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Supporting Information

A rapid room-temperature DNA amplification and detection strategy based on nicking endonuclease and catalyzed hairpin assembly.

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Table S1: The sequences of DNA used in the experiments.

DNA name	DNA sequence (5' to 3')
H1	GCACAGAGACTACCAGAGTCACACAACAGACGGGCACTCTGG TATCTGGTCGCCCGTCTGTTGTGTGACCCTTGAGTTAGAGCAG
H2	CAGACGGGCGACCAGATACCAGAGTGCCCGTCTGTTGTGTGAC TCTGGTATCTGGTCGC
H3	TACCAGAGTCTCTGTGCCCGTCTGTTGTGTGACTCTGGTA
F	(FAM)GCACTGCTCTAACTCAAGGGTCACACAA
Q	CCTTGAGTTAGAGCAGTGC(Dabcyl)
Perfectly matched Target	GTGCCCGTCTGTTGTGTGACTCTGGTA
Deleted Target	GTGCCCGTCTGTT_TGTGACTCTGGTA
Inserted Target	GTGCCCGTCTGTTGTTGTGACTCTGGTA
Mismatched Target	GTGCCCGTCTGTTTTGTGACTCTGGTA

Table S2: Different DNA amplification methods.

Method	Reaction temperature (°C)	Detection time	Ref.
Loop-mediated isothermal amplification (LAMP)	65	1 h	[1]
Helicase-dependent isothermal DNA amplification (HDA)	37	2 h	[2]
Strand displacement amplification (SDA)	40	2 h	[3]
Cross-priming amplification (CPA)	63	28 min	[4]
Strand exchange amplification (SEA)	65	1 h	[5]
Beacon assisted detection amplification (BAD AMP)	40	40 min	[6]
DNA sequence-based amplification (NASBA)	37	1 h	[7]
Rolling circle amplification (RCA)	30	12 h	[8]
Exonuclease-assisted isothermal amplification (Exo-NAT)	65	1.5 h	[9]
Self-replicating CHA	room temperature	10 min	this work

Table S3: Different signal amplification approaches for DNA assay.

Strategy	Signal readout	Detection limit	Detection time	Ref.
Molecular beacon-based junction sensing system	Fluorescence detection	1.6 nM	30 min	[10]
Autocatalytic and E6 Mg^{2+}- dependent DNAzyme mediated process	Fluorescence detection	1 pM	12 h	[11]
DNA machine	Colorimetric detection	0.2 nM	19 h	[12]
Y-shaped junction scaffold mediated modular and cascade amplification strategy	Fluorescence detection	28.2 fM	1 h	[13]
Hybridization chain reaction (HCR)	Colorimetric detection	50 pM	1 h	[14]
G-quadruplex integrated hybridization chain reaction (GQ-HCR)	Fluorescence detection	4 nM	1 h	[15]
Target-catalyzed autonomous assembly of dendrimer-like DNA nanostructures	Colorimetric detection	9 pM	1.5 h	[16]
Target-driven DNA association	Fluorescence detection	21.6 pM	1 h	[17]
Self-replicating CHA	Fluorescence detection	2.6 pM	10 min	This work

Table S4: Determination of HIV target DNA added in human blood serum with proposed strategy.

sample number	added/pM	found/pM	recovery/%	RSD/%
1	50.0	50.4	100.8	2.42
2	100.0	98.9	98.9	3.21
3	200.0	202.6	101.3	4.38
4	300.0	309.0	103.0	2.84

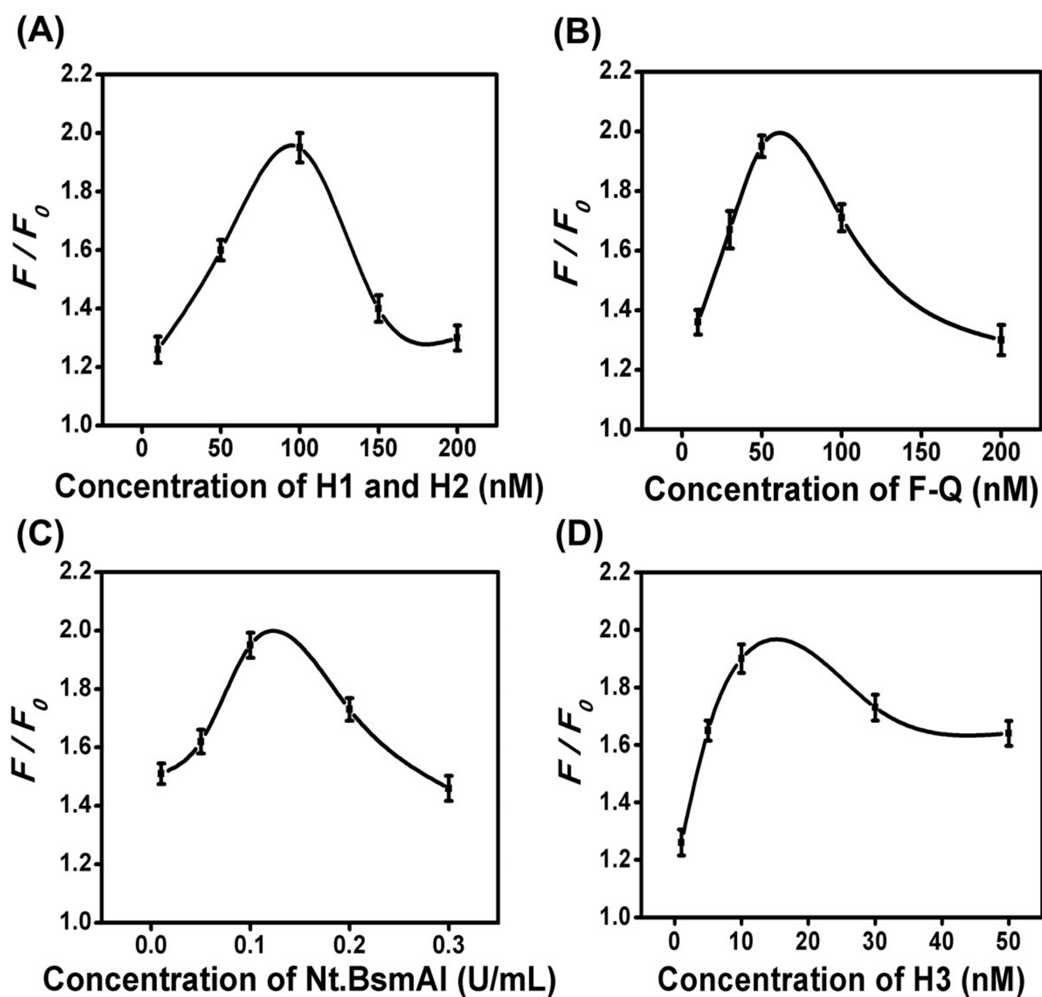

Fig. S1 Conditions optimization for HIV target DNA detection: concentrations of hairpin probes H1 and H2 (A), F-Q duplex (B), Nt.BsmAI (C) and H3 (D). F/F_0: The fluorescence intensity at 520 nm of the detection system in the presence of 5 nM target DNA versus the fluorescence intensity at 520 nm of the detection system in the absence of target DNA.

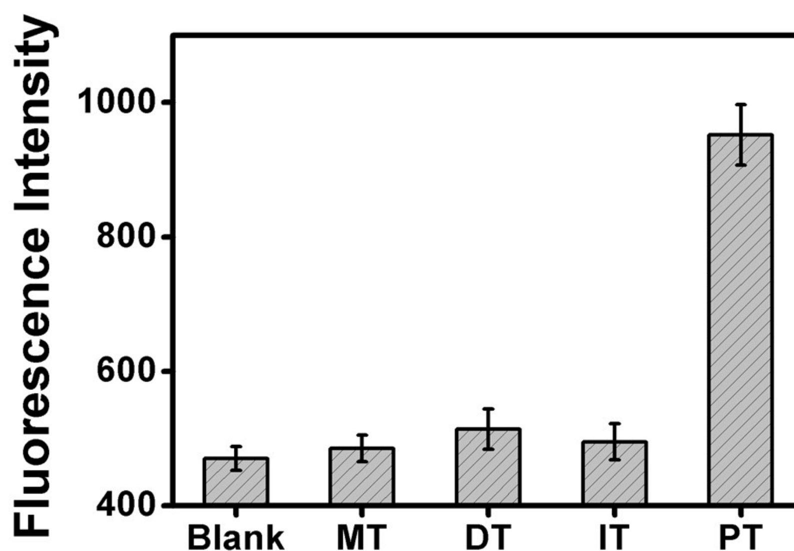

Fig. S2 Fluorescence spectra of the detection system in the presence of mismatched HIV target (MT), deleted HIV target (DT), inserted HIV target (IT), and perfectly matched HIV target (PT). The concentrations of H1, H2, H3, F-Q and Nt.BsmAI were 100, 100, 10, 50 nM and 0.1 U/mL; MT, DT, IT, and PT concentration: 5 nM.

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