Supplementary information:

Single-digit *Salmonella* detection with the naked eye using bio-barcode immunoassay coupled with recombinase polymerase amplification and CRISPR-Cas12a system

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Materials and methods

Bacterial growth

The stock culture of *S*. Typhimurium, *E*. *coli* and *L*. *monocytogenes* were grown in Brain Heart Infusion (BHI) broth. After 18 h incubation, 1 mL of bacteria solution was collected by centrifugation at 6000 rpm for 8 min to remove the supernatant and washed with PBST. By plating bacteria on Xylose-Lysine-Desoxycholate (XLD) agar plates, the number of bacteria per milliliter can be acquired via counting related colony forming unit (CFU) after overnight incubation at 37°C.

Standard RPA reaction assay

Standard RPA reactions were assembled according to the instructions of the manufacturer (TwistAmp Basic kit, TwistDx). Briefly, each 25 μL reactions contained 14.75 μL of rehydration buffer, 1.2 μL of 10 μM forward primer, 1.2 μL of 10 μM reverse primer, 3 μL of bio-barcode DNA, 1.25 μL of 280 mM magnesium acetate (MgOAc), a lyophilized enzyme pellet, and sterile water up to 25 μL. The mixture was incubated at 37°C for 5 min.

ssDNA-triggered CRISPR-Cas12a system

The ssDNA-triggered CRISPR-Cas12a system contained 1 μL 10 μM crRNA, 5 μL 1 μM Cas12a, 2 μL 2 μM quenched fluorescent ssDNA reporter, 10 U of RNase inhibitor, and 2 μL of NEBuffer 2.1 with 3 μL of amplified products or ssDNA and RNase free ddH_2O up to 20 μL. The reaction was performed at 37 °C for 10 min.

Characterization of the AuNP probes

Transmission electron microscope (TEM) imaging showed that both unmodified and barcode AuNPs exhibit great dispersion with diameter of 12.5 nm (Fig. S1). A shadow coating was observed after bare AuNPs (Fig. S1A) being coated with antibody and DNA (Fig. S1B) due to the presence of a coating material with a lower electron density.

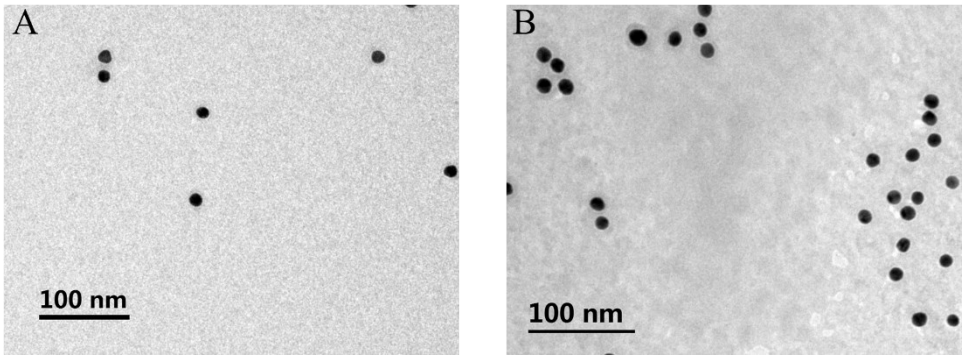


Fig. S1 TEM imaging of bare AuNPs(A) and barcode AuNPs(B).

The impact of MNPs, AuNPs, and bacteria to RPA and CRISPR-Cas12a system

To testify the impact of BCA components on the RPA and CRISPR-Cas12a system, we added individual components of BCA reagents, including MNPs, AuNPs, and bacteria into the standard RPA reaction system and CRISPR-Cas12a system, respectively. Agarose gel electrophoresis revealed that all samples contained the expected amplified DNA bands (Fig. S2A), illustrating the components of BCA have no effect on RPA. Next, we investigated the effects on the CRISPR-Cas12a system. As shown in Fig. S2B, there were almost no difference between standard CRISPR-Cas12a system and with different components from BCA, which proved the MNPs, bacteria, and AuNPs would not affect the CRISPR-Cas12a system.

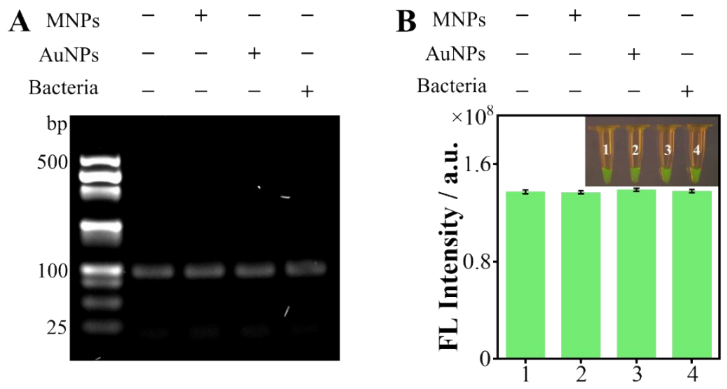


Fig. S2 The impact of MNPs, AuNPs, and bacteria on RPA and CRISPR-Cas12a system. (A) Agarose gel electrophoresis of RPA products with different components from BCA. (B) Fluorescence intensity of CRISPR-Cas12a system with different components of BCA and visual detection under blue light, photographed by smart phone.

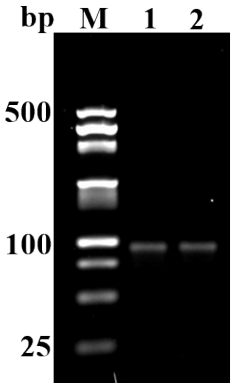


Fig. S3 The effect of DTT on RPA reaction. Lane M: the molecular weight marker. Lane 1: standard RPA reaction. Lane 2: standard RPA reaction with DTT.

Optimization of antibody concentration

The antibody concentration for preparation of barcode AuNPs has a great influence on their affinity toward target bacteria due to the site competition between antibody and DNA. According to the previous report[1], the concentration of bio-barcode DNA was fixed at 3 μM (ratio of ~300:1) which is excess for AuNPs. At a constant DNA concentration, the different concentrations of antibody (0.05, 0.1, 0.15, and 0.2 mg mL^{-1}) were tested. As shown in Fig. S4, the fluorescence intensity of positive sample was the highest at the concentration of 0.1 mg mL^{-1} and gradually decreased with continually increase of the antibody concentration, which may be caused by the decreasing content of bio-barcode DNA on AuNPs. Thus, 0.1 mg mL^{-1} was identified as the optimum antibody concentration for preparation of barcode AuNPs.

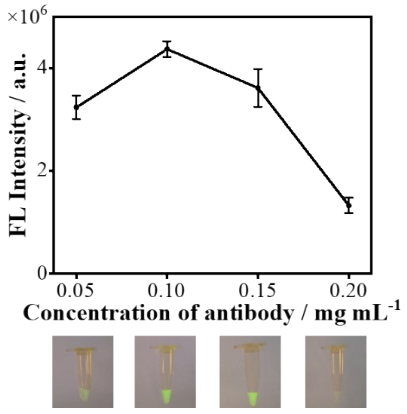


Fig. S4 Optimization of the concentration of antibody.

Optimization of the concentration of barcode AuNPs

In the formation of sandwich structure, the concentration of barcode AuNPs could directly affects the amount of barcode AuNPs attaching on the surface of bacteria and the efficiency of subsequent signal amplification. Therefore, the e□ect of the amount of barcode AuNPs ranging from 5 to 35 μL on the fluorescent signal was investigated. As shown in Fig. S5, the fluorescence intensity of positive sample increased with the amount of barcode AuNPs. For negative control, the fluorescent intensity was negligible until the amount of barcode AuNPs was up to 35 μL, which means the nonspecific adsorption happened when the amount of barcode AuNPs was too high. Furthermore, the F/F_0 value (fluorescent intensity of positive vs. its negative control) also demonstrated the amount of 20 μL was optimal. Therefore, 20 μL of barcode AuNPs was used in the following study.

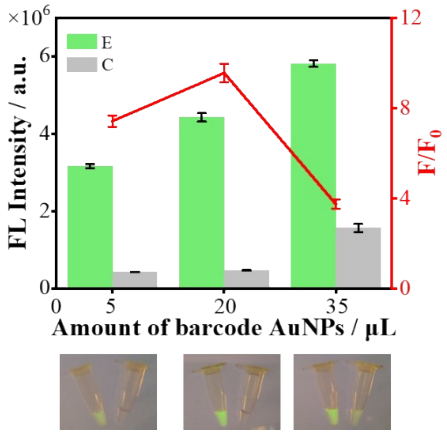


Fig. S5 Optimization of the amount of barcode AuNPs. The concentration of *S.* Typhimurium was 10^3 CFU mL^{-1}.

Optimization of the concentration of enzyme for RPA reaction

The concentration of enzyme for RPA reaction was optimized. Considering that no detailed concentration of enzyme was provided in the RPA kit, one lyophilized enzyme pellet was used as an enzyme unit. Different amount of lyophilized enzyme (1/10, 1/8, 1/6, 1/4, and 1/2 unit) were employed in the optimized experiments. As shown in Fig. S6, the fluorescence intensity of both positive sample and negative control are positively correlated to the concentration of lyophilized enzyme. When the amount of lyophilized enzyme was up to 1/4 unit, the fluorescence signal of negative control sample can be visualized, which means the concentration of 1/4 or more will lead to obvious background signal and have adverse effect on the sensitivity of detection. In addition, the F/F_0 value of 1/6 unit group was the highest. Therefore, 1/6 unit was selected as the optimal enzyme concentration and used in the following experiments.

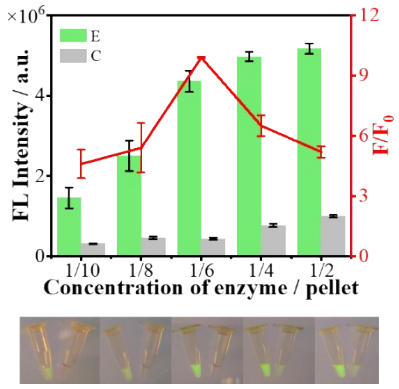
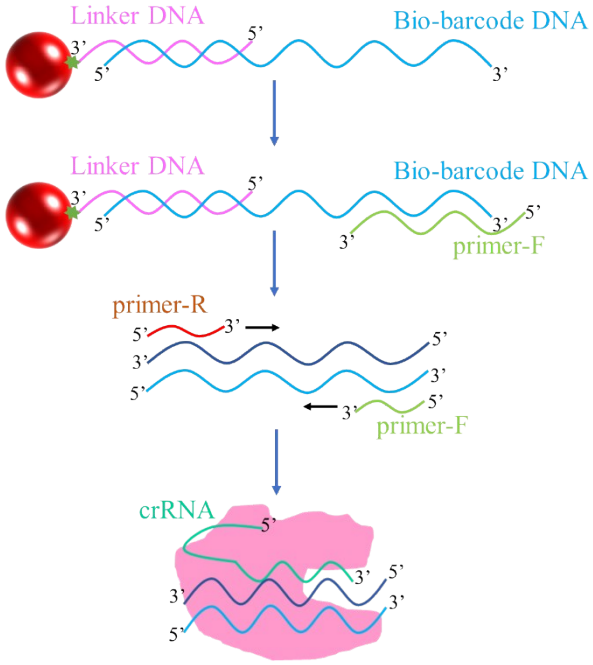


Fig. S6 Optimization of the concentration of enzyme of RPA reaction. The concentration of *S.* Typhimurium was 10^3 CFU mL^{-1}.



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121 **Fig. S7** The scheme RPA reaction of barcode AuNPs. The colors of different DNA

122 are same with their sequences colors in Table S1.

123 **Table S1** Sequence information for the nucleic acids used in this study.

Nucleic acids name	Sequences (5'-3')	Length (bp)
Linker DNA	ACAACATACGAGCCGGAAGCATAAAGT-C6-SH	27
Bio-barcode DNA	ACTTTATGCTTCCGGCTCGTATGTTGTGTGTTTGTTATCGCAAC-TTTCTACTGAATTCGTCGTATTACAATTCACTGGCCGTCGTTTTA	89
ssDNA	ACTTTATGCTTCCGGCTCGTATGTTGTGAATTCAGTA-GAAAGTTGCGATAA	51
RPA primer-F	TAAAACGACGGCCAGTGAATTGTAATACGAC	31
RPA primer-R	ACTTTATGCTTCCGGCTCGTATGTTGTGTG	30
crRNA	GAAUUUCUACUGUUGUAGAUUAUCGCAACUUUCUACUGAAUUC	43
ssDNA reporter	6-FAM-TTATT-BHQ1	5

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Table S2 Compare of detection limit of foodborne pathogen using different methods.

Method	Detection limit	Signal output	Target	References
BCA	86 CFU mL^{-1}	Fluorescence spectrophotometry	*Staphylococcus aureus*	2
BCA	90 CFU mL^{-1}	Fluorescence spectrophotometry	*Shigella spp*	3
BCA	1.07×10^2 CFU mL^{-1}	Fluorescence detector	*B. melitensis 16M*	4
BCA	0.5 ng mL^{-1} (*Bacillus Anthracis*) 50 pg mL^{-1} (*Salmonella enteritidis*)	Electrochemical element	Protective antigen A gene of *Bacillus anthracis* and the insertion element gene of *Salmonella enteritidis*	5
RPA-Cas12a-FS	10 copies	Microplate reader or handheld fluorometer	*E. coli; L. monocytogenes; S. aureus; V. parahaemolyticus*	6
RPA-Cas12a	1 CFU mL^{-1}	Microplate reader	*E. coli* O157:H7	7
RPA-Cas12a	4.48 fM	Fluorescence detector	*Mycobacterium tuberculosis*	8
BCA-RPA-Cas12a	1 CFU mL^{-1}	Naked eye under blue light LED	*S.* Typhimurium	This work

Notes and references

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