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

Simultaneous Amplification of DNA in a Multiplex Circular Array Shaped Continuous Flow PCR Microfluidic Chip for On-site Detection of Bacterial

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Experimental section

Capillary electrophoresis system

All the PCR products were validated in a self-built capillary electrophoresis (CE) system, which has been detailed described in Ref[1, 2]. In brief, it was composed of a confocal optical system, a MODEL 610E high voltage power supply (TREK, USA), and a photomultiplier (PMT) (R928, Hamamatsu photonics, Japan). The confocal optical system was based on a BX51 epi-illumination microscope (Olympus, Japan). The light centered at 490 nm was achieved from a mercury lamp. Then it passed through the mixture of DNA and SYBR Green I in the capillary. The fluorescence from the mixture was collected by a PMT. NI-USB-6212 card (National Instrument, USA) and a self-built LabVIEW software were used for input signal generation and output signal detection. The total length and the effective length of the capillary (Ruifeng Chromatography Ltd., Handan, China) were 12 cm and 8 cm, respectively. The samples were electrokinetically introduced into the capillary. The capillary was cleaned by deionized water after each run. All the separations were performed in dark house at room temperature.

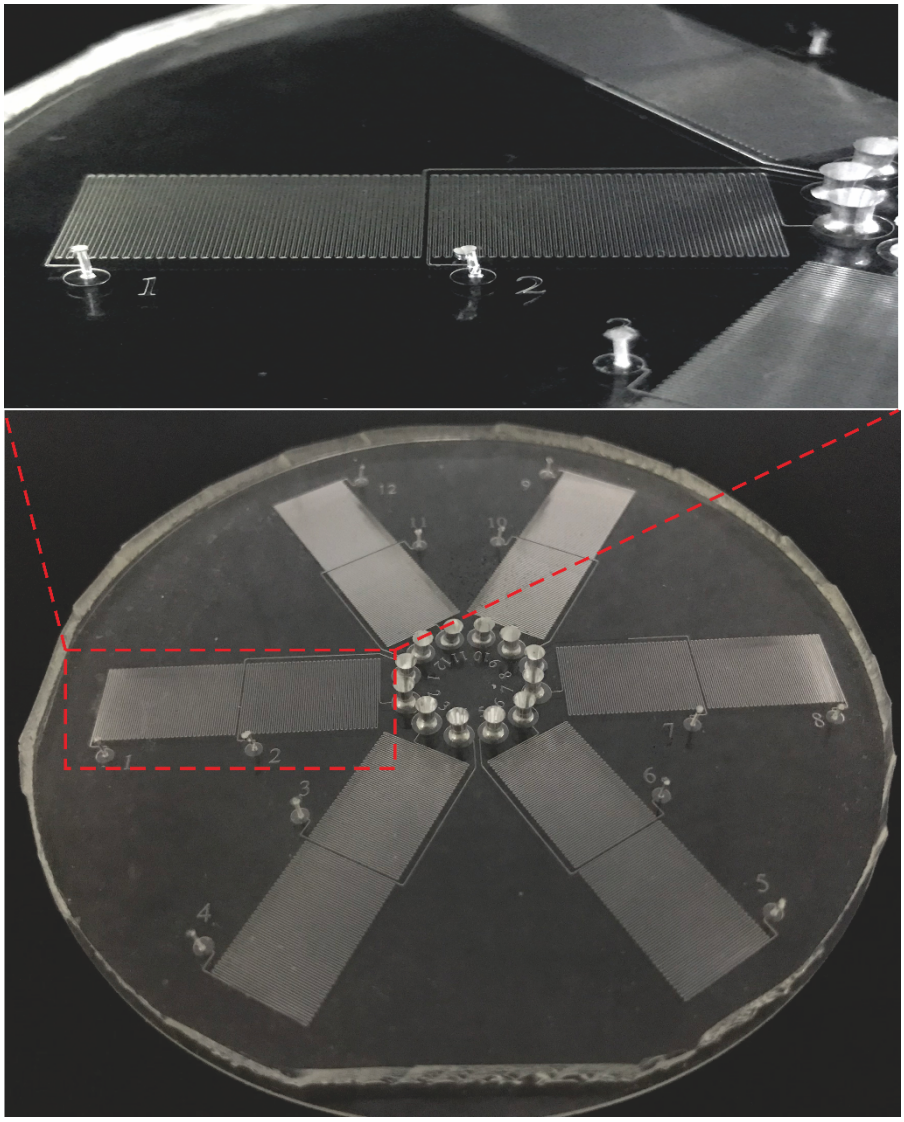

Fig.S1 The photo of the high throughput CF-PCR microfluidic chip.

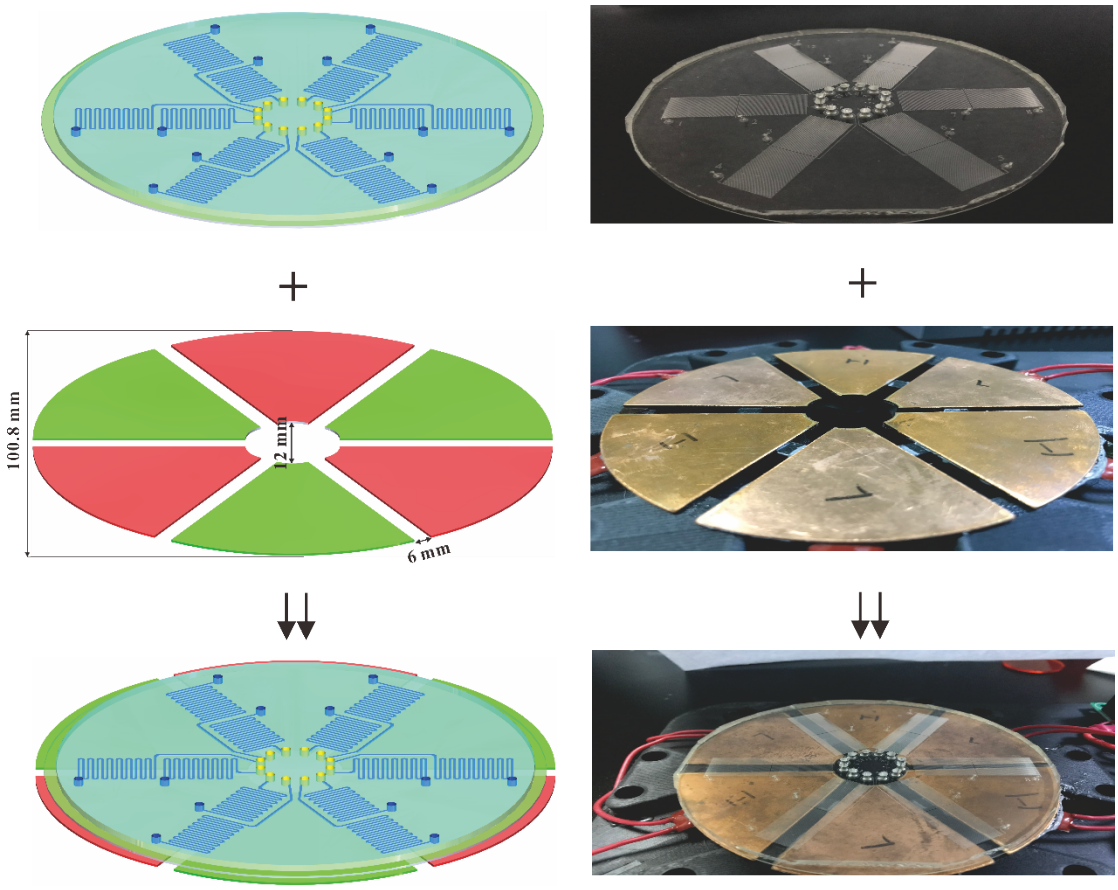

Fig.S2 The left side showed a schematic diagram of the heating block, PDMS-glass chip, and chip system. There was a corresponding physical picture on the right side.

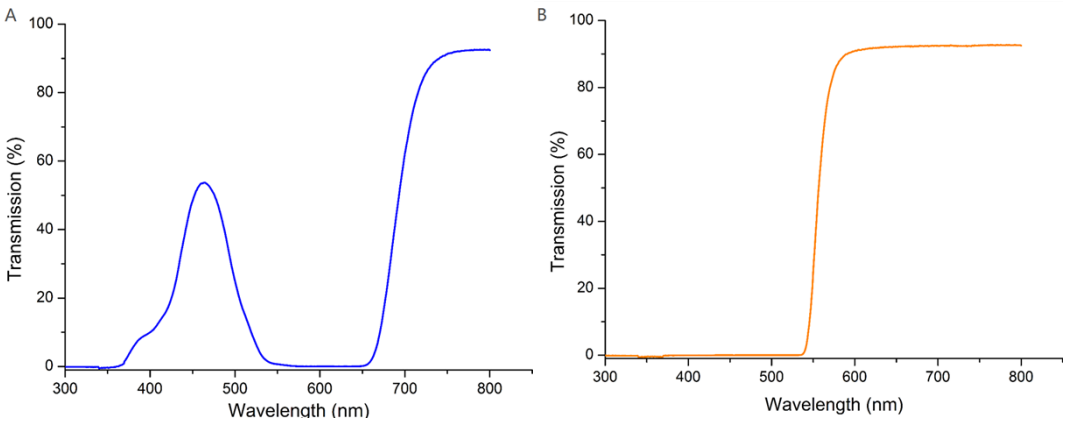

Fig.S3 (A) The transmission rate of the plastic excitation filter above the LED array. (B) The transmission rate of the plastic emission filter below the CMOS.

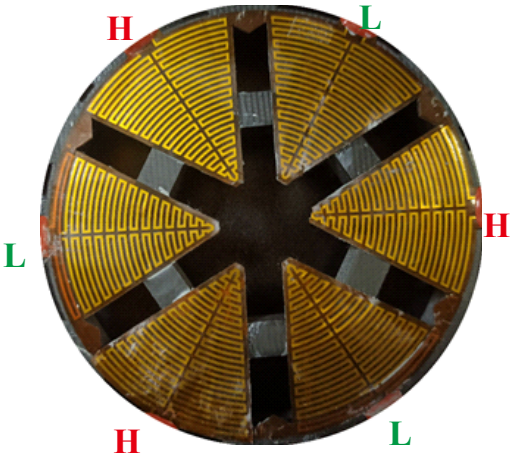

Fig.S4 This is the Pi heating film. The six wedge-shaped areas were divided into two groups to provide different temperatures. The resistance of the metal heating wire in each wedge was 46 ± 0.2 Ω, and the working voltage was 12 V. Silver silicone grease will be added in the middle of the heating film and the copper block to improve the thermal conductivity.

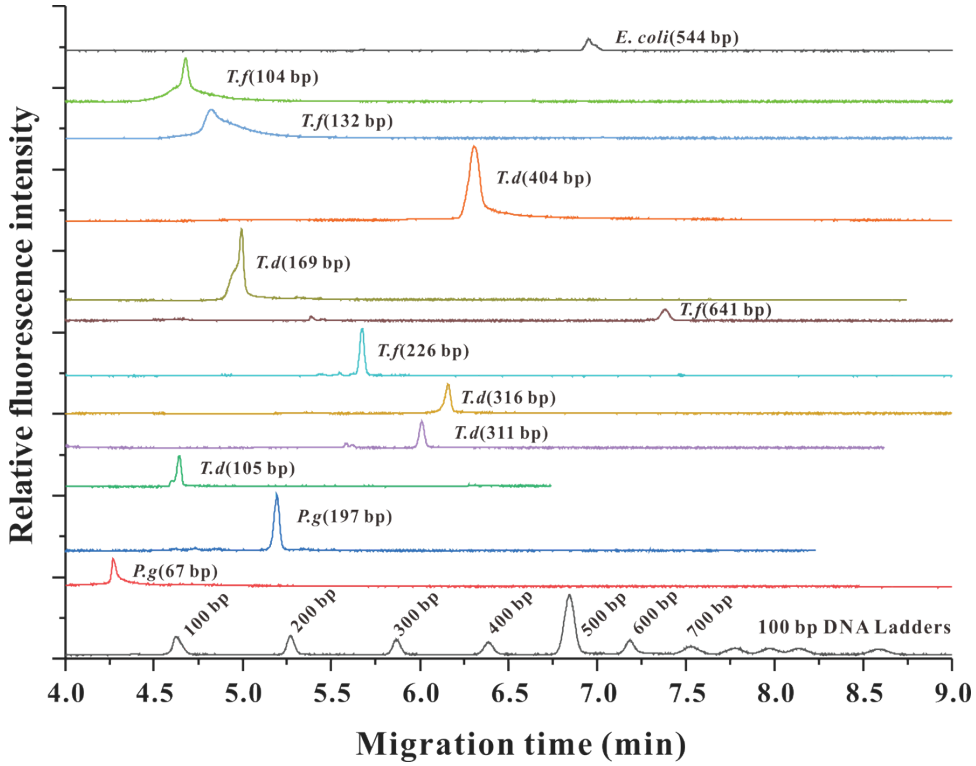

Fig.S5 Capillary electrophoresis of 100 bp DNA Ladders and the reaction solution of the microchip collecting tanks under the same electrophoretic conditions. Electrophoretic conditions: 100 V·cm^{-1} of electric field strength, 0.5% HEC (1300k) as sieving matrix, effective length of the capillary quartz tube: 8 cm; total length of the capillary quartz tube: 12 cm. The minor migration time shift was possibly caused by the little position shift of the capillary.

Table.S1 The primers of *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerela forsythia* and *Escherichia coli*

Primers	The nucleotide sequence (5'---3')	Amplicons	Bacteria
PG197-F	TGTAGATGACTGATGGTGAAAACC	197 bp	*Porphyromonas gingivalis*[3-6]
PG197-R	ACGTCATCCCCACCTTCCTC		
PG67-F	GCGCTCAACGTTCAGCC	67 bp	
PG67-R	CACGAATTCCGCCTGC		
PG169-F	TGACCTTAAGCCTGTAG	169 bp	
PG169-R	TCATCTCGTGTCCGATCACT		
PG404-F	AGGCAGCTTGCCATACTGCG	404 bp	
PG404-R	ACTGTTAGCAACTACCGATGT		
TD311-F	AAGGCGGTAGAGCCGCTCA	311 bp	*Treponema denticola*[3, 5, 7]
TD311-R	AGCCGCTGTCGAAAAGCCCA		
TD316-F	TAATACCGAATGTGCTCATTTACAT	316 bp	
TD316-R	TCAAAGAAGCATTCCCTCTTCTTCTTA		
TD105-F	AGAGCAAGCTCTCCCTTACCGT	105 bp	
TD105-R	TAAGGGCGGCTTGAAATAATGA		
TF641-F	GCGTATGTAACCTGCCCGCA	641 bp	
TF641-R	TGCTTCAGTGTCAGTTATACCT		
TF226-F	ATCCTGGCTCAGGATGAACG	226 bp	*Tannerela forsythia*[3, 8-10]
TF226-R	TACGCATACCCATCCGCAA		
TF132-F	TCACTATTGTGTCTCGCTG	132 bp	
TF132-R	TCTCTCCGATTGTGGTTA		
TF104-F	GACAACCGGATCAGCGAAAT	104 bp	
TF104-R	TCATTGACTTGGCGGATCG		
E.coli544-F	GGAAGAAGCTTGCTTCTTTGCTGAC	544 bp	*Escherichia coli*[11]
E.coli544-R	AGCCCGGGGATTTCACATCTGACTTA		

Table.S2 The time consumption for the volume of solution collection in the collection tank and DNA amplification in the channel

Inner-ring channels										
Flow rate	0.1 mL/hr		0.25 mL/hr		0.5 mL/hr		0.75 mL/hr		1 mL/hr	
Volume	Average Time(min)	Standard Deviation	Average Time(min)	Standard Deviation	Average Time(min)	Standard Deviation	Average Time(min)	Standard Deviation	Average Time(min)	Standard Deviation
0	12.55	0.39	9.23	0.27	7.65	0.07	6.85	0.13	5.26	0.11
5	21.71	0.82	17.38	0.46	14.39	0.84	12.17	0.65	9.85	0.40
10	30.81	2.04	27.32	2.93	23.53	1.76	17.33	3.00	14.31	1.27
15	34.99	2.30	30.91	4.02	28.69	1.54	19.50	2.94	16.09	1.40
20	38.77	3.75	33.81	4.07	30.60	1.66	22.12	3.11	17.81	1.71
25	42.16	4.32	37.41	4.55	33.73	1.85	23.93	3.02	19.41	2.34
30	47.34	3.69	41.27	5.69	36.96	2.10	26.08	3.33	20.45	2.72
35	51.30	4.72	45.97	6.65	40.37	2.27	28.47	3.97	21.99	3.26
40	56.80	5.52	50.65	7.01	43.33	2.75	30.34	4.15	23.64	3.70
Outer-ring channels										
Flow rate	0.1 mL/hr		0.25 mL/hr		0.5 mL/hr		0.75 mL/hr		1 mL/hr	
Volume	Average Time(min)	Standard Deviation	Average Time(min)	Standard Deviation	Average Time(min)	Standard Deviation	Average Time(min)	Standard Deviation	Average Time(min)	Standard Deviation
0	11.82	0.20	8.86	0.27	7.32	0.16	6.53	0.20	5.51	0.20
5	22.07	0.30	16.60	0.26	13.80	0.46	11.88	0.39	9.49	0.33
10	27.13	0.63	20.34	1.47	17.67	0.44	13.84	0.46	11.05	1.42
15	31.78	0.72	23.45	1.38	19.60	0.77	16.67	1.48	13.01	1.60
20	36.19	1.22	26.77	1.37	22.21	1.26	19.03	1.80	14.58	2.26
25	40.28	2.07	29.25	1.52	24.31	1.81	20.85	1.71	16.05	3.16
30	44.23	2.32	32.05	2.04	26.13	1.82	22.36	1.52	17.35	4.00
35	47.92	2.11	34.03	2.21	28.15	2.40	23.90	1.83	18.74	4.89
40	52.35	2.55	38.00	3.32	30.37	2.85	25.57	2.22	20.04	5.75

(Volume equal 0 in the table indicated the time consumption of the reaction solution flowing from the inlet to the outlet in the microchannel)

Table.S3 The relationship between the flow rates and the fluorescence intensity of *P.g*(169 bp), *T.d* (311 bp), *T.f* (226 bp) and *E. coli* (544 bp)

P.g（169 bp）	Flow rate	Fluorescence intensity		*T.d*（311 bp）	Flow rate	Fluorescence intensity	
		Mean	Standard Deviation			Mean	Standard Deviation
	0.10	116.50	5.86		0.10	111.18	5.99
	0.25	26.63	1.48		0.25	22.26	3.29
	0.50	10.49	2.56		0.50	12.96	3.55
	0.75	5.51	0.96		0.75	10.30	2.43
	1.00	4.15	1.04		1.00	7.55	2.04
T.f（226 bp）	Flow rate	Fluorescence intensity		*E.coli*（544 bp）	Flow rate	Fluorescence intensity	
		Mean	Standard Deviation			Mean	Standard Deviation
	0.10	89.89	4.59		0.10	66.90	7.03
	0.25	46.44	4.63		0.25	17.57	4.04
	0.50	36.79	2.70		0.50	11.56	2.97
	0.75	21.30	3.45		0.75	9.77	1.30
	1.00	7.76	2.83		1.00	6.68	1.37

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