# SUPPLEMENTARY INFORMATION

## Single primer-triggered isothermal amplification for double-stranded DNA detection

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## Materials and methods:

## Materials.

The molecular beacons used in this work were designed by using NUPACK software (http://www.nupack.org/) and the nucleic acid melting prediction by DINAMelt web server (http://mfold.rit.albany.edu/?q=DINAMelt). All oligonucleotides (Supplementary Table S1) were produced by Shanghai Bio-Engineering Company (Shanghai, China). The *Bst 2.0 WarmStart*$^{TM}$ DNA polymerase (8 U/μL), *Nt.BstNBI* nicking enzyme (10 U/μL) and a mixture of deoxynucleoside triphosphates (dNTPs) were purchased from New England Biolabs. SsoAdvanced$^{TM}$ SYBR Green Supermix kit was purchased from Bio-Rad Laboratories, Inc.

## The Reaction System.

The SAMP reaction in 10 μL contained $5.0\times10^{-7}$ M molecular beacon, $2.5\times10^{-4}$ M dNTPs, 0.4 U *Bst 2.0 WarmStart*$^{TM}$ DNA polymerase, 4 U *Nt.BstNBI* nicking enzyme, $2.0\times10^{-7}$ M primer and 1×NEBuffer 3.1 (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/mL BSA pH 7.9 @ 25℃). The reaction was initiated by adding different concentrations of the target and incubated at 55.0℃. The real-time fluorescence was measured in a CFX96$^{TM}$ Real-Time PCR detection system (Bio-Rad) at 1 min intervals. Polyacrylamide gel electrophoresis (PAGE) was carried out on 17.5% polyacrylamide using tris-acetate-EDTA (TAE) buffer (pH 8.0) for 1 h 10 min at 135 V.

## Preparation of DNA-Modified AuNPs.

Gold nanoparticles with an average diameter of 14 nm were synthesized using the citrate reduction protocol previously reported[1]. The AuNPs were functionalized with thiol-modified oligonucleotides according to the described in the literature[2].

## Isolation of HBV DNA From Serum.

HBV DNA was extracted from 300 μL patient serum obtained from the Affiliated Hospital of Qingdao University Medical College, with proteinase K in lysis buffer followed by phenol/chloroform/isoamyl alcohol extraction[3]. The DNA pellet was dissolved in 20 μL sterile distilled water.

## Isolation of Genomic DNA and Plasmid DNA.

The genomic DNAs of *Cyprinus carpio*, *Gallus gallus*, *Escherichia coli* and pBlu2KSP plasmid DNA were extracted according to the method described in the literature[4].

1.J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 1959-1964.

2.S. J. Hurst, A. K. Lytton-Jean and C. A. Mirkin, *Anal. Chem.*, 2006, **78**, 8313-8318.

3.P. Tangkijvanich, P. Komolmit, V. Mahachai, P. Sa-Nguanmoo, A. Theamboonlers and Y. Poovorawan, *Hepatol. Res.*, 2010, **40**, 269-277.

4.M. R. Green and J. Sambrook, *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratory Press New York, 2012.

## Supplementary Figures

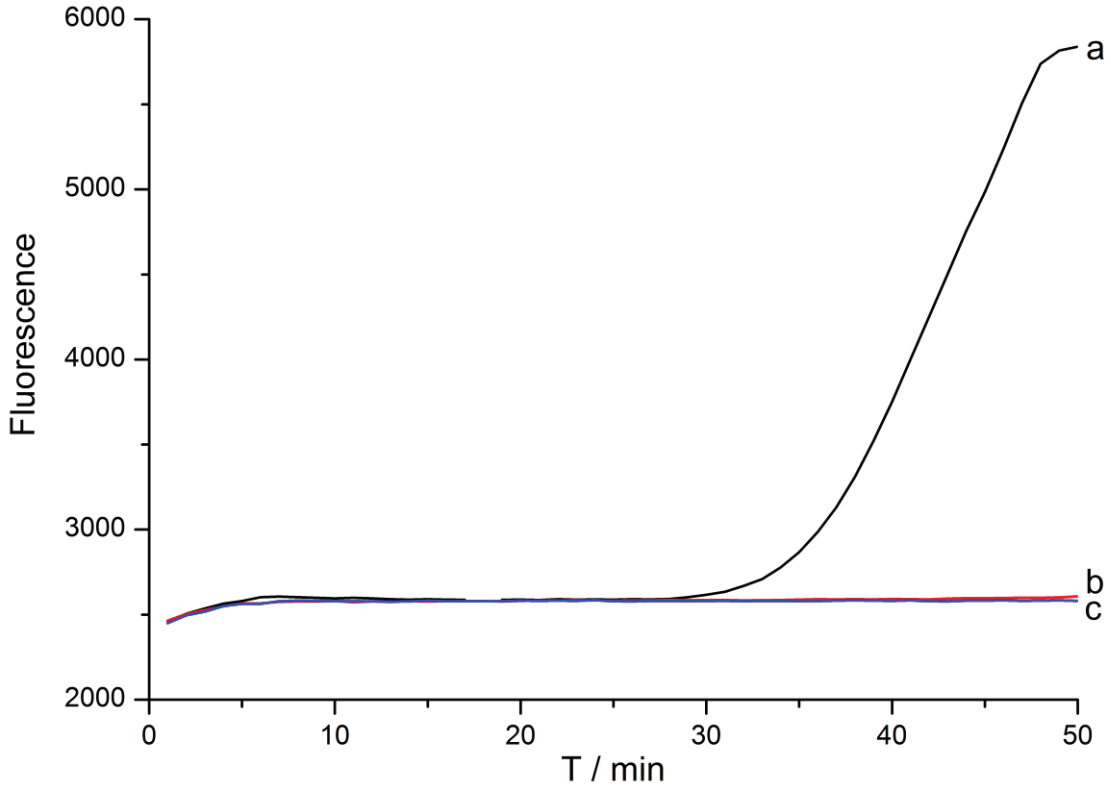


**Fig. S1** The specificity of SAMP.

(a) The fluorescence signal from complete complementary primer of pBlu2KSP. (b) The fluorescence signal from one-base mismatched primer (GGTTCCCAACGATCA GATCCTGGTGAGACTCAACTATGCTATTTC***T***TTCATC) of pBlu2KSP. (c) The fluorescence signal from two-base mismatched primer (GGTTCCCAACGATCAGAT CCTGGTGAGACTCAACTATGCTATTTC***T***TT***A***ATC) of pBlu2KSP.The mismatch bases were shown in bold and italic. The concentration of pBlu2KSP was $1.0\times10^{-13}$ M.

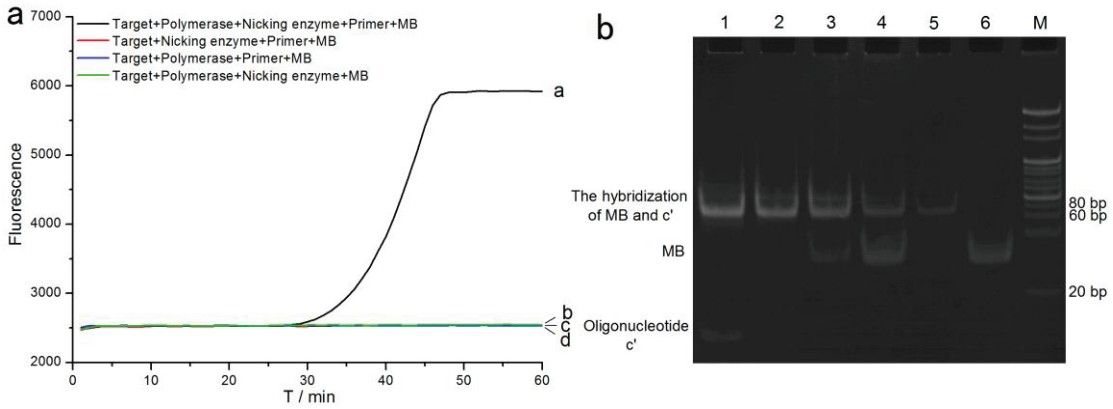


**Fig. S2** Verification of SAMP.

(a) Verification by real-time fluorescence curves. a. The reaction mixture contained $1.0\times10^{-13}$ M pBlu2KSP, $5.0\times10^{-7}$ M molecular beacon, $2.0\times10^{-7}$ M primer, 0.4 U *Bst 2.0 WarmStart*$^{TM}$ DNA polymerase, 4.0 U *Nt.BstNBI* nicking enzyme, and 1×NEBuf3.1. b. The polymerase was omitted from the reaction. c. The nicking enzyme omitted. d. The primer omitted. (b) 17.5% PAGE of the reaction products from different concentrations of pBlu2KSP. Lane 1: $1.0\times10^{-9}$ M pBlu2KSP Lane 2: $1.0\times10^{-10}$ M pBlu2KSP Lane 3: $1.0\times10^{-11}$ M pBlu2KSP Lane 4: $1.0\times10^{-12}$ M pBlu2KSP Lane 5: $2.0\times10^{-7}$ M primer Lane 6: $5.0\times10^{-7}$ M molecular beacon M: 20-bp DNA ladder. The reactions were incubated for 30 min at 55℃.

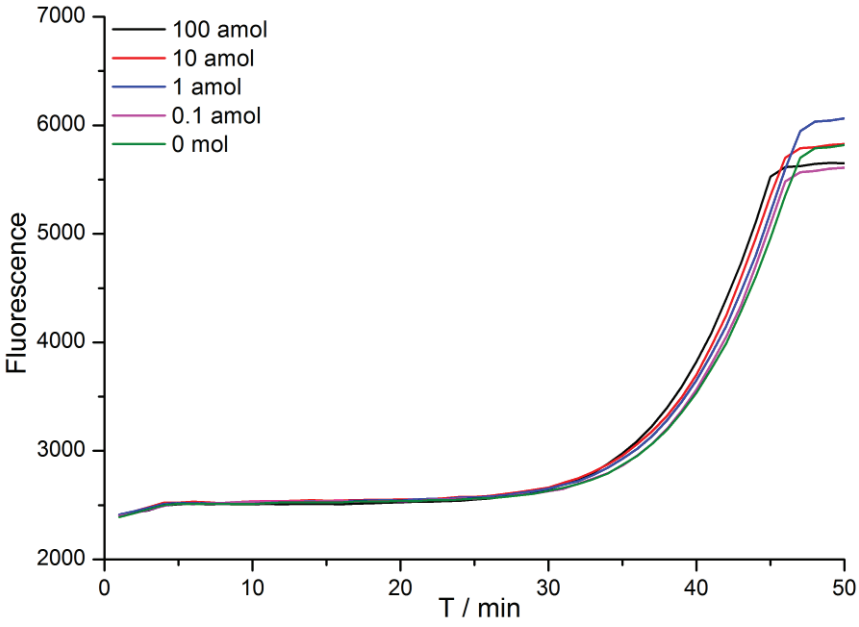


**Fig. S3** Anti-jamming of SAMP. The real-time fluorescence curves were triggered by 1 amol pBlu2KSP plasmid DNA plus different amounts of *Escherichia coli* genomic DNA.

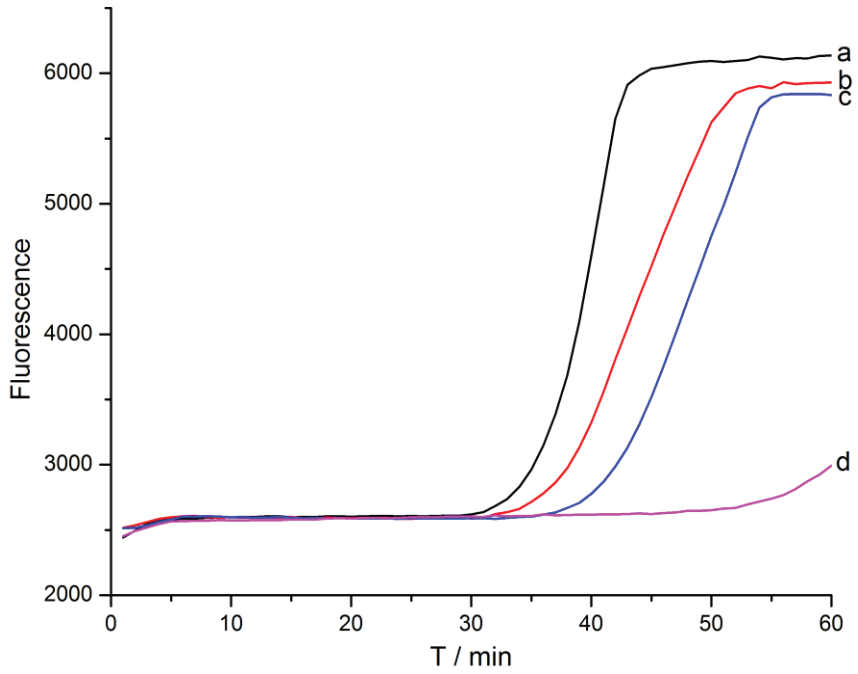


**Fig. S4** Real-time fluorescence curves for multiple targets.

(a). 1 amol *Cyprinus carpio* genomic DNA+1 amol *Gallus gallus* genomic DNA+200 nM Pcyp+200 nM Pgal1+500 nM MB1 (b). 1 amol *Gallus gallus* genomic DNA+200 nM Pgal1+500 nM MB1 (c). 1 amol *Cyprinus carpio* genomic DNA+200 nM Pcyp+500 nM MB1 (d). Negative control.

## Supplementary Tables

## Table S1: Sequences of SAMP

| Oligonucleotides | Sequence(5'-3') |
|---|---|
| specific sequence of *Cyprinus carpio* ([a]NC_001606.1 [b]3418-3443) | GAGTCCATATCGACGAGGGGGTTTAC |
| specific sequence of *Gallus gallus* ([a]AY235570.1 [b]8668-8694) | AGACTTCAAGGACCTCTCATTTGACTC |
| specific sequence of plasmid *pBluescript II KS(+)* ([a]X52327.1 [b]2016-2045) | CTATTTCGTTCATCCATAGTTGCCTGACTC |
| specific sequence of *Hepatitis B virus* ( [a]U95551.1 [b]2029-2055) | GAGTCTCCTGAGCATTGTTCACCTCAC |
| **MB1:** Molecular beacon1 | FAM-CGCTTGGTAGGCTCCGGTTCCCAACGA TCAGATCCTGCTACCAAGCG-DABCYL |
| **MB2:** Molecular beacon 2 | HEX-CCGATCGAACGCTCCCGTGTGTGCAGC CTACAACCAAGTTCGATCGG-DABCYL |
| Primer for plasmid *pBluescript II KS(+)* | GGTTCCCAACGATCAGATCCTGGTGAGACTC AACTATGCTATTTCGTTCATC |
| **$P_{cyp}$:** Primer for *Cyprinus carpio* | GGTTCCCAACGATCAGATCCTGGTGAGACTC TCGACGGTAAACCCCCTC |
| **$P_{gal}$1:** Primer 1 for *Gallus gallus* | GGTTCCCAACGATCAGATCCTGGTGAGACTC GAGAGGTAGACTTCAAGGAC |
| **$P_{gal}$2:** Primer 2 for *Gallus gallus* | CGTGTGTGCAGCCTACAACCAAGTGAGACT CGAGAGGTAGACTTCAAGGAC |
| Primer for *Hepatitis B virus* | GGTTCCCAACGATCAGATCCTGGTGAGACTC GAGCATTCGTGAGGTGAACA |
| Forward primer of HBV | CCGATCCATACTGCGGAAC |
| Reverse primer of HBV | GCAGAGGTGAAGCGAAGTGC SH-GGTTCCCAACGT ATCAGATCCTG-SH |

[a] Genbank accession number. [b] The position of specific sequence in genomic DNA.
The underlined sequence represented recongnizing sequences of nicking endonuclease *Nt.BstNBI*.