

Weekly Sars-Cov-2 one tube reaction test.

(DATA FROM 22.09.20)

Using 7 different primer sets with TaqMan probe. Same template (from Trondheim) with the following concentrations in 20 microliter reaction volume.

Fragment: Sars CoV-2	PCR			Date	29.09.2020
PCR volum, µl	20		# of reactions		1
	Working				
	solutions		Total volume 20 µl		Desired
	concentration		Volum		concentration
H2O			16,04		
10X Thermopol no MgSO4	0 mM MgCl		2,00		
MgSO4	200 mM		0,20		2 mM
Primer forward	100 µM		0,08		0,4 µM
Primer reverse	100 µM		0,08		0,4 µM
Probe	100 µM		0,08		0,4 µM
dNTP	100 mM		0,08		400 µM
RNA	10 ng		1,00		0,5 ng/µl
BSA (10mg/ml)	100 %		0,20		1 %
Taq native	37,5 U/µl		0,04		0,075 U/µl
Mashup	7,5 U/µl		0,20		0,075 U/µl

Reverse transcriptase and PCR is performed in a 96 well PCR plate. To accommodate different annealings temperatures for each primer set a gradient is introduced. The temperature gradient runs from high to low (A –H). Thus, well A01 to A12 is keep at 62°C. The row contains 1 blank and 11 technical replicates.

Program; 42°C 5 min, 95°C 15 sec, 62-55°C 30 sec, read the plate, goto 95°C 50 times.

96 well PCR plate, with gradient													
	1	2	3	4	5	6	7	8	9	10	11	12	
A	Blank #21	#21	#21	#21	#21	#21	#21	#21	#21	#21	#21	#21	62 °C
B	Blank #1	#1	#1	#1	#1	#1	#1	#1	#1	#1	#1	#1	61,6 °C
C	Blank #5	#5	#5	#5	#5	#5	#5	#5	#5	#5	#5	#5	60,9 °C
D	Blank #19	#19	#19	#19	#19	#19	#19	#19	#19	#19	#19	#19	59,5 °C
E	Blank #20	#20	#20	#20	#20	#20	#20	#20	#20	#20	#20	#20	57,8 °C
F	Blank #6	#6	#6	#6	#6	#6	#6	#6	#6	#6	#6	#6	56,4 °C
G	Blank #7	#7	#7	#7	#7	#7	#7	#7	#7	#7	#7	#7	55,5 °C
H													

Primer set #	Mean Cq	1 STD	Forward	Reverse	Probe
21	26,84	0,41	ACAGGTAACGTTAATAGTTAATAGCGT	ATATTGCAGCAGTACGCACACA	FAM-ACACTAGCCATCCTTACTGCGCTTCGBHQ1
1	33,02	0,14	CCCTGTGGGTTTTACACTTAA	ACGATTGTGCATCAGCTGA	FAM-CCGTCTGCGGTATGTGAAAGGTTATGGBHQ1
5	27,89	0,17	TGGGGYTTTACRGGTAACCT	AACRCGCTTAACAAGCACTC	FAM-TAGTGTGTGATGCWATCATGACTAGTAMRA
19	26,33	0,10	ATGAGCTTAGTCCTGTTG	CTCCCTTTGTTGTGTTGT	FAM-AGATGTCTTGTGCTGCCGTABHQ1
20	26,18	0,05	GGTAAGTGGTATGATTTG	CTGGTCAAGGTTAATATAGG	FAM-TCATACAAACCACGCCAGGBHQ1
6	26,98	0,34	TAATCAGACAAGGAAGTATTA	CGAAGGTGTGACTTCCATG	FAM-GCAAATTTGTGCAATTTGCGGTAMRA
7	27,15	0,10	CGTTTGGTGGACCCAGAT	CCCCACTGCGTTCTCCATT	FAM-CAACTGGCAGTAACCBHQ1







