

**iso- $\mu$ Gene: Isothermal amplification-based portable microfluidic system for simple, reliable and flexibly multiplexed Genetic identification and quantification**

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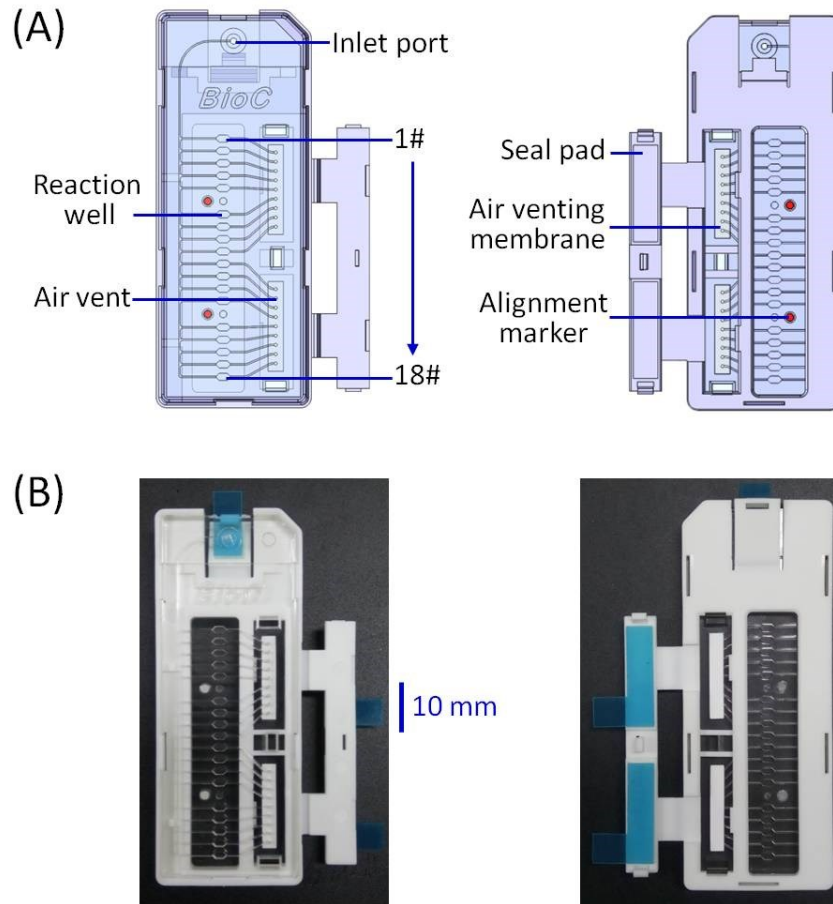
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**Note added after first publication:** This Supplementary Information file replaces that originally published on 1<sup>st</sup> May 2020. Additional information has been included in Figure S1 regarding chip fabrication.

**Table S1** LAMP primers used in this study

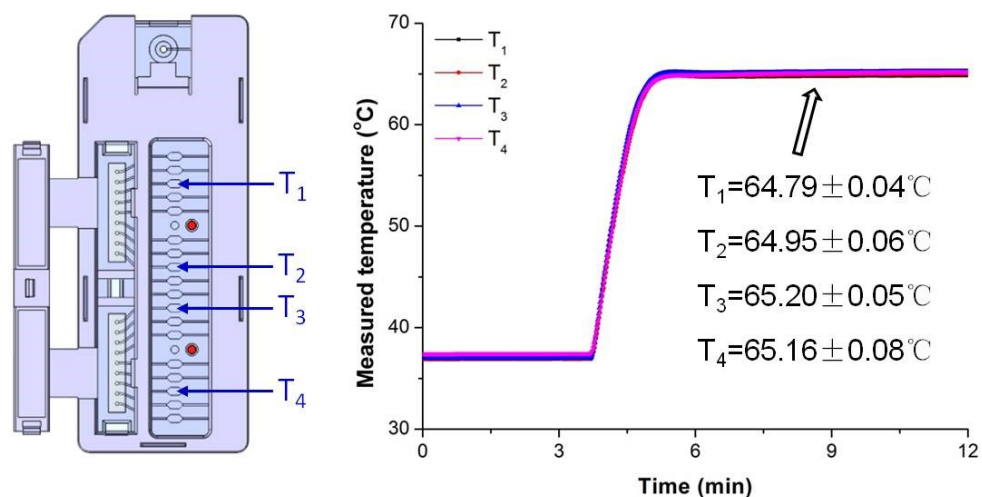
Bacteria	Target gene (Assay ID)	Primer sequence(5'-3')	Source
<i>E.tarda</i>	EsrB CP001135.1 (Et-EsrB)	<b>F3</b> : ACTCCAGAACCCCCAGG <b>B3</b> : CCTCGTCCGATATGGCTCA <b>FIP</b> :ACCCACCGGCTCAACCTGATTTTTGTTGCTCAGCTCCGCC <b>BIP</b> :GATGGACAGCAGCTCCGCGATTTTTGACCCTGCGCGAACG <b>LF</b> : CGCAAGCTGGATGCCCA <b>LB</b> : TCGGCGACCAGCTTGAGA	Xie <i>et al.</i> (2012)
	Hemolysin (HlyB) CP001135.1 (Et-Hlyb)	<b>F3</b> : TCAAGATTAGCGCATCCTT <b>B3</b> : CATCAAAAAGAAGGCTGGTG <b>FIP</b> :AGAAATAAGCGTCCGTGTTTTGATGGGTTACCCGTCGTC <b>BIP</b> :CGGGCTAGTGCCCCAAAATATTTTCGCTATTTGTCGTGCTCG <b>LF</b> : GCTTATTCCTTATGTCAGTGTGAT <b>LB</b> : TCCCCGTATTTACAGGGCG	Xie <i>et al.</i> (2013)
	OmpA EF528483 (Et-ompA)	<b>F3</b> : CATTAGCAGTGGCACTGG <b>B3</b> : TGCCTTGAACCTACCGTTC <b>FIP</b> :TCGGATGAGACTTCGTGGAGTTTTTGTAGGTGGTAAACTGG GTTG <b>BIP</b> :CGGTGCTTTCTTCGGTTACCATTTCCAGTCGTAGCCCATT C <b>LF</b> : AAGCTGTTACCGATGTAGTGG <b>LB</b> : CTAATCCGTACCTGGGCTTC	Cai <i>et al.</i> (2017)
<i>V. harveyi</i>	vhhP2 FJ025787.1 (Vh-vhhP2)	<b>F3</b> : CAATTCGAAACAGGCGTG <b>B3</b> : AGTAAAGCTTGCCACACG <b>FIP</b> :CGCCACCACCATATCCATCGTTTTTGGTTAGTCAATGGTGGA ACA <b>BIP</b> :GGATGTAAATGAGTTTGGCTTTCCGTTTTTTGTCCTATGTTA TACGGGTTG	Thongkao <i>et al.</i> (2013)
	Hemolysin (vhhA) AF293430 (Vh-Vha)	<b>F3</b> : AACCAATACATCGCTCTGAC <b>B3</b> : TCATTTCCACGATCTTCGC <b>FIP</b> :GGCACGCTACGGTTGTAGTTTTTTAACTACAAGCCTGCTAAT ACC <b>BIP</b> :GACTACGCGGAAGCCTTGATTTTTTTCTGGTAGTGTCAATCAA CAAC <b>LF</b> : AGACCAAATCAAGGGTAAACA <b>LB</b> : TGCAGGTTAGCGCATCCTT	Zhou <i>et al.</i> (2014)



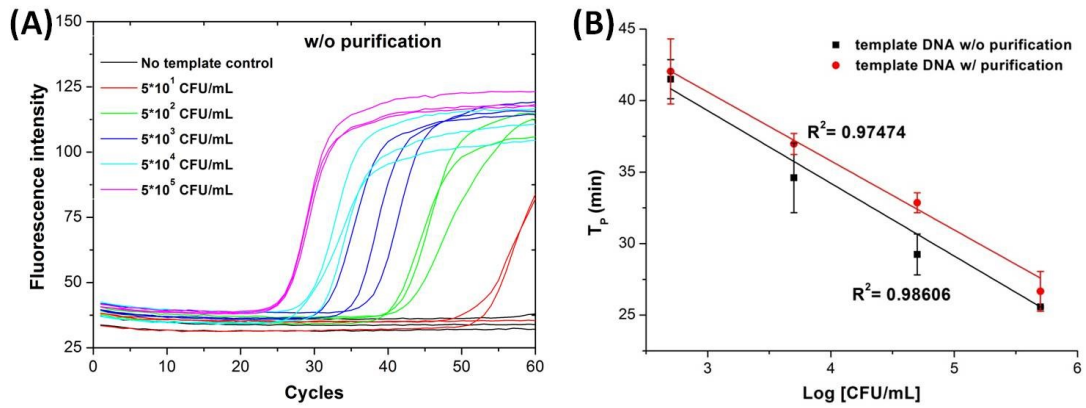
**Fig. S1** Schematics (A) and photographs (B) of the front and back side of the assembly of an empty 18-well chip with an unbuckled plastic shell. The well-array chip and the dedicated shell was injection molded with the materials of polycarbonate (PC-110, Qimei Corp.) and nylon (PA66 70G30L, Dupont Company), respectively. After chip cleaning and drying (QX-002, Xitu, China), an appropriate amount of LAMP primers was subsequently dispensed and dehydrated in each reaction well. The air vents were then covered with the gas-permeable hydrophobic membrane (GVHP 09050, Millipore) and the chip was bonded to a polymer film according to the method described by Stedtfeld et al.<sup>1</sup> Finally, the chip and the shell was assembled manually.

## References

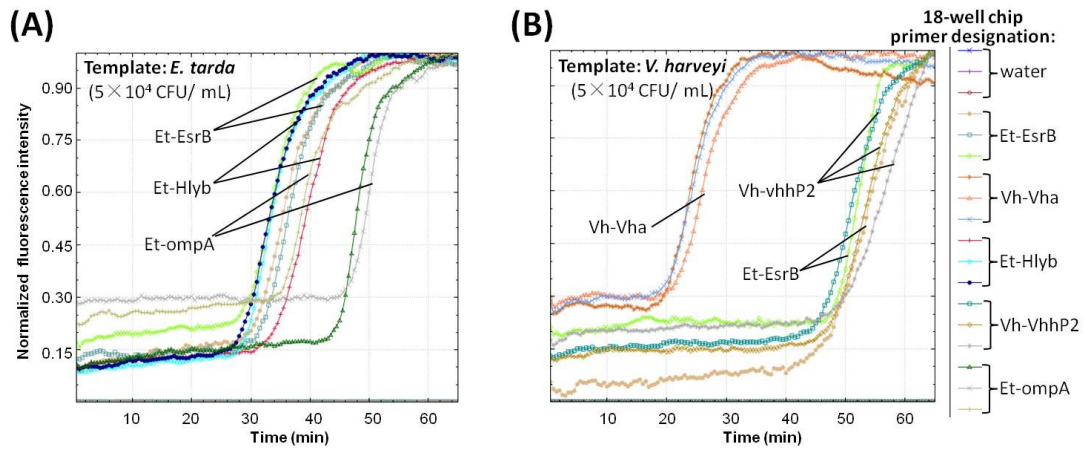
1. R. D. Stedtfeld, D. M. Tourlousse, G. Seyrig, T. M. Stedtfeld, M. Kronlein, S. Price, F. Ahmad, E. Gulari, J. M. Tiedje and S. A. Hashsham, *Lab on a chip*, 2012, 12, 1454-1462.



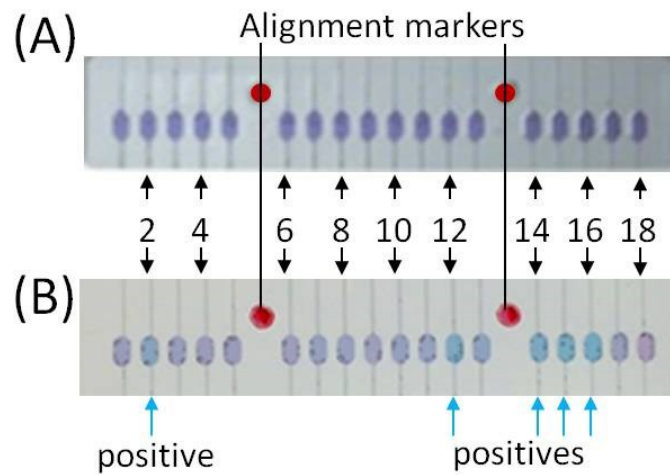
**Fig. S2** A 18-well chip shows placement of four thermocouples to test temperature uniformity. The graph shows temperatures of the four thermocouples as the heater in the device is ramped to reaction temperature (65 °C) and the average temperatures once the set-point is reached. It should be noted that the standard errors of measurement, determined by placing thermocouples propinquity on a heat plate or into reaction wells are less than 0.1 °C.



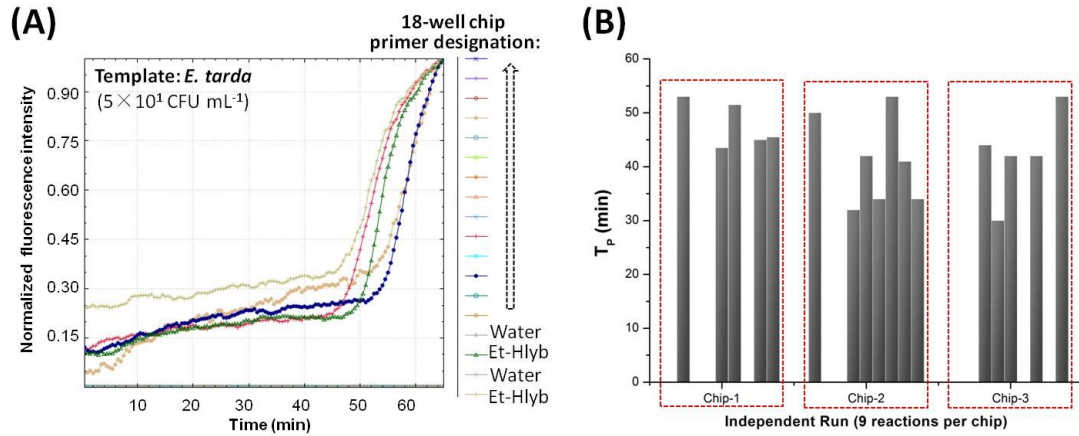
**Fig. S3** Comparison of amplification efficiency of in-tube Et-Hlyb LAMP assay for diluted lysate as template with and without DNA purification. (A) Typical amplification curves for a 10-fold serially diluted template samples from thermal lysate of *E. tarda* without DNA purification. (B) Corresponding standard curve based on the average  $T_P$  values from at least three of the replicates, as determined by using the 10- $\mu$ L in-tube LAMP assay without (black squares) and with (red circles) DNA purification. Purification of genomic DNA from bacterial lysate was performed by using a MagicMag Beads Bacteria gDNA extraction Kit (Sangon Biotech, Shanghai, China) according to the manufacturer's instruction.



**Fig. S4** Real-time fluorescence curves of on-chip LAMP with DNA templates from *E. tarda* (A) and *V. harveyi* (B), respectively, testing the cross-reactivity and amplification efficiency of LAMP primers. Five sets of LAMP primers targeting different genes of the two pathogenic bacteria were pre-dispensed and dehydrated in reaction wells on the 18-well chip. The layout of the primer set designation (each in triplicate in neighboring wells, along with DNA-free water as negative control) is indicated on the right of (B). Once assembled, the chip was loaded with LAMP reagents containing the diluted thermal lysate of cultured bacterium as template ( $5 \times 10^4$  CFU mL<sup>-1</sup>, equivalent to 200 CFU per reaction).



**Fig. S5** Images of an 18-well chip loaded with LAMP reaction mixtures before (A) and after (B) isothermal amplification. For this experiment, primers (for positive control) and DNA-free water (for negative control) were pre-dehydrated in different wells, and the chip was then loaded with LAMP reagents plus template DNA ( $10^4$  copies  $\text{mL}^{-1}$ ) and hydroxynaphthol blue (HNB, Sigma-Aldrich) served as a colorimetric indicator. The color of the reaction mixture turned from violet to blue (well 2#, 12# and 14-16#) after LAMP reaction indicating positive results.



**Fig. S6** Test of sensitivity for on-chip LAMP assay. Three separate 18-well chips were dehydrated with primers targeting the *E. tarda hlyb* gene and DNA-free water in alternating reaction wells, as indicated on the right of (A). Once assembled, the chip was loaded with sample contained LAMP reagents and diluted thermal lysate of cultured *E. tarda* as template ( $5 \times 10^1$  CFU mL<sup>-1</sup>, equivalent to 0.2 CFU per well). (A) Amplification plot of all positive reaction wells in a single representative chip. (B)  $T_P$  (time to positive amplification) values for all positive reaction wells in three chips.