

MATERIALS AND METHODS

Nanogap fabrication

The nanogap was fabricated using anisotropic wet etching of a boron-doped p-type (100) SOI wafer (IBIS Technology, USA). Initial 400-nm window line patterns were defined by optical lithography onto the SOI wafer through 10-nm-thick silicon nitride (Si_2N_4) deposition by low pressure chemical vapor deposition (LPCVD) and reactive ion etching (RIE). The anisotropic wet etching of silicon was performed with 20 wt% KOH solution for 20 sec at 80°C to create an inverse trapezoidal shape. The nanogap electrodes were finally constructed by removing the SiO_2 layer using hydrofluoric acid (HF, 49%) for 20 sec. Finally, gold electrodes on the silicon nanogap were fabricated with Cr/Au evaporation using a shadow mask that was patterned with 10- μm diameter after insulation with 80~120 nm of aluminum oxide (Al_2O_3) (Fig. S1).

Target DNA preparation

Single-stranded target DNA (300 nt) (partial hemagglutinin sequence of H1N1/influenza A virus) and complementary helper DNA (257 nt) for stretching the target DNA were prepared with asymmetric PCR (uni pfu polymerization system/BIOPROGEN/Korea). Each PCR reaction was conducted using 0.8 μM forward and 80 pM reverse primers (Fig. S2). The reaction mixture was incubated for 30 sec at 94°C. Forty cycles of PCR were performed at 94°C for 30 sec, 50°C for 30 sec, and 72°C for 20 sec followed by incubation at 72°C for 2 min. After each reaction, the products were gel purified (Bioneer/Korea). Two single-stranded DNA segments were pre-hybridized through

incubation at 94°C for 10 min. Finally, the sample was slowly cooled to room temperature before hybridization with the capture DNA immobilized on the nanogap electrodes.

Capture DNA immobilization on the nanogap electrode

All single-stranded DNAs were prepared by Bioneer (Korea) (Table S1). Two thiol-modified capture DNAs (1 μ M, 1X Saline-Sodium Citrate (SSC), 0.02% Sodium Dodecyl Sulfate (SDS)) with different sequences were immobilized at the same time on the nanogap electrode for 3 hr. After washing the nanogap device with 0.2% SDS and distilled water (DW), the device was reacted with mercaptohexanol (1%, ethanol) for 3 hr to reduce the nonspecific enhancement of the gold surface.

Capture DNA immobilization on the slide glass chip and target hybridization

Surface modification of a slide glass chip was completed in the following order: Piranha solution ($\text{H}_2\text{O}_2:\text{H}_2\text{SO}_4 = 1:3$, 30 min), 3-aminopropyltrimethoxysilane (APTMS, 2%, ethanol, 2 hr), and Ethyl (Dimethylaminopropyl) Carbodiimide (EDC) / N-HydroxySuccinimide (NHS) (0.4 M / 0.1 M, 1 hr). Next, an amine-modified target capture DNA mixture (10 μ M, 1X SSC, 0.02% SDS) was spotted with a microarrayer machine (Proteogen/Korea), and the chip was incubated for 1 hr. Finally, the glass chip was blocked with ethanolamine (1 M, pH 8.5). The pre-hybridized target samples (5X SSC, 0.1% SDS) were poured onto the capture DNA-hybridized glass chip, and the chip was then incubated for 1 hr. After washing the chip (2X SSC, 0.1% SDS), the Cy5-modified capture DNA (10 nM, 5X SSC, 0.1% SDS) was applied, and the chip was incubated for 1 hr (Fig. S3).

DNA hybridization and fabrication of conducting DNA-templated gold nanowire bridges

The pre-hybridized (5X SSC, 0.1% SDS) target DNA was introduced with capture DNAs immobilized on the gold surface of the nanogap for 2 hr at room temperature. DNA-templated conducting gold nanowires were fabricated by deposition of positively charged, 1.4-nm gold particle and gold enhancing. A 100- μ l volume of gold particles (Nanoprobes/USA) diluted in buffer solution (2 ml, 2X SSC, 0.1% Tween 20) was deposited on the negatively charged DNA backbones connecting

the two electrodes for 10 min at room temperature by gentle shaking. After washing to remove the remaining gold seed particles, the gold enhancing solution (Nanoprobes/USA) was poured onto the device, which was then incubated for the indicated time.

Table S1. All synthetic oligomers

Synthetic oligomers	Sequence and modification
5' control capture	SH-TTTTTTTTTTGATACCCATGTTTT
3' control capture	AGCGATGGCAACCATTTTTTTTTT-SH
5' target capture	SH-TTTTTTTTTTAGCTCCTCATAATCGAT
3' target capture	ATAACCTATACATAATGTTTTTTTTT-SH
5' HA primer (300)	ACATTATGTATAGTTATCAT
3' HA primer (300)	TAGCTCCTCATAATCGATGAA
5' helper primer (257)	CATGCGAACAATTCAACAGAC
3' helper primer (257)	GAAATCTCCTGGGTAACACGT
5' cy5-target capture	Cy5-TTTTTTTTTTAGCTCCTCATAATCGAT
5' target capture (NH)	NH-TTTTTTTTTTAGCTCCTCATAATCGAT
3' target capture (NH)	ATAACCTATACATAATGTTTTTTTTT-NH
5' control capture (NH)	NH-TTTTTTTTTTGATACCCATGTTTT
3' control capture (NH)	AGCGATGGCAACCATTTTTTTTTT-NH
5' Single mismatched capture	SH-TTTTTTTTTTAGCTCCTC <u>T</u> TAATCGAT
3' Single mismatched capture	ATAACCTA <u>A</u> ACATAATGTTTTTTTTT-SH

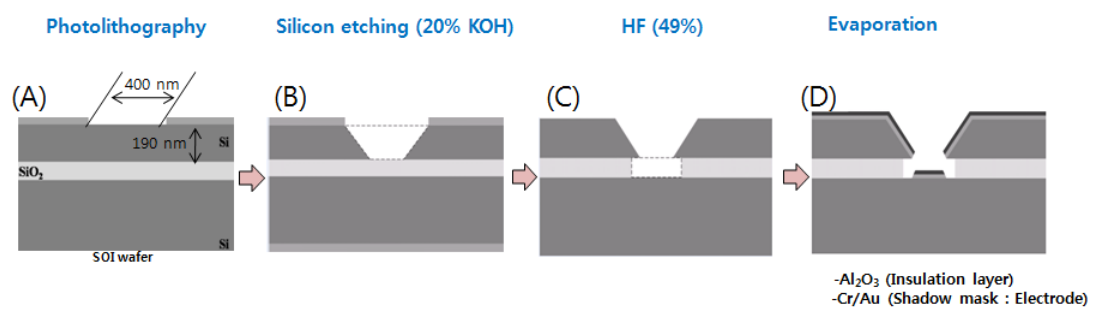


Fig. S1 Fabrication process of the nanogap electrodes

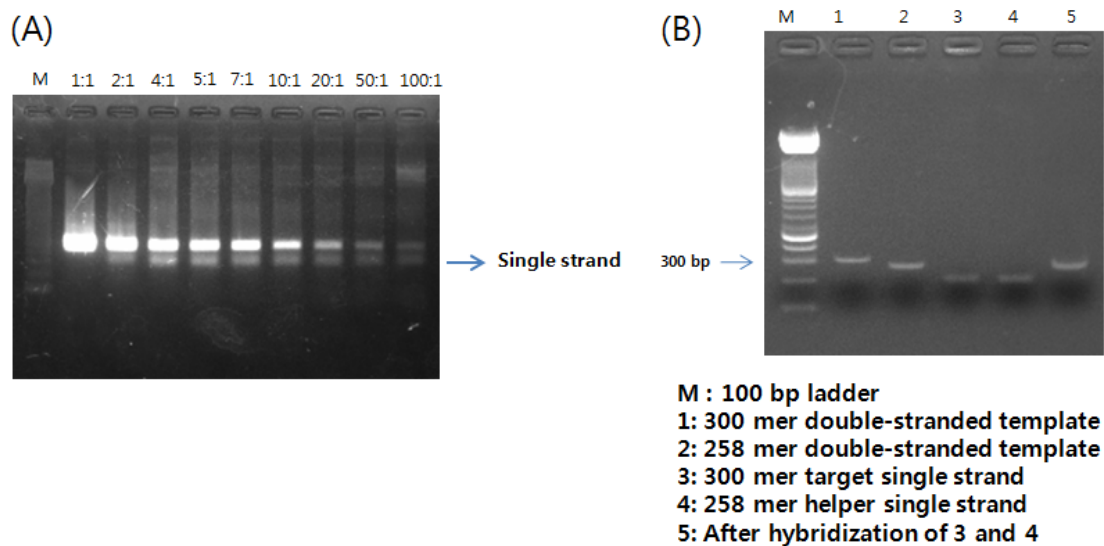


Fig. S2 Asymmetric PCR products prepared with varying concentrations of primers (A), and Gel-purified ss DNA & ds DNA PCR products (B)

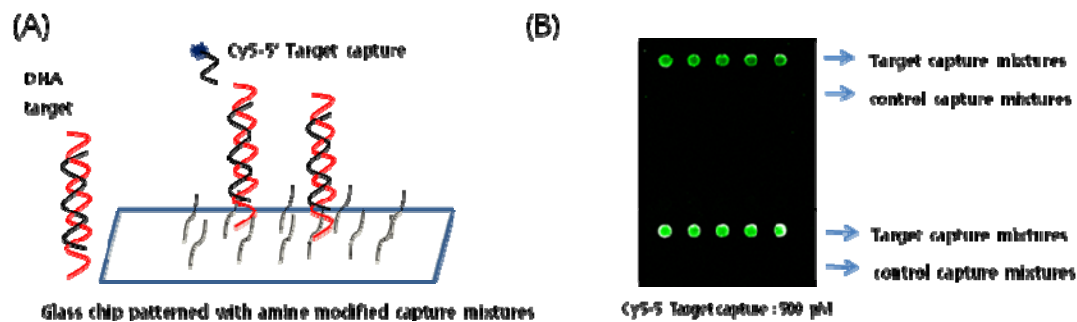


Fig. S3 Illustration of a fluorescence test on a glass chip to verify the hybridization of the target DNA and capture DNA mixtures (A), and result of hybridization with Cy5-5' target capture DNA (500 pM, 5XSSC, 0.2% SDS) (B)

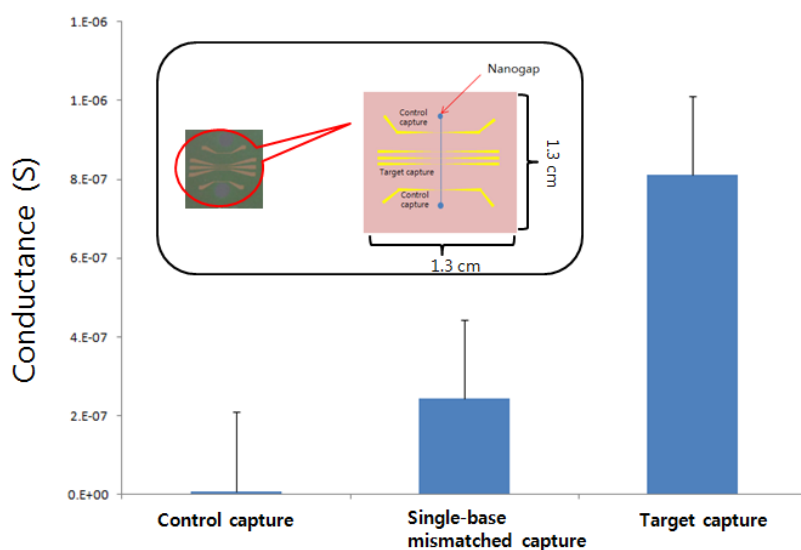


Fig. S4 Target DNA selectivity of nanogap electrodes immobilized with control capture, single-base mismatched capture, and target capture probes, respectively. 1 nM of target DNA was added to differently modified nanogap electrodes and nanobridges were formed via 30 sec enhancing reaction. The error bars show the relative standard deviations from ten measurements. Inset picture shows the nanogap electrode used in this study with locations of control and target capture probes on one device. (Single mismatched capture probe was immobilized in place of target capture probe)