

Supplementary Information for:

Development and Evaluation of RT-LAMP Assays to Identify Variants of SARS-CoV-2

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Supplementary Table 1. Sequences of LAMP primers and OSD probes

Set Name	Primer	Sequence [^]
SGF	F3	5' – GCGTATTATGACATGGTTGG – 3'
	B3	5' – ACTTTATAAACGAGTGTCAAGAC – 3'
	FIP	5' – ACAGCTGATGCATACATAACACA TATGGTTGATACTAGTTT <u>GTCTGG</u> – 3'
	FIPdel*	5' – ACAGCTGATGCATACATAACACA TATGGTTGATACTAGTTTGAAGC – 3'
	BIP	5' – TAATCCTTATGACAGCAAGAACTGT ATTCATAAGTGTCCACACTCTC – 3'
	LB	5' – GTATGATGATGGTGCTAG – 3'
EFR	F3	5' – GTGAATTTCAATTTTGAATGATCC – 3'
	B3	5' – AAACCCTGAGGGAGATCA – 3'
	FIP	5' – CCATAAGAAAAGGCTGAGAGACATA GTTGGATGGAAAGT <u>GAGTTCA</u> – 3'
	BIP	5' – ACCTTGAAGGAAAACAGGGTAATT AATTAATAGGCGTGTGCTTAG – 3'
	LF	5' – CAAAAGTGCAATTATTCGCACTAG – 3'
	LB	5' – TCAAAAATCTTAGGGAATTTGTGTT – 3'
GVY	F3	5' – CTAATGTTGTTATTAAGTCTGTGA – 3'
	B3	5' – ATTCTTAAACACAAATTCCTAAG – 3'
	FIP	5' – CACTTTCCATCCAACCTTTGTTG GTAATGATCCATTTTTGGGT <u>GTI</u> – 3'
	FIPdel	5' – AACTCTGAACTCACTTTCCATCC GTAATGATCCATTTTTGG <u>ACCA</u> – 3'
	BIP	5' – TCTAGTGCGAATAATTGCACTTTTG ACCCTGTTTTCTTCAAGGT – 3'
	LB	5' – ATGTCTCTCAGCCTTTTCTTATGG – 3'
S501	F3	5' – CTGTATAGATTGTTTAGGAAGTCT – 3'
	B3	5' – CTTTTTAGGTCCACAAACAGT – 3'
	FIP	5' – TYAACACCATTACAAGGTGTGCT AATCTCAAACCTTTTGAGAGAG – 3'
	BIP	5' – TACAATCATATGGTTTCCAACC CTGGTGCATGTAGAAGTTCAA – 3'
	LF	5' – CCGGCCTGATAGATTTCAAGTTG – 3'
	LB	5' – CCAACCATACAGAGTAGTAGTACT – 3'
	Probe_N	5' – /6-FAM/ ACTACTACTCTGTATGGTTGGTAACCAACACCATA <u>A</u> AGTG /Inverted dT/ – 3'
	Probe_Y	5' – /6-FAM/ ACTACTACTCTGTATGGTTGGTAACCAACACCATT <u>A</u> AGTG /Inverted dT/ – 3'
	Quencher	5' – GTTGGTTACCAACCATACAGAGTAGTAGT /BHQ1/ – 3'

[^] Residues affected by mutations are designated by underline.

* Mismatching nucleotide residue to Omicron-BA.1 was designated by *italic*.

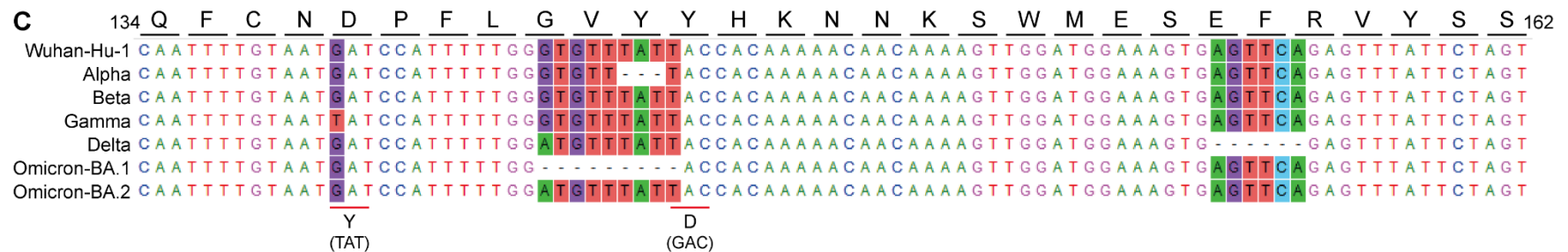
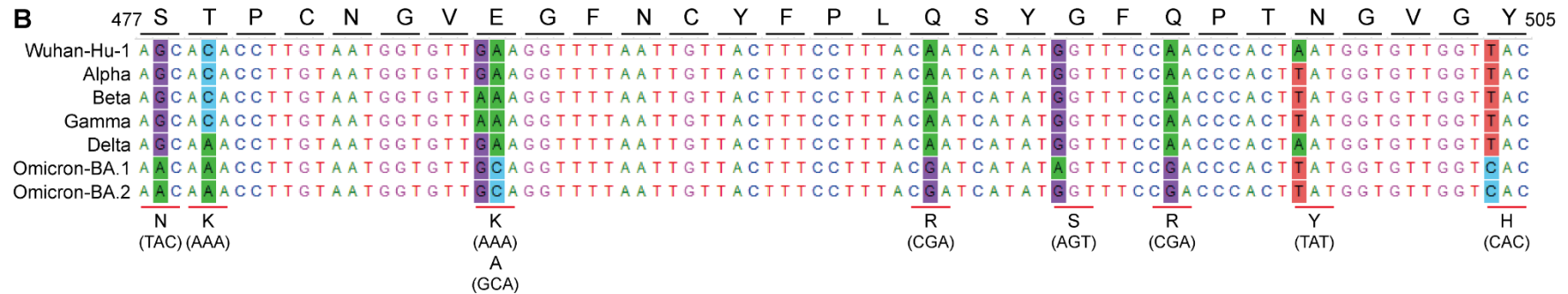
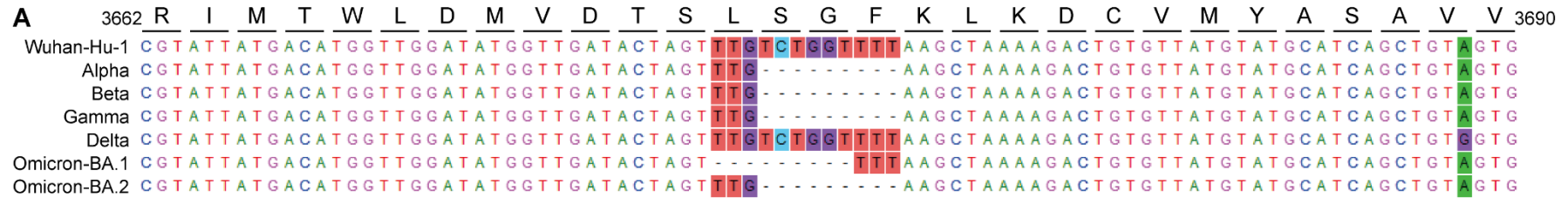
Supplementary Table 2. Sequences of qRT-PCR primers and probes

Set Name	Primer	Sequence	Working Concentration (nmol/L)
Nsp3[^]	F	5' – GAAGAGCAAGAAGAAGATTGGT – 3'	400
	R	5' – TGGTGTAAGTTCCATCTCTAATTG – 3'	400
	P	5' – /6-FAM/ ACGGCAGTGAGGACAATCAGACA /BHQ1/ – 3'	200
S3[*]	F	5' – GGTGATTCTTCTTCAGGTTGGA – 3'	400
	R	5' – GTACACTTTGTTTCTGAGAGAGG – 3'	400
	P	5' – /6-FAM/ CTGGTGCTGCAGCTTATTATGTGGG /BHQ1/ – 3'	200

[^] Nsp3 set was used to titrate Orf1 copy number of viral RNAs corresponding to SGF/SGFdel LAMP.

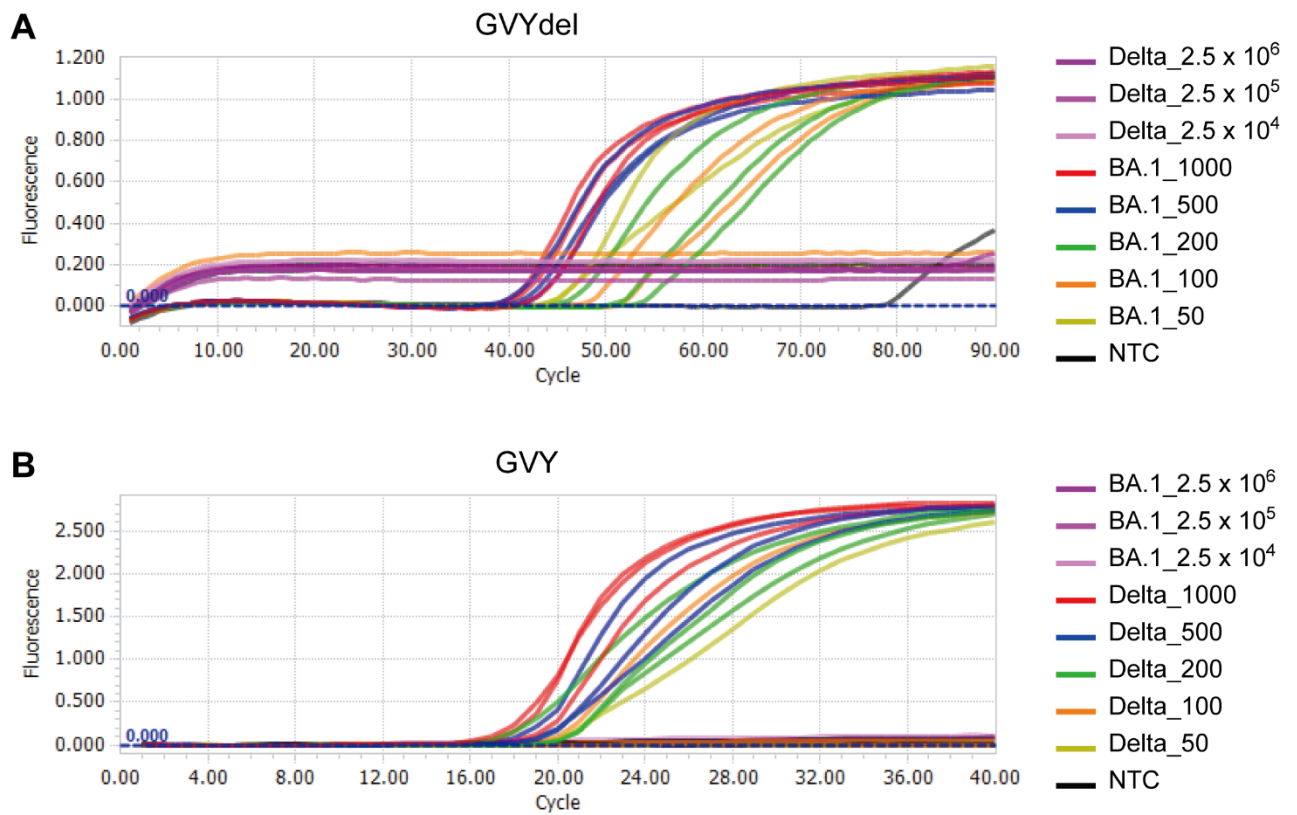
^{*} S3 set was used to titrate Spike copy number of viral RNAs corresponding to EGR/GVY/GVYdel/S501 LAMP.

Supplementary Figure 1



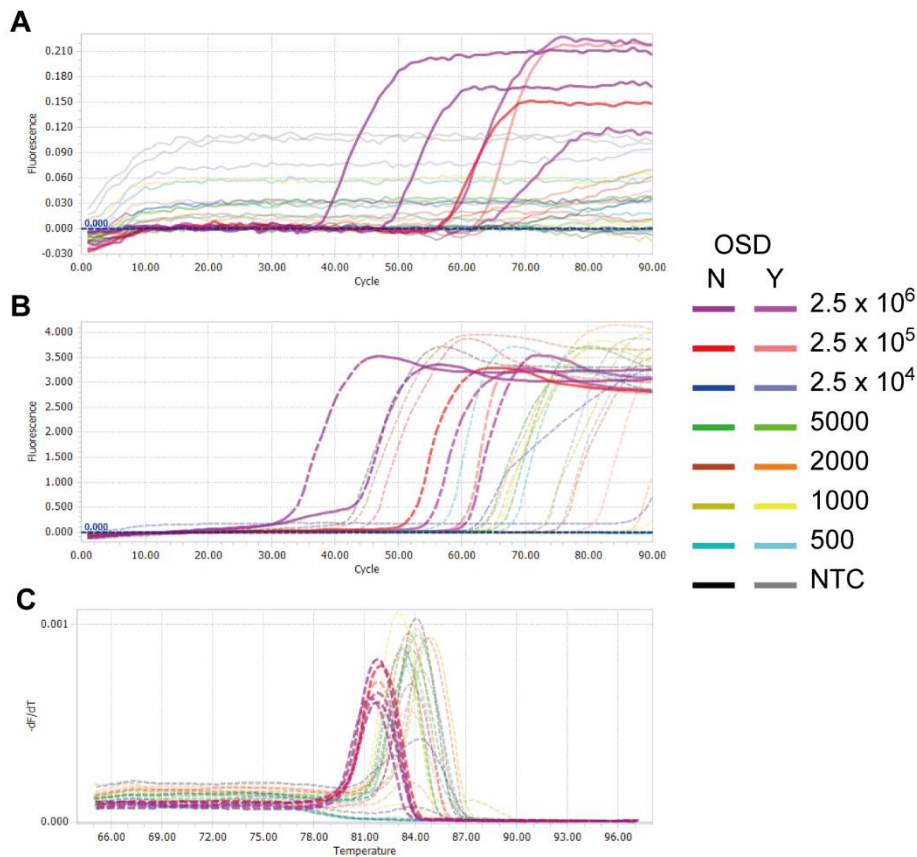
Supplementary Figure 1. Aligned sequences (A) around Orf1a SGF3675-3677del mutation, (B) of Spike S477 to Y505 related to N501Y targeting OSD-probe RT-LAMP, and (C) around Spike EF156-157del+R158G and GVV142-144del+Y145D mutations. Corresponding amino acids are designated above of each panel. Changed amino acids by mutations are designated by red lines with corresponding codons. Wuhan-Hu-1 sequence is the NCBI reference sequence (NC_045512.2). Sequences of variants are obtained from GISAID (<https://www.gisaid.org/>) and accession ID are as follow: Alpha – EPI_ISL_601443, Beta – EPI_ISL_660190, Gamma – EPI_ISL_833137, Delta – EPI_ISL_1409773, Omicron-BA.1 – EPI_ISL_7545782, and Omicron-BA.2 – EPI_ISL_7190366.

Supplementary Figure 2



Supplementary Figure 2. Real-time SYTO-9 fluorescence signal of GYdel (A) and GY (B) primer sets corresponding to panel (G) and (H) of main Figure 1, respectively.

Supplementary Figure 3



Supplementary Figure 3. Real-time fluorescence signal of (A) FAM from OSD-probes, (B) SYTO-82, and (C) melting curve of SYTO-82 from OSD-probe RT-LAMP test with Omicron-BA.1 viral RNA. Copy number per reaction and OSD-probe targets are indicated for each color of lines. Template-derived proper amplifications are indicated by bolded lines.