Electronic Supplementary Material (ESI) for Analyst.

Integration of CRISPR Cas12a-assisted multicolor biosensor and micropipette-tip enables visible point-of-care testing of foodborne *Vibrio vulnificus*

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Supporting Information

Table S1 Oligonucleotides used for establishing the detection system in this study.

Name	Sequence(5'→3')
vvhA gene NTS	TCTGCGGGCTCGTCAACCAACAGCAGTACTGT GAAACAACGTATTCGCATCGACTGGAATCATC CACTGTTTGAAGCGGAAGCACACGTTACACTG CAGTCATTGAGCAACAACGATCTCTGCCTAGA TGTTTATGGTGAGAACGGTGACAAAACGGTTG CGGGTGGTTCGGTTAACGGCTGGAGCTGTCAC GGCAGTTGGAACCAAGTTTGGGGCCTAGATA AAGAAGAACGTTATCGTAGCCGAGTGGCATC CGATCGTTGTTTGACC
vvhA gene TS	GGTCAAACAACGATCGGATGCCACTCGGCTA CGATAACGTTCTTCTTTATCTAGGCCCCAAAC TTGGTTCCAACTGCCGTGACAGCTCCAGCCGT TAACCGAACCACCCGCAACCGTTTTGTCACCG TTCTCACCATAAACATCTAGGCAGAGATCGTT GTTGCTCAATGACTGCAGTGTAACGTGTGCTT CCGCTTCAAACAGTGGATGATTCCAGTCGATG CGAATACGTTGTTTCACAGTACTGCTGTTGGT TGACGAGCCCGCAGA
16S rDNA NTS	ACTCCGTGCCAGCAGCCGCGGTAATACGGAG GGTGCGAGCGTTAATCGGAATTACTGGGCGTA AAGCGCATGCAGGTGGTTTGTTAAGTCAGATG TGAAAGCCCGGGGCTCAACCTCGGAACTGCA TTTGAAACTGGCAGACTAGAGTACTGTAGAG GGGGGTAGAATTTCAGGTGTAGCGGTGAAAT GCGTAGAGATCTGAAGGAATACCGGTGGCGA AGGCGGCCCCCTGGACAGATACTGACACTCA

	GATGCGAAAGCGTGGGGAGC
16S rDNA TS	GCTCCCCACGCTTTCGCATCTGAGTGTCAGTA TCTGTCCAGGGGGCCGCCTTCGCCACCGGTAT TCCTTCAGATCTCTACGCATTTCACCGCTACA CCTGAAATTCTACCCCCCTCTACAGTACTCTA GTCTGCCAGTTTCAAATGCAGTTCCGAGGTTG AGCCCCGGGCTTTCACATCTGACTTAACAAAC CACCTGCATGCGCTTTACGCCCAGTAATTCCG ATTAACGCTCGCACCCTCCGTATTACCGCGGC TGCTGGCACGGAGT
vvhA-RAA-F	CCACTGTTTGAAGCGGAAGCACACGTTACACT
vvhA-RAA-R	TCCAACTGCCGTGACAGCTCCAGCCGTTAA
16S-RAA-F	ATACGGAGGGTGCGAGCGTTAATCGGAATT
16S-RAA-R	CCCACGCTTTCGCATCTGAGTGTCAGTATC
vvhA-crRNA	UAAUUUCUACUAAGUGUAGAUUGGUGAGAA CGGUGACAAAA
16S-crRNA	UAAUUUCUACUAAGUGUAGAUAAACUGGCA GACUAGAGUAC
Fluorescence reporter	5’-HEX-TTATTATTATTA-BHQ_1-3’
Thiol-ssDNA	5’-Thiol-$(T)_{74}$-TCAGTGAATCGATCTAGTCAGTCAG-3’
Biotin-ssDNA	5’-Biotin-$(T)_{74}$-CTGACTGACTAGATCGATTCACTGA-3’
vvhA-qPCR-F	TTCCAACTTCAAACCGAACTATGAC
vvhA-qPCR-R	ATTCCAGTCGATGCGAATACGTTG
vvhA-qPCR-Probe	5’-FAM-AACTATCGTGCACGCTTTGGTACCGT-MGB-3’

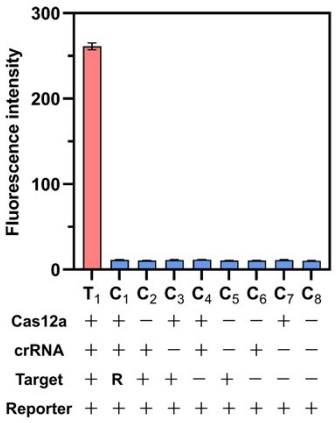

Fig S1 Feasibility of fluorescence-CRISPR assay (each point showed the average result of three replicates; error bars represent the mean ± S.D.). The Cas12a, crRNA, and target (100 nM) were selected as variables to verify the feasibility of fluorescence-CRISPR assay.

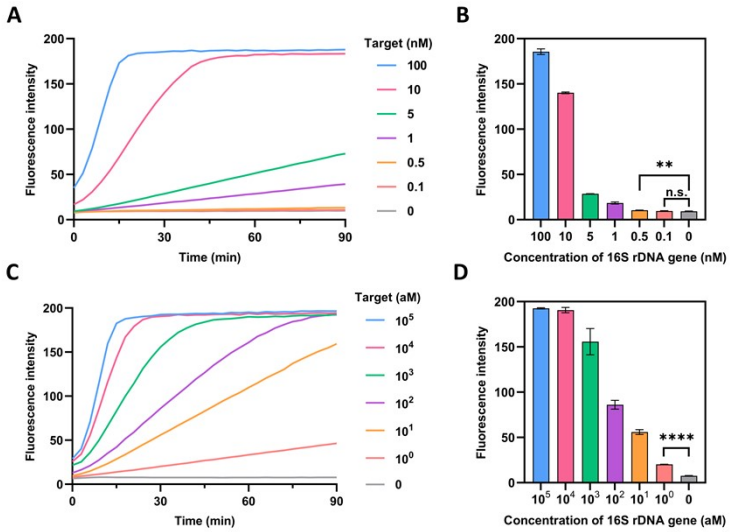

Fig S2 Sensitivity of fluorescence-CRISPR assay. (A) and (B) Reaction kinetics and sensitivity of the fluorescence-CRISPR assay for 16S rDNA gene at 30 min (each curve and each point showed the average result of three replicates; error bars represent the mean ± S.D.; **p < 0.01, n.s.: no significance in "Student's t test"). (C) and (D) Reaction kinetics and sensitivity of the RAA-based fluorescence-CRISPR assay for 16S rDNA gene at 30 min (each curve and each point showed the average result of three replicates; error bars represent the mean ± S.D.; ****p < 0.0001 in "Student's t test").

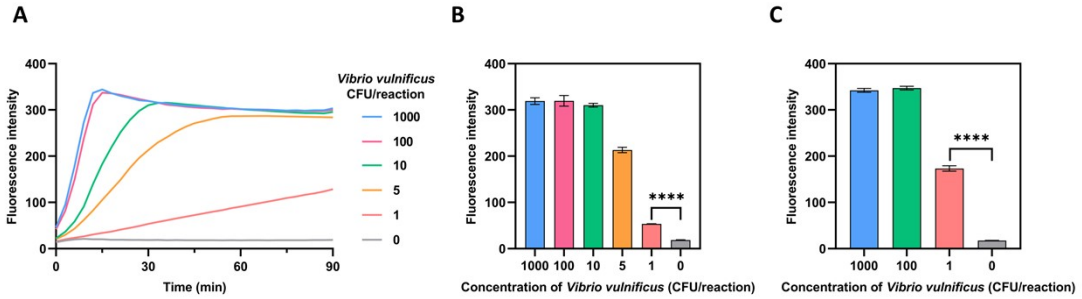

Fig S3 Sensitivity of fluorescence-CRISPR assay in *Vibrio vulnificus* strain. (A) and (B) Reaction kinetics and sensitivity of the RAA-based fluorescence-CRISPR assay for *vvhA* gene at 30 min (each curve and each point showed the average result of three replicates; error bars represent the mean ± S.D.; ****p < 0.0001 in "Student's t test"). (C) Sensitivity of the RAA-based fluorescence-CRISPR assay for 16S rDNA gene at 30 min (each curve and each point showed the average result of three replicates; error bars represent the mean ± S.D.; ****p < 0.0001 in "Student's t test").

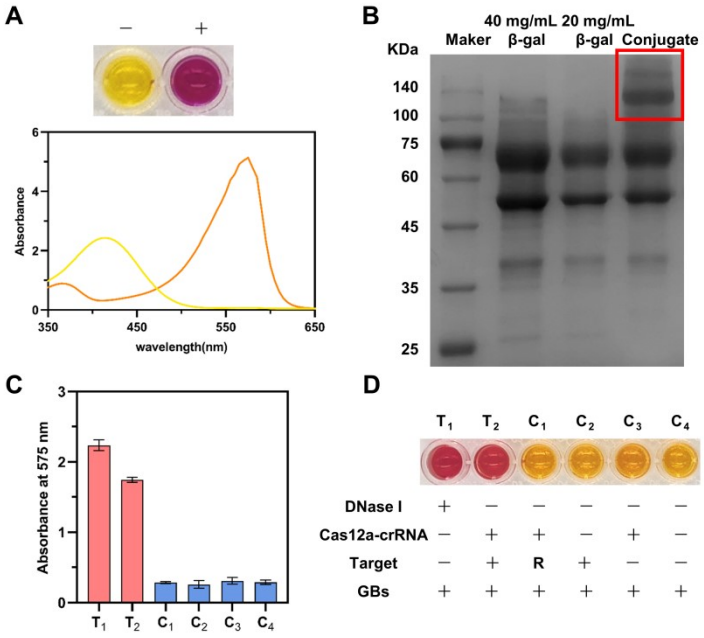

Fig S4 Feasibility of GBs-CRISPR assay. (A) The absorption curve of CPR, and there was an absorption peak at 575 nm. The "+" solution was added with β-galactosidase, and "-" solution was added with the equal amount of water. (B) SDS-PAGE analysis of ssDNA and β-galactosidas conjugate and the larger molecular weight band represented the conjugate. (C) and (D) The value of the CPR absorption peak and the

color of detetion solution after DNase I or Cas12a reaction in the visible assay using β-galactosidase-modified sepharose beads (each point showed the average result of three replicates; error bars represent the mean ± S.D.). The DNase I (100 U/μL), Cas12a-crRNA, and target (100 nM) were selected as variables to verify the feasibility of GBs-CRISPR assay.

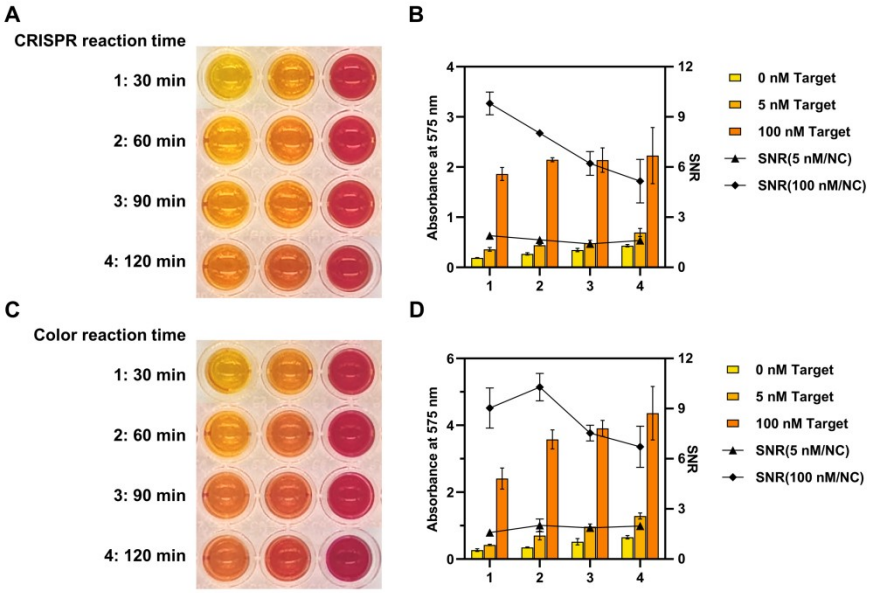

Fig S5 Optimization of the GBs-CRISPR assay. (A) and (B) Optimization of the CRISPR reaction time. The CRISPR system was incubated at 37 ℃ for different times, and then CPRG was catalyzed by the filtered solution at 37 ℃ for 30 min. According to the results, 1 h was the relatively optimal CRISPR reaction time for this assay (each point showed the average result of three replicates; error bars represent the mean ± S.D., SNR: Signal-to-Nosie Ratio, NC: Negative Control). (C) and (D) Optimization of the color reaction time. The CRISPR system was incubated at 37 ℃ for 1 h, and then CPRG was catalyzed by the filtered solution at 37 ℃ for different times. According to the results, 1 h was the relatively optimal color reaction time for this assay (each point showed the average result of three replicates; error bars represent the mean ± S.D., SNR: Signal-to-Nosie Ratio, NC: Negative Control).

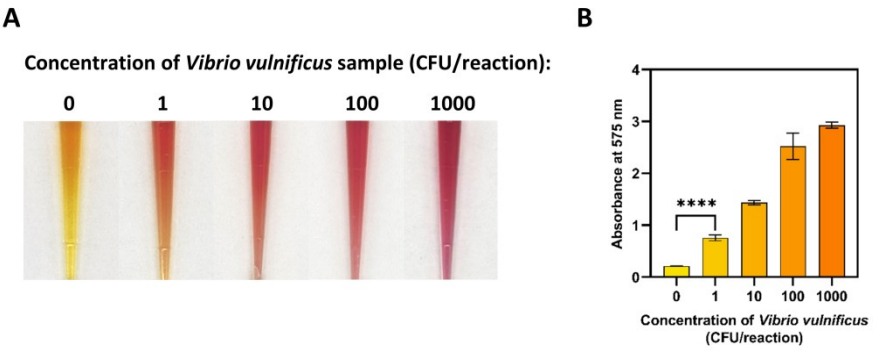

Fig S6 Sensitivity of GBs-CRISPR assay. (A) The degree of color change in the solution was observed by the naked eye in the micropipette-tip to detect the 16S rDNA gene in *Vibrio vulnificus* strain. (B) The value of the CPR absorption peak was measured to detect the 16S rDNA gene in *Vibrio vulnificus* strain (each point showed the average result of three replicates; error bars represent the mean ± S.D.; ****p < 0.0001 in "Student's t test").

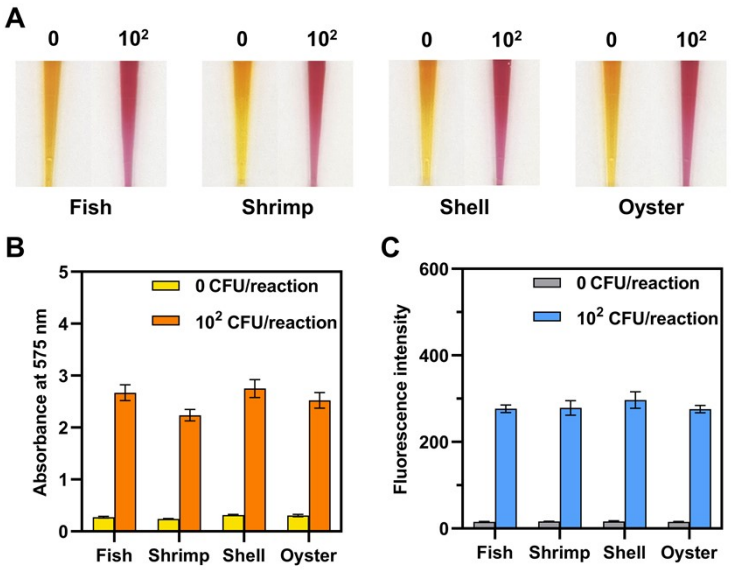

Fig S7 Feasibility of GBs-CRISPR assay and fluorescence-CRISPR assay in the artificially contaminated seafood. (A) The degree of color change in the solution was observed by the naked eye in the micropipette-tip to detect the 16S rDNA gene in 4 kinds of seafood. (B) The value of the CPR absorption peak was measured to detect the 16S rDNA gene in 4 kinds of seafood (each point showed the average result of three replicates; error bars represent the mean ± S.D.). (C) The fluorescence intensity of the RAA-based fluorescence-CRISPR assay for 16S rDNA gene in 4 kinds of seafood at 30 min (each point showed the average result of three replicates; error bars

represent the mean ± S.D.).

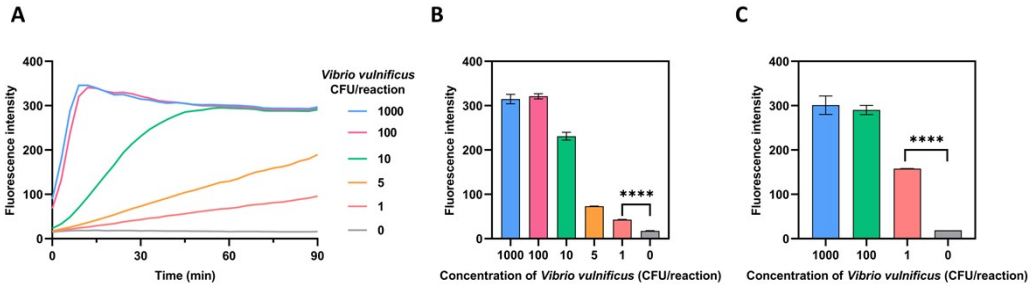

Fig S8 Sensitivity of fluorescence-CRISPR assay in the artificially contaminated seafood.(A) and (B) Reaction kinetics and sensitivity of the RAA-based fluorescence-CRISPR assay for *vvhA* gene in the artificially contaminated fish sample at 30 min (each curve and each point showed the average result of three replicates; error bars represent the mean ± S.D.; ****p < 0.0001 in "Student's t test"). (C) Sensitivity of the RAA-based fluorescence-CRISPR assay for 16S rDNA gene in the artificially contaminated fish sample at 30 min (each curve and each point showed the average result of three replicates; error bars represent the mean ± S.D.; ****p < 0.0001 in "Student's t test").

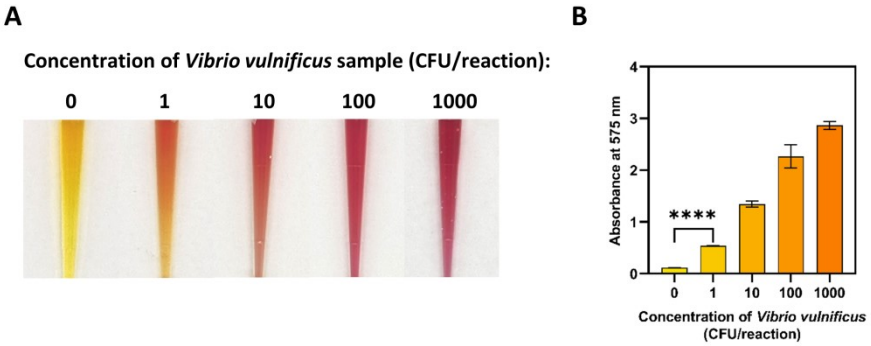

Fig S9 Sensitivity of GBs-CRISPR assay in the artificially contaminated seafood. (A) The degree of color change in the solution was observed by the naked eye in the micropipette-tip to detect the 16S rDNA gene in the artificially contaminated fish samples. (B) The value of the CPR absorption peak was measured to detect the 16S rDNA gene in the artificially contaminated fish sample (each point showed the average result of three replicates; error bars represent the mean ± S.D.; ****p < 0.0001 in "Student's t test").

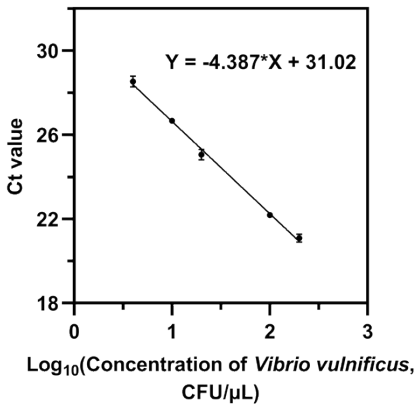

Fig S10 Response of the Ct values vs. the concentrations of *Vibrio vulnificus* (CFU/μL) (each point showed the average result of three replicates; error bars represent the mean ± S.D.; R^2=0.993).

Table S2 Detection results from GBs-CRISPR and qPCR.

Detection system	Sample number	Spiked *Vibrio vulnificus* (CFU/μL)	qPCR		GBs-CRISPR	
			Quantitative results	Recovery (%)	Qualitative Results	Visible results
Vibrio vulnificus	S-1	0	-	-	N	
	S-2	0	-	-	N	
	S-3	0	-	-	N	
	S-4	0	-	-	N	
	S-5	4	3.73	93.10	P	
	S-6	6	5.53	92.12	P	
	S-7	8	9.82	122.78	P	
	S-8	10	9.85	98.49	P	
	S-9	14	16.42	117.32	P	
	S-10	16	17.13	107.09	P	
	S-11	18	19.41	107.84	P	
	S-12	20	23.08	115.39	P	
	S-13	40	47.11	117.77	P	
	S-14	60	73.13	121.89	P	

	S-15	80	85.11	106.39	P	
	S-16	100	103.27	103.27	P	
	S-17	120	125.88	104.90	P	
	S-18	140	134.15	95.82	P	
	S-19	160	166.33	103.96	P	
	S-20	200	184.97	92.49	P	

Notes: N means “Negative”, P means “Positive” in Table S2. The value of the CPR absorption peak < 0.35 was negative and ≥ 0.35 was positive.

Table S3 The comparison of different detection methods.

	Detection method	Detection sensitivity	Detection time	Reference
Immunoassay detection	Chromatographic lateral flow assay	10 CFU/mL	＜7 h	[29]
	DNAzyme-based biosensor	2.2×10^3 CFU/mL	5-10 min	[30]
	Dot-enzyme-linked immunosorbent assay	1 CFU/mL	＞6 h	[31]
Nucleic acid detection	LAMP	4 CFU/reaction	16.5 h	[32]
	qPCR	10^2 CFU/g	9-12 h	[33]
	RAA-CRISPR/Cas12a	2 copies/mL	40 min	[34]
	multiplex PCR	10 CFU/mL	＞6 h	[35]
	Our assay (GBs-CRISPR)	10^2 CFU/mL (1 CFU/reaction)	3 h	-