Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Electrochemical Detection of DNA Mutations on a PNA-Modified Electrode Utilizing a Single-Stranded DNA Specific Endonuclease

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Experimental Section

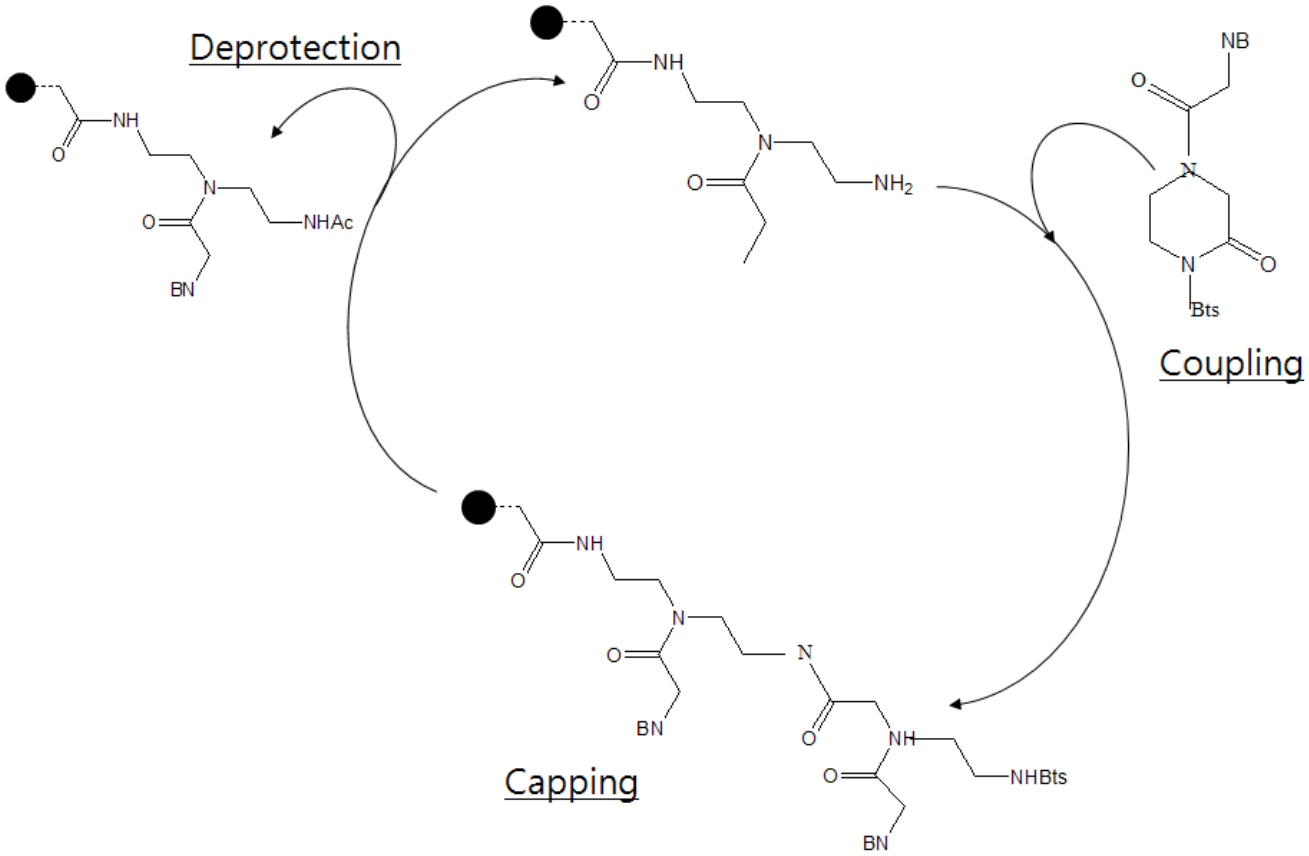
Chemicals and reagents. Potassium ferricyanide and potassium hexacyanoferrate trihydrate were purchased from Sigma-Aldrich. Fifty mM PBS (pH 7.2) was prepared using a phosphate buffered saline pack from Pierce (Rockford, IL). The PNA capture probes were synthesized (scheme S1 and table S1), purified by HPLC and confirmed by using MALDI-TOF by Panagene® (Daejeon, Korea) (figure S1). The PNA probes used in this study were thiolated with mecaptoundecanoic acid at their N-terminal positions. Nuclease S1 endonuclease (762 U/μL), purified from *Aspergillus oryzae,* was purchased from Invitrogen™. Triply distilled water with a specific resistance over 18 MΩcm was used and all other materials used were of the highest quality available.

Genomic DNA isolation. Genomic DNA was isolated from whole blood of healthy subjects and breast cancer patients by using a genomic DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. After PCR amplification, direct sequencing was performed to confirm sequence information on the mutation sites using ABI Dye Terminator Cycling Sequencing kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction, followed by analysis on ABI3700 DNA sequencer (Applied Biosystems).

Preparation of PCR product. Specific site of *BRCA 1* gene was amplified by performing PCR on a DNA engine-Peltier thermo cycler (Bio-Rad, Hercules, CA) in a 50 μL solution containing 100 ng of genomic DNA, 0.25 μM of each primer, 10× PCR reaction buffer (300 mM Tris-HCl (pH 9.3), 300 mM salts consisting of K^+ and NH_4^+, 20 mM Mg^{2+}), 0.2 mM dNTPs, and 1 U of i-StarMAX™ II DNA polymerase (iNTRON BIOTECHNOLOGY). The primer sequences were designed to have similar G/C contents (50%) and T_m around 60ºC. (1) Forward primer: 5'-TGGAAGTAATTGTAAGCATCCTGAAATAAAAA-3' and reverse primer: 5'-GGGAAGCTCTTCATCCTCACTAGATAA-3' were used to amplify target samples for mutation detection at 3459 and 3746 sites (We used the same PCR-product for both 3459 and 3746 sites), and (2) forward primer: 5'-GCAGATTTGGCAGTTCAAAAGACTC-3' and reverse primer: 5'-GCATGAATATGCCTGGTAGAAGACTTCC-3' were used to amplify target samples for mutation detection at 1942 site. PCR was programmed for 5 min at 94 ºC, followed by 35 cycles of 20 s at 94 ºC, 30 s at 55 ºC, and 1 min at 72 ºC, and ended with 5 min at 72 ºC. After amplification, the PCR products were confirmed by agarose-gel electrophoresis and purified

with a QIAquick® PCR Purification Kit (QIAGEN). The concentrations of PCR products were determined by using a nanodrop® ND-1000 (Wilmington, DE) spectrophotometer based on the generally accepted extinction coefficient of double-stranded DNA (50 ng cm/μL). PCR products was diluted with 1 × SSPE buffer to 100 nM and used immediately after denaturation by heating at 98 ºC.

PNA synthesis and its characterization. Panagene® (Daejeon, Korea) had developed the proprietary Bts PNA monomers (Bts: benzothiazole-2-sulfonyl group) and the proprietary oligomerization process. Panagene® synthesized PNA oligomers utilizing self-activated Bts PNA monomers and the PNA oligomerization protocol consisting of repetitive cycles of deprotection, coupling and capping steps as shown in Scheme S1. The synthesized PNA oligomers were analyzed with using HPLC and confirmed by using MALDI-TOF mass spectrometry by Panagene® (Figure S1).



Scheme S1 Solid phase synthesis of PNA oligomer by cyclic Bts monomer.

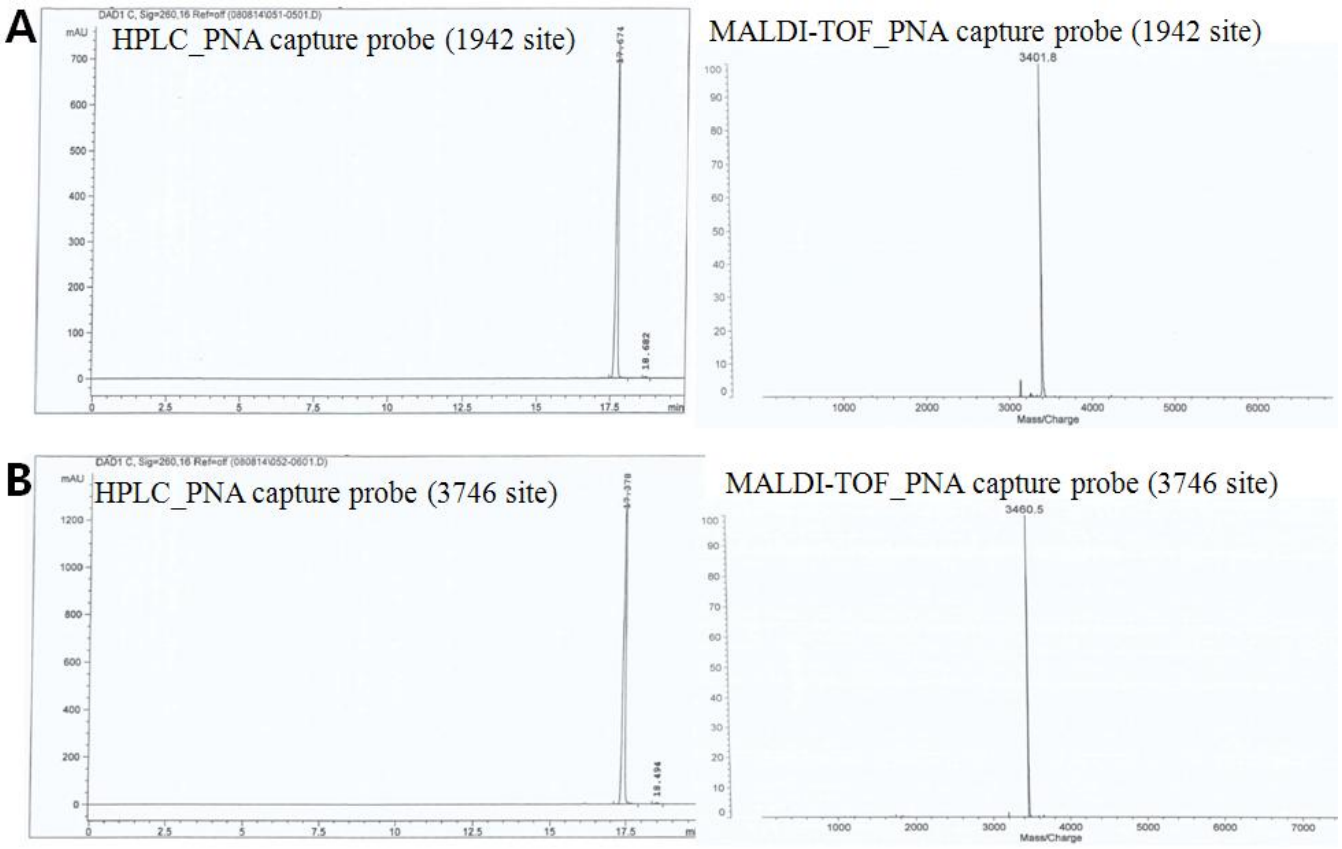


Fig. S1 PNA captures probe characterization data supported from Panagene®. (A) The HPLC and MALDI-TOF mass spectrometric analysis data for the PNA capture probe for the detection of the 1942 site in *BRCA 1* gene, (B) The HPLC and MALDI-TOF mass spectrometric analysis data for the PNA capture probe for the detection of the 3746 site in *BRCA 1* gene.

Table S1. Sequences of PNA capture probes used in this work

Probes	Sites[a]	Capture probe sequences[b] (N → C)
Capture probes	3746	C11 thiol-GGACTC_TAATTT
	1942	C11 thiol-CTATTC**T**TTTTA
	3459	C11 thiol- GAATAT**G**AAGAA

[a]Base sites in *BRCA 1* exon 11.

[b]The mutation points are underlined and bold. The wild type targets are complementary with the corresponding PNA capture probes while the mutant targets have single A base insertion ('_' → 'A') at 3746 site, single A base deletion ('A' → ' ') at 1942 site, and single C base substitution ('C' → 'A') in 3459 site.