Surface ligation-based resonance light scattering analysis of methylated genomic DNA on microarray platform

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Contents:

Experimental section

Preparation of DNA-functionalized gold nanoparticle probes

The citrate stabilized 13 nm gold nanoparticles (GNPs) were synthesized by classical Turkevich–Frens method (Frens 1973). DNA-functionalized GNP (named as DNA-GNP) probes were prepared referred to a reported procedure (Wang et al. 2004). Generally, GNP solution (8 nmol L^{-1}, 300 μL) was incubated with oligonucleotide (Label 1, 100 μmol L^{-1}, 2.5 μL) in aqueous solution overnight, then diluted with 300 μL PBS buffer (10 mmol L^{-1} PB, 0.2 mol L^{-1} NaCl, pH7.5). After further incubation for another 10 h, the solution was evaporated to 100 μL by a vacuum concentrator (Eppendorf AG Co., Germany). Excess oligonucleotides were removed by repeated centrifugations (9000 rpm, 3 times). Finally, the DNA-GNP probes were dispersed in probe reaction buffer (0.67×SSC, 0.1% (w/v) SDS).

DNA microarray Fabrication and hybridization

Probe DNAs in Table S1 (30 μmol L^{-1} in spotting buffer (3×SSC, 1.5 mol L^{-1} betaine, 0.005% (w/v) SDS)) were spotted onto aldehyde 3-D glass slide by a SmartArrayer 96 system (Capitalbio Ltd., Beijing, China). After an overnight incubation under 70% humidity at 37 °C, the slide was orderly rinsed with washing buffer (1×SSC, 0.01% (w/v) SDS) and water to remove excess probe DNAs. After further treatment with 0.5% (w/v) $NaBH_4$ solution for 30 min, the slide was dried by centrifugation (480 g for 1 min) and separated into independent subarrays by PTFE grids (Capitalbio Ltd., Beijing, China). Subsequently, the subarrays were incubated with blocking solution (4 mg mL^{-1} PEG-NH_2 in PBS buffer (50 mmol L^{-1} PB, 0.15 mol L^{-1} NaCl, pH7.5)) for 1 h at 30 °C to inactivate remaining aldehyde groups. After that, the slide was washed with PBS buffer and water successively and dried by centrifugation.

After blocking step, target DNAs were dissolved in 25 μL hybridization buffer (4 ×SSC, 0.1% (w/v) SDS) with desired concentration and incubated with subarrays at 45 °C for 1 h. Then, the slide was subjected to a series of rinses: (1) 30 mL hybridization buffer at 45 °C for 5 min (3 times), (2) 30 mL washing buffer at 45 °C

for 5 min (3 times), and (3) 30 mL Milli-Q water for 3 min (3 times), respectively. Subsequently, the subarrays were further incubated with 25 μL assistant DNAs (100 nmol L^{-1} in hybridization buffer) at 45 °C for 1 h. Then, the subarrays were washed and dried as previously described.

DNA-GNP probe labeling

After RCA reaction, PTFE grids were firstly removed from the slides. The slides were incubated with 5 nmol L^{-1} DNA-GNP probes (in 500 μL probe reaction buffer) at 30 °C for 1 h, and washed as previously described. Then, 1 mL silver enhancer solution (solution A ($AgNO_3$) and solution B (hydroquinone) were mixed with the volume ratio of 1:1) was transferred to slide and incubated for 8 min. Finally the slide was washed with water (3 times) and dried by centrifugation.

Data acquisition and analysis

RLS signal and fluorescence signal were acquired by ArrayIt SpotWare Colorimetric Microarray Scanner (Telechem. International Inc., USA) and LuxScan-10K fluorescence microarray scanner (Capitalbio, Beijing) according to manufacturer's preset parameters, respectively. The background signal originating from the slide was recorded and subtracted prior to evaluation. The mean value and standard deviation of the signal intensity were determined from 6 spot replicates per sample.

Table S1. Sequences of oligonucleotides used in experiment[a]

Name	Sequence
Circle template	5'-P-CGATAGTCTGTTTAGTCTTTGCTACTTCACGATTGCTTCATCTACGCTGATTTCCGCTTCCTTTGC-3'
Link template	5'-AACAGACTATCGGCAAAGGAAGCG-3'
Probe 1	5'-NH_2-C_6-T_{10}AACCCACCGCGGGTTTGCG-3'
Probe 2	5'-NH_2-C_6-T_{10}AACCCACCGCGGGTTTACG-3'
Probe 3	5'-NH_2-C_6-T_{10}AACCCACCGCGGGTTTTCG-3'
Probe4	5'-NH_2-C_6-T_{10}AACCCACCGCGGGTTTCCG-3'
Probe 5	5'-NH_2-C_6-T_{10}AACCCACCGCGGGTCTGCG-3'
Probe 6	5'-NH_2-C_6-T_{10}AACCCACCGCGGATTTGCG-3'
Target 1C	5'-CTGTCCTCCAGGAATCAAGGGAAATACGCAAACCCGCGGTGGGTT-3'
Target 1T	5'-CTGTCCTCCAGGAATCAAGGGAAATATGCAAACCCGCGGTGGGTT-3'
Assistant 1-Cy5	5'-P-TATTTCCCTTGATTCCTGGAGGACAGATCAGCGTAGATGAAGCA-Cy5-3'
Assistant 1	5'-P-TATTTCCCTTGATTCCTGGAGGACAGATCAGCGTAGATGAAGCA-3'
Probe 14	5'-NH_2-C_6-T_{10}AAACACAACTCCAAACAACCCG-3'
Target 14T	5'-GGTGATGGATGTAGTTAGGGGGTGAGTTGTTTGGAGTTGTGTTT-3'
Target 14C	5'-GGTGATGGATGTAGTTAGGGGGCGAGTTGTTTGGAGTTGTGTTT-3'
Assistant 14	5'-P-CCCCCTAACTACATCCATCACCATCAGCGTAGATGAAGCA-3'
Probe 26	5'-NH_2-C_6-T_{10}TCTCCCTCCCACCTATCG-3'
Target 26T	5'-GTGTATATAGGGTGGGAAAGTGGTGGTAGGTGGGAGGGAGA-3'
Target 26C	5'-GTGTATATAGGGTGGGAAAGTGGCGGTAGGTGGGAGGGAGA-3'
Assistant 26	5'-P-CCACTTTCCCACCCTATATACACATCAGCGTAGATGAAGCA-3'
Probe 44	5'-NH_2-C_6-T_{10}CACCCCCACTCCCACGCG-3'
Target 44T	5'-AGTTGTTTTGAGATTTTGGAGGTTTGGGTGGGAGTGGGGGTG-3'
Target 44C	5'-AGTTGTTTTGAGATTTTGGAGGTTCGGGTGGGAGTGGGGGTG-3'
Assistant 44	5'-P-AACCTCCAAAATCTCAAAACAACTATCAGCGTAGATGAAGCA-3'
Probe 48	5'-NH_2-C_6-T_{10}CTCCACCCCACAAACTCG-3'

Target 48T	5'-GTGTTTAGGGAAGGTGGGTG**T**GTGTTTGTGGGGTGGAG-3'

Continued from Table S1

Target 48C	5'-GTGTTTAGGGAAGGTGGGTG**C**GTGTTTGTGGGGTGGAG-3'
Assistant 48	5'-P-CACCCACCTTCCCTAAACAC<u>ATCAGCGTAGATGAAGCA</u>-3'
Probe 53	5'-NH_2-C_6-T_{10}CCTTAACTACAAACTAAAACCCCCG-3'
Target 53T	5'-GATGGGTAGGGGGTGGTG**T**GTGGGTTTTAGTTTGTAGTTAAGG-3'
Target 53C	5'-GATGGGTAGGGGGTGGTG**C**GTGGGTTTTAGTTTGTAGTTAAGG-3'
Assistant 53	5'-P-CACCACCCCCTACCCATC<u>ATCAGCGTAGATGAAGCA</u>-3'
Probe 54	5'-NH_2-C_6-T_{10}CCCTTTAACACCAAAAATAAACACCG-3'
Target 54T	5'-CAGTTAAGGGGGTAGGAGTGG**T**GTTGTTTATTTTTGGTGTTAAAGGG-3'
Target 54C	5'-CAGTTAAGGGGGTAGGAGTGG**C**GTTGTTTATTTTTGGTGTTAAAGGG-3'
Assistant 54	5'-P-CCACTCCTACCCCCTTAACTG<u>ATCAGCGTAGATGAAGCA</u>-3'
Probe 48-1	5'-NH_2-C_6-T_{10}CACCACCCCCTACCCATCTCCACCCCACAAACTCG-3'
Probe 48-2	5'-NH_2-C_6-T_{10}CACCGCCCCCTACCCATCTCCGCCCCGCAAACTCG-3'
Probe 53-1	5'-NH_2-C_6-T_{10}CCACTCCTACCCCCTTAACTACAAACTAAAACCCCCG-3'
Probe 54-1	5'-NH_2-C_6-T_{10}CAACCACTACACCACCCTTTAACACCAAAAATAAACACCG-3'
Label 1	5'-SH-C_6-T_{10}TCTTTGCTACTTCACGAT-3'

[a] The underline fragment is complementary with circular template. Probes 1 to 6 are employed to investigate the effect of mismatched base pair on the fidelity of Taq DNA ligase. The red and bold bases in Probe 2 to 6 are different from the corresponding base in Probe 1. Assistant DNAs are phosphorylated at 5' terminal.

Table S2. Components of mimic samples and assistant DNAs mixture used in Fig. 2.

Name	Component
Control	100% Target 14T, 100% Target 26T, 100% Target 44T, 100% Target 48T, Target 53T, and Target 54T
Sample 1	0.01% Target 14C, 99.99% Target 14T, 0.01% Target 26C, 99.99% Target 26T, 0.01% Target 44C, 99.99% Target 44T, 0.01% Target 48C, 99.99% Target 48T, 0.01% Target 53C, 99.99% Target 53T, 0.01% Target 54 C, and 99.99% Target 54T
Sample 2	0.03% Target 14C, 99.97% Target 14T, 0.03% Target 26C, 99.97% Target 26T, 0.03% Target 44C, 99.97% Target 44T, 0.03% Target 48C, 99.97% Target 48T, 0.03% Target 53C, 99.97% Target 53T, 0.03% Target 54 C, and 99.97% Target 54T
Sample 3	0.10% Target 14C, 99.9% Target 14T, 0.10% Target 26C, 99.9% Target 26T, 0.10% Target 44C, 99.9% Target 44T, 0.10% Target 48C, 99.9% Target 48T, 0.10% Target 53C, 99.9% Target 53T, 0.10% Target 54 C, and 99.9% Target 54T
Sample 4	0.30% Target 14C, 99.7% Target 14T, 0.30% Target 26C, 99.7% Target 26T, 0.30% Target 44C, 99.7% Target 44T, 0.30% Target 48C, 99.7% Target 48T, 0.30% Target 53C, 99.7% Target 53T, 0.30% Target 54 C, and 99.7% Target 54T
Sample 5	1.0% Target 14C, 99% Target 14T, 1.0% Target 26C, 99% Target 26T, 1.0% Target 44C, 99% Target 44T, 1.0% Target 48C, 99% Target 48T, 1.0% Target 53C, 99% Target 53T, 1.0% Target 54 C, and 99% Target 54T
Sample 6	3.0% Target 14C, 97% Target 14T, 3.0% Target 26C, 97% Target 26T, 3.0% Target 44C, 97% Target 44T, 3.0% Target 48C, 97% Target 48T, 3.0% Target 53C, 97% Target 53T, 3.0% Target 54 C, and 97% Target 54T
Sample 7	10% Target 14C, 90% Target 14T, 10% Target 26C, 90% Target 26T, 10% Target 44C, 90.00% Target 44T, 10% Target 48C, 90% Target 48T, 10% Target 53C, 90% Target 53T, 10% Target 54 C, and 90% Target 54T
Sample 8	30% Target 14C, 70.00% Target 14T, 30% Target 26C, 70% Target 26T, 30% Target 44C, 70% Target 44T, 30% Target 48C, 70% Target 48T, 30% Target 53C,

	70% Target 53T, 30% Target 54 C, and 70% Target 54T
Sample 9	100% Target 14 C, 100% Target 26C, 100% Target 44C, 100% Target 48C, 100% Target 53C, and 100% Target 54 C
assistant mixture	Assistant 14: Assistant 26: Assistant 44: Assistant 48: Assistant 53: Assistant 54=1:1:1:1:1:1

The total concentration of target DNA in mimic sample is 120 nmol L^{-1}, and the total concentration of assistant DNAs mixture is 600 nmol L^{-1}, repectively. The percentage indicates the proportion of target DNA in mixed homologous DNAs (i.e. mixture of corresponding C-allele target and T-allele target).

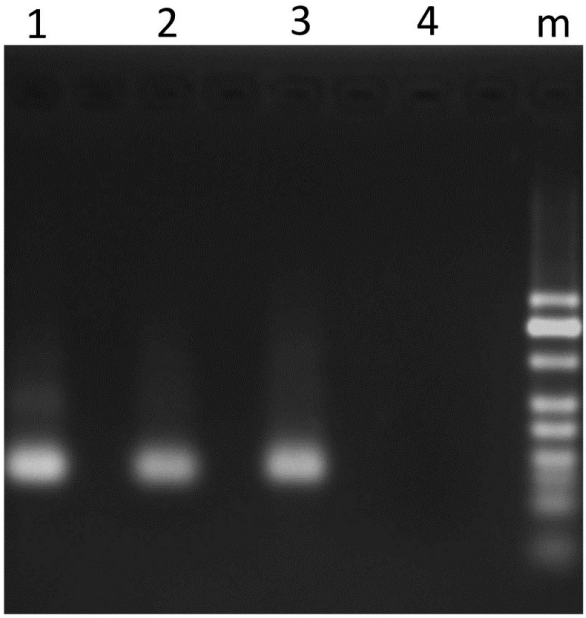

Fig. S1 Electrophoresis analysis of circle template after different treatments. Lane 1: circle template and link template were treated with Taq DNA ligase. Lane 2: sample in lane 1 was further digested with Exonuclease III and Exonuclease I. Lane 3: circle template and link template without treatment. Lane 4: sample in lane 3 was further digested with Exonuclease III and Exonuclease I. Lane m indicates the Low MW DNA Marker-A containing 9 individual DNA fragments: 500, 400, 300, 200, 150, 100, 75, 50 and 25 bp.

Prior to RCA reaction, circular DNA was prepared and then analyzed with agarose electrophoresis. Noticeably, after the reaction with Exonuclease I and III, only small amount of Taq DNA ligase treated circle templates were digested, while untreated circle templates were completely digested. These results demonstrated successful preparation of circular DNA which can be served as circular template in RCA reaction.

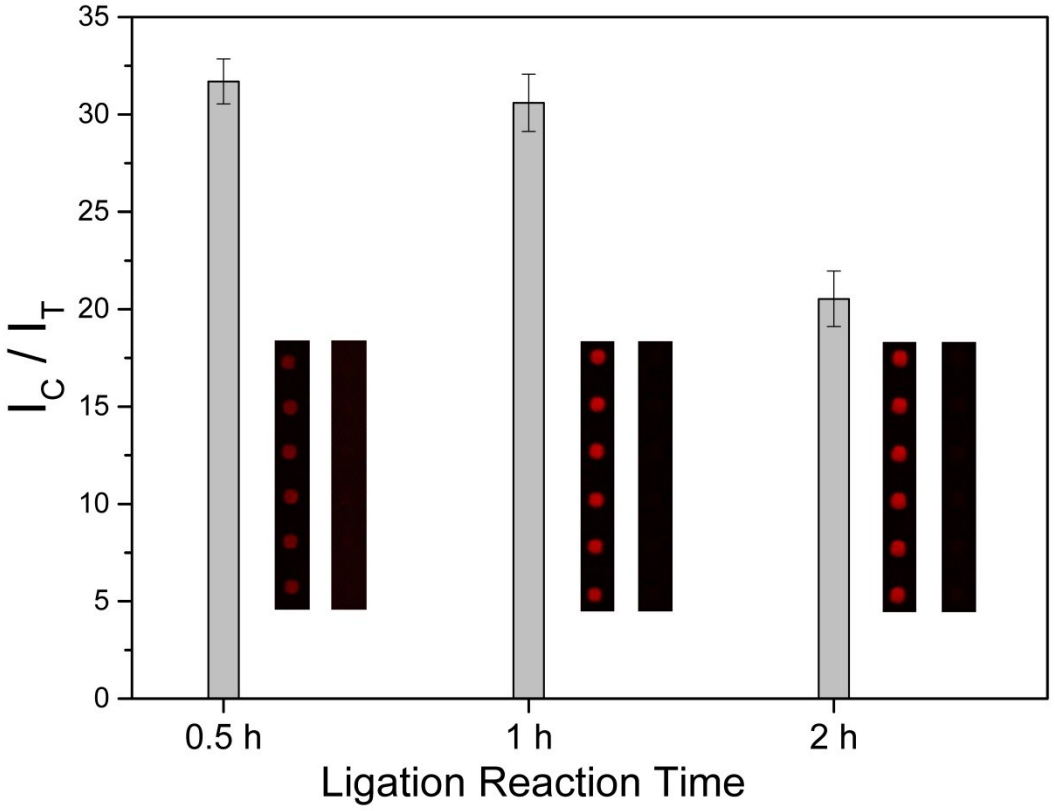

Fig. S2 Effect of ligation reaction time. The immobilized Probe 2 (30 μmol L^{-1} in spotting solution) were hybridized with 200 nmol L^{-1} Target 1C (left columns of insets) or Target 1T (right columns of insets), following reacted with 100 nmol L^{-1} Assistant 1-Cy5, ligated by 500 U mL^{-1} Taq DNA ligases and denatured by heating treatment, respectively.

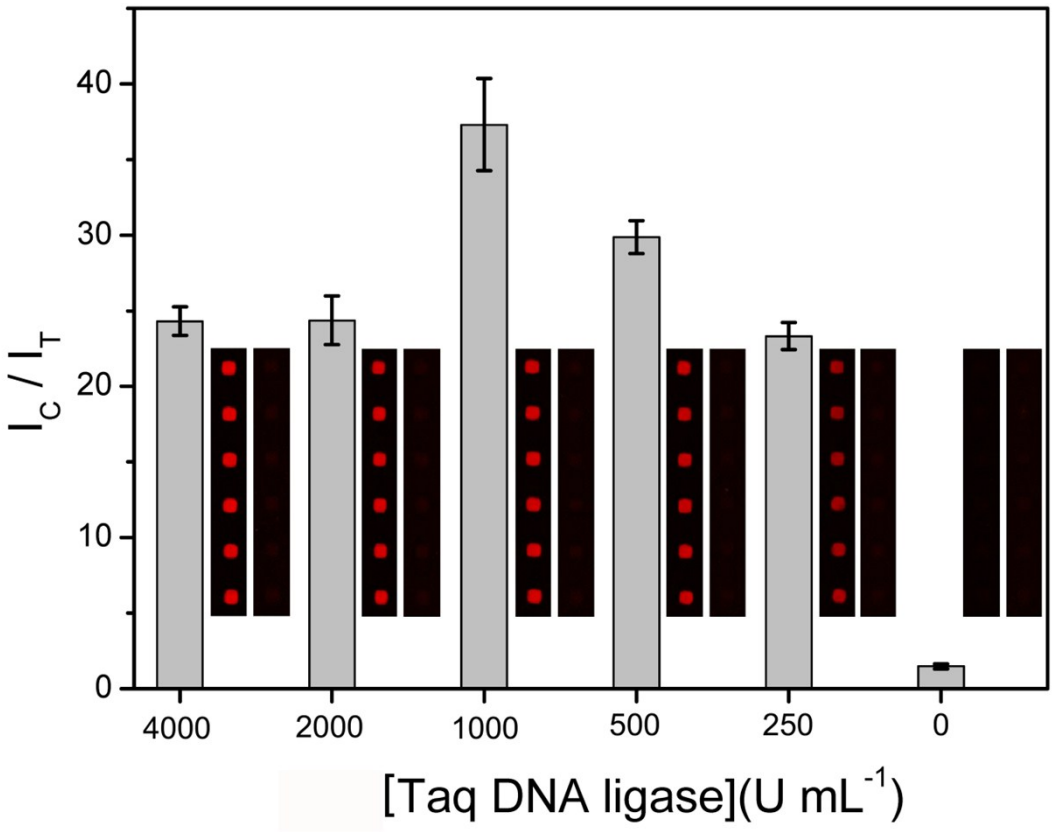

Fig. S3 Effect of Taq DNA ligase concentration. The immobilized Probe 2 (30 μmol L^{-1} in spotting solution) were hybridized with 200 nmol L^{-1} Target 1C (left columns of insets) or Target 1T (right columns of insets), following reacted with 100 nmol L^{-1} Assistant 1-Cy5, ligated by desired concentration of Taq DNA ligases and denatured by heating treatment, respectively.

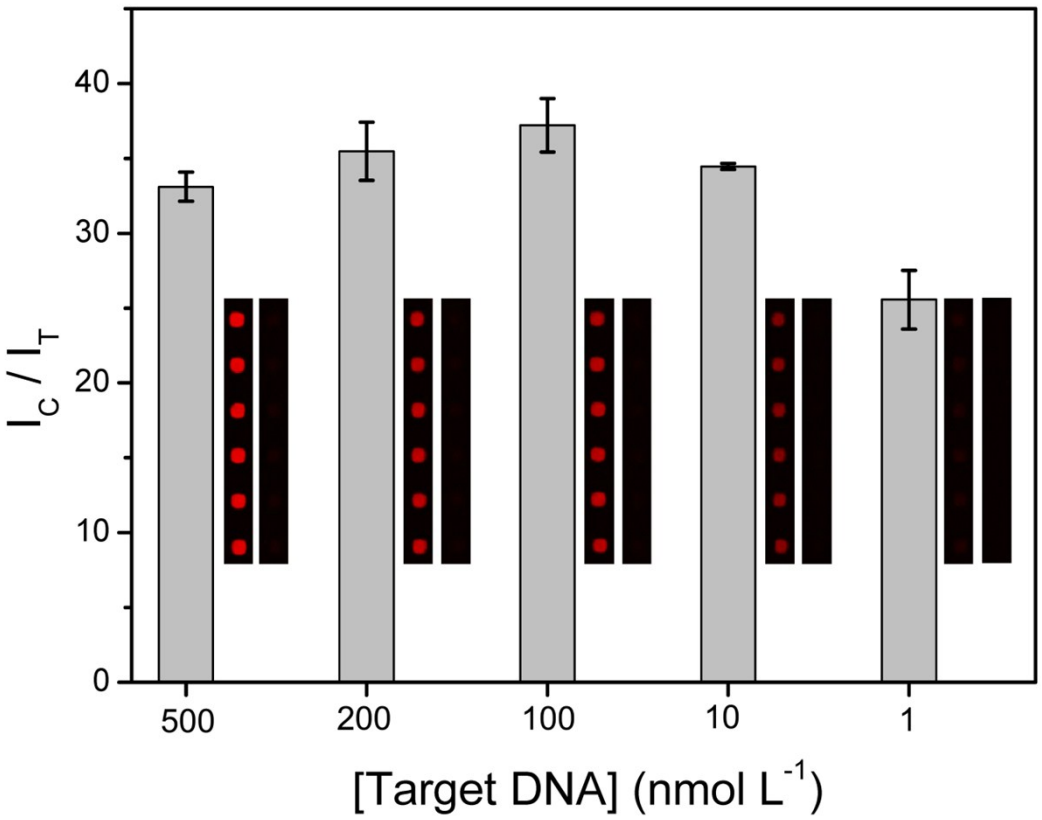

Fig. S4 Effect of target DNA concentration. The immobilized Probe 2 (30 μmol L^{-1} in spotting solution) were hybridized with desired concentration of Target 1C (left columns of insets) or Target 1T (right columns of insets), following reacted with 100 nmol L^{-1} Assistant 1-Cy5, ligated by 1000 U mL^{-1} concentration of Taq DNA ligases and denatured by heating treatment, respectively.

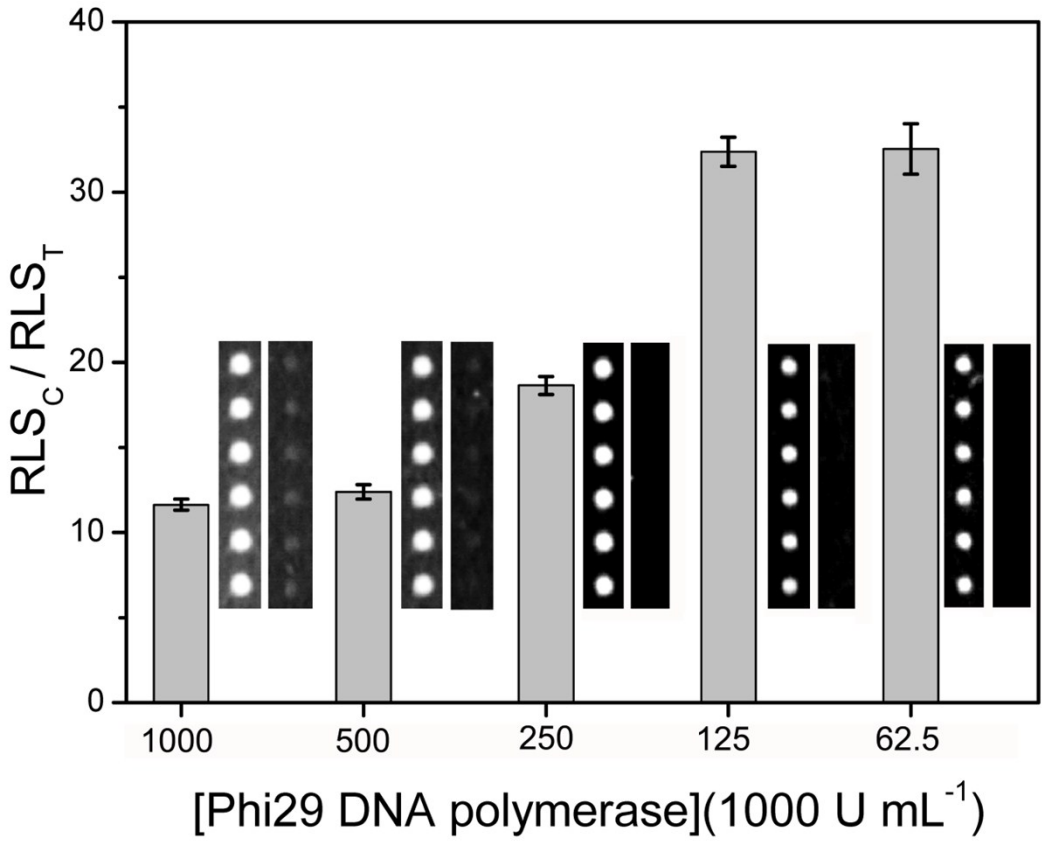

Fig. S5 Effect of the concentration of Phi29 DNA polymerase. The immobilized Probe 2 (30 μmol L^{-1} in spotting solution) were hybridized with 100 nmol L^{-1} Target 1C (left columns of insets) or Target 1T (right columns of insets), following reacted with 100 nmol L^{-1} Assistant 1-Cy5, ligated by 1000 U mL^{-1} concentration of Taq DNA ligases and denatured by heating treatment, respectively. The denatured microarrays were further reacted with various concentrations of phi29 DNA polymerase for 30 min, labeled by 5 nmol L^{-1} DNA-GNPs, and amplified by silver enhancement, respectively.

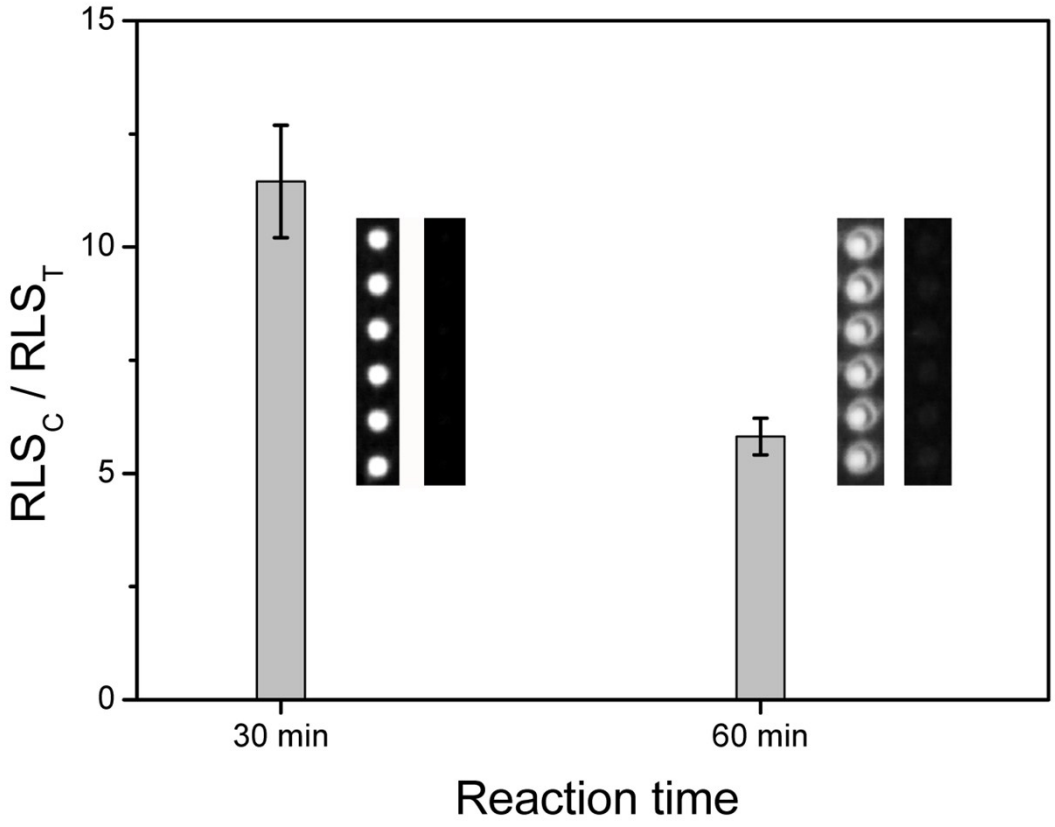

Fig. S6 Effect of RCA reaction time. The immobilized Probe 2 (30 μmol L^{-1} in spotting solution) were hybridized with 100 nmol L^{-1} Target 1C (left columns of insets) or Target 1T (right columns of insets), following reacted with 100 nmol L^{-1} Assistant 1-Cy5, ligated by 1000 U mL^{-1} concentration of Taq DNA ligases and denatured by heating treatment, respectively. The denatured microarrays were further reacted with 500 U mL^{-1} phi29 DNA polymerase for 30 min or 60 min, labeled by 5 nmol L^{-1} DNA-GNPs, and amplified by silver enhancement, respectively.

With the increase of RCA reaction time, dim circles are formed around the signal spots due to the elongated tandem single-stranded DNA (as shown in inset of Fig. S6), resulting in decreased RLS_C/RLS_T.

Fig. S7 Segmental sequence of the promoter and exon 1 regions of p16 gene. CpG dinucleotides are numbered orderly according to their position in the sequence. The colored CpG dinucleotides are #14 (red), #26 (green), #44 (blue), #48 (orange), #53 (purple) and #54 (pink) CpG sites which are the target sites in mimic sample analysis. The underlined portion is the target fragment in real sample analysis.

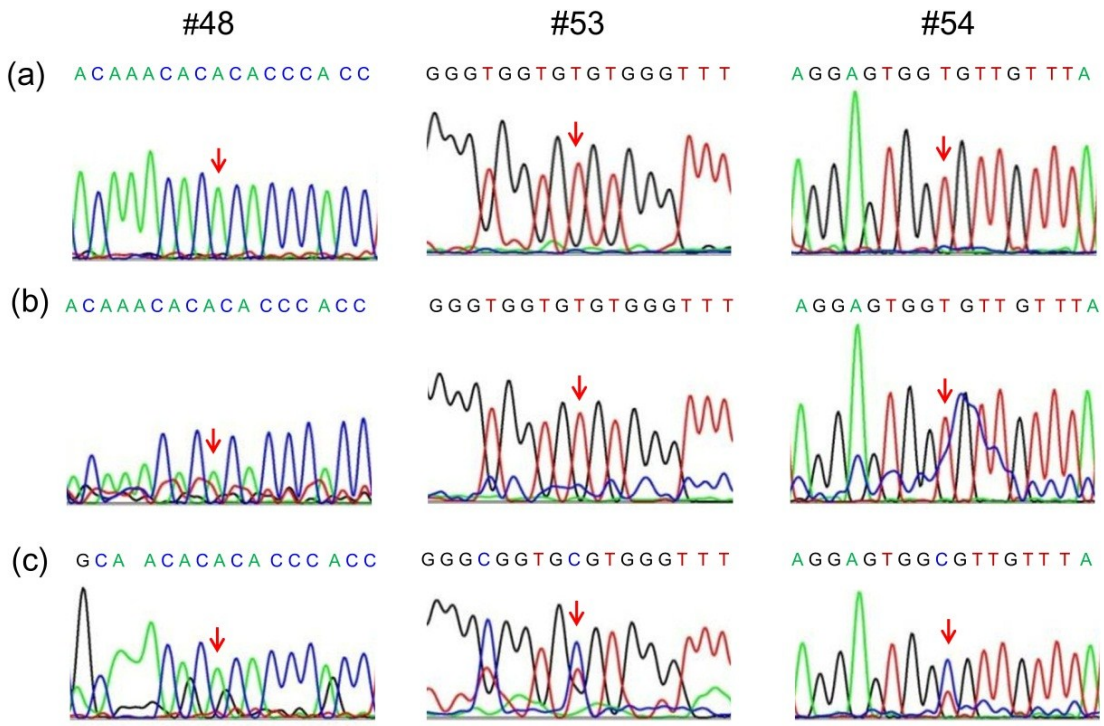

Fig. S8 The sequencing results of symmetric PCR fragment: (a) HCT 116, (b) SW480 and (c) LS 174T. The target sites are marked by red arrows.

Supplementary reference

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Wang, Z., Kanaras, A.G., Bates, A.D., Cosstick, R., Brust, M., 2004. J. Mater. Chem. 14, 578-580.