



## SARS-CoV-2 and Beta Coronavirus detection using GeneFinder RT-qPCR kit.

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes the respiratory illness responsible for the COVID-19 (coronavirus disease 2019) pandemic. It was previously referred to as 2019 novel coronavirus (2019-nCoV, human coronavirus 2019 (HCoV-19 or hCoV-19) and Wuhan Coronavirus. On January 30, 2020, the World Health Organization declared the outbreak a Public Health Emergency of International Concern and a pandemic on 11 March 2020. GeneFinder™ COVID-19 PLUS RealAmp Kit is used for the detection of SARS-CoV-2 virus causing Coronavirus Disease 2019 (COVID-19) through a one-step reverse transcription real-time Polymerase Chain Reaction (RT-qPCR) approach using viral RNA extracted from respiratory specimens such as nasopharyngeal swab, alveolar lavage fluid, throat swab and sputum. This product targets the viral RNA-dependent RNA-polymerase encoding gene (RdRp), the spike protein gene (E), the nucleocapsid encoding (N) gene as well as Ribonuclease P (RNaseP) as an internal control<sup>1,2</sup>. Betacoronavirus are one of four genera (Alpha-, Beta-, Gamma-, and Delta-) of coronaviruses, subfamily Orthocoronavirinae, Coronaviridae family, Nidovirales order. They are enveloped viruses having positive-strand RNA which infect mammals. The natural reservoirs for betacoronaviruses are bats and rodents. The betacoronaviruses of greatest clinical relevance are OC43 and HKU1 (which can cause common cold and belong to lineage A), SARS-CoV and SARS-CoV-2 belonging to lineage B, and MERS-CoV of lineage C<sup>1</sup>.

### Procedure

1. Remove GeneFinder™ COVID-19 PLUS RealAmp Kit box (GeneFinder, OSANG Healthcare Co., Ltd. Korea, Reference number IFMR-45) from the lower door shelf of main lab -20°C freezer and thaw on thermomixer at 40°C for no more than 1 minute.
2. Remove SARS-CoV-2 Negative RNA (Non-template Control) from COVID-19 NTC (non-template control, negative RNAs), place in refrigerated microtube rack.
3. Place kit contents (2x Reaction tube, Probe Mixture, NC, and PC) in refrigerated microtube rack along with NTC and place rack inside the RT-PCR workstation.





4. The following table provides reagent volumes required for the processing of 1 to 93 patient samples or pools (which occupy 96 wells in the Applied Biosystems 7500 Cyclers) while considering the 3 additional control reactions required (NC, NTC and PTC) along with a 1% additional volume to compensate for pipetting errors.

Samples	1	2	3	4	5	6	7	8	9	10	11	12
Total Rx	4	5	6	7	8	9	10	11	12	13	14	15
2x RXN	20.2	25.3	30.3	35.4	40.4	45.5	50.5	55.6	60.6	65.7	70.7	75.8
Probe	10.1	12.6	15.2	17.7	20.2	22.7	25.3	27.8	30.3	32.8	35.4	37.9

Samples	13	14	15	16	17	18	19	20	21	22	23	24
Total Rx	16	17	18	19	20	21	22	23	24	25	26	27
2x RXN	80.8	85.9	90.9	96.0	101.0	106.1	111.1	116.2	121.2	126.3	131.3	136.4
Probe	40.4	42.9	45.5	48.0	50.5	53.0	55.6	58.1	60.6	63.1	65.7	68.2

Samples	25	26	27	28	29	30	31	32	33	34	35	36
Total Rx	28	29	30	31	32	33	34	35	36	37	38	39
2x RXN	141.4	146.5	151.5	156.6	161.6	166.7	171.7	176.8	181.8	186.9	191.9	197.0
Probe	70.7	73.2	75.8	78.3	80.8	83.3	85.9	88.4	90.9	93.4	96.0	98.5

Samples	37	38	39	40	41	42	43	44	45	46	47	48
Total Rx	40	41	42	43	44	45	46	47	48	49	50	51
2x RXN	202.0	207.1	212.1	217.2	222.2	227.3	232.3	237.4	242.4	247.5	252.5	257.6
Probe	101.0	103.5	106.1	108.6	111.1	113.6	116.2	118.7	121.2	123.7	126.3	128.8

Samples	49	50	51	52	53	54	55	56	57	58	59	60
Total Rx	52	53	54	55	56	57	58	59	60	61	62	63
2x RXN	262.6	267.7	272.7	277.8	282.8	287.9	292.9	298.0	303.0	308.1	313.1	318.2
Probe	131.3	133.8	136.4	138.9	141.4	143.9	146.5	149.0	151.5	154.0	156.6	159.1

Samples	61	62	63	64	65	66	67	68	69	70	71	72
Total Rx	64	65	66	67	68	69	70	71	72	73	74	75
2x RXN	323.2	328.3	333.3	338.4	343.4	348.5	353.5	358.6	363.6	368.7	373.7	378.8
Probe	161.6	164.1	166.7	169.2	171.7	174.2	176.8	179.3	181.8	184.3	186.9	189.4

Samples	73	74	75	76	77	78	79	80	81	82	83	84
Total Rx	76	77	78	79	80	81	82	83	84	85	86	87
2x RXN	383.8	388.9	393.9	399.0	404.0	409.1	414.1	419.2	424.2	429.3	434.3	439.4
Probe	191.9	194.4	197.0	199.5	202.0	204.5	207.1	209.6	212.1	214.6	217.2	219.7

Samples	85	86	87	88	89	90	91	92	93
Total Rx	88	89	90	91	92	93	94	95	96
2x RXN	444.4	449.5	454.5	459.6	464.6	469.7	474.7	479.8	484.8
Probe	222.2	224.7	227.3	229.8	232.3	234.8	237.4	239.9	242.4





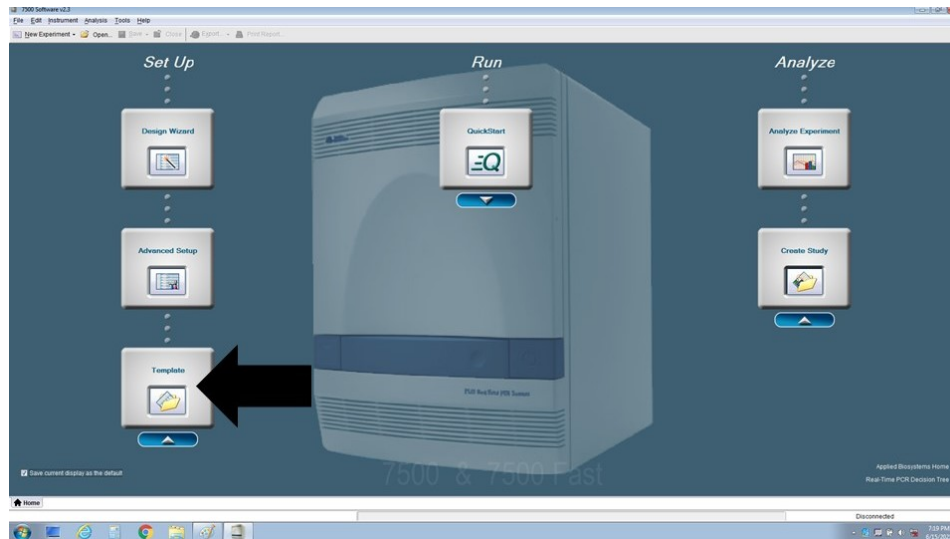
5. Always provide NC, NTC and PTC with all runs.
  - a. **NC**= Negative control (distilled water, stored with kit in main lab -20°C freezer).
  - b. **NTC**=Negative Template Control (negative RNA, stored in main lab -80°C BSL2 freezer in cardboard box labelled as #6 in lower-most shelf, any sample having initials is negative).
  - c. **PTC**=Positive Template Control (SARS-CoV-2 positive RNA, stored in main lab -80°C BSL2 freezer, second shelf from top, purple plastic box labelled “GeneFinder SARS-CoV-2 PTC” in pink coloured screw-top tubes).
6. Prepare the RT-qPCR Mastermix in the RT-PCR workstation according to previous table. Vortex and spin tube down.
7. In the RT-PCR workstation, distribute 7.5 µL of mastermix to each PCR tube (optical caps and tubes required). Avoid the use of refrigerated PCR tube racks as water condensation on PCR tube external surface may interfere with laser readout.
8. In the RT-PCR workstation, add 2.5 µL of NC and NTC to the corresponding tubes (first two tubes, in this order).
9. In the RT-PCR workstation, add 2.5 µL of sample or pool RNA to each of the remaining tubes.
10. Cap all reaction tubes with optical strips except that of the PTC. Leave RT-PCR workstation.
11. In the Post-PCR or Instrument workstation, add 2.5 µL of PTC to the corresponding (final) tube.
12. Cap all tubes securely, vortex briefly and spin down.
13. Place in Applied Biosystems 7500 tube tray (make sure the plate tray has been replaced with tube tray).

### Applied Biosystems 7500 operation

1. Turn Applied Biosystems thermal cycler and laptop on.
2. Log into laptop account **INSTR\_USR** with the corresponding password.
3. Click on the “7500 Software” **icon** on top left corner of screen.
4. Log into instrument as **Guest**.



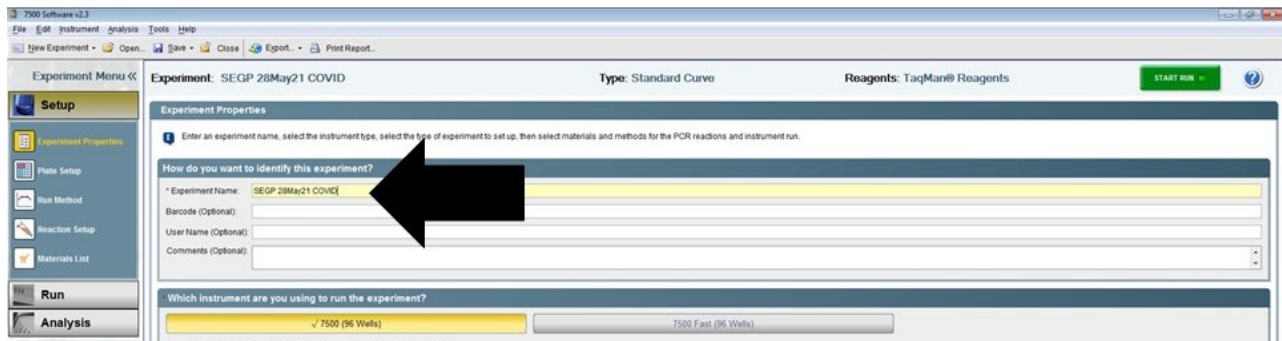
5. Select “**Template**” Set up.



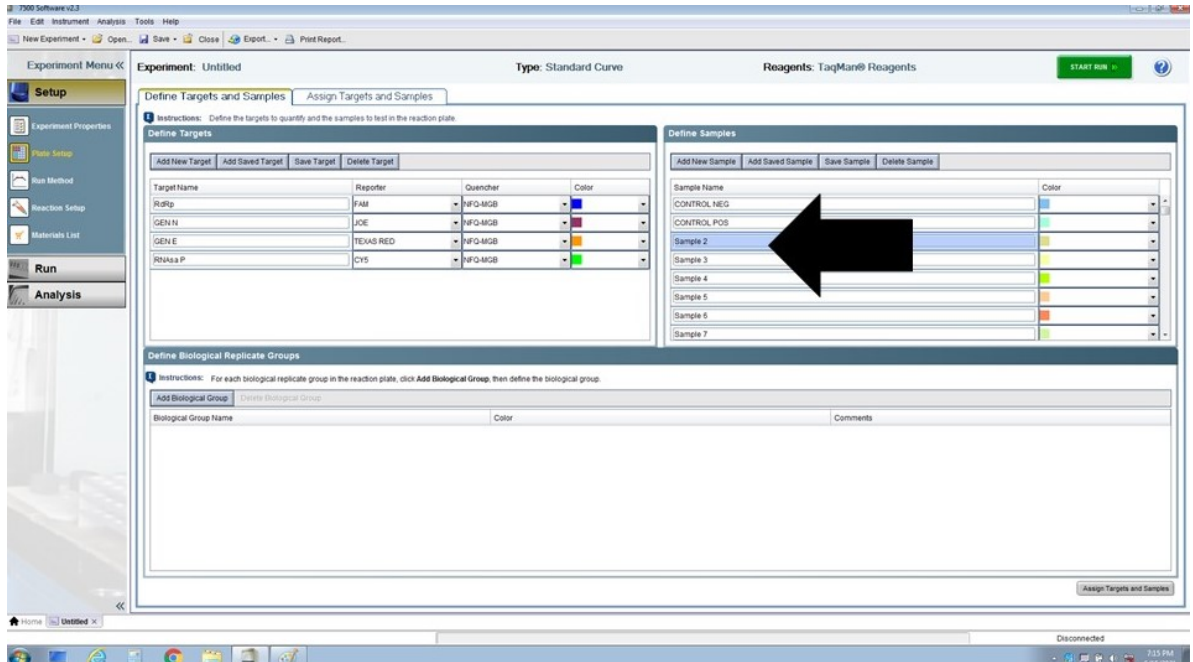
6. Select “**genefinder templates**” folder.

7. Select “vertical 94 sample layout”.

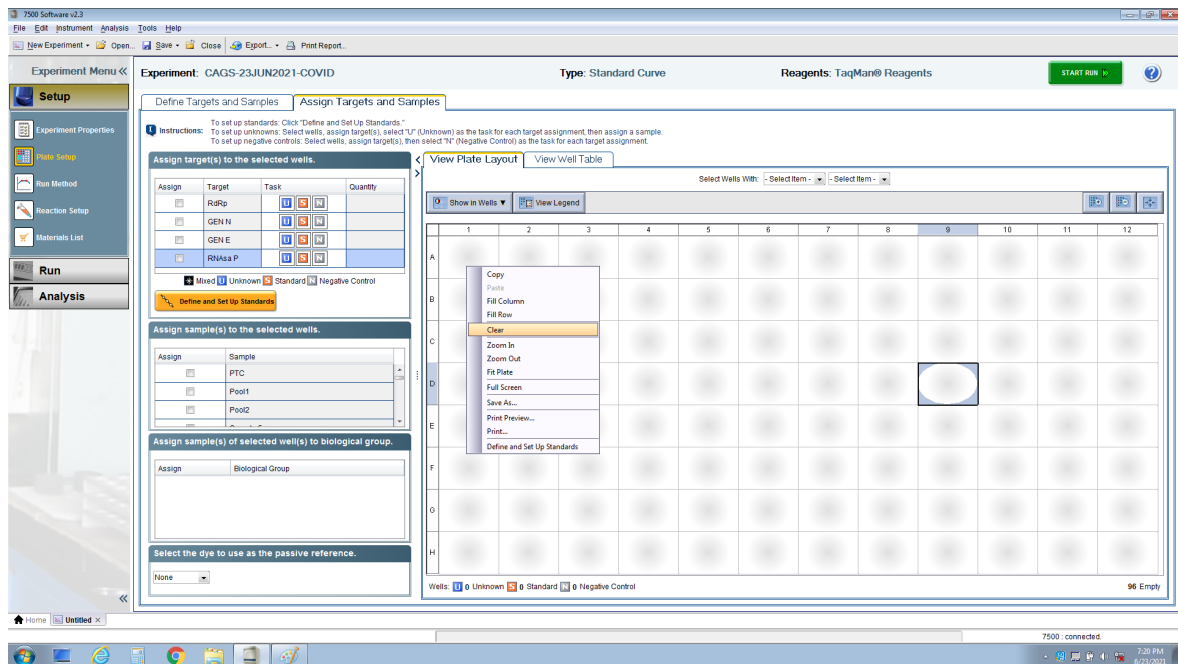
8. Go to “**Setup**” tab on upper-left and change **experiment name** with proper ID. At the Viral & Human Genomics Laboratory we prefer to name experiments with the operators ID followed by two-digit day, three letter month, two-digit year and type of test. I.e., “SEGP 28May21 COVID”



- Go to “**Define Samples**” box on upper-right and change definition of first line to Negative Control (NC for dH<sub>2</sub>O), change the second line to Negative Template Control (NTC), change Sample 2 to Positive Template Control (PTC) and subsequently Sample 3 to whatever ID you are using for your first sample or pool to be tested, etc.



- Clear all cell designations by clicking on right button and then “clear”.



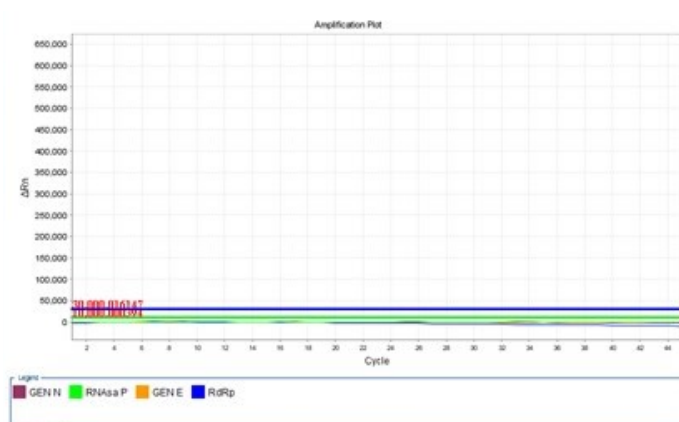


## Analysing results

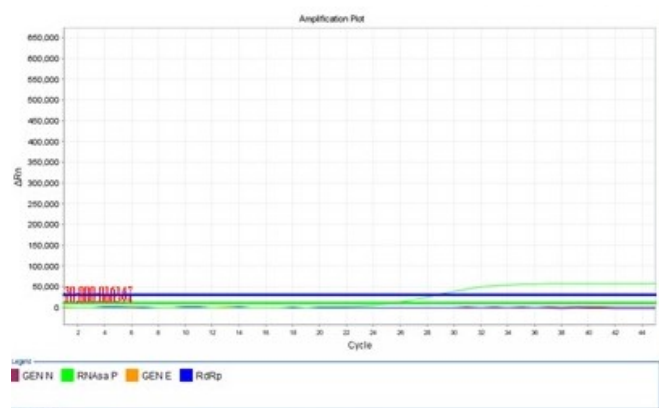
1. When instrument has finished a run, select “Analyse” button on top-right corner of screen.
2. Go to “Analysis” tab on upper-left corner.
3. In “Amplification Plot, plot Settings” change Plot Color to “Target” and graph type from log to linear (as GeneFinder kit does not have internal ROX calibration dye, linear plots are easier to visualize).
4. The manufacturer (GeneFiner) indicates the following criteria should be met to diagnose or discard SARS-CoV-2 infection.

#	Ct range				Result
	RdRp (FAM)	E (Texas Red)	N (JOE)	IC (Cy5)	
1	≤43	≤43	≤43	≤35*	COVID-19 Positive
2	≤43	≤43	U.D	≤35	
3	≤43	U.D	≤43	≤35	
4	≤43	U.D	U.D	≤35	Repeat the test (COVID-19 Positive if RdRp≤43)
5	U.D	≤43	≤43	≤35	Repeat the test (COVID-19 Positive if E and N≤43)
6	U.D	U.D	≤43	≤35	Repeat the test (COVID-19 Positive if N≤43)
7	U.D	≤43	U.D	≤35	Beta coronavirus
8	U.D	U.D	U.D	≤35	Negative
9	U.D	U.D	U.D	U.D	Invalid (re-test)

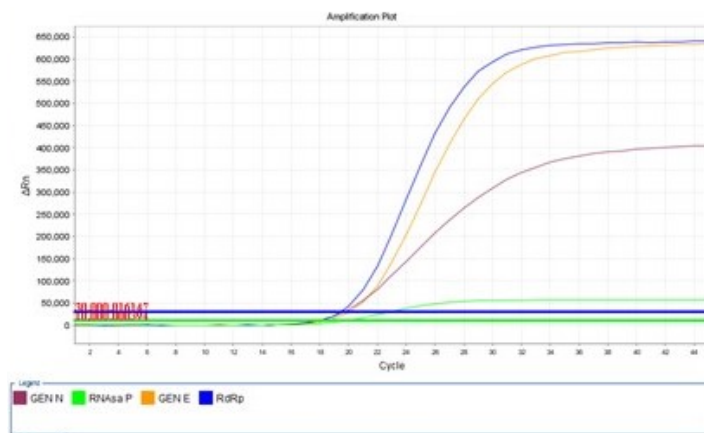
5. Negative Control (NC) should not exhibit any amplification curve unless contaminated.



6. Non-Template Control (NTC) should **ONLY** exhibit RNaseP amplification.



7. The Positive Template Control (PTC) should exhibit all four amplification curves (RdRp, E, N and RNaseP).



8. According to the manufacturer, for the PTC to be considered truly positive, RdRp gene Ct (FAM channel on ABI 7500), N gene Ct (JOE channel on the ABI 7500) and the E gene Ct (Texas Red channel on ABI 7500) should all rise  $\geq 22$  cycles with the internal control (Cy5 channel on ABI 7500) rising at or before cycle number 21. NC should not exhibit any amplification curve at any Ct.

## Notes

1. All kit reagents should be stored at  $-20^{\circ}\text{C}$  or below and protected from light.





2. The GeneFinder™ COVID-19 PLUS RealAmp Kit is to be used with RNA extracted using the QIAamp Viral RNA kit (Qiagen, Germany, Cat. # 52904) as indicated by the manufacturer and used at the Viral & Human Genomics Laboratory ([http://www.genomica.uaslp.mx/Protocolos/Resp\\_COVID\\_RNA\\_Extraction\\_ENG.pdf](http://www.genomica.uaslp.mx/Protocolos/Resp_COVID_RNA_Extraction_ENG.pdf))
3. Thaw all components thoroughly at room temperature before using. Mix gently, spin down the contents for 5 seconds.

## References

1. Instructions for Use GeneFinder™ COVID-19 PLUS RealAmp Kit, Cat Num. IFMR-45 OSANG Healthcare Co., Ltd 132, Anyangcheondong-ro, Dongan-gu, Anyang-si, Gyeonggi-do, 14040 Korea.
2. Spearman, P. Diagnostic testing for SARS-CoV-2/COVID19. Current opinion in pediatrics vol. 33 122-128 (2021).
3. Ong, D. S. Y., Claas, E. C. J., Breijer, S. & Vaessen, N. Comparison of the GeneFinder™ COVID-19 Plus RealAmp Kit on the sample-to-result Platform ELITE InGenius to the national reference method: An added value of N gene target detection? J. Clin. Virol. 132, (2020).

## Revision history

- 1.0 Original document.
- 2.0 Changes relating RT-PCR workstation use, Instrument operation and interpretation of results.
- 3.0 Eliminated recommendation to use refrigerated PCR tube rack and added comment on expected PTC Ct rise.

