



Supplemental figure 1. Limit of detection (LoD) from N1 and N2 targets using commercial plasmids. Commercial plasmids provided by Integrated DNA Technologies (IDT) contained nucleocapsid phosphoprotein gene of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were used to determinates the LoD of N1 and N2 regions targets by primers sets and probes designed by United States' Centers for Disease Control and Prevention. All samples' Ct were determined using the same threshold value acquired from the standard curves. left panels show the linear regression and right panels show the amplification plots of plasmids dilutions detected by (A) N1 and (B) N2 primer/probe sets. (C) shows pairwise analyses of N1 and N2 Ct values of samples with 5 GC/ μ L of N gene. GC: genome copies.

A

>NC_045512.2:28274-29533 severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, Nucleocapsid Phosphoprotein gene
 ATGTCTGATAATGGACCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCCTCAGATTCAA
 CTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGATCAAAACAACGTCGGCCCCAAGGTTTACCCAA
 TAATACTGCGTCTTGGTTCACCGCTCTCACTCAACATGGCAAGGAAGACCTTAAATTCCTTCGAGGACAA
 GCGGTTCCAATTAACACCAATAGCAGTCCAGATGACCAAATTTGGCTACTACCGAAGAGCTACCAGACGAA
 TTCGTGGTGGTGACGGTAAAATGAAAGATCTCAGTCCAAGATGGTATTTCTACTACCTAGGAACCTGGGCC
 AGAAGCTGGACTTCCCTATGGTGTAAACAAAGACGGCATCATATGGGTTGCAACTGAGGGAGCCTTGAAT
 ACACCAAAAAGATCACATTTGGCACCCGCAATCCTGCTAACAAATGCTGCAATCGTGTACAACCTTCTCAAG
 GAACAACATTTGCCAAAAGGCTTCTACGCAGAAAGGAGCAGAGGGCGGCAGTCAAGCCTTCTCTCGTTCCCTC
 ATCACGTAGTCGCAACAGTTCAAGAAATTTCAACTCCAGGCAGCAGTAGGGGAACCTTCTCCTGCTAGAATG
 GCTGGCAATGGCGGTGATGCTGCTCTTGTCTTGTGCTGCTTGGACAGATTGAACCAGCTTGAGAGCAAAA
 TGCTTGGTAAAGGCCAACAAACAAGGCCAAACTGTCACATAAGAAATCTGCTGCTGAGGCTTCTAAGAA
 GCCTCGGCAAAAACGTACTGCCACTAAAAGCATAACAATGTAACACAAGCTTTCGGCAGACGTGGTCCAGAA
 CAAACCCAAGGAAAATTTTGGGGACCAGGAACTAATCAGACAAGGAAGTATTACAAACATTTGGCCGCAAA
 TTGCACAATTTGCCCCAGCGCTTTCAGCGTTCTTTCGGAAATGTCGCGCATTTGGCATGGAAGTACACCTTC
 GGAACGTGGTTGACCTACACAGGTGCCATCAAAATTTGGATGACAAAGATCCAAATTTCAAAGATCAAGTC
 ATTTTGTGAATAAGCATATTGACGCATACAAAACATTTCCACCAACAGAGCCTAAAAAGGACAAAAAGA
 AGAAGGCTGATGAAACTCAAGCCTTACCAGAGACAGAAAGAAACAGCAAACCTGTGACTCTTCTTCTCTGC
 TGCAGATTTGGATGATTTCTCCAAAACATTTGCAACAATCCATGAGCAGTGTGACTCAACTCAGGCCTAA

B

	10	20	30	40	50	60	70
MW494424.1_Alpha_B.1.1.7_South	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
OK066139.1_Alpha_B.1.1.7_North	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
MW494127.1_Alpha_B.1.1.7_Centr	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
OU564105.1_Alpha_B.1.1.7_Europ	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
MZ413358.1_Alpha_B.1.1.7_Asia	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
MZ150530.1_Alpha_B.1.1.7_Afric	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
MW598419.1_Beta_B.1.351_Africa	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
MZ314964.1_Beta_B.1.351_Asia	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
OU549646.1_Beta_B.1.351_Europe	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
OK138468.1_Beta_B.1.351_North	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
MZ477746.1_Gamma_P.1_South_Ame	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
MZ621913.1_Gamma_P.1_Central_A	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
MZ277386.1_Gamma_P.1_Asia	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
OK153352.1_Gamma_P.1_North_Ame	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
OU572353.1_Gamma_P.1_Europe	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
NC_045512.2:28274-29533_Wuhan	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
OU754228.1_Kappa_B.1.617.2_Eur	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
MZ131947.1_Kappa_B.1.617.2_Nor	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
OK037152.1_Kappa_B.1.617.2_Asi	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						
MZ707692.1_Kappa_B.1.617.2_Afr	GACCCCAAAATCAGCGAAATGCACCCCGCATTAACGTTTGGTGGACCCCTCAGATTCAACTGGCAGTAAACGAGA						

C

	10	20	30	40	50	60
MW494424.1_Alpha_B.1.1.7_South	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
OK066139.1_Alpha_B.1.1.7_North	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
MW494127.1_Alpha_B.1.1.7_Centr	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
OU564105.1_Alpha_B.1.1.7_Europ	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
MZ413358.1_Alpha_B.1.1.7_Asia	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
MZ150530.1_Alpha_B.1.1.7_Afric	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
MW598419.1_Beta_B.1.351_Africa	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
MZ314964.1_Beta_B.1.351_Asia	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
OU549646.1_Beta_B.1.351_Europe	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
OK138468.1_Beta_B.1.351_North	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
MZ477746.1_Gamma_P.1_South_Ame	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
MZ621913.1_Gamma_P.1_Central_A	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
MZ277386.1_Gamma_P.1_Asia	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
OK153352.1_Gamma_P.1_North_Ame	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
OU572353.1_Gamma_P.1_Europe	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
NC_045512.2:28274-29533_Wuhan	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
OU754228.1_Kappa_B.1.617.2_Eur	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
MZ131947.1_Kappa_B.1.617.2_Nor	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
OK037152.1_Kappa_B.1.617.2_Asi	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					
MZ707692.1_Kappa_B.1.617.2_Afr	TTACAAACATTGGCCGCAAAATTCACAAATTTGCCCCAGCGCTTCAGCGTTCTTCGGAATGTCGCGC					

Supplemental figure 2. Melting regions of nucleocapsid phosphoprotein (NP) gene in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants. (A) Sequence of the first case of SARS-CoV-2 infection was compared with dispensable NP gene sequences from new variant isolates until September 2021. (B-C) shows a single mutation in the region of N1 and no mutations in region of N2 targets in the analyzed sequences.