BIOGENIX

POLICY & PROCEDURE FOR QPCR PROCESSING AND REPORTING

	NAME	DESIGNATION	SIGNATURE	DATE
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1 REVISION HISTORY

#	Version	Date	Changes Made by	Reason for Changes	Clause Changed
1	1.0				

2 REVIEW HISTORY

#	Version	Date	Changes Made by	Reason for Changes	Clause Changed
1	1.0				

3 STATEMENT

This procedure describes the steps of performing the qPCR step of SARS-Cov-2 Nucleic Acid Detection (RT-PCR), data analysis, and result reporting standard.

4 PURPOSE

To show the steps of performing the qPCR on the extracted nucleic acid materials that was performed in the extraction room.

5 SCOPE

5.1 This policy covers the process of performing the qPCR on the extracted sample RNA

5.2 The steps of interpreting the readings of the qPCR run

5.3 The criteria for validating the quality control of the run

5.4 The criteria for performing repeating qPCR run, re-extraction, or repeating sample collection

6 DEFINITION

6.1 Extraction: The process of extracting the total RNA of the sample. Next, mRNA is reverse transcribed into cDNA by using oligo dT primers and reverse transcriptase, the resulting cDNA can be used as the template for quantitative PCR amplification to detect SARS-Cov-2

6.2 Re-extraction: The process of repeating the extraction step from the stored patient sample

7 ACRONYMS

7.1 COVID-19: CORONAVIRUS DISEASE 2019
7.2 SARS-COV-2: SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2
7.3 Real time RT-PCR: real-time reverse transcriptase polymerase chain reaction.
7.4 qPCR: quantitative polymerase chain reaction
7.5 BSC: Biosafety Cabinet
7.6 Ct value (cycle threshold): The number of cycles required for the fluorescent signal to cross the threshold (i.e. exceeds background level)
7.7 VIC/HEX : Human House-keeping gene β-actin for control with HEX/VIC channel
7.8 FAM :COVID 19 specific gene ORF-1ab for SARS-CoV-2 with FAM channel
7.9 PBS: Phosphate–buffered saline or solution
7.10 bp : base pair
7.11 ng: nanogram
7.12 Rxn: Reagent

8 RESPOSIBILITIES

8.1 It is the responsibility of the laboratory technologists to follow the steps described in this procedure

9 PROCEDURE

9.1 PRINCIPLE

The kit is based on in vitro RT-PCR combining fluorescent probing. Primers and a sequence-specific fluorescence probes were designed tailored to high conservative region in 2019-nCoV genome. The probes are oligonucleotide attached fluorophores at the 5' end with FAM as reporter and 3’ end with quencher. In a meantime, specific primers and probes were developed as internal reference with fluorophores VIC/HEX attached at 5’ end as reporter. During the PCR procedures, the DNA polymerase cleaves the probe at the 5’ end and separates the reporter dye from the quencher dye when the probes hybridize to the target DNA. This cleavage results in the fluorescent

signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. Monitoring the fluorescence intensities during Real Time allows the qualitative detection of 2019-nCoV in the test samples.

9.2 qPCR Experiment Requirements

9.2.1 Reagents

Table 1. The reagents used to perform the qPCR step in the

Reagent Name	Lot No.	Size	Brand
Real-Time Fluorescent RT-PCR kit for detecting 2019-nCoV）	-	50rxn	BGI

9.2.2 Main instruments

Table 2. The instruments required in performing the qPCR

Equipment name	parameter	Usage	Recommended brand	Recommended models
Refrigerator	4℃ and -20℃	Reagent and sample storage	—	—
Temperature and humidity recorder	—	Record temperature and humidity	—	—

UV Disinfection Vehicle	wave length 254nm	sterilization	—	—
QPCR machine	FAM/VIC/HEX fluorescent channel	PCR	ABI	Stepone7500

9.2.3 Temperature and Humidity standards

9.2.3.1 Laboratory temperature range: 20 ℃ ~ 25 ℃; Humidity range: 30% ~ 70%.

9.2.3.2 -20 ℃ freezer temperature range: -15 ℃ ~ -25 ℃; 4 ℃ refrigerator temperature range: 2 ℃ ~ 8 ℃

9.2.3.3 The laboratory management team should be contacted in time if the environment exceeds the required range;

9.2.3.4 Only those who have an employment permit can sign on the logbook. Those who have not been issued with the employment permit should sign the logbook under the supervision of experienced technician.

9.3 qPCR

9.3.1 Pre-qPCR Preparation

9.3.1.1 Turn on the qPCR instrument and the connected computer.

9.3.1.2 Check and confirm plate ID on the 96-well plate and info in《COVID-19 Sample Nucleic Acid Test Record》task list are consistent, confirm the samples have no visible residual beads and bubbles, if PCR plate foil was completed, if the liquid

volume is 30 uL , no liquid remains on the well wall, then spray 75% Ethanol to the printed《COVID-19 Sample Nucleic Acid Test Record 》.

9.3.1.3 After confirm the information above, then put the sample plate (or tube) in a PCR plate mini-centrifuge and centrifuge briefly for 10s.

9.3.1.4 If there was any abnormal, contact the extraction lab technicians to confirm the process, if the plate will continue to be tested, remark the problem in the《COVID-19 Sample Nucleic Acid Test Record 》

9.3.2 qPCR Experiment

9.3.2.1 Turn on the software and the qPCR instrument, as shown in Figure 1.

Figure 1. qPCR Software

9.3.2.2Click on COVID-2019 in the red box in the Figure 2

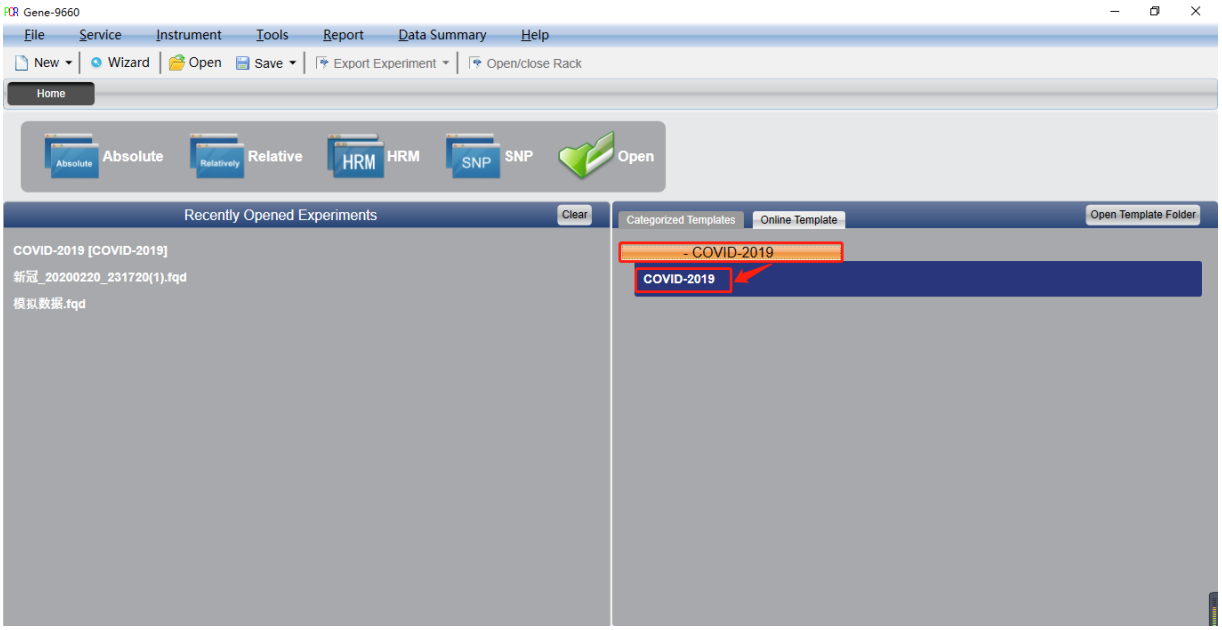


Figure 2. Second step Click on COVID-2019 in the red box

9.3.2.3 Name the experiment, as shown in Figure 3. In the software interface, click Detector, then click Experiment Name, experiment naming rules is ：Plate ID- the machine number, such as 20200318-1-G01.

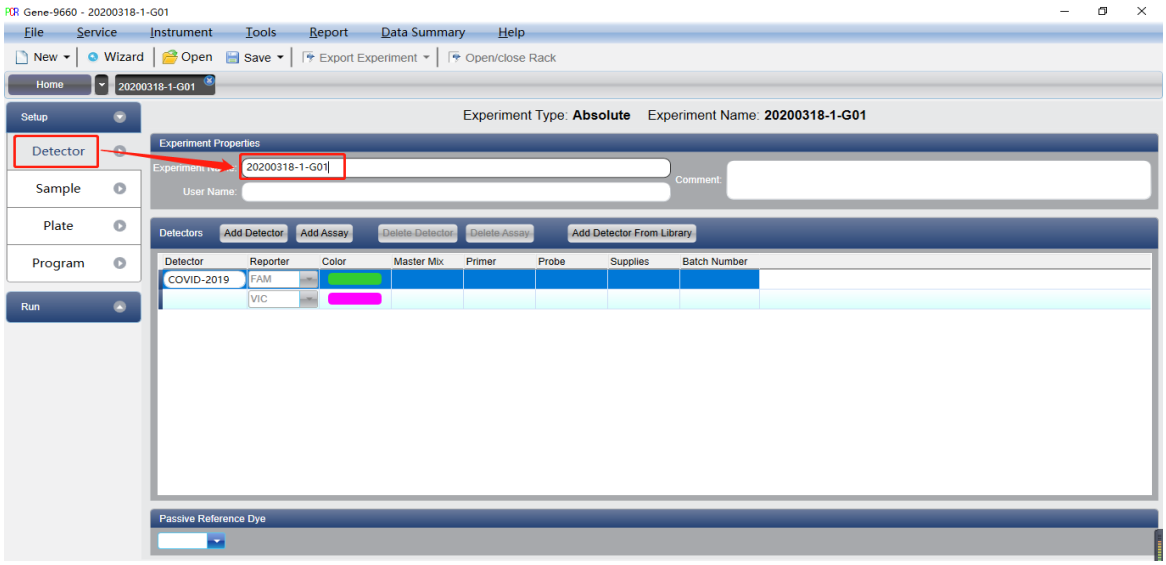
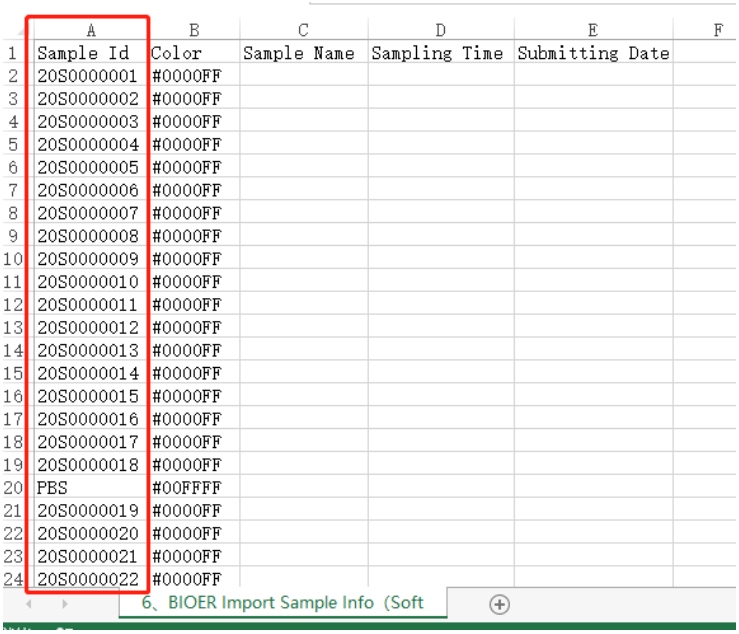


Figure 3. Third step, creating experiment name.

9.3.2.4 Input the sample information. Copy the Sample ID of the softcopy of the 《COVID-19 Sample Nucleic Acid Test Record》 to the Sample ID of the 《BIOER Import Sample Info》, as shown in Figure 4.



	A	B	C	D	E	F
1	Sample Id	Color	Sample Name	Sampling Time	Submitting Date	
2	20S0000001	#0000FF				
3	20S0000002	#0000FF				
4	20S0000003	#0000FF				
5	20S0000004	#0000FF				
6	20S0000005	#0000FF				
7	20S0000006	#0000FF				
8	20S0000007	#0000FF				
9	20S0000008	#0000FF				
10	20S0000009	#0000FF				
11	20S0000010	#0000FF				
12	20S0000011	#0000FF				
13	20S0000012	#0000FF				
14	20S0000013	#0000FF				
15	20S0000014	#0000FF				
16	20S0000015	#0000FF				
17	20S0000016	#0000FF				
18	20S0000017	#0000FF				
19	20S0000018	#0000FF				
20	PBS	#00FFFF				
21	20S0000019	#0000FF				
22	20S0000020	#0000FF				
23	20S0000021	#0000FF				
24	20S0000022	#0000FF				

6、BIOER Import Sample Info（Soft

Figure 4. Copying sampe ID to " BIOER Import Sample Info".

9.3.2.5 Input《BIOER Import Sample Info. Click **Sample** - **Import Sample info** - Select 《 BIOER Import Sample Info》 file – open the file, the procedure as shown in Figure 5.

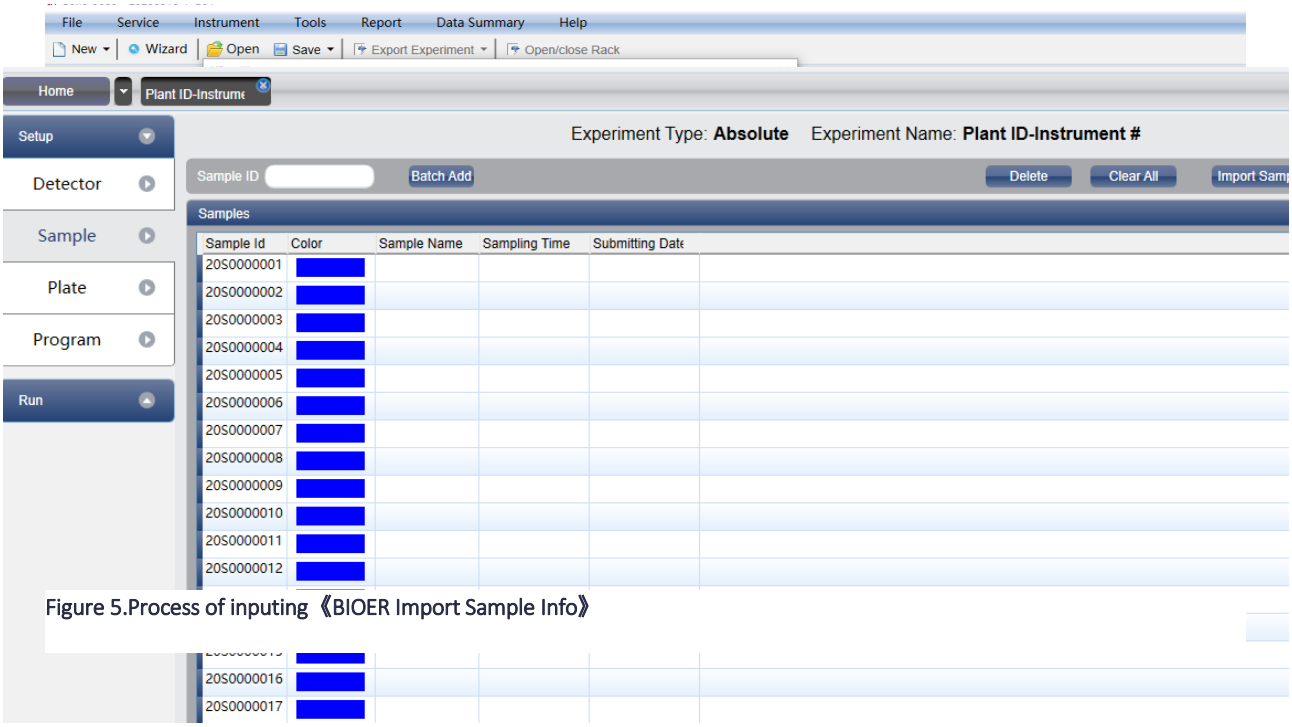


Figure 5.Process of inputing《BIOER Import Sample Info》

Figure 6. The presentation of program interface following to importing sample ID

9.3.2.6 After the sample ID is successfully imported, the program interface is as shown in Figure 6

9.3.2.7 Sample position information setup in plate. Click Plate and select all the 96 samples at plate block, then click **Sample Auto Arrange—Vertical/Horizontal—OK**, the samples will be arranged automatically, the procedure is as shown in Figure 7

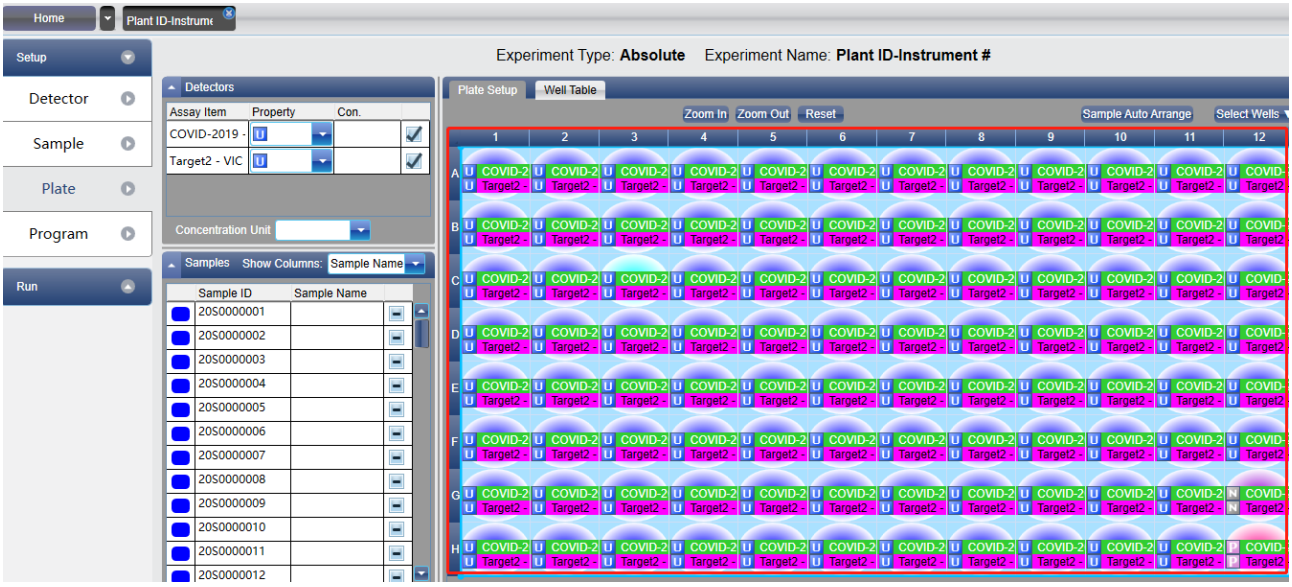


Figure 7.Arrangement of the samples in the 96 samples at plate block after clicking on "Sample Auto Arrange".

9.3.2.8 After sample arranged, check and confirm blank control, the positive control, and 3 random test samples' ID are consistent with the《COVID-19 Sample Nucleic Acid Test Record》, as shown in Figure 8.

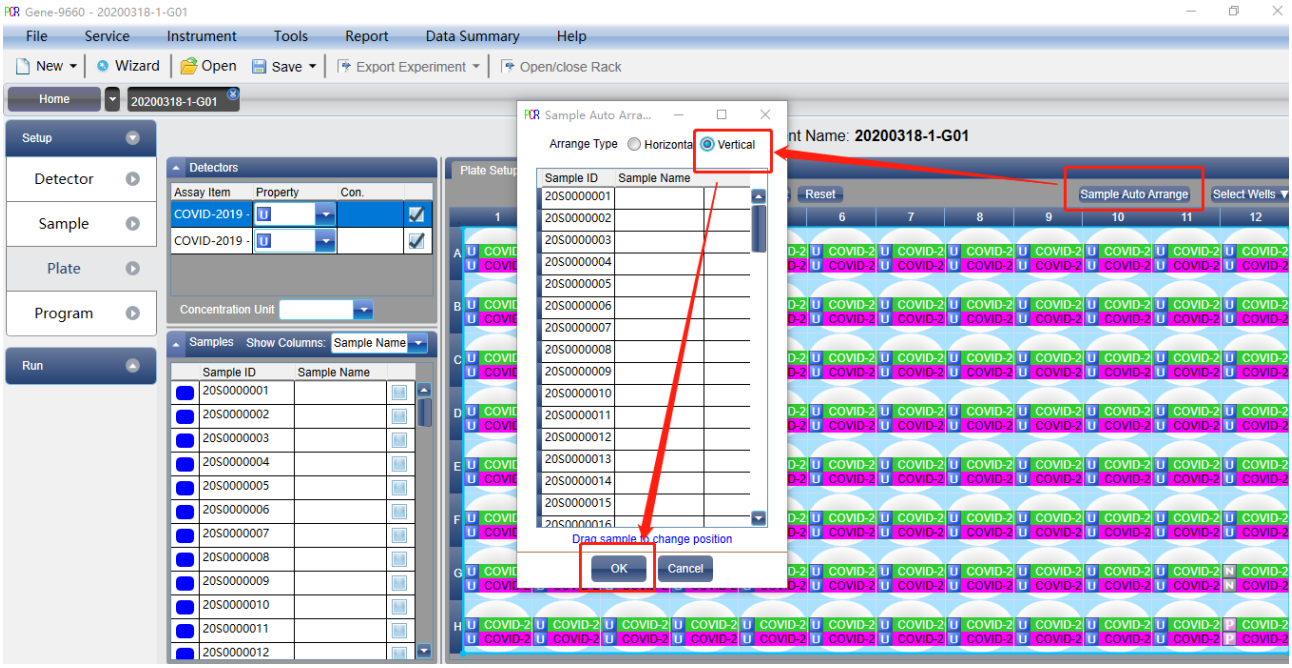


Figure 8. Confirm the positions of samples to be compliant with "COVID-19 Sample Nucliec Acid Test Record".

9.3.2.9 Click **Program**, confirm PCR reaction procedure as Table 3.

Table 3. The required PCR reaction proceduer program.

Temp.	Duration	Cycle	Collecting fluorescence signals
50 ℃	20 min	1	No
95 ℃	10 min	1	No
95 ℃	15 s	40	No
60 ℃	30 s		Yes

【NOTE】 At the last procedure, check Sampling √ as well as Tube √, confirm the procedures should be like the Figure 9 interface shown later.

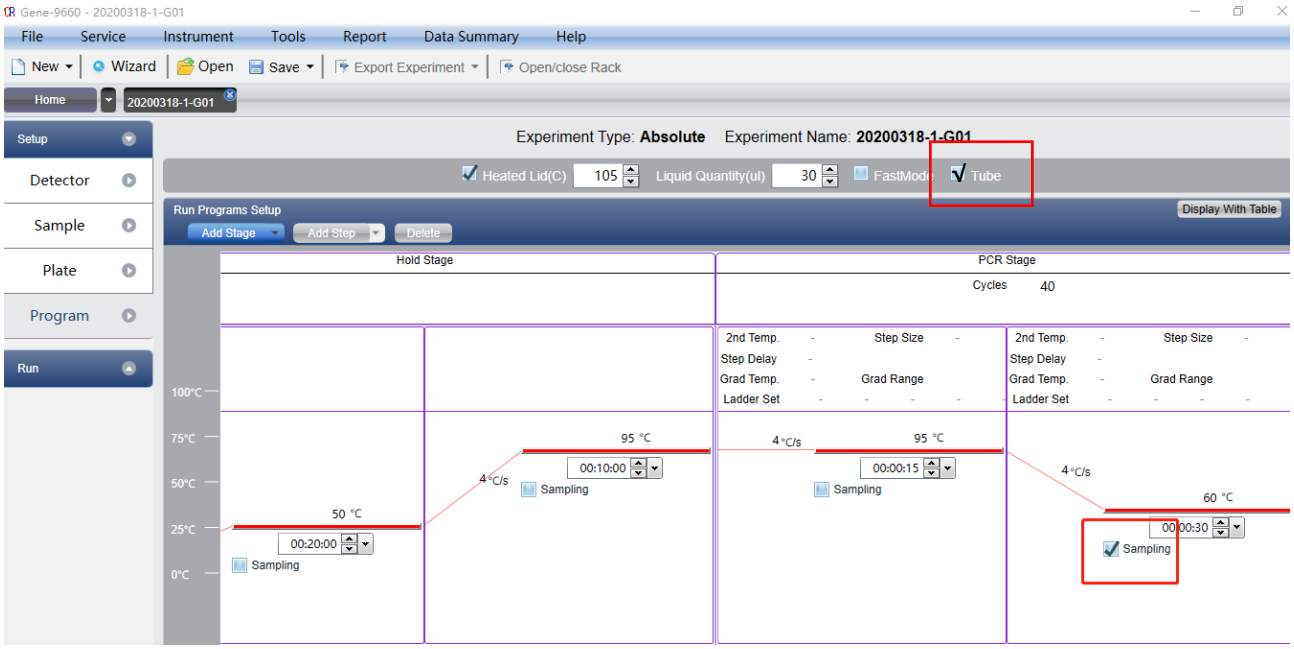


Figure 9. PCR program procedure.

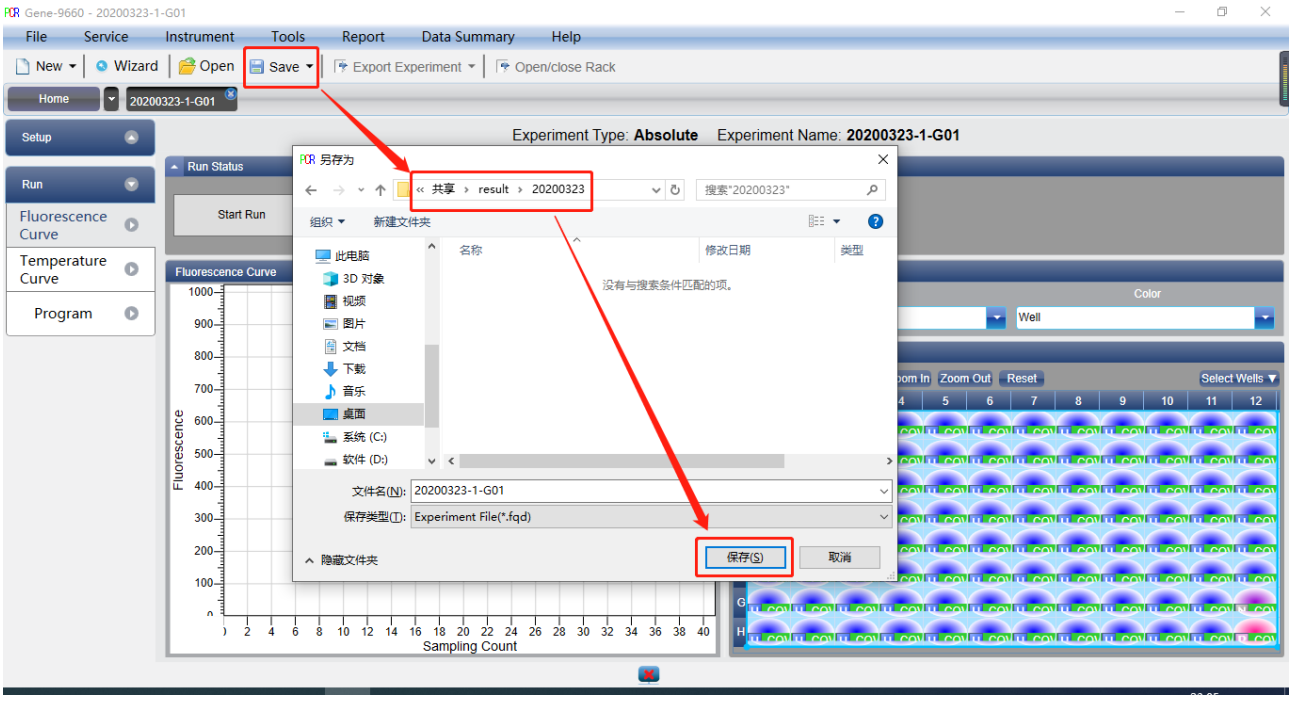


Figure 10. Export 《qPCR Result Export Excel》 and save as a specific shared file location in NAS

9.3.2.10 Click Export experiment to export 《qPCR Result Export Excel》, save as a specific shared file location in the result folder of the day in qPCR folder, as shown in Figure10.

9.3.2.11 Select Save as to a specific shared qPCR folder in the result folder of the day, file naming rule is plate ID - the instrument number, same as the experiment name rule. as shown in Figure 11.

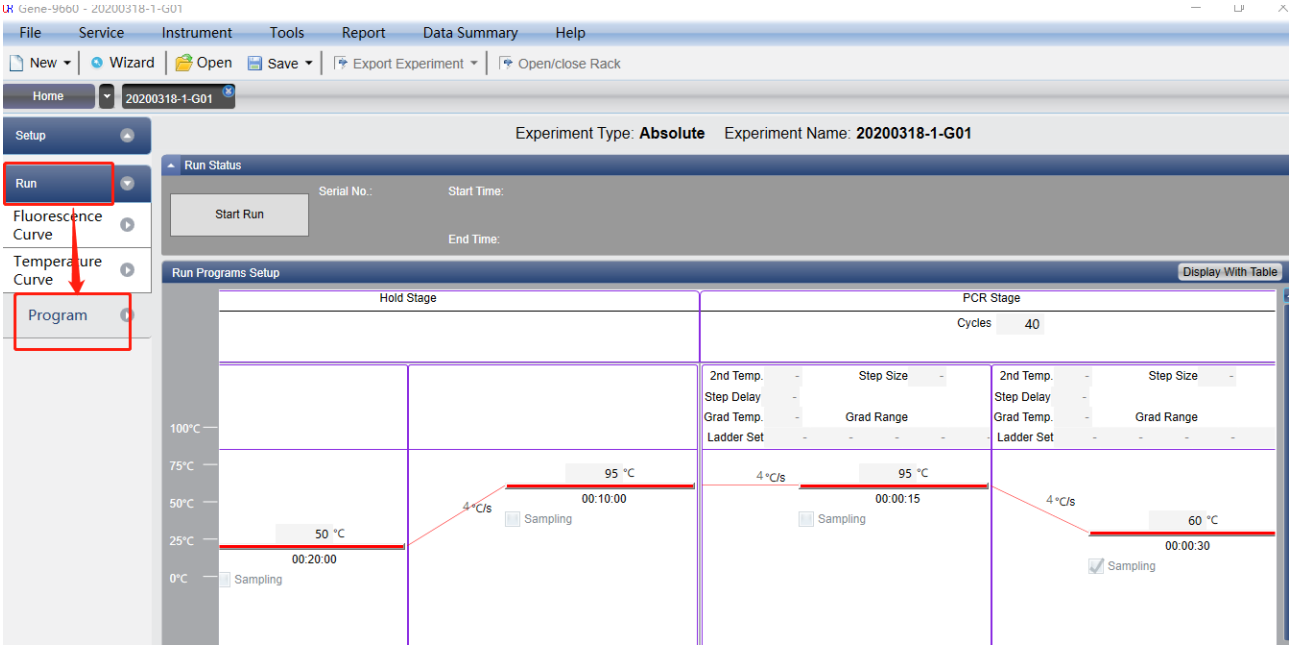


Figure 11. Save to specific shared qPCR folder in the result folder of the day, file naming rule is plate ID - the instrument number, same as the experiment name rule.

9.3.2.12 Click the **Run**, then click **Program**, check again to confirm the procedure set is correct.

9.3.2.13 Open qPCR instrument lid, put the PCR plate into the corresponding qPCR instrument, ensure the correct placement which means the upper left corner toward the cutaway, check again for correct plate label, close qPCR instrument lid, click Start Run to run qPCR program. After confirming the program can run normally, fill in the printed 《COVID-19 Sample Nucleic Acid Test Record》and《COVID-19 qPCR Summary Record》.

9.3.2.14 qPCR instrument running duration is around 1.5h.

9.3.3 Post-experiment treatment

9.3.3.1 Open 《COVID-19 qPCR Summary Record 》 and fill in the end time of experiment.

9.3.3.2 Take out the 96-well plate, check the insistence of 96-well plate ID on task list and on board, after confirmation, handle the used 96-well plate in accordance with the medical waste.

9.3.3.3 After the final batch experiment in a day, turn off the qPCR room instrument power, spray the qPCR instrument surface with 75% Ethanol, place and switch on the ultraviolet lamp to qPCR instrument surface above 0.5m in the range of UV sterilized for at least 30 min .

9.4 Sample Analysis

9.4.1 Baseline and Threshold setting

Set the Bioer9660 fluorescence quantitative qPCR instrument baseline and threshold settings as bellow:

Baseline setting: the start cycle of the baseline is 3, and the end cycle is 15.

Threshold setting: The threshold value of each channel should be set separately. When setting the threshold value of a channel, firstly select the negative control for detection, remove the automatic checked threshold, check " Auto" , and manually adjust the threshold line so that the threshold line just exceeds the highest point of the blank control amplification curve in FAM channel (the random noise line).

9.4.2 Quality Control and Retest Criteria

9.4.2.1 Quality Control Analysis Criteria

9.4.2.1.1 First step: Blank/Positive control should pass following criteria

9.4.2.1.2 Blank control (VIC＞32，FAM＞38) while Positive control (VIC＜32，FAM＜32)

9.4.2.1.3 If VIC of Blank control fails in quality control, perform whole plate sample RE-extraction;

9.4.2.1.4 If FAM of Blank control fails in quality control, perform RE-extraction for sample with positive results;

9.4.2.1.5 If Positive control fails in quality control, perform whole plate sample RE-extraction.

9.4.2.1.6 If VIC of whole plate appear style of “Horse Tail (VIC curve raised at same PCR cycle but separate from each other widely at 40 cycle (Figure 12), which may cause the false negative results)”, perform whole plate sample RE-extraction.

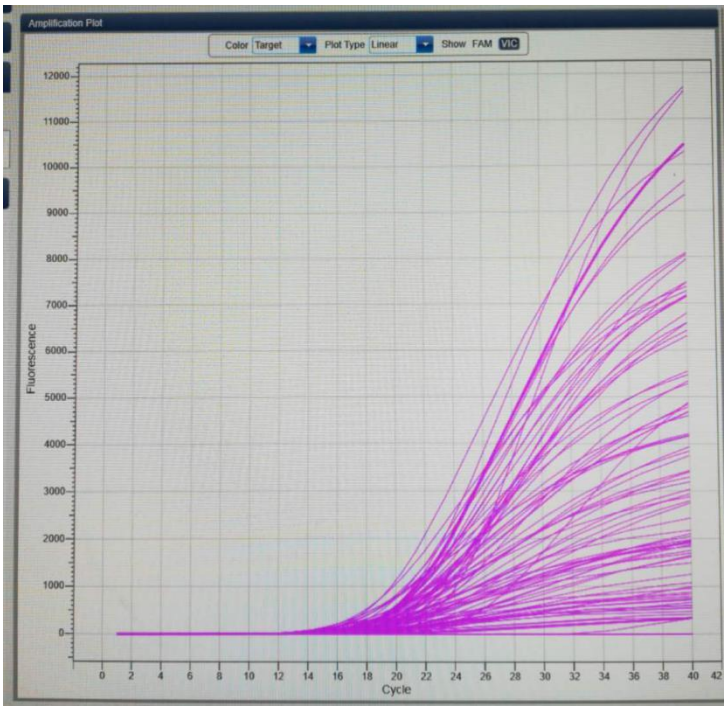


Figure 12. Horse Tail style in plate reading

9.4.3 Patient Sample Reporting Logic

9.4.3.1 For each test specimen, VIC must present “S” curve with VIC Ct value ≤32;

9.4.3.2 When Ct value at VIC channel are higher than 32, or with no Ct value at VIC, re-extraction is required.

9.4.3.3 Table 4 outlines the interpretation on positive, grey zone, and negative results.

Table 4. Criteria of reporting logics of patient sample results.

Interpretation on positive, grey zone and negative (Ct at FAM)	Action on 1st test result	No. of reference result	Action after re-test
No "S" curve and No Ct value at FAM: Negative	Negative report	1	
"S" curve and FAM Ct≤35: High positive	Positive report	1	
"S" curve and 35<FAM Ct≤38: Weak positive	RE	2	If "S" curve and FAM Ct≤38 in RE results, release **Positive** report.
		2	If negative RE result, **re-collect new sample**.
		2	If "S" curve and FAM Ct>38 in RE results, **re-collect new sample**.
"S" curve and FAM CT>38: Grey zone	RE	2	If no Ct at FAM for RE result, release **Negative** report
		2	If "S" curve and FAM Ct≤38 in RE results, release **Positive** report.
		2	If "S" curve and FAM Ct>38 in RE results, **re-collect new sample**.
No "S" curve but with Ct value at FAM: Re-extraction	RE	2	If result remains as 1st test, perform Re-collection

9.4.4 qPCR Results Records

9.4.4.1 Open《COVID-19 Testing Report Summary Record》and《qPCR Result Export Excel 》

9.4.4.2 Copy《qPCR Result Export Excel》-Detectors-Experiment Name Sample ID to《COVID-19 Testing Report Summary Record》- Plate ID ,Sample ID

9.4.4.3 Copy《qPCR Result Export Excel》-Quan. Result-Ct to《COVID-19 Testing Report Summary Record》-FAM Ct value, VIC Ct value

9.4.4.4 According to the data analysis metrics, fill in the results, report released? And remarks in《COVID-19 Testing Report Summary Record》.

9.4.4.5 Fill in the quality control results in the 《Real Time Fluorescent RT-PCR Quality Control Monitoring Summary Log Sheet》

9.4.4.6 And according to the result, to fulfill the re-test samples information to《COVID-19 Retest Sample Record》, then inform the lab coordinator and extraction lab technicians in time.

10CROSS REFERENCES:

10.1Manual operating producer for the machine MGI -960

10.2MGI Easy Nucleic Acid Extraction Kit User Manual

10.3kit insert of real time fluorescent

10.4Real time Fluorescent RT-PCR method sheet insert sheet

11.RELEVANT DOCUMENTS & RECORDS

11.1 BG/REC/MOL/022 COVID-19 Test Report Summary Records

11.2 BG/REC/MOL/023 COVID-19 Test Retest Records

11.3 BG/REC/MOL/025 Real Time Fluorescent RT-PCR Quality Control Monitoring Summary Log Sheet