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

Supporting Information

Parallel Multistep Digital Analysis SlipChip Demonstrated with the Quantification of Nucleic Acid by Digital LAMP-CRISPR

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MATERIALS

All solvents and salts obtained from commercially available sources were used as received unless otherwise stated. Soda-lime glass plates coated with chromium and photoresist were purchased from Telic Company (Valencia, CA) and MicroCAD (Shenzhen, China). Sodium hydroxide, ammonium ceric nitrate, perchloric acid, nitric acid, ammonium fluoride, chloroform, acetone, ethanol, hydrogen peroxide and sulfuric acid were ordered from SinoPharm Chemical Reagent Co., Ltd. (Beijing, China). SARS-CoV-2 pseudovirus was purchased from Yeasen Biotechnology Co., Ltd. (Shanghai, China). The RevertAid First Strand cDNA Synthesis Kit was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Bst 2.0 WarmStart™ DNA polymerase and dNTPs were ordered from New England Biolabs (Ipswich, MA, USA). Bovine serum albumin (BSA) was purchased from Sangon Biotech (Shanghai, China). All these primers were synthesized by Sangon Biotech (Shanghai, China). Cas12a Nuclease and 10 × TOLO Buffer3 were purchased from TOLOBIO (Shanghai, China). crRNA was synthesized by GenScript (Nanjin, China). A fluorescent probe was synthesized by GENEWIZ (Suzhou, China). Hydrofluoric acid, dichlorodimethylsilane, PMX-200 and tetradecane were obtained from Aladdin Co., Ltd. (Shanghai, China). Tween-20 was purchased from Sigma-Aldrich (Sternheim, Germany).

DESIGN AND FABRICATION OF THE PAMDA-SLIPCHIP DEVICE

The PAMDA-SlipChip device was designed using AutoCAD (Autodesk, San Rafael, CA, USA) and corresponding photo-mask was ordered from MicroCAD (Shenzhen, China). The PAMDA-SlipChip is 50 mm×55 mm, containing 2400 microwells in it. The diameter of pearl microwell is 300 μm and the depth is 50 μm. The width of "chain" is 20 μm and the depth is 20 μm. Here we use extension microwells rather than extension channel to provide additional space for droplet formation. The diameter of extension microwell is 260 μm and the depth is 100 μm. The volume of microwell was simulated using SolidWorks software (Dassault Systèmes, MA, USA). Each "chain of pearl" section could hold 3.2 nL liquid. The volume for each extension microwell is 6.2 nL. The fabrication process has been described previously.[1]

NUCLEIC ACID PREPARATION

The RNA template was extracted from a 200 μL SARS-CoV-2 pseudovirus solution using a quick-RNA viral kit purchased from Zymo Research (Irvine, CA, USA) and was eluted in 20 μL water. A reverse-transcription mix was used to convert the RNA template into cDNA. The 20 μL reverse-transcription mix consisted of 4 μL of 5× reaction buffer, 2 μL of 10 mM dNTPs, 200 U of RevertAid M-MuLV RT, 1 μL of 20 μM primer, 10 μL of RNA template and 2 μL of nuclease-free water. The reaction mixture was incubated at 42°C for 60 min followed by heating at 70°C for 5 min on a thermal cycler. All RNA templates and cDNA were stored at -20 °C.

PAMDA REACTION ON THE CHIP

A LAMP reaction mix was delivered into PAMDA SlipChip in the first step. A 25 μL LAMP

reaction master mix consisted of 2.5 μL of 10× Isothermal Amplification Buffer, 6 mM MgSO4, 8 U of Bst 2.0 WarmStart® DNA Polymerase, 1.4 mM each of the dNTPs, 1 mg/mL BSA, 1% Tween-20, 5 μL cDNA template, 1.6 μM forward inner primer, 1.6 μM backward inner primer, 0.8 μM forward loop primer, 0.8 μM backward loop primer, 0.2 μM forward outer primer, and 0.2 μM backward outer primer. The FIP, BIP, 0.7 μL 10× Isothermal Amplification Buffer and 5 μL template were preliminarily mixed and incubated at 95 ℃ for 8 min and cooled to 30 ℃ for 3 min before being added to the LAMP reaction master mix. The master mix was injected into the PAMDA SlipChip to form droplets and incubated at 65°C for 30 min.

A CRISPR-Cas12a reaction mix was delivered into the PAMDA SlipChip in the second step. The 20 μL CRISPR reaction mix consisted of 4 μL of 10 × TOLO Buffer3, 1% Tween-20, 1 μM fluorescent probe, 500 nM crRNA and 1 μM Cas12a nuclease. The CRISPR-Cas12a reaction mix was injected into the PAMDA SlipChip and fused to relative LAMP droplets. The PAMDA SlipChip was incubated at 40°C for 30 min. The results were acquired using Nikon Ti2 fluorescence microscope and analyzed using NIS-ElementsS-7 ver. 5.01.

TABLE S1 SEQUENCE FOR PAMDA TEST

Name	Sequence (5'-3')
SARS-CoV-2 LAMP FIP	CCTACTGCTGCCTGGAGTTGAAGCCTCTTCTCGTTCCTCATC
SARS-CoV-2 LAMP BIP	GGCAATGGCGGTGATGCTCCAGACATTTTGCTCTCAA
SARS-CoV-2 LAMP LF	TTTCTTGAACTGTTGCGACTACGT
SARS-CoV-2 LAMP LB	CTCTTGCTTTGCTGCTG
SARS-CoV-2 LAMP F3	GGCTTCTACGCAGAAGGGA
SARS-CoV-2 LAMP B3	CTTAGTGACAGTTTGGCC
SARS-CoV-2 CRISPR crRNA	AAUUUCUACUCUUGUAGAUCUGCUGCUUGACAGAUUGAACCAG
SARS-CoV-2 CRISPR probe	FAM-NNNNNNNNNNN-Eclipse

REFERENCE

1 Z. Yu, W. Lyu, M. Yu, Q. Wang, H. Qu, R. F. Ismagilov, X. Han, D. Lai and F. Shen, *Biosens. Bioelectron.*, 2020, **155**, 112107.