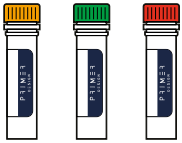
SNPSIG

PRIMER DESIGN

SNPsig® VariPLEX (SARS-CoV-2) Research Use, Procedure Card

SNPsig® VariPLEX (SARS-CoV-2) Research Use is intended for use as a **reflex test** only. Thus, a primary confirmation test for SARS-CoV-2 would be carried out using suitable methodology, and the extracted RNA from patient samples (or any material suited for PCR amplification) thereafter applied to this test.

Each genotyping primer/probe mix contains labelled probes homologous to the genotypes under investigation as specified in IFU.



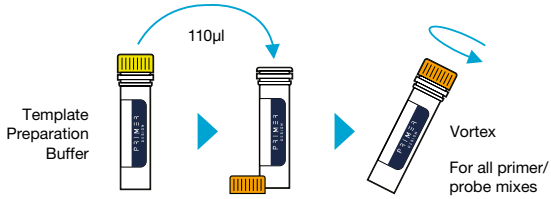
SNPsig® VariPLEX (Covid-19) genotyping primer probe mix

1. Primer/Probe Mix

Resuspend each primer/probe mix in 110µl Template Preparation Buffer supplied (tube 1 shown).

To ensure complete resuspension, vortex each tube thoroughly.

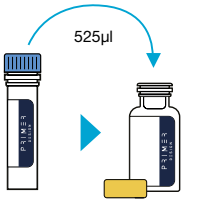
Repeat for all primer/probe mixes.



2. Master Mix

Resuspend the mastermix in 525µl mastermix resuspension buffer supplied.

Repeat for other vials as required.

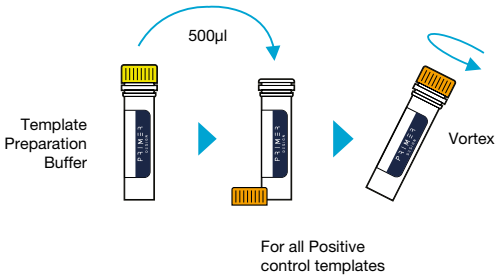


3. Positive Control

Resuspend each positive control templates in 500µl template preparation buffer supplied (tube 1 shown).

To ensure complete resuspension, vortex each tube thoroughly.

Repeat for all positive control templates.



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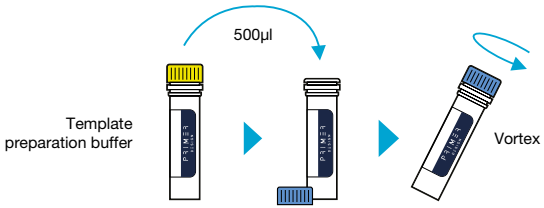
4. Internal Control Template

Resuspend the internal control template in 500µl template buffer supplied.

To ensure complete resuspension, vortex each tube thoroughly.



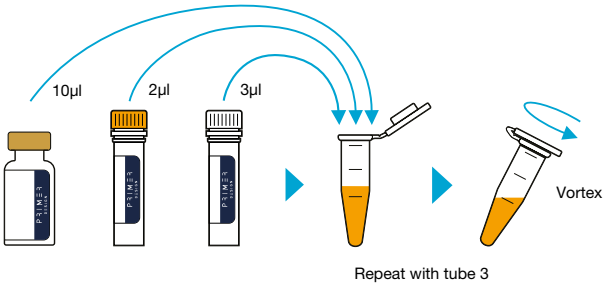
5. Prepare Genotyping Reaction Mixes

For tubes 1 and 3:

Mix 10µl complete RT-qPCR Master Mix.

2µl primer/probe mix.

3µl RNase/DNase free water.



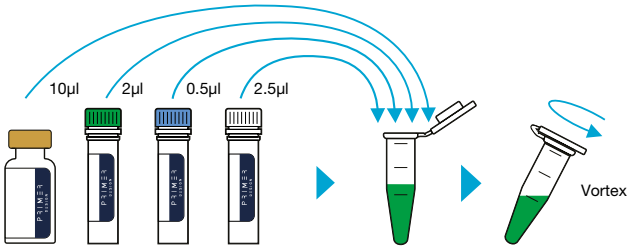
For tube 2:

Mix 10µl complete RT-qPCR Master Mix.

2µl primer/probe mix.

0.5µl internal control RNA template.

2.5µl RNase/DNase free water.



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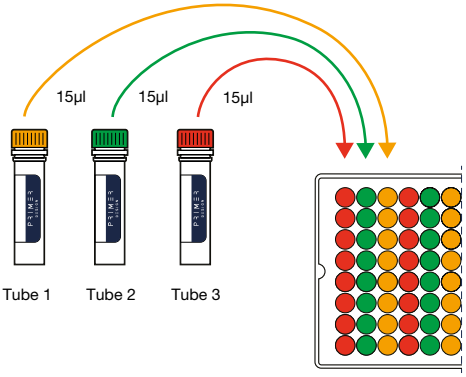
6. Prepare 96 Well Plate with Genotyping Mixtures

Add 15µl of each genotyping mixture to wells in sets of three as shown.

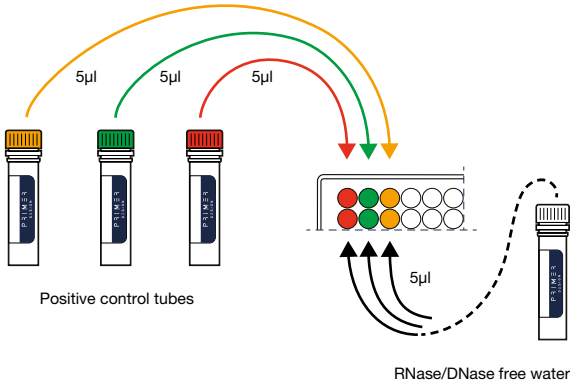


Use of coloured solutions purely for illustrative purposes.

7. Add Positive and Negative Controls

Add 5µl of the positive control, to the genotyping mixture.

For negative control, add 5µl of RNase/DNase free water to each of three genotyping mixtures for total of three negative controls.



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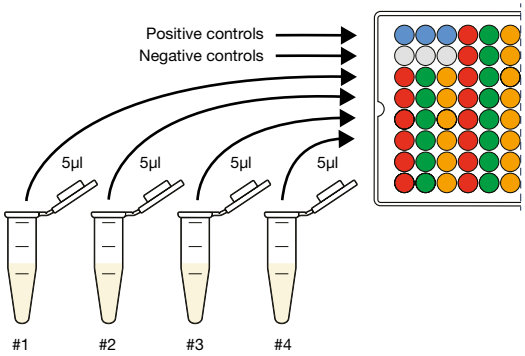
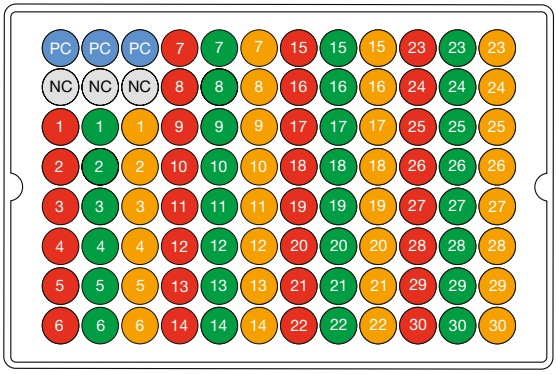
8. Add Samples

Add 5µl of RNA1, RNA2, RNA3, …RNAn from each of the samples to be processed into each group of three wells containing each of the genotyping mixtures.



E.g. samples 1, 2, 3, and 4

If using all genotype mixtures, one 96 well plate will fit 30 samples as shown.



Use of coloured solutions purely for illustrative purposes.

Follow the qPCR amplification protocol as stated in the Handbook

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