Supplementary Table 1: Primers and probes for common SARS-CoV-2 RT-qPCR diagnostic assays.

Institute	Target	Primer/Probe	Sequence	Ref
Charité	E	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	1
		E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	
		E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1	
	RdRp	RdRp_SARSr-F	GTGA**R**ATGGTCATGTGTGGCGG	
		RdRp_SARSr-R	CARATGTTAAA**S**ACACTATTAGCATA	
		RdRp_SARSr-P1	FAM-CCAGGTGG**W**AC**R**TCATC**M**GGTGATGC-BHQ1	
		RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BHQ1	
HKU	N	HKU-N-F	TAATCAGACAAGGAACTGATTA	2
		HKU-N-R	CGAAGGTGTGACTTCCATG	
		HKU-N-P	FAM-GCAAATTGTGCAATTTGCGG-BHQ1	
	nsp14	HKU-ORF1-F	TGGGG**Y**TTTACRGGTAACCT	
		HKU-ORF1-R	AAC**R**CGCTTAACAAAGCACTC	
		HKU-ORF1-P	FAM-TAGTTGTGATGC**W**ATCATGACTAG-BHQ1	
China CDC	N	CCDC-N-F	GGGGAACTTCTCCTGCTAGAAT	3
		CCDC-N-R	CAGACATTTTGCTCTCAAGCTG	
		CCDC-N-P	FAM-TTGCTGCTGCTTGACAGATT-BHQ1	
	nsp10	CCDC-ORF1-F	CCCTGTGGGTTTTACACTTAA	
		CCDC-ORF1-R	ACGATTGTGCATCAGCTGA	
		CCDC-ORF1-P	FAM-CCGTCTGCGGTATGTGGAAAGGTTATGG-BHQ1	
US CDC	N	2019-nCoV_N1-F	GACCCCAAAATCAGCGAAAT	4
		2019-nCoV_N1-R	TCTGGTTACTGCCAGTTGAATCTG	
		2019-nCoV_N1-P	FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1	
	N	2019-nCoV_N2-F	TTACAAACATTGGCCGCAAA	
		2019-nCoV_N2-R	GCGCGACATTCCGAAGAA	
		2019-nCoV_N2-P	FAM-ACAATTTGCCCCCAGCGCTTCAG-BHQ1	
	N	2019-nCoV_N3-F	GGGAGCCTTGAATACACCAAAA	
		2019-nCoV_N3-R	TGTAGCACGATTGCAGCATTG	
		2019-nCoV_N3-P	FAM-A**Y**CACATTGGCACCCGCAATCCTG-BHQ1	
	Human RNase P	RP-F	AGATTTGGACCTGCGAGCG	
		RP-R	GAGCGGCTGTCTCCACAAGT	
		RP-P	FAM-TTCTGACCTGAAGGCTCTGCGCG-BHQ1	

Degenerate nucleotides are shown in bold.

Supplementary Table 2: Primers for generation of T7 RNA transcript standards for SARS-CoV-2.

Target	Primer	Sequence
nsp10	nsp10-Std-T7-F	TAATACGACTCACTATAGGGGTGGGGGACAACCAATCACT
	nsp10-Std-R	AGACGAGGTCTGCCATTGTG
RdRp	RdRp-Std-T7-F	TAATACGACTCACTATAGGGAATAGAGCTCGCACCGTAGC
	RdRp-Std-R	CATCTACAAAACAGCCGGCC
nsp14	nsp14-Std-T7-F	TAATACGACTCACTATAGGGTAGTGCTAAACCACCGCCTG
	nsp14-Std-R	AACTGCCACCATCACAACCA
E	E-Std-T7-F	TAATACGACTCACTATAGGGGCGTGCCTTTGTAAGCACAA
	E-Std-R	GGCAGGTCCTTGATGTCACA
N	N-Std-T7-F	TAATACGACTCACTATAGGGGAATTGTGCGTGGATGAGGC
	N-Std-R	TGTCTCTGCGGTAAGGCTTG

Supplementary Table 3: RNA transcript standards for common SARS-CoV-2 diagnostic assays (see genomic context on Figure 1a).

Gene	Length	Sequence
nsp10	704nt (13,122 - 13,825)	GUGGGGGACAACCAAUCACUAAUUGUGUUAAGAUGUUGUGUACACACACUG GUACUGGUCAGGCAAUAACAGUUACACCGGAAGCCAAUAUGGAUCAAGAAUC CUUUGGUGGUGCAUCGUGUUGUCUGUACUGCCGUUGCCACAUAGAUCAUCC AAAUCCUAAAGGAUUUUGUGACUUAAAAGGUAAGUAUGUACAAAUACCUACA ACUUGUGCUAAUGACCCUGUGGGUUUUACACUUAAAAACACAGUCUGUACCG UCUGCGGUAUGUGGAAAGGUUAUGGCUGUAGUUGUGAUCAACUCCGCGAAC CCAUGCUUCAGUCAGCUGAUGCACAAUCGUUUUUAAACGGGUUUGCGGUGU AAGUGCAGCCCGUCUUACACCGUGCGGCACAGGCACUAGUACUGAUGUCGU AUACAGGGCUUUUGACAUCUACAAUGAUAAAGUAGCUGGUUUUGCUAAAUUC CUAAAAACUAAUUGUUGUCGCUUCCAAGAAAAGGACGAAGAUGACAAUUUAA UUGAUUCUUACUUUGUAGUUAAGAGACACACUUUCUCUAACUACCAACAUGA AGAAACAAUUUAUAAUUUACUUAAGGAUUGUCCAGCUGUUGCUAAACAUGAC UUCUUUAAGUUUAGAAUAGACGGUGACAUGGUACCACAUAUAUCACGUCAAC GUCUUACUAAAUACACAAUGGCAGACCUCGUCU
RdRp	883nt (15,094 - 15,976)	AAUAGAGCUCGCACCGUAGCUGGUGUCUCUAUCUGUAGUACUAUGACCAAU AGACAGUUUCAUCAAAAAUUAUUGAAAUCAAUAGCCGCCACUAGAGGAGCUA CUGUAGUAAUUGGAACAAGCAAAUUCUAUGGUGGUUGGCACAACAUGUUAAA AACUGUUUAUAGUGAUGUAGAAAACCCUCACCUUAUGGGUUGGGAUUAUCC UAAAUGUGAUAGAGCCAUGCCUAACAUGCUUAGAAUUAUGGCCUCACUUGUU CUUGCUCGCAAACAUACAACGUGUUGUAGCUUGUCACACCGUUUCUAUAGAU UAGCUAAUGAGUGUGCUCAAGUAUUGAGUGAAAUGGUCAUGUGUGGCGGUU CACUAUAUGUUAAACCAGGUGGAACCUCAUCAGGAGAUGCCACAACUGCUUA UGCUAAUAGUGUUUUUAACAUUUGUCAAGCUGUCACGGCCAAUGUUAAUGCA CUUUUAUCUACUGAUGGUAACAAAAUUGCCGAUAAGUAUGUCCGCAAUUUAC AACACAGACUUUAUGAGUGUCUCUAUAGAAAUAGAGAUGUUGACACAGACUU UGUGAAUGAGUUUUACGCAUAUUUGCGUAAACAUUUCUCAAUGAUGAUACUC UCUGACGAUGCUGUUGUGUGUUUCAAUAGCACUUAUGCAUCUCAAGGUCUA GUGGCUAGCAUAAAGAACUUUAAGUCAGUUCUUUAUUAUCAAAACAAUGUUU UUAUGUCUGAAGCAAAAUGUUGGACUGAGACUGACCUUACUAAAGGACCUCA UGAAUUUUGCUCUCAACAUACAAUGCUAGUUAAACAGGGUGAUGAUUAUGUG UACCUUCCUUACCCAGAUCCAUCAAGAAUCCUAGGGGCCGGCUGUUUUGUA GAUG
nsp14	848nt (18,447-19,294)	UAGUGCUAAACCACCGCCUGGAGAUCAAUUUAAACACCUCAUACCACUUAUG UACAAAGGACUUCCUUGGAAUGUAGUGCGUAUAAAGAUUGUACAAAUGUUAA GUGACACACUUAAAAAUCUCUCUGACAGAGUCGUAUUUGUCUUAUGGGCACA UGGCUUUGAGUUGACAUCUAUGAAGUAUUUUGUGAAAAUAGGACCUGAGCG CACCUGUUGUCUAUGUGAUAGACGUGCCACAUGCUUUUCCACUGCUUCAGA CACUUAUGCCUGUUGGCAUCAUUCUAUUGGAUUUGAUUACGUCUAUAAUCC GUUUAUGAUUGAUGUUCAACAAUGGGGUUUUACAGGUAACCUACAAAGCAAC CAUGAUCUGUAUUGUCAAGUCCAUGGUAAUGCACAUGUAGCUAGUUGUGAU GCAAUCAUGACUAGGUGUCUAGCUGUCCACGAGUGCUUUGUUAAGCGUGUU GACUGGACUAUUGAAUAUCCUAUAAUUGGUGAUGAACUGAAGAUUAAUGCGG CUUGUAGAAAGGUUCAACACAUGGUUGUUAAAGCUGCAUUAUUAGCAGACAA AUUCCCAGUUCUUCACGACAUUGGUAACCCUAAAGCUAUUAAGUGUGUACCU CAAGCUGAUGUAGAAUGGAAGUUCUAUGAUGCACAGCCUUGUAGUGACAAA GCUUAUAAAAUAGAAGAAUUAUUCUAUUCUUAUGCCACACAUUCUGACAAAU UCACAGAUGGUGUAUGCCUAUUUUGGAAUUGCAAUGUCGAUAGAUAUCCUG CUAAUUCCAUUGUUUGUAGAUUUGACACUAGAGUGCUAUCUAACCUUAACUU GCCUGGUUGUGAUGGUGGCAGUU
Envelope (E)	808nt (26,207 - 27,116)	GCGUGCCUUUGUAAGCACAAGCUGAUGAGUACGAACUUAUGUACUCAUUCG UUUCGGAAGAGACAGGUACGUUAAUAGUUAAUAGCGUACUUCUUUUUCUUG CUUUCGUGGUAUUCUUGCUAGUUACACUAGCCAUCCUUACUGCGCUUCGAU UGUGUGCGUACUGCUGCAAUAUUGUUAACGUGAGUCUUGUAAAACCUUCUU

		UUUACGUUUACUCUCGUGUUAAAAAUCUGAAUUCUUCUAGAGUUCCUGAUCU UCUGGUCUAAACGAACUAAAUAUUAUAUUAGUUUUUCUGUUUGGAACUUUAA UUUUAGCCAUGGCAGAUUCCAACGGUACUAUUACCGUUGAAGAGCUUAAAAA GCUCCUUGAACAAUGGAACCUAGUAAUAGGUUUCCUAUUCCUUACAUGGAUU UGUCUUCUACAAUUUGCCUAUGCCAACAGGAAUAGGUUUUUGUAUAUAAUUA AGUUAAUUUUCCUCUGGCUGUUAUGGCCAGUAACUUUAGCUUGUUUUGUGC UUGCUGCUGUUUACAGAAUAAAUUGGAUCACCGGUGGAAUUGCUAUCGCAA UGGCUUGUCUUGUAGGCUUGAUGUGGCUCAGCUACUUCAUUGCUUCUUUCA GACUGUUUGCGCGUACGCGUUCCAUGUGGUCAUUCAAUCCAGAAACUAACA UUCUUCUCAACGUGCCACUCCAUGGCACUAUUCUGACCAGACCGCUUCUAG AAAGUGAACUCGUAAUCGGAGCUGUGAUCCUUCGUGGACAUCUUCGUAUUG CUGGACACCAUCUAGGACGCUGUGACAUCAAGGACCUGCC
Nucleocapsid (N)	1363nt (28,068 - 29,430)	GAAUUGUGCGUGGAUGAGGCUGGUUCUAAAUCACCCAUUCAGUACAUCGAU AUCGGUAAUUAUACAGUUUCCUGUUUACCUUUUACAAUUAAUUGCCAGGAAC CUAAAUUGGGUAGUCUUGUAGUGCGUUGUUCGUUCUAUGAAGACUUUUUAG AGUAUCAUGACGUUCGUGUUGUUUUAGAUUUCAUCUAAACGAACAAACUAAA AUGUCUGAUAAUGGACCCCAAAAUCAGCGAAAUGCACCCCGCAUUACGUUUG GUGGACCCUCAGAUUCAACUGGCAGUAACCAGAAUGGAGAACGCAGUGGGG CGCGAUCAAAACAACGUCGGCCCCAAGGUUUACCCAAUAAUACUGCGUCUUG GUUCACCGCUCUCACUCAACAUGGCAAGGAAGACCUUAAAUUCCCUCGAGGA CAAGGCGUUCCAAUUAACACCAAUAGCAGUCCAGAUGACCAAAUUGGCUACU ACCGAAGAGCUACCAGACGAAUUCGUGGUGGUGACGGUAAAAUGAAAGAUC UCAGUCCAAGAUGGUAUUUCUACUACCUAGGAACUGGGCCAGAAGCUGGAC UUCCCUAUGGUGCUAACAAAGACGGCAUCAUAUGGGUUGCAACUGAGGGAG CCUUGAAUACACCAAAAGAUCACAUUGGCACCCGCAAUCCUGCUAACAAUGC UGCAAUCGUGCUACAACUUCCUCAAGGAACAACAUUGCCAAAAGGCUUCUAC GCAGAAGGGAGCAGAGGCGGCAGUCAAGCCUCUUCUCGUUCCUCAUCACGU AGUCGCAACAGUUCAAGAAAUUCAACUCCAGGCAGCAGUAGGGGAACUUCUC CUGCUAGAAUGGCUGGCAAUGGCGGUGAUGCUGCUCUUGCUUUGCUGCUG CUUGACAGAUUGAACCAGCUUGAGAGCAAAAUGUCUGGUAAAGGCCAACAAC AACAAGGCCAAACUGUCACUAAGAAAUCUGCUGCUGAGGCUUCUAAGAAGCC UCGGCAAAAACGUACUGCCACUAAAGCAUACAAUGUAACACAAGCUUUCGGC AGACGUGGUCCAGAACAAACCCAAGGAAAUUUUGGGGACCAGGAACUAAUCA GACAAGGAACUGAUUACAAACAUUGGCCGCAAAUUGCACAAUUUGCCCCCAG CGCUUCAGCGUUCUUCGGAAUGUCGCGCAUUGGCAUGGAAGUCACACCUUC GGGAACGUGGUUGACCUACACAGGUGCCAUCAAAUUGGAUGACAAAGAUCC AAAUUUCAAAGAUCAAGUCAUUUUGCUGAAUAAGCAUAUUGACGCAUACAAA ACAUUCCCACCAACAGAGCCUAAAAAGGACAAAAAGAAGAAGGCUGAUGAAA CUCAAGCCUUACCGCAGAGACA

Supplementary Table 4: Degenerate bases in common SARS-CoV-2 RT-qPCR assay primers and probes.

Primer	Degenerate base, and its purpose	Position in primer (5'-3')	Genomic position (5'-3')	Pairing base in genomes (frequency)
RdRp-SARSr-F	R, to pair with T or C	5	15,435	T (992/992; 100.0%)
RdRp-SARSr-R	**S, to pair with C or G**	12	15,519	T (990/992; 99.8%)
RdRp-SARSr-R	R, to pair with T or C	3	15,528	T (992/992; 100.0%)
HKU-ORF1-F	Y, to pair with A or G	6	18,783	A (992/992; 100.0%)
HKU-ORF1-F	R, to pair with T or C	12	18,789	T (989/992; 99.7%)
HKU-ORF1-P	W, to pair with T or A	13	18,861	T (992/992; 100.0%)
HKU-ORF1-R	R, to pair with T or C	4	18,906	T (992/992; 100.0%)
2019-nCoV_N3-P	Y, to pair with A or G	2	28,705	A (992/992; 100.0%)

Supplementary File 1: Research Protocol SARS-COV-2 RT-qPCR

Primer/probe sets Link to CDC assay
- CDC N1 (nucleocapsid)
- CDC N2
- CDC RP (RNase P)

Reagents: NEB Luna® Universal Probe One-Step RT-qPCR Kit

Protocol

1. On ice, prepare a master mix containing the following (account for 10% extra lost during pipetting), *except RNA*:

Component	**Volume in 20 µL reaction**
Luna Universal Probe One-Step Reaction Mix, 2X	10 µL
Luna WarmStart® RT Enzyme Mix (20X)	1 µL
Forward Primer (10 µM)	1 µL
Reverse primer (10 µM)	1 µL
Probe (10 µM)	0.5 µL
Nuclease-free water	1.5 µL
RNA	*5 µL (do not add to master mix)*

2. Add 15 µL of mastermix to each designated well (on ice).
3. Add 5 µL of sample RNA, water (no-template control), or viral RNA (positive control, e.g. 10^3 copies/ µL) to each designated well (on ice).
4. Cover with plate sealer. Centrifuge to remove bubbles, if present.
5. Run the following thermocycler conditions:

Step	Temp	Time
1	55℃	10 min
2	95℃	1 min
3	95℃	10 sec
4	55℃	30 sec
5	Read plate	
Repeat steps 3-5 for **45** cycles.		

Example plate set-up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1	S1	S9	S9	S9	S17	S17	S17	S25	S25	S25
B	S2	S2	S2	S10	S10	S10	S18	S18	S18	S26	S26	S26
C	S3	S3	S3	S11	S11	S11	S19	S19	S19	S27	S27	S27
D	S4	S4	S4	S12	S12	S12	S20	S20	S20	S28	S28	S28
E	S5	S5	S5	S13	S13	S13	S21	S21	S21	NEC	NEC	NEC
F	S6	S6	S6	S14	S14	S14	S22	S22	S22	NTC	NTC	NTC
G	S7	S7	S7	S15	S15	S15	S23	S23	S23	RNA 10^3	RNA 10^3	RNA 10^3
H	S8	S8	S8	S16	S16	S16	S24	S24	S24	RNA 10^3	RNA 10^3	RNA 10^3
	N1	N2	RP	N1	N2	RP	N1	N2	RP	N1	N2	RP

Sx = Sample RNA, NEC = Negative Extraction Control, NTC = Negative Template Control, RNA 10^3 = SARS-CoV-2 RNA at 10^3 GE/uL.

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