BaseTyper™ 48.4

Quiet HRM Real-Time PCR System

Silent, rapid and accurate PCR analyses

INSTRUCTIONS FOR USE

PentaBase

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CE IVD

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1 Intended purpose

The BaseTyper™ 48.4 Quiet HRM Real-Time PCR System is intended for performing silent, rapid, and accurate Polymerase Chain Reactions (PCR), Reverse Transcriptase PCR (RT-PCR), quantitative PCR (qPCR), and fluorescence-based melt and High-Resolution Melt (HRM) analyses. The BaseTyper™ 48.4 Quiet HRM Real-Time PCR System obtains real-time measurements of signals from up to 48 samples in each run. The sample material is normally genetic material like RNA and DNA. The BaseTyper™ 48.4 Quiet HRM Real-Time PCR System can be used in medical institution laboratories and clinical laboratories for infectious pathogen (such as viruses, bacteria, mycoplasma, etc.) detection, or multiple tumor marker tests of neoplastic diseases, etc. For scientific research, it could be used for the fluorescence quantitative or qualitative analysis of genetic material (DNA, RNA, and derivatives of both) in fields of immunology, molecular biology, forensic science, genetics, archeology, zoology, phytology, etc. Each well can be analysed using fluorescence in up to four different fluorescent channels. The fluorescent signals can for example originate from DNA-binding fluorescent dyes or labeled probes. Signals can be converted to presence, comparative quantities, and/or physical-chemical property readouts. The instrument is intended for use by healthcare professionals or qualified laboratory personnel instructed and trained in the techniques of PCR.

2 User requirements

The BaseTyper™ must only be operated by laboratory professionals who have been trained in the relevant laboratory techniques and who have carefully read this manual.

3 Safety symbols and labels

3.1 Safety labels on the BaseTyper™

Icon	Description
	**High Temperature** Indicates areas with very high temperatures. Do not touch these areas.
	**IVD Equipment** The BaseTyper™ belongs to In Vitro Diagnostic equipment.
	**CE Mark** Indicates this BaseTyper™ is in conformity with the essential health and safety requirements set out in the REGULATION (EU) 2017/746 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU.
	**Serial Number** Indicates the serial number of this BaseTyper™.
	**Manufacturer** Indicates the manufacturer of this BaseTyper™.
	**Manufacture Date** Indicates the manufacture date of this BaseTyper™.
	**Caution** Indicates the “caution” of this BaseTyper™.
	**Consult Instructions for Use** Indicates the consult instructions for the use of this BaseTyper™.
	**Separate Collection for this Equipment** Indicates that when the end-user wishes to discard this electrical and electronic equipment, it should not be discarded as unsorted waste but must be sent to separate collection facilities for recovery and recycling.

3.2 Symbols on the transport package

Icon	Description
	**Fragile** The items inside are fragile, please handle with care.
	**This Way Up** Indicates the upward side of the transport package.
	**Keep Dry** Keep the transport package away from rain or any liquid.
	**IVD Equipment** The BaseTyper™ belongs to In Vitro Diagnostic equipment.
	**Temperature Limit** Indicates the temperature limits for the storage and transportation of package.
	**Humidity Limit** Indicates the humidity limits for the storage and transportation of package.

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	**Atmospheric Pressure Limit** Indicates the atmospheric pressure limits for the storage and transportation of package.
	**Max 4 Packages** It is prohibited to stack more than 4 layers for the storage and transportation of packages.
	**Recycle** Indicates the packaging materials are recyclable.

3.3 Symbols used in this manual

Icon	Description
	**Caution** Reminding the user to pay attention to a certain operation.
	**Attention** Providing important information needed for successful use of the BaseTyper™.
	**Prohibit** Prohibiting the user to perform a dangerous operation.

3.4 Safety and regulatory compliance

Operation, maintenance, and repair of the BaseTyper™ instrument shall strictly follow the safety specifications listed in this section and throughout this manual. The BaseTyper™ is designed to withstand biological contamination, it has electrical safety protection and mechanical motion protection. Non-observance of the instructions or performing any operations not stated herein may affect the safety protection provided and may also destroy the safety standards of design and manufacturer as well as the expected application scope of BaseTyper™ 48.4 Quiet HRM Real-Rime PCR System.

***PentaBase A/S** will not be responsible for any possible consequence caused by either not having read the manual or violation of the instructions mentioned herein.*


Caution: Carefully read this manual before operating the BaseTyper™. Incorrect understanding or operations may cause instrument damage or inefficient use, laboratory damage, or personal injury.


Attention: Pay attention to descriptions marked with “Caution”, “Attention” and “Prohibit” symbols along with the safety labels on the instrument and the transport box.

3.5 General instrument safety and precautions


Caution: Users are not allowed to open the instrument body to replace any components or to debug the BaseTyper™.


Caution: Handle the BaseTyper™ with care.


Caution: In case any of the following conditions occur, immediately cut off the power supply and contact the distributor or manufacturer:

- Any liquid has entered the inside of the BaseTyper™.
- Abnormal sound or smell appears while the BaseTyper™ is running.
- The BaseTyper™ is soaked with water.
- Obvious functional changes of the BaseTyper™.


Prohibit: Never handle or move the instrument while it is running.


Caution: The BaseTyper™ has openings for ventilation to protect the instrument from overheating. Do not block these ventilations openings and do not cover the instrument with the dust cover or any other materials while it is running.


Caution: Transportation and installation of the BaseTyper™ should be performed by a trained laboratory professional or under from the Technical department of PentaBase A/S.


Caution: Do not open the top lid while the instrument is running, this may break the biological safety and electromagnetic radiation protection measures of the instrument.


Caution: Do not try to force unsuitable consumables into the sample block.

3.6 Electrical safety and precautions


Prohibit: The voltage of the BaseTyper™ can cause harm to the human body, cut off the power supply before opening the instrument shell. It is prohibited to replace any parts of the instrument while it is connected to power.


Caution: The BaseTyper™ should be properly grounded, any damage to the internal or external grounding path may cause danger. The power cord provided is a standard three-pin plug, plug it into an appropriate three-wire grounded power socket to ensure safety.


Caution: In case of electric leakage, immediately unplug the BaseTyper™ and stop using it.

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Caution: Unplug the power cord before moving the BaseTyper™.

Attention: Use the power cord attached to the instrument. If the original power cord breaks, replace it with a similar one.

Caution: The power grid environment of the BaseTyper™ must have a grounded wire.

Caution: Check the power connection carefully. Hold the power plug when you plug the power cord and make sure the power plug is perfectly inserted into the socket. Do not pull the power cord to pull out the plug.

Caution: Keep the power cord away from heaters or other objects with high temperatures. Do not place anything on the power cord and keep it away from places where people move around.

Caution: The BaseTyper™ fuse tube (250V /F10AH) is located in the fuse tube box near the power outlet at the rear of the instrument. Using an improper fuse tube may lead to system circuit damage. Ensure that the fuse tube has been properly installed before the instrument is turned on.

Caution: Before replacing the fuse tube, cut off the instrument power supply, take out the power plug and use a screwdriver to open the fuse box. Then replace the old fuse tube with a similar one.

3.7 Environmental safety and precautions

Caution: The BaseTyper™ is for indoor use only and should be placed in a well-ventilated room without exposure to corrosive gases.

Prohibit: Never run the BaseTyper™ in a room with flammable and explosive gases.

Prohibit: Do not use sprays close to the BaseTyper™ as spraying liquid on electrical parts may cause a fire.

Attention: The working environment temperature of the BaseTyper™ should be between 10˚C and 30˚C. The relative humidity should be between 20% and 85%.

Attention: The working environment of the BaseTyper™ should be under normal atmospheric pressure (altitude below 2000m).

3.8 Biological safety and precautions

Biohazard: The BaseTyper™ is intended for use with samples containing nucleic acids. Consider the biological hazard of all samples and handle them with care. If necessary, wear protective clothing, glasses, and gloves, while processing the samples.

Biohazard: Do only use reagents and consumables that have not expired.

Biohazard: In case of liquid overflow during a PCR run, immediately disinfect the contaminated area with appropriate detergent to avoid the spreading of contaminants.

Biohazard: **C**omply with the local or national regulations to complete the disposal of waste samples and contaminated materials.

Biohazard: An abandoned BaseTyper™ should be considered as biologically contaminated material. Comply with the local or national regulations to complete the disposal of the instrument.

4 Overview

4.1 Principle of operation and instrument structure

4.1.1 Principle of operation

The main components of the BaseTyper™ are a control system, a power system, a temperature control system, a detection system, a user-machine interface system, and an instrument shell.

The temperature control system includes a Peltier heating module, sample block, and hot lid. The detection system includes a LED excitation light source, photodiodes, and light filters. The user-machine interface system includes data acquisition and analysis software, which is responsible for collecting real-time data, fluorescence diagram formation, data processing, and diagram analysis, to quantify or characterise the target nucleic acid and obtain other test report information.

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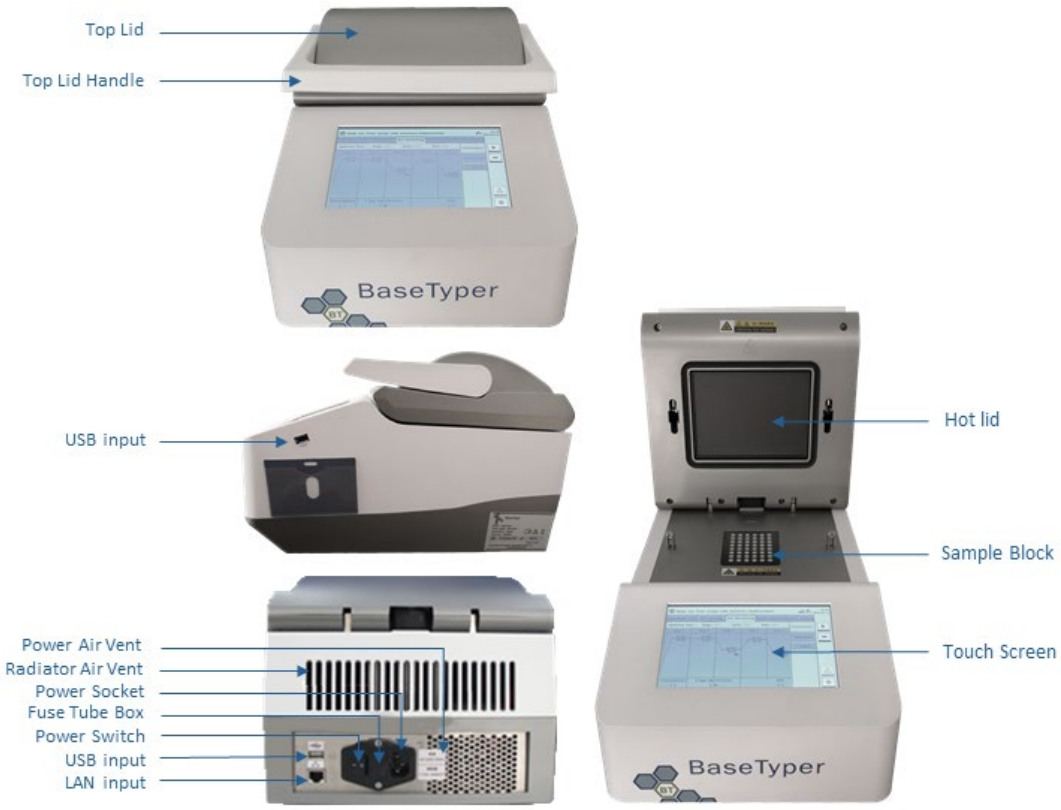
4.1.2 Instrument structure

The structure of the BaseTyper™ is shown below:



5 Product introduction

Table 1. General instrument parameters of the BaseTyper™ 48.4 Quiet HRM Real-Rime PCR System.

General instrument parameters	
Instrument specifications	Dimensions: 400 mm (L) x 260 mm (W) x 260 mm (H) Weight: 11 kg
Package specification	Dimensions: 500 mm (L) x 350 mm (W) x 360 mm (H) Weight: 13 kg
Power specification	Voltage: AC 100-240V Frequency: 50-60Hz Rated power: 600VA
Communication specification	Network port: TCP/IP protocol Ethernet connection
Application environment	Temperature: 10˚C - 30˚C Relative humidity: 20% - 85%, non-condensing Atmospheric pressure: 85.0 kPa – 106.0 kPa Altitude: Below 2000 m
Storage and transportation environment	Temperature: -20˚C to 55˚C Relative humidity: less than 93%
Running noise	The noise level of a running BaseTyper™ does not exceed 65 decibels

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Table 2. Technical instrument parameters of the BaseTyper™ 48.4 Quiet HRM Real-Rime PCR System.

Thermal parameters	
Temperature accuracy	≤ 0.1˚C
Temperature uniformity	± 0.1˚C
Temperature precision	≤ 0.1˚C
Max heating and cooling ramp	8˚C/s
Optical parameters	
Excitation light source	LED light source
Detect system	Photodiodes **Optical Channel** / **Dyes** 1 – FAM, SYBR Green I, etc. 2 – VIC®[1], HEX, TET, JOE, etc. 3 – ROX, Texas Red, etc. 4 – Cy5, etc.
Detective parameters	
Throughputs	Simultaneously detects 48 samples
Repeatability	CV ≤ 0.5%
Linear correlation	|r| ≥ 0.999, within the scope of no less than five magnitudes concentration gradients.
User-machine interactive system parameters	
Touch screen	A built-in 7.0-inch full-colour touch screen for easy operation of the instrument without a connected computer
Main control computer	The recommended minimum system requirements of the main control computer are list below: ○ CPU: 2.4 GHz, dual-core ○ Internal storage: ≥ 2 GB ○ Hard Disk: 500 GB ○ Operating system: Windows XP Pro or higher
Network control	Up to ten BaseTyper™(s) can be connected to one computer at the same time ⚠ The main control computer of the BaseTyper™ instrument is not designed for online use, connecting it to the internet may cause risks of computer virus infections or hacker attacks. PentaBase A/S will not be responsible for any damages caused by connecting the computer to the internet. ⚠ It is not recommended to install other software on the main control computer of the BaseTyper™. This could potentially cause a software module conflict and may also influence the reliability of results. ⚠ PentaBase A/S will not provide any anti-virus software. Therefore, if necessary, take precautions to prevent the main control computer from viruses.

[1] VIC is a registered tradename of Applied Biosystems, Inc

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Table 3. Instrument characteristics of the BaseTyper™ 48.4 Quiet HRM Real-Rime PCR System.

Instrument	
Touch screen operation	The BaseTyper™ has a built-in 7.0-inch touch screen which allows the BaseTyper™ to run without a connected computer
Independent temperature control	The BaseTyper™ uses an independent temperature control technology and a high repeatability temperature zone
Power-off protection	The BaseTyper™ has a power-off protection function which can protect all configuration settings in case the power is turned off. This allows the interrupted experiment to continue when the power is turned back on
Multiple PCR step modes	The BaseTyper™ provides multiple PCR step modes, including a touchdown step, a long step, a gradient step, and a melting step
Remote running	Edit experiment settings on a computer and control the connected BaseTyper™
Software	
Software interface	A wizard-style interface with an intuitive layout and program settings that make the software easy to operate
Software language	Default languages are English and Chinese
Multiple functions	The BaseTyper™ has multiple analysis functions that suit a variety of experimental requirements. These include absolute quantification analysis, relative quantification analysis, melting curve analysis, high resolution melting (HRM) analysis, genotyping analysis, and endpoint fluorescence analysis
Data transmission	Data can be transmitted between the BaseTyper™ and the control computer. Real-time data will automatically be transmitted to the computer during the experiment run
Data storage	The BaseTyper™ can store more than 1000 experiment settings/experiment data files
Program setting	Each stage can contain 99 steps, and a maximum of 99 cycles. A cooling step can be added at the end of the program for long-term preservation after the experiment

Table 4. Reagent specification for the BaseTyper™ 48.4 Quiet HRM Real-Rime PCR System.

PCR reagent	The open reagent platform can be used with all qualitative and quantitative PCR reagents
Dyes	FAM/SYBR Green I, VIC/HEX/TET/JOE, ROX/Texas Red, Cy5, etc.
Consumables	0.2 mL single PCR tubes and 0.2 mL 8-strip PCR tubes **Biohazard:** Reagents and consumables should only be used within their expiration date.

6 Installation of the BaseTyper™

6.1 Unpackaging instructions

The BaseTyper™ and its accessories are well protected in a carton case. To prevent shocks and shakes during transportation, the BaseTyper™ is sealed with a plastic bag and well supported by protective foams as shown in the figure below:

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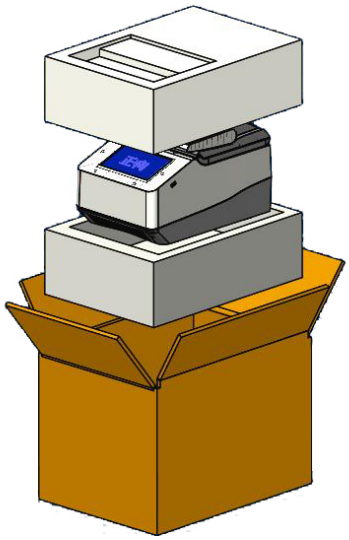
6.1.1 Unpackaging steps



1. Take out the BaseTyper™ accessories and remove the protective foam.
2. Remove the plastic bag and place the BaseTyper™ on a steady surface.

Attention: To prevent the formation of condensing water, do not open the transport package until it reaches room temperature.

Attention: Check the condition of the package before opening it. In case of any damage or watermarks, contact PentaBase A/S.

Attention: Check that the instrument and its accessories are in accordance with the packing list and ensure that all components are present and intact. Report any missing items to PentaBase A/S.

Caution: Please keep the original packing materials for future use. The transport package of the BaseTyper™ is designed to reduce instrument damage and ensure safety during transportation. Using other packaging materials will break the warranty, and PentaBase A/S will not be responsible for damages that occurred as consequences of improper packaging. Please also keep instrument-related documents provided by PentaBase A/S.

6.2 Materials Provided

1. BaseTyper™ 48.4 instrument (x1)
2. Power Cord (x1)
3. Instructions for use (x1)
4. Electrical fuses (x2)
5. Quality Certificate (x1)
6. USB storage stick (x1)

6.3 Materials required but not provided

Use non-skirted 0.2 ml clear or frosted PCR tubes, either single or 8-strip with appropriate PCR assays along with appropriate sample material. The PCR assay must be prepared according to the manufacturer's instructions.

6.4 Requirements for working environment

6.4.1 Instrument space requirements

1. The BaseTyper™ is for indoor use only and should be placed in a room with low humidity (between 20% and 85% RH) and an appropriate temperature (between 10°C and 30°C). The room should be well ventilated and without corrosive gases.
2. The BaseTyper™ should be placed on a steady surface.
3. Keep the BaseTyper™ away from heat sources (direct sunshine, heaters, stoves, etc.) and water sources (sinks, water tubes, etc.).
4. The working environment of the BaseTyper™ instrument should be without electromagnetic interference, vibration, and high-frequency wave electrical equipment.
5. Leave at least 30 cm of free space in front of the ventilation openings when the BaseTyper™ is running. The ventilation openings prevent the machine from overheating and should never be covered while the machine is running.

6.4.2 Instrument power requirements

1. The power grid environment of BaseTyper™ should possess ground wire and the instrument should be properly grounded.
2. Ensure that the power cord of the BaseTyper™ has 3~4 three-phase plugs in order to meet the demands for the instrument and control computer.
3. The power specifications of the BaseTyper™ are listed in **Table 1**. It is recommended to use a UPS power supply. The use of improper power may damage the system circuit and potentially cause a fire.

Attention: Before connecting the AC power supply, ensure that the supplied voltage corresponds to the required voltage of the BaseTyper™ (allowable deviation ± 10%). Also make sure that the provided current does not exceed the rated current of the BaseTyper™.

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Attention: If the power supply system of the BaseTyper™ working environment is unstable, do not connect other electrical equipment to the same power circuit.

Prohibit: Spraying liquid on electrical parts may cause a short circuit and result in fire, do not use sprays around the instrument.

Caution: Do not place anything on the power cord and keep it away from places where people move around. Hold the power plug when plugging the power cord and make sure that the power plug is perfectly inserted into the socket. Do not pull the power cord to pull out the plug.

Attention: Under normal circumstances, use the instrument attached power cord. If the original power cord is destroyed, replace it with a similar one.

6.5 Instrument installation

6.5.1 External device connection

Plug the power cords of the BaseTyper™ and control computer into the power supply. Make sure that the necessary external devices are connected to the control computer, such as display, keyboard, mouse etc.

6.5.2 Installation of application software

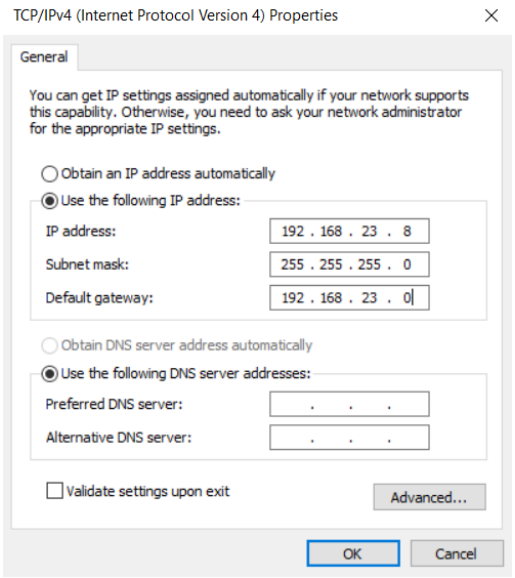
The application system software is required on the control computer for controlling the BaseTyper™ and analysing the experimental data. The software is provided on a USB stick and can be installed as described below:

1. Switch on the control computer and turn off its firewall.
2. Place the USB stick in the USB port of the computer.
3. Click on the BaseTyper™ software file and install the software.

6.5.3 Computer network setting

The network address of the control computer should be set according to the default network information of the BaseTyper™ allowing the computer to connect to the instrument via LAN.

1. Check the network information of the BaseTyper™ via the touchscreen interface on the instrument, by clicking the Edit button in the network information window (General Setting > Configuration).
2. Open the Control Panel > Network and Sharing Center > Local Area Connection > Properties > Internet Protocol Version 4 (TCP/IPv4) on the control computer, to open the setting window shown below:



3. The last number of the IP address must differ from the last number of the IP address on the BaseTyper™ – the rest of the numbers must be identical. The Subnet mask and Default gateway must be identical to the numbers shown in the BaseTyper™ network information window. Press OK.
4. Open the BaseTyper™ software on the control computer and click the **Instrument management** icon. Click **Add** in the **Instrument Management** window. The BaseTyper™ should appear on the **Instrument List**. Select the instrument and press **OK**. The BaseTyper™ is then connected to the control computer. In case you have any difficulties connecting the BaseTyper™, please contact PentaBase A/S.

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7 Preparation before an experiment

7.1 Instrument self-inspection

The BaseTyper™ has a self-inspection function, and it is necessary to let the instrument conduct the self-inspection before running any experiments, to ensure that it runs normally.

1. Switch on the power switch of the BaseTyper™.
2. The BaseTyper™ will automatically conduct the self-inspection when it is turned on. The self-inspection will inspect the instrument version, electric system, power supply and initialise motor positions.
3. After the self-inspection, the BaseTyper™ will enter standby mode.

Caution: Before turning on the BaseTyper™, ensure that external devices and the power supply are properly connected.

Caution: In case the BaseTyper™ fails the self-inspection, please contact the distributor or PentaBase A/S.

7.2 Installation validation

In addition to the self-inspection (Section 7.1) an installation kit (*Real Time PCR System Installation Kit*, Xi'an TianLong Science and Technology Co., Ltd., Ref. No S3010000007) can be obtained. Please follow the instructions for use provided with the kit for proper use. Always contact your local distributor or PentaBase A/S (Section □) for questions or clarification.

7.3 Reagent preparation

Follow the instructions of the PCR kit to prepare the PCR reagents. Add the sample and PCR reagents into suitable consumables and seal the tubes.

Loading the instrument

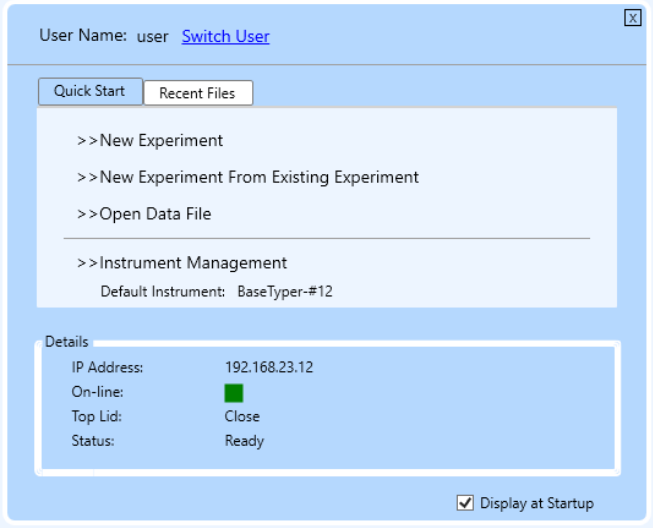
1. Lift and open the top lid to get access to the sample block.
2. Place the consumables containing the sample and PCR mix in the sample block and close the top lid.
3. Select or edit an experimental program and start the run.

8 Operating the application software

The following instructions for operating the BaseTyper™ software include an introduction of the basic software functions and operation descriptions, such as user account management, design or editing of experiments, real-time monitoring, and analysis of experimental data.

8.1 Start the software

After installing the software, double-click on the icon to start the software. The start-up interface will display a quick start bar as shown below. In the quick start bar, the user can sign in, create a new experiment, open a data file, set a default instrument, view instrument information, and conduct other operations.



8.1.1 Introduction of the quick start bar

- **Username:** The current Username is displayed at the top of the quick start bar. Click **Switch User** to change the current user account.

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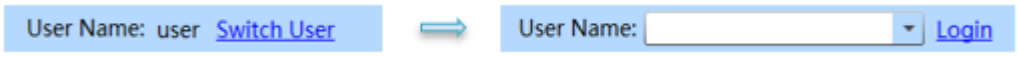
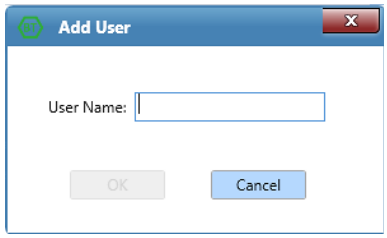
- **Login:** Click **Switch User** and enter a registered account name in the input box or select it from the drop-down menu. Then click **log in**.

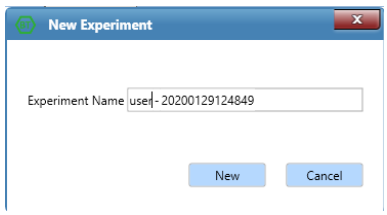
  Attention: The software provides two usernames by default: **User** and **Admin**.



- **Register a New User Account:** Click **Switch User** and select the **Add User** option in the drop-down menu of the **Username** input box. The software will automatically pop up the Add User dialog box. Enter a new user account name in the **Username** input box and click **OK** to register a new user account.



- **Quick Start** tab**:** Includes four shortcut keys:
  - **New Experiment:** A new window will pop up, where the user can enter the name of a new experiment. Press New to create the new experiment file.



  - **New Experiment from Existing Experiment:** Choose an existing experiment file and use the settings of this file as a template for a new experiment.
  - **Open data file:** Open an experiment file from the run files folder.
  - **Instrument Management:** Manage all instruments connected to the LAN.
- **Recent files** tab: Display the latest experiments. Click on a file from the list to open it.
- **Details:** The default instrument, IP address, Online status, and information about the Top-lid and Status are displayed.
- Choose not to see the **Quick Start** bar window by unticking the box **Display at Startup**.
- To open the **Quick Start** bar from the software main interface, click on the **Quick Start** icon in the toolbar.

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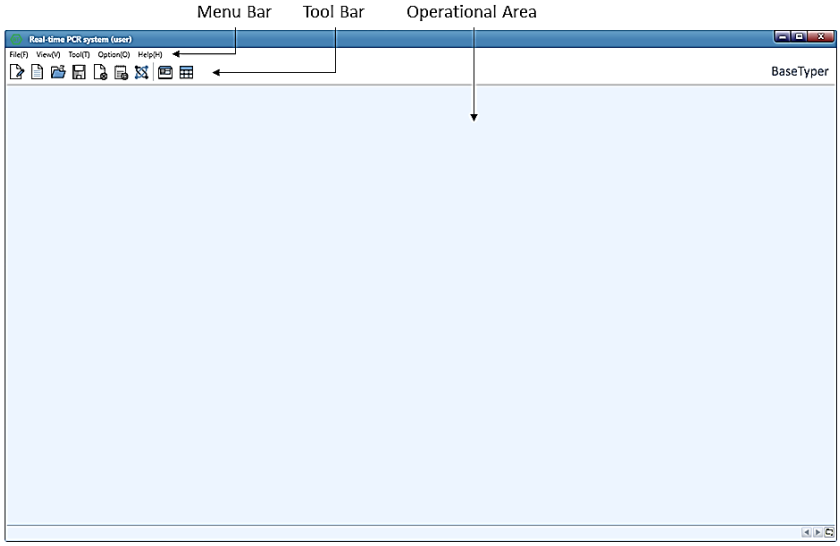
8.2 Main interface

When closing the Quick Start bar, the application software will automatically enter the main interface, which consists of a menu bar, toolbar, and an operational area as shown in the figure below:



8.3 Menu bar

The menu bar in the software includes the submenus: **File (F), View (V), Tool (T), Option (O),** and **Help (H).**

File (F) tab functional descriptions:

- **New Experiment(N):** Create a new experiment.
- **New Experiment from Existing Experiment:** Choose an existing experiment file and use the settings of this file as a template for a new experiment.
- **Open data file:** Open an experiment data file to view or analyse the data.
- **Recent files:** Show the latest files, which can be opened directly from the list.
- **Close Experiment**.
- **Save:** Save the experiment file in the default file path set in configuration management.
- **Save As:** Click to save the experiment file in a selected folder.
- **Export Raw Data:** Export experiment raw data file to a selected folder.
- **Export All Data Sheets to Excel:** Export experiment data as an Excel file to a selected folder.
- **Exit:** Exit and close the software.

View (V) tab functional descriptions

- **Quick Start:** Open the **Quick Start** bar.
- **Show Toolbar:** Decide whether to show the **Toolbar** on the main interface of the software.
- **Show Instrument Information:** Decide whether to show **Instrument Information** on the main interface of the software. This will be displayed in the top right corner like this:

On-line: Top Lid: Close Status: Ready

  - **On-line:** indicates disconnection to the instrument. Indicates connection to the instrument.
  - **Top Lid:** Close/open refers to the lid.
  - **Status:** Ready/running refers to the running status.

Tool (T) tab functional descriptions

- **Instrument Management:** This interface is shown below and displays:

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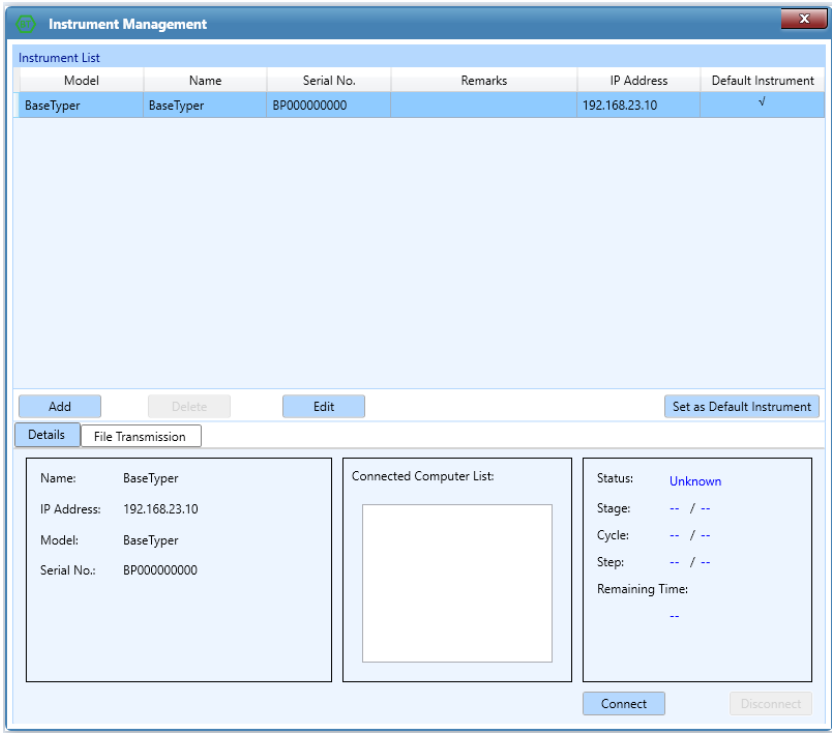
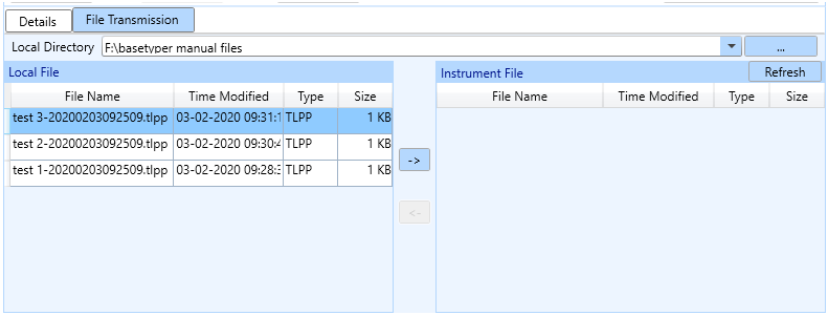


- **Instrument list:** Shows information on connected instruments.
- **Details:** Shows information about the connected instrument including Name, IP Address, Model, and Serial number.
- **File Transmission:** Shows experiment files saved on the instrument (Instrument File) or the computer (Local File). Select the destination for Local files in the “Local Directory” drop-down menu.

  Click the -> button to transfer the selected local file to the instrument.

  Click the <- button to transfer the selected instrument file to the Local Directory.



- **Tm Calculator:** Open the Tm calculator interface where the melting temperature of primers can be calculated.

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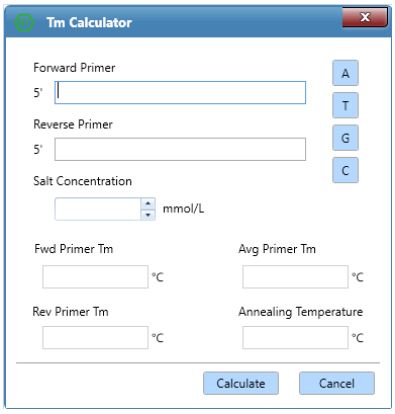
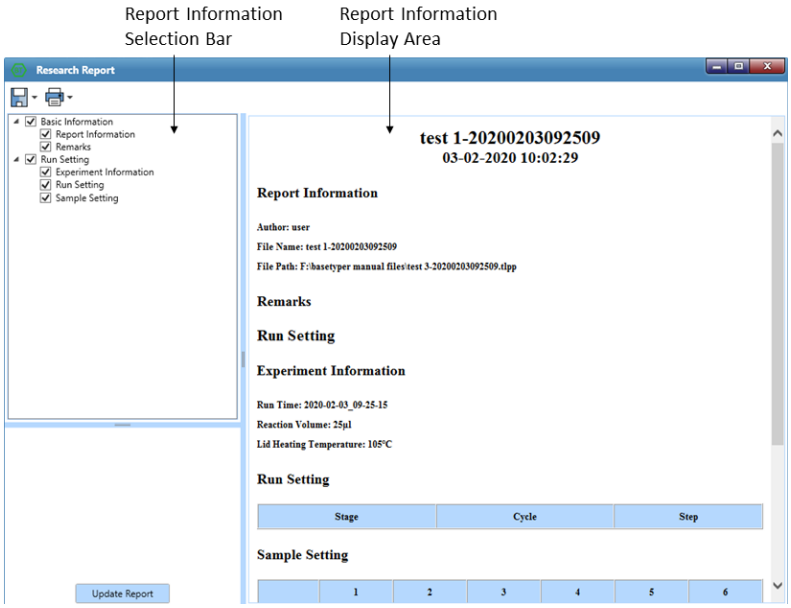


Use the A T G C keys to put in the sequence of the forward and the reverse primer. Put in the salt concentration and click **Calculate**. The software will automatically calculate the **Forward Primer Tm**, **Reverse Primer Tm**, **Average Primer Tm,** and **Annealing Temperature**.

- **Research Report:** The Research Report will contain all information about an experiment. In the information selection box, the user can choose experiment information to be displayed in the Display area. Click **Update Report** to refresh the information in the research report. The research report can be printed or saved as a PDF or HTML file in a selected folder.



Option (O) tab functional descriptions

- **Configuration Management:** The Configuration Management consists of three tabs:

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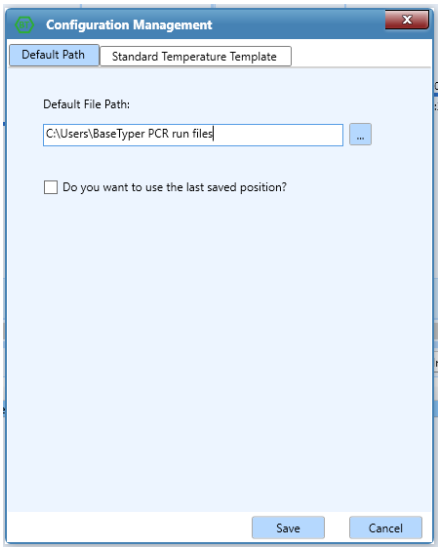
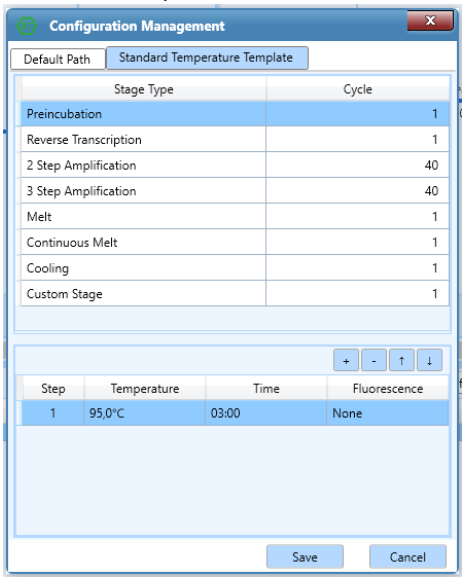


- **Default Path** tab: Experiment files are saved in this path by default. To change this folder, click on the ... icon and select a path, or manually type in the path. Tick the **Do you want to use the last saved position** to save future experiments in the selected folder. To save an experiment in a different path, click **Save As** and select a different path.



- **Standard Temperature Template** tab: Seven pre-defined standard temperature templates are displayed. The templates can be modified by double-clicking on the cycle number, step, temperature, time, or fluorescence.
- **Analysis Parameter Management:** Pre-define settings for Abs Quant Analysis and HRM curve analysis. This interface consists of two tabs;
- **Abs Quant:** Add a Gene Name and its corresponding Ct value. These settings can be saved and used for absolute quantification analyses of future experiments with the same gene.

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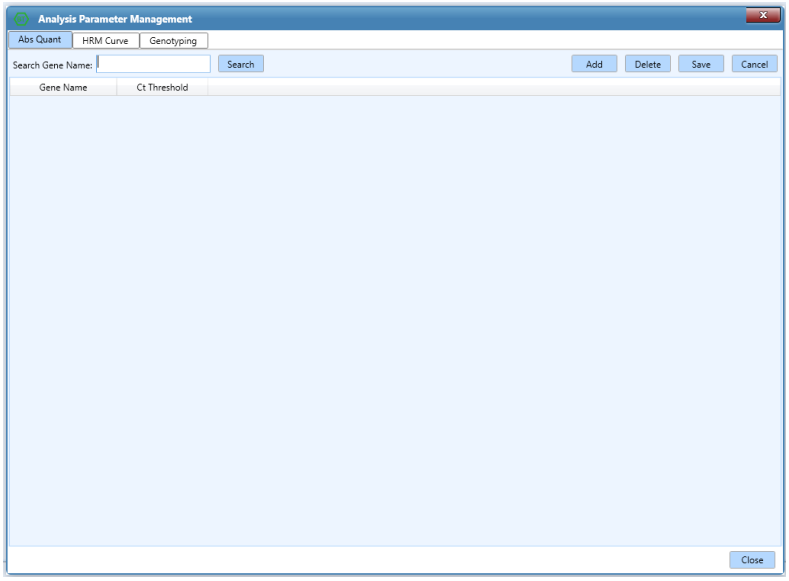
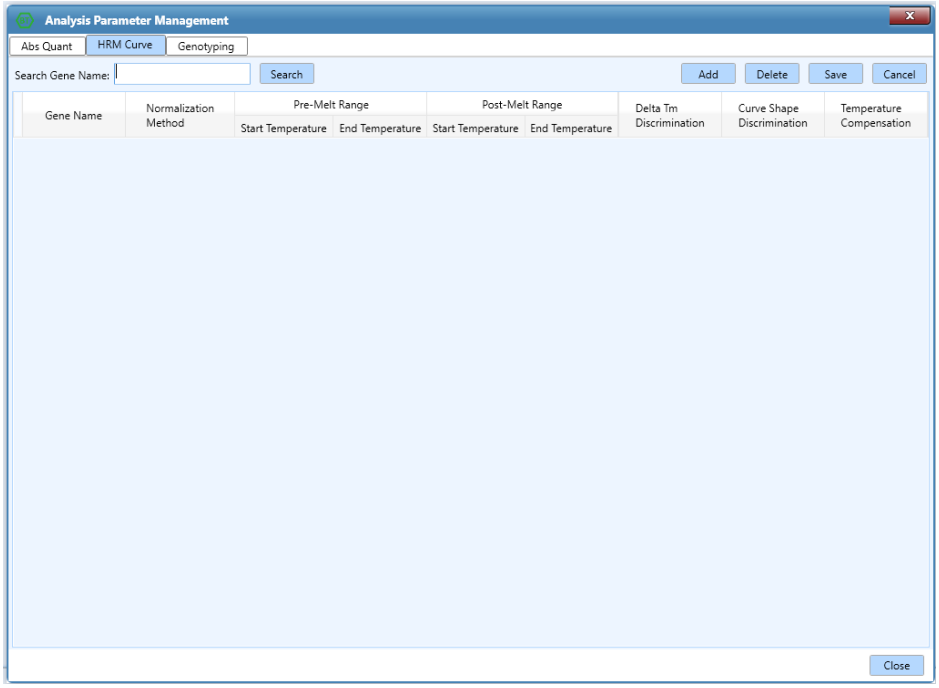


- **HRM Curve:** Add a Gene Name and its corresponding Normalisation method, Pre-Melt Range, Post-Melt Range, Delta Tm Discrimination, Curve Shape Discrimination, and Temperature Compensation. These settings can be saved and used for HRM curve analyses of future experiments with the same gene.



- **Genotyping:** Management of the analysis parameters of specific or multiple genes for genotyping experimental results analysis. Add an analysis parameter setting for a specific gene, and fill in the Gene Name and manually input the Allelic Gene X, Allelic Gene Y and Heterozygote. Any gene in the list can be deleted and the settings will be removed from the analysis parameter setting.

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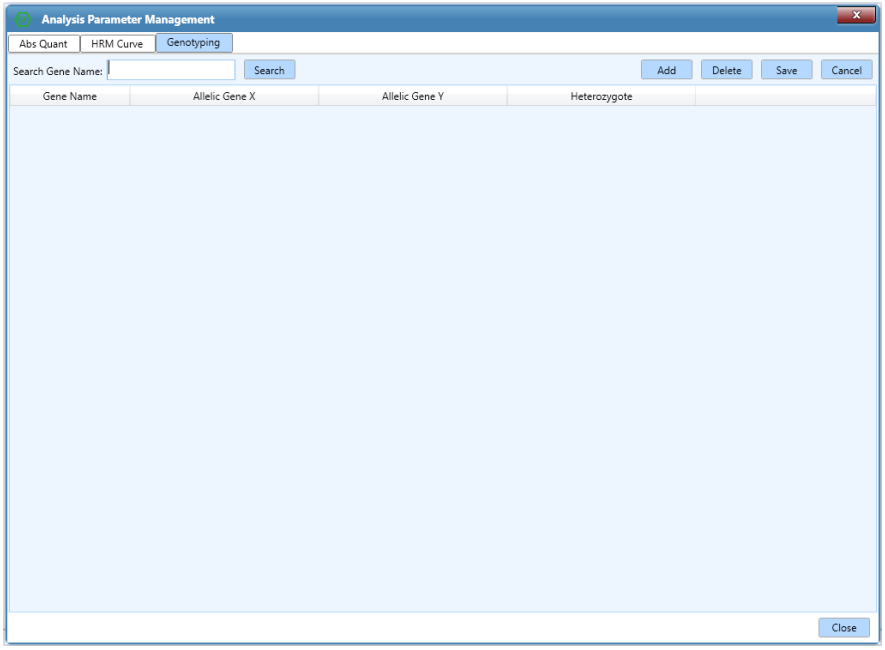
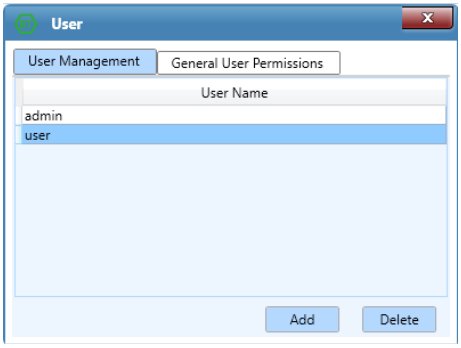
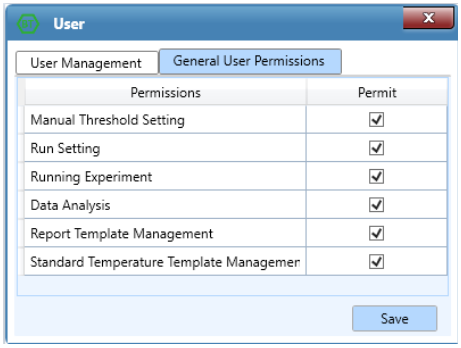


- **User:** When logged in as Admin (password is “admin”), the general user accounts can be managed.
  - **User Management:** Add or Delete user accounts.



  - **General User Permissions:** Set the relevant permissions for the general user account.



Help (H) tab functional descriptions

- **About:** Displays the software version and copyright information.

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8.4 Toolbar

The toolbar consists of eight commonly used function icons.
Toolbar functional descriptions

 **New Experiment:** Create a new empty experiment.

 **New Experiment from Existing Experiment:** Select an existing experiment and use the settings from this experiment to create a new experiment.

 **Open Data File:** Open a data file to view or analyse the data.

 **Save Experiment:** Click to save the experiment file in the default file path set in configuration management.

 **Close Experiment:** Close an opened experiment file.

 **Export:** Export raw experiment data as an Excel file.

 **Instrument Management:** Click to enter the instrument management interface.

 **Quick Start:** Click to open the quick start bar.

8.5 Operation area

After creating a new experiment file or opening an existing experiment file, the operation area on the main interface of the software will be activated. The operation area is divided into four tabs: **Run Setting, Sample Setting, Run Monitoring,** and **Analysis**.

Run Setting tab: When this tab is selected, two additional function icons will appear in the toolbar:

-  **Choose Run Parameter Template:** Choose a saved run parameter template to run an experiment from.
-  **Save Run Parameter Template:** Save the run settings of the experiment as a template.

Sample Setting tab**:** When this tab is selected, two additional function icons will appear in the toolbar:

-  **Choose Sample Parameter Template:** Choose a saved sample parameter template to run an experiment from.

   **Save Sample parameter Template:** Save the sample settings of the experiment as a template.

Analysis tab: When this tab is selected, four additional function icons will appear in the toolbar:

-  **New Analysis:** After running an experiment, click this icon in the toolbar to create a new analysis. The software provides six analysis methods: **Abs Quant, Rel Quant, Melting Curve, High-Resolution Melting, Genotyping,** and **End Point Fluorescence.**
-  **Analysis Setting:** Click this to set the analysis parameters for the current experiment data analysis method.
-  **"Delete Analysis":** Click to delete the chosen analysis.
-  **"Export List":** Click to export current experiment data in .csv, .txt or .xlsx file format.

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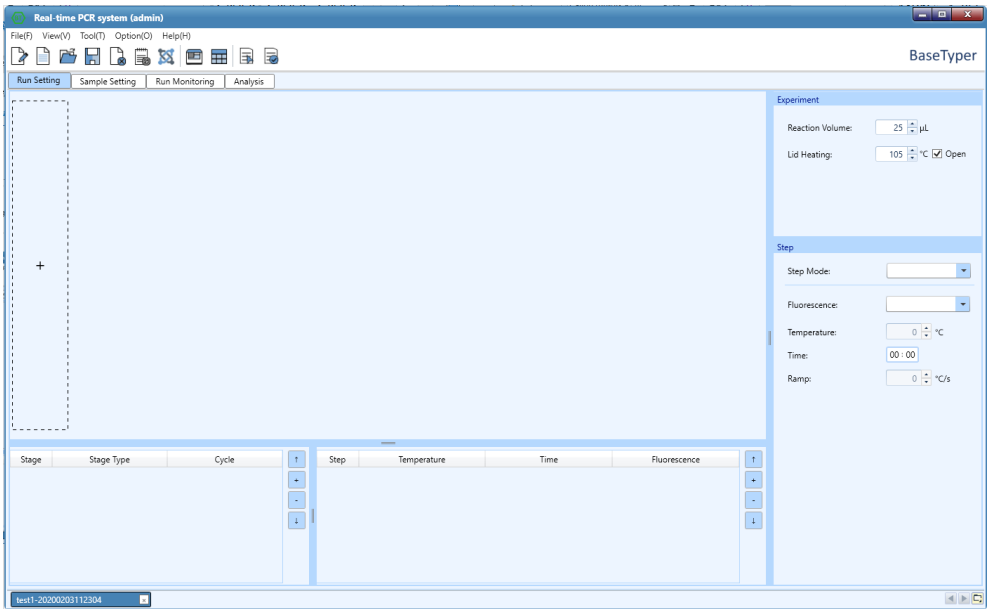
8.6 Run setting

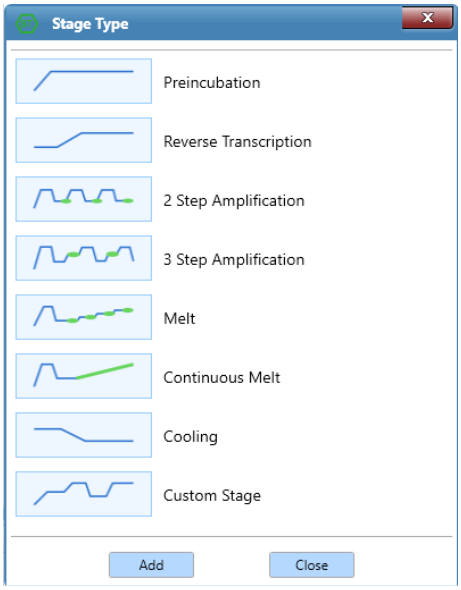
The operation area on the main interface displays **Run Setting** tab by default as shown below. The Run Setting Interface consists of five parts: **Temperature Program Area, Stage and Step lists, Experiment** and **Step editing** area.



8.6.1 Run setting functional descriptions

Temperature Program Area

Press the + icon in the temperature program area in the stage list to create a new temperature program. A list of stage types will pop up as shown below. Select a stage and click Add or double click on the stage to add it to the program.



The selected stages are displayed in the Temperature Program Area.

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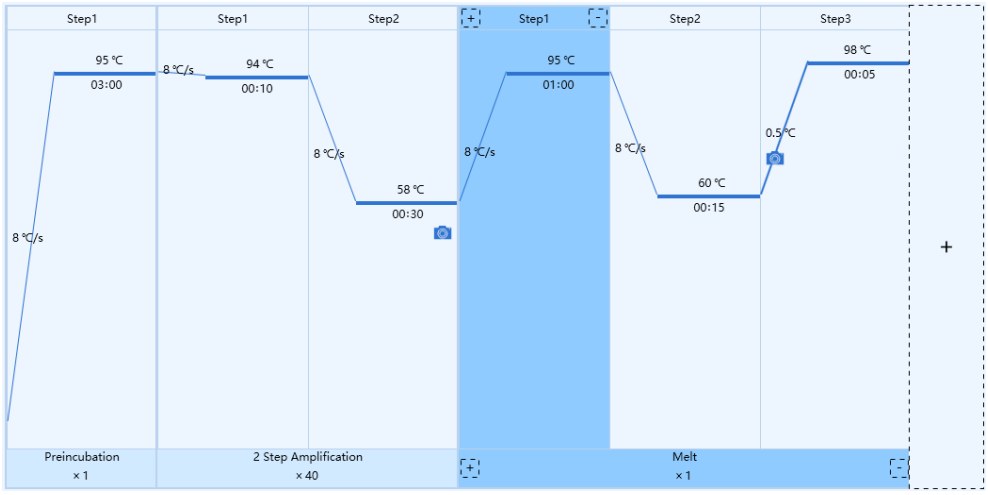
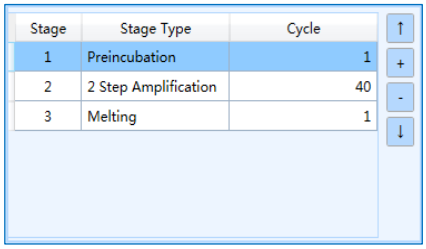


- Stages are longitudinally separated. If a stage contains several steps, these steps are separated by a blue line.
- The corresponding stage type and cycle number are displayed below each stage box and the corresponding step number is displayed above the step box.
- Inside each step box, the corresponding temperature of the current step is shown above the solid blue line and the corresponding time of the current step is shown below the solid blue line.
- The solid blue line between two stages/steps represents the temperature ramp or increment or readings of a melting step.
- The  icon is displayed at the steps where fluorescence is being measured.
- Hold the mouse on a temperature or time to see the setting details.

Stage List
The stage list consists of Stage, Stage Type, and Cycle, as shown below:



Stage	Stage Type	Cycle
1	Preincubation	1
2	2 Step Amplification	40
3	Melting	1

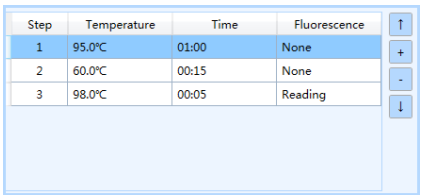
Stages can be added, deleted, or moved by using the control buttons on the right side.

Attention: The user can also double click on the cycle number below the stage box in the temperature program area to set the cycle number which can range between 1 and 99.

Attention: Click the + icon in the dotted box in the temperature program area or click the + icon below any stage box to add a new stage. Click the - icon below any stage box in the temperature program area to delete the stage.

Attention: At least one stage should be included in the temperature program.

Step List
The step list shows the temperature, time, and fluorescence settings for each step as shown below:



Step	Temperature	Time	Fluorescence
1	95.0℃	01:00	None
2	60.0℃	00:15	None
3	98.0℃	00:05	Reading

Steps can be added, deleted, or moved by using the control buttons on the right side.

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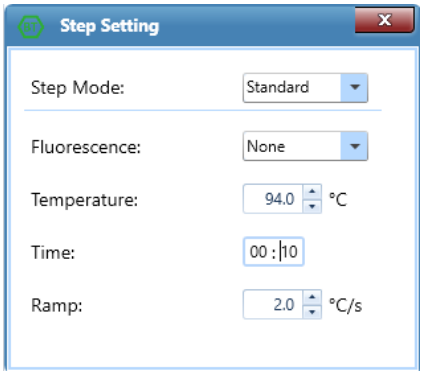
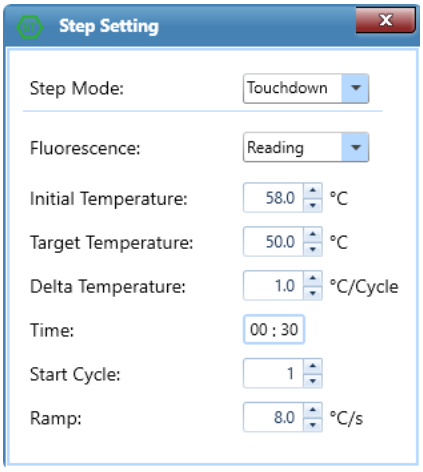
- Click the [+] or [-] icon above any step box in the temperature program area to add or delete a temperature step.

Step Setting
Double click on any step in the step list or the temperature program area to edit the step in the Step Setting box. The Step Setting box for preincubation, reverse transcription, amplification, and cooling steps is shown below:



- **Step mode:** The step mode can be set to Standard, Touchdown, Long, and Gradient.
  - **Standard Step Mode:** Set the Temperature, Time, and Ramp for the standard step and decide whether to read fluorescence.
    - **Fluorescence:** Choose whether to read fluorescence at the current step.
    - **Temperature:** Enter the temperature at ˚C.
    - **Time:** The time for which the temperature should be held. The time can be set from 1 s to 60 min.

      Attention: If the current step is the last step of the temperature program, check the ∞ check box to set the current step time to infinite.
    - **Ramp:** The rate of temperature change(°C) per second. The ramp can range from 0.1 °C/s to 8 °C/s.
  - **Touchdown Step Mode:** This mode allows the temperature program to change the annealing step temperature from the initial temperature to the target temperature as the cycling proceeds.



    - **Initial temperature:** The initial value of the annealing temperature change range. The initial temperature can range from 0.0 °C to 100.0 °C.
    - **Target Temperature:** The target value of the annealing temperature change range. The target temperature can range from 0.0 °C to 100.0 °C.
    - **Delta Temperature:** The temperature change (°C) per cycle. The Delta Temperature can range from 0.1 °C to 5.0 °C.
    - **Start Cycle:** The cycle number after which the temperature change is started. The start cycle range is 1 to the maximum cycle number of the current stage.

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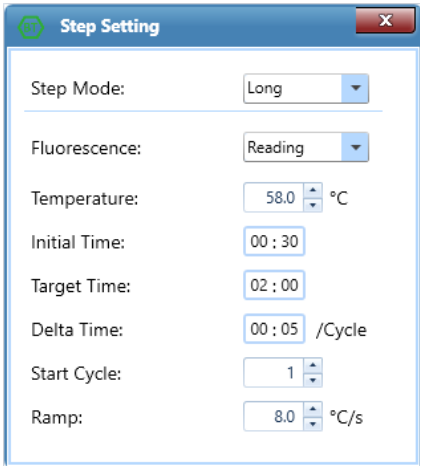
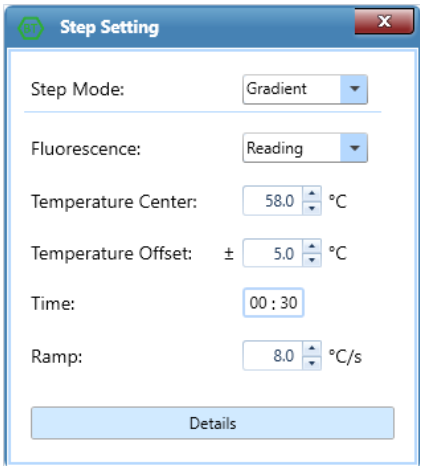
- **Long Mode:** This mode allows the temperature program to change the elongation step temperature holding time from the initial time to the target time as the cycling proceeds.



  - **Initial Time:** The initial value of the elongation time change range. The Initial time can range from 1 s to 60 min.
  - **Target Time:** The target value of the elongation time change range. The target time can range from 1 s to 60 min.
  - **Delta Time:** The time change per cycle. The delta time can range from 1s to 10 min.
  - **Start Cycle:** The cycle number after which the time change is started. The start cycle range is 1 to the maximum cycle number of the current stage.
- **Gradient Step Mode:** This mode allows the sample block to adopt different temperatures and set the **Temperature Center** and **Temperature Offset** values for the current gradient step, as shown below. The system will automatically calculate the corresponding gradient temperatures.



  - **Temperature Center:** This can range from 35.5 °C to 99.5 °C.
  - **Temperature Offset:** Can range from 0.5 °C to 20.0 °C.
  - **Details:** Click the detail button to see the specific gradient temperatures.

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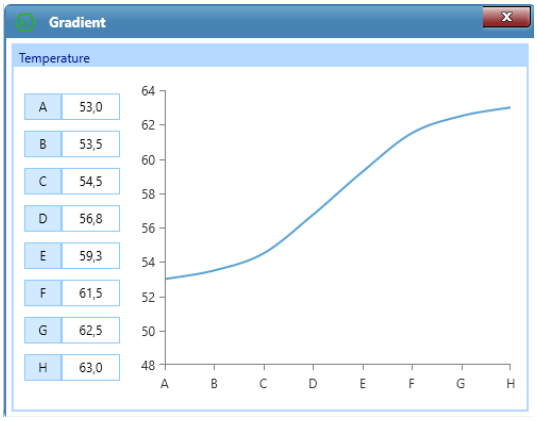
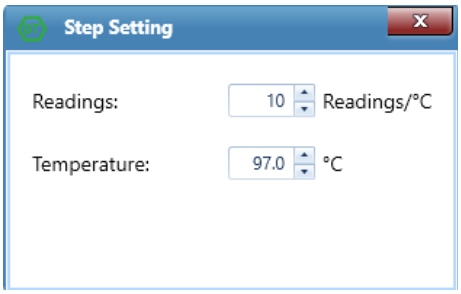


Attention: The gradient step mode is ideal for the optimisation of primer performance.

- **Melt Step:** The Step Setting box for Melt steps is shown below:

- **Continuous Melt:** This step is for adjusting the number of fluorescence readings. Set the fluorescence readings per ˚C in the Continuous Melt Step Setting box:



- **Attention:** The frequency of fluorescence readings can range from 2 to 15 readings/˚C.

Experiment Editing Area
Edit the parameters of the current experiment.

Tube Type: Select the type of consumable used in the current experiment. Select between **Clear, White,** and **Frosted.**

Reaction Volume: Enter the total reaction volume in the tubes of the current experiment.

Attention: The reaction volume setting can range from 0µL to 100µL.

- **Hot Lid:** Enter the Hot Lid temperature for the current experiment. Tick the **Open** check box to use the hot lid heating function.

Attention: The hot lid temperature setting range is 40.0 ˚C to 110 ˚C.

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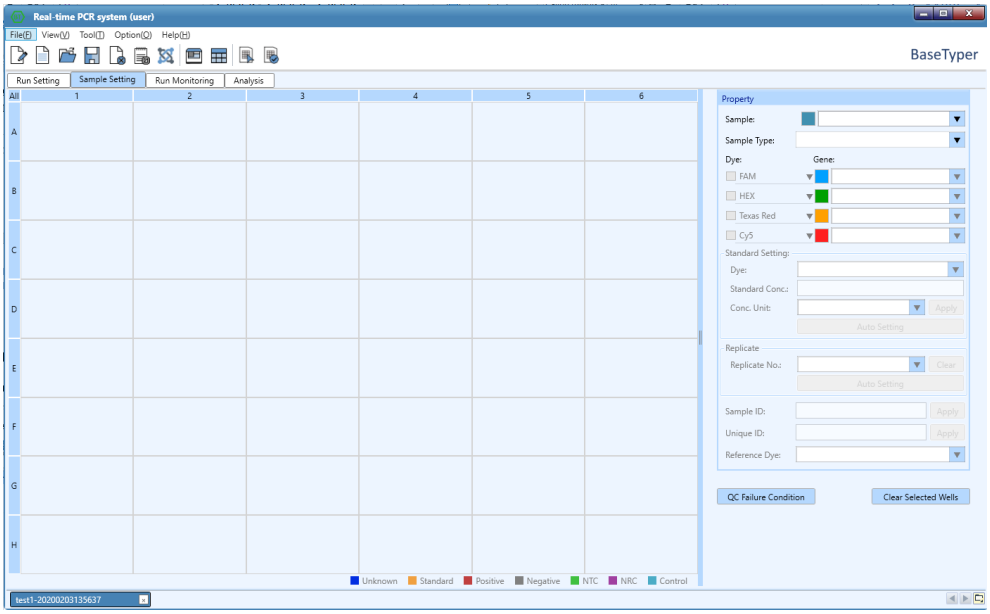
8.7 Sample setting

After completing the experiment run settings, proceed to the Sample Setting tab shown below:



This interface consists of a **Sample Settings** Area and a **Sample Property Setting** Area.

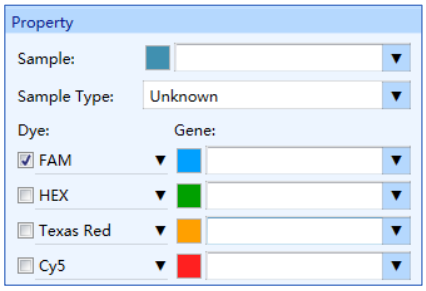
Sample Setting Area

- Click on wells containing samples.
  - To select more sample wells, press **[Crtl]** on the computer keyboard and click on the wells of interest.
  - To select a row or column of wells, click on the row or column number.
  - Hold down the left mouse button and drag the mouse over wells to select them.
  - Click **All** in the top left corner to select all sample wells.

Sample Property Setting Area

Select one or more sample wells from the Sample Setting area and set the properties for the wells.

- **Property Box:** Edit the **Sample**, **Sample Type, Dye,** and **Gene** for the selected wells, as shown below:



- **Sample:** Enter a new relevant sample name for the selected well or select a sample name from the drop-down list.
  - **Sample Type:** Set the relevant sample type for the selected wells from the drop-down list. Choose between Unknown, Standard, Positive, Negative, NTC, NRC, and Control.
- **Dye:** Check the relevant dye check box to determine the detection dyes for the selected wells.
- **Gene:** Enter a new relevant gene name for the selected wells or select a gene name from the drop-down list.

  Attention: Click the colour block to select a data analysis colour for all samples with the current gene name.
- **Standard Setting:** When the Sample Type is set to Standard, the Standard Setting box is activated as shown below:

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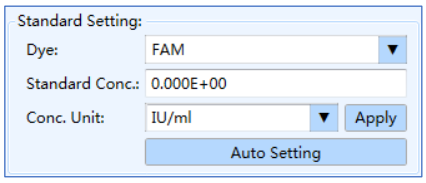
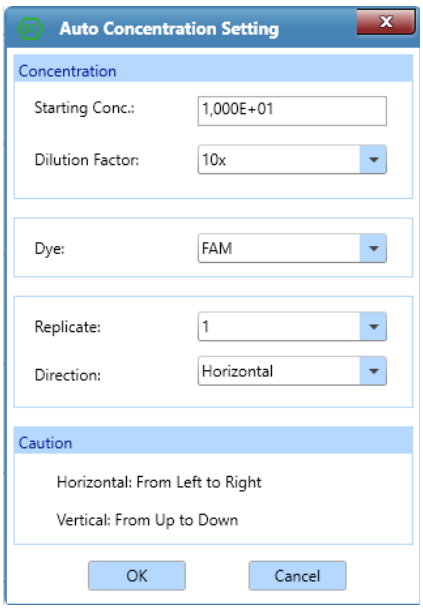
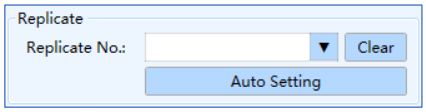


- **Dye:** Set the dye for the corresponding standard curve from the drop-down list.
- **Standard Conc:** Select a single standard sample and enter its standard concentration.
- **Conc. Unit:** Select a single standard sample and set its concentration unit from the drop-down list. The software has two default concentration units: IU/ml and Copies/ml.
- After entering the settings, click **Apply** or press **[Enter]** to confirm and register the settings.

For a series of standard samples diluted according to a certain dilution factor, select the corresponding standard sample wells in the sample setting area and let the application software automatically calculate and set the relevant standard concentration for this series of standard samples. Click **Auto Setting** and the auto concentration setting window will pop up automatically, as shown below:



- **Starting Conc.:** Set the start concentration for the series of diluted standard samples.
- **Dilution Factor:** Select the dilution factor for the series of diluted standard samples.
- **Dye:** Select the dye for the corresponding standard curve of a series of diluted standard samples.
- **Replicate:** Select the number of standard samples from the drop-down list.
- **Direction:** Set the auto concentration setting direction from the drop-down list to Horizontal (from left to right) or Vertical (from up to down).
    - **Replicate:** Classify similar samples into one replicate group.



    - **Replicate no.:** Select sample wells in the sample setting area and select the replicate number from the drop-down list to classify the selected samples into one replicate group.
    - **Auto Settings:** The software can automatically divide multiple replicate groups according to user requirements. Select all wells of the same samples in the sample setting area and click **Auto Settings:** The auto setting dialog box will pop up automatically, as shown below:

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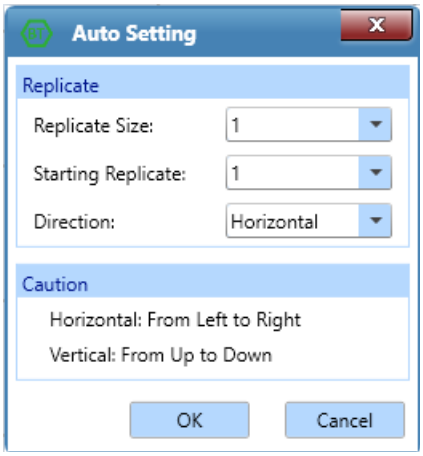
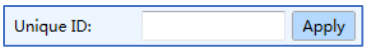
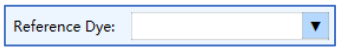
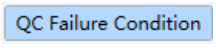


- **Replicate size:** Select the number of samples in each replicate group.
- **Starting Replicate:** Select a start value for the replicate numbers.
- **Direction:** Set the auto concentration setting direction from the drop-down list to Horizontal (from left to right) or Vertical (from up to down).

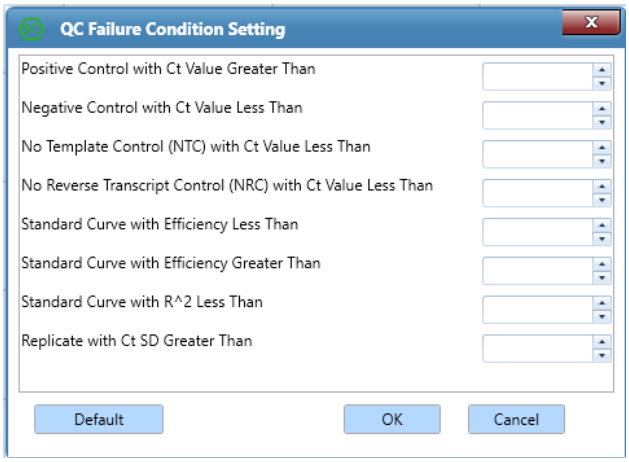
- **Sample ID:** 

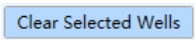
Select one or more wells in the sample setting area and enter a Sample ID. Press **[Enter]** or click **Apply** to confirm and save the sample ID.
- **Unique ID:** 

Select one or more wells in the sample setting area and enter a Unique ID. Press **[Enter]** or click **Apply** to confirm and save the Unique ID.
- **Reference Dye:** 

Select a reference dye from the drop-down list.
- **QC Failure Condition Setting:** After completing the sample well settings, click on the 

button, which opens the QC failure condition setting dialog box as shown below. Enter the QC failure condition settings. Click **Default** to clear all QC failure condition settings.



- **Clear Selected Wells:** Click on the 

button to clear the settings for the selected wells.

8.8 Run monitoring

After finishing the experiment run settings and sample settings, click on the **Run Monitoring** tab to enter the run monitoring interface where the experiment can be started and monitored. This interface is divided into four functional modules where **Real-time Fluorescence, Temperature Program, Sample Setting, and Run Info** are displayed by default, as shown below:

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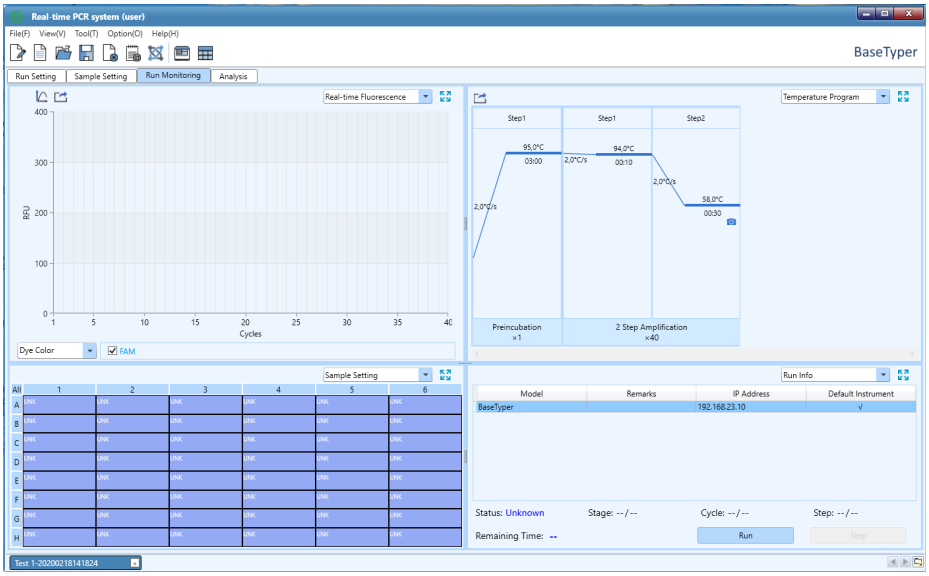
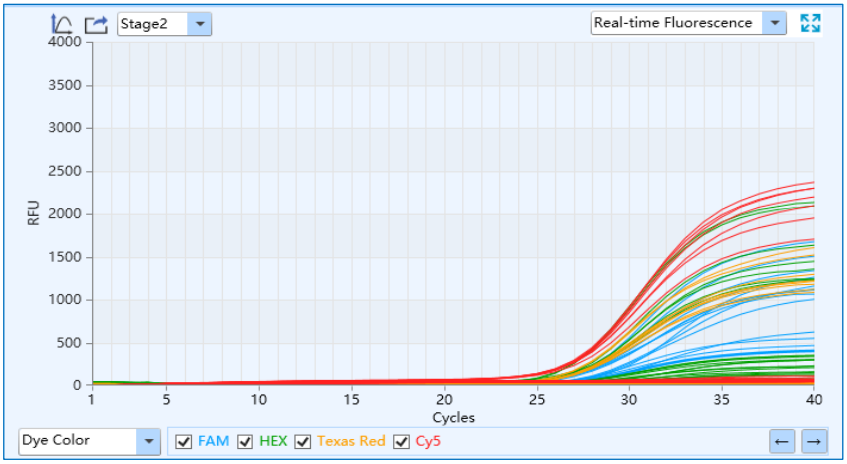


In each functional module, it is possible to select one of six functional modules: **Real-time Fluorescence, Temperature Program, Sample Setting, Run Info, Sample Info,** and **Heat Map** from the drop-down list.

Real-time fluorescence

The Real-time fluorescence module displays the real-time fluorescence intensity against the cycle number of the running experiment, as shown below:



The real-time fluorescence curve can be displayed according to **Well Colour, Dye Colour, Sample Colour,** or **Gene Colour.**

- **Y-axis Coordinate Adjustment:** Click on the icon to adjust the Y-axis coordinate in the coordinate range setting box. Select Automatic or Manual option.
  - **Automatic:** The software automatically adjusts the Y-axis coordinate.
  - **Manual:** Allows for entering a maximum and a minimum value for the Y-axis.
- **Export:** Click to export the current real-time fluorescence monitoring diagram. The diagram is exported as a picture (PNG).

Temperature Program

The Temperature Program module displays the temperature program of the running experiment.

- **Export:** Click to export the current temperature program monitoring diagram.

Sample Setting

The Sample Setting module displays the sample well settings of the currently running experiment.

- Select one or more sample wells in the Sample Setting function module to display the corresponding fluorescence curve and relevant data in the **Real-time Fluorescence** and **Sample Info** function modules.

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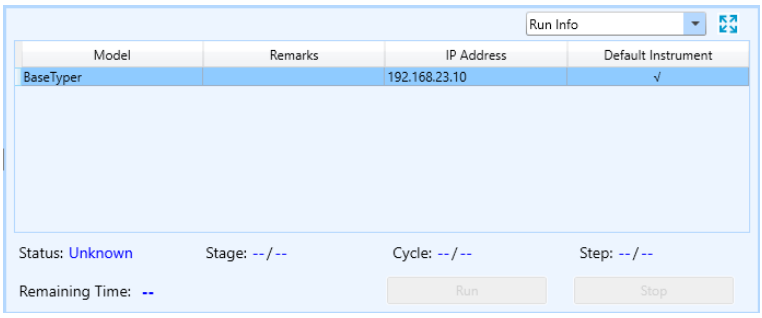
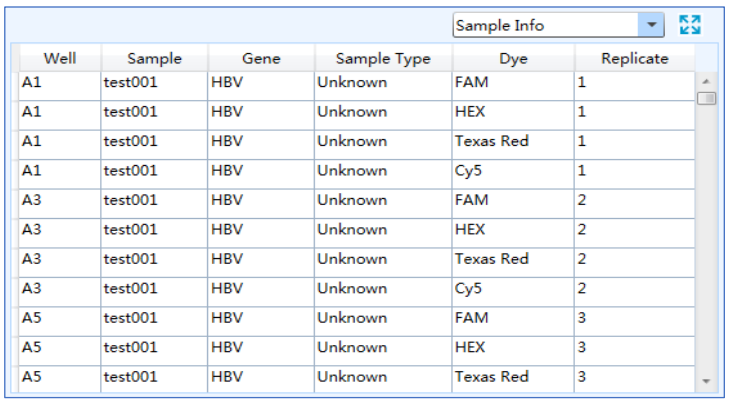
Run Info
The Run Info module displays the current instrument information and the real-time running status, such as the **Stage, Step, Cycle,** and **Remaining Time** of the current experiment, as shown below:



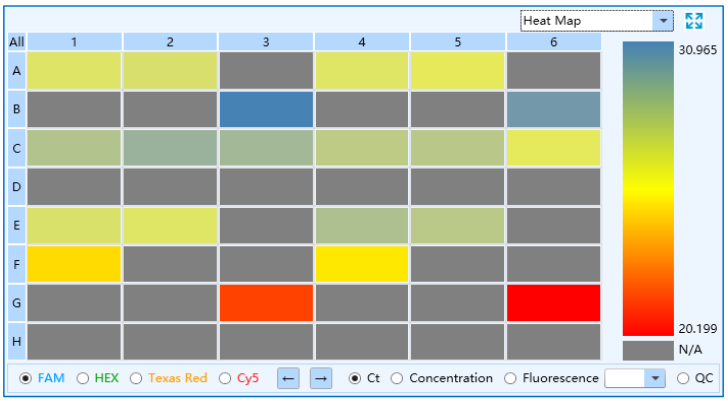
- Click on the instrument and click **Run** to start the experiment. Click **Stop** to stop the experiment.

Sample Info
The Sample Info module displays detailed sample information of the currently running experiment.



Well	Sample	Gene	Sample Type	Dye	Replicate
A1	test001	HBV	Unknown	FAM	1
A1	test001	HBV	Unknown	HEX	1
A1	test001	HBV	Unknown	Texas Red	1
A1	test001	HBV	Unknown	Cy5	1
A3	test001	HBV	Unknown	FAM	2
A3	test001	HBV	Unknown	HEX	2
A3	test001	HBV	Unknown	Texas Red	2
A3	test001	HBV	Unknown	Cy5	2
A5	test001	HBV	Unknown	FAM	3
A5	test001	HBV	Unknown	HEX	3
A5	test001	HBV	Unknown	Texas Red	3

Heat Map
The Heat Map displays the real-time fluorescence heat map of the currently running experiment.



8.9 Analysis

When the experiment run has finished, click on the **Analysis** tab to enter the analysis interface.

- **New Analysis:** Click on this icon to select a suitable data analysis method from the list shown below. Choose from six analysis methods depending on the temperature program: **Abs Quant, Rel Quant, Melting Curve, High Resolution Melting, Genotyping,** and **End Point Fluorescence.**

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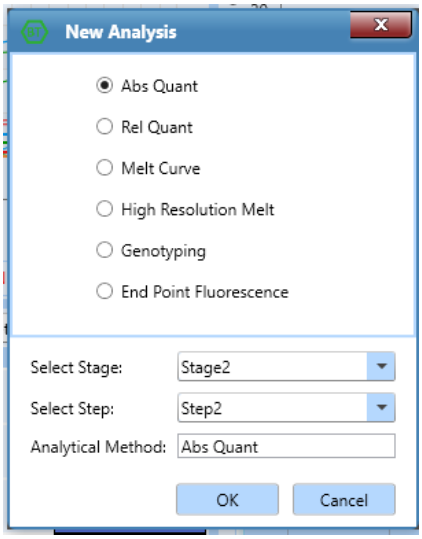

- **Select Stage:** Select the stages that need to be analysed by the selected analysis method.
- **Select Step:** Choose the steps that need to be analysed.
- **Analytical Method:** Shows the selected analysis method.

8.9.1 Absolute quantification

The absolute quantification analysis is intended to quantify the number of nucleic acids of interest. Samples with unknown initial nucleic acid quantities are amplified as is dilution series of gene-specific standard samples with known concentrations. The measured Ct values of the standard samples are plotted against their known concentrations to obtain a regression line named standard curve. The initial nucleic acid quantities of the samples can be obtained by plotting their Ct values on the standard curve.

The absolute quantification analysis interface consists of seven functional modules: **Amplification Curve, Standard Curve, Sample Setting, Result Table, Raw Curve, Raw Fluorescence,** and **Heat Map.** The relative quantification analysis interface is divided into four areas. In the analysis interface, the application software displays four functional modules **Amplification Curve, Standard Curve, Sample Setting, and Result Table** by default.

Attention: Select the functional module to be displayed on the analysis interface from the drop-down list on the top right corner of each area.

Attention: Click the icon on the top right corner of each area to display the current functional module on full screen.

Introduction of general function keys

Depending on the selected analysis module, the following icons can be displayed in the top right corner of the module:

- **Y-axis Coordinate Adjustment:** See Run monitoring section.
- **Export:** Export the amplification curve diagram.
- **Log View:** View the Log image of the amplification curve diagram.

Amplification Curve

The Amplification Curve displays the diagram of fluorescence intensity against cycle number, as shown below:

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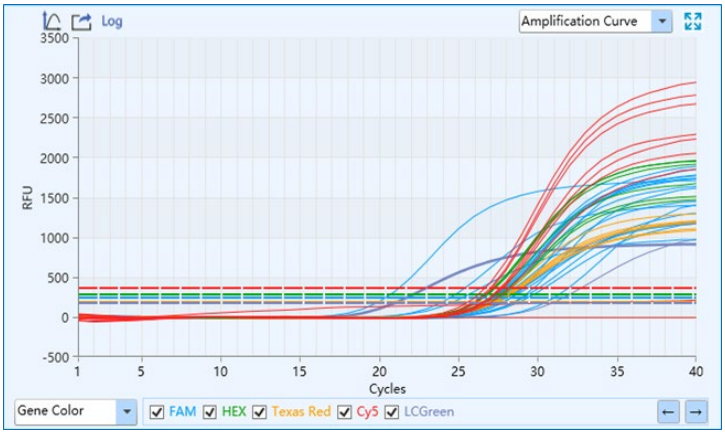
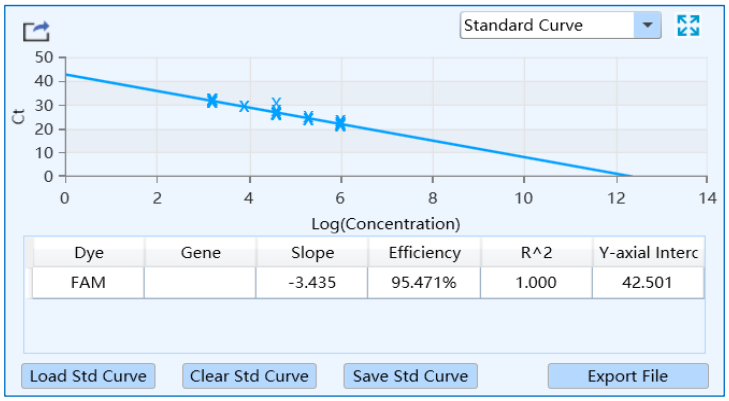


- **X-axis:** Represents the cycle number.
- **Y-axis:** Represents the Relative Fluorescence Unit (RFU)

Attention: When hovering the mouse over an amplification curve, the curve will be highlighted and its corresponding channel and well coordinates will be displayed.

8.9.2 Standard curve

The Standard Curve displays the threshold Ct value against the initial nucleic acid quantity of each standard sample, as shown below. For absolute quantification, the standard curve is used to assign initial nucleic acid quantities to unknown samples.



- **X-axis:** Represents the log concentration of samples.
- **Y-axis:** Represents the threshold Ct value.
- **Dye:** Displays the relevant dye of the standard curve.
- **Target Gene:** Displays the target gene of the standard curve.
- **Slope:** Displays the slope of the standard curve.
- **Efficiency:** Displays the amplification efficiency of the standard curve.
- **R^2:** Displays the linear regression coefficient square value of the standard curve.
- **Y-axial Intercept:** Display the maximum Y-value.
- **Load Std Curve:** Click to open the standard curve window which contains three areas:
  - **Import Standard Curve:** Click **Import** to select and import a saved standard curve file to the standard curve list. The standard curve diagram will be displayed in the **Standard Curve Preview** area below.
  - **Select Standard Curve:** Select a standard curve, which corresponds to the current experiment setting from the Standard Curve drop-down list. The selected standard curve will be loaded to the standard curve functional module.
  - **Standard Curve Preview:** Displays the preview of selected standard curves.

8.9.3 Sample setting

Display the well settings of the current experiment. Click on a well to see the corresponding amplification curve and data in the Amplification Curve and Sample Info functional modules.

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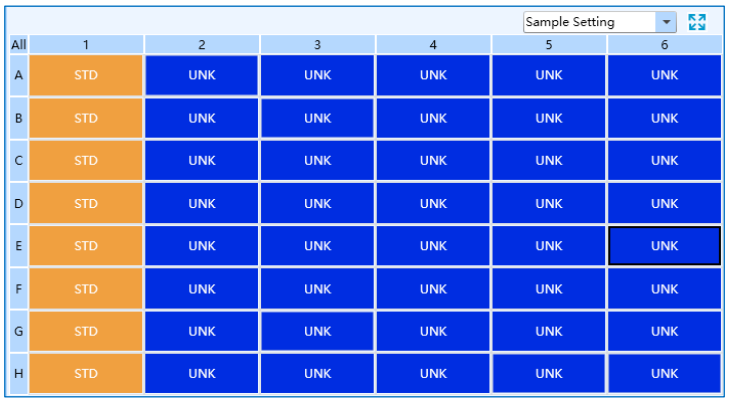


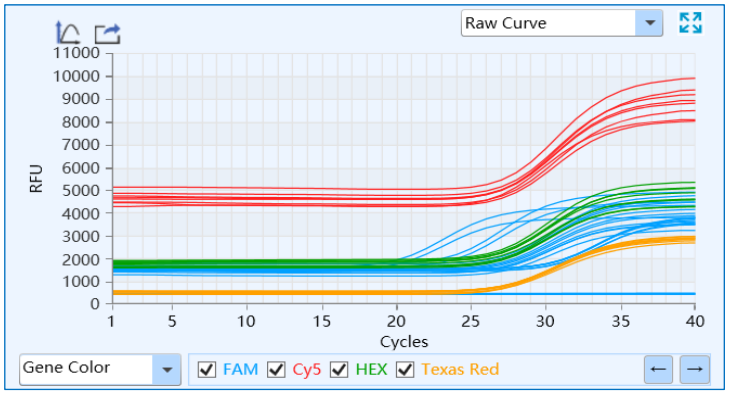
8.9.4 Result table

The Result Table displays the sample details and experiment data of the current experiment. The result table module consists of **Result** and **Statistics** sub-tabs.

- **Result** tab: This tab is selected by default on the result table module, which displays the sample details and results of the experiment. Double click on the title of a column to sort the samples after the content in the column. Click and drag on the column name to move columns in the table.
- **Statistics** tab: Click on this tab to view the statistical data of the experiment.

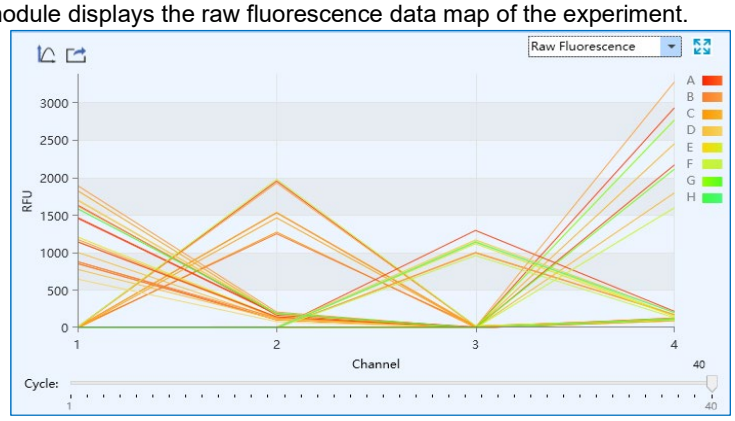
8.9.5 Raw curve

The Raw Curve displays the raw amplification curves without a subtracted baseline, as shown below:



8.9.6 Raw fluorescence

The Raw Fluorescence module displays the raw fluorescence data map of the experiment.



X-axis: Represents the channels of the BaseTyper™.
Y-axis: Represents the relative fluorescence unit (RFU)
The colours of the curves represent the A-H rows of samples.
Slide the **Cycle** slider at the bottom of the raw fluorescence module to view the raw fluorescence value of different wells at different cycles.

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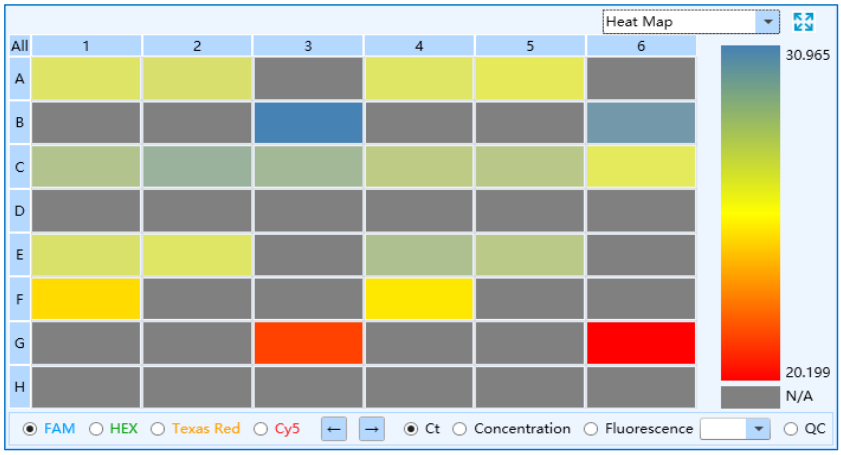
8.9.7 Heat map

The Heat Map module displays the heat map and the QC diagram of the experiment.



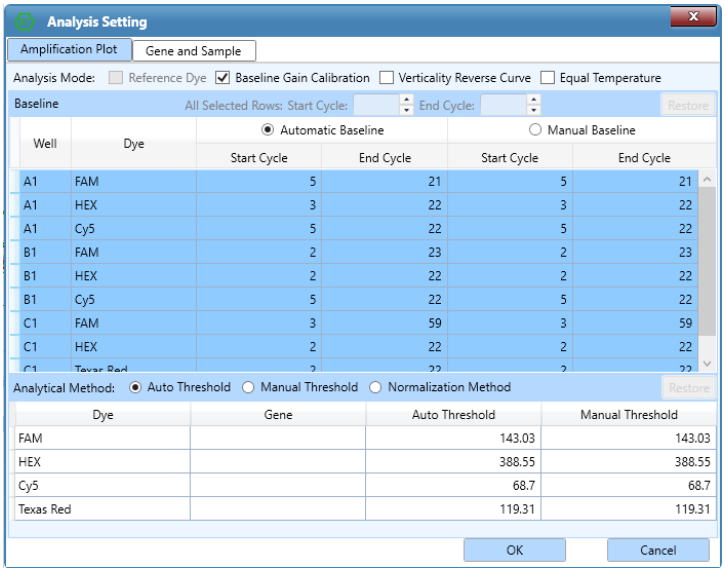
- **Ct:** Check the Ct box and click on any dye to view the corresponding heat map.
- **Concentration:** Check the Concentration box and click on any dye to view the corresponding heat map.
- **Fluorescence:** Check the fluorescence box and select a cycle number from the drop-down list, then select any dye to view the corresponding heat map.
- **QC:** Check the QC box to see samples that conform to the QC failure criteria set in the Sample Setting interface. These samples will be displayed with a **N/A** on the QC diagram.

8.10 Absolute quantification analysis settings

Click on the **Analysis Setting** icon in the Tool Bar to open the Analysis Setting window which has two tabs: **Amplification Plot** and **Gene and Sample**. Different parameters for the experiment data analysis can be set.

8.10.1 Amplification Plot

Set the Analysis mode and Analytical method of the experiment.



- **Analysis mode:** Check the analysis mode checkbox of **Reference Dye**, **Baseline Gain Calibration, Vertically Reverse Curve,** or **Equal Temperature** according to the experiment requirements and set the corresponding parameters in the **Baseline** list. The **Baseline Gain Calibration** analysis mode is selected by default and gives the user two baseline setting methods: **Automatic Baseline** and **Manual Baseline.**
    - **Automatic Baseline:** The software will automatically set the baseline for the amplification curve. The **Start Cycle** and **End Cycle** of the automatic baseline are shown in the **Baseline** list.

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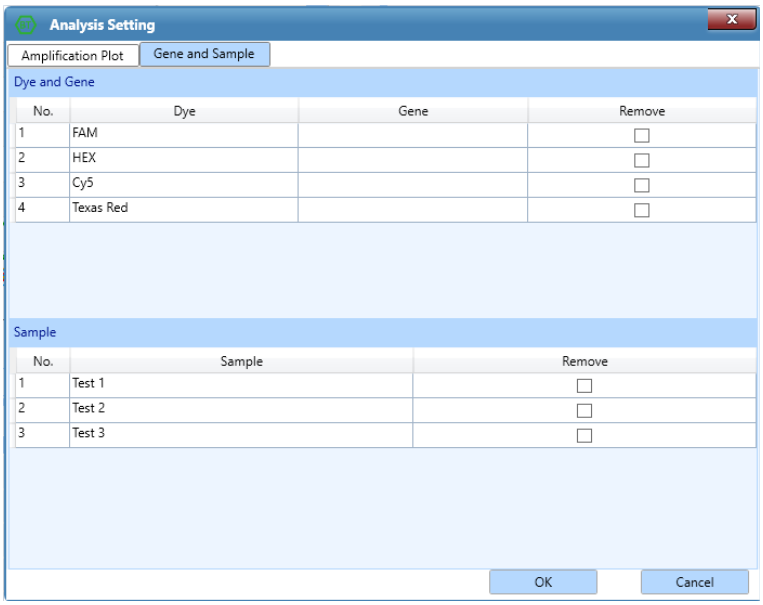
- **Manual Baseline:** Select one or more wells in the **Baseline** list and use and keys to manually set the **Start Cycle** and **End Cycle** of the baseline setting for the samples. Click **Restore** in the **Baseline** list to reset the **Start Cycle** and **End Cycle** settings to the automatic baseline setting.
- **Reference Dye:** If a reference dye was set in the Sample Setting interface, the user can check the **Reference Dye** checkbox.

- **Analytical Method:** The software has three Ct values analytical methods:
  - **Auto Threshold:** The software automatically set and show the threshold value for all dyes.
  - **Manual Threshold:** Click on the value in the Manual Threshold column and use the and keys to set the manual threshold value. Click **Restore** on the top right corner of the threshold list to restore the **Auto Threshold** settings.
  - **Normalization Threshold:** The software will automatically calculate the Ct value according to the normalisation value of the amplification curve.

8.10.2 Gene and sample

Select the dye, gene, and sample required for the data analysis of the experiment.



- **Dye and Gene:** Display the name of the target gene and its dye. Check a box in the Remove column to remove the data of this gene and target.
- **Sample:** Display all sample names. Check a box in the Remove column to remove the data of these samples.

8.11 Relative quantification

The relative quantification experiment compares the expression level of two or more genes within the same individual. Usually, an endogenous housekeeping gene with a constant expression level is used as the internal reference gene. The relative quantification is calculated by measuring the changes of the target genes expression level relative to the internal reference genes.

The relative quantification analysis interface consists of eight operating modules: **Amplification Curve, Bar Chart, Sample Setting, Result Table, Standard Curve, Raw Curve, Raw Fluorescence,** and **Heat Map**. This interface is divided into four areas and four functional modules **Amplification Curve, Bar Chart, Sample Setting, Result Table** are displayed by default, as shown below:

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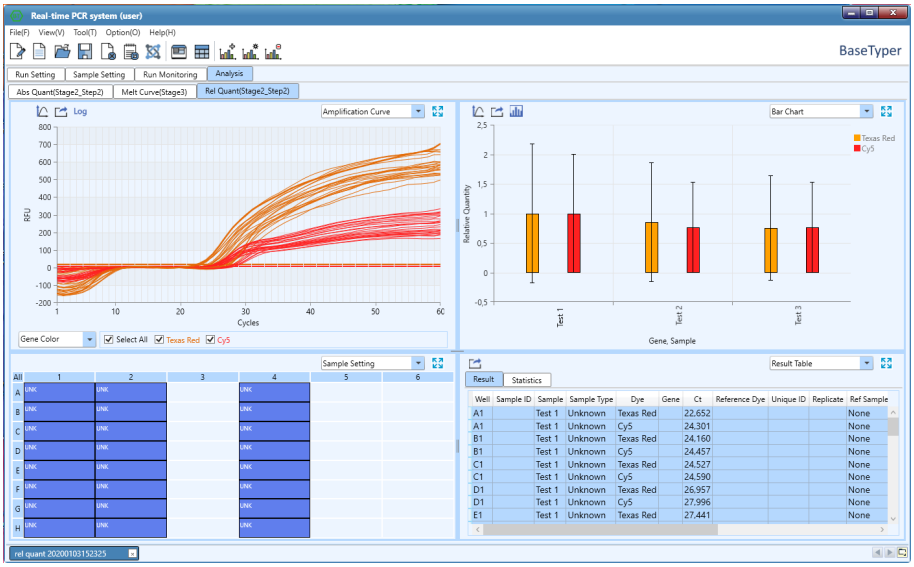
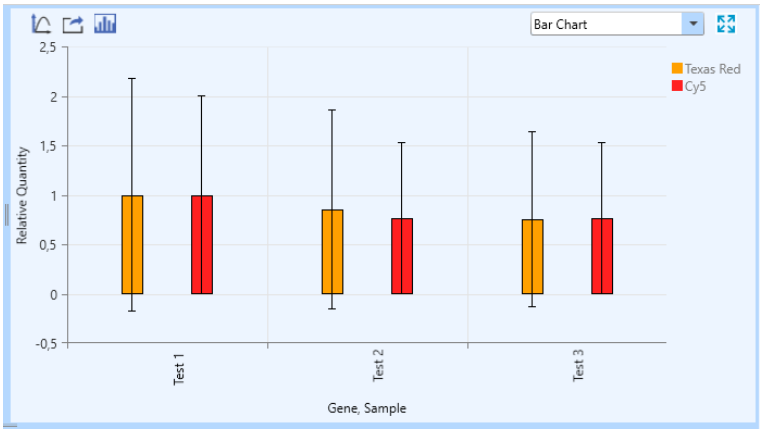


Attention: The parameter descriptions of Amplification Curve, Sample Setting, Result Table, Standard Curve, Raw Curve, Raw Fluorescence, and Heat Map functional modules are the same as those introduced in the previous section.

Bar Chart

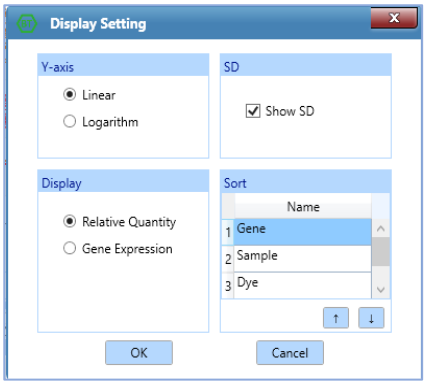
Displays the relative ratio between the target gene and reference gene in the sample, as shown below:



- **X-axis:** Shows the gene and sample to be compared
- **Y-axis:** Shows the **Relative Quantity** ratio of the compared gene and sample.

  Attention: The relative quantity ratio of the reference sample is set to 1 by default.

  Display Setting: Click on the icon to open the Display Setting window as shown below:



  - **Y-axis:** Select linear or logarithm scale for the y-axis.
  - **SD:** Select whether to show the standard deviation values on the bar chart.

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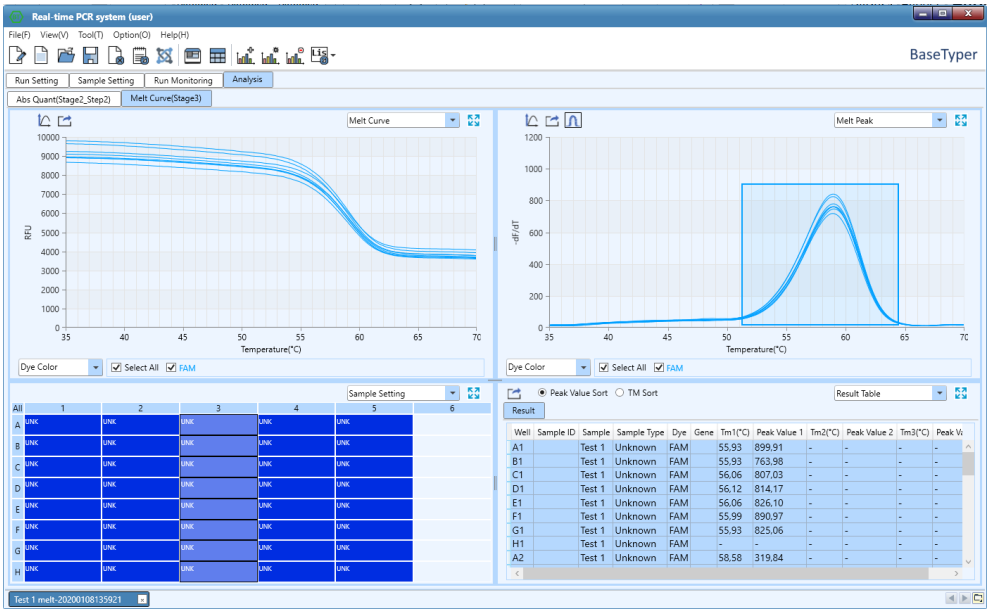
- **Display:** Select **Relative Quantity** to display the relative quantity ratio of the target gene and reference gene. Select **Gene Expression** to display the relative change in gene expression.
- **Sort:** Use the use ↑ and ↓ keys to sort the order of **Gene, Sample,** and **Dye.**

Relative Quantification Analysis Setting
When entering the relative quantification analysis interface, an Analysis Setting window will automatically pop up (Section 8.10)

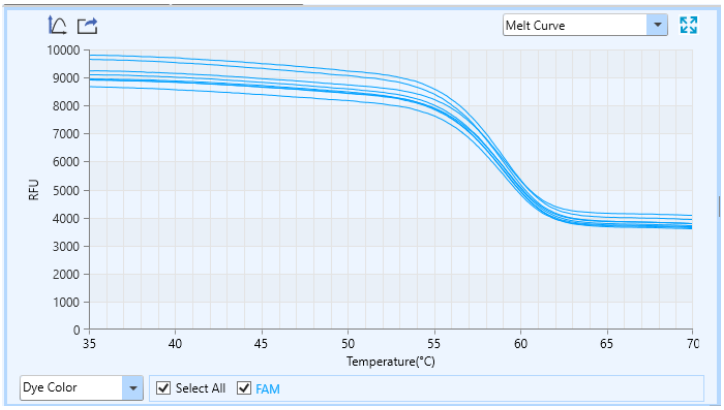
8.12 Melt curve

For intercalating dyes (such as SYBR Green) and non-cleavable hybridisation probes, the fluorescence intensity is proportional to the amount of double-stranded DNA (dsDNA). However, these dsDNA include specific and non-specific PCR products, which means that the presence of primer-dimers and other non-specific products can affect the quality of real-time PCR data.
The melting curve shows that the melting degree of dsDNA varies with the rising temperature. The melting temperature T_m (the temperature at which 50% of the dsDNA has dissociated to single-stranded DNA) of the dsDNA depends on the base sequence, fragment length, and GC content. Therefore, the melting curve can be used to check for non-specific PCR products.
The melting curve analysis interface consists of five operating modules: **Melting Curve, Melting Peak, Sample Setting, Result Table,** and **Heat Map**. The melting curve analysis interface is divided into four areas, where the **Melting Curve, Melting Peak, Sample Setting** and **Result Table** modules are displayed by default, as shown below:



Attention: For parameter descriptions of **Sample Setting, Result Table** and **Heat Map** operating modules, see section 8.9.1.

Melting curve
The melting curve displays the fluorescence intensity against the temperature as shown below:



The melting curve display mode can be selected from the drop-down list on the bottom left of **Melting Curve** and can be

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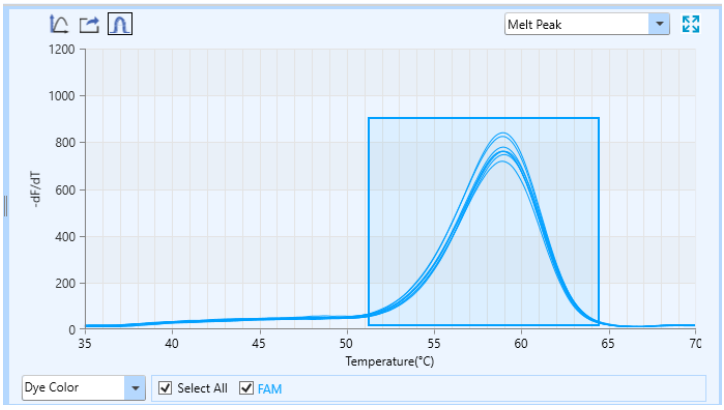
displayed according to **Well Colour**, **Dye Colour, Sample Colour,** and **Gene Colour**.

Attention: Hold the mouse over a certain melting curve to highlight the curve and see its corresponding channel and well coordinates.

Attention: Drag and drop the mouse button to zoom in on or click the right mouse button to zoom out.

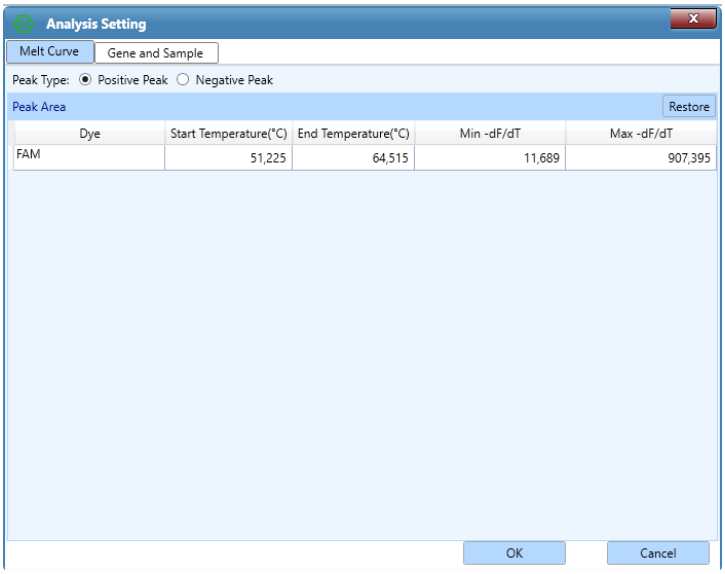
Melt peak

The melt peak displays the change of fluorescence intensity with respect to the per unit change in temperature (-dF/dT), as shown below. If a curve only has a single melting peak, it means that no non-specific PCR products are detected in the experiment.



Click the **Analysis Setting** icon in the Tool Bar to open the melting curve analysis setting window, where the relevant parameters can be set for the melt curve analysis. The melting curve analysis setting window consists of two tabs; **Melt Curve** and **Gene and Sample**.

- **Melt curve** tab:



  - Choose between negative peak and positive peak.
  - **Dye:** Displays the dye and corresponding gene name.
  - **Start Temperature** and **End Temperature:** The temperature range in which the melting peaks are found. Click on a temperature and use the and keys to change it.
  - **Min -df/dT** and **Max -df/dT:** The fluorescence range in which the melting peaks are found. Click on the values and use the and keys to change them.

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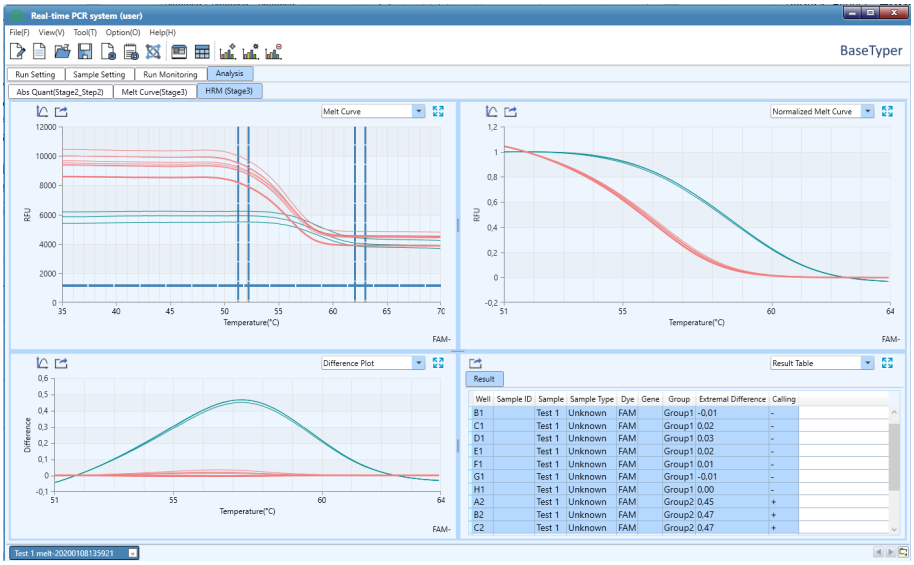
8.13 High-resolution melting

The melting temperature T_m of dsDNA depends on its base sequence, fragment length, and GC content. In theory, any base change can cause a difference in melting temperature. However, the difference caused by a single base change is tiny and usually less than 1 degree. It is therefore required to read the fluorescence signal several times within one degree of temperature change to accurately detect the difference in melting temperature. Therefore, high resolution melting (HRM) is often used for single nucleotide polymorphism (SNP) analysis, mutation scanning, methylation research and genotyping, etc.

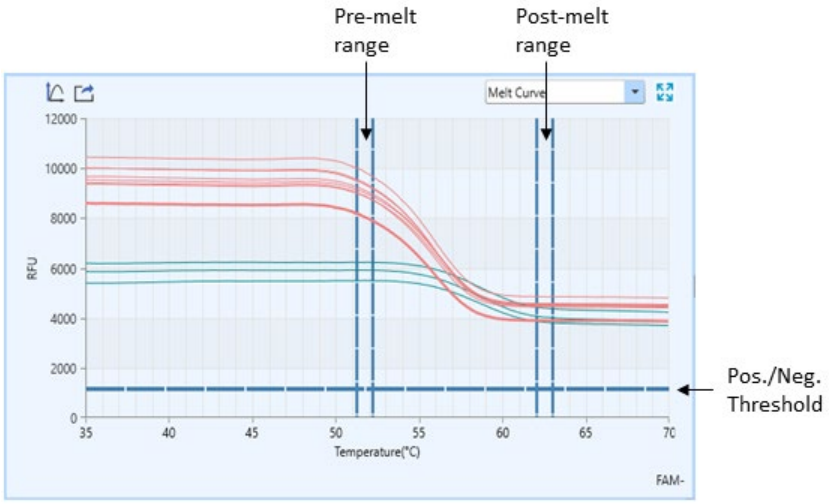
The high-resolution melting analysis interface consists of seven operating modules: **Melting Curve, Normalized Melting Curve, Difference Plot, Result Table and Sample Setting, Normalized Peak Melting,** and **Heat Map**. The HRM analysis interface is divided into four areas, and the four operating modules (**Melting Curve, Normalized Melting Curve, Difference Plot,** and **Result Table**) are displayed by default.



Attention: For parameter descriptions of **Sample Setting, Result Table,** and **Heat Map** operating modules, see section 8.9.1.

Melting Curve

The Melting curve displays the fluorescence intensity against the temperature as shown below:



The software will automatically determine the normalised area of the melting curve. The two vertical sliders on the left are the **Pre-melting Range** threshold lines, which are used to specify the pre-melting temperature range. The two vertical sliders on the right are the **Post-melting Range** threshold lines, which are used to specify the post-melting temperature range. The single line at the bottom is the positive/negative (**Pos/Neg**) **Threshold** line, which is used to determine the positivity or negativity of the samples.

The threshold lines can manually be dragged, or the values changed in the melting analysis setting window to specify the normalisation area.

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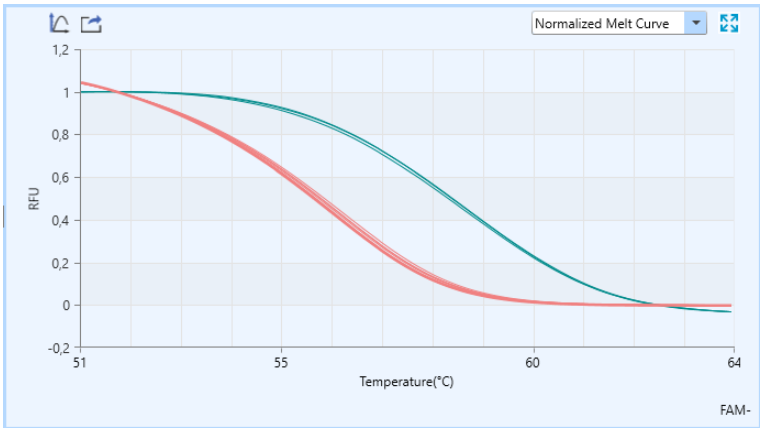
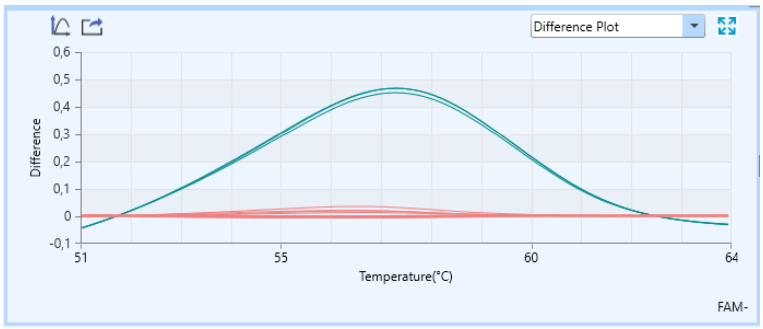
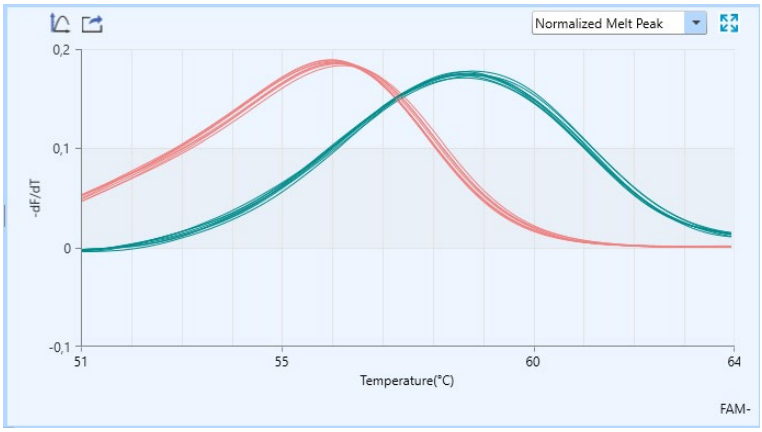
Normalised Melting Curve
Displays the normalised melting curve according to the normalisation method and fluorescence normalisation settings. For more details, see section 0.



Difference Plot
Displays the curve after subtracting the reference or baseline group curve. The appearance of the curve on the **Difference Plot** functional module depends on the selected reference or baseline group. The reference or baseline group can be either a sample set as standard, a specific well, or a baseline group. For more details, please see section 0.



Normalised Melting Peak
The Normalised melting peak displays the first negative derivative of the normalised melting curve, as shown below. The melting temperature range of each sample appears as a peak after normalisation, and optionally temperature shift.



High-Resolution Melt Analysis Setting

Click the  **Analysis Setting** icon in the Tool Bar to open the High-Resolution Melting analysis setting window.

The melting curve analysis setting window consists of two tabs; **High-Resolution Melt** and **Gene and Sample**.

High-Resolution Melt

- **Normalisation:** Choose between two normalisation methods: **Ratio Method** or **Exponential Method**. The ratio method will be selected by default.

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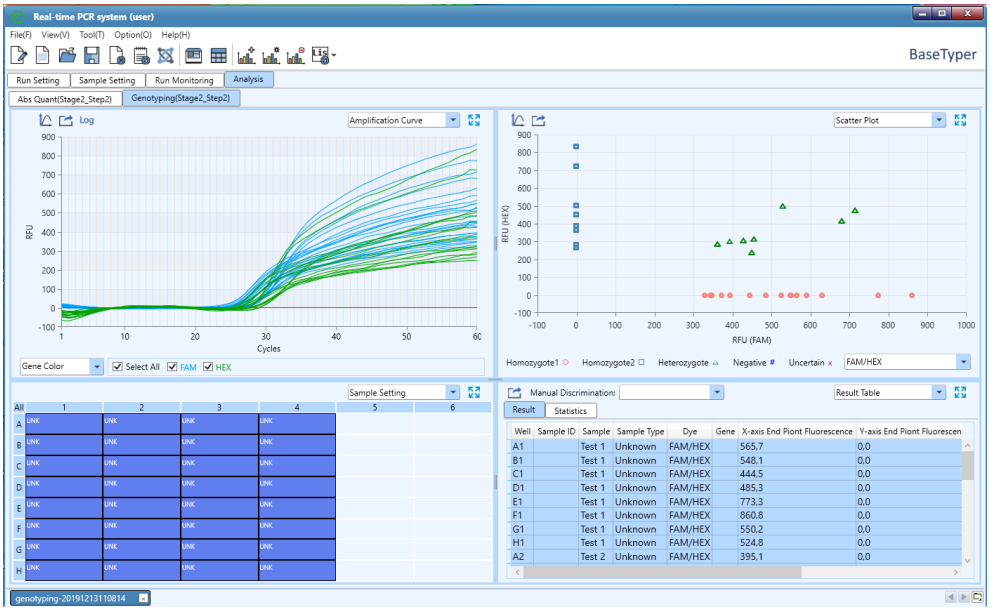
- **Fluorescence Normalization:** Defines the temperature range for the fluorescence normalisation of melting curves. The temperatures can be edited by typing a new temperature or using the and keys.
- **Sensitivity:** Set the discrimination sensitivity of the melting curve.
  - The **Delta Tm discrimination** is set to 0.5 by default. Reduce this value to group the melting curves at a higher temperature resolution.
  - The **Curve Shape Discrimination** is set to 0.5 by default. Reduce this value to group the melting curves at a higher shape resolution.
- **Reference:** Set the Reference, which is the curve that is subtracted from other curves to create the Difference Plot.
- **Other:**
  - **Pos/Neg Threshold:** Use and keys to set the threshold value to determine the positivity or negativity of samples.
  - **Temperature compensation:** Check the **Temperature Compensation** checkbox and the software will automatically calculate the temperature compensation value between sample block wells. Use and keys to change the temperature compensation value.
- **Gene and Sample** tab: See section 8.10.

8.13.1 Genotyping

The Genotyping analysis adopts two sequence-specific probes labelled with different dyes to identify the wild type and mutation alleles, respectively. After running the experiment program, the software will automatically detect the endpoint fluorescence and distinguish different genotypes based on the distribution of the two dyes on the scatter plot.

The Genotyping analysis interface consists of seven operating modules: **Amplification Curve, Scatter Plot, Sample Setting, Result Table, Raw Curve, Raw Fluorescence,** and **Heat Map**. The Genotyping analysis interface is divided into four areas and the software displays four functional modules (**Amplification Curve, Scatter Plot, Sample Setting, Result Table**) by default, as shown below:



Attention: For parameter descriptions of **Sample Setting, Result Table,** and **Heat Map** operating modules, see Section 8.9.1.

Scatter Plot

Displays the endpoint fluorescence distribution of two dyes, as shown below:

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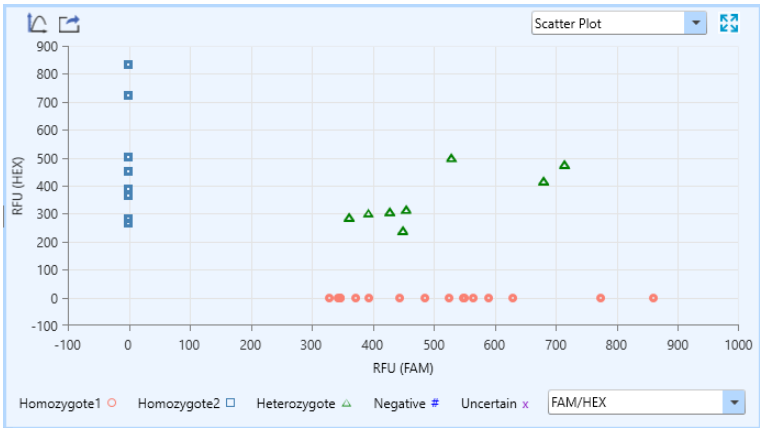
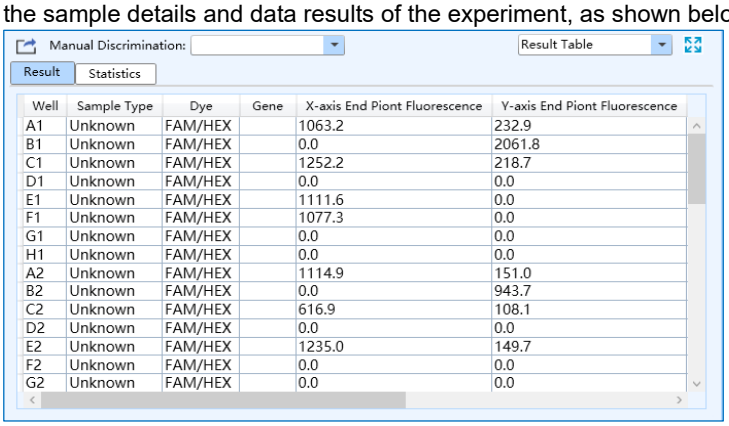


- **X axis and Y axis:** Represent the Relative Fluorescence Unit (RFU) of different dyes, respectively. Each point represents a sample and different icons represent different genotypes or analysis results.

Result Table

The Result Table displays the sample details and data results of the experiment, as shown below:



Well	Sample Type	Dye	Gene	X-axis End Piont Fluorescence	Y-axis End Piont Fluorescence
A1	Unknown	FAM/HEX		1063.2	232.9
B1	Unknown	FAM/HEX		0.0	2061.8
C1	Unknown	FAM/HEX		1252.2	218.7
D1	Unknown	FAM/HEX		0.0	0.0
E1	Unknown	FAM/HEX		1111.6	0.0
F1	Unknown	FAM/HEX		1077.3	0.0
G1	Unknown	FAM/HEX		0.0	0.0
H1	Unknown	FAM/HEX		0.0	0.0
A2	Unknown	FAM/HEX		1114.9	151.0
B2	Unknown	FAM/HEX		0.0	943.7
C2	Unknown	FAM/HEX		616.9	108.1
D2	Unknown	FAM/HEX		0.0	0.0
E2	Unknown	FAM/HEX		1235.0	149.7
F2	Unknown	FAM/HEX		0.0	0.0
G2	Unknown	FAM/HEX		0.0	0.0

- Double click on the title of each column to order the samples according to the specific column content.
- Change the order of the columns by clicking on the title of a column and drag it to the left or right.
- The software will automatically discriminate the different genotypes. It is also possible to select any sample in the **Result Table** list and set the genotype for the selected sample from the **Manual Discrimination** drop-down list.

Genotyping Analysis Settings

Click the **Analysis Setting** icon in the Toolbar to open the genotyping analysis setting window, where the relevant parameters can be set for the genotyping analysis.

The Genotyping analysis setting window consists of two tabs (Section 8.10).

- **Genotyping** tab
  - **Analysis Mode:** Select one or more of the three following analysis modes;
    - **Subtract Baseline**
    - **Reference Dye**
    - **Vertically Reverse Curve**

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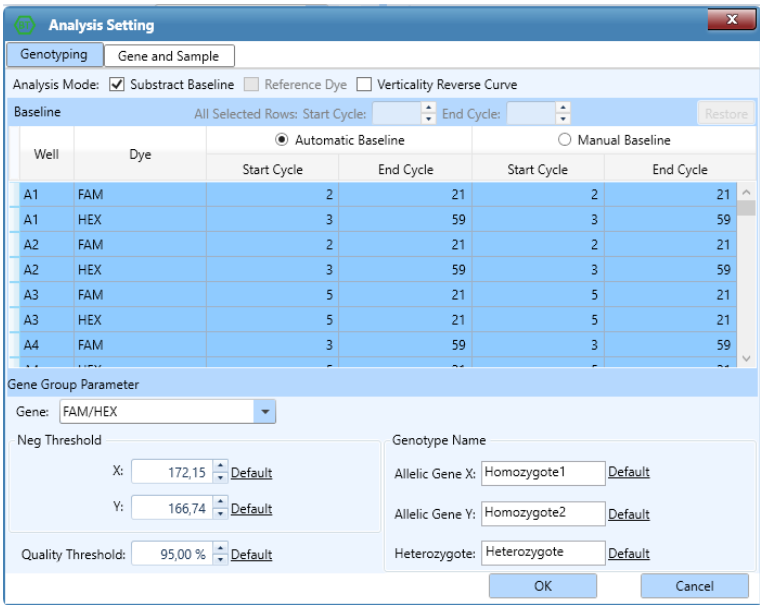
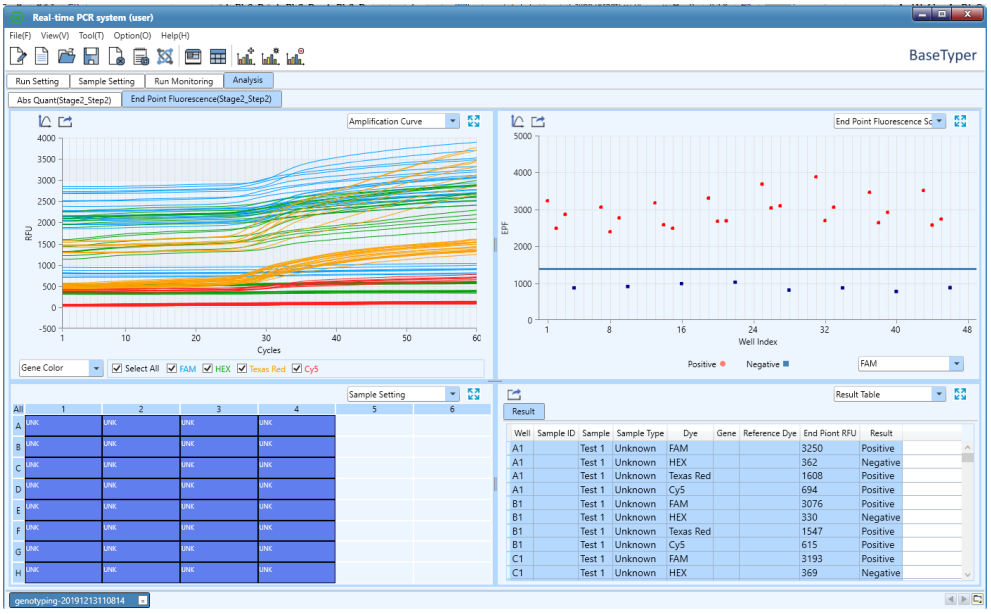


- **Gene Group Parameter**
  - **Gene:** Displays the detective dyes of different genotypes.
  - **Neg. Threshold:** Use the and keys to set the negative threshold for the X-axis and Y-axis of the scatter plot.
  - **Quality Threshold:** Use the and keys to set the quality threshold of the software algorithm. Increasing the quality threshold value can improve the credibility of the grouping result.
  - **Genotype Name:** Change the group name for allelic genes and heterozygous genes.
- **Gene and Sample** tab: See section 8.10.

End Point Fluorescence

The endpoint fluorescence analysis displays the final detection results based on the fluorescence intensities measured at the endpoint of the amplification plateau phase.

The endpoint fluorescence analysis interface consists of seven operation modules: **Amplification Curve, End Point Fluorescence Scatter Plot, Sample Setting, Result Table, Raw Curve, Raw Fluorescence,** and **Heat Map**. The endpoint fluorescence analysis interface is divided into four areas, and four functional modules (**Amplification Curve, End Point Fluorescence Scatter Plot, Sample Setting, Result Table**) are displayed by default, as shown below:



Attention: For descriptions of **Amplification Curve, Sample Setting, Result Table, Raw Curve, Raw Fluorescence and Heat Map** functional modules see section 8.9.1.

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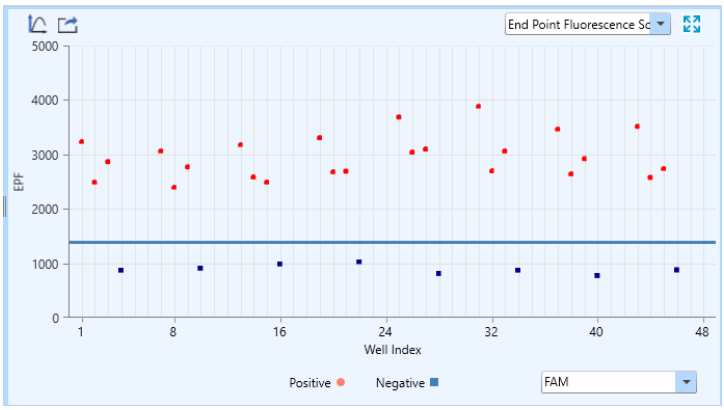
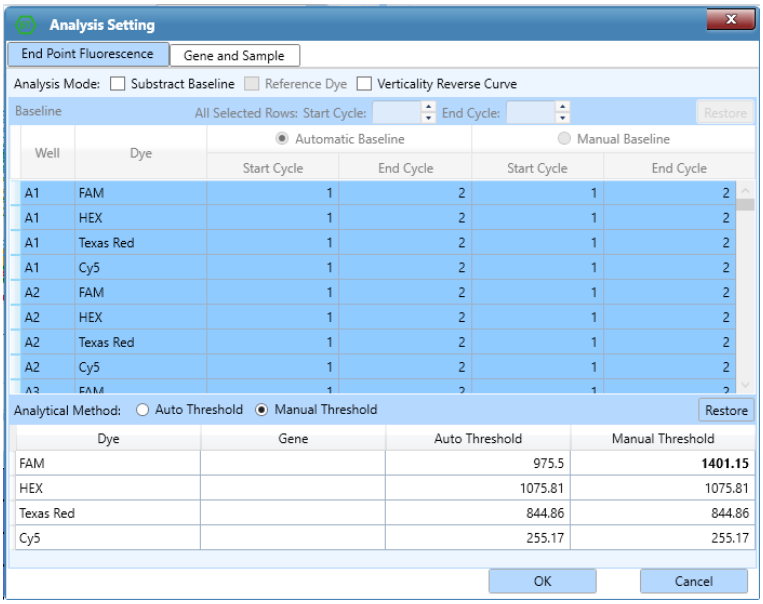
End Point Fluorescence Scatter Plot
The End Point Fluorescence Scatter Plot displays the scatter plot of fluorescence intensities measured at the endpoint of the amplification plateau phase, as shown below:



- **X-axis:** Represents the sample well index of the current experiment samples.
- **Y-axis:** Represents the End Point Fluorescence (EPF) intensity.
- Each point represents a sample and different icons represent the negative or positive interpretation results.

Click the  **Analysis Setting** icon in the Toolbar to open the endpoint fluorescence analysis setting window, which consists of two tabs: **End Point Fluorescence and Gene and Sample**.



- **End Point Fluorescence**
  - **Analysis Mode:** See section 8.10.
  - **Analytical Method:** See section 8.10.
  - **Gene and Sample** tab: See section 8.10.

9 Operating the instrument software

The BaseTyper™ can run independently without a connection to a control computer. Use the touch screen or an external mouse to operate the instrument software. Switch on the power switch of the BaseTyper™. The instrument system will automatically conduct self-inspection, and the touch screen will light up and display the instrument software boot screen.

9.1 Instrument software main interface

After the self-inspection, the instrument software will enter the main interface, which consists of a status bar, an operation area, and main function keys as shown below:

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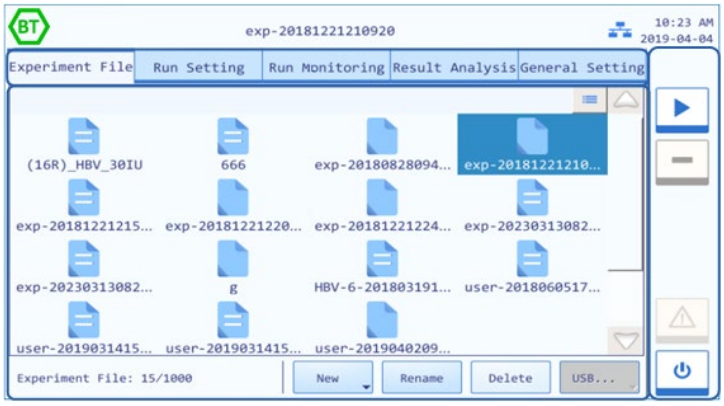


9.1.1 Status bar

The Status Bar displays the system status, current file name, date, and time. The following icons show the instrument status:

Not connected: The instrument is not connected to the network.

Connected: The instrument is connected to the network.

USB: An USB device connected to the instrument.

Hot Lid Used: The hot lid heating function is enabled.

Hot Lid Unused: The hot lid heating function is not enabled.

Top Lid Open: The top lid is open.

Error: An instrument hardware or software error has occurred, and the instrument is unable to proceed with any operations.

9.1.2 Operation area

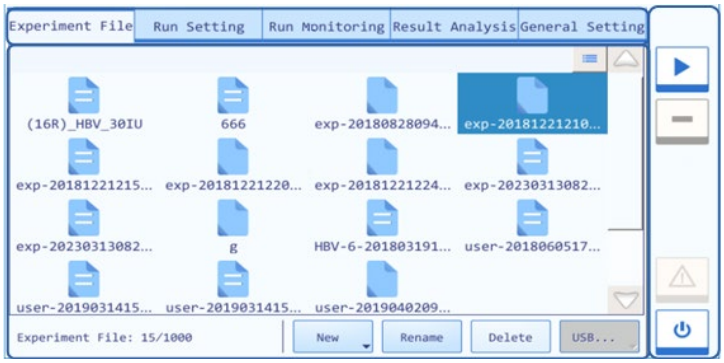
The operation area includes four tabs: **Experiment File**, **Run Setting, Run Monitoring,** and **General Setting**.

9.1.3 Experiment file

The **Experiment File** tab will be selected by default and consists of three parts; **Experiment file display area**, **experiment file information window,** and **experiment file action bar**, as shown below:

- **Experiment file display area:** Display the pre-existed experiment files or file folders within the instrument system. Pres the file icon to open a file.

  Press the icon to display the experiment file as a file detail list or press the icon to display the files as file icons.



- **Experiment file information window:** Displays the number of existing experiments within the instrument system.

- **Experimental file action bar:** Consists of four tabs; **New**, **Rename**, **Delete,** and **USB**.

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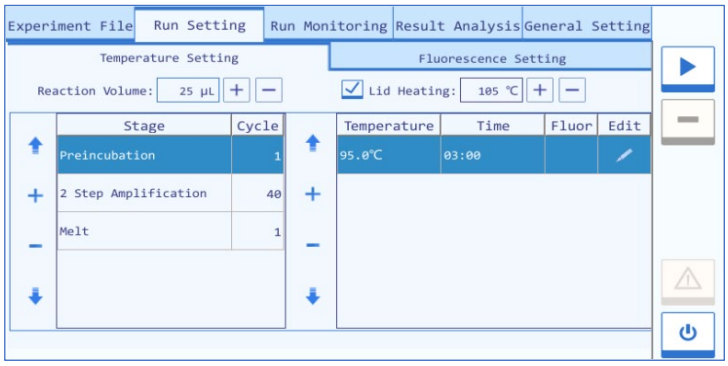
- **New:** There are three options when pressing the **New** button: Press **New folder** to create a new experiment folder, press **New Experiment** to create a new experiment file, or press N**ew Experiment from Selected Experiment** to create a new experiment file using an existing file as a template.
- **Rename:** Rename the selected experiment file or folder. This key is inactivated when the BaseTyper™ is running.
- **Delete:** Delete the selected experiment file or folder. This key is inactivated when the BaseTyper™ is running.
- **USB:** When a USB stick is connected to the instrument, press the **USB** button. Select either **Export Experiment** to transfer experiment files or folders from the instrument to the USB or select **Import Experiments** to import a selected experiment file or folder from the USB to the instrument.

  Attention: If more than one USB device is connected to the BaseTyper™, the USB device of interest can be selected from a pop-up USB device list.

9.1.4 Run setting

The **Run Setting** tab contains two sub-tabs; **Temperature Setting** and **Fluorescence Setting**.



Temperature Setting

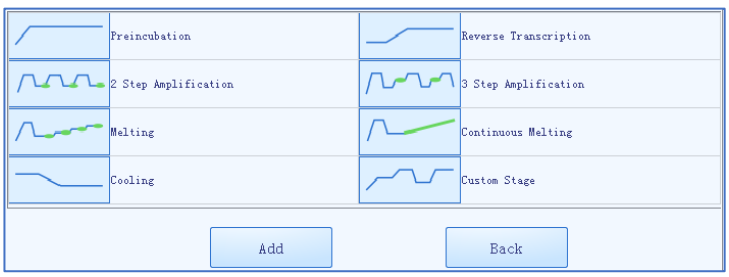
The Temperature Setting tab consists of the Stage Setting box and the Step Setting box. Edit the relevant experiment settings and temperature program.

- Set the **Reaction Volume** by using the + or − buttons.

  Attention: The reaction volume can range between 0 µL and 100 µL.
- Check the **Hot Lid** checkbox to utilise the hot lid function. Use the + or − buttons to set the hot lid temperature.

  Attention: The hot lid temperature settings can range from 40 ˚C to 110 ˚C.
- The **Stage Setting Box** displays the stage type of the temperature program and the Cycle number of the current stage.
  - Press + button to open the stage selection window where stages can be added. The instrument software provides seven predefined stage types: **Preincubation, Reverse Transcription, 2 Step Amplification, 3 Step Amplification, Melting, Continuous Melting,** and **Cooling**. Select the **Custom Stage** to define a stage according to the requirements of the experiments.

    Attention: At least one stage needs to be included in the temperature program.



- Click on the cycle number of a stage to edit it.

  Attention: The number of cycles can range from 1 to 99.

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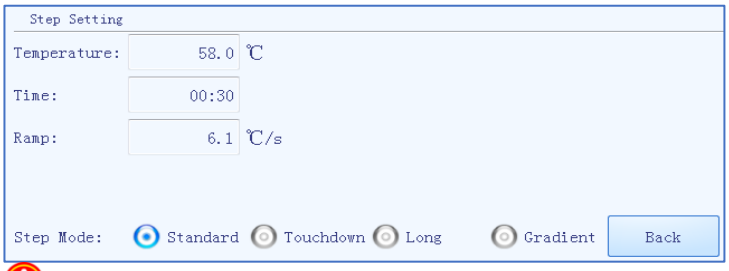
- The **Step Setting Box** displays the target temperature, temperature holding time, and whether to read the fluorescence of the current step.
    - Press the **Flour** column of a step to set whether to read fluorescence. The  icon will appear in the fluorescence reading step.

      Attention: There can only be one fluorescence reading step per stage.
    - Press the  icon to edit a step. A pop-up window will open where the Temperature, Time, Ramp, and Step Mode can be edited. Select from four different Step Modes; **Standard, Touchdown, Long,** and **Gradient**.
        - **Standard:** This mode is selected by default**.**

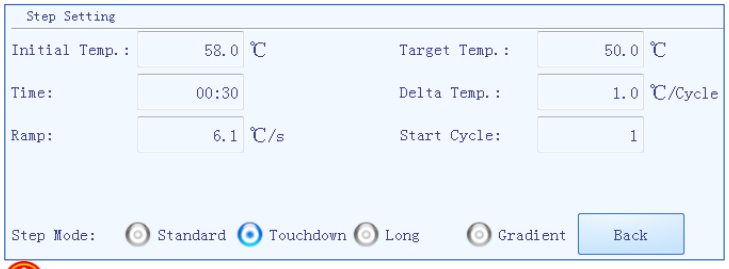


Attention: The temperature can range from 0.0°C to 100.0°C.

Attention: The time can range from 1 s to 60 min.

Attention: The ramp can range from 0.1 °C/s to 8 °C/s.

- **Touchdown:** This mode allows the temperature program to change the annealing step temperature from the initial temperature to the target temperature as the cycling proceeds.

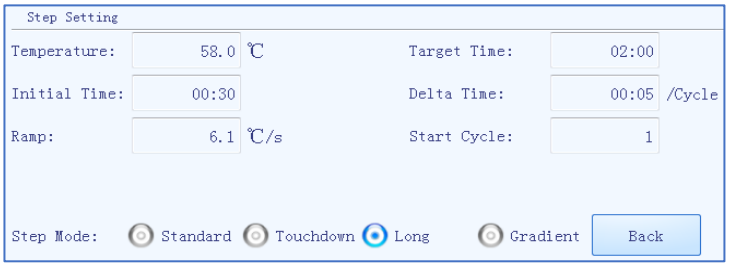


Attention: The initial and target temperature can range from 35.0°C to 100.0°C.

Attention: The delta temperature can range from 0.1°C to 5.0°C.

Attention: The start cycle can range from 1 to the highest cycle number of the current stage.

- **Long:** This mode allows the temperature program to change the elongation step temperature holding time from the initial time to the target time, as the cycling proceeds.



Attention: The initial and target time can range from 1 s to 60 min.

Attention: The delta time can range from 1 s to 10 min.

Attention: The star cycle range is 1~ max cycle number of the current stage.

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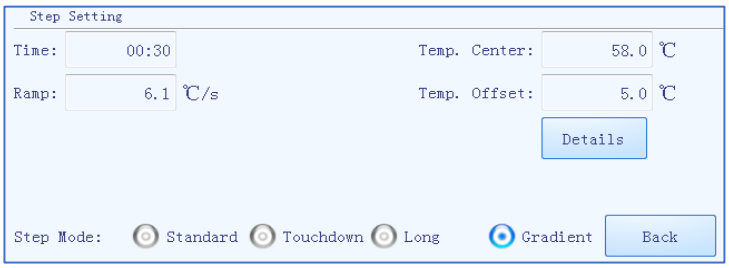
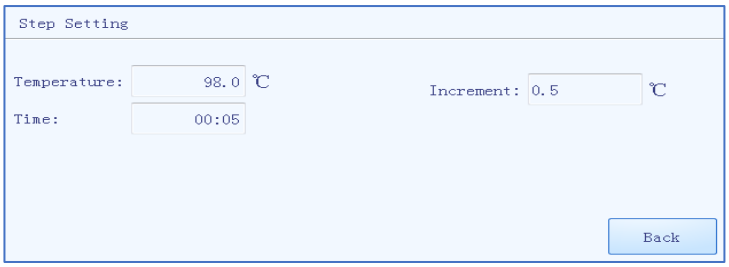
- **Gradient:** This mode allows the BaseTyper™ to adopt different temperatures. When the temperature center value and the temperature offset value are set, the software will automatically calculate the gradient temperatures. In the melting stage, the fluorescence will be read after each temperature increment.



Attention: The Temperature Center can range from 35.5°C to 99.5°C.

Attention: The Temperature Offset can range from 0.5°C to 20.0°C.

- **Melt:** The melting stage allows the instrument to read fluorescence signals after each temperature increment.



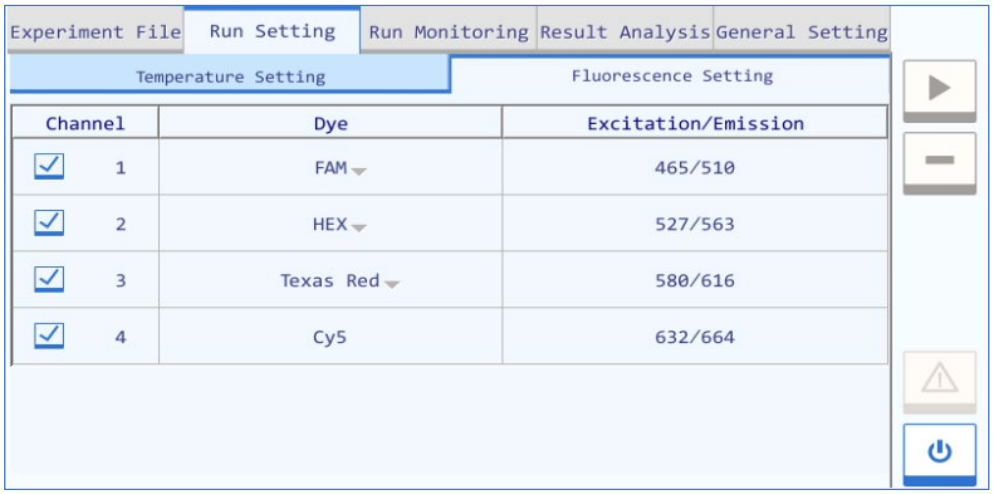
Attention: The temperature Increment can range from 0.1 ˚C to 5.0 ˚C.

- **Continuous Melt:** The continuous melt stage allows the instrument to read the fluorescence more frequently.

Attention: The reading frequency can range from 2 readings/ ˚C to 15 readings/ ˚C.

Fluorescence Setting

Select the fluorescence channel and dyes for the current experiment. Press the button in the Dye column to select a dye from the drop-down menu. The excitation and emission wavelengths of the channels are displayed in the Excitation/Emission column.



9.1.5 Run monitoring

After finishing the experiment settings press the **Run Experiment** button to start running the current experiment. The system will enter the run monitoring interface, which consists of three parts: **Run status bar, run monitoring option** tab, and **run monitoring diagram**, as shown below:

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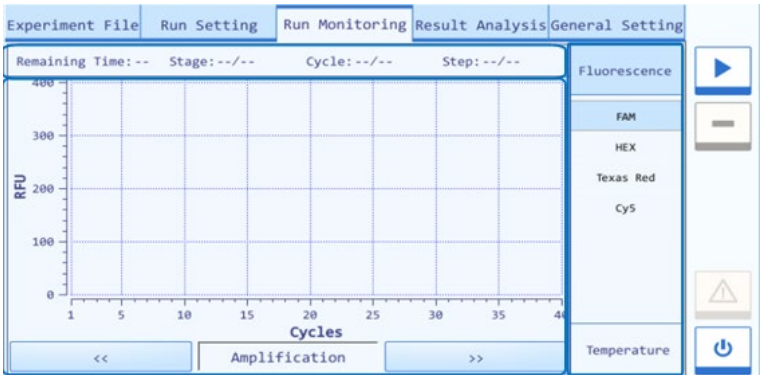


Run Status Bar
Displays the real-time running status of the current experiment including the Remaining Time, Stage, Cycle, and Step.

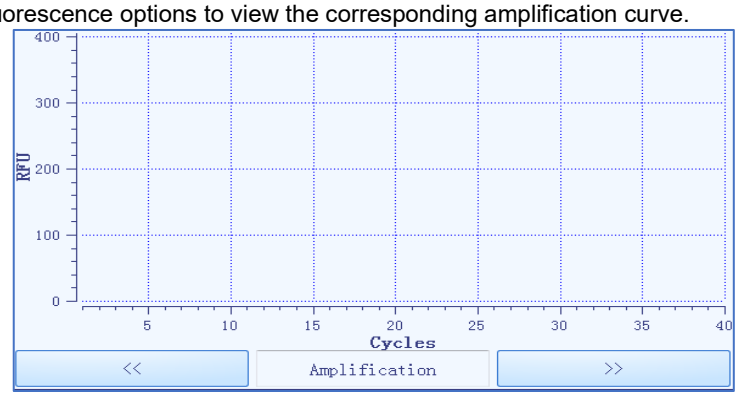
Run Monitoring Option Bar
Select the **Fluorescence** or **Temperature** option in the run monitoring option bar to monitor the corresponding content of the current experiment.

- **Fluorescence:** Select to monitor the real-time amplification curve, melting curve, and the fluorescence heat map of the experiment.
- **Temperature:** Select to monitor the real-time temperature program of the experiment.

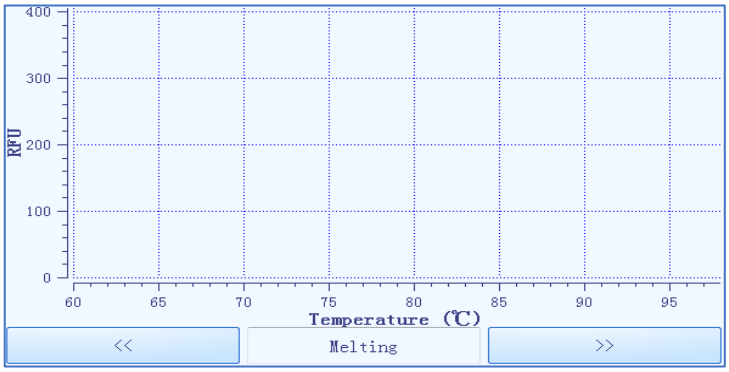
Run Monitoring Diagram

The run monitoring diagram will display the real-time Amplification curve by default. Use the << or >> buttons below the run monitoring diagram to select the content to monitor. Select between **Amplification**, **Melting, Temperature,** or **Heat Map**.

- **Amplification:** The real-time amplification curve will be displayed in the run monitoring diagram. The cycle number is displayed on the X-axis and the Relative Fluorescence Unit (RFU) is displayed on the Y-axis. Press any dye under the fluorescence options to view the corresponding amplification curve.



- **Melting:** The real-time melting curve will be displayed in the run monitoring diagram. The temperature is displayed on the X-axis and the RFU is displayed on the Y-axis. Press any dye under the fluorescence options to view the corresponding amplification curve.



- **Fluorescence Heat Map:** The Fluorescence Heat Map displays the 48 sample wells corresponding to the sample block. The colour bar on the right side displays the colour corresponding to the fluorescence intensity.

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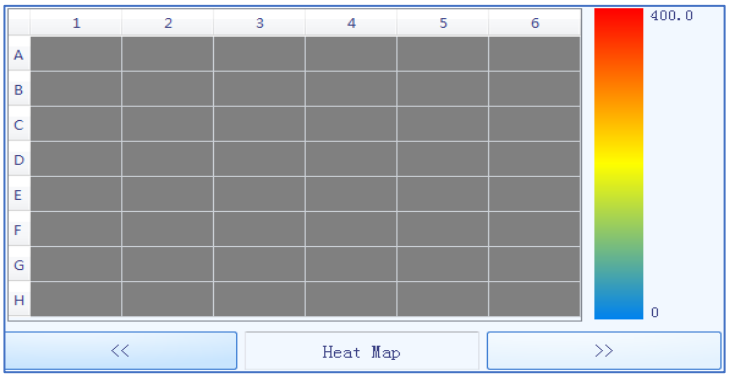
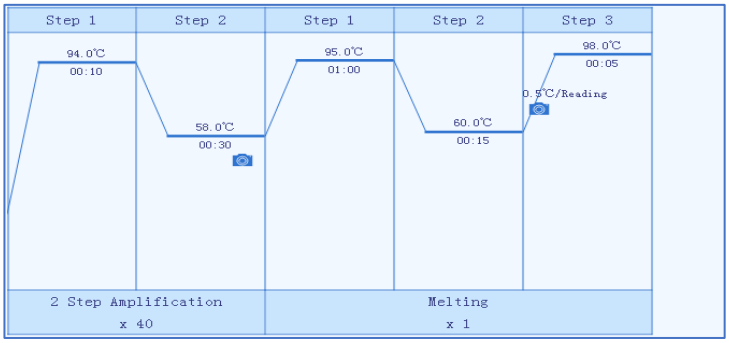


- **Temperature:** The temperature program is displayed, and the current step will be highlighted.



9.1.6 Main function keys

Run Experiment: The key is activated when the instrument status is **Ready** or **Pause**.

Pause Experiment: This key pause a run.

Stop Experiment: This key stops a run.

Shutdown/Restart: This key shuts down the BaseTyper™.

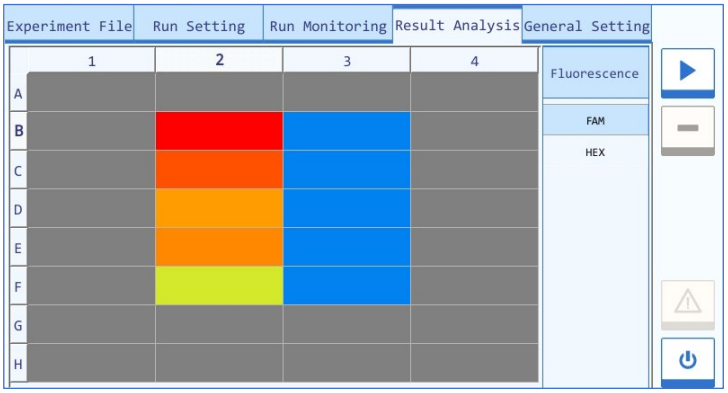
Warning: Inactivated warning icon when the system status is normal.

Warning: A system error has occurred. The instrument can still execute the current operation.

Warning: A system error has occurred. The instrument cannot execute the current operation.

9.2 Result analysis

After a finished experiment run, click the **Result Analysis** tab to analyse the experiment results. The fluorescence heat map will be displayed by default.



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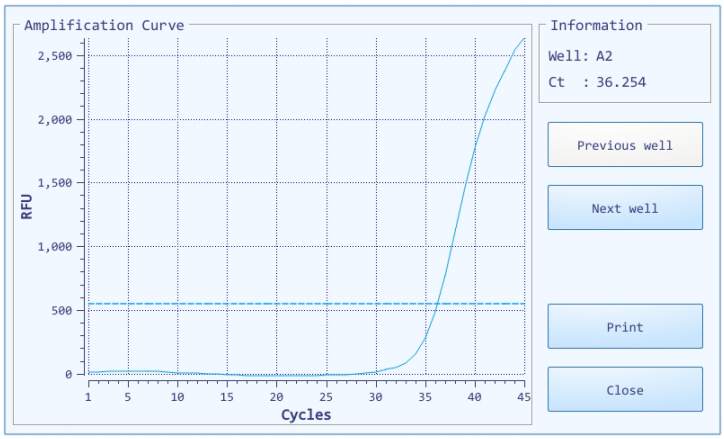
Press any well of the fluorescence heat map to view the amplification result of that sample. The amplification result interface displays the Amplification Curve, Well number, and Ct value for the selected sample.



- Press **Previous well** to see the data for the previous well.
- Press **Next well** to see the data for the next well.
- Press **Print** to print the amplification data for the current sample.
- Press **Close** to exit the amplification result interface.

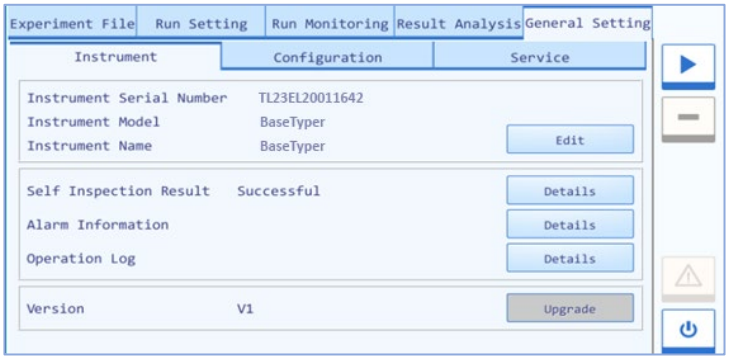
9.3 General setting

The **General Setting** tab consists of three sub-tabs; **Instrument, Configuration,** and **Service**.

9.3.1 Instrument

The **Instrument** tab contains information about:

- **Instrument Serial Number**
- **Instrument Model**
- **Instrument Name**
- **Self-Inspection**
- **Alarm Information**
- **Operation log**
- **Version**



9.3.2 Configuration

The Configuration tab contains information about:

- **Network Information:** Press **Edit** to view or edit the IP address, Subnet Mask and Default Gateway.
- **LCD Brightness**
- **Touch Screen Sound**
- **Current Date/Time:** Press **Set** to edit date and time and choose between a 12-hour or 24-hour time format.
- **Language Setting:** The instrument language can be set to English or Simplified Chinese.

9.3.3 Service

The Service tab includes the options to:

- **Clear Memory:** Press **Clear** to delete all experiment files from the instrument.

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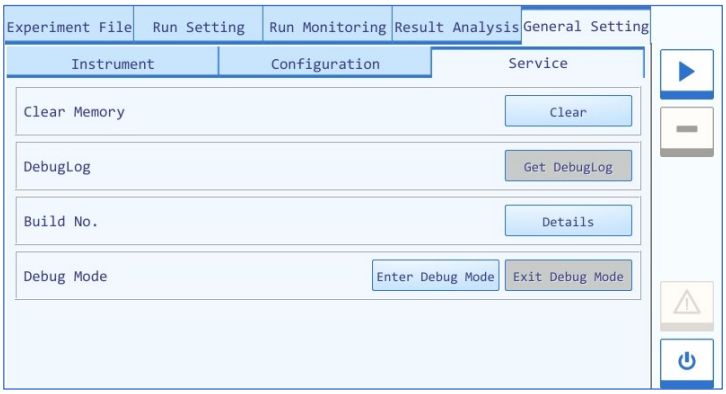
- **DebugLog:** Connect a USB drive to the BaseTyper™ and press **Get DebugLog** to download all the log files from the BaseTyper™. This will help the professional engineer to get information in case the instrument requires service.
- **Build No.:** Press **Details** to view the build number of the instrument.
- **Debug Mode:** Press **Enter Debug Mode** and enter the password to pop-up the debug interface (for qualified persons only).



10 Cleaning and Maintenance of the instrument

Under proper use conditions, the BaseTyper™ instrument requires little maintenance. However, the BaseTyper™ instrument should be cleaned and maintained on a regular schedule, to ensure continuous functionality.

10.1 Instrument cleaning instructions

The BaseTyper™ should be cleaned regularly (every other month). Carefully read the following instructions before cleaning the instrument.

Prohibit: Never clean the instrument while it is connected to power.

Prohibit: Never pour water or other solutions into the sample block or any interior parts of the instrument. Fluids can cause electrical shock when the instrument is connected to power.

Caution: Ethanol is a flammable and volatile liquid. Wear appropriate protection when cleaning with ethanol.

High Temperature: The sample block and the hot lid may be hot after running an experiment. Allow the sample block and hot lid to cool to room temperature before cleaning it.

Biohazard: Consider all samples as potential biohazardous materials. Universal precautions should be taken when handling or processing samples. Sample spill should be disinfected immediately with an appropriate disinfectant to avoid contamination of the instrument.

10.1.1 Clean the instrument shell

1. Switch off the instrument and unplug the power cord.
2. Wipe the instrument shell with a piece of damp, soft cloth, and if needed, use a mild detergent for cleaning.

   Prohibit: Do not spray detergent directly on the instrument.

Caution: Do not use organic or strong detergent to clean the instrument shell, as it may ruin the surface coating.

10.1.2 Clean the touch screen

1. Switch off the instrument and unplug the power cord.
2. Wipe the touch screen with a dry soft cloth to remove dust, oil, or fingerprints.
3. If more cleaning is needed, use a damp soft cloth with a low concentration of isopropanol or ethanol to clean the touch screen.

   Prohibit: Do not spray detergent directly on the touch screen, as malfunctions of the electronics may occur.

   Caution: Do not use abrasive or strong detergent to clean the touch screen.

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10.1.3 Clean the sample block

1. Switch off the instrument and unplug the power cord.
2. Open the top lid and clean the sample block surface with a damp soft cloth. If needed, use a mild commercial detergent.
3. Clean the sample block wells with cotton swabs, first clean using dry swabs and if necessary clean with swabs that are slightly damp with 70% ethanol.

Prohibit: Do not spray detergent directly on the thermal cycler block.

Caution: Do not close the top lid before the sample block is completely dry.

10.2 Instrument maintenance instructions

10.2.1 Maintain a sufficient airflow

The location of the BaseTyper™ should be checked regularly, as it requires enough airflow to precisely reach the correct target temperature. Ensure that the airflow is unobstructed.

10.2.2 Maintain a stable power supply

The BaseTyper™ instrument requires a stable power supply (allowable AC voltage deviation ± 10%) for proper functioning, the power supply should therefore be checked regularly.

10.2.3 Maintain instrument cleanliness

Contamination of the sample block or the optical components can interfere with the thermal cycling and collection of data. To avoid contaminating the BaseTyper™ Instrument:

- Make sure that the outside surfaces of consumables are clean before placing them in the sample block.
- Clean the sample block regularly to prevent the build-up of dirt.

Caution: Never place a consumable with an open or leaking cap in the sample block. The reagents may vaporise during heating and contaminate the sample block and hot lid.

Caution: Never run a PCR reaction with volatile reagents that could contaminate the sample block and hot lid.

Caution: In case the instrument will not be used for a long time, unplug it and cover the instrument with a cover or a plastic bag to protect it from dust.

10.3 Replace the fuse tube

The fuse tube (type 250V~/F10AH) of the BaseTyper™ is located in the fuse tube box near the power outlet at the back of the instrument. Before replacing the fuse tube, switch off the instrument and unplug the power plug. Use a flathead screwdriver, tweezers or similar to pry open the fuse tube box and substitute the old fuse tube with an equal one, as shown below:

Attention: In case the screen is empty when starting up the BaseTyper™, please check the fuse tube.

Caution: An improper fuse tube may lead to system circuit damage or even fire.

11 Transportation or Return to Manufacturer

In case the BaseTyper™ has to be moved to another lab, please follow the Product packing instruction. If the instrument needs to be returned to PentaBase A/S for maintenance or repair, please first disinfect the instrument and fill in the disinfection certificate (obtained by contacting tech@pentabase.com).

11.1 Product packaging

Please use the original packaging materials to pack the BaseTyper™ and its accessories to prevent shaking or punches of the instrument during transportation. Pack the BaseTyper™ as listed and shown below.

1. Find the transport box for the Basetyper™ 48.4, and place the bottom protective foam in the box.
2. Pack the instrument in a plastic bag and place the instrument in the box with the protective foam.
3. Place the top protective foam on top of the instrument

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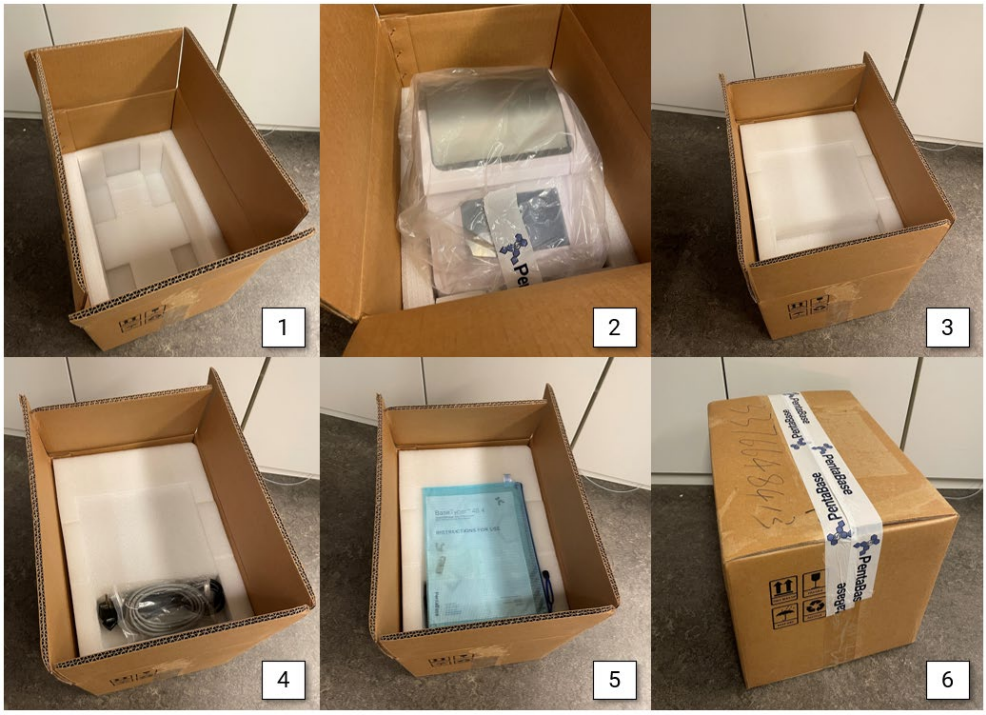
4. Put the accessories on top of the foam.
5. Place the related documents on top of the foam.
6. Seal the carton cover with wide tape.



11.2 Instrument disinfection

The disinfection of the BaseTyper™ should be performed as listed:

- Wear protective clothing and medical disposable gloves.
- Open the top lid and remove all consumables from the sample block.
- Switch off the instrument and unplug the power cord.
- Wipe all surfaces with 70% ethanol on a damp cloth.
- Leave the top lid open until it is dry.
- Open the top lid and leave the instrument under UV light for 2 hour for disinfection.
- Place 1 empty 0.2 ml tube in each corner of the sample block. If single tubes are unavailable place 1 0.2 ml 8-strip in row 1 and row 6.
- Close the lid and pack according to 11.1.

⚠ **Caution:** The original transport package of the BaseTyper™ is designed to reduce the risk of instrument damage and ensure its safety during transport. The use of other packaging materials will break the warranty, and PentaBase A/S will not be responsible for damages due to improper packaging.

Attention: The BaseTyper™ instrument should be transported with a courier/shipping company with tracking and coverage in case of damaged/missing items.

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12 Troubleshooting

In general, corrective instructions will be displayed along with the error messages by the software. Software running errors can under normal circumstances be solved by restarting the computer or the instrument system. This section describes the main possible errors of the BaseTyper™ together with possible causes and corrective instructions.

No.	Error	Possible Cause	Corrective Instructions
1	No display on the screen	No power supply	Plug the power supply.
		The power switch is on “off”	Switch the power switch to “on”.
		Unstable power cord connection	Connect the power cord again or renew the power cord.
		Inappropriate power voltage	Adjust the scope of power voltage into the normal range.
		Damaged fuse tube	Replace the fuse tube.
		Others	Contact us.
2	The boot screen displays an error message	The activation of the system failed	Please contact us and consult the maintenance engineer.
		The power voltage is too low	Ensure there is no other appliance or circuit in the same electric circuit.
3	The system crashed or is out of control	Improper operation	Restart the instrument system.
		Others	Contact us.
4	The temperature does not rise while heating	Check the temperature control settings	Start the temperature control.
		Others	Contact us.
5	No experiment results	Wrong operation process	Check the operation process and test again.
		Reagents of bad quality	Renew the reagents and run the experiment again.
		Experiment settings do not meet the requirements (wrong temperature or low cycle number)	Reset the experiment procedure.
6	Abnormal ramp or incorrect temperature	The air vent is blocked	Clean the air vent.
		Loose connection	Contact your local distributor or PentaBase A/S.

⚠ **Caution:** In case you cannot judge and eliminate these errors by yourself, please contact PentaBase A/S.

⚠ **Caution:** In case any of the following situations occurs, immediately cut off the power supply and contact PentaBase A/S. We will arrange qualified maintenance personnel for processing:

- Any liquid has entered the instrument.
- Abnormal sounds or smells appears inside the instrument.
- The instrument is soaked with water or rain.
- Any housing damages.
- Obvious functional changes in the instrument.

13 Legal manufacturer

PentaBase A/S is the legal manufacturer of this device. For technical assistance, please contact your local distributor or PentaBase A/S. Look up distributors at the homepage (https://pentabase.com/distributor/).

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Email: info@pentabase.com (General customer support) / tech@pentabase.com (Technical assistance)
Webpage: www.pentabase.com

NOTICE TO USERS: Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

14 Change history

Version	Date	Description of significant changes
2.0	2022-10-05	New setup and layout, edits in all chapters/sections.
3.0	2022-10-06	Corrected version number. No other changes.
4.0	2023-01-23	Added change history. Removed Biohazard safety label from section 3.1. Minor corrections to layout in connection with Instructions for Use review.

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