One-pot synthesized three-way junction based multiple strand displacement amplification for sensitive assay of H5N1 DNA

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Table S1 Sequences of oligonucleotides used in this study

Name	Sequences (5'-3')
H1	TCAAGTCGAGAGTTAGGCTGAGGCACTGG AGACTCTTGAGTTCTCAGTATGATGGGTCA GAACTCAAGAGTCTTTACGC
H2	TCAAGTCGAGAGTTAGGCTGAGGGCGTAA AGACTCTTGAGTTCTGACCCATCAAGAGTA GAACTCAAGAGTCTGGTACT
H3	TCAAGTCGAGAGTTAGGCTGAGGAGTACC AGACTCTTGAGTTCTACTCTTGCATACTGA GAACTCAAGAGTCTCCA
H5N1 DNA	CATACTGAGAACTCAAGAGTCT
Single-base mismatch (SM)	CATACTGAGAATTCAAGAGTCT
Double-base mismatch (DM)	CATACTTAGAATTCAAGAGTCT
Three-base mismatch (TM)	CATACTTAGAATTCAAGATTCT
Non-complementary (NC)	TGCGACTGTCCAGTGGACTCAC
H7N9 DNA	CATTGGAATAGCAAACCTAGGATT
H1N1 DNA	GACTACACTCTCGATGAAGAA
activator	TCAGCCTAACTCTCGACTTGA
crRNA	UAAUUUCUACUAAGUGUAGAUCAAGUCG AGAGUUAGGCUGA
FQ	FAM-TTATT-BHQ1

Note: The H5N1 DNA binding sequence in H1 is red. The nicking enzyme sites are highlighted in yellow. The underlined bases of SM, DM and TM indicate mismatched bases

Table S2 Detection of H5N1 in fetal bovine serum through the system

Samples	Spiked(pM)	Measured(pM)	Recovery(%)	RSD(%)
Serum	0.4	0.54	135	1.8
	200	156.17	78.09	1.3
	500	499.04	99.81	9.5
	800	804.7	100.59	0.77

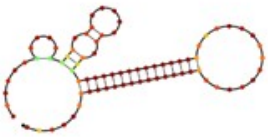
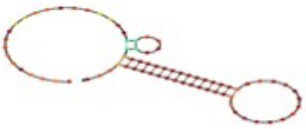
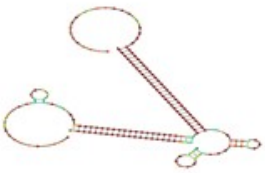
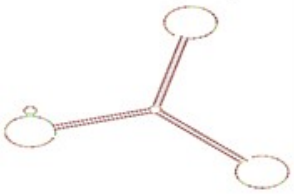
Name	H1	H2	H3
Schematic			
ΔG/kcal/mol	-16.25	-17.79	-15.08
Name	H1+H5N1 DNA	H1+H2+H5N1 DNA	H1+H2+H3(TWJ)
Schematic			
ΔG/kcal/mol	-31.43	-72.27	-104.52

Fig S1. Secondary structures and Gibbs free energy (ΔG) of hairpin and reaction products from NUPACK

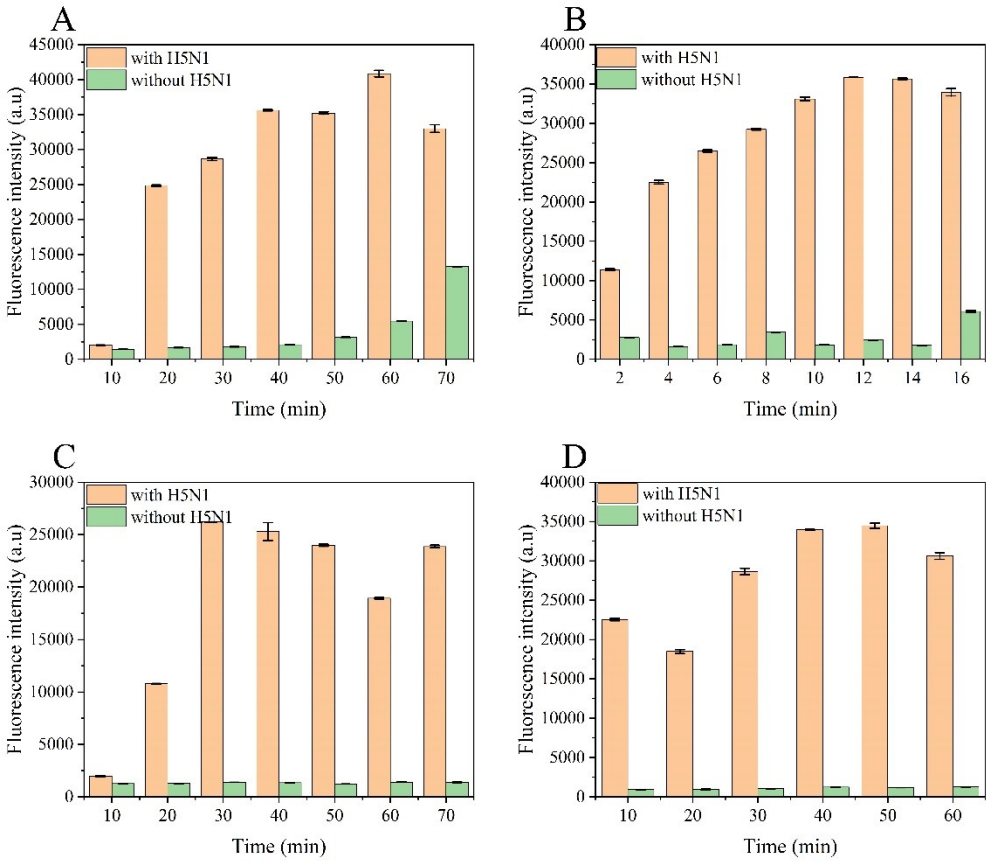


Fig S2. (A) Cycle2 reaction time in the stepwise reaction; (B) Cycle 1 reaction time per step in the stepwise reaction; (C) Cycle 2 reaction time in one-pot reaction; (D) Cycle 1 reaction time in one-pot reaction. The error bar represents the standard deviation of three measurements.

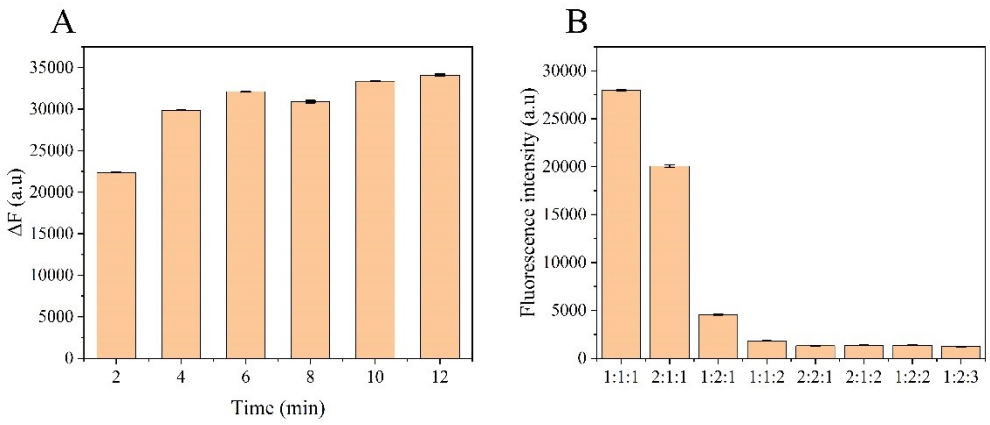


Fig S3. (A) Cycle 2 post-reaction inactivation time($\Delta F=F-F_0$， F_0 is the fluorescence signal without H5N1 DNA); (B) Hairpin H1:H2:H3 ratio in cycle 1. Data are expressed as mean ±SD (n = 3).