Electronic supplementary information

Materials

All oligonucleotides purified by HPLC were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences were listed in Table S1.

SYBR Green I/II purchase from Thermo Fisher Scientific. Ammonium persulfate、TEMED,5x TBE,6x loading buffer were purchased from Shanghai Sangon.

DNA Sequence

Table S1. Sequences of oligonucleotides used in the present work.

Name	Sequences（from 5’ to 3’）
Target	GGAATGTTGTCTGGCTCGAGG
SNV1	GGACTGTTGTCTGGCTCGAGG
SNV2	GGAATGTTGTCTGGCACGAGG
SNV3	GGAATGTTGTTTGGCTCGAGG
B	CTTCTACCACT-FAM-GCGCACAATATAACCAGACAACATTCC
C	TGTCTGGTTATATTGTGCGCA-BHQ1
Helper1	TGCGCACAATATAACCAGACA
G	GCCTTATCTGT-ROX-AACCAACCAACAACCTCGAGCCAGACA
L	CTCGAGGTTGTTGGTTGGTTA-BHQ2
Helper2	TAACCAACCAACAACCTCGAG
B’	CTTCTACCACTGCGCACAATATAACCAGACAACATTCC
C’	TGTCTGGTTATATTGTGCGCATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
G’	GCCTTATCTGTAACCAACCAACAACCTCGAGCCAGACA
L’	CTCGAGGTTGTTGGTTGGTTATTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

The DNA sequences are marked with colors in the corresponding areas in Figure 1/2.

Experimental Section

DNA powder was centrifuged and dissolved with PBS buffer solution (pH=7.4, c (NaCl) =1300 mM).

Native polyacrylamide gel electrophoresis (native-PAGE)

Prepare 10 mL of 12.0% native polyacrylamide gel in room temperature with 4 mL of 30% acrylamide (Acryl/Bis solution (29:1), 30% (w/v)), 4 mL ultrapure water (18.25 MΩ), 2 mL 5x TBE，5 μL TEMED，50 μL of 10% ammonium persulfate and put it on the gel plate for 45 min standing to form 12.0% polyacrylamide gel.

Different mixtures of the DNA solution were incubated for at room temperature; the concentration of each oligonucleotide was 1 μM. 10 μL of each sample was mixed with 2 μL of 6x loading buffer, and then the mixture was

added into the gel for electrophoresis. A 12.0% native polyacrylamide gel was prepared using 1x TBE buffer (89 mM Tris base, 89 mM Boric acid, 2 mM EDTA, pH=8.3). The NATIVE-PAGE was carried out in 0.1x TBE buffer at a constant voltage of 120 V for about 90 min at room temperature (using Bio-Rad Mini-Protean Tetra Electrophoresis System). The gel was scanned by Tanon-2500 automatic digital gel image analysis system.

Fluorescence spectra measurement

To prepare the B-C and G-L, C strand was mixed with B strand in assay buffer. And L strand was mixed with G strand in assay buffer. Then, the mixtures were annealed by heating to 95℃ for 10 min and slowly cooled down to room temperature to obtain DNA duplexes to form the key logic device of the encoders. The prepared B-C and G-L were used was used as the platform directly for Fluorescence assays.

Control experiments using the B-C/SNV3/Helper1 system, the B-C/SNV2/Helper1 system, the B-C/SNV1/Helper1 system the B-C/Target/Helper1 or the G-L/SNV3/Helper2 system, the G-L/SNV2/Helper2 system, the G-L/SNV1/Helper2 system, the G-L/Target/Helper2 system were carried out under identical conditions. The final concentration of 26.9 nM B-C, 26.9 nM G-L, 26.9 nM Target, 26.9 nM SNV1, 26.9 nM SNV2, 26.9 nM SNV3, 134 nM Helper1, 134 nM Helper2.

The outputs of the 2-to-1 encoder are mainly the fluorescence produced by FAM and the outputs of the 4-to-2 encoder are mainly the fluorescence produced by ROX and FAM. The emission spectra of FAM and ROX were collected at 520 nm with the excitation wavelength of 495 nm and 604 nm with the excitation wavelength of 595 nm, respectively. The slit widths for the excitation and emission were 2 nm. FluoroMax-4 fluorescence spectrometer was used for the detection.

Optimization of experimental condition

1. Optimization of reaction time.

Experiments were conducted to select the appropriate reaction time.

The reason why the lane 5 for B'-C' in Figure 3C are brighter than the lane 5 for G'-L' in Figure 3D, based on our conjecture, is that reactions of Target with G'-L' is faster than that with B'-C', resulting in more consumption of G'-L' within the same time length. The kinetic curve tests we have done on Target with B'-C' and G'-L' respectively as shown in following Figure S1A have proved our guess.

In order to determine the reason for this reaction kinetic difference, we further used Gel Electrophoresis and Nucleic Acid Package (Nupack) software to analyze the target. As shown in Figure S1B and S1C, the bright bands of Target is present when Target is anteriorly stained by SYBR Green I (a dye for dsDNA). When post stained by SYBR Green II (a dye for ssDNA), the band appears in the same level. As shown in Figure S1D, the analysis result of Nupack shows that target folded itself near the 5 end of domain 1*, which explains it. In our design, domain 1* is designed to hybridize with B' and domain 3* is designed to hybridize with G'. Thus, the self-folding occurred in domain 1 affects B'-C' system more seriously than G'-L'. This can prove that why the lane 5 for B'-C' in Figure.3 are brighter than the lane 5 for G'-L' in Figure 3. The self-folding property is commonly seen in nucleic acids which decreases the basic reaction rate of strand displacement, but it does not affect the identification ability of the encoder since its function is realized through its structure design, which has been proved by the experiment results.

Besides, Target and SNV1 was introduced in 2-to-1 encoder B-C mentioned in Figure.1, respectively. The experiment results (Figure S1E) showed that the ratio of normalized fluorescence intensity between Target and SNV1 introduced 2-to-1 encoder was 1:0.25 regardless of 1 h or 12 h reaction time and thus we used 1h as the reaction time.

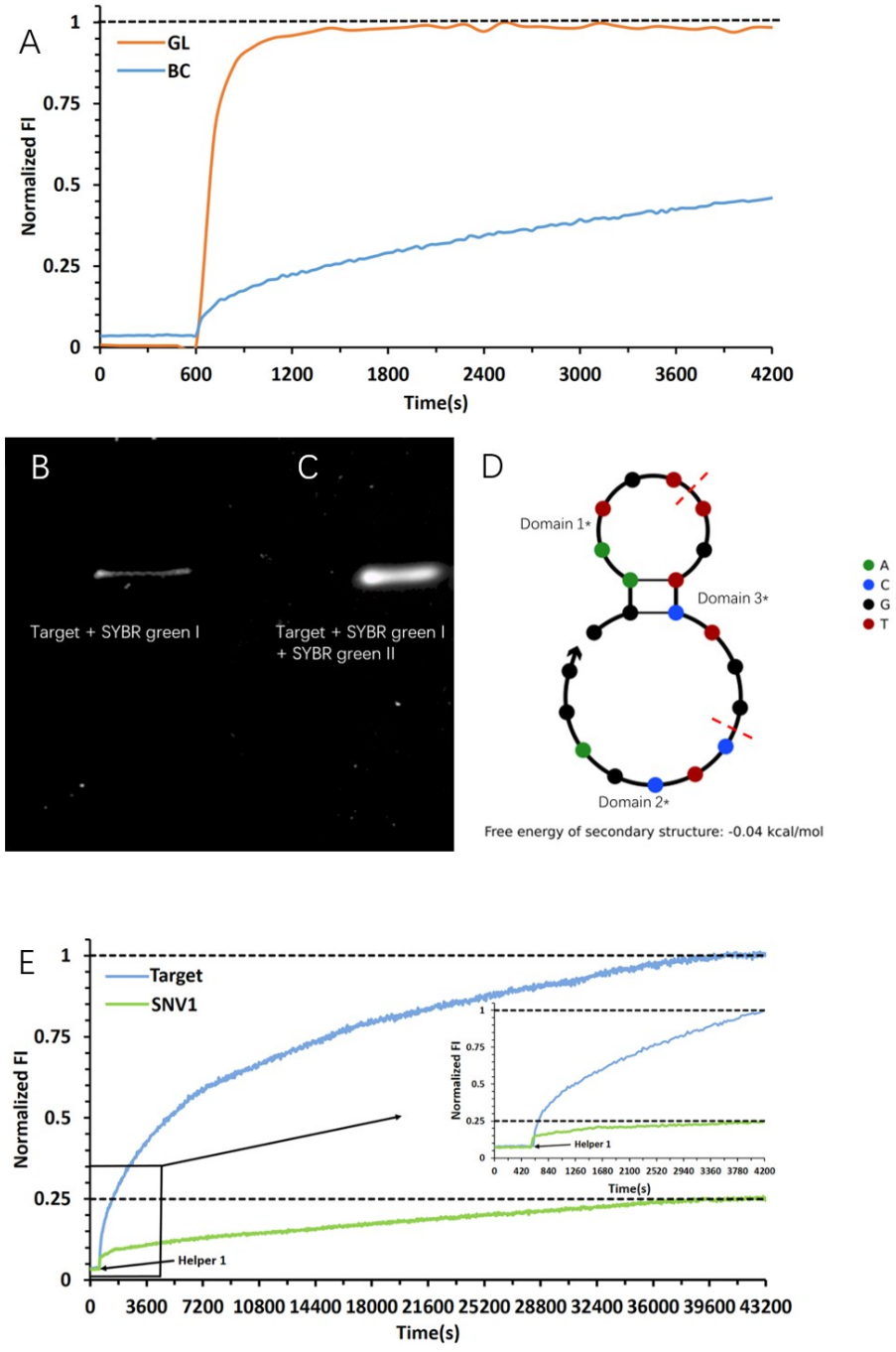

Figure S1: (A) The kinetic curve of Target inputting BC and GL systems. (B) The 12% native-PAGE results of target anterior staining by SYBR Green I. (C) The image of the gel (B) stained with SYBR green II for 30 min. (D) The analysis result of Nucleic Acid Package software to target at roommate temperature. (E) The kinetic curve of identification of SNV based on 2-to-1 encoder B-C in 1 h or 12 h.

2.Optimization of NaCl concentration.

The increase of NaCl concentration is beneficial to the formation of DNA double structure, which can accelerate the operation of the 4-to-2 encoder. SNV1, SNV2, SNV3 and Target were introduced in the 4-to-2 encoder where experiments were carried out at different salt concentrations in 100 mM, 500 mM, and 1300 mM, respectively. With the increase of salt concentration, the normalized fluorescence intensity of the input participating system also increased and thus we need to choose an appropriate salt concentration of the system. Comparing the experimental results, we can get that the normalized fluorescence intensity difference between 1 and 0 is more obvious at 1300 mM NaCl.

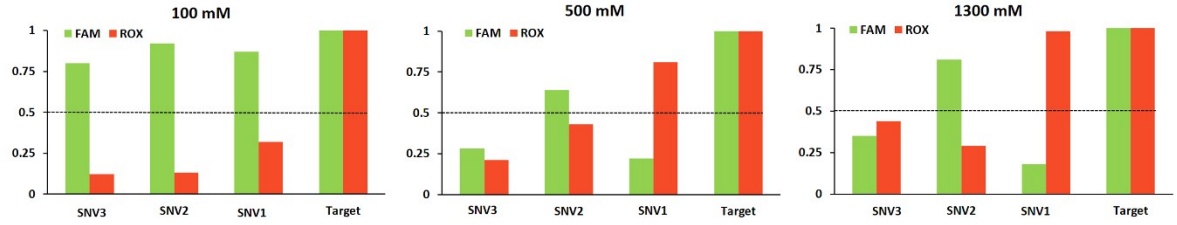

Figure S2: The optimization of 4-to-2 encoder by changing NaCl concentration.

3. Optimization of DNA concentration

SNV1, SNV2, SNV3 and Target were introduced in the 4-to-2 encoder where experiments were carried out at different DNA concentrations radio Target: Helper1 in 1:4, 1:5, 1:6, 1:8, and 1:10. The normalized Fluorescence intensity will increase with the increase of Helper concentration while excessive growth is not conducive to distinguish SNV and non-SNV. Comparing the experimental results, when Target: Helper1 was 1:5, the normalized fluorescence intensity difference between 1 and 0 was more obvious.

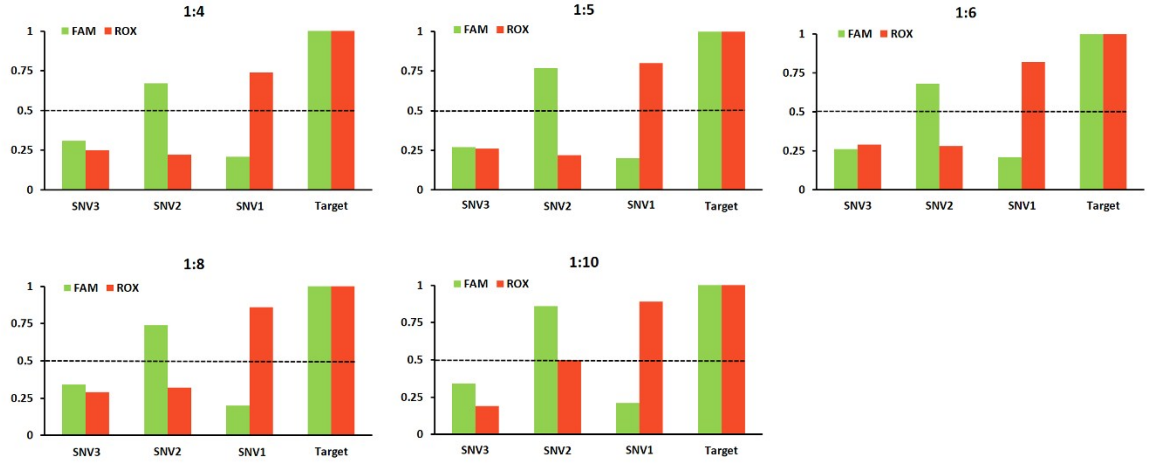

Figure S3: The optimization of 4-to-2 encoder by changing the radio of Target :Helper in reaction.

4. Optimization of reaction temperature

Temperature is also the factor that affects DNA hybridization. SNV1, SNV2, SNV3 and Target were introduced in the 4-to-2 encoder where experiments were carried out at different reaction temperature in room temperature and 37℃.And the result showed that the difference of normalized fluorescence intensity between 1 and 0 in room temperature or 37℃ were both suitable. Therefore, we choose room temperature as experimental condition since it's closer to room temperature.

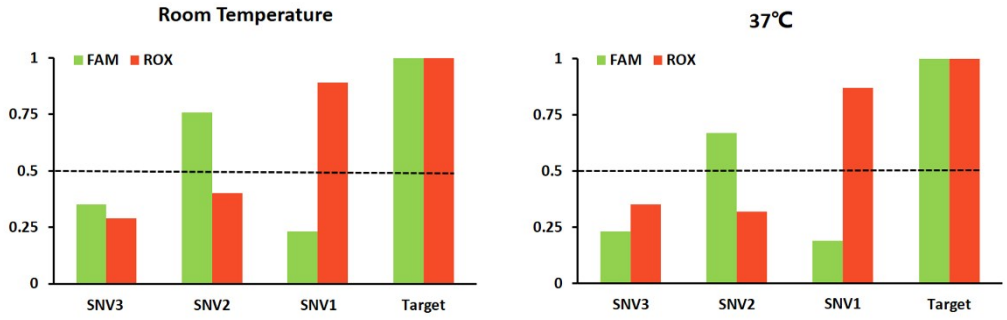

Figure S4: The optimization of 4-to-2 encoder by changing the reaction temperature.

5. The detection range of 4-to-2 encoder

When the concentration is increased to 1X, 2X, or 8X (X=26.9 nM), the Target or SNV1, SNV2, SNV3 can still be detected.

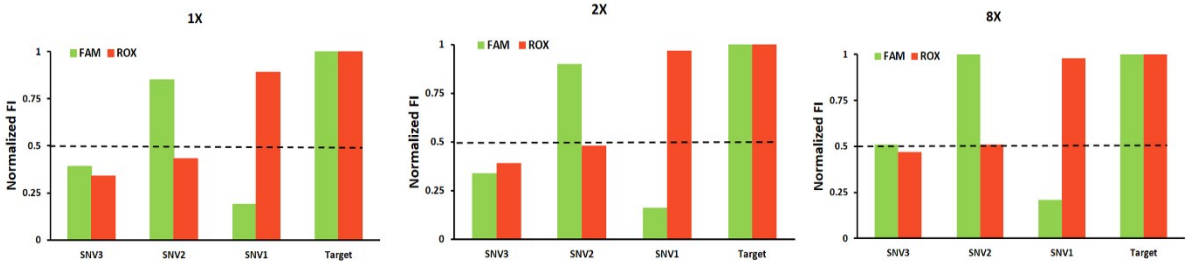

Figure S5: The normalized fluorescence spectroscopy results of 4-to-2 encoder in different concentration (1X, 2X, 8X, X=26.9 nM) of inputs (SNV3, SNV2, SNV1, Target).