**Electronic Supplementary Information**

# The construction of a novel DNA-based comparator and its application in intelligent analysis

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.

## 1. Experimental section

### Chemicals and instruments

All chemicals (analytical grade) were purchased from Titan technology Co. Ltd. (Shanghai, China) and used without further purification. Synthetic oligonucleotides were purchased from Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). And DNA stock samples were dissolved by physiological mimic solution (10 mM $K_2HPO_4/KH_2PO_4$, 140 mM KCl, 5 mM $MgCl_2$ and 10 mM NaCl, termed PBS) and quantified by measuring their UV-visible absorption at 260 nm with an EVOLUTION 201 spectrophotometer (Thermo SCIENTIFIC, USA) at 25 °C in a 10 mm quartz cell. The water was prepared by an ULUPURE (Chengdu, China) ultrapure water system and used throughout the experiments.

### Fluorescent assays

Fluorescence spectra were recorded with a F-4600 spectrophotometer (HORIBA, Japan) in a 10 mm quartz cell at the room temperature, Xenon arc lamp was used in the excitation light source in fluorescence measurement. The excitation wavelength was set at 485 nm and 580 nm for the fluorescence assays of FAM and ROX, respectively. Both excitation and emission slits were 5 nm, and the voltage was 400 V with a scan speed of 1200 nm/min.

### The comparator logic operation

Logic circuit was constructed by using commercial oligonucleotides as described (Table S1). A total of four DNA mixtures were prepared, without adding **A** and **B**, adding 200 nM **A**and 10 nM **B**, adding 10 nM **A** and 200 nM **B**, and adding 200 nM **A** and 200 nM **B** to a solution containing 200 nM **Reg1**-**Tran1**, **Reg2**-**Tran2**, **MB1**, and **MB2**. Each sample was incubated at 37 °C for 20 min, and then the fluorescence spectra of FAM and ROX were collected. In *E. coli* lysate, the samples were prepared by diluting DNA using *E. coli* lysate, other operations were same as the one in PBS. The final volume of the sample was 500 μL.

### Detection of A (SA) and B (SE) in *E. coli* lysate

The pure culture of *E. coli* strains Top 10 was grown in 5 mL Agarose broth with 0.1% Ampicillin at 37°C overnight under 270 rpm rotation. Then the resultant bacterial cells were separated and washed three times in ultrapure water by centrifugation (12000 rpm, 5 min). Then, the lysate was prepared by incubated bacterial cells at 100°C for 15 min. For the dominant pathogenic bacteria experiment, different concentrations ratios of **A (SA)** and **B (SE)** gene was added into mixture of 150 nM **Reg1**-**Tran1**, **Reg2**-**Tran2**, 200 nM **MB1** and **MB2**, and then incubated for 20 min at 37°C before the fluorescence measurement. The final volume of the sample was 500 μL in PBS. The excitation wavelength was set at 485 nm and 580 nm for the fluorescence assays of FAM and ROX, respectively. Both excitation and emission slits were 5 nm, and the voltage was 400 V with a scan speed of 1200 nm/min.

For the LODs experiment, different concentrations of **A (SA)** or **B (SE)** gene was added into mixture of 150 nM **Reg1**-**Tran1**, **Reg2**-**Tran2**, 200 nM and **MB1** and **MB2**, and then incubated for 20 min at 37°C before the fluorescence measurement.

### Native polyacrylamide gel electrophoresis (PAGE)

Non-denaturing polyacrylamide gel (20%) was prepared by was prepared by mixing 5 mL 30% crylamide/bis-acrylamide gel solution (29:1), 1.5 mL 5×TAE buffer (50 mM Tris-Ac, pH 7.4), 950 μL ultrapure water, 45 μL 10% ammonium persulfate, 5 μL 8 N,N,N',N'-tetramethylethylenediamine. Each sample was prepared accorrding to the logic operation and was subjected to heat at 37°C for 20 min. A total of 3 μL from each sample was mixed with loading buffer (3 μL) before loading onto the gel. In each sample, the concentration of each DNA strand was 5 μM. And Gel electrophoresis was run in the same buffer at the room temperature for 120 min under a voltage of 90 V. The gel was then stained by Stains-all overnight, faded with ultrapure water, and photographed by camera.

## 2. Oligonucleotide sequences used in this work and analysis of the complementary condition.

**Table S1.** The oligonucleotide sequences used in constructing comparator and specificity experiment in this work.

| Name | Sequence (from 5' to 3') |
|---|---|
| **A=SA** | GCT CAT CTA AAC TTG ACT TAC CTG ACT CAC TACT TCT GAA CCT CTG |
| **B=SE** | GTC AGA GTG AGT CAG GTA AGT TGA TGT TGA AGA GTG ATA |
| **Reg1** | TAG GAG GTT CAG AGT GAG TCA GGT AAG TCA AGT TTA GAT GAG |
| **Reg2** | T TAT CAC TCT TCA ACA TCA ACT TAC CTG ACT CAC TCT GAC TTT TTT |
| **Tran1** | CTG ACT TAC CTG ACT CAC TCT GAA CCT CCT AGC ACT G |
| **Tran2** | GTC AGA GTG AGT CAG GTA AGT TGA TGT TGA GAT C |
| **MB1** | **(FAM)** ACTACGA GGTTCAGAGTGA TCGTAGT **(Dabcyl)** |
| **MB2** | **(ROX)** CCTTGAG AACATCAACTTAC CTCAAGG **(BHQ-2)** |
| **MASA** | GTATGCTTTGGTCTTTCTGCATTCCTGGAA |
| ***E. coli*** | TGCCGCTCATCCGCCACATATCCT |
| **A-13** | GCT CAT CTA AAC GTG ACT TAC CTG ACT CAC TACT TCT GAA CCT CTG |
| **A-8-13** | GCT CAT CGA AAC GTG ACT TAC CTG ACT CAC TACT TCT GAA CCT CTG |
| **B-28** | GTC AGA GTG AGT CAG GTA AGT TGA TGT CGA AGA GTG ATA |
| **B-28-33** | GTC AGA GTG AGT CAG GTA AGT TGA TGT CGA AGT GTG ATA |
| **mec A** | TGGTCTTTCTGCATTCCTGG |

The **FAM** and **ROX** represent fluorophores modified at 5’ of **MB1** and **MB2**, respectively. And **Dabcyl** and **BHQ-2** represent the fluorescence quencher labeled in 3’ of **MB1** and **MB2**, respectively.

The underline bases represent the mutation bite.

**Table S2.** The analysis of complementary base numbers and free energy of each duplexes.

| Name of duplex | Number of complementary bases | Free energy ($kcal.mol^{-1}$) |
|---|---|---|
| Bac1-Reg1 | 29+10 | -46.31 |
| Bac2-Reg2 | 39 | -49.94 |
| Reg1-Tran1 | 29 | -39.60 |
| Reg2-Tran2 | 29 | -39.07 |
| Tran1-Tran2 | 19 | -26.70 |
| Tran1-Sig1 | 13+3 | -19.39 |
| Tran2-Sig2 | 15 | -19.52 |
| MB1 | 7 | -4.26 |
| MB2 | 7 | -4.25 |

The free energy was obtained by NUPACK analysis.

## 3. The fluorescence change “close” and “open” of MB1 and MB2

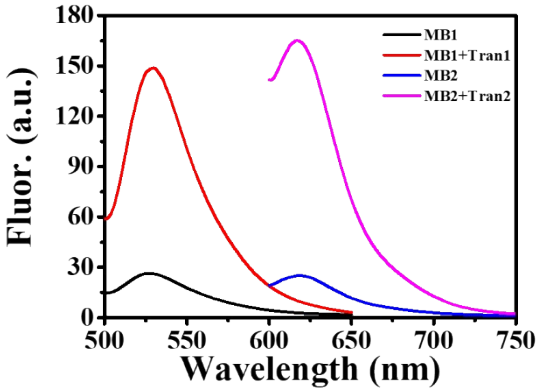


**Figure S1.** The fluorescence change of FAM and ROX in the absence and presence of **Tran1** or **Tran2** in the solution containing **MB1** and **MB2**.

## 4. DNA secondary structure of each complexes in this work by NUPACK analysis

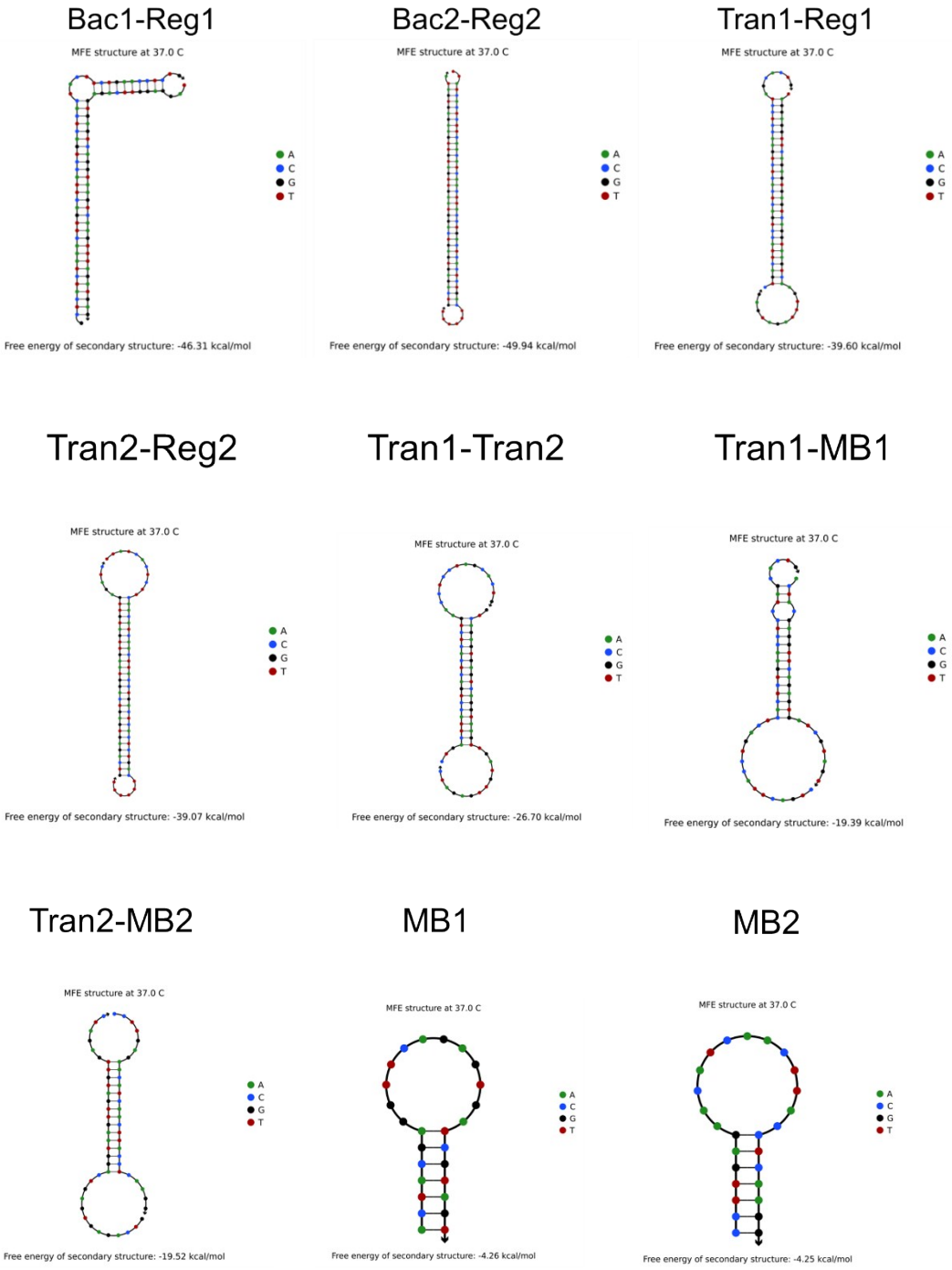


**Figure S2.** The secondary structures of different DNA complexes by NUPACK analysis.

## 5. The validation of the comparator: polyacrylamide gel electrophoresis (PAGE) experiments

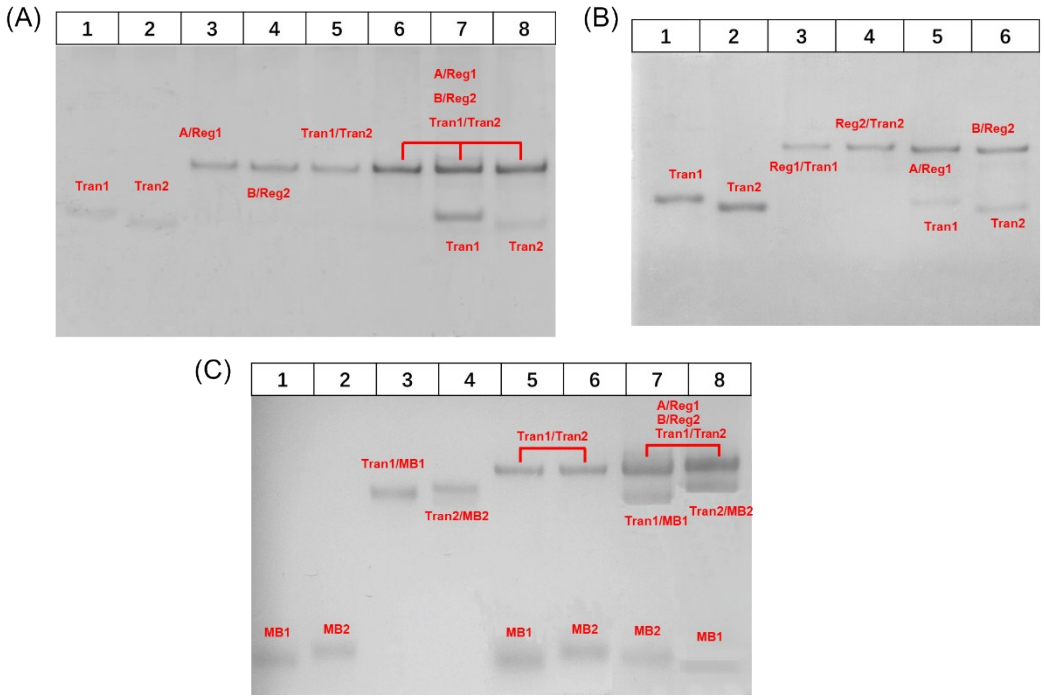


**Figure S3.** Polyacrylamide gel analysis of the strands used in the comparator. (A) Lane 1: **Tran1**; Lane 2: **Tran2**; Lane 3: **Reg1** + **Tran1**; Lane 4: **Reg2** + **Tran2**; Lane 5: **A** + **Reg1** + **Tran1**; Lane 6: **B** + **Reg2** + **Tran2**. (B) Lane 1: **Tran1**; Lane 2: **Tran2**; Lane 3: **A** + **Reg1**; Lane4: **B** + **Reg2**; Lane 5: **Tran1** + **Tran2**; Lane 6: **A** + **B** + **Reg1** + **Reg2** + **Tran1** + **Tran2**; Lane 7: **A** + **B** + **Reg1** +**Reg2** + 2 **Tran1** + **Tran2**; Lane 8: **A** + **B** + **Reg1** + **Reg2** + **Tran1** + 2 **Tran2**. (C) Lane 1: **MB1**; Lane 2: **MB2**; Lane 3: **Tran1** + **MB1**; Lane 4: **Tran2** + **MB2**; Lane 5: **Tran1** + **Tran2** + **MB1**; Lane 6: **Tran1** + **Tran2** + **MB2**; Lane 7: **A** + **Reg1** + **B** + **Reg2** + 2 **Tran1** + **Tran2** + **MB1** + **MB2**; Lane 8: **A** + **Reg1** + **B** + **Reg2** + **Tran1** + 2 **Tran2** + **MB1** + **MB2.** Concentrations for each DNA in PAGE are all 5μM.

The feasibility of the DNA hybridizations in Figure 2 was characterized by native polyacrylamide electrophoresis (PAGE) experiments. In sensing module (Figure S3A), the results of lane 1-2 demonstrated the individual single strands, **Tran1** and **Tran2**, respectively. Lane 3-4 in Figure S1B, no toehold-mediated displacement reaction occurred without addition of targets and thus the duplexes of **Tran1**/**Reg1** and **Tran2**/**Reg2** can be observed. However, in the presence of target **A** or **B**, the gel band of **Tran1**/**Reg1** or **Tran2**/**Reg2** disassembled to produce a new band of **A**/**Reg1** or **B**/**Reg2** and **Tran1** or **Tran2** was released (lane 5-6), indicating the oeders of stability: **A-Reg1** or **B-Reg2** > **Tran1-Tran2**.

In comparing module, the bands represented the individual single strands of **Tran1** and **Tran2** in lane 1-2, respectively. It was observed that the new bands in lane 3-4 validated the formation of duplexes of **A**/**Reg1**, **B**/**Reg2**, respectively. In the mixure of **Tran1** and **Tran2**, a new band appeared at a different position, validating the formation of duplex of **Tran1-Tran2** (Lane 5). If lane 5 and lane 6 in Figure S1A were mixed, namely lane 6 in Figure S1B, it could be seen that the released **Tran1** and **Tran2** have hybridized with each other. Although the locations of **A-Reg1**, **B-Reg2**, and **Tran1-Tran2** cannot disdinguished, the bands in lane 6 became dark and bold, indicating the formation of **A-Reg1**, **BReg2**, and **Tran1-Tran2** complexes. The results showed that **A** and **B**were equal, the displaced **Tran1** and **Tran2** could completely offset as expected. When **A** > **B**, namely the displaced **Tran1** > **Tran2**, the lower band in lane 7 showed the excess **Tran1**. In contrast, if **A** < **B** the excess **Tran2** would be remained (lane 8).

In signal module, the results of lane 1-2 in Figure S1C showed the locations of **MB1** and **MB2**. Lane 3-4, no toehold-mediated displacement reaction occurred without addition of **Tran1(2)** strands and thus the duplexes of **Tran1**/**MB1** and **Tran2**/**MB2** can be observed. However, in the presence of target **Tran1** and **Tran2**, the gel band of **Tran1**/**Tran2** disassembled to produce a new band of **Tran1-Tran2**, and **MB1** or **MB2** was released (lane 5-6), indicating that the oeders of stability: **Tran1-Tran2** > **Tran1-MB1** or **Tran2-MB2**. Lane 7-8 tested whether integration of three modules could be realized as expected. It was observed there was the bands of **Tran1-MB1** and **MB2** (Lane 7), likewise the bands of **Tran2-MB2** and **MB1** were also obtained, which were consistent with the Figure 2.

To summarize, the orders of stability as follows: **A-Reg1** or **B-eg2** > **Tran1-Tran2** > **Tran1-MB1** or **Tran2-MB2**.

## 6. UV temperature experiments

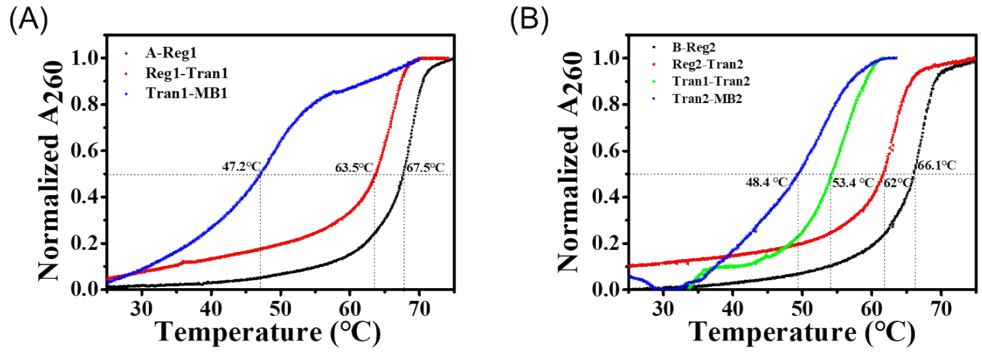


**Figure S4.** Melting profiles of 1 μM of four duplexes in PBS in pH 7.12 buffer. The absorbance was monitored at 260 nm and normalized.

To further verified the stability order of these DNA complexes, the absorbance of them at 260 nm were monitored ranging from 25 ℃ to 75 ℃. The results showed that melting point of **A-Reg1**, **B-Reg2**, **Reg1-Tran1**, **Reg2-Tran2**, **Tran1-Tran2**, **Tran1-MB1** and **Tran2-MB2** was 67.5 ℃, 66.1 ℃, 63.5 ℃, 62 ℃, 53.4 ℃, 48.4 ℃ and 47.2 ℃, respectively, indicating the stability order is **A-Reg1** or **B-Reg2** > **Tran1-Tran2** > **Tran1-MB1** or **Tran2-MB2**.

## 7. The kinetics study of the system

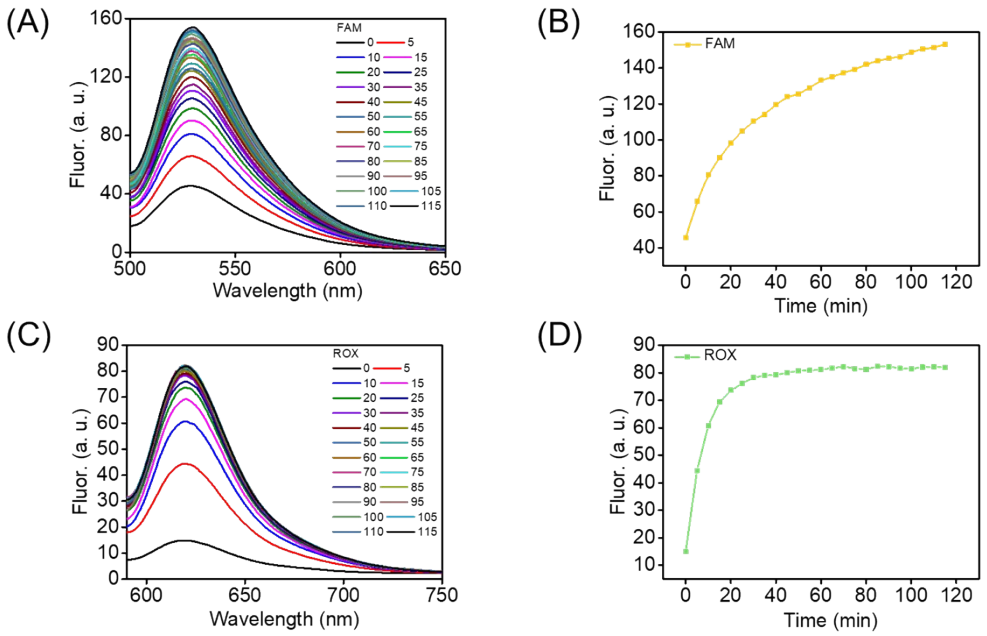


**Figure S5.** The dynamic analysis of the system. (A) The fluorescence spectra of FAM when **SA** is in excess. (B) The fluorescence spectra of ROX when **SE** is in excess (C) The kinetics of the comparator at 528 nm when **SA** is in excess. (D) The plots of ROX when **SE** at 616 nm is in excess upon time titration. The study was carried out at 37℃.

We determined the response kinetics of the comparator (Figure S5). As shown in Figure S5C, when **A (SA)** was in excess, the maximum fluorescence signal of FAM was reached within 20 min. Similarly, when **B (SE)** was in excess, the 90% fluorescence signal of ROX was reached within 20 min. The data indicated that the DNA logic system can provide a fast and effective response toward the targets. [1]

## 8. Determining the quantitative relationship between inputs

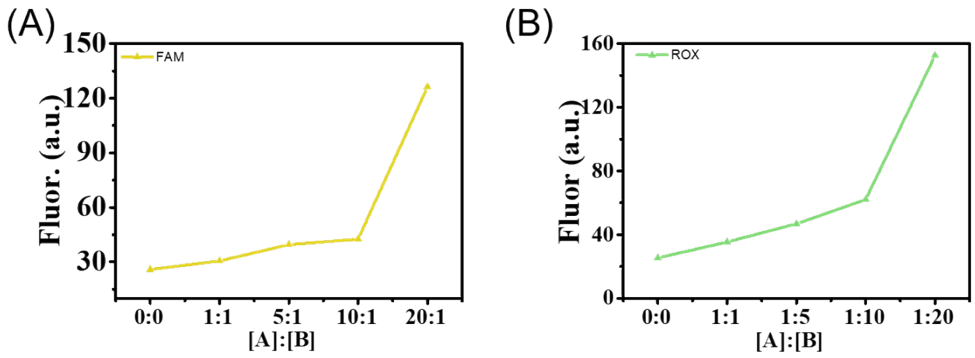


**Figure S6.** The plots of FAM at 526 nm (A) and ROX at 616 nm (B) fluorescence intensities in response to increasing different concentration ratios of A : B.

The fluorescence change were monitered after adding the target different ratios **A** and **B** into the solution containing 200 nM **Reg1-Tran1**, **Reg2-Tran2**, **MB1** and **MB2**. As shown in Figure S6, when the ratio between inputs was 20, the fluorescence of FAM and ROX was significantly enhanced. Thus, 20-fold was selected to construct the comparator.

## 9. The influence of buffer pH and salt on the comparator in *E. coli* lysate.

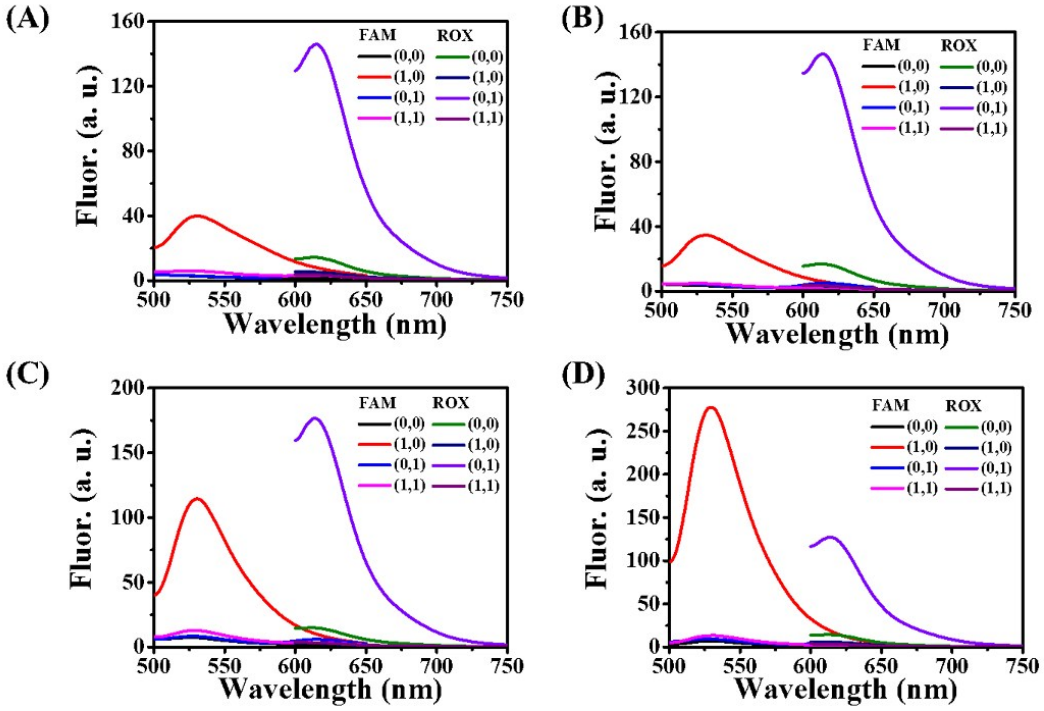


**Figure S8.** The fluorescence spectra of different buffer pH: 5.0 (A), 6.0 (B), 7.0 (C) and 8.0 (D).

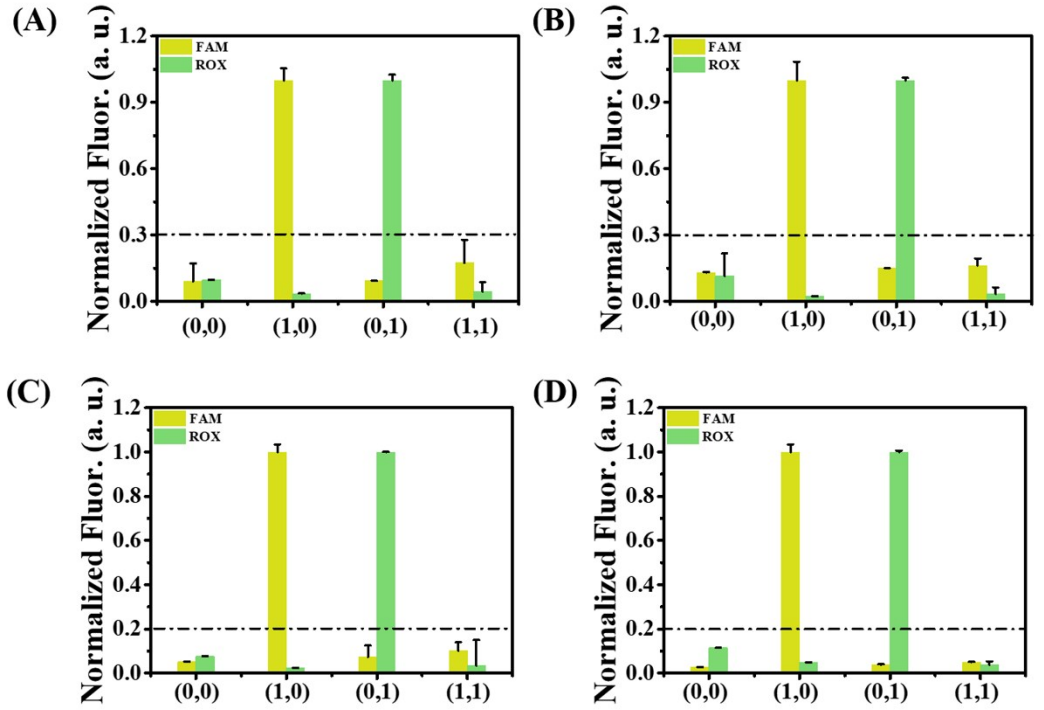


**Figure S9.** The column diagram of the normalized fluorescence of FAM (526 nm) and ROX (616 nm) under different buffer pH: 5.0 (A), 6.0 (B), 7.0 (C) and 8.0 (D).

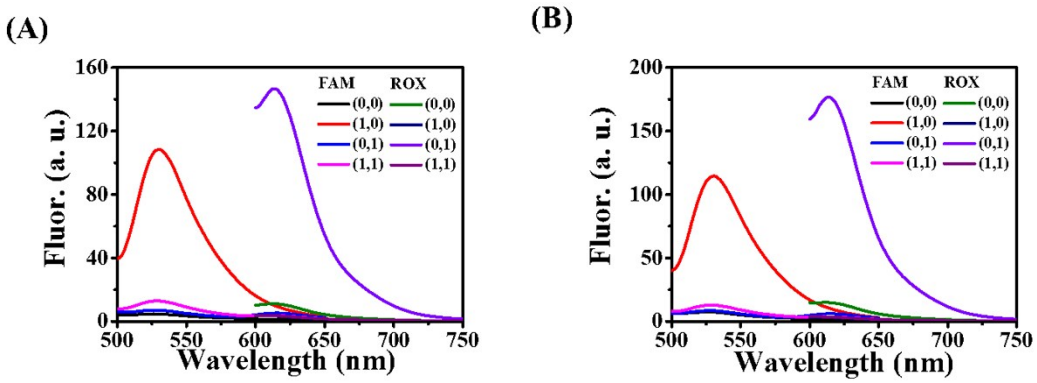


**Figure S10.** The fluorescence spectra of the absence (A) or presence (B) of $Mg^{2+}$.

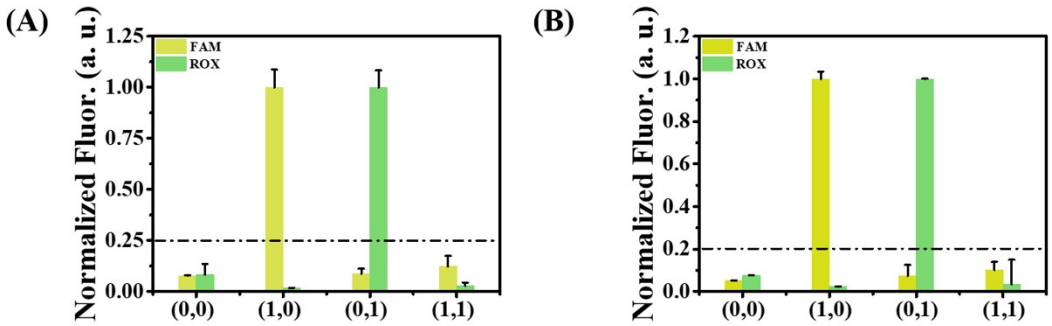


**Figure S11.** The column diagram of the normalized fluorescence of FAM (526 nm) and ROX (616 nm) in the absence (A) or presence (B) of $Mg^{2+}$.

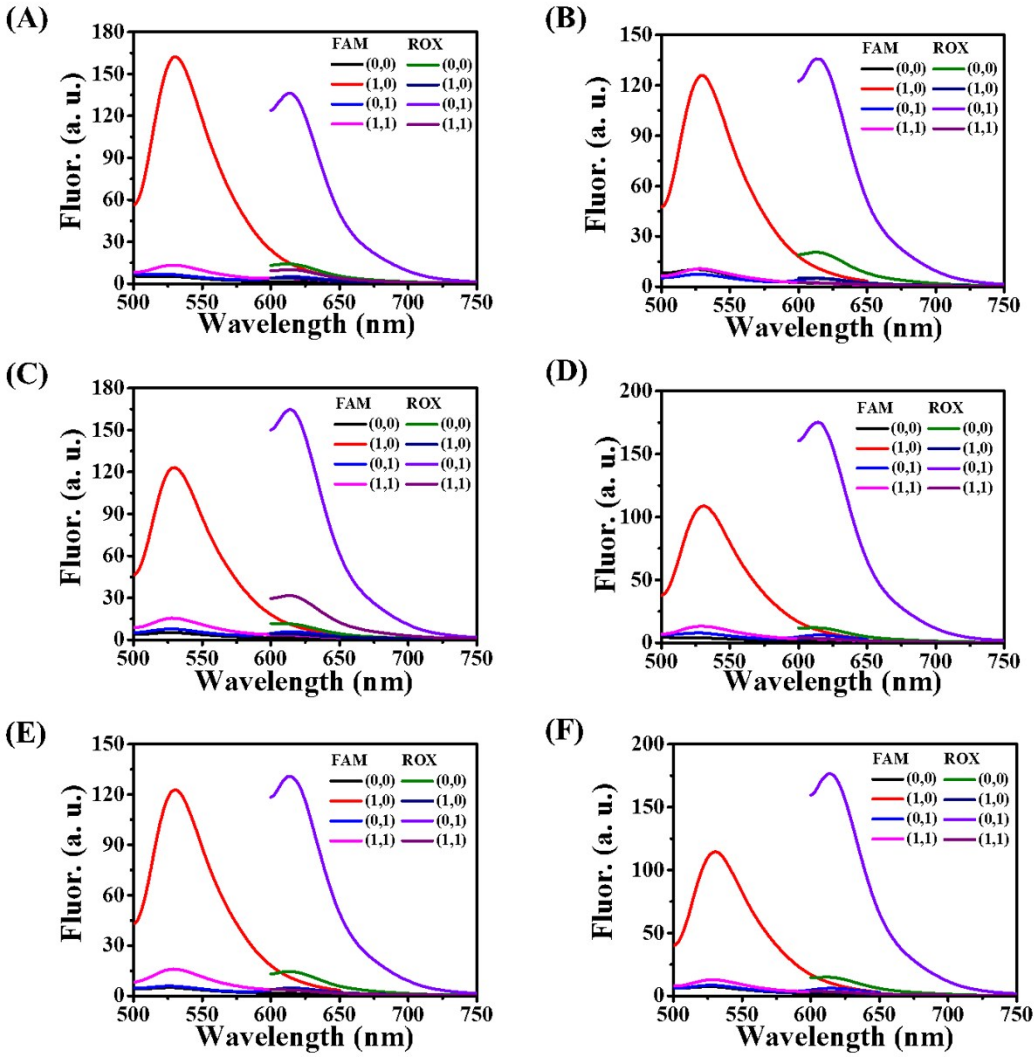


**Figure S12.** The fluorescence spectra of different $K^+$: 0 mM (A), 30 mM (B), 60 mM (C), 90 mM (D), 120 mM (E) and 140 mM (F).

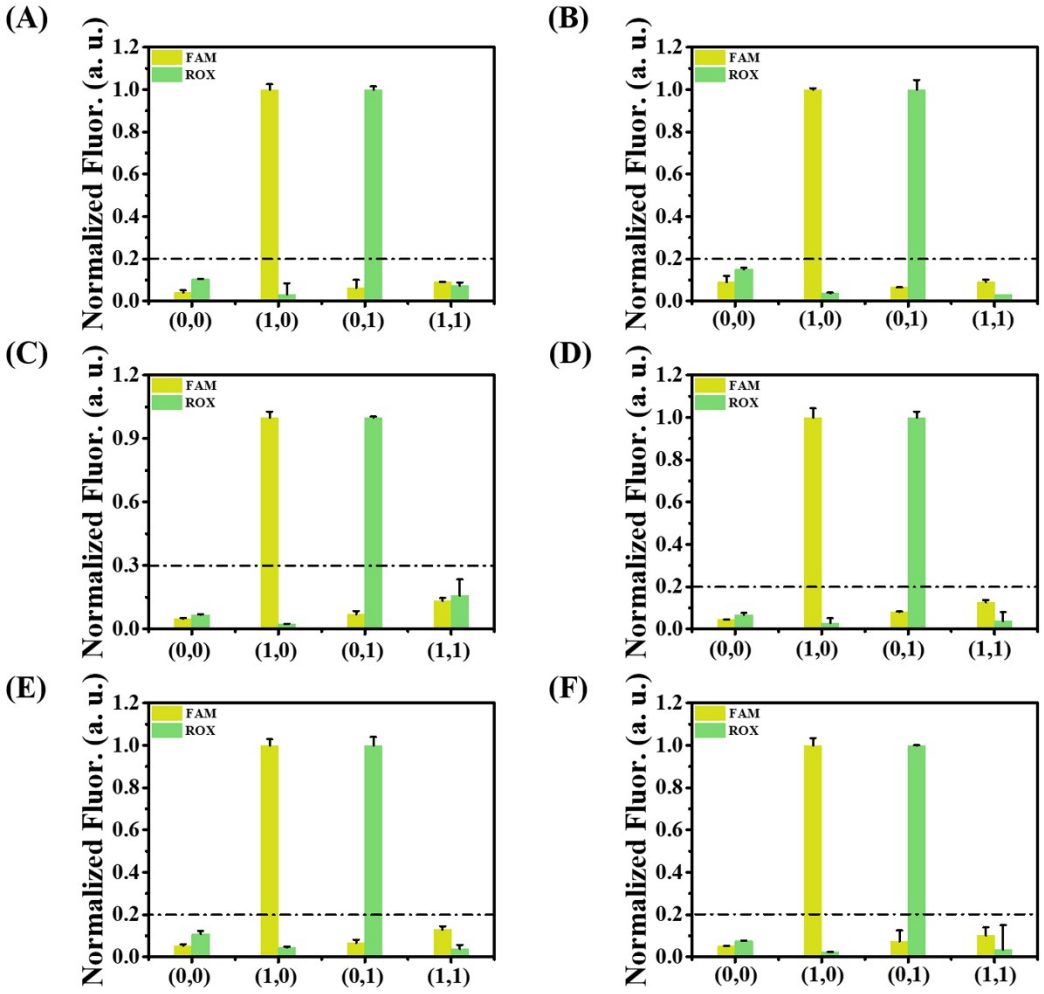


**Figure S13.** The column diagram of the normalized fluorescence of FAM (526 nm) and ROX (616 nm) under different $K^+$: 0 mM (A), 30 mM (B), 60 mM (C), 90 mM (D), 120 mM (E) and 140 mM (F).

## 10. The original fluorescence spectra of FAM and ROX in *E.coli* lysate

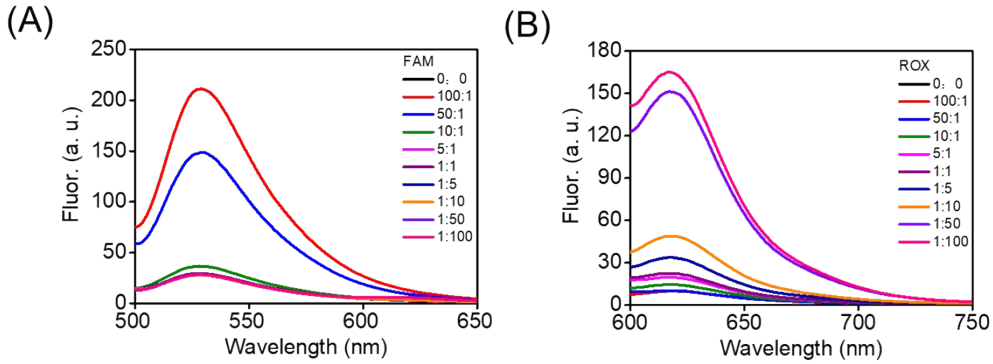


**Figure S14.** The original fluorescence spectra of FAM and ROX in *E. coli* lysate.

## 11. The limits of detection (LODs) of SA and SE in *E. coli* lysate

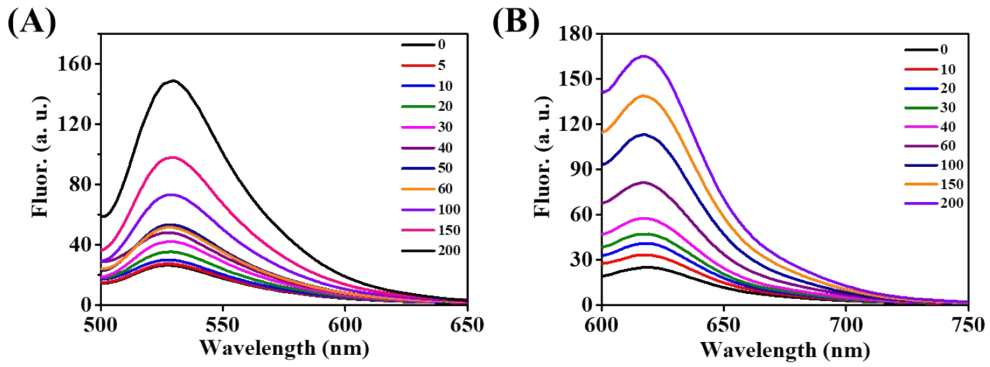


**Figure S15.** (A) The fluorescence spectra in response to increasing concentrations of the **SA** gene: 0, 5, 10, 20, 30, 40, 50, 60, 100, 150, 200 nM (C) The fluorescence spectra in response to increasing concentrations of the **SE** gene: 0, 10, 20, 30, 40, 60, 100, 150, 200 nM.

## 12. The specificity of the comparator

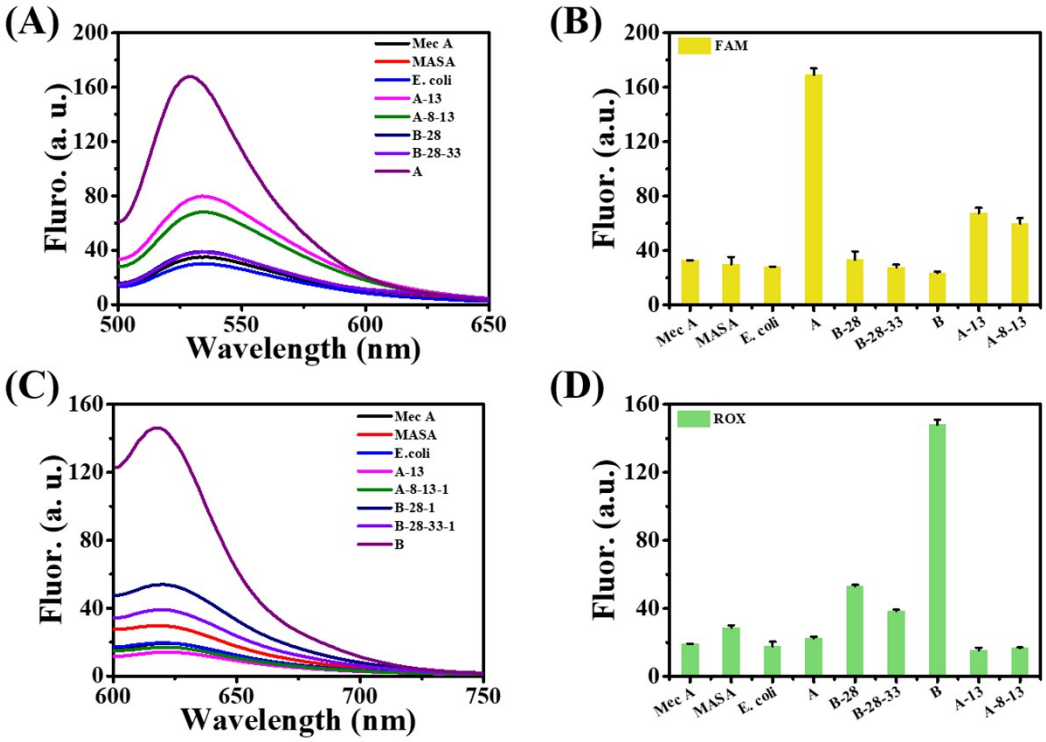


**Figure S16.** The fluorescence spectrum of FAM (A) and ROX (C) Under the interference sequence. The column diagram of the fluorescence of FAM (526 nm) (B) and ROX (616 nm) (D). The number represents the mutation site from 5' to 3'.

## Reference


1 M. Jauset-Rubio, H. Tomaso, M. S. El-Shahawi, A. S. Bashammakh, A. O. Al-Youbi and C. K. O'Sullivan, *Anal. chem.,* 2018, **90**, 12745.