

**Supplementary Table S1. Detailed information of SARS-CoV-2 genomes used for multiple alignment**

Accession	Pangolin lineage	WHO classification	Category	Length (bp)	Location
MN908947	B			29903	China
MW913362	B.1.1.7	Alpha	variant of concern	29763	Austria: Upper Austria, Voecklabruck
OU514166	B.1.1.7	Alpha	variant of concern	29810	Germany:Europe/Germany/Baden-Wurttemberg
OU514176	B.1.1.7	Alpha	variant of concern	29813	Germany:Europe/Germany/Baden-Wurttemberg
MZ350105	B.1.1.7	Alpha	variant of concern	29756	Ghana
MZ350109	B.1.1.7	Alpha	variant of concern	29773	Ghana
MZ266365	B.1.1.7	Alpha	variant of concern	29763	Hong Kong
BS001137	B.1.1.7	Alpha	variant of concern	29863	Japan: Tochigi
BS001138	B.1.1.7	Alpha	variant of concern	29862	Japan: Tochigi
BS001139	B.1.1.7	Alpha	variant of concern	29877	Japan: Tochigi
BS001140	B.1.1.7	Alpha	variant of concern	29862	Japan: Tochigi
OU538849	B.1.1.7	Alpha	variant of concern	29884	Switzerland:SO
OU538851	B.1.1.7	Alpha	variant of concern	29884	Switzerland:BE
MZ888516	B.1.1.7	Alpha	variant of concern	29882	Thailand: Kamphaeng Phet
OU562607	B.1.1.7	Alpha	variant of concern	29884	United Kingdom:Scotland
OU562608	B.1.1.7	Alpha	variant of concern	29884	United Kingdom:Scotland
MZ895877	B.1.1.7	Alpha	variant of concern	29521	USA: Washington
MZ298839	B.1.351	Beta	variant of concern	29764	Hong Kong
MZ298840	B.1.351	Beta	variant of concern	29764	Hong Kong
MW981442	B.1.351	Beta	variant of concern	29848	South Africa
MZ202314	B.1.351	Beta	variant of concern	29783	South Africa
MZ376663	B.1.351	Beta	variant of concern	29848	South Africa
OU539352	B.1.351	Beta	variant of concern	29885	Switzerland:AG

OU525106	B.1.351	Beta	variant of concern	29885	United Kingdom:England
MZ879365	B.1.351	Beta	variant of concern	29782	USA: Colorado
MZ913100	B.1.351	Beta	variant of concern	29712	USA: New Mexico
LC643036	B.1.617.2	Delta	variant of concern	29875	Japan: Tokyo
LC643044	B.1.617.2	Delta	variant of concern	29878	Japan: Tokyo
MZ854386	B.1.617.2	Delta	variant of concern	29830	Sierra Leone
MZ854387	B.1.617.2	Delta	variant of concern	29831	Sierra Leone
MZ854389	B.1.617.2	Delta	variant of concern	29830	Sierra Leone
MZ853946	B.1.617.2	Delta	variant of concern	29890	South Korea
OU539261	B.1.617.2	Delta	variant of concern	29893	Switzerland:TG
OU539690	B.1.617.2	Delta	variant of concern	29890	Switzerland:ZH
MZ888532	B.1.617.2	Delta	variant of concern	29835	Thailand: Kamphaeng Phet
MZ888533	B.1.617.2	Delta	variant of concern	29809	Thailand: Kamphaeng Phet
OU562629	B.1.617.2	Delta	variant of concern	29890	United Kingdom:England
MZ896136	B.1.617.2	Delta	variant of concern	29516	USA: Oregon
MZ477746	P.1	Gamma	variant of concern	29741	Brazil: Parana
MZ477748	P.1	Gamma	variant of concern	29738	Brazil: Parana
MW938104	P.1	Gamma	variant of concern	29874	Peru
OU539784	P.1	Gamma	variant of concern	29894	Switzerland:AG
OU539830	P.1	Gamma	variant of concern	29894	Switzerland:GR
OU539894	P.1	Gamma	variant of concern	29894	Switzerland:ZH
OU492255	P.1	Gamma	variant of concern	29898	United Kingdom:England
OU565182	P.1	Gamma	variant of concern	29898	United Kingdom:Scotland
MZ896179	P.1	Gamma	variant of concern	29531	USA: Oregon
MZ896359	P.1	Gamma	variant of concern	29827	USA: Minnesota

MZ896669	P.1	Gamma	variant of concern	29808	USA: California
MZ911221	B.1.427	Epsilon	variant of interest	29864	USA: Arizona, Pima
MZ375854	B.1.427	Epsilon	variant of interest	29870	Mexico: B.C.
MZ375858	B.1.427	Epsilon	variant of interest	29833	Mexico: B.C.
MZ943834	B.1.429	Epsilon	variant of interest	29780	USA: California
MZ687447	B.1.429	Epsilon	variant of interest	29838	USA: Tennessee, Davidson county
OU282717	B.1.429	Epsilon	variant of interest	29903	United Kingdom:Wales
OU235436	B.1.429	Epsilon	variant of interest	29903	United Kingdom:England
MZ277390	B.1.429	Epsilon	variant of interest	29891	Taiwan
MZ746235	B.1.617.1	Kappa	variant of interest	29791	USA: Utah
MZ724425	B.1.617.1	Kappa	variant of interest	29788	India
MZ571142	B.1.617.1	Kappa	variant of interest	29818	Morocco
MZ562746	B.1.617.1	Kappa	variant of interest	29837	India: Madhya Pradesh
MZ538882	B.1.617.1	Kappa	variant of interest	29823	USA: FL
OU322235	B.1.617.1	Kappa	variant of interest	29559	Germany:Europe/Germany/Berlin
OU290035	B.1.617.1	Kappa	variant of interest	29893	United Kingdom:England
MZ487800	B.1.617.1	Kappa	variant of interest	29809	USA: DC
OU265178	B.1.617.1	Kappa	variant of interest	29902	United Kingdom:Wales
OU247018	B.1.617.1	Kappa	variant of interest	29902	United Kingdom:England
OU254949	B.1.617.1	Kappa	variant of interest	29902	United Kingdom:Wales
MZ397173	B.1.617.1	Kappa	variant of interest	29830	Myanmar
MZ397175	B.1.617.1	Kappa	variant of interest	29812	Myanmar
MZ397176	B.1.617.1	Kappa	variant of interest	29822	Myanmar
MZ397177	B.1.617.1	Kappa	variant of interest	29823	Myanmar
OU168413	B.1.617.1	Kappa	variant of interest	29795	Germany:Europe/Germany/Saxony

OU172184	B.1.617.1	Kappa	variant of interest	29835	Switzerland:SO
OU182966	B.1.617.1	Kappa	variant of interest	29850	Switzerland:BL
MZ363839	B.1.617.1	Kappa	variant of interest	29801	India
MZ332524	B.1.617.1	Kappa	variant of interest	29801	India
MZ332527	B.1.617.1	Kappa	variant of interest	29801	India
OU061269	B.1.617.1	Kappa	variant of interest	29902	United Kingdom:Scotland
LC633761	B.1.617.1	Kappa	variant of interest	29890	Japan
MZ026886	B.1.617.1	Kappa	variant of interest	29525	Bahrain
MZ953185	C.37	Lambda	variant of interest	29805	USA: California
MZ953277	C.37	Lambda	variant of interest	29801	USA: Massachusetts
MZ937666	C.37	Lambda	variant of interest	29379	USA: Illinois
MZ942542	C.37	Lambda	variant of interest	29653	USA: Kentucky
MZ908711	C.37	Lambda	variant of interest	29785	USA: Virginia
MZ857990	C.37	Lambda	variant of interest	29750	USA: Tennessee
OU517038	C.37	Lambda	variant of interest	29795	Germany:Europe/Germany/Hesse
OU356036	C.37	Lambda	variant of interest	29873	United Kingdom:England
OU351967	C.37	Lambda	variant of interest	29873	Switzerland:GE
MZ496613	C.37	Lambda	variant of interest	29794	Peru
OU268528	C.37	Lambda	variant of interest	29873	Switzerland:GE
OU100999	C.37	Lambda	variant of interest	29752	Germany:Europe/Germany/Berlin
OU053018	C.37	Lambda	variant of interest	29873	United Kingdom:England
MZ275295	C.37	Lambda	variant of interest	29781	Peru
MZ275301	C.37	Lambda	variant of interest	29798	Peru

**Supplementary Table S2. Summary of DNA target sequences used for plasmid construction**

Gene fragments	Length (bp)	Sequence (5'→3')
Wild-type <i>Se</i>	3822	ATGTTTGTTTTTTTTGTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCAGAACTCAATTACCCCCTGCATACAC TAATTCCTTTCACACGTGGTGTATTACCCTGACAAAGTTTTTCAGATCCTCAGTTTTACATTCAACTCAGGACTTGTCTTACCTT TCTTTTCCAATGTTACTTGGTTCATGCTATAACATGTCTCTGGGACCAATGGTACTAAGAGGTTTGCTAACCCCTGTCCTACCATT AATGATGGTGTATTGTTGCTTCCACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTTCGAAGACCC AGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAGTCTGTGAATTTCAATTTTGTAAATGATCCATTTTTGGGTGTT TATTACCACAAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTC TCTCAGCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCAAAAATCTTAGGGAATTTGTGTTAAGAATATTGATGGTT ATTTTAAAATATATTCTAAGCACACGCCTATTAATTTAGTGCCTGGTCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTGGTAGA TTTGCCAATAGGTATTAACATCACTAGGTTTCAAACCTTACTTGCTTTACATATAAGTTATTTGACTCCTGGTGATTCTTCTTCAG GTTGGACAGCTGGTGCTGCAGCTTATTATGTGGGTTATCTTCAACCTAGGACTTTTCTATTAAAATATAATGAAAATGGAACCAT TACAGATGCTGTAGACTGTGCACTTGACCCTCTCTCAGAAACAAAGTGTACGTTGAAATCCTTCACTGTAGAAAAGGAATCTA TCAAACCTTCTAACTTTAGAGTCCAACCAACAGAATCTATTGTTAGATTTCTAATATTACAAACTTGTGCCCTTTTGGTGAAGTT TTTAACGCCACCAGATTTGCATCTGTTTATGCTTGGAACAGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGTCCTATATA ATTCGCATCATTTTCCACTTTTAAAGTGTTATGGAGTGTCTCCTACTAAATTAATGATCTCTGCTTTACTAATGTCTATGCAGAT TCATTTGTAATTAGAGGTGATGAAGTCAGACAAATCGCTCCAGGGCAAACCTGGAAACATTGCTGATTATAATTATAAATTACCA GATGATTTTACAGGCTGCGTTATAGCTTGGAAATCTAACAATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCTGTTTAGAT TGTTTAGGAAGTCTAATCTCAAACCTTTGAGAGAGATATTTCAAACCTGAAATCTATCAGGCCGGTAGCACACCTTGTAATGGTG TTAAAGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCAAACCCACTTATGGTGTTGGTTACCAACCATAACAGAGTAGT AGTACTTCTTTTGAACCTTCTACATGCACCAGCAACTGTTTGTGGACCTAAAAGTCTACTAATTTGGTTAAAACAATGTGTC AATTTCAACTTCAATGGTTTAAACAGGCACAGGTGTTCTTACTGAGTCTAACAAAAAGTTTCTGCCTTTCCAACAATTTGGCAGAG

ACATTGATGACACTACTGATGCTGTCCGTGATCCACAGACACTTGAGATTCTTGACATTACACCATGTTCTTTTTGGTGGTGTTCAG  
TGTTATAACACCAGGAACAAATACTTCTAACCAGGTTGCTGTTCTTTATCAGGGTGTAACTGCACAGAAGTCCCTGTTGCTATT  
CATGCAGATCAACTTACTCCTACTTGGCGTGTTTATTCTACAGGTTCTAATGTTTTTCAAACACGTGCAGGCTGTTTAATAGGGG  
CTGAACATGTCAACAACCTCATATGAGTGTGACATACCCATTGGTGCAGGTATATGCGCTAGTTATCAGACTCAGACTAATTCTC  
ATCGGCGGGCACGTAGTGTAGCTAGTCAATCCATCATTGCCTACACTATGTCACCTGGTGTAGAAAATTCAGTTGCTTACTCTAA  
TAACTCTATTGCCATACCCATAAATTTTACTATTAGTGTACCACAGAAATTCTACCAGTGTCTATGACCAAGACATCAGTAGAT  
TGTACAATGTACATTTGTGGTGATTCAACTGAATGCAGCAATCTTTTGTGCAATATGGCAGTTTTTGTACACAATTAACCGTG  
CTTTAACTGGAATAGCTGTTGAACAAGACAAAAACCCCAAGAAGTTTTTGCACAAGTCAAACAAATTTACAAAACACCACCA  
ATTAAGATTTTTGGTGGTTTTAATTTTTCACAAATATTACCAGATCCATCAAAACCAAGCAAGAGGTCATTTATTGAAGATCTAC  
TTTTCAACAAAGTGACACTTGCAGATGCTGGCTTCATCAAACAATATGGTGATTGCCTTGGTGATATTGCTGCTAGAGACCTCAT  
TTGTGCACAAAAGTTTAAACGGCCTTACTGTTTTGCCACCTTTGCTCACAGATGAAATGATTGCTCAATACACTTCTGCACTGTTA  
GCGGGTACAATCACTTCTGGTTGGACCTTTGGTGCAGGTGCTGCATTACAAATACCATTTGCTATGCAAATGGCTTATAGGTTTA  
ATGGTATTGGAGTTACACAGAATGTTCTCTATGAGAACCAAAAATTGATTGCCAACCAATTTAATAGTGCTATTGGCAAAATTC  
AAGACTCACTTTCTTCCACAGCAAGTGCACCTTGGAAAACCTCAAGATGTGGTCAACCAAAAATGCACAAGCTTTAAACACGCTTG  
TTAAACAACCTTAGCTCCAATTTTTGGTGCAATTTCAAGTGTTTTAAATGATATCCTTGCACGTCTTGACAAAGTTGAGGCTGAAGT  
GCAAATTGATAGGTTGATCACAGGCAGACTTCAAAGTTTGCAGACATATGTGACTCAACAATTAATTAGAGCTGCAGAAATCA  
GAGCTTCTGCTAATCTTGCTGCTACTAAAATGTCAGAGTGTGTACTTGGACAATCAAAAAGAGTTGATTTTTGTGGAAAGGGCT  
ATCATCTTATGTCCTTCCCTCAGTCAGCACCTCATGGTGTAGTCTTCTTGCATGTGACTTATGTCCTTGCACAAGAAAAGAACTT  
CACAACCTGCTCCTGCCATTTGTCATGATGGAAAAGCACACTTTCCTCGTGAAGGTGTCTTTGTTTCAAATGGCACACACTGGTTT  
GTAACACAAAGGAATTTTTATGAACCACAAATCATTACTACACACAACACATTTGTGTCTGGTAACTGTGATGTTGTAATAGGA  
ATTGTCAACAACACAGTTTATGATCCTTTGCAACCTGAATTAGACTCATTCAAGGAGGAGTTAGATAAATATTTTAAGAATCAT  
ACATCACCAGATGTTGATTTAGGTGACATCTCTGGCATTAAATGCTTCAGTTGTAAACATTCAAAAAGAAATTGACCGCCTCAAT  
GAGGTTGCCAAGAATTTAAATGAATCTCTCATCGATCTCCAAGAACTTGGAAAGTATGAGCAGTATATAAAATGGCCATGGTAC  
ATTTGGCTAGGTTTTATAGCTGGCTTGATTGCCATAGTAATGGTGCACAATTATGCTTTGCTGTATGACCAGTTGCTGTAGTTGTC  
TCAAGGGCTGTTGTTCTTGTGGATCCTGCTGCAAATTTGATGAAGACGACTCTGAGCTAGTGCTCAAAGGAGTCAAATTACATT  
ACACATAA

<b>Mu tant S gen e</b>	<b>38 22</b>	ATGTTTGTTTTTTTTGTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCAGAACTCAATTACCCCCTGCATACAC TAATTCCTTTCACACGTGGTGTATTACCCTGACAAAGTTTTAGATCCTCAGTTTTACATTCAACTCAGGACTTGTCTTACCTT TCTTTTCCAATGTTACTTGGTTCATGCTATAACATGTCTCTGGGACCAATGGTACTAAGAGGTTTGCTAACCCCTGTCCTACCATT AATGATGGTGTATTATTTGCTTCCACTGAGAAGTCTAACATAATAAGAGGCTGGATTTTTGGTACTACTTTAGATTTCGAAGACCC AGTCCCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAGTCTGTGAATTTCAATTTTGTAAATGATCCATTTTTGGGTGTT TATTACCACAAAACAACAAAAGTTGGATGGAAAGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTC TCTCAGCCTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCAAAAATCTTAGGGAAATTTGTGTTAAGAATATTGATGGTT ATTTTAAAATATATTCTAAGCACACGCCTATTAATTTAGTGCCTGGTCTCCCTCAGGGTTTTTTCGGCTTTAGAACCATTGGTAGA TTTGCCAATAGGTATTAACATCACTAGGTTTCAAACCTTACTTGCTTTACATATAAGTTATTTGACTCCTGGTGATTCTTCTTCAG GTTGGACAGCTGGTGCTGCAGCTTATTATGTGGGTTATCTTCAACCTAGGACTTTTCTATTAATAATAATGAAAATGGAACCAT TACAGATGCTGTAGACTGTGCACTTGACCCTCTCTCAGAAACAAAGTGTACGTTGAAATCCTTCACTGTAGAAAAGGAATCTA TCAAACCTTCTAACTTTAGAGTCCAACCAACAGAATCTATTGTTAGATTTCCCTAATATTACAAACTTGTGCCCTTTTGGTGAAGTT TTTAAACGCCACCAGATTTGCATCTGTTTATGCTTGGAACAGGAAGAGAATCAGCAACTGTGTTGCTGATTATTCTGTCTATATA ATCCGCATCATTTTCCACTTTTAAAGTGTATGGAGTGTCTCCTACTAAATTAATGATCTCTGCTTACTAATGTCTATGCAGAT TCATTTGTAATTAGAGGTGATGAAGTCAGACAAATCGCTCCAGGGCAAACCTGGAAATATTGCTGATTATAATTATAAATTACCA GATGATTTTACAGGCTGCGTTATAGCTTGGAAATCTAACAATCTTGATTCTAAGGTTGGTGGTAATTATAATTACCGGTTTAGAT TGTTTAGGAAGTCTAATCTCAAACCTTTGAGAGAGATATTTCAAACCTGAAATCTATCAGGCCGGTAGCAAACCTTGTAATGGTG TTCAAGGTTTTAATTGTTACTTTCCTTTACAATCATATGGTTTCCAACCCACTTATGGTGTGGTTACCAACCATAACAGAGTAGT AGTACTTTCTTTTGAACCTTCTACATGCACCAGCAACTGTTTGTGGACCTAAAAAGTCTACTAATTTGGTTAAAAACAATGTGTC AATTTCAAACCTCAATGGTTTAAACAGGCACAGGTGTTCTTACTGAGTCTAACAAAAAGTTTCTGCCTTTCCAACAATTTGGCAGAG ACATTGATGACACTACTGATGCTGTCCGTGATCCACAGACACTTGAGATTCTTGACATTACACCATGTTCTTTTGGTGGTGTGTCAG TGTTATAACACCAGGAACAAATACTTCTAACCAGGTTGCTGTTCTTTATCAGGGTGTAACTGCACAGAAGTCCCTGTTGCTATT CATGCAGATCAACTTACTCTACTTGGCGTGTATTCTACAGGTTCTAATGTTTTTCAAACACGTGCAGGCTGTTAATAGGGG CTGAACATGTCAACAACCTCATATGAGTGTGACATACCCATTGGTGCAGGTATATGCGCTAGTTATCAGACTCAGACTAATTCTC ATCGGCGGGCACGTAGTGTAGCTAGTCAATCCATCATTGCCTACACTATGTCACCTGGTGTAGAAAATTCAGTTGCTTACTCTAA TAACTCTATTGCCATACCCATAAATTTTACTATTAGTGTACCACAGAAATCTACCAGTGTCTATGACCAAGACATCAGTAGAT
--	------------------	---

		<p>TGTACAATGTACATTTGTGGTGATTCAACTGAATGCAGCAATCTTTTGTGCAATATGGCAGTTTTTGTACACAATTAACCGTG  CTTTAACTGGAATAGCTGTTGAACAAGACAAAAACCCCAAGAAGTTTTTGCACAAGTCAAACAAATTTACAAAACACCACCA  ATTAAGATTTTGGTGGTTTTAATTTTTCACAAATATTACCAGATCCATCAAACCAAGCAAGAGGTCATTTATTGAAGATCTAC  TTTTCAACAAAGTGACACTTGCAGATGCTGGCTTCATCAAACAATATGGTGATTGCCCTGGTGATATTGCTGCTAGAGACCTCAT  TTGTGCACAAAAGTTTAACGGCCTTACTGTTTTGCCACCTTTGCTCACAGATGAAATGATTGCTCAATACACTTCTGCACTGTTA  GCGGGTACAATCACTTCTGGTTGGACCTTTGGTGCAGGTGCTGCATTACAAATACCATTTGCTATGCAAATGGCTTATAGGTTTA  ATGGTATTGGAGTTACACAGAATGTTCTCTATGAGAACCAAAAATTGATTGCCAACCAATTTAATAGTGCTATTGGCAAAATTC  AAGACTCACTTTCTTCCACAGCAAGTGCACCTTGGAAAACCTCAAGATGTGGTCAACCAAAAATGCACAAGCTTTAAACACGCTTG  TTAAACAACCTTAGCTCCAATTTTGGTGCAATTTCAAGTGTTTTAAATGATATCCTTGCACGTCTTGACAAAGTTGAGGCTGAAGT  GCAAATTGATAGGTTGATCACAGGCAGACTTCAAAGTTTGCAGACATATGTGACTCAACAATTAATTAGAGCTGCAGAAATCA  GAGCTTCTGCTAATCTTGCTGCTACTAAAATGTCAGAGTGTGTACTTGGACAATCAAAAAGAGTTGATTTTTGTGGAAAGGGCT  ATCATCTTATGTCCTTCCCTCAGTCAGCACCTCATGGTGTAGTCTTCTTGCATGTGACTTATGTCCTTGCACAAGAAAAGAACTT  CACAACCTGCTCCTGCCATTTGTCATGATGGAAAAGCACACTTTCCTCGTGAAGGTGTCTTTGTTTCAAATGGCACACACTGGTTT  GTAACACAAAGGAATTTTATGAACCACAAATCATTACTACACACAACACATTTGTGTCTGGTAACTGTGATGTTGTAATAGGA  ATTGTCAACAACACAGTTTATGATCCTTTGCAACCTGAATTAGACTCATTCAAGGAGGAGTTAGATAAATATTTTAAGAATCAT  ACATCACCAGATGTTGATTTAGGTGACATCTCTGGCATTAAATGCTTCAGTTGTAAACATTCAAAAAGAAATTGACCGCCTCAAT  GAGGTTGCCAAGAATTTAAATGAATCTCTCATCGATCTCCAAGAACTTGGAAAGTATGAGCAGTATATAAAATGGCCATGGTAC  ATTTGGCTAGGTTTTATAGCTGGCTTGATTGCCATAGTAATGGTGACAATTATGCTTTGCTGTATGACCAGTTGCTGTAGTTGTC  TCAAGGGCTGTTGTTCTTGTGGATCCTGCTGCAAATTTGATGAAGACGACTCTGAGCTAGTGCTCAAAGGAGTCAAATTACATT  ACACATAA</p>
<b>S- Orf 8 gene</b>	<b>36 6</b>	<p>ATGAAATTTCTGTTTTCTTAGGAATCATCACAACCTGTAGCTGCATTTACCAAGAATGTAGTTTACAGTCATGTAACATC  AACCATATGTAGTTGATGACCCGTGTCCTATTCACCTTCTATTCTAAATGGTATATTAGAGTAGGAGCTAGAAAATCAGCACCTTT  AATTGAATTGTGCGTGGATGAGGCTGGTTCTAAATCACCCATTCAGTACATCGATATCGGTAATTATACAGTTTCTGTTACCT  TTTACAATTAATTGCCAGGAACCTAAATGGGTAGTCTTGTAGTGCGTTGTTCTGTTCTATGAAGACTTTTTAGAGTATCATGACG  TTCGTGTTGTTTTAGATTCATCTAA</p>



L-Orf8 gene	36 6	ATGAAATTTCTTGTTTTCTTAGGAATCATCACAACCTGTAGCTGCATTTACCAAGAATGTAGTTTACAGTCATGTACTCAACATC AACCATATGTAGTTGATGACCCGTGTCCTATTCACCTTCTATTCTAAATGGTATATTAGAGTAGGAGCTAGAAAATCAGCACCTTT AATTGAATTGTGCGTGGATGAGGCTGGTTCTAAATCACCCATTCAGTACATCGATATCGGTAATTATACAGTTTCCTGTTTACCT TTTACAATTAATTGCCAGGAACCTAAATTGGGTAGTCTTGTAGTGCGTTGTTTCGTTCTATGAAGACTTTTTAGAGTATCATGACG TTCGTGTTGTTTTAGATTTTCATCTAA
NP gene	12 60	ATGTCTGATAATGGACCCCAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAACTGGCAGTAACCAG AATGGAGAACGCAGTGGGGCGCGATCAAAACAACGTCCGGCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCACCGCTCTC ACTCAACATGGCAAGGAAGACCTTAAATTCCTCGAGGACAAGGCGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAAT TGGCTACTACCGAAGAGCTACCAGACGAATTCGTGGTGGTGACGGTAAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTA CCTAGGAACTGGGCCAGAAGCTGGACTTCCCTATGGTGCTAACAAAGACGGCATCATATGGGTTGCAACTGAGGGAGCCTTGA ATACACCAAAGATCACATTGGCACCCGCAATCCTGCTAACAAATGCTGCAATCGTGCTACAACCTTCCCTCAAGGAACAACATTGC CAAAGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACGTAGTCGCAACAGTTCAAGA AATTCAACTCCAGGCAGCAGTAGGGGAACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCTG CTTGACAGATTGAACCAGCTTGAGAGCAAAATGTCTGGTAAAGGCCAACAAACAAGGCCAAACTGTCACTAAGAAATCTGC TGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGCCACTAAAGCATAACAATGTAACACAAGCTTTTCGGCAGACGTGGTC CAGAACAACCCAAGGAAATTTGGGGACCAGGAACTAATCAGACAAGGAACTGATTACAAACATTGGCCGCAAATTGCACA ATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCCGCGCATTGGCATGGAAGTACACCTTCGGGAACGTGGTTGACCTACAC AGGTGCCATCAAATTGGATGACAAAGATCCAAATTTCAAAGATCAAGTCATTTTGCTGAATAAGCATATTGACGCATACAAAA CATTCCCACCAACAGAGCCTAAAAAGGACAAAAAGAAGAAGGCTGATGAAACTCAAGCCTTACCGCAGAGACAGAAGAAACA GCAAACCTGTGACTCTTCTCCTGCTGCAGATTTGGATGATTTCTCCAAACAATTGCAACAATCCATGAGCAGTGCTGACTCAACT CAGGCCTAA

**Note:** NP gene, the gene encoding the nucleoprotein of SARS-CoV-2 (nt 28,274-29,533, NCBI accession number MN908947); Wild-type S gene, gene fragment of SARS-CoV-2 spike protein (nt 21,563-25,384, NCBI accession number MN908947); Mutant S gene, gene fragments of SARS-CoV-2 spike protein including mutations: L5F, D80A, D215G, R246I, K417N, L452R/Q, Y453F, E484Q, N501Y, A570D, D614G, P681H, A701V, T716I, S982A, D1118H, P1263L; S-Orf8 gene, S lineage gene fragment of open reading frame 8 (ORF8, nt 27,894-28,259, NCBI accession number MN908947); L-Orf8 gene, L lineage fragment of SARS-CoV-2 ORF8 with mutation of S84L.

**Supplementary Table S3. Information of clinical samples**

<b>SARS-CoV-2 variants</b>	<b>Sample ID</b>	<b>Cycle threshold value (ORF1a/b)</b>	<b>Cycle threshold value (N)</b>
<b>Wild-type</b>	20SF5770	37	36
	20SF5783	28	28
	20SF5774	21	21
	20SF5787	21	22
	20SF5788	28	28
<b>Aplha</b>	XG01774	21	20
	XG02064	24	23
	XG03594	17	16
	XG03595	26	26
	XG03596	15	15
	XG03607	18	16
	XG03623	16	15
	XG04704	18	17
	XG04850	21	22
	XG08145	24	22
	XG08337	17	16
<b>Beta</b>	XG02289	23	23
	XG03246	18	18
	XG03255	19	19
	XG03240	14	13
	XG05824	18	17
	XG07753	27	26
	XG07933	22	22

	XG08741	31	31
<b>Delta</b>	XG08547	20	19
	XG08859	18	19
	XG09082	16	17
	XG08688	21	21
	XG08689	15	15
	XG08692	16	16
	XG08693	16	16
	XG08694	18	16

**Supplementary Table S4. Summary of guide RNA (crRNA) sequences**

<b>crRNA</b>	<b>Mutations detected</b>	<b>On-target</b>	<b>Off-target</b>	<b>Sequence (5'→3')</b>
crRNA-N1	NA	NP gene	NA	UAAUUUCUACUAAGUGUAGACCCCCAGCGCUUCAGCGUUC
crRNA-N2	NA	NP gene	NA	UAAUUUCUACUAAGUGUAGAGUGGACCCUCAGAUUCAACU
crRNA-N3	NA	NP gene	NA	UAAUUUCUACUAAGUGUAGACCCAAUAAUACUGCGUCUUG
crRNA-N4	NA	NP gene	NA	UAAUUUCUACUAAGUGUAGAUACUACCUAGGAACUGGGCC
crRNA-N5	NA	NP gene	NA	UAAUUUCUACUAAGUGUAGACUGCUGCUUGACAGAUUGAA
crRNA-S	NA	Both wild-type and mutant S gene	NA	UAAUUUCUACUAAGUGUAGAAUAGGGGCUGAACAUUGUCA
crRNA-S-5F	L5F (Spike protein)	Mutant S gene (5F)	Wild-type S gene (5I)	UAAUUUCUACUAAGUGUAGAUUUUAUUGCCACUAGUCUCU
crRNA-S-80A	D80A (Spike protein)	Mutant S gene (80A)	Wild-type S gene (80D)	UAAUUUCUACUAAGUGUAGACUAACCCUGUCCUACCAUUU
crRNA-S-215G	D215G (Spike protein)	Mutant S gene (215G)	Wild-type S gene (215D)	UAAUUUCUACUAAGUGUAGAGUGCGUGGUCUCCCUCAGGG
crRNA-S-246I	R246I (Spike protein)	Mutant S gene (246I)	Wild-type S gene (246R)	UAAUUUCUACUAAGUGUAGACAUAUAAGUUAUUUGACUCC
crRNA-S-417K	K417N (Spike protein)	Wild-type S gene (417K)	Mutant S gene (417N)	UAAUUUCUACUAAGUGUAGAAACGAUUGCUGAUUAUAAU
crRNA-S-417N	K417N (Spike protein)	Mutant S gene (417N)	Wild-type S gene (417K)	UAAUUUCUACUAAGUGUAGAAACUAUUGCUGAUUAUAAU
crRNA-S-452R	L452R (Spike protein)	Mutant S gene (452R)	Wild-type S gene (452L)	UAAUUUCUACUAAGUGUAGACCGAUUAUAGAUUGUUUAGGA
crRNA-S-452Q	L452Q (Spike protein)	Mutant S gene (452Q)	Wild-type S gene (452L)	UAAUUUCUACUAAGUGUAGACCAUAUAGAUUGUUUAGGA

crRNA-S-453F	Y453F (Spike protein)	Mutant S gene (453F)	Wild-type S gene (453Y)	UAAUUUCUACUAAGUGUAGAGAUUGUUUAGGAAGUCUAAU
crRNA-S-478T	T478K (Spike protein)	Wild-type S gene (478T)	Mutant S gene (478K)	UAAUUUCUACUAAGUGUAGAAAGGGGUGCUACCGGCCUGA
crRNA-S-478K	T478K (Spike protein)	Mutant S gene (478K)	Wild-type S gene (478T)	UAAUUUCUACUAAGUGUAGAAAGGGUUGCUACCGGCCUGA
crRNA-S-484K	E484K (Spike protein)	Mutant S gene (484K)	Wild-type S gene (484E)	UAAUUUCUACUAAGUGUAGAACACCAUACAAGGUGUGCU
crRNA-S-484Q	E484Q (Spike protein)	Mutant S gene (484Q)	Wild-type S gene (484E)	UAAUUUCUACUAAGUGUAGAAAUGGUGUACAAGGUUUUAA
crRNA-S-501N	N501Y (Spike protein)	Wild-type S gene (501N)	Mutant S gene (501Y)	UAAUUUCUACUAAGUGUAGACAACCCACUAAUGGUGUUGG
crRNA-S-570D	A570D (Spike protein)	Mutant S gene (570D)	Wild-type S gene (570A)	UAAUUUCUACUAAGUGUAGAGCAGAGACAUUGAUGACACU
crRNA-S-614D	D614G (Spike protein)	Wild-type S gene (614D)	Mutant S gene (614G)	UAAUUUCUACUAAGUGUAGAUCAGGACGUU AACUGCACAG
crRNA-S-701V	A701V (Spike protein)	Mutant S gene (701V)	Wild-type S gene (701A)	UAAUUUCUACUAAGUGUAGAUACACCAAGUGACAUAGUGU
crRNA-S-716I	T716I (Spike protein)	Mutant S gene (716I)	Wild-type S gene (716T)	UAAUUUCUACUAAGUGUAGAAUGGGUAUGGCAAUAGAGUU
crRNA-S-982S	S982A (Spike protein)	Wild-type S gene (982S)	Mutant S gene (982A)	UAAUUUCUACUAAGUGUAGAACGUCUUGACAAAGUUGAGG
crRNA-S-1263L	P1263L (Spike protein)	Mutant S gene (1263L)	Wild-type S gene (1263P)	UAAUUUCUACUAAGUGUAGAAGCACUAGCUCAGAGUCGUC
crRNA-Orf8	S84L (ORF8 protein)	L-Orf8 gene (84L)	S-Orf8 gene (84S)	UAAUUUCUACUAAGUGUAGACCUUUUACAAUAAUUGCCA

Note: NA, not available; NP gene, the gene encoding the nucleoprotein of SARS-CoV-2 (nt 28,274-29,533, NCBI accession number MN908947); Wild-type S gene, gene fragment of SARS-CoV-2 spike protein (nt 21,563-25,384, NCBI accession number MN908947); Mutant S gene, gene fragments of SARS-CoV-2 spike protein including mutations: L5F, D80A, D215G, R246I, K417N, L452R/Q, Y453F, T478K, E484K/Q, N501Y, A570D, D614G, P681H, A701V, T716I, S982A, D1118H, P1263L; S-Orf8 gene, S lineage gene fragment of open reading frame 8 (ORF8, nt 27,894-28,259, NCBI accession number MN908947); L-Orf8 gene, L lineage fragment of SARS-CoV-2 ORF8 with mutation of S84L.

**Supplementary Table S5. Summary of primer sequences**

Primer	Sequence (5'→3')
N-gene_F	ATGTCTGATAATGGACC
N-gene_R	GGTGTGACTTCCATGCC
S-gene_F1	CTAGTGATGTTCTTGTT
S-gene_R1	TGCACAGTCTACAGCATC
S-gene_F2	ACTTGTGCCCTTTTGGTGAAG
S-gene_R2	GCTATTCCAGTTAAAGCACGGT
S-gene_F3	AGACTCACTTTCTTCCACAGCAA
S-gene_R3	GTATCGTTGCAGTAGCGCGA
Orf8-gene_F	AGGAATCATCACAACCTGTAGC
Orf8-gene_R	AACGAACAACGCACTACAAGAC
K417N_F	ATCGCTCCAGGGCAAATTTGAAA
K417N_R	GCTATTCCAGTTAAAGCACGGT
L452R_F	AGGTTGGTGGTAATTATATTTAC
L452R_R	AGTACTACTACTCTGTATGGTTGGT
T478K-R	AACACCATTTC AAGGT
E484Q_F	AGCACACCTTTTAATGGTGTT
E484Q_R	AGTACTACTACTCTGTATGGTTGGT
HCoV 229E NP gene_F	CTACAGTCAAATGGGCTGATGCATCT
HCoV 229E NP gene_R	TGACTTCATCAATTATGTCAG
HCoV OC43 NP gene_F	TCCAGTAGTAGAGCGTCCTCTGGA
HCoV OC43 NP gene_R	GCTGATGTCCTCTGCAGTCAACTCTA
HCoV HKU1 NP gene_F	ATACTCCCGGTCATTATGCTGGAA
HCoV HKU1 NP gene_R	ACATAAGGATCATCAAGAGTAGC

HCoV 229E S gene outer_F	TTGTTGCATATGCCTTGTTGCATATTGC
HCoV 229E S gene outer__R	ATCTTTTCAACGTCGTAATAAGGAAGTTTAG
HCoV 229E S gene inner_F	ACTCTGTTTGCAACGGCTGTGTTGGT
HCoV 229E S gene inner__R	ACTCACCACAAAGATGAGCACGACTG
HCoV OC43 S gene outer_F	TATAAGTATTGATACTGTTGATGTTACTA
HCoV OC43 S gene outer__R	GTCCTTGAGATTGATGTAGCTATGA
HCoV OC43 S gene inner_F	CAGGTTCCACATATCGTAATAT
HCoV OC43 S gene inner__R	AGCTATACCTCTATTACCAGCAATGCA
HCoV HKU1 S gene outer_F	GCCTACAACATTAGCTGTTATA
HCoV HKU1 S gene outer__R	ACACACTCATTAACCTTCTC
HCoV HKU1 S gene inner_F	GTTAAGAATACTAAGTTGTATGT
HCoV HKU1 S gene inner__R	AGCCTATCAATCTGAACCTGA
HCoV 229E Orf3 gene_F	CTCTAGGTTTGTTCACATTGCA
HCoV 229E Orf3 gene_R	TCTGTGTTGACTATTCCAACAGC
HCoV OC43 Orf5 gene_F	ACTTCACATGACGACTAAGTT
HCoV OC43 Orf5 gene_R	ACAATCCACCATCACATGA
HCoV HKU1 Orf4 gene_F	ATGGACGTTTGGAGACCTAGC
HCoV HKU1 Orf4 gene_R	TCAACCATACTTATTAAGTGC



**Supplementary Table S6. Comparison of CRISPR-Cas12a, RT-PCR and whole genome sequencing in detecting SARS-CoV-2 variants**

<b>Characteristics</b>	<b>CRISPR-Cas12a</b>	<b>RT-PCR(1, 2)</b>	<b>Whole genome sequencing<sup>§</sup></b>
<b>Procedure</b>	<ol style="list-style-type: none"> <li>1. Amplification plus Cas12 cleavage (1.0-2h)</li> <li>2. Amplification-free Cas12 system (20-30 min)</li> </ol>	<ol style="list-style-type: none"> <li>1. Amplification of target gene (1.5-2h)</li> <li>2. Detection (0-30min) depending on different assays</li> </ol>	<ol style="list-style-type: none"> <li>1. Library preparations (5-7 h)</li> <li>2. Sequencing (17-32 h)</li> <li>3. Bioinformatic analysis (1-2 h)</li> </ol>
<b>Assay results</b>	Qualitative/Semi-quantitative / diagnosis / genotyping	Quantitative / diagnosis / genotyping	Qualitative / genotyping / characterizing mutations
<b>Limits of detection</b>	1-10 copies per $\mu\text{L}$ or higher	$\sim 1$ copies per $\mu\text{L}$	Not applicable
<b>Estimated cost</b>	\$4 per sample	\$3 per sample	\$87 per sample
<b>Benefits</b>	<ol style="list-style-type: none"> <li>1. Isothermal amplification and reaction at room temperature.</li> <li>2. Easily to find conserved primer sequences to ensure sensitivity.</li> <li>3. Specificity relies on crRNA or gRNA.</li> </ol>	<ol style="list-style-type: none"> <li>1. Matured technology and products.</li> <li>2. PCR equipment and facilities available.</li> </ol>	<ol style="list-style-type: none"> <li>1. Identify novel mutations and variants.</li> <li>2. Provide complete genome sequence information.</li> </ol>
<b>Challenges</b>	<ol style="list-style-type: none"> <li>1. Limited to known mutations or variants with signature mutations.</li> <li>2. Multiple readouts and mixed mutations need an integrated testing system and algorithm.</li> </ol>	<ol style="list-style-type: none"> <li>1. PCR equipment and facility needed;</li> <li>2. Limited to known mutations or variants with signature mutations.</li> <li>3. Difficult to find appropriate primers for specific mutations.</li> <li>4. Not suitable for point-of-care testing.</li> </ol>	<ol style="list-style-type: none"> <li>1. Time-consuming for sequencing.</li> <li>2. Complex bioinformatics pipeline.</li> <li>3. High-end facilities and trained specialists.</li> </ol>

1. **Wang H, Miller JA, Verghese M, Sibai M, Solis D, Mfuh KO, Jiang B, Iwai N, Mar M, Huang C, Yamamoto F, Sahoo MK, Zehnder J, Pinsky BA.** 2021. Multiplex SARS-CoV-2 Genotyping RT-PCR for Population-Level Variant Screening and Epidemiologic Surveillance. *Journal of clinical microbiology*:Jcm0085921.
2. **Zelyas N, Pabbaraju K, Croxen MA, Lynch T, Buss E, Murphy SA, Shokoples S, Wong A, Kanji JN, Tipples G.** 2021. Precision Response to the Rise of the SARS-CoV-2 B.1.1.7 Variant of Concern by Combining Novel PCR Assays and Genome Sequencing for Rapid Variant Detection and Surveillance. *Microbiology spectrum* **9**:e0031521.

**§Based on Illumina MiSeq sequencing platforms.**

























	670	680	690	700	710	720
MN908947/Wuhan-Hu-1	EC	DI	PI	GA	GI	CA
BS001137/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
BS001138/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
BS001139/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
BS001140/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
MW913362/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
MZ266365/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
MZ350105/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
MZ350109/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
MZ888516/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
OU562607/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
OU514166/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
OU538851/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
OU538849/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
MZ895877/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
OU514176/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
OU562608/B.1.1.7/Alpha	EC	DI	PI	GA	GI	CA
MW981442/B.1.351/Beta	EC	DI	PI	GA	GI	CA
MZ298839/B.1.351/Beta	EC	DI	PI	GA	GI	CA
MZ913100/B.1.351/Beta	EC	DI	PI	GA	GI	CA
MZ879365/B.1.351/Beta	EC	DI	PI	GA	GI	CA
OU525106/B.1.351/Beta	EC	DI	PI	GA	GI	CA
OU539352/B.1.351/Beta	EC	DI	PI	GA	GI	CA
MZ202314/B.1.351/Beta	EC	DI	PI	GA	GI	CA
MZ298840/B.1.351/Beta	EC	DI	PI	GA	GI	CA
MZ376663/B.1.351/Beta	EC	DI	PI	GA	GI	CA
LC643036/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
LC643044/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
MZ853946/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
MZ854386/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
MZ888532/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
MZ888533/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
MZ896136/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
OU539261/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
MZ854389/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
OU562629/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
OU539690/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
MZ854387/B.1.617.2/Delta	EC	DI	PI	GA	GI	CA
OU539784/P.1/Gamma	EC	DI	PI	GA	GI	CA
OU539830/P.1/Gamma	EC	DI	PI	GA	GI	CA
OU539894/P.1/Gamma	EC	DI	PI	GA	GI	CA
MZ477748/P.1/Gamma	EC	DI	PI	GA	GI	CA
MZ477746/P.1/Gamma	EC	DI	PI	GA	GI	CA
MW938104/P.1/Gamma	EC	DI	PI	GA	GI	CA
MZ896179/P.1/Gamma	EC	DI	PI	GA	GI	CA
MZ896359/P.1/Gamma	EC	DI	PI	GA	GI	CA
MZ896669/P.1/Gamma	EC	DI	PI	GA	GI	CA
OU565182/P.1/Gamma	EC	DI	PI	GA	GI	CA
OU492255/P.1/Gamma	EC	DI	PI	GA	GI	CA
MZ911221/B.1.427/Epsilon	EC	DI	PI	GA	GI	CA
MZ375854/B.1.427/Epsilon	EC	DI	PI	GA	GI	CA
MZ375858/B.1.427/Epsilon	EC	DI	PI	GA	GI	CA
MZ943834/B.1.429/Epsilon	EC	DI	PI	GA	GI	CA
MZ277390/B.1.429/Epsilon	EC	DI	PI	GA	GI	CA
OU282717/B.1.429/Epsilon	EC	DI	PI	GA	GI	CA
OU235436/B.1.429/Epsilon	EC	DI	PI	GA	GI	CA
MZ687447/B.1.429/Epsilon	EC	DI	PI	GA	GI	CA
MZ538882/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ724425/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ363839/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ332524/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ332527/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
LC633761/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ562746/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ571142/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
OU168413/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ026886/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
OU172184/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ397173/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ397176/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ397175/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ397177/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
OU290035/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
OU265178/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
OU254949/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
OU247018/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
OU061269/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
OU182966/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ746235/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ487800/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
OU322235/B.1.617.1/Kappa	EC	DI	PI	GA	GI	CA
MZ953185/C.37/Lambda	EC	DI	PI	GA	GI	CA
MZ937666/C.37/Lambda	EC	DI	PI	GA	GI	CA
OU356036/C.37/Lambda	EC	DI	PI	GA	GI	CA
OU351967/C.37/Lambda	EC	DI	PI	GA	GI	CA
OU053018/C.37/Lambda	EC	DI	PI	GA	GI	CA
OU268528/C.37/Lambda	EC	DI	PI	GA	GI	CA
OU100999/C.37/Lambda	EC	DI	PI	GA	GI	CA
MZ275295/C.37/Lambda	EC	DI	PI	GA	GI	CA
MZ275301/C.37/Lambda	EC	DI	PI	GA	GI	CA
MZ908711/C.37/Lambda	EC	DI	PI	GA	GI	CA
MZ496613/C.37/Lambda	EC	DI	PI	GA	GI	CA
OU517038/C.37/Lambda	EC	DI	PI	GA	GI	CA
MZ953277/C.37/Lambda	EC	DI	PI	GA	GI	CA
MZ942542/C.37/Lambda	EC	DI	PI	GA	GI	CA
MZ857990/C.37/Lambda	EC	DI	PI	GA	GI	CA

















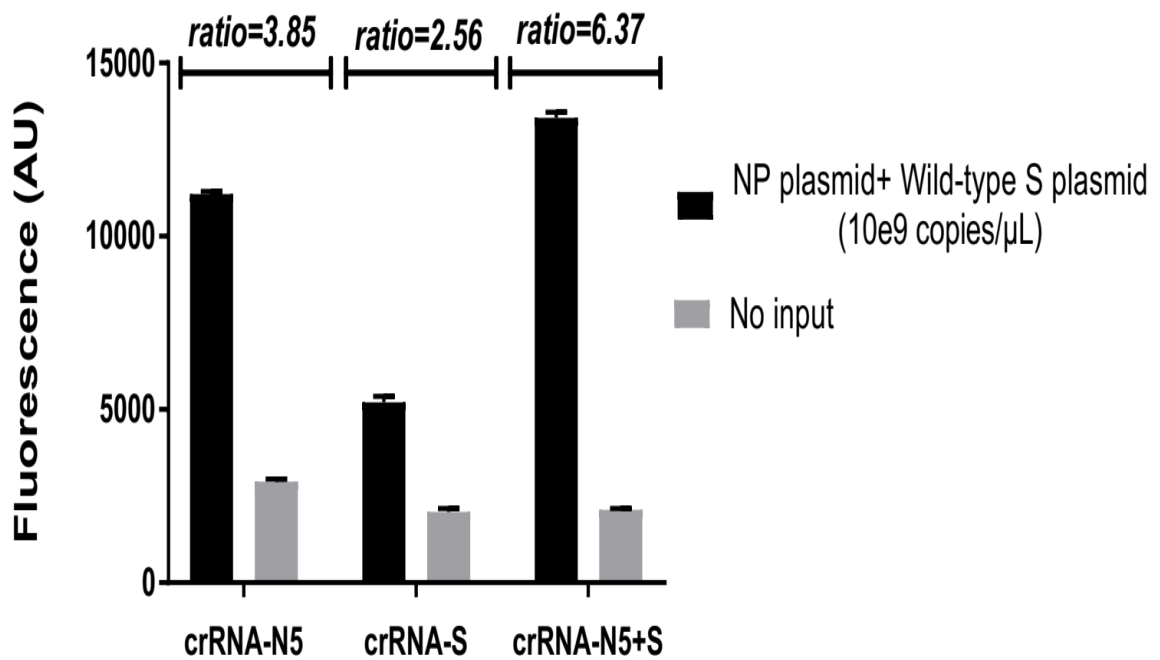




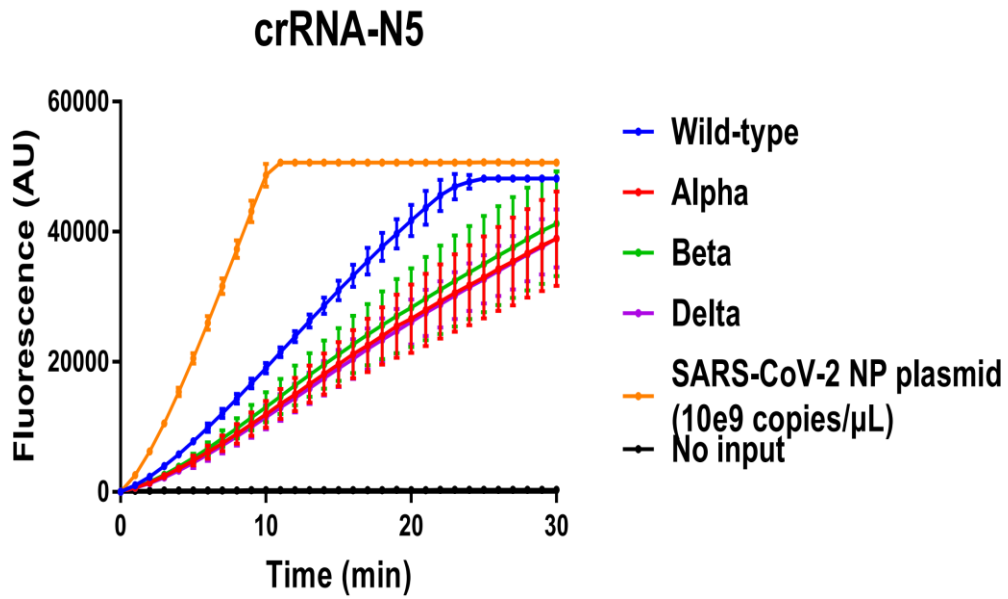
MN908947/Wuhan-Hu-1	SEPVLKGVKLLHYY
BS001137/B.1.1.7/Alpha	SEPVLKGVKLLHYY
BS001138/B.1.1.7/Alpha	SEPVLKGVKLLHYY
BS001139/B.1.1.7/Alpha	SEPVLKGVKLLHYY
BS001140/B.1.1.7/Alpha	SEPVLKGVKLLHYY
MW913362/B.1.1.7/Alpha	SEPVLKGVKLLHYY
MZ266365/B.1.1.7/Alpha	SEPVLKGVKLLHYY
MZ350105/B.1.1.7/Alpha	SEPVLKGVKLLHYY
MZ350109/B.1.1.7/Alpha	SEPVLKGVKLLHYY
MZ888516/B.1.1.7/Alpha	SEPVLKGVKLLHYY
OU562607/B.1.1.7/Alpha	SEPVLKGVKLLHYY
OU514166/B.1.1.7/Alpha	SEPVLKGVKLLHYY
OU538851/B.1.1.7/Alpha	SEPVLKGVKLLHYY
OU538849/B.1.1.7/Alpha	SEPVLKGVKLLHYY
MZ895877/B.1.1.7/Alpha	SEPVLKGVKLLHYY
OU514176/B.1.1.7/Alpha	SEPVLKGVKLLHYY
OU562608/B.1.1.7/Alpha	SEPVLKGVKLLHYY
MW981442/B.1.351/Beta	SEPVLKGVKLLHYY
MZ298839/B.1.351/Beta	SEPVLKGVKLLHYY
MZ913100/B.1.351/Beta	SEPVLKGVKLLHYY
MZ879365/B.1.351/Beta	SEPVLKGVKLLHYY
OU525106/B.1.351/Beta	SEPVLKGVKLLHYY
OU539352/B.1.351/Beta	SEPVLKGVKLLHYY
MZ202314/B.1.351/Beta	SEPVLKGVKLLHYY
MZ298840/B.1.351/Beta	SEPVLKGVKLLHYY
MZ376663/B.1.351/Beta	SEPVLKGVKLLHYY
LC643036/B.1.617.2/Delta	SEPVLKGVKLLHYY
LC643044/B.1.617.2/Delta	SEPVLKGVKLLHYY
MZ853946/B.1.617.2/Delta	SEPVLKGVKLLHYY
MZ854386/B.1.617.2/Delta	SEPVLKGVKLLHYY
MZ888532/B.1.617.2/Delta	SEPVLKGVKLLHYY
MZ888533/B.1.617.2/Delta	SEPVLKGVKLLHYY
MZ896136/B.1.617.2/Delta	SEPVLKGVKLLHYY
OU539261/B.1.617.2/Delta	SEPVLKGVKLLHYY
MZ854389/B.1.617.2/Delta	SEPVLKGVKLLHYY
OU562629/B.1.617.2/Delta	SEPVLKGVKLLHYY
OU539690/B.1.617.2/Delta	SEPVLKGVKLLHYY
MZ854387/B.1.617.2/Delta	SEPVLKGVKLLHYY
OU539784/P.1/Gamma	SEPVLKGVKLLHYY
OU539830/P.1/Gamma	SEPVLKGVKLLHYY
OU539894/P.1/Gamma	SEPVLKGVKLLHYY
MZ477748/P.1/Gamma	SEPVLKGVKLLHYY
MZ477746/P.1/Gamma	SEPVLKGVKLLHYY
MW938104/P.1/Gamma	SEPVLKGVKLLHYY
MZ896179/P.1/Gamma	SEPVLKGVKLLHYY
MZ896359/P.1/Gamma	SEPVLKGVKLLHYY
MZ896669/P.1/Gamma	SEPVLKGVKLLHYY
OU565182/P.1/Gamma	SEPVLKGVKLLHYY
OU492255/P.1/Gamma	SEPVLKGVKLLHYY
MZ911221/B.1.427/Epsilon	SEPVLKGVKLLHYY
MZ375854/B.1.427/Epsilon	SEPVLKGVKLLHYY
MZ375858/B.1.427/Epsilon	SEPVLKGVKLLHYY
MZ943834/B.1.429/Epsilon	SEPVLKGVKLLHYY
MZ277390/B.1.429/Epsilon	SEPVLKGVKLLHYY
OU282717/B.1.429/Epsilon	SEPVLKGVKLLHYY
OU235436/B.1.429/Epsilon	SEPVLKGVKLLHYY
MZ687447/B.1.429/Epsilon	SEPVLKGVKLLHYY
MZ538882/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ724425/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ363839/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ332524/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ332527/B.1.617.1/Kappa	SEPVLKGVKLLHYY
LC633761/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ562746/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ571142/B.1.617.1/Kappa	SEPVLKGVKLLHYY
OU168413/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ026886/B.1.617.1/Kappa	SEPVLKGVKLLHYY
OU172184/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ397173/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ397176/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ397175/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ397177/B.1.617.1/Kappa	SEPVLKGVKLLHYY
OU290035/B.1.617.1/Kappa	SEPVLKGVKLLHYY
OU265178/B.1.617.1/Kappa	SEPVLKGVKLLHYY
OU254949/B.1.617.1/Kappa	SEPVLKGVKLLHYY
OU247018/B.1.617.1/Kappa	SEPVLKGVKLLHYY
OU061269/B.1.617.1/Kappa	SEPVLKGVKLLHYY
OU182966/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ746235/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ487800/B.1.617.1/Kappa	SEPVLKGVKLLHYY
OU322235/B.1.617.1/Kappa	SEPVLKGVKLLHYY
MZ953185/C.37/Lambda	SEPVLKGVKLLHYY
MZ937666/C.37/Lambda	SEPVLKGVKLLHYY
OU356036/C.37/Lambda	SEPVLKGVKLLHYY
OU351967/C.37/Lambda	SEPVLKGVKLLHYY
OU053018/C.37/Lambda	SEPVLKGVKLLHYY
OU268528/C.37/Lambda	SEPVLKGVKLLHYY
OU100999/C.37/Lambda	SEPVLKGVKLLHYY
MZ275295/C.37/Lambda	SEPVLKGVKLLHYY
MZ275301/C.37/Lambda	SEPVLKGVKLLHYY
MZ908711/C.37/Lambda	SEPVLKGVKLLHYY
MZ496613/C.37/Lambda	SEPVLKGVKLLHYY
OU517038/C.37/Lambda	SEPVLKGVKLLHYY
MZ953277/C.37/Lambda	SEPVLKGVKLLHYY
MZ942542/C.37/Lambda	SEPVLKGVKLLHYY
MZ857990/C.37/Lambda	SEPVLKGVKLLHYY



**Supplementary Figure S1.** Multiple alignment of amino acid sequences of spike protein from different variants of concern or variants of interest from different regions, and compared with wild-type SARS-CoV-2 strain isolated from Wuhan, China (accession number MN908947). SARS-CoV-2 genomes used for alignment were download from NCBI GenBank database and its detailed information was listed in Supplementary Table S1. Multiple alignment was performed and visualized using MAFFT version 7 and ESPript 3.0 respectively. The accession number, pangolin lineage and WHO classification are shown on the left side of the sequences. The red boxes represent identical residues while similar residues are indicated by red letters.



**Supplementary Figure S2.** Enhanced detection sensitivity for the combination of different guide RNAs in CRISPR-Cas12a-based detection.  $10^9$  copies/ $\mu$ L of plasmid DNA for SARS-CoV-2 nucleoprotein (NP) and wild-type spike (S) gene were detected by CRISPR-Cas12a-based assay using crRNA-N5, crRNA-S and the mixture of crRNA-N5 and crRNA-S, respectively. The ration of fluorescence signal for SARS-CoV-2 plasmid DNA over no input control was presented at the top of the figure.



**Supplementary Figure S3.** CRISPR-Cas12a-based detection of SARS-CoV-2 NP gene. Viral RNA templates were extracted from SARS-CoV-2 wild-type strain as well as Alpha, Beta and Delta SARS-CoV-2 variants of concern and amplified by RT-PCR followed by CRISPR-Cas12a-mediated detection by using crRNA-N5. The SARS-CoV-2 NP plasmid DNA ( $10^9$  copies/ $\mu\text{L}$ ) was used as positive control. The fluorescence was measured at different time points and presented as the mean  $\pm$  standard deviation (SD) from 3 replicates. No input, negative control with no plasmid DNA.