Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

A New Isothermal Nucleic Acids Detection Strategy Mediated by Double-Nicked Beacon

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Experimental Section

Materials

The molecular beacon used in this work was designed by using NUPACK software (http://www.nupack.org/) and produced by Shanghai Bio-Engineering Company (Shanghai, China), as shown in Table S1. The Klenow fragment polymerase (exo$^-$) (5 U/μL), Nb.BbvCI nicking enzyme (10 U/μL) and a mixture of deoxynucleoside triphosphates (dNTPs) were purchased from New England Biolabs. The total RNA of human lung carcinoma cell (H1299) was extracted by RNApure High-purity Total RNA Rapid Extraction Kit (Spin-column; Bioteke, Beijing, China).

Methods

The BAMP reaction in 10 μL contained 2.0×10^{-7} M molecular beacon, 1.0×10^{-4} M dNTPs, 1 U Klenow fragment DNA polymerase (exo$^-$), 2 U Nb.BbvCI nicking enzyme, 1×NEB Cutsmart™ buffer (50 mM potassium acetate, 20 mM tris-acetate, 10 mM magnesium acetate, 100 μg/mL BSA, pH 7.9). The reaction was initiated by adding the different concentration of the target and incubated at 37.0℃. The real-time fluorescence measure of miRNAs was made in a CFX96™ Real-Time PCR detection system (Bio-Rad) at 1 min intervals.

S1：Supporting Figures

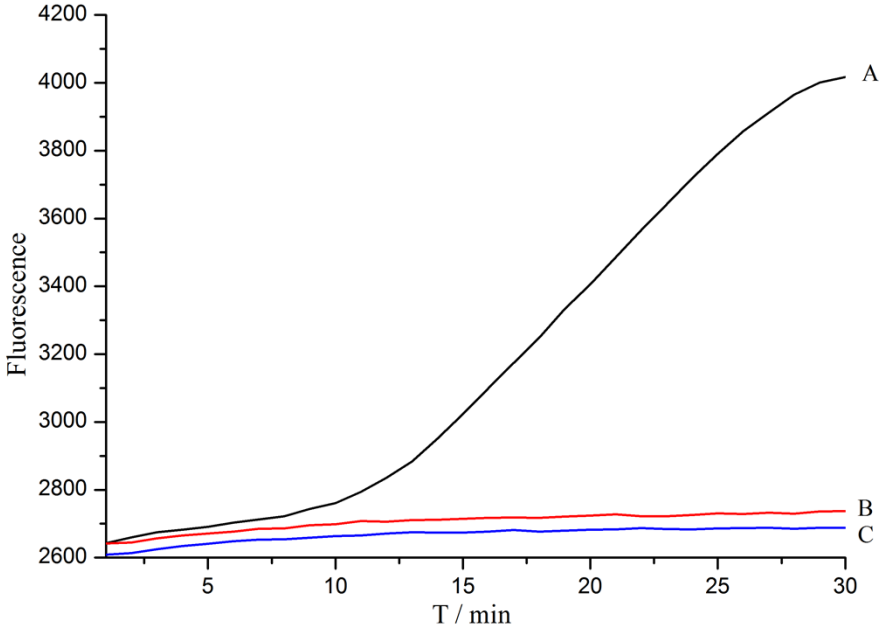

Figure S1. The BAMP reaction was prepared, and each reaction was initiated with 10 fmol of the Let-7a in a final 10 μL volume containing 2.0 $\times 10^{-7}$ M beacon. A. The reaction was added 0.2 μL Nb.BbvCI and 0.2 μL Klenow fragment polymerase. B. Only 0.2 μL Klenow fragment polymerase added C. Only 0.2 μL Nb.BbvCI added.

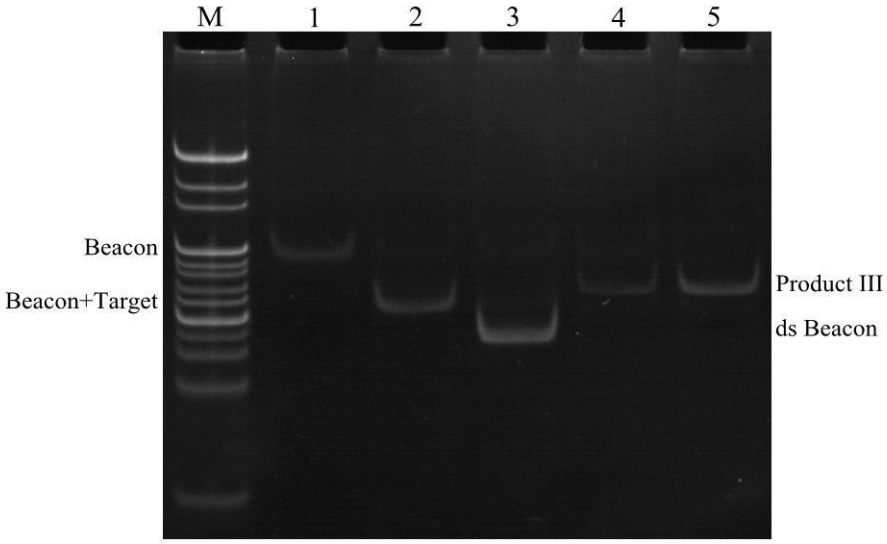

Figure S2. 17.5% PAGE of amplification reaction products. M：20 bp DNA Marker 1: 2.0×10^{-7} M molecular beacon; 2: added 1.0×10^{-7} M Let-7a on the basis of Lane 1; 3: added 0.2 μL polymerase on the basis of Lane 2; 4: The reaction containing 1.0×10^{-9} M Let-7a, 2.0×10^{-7} M molecular beacon, 0.2 μL polymerase and 0.2 μL *Nb.BbvCI* incubated for 20 min; 5: Lane 4 amplification reaction incubated for 30 min.

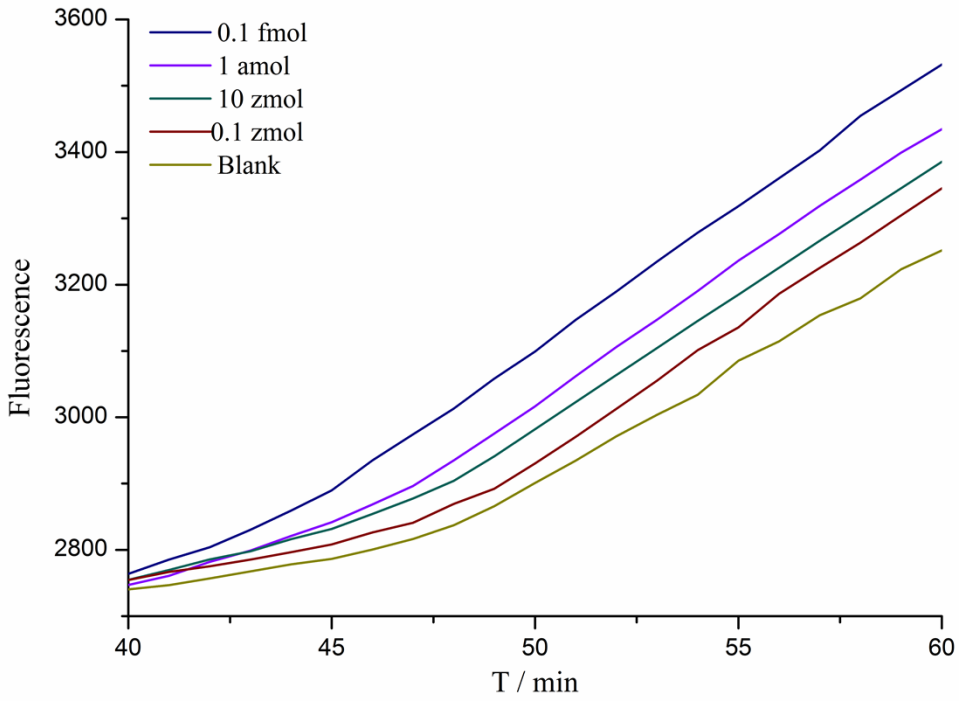

Figure S3. The enlarge figure of the concentration range from 0.1 zmol to 0.1 fmol from Figure 3.

We have discovered an interesting phenomenon from Figure S4. The amount of the nicking enzyme could affect the time and the rate of signal increase. In a given concentration of the target, the more nicking enzyme was, the time of signal increase was instead postponed, but the rate of signal increase was accelerated. This is different with polymerase. An increase in polymerase concentration caused the appearance of the signal in advance. Thus, we deduced that the nicking enzyme and polymerase was relations of rivals because that the extension site of polymerase and the nicking site were located in the same place. When the amount of the nicking enzyme was increased, the competitive power of nicking enzyme was larger than that of polymerase, the polymerization was inhibited, so the time of signal increase was postponed. With the increase of the products, the nicking site was increased, the competitive power of nicking enzyme weakened, and the polymerization was released to increase fluorescence signal.

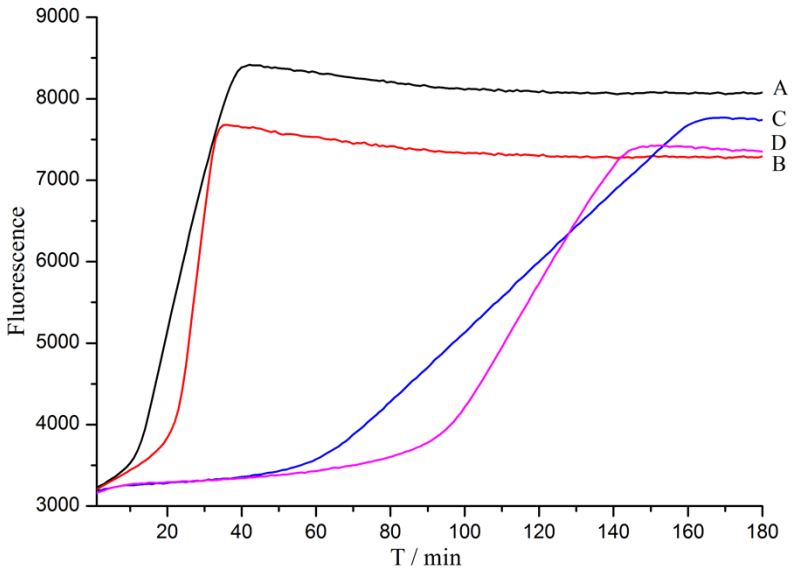

Figure S4. Effect of different concentrations of the nicking enzyme on BAMP reaction. 10 μL reaction system containing 500 nM beacon and 0.1 μL Klenow polymerase were prepared and different amounts of *Nb.BbvCI* and Let-7a were added to each reaction. A. 0.1 pmol Let-7a+0.3 μL *Nb.BbvCI* B. 0.1 pmol Let-7a+0.5 μL *Nb.BbvCI* C. 1 fmol Let-7a+0.3 μL *Nb.BbvCI* D. 1 fmol Let-7a+0.5 μL *Nb.BbvCI* .The progress of each reaction was monitored by real-time PCR.

S2: Supporting Tables

***Table S1*. Sequences of oligonucleotide [a]**

Strands	Sequence (5′ to 3′)
Let-7a	UGAGGUAGUAGGUUGUAUAGUU
Let-7b	UGAGGUAGUAGGUUGU*G*U*G*GUU
Let-7c	UGAGGUAGUAGGUUGUAU*G*GUU
Let-7e	UGAGGUA*G*GAGGUUGUAUAGUU
Let-7i	UGAGGUAGUAG*U*UUGU*GCU*GUU
Beacon	FAM- ~~AGGTAGTAG~~CCATCCTCAGCACTCCGAATCCTC AGCA*AACTATACAAC~~CTACTACCT~~*-DABCYL

[a]The two nicking sites of *Nb.BbvCI* in beacon are underlined. The strikethrough portion indicates stem of the beacon. The italic portion in beacon is the complementary sequence of the target. The bases that differ from those in Let-7a are marked in red and italic in Let-7b, 7c, 7e and 7i.