

Supplementary Information

PAM-Free Activation of CRISPR/Cas12a via Semi-Nested Asymmetric RPA: Ultra-Sensitive and High Specific Detection of HPV16 dsDNA

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15 **1 Experimental reagents and DNA sequences**

16 All reagents(Table S1) and DNA sequences(Table S2) are shown with information
17 in the Appendix.

18 Table S1 Regents

Name of the reagent	Specification	Manufacturer
LbaCas12a	20 μM	MAGIEN (Guangzhou, China)
The ERA nucleic acid amplification kit	24 T	GenDx Biotechnology(Suzhou, China)
DNase/RNase-free Water	250 ml	TIANGEN(Beijing, China)
APS	25 g	Sigma-Aldrich(Shanghai, China)
TEMED	500 mL	Macklin(Shanghai, China)
DEPC-treated Water	500 mL	Sangon Biotech(Shanghai, China)
5×TBE nucleic acid electrophoresis buffer	500 mL	Sangon Biotech(Shanghai, China)
Tris-EDTA buffer	500 mL	Sangon Biotech(Shanghai, China)
Double distilled water	500 mL	Sangon Biotech(Shanghai, China)
TS-GelRED (10000×)	500 μL	Sangon Biotech(Shanghai, China)
DNA Molecular Weight Marker	25-500 bp	Sangon Biotech(Shanghai, China)
6X Glycerol Gel Loading Buffer IX	1 mL	Sangon Biotech(Shanghai, China)
30% Acr-Bis (29:1)	500 mL	Sangon Biotech(Shanghai, China)
Agarose	100 g	Sangon Biotech(Shanghai, China)
10×Reaction Buffer	1.2 ml	Sangon Biotech(Shanghai, China)

19 Table S2 DNA sequence(5'-3')

HPV-16	GAATGCAGGTGACTTTTATTTACATCCTAGTTATTACATGTT ACGAAAACGACGTAAACGTTTACCATATTTTTTTTCAGATGT CTCTTTGGCTGCCT
R	AGGCAGCCAAAGAGACATCTGAAAAAAAT
F1	GAATGCAGGTGACTTTTATTTACATCCTAG
F2	TTTACATCCTAGTTATTACATGTTACGAAA

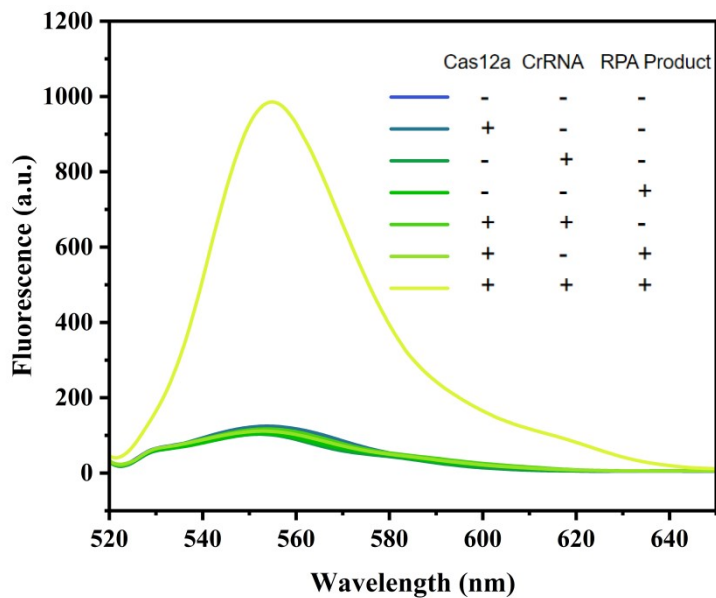
crRNA	UAAUUUCUACUAAGUGUAGAU AUG GUA AAC GUU UAC GUC GU
DNA-FQ	HEX-TATTATT-BHQ1
HPV-18	TCATAACAATGGTGTGGCTGGCATAATCAATTATTTGTTAC TGTGGTAGATACCACTCGCAGTACCAATTTAACAATATGTG CTTCTACACAGTCTCCTGTACCTGGG
Random 2	TTCCAACCACGTCTTCAAAGC
Random 3	GGAAGGGTCTTGCGAAGGATA
Random 4	TGAATCCTGTTGCCGGTCTTGCGATGATTATC

20 2 Other peers' work

21 Table S3 Other peers' work

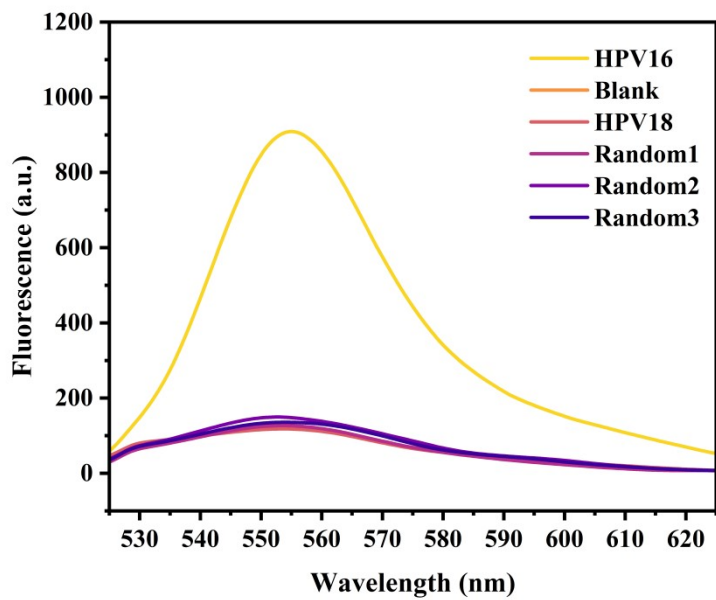
Method	Target	Detection time	Limit of detection (L OD)	References	PAM-FREE
LAMP-CRISPR	Pyogenes streptococcus	60 min	100CFU mL ⁻¹	1	NO
HCR-CRISPR	miRNA	35 min	0.3 fM	2	NO
RPA-CRISPR	Lumpy Skin Disease Virus (LAD V)	50 min	1.21 × 10 ¹ copies μ L ⁻¹	3	NO
RCA-CRISPR	HPV16 ssDNA	90 min	38.02 fM	4	NO
light-operated-CRISPR	HPV16 dsDNA	-	3.3 pM	5	YES
DNA tetrahedral-CRISPR	HPV16 dsDNA	100 min	8.86 fM	6	YES
RPA-CRISPR	HPVds DNA	45 min	50 copies/ μ L	7	YES
This Work	HPV16 dsDNA	70 min	18 aM		NO

3 Supplementary images in the appendix



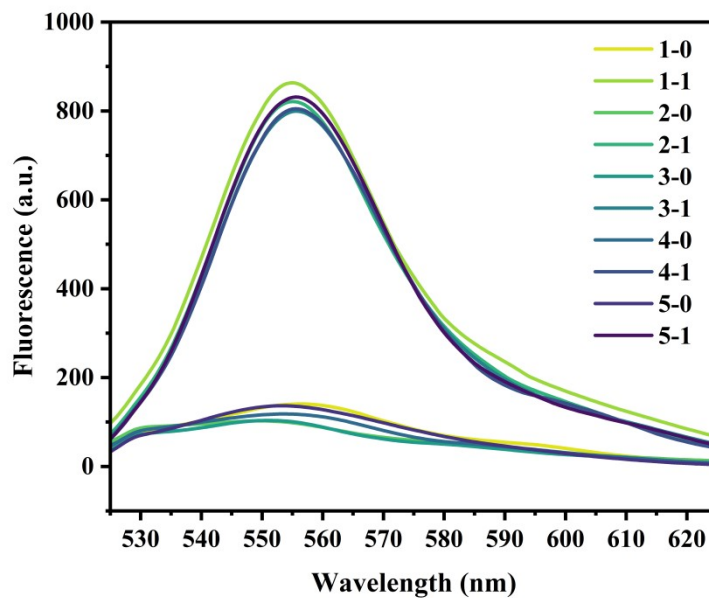
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25 Figure S1 Fluorescence feasibility when different Cas12a components are
26 added



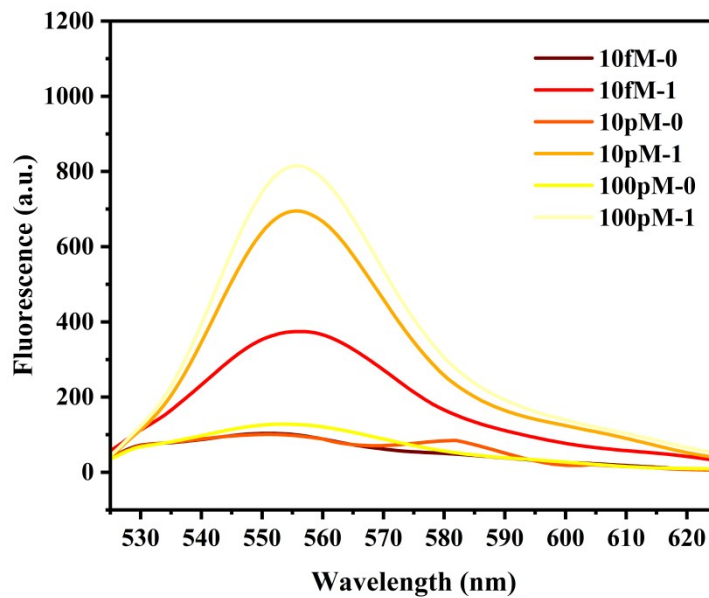
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28 Figure S2 SNA-RPA-CRISPR was used to detect the fluorescence curves of
29 HPV16, blank group, HPV18 and three random sequences



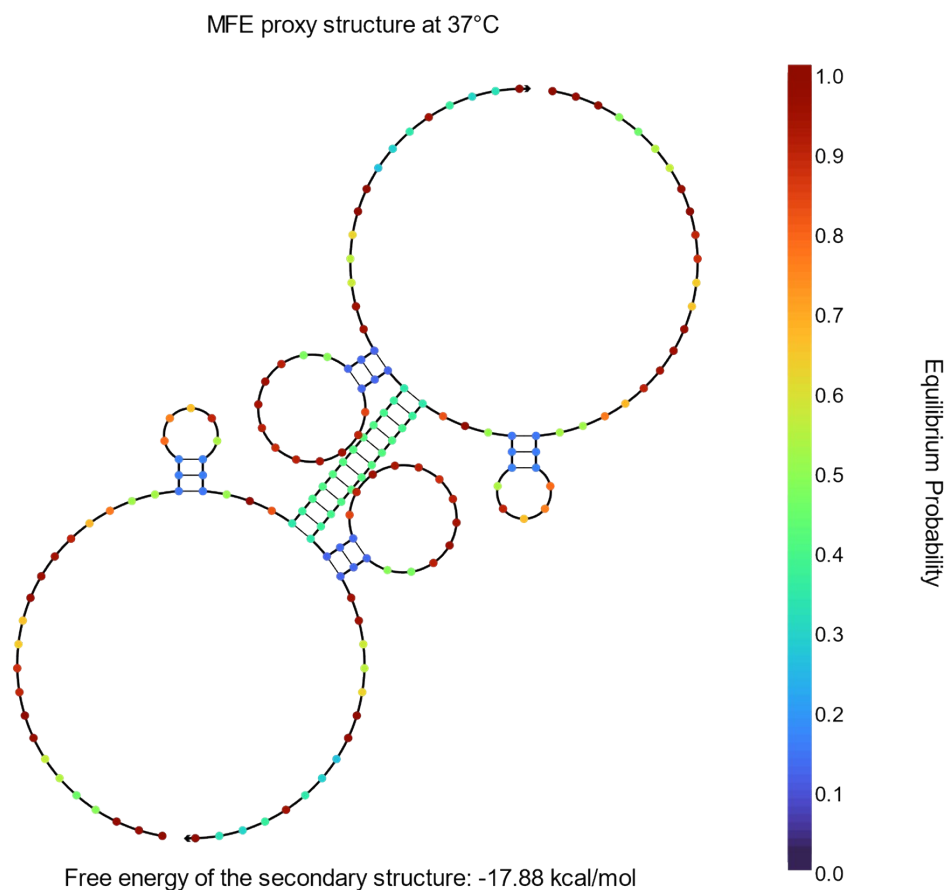
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31 Figure S3 Fluorescence curves of five independent groups of samples of
 32 HPV16 dsDNA were detected by SNA-RPA-CRISPR



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34 Figure S4 Detection of HPV16 dsDNA with 10% serum addition using
 35 ASN-RPA-LCRISPR



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37 Figure S5 ssDNA self-pairing analysis based on NUPACK software

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