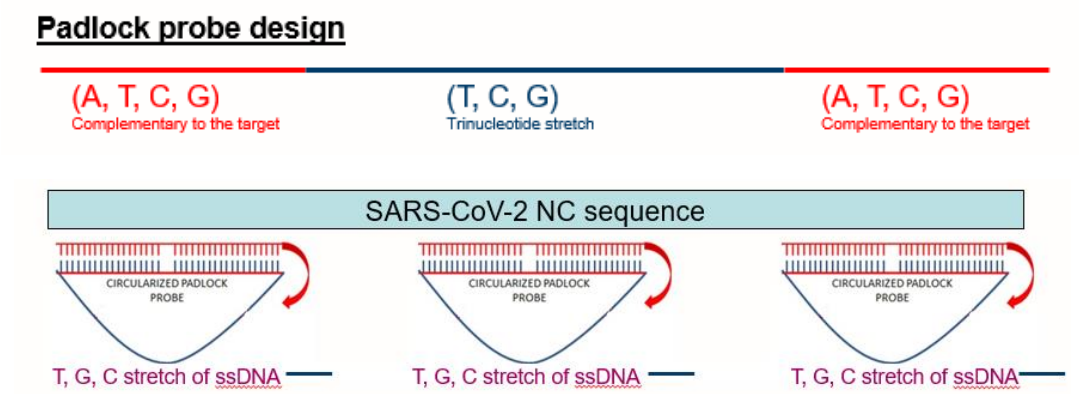
Dual RCA for SARS-Cov-2 nucleic acid testing

1. The following reactions all happen sequentially in one tube for ~60-70 mins.

2. ssDNA padlock probes are designed to target different conserved but specific regions of the SARS-CoV-2 RNA sequence.

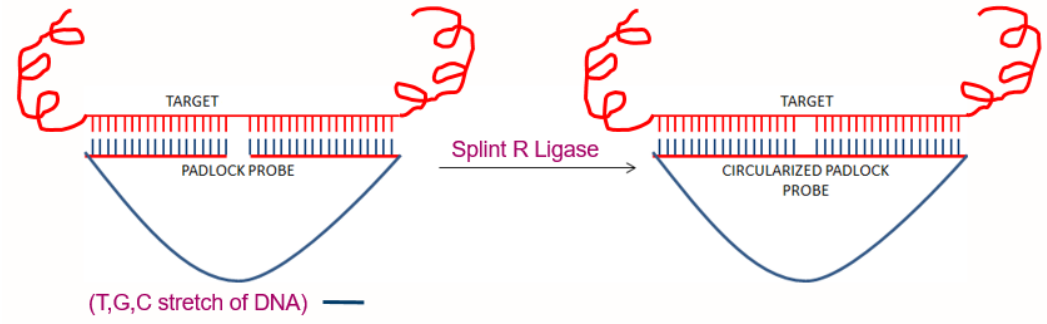
3. The two ends of the padlock probe are complementary to the ssRNA region of the SARS-CoV-2 sequence and consist of all 4 nucleotides (A,T,G,C). The spacer region between the two target specific ends consists of a trinucleotide stretch (T,G,C)



Probe-1 Probe-2 Probe-3

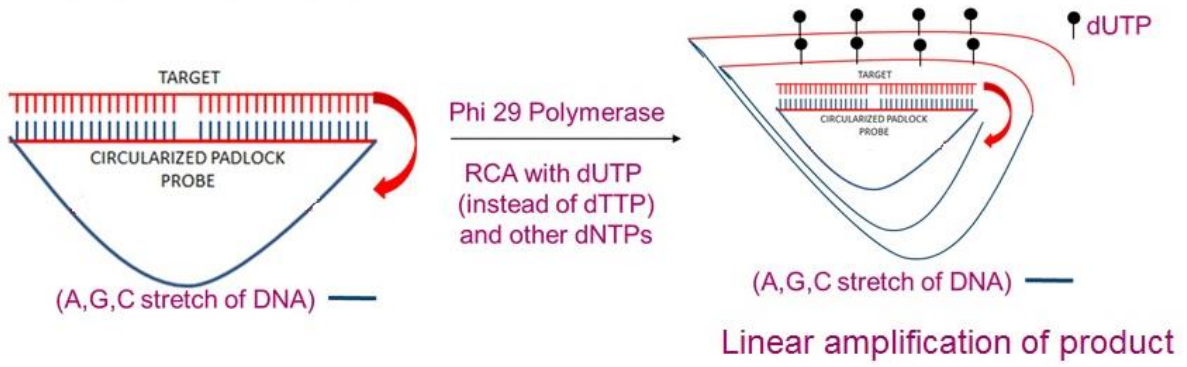
The two target-specific ends (in red) for probes 1, 2 and 3 would be different as they are complementary to the corresponding target sequence. However, the central trinucleotide stretch (in blue) sequence will be the same.

4. The probes bind to the NC region as depicted below and can be ligated using Splint R ligase which specifically ligates ssDNA in an RNA:DNA Duplex. This reaction takes place at room temperature for 15 mins. Both, viral lysates prepared from Nasal/Oral swabs or purified RNA could be employed initially to test whether viral nucleic acid could be detected sensitively in either prep.

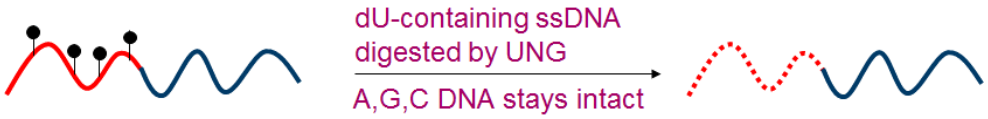


5. After circularization of the padlock probe, Phi29 DNA polymerase along spacer-specific trinucleotide primer is added to the tube.

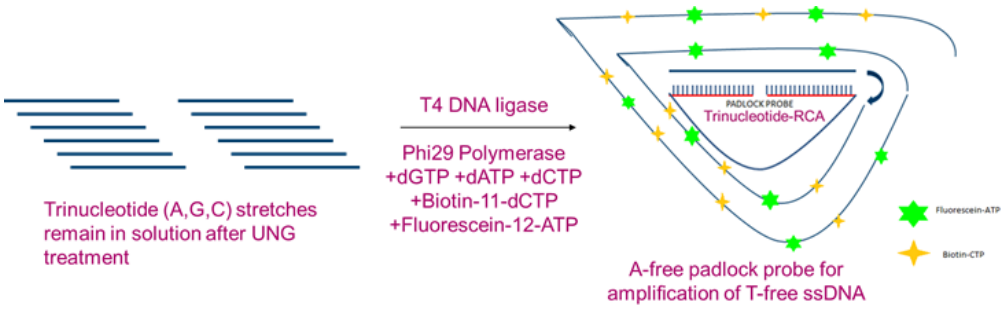
6. **1st RCA reaction:** RCA reaction is carried out by adding all nucleotides except dTTP, which is substituted with dUTP. dUTP will get incorporated into the newly synthesised ssDNA after RCA instead of dTTP. The temperature for this reaction will be determined empirically and the reaction will be carried out for 15-30 mins.



7. Uracil N-Glycosylase (UNG) is added to the reaction mixture for 10 mins at room temperature to digest the dUTP containing DNA. Subsequently, the reaction is heated up to 95˚C for 2 mins to inactivate the UNG and denature the DNA and form ssDNA.

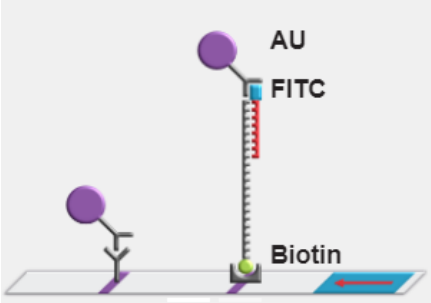


8. A 2nd tri-nucleotide padlock probe is added to the mixture to target the trinucleotide (TN) sequence amplified by the first probe. The padlock probe ligation is carried out in presence of T4 DNA ligase (for ssDNA) at room temperature for 15mins.

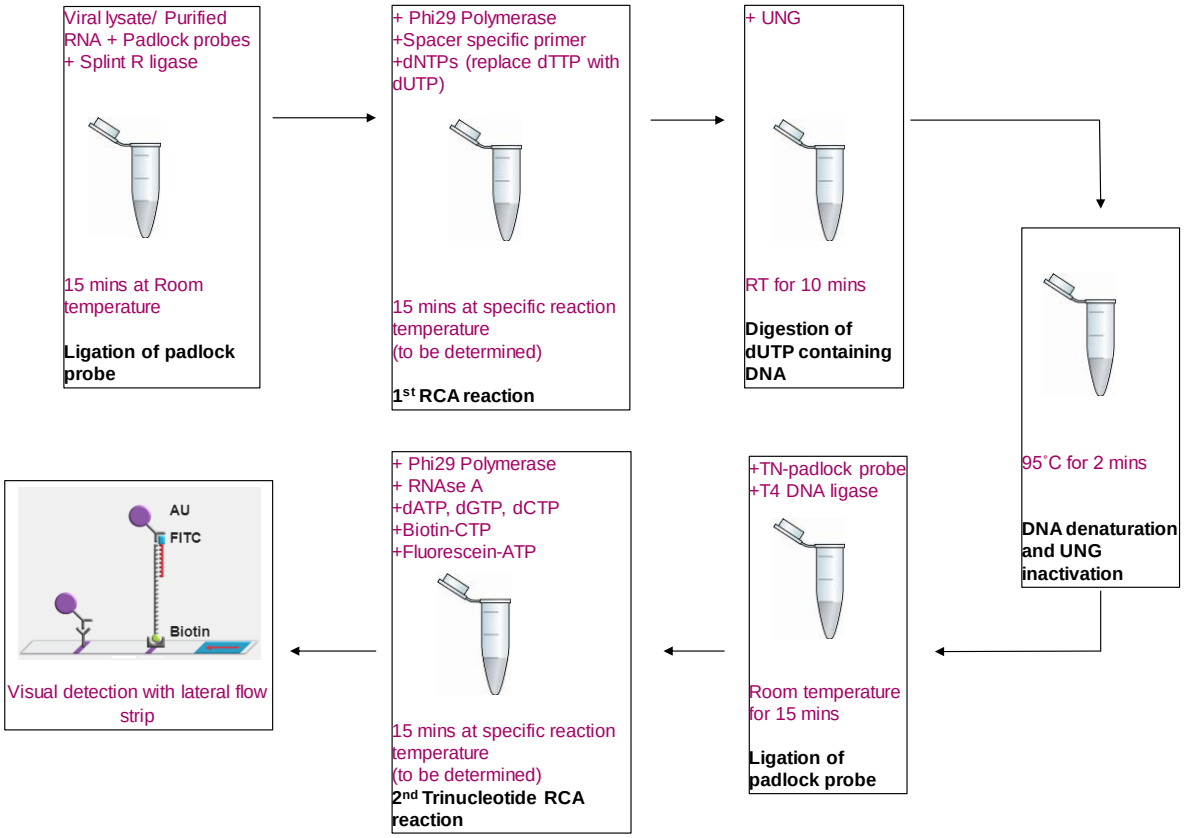


9. **<u>2nd tri-nucleotide RCA reaction:</u>** The RCA reaction is carried out in the presence of dATP+ dGTP + dCTP (no dTTP- hence trinucleotide RCA, which increases specificity of amplification). Also, Biotin-11-dCTP and Fluorescein-12P are added to the reaction. They get incorporated into the newly synthesised TN-RCA ssDNA sequence. The temperature for this reaction will be determined empirically and the reaction will be carried out for 15-30 mins.

10. Visual detection of Fluorescein and Biotin labelled DNA is carried out with lateral flow assay strips. The double labelled DNA (Biotin and Fluorescein) forms a test band in the place of the Biotin ligand to give a positive result.



Experimental workflow on the bench-



References:

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