Hybrid Opto-Thermocycler for RT-qPCR using a Bubble-free Microfluidic Device detects SARS-CoV-2

Pablo E Guevara-Pantoja[1], Roberto Rodriguez-Moncayo[1], Oriana G Chavez-Pineda[1], Diana F Cedillo-Alcantar[1], Keziah B Reynoso-Hernandez[1], Jose A Ramirez-Pool[2], Leandro A Nuñez-Muñoz[2], Beatriz Xoconostle-Cazares[2], Jose L Garcia-Cordero*[,1]

[1]Laboratory of Microtechnologies Applied to Biomedicine
Centro de Investigación y de Estudios Avanzados (Cinvestav), Monterrey, NL,Mexico.

[2]Department of Biotechnology and Bioengineering, Cinvestav, Mexico City

*e-mail: jlgarciac@cinvestav.mx

Table S1. List of electronic and optical components used to assemble the HybOT cycler.

Label	Component	Model	Manufacturer	Quantity	Cost	Total
1	Fan	THA0412AD	Delta electronics	2	21.65	43.3
2	Power supply	ALM150PS12	XP Power	1	75	75
3	Peltier module +Heatsink Assembly	1335	Adafruit	1	34.95	34.95
4	Infrared Thermometers	MLX90615	Melexis	1	20.66	20.66
5	Microcontroller	Nano	Arduino	1	22	22
6	Driver H-Bridge PWM For Arduino	BTS7960B	Infineon	1	9.89	9.89
7	Bluetooth module	HC-06	Wavesen	1	10	10
8	Vaccum Pump	SC3710PM	SKOOCOM	1	8	8
9	DC-DC Voltage Boost Step Up 20w	XL6019	Xlsemi	1	10	10
10	Fluorescence Emission filter	867-028	Edmund Optics	1	280	280
11	Fluorescence Excitation filter	867-031	Edmund Optics	1	280	280
12	Blue excitation LED	LXML-PX02-A900	Lumileds	4	2.8	11.2
13	Light sensor	VELM6030	Vishay	4	2.7	10.8
14	LED driver	COM-13705	SparkFun	1	15.95	15.95
	Total					**$713.45**

Table S2. Intra-assay precision(repeatability) for the SARS-CoV-2 N1 gene amplification experiments in the HybOT Cycler.

Standard copies/µL	Mean Ct n=3	STD	C.V. (%)
10^7	21	2.64	12.5
10^6	24.66	0.57	2.3
10^5	27	2.08	7.5
10^4	31	2.64	8.5
10^3	36.66	2.3	6.2

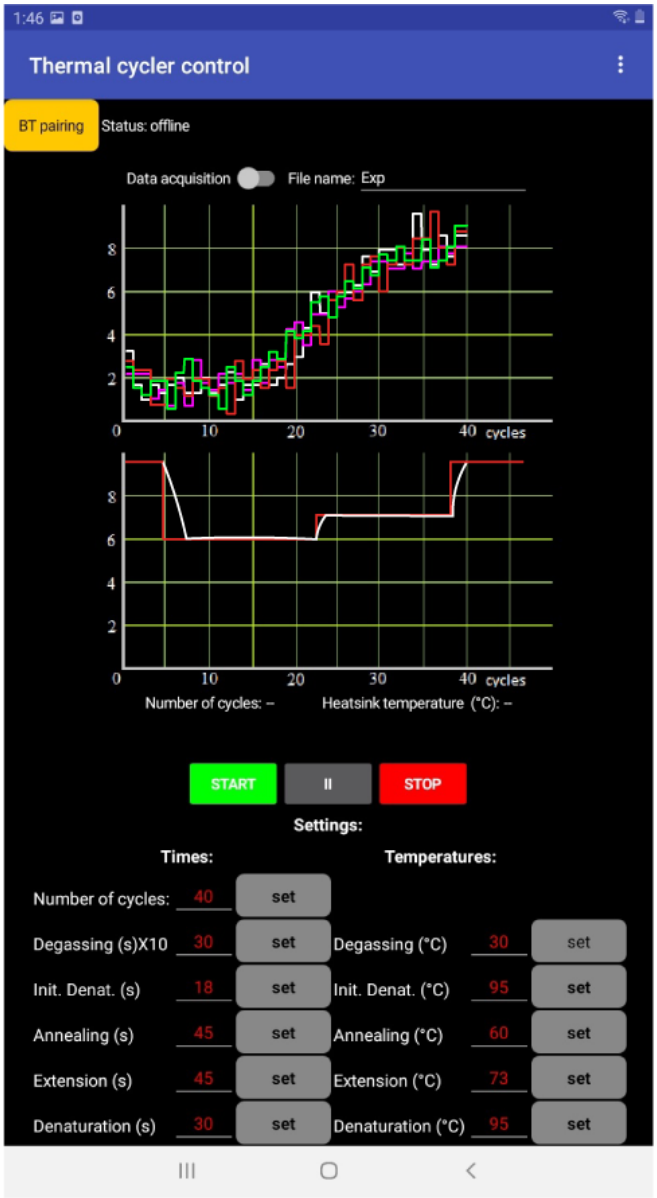

Figure S1. Tablet interface. Interface developed in App inventor for the control of HybOT thermocycler. The display shows fluorescence intensity and temperature plots in real time

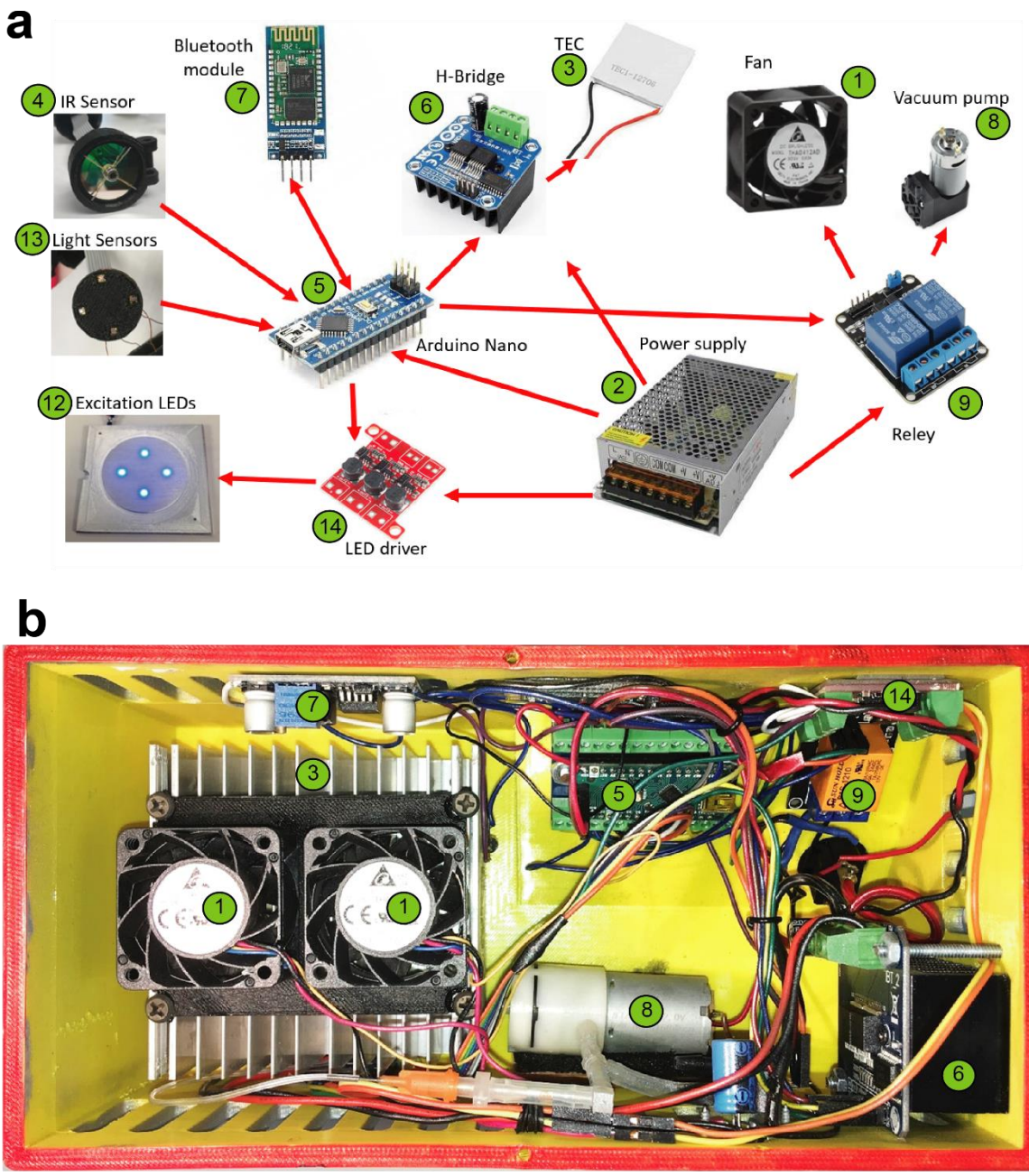

Figure S2. HybOT instrument. **a**) Block diagram of the electronic components. **b**) Photograph of the components inside the HybOT Cycler. Green labels point to numbers itemized in **Table S1.**

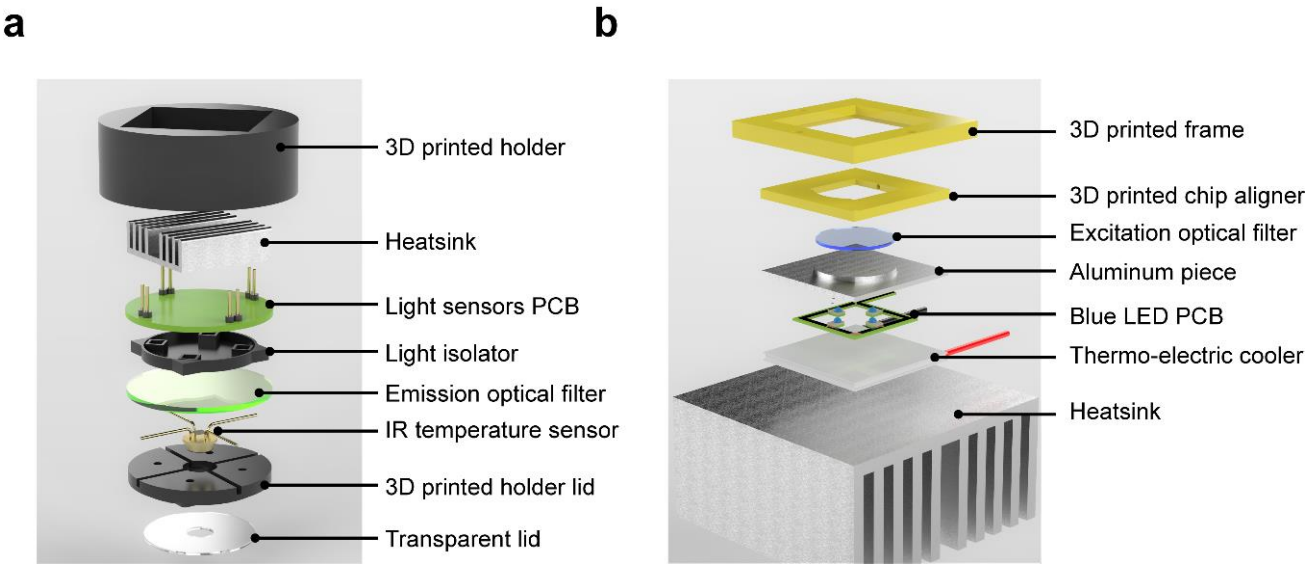

Figure S3. Design and assembly of the main parts that make the HybOT. (**a**) Exploded view of the sensors lid. (**b**) Exploded view of the thermal and excitation module.

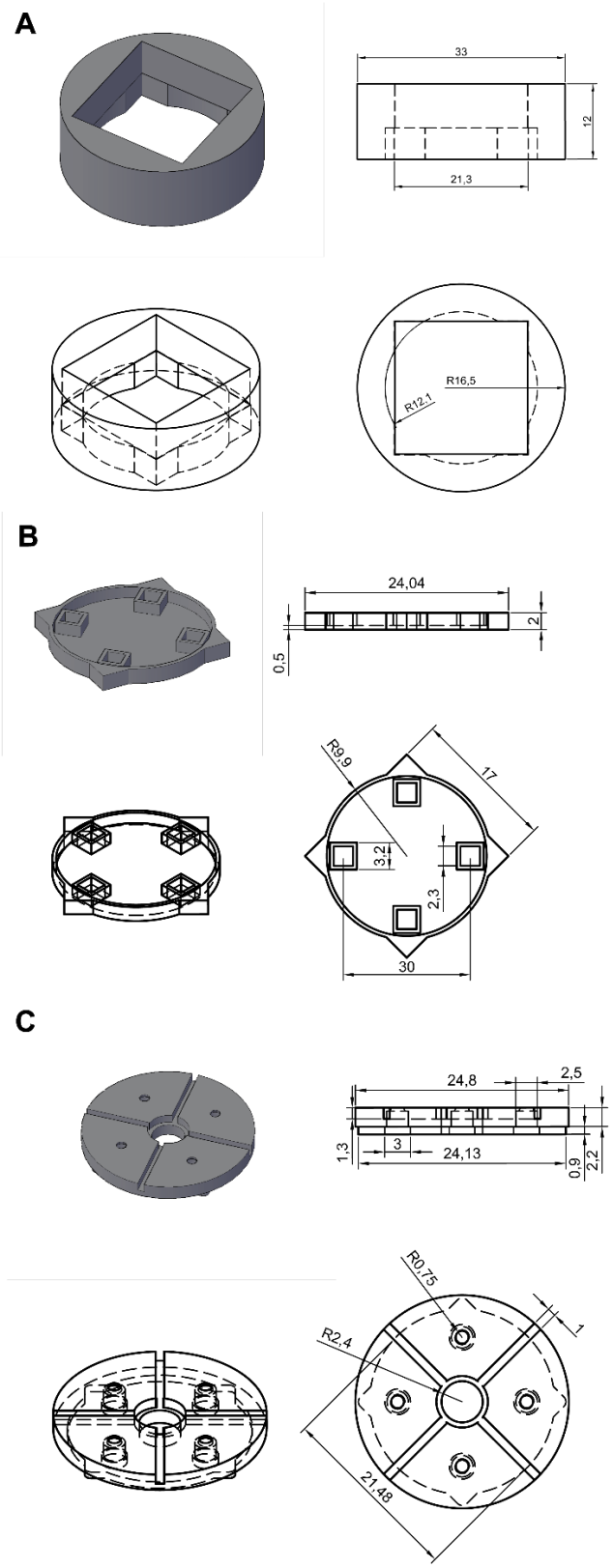

Figure S4. Drawings of lid parts. **A**) 3D printed holder. **B**) Light isolator. **C**) Holder lid. Dimensions are in millimeters.

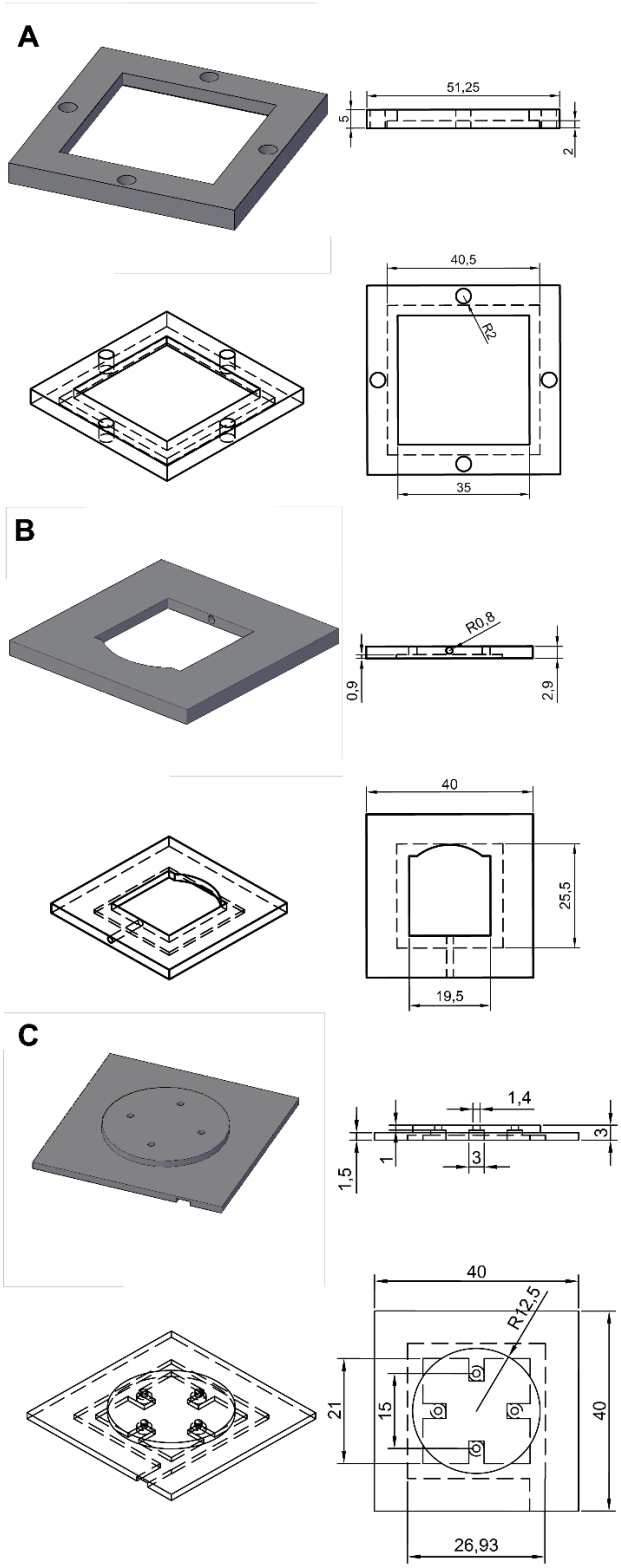

Figure S5. Drawings of the HybOT parts. **A**) 3D printed frame. **B**) 3D printed chip aligner. **C**) Aluminum piece. Dimensions are in millimeters.

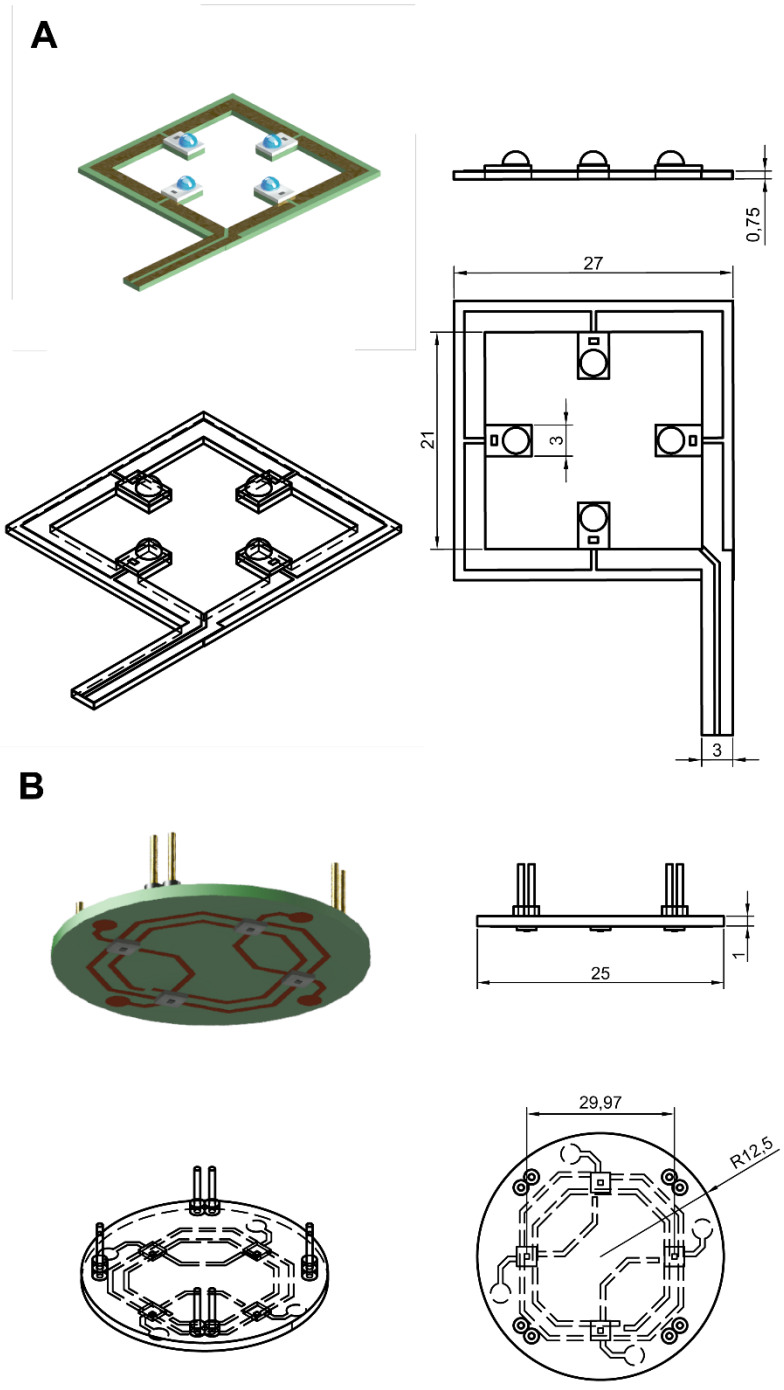

Figure S6. Designs of the Printed Circuit Boards (PCB). **A**) PCB of excitation LEDs. **B**) PCB of the lid light sensors. Dimensions in millimeters.

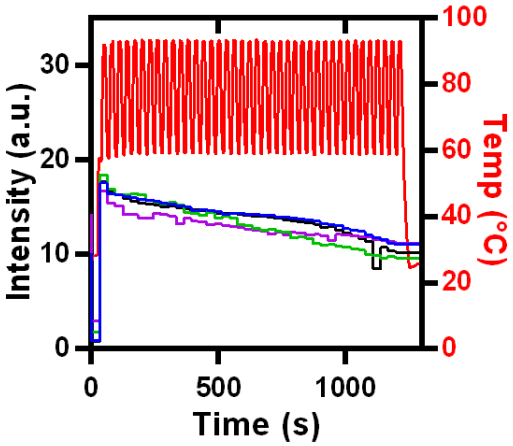

Figure S7. Variations of the light sensor over a PCR assay without active cooling.

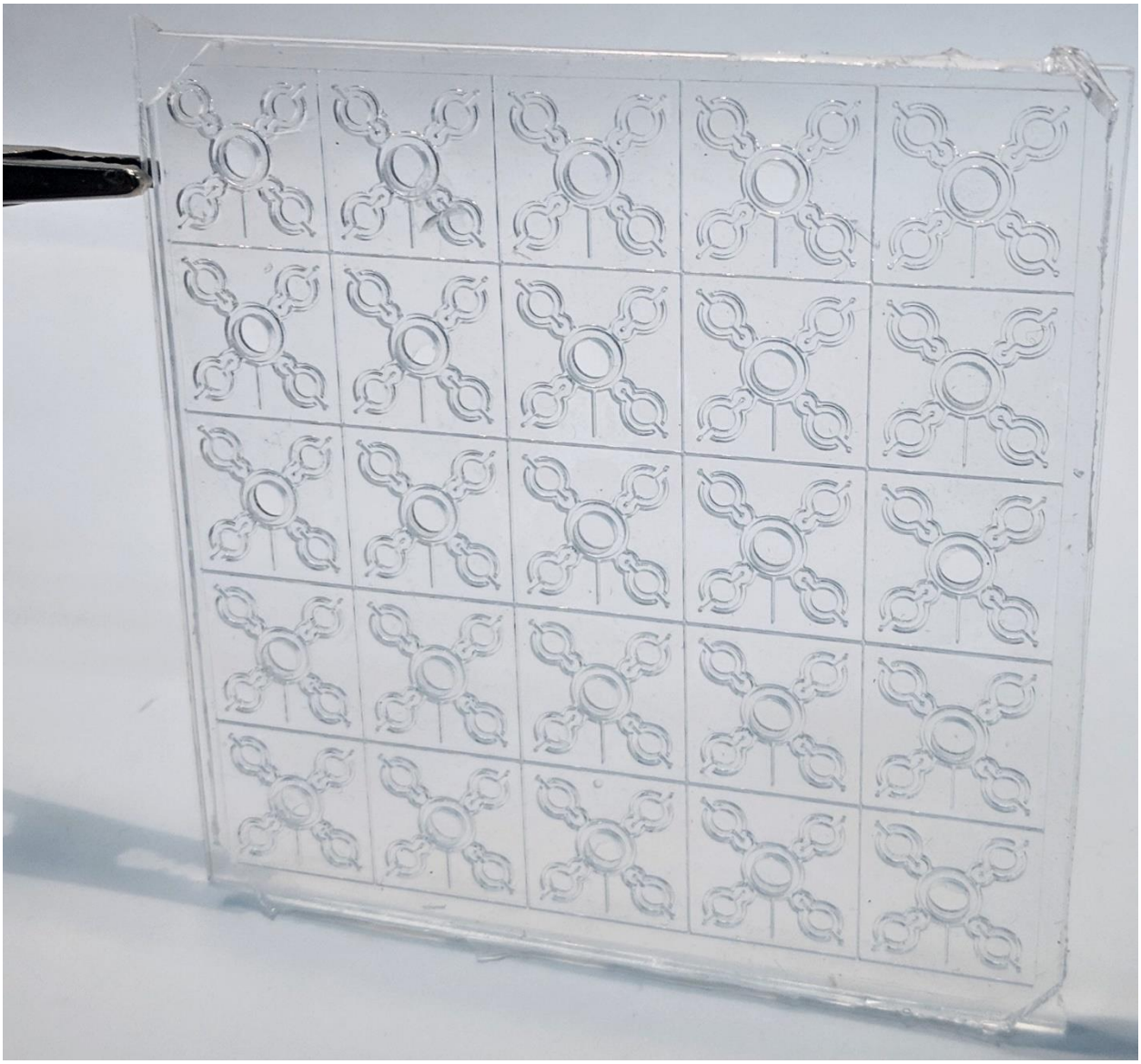

Figure S8. Acrylic mold for 25 PCR microfluidic devices.

Figure S9. Representative photographs of the microfluidic devices for three different degassing conditions (top to bottom). The assay conditions are shown to the right of each row. Left and right photographs show the beginning of the assay and the end of the initial denaturation stage, respectively. The red arrows point to the formation of bubbles in the chambers.

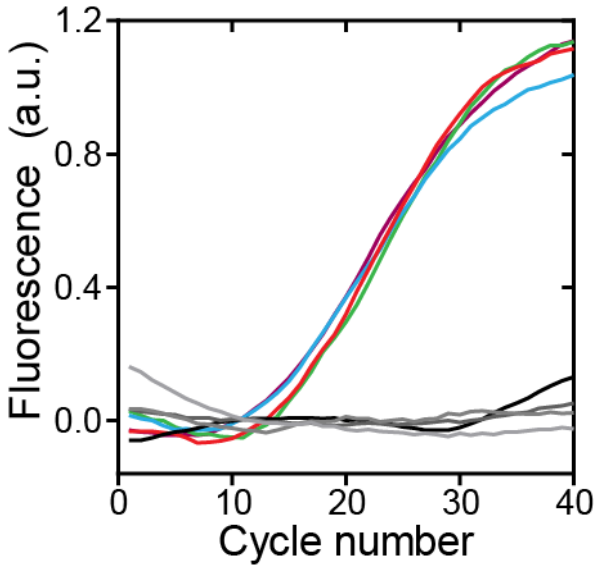

Figure S10. An initial concentration of 10^7 copies of the gene RNase P gene was amplified in the four chambers of the device at the same time. Resulting curves are shown. Four negative controls were also amplified (grey colors).

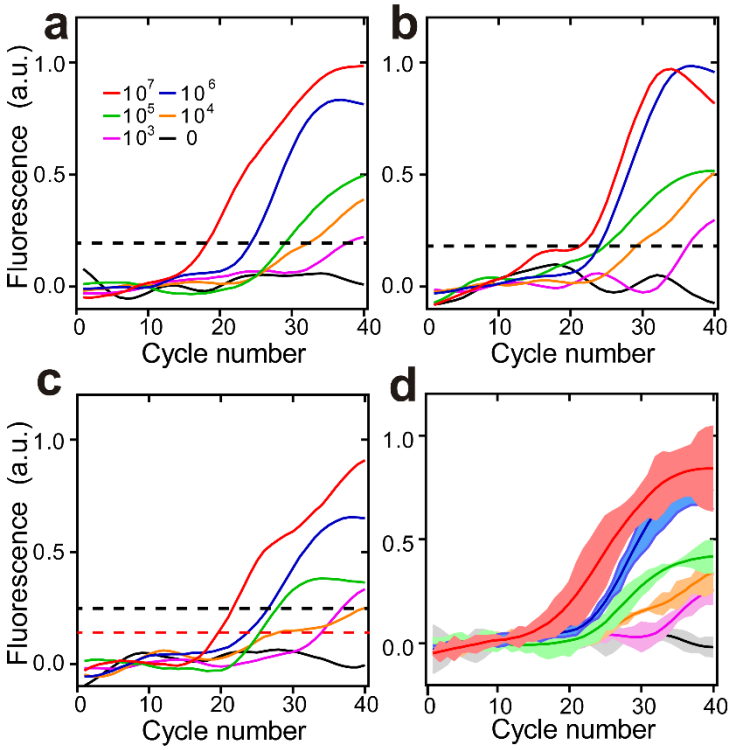

Figure S12. (**a,b,c**) Amplification curves for the SARS-CoV-2 N1 gene performed on different days. Black dotted lines represent threshold calculated by the mean plus 10 times the standard deviation of the baseline. Red dotted line in (**c**) represents a manually defined threshold. (**d**) Plot shows the average of panels a, b, and c, with the standard deviation highlighted