

TABLE S1- PRIMERS ASSIGNMENT

No	Gene	Type	Orient	Nt	Sequence	Amplicon (bp)	Region
1	GAPDH	Normal	Sense	20	GGAAGGTGAAGGTCGGAGTC	135	
1'	GAPDH	Normal	AS	25	ACATGTAAACCATGTAGTTGAGGTC	135	
2	GAPDH	D-B	Sense	30	cacctatggg GGAAGGTGAAGGTCGGAGTC	152	
2'	GAPDH	D-B	AS	32	atgggttACATGTAAACCATGTAGTTGAGGTC	152	
3	"N"	Normal	Sense	21	GCAGAGACAGAAGAAACAGCA	96	1
3'	"N"	Normal	AS	20	TCAGCACTGCTCATGGATTG	96	1
4	"N"	D-B	Sense	30	ctgtct gacGCAGAGACAGAAGAAACAGCA	115	1
4'	"N"	D-B	AS	30	gcagtgactgTCAGCACTGCTCATGGATTG	115	1
5	"N"	Normal	Sense	20	CAAGCCTTACCGCAGAGACA	119	1
5'	"N"	Normal	AS	20	GCCTGAGTTGAGTCAGCACT	119	1
6	"N"	Normal	Sense	30	TGATGAAACTCAAGCCTTACCGCAGAGACA	139	1
6'	"N"	Normal	AS	30	ATGAGTTTAGGCCTGAGTTGAGTCAGCACT	139	1
7	"N"	Normal	Sense	30	ataaccaccaCAAGCCTTACCGCAGAGACA	139	1
7'	"N"	Normal	AS	30	atatttgactGCCTGAGTTGAGTCAGCACT	139	1
8	"N"	D-B	Sense	30	taaggccaaaCAAGCCTTACCGCAGAGACA	139	1
8'	"N"	D-B	AS	30	aactcagctcGCCTGAGTTGAGTCAGCACT	139	1
9	"N"	Normal	Sense	20	TCTTGCTTTGCTGCTGCTTG	138	2
9'	"N"	Normal	AS	20	GCAGTACGTTTTTGCCGAGG	138	2
10	"N"	D-B	Sense	30	aagcaatgggTCTTGCTTTGCTGCTGCTTG	158	2
10'	"N"	D-B	AS	30	cgtacgacgaGCAGTACGTTTTTGCCGAGG	158	2
11a	"S"	D-B	Sense	31	aaaccacgcgATATGGTTTCCAACCTACTa	111	3
11b	"S"	D-B	Sense	30	aaaccacgcgATATGGTTTCCAACCTACTt	111	3
11c	"S"	D-B	AS	30	gtggacgacgTAGGTCCACAAACAGTTGCT	111	3
12	"N"	TqM	Sense	45	VIC-ACTCTTCTCTGCTGCTGAGATTGGATGATTCTCCAAACAATTG -NFQ-MGB		1
13	"N"	TqM	Sense	44	FAM-CAAGGCCAAACTGTCACTAAGAAATCTGCTGCTGAGGCTTCTAA -NFQ-MGB		2

Table S1

Primers assignment. GAPDH- Glyceraldehyde 3-Phosphate Dehydrogenase. "N"- SARS-COV-2 nucleocapsid gene. "S"- Spike gene. Normal Type primer - conventional primer usually 20bp. D-B Type primer- Double-Bubble primer with stem-loop and homo-dimer configurations. TqM- TaqMan. Sense- 5'→3' primer orientation, AS- anti- sense 3'→5' primer orientation. Nt- nucleotide numbers in primer. Amplicon- size of PCR product in base pairs (bp). Region 1: primers # 3-8 in SARS-COV-2 gene "N". Region 2: primers #9 and #10 in SARS-COV-2 gene "N". Region 3: primer #11 in SARS-COV-2 gene "S". We also designed 2 TaqMan (TqM) probes with non-fluorescent quencher (NFQ) and 2 different reporter dyes for each region (see also **Fig. S3**; probes #12 and #13, 45nt and 44nt, VIC and FAM dyes, regions 1 and 2,

respectively). The TqM probes were longer than the normal TqM probes to afford binding at higher annealing temperatures to take advantage of the longer D-B primers, for improved specificity and shorter PCR cycling. However, the TqM probe could not exceed the 45nt length since at longer molecular distance, the NFQ quenching capabilities of the reporter dye are not effective. In addition, the TqM probes included a minor groove binder (MGB) moiety at the 3'-end that increases the melting temperature (T_m) of the probe and stabilizes probe/target hybrids (ThermoFisher Scientific). Regions in D-B primers: Uppercase letters- sequence-specific. Uppercase black letters- 3'-overhang. Uppercase red bold letters and lowercase red letters- stem region. Uppercase red letters and lowercase green letters- loop region. Lowercase blue background letters in 3'-end of primer 13a: wild type "a", and primer 13b- mutant "t". Lowercase blue letter "t" in position minus 5 in primers 11a and 11b represents mismatch c→t mutation introduced to weaken the primer annealing to its target. Locations of primers in the SARS-COV-2 genome are shown in **Figure S3**. Primer analysis was performed using IDT's Oligo Analyzer Version 3.1. Care was taken to avoid primer-hetero-dimers.