Quantification of viable bacteria in fermented foods using an enzymatic cascade-based pre-amplification-free RNA assay

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Experimental Procedures

Materials and reagents

All DNA oligonucleotide probes with sequences shown in Table S3 were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). The RNA sequences were synthesized by Takara Bio Inc (Dalian, China) and purified by HPLC. The LbuCas13a and LbCas12a proteins were expressed by our laboratory using the pGJK-His-SUMO-LbuCas13a and pMBP-LbCas12a vectors (Figure S5). T7 RNA Polymerase (20 U/μL) (cat. no. EP0111), phi29 DNA Polymerase (10 U/μL) (cat. no. EP0094), DNase I (1000 U/mL) (cat. no. EN0521), dNTP solution mix (cat. no. R0192), and rNTP solution mix (cat. no. R0481) were purchased from Thermo Fisher Scientific (Waltham, USA). LB broth medium (A507002), and LB Broth Agar (A507003) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). MRS Broth medium (027319) was purchased from Guangdong Huankai Microbial Sci.&Tech.Co., Ltd (Guangdong, China). NaCl (S9888), and Glycerol (G7893) were purchased from Sigma-Aldrich Trading Co., Ltd (Shanghai, China). All fermented dairy products were purchased from local supermarkets. M5 HiPer Universal RNA Mini Kit (MF036) was purchased from Mei5 Biotechnology Co., Ltd. Bacterial genomic DNA Extraction kit was purchased from Tiangen Biotech Co., Ltd (Beijing, China).

Bacterial culture

The *L. plantarum* (ATCC 202195), *P. pentosaceus* (ATCC 25745), *C. farciminis* (ATCC 29644), and *B. licheniformis* (ATCC 14580) were provided by the Guangdong Microbial Culture Collection Center (GDMCC) and propagated in MRS Broth medium without antibiotics. Their concentrations were determined by the traditional plate counting method. The bacteria were purified by centrifugation at 4000 rpm for 10 min and then dissolved by sterile NaCl solution (0.85% (w/v)) to specified concentrations.

Preparation of viable and Dead Bacteria

To prepare viable and dead bacteria for analysis, freshly cultured bacteria were first harvested by centrifugation at 4,000 rpm for 10 min and resuspended in sterile 0.85% NaCl solution. The resulting suspension was divided into two equal aliquots with one group maintaining activity in physiological saline, while the other was treated with 100% isopropanol to reach a final

concentration of 70% to induce bacterial inactivation. Both groups were incubated at room temperature for 2 h with periodic mixing every 15 min followed by another centrifugation at 4,000 rpm for 10 min. Finally, the cells were washed three times with sterile physiological saline to ensure the complete removal of residual isopropanol. Before mixing at different ratios for the bacterial viability proportion assay, both the viable and dead bacterial suspensions were individually adjusted and standardized to a cell density of 5×10^6 CFU/mL using sterile 0.85% NaCl solution, thereby ensuring that the final total LAB concentration of the mixture in each group remained constant at 5×10^6 CFU/mL. Subsequently, the live and dead bacteria, mixed in the desired proportions, were prepared for use in subsequent sensing experiments.

Extraction of bacterial RNA

For the extraction of bacterial RNA, bacteria were cultured in MRS broth at 35°C for 12 h. A 4 mL aliquot of the culture was centrifuged at 4,000 rpm for 10 min at 25°C to collect the cell pellet. The cells were resuspended in 200 μL of lysozyme buffer (20 mg/mL lysozyme, 20 mM Tris, 2 mM Na_2-EDTA, and 1.2% Triton) and incubated at 37°C for 30 min to facilitate cell wall digestion. Total RNA was then isolated using the M5 HiPer Universal RNA Mini Kit (Polymag, China) following the manufacturer's protocol. For fermented dairy products, the matrix was pre-diluted to 10^2–10^4 CFU/μL, and 10 μL of proteinase K (20 mg/mL) was added during the lysis step to remove protein interferences. The concentration and purity of the extracted RNA were quantified by measuring the absorbance at 260 nm using a Synergy H1 microplate reader. All RNA samples were stored at -80°C for subsequent analysis.

Preparation of crRNA and gRNA

Both the crRNA for LbuCas13a and the gRNA for LbCas12a were obtained via in vitro transcription. The specific transcription procedure is as follows. Initially, the double-stranded DNA transcription template was prepared in a reaction mixture containing 4 μL of 10×phi29 buffer, 4 μL of the transcription template (10 μM), 4 μL of T7 promoter (10 μM), and 16.1 μL of deionized water. The mixture was denatured at 90°C for 5 min and annealed at room temperature for 30 min to facilitate efficient hybridization between the T7 promoter and the template. Subsequently, 0.5 μL of phi29 DNA polymerase (10 U/μL) and 2 μL of dNTP mix (25 mM of dATP, dGTP, dTTP, dCTP,

respectively) were added, and the mixture was incubated at 37°C for 30 min to generate full-length double-stranded DNA templates. Following template preparation, the transcription reaction was initiated by adding 8 μL of 5×Transcription buffer, 4 μL of rNTPs (25 mM of rATP, rGTP, rTTP, rCTP, respectively), and 1 μL of T7 RNA polymerase, followed by incubation at 37°C for 16 h. To eliminate the DNA templates, 8 μL of 5×DNase I buffer and 4 μL of DNase I (10 U/μL) were added and incubated at 37°C for 2 h, followed by heat inactivation at 75°C for 15 min. The concentration of the resulting RNA transcripts was quantified using a microplate reader. Finally, the purified RNA was diluted to 2 μM with deionized water based on its molecular mass and stored at -80°C for subsequent use.

LbuCas13a-LbCas12a cascade sensor based bacteria detection

The LbuCas13a-LbCas12a cascade detection assay was established using target RNA obtained through either in vitro synthesis or extraction from bacteria. The reaction was performed in a 30 μL mixture containing 3 μL of 10×Reaction buffer-A, 1.2 μL of LbuCas13a (1μM), 0.6 μL of crRNA (2 μM), 1.5 μL of LbCas12a (2 μM), 1.5 μL of gRNA (2 μM), 0.3 μL of Target-12 (10 μM), 1.5 μL of TA-Reporter (4 μM), 0.6 μL of RNA sample, and 19.8 μL of ddH_2O. Upon completion of the reaction, the mixture was immediately subjected to fluorescence spectroscopic analysis, with the excitation wavelength set to 480 nm and the emission wavelength range spanning 510 to 600 nm. The fluorescence signal of the blank or control group was defined as F_0, while the fluorescence of the experimental group was recorded as F. The fluorescence difference was recorded as ΔF ($|F - F_0|$). All measurements were performed in three independent experiments to ensure the accuracy and reliability of this cascade sensing system.

LbuCas13a based bacteria detection

The LbuCas13a detection assay was established using target RNA obtained through either in vitro synthesis or extraction from bacteria. The reaction was performed in a 30 μL mixture containing 3 μL of 10×Reaction buffer-B, 1.2 μL of LbuCas13a (1μM), 0.6 μL of crRNA (2 μM), 1.5 μL of U_5-Reporter (4 μM), 0.6 μL of RNA sample, and 23.1μL of ddH_2O.

Detection of the ratio of viable and dead bacteria

To evaluate the ability of the sensor to differentiate bacterial viability, mixed populations of viable and dead lactic acid bacteria were prepared. Initially, viable and isopropanol-inactivated bacterial suspensions were adjusted to a concentration of 5×10^3 CFU/μL in 0.85% NaCl solution. These suspensions were then blended to create heterogeneous samples with viable bacteria proportions of 0%, 1%, 5%, 10%, 20%, 40%, 60%, 80%, and 100%. After thorough mixing, 4 mL of each sample was subjected to total RNA extraction using the previously established protocol. The extracted RNA was subsequently introduced into the LbuCas13a-LbCas12a cascade reaction system. Finally, the fluorescence signals were recorded and analyzed as ΔF to determine the correlation between the viable cell ratio and the sensor output.

Table S1. Sequencing results and comparison information of the bacterial strain.

Strain number	16S RNA sequencing results	Strain	ID
1	ATGCAAGTCGAACGAACTTTCCGTTAATTGATTATGAC GTACTTGTACTGATTGAGATTTTAACACGAAGTGAGTG GCGAACGGGTGAGTAACACGTGGGTAACCTGCCCAGA AGCAGGGGATAACACCTGGAAACAGATGCTAATACCG TATAACAGAGAAAACCGCATGGTTTTCTTTTAAAAGAT GGCTCTGCTATCACTTCTGGATGGACCCGCGGCGTATT AGCTAGTTGGTGAGGCAAAGGCTCACCAAGGCAGTGA TACGTAGCCGACCTGAGAGGGTAATCGGCCACATTGG GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCA GTAGGGAATCTTCCACAATGGACGCAAGTCTGATGGA GCAACGCCGCGTGAGTGAAGAAGGGTTTCGGCTCGTA AAGCTCTGTTGTTAAAGAAGAACGTGGGTAAGAGTAA CTGTTTACCCAGTGACGGTATTTAACCAGAAAGCCACG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGT GGCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGC GCAGGCGGTCTTTTAAGTCTAATGTGAAAGCCTTCGGC TCAACCGAAGAAGTGCATTGGAAACTGGGAGACTTGA GTGCAGAAGAGGACAGTGGAACTCCATGTGTAGCGGT GAAATGCGTAGATATATGGAAGAACACCAGTGGCGAA GGCGGCTGTCTGGTCTGCAACTGACGCTGAGGCTCGAA AGCATGGGTAGCGAACAGGATTAGATACCCTGGTAGT CCATGCCGTAAACGATGATTACTAAGTGTTGGAGGGTT TCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGTAATC CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAA GAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTG GTTTAATTCGAAGCTACGCGAAGAACCTTACCAGGTCT TGACATCTTCTGACAGTCTAAGAGATTAGAGGTTCCCT TCGGGGACAGAATGACAGGTGGTGCATGGTTGTCGTC AGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACG AGCGCAACCCTTATTACTAGTTGCCAGCATTAAGTTGG GCACTCTAGTGAGACTGCCGGTGACAAACCGGAGGAA GGTGGGGACGACGTCAAATCATCATGCCCCTTATGACC TGGGCTACACACGTGCTACAATGGATGGTACAACGAG TCGCGAGACCGCGAGGTTAAGCTAATCTCTTAAAACCA TTCTCAGTTCGGACTGTAGGCTGCAACTCGCCTACACG AAGTCGGAATCGCTAGTAATCGCGGATCAGCATGCCG CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGT CACACCATGAGAGTTTGTAACACCCAAAGCCGGTGGG GTAACCTTTTAGGAGCTAGCCGTCTAAGGTGGGACAGA	Pediococcus. pentosaceus strain DSM 20336	NR 042058.1

Strain number	16S RNA sequencing results	Strain	ID
	TGATTAGGGTGAAGT		
2	TGGTATTGATTGGTGCTTGCATCATGATTTACATTTGAG TGAGTGGCGAACTGGTGAGTAACACGTGGGAAACCTG CCCAGAAGCGGGGGATAACACCTGGAAACAGATGCTA ATACCGCATAACAACTTGGACCGCATGGTCCGAGCTTG AAAGATGGCTTCGGCTATCACTTTTGGATGGTCCCGCG GCGTATTAGCTAGATGGTGGGGTAACGGCTCACCATGG CAATGATACGTAGCCGACCTGAGAGGGTAATCGGCCA CATTGGGACTGAGACACGGCCCAAACTCCTACGGGAG GCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCT GATGGAGCAACGCCGCGTGAGTGAAGAAGGGTTTCGG CTCGTAAAACTCTGTTGTTAAAGAAGAACATATCTGAG AGTAACTGTTCAGGTATTGACGGTATTTAACCAGAAAG CCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGT AGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGC GAGCGCAGGCGGTTTTTTAAGTCTGATGTGAAAGCCTT CGGCTCAACCGAAGAAGTGCATCGGAAACTGGGAAAC TTGAGTGCAGAAGAGGACAGTGGAACTCCATGTGTAG CGGTGAAATGCGTAGATATATGGAAGAACACCAGTGG CGAAGGCGGCTGTCTGGTCTGTAACTGACGCTGAGGCT CGAAAGTATGGGTAGCAAACAGGATTAGATACCCTGG TAGTCCATACCGTAAACGATGAATGCTAAGTGTTGGAG GGTTTCCGCCCTTCAGTGCTGCAGCTAACGCATTAAGC ATTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTC AAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCA TGTGGTTTAATTCGAAGCTACGCGAAGAACCTTACCAG GTCTTGACATACTATGCAAATCTAAGAGATTAGACGTT CCCTTCGGGGACATGGATACAGGTGGTGCATGGTTGTC GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCA ACGAGCGCAACCCTTATTATCAGTTGCCAGCATTAAGT TGGGCACTCTGGTGAGACTGCCGGTGACAAACCGGAG GAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATG ACCTGGGCTACACACGTGCTACAATGGATGGTACAAC GAGTTGCGAACTCGCGAGAGTAAGCTAATCTCTTAAAG CCATTCTCAGTTCGGATTGTAGGCTGCAACTCGCCTAC ATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCATG CCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCC CGTCACACCATGAGAGTTTGTAACACCCAAAGTCGGTG GGGTAACCTTTTAGGAACCAGCCGCC	Lactiplantibacillus. plantarum strain JCM 1149	NR 1156 05.1

Strain number	16S RNA sequencing results	Strain	ID
3	GAAGCTTGCTTCTTGATTCAGACCTTGGTGAGTGGCGG ACGGGTGAGTAACACGTGGGTAACCTGCCCAAAAGTG GGGGATAACATTTGGAAACAAGTGCTAATACCGCATA ACAACTACTTTCACATGATCGTAGCTTGAAAGATGGCT CTGCTATCGCTTTTGGATGGACCCGCGGCGTATTAGCT AGTTGGTGAGGTAATAGCTCACCAAGGCAATGATACG TAGCCGACCTGAGAGGGTAATCGGCCACATTGGGACT GAGACACGGCCCAAACTCCTACGGGAGGCAGCAGTAG GGAATCTTCCACAATGGGCGAAAGCCTGATGGAGCAA TGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACT CTGTTGTTGAAGAAGAACATGCGTGAGAGTAACTGTTC ACGTACTGACGGTATTCAACCAGAAAGCCACGGCTAA CTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAA GCGTTGTCCGGATTTATTGGGCGTAAAGAGAATGTAGG CGGTTTATTAAGTTTGAAGTGAAAGCCCTCGGCTCAAC CGAGGAAGTGCTTCGAAAACTGGTAAACTTGAGTGCA GAAGAGGAAAGTGGAACTCCATGTGTAGCGGTGGAAT GCGTAGATATATGGAAGAACACCAGTGGCGAAGGCGG CTTTCTGGTCTGTAACTGACGCTGAGATTCGAAAGCAT GGGTAGCAAACAGGATTAGATACCCTGGTAGTCCATG CCGTAAACGATGAGTGCTAAGTGTTGGAGGGTTTCCGC CCTTCAGTGCTGCAGCTAACGCATTAAGCACTCCGCCT GGGGAGTACGATCGCAAGATTGAAACTCAAAGGAATT GACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTA ATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGAC ATACCATGACAAACTAAGAGATTAGTCTTTCCCTTCGG GGACATGGATACAGGTGGTGCATGGTTGTCGTCAGCTC GTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC AACCCTTATTATCAGTTGCCAGCATTCAGTTGGGCACT CTGGTGAGACTGCCGGTGACAAACCGGAGGAAGGTGG GGACGACGTCAAATCATCATGCCCCTTATGACCTGGGC TACACACGTGCTACAATGGTCGGTACAACGTGTTGCGA ACTCGCGAGGGCAAGCAAATCACTTAAAACCGATCTC AGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCT GGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTG AATACGTTCCCGGGCCTTGTACACACCGCCCGTCACAC CATGAGAGTTTGTAACACCCAAAGTCGGTGGGGGTAA CCCTTCGGGGAA	Companilactobacillus. farciminis strain BCRC 14043	NR 1143 98.1

Strain number	16S RNA sequencing results	Strain	ID
4	GGGGGTGGCTCCAAAGGTTACCTCACCGACTTCGGGTG TTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAG GCCCGGGAACGTATTCACCGCGGCATGCTGATCCGCGA TTACTAGCGATTCCAGCTTCACGCAGTCGAGTTGCAGA CTGCGATCCGAACTGAGAACAGATTTGTGGGATTGGCT TAGCCTCGCGGCTTCGCTGCCCTTTGTTCTGCCCATTGT AGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGA TTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGC AGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAA GATCAAGGGTTGCGCTCGTTGCGGGACTTAACCCAACA TCTCACGACACGAGCTGACGACAACCATGCACCACCTG TCACTCTGCCCCCGAAGGGGAAGCCCTATCTCTAGGGT TGTCAGAGGATGTCAAGACCTGGTAAGGTTCTTCGCGT TGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGG GCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGT ACTCCCCAGGCGGAGTGCTTAATGCGTTTGCTGCAGCA CTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCG TTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTCG CTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACC AGAGAGTCGCCTTCGCCACTGGGGTTCCTCCACATCTC TACGCATTTCACCGCTACACGTGGAATTCCACTCTCCT CTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCC CGGTTGAGCCGGGGGCTTTCACATCAGACTTAAGAAAC CGCCTGCGCGCGCTTTACGCCCATAATTCCGGACAACG CTTGCACCTACGTATAACGGGGCTGCTGGCACGAATTA TCCGTGGCTTTCTGTTAGGAA	Bacillus. licheniformis strain BCRC 11702	NR 1160 23.1

Table S2. The sequences of crRNAs of the LbuCas13a.

Oligonucleotides	Sequences (5’ to 3’)
LAB-1	UAGACCACCCCAAAAAUGAAGGGGACUAAAACUUUCUG GUUAAAUACCGUCA
LAB-2	UAGACCACCCCAAAAAUGAAGGGGACUAAAACCUGGUG UUCUUCCAUAUAUC
LAB-3	UAGACCACCCCAAAAAUGAAGGGGACUAAAACCUUGCC ACCUACGUAUUACC
LAB-4	UAGACCACCCCAAAAAUGAAGGGGACUAAAACUUACGC CCAAUAAAUCCGGA
LAB-5	UAGACCACCCCAAAAAUGAAGGGGACUAAAACCGCCUG CGCUCGCUUUACGC
LAB-6	UAGACCACCCCAAAAAUGAAGGGGACUAAAACAUGCAC UUCUUCGGUUGAGC
LAB-7	UAGACCACCCCAAAAAUGAAGGGGACUAAAACGUCCUC UUCUGCACUCAAGU
LAB-8	UAGACCACCCCAAAAAUGAAGGGGACUAAAACCACAUG GAGUUCCACUGUCC
LAB-9	UAGACCACCCCAAAAAUGAAGGGGACUAAAACUCUUCC AUAUAUCUACGCAU
LAB-10	UAGACCACCCCAAAAAUGAAGGGGACUAAAACUUCGCC ACUGGUGUUCUUCC

Table S3. Oligonucleotide sequences used in the experiment.

Oligonucleotides	Sequences (5' to 3')
T7 promoter	TAATACGACTCACTATAGGG
LAB-template1	TGACGGTATTTAACCAGAAAGTTTTAGTCCCCTTCATTTTTGGG GTGGTCTACCCTATAGTGAGT CGTATTA
LAB-template2	GGTAATACGTAGGTGGCAAGGTTTTAGTCCCCTTCATTTTTGGG GTGGTCTACCCTATAGTGAGTCGTATTA
LAB-template3	GATATATGGAAGAACACCAGGTTTTAGTCCCCTTCATTTTTGGG GTGGTCTACCCTATAGTGAGTCGTATTA
LAB-template4	TCCGGATTTATTGGGCGTAAGTTTTAGTCCCCTTCATTTTTGGGG TGGTCTACCCTATAGTGAGTCGTATTA
LAB-template5	GCGTAAAGCGAGCGCAGGCGGTTTTAGTCCCCTTCATTTTTGGG GTGGTCTACCCTATAGTGAGTCGTATTA
LAB-template6	GCTCAACCGAAGAAGTGCATGTTTTAGTCCCCTTCATTTTTGGG GTGGTCTACCCTATAGTGAGTCGTATTA
LAB-template7	ACTTGAGTGCAGAAGAGGACGTTTTAGTCCCCTTCATTTTTGGG GTGGTCTACCCTATAGTGAGTCGTATTA
LAB-template8	GGACAGTGGAACTCCATGTGGTTTTAGTCCCCTTCATTTTTGGG GTGGTCTACCCTATAGTGAGTCGTATTA
LAB-template9	ATGCGTAGATATATGGAAGAGTTTTAGTCCCCTTCATTTTTGGG GTGGTCTACCCTATAGTGAGTCGTATTA
LAB-template10	GGAAGAACACCAGTGGCGAAGTTTTAGTCCCCTTCATTTTTGGG GTGGTCTACCCTATAGTGAGTCGTATTA
gRNA-template	GGTCGAGCTGGACGGCGACGATCTACACTTAGTAGAAATTACC CTATAGTGAGTCGTATTA
target DNA	CGAGAATGGCTGCGCCATAC
TA-Reporter	FAM-TTATT-BHQ1
crRNA	UAGACCACCCCAAAAAUGAAGGGGACUAAAACGGUCCACCAA ACGUAAUGCG
U_5-Reporter	FAM-UUUUU-BHQ1
gRNA	UAAUUUCUACUAAGUGUAGAUCGUCGCCGUCCAGCUCGACC
Randem RNA	CGCAUUACGUUUGGUGGACC

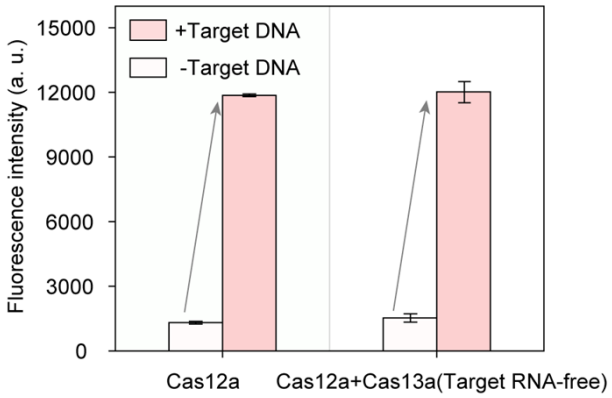

Figure S1. Fluorescence analysis of Cas12a, Cas12a+Cas13a (Target RNA-free) for target DNA detection, respectively. Data are means ± SD (n = 3).

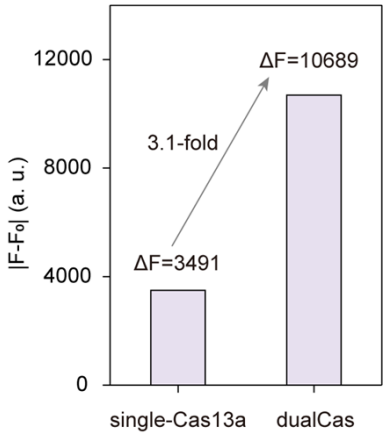

Figure S2. Fluorescence difference analysis of non-cascade and cascade systems for target RNA detection, respectively. The fluorescence signal of the blank or control group was defined as F_0, while the fluorescence of the experimental group was recorded as F. The fluorescence difference was recorded as ΔF ($|F - F_0|$).

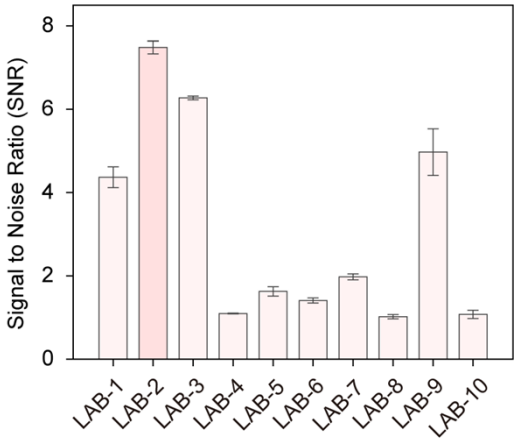

Figure S3. Signal to noise ratio (SNR) of fluorescence analysis of crRNA designed based on different sites of conserved sequences of LAB. Data are means ± SD (n = 3).

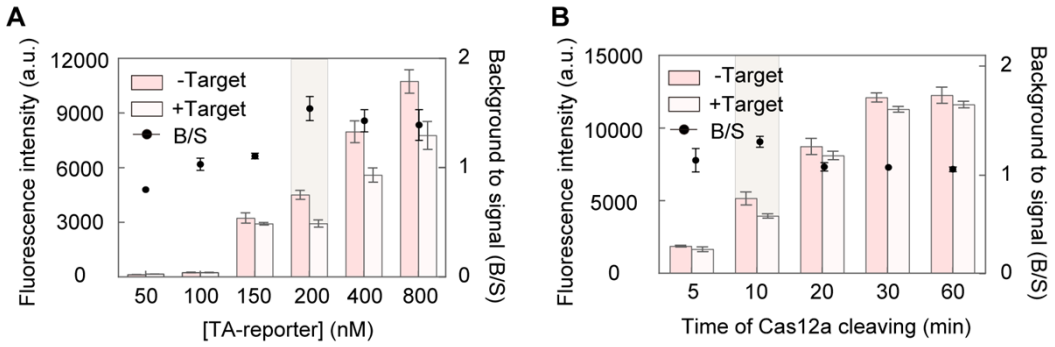

Figure S4. (A-B) Effects of TA-reporter concentration (A), and LbCas12a cleavage time (B) on assay performance. Data in panel A, and B are means ± SD (n = 3).

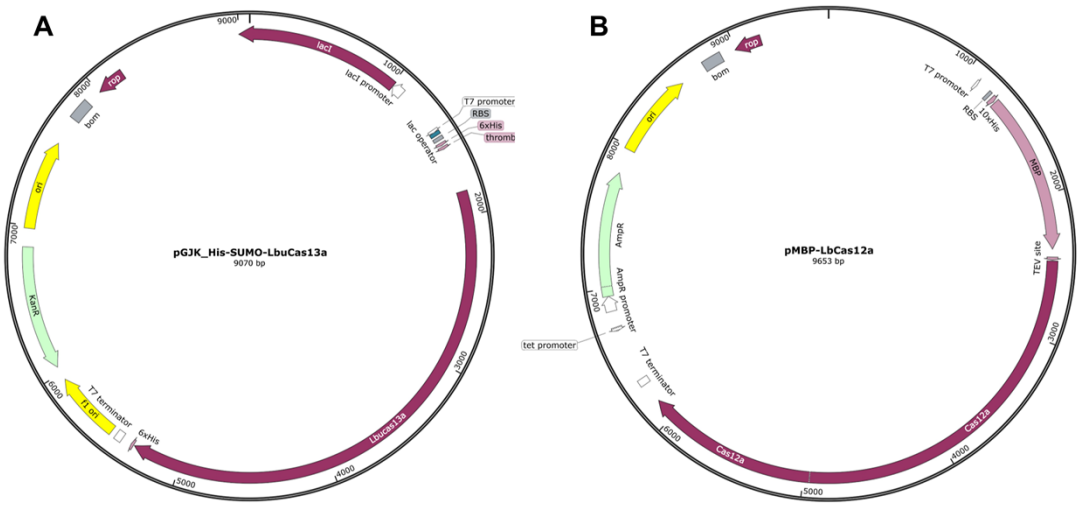

Figure S5. Plasmid profile of the CRISPR proteins. (A) Plasmid profile of LbuCas13a. (B) Plasmid profile of LbCas12a.