Fluorescent Quantitative Detection system

LineGene 9600 Series

User's 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.

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Thank you for your purchase of this product. Before initial use of this instrument, please read this manual thoroughly !

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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/IEC61010-1, is a general instrument of class I, the protection degree is IP20. It is intended for indoor use.

Note: The instrument, complying with the Standard YY0648/IEC61010-2-101, is used for IVD medical 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 to the mains and switching the instrument on, make sure the voltage is consistent with the instruments requirements (100-240V, 50/60Hz). The rated load for the power socket must not be less than the instruments maximum load of 600W

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 overheating of the instrument they shall not be blocked or covered. When a single set or several sets of instruments are used, the space between its ventilation openings and the nearest object should not be less than 30cm.

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 a test, care should be taken to prevent liquid from dropping onto the instrument.

The waste remaining following a test, such as consumables, reagents, etc. should be treated as advised in your local disposal procedures, and should not be thrown into normal waste or poured down a drain.

If hazardous substances are used in a test, the user must be adequately trained before use. Waste hazardous substances, must be disposed of according to local disposal instructions.

The instrument operator, must be appropriately trained in its use.

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 are handling potentially hazardous biological substances such as human or animal derived tissues or fluids, appropriate protective clothing and gloves need to be used.

h) Transportation

If transporting the instrument again, the instrument and its detection wells need be empty and thoroughly cleaned before transportation, and should preferably be disinfected by UV light. The power should be switched off and the unit un-plugged before commencing cleaning procedures.

i) Warning Sign

Warning identification

DANGER!	Indicates danger if the instrument is used incorrectly.
SCALDING!	Indicates a scalding hazard as this area will be hot during use.
BIOHAZARD	Indicates a biohazard during use.
PROTECTIVE EARTH	Indicates the location of the protective earth on the instrument

Warning mark



Warning! 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



Maintain in an upright position as indicated by the arrows facing upwards.

Fragile		Care should be taken during transportation, as there are breakable items contained in the transport package.
Keep Dry		Transport package must be kept dry
Stacking limit	2	Maximum number of stacking layers of the same package is 2.
Temperature limit	-370=	The transport package should be maintained at a temperature between -20C to 55C.

3. EMC Consideration

Note: This is a class A equipment, only suitable for use in establishments other than domestic, and those directly connected to a low voltage power supply network.

4. 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.

5. 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 96-well Fluorescent Quantitative Detection System.

1. Applications

The 96-well fluorescent quantitative PCR detection system allows real-time detection of amplified DNA.

Application areas include research into the human genome, forensics, cancer, tissue, population biology, palaeontology, zoology and botany and in clinical diagnosis of virus, cancer and genetic diseases.

PCR detection system belongs to IVD medical equipment, which is to use the polymerase chain reaction to perform quantitative analysis of different genes in a clinical laboratory.

2. Features

- 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.
- Multi-point temperature control ensures consistent temperature of 96 sample wells.
- It can create a temperature gradient with 4 Thermo electric modules.
- Stable and accurate 1~36C gradient function makes optimising PCR conditions simple and easy.
- The constant temperature function of SOAK enables low-temperature storage of PCR reagents.
- It uses maintenance-free long life LED excitation light source.
- The advanced fibre optic transmission technology makes the photo-electric detection system very sensitive and reliable.
- 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.
- Automatic hot-lid technology needs no manual opening/closing and ensures constant pressure of the hot-lid used with different height reaction tubes or plates.
- 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.
- Unique patented advanced module bottom fluorescent detection technology.

3. Notes to model and description





Description:



4. Performance parameters

Specification/model		FQD-96A						
Sample capacity	96x0.2ml (suitable for single tube, 8-row tubes and 96-well							
	fully-skirted plate)							
Detection channel	F1	F2	F3	F4	F5			
Applicable dye	FAM, SYBR Green I	VIC, HEX, TET, JOE, Cy3, TAMR A	ROX, TEXAS -RED,	Cy5 Quasar -670	Cy5.5 Quasar -705			
Temperature range of block working	4∼105℃ (Minimum division:0.1℃)							
Heating/cooling rate				4.0°C/s	s (max)			
Temperature fluctuation	<pre></pre>	±0.1°C	(full-ran	ge), (55	°C typic	al value	≤±0.1°0	C)
Temperature accuracy	≤	±0.2°C	(full-ran	ge), (55	°C typic	al value	≤±0.1°0	C)
Temperature uniformity	≤	±0.4°C	(full-ran	ge), (55	°C typic	al value	≤±0.3°0	C)
Temperature range of hot-lid		30~	~110°C	(adjusta	able, de	fault 105	5°C)	
working							,	
Repeatability of fluorescent				5	%			
intensity detection								
Running mode			С	ontinuou	us runni	ng		
Operation system	W	/indows	XP/Win	dows V	ista/Win	dows7/\	Window	s8
Power supply			100-24	40V \sim 5	50/60Hz	600W		
Dimensions			410n	1m×386	mm×35	2 mm		
Weight				28	kg			

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 LineGene 9600 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: 10°C~30°C Environmental RH: ≤70% Altitude: < 2000 m Pollution degree: 2 Power supply: 100-240V ~50/60Hz 600W

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

3.1 Connection of power wire and communication wire

Connection of power wire: Only the power cable 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.

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.

4. System Installation and Unloading

4.1 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

System installation

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

Double click LineGene9600 installation file (LineGene9600ScientificSetup.exe) ► display the installation interface (select installation language) ► set up installation

Operating system

1. Double click the LineGene9600 shortcut on the desktop Or Click the start menu ► Program ► LineGene9600

2. Double click the PcrServer shortcut on the desktop Or Click the start menu ► Program
 ► PcrServer

4.2 System Unloading

Control Panel ► Add/Delete Program ► PcrServer ► Unloading Control Panel ► Add/Delete Program ► LineGene9600 ► 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:

 $1_{\mbox{\scriptsize st}}$ step: Start computer display and host.

2nd step: Turn on power using the switch on the back of the instrument.

3rd step: Press the Run Switch on the front cover of the instrument to set the system ready to run.

4th step: On the computer after entering operation system, start the LineGene 9600 fluorescent quantitative detection system. To start the software, click "LineGene9600" from [Start]/ [programme] menu or double click the short-cut icon on desktop.

3. Starting software interface

Run the programme and it will display the system window. - 6 File Service Instrument Tools Report Data Summary Help 🗋 New 🔹 📄 Open 🔚 Save 🔹 🕞 Export Experiment 🔹 Home Palatinaka Recently Opened Experiments Clear Open Template Folder ed Templates Online Template Feedeniny O 宇宙, 9660_20140825_091214 - 副本.1qd 124.fqd 本底測试_20140824_090741.fqd 宇壇_20140824_121030.fqd 600199-1 2.4 5+桜度.fqd IntestinalBac_20140815_異常.fqd 納伯基因20121120.fqd IntestinalBac_20140815_異常.fqd 圖管双色_20140808_165937_3号机.fqd 圖管双色_20140808_165937_3号机.fqd + 2222 + melt + Test は言葉がピュンロ44888。185537_35-96-1874 重量性HEX ROX CY5_133949.fqd 96説明ば溺fam垂重性_20140810_101546.fqd 96説明ば溺fam接度_20140810_141843.fqd 移版訳ws.fql.fdd Crosstalk Parameter Measurement 20140630 182026 600402.fod -

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.

NOTE: The Absolute quantitative experiment can be also created by:

- a. Clicking File > New > Absolute 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.

Experiment Prope	erties		
Experiment Name:	20111117_Experiment		remark
User Name:	user	Comment:	

3. Detector Setting

Set up the Detector, Assay, Dye and Colour.

If necessary, the user can also:

- a. Add detector
- b. Add assay
- c. Delete detector
- d. Delete assay

e. 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.

Add Modify Delete Detector Reporter Color Master Mix Primer Probe Supplies Batch Number Target1 FAM Image: 1 transmitted in transmit	Detecto	r Library							
Detector Reporter Color Master Mix Primer Probe Supplies Batch Number Target1 FAM Target2 FAM Select	Add	Modify	Delete						
Target1 FAM Target2 FAM Select Clo	Detector	Reporter	Color	Master Mix	Primer	Probe	Supplies	Batch Number	
Target2 FAM	Target1	FAM							
Select Co	Target2	FAM							
Select Clo									
Select Clo									
Select Clo									
Select Clo									
Select									
Select Clo									
Select									
Select									
Select									
Select Clo									
Select Clo									
Select Clo									
Select Clo									
Select Clo									
								Sele	ct Close

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

Detector	s Add Detector	Add Assay	Delete Detector	Delete Assay	Add	Detector From Lil	brary	
Detecto	or Reporter	Color	Master Mix	Primer	Probe	Supplies	Batch Number	
Target	1 FAM							
Target	2 FAM	-						

4. Set up reference dye

Reference Dye		
VIC		

1.3 Sample Information Setting

1. Click Setup **Sample**

Setup	0
Detector	0
Sample	0
Plate	0
Program	0

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** I the Batch Add window will pop up

C Batch Add	X
Start Sample Id a	Sample Count 5 💌
	Add Cancel

3. Delete sample information

a. Itemized deletion: select one sample \blacktriangleright click **Delete** \blacktriangleright 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

Sample ID Batch Add	Delete	Clear All	Import Samples Info	Export Samples Info
---------------------	--------	-----------	---------------------	---------------------

5. Set up sample information

Samples	_	_	_	_
Sample Id	Color	Sample Name	Sampling Time	Submitting Date
al		Sample1	2013-12-06	2013-12-06
a2		Sample2	2013-12-06	2013-12-06
a3		Sample3	2013-12-06	2013-12-06
a4		Sample4	2013-12-06	2013-12-06
a5		Sample5	2013-12-06	2013-12-06

1.4 Reaction Plate Setting

1. Click Setup	Plate
Setup	۲
Detector	0
Sample	0
Plate	0
Program	0

- 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.

CR Edit Det	ector Li	brary						×
Detector Name	e: Target3 Delete							
Reporter	Color	Master Mix	Primer	Probe	Supplies	Batch Number		
FAM	-							
						ОК	Cance	1

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
Z	Negative	NO	Fg/ml
Ρ	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	Plate S	Setup	Well Tal	ble				_
	#	Well	Sample Id	Assay Item	Property	Dye	Con.	
	1	A01		Target1	Unknown	FAM		
	2	A02						
	3	A03						
	4	A04						
	5	A05						
	6	A06						
	7	A07						
	8	A08						
	9	A09						
	10	A10						
	11	A11						
	12	A12						

1.5 Programme Setting

1. Click Setup **Programme**

Setup	•
Detector	0
Sample	0
Plate	0
Program	0

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

Start
\checkmark
Design experiment
\checkmark
Prepare for the
reaction
▼
Run the experiment
▼
Experiment analysis
▼
Experiment report
V
Data export
\checkmark
End

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

Start
▼
Design experiment
▼
Prepare for the reaction
▼
Run the experiment
▼
Experiment analysis
▼
Experiment report
▼
Data export
V
End

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.

Caution: Before running the programme, push the module smoothly until the locking sound is heard and the alarm lamp is switched off.

If the module is pulled out or is improperly closed, the software will produce a pop-up warning and the alarm lamp will light up.

In this case, the temperature programme can be run but the fluorescence scanning data would become invalid.

3.1 Preparation for reagent sample

- Preparation for reagent: The LineGene 9600 series fluorescent quantitative PCR detection system uses 0.2ml PCR tubes, strip tubes or 96 well PCR plates to conduct the reaction. The recommended reaction volume is 10µl~50µl for an optimal reaction system.
- The tube, strip tube or 96 well PCR plate, must have an optically clear bottom.
- 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.
- To insert test tube: If individual tubes or strip tubes are used, and the sample quantity is less than the maximum capacity of the instrument, it is recommended the sample tubes should be evenly distributed across the block as far as possible. This will create even pressure across the hot-lid, ensuring consistent pressure on all the tubes during running, which greatly improves temperature consistency across all sample tubes.

Correct. The sample is at the bottom of the PCR tube



Incorrect

- 1. Requires a greater spin speed
- 2. Requires a longer spin time

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 (baseline) parameter setting
 - c. Target fluorescence value setting

Warning	
Without the baseline para will not be calibrated.	meters, the fluorescence data
Reference gain of Dye "F replaced with the default	'AM" does not exist, has been : gain.
🖌 Hotlid: 105 🚔	Liquid Quantity: 40
Gain	
📕 Auto Gain	
Used Gain	
F1 (FAM) 7 💌 Մ 🕫	e Reference Gain
	Contraction of Contraction

- 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 (baseline) parameter setting
- c. Target fluorescence value setting

ithout the baseline parame ill not be calibrated.	ters, the fluorescend	a data
		a data
eference gain of Dye "FAM eplaced with the default g	" does not exist, ha ain.	is been
Hotlid: 105 🚔	Liquid Quantity:	40 🔦
ain		
🔜 Auto Gain		
Used Gain		
F1(FAM) 7	Reference Gain	
	OK	Cancal

- 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.

Caution: 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 front cover of the instrument is fixed with a self-locking key to control energizing of its internal control system:

- Run Switch: running/standby switch.
- After pressing this key, the green indicator lamp is lit on the instrument, the internal system is energized and the instrument is ready to run the programme.
- After pressing this key again, 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.

3.5.1. 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



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



4.1.3 Check Melting Curve

1. Click Analysis Melting 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 well table information
- d. Check results summary



a. Set up assay b. Set up colour Melting Curve Assay target 1 Color Well

4.2 Adjusting Parameters and Re-analysis

- 1. 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

O 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 target1 - SYBR Auto Auto Image: Cycle Auto Image: Cycle Cycle<
Threshold: 293.41 💌
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

1. 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, Amplification curve and Quantification Analysis Results.

📱 Report Designer - D:\LineGene9600\Scientific\config\report\Absolute\default.rpt										
🕴 🗋 New 🤗 Open 🔲 Save 🛛 🗋 Preview	📓 View 🔹 🗙 Delete Selected Controls 🖳 🗐 💼 🚆 🧃	🛞 Settings								
Available controls Used controls										
Common Controls Static Text Oynamic Text Static Image Line Amplification Curve Quantification Analysis Result Known Controls	[Hospita]] [Report] Name:[Name] Sec[Sed] (Age:[Age]) (HospitalNo.1[HospitalNo.1])									
ia - Static Text Controls ⊡ - Dynamic Text Controls	Test Item Test Result Reference Conclusion Amplification Curve									
	4000									
₽ ∎ 2 ↓	<u>a</u> 3000									
Appearance										
Alignment MiddleRight	1 ⁶ 2000									
BackColor White										
Border Solid, 1, False, False, False										
Color Black	1000									
Taut Tanoma, 8.25pt										
lext										
Tag										
🖻 Design	Cvcle									
DesignVisible True		<u> </u>								
Name Label10										
🗉 Layout	[Submitting Date] Report Date:[ReportDate] Tester: [Tester] Checker; [Checker]									
E Location 93, 62										
Padding 0, 0, 0, 0										
■ Size 100, 20										
Type Label										
Text text of the element										

5.2 Print Setting

1. Click **Report > Print Template Setting >** 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.

C Print Templa	ate Settings(Absolute)	\mathbf{X}
_ Template Setup —		
Hospital		
Report		
Reference	100	
Tester		
Checker		
Amplification Plo	ot Setup	-
Legend: OColor	U LineStyle	
Print Setup		
Default Report T	emplate default	
Paper Size A4	-	
Printer		ר
Use Default 1	Printer	
Use Custom P	rinter	
	OK	1

5.3 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, standard curve, plate information, etc..

solidat	ed Report											
ÐQ	* 2 8 8											Report Items
												J Basic Information
												Run Program
LineConeQ600 Concolidated Report												Detectors
LineGeneapoo Consolidated Kepoit										Table Plate		
Experiment Name: 20111104 1F1-600183												Ann Curve(Linear)
Experiment Type: Absolute												Amp. Curve(Log)
File Name: F:\LineGene9600\20111104_1F1-600183.fqd												💙 Quan. Analysis Result
Run Tim	ie: 2011	/11/04	15:22:0	05 - 2011	/11/04 1	.6:22:10	8					🚺 Standard Curve
Gain:	F1:1	0, F2:6	, F3:2, F	4:4,F5:7								
R	un Program											Create Report
Hold Star	re											
Target	Incubation Time	Rate	Samplin	e								
94	120	4										
PCR Star	e Cycles:40											
Target	Incubation Time	Rate	2nd Tem	p. Step :	Size Ster	o Delav	Grad Temp.	Grad Range	Sampling			
95	5	4										
60	30	4							v			
Target 1		AS	say		F	ye AM EX y3		010	Ŷ			
					R	ox						
					C	y5						
F	lot Plate —											
. 1	2	5	4	6		7	8	9	10 11	12		
A 🚺 Tar	get 🚺	Target		U Target		U Target		U Target	U Target			
5	U Target	l	Target	-	1 Target		U Target	U	larget.	U Target		
Tar	7.01 III	Target		Target		Target		Target	Target	-		
			-		_		_					
D	U larget		larget	_	larget		U larget	U	larget	U larget		
I U Tar	get U	Target		U Target		U Target		U Target	U Target	100		
7 S Tar	get 🚺 Target		Target	S Target		S Target	t S Target	S	farget 🛐 Target			
6										-		
-												
×												
т	ahle Plate											
# ¥_1	1 Assav Item	Property	Dve	Std Cor	Sample N	ane						
1 A01	Target 1	Unknown	FAM	ana. cost.	compro in							
	-											

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 items to print
 - d. Print preview e. Print the report

Report T	emplate: de	fault				-	Prin	t Setting							Print Preview
Select	/UnSelect	Select All Sa	mples												Print One Assay PerReport
Print	Sample Id	Sample Name	Test Item	Name	Sex	Age	Case No.	Outpatient No.	Bed No.	Hospital No.	Nationality	Sampling Time	Diagnosis	Notes	
	04		target1									2011/12/15			
	Assay Item:SYBR Detect Concentration:7.82e+06 Conclusion:Positive														

5.5 QC Summary

1. Click **Report > QC Summary**



2. Check the QC summary

Amplification Plot										I	QC Summary					
ſ	Well		- Pl	otType	Line	ar	-	Show	FI E	2 F3	F4	111	Description	Value	Use	Result
<u> </u>	1												Negative control with a Ct less than	38		
5	000									T			Positive control with a Ct greater than	30	\checkmark	
g 4	000-		_					-/		1	1		Unknown without a Ct	N/A	\checkmark	
Cenc	000						_						Standard without a Ct	N/A	\checkmark	
Linore 1	000	0	1 4	8	12 1	I I 6 2 Cyc	0 24 cle	1 28	1 32	1 36	1 40					
A01	A02	A03	A04	A05	A06	A07	A08	A09	A10	A11	A12					
B01	B02	B03	B04	B05	B06	B07	B08	B09	B10	B11	B12					
C01	C02	C03	C04	C05	C06	C07	C08	C09	C10	C11	C12					
D01	D02	D03	D04	D05	D06	D07	D08	D09	D10	D11	D12					
E01	EU2	E03	E04	E05	E06	E07	E08	E09	E10	E11	E12					
F01	F02	FU3	F04	F05	F06	F07	F08	C00	F10	C11	F12					
801	H02	903	004 H04	905	HOG	H07	908	909	010 H10	911	612 H12					
1101	1102	1103	1104	1105	1100	1107	1108	1109	1110		112					

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

🕸 Experimental archive storage directory	
Experiments in the following archive directory:	
D:\LineGene9600	
Change	
OK Cancel	

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

1. 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



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.

C Detecto	r Library							
Add	Modify	Delete						
Detector	Reporter	Color	Master Mix	Primer	Probe	Supplies	Batch Number	
Target1	FAM							
Target2	FAM							
							Selec	tClose

Detectors	Add	Detector	Delete Detector	Add Detector	From Library				
Detector		Reporter	Color	Endogenous Contrc	Master Mix	Primer	Probe	Supplies	Batch Num
Target1		FAM							
Target2		HEX		\checkmark					

4. Set up reference dye

Reference Dye	
VIC -	

1.3 Sample Information Setting

1. Click Setup **>** Sample

Setup	0
Detector	0
Sample	0
Plate	0
Program	0

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 \blacktriangleright click **Delete** \blacktriangleright delete the selected 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

Sample ID	Batch Add	1 Delete	Clear Al	l Import Samples Inf	Export Samples Info

5. Set up sample information

1	Samples									
ľ	Sample Id	Color	Sample Name	Sampling Time	Submitting Date					
I	al			2013-12-06	2013-12-06					
I	a2			2013-12-06	2013-12-06					
I	a3			2013-12-06	2013-12-06					
I	a4			2013-12-06	2013-12-06					
l	a5			2013-12-06	2013-12-06					

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.

C Edit Dete	ctor Lib	rary						X
Detector Name:	Target3							
Reporter	Color	Master Mix	Primer	Probe	Supplies	Batch Number		
FAM 🗾								
						ок	Cancel	

b. Select inspection 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/111
			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

 Detectors 	Plate Setup	Well Tab	le									l
Assay Item Property Con.			Zoom In	Zoom Out	Reset		Sample A	uto Arran	ge			
Target1 - FAM(GOI) 🔟 🕌	1	2 3	4	5	6	7	8	9	10	11	12	
Targeti - HEX(HKG) 🚺 💌 🐼	A U Targe U Targe											
Concentration Unit copies/ml 💌	в											
Samples Show Columns: Sample Name	с											
a2 Sample 2 a3 Sample 3 a4 Sample 4	D											
a5 Sample 5	E											
	F											
	G											
	н					8	8			8		

Plate Setup		Setup	Well Ta	ble	_			
	#	Well	Sample Id	Assay Item	Property	Dye	Con.	
	1	A01		Target1	Unknown	FAM		
	1	A01		Target2	Unknown	HEX		
	2	A02						
	3	A03						
	4	A04						
	5	A05						
	6	A06						
	7	A07						
	8	A08						
	9	A09						
	10	A10						
	11	A11						
	12	A12						

1.5 Programme Setting

1. Click Setup **>** Programme

Setup	•
Detector	0
Sample	0
Plate	0
Program	0

2. Run Programme Setup

a. Čreate 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

	V Hot	lid(C) 105 🔽 Liquid Quant.(ul) 40 😴						
Run Programs Setup Add Stage Add Step Delete								
	Hold Stage	PCR Stage	1					
		Cycles 40	-					
		2nd Temp Step Size - <mark>2nd Temp Step Size -</mark>						
		Step Delay - Step Delay -						
		Grad Temp Grad Range Grad Temp Grad Range						
100°C —								
	95 °C	4°C/s 95°C						
	00:00:20 🔷 🗨	00:00:15						
	Sampling	Sampling Sampling						
75°C —		4°C/s						
	4 °C/s	D" 00						
50°C —		00:00:20 🚔 🗸	J					
		Sampling						
2530	/							
250								
orc —								

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

2. Prepare for Reaction

Start
▼
Design experiment
▼
Prepare for the
reaction
▼
Run the experiment
▼
Experiment analysis
\checkmark
Experiment report
▼
Data export
\checkmark
End

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 (sample volume)
- b. Gain (baseline) 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

Run	•
Fluorescence Curve	0
Temperature Curve	0
Program	0

2. Click Run 🕨 Start



3. Operating confirmation

- a. Modify hot-lid temperature and liquid quantity (sample volume)
- b. Gain (baseline) parameter setting
- c. Target fluorescence value setting

Warning	
Without the baseline p will not be calibrated	marameters, the fluorescence data
Reference gain of Dye replaced with the defa	"FAM" does not exist, has been ault gain.
✔ Hotlid: 105 🗧	Liquid Quantity: 40 💌
Gain	
🔜 Auto Gain	
Used Gain	
F1 (FAM) 7	Use Reference Gain

- 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

Assay	
Assay Target1-FAM 🔽 Threshold 📃 Au	267 🚔 🔝 Auto Baseline

4.1.2 Check Standard Curve

1. Click Analysis > Standard Curve



2. Check standard curve



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



4.2 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

1. Click **Analysis Settings** I 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
- h. Relative quantification setting

10	Analysis Setti	ngs									
ſ	Ct Settings Adva	unced Setting	s Standard	Curve Setting	gs Relative Quantification Settings						
	The stage to use fo	r Ct analysi	s: Stage 2								
	The algorithm to calculate Ct: Baseline Threshold 🔽 🔟 S Fitting										
					target1 - SYBR						
	Assay Item target1 - SYBR	Threshold 230.6	Auto	End Cycle Auto	Auto Threshold						
	target1 - HEX	Auto	Auto	Auto	Threshold: 230.6						
					Auto Baseline						
					Start Cycle: 3 📥 End Cycle: 15 📥						
	L										
					Apply Analysis Settings Cancel						

5. Experiment Report

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



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

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, standard curve, plate information, etc..

Ð 🖯												
	# 2 8 8]									Rep	ort Items
			LineGer	ne9600 C	Consolida	ated Rep	ort		1 / 12	Ì	イント	Basic Information Run Program Detectors Plot Plate Table Plate
Experime Experime File Nam Run Time Gain:	ent Name: 2011 ent Type: Rela me: F:\L e: 2011 F1:6	1123 tive ineGen /11/23 ,F2:7	e9600\2011 09:47:00	1123.fqd - 2011/11,	/23 10:45:4	9					111	Samples Amp. Curve(Linear) Amp. Curve(Log) Quan. Analysis Result Standard Curve
R1	un Program —										Ż	Relative Plot(Linear) Relative Plot(Log)
Hold Stag	e										1	Relative Analysis Resu
Target	Incubation Time	Rate	Sampling									
94	120	4										Create Report
PCR Stage	Cycles:40											
Target	Incubation Time	Rate	2nd Temp.	Step Size	Step Delay	Grad Temp.	Grad Range	Sampling				
95	5	4										
60	30	4						~				
Detector 项目1		GO	I/HKG		Dye		Colo	r				
- H A		GO	I		FAM		•					
-28*		GO	I G		FAM HEX							
	lot Plate	HE	G 4	s •	FAM HEX	•	,	20 31				
P]	lot Plate	GO HE	I G (() () () () () () () () () () () () (5 6	FAM HEX I STUL - MARK		,	- 10 11 12 21 (m) 12 22 22 22	11			
P]	lot Plate	GO HE	I G 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	1 1	FAM HEX T	= 11 21 81 = 9as(sca) 12 37 81 = ext (sca)	,	- 10 11 - 200 (cm) - 2				
	lot Plate			т та 1 - ма (40) 1 - та та та 1 - та та та 1 - та та та	FAM HEX U 101 1000 101 101 1000 101 101 1000 101 101	11 2(1) - Parijan 11 2(1) - Parijan 11 2(1) - Parijan 11 2(1) - Parijan 12		- - 21 (00) - 22 (00) - 22 (00) - 22 (00) - 22 (00) - 22 (00) - 22 (00)	12 12 13 14 14 15 15 15 15 15 15 15 15 15 15 15 15 15			
P 2 He *	lot Plate			• • • • • • • • • • • • • • • • • • • •				- - - - - - - - - - - - - -				
P P P	lot Plate			1 - 740 (000) 2 - 740 (000) 2 - 740 (000) 2 - 740 (000) 1 - 74								
	lot Plate	GO HK										
P.	lot Plate											
P:	lot Plate											

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

C Experimental	archive storage directory	
Experiments in the	following archive directory:	
D:\LineGene9600		
Change		
	OK Cancel	

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 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

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

An SNP experiment can be also created by:



1.2 Detector Setting

1. Click Setup **>** Detector

Setup	
Detector	0
Sample	0
Plate	0
Program	0

2. Input basic information

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

Experiment Properties								
Experiment Name:	20111117_Experiment		remark					
User Name:	user	Comment:						

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.

O Detecto	r Library								
Add	Modify	Delete							
Detector	Allele	Reporter	Color	Master Mix	Primer	Probe	Supplies	Batch Number	
Target1	Allele1	FAM							
	Allele2	HEX							
Target2	Allele1	FAM							
	Allele2	HEX							
								Select	Close

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

	Detectors	Add Detector	Delete Detector		Add Detector From Librar	У	_	_	_
l	Detector	Allele	Reporter	Colo	Master Mix	Primer	Probe	Supplies	Batch Number
l	Target1	Allele1	FAM						
I		Allele2	HEX						

4. Set up reference dye

Reference Dye

1.3 Sample Information Setting

1. Click Setup **Sample**

Setup	\odot
Detector	0
Sample	0
Plate	0
Program	0

- 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

C Batch Add	
Start Sample Id a	Sample Count 5
	Add

- 3. Delete sample information
 - a. Itemized deletion: select one sample \blacktriangleright click **Delete** \blacktriangleright 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

Sample ID	Batch Add	Delete	ł	Clear All	Import Samples Info	Export	Samples Info	
								<i>.</i>

5. Set up sample information

Samples										
Sample Id	Color	Sample Name	Sampling Time	Submitting Date						
al		Sample1	2013-12-06	2013-12-06						
a2		Sample2	2013-12-06	2013-12-06						
a3		Sample3	2013-12-06	2013-12-06						
a4		Sample4	2013-12-06	2013-12-06						
a5		Sample5	2013-12-06	2013-12-06						

1.4 Reaction Plate Setting



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.

C Edit Dete	ctor Libr	ary						×
Detector Name:	Target2							
Allele	Reporter	Color	Master Mix	Primer	Probe	Supplies	Batch Number	
Allele1	FAM							
Allele2	HEX							
•								
							OK Cance	el

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

Property	Name	Concentration	Concentration unit
U	Unknown	NO	
z	Negative	NO	Copies/ml
1	Positive Allelic gene 1	NO	Fg/ml
12	Positive Heterozygous	NO	Pg/ml
22	Positive Allelic gene 2	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

▲ Detectors	Plate Setup	Well T	able								
Detector Property Con.				Zoom In	Zoom Out	Reset	Sa	mple Auto	Arrange		
Target1 🗾 🚽	1	2		4					9	10	12
FAM W HEX	A U Target1 U Target1										
Concentration Unit copies/ml	в										
Samples Show Columns: Sample Name	c										
al Samplel											
a3 Sample3	<i>b</i>										
a4 Sample4	F										
a5 Sample5	2										
	F										
	G										
	н										

	F	Plate 9	Setup	Well Ta	ble			
I		#	Well	Sample Id	Assay Item	Property	Dye	
I		1	A01	a1	Target1	Unknown	FAM	
I		1	A01	al	Target1	Unknown	HEX	
I		2	A02					
I		3	A03					
I		4	A04					
I		5	A05					
I		6	A06					
I		7	A07					
I		8	A08					

1.5 Programme Setting

1. Click Setup **Programme**

Setup	•
Detector	0
Sample	0
Plate	0
Program	0

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

	V Hot.	lid(C) 105 🚔 Liquid Quan	nt.(ul) 40 😴	
Run Prog	rams Setup I Stage - Add Step - De	elete		Display With Table
	Hold Stage	PCR S	Stage	
		Cycles	40]
		2nd Temp Step Size -	2nd Temp Step Size -	
		Step Delay -	Step Delay -	
		Grad Temp Grad Range	Grad Temp Grad Range	
100°C	95 °C 00:00:20 🗭 v Sampling	4℃/s 95℃ 00:00:15 ↔ Sampling	4℃/s 60℃ 00:00:20 ♀ ✓ Sampling	

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.



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

Run	\odot
Fluorescence Curve	0
Temperature Curve	0
Program	0

2. Click Start Run



3. Operating confirmation

- a. Modify hot-lid temperature and liquid quantity (sample volume).
- b. Gain (baseline) parameter setting
- c. Target fluorescence value setting

Warning				
Without the bas will not be ca	seline para librated.	meters, th	e fluorescen	ce data
Reference gain replaced with	of Dye "F the default	AM" does gain.	not exist, h	as been
🖌 Hotlid:	105 💌	Liquid	Quantity:	40 🔦
Gain				
📕 Auto Gain				
Used Gain				
F1 (FAM)	7 🚔 🛛 🛙 🖬	e Referenc	e Gain	
			0	0 ⁻

- 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 (sample volume).
- b. Gain (baseline) parameter setting
- c. Target fluorescence value setting

arming					
Without the baseline p will not be calibrated	aram(eters,	the flu	orescen	ce data
Reference gain of Dye replaced with the defa	"FAD ult g	(" doe gain.	s not e	xist, h	as been
🖌 Hotlid: 105		Liqui	d Quant	ity:	40 🖍
ain					
Auto Gain					
Used Gain					
F1 (FAM) 7	Use	Refere	ice Gai	n	

- 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.





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

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

a. Adjust analysis data

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

C Analysis	Settings						
Ct Settings	Advanced Settings	SNP Settings					
Data Analysis	Settings Sample Flu	orescence 🔽					
Assay Item	Keep Manual Calls						
Target 1							
					Apply Analysi	s Settings	Cancel

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

1. 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.

🛃 Report	Designer - D:\LineG	ene9600\Scientific\config\report\Snp\default.rpt						
🗄 🗋 New 🔗	Open 🔚 Save 🛛 🗋 Preview	🛛 🖓 View 👻 🗡 Delete Selected Controls 🖹 🗐 🔟 📲 🗍						
Available cont	rols Used controls							
Common Controls		[Hospital] [Report] Name: [Name] Sec: [Sex] Age: [Age] HospitalNo.: [HospitalNo]						
State State Dyna	: lext Controls mic Text Controls	Test Item: [Test Item] 30 ^ SNP Typing Result Plot Gene Typing: [Gene Typing] 25 5 10						
<u>ĕ</u> ≣ 2 +								
Appearance	e as della set	Allele, 2						
Alignment	Maite							
E Render	Solid 1 Ealeo Ealeo Ealeo	Submitting Date: [Submitting Date] Report Date: ReportDate] Tester: Tester] Checker: [Checker]						
Color	Black							
E Font	Taboma 8 25at							
E Data	ranoma, o.z.spc							
DataField	Sev							
Tag	JCA							
E Design								
DesignVisible	True							
Name	DataField6							
🗆 Lavout								
Location	266, 109							
	0, 0, 0, 0							
⊞ Size	49, 20							
Туре	DataField							
DataField data field of th	e element							

5.2 Print Setting

1. 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.

© Print Template Settings(SMP)	×
CTemplate Setup	
Hospital	
Report	
Tester	
Checker	
Print Setup	
Default Report Template default	
Paper Size A4	
Printer	
Use Default Printer	
Use Custom Printer	
OK Cance	1

5.3 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, SNP, plate information, etc..

solidat	ed Report									
Ð Q										Report Items
Experim Experim File Na	ent Name: 2011; ent Type: SNP me: F:\L;	1125 ineGen	LineGer	ne9600 C	Consolida	ated Rep	ort		1 / 11	Basic Information Nun Program Detectors Flot Plate Table Plate Samples Amp. Curve(Linear) Amp. Curve(Log)
Gain:	F1:6,	, F2:7	13:42:43	- 2011/11/	20 14:41:2	5				Quan. Analysis Result
-										SNP Analysis Result
Hold Star	un Program —— *									
Target	Incubation Time	Rate	Sampling							Create Report
94	120	4								
PCR Stage	Cycles:40									
Target	Incubation Time	Rate	2nd Temp.	Step Size	Step Delay	Grad Temp.	Grad Range	Sampling		
95	5	4								
60	30	4							2	
D	etectors									
Detector 项目1		A1	10101		Dye FAM		010	r		
		A1	lele2		HEX					
P	lot Plate —									
. 1	2 3		4	5 6	7	8	9	10 11	1 12	
A										
-										
в	须目1 - FAM 🔲 项目1 -	FAX 3	美目1 - FAM 🛄 3	项目1 - FAM	² 2 项目1 - FAM ²	2 项目1 - FAM ²	2 项目1 - FAM ² 2	项目1 - FAM		
-			201 - AL				4 - x 0 + 7 0+4 24	AND I THIN		
с										
			_	_						
D	原目1 - FAM <mark>F2</mark> 項目1 - 原目1 - HEX <mark>F2 项目1 -</mark>	FAM 2 I	原目1 - FAM 1/2 : 原目1 - HEX 1/2 :	项目1 - FAM 项目1 - HEM	2 项目1 - FAM 2 项目1 - HEM	2 项目1 - FAM // 2 项目1 - HEX //	2 项目1 - FAM 52 项目1 - HEX 52	项目1 - FAM 项目1 - HEX	N 项目1 - FAM	
E			100	100	100		1.00	100		

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

Rep	ort Te	mplate: de	fault					Prin	t Setting							Print Preview
S	elect/	UnSelect	Select All Sa	mples												🔛 Print One Assay PerReport
, P	rint	Sample Id	Sample Name	Test Item	Name	Sex	Age	Case No.	Outpatient No.	Bed No.	Hospital No.	Nationality	Sampling Time	Diagnosis	Notes	
		04		target1									2011/12/15			
1.0		🔜 Assay It	em:SYBR Detec	t Concentrat:	ion:7.82	2e+06	Conclus	ion:Positiv	re l							

5.5 QC Summary

1. Click Report **>** QC Summary



2. Check the QC summary

Amplification Plot	QC Summary
Color Well PlotType Linear Show 🛐 F2	Description Value Use Result
7000-	Negative control with a Ct less 38
6000	Positive control with a Ct 30
9 5000	Unknown without a Ct N/A
¥ 4000	Standard without a Ct N/A 🚺
3000	
A01 A02 A03 A04 A05 A06 A07 A08 A09 A10 A11 A12	
B01 B02 B03 B04 B05 B06 B07 B08 B09 B10 B11 B12	
C01 C02 C03 C04 C05 C06 C07 C08 C09 C10 C11 C12	
D01 D02 D03 D04 D05 D06 D07 D08 D09 D10 D11 D12	
E01 E02 E03 E04 E05 E06 E07 E08 E09 E10 E11 E12	
F01 F02 F03 F04 F05 F06 F07 F08 F09 F10 F11 F12	
G01 G02 G03 G04 G05 G06 G07 G08 G09 G10 G11 G12	
H01 H02 H03 H04 H05 H06 H07 H08 H09 H10 H11 H12	

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

R Experimental archive storage directory	
Experiments in the following archive directory:	
D:\LineGene9600	
Change	
OK Cancel	

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**

Setup	
Detector	0
Sample	0
Plate	0
Program	0

2. Input basic information

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

Experiment Prope	erties		
Experiment Name:	20111117_Experiment		remark
User Name:	user	Comment:	

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.

© Detector Libr	ary			
Add Modify Del	ete			
Detector	Assay	Dye	Color	
Target3	GOI	FAM		
	HKG	HEX		
Target4		FAM		
		HEX		
			Select	Close

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

Detectors	Add Detector	Add Assay	Delete Detecto	r Delete Assay	Add Detector From Librar
Detector	Ass	ay	Dye	Color	
Target1	GC	Я	FAM	••	

4. Set up reference dye

Reference Dye

1.3 Sample Information Setting

1. Click Setup **Sample**

Setup	\odot
Detector	0
Sample	0
Plate	0
Program	0

- 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

C Batch Add	
Start Sample Id a	Sample Count 5 🔦
	Add Cancel

- 3. Delete sample information
 - a. Itemized deletion: select one sample \blacktriangleright click **Delete** \blacktriangleright 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

	Sample ID		Batch Add	Dele	te	Clear All		Import Samples Info	Export Samples Info
--	-----------	--	-----------	------	----	-----------	--	---------------------	---------------------

5. Set up sample information

1	Samples	_				
	Sample Id	Color	Sample Name	Sampling Time	Submitting Date	
	a1		Sample1	2013-12-06	2013-12-06	
I	a2		Sample2	2013-12-06	2013-12-06	
I	a 3		Sample3	2013-12-06	2013-12-06	
I	a4		Sample4	2013-12-06	2013-12-06	
	a5		Sample5	2013-12-06	2013-12-06	

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.

C Edit Detector 1	Library		×
Detector Name: Targ	get4		
Add Delete			
Assay	Dye	Color	
	FAM		
			OK Cancel

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

Property	Name	Concentration	Concentration unit
U	Unknown	NO	Copies/ml
S	Standard	YES	IU/ml
Z	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

 Detectors 	Plate Setup	Well Table	-								
Detector Property Con.			Zoom In	Zoom Out	Reset	Sa	mple Auto	Arrange			
Targetl 🚺 🕶 🏹	1								10	11	12
FAM / HEX	A U Targeti U Targeti										
Concentration Unit copies/ml	в										
Samples Show Columns: Sample Name	с										
a1 Sample1 a2 Sample2	D										
a3 Sample3											
a4 Sample4	P										
a5 Sample5 🔲	E										
	F										
	G										
	н										

	F	Plate 9	Setup	Well Ta	ble			
I		#	Well	Sample Id	Assay Item	Property	Dye	
I		1	A01	al	Target1	Unknown	FAM	
I		1	A01	al	Target1	Unknown	HEX	
I		2	A02					
I		3	A03					
I		4	A04					
I		5	A05					
I		6	A06					
I		7	A07					
I		8	A08					

1.5 Programme Setting

1. Click Setup **Programme**

Setup	•
Detector	0
Sample	0
Plate	0
Program	0

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

🚺 Hotlid(C) 105 🔺 Liquid Quant. (ul) 40 🖍 Run Programs Setup Display With Table - Delete Add St Add Ste Hold Stage PCR Stage Melting Stage Cycles 40 Step 0.1 Step Holding Sec. 5 Step Size 2nd Temp. Step Size 2nd Temp. Step Delay Step Delay Grad Temp. Grad Range Grad Temp. Grad Range 95 °C 95 °C 4 22/2 00:00:15 🜩 🕶 00:00:15 🗬 🕶 85 °C Sampling Sampling 00:00:20 🔷 🕶 4 ℃/s 4 ℃/s AC/s Sampling 60 °C 60 % °C/s 00:01:00 🜩 🕶 00:00:20 🗬 🕶 🗸 Sampling Sampling

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.



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

Run	•
Fluorescence Curve	0
Temperature Curve	0
Program	0

2. Click Start Run



3. Operating confirmation

- a. Modify hot-lid temperature and liquid quantity (sample volume).
- b. Gain (baseline) parameter setting c. Target fluorescence value setting

larning		
fithout the baseline param will not be calibrated.	eters, the fluorescer	ice data
Reference gain of Dye "FA replaced with the default	M" does not exist, h gain.	uas been
🖌 Hotlid: 105 🗲	Liquid Quantity:	40 🖍
ain		
🔛 Auto Gain		
Used Gain		
F1 (FAM) 7	Reference Gain	
		6

- 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 (sample volume).
- b. Gain (baseline) parameter setting
- c. Target fluorescence value setting

arning					
Without the baseline will not be calibrate	param ad.	eters, tl	e fluore	scence	data
Reference gain of Dye replaced with the def	• "FA Fault	M" does gain.	not exis	t, has	been
🖌 Hotlid: 105	▲ ▼	Liquid	Quantity	:	40 🖍
ain					
🔛 Auto Gain					
Used Gain					
F1 (FAM) 7	Use	Referenc	e Gain		

- 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





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 Analysis Amplification Plot Standard Curve 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 I 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.

— P	lot Plate —										_	Detectors	
	3 Gargeti - FAM (1)	4 target1 - FAM	5) target) target	1 - FAM U ta	6 rgeti - FAM	7 U targett - FAM	8 1) Largert - FAM	10 argeti - FAM	11 U targe	1 11 - FAM U		Table Plate Amplification Curve Plot Type Linear Quan. Analysis Resul Metting Curve Metting Curve(Derivat Metting Analysis Resul Metting Analysis Resul HRM(Aligned) HRM(Aligned) Create Report Print Report	ive It
Ta	able Plate –	Property	Dvo	Std. Con	Sample								
A05	target1	Unknown	EAM	otu. com.	Jampie						- 1		
A05	target1	Unknown	EAM								- 1		
A07	target1	Unknown	FAM								- 1		
0 A10	target1	Unknown	FAM										
1 A11	target1	Unknown	FAM										
2 A12	target1	Unknown	FAM										
5 B03	target1	Unknown	FAM										
.6 B04	target1	Unknown	FAM										
.7 B05	target1	Unknown	FAM										
0 B08	target1	Unknown	FAM										
0 A10 1 A11 2 A12 5 B03 6 B04 7 B05 9 B08	target1 target1 target1 target1 target1 target1 target1 target1	Unknown Unknown Unknown Unknown Unknown Unknown	FAM FAM FAM FAM FAM FAM										

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

R Experimental archive storage directory	
Experiments in the following archive directory:	
D:\LineGene9600	
Change	
OK Cancel	

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 8 Service

1. User Management

User management is used to manage user information Click **Service > User Management** on the menu bar



. .	
	Change password
	Old password: New password: Confirm new password: OK Reset Cancel
d. add user	
	Add user
	User name:
	User name: Password: Confirm password:
	Add user User name: Password: Confirm password: Permission: User manage Run experiment View experiment Manage experiment
	Add user User name: Password: Confirm password: Permission: User manage Run experiment View experiment OK Cancel

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. query
- 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

Delete experiment		
· 直询条件		Clear Condition Query
	Delete Recover Clear	
Experiment type Experiment name Executor Notes Seria	I No Start time End time Purpose Creator name Create time Updater name U	Jpdale lime

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

Template manger	nent							
				Downloa	Delete			
Template category	Template name	Create user name	CreateTime					
8	20140416_135107	admin	2014-05-04					
8	参考增益测量	admin	2014-05-04					
6	2	admin	2014-05-04					
6	20140428_155955	admin	2014-05-04					
5	3	admin	2014-05-04					
6	7项_20120522_123347	admin	2014-05-04					

4. User Login Click Service ► User Login on the menu bar

🔳 User Login	×
Upper Service:	127.0.0.1
UserName:	admin
Password:	
	Automatic login
_	
OK	Cancel

5. Change Password

Click Service ► Change Password on the menu bar

Change password
Old password:
New password:
Confirm new password:
OK Reset Cancel

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 Usage

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.

C Gain	Settin	g								X	
Reference	ence Gain		(🔘 Custom	Gain			\bigcirc	Auto Gain		
F1	F2		F3	F4		F5		F6			1
				ОК		Can	ncel				-

2. Block Scan Method

Click **Tools** \blacktriangleright **Block Scan Method** \blacktriangleright the following window will pop up. The user can select Whole Block Scan or Line Scan.



3. 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

C Detecto	or Library							
Add	Modify	Delete						
Detector	Reporter	Color	Master Mix	Primer	Probe	Supplies	Batch Number	
Target1	FAM							
Target2	FAM							
								Close

4. 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.

C Customized Dy	es	
Dye	Channel	
FAM	1	Dye
SYBR	1	FAM
HEX	2	
TET	2	Channel
VIC	2	1 (470nm -525nm)
JOE	2	
Cy3	3	Delete
TAMRA	3	
NED	3	
ROX	4	
TexRed	4	
Cy5	5	
LCRed	6	
	•	MoveUp
		MoveDown
Dye after you add measurement.	or modify para	meters for crosstalk
	Create	OK Cancel

5. 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

Column Name	
New Item	
	-
	Add
	Dates
	Delete
	ОК

6. 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

Select Columns						
Unselected Columns		Current Column	S			
Item 1(Item00)	Query Query Condition					
	Absolute Report Setting					
		SNP Report Settin	ıg			
	Sample	Absolute Report	SNP Report			
	(*) Sample I	d(SampleId)				
	(*) Color(Co	lor)				
	(*) Sample N	ame (SampleName)				
	(*) Name (Nam	e)				
	(*) Sex(Sex)					
	(*) Age (Age)					
	Case No. (CaseNo)					
	Outpatient No. (OutpatientNo)					
	Bed No. (BedN	o)				
	Hospital No.	(HospitalNo)				
	Nationality()	Nationality)				
	(*) Sampling Time(SamplingTime)					
	(*) Doctor(Doctor)					
	(*) Dept. (Department)					
	(*) Submittin	ng Date(SubmittingTime)				
		OK	Cancel			

7. 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				
© Sample Column Library				
Auto Complete Co	lumns Management			
Auto Complete Columns	Current Values			
Nationality (Nationality)	China			
Diagnosis (Diagnosis)				
Doctor(Doctor)				
Dept. (Department)				
Tester(Tester)				
Notes (Notes)				
Add Delete	One Value per Line			
	OK Cancel			

8. 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

Sel	ect Instrument 600254
Baseline Parameters	Measured
Reference Gain Parame	Cy3,Cy5,FAM,HEX,ROX
Proportion Parameters	Measured
Crosstalk Correction Pa	Cy5,FAM,HEX,ROX,TAMRA
Crosstalk Gain Paramet	F1,F2,F3,F4,F5
9. 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.



10. Crosstalk Gain Parameter Measurement

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

Click **Tools** \blacktriangleright **Measure Crosstalk Gain Parameters** \blacktriangleright 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.

	E	xperimen	t Type: Abs	olute Expe	eriment Nam	ie: Crosstal	k Gain Mea	surement	
Experiment Properties									
Experiment Name: Crosstalk Gain Measurement									
User Name:									
Detectors	Add Det	tector Ad	d Assay	elete Detector	Delete Assay	Add Dete	ector From Libra	rv .	
Detector	Re	eporter	Color	Master Mix	Primer	Probe	Supplies	Batch Number	
F1	F/	AM							
F2	Н	EX 🚽							
Reference D	ye								
	-								

11. 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

С	Upgrade	
υ.	opgraduc	

0. Opgiuuo	
O Firmware Upgrade	×
Choose Port: COM1	
Upgrade File	
E:\PCR.bin	Select
	Upgrade

12. 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

File			State	
Add upgrading files	Remove selecte	ed files		
Add upgrading files	Remove selecte	ed files		

13. 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.

O Ta Calculator	
Forward Primer	
Reverse Primer	
Forward Temperature C	
Reverse Temperature C	
Average Temperature C	
Anneling Temperature C	
Calculate Close	

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 **L**; if the instrument is disconnected, the icon on the status bar will be **L**

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 conditionc. Queryd. Clear all query conditions

6 Data Query	
Path:	Browser
Very Condition	
	Clear Condition
	Query
Query Result	
# File Name Sample Id Sample Name Test Item Name Sex Age Case No. Outpatient No. Bed No. Hospital No. Nationality Sampling Time Diagnosis Doct	or Dept. Test Result H
L	

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: 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 power switch at the rear of the instrument is set ON, but the instrument makes no response.	The run switch at the front of the instrument is not switched on. The internal switching power will have no voltage output.	For working the instrument, its run switch must be pressed and the green indicator lamp should be lit green to indicate energizing of the control system.
2	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.
3	After turning the power switch on, the power lamp fails to light up	The run switch is not pressed	This switch is used for temporary turning on/off of the output power and is equivalent to "standby" mode. For prolonged shutdown, the rear power switch should be switched "Off".
		Power is not switched on.	Check and switch on power
		Burnt fuse	Replace fuse (250V 8A Φ5x20)
		Damaged switch	Replace switch
		Other	Contact the supplier or manufacturer

4	At detecting sample position, the step motor fails	Poor contact or damage of the interface wire	Check, connect or replace interface wire
	to work and the communication fails.	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
		The fixing clamp is not fully inserted	Tighten it into the UNLOCKER port, and switch on power again after shutdown
5	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
6	Module temperature heating or cooling rate obviously	The ventilation opening is blocked.	Clear the ventilation opening
	decreases or temperature control is incorrect.	Loose connection wire	Contact the supplier or manufacturer
		The refrigerating sheet is damaged	Contact the supplier or manufacturer
		Fan is damaged or fails to run	Contact the supplier or manufacturer
		Fan is damaged or fails to run The temperature sensor is damaged	Contact the supplier or manufacturer Contact the supplier or manufacturer
7	The module fails to heat and refrigerate.	Fan is damaged or fails to run The temperature sensor is damaged The inside of the instrument is damaged	Contact the supplier or manufacturer Contact the supplier or manufacturer Contact the supplier or manufacturer
7	The module fails to heat and refrigerate.	Fan is damaged or fails to run The temperature sensor is damaged The inside of the instrument is damaged The refrigerating sheet is damaged	Contact the supplier or manufacturer Contact the supplier or manufacturer Contact the supplier or manufacturer Contact the supplier or manufacturer
7	The module fails to heat and refrigerate.	Fan is damaged or fails to run The temperature sensor is damaged The inside of the instrument is damaged The refrigerating sheet is damaged During hot-lid heating-up	Contact the supplier or manufacturer Contact the supplier or manufacturer Contact the supplier or manufacturer Contact the supplier or manufacturer Waiting until the hot-lid temperature comes to the target value. When stopping running, module temperature holds down 30C automatically.
7	The module fails to heat and refrigerate. Abnormal temperature or fluorescence curve: straight line or loss of partial data	Fan is damaged or fails to run The temperature sensor is damaged The inside of the instrument is damaged The refrigerating sheet is damaged During hot-lid heating-up The running programme is infected by a virus	Contact the supplier or manufacturer Contact the supplier or manufacturer Contact the supplier or manufacturer Contact the supplier or manufacturer Waiting until the hot-lid temperature comes to the target value. When stopping running, module temperature holds down 30C automatically. After removing the virus, re-install the application software

		or the setup of communication port is not appropriate.	
9	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
10	The hot-lid is will not heat	Thermal-sensitive fuse is damaged	Contact the supplier or manufacturer
		Loose plug-pieces	Contact the supplier or manufacturer
		Heating elements of hot-lid is damaged	Contact the supplier or manufacturer
		Temperature sensor of hot- lid is damaged	Contact the supplier or manufacturer
11	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******.b96 background parameters are set incorrectly.	Eliminate contamination. Each instrument shall correspond to baseline96 document. After perennial use, offset would occur in the optical elements. In this case, contact the manufacturer to re- calibrate the background value.
12	Reagent evaporation	The PCR tube cap does not sealing tightly enough.	Change consumable to one with a tighter fitting cap.
13	Signal crosstalk among channels	Dye signal crosstalk among channels can happen.	You can measure by using "Crosstalk Measurement", and save parameters to modify.
14	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: LineGene 9600 series wiring



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Note: