
The resulting resin of loading c.a. 0.2 mmol/g was deprotected again (20% piperidine in DMF, 10 min.). Fmoc-Lys(Boc)-OH (186 mg, 0.4 mmol, 4.0 eq) was dissolved in NMP (5 mL) and HBTU (133 mg, 0.35 mmol, 3.5 equiv) followed by EtPr₂N (66 μL, 0.4 mmol, 4.0 eq) and 2,6-lutidine (71 μL, 0.6 mmol, 6.0 equiv) were added. The mixture agitated 15 min prior to addition to the resin and agitated 2 hr with the resin. The resin was then washed with DMF and CH₂Cl₂ and capped (30 min., acetic anhydride/2,6-lutidine, as previously), washed again with DMF and CH₂Cl₂, and dried by washing with diethyl ether and drying under reduced pressure on a filter for 30 min. The resulted Fmoc-Lys(Boc)-Lys(Boc)-Rink resin (loading c.a. 0.2 mmol/g) was stored at -20°C for further use. Conversely, the same resin could be obtained using 0.2 mmol/g Rink resin using two HBTU couplings.

**preparation of the serine-PNA-functionalized solid support:** Rink resin (500 mg, 0.7 mmol/g, 0.35 mmol, 3.5 equiv 100-200 mesh, Novabiochem) was swollen 20 min. in CH₂Cl₂ (5 mL). Fmoc protecting group was removed (20% piperidine/DMF, 20 min.) and the resin washed extensively with DMF and CH₂Cl₂. Monomer 4-S (73.6 mg, 0.1 mmol, 1.0 equiv) and HOBt (67.5 mg, 0.5 mmol, 5.0 equiv) were dissolved in anhydrous NMP (5 mL), DIC (155 μL, 1 mmol, 10 equiv) was added and the mixture was stirred for 15 min prior to addition to the resin. After 3 h, the resin was washed with DMF and CH₂Cl₂ and the unreacted amino groups were capped with solution of acetic anhydride (0.92 mL) and 2,6-lutidine (1.3 mL) in DMF (20 mL) for 30 min. The resin was subsequently washed with DMF and CH₂Cl₂ and dried by washing with diethyl ether and drying under reduced pressure on a filter for 30 min. The resulted Fmoc-2-(O-TBS)-C(Boc)PNA-Rink resin (loading c.a. 0.2 mmol/g) was stored at -20°C for further use. Conversely, the same resin could be obtained by HBTU coupling with a 0.2 mmol/g Rink resin.

**Automated synthesis of the C-azidocoumarin-terminated PNA probes:** Fmoc-2-(O-TBS)-C(Boc)PNA-Rink resin (0.2 mmol/g, 10 mg, 2 μmol, preparation described above) was loaded to the automatic PNA-oligomer synthesizer (Expedite, Applied Biosystems), deprotected (20% piperidine/DMF), coupled with Fmoc-protected PNA monomers 4 (0.2 M soln. in NMP, 4.0 equiv) using HBTU (0.175 M soln. in NMP, 3.5 equiv) and bases (4.0 equiv EtPr₂N with 6.0 equiv 2,6-lutidine in NMP-0.2/0.3 M). The resin was capped after every step (acetic anhydride/2,6-lutidine) and the N-terminal Fmoc protecting group was removed (20% piperidine/DMF). When the desired sequence was obtained and characterized (Analysis of polymer bound sequences was performed by cleaving a few beads with 4:1 TFA/cresol or 19:1 TFA/thiethylsilane for 30 min then precipitate the product from 10 volumes of diethyl ether, and pellet the product by centrifugation - 5 min at 19 000 g. The pellet was redissolved in MeCN:water 1:1 and analyzed by LCMS), two subsequent couplings with lysine residues were performed using semi-automatic equipment for solid-phase synthesis. The resin was transferred into a reactor tube with a filter, swelled for 20 min. in CH₂Cl₂ and the Fmoc protecting group removed if necessary (20% piperidine/DMF, 5 min.). Fmoc-Lys(Boc)-OH (18.8 mg, 0.04 mmol, 20 equiv) was dissolved in 0.175 M soln. of HBTU in NMP (200 μL, 0.035 mmol, 17.5
equiv) and 200 μL of EtPr2N /2,6-lutidine solution (0.2 M/0.3 M, 0.04 /0.06 mmol, 20/30 equiv) was added. The coupling mixture was added to the resin after a few minutes and the resin was agitated for 30 min. The resin was filtered off, washed with DMF, deprotected (20% piperidine/DMF) and the second lysine residue (BocLys(Boc)OH) was introduced using the same procedure. The silyl protecting group was removed with 1M tetrabutylammonium fluoride (TBAF) solution in DMF (1 mL, 1h, twice) and the resin was washed extensively with DMF and CH2Cl2. 7-azidocoumarin-4-acetic acid (9.8 mg, 0.04 mmol, 20 eq) was dissolved in 0.175 M soln. of HBTU in NMP (200 μL, 0.035 mmol, 17.5 equiv). 200 μL of EtPr2N /2,6-lutidine solution (0.2 M/0.3 M, 0.04 /0.06 mmol, 20/30 equiv) was added. The coupling mixture was added after 15 min preincubation to the resin and stirred overnight. If the subsequent LC/MS analysis revealed unreacted substrate, coupling with the coumarin derivative was repeated. When coupling was complete as judged by LC/MS analysis from the cleavage of an analytical sample, the resin was filtered off, washed extensively with DMF and CH2Cl2, cleaved with 4:1 TFA/cresol or 19:1 TFA/thiethylsilane (0.5-1 mL) for 2 h. The resulting mixture was separated from the resin and precipitated from 10 volumes of ether. The resin was cleaved again (0.5 volume of the previous cleaving mixture, 30 min., then the filtrate precipitated from 10 vol. Et2O). The suspensions were centrifuged (9600 rpm, 4 ºC, 20 min.), resuspended in fresh Et2O of the same volume and centrifuged again under the same conditions. The crude precipitates were redissolved in water and purified with reverse-phase chromatography using gradient of MeCN in water (with 0.1% TFA). Purity of the fractions were analyzed with MALDI and LCMS. The pure product 7 was collected at 20-30% MeCN in water. The pure fractions were combined and lyophilized. Stored under N2 at -20ºC as solid.

**Automated synthesis of the N-phosphine-terminated PNA probes 9:** Fmoc-Lys(Boc)-Lys(Boc)-Rink resin (0.2 mmol/g, 10 mg, 2 μmol) was loaded to the automatic PNA-oligomer synthesizer (Expedite, Applied Biosystems), deprotected (20% piperidine/DMF), coupled with Fmoc PNA monomers 4 (0.2 M soln. in NMP, 4.0 equiv) using HBTU (0.175 M soln. in NMP, 3.5 equiv) and bases (4.0 equiv EtPr2N with 6.0 equiv 2,6-lutidine in NMP-0.2/0.3 M). The resin was capped after every step (acetic anhydride/2,6-lutidine) and the N-terminal Fmoc protecting group was removed (20% piperidine/DMF). The proper synthesis of the sequence was verified by cleavage of an analytical sample as described above. Coupling of 2-diphenylphosphinobenzoic acid was performed using semi-automatic equipment for solid-phase synthesis. The resin was transferred into a reactor tube with a filter, swelled for 20 min. with CH2Cl2, the Fmoc protecting group removed if necessary (20% piperidine/DMF, 5 min.) and 2-diphenylphosphinobenzoic acid (12.2 mg, 0.04 mmol 20 equiv) was dissolved in 0.175 M soln. of HBTU in NMP (200 μL, 0.035 mmol, 17.5 equiv). 200 μL of ETIPR2N/2,6-lutidine solution (0.2 M/0.3 M, 0.04 /0.06 mmol, 20/30 equiv) was added. The coupling mixture was directly mixed with the resin and stirred 30 min. The resin was filtered off, washed with DMF and the coupling was repeated for another 30 min. The resin was filtered off, washed extensively with DMF and CH2Cl2, cleaved with 4:1 TFA/cresol or 19:1 TFA/thiethylsilane (0.5-1 mL) as
described above. The crude precipitates were redissolved in water and purified with reverse-phase chromatography using gradient of MeCN in water (with 0.1% TFA). Purity of the fractions were analyzed with MALDI and LCMS, the pure product being eluted at 20-30% MeCN in water. The most abundant by-product (phosphine oxide) was usually well separated. The pure fractions were combined, lyophilized and stored under N₂ at -20°C as solid.

Oligomers – MALDI analysis

C'- N₃Coum-CGTGCA-KK-NH₂ (7) MALDI [M+H]⁺ calc. 2156.90, found 2156.62.
C'- KK-ATAGGC-PPh₃ (9) MALDI [M+H]⁺ calc. 2211.94, found 2212.24
C'- N₃Coum-CGTGCAAGC-KK-NH₂ (7) MALDI: [M+H]⁺ calc. 2699.11, found 2699.10
C'- KK-CCATAGGC-PPh₃ (9) MALDI: [M+H] calc. 2714.14, found 2715.39.

Absorption/emission quenched azidocoumarin and its reduced product

Absorption (top) and emission (bottom) maxima of azidocoumarin-PNA conjugate 7 (5 μM in 50% formamide for 455 nm emission and 360 nm excitation respectively)
Hybridization reactions:
Commercially available DNA templates were purchased as desalted 100 μM solutions in deionized water (Sigma Proligo, 5 O.D.) and diluted to the concentrations of 10, 2 and 0.2 μM. The PNA probes were dissolved in deionized water to 1 mM concentration which was the basic stock solution. The probes were diluted to their final concentrations with 1 mM PBS buffer solution (pH = 7.0) or with a 50% formamide solution in 1 mM PBS buffer (pH = 7.0). The stock solutions were stored in the buffer at -20°C.

Black 96-well plates (500 μL/well, NUNC) for fluorescent readout were used to perform the templated reactions. 240 μL of the azide-PNA solution and DNA template solution (1.2-12 μL of one of the stock solutions, depending on the desired final DNA percentage and concentration) were mixed. 240 μL of the phosphine-PNA (two fold higher concentration than the azide-PNA) was added using a multichannel pipette and the fluorescence readout was recorded immediately. Negative controls – equal volumes of the azide- and phosphine-PNA with 1mM PBS buffer of the volume equal to the DNA solutions used in the experiment. Background level for the buffer and reagents – 480 μL 1 mM PBS (buffer), 240 μL 1mM PBS + 240 μL azide-PNA solution (azide-PNA background), 240 μL 1mM PBS + 240 μL phosphine-PNA solution (phosphine-PNA background). The level of full conversion of the fluorogenic substrate to the fluorescent product was measured using 240 μL azide-PNA + 240 μL of 1mM TCEP solution. The optimal measurement wavelengths were found by scanning the ranges 300-400 nm (excitation) and 400-500 nm (emission) for the biggest difference between the fluorescence level of the starting azide and the amine product yielding 360 nm (excitation) and 455 nm (emission) as the optimal wavelength.

The fluorescence readout of the templated reactions was performed for 45-120 min. in duplicates and the average of both the results were plotted.

Linear dependence of fluorescence level on the product concentration was initially confirmed within the range of 4-0.03 μM of azide-PNA concentration (error within 5.4-6.9 %, as standard deviation) by reaction with 1 mM TCEP solution at 37 °C.

The untemplated Staudinger reaction between the PNA probes was taking place down to 1 μM azide-PNA concentration. At 500 nM and below no background reaction was observed within the experiment times. The templated process was investigated in terms of sensitivity over mutations introduced to the DNA sequence and fidelity of the reaction in both directions of hybridization (PNA may hybridize with DNA both directions).

<table>
<thead>
<tr>
<th>Symbol</th>
<th>DNA Sequence (5'-3')</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>GGG TAT CCG GCA CGT CGG</td>
<td>perfect match</td>
</tr>
<tr>
<td>T2</td>
<td>GGG TAT CCG AA GCA CGT CGG</td>
<td>match with 2 abasic sites</td>
</tr>
<tr>
<td>T3</td>
<td>GGG TAT CCG AAC A GCA CGT CGG</td>
<td>match with 4 abasic</td>
</tr>
<tr>
<td>Probe</td>
<td>Sequence</td>
<td>Matches with</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>T4</td>
<td>GGG TAT CCG AAC AAC GCA CGT CGG</td>
<td>6 abasic sites</td>
</tr>
<tr>
<td>T5</td>
<td>GGC TGC ACG GCC TAT GGG</td>
<td>reversed perfect match</td>
</tr>
<tr>
<td>T6</td>
<td>GGC TGC ACG GG GCC TAT GGG</td>
<td>reversed match with 2 abasic sites</td>
</tr>
<tr>
<td>T7</td>
<td>GGC TGC ACG GGG GCC TAT GGG</td>
<td>reversed match with 3 abasic sites</td>
</tr>
<tr>
<td>T1mG</td>
<td>GGG TAT GCG GCA CGT CGG</td>
<td>match with a C-&gt;G mutation</td>
</tr>
<tr>
<td>T1mA</td>
<td>GGG TAT AAC GCA CGT CGG</td>
<td>match with a C-&gt;A mutation</td>
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<tr>
<td>T1mT</td>
<td>GGG TAT TCG GCA CGT CGG</td>
<td>match with a C-&gt;T mutation</td>
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<td>T2mG</td>
<td>GGG TAT GCG AA GCA CGT CGG</td>
<td>2 abasic sites and a C-&gt;G mutation</td>
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<tr>
<td>T2mA</td>
<td>GGG TAT AAC AA GCA CGT CGG</td>
<td>2 abasic sites and a C-&gt;A mutation</td>
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<tr>
<td>T2mT</td>
<td>GGG TAT TCG AA GCA CGT CGG</td>
<td>2 abasic sites and a C-&gt;T mutation</td>
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<td>match with other C-&gt;G mutation</td>
</tr>
<tr>
<td>T2mN</td>
<td>GGG TAT CGG AA GCA GGT CGG</td>
<td>2 abasic sites and other C-&gt;G mutation</td>
</tr>
<tr>
<td>Tx</td>
<td>A A CCG GT A CG</td>
<td>random template</td>
</tr>
</tbody>
</table>

**Figure S-1.** Selectivity of the octameric probes in 1mM PBS buffer (37°C, 250nM azide-PNA) with the perfect match template and templates containing single nucleotide mismatch (20% template load).
Figure S-2. Conversion as a function of time for various DNA concentrations (PNA-octamers, 500 nM in 50% formamide, matching T1 (left) and T2 (right) templates)

Figure S-3. Conversion as a function of time for various DNA concentrations (PNA-octamers, 100 nM in 50% formamide, matching T1 (left) and T2 (right) templates).

Figure S-4. Influence of abasic sites for hybridization of hexameric (left) and octameric (right) PNA probes on DNA templates (500 nM probes, 100% DNA, 50% formamide, 37 °C)
Figure S-5. Conversion as a function of time for matching T2 DNA (1% DNA) and single mutations (T2mG, T2mA, T2mT) (PNA-octamers, 500 nM in 50% formamide)

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<table>
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<tr>
<td>1%T2</td>
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<tr>
<td>1%T2mG</td>
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<tr>
<td>1%T2mA</td>
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<td>1%T2mT</td>
<td>0.028</td>
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Table S-2. The initial reaction rates\(^3\) (t < 15 min.; v [nmol/s]) for matching T2 DNA (1% DNA) and single mutations (T2mG, T2mA, T2mT) (PNA-octamers, 500 nM in 50% formamide)

Figure S-6. Conversion as a function of time for matching templates T1 and T2 as well as reversed templates of the same sequences T5 and T6 (20% DNA, 500 nM PNA-octamers in 50% formamide)

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<table>
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</thead>
<tbody>
<tr>
<td>T1</td>
<td>0.63</td>
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<tr>
<td>T2</td>
<td>0.61</td>
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<tr>
<td>T5</td>
<td>0.054</td>
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<tr>
<td>T6</td>
<td>0.059</td>
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</tbody>
</table>

Table S-3. The initial reaction rates\(^3\) (t < 3 min.; v [nmol/s]) for the octameric PNA probes on matching and reversed templates (20% DNA, 500 nM PNA-octamers in 50% formamide)

[3] The initial reaction rates were calculated using the approximation valid at the initial, linear part of the kinetic plot:

\[ v = -\frac{d[A]}{dt} \approx \frac{\Delta[A]}{\Delta t} \]