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Integration of logic gates to CRISPR/Cas12a system for rapid and sensitive detection of pathogenic bacterial genes

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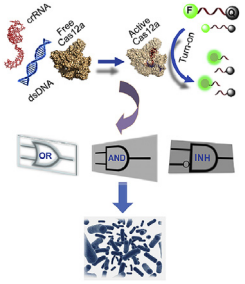
HIGHLIGHTS

- For the first time, three 2-input elementary AND, OR, INHIBIT logic gates have been constructed by CRISPR-Cas12a system.
- The AND gate responded rapidly to *Staphylococcus aureus* with high sensitivity and specificity.
- The total time was 2.0 h, the limit of detection (LOD) was 10^3 CFU/mL, and the dynamic range was 10^3–10^7 CFU/mL.
- The sequence addressability enabled this AND logic gate to accurately trace back and distinguish input genes.
- These above-mentioned features were highly ideal to incur a rapid response to pathogenic bacteria for decision making.

GRAPHICAL ABSTRACT



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ABSTRACT

For the first time, three 2-input elementary AND, OR, INHIBIT logic gates have been constructed by using CRISPR-Cas12a system. These logic gates utilised the intrinsic advantages of programmability, sequence specificity and high base resolution of CRISPR-Cas12a system. Among them, the AND gate owned the potentials as a built-in biosensor that responded rapidly to external pathogenic bacteria such as *Staphylococcus aureus* with high sensitivity and specificity. We applied the CRISPR-Cas12a based bacterial detection after a target-amplification using PCR. The total sample-to-answer time was appropriately 2.0 h, the limit of detection (LOD) was 10^3 CFU/mL, and the dynamic range was 10^3–10^7 CFU/mL. Also, the sequence addressability enabled this AND logic gate to accurately trace back and distinguish input genes. These above-mentioned features were highly ideal to incur a rapid response to pathogenic bacteria for decision making. Our results not only validated the possibility of using CRISPR-Cas systems for constructing bio-computing devices but also provided a prototype of biosensor for rapid and intelligent pathogenic bacteria detection.

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1. Introduction

Biomolecular logic gates have attracted great attentions for performing computational operations, digital data storage and information processing that are central for complex man-made machinery [1,2]. They utilise biomolecules including nucleic acids, proteins, peptides and bacteria within logic functions to fulfill these purposes in test tubes or living cells [3–6]. This spawns intelligent computation at molecular levels to mimic their electronic counterparts. Also this creates unprecedented potentials for developing unconventional computing devices with rational control capability in a range of applications such as sensing and diagnosis [7–12]. In particular, DNA, as a carrier of genetic information, is promising to be used for constructing high-capacity and low-maintenance digital information processing devices due to their dynamic structures, modularity, complexity and sequence specificity [13–15]. It is noted that binary Boolean logical operations are the basis of current electronic computation [16]. Simply, such logic gates generate output states (0 or 1) depending on the input conditions (0 or 1) that use simplified 'true' (1, high value) or 'false' (0, low value).

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated proteins (Cas), together referred to as CRISPR-Cas systems. CRISPR-Cas systems act as adaptive immune systems in bacteria and archaea [17]. Over the last few decades, several CRISPR-Cas systems have been discovered [18]. It is recently found that Cas12a (also called Cpf1), as a member of class 2 type V-A CRISPR-Cas effector, is an RNA-guided DNA endonuclease. At present, Cas12a is an eye-catching research topic owing to its simplicity and huge potentials for high DNA targeting specificity *in vivo*; thereby it could be an alternative to Cas9 in gene editing [19]. Cas12a is guided by a single CRISPR RNA (crRNA), and unlike Cas9, it does not need *trans*-activating crRNA (tracrRNA). Together with a T-rich protospacer adjacent motif (PAM) sequence, Cas12a is able to cleave double-stranded DNA (dsDNA) targets [20]. However, the cleavage of single-stranded DNA (ssDNA) targets by Cas12a is PAM-independent [21]. Besides, Cas12a is capable of collateral cleavage (also termed as *trans*-cleavage) on non-targeted ssDNAs upon the formation of the Cas12a-crRNA-target DNA ternary complex. This striking feature has already been explored in terms of nucleic acid detection to provide CRISPR-Cas based diagnostic such as DETECTR and HOLMES [22,23]. Other recent work by using the *trans*-cleavage feature of Cas12a for bio-sensing includes the detection of virus, mycoplasma, single nucleotide polymorphism (SNP), exosome, crop disease diagnosis and genetically modified organisms (GMOs) as well as small molecules [23–31]. Furthermore, a recent paper describes the programmability of Cas12a-based DNA processing; and combining it with strand displacement-based reaction circuits give rise to logic gated transcriptional control of gene expression in *E. coli* cells [32]. All these achievements highlight the importance and potential applications of CRISPR-Cas12a as a new generation of detection platform. CRISPR-Cas12a system is also reminiscent of other novel CRISPR-Cas systems with regard to the applications in biosensing [33–39].

2. Experimental

2.1. Materials and reagents

Staphylococcus aureus (S. aureus), Escherichia coli (E. coli), Salmonella spp., *Lactobacillus, Acetobacterium Balch, Agrobacterium tumefaciens,* human colonic adenocarcinoma cells HT29, human gastric carcinoma MGC803 cells and human cervical carcinoma HeLa cells were stored within the group. RNA and DNA oligonucleotides were synthesized by Synbio Technologies (China). Baird-Parker agar base was purchased from Hopebio-Technology Co., Ltd. (China). Common chemicals were obtained from Sigma-Aldrich (USA) or Aladdin Biochemical Technology Co., Ltd. (China) unless otherwise specified. They were analytical grade and used without further purification. Ribonuclease (RNase) inhibitor (#M0314S) was purchased from New England Biolabs (USA). 2 x Taq PCR MasterMix II was purchased from Tiangen Biotech Co., Ltd. (China). Distilled deionized water (ddH_2O) was used throughout the investigation. The DNA and RNA concentrations were quantified using 260 nm absorbance by a UV–Vis spectrophotometer with the extinction coefficients calculated using online IDT OligoAnalyzer 3.1 (http://www.idtdna.com/calc/analyzer#).

2.2. Bacterial genomic DNA extraction and PCR amplification of femA gene of S. aureus

Bacterial genomic DNA was extracted with MiniBEST Bacteria Genomic DNA Extraction Kit Ver.3.0 (TaKaRa Biotechnology) according to the manufacturer's guidelines. The PCR cycling protocol for *femA* gene amplification was pre-denaturing at 95 °C for 5 min, followed by 21 cycles with denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 5 s.

2.3. LbCas12a protein purification

The protein expression vectors having *Lachnospiraceae bacterium* ND2006 Cas12a protein (LbCas12a, GI number 917059416) gene cloned were transformed into BL21 (DE3) competent cells using the heat shock method. Simply, a mixture of chemically competent bacteria and plasmid DNA was placed at 42 °C for 90 s and then placed back on ice. BL21 (DE3) cells, transformed with the plasmids, were grown overnight in Luria-Bertani (LB) medium supplemented with 100 μg/mL kanamycin, which was used to inoculate in LB for growth at 37 °C and 160 rpm. Isopropylb-D-1-thiogalactopyranoside was added to a final concentration of 0.5 mM to initiate protein expression after the cell optical density (OD_{600}) reached 0.4–0.6. The cultures were then shaken for an additional 25 h at 16 °C, and then harvested by centrifugation at 8,000 rpm for 15 min at 4 °C. Cell pellets were harvested and stored at −20 °C for later purification. All subsequent steps of the protein purification were performed at 4 °C. The collected cells were crushed and resuspended in lysis buffer (50 mM Tris-HCl, 1.5 M NaCl, 1 mM DTT, pH 8.0) supplemented with 1 mM phenylmethylsulfonyl fluoride (PMSF) followed by sonication (XO-650D, Atpio, Nanjing, China). The lysate was centrifugated for 30 min at 12000 rpm and the supernatant was filtered through a 0.22 μm filter. Filtered supernatant was applied to column containing Ni Sepharose™ 6 Fast Flow (GE) and incubated with rotation for 2 h followed by washing three times by using lysis buffer. The target protein was eluted with a stepwise gradient of 50, 100, 200 mM imidazole. The collected eluted protein was dialysed against buffer (50 mM Tris-HCl, 200 mM NaCl, 1 mM DTT, 5% glycerol, pH 8.0) and then concentrated using Millipore concentrators. The protein concentration was measured based on Lambert-Beer law and frozen at −80 °C.

2.4. Preparation of crRNA

For preparation of crRNA, a single-stranded cognate DNA oligonucleotide with T7 promoter sequence was annealed with a primer sequence having a complementary T7 primer sequence (final concentrations 5 μM), which was then transcribed to crRNA by using the HiScribe T7 Quick High Yield RNA Synthesis Kit (New England Biolabs). This transcription reaction was carried out at 37 °C overnight. Subsequently, the product crRNA was purified using Monarch® RNA Cleanup Kit (New England Biolabs).

2.5. Construction of OR, AND and INHIBIT gates

For each gate, it has 200 nM purified LbCas12a, 250 nM crRNA, 50 nM quenched fluorescent reporter and input 10 nM ssDNA, dsDNA or RNA where necessary, in a 100 μL reaction buffered with 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 10 mM $MgCl_2$,1 mM DTT and 1 U/μL RNase inhibitor.

2.6. Standard curves of LbCas12a for S. aureus and selectivity

The extracted genomic DNA was used as a template for setting up the standard curve for LbCas12a in terms of *S. aureus* detection. Assays were performed with 200 nM purified LbCas12a, 250 nM crRNA, 50 nM quenched fluorescent ssDNA reporter and varying concentrations of input nucleic acid target, in a 100 μL reaction buffered with 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT and 1 U/μL RNase inhibitor. Assays were carried out at 37 °C and kinetic data were recorded in a Tecan Infinite® 200 Pro plate reader (Tecan, Switzerland) with excitation and emission wavelengths at 484 nm and 529 nm respectively and fluorescent intensities were measured every 30 s. The different bacterial concentrations expressed as ($\log^{CFU/mL}$) were plotted against the corresponding fluorescent values. Each data point was tested in triplicate. The LOD was defined as lowest detectable quantities of bacteria. The selectivity for *S. aureus* detection by using CRISPR-Cas12a was evaluated over some other strains including *Escherichia coli (E. coli), Salmonella* spp., *Lactobacillus, Acetobacterium Balch, Agrobacterium tumefaciens,* human colonic adenocarcinoma cells HT29, human gastric carcinoma MGC803 cells and cervical carcinoma HeLa cells.

2.7. Real time quantitative PCR (qPCR)

For qPCR experiments, the genomic DNA extraction step was described in 2.2 Bacteria genomic DNA extraction and PCR amplification of *femA* gene of *S. aureus*. Each reaction was performed in a 20 μL mixture containing 10 μL 2 × SYBR Green qPCR Master Mix (Bimake.com, USA), 0.5 μL gDNA of *S. aureus* (template), 0.4 μL 50 × ROX reference dye and 200 nM of each primer. The cycling protocol was 95 °C for 20 min, 40 cycles of denaturation (95 °C, 3 s), and annealing and extension (60 °C, 30 s), using an Applied Biosystems StepOne™ Real-Time PCR System. The different bacterial concentrations (expressed as $\log^{CFU/mL}$) were plotted against the corresponding quantification cycle (Cq) values.

2.8. Detection of S. aureus in milk samples

Milk samples were purchased from the local supermarket. Varied unknown amount of *S. aureus*, in the range of 10 to 1×10^4 CFU/mL, was randomly spiked into 10 milk samples, and then messed with 10 sterilized milk samples. These 20 milk samples were prepared in a double-blind preparation. Samples were then used directly after dilution without any further process. Cas12a based method and real-time PCR were used to detect each sample, respectively.

3. Results and discussions

Here, we reported the progress toward the goal to create elementary OR, AND, INHIBIT logic gates by using CRISPR-Cas12a and its potential application in detecting the gene of pathogenic bacteria. The fundamental principle has been depicted in Fig. 1, simply, *Staphylococcus aureus* (*S. aureus*) is used as a model, the genomic DNA (gDNA) is firstly extracted, and a marker gene of interest is amplified accordingly using a conventional PCR instrument. In this investigation, we have chosen a species-specific *femA* gene (694 bp in length; GenBank accession No. AF144661) that encodes the factor necessary for methicillin resistance (Fig. 1A) [40]. The PCR product is then readily subjected to Cas12a recognition with the aid of crRNA, which triggers the *trans*-cleavage of a pre-added doubly labeled ssDNA reporter. The cleavage of ssDNA reporter results in de-quenching of FAM fluorophore from BHQ1 (black hole quencher 1), giving much enhanced fluorescence. The changes of fluorescence intensity could be easily recorded and processed for quantification (Fig. 1B). The purified *Lachnospiraceae bacterium* Cas12a protein (LbCas12a) used throughout this study is shown in Fig. S1; the *femA* gene amplification and crRNA preparation are shown in Fig. S2. More importantly, this proposed strategy can be incorporated into an AND logic gate (Fig. 1C). Specifically, the dsDNA obtained using PCR works as the input 1 and with input 2 (cognate crRNA in this case), they are able to perform the AND gate computation, which could be used as an intelligent decision making machine. This decision making machine rapidly responds to external pathogenic bacterial genes. It can also be reasonably estimated that the entire sample-to-answer time consisting of gDNA extraction, PCR-based gene amplification and *trans*-cleavage is approximately 2.0 h, which is far less than the conventional culture-based methods.

To be detail, for the AND gate (Fig. 2A), it started from a solution composed of LbCas12a and ssDNA reporter, and the individual addition of crRNA or dsDNA did not trigger *trans*-cleavage at all. Only if all four elements were completely presence, namely LbCas12a, crRNA, target and ssDNA reporter such as what happened in (1,1), the enhanced fluorescence resulted from the cleavage of ssDNA reporter can be executed. For the OR gate (Fig. 2B), the initial state in solution phase consisted of LbCas12a, crRNA and fluorescent ssDNA reporter, which could be defined as (0,0). In this situation, there was only basal level of fluorescence, as LbCas12a would not perform *trans*-cleavage on ssDNA reporter in the absence of correct input target. Therefore a '0' output can be achieved. For (0,1) and (1,0) combinations, 1 denoted the separate addition of corresponding dsDNA and ssDNA targets respectively,

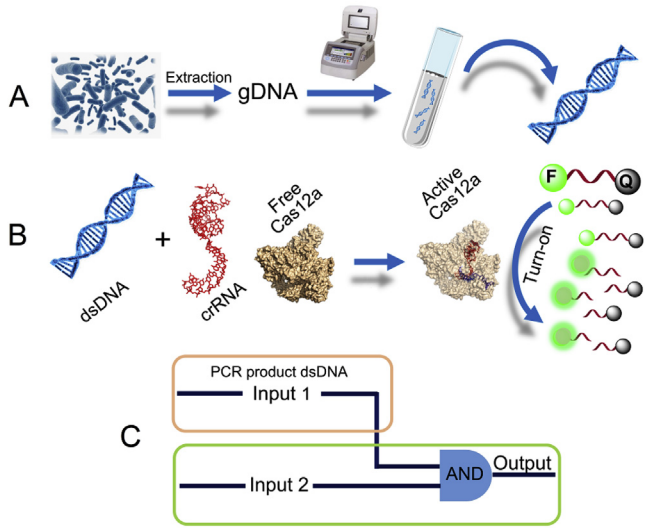

Fig. 1. The schematic illustration of CRISPR-Cas12a based logic gates with the function for detecting *S. aureus*. (A) Gene amplification by using conventional PCR. (B) The amplified *femA* gene of *S. aureus* is served as input 1, and with input 2 (cognate crRNA) to initiate *trans*-cleavage of the reporter, cutting off a fluorophore and a quencher modified ssDNA. This *trans*-cleavage produces 'turn-on' fluorescent signals. (C) The AND logic gate fabricated using CRISPR-Cas12a enables the detection of *S. aureus*. Please note in this study, F = FAM and Q = BHQ1.

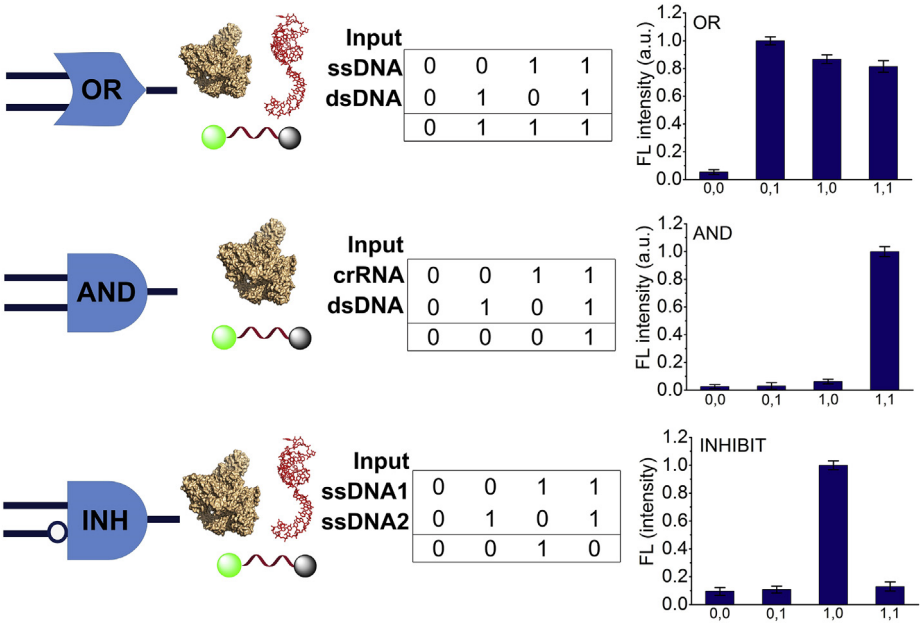

Fig. 2. Three logic gates constructed by using CRISPR-Cas12a system. (A) to (C) are AND, OR and INHIBIT logic gates, respectively. Form leftmost to right for each gate are the electrical symbol, initial components in the solution, the truth table and the bar graph representing the fluorescent output. The error bars represent the standard deviations in three individual experiments.

which can be recognised by crRNA. In both cases, *trans*-cleavage can be activated and this resulted in high fluorescence '1'. The (1,1) indicated high fluorescence output, which can be attributed to the addition of both dsDNA and ssDNA in the same test tube. For the INHIBIT gate (Fig. 2C), it was composed of LbCas12a, crRNA and ssDNA reporter, the (0,1) represented the addition of ssDNA2 strand that was not supposed to be recognised by crRNA, as it was not the correct substrate for LbCas12a. Thereby, this did not give rise to any obvious influence on fluorescence intensity. While the correct ssDNA1 input (1.0) was capable of *trans*-cleavage stimulation, since it can be recruited to form Cas12a-crRNA-dsDNA complex. This generated an output as '1'. Then, we added both ssDNA input in (0,1) and (1,0) to execute the (1,1) combination. These two ssDNAs (ssDNA1 and ssDNA2) were complementary to each other and we pre-annealed them to make them double-stranded, since this dsDNA did not have a PAM, it was not capable of *trans*-cleavage activation an a low fluorescence '0' can be reached.

We tried to test if our proposed AND logic gate design could be further explored to detect *S. aureus*. and the possibility toward its real use. Foodborne diseases are the major concern worldwide. Bacteria are the causative factors of nearly two thirds of foodborne outbreaks. *S. aureus* is arguably a predominant cause of gastroenteritis, resulting from the consumption of contaminated food [41]. The sensitive and selective detection of *S. aureus* is urgently needed. To begin with, we scrutinized the feasibility of the *trans*-cleavage of LbCas12a. It was found that LbCas12a was capable of *trans*-cleavage (Fig. S3A); more importantly, It was also confirmed that there was a dsDNA target concentration-dependent fluorescent signal increment for LbCas12a *trans*-cleavage (Fig. S3B), implying a huge possibility for LbCas12a to quantitatively sense dsDNA.

In order to use CRISPR-Cas12a for *S. aureus* detection, some factors that are in correlation with the performance of the fluorescence measurements, have been investigated in detail. As shown in Fig. 3, the results were represented by using fluorescence intensity (FL) increase rate *I*; $I = \frac{\text{FL of group with target DNA}}{\text{FL of group without target DNA}}$ for this optimization. In this scenario, pH value as well as the concentrations of Tris-buffer, Na^+ and Mg^{2+} were all tested. We decided to choose pH value at 8, since it not only gave a good *I* value but also it had the highest FL upon target DNA recognition (Fig. 3A). It was found that for the most previous publications that Tris-HCl was adopted for CRISPR-Cas12a [21–23], thus we intended to use the same buffer. The optimal concentrations of Tris-HCl, Na^+ and Mg^{2+} were determined at 50, 100 and 10 mM, respectively (Fig. 3B–D).

The schematic of illustrations of dsDNA target and crRNA used in current study are shown in Fig. 4A. Upon the completion of the experimental conditions optimization, the relationship between fluorescence intensity and the concentrations of spiked *S. aureus* was tested. With incremental amounts of *S. aureus*, more PCR product dsRNA can be eventually obtained and thus more LbCas12a mediated *trans*-cleavage on reporter was expected. This gave rise to a detectable increase in fluorescence. There was a linear relationship ($R = 0.999$) can be obtained over the range from 10^3 to 10^7 CFU/mL and a 10^3 CFU/mL limit of detection (LOD) can be noticed (Fig. 4B). The LOD and dynamic range of Cas12a based method is better of comparable than the most of reported methods using techniques including electrochemistry, SPR (surface plasmon resonance), SERS (surface enhanced raman spectroscopy), colorimetric methods, lateral flow immunoassay, *etc*. A summary of this comparison can be found in Table S2. The concentrations of spiked *S. aureus* were detected using traditional plate counting method that was considered as a 'gold standard' method. The results were shown in Fig. S4. The selectivity for *S. aureus* over some other common prokaryotic and eukaryotic cells was crucial for its practical use, which needed careful evaluation. Fig. 4C presented the histograms of fluorescence intensities for *S. aureus* and other cell samples. It can be observed that compared with *S. aureus*, the other interference (both prokaryotic and eukaryotic cells) did not influence the system much, as the fluorescence intensities were almost undisturbed. It hence can be concluded that these competing cells had only a negligible effect on our detection system. This approach exhibited high selectivity for *S. aureus*, which enabled its scope for future use.

Lastly, a side-by-side comparison was performed in order to compare our proposed Cas12a based method with a well-proved

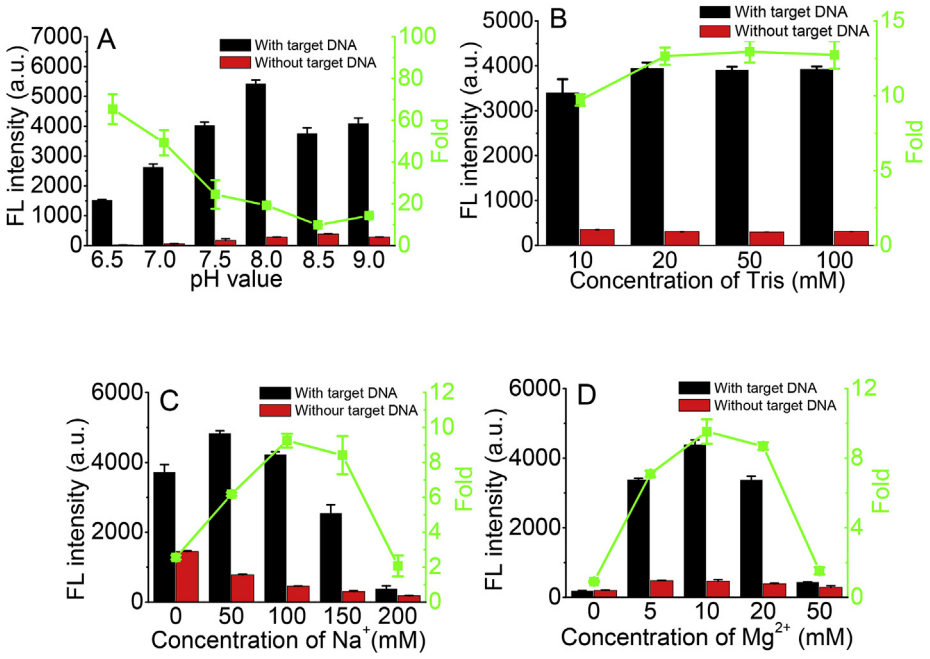

Fig. 3. The optimization of experimental conditions of CRISPR-Cas12a biosensor for *S. aureus* detection. Please note 200 nM Lbcas12a, 250 nM crRNA, 10 nM target ssDNA and 50 nM reporter were mixed in the reaction with varied conditions. The buffer used was Tris-HCl with different pH values, NaCl, $MgCl_2$ and 1 mM DTT. The pH value as well as the concentrations of Tris-buffer, Na^+ and Mg^{2+} were subjected to optimization in Fig. 3A–D, respectively. The error bars represent the standard deviation in three individual experiments. The fluorescence intensity (FL) increase rate (green square) was used as indicator for this optimization. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

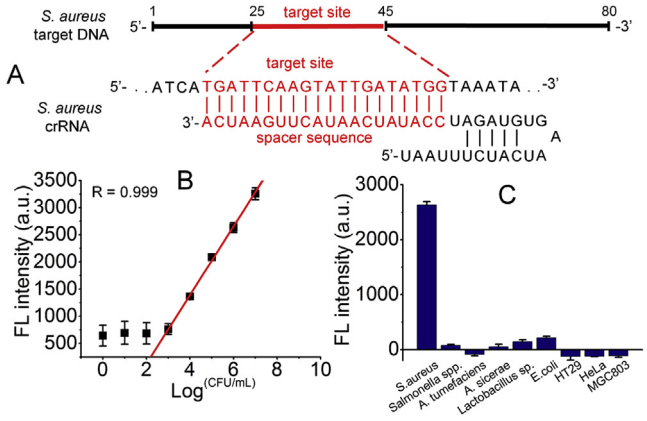

Fig. 4. Schematic illustration of the detection of pathogenic bacterial genes using AND logic gate. (A) dsDNA target and crRNA used in the current study. (B) The dependence of fluorescence on *S. aureus* concentrations and the linear relationship for our proposed Cas12a method. (C) Selectivity of proposed AND logic gate for *S. aureus* sensing over other common cells. Please note that a 10^4 CFU/mL concentration was adopted for each type of cell in this test. The error bars represent the standard deviation in three individual experiments.

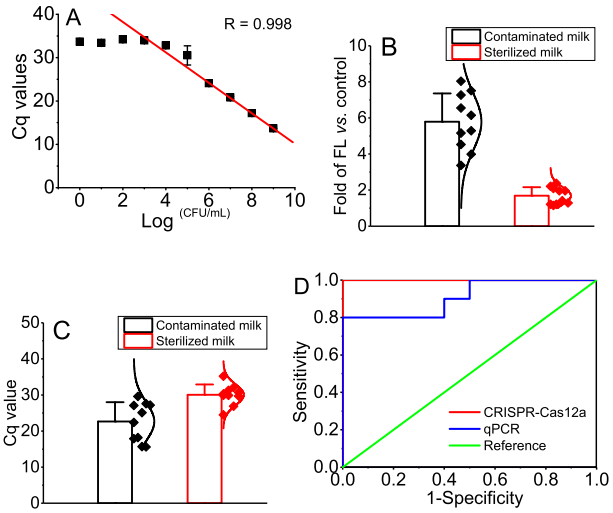

Fig. 5. The comparison of between qPCR and CRISPR-Cas12a based methods for *S. aureus* detection. (A) The dependence of Cq values on *S. aureus* concentrations and the linear relationship derived from qPCR method. (B) Fluorescence data derived from CRISPR-Cas12a and qPCR methods for both sterilized and contaminated milk samples in the double-blind experiment. (C) Cq vaules derived from qPCR methods for both sterilized and contaminated milk samples in the double-blind experiment. (D) ROC curve analysis of a double-blind experiment for contaminated and sterilized milk samples. The error bars represent the standard deviations in three individual experiments.

and perhaps the most widely used one, namely real time quantitative PCR (qPCR). As shown in Fig. 5A, it seemed to us that qPCR method demonstrated comparable competency in *S. aureus* detection in spiked samples. In addition, we would like to further compare the sensitivity and specificity between our method with qPCR. In order to so, we designed a double-blind experiment for distinguishing contaminated and sterilized milk samples using both methods (Fig. 5B and C). The ROC (receiver operating characteristic) curves were accordingly employed. As shown in Fig. 5D, the ROC curve of Cas12a based method showed an AUC of 1.0. By contrast, qPCR was inferior in separating contaminated milk samples from the sterilized ones, evidence by a lower AUC value (0.90).

In conclusion, for the first time, we have successfully constructed three 2-input elementary logic operations by using CRISPR-Cas12a system. This choice of logic gates was selected as it represented one example of a complete set of logic gates, indicating

that they can be wired together into multi-level or cascade circuitry to execute more complex purposes [42]. There are several achievements of our current work listed as follows. Firstly, there are a number of previous publications about building logic gates using functional nucleic acids such as aptamer, DNAzyme or structure-specific DNA. But a limitation of the current DNA-based logic gate designs is a short of direct interfacing with biological components other than oligonucleotides [43]. Enzyme–DNA interactions can be found in a variety of important biological events such as DNA replication, repair as well as gene expression [44]. But it is rare to see they are adopted for perform enzyme-interfaced DNA computing. In fact, enzyme–DNA interactions bear a high substrate specificity, as these nucleic acid manipulating enzymes are either structure- or sequence-dependent [45,46]. In addition to this, the diversities of nucleic acids resulted from their structures, dynamic, or base combinations are rather striking [47,48]. The specificity and diversity of enzyme–DNA interactions would be considerably beneficial for logic gate construction in terms of reducing background signal, increasing signal to noise, minimising false positive, enhancing sensitivity. Our results, in this regard, validated the applications of enzyme−DNA interactions as platforms for the fabrication of addressable elementary arithmetic operations. The CRISPR-Cas12a could be replaced in potential by other DNA interacting enzymes, which greatly expands the input and output possibilities. The possible future direction of the CRISPR-Cas12a based logic gates could be the applications of them in DNA process machines such as biocomputing security system, DNA steganography, *etc*. Secondly, our endeavour also expands the applications of CRISPR-Cas that has already been an intensively researched area [49−51]. Thirdly, our logic gate design is not only conceptually proved, it also has practical applications in terms of rapid and sensitive detection of pathogenic bacterial genes. We applied the CRISPR-Cas12a based bacterial detection after a target-amplification using PCR. In terms of rapidity, traditional bacterial culture experiments usually take about 2−3 days or even longer [52]. With regard to some conventional detection methods, for examples, electrochemistry requires ~4.5 h to complete [53]; SERS (Surface Enhanced Raman Spectroscopy) and PCR need 3 h [54,55]. The LOD is low, the entire sample-to-answer time is rather short and the selectivity is high, in comparison to the most widely used methods. The sequence addressability enables this AND logic gate to accurately trace back and distinguish input genes. These features are highly ideal to incur a rapid response to pathogenic bacteria for decision making. In terms of the limitation, we believe every logic gate design has its own. For what we have proposed, the limitation could be that the readouts of these logic gates are outputted via fluorescence signals only and varied other signals could be applied in the future design. All in all, our study will open up new avenues and provide a straightforward way to construct logic gates based on CRISPR-Cas system. This, as we believe, will drive the development of expanding fields such as DNA programming and biological computing, which can be applied in areas such as food safety inspection, medical diagnosis, environment monitoring, *etc*.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2020.05.017.

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