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Supplementary Protocol - cDNA Synthesis for SARS-CoV-2 ARTIC Panel

This protocol outlines the conditions and steps for first strand cDNA synthesis in 10 µl reaction volume. The product of the reaction can be directly inserted into the ARTIC Panel PCR. We recommend SuperScript™ Reverse Transcriptase (Thermo Fisher Scientific, Cat. No. 18091200) for cDNA synthesis.

First strand cDNA is generated using a mix of oligo(dT) primer and random hexamers. The final concentration per reaction should be 2.5 µM for the oligo(dT) primer and 2.5 µM for the random hexamer primer.

1	Mix 4.5 µl RNA with 0.5 µl 50 µM random hexamer primers and 0.5 µl 50 µM OligodT primer in a total volume of 5.5 µl. Fill up to 5.5 µl if using a lower RNA volume.
2	Heat to 65 °C for 5 minutes.
3	Place the reaction on ice.
4	Prepare a mastermix of 2 µl 5x SSIV Buffer, 0.5 µl of Dithiothreitol (100 mM), 1 µl dNTPs mix (10 mM each), and 1 µl SuperScript™ IV per sample. Mix thoroughly and spin down briefly.
5	Spin down samples from step 3 and add 4.5 µl of the mastermix prepared in step 4. Mix thoroughly and spin down.
6	Incubate with the following temperature program: 10 minutes at 23 °C, 10 minutes at 55 °C, 10 minutes at 80 °C, then cool to 25 °C and hold for 1 minute. The cDNA can now be split and inserted into SARS-CoV-2 ARTIC Panel PCR following the protocol provided in **SR9042UG358**. ☞ Safe stopping point, cDNA can be stored at ≤-20 °C at this point.