

Supplementary Materials

Ultr sensitive and Rapid Visual Detection of *Escherichia coli* O157:H7 Based on RAA–CRISPR/Cas12a System

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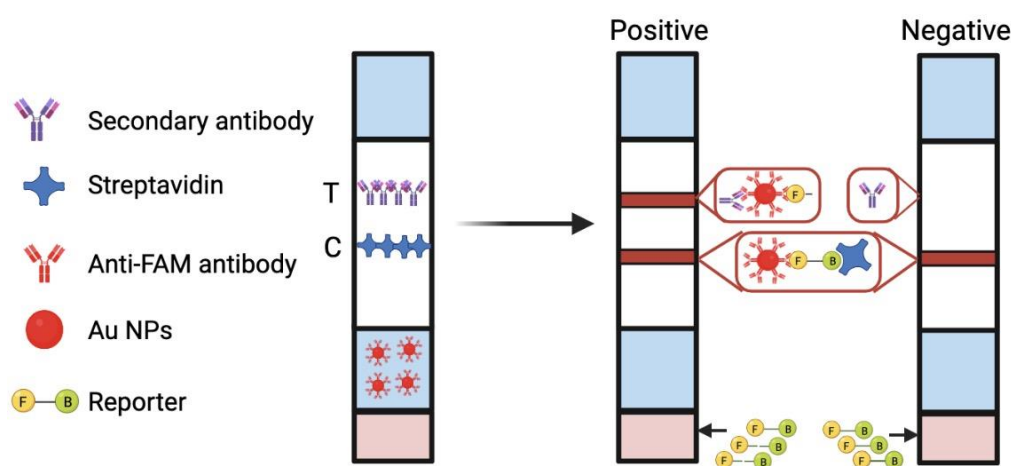


Fig. S1. Principle of visual detection with LFA.

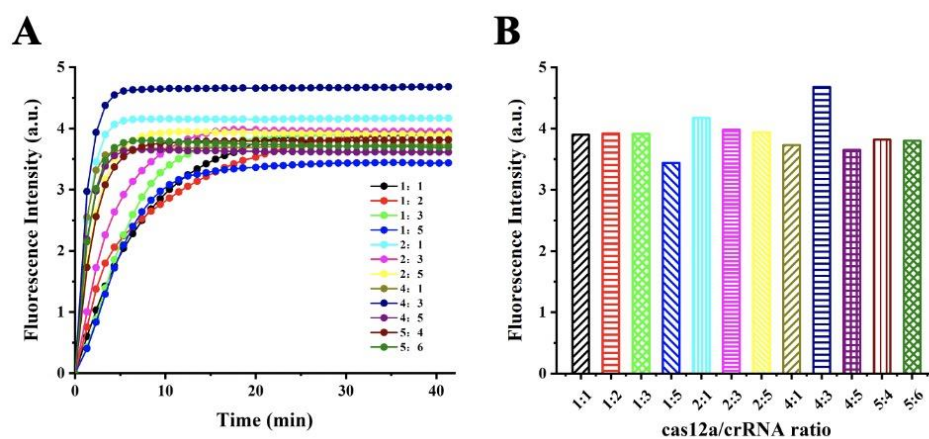


Fig. S2. Effects of different Cas12a/crRNA ratios on fluorescence intensity. A) Real-time fluorescence detection assay using the CRISPR/Cas12a-FQ detector for different Cas12a/crRNA ratios. B) Fluorescence generated from different Cas12a/crRNA ratios.

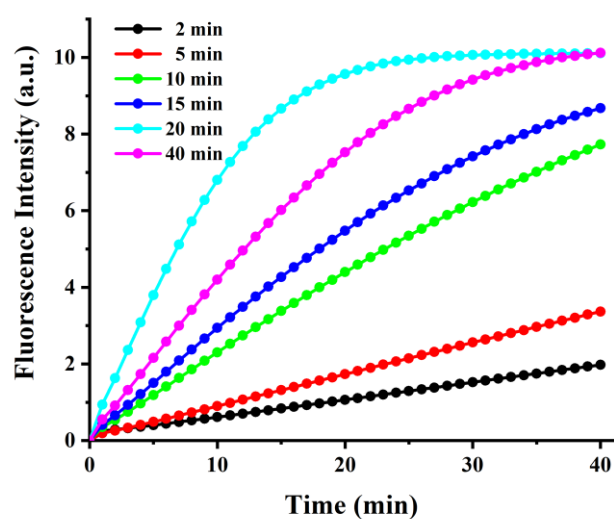


Fig. S3. Effect of different amplification times of RAA on fluorescence intensity.

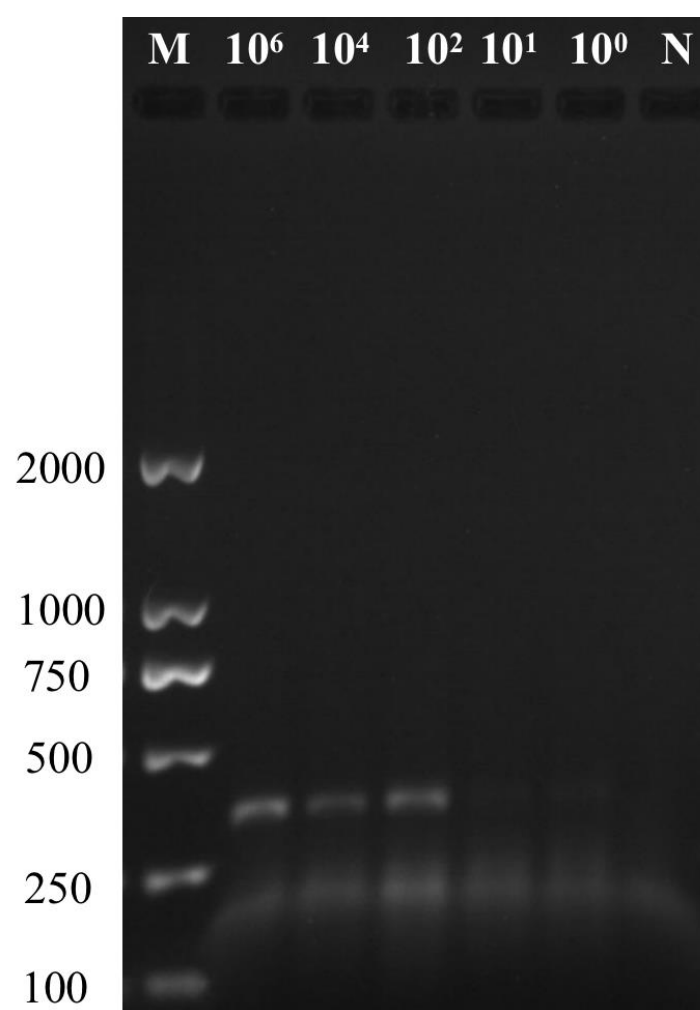


Fig. S4. The gel electrophoresis result of RAA for different bacterial concentration.

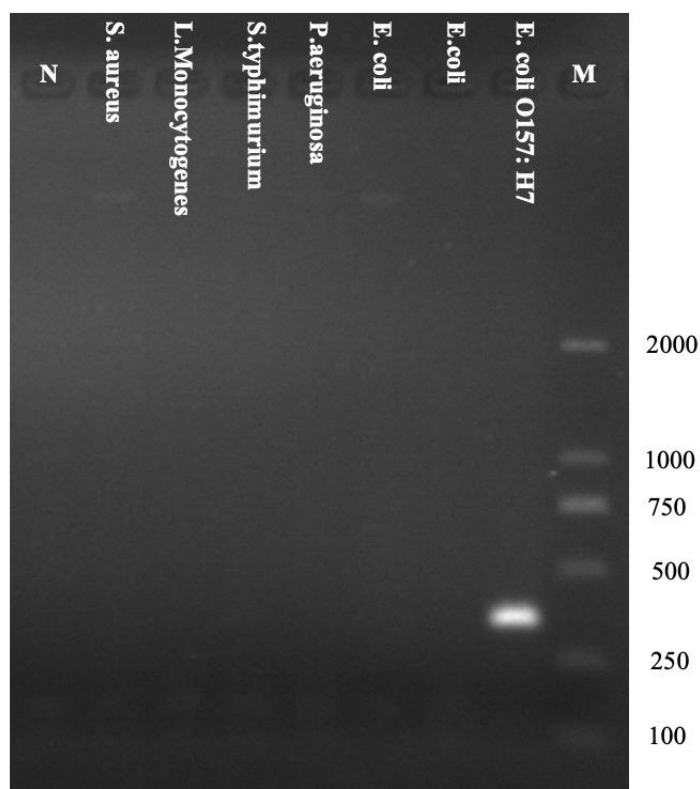


Fig. S5. The gel electrophoresis result of RAA for different bacterial strains.

Table S1. Sequences used in this study ^[1]

Method	Primer's name	Sequence (5' to 3')
RAA	rfbE-F1	AACATATCGATAGACAGTTAAATATAAGAG
	rfbE-R1	TTTGCCAAGTTTCATTATCTGAATCAACG
CRISPR/Cas12a	LbCas12a crRNA	GGGUAAUUUCUACUAAGUGUAGAUCCAACCGUCAUUGACAGGAA
	ssDNA-FB reporter	6-FAM-TTTTTT-Biotin
	ssDNA-FQ reporter	6-FAM-TTTTTT-BHQ1

Table S2. Verification of CRISPR/Cas12a specificity

No.	Bacterial species	Strains	CRISPR/Cas12 system results
1	<i>E. coli</i> O157: H7	CICC 24187	+
2	<i>E. coli</i>	ATCC25922	-
3	<i>E. coli</i>	ATCCBAA2452	-
4	<i>S. aureus</i>	CICC10306	-
5	<i>P. aeruginosa</i>	CICC21636	-
6	<i>S. typhimurium</i>	CICC22956	-
7	<i>L. monocytogenes</i>	CICC21635	-

CICC: China Center of Industrial Culture Collection; ATCC: American Type Culture Collection. “+/-” indicates positive and negative results.

Table S3 Detection limit of E. coli O157:H7 with different method

No.	Method	LOD (CFU/mL)	Time (min)	Ref.
1	RPA-CRISPR/Cas12a	6.5×10^4	> 100	[1]
2	ELISA	1×10^4	> 480	[2]
3	p-ELISA	1×10^4	< 180	[3]
4	I-IMS-FLFI	2.39×10^2	90	[4]
5	ICT	1.8 (Minimum concentration)	/	[5]
6	RT-PCR	6.4×10^3	/	[6]
7	A One-Pot Toolbox	1	< 50	[7]
8	RAA-CRISPR/Cas12a	1 (Minimum concentration)	< 55	This work

Table S4 Detection of *E. coli* O157:H7 in milk and drinking water samples using fluorescence signal

samples	spiked (CFU/mL)	fluorescence	RSD (n=3)
DEPC	0	0.57	0.016
	1.0×10^0	1.21	0.0084
	1.0×10^1	1.87	0.58
	1.0×10^2	2.68	0.51
	1.0×10^4	2.61	0.42
Drinking water	0	0.43	0.052
	1.0×10^0	1.31	0.12
	1.0×10^1	1.26	0.31
	1.0×10^2	2.76	0.35
	1.0×10^4	2.83	0.58
Skim milk	0	0.23	0.0069
	1.0×10^0	1.70	0.47
	1.0×10^1	1.67	0.059
	1.0×10^2	2.21	0.18
	1.0×10^4	2.75	0.41

References

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