

Linear DNA probe as alternative to molecular beacon for improving sensitivity of homogenous fluorescence biosensing platform for DNA detection using target-primed rolling circle amplification

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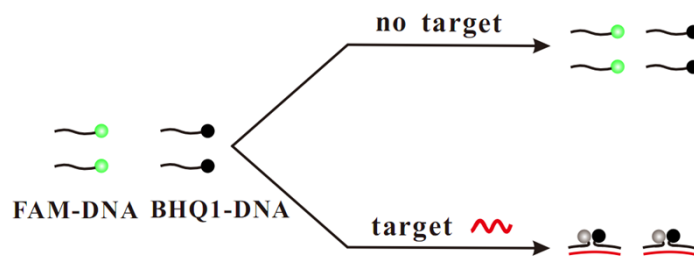
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Table S1. Sequences of the Used Oligonucleotides

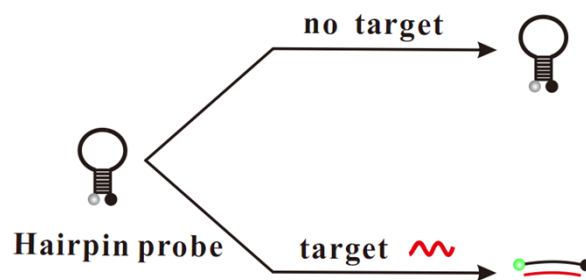
Oligonucleotide	Sequence (5' to 3')
padlock probe	5'-phosphate- CTC AGC TGT GTG CTT CCA TCT GCC AAC CTC TTT CAG ATG TCC TAT C -3'
P1	5'-FAM-CAA CCT CTT TCA G-3'
P2	5'-GCT TCC ATC TGC-BHQ ₁ -3'
MB	5'-6-FAM- CAG CTA ACC ATC TGC CAA CCT CTT TAG CTG -BHQ ₁ -3'
Target DNA	5'-ACA CAG CTG AGG ATA GGA CAT-3'
T1 ^a	5'-ACA CAG CTG AGG ATA GGA CAA-3'
T1 ^b	5'- <u>T</u> CA CAG CTG AGG ATA GGA CAT-3'
T1 ^c	5'-ACA CAG CTG A <u>T</u> G ATA GGA CAT-3'
T1 ^d	5'-ACA CAG CTG AG <u>A</u> ATA GGA CAT-3'
T2 ^a	5'-ACA CAG CTG A <u>CA</u> ATA GGA CAT-3'
T2 ^b	5'- <u>C</u> CA CAG CTG AGG ATA GGA CAA-3'
T2 ^c	5'-ACA C <u>T</u> G CTG AGG ATA <u>C</u> GA CAT-3'
Tn	5'- <u>CTG GCA TAC GAA CAT TTG GTA</u> -3'

Table S2. Sequences of the used oligonucleotides

Oligonucleotide	Sequence (5' to 3')
MB	5'-6-FAM-CAG CTA ACC ATC TGC CAA CCT CTT TAG CTG-BHQ ₁ -3'
complementary sequence of MB (cMB)	5'-CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG-3'
cMB-cMB	5'-CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG-3'
cMB-A ₃ -cMB	5'-CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG AAA CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG-3'
cMB-A ₅ -cMB	5'-CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG AAAAA CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG-3'
cMB-A ₁₀ -cMB	5'-CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG AAAAA AAAAA CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG-3'
cMB-A ₁₅ -cMB	5'-CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG AAAAA AAAAA AAAAA CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG-3'
cMB-A ₂₀ -cMB	5'-CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG AAAAA AAAAA AAAAA AAAAA CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG-3'
cMB-A ₃₀ -cMB	5'-CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG AAAAA AAAAA AAAAA AAAAA AAAAA AAAAA CAG CTA AAG AGG TTG GCA GAT GGT TAG CTG-3'
P1	5'-FAM-CAA CCT CTT TCA G-3'
P2	5'-GCT TCC ATC TGC-BHQ ₁ -3'
complementary sequence of P1/P2 (cDNA)	5'-C TGA AAG AGG TTG GCA GAT GGA AGC-3'
cDNA-cDNA	5'-C TGA AAG AGG TTG GCA GAT GGA AGC C TGA AAG AGG TTG GCA GAT GGA AGC-3'
cDNA-A ₅ -cDNA	5'-C TGA AAG AGG TTG GCA GAT GGA AGC AAAAA C TGA AAG AGG TTG GCA GAT GGA AGC-3'
cDNA-A ₁₀ -cDNA	5'-C TGA AAG AGG TTG GCA GAT GGA AGC AAAAA AAAAA C TGA AAG AGG TTG GCA GAT GGA AGC-3'



Scheme S1. Schematic illustration of the strategy without RCA for homogenous fluorescence detection of DNA with linear DNA probe as signal probe.



Scheme S2. Schematic illustration of the strategy without RCA for homogenous fluorescence detection of DNA with hairpin probe as signal probe.

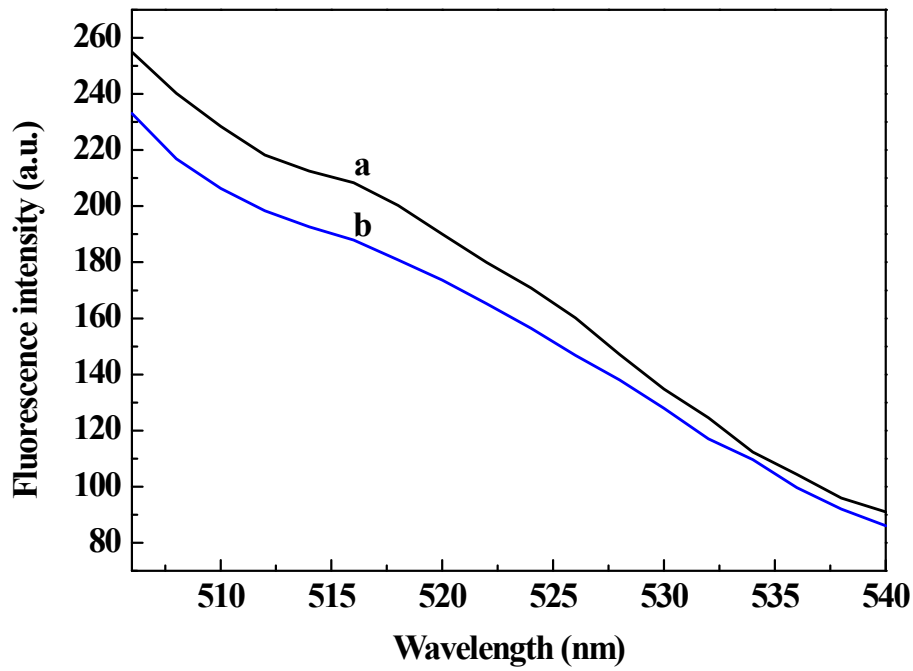


Figure S1. Fluorescence spectra of the fluorescence system without RCA using linear DNA probe as signal probe in the absence (a) and presence (b) of 1×10^{-11} M target DNA. Experimental condition: 20 μ L FAM-labeled P1 (1×10^{-10} M) + 20 μ L BHQ1-labeled P2 (1×10^{-10}) + 150 μ L 100 mM PBS buffer (pH 7.4, 100 mM KCl, and 20 mM MgCl_2).

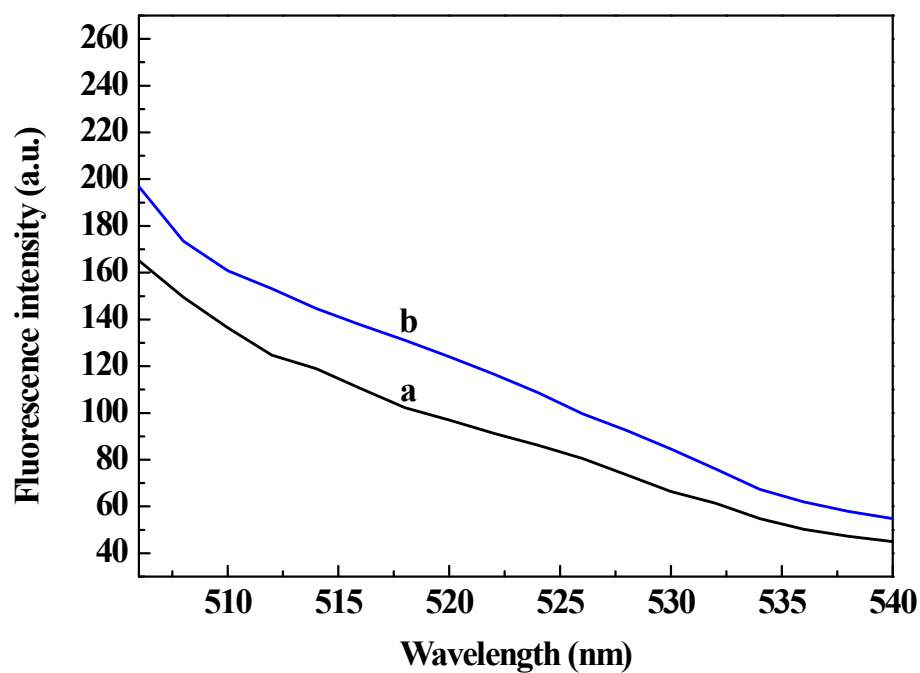


Figure S2. Fluorescence spectra of the fluorescence system without RCA using hairpin probe as signal probe in the absence (a) and presence (b) of 1×10^{-11} M target DNA. Experimental condition: 20 μ L hairpin probe (1×10^{-10} M) + 170 μ L 100 mM PBS buffer (pH 7.4, 100 mM KCl, and 20 mM MgCl_2).

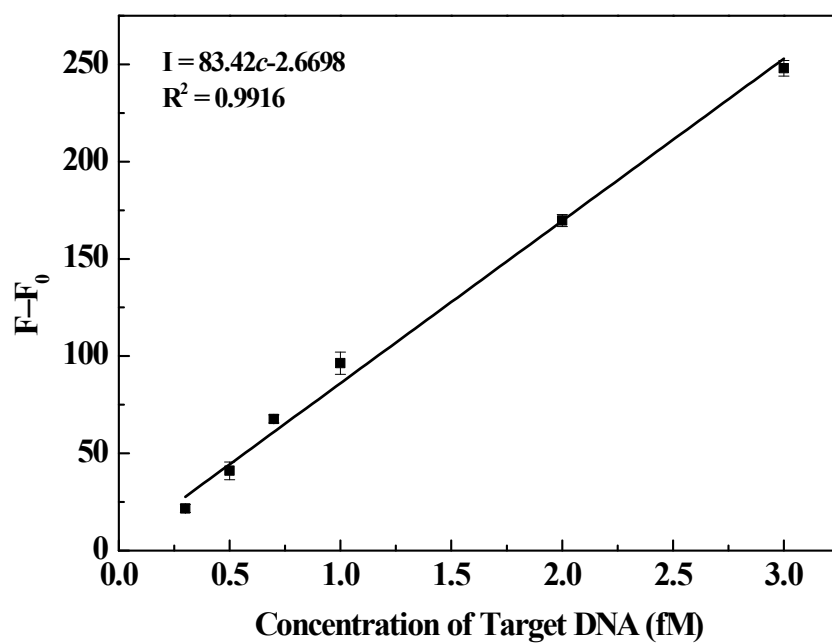


Figure S3. Linear relationship between the fluorescence intensity change (F_0-F) with MB as signal probe and target DNA concentration from 0.3 to 3 fM. Error bars represent the standard deviations of three independent measurements.

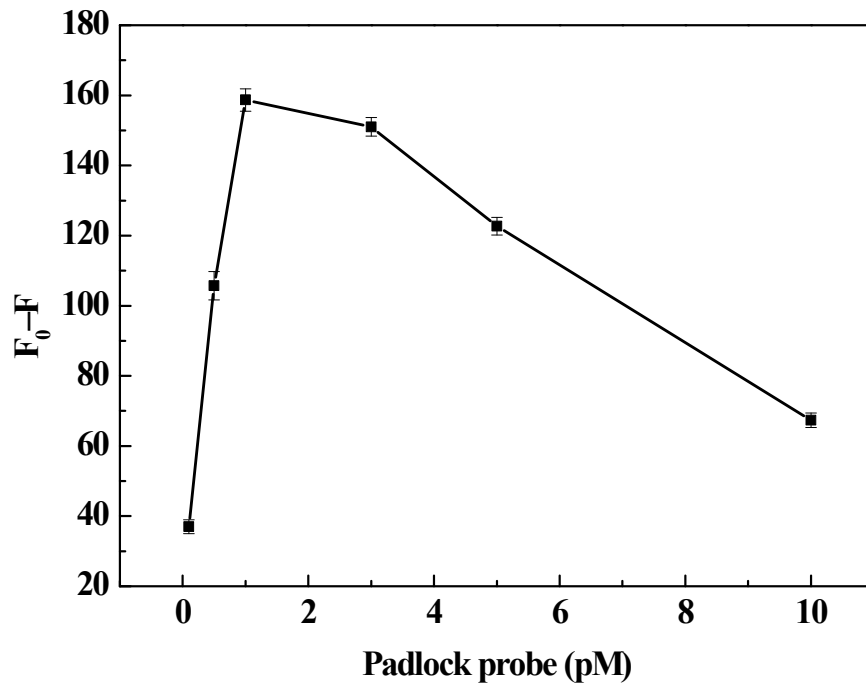


Figure S4. Effect of padlock probe concentration on the fluorescence signal. Experimental conditions: T4 DNA ligase 4 U, phi29 DNA polymerase 4 U, dNTPs (2.5 mM) 8 μ L, RCA reaction time 150 min, FAM-DNA 30 pM, BHQ₁-DNA 60 pM.

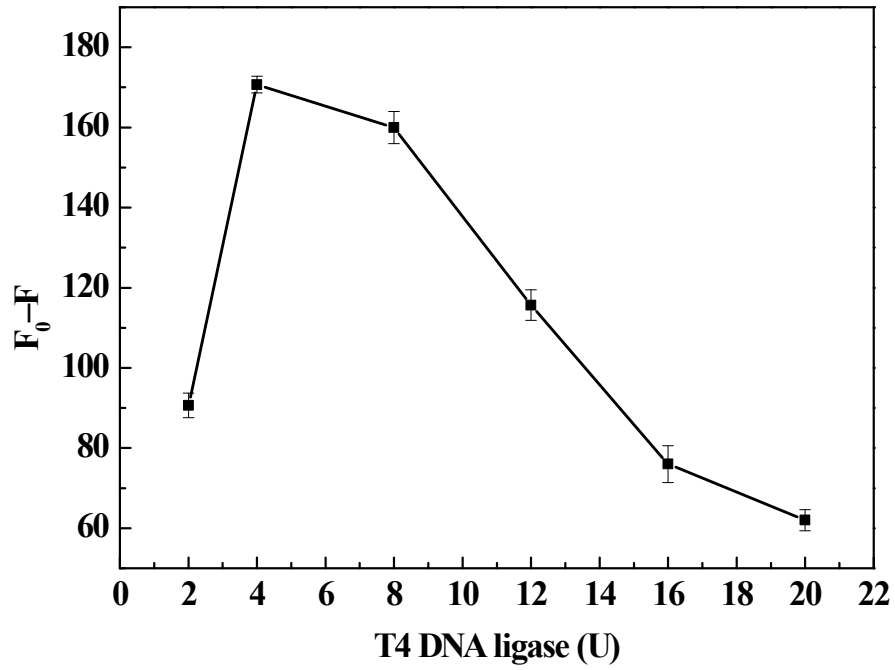


Figure S5. Effect of T4 DNA ligase on the fluorescence signal. Experimental conditions: Padlock probe 1 pM, phi29 DNA polymerase 4 U, dNTPs (2.5 mM) 8 μ L, RCA reaction time 150 min, FAM-DNA 30 pM, BHQ₁-DNA 60 pM.

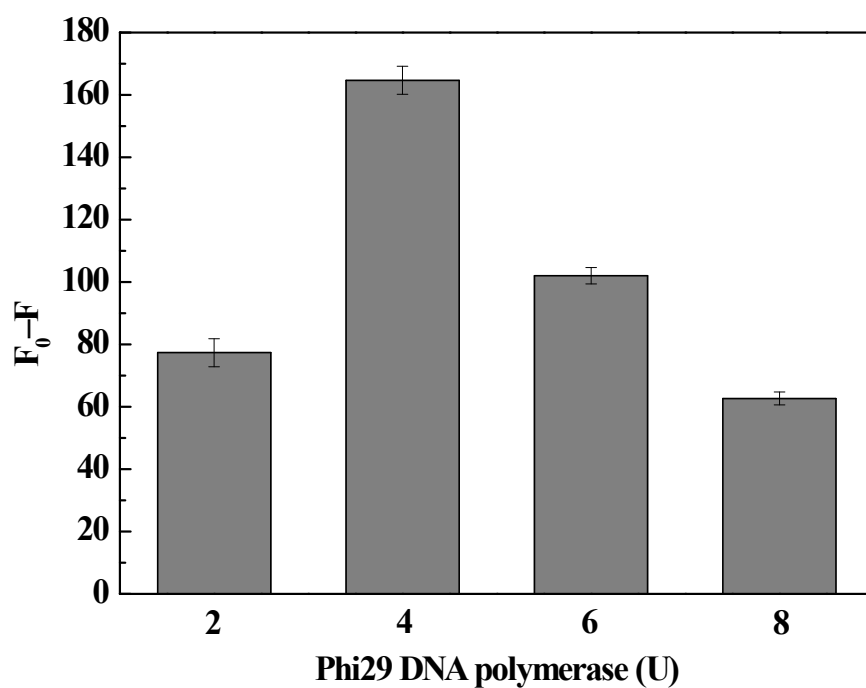


Figure S6. Effect of Phi29 DNA polymerase on the fluorescence signal. Experimental conditions: Padlock probe 1 pM, T4 DNA ligase 4 U, dNTPs (2.5 mM) 8 μ L, RCA reaction time 150 min, FAM-DNA 30 pM, BHQ₁-DNA 60 pM.

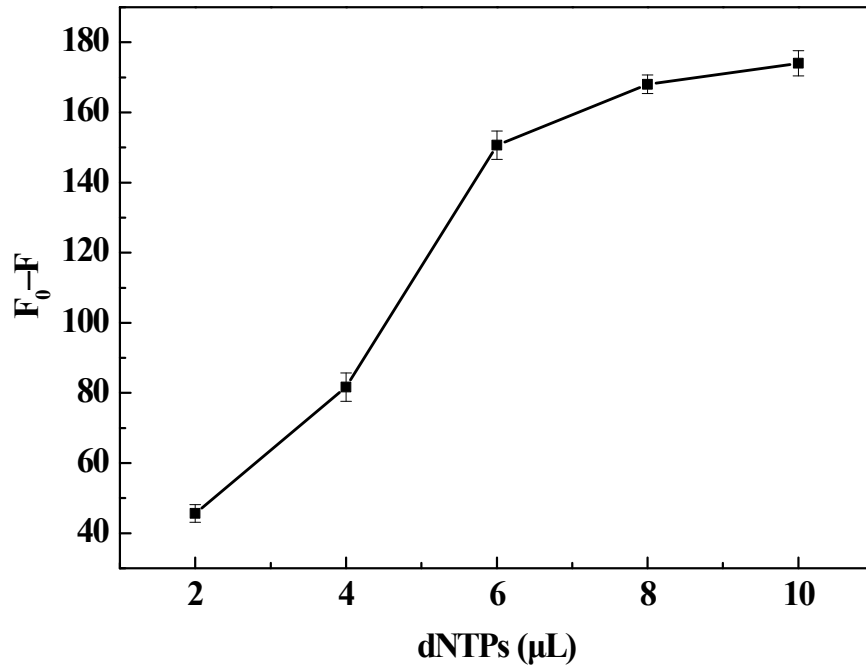


Figure S7. Effect of dNTPs on the fluorescence signal. Experimental conditions: Padlock probe 1 pM, T4 DNA ligase 4 U, phi29 DNA polymerase 4 U, RCA reaction time 150 min, FAM-DNA 30 pM, BHQ₁-DNA 60 pM.

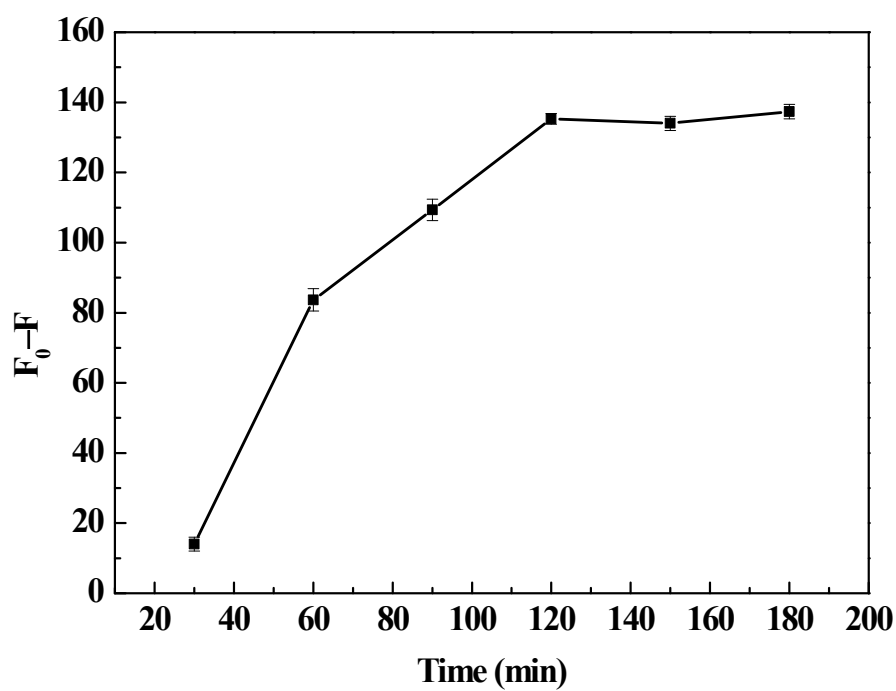


Figure S8. Effect of RCA reaction **time** on the fluorescence signal. Experimental conditions: Padlock probe 1 pM, T4 DNA ligase 4 U, phi29 polymerase 4 U, dNTPs (2.5 mM) 8 μ L, FAM-DNA 30 pM, BHQ₁-DNA 60 pM.

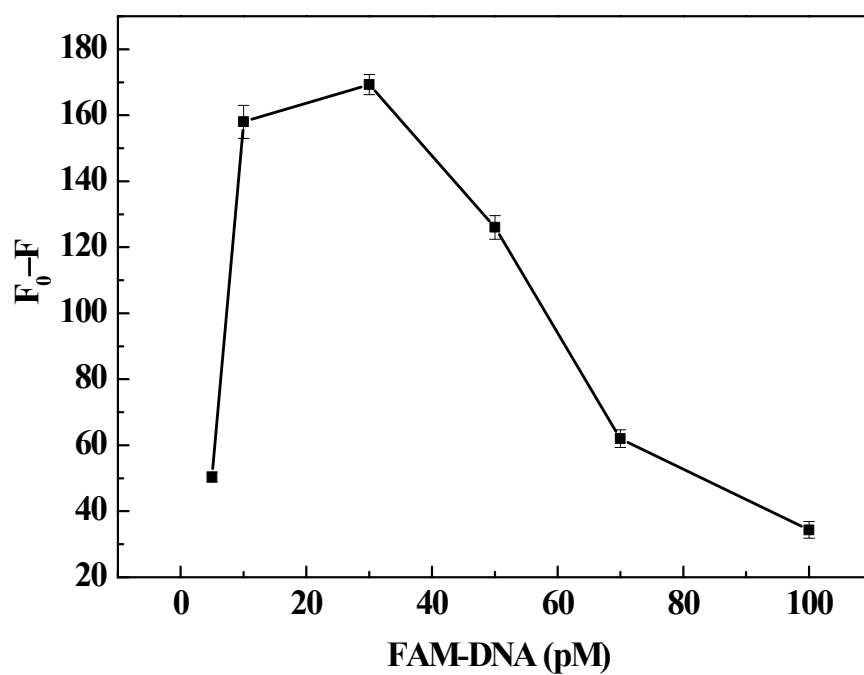


Figure S9. Effect of FAM-DNA on the fluorescence signal. Experimental conditions: Padlock probe 1 pM, T4 DNA ligase 4 U, phi29 polymerase 4 U, dNTPs (2.5 mM) 8 μ L, RCA reaction time 150 min, BHQ₁-DNA 60 pM.

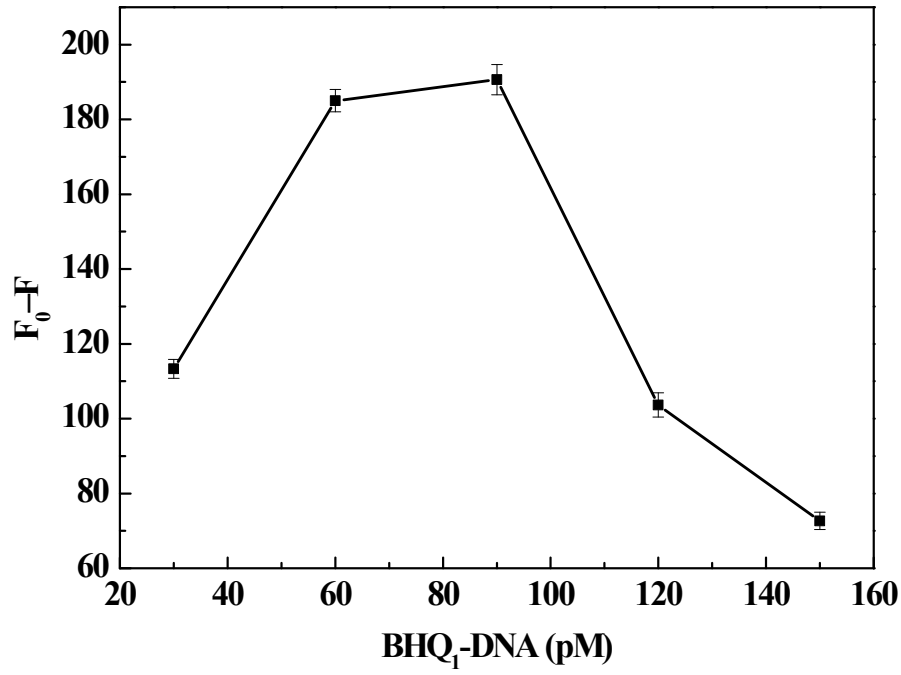


Figure S10. Effect of BHQ₁-DNA on the fluorescence signal. Experimental conditions: Padlock probe 1 pM, T4 DNA ligase 4 U, phi29 polymerase 4 U, dNTPs (2.5 mM) 8 μ L, RCA reaction time 150 min, FAM-DNA 30 pM.