Electronic Supplementary Material (ESI) for Analytical Methods.

Electronic Supporting Information

A novel reverse transcription-multiple inner primer loop-mediated isothermal amplification (RT-MIPLAMP) for visual and sensitive detection of SARS-CoV-2

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1. Table S1 The sequences of the primers used in this work.

Name		Sequences (5′-3′)
N gene	FIP-1	TCTGTCAAGCAGCAGCAAAGCACTCCTGCTAGAATGGCTGGCAAT
	BIP-1	GAACCAGCTTGAGAGCAAAATGTCTAGCAGCAGATTTCTTAGTGACAG
	F3-1	CAACTCCAGGCAGCAGTA
	B3-1	TTTGCCGAGGCTTCTTAGA
	FIP-2	TACTGCTGCCTGGAGTTGACTCATCACGTAGTCGCAACA
	BIP-2	TCTAAGAAGCCTCGGCAAAAACTGTGTTACATTGTATGCTTTAGTGG
	F3-2	CGGCAGTCAAGCCTCTT
	B3-2	TTCTGGACCACGTCTG
	FIP-3	AAGAGGCTTGACTGCCGCCAAAAGGCTTCTACGCAG
	BIP-3	GCAGACGTGGTCCAGAACAAATTAGTTCCTGGTCCCCAA
	F3-3	CAACTTCCTCAAGGAACAACA
	B3-3	CCAATGTTTGTAATCAGTTCCTT
	LF	AGAGCAGCATCACCGCC
	LB	AGGCCAACAACAACAAGG
ORF1ab gene	FIP-1	ACCATCAACAAATATTTTTCTCACTAGTTTATTCTCTACAGTGTTCCCAC
	BIP-1	TTGTAGTTTCAACTGGATACCACTACATCCTGATTATGTACAACACC
	FIP-2	AAGTTTGCACAATGCAGAATGCAAATTGTGTTAACTGTTTGG
	BIP-2	CTTACATAGCTCTAGACTTAGTTTTAAGGGGGTCAGCAGCATACAC
	F3-2	TTTGACCGTTATTTTAAATATTGG
	B3-2	TAGTAATAGATTACCAGAAGCA
PCR primer for N gene of SARS-CoV-2	FP	TAATACGACTCACTATAGGATGTCTGATAATGGACCCCAAAATCA
	RP	TTAGGCCTGAGTTGAGTCAGC
PCR primer for ORF1ab gene of SARS-CoV-2	FP	TAATACGACTCACTATAGGGTGTTAATGCCTATATTAACCTTGACCA
	RP	GCAACATTGTTAGTAAGTGCAGC
PCR primers for N gene of Bat SARS-like coronavirus	FP	TAATACGACTCACTATAGGATGTCTGATAATGGACCCCAAAACC
	RP	TTAAGCCTGGGTTGAATCAGTAC

isolate bat-SL-CoVZC45		
PCR primers for N gene of SARS coronavirus Tor2	FP	TAATACGACTCACTATAGGATGTCTGATAATGGACCCCAATCAAA
	RP	TTATGCCTGAGTTGAATCAGCAGAAG
PCR primers for N gene of human coronavirus HKU1	FP	TAATACGACTCACTATAGGATGTCTTATACTCCCGGTCATTATGCT
	RP	TTAAGCAACAGAGTCTTCTACATAAGGATC

2. The optimization of the SYBR Green I concentration in the visual RT-MIPLAMP assay

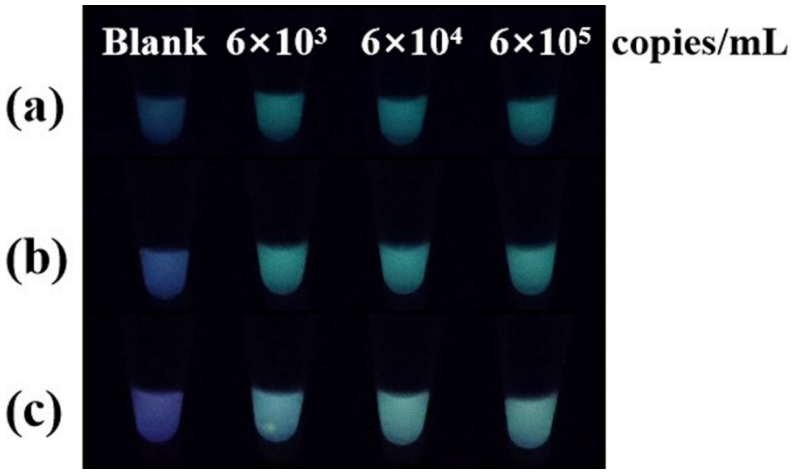

Figure S1 Visual RT-MIPLAMP detection of N gene with (a) 2 ng/μL, (b) 4 ng/μL and (c) 6 ng/μL SYBR Green I.

In order to make the RT-MIPLAMP universally applicable in all areas including field-testing, SYBR Green I is employed to identify the DNA double strand products to enable the visual detection of SARS-CoV-2. The concentration of the SYBR Green I is optimized in the visual RT-MIPLAMP assay. When 2 ng/μL SYBR Green I is used, 6×10^3, 6×10^4 and 6×10^5 copies/mL N gene change the color to green, while blank is still colorless, indicating 2 ng/μL SYBR Green I can discriminate the blank and samples. When 4 ng/μL SYBR Green I is used, the color difference between samples and blank is more obvious. When the SYBR Green I concentration increases to 6 ng/μL, the green color of samples is oversaturated, indicating the SYBR Green I is excessive. Therefore, 4 ng/μL SYBR Green I is used in the following visual RT-MIPLAMP assay.

3. The sensitivity of RT-MIPLAMP for assaying ORF1ab gene

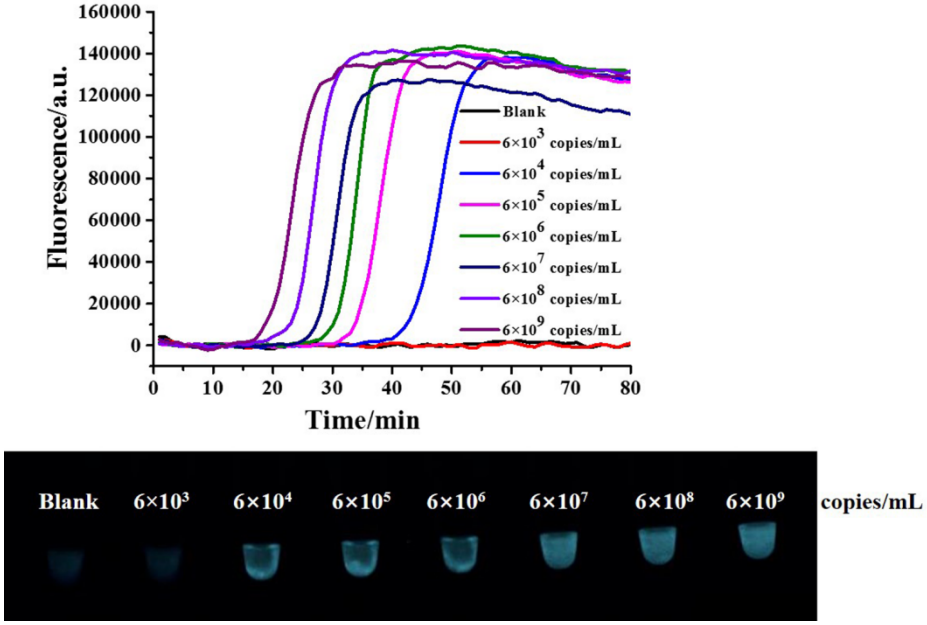

Fig. S2 Sensitivity determination of the RT-MIPLAMP assay on ORF1ab gene. ORF1ab gene in different concentrations are respectively detected with (a) real-time fluorescence monitoring and (b) visual method. The concentrations of the ORF1ab gene are 0, 6×10^3, 6×10^4, 6×10^5, 6×10^6, 6×10^7, 6×10^8 and 6×10^9 copies/mL, respectively.

4. The effect of loop primers on RT-MIPLAMP for assaying N gene

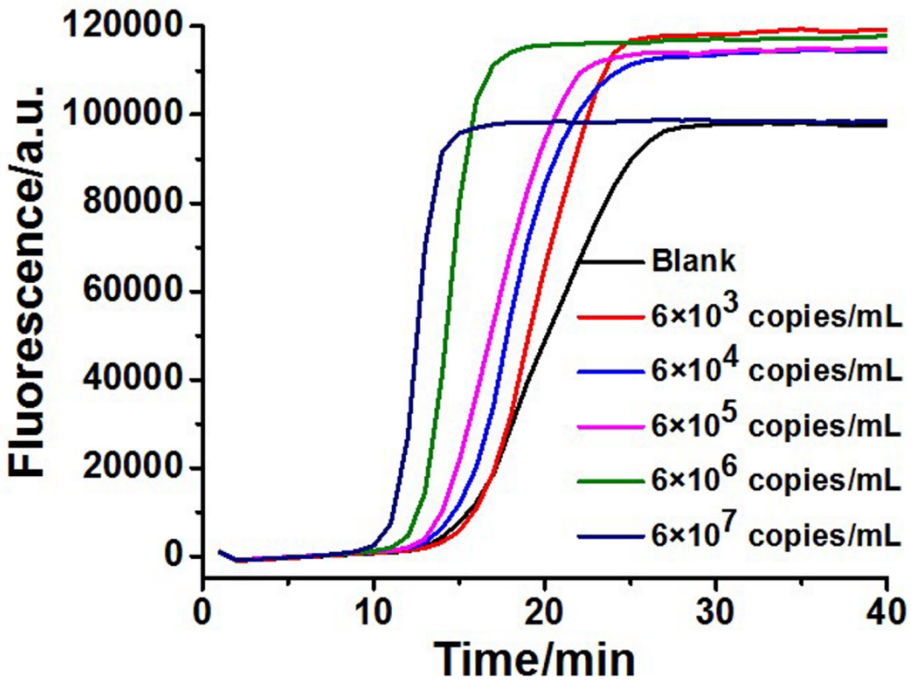

Fig. S3 The fluorescent curves are produced from 0, 6×10^3, 6×10^4, 6×10^5, 6×10^6 and 6×10^7 copies/mL N gene by using RT-MIPLAMP primers and loop primers for the FIP-1 and BIP-1.