Order: 010-59822688
Toll-free: 800-990-6057 /400-810-6057
TIANGEN BIOTECH (BEIJING) Co., LTD.

Version No.: OS240709

TGreat Real 96 qPCR User Manual



Catalog Number	Product Name
OSE-R96-01-AM	TGreat Real 96 qPCR (4-channel)
OSE-R96-02-AM	TGreat Real 96 qPCR (5-channel)
OSE-R96-03-AM	TGreat Real 96 qPCR (6-channel)

For Research Use Only. Not for use in diagnostic procedures.

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Chapter 1 Attentions

1. Routine practice

Statement	Note that the program contains particularly important information, so please read it carefully. Failure to follow the instructions in the user's manual may result in damage to the instrument or prevent it from working properly.

 **Warning!**	Warning messages require that users take special care in handling a particular step or method. Failure to use the equipment in accordance with the methods specified by the manufacturer may jeopardize the protection provided by the equipment.

2. Safety

The following basic safety measures must be observed during all stages of operation, maintenance and repair of this instrument. Failure to observe these measures or the warnings, Cautions indicated in this user's manual may affect the basic protection provided by the instrument. It also undermines the safety standards for the design and manufacture of the instrument as well as the intended use of the instrument.

TIANGEN Biotech (Beijing) Co., Ltd. shall not be liable for any consequences resulting from the user's failure to comply with the following requirements.

Attention	**This instrument is a class I equipment in accordance with GB4793.1 standard with protection level IP20. This instrument is a product for indoor use.**

2.1 Instrument grounding

In order to avoid electric shock accidents, the input power cord of the instrument must be reliably grounded. This instrument uses a 10A three-pronged grounding plug, which has a 3rd (grounding) prong and can only be used with a grounding type power outlet, which is a safety device. If the plug cannot be inserted into the socket, then an electrician should be asked to install the correct socket and not to make the grounded plug lose its safety protection.

2.2 Stay away from electrically charged circuits

Operators are not allowed to unauthorized disassembly of the instrument protection, replacement of components or in-machine adjustment, if necessary must be completed by licensed professional maintenance personnel, is strictly prohibited in the case of power supply replacement components.

2.3 Power supply used

Before connecting the AC power supply, make sure that the power supply is consistent with the conditions required by the instrument (220 V~,50 Hz), and make sure that the rated load of the power outlet is not less than the maximum load of the instrument of

1,000VA required.

2.4 Power cord

The instrument should normally be used with the supplied power cord. If the power cord is damaged, it must be replaced and not repaired. Replacement must be made with a power cord of the same type and size. When this instrument is in use, do not press anything on the power cord, and do not place the power cord in a place where there is a flow of people. If the power cord will come into contact with a hot surface when used, added protection is required to prevent damage to the power cord insulation.

2.5 Power cord plugging and unplugging

When plugging and unplugging the power cord, be sure to hold the operating part of the plug correctly, and make sure that the plug is inserted completely and tightly into the socket when the plug is inserted, and don't pull or tug on the power cord when pulling out the plug.

2.6 Caution: Placement of the instrument

When handling the instrument, use both hands on the bottom of the instrument's two side panels to pick up the instrument and place it in a suitable position.

This instrument should not be placed in a location where it is difficult to disconnect the power supply.

This instrument should be placed in a place with low humidity, less dust and far away from water sources (e.g., pools, water pipes, etc.), and the room should be well ventilated and free from corrosive gases or strong magnetic field interference. Do not locate the instrument in a damp or dusty place. The table where the instrument is placed should be horizontal and stable.

The openings on this instrument are provided for ventilation circulation; to avoid overheating the body temperature, be sure not to block or cover these vents. When a single instrument is used, the distance between the vents around the instrument and the nearest object should be not less than 30 cm, and when more than one instrument is used at the same time, the distance between each instrument should be not less than 50 cm.

High ambient temperatures can affect the instrument test performance or cause malfunctions. Do not use the instrument in direct sunlight and strong light sources, so as not to affect the instrument fluorescence test, and should be kept away from heaters, stoves and all other heat sources.

When stop working, the power should be turned off. When the instrument is not used for a long time, the power should be cut off, unplugged, and the instrument covered with soft cloth or plastic film to prevent dust and foreign objects from entering.

Warning!	Do not install equipment on combustible material surfaces!

2.7 Caution: Operation

Within 5 minutes of the experimental run, pay attention to whether the temperature of the hot cover reaches the set temperature or not, if there is any abnormality, please

contact the supplier in time.

Keep an operating distance of 30 cm from the front panel of the instrument during test operation.

Avoid dripping liquid on the instrument during test operation. Consumables, reagents and other wastes used in the test should be disposed of in accordance with the relevant requirements, and should not be discarded or dumped arbitrarily.

If there are hazardous substances in the test, users must be trained to operate them before use.

Hazardous substances after use should be properly handled and stored in strict accordance with their instructions for use.

The testers operating the instrument need to be trained and qualified.

Attention	In the following cases, immediately disconnect the power supply, unplug this instrument from the electrical outlet, and contact the supplier or have qualified service personnel handle the situation: • Spilling of liquid into the instrument; • Instruments exposed to rain or water; • The instrument is not working properly, and any unusual sounds or odors are present; • Dropped instruments or damaged cases; • There is a significant change in instrument function.

Attention	Handling potentially infectious substances requires the use of protective gloves or other protective measures if there is a risk of skin contact.

2.8 Re-transportation

If the instrument has to be transported again, the well position and the instrument have to be cleaned and sterilized by UV light before transportation, and when transporting, please use the original packing box and pack it properly for transportation.

2.9 Warning label

2.9.1 Warning label

Caution, danger	Avoid improper use and beware of danger during use by avoiding inappropriate use in locations where this marking is affixed in the instrument.
Caution, burn	The locations in the instrument where this logo is affixed generate high temperatures during use, so be careful of burns.
Biohazard	Where this symbol is affixed in the instrument, the process of using the reagent is biohazardous, so pay attention to the protection.

2.9.2 Warning label 

Warning!	"HOT SURFACE!" is affixed to the instrument. The metal parts (modules) in the vicinity of this mark are strictly forbidden to be touched with any part of the body during the operation of the instrument or for a certain period of time just after the program operation to avoid burns!

Warning!	The instrument may be used in a manner that exposes the operator to or leaves residues of substances that are harmful to organisms or infectious. The operator should be aware of the hazards and strictly follow the environment of this instrument should be in accordance with the relevant regulations of the national PCR laboratory. Operators need to be trained and qualified.

3. Instrument maintenance

If there is any stain on the surface of this instrument, use a soft cloth with cleaning paste to clean it.

The use of heat transfer oil media is prohibited in the module well of this instrument.

This instrument should be stored normally and the drawer should be closed at the end of use to prevent the accumulation of dust.

Warning!	The power supply must be disconnected while the instrument is being cleaned. The instrument surface is strictly prohibited to be cleaned with corrosive cleaning agents. The modules of the instrument contain precision optics and should be protected from dust, foreign matter and residual liquid.

Chapter 2 Instrument transportation and installation

1. Instrument transportation and storage conditions

Ambient temperature: -20°C to 55°C

Relative humidity: ≤ 93 %.

Atmospheric pressure: 75 kPa to 106 kPa.

2. Normal working conditions

Ambient temperature: 10°C to 30°C

Ambient humidity: ≤80%

Power supply: 220V~50Hz 1000VA

Attention	Before using the instrument, make sure that the operating conditions meet the above requirements. Pay special attention to the fact that the power socket is a 3-hole socket and that the grounding is reliable.

3. Preparation of the instrument before startup

Connection of power cord: The power cord supplied with the instrument should be used. When connecting, the instrument power switch should be off; after connecting, check whether the power cord and the instrument socket are too loose, otherwise replace a new one.

Network cable connection: The instrument itself comes with its own operating system. However, if users need to use a computer for experimental operation, use a network cable to connect the network port at the rear of the instrument to the computer.

Attention	The supplied power cord is reliable, but after many plugs and unplugs, it may cause the plug to become too loose. In this case, the power cord should be replaced. The power cord should be replaced with one of the same size.

4. Installation of software

4.1 Selection of computer systems

4.1.1 System environment

Operating System: Windows XP/Windows Vista/Windows 7/Windows 8

Running environment: Net Framework 4.0

Other software: PDF Reader

4.1.2 Minimum configuration

Processor: Intel Core i3

Memory: 2 GB

Hard disk: 10 GB

4.2 Installation of software

Double-click the Real 96 Server installation file to display the installation screen (select the installation language) Set the installation path to install

Double-click the TGreat Real 96 installation file to display the installation screen (select the installation language) Set the installation path to install

4.3 Uninstallation of software

Control Panel Add/Remove Programs Real 96 Server Uninstallation

Control Panel Add/Remove Programs TGreat Real 96 Uninstallation

5. Startup

5.1 Pre-startup inspection

Before plugging in the power-on and applying power to this inspection system, the

following should be verified:

Is the power supply compatible with the voltage required by the system;

Verify that the power cord plug is properly and reliably inserted into the electrical outlet;

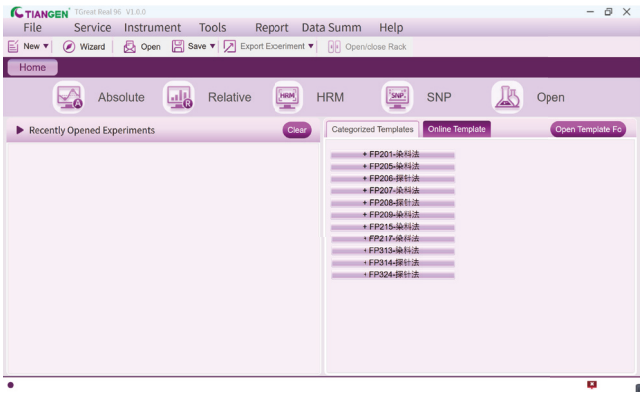
Whether the surrounding working environment and the placement conditions of the instrument meet the requirements.

5.2 Startup

The following sequence should be followed for startup of the system:

Step 1: Turn on the power switch on the rear bottom panel of the instrument;

Step 2: After entering the operating system, the TGreat Real 96 software will start automatically, and the screen of the software after starting is shown below:



Chapter 3 Instrument introduction and packing content

1. Scope of application of product

The product is based on the principle of fluorescence quantitative polymerase chain reaction and is used together with the accompanying test reagents for qualitative and quantitative test of analytes in DNA/RNA of nucleic acid samples.

2. Product features

- 10.1 large screen design, touch software operation, new, humanized operation, operation interface, smoother operation
- Real-time fluorescence test allows simultaneous amplification and test in the same tube without post-processing.
- Advanced thermoelectric refrigeration technology ensures ultra-high-speed thermal cycling system heating, rapid and stable cooling

- Multi-point temperature control to ensure higher temperature uniformity across 96 sample wells
- 6 zones temperature control function, each zone temperature difference range 0-6°C
- SOAK's thermostat function enables cryopreservation of PCR reagents.
- Adoption of long-life white LED excitation light source, no need for maintenance
- Advanced fiber optic conduction technology, making the photoelectric inspection system more sensitive and reliable
- Large linear range up to 10 orders of magnitude of starting DNA copy number without gradient dilution
- Built-in high sealing hot cover, can be automatically adjusted up and down, to realize the constant pressure of the test tube, and effectively prevent the reagents from volatilization
- Flexible program settings, comprehensive analysis and reporting functions, all parameters can be stored
- Multiple or single sample reports can be printed

3. Structural composition of product

This product is mainly composed of control parts, hot cover parts, heat cycle parts, photoelectric parts, transmission parts, power supply parts and operation software.

4. Technical parameters

Cat. No.	OSE-R96-01-AM	OSE-R96-02-AM	OSE-R96-03-AM
Sample capacity	96×0.2 ml, suitable for single tube (top transparent), 8-stripe (top transparent) and 96-well plate (no skirt, half skirt).		
Reaction system	5~100 μl		
Detection channel	4	5	6
Fluorescent dye	F1: FAM,SYBR, Green I F2: HEX, VIC, TET, JOE, CY3, TAMRA F3: ROX, TEXAS-RED F4: Cy5, Quasar-670	F1: FAM,SYBR, Green I F2: HEX, VIC, TET, JOE, CY3, TAMRA F3: ROX, TEXAS-RED F4: Cy5, Quasar-670 F5: Cy5.5,Quasar-705	F1: FAM,SYBR, Green I F2: HEX, VIC, TET, JOE, CY3, TAMRA F3: ROX, TEXAS-RED F4: Cy5, Quasar-670 F5: Cy5.5,Quasar-705 F6: Customized
Fluorescence detection wavelength	500~800 nm		
Excitation light wavelength	300~800 nm		

Excitation light source	Long-life LED light source, maintenance-free
Resolution	Differentiate copy number differences as low as 1.5-fold in a single reaction.
Dynamic range	$1\sim10^{10}$
Repeatability	CV value CV≤0.2%
Maximum ramp-up rate	6.5°C/sec
Temperature control precision	≤±0.1°C
Temperature uniformity	≤±0.2°C
Sample compartment	Full-automatic probe-out sample bin design for easy operation
Temperature control mode	Block control and analog tube control
Thermal cover temperature range	30~110°C (adjustable, default 105°C)
Module operating temperature range	4~105°C (increment of 0.1°C)
Gradient control	6-zone temperature control
Interface	USB, Bluetooth
Input power supply	100~240VAC, 50/60Hz, 1000VA
Weight	28 kg
Safety protection and alarms	Over-temperature protection and alarm for the hot cover and over-temperature protection for the switching power supply.

5. Packing content

Name	Quantity	Unit
TGreat Real 96 Fluorescent Quantitative PCR Instrument	1	Set
Pressure balance plate	1	Nos.
Wireless mouse	1	Pcs
Wireless keyboard	1	Pcs
Power cord	1	Pcs
Fuse	2	Nos.
User's manual	1	Copy
Performance test report	1	Copy
Product warranty card	1	Copy
Certificate of conformity	1	Copy

6. Quality assurance

The warranty period of our TGreat Real 96 Fluorescence PCR Instrument is one year. Damage to the instrument caused by the user's improper use and operation, use under non-compliant conditions, and unauthorized repair or modification is not covered by the warranty. The warranty does not cover normal wear and tear and damage due to radioactive contamination and cleaning.

Chapter 4 Absolute quantification

1. Design of experiments

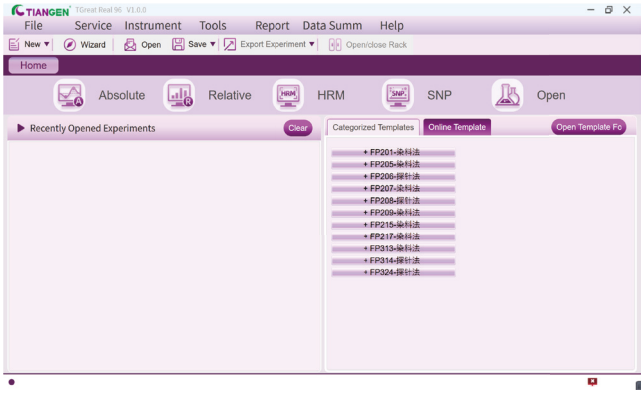
1.1 New absolute quantification experiment

There are three ways to create a new absolute quantitative experiment:

(1) Click "File" in the toolbar, select "New", and choose "Absolute".

(2) Click on the "New Experiment" drop-down menu in the menu bar and select "Absolute".

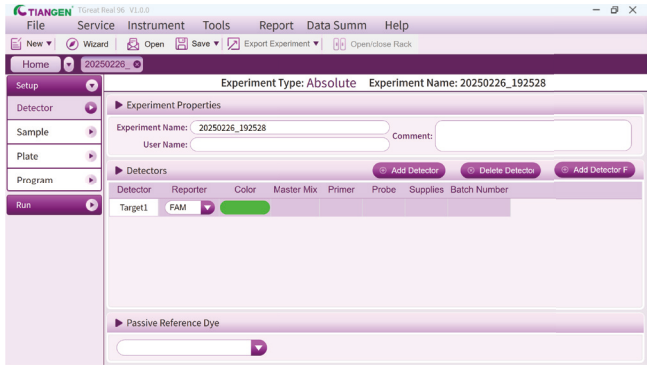
(3) Click "Absolute" on the home screen.



1.2 Setting

After creating a new experiment, users can set the target gene, sample information, reaction plate, and program settings.

1.2.1 Click "Detector", the screen will be shown as below:



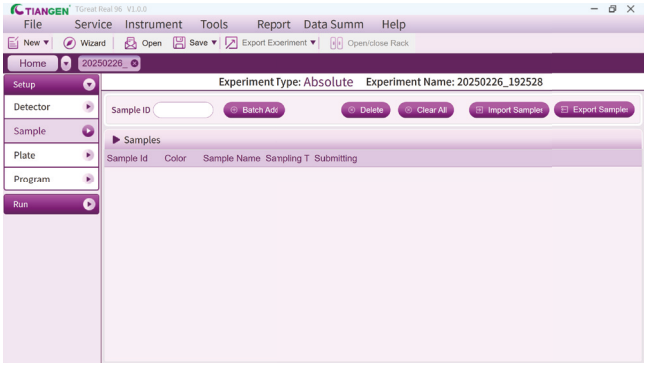
In the "Experiment Properties" field, users can change the experiment name, user name, and note information. The default experiment name is the time when the new experiment was created. In the "Detector" field, users can edit the gene name, reporter fluorescence, color, Master Mix, primers, probes, consumables, and batch number. If users have more than one test gene, users can click "Add Detector".

To delete a detector, select the row where the gene is located and click "Delete Detector".

By clicking "Add library item", users can directly add detectors in the library, or save frequently used detectors into the library.

In the "Passive Reference Dye" field, users can select a reference fluorescence, such as ROX, or users do not need to select one if there is no reference fluorescence.

1.2.2 Click "Sample", the screen is shown below:



To add sample information, users can choose to add them one by one or in a batch.

(1) Add one by one: Enter the sample number in Sample ID and press the Enter key, then one sample information will be added.

(2) Batch Add: Click "Batch Add" to bring up the batch add window, enter the starting

sample number and the number of samples, then users can add them in batch.

Deleting sample information can be done either line by line or all together.

(1) Delete item by item: Select the samples to be deleted and click "Delete" to delete the selected samples.

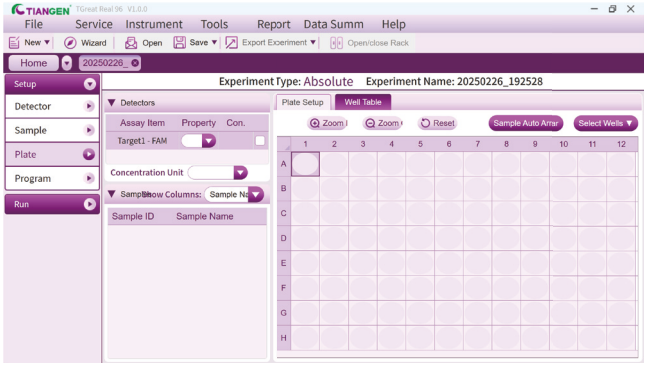
(2) Clear All: Click "Clear All" to delete all sample information.

Import/export sample information.

(1) Click "Import Sample Information" to import a CSV format sample information file in the pop-up file import window.

(2) Click "Export Sample Information" to export the sample information file in CSV file format in the Save As window.

1.2.3 Click "Plate", the screen is shown as below:



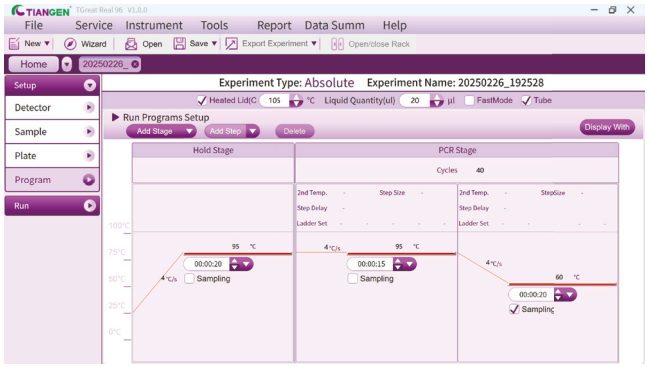
Select the corresponding well position in the reaction plate list, check the corresponding detector in the detector column on the left, and select the properties of the detector. The attributes, concentrations and concentration units of the detectors are shown in the table below, and the user can set the corresponding attributes of the detectors as well as the concentrations according to his/her own experiments.

Attribute	Name	Concentration	Concentration unit
U	Unknown	None	Copies/ml
S	Standard	Yes	IU/ml
N	Negative	None	Fg/ml
P	Positive	None	Pg/ml

If "Standard" is selected for the target gene attribute, the corresponding gradient concentration can be entered in the concentration field so that the standard curve can be viewed directly in the software for subsequent experimental analysis.

Right-click on a reaction plate well position to copy, paste, or add a new target gene.

1.2.4 Click "Program", the screen is shown below:



The new run program setup allows users to add run segments and run sections. Run segments include thermostatic segments, cycle segments, melting segments, and infinite cycle segments. Run sections are contained in individual run segments and can be added and deleted depending on the experiment.

The default program has only thermostat and cycle segments. To create a new segment, select the segment in front of the location users want to add, click the "Add Stage" drop-down menu, and select the segment users want to add. To create a new runtime section, select the location where users want to add it, click on the "Add Step" drop-down menu, and select "Before" or "After". If users want to delete a segment or section, select the segment or section to be deleted and click "Delete".

Heated Lid: the default is 105°C, users can directly input the number or click the arrow after the number to change the temperature, the hot cover temperature range is 30~110°C.

Liquid Quantity: default is 20 µl, can be modified according to the actual reaction system.

Fast Mode: Check the "Fast Mode" box to run the program at the maximum rate of temperature rise and fall, reducing the run time.

Tube: The default check box is reaction tube mode temperature control, if unchecked, it will be module temperature control.

Temperature: Users can change the temperature by directly entering a number, or by clicking the up and down arrows next to the temperature.

Time: The time is displayed in hours: minutes: seconds, users can directly enter the time, click the up and down arrows, users can change the time in "1 sec" as a progress, users can also directly click the drop-down menu to select the time.

Read Fluorescence: Checking "Sampling" will result in fluorescence acquisition in this run section, with fluorescence being read by default in the extension section of the cycle section, and by default in the last section of the melting section.

Cycles: the default is 40 cycles, if users want to change it, directly input the cycle number to change, or click the up and down arrows to change the cycle number.

Gradient setting: Enter the gradient difference value in the step setting in the constant temperature section, the instrument is 6-zone temperature control, users can set 6 gradient difference values respectively. The temperature range of the gradient setting is 0-6°C.

2. Prepare to react

2.1 Preparation of reagent sample

System: TGreat Real 96 fluorescence quantitative PCR instrument using 0.2 ml centrifuge tube to place the reagent samples, the sample optimal reaction system is recommended 10~50 μl.

Consumables: The instrument allows the use of standard 0.2 ml single tubes (clear top), 0.2 ml 8-stripes (clear top), 0.2 ml semi-skirted 96-well PCR plates, and 0.2 ml unskirted 96-well PCR plates.

Centrifugation: The PCR tube or 96-well PCR plate in which the reagent samples are placed should be centrifuged in a centrifuge before placing it in the instrument to ensure that the reagent samples are at the bottom of the centrifuge tube or 96-well PCR plate and that the interior is free of air bubbles.

2.2 Installation of reaction plate

Click "Open/Close Hatch" in the menu bar to open the sample chamber.

96-well PCR plate placement: 96-well PCR plate can be placed directly and smoothly.

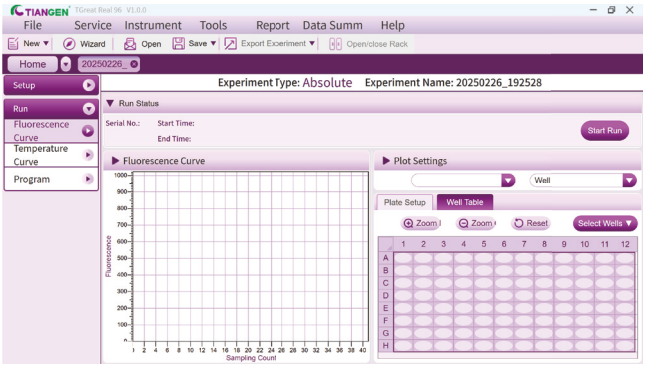
8-stripes: An empty 8-stripe needs to be placed in each of columns 1 and 12 to equalize the hot cover pressure.

Pressure balance. When placing the reaction tubes, try to evenly distribute the tubes in the module well to ensure that the hot cover presses smoothly on the top of the tubes during operation, and that the module is evenly loaded to ensure uniform and consistent temperature changes across the tubes.

Note: Make sure that the reaction tubes are lined up in the same layout as in the reaction plate setup.

3. Running program

Click "Run", the screen is shown below:



Click "Start Run", a dialog box will pop up to confirm the path to save the experiment and confirm the hot cover temperature and sample volume.

3.1 Fluorescence curve

After the run starts, the real-time fluorescence curve is displayed on this screen, and users can view the program start time, total run time, remaining time, and run status.

Users can also set "Skip Current Segment", "CyclePlus One", "Cycle Minus One" and "Stop Run". The following settings are also available.

3.2 Temperature curve

Once the run starts, this screen displays the real-time temperature change of the hot cover and module, and allows users to view the program start time, total run time, remaining time, and run status.

Users can also set "Skip Current Segment", "CyclePlus One", "Cycle Minus One" and "Stop Run". The following settings are also available.

3.3 Running program

This screen allows users to view the running program, but not to change it.

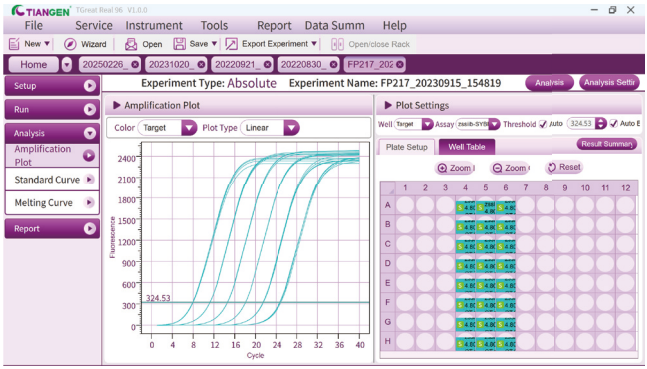
4. Experimental analysis

At the end of the run, the results of this experiment can be analyzed.

If users want to open the results of previous experiments and analyze them, click "File" in the toolbar, select "Open" and find the file users need to open; or click "Open" in the menu bar to find the file users need to open; or users can directly click "Open Experiment" in the home interface to find the file users need to open. or click "Open" in the menu bar to find the file users need to open; users can also directly click "Open Experiment" in the home screen to find the file users need to open.

4.1 Amplification curve

Click on Amplification Curve in Analysis to view the amplification curve, as shown below:



4.1.1 Amplification curve

Color: Set the color of the amplification curve, which can be well position color, target gene color or sample information color.

Linear: Amplification curves can be selected as linear or logarithmic.

4.1.2 Curve display setting

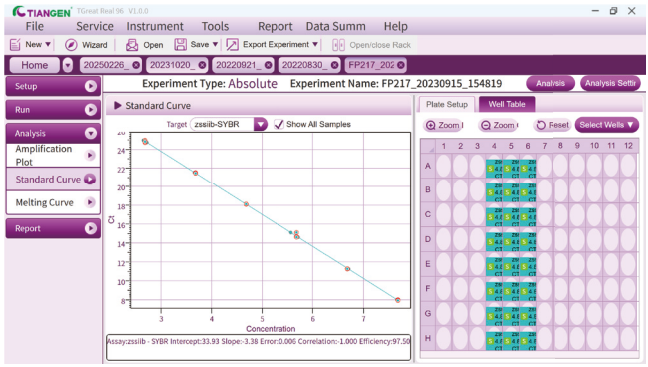
Threshold: Default automatic threshold, if users need to adjust the threshold, uncheck "Automatic" and adjust the threshold manually.

Reaction Plate: Select the reaction plate to display the corresponding amplification curve in the Amplification Curve.

List information: display well position, sample information, target gene, Ct value and other information in list form.

4.2 Standard curve

If the target gene is a standard and the concentration of the target gene has been set in the settings, users can view the standard curve as shown below:

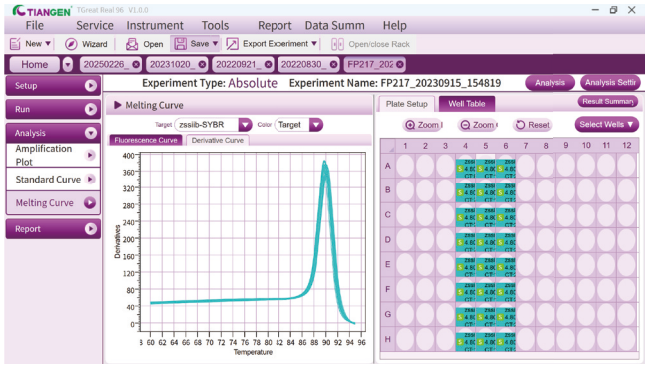


The software can calculate the correlation coefficient of the standard curve and amplification efficiency based on the set sample concentration.

When there are multiple target genes, users can view the standard curves of all target genes at the same time, or users can view the standard curve of only one of the target genes.

4.3 Melting curve

If the dye method is used, the melting curve can be viewed as shown below:

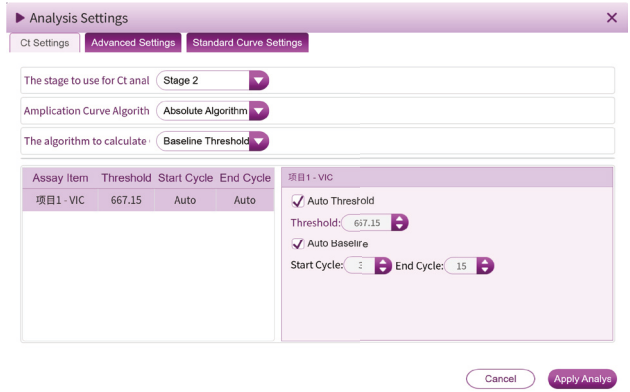


Both fluorescence and derivative curves can be viewed in the melting curves.

4.4 Re-adjust parameter and re-analyze

Click "Analyze Settings", users can set the analysis parameters in the pop-up dialog box.

4.4.1 Ct settings



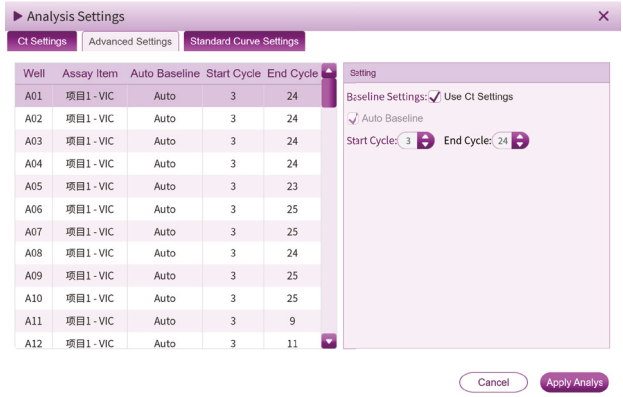
Amplification curve algorithms: absolute fluorescence value method and relative fluorescence value method.

Ct analysis algorithms: baseline threshold method and maximum second order derivative method.

Threshold: the threshold can be adjusted manually.

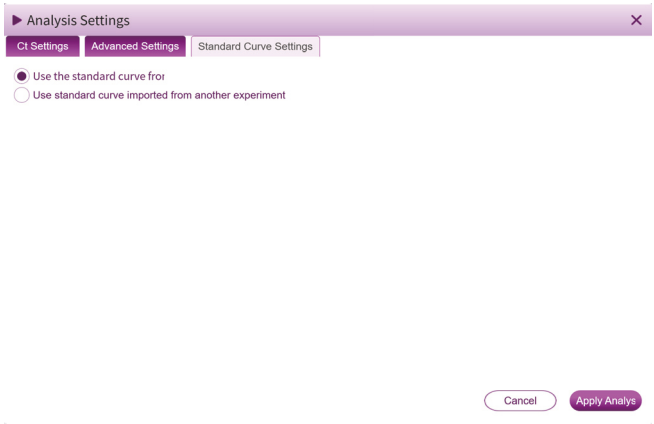
Baseline: Users can change the number of starting cycles of the baseline.

4.4.2 Advanced setting



If the baseline settings cancel the use of the Ct setting, the number of baseline start cycles can be adjusted manually.

4.4.3 Standard curve setting



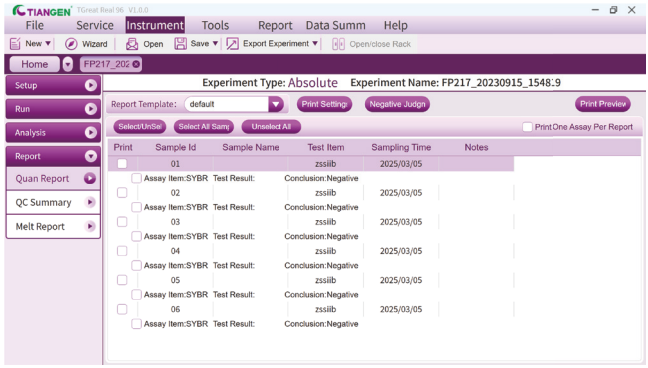
By default, it uses the standards from the experimental setup, or users can choose an externally imported standard curve.

When the analysis settings are complete, click "Analyze" to re-analyze the results.

5. Report

5.1 Quantitative report

The Quantitative Report screen is shown as below:



Select/Uncheck: Click to check or uncheck the currently selected sample.

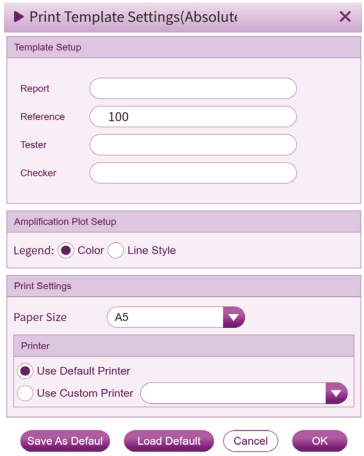
Select All Samples: Click to select all samples.

Deselect All: Click to deselect all samples.

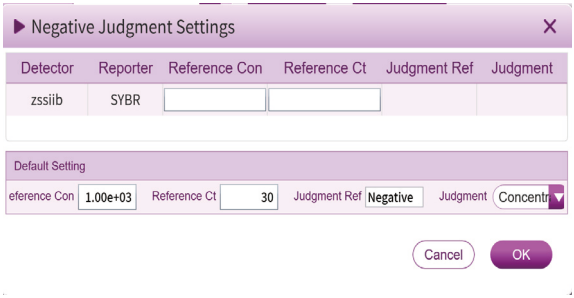
Report template: default report template default, users can also design users own report template.

Print Settings: Users can set the name, paper size, printer, etc. of the printed report, as

shown in the following figure:



Negative Judgment Settings: Users can customize the determination reference concentration, Ct value, and the object of the determination; if not set, the default settings will be used. As shown in the figure below:

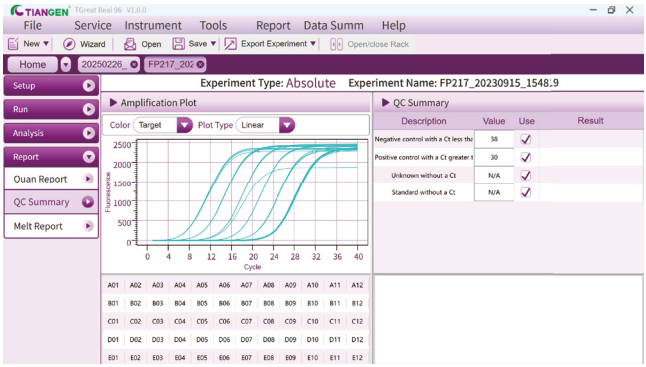


Print Preview: Click to preview the quantitative report as shown below:



5.2 Quality control report

Click "QC Report", the screen is shown below:

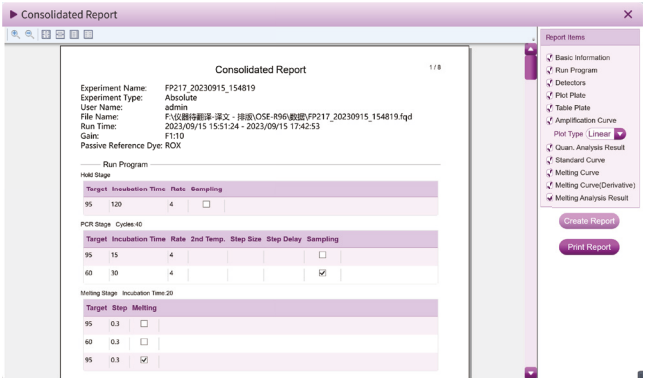


5.3 Melting report

If the dye method is used, there is a melting report, which is similar to the quantitative report, except that the reported curve is a melting curve.

5.4 Comprehensive report

Click "Reports" in the toolbar, select "Comprehensive Report", the pop-up window is shown in the figure below:



The content of the comprehensive report for the above figure "report items" in the check all the content, such as do not need to reflect the project in the report, users can uncheck the check box, click "Generate Report".

Note: The Experiment Basic Information item cannot be unchecked.

Click "Print Report" to print the comprehensive report.

6. Data export

6.1 Export experimental data to EXCEL

Click on the toolbar "Export Experiment" drop-down menu, select "Export to EXCEL", select the export path, click "Confirm", the experimental data exported to generate The experimental data will be exported to an excel file. Users can also click "Data Summary" in the menu bar, select "Export Experiment", select "Export to EXCEL", select the export path and click "Confirm". "Confirm", the experimental data is exported to excel file.

6.2 Exporting experimental data to text

Click the drop-down menu of "Export Experiment" in the toolbar, select "Export to Text", choose the export path, and click "Confirm", the experimental data will be exported to a text file. Users can also click "Data Summary" in the menu bar, select "Export Experiment", select "Export to Text", choose the export path, and click "Confirm". The experimental data will be exported to a text file.

Chapter 5 Relative quantification

1. Design of experiments

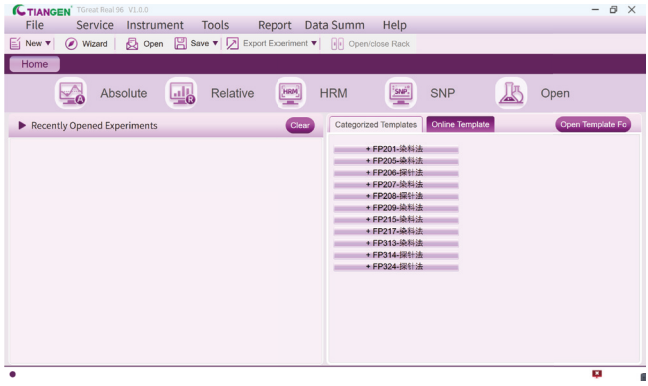
1.1 New relative quantification experiment

There are three ways to create a new relative quantitative experiment:

(1) Click "File" in the toolbar, select "New", and choose "Relative Quantity".

(2) Click "New Experiment" in the menu bar and select "Relative Quantification".

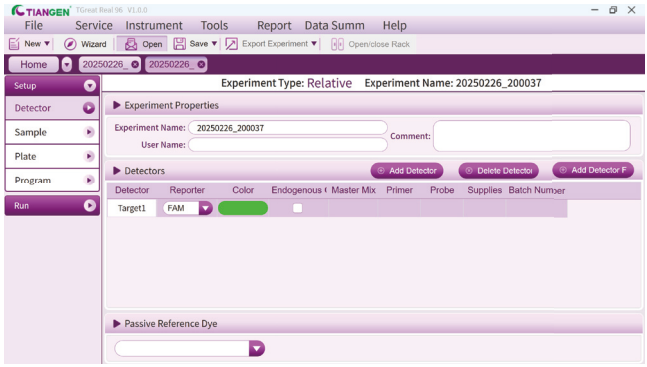
(3) Click on "Relative Quantification" on the home screen.



1.2 Setting

After creating a new experiment, users can set the detector, sample information, reaction plate, and program settings.

1.2.1 Click "Detector", the screen will be shown as below:



In the "Experiment Properties" field, users can change the experiment name, user name, and note information. The default experiment name is the time when the new experiment was created.

In the "Detector" field, users can edit the gene name, reporter fluorescence, color, housekeeping gene, Master Mix, primers, probes, consumables, and batch number.

If users have more than one test gene, users can click "Add Detector".

To delete a target gene, select the row where the gene is located and click "Delete Detector".

By clicking "Add library item", users can directly add target genes in the library, or save frequently used target genes into the library.

2. Prepare to react

2.1 Preparation of reagent sample

System: TGreat Real 96 fluorescence quantitative PCR instrument using 0.2 ml centrifuge tube to place the reagent samples, the sample optimal reaction system is recommended 10~50 μl.

Consumables: The instrument allows the use of standard 0.2 ml single tubes (clear top), 0.2 ml 8-stripe (clear top), 0.2 ml semi-skirted 96-well PCR plates, and 0.2 ml unskirted 96-well PCR plates.

Centrifugation: The PCR tube or 96-well PCR plate in which the reagent samples are placed should be centrifuged in a centrifuge before placing it in the instrument to ensure that the reagent samples are at the bottom of the centrifuge tube or 96-well PCR plate and that the interior is free of air bubbles.

2.2 Installation of reaction plate

Click "Open/Close Hatch" in the menu bar to open the sample chamber.

96-well PCR plate placement: 96-well PCR plate can be placed directly and smoothly.

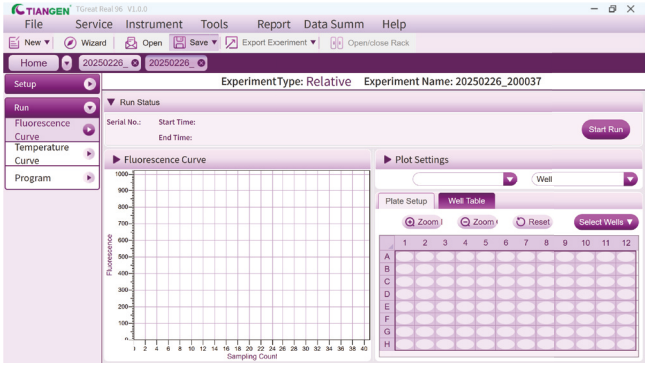
8-stripe placement: An empty 8-stripe needs to be placed in each of columns 1 and 12 to equalize the hot cover pressure.

Pressure balance. When placing the reaction tubes, try to evenly distribute the tubes in the module well to ensure that the hot cover presses smoothly on the top of the tubes during operation, and that the module is evenly loaded to ensure uniform and consistent temperature changes across the tubes.

Note: Make sure that the reaction tubes are lined up in the same layout as in the reaction plate setup.

3. Running program

Click "Run", the screen is shown below:



Click "Start Run", a dialog box will pop up to confirm the path to save the experiment and confirm the hot cover temperature and sample volume.

3.1 Fluorescence curve

After the run starts, the real-time fluorescence curve is displayed on this screen, and users can view the program start time, total run time, remaining time, and run status.

Users can also set "Skip Current Segment", "CyclePlus One", "Cycle Minus One" and "Stop Run". The following settings are also available.

3.2 Temperature curve

After the run starts, the real-time fluorescence curve is displayed on this screen, and users can view the program start time, total run time, remaining time, and run status.

Users can also set "Skip Current Segment", "CyclePlus One", "Cycle Minus One" and "Stop Run". The following settings are also available.

3.3 Running program

This screen allows users to view the running program, but not to change it.

4. Experimental analysis

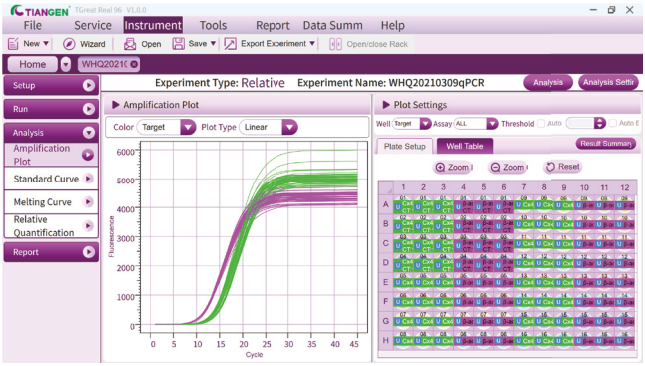
At the end of the run, the results of this experiment can be analyzed.

If users want to open the results of previous experiments and analyze them, click "File" in

the toolbar, select "Open" and find the file users need to open; or click "Open" in the menu bar to find the file users need to open; or users can directly click "Open Experiment" in the home interface to find the file users need to open. Or click "Open" in the menu bar to find the file users need to open; users can also directly click "Open Experiment" in the home screen to find the file users need to open.

4.1 Amplification curve

Click on Amplification Curve in Analysis to view the amplification curve, as shown below:



4.1.1 Amplification curve

Color: Set the color of the amplification curve, which can be well position color, target gene color or sample information color.

Linear: Amplification curves can be selected as linear or logarithmic.

4.1.2 Curve display setting

Threshold: Default automatic threshold, if users need to adjust the threshold, uncheck "Automatic" and adjust the threshold manually.

Reaction Plate: Select the reaction plate to display the corresponding amplification curve in the Amplification Curve.

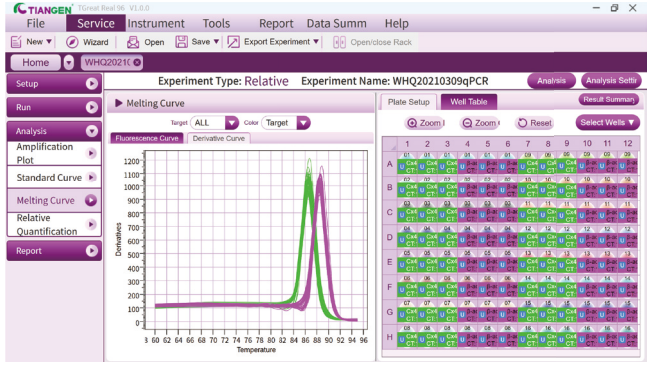
List information: display well position, sample information, target gene, Ct value and other information in list form.

4.2 Standard curve

If the target gene is a standard and the concentration of the target gene has been set in Setup, users can view the standard curve in this screen.

4.3 Melting curve

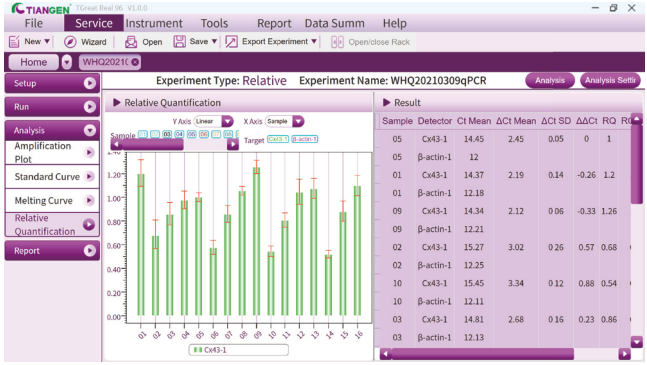
If the dye method is used, the melting curve can be viewed as shown below:



Both fluorescence and derivative curves can be viewed in the melting curves.

4.4 Relative quantification

Click on "Relative Quantification" as shown below:



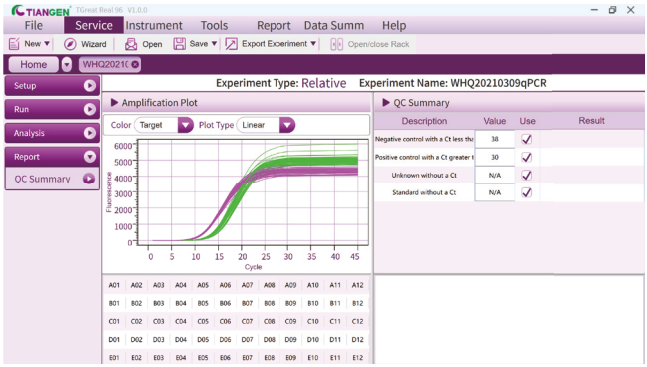
Relative Quantification: In this column users can view the relative expression of individual samples.

Analyze the results: users can view the results of the mean, standard variance, and relative discovery of the Ct values for each sample.

5. Report

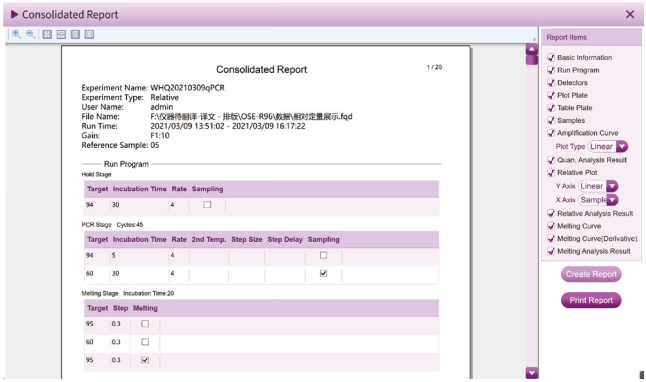
5.1 Quantitative report

The Quantitative Report screen is shown as below:



5.2 Comprehensive report

Click "Reports" in the toolbar, select "Comprehensive Report", the pop-up window is shown in the figure below:



The content of the comprehensive report for the above figure "report items" in the check all the content, such as do not need to reflect the project in the report, users can uncheck the check box, click "Generate Report".

Note: The Experiment Basic Information item cannot be unchecked.

Click "Print Report" to print the comprehensive report.

6. Data export

6.1 Export experimental data to EXCEL

Click on the toolbar "Export Experiment" drop-down menu, select "Export to EXCEL", select the export path, click "Confirm", the experimental data exported to generate The experimental data will be exported to an excel file. Users can also click "Data Summary" in the menu bar, select "Export Experiment", select "Export to EXCEL", select the export path and click "Confirm". "Confirm", the experimental data is exported to excel file.

6.2 Exporting experimental data to text

Click the drop-down menu of "Export Experiment" in the toolbar, select "Export to Text", choose the export path, and click "Confirm", the experimental data will be exported to a text file. Users can also click "Data Summary" in the menu bar, select "Export Experiment", select "Export to Text", choose the export path, and click "Confirm". Confirm", the experimental data will be exported to a text file.

Chapter 6 High resolution

1. Design of experiments

1.1 New high-resolution experiment

There are three ways to create a new high-resolution experiment:

(1) Click on the toolbar "File", select "New", select "High Resolution".

(2) Click "New Experiment" in the menu bar and select "High Resolution".

(3) Click "High Resolution" on the home screen.

Refer to Chapter 4, Section 1 for target genes, sample information, reaction plates, and program settings.

2. Prepare to react

Refer to Chapter 4, part 2.

3. Running program

Refer to Chapter 4, part 3.

4. Experimental analysis

4.1 Amplification curve

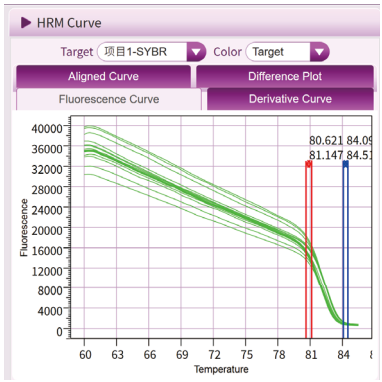
Refer to Chapter 4, part 4.4.1.

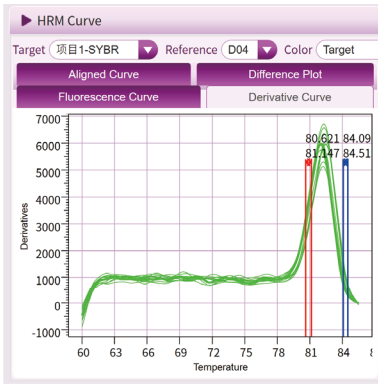
4.2 Standard curve

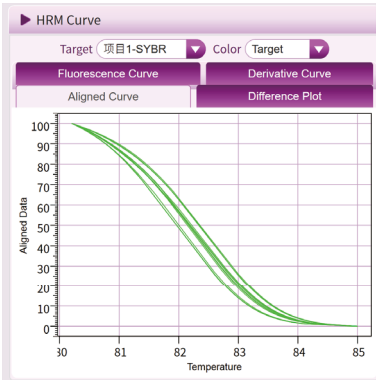
Refer to Chapter 4, part 4.4.2.

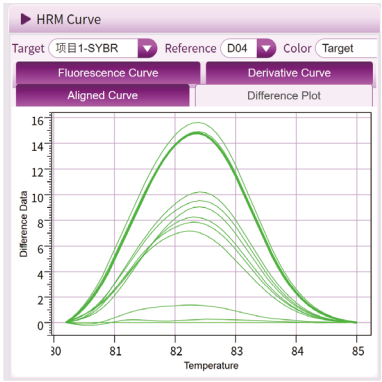
4.3 High resolution curve

Fluorescence curves, derivative curves, normalized curves, and difference plots can be viewed in high resolution. Users can set the target gene to be displayed as well as the curve display color. As shown in the figure below:









5. Report

Refer to Chapter 5, part 5.

6. Data export

Refer to Chapter 5, part 6.

Chapter 7 SNP

1. Design of experiments

1.1 New SNP experiments

There are three ways to create a new SNP experiment:

(1) Click "File" in the toolbar, select "New" and choose "SNP".

(2) Click "New Experiment" in the menu bar and select "SNP".

(3) Click "SNP" on the home screen.

Refer to Chapter 4, Section 1 for target genes, sample information, reaction plates, and program settings.

2. Prepare to react

Refer to Chapter 4, part 2.

3. Running program

Refer to Chapter 4, part 3.

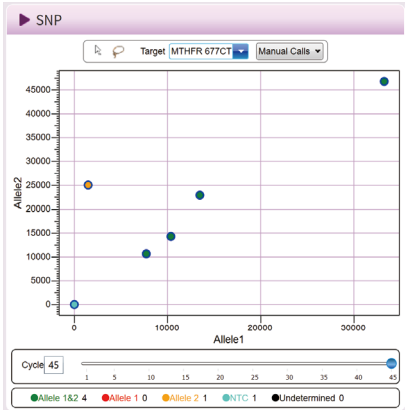
4. Experimental analysis

4.1 Amplification curve

Refer to Chapter 4, part 4. 4.1.

4.2 SNP

Click on "SNP" to view the scatterplot as shown below:



5. Report

Refer to Chapter 4, part 5.

6. Data export

Refer to Chapter 4, part 6.

Chapter 8 Other functions

1. File

1.1 Import setup parameters

Click "Import Setting Parameters" to select a previously saved experiment and import the parameters of that experiment.

1.2 Save and save as

Click "Save" and select the save path to save the original file.

Click "Save As" and select the save path to save the original file.

1.3 Save as template

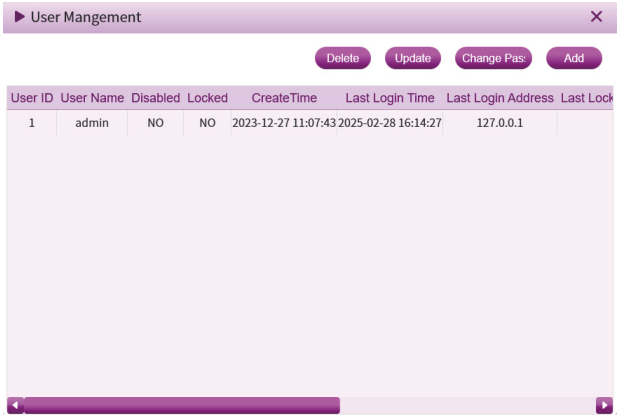
Click "Save as template", enter the save type and template name in the pop-up window, choose to save the template content, the destination gene and runtime program settings are mandatory, the sample information and reaction plate can be checked or unchecked.

1.4 Exporting standard curve

If users want to export and save the standard curve in the experimental data, open the experimental data, click "File", select "Export Standard Curve", and choose the save path to export the standard curve.

2. Service

2.1 User management



Delete: Select the user users want to delete and click Delete to remove the user.

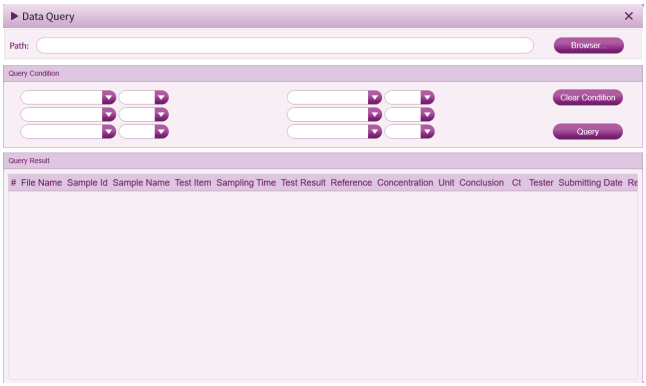
Update: Check the user to update the user name and permissions.

Change password: change the user's password.

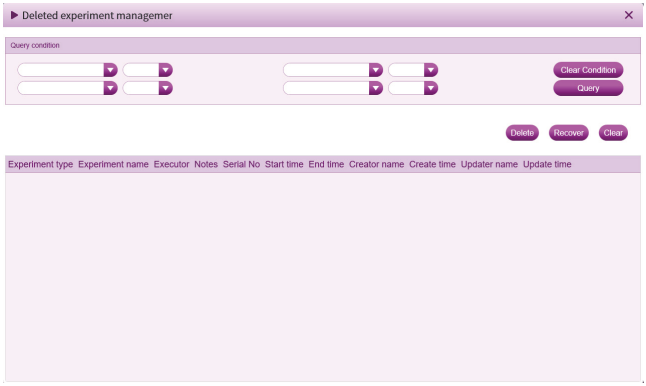
Add more: new users can be added.

2.2 Experimental management

Manage Experiments: Users can query, edit, delete and download the run experiments. As shown in the figure below:

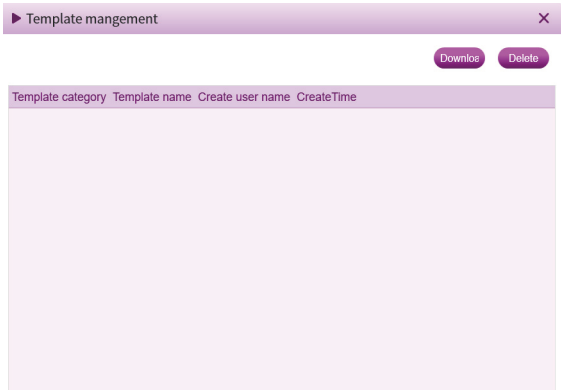


Manage deleted experiments: Users can query, delete, restore and empty deleted experiments. As shown in the figure below:



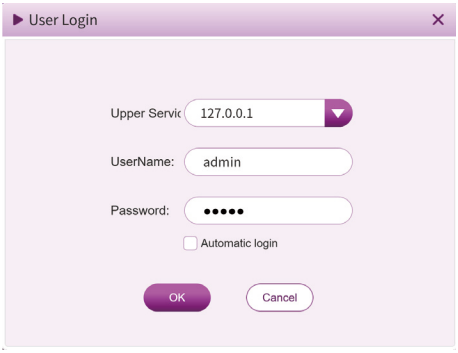
2.3 Template management

Download or delete the experiment template.



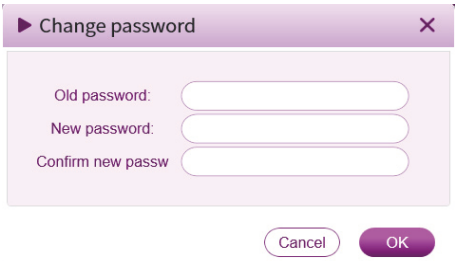
2.4 User login

Click "User Login" and the following screen will be displayed:



2.5 Change password

Users can change the password of the currently logged in user.



2.6 View running experiment

If there are currently running experiments, users can click to view them.

3. Instrument

3.1 Connect instrument

Click "Connect Instrument", users can select "Auto Connect" or "Search Connection" to connect the machine.

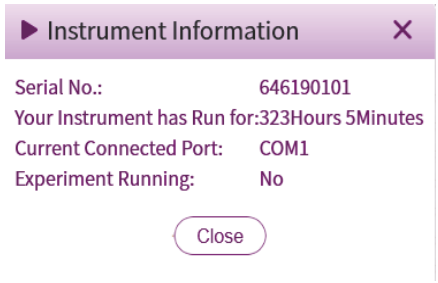
3.2 Disconnect

Click "Disconnect" to disconnect the currently connected instrument.

3.3 Instrument information

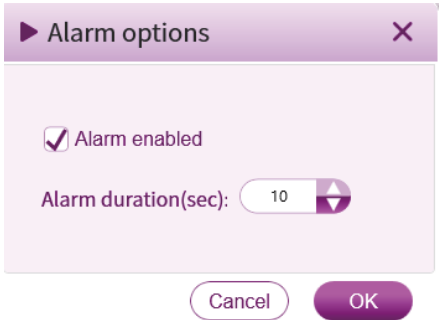
When the instrument is connected, users can view instrument information.

Instrument information includes: instrument serial number, time run, currently connected port, and whether or not the experiment is running. As shown in the figure below:



3.4 Alarm options

Users can select whether or not to use the beep alarm and how long the alarm lasts. As shown in the figure below:



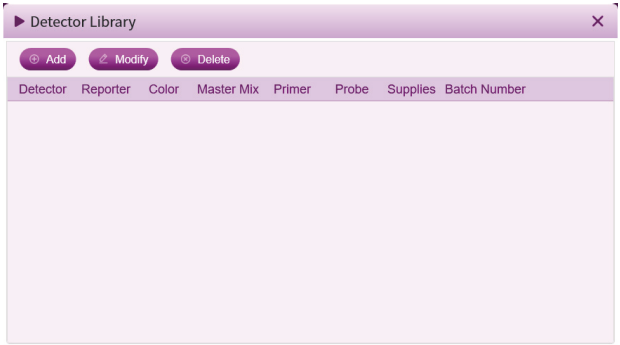
4. Tools

4.1 Setting gain

The instrument is an automatic gain version, eliminating the need for human gain setting.

4.2 Detector library

Click Tools and select Detect Project Library, the pop-up window will appear as follows:



Click "Add" to add test items;

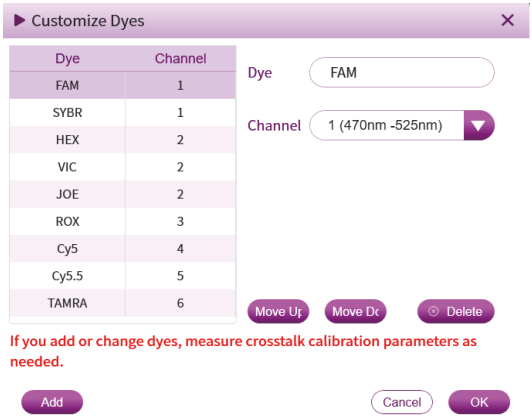
Click "Delete" to delete the test items in the item library;

Click "Modify" to edit the items in the test item library.

4.3 Customize Dyes

Used to set up existing dyes and add new dyes.

Click on Tools, select Customize Dyes and the pop-up window will appear as follows:



Selecting the dye on the left allows users to change the dye name and channel on the right.

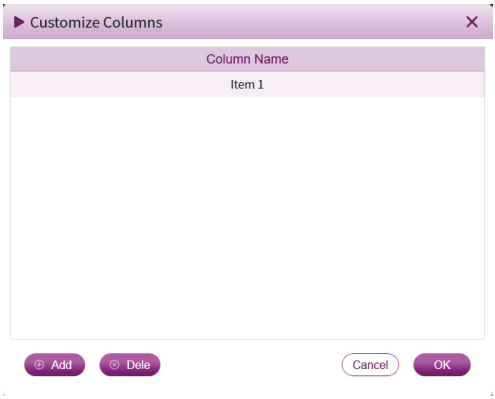
Click "Create" to add a new dye. After creating a new dye, enter the name of the dye on the right and select the number of channels.

Users can delete the selected dyes by selecting them and clicking "Delete".

4.4 Customize Columns

For customized columns

Click on Tools, select Customize Columns and the pop-up window will appear as follows:



Users can add columns, delete columns and change column names.

4.5 Column selection

Used to add new columns added in the column customization to the currently existing columns, or remove existing columns from the current columns.

Click on Tools, select Column Selection and the pop-up window will appear as follows:

Currently available column items include Samples, Reports, Report Settings, Data Queries, and Data Query Conditions, and columns with (*) indicate that they cannot be removed.

Double-click on an item in the "Unselected Columns" to add it to the "Current Columns".

Double-click an item without (*) in the "Current Column" to remove it to the "Unselected Columns".

4.6 Definition of automatic sample prompts

Used in the design of experiments stage to define the contents of the drop-down box selections when setting up sample information.

Click Tools and select "Sample Column Library" to bring up the following window:

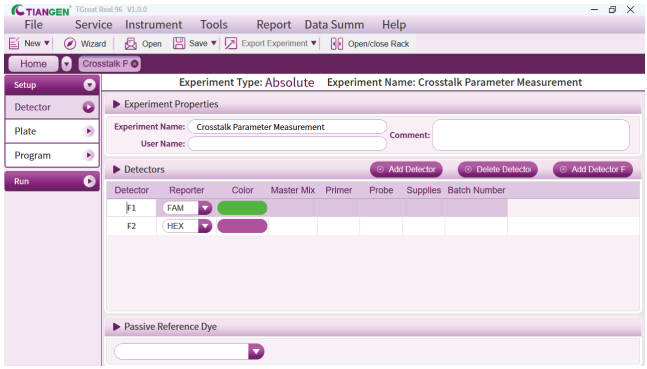


Columns can be added, deleted and edited

4.7 Crosstalk correction parameter measurement

Used to measure crosstalk correction parameters.

Click Tools and select "Crosstalk Parameter Measurement", the pop-up window will appear as follows:

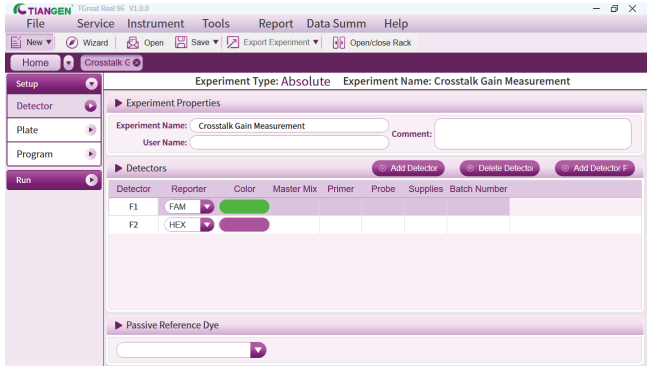


Users can add and change the channels and dyes to be tested according to their needs; load the corresponding reaction plate and run the test, and the system automatically saves the crosstalk correction parameters after the test is completed.

4.8 Crosstalk gain parameter measurement

Used to measure crosstalk gain parameters.

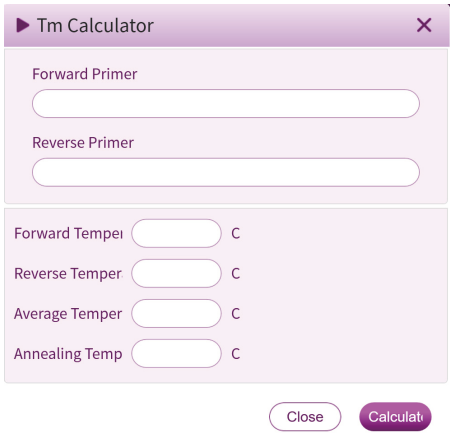
Click Tools and select "Crosstalk Gain Measurement", the pop-up window will appear as follows:



Users can add and change the channels and dyes to be tested according to their needs; load the corresponding reaction plate and run the test, and the system automatically saves the crosstalk gain parameters after the test is completed.

4.9 Tm calculator

Click Tools and select "Tm Calculator", the popup window will appear as follows:



Enter the forward primer, reverse primer, and click Calculate to obtain the forward temperature, reverse temperature, average temperature, and annealing temperature.

Article 9 Maintenance

1. Regular cleaning

In order to ensure the normal operation and testing of the instrument, it is recommended that the instrument be cleaned on a regular basis.

- Cleaning of external surfaces: Clean with a soft rag, dipped in alcohol, distilled water or cleaning paste if necessary;
- Cleaning of module wells: Use a dust-free cleaning swab for this purpose, and dip a small amount of 95% anhydrous ethanol or distilled water if necessary. It is recommended to confirm whether the module wells are dirty or contaminated before daily experiments, and use this method for cleaning.

Warning!	1. When cleaning this instrument, the power supply must be disconnected. 2. The surface of this instrument should not be cleaned with corrosive cleaning agents, if in doubt, consult the manufacturer or its agent.

2. Failure Analysis and Handling

S/N	Fault description	Cause analysis	Solution
1	The power switch at the rear of the instrument is set to ON, but the instrument does not respond.	The RUN SWITCH on the front of the instrument is not depressed	Press the RUN SWITCH

2	System Parameters menu shows the need to enter a "password".	The system parameters are for internal calibration by the instrument manufacturer and require a special password to access.	The user does not need to use this function. For calibration, please contact factory service personnel
3	Module temperature rise and fall speed significantly slower or temperature control inaccurate	Vent blocked	Remove obstructions from vent
		Loose connection cable	Contact the supplier or the manufacturer
		Damaged refrigeration sheet	
		Damaged fan or fan stops running	
		Damaged temperature sensor	
4	Module neither heats nor cools	Internal failure of instrument	Contact the supplier or the manufacturer
		Damaged refrigeration sheet	
		Hot cover warming up process	Wait for the instrument hot cover temperature to reach the target value Module temperature is automatically controlled at 30°C when it stops running.
5	Abnormal temperature or fluorescence curve	Running a program infected with a virus and the computer's CPU is heavily occupied	Reinstall the application after antivirus
6	Hot cover cannot be heated up.	Damaged thermal fuse	Contact the supplier or the manufacturer
		Loose connector	
		Damaged heating element in hot cover	
		Damaged temperature sensor in hot cover	
7	Increased differences in fluorescence values across wells in the no-tube state or a large background	Contaminated test tube well or hot cover Baseline background parameters are misused Test tube quality problem, failed sealing	Decontamination Each instrument needs to correspond to the Baseline file. Optics shifted after long-term use, need to contact the manufacturer to recalibrate the background
8	Reagent evaporation	Incorrect or inappropriate test tube cover or film covering	Selection of suitable consumables with better sealing properties

9	Inter channel signal crosstalk	Objective crosstalk between the dye signals of the channels	Measurement can be performed by the "Crosstalk Coefficient Measurement" function in the software, and the calibration parameters can be saved and corrected.
10	Abnormal fluorescence test value	External strong light source irradiation	Turn off the external light source
		Damaged optoelectronic system	Contact the supplier or the manufacturer

Attention	During the warranty period, it is strictly prohibited for users to open the amplifier shell privately to check by themselves. If a malfunction occurs that requires opening the instrument case for inspection in the table above, contact the supplier or manufacturer. It is strictly prohibited for users to check or replace parts privately, and parts can only be checked or provided by the manufacturer or agent.