Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Mismatch DNA-specific enzymatic cleavage employed in a new method for the electrochemical detection of genetic mutations

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General experimental

Reagents. CEL I nuclease was purchased from Transgenomics (Crewe, UK) as a Surveyor™ mutation detection kit. Glucose oxidase (GOX)-avidin conjugate was purchased from Vector laboratories (Brulingame, CA). Biotinamidocaproic acid 3-sulfo-N-hydroxysuccinimide ester and β-D-glucose were purchased from Sigma and polyamidoamine dendrimer (generation 4) was purchased from Aldrich. NAP™ 5 column containing Sephadex™ G-25 medium was purchased from GE Healthcare. 50 mM PBS (pH 7.2) was prepared with phosphate buffered saline pack (Pierce) and 1x SSPE (saline sodium phosphate EDTA) buffer was prepared by diluting 20xSSPE (Sigma). The DNA oligonucleotides were obtained from Bioneer® (Daejeon, Korea), and had sequences as shown in Table S1. All the synthesized oligonucleotides were purified by HPLC and their identities were confirmed by MALDI-TOF. All other materials used were of the highest quality available and doubly distilled water was used with a specific resistance over 18 MΩ·cm.

Apparatus. Cyclic voltammetric (CV) analysis was conducted with a CH instrument 620B electrochemical analyzer (Austin, TX) coupled with desktop computer for data acquisition. The voltammetry was performed with three-electrode system including a gold working electrode, platinum counter electrode and silver/silver chloride reference electrode.

Quartz crystal microbalance (QCM) measurements were performed with Stanford Research System QCM200 (Sunnyvale, CA) coupled with syringe pump. Titanium/gold coated quartz crystal (5 MHz, 1" diameter) was used.

Electrode fabrication. The coating of titanium (20 nm) on Si wafer (100) was followed by 200 nm gold (99.999%) thin layer formation by an e-beam evaporator. The gold-coated electrode surface was immersed into piranha solution (H_2SO_4 : H_2O_2 = 4 : 1) for 5 min. The synthesized capture probe was pretreated with 100 mM dithiothreitol,

purified using NAP^{TM} 5 column, and dissolved in PBS. The prepared electrode surfaces were washed with PBS thoroughly, and immersed into 1 μM aqueous capture probe solution for 2 h. After washing with PBS, the surfaces were moved into 1 mM aqueous mercaptohexanol solution for 30 min. Finally, the fabricated electrode surfaces were washed with PBS and water, and dried under nitrogen atmosphere.

Dendrimer-biotin conjugation. Biotinamidocaproic acid 3-sulfo-N-hydroxysuccinimide ester (45 μmol) was dissolved in 150 μL of water, and polyamidoamine dendrimer solution (10 μL in 140 μL methanol) was added. The suspension was incubated at RT overnight. The product was purified with Amicon® (MWCO 10,000) and confirmed by ^{1}H NMR spectroscopy (500 MHz, AMX 500). ^{1}H NMR ($CDCl_3$) δ 7.47 (s, 1H), 7.04 (S, 1H), 5.06 (bs, 2H), 3.49 (m, 1H), 2.57 (m, 1H), 2.27 (m, 4H), 1.37 (m, 8H), 1.13 (m, 4H), 3.78~1.25 (dendrimer protons).

Genomic DNA isolation and PCR amplification. Genomic DNA was isolated from whole blood of an apparently healthy subject 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 using ABI Dye Terminator Cycling Sequencing kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions, followed by analysis on an ABI3700 DNA sequencer (Applied Biosystems).

Each PCR amplification was carried out by using the following procedure. Two pairs of PCR primers were designed to have similar G/C contents (~50%) and T_m of ~60℃, resulting in 376 bp long amplicon containing 3459 and 3746 mutation site and 305 bp containing 1942 mutation site. (We used the same PCR-product for both 3459 and 3746 mutants and we designed the capture probes to contain only its corresponding mutation site.) The amplicons were designed to contain the target DNA sequence that binds to the capture probe DNA sequence. PCR amplification was carried out in a thermocycler (Applied Biosystems) using 100 ng of genomic DNA, 0.2 mM of dNTPs, 2.5 μM of

each primer for 3459 and 3746 position (forward: 5'-TGGAAGTAATTGTAAGCATCCTGAAATAAAAA-3' and reverse: 5'-GGGAAGCTCTTCATCCTCACTAGATAA-3') or for 1942 position (forward: 5'-GCAGATTTGGCAGTTCAAAAGACTC-3' and reverse: 5'-GCATGAATATGCCTGGTAGAAGACTTCC-3'), 30 mM KCl, 30 mM Tris-HCl, 2 mM $MgCl_2$ and 1 U of *i-StarMAX*^{TM} II DNA polymerase (iNtRON BIOTECHNOLOGY) in a volume of 50 μL. After denaturing at 94℃ for 5 min, the reaction was carried out for 35 cycles of 94℃ for 20 s, 55℃ for 30 s, and 72℃ for 1 min. This was followed by a final extension for 5 min at 72℃. After completion of the reaction, the PCR products were purified using a QIAquick® PCR Purification Kit (QIAGEN). The concentration of PCR product was determined with nanodrop® ND-1000 (Wilmington, DE) spectrophotometer based on the extinction coefficient of double-stranded DNA (50 ng·cm/μL). And the PCR product was used immediately after denaturation by heating at 98℃.

Detection procedure. On the capture probe-modified electrode surfaces, 1 μM target probe solutions (1X SSPE) were applied and allowed to hybridize at RT for 30 min. After washing with 1X SSPE and PBS, CEL I nuclease was introduced onto the electrode surfaces and reacted at 42℃ for 30 min according to the manufacturer's protocol. After washing with PBS, 50 μL of GOX-avidin solution (1 mg/mL PBS) was added onto the electrode surface and reacted for 30 min. Then, 50 μL biotin-modified dendrimer (1 nmol) was added and reacted with excess GOX-avidin for another 30 min. Finally, the electrode surfaces were washed with PBS thoroughly. Cyclic voltammetric analysis was performed in the electrolyte containing 0.1 mM ferrocenemethanol and 10 mM glucose in 50 mM PBS with the measurement conditions of 0 ~ 0.5 V scan range and 5 mV/s scan rate.

Table S1 Sequences of DNA probes used in this work.

Probes	Position[b]	Sequences (5' → 3')
Capture probes[a]	3459	C6 thiol-AATTGTAAGCATCCTGAAATAAAAAAGCAAGAATAT**G**AAGAAGTAGTTCA-biotin
	1942	C6 thiol-ATATGCCTGGTAGAAGACTTCCTCCTCAGCCTATTC**T**TTTTAGGTGCTTT-biotin
	3746	C6 thiol-CTTCATCCTCACTACATAACTTCTCTTCTGAGGACTC_TAATTTCTTGGCC-biotin
Perfectly matched target probe for 3459	TGAACTACTTCTT**C**ATATTCTTGCTTTTTTATTTCAGGATGCTTACAATT	
Single-base mismatched target probe for 3459	TGAACTACTTCTT**A**ATATTCTTGCTTTTTTATTTCAGGATGCTTACAATT	

[a]The mutation points are underlined and bold. The wild type targets are complementary with the corresponding capture probe while the mutant targets have single-base substitution ('C'→'A'), deletion ('A'→'_'), and insertion ('_'→'A') in 3459, 1942 and 3746 position, respectively.

[b]Base positions in *BRCA I* exon 11.

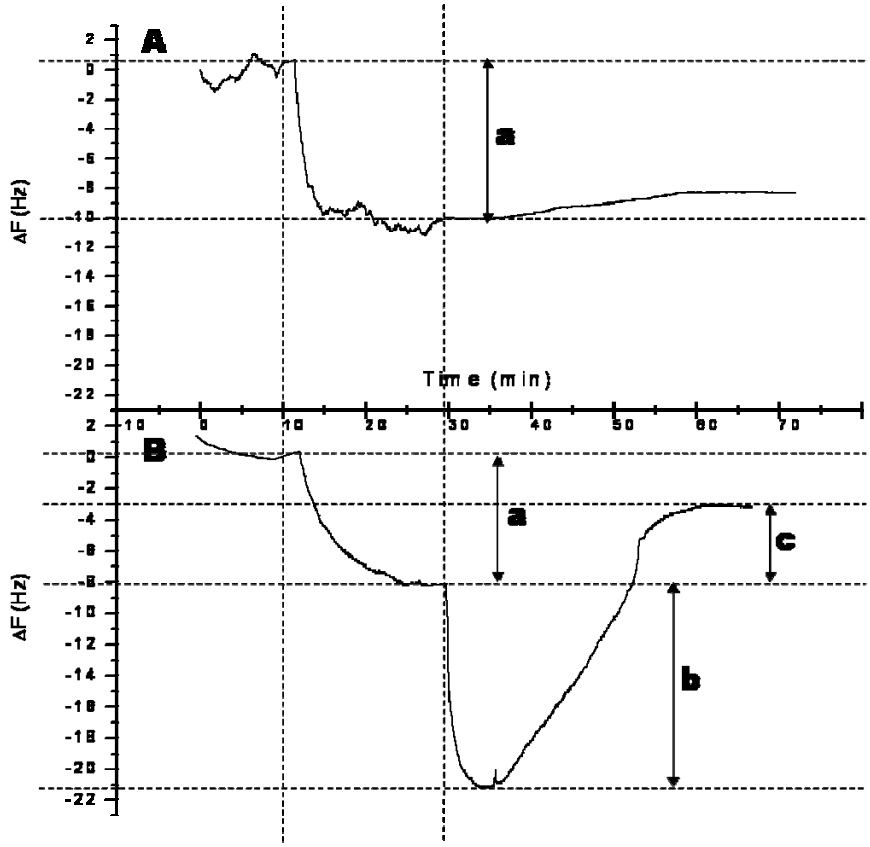

Fig. S1 Frequency shift of Au-coated quartz crystal electrode, modified with biotin-tagged capture probe, after addition of target samples (10 min) and CEL I nuclease (30 min) with perfectly matched target (A) and single-base mismatched target (B). The data indicate target hybridization with capture probe (a), CEL I binding to the mismatched site (b), and release of cleaved fraction (c).

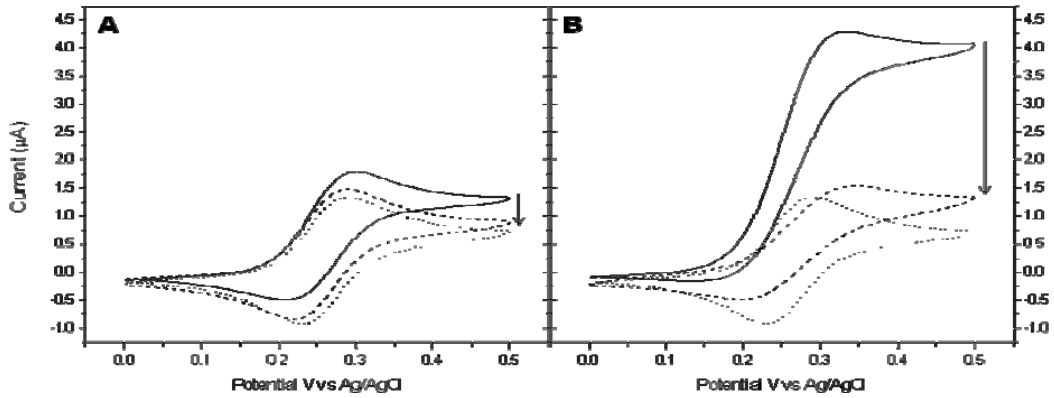

Fig. S2 Cyclic voltammograms before (A) and after (B) the addition of biotin-dendrimer on the sensing surface with artificial wild type sample (solid line), or artificial mutant sample (single-base substitution in position 3459, dashed line), and without electrocatalysis (control, dotted line).