Electronic Supplementary Information for

A Handheld Flow Genetic Analysis System (FGAS): Towards Rapid, Sensitive, Quantitative, Multiplex Molecular Diagnosis at the Point-of-Care Level **

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Content:

1. Materials and reagents

Three bacterial strains used in this study included *Salmonella enterica* CMCC50040 (*Sal*), *Listeria monocytogenes* CMCC54007 (*Lis*), and *Staphylococcus aureus* CMCC26003 (*Sta*), which were obtained from Guangzhou Institute of Microbiology (Guangzhou, China). The genomic DNAs were isolated from these bacterial cultures using the TIAMamp Bacterial Genomic DNA Extraction Kit (Tiangen Biotech Co., Ltd. Beijing, China) according to the manufacturer's instructions. The isolated DNAs were quantified on the Eppendorf Bio-Photometer and were then adjusted to 10^7 copies/μL. The sequences of oligonucleotide primer pairs used in the experiments are

listed in **Table S1**, which were synthesized by Sangon Biotech (Shanghai) Co. Ltd. The three target amplicons included 212-bp fragment of *invA* gene for Sal, 254-bp fragment of *hlyA* gene for Lis, and 111-bp fragment of *nuc* gene for Sta. The PCR chemicals, including 10 × PCR buffer (100 mM Tris-HCl (pH 8.3), 15 mM MgCl2, 500 mM KCl), deoxynucleotide triphosphates (dNTPs, 2.5 mM each of dATP, dGTP, dCTP, and dTTP) mixture, and Taq polymerase, were all purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. Bovine serum albumin (BSA, molecular biology grade) was bought from Thermo Fisher Scientific (China) Co., Ltd.. The carrier fluid in all experiments is light mineral oil (0.84 g/mL) supplemented with 0.1% w/w Triton X-100, and these two reagents were purchased from Sigma-Aldrich Shanghai Trading Co., Ltd..

Table S1 Oligonucleotides used as primers in continuous-flow PCR

Pathogen (VMGs)	**Oligonucleotide Sequence(5' to 3')**
S. enteric (invA)	Sal-F: TGCTCAGACATGCCACAGT
	Sal-R: TGCTCGTAATTCACCACCATTG
L. monocytogenes (hlyA)	Lis-F: CAAGTCCTAAGACGCCAATC
	Lis-R: CAAGTCCTAAGACGCCAATC
S. aureus (nuc)	Sta-F: GGTCCTGAAGCAAGTGCATT
	Sta-R: ATACGCTAAGCCACGTCCAT

Note: VMGs, virulence and marker genes.

2. Self-containing microflow actuator

The microflow actuator mainly comprises a decelerated stepper motor (5 V, 20 Ω, step angle = 0.18^{o}, gear ratio = 1/100), a linear motion guide with a ball feed screw, a 1-mL disposable syringe, and a programmable microcontroller-based driver board (**Figure S1**). With the support of the driver board, the fluidic actuator can implement the operations of infuse,

withdraw, speed adjustment, and saving of parameters with the on-board keyboard. To obtain a standard linear speed, the actuator was calibrated with a precision syringe pump (KDS210, KD Scientific Inc., MA, USA).

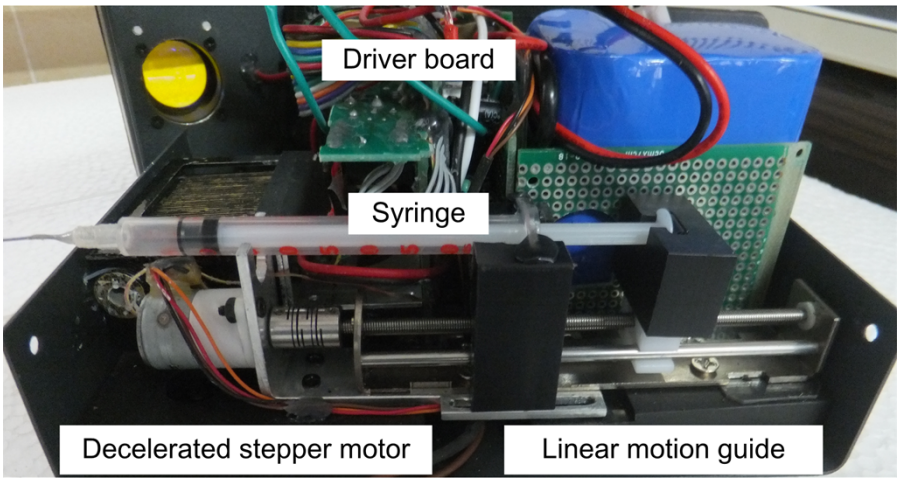

Figure S1. Photograph of the microflow actuator that is self-contained in the FGAS.

3. Electronic system

The electronics in the FGAS is designed for control of temperature, fluid and power. Commercial integrated circuit (IC) chips were assembled to create a cost-effective ($25) modular platform. This platform consists of four functional models (**Figure S2**): a fuzzy PID temperature-control circuit (XMTG-6311, Ningbo LongWay Electronic Co., Ltd., China), a microcontroller-based stepper motor driver board, a constant voltage/constant current circuit, and a step-down circuit. Briefly, the temperature signal from the thermocouple is input to the temperature controller that determines the power input to the heater through the fuzzy PID control algorithm. And, temperature setting and real-time displaying can be performed in a

user-friendly way via the on-board keys and the LED segment displays, without need of any additional external terminals (e.g., computer). The voltage/current circuit is used to drive the fluorescence excitation source—the high-intensity blue LED, thus achieving a stable illumination. A chargeable lithium battery (12 V, 4400 mAh) is used as the power supply of the whole system, which can last 3.5 h for typical applications. Its output voltage is converted into 5.0 V via step-down circuit, to supply the stepper motor driver board and the cooling fan. The applied voltage of the fan is simply regulated by a potentiometer with the LED segment displays, and the voltage displaying can be further used as the reference of the cooling side's temperature.

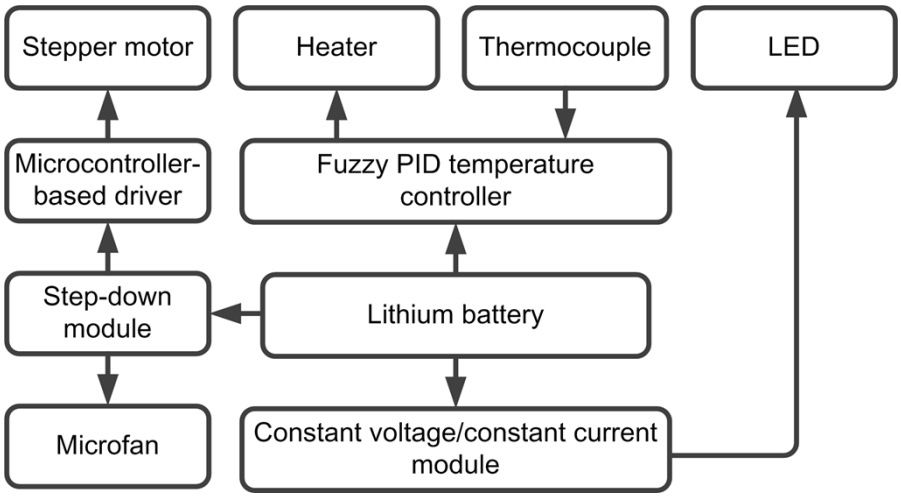

Figure S2. Block diagram of the electronics for the FGAS. The electronics is self-contained for standalone operation.