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Supporting information for

Coupling the Nucleic Acid Circuitry with CRISPR-Cas12a System for Universal and Signal-On Detection

Rujian Zhao [1, 2], Chunxu Yu [1, 2], Baiyang Lu [1 *], Bingling Li [1, 2*]

[1]State Key Lab of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Science, Changchun, Jilin, 130022, China.

[2]School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei, Anhui, 230026, China.

Corresponding Author

* E-mail:binglingli@ciac.ac.cn (Prof. B. Li)

* E-mail:bylv@ciac.ac.cn

1. Experimental procedures

1.1 Materials

All the oligonucleotides were synthesized by Sangon Biotech Co., Ltd (Shanghai, China), and the sequences are presented in Table S1. All oligonucleotides were stored in H_2O at -20℃. All the modified sequences were purified with high-pressure liquid chromatography and the unmodified sequences were purified with ultra-polyacrylamide gel electrophoresis. dsDNA-ASFV templates (plasmid number: 190111HT8163-1) were obtained from Sangon Biotech Co., Ltd (Shanghai, China). The crRNAs were designed with a recognition sequence that was complementary to the target DNAs. HPLC-purified crRNAs were obtained from Hippo Biotechnology (Huzhou, China). The storing solution of crRNA were obtained by dispersing them with nuclease-free water and stored at -80℃.

1.2 Reagents

EnGan Lba Cas12a and 10 × NEBuffer 2.1 (500 mM NaCl, 100mM Tris-HCl, 100 mM $MgCl_2$, 1ng/ml BSA, pH 7.9) were obtained from New England Biolabs (Ipswich, MA, USA). RNase inhibitor was obtained from Takara Biotech. Inc. (Beijing, China). RNase free water and TIANprep Mini Plasmid Kit were produced by Tiangen Biotech Co. Ltd (Beijing, China). The pig serum was obtained from Jilin Province animal husbandry administration.

1.3 Apparatus

The concentrations of the sequences were determined by measuring the absorbance at 260 nm using a DeNovix DS-11+FX spectrophotometer (DeNovix Inc., Wilmington, DE, USA). Figure 1 and Figure 2 were monitored with the Cytation™ 5 imaging mult-mode plate reader (Biotek, Winooski, USA.) and Figure 3 was monitored with the LightCycler® 96 Instrument (Roche Diagnostics GmbH, Germany).

1.4 ASFV LAMP reaction

A LAMP reaction was prepared against African swine fever virus p72 gene (dsDNA-ASFV) (GenBank: MK554698.1). Briefly, five primers mixture and 3μL different copies of the target gene were added to ASFV LAMP buffer. The final reaction concentrations were 1.6 μM FIP and BIP, 0.4 μM F3 and B3, 0.8 μM LP, 1 M Betaine, 0.8 mM dNTPs, 4 mM $MgCl_2$, 1 × Isothermal buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8). The reaction solution was annealed at 95 ℃ for 2 min, followed by chilling in ice for another 2 min. Then 1.5 μL of Bst 2.0 DNA polymerase was added to 23.5 μL of above reaction solution to initiate the LAMP reaction. The LAMP reactions were incubated at 60℃ for 90 min, followed by heating to 80 ℃ for 20 min to denature the polymerase. Afterwards the products were stored at -20℃ for the next experiments. The electrophoretic analysis of the ASFV LAMP products as shown in Figure S3.

1.5 CRISPR-Cas12a cutting reaction

5 μL of 1 μM Initiator were diluted into 5 μL of cleavage buffer (300 nM Cas12a, 500 nM crRNA, 1 × NEbuffer 2.1) with different concentration of Target. Then incubated the mixture at 37 ℃ for 1 h, afterwards, the Cas12a reaction solutions were kept at 4 ℃ until they were used by HCR.

1.6 HCR reaction

Fluorescence data were obtained by using CytationTM 5 imaging mult-mode plate reader (Biotek, Winooski, USA.), with the temperature controller set to 25 ℃. Excitation and emission wavelengths were 520 nm and 485 nm, respectively, with 5-nm bandwidths. H1-FAM and H2-BlackQ1 were separately refolded in 1 × Binding buffer (10 mM Tris, 100 μM $MgSO_4$, pH 7.5). This and other involved refolding reaction heating to 95 ℃ for 5 min followed by slowly decreasing the temperature to 37 ℃ at a rate of 0.1℃/s to ensure the formation of hairpin structure. Then 4.8 μL of the prepared 2 μM H1 and 4.8 μL of the prepared 2 μM H2 were mixed with 10 μL of the 1 M NaCl, 10 μL of 1 × Binding buffer, 4 μL of the BL Oligo Dt (IDT, Coralville, IA, USA.). The reactions were started by the addition of 6.4 μL of the Cas12a reaction solution, forming the 40 μL HCR liquid. The HCR liquid were added to different wells of a 384-well plate, which was immediately transferred to the CytationTM 5 imaging mult-mode plate reader for fluorescence measurements.

1.7 Analyst the real serum samples

The reference serum was collected from domestic pigs in China. In order to prepare serum samples, we added 10 μL 2×10^{15} copies/μL dsDNA-ASFV template to 190 μL uninfected pig serum as the positive sample. And then DNA was extracted from serum using TIANamp Genomic DNA Kit (TIANGEN BIOTECH (BEIJING) CO., LTD). We next diluted the extracted DNA solution to obtain a series of serum samples. Other procedures were the same as the above analysis.

1.8 CHA reaction

The reaction was performed at 37 ℃ for 90 min on LightCycleR 96 Instrument (Roche Diagnostics GmbH, Germany) with fluorescence collections every 1 min. H3, H4-FAM and Probe mixture were separately refolded in 1 × TNaK buffer ((20 mM Tris, 140 mM NaCl, 5 mM KCl, 20 mM $MgSO_4$, pH 7.5). Then 6 μL of the prepared 2 μM H3 and 6 μL of the prepared 1 μM H4-FAM as well as 7.5 μL of the prepared Probe mixture (800 nM P-BlackQ1, 1.6 μM cP) were mixed in 1× TE (10 mM Tris-HCl, 1 mM EDTA, pH=8.0), then added 4 μL of the BL Oligo Dt (IDT, Coralville, IA, USA.). The reactions were started by the addition of 10 μL of the Cas12a reaction solution, forming the 40 μL CHA liquid.

2. Supporting Figures and Tables

Name	Sequence (5' to 3')	modification
LAMP-ASFV-FIP	AAGTCGTTCTCCGGGGTATTTTCGTCTAGGGAATTTCCATTTA	
LAMP- ASFV-BIP	GGTACGCATTCTTTGTGCCGGGGCGCCCTCTAAGGGTG	
LAMP- ASFV-F3	ACAAGCGTTGTGACATCC	
LAMP- ASFV-B3	TGGTTGTCCCAGTCATATC	
LAMP- ASFV-LP	AATCTTACGTTTTCATAAAGTC	
LAMP-MERS-FIP	AAGCATTAGTGGGGGCAAGCCCCACTACTCCCATTTCG	
LAMP-MERS-BIP	ATGCGCACTACACATACTGATATTTGTACAATCTCTTCACTACAATGA	
LAMP-MERS-F3	TCAACGTCTGCGGTGTGATCGCCTGCACG	
LAMP-MERS-B3	CGCAAAGTTAGAAAGTGATGG	
LAMP-M13mp18-FIP	CGACTCTAGAGGATCCCCGGGTACTTTTTGTTGTGTGGAATTGTGAGCGGAT	
LAMP-M13mp18-BIP	ACAACGTCGTGACTGGGAAAACCCTTTTTGTGCGGGCCTCTTCGCTATTAC	
LAMP-M13mp18-F3	ACTTTATGCTTCCGGCTCGTA	
LAMP-M13mp18-B3	GTTGGGAAGGGCGATCG	
crRNA-T1	UAAUUUCUACUAAGUGUAGAUCAUCGAAUCUUACGUUUUCAU	
crRNA-T2	UAAUUUCUACUAAGUGUAGAUUGAAGUAGAUAUGGCAGCAC	
crRNA-ssT	UAAUUUCUACUAAGUGUAGAUAAGGUUUGUGUGUUUACCUG	
HCR-H1	ATAGTGAGGGCCGAATCCTAGAGTCAAAGTACTCTAGGATTCGGCCCT	3'FAM
HCR-H2	ACTCTAGGATTCGGCCCTCACTAT-BHQ1-AGGGCCGAATCCTAGAGTACTTTG	Intermediate modification BHQ1
HCR-I_0	ACTCTAGGATTCGGCCCTCACTAT	
CHA-C1	CGACATCTAACCTAGCTCACTGAC	
CHA-H3	GTCAGTGAGCTAGGTTAGATGTCGCCATGTGTAGACGACATCTAACCTAGCCCTTGTCATAGAGCAC	
CHA-H4	AGATGTCGTCTACACATGGCGACATCTAACCTAGCCCATGTGTAGA	5'FAM
CHA-P	TGACAAGGGCTAGGTT	3'BHQ1
CHA-cP	CCTTGTCATAGA	

Table S1. Sequence of oligonucleotides used in this work.

Name	Sequences (5' to 3')
dsDNA-ASFV/ (dsDNA-T1 is underlined and the italics one is PAM)	AACGCAGCACAGCTGAACCGTTCTGAAGAAGAAGAAAGTTAATAGCA GATGCCGATACCACAAGATCAGCCGTAGTGATAGACCCCACGTAATCCG TGTCCCAACTAATATAAAATTCTCTTGCTCTGGATACGTTAATATGACCA CTGGGTTGGTATTCCTCCCGTGGCTTCAAAGCAAAGGTAATCATCATCG CACCCGGATCATCGGGGGTTTTAATCGCATATGTAAGAGCTGCAGAACT TTGATGGAAATTTATCGATAAGATTGATACCATGAGCAGTTACGGAAATG TTTTTAATAATAGGTAATGTGATCGGATACGTAACGGGGCTAATATCAGA TATAGATGAACATGCGTCTGGAAGAGCTGTATCTCTATCCTGAAAGCTTA TCTCTGCGTGGTGAGTGGGCTGCATAATGGCGTTAACAACATGTCCGAA CTTGTGCCAATCTCGGCGTTAACAACATGTCCGAACTTGTGCCAATCTC GGTGTTGATGAGGATTTTGATCGGAGATGTTCCAGGTAGGTTTTAATCCT ATAAACATATATTCAATGGGCCATTTAAGAGCAGACATTAGTTTTTCATC GTGGTGGTTATTGTTGGTGTGGGTCACCTGCGTTTTATGGACACGTATCA GCGAAAAGCGAACGCGTTTTACAAAAAGGTTGTGTATTTCAGGGGTTA CAAACAGGTTATTGCGCACAAGCGTTGTGACATCCGAACTATATTCGTC TAGGGAATTTCCA*TTTA*CATCGAATCTTACGTTTTCATAAAGTCGTTCTC CGGGGTATTCGCAGTAGTAAACCAAGTTTCGGTACGCATTCTTTGTGCC GGGTACAATGGGTCTTCCAAAAGGATCTACAAGCGTGTAAACGGCGCC CTCTAAGGGTGTTTGGTTGTCCCAGTCATATCCGTTGCGAGGAAACG
dsDNA-HPV/ dsDNA-T2	GGGTAACCAACTATTTGTTACTGTTGTTGATACTACACGCAGTACAAATA TGTCATTATGTGCTGCCATATCTACTTCAGAAACTACATATAAAAATACTA ACTTTAAGGAGTACCTACGACATGGGGAGG
ssDNA-T	CCCAGGTAAACACACAAACCTT
dsDNA-MERS template	CACCTTTTTGACACTTTTCTTGTTGCCTGTGGCTATTTGTTTGACTTATG CAAACATAGTCTACGAGCCCACTACTCCCATTTCGTCAGCGCTGATTGC AGTTGCAAATTGGCTTGCCCCCACTAATGCTTATATGCGCACTACACATA CTGATATTGGTGTCTACATTAGTATGTCACTTGTATTAGTCATTGTAGTGA AGAGATTGTACAACCCATCACTTTCTAACTTTGCGTTAGCATTGTGCAGT GGTGTAATGTGGTTGTACACTTATAGCAT
ds-M13mp18 template	CACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATG TTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTAT GACCATGATTACGAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTC GACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTTACAACGTCG TGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACA TCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCG CCCTTCCCAAC

Table S2. DNA templates used for isothermal amplifications.

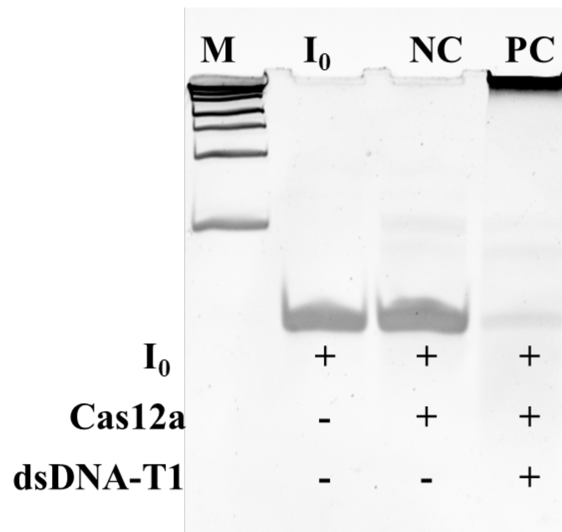

Figure S1: The electrophoresis of the CRISPR Cas12a cleaving I_0. Gel images of ssDNA marker (M), and pure I_0 control, NC (without the dsDNA-T1 targets), PC (with 12.5 nM dsDNA-T1 targets). It can be seen that with the dsDNA-T1 targets, the I_0 was cleaved by Cas12a, but no cutting reaction occurred in NC.

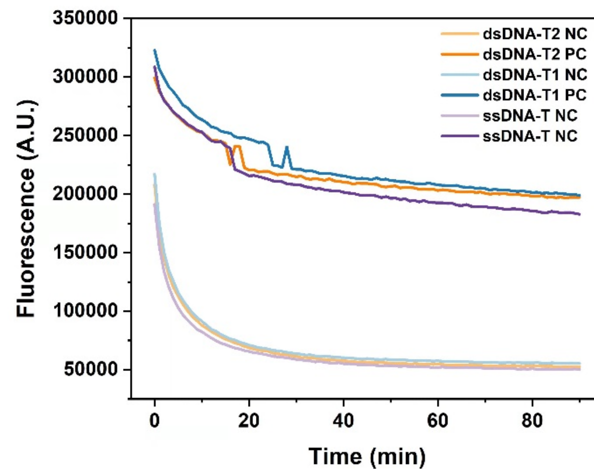

Figure S2: The kinetic fluorescence signals of CRISPR-HCR system, to validate the universality of the CRISPR-HCR with different targets, including dsDNA-T1 (blue,), dsDNA-T2 (orange) and ssDNA-T (purple). "NC" and "PC", in respective, represents "0 nM" and "50 nM" target is added. Note that for the detection of dsDNA-T1, dsDNA-T2 and ssDNA-T, the merely different component is the crRNA sequence. The fluorescence was performed on the CytationTM 5 imaging multi-mode plate reader (Biotek, Winooski, USA.).

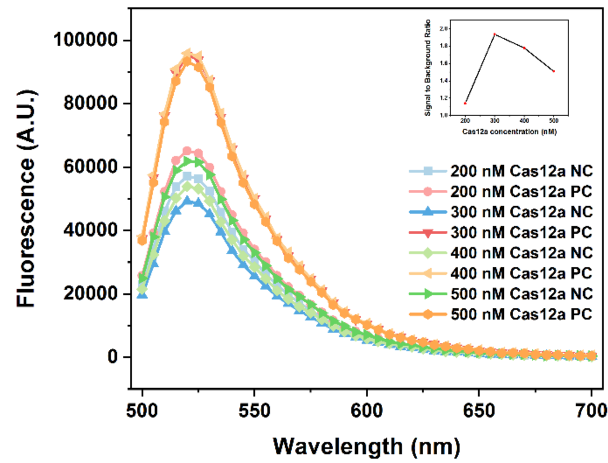

Figure S3: The optimization of the concentration of Cas12a in CRISPR-HCR system. Fluorescence assay of 50 nM crRNA using different concentrations of LbCas12a. PC and NC is, in respective, the detection with and without 50 nM dsDNA-T1. Finally, the best concentrations for LbCas12a was optimized as 300 nM. The fluorescence was performed on the CytationTM 5 imaging multi-mode plate reader (Biotek, Winooski, USA.).For all the fluorescence assay, λ ex is 520 nm and λ em is 485 nm.

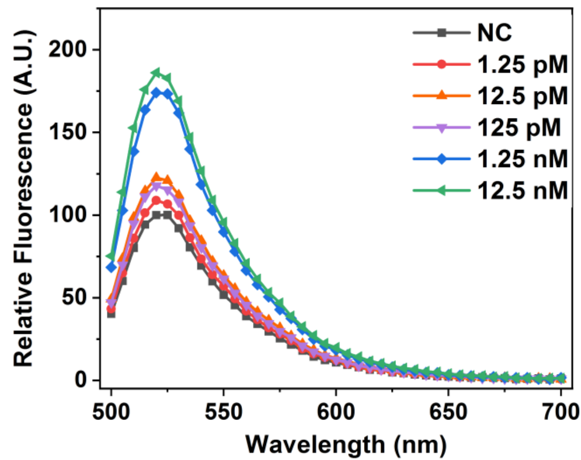

Figure S4: The kinetic fluorescence signals of CRISPR-HCR system, Fluorescence spectra of the CRISPR-HCR with different concentrations of dsDNA-T1.

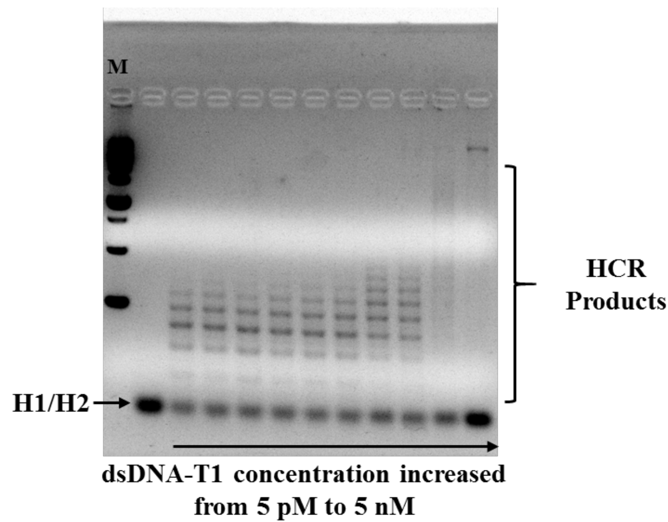

Figure S5: The electrophoresis of the different amounts of dsDNA-T1 in the CRISPR-HCR assays. Gel images of dsDNA marker (M), and from left to right the concentration of dsDNA-T1 increased from 5 pM to 5 nM. It can be seen that with the increase of dsDNA-T1 concentration, the higher the efficiency of Cas12a cleaving I_0, the less the HCR products are produced.

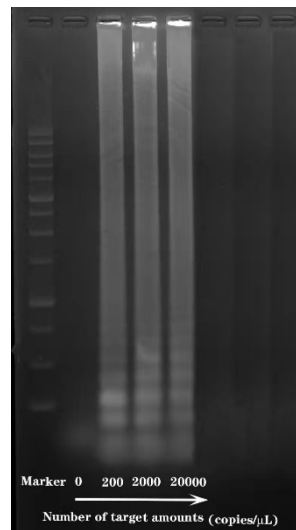

Figure S6: The electrophoresis of the different amounts of ASFV-LAMP Products. Gel images of dsDNA marker (M), and the ASFV-LAMP Products amplified from nuclease-free water (0), 200 copies/μL of synthetic dsDNA-ASFV, 2000 copies/μL of synthetic dsDNA-ASFV and 20000 copies/μL of synthetic dsDNA-ASFV.

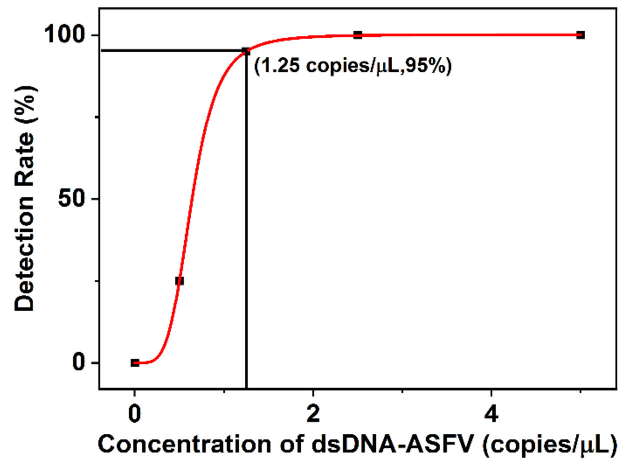

Figure S7: The detection limit of LAMP reaction. The concentration of dsDNA-ASFV was changed from 0 to 5 copies/μL. 20 LAMP reactions were performed for each concentration target, and the detection results were counted to draw this curve. According to the curve, when the detection rate was 95%, the detection limit of this LAMP reaction was 1.25 copies/μL.

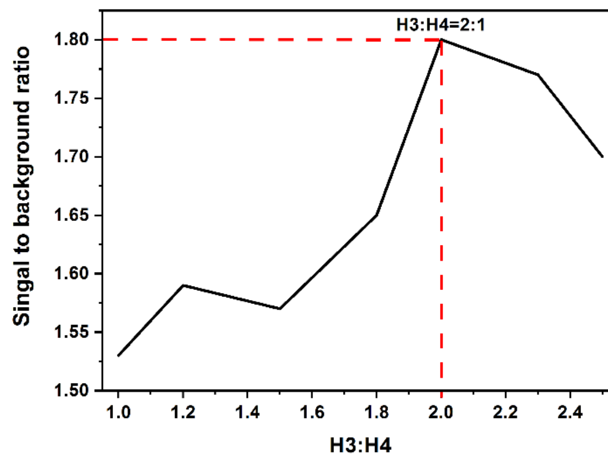

Figure S8: The kinetic fluorescence signals of CHA reaction, to optical the ratio of H3:H4. The ratio of H3:H4 was changed from 1:1 to 2.5:1 by changing the concentration of H3. H4 was always 50 nM, and P:cP was 1:2, which were 50 nM and 100 nM respectively. PC and NC is, in respective, the detection with and without 5 nM dsDNA-T1. Finally, the best ratio of H3:H4 was optimized as 2:1.

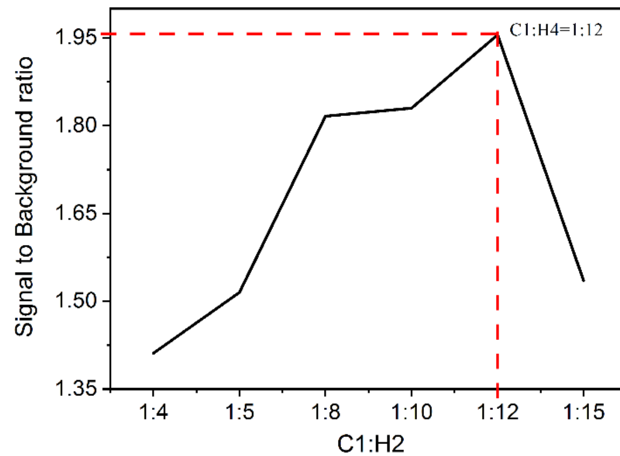

Figure S9: The kinetic fluorescence signals of CRISPR-CHA system, to optical the ratio of C1: H4. The ratio of C1:H4 was changed from 1:4 to1:12 by changing the concentration of C1. H4 was always 50 nM, and P:cP was 1:2, which were 50 nM and 100 nM respectively. The concentration of LbCas12a was 300 nM and the crRNA was 500 nM. PC and NC is, in respective, the detection with and without 50 nM dsDNA-T1. Finally, the best ratio of C1:H4 was optimized as 1:12.

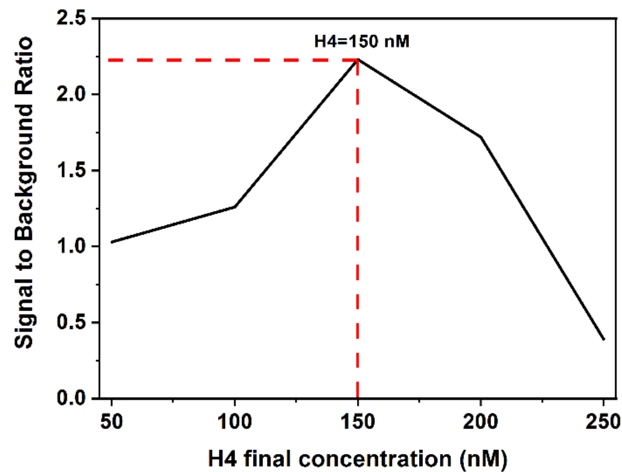

Figure S10: The kinetic fluorescence signals of CRISPR-CHA system, to optical the final concentration of H4. The concentration of H4 was changed from 50 nM to 250 nM, and C1:H3:H4:P:cP is 1/12:2:1:1:2. The concentration of LbCas12a was 300 nM and the crRNA was 500 nM. PC and NC is, in respective, the detection with and without 50 nM dsDNA-T1. Finally, the best concentrations for H4 was optimized as 150 nM.