

## Supporting Information for

### **MS<sup>2</sup> device: smartphone-facilitated mobile nucleic acid analysis on microfluidic device**

Xiaosong Wu<sup>‡a,b</sup>, Jingyu Pan<sup>‡a</sup>, Xinchao Zhu<sup>a,b</sup>, Chenggang Hong<sup>a</sup>, Anzhong Hu<sup>a</sup>,  
Cancan Zhu<sup>a</sup>, Yong Liu<sup>a</sup>, Ke Yang<sup>\*a</sup> and Ling Zhu<sup>\*a</sup>

<sup>a</sup> Anhui Institute of Optics and Fine Mechanics, Hefei Institutes of Physical Science,  
Chinese Academy of Sciences, Hefei, Anhui, PR China. E-mail addresses:  
[keyang@aiofm.ac.cn](mailto:keyang@aiofm.ac.cn) (Ke Yang), [zhul@aiofm.ac.cn](mailto:zhul@aiofm.ac.cn) (Ling Zhu)

<sup>b</sup> University of Science and Technology of China, No. 96, JinZhai Road Baohe District,  
Hefei 230026, PR China

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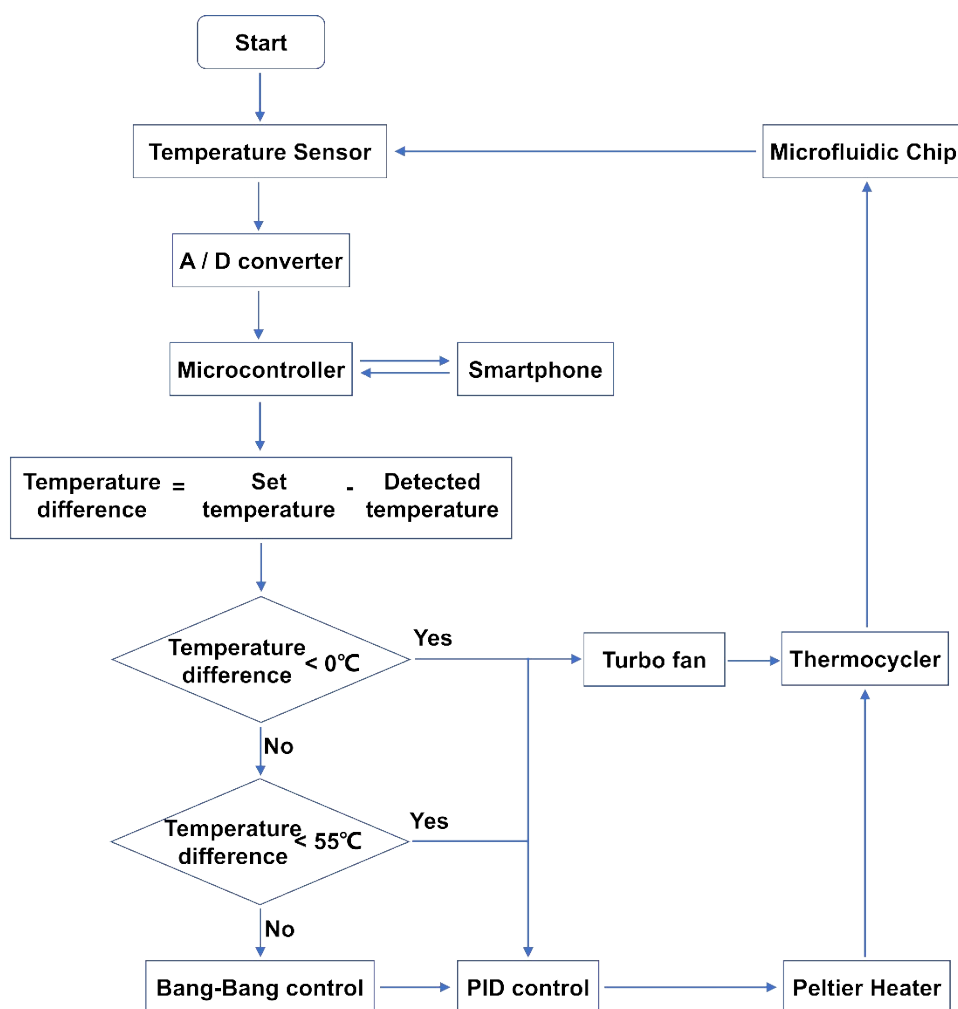
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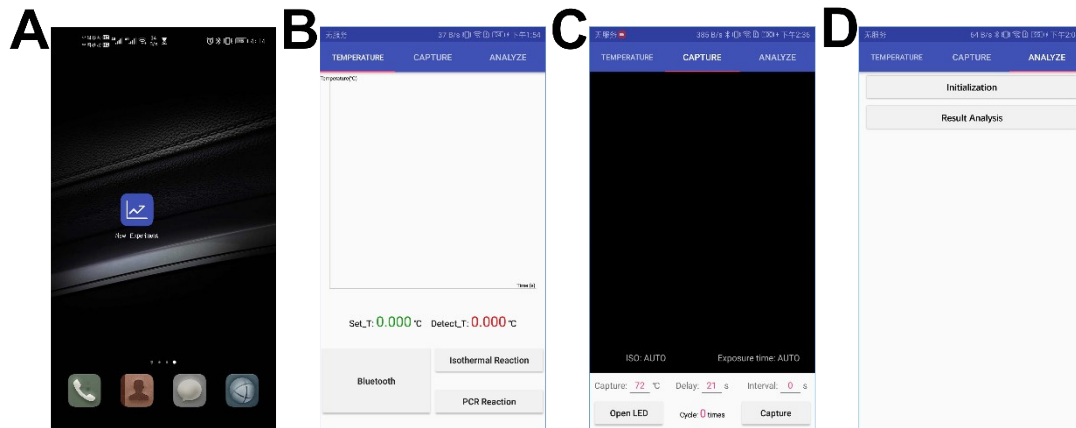
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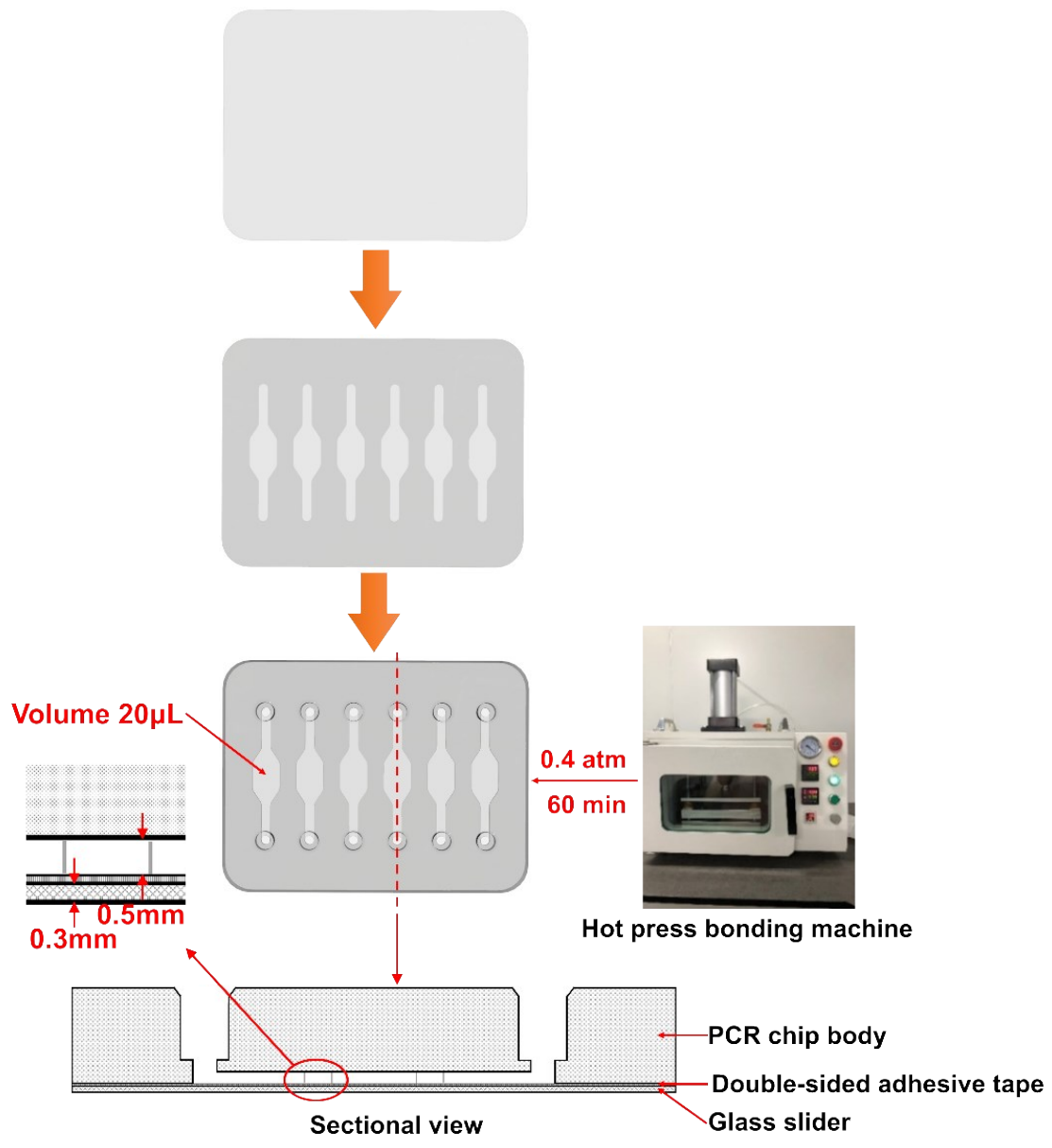
**Supporting Information 1** Operation steps of qPCR when using the smartphone App



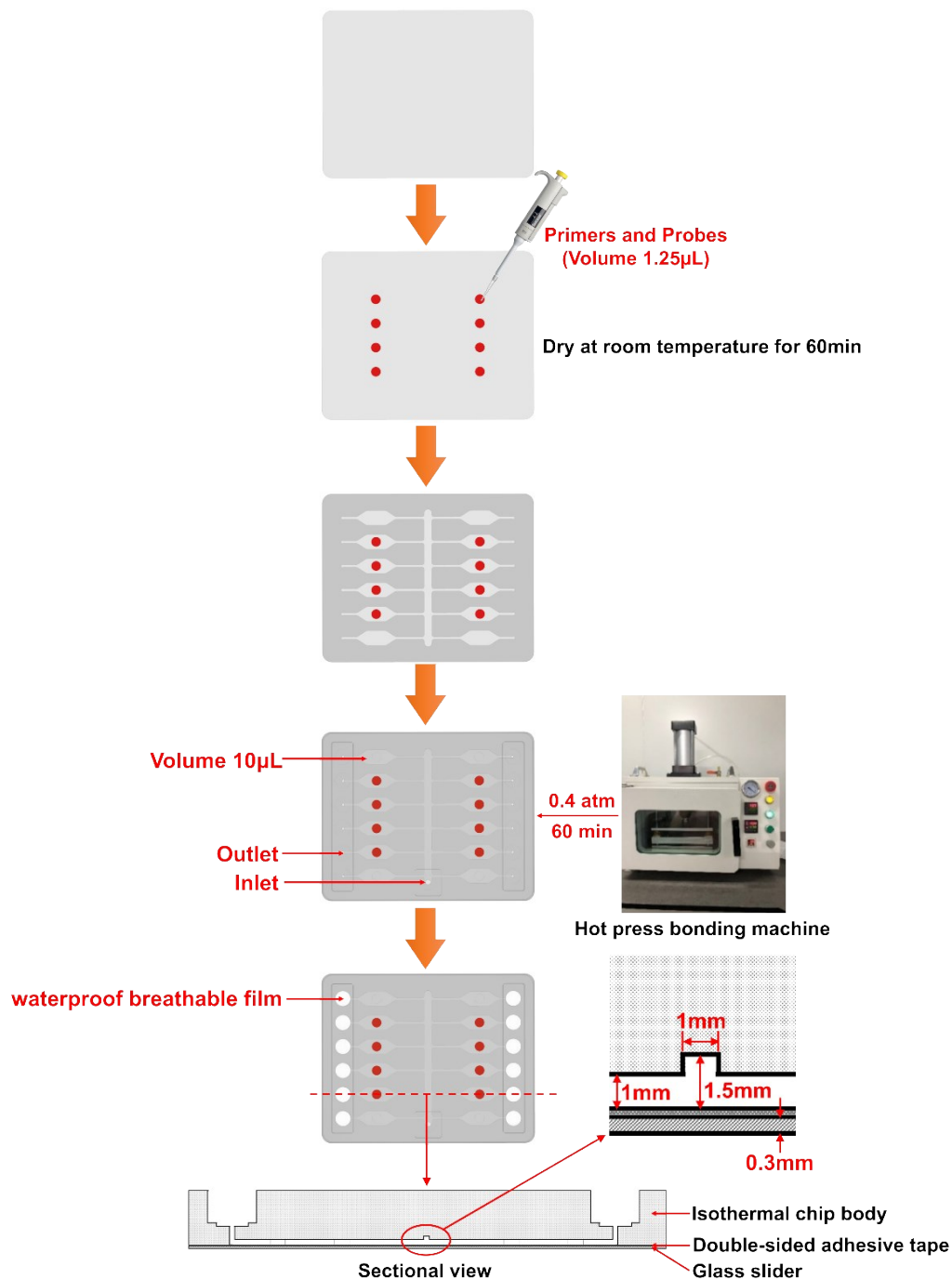
**Fig. S-1** Schematic diagram of the temperature control process. The thermocouple located in the copper heat sink of the thermocycler monitors the temperature of the reaction chamber in the microfluidic chip in real-time. A change in temperature causes a change in its resistance, which in turn causes a change in the current in the circuit. A/D converter (Analog-to-digital converter) converts the analog signal of current change into a digital signal and passes it to the microcontroller. The microcontroller calculates the temperature value according to the current-temperature conversion algorithm and the temperature compensation algorithm and sends it to the smartphone through the Bluetooth module for real-time display and drawing of the temperature curve. When the temperature needs to be adjusted, the smartphone sends a temperature control command to the microcontroller through the Bluetooth module. The microcontroller calculates the difference between the set temperature and the temperature detected by the temperature sensor. When the temperature difference is less than zero, it is a cooling process. At this time, the microcontroller calls a PID control algorithm to adjust the input current of the Peltier, and at the same time, the turbofan is started to realize the cooling process of the thermocycler. When the temperature difference is greater than zero and less than 55 °C, it is a heating process. At this time, the microcontroller calls the PID control algorithm to realize the healing process of the thermocycler. When the temperature difference is greater than 55 °C, it is a rapid heating process. At this time, the microcontroller first calls the Bang-Bang control algorithm and then the PID control algorithm to realize the rapid heating process of the thermocycler. The temperature sensor detects the temperature in real-time, and the microcontroller controls the temperature in real-time.



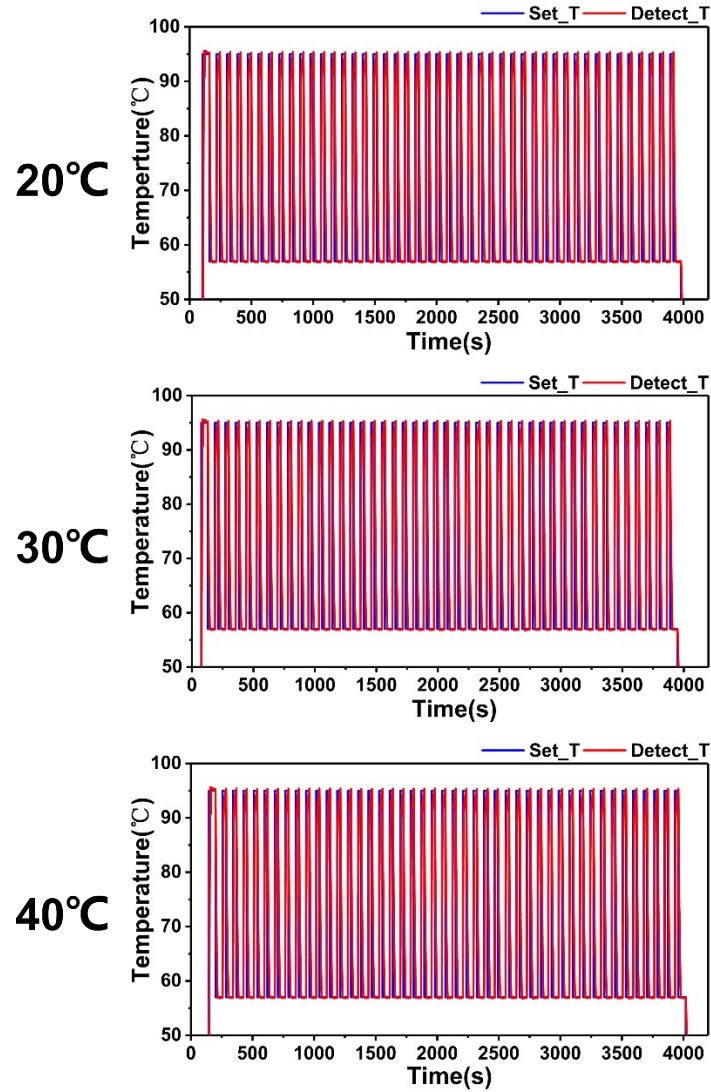
**Fig. S-2** Smartphone App interface. (A) App icon on the smartphone desktop; (B) The TEMPERATURE interface. (C) The CAPTURE interface. (D) The ANALYZE interface.



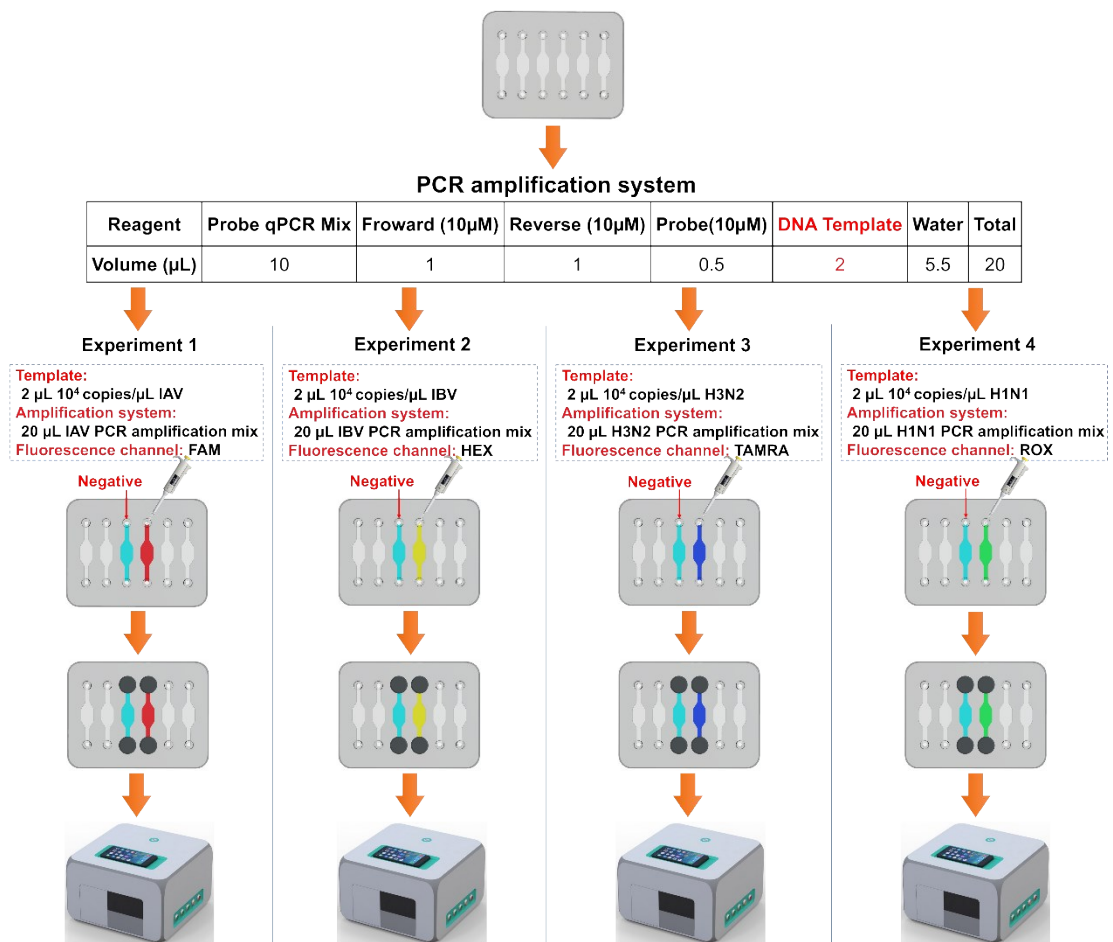
**Fig. S-3** Production steps for PCR chip. Firstly, the glass slide was cleaned with deionized water and air-dried. Secondly, one side of the custom-sized double-sided adhesive tape was completely attached to one side of the glass slide. Finally, the bottom of the chip body was completely attached to the other side of the custom-sized double-sided adhesive tape, and then the chip was placed in the hot press bonding machine for bonding at 0.4 atm for 60 min.



**Fig. S-4** Production steps for the isothermal chip. Firstly, the glass slide was cleaned with deionized water and air-dried. Secondly, a fixed volume of dots containing target primers and probes were loaded on one side of the glass slide using a pipette (the position of dots corresponded to the position of the chip reaction chamber, and the number of dots was set according to the experimental arrangement). Then the glass slide was dried at room temperature for 60 min. Thirdly, one side of the custom-sized double-sided adhesive tape was completely attached to the side of the glass slide containing the target primers and probes. Fourthly, the bottom of the chip body was completely attached to the other side of the custom-sized double-sided adhesive tape, and then the chip was placed in the hot press bonding machine for bonding at 0.4 atm for 60 min. Finally, the chip outlet was sealed with the waterproof breathable film. The prepared chip was stored at 4 °C for later use.

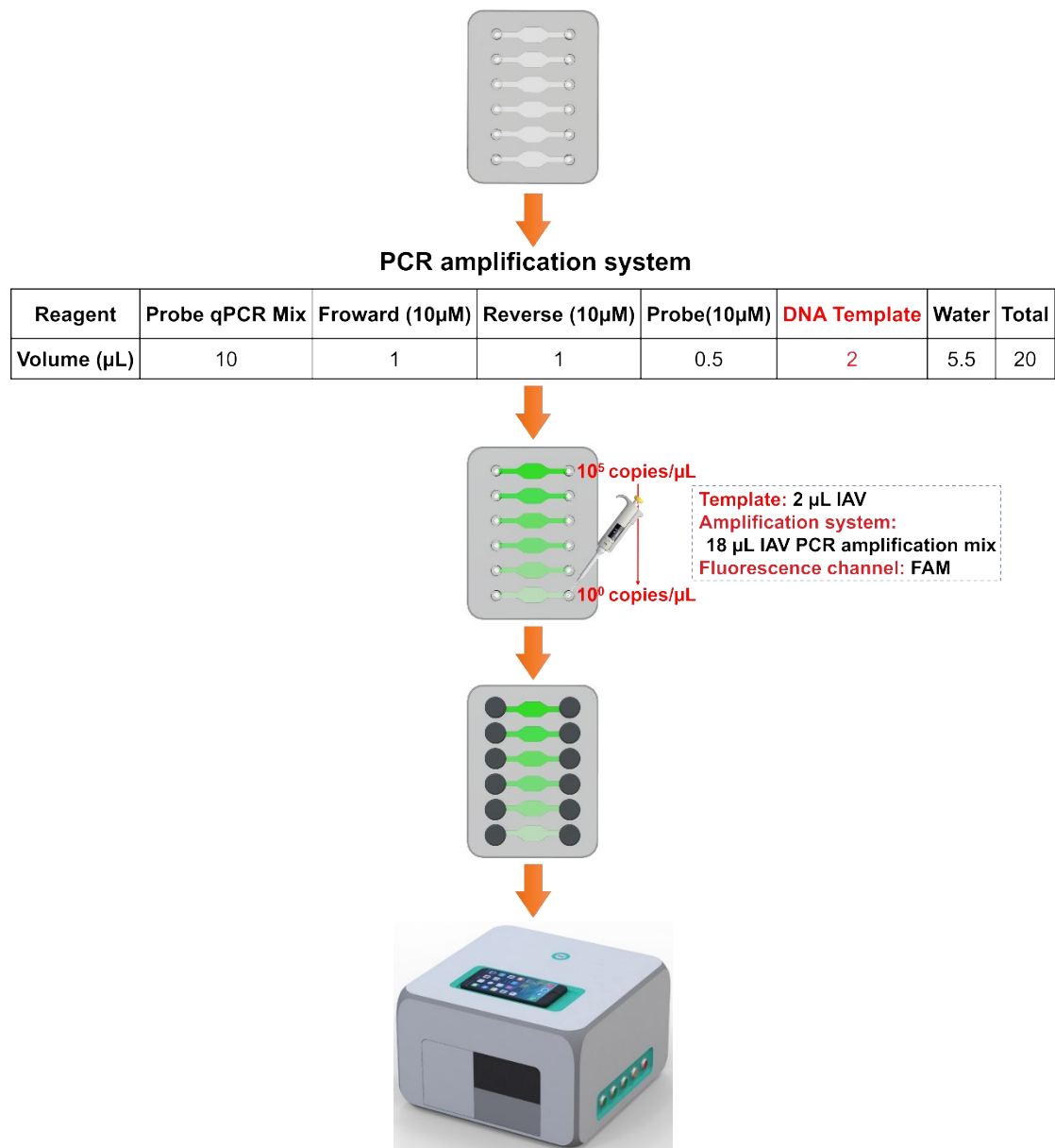


**Fig. S-5** Measured temperature profiles of the thermocycler at different exterior temperatures: 20, 30, and 40 °C. It can be seen from the figure that the temperature control curve of the thermocycler does not change significantly under different ambient temperatures.

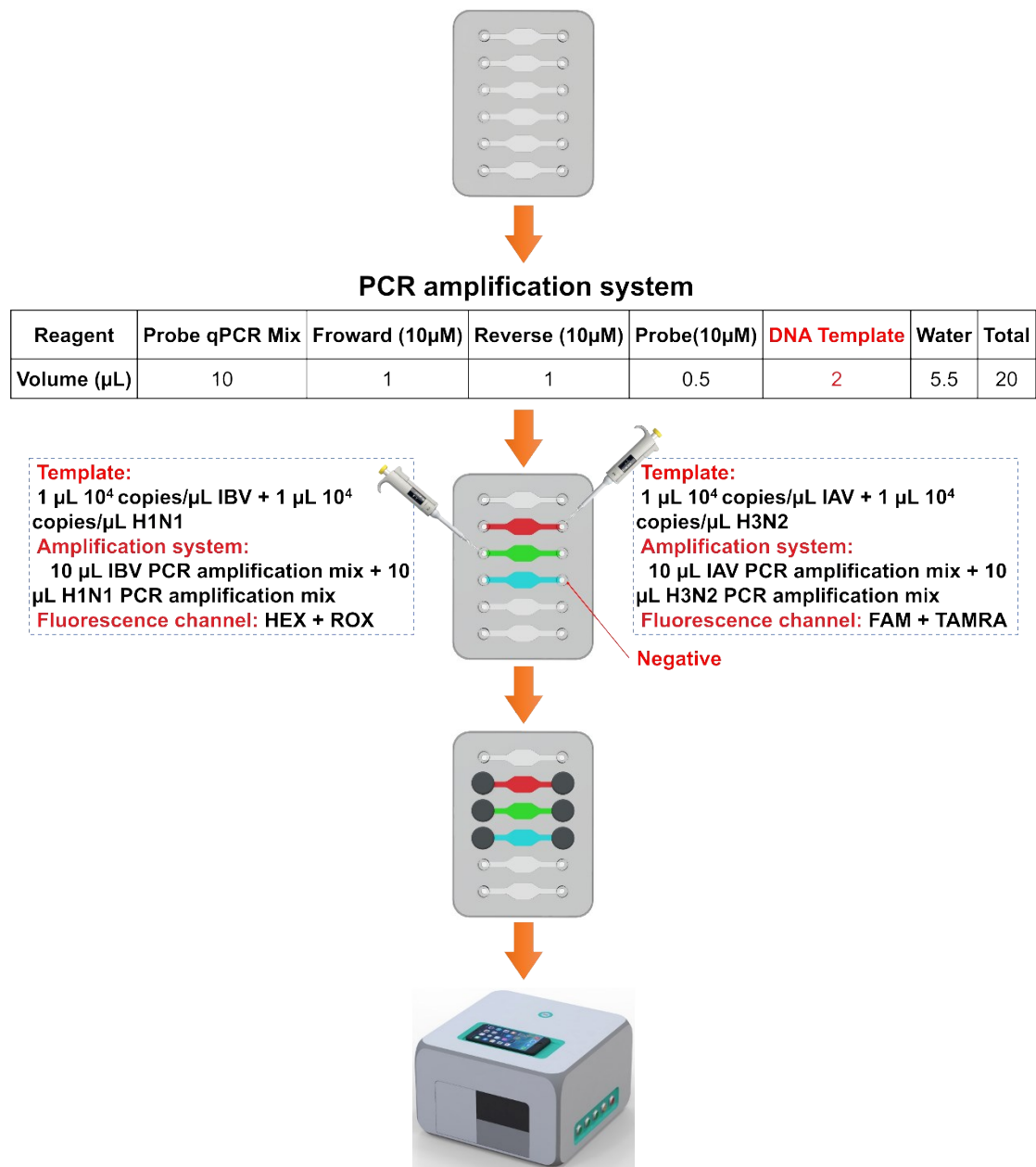


**Fig. S-6** Operation steps of qPCR. Firstly, the PCR chip was prepared. Secondly, the PCR reaction solution was configured according to the PCR amplification system and experimental arrangement. Thirdly, the configured PCR reaction solution was loaded into the reaction chamber of the PCR chip using a pipette. Fourthly, the inlet and outlet of the corresponding reaction chamber of the PCR chip were sealed by the sealing plug. Finally, the chip was placed on the MS<sup>2</sup> device for PCR experiments.

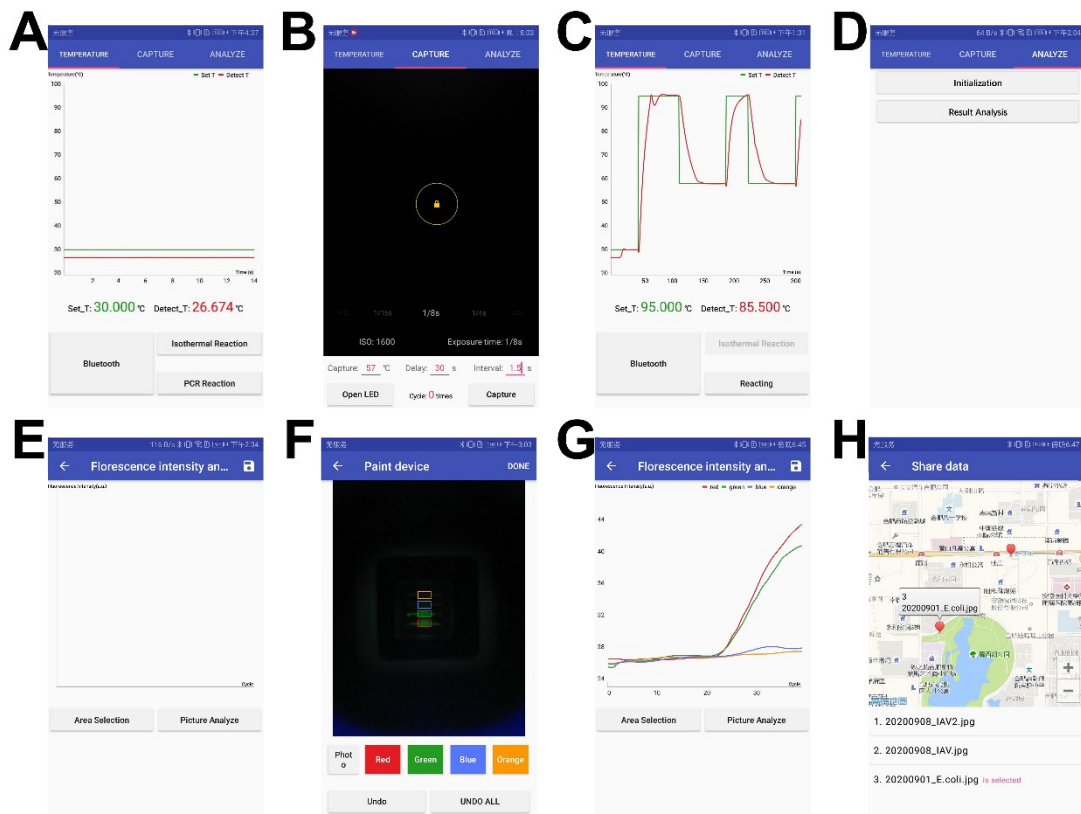




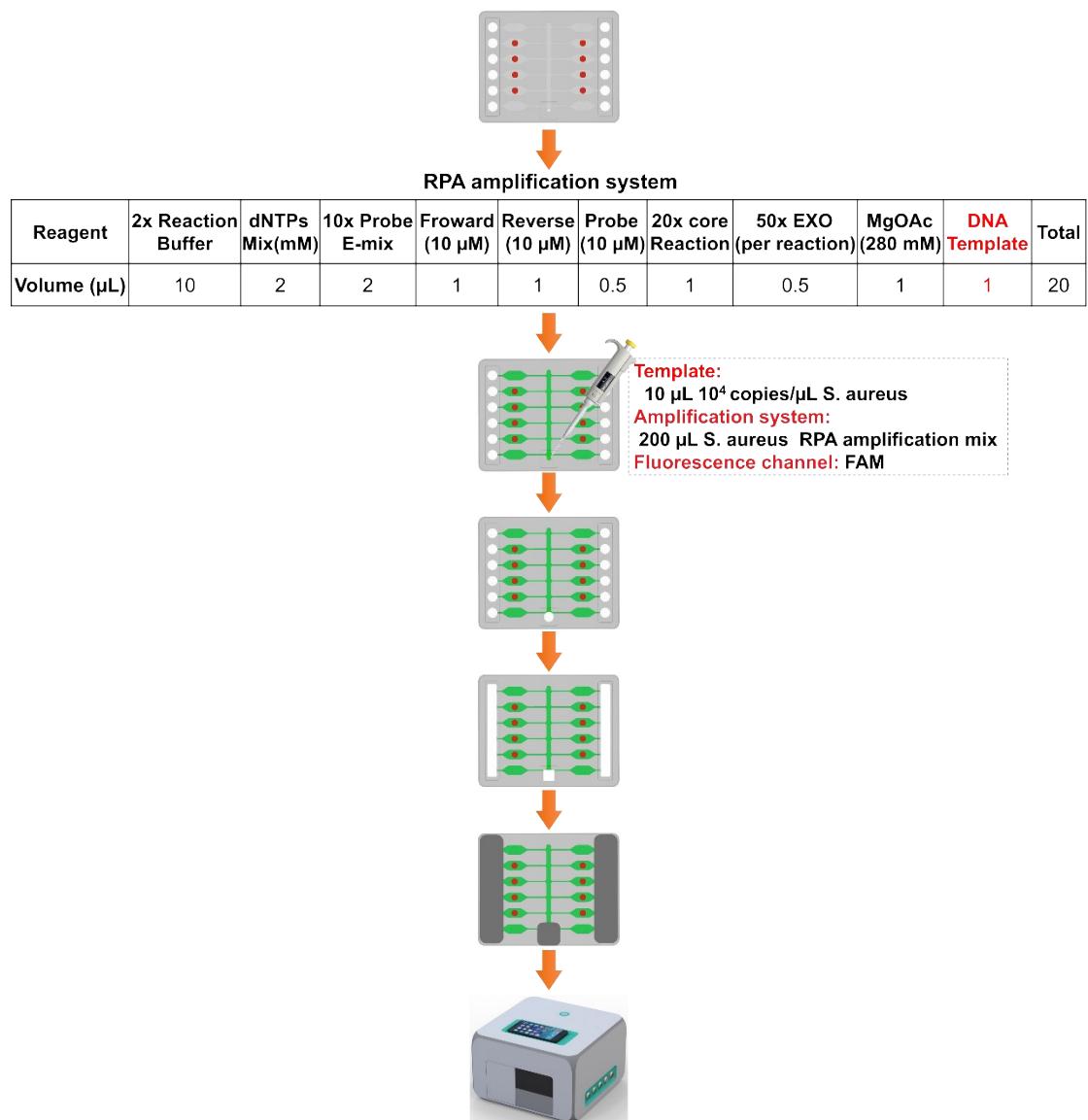
**Fig. S-7** Operation steps for LOD test. Firstly, the PCR chip was prepared. Secondly, the PCR reaction solution was configured according to the PCR amplification system and experimental arrangement. Thirdly, the configured PCR reaction solution was loaded into the reaction chamber of the PCR chip using a pipette. Fourthly, the inlet and outlet of the corresponding reaction chamber of the PCR chip were sealed by the sealing plug. Finally, the chip was placed on the MS<sup>2</sup> device for qPCR experiments.



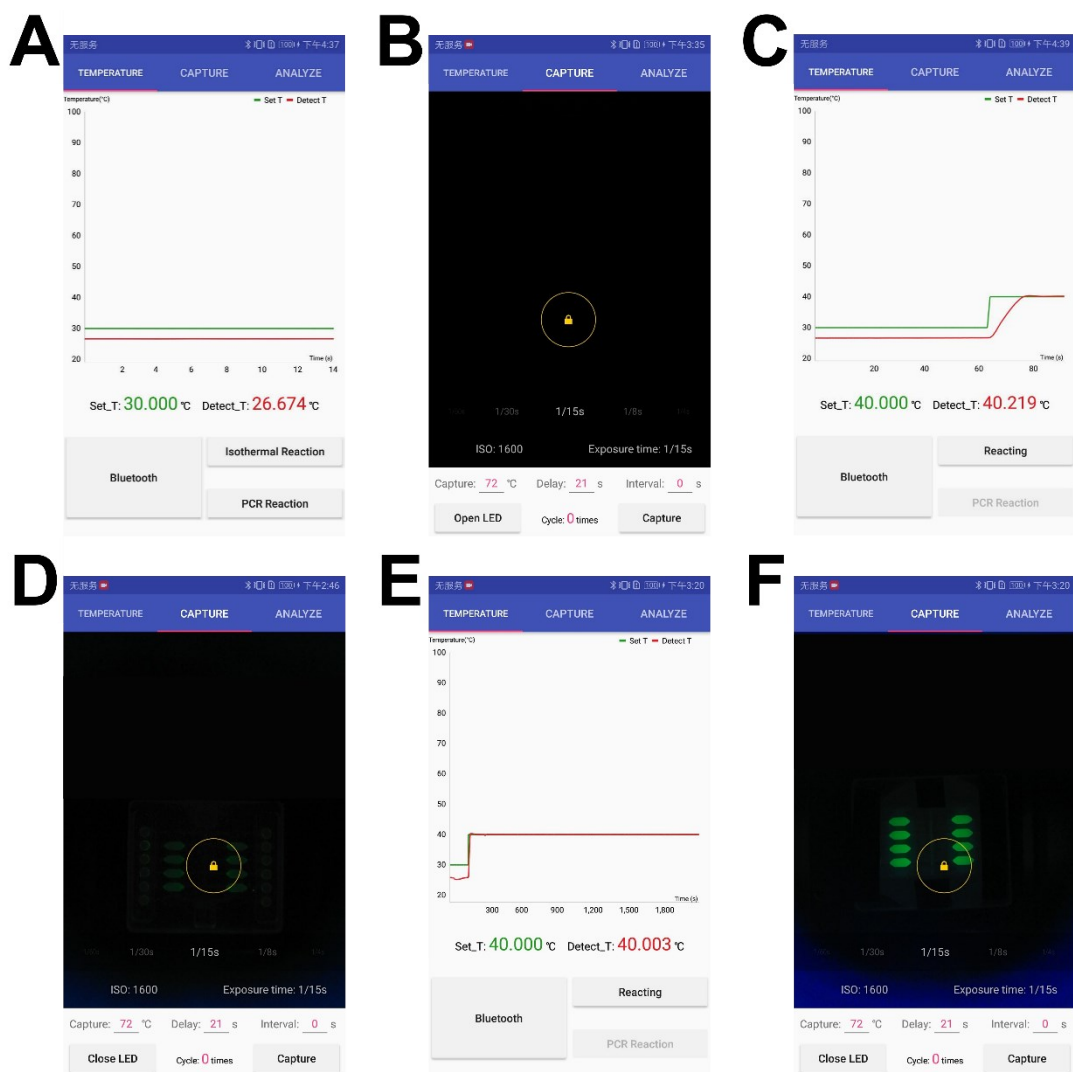
**Fig. S-8** Operation steps for Multiplex PCR. Firstly, the PCR chip was prepared. Secondly, the PCR reaction solution was configured according to the PCR amplification system and experimental arrangement. Thirdly, the configured PCR reaction solution was loaded into the reaction chamber of the PCR chip using a pipette. Fourthly, the inlet and outlet of the corresponding reaction chamber of the PCR chip were sealed by the sealing plug. Finally, the chip was placed on the MS<sup>2</sup> device for PCR experiments.



**Fig. S-9** Operation steps of multiplex PCR when using the smartphone App. After the smartphone App and MS<sup>2</sup> device are connected via Bluetooth. Follow the steps in Supporting Information 1 to set the focal length, gain, and exposure time of the smartphone camera, as well as the acquisition temperature and delay time when acquiring fluorescent images in each PCR cycle. In each multiplex PCR cycle, the interval time “Interval” of fluorescence image acquisition between the four fluorescence channels is set according to the switching speed of the fluorescence channels. The subsequent experimental operation is the same as that shown in Supporting Information 1.



**Fig S-10** The operation steps for RPA. Firstly, the isothermal chip was prepared. Secondly, the RPA reaction solution was configured according to the RPA amplification system and experimental arrangement. Thirdly, the configured RPA reaction solution was loaded into the reaction chamber of the isothermal chip using a pipette. Fourthly, the waterproof-breathable film was loaded onto the inlet of the isothermal chip. Fifthly, the inlet and outlet of the isothermal chip were sealed by the sealing plug. Finally, the chip was placed on the MS<sup>2</sup> device for PCR experiments.



**Fig. S-11** Operation steps of RPA when using the smartphone App. After the smartphone App and MS<sup>2</sup> device are connected via Bluetooth. Follow the operations in Supporting Information 1 to set the focal length, gain, and exposure time of the smartphone camera. On the main interface of temperature control, click the "Isothermal Reaction" button to perform RPA isothermal amplification temperature control. When the temperature control curve in the interface rises from the ambient temperature to 40 °C and remains constant, turn on the LED light on the fluorescence image acquisition main interface and click the "Capture" button to capture the fluorescent image. After the image capture is completed, turn off the LED light. After the temperature remained stable at 40 °C for about 30 minutes, the fluorescence image was captured again.

**Table. S-1** Template sequences for biological experiments to verify system performance.

<b>Template name</b>	<b>Template sequence</b>
<b>IAV</b>	AGCAAAAGCAGGTAGATGTTTAAAGATGAGTCTTCTAACCGAGGTCGAAACGTATG TTCTCTCTATCGTTCCGTCAGGCCCTCAAAGCCGAGATAGCGCAGAGACTTGAA GATGTTTTTGCAGGGAAAAACACCGATCTTGAGGCACTCATGGAATGGCTAAAAGAC AAGACCAATCCTGTACCTCTGACTAAGGGGATTTAGGATTTGTGTTACAGCTCAC CGTGCCAGTGAGCGAGGACTGCAGCGTAGACGCTTTGTCCAGAATGCCCTCAATG GGAATGGTGACCCGAACAACATGGACAAAGCGGTCAAAGTGTACAGAAAACCTTAA AAGGGAAATAACATTCCACGGGGCCAAAGAAGTAGCGCTCAGTTACTCTGCTGGTG CACTTGCCAGTTGCATGGGCCTCATATACAACAGAATGGGGACTGTCACCACTGAG GTGGCCTTTGGTCTAGTATGCGCAACCTGTGAACAGATTGCTGATTCCCAG
<b>IBV</b>	ATGGCGGACAACATGACCACAACAAAATTGAGGTGGGTCCGGGAGCAACCAATG CCACCATAAACTTTGAAGCAGGAATTCTGGAGTGCTATGAAAGGCTTTCATGGCAA AGAGCCCTTGACTACCCTGGTCAAGACCGCCTAACAGACTAAAGAGAAAATTAG AGTCAAGAATAAAGACTCACAAACAAAAGTGAGCCTGAAAGTAAAAGGATGTCTCT TGAAGAGAGAAAAGCAATTGGAGTAAAAATGATGAAAGTGCTCCTATTTATGGAC CCATCTGCTGGAATTGAAGGGTTTGAGCCATACTGTATGAAAAGTTCCTCAAATAG CAACTGTCTAAATACAATTGGACCGATTACCCTTCAACACCAGGAAGGTGCCTTG ATGACATAGAAGAAGAACCAGAGGATGTTGATGGCCCAACTGAAATAGTATTAAG GGACATGAACAACAAGATGCAAGGCAAAAAGATAAAGGAGGAAGTAA ACACTC
<b>H1N1</b>	AGAGACACCAAACCCTGAGAATGGAACATGTTACCCAGGGTATTTCCGCCACTATG AGGAACTGAGGGAGCAATTGAGTTCAGTATCTTCATTTGAGAGGTTCCGAAATATTC CCCAAAGAAAGCTCATGGCCCAACCACCCGTAACCGGAGTATCGGCATCATGCTC CCATAATGGGAAAAGCAGTTTTTACAGAAATTTGCTATGGCTGACGGAGAAGAATG GCTTGTACCCAAATCTGAGCAAGTCTATGTAAACAACAAGGAGAAAAGAAGTCCTT GTACTATGGGGTGTTCATCACCCGTCTAACATAGGGGTCCAAAGGGCCATCTATCA TACAGAAAATGCTTATGTCTCTGTAGTGTCTTCACATTATAGCAGAAGATTCACCCC AGAAATAGCAAAAAGACCCAAAGTAAGAGATCAGGAAGGAAGAATCAACTACTAC TGGACTCTGCTGGAACCCGGGGACA
<b>H3N2</b>	GCAGAATAAGCATCTATTGGACAATAGTAAAACCGGGGACATACTTTTGATTAAC AGCACAGGGAATCTAATTGCTCCTAGGGGTTACTTCAAATACGAAGTGGGAAAAG CTCAATAATGAGATCAGATGCACCCATTGGCAAATGCAATTCTGAATGCATCACTC CAAATGGAAGCATTCCAATGACAAACCATTTCAAATGTAAACAGGATCACATAT GGGGCCTGTCCCAGATATGTTAAGCAAAACACTCTGAAATTGGCAACAGGGATGCG AAATGTACCAGAGAAAACAACTAGAGGCATATTTGGCGCAATCGCGGGTTTCATAG AAAATGGTTGGGAGGGAATGGTGGATGGTTGGTACGGTTTCAGGCATCAAATTTCT GAGGGAATAGGGCAAGCAGCAGATCTCAAAGCACTCAAGCAGCAATCAATCAAA TCAATGGGAAGCTGAATAGGTTGATCGG
<b>S. aureus</b>	CCAAAGAATGTATCTAAAAAAGATTATAAAGCAATCGCTAAAGAACTAAGTATTTT TGAAGACTATATCAAACAACAATGGATCAAATTTGGGTACAAGATGATACCTTCG TTCCACTTAAAACCGTTAAAAAATGGATGAATATTTAAGTGATTTTCG

**Table. S-2** Primer and probe sequences for biological experiments to verify system performance.

<b>Virus name</b>	<b>Primer name</b>	<b>Sequence (5'-3')</b>	<b>Amplicon size (bp)</b>
<b>IAV</b>	Forward	CCGAGGTCGAAACGTATGTTC	67
	Reverse	CTCTGCGCTATCTCGGCTTT	
	Probe (FAM)	CTCTATCGTTCCGTCAGGCCCCCT	
<b>IBV</b>	Forward	GGACAACATGACCACAACACAAAT	81
	Reverse	TCCAGAATTCCTGCTCAAAGTTTA	
	Probe (HEX)	CCGGGAGCAACCAATGCCACC	
<b>H1N1</b>	Forward	CATAATGGGAAAAGCAGTTTTTACAG	124
	Reverse	ATAGGACTTGCTCAGATTTGGGTAC	
	Probe (ROX)	TGCTATGGCTGACGGAGAAGAATGGCT	
<b>H3N2</b>	Forward	AAGCATTCCAATGACAAACC	147
	Reverse	ATTGCGCAAATATGCCTCTAGT	
	Probe (TAMRA)	CAGGATCACATATGGGGCCTGTCCCAG	
<b>S. aureus</b>	Forward	CCAAAGAATGTATCTAAAAAAGATTATAAAGCAAT C	160
	Reverse	CGAAATCACTTAAATATTCATCCATTTTTTTAACGG	
	EXO probe (FAM)	ACTAAGTATTTCTGAAGACTATATCAAACAACAAA/i 6famdt/G/idsp/A/ibhq1dt/CAAAATTGGGTACAA/ispc3/	

**Table. S-3** Reaction mixture system of isothermal amplification experiment based on RPA technology.

<b>Reagent</b>	<b>Volume (<math>\mu\text{L}</math>)</b>
2x Reaction Buffer	10
dNTPs Mix(mM)	2
10x Probe E-mix	2
Froward (10 $\mu\text{M}$ )	1
Reverse (10 $\mu\text{M}$ )	1
Probe(10 $\mu\text{M}$ )	0.5
20x core Reaction	1
50x EXO (per reaction)	0.5
MgOAc (280 mM)	1
DNA Template	1
<b>Total</b>	<b>20</b>

**Table. S-4** Reaction mixture system for PCR experiments.

<b>Reagent</b>	<b>Volume (<math>\mu\text{L}</math>)</b>
Probe qPCR Mix	10
Froward (10 $\mu\text{M}$ )	1
Reverse (10 $\mu\text{M}$ )	1
Probe(10 $\mu\text{M}$ )	0.5
DNA Template	2
Water	5.5
<b>Total</b>	<b>20</b>



**Table. S-5** Parameters of a focusing lens for a collimating optical path module.

<b>Lens</b>	<b>Curvature radius (mm)</b>	<b>Diameter (mm)</b>	<b>Thickness (mm)</b>	<b>Focal length (mm)</b>
<b>1</b>	6.19	9	3.44	12
<b>2</b>	10.3	12.7	4	20
<b>3</b>	18.03	25.4	7.2	35
<b>4</b>	25.75	25.4	5.3	50
<b>5</b>	38.63	25.4	4.1	75
<b>6</b>	51.51	25.4	3.6	100

**Table. S-6** Parameters of filters and dichroic mirrors.

<b>Channel</b>	<b>Excitation filter</b>		<b>Emission filter</b>		<b>Dichroic mirror</b>	
	Central wavelength (nm)	Bandwidth (nm)	Central wavelength (nm)	Bandwidth (nm)	Reflected wavelength (nm)	Transmitted wavelength (nm)
<b>FAM</b>	480	30	530	15	480	530
<b>HEX</b>	530	15	570	15	530	570
<b>TAMR A</b>	550	15	590	15	550	590
<b>ROX</b>	575	15	620	15	575	620

**Supporting Information 1** Operation steps of qPCR when using the smartphone App. After the smartphone App and MS<sup>2</sup> device are connected via Bluetooth. The main interface of fluorescence image acquisition will be opened by clicking the "Capture" tab of the main interface of temperature control. When you click the "Open LED" button at the bottom of the interface, the LED light of the MS<sup>2</sup> device will be turned on. Observe the brightness and clarity in the field of view of the smartphone camera in the central area of the interface, tap and hold the fluorescent area that needs to be collected, the smartphone camera will automatically focus on the area and lock the focus, and then tap "ISO" respectively Button and "Exposure Time" button, select the gain value and exposure time of the smartphone camera until a clear and bright fluorescent image can be seen on the interface, and finally click the "Close LED" button to turn off the LED light of the MS<sup>2</sup> device. The acquisition temperature "capture" and delay time "delay" when acquiring fluorescence images in each cycle are set according to the temperature conditions of the qPCR experiment. On the main interface of temperature control, click the "PCR Reaction" button to perform PCR temperature control. When the PCR amplification is over, the temperature control is closed, and the temperature control curve gradually returns to the ambient temperature. Click the "Analysis" tab to enter the main analysis interface, click the "Result Analysis" button in the interface to open the fluorescence intensity curve drawing sub-interface, click the "area selection" button to open the fluorescence area frame selection sub-interface, and click the "Photo" button to open Picture selection sub-interface, click to select the first fluorescence picture, click the "DONE" button to return to the fluorescence area frame selection sub-interface, and then click the "Red", "Green", "Blue" and "Orange" buttons to add the fluorescence image The effective fluorescent area is selected by the rectangular frames of these four colors. If the frame selection is wrong, click "Undo" or "UNDO ALL" to cancel. After the frame selection is completed, click the "DONE" button to return to the fluorescence intensity curve drawing sub-interface, and click the "Image Analysis" button to draw the fluorescence intensity curve on the interface. Click the save icon to save the graph in .jpg format. Click the "Save Data" button on the main analysis interface to open the data storage sub-interface, where you can see the saved graph, modify the name of the picture "Title", and click the save icon to save the renamed graph to the smartphone. Click the "Share Data" button in the main analysis interface to open the data sharing sub-interface, you can see all the renamed experimental data and the geographic location of each experiment. Click the name of any experimental data to view the experimental data, and click the sharing icon on the data viewing interface to share the viewed experimental data through the wireless transmission function of the smartphone.