Simple, Visual, Point-of-Care SARS-CoV-2 Detection Incorporating Recombinase Polymerase Amplification and Target DNA–Protein Crosslinking Enhanced Chemiluminescence

CONTENTS

Table S1. Detailed information of the oligonucleotide sequences used in this study.

Names	Sequences (5'-3')
SARS-CoV-2 N gene	ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGA CCCTCAGATTCAACTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGATCAAA ACAACGTCGGCCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCACCGCTCTCAC TCAACATGGCAAGGAAGACCTTAAATTCCCTCGAGGACAAGGCGTTCCAATTAACA CCAATAGCAGTCCAGATGACCAAATTGGCTACTACCGAAGAGCTACCAGACGAATT CGTGGTGGTGACGGTAAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTA GGAACTGGGCCAGAAGCTGGACTTCCCTATGGTGCTAACAAAGACGGCATCATATG GGTTGCAACTGAGGGAGCCTTGAATACACCAAAAGATCACATTGGCACCCGCAATC CTGCTAACAATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAACATTGCCAAAAG GCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCAC GTAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTCCT GCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGA TTGAACCAGCTTGAGAGCAAAATGTCTGGTAAAGGCCAACAACAACAAGGCCAAAC TGTCACTAAGAAATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGC CACTAAAGCATACAATGTAACACAAGCTTTCGGCAGACGTGGTCCAGAACAAACCC AAGGAAATTTTGGGGACCAGGAACTAATCAGACAAGGAACTGATTACAAACATTGG CCGCAAATTGCACAATTTGCCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGCATT GGCATGGAAGTCACACCTTCGGGAACGTGGTTGACCTACACAGGTGCCATCAAATT GGATGACAAAGATCCAAATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGA CGCATACAAAACATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAAGAAGGCT GATGAAACTCAAGCCTTACCGCAGAGACAGAAGAAACAGCAAACTGTGACTCTTCT TCCTGCTGCAGATTTGGATGATTTCTCCAAACAATTGCAACAATCCATGAGCAGTGC TGACTCAACTCAGGCCTAA
SARS-2 FP	CAACTTCCTCAAGGAACAACATTGCCAAAA
SARS-2 RP	TGGAGTTGAATTTCTTGAACTGTTGCGACT
SARS-2 FP-biotin	biotin-CAACTTCCTCAAGGAACAACATTGCCAAAA
SARS-2 RP-biotin	biotin-TGGAGTTGAATTTCTTGAACTGTTGCGACT

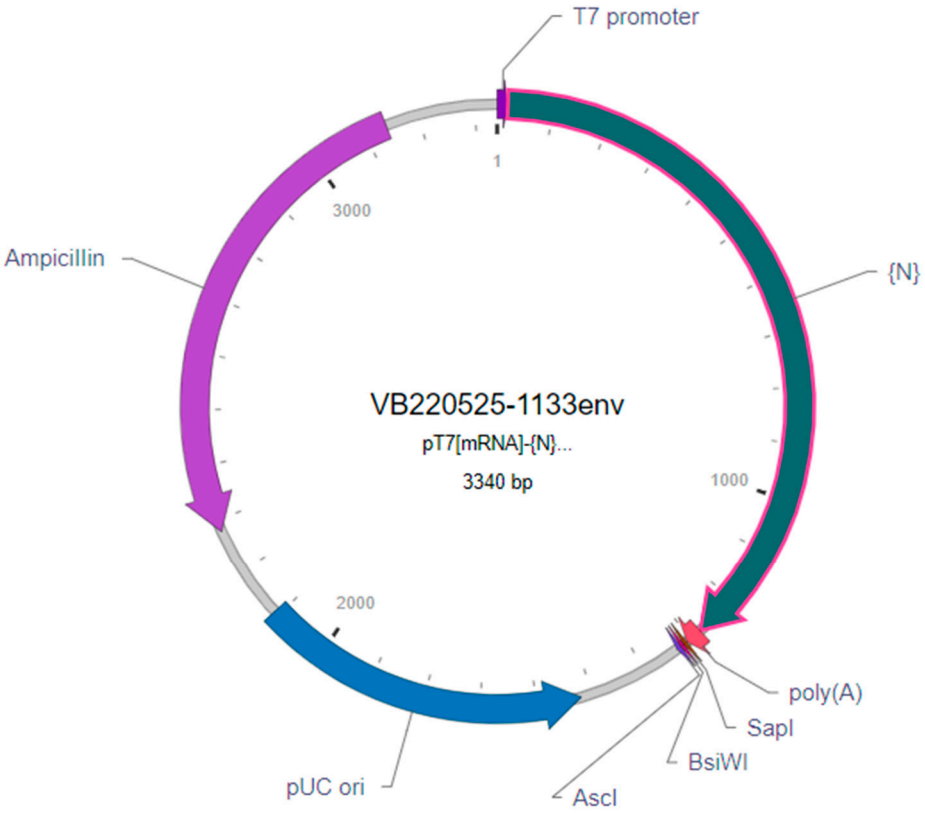

Figure S1. The designed SARS-CoV-2 N gene plasmid map.

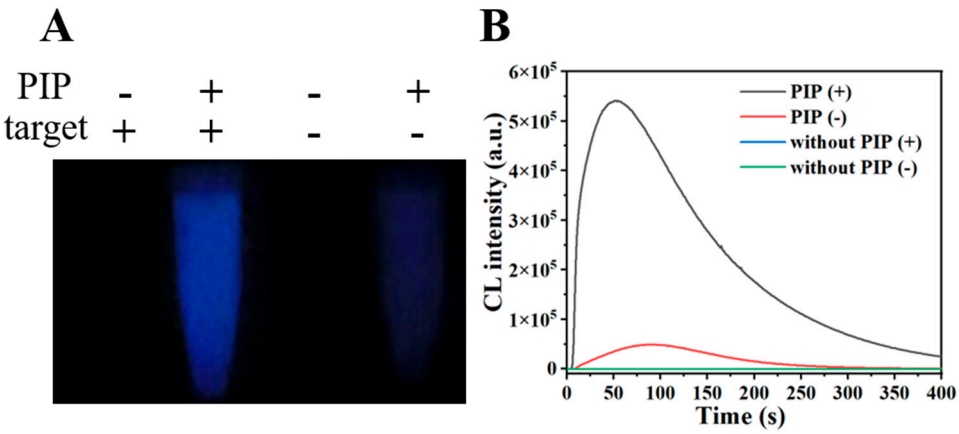

Figure S2. Effect of the PIP enhancer on RPADPCL chemiluminescent signal strength.

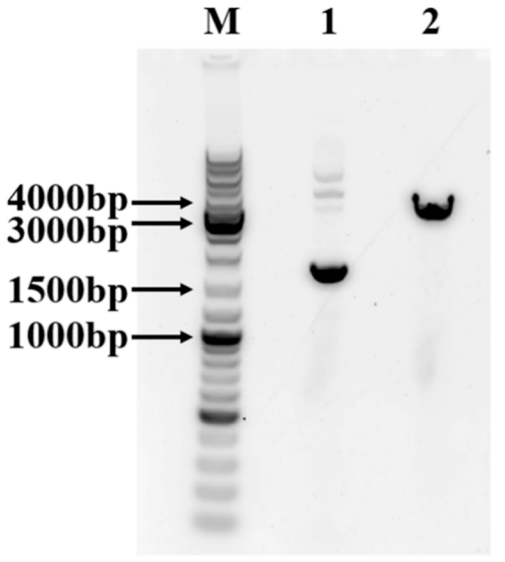

Figure S3. Identification of SARS-CoV-2 N gene plasmid by 1% agarose gel electrophoresis. M：DNA marker; Lane 1：The designed SARS-CoV-2 N gene plasmid. Lane 2：The plasmid after AscI enzyme digestion.

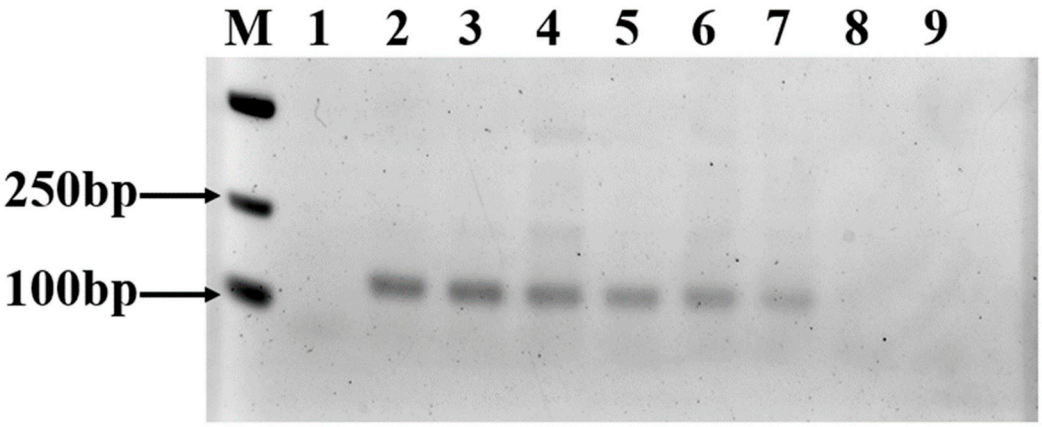

Figure S4. Identification of RPA products for SARS-CoV-2 N gene plasmid detection by 1% agarose gel electrophoresis. M：DNA marker; Lane 1: negative control; Lane 2-9: The concentration of SARS-CoV-2 N gene plasmid was 1000, 500, 300, 200, 100, 50, 10, 1 copies, respectively.

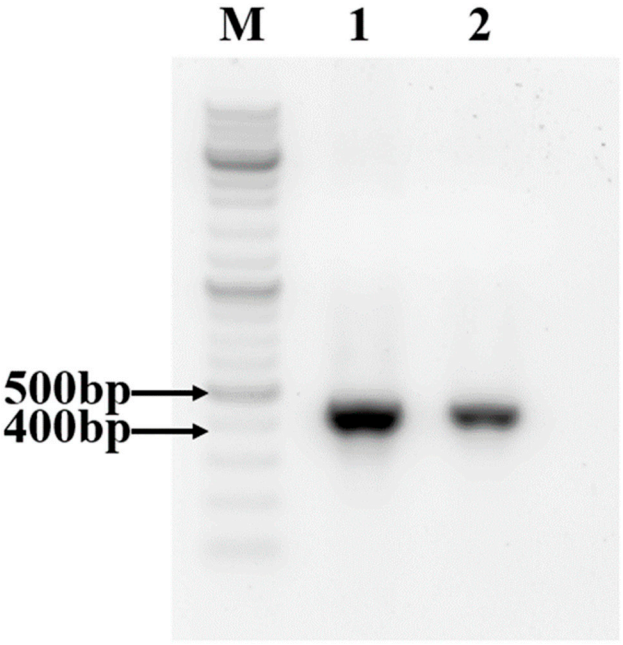

Figure S5. Identification of SARS-CoV-2 IVT RNA by 1% agarose gel electrophoresis. M：DNA marker; Lane 1：The IVT RNA synthetized by RiboMAX Large Scale RNA Production System Kit. Lane 2：The IVT RNA synthetized byT7 Quick High Yield RNA Transcription Kit.

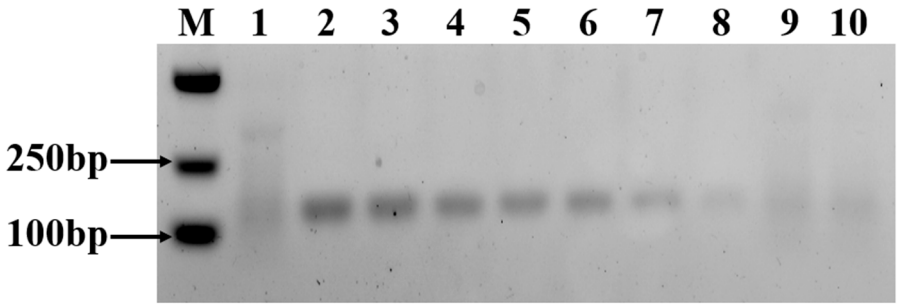

Figure S6. Identification of RPA products for IVT RNA detection by 1% agarose gel electrophoresis. M： DNA marker; Lane 1: negative control; Lane 2-10: The concentration of SARS-CoV-2 N gene plasmid was 2000, 1000, 500, 300, 200, 100, 50, 10, 1 copies, respectively.

Table S2. Comparison between the proposed method and other RPA-based SARS-CoV-2 detection methods.

No.	Methods	Target genes	Sensitivity (LOD)	Quantification	Visualization	Detection time	Ref.
1	Strip	N/ORF1ab gene	10 copies	×	√	60 min	[1]
2	CRISPR/Cas9-mediated strip	E/ORF1ab gene	100 copies	×	√	58 min	[2]
3	Strip	N/S gene	10 copies	×	√	45 min	[3]
4	Microfluidic-integrated strip	N gene	30 copies	×	√	20 min	[4]
5	Strip	N gene	35.4 copies	×	√	45 min	[5]
6	CRISPR/Cas fluorometry	N/ORF1ab gene	2 copies	√	×	50 min	[6]
7	Colorimetric CRISPR/Cas12a assay	N/ORF1ab gene	1 copy	√	×	240 min	[7]
8	Real-time RPA	N gene	10 copies	√	×	27 min	[8]
9	Real-time RPA	ORF1ab/S gene	10 copies	√	×	24 min	[9]
10	Real-time RPA	N/E/RdRP gene	15 copies	√	×	15 min	[10]
11	Fluorescent strip	E/RdRP gene	9.5 copies	√	×	30 min	[11]
12	CRISPR/Cas-based lab-on-paper	N/S gene	100 copies	√	×	60 min	[12]
13	Chemiluminometry	N gene	15 copies	√	√	60 min	This work

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