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Supplemental information

Centrifugal microfluidic-based multiplex recombinase polymerase amplification assay for rapid detection of SARS-CoV-2

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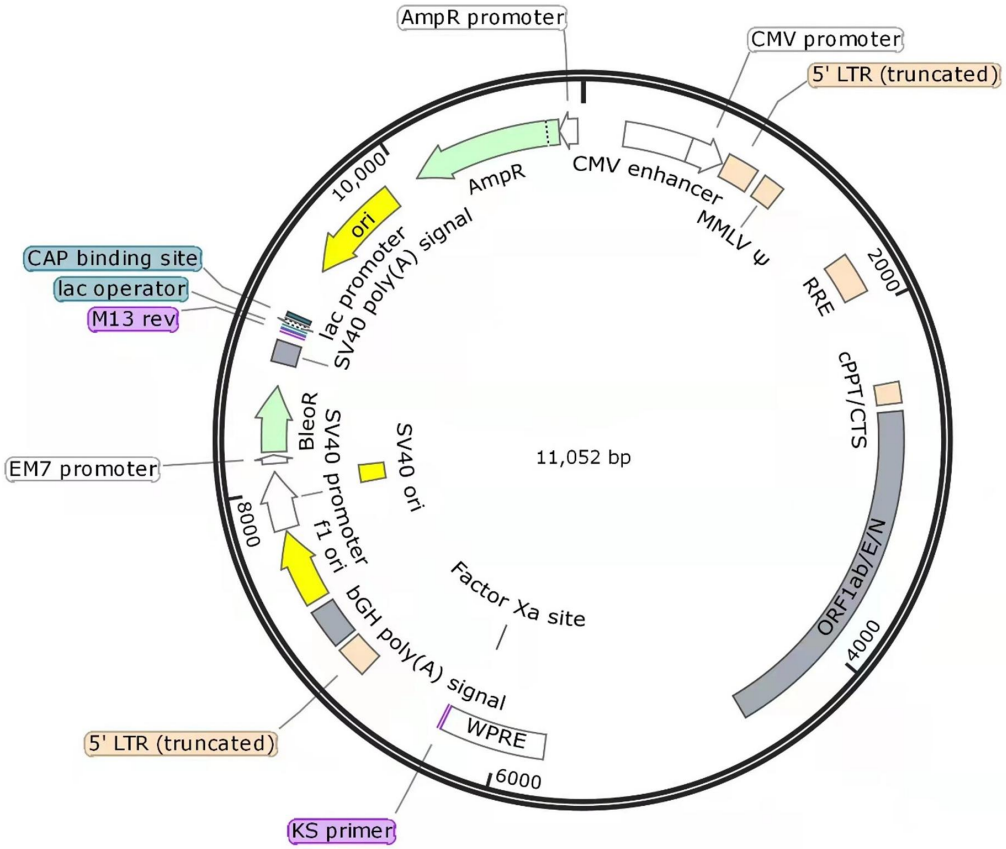

Figure S1. Schematic map of the transfer plasmid of retroviral vector containing the target genes of SARS-CoV-2, related to STAR methods. The complete N gene, complete E gene, and partial ORF1ab gene of SARS-CoV-2 contained on the retroviral vector.

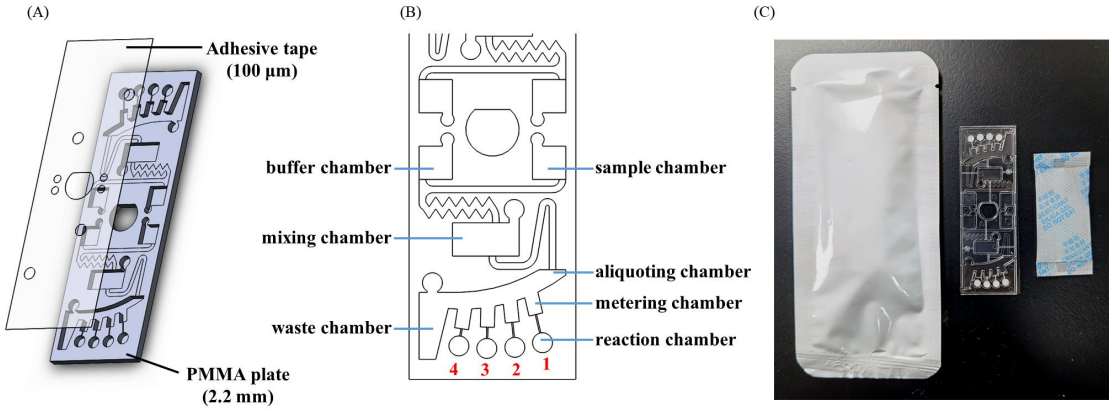

Figure S2. Illustrations of the microscope slide-shaped microfluidic chip, related to Figure 4.

(A) Schematic of the 3D structures of the microfluidic chip consisted of a PMMA plate and a pressure-sensitive adhesive layer.

(B) An enlarged schematic of each unit contained a buffer chamber, a sample chamber, a mixing chamber, an aliquoting chamber, a metering chamber, and four reaction chambers.

(C) Pictures of chip packaging.

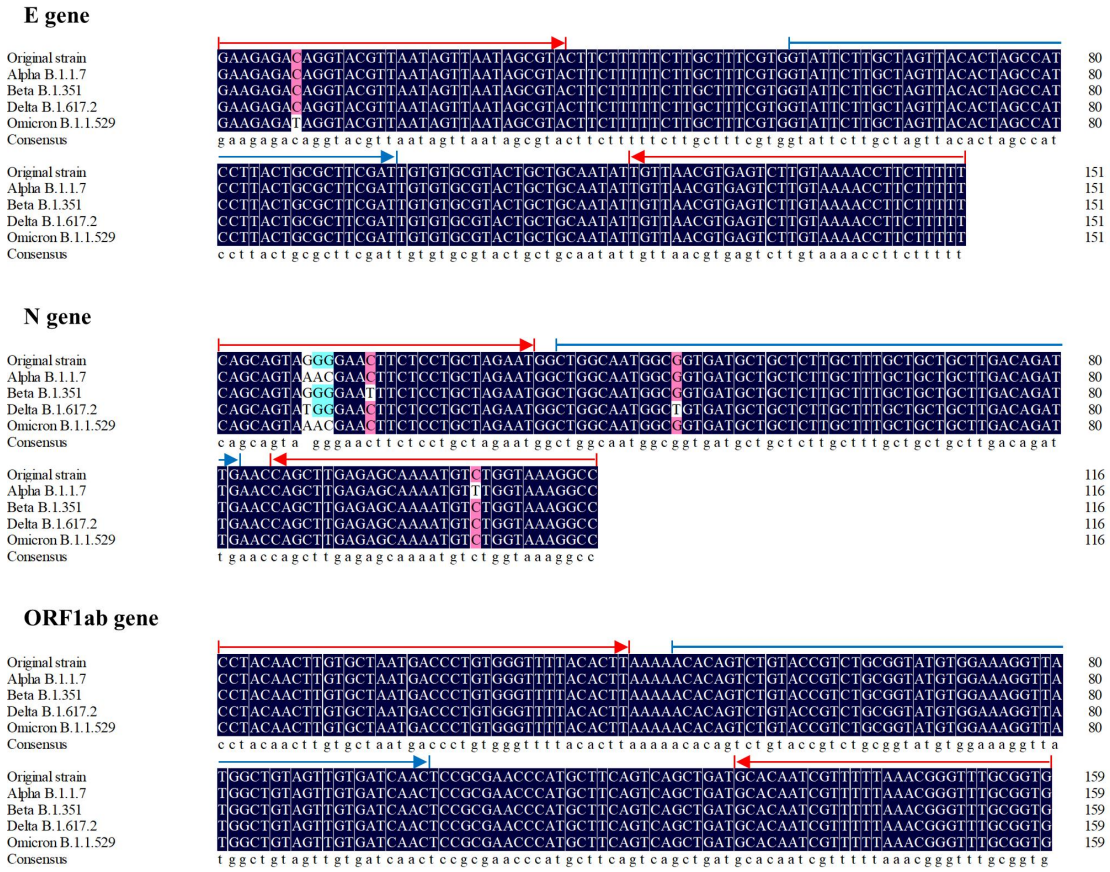

Figure S3. Multiple sequence alignments of the original strain of SARS-CoV-2 with the other four common VOCs, related to Figure 3. Matched bases were highlighted in black; partially matched bases were highlighted in red or blue color, and mismatched bases were shown with a white background. A consensus sequence among all five sequences was shown. The red and blue arrows above the aligned sequences indicated the primers and probes used in this study, respectively.

Table S1. Sequences of the SARS-CoV-2 pseudovirus with N, E, and ORF1ab genes, related to STAR methods.

Gene	Sequence (5'–3')
N	ATGTCTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTG GACCCTCAGATTCAACTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGAT CAAAACAACGTCGGCCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCACCGC TCTCACTCAACATGGCAAGGAAGACCTTAAATTCCCTCGAGGACAAGGCGTTCC AATTAACACCAATAGCAGTCCAGATGACCAAATTGGCTACTACCGAAGAGCTACC AGACGAATTCGTGGTGGTGACGGTAAAATGAAAGATCTCAGTCCAAGATGGTATT TCTACTACCTAGGAACTGGGCCAGAAGCTGGACTTCCCTATGGTGCTAACAAAGA CGGCATCATATGGGTTGCAACTGAGGGAGCCTTGAATACACCAAAAGATCACATT GGCACCCGCAATCCTGCTAACAATGCTGCAATCGTGCTACAACTTCCTCAAGGAA CAACATTGCCAAAAGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCC TCTTCTCGTTCCTCATCACGTAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCA GCAGTAGGGGAACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCT TGCTTTGCTGCTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTGGTAAA GGCCAACAACAACAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGAGGCTTCT AAGAAGCCTCGGCAAAAACGTACTGCCACTAAAGCATACAATGTAACACAAGCT TTCGGCAGACGTGGTCCAGAACAAACCCAAGGAAATTTTGGGGACCAGGAACT AATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGCACAATTTGCCCCC AGCGCTTCAGCGTTCTTCGGAATGTCGCGCATTGGCATGGAAGTCACACCTTCGG GAACGTGGTTGACCTACACAGGTGCCATCAAATTGGATGACAAAGATCCAAATTT CAAAGATCAAGTCATTTTGCTGAATAAGCATATTGACGCATACAAAACATTCCCA CCAACAGAGCCTAAAAAGGACAAAAAGAAGAAGGCTGATGAAACTCAAGCCTT ACCGCAGAGACAGAAGAAACAGCAAACTGTGACTCTTCTTCCTGCTGCAGATTT GGATGATTTCTCCAAACAATTGCAACAATCCATGAGCAGTGCTGACTCAACTCAG GCCTAA
E	ATGTACTCATTCGTTTCGGAAGAGACAGGTACGTTAATAGTTAATAGCGTACTTCT TTTTCTTGCTTTCGTGGTATTCTTGCTAGTTACACTAGCCATCCTTACTGCGCTTCG ATTGTGTGCGTACTGCTGCAATATTGTTAACGTGAGTCTTGTAAAACCTTCTTTTT ACGTTTACTCTCGTGTTAAAAATCTGAATTCTTCTAGAGTTCCTGATCTTCTGGTC TAA
ORF1ab	ATCGTGTTGTCTGTACTGCCGTTGCCACATAGATCATCCAAATCCTAAAGGATTTT GTGACTTAAAAGGTAAGTATGTACAAATACCTACAACTTGTGCTAATGACCCTGT GGGTTTTACACTTAAAAACACAGTCTGTACCGTCTGCGGTATGTGGAAAGGTTAT GGCTGTAGTTGTGATCAACTCCGCGAACCCATGCTTCAGTCAGCTGATGCACAAT CGTTTTTAAACGGGTTTGCGGTGTAAGTGCAGCCCGTCTTACACCGTGCGGCACA GGCACTAGTACTGATGTCGTATACAGGGCTTTTGACATCTACAATGATAAAGTAGC TGGTTTTGCTAAATTCCTAAAAACTAATTGTTGTCGCTTCCAAGAAAAGGACGAA GATGACAATTTAATTGATTCTTACTTTGTAGTTAAGAGACACACTTTCTCTAACTA CCAACATGAAGAAACAATTTATAATTTACTTAAGGATTGTCCAGCTGTTGCTAAAC AT

Table S2. RPA primers and probes used in this study, related to STAR methods.

Gene	Oligonucleotide	Sequence (5'–3')[a]
N	forward primer	CAGCAGTAGGGGAACTTCTCCTGCTAGAAT
	reverse primer	GGCCTTTACCAGACATTTTGCTCTCAAGCTG
	probe	CTGGCAATGGCGGTGATGCTGCTCTTGCTT[HEX-dT]G[THF][BHQ1-dT]GCTGCTTGACAGATT GTGTGCAACTTTAG[phosphate]
ORF1ab	forward primer	CCTACAACTTGTGCTAATGACCCTGTGGGTTTTACACTT
	reverse primer	CACCGCAAACCCGTTTAAAAACGATTGTGC
	probe	ACACAGTCTGTACCGTCTGCGGTATGTGGAAAGG[HEX-dT][THF]A[BHQ1-dT]GGCTGTAGTTG TGATCAAC[phosphate]
E	forward primer	GAAGAGACAGGTACGTTAATAGTTAATAGCGTA
	reverse primer	AAAAAGAAGGTTTTACAAGACTCACGTTAACA
	probe	ATCGAAGCGCAGTAAGGATGGCTAG[HEX-dT][THF][BHQ1-dT]AACTAGCAAGAATAC[phosphat e]
ACTB	forward primer	CTCCATCCTGGCCTCGCTGTCCACCTTCCAG
	reverse primer	AATCTCATCTTGTTTTCTGCGCAAGTTAGG
	probe	GTCAAGAAAGGGTGTAACGCAACTAAGTCA[HEX-dT][THF]G[BHQ1-dT]CCGCCTAGAAGCAT [phosphate]

[a] HEX-dT, thymidine nucleotide carrying fluorescein ROX; THF, tetrahydrofuran spacer; BHQ1-dT, thymidine nucleotide carrying Black Hole Quencher 1; phosphate, 3' phosphate to block elongation.

Table S3. The analytical specificity of three SARS-CoV-2 RT-RPA assay, related to Figure 3 and 6.

Nucleic acid	N	E	ORF1ab
SARS-CoV-2	+	+	+
SARS-CoV	−	−	+
human coronavirus 229E	−	−	−
human coronavirus NL63	−	−	−
human coronavirus OC43	−	−	−
human coronavirus HKU1	−	−	−
MERS-Coronavirus	−	−	−
influenza A virus	−	−	−
influenza B virus	−	−	−
respiratory syncytial virus A and B	−	−	−
human parainfluenza virus	−	−	−
adenovirus	−	−	−
enterovirus	−	−	−
epstein-barr virus	−	−	−
human cytomegalovirus	−	−	−
human genome	−	−	−

Table S4. The burst frequency of each capillary valve on the microscope slide microfluidic chip, related to Figure 4.

Capillary valve	Calculated burst frequency (RPM)	working spin speed (RPM)
the inlet to the mixing chamber	1054.95	3000
the inlet to the aliquoting chamber	981.69	1500
the inlet to the reaction chamber	1720.38	7000