Supporting Information

A Microfluidic Chip for Rapid Analysis of DNA Melting Curves for BRCA2 Mutation Screening

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Experimental Procedures

1. Microheater and sensor layer fabrication and measurement

A photoresist HPR 506 (Fujifilm, Tokyo, Japan) was deposited on Pyrex glass at 4000 rpm using a spin coater (PHT-SC1, Suss, Munich, Germany). It was patterned by photolithography in a mask aligner (ABM-USA, San Jose, USA). The photoresist was developed using FHD-5 (Fujifilm, Tokyo, Japan) and washed with double deionized (DDI) water three times. A layer of 10 nm Titanium (Ti) which acted as an adhesive layer and 200 nm Platinum (Pt) was sputtered, then deposited on the designed patterned glass surface of the glass. The glass was soaked in acetone overnight and sonicated to lift off the residues. Similarly, a 200 nm Au layer was deposited to form the heater electrodes. The glass with microheater was by rinsing with DDI water for four times and spun dry in N_2 gas.

To prepare the temperature sensor layer, 5 mg tris(1,10-phenanthroline) ruthenium (II) dichloride ($Ru(phen)_3$) and 500 mg poly(styrene-co-acrylonitrile) (PSAN) were mixed in chloroform (3.4 mL, all from Aldrich, St. Louis, USA) and stirred overnight at room temperature, as the coating solution. The $Ru(phen)_3$-PSAN mixture was spread by blade coating (BDG218, Biuged, Guangzhou, China) on a glass surface. A thin film was generated after evaporation of the solvent. The heights were measured by a surface profiler (Tencor P-7 Surface Profiler, KLA, Milpitas, USA) at 50 μm* min^{-1}. The sensor film was torn off manually at a corner for measurement. The result showed the microheater was around 200 μm width and 200 nm height (Fig. S1a) and the blade-coated temperature sensor layer showed an average height of 5.5 μm (Fig. S1b).

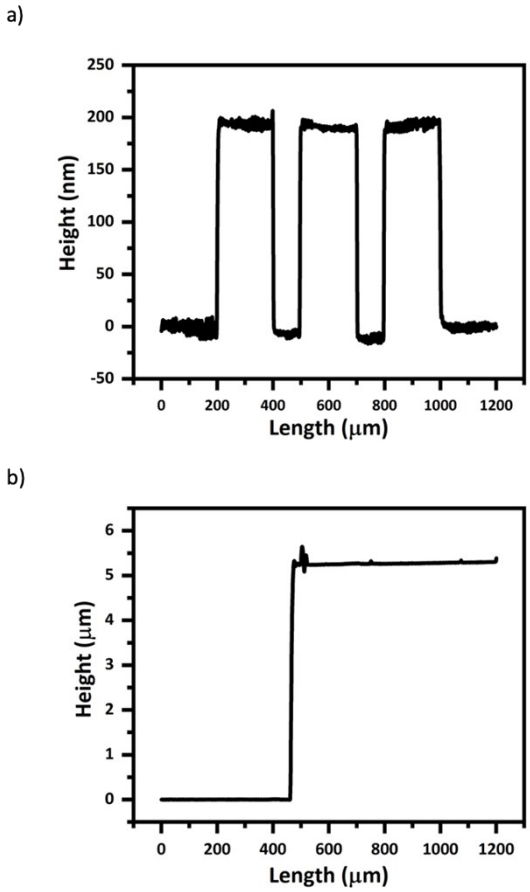

Figure S1. a) The width and height of the deposited Pt microheater structure. b) The height of the Ru(phen)$_3$-PSAN temperature sensor film.

2. Microchip assembly

To observe the binding of PDMS and the Ru(phen)$_3$-PSAN layer, one-third of the PDMS slab was cut and removed carefully from the sensor layer (Fig. S2a). The cut-off chip was place on a microscope (Nikon AZ100, Tokyo, Japan) vertically under an 2x objective (AZ Plan Fluor, Nikon, Tokyo, Japan) which showed that the PDMS slab was sealed tightly with the sensor film coating on the glass substrate (Fig. S2b & c).

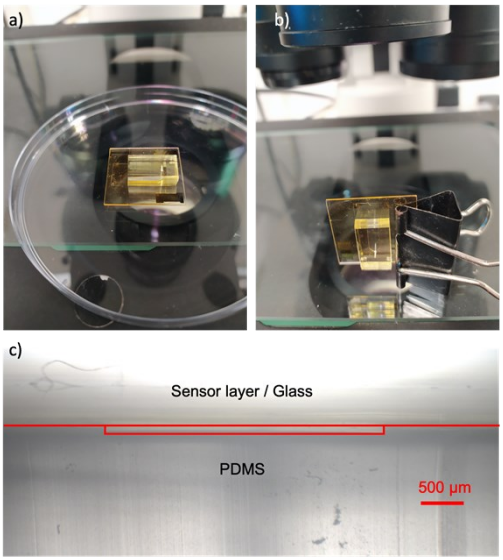

Figure S2. a) Camera picture of a microfluidic chip, cut for inspection in c), b) side view of the microchip, c) cross-section of the micro-chamber, imaged with a brightfield microscope with 2x objective.

3. Melting temperature measurements

Six oligonucleotides, BRCA2 exon 5 and its five base pair (bp) deletion fragment (rs80359463), 51 bp BRCA2 exon 11 and its two base pair deletion fragment (rs276174826), 45 bp BRCA2 exon 11 and its five-base-pair deletion fragments (rs876660311) were dissolved and diluted in the buffer solution respectively. The buffer (pH 8.3) contained 10 mM Tris-HCl, 50 mM KCl and 2.5 mM $MgCl_2$. 11.1 µL of 10x LCGreen Plus dye (Idaho Technology, Salt Lake City, USA) was added to the 100 µL DNA mixture, resulting in a final concentration of 1x for LCGreen Plus and 20 µM for the double stranded DNA. A 20 µL sample containing 20 µM dsDNA sample fragment and 1x LCGreen dye was manually injected into the microfluidic chamber and 3 minutes were allowed after applying voltage for thermal equilibrium. The fluorescence of the solution in the microchamber was measured using CCD camera in the imaging assembly at room temperature and after switching on the micro-heater. The measurements provided the spatial distribution of temperature via lifetimes of Ru(phen)3-PSAN film and the fluorescence of the DNA. Shown in the following are measurements with. The repeatability of melting temperature was investigated with different microchips in the same dsDNA fragment independently and the results are displayed in Figs. S3-S8. The measurements for each DNA fragment were repeated for three times and microchips were disposed after each shot of the test. The mean values and standard deviations of melting temperatures were obtained.

a) Melting temperature measurements of BRCA2 exon 5

Melting curves were acquired with different microchips and the average melting temperature of BRCA2 exon 5 50 bp fragment was determined to be 65.3 ± 0.1 °C (Fig. S3).

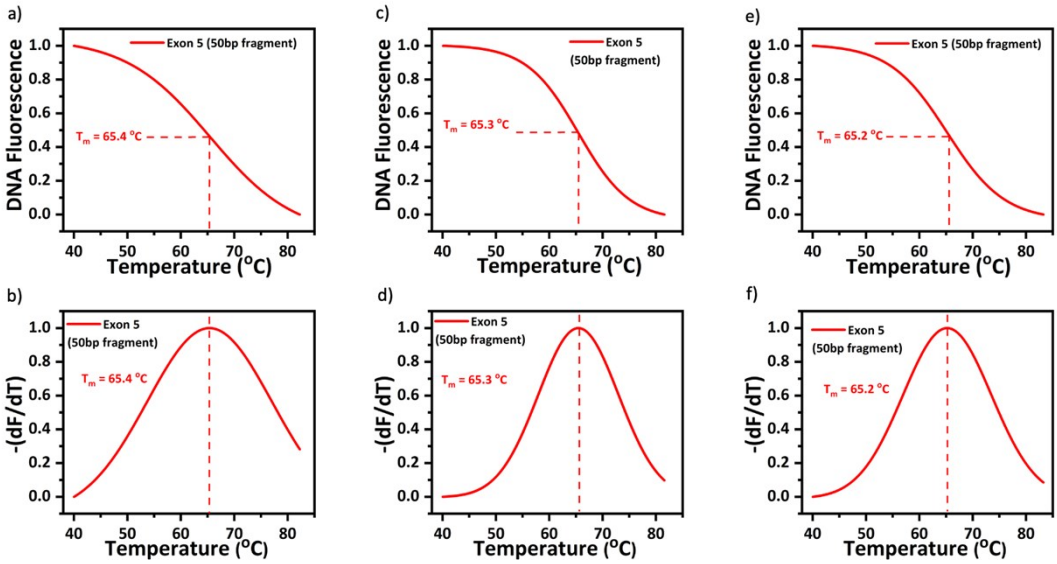

Figure S3. a, c, e) Plot of the DNA mixture as a function of DNA fluorescence to temperature of BRCA2 exon 5 (50bp), b, d, f) Plot of the derivative function of DNA fluorescence to temperature and its derivative function of the fragment of f BRCA2 exon 5 (50bp).

b) Melting temperature measurements of rs80359463

Melting curves were acquired with different microchips and the average melting temperature of BRCA2 exon 5 rs80359463 fragment was 64.2 ± 0.5 °C (Fig. S4).

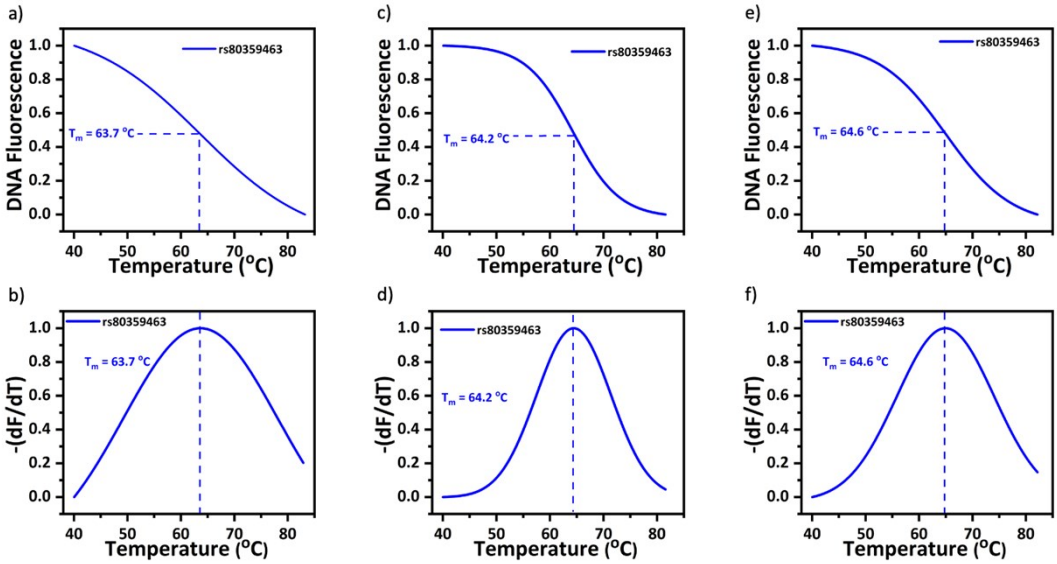

Figure S4. a, c, e) Plot of the DNA mixture as a function of DNA fluorescence to temperature of rs80359463 (45bp), b, d, f) Plot of the derivative function of DNA fluorescence to temperature and its derivative function of the fragment of rs80359463 (45bp).

c) Melting temperature measurements of the 51bp BRCA2 exon 11 fragment

Melting curves were acquired with different microchips and the average melting temperature of BRCA2 exon 11 51 bp fragment was 72.8 ± 0.2 °C (Fig. S5).

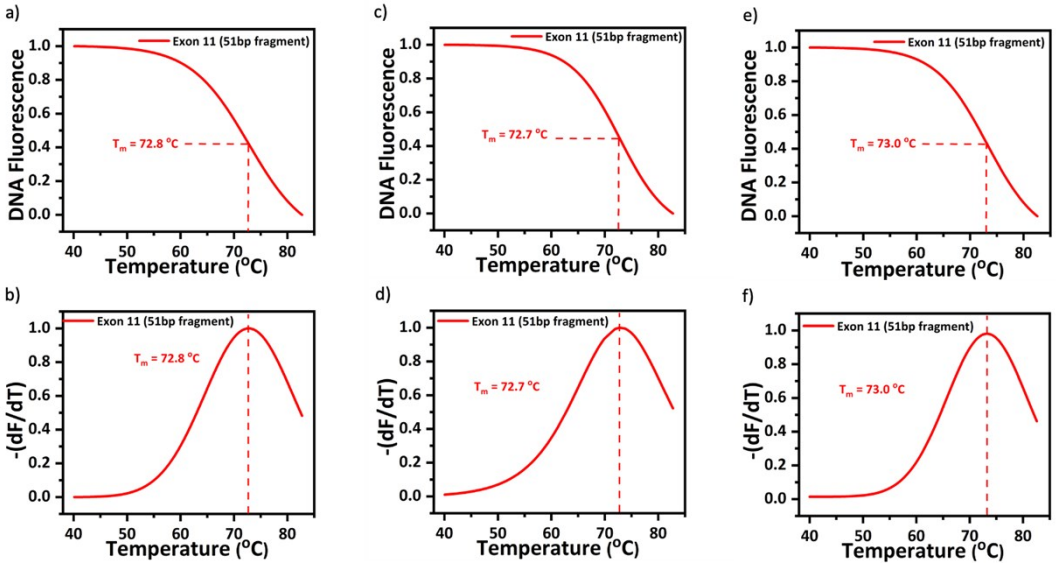

Figure S5. a, c, e) Plot of the DNA mixture as a function of DNA fluorescence to temperature of BRCA2 exon 11 (51bp), b, d, f) Plot of the derivative function of DNA fluorescence to temperature and its derivative function of the fragment of BRCA2 exon 11 (51bp).

d) Melting temperature measurements of rs276174826

Melting curves were acquired with different microchips and the average melting temperature of BRCA2 exon 11 rs276174826 fragment was 72.0 ± 0.2 °C (Fig. S6).

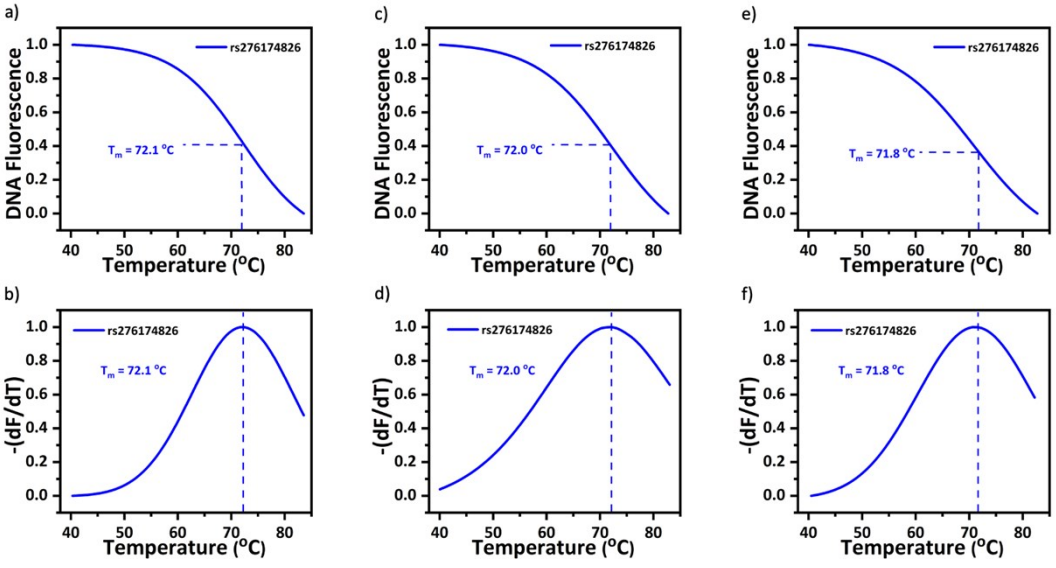

Figure S6. a, c, e) Plot of the DNA mixture as a function of DNA fluorescence to temperature of rs276174826 (49bp), b, d, f) Plot of the derivative function of DNA fluorescence to temperature and its derivative function of the fragment of rs276174826 (49bp).

e) Melting temperature measurements of 45bp BRCA2 exon 11 fragment

Melting curves were acquired with different microchips and the average melting temperature of BRCA2 exon 11 45 bp fragment was determined to be 77.0 ± 0.2 °C (Fig. S7).

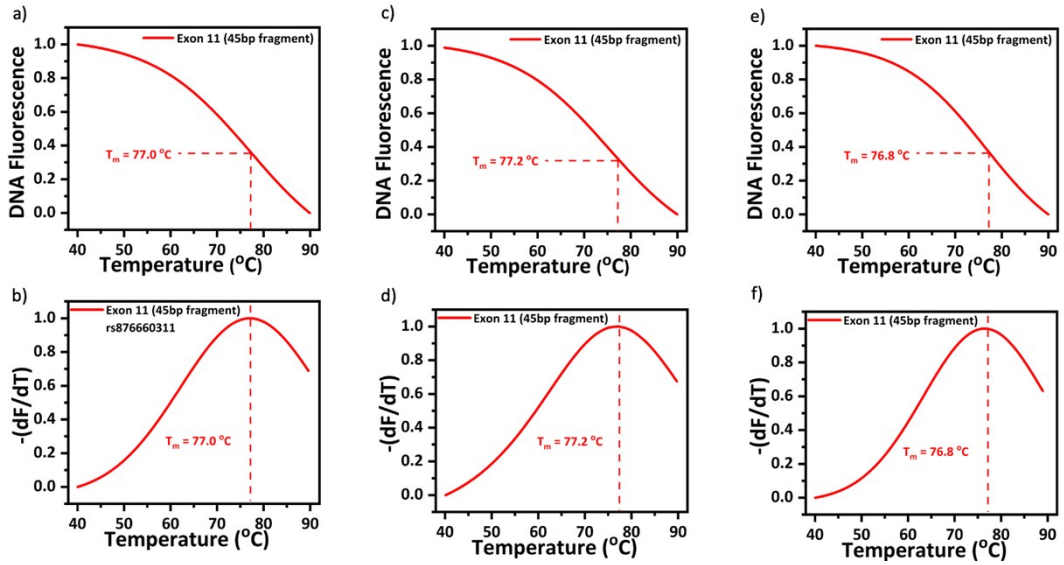

Figure S7. a, c, e) Plot of the DNA mixture as a function of DNA fluorescence to temperature of BRCA2 exon 11 (45bp), b, d, f) Plot of the derivative function of DNA fluorescence to temperature and its derivative function of the fragment of BRCA2 exon 11 (45bp).

f) Melting temperature measurements of rs876660311

Melting curves were acquired with different microchips and the average melting temperature of BRCA2 exon 11 rs876660311 fragment was determined to be 75.3 ± 0.1 °C (Fig. S8).

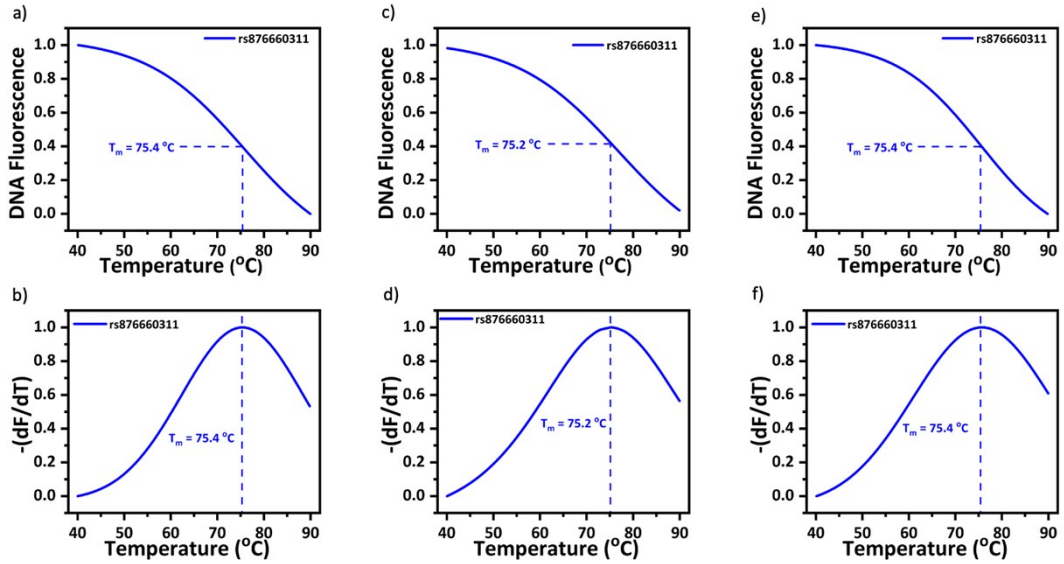

Figure S8. a, c, e) Plot of the DNA mixture as a function of DNA fluorescence to temperature of rs876660311 (40bp), b, d, f) Plot of the derivative function of DNA fluorescence to temperature and its derivative function of the fragment of rs876660311 (40bp).