Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

A primerless molecular diagnostic: phosphorothioated-terminal hairpin formation and self-priming extension (PS-THSP)

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Table S1 Oligonucleotide sequences used in this study

Oligo name	Sequence (5' to 3')
Template 1-1	AAGAATTCTTAAGAATTCTTAAGTTAGTGGAAAACCACTAAC
Template 1-2	AAGAATTCTTAAGAATTCTTAAGTTAGTGGAAAACCACTAAC
Template 1-3	AAGAATTCTTAAGAATTCTTAAGTTAGTGGAAAACCACTAAC
Template 1-4	AAGAATTCTTAAGAATTCTTAAGTTAGTGGAAAACCACTAAC
Template 1-4P	AAGAATTCTTAAGAATTCTTAAGTTAGTGGAAAACCACTAAC-phosphate
Template 2-1	CAGAACTCAAAAGAGTTCTGAAGTTAGTGGAAAACCACTAAC
Template 2-2	CAGAACTCAAAAGAGTTCTGAAGTTAGTGGAAAACCACTAAC
Template 2-3	CAGAACTCAAAAGAGTTCTGAAGTTAGTGGAAAACCACTAAC
Template 2-4	CAGAACTCAAAAGAGTTCTGAAGTTAGTGGAAAACCACTAAC
Wild-type template	TCCTGTGAATATGTGAGCACTCACTGCTGCTACTGCAAAAAAAA
Mutant template	TCCTGTGAATATGTGAGCACCCACTGCTGCTACTGCAAAAAAAA
Ligation substrate 1	AAGAATTCTTAAGAATTCTT GCAGTAGCAGCAGTGG
Ligation substrate 1 (0 PS)	AAGAATTCTTAAGAATTCTT GCAGTAGCAGCAGTGG
Ligation substrate 2	Phosphate-GTGCTCACATATTCACAGGA GTTAGTGGAAAACCACTAAC

*Underlining indicates phosphorothioate (PS) modifications.

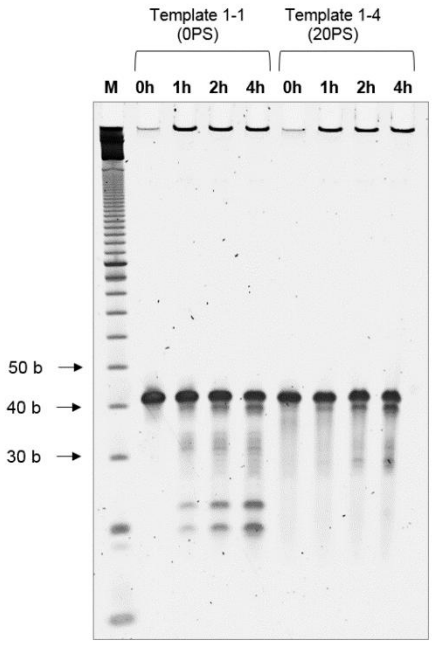
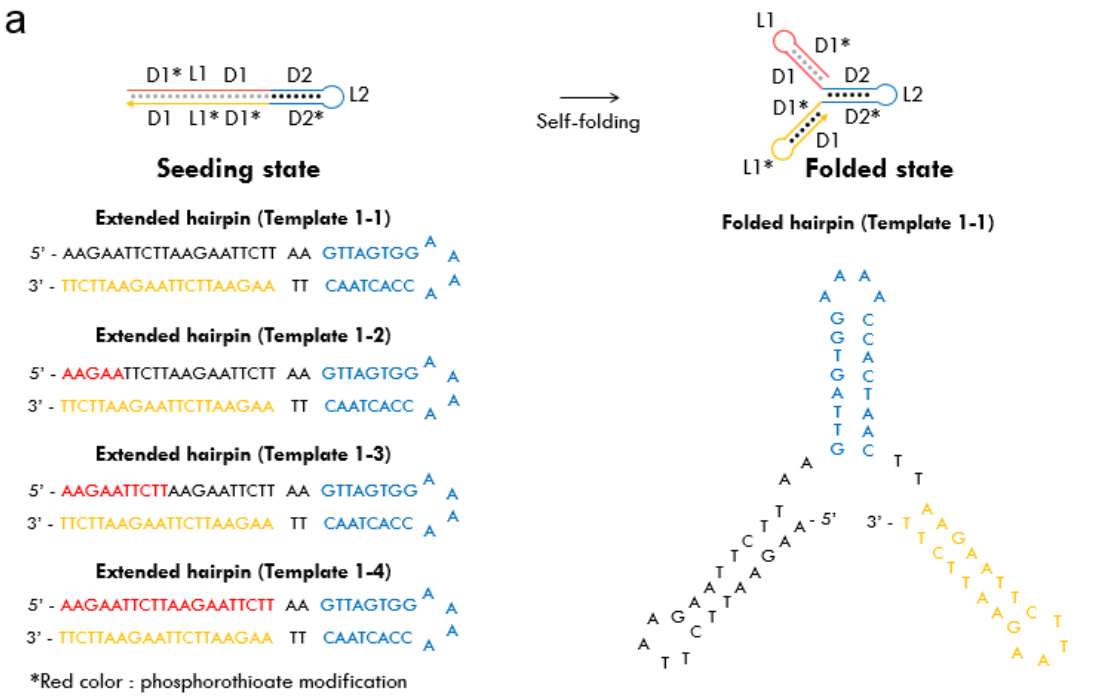
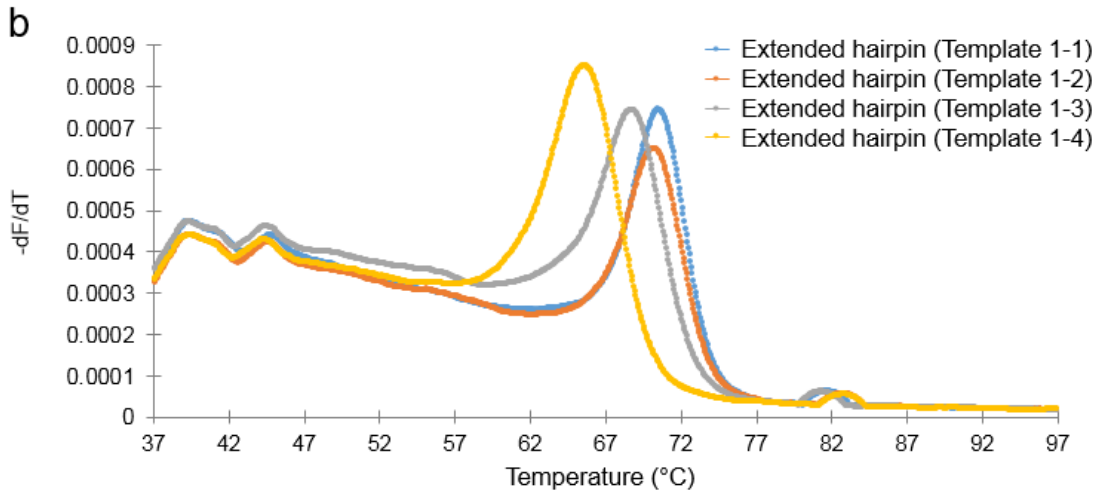


Fig. S1 PAGE analysis of human serum degradation assay for template 1-1 (0PS) and template 1-4 (20PS). 18 μl reactions were prepared with a 1X TM buffer (10 mM Tris-HCl, 5 mM $MgCl_2$, pH 8.0) and 1 μM templates. To this mixture, a fresh sample of undiluted human serum (2 μl) was added to make the overall % of human serum 10% (v/v), followed by incubation at 37 °C during different times (1 h, 2 h and 4 h). To stop the reaction, the samples were heated at 95 °C for 10 min followed by incubation at 4 °C. For the case of 0 h incubation, distilled water (2 μl) was added instead of human serum. Digested products were analyzed by denaturing PAGE (10%, 350 V for 60 min). M (Marker): 10 bp ladder.



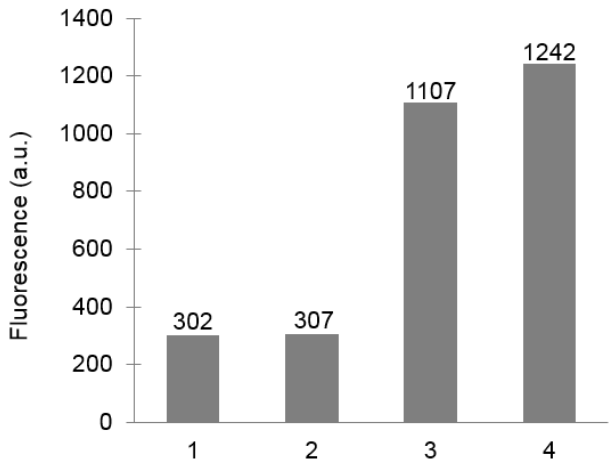


Extended hairpin	Measured Tm (°C)
Template 1-1	70.50
Template 1-2	70.18
Template 1-3	68.71
Template 1-4	65.51

Folded hairpin	*Calculated Tm (°C)
Template 1-1	60.7

* Tm was calculated using OligoAnalyzer 3.1 under 1X isothermal amplification buffer condition.

Fig. S2 T_m analysis of extended hairpins in the seeding state and folded state. **(a)** The predicted structures of the extended hairpins in the seeding state and folded state. **(b)** Melting temperatures of the extended hairpins (template 1) were experimentally measured and the melting temperature of a folded hairpin (template 1-1) was calculated using OligoAnalyzer 3.1. To make the extended hairpins, each PS-THSP reaction was performed at 37 °C for 10 min with Bst 2.0 DNA polymerase (the lower temperature, 37 °C, ensured a single cycle of extension). The products were purified through a Qiaquick nucleotide removal kit (Qiagen; Valencia, CA). Samples for T_m measurement were prepared in a 1X isothermal buffer that included dNTPs and 1X EvaGreen. T_m curves were obtained through the 'High Resolution Melting' program of a LightCycler 96 System (Roche).



	1	2	3	4
Template 1-1 (100 pM)	-	+	-	+
Bst 2.0 WarmStart DNA polymerase	-	-	+	+

Fig. S3 Fluorescence due to non-specific amplification. PS-THSP reactions (containing various components according to the table above) were carried out at 60 °C for 1 h. End-point fluorescence intensities were recorded after incubation with an EvaGreen intercalating dye. The control experiments (1) without template 1-1 and Bst 2.0 WarmStart DNA polymerase and (2) only with template 1-1 did not yield a significance increase in fluorescence intensity. However, the addition of the DNA polymerase alone (3) led to an increase in fluorescence intensity, likely due to the embedded aptamer used for warm start. An additional small increase in fluorescence is seen when a template that does not contain phosphorothioates (template 1-1) was also added, presumably due to a single round of extension. It should be noted that all of these background fluorescence values are far smaller than the values obtained with PS-THSP (compare with the y axis of **Fig. 2a**, for example)

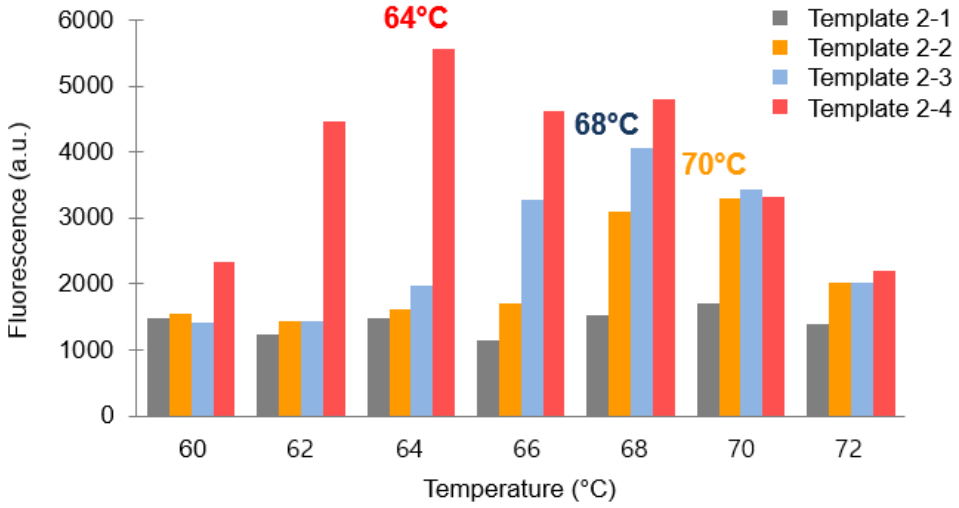


Fig. S4 Effects of phosphorothioate (PS) modification and temperature on template 2 amplification. PS-THSP templates (100 pM) with different numbers of PS modifications (template 2-1: 0PS; template 2-2: 8PS; template 2-3: 12PS; template 2-4: 20PS) were assayed at different temperatures (from 60 °C to 72 °C) for 1 h. The final fluorescence intensities reflect the effects of varying PS modifications and temperatures on PS-THSP amplification of template 2. Optimal reaction temperatures are noted above the bars

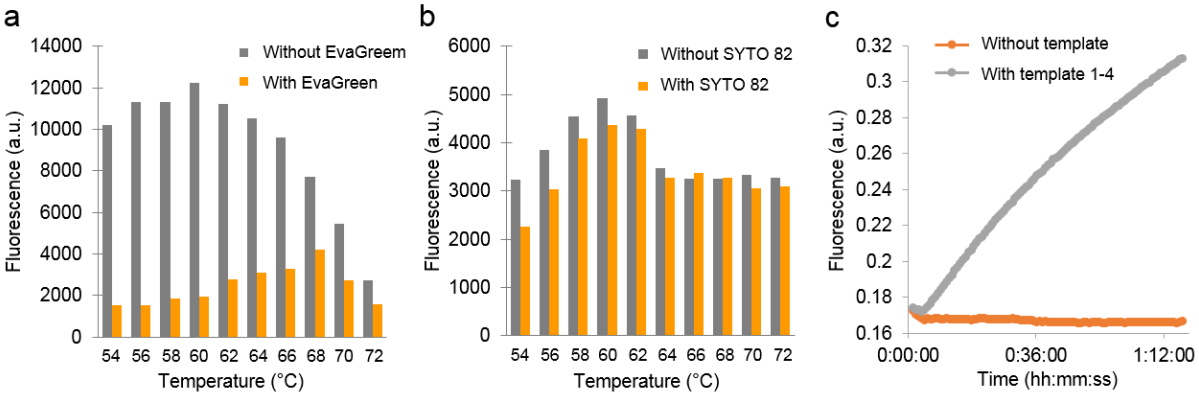


Fig. S5 Impact of different intercalating dyes on PS-THSP. End-point fluorescence detection for **(a)** EvaGreen and **(b)** SYTO 82 dyes. PS-THSP reactions with template 1-4 (100 pM) were performed in the absence of dye (grey) as well as in the presence of dye (orange) at different temperatures (from 54 °C to 72 °C) for 1 h. For PS-THSP reactions without dye, dye was added to the samples after PS-THSP was complete in order to measure the amount of product. **(c)** Real-time fluorescence detection. PS-THSP reactions without template (orange) and with template 1-4 (100 pM) (grey) were monitored in presence of SYTO 82 dye at 60 °C using a LightCycler 96 System (Roche)

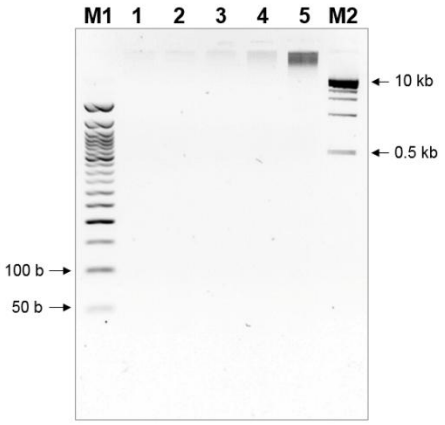


Fig. S6 Gel electrophoretic analysis of PS-THSP products resulting from SNP templates. The PS-THSP/SNP detection method was tested with 5000 pM mixed wild-type and mutant templates. The PS-THSP products were analysed by gel electrophoresis on a 2% agarose gel. M1: 50 bp DNA ladder; lane 1: 0:5000; lane 2: 5:4995; lane 3: 50:4950; lane 4: 500:4500; lane 5: 5000:0; M2: 1 kb DNA ladder, where the underlined ratios represent Mutant (pM):Wild type (pM)

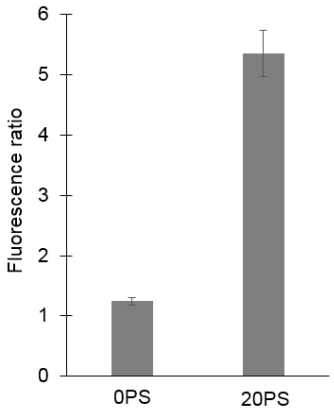


Fig. S7 Effect of PS modifications on SNP detection. SNP detection experiments were performed using template 1 ligation substrates with and without PS modifications. Fluorescence ratios for each case were calculated by dividing the fluorescence intensity in the presence of the mutant template (5 nM) by the fluorescence intensity in the absence of template

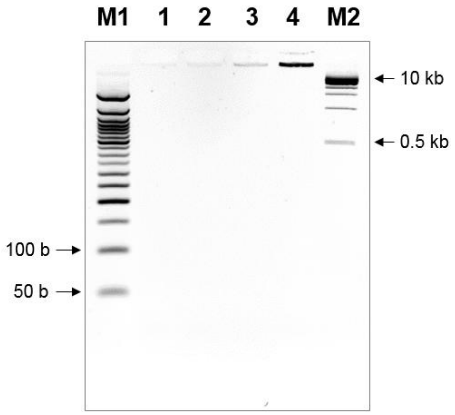


Fig. S8 Gel electrophoretic analysis of PS-THSP products resulting from templates treated with alkaline phosphatase. Different concentrations of alkaline phosphatase were added to dephosphorylate the 3' end of template 1-4P (10 nM), which was then used to initiate a PS-THSP reaction. The PS-THSP products were analysed by gel electrophoresis. M1: 50 bp DNA ladder; lane 1: 0 mU/mL; lane 2: 0.05 mU/mL; lane 3: 0.5 mU/mL; lane 4: 5 mU/mL; M2: 1 kb DNA ladder