

Supplemental information

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

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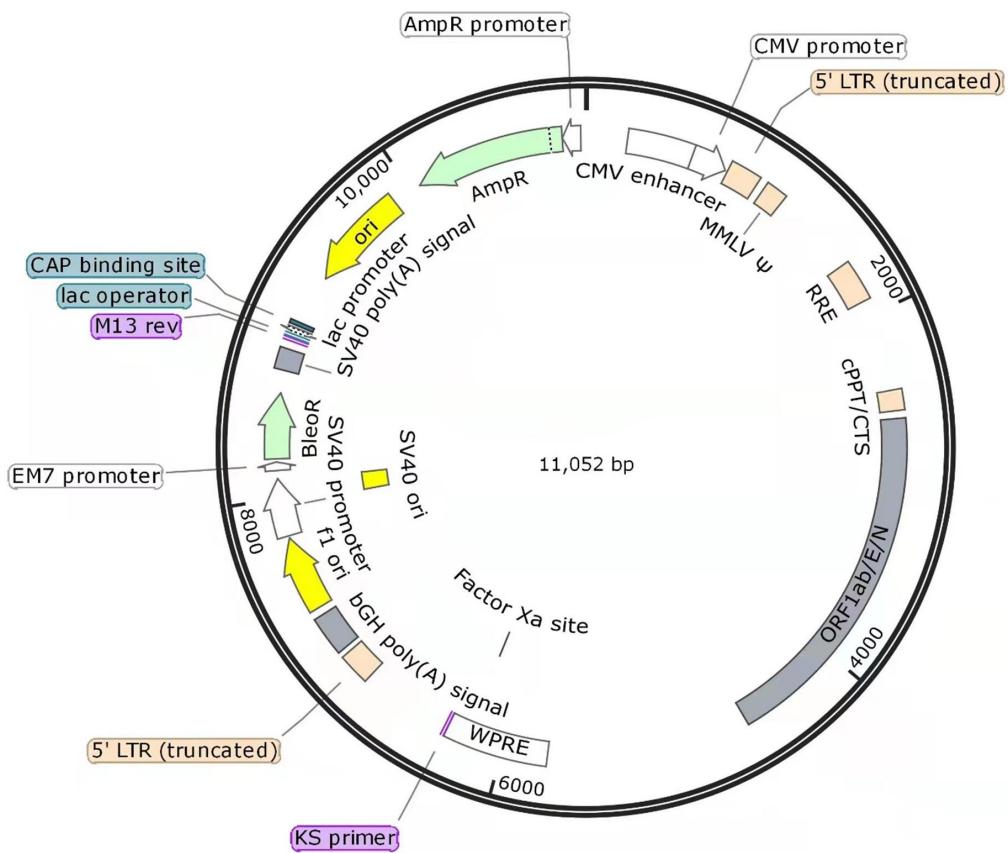


Figure S1. Schematic map of the transfer plasmid of retroviral vector containing the target genes of SARS-CoV-2, related to STAR methods. The complete N gene, complete E gene, and partial ORF1ab gene of SARS-CoV-2 contained on the retroviral vector.

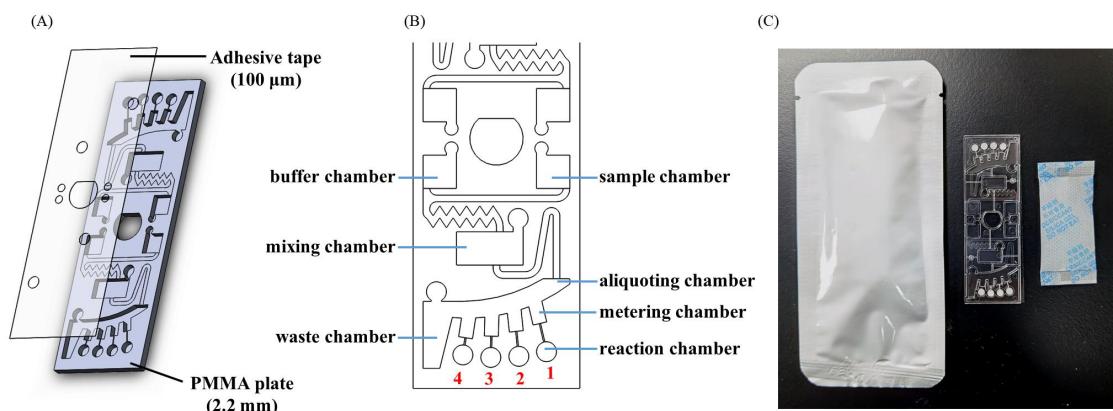


Figure S2. Illustrations of the microscope slide-shaped microfluidic chip, related to Figure 4.

(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.

E gene

Original strain				
Alpha B.1.1.7	GAAGAGA <ins>CAGGT</ins> ACGTTAAATAGTTAAATAGCGTACT	CTTTCTTGCTTTCTGGTATTCTTGCTAGTTACACT	AGCCAT	80
Beta B.1.351	GAAGAGA <ins>CAGGT</ins> ACGTTAAATAGTTAAATAGCGTACT	CTTTCTTGCTTTCTGGTATTCTTGCTAGTTACACT	AGCCAT	80
Delta B.1.617.2	GAAGAGA <ins>CAGGT</ins> ACGTTAAATAGTTAAATAGCGTACT	CTTTCTTGCTTTCTGGTATTCTTGCTAGTTACACT	AGCCAT	80
Omicron B.1.1.529	GAAGAGA <ins>CAGGT</ins> ACGTTAAATAGTTAAATAGCGTACT	CTTTCTTGCTTTCTGGTATTCTTGCTAGTTACACT	AGCCAT	80
Consensus	gaagaga <ins>caggta</ins> acgttaatagttaaatagcgtaacttcttttcgttcttgcgttggattttcttgcgttagttacaactagccat			
Original strain				
Alpha B.1.1.7	CTTTACTGGCCTTCGATTGTTGCGTACTGCTGCAATATTGTTAACGTGAGTCCTGAAAACCTTCCTTT	AACGTGAGTCCTGTTGAAAACCTTCCTTT	151	
Beta B.1.351	CTTTACTGGCCTTCGATTGTTGCGTACTGCTGCAATATTGTTAACGTGAGTCCTGTTGAAAACCTTCCTTT	AACGTGAGTCCTGTTGAAAACCTTCCTTT	151	
Delta B.1.617.2	CTTTACTGGCCTTCGATTGTTGCGTACTGCTGCAATATTGTTAACGTGAGTCCTGTTGAAAACCTTCCTTT	AACGTGAGTCCTGTTGAAAACCTTCCTTT	151	
Omicron B.1.1.529	CTTTACTGGCCTTCGATTGTTGCGTACTGCTGCAATATTGTTAACGTGAGTCCTGTTGAAAACCTTCCTTT	AACGTGAGTCCTGTTGAAAACCTTCCTTT	151	
Consensus	ccttactg <ins>cgcc</ins> tccgattgtgtgcgtactgcgtcaatattgttaacgtgagtcctgtaaaaccccttccttt			

N gene

Original strain				
Alpha B.1.1.7	CAGCAGT <ins>AGGGAA</ins> CTTCCTGCTAGAAATGGCTTGGCAATGGCCGTGATGCTGCTCTTGCTTGCTTGACAGAT	GCTCTTGCTTGCTTGCTTGACAGAT	80	
Beta B.1.351	CAGCAGT <ins>AGGGAA</ins> CTTCCTGCTAGAAATGGCTTGGCAATGGCCGTGATGCTGCTCTTGCTTGCTTGACAGAT	GCTCTTGCTTGCTTGACAGAT	80	
Delta B.1.617.2	CAGCAGT <ins>AGGGAA</ins> CTTCCTGCTAGAAATGGCTTGGCAATGGCCGTGATGCTGCTCTTGCTTGCTTGACAGAT	GCTCTTGCTTGCTTGACAGAT	80	
Omicron B.1.1.529	CAGCAGT <ins>AGGGAA</ins> CTTCCTGCTAGAAATGGCTTGGCAATGGCCGTGATGCTGCTCTTGCTTGCTTGACAGAT	GCTCTTGCTTGCTTGACAGAT	80	
Consensus	cagcagta <ins>gggaa</ins> cttcctcgttagaatggctggcaatgggggtgatgctcttgtcttgctgtcttgacagat			
Original strain				
Alpha B.1.1.7	TGAACCAGCTTGAGAGCAAATGTCGGTAAAGGCC	TGGTAAAGGCC	116	
Beta B.1.351	TGAACCAGCTTGAGAGCAAATGTCGGTAAAGGCC	TGGTAAAGGCC	116	
Delta B.1.617.2	TGAACCAGCTTGAGAGCAAATGTCGGTAAAGGCC	TGGTAAAGGCC	116	
Omicron B.1.1.529	TGAACCAGCTTGAGAGCAAATGTCGGTAAAGGCC	TGGTAAAGGCC	116	
Consensus	tgaaccagct <ins>tgagagcaaaatgtctgttaaaggcc</ins>			

ORF1ab gene

Original strain				
Alpha B.1.1.7	CCTACAACCTTGCTTAATGACCCCTGGGGTTAACACTTAAAACACAGTCGTACCGCTTGCGGTATGIGGAAGGTAA	GTACCGCTTGCGGTATGIGGAAGGTAA	80	
Beta B.1.351	CCTACAACCTTGCTTAATGACCCCTGGGGTTAACACTTAAAACACAGTCGTACCGCTTGCGGTATGIGGAAGGTAA	GTACCGCTTGCGGTATGIGGAAGGTAA	80	
Delta B.1.617.2	CCTACAACCTTGCTTAATGACCCCTGGGGTTAACACTTAAAACACAGTCGTACCGCTTGCGGTATGIGGAAGGTAA	GTACCGCTTGCGGTATGIGGAAGGTAA	80	
Omicron B.1.1.529	CCTACAACCTTGCTTAATGACCCCTGGGGTTAACACTTAAAACACAGTCGTACCGCTTGCGGTATGIGGAAGGTAA	GTACCGCTTGCGGTATGIGGAAGGTAA	80	
Consensus	cctaaca <ins>ttgtctaatgaccctgtgggtttacacttaaaaaacacagtctgtaccgtctgcggatgtggaaaggta</ins>			
Original strain				
Alpha B.1.1.7	TGGCTGTAGTTGATCAACTCCGGAAACCATGCTTCAGTCAGCTGATGCCAAATCGTTTAAACGGGTTTGCCTG	GGGTTTGCCTG	159	
Beta B.1.351	TGGCTGTAGTTGATCAACTCCGGAAACCATGCTTCAGTCAGCTGATGCCAAATCGTTTAAACGGGTTTGCCTG	GGGTTTGCCTG	159	
Delta B.1.617.2	TGGCTGTAGTTGATCAACTCCGGAAACCATGCTTCAGTCAGCTGATGCCAAATCGTTTAAACGGGTTTGCCTG	GGGTTTGCCTG	159	
Omicron B.1.1.529	TGGCTGTAGTTGATCAACTCCGGAAACCATGCTTCAGTCAGCTGATGCCAAATCGTTTAAACGGGTTTGCCTG	GGGTTTGCCTG	159	
Consensus	tggctgtatgtgtatcaactccggaaaccatgcttcagtcagctgtgcacaaatcgttttaaacgggtttgcgggt			

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	ATGCTGATAATGGACCCAAAATCAGCGAAATGCACCCCGCATTACGTTGGTG GACCCTCAGATTCAACTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGAT CAAAACAACGTCGGCCCCAAGGTTACCCAATAACTGCGTCTGGTTACCGC TCTCACTCAACATGGCAAGGAAGACCTTAAATTCCCTCGAGGACAAGGCCTTCC AATTAACACCAATAGCAGTCCAGATGACCAAATTGGCTACTACCGAAGAGCTACC AGACGAATTCTGGTGGTGACGGTAAAATGAAAGATCTCAGTCCAAGATGGTATT TCTACTACCTAGGAACTGGGCCAGAAGCTGGACTTCCCTATGGTGCTAACAAAGA CGGCATCATATGGGTGCAACTGAGGGAGCCTGAATACACCAAAAGATCACATT GGCACCCGCAATCCTGCTAACAAATGCTGAATCGTGTACAACCTCCTCAAGGAA CAACATTGCCAAAAGGCTTCTACGCAGAAGGGAGCAGAGGCCAGTCAGGCC TCTTCTCGTTCCATCACGTAGTCGAACAGTTCAAGAAATTCAACTCCAGGCA GCAGTAGGGGAACCTCTCTGCTAGAATGGCTGGCAATGGCGGTATGCTGCTCT TGCTTGCTGCTGCTTGACAGATTGAACCAGCTTGAGAGCAAAATGCTGGTAAA GGCCAACAACAACAAGGCCAAACTGTCACTAACAGAAATCTGCTGCTGAGGCTT AAGAAGCCTCGGAAAAACGTACTGCCACTAACAGCATACAATGTAACACAAGCT TTCGGCAGACGTGGTCCAGAACAAACCAAGGAAATTGGGGACCAGGAAC AATCAGACAAGGAACGTGATTACAAACATTGGCGCAAATTGACACAATTGCCCC AGCGCTTCAGCGTTCTCGGAATGTCGCGATTGGCATGGAAGTCACACCTCGG GAACGTGGTTGACCTACACAGGTGCCATCAAATTGGATGACAAAGATCCAATT CAAAGATCAAGTCATTTGCTGAATAAGCATATTGACGCATAAAACATTCCA CCAACAGAGCTAAAAGGACAAAAAGAAGAAGGCTGATGAAACTCAAGCCTT ACCGCAGAGACAGAACAGCAAACAGTGTGACTCTTCTGCTGCTGAGATT GGATGATTCTCCAAACAATTGCAACAATCCATGAGCAGTGACTCAACTCAG GCCTAA
E	ATGTACTCATTCTCGGAAGAGACAGGTACGTTAATAGTTAATAGCGTACTTCT TTTCTGCTTCGTGGTATTCTGCTAGTTACACTAGCCATCCTACTGCGCTTCG ATTGTGTGCGTACTGCTGCAATATTGTTAACGTGAGTCTGTAAAACCTCTTTT ACGTTACTCTCGTGTAAAAATCTGAATTCTCTAGAGTTCTGATCTCTGGTC TAA
ORF1a b	ATCGTGTGCTGTACTGCCGTTGCCACATAGATCATCCAAATCCTAAAGGATT GTGACTTAAAGGTAAGTATGTACAAATACCTACAACCTGTGCTAATGACCCTGT GGGTTTACACTAAAAACACAGTCTGTACCGTCTGCGGTATGTGGAAAGGTTAT GGCTGTAGTTGTGATCAACTCCCGAACCCTGCTCAGTCAGCTGATGCACAAT CGTTTTAAACGGGTTGCGGTGTAAGTGCAGCCGTCTTACACCGTGGCACA GGCACTAGTACTGATGTCGTATACAGGGCTTGTACATCTACAATGATAAGTAGC TGGTTTGCTAAATTCTAAAAACTAATTGTTGTCGCTCCAAGAAAAGGACGAA GATGACAATTAAATTGATTCTTACTTGTAGTTAAGAGACACACTTCTCTAACTA CCAACATGAAGAAACAATTATAATTACTTAAGGATTGTCAGCTGTTGCTAAAC AT

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

Gene	Oligonucleotide	Sequence (5'-3') ^a
N	forward primer	CAGCAGTAGGGGAACCTCTCCTGCTAGAAT
	reverse primer	GGCCTTACCAGACATTTGCTCTCAAGCTG
	probe	CTGGCAATGGCGGTGATGCTGCTCTGCTT[HEX-dT]G[THF][BHQ1-dT]GCTGCTTGACAGATT GTGTGCAACTTAG[phosphate]
ORF1ab	forward primer	CCTACAACTTGTGCTAACATGACCCCTGTGGGTTTACACTT
	reverse primer	CACCGCAAACCCGTTAAAACGATTGTGC
	probe	ACACAGTCTGTACCGTCTGCGGTATGTGGAAAGG[HEX-dT][THF]A[BHQ1-dT]GGCTGTAGTTG TGATCAAC[phosphate]
E	forward primer	GAAGAGACAGGTACGTTAATAGTTAATAGCGTA
	reverse primer	AAAAAGAAGGTTTACAAGACTCACGTTAACAA
	probe	ATCGAAGCGCAGTAAGGATGGCTAG[HEX-dT][THF][BHQ1-dT]AACTAGCAAGAATAC[phosphat e]
ACTB	forward primer	CTCCATCCTGGCCTCGCTGTCCACCTTCCAG
	reverse primer	AATCTCATCTGTTTCTGCGCAAGTTAGG
	probe	GTCAAGAAAGGGTGTAAACGCAACTAAGTCA[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