Electronic Supplementary Material (ESI) for Lab on a Chip.

LoMA-B: A simple and versatile lab on a chip system based on single-channel bisulfite conversion for DNA methylation analysis

Jaeyun Yoon[1,2], Mi Kyoung Park[1], Tae Yoon Lee[1], Yong-Jin Yoon[2,*], Yong Shin[1,*]

[1]Institute of Microelectronics, A*STAR (Agency for Science, Technology and Research), 11 Science Park Road, Singapore Science Park II, Singapore117685

[2]School of Mechanical & Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639789

[*] Corresponding Author, E-mail Address to Y.S: shiny@ime.a-star.edu.sg, Tel: +65 6770 5764 or

Y.J.Y: yongjiny@ntu.edu.sg, Tel: +65 6790 5033

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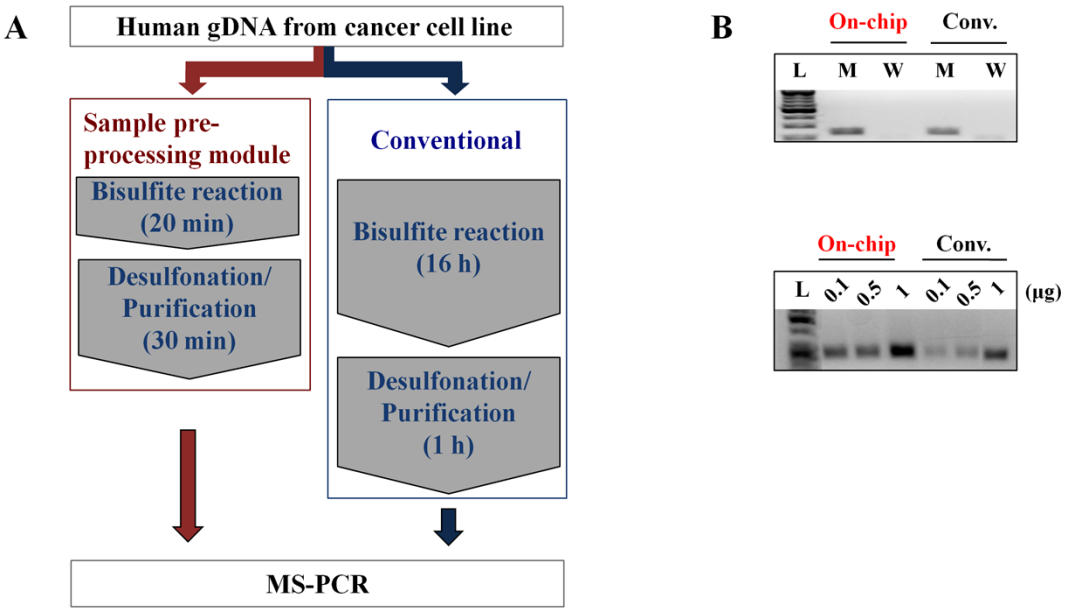

Fig. S1: Comparison of bisulfite conversion efficiency by sample pre-processing module and conventional method. (A) Comparison of bisulfite reaction time. By using proposed sample pre-processing module, overall reaction time is less than 50min compared to 17 h by conventional method. (B) MS-PCR using bisulfite converted gDNA (1 μg) from MCF-7 by sample pre-processing module (On-chip) or conventional method (Conv.), methyl-specific (M) and wild-type (W) primer of RARβ gene. (Top) MS-PCR using bisulfite converted DNA by two methods (On-chip or Conv.) and methyl-specific primer performed on various amounts of DNA converted (0.1, 0.5, and 1 μg from left) (Bottom).

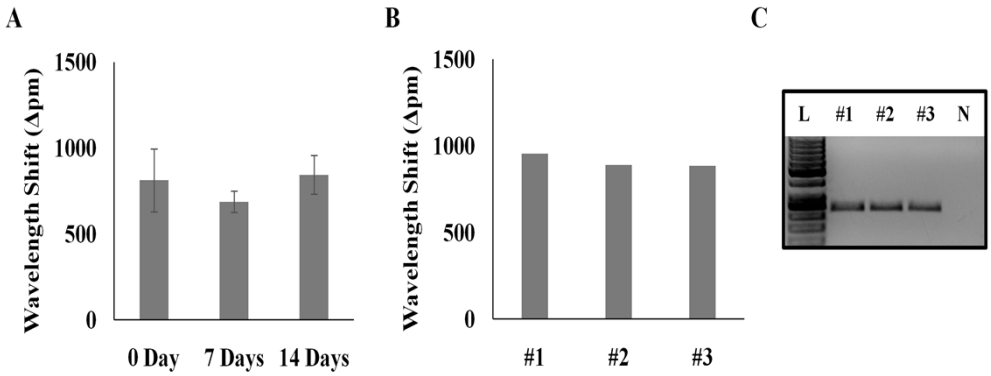

Fig. S2. Repeatability of LoMA-B system (sample pre-processing & detection module). (A) Long-term stability of grafting primers on silicon microring resonator (SMR) for storage possibility of detection module. (B) Reusability of detection module. All experiments were performed using the same SMR chip for detection of the bisulfite converted gDNA. The used chip was washed with NaOH to get rids of all bound molecules on the surface. (C) Reusability of sample pre-processing module. All experiments were performed using the same sample pre-processing module for bisulfite conversion of gDNA from MCF-7. The used chip was washed with dH_2O to get rids of all bound molecules on the glass substrate. The DNA modified was analyzed by MS-PCR using the methyl-specific (M) primer.

Table S1. Primer sequences for LoMA-B system and conventional methods (MS-PCR/RT-PCR)

Method	Primer set	**Sequence**
LoMA-B	Methyl-specific-F	5'-NH_2-$(CH_2)_6$-AAAAAATTTGAAGGTTAGTAGTTCGGGTAGGGTTTATC-3'
	Methyl-specific-R	5'-NH_2-$(CH_2)_6$-AAAAATTCCGAATCCTACCCCGACGGTGCCCAGACAAA-3'
	Unmethyl-specific-F	5'-NH_2-$(CH_2)_6$-AAAAAATTTGAAGGTTAGTAGTTTGGGTAGGGTTTATT-3'
	Unmethyl-specific-R	5'-NH_2-$(CH_2)_6$-AAAAATTCCAAATCCTACCCCAACAATACCCAAACAAA-3'
MS-PCR & RT-PCR	Methyl-specific-F	5'-GGTTAGTAGTTCGGGTAGGGTTTATC-3'
	Methyl-specific-R	5'-CCGAATCCTACCCCGACG-3'
	Unmethyl-specific-F	5'-TTAGTAGTTTGGGTAGGGTTTATT-3'
	Unmethyl-specific-R	5'-CCAAATCCTACCCCAACA-3'
	Wild-type-F	5'-ATTTGAAGGTTAGCAGCCCG-3'
	Wild-type-R	5'-GCATCCCAGTCCTCAAACAG-3'

Table S2. Comparison of LoMA-B and existing methods based on PCR technique

	LoMA-B	**MS-PCR**	**MethyLight**	**MS-HRM**
Assay Time	80 min	> 3h*	> 2h*	> 2h*
Detection limit	1%	0.1-1%	0.1%	0.1-1%
Quantitative	Yes	No	Yes	Yes
Advantages	Cost-effective Label-free Automated sample processing	Cost-effective	High sensitivity	Overcomes false-positive
Disadvantages	Requires primer immobilization steps on detection module	Labor-intensive, False-positive	Limited to detection of fully methylated/unmethylated alleles	Limited amplicon length/number of methylated sites for high sensitivity

*Excluded reaction time for bisulfite conversion