Supplementary Information

A three-in-one microfluidic droplet digital PCR platform for absolute quantitative analysis of DNA

Yulin Ren[a,b] Jingcheng Ji[a,b], Haoqing Zhang[a,b], Lei Cao[a,b], Jie Hu[c], Feng Xu[a,b], Zedong Li[a,b*]

[a] *The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, 710049, P.R. China*

[b] *Bioinspired Engineering and Biomechanics Center (BEBC), Xi'an Jiaotong University, Xi'an, 710049, P.R. China*

[c] *Suzhou DiYinAn Biotechnology Co., Ltd, Suzhou, 215010, P.R. China*

[*] *Corresponding author: The Key Laboratory of Biomedical Information Engineering of Ministry of Education, Xi'an Jiaotong University, Xi'an, 710049, P.R. China.*
E-mail addresses: zedong@xjtu.edu.cn

The theoretical value of dynamic detection range

Appropriately increasing the number of dPCR partition is beneficial to the quantitative accuracy and can expand the dynamic detection range.[1, 2] Our chip can collect 80,000-100,000 droplets with diameter of 57.8 μm. The number of droplets is larger than the conventional commercial chip of 20,000 droplets, so the dynamic detection range can be increased. According to the quantitative characteristics of dPCR, the average copy number λ contained in the partition and the ratio q of the negative partition meet the following relationship:

$$\lambda=-\ln (b/n)=-\ln q$$

The upper detection limit and lower detection limit of digital PCR are λ_u and λ_l respectively, and the dynamic range R is used to characterize the range of deplorable detection concentrations. Then R can be expressed in the following logarithmic form:

$$R=\lg (\lambda_u/\lambda_l)$$

The theoretical dynamic detection range of our platform can reach 6log of magnitude, which is higher than the commercial platform of 5log.

Application of three-in-one ddPCR platform for *Helicobacter pylori* 23S rRNA gene detection

Helicobacter pylori (*H. pylori*) is one of the most prevalent infectious bacteria, affecting about 50% of the world's population and is closely associated with peptic ulcers and gastric cancer.[3] The 23S rRNA gene is commonly used as a recognition gene for *H. pylori* detection. Therefore, to verify the applicability of our platform for pathogen detection, we tested *H. pylori* 23S rRNA gene using our three-in-one ddPCR platform. Specifically, the ddPCR reaction reagent (20 μL) for *H. pylori* 23S rRNA gene was prepared by mixing the following reagents: 10 μL of ddPCR mix (BR186-3024, Biorad), 3.2 μL total of a pair of ptimers (F1, R1, 10 μM), 1 μL of probes (10 μM), 2 μL of purified plasmid template and 3.8 μL of double-distilled water (ddH_2O). ddH_2O was used as the template in negative controls. The primer, probe, and template sequences were listed in **Table S1**. The thermocycling protocol was as follows: 95 °C

for 5 min, followed by 45 cycles of 95 °C for 15 s and 58 °C for 25 s. The detection results were displayed in **Fig. S8**, demonstrating that our three-in-one ddPCR platform can be used for pathogen detection.

References

1. N. Majumdar, S. Banerjee, M. Pallas, T. Wessel and P. Hegerich, *Sci. Rep.*, 2017, **7**, 9617.
2. N. Majumdar, T. Wessel and J. Marks, *PloS one*, 2015, **10**, e0118833.
3. F. Khadangi, M. Yassi and M. A. Kerachian, *Helicobacter*, 2017, **22**.

Supporting Figures and Tables

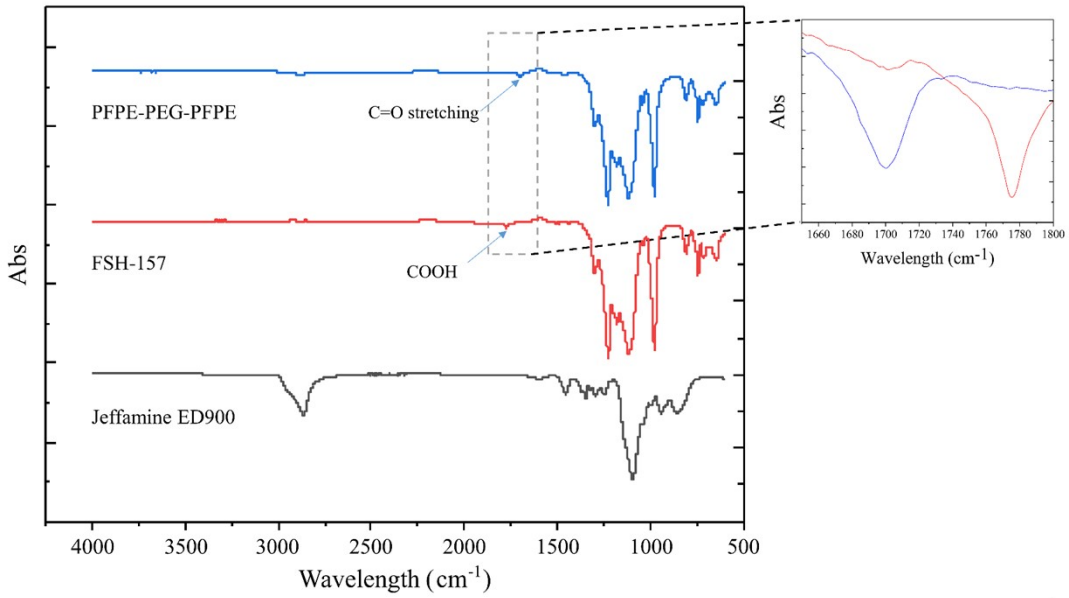

Figure S1. FT-IR analysis of PFPE-PEG-PFPE surfactant. In the IR spectrum of the surfactant, new peaks appear at 1700 cm^{-1}, representing C=O stretching of the carbonyl group and amide II band. There is no indication of unreacted PFPE.

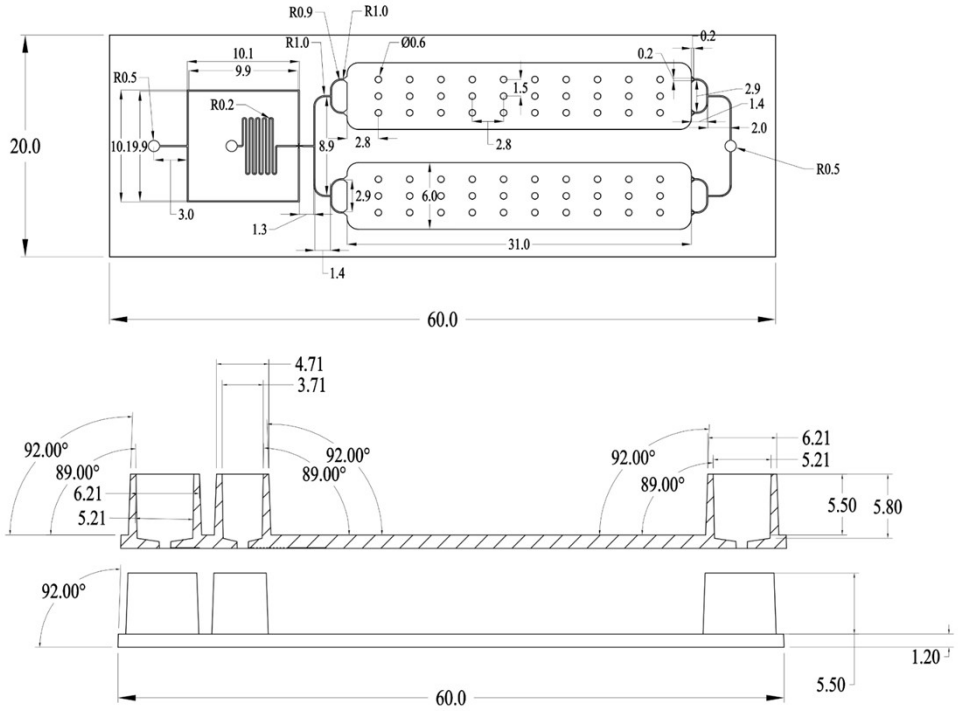

Figure S2. Detailed size of chip. Size unit is mm.

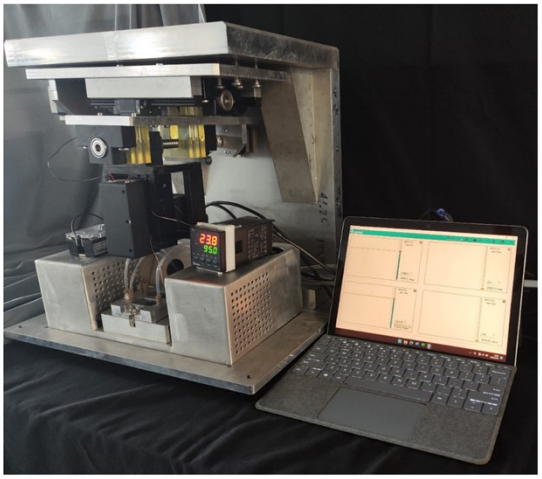

Figure S3. Photograph of the three-in-one ddPCR platform. The platform has dimensions of 40 cm in length, 40 cm in width, and 38.9 cm in height. The current instrument prototype has a weight of 33.25 kg.

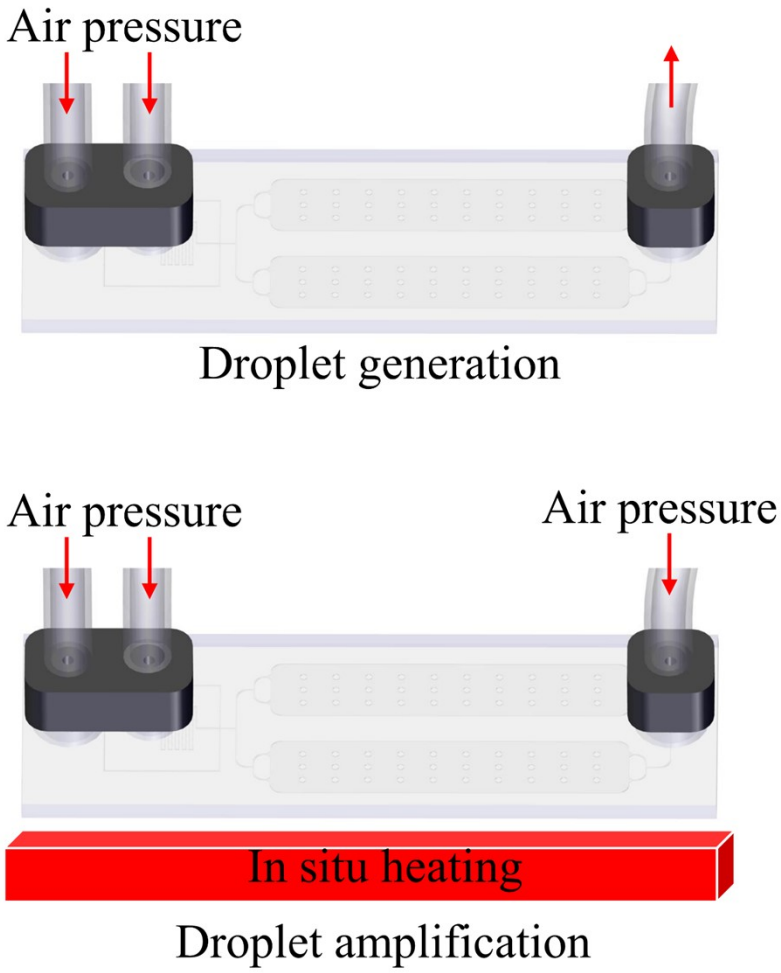

Figure S4. The air pressure was added to the chip from droplet generation to in situ heating process.

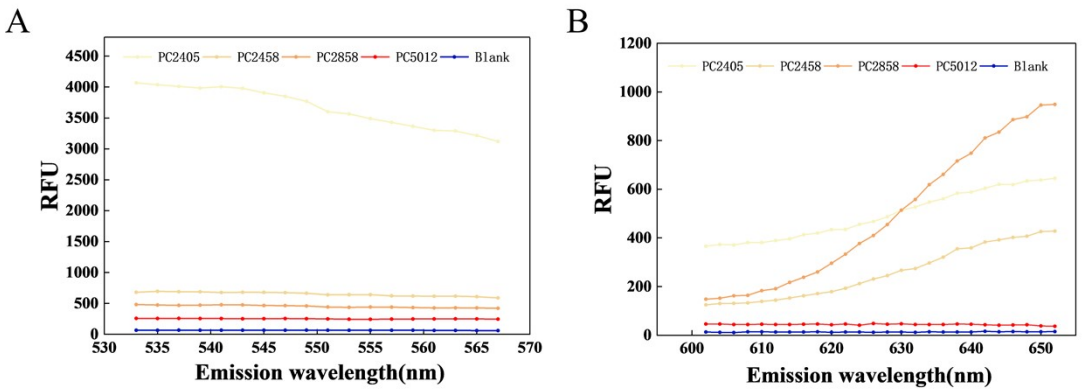

Figure S5. Fluorescence emission spectra of different materials at 488 nm excitation light (A) and at 538 nm excitation light (B).

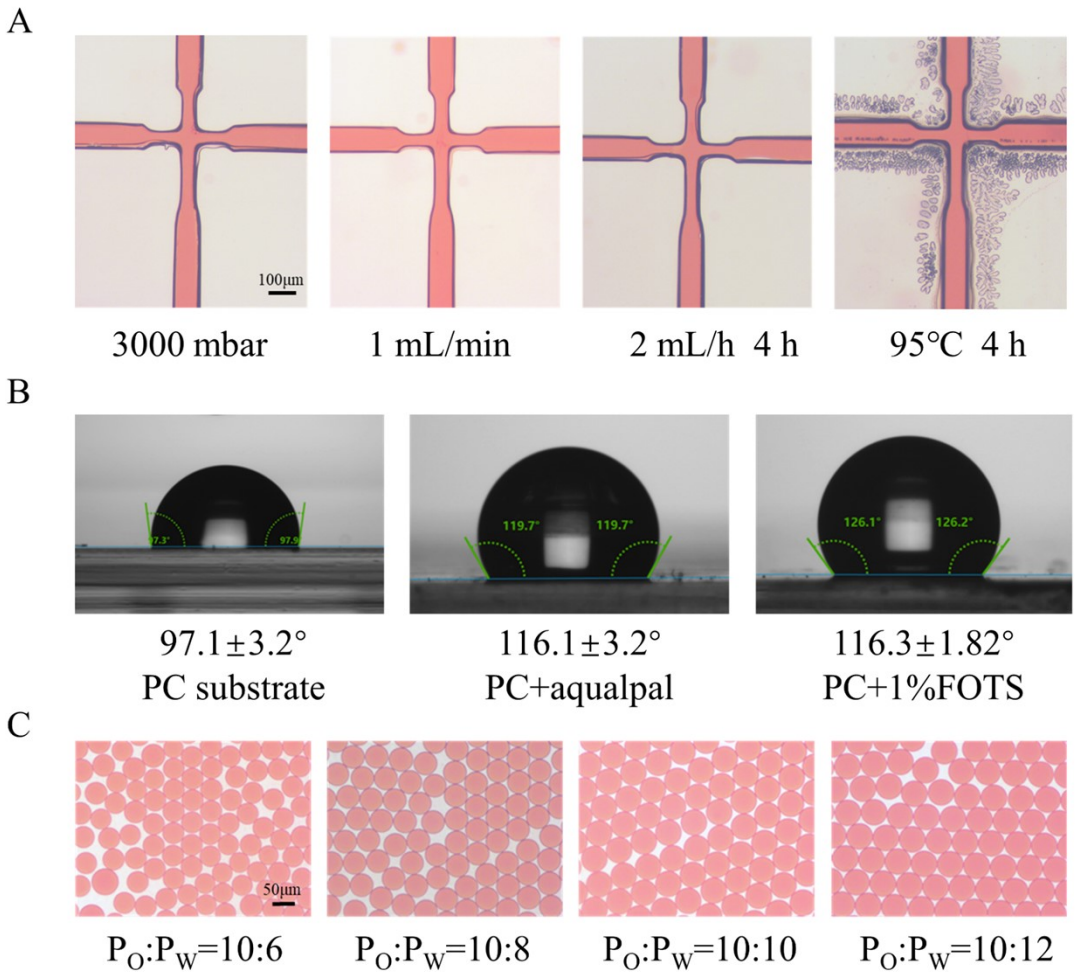

Figure S6. Chip performance characterization. (A) Flow channel bonding stability of chips under different conditions: 3000 mbar high pressure, 1 mL/min high flow rate, 2 mL/h continuous perfusion for 4 h, 95 ℃ for 4 h high temperature resistance. (B) Measurement of contact angle after hydrophobic treatment of chip surface with

aqualpal and with 1% FOTS. (C) Droplets with different sizes generated by different oil-water phase pressure ratios.

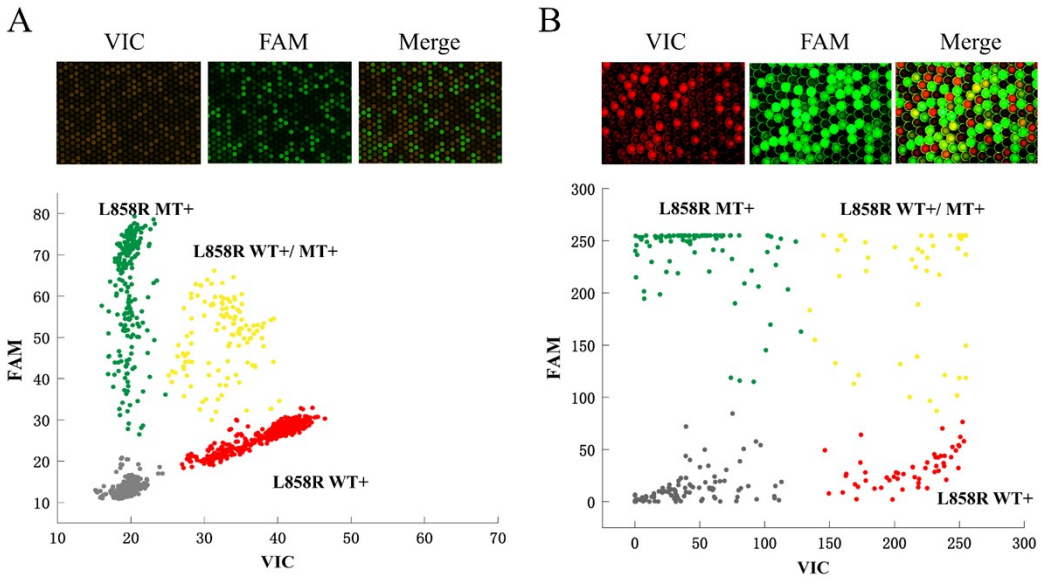

Figure S7. Two channels detection results of mixed samples of L858R WT and MT. (A) The detection results obtained from QuantStudio^{TM} 3D dPCR system. (B) The detection results from our three-in-one ddPCR platform.

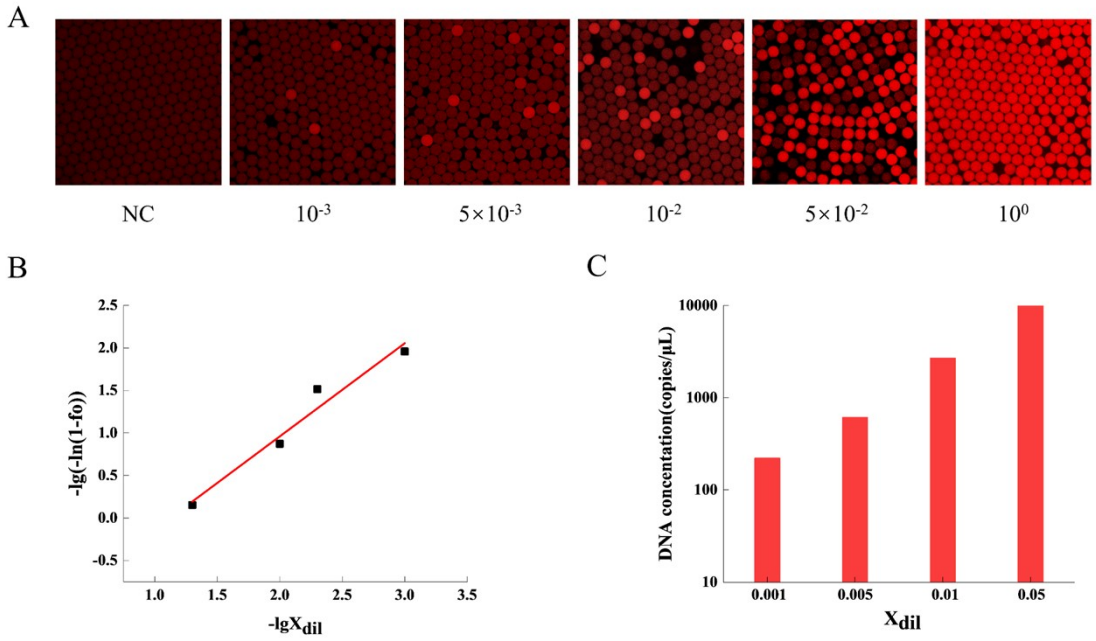

Figure S8. Application of three-in-one ddPCR platform for *Helicobacter pylori* 23S rRNA gene detection. (A) Fluorescent images were recorded with a serial

dilutions of 23S rRNA gene ranging from 10^{-3} to 10^{0} dilution. NC refers to negative control. (B) The linear fitting curve of 23S rRNA gene was obtained by plotting -lg (-ln (1-f_0)) against -lgX_{dil}. (C) The copy number results with a serial dilutions of 23S rRNA gene by three-in-one ddPCR platform.

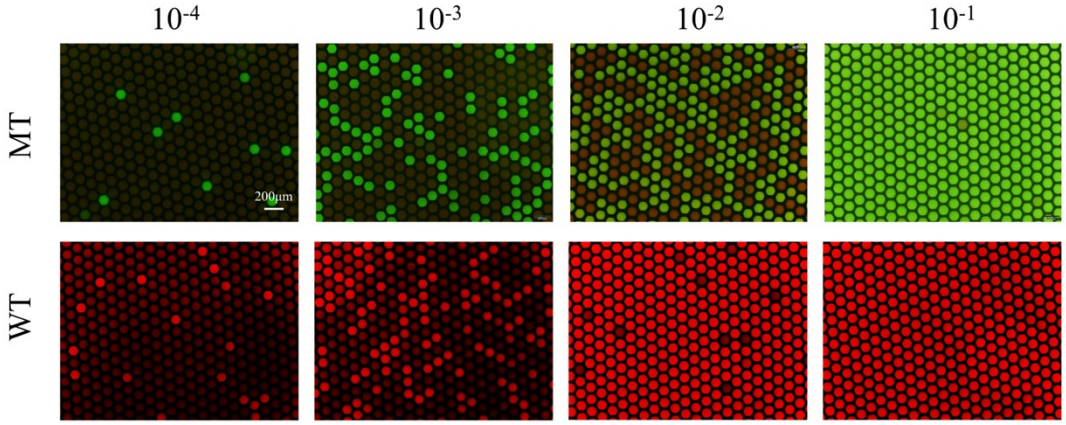

Figure S9. dPCR fluorescent image with a serial dilution of the plasmid DNA template with L858R gene MT and WT ranging from 10^{-4} to 10^{0} dilution. When the dilution concentration is 10^{-1}, the QuantStudio™ 3D dPCR test results showed that all the microwells in the chip are positive, which is beyond the detection range. Our three-in-one ddPCR platform can still work at this concentration.

Table S1. The squence of primers, probes and templates for dPCR

Types	Name	Squence (5' to 3')
Primer	EGFR L858R F	GCAGCATGTCAAGATCACAGATT
	EGFR L858R R	CCTCCTTCTGCATGGTATTCTTTCT
	23S rRNA gene F	GTGAAAATTCCTCCTACCC
	23S rRNA gene R	AAGAGCCAAAGCCCTTAC
Probe	L858R MP	FAM-AGTTTGGCCCGCCCAA-MGB-NFQ
	L858R WP	VIC-AGTTTGGCCAGCCCAA-MGB-NFQ
	23S rRNA gene P	VIC-CCCACCTATCCTGCG-MGB
Template	L858R MT	TCGCCAGCCATAAGTCCTCGACGTGGAGAGGCTCAGAGCCTGGCATGAACATGACCCTGAA TTCGGATGCAGAGCTTCTTCCCATGATGATCTGTCCCTCACAGCAGGGTCTTCTCTGTTTCAG GGCATGAACTACTTGGAGGACCGTCGCTTGGTGCACCGCGACCTGGCAGCCAGGAACGTAC TGGTGAAAACACCGCAGCATGTCAAGATCACAGATTTTGGGCGGGCCAAACTGCTGGGTGC GGAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTAAGGAGGTGGCTTTAGGTCAGCCA GCATTTTCCTGACACCAGGGACCAGGCTGCCTTCCCACTAGCTGTATTGTTTAACACATGCA GGGGAGGATGCTCTCCAGACATTCTGGGTGAGCTCGC

L858R WT	GCAGGGTCTTCTCTGTTTCAGGGCATGAACTACTTGGAGGACCGTCGCTTGGTGCACCGCGA CCTGGCAGCCAGGAACGTACTGGTGAAAACACCGCAGCATGTCAAGATCACAGATTTTGGG CTGGCCAAACTGCTGGGTGCGGAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTAAGG AGGTGGCTTTAGGTCAGCCAGCATTTTCCTGACACCAGGGACCAGGCTGCCTTCCCACTAGC
23S rRNA gene	GCGTCAGTCGCAAGATGAAGCGTTGAATTGAAGCCCGAGTAAACGGCGGCCGTAACTATAA CGGTCCTAAGGTAGCGAAATTCCTTGTCGGTTAAATACCGACCTGCATGAATGGCGTAACGA GATGGGAGCTGTCTCAACCAGAGATTCAGTGAAATTGTAGTGGAGGTGAAAATTCCTCCTAC CCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAACTTAGCACTGCTAATGGGAAT ATCATGCGCAGGATAGGTGGGAGGCTTTGAAGTAAGGGCTTTGGCTCTTATGGAGCCATCCT TGAGATACCACCCTTGATGTTTCTGTTAGCTAACTGGCCTGTGTTATCCACAGGCAGGACAA TGCTTGGTGGGTAGTTTGACTGGGGCGGTCGCCTCCTAAAAAGTAACGGAGGCTTGCAAAG GTTGGCTCATTGCGGTTGGAAATCGCAAGTTG

Table S2. Comparison of performance between three-in-one ddPCR and existing dPCR platforms

dPCR Platform	Manufacturer	Integration	Detection time	Dynamic range	Cost
QX100™/200™	Biorad	3 instruments	3-5h	5log	500K-700K RMB + 80 RMB
QuantStudio™3D	Thermo Fisher	3 instruments	4h	5log	300K-400K RMB + 400 RMB
Naica™ Crystal	Stilla	2 instruments	2.5h	5log	500K-700K RMB + 286 RMB/4 samples
TD-1/TD-2	TargetingOne	2 instruments	3h	5log	500K-1000K
QX One	Biorad	1 instrument	2-3h	5log	2000K-5000K RMB + 80 RMB
Three-in-one ddPCR	This work	1 instrument	2h	6log	100K RMB + 13.3 RMB