

Supplementary figures, 2019 novel coronavirus RT-PCR development

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E_Sarbeco_assay

WH-Human_1 [China] 2019-Dec
BetaCoV/Wuhan/IPBCAMS-WH-01/2019 [EPI_ISL_402123]
BetaCoV/Wuhan/IVDC-HB-01/2019 [EPI_ISL_402119]
BetaCoV/Wuhan/IVDC-HB-04/2020 [EPI_ISL_402120]
BetaCoV/Wuhan/IVDC-HB-05/2019 [EPI_ISL_402121]
BetaCoV/Wuhan/WIV04/2019 [EPI_ISL_402124]
NC_004718 (SARS coronavirus, complete genome)
EU371564 (SARS coronavirus BJ182-12, complete genome)
AY559095 (SARS coronavirus Sin847, complete genome)
FJ882956 (SARS coronavirus ExoN1 isolate P3pp5, complete genome)
FJ882960 (SARS coronavirus ExoN1 isolate P3pp34, complete genome)
FJ882961 (SARS coronavirus MA15 isolate P3pp5, complete genome)
GQ153542 (Bat SARS coronavirus HKU3-9, complete genome)
KY552407 (Severe acute respiratory syndrome-related coronavirus strain BKY72, comp...
NC_014470 (Bat coronavirus BM48-31/BGR/2008, complete genome)

ACAGGTACGTTAATAGTTAATAGCGT--ACACAGCCATCCTTACTGCGCTTCG--TGTGTCGCTACTGCTGCAATAT
E_Sarbeco_E E_Sarbeco_P E_Sarbeco_R

Sequence alignment for E_Sarbeco assay showing gaps and matches for various SARS-related CoV sequences.

RdRp_SARS_P2

RdRp_SARS_Oligo

WH-Human_1 [China] 2019-Dec
BetaCoV/Wuhan/IPBCAMS-WH-01/2019 [EPI_ISL_402123]
BetaCoV/Wuhan/IVDC-HB-01/2019 [EPI_ISL_402119]
BetaCoV/Wuhan/IVDC-HB-04/2020 [EPI_ISL_402120]
BetaCoV/Wuhan/IVDC-HB-05/2019 [EPI_ISL_402121]
BetaCoV/Wuhan/WIV04/2019 [EPI_ISL_402124]
NC_004718 (SARS coronavirus, complete genome)
DQ322305 (Bat SARS coronavirus HKU3-1, complete genome)
AB585995 (SARS bat coronavirus Rf10r gene for RNA-dependent RNA polymerase, p...
AB585996 (SARS bat coronavirus RdRp gene for RNA-dependent RNA polymerase, p...
DQ412043 (Bat SARS coronavirus Rm1, complete genome)
JN93887 (Bat coronavirus Rp/Shaanxi2011, complete genome)
KF294442 (SARS-related bat coronavirus isolate Langshan-4 RNA-dependent RNA pol...
KF294451 (SARS-related bat coronavirus isolate Arlong-11 orf1ab polyprotein end...
KF794456 (SARS-related bat coronavirus isolate Jiyuan-331 orf1ab polyprotein gene...
KJ473811 (Bt-BetaCoV/YN2013, complete genome)
KJ473813 (Bt-BetaCoV/SJ2012, complete genome)
KJ473814 (Bt-BetaCoV/HuB2013, complete genome)
KJ972696 (SARS-related coronavirus isolate T29 RdRp mRNA, partial cds)
MG772848 (Bat SARS-like coronavirus isolate bat-SL-CoVUKC6 RNA-dependent RNA...
MG772849 (Bat SARS-like coronavirus isolate bat-SL-CoVUKC6 RNA-dependent RNA...
MG772852 (Bat SARS-like coronavirus isolate bat-SL-CoVZ2_113 RNA-dependent RNA...
MG772857 (Bat SARS-like coronavirus isolate bat-SL-CoVZ2_39 RNA-dependent RNA...
MG772870 (Bat SARS-like coronavirus isolate bat-SL-CoVZ2_76 RNA-dependent RNA...
MG772879 (Bat SARS-like coronavirus isolate bat-SL-CoVZ2_46 RNA-dependent RNA...
MG772886 (Bat SARS-like coronavirus isolate bat-SL-CoVZ2_3 RNA-dependent RNA p...
MG772891 (Bat SARS-like coronavirus isolate bat-SL-CoVZ2_1 RNA-dependent RNA p...
MG772892 (Bat SARS-like coronavirus isolate bat-SL-CoVZ2_34 RNA-dependent RNA...
MG772933 (Bat SARS-like coronavirus isolate bat-SL-CoVZC45, complete genome)
MG772934 (Bat SARS-like coronavirus isolate bat-SL-CoVZC21, complete genome)
KY552407 (Severe acute respiratory syndrome-related coronavirus strain BKY72, co...
NC_014470 (Bat coronavirus BM48-31/BGR/2008, complete genome)
KC533199 (Betacoronavirus BtCoV/ShiJie/11/17/17AZ/09 RNA-dependent RNA polyme...
KC533202 (Betacoronavirus BtCoV/ShiJie/11/17/17AZ/09 RNA-dependent RNA polyme...
KC533203 (Betacoronavirus BtCoV/ShiJie/11/17/17AZ/09 RNA-dependent RNA polyme...
KC533208 (Betacoronavirus BtCoV/ShiJie/11/17/17AZ/09 RNA-dependent RNA polyme...
KC533211 (Betacoronavirus BtCoV/ShiJie/11/17/17AZ/09 RNA-dependent RNA polyme...
KC533213 (Betacoronavirus BtCoV/ShiJie/11/17/17AZ/09 RNA-dependent RNA polyme...
KC533220 (Betacoronavirus BtCoV/ShiJie/11/17/17AZ/09 RNA-dependent RNA polyme...)

RdRp_SARS_P2
GTGARATGGTCATGCTGTGGCG--CCAGGTGGWACRTGATCRGGTGATGC--TATGCTAATAGTGTATTTAAACATYTG
RdRp_SARSE E RdRp_SARSP1 RdRp_SARSER

Sequence alignment for RdRp_SARS_P2 assay showing gaps and matches for various SARS-related CoV sequences.

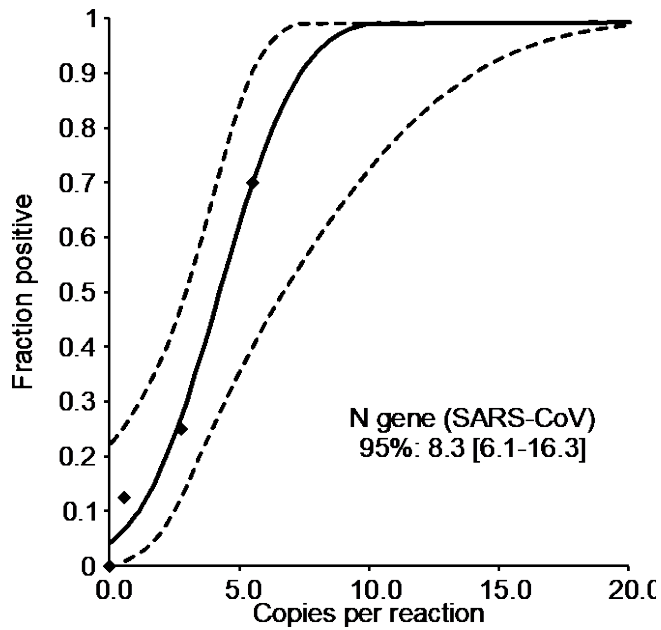
N_Sarbeco_Oligos

WH-Human_1 [China] 2019-Dec
BetaCoV/Wuhan/IPBCAMS-WH-01/2019 [EPI_ISL_402123]
BetaCoV/Wuhan/IVDC-HB-01/2019 [EPI_ISL_402119]
BetaCoV/Wuhan/IVDC-HB-04/2020 [EPI_ISL_402120]
BetaCoV/Wuhan/IVDC-HB-05/2019 [EPI_ISL_402121]
BetaCoV/Wuhan/WIV04/2019 [EPI_ISL_402124]
NC_004718 (SARS coronavirus, complete genome)
DQ022305 (Bat SARS coronavirus HKU3-1, complete genome)
DQ412042 (Bat SARS coronavirus Rf1, complete genome)
EU371564 (SARS coronavirus BJ182-12, complete genome)
GQ153542 (Bat SARS coronavirus HKU3-9, complete genome)
JX162087 (SARS coronavirus ExoN1 isolate cSP10, complete genome)
JX93987 (Bat coronavirus Rp/Shaanxi2011, complete genome)
KC881005 (Bat SARS-like coronavirus R5HC014, complete genome)
KF294457 (SARS-related bat coronavirus isolate Longquan-140 orf1ab polyprotein, s...
KJ473814 (BtRS-BetaCoV/HuB2013, complete genome)
KJ473815 (BtRS-BetaCoV/GX2013, complete genome)
KJ473816 (BtRS-BetaCoV/YN2013, complete genome)
KP886808 (Bat SARS-like coronavirus YNLF_31C, complete genome)
KY417143 (Bat SARS-like coronavirus isolate Rs4081, complete genome)
KY417144 (Bat SARS-like coronavirus isolate Rs4084, complete genome)
KY417146 (Bat SARS-like coronavirus isolate Rs4231, complete genome)
MG772933 (Bat SARS-like coronavirus isolate bat-SL-CoVZC45, complete genome)
KY552407 (Severe acute respiratory syndrome-related coronavirus strain BKY72, co...
NC_014470 (Bat coronavirus BM48-31/BGR/2008, complete genome)

CACATTGGCACCCGCAATC--ACTTCCTCAAGGAACAACATTGCCA--CAAGCCTCTTCGTTCTCC
N_Sarbeco_E N_Sarbeco_P N_Sarbeco_R

Sequence alignment for N_Sarbeco_Oligos assay showing gaps and matches for various SARS-related CoV sequences.

Supplementary Figure 1. Non-redundant alignments of SARS-related CoVs focused on oligonucleotide binding sites of all assays (top to bottom: RdRp, E, N). Viruses not present in these alignments have been removed because their binding sites are 100% identical to one of the members of the alignment. ("--") means sequence gaps not covered by oligonucleotides.



Supplementary Figure 2: Additional confirmatory assay: N gene. Limit of detection study for N gene assay. Technical LOD = 8.3 RNA copies/reaction, at 95% hit rate; 95% CI: 6.1-16.3 RNA copies/reaction. Y-axis shows positive results in all parallel reactions performed, squares are experimental data points resulting from replicate testing of given concentrations (x-axis) in parallel assays (8 replicate reactions per datum point). The inner line is a probit curve (dose-response rule). The outer dotted lines are 95% confidence intervals.