Supporting Information

for

μ-eLCR: A Microfabricated Device for Electrochemical Detection of DNA Base Changes in Breast Cancer Cell Lines

Eugene JH Wee,[¶,†] Sakandar Rauf,[¶,†] Kevin MS Koo,[†] Muhammad JA Shiddiky,[†,] and Matt Trau[†,‡,*]*

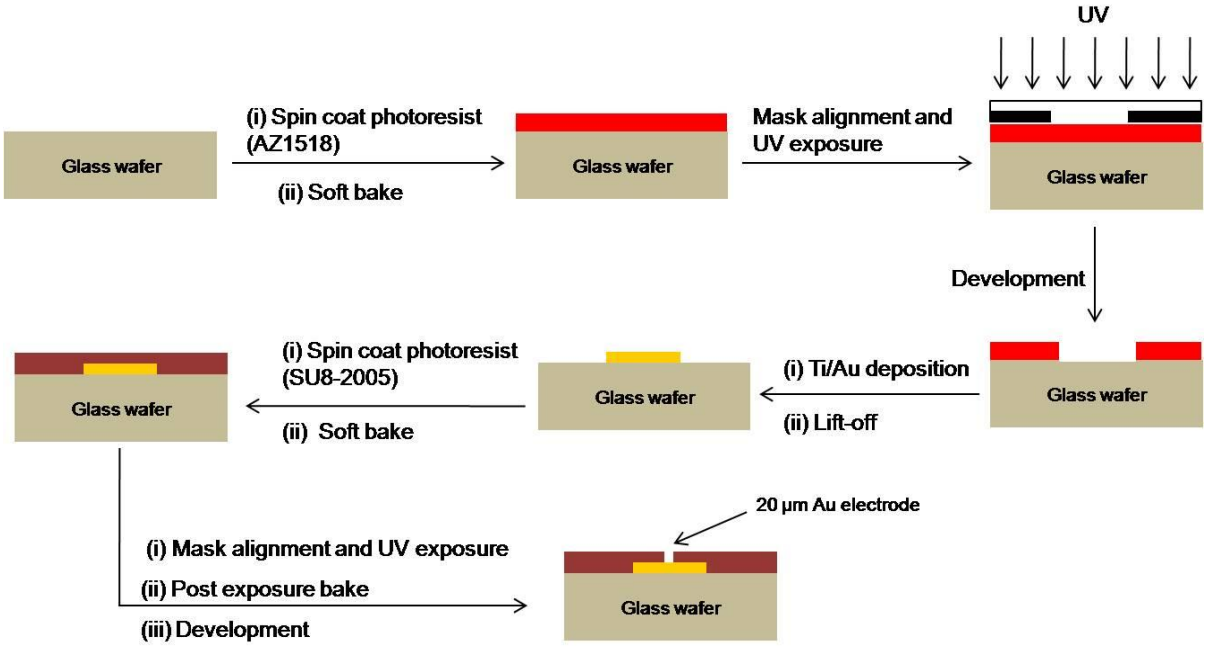

Fig S1. Shows scheme for the fabrication of microelectrodes.

Fabrication of Microchips

Pyrex glass wafers (4", 500 µm thick, double-side polished) were obtained from University wafers, Boston, USA. The chip was designed using Layout Editor (L-Edit V15, Tanner Research Inc., CA) and 5" X 5" sodalime chrome masks for these designs were obtained from Qingyi Precision Maskmaking (Shenzhen) Ltd, China. Briefly, the glass wafer was first cleaned with sonication in acetone and Isopropanol (IPA). The cleaned glass wafer was coated with positive photoresist (AZ1518 Microchemicals, Germany) to obtain a 2 µm thick resist layer (4000 rpm for 30s), followed by a soft bake for 6 min at 95 °C. The positive photoresist coated wafer was then UV exposed (150 mJ/cm^2) using a mask aligner (EVG620, EV Group GmbH, Austria) and developed in AZ 726 developer solution (Microchemicals, Germany) for 25s, followed by rinsing with deionized (DI) water (Millipore Pvt. Ltd., Australia) and dryed under the flow of nitrogen (N_2) gas. The glass wafer was then treated in oxygen plasma (30 watt for 30s) to remove any residual resist layer in the developed patterns. A 200 nm of gold with 10 nm titanium adhesion layer was then deposited on the wafers using an e-beam evaporation method (Temescal BJD-2000 E-beam), and gold electrode pattern was obtained with a lift off process. Finally, the wafer was rinsed with acetone and IPA, and dried under the flow of N_2 gas. Typical gold patterns were 200 µm in width (w) and 6 mm in length (l) connected with relatively larger pads of 1×1 mm^2.

The wafer was then coated with SU8-2005 (Microchemicals, Germany) at 500 rpm for 10s and 2000 rpm for 30s to obtain 5µm thick resist layer to cover the gold electrode patterns. This resist layer acts as an insulator. The glass wafer was soft baked at 65 °C for 2 min, 95 °C for 4 min followed by exposure with UV light (200 mJ/cm^2) using the mask aligner. The photomask was designed such that on each electrode, a 20 µm diameter working electrode and a 1×1 mm gold connecting pad were exposed after development in Propylene glycol

monomethyl ether acetate (PGMEA). The gold alignment marks were used to align the wafer with the second mask. The rest of the electrode areas were insulated with SU8-2005.

Fabrication of microfluidic Channels and Wells

For the second part of the chip, an SU8 master was fabricated on a silicon wafer using SU8-2150 (Microchem, SU-8 2150). Briefly, silicon wafer was cleaned by sonication in acetone and IPA. The wafer was then further cleaned in oxygen plasma (100 watt for 1 min). SU8-2150 was spin coated at 1800 rpm for 30s, soft baked at 65 °C for 7 min, 95 °C for 60 min and 65 °C for 5 min. The wafer was then exposed to UV light (380 mJ/cm^2). Following the post exposure bake (65 °C for 5 min, 95 °C for 20 min and 65 °C for 3 min), the wafer was developed in propylene glycol methyl ether acetate (PGMEA) to reveal the photoresist pattern which was used as master.

For the preparation of PDMS microchannels, PDMS elastomer Sylgard 184 kit (Dow Corning) and master prepared above were used. The PDMS precursor was mixed with curing agent (10:1), after degassing poured on top of the master. After curing at 65°C for 4 h, 500 µm wide and 300 µm deep channels were obtained. Four and five mm holes were punched into the PDMS to open inlet, outlet and deep wells for the electrodes. These were then permanently bound to the chips prepared above after treatment with oxygen plasma (20 Watt for 10 s). In case of PDMS wells, the PDMS along with the precursor poured on top of the plane silicon wafer. After curing at 65°C for 4 h, the PDMS was peeled off from the wafer and cut into the same size as the chip. After this, five mm holes were punched into the PDMS and then it was bonded to the chip to open deep wells for the electrodes.

Estimation of the Effective Working Area

Cyclic volammetry were conducted in 100 mM PBS solution containing 2.5 mM $K_3[Fe(CN)_6]$, 2.5 mM $K_2[Fe(CN)_6]$ and 0.1 M KCl.

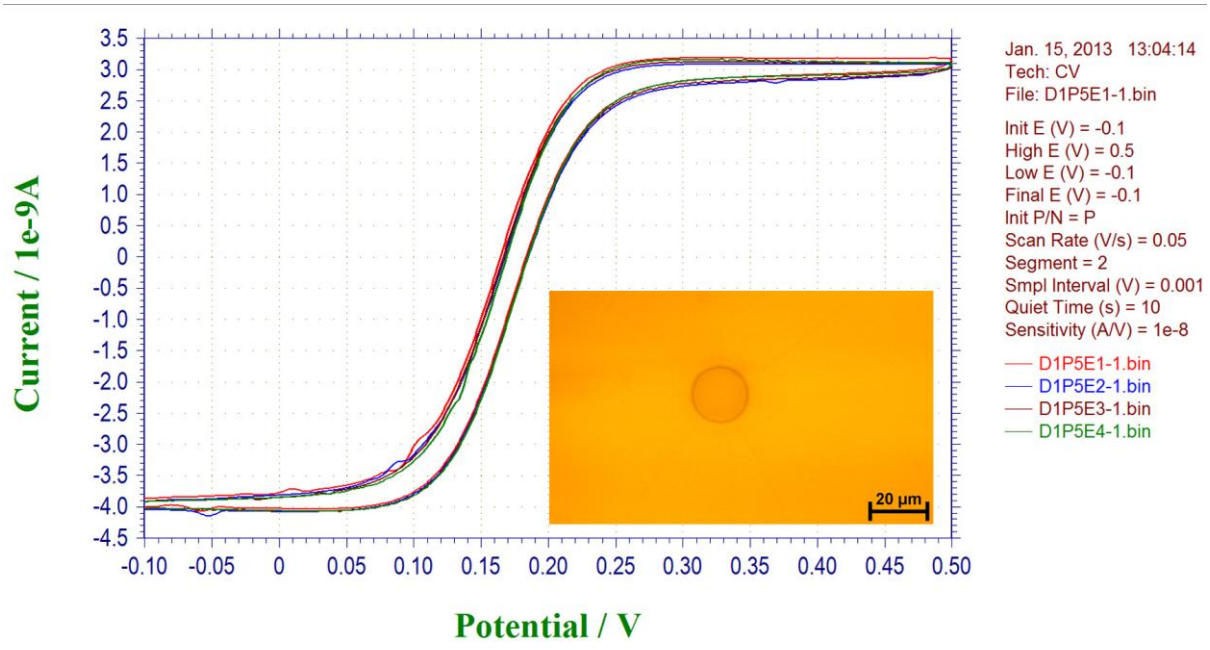

Fig. S2. Representative CV profiles of four microelectrodes in a well. Insert: bright field microscopy images of one electrode. 20µm scale is given.

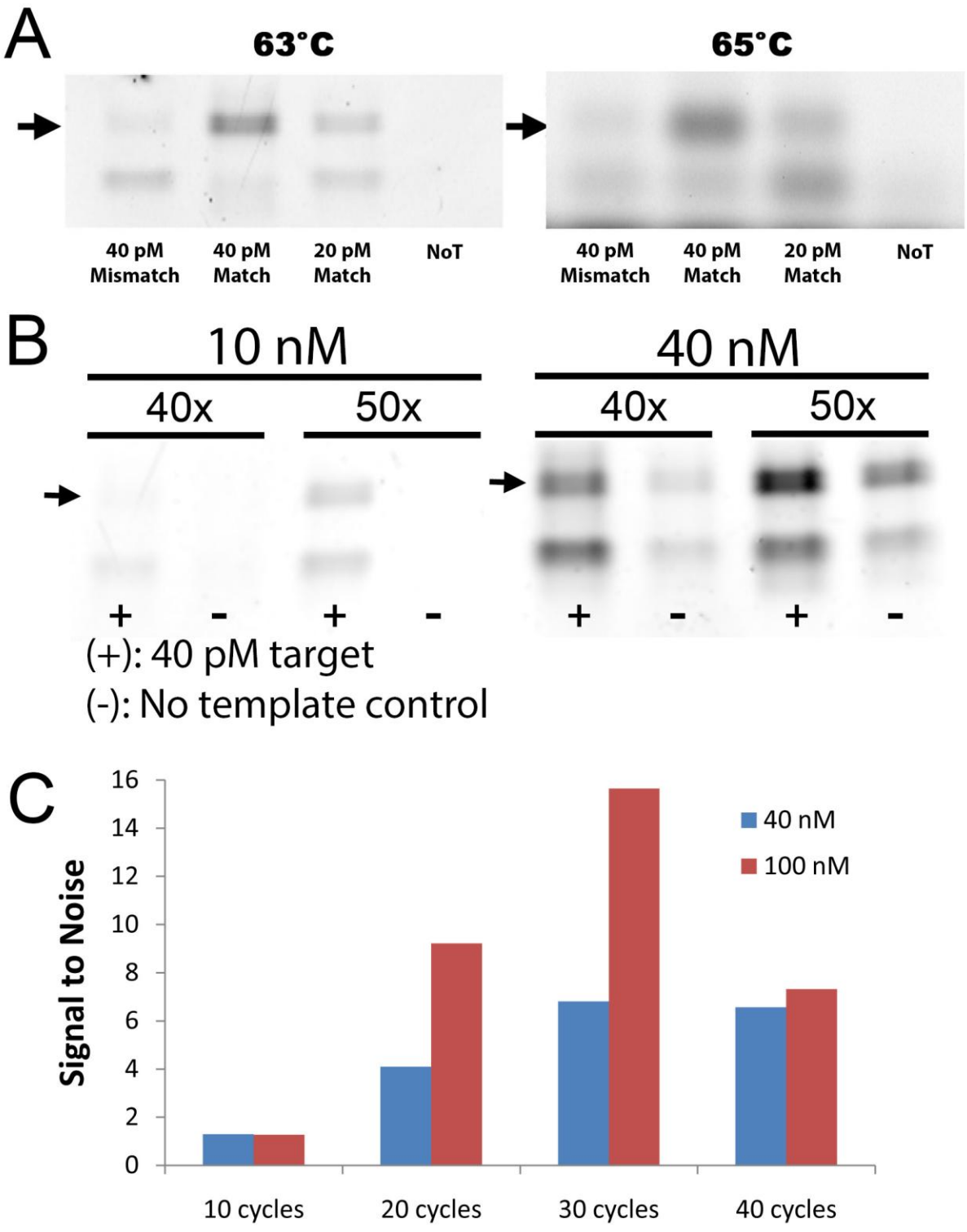

Fig. S3. (A) 10 nM of fluorescence-labelled probes were used in the LCR reaction at 63°C and 65°C to optimize annealing/ligation temperature for 40 pM of mismatch target, 40 pM and 20 pM matched target, and a no template (NoT) control. After 50 cycles, specific amplification was observed at 65°C. Arrows indicate the amplified bands. (B) When 40 pM

of probes was used, ligation product (arrowed) could be detected after 40 cycles indicating that higher probe concentrations can reduce cycle numbers which leads to shorter run times. (C) To further reduce run times, 100 nM of probes were used for LCR where specific amplification could be detected as soon as 20 cycles. To evaluate the approach, S/N was used to determine the optimal LCR condition. The best condition was seen at 100 nM of probes with 30 LCR cycles. But to avoid signal saturation, 100 nM with 20 cycles was used for subsequent experiments.

LCR Products Amplified from Serum DNA

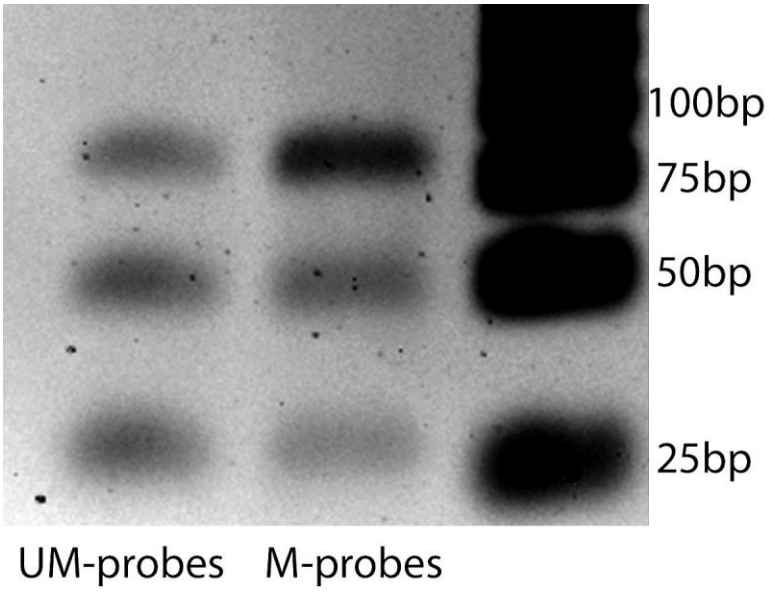

Fig. S4. DNA gel electrophoresis stained with ethidium bromide of LCR products amplified from serum DNA using both the methylated specific probes (M-probes) and unmethylated specific probes (UM-probes). Successfully ligated products are of approximately 75bp in length. The darker 75bp band intensity using M-probes suggests higher amounts of LCR products compared to that of UM-probes.