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IdPath™ COVID-19 Real-Time RT-PCR Kit

(Cat no. IP2000)

Software Setting Guide

Rev. Version: Nov 26, 2021 V6.3

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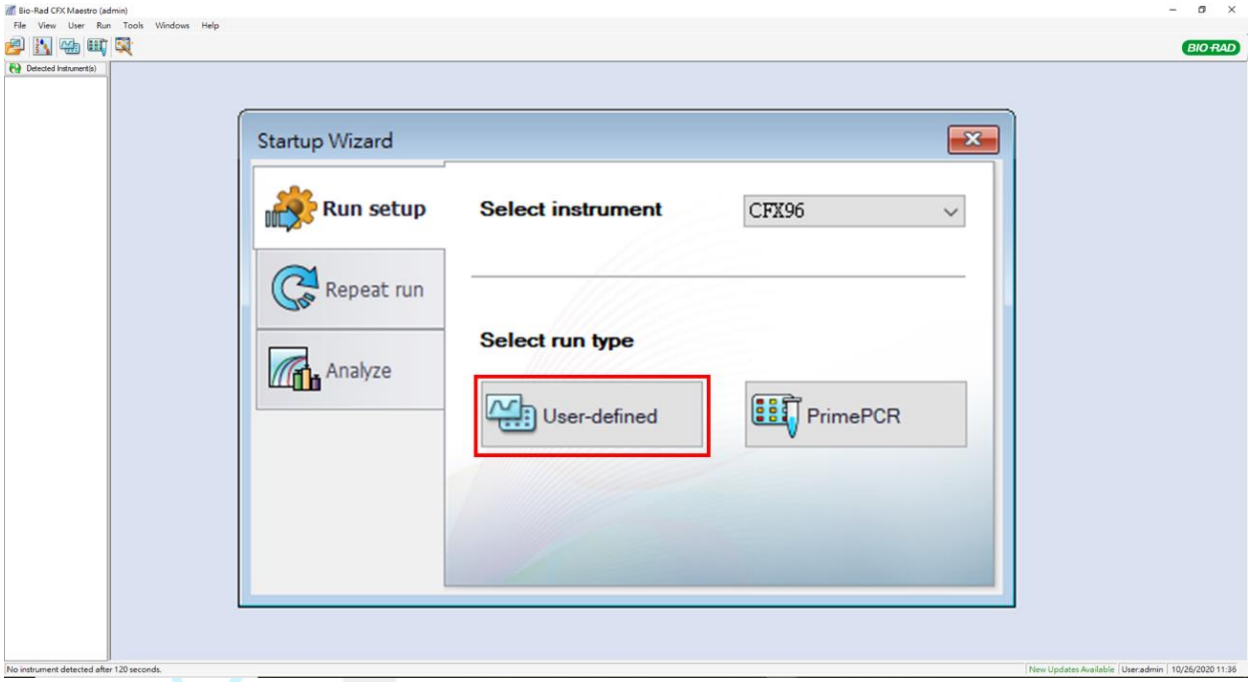
Software Setting

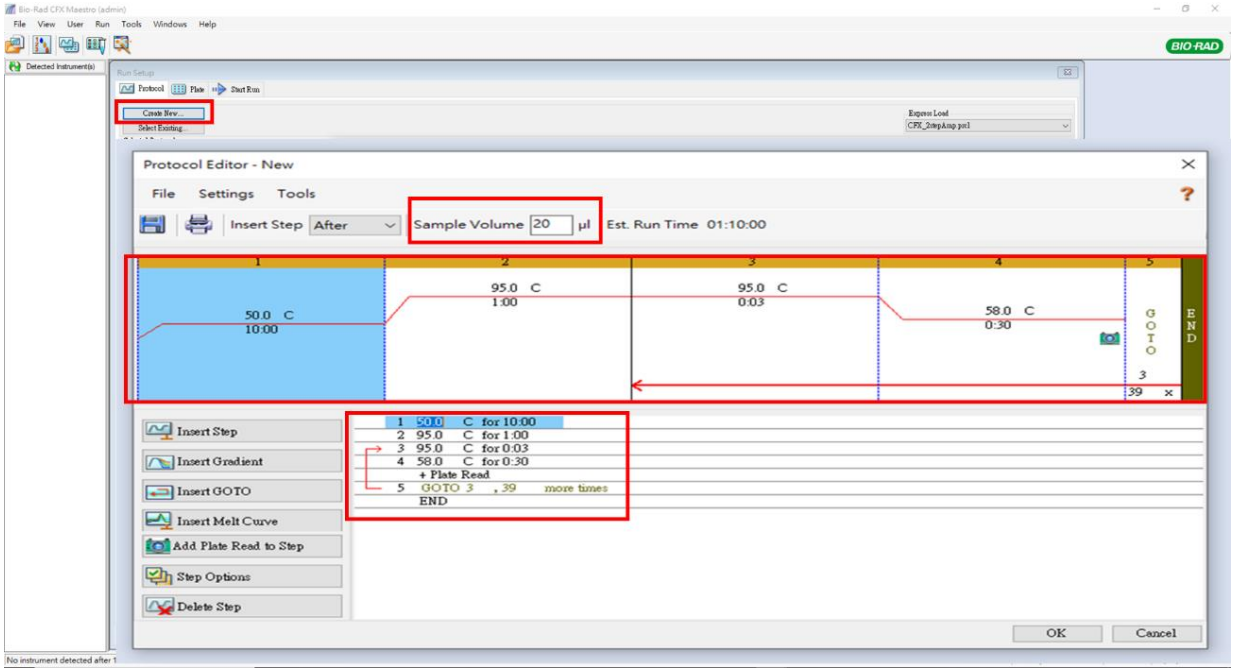
1. CFX96 Touch™ Real-Time PCR Detection System (BIO-RAD, Product No. 1855196, Software Bio-Rad CFX Maestro version 4.1)

i. Run a software and click “User-defined”

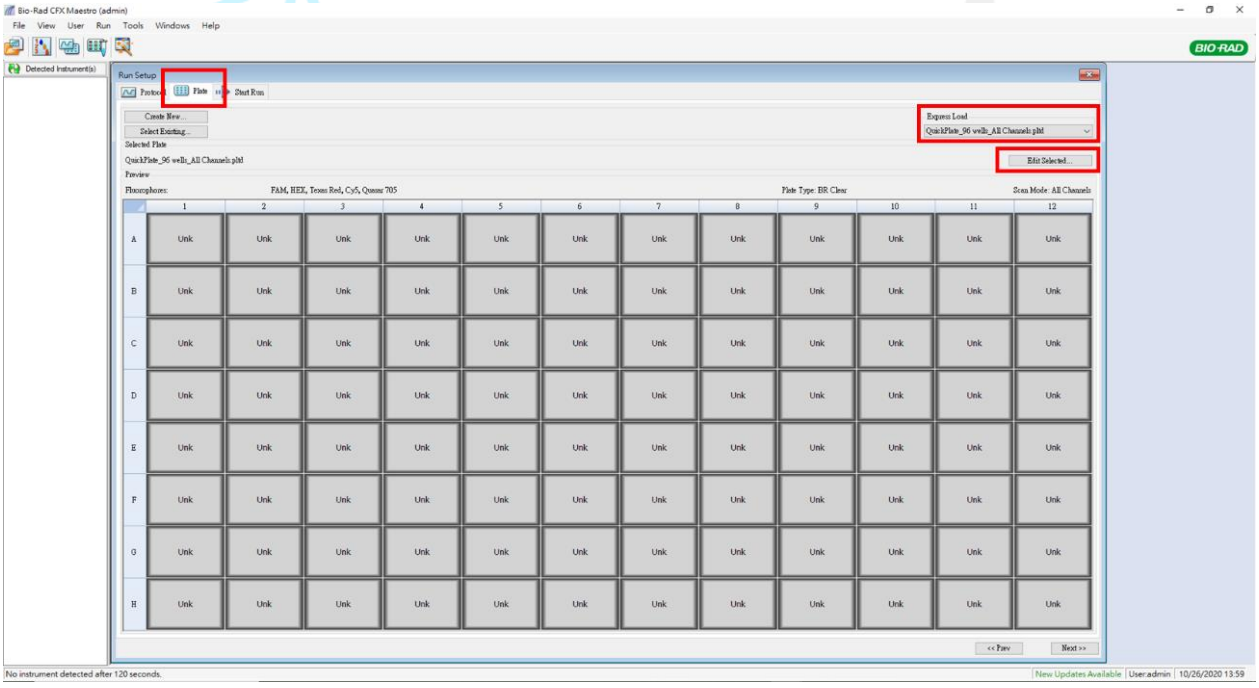


ii. Click “Create New” and enter the reaction volume as 20 µl and modify PCR reaction conditions as below.

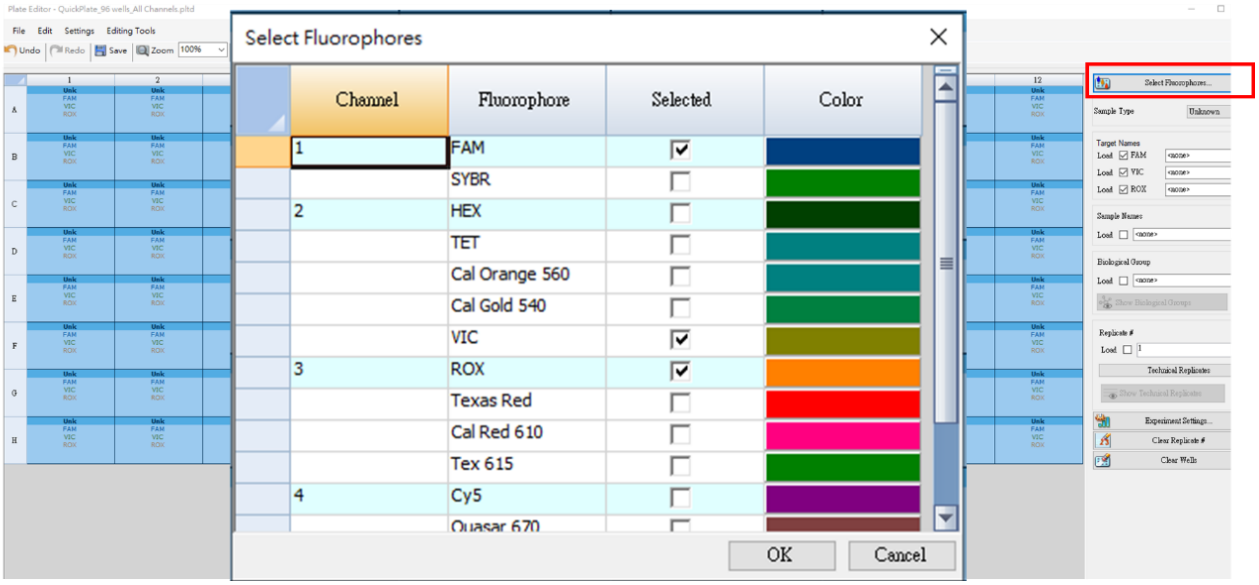
Step	Temperature	Time	Cycle
RT	50°C	10 min	1
Incubation	95 °C	1 min	1
Amplification	95 °C	3 sec	40
	58 °C	30 sec	



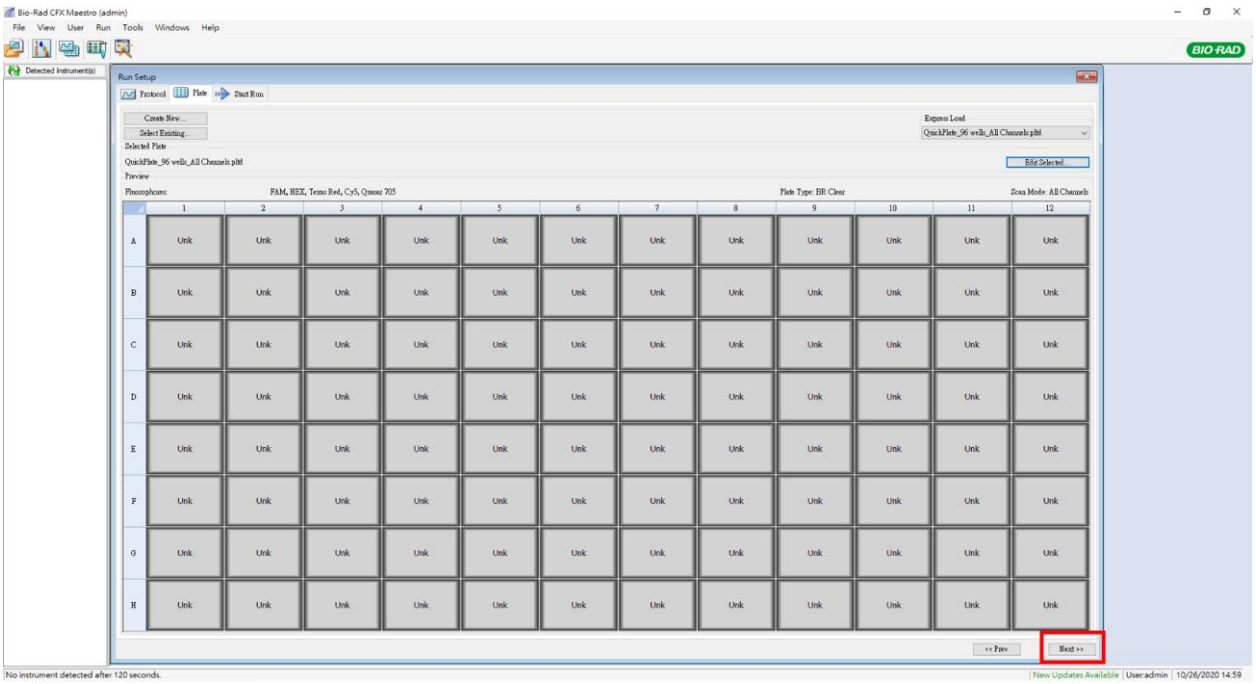
iii. Click “Plate” and check the “Express Load: QuickPlate_96 wells_All Channels.pltd” and click “Edit selected”.

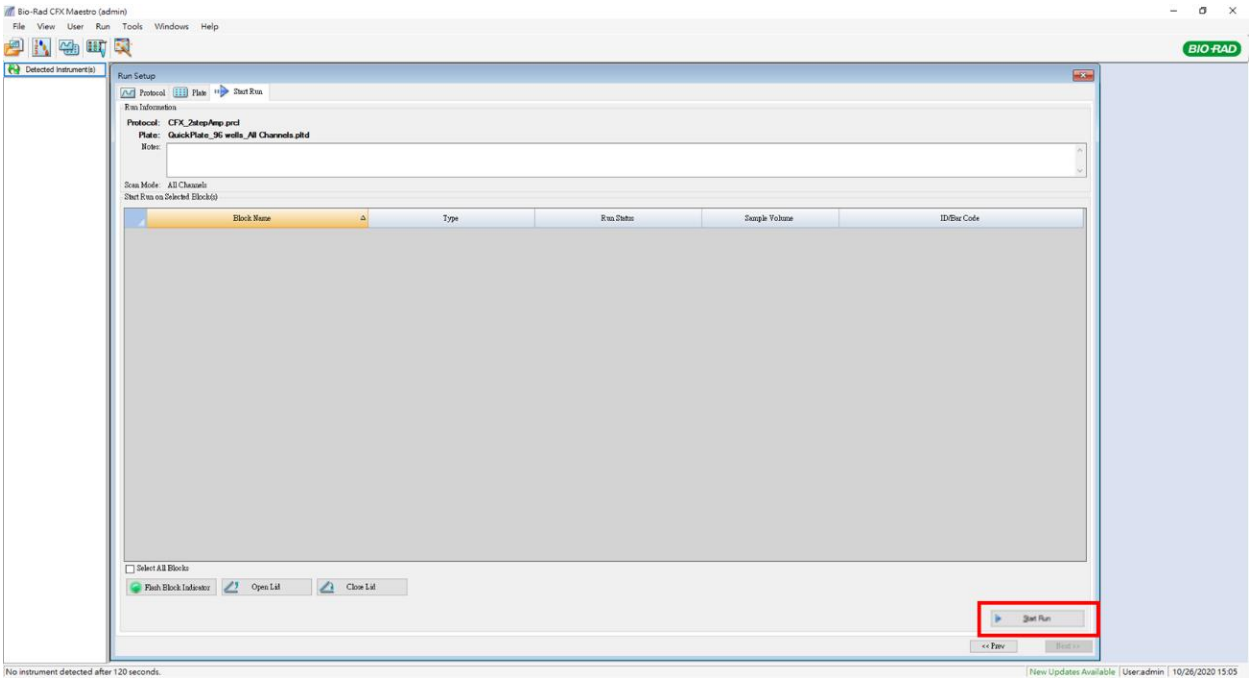


iv. Click “Select Fluorophores” and tick FAM, VIC and ROX. Also, define 96 well PCR plate layout on program.



v. Click “Next” and click “Start Run”.



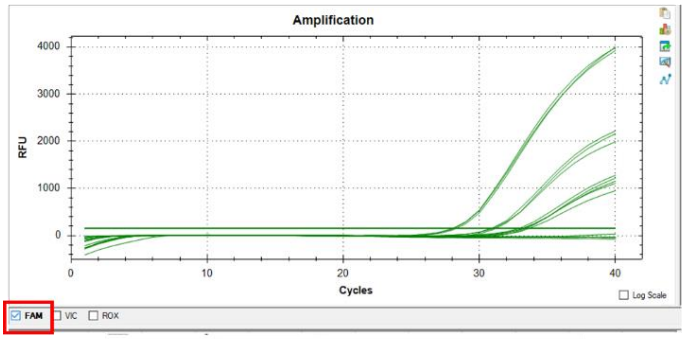


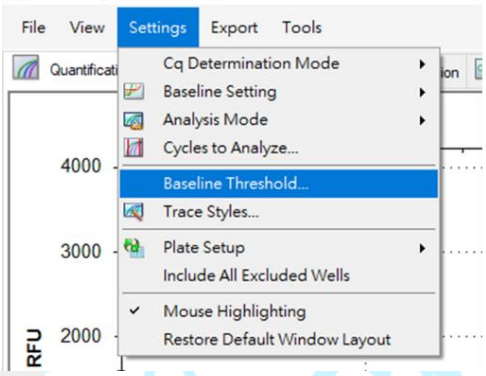
vi. For data analysis, follow the settings summarized in the table below. More detailed instructions for setting FAM, VIC, ROX channels are also demonstrated as below.

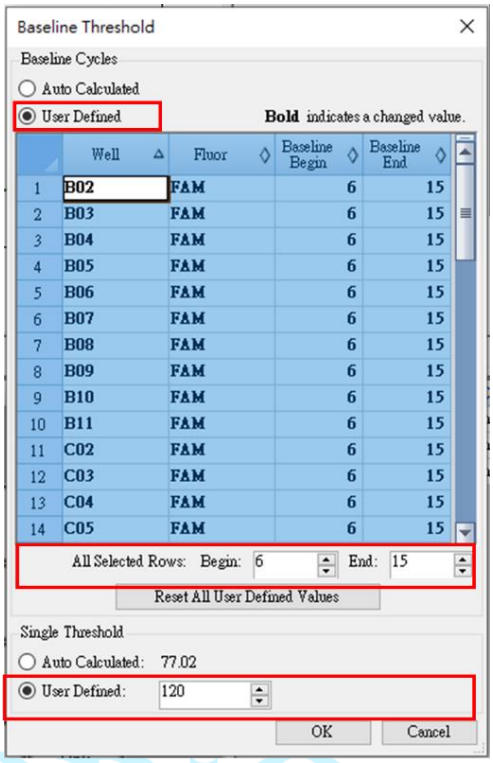
For 96 well PCR plate:

CFX96 (Bio-Rad)- 96 well PCR plate			
Channel	**Threshold**	**Baseline**	
		Begin	End
FAM	120	6	15
VIC	200		
ROX	200		

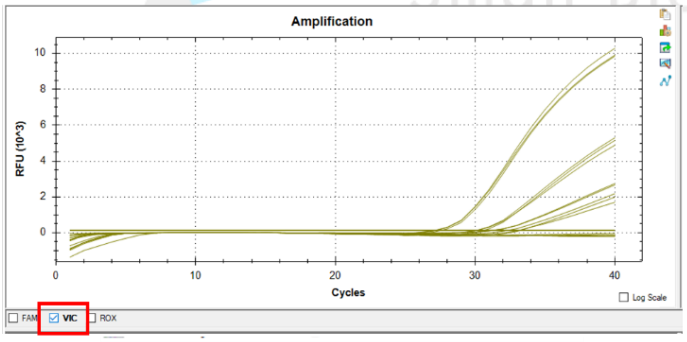
Channel: FAM

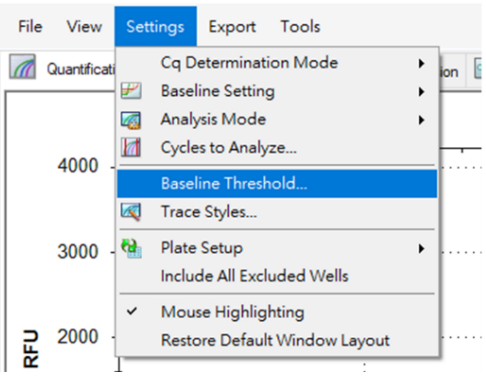


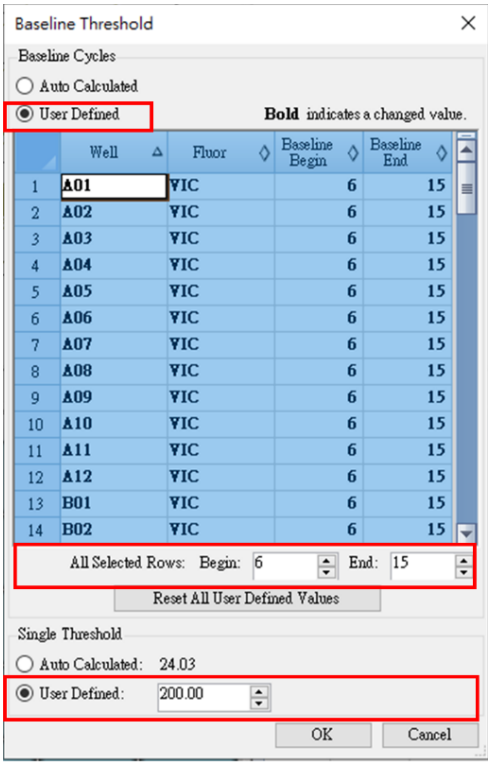




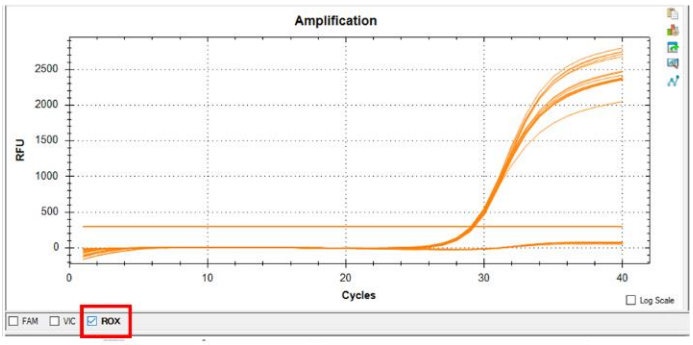
Channel: VIC

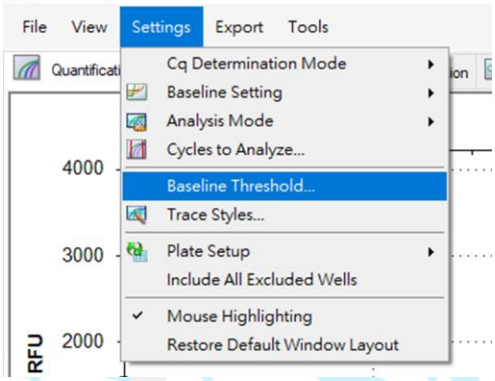


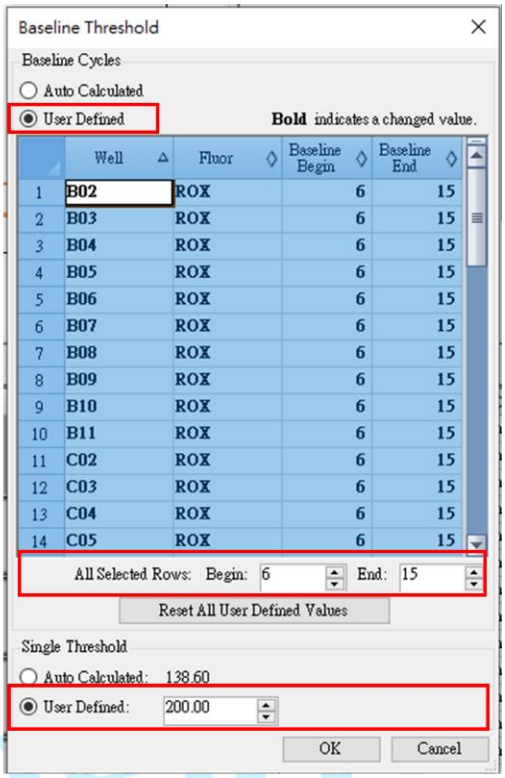




Channel: ROX

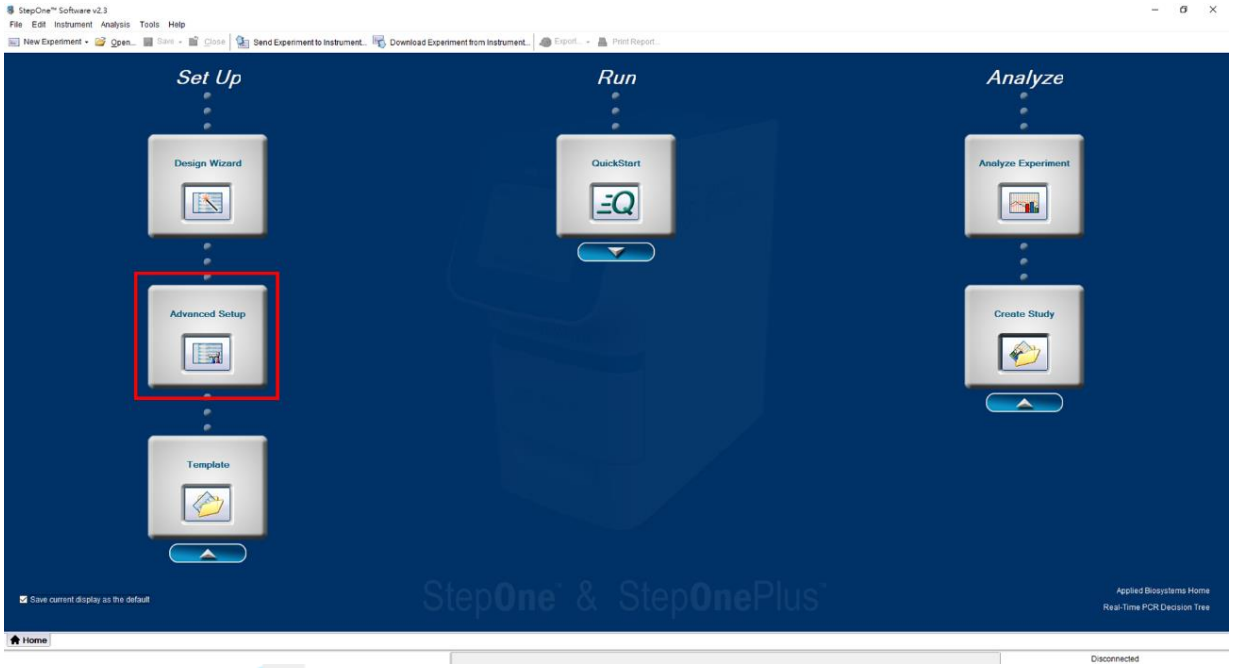

Amplification
RFU
2500
2000
1500
1000
500
0
0
10
20
30
40
Cycles
Log Scale
FAM
VIC
ROX


File
View
Settings
Export
Tools
Quantificati
Cq Determination Mode
Baseline Setting
Analysis Mode
Cycles to Analyze...
Baseline Threshold...
Trace Styles...
Plate Setup
Include All Excluded Wells
Mouse Highlighting
Restore Default Window Layout
4000
3000
2000
RFU

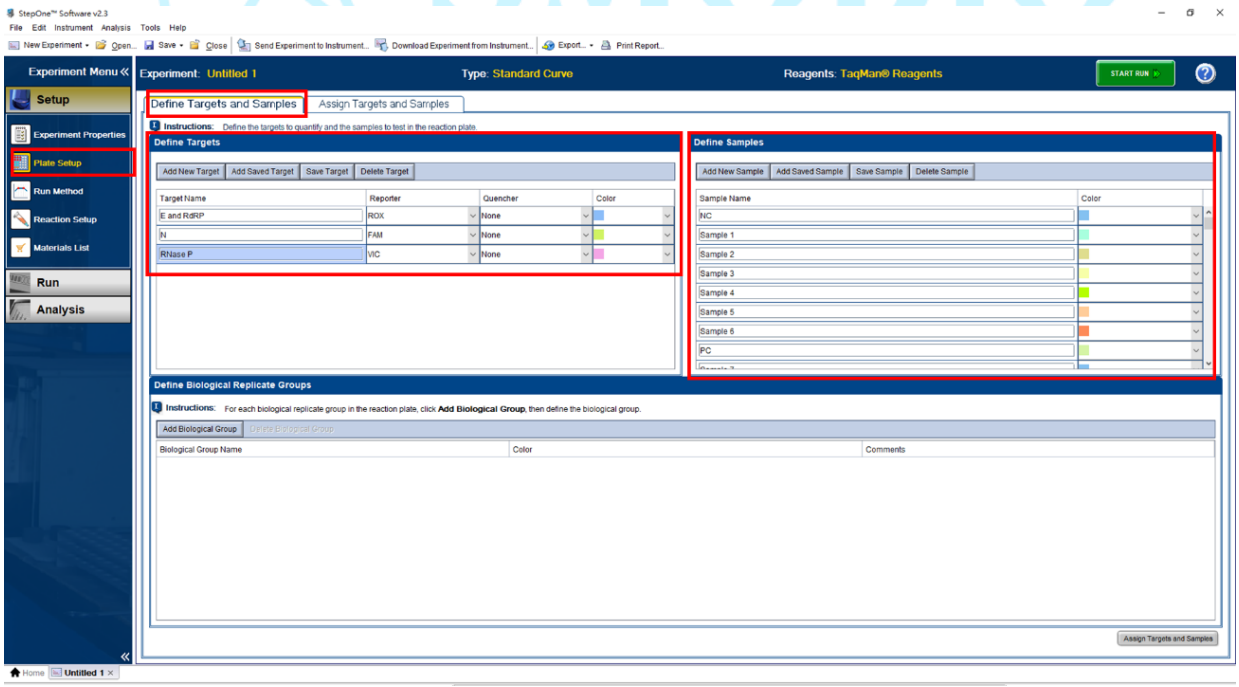

Baseline Threshold
Baseline Cycles
Auto Calculated
User Defined
Bold indicates a changed value.
Well
Fluor
Baseline Begin
Baseline End
1 B02 ROX 6 15
2 B03 ROX 6 15
3 B04 ROX 6 15
4 B05 ROX 6 15
5 B06 ROX 6 15
6 B07 ROX 6 15
7 B08 ROX 6 15
8 B09 ROX 6 15
9 B10 ROX 6 15
10 B11 ROX 6 15
11 C02 ROX 6 15
12 C03 ROX 6 15
13 C04 ROX 6 15
14 C05 ROX 6 15
All Selected Rows: Begin: 6 End: 15
Reset All User Defined Values
Single Threshold
Auto Calculated: 138.60
User Defined: 200.00
OK
Cancel

2. Applied Biosystems StepOnePlus Real-Time PCR Instrument System (Thermo Fisher Scientific, Product No. 4376357, Software version 2.3)

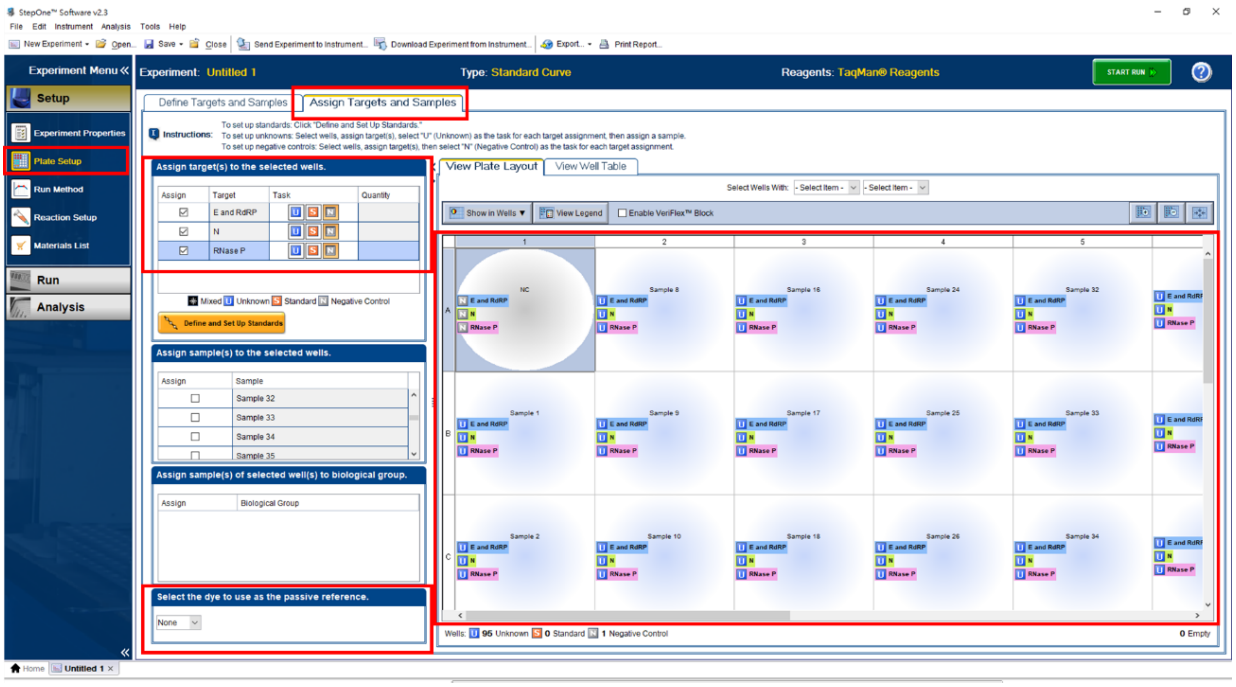
i. Run a software and click “Advanced setup”.



ii. Click “Plate setup” and select “VIC” for E and RdRP target, “FAM” for N target and “ROX” for RNaseP target in “Define Targets and Samples”.

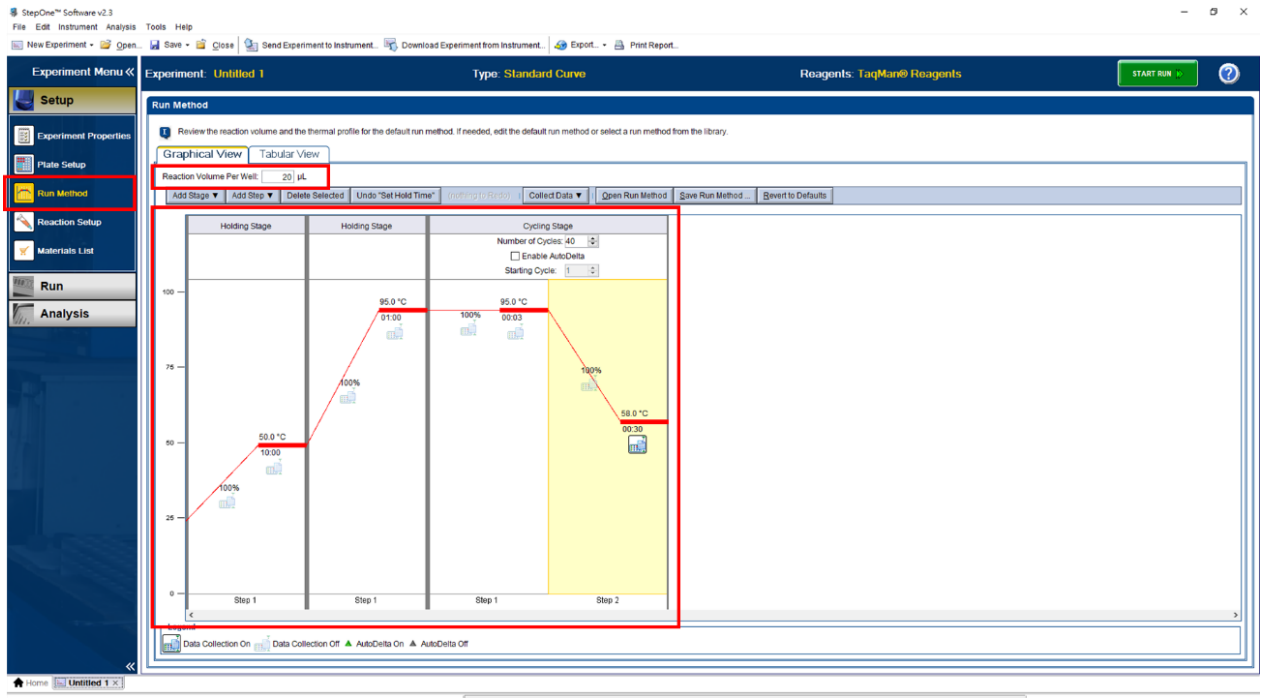


iii. Click “Assign Targets and Samples” and define 96 well PCR plate layout on program. Also, select “None” in the “Select the dye to use as the passive reference”.

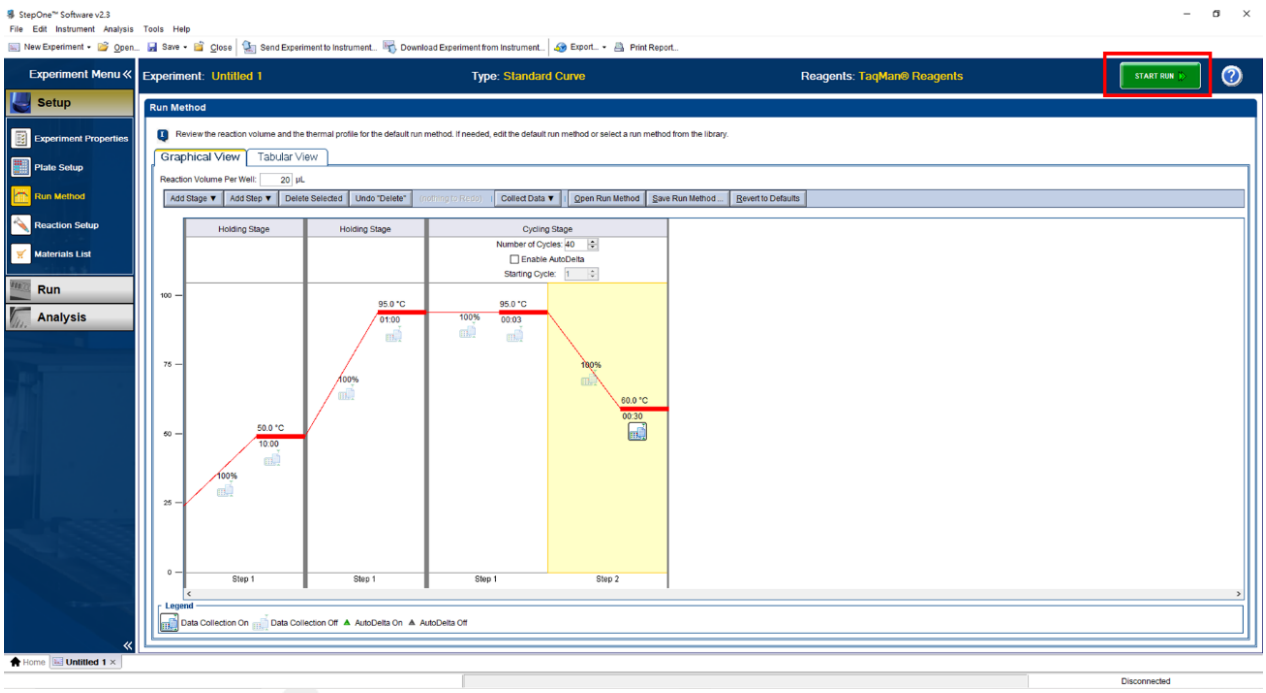


iv. Click “Run Method” and enter the reaction volume as 20 µl and modify PCR reaction conditions as below.

Step	Temperature	Time	Cycle
RT	50°C	10 min	1
Incubation	95 °C	1 min	1
Amplification	95 °C	3 sec	40
	58 °C	30 sec	

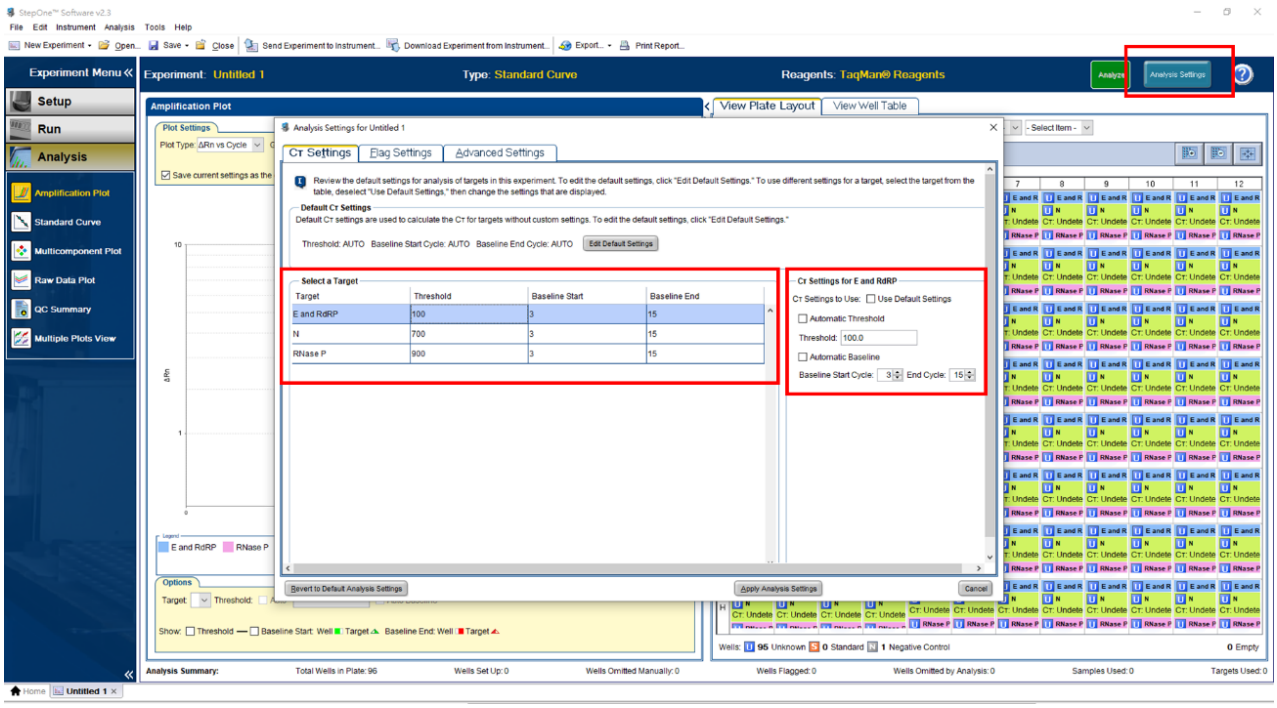


v. Click ”Start Run ”.



vi. For data analysis, set settings as shown in the table below.

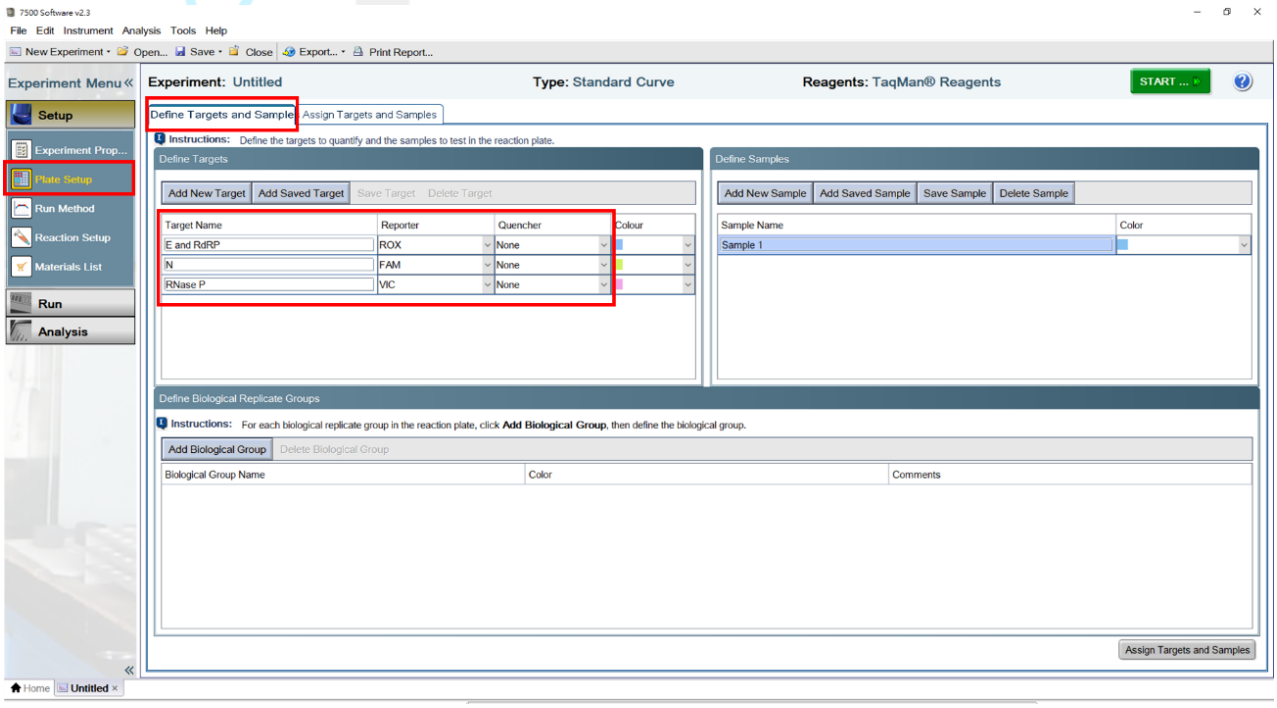
ABI StepOnePlus			
Channel	Threshold	Baseline	
		Begin	End
FAM	3900	6	15
VIC	4100		
ROX	1100		



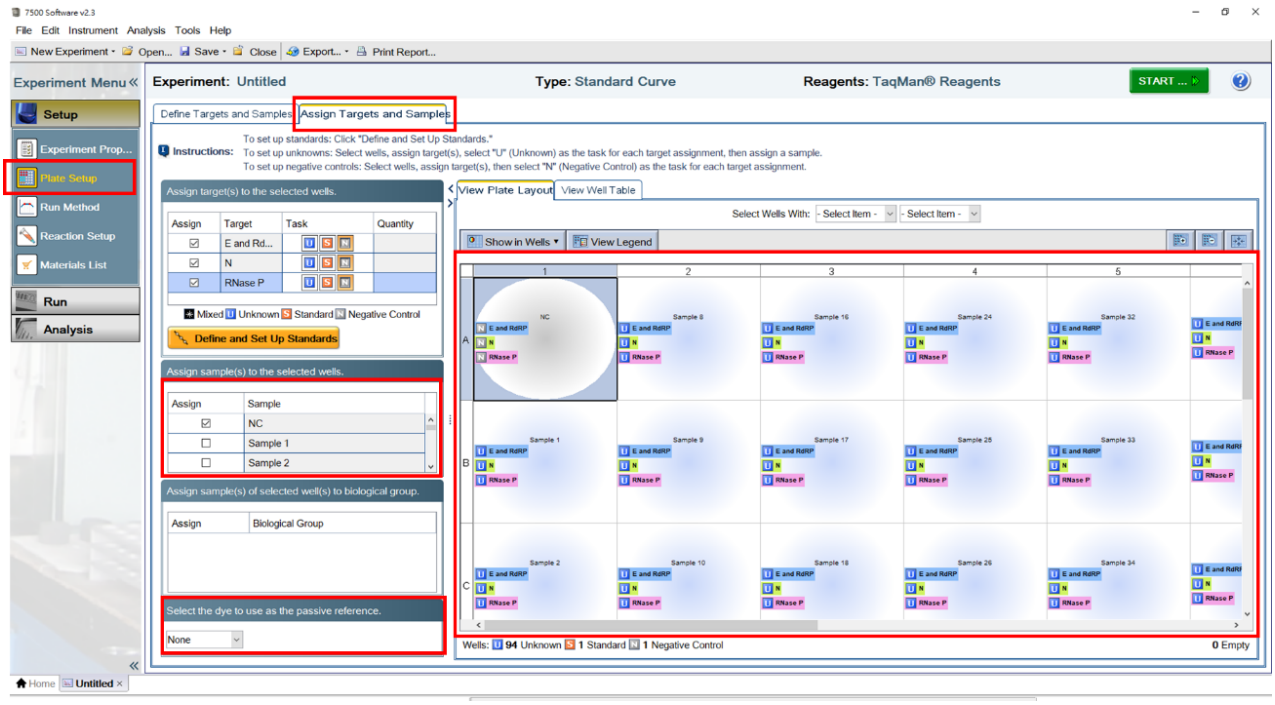
3. Applied Biosystems 7500 Fast Real-Time PCR Instrument System (Thermo Fisher Scientific, Product No. 4345241, Software version 2.0.6)

i. Run a software and click “Advanced setup”

ii. Click “Plate setup” and select “VIC” for E and RdRP target, “FAM” for N target and “ROX” for RNaseP target in “Define Targets and Samples”.

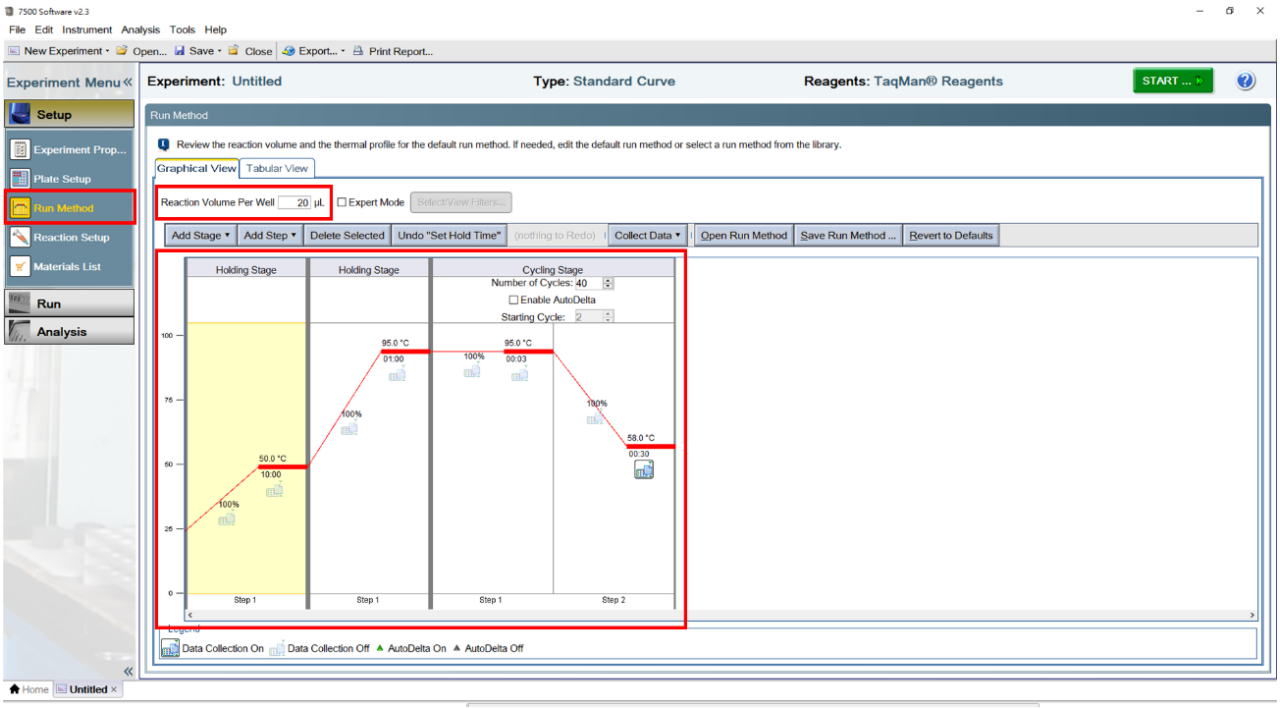


iii. Click “Assign Targets and Samples” and define 96 well PCR plate layout on program. Also, select “None” in the “Select the dye to use as the passive reference”.

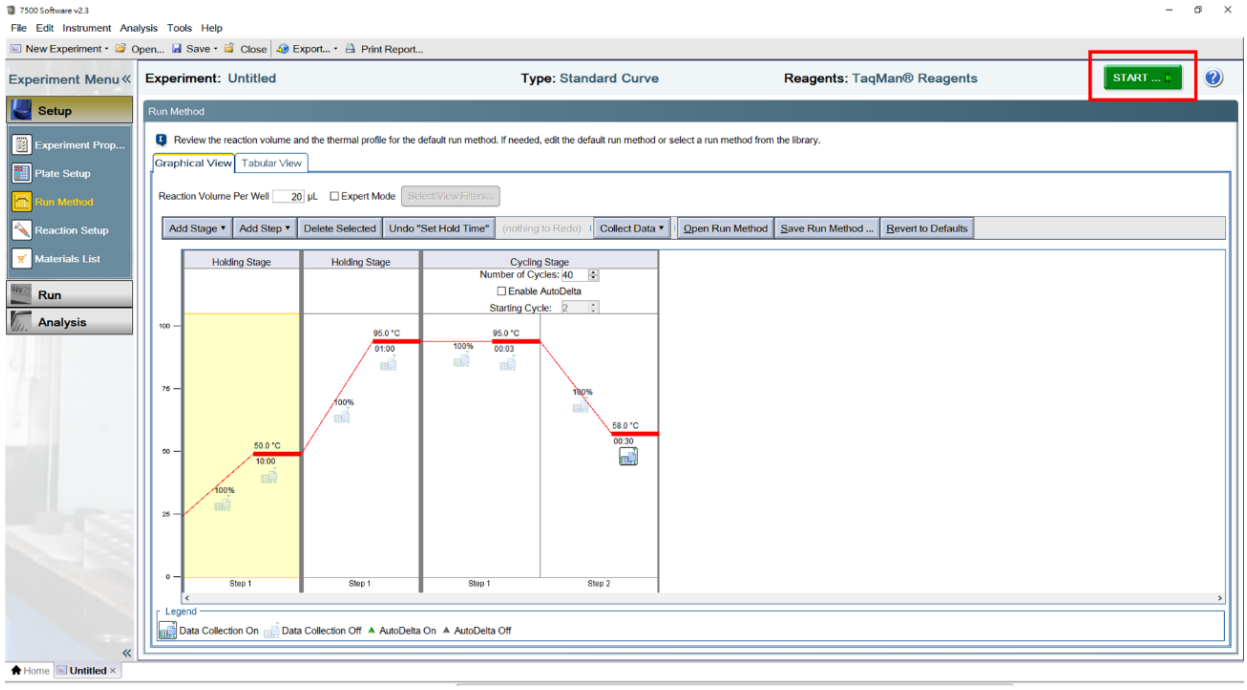


iv. Click “Run Method” and enter the reaction volume as 20 µL and modify PCR reaction conditions as below.

Step	Temperature	Time	Cycle
RT	50°C	10 min	1
Incubation	95 °C	1 min	1
Amplification	95 °C	3 sec	40
	58 °C	30 sec	

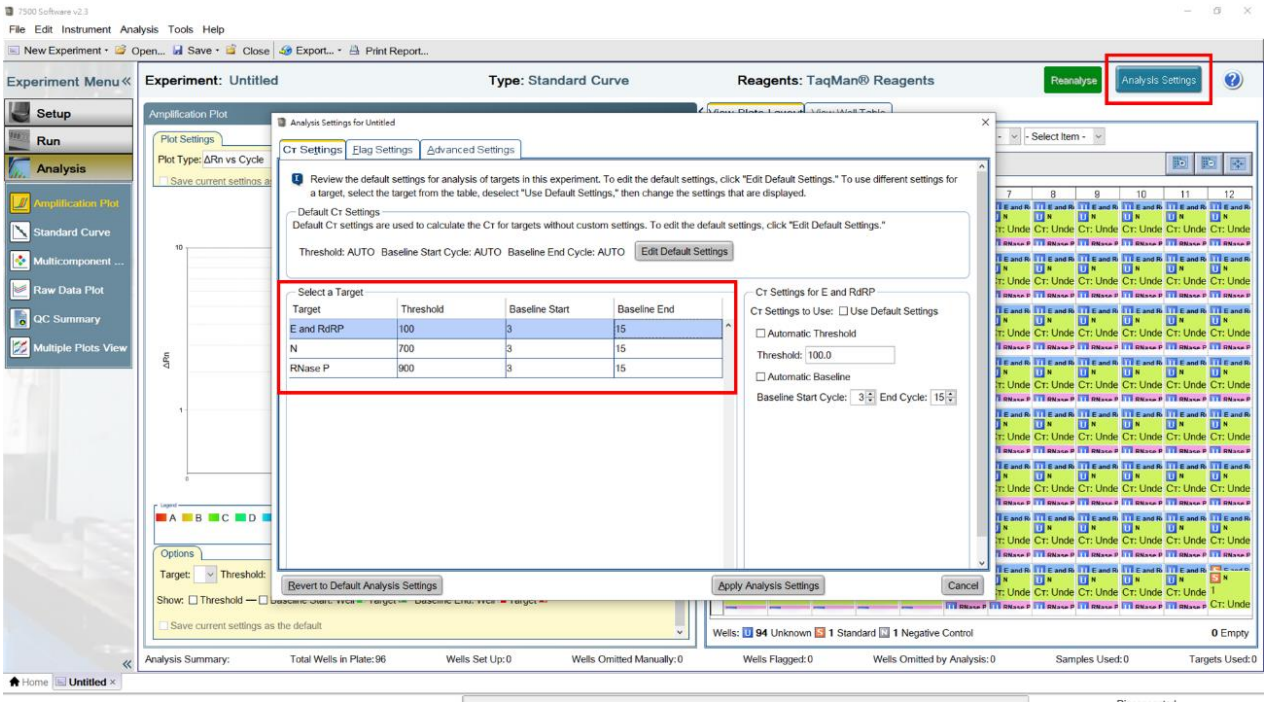


v. Click ”Start Run ”.



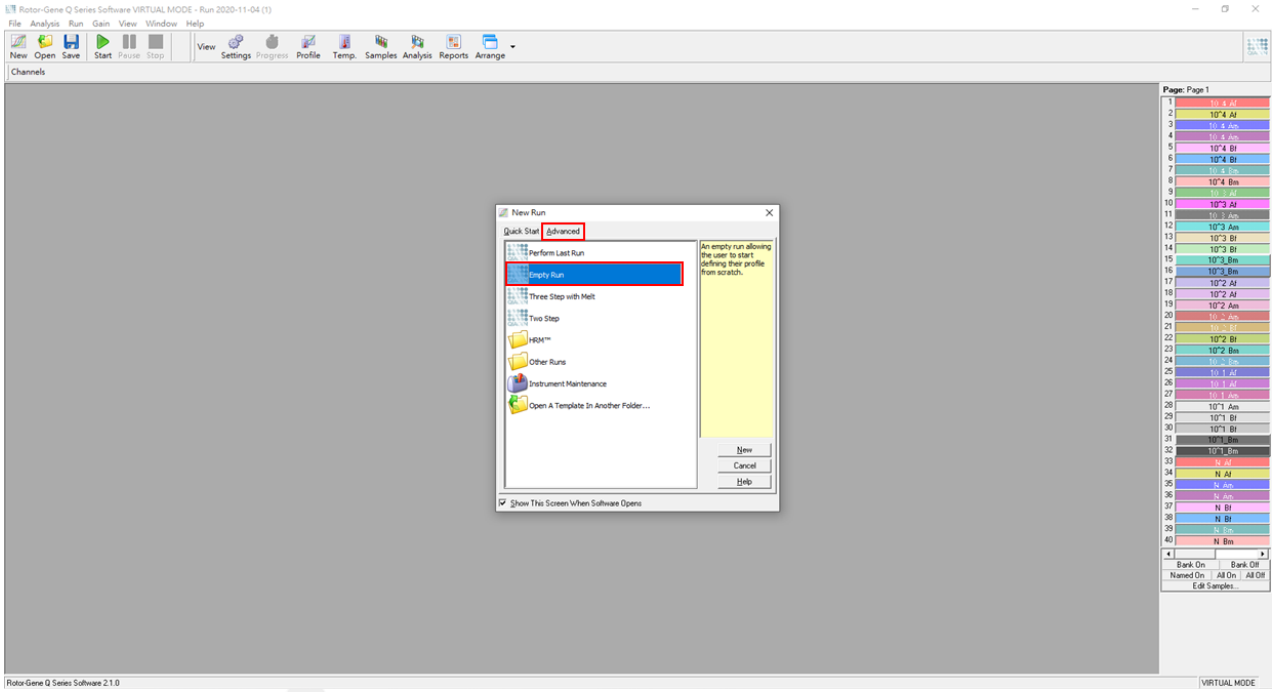
vi. For data analysis, set settings as shown in the table below.

ABI 7500			
Channel	**Threshold**	**Baseline**	
		Begin	End
FAM	95000	6	15
VIC	45000		
ROX	33000		

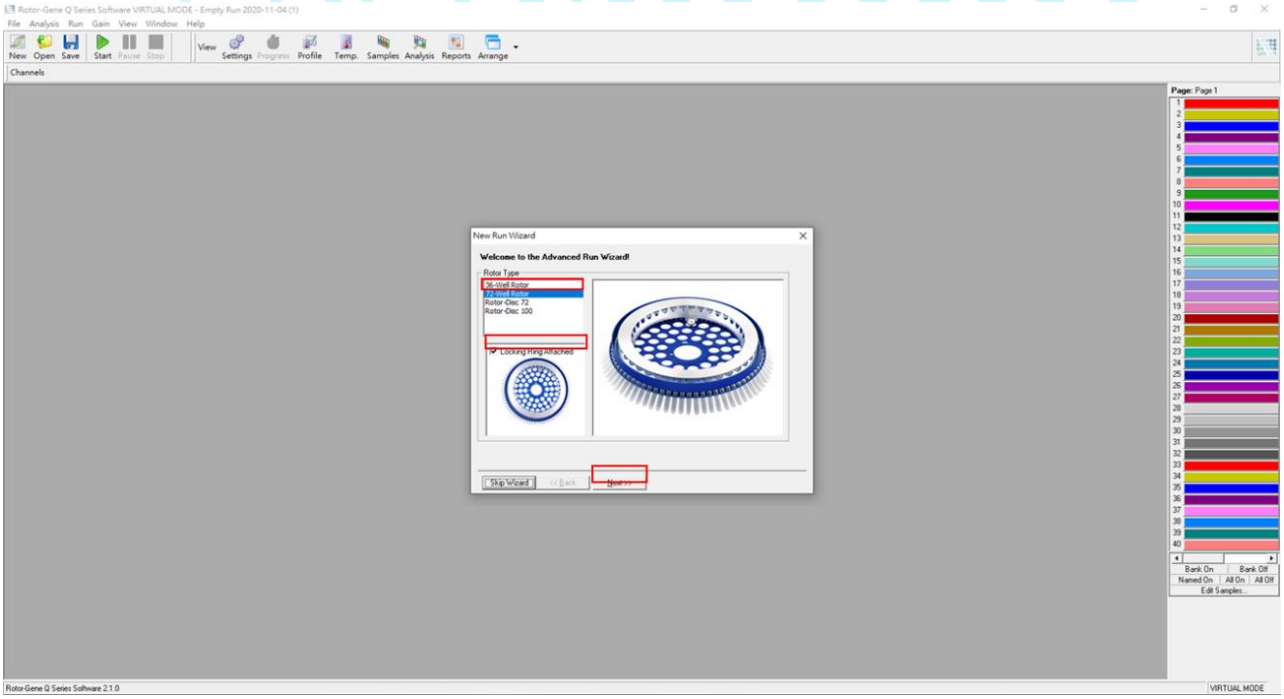


4. Rotor-Gene Q 5plex HRM (Qiagen, Product No. 9001580, Software version 2.3.4)

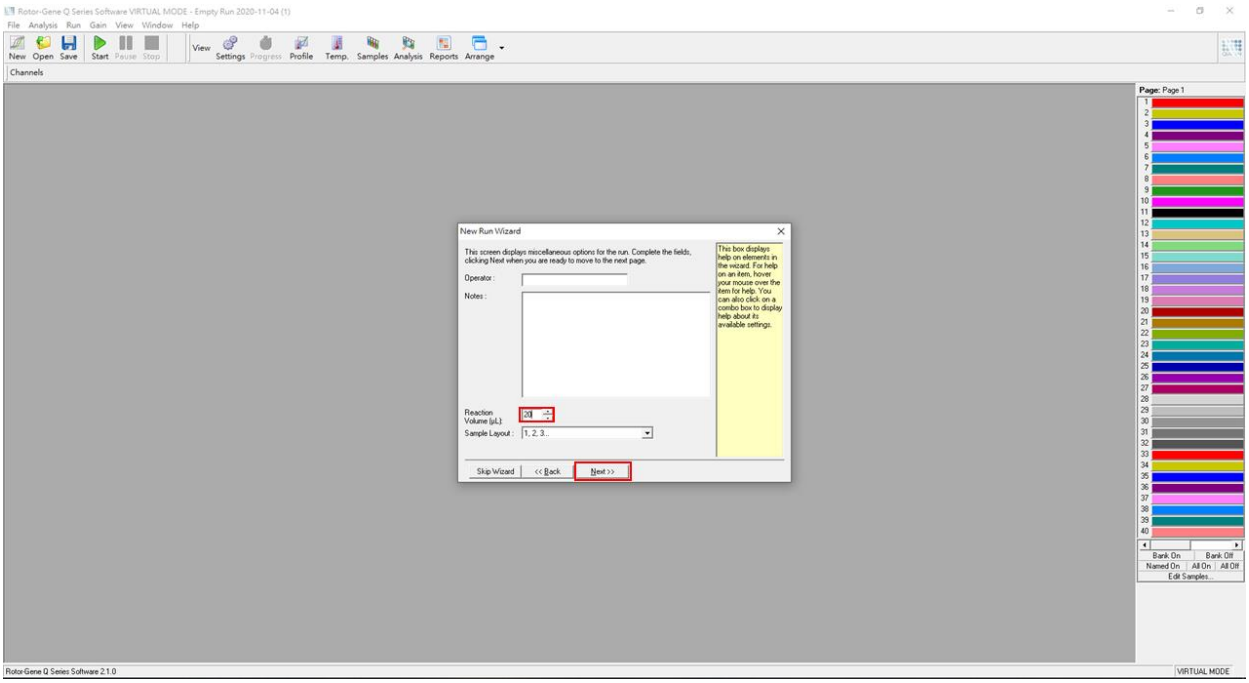
i. Run a software and click “Advance” label and select “Empty Run”.



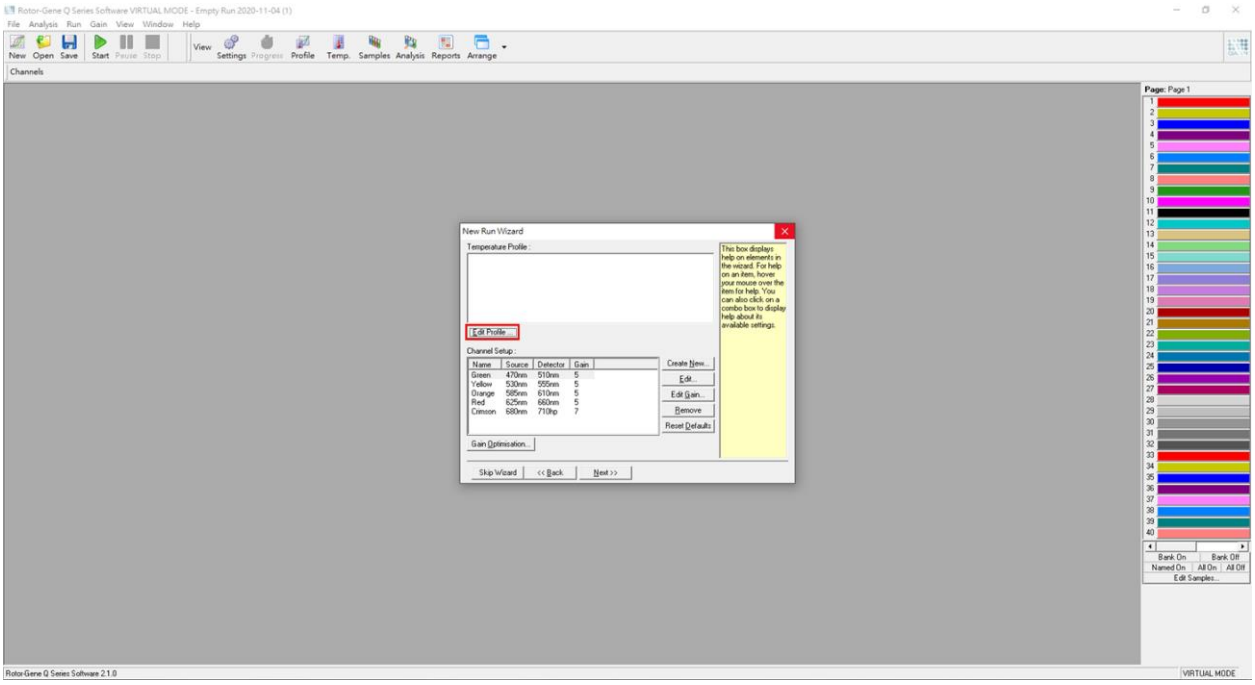
ii. Choose “72-well rotor” and tick check of “Locking ring attached”. Click “Next”.



iii. Enter the reaction volume as 20 (µL) and click “Next”.



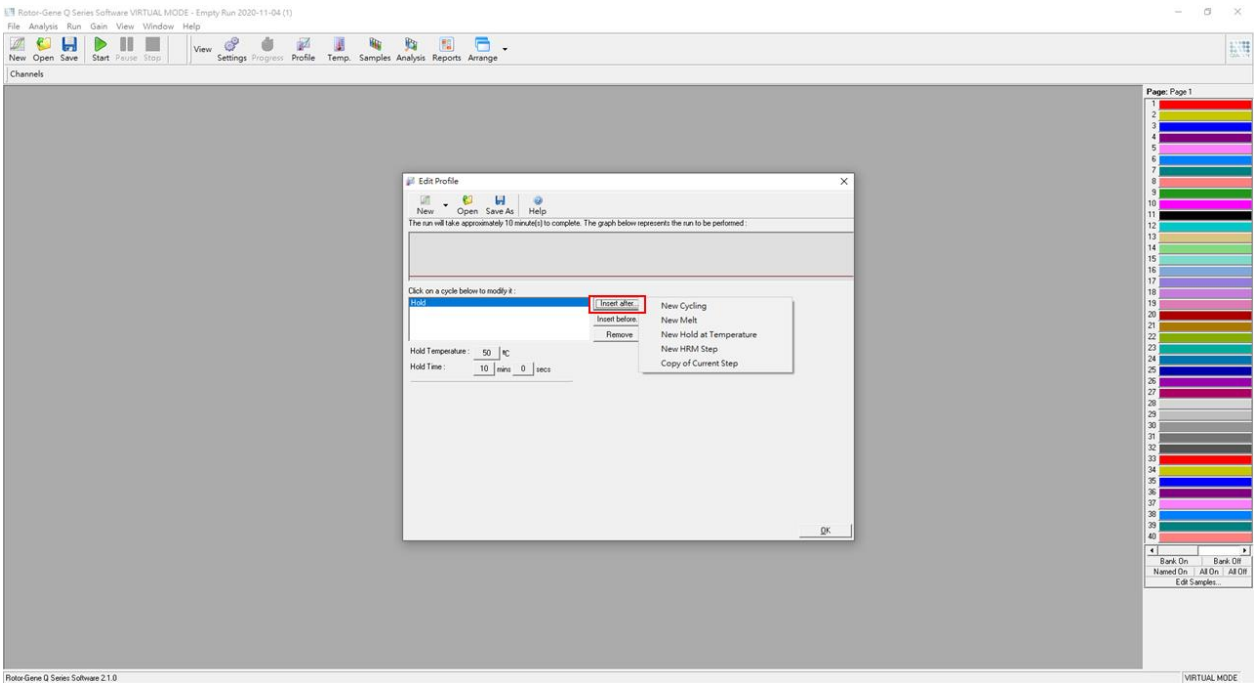
iv. Click “Edit profile”.



v. v. Click “Insert after”, and select hold program as below.

hold : 50℃ 10mins

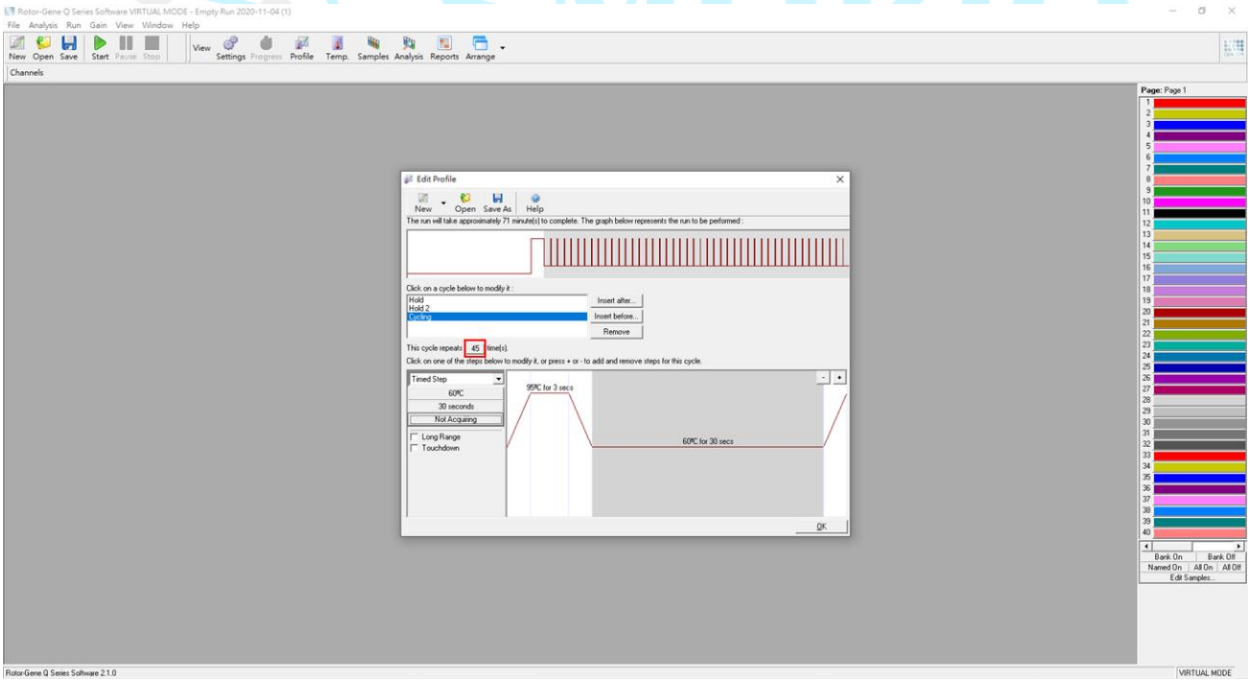
hold2 : 95℃ 1mins



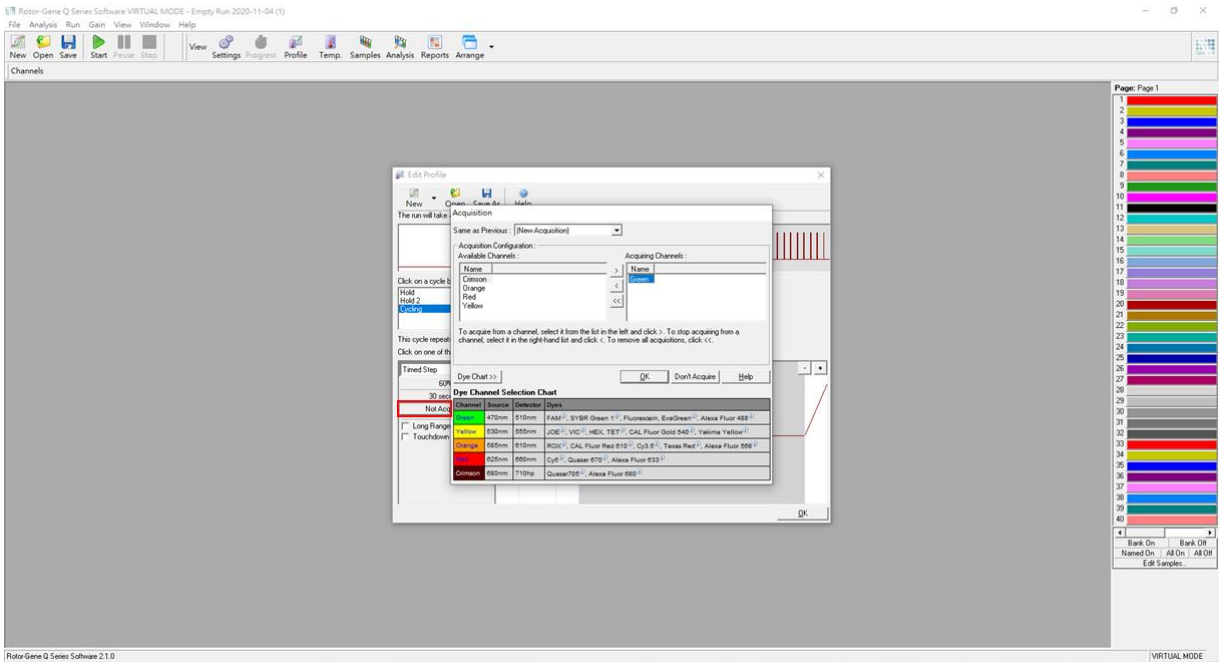
vi. Click “Insert after”, and select cycling program as below for 40 time(s).

cycling : 1. 95℃ 3 sec

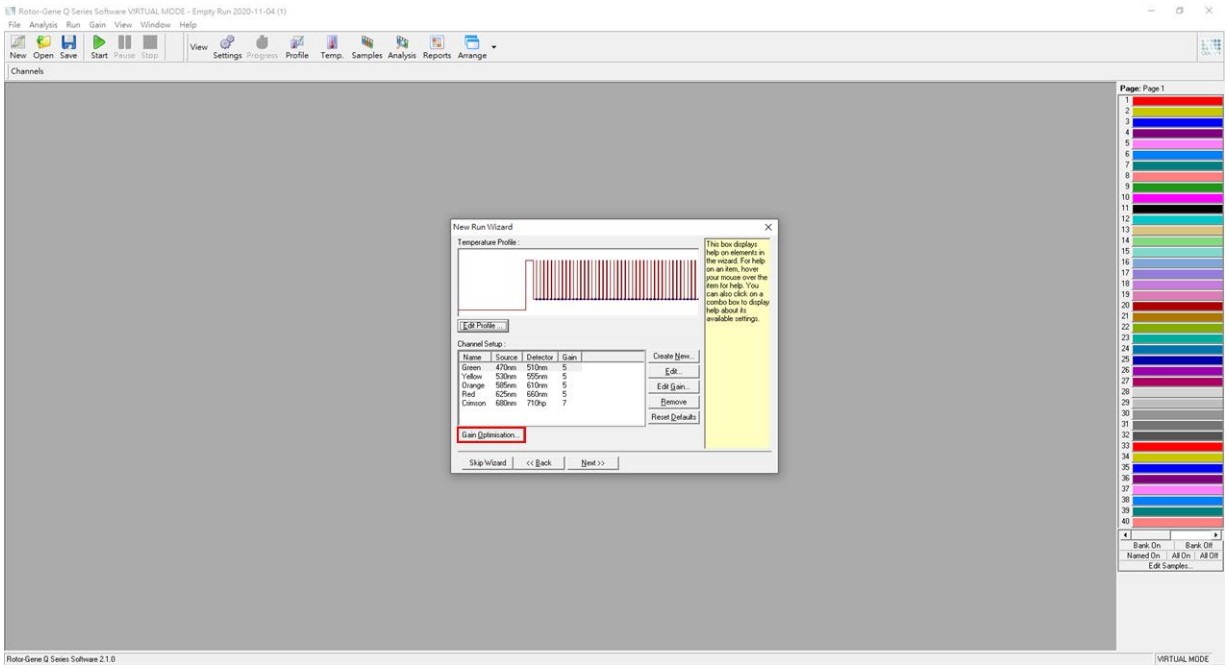
2. 58℃ 30 sec



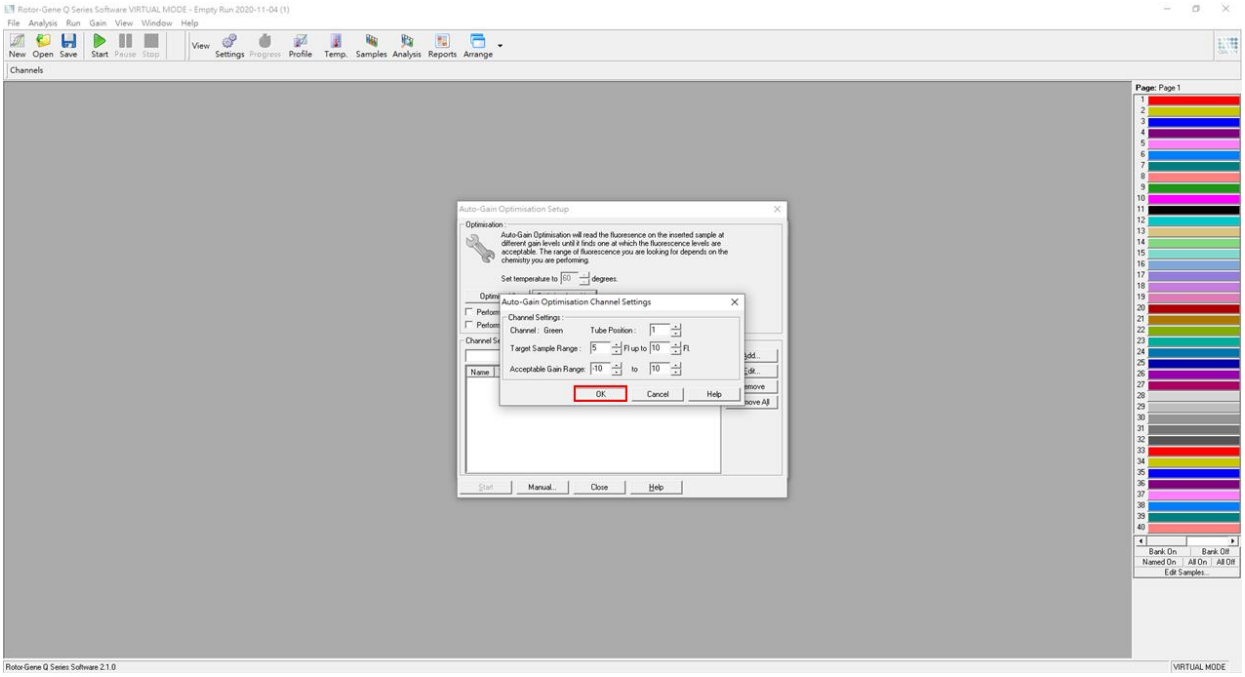
vii. Click “Not acquiring”, and select green (FAM), yellow (VIC) and orange (ROX) for multiplex into Acquiring channels column and click “OK”.



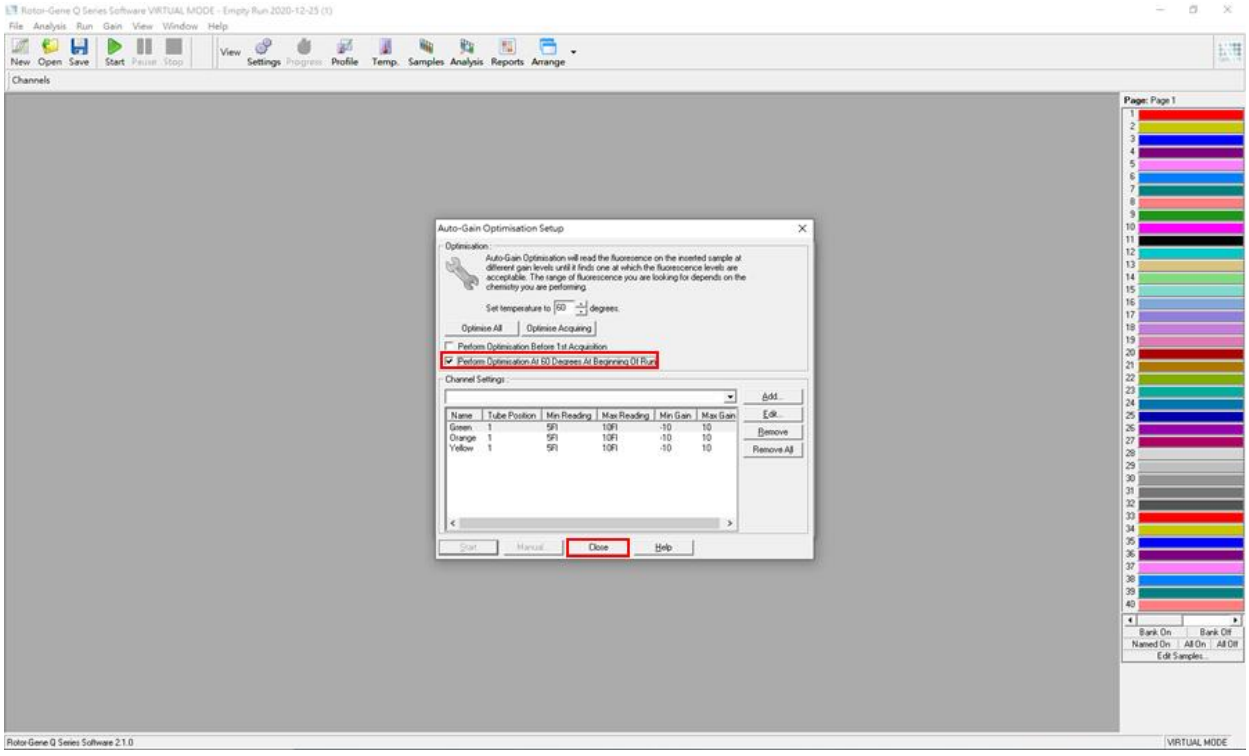
viii. Click “Gain Optimisation”.



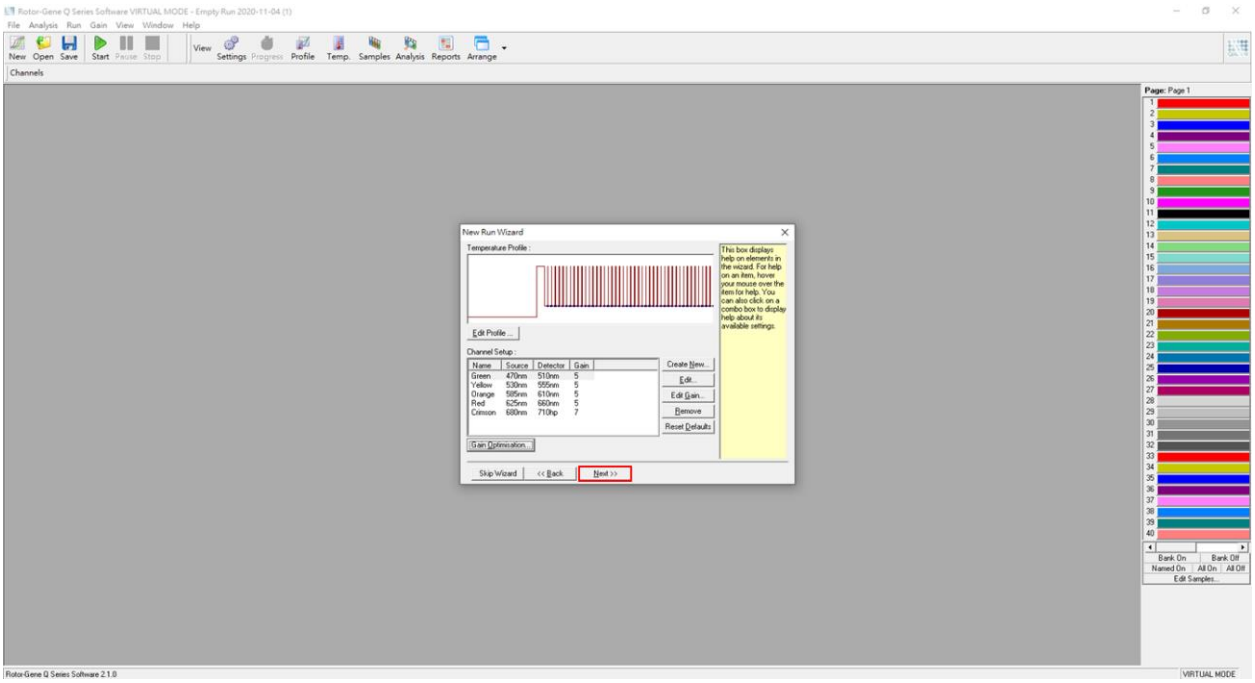
ix. Click “Optimise Acquiring” and click “OK”.



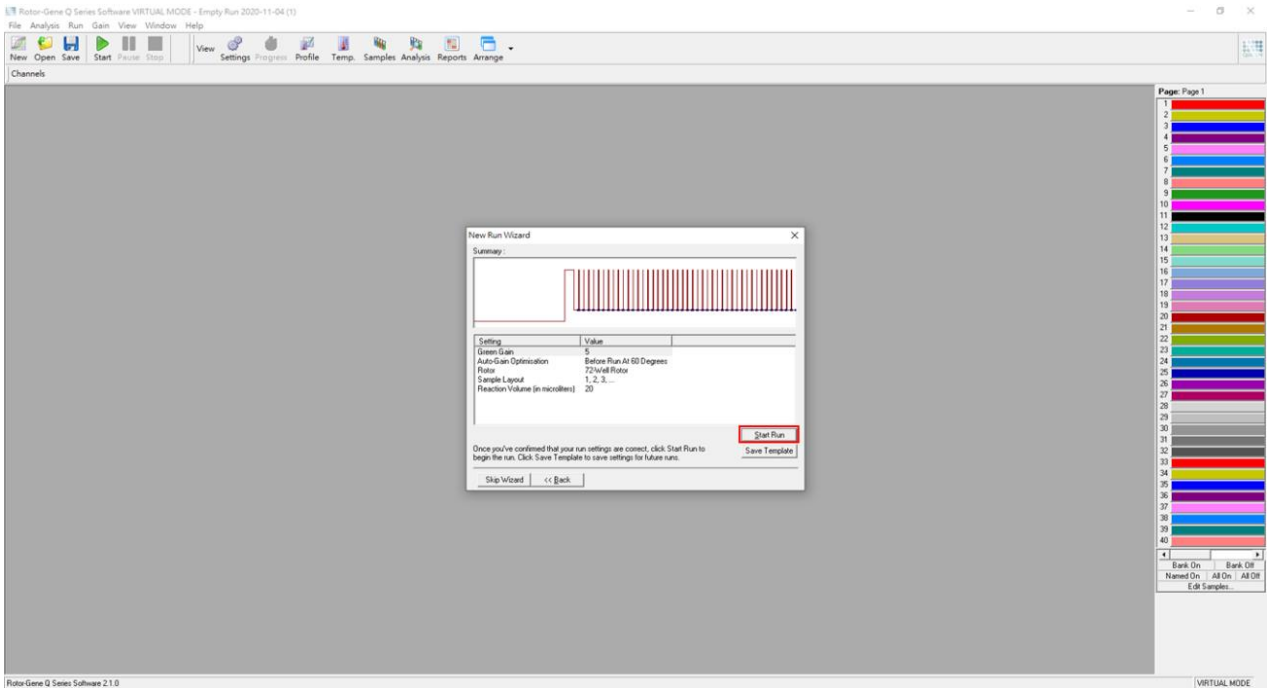
x. Tick “Perform Optimisation at 60 Degrees At Beginning of Run” and click “Close”.



xi. Click “Next”.



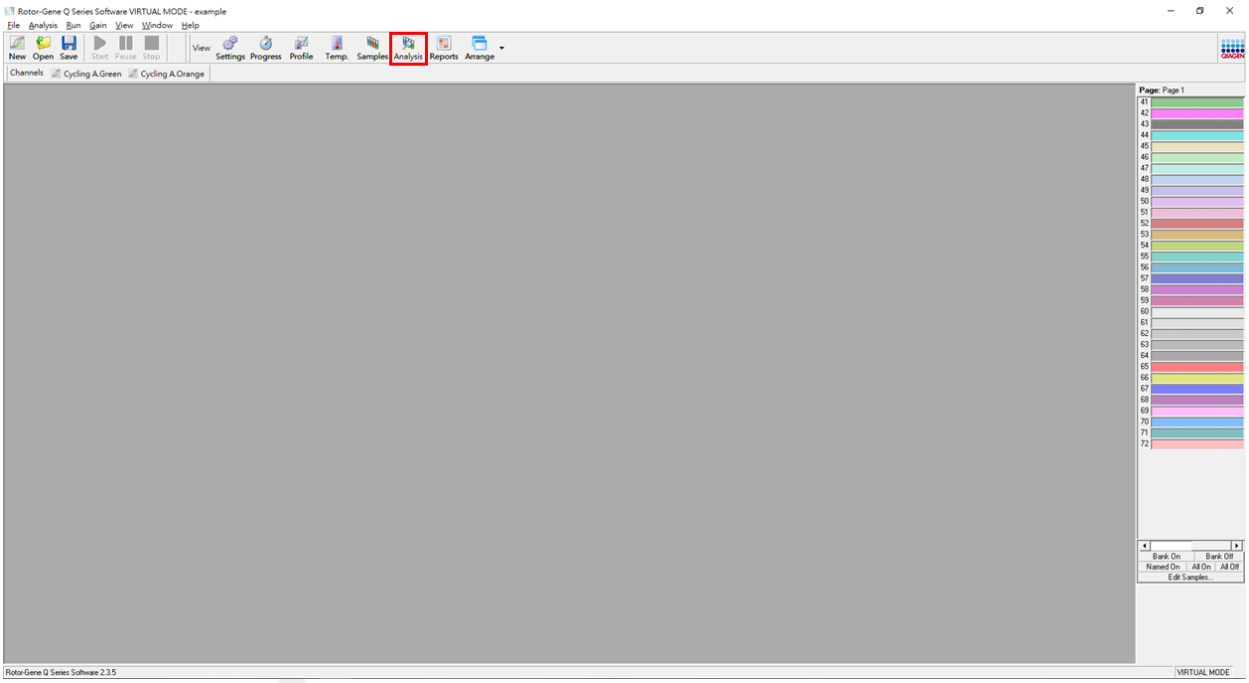
xii. Click ”Start Run”.



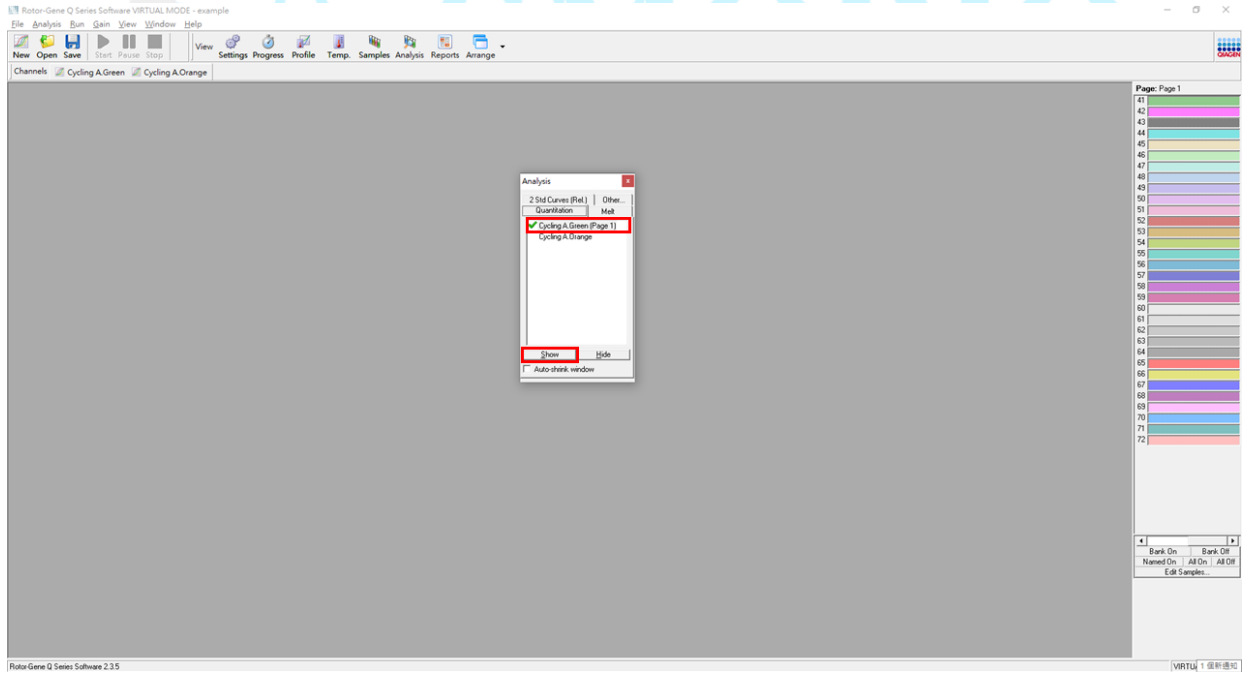
xiii. For data Analysis follow the settings below.

Rotor-Gene Q 5plex HRM (Qiagen)			
Channel	**Threshold**	**Baseline**	
		Begin	End
FAM	0.01	6	15
VIC	0.01		
ROX	0.12		

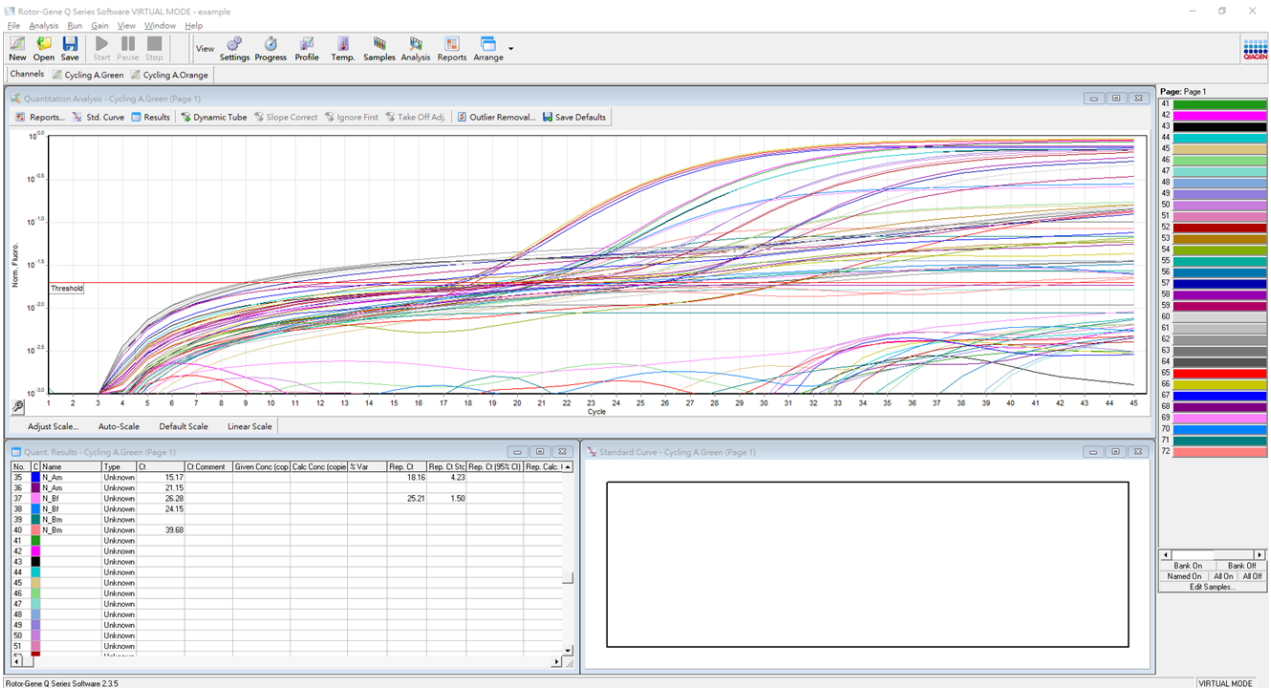
A. Run a file and click “Analysis”



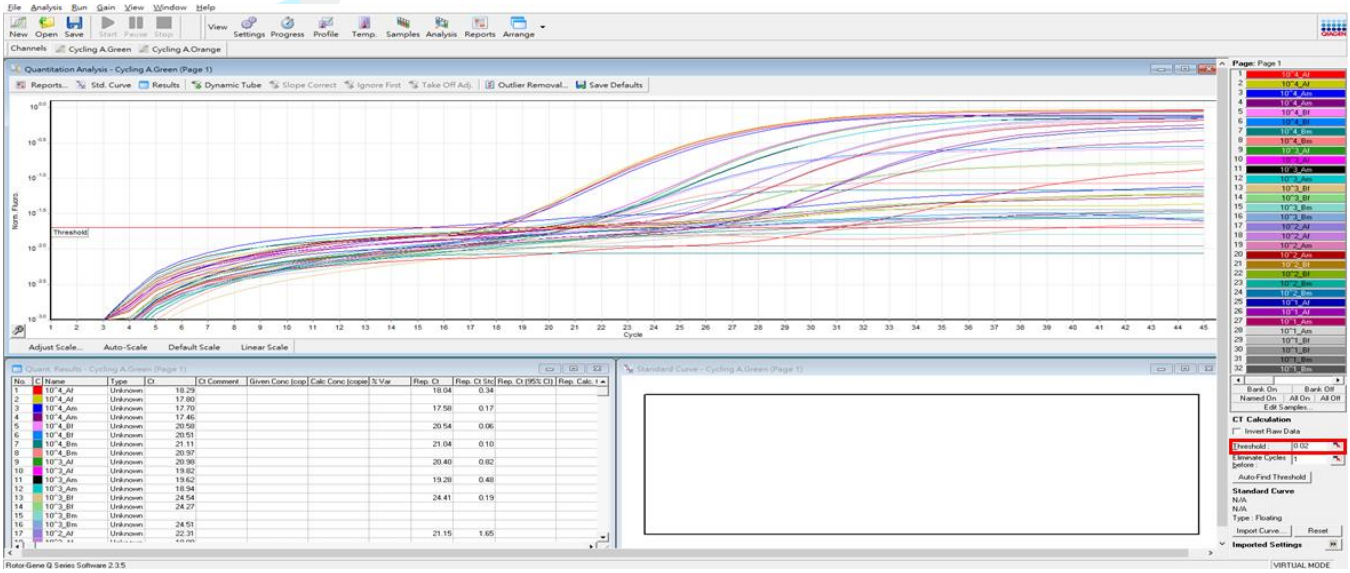
B. Select a cycling or and click “show”



C. The information will show as below.

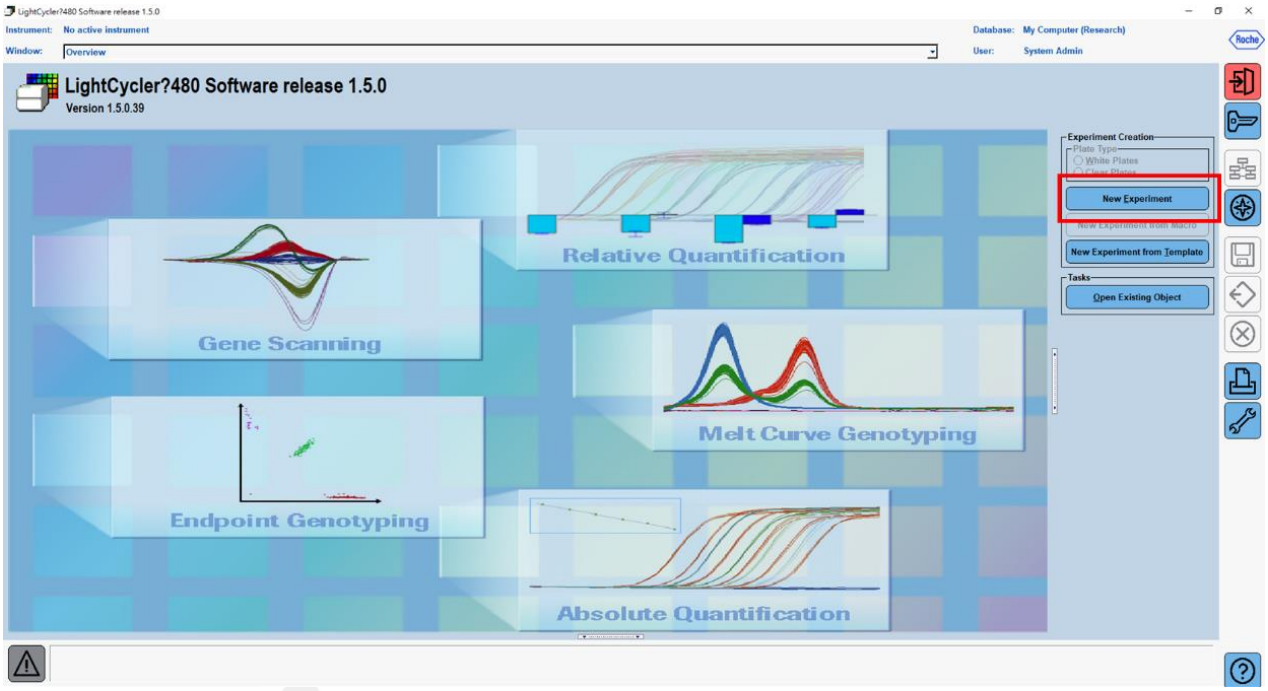


D. Threshold adjustment: Click Quantitation Analysis window, and CT calculation will show up on the right side and enter threshold.

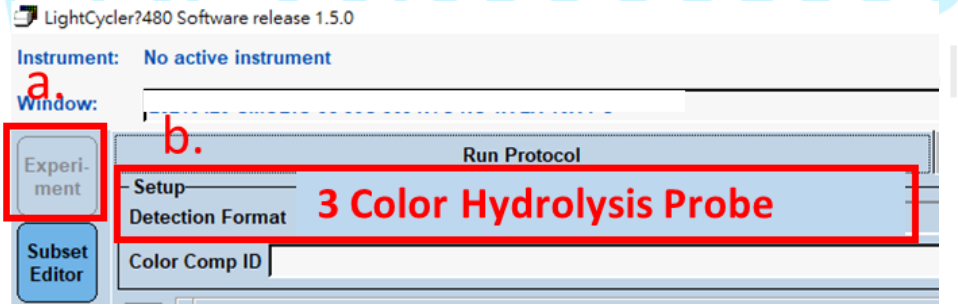


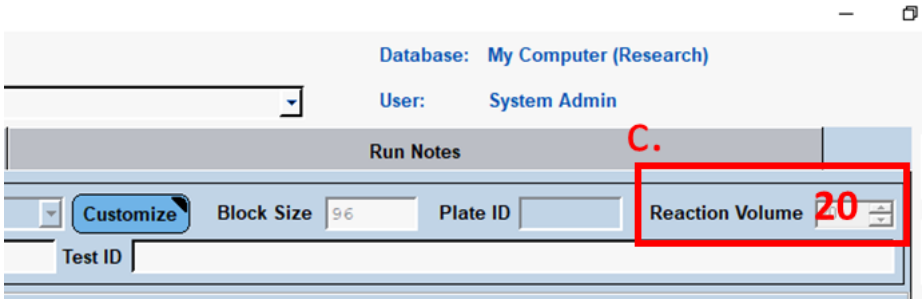
5. LightCycler 480 System (Roche, Product No. 05015278001, Software version 1.5)

i. Run a software and click “New Experiment”.

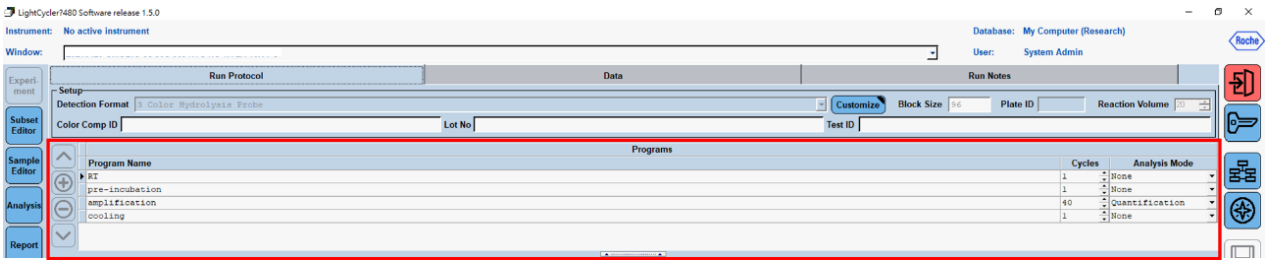


ii. Click” Experiment” and Select “3 color hydrolysis probe” for the detection format, and enter the reaction volume as 20 ㎕.



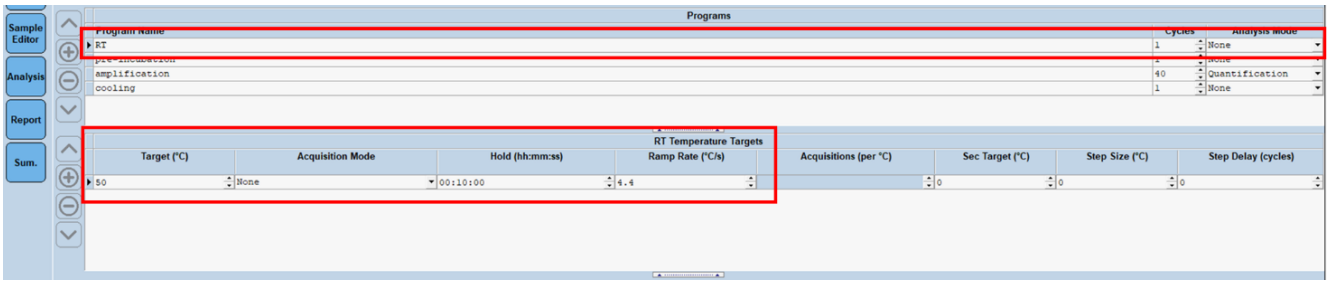


iii. Modify Program conditions as below.



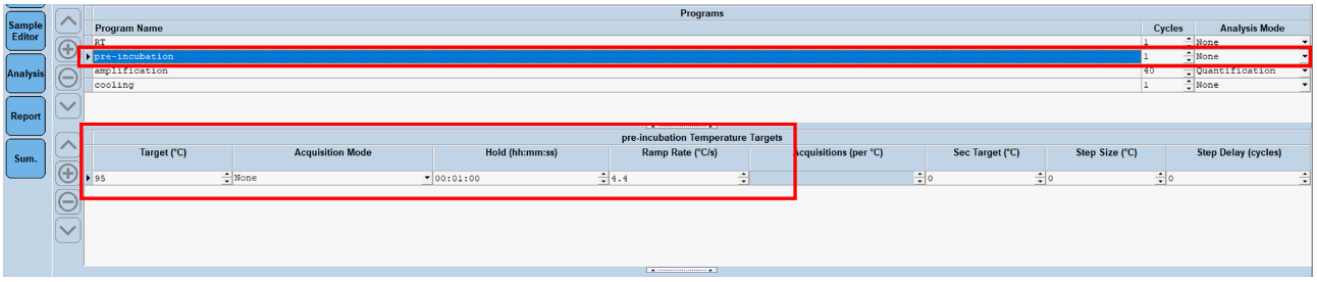
Program		
Program Name	Cycles	Analysis Mode
RT	1	None
pre-incubation	1	None
amplification	40	Quantification
cooling	1	None

iv. Modify RT amplification Temperature Targets as below.



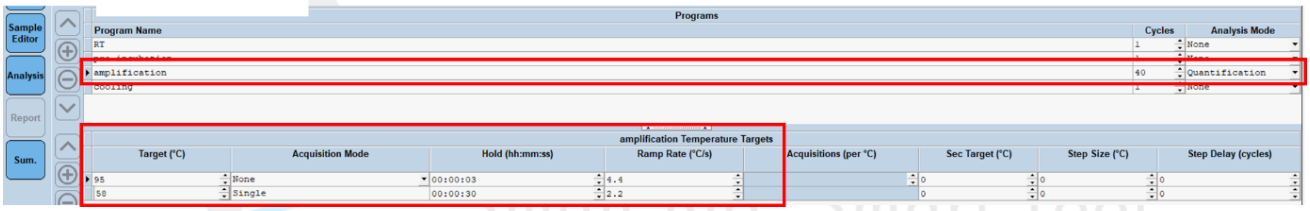
RT Program			
Target (℃)	Acquisition Mode	Hold	Ramp Rate
50	None	00:10:00	4.4

v. Modify pre-incubation Temperature Targets as below.



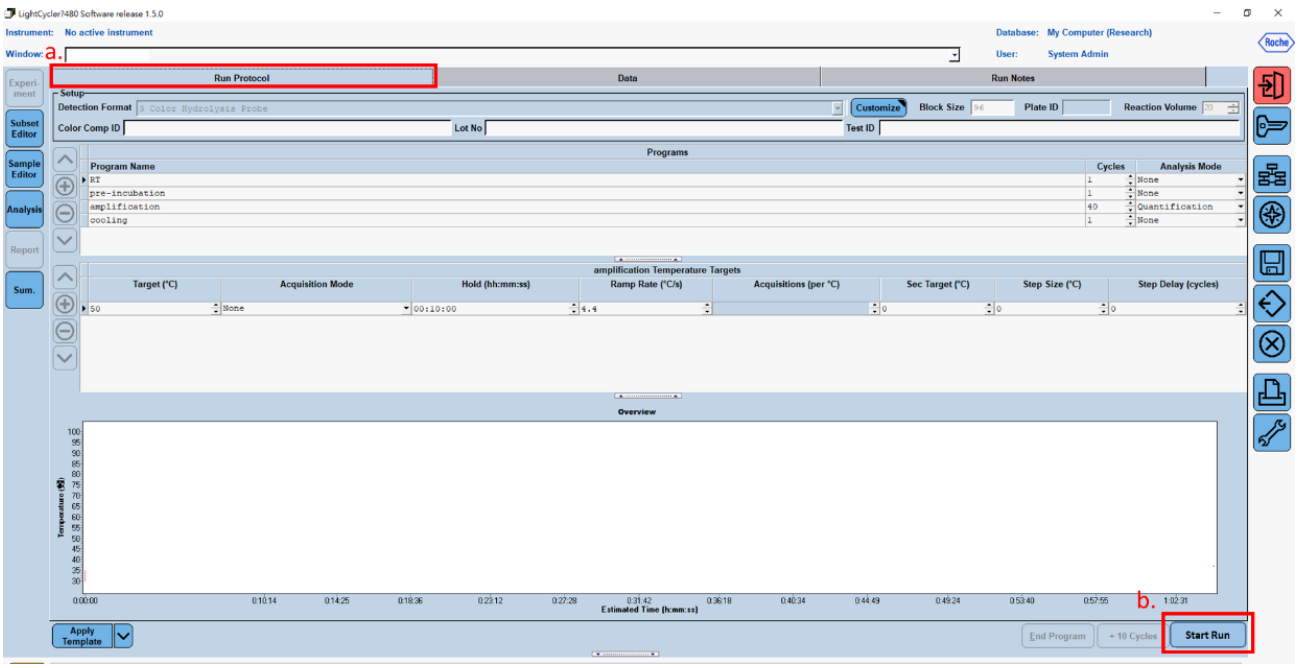
pre-incubation Program			
Target (℃)	Acquisition Mode	Hold	Ramp Rate
95	None	00:01:00	4.4

vi. Modify amplification Temperature as below.

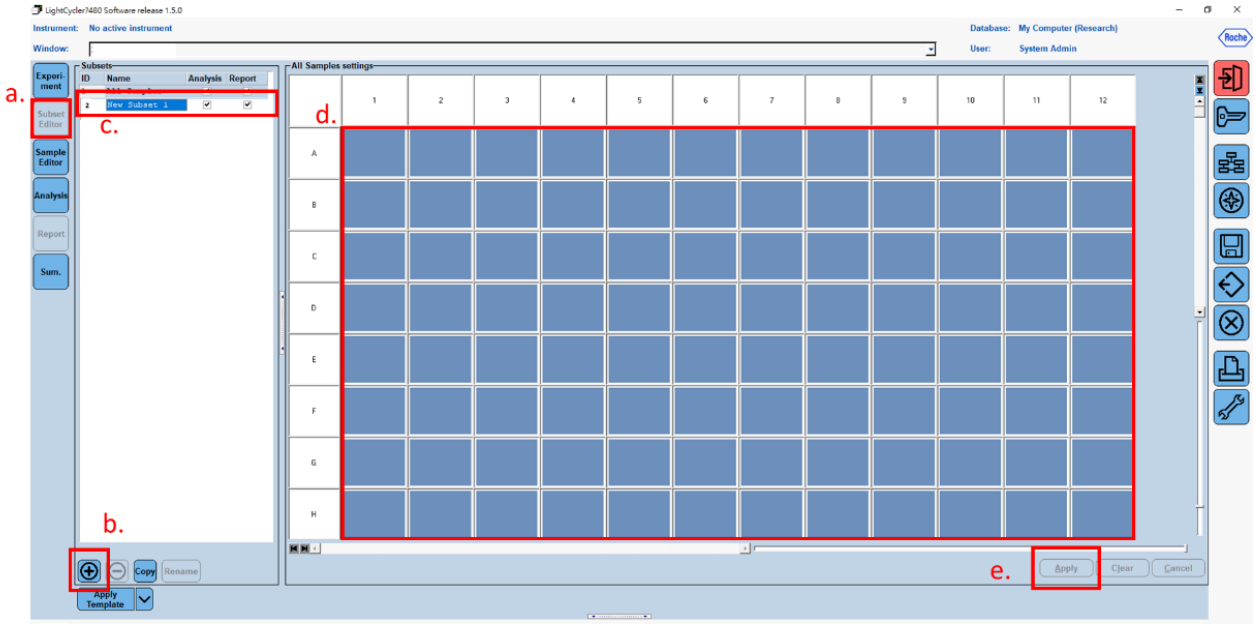


Amplification Program			
Target (℃)	Acquisition Mode	Hold	Ramp Rate
95	None	00:0:03	4.4
58	Single	00:0:30	2.2

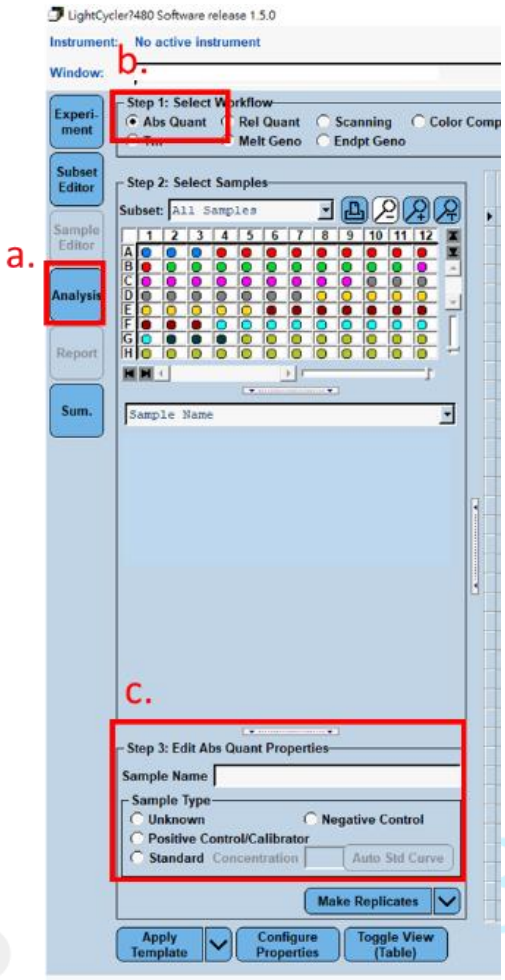
vii. Click “Run Protocol” on the above menu bar and then “Start Run”.



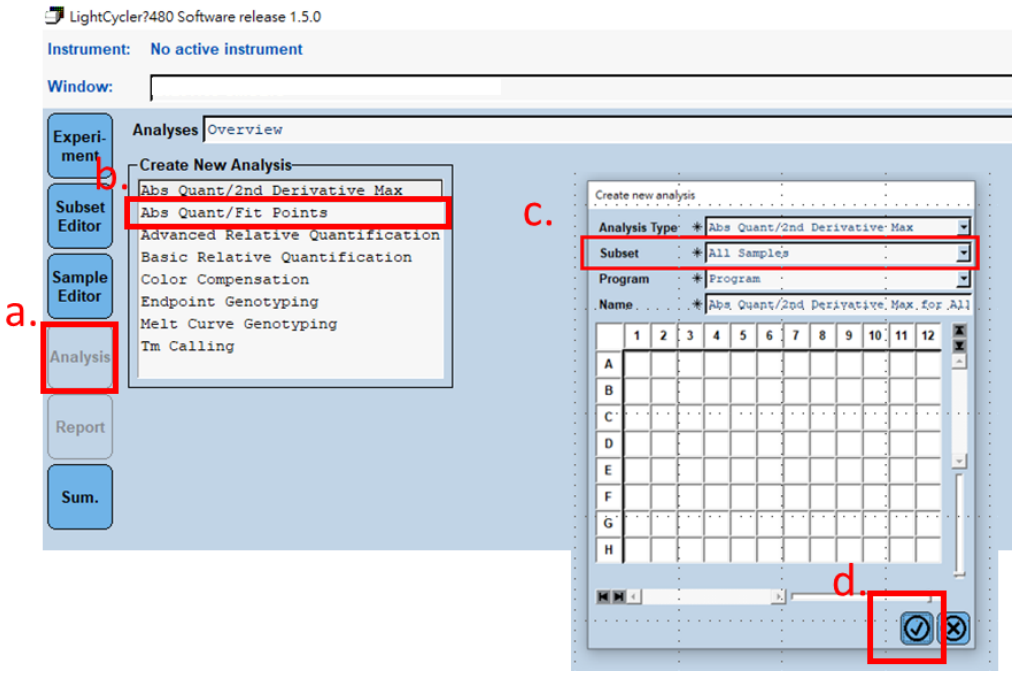
viii. Click “Subset Editor” and define 96 well PCR plate layout on the new subset.



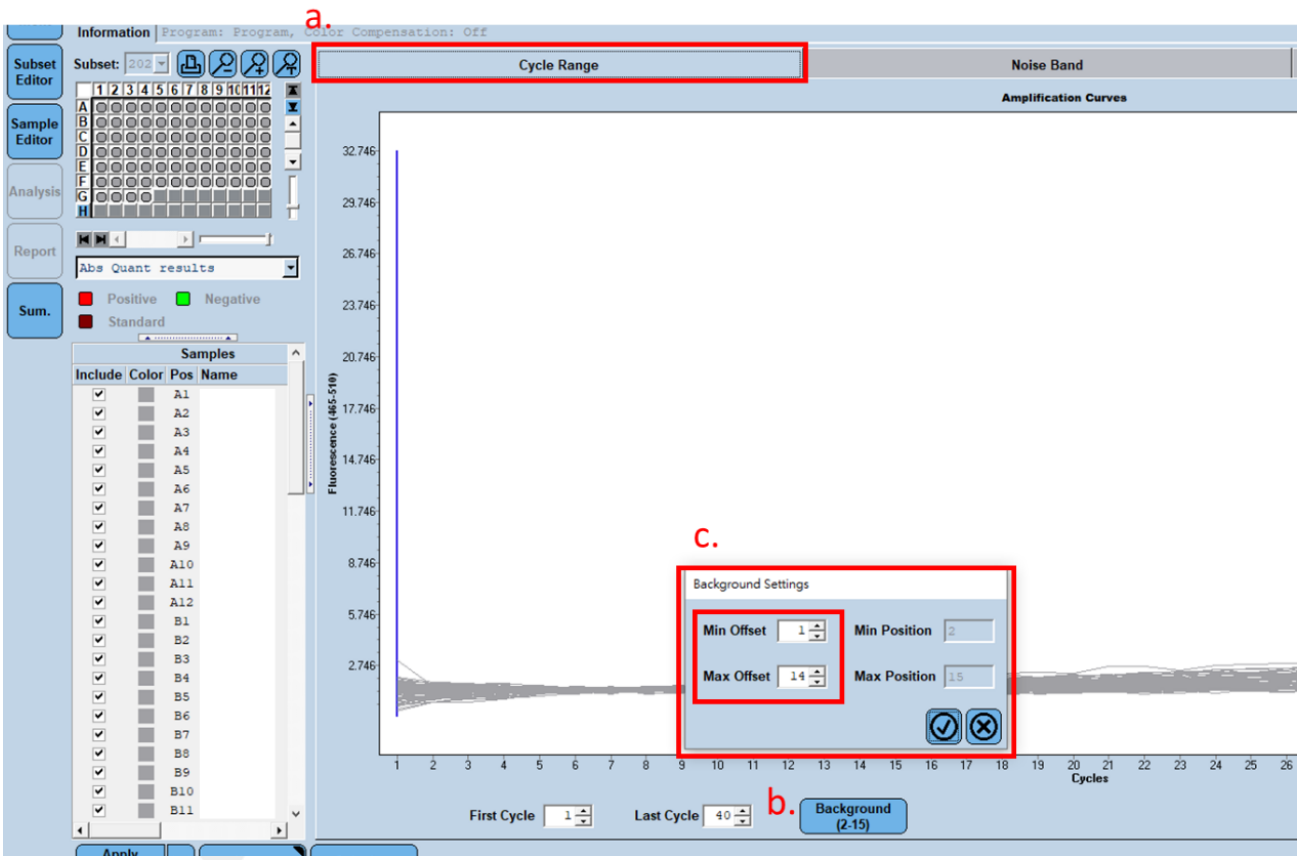
ix. Click “Analysis”, select “Abs Quant” for workflow and edit “Abs Quant Properties” for sample type.



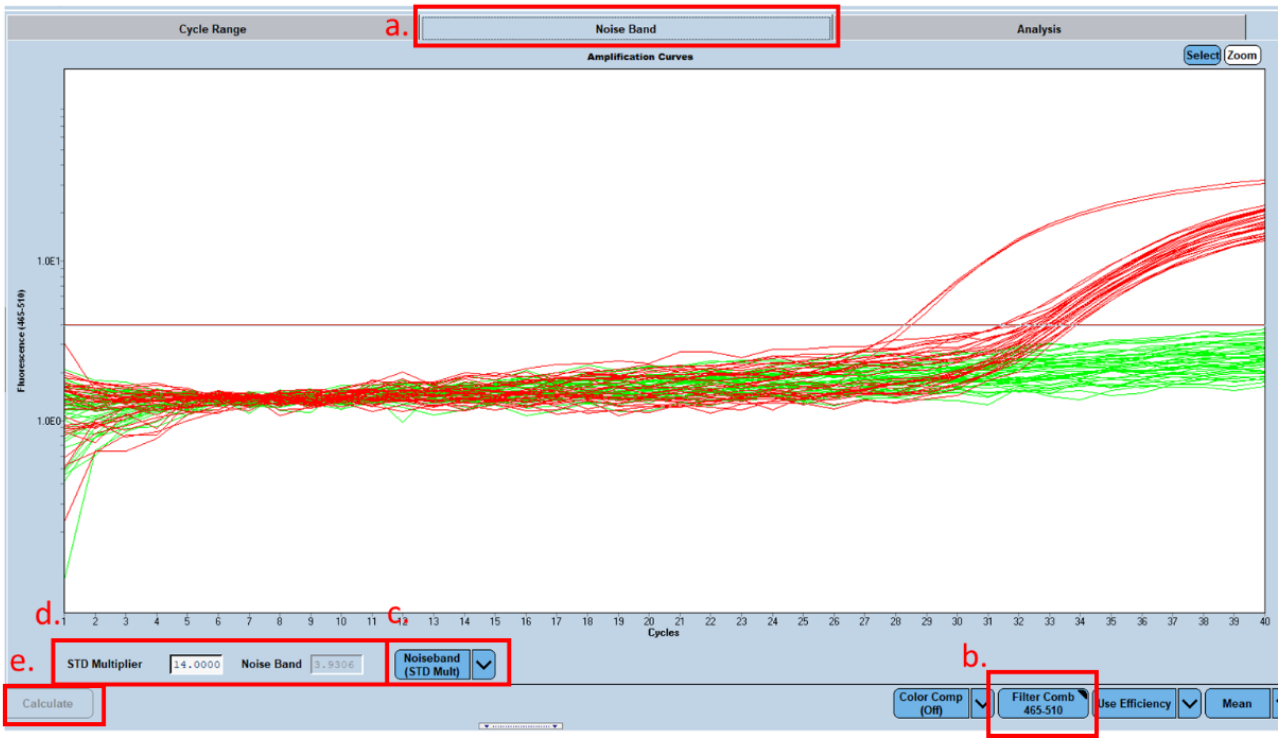
x. Click “Analysis”, “Create new analysis” in Abs Quant/Fit Point analysis, and choose the “subset”.



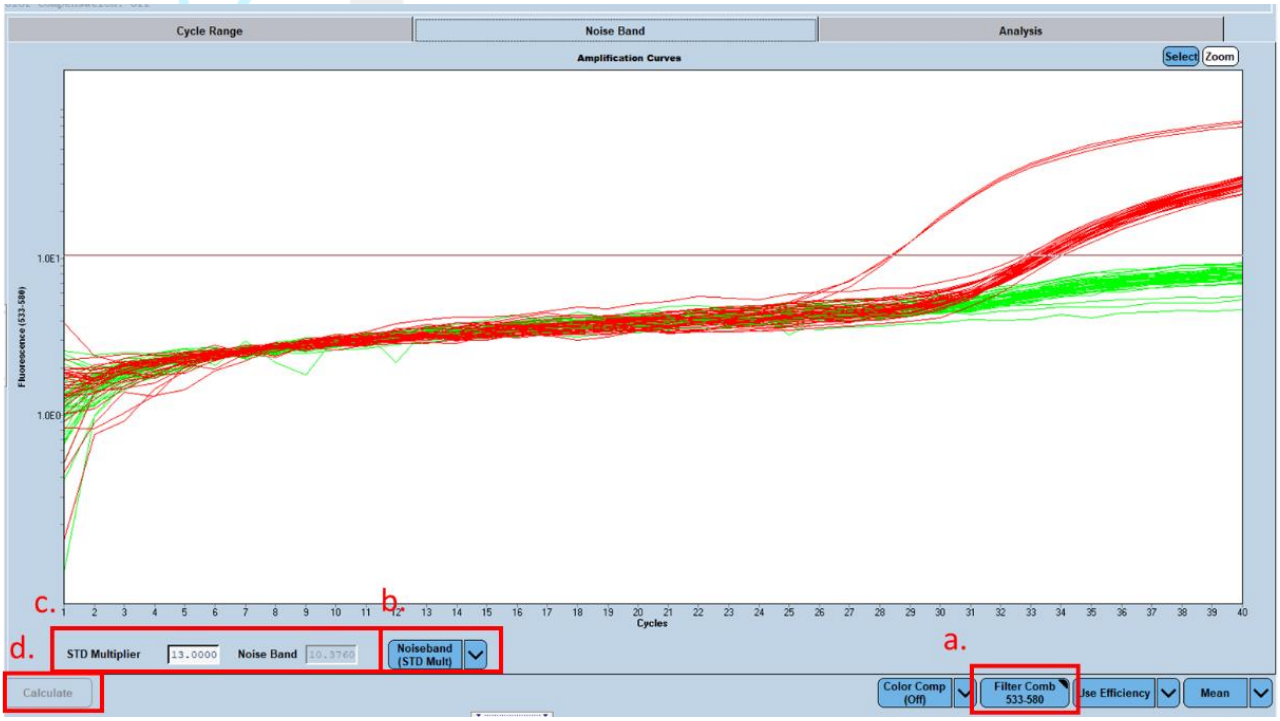
xi. Click “Cycle Range”, “Background” and set the Offset from 1 to 14.



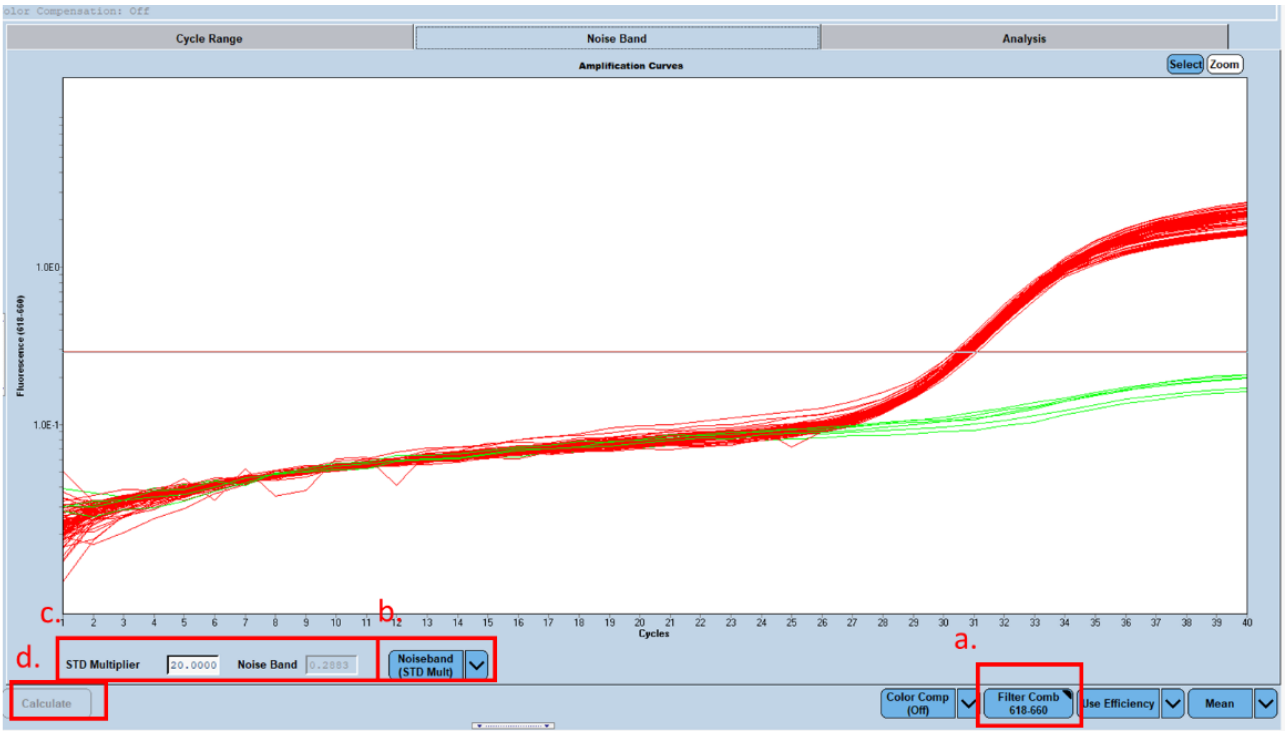
xii. Click “Noise Band”, “Filter Comb 465~510”, “Noise band (STD Multi)” and set “14” in the “STD Multiplier”, and then calculate the data.



xiii. Click “Filter Comb 533~580”, “Noise band (STD Multi)” and set “13” in the STD Multiplier, and then calculate the data.

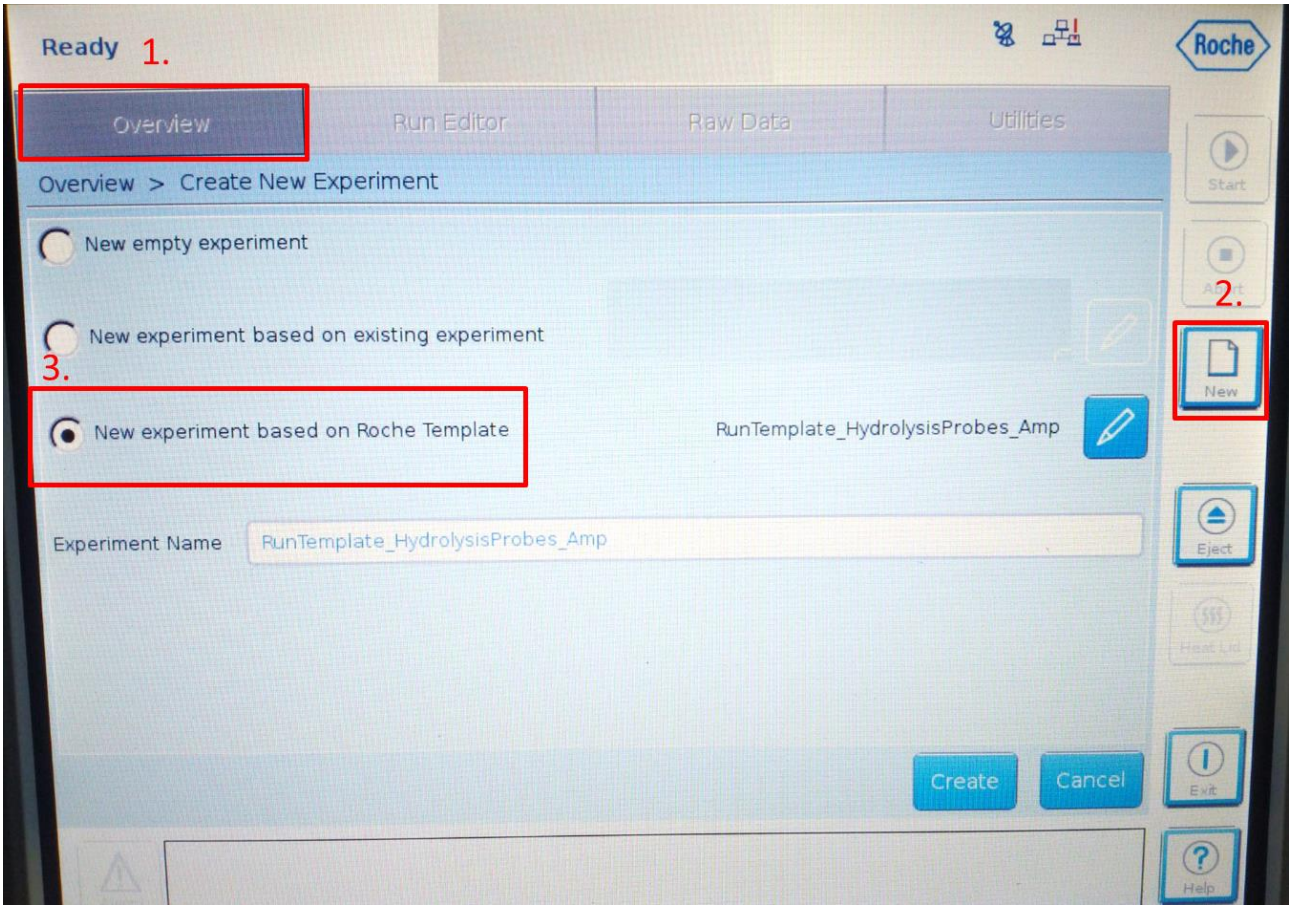


xiv. Click “Filter Comb 618~660”, “Noise band (STD Multi)” and set “20” in the STD Multiplier, and then calculate the data

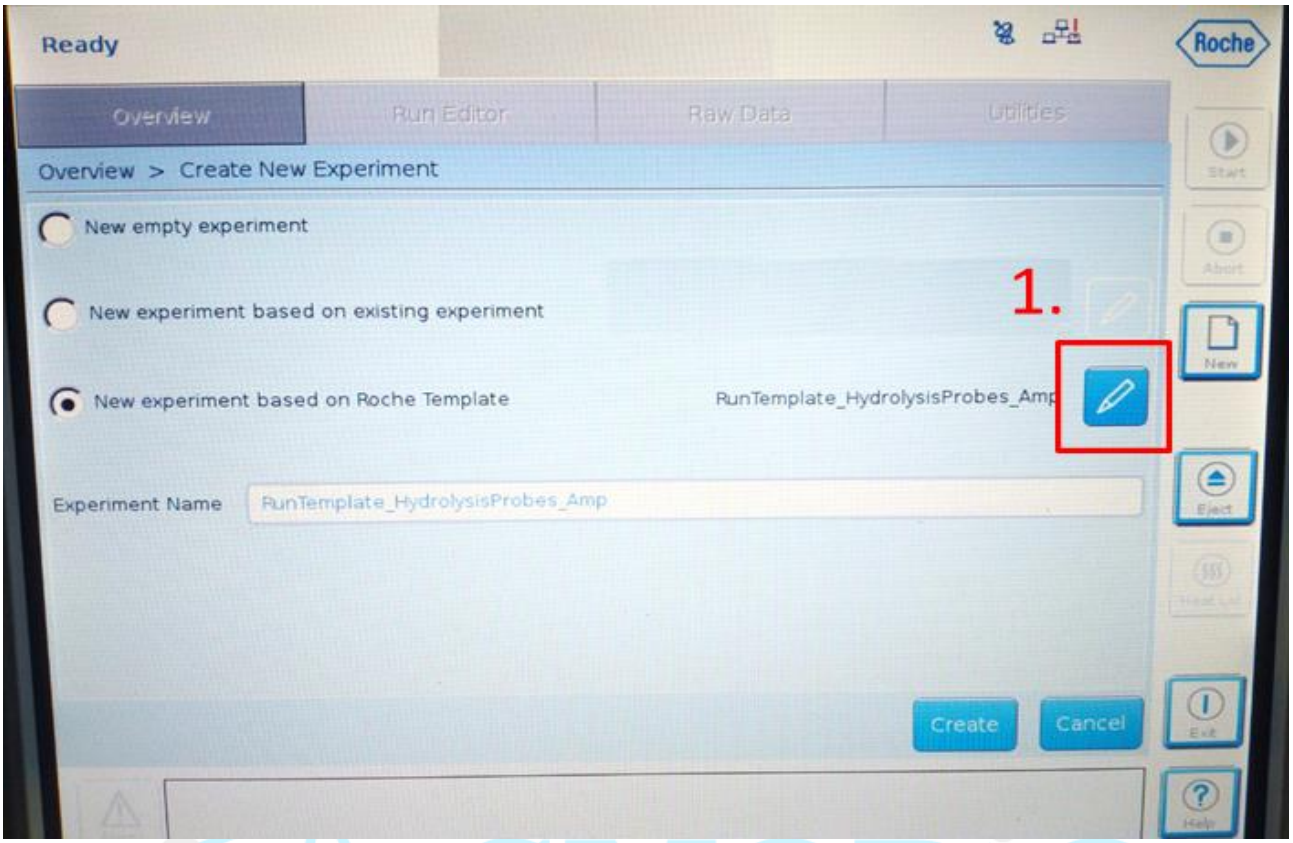


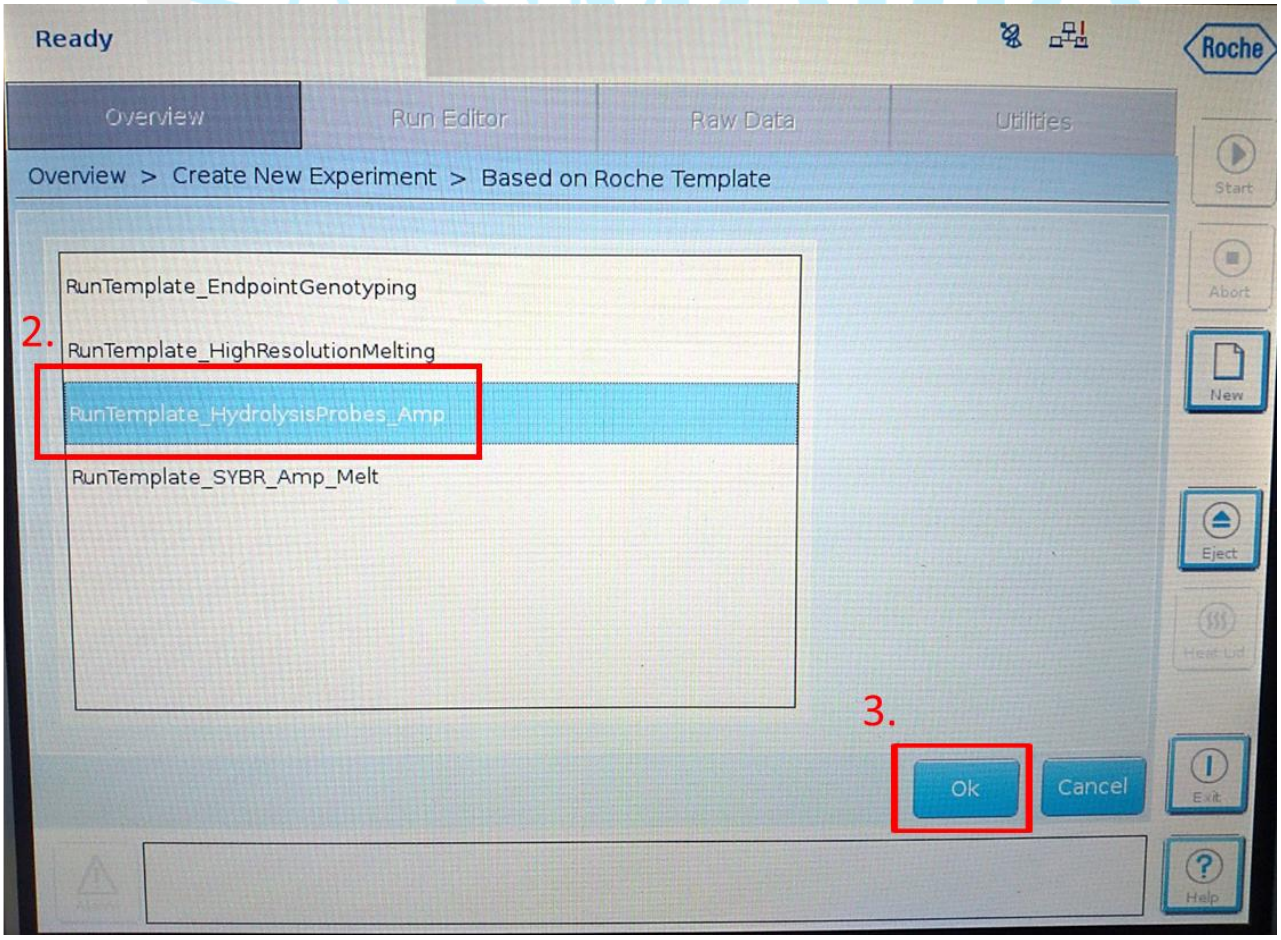
6. LightCycler 96 System (Roche, Product No. 05815916001, LightCycler® 96 Application Software Version 1.1)

i. On the machine panel, click “New” and select “New experiment based on Roche Template” in Overview.

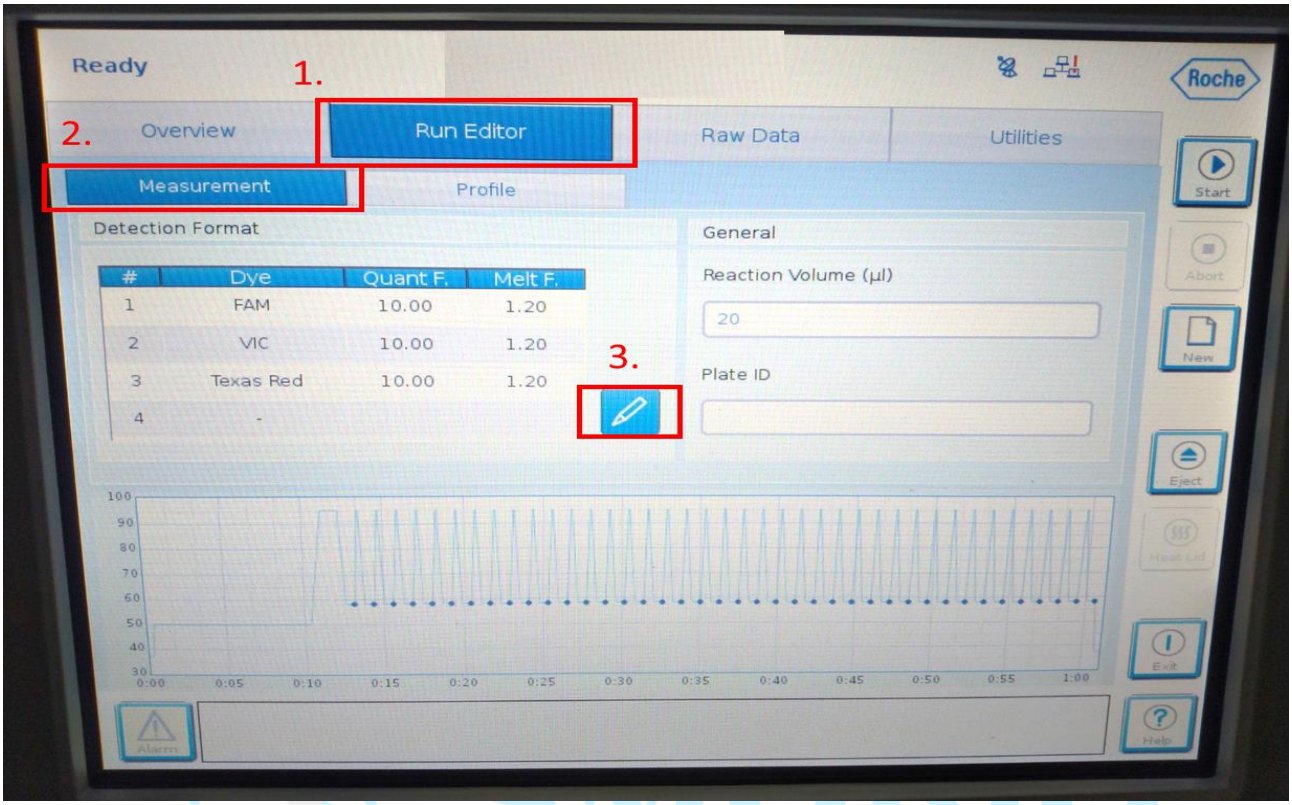


ii. Click the “pencil button” and select the “RunTemplate_HydrolysisProbes_Amp” in Roche Template.

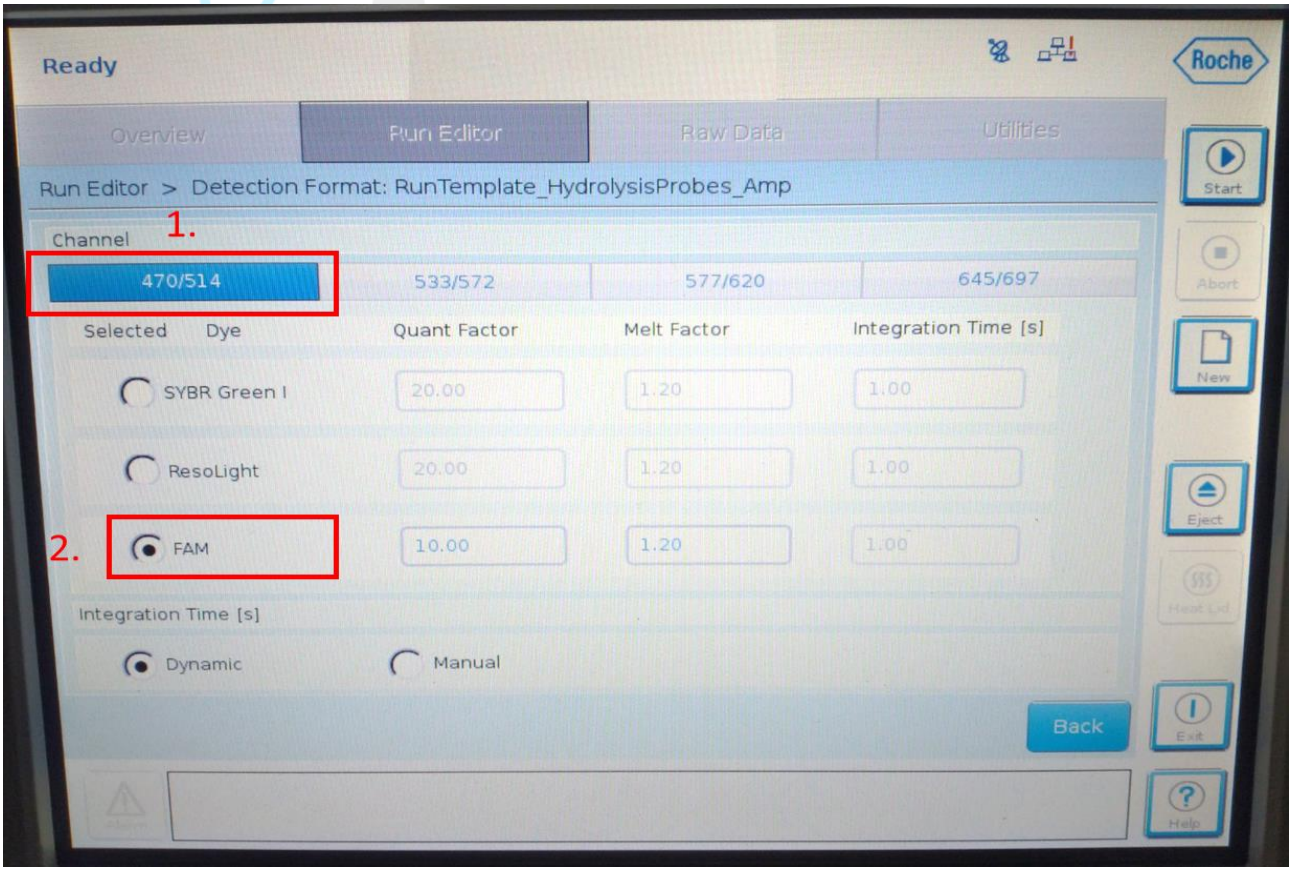

Ready
Roche
Overview
Run Editor
Raw Data
Utilities
Overview > Create New Experiment
New empty experiment
New experiment based on existing experiment
New experiment based on Roche Template
RunTemplate_HydrolysisProbes_Amp
1.
Experiment Name
RunTemplate_HydrolysisProbes_Amp
Create
Cancel
Start
Abort
New
Eject
Exit
Help


Ready
Roche
Overview
Run Editor
Raw Data
Utilities
Overview > Create New Experiment > Based on Roche Template
RunTemplate_EndpointGenotyping
2.
RunTemplate_HighResolutionMelting
RunTemplate_HydrolysisProbes_Amp
RunTemplate_SYBR_Amp_Melt
3.
Ok
Cancel
Start
Abort
New
Eject
Exit
Help

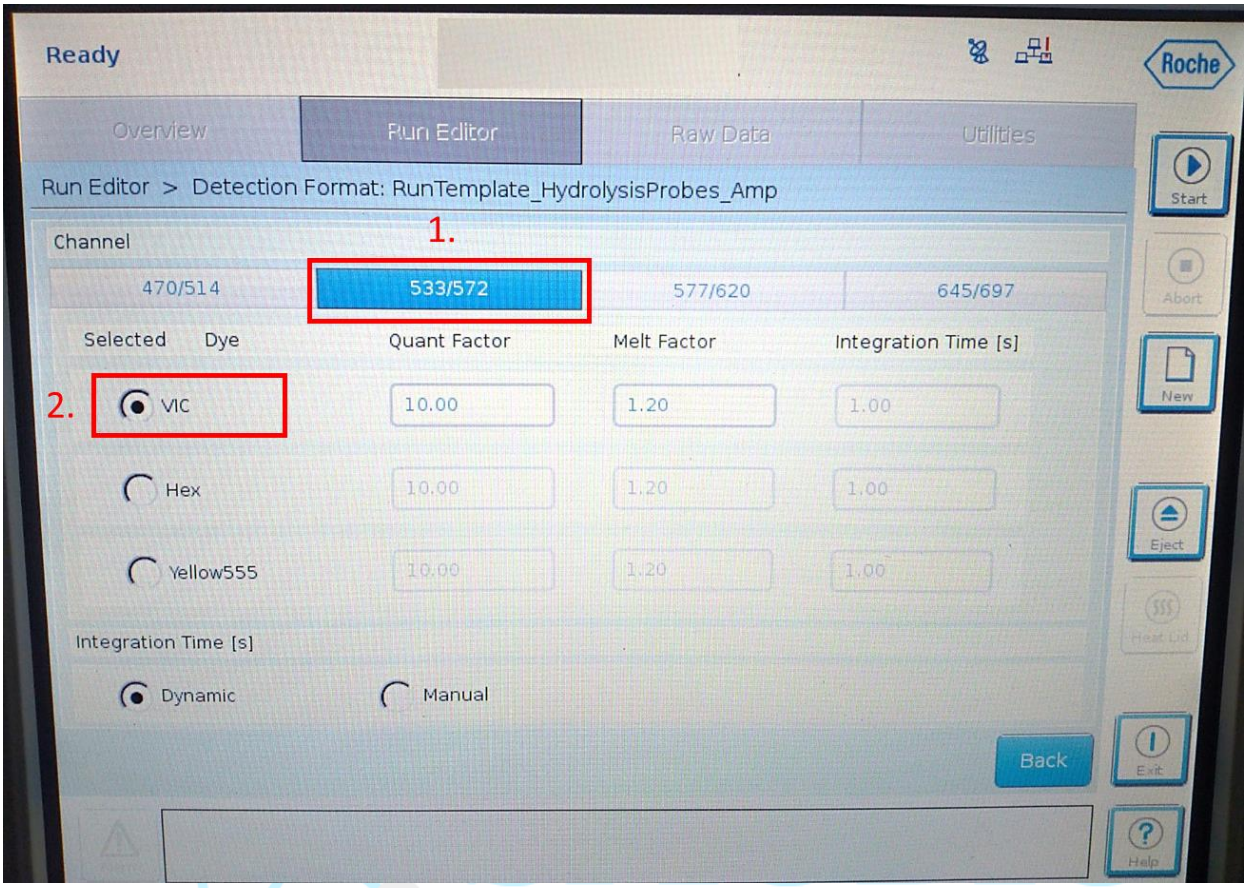
iii. Click the Measurement and click the “pencil button” and select the Detection Format in Run Editor.



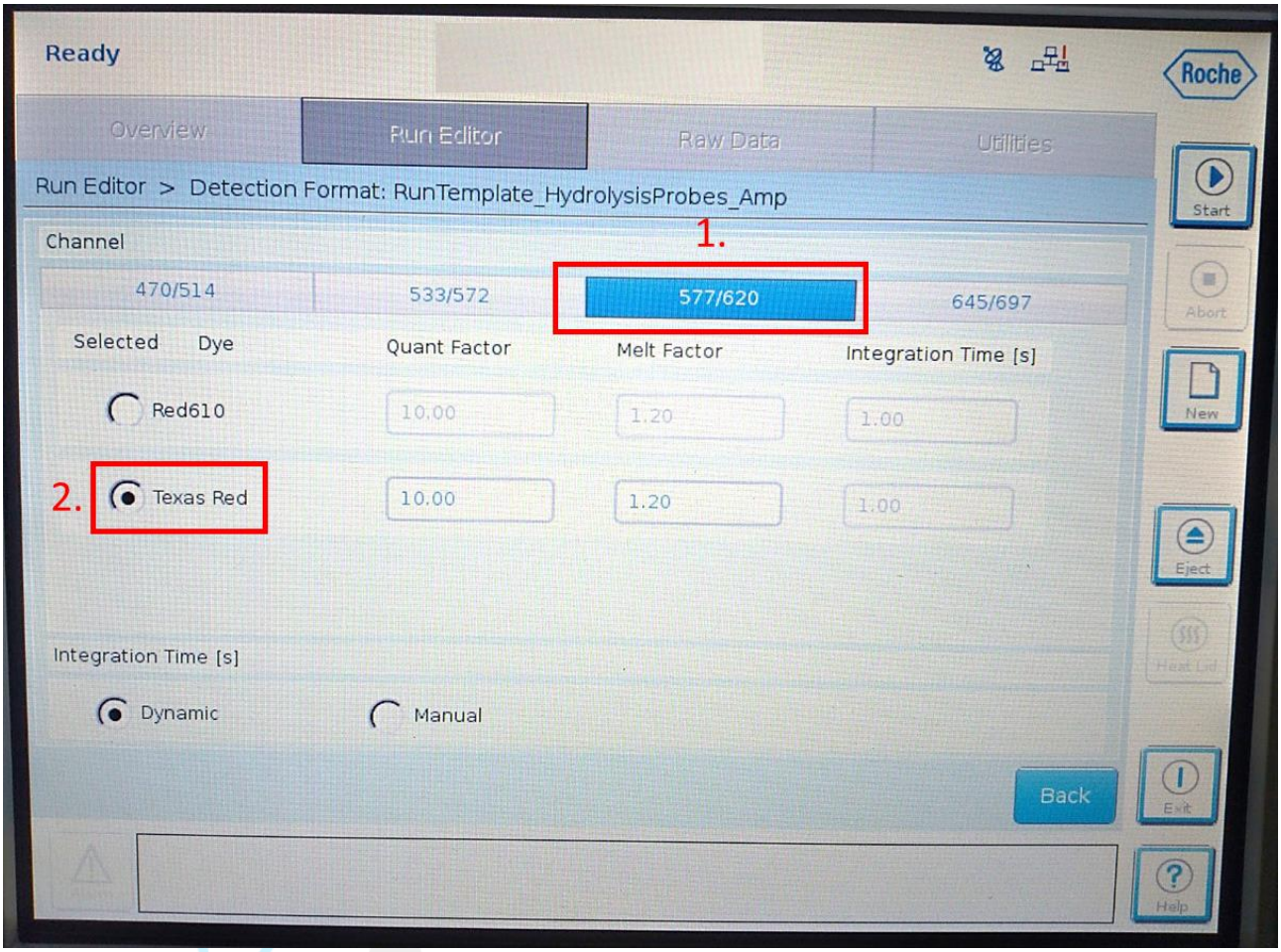
iv. Select the “FAM” for Channel 470/514.



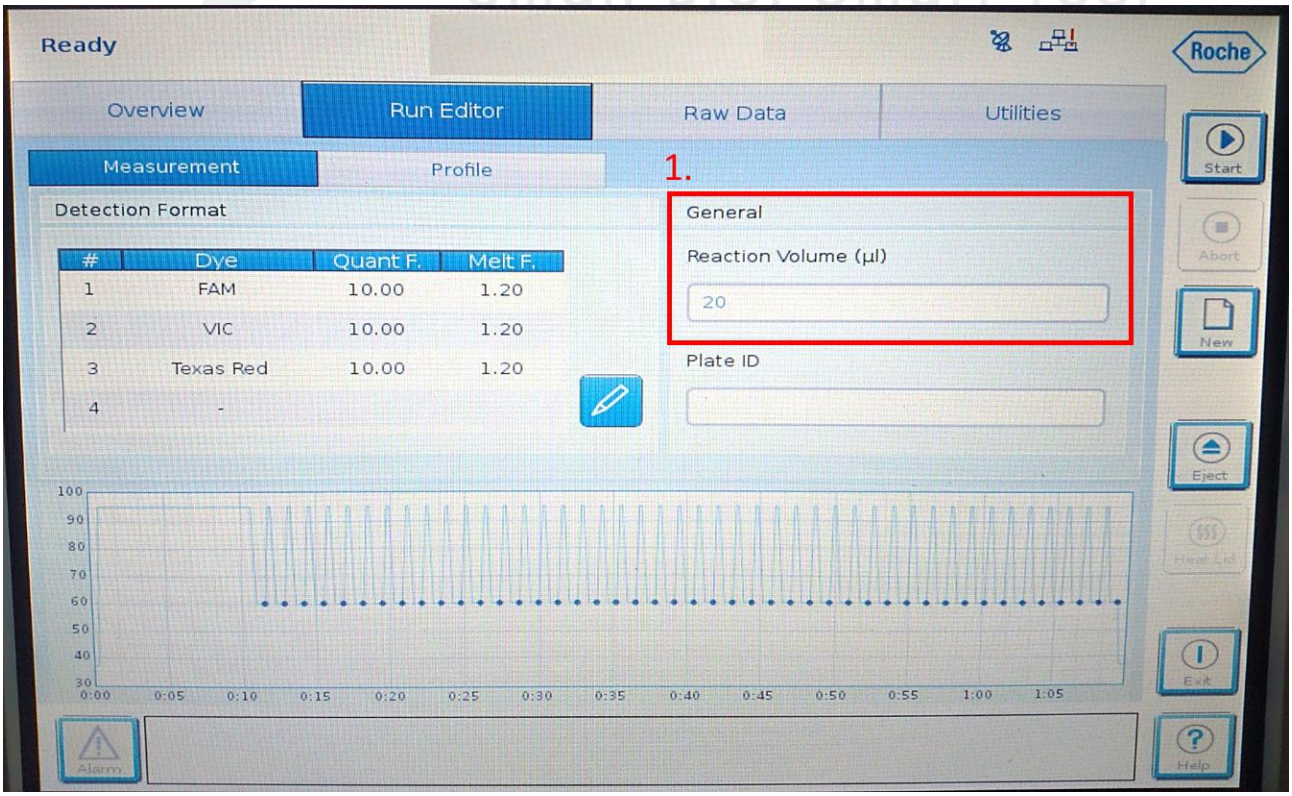
v. Select the “VIC” for Channel 533/572.



vi. Select the “Texas Red” for Channel 577/620



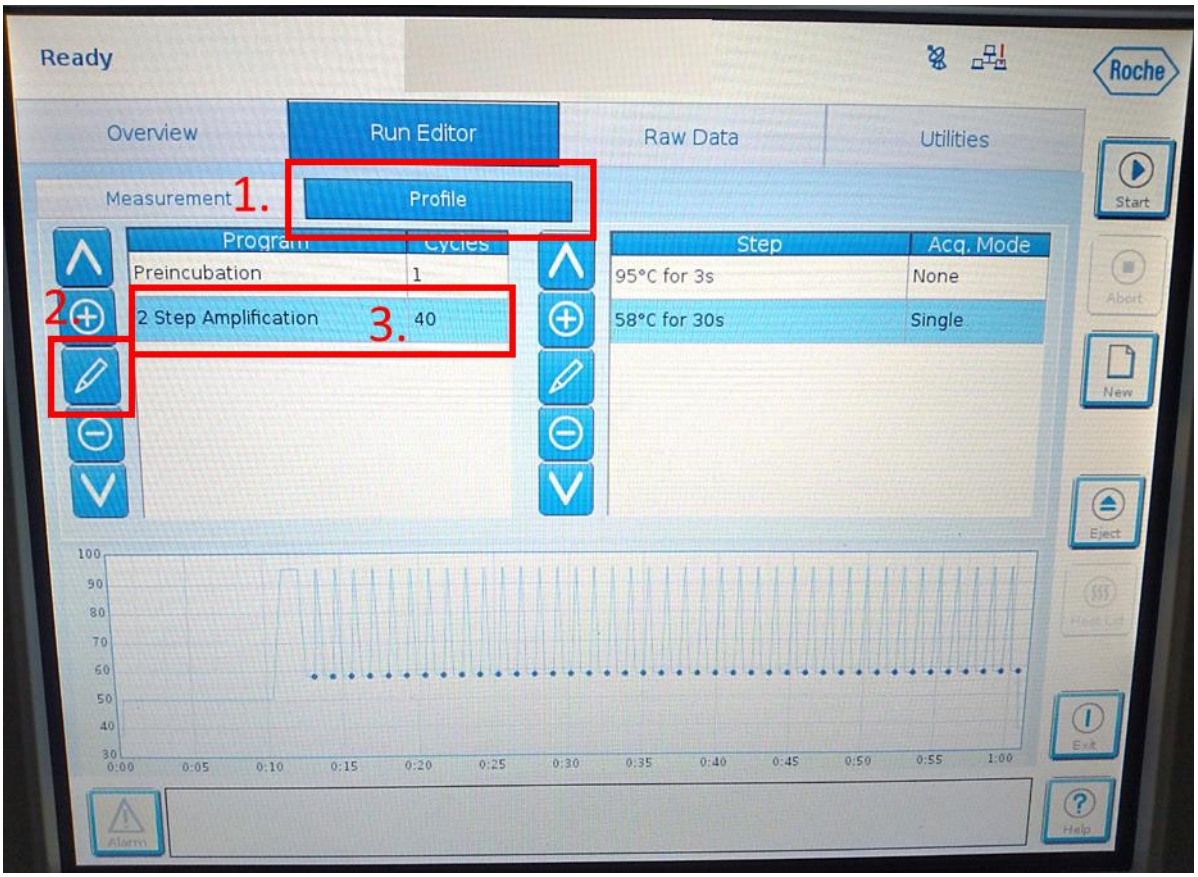
vii. Setup the Reaction Volume as 20 µl.

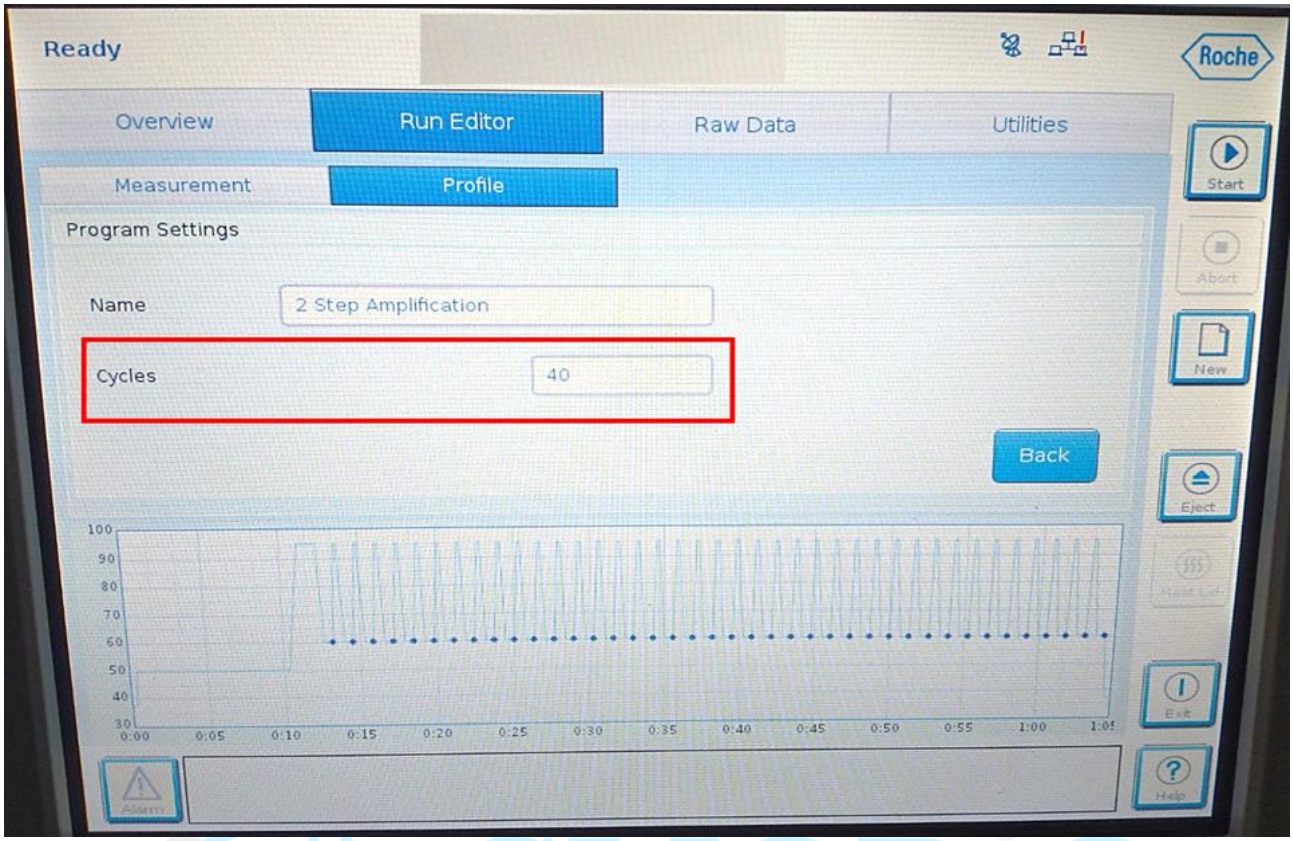


viii. Setup the PCR program according to the table below.

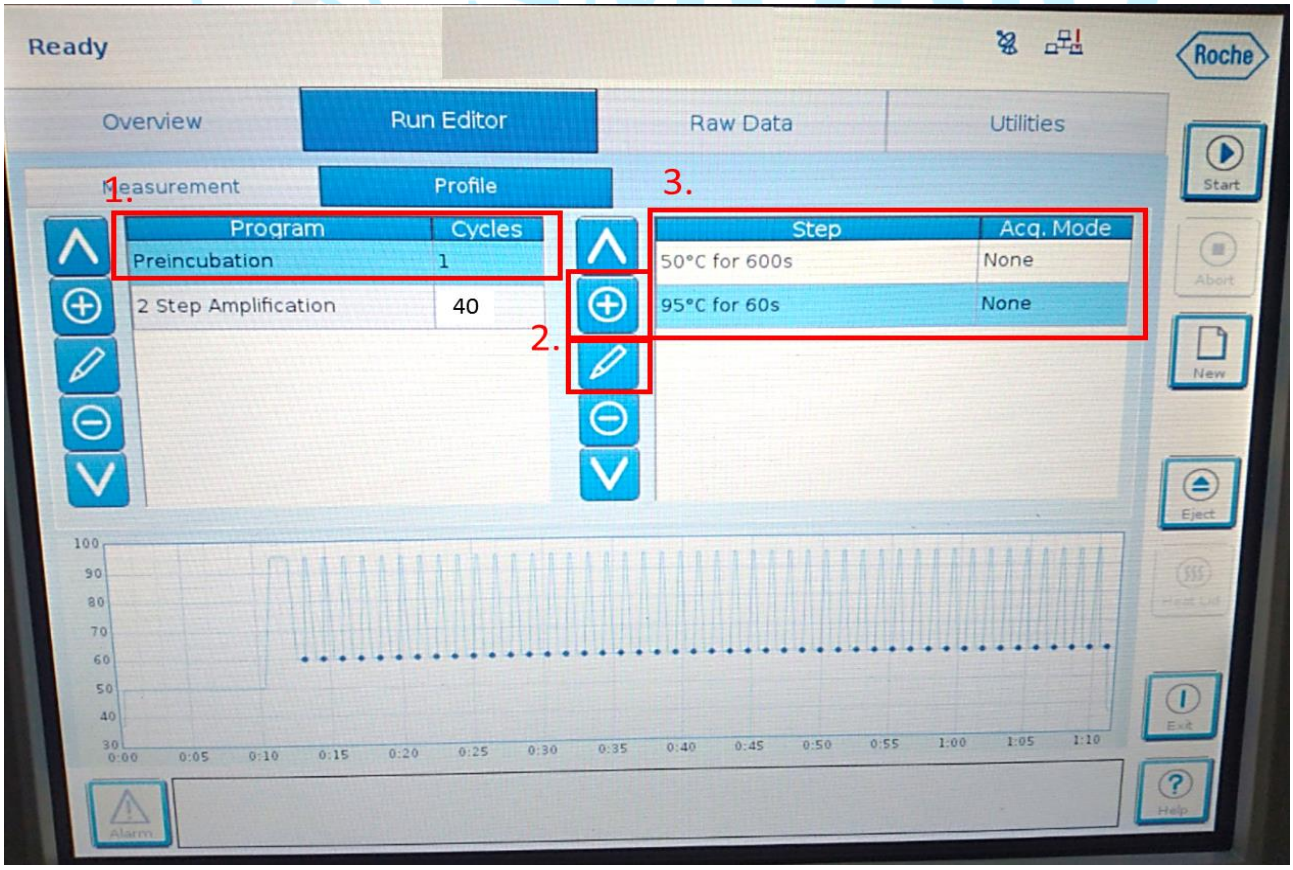
Step	Temperature	Time	Cycle
RT	50℃	10 min	1
Incubation	95℃	1 min	1
Amplification	95℃	3 sec	40
	58℃	30 sec	

ix. Click the Profile and set the cycles as 40 by pencil button".

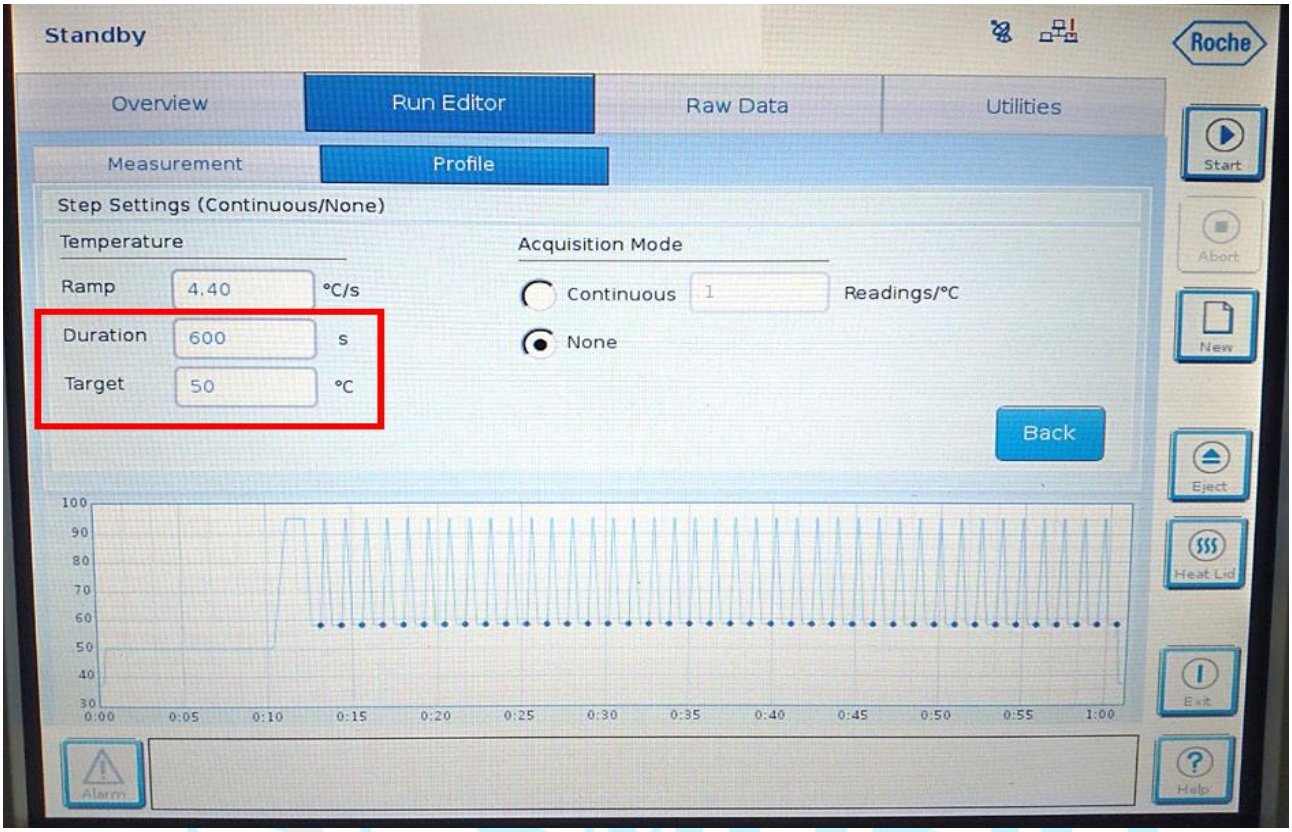


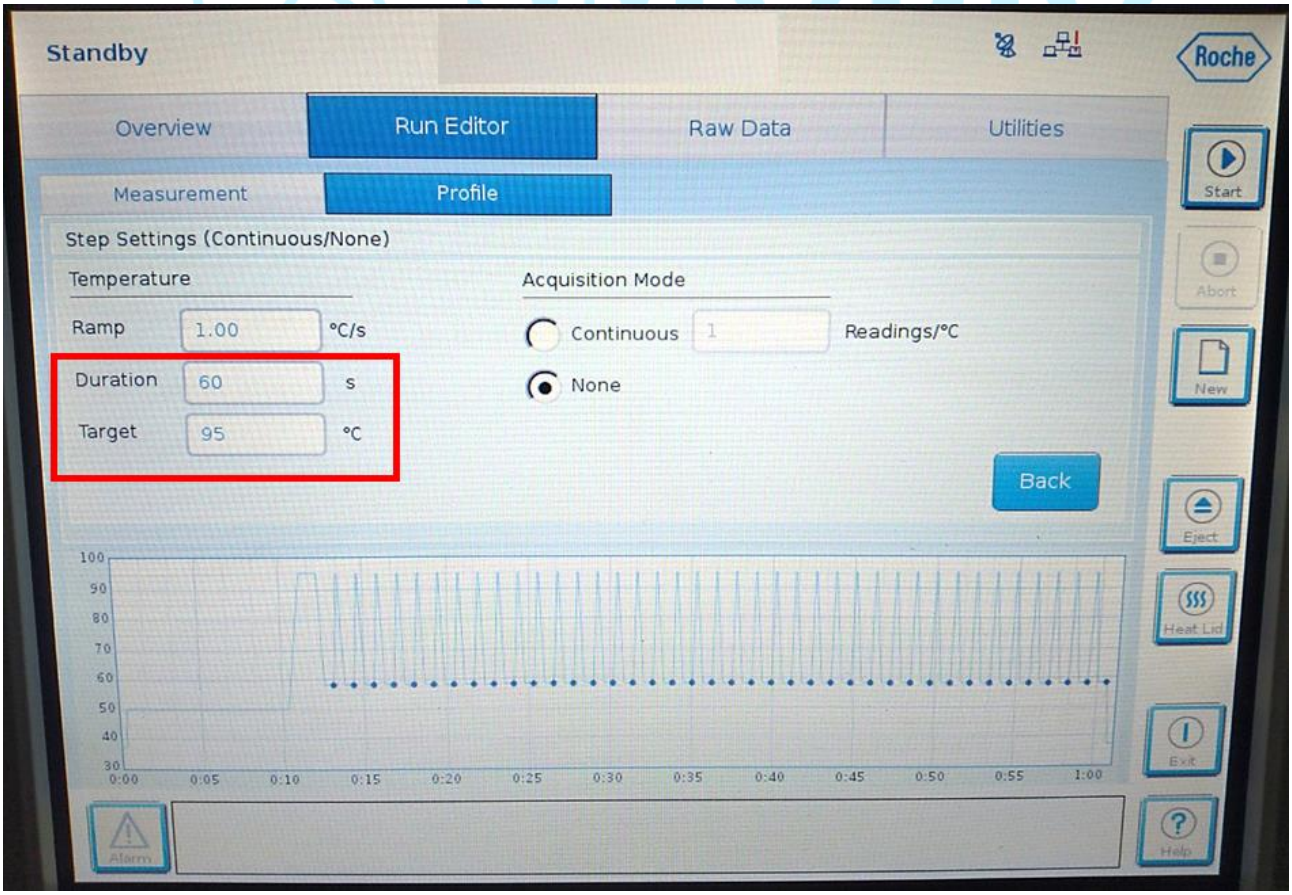


x. Click the Profile and set the Preincubation by adding one more step.

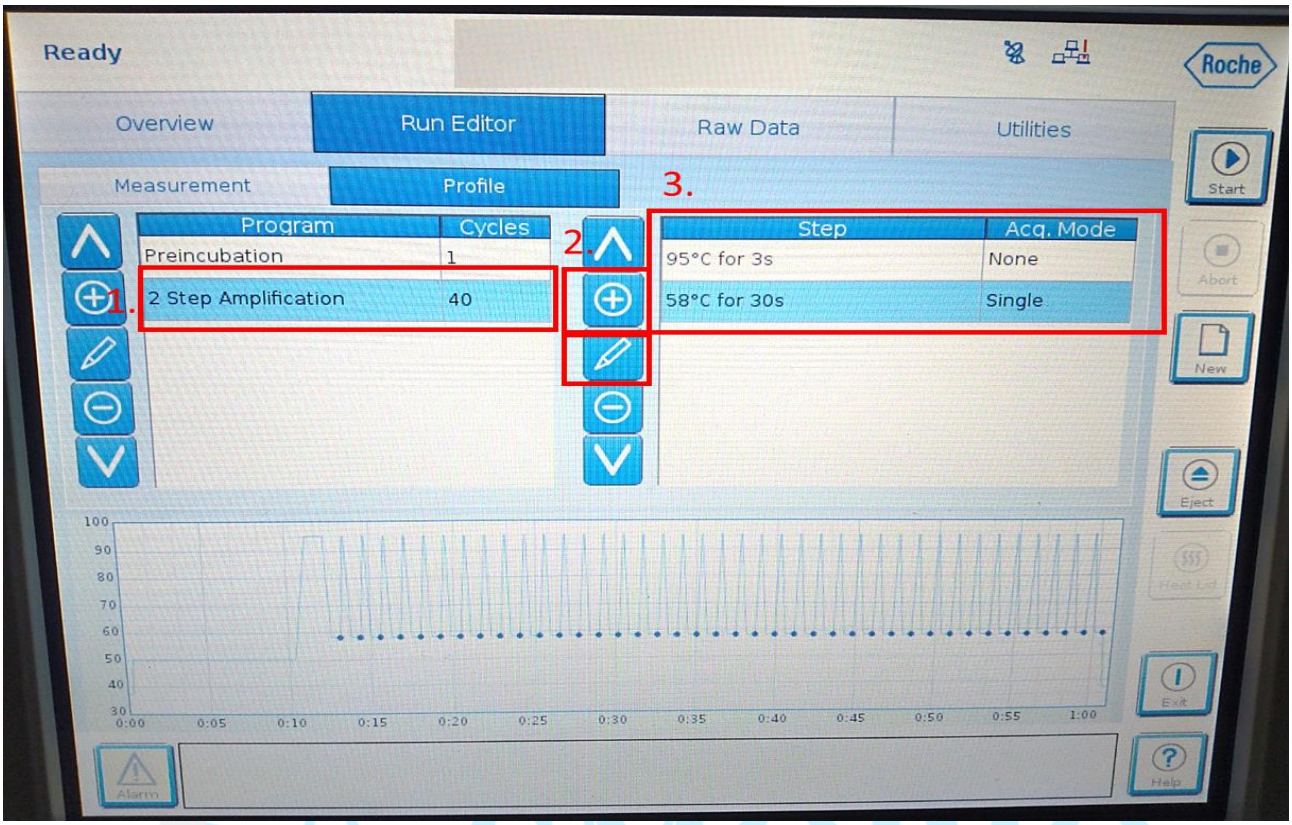


xi. Click the Profile and set the Preincubation by modifing the Target temperature and Duration.

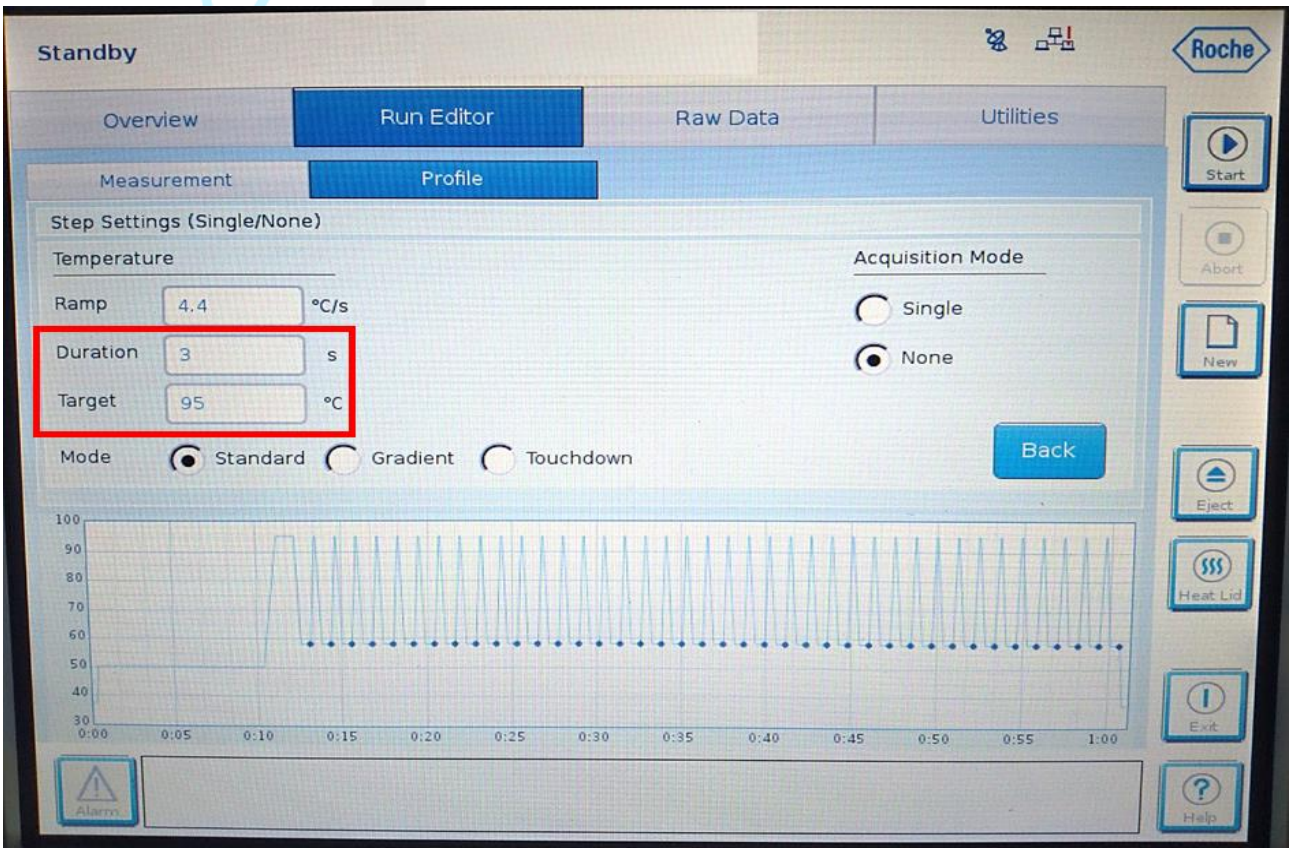


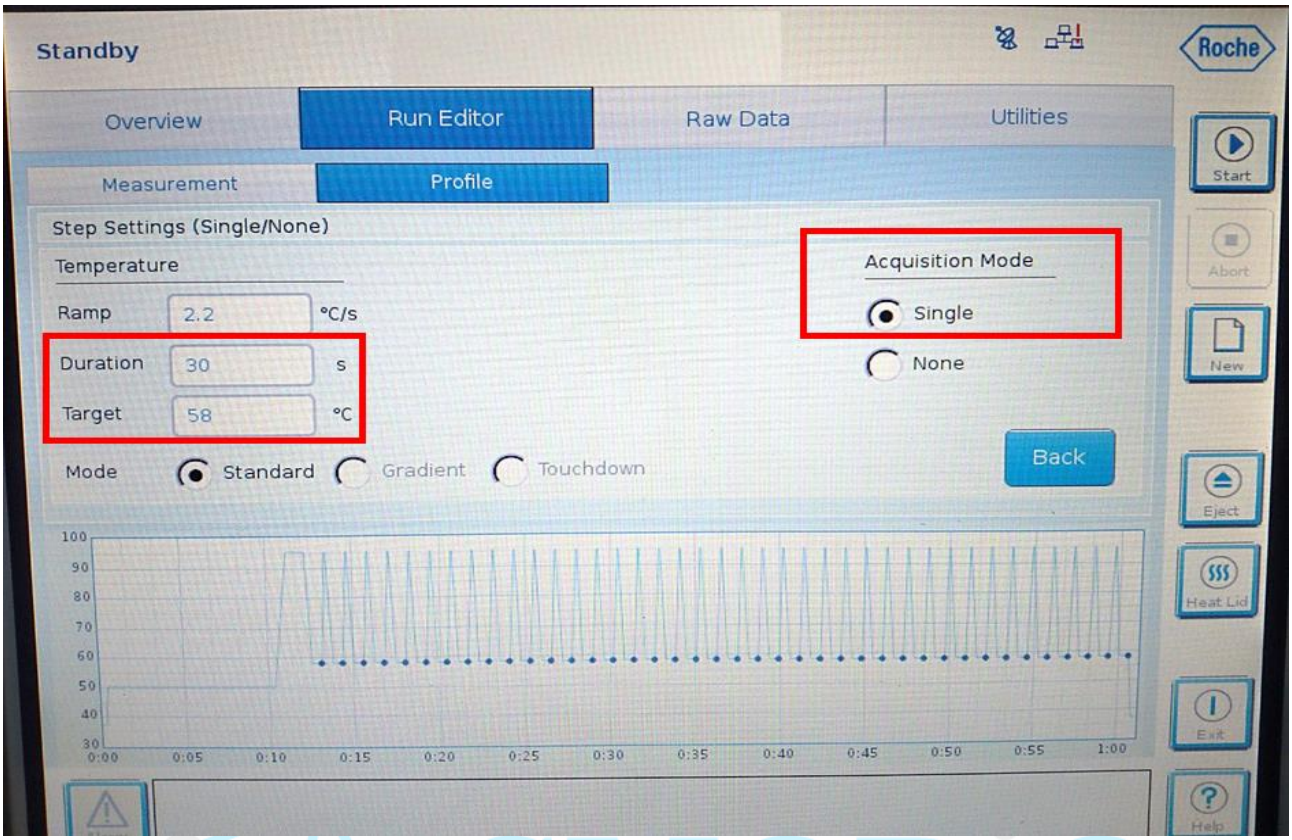


xii. Click the Profile and set the 2 Step Amplification.

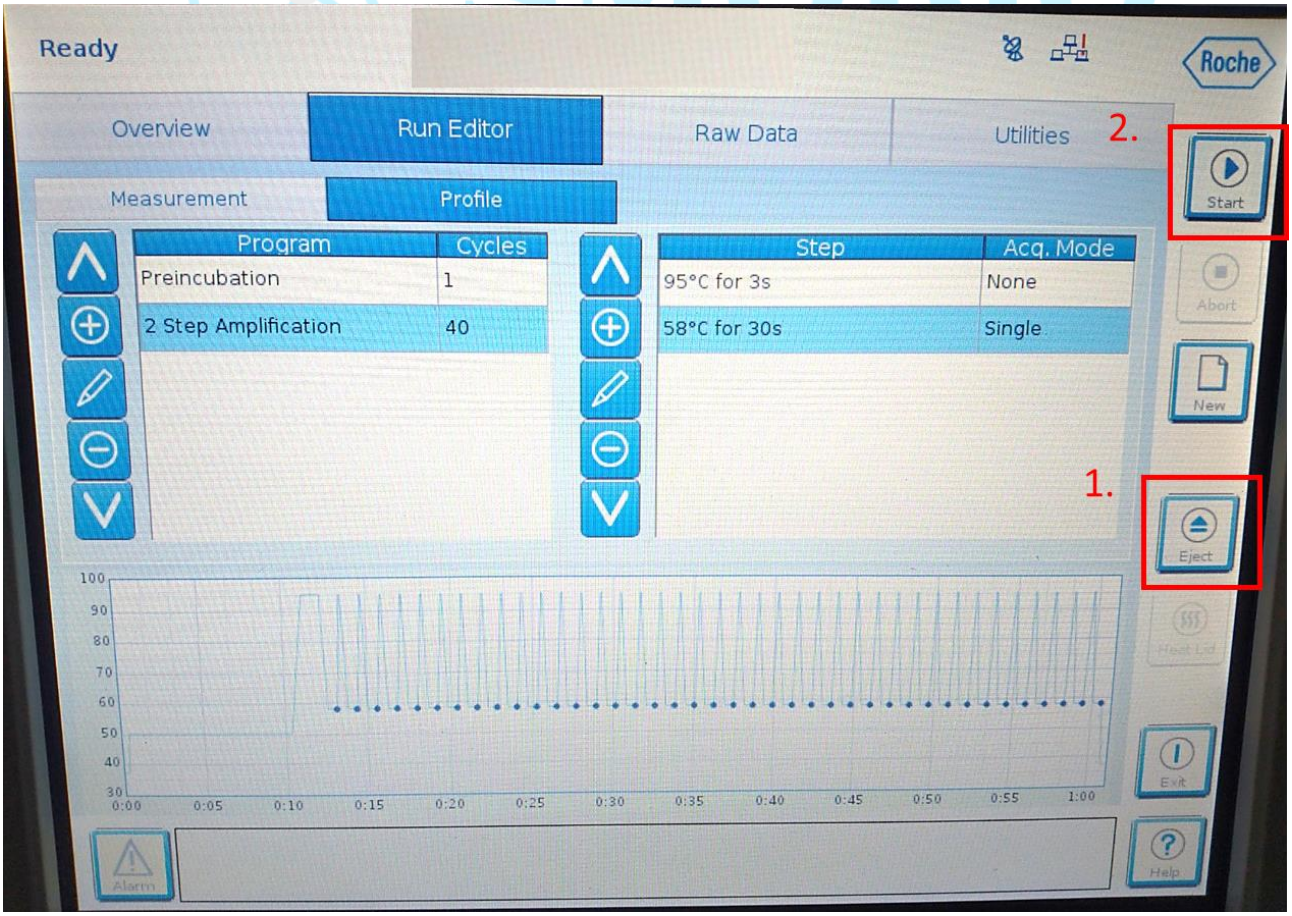


xiii. Click the Profile and set the 2 Step Amplification by modifing the Target temperature and Duration, and check the Acquisition mode.

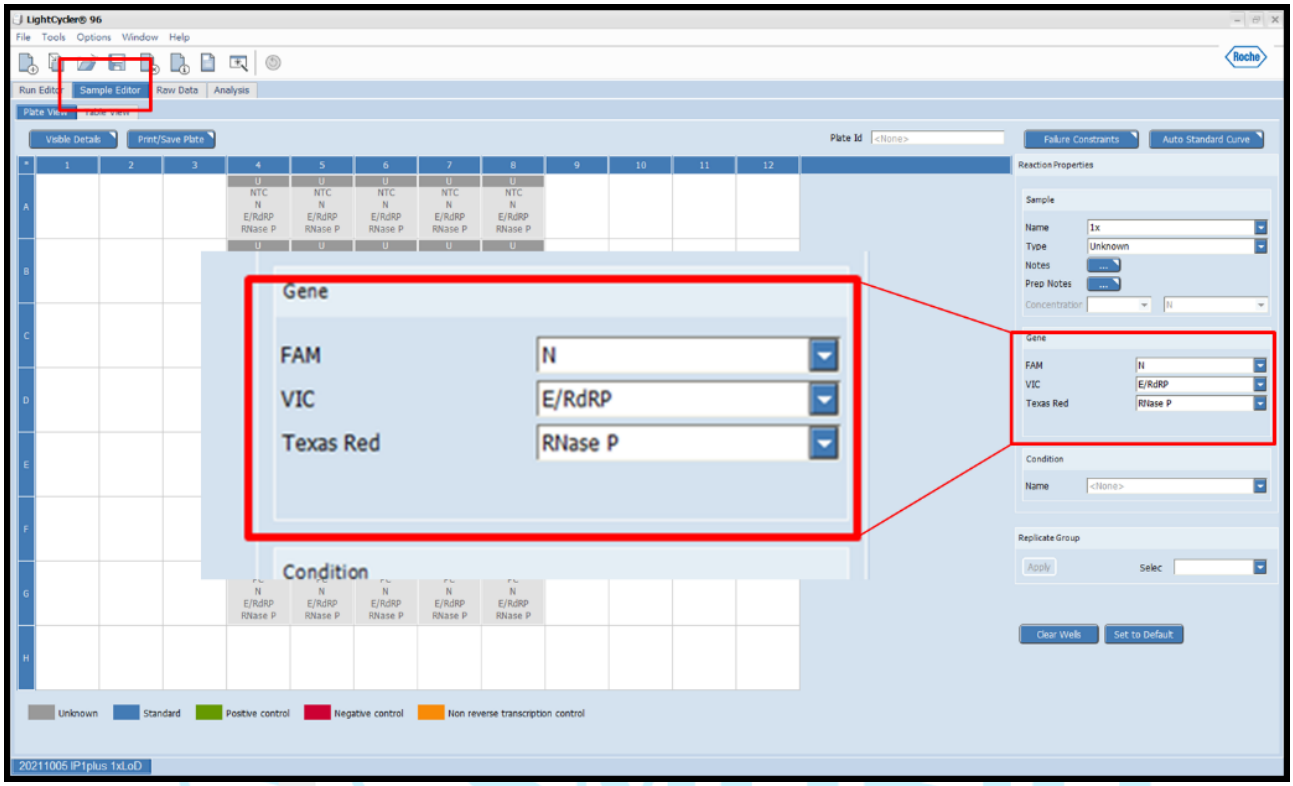




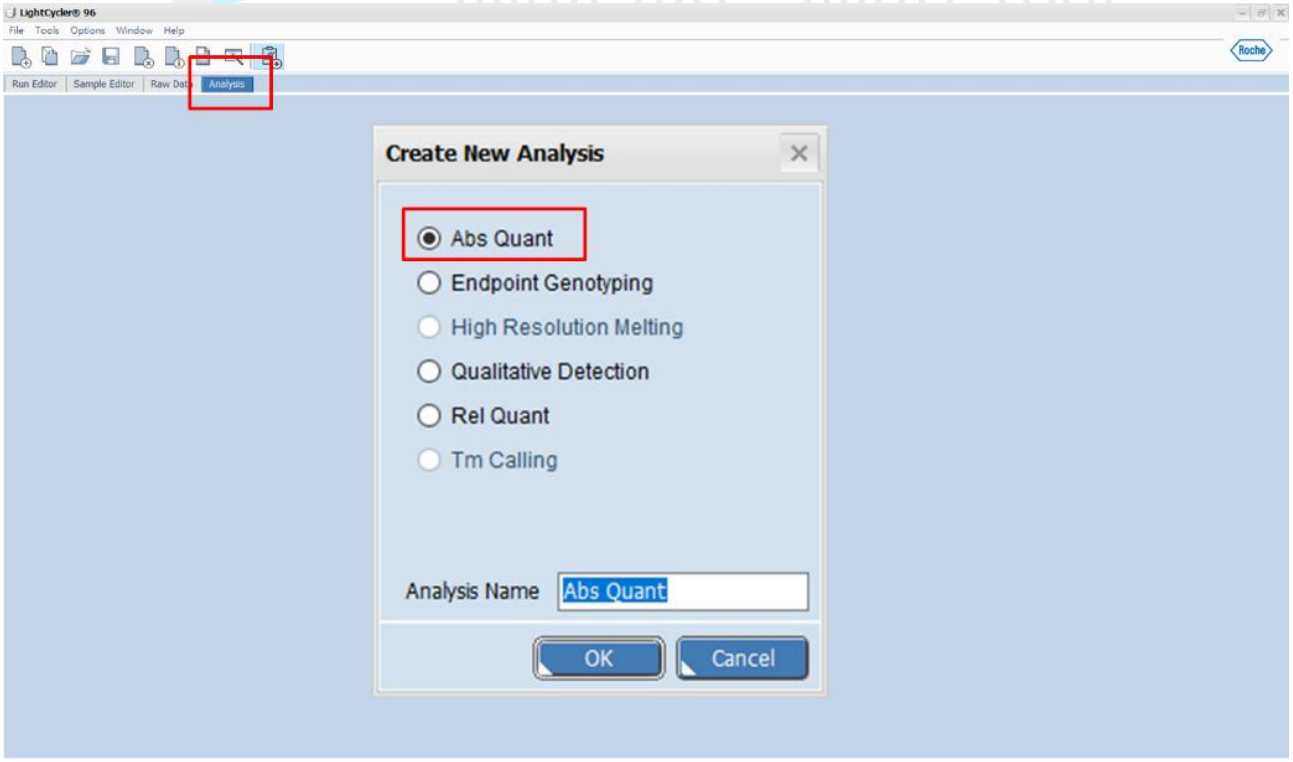
xiv. Eject the drawer, put the reaction plate in the drawer and click “Start” to initiate the reaction.



xv. For data analysis after PCR, select “FAM” channel for N target, “VIC” channel for E/RdRP target and “Texas Red” channel for RNase P target.



xvi. Select “Analysis” and use the default analysis mode “Abs Quant”.



xvii. Select “OK” to run analysis automatically and get the results.