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Luminescent DNAzyme and universal blocking linker Super Polymerase Chain Reaction visual biosensor for the detection of *Salmonella*

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ABSTRACT

Here, we constructed a fast, universal, "turn-on" biosensor for the highly sensitive visual detection of *Salmonella* based on luminescent DNAzyme and a universal blocking linker Super Polymerase Chain Reaction (S-PCR). The primer in this biosensor was specially designed. The G-quadruplex sequence is attached to the 5' end of the primer by a blocking linker and is blocked in the stem part. The S-PCR amplification releases the G-quadruplex, which is incubated with hemin to form DNAzyme to exert peroxide-like activity. The visible colored products are generated by the addition of 3,3′,5,5′-Tetramethylbenzidine. Detection sensitivity is high and, even when the concentration of the *Salmonella* genome in a sample is as low as 1.5 copies/μL, this color change can be seen with the naked eyes. Moreover, this method is simple and fast, as the S-PCR can be completed in less than 10 min using the newly built equipment and without the need for large instruments.

1. Introduction

Salmonella, a member of the family *Enterobacteriaceae*, is a gram-negative bacterium (Zhang & Zhu, 2002). *Salmonella* is a major cause of foodborne disease outbreaks (Ximenes, Ku, Hoagland, & Ladisch, 2019), with salmonellosis ranking highest in symptomatic food poisoning infections throughout the year. In its onset in animals, *Salmonella* can invade the blood circulatory system through the intestinal lymph nodes, thereby infecting the whole body. The detection of *Salmonella* is, thus, critical to prevent its spread and detection technologies have, therefore, received widespread attention in the scientific communities (Li, 2009; Liu, 2010).

At present, methods for the detection of *Salmonella* include predominantly traditional bacterial culture detection, immunodetection technology relying on the specific binding of antigen and antibody, and molecular biological detection, such as that using Polymerase Chain Reaction (PCR). Bacterial culture methods are disadvantaged by their long detection period and cumbersome process. Furthermore, it can't easily detect strains that are either difficult to culture or damaged by processing techniques or other food ingredients, so detection by these methods are not entirely effective. Immunological detection methods, including Immunomagnetic Separation (IMS) (Yu et al., 2009), Immuno Diffusion Technique (IDT) (Dudman, 1964), and Enzyme-Linked Immunosorbent Assay (ELISA) (Li, Xia, & Yu, 2013), have become relatively mature detection technologies in the laboratory. However, limitations in these systems, such as insufficient information, complicated methods, and false positive results caused by competitive substances or the structural analogues of target bacteria in samples (Li, Lv, Liu, Huang, & Yang, 2018), restrict the promotion and application of these immunological methods in the effective detection of foodborne pathogenic bacteria.

The molecular biology detection method is based on nucleic acid detection. The detection of strain is realized according to the genetic information of such strain. And results are, therefore, more accurate than the abovementioned methods. PCR technology is a milestone in the field of molecular biology and has dramatically altered how molecular studies are conducted. PCR technology has been widely used in various fields because of its sensitivity, specificity and simple operation. Li et al. (Li et al., 2012) proposed a strategy for the detection of *Salmonella* by integrating simple DNA extraction, specific PCR with an invA gene-based electrochemical DNA sensor. Zhang et al. (Zhang et al. (2018)) constructed serovar-specific PCR for *Salmonella* enterica serovar Indiana. In 2019, Xu et al. (Xu et al., 2019) established a multiplex PCR assay for detection of three *Salmonella* serovars, and the products were analyzed using 2% agarose gel electrophoresis. Furthermore, there are still many scientists designing biosensors to detect *Salmonella*

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based on PCR (Bai et al., 2013; Pham, Kieu The Loan, Jung, & Lee, 2018; Verdoy et al., 2012). These strategies can be used to detect *Salmonella*, but cannot be applied for rapid testing because it usually takes relatively long time for PCR amplification. Furthermore, the application of direct visual output based on PCR is seldom.

G-quadruplex DNA is a type of nucleic acid with special structure and function, which can convert nucleic acid signals into color (Zhang et al., 2011), fluorescent (Doria et al. 2015), electrochemical (Li, Li, Xia, & Wang, 2013) and optical signals (Lu, Li, Wang, & Tang, 2013). It can form DNAzyme with hemin that has weak horseradish peroxidase activity. The catalytic activity of G4/hemin DNAzyme is approximately ten times higher than that of hemin alone (Travascio, Li, & Sen, 1998) and it can facilitates the redox reaction between H_2O_2 and molecules such as 3,3′,5,5′-Tetramethylbenzidine (TMB) (Zhou, Kong, & Shen, 2009) and 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) (Yang, Li, Li, & Wang, 2010), which results in the appearance of colored product. There have been literatures reviewed the applications of G4/hemin in sensor design (Han, Feng, & Chen, 2015; Kong, 2011; Kosman & Juskowiak, 2011). Therefore, the applications of G4/hemin DNAzymes have been relatively mature.

In order to shorten the time of traditional PCR amplification reaction, and directly use PCR products for visual signal output and analysis. Based on the above, a Super PCR (S-PCR) prototype facility for enhancing the PCR speed was set up in this study. And a universal G-quadruplex-blocking hairpin primer with a blocking linker was designed and applied it to S-PCR to achieve rapid, highly sensitive and visual detection of *Salmonella*. In this biosensor, the primer was specially designed to form a hairpin structure, while the G-quadruplex sequence was attached to 5' end of the primer by spacer 18, a blocking linker, and blocked in the stem of the primer. The function of the blocking linker was to prevent the extension of the DNA polymerase to form a nucleic acid product with a single-stranded DNA sequence. After the extension of the S-PCR, the hairpin was opened and the G-quadruplex was released, combining with the hemin to exert peroxidase-like catalytic activity, which catalyzed the formation of colored substances by TMB. The DNAzymes could be used not only as a signal output element, to convert nucleic acid signals into color signals, but also for its catalytic activity, which could play a role in signal amplification. This method was an improvement on PCR technology, realized rapid PCR amplification and visual "turn on" detection of *Salmonella*.

2. Materials and methods

2.1. Materials

All oligonucleotide sequences, listed in Table S-1, were synthesized by Invitrogen (Shanghai, China). The *Salmonella* spp. were the preserved strain in the laboratory (Laboratory of Food Safety, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China). The 10× PCR Buffer (20 mM Mg^{2+} Plus), dNTPs mixture (2.5 mM each) and rTaq DNA polymerase were purchased from TaKaRa (Dalian, China). The hemin was bought from Sigma-Aldrich (St. Louis, MO, USA). TMB was purchased from Beijing Biyuntian Co., Ltd. (Beijing, China). The multimode microplate reader and NanoDrop 2000 ultra-micro nucleic acid/protein analyzer were supplied from Thermo Fisher Scientific (Life Technologies, Beijing, China). Other chemicals were of analytical grade and used without further purification.

2.2. DNA extraction from samples

First, 1.5 mL of the bacterial solution was placed in a 2 mL centrifuge tube and centrifuged at 10,000 r/min for 10 min to precipitate the cells. The supernatant was set aside for sample pretreatment. Subsequently, the *Salmonella* genome was extracted using improvised lysates, lotion A and lotion B, as well as a kit purchased from Beijing Fuan Technology Co., Ltd., used as per the manufacturer's instructions.

2.3. Primer design

The specific genomic sequence of *Salmonella* (invA gene, NC_003197.2) was selected through the GenBank website (https://www.ncbi.nlm.nih.gov/). Primer design was performed using Primer 5.0 design software. G-quadruplex sequences were introduced into the 5' end of the forward and reverse primers by spacer 18 blocking linker for functional design.

2.4. DNAzymes color rendering experiment

80 μL reaction buffer, 10 μL of 40 μM hemin, and 10 μL G-quadruplexes solution with 10 μM concentration were incubated at 37 ℃ for 30 min. Thereafter, 30 μL TMB chromogenic solution was added and incubation continued for another 20 min. Whereafter, 30 μL of 2 M H_2SO_4 solution was added to terminate the reaction. Absorbance values of 400–550 nm were determined by microplate reader and the absorbances at 450 nm was used to analyze the results.

2.5. Fabrication of Super PCR

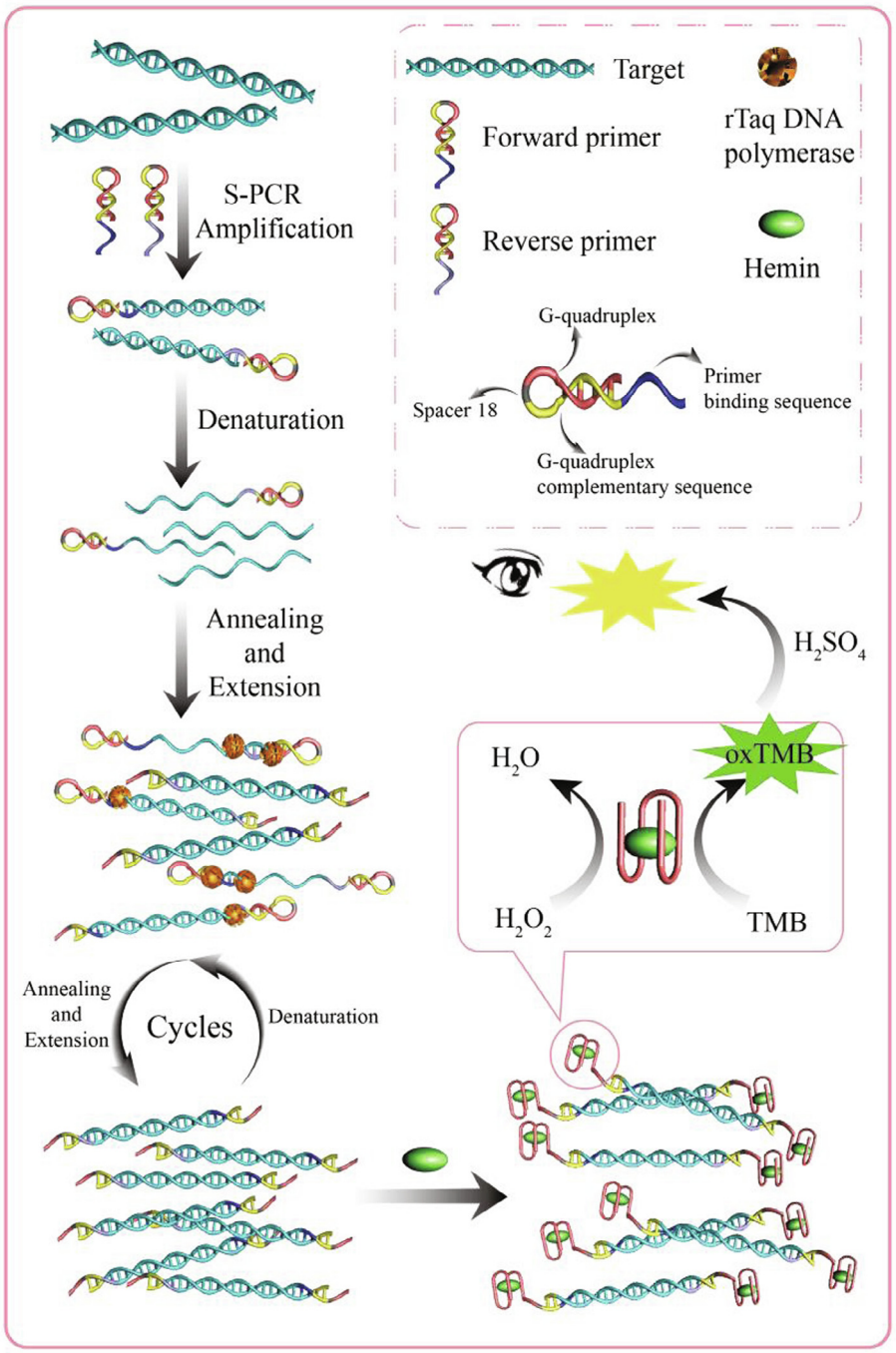
A hot water bath (HH-1, Junsi) of 96 ℃ and a warm water bath of 60 ℃ were used to change the temperature of reaction containers. A stepper motor (42JSF630AS-1000, Just Motion Control) was powered by switching the power supply (S-100-24, Elecall) and controlled using an AC Servo Motor Driver (YZ-ACSD608, Moving) and LabVIEW Software (version 2014). A thermocouple (T-type, HXW) centered in a dedicated control tube was used to measure the temperature. Thermocouple amplification and linearization were transmitted using a temperature transmitter (SBWR-2260, K, Yuancheng) and performed using an Arduino UNO v1.0 chip. The analog signal was digitized using the Arduino UNO chip and processed with Arduino IDE (version 1.8.1). Sample containers for the PCR were Light Cycler capillaries (20 μL, 04 929 292 001, Roche). Samples were spun down to the bottom of each tube by brief centrifugation and fixed on the 3d-printed plastic stent (Tian et al., 2018). The 20 μL S-PCR reaction system contained 1 × PCR Buffer, 250 μM of dNTP mixture, serial dilutions of genomic DNA extracted from pure *Salmonella* cultures, 1.5 U/μL rTaq DNA polymerase, and 1.4 μM of primers. S-PCR was triggered at 96 ℃ for 8 s and at 60 ℃ for 12 s for annealing and extension in one thermal cycle. The amplification results were analyzed by means of 2% agarose gel electrophoresis stained with ethidium bromide (EB) at 120 V for 25 min, performed on a Molecular Imager Gel Doc XR (Bio-Rad, Beijing, China).

2.6. Calculation of the limit of detection

The genomic mass concentration was measured using a micro-ultraviolet analyzer and converted according to the following equation:

$$\text{Copy number}(\text{copies}/\mu\text{L}) = \frac{6.02\times10^{23} \times 10^{-9} \times \text{concentration}(\text{ng}/\mu\text{L})}{\text{number of haploid genomic bases} \times 660}$$

Salmonella genome samples were prepared at concentrations of 1.5 copies/μL, 1.5×10^1 copies/μL, 1.5×10^2 copies/μL, 1.5×10^3 copies/μL, 1.5×10^4 copies/μL, 1.5×10^5 copies/μL, and 1.5×10^6 copies/μL, respectively. The amplifications and color reactions were carried out under the optimal experimental conditions. After the color rendering experiments were completed, the absorbances at 450 nm were detected by a microplate reader and the results were analyzed.



Scheme 1. The principle of the *Salmonella* detection system based on luminescent DNAzymes and universal blocking linker S-PCR.

3. Results and discussion

3.1. The principle of the assay

The principle of the "turn on" *Salmonella* detection system is shown in Scheme 1. The assay consisted mainly of two parts. In the first, S-PCR was used for target recognition and signal amplification to generate a large number of free G-rich nucleic acid sequences. Compared with conventional PCR, the forward and reverse primers for target recognition were wisely designed to be hairpin structures. A G-quadruplex sequence was attached to the 5' end of the primer through a Spacer 18 blocking linker, and was blocked in the stem by DNA hybridization to inhibit its ability to perform catalytic function, thereby reducing the background signal. Within ten minutes of the S-PCR reaction, the 20 μL samples trapped in prototype devices were dipped continuously in alternating hot water and warm water baths, while specific segments of the target were amplified with the unraveling of the hairpin structure and the chain extension blocked at the position of the Spacer 18. Following the S-PCR reaction, a large number of dsDNA products with ssDNA tails (G-quadruplex sequences) accumulated in the system. Subsequently, the released G-quadruplex sequences interacted with the hemin to form DNAzymes with strong peroxidase-like catalytic activity, in the presence of which the chromogenic substrate TMB was catalyzed to form oxTMB and the previous colorless system turned a visible blue. Thereafter, H_2SO_4 was added to terminate the chromogenic reaction, as a result of which the blue color turned yellow. The distinct absorption peak was measured at 450 nm and the visual detection of *Salmonella* was, thus, realized.

3.2. Screening of G-quadruplex sequences

According to literature reports, the catalytic activity of DNAzymes is closely related to the structure of G-quadruplex. Generally, a parallel structure has strong catalytic activity, a mixed parallel structure is less strong, while an anti-parallel structure is relatively weak. The structure of G-quadruplex is mainly determined by its sequence and, therefore, it is necessary to screen and optimize the G-quadruplex sequence. In addition, the catalytic ability of the G-quadruplex is affected by numerous other factors, such as ion concentration, and pH value.

Therefore, in order to ensure good experimental results and improved detection sensitivity, the screening experiment of the G-quadruplex sequence was first carried out under the conditions that the chromogenic system and temperature were completely identical. Five commonly used G-quadruplex sequences, namely PS 2.M, PS, PW17, FW and T3065, were selected for the G-quadruplex color rendering experiments. The specific sequences and results are shown in Table S-1 and Fig. S-1. From Fig. S-1, it can be seen that the FW sequence had the highest absorbance at 450 nm, and the TMB color rendering effect of the FW sequence was optimal. The FW sequence was, therefore, used for primer design.

3.3. Super PCR fabrication and feasibility verification

The prototype facility for Super PCR was constructed by our lab, as illustrated in Fig. 1(a). The main components of the Super PCR amplifier include: real-time temperature monitoring system that real-time temperature monitor in the container with fast heat conduction senses the real-time temperature; ultrathin PCR reaction container with fast heat conduction function; a stepper motor powered by power supply and controlled by program and an AC Servo Motor Driver, that can control the movement of the robotic arm; two temperature difference vessels that control the temperature of the reaction system; and a control host for real-time temperature monitoring and control of the stepper motor. Samples in ultrathin reaction containers fixed on the robotic arm were continuously flipped between different temperature vessels driven by stepper motor-powered robotic arm to obtain the predesigned temperature cycling program and realize amplification. Therefore, the Super PCR amplifier is very different from commercially available PCR thermal cyclers. Furthermore, the concentrations of DNA polymerase and primer pairs are the two key factors influencing the time required for PCR (Farrar & Wittwer, 2015). By optimizing the reaction system of Super PCR to achieve the maximum collision rate of reactants, 30 cycles of amplification can be completed in ten minutes, greatly reducing the reaction time of ordinary PCR.

To verify the rationality of primer design, the hairpin primer with the G-quadruplex and blocking linker were separately subjected to a S-PCR reaction with the common primer, and the reaction products were subjected to 2% agarose gel electrophoresis analysis. The results are shown in Fig. 1(b). The S-PCR products amplified by the common unmodified primers were 108 bp (lanes 1, 2). Due to the introduction of hairpin primers, the molecular weight of the PCR products increased, making the products migrate more slowly and closer to the sample holes (lanes 3, 4). Thus, the feasibility of primer design in this study was illustrated.

3.4. Optimization of the complementary sequence length of G-quadruplex

In the hairpin-shaped primer, the length of the G-quadruplex complementary sequence is one of the key points for controlling the background value of the experiment. If the length is too short, it will produce a high background signal. Therefore, this section explored the optimal number of nucleotides complementary to the G-quadruplex. The FW sequence has a total of 18 bases. 18, 16, 14, 12 and 10 complementary bases were considered for further investigation.

The results shown in Fig. 2 indicate that the nucleic acid sequences containing the complementary sequences to the G-quadruplex could reduce the color effect more effectively than a single G-quadruplex sequence. Furthermore, it was determined that the longer the complementary sequence, the lower the absorbance value at 450 nm, the

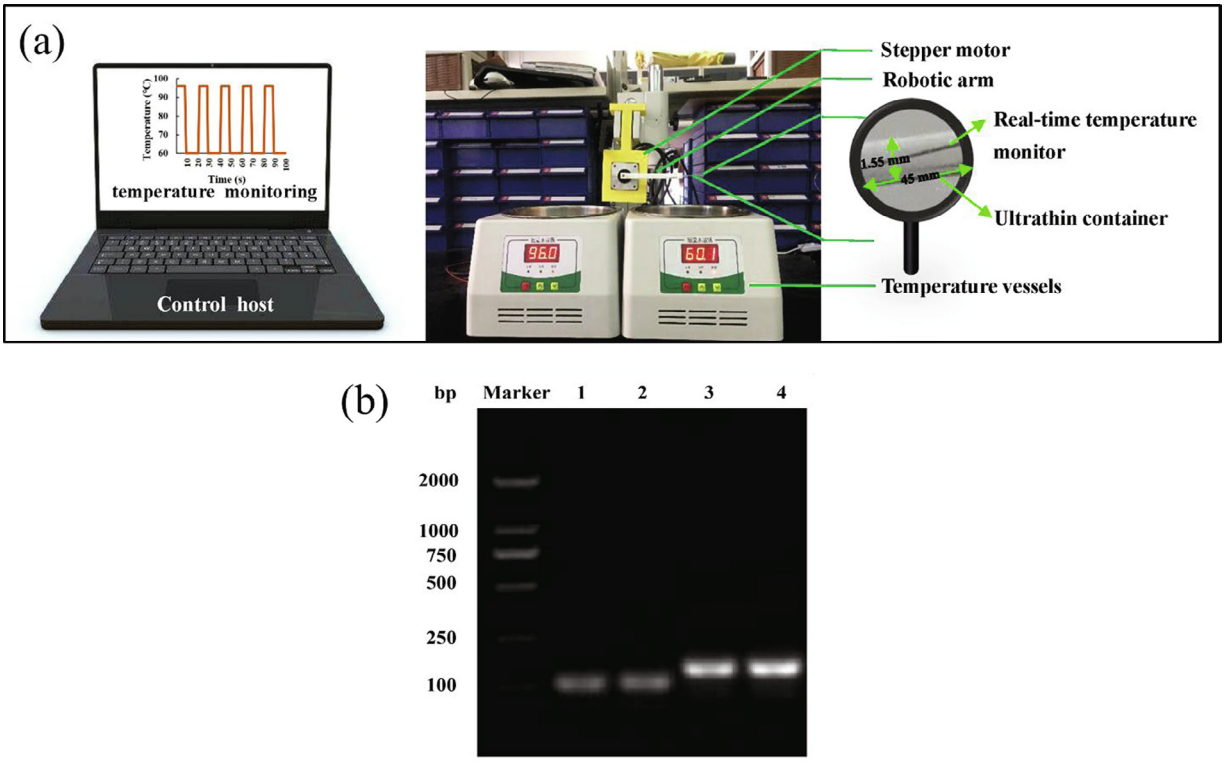


Fig. 1. (a) Picture and description of the S-PCR reaction device; (b) Analysis of S-PCR products by agarose gel electrophoresis (line 1 and line 2 are S-PCR products with common primers, line 3 and line 4 are S-PCR products with G-quadruplex-blocking hairpin primers).

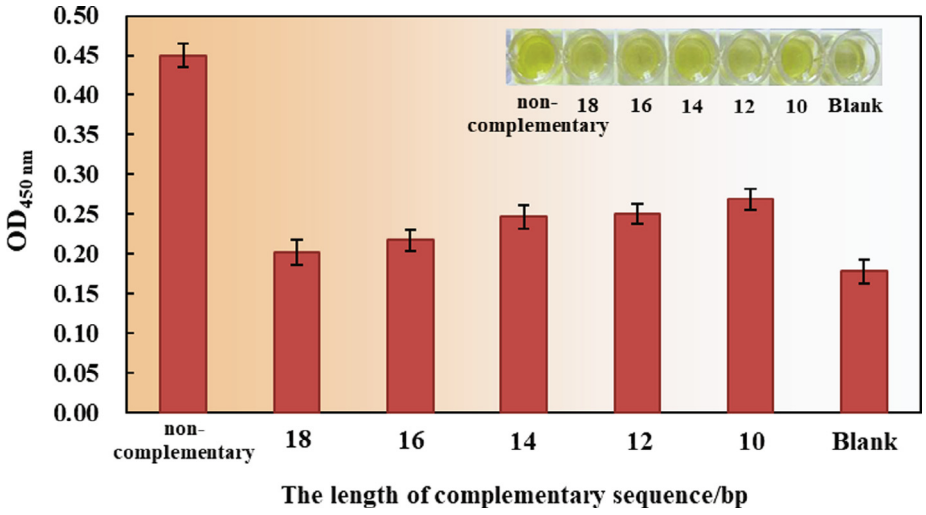


Fig. 2. Optimization results of the complementary sequence length of G-quadruplex, with an inset photograph of the corresponding system. Blank represents that the system did not contain any G-rich sequence.

lighter the color, and the better blocking effect of the G-quadruplex. This was because the longer the complementary sequence, the higher the Tm of the double-stranded fragment and the more difficult it became to destroy the structure. This, in turn, means that was harder to combine with hemin to form catalytically active complex, resulting in a decrease in colored product. In order to reduce the interference of the background signal, therefore, the G-quadruplex was completely blocked in the stem of the hairpin primer with a complementary length of 18 bp.

3.5. Optimization of primer concentration

In this strategy, the opening of the 5' end of the primers is the key to signal output. The exertion of DNAzymes activity depends on the release of G-quadruplex during PCR amplification. Therefore, the concentration of the primer with the G-quadruplex has a significant influence on the detection performance of the biosensor. If the primers are too concentrated, it will lead to too many unused hairpin primers, which make primer dimers easier to form, thus, affecting the amplification effect. Conversely, if the primer concentration is too low, the collision efficiency between molecules will be reduced, the amplification will also be affected, and it will lead to a small number of amplification products, insufficient DNAzymes, a poor color rendering effect, and the reduced detection performance of this biosensor. As a consequence, it was necessary to optimize the primer concentration. As can be seen from the electropherogram in Fig. 3(a), the bands of the S-PCR amplification products gradually brightened with the increase in the primer concentration. The change in brightness was not obvious after 1.2 μM, however the primer dimers began to appear when the concentration reached 1.6 μM. Fig. 3(b) shows that the absorbance at 450 nm changed with the concentration of primer. With the increasing of primer concentration, the absorbance increased continuously and slowly after 1.4 μM, which was consistent with the results of electrophoresis. Based on the above results, the primer concentration of 1.4 μM was selected for subsequent experiments.

3.6. Optimization of S-PCR reaction time

PCR detection is a traditional method of detecting *Salmonella*, but it requires relatively long reaction times. In order to shorten the reaction time of PCR and achieve rapid detection, a prototype device was developed in the laboratory. Samples in capillaries were continuously flipped between a 96 °C hot water bath and a 60 °C warm water bath for 30 cycles. In addition, the concentrations of two crucial factors (the primers and the DNA polymerase) in the S-PCR reaction system were also increased.

To meet the requirements of a shorter reaction time and higher amplification efficiency, the reaction time of S-PCR was optimized to complete 30 cycles in 11 min, 10 min, 9 min, 8 min and 7 min, respectively. The electropherogram results, in Fig. 4(a), show that, within the reaction times from 7 to 10 min, the amount of target amplification product increased rapidly, while the amount of primer dimers decreased sharply. However, at 10 min, the amplification product reached a maximum level and no primer dimers appeared. When the reaction time was extended a further 1 min, the amount of the product was significantly reduced and the primer dimers reappeared. These results were in agreement with the changes of absorbance at 450 nm with reaction time, shown in Fig. 4(b). When the reaction time was 10 min, the absorbance value was at its maximum. Therefore, 10 min was considered the optimal reaction time.

3.7. Sensitivity analysis

In order to explore the detection ability of the novel method for detecting *Salmonella*, samples containing different concentrations of the *Salmonella* genome were tested under the optimal experimental conditions, and the results are shown in Fig. 5. It can be seen from Fig. 5 that there was no significant linear relationship between the change in absorbance at 450 nm and the concentration of *Salmonella* genome. It is well known that, in theory, PCR is an exponential growth process, but the actual PCR amplification curve is an S-shaped curve. This is because with the increase in the number of PCR amplification cycles, the amplification scale increases rapidly, and various elements such as Taq polymerase, dNTP, primers, and even templates gradually fail to meet the demand, resulting in lower and lower PCR amplification efficiency and slower product generation speed. When all Taq polymerase is saturated, the PCR enters the plateau phase. Due to the complex interaction of various environmental factors, the time at which different reaction systems enter the plateau and the height of the plateau are greatly changed, which are difficult to accurately control. Even if the experiment is repeated, all conditions are basically the same, the number of DNA copies finally obtained is often different. Therefore, the amount of DNA products obtained by PCR amplification cannot reflect the actual amount of the initial templates, and there is no linear relationship between them. Hence, in this work, there was no linear relationship between the amount of S-PCR products and the concentration of *Salmonella* genome, making the final color signal independent of target concentration.

However, it is worth noting that, compared with the control group,

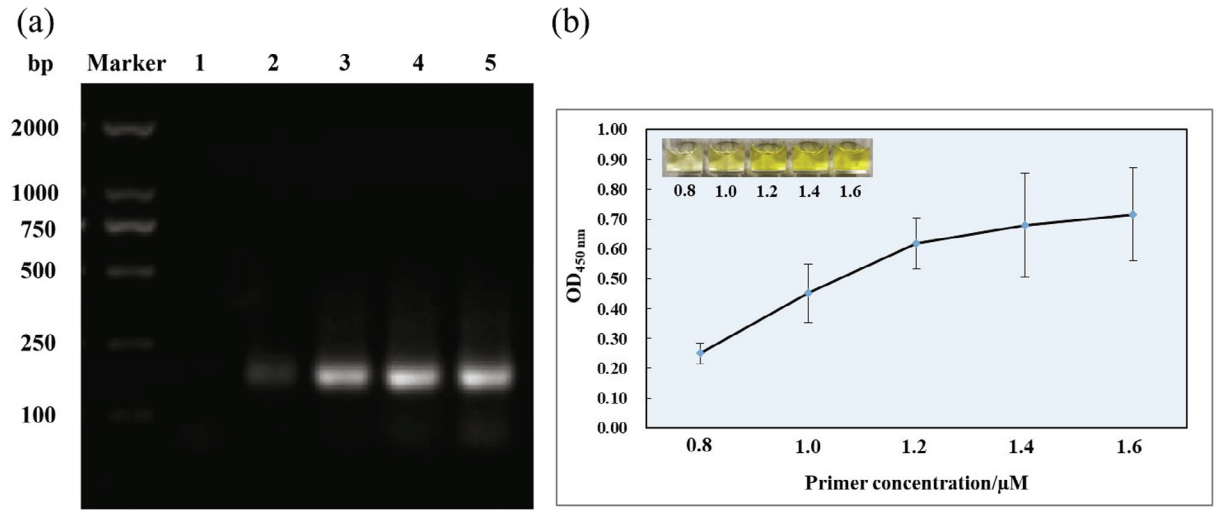


Fig. 3. Optimization results of primer concentration: (a) 2% agarose gel electropherogram (the primer concentrations from lane 1 to lane 5 are 0.8 μM, 1 μM, 1.2 μM, 1.4 μM, and 1.6 μM, respectively); (b) the changes in absorbance at 450 nm with the primer concentration. The inset is a photograph of the corresponding system.

a yellow change could be seen with the naked eyes when the concentration of *Salmonella* genome was as low as 1.5 copies/μL. Therefore, the visual biosensor constructed in this study did successfully detect 1.5 copies/μL of *Salmonella*, and the highly sensitive visual detection of *Salmonella* was realized. It is emphasized in relevant national standards that *Salmonella* should not be detected in food samples. This method can, therefore, be considered to have great potential for screening samples containing *Salmonella*. Furthermore, in comparison with the reported *Salmonella* detection methods summarized in Table S-2, the strategy in our study is not only sensitive but also fast and visual.

3.8. Specificity analysis

To investigate the specificity of this biosensor, some typical pathogens, including three gram-negative bacteria (*Shigella*, *Vibrio Parahaemolyticus*, and *Escherichia Coli*) and two gram-positive bacteria (*Staphylococcus Aureus* and *Bacillus Cereus*), were selected for comparison. The genomes of each strain were extracted using the same method as that of *Salmonella*, and *Salmonella* sample with a concentration of 10^5 copies/μL and samples of other non-specific strains with a concentration of 10^6 copies/μL were prepared for analysis. It can be seen from Fig. S-2 that the absorbance value of the *Salmonella* experimental group at 450 nm was much higher than that of other bacteria, and the visible yellow color was deeper. The results show that this method has high specificity and can be discriminated by the naked eyes alone, thus satisfying the detection requirements.

3.9. Real samples analysis

Using the newly constructed biosensor for actual sample detection is an important means to further evaluate whether the method has practical application potential. Therefore, we selected raw milk free of pathogens to verify. We added different amounts of *Salmonella* to the raw milk without *Salmonella*, and tested them with the biosensor to see whether they could cause the color change of the final reaction systems and be judged with the naked eyes. The results are summarized in Fig. 6. *Salmonella* genomes with concentrations of 1.5 copies/μL, 1.5×10^2 copies/μL and 1.5×10^4 copies/μL were added to raw milk, and the final reaction systems had more distinct yellow color than that of unadded milk, the absorbance values were also higher. Showing that this sensing system provides an acceptable analysis performance.

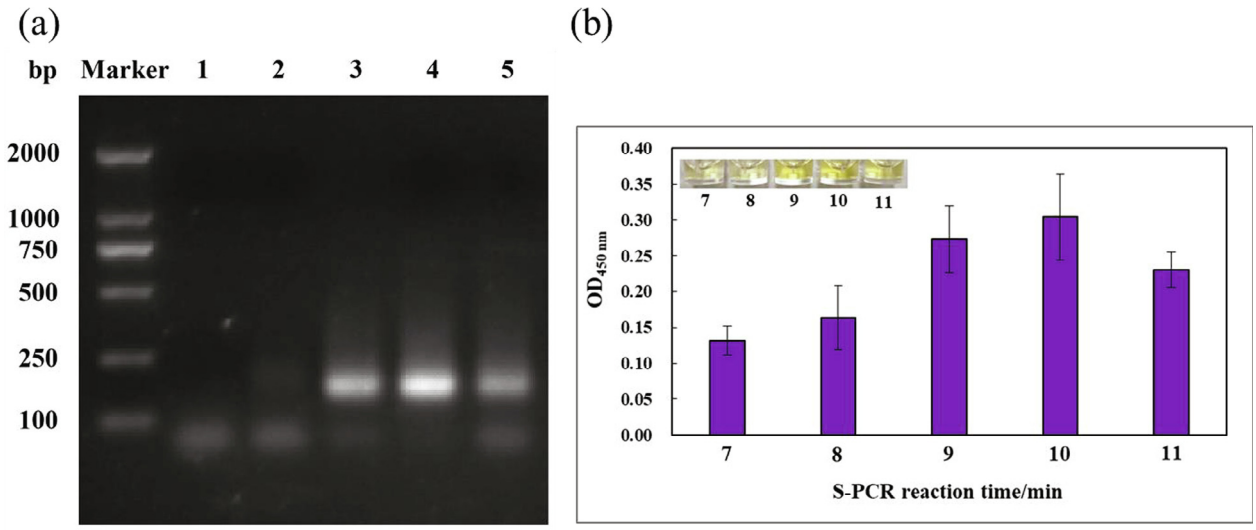


Fig. 4. Optimization results of S-PCR reaction time: (a) 2% agarose gel electropherogram (the S-PCR reaction times from lane 1 to lane 5 are 7 min, 8 min, 9 min, 10 min and 11 min, respectively); (b) the changes in absorbance at 450 nm with S-PCR reaction time. The inset is a photograph of the corresponding system.

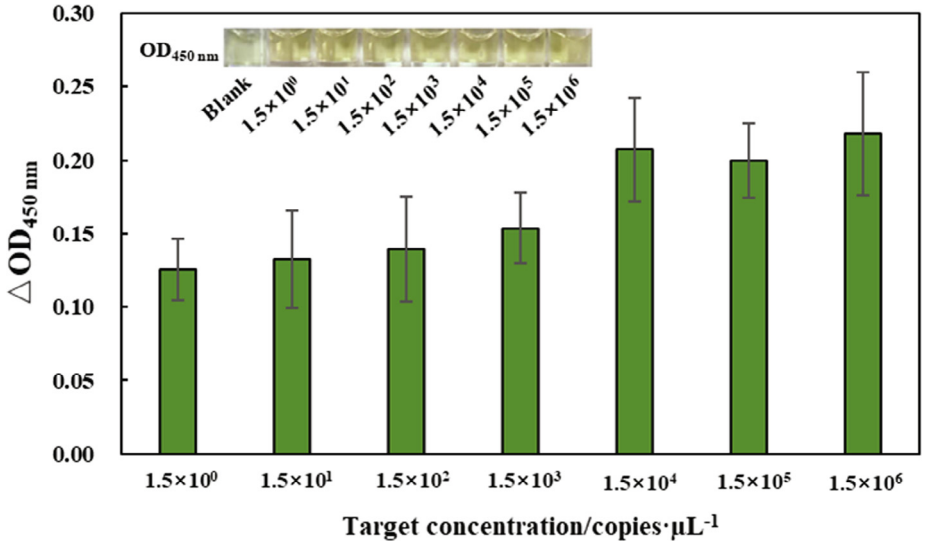


Fig. 5. Results of sensitivity analysis: the difference between the absorbance of the experimental group and the control group at 450 nm varies with the concentration of *Salmonella* genome. The inset is a photograph of the corresponding system.

4. Conclusion

In general, a super-fast, universal, "turn-on" visual biosensor for *Salmonella* detection based on luminescent DNAzymes and universal blocking linker Super Polymerase Chain Reaction was constructed. In this strategy, primers are specially designed and a G-quadruplex sequence is attached to the 5' end of the primer by Spacer 18, a blocking linker, and blocked in the stem of the primer. Through S-PCR, a large number of products with G-quadruplex single-strand sequences can be produced. Co-incubation with hemin stimulates the activity of peroxidase-like, and colored products are formed following the addition of TMB to achieve a "turn-on" visual signal output. The S-PCR can be completed in a short time of 10 min and the entire experiment process is simple to operate. The color variation of the samples containing 1.5 copies/μL *Salmonella* genome can also be observed by the naked eyes. Therefore, this method offers excellent potential for screening samples that may contain *Salmonella*. Moreover, the binding sequence in the primers can be slightly modified to facilitate the rapid detection of other target substances as well.

CRediT authorship contribution statement

ShuTing Li: Methodology, Investigation, Writing - original draft, Software. **Yuan Zhang:** Investigation, Validation. **JingJing Tian:** Investigation. **WenTao Xu:** Resources, Supervision, Conceptualization, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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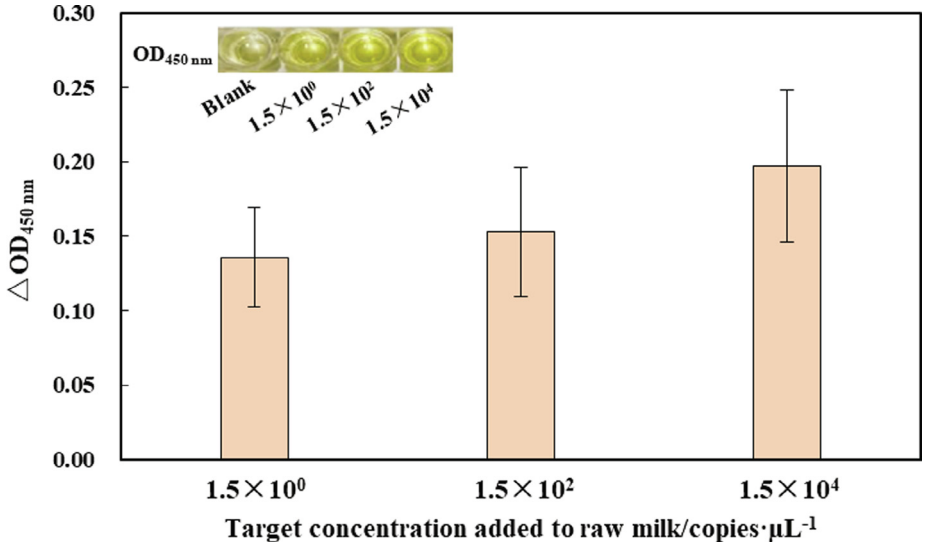

Fig. 6. Results of actual sample analysis: the difference between the absorbance of the experimental group and the control group at 450 nm varies with the concentration of added *Salmonella* genome. The inset is a photograph of the corresponding system.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.126859. The figures of the results of optimization of the G-quadruplex sequences and verification of the system specificity, and the tables of the DNA sequences used in this work and the comparison of detection limits for *Salmonella* sensors.

References

Bai, Y., Song, M., Cui, Y., Shi, C., Wang, D., Paoli, G. C., & Shi, X. (2013). A rapid method for the detection of foodborne pathogens by extraction of a trace amount of DNA from raw milk based on amino-modified silica-coated magnetic nanoparticles and polymerase chain reaction. *Analytica Chimica Acta, 787*, 93–101.

Doria, F., Oppi, A., Manoli, F., Botti, S., Kandoth, N., Grande, V., ... Freccero, M. (2015). A naphthalene diimide dyad for fluorescence switch-on detection of G-quadruplexes. *Chemical Communications, 51*(44), 9105–9108.

Dudman, W. F. (1964). Immune diffusion analysis of the extracellular soluble antigens of two strains of rhizobium meliloti. *Journal of Bacteriology, 88*(88), 782–794.

Farrar, J. S., & Wittwer, C. T. (2015). Extreme PCR: Efficient and specific DNA amplification in 15–60 seconds. *Clinical Chemistry, 61*(1), 145–153.

Han, G. C., Feng, X. Z., & Chen, Z. (2015). Hemin/G-quadruplex DNAzyme for designing of electrochemical sensors. *International Journal of Electrochemical Science, 10*(5), 3897–3913.

Kong, D. (2011). Applications of G-quadruplex-hemin DNAzymes in sensor design. *Progress in Chemistry, 23*(10), 2119–2131.

Kosman, J., & Juskowiak, B. (2011). Peroxidase-mimicking DNAzymes for biosensing applications: A review. *Analytica Chimica Acta, 707*(1), 7–17.

Li, F., Lv, Z., Liu, T., Huang, X., & Yang, H. (2018). Research progress in detection technology of foodborne pathogens. *China Animal Husbandry and Veterinary Abstracts, 34*(5), 61.

Li, J., Xia, K., & Yu, C. (2013). Detection of *Alicyclobacillus acidoterrestris* in apple juice concentrate by enzyme-linked immunosorbent assay. *Food Control, 30*(1), 251–254.

Li, Q., Cheng, W., Zhang, D. C., Yu, T. X., Yin, Y. B., Ju, H. X., & Ding, S. J. (2012). Rapid and sensitive strategy for *Salmonella* detection using an InvA gene-based electrochemical DNA sensor. *International Journal of Electrochemical Science, 7*(1), 844–856.

Li, X. (2009). Clinical symptoms and preventive measures of *Salmonella* food poisoning. *Chinese Medical Innovation, 6*(35), 194–195.

Li, Y., Li, S., Xia, G. Q., & Wang, Y. (2013). Label free detection of adenosine based on hemin/G-quadruplex peroxidase-mimicking DNAzyme as bioelectrocatalyst. *Asian Journal of Chemistry, 25*(18), 10541–10545.

Liu, Y. Y. (2010). On-site first-aid treatment and preventive measures for *Salmonella* food poisoning. *China Health Nutrition: Journal of Clinical Medicine,* (1), 115–116.

Lu, Y., Li, X., Wang, G., & Tang, W. (2013). A highly sensitive and selective optical sensor for Pb2+ by using conjugated polymers and label-free oligonucleotides. *Biosensors & Bioelectronics, 39*(1), 231–235.

Pham, Q. N., Kieu The Loan, T., Jung, S. W., & Lee, N. Y. (2018). Microdevice-based solid-phase polymerase chain reaction for rapid detection of pathogenic microorganisms. *Biotechnology and Bioengineering, 115*(9), 2194–2204.

Tian, J., Huang, K., Luo, Y., Zhu, L., Xu, Y., & Xu, W. (2018). Visual single cell detection of dual-pathogens based on multiplex super PCR (MS-PCR) and asymmetric tailing HCR (AT-HCR). *Sensors and Actuators B: Chemical, 260*, 870–876.

Travascio, P., Li, Y., & Sen, D. (1998). DNA-enhanced peroxidase activity of a DNA-aptamer-hemin complex. *Chemistry and Biology, 5*(9), 505–517.

Verdoy, D., Barrenetxea, Z., Berganzo, J., Agirregabiria, M., Ruano-Lopez, J. M., Marimon, J. M., & Olabarria, G. (2012). A novel Real Time micro PCR based Point-of-Care device for *Salmonella* detection in human clinical samples. *Biosensors & Bioelectronics, 32*(1), 259–265.

Ximenes, E., Ku, S., Hoagland, L., & Ladisch, M. R. (2019). Accelerated sample preparation for fast *Salmonella* detection in poultry products. *Foodborne Bacterial Pathogens, 1918*, 3–20.

Xu, J., Zhang, P., Zhuang, L., Zhang, D., Qi, K., Dou, X., ... Gong, J. (2019). Multiplex polymerase chain reaction to detect *Salmonella* serovars Indiana, Enteritidis, and Typhimurium in raw meat. *Journal of Food Safety, 39*(5).

Yang, X., Li, T., Li, B., & Wang, E. (2010). Potassium-sensitive G-quadruplex DNA for sensitive visible potassium detection. *Analyst, 135*(1), 71–75.

Yu, Z., Mingqiang, Y., Qiangguo, C., Nengqin, J., Yu, G., & Hebai, S. (2009). Simultaneous detection of multifood-borne pathogenic bacteria based on functionalized quantum dots coupled with immunomagnetic separation in food samples. *Journal of Agricultural and Food Chemistry, 57*(2), 517–524.

Zhang, J., Gao, Q., Chen, P., Chen, J., Chen, G., & Fu, F. (2011). A novel Tb3+-promoted G-quadruplex-hemin DNAzyme for the development of label-free visual biosensors. *Biosensors & Bioelectronics, 26*(10), 4053–4057.

Zhang, P., Zhuang, L., Zhang, D., Xu, J., Dou, X., Wang, C., & Gong, J. (2018). Serovar-specific polymerase chain reaction for detection of *Salmonella* enterica Serovar Indiana. *Foodborne Pathogens and Disease, 15*(12), 776–781.

Zhang, Y., & Zhu, C. (2002). Overview of *Salmonella* and fungal distribution in China. *Preventive Medicine, 29*(3), 400–401.

Zhou, X. H., Kong, D. M., & Shen, H. X. (2009). Ag^+ and cysteine quantitation based on G-quadruplex–hemin DNAzymes disruption by Ag^+. *Analytical Chemistry, 82*(3), 789–793.