Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting information

Single Bacteria Detection by Droplet DNAzyme-Coupled Rolling Circle Amplification

Yanchen Chi,[‡,a] Meng Shi,[‡,a] Yanfang Wu,[b] Yunping Wu,[a] Yangyang Chang,[a] and Meng Liu[*,a]

a. School of Environmental Science and Technology, Key Laboratory of Industrial Ecology and Environmental Engineering (Ministry of Education), Dalian University of Technology, Dalian POCT Laboratory, Dalian, 116024 (China).

b. School of Chemistry and Australian Centre for NanoMedicine, The University of New South Wales, Sydney, NSW, 2052 (Australia).

‡These authors made equal contributions.

Corresponding author: mliu@dlut.edu.cn

Oligonucleotides and other materials. All DNA oligonucleotides (Table S1) were obtained from Sangon Biotech (Shanghai, China) and purified by standard 10% denaturing (8 M urea) polyacrylamide gel electrophoresis (dPAGE) or high-performance liquid chromatography (HPLC). T4 polynucleotide kinase (PNK) was purchased from Takara Biotechnology Co. (Dalian, China). T4 DNA ligase, dNTP, and thioflavin T (ThT) were purchased from Sangon Biotech (Shanghai, China). Phi29 DNA polymerase was obtained from Thermo Scientific (Waltham, MA, USA). All other chemicals were purchased from Sangon Biotech (Shanghai, China) and used without further purification.

Instrumentation. The fluorescence images of gels were obtained using a Typhoon 9200 variable mode imager (GE Healthcare, Chicago, IL, USA) and analyzed using Image Quant software (Molecular Dynamics, Chicago, IL, USA). Quantitative detection of the bacterial cells was carried out on the Naica digital PCR system (Stilla Technologies, Villejuif, France) with Sapphire chips (Stilla Technologies, Villejuif, France). Fluorescence measurements were performed with a Cary Eclipse fluorescence spectrophotometer (Agilent, Malaysia).

Cell culture and cell lysate preparation. *E. coli* BL21 (B541012) was plated onto a Luria Broth (LB) agar plate and grown for 18 h at 37 °C. A single colony was taken and inoculated into 5 mL of Super Optimal Broth (SOB) and grown with shaking at 37 °C until OD_{600} reached ~ 1, the bacterial culture was serially diluted in 10-fold intervals. 10 μL of each diluted solution were plated on the LB agar plates (3 repeats) and cultured at 37°C for 18 h to determine the number of colony forming units per milliliter in the original bacteria cultures. 1 mL of these cultures were centrifuged at 11,000 g for 5 min at 4°C, the supernatant was removed. The cell pellet was suspended in 200 μL of 1× reaction buffer (1× RB; 50 mM HEPES, 150 mM NaCl, 15 mM $MgCl_2$, pH 7.5) and heated at 50 °C for 15 min. The heat-treated cell suspension was then centrifuged at 11,000 g for 5 min at 4 °C. The supernatant was taken as the cell lysate for the following experiment.

Preparation of circular DNA template (CDT). A total of 200 pmol of linear DNA templates (LDT) was first mixed with 10 U (U: unit) T4 polynucleotide kinase (PNK) and 2 mM ATP in 50 μL of 10× PNK buffer A (50 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine). The mixture was incubated at 37 °C for 40 min. Subsequently, 220 pmol of template primer (TP) and 10 μL of 10× T4 DNA ligase buffer (400 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM DTT, 5 mM ATP, pH 7.8 at 25°C) were added and heated at 90 °C for 3 min. After cooling to room temperature (RT) the mixture was left for 15 min, after which 10 U T4 DNA ligase was added. The resulting mixture (total volume of 100 μL) was incubated at RT

for 2 h before heating at 90 °C for 5 min to deactivate the ligase. The obtained CDT was concentrated by standard ethanol precipitation and purified by 10% denaturing (7 M urea) gel electrophoresis (dPAGE).

DNAzyme-coupled RCA reaction in solution. A total of 10 μL of 100 mM thioflavin T (ThT), 5 μL of 10× RB, 5 μL of cell lysate, 2.5 μL of 100 μM RFD-EC1, 1 μL of 50 μM RDS, 5 μL of 10× RCA reaction buffer (330 mM Tris acetate, 100 mM magnesium acetate, 660 mM potassium acetate, 1% (v/v) Tween-20, 10 mM DTT, pH 7.9), 5 U phi29 DNA polymerase (phi29DP), 5 U PNK, 5 μL of 1 μM CDT and 5 μL of dNTPs (10 mM) were introduced to above mixture (total volume: 50 μL). The reaction mixture was incubated at 30 °C for 1 h. The resultant RCA products were analyzed by 0.6% agarose gel electrophoresis. Fluorescence measurements were performed with a Cary Eclipse fluorescence spectrophotometer with an excitation wavelength (λ_{ex}) of 420 nm.

Digestion of RCA products. LDT2 was encoded with an EcoRV recognition site (GATATC) and its sequence was shown in Table S1. 200 pmol of LDT2 was first used to prepare CDT2 for performing the RCA reaction. Then, 50 μL of the resulting solution were mixed with 10 μL of digestion template (DT, 100 μM), 10 μL of 10× EcoRV buffer and 25 μL of water, and heated at 90 °C for 5 min. After cooling to RT for 15 min, 5 μL of EcoRV (15 U/μL) was added, and the mixture was incubated at 37°C for 3 h. The digested products were analyzed by 0.6% agarose gel electrophoresis.

Droplet DNAzyme-coupled RCA (dDRCA) reaction. 25 μL of the above mixture was pipetted into each hole of the Sapphire chips and then loaded into the Naica Geode thermocycler. The temperature was set at 40 °C (Sapphire chips) and the pressure increased from atmospheric pressure (AP) to AP + 1000 mbar (followed by a drop to AP + 950 mbar at the end) in order to enable droplet generation (12 min). Following 43 min incubation at 30 °C, the Sapphire chips were removed from the Naica Geode thermocycler and imaged using the Naica Prism3 reader.

Statistical analysis. The Crystal Miner software was used for data analysis. The image processing algorithms including a neural network automatically excludes artefacts and non-homogenous droplets in crystal images with high reliability. The counting result is derived from the Poisson distribution. By default, the fluorescence threshold values are automatically estimated by the software, which can best discriminate the positive and negative droplet populations in each channel. More precisely, the automated threshold maximizes the inter-class variance and minimizes the intra-class variance by considering the fluorescence points

aggregated from all chambers. Therefore, the droplets are deemed as “positive” if the fluorescence of the droplet is higher than the defined threshold, and “negative” if it is lower. And the counting results are calculated and analyzed by the Crystal Miner software.

Characterization of droplet generation. 1 mL of original bacteria culture was centrifuged at 11,000 g for 5 min at 4°C. After removing the supernatant, the cell pellet was suspended in 1 mL of 1× phosphate-buffered saline (PBS) buffer (containing 137 mM NaCl, 3 mM KCl, and 1 mM $MgCl_2$, pH 7.4). And 5μL of DAPI dye was added into the above mixture (100μL) and then incubated at room temperature for 20 min. 25 μL of mixture was pipetted into the Sapphire chips and then loaded into the Naica Geode thermocycler. After the partition and release program (45 min), the chip was observed under a 10× objective lens by confocal laser scanning microscopy (CLSM) and fluorescence microscopy.

***E. coli* detection from clinical urine samples by dDRCA.** 1 mL urine sample was first centrifuged at 11,000 g for 5 min. The cell pellet was suspended in 200 μL of 1× RB and heated at 50 °C for 15 min. The heat-treated cell suspension was then centrifuged at 11,000 g for 5 min at 4 °C. The supernatant was taken as the cell lysate for the following experiment as described above.

Table S1. Sequences of DNA oligonucleotides used in this work.

Name of DNA oligonucleotide	**Sequence(5'-3')**
RFD-EC1	GATGTGCGTTGTCGAGACCTGCGACCGGAACACTACACTG
RNA-containing DNA sequence (RDS)	ACTCTTCCTAGCTRTGGTTCGATCAAGA (R = adenosine ribonucleotide.)
linear DNA template (LDT1)	TAGCTAGGAAGAGTCCCAACCCGCCCTACCCAAAATGTCTCGGAT
template primer (TP)	TTCCTAGCTAATCCGAGACA
linear DNA template (LDT2)	TAGCTAGGAAGAGTCCCAACGATATCCCGCCCTACCCAAAATGTCTCGGAT
digestion template (DT)	GTAGGGCGG GATATCGTTGGGACT

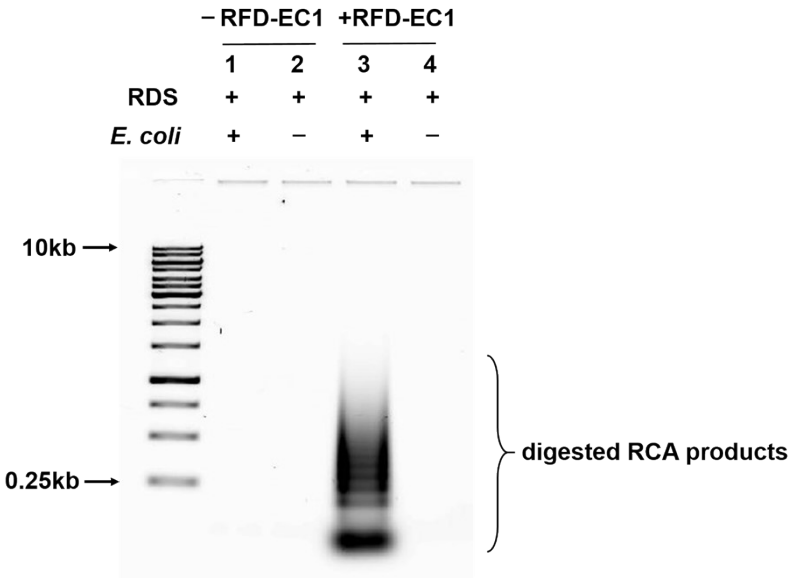

Figure. S1. Analysis of digested RCA products by 0.6% agarose gel electrophoresis.

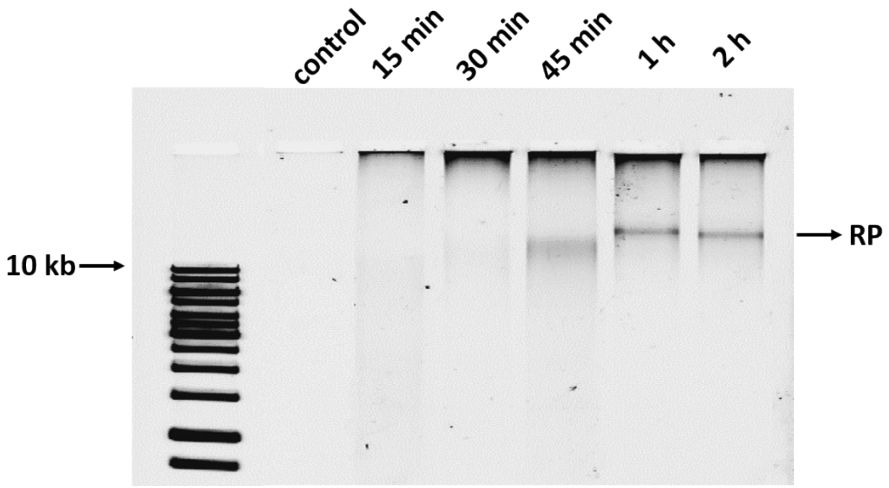

Figure. S2. Time-dependent DNAzyme-coupled RCA reaction in solution. Control lane: with RDS and RFD-EC1 but without *E. coil*.

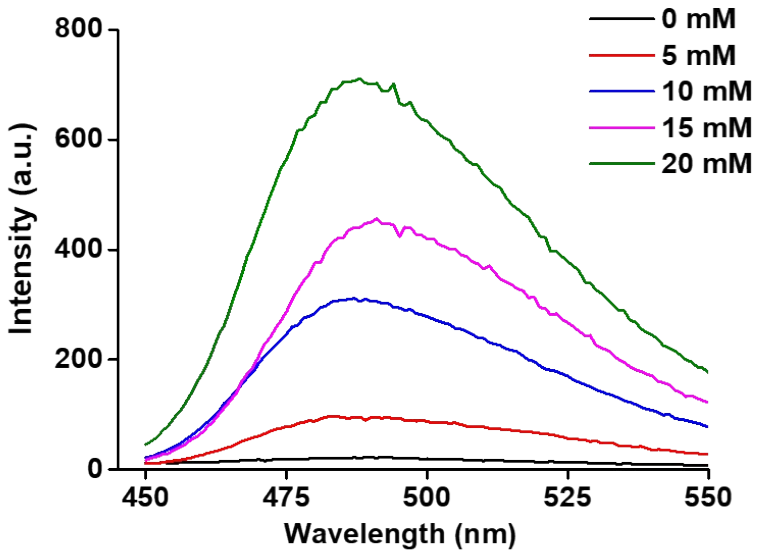

Figure. S3. ThT concentration-dependent fluorescence signaling of RP in solution.

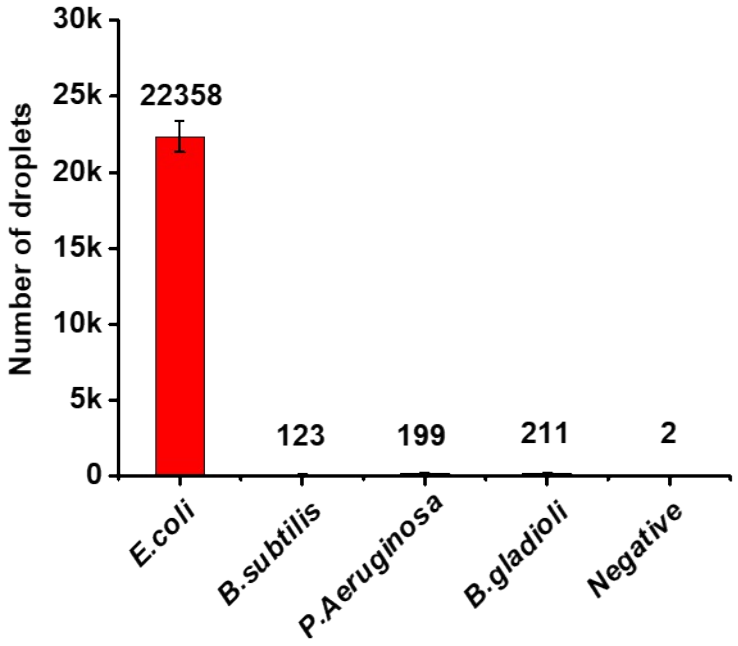

Figure. S4. Specificity of dDRCA in urine samples. The error bars represent standard deviations of three independent experiments.