LyteStar 2019-nCoV RT-PCR Kit 2.0 Workflow

Kit Components

What is in the kit?

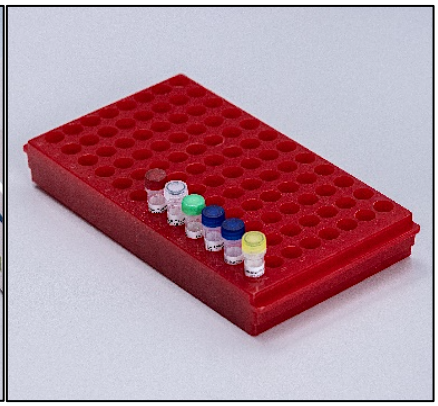
2x Master A (yellow cap)
4x Master B (blue cap)
1x Internal Control (green cap)
1x Positive Control (red cap)
1x Negative Control (clear cap)

1x Master A + 2x Master B
= 48 reactions

Master Mix Setup for 48 reactions

1. Remove the following tubes from the box and let thaw completely:

1x Master A (yellow cap)
2x Master B (blue cap)
1x Internal Control (green cap)
1x Positive Control (red cap)
1x Negative Control (clear cap)

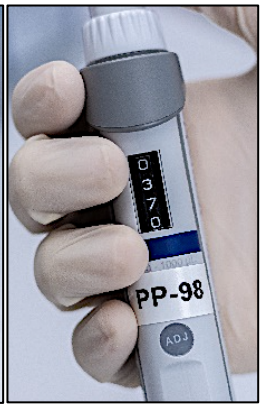


2. After all components have thawed completely, briefly vortex and centrifuge.

3. Prepare PCR Master Mix by adding both tubes Master B to Master A.

Set Pipette to **370** µl and pipette the complete content of the 1st tube Master B (blue cap) into Master A (yellow cap).

Repeat the process with the 2nd tube of Master B.

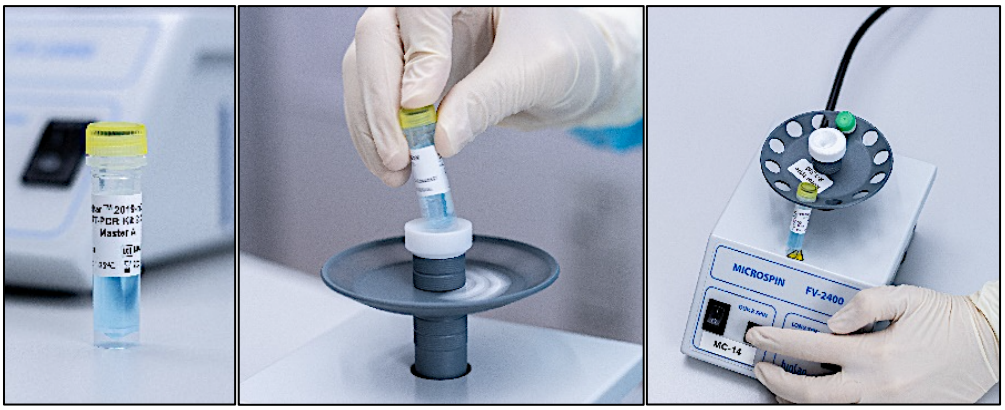


4. Add Internal Control into PCR Master Mix.

Set Pipet to **27** µl

and pipette 27 µl of the Internal Control (green cap) into the PCR Master Mix.

5. Briefly vortex and centrifuge the PCR Master Mix.



PCR reactions Setup

6. Pipette 20 µl of PCR Master Mix into each PCR tube.

Set the Pipet to **20** µl

and add 20 µl of PCR Master Mix to each required Mic PCR tube.

7. Add the Negative Control

Set the Pipet to **5** µl

and add 5 µl of PCR grade water to the appropriate PCR tubes for NTC.

Make sure to use a fresh pipet tip for each Negative Control.

8. Add the Samples

Add 5 µl of each sample to the appropriate PCR tubes.

Make sure to use a fresh pipet tip for each sample.

9. Add the Positive Control

Add 5 µl of Positive Control to the appropriate PCR tubes.

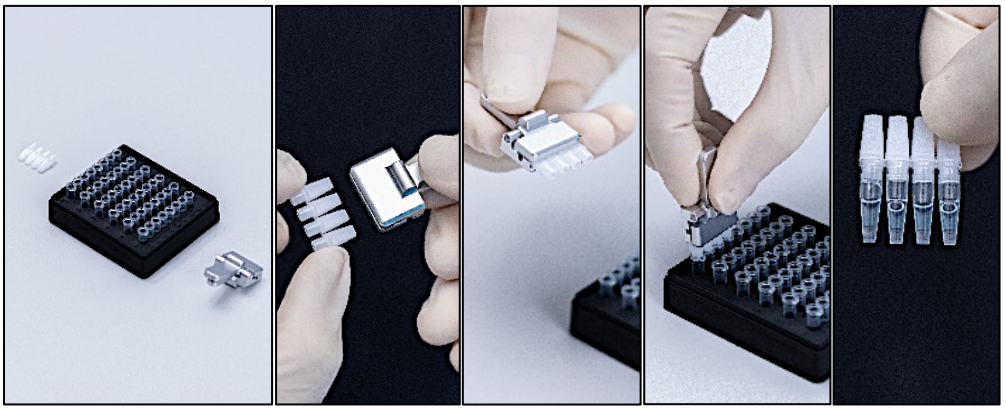
Make sure to use a fresh pipet tip.

10. Close the PCR tubes

Tightly close the PCR tubes with the appropriate caps.

For Mic PCR tubes, use the provided capping tool to fit the caps properly.

Only for ultraSMBS24, add one drop of PCR mineral oil into each PCR tube before closing the tube.

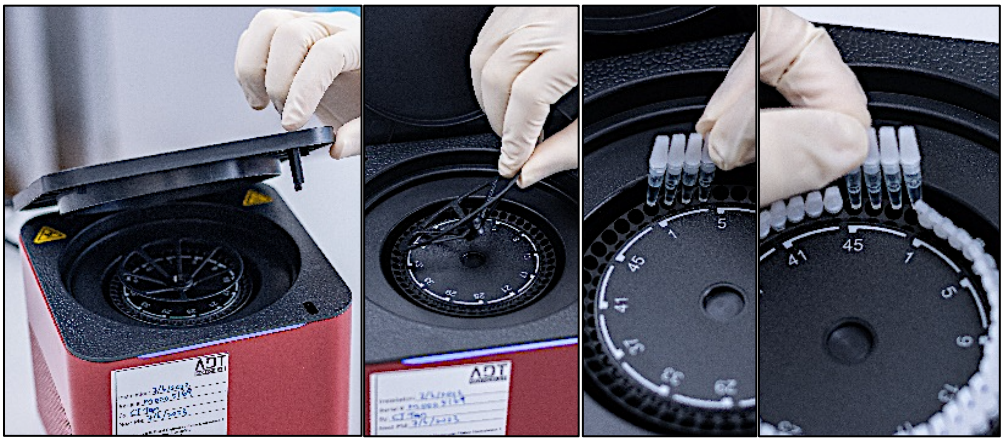


11. Load the PCR cycler

Open the Mic qPCR cycler and remove the tube clamp.

Starting from **Position 1**, place the Mic tubes in the Mic qPCR cycler. Fill the unused wells with Mic tubes that are pre-loaded with 25 µl of water.

Place the tube clamp back on and close the lid of the instrument.



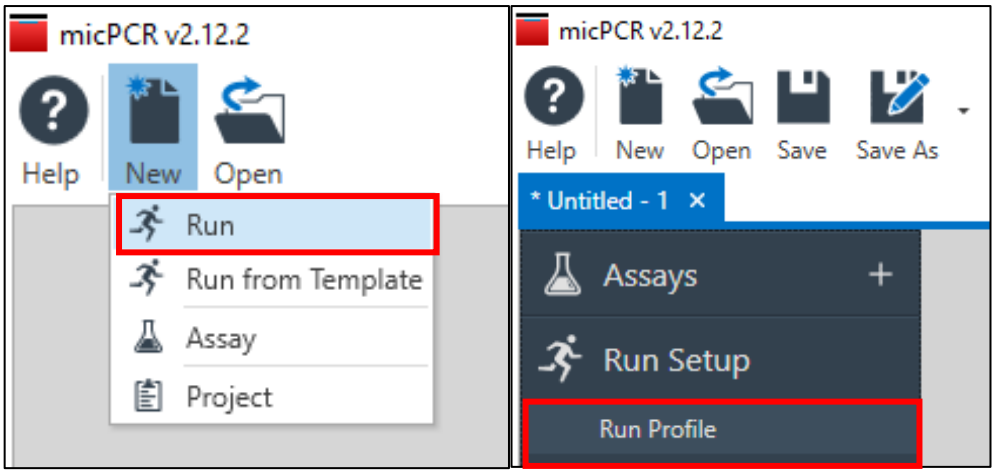
Programming the Mic

Programming the Mic real-time PCR instrument

1.

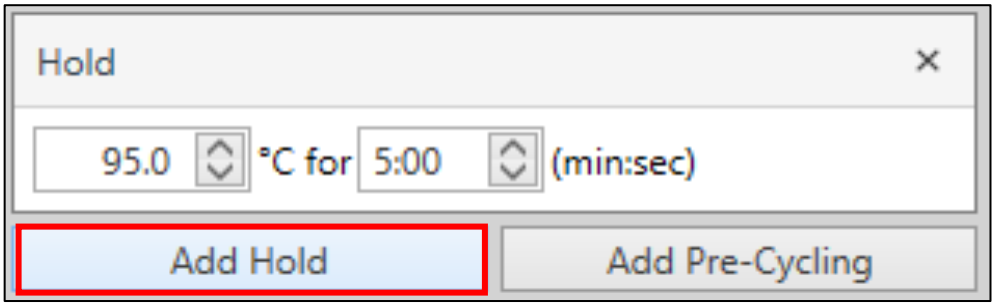
Select *New* from the tool bar menu and then *Run* from the drop-down list.

Select *Run Profile* from the navigator bar.



2.

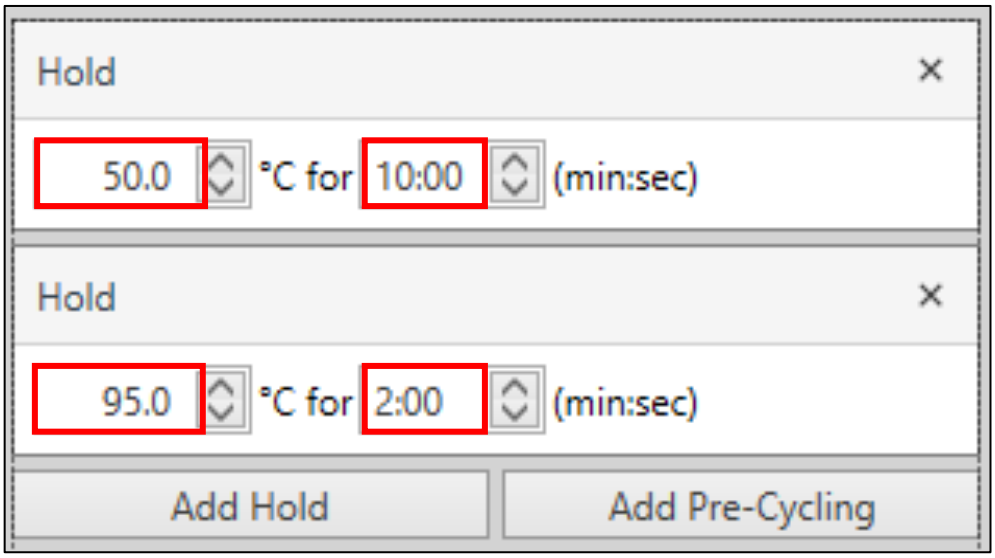
Under the *Hold* section, use the *Add Hold* button to add an additional Hold step.



3.

Set the temperature and time of the first Hold step as *50* degrees for *10* minutes. (Reverse Transcription step)

Set the temperature and time of the second Hold step as *95* degrees for *2* minutes. (Initial Activation step)



4.

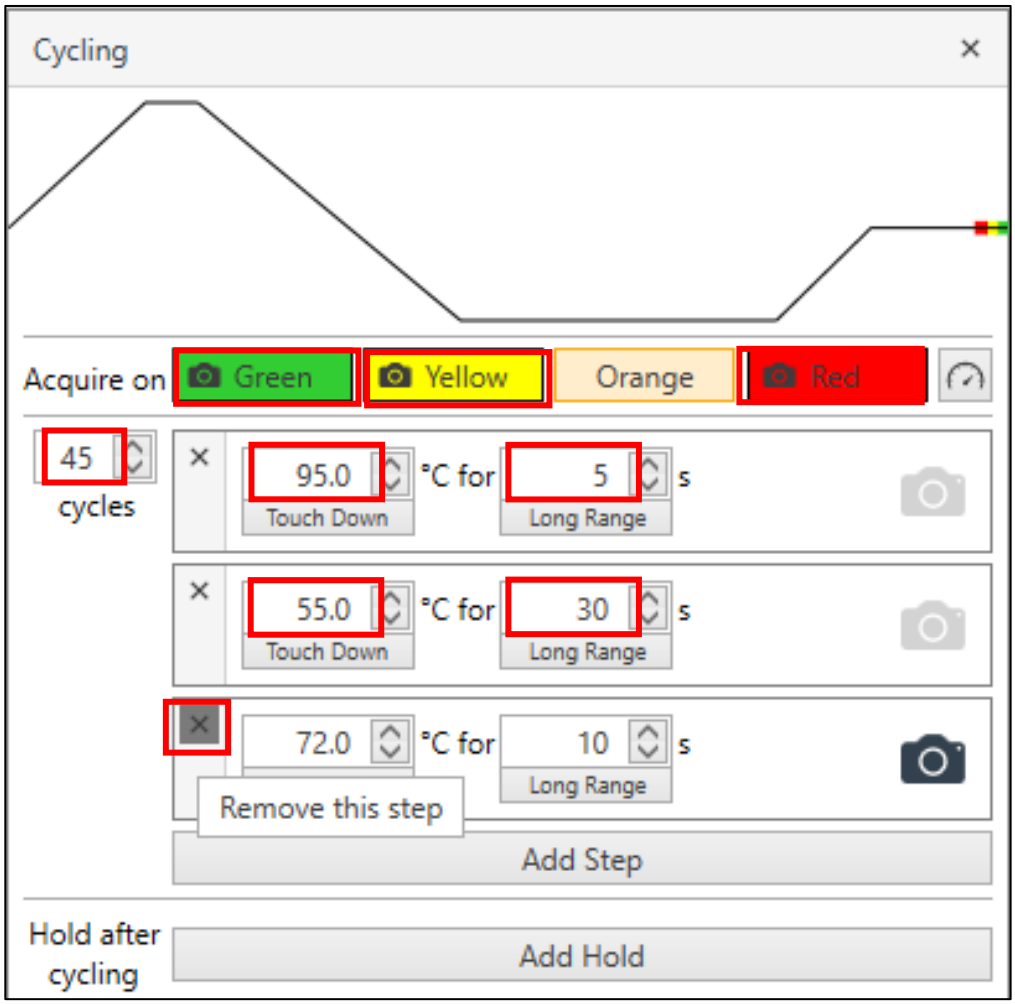
Under the *Cycling* section, enter the number of cycles as *45.*

Select *Green*, *Yellow* and *Red* channels to *Acquire on*.

Set the temperature and time of the first cycling step as *95* degrees for *5* seconds. (Denaturation step)

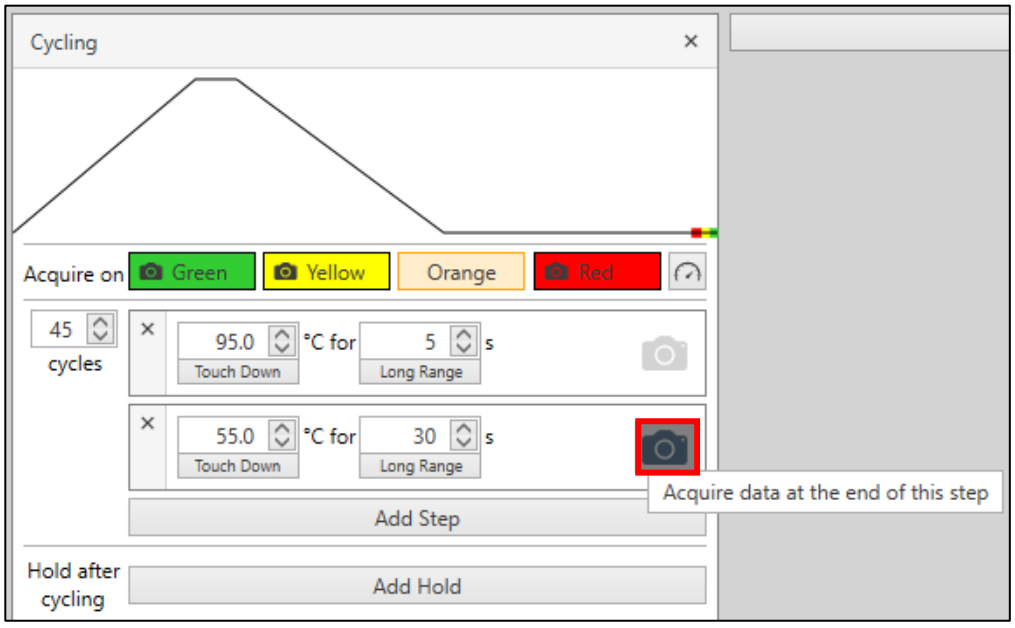
Set the temperature and time of the second cycling step as *55* degrees for *30* seconds.
(Annealing/Extension step)

Remove the third cycling step as it is not required.



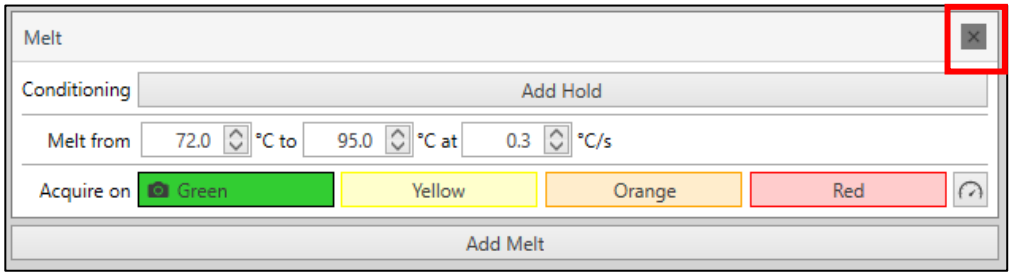
5.

Select the Annealing/Extension step to Acquire on by clicking the camera icon.



6.

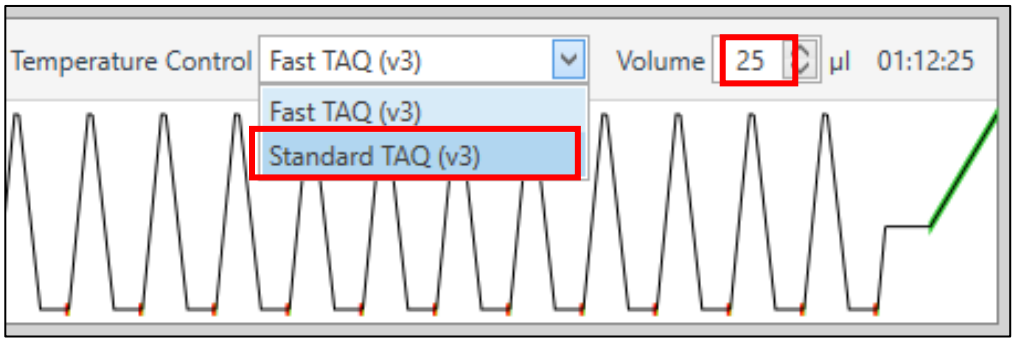
Remove the *Melt* step from the run profile.



7.

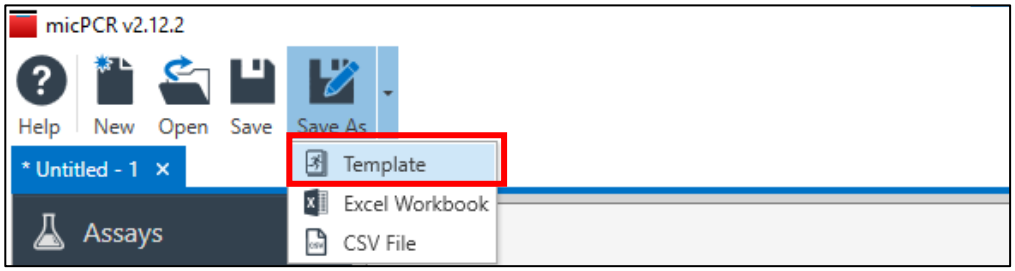
Select the *Standard TAQ (v3)* as the Temperature Control option.

Insert *25* µl as the volume.



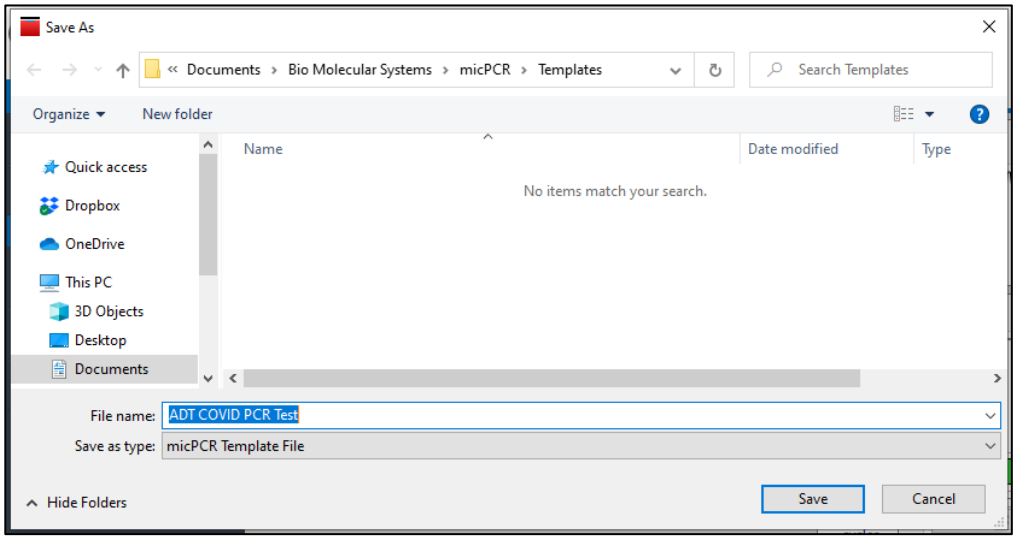
8.

Select the arrow next to the *Save As* button, and then select *Template.*



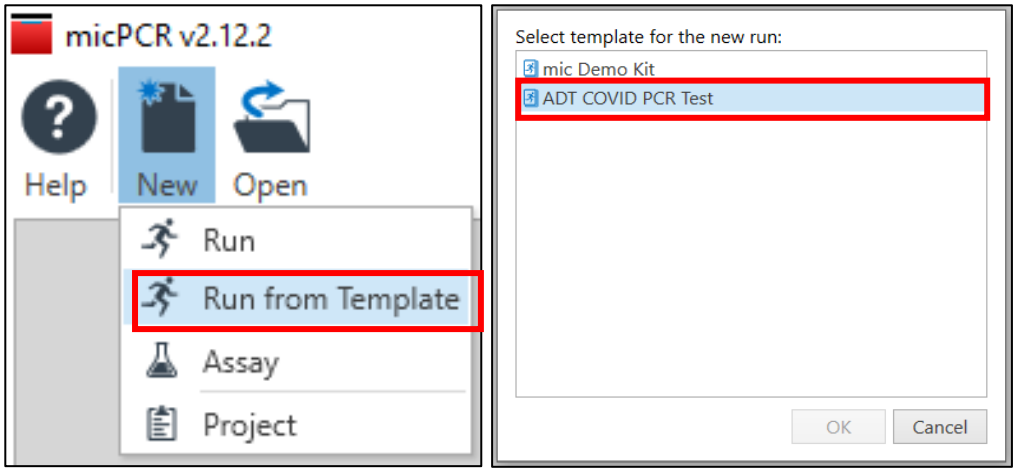
9.

Save the template in the Template library located in *Documents/BioMolecularSystems/micPCR/Templates*



10.

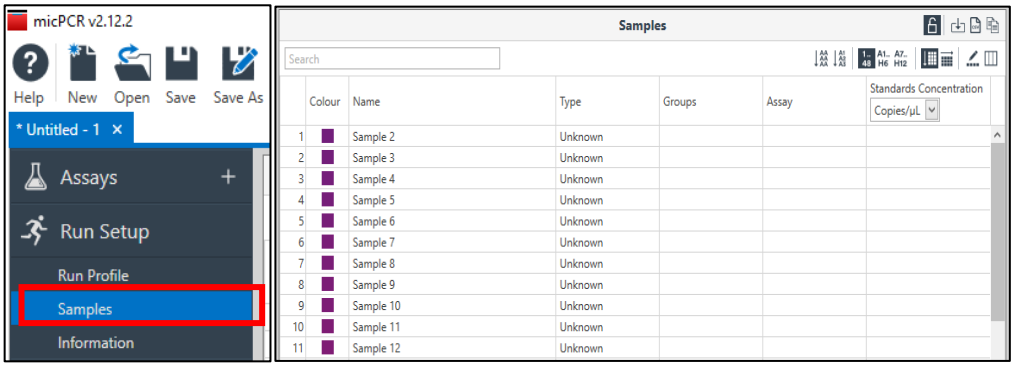
Once the run profile has been saved as a template you do not need to repeat the programming of the Mic qPCR cycler but can simply open the template from the *Template* folder for any new PCR runs.



11.

Select *Samples* from the navigator bar.

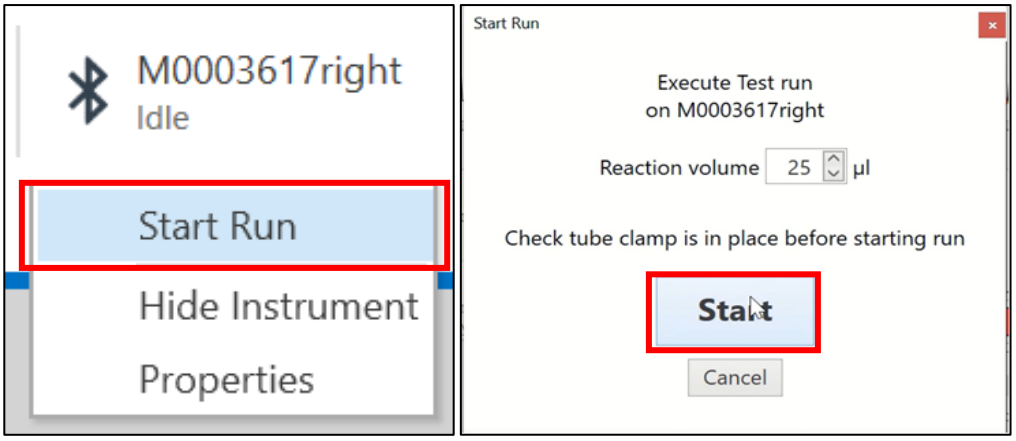
Enter the sample information in the *Samples* editor. This step can be performed before, during or after a run.



12.

Select the *Instrument* to be used for the run in the tool bar and then select the *Start Run* option from the drop-down list.

Start the run by clicking the *Start* button in the confirmation dialogue box.

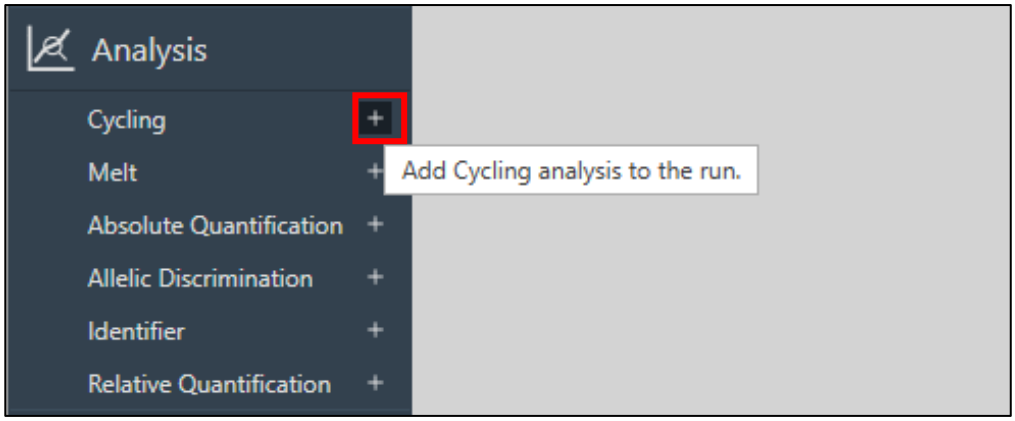


Data Analysis on the Mic

Data Analysis

1.

Once the run has completed, under the Analysis section of the navigator bar, select the *Add* button next to *Cycling*.

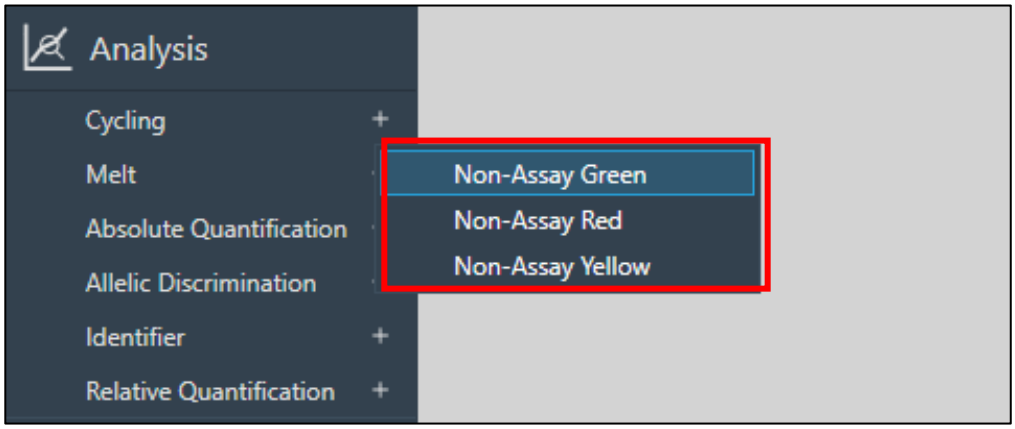


2.

Select the targets to analyse from the list of options.

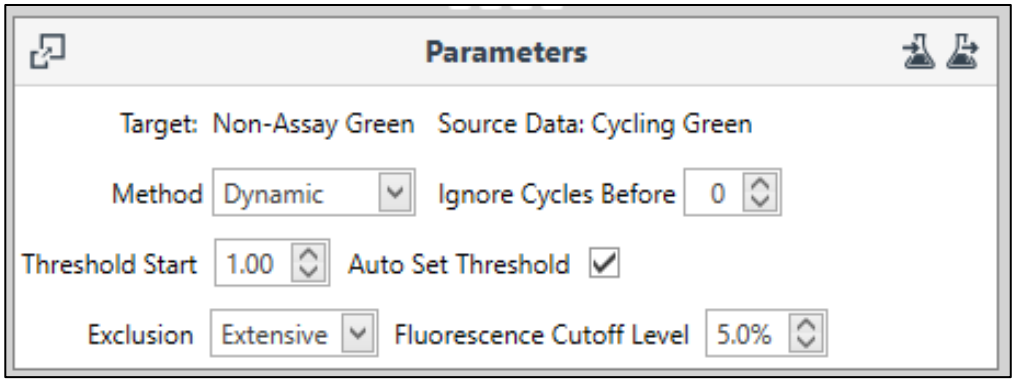
Select *Non-Assay Green*, *Non-Assay Red* and *Non-Assay Yellow*.

Select the *Non-Assay Green* to begin analysis.



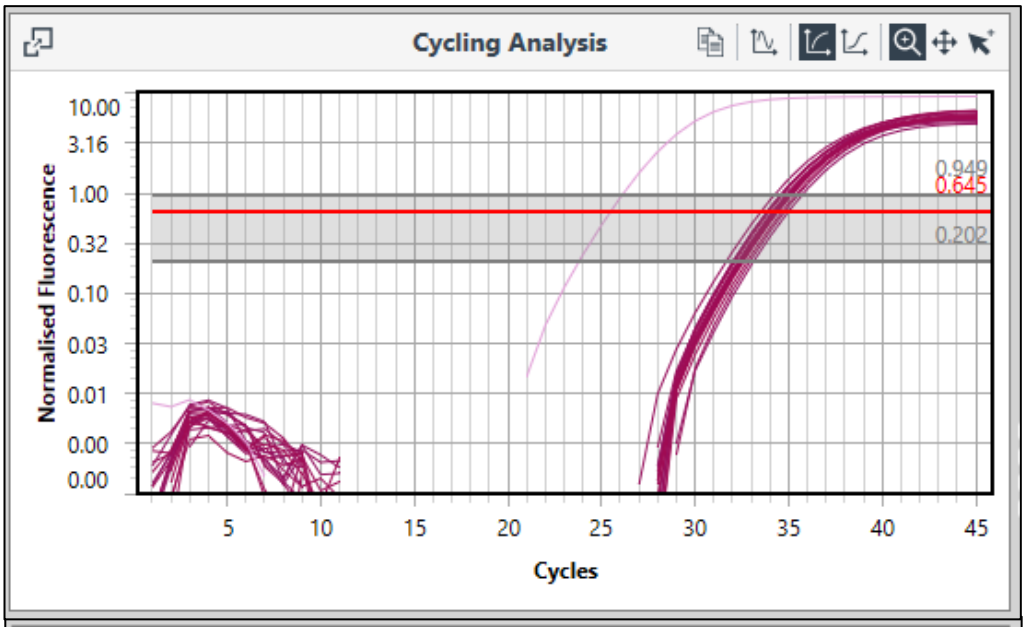
3.

Use the default set of analysis parameters.



4.

The quantification cycle (Cq) of each sample is displayed in the results table; a positive result is indicated by an amplification signal in the plot area and a Cq value in the table. A negative result is indicated by the absence of both.

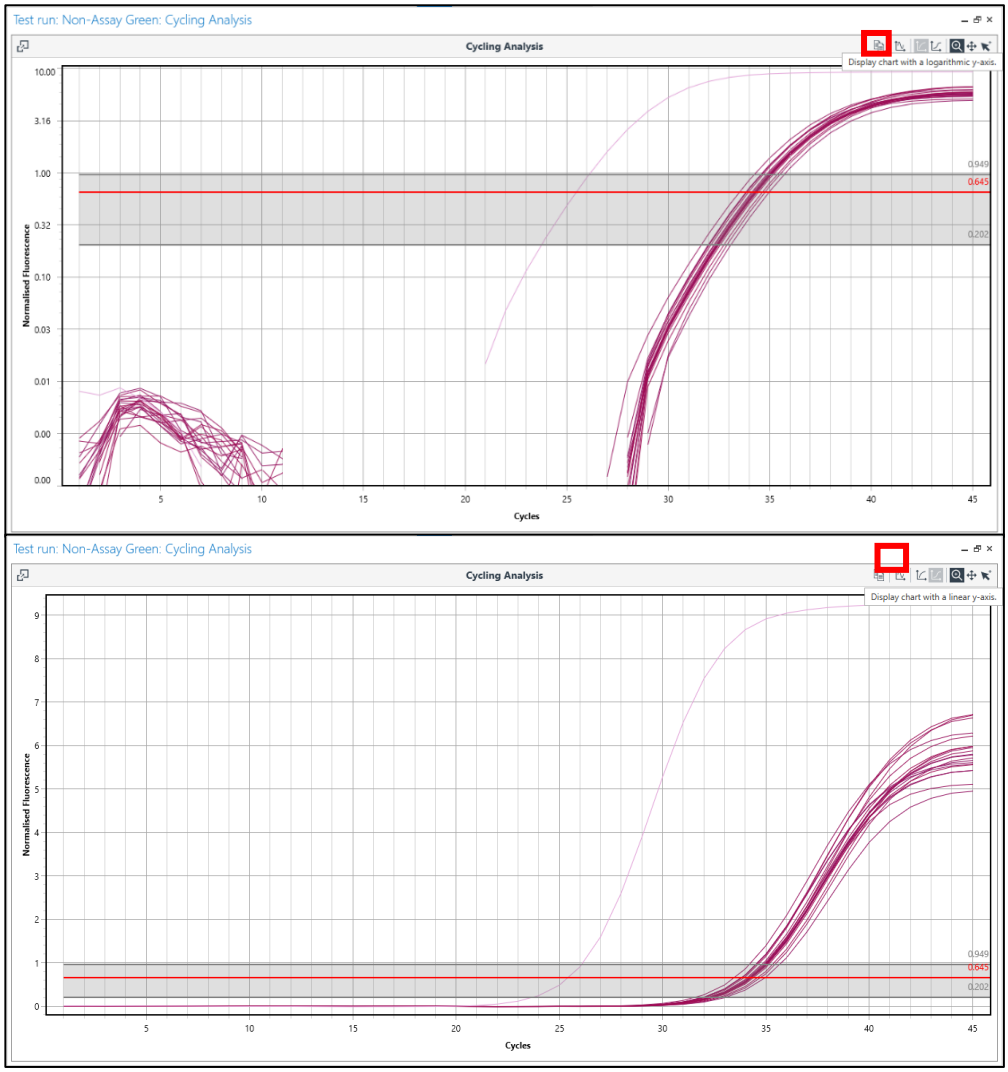


Results

Well	Sample	Cq	Efficien...	R^2	Result
28	Sample 28	34.12	0.81	0.99805	
29	Sample 29	34.21	0.81	0.99820	
30	Sample 30	33.80	0.83	0.99833	
31	Sample 31	33.77	0.83	0.99831	
32	Sample 32	34.93	0.81	0.99787	
33	Sample 33	34.26	0.81	0.99848	
34	Sample 34	34.22	0.82	0.99808	
35	Sample 35	34.22	0.82	0.99810	
36	Sample 36	33.43	0.82	0.99869	
37	Sample 37	34.25	0.81	0.99858	
38	Sample 38	34.70	0.83	0.99854	
39	Sample 39	33.81	0.83	0.99789	
40	Sample 40	34.07	0.82	0.99816	
41	Sample 41	34.30	0.81	0.99866	
42	Sample 42	34.21	0.83	0.99848	
43	Sample 43	33.91	0.83	0.99823	
44	Sample 44	34.56	0.81	0.99864	
45	Sample 45	34.17	0.82	0.99827	
46	Sample 46	34.36	0.81	0.99868	
47	PC	25.38	0.94	0.99938	
48	NTC				Excluded

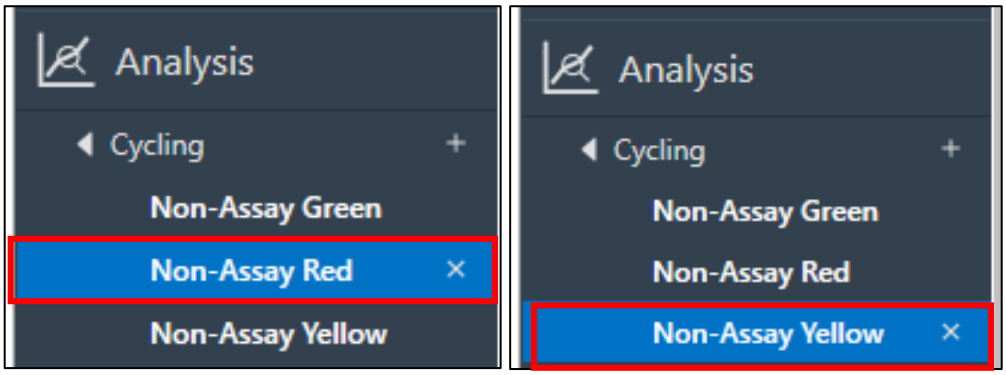
5.

Click on the buttons at the top of the Cycling Analysis bar to choose between *display chart with a logarithmic y-axis* or *display chart with a linear y-axis*.



6.

Repeat the Data Analysis steps for the Non-Assay Red and Non-Assay Yellow targets.



7.

Analyse the PCR results for each individual sample and the validity of the overall PCR experiment as per the validation criteria set out in detail in the Instructions for Use; in brief,

A. The No Template Control must always be negative in the green and red channels, and positive in the yellow (Internal Control) channel **(Well 48 in image).**

B. The Positive Control must be positive in all three channels **(Well 47 in image).**

C. A positive sample is indicated by amplification in the green and red channels **(Well 46 in image).**

D. A negative sample is indicated by an absence of signals in the green and red channels, but must have amplification in the yellow (Internal Control) channel to rule out PCR inhibition **(Well 25 in image)**.