Fluorescent Quantitative PCR Detection system

LineGene 1600 Series

Operation Manual

Attention

Users are recommended to read the contents of this manual thoroughly before operating the Bioer Fluorescent Quantitative PCR Detection System.

To carefully observe all special Warnings and Cautions outlined in this manual.

This manual should be maintained properly in good condition for reference.

杭州博日科技有限公司
HANGZHOU BIOER TECHNOLOGY CO., LTD

Thank you for your purchase of this product.

Before initial use of this instrument, please read this manual thoroughly ！

Technical requirement No. of registered product: YZB/G.0432-2015
Product registration No.: **********
License for Medical Instruction Manufacturing Enterprise: Z.S.Y.J.X.S.C.X.No.20150033

File No.: BYQ661800000000SM
File Version: July 2017, Version 1.1

Important Notes

1. Usual practice

Note: Very important information is contained within this manual and it should be carefully read before first use of the instrument. Failure to operate instrument according to the instruction could result in damage or abnormal functioning of the instrument.

Warning! The warning message requires extremely careful operation of a certain step.
Failure to observe the instruction could result in serious personal injury.

2. Safety

During operation, maintenance and repair of this instrument, the following basic safety notes must be observed. In case of failure to follow these measures or the warnings or notes indicated herein, the basic protection provided by the instrument, its safety criteria of design and manufacture, and its predicted use range would be impaired.

Hangzhou Bioer Technology Co., Ltd. shall not be held responsible for any consequences resulting from the user's failure to observe the following requirements.

Note: The instrument, complying with the Standard GB4793.1, is a general instrument of class III, the protection degree is IP20. It is intended for indoor use of Elevation 200 meters below.
Note: The instrument. complying with the Standard YY0648 is used for IVD
edical equipment
a) Instrument earth
In order to avoid an electric shock, the input power cable of the instrument must be properly earthed. This instrument uses a 10A 3-core earthed plug, which is provided with a third (earth) pin. It is for use with an earth type power socket and is a safety unit. If the plug cannot be inserted into the socket, the socket must be fixed by a qualified electrician, to maintain the safety function of the plug and the protection it provides.

b) Keeping apart from the live circuit
Internal maintenance or replacement of any part of the thermal cycler must only be carried out by qualified personnel. The instrument must be disconnected from the mains circuit prior to any maintenance being carried out.

c) Use of power supply
Before connecting the power adapter, please confirm the maximum load of the adapter power shall not be less than 180W, the voltage is DC24V and the connector is consistent with the instruction power supply input.

Before connecting to the mains and switching the instrument on, make sure it is consistent with the adapter requirements (100-240V, 50/60Hz). The rated load for the power socket must not be less than the instruments maximum load of 180W

d) Power wire
The instrument is supplied with a power cable which should be used at all times when operating the instrument. If the power cable is damaged it should be replaced with a new one of the same specification. Care must be taken that the power cable does not get compressed or tightly bent and that it does not lie across areas where it may cause a trip hazard to personnel.

e) Insertion and withdrawal of power cable

At insertion and withdrawal of power cable, the back of the plug shall be firmly held with the hand. The plug must be completely and tightly inserted into the socket and must not be removed by pulling the cable. The back of the plug should be grasped in the hand and pulled directly backwards to remove from the socket.

f) Placement of instrument
This instrument should not be positioned in a place where it is difficult to cut off the power supply.

This instrument should be placed in a low relative humidity (RH) and low dust environment well away from any water (e.g. sinks and pipes). The room should be well ventilated, and free from corrosive gas, or interference from a strong magnetic field. The instrument should not be placed in a wet or dusty location, but should be positioned on a sturdy, level and secure table appropriate to its weight.

The openings on this instrument are for ventilation purposes and in order to avoid over-heating of the instrument they shall not be blocked or covered. When a single set of instrument is used, the space between its ventilation openings and the nearest object should not be less than 30cm. When several sets of instruments are used, it should not be less than 50cm.

Excessive environmental temperature would impair the test performance and could result in failure of the instrument. This instrument should not be used in locations subjected to direct sunlight or strong radiation or light source, as this could impair the fluorescence detection.

The instrument should be kept away from hot gas, furnaces, stoves and all other sources of heat.

When switched off, the power should also be switched off. If the instrument is not going to be used for a long time, the power should be switched off, the power plug withdrawn and the instrument covered with soft cloth or plastic film to prevent dust or foreign bodies entering the machine.

g) Notes during operation
During test, cares shall be taken to prevent liquid from dropping onto the instrument. The castoff used in test , such as consumables, reagent, and so on, should be treated as require, and should not be thrown away or poured.
During test, if there is hazardous substances, user must be trained before using.
Hazardous substances, which has been used, should be coped with and saved according to derection for use.
User, who operates the instrument, must be trained and has relevant quantification.

Caution: If any of the following should occur, you should immediately switch off the power supply, withdraw the power plug from the power socket, and contact the supplier to effect a repair: Repairs can only be carried out by suitably qualified engineers.

- Liquid gets inside the instrument.
- The instrument is rained upon or water is spilled over it.
- The instrument works abnormally, or generates an abnormal sound/s or generates a strange odour.
- The instrument is dropped or its casing is damaged.
- There is an obvious change in the function of the instrument.

Caution: When you deal with potential contagious matter such as flesh sample or reagent, which is likely to touch skin, protecting glove or other protecting measures are need to be used.

h) Transportation again

If transporting the instrument again, the instrument and its detection wells need be cleared wholly before transportation, and be disinfected by UV light.

i) Warning Sign

Warning identification

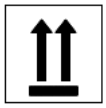
DANGER!		Place pasted this mark in instrument, is danger, if the instrument is used irrelevancy.
SCALDING!		Place pasted this mark in instrument, causes high temperature and is scalding during use.
BIOHAZARD		Place pasted this mark in instrument, caused biohazard during use.
PROTECTIVE EARTH		Protective earth is near to the place pasted this mark in instrument

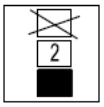
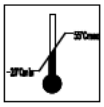
- Warning mark

Warning! There is a sign reading “HOT SURFACE”. This indicates a surface which will be hot during and immediately following the running of a programme. Contact with this metal area will cause burns.

Warning! During the use of the instrument the user may come into contact with biologically hazardous materials. Rules for safe handling of such materials must be followed. The operator must be appropriately trained.

j) Signs on the external packaging

Up		Correct position of transport package is vertical upwards.
Breakables		Be treated with care when transportation, there is breakables in transport package,
Fear rain		Transport package is not rained.

The limit of stacking layer		Maximum stacking layer of the same package is 2.
Temperature limit		Temperature limit that transport package should keep is form -20C to 55C.

3. Maintenance of instrument

Any stains on the instrument can be cleaned with soft cloth soaked with a gentle cleaning solution.

Heat conductive oil medium should not be used in the block wells of this instrument.

Module should not be left open for any period of time as this may allow dust to enter the instrument.

Warning !

- When cleaning the instrument, the power should be turned off.
- The instrument surface should not be cleaned with corrosive cleaning agents.
- The instrument module includes precise optics, dust, foreign matter and residue should be avoided.

4. After-sales services

The warranty content and scope are shown in the warranty sheet.\

Note:

- After unpacking, immediately check the goods against the packing list. If any parts are damaged or missing, please contact the supplier immediately.
- After qualification of acceptance, complete the product acceptance sheet and send (or fax) the copied sheet to the supplier for filing and maintenance.
- Before first use of the product, the user shall complete the instrument registration form and send to Hangzhou Bioer Technology Co., Ltd. to obtain the correct operation password.
- After unpacking, the packing box and packing materials should all be kept in case it is required for transportation or service in the future.
- In the event that a repair is required, the instrument must be disinfected before being sent to the repair department. A decontamination sheet should be completed and sent together with the instrument. These are available on request from your local supplier.
- It is recommended that service personnel disinfect the instrument on receipt in the service department, before commencing any scheduled work.
- Hangzhou Bioer Technology Co., Ltd. shall bear no liability in the event of any damage to the instrument occurring during transportation to the service department due to improper packaging.

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Chapter 1 General description

This chapter mainly describes the applications, features, specification, model, performance parameters and software functions of this 16-well Fluorescent Quantitative Detection System.

1. Main Applications of 16-well Fluorescent Quantitative Detection System

This 16-well detection system adopts fluorescent real-time detection method to analyse PCR template amplification and is suitable for polymerase chain reaction fluorescent quantitative detection in research fields of human gene group engineering, forensic medicine, oncology, tissue and community biology, paleontology, zoology, botany and clinical diagnosis fields of virus, tumor, hereditary disease.

This PCR detection system is a kind of in vitro diagnosis (IVD) equipment. It could be used for quantitative analysis of copies of different genes in clinical laboratory by adopting fluorescence polymerase chain reaction.

2. Features of 16-well Fluorescent Quantitative Detection System

- Novel and human-orientated running interface for smooth operation.
- The adopted fluorescent real-time detection mode realizes simultaneous amplification and detection in the same tube without need of post experimental treatment.
- Advanced thermoelectric technology ensures fast and steady heating and cooling of the ultra-fast heat cycling system.
- Two-point TE temperature control ensures consistent temperature of 16 sample wells.
- It uses maintenance-free long life LED excitation light source.
- Precise optical path system and ultra-sensitive PMT system provide the most accurate and sensitive fluorescent detection.
- It can create a real-time dynamic monitor of the entire PCR amplification process.
- It has high linear range up to 10 orders of start DNA copies without serial dilution.
- It is unnecessary to open the PCR reaction tube, ensuring samples are protected from contamination during and after PCR and ensures accurate results.
- Multiplexing is possible.
- The hot-lid technology allows for oil-free operation of PCR.
- User friendly interface with flexible programme setting and analysis and reporting using the stored parameters.
- It can print out one or more sample report(s).
- Automatic, correct and timely remote networked services provide the latest technical support.
- Advanced top fluorescent detection technology brings fast and convenient scanning.
- Support RS232, USB and Bluetooth interface.

3. Notes to model and description

Model：FQD – 16 A

- A — Design series number
- 16 — Specification code, 16 denoting maximum sample quantity 16
- FQD — Abbreviation for Fluorescent quantitative polymerase chain reaction (PCR) detection system

Mode

FQD – 16 A

- A → Design series number
- 16 → Specification code, 16 denoting maximum sample quantity 16
- FQD → Abbreviation for Fluorescent quantitative polymerase chain reaction (PCR) detection system

Specification：LineGene 16 ××

- ×× — Code realted with optical filter group
  - 20 （matched with F1, F2 optical filter）
  - 30 （matched with F1, F3 optical filter）
  - 40 （matched with F1, F2, F3, F4, optical filter）
- 16 — Specification code, 16 indicates the maxium sample No. is 16
- LineGene — English name of description of Bioer Fluorescent quantitative polymerase chain reaction (PCR) analysis system series.

4. Performance parameters

Model	FQD-16A							
Sample capacity	16×0.2ml (suitable for single tube, 8-row tubes and 96-well fully-skirted plate)							
Detection channel	F1	F2	F3	F4				
Applicable dye	FAM, SYBR Green I	VIC, HEX, TET, JOE, Cy3,	ROX	Cy5				
Temperature range of block working	0～100℃ (Minimum division:0.1℃)							
Heating/cooling rate	5.0 ℃/s (max)							
Temperature fluctuation	≤ 0.1°C (full-range), (55°C typical value ≤ 0.1°C)							
Temperature accuracy	≤ ±0.1 ℃ (full-range), (55°C typical value ≤ 0.1°C)							
Temperature uniformity	≤ 0.2 ℃ (full-range), (55°C typical value ≤ 0.15 ℃)							
Temperature range of hot-lid working	70～110 ℃ (adjustable, default 105 ℃)							
Repeatability of fluorescent intensity detection	5%							
Running mode	Continuous running							
Operation system	Windows XP/Windows Vista/Windows7/Windows8							
Power supply	DC24V 180W							
Dimensions	280mm×240mm×220mm							
Weight	6.5kg							

5. General description of functions of the software

a) Parameters setting-up function (including temperature, time, cycles, heating/cooling rate, selection of detection channel and yield of photo-electric amplification tube).

b) Note function of text contents.

c) Sample material record function (sample No., sample name and sample data).

d) Document running display function (PCR heat cycle data display, fluorescence detection data display and real-time display of each data during running of instrument).

e) Detection data analysis function (The analysis function may be independently used without connection to the instrument).

f) Analysis result output function. It may output the analysis result to various types of document, e.g.: EXCEL, TXT document. It is possible to run an enquiry and print out analysis result, modify the printing format and select/de-select items to print.

g) Document storage function (setting up data, running data and analysis results).

h) Fault protection and alarm function

Caution:	The above-mentioned software functions are merely for reference. The software functions may be modified without notice.

Chapter 2 Preparations

This chapter mainly describes use, transport and storage condition, structural composition, installation/unloading of software and preparations before first using the LineGene16xx series fluorescent quantitative detection system.

1. Transport and storage conditions of the instrument

Environmental temperature: -20℃～55℃
Relative Humidity: ≤80％

2. Normal working conditions

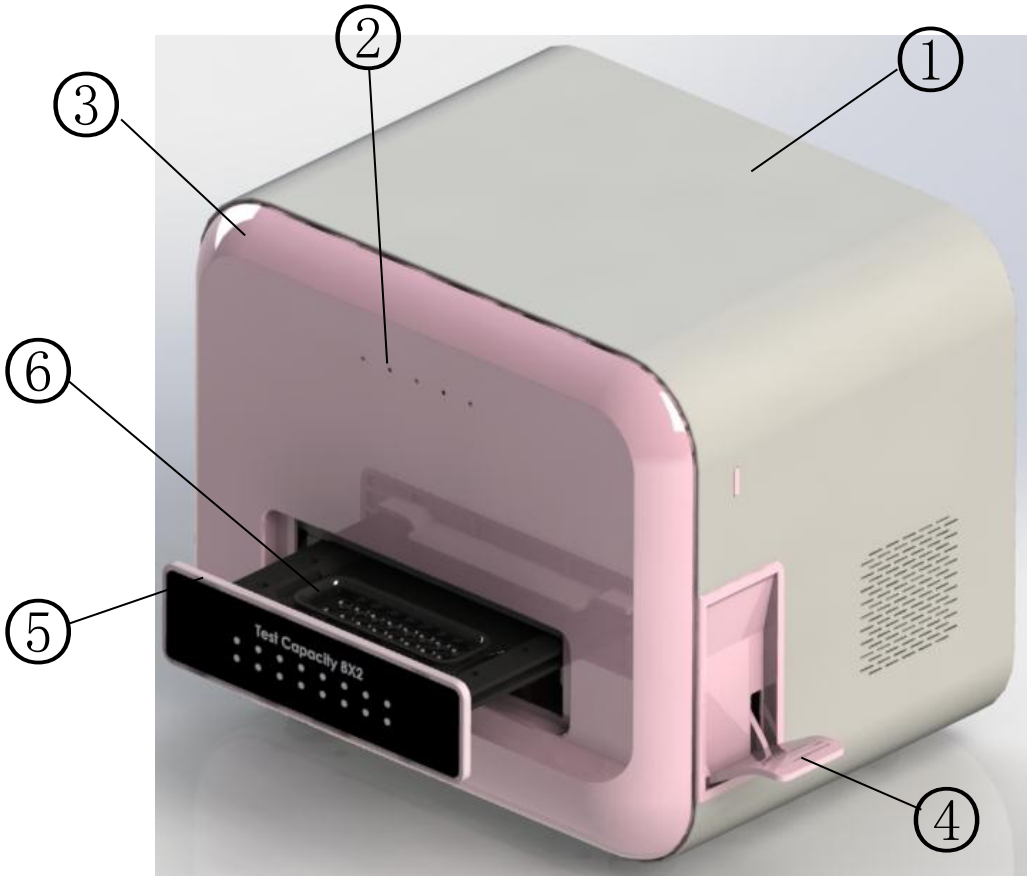
Environmental temperature: 5℃～35℃
Environmental RH: ≤80％
Altitude: < 2000 m
Power supply: DC24V 180W

Caution:	Before using the instrument, please make sure the working conditions meets the above requirements. The power socket shall be a 3-hole socket and properly earthed.

3. Preparations before starting the instrument

4. Preparation before starting

4.1 Skematic diagram of the structure



Back of Mini PCR

①Shell ②Status Indicator ③ Front Cover ④ Handle ⑤ Tube Shelf

⑥ Tube Tray ⑦I/O Series Port ⑧ Power Swtich ⑨ Adapter Socket

4.2 Connection of power wire and communication wire

Connection of power wire: Only the power adapter supplied with the instrument should be used. At connection, make sure the instrument power switch is in the "OFF" position and after connection the power cable should be checked to ensure a tight contact with the instrument socket; otherwise it should be replaced.

Connection of communication wire: The supplied communication wire and communication conversion box should be used. One end of the communication conversion box is connected with the DB15 communication interface at the back of the instrument, and the other end connected with the computer USB, RS232C or Bluetooth interface. After connection, screws should be securely tightened.

For Bluetooth communication conversion box, it is necessary to install Bluetooth manager in the computer. The Bluetooth manager in Windows could be used with Bluetooth virtual serial port installed and configured. A third party management software could also be adopted. When installing Bluetooth

manager, please operate as per the software installation tips.

Caution: If with prolonged use the supplied power cable connection becomes loose, it should be replaced with one of the same type and specification.
The communication conversion box is built with special circuits and must not be opened.
If USB interface is used, the computer shall install USB driver.

5. System Installation and Unloading

5.1 System Installation

System Environment
Operating system: Windows XP/Windows Vista/Windows7/Windows8
Runtime environment: Net Framework 4.0
Other software: PDF reader

Minimum configuration:
Processor: Intel Core i3
Memory: 2GB
Hard Disc: 10GB

5.2 LineGene16xx software installation

Double click PcrServer installation file (PcrServerSetup.exe) ▶ display the installation interface (select installation language) ▶ set up installation path ▶ install

Double click LineGene1600 installation file (LineGene1600DiagnosisSetup.exe) ▶ display the installation interface (select installation language) ▶ set up installation

5.3 LineGene16xx Software Unloading

Control Panel ▶ Add/Delete Program ▶ PcrServer ▶ Unloading
Control Panel ▶ Add/Delete Program ▶ LineGene1600s ▶ Unloading

Chapter 3 Start

1. Checks before start

After inserting the power plug and switching on this detection system, the following should be checked:

- Check the voltage of the power supply is consistent with the system-required one.
- Check the power cable plug for correct and reliable insertion into the power socket.
- Check the communication converter for correct insertion into the host and tightening and the cable plug for correct and reliable connection to the computer.
- Check the update shift switch MODE of communication converter is set to “normal” state.
- Check the environmental conditions meet the required tolerances.

2. Start

In order to ensure effective connection and communication between the instrument and the computer system, the system shall be started in the following sequence:

1st step: Start computer display and host.

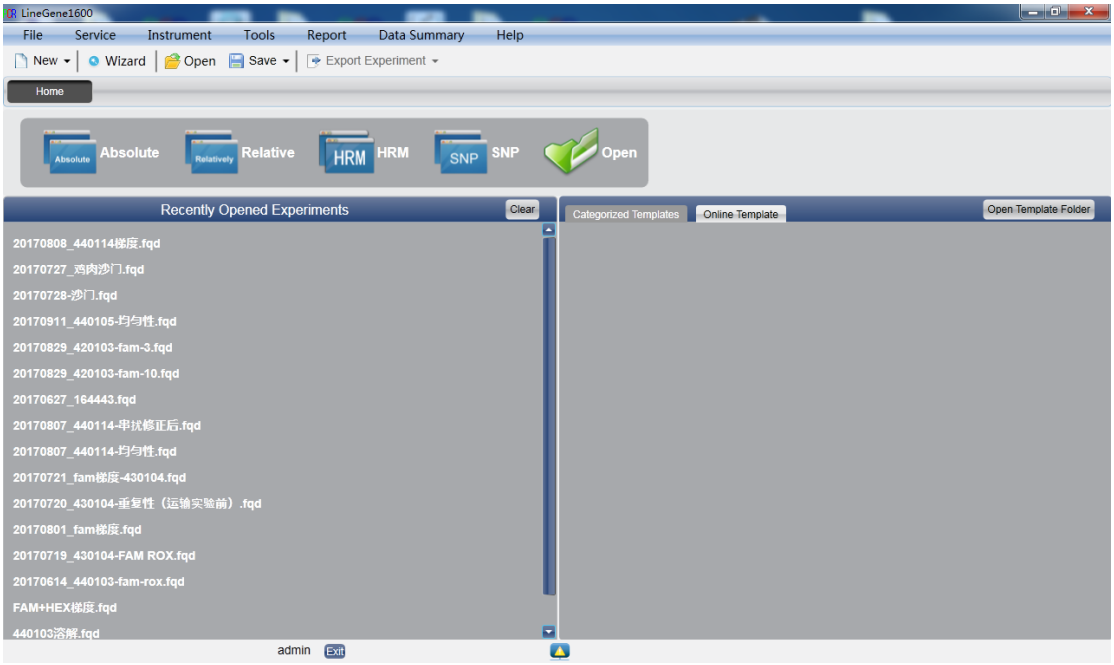
2nd step: Turn on power using the switch on the back of the instrument to set the system ready to run.

3rd step: When the computer enters operation system, start the LineGene16xx fluorescent quantitative detection system.

To start the software, click “LineGene16xx” from [Start]/ [programme] menu or double click the short-cut icon on desktop.

3. Starting software interface

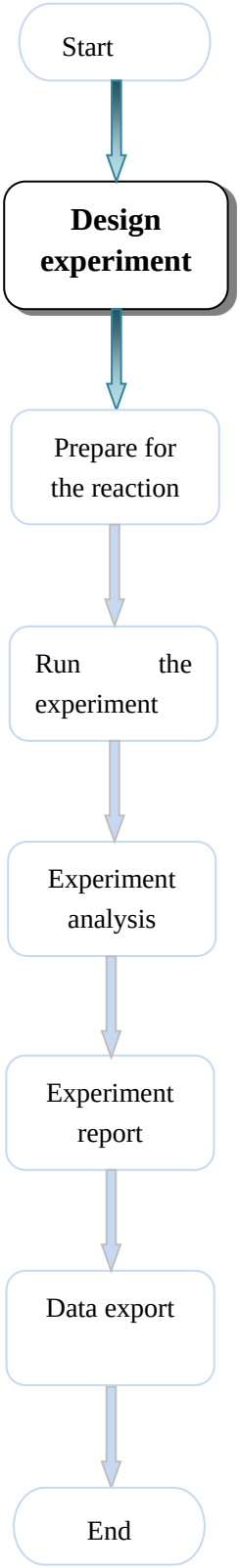
Double click any software running fast icon of LineGene1600 Series Fluorescent Quantitative PCR Detection system and it will display the start window.



The system window consists of the menu bar, the toolbar and the main page.

Chapter 4 Absolute Quantification

1. Design Experiment



This section describes how to design a new absolute quantification experiment and covers inspection item setting, sample information setting, reaction plate setting and programme setting.

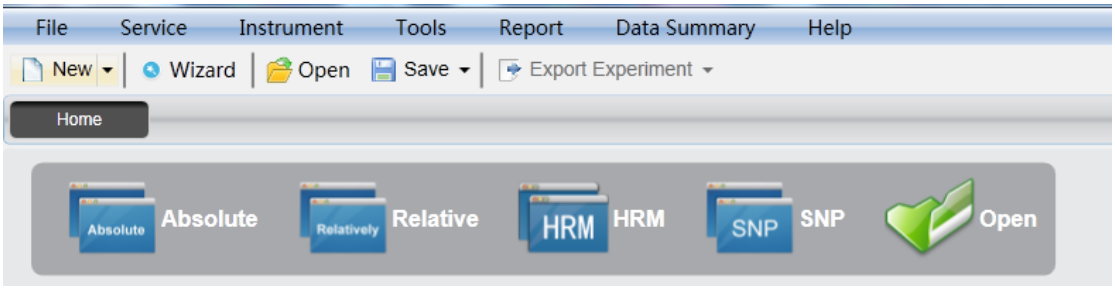
1.1 Create New Absolute Quantitative Experiment

1. Click build **Absolute** on the **Home** interface and this will open the absolute quantitative experiment window.

※The Absolute quantitative experiment can be also created by:

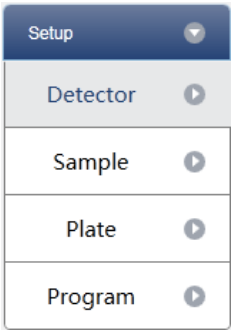
a. Clicking **File** ▶ **New** on the menu bar

b. Clicking **New** ▶ **Absolute** on the toolbar



1.2 **Detector Setting**

1) Click **Setup** ▶ **Detector**



2) Input experiment properties

Input the experiment name, user name and any comments in the experiment properties column.

3) Detector Setting

Set up the Detector, Dye, Colour, Master Mix etc.

※If necessary, the user can also:

a. Add detector

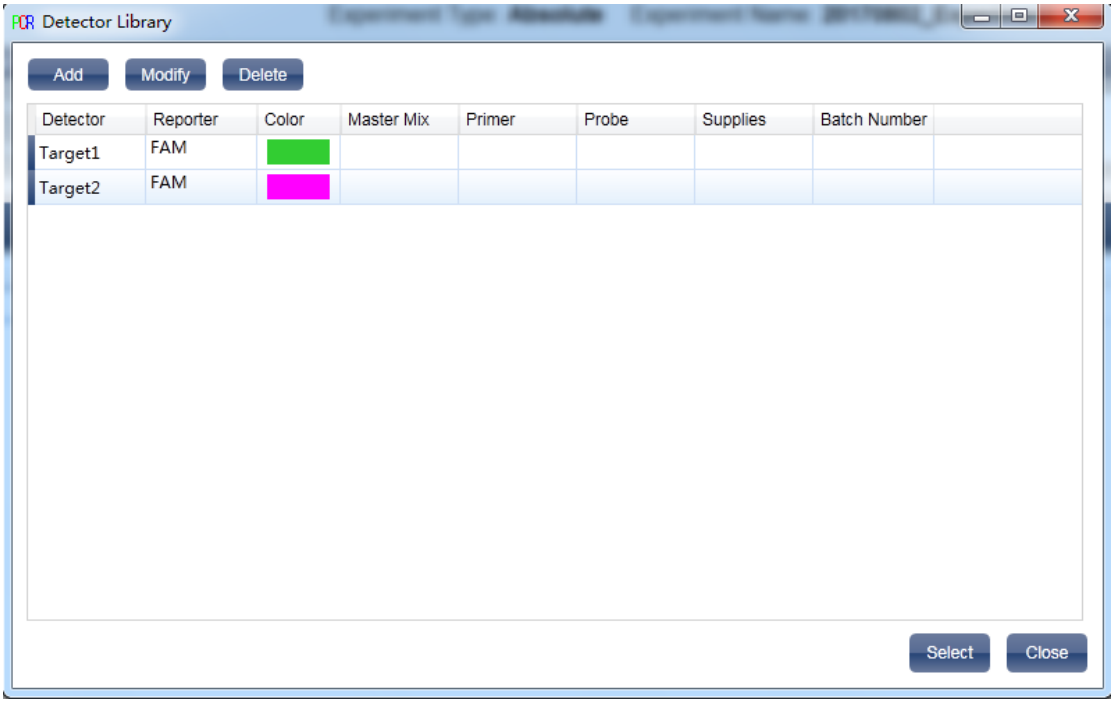
b. Add assay

c. Delete detector

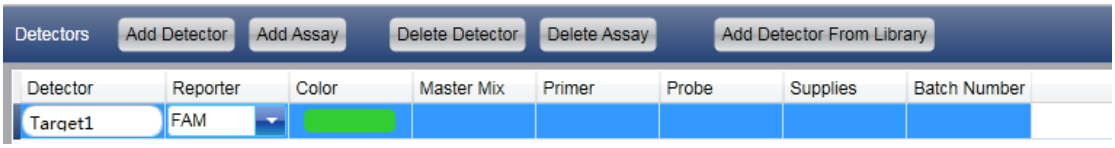
d. Delete assay

Add the detector in the Detector Library: click **Add Detector From Library** ▶ the **Detector Library** window will pop up ▶ select the Detector in the window to be added

※The user can also conduct Add, Modify and Delete operations in the item library.



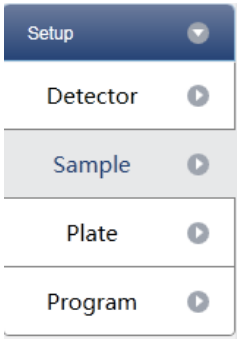
f. Set up the detector, set up the assay, set up the dye name and set up the colour



4) Set up reference dye

1.3 **Sample Information Setting**

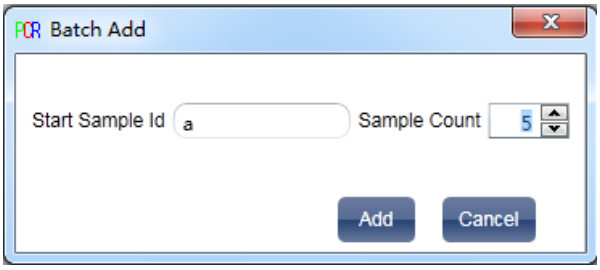
1) Click **Setup** ▶ **Sample**



2) Add sample information

a. Itemized addition: input ID in **Sample ID** ▶ press **Enter** ▶ add information for one sample

b. Batch addition: click **Batch Add** ▶ the Batch Add window will pop up



3. Delete sample information

a. Itemized deletion: select one sample ▶ click **Delete** ▶ delete the selected sample information

b. Delete all: click **Clear All** ▶ delete all sample information

4. Import/Export sample information

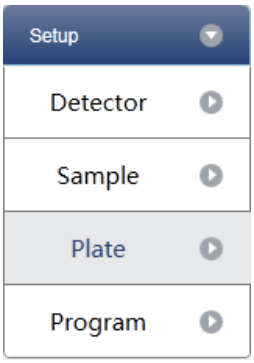
a. Click **Import Sample Info** ▶ the File Import window will pop up ▶ import sample information file in CSV format

b. Click **Export Sample Info** ▶ the Save As window will pop up ▶ the sample information will be exported in CSV file format

5) Set up sample information

1.4 **Reaction Plate Setting**

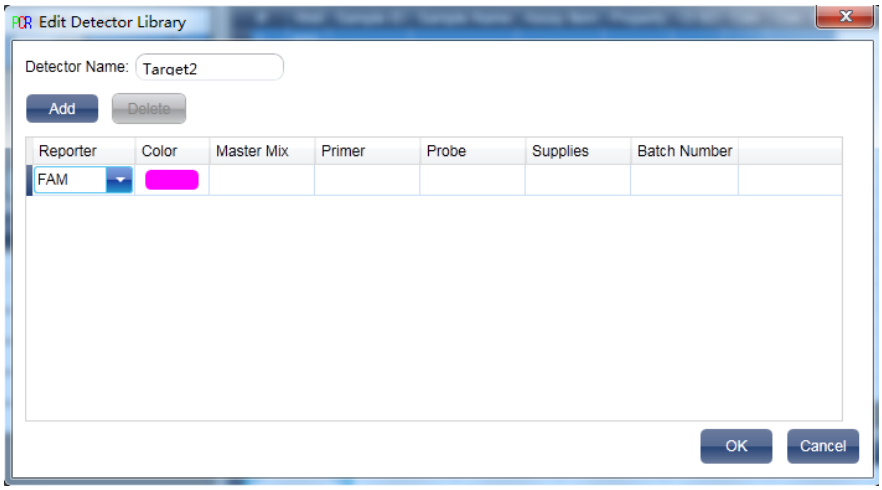
1) Click **Setup** ▶ **Plate**



2) Set up the inspection criteria of the reaction plate

a. Select reaction plate well site: click Reaction Plate well Site

The user can also right click the reaction plate well site to Copy, Paste and Add New Detector. Adding a new detector will open the **Edit Detector Library** window.



b. Select Assay item and modify the property, concentration and concentration unit.

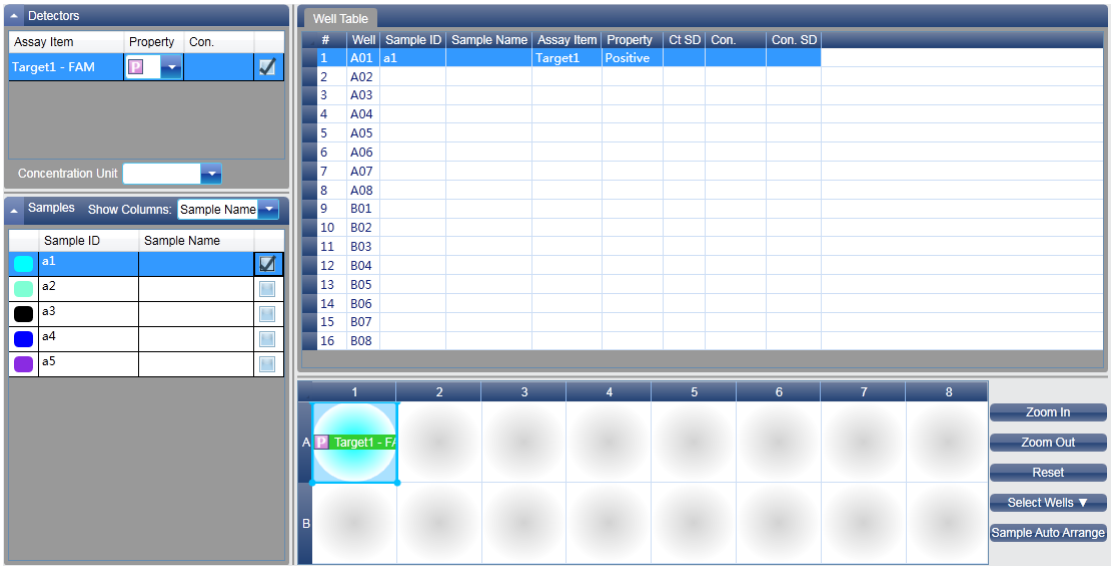
Property	Name	Concentration	Concentration unit
U	Unknown	no	Copies/ml
S	standard	yes	IU/ml
N	Negative	no	Fg/ml
P	Positive	no	Pg/ml

c. Select a sample and the list displayed will change

d. Zoom-In, Zoom-Out and reset the reaction plate.

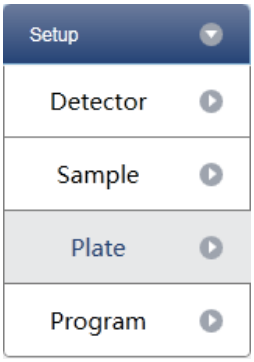
e. Sample Auto Arrange

f. Check Well Table



1.5 **Programme Setting**

1) Click **Setup** ► **Programme**



2. Run Programme Setup

a. Create new stage: the user can create a new **Hold Stage**, **Cycling Stage** or **Melting Stage**

※The user can also click **Add Stage** directly and the default will be creating a new **Cycling Stage**.

b. Create new step: the user can create a new step **Before** or **After** the currently selected step

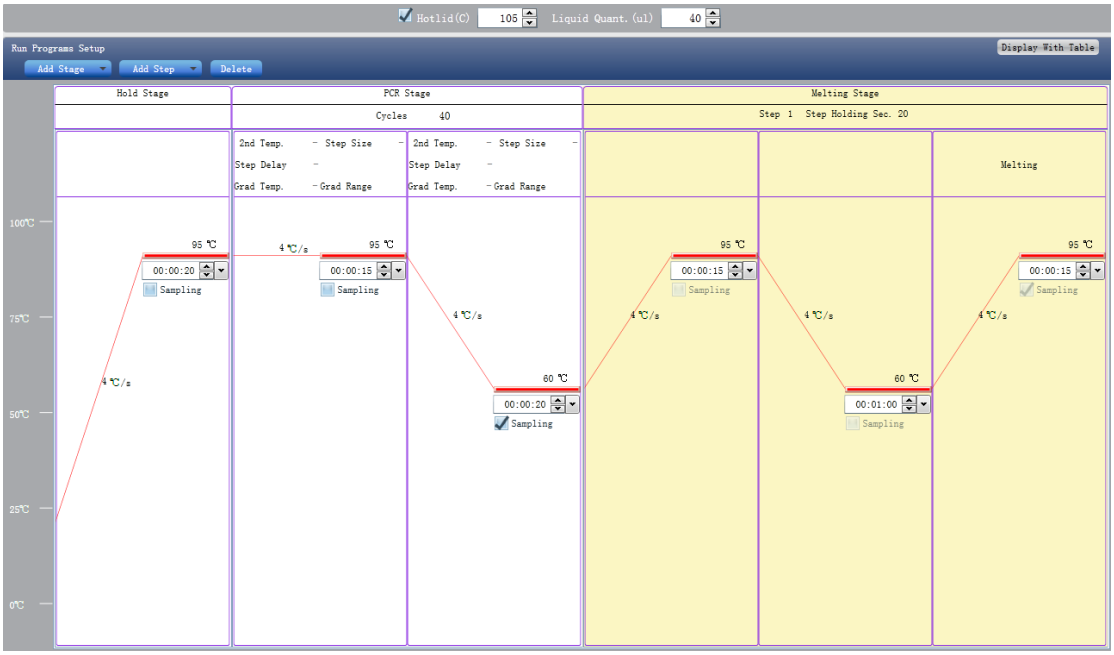
※The user can also click **Add Step** and the default will be adding a new Step at the end of the currently selected stage or after the currently selected step.

c. Delete: the user can delete the currently selected step or stage

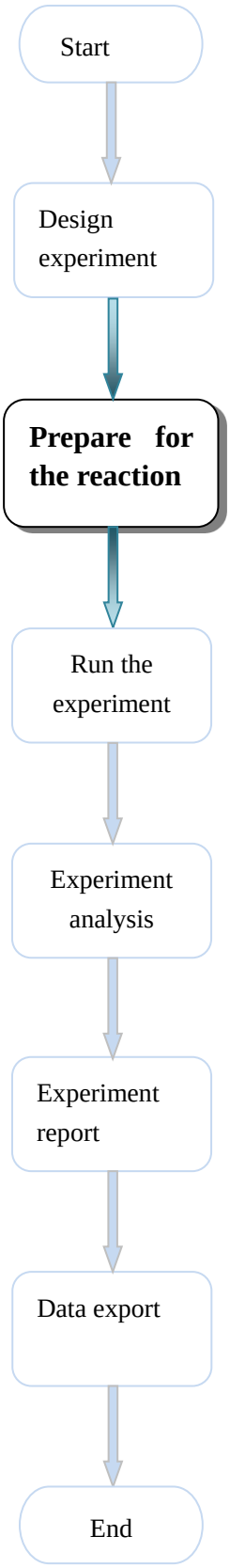
d. Display form: click **Display With Table** ▶ new window will pop up ▶ the details of the current experiment will be displayed in a table.

e. Set up the experimental data of the hold stage, cycling stage and melting stage melting section

f. Set up the hot-lid temperature and liquid volume



2. Prepare for Reaction

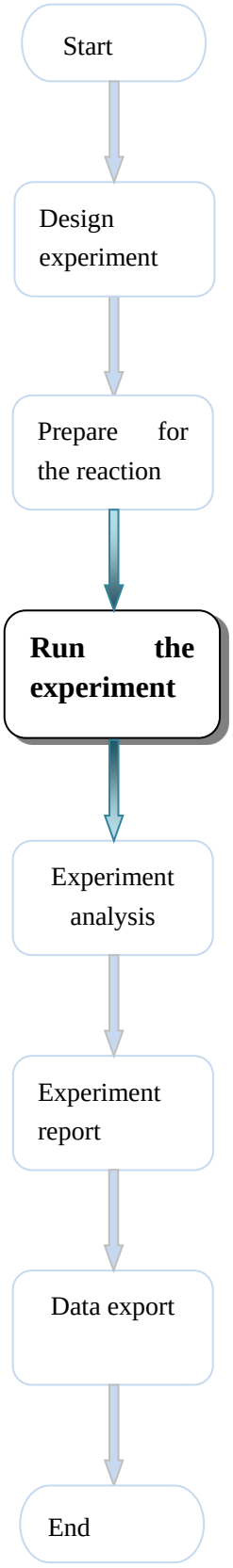


The user should make full preparations prior to the experiment:

• Ensure appropriate materials are used.

• Ensure the arrangement of the PCR reaction plate is consistent with the setting layout of the reaction plate in Section 1.4.

3. Run the Experiment

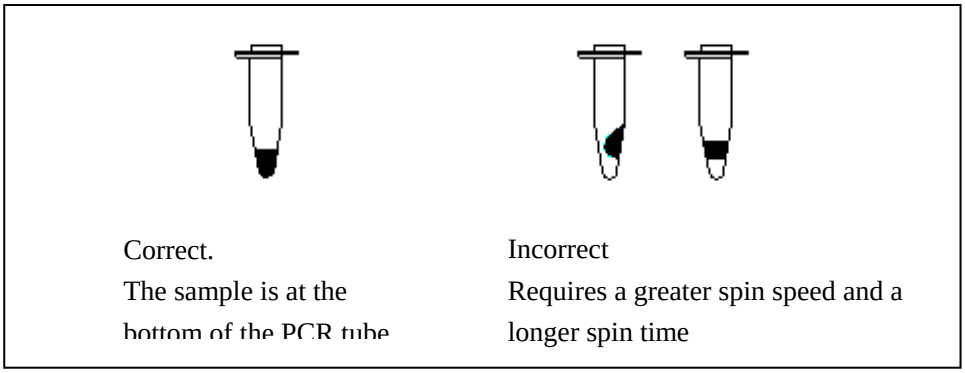


This section describes how to run/operate the experiment after loading the reaction plate and includes how to operate the fluorescence curve, the temperature curve and programming

Caution: Before starting, make checks and follow the procedure for correct start up of the system.
The green lamp of the run switch will be lit and the system will be ready to run.

3.1 **Preparation for reagent sample**

- Preparation for reagent: The LineGene1600 series fluorescent quantitative PCR detection system uses 0.2ml PCR tubes to conduct the reaction. The recommended reaction volume is 10μl～50μl for an optimal reaction system.
- The PCR tube must have an optically clear top.
- Centrifugal operation: Before placing reactions into the instrument, it is recommended that a short centrifugal spin is used to ensure that the reagent is at the bottom of the reaction tube and the reagent/sample mix is free from bubbles.

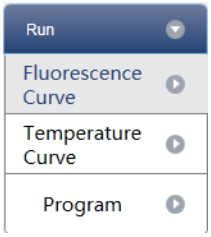


Correct position of the sample in the test tube

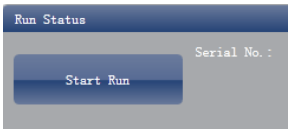
- Insert the test tube: if the sample number is less thant the hole number of the mold, then try to spread the sample test tube in the hole of the mold evenly to ensure the heat lid could reliably press at the top of the test tube during operaiton. At the same time, make the load of the mold to be even to ensure the temparature change of each test tube is consistent.

3.2 **Run Fluorescence Curve**

1) Click **Run** ▶ **Fluorescence Curve**



2) Click **Start Run**

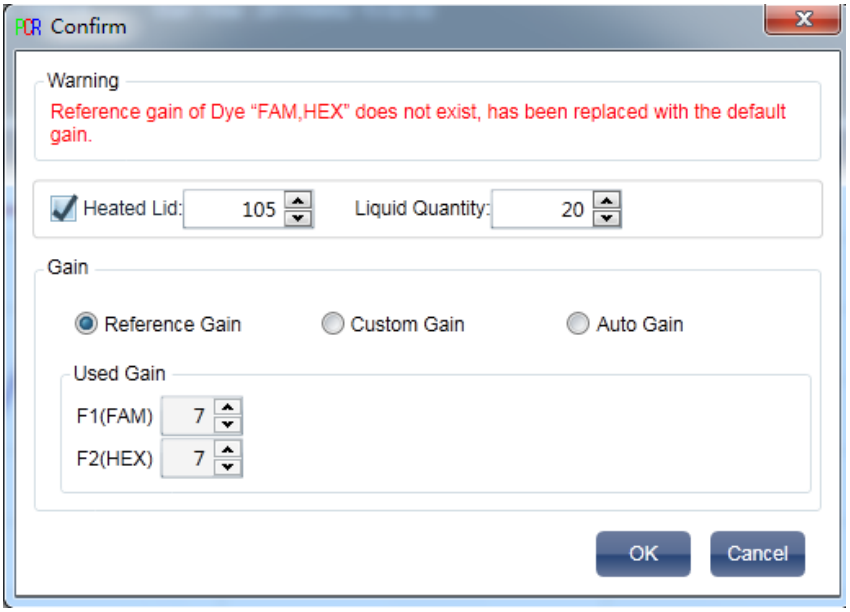


3) Operating confirmation

a. Modify hot-lid temperature and liquid quantity (sample volume).

b. Gain parameter setting

c. Target fluorescence value setting



4) After it starts operating, the user can:

a. Skip the current stage

b. Add a cycle

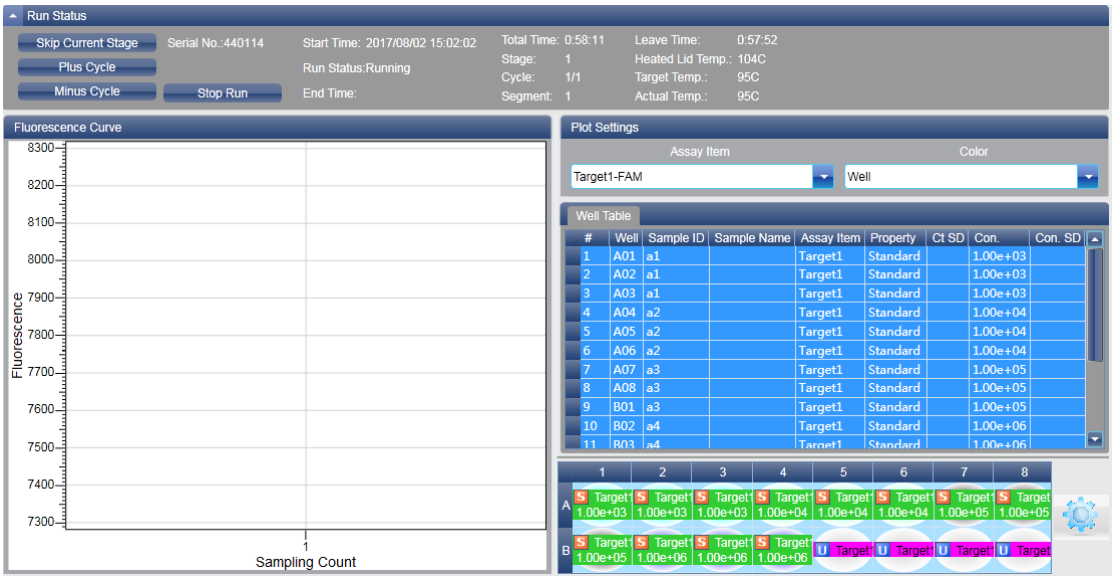
c. Delete a cycle

d. Stop run

5) Plot display setting

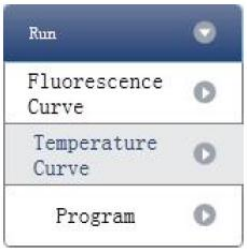
a. Assay item

b. Plot colour

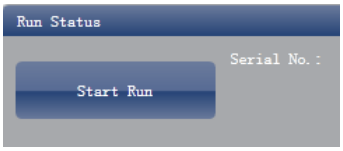


3.3 **Run Temperature Curve**

1) Click **Run** ▶ **Temperature Curve**



2) Click **Start Run**

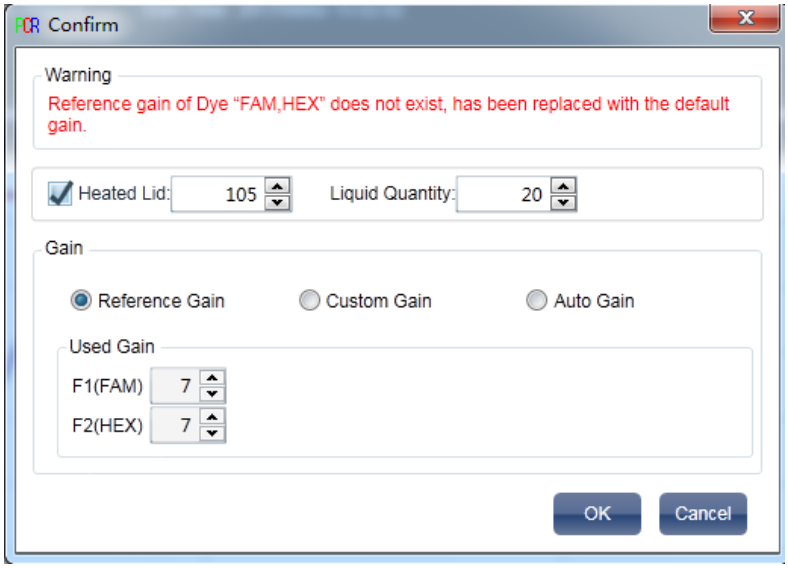


3) Operating confirmation

a. Modify hot-lid temperature and liquid quantity (sample volume).

b. Gain parameter setting

c. Target fluorescence value setting



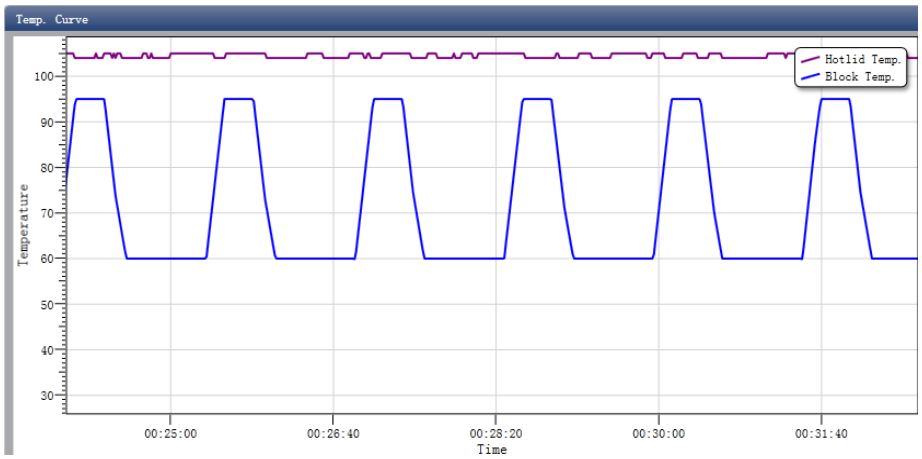
4) After it starts running, the user can:

a. Skip the current stage

b. Add a cycle

c. Delete a cycle

d. Stop run



3.4 **Programme Setting**

The user can only check the programme setting but cannot make modifications.

3.5 **Working state indication lamps on instrument**

The panel at the right of the instrument is fixed with 1 lamp and the colors related to the system state during the running of a programme:

- **Standby**: The indicator lamp lights **blue**, which denotes that the entire machine is ready to operate.
- **Running**: The indicator lamp lights **green**, which denotes that the entire machine is running a programme.
- **Error**: The indicator lamp lights **red**, which denotes that the instrument has detected a fault.

注意：	For prolonged shutdown, switch off the power at the back of the instrument and at the socket. When switched on again, the hot-lid and module will revert to the default settings.

The rear cover of the instrument is fixed with a switch to control energizing of its internal control system:

- Turning on the key, the green indicator lamp is lit on the instrument, the internal system is energized and the instrument is ready to run the programme.
- Turning off the key, the key will spring out, the green indicator lamp goes off, the instrument internal system is de-energized and the system is under standby.

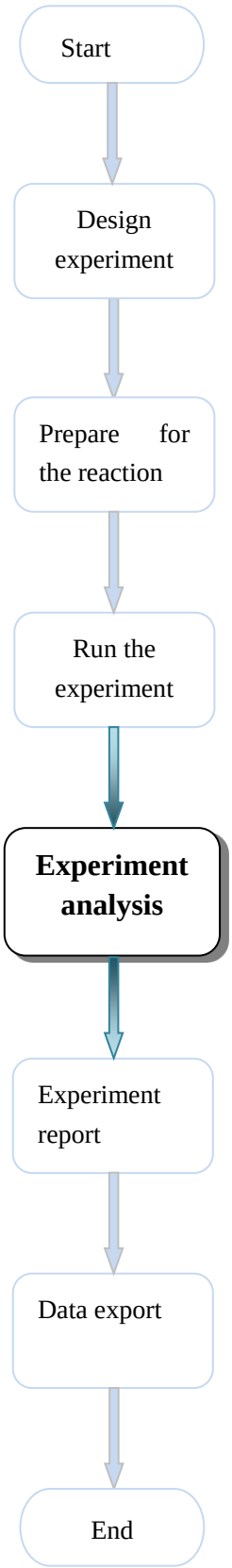
Caution:	The run switch is for ease of operation and is merely used for temporary or short term closing down of the control system. When the system is under the standby state, the instrument internal AC circuit remains live.

3.6 **Prompts which may occur during running**

- Hot-lid temperature sensor alarm prompt
- Sink temperature sensor alarm prompt

- Environmental temperature sensor alarm prompt
- Module temperature sensor alarm prompt
- Module sensor short-circuit or short-circuit alarm prompt

Caution: In case the temperature alarm displays during the running of a programme, the PCR detection system will terminate the current programme. The instrument should be switched off and then re-started.



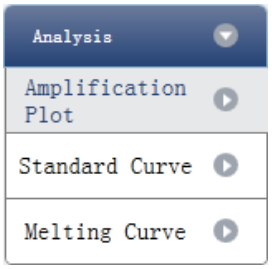
4. Experiment Analysis

This section describes how to view the experiment analysis results after running an experiment and adjusting parameters for re-analysis. This section covers the analysis of amplification curves and standard curves, adjusting parameters for re-analysis and importing parameters.

4.1 **Check Results**

4.1.1 Check the Amplification Plot

1) Click **Analysis ▶ Amplification Plot**



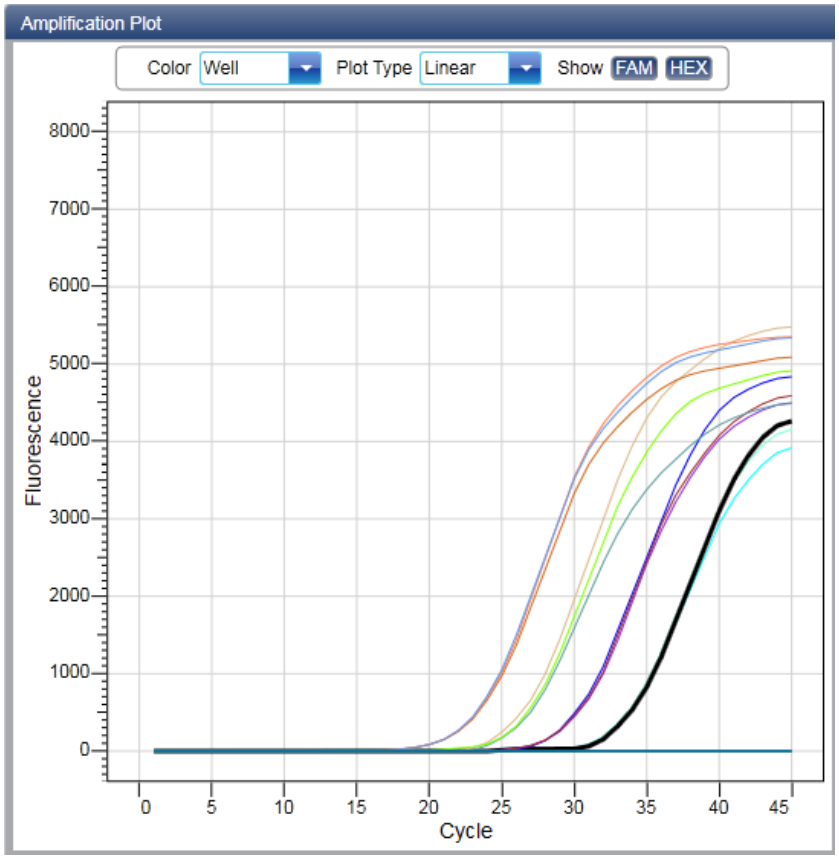
2) Check the amplification curve

a. Set up colour

b. Set up plot type

c. Set up show dye

※When the background colour of a dye name is blue, it will be displayed; while white indicates it will not be displayed.



3) Check the reaction plate

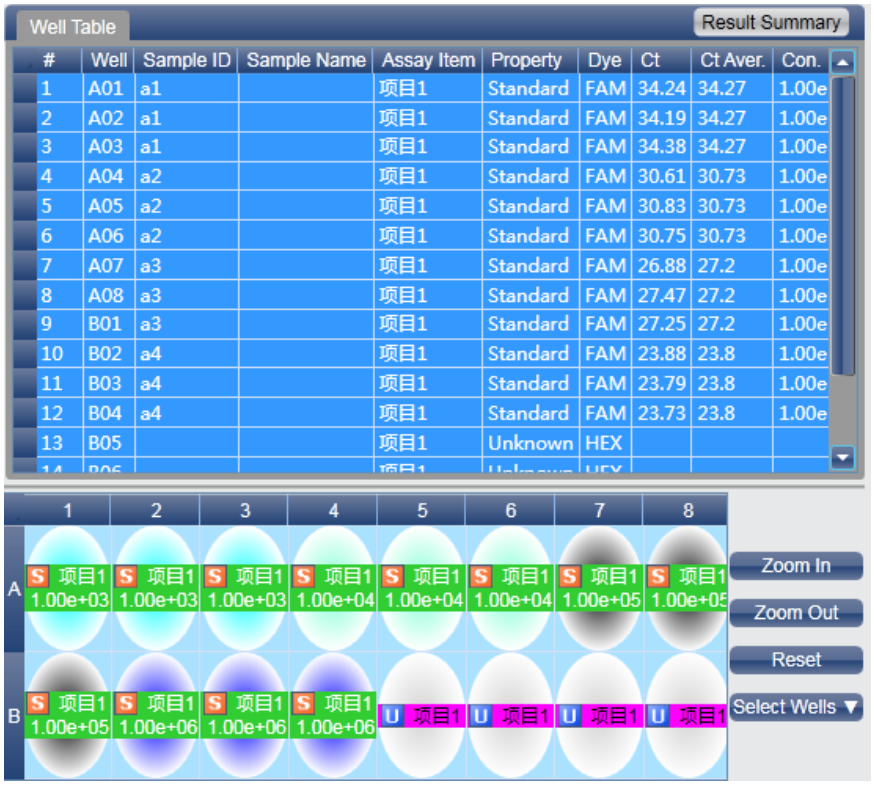
a. Select reaction plate well site and check corresponding well site curve

※The default is all wells are selected

b. Zoom-In, Zoom-Out and reset the reaction plate

c. Check well table

d. Check results summary



4) Set up assay

a. Set up assay

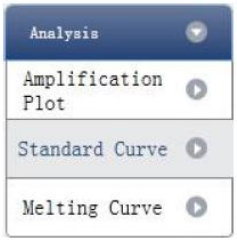
b. Set up threshold

c. Set up automatic baseline

※When the threshold value is not automatic, the user cannot set up the automatic baseline

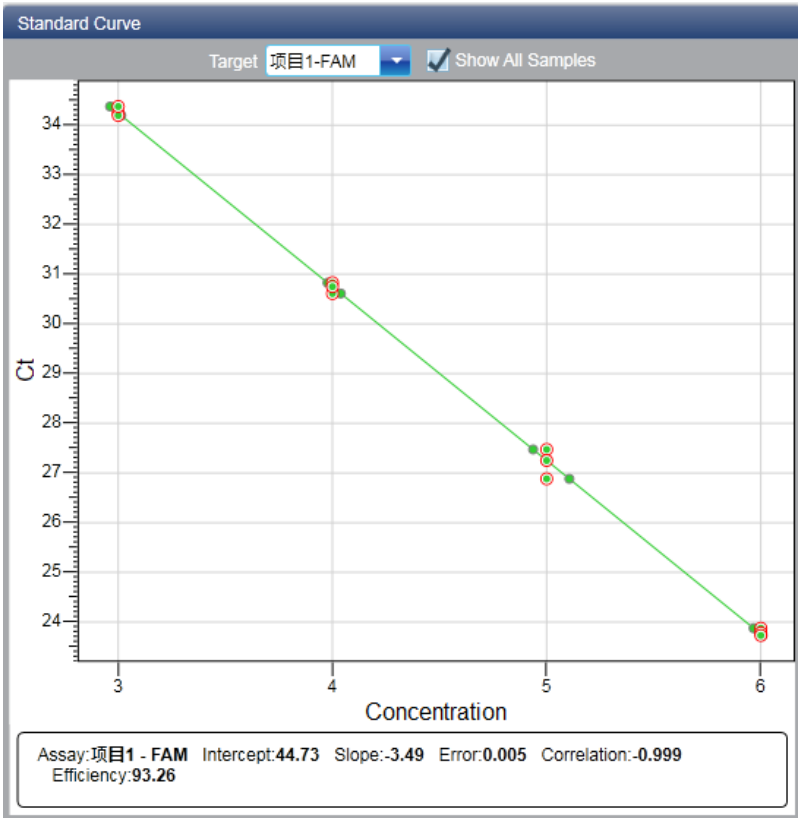
4.1.2 Check Standard Curve

1) Click **Analysis ▶ Standard Curve**



2) Check standard curve

a. Set up assay



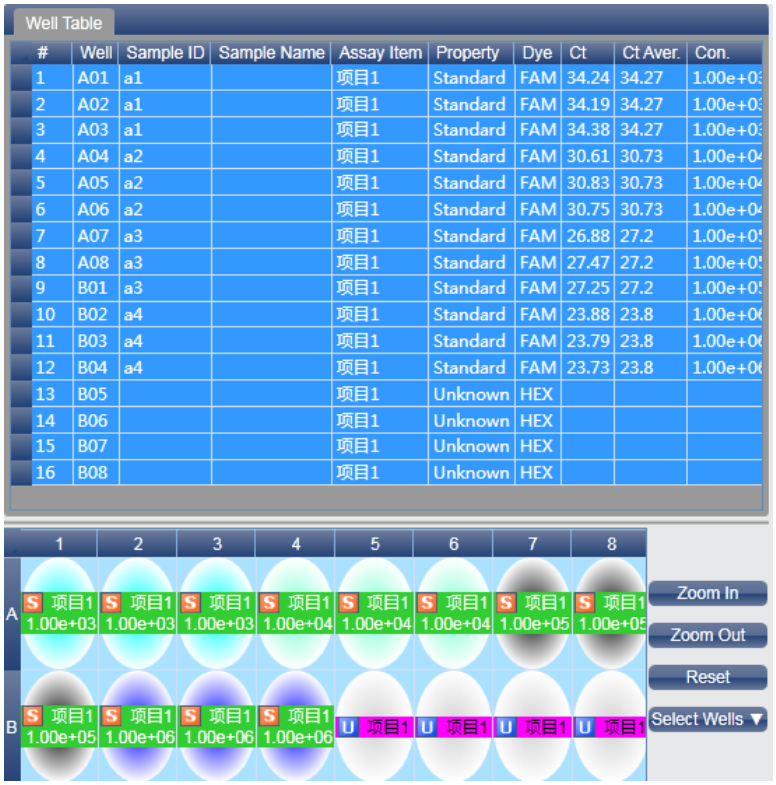
3) Check the reaction plate

a. Select reaction plate well site and check corresponding well site curve

※The default is all wells are selected

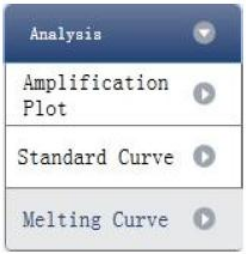
b. Zoom-In, Zoom-Out and reset the reaction plate

c. Check table information



4.1.3 Check Melting Curve

1) Click **Analysis ▶ Melting Curve**

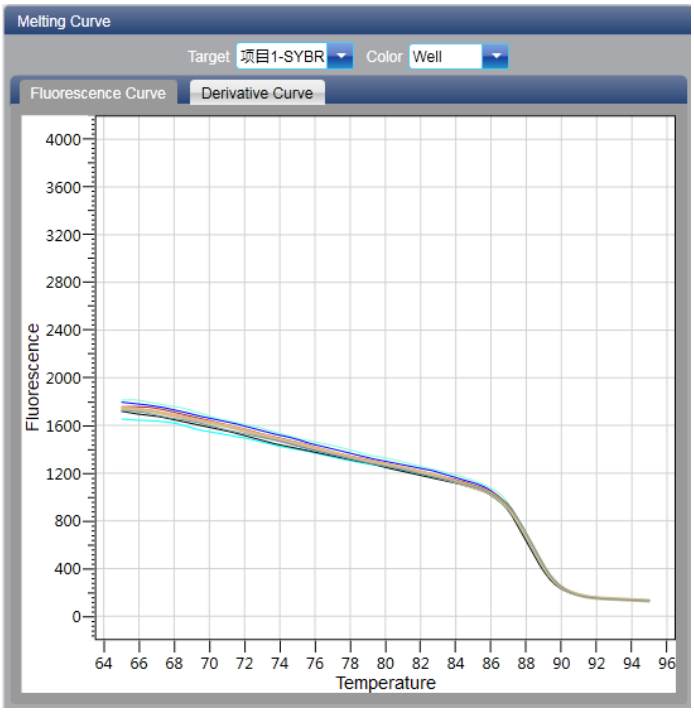


2) Check the melting curve

a. Check the fluorescence curve

b. Check the derivative curve

c. Set up colour



3) Check the reaction plate

a. Select reaction plate well site and check corresponding well site curve

※The default is all wells are selected

b. Zoom-In, Zoom-Out and reset the reaction plate

c. Check table information

4)Set up assay

a. Set up assay

b. Set up colour

4.2 **Adjusting Parameters and Re-analysis**

Click **Analysis Settings** ▶ the Analysis Settings dialog box will pop up

a. Adjust the start cycle and end cycle of the baseline

b. Adjust Ct analysis algorithm

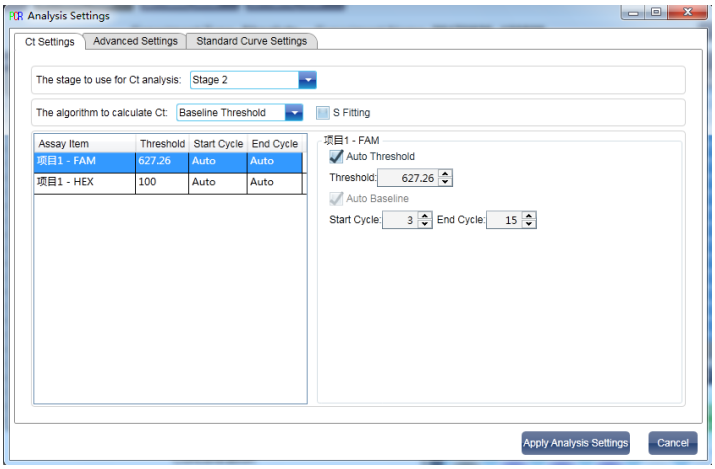
c. Set up the use of S fitting

d. Set up the stage to use for Ct analysis

e. Set up the automatic threshold value

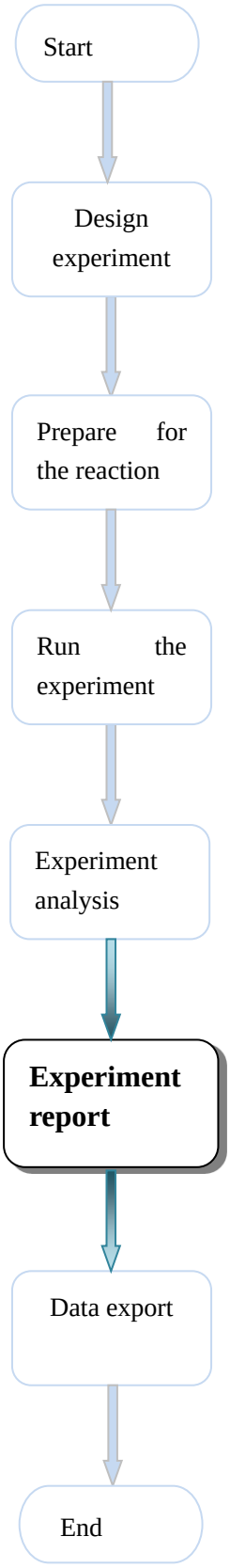
f. Advanced setting

g. Standard curve setting


Analysis Settings
Ct Settings
Advanced Settings
Standard Curve Settings
The stage to use for Ct analysis:
Stage 2
The algorithm to calculate Ct:
Baseline Threshold
S Fitting
Assay Item
Threshold
Start Cycle
End Cycle
项目1 - FAM
627.26
Auto
Auto
项目1 - HEX
100
Auto
Auto
项目1 - FAM
Auto Threshold
Threshold:
627.26
Auto Baseline
Start Cycle:
3
End Cycle:
15
Apply Analysis Settings
Cancel

5. Experiment Report

This section describes how to print an experiment report and covers designing of a report template and print settings.

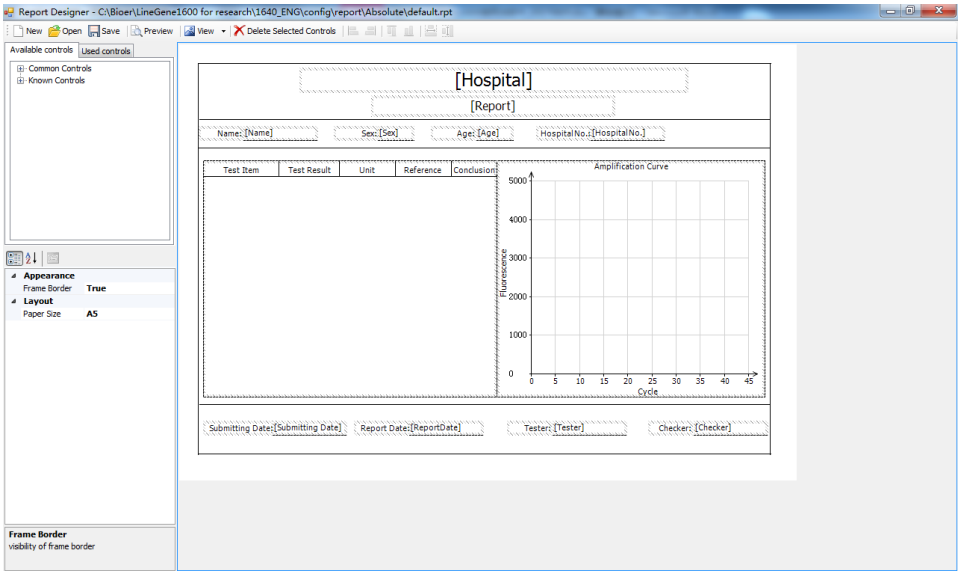


5.1 **Designing a Report Template**

Click **Report ▶ Report Template Editor ▶Select expeiremnt type ▶**the Report Designer window will pop up

The report consists of controls and the user can add, modify and delete controls.

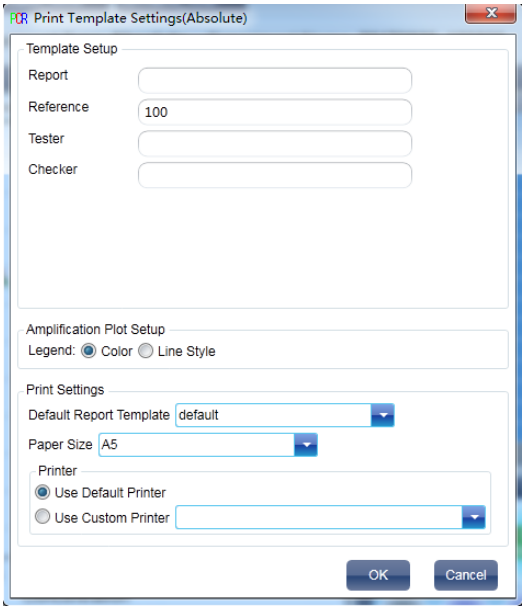
Available controls include Static Text, Dynamic Text, Line, Static Image, Amplification curve and Quantification Analysis Results.



5.2 **Print Setting**

Click **Report ▶ Print Template Setting ▶Select Expreiment Type ▶**the Print Template Setting window will open

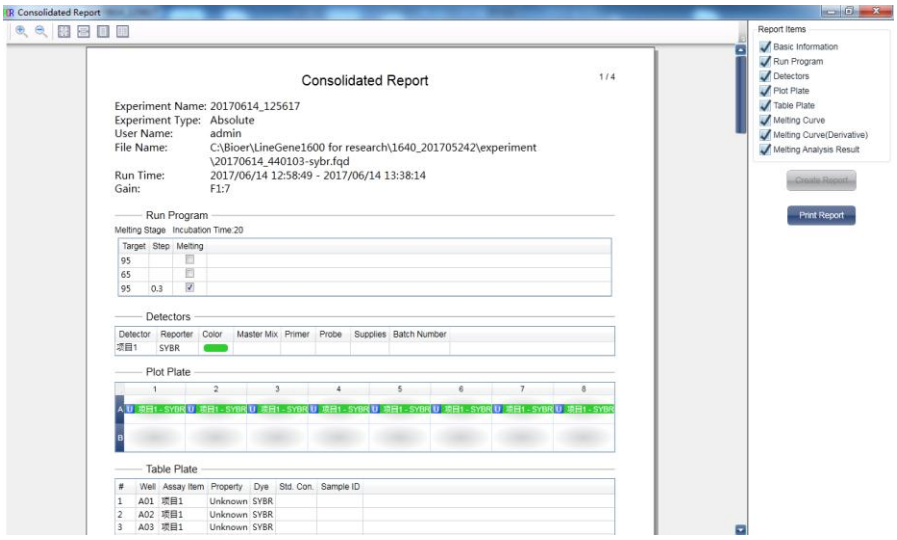
The user can set up the laboratory name, report name, reference value, tester, checker, amplification plot, default report template and paper size.



5.3 **Comprehensive Report**

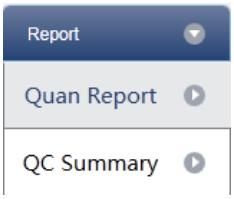
Click **Report ▶ Consolidated Reports ▶** the Consolidated Report window will pop up

The Consolidated Report includes the basic information, sample information, amplification curve, standard curve, plate information, etc.



5.4 **Report Printing**

1) Click **Report ▶ Quantitative Report**



2) Report print setting

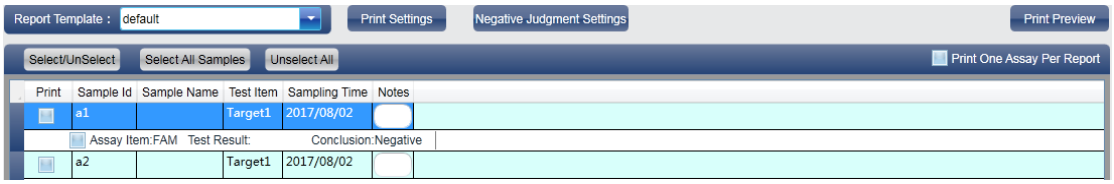
a. Set up report template

b. Print setting (please refer to Section 5.2)

c. Select items to print

d. Print preview

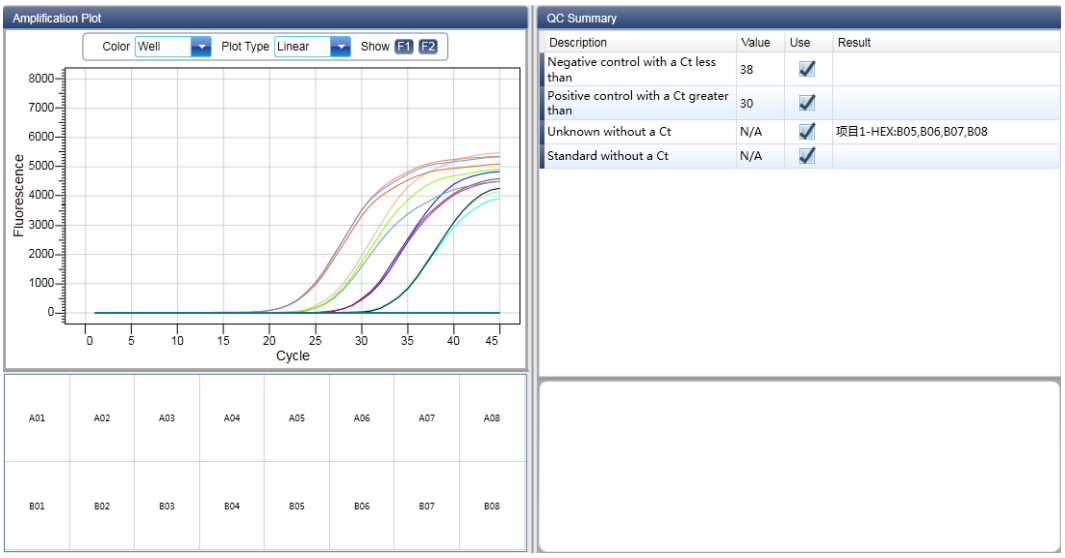
e. Print the report

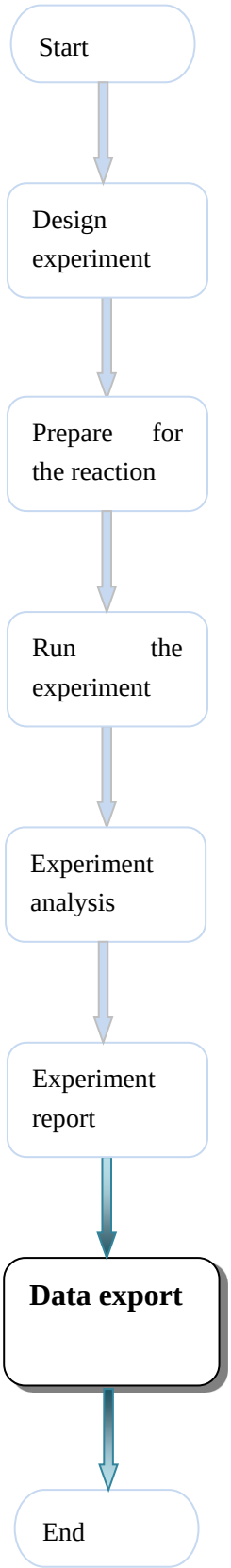


5.5 **QC Summary**

1) Click **Report ▶ QC Summary**

2) Check the QC summary





6. Data Export

This section describes how to export data and covers exporting to a database, experiment filing and exporting the experiment data to EXCEL

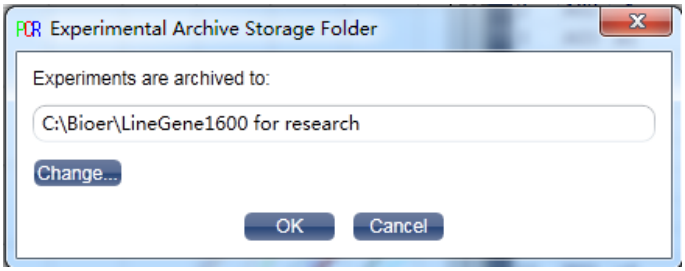
6.1 Export to Database

Click **Data Summary** ▶ **Export to Database** ▶ the Save File dialog box will pop up ▶ save the exported database file

6.2 Experiment Filing

1) Set filing experiment storage folder

Click **Data Summary** ▶ **Archived Experiment Directory** ▶ the Experimental archive storage directory window will pop up ▶ set up the storage path of file.



2) Experiment filing

Click **Data Summary** ▶ **Archived Experiment** ▶ export the filed experiment file

※The suffix of the filed experiment file is .fqh

6.3 Export Experiment Data to EXCEL

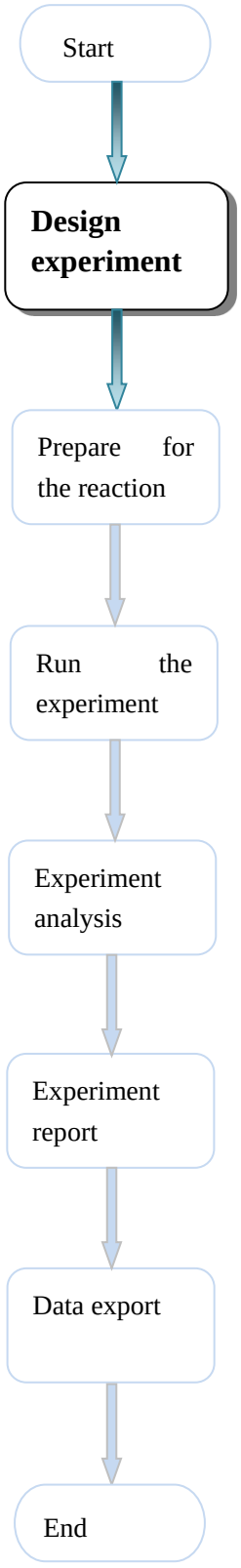
Click **Data Summary**▶**Export Experiment** ▶ **Export Experiment to Excle** ▶the exported experiment data will generate EXCEL file

6.4 Export Experiment Data to TEXT

Click **Data Summary**▶**Export Experiment** ▶ **Export Experiment to Text**▶ the exported experiment data will generate TEXT file

Chapter 5 Relative Quantitative

1. Design Experiment



This section describes how to design a relative quantitative experiment and covers creating new relative quantitative experiment, inspection item setting, sample information setting, reaction plate setting and programme setting

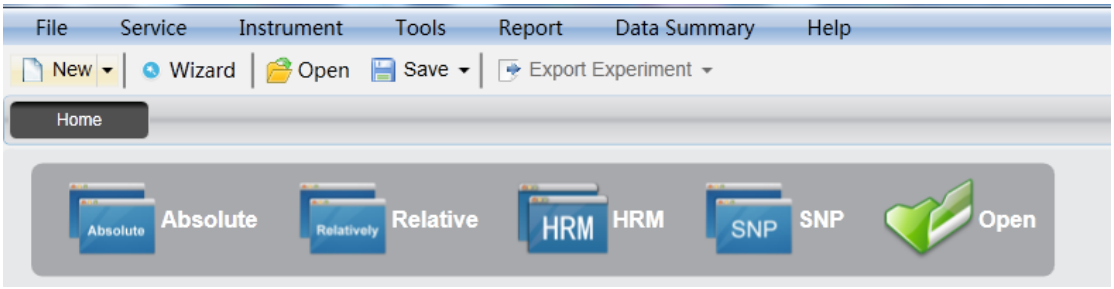
1.1 Create New Relative Quantitative Experiment

Click **Relative** on **Home** interface and create Relative Quantitative Experiment window.

※Relative quantitative experiment can be also created by:

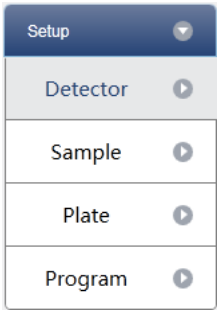
a. Clicking **New** ▶ **Relative** on the toolbar

b. Clicking **File** ▶ **New** ▶ **Relative** on the menu bar



1.2 Detector Setting

1) Click **Setup** ▶ **Detector**



2) Input Experiment Properties

Input the Experiment name, User name and Comment in the basic information column

3) Inspection Item Setting

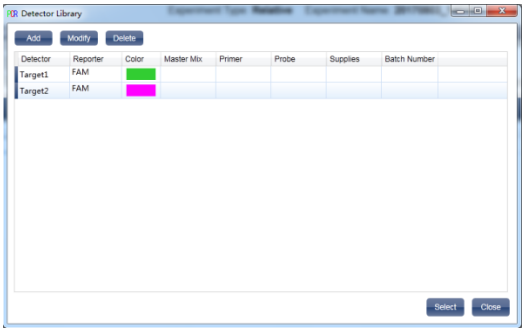
a. Set up the Detector, Assay, Dye and Colour.

b. Add detector

c. Delete detector

d. Add detector from library

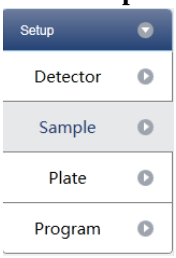
※The user can also conduct Add, Modify and Delete operations in the item library.



4) Set up reference dye

1.3 **Sample Information Setting**

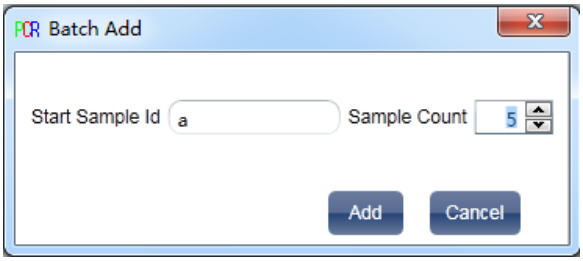
1) Click **Setup** ▶ **Sample**



2) Add sample information

a. Itemized addition: input ID in **Sample ID** ▶ press **Enter** ▶ add information for one sample.

b. Batch addition: click **Batch Add** ▶ the Batch Add window will pop up



3) Delete sample information

a. Itemized deletion: select one sample ▶ click **Delete** ▶ delete the selected sample information

b. Delete all: click **Clear All** ▶ delete all sample information

4) Import/Export sample information

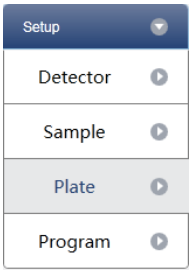
a. Click **Import Sample Info** ▶ the File Import window will pop up ▶ import sample information file in CSV format

b. Click **Export Sample Info** ▶ the Save As window will pop up ▶ the sample information will be exported in CSV file format

5. Set up sample information

1.4 **Reaction Plate Setting**

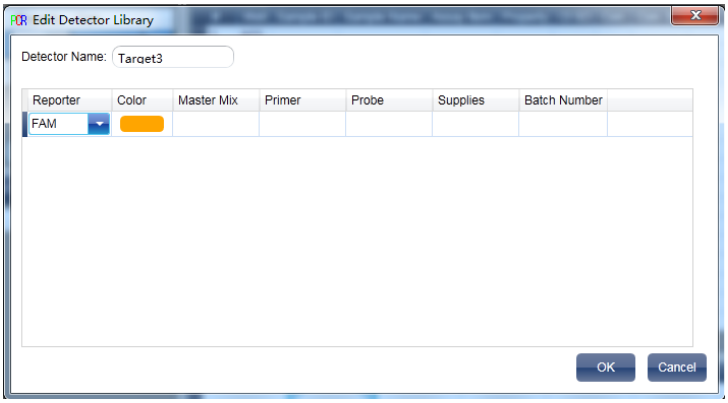
1) Click **Setup ▶ Plate**



2) Set up the inspection criteria of the reaction plate

a. Select reaction plate well site: click Reaction Plate well Site

The user can also right click the reaction plate well site to Copy, Paste and Add New Detector. Adding a new detector will open the **Edit Detector Library** window.



b. Select inspection item and modify the property, concentration and concentration unit.

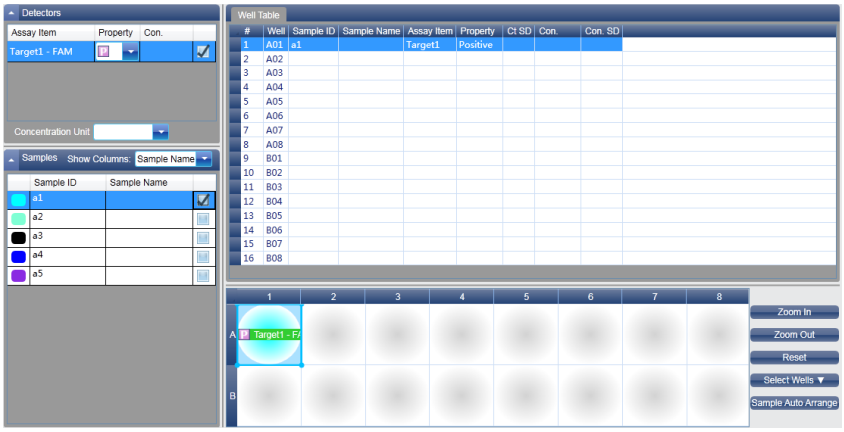
Property	**Name**	**Concentration**	**Concentration unit**
U	Unknown	NO	Copies/ml IU/ml Fg/ml Pg/ml
S	Standard	YES	
N	Negative	NO	

c. Select a sample and the list displayed will change

d. Zoom-In, Zoom-Out and reset the reaction plate.

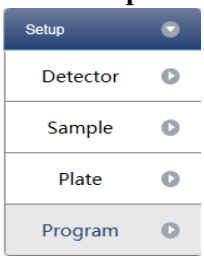
e. Sample Auto Arrange

f. Check Well Table



1.5 **Programme Setting**

1) Click **Setup** ▶ **Programme**



2)Run Programme Setup

a. Create new stage: the user can create a new **Hold Stage**, **Cycling Stage** or **Melting Stage**

※The user can also click **Add Stage** directly and the default will be creating a new **Cycling Stage**.

b. Create new step: the user can create a new step **Before** or **After** the currently selected step

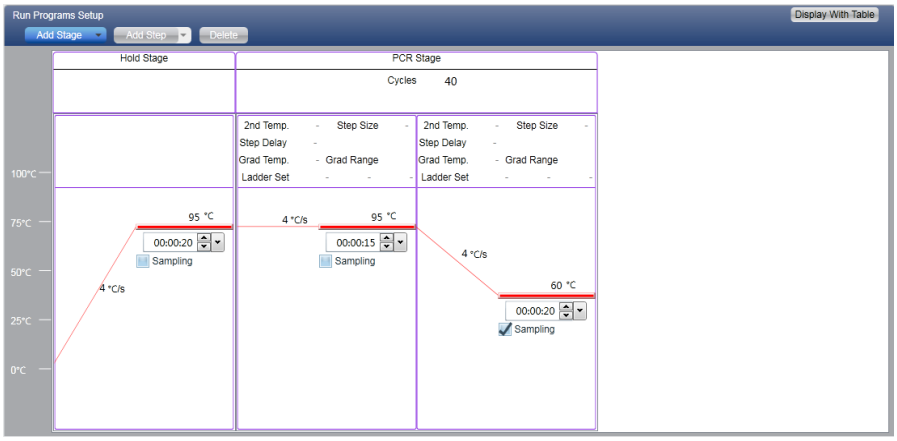
※The user can also click **Add Step** and the default will be adding a new Step at the end of the currently selected stage or after the currently selected step.

c. Delete: the user can delete the currently selected step or stage

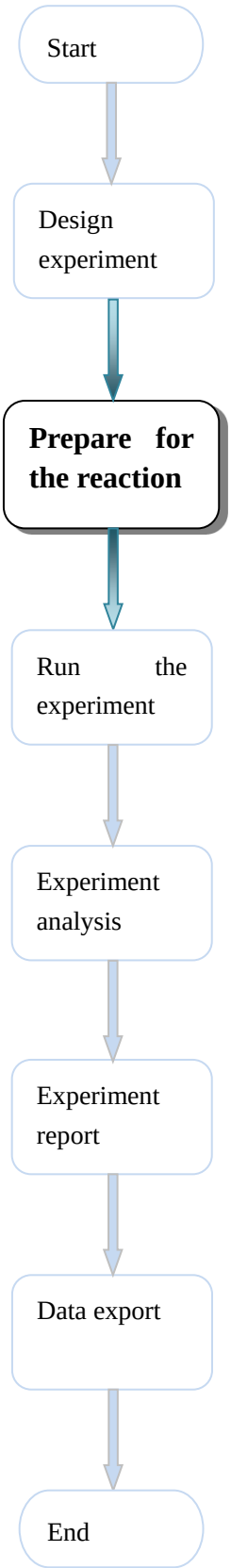
d. Display form: click **Display With Table** ▶ new window will pop up ▶ the details of the current experiment will be displayed in a table.

e. Set up the experimental data of the hold stage, cycling stage and melting stage melting section

f. Set up the hot-lid temperature and liquid volume



2. Prepare for Reaction

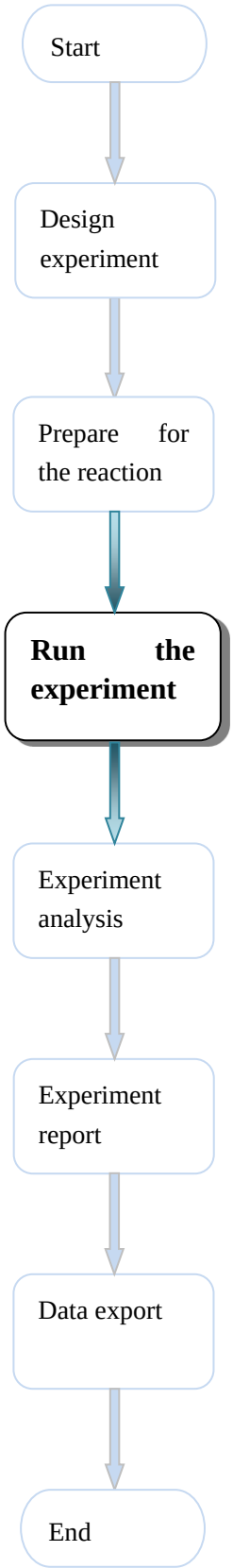


The user should make full preparations prior to the experiment

• Ensure appropriate materials are used.

• Ensure the arrangement of the PCR reaction plate is consistent with the setting layout of the reaction plate in Section 1.4.

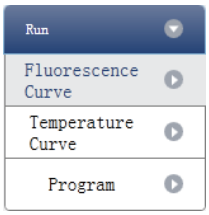
3. Run the Experiment



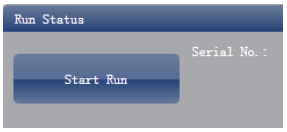
This section describes how to run/operate the experiment after loading the reaction plate and includes how to operate the fluorescence curve, the temperature curve and programming

3.1 **Run Fluorescence Curve**

1) Click **Run ▶ Fluorescence Curve**



2) Click **Start Run**

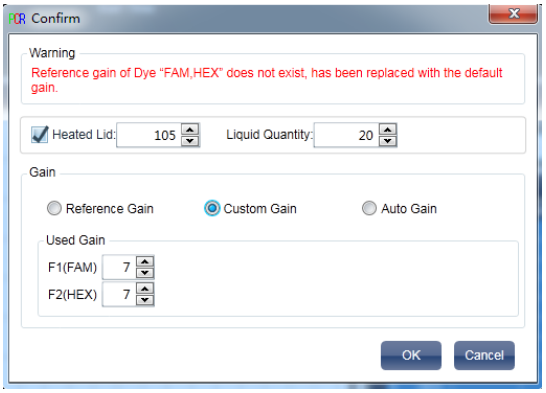


3) Operating confirmation

a. Modify hot-lid temperature and liquid quantity

b. Gain parameter setting

c. Target fluorescence value setting



4)After it starts running, the user can:

a. Skip the current stage

b. Add a cycle

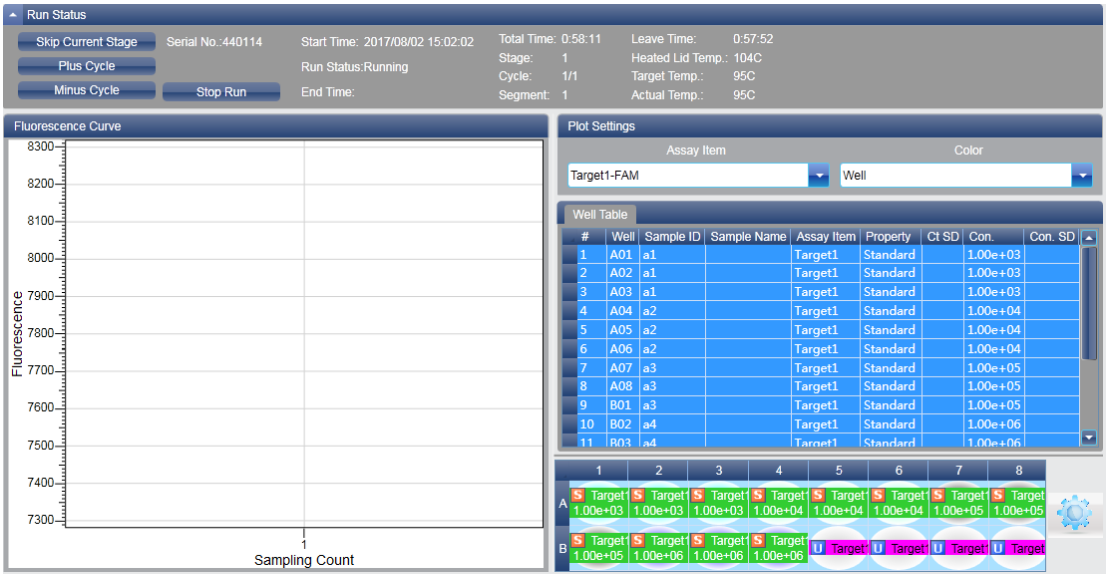
c. Delete a cycle

d. Stop run

5) Plot display setting

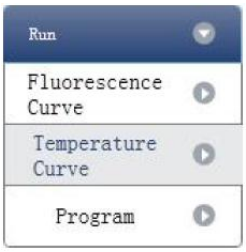
a. Assay item

b. Plot colour

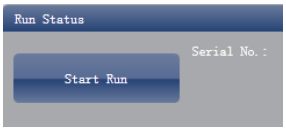


3.2 **Run Temperature Curve**

1) Click **Run** ▶ **Temperature Curve**



2) Click **Run** ▶ **Start**

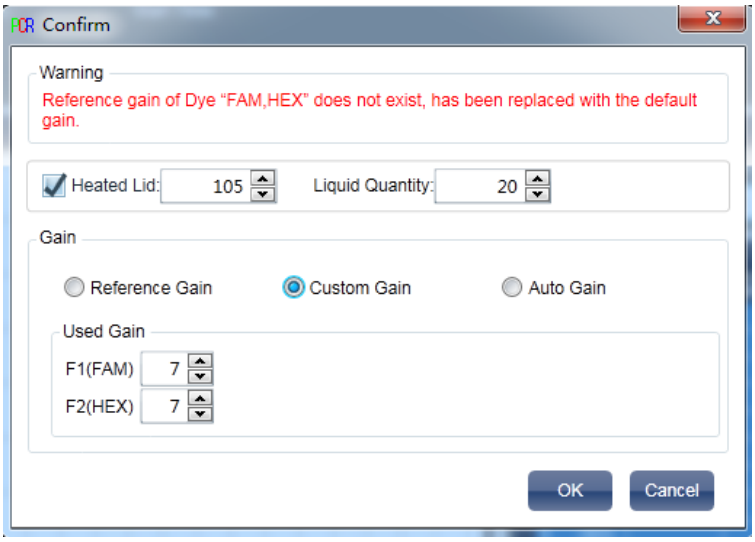


3)Operating confirmation

a. Modify hot-lid temperature and liquid quantity

b. Gain parameter setting

c. Target fluorescence value setting



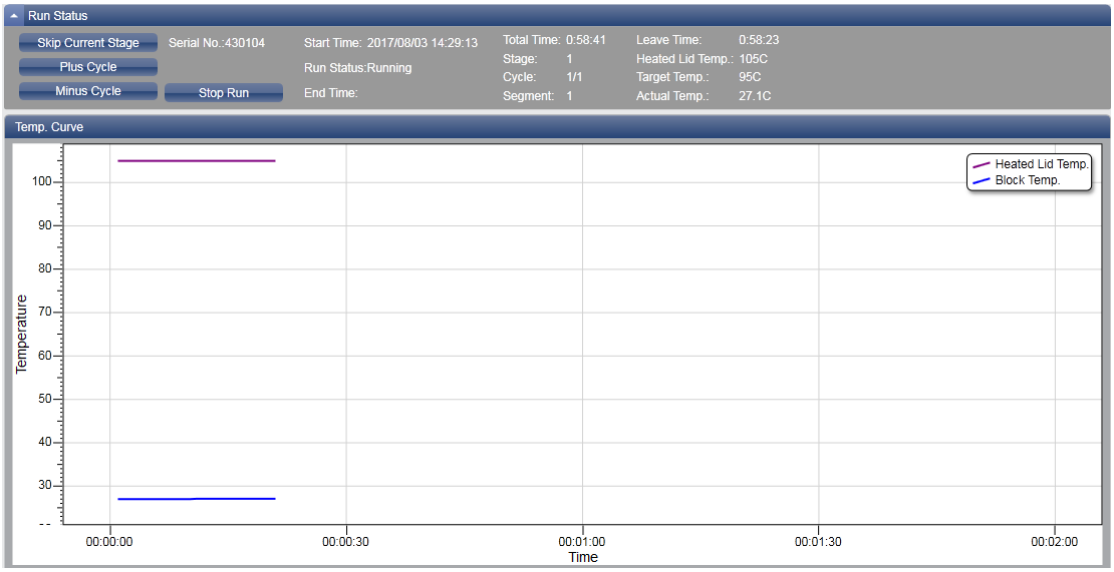
4) After it starts running, the user can:

a. Skip the current stage

b. Add a cycle

c. Delete a cycle

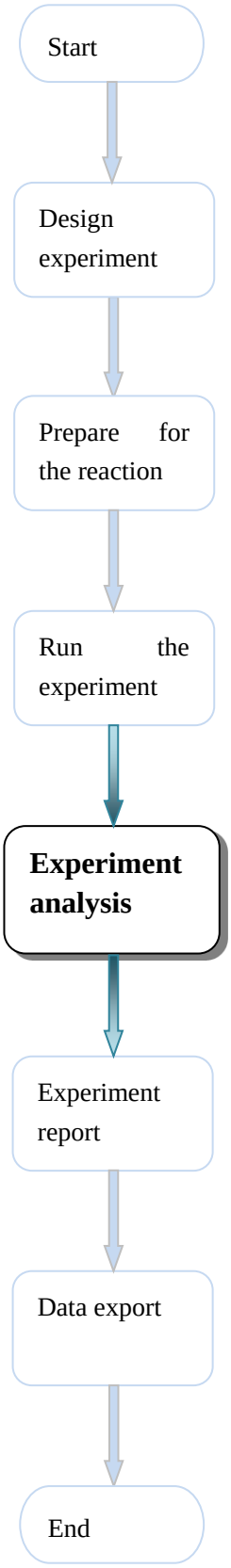
d. Stop run



3.3 **Programme Setting**

The user can only check the programme setting but cannot make modifications.

4. Experiment Analysis

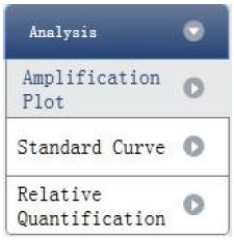


This section describes how to view the experiment analysis results after running an experiment and adjusting parameters for re-analysis. This section covers the analysis of amplification curves and standard curves, the analysis of relative quantification, adjusting parameters for re-analysis and importing parameters.

4.1 **Check Results**

4.1.1 Check the Amplification Plot

1) Click **Analysis ▶ Amplification Plot**



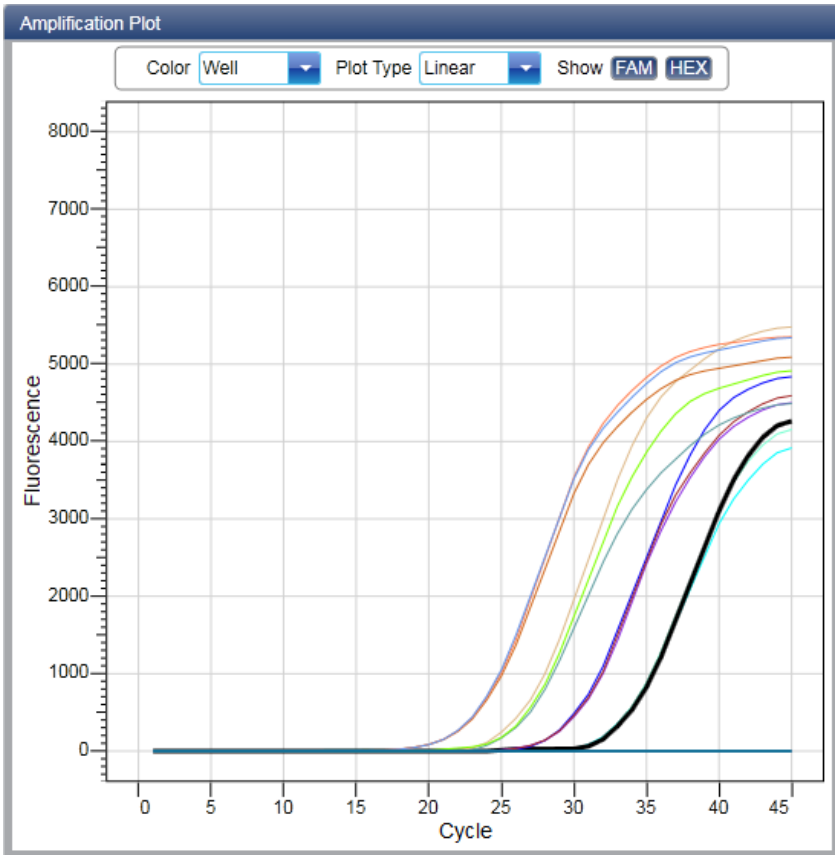
2)Check the amplification curve

a. Set up colour

b. Set up plot type

c. Set up show dye

※When the background colour of a dye name is blue, it will be displayed; while white indicates it will not be displayed.



3) Check the reaction plate

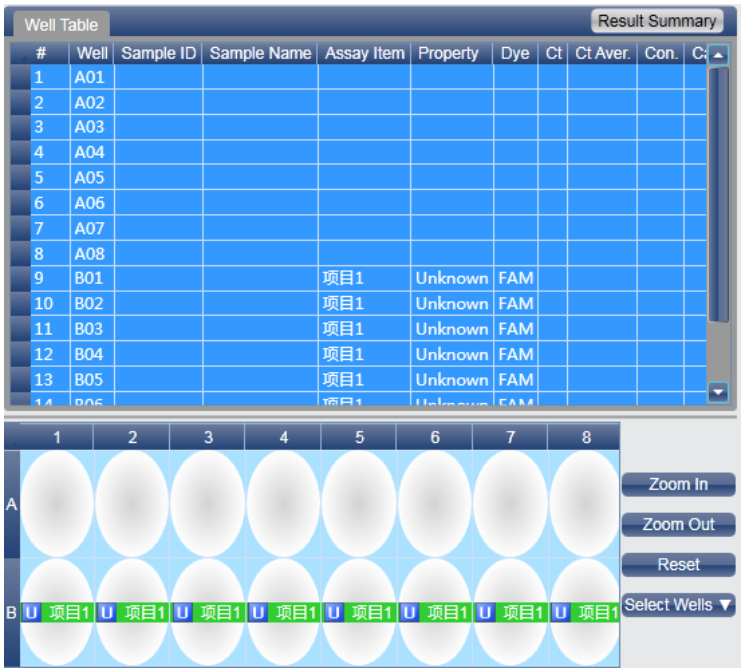
a. Select reaction plate well site and check corresponding well site curve

※The default is all wells are selected

b. Zoom-In, Zoom-Out and reset the reaction plate

c. Check well table

d. Check results summary



4) Set up assay

a. Set up assay

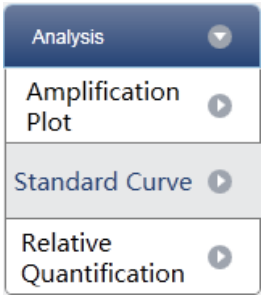
b. Set up threshold

c. Set up automatic baseline

※When the threshold value is not automatic, the user cannot set up the automatic Baseline

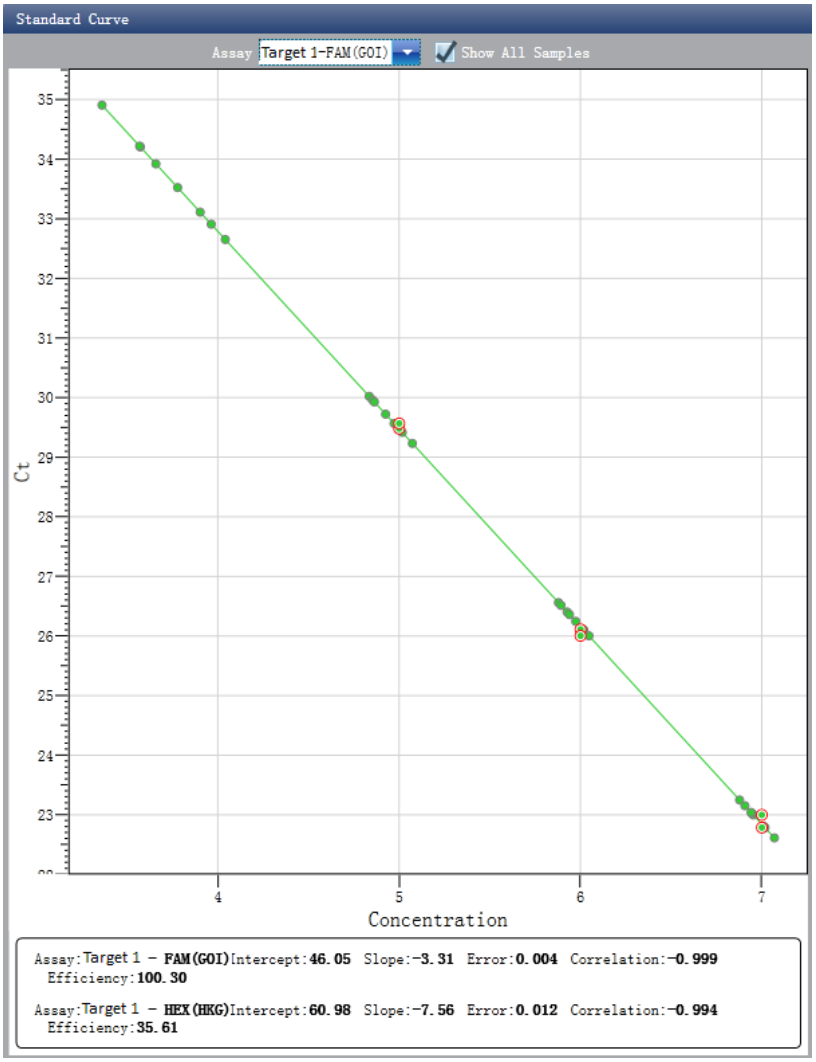
4.1.2 Check Standard Curve

1) Click **Analysis ▶ Standard Curve**



2) Check standard curve

a. Set up assay



3)Check the reaction plate

a. Select reaction plate well site and check corresponding well site curve

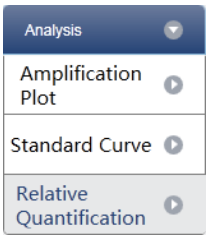
※The default is all wells are selected

b. Zoom-In, Zoom-Out and reset the reaction plate

c. Check table information.

4.2 **Check Relative Quantification**

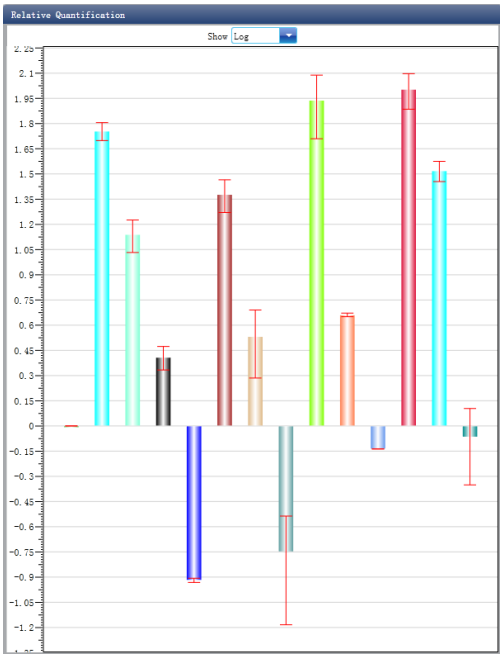
1) Click **Analysis** ▶ **Relative Quantification**



2)Check relative quantitative

a. Set up the show type

b. Check the analysis results



Result

Sample Id	Assay Item	Property	GOI Aver. Con.	GOI Con. SD	HKG Aver. Con.	HKG Con. SD	Max	Min	Aver.
	target1	Comparison	7.99e+03	0.00e+00	1.37e+04	0.00e+00	1	1	1
01	target1	Unknown	1.10e+07	1.05e+06	1.93e+05	1.48e+04	63.92	49.95	56.94
02	target1	Unknown	8.48e+05	1.31e+05	6.14e+04	9.61e+03	16.84	10.78	13.81
03	target1	Unknown	9.40e+04	1.40e+04	3.67e+04	2.06e+03	2.97	2.15	2.56
04	target1	Unknown	3.72e+03	2.66e+01	3.08e+04	8.82e+02	0.12	0.12	0.12
06	target1	Unknown	9.44e+05	1.43e+05	3.95e+04	6.33e+03	29.18	18.63	23.9
07	target1	Unknown	9.33e+04	3.53e+04	2.73e+04	5.86e+03	4.9	1.93	3.41
08	target1	Unknown	4.14e+03	2.62e+03	2.33e+04	8.42e+02	0.29	0.07	0.18
09	target1	Unknown	8.44e+06	5.34e+05	9.71e+04	3.93e+04	122.5	51.28	86.89
11	target1	Unknown	7.21e+04	1.20e+03	1.57e+04	2.97e+02	4.7	4.47	4.58
12	target1	Unknown	1.10e+04	0.00e+00	1.51e+04	0.00e+00	0.73	0.73	0.73
13	target1	Unknown	8.12e+06	8.33e+05	8.05e+04	1.74e+04	125.02	76.77	100.89
14	target1	Unknown	8.25e+05	6.25e+04	2.50e+04	2.87e+03	37.59	28.5	33.05
16	target1	Unknown	6.87e+03	3.28e+03	8.01e+03	4.28e+02	1.27	0.45	0.86

4.3 **Adjust Parameter Reanalysis**

Click **Analysis Settings** ▶ the Analysis Settings dialog box will pop up

a. Adjust the start cycle and end cycle of the baseline

b. Adjust Ct analysis algorithm

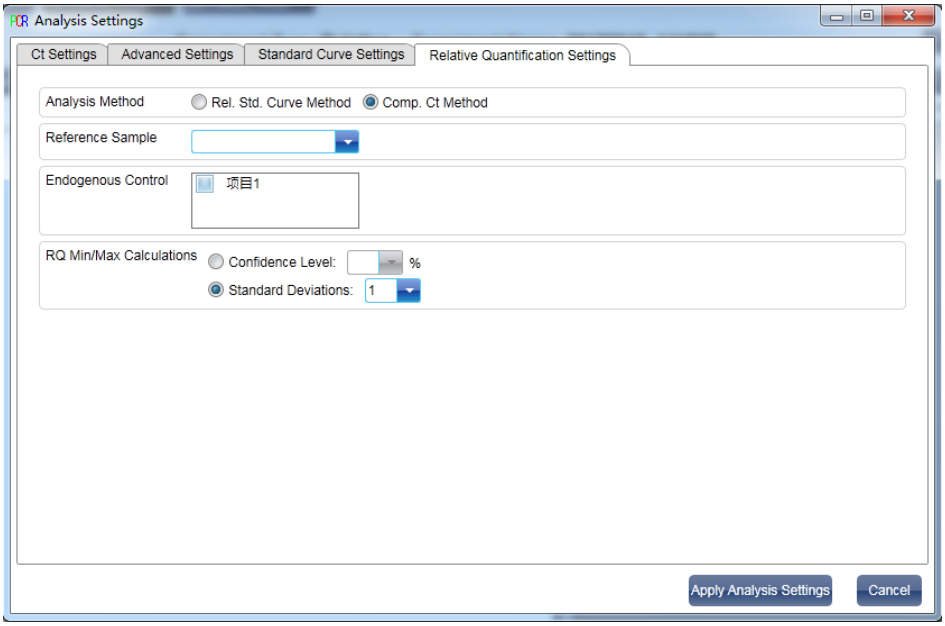
c. Set up the use of S fitting

d. Set up the stage to use for Ct analysis

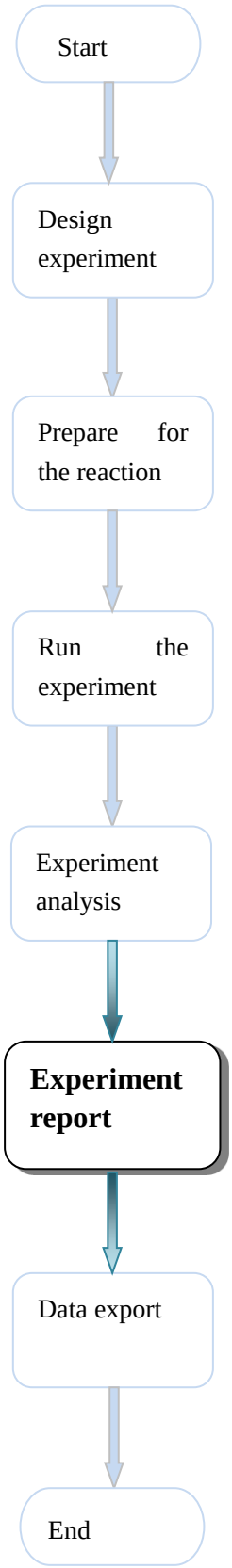
e. Set up the automatic threshold value

f. Advanced setting

g. Relative quantification setting


Analysis Settings
Ct Settings
Advanced Settings
Standard Curve Settings
Relative Quantification Settings
Analysis Method
Rel. Std. Curve Method
Comp. Ct Method
Reference Sample
Endogenous Control
项目1
RQ Min/Max Calculations
Confidence Level:
%
Standard Deviations:
1
Apply Analysis Settings
Cancel

5. Experiment Report

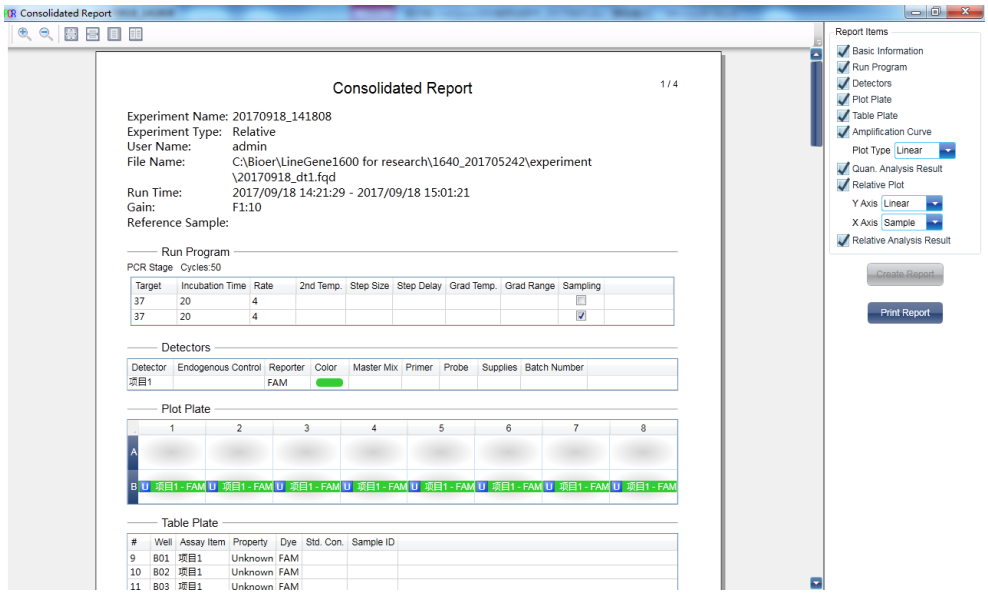


This section describes how to print an experiment report and covers designing of a report template and print settings.

5.1 **Comprehensive Report**

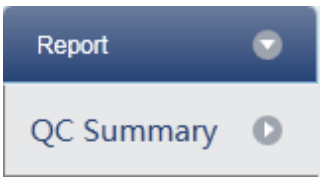
Click **Report** ▶ **Consolidated Reports** ▶ the Consolidated Report window will pop up

The Consolidated Report includes the basic information, sample information, amplification curve, standard curve, plate information, etc.

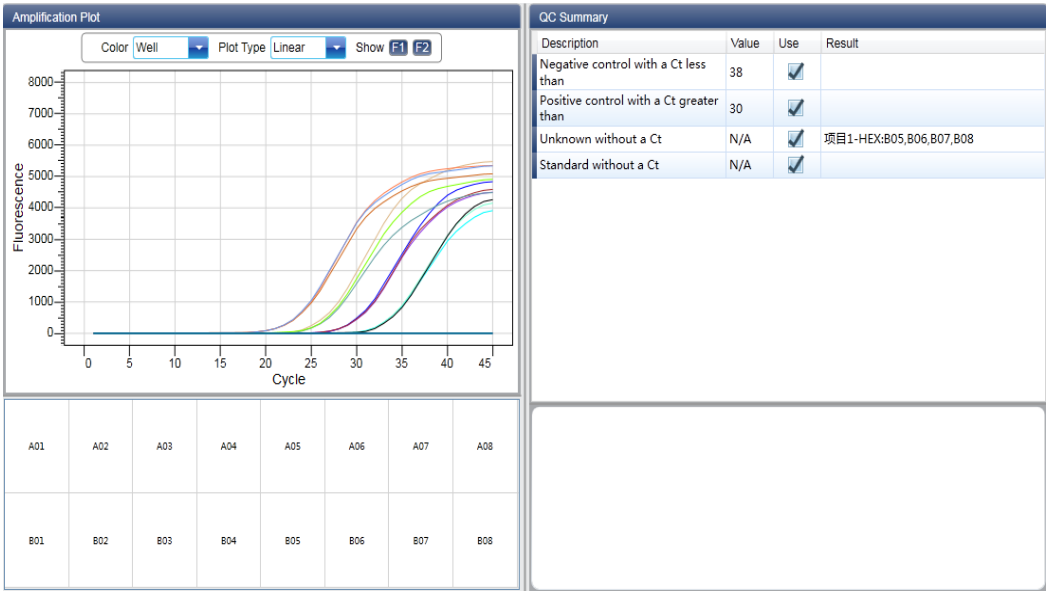


5.2 **QC Summary**

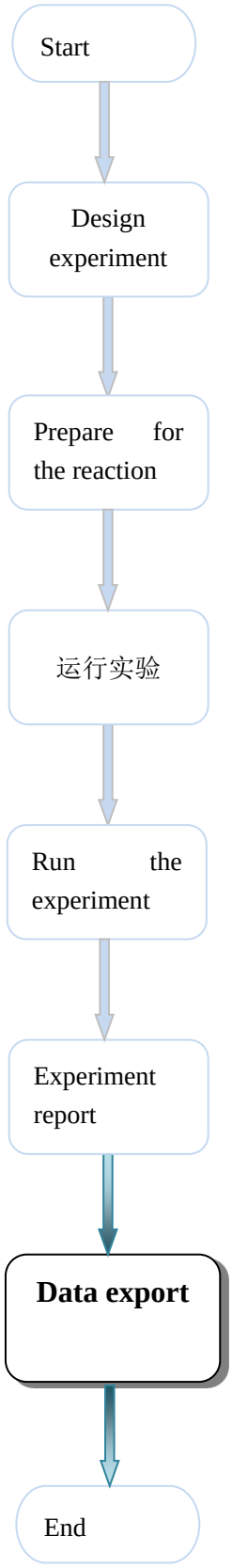
1) Click **Report** ▶ **QC Summary**



2) Check the QC summary


Amplification Plot
Color Well
Plot Type Linear
Show F1 F2
Fluorescence
Cycle
QC Summary
Description
Value
Use
Result
Negative control with a Ct less than
38
Positive control with a Ct greater than
30
Unknown without a Ct
N/A
项目1-HEX:B05,B06,B07,B08
Standard without a Ct
N/A
A01 A02 A03 A04 A05 A06 A07 A08
B01 B02 B03 B04 B05 B06 B07 B08

6. Data Export



This section describes how to export data and covers exporting to a database, experiment filing and exporting the experiment data to EXCEL.

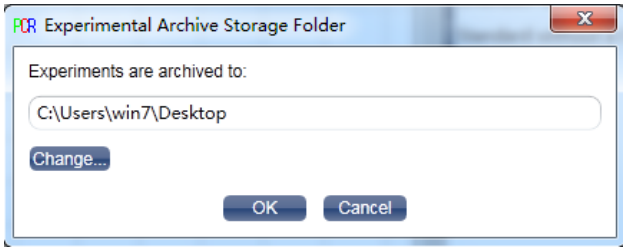
6.1 **Export to Database**

Click **Data Summary** ▶ **Export to Database** ▶ the Save File dialog box will pop up ▶ save the exported database file

6.2 **Experiment Filing**

1) Set filing experiment storage folder

Click **Data Summary** ▶ **Archived Experiment Directory** ▶ the Experimental archive storage directory window will pop up ▶ set up the storage path of file



2) Experiment filing

Click **Data Summary** ▶ **Archived Experiment** ▶ export the filed experiment fil

※The suffix of filed experiment file is .fqh

6.3 Export Experiment Data to EXCEL

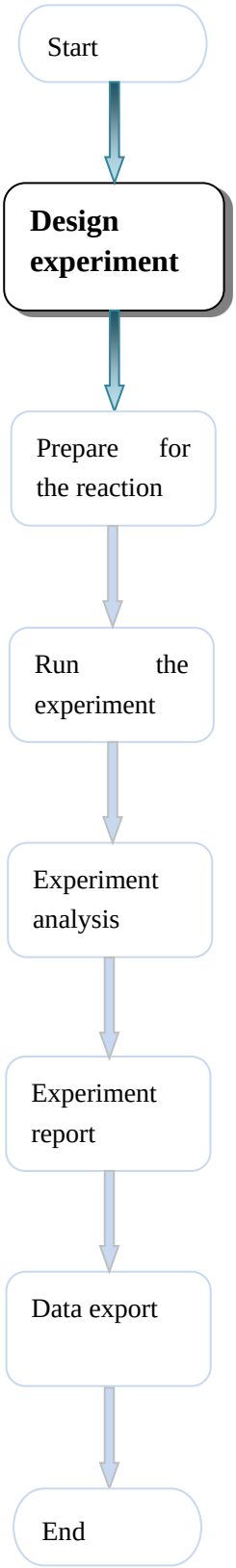
Click **Data Summary**▶**Export Experiment** ▶ **Export Experiment to Excle** ▶the exported experiment data will generate EXCEL file.

6.4 Export Experiment Data to TEXT

Click **Data Summary**▶**Export Experiment** ▶ **Export Experiment to Text**▶ the exported experiment data will generate TEXT file.

Chapter 6 SNP

1. Design Experiment



This section describes how to design an SNP experiment and covers creating a new SNP experiment, inspection item setting, sample information setting, reaction plate setting and programme setting.

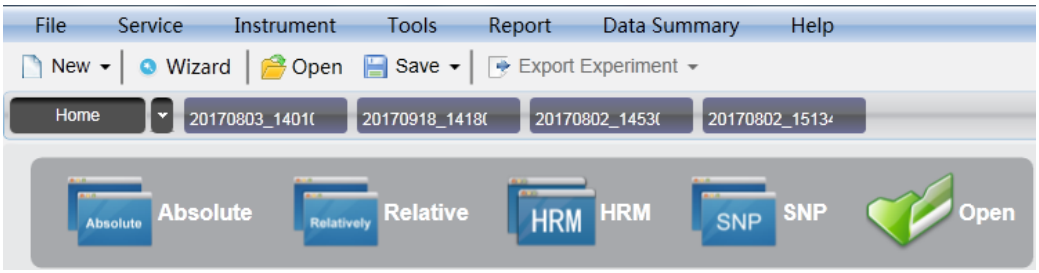
1.1 Create SNP Experiment

Click **SNP** on **Home** interface and create SNP Experiment window.

※An SNP experiment can be also created by:

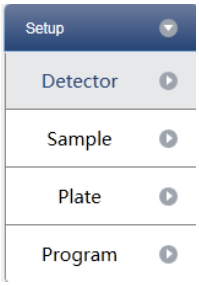
a. Clicking **New** ▶ **SNP** on the toolbar

b. Clicking **File** ▶ **New** ▶ **SNP** on the menu bar



1.2 Detector Setting

1) Click **Setup** ▶ **Detector**



2) Input basic information

Input the experiment name, user name and any comments in the experiment properties column.

3) Inspection Item Setting

Set up the Detector, Allele, Dye and Colour.

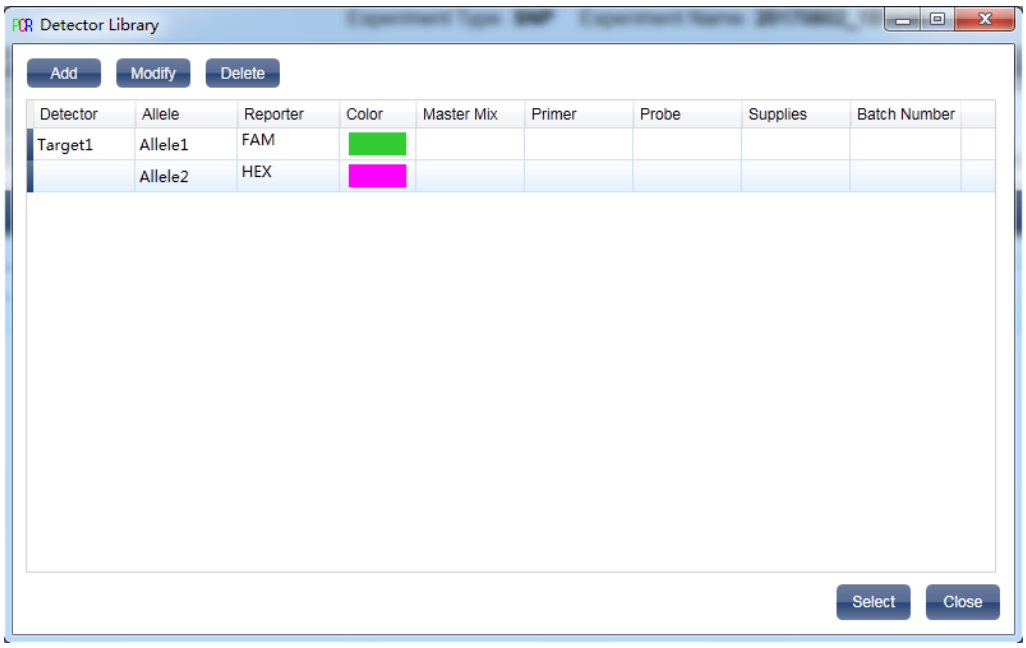
※If necessary, the user can also:

a. Add Detector

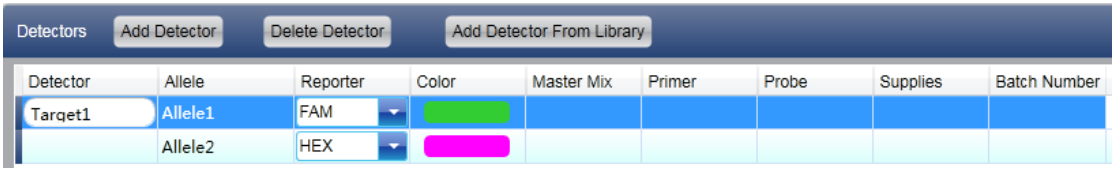
b. Delete Detector

c. Add the Detector in the Detector library: click **Add Detector From Library** ▶ the **Detector Library** window will pop up ▶ select the Detector on the window to be added

※The user can also conduct Add, Modify and Delete operations in the item library.



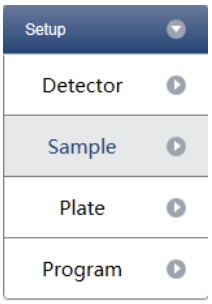
f. Set up the item name, set up the dye name and set up the colour, Msdter Mix etc.



4) Set up reference dye

1.3 **Sample Information Setting**

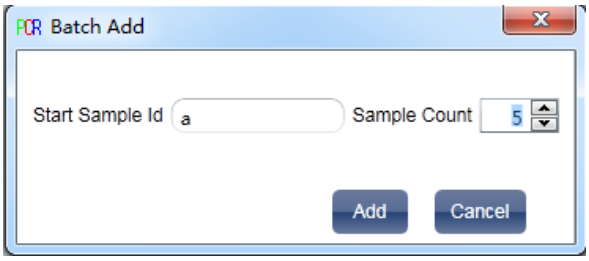
1) Click **Setup** ▶ **Sample**



2)Add sample information

a. Itemized addition: input ID in **Sample ID** ▶ press **Enter** ▶ add information for one sample

b. Batch addition: click **Batch Add** ▶ the Batch Add window will pop up



3)Delete sample information

a. Itemized deletion: select one sample ▶ click **Delete** ▶ delete the selected sample information

b. Delete all: click **Clear All** ▶ delete all sample information

4) Import/Export sample information

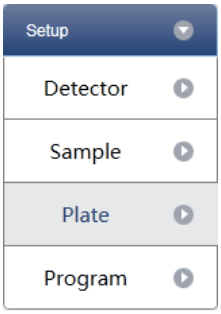
a. Click **Import Sample Info** ▶ the File Import window will pop up ▶ import sample information file in CSV format

b. Click **Export Sample Info** ▶ the Save As window will pop up ▶ the sample information will be exported in CSV file format

5) Set up sample information

1.4 **Reaction Plate Setting**

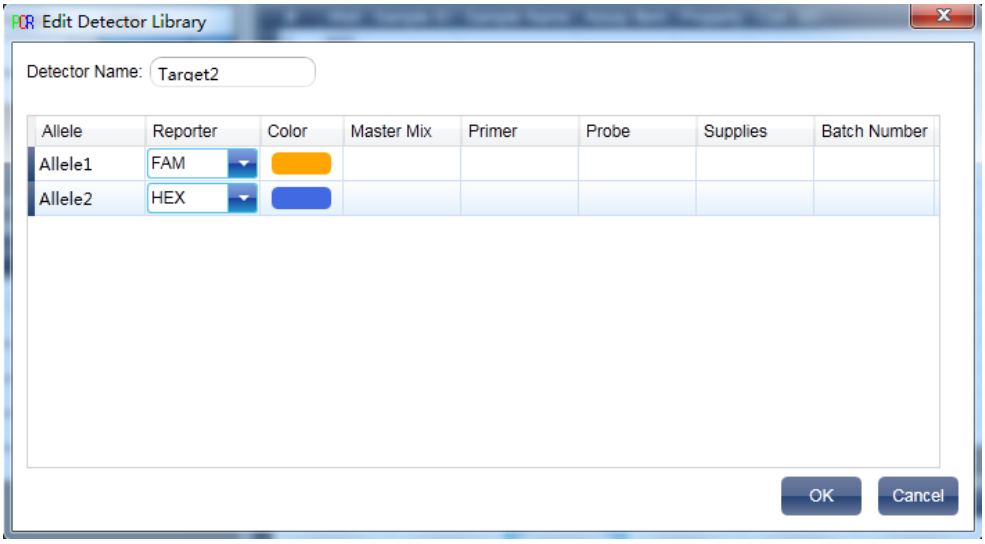
1) Click **Setup** ▶ **Plate**



2)Set up the inspection criteria of the reaction plate

a. Select reaction plate well site: click Reaction Plate well Site

The user can also right click the reaction plate well site to Copy, Paste and Add New Detector. Adding a new detector will open the **Edit Detector Library** window.



b. Select inspection item and modify the property, concentration and concentration unit.

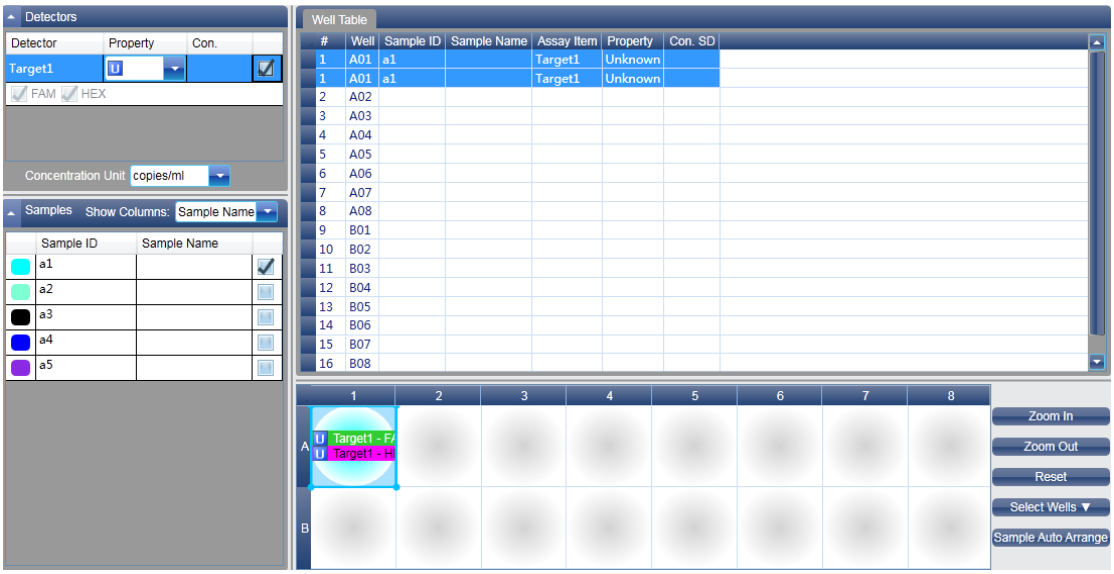
Property	Name	Concentration	Concentration unit
U	Unknown	无	Copies/ml IU/ml Fg/ml Pg/ml
N	Negative	无	
1_1	Positive Allelic gene 1	无	
1_2	Positive Heterozygous	无	
2_2	Positive Allelic gene 2	无	

c. Select a sample and the list displayed will change

d. Zoom-In, Zoom-Out and reset the reaction plate.

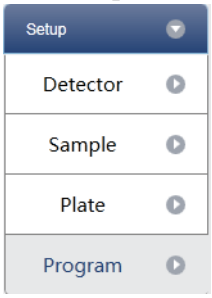
e. Sample Auto Arrange

f. Check Well Table



1.5 **Programme Setting**

1) Click **Setup ▶ Programme**



2) Run Programme Setup

a. Create new stage: the user can create a new **Hold Stage**, **Cycling Stage** or **Melting Stage**

※The user can also click **Add Stage** directly and the default will be creating a new **Cycling Stage**.

b. Create new step: the user can create a new step **Before** or **After** the currently selected step

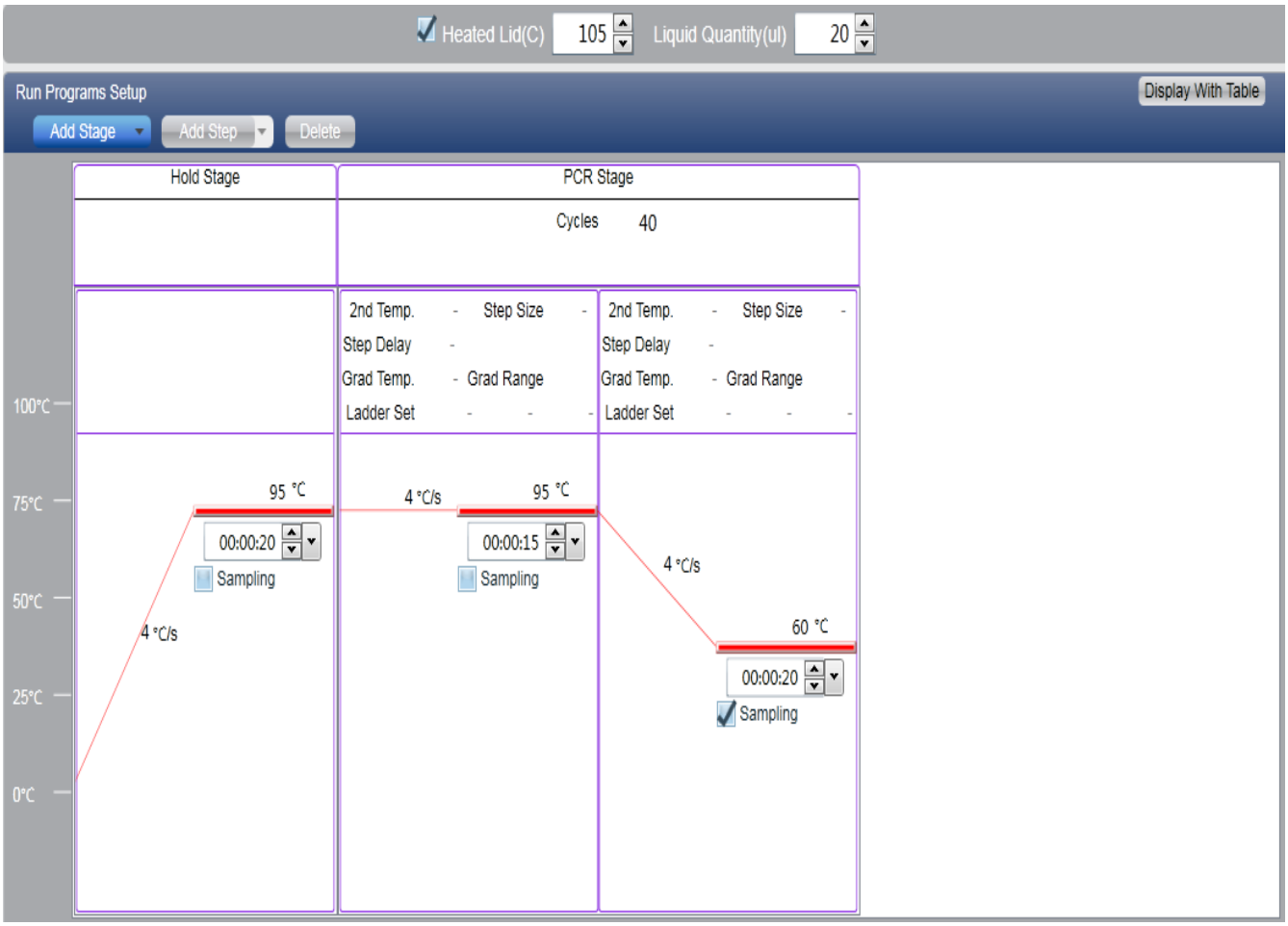
※The user can also click **Add Step** and the default will be adding a new Step at the end of the currently selected stage or after the currently selected step

c. Delete: the user can delete the currently selected step or stage

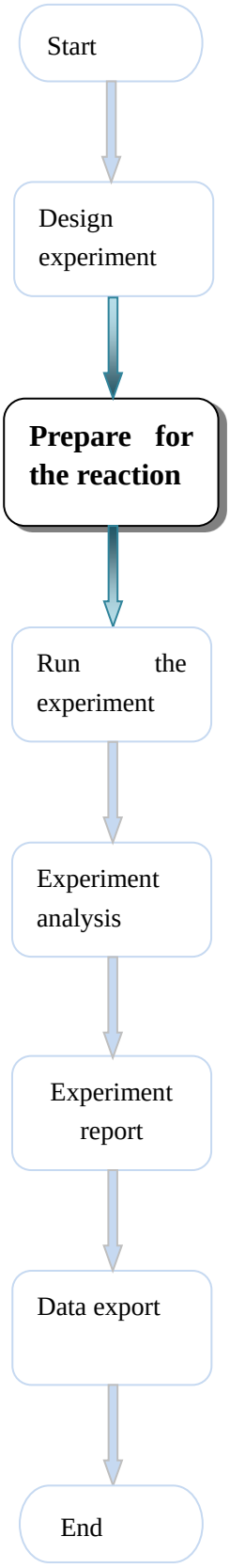
d.Display form: click **Display With Table ▶** new window will pop up **▶** the details of the current experiment will be displayed in a table.

e. Set up the experimental data of the hold stage, cycling stage and melting stage melting section

f. Set up the hot-lid temperature and liquid volume


Heated Lid(C)
105
Liquid Quantity(ul)
20
Run Programs Setup
Display With Table
Add Stage
Add Step
Delete
Hold Stage
PCR Stage
Cycles
40
2nd Temp.
Step Size
Step Delay
Grad Temp.
Grad Range
Ladder Set
100℃
75℃
50℃
25℃
0℃
95 ℃
00:00:20
Sampling
4 ℃/s
4 ℃/s
95 ℃
00:00:15
Sampling
4 ℃/s
60 ℃
00:00:20
Sampling

2. Prepare for Reaction

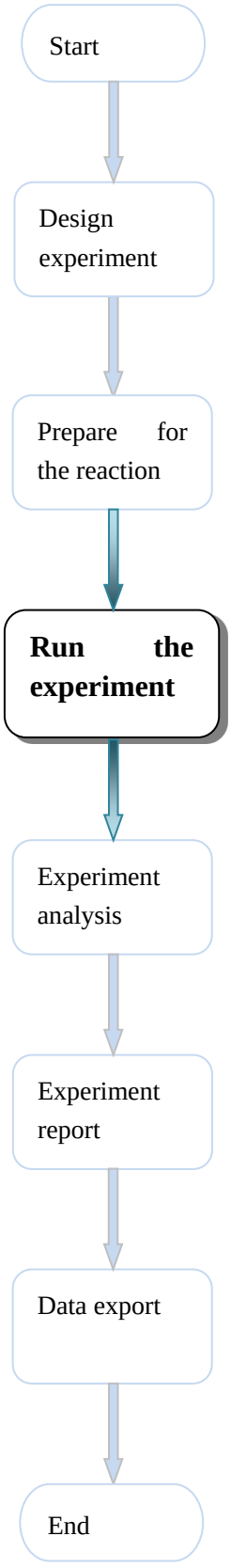


The user should make full preparations prior to the experiment:

• Ensure appropriate materials are used.

• Ensure the arrangement of the PCR reaction plate is consistent with the setting layout of reaction plate in Section 1.4.

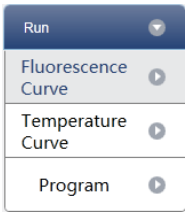
3. Run the Experiment



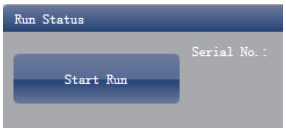
This section describes how to run the experiment after loading the reaction plate and covers the operating of fluorescence curve, the operating of temperature curve and programme setting.

3.1 **Run Fluorescence Curve**

1) Click **Run** ▶ **Fluorescence Curve**



2) Click **Start Run**

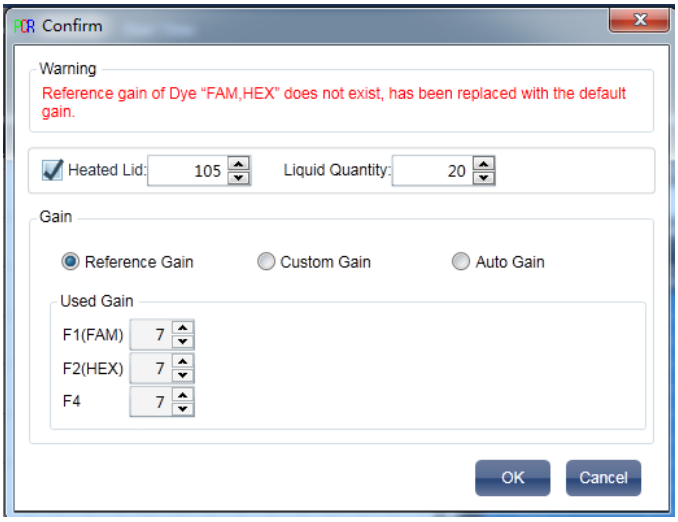


3)Operating confirmation

a. Modify hot-lid temperature and liquid quantity

b. Gain parameter setting

c. Target fluorescence value setting



4. After it starts running, the user can:

a. Skip the current stage

b. Add a cycle

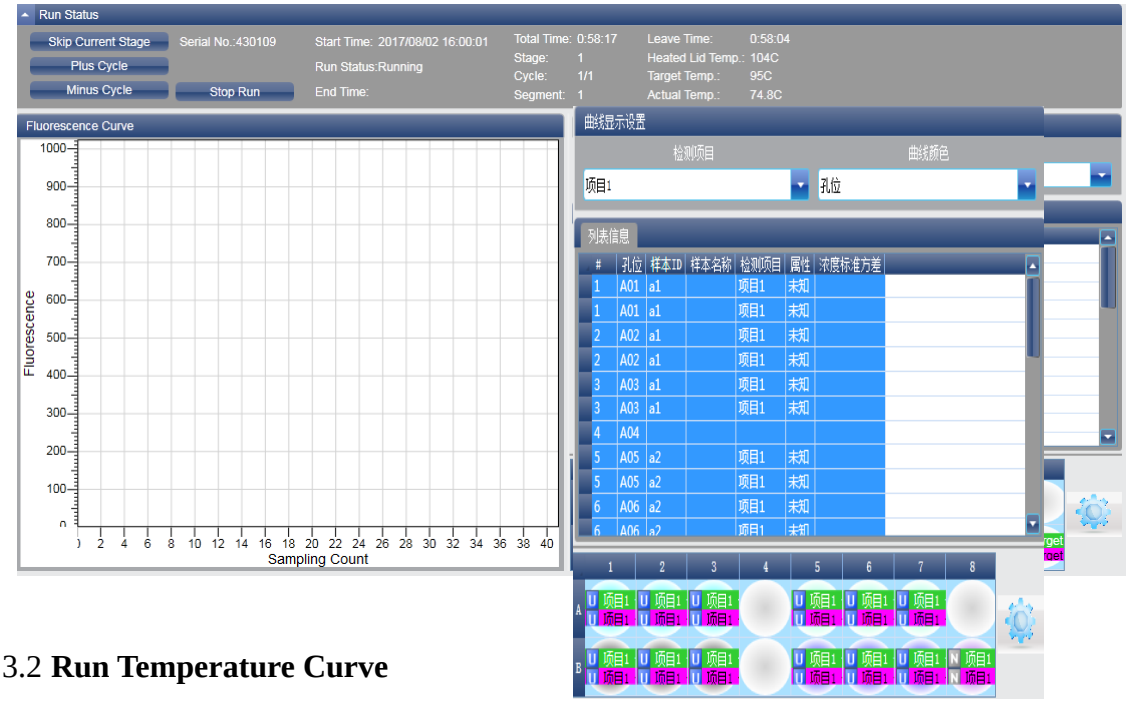
c. Delete a cycle

d. Stop run

5. Plot display setting

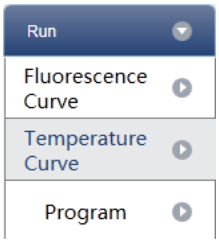
a. Assay item

b. Plot colour

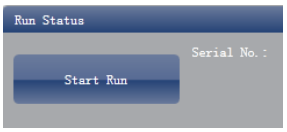


3.2 **Run Temperature Curve**

1) Click **Run** ▶ **Temperature Curve**



2) Click **Start Run**

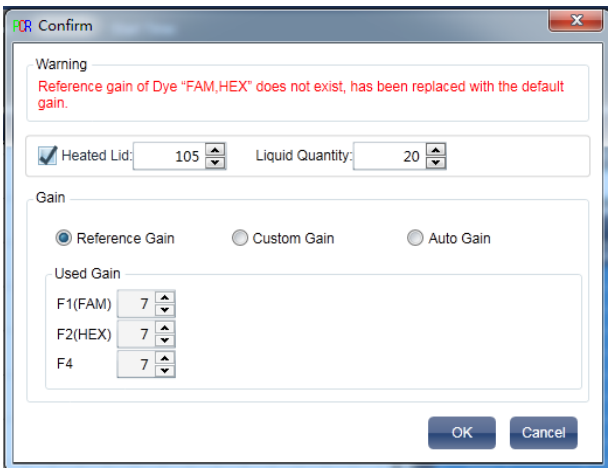


3) Operating confirmation

a. Modify hot-lid temperature and liquid quantity

b. Gain parameter setting

c. Target fluorescence value setting



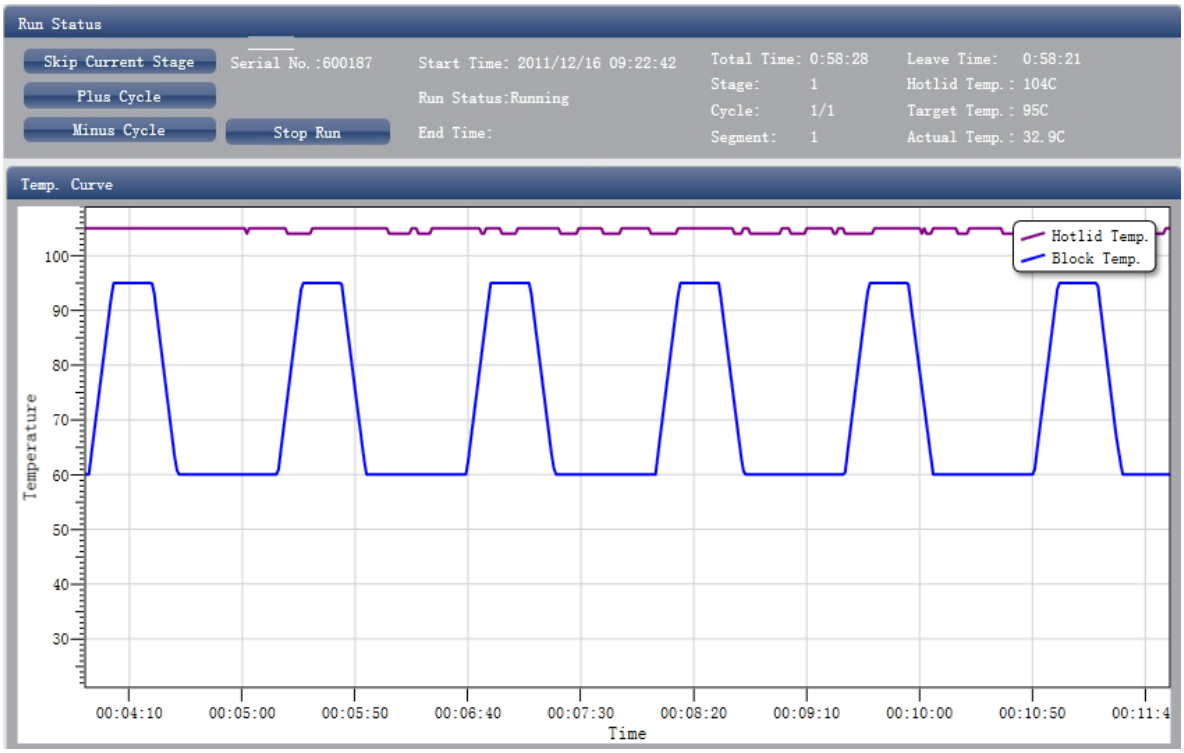
4) After it starts running, the user can:

a. Skip the current stage

b. Add a cycle

c. Delete a cycle

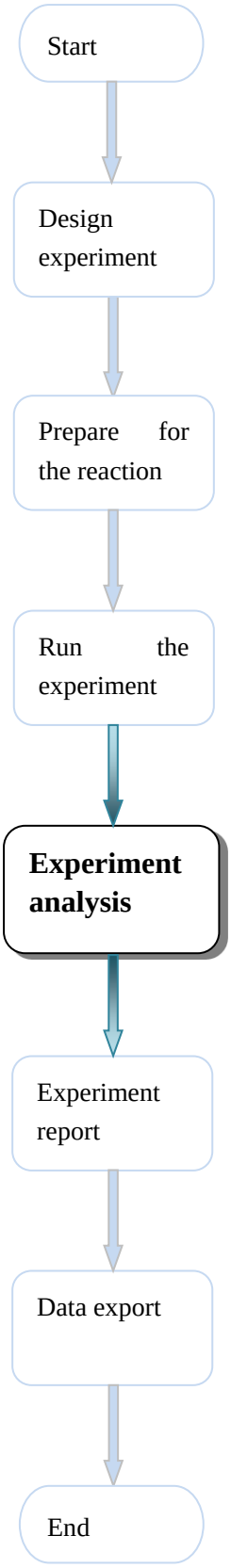
d. Stop run



3.3 **Programme Setting**

The user can only check the programme setting but cannot make modifications.

4. Experiment Analysis

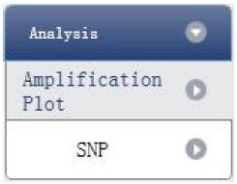


This section describes how to view the experiment analysis results after running an experiment and adjusting parameters for re-analysis. This section covers the analysis of amplification curves and standard curves, adjusting parameters for re-analysis and importing parameters.

4.1 **Check Results**

4.1.1 Check the Amplification Plot

1) Click **Analysis ▶ Amplification Plot**



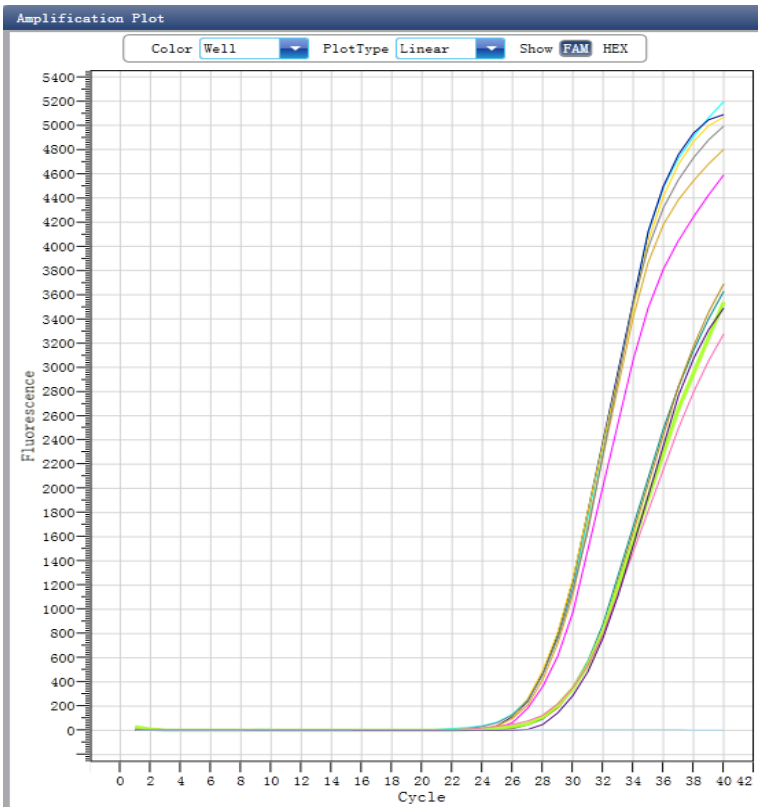
2)Check the amplification curve

a. Set up colour

b. Set up plot type

c. Set up show dye

※When the background colour of a dye name is blue, it will be displayed; while white indicates it will not be displayed.



3) Check the reaction plate

a. Select reaction plate well site and check corresponding well site curve

※The default is all wells are selected

b. Zoom-In, Zoom-Out and reset the reaction plate

c. Check well table

d. Check results summary

4) Set up inspection item

a. Set up assay

b. Set up threshold

c. Set up automatic baseline

※When the threshold value is not automatic, the user cannot set up the automatic baseline

4.1.2 Check SNP

1) Click **Analysis** ▶ **SNP**

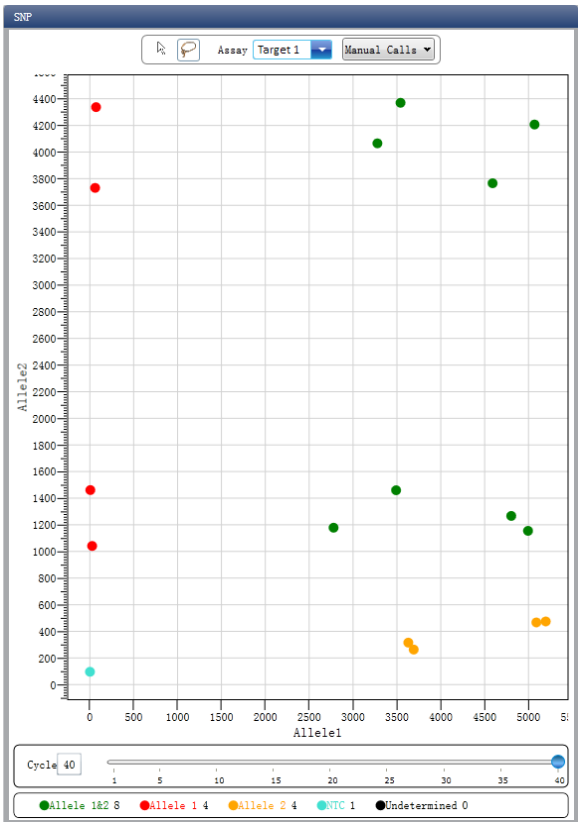
2) Check SNP

a. Select well site

※The user can select well site by dragging a rectangle with the mouse around the wells of interest or select wells one by one.

b. Set up Assay

c. Set up manual calls



3) Check the reaction plate

a. Select reaction plate well site and check corresponding well site curve

※The default is all wells are selected

b. Zoom-In, Zoom-Out and reset the reaction plate

c. Check well table information

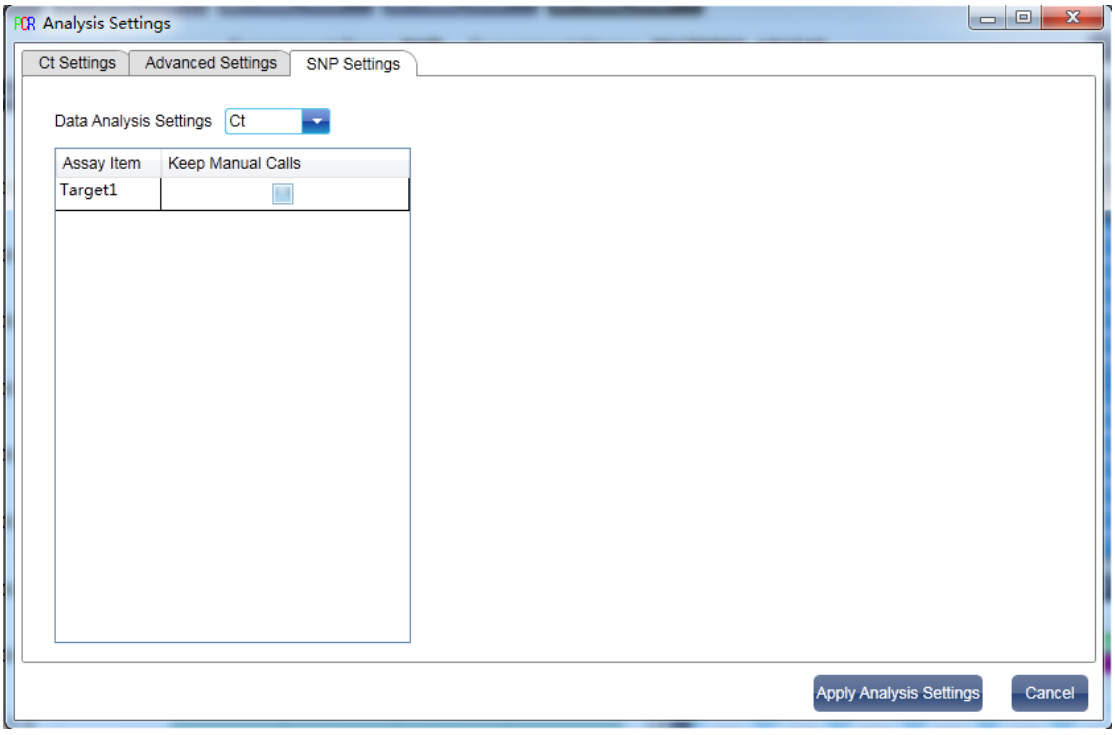
d. Check results summary

4.2 **Adjust Parameter Re-analysis**

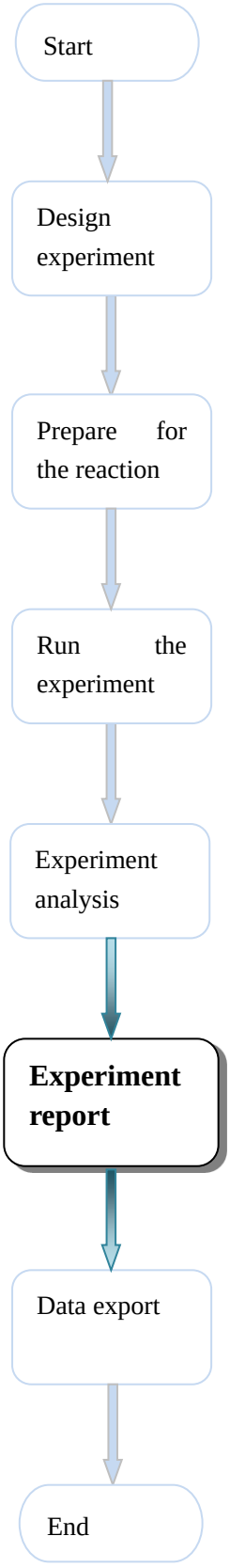
Click **Analysis Settings** ▶ the Analysis Settings dialog box will pop up

a. Adjust analysis data

b. Adjust whether the inspection item will retain manual recognition genotype



5. Experiment Report



This section describes how to print an experiment report and covers designing of a report template and print setting.

5.1 **Designing a Report Template**

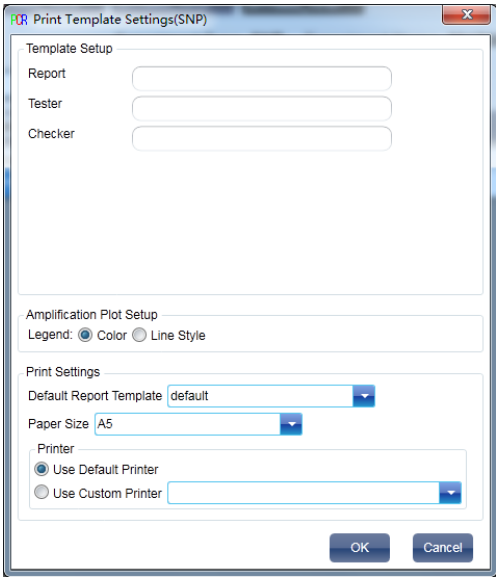
Click **Report** ▶ **Report Template Editor** ▶ the Report Designer window will pop up

The report consists of controls and the user can add, modify and delete controls. Available controls include Static Text, Dynamic Text, Line, Static Image and SNP Typing Curve.

5.2 **Print Setting**

Click **Report** ▶ **Print Template Setting** ▶ the Print Template Setting window will pop up

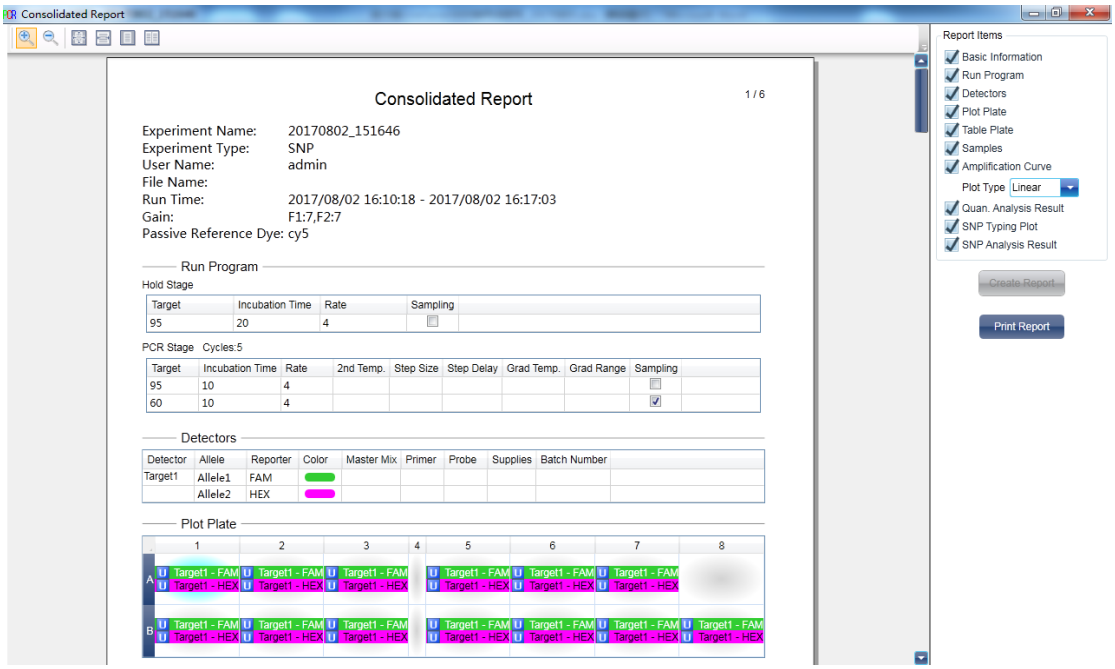
The user can set up the laboratory name, report name, reference value, tester, checker, amplification plot set up, default report template and paper size.



5.3 **Comprehensive Report**

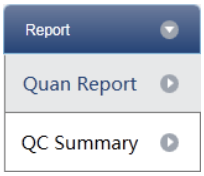
Click **Report** ▶ **Consolidated Reports** ▶ the Consolidated Report window will pop up.

The Consolidated Report includes the basic information, sample information, amplification curve, SNP, plate information, etc.



5.4 **Report Printing**

1) Click **Report** ▶ **Report Print**



2) Report print setting

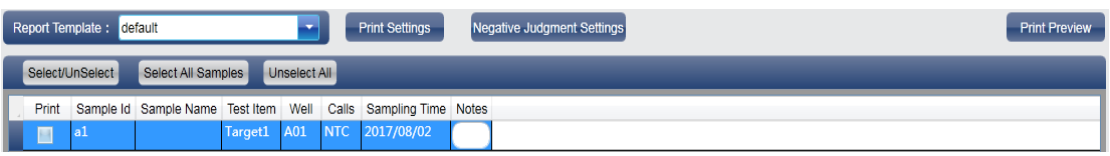
a. Set up report template

b. Print setting (please refer to Section 5.2)

c. Select print items

d. Print preview

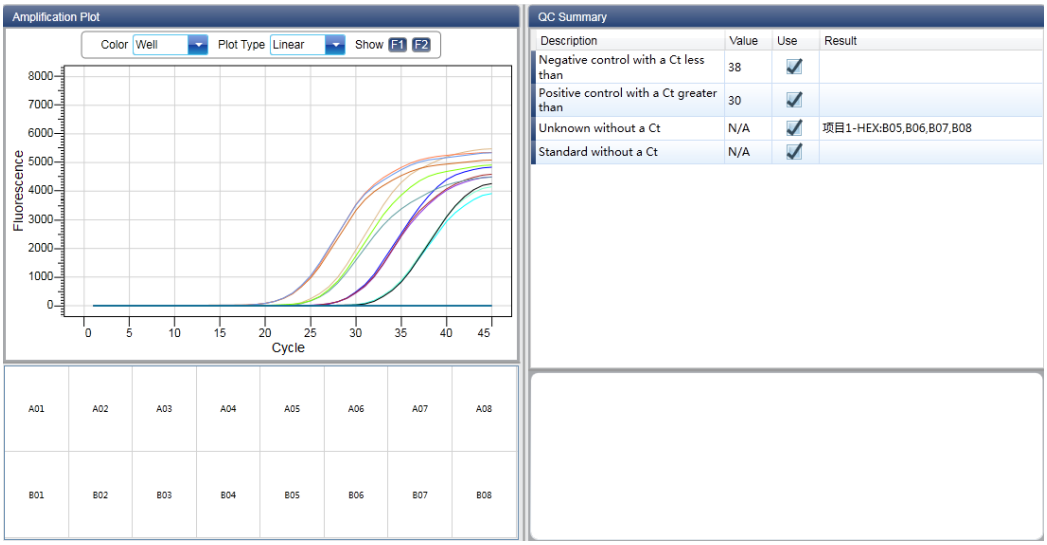
e. Print the report



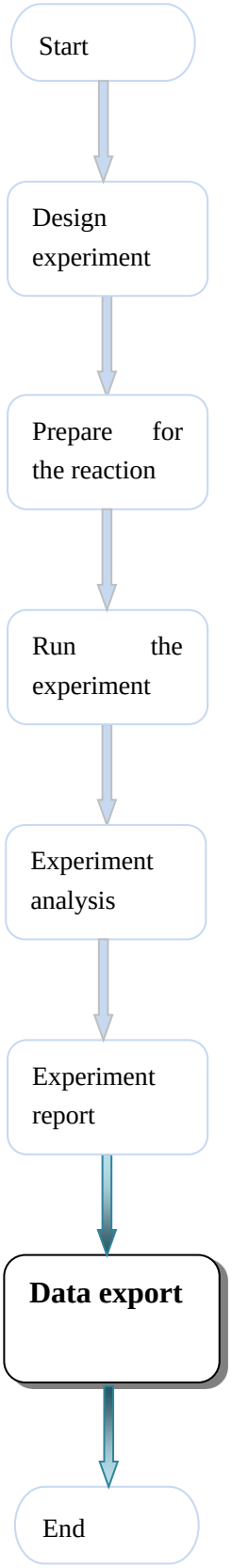
5.5 **QC Summary**

1) Click **Report ▶ QC Summary**

2) Check the QC summary



6. Data Export



This section describes how to export data and covers exporting to a database, experiment filing and exporting the experiment data to EXCEL.

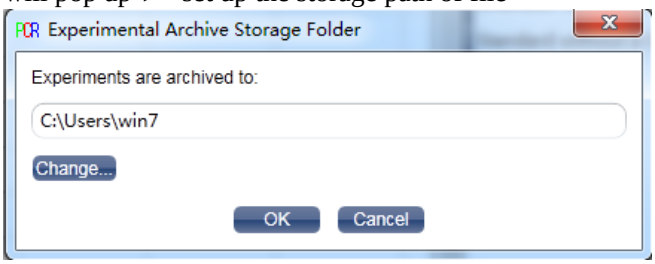
6.1 **Export to Database**

Click **Data Summary** ▶ **Export to Database** ▶ the Save File dialog box will pop up ▶ save the exported database file

6.2 **Experiment Filing**

1) Set filing experiment storage folder

Click **Data Summary** ▶ **Archived Experiment Directory** ▶ the Experimental archive storage directory window will pop up ▶ set up the storage path of file



2) Experiment filing

Click **Data Summary** ▶ **Archived Experiment** ▶ export the filed experiment file

※The suffix of filed experiment file is .fqh

6.3 Export Experiment Data to EXCEL

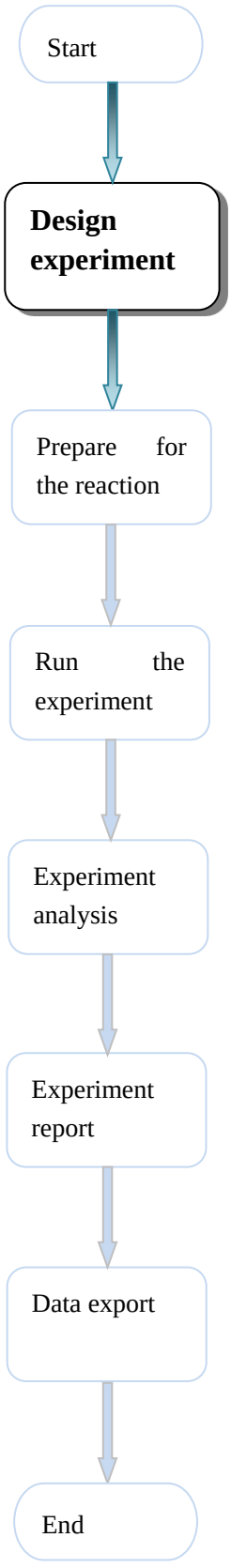
Click **Data Summary**▶**Export Experiment** ▶ **Export Experiment to Excle** ▶the exported experiment data will generate EXCEL file.

6.4 Export Experiment Data to TEXT

Click **Data Summary**▶**Export Experiment** ▶ **Export Experiment to Text**▶ the exported experiment data will generate TEXT file.

Chapter 7 High Resolution Melting

1. Design Experiment



This section describes how to design an SNP experiment and covers creating a new SNP experiment, inspection item setting, sample information setting, reaction plate setting and programme setting

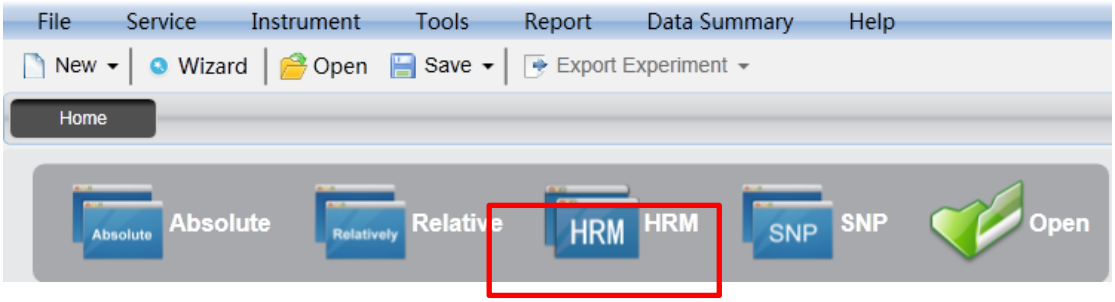
1.1 Create High Resolution Melting Experiment

1. Click **HRM** on **Home** interface and create SNP Experiment window.

※An SNP experiment can be also created by:

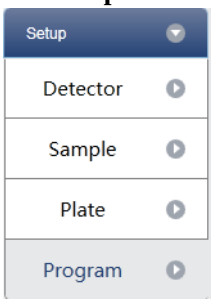
a. Clicking **New** ▶ **HRM** on the toolbar

b. Clicking **File** ▶ **New** ▶ **HRM** on the menu bar



1.2 Detector Setting

1. Click **Setup** ▶ **Detector**



2.Input basic information

Input the experiment name, user name and any comments in the experiment properties column.

3. Inspection Item Setting

Set up the Detector, Allele, Dye and Colour.

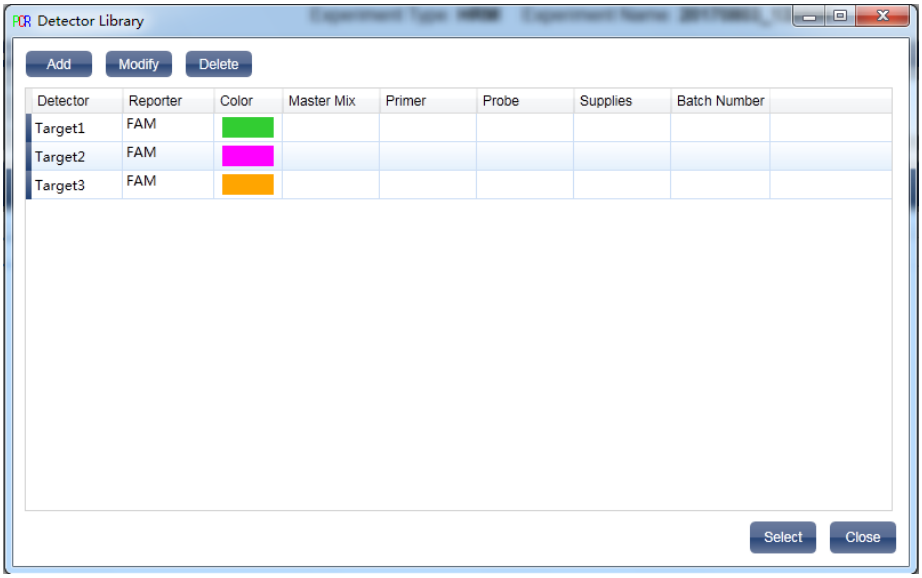
※If necessary, the user can also:

a. Add Detector

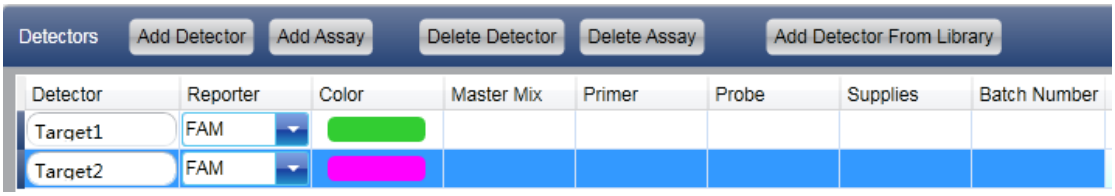
b. Delete Detector

c. Add the Detector in the Detector library: click **Add Detector From Library** ▶ the **Detector Library** window will pop up ▶ select the Detector on the window to be added

※The user can also conduct Add, Modify and Delete operations in the item library.



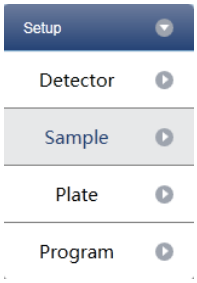
f.Set up the item name, set up the dye name and set up the colour



4.Set up reference dye

1.3 **Sample Information Setting**

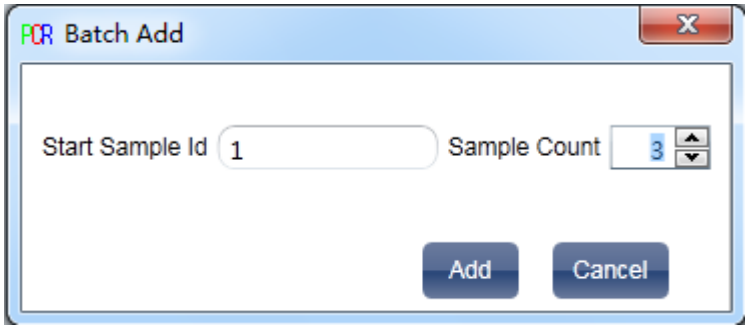
1. Click **Setup** ▶ **Sample**



2.Add sample information

a. Itemized addition: input ID in **Sample ID** ▶ press **Enter** ▶ add information for one sample

b. Batch addition: click **Batch Add** ▶ the Batch Add window will pop up



3. Delete sample information

a. Itemized deletion: select one sample ▶ click **Delete** ▶ delete the selected sample information

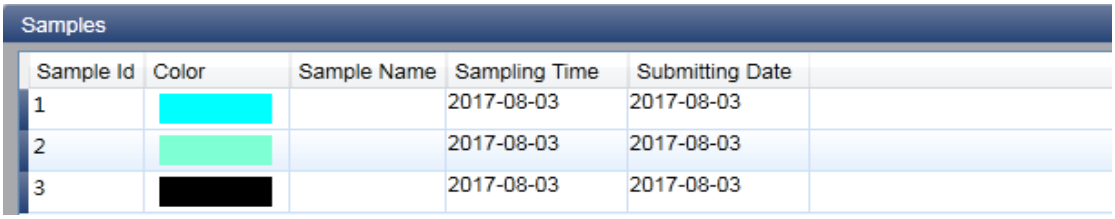
b. Delete all: click **Clear All** ▶ delete all sample information

4. Import/Export sample information

a. Click **Import Sample Info** ▶ the File Import window will pop up ▶ import sample information file in CSV format

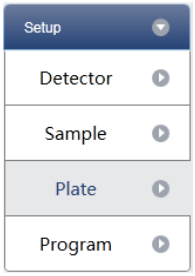
b. Click **Export Sample Info** ▶ the Save As window will pop up ▶ the sample information will be exported in CSV file format

5. Set up sample information



1.4 Reaction Plate Setting

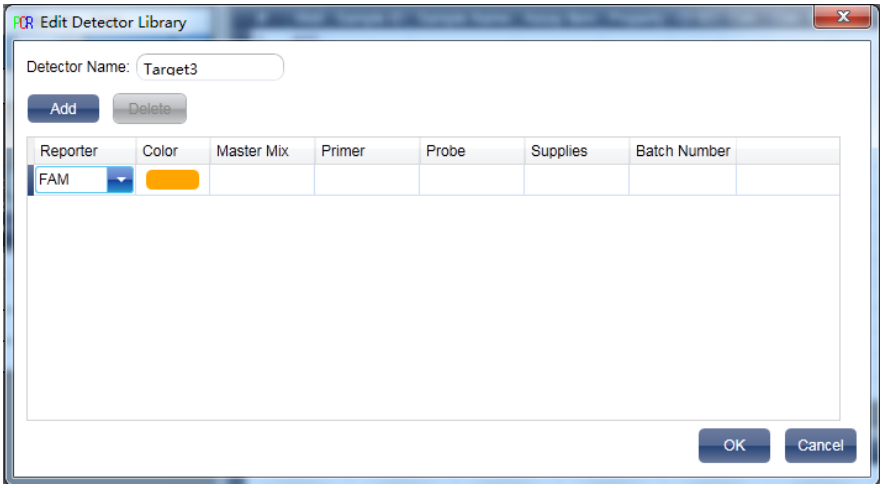
1. Click **Setup** ▶ **Plate**



2. Set up the inspection criteria of the reaction plate

a. Select reaction plate well site: click Reaction Plate well Site

The user can also right click the reaction plate well site to Copy, Paste and Add New Detector. Adding a new detector will open the **Edit Detector Library** window.



b. Select inspection item and modify the property, concentration and concentration unit.

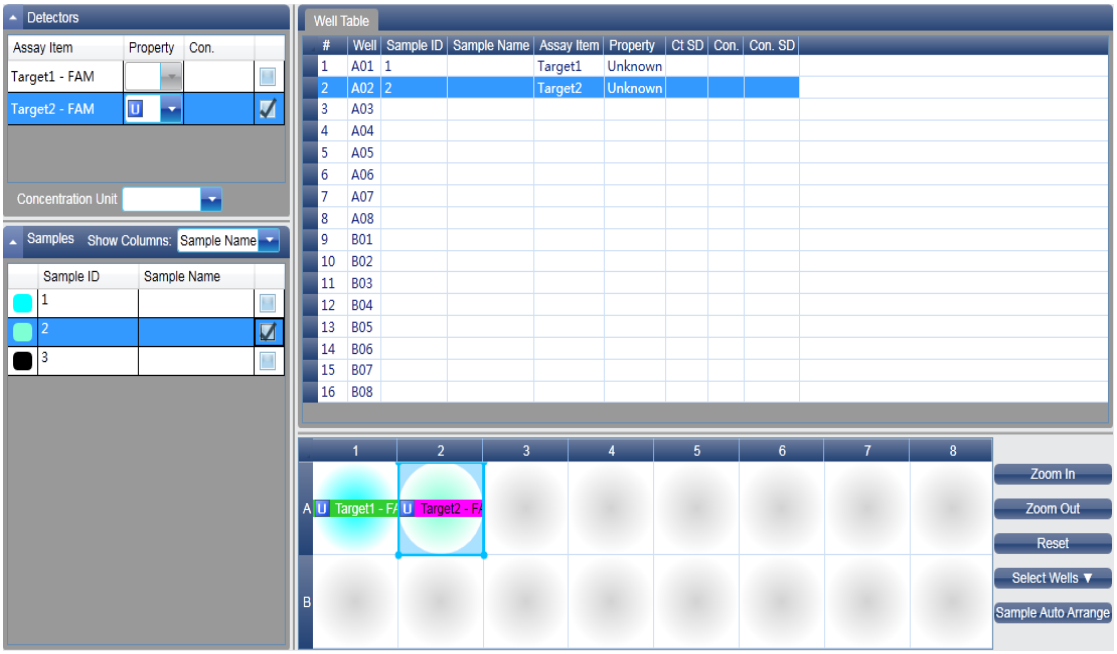
Property	Name	Concentration	Concentration unit
U	Unknown	NO	Copies/ml IU/ml
N	Negative	NO	

c. Select a sample and the list displayed will change

d. Zoom-In, Zoom-Out and reset the reaction plate.

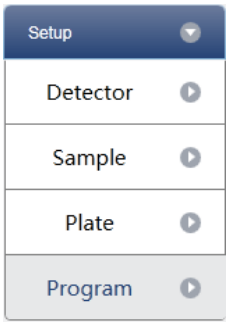
e. Sample Auto Arrange

f. Check Well Table



1.5 **Programme Setting**

1. Click **Setup ▶ Programme**



2. Run Programme Setup

a. Create new stage: the user can create a new **Hold Stage**, **Cycling Stage** or **Melting Stage**

※The user can also click **Add Stage** directly and the default will be creating a new **Cycling Stage**.

b. Create new step: the user can create a new step **Before** or **After** the currently selected step

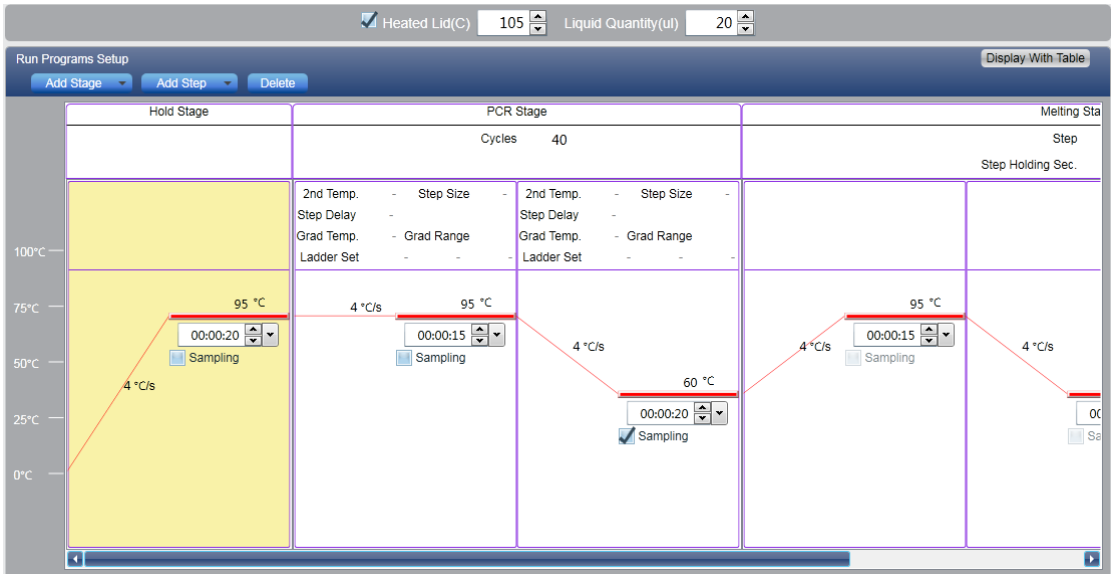
※The user can also click **Add Step** and the default will be adding a new Step at the end of the currently selected stage or after the currently selected step.

c. Delete: the user can delete the currently selected step or stage

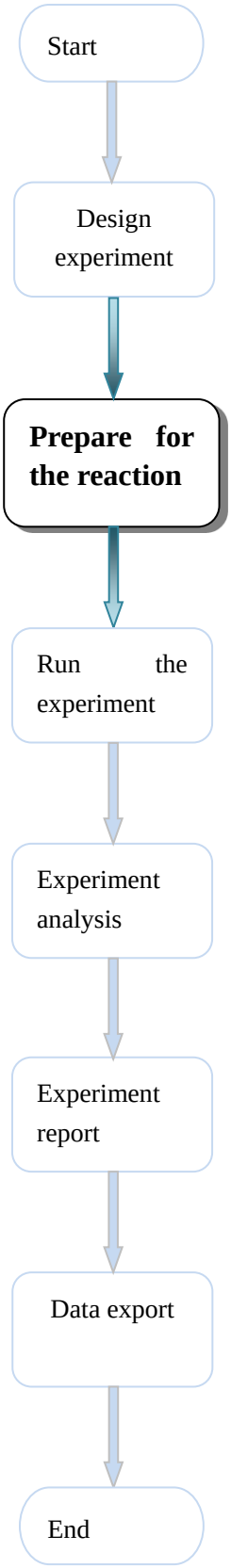
d. Display form: click **Display With Table ▶** new window will pop up **▶** the details of the current experiment will be displayed in a table.

e. Set up the experimental data of the hold stage, cycling stage and melting stage melting section

f. Set up the hot-lid temperature and liquid volume



2. Prepare for Reaction

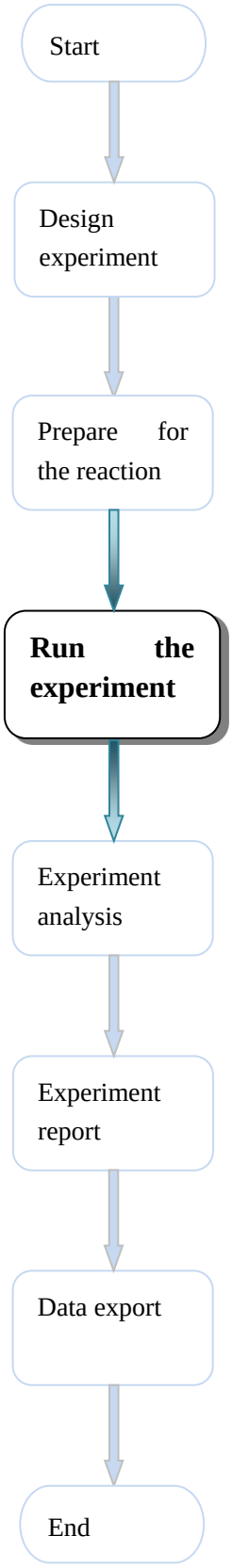


The user should make full preparations prior to the experiment:

• Ensure appropriate materials are used.

• Ensure the arrangement of the PCR reaction plate is consistent with the setting layout of reaction plate in Section 1.4.

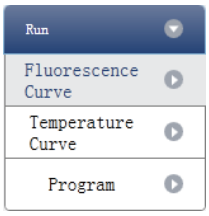
3. Run the Experiment



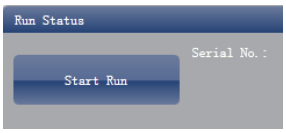
This section describes how to run the experiment after loading the reaction plate and covers the operating of fluorescence curve, the operating of temperature curve and programme setting.

3.1 **Run Fluorescence Curve**

1. Click **Run ▶ Fluorescence Curve**



2. Click **Start Run**

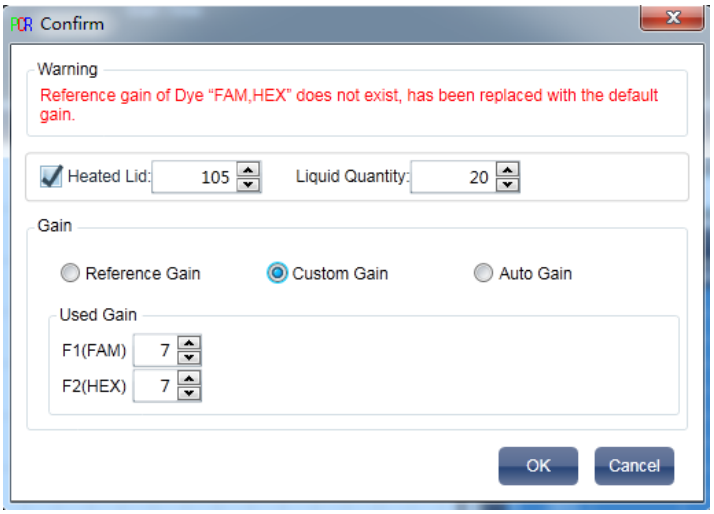


3.Operating confirmation

a. Modify hot-lid temperature and liquid quantity

b. Gain parameter setting

c. Target fluorescence value setting



4. After it starts running, the user can:

a. Skip the current stage

b. Add a cycle

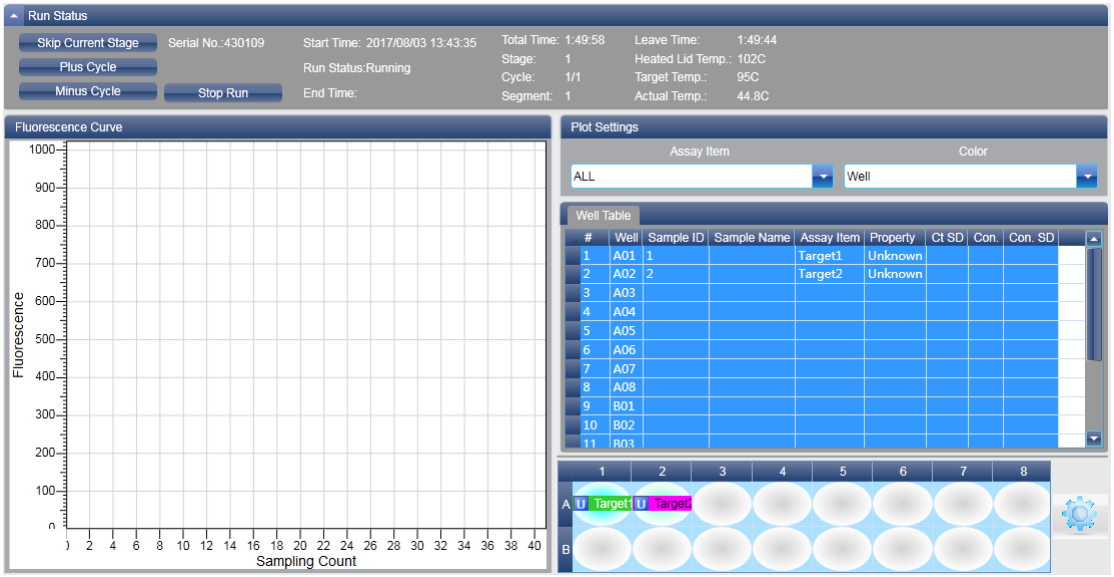
c. Delete a cycle

d. Stop run

5. Plot display setting

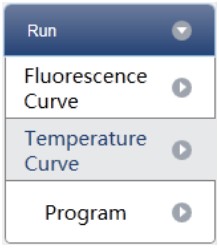
a. Assay item

b. Plot colour

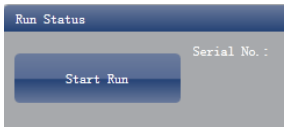


3.2 **Run Temperature Curve**

1. Click **Run** ▶ **Temperature Curve**



2. Click **Start Run**

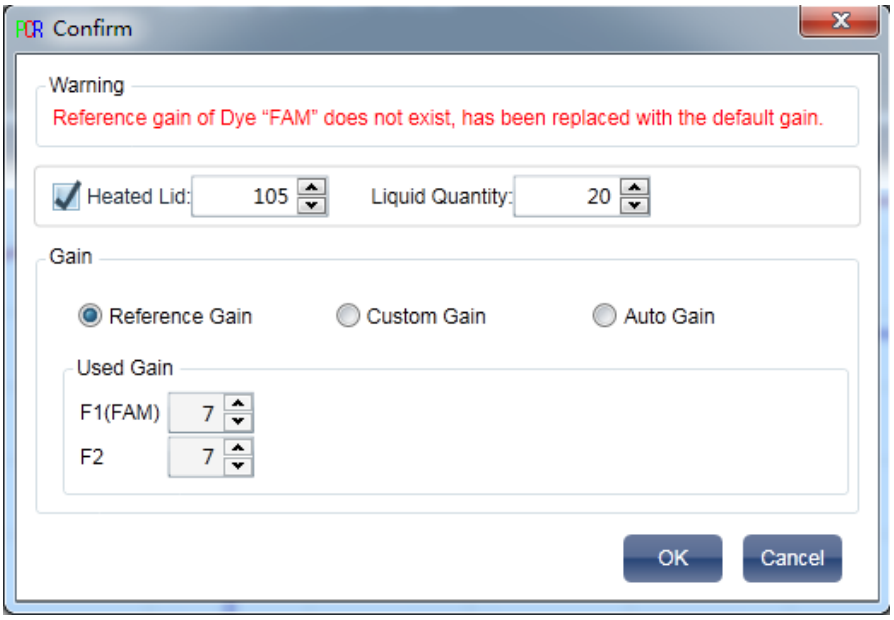


3.Operating confirmation

a. Modify hot-lid temperature and liquid quantity

b. Gain parameter setting

c. Target fluorescence value setting



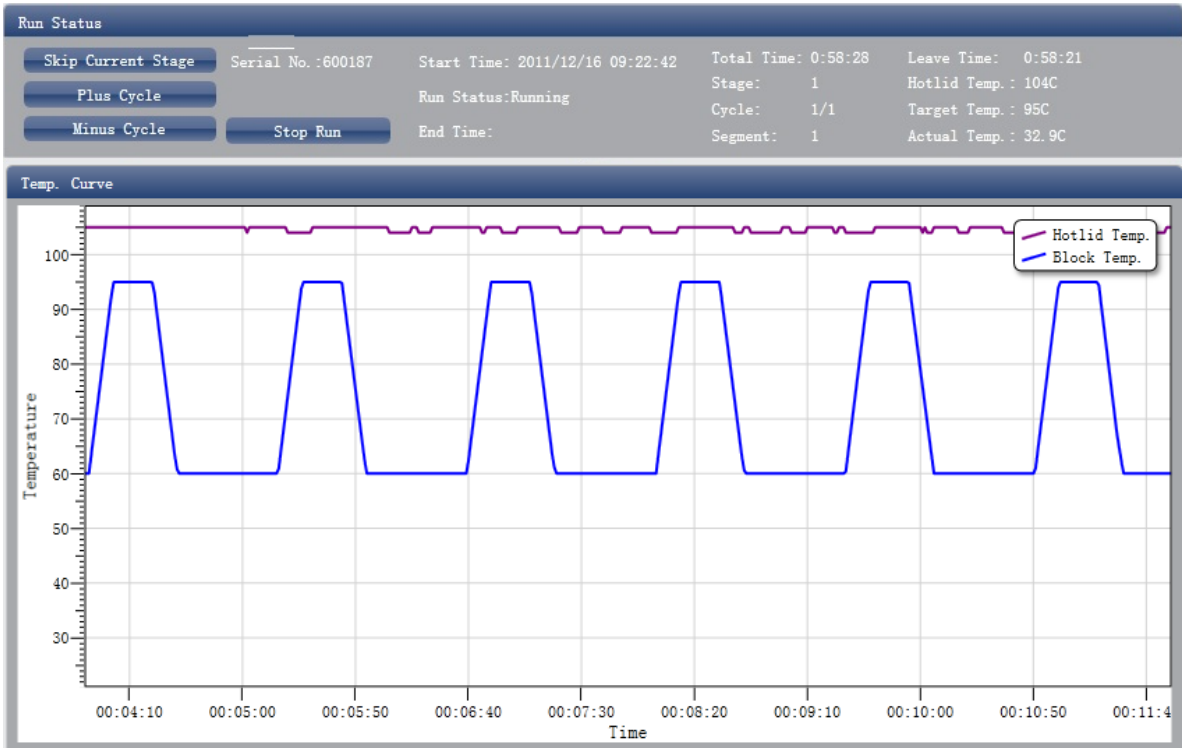
4.After it starts running, the user can:

a. Skip the current stage

b. Add a cycle

c. Delete a cycle

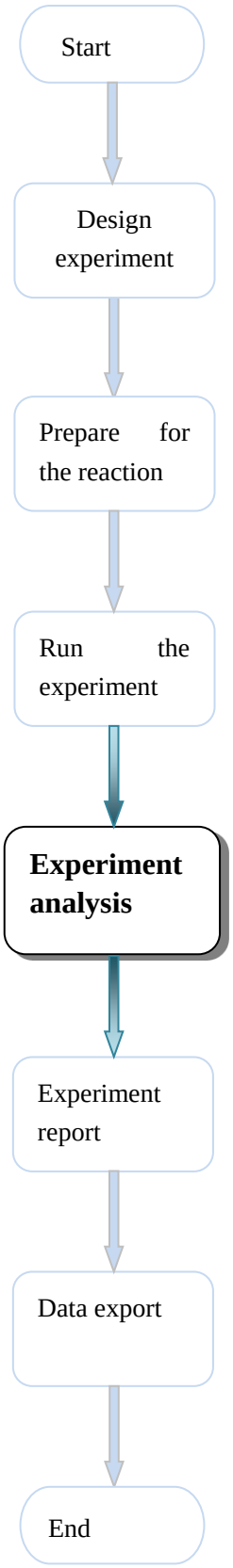
d. Stop run



3.3 **Programme Setting**

The user can only check the programme setting but cannot make modifications

4. Experiment Analysis

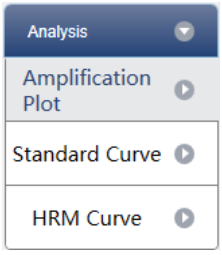


This section describes how to view the experiment analysis results after running an experiment and adjusting parameters for re-analysis. This section covers the analysis of amplification curves and standard curves, adjusting parameters for re-analysis and importing parameters.

4.1 Check Results

4.1.1 Check the Amplification Plot

1. Click **Analysis ▶ Amplification Plot**



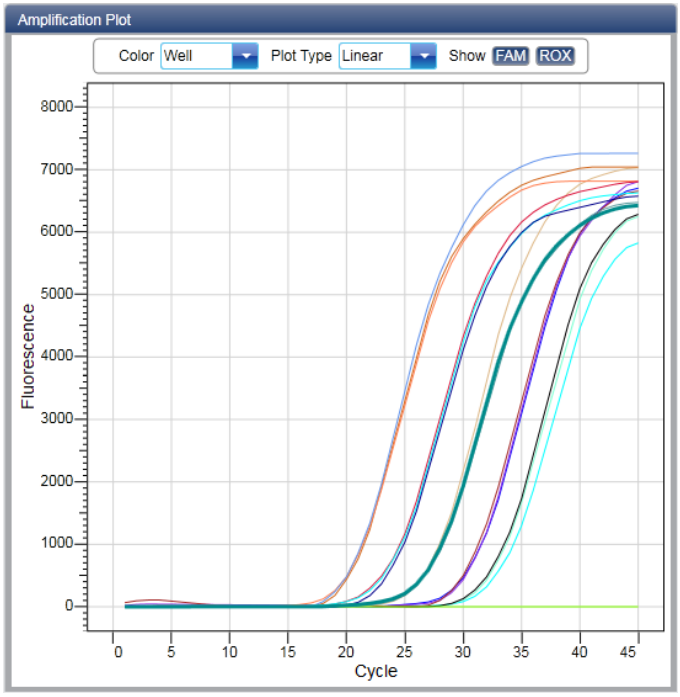
2. Check the amplification curve

   a. Set up colour

   b. Set up plot type

   c. Set up show dye

※When the background colour of a dye name is blue, it will be displayed; while white indicates it will not be displayed.



3. Check the reaction plate

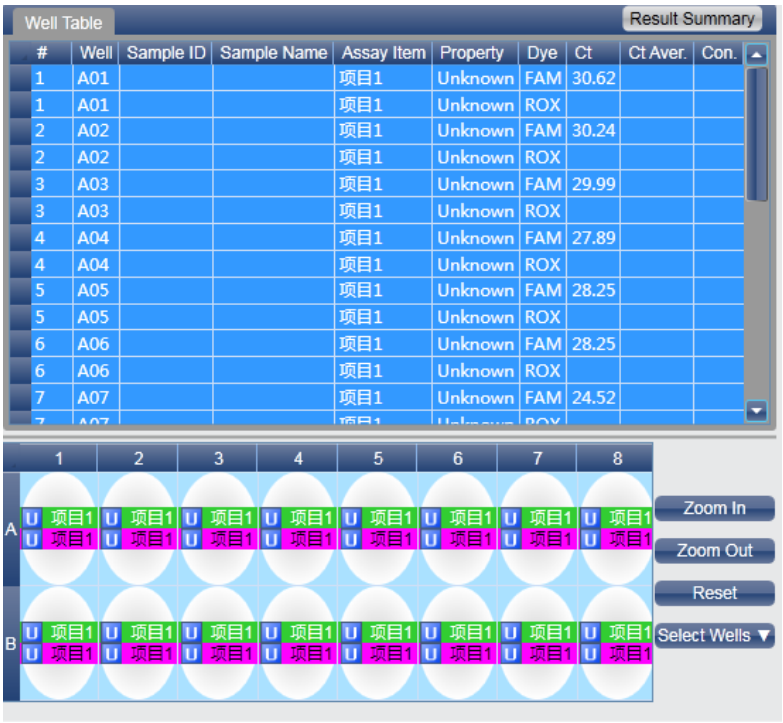
   a. Select reaction plate well site and check corresponding well site curve

   ※The default is all wells are selected

   b. Zoom-In, Zoom-Out and reset the reaction plate

   c. Check well table

   d. Check results summary



4. Set up inspection item

a. Set up assay

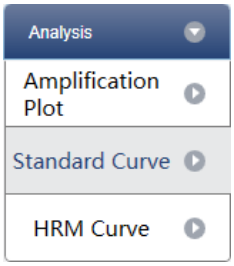
b. Set up threshold

c. Set up automatic baseline

※When the threshold value is not automatic, the user cannot set up the automatic baseline

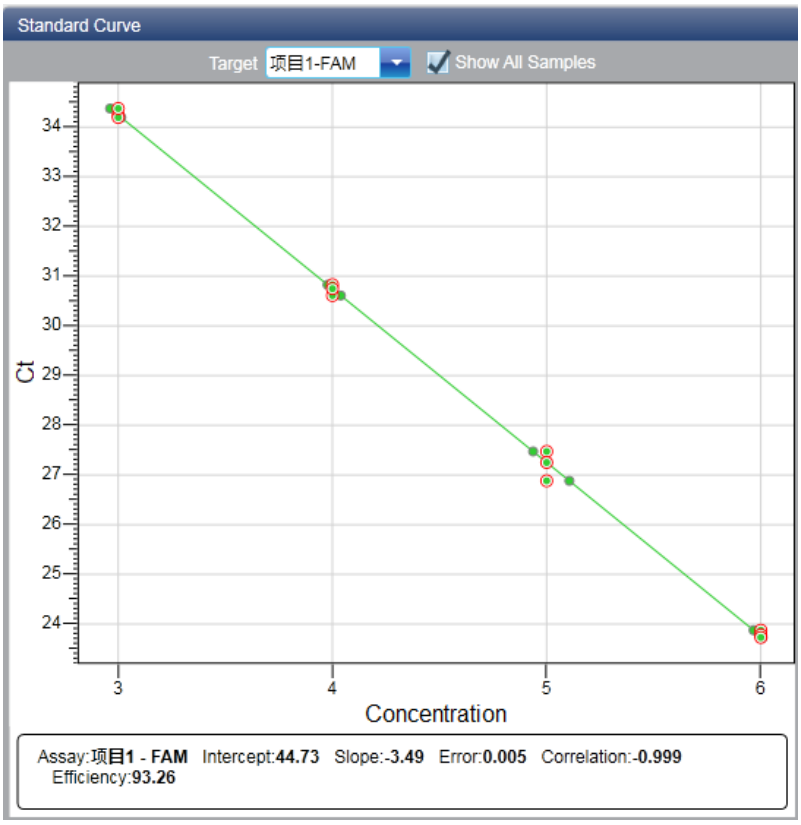
4.1.2 Check the Standard Curve

1. Click **Analysis ▶ Standard Curve**



2. Check the Standard Curve

a. Set up array



3. Check the reaction plate

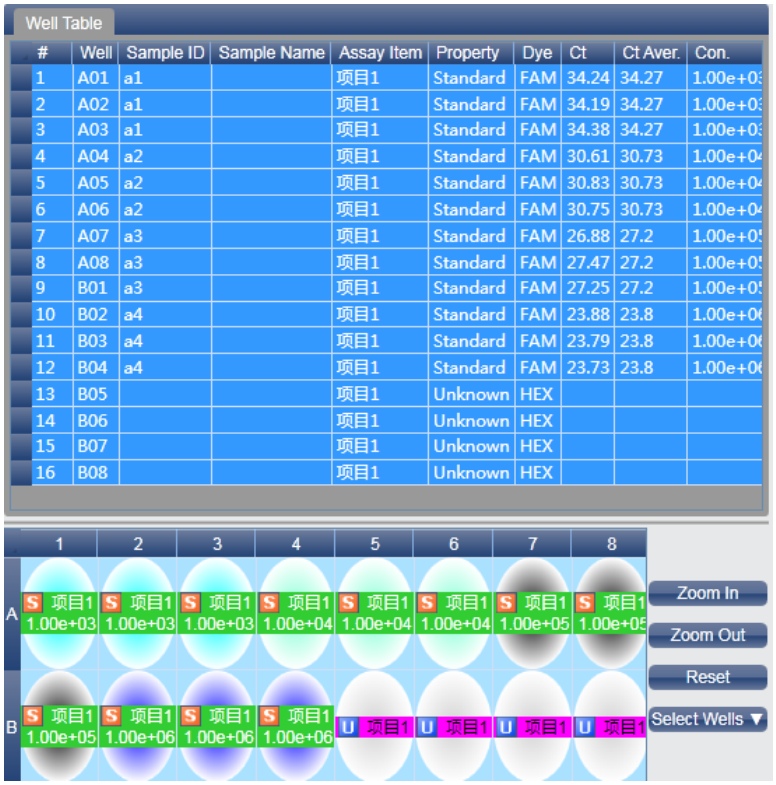
a. Select reaction plate well site and check corresponding well site curve

※The default is all wells are selected

b. Zoom-In, Zoom-Out and reset the reaction plate

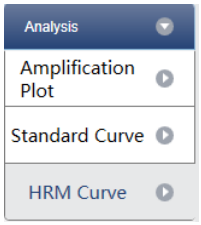
c. Check well table

d. Check results summary

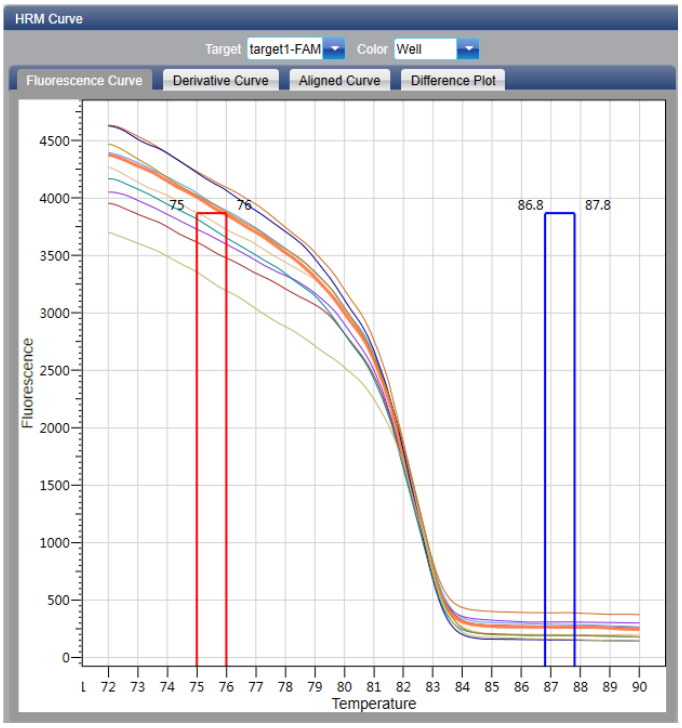


4.1.3 Check HRM

1. Click **Analysis** ▶ **HRM Curve**



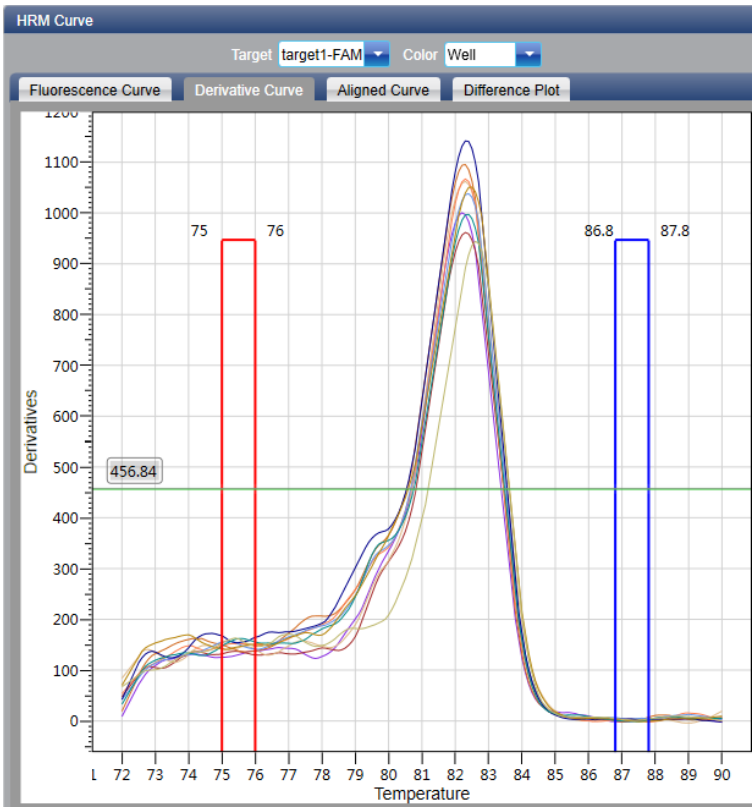
2. Check the fluorescence curve
   a. Set up target
   b. Set up color



3. Check the derivative curve

a. Set up target

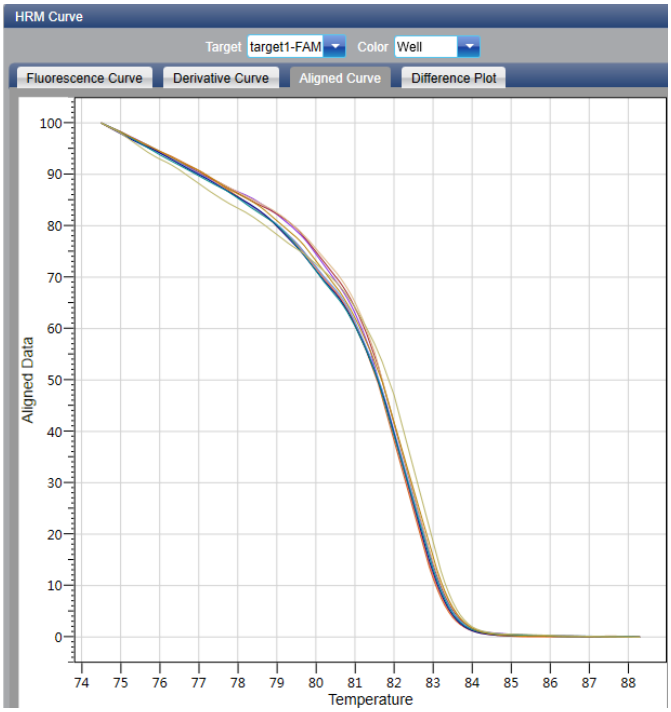
b. Set up color



4.Check the aligned curve

a. Set up target

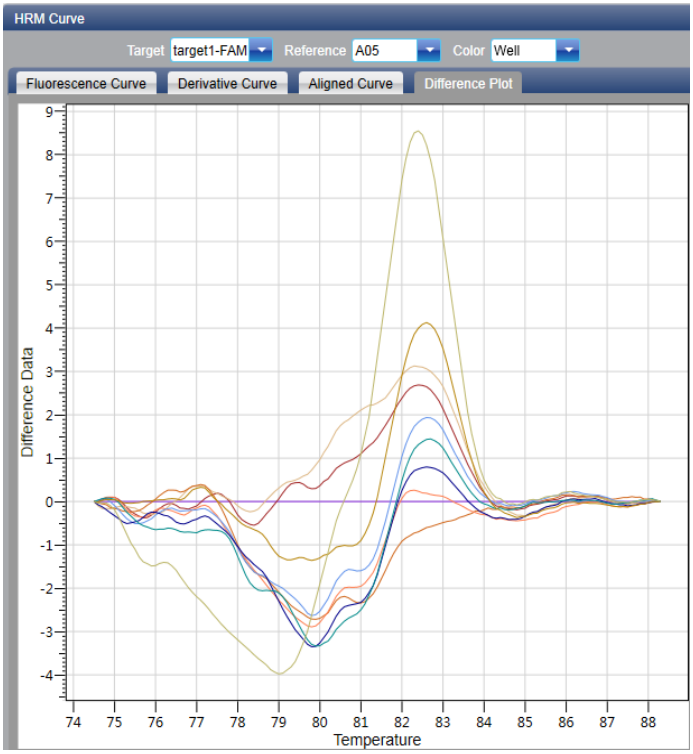
b. Set up color



5. Check the Different Pilot

a. Set up target

b. Set up color



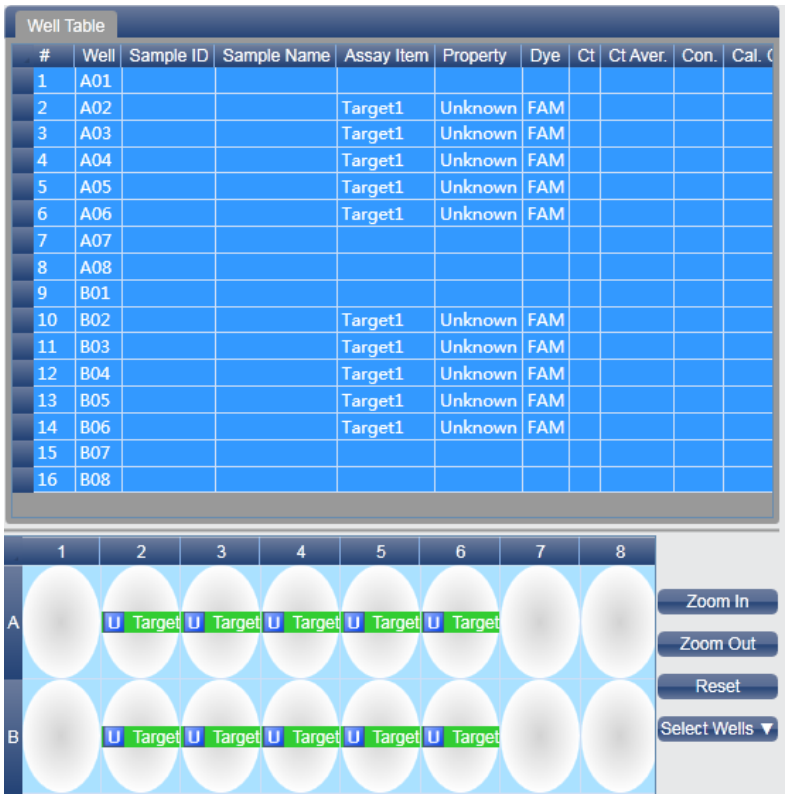
6.Check the reaction plate

a. Select reaction plate well site and check corresponding well site curve

※The default is all wells are selected

b. Zoom-In, Zoom-Out and reset the reaction plate

c. Check well table

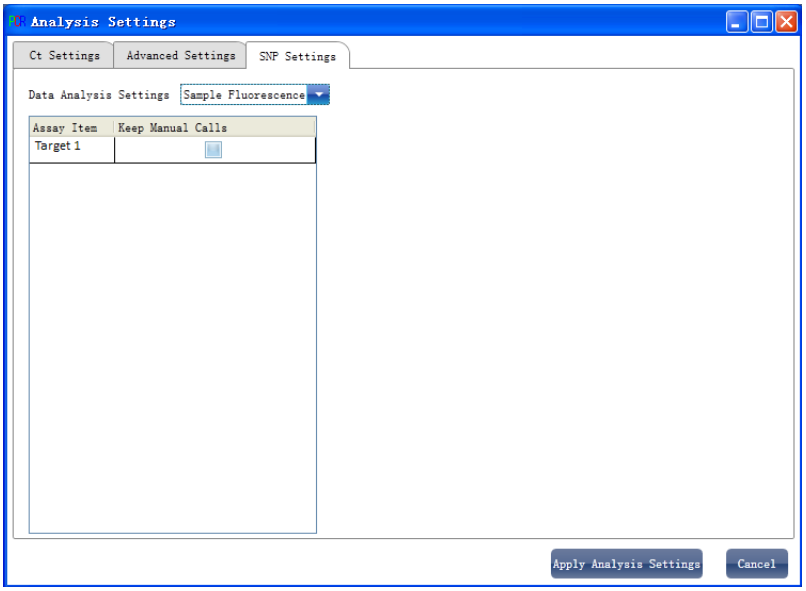


4.2 **Adjust Parameter Re-analysis**

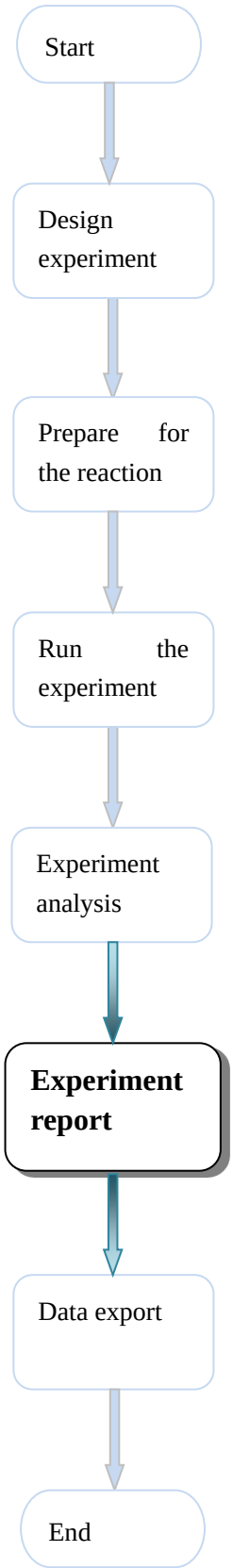
1. Click **Analysis Settings** ▶ the Analysis Settings dialog box will pop up

a. Adjust analysis data

b. Adjust whether the inspection item will retain manual recognition genotype



5. Experiment Report



This section describes how to print an experiment report and covers designing of a report template and print setting.

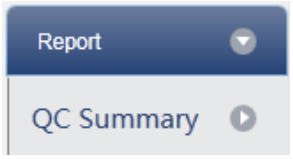
5.1 **Comprehensive Report**

1. Click **Report** ▶ **Consolidated Reports** ▶ the Consolidated Report window will pop up.

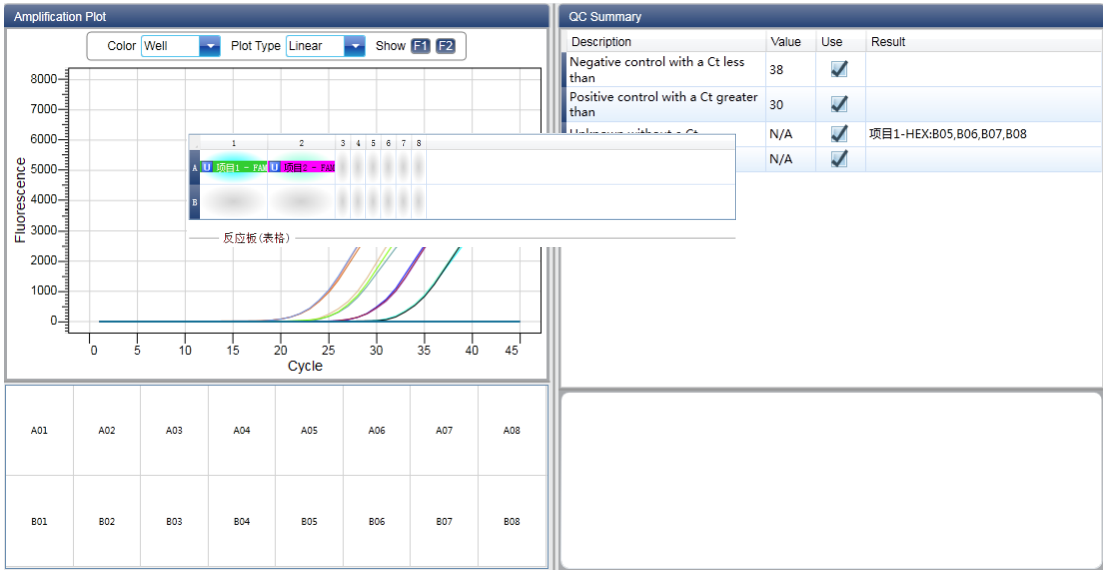
The Consolidated Report includes the basic information, sample information, amplification curve, HRM curve, plate information, etc..

5.2 **QC Summary**

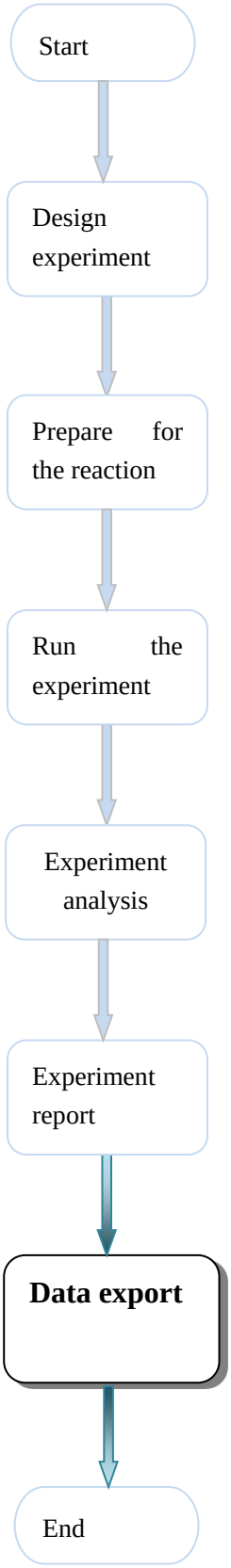
1. Click **Report** ▶ **QC Summary**



2. Check the QC summary



6. Data Export



This section describes how to export data and covers exporting to a database, experiment filing and exporting the experiment data to EXCEL.

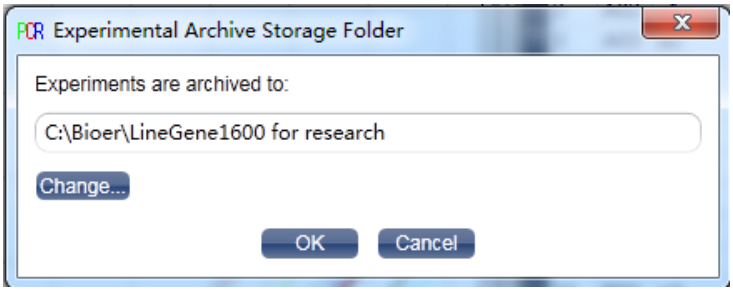
6.1 **Export to Database**

Click **Data Summary** ▶ **Export to Database** ▶ the Save File dialog box will pop up ▶ save the exported database file

6.2 **Experiment Filing**

1. Set filing experiment storage folder

Click **Data Summary** ▶ **Archived Experiment Directory** ▶ the Experimental archive storage directory window will pop up ▶ set up the storage path of file



2. Experiment filing

Click **Data Summary** ▶ **Archived Experiment** ▶ export the filed experiment file

※The suffix of filed experiment file is .fqh

6.3 Export Experiment Data to EXCEL

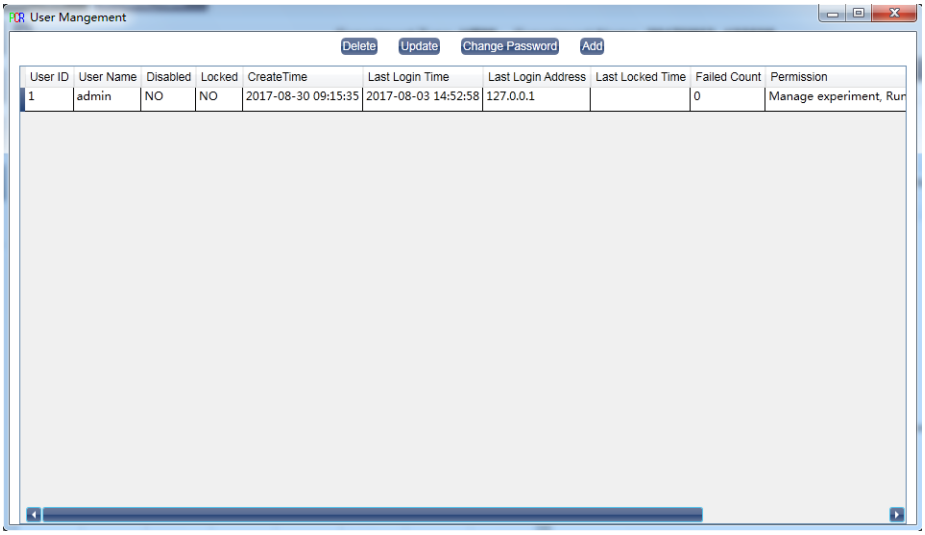
Click **Data Summary**▶**Export Experiment** ▶ **Export Experiment to Excle** ▶the exported experiment data will generate EXCEL file.

Chapter 8 Upper Machine Service

1. User Management

User management is used to manage user information

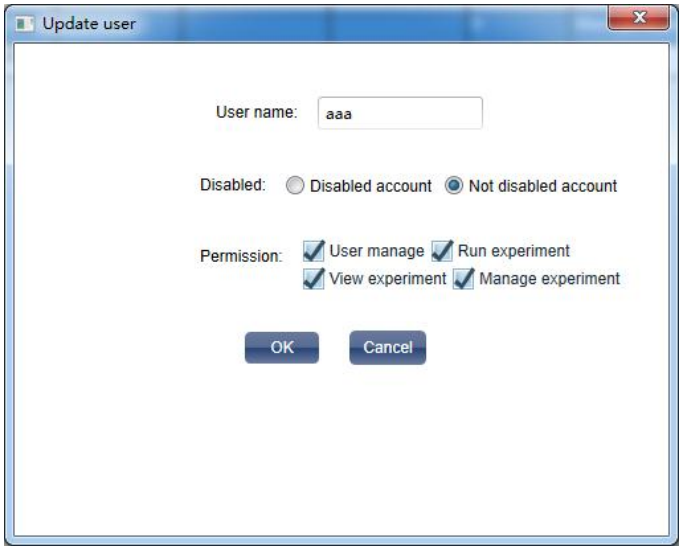
Click **Service►User Management** on the menu bar



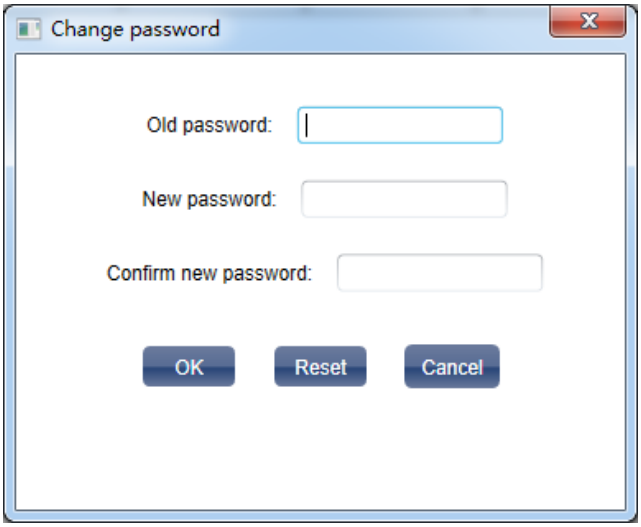
The user can:

a. delete user

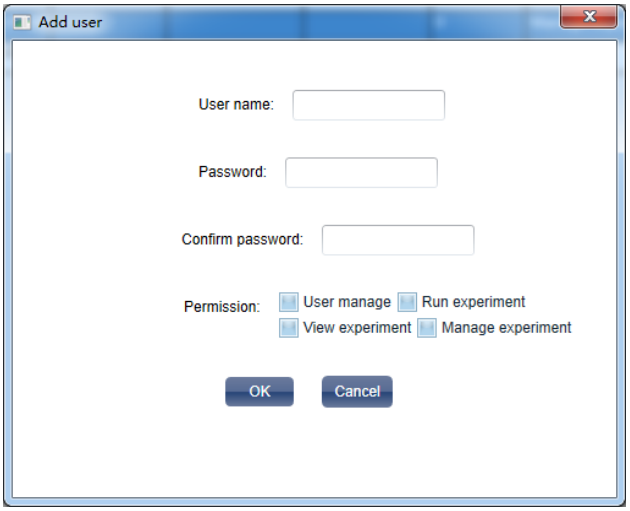
b. update user



c. change password



d. add user



2. 2. Experiment Management

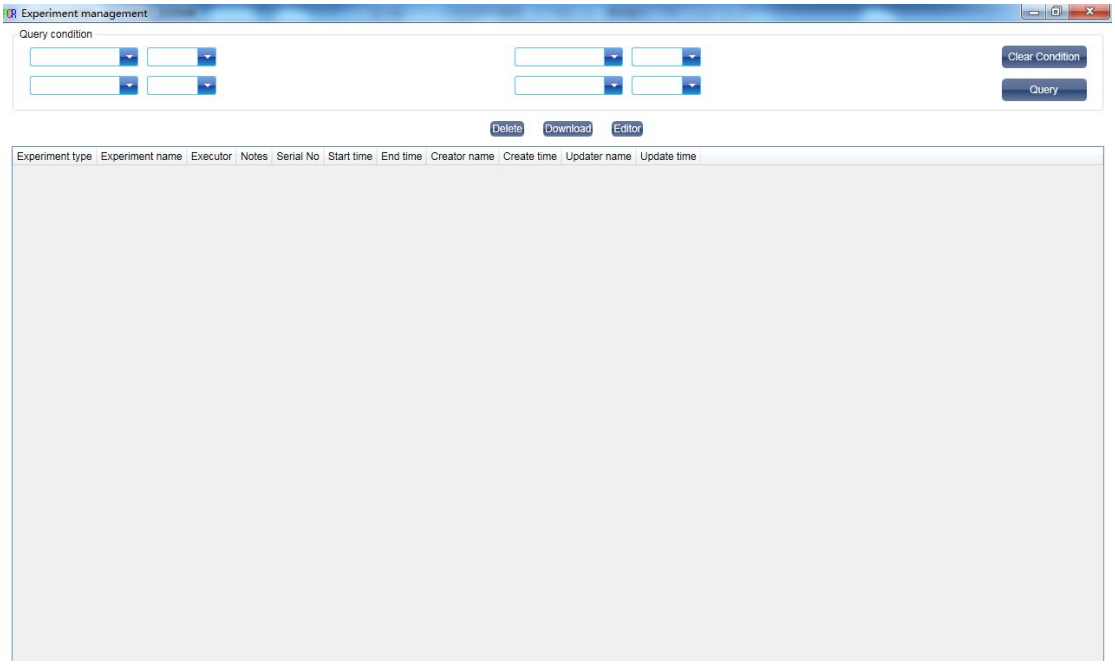
Experiment Management is used to manage experiment information and deleted experiment information

2.1 Experiment Management

Click **Service►Experiment management► Experiment management** on the menu bar, the user can：

a. clear query condition
b. set query condition
c. cquery

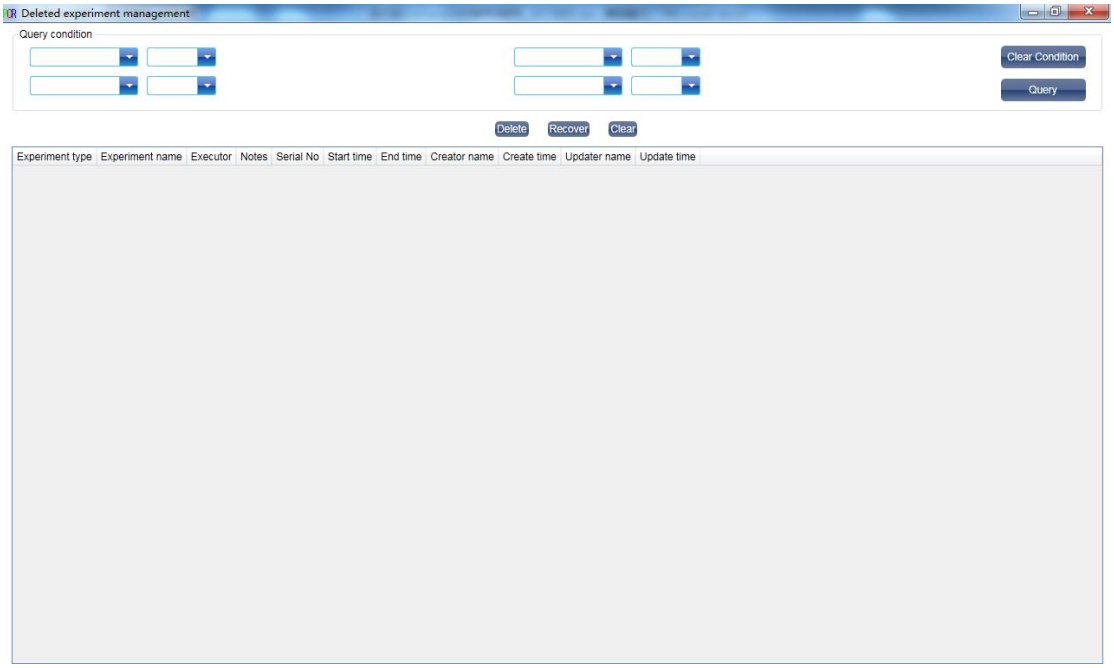
d. delete experiment
e. download experiment
f. edit experiment



2.2 **Deleted Experiment Management**

Click **Service►Experiment Management►Deleted Experiment Management** on the menu bar
The user can：

a. clear query condition
b. set query condition
c. query
d. delete experiment
e. recover experiment
f. clear experiment



3. Template Management

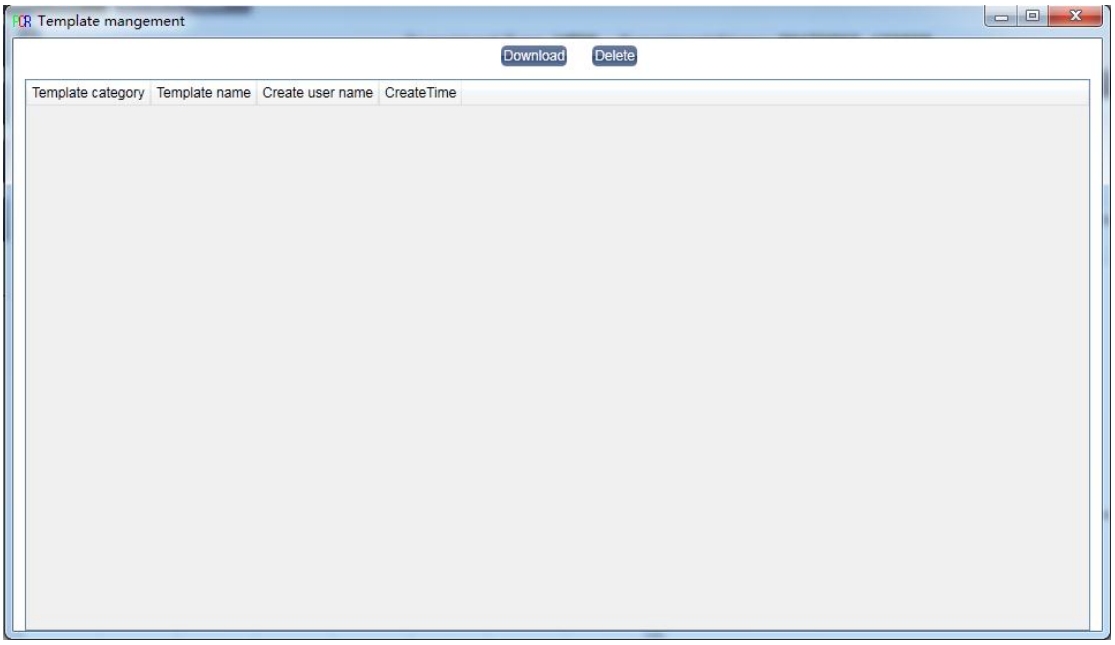
Template Management is used to manage template information。

Click **Service►Template Management** on the menu bar

The user can：

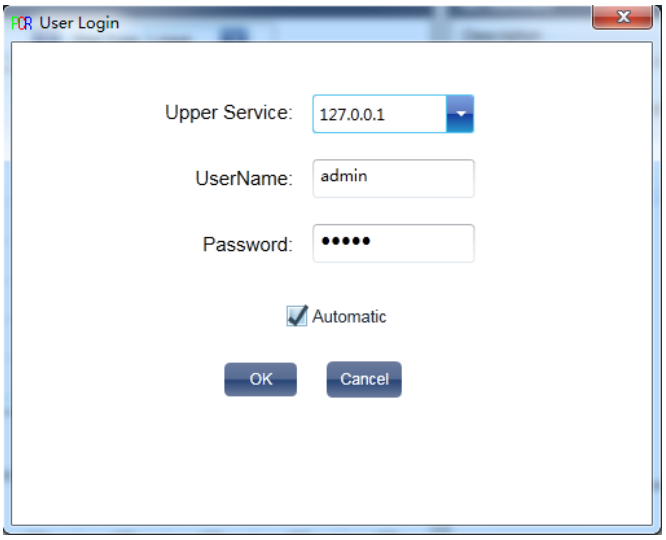
a. download template

b. delete template



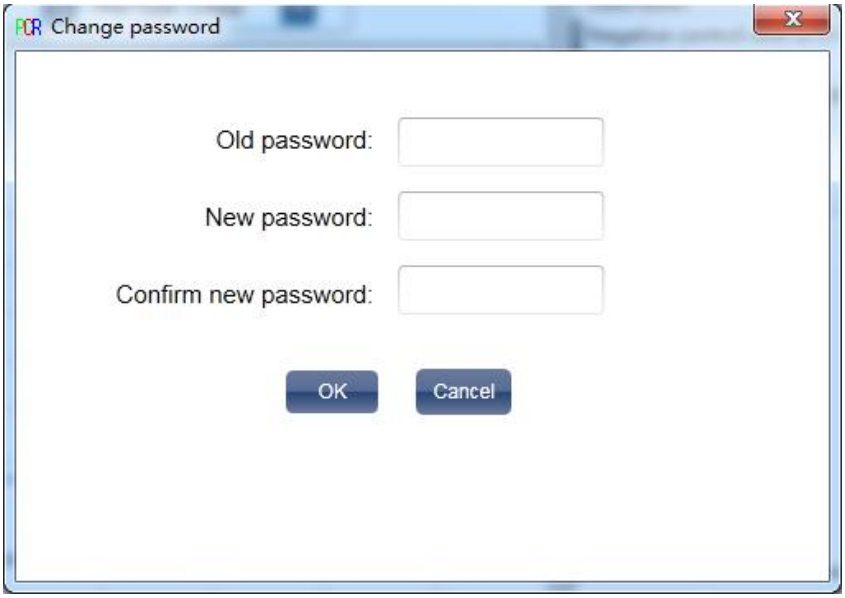
4. User Login

Click **Service►User Login** on the menu bar



5. Change Password

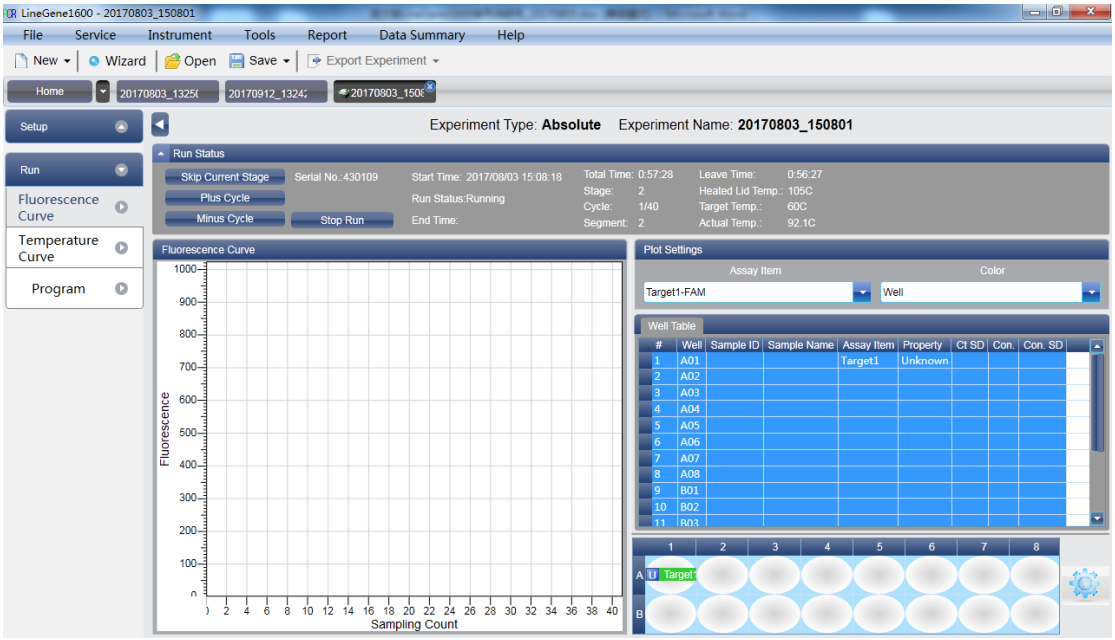
Click **Service►Change Password** on the menu bar



6. See Running Experiment

See Running Experiment is used to see running experiment which is running on connected instrument。

Click **Service► See Running Experiment** on the menu bar



Chapter 9 Tool Use

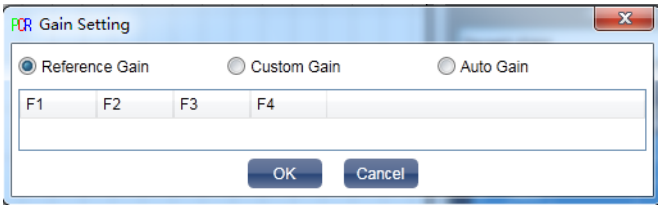
1. Gain Setting

The **Gain Setting** tool is used to set up gain modes.

Click **Tools** ▶ **Gain Setting** ▶ the following window will pop up

Gain setting can be set up as: **reference gain, custom gain and auto gain**

In **Custom Gain** mode, the user can modify the gain value.



2. Detector Library

The **Detector Library** tool is used to set up the inspection libraries of absolute quantitative, relative quantitative and SNP analysis.

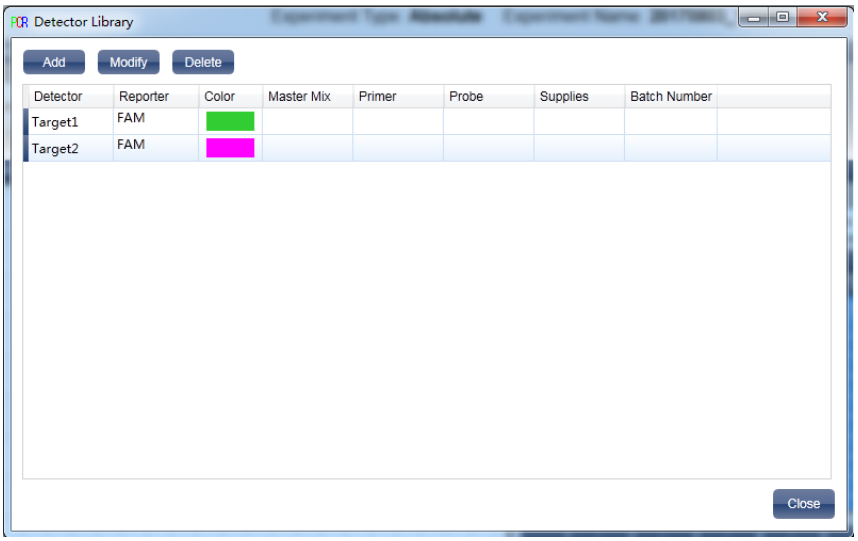
Click **Tools** ▶ **Detector Library** ▶ **(Absolute /Relative/SNP)** ▶ open the following window

The user can:

a. Add Detector

b. Modify Detector

c. Delete Detector



3. Customized Dyes

The **Customized Dyes** tool is used to set up existing dyes and newly added dyes.

Click **Tools** ▶ **Customize Dyes** ▶ open the following window

The user can:

a. Create dye

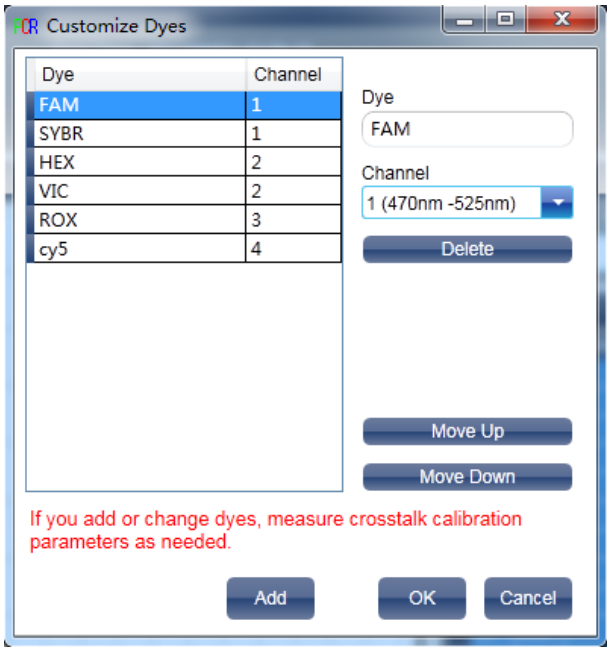
b. Modify dye name and channel

c. Delete dye

d. Move dye upward

e. Move dye downward

※After adding new dyes or modifying dyes, the user should conduct crosstalk parameter measurements.



4. Customize Columns

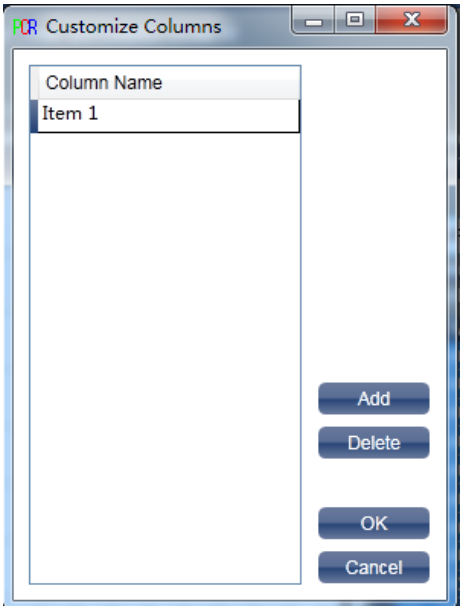
Click **Tools** ▶ **Customize Columns** ▶ the following window will pop up

The user can:

a. Add columns

b. Delete columns

c. Modify column name



5. Column Selection

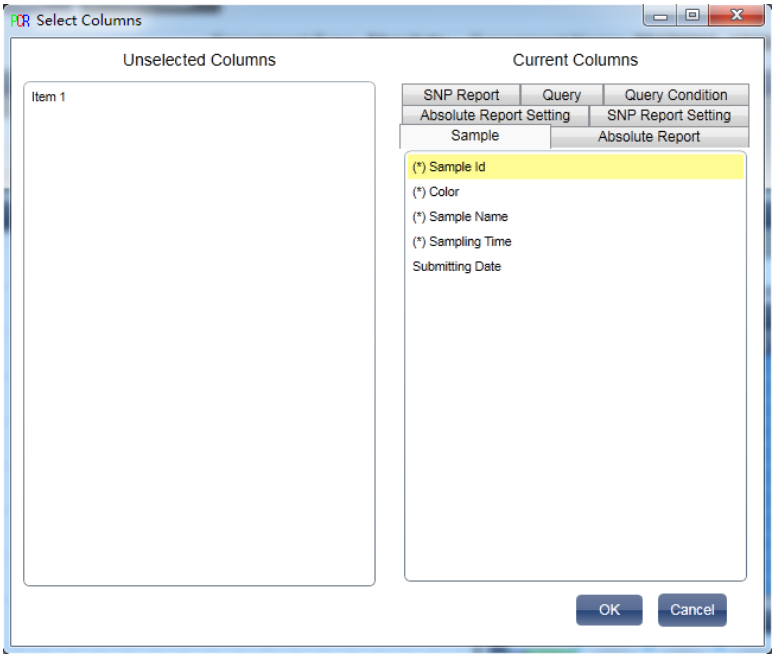
The **Select Columns** tool is used to add the new columns in above section into current existing columns, or remove existing columns in current column.

Click **Tools** ▶ **Select Columns** ▶ the following window will pop up

※1. Current existing column items include sample, report, report setting, query and query condition

2. Double click column can add or remove a column

3. Column with (*) indicates it cannot be removed



6. Sample Column Library

The **Sample Column Library** tool is used in the experiment design phase. The user can select the definition of contents in the drop-down box when setting up sample information.

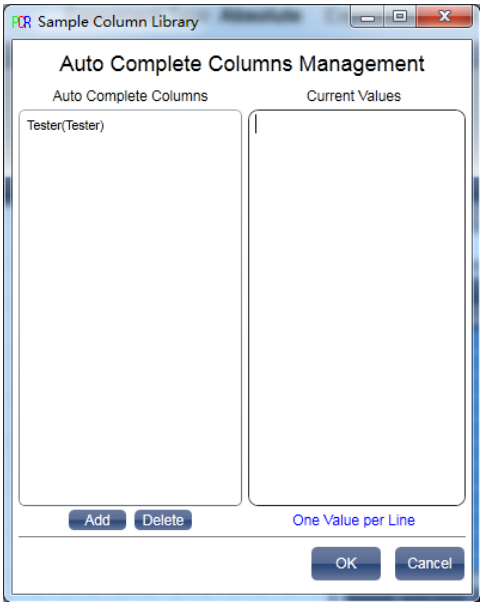
Click **Tools** ▶ **Sample Column Library** ▶ the following window will pop up

The user can:

a. Add columns

b. Delete columns

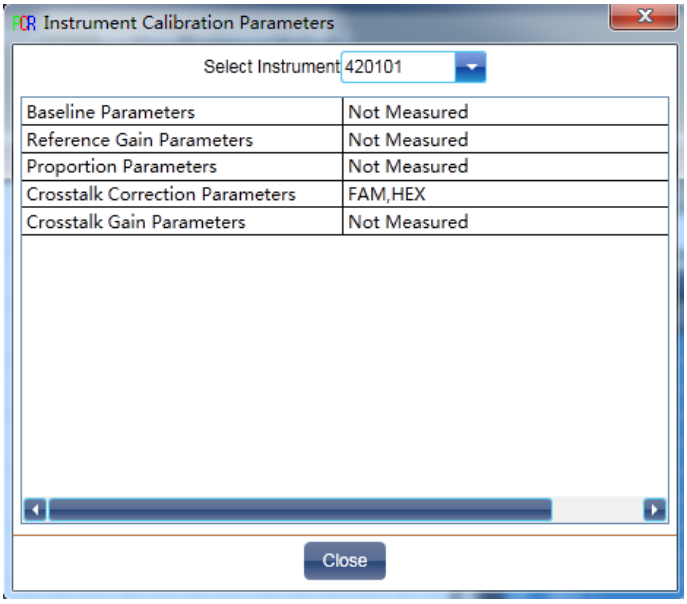
c. Edit the columns content



7. Instrument Calibration Parameters

The **Instrument Calibration Parameters** tool is used to calibrate the instrument parameters.

Click **Tools** ▶ **Instrument Calibration Parameters** ▶ the following window will pop up

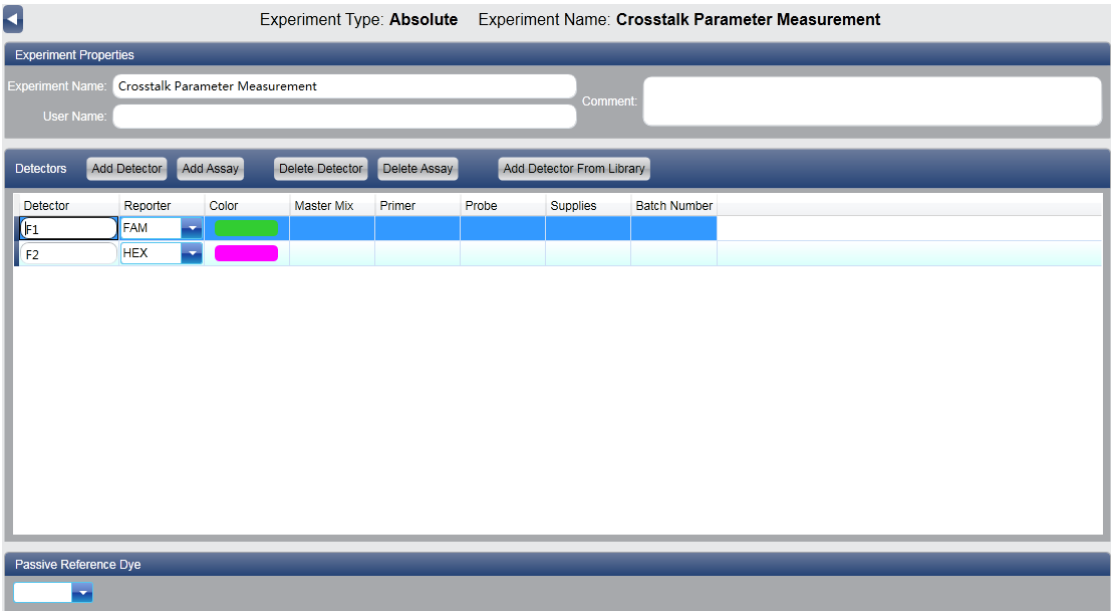


8. Measure Crosstalk Calibration Parameters

The **Measure Crosstalk Calibration Parameters** tool is used to measure crosstalk correction parameters.

Click **Tools** ▶ **Measure Crosstalk Calibration Parameters** ▶ the following window will pop up

The user can add and modify the channels to be tested and dyes according to his needs; upload corresponding reaction plates and operate the experiment. When the experiment is over, the system will automatically save the crosstalk correction parameters.

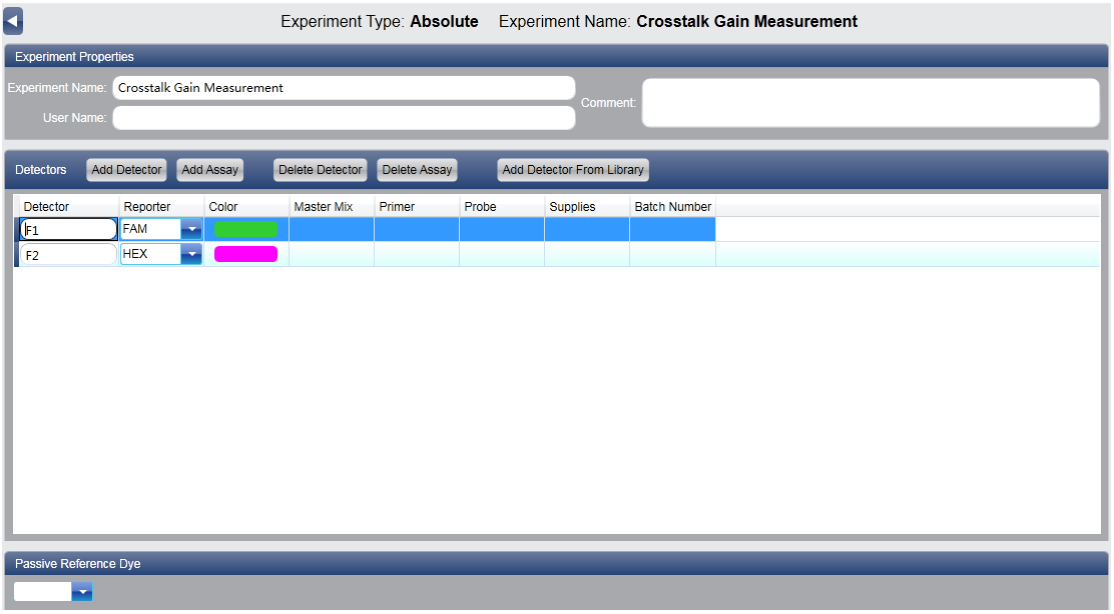


9. Crosstalk Gain Parameter Measurement

The **Crosstalk Gain Parameter Measurement** tool is used to measure crosstalk gain parameters.

Click **Tools** ▶ **Measure Crosstalk Gain Parameters** ▶ the following window will pop up.

The user can add and modify the channels to be tested and dyes according to his needs; upload corresponding reaction plates and operate the experiment. When the experiment is over, the system will automatically save the crosstalk gain parameters.

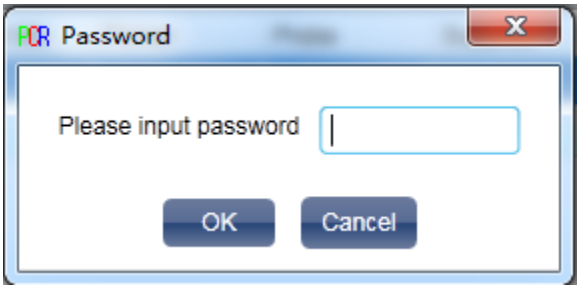


10. System Maintenance

The **System Maintenance tools** are used for system maintenance.

Click **Tools** ▶ **System Maintenance** ▶ the Password Input box will pop up ▶ input the correct Password ▶ conduct the following settings:

a. Y-axis commissioning
b. X-axis origin calibration
c. Machine serial number setting
d. Photomultiplier setting
e. Runtime zero clearing
f. Background measurement
g. Reference gain measurement
h. Fluorescence incremental calibration
i. Firmware Upgrades



Firmware Upgrade tools are used to upgrade the firmware.

Software updates are achieved by connecting to the computer with the RS232 interface supplied with the instrument.

- Set the MODE update switch of the communication box on the back of the instrument to the right hand side ▶Update.Switch the power on and connect the serial port line.The instrument is in update status.In the panel, indicator light flashes green and red at the same time, which is normal.
- Click **Tools** ▶ **System Maintenance** ▶**Firmware Upgrade** ▶ the following window will pop up.

The user can:

a. Select serial ports

b. Select the BIN file to be upgraded

c. Upgrade

11. Upgrade Experiment File Format

The Upgrade Experiment File Format tools are used to convert old files with the suffix of .fqj or .fqs into new files with the suffix of .fqd.

Click **Tools** ▶ **Upgrade Experiment File Format** ▶ the following window will pop up.

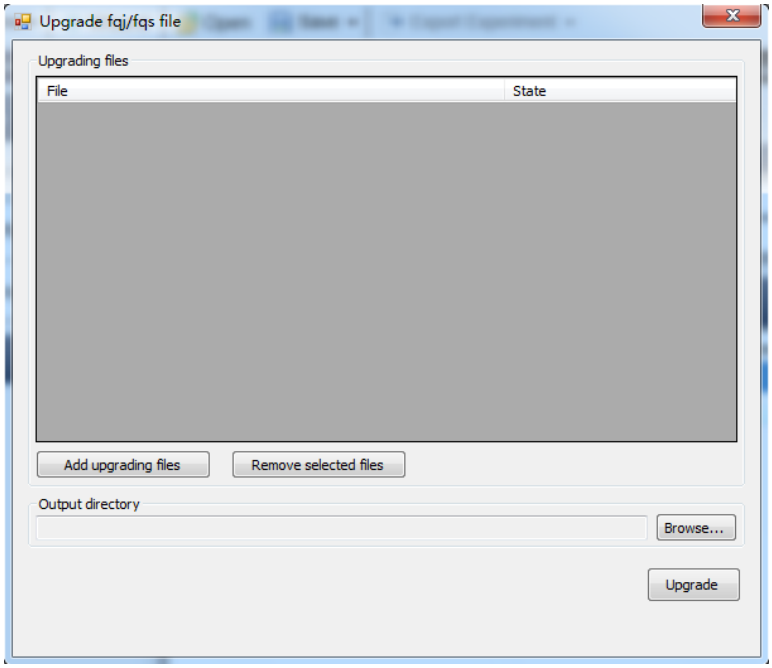
The user can:

a. Add files to be upgraded

b. Remove selected files

c. Select the output directory of new files

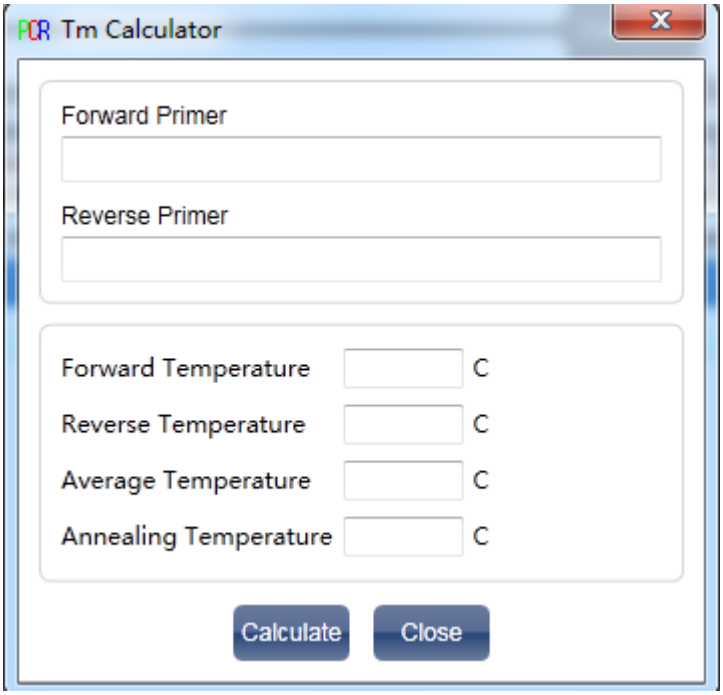
d. Upgrade



12. Ta Calculator

Click **Tools** ▶ **Ta Calculator** ▶ the following window will pop up.

Input Forward Primer and Reverse Primer,click Calculate to gain Forward Tempperature、Reverse Tempperature、Average Tempperature and Anneling Tempperature.



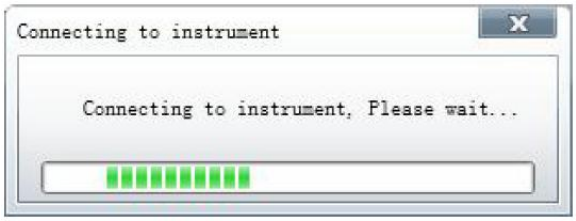
Chapter 10 Other Functions

1. Instrument Operation

The Instruments operations include **Connect** instrument, **Disconnect** instrument and **Instrument Information**.

1.1 Connect

Click **Instrument** ▶ **Connect** ▶ select port number or select automatic port matching.



When the instrument is connected, the icon on the status bar will be  ; if the instrument is disconnected, the icon on the status bar will be  .

1.2 Disconnect

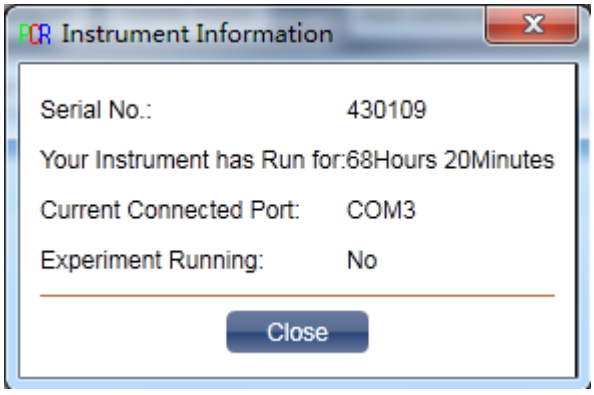
Click **Instrument** ▶ **Disconnect** ▶ disconnect currently connected instrument

1.3 Instrument Information

When the instrument is connected, the user can check the instrument information.

Click **Instrument** ▶ **Instrument Information** ▶ the following dialog box will pop up

Instrument information includes instrument serial number, runtime, currently connected ports, and whether an experiment is in operation.



2. Data Query

Data Query is used to query the data already exported to the database.

Click **Data Summary** ▶ **Data Query** ▶ the following window will pop up

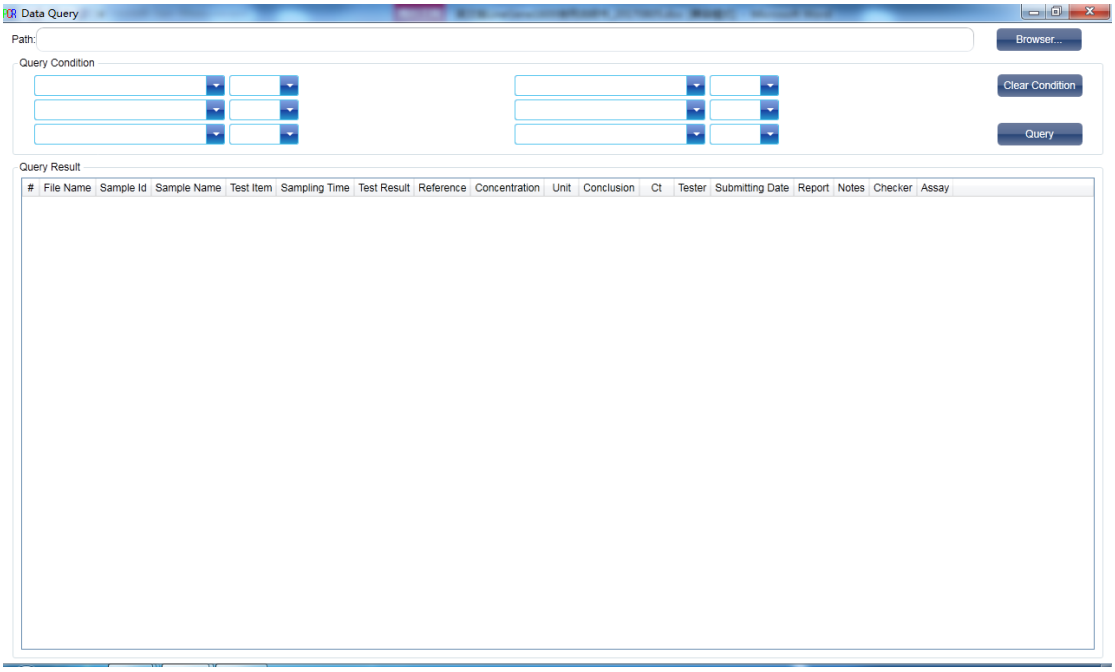
The user can:

a. Select database files

b. Set up query condition

c. Query

d. Clear all query conditions



3. System Help

Click **Help** ▶ **Help Topics**

Chapter 11 Maintenance

1. Regular cleaning

In order to ensure normal operation, detection and use, the instrument needs to be cleaned regularly.

- To clean the outer surface: Clean only with a soft cloth, and if necessary, the cloth may be soaked with alcohol, distilled water or a mild detergent.
- To clean the module wells: Loosen the 2 screws at the bottom of the plate, and then press the buttons on the two sides of the shell to take off the front cover. Wells may be cleaned with nail wipes which does not bring dust and if necessary, they may be soaked with 95 percent of absolute ethyl alcohol used in medicine or distilled water.

Warning! 1. Before cleaning this instrument, the power supply must be switched off.
2. When cleaning the conical wells of the module, care must be taken to prevent any cleaning agents from dropping into the wells.
3. The surface of this instrument **MUST NOT** be cleaned with corrosive cleaning agents.
4. In order to avoid scratches or damage to the optics in the wells, **NEVER** use sharp or hard objects to clean the wells.

2. Analysis and Troubleshooting

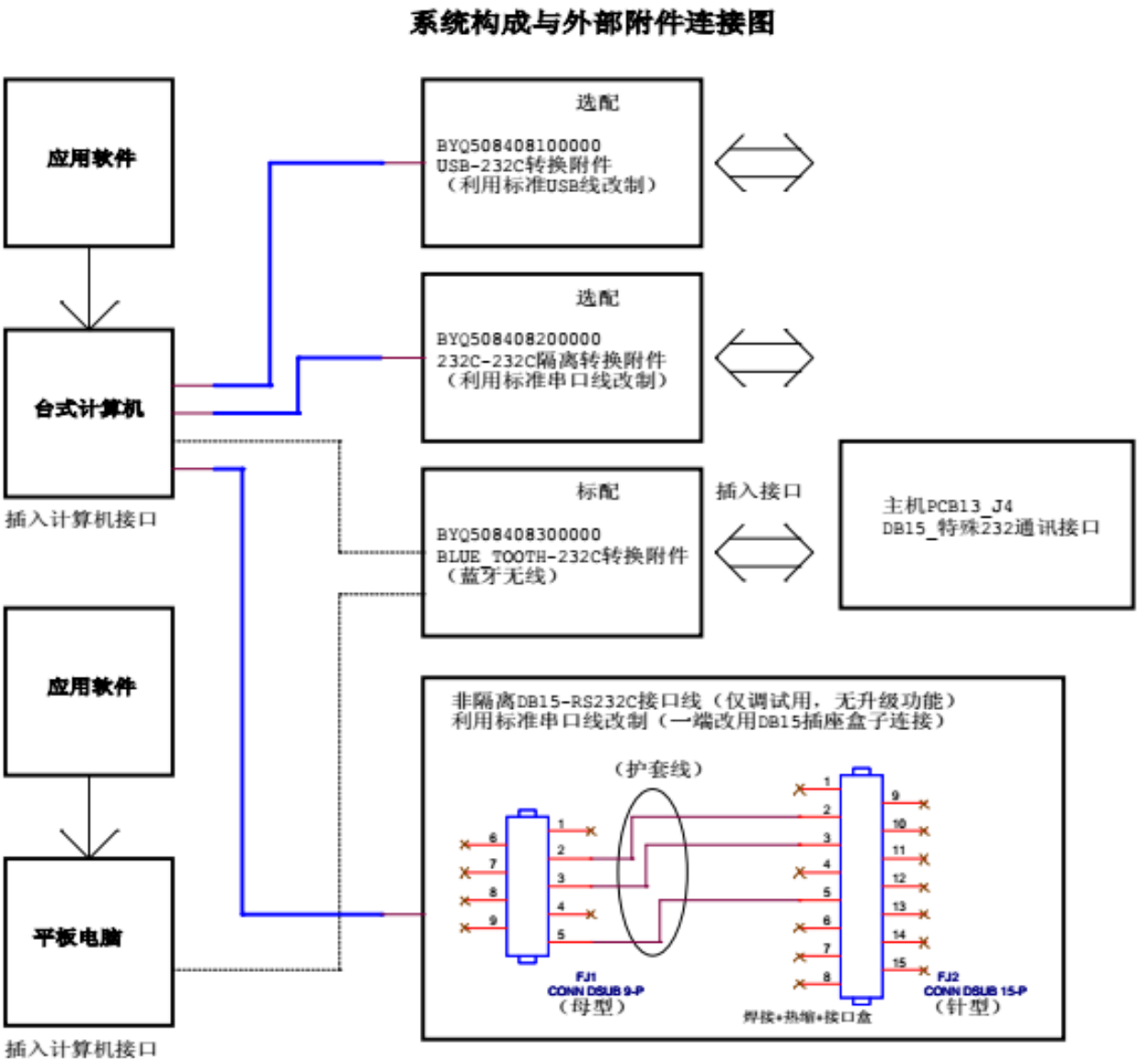
No.	Problem	Possible Cause	Correction
1	The display of system parameters menu requires input of "Password".	The system parameters are for instrument manufacturer's internal calibration and require special accession password.	The function is not required for the end user and for calibration contact the manufacturer or supplier's service personnel.
2	At detecting sample position, the step motor fails to work and the communication fails.	Poor contact or damage of the interface wire	Check, connect or replace interface wire
		The power switch is not turned on or is turned on only after the programme starts running	Turn on the power switch and restart the programme
		The step motor or the drive is damaged	Contact the supplier or manufacturer
3	After detecting sample position, the actual temperature displays 0C or 100C	The module temperature sensor is damaged. It accompanies panel red lamp alarm and a software prompt, and the instrument automatically stops running.	Contact the supplier or manufacturer

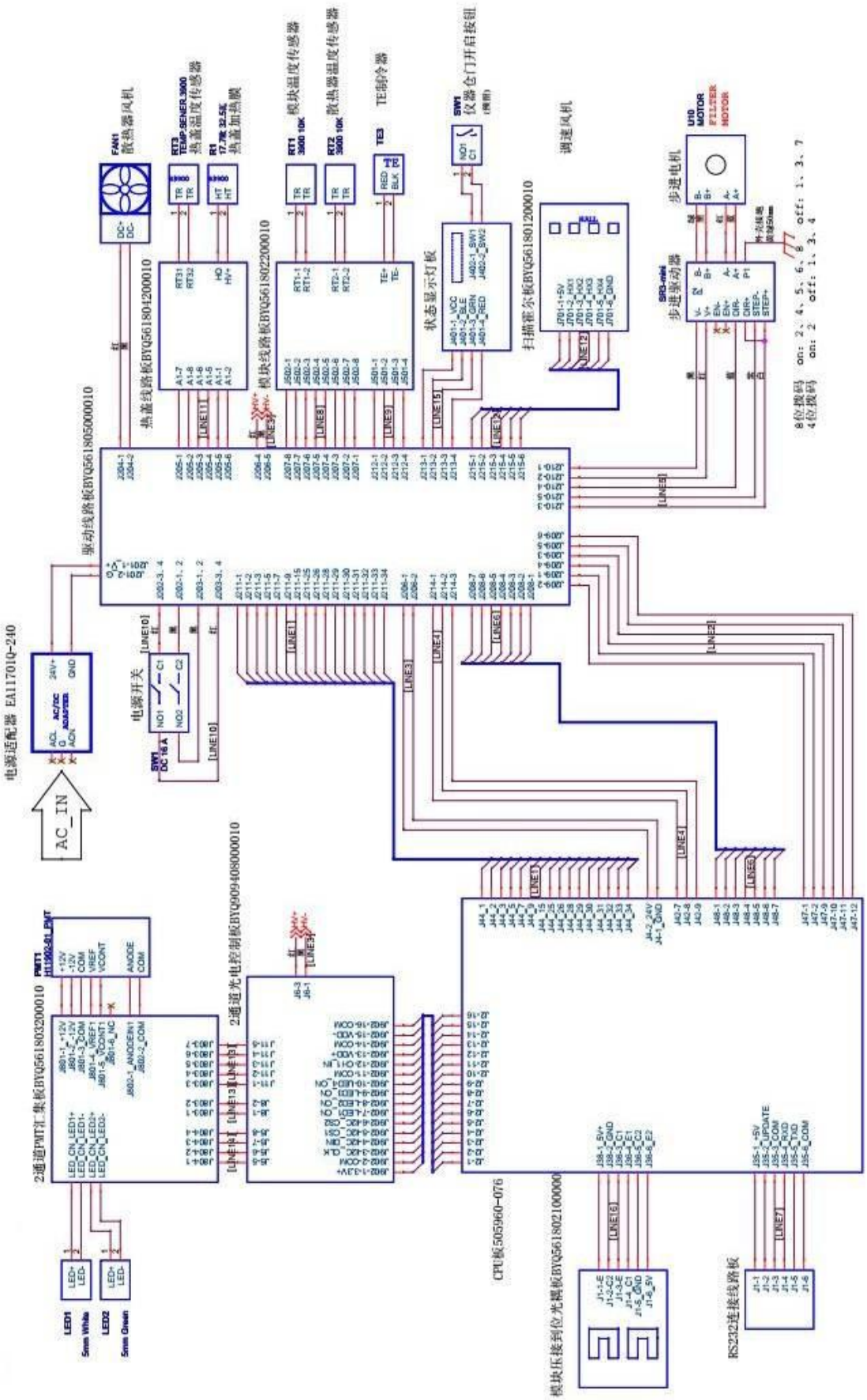
		The power switch is turned on only after the programme starts running	Switch on the power and restart the programme
		The programme is searching communication port and during this period data would be not sent.	If the trouble still exists after researching, contact the supplier or manufacturer
4	Module temperature heating or cooling rate obviously decreases or temperature control is incorrect.	The ventilation opening is blocked.	Clear the ventilation opening
		Loose connection wire	Contact the supplier or manufacturer
		The refrigerating sheet is damaged	
		Fan is damaged or fails to run	
		The temperature sensor is damaged	
5	The module fails to heat and refrigerate.	The inside of the instrument is damaged	Contact the supplier or manufacturer
		The refrigerating sheet is damaged	
		During hot-lid heating-up	Waiting until the hot-lid temperature comes to the target value. When stopping running, module temperature holds down 30C automatically.
6	Abnormal temperature or fluorescence curve: straight line or loss of partial data	The running programme is infected by a virus	After removing the virus, re-install the application software
		Computer configuration fails to meet requirements or the setup of communication port is not appropriate.	Configure as per requirements
7	Yellow lamp on panel lights on	The module is not fully pushed in and the optic coupler fails to detect the module	Push in again, if the light is still on, contact the supplier or manufacturer
8	The hot-lid is will not heat	Thermal-sensitive fuse is damaged	Contact the supplier or manufacturer
		Loose plug-pieces	
		Heating elements of hot-lid is damaged	

		Temperature sensor of hot-lid is damaged	
9	Under no test tube state, the fluorescence value difference between wells increases or the background value is very high.	The test tube well or hot-lid is contaminated, or baseline******.b16 background parameters are set incorrectly.	Eliminate contamination. Each instrument shall correspond to baseline document. After perennial use, offset would occur in the optical elements. In this case, contact the manufacturer to re-calibrate the background value.
10	Reagent evaporation	The PCR tube cap does not sealing tightly enough.	Change consumable to one with a tighter fitting cap.
11	Signal crosstalk among channels	Dye signal crosstalk among channels can happen.	You can measure by using “Crosstalk Measurement”, and save parameters to modify.
12	Fluorescence detection value -abnormal	Irradiation by external strong light	Switch off external light source, or remove instrument from external light source
		During a programme run, the hot-lid is opened	Close the hot-lid (detection result unreliable)
		The photo-electric system is damaged	Contact the supplier or manufacturer

Caution: During the warranty period, opening the instrument casing to inspect the internal workings will invalidate the warranty. If any problems should arise please contact the supplier or manufacturer in the first instance.

Appendix: **LineGene1600** series wiring




电源适配器 EA1170IQ-240
AC_IN
电源开关
驱动线路板BYQ56180500010
热盖线路板BYQ56180400010
模块线路板BYQ56180200010
状态显示灯板
扫描霍尔板BYQ56180120010
调速风机
步进电机
步进驱动器
散热器风机
热盖温度传感器
热盖加热膜
模块温度传感器
散热器温度传感器
TE制冷器
仪器仓门开启按钮
2通道PMT汇集板BYQ56180320010
2通道光电控制板BYQ09408000010
CPU板5059G0-076
模块压接到位光耦板BYQ56180210000
RS232连接线路板
8位拨码 on: 2、4、5、6、8 off: 1、3、7
4位拨码 on: 2 off: 1、3、4

Note:

Note:

Note: