

Supplementary Material

Supplementary Table S1. Strain number of the selected human coronaviruses (HCoVs).

Virus Species	No. GenBank
HCoV-229E	NC_002645 / KF514432 / KF514430 / KF514433 / JX503061 / JX503060
HCoV-OC43	NC_003045 / AY585229 / AY903459 / JN129834 / KP198610 / KP198611 / KJ958218 / KF530060 / KF530099 / KF530096
HCoV-NL63	NC_005831 / JX524171 / JQ765563 / JQ765569 / JQ765566 / DQ445911 / JX504050 / KF530104 / KF530110
HCoV-HKU1	NC_006577 / AY884001 / DQ415896 / DQ415897 / DQ415902 / HM034837 / KF686340 / KF430201
SARS-CoV	AY278489 / AY772062 / DQ182595 / AY278741 / AY304486 / AY304488 / AY278488
MERS-CoV	JX869059 / KC667074 / KC164505 / KF192507 / KF186567 / KC776174
SARS-CoV-2	MW134174 / MW134155 / MW130867 / LC581375 / LC581368 / LC581364 / MW086898 / MW065480 / MW064953 / MW064875

Supplementary Table S2. Related values of mass probe extension (MPE) amplification.

Target Gene	MPE Sequence	Direction	MPE Mass (Da)	Extension base	Product Sequence	Product Mass (Da)
HCoV-229E-N	CCAACCAGAGATACCACAC-TTCAA	F ¹	7243.8	T	CCAACCAGAGATACCACAC-TTCAAT	7585.8
HCoV-229E-RdRp	CCCCATGTATAACTTACTTA AAGG	F	7295.8	C	CCCCATGTATAACTTACTTAA AGGC	7568.8
HCoV-NL63-N	TACCAGTCGAAGTCACCT	F	5443.6	A	TACCAGTCGAAGTCACCTA	5740.6
HCoV-NL63-RdRp	GAAAGCAATTAGGTTTGGT	F	5906.8	T	GAAAGCAATTAGGTTTGGTT	6248.8
HCoV-OC43-N	ATTCGCTACTT-GGGTCCCGAT	R ²	6388.2	G	ATTCGCTACTT-GGGTCCCGATC	6661.2
HCoV-OC43-RdRp	CGCATGACACATGGTCAG	R	5508.6	G	CGCATGACACATGGTCAGC	5781.6
HCoV-HKU1-N	GGAGAAGTTTTCTTGAG-GATT	R	6531.2	G	GGAGAAGTTTTCTTGAG-GATTTC	6804.2
HCoV-HKU1-RdRp	GGGTGCATAGCAG-GATCTGCTGCATA	R	8051.2	T	GGGTGCATAGCAG-GATCTGCTGCATAA	8348.2
MERS-CoV-N	CCAAAATTAATACCGG-GAATGGA	F	7089.6	A	CCAAAATTAATACCGG-GAATGGAA	7386.6
MERS-CoV-E	CCGGCTACTAGAT-TATGTGTGCAAT	F	7672.0	G	CCGGCTACTAGAT-TATGTGTGCAATG	7985.0
MERS-CoV-RdRp	AGCCAAGCTATCTTAAACA	F	5764.8	C	AGCCAAGCTATCTTAAACAC	6037.8
MERS-CoV-ORF1b	CCCCACAGGGTCATCAACA AT	R	6344.2	T	CCCCACAGGGTCATCAACAA TA	6641.2
SARS-CoV-N	GGGCAGAGACAAAAGAA-GCAGCCC	F	7438.8	A	GGGCAGAGACAAAAGAA-GCAGCCCA	7735.8
SARS-CoV-E	TCTTGTTAACGTGAGTTTA GGGTTTTAA-	F	5823.8	G	TCTTGTTAACGTGAGTTTAG GGGTTTTAA-	6136.8
SARS-CoV-ORF1b	TAACAGAAGGTTTCAGT	F	7760.0	C	TAACAGAAGGTTTCAGTC	8033.0
SARS-CoV2-ORF1b	CCCATCTTAACACAATTAG-TGATTGG	R	7929.2	A	CCCATCTTAACACAATTAG-TGATTGGT	8271.2
SARS-CoV2-N1	CGACGTTGTTTTGATCG	R	5207.4	G	CGACGTTGTTTTGATCGC	5480.4
SARS-CoV2-N2	TCTGGCTGG-CAATGGCGGTGAT	F	6822.4	G	TCTGGCTGG-CAATGGCGGTGATG	7135.4
SARS-CoV2-S	CTCAATTTGGCAGAGACATT	F	6116.0	G	CTCAATTTGGCAGAGACATTG	6429.0

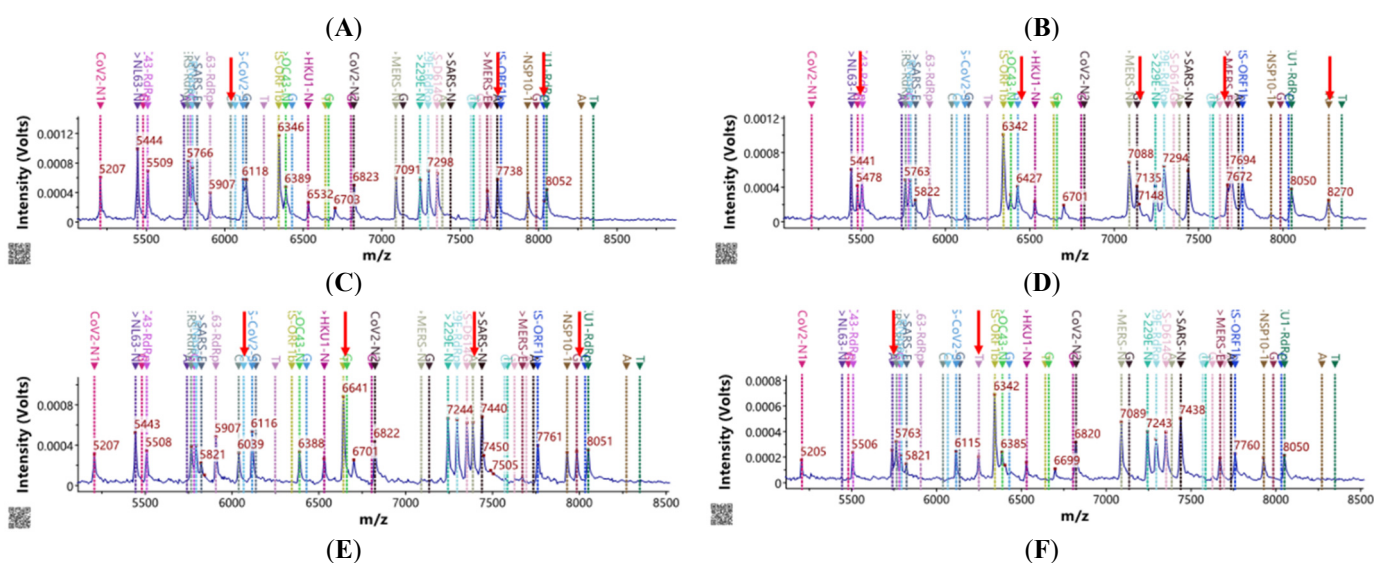
SARS-CoV2-S (D614G)	CCAGGGACTTCTGTGCAG- TTAACA	R	7352.8	A/G	CCAGGGACTTCTGTGCAG- TTAACAT/ CCAGGGACTTCTGTGCAG- TTAACAC	7694.8/ 7625.8
------------------------	-------------------------------	---	--------	-----	---	-------------------

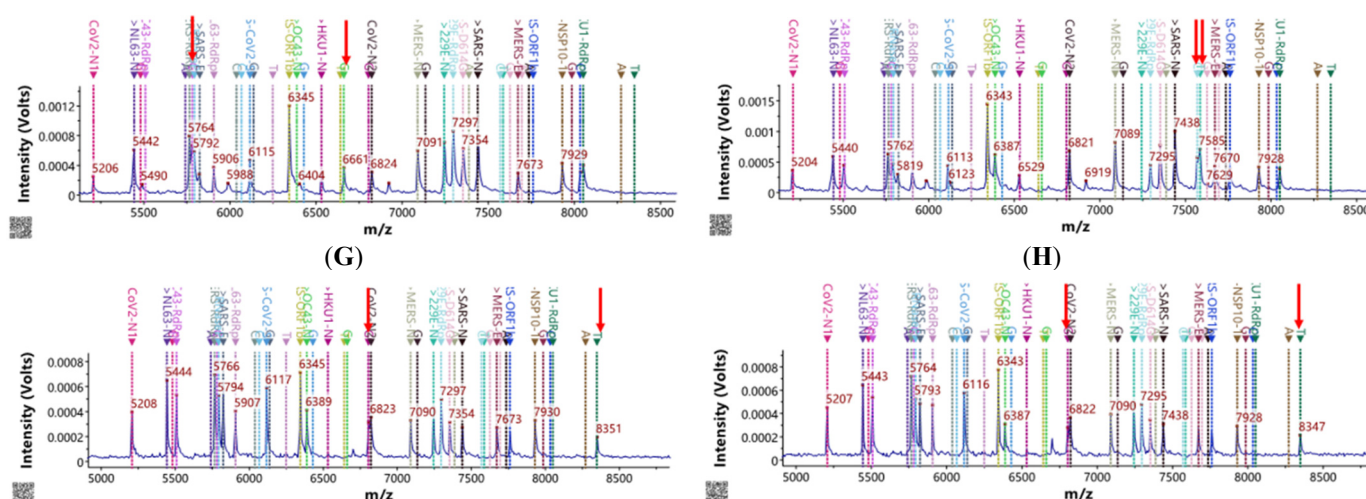
¹. F: Forward. ². R: Reverse.

Supplementary Table S3. Sample Information and Identification Results of United Nations Coronavirus (UN-CoV)-2020.

Sample Set ID	Sample ID	Sample Type	Sample Description	Result
A	RKI-SW-1.1	Human throat swab	possibly contaminated (CoV pos ¹ or neg ²)	CoV pos: <i>SARS-CoV-2</i>
	RKI-SW-1.2	Human throat swab	possibly contaminated (CoV pos or neg)	CoV pos: <i>HCoV-NL63</i>
	RKI-SW-1.3	Human throat swab	Uncontaminated (neg control)	CoV neg
B	RKI-SW-2.1	Human throat swab	possibly contaminated (CoV pos or neg)	CoV pos: <i>SARS-CoV-2</i>
	RKI-SW-2.2	Human throat swab	possibly contaminated (CoV pos or neg)	CoV neg
	RKI-SW-2.2	Human throat swab	Uncontaminated (neg control)	CoV neg
C	RKI-SW-3.1	Livestock throat swab	possibly contaminated (CoV pos or neg)	CoV pos: <i>SARS-CoV-2</i>
	RKI-SW-3.2	Livestock throat swab	possibly contaminated (CoV pos or neg)	CoV pos: <i>SARS-CoV</i>
	RKI-SW-3.3	Livestock throat swab	Uncontaminated (neg control)	CoV neg
D	RKI-SW-4.1	Cell culture supernatant	possibly contaminated (CoV pos or neg)	CoV pos: <i>SARS-CoV-2</i>
	RKI-SW-4.2	Cell culture supernatant	possibly contaminated (CoV pos or neg)	CoV pos: <i>SARS-CoV</i>
	RKI-SW-4.3	Cell culture supernatant	Uncontaminated (neg control)	CoV neg

¹. pos: positive. ². neg: negative.





Supplementary Figure S1. Amplification results of each plasmid target gene when 45 multiplex PCR (mPCR) cycles were used. (A) The amplification results of each target gene of severe acute respiratory syndrome (SARS-CoV), the red arrows represent the *E*, *N*, and *ORF1ab* genes respectively. (B) The amplification results of each target gene of SARS-CoV-2, the red arrows represent the *N1*, *S*, *N2*, *S* (including the D614G mutation site), and *ORF1b* genes, respectively. (C) The amplification results of each target gene of Middle East respiratory syndrome-related coronavirus (MERS-CoV), the red arrows represent the *RdRp*, *ORF1b*, *N*, and *E* genes, respectively. (D) The amplification results of each target gene of human coronavirus (HCoV)-NL63, the red arrows represent the *N* and *RdRp* genes, respectively. (E) The amplification results of each target gene of HCoV-OC43, the red arrows represent the *RdRp* and *N* genes, respectively. (F) The amplification results of each target gene of HCoV-229E, the red arrows represent the *N* and *RdRp* genes, respectively. (G) The amplification results of each target gene of HCoV-HKU1-1, the red arrows represent the *N* and *RdRp* genes, respectively. (H) The amplification results of each target gene of HCoV-HKU1-2, the red arrows represent the *N* and *RdRp* genes, respectively.