

Supporting information

Supplemented experimental section

1. Bacterial culture and DNA genome extraction

Each of the bacteria strains was streaked onto LB plates from freezer stocks held at –80 °C and grown at 37 °C for 16 h to form single colonies. Single colonies were picked and grown in tubes containing 2 mL of liquid LB medium and were shaken for 16 h at 37 °C (180 rpm). Kit-based method and heat-treated method were employed to obtain bacterial genomic DNA. As to the kit-based method, genomic DNA was extracted following the instruction of bacterial DNA extraction Kit (No. DP302, Tiangen, China). Bacterial cells were harvested by centrifugation (13400g, 1 min) of 1 mL of cell suspension. The purified DNA was eluted twice with 50 µL deionized water. The heat-treated method was employed for crude DNA extractions from clinical samples. All swabs were first blistered into respective sterilized EP tube and gently shaken. The instrument used for heating was a metal bath, the instrument was preheated to 100 °C, then 300 µL of nucleic-free water that partially dissolved the swab was immediately inserted into metal bath. After 8 min, the lysate containing crude genomic DNA was collected by centrifugation at 11,000g for 1 min and supernatant was used as a template for following experiments.

2. Preparation of the dCas9/sgRNA@MBs

To covalently attach an oligonucleotide (linker sequence) to the dCas9 protein, 10 µL of the linker sequence (10 µM) with a 5' O₆-benzylguanine (BG) group was incubated with the 2 µL of dCas9 protein (1 mg) at 37 °C for 60 min. The dCas9/linker complex were added to 20 µL solution containing streptavidin-coated magnetic beads (MBs) (0.4 mg). After 30 min incubation at room temperature, the solution was treated by the magnet for 10 min to enrich the dCas9 @MBs. The precipitate was washed by the PBS buffer solution for 3 times to remove the free dCas9/linker complex. We then assembled 2 µL of sgRNA (10 µM) and dCas9@MBs in a 1× NEBuffer 3.1 Reaction Buffer (100 mM NaCl, 50 mM Tris-HCl, 10 mM MgCl₂, 100 µg/mL of BSA, pH 7.9 at 25 °C) for 30 min to construct the dCas9/sgRNA@MBs.

3. Assembly of the sensing probe (SP)

20 µL of the synthesized SP probe (10 µM) in linear state was heated to 90 °C for 10 min. The mixture was then cooled to room temperature (1 °C /min) to assemble the SP. The obtained SP solution was then diluted to 500 nM before applying in the proposed method for *P. aeruginosa* detection.

Table S1. Oligonucleotide sequences used in this work.

Name	Sequences (5' to 3')
sgRNA	UAA UUU CUA CUA AGU GUA GAU AGC GUA GGA CAG CUU GCC AU
SP	AACT ATA CAA CCT ACT ACC TCA ATA TTA AAT GGC AAG CTG TCC TAC GCT TTT TTT TTT AGC GTA GGA CAG CTT GCC ATT

FP	TAA CG ACC TTC CAC CGA GCT AGA TCC CTG GAC GAC TTG AAA AAC TAT ACA ACC TAC TAC CTC A TTT CAA GTC GTC CAG TTG AAA
“1*”	TGA GGT AGT AGG TTG TAT AGT T

Table S2. Bacterial strains used in this study.

Bacteria species	Strain
<i>Listeria monocytogenes</i> (<i>L. monocytogenes</i>)	ATCC 19606
<i>Klebsiella pneumonia</i> (<i>K. pneumoniae</i>)	clinical strain identified by our lab
<i>Escherichia coli</i> (<i>E. coli</i>)	ATCC 19433
<i>Staphylococcus aureus</i> (<i>S. aureus</i>)	ATCC 25923
<i>Pseudomonas aeruginosa</i>	PAO1

Table S3. Comparison of the method with traditional methods.

Title	Mechanism	Specificity	Cost	Thermal cycles	Equipment	Primer design
The method	dCas9/sgRNA+ self-priming mediated chain extension	High	Low	No	No	Simply change the sgRNA
RT-PCR	Chain extension	False positive results from non-specific hybridization and primer binding	High	Yes	PCR instrument	Complicated
Colony culture and count	Dilute the bacteria to a single colony	Low (morphological observation)	Low	No	No	No primer design

RT-PCR, Reverse Transcription-Polymerase Chain Reaction.

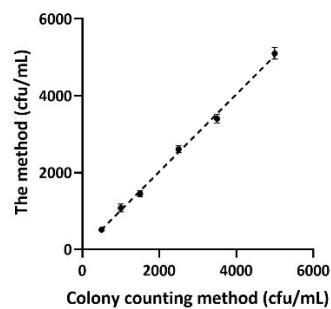


Figure S1. The correlation between the calculated *P. aeruginosa* concentrations by the method and the standard colony counting method.