



Generation of SARS-COV-2 RNA transcript standards for qRT-PCR detection assays

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1 Works for me dx.doi.org/10.17504/protocols.io.bdv6i69e

Coronavirus Method Development Community



🔔) Nathan Grubaugh 🚱



ABSTRACT

The protocol describes how to generate high-quality single-stranded RNA transcript standards (starting from a virus RNA stock) targeting the nsp10, RdRp, nsp14, envelope (E), and nucleocapsid (N) coding regions for use with China CDC, Hong Kong University (HKU), Corman et al. (Berlin), and US CDC SARS-CoV-2 primer and probe sets for qRT-PCR. (Sequences for transcripts generated, along with their corresponding assays, are provided under 'Guidelines.')

GUIDELINES

RNA transcript sequences

Transcript name	Length (nt)	Genome position	Assays	Sequence
SARS-COV-2 nsp10 RNA	704	13,122 - 13,825	China CDC-ORF1	GUGGGGGACA
				ACCAAUCACUA
				AUUGUGUUAAG
				AUGUUGUGUAC
				ACACACUGGUA
				CUGGUCAGGCA
				AUAACAGUUAC
				ACCGGAAGCCA
				AUAUGGAUCAA
				GAAUCCUUUGG
				UGGUGCAUCGU
				GUUGUCUGUAC
				UGCCGUUGCCA
				CAUAGAUCAUC
				CAAAUCCUAAA
				GGAUUUUGUGA
				CUUAAAAGGUA
				AGUAUGUACAA
				AUACCUACAAC
				UUGUGCUAAUG
				ACCCUGUGGGU
				UUUACACUUAA
				AAACACAGUCU
				GUACCGUCUGC
				GGUAUGUGGAA
				AGGUUAUGGCU
				GUAGUUGUGAU
				CAACUCCGCGA

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				ACCCAUGCUUC
				AGUCAGCUGAU
				GCACAAUCGUU
				UUUAAACGGGU
				UUGCGGUGUAA
				GUGCAGCCCGU
				CUUACACCGUG
				CGGCACAGGCA
				CUAGUACUGAU
				GUCGUAUACAG
				GGCUUUUGACA
				UCUACAAUGAU
				AAAGUAGCUGG
				UUUUGCUAAAU
				UCCUAAAAACU
				AAUUGUUGUCG
				CUUCCAAGAAA
				AGGACGAAGAU
				GACAAUUUAAU
				UGAUUCUUACU
				UUGUAGUUAAG
				AGACACACUUU
				CUCUAACUACC
				AACAUGAAGAA
				ACAAUUUAUAA
				UUUACUUAAGG
				AUUGUCCAGCU
				GUUGCUAAACA
				UGACUUCUUUA
				AGUUUAGAAUA
				GACGGUGACAU
				GGUACCACAUA
				UAUCACGUCAA
				CGUCUUACUAA
				AUACACAAUGG
				CAGACCUCGUC
				U
SARS-COV-2 RdRp RNA	883	15,094 - 15,976	Berlin-RdRp	AAUAGAGCUCG
				CACCGUAGCUG
				GUGUCUCUAUC
				UGUAGUACUAU
				GACCAAUAGAC
				AGUUUCAUCAA
				AAAUUAUUGAA
				AUCAAUAGCCG
				CCACUAGAGGA GCUACUGUAGU
				AAUUGGAACAA
				GCAAAUUCUAU
				GGUGGUUGGC
				ACAACAUGUUA
				AAAACUGUUUA
				UAGUGAUGUAG
				AAAACCCUCAC
				CUUAUGGGUUG
				GGAUUAUCCUA
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AAUGUGAUAGA **GCCAUGCCUAA** CAUGCUUAGAA UUAUGGCCUCA CUUGUUCUUGC UCGCAAACAUA CAACGUGUUGU AGCUUGUCACA CCGUUUCUAUA GAUUAGCUAAU GAGUGUGCUCA AGUAUUGAGUG **AAAUGGUCAUG** UGUGGCGGUUC ACUAUAUGUUA AACCAGGUGGA ACCUCAUCAGG AGAUGCCACAA CUGCUUAUGCU AAUAGUGUUUU UAACAUUUGUC AAGCUGUCACG GCCAAUGUUAA UGCACUUUUAU CUACUGAUGGU AACAAAAUUGC CGAUAAGUAUG UCCGCAAUUUA CAACACAGACU UUAUGAGUGUC UCUAUAGAAAU AGAGAUGUUGA CACAGACUUUG UGAAUGAGUUU UACGCAUAUUU **GCGUAAACAUU** UCUCAAUGAUG AUACUCUCUGA CGAUGCUGUUG UGUGUUUCAAU AGCACUUAUGC AUCUCAAGGUC UAGUGGCUAGC AUAAAGAACUU UAAGUCAGUUC UUUAUUAUCAA AACAAUGUUUU UAUGUCUGAAG CAAAAUGUUGG ACUGAGACUGA CCUUACUAAAG GACCUCAUGAA UUUUGCUCUCA ACAUACAAUGC UAGUUAAACAG

				GGUGAUGAUUA
				UGUGUACCUUC
				CUUACCCAGAU
				CCAUCAAGAAU
				CCUAGGGGCCG
				GCUGUUUUGUA
0400 001/0	0.40	10.447. 10.004	LIKI ODE1	
SARS-COV-2 nsp14 RNA	848	18,447 - 19,294	HKU-ORF1	UAGUGCUAAAC
				CACCGCCUGGA GAUCAAUUUAA
				ACACCUCAUAC CACUUAUGUAC
				AAAGGACUUCC
				UUGGAAUGUAG
				UGCGUAUAAAG
				AUUGUACAAAU
				GUUAAGUGACA
				CACUUAAAAAU
				CUCUCUGACAG
				AGUCGUAUUUG
				UCUUAUGGGCA
				CAUGGCUUUGA
				GUUGACAUCUA
				UGAAGUAUUUU
				GUGAAAAUAGG
				ACCUGAGCGCA
				CCUGUUGUCUA
				UGUGAUAGACG
				UGCCACAUGCU
				UUUCCACUGCU
				UCAGACACUUA
				UGCCUGUUGGC
				AUCAUUCUAUU
				GGAUUUGAUUA
				CGUCUAUAAUC
				CGUUUAUGAUU
				GAUGUUCAACA
				AUGGGGUUUUA
				CAGGUAACCUA
				CAAAGCAACCA
				UGAUCUGUAUU
				GUCAAGUCCAU
				GGUAAUGCACA
				UGUAGCUAGUU
				GUGAUGCAAUC
				AUGACUAGGUG UCUAGCUGUCC
				ACGAGUGCUUU
				GUUAAGCGUGU
				UGACUGGACUA
				UUGAAUAUCCU
				AUAAUUGGUGA
				UGAACUGAAGA
				UUAAUGCGGCU
				UGUAGAAAGGU
				UCAACACAUGG

USULAAAGGU					THICHHAAACCH
### AGACAAAUUCC CAGUUCIUCAC GAGUUCIUCAC GAGUUCIUCAC GACAUUGUUA CCCUAAGCUA UUAAGGUGGUA CCUCAAGCUA GCACAAUGCU GACAAUGCU GACAAUGCU GACAAUUCC GACACAUUCU GACACAUUCU GACACAUUCC GACAAUUCC GAUGCUUAUUG GACAAUUCC GAUGCUUAUUG GACAAUUCC GAUGCUUAUUG GACAAUUCC GAUGCUUAUUG GAAAGACAG GUAUUGUUUC GGAAAGAACAG GUAUUUUUG GAAAGACAG GUAUCCUUACU GGGGUUGGAAU GUUAACCUUACU GGGGUUGGAAU GUUAACCUUACU GGGGUUGGAAU GUUAACCUUACU GGGGUUGGAAU GUUAACCUUACU GGGGUUGGAAA GUUAACCUAACU GGGGUUGGAAAU GUUAACCUAACU GGGGUUGGAAU GUUAACCUACU GGGGUUGGAAA GUUAACCUACU GGGGUUGGAAAU GUUAACCUACU GGGGUUGGAAAU GUUAACCUACU GGGGUUGGAAAU GUUAACCUACU GGGGUUGGAAAU GUUAACCUACU GGGGUUGGAAA CUUUCUUUACCU GGGGUUGGAAA CUUCUUAACCUACU GGGGUUGGAAA CUUCUUAACCUACU GGGGUUGGAAAAU GUUAACCUACU GGGGUUGGAAAAU GUUAACCUACU GGGGUUGGAAAU GUUAACCUACU GGGGUUGGAAAAU GUAAACCUACUACU GGGGUUGGAAAAU GUAACGAACLAA AAAUUACUAAA AAAUUCACUAAA AAAUUCACUAAAA AAAUUACUAAAAAUAAAAAUAAAAAUAAAAAAAA					
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SARS-COV-2 envelope (E) 808 26,207-27,116 Serlin-E GACAGUGGAUAG GACAGAGGA GACAGAGGA GACAGAGGA GACAGAGGA GUULAUAGAGGAGGA GUULAUAGAGAGGA GUULAUAGAAUAGA					
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CUUAUAAAAIA GAAGAAUJAAU CUAUUUAUG GAAGAAUJAAU CUAUUUGGAAGAUJAGU CUAUUAUGGAAGAAUJAGU CAACAAUUCAC GACAAAUUCAC CAGAUGGUAU CUGCUAAUUCC CUGCUAUUUGG AAUUGAAACCUAAUCAC AGAUGGUGGCAGU CUGCUAAUUCC CUGCUAAUUCC CAUUGAUGACACUA GASUGCUACUU GCCUGGUUGUG AUGGUGGCAGU II II CUAUCCUUUC GCAGAAGACAGC UGAUGAGAACCUAAUCAC UCAUCCUUUCG CAACAAGC UGAUGAGAACAG CUAUCCUUUCG GAAGAGAAGA					
### GAAGAAUUAUU CUAUUCUUGUG CCACACAUUCO GACAAAUUCAC AGAUGGUGUAU GCCUAUUUUGG AAUUGCAAUGU CCGAUAGAUAUC CUGGUAAUUC CUGGUAAUUC AAUUGUUGUAG AUUUGAACAUA GAGUGCUAUCU AACCUUAACU AACCUUAACU GCCUGGUUGUG AUGGUGCAGU UAACACAAGC UGAUAACUA GAGUGCUAUCU CGCAACAAGC UGAUAAUC CUAUUCUUUUC GGAAGAACAAG GUACCUUAAU GUUAAUAGCGU AGUUAAUAGGGU AUUUCUUUUC GGAAGAAAAC ACUUAAUAGGGU AGUUAAUAC GGCCUUGAUC GGCCAUCAUAU GUUAACCUAC GGCCUUGAUC GGCCAUAAUU GUUAACCUAC GGCCUUGAUC GGCCAUCCUUACU GGCCAAAAAU GUUAACCUAC GCUGCAAAAUU GUUAACCUAC GCUGCAAAUAU GUUAACCUAC GCUGCAAAUAU GUUAACCUAC GCUGCAAUAUU GUUAACCUCC GCUGCCAUCAU GCUGCCAUCAU GUUACCCUCC AAACCAACUAA AUAUUAUAUAUAUAAUUA AUAUUAUAUAUA					
### CUAUUCUIUAUG GACAAAUUCAU GACAAAUUCAU GACAAAUUCAU GACAAAUUCAU GACAAAUUCAU GACAAAUUCAU GACAAAAUAU GAUUGACAUA GAUGUUUUUG AAUUGACACUA GAUGUUAAUUC AACCUUAACUU AACCUUAACUU AACCUUAACUU AACCUUAACUU AACCUUAACUU AACCUUAGCU UAAGCACAAGC UGAUGAGAGAAGA AAUUAAUUCAUUC UAAGCACAAGC UGAUGAGAGAAGA GUAAGCACAAGC UGAUGAGUAUC GAAGAGACAG GAAGAGACAG GUAGUUUGUUUC GGAAGAGACAG GUAGUUAACU UUGAUUCGUUUC GGAAGAGACAG GUAGUUAAAA GUUAAAAAUC GUAGUUAAUG GUAGUAAAAA GUUAAAAAUC GUUAACCUAC GCGCUUCAAU GUGAAAAAUC GUUAAAAAUC GUUAAAAAUC UUCUUUUUUA GGAAGACUAA AAUAUUAUUAU AGAGACUAA AUAUUAUUAU AAACCACAACAA AUAUUAUUAU AAACACACAUAA AUAUUAUUAU AAACACACAUAA AUAUUAUUAU AAACACACAUAA AUAUUAUUAUUAAAAAUC AAACACACAUAA AUAUUAUUAUUAAAAAUC AAACACACAUAA AUAUUAUUAUUAAAAAUC AAACACACAUAA AUAUUAUUAUUAAAAAAC AAAAUACUUACACACAAC AAACAACAACAAAAAAC AAAAUAAAAAUC AAACAACAACAAAAAAAC AAAAAAAA					
CCACACAUUCU GACAAAUUCA AGAUGUGUAU GCCUAUUUUGG AAUUGCAAUGU CCUGCUAAUUCC CUGCUAAUUCC AUUGUUGUAG AUUGCAACUUA GAUGCUAACUU ACCCUUAACUU ACCCUUAACUU ACCCUUAACUU ACCCUUAGUUGG AUUGUGGCAGU UAAGGCAACAG AUGUGGCAGUUG AUGUGGCAGUUG AUGUGGCAGUUG AUGUGGCAGUUG AACCUUAAUAC CUAAUAGAACAG GUAAGGAACAG GUAAGGAACAG GUAACGUUAAUA GUUAAUAGCU AGGUACAGUA ACUUCUUCUC UGCCUUGUUGG GGAAGAACAG GUAACCUUCUUUUUC CUGCCUUCGU GGCCUUCGAUU GCCACCCUUAG GUAUCCUUCCU GGCGCUUCGAUU GUUAAAAAC CUUCUUUUUAC GGAAGAACG GUAACCGAAUAU GUUAAAAAC CUUCUUUUUAC GGAAGAACAG GUUAACACUG GCGCUUCGAAU GUUAAAAAC CUUCUUUUUAC GAAGUGCGAAU GUUAAAAAC CUUCUUUUUAC GUUAAAAAC CUUCUUUUUAC GUUAAAAAC CUUCUUUUUAC GUUAAAAAC CUUCUUUUUAC GUUAAAAAC CUUCUUUUUAC GUUAAAAAC CUUCUUUUAAAAC CUUCUUUUAAAAAC CUUCUUUUAAAAAC CUUCUUUUAAAAAC CUUCUUUUAAAAAC CUUCUUUUAAAAAC CUUCUUUUAAAAAC CUUCUUUUAAAAAC CUUCUUUUAAAAAC CUUCUUUAAAAAAC CUUCUUAAAAAAC CUUCUUUAAAAAAC CUUCUUUAAAAAC CUUCUUAAAAAC CUUCUUAAAAAAC CUUCUUAAAAAAC CUUCUUAAAAAAC CUUCUUAAAAAAC CUUCUUCUGGAAU AAACGAACUAA AUAUUAUAUAUAUAAAAAAAC AAAGAACUAA AAAAAAAAC AAAGAACUAA AAAAAAAAAA					
AGAUGGUGUAU GCCUJAUJUUGG AAUUGCAAUGU CGAUAGAUJUC CGAUAGAUJUC CAJUGUUGUAG AUUUGUAGACAUA GAGUGCUAUCU AACCUJAACUU GCCUGGUUGUG AUGGGGGGAU II I					
GCCUAUUUUGE AAUUGAAAUGU CGHAAAUUC CUGCUAAUUCC CUGCUAAUUCC AAUUUUAGA AUUUGAACAUA GAGUGCUAUCU AACCUUAACUU GCCUGGUUGG AUGGCAGU II SARS-COV-2 envelope (E) RNA Berlin-E GGGUGCCUUUG UGAAUGAGUAACU UGAUGAGUUC GGAAGAGACAG UGAUGAGUUC GGAAGAGACAG GUAUUUUUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCUUC UUGCUUUUUC UUGCUUUCGUG GUAAUAACACAAC CUUCUUUUUAC GCGCCUUCGAUU GUUAAAAGC UCUUCUUUUUAC GCGCCUUCGAUU GUUAAAAAC UUCUUUUUAAAAC GUUAAAAAUC GUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAGAGUUCCUGA UCUUCGGUCUAAAAAAA AUAUAUAUAUAAAAAAAAAA					GACAAAUUCAC
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AUUUGACACUA GAGUGCUAUCU AACCUUAACUU GCCUGGUUGUG AUGGUGGCAGU II SARS-COV-2 envelope (E) RNA Berlin-E GCGUGCCUUUG GAAGCACAAGC UAAGCACAAGC UAAGCACAAGC UGAUAGUUAC UCAUUCGUUUC GGAAGACACA GUAAUAUGUUC UUGCUUUCGUG GUAAUAUCUGCU UUGCUUCGUG GUAACACACAC GUAACCUAAC CCAUCCUACU GCGCUUCGAUU GUUAACGUAAC CUUCUUUUUAC GUUAACACAAC CUUCUUUUUAC GUUAACACACA CUUCUUUUUAC GUUAACAAAAC CUUCUUUUUAC GUUUAACAAAC CUUCUUUUUAC GUUUAACAAAA CUUCUUUUUAAC GUUUAACAAAA CUUCUUCUUCG UGAAUUCUUCCU AGAAUUCUUCCU AGAAUUCUUCCU AAACGAACUAA AUAUUAUAUAUA					CUGCUAAUUCC
GAGUGCUAUCU AACCUUAACUU GCCUGGUUGUG GCCUGGUUGUG AUGGUGGCAGU II SARS-COV-2 envelope (E) RNA Berlin-E GCGUGCCUUUG GAAGCACAAGC UGAUGAGUACG AACUUAUGUUC GGAAGACAG GUACGUUAUL GUUAAUUAUGUUC GGAAGACAG GUAAUUAUGUUC GGAAGACAG GUAAUUAUGUUC GGAAGACACG GUAAUUAUGUUC GGAAGACACG CCAUCCUUACU GCGCUUCGAUU GUUAACCACAG CCAUCCUUACU GCGCUUCGAUU GUUAACACUAC GCGCUUCGAUU GUUAACACUAC GUUAACUACC GCGCUUCGAUU GUUAACACCAC GUUCUUUUUAC GUUAACACCAC CUUCUUUUUAC GUUAACACCAC GUUCUUUUUAC GUUAACACCAC GUUCUUUUUAC GUUAACACCACACACACACACACACACACACACACACACA					AUUGUUUGUAG
SARS-COV-2 envelope (E) 808 26,207 - 27,116 Berlin-E GCGUGCCUUUG UAAGCACAAGC UGAUGAGUUCG AACUUAUUUUCGUUUCG					AUUUGACACUA
SARS-COV-2 envelope (E) RNA SARS-COV-2 envelope (E) RNA SOB SOB SOB SOB SOB SOB SOB SO					GAGUGCUAUCU
SARS-COV-2 envelope (E) RNA 26,207 - 27,116 Berlin-E GCGUGCCUUUG UAAGCACAAGC UGAUGGUUUC GGAAGAGACAG GUACGUUAUU GGAAGAGACAG GUACGUUAUU ACUUCUUUUC GUAAUAUGUU ACUUCUUUU GUUAACACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUC GGAAGACAG GUACGUUACU GCGCUUCGAU GUGGCGAUAUU GUUAACGGUAC GCGCUUCGAUU GUUAACGUGAG UCUUGUAAAAAC CUUCUUUUUAC GUUAACAUAA UCUUCUGGUCU AAGGUUCCUGA UCUUCUGGUCU AAAGGAACUAA AUAUUAUAUU					AACCUUAACUU
SARS-COV-2 envelope (E) 808 26,207 - 27,116 Berlin-E GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUAC UCAUUCGUUUC GGAAGAACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUUC UUGCUUUCGUG GUAUUCGUUCGUG GUAUUCUUGGU GGAUUACACAAG CCAUCCUUACU GCGCUUCCAUU GUGGCGUACU GCUGCAAUAUU GUUAACACAG GUAACACAAG CCAUCCUUUUUUAC GUUAACACACAG CUUCUUUUUUAC GUUAACAGAG UCUUGUAAAAAC CUUCUUUUUAAC GUUAAAAAAC CUUCUUUUUAAC GUUAAAAAUC UGAAUUCUUCG AGAGUUCCUGA UCUUCUUGGUCU AAACGAACUAA AUAUUAUAAUUA AUAUUAAUAAUUA					GCCUGGUUGUG
SARS-COV-2 envelope (E) RNA 26,207 - 27,116 Berlin-E GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGAUAC GGAAGACAG GUAAGGAGACAG GUAAGUUAUUAU GUUAAUAGCGU AGUUAUUUCUUUUU UUGCUUUCGUG GUAAUACACUAG CCAUCCUUACU GGCGUUCGAUU GUUGAACACAG CCAUCCUUACU GCGCAAUAUU GUUAACGGG GUUAACACACA CCUUCUUUUUC GCUGCAAUAUU GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUAC GUUAACACUAG UCUUGUAAAAAC CUUCUUUUUAC GUUAACGAG UCUUGUAAAAAC CUUCUUUUUAC GUUAACGAG UCUUGUAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUU					AUGGUGGCAGU
RNA UAAGCACAAGC UGAUGAGUACG AACUUAUGUAC UCAUUCGUUUC GGAAGACAAG GUACGUUAAUA GUUAAUAGCGG ACUUCUUUUUC UUGCUUUCGUG GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUUAACGUAC GUUAACGUAC GUUAACGUAC GUUAACAUAC UUCUUUUUAAAAAUC UUCUUUUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUU					710000007100
UGAUGAGUACG AACUUAUGUAC UCAUUCGUUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUCGUG GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUGGGCGUACU GCUGCAAUAUU GUUAACAGAG UCUUGUUUUAC GUUAAACAC CUUCUUUUUAC GUUAAAAAUC UUCUUUUUUAC GUUUACUCGGUCU AAACGAAUAUU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUU	CARC COV 2 anyelene (E)	000	06 207 27 116	Dodin F	П
AACUUAUGUAC UCAUUCGUUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUCGUG GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUGGGCGUACU GCUGCAAUAUU GUUAACGUGAG UCUUGUUUUAC GUUUACUCGGUCU GCUCCAUUUUUAC GUUAAAAAC CUUCUUUUUUAC GUUUACUCGGUCU AGAUUCUUCUAAAAAC CUUCUUUUUUAC		808	26,207 - 27,116	Berlin-E	GCGUGCCUUUG
UCAUUCGUUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUACCUAG CCAUCCUUACU GCGCUUCGAUU GUGUGCGUACU GCUGCAAUAUU GUUAACGUGAG UCUUGUUAAAAAC CUUCUUUUUAC GUUAAAAAAUC UGAAUUCUCG UGAAUUCUCUCA AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAAUAUUA		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC
GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUGGCGUACU GUUAACGUGAG UCUUGUAAAAAC CUUCUUUUUAC GUUUACUCG UGUUAAAAAUC UGAAUUCUCG UGAAUUCUCUCA AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUU		808	26,207 - 27,116	Berlin-E	GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG
GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUUGCAAUAUU GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUAC GUUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUU		808	26,207 - 27,116	Berlin-E	GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUAC
GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUGGCAAUAUU GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUAC GUUUAAAAAC CUUCUUUUUAC GUUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUU		808	26,207 - 27,116	Berlin-E	GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUAC UCAUUCGUUUC
ACUUCUUUUUC UUGCUUUCGUG GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUGACGUACU GCUGCAAUAUU GUUAACGUGAG UCUUGUUUUUAC GUUUACUCUCG UGUUAAAAAC CUUCUUUUUAC GUUUACUCUCG UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUU		808	26,207 - 27,116	Berlin-E	GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUAC UCAUUCGUUUC GGAAGAGACAG
UUGCUUUCGUG GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUUGCGUACU GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUAC GUUUACUCUGG UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA		808	26,207 - 27,116	Berlin-E	GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUAC UCAUUCGUUUC GGAAGAGACAG GUACGUUAAUA
GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUGUGCGUACU GCUGCAAUAUU GUUAACGUGAG UCUUGUAAAAAC CUUCUUUUUAC GUUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAUA		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUAC UCAUUCGUUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU
AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUUGCGAUACU GCUGCAAUAUU GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUAC GUUACUCUCG UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUU		808	26,207 - 27,116	Berlin-E	GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUAC UCAUUCGUUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC
GCGCUUCGAUU GUGUGCGUACU GCUGCAAUAUU GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUAC GUUUACUCUCG UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAUA		808	26,207 - 27,116	Berlin-E	GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUC UCAUUCGUUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG
GUGUGCGUACU GCUGCAAUAUU GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUAC GUUUACUCUCG UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAUA		808	26,207 - 27,116	Berlin-E	GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCUUGCU
GCUGCAAUAUU GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUAC GUUUACUCUCG UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAUA		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCUUGCU AGUUACACUAG
GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUAC GUUUACUCUGG UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUA		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUAC UCAUUCGUUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCCUGCU AGUUACACUAG CCAUCCUUACU
UCUUGUAAAAC CUUCUUUUUAC GUUUACUCUCG UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAUA		808	26,207 - 27,116	Berlin-E	GCGCUUCGAUU GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCGUG GUAUUCCUUGCU AGUUACACUAG CCAUCCUUACU
CUUCUUUUUAC GUUUACUCUCG UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAUA		808	26,207 - 27,116	Berlin-E	GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU
GUUUACUCUCG UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAUA		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUUC GGAAGAGACAG GUACGUUAAUA GUUAUUGUUUC UUGCUUUCGUG GUAUUCGUG GUAUUCUUGCU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUGUGCGUACU GCUGCAAUAUU
UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAUA		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCACUAG CCAUCCUUACU GCGCUUCGAUU GUGUGCGUACU GCUGCAAUAUU GUUAACGUGAG
UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAU		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUAC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUCCUUACU AGUUACACUAG CCAUCCUUACU GCGCUUCGAUU GUGCGAUAUU GUUAACGUGAG UUAACGUGAG UUUAACGUGAG UUUAACGUGAG UCUUGUUACU
AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAU		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCACUAG CCAUCCUUACU GCGCUUCGAUU GUGCGUACU GCUGCAAUAUU GUUAACGUGAG UCUUGUACC GUUCGAUU GUUAACGUGAG UCUUGUACC
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AAACGAACUAA AUAUUAUAUUA		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCCUUACU GCGCUUCGAUU GUGUGCGUACU GCUGCAAUAUU GUUAACGUGAG UCUUGUUACU GCUUCGAUU GUUAACGUGAG UCUUGUACU GCUUCGAUU GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUUAC GUUAACGUGAG UCUUGUAAAAAC CUUCUUUUUAC
AUAUUAUAUA		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUC UUGCUUUCGUG GUAUCCUUACU GCGCCUUCGAUU GUUGCAAUAUU GUUAACGUGAG UCUUGUUUUC GCUGCAAUAUU GUUAACGUGAG UCUUGUUUUAC GUUGUAAAAAC CUUCUUUUUAC GUUGAAUACC GUUGAAUACC GUUGAAAAAC CUUCUUUUUAC GUUAAAAAAC CUUCUUUUUAC GUUAAAAAAUC UGAAUUCUCG UGAAUUCUCU
		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACG AACUUAUGUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCGUGC GUAUCCUUACU GCGCUUCGAUU GUGCAAUAUU GUUAACGUGAG UCUUGUACU GUUGCAAUAUU GUUAACGUGAG UCUUGUACU GUUAACGUGAG UCUUGUUUUUAC GUUAACGUGAG UCUUGUACU GUUAACGUGAG UCUUGUACU GUUAACGUGAG UCUUGUACUCG UGUUAACACC UUCUUUUUUAC GUUAACACC GUUACUCG UGUUAACACC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU
GUUUUUCUGUU		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACC ACUUAUGUUUC GGAAGAGACAG GUACGUUAAUA GUUAUUGUUUC UUGCUUUUUUC UUGCUUUCGUG GUAUUCCUUACU AGUUACACUACU GCGCUUCGAUU GUUGCGUACU GUUAACGUGAG UCUUGUUACU GUUAACGUGAG UCUUGUUACU GUUAACGUGAG UCUUGUUACU GUUAACGUGAG UCUUGUACU GUUCUUUUUAC GUUAACACUGAG UCUUGUAAAAC CUUCUUUUUAC GUUUACUCUG UGUUAAAAAC CUUCUUUUUAC GUUUACUCUGA UCUUCUGGUCU AGAGUUCCUGA
		808	26,207 - 27,116	Berlin-E	II GCGUGCCUUUG UAAGCACAAGC UGAUGAGUACC ACUUAUGUUUC GGAAGAGACAG GUACGUUAAUA GUUAAUAGCGU ACUUCUUUUUC UUGCUUUCGUG GUAUUCCUUACU GCGCUUCGAUU GUUGCAUUCGUUCGU GCUGCAAUAUU GUUAACGUGAG UCUUGUUUUUC GUUGCAUUC GCGCUUCGAUU GUUAACGUGAG UCUUGUACU GUUAACGUGAG UCUUGUACUCG UGUUAACACC UUCUUUUUUAC GUUGCAAUAUU GUUAACGUGAG UCUUGUAAAAC CUUCUUUUUUAC GUUUACUCCG UGUUAAAAAUC UGAAUUCUUCU AGAGUUCCUGA UCUUCUGGUCU AAACGAACUAA AUAUUAUAUAUA

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				GGAAUAGGUUU
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				GCUGCUGUUUA CAGAAUAAAUU
				GGAUCACCGGU
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				CGCAAUGGCUU
				GUCUUGUAGGC
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				UUGCUUCUUUC
				AGACUGUUUGC
				GCGUACGCGUU
				CCAUGUGGUCA
				UUCAAUCCAGA
				AACUAACAUUC
				UUCUCAACGUG
				CCACUCCAUGG
				CACUAUUCUGA
				CCAGACCGCUU
				CUAGAAAGUGA
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SARS-COV-2	1363	28,068 - 29,430	China CDC-N; HKU-N; US	GAAUGUGCGU
nucleocapsid (N) RNA			CDC-N1; US CDC-N2; US	GGAUGAGGCU
			CDC-N3	GGUUCUAAAUC
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AUUAAUUGCCA **GGAACCUAAAU** UGGGUAGUCUU GUAGUGCGUUG UUCGUUCUAUG AAGACUUUUUA GAGUAUCAUGA CGUUCGUGUUG UUUUAGAUUUC AUCUAAACGAA CAAACUAAAAU GUCUGAUAAUG GACCCCAAAAU CAGCGAAAUGC ACCCCGCAUUA CGUUUGGUGGA CCCUCAGAUUC AACUGGCAGUA ACCAGAAUGGA GAACGCAGUGG GGCGCGAUCAA AACAACGUCGG CCCCAAGGUUU ACCCAAUAAUA CUGCGUCUUGG UUCACCGCUCU CACUCAACAUG GCAAGGAAGAC CUUAAAUUCCC UCGAGGACAAG **GCGUUCCAAUU** AACACCAAUAG CAGUCCAGAUG ACCAAAUUGGC UACUACCGAAG AGCUACCAGAC GAAUUCGUGGU GGUGACGGUAA AAUGAAAGAUC UCAGUCCAAGA UGGUAUUUCUA CUACCUAGGAA CUGGGCCAGAA GCUGGACUUCC CUAUGGUGCUA ACAAAGACGGC AUCAUAUGGGU UGCAACUGAGG GAGCCUUGAAU ACACCAAAAGA UCACAUUGGCA CCCGCAAUCCU GCUAACAAUGC UGCAAUCGUGC UACAACUUCCU CAAGGAACAAC

AUUGCCAAAAG
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CAAGCUUUCGG CAGAACAAACC CAAGGAAAUUU UGGGGACCAG GAACUAAUCAG ACAAGGAACUG AUUACAAACAU UGGCCGCAAAU UGCACAAUUUG
CAAGCUUUCGG CAGAACGUGGUC CAGAACAAACC CAAGGAAAUUU UGGGGACCAG GAACUAAUCAG ACAAGGAACUG AUUACAAACAU UGGCCGCAAAU UGCACAAUUUG CCCCCAGCGCU
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CAAGCUUUCGG CAGACGUGGUC CAGAACAAACC CAAGGAAAUUU UGGGGACCAG GAACUAAUCAG ACAAGGAACUG AUUACAAACAU UGGCCGCAAAU UGCACAAUUUG CCCCCAGCGCU UCAGCGUUCUU CGGAAUGUCGC GCAUUGGCAUG GAAGUCACACC UUCGGGAACGU GGUUGACCUAC ACAGGUGCCAU CAAAUUGGAUG ACAAAGAUCCA
CAAGCUUUCGG CAGACGUGGUC CAGAACAAACC CAAGGAAAUUU UGGGGACCAG GAACUAAUCAG ACAAGGAACUG AUUACAAACAU UGGCCGCAAAU UGCACAAUUUG CCCCCAGCGCU UCAGCGUUCUU CGGAAUGUCGC GCAUUGGCAUG GAAGUCACACC UUCGGGAACGU GGUUGACCUAC ACAGGUGCCAU CAAAUUGGAUG ACAAAGAUCCA AAUUUCAAAGA
CAAGCUUUCGG CAGACGUGGUC CAGAACAAACC CAAGGAAAUUU UGGGGACCAG GAACUAAUCAG ACAAGGAACUG AUUACAAACAU UGGCCGCAAAU UGCACAAUUUG CCCCAGCGCU UCAGCGUUCUU CGGAAUGUCGC GCAUUGGCAUG GAAGUCACACC UUCGGGAACGU GGUUGACCUAC ACAGGUGCCAU CAAAUUGGAUG ACAAAGAUCCA AAUUUCAAAGA UCAAGUCAUUU
CAAGCUUUCGG CAGACGUGGUC CAGAACAAACC CAAGGAAAUUU UGGGGACCAG GAACUAAUCAG ACAAGGAACUG AUUACAAACAU UGGCCGCAAAU UGCACAAUUUG CCCCAGCGCU UCAGCGUUCUU CGGAAUGUCGC GCAUUGGCAUCG GAAGUCACACC UUCGGGAACGU GGUUGACCUAC ACAGGUGCCAU CAAAUUGGAUG ACAAAGAUCCA AAUUUCAAAGA UCAAAGUCAUUU UGCUGAAUAAG
CAAGCUUUCGG CAGACGUGGUC CAGAACAAACC CAAGGAAAUUU UGGGGACCAG GAACUAAUCAG ACAAGGAACUG AUUACAAACAU UGGCCGCAAAU UGCACAAUUUG CCCCCAGCGCU UCAGCGUUCUU CGGAAUGUCGC GCAUUGGCAUG GAAGUCACACC UUCGGGAACGU GGUUGACCAU ACAGGUGCCAU CAAAUUGGAUG ACAAGUCACAC AAUUUCAAAGA UCAAGUCAUUU UGCUGAAUAAG CAUAUUGACGC

		GGACAAAAAGA
		AGAAGGCUGAU
		GAAACUCAAGC
		CUUACCGCAGA
		GACA

RNA transcripts generated by this protocol, along with their length (in nucleotides), their position on the SARS-COV-2 reference genome, and the assays they serve as positive controls for (since all assays are multiplexed, the target is specified).

MATERIALS

NAME ~	CATALOG #	VENDOR >
Q5 High-Fidelity 2X Master Mix - 100 rxns	M0492S	New England Biolabs
Agilent High Sensitivity DNA Kit	5067-4626	Agilent Technologies
Bioanalyzer RNA 6000 Nano Kit	5067	Agilent Technologies
SuperScript™ IV VILO™ Master Mix	11756500	Thermo Fisher
MEGAscript™ T7 Transcription Kit	AM1334	Thermo Fisher
Qubit™ dsDNA HS Assay Kit	Q32851	Thermo Fisher
Qubit™ RNA HS Assay Kit	Q32852	Thermo Fisher
Mag-Bind® TotalPure NGS	M1378-01	Omega Biotek
STEPS MATERIALS		
STEPS MATERIALS NAME	CATALOG # ×	VENDOR ~
	CATALOG # \(\times \) 11756500	VENDOR V
NAME ~		
NAME SuperScript™ IV VILO™ Master Mix	11756500	Thermo Fisher
NAME SuperScript™ IV VILO™ Master Mix Q5 High-Fidelity 2X Master Mix - 100 rxns	11756500 M0492S	Thermo Fisher New England Biolabs
NAME SuperScript™ IV VILO™ Master Mix Q5 High-Fidelity 2X Master Mix - 100 rxns Mag-Bind® TotalPure NGS	11756500 M0492S M1378-01	Thermo Fisher New England Biolabs Omega Biotek
SuperScript™ IV VILO™ Master Mix Q5 High-Fidelity 2X Master Mix - 100 rxns Mag-Bind® TotalPure NGS Qubit™ dsDNA HS Assay Kit	11756500 M0492S M1378-01 Q32851	Thermo Fisher New England Biolabs Omega Biotek Thermo Fisher
SuperScript™ IV VILO™ Master Mix Q5 High-Fidelity 2X Master Mix - 100 rxns Mag-Bind® TotalPure NGS Qubit™ dsDNA HS Assay Kit Agilent High Sensitivity DNA Kit	11756500 M0492S M1378-01 Q32851 5067-4626	Thermo Fisher New England Biolabs Omega Biotek Thermo Fisher Agilent Technologies

SAFETY WARNINGS

Please be aware that full length single-stranded positive sense SARS-CoV-2 RNA is considered infectious and must be handled in BSL-2 or above conditions. Please consult with your institutional biosafety team before handling SARS-CoV-2 RNA.

BEFORE STARTING

Basic outline of steps: cDNA synthesis > PCR > Purification > Quality + Quantity check > T7 RNA transcription > Purification > Quality + Quantity check

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Preparation of cDNA

- Isolate viral RNA using Omega Viral DNA/RNA kit, Trizol, or equivalent.
- Many different cDNA synthesis kits can be used, but choose something that is relatively high-fidelity. The current protocol uses SuperScript IV VILO Master Mix because the enzyme has low error rates and the protocol is fast and easy.



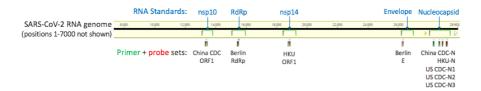
Component	Volume in 20 μL reaction
SSIV VILO Master Mix	4 μL
Nuclease-free water	11 μL
Virus RNA	5 µL

Run the following cycles on a thermocycler:

Temperature	Time
25°C	10 minutes
50°C	10 minutes
85°C	5 minutes
4°C	∞

Store samples at § 4 °C (same day) or § -20 °C (up to a week) until ready for PCR.

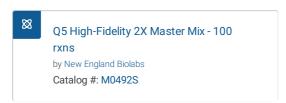
Prepare the following primers at a concentration of 10 μM, depending on which assay is needed. The nucleocapsid (N) transcript is for the US CDC-N1, N2, and N3 (N3 is not currently in use), HKU-N, and China CDC-N assays. The envelope (E) transcript is for use with the Berlin-E assay, nsp14 transcript is for use with the HKU-ORF1 assay, the RdRp transcript is for use with the Berlin RdRp assay (low sensitivity), and the nsp10 transcript is for use with the China CDC-ORF1 assay. See here for assay primer sequences and references.



Target	Primer	Sequence	Position	Amplicon size
N	N-Std-T7-Fwd	TAATACGACTCACTAT AGGGGAATTGTGCGT GGATGAGGC	28068	1363
	N-Std-Rev	TGTCTCTGCGGTAAG GCTTG	29411	
E	E-Std-T7-Fwd	TAATACGACTCACTAT AGGGGCGTGCCTTTG TAAGCACAA	26207	808
	E-Std-Rev	GGCAGGTCCTTGATG TCACA	27016	
nsp14	nsp14-Std-T7-Fwd	TAATACGACTCACTAT AGGGTAGTGCTAAAC CACCGCCTG	18447	848
	nsp14-Std-Rev	AACTGCCACCATCACA ACCA	19275	
RdRp	RdRp-Std-T7-Fwd	TAATACGACTCACTAT AGGGAATAGAGCTCG CACCGTAGC	15094	883
	RdRp-Std-Rev	CATCTACAAAACAGCC GGCC	15957	
nsp10	nsp10-Std-T7-Fwd	TAATACGACTCACTAT AGGGGTGGGGGACAA CCAATCACT	13122	704
	nsp10-Std-Rev	AGACGAGGTCTGCCA TTGTG	13806	

Primers for PCR amplification. Note that forward primers include a T7 promoter sequence.

6 Prepare the following PCR reaction for each sample:



Component	Volume in 25 μL reaction
Q5 2x Master Mix	12.5 µL
10 μM Primer F-T7	1.25 µL
10 μM Primer R	1.25 µL
Nuclease-free water	8 μL
cDNA	2 μL

7 Run the following cycles on a thermocycler:

Temperature	Time	
98°C	30 seconds	
98°C	10 seconds	
55°C	30 seconds	
72°C	90 seconds	
Repeat steps 2-4 for a total of 35 cycles		
72°C	2 minutes	
4°C	∞	

Post-PCR cleanup

8 Allow Mag-Bind TotalPure NGS beads to equilibrate to room temperature, vortex until homogenous.



9 Bring PCR product volume up to $25 \,\mu L$ with water (if not at volume already).

10 Add 20 µL of beads to 25 µL of PCR product, mix well, and incubate at room temperature for 10 minutes.

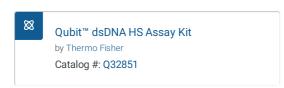


If your lab has a KingFisher extractor to use for post-PCR cleanup steps, our automated protocols are available here: https://github.com/grubaughlab/Kingfisher_protocols (use 'purification.bdz' for this cleanup)

- 11 Place tubes on a magnetic stand and incubate until solution appears clear.
- 12 Discard supernatant without disturbing the beads.
- 13 While tubes are on the magnet, add 200 µL of 80% ethanol, incubate for 30 seconds, and discard the ethanol wash.
- 14 Repeat previous 80% ethanol wash and remove as much ethanol as possible.
- 15 Leave tubes on magnet and air dry for 5 minutes.
- $\,$ 16 $\,$ Remove tubes from magnet and add 20 μL of nuclease-free water. Mix well by pipetting.
- 17 Place tubes on magnet stand. When solution appears clear, remove supernatant without disturbing the beads and place into new tubes.

Intermediate quantification

18 Quantify the DNA concentration using the Qubit High Sensitivity DNA kit (or equivalent) from 1 μL of each product, following manufacturer's protocol.



19 Check DNA fragment distributions of the samples using the BioAnalyzer DNA 1000 kit, following manufacturer's protocol.



Reverse Transcription (Megascript T7 kit)

20 Important: thaw reagents on ice, but keep the 10X reaction buffer at room temperature while assembling the reaction. Add the 10X reaction buffer after water and NTPs have been added to the tube.



- Mix equal volumes ($2 \mu L$ /sample) of the four ribonucleotide solutions together and add 8 μL of the mixture for each sample.
- 22 Use 2 µL of pTRI-Xef in a standard reaction as a transcription control. This should yield a transcript of 1.89 kb.

Component	Volume in 20 µL reaction
Nuclease-free water	Add to 20 µL
NTP mixture (ATP + CTP + GTP + UTP)	8 µL
10X reaction buffer	2 μL
Purified PCR product	0.1-0.2 μg PCR product
Enzyme mix	2 μL

- 23 Mix by pipetting up and down and microfuge briefly to collect reaction mixture at the bottom of the tube.
- 24 Incubate at § 37 °C for 4 hours.
- 25 Add 1 μ L of TURBO DNase, mix well and incubate $\, \uplambda$ 37 °C for 15 minutes .

RNA product purification

26 Add the following components to a 1.5 mL tube.

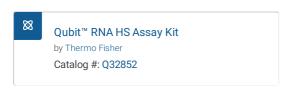
Component	Volume in 81 µL reaction
Nuclease-free water	30 μL
Lithium Chloride	30 μL
T7 transcription product	21 μL

- 27 Mix and chill at & -20 °C for >30 minutes.
- 28 Centrifuge at § 4 °C for 15 minutes at maximum speed to pellet the RNA.

- Carefully remove supernatant. Wash pellet with 1 mL of 70% ethanol and re-centrifuge at 8 4 °C for 15 minutes at maximum speed to maximize removal of unincorporated nucleotides.
- 30 Carefully remove the ethanol and resuspend in 20 µL of nuclease-free water.

Final quantification

Quantify the RNA concentration using the Qubit High Sensitivity RNA kit from 1 μL of each product, following manufacturer's protocol.



32 Check RNA fragment distributions of the samples using the BioAnalyzer RNA 6000 pico kit, following manufacturer's protocol.



33 Use the following RNA copy number calculator to calculate genome equivalents per uL and prepare standards.

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