# Supplementary materials

## Materials and method

### Preparation of bacterial strains and DNA

*Vibrio parahaemolyticus*, *Vibrio splendidus*, *Vibrio natriegens*, *Vibrio mimicus*, *Vibrio harveyi*, *Vibrio shilonii*, and *Vibrio chagasii* were kindly provided by Lianyungang Institute of Food and Drug Control (Lianyungang, China). *Campylobacter pylori*, *Bacillus cereus*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Listeria monocytogene* were kindly provided by the Wuhan Institute for Food and Cosmetic Control (Wuhan, China). Bacterial genomic DNA was extracted using the MolPure Bacterial DNA Kit (Yeasen Biotechnology (Shanghai) Co., Ltd., Shanghai, China), in accordance with the manufacturer's instructions. DNA concentrations were measured using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc, USA) and then stored at -20ºC for later use.

### Primer and sgRNA

The LAMP primer sets (Supplementary Materials Table S1) were designed on the NEB LAMP Primer Design Tool v1.3.0 (https://lamp.neb.com/) using the *V. parahaemolyticus TL* virulence gene (GenBank accession No. M36437.1) as the template, and synthesized by General Biol (Anhui) Co., Ltd. (Chuzhou, China).

After confirming the best LAMP primer sets, we selected 20 nt spacer sequence following each possible protospacer adjacent motif (PAM) in the LAMP F2−B2 region as the sgRNA candidates. A 111 bp DNA fragment containing the conserved AacCas12b sgRNA scaffold was synthesized and ligated into a pBAD vector by LightNing™ DNA Assembly Mix Plus (BestEnzymes Biotech Co., Ltd., Lianyungang, China). The DNA template for in vitro transcription of sgRNA was amplificated by overlapping PCR of two primers, one contained the T7 promoter sequence and the other contained the corresponding spacer sequence. Besides, primer and spacer sequence cross-interference with other pathogens should be avoided with NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The purified PCR product was transcribed with T7 RNA polymerase (BestEnzymes Biotech) at 37°C for 16 h following the manufacturer's instruction for short RNA transcripts. The in vitro transcription product was treated with DNase I (BestEnzymes Biotech) for 15 min at 37°C, and then purified using Tiangen RNA Cleanup Kit (Tiangen Biotech (Beijing) Co., Ltd., Beijing, China). The sgRNA sequences are presented in Supplementary Materials Table S2.

### Standard LAMP system

LAMP analysis was performed in a 25 μL reaction mixture containing 8U Bst 2.0 DNA polymerase (New England Biolabs, Ipswich, USA), 1× isothermal amplification buffer, 6 mM magnesium sulfate, 0.2 μM F3/B3,1.6 μM forward and backward internal primers (FIP/BIP), 1.4

mM dNTP (0.35 mM for each; therein, dTTP was replaced with a mixture of dTTP and dUTP at a certain proportion), and 1 ng *V. parahaemolyticus* genomic DNA.

**CRISPR/Cas12b system**

The target gene was determined with CRISPR/Cas12b system reactions using AapCas12b (BestEnzymes Biotech). The reaction mixture was 20 μL, including 1x Cas reaction buffer, 0.6 μM Cas12b, 20 U RNase inhibitor, 500 nM FQ ssDNA reporter (FAM-TTATT-Quencher, Takara Biotechnology), 1 ng genome DNA, and 250 nM sgRNA. The reaction was performed in a CFX96 fluorescent quantitative PCR system (Bio-Rad Laboratories Inc., Hercules, CA) at 60°C for 45 min. Fluorescence signals were collected every 30 seconds until the end.

**LAMP-CRISPR/Cas12b system**

The LAMP amplification and CRISPR/cas12b reaction were combined in a single tube for the concatenation of systems. The reaction system was as follows: 1x Cas reaction buffer, 6 mM MgSO4, 1.4 mM dNTP (0.35 mM for each; therein, dTTP was replaced with a mixture of dTTP and dUTP in a certain proportion), 0.2 μM F3/B3, 1.6 μM FIP/BIP, 8 U Bst 2.0 DNA polymerase, 250 nM sgRNA, 500 nM FQ ssDNA reporter, 0.6 μM Cas12b, and 1 ng genomic DNA. The reaction tubes were reacted in a CFX96 qPCR System at 60°C for 30 min. Fluorescence signals were collected every 30 seconds until the end. The results also could be monitored with the naked eye under blue light after 30 minutes of reaction.

**LAMP-CRISPR/Cas12b combined with HL-UDG in one tube**

The UDG-LAMP-CRISPR/Cas12b system was performed in a 50 μL reaction mixture, including 5 μL 10x Cas reaction, 4 μL dNTP mixture (2.5 mM for each; therein, dTTP was replaced with a mixture of dTTP and dUTP at a certain proportion), 1 U HL-uracil DNA glycosylase (BestEnzymes Biotech), 2 μL DNA template, 6 mM MgSO4, 8 U Bst 2.0 DNA polymerase, 5 μL 10x LAMP primer pool, 1 μL Cas12b, 250 nM sgRNA, 500 nM fluorescent probe, and 5 μL taurine. The reaction tubes were incubated at 25°C for 5 min to remove carry-over contamination. The reaction tubes were then incubated in a qPCR system at 65°C for 30 min, followed by 2 min at 80ºC to terminate the reaction. Fluorescence signals were collected every 30 seconds until the end. The results also could be monitored with the naked eye under blue light after 30 minutes of reaction.

**Evaluation of the limit of detection of UDG-LAMP-CRISPR/Cas12b system**

The limit of detection (LOD) of UDG-LAMP-CRISPR/Cas12b was assayed through a gradient dilution of the *V. parahaemolyticus* suspension. The concentration of the *V. parahaemolyticus* culture solution was determined following the Chinese National Standard GB4789.7-2013 and diluted in a gradient of $1\times10^6$, $1\times10^5$, $1\times10^4$, $1\times10^3$, $1\times10^2$, and $1\times10^1$ CFU/mL with sterile 0.85% (*w*/*w*) NaCl. Bacterial DNA was extracted from 1 mL of each culture solution with the MolPure Bacterial DNA Kit (Yeasen Biotech) and eluted in 50 μL. To evaluate the LOD of UDG-LAMP-CRISPR/Cas12b in an artificially contaminated sample, commercial fresh shrimps were purchased from a local seafood market (Lianyungang, China). A sample of 10 g of shrimp was homogenized

with 90 mL of sterile 0.85% (w/w) NaCl. One milliliter of different concentrations of *V. parahaemolyticus* ($1\times10^6$ to $1\times10^2$ CFU/mL) was mixed with 9 mL of sample homogenate to prepare artificially contaminated samples (no incubation). At each gradient, 1 mL aliquots were collected, and DNA was extracted as described earlier. The 2 μL of extracted DNA from pure cultures or artificially contaminated samples was used as the template for the UDG-LAMP-CRISPR/Cas12b assay, and the experiment was repeated three times.

**Determination of the specificity of the UDG-LAMP-CRISPR/Cas12b system**

For the specificity assay, six common strains of other *Vibrio* species and six different pathogenic bacteria of seafood were included to determine the specificity of the UDG-LAMP-CRISPR/Cas12b method. Genomic DNA was extracted from these strains as a template. The remaining steps were similar to the process described earlier.

**Comparison of UDG-LAMP-CRISPR/Cas12b with qPCR assay on real samples**

To test real samples, 20 fresh samples of aquatic foods (10 samples of shrimp and 10 samples of fish) were obtained from the local market (Lianyungang, China). Ten grams of each sample was homogenized with 90 mL of sterile 0.85% (w/w) NaCl and incubated at 37°C for 2 h. Total DNA was extracted from 1 mL of homogenate using the MolPure Marine Animals DNA Kit (Yeasen Biotech) and eluted in 50 μL. As a template for UDG-LAMP-CRISPR/Cas12b or qPCR assays (Liu et al., 2012), 2 μL of purified DNA was used.

**Data analyses**

The results are expressed as mean ± standard deviation. All statistical analyses and graphs were performed using GraphPad Prism 9.3. A $p$-value of $<0.05$ was considered statistically significant.

**Table S1 Primer sequences used throughout the text**

| Oligo names | Sequences (5'-3') |
|---|---|
| TL-LAMP-P1 | F3: CGCTGACAATCGCTTCTCAT<br>B3: GTTCTTCGCTTTGGCAATGT<br>FIP: CTGTCACCGAGTGCAACCACTT AACCACACGATCTGGAGCA<br>BIP: GCATCACAATGGCGCTTCCC ACCGTTGGAGAAGTGACCTA |
| TL-LAMP-P2 | F3: GCCGAAGAGCCAACCTTA<br>B3: AACGTTTTGCGACGTGTT<br>FIP: TGCGATACCAACAGCGAACATA GTTTCAGCGTCTGAAGTGAT<br>BIP: GACCGATTGGGAATGGGCAA ATGAAACGGAGCTCCACC |
| TL-LAMP-P3 | F3: GCGCAAGGTTACAACATCAC<br>B3: CGGCGAAGAACGTAATGTCT<br>FIP: CCACCAGTAGCCGTCAATGGTG AAGATGATCCAGCGACCGAT<br>BIP: ACACCAACACGTCGCAAAACG GCGTTCTCGTTCGCCAAAT |
| TL-F | TCGACTGTCTGGAGTATTTACTCAG |
| TL-R | GCTTCTTTGTTGGAGAACCGCTACA |
| 111-dsDNA-F | TAATACGACTCACTATAGGGGTCTA |
| 111-dsDNA-R | TCTCAAATCTGAGAAGTGGCAC |

**Table S2 sgRNA sequences**

| Names | Sequences (5'-3') |
|---|---|
| sgRNA1 | GGGGUCUAGAGGACAGAAUUUUUCAACGGGUGUGCCAAUGGCCACUUUCC<br>AGGUGGCAAAGCCCGUUGAGCUUCUCAAAUCUGAGAAGUGGCAC<br>*GAGACGCUAACUUCUGCGCC* |
| sgRNA2 | GGGGUCUAGAGGACAGAAUUUUUCAACGGGUGUGCCAAUGGCCACUUUCC<br>AGGUGGCAAAGCCCGUUGAGCUUCUCAAAUCUGAGAAGUGGCAC<br>*UGCGCCCGAAGAGCACGGUU* |
| sgRNA3 | GGGGUCUAGAGGACAGAAUUUUUCAACGGGUGUGCCAAUGGCCACUUUCC<br>AGGUGGCAAAGCCCGUUGAGCUUCUCAAAUCUGAGAAGUGGCAC<br>*GUGAACGCGAGCGAUCCUUG* |
| sgRNA4 | GGGGUCUAGAGGACAGAAUUUUUCAACGGGUGUGCCAAUGGCCACUUUCC<br>AGGUGGCAAAGCCCGUUGAGCUUCUCAAAUCUGAGAAGUGGCAC<br>*UUUGGACAUCAACCGCUCAU* |
| sgRNA5 | GGGGUCUAGAGGACAGAAUUUUUCAACGGGUGUGCCAAUGGCCACUUUCC<br>AGGUGGCAAAGCCCGUUGAGCUUCUCAAAUCUGAGAAGUGGCAC<br>*CAUGUACACCCACGCAUUGC* |
| sgRNA6 | GGGGUCUAGAGGACAGAAUUUUUCAACGGGUGUGCCAAUGGCCACUUUCC<br>AGGUGGCAAAGCCCGUUGAGCUUCUCAAAUCUGAGAAGUGGCAC<br>*CGCUCUGAGUGUGCAGCGUC* |

The italic letters present the spacer sequences of sgRNAs.

(a)


(b)

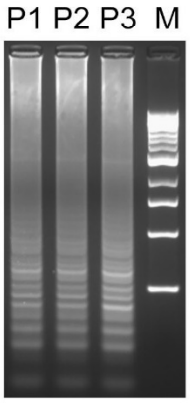


**Fig. S1 LAMP primers screening. (a) The position of three primer combinations on gene. (b) LAMP amplification results with different primer combinations.**