User Manual

Real-Time PCR System

E-QPCR4-CI

E-QPCR6-CI

Please carefully read this user manual in advance to use the instrument at its full potential

CONTENTS

COPYRIGHT 5
SAFETY NOTICE 6
Common safe type cautions 6
Chapter1. Introduction 9
1.1 Instrument introduction 9
1.1.1 Intended use 9
1.1.2 Specifications 9
1.1.3 Characteristics 10
1.1.4 Software functions 11
1.2 Declaration of conformity 11
Chapter2. INSTRUMENT STRUCTURE 13
2.1 Equipment Overview 13
2.1.1 Structure 13
2.1.2 Status light 13
2.1.3 Entrance and exit button 14
2.1.4 Instrument switch 14
2.1.5 Data Interface 14
2.1.6 Air inlet 14
2.2 Temperature control system 14
2.2.1 Heated lid 14
2.2.2 Reaction plate 15
2.2.3 Applicable supplies 15
Chapter3. INSTALLATION 16
3.1 Installation preparation 16
3.1.1 Visual inspection 16
3.2 Instrument installation 16

3.2.1 Required environment conditions 16
3.2.2 Electrical requirements 17
3.2.3 Unlock 18
3.2.4 Connect to computer 19
3.3 Software Installation 19
3.4 System startup 21
3.5 Instrument transportation 22
Chapter4. SOFTWARE INTRODUCTION 24
4.1 Software startup 24
4.2 Interface introduction 24
4.2.1 Menu Commands 24
4.2.2 Toolbar 28
4.2.3 Status bar 28
4.3 Properties Setting 28
4.3.1 Instrument Type 28
4.3.2 Experiment Setting 29
4.3.3 System Setting 29
4.3.4 Instrument Setting 29
4.4 Program setting 30
4.4.1 Interface setting 30
4.4.2 Parameter modification 30
4.4.3 Save method 33
4.5 Sample Setting 33
4.5.1 Select sample location 33
4.5.2 Sample information setting 33
4.6 Start running 36
4.7 Stop running 37
4.8 Data export 37

4.8.1 View Amplification Curves and Ct Value 37
4.8.2 View melting curves and Tm value 41
Chapter5. DATA ANALYSIS 43
5.1 Absolute quantification 43
5.1.1 Experiment setting 43
5.1.2 Data analysis 43
Chapter6. APPLICATIONS 45
6.1 SYBR Green dye-based assays- GM soybean identification 45
6.1.1 Genomic DNA extraction 45
6.1.2 Primer design 46
6.1.3 Preparation of standards 46
6.1.4 Preparation of qPCR Reaction Solution 46
6.1.5 Power on 47
6.1.6 Software startup 47
6.1.7 New experiment 47
6.1.8 Program Setting 48
6.1.9 Sample Setting 48
6.1.10 Save method 49
6.1.11 Sample entry 49
6.1.12 Start running 49
6.1.13 Stop running 50
6.1.14 Data analysis 50
6.2 Taqman Assays- Liver cancer gene expression 51
6.2.1 Preparation of template 52
6.2.2 Primer design 52
6.2.3 Preparation of qPCR Reaction Solution 52
6.2.4 Power on 53
6.2.5 Software startup 53

6.2.6 New experiment 53
6.2.7 Program Setting 53
6.2.8 Sample Setting 54
6.2.9 Save method 54
6.2.10 Sample entry 55
6.2.11 Start running 55
6.2.12 Stop running 55
6.2.13 Data analysis 55
Chapter7. TROUBLE SHOOTING 56
Chapter8. DAILY MAINTENANCE 57
8.1 Computer and software maintenance 57
8.2 Reaction plate cleaning 57
8.3 Fan cleaning 57
8.4 Fuse 58
8.4.1 Model 58
8.4.2 Inspection and replacement 58

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SAFETY NOTICE

Common safe type cautions

Carefully read the following safety precautions for a thorough understanding.

- Follow the instructions and procedures described in this manual to operate this instrument safely.
- Carefully read all safety messages in this manual and the safety instructions on the instrument.
- Safety messages are labeled as indicated below. They are in combination with signal words of “WARNING” and “CAUTION” with the safety alert symbol ⚠ to call your attention to items or operations that could be dangerous to you or other persons using this instrument.

⚠ WARNING: Personal Danger

Warning notes indicate any condition or practice, which if not strictly observed, could result in personal injury.

⚠ CAUTION: Possible damage to instrument

Caution notes indicate any condition or practice, which if not strictly observed or remedied, could result in damage or destruction of the instrument.

⚠ NOTE: Notes indicate an area or subject of special merit, emphasizing either the function of the product or common errors in operation or maintenance.

- Do not operate this instrument in any manner not described in this manual. When encounter trouble with this instrument, ASK FOR HELP from original manufacturer or authorized distributors.
- The precautions described in this manual are carefully developed in an attempt to cover all the possible risks. However, it is also important that you are alert for unexpected incidents. Please operate with care.

⚠ WARNING

- The instrument must be grounded properly to avoid electric shock.
- Keep away from live circuits: The operator must not disassemble the plastic case, replace components or adjust the machine. It is forbidden to disassemble the machine when the power is on. Please contact the professional after-sales engineer for instrument maintenance and repair If necessary.
- Power cord: The supplied power cord should be used. It must be replaced with a power cord of the same type and size if the power cord is damaged. Make sure that the plug is fully and securely inserted into the socket.
- Placement of the instrument: Do not place the instrument in a location where it is difficult to turn off the power. The instrument should be placed in a place with low humidity, low dust and away from water sources (pools, water pipes, etc.). The laboratory should be well ventilated and free from corrosive gases or strong magnetic fields. Do not place the instrument in a location that is wet or dusty.
- The vents at the lower end of the front door, the back panel, and the bottom plate are all set up for the ventilation and heat dissipation. Do not block or cover these vents to ensure normal operation and accurate experimental data of the instrument.
- Excessive ambient temperatures can affect test performance and even cause operational failures. Keep away from heating, hot gases, and all other sources of heat. Do not use the instrument in direct sunlight or strong light, so as not to affect the reliability of fluorescence detection.
- The power supply should be turned off when the instrument stops working. Please cut off the power supply and unplug the power plug when it is not used for a long time. Cover the instrument with soft cloth to prevent dust and foreign matter into it.

⚠ NOTE

- If any of the following occurs, please cut off the power supply immediately, unplug the power cord, and contact our after-sales service department as soon as possible. The company will arrange for professionals to repair.
- The liquid falls into the inside of the instrument.
- The instrument is rained or watered.
- There is abnormal sound or smell after the instrument is powered on.

- The instrument falls or the casing is damaged.
- The function of the instrument has changed significantly.

Chapter1. Introduction

1.1 Instrument introduction

1.1.1 Intended use

The Eins-Sci E-QPCR real-time quantitative PCR system with 96-well is based on Fresnel lens optical signal acquisition technology, time-resolved signal separation technology and unique temperature control technology. It reaches international advanced level in sensitivity, multi-color crosstalk, temperature uniformity and accuracy. The instrument has 4/6 detecting channels and conform to multiplex PCR application.

The instrument currently supports the application of all common real-time PCR assays, including relative quantification, absolute quantification, allelic discrimination assays, genotyping, and gradient PCR. It can be applied to multiple areas of basic research in medicine and biology, such as early clinical diagnosis of diseases, pathogen detection, tumor-related genes and drug resistance genes detection, gene expression levels in tissues or cells, transgenic animal and plant testing.

1.1.2 Specifications

Temperature control system	
Sample capacity	0.1ml PCR tubes×96, 8×12 PCR plate or 96 well plate ×1
Reaction volume	10-50 μl
Thermal cycle technology	Peltier
Max. Heating/Cooling rate	6.0° C/s
Heating temperature range	4 – 100 °C
Temperature accuracy	± 0.2°C
Temperature uniformity	±0.2℃ @60℃ , ±0.2℃ @95℃
Temperature gradient setting range	30–100°C
Temperature gradient difference setting range	1 – 36 °C
Detection system	
Excitation light source	4/6 monochrome high efficiency LEDs

Detection device	PMT
Detection mode	Time-resolved signal separating technology
Excitation/detection wavelength range	455-650nm/510-715nm
Fluorescent channels	4/6 channels
Supported dye	FAM/SYBR Green, VIC/JOE/HEX/TET, ABY/NED/TAMRA/Cy3, JUN, ROX/Texas Red, Mustang Purple, Cy5/LIZ
Sensitivity	Single copy gene
Resolution	1.33 folds copy number difference can be distinguished in single-plex qPCR
Dynamic range	10 orders of magnitude copies
Analysis mode	
Absolute quantification, Melting curve analysis	
Data output	
Original file, Excel data file, results include run settings, graph and tabular data results	

1.1.3 Characteristics

1. High sensitivity

New optical signal detecting technology based on Fresnel lens, special high-efficiency PMT and long-life maintenance-free LED, further improves the detection sensitivity of the prior technology.

2. High accuracy

Innovative scanning mode and time-resolved signal separating technology can effectively avoid cross-interference of inter-hole signals and multi-color fluorescence.

3. Multiple detection

Up to 4/6 fluorescence detection channels meet the requirement of multiplex PCR.

4. Fast and stable

Owing to unique edge temperature compensation technology, the thermal cycle rate, temperature uniformity and accuracy of the system reach a new height.

5. Use-friendly software

Humanized and fully featured software provides complete solutions for sample testing, data calculating, and result analysis.

6. Pursuing quality

Internationalized product design ensures the superior performance of each instrument.

1.1.4 Software functions

1. Parameter setting function (fluorescence channel, temperature, time, cycle number, data processing method).
2. Sample information recording function (experiment time, sample number, sample type, sample name).
3. Detection data output function (threshold, fluorescence value, CT value, Tm value).
4. Data result analysis function (absolute quantification, relative quantification).
5. File storage function (setting data, running data, analysis results).
6. Fault reminder function.

1.2 Declaration of conformity

In compliance with the following safety standards:
EN 61010-1
EN 61010-2-020
EN 61010-2-101
In compliance with the following EMC standards:
EN 61326-1/FCCPart15Subpart B/ IECS 001
EN 61326-2-6:2006
Associated EU guidelines:
EMCguidelines:2004/108/EC
LVD guidelines: 2006/95/EC
This ISM device complies with Canadian ICES-001.

Changes or modifications not expressly approved by the party responsible for compliance could void the user's authorization granted by the manufacturer to operate

the equipment.

NOTE: This instrument has been tested and found to comply with the limits for a Class a digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the instrument is operated in a commercial environment. The instrument generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the user manual, may cause harmful interference to radio communications. Operation of instrument in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his or her own expense.

Chapter2. INSTRUMENT STRUCTURE

2.1 Equipment Overview

2.1.1 Structure

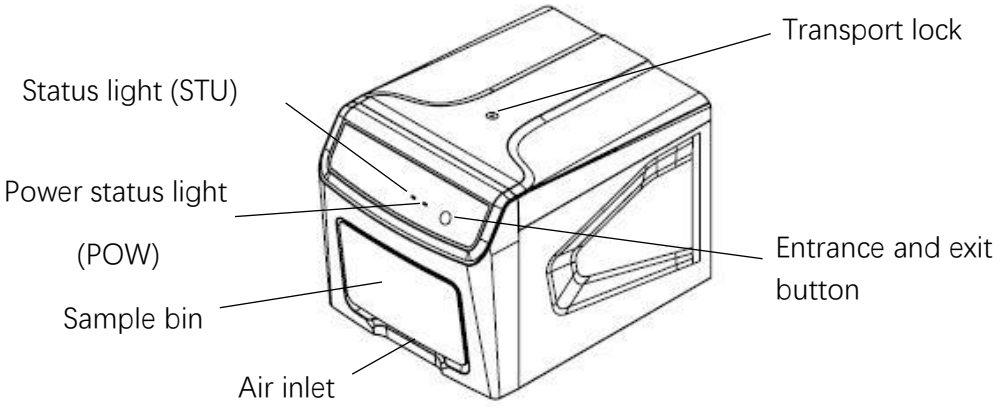


Figure 2-1 Side view of the instrument

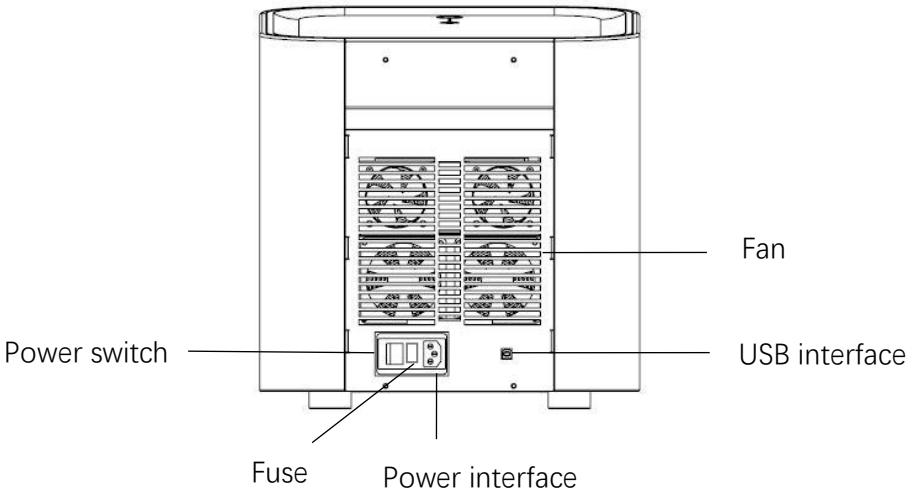


Figure 2-2 Back view of the instrument

2.1.2 Status light

The meaning of the POW and STU lamp on the instrument is shown in the table below.

	State	Meaning
POW	Blue steady bright	The system is powered on normally.
STU	Green flash slowly	Reaction plate entrance and exit
	Green flash quickly	The system is working or self-inspection
	Green steady bright	The system is idle
	Red steady bright	Failure, stop working

2.1.3 Entrance and exit button

The extending and retracting of the reaction plate are controlled by the entrance and exit button.

> ⚠ CAUTION:
>
> The extending and retracting of sample bin can operation only in the idle state of the instrument. The sample bin movement is not allowed in work process or self-inspection process.

2.1.4 Instrument switch

The instrument switch is located on the back of the instrument. The ship-type switch is used. The "I" button is on and the "O" button is off.

2.1.5 Data Interface

Data (USB) interface is used to connect computer with device.

2.1.6 Air inlet

The lower gap on the front panel is the air inlet. Blockage of objects should be avoided during the work.

> ⚠ WARNING:
>
> The air inlet is strictly forbidden to be used as a handle during transportation.

2.2 Temperature control system

2.2.1 Heated lid

The heated lid is located above the reaction plate. The holes position is correspond

to the reaction plate one by one. It is automatically positioned and pressing the reaction tube after the reaction plate enter the sample bin.

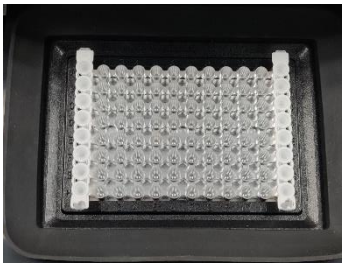
2.2.2 Reaction plate

The 96-well reaction plate is suitable for 96-well plates, eight-row tubes, and 100μl /200μl PCR tubes. Both white tube and transparent tube can be used.

⚠ NOTE:

1) Only the same specification PCR reaction tube can be used in one experiment to ensure the accuracy of the experiment.

2) Empty reactor tubes need to be placed at the sides of the reactor plate to support the heat lid when there are only several PCR reaction tubes in a single experiment, as shown in the figure below.



2.2.3 Applicable supplies

It is recommended to use 0.1ml PCR tube or 0.2ml Low-Profile PCR tube, as shown in the table below.

Index	Name	Manufacturer	Model
1	Low-Profile PCR Tubes 8-tube strip, white	BioRad	TLS0851
2	0.1ml LOW PROFILE, CLEAR PCR TUBES＆CAPS 8 STRIP TUBES＆REAL TIME CAPS	Axgen	PCR-0108-LP-RT-C
3	0.1ml No Skirt H12 White /Transparent	Nest	402111、402101

Chapter3. INSTALLATION

Users are requested not to open the package privately after receiving the instrument. Please call the after-sales service department and make an appointment for installation.

> ⚠ NOTE:
>
> The company does not take any responsibility for (including but not limited to the following) the damage of the instrument, the malfunction of the instrument and personal injury caused by open the package and installation privately without the written permission of the company. And the company will not provide free maintenance services. Please understand.

3.1 Installation preparation

3.1.1 Visual inspection

Please check the appearance of the instrument carefully for damage after receiving it. Check the entry as follows (including but not limited to the following items):

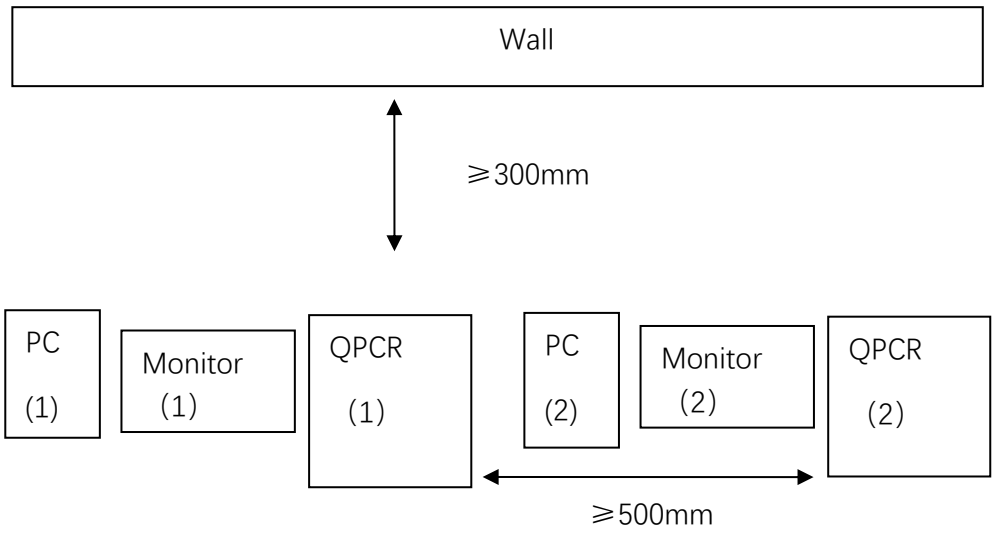
1) There is no obvious damage, bumping, deformation or rain immersion in the outer packing box;
2) No damage or scratch of packaging bags (or film);
3) No scratch or dirt on the shell;
4) No scratch or rust on bare metal parts of equipment;
5) No damage of random accessories or packaging bags.

3.2 Instrument installation

3.2.1 Required environment conditions

1) The instrument is for indoor installation only. Place the instrument on a firm, flat and horizontal platform, ensure the four feet of this instrument stand on the platform firmly. Avoid install on a slippery surface.
2) Appropriate ambient temperature range: 5-30℃. The recommended ambient temperature is 20℃±5℃. Avoid temperature over 30℃ or direct exposure to sunlight. Ambient humidity: 20-80%.
3) Keep away from heat source or liquid.

4) Keep clear of the instrument to guarantee the cooling efficiency, the space requirements for installation are as follows:



⚠ NOTE:

The reliability of data will not be guaranteed if the instrument does not work under above environmental conditions. Indoor air conditioning should be used If the temperature and humidity exceed the appropriate range. However, direct air blowing instruments should be avoided, otherwise the reliability of operation data may be affected.

3.2.2 Electrical requirements

1. Power supply requirements: 220 V, 50/60 Hz, voltage fluctuation <10%.
2. The maximum power consumption of the power outlet is 600W, and the grounding voltage is ≤5V.
3. The instrument must be grounded properly.

⚠ WARNING:

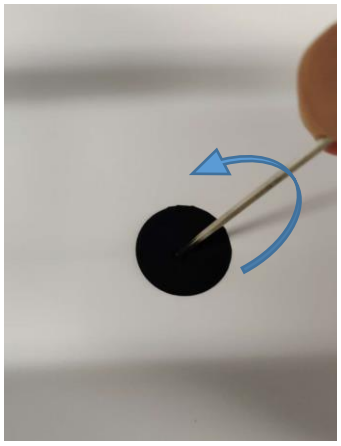
1) Improper grounding may result in personnel electric shock or instrument damage.

2) Verify that the input voltage meets the requirements of the instrument and that a qualified fuse has been installed.

3.2.3 Unlock

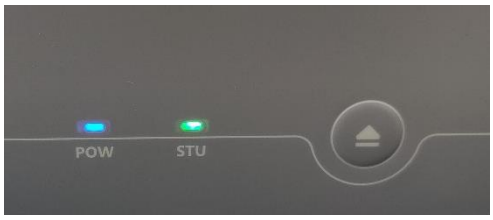
The transport lock is locked when each instrument is packing from the factory, and the installer needs to open the transport lock as follows.

1) Before first power up, insert the M4 inner hexagonal wrench attached to the instrument into the rubber hole on the top of the instrument, press down slightly and rotate 90 degrees counterclockwise, then loosen the wrench. The transport lock will pop out and the mechanical unlock is completed.



Unlock operation

2) When the instrument is powered on, the internal motor of the instrument performs the reset action automatically. The STU green indicator light is always on, and the unlocking is successful.



3.2.4 Connect to computer

Inserted one end of the black power line into the power interface of the QPCR instrument, and the other end to the power supply outlet. Connect the white data line to the computer.

3.3 Software Installation

The computer should be installed win10 system to adapt to the QPCR software.

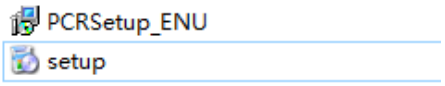
The software installation steps are as follows.

1. Double click the left button to install the zip package.

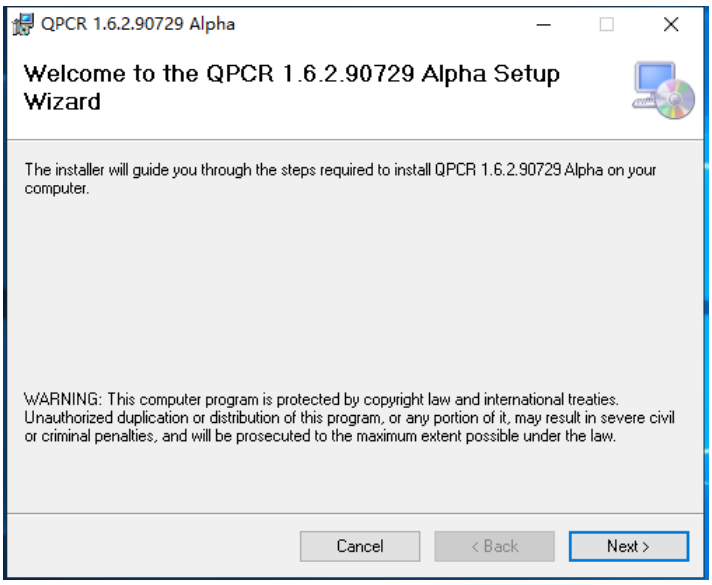
2. Unzip the file to the computer (recommend to install to D drive). Get the file installation package.

3. Click to open the folder.

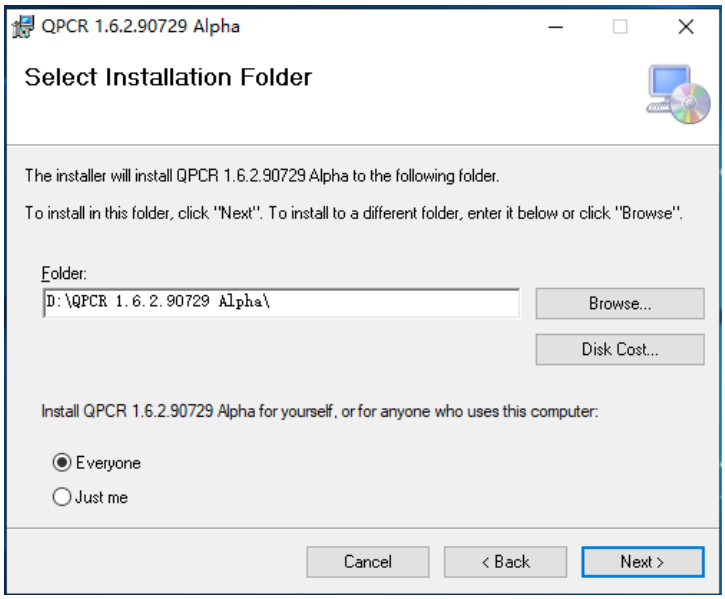
4. Double-click "setup" to enter the installation guide.



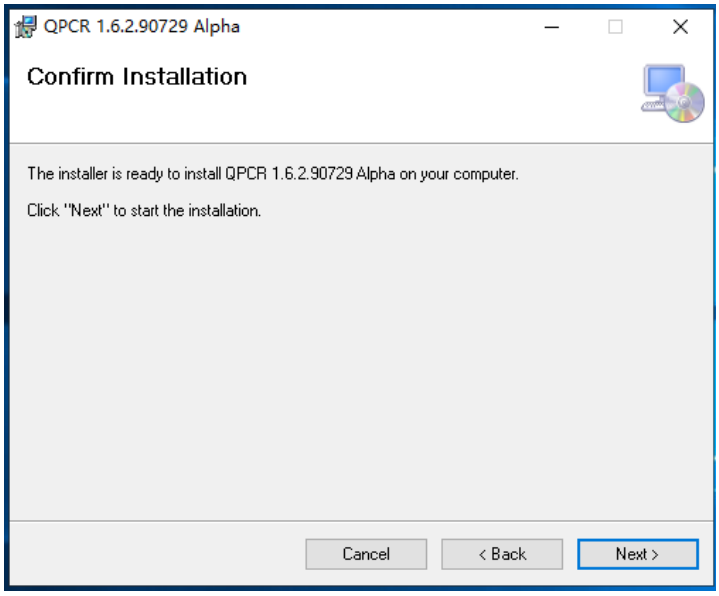
5. Click next.



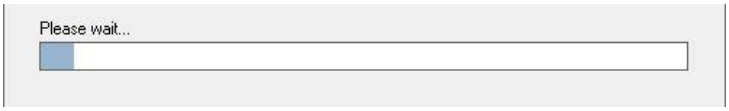
6. Click next, you can click Browse to change the installation location.



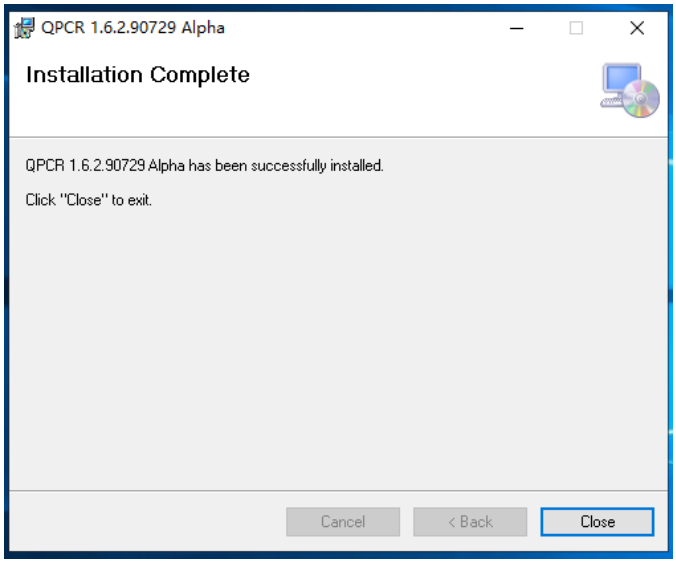
7. Click next.



8. Installing.

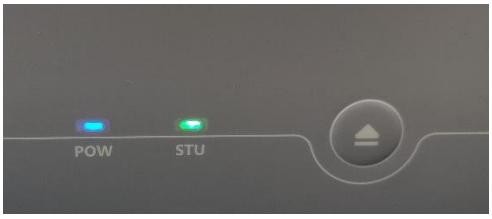


9. Click close to complete.

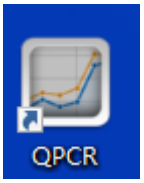


3.4 System startup

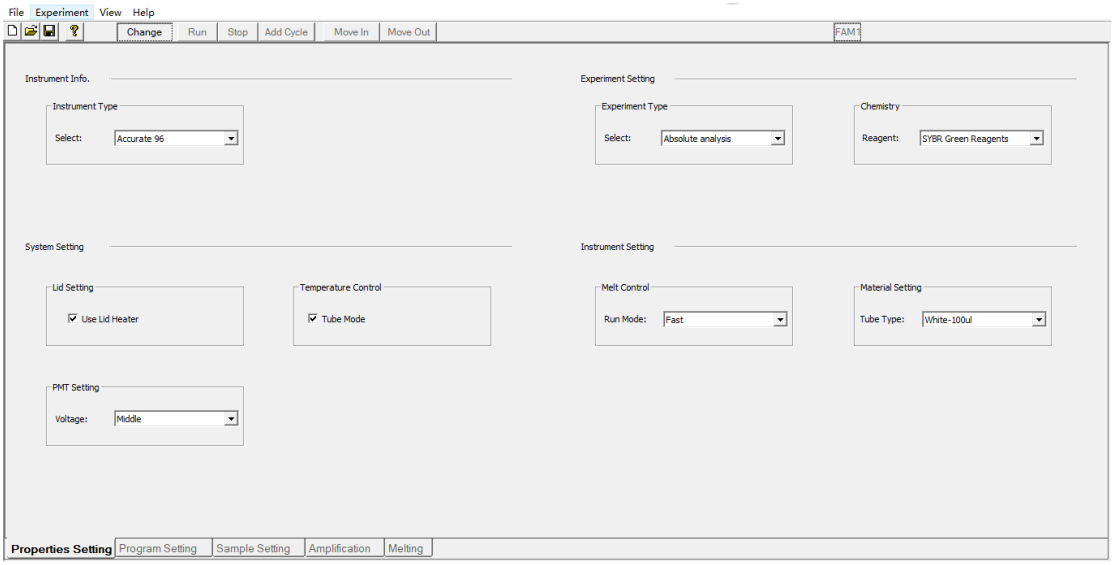
1. Start the computer monitor and host.
2. Turn on the instrument power switch, the POW is steady blue; the STU is green flashing during the self-test of the instrument, then it is steady green;



3. Double-click the desktop software icon to launch the software;



4. The interface after software start-up is as follows:

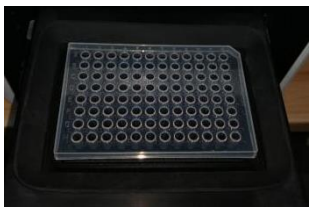


3.5 Instrument transportation

Please call our after-sales service telephone for consultation if you need to move the instrument. The following operations should be performed before transportation:

1) Power on the instrument.

2) Press the entrance and exit button. Then put the 96-hole plate into the reaction plate for protection as shown below. Press it slightly to fit the hole. At last, press the entry and exit control button again to retract the reaction plate.

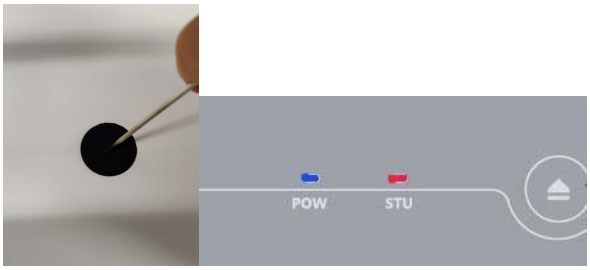


Put the 96-hole plate for protection

3) Insert the M4 inner hexagonal wrench into the rubber hole on the top of the instrument, press down slightly and rotate 90 degrees clockwise, then loosen the wrench, and the spring does not pop out, the STU indicator status is red and green alternately. Now the mechanical lock is completed.

The locking is failed if the indicator is steady red and the 3) step must be repeated.

Lock verification: Press the entrance and exit button, if the sample bin does not extend, the lock is successful.



Red light is always on when lock failure

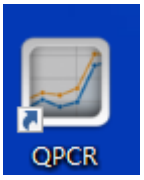
4) Turn off the power and unplug the power cord and data cable.

> ⚠ NOTE:
>
> It is necessary to lock the transport lock to avoid bumping damage to important parts of the instrument before transporting, especially fluorescent detectors.

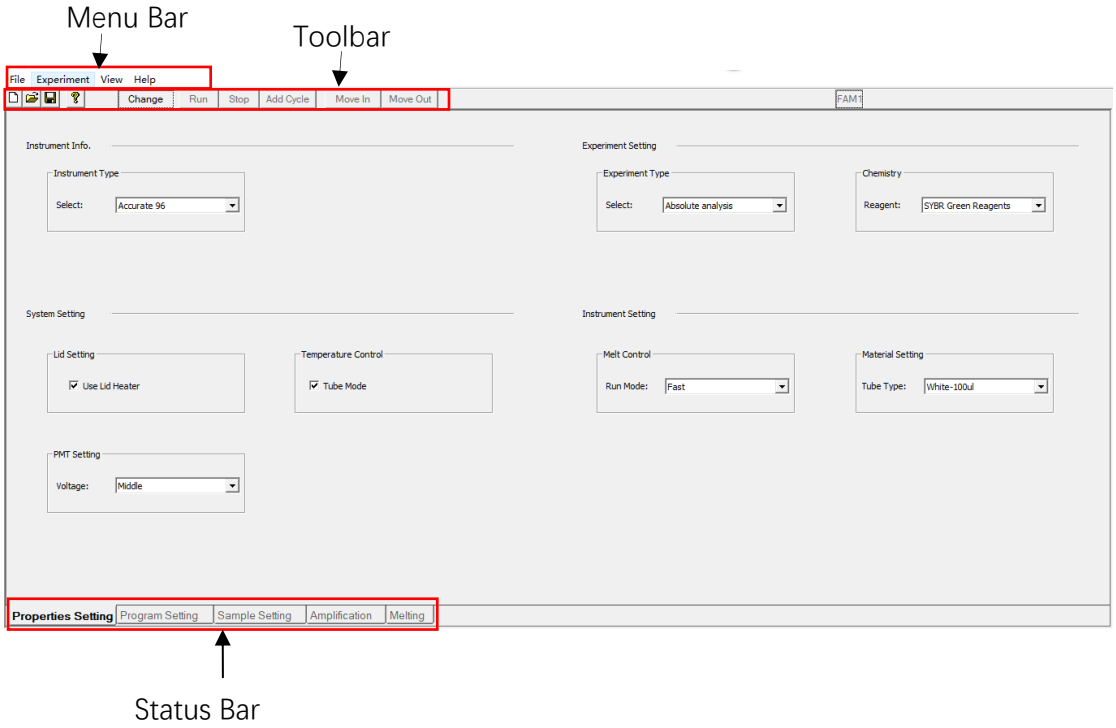
Chapter4. SOFTWARE INTRODUCTION

4.1 Software startup

Double-click the desktop icon to launch the application.



4.2 Interface introduction



4.2.1 Menu Commands

4.2.1.1 File menu

The File menu contains commands used to new, open, save, save as and exit files. The menu also contains commands for exporting files as well as a list of recently-opened experiments.

1. New：Create a new experiment.

To create a new experiment, click New in the File menu, press Ctrl+N, or click the new experiment button on the toolbar. The Properties Setting screen is displayed.

2. Open：Open an existing experiment

To open an existing experiment, click open in the File menu, press Ctrl+O, or click the open button on the toolbar. The open dialog box will appear. Select the experiment to be opened. Click the open button at the bottom of the dialog box.

3. Save：Experiment save

To save an experiment, click save in the File menu, press Ctrl+S, or click the save button on the toolbar. If the experiment has already been saved, no dialog box will be displayed and the experiment will be saved under its current name. If the experiment has not already been saved, the save as dialog box will open, allowing you to specify a file name and directory for storing the experiment.

4. Save As：Save the file as

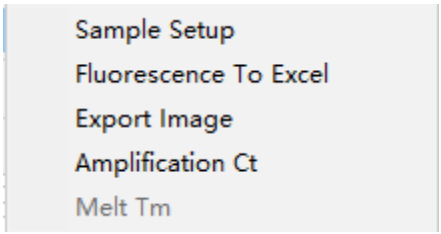
To save a new experiment or to save an existing experiment under a new name, click save as in the file menu, and the save as dialog box will open. If the experiment was previously saved, the name of the experiment will appear in the File name field where it may be modified. If the experiment has not yet been saved, a default file name will appear in the File name field. You can select to either use the default file name or specify another file name.

5. New Frame: You can create a new method file in a new window.

6. Open in New Frame：When you are running an experiment, you can open a file that was saved in the past at the same time.

7. Save As Template：Save the file as a method file without saving the results. The storage location is User folder.

8. Export：Exporting data to other applications



To export the sample setup, fluorescence, amplification Ct, melt Tm and temperature to Microsoft Excel file, click export in the file menu. It can also export image of amplification curve.

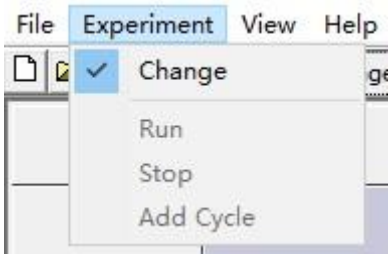
9. Recent experiments

The File menu displays a list of the four most recently used experiments. Any experiment in the list may be opened by clicking on its file name.

10. Exit：exit the program

To exit the program, click the exit command in the File menu . If the open experiment has not been saved, the save current Experiment dialog box appears.

4.2.1.2 Experiment menu



1. Change: Modify/lock settings parameters

Click the change in the Experiment or the change button on the toolbar before modifying the settings. The page parameters convert between locked and modified when click change.

After clicking change, please note that the new file needs to be saved as a different file name, otherwise the original experimental data results will be overwritten and lost.

2. Run: Run the experiment

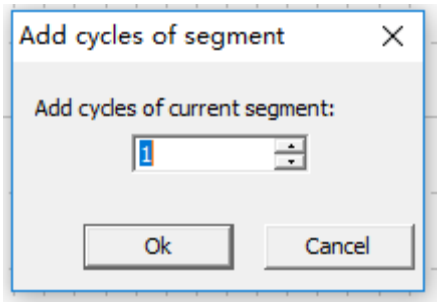
To start running the experiment, click run in the experiment menu or the run button on the toolbar.

3. Stop: Stop running

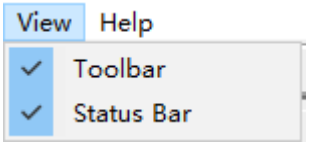
   To stop the experiment while the experiment is running, click stop in the experiment menu or the stop button on the toolbar.

4. Add cycle

   Add cycles of segment when the experiment is running. Click OK.

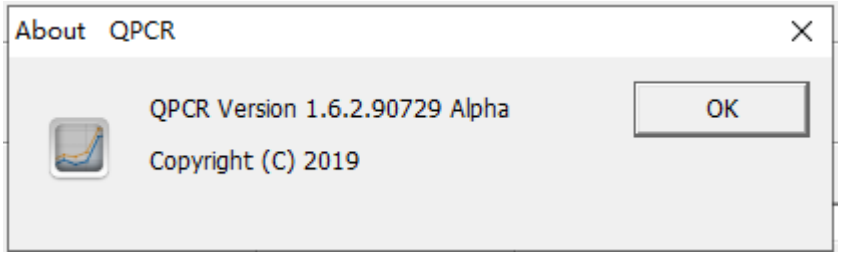


4.2.1.3 View menu



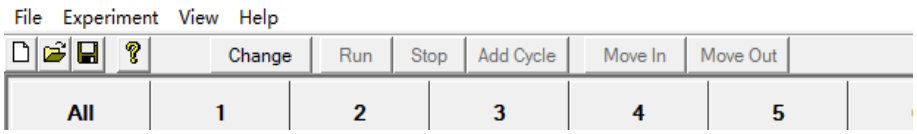
1. Check the toolbar, the toolbar button is displayed. Unchecked, and the toolbar button is hidden;
2. Check the status bar, the status bar button is displayed. Unchecked, and the status bar button is hidden.

4.2.1.4 Help menu



To display the software version number, click About QPCR in the Help menu or click the button  on the toolbar.

4.2.2 Toolbar



From left to right are new , open, save, about QPCR, change (switch lock/modify status), run (start running), stop (stop running), Add Cycle. See 4.2.1 for details.

Move in is the entry position. Move out is the out position. The function is equivalent to the entrance and exit button of the instrument top board.

4.2.3 Status bar

Properties Setting: Set experimental methods, instrument parameters, etc.

Sample Setting: Set the name, type, location and fluorescent dye of the sample, etc.

Program Setting: Set PCR reaction program.

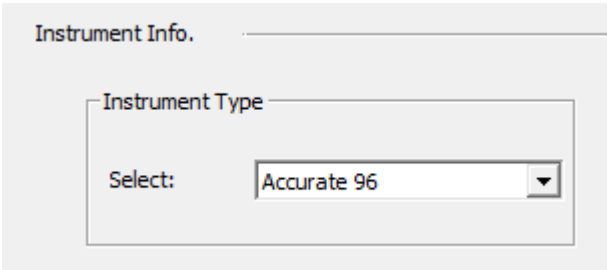
Amplification: Amplification curve.

Melting: Melting curve.

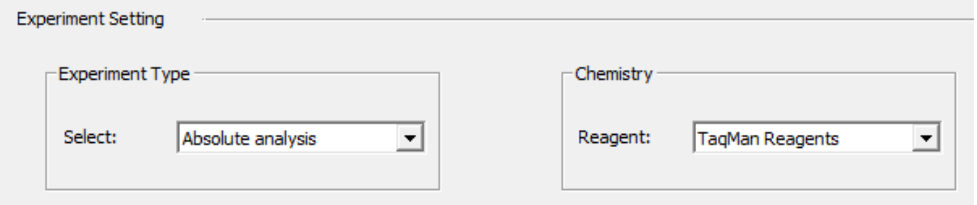
4.3 Properties Setting

4.3.1 Instrument Type

Select instrument type of Accurate 96.



4.3.2 Experiment Setting

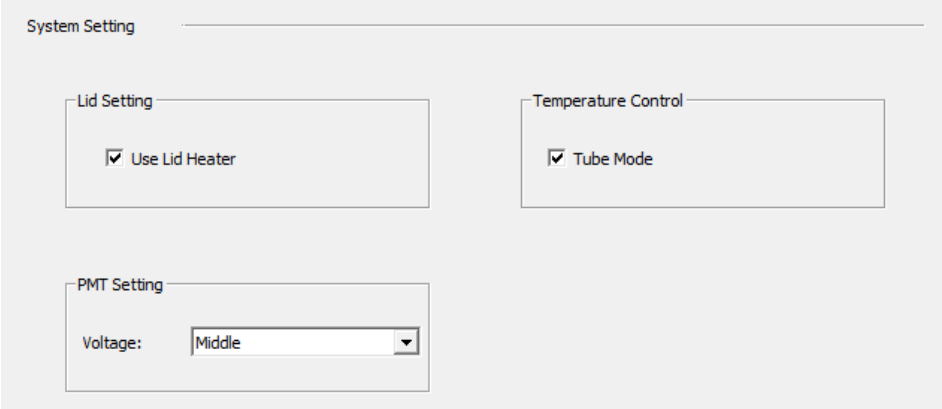


1) Choose Experiment Type: Absolute analysis is suitable for absolute quantitative analysis or other analysis options. Melting Analysis just for melting analysis.

2) Choose Chemistry. Select SYBR Green Reagents or TaqMan Reagents according to you experiment.

4.3.3 System Setting

Set the basic parameters of the system.



1) Lid Setting. Choose “Use Lid Heater” means the hot lid is automatically heated to 100℃ in the experiment.

2) Temperature Control. “Tube Temperature Mode” means that the temperature is based on the actual temperature in the tube instead of block temperature.

3) PMT setting. Voltage is available in High, Middle, and Low. It is selected according to the amplification efficiency and defaults to Middle.

4.3.4 Instrument Setting

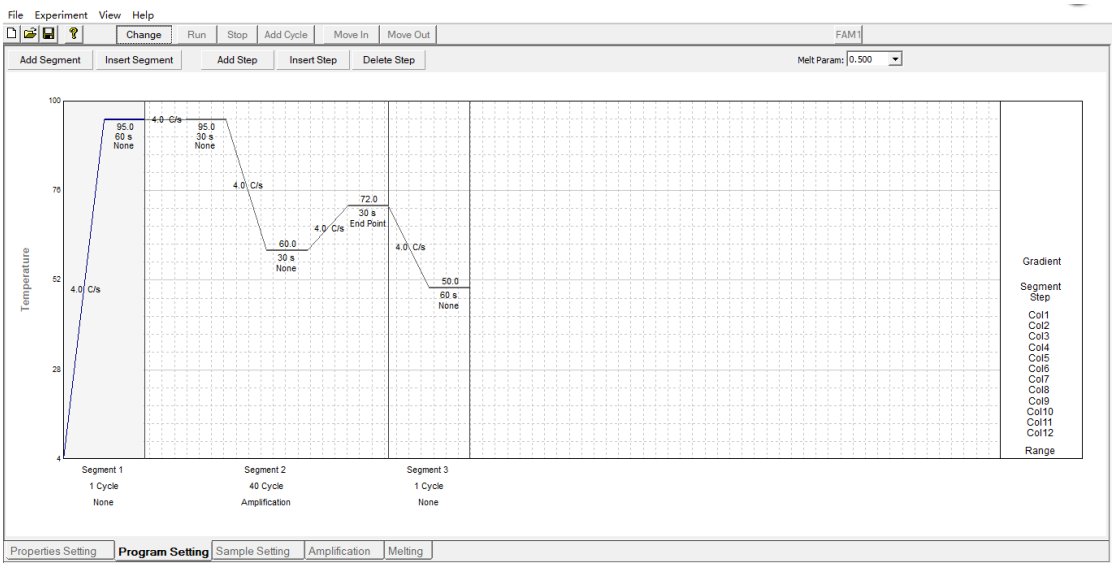
Set Melt Control and Material Setting

1) Melt Control. The run mode includes Fast and Standard. The setting is valid when the experiment contains a melting curve.

2) Material Setting. Tube Type include White tube and Transparent tube. Select according to the consumables used.

4.4 Program setting

4.4.1 Interface setting



Add Segment: Add a new segment at the end of the reaction step.

Insert Segment: Insert a new segment in front of the current selected segment.

Add Step: Within this segment, add a new step after the current selected step.

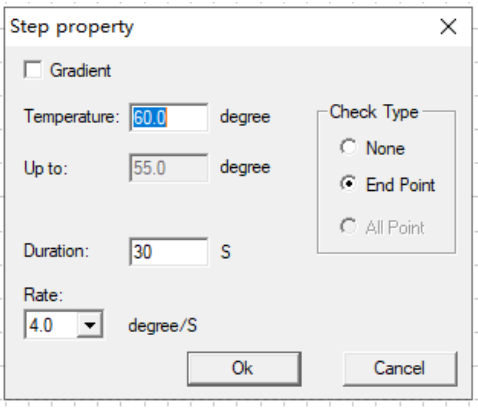
Insert Step: Within this segment, add a new step in front of the current selected step.

Delete Step: Delete the currently selected reaction step.

Melt Param: Signal acquisition frequency of Melting curve (Recommended set to 0.2)

4.4.2 Parameter modification

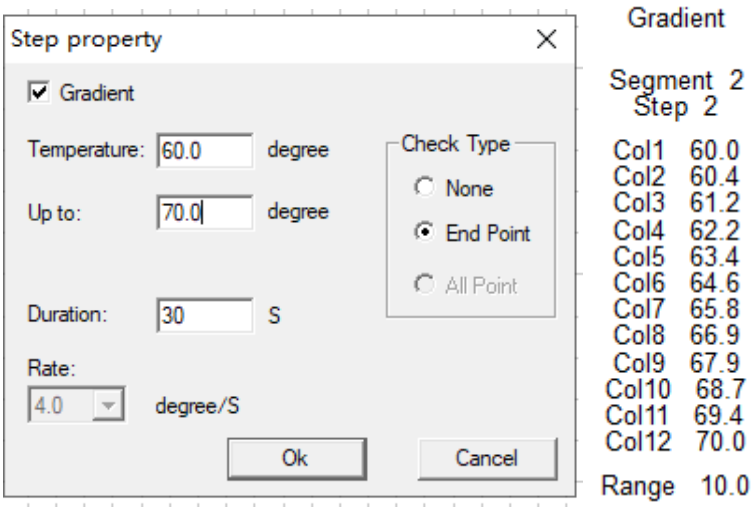
To modify the reaction temperature, time, cycle number, heating/Cooling rate, fluorescence collection point (End Point), click on the value to modify, or double-click anywhere on the current step and modify in the pop-up text box, as shown below.



1. Temperature

Fill in the temperature setting value of this step in the temperature item.

If you need to set the temperature gradient, check Gradient. “Temperature” is the lowest temperature, “Up to” refers to the highest temperature. The temperature distribution increases from 1st to 12th column. For example, set the temperature gradient from 60°C to 70 °C, the specific temperature of each column will display in the lower right corner of the interface, as shown below.



When viewing the data, to select the annealing step the temperature gradient will display on the right side of the interface.

2. Time

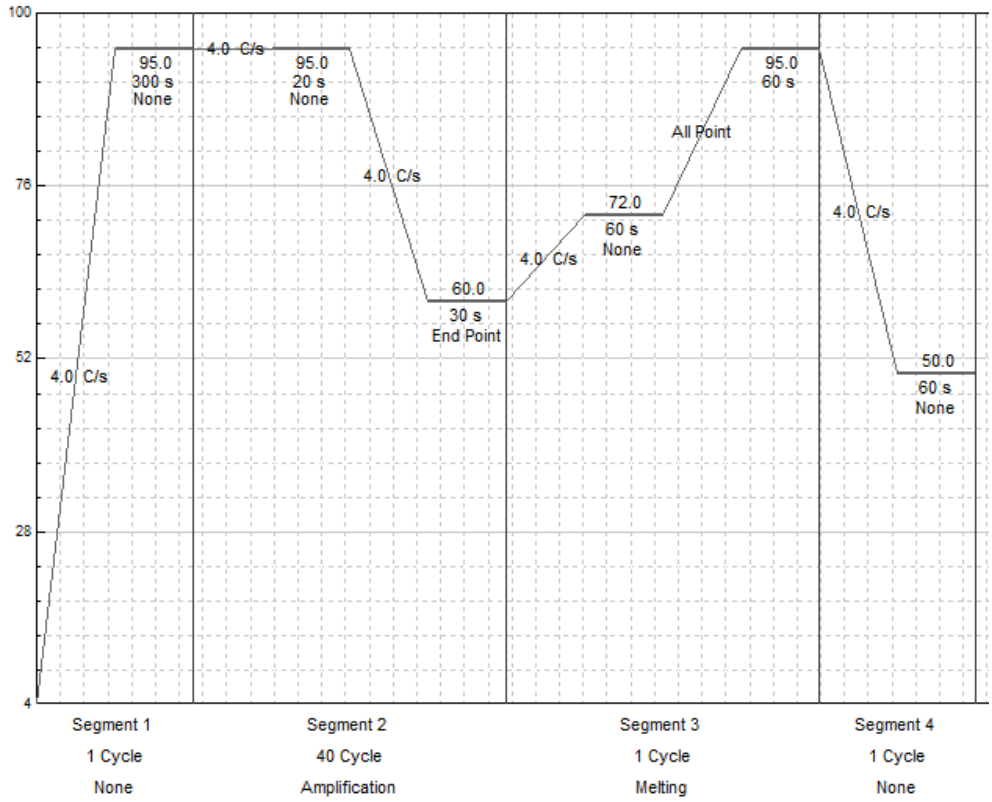
Fill in the set time in the Duration item.

3. **Heating/Cooling rate**

Fill in the Heating/Cooling rate in the Rate item. It is recommended 4.0 ℃/sec which is system default value.

4. End Point

None in the Check Type means no fluorescence will be collected, and End Point means the fluorescence is collected in this step. Normally you need to select End Point in the extension step of PCR. And select All Point in heating process of dissolution curve. For example, the settings are shown as below.



4.4.3 Save method

File-save/save as, select the file storage location, and the default format is ".fsd".

4.5 Sample Setting

4.5.1 Select sample location

Click or drag the mouse to select the sample position. Click on the bar of row or column to select this row/column. You can also use the shortcut buttons: Select All and Clear Wells. After selecting the sample location, select the corresponding sample type and load.

4.5.2 Sample information setting

4.5.2.1 Sample Type

Select the sample type corresponding after selecting the hole position. There are six sample type to be seletect.

Unknow: It refers to the sample to be tested in the experimental group;

Standard: A sample of the target of interest that the copy number must be known

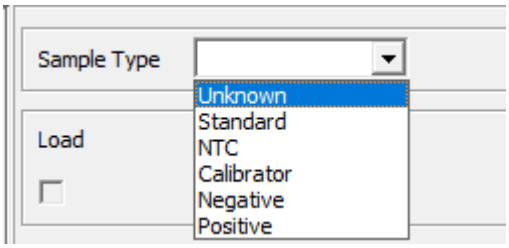
to setup an absolute standard curve, include DNA standards and RNA standard.

NTC: No template control;

Calibrator: Generally used for comparative quantification.

Negative: Negative control, or no template control, used to exclude template contamination and test the reliability of the experiment.

Positive: Positive control for testing the reliability of reagent and method.

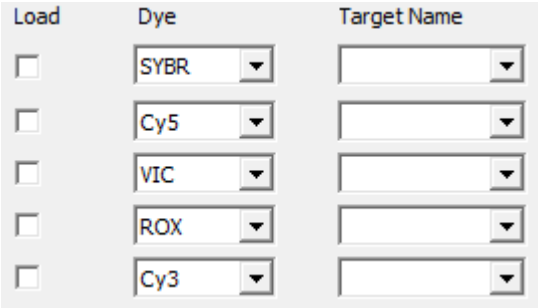


4.5.2.2 Load

It means fluorescent channel, selected based on fluorescent PCR chemistries.

The instrument is available in four/five channels. The channels are classified according to the absorption and emission wavelengths of the different fluorophores.

Load	Dye
1	SYBR/LC/EVA/FAM1
2	Cy5
3	VIC/HEX/JOE/TET
4	ROX/Texas/Rad
5	Cy3/NED/TAMRA

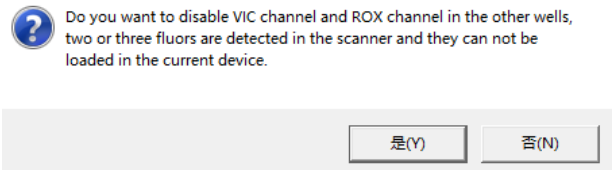


Multiple channels can be selected simultaneously for multi-probe experiments.

⚠ NOTE:

When the fifth channel (Cy3/NED/TAMRA) is selected, the third channel (VIC/HEX/JOE/TET) and the fourth channel (ROX/Texas/Rad) cannot be selected at the same time. The instrument will display an error message. Click “No” to reselect the

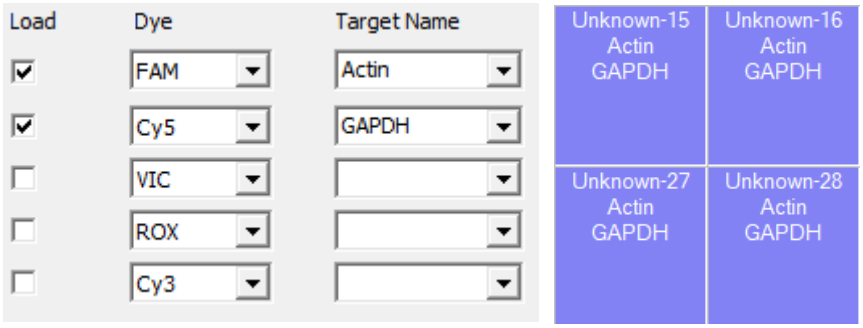
fluorescence channel.



4.5.2.3 Target Name

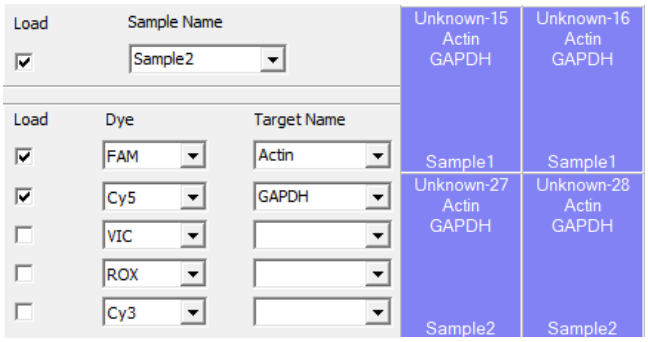
The gene name corresponding to each channel can be selected after the fluorescence channel is selected.

Fill in the gene name or click to select the edited gene name. Click on the Target Name in the View and the gene name will be displayed in the corresponding hole position.



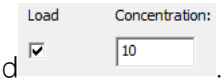
4.5.2.4 Sample Name

Check the Sample in the view, select the corresponding hole, fill in the sample name or click to select the edited sample name. The sample name is displayed in the corresponding hole position.

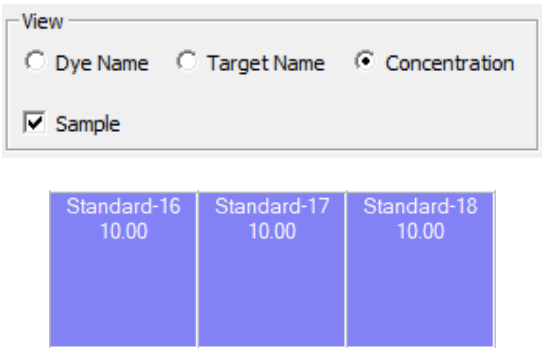


4.5.2.5 Concentration

Absolute quantification requires setting the concentration of standards.

Select the hole position of the same concentration, fill in the concentration value in the concentration box, check Load 

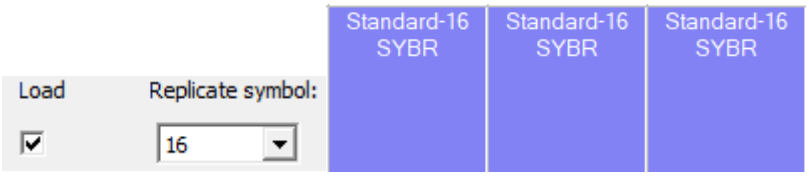
. At this point, click 'Concentration' in View and the concentration is displayed in the corresponding hole position.



4.5.2.6 Replicate symbol

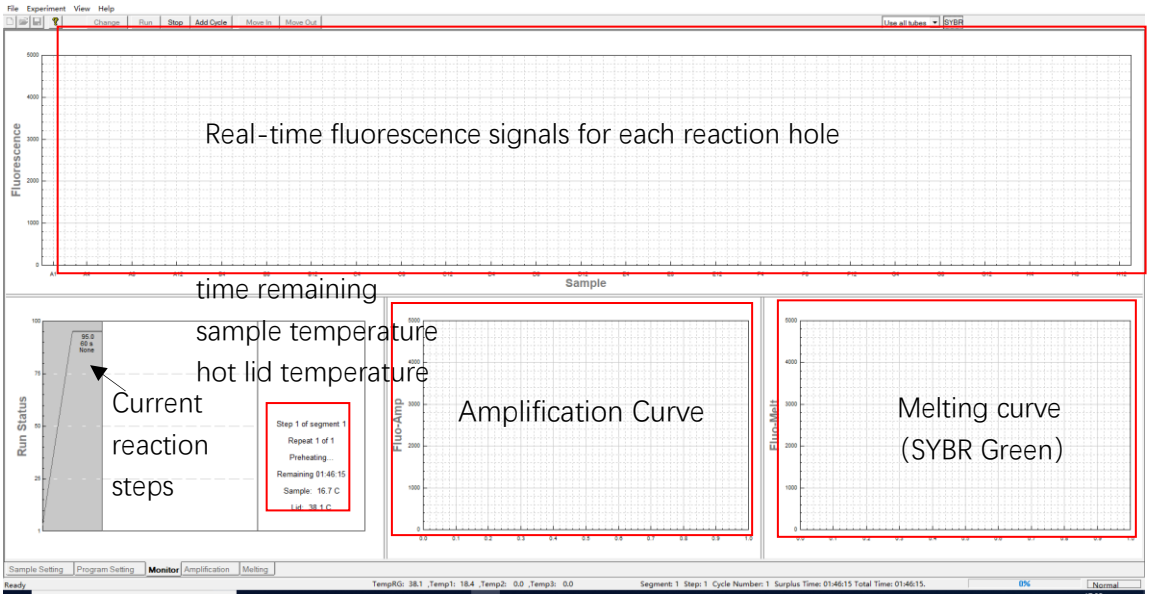
Repeated wells of the same reaction need to be set to repeat for subsequent analysis.

Select the repeating hole position, the serial number of the selected hole will appear in the selection box, select one of the serial number names, and check Load.



4.6 Start running

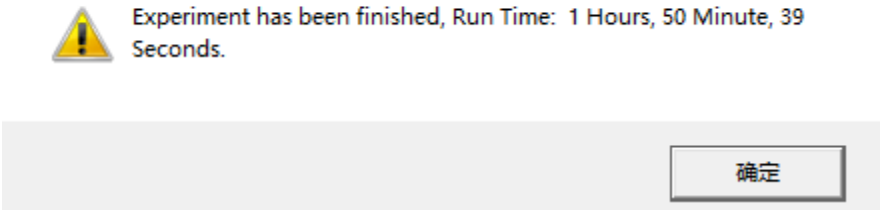
Click run to start running. The running interface in shown as blow.



> ⚠ Note:
> The computer screen must remain on while the instrument is running, and both the "screen off setting" and "sleep setting" of computer must be off.

4.7 Stop running

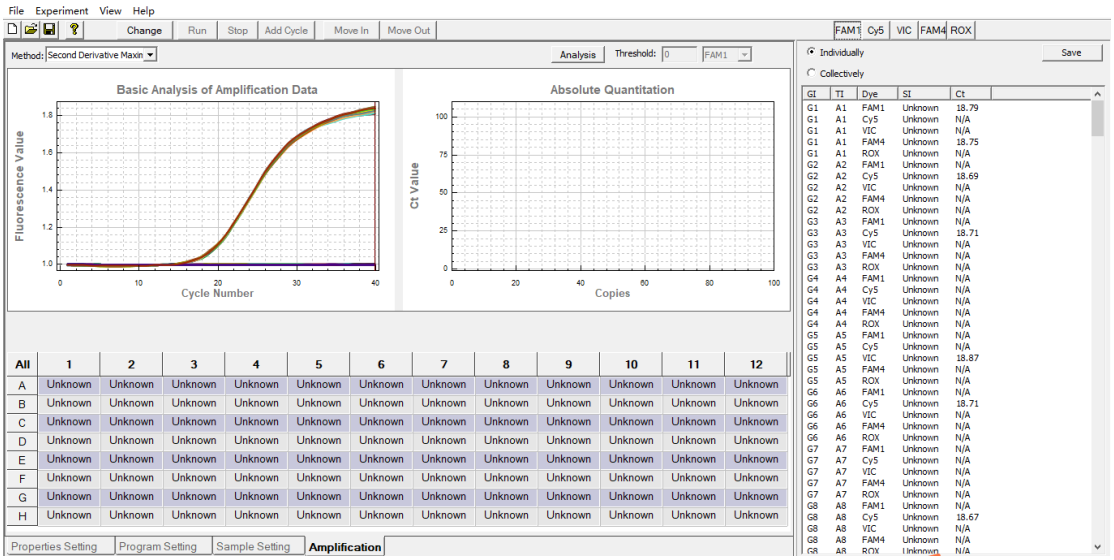
The following prompt message will pop-up after the program finished. Click OK and the result data will be saved to the created file automatically.



4.8 Data export

4.8.1 View Amplification Curves and Ct Value

To open the existing experiment, click open in the File menu, press Ctrl+O, or click the open button  on the toolbar. Click Amplification in the status bar, the interface is as follows. To export the sample Setup, fluorescence and amplification Ct to Microsoft Excel file, click export in the file menu. It can also export image of amplification curve.



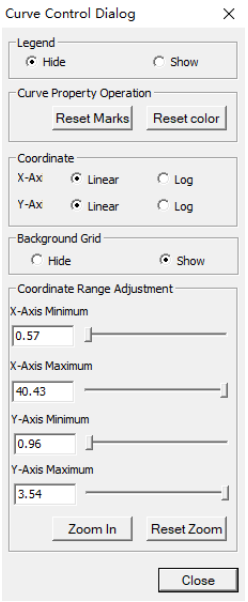
4.8.1.1 Basic Analysis of Amplification Data: Amplification Curves.

1) Analysis: Absolute quantitative analysis function, click the button to get the standard curve, see 5.1.

   Threshold: It can be adjusted when the spline method is selected. See Spline for details.

2) Curve adjustment

   Double-click the left button in the amplification curve block diagram to display the curve control dialog box.



a) Legend：Hide or Show the sample name of the curve.

b) Curve Property Operation

For each cycle of fluorescence collection point options, click Reset Marks to display, Clear Marks to hide.

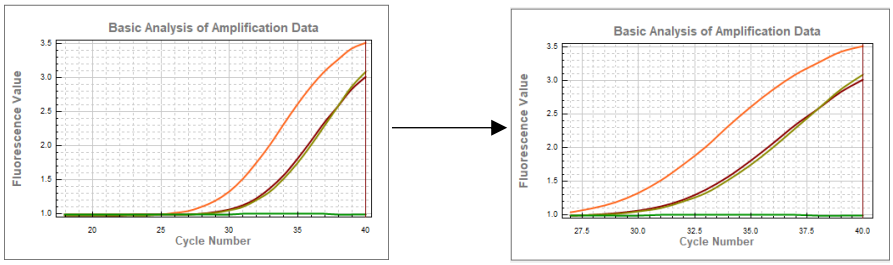
c) Coordinate

Both X-Ax and Y-Ax can be switched between Linear and logarithmic（Log）. The Y-axis is recommended to select the log value when adjust the fluorescence threshold with spline method.

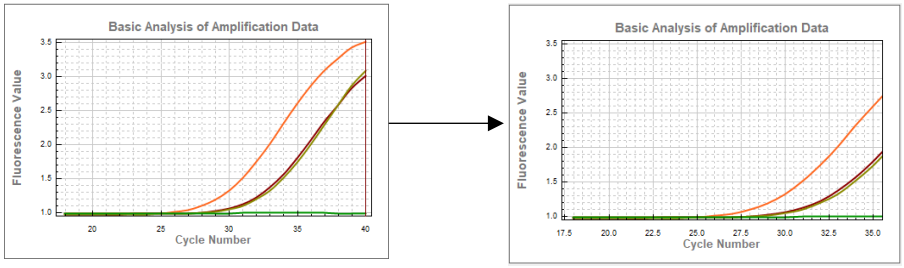
d) Background Grid：Select hide or show.

e) Coordinate Range Adjustment：It can be modified by entering data or pulling the adjustment button.

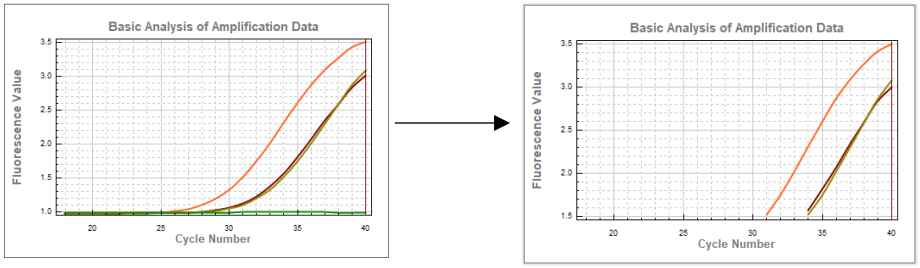
X-Axis Minimum



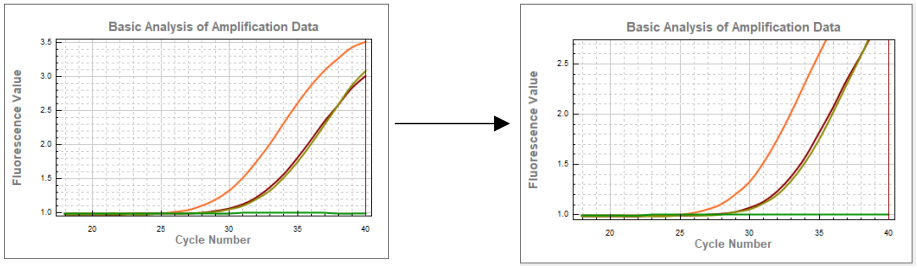
X-Axis Maximum



Y-Axis Minimum



Y-Axis Maximum



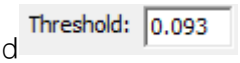
4.8.1.2 Method

The analysis methods include the following two kinds:

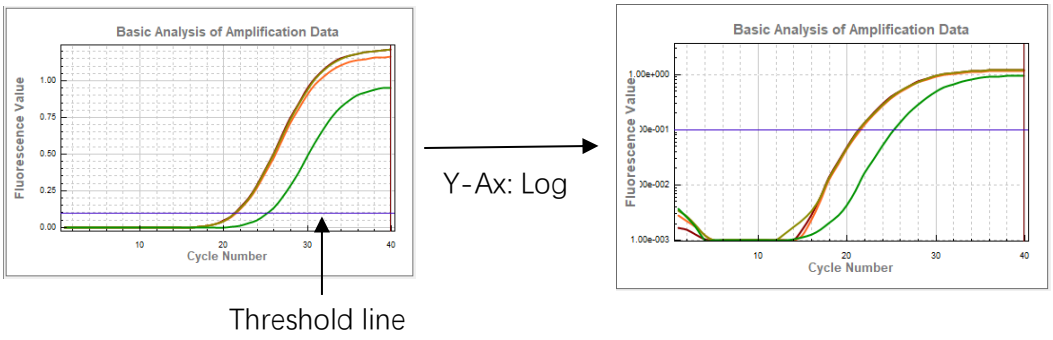
1) Second Derivative Maximum

The second derivative Maximum method gives the fluorescence threshold obtained by the optimization algorithm through the second derivative of the amplification curve, and then gets the corresponding CT value. It is the default method.

2) Spline

When the spline method is selected, and the threshold line appears. The threshold can be adjusted appropriately, curve coordinate Y-Ax selects Log, the amplification curve becomes a logarithmic curve as shown blow(right). Click on the threshold line to move the position or manually enter the threshold 

.The corresponding CT value changes accordingly, which is displayed in the right information table.



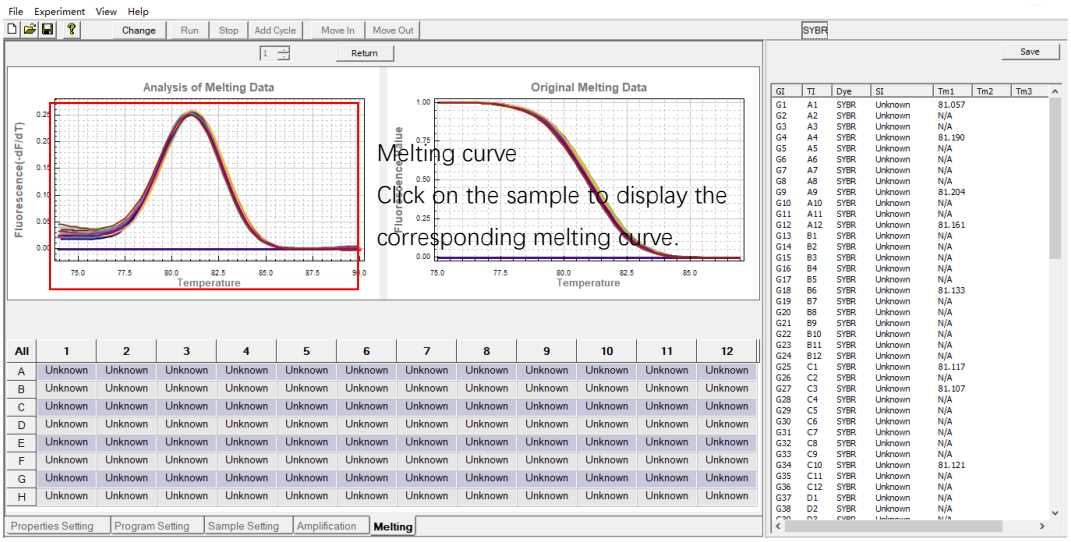
4.8.1.3 Ct value

On the right side of the interface, there are information of position, type, channel and CT value of the sample. To save the CT value to the required folder, click save in the upper right corner and export it in Excel format.

The software defaults to Individually, showing the amplification curve and Ct value for each sample. When Collectively is clicked, the average Ct value of repeated samples were displayed.

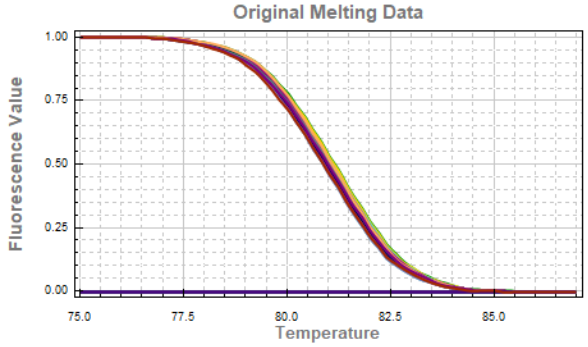
4.8.2 View melting curves and Tm value

To open an existing experiment, click open in the File menu, press Ctrl+O, or click the open button  on the toolbar. Click Melting in the status bar, the interface is as follows. To export the fluorescence, melt Tm and temperature to Microsoft Excel file, click export in the file menu. It can also export image of melting curve.



4.8.2.1 Analysis of Melting Data: Melting curve

Click on Original Data, the original image appears on the right side of the melting curve derivative graph, as shown below.



Double-click the left button in the melting curve block diagram, the curve control dialog box appears, and the adjustment method is adjusted with the amplification curve2).

4.8.2.2 Tm value

On the right side of the interface, there are information of position, type, channel and Tm value of the sample. To save the Tm value to the required folder, click save in the upper right corner and export it in Excel format.

Chapter5. DATA ANALYSIS

5.1 Absolute quantification

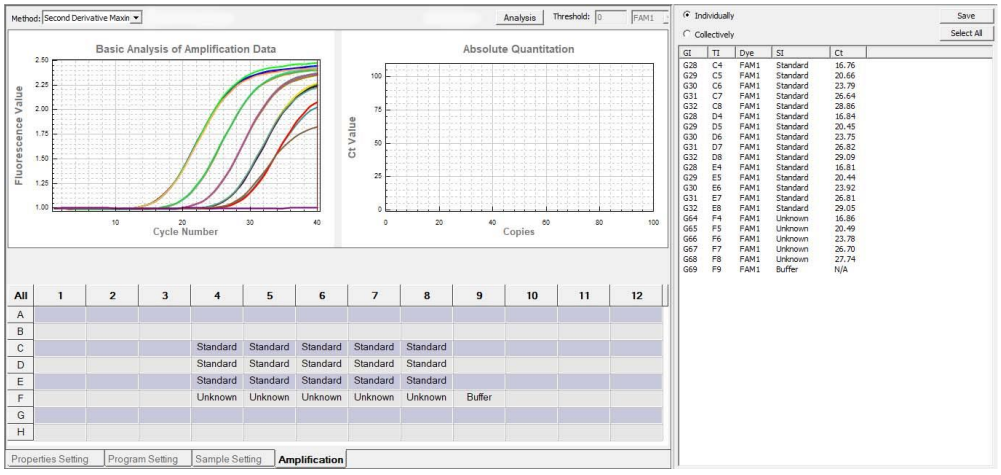
5.1.1 Experiment setting

Setting method refers to software introduction.

> ⚠ Note:
>
> 1) In the properties setting, the experiment type must select absolute quantification, see 4.3.2.
>
> 2) In the sample setting, the concentration of standards (see 4.5.2.5)and replicate symbol must be set (see 4.5.2.6).

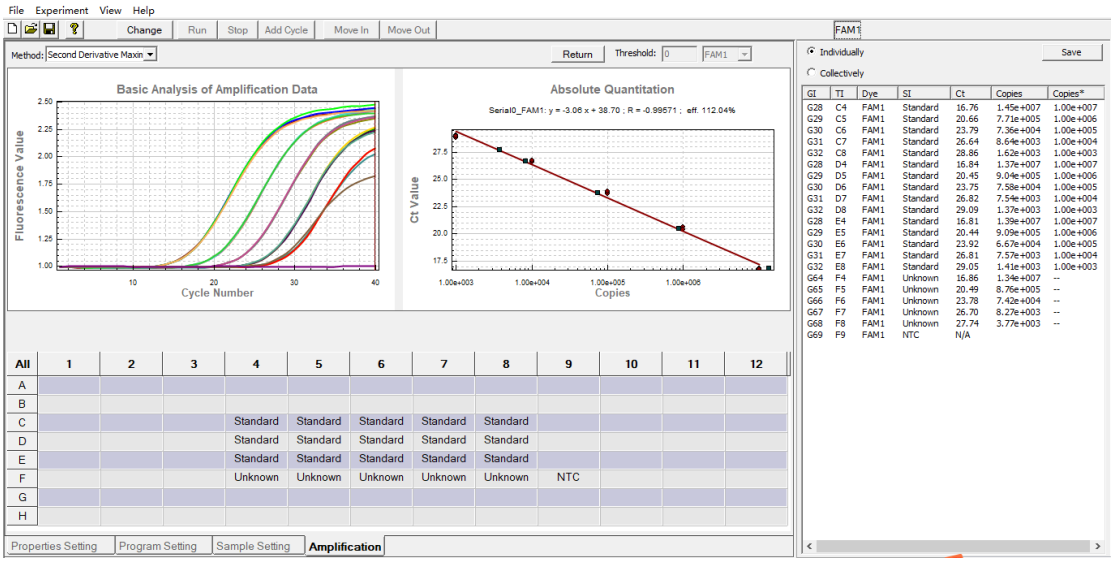
5.1.2 Data analysis

1. After the end of the experiment, the status bar of the Amplification displays the amplification curve of the standards and samples. And the right interface shows the corresponding CT value.



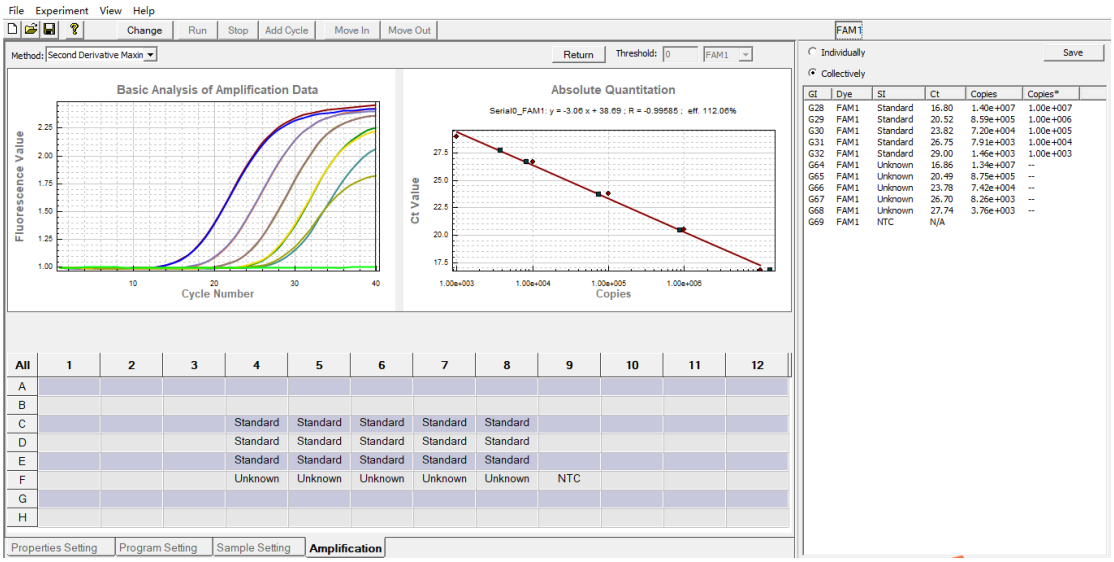
2. Analysis

   Click Analysis to display the standard curve. The standard curve equation, the R value and the amplification efficiency（eff） are shown above the standard curve. The copy number calculated from the standard curve is also displayed in the message box on the right surface. The Copies* refers to the setting concentration of standard.



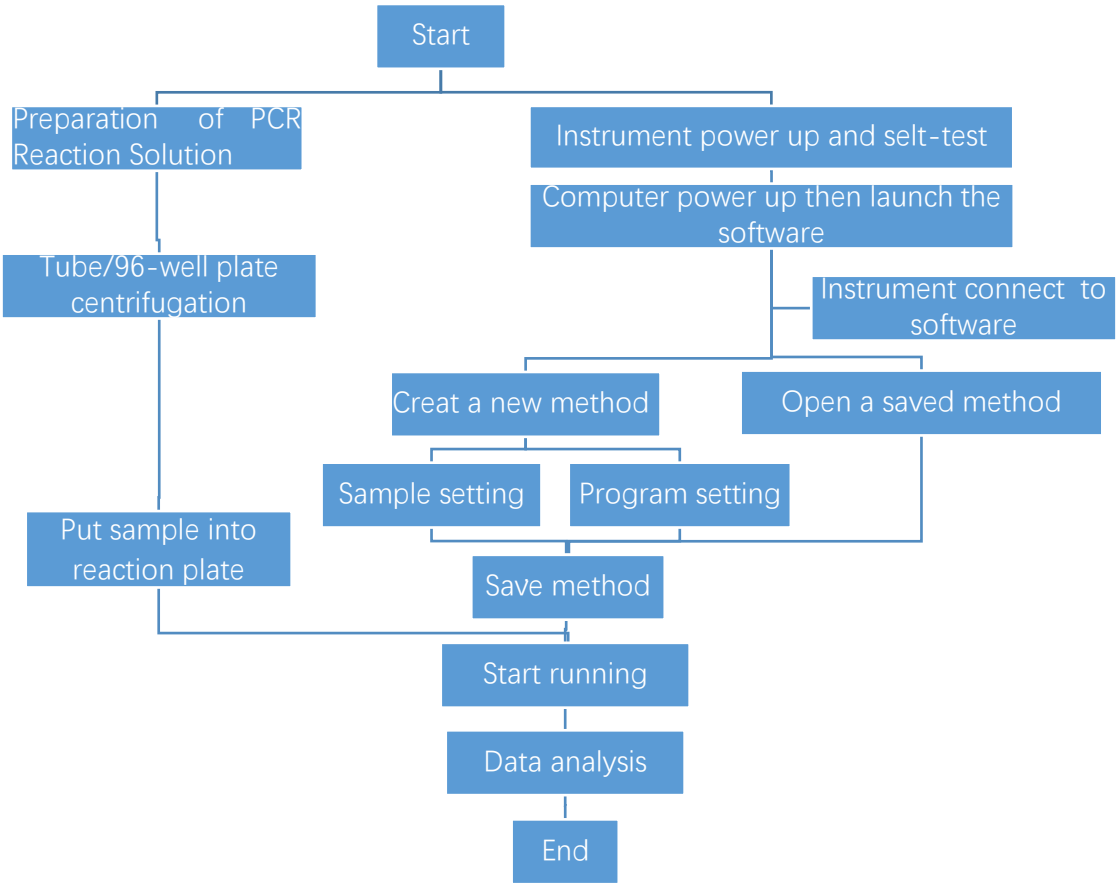
3. Repeated hole data integration

When the software defaults to select Individually, the amplification curve and CT value of single sample is shown. When select Collectively, the average data of the repeated samples are calculated, as shown in the following figure.



Chapter6. APPLICATIONS

Flow chart



6.1 SYBR Green dye-based assays- GM soybean identification

SYBR Green I dye is a fluorescent DNA-binding dye that binds to the minor groove of any dsDNA. Excited DNA-bound SYBR Green dye produces a much stronger fluorescent signal than unbound dye. If the target was present in the sample, sufficient accumulated PCR product will be produced at some point so that an amplification signal becomes visible.

Taking the detection of genetically modified soybeans as an example, the absolute quantitative method was used to detection.

6.1.1 Genomic DNA extraction

Genomic DNA was extracted from GM soybean and soybean samples by plant genome extraction kit. The concentration and purity of DNA were detected by micro-spectrophotometer, OD260/OD280 = 1.7-2.0.

6.1.2 Primer design

Primer design for the 35S promoter which can be commonly found in GM soybeans.

6.1.3 Preparation of standards

1. PCR

Set up 20μL PCR as detailed in the table below.

Reagent	Volume（μL）
2×PCR mix	10
F-primer（10μM）	0.5
R-primer（10μM）	0.5
GM soybean genomic DNA	1
ddH_2O	8

Program：

Pre-denaturation at 94 °C for 3 min; (denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s, extension at 72 °C for 30 s) 35 cycles; extension at 72 °C for 2 min. The replacement of the genome with ddH_2O was negative.

2. Purification of PCR products

The amplified products were detected by agarose gel electrophoresis of 2%. The target fragments in gel were purified and recovered by gel recovery kit.

The concentration was determined by nucleic acid protein analyzer. The dilution concentration of 10-fold series is 0.01-1 nmol/L, which is used as standard DNA.

6.1.4 Preparation of qPCR Reaction Solution

Set up 20μL qPCRs as detailed in the table below.

Reagent	Volume（μL）
2XSuper PreMix Plus(with SYBR Green I)	10
F-primer（10μM）	0.5
R-primer（10μM）	0.5
Standard DNA/test sample DNA	3
ddH_2O	6

(2 X Super PreMix Plus(with SYBR Green I) was purchased from TIANGEN Biochemical Technology Co., Ltd.)

Repeat three copies of each sample. DdH_2O was used instead of DNA as a negative control.

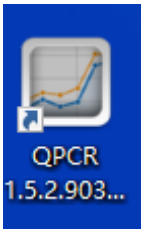
> ⚠ NOTE:
>
> Reaction solution is prepared at once and then sub-pack. The reaction tube needs to be centrifuged instantaneously.

6.1.5 Power on

Connect the power cable for the instrument. Turn on the instrument power switch and the instrument will start self-test. At the end of the self-test, the STU status light is steady green.

6.1.6 Software startup

Make sure the data cable is connected. Start the computer and double-click the desktop icon to launch the application.



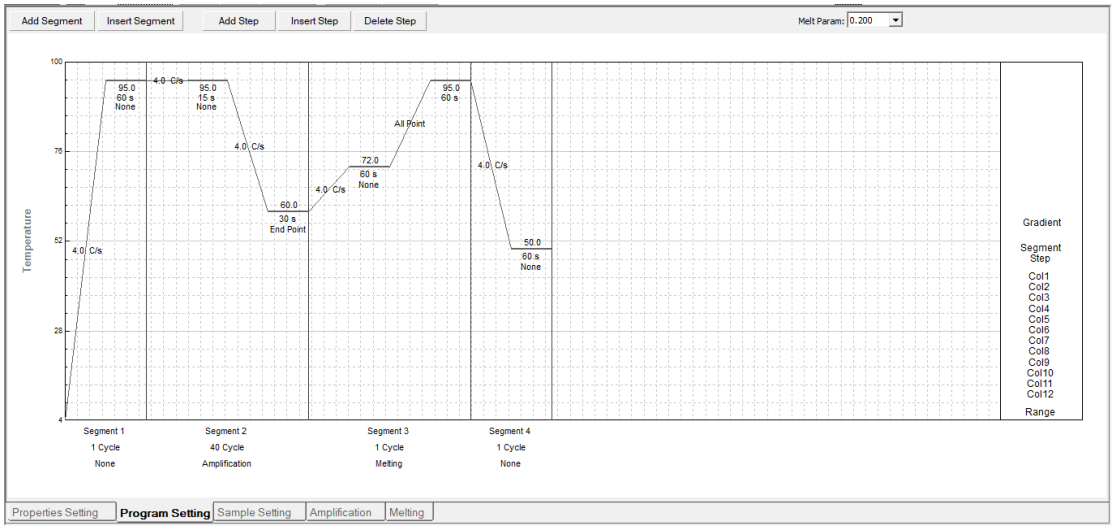
6.1.7 New experiment

To create a new experiment, click New in the File menu, press Ctrl+N, or click the new experiment button on the toolbar. Then set the properties as shown below.

6.1.8 Program Setting

Click Program Setting in the status bar to enter the program setting interface.

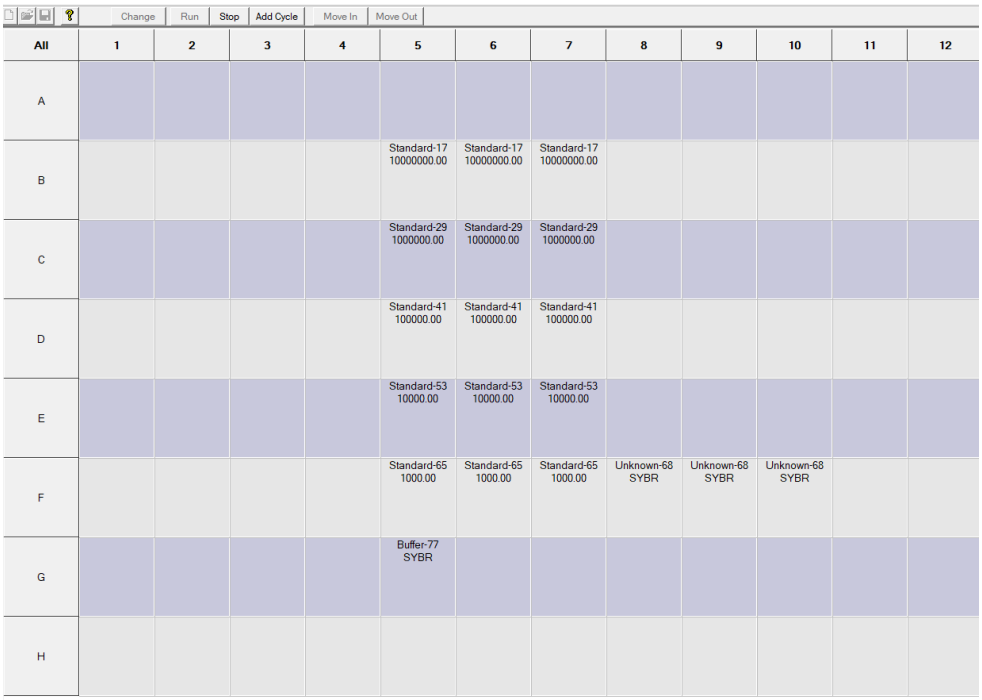
Set the temperature, time, fluorescence collection point and cycles, as shown below. Note that the fluorescence acquisition in the extension phase （60℃30s） of the amplification curve should be set to End Point, and the fluorescence acquisition of the melting curve（72℃-95℃） should be set to All Point. Melt Param is recommended to 0.2. For details, see4.4.



6.1.9 Sample Setting

Click Sample Setting in the status bar to enter the sample setting interface.

The standard has five concentration gradients, three repetitions for each concentration, select Standard type, SYBR channel, set the concentration and replicate symbol; Three replicates of the sample to be tested select Unknown type, SYBR channel, set the replicate symbol; No template control select NTC, SYBR channel, as shown below.



6.1.10 Save method

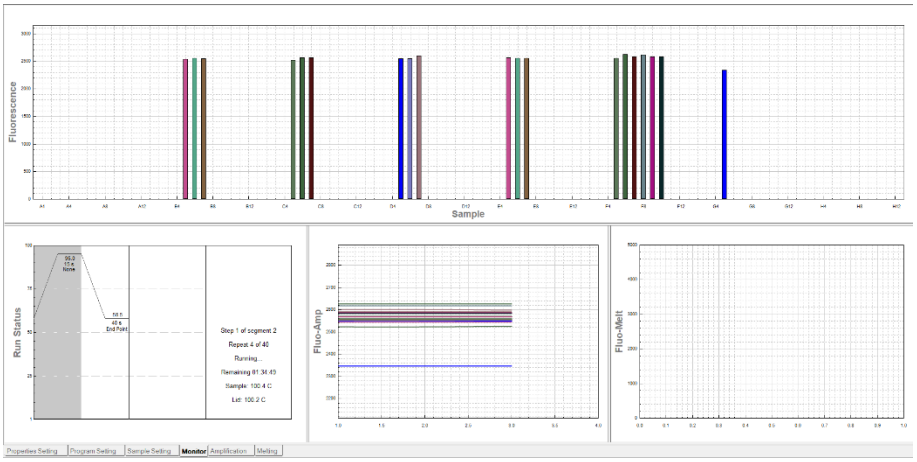
To save method, click save as in the File menu. Select the file storage location as “D disk”, named 2019-SYBR-GMO. Or the storage path settings will automatically pop up after clicking Run.

6.1.11 Sample entry

Click entrance and exit control button， and put the reaction tube into the reaction plate. The sample position is consistent with the set position. Empty reactor tubes need to be placed at four corners of the reactor plate to support the heat lid. See 2.2.2. Click the button again, and the reaction plate moves in.

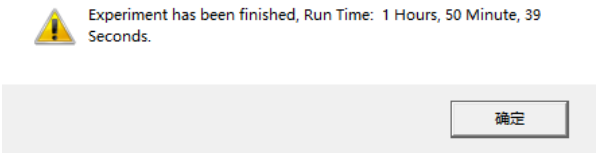
6.1.12 Start running

Click "run" to start running. The running interface is shown below.



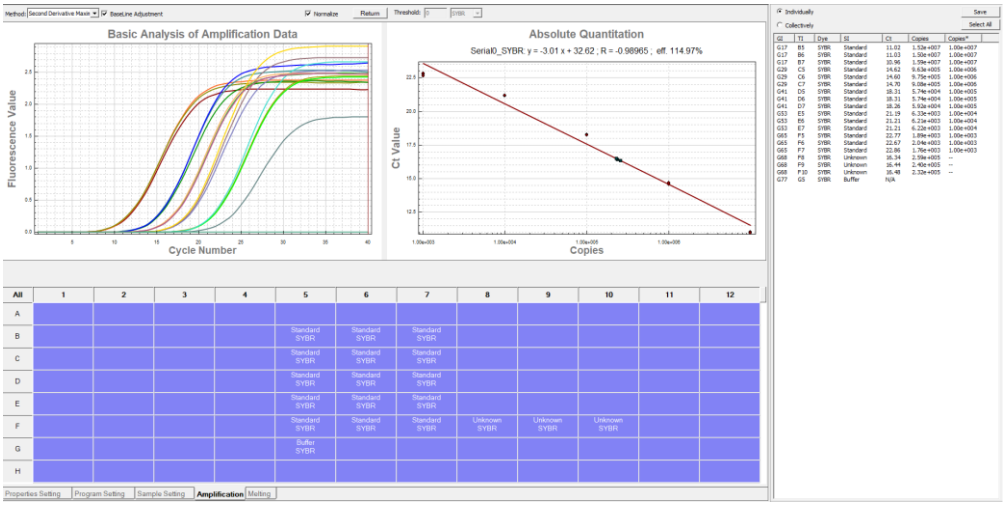
6.1.13 Stop running

The following prompt message will appear after the program finished. Click OK and the result data will be saved to the created file automatically.

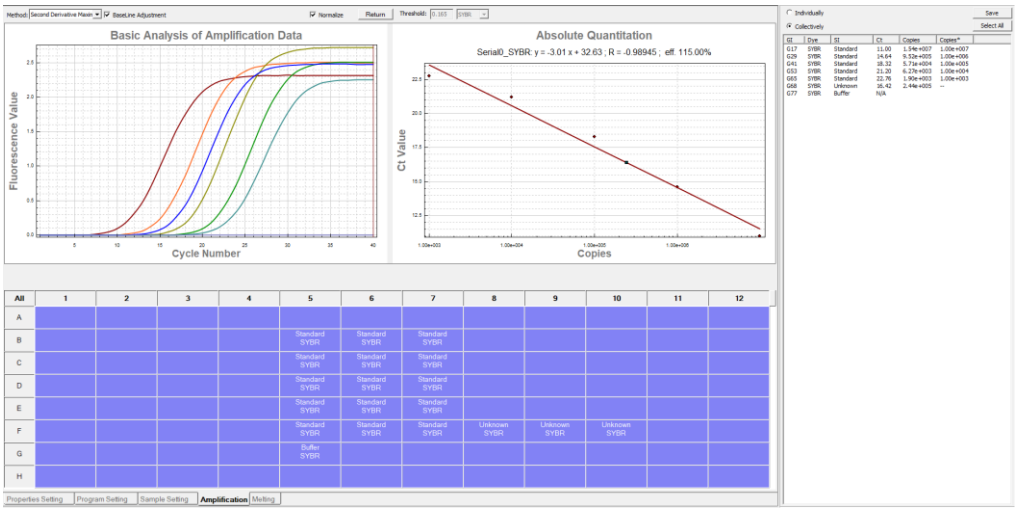


6.1.14 Data analysis

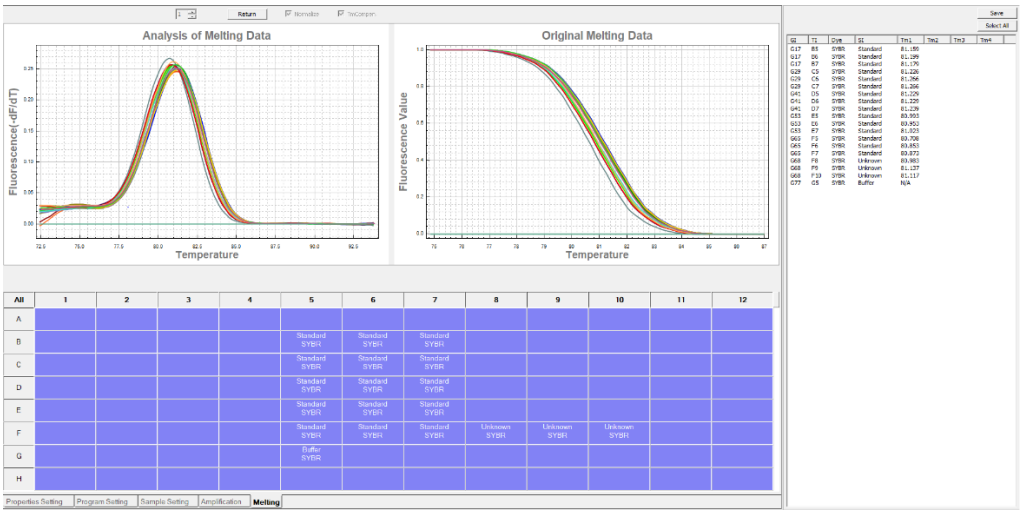
Click Amplification in the status bar to enter the amplification interface. Click Analysis to get the standard curve. The copy numbers of the samples to be tested is displayed in the right information box.



Click Collectively, the average data of the repeated samples are calculated, as shown in the following figure.



Click Melting in the status bar to enter the melting interface. The interface displays the derivative curve of the melting curve. Click on Original Data to display the original map of the melting curve. The Tm value is displayed in the information box on the right.



6.2 Taqman Assays- Liver cancer gene expression

Taqman assays for target detection or quantification typically consists of two specific PCR primers and a Taqman probe. During PCR, the primers and probe hybridize with the target. DNA polymerase extends the primer upstream of the probe. If the probe is bound to the correct target sequence, the polymerase's 5′ nuclease cleaves the probe activity, releasing a fragment containing the reporter dye.

Taking the expression of the cell cycle kinase 4 *CDK4* gene in primary carcinoma of the liver as an example, the important role of *CDK4* in the development of liver cancer was studied by comparing the expression of this gene in liver cancer tissues and normal tissues.

6.2.1 Preparation of template

1. RNA extraction

Extraction of total RNA from cryopreserved surgical specimens of liver cancer tissues and normal tissues using tissue RNA extraction kit. Assess RNA integrity on a denaturing gel or on an instrument such as micro-spectrophotometer. Ensure that the ratio of UV absorbance at 260 nm to the absorbance at 280 nm (A260/A280 ratio) is between 1.8 and 2.0.

2. Reverse transcription

Reverse transcription of RNA to cDNA using a reverse transcription kit.

6.2.2 Primer design

Primer design for reference gene *β-actin* and target gene *CDK4*. Reporter is FAM, and quencher is TAMRA.

6.2.3 Preparation of qPCR Reaction Solution

Set up 20μL qPCR as detailed in the table below.

Reagent	Volume（μL）
2XGoldStar TaqMan Mixture(UNG)	10
F-primer（10μM）	0.5
R-primer（10μM）	0.5
probe（10μM）（FAM-TAMRA）	0.5
cDNA	3
ddH_2O	5.5

(2XGoldStar TaqMan Mixture(UNG) was purchased from Beijing ComWin Biotech Co.,Ltd)

The number of samples prepared is shown in the table below. cDNA was replaced with ddH_2O as a negative control.

	liver cancer tissues	normal tissues
Target gene *CDK4*	3	3
Reference gene *β-actin*	3	3

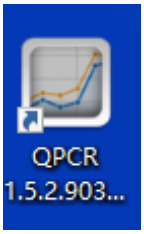
> ⚠ NOTE:
>
> Reaction liquid is prepared and then subpack. The reaction tube needs to be centrifuged instantaneously.

6.2.4 Power on

Connect the power cable for the instrument. Turn on the instrument power switch and the instrument will start self-test. At the end of the self-test, the STU status light is steady green.

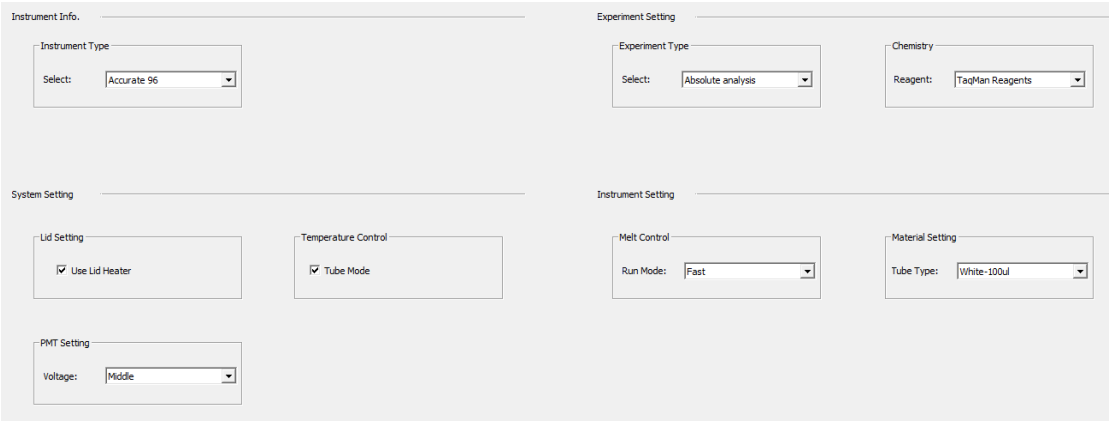
6.2.5 Software startup

Make sure the data cable is connected. Start the computer and double-click the desktop icon to launch the application.



6.2.6 New experiment

To create a new experiment, click New in the File menu, press Ctrl+N, or click the new experiment button on the toolbar. Then set the properties as shown below.

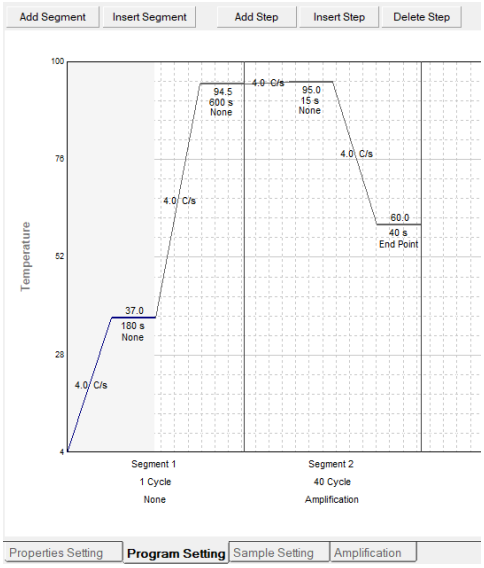


6.2.7 Program Setting

Click Program Setting in the status bar to enter the program setting interface.

Set the temperature, time, fluorescence collection point and cycles, as shown below.

Note that the fluorescence acquisition in the extension phase of the amplification curve should be set to End Point.



6.2.8 Sample Setting

Click Sample Setting in the status bar to enter the sample setting interface.

The normal tissue was set to Calibrator, the liver cancer tissue was Unknown, *CDK4* was the target gene, and *β-actin* was the Reference gene. There were four tests, three repetitions for each test. Select the sample position and FAM fluorescence channel. As shown below.

Unknown-29 Actin liver cancer	Unknown-30 CDK4 liver cancer	Calibrator-31 Actin Normal	Calibrator-32 CDK4 Normal
Unknown-29 Actin liver cancer	Unknown-30 CDK4 liver cancer	Calibrator-31 Actin Normal	Calibrator-32 CDK4 Normal
Unknown-29 Actin liver cancer	Unknown-30 CDK4 liver cancer	Calibrator-31 Actin Normal	Calibrator-32 CDK4 Normal

6.2.9 Save method

To save method, click save as in the File menu. Select the file storage location as "D disk", named 2019-FAM-liver. Or the storage path settings will automatically pop up after clicking Run.

6.2.10 Sample entry

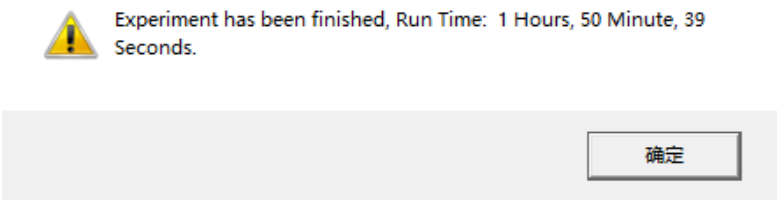
Click entrance and exit control button， and put the reaction tube into the reaction plate. The sample position is consistent with the set position. Empty reactor tubes need to be placed at four corners of the reactor plate to support the heat lid. See 2.2.2. Click the button again, and the reaction plate moves in.

6.2.11 Start running

Click "run" to start running.

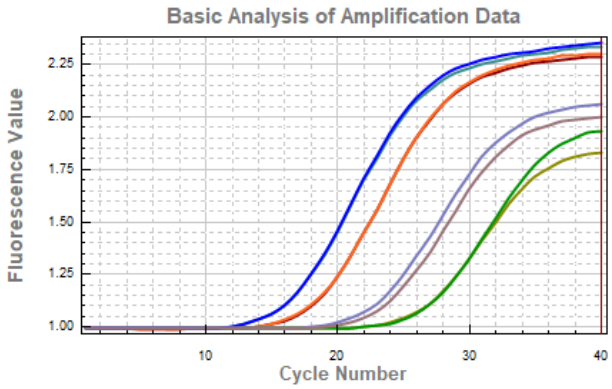
6.2.12 Stop running

The following prompt message will appear after the program finished. Click OK and the result data will be automatically saved to the created file.



6.2.13 Data analysis

The amplification curves and Ct values are as follows. The expression level of the CDK4 gene relative to β-actin can be obtained from the Ct value.



Ct value	liver cancer tissues	normal tissues
Target gene *CDK4*	22.79	25.90
Reference gene *β-actin*	15.96	18.09

Chapter7. TROUBLE SHOOTING

Common failures.

Index	Trouble	Possible reason	Solution
1	The power switch of the instrument is turned on, but the instrument does not respond.	1. The power cord is not connected correctly. 2. The power supply is not energized. 3. The fuse blows or bursts.	1. Reconnect the instrument power cord 2. Replace the fuse, see 8.4
2	Power status indicator does not light after turning on the power switch	Indicator failure	Contact the producer
3	Software and computer connection failed	Incorrect data cable connection	Close the software and reconnect the data cable
4	Abnormal fluorescence curve: straight line, partial data loss, etc.	No amplification of the reaction, or the fluorescence collection point is set incorrectly	1. Fluorescence collection point is set in the extension step 2. Optimize reaction conditions
5	The difference in fluorescence value of each well in the absence of test tube is large, or the background is large	Test tube hole or hot lid is contaminated	Clean the reaction plate twice with 70% ethanol and purified water.
6	Reagent evaporation	The PCR tube has quality problems such as poor sealing, and the test tube cover or the sealing film is not used correctly.	1. Replacement of qualified PCR 2. Correct coating or capping
7	Abnormal fluorescence detection value	1. Opening of hot lid during program running 2. Photoelectric system damage	1. Close the hot lid and re-test it. 2. Contact the producer 3. Others

Chapter8. DAILY MAINTENANCE

8.1 Computer and software maintenance

Maintenance project	Requirement
Use original computer and software	Do not replace the computer yourself
	Do not install other third-party software yourself.
Prevention of computer viruses	Do not use mobile memory unrelated to the experiment
	It is best to format the USB flash drive on your computer before using a USB flash drive.
	It is best to use a dedicated USB flash drive.
Insertion and removal of data lines	Avoid inserting and removing connection data lines frequently
	Unplug the data cable with the computer and the host turned off

8.2 Reaction plate cleaning

In order to ensure the accuracy of experimental data and reduce pollution, the QPCR reactor plate needs to be cleaned regularly every month.

1. Click the entrance and exit button to open the sample bin.

2. Dip 70% ethanol with a clean cotton swab and wipe the reaction well twice.

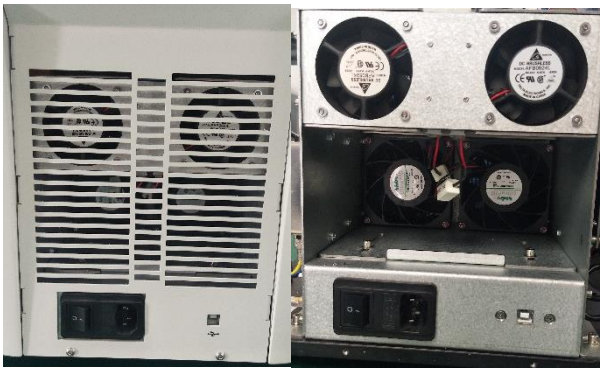
3. Use a new cotton swab to extract pure water after the alcohol is completely evaporated, and then wipe the reaction well twice.

4. Dry naturally;

5. Close the sample bin.

8.3 Fan cleaning

According to the frequency of use of QPCR, fans need to be cleaned regularly for half a year to one year. The fan is located inside the instrument backplane as shown below. After the instrument is powered off, use a Phillips screwdriver to open the backplane housing. The two sets of fans in the figure below can be cleaned with a brush. After cleaning, reinstall the backplane.



8.4 Fuse

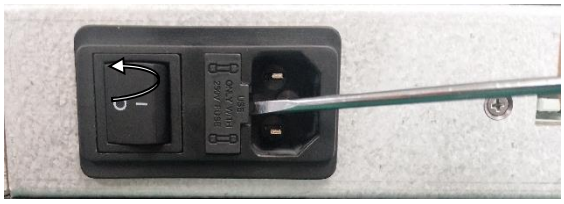
8.4.1 Model

Fuse diode，F5AL 250V

8.4.2 Inspection and replacement

1. **Remove the fuse**

The fuse is located between the instrument switch and the power plug. Use a flat-blade screwdriver to move the fuse out, as shown in the figure below.



2. Pull out the fuse diode

3. Detect fuse performance

Adjust the multimeter to the diode position, the electrodes touch the ends of the fuse respectively, and the multimeter emits a “drop” sound, indicating that the fuse is normal. Otherwise, switch to a new fuse and re-test.

4. Put the fuse back into the instrument.

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