NIPPON Genetics EUROPE
INNOVATION FOR YOU

Your distributor in Switzerland
LubioScience GmbH
Baumackerstrasse 24
8050 Zürich
+41 (0)41 417 02 80
info@lubio.ch
www.lubio.ch

FastGene®
qFYR

Real-Time PCR System

# FastGene® qFYR Real-Time PCR System

## User Manual

Version 1.0

# Copyright & Disclaimer, NIPPON Genetics EUROPE GmbH

The copyright of this manual is owned by NIPPON Genetics EUROPE GmbH. No individual or organization shall, in any form, copy, edit, publish, or translate the contents of this manual (including but not limited to words, trademarks, logos, keystroke icons, lists, images, data, etc.) into other languages without the authorization or written permission of the company. In violation of the above statement, the Company will investigate its relevant legal liability.

NIPPON Genetics EUROPE GmbH strives to ensure that the contents of this manual are correct when printed, but the company still has the right to modify the contents of this manual at any time without prior notice. The contents of the manual shall be subject to the changed contents.

| | |
|---|---|
| **User manual version number:** | **V1.0** |
| **User manual revision time:** | **September 2022** |
| **Type of instrument applicable:** | **FastGene® qFYR Real-Time PCR System** |
| **Software Release Number:** | **V 1.0** |
| **Instrument production date:** | **See production label** |

# Introduction

Welcome to the FastGene® qFYR Real-Time PCR System. Please read this instruction carefully so that you can use this instrument correctly. After careful reading, please keep it for future inquiry when necessary.

# Product information

| | |
|---|---|
| **Description**: | Real-Time PCR System |
| **Product model:** | FastGene® qFYR Realtime PCR System |
| **Software Name:** | FastGene®qFYR Analysis Studio |
| **Software Release Number:** | V1 |
| **Product Size:** | 350 x 520 x 370 mm (length x wide x height) |
| **Net Weight:** | 25 Kg |

# Enterprise information

**Statement of intended use:**

This product is used to run real-time fluorescence PCR experiments (Real-time PCR) and to analyze the experimental data. The instrument is operated in a laboratory with the corresponding reagents, to carry out rapid and accurate quantitative and qualitative detection of target nucleic acids sequences from human and animal samples (such as blood, oral swabs, nasopharyngeal swabs, body fluids, etc.) or other analytes, or to conduct melting curves, genotype analysis, etc.

**Special statement:**

Before installing and using this instrument for the first time, please read the instructions in this manual carefully. Please take into consideration the possible scope of maloperation or non-recommended usage and pay special attention to the possible consequences.

**Intended Usage:**

This product is for ***Research-Use-Only*** (RUO).

**User requirements:**

This product must be operated by trained professionals, who have studied this user manual.

# Table of Contents

Table of Contents 6

Chapter 1 Security and considerations 10

1.1 Security Symbols and Markings 10
1.2 General Instrument Safety and Precautions 10
1.3 Personal Safety and Precautions 11
1.4 Electrical Safety and Precautions 11
1.5 Environmental Safety and Precautions 12
1.6 Biosafety and Precautions 13
1.7 Electromagnetic Compatibility (EMC) Standards 13

Chapter 2 Product Overview 15

2.1 Scope of application 15
2.1.1 Intended Use 15
2.1.2 Product Intended Use Environment 15
2.2 Application Areas 15
2.3 Basic Principles 16
2.4 Main Functions 16
2.5 Product Composition 16
2.6 Product Structure 17
2.7 Instrument Parameters and Characteristics 19
2.7.1 Physical Specifications 19
2.7.2 Instrument Technical Parameters 20
2.7.3 Software Features 21
2.7.4 Chemistry Reagent Features 21
2.7.5 Consumables Specifications 21

Chapter 3 Installation and Transportation of Instruments 22

3.1 Transport and Storage 22
3.2 Unboxing 22
3.3 Packing List 23
3.4 Installation Requirements 23

3.4.1 Instrument Working Environment Requirements 23
3.4.2 Computer Configuration Requirements 24
3.5 Equipment Installation 25
3.5.1 Equipment Appearance 25
3.5.2 Release Instrument Transport Lock 27
3.5.3 First Installation and Run Protocol 28
3.6 Instrument Software Installation 29
3.6.1 FastGene® qFYR Analysis Studio Software Installation 29
3.7 Pre-preparation for boot 32
3.7.1 Instrument Self-inspection 32
3.7.2 System Start-up 32
3.7.3 Experimental Preparation 33

Chapter 4 Operation to Run the Experiment 34

4.1 Software Start 34
4.2 Create New Experiment 36
4.2.1 New Experiment or Loading existing files 36
4.3 Experiment Properties 37
4.3.1 Name 37
4.3.2 Plastic Type 37
4.3.3 Experiment type 38
4.3.4 Chemistry 39
4.4 Experiment Method 40
4.4.1 Reaction volume 40
4.4.2 Thermal lid temperature 40
4.4.3 Basic setting 40
4.5 Plate Setup 42
4.5.1 Setup 42
4.5.2 Quick Setup 44
4.5.3 Remove Wells from the Reaction 44
4.6 Run an Experiment 45
4.6.1 Insert a Plate or Reaction tubes 45
4.6.2 Run the Experimental Program 45
4.7 Save Experiment 47

Chapter 5 Operation of Different Experiment Types 48

5.1 Absolute Quantification (Standard Curve) Experiment 48
5.1.1 Setup of a Standard curve 49
5.2 Experiment of Melting Curve 52
5.2.1 Setup of Melt Curve Analysis 52
5.3 Relative Quantification Experiment（ΔΔCq） 53
5.3.1 Setup of comparative Cq (ΔΔCq) Analysis 53
5.4 Genotyping Experiments 54
5.4.1 Set Up of a Genotyping analysis 54
5.4.2 Set Up of a High-Resolution Melt Analysis (HRM) 55
5.5 Presence / Absence Experiments 56
5.5.1 Set Up of a Presence / Absence analysis 56

Chapter 6 Result Analysis and Data Export 57

6.1 Result Tab 57
6.1.1 Buttons in Toolbar 57
6.1.2 Other Toolbars 58
6.2 Result Overview 59
6.2.1 Amplification plot 59
6.3.1 Plot Properties 59
6.2.2 Plot Settings 60
6.2.3 Threshold Settings 60
6.2.4 Baseline Settings 61
6.2.5 Target Settings 61
6.2.6 Results as Reaction Board 62
6.2.7 Results as List 63
6.2.8 Raw Amplification Plot and Raw Melt Peak Plot 64
6.2.9 Standard Curve 65
6.2.10 Melt Curve 66
6.2.11 Gene Expression Map 67
6.2.12 Analysis Settings 68

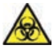
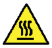
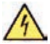
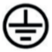
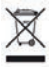
6.2.13 Genotyping Maps 69
6.2.14 Raw Melt Curves of the High-Resolution Melt analysis 70
6.2.15 Aligned Melt Curves of the High-Resolution Melt analysis 71
6.2.16 Difference plot of the High-Resolution Melt analysis 72
6.2.17 Presence/Absence Plot 74
6.2.18 QC Summary 75
6.3 Data Export 77
6.3.1 Toolbars in Export Tab 77
6.3.2 Data Export 77
6.3.3 Printed report 79

Chapter 7 Instruments Maintenance 81

7.1 Instrument Cleaning 81
7.1.1 Cleaning the Instrument 82
7.1.2 Clean Sample-tray and Reaction-holes 82
7.2 Instrument maintenance 83
7.2.1 Keep Air-Circulation 83
7.2.2 Keep Stable Electricity Supply 83
7.2.3 Keep Clean 83
7.2.4 Waste Disposal 83
7.2.5 Fuse Replacement 84
7.2.6 Overheating Protection 84

Chapter 8 Troubleshooting 85

**Safety markings used on instruments**

| Symbol | Heading | Description |
|---|---|---|
| | Caution | This symbol is used to indicate that non- compliance with instructions or procedures may lead to physical injury or even death or could cause damage to the instrument. Consult the Operator's Manual. |
|  | Biohazard | This symbol is used to indicate that certain precautions must be taken when working with potentially infectious material. |
|  | Hot surface | Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution. |
|  | Electricity hazard | Indicates the presence of high voltage at the instrument. |
|  | Appliance class 1 | Indicates that insulation of voltage-carrying parts (= basic insulation) and connection of touchable metal parts to the protective conductor (= grounding) protects against excessive touch voltages |
| CE | CE symbol | This symbol indicates European conformity. |
| SN | Serial number | Identification of the serial number of the instrument. |
| | Date of Manufacturing | The date of manufacturing will appear next to this symbol. |
| | Manufacturer | The name and the address of the manufacturer will appear next to this symbol. |
|  | Disposal | Do not dispose of this product as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). |

## 1.2 General Instrument Safety and Precautions

The operator shall not disassemble the instrument, replace components or debug

the instrument without the authorization of our company. If one of the above is needed, it must previously be approved by our company, and completed by trained professionals, otherwise unexpected consequences will occur.

Please avoid collision, damage the instrument and store carefully.

The user should immediately unplug the instrument from the power outlet and contact the supplier or ask our professional maintenance staff if one of the following is to happen:

- The instrument is subjected to rain or flooding.
- Abnormal sound or smell in the instrument.
- Collision or damage to the enclosure.
- The function of the instrument changed.

**It is forbidden to carry or move the instrument during the operation of the instrument.**

The openings on this instrument are ventilated. In order to avoid excessive temperature, **do not block or cover these vents during the operation of the instrument**, or use dust shields and any other substances to cover the surface of the instrument.

If you need to install or transport this instrument, please contact our company to request professional personnel or professional guidance. Otherwise, we will not bear any responsibility for the damage caused by the instrument.

After the installation of the instrument, the transport lock should be properly retained in accordance with the requirements of the technical personnel.

During the operation of the instrument, do not forcibly open the sample draw, otherwise it will destroy the biological safety of the instrument, its electromagnetic radiation, and other protective measures.

Do not force consumables (8 tubes / test tubes /96 well plates, etc.) not matched with this instrument into the sample draw.

Failure to use this instrument in accordance with the manufacturer's prescribed method may damage the protection provided by the instrument.

## 1.3 Personal Safety and Precautions

The weight of the instrument is heavy. Moving or lifting the instrument requires appropriate tools, methods, or cooperation with others to complete. Please avoid moving the instrument alone without the cooperation of others. Improper lifting of this instrument may cause physical injury, pain and/or instrument damage.

Do not touch the power plug, power cord or switch with wet hands.

**High surface temperature:**

During the operation of the instrument, the heating module of the sample draw may become very hot! During the experiment and after the experiment, do not touch the heating block directly with your hand or any part of the body to avoid scalding. Please open the sample draw after the heating block temperature drops to standby temperature and take out the sample.

## 1.4 Electrical Safety and Precautions

**Prohibition**

This instrument has a voltage harmful to the human body! At any time, before disassembling the instrument shell, please cut off the instrument power supply. It is forbidden to replace components when power lines are connected.

The shell of this instrument should be properly grounded through the power ground wire. Any damage to the internal or external grounding path of the instrument may be dangerous.

**Note:** If leakage is found, please cut off the power immediately and stop using the instrument.

**Note:** Please cut off the power supply before moving the instrument.

Use the power cord provided with this instrument. If the original power cord is broken, worn, or disconnected, the same power cord should be replaced as soon as possible.

In order to avoid the danger of electric shock, the power cord of this instrument must be reliably grounded. The power cord of this instrument is a standard plug power cord. Please insert the power plug into the appropriate three-wire grounding socket (the external network power supply connected by the socket must be reliably grounded) to ensure the safe use of the instrument.

The electrical supply in which the instrument is located must be connected with ground wire.

Please carefully check whether the power connection is firm. Do not hard pull the power cord. When the plug is plugged into the power supply, make sure the plug is fully plugged into the socket.

Please keep the power plug and the power cable away from high temperature objects such as heater. When using this instrument, please do not place any items on the power cord, do not put the power cord in the place where people often walk.

The fuse type of this instrument is T10AH250VP, placed in the backup box at the power outlet at the back of the instrument. The use of improper fuses may damage the instrument's line system and cause fire. Before turning on the instrument power switch, check and make sure the fuse is properly installed.

In order to prevent fire hazards, use this instrument only with the indicated fuse. When replacing the fuse, please cut off the power supply, pull out the power cord, pry open the fuse box with a word screwdriver, and replace the old fuse with the same new fuse.

## 1.5 Environmental Safety and Precautions

This instrument is only suitable for indoor use, indoor ventilation should be good, and free of corrosive gas.

EDM may cause explosion hazard and is prohibiting the use of this instrument in the presence or possible presence of flammable and explosive gases.

The working environment temperature of this instrument should be between 10℃~30℃ and the relative humidity should ≤85%.

The working environment pressure of this instrument should be normal atmospheric pressure (altitude less than 2000 meters).

## 1.6 Biosafety and Precautions

**Biological hazard**

The sample object of this instrument is nucleic acid. In practice, please regard it as a biological sample with potential biological hazard. When handling and operating samples, generally applicable safety precautions shall be taken and appropriate protective goggles, clothing and gloves shall be worn.

Users are requested to comply with all applicable local or national regulations to complete the disposal of waste samples and contaminated materials.

This instrument shall be treated as waste with biological pollution hazard. Before reuse, recycling, or disposal, the instrument needs to be purified (including cleaning, disinfection and/or sterilization). This instrument shall be handled in accordance with relevant local or local regulations.

During any operation, if liquid samples overflow, appropriate disinfectants should be quickly used for disinfection, to avoid the spread of pollutants to laboratory personnel or contaminated instruments.

## 1.7 Electromagnetic Compatibility (EMC) Standards

This instrument should be installed and used according to the electromagnetic compatibility information in this chapter.

The electromagnetic compatibility of this instrument is classified into categories A and groups I.

1. This instrument meets the emission and immunity requirements specified in the EN 61326-1:2013 and EN 61326-2-6:2013
2. This instrument is designed and tested according to A kinds of instruments in the CISPR 11:2016
3. Portable and mobile RF communication instruments may affect the use of this instrument. It is recommended to stay away from or close the portable and mobile RF communication instruments when using this instrument normally.
4. This instrument must be used with the power cord provided by our company, the length of the power cord is not more than 1.5 m

Except the accessories provided by our company, the use of other manufacturer accessories may lead to an increase in the emission of this instrument or a decrease in immunity.

1. Please evaluate the electromagnetic environment before this instrument is used.
2. Do not use this instrument next to strong radiation sources (such as unshielded RF sources), otherwise it may interfere with the normal operation of the instrument.

**Note:** It is the responsibility of the user to ensure the EMC environment of the instrument so that the instrument can work properly.

# Chapter 2 Product Overview

## 2.1 Scope of application

### 2.1.1 Intended Use

FastGene® qFYR Real-Time PCR Systems are used to run real-time fluorescence PCR experiments (Real-time PCR) and to analyze the experimental data. The instruments are operated in a laboratory, in conjunction with the corresponding reagents, for rapid and accurate quantitative and qualitative detection of target nucleic acids from human samples (e.g., blood, oral swabs, nasopharyngeal swabs, body fluids, etc.) or other analytes in the patient's body, or for melting curves, genotype analysis.

### 2.1.2 Product Intended Use Environment

Institutions such as medical institutions, laboratories, and companies that meet the requirements of the product operating environment.

1. diagnostic laboratory use
2. General laboratory research use. Its users are professional and technical personnel of the hospital laboratory or general laboratory and need specialized training for PCR laboratory technology and instrument, software operation, and have skilled relevant operation skills.

## 2.2 Application Areas

- Basic scientific research
- Clinical detection of pathogens
- Genetic screening
- Cancer diagnosis
- Diagnosis and treatment of cardiovascular diseases
- The drug genome
- Public Health and Safety Surveillance
- Food Hygiene and Quarantine
- Customs Import and Export Quarantine
- Transgenic and Pathogen Detection in Animals and Plants
- and others

## 2.3 Basic Principles

PCR principle is similar to the natural replication process of DNA. Its specificity depends on oligonucleotide primers complementary to both ends of the target sequence. PCR consists of three basic reaction steps: denaturation-annealing-extension. Repeated cyclic denaturation-annealing-extending processes can amplify the target gene by millions of times in 1~2 hours, for a high detection sensitivity.

The principle of real-time fluorescence PCR is to add a fluorescent reporter (probe or dye) to the PCR reaction system. The target nucleic acid derived from DNA samples is qualitatively or quantitatively detected by fluorescence detection system and real-time monitoring of the fluorescence value. Real-time fluorescence PCR detection has higher specificity and sensitivity than end-point PCR detection.

## 2.4 Main Functions

FastGene® qFYR Real-Time PCR Systems are special instruments for real-time fluorescence detection. It integrates PCR amplification, fluorescence detection and data analysis. It can monitor the increase of fluorescence in each test tube in real time as the amplification increases. After the amplification, the software automatically processes the experimental data, carries on the quantitative / qualitative, melting curve or genotyping analysis to the sample, displays and prints the experimental results.

## 2.5 Product Composition

This product mainly consists of electronic control system, power module, temperature control system, detection system, shell components and software(V1).

## 2.6 Product Structure

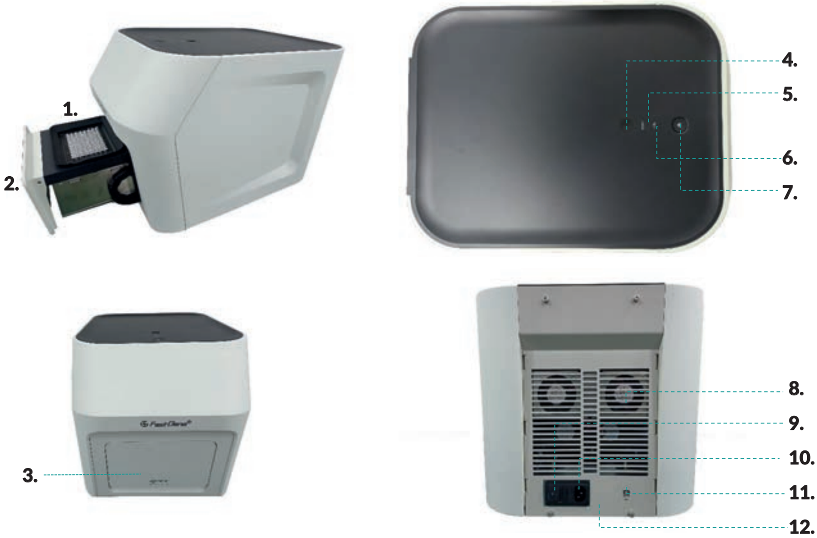
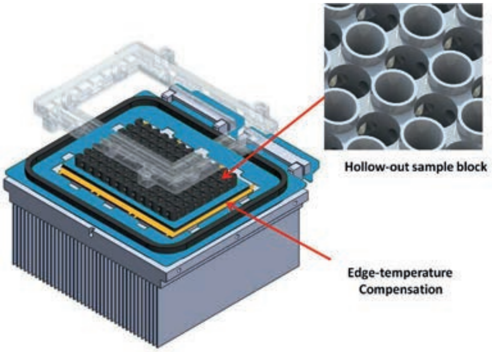
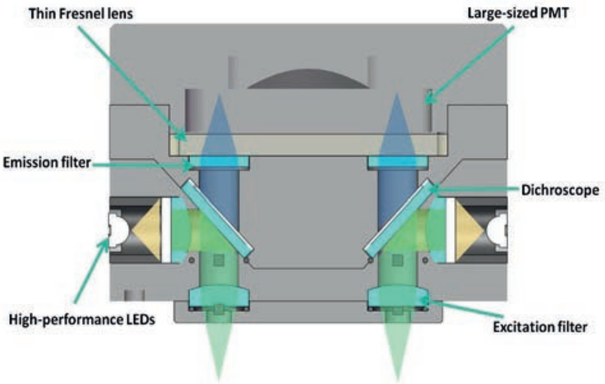


Diagram of the Structure of the Machine

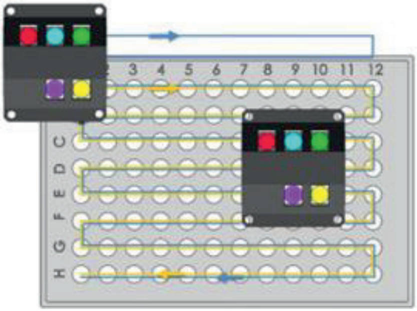
1. Sample module
2. Sample draw
3. Inlet
4. Transport lock
5. Power indicator
6. Status indicator
7. In/Out button
8. Vent
9. Power switch
10. power cord socket
11. USB interface
12. Fuse


Hollow-out sample block
Edge-temperature Compensation

Schematic diagram of temperature control module


Thin Fresnel lens
Large-sized PMT
Emission filter
Dichroscope
High-performance LEDs
Excitation filter

Schematic diagram of optical path module


2
3
4
5
6
7
8
9
10
11
12
C
D
E
F
G
H

Schematic illustration of optical scanning patterns

## 2.7 Instrument Parameters and Characteristics

### 2.7.1 Physical Specifications

| | |
|---|---|
| **Instrument specifications** | Size: 350 mm(L) × 520 mm (W) × 370 mm (H).<br>Weight：25 kg |
| **Packing specifications** | Size: 710 mm(L) × 540 mm (W) × 580 mm (H).<br>Weight：36 kg |
| **Power supply specification** | power supply voltage：AC 110-230 V<br>Frequency：50 Hz.<br>Power：750 w |
| **Communication specifications** | USB ports |
| **Environmental conditions** | Temperature：10℃ ~ 30℃.<br>Humidity：≤85% RH no condensation.<br>Atmospheric pressure：85.0 kPa ~ 106.0 kPa.<br>Altitude：< 2000m |
| **Storage and transportation environment** | Temperature：-20℃ ~ 55℃， in the transport box.<br>Humidity：≤85% RH |
| **Running noise** | Maximum noise when running instrument does not exceed 60dB（A） |

## 2.7.2 Instrument Technical Parameters

### Thermal parameters

| | |
|---|---|
| **Heat cycle** | Peltier semiconductor |
| **Temperature control mode** | Temperature control module |
| **Maximum ramp rate** | 3.6 ℃/s |
| **Temperature accuracy** | ± 0.2 °C |
| **Temperature uniformity** | ± 0.2 ℃ |

### Optical parameters

| | | |
|---|---|---|
| **Excitation source** | Single color LED | |
| **Detection device** | High sensitivity MPPC（silicon photomultiplier tube） | |
| **Fluorescent channel number** | 4 channels (2x FAM) | |
| **Applicable to fluorescent** | Channel 1 | FAM/SYBR Green etc. |
| | Channel 2 | VIC/JOE/HEX/TET etc. |
| | Channel 3 | ROX/Texas Red etc. |
| | Channel 4 | Empty |
| | Channel 5 | Cy5 etc. |
| | Channel 6 | FAM/SYBR Green etc. |

### Detection parameters

| | |
|---|---|
| **Sample size** | 1-96 wells |
| **Sample volume** | 10-50 µl |
| **Repeatability of fluorescence intensity detection** | CV ≤3% |
| **Detection of fluorescence intensity** | CV ≤5% |
| **Sample repeatability** | CV ≤ 3% |
| **Linear fluorescence intensity detection** | A linear regression coefficient ｜r｜≥0.990 in 5 concentration gradients |
| **Sample linear correlation** | A linear regression coefficient｜≥0.980 in 5 concentration gradients |

### 2.7.3 Software Features

**Software interface:** Wizard interface, intuitive sample plate layout and program settings, easy to use operation editing.

**Software functions:** absolute quantitation, relative quantitation, genotyping, HRM-analysis, and other analytical functions to meet the requirements of a variety of experiments.

**Language Support:** English.

**Data transmission:** The instrument can transmit the experimental data to the connected computer in real time during the experiment.

**Overlong program settings:** Each stage may include up to 100 steps and up to 10 cycles.

### 2.7.4 Chemistry Reagent Features

**PCR reagent:** Open reagent platform, all quantitative, qualitative PCR reagents are applicable.

**Fluorescent dyes:** Suitable for fluorescent dyes such as

FAM/SYBR Green/ FastGene® IC Green
VIC/HEX/TET/JOE
ROX/Texas Red
Cy5

### 2.7.5 Consumables Specifications

0.1 ml clear/white PCR thin-walled single tube, flat-topped transparent optical tube cover.
0.1 ml clear/white PCR thin-walled 8-well tubes, flat-top transparent optical tube cover.
0.1 ml clear/white PCR thin-walled 96-well plate with no hemline or hemline.

**Note:** FastGene® qFYR Realtime PCR System is compatible with low 0.1 mL PCR flat top transparent tube cover reaction tube /8-well joint tube, no skirt or half skirt edge 96-well PCR reaction plate.

**Do not use 0.2 mL PCR reaction tube and convex cover.**

If the sample tubes are just a few, please use 8-well tubes for support on both sides, or use empty single tubes in the four corners to prevent uneven force on the plate slot.

# Chapter 3 Installation and Transportation of Instruments

## 3.1 Transport and Storage

Product transportation is carried out in accordance with the requirements of the product. Original packaging must be used in transportation to avoid damage to the instrument.

The instrument in the packaging and transport state, should be stored in the ambient temperature of -20℃~55℃, relative humidity less than 85%, and the air cannot contain corrosive agents.

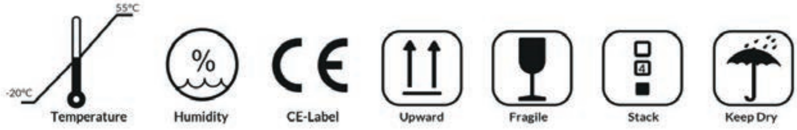
The outer packaging of the product specifies the transport instructions allowed for this product, which are:

**Packaged products are only allowed to be placed in the direction indicated by the arrow.**

Please hold the handling process lightly.

Maximum stack number is limited to four layers, do not exceed four layers stack.

**Do not transport or store this product in rain or wet environment.**



## 3.2 Unboxing

The outer packing of the product is cardboard box, filled with shock absorber foam. Please do not rush to open the equipment packaging after receiving the equipment. Carefully check the packaging´s appearance for damage. Please check the content you received if parts are missing and damaged after unpacking. If the product is damaged during transportation, please do not use it, and contact NIPPON Genetics EUROPE or the distributor!

## 3.3 Packing List

| | NAME | Unit |
|---|---|---|
| 1 | Real-Time PCR System FastGene® qFYR | 1 |
| 2 | Instrument power cable | 1 |
| 3 | Instrument data cable | 1 |
| 4 | Fuse | 2 |
| 5 | hexagon spanner | 1 |
| 6 | Packing List | 1 |
| 7 | USB Stick (including software and electronic user manual) | 1 |

## 3.4 Installation Requirements

### 3.4.1 Instrument Working Environment Requirements

**Location:** The main unit must be placed on a stable, horizontal working table, avoiding direct sunlight, not near heating equipment. The instrument´s environment must be at ambient temperature of 10~30℃, with a relative humidity less than 85%. For indoor use only.

**Surrounding environment:** Do not install the main unit next to instruments with strong electromagnetic interference or high induction factors, such as refrigerators, high-speed centrifuges, oscillators, etc.

The mainframe must be placed **at least 15 cm from the surrounding object or wall** for heat dissipation and ventilation of the instrument, and for the convenience of users to turn on and off the instrument power supply.

**Do not place the mainframe in a narrow space where it is difficult to operate and disconnect the power supply!**

**Power requirements:** 110-220 VAC,50/60Hz, voltage fluctuations <10 V.

**Power outlet:** To prevent electrical shock, the instrument mainframe must be connected to a three-core grounding socket that meets the safety standards. The voltage is ~110-220 V (50-60 Hz) and the zero-ground voltage ≤5 V. If conditions permit, connect the instrument to a dedicated power outlet.

**Warning:** Incorrect grounding may cause electrical shock or instrument damage. Confirm that the input voltage meets the requirements of the instrument and that the fuse has been installed in accordance with the specifications.

### 3.4.2 Computer Configuration Requirements

The computer installed with the FastGene® qFYR Analysis Studio must have the following configuration:

| | |
|---|---|
| **Processor:** | Intel or AMD dual-core CPU,2.8GHz |
| **Memory:** | 4 GB |
| **Hard disk:** | 500 GB |
| **Network card:** | 10 M/100M adaptive network card (optional) |
| **Display resolution:** | 1440×900 or higher |
| **Operating system:** | Windows 10 or above & Office Word/Excel 2007 or above. |

**Note:** The control computer of this instrument is not designed for online use. Connecting it to the network will have the risk of acquiring viruses and becoming the target of hacker attacks. NIPPON Genetics EUROPE is not responsible for any damage caused by an internet connection. It is not recommended to install other software in the control computer. There will be a potential risk of conflict with the instrument software module and may affect the reliability of the results. NIPPON Genetics EUROPE does not provide antivirus software. Therefore, if necessary, take preventive measures to ensure that there is no virus on the computer of this instrument.

## 3.5 Equipment Installation

The following contents are convenient for users to quickly complete the installation of the FastGene® qFYR Real-Time PCR System. Please be sure to operate according to the following contents. If you have any questions during the installation operation, please contact our support.

### 3.5.1 Equipment Appearance

FastGene®
qFYR

### Status Lamp

There are power status light “POW” and system status light “STU” on the equipment´s top part. The meaning of status lights is shown in the following:

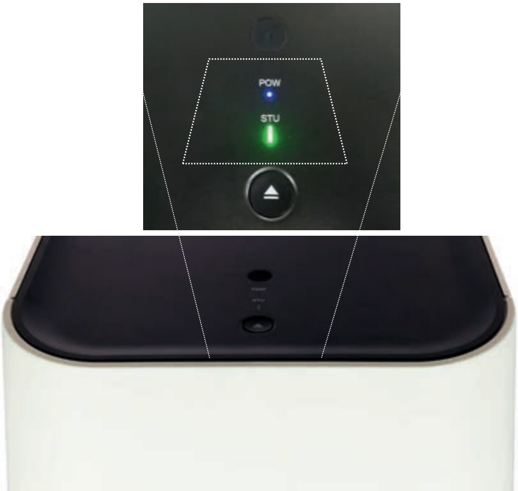
| Name | Status | Means |
|---|---|---|
| POW | Blue light | Ready |
| STU | Green light slowly flashing | Self-checking |
| | Green light quickly flashing | Working |
| | Green light on | In idle condition |
| | Yellow | Caution, able to continue working |
| | Red | Malfunction, stop working |

### Equipment switch

The device has a power switch in the back. Turn on the instrument by turning the switch to the "I" position and switch off at the "O" position.

### Accessing the Plate

The draw of the FastGene® qFYR Real-Time PCR System is controlled by the top button ⏏. The plate holder draw will open as soon as you press the top button. The in-and-out operation needs to be carried out in the idle state. It will not work during a PCR and during the self-inspection. The system does not respond to the in-and-out request.



**Data communication**

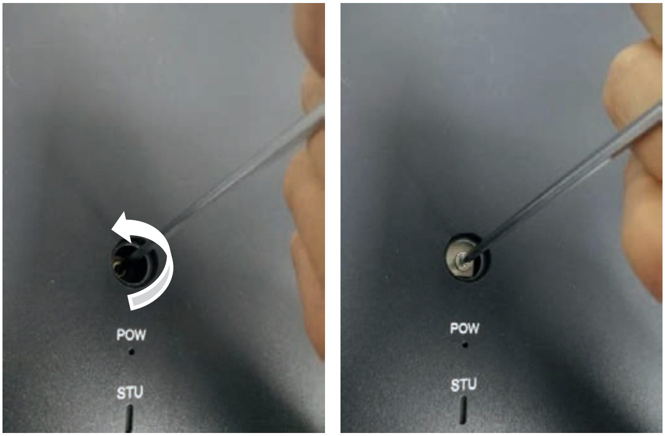
The interface of the device's back panel is the USB, which is used for communication between the computer and the device.

**Air inlet**

The lower gap of the front panel of the equipment is a set of air intakes. Blocking must be prevented when running. Pay special attention to this gap for air inlet, **it is strictly prohibited to access this area with your hands, as severe injuries can happen!**

### 3.5.2 Release Instrument Transport Lock

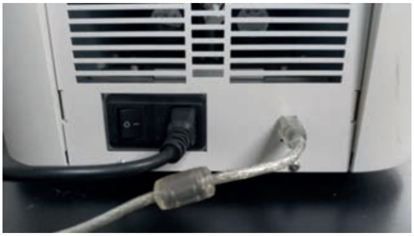
In order to avoid the damage of important parts of the instrument during transportation, a transport lock is added. While the instrument is switched off, find the locking hole at the top of the instrument and remove the soft rubber cap. Use the M4 hexagonal wrench to the inner bolt, press down slightly to the bottom and rotate 90 degrees counterclockwise. This will loosen the wrench, and the transport lock will pop up due to the of an internal spring mechanism of the instrument (as shown below).



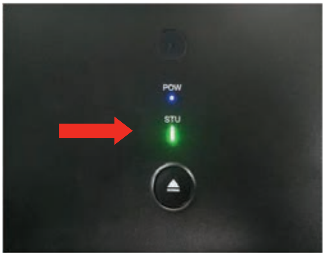
STU light status will change from slow flicker to green light on, unlocked successfully.

### 3.5.3 First Installation and Run Protocol

Insert the USB data cable and the power cable (as shown in figure A below) and connect data cable to the computer. After turning on the power supply, press the power switch at the back end of the instrument to turn on the instrument, and wait until the power indicator light (POW) is constantly on, and the system begins self-checking process. When the system self-test is completed, the instrument´s normal state lamp (STU) is green (as shown in figure B). Press the plate remove button and remove the support plate in the sample platform (as shown in figure C). The installation steps are completed.



A



B

C

## 3.6 Instrument Software Installation

### 3.6.1 FastGene® qFYR Analysis Studio Software Installation

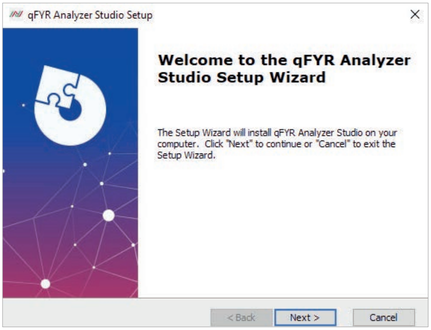
You need to install the FastGene® qFYR Analysis Studio software in your computer before using the FastGene® qFYR Real-Time PCR System. You can find the installation software in the provided USB-drive.

Please refer to the following steps to complete the installation process:

1. Double-click the Setup icon

qFYRAnalyzerStudioInstall-V1.0

2. Unzip file interface by clicking on "Extract" extract file
3. At the pop-up installation interface, click on next to continue

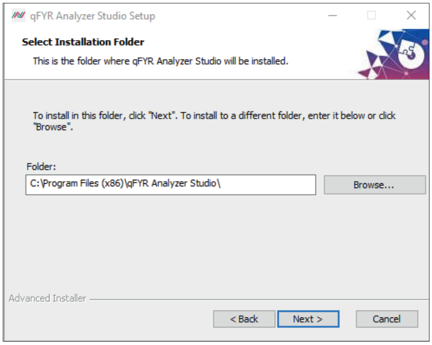


qFYR Analyzer Studio Setup
< Back
Next >
Cancel

**Welcome to the qFYR Analyzer Studio Setup Wizard**

The Setup Wizard will install qFYR Analyzer Studio on your computer. Click "Next" to continue or "Cancel" to exit the Setup Wizard.

4. Select the destination folder of the installation



qFYR Analyzer Studio Setup
Browse...
Advanced Installer
< Back
Next >
Cancel

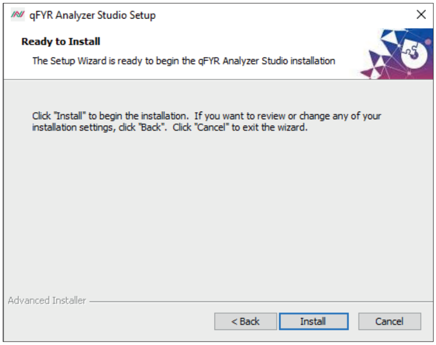
**Select Installation Folder**

This is the folder where qFYR Analyzer Studio will be installed.

To install in this folder, click "Next". To install to a different folder, enter it below or click "Browse".

Folder: C:\Program Files (x86)\qFYR Analyzer Studio\

5. Click install to complete the program installation



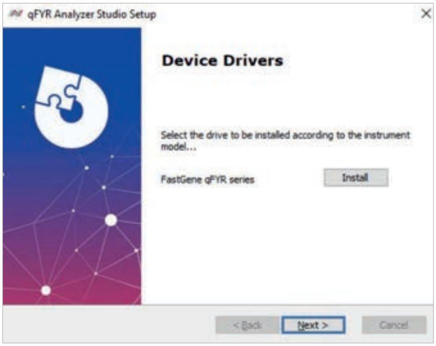
qFYR Analyzer Studio Setup
Advanced Installer
< Back
Install
Cancel

**Ready to Install**

The Setup Wizard is ready to begin the qFYR Analyzer Studio installation

Click "Install" to begin the installation. If you want to review or change any of your installation settings, click "Back". Click "Cancel" to exit the wizard.

6. You need to pre-install drivers in the system before using FastGene® qFYR Real-Time PCR System and the FastGene® qFYR Analysis Studio software. After installation of the FastGene® qFYR Analysis Studio software, please click on the install button in the window below:



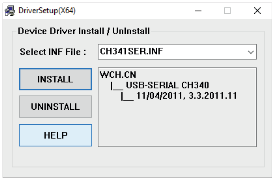
qFYR Analyzer Studio Setup
Install
< Back
Next >
Cancel

**Device Drivers**

Select the drive to be installed according to the instrument model...

FastGene qFYR series

7. Please click on install:



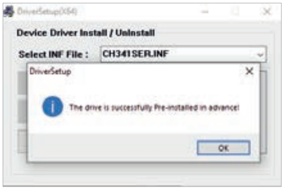
DriverSetup(X64)
INSTALL
UNINSTALL
HELP

Device Driver Install / Uninstall

Select INF File : CH341SER.INF

```
WCH.CN
  |__ USB-SERIAL CH340
     |__ 11/04/2011, 3.3.2011.11
```

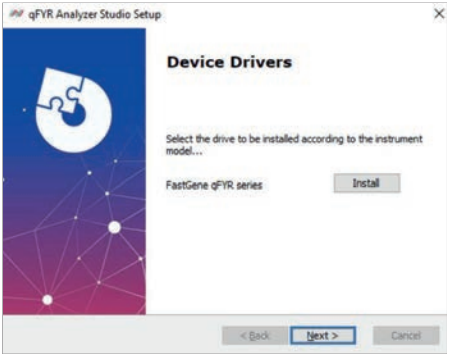
8. The installation will be confirmed:


DriverSetup(X64)
Device Driver Install / Uninstall
DriverSetup
OK

Select INF File : CH341SER.INF

The drive is successfully Pre-installed in advance!

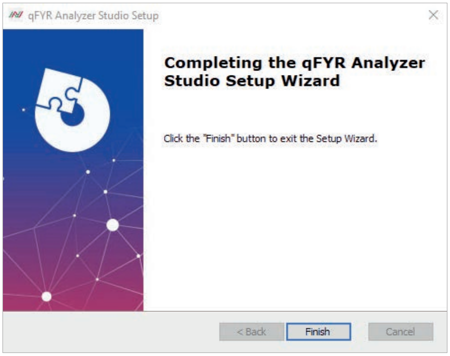
9. Please close the window of the driver installer and press Next on the window below:


qFYR Analyzer Studio Setup
Device Drivers
Install
< Back
Next >
Cancel

Select the drive to be installed according to the instrument model...

FastGene qFYR series

10. The installation is complete by clicking Finish


qFYR Analyzer Studio Setup
Completing the qFYR Analyzer Studio Setup Wizard
< Back
Finish
Cancel

Click the "Finish" button to exit the Setup Wizard.

## 3.7 Pre-preparation for boot

### 3.7.1 Instrument Self-inspection

This instrument has the function of self-inspection. Before use, the user should run the instrument´s self-inspection to ensure that the instrument can work normally during the experiment.

1. Turn the power switch on at the back of the instrument and turn on the instrument.
2. The instrument automatically enters the self-inspection mode: The automatic inspection includes the instrument version, whether the electricity is in normal working condition, whether the power supply is normal or not, and the position of the initializing motor, etc.
3. After the self-inspection, the top light will constantly show a green color. The instrument will be on standby until used.

**Note**: Make sure that instrument transport lock has been removed, the control computer and other external instruments have been correctly connected and that instrument´s power supply has been connected correctly.

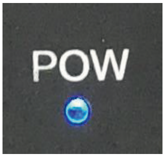
**Note:** In case of not passing the self-inspection, please turn the machine off and again on for self-inspection. If the instrument still cannot pass self-inspection, please contact the support.

**Do not shake the instrument.**

### 3.7.2 System Start-up

a) Start the computer

b) Turn on the power switch of the backboard of the instrument, and wait until the power status light POW is blue and always on



c) Wait for the system to self-check. During the self-check, the status light STU is green flashing state. After the successful self-check, green light will shine constantly.

### 3.7.3 Experimental Preparation

**Good laboratory practice for PCR and RT-PCR**

When preparing PCR or RT-PCR amplification samples：

☑ Use only clean personal protection equipment and lab coat

☑ Whenever you suspect that the gloves are contaminated, it is recommended to change them

☑ Set up separate areas and special equipment and supplies for the following purposes:

- Sample preparation and reaction settings
- Product amplification and analysis
- To avoid contamination, do not bring amplified products into the reaction setting area

☑ Carefully open and cap all sample tubes to avoid splashing or spraying samples

☑ Regularly clean the experimental table with proper disinfectants

☑ Use calibrated pipettes and anti-aerosol tips

☑ Prepare the reaction mixture according to the manufacturer's recommendations for the main mixture and the mixture to be tested

☑ Include excess volume in the calculation to provide excess volume of master mixes for losses during reagent transfer

☑ Dilute the sample and any standards with TE buffer or water

☑ Be careful when diluting samples and standards, errors or inaccuracies in the dilution process directly affect the accuracy of the data

☑ Place the diluent and assay mixture in the refrigerator away from light until they are ready for use. Overexposure may affect fluorescent probes or dyes

☑ Before use:

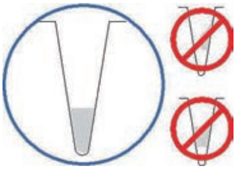
- Thoroughly mix the master mix by rotating the bottle
- Resuspend the assay mixture by vortexing and then centrifuge briefly.
- Thaw any frozen samples on ice. After thawing, resuspend the sample by vortexing, and then centrifuge the tube briefly.

**Guide to set reaction in plate or tube**

☑ Make sure that the liquid in each tube is at the bottom of the tube without bubbles. Otherwise centrifuge the well plate again

☑ Ensure that the plate or tube is properly sealed

☑ Keep the reaction plate or reaction tube in a dark environment at 4° C until it is ready to be loaded into the instrument



☑ Keep the bottom of the plate clean. Liquid and other contaminants on the bottom of the plate will contaminate the sample and cause abnormally high background signals

☑ If necessary, use permanent markers or pens to mark on the side of the test tube and plate, do not use highlighters

# Chapter 4 Operation to Run the Experiment

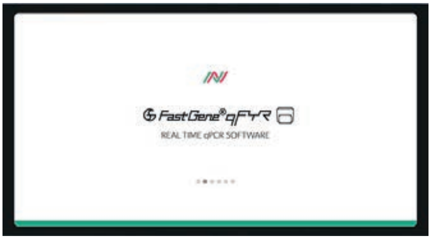
This section will describe in detail how to set up and run experiments using FastGene® qFYR Analysis Studio software.

## 4.1 Software Start

1. Double-click the desktop software icon to launch the software

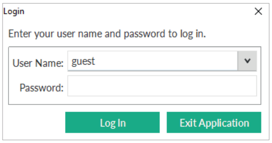
qFYR Analyzer Studio
V1.0

2. Interface of the software startup


FastGene® qFYR
REAL TIME qPCR SOFTWARE

3. User login

After the software starts, the system Login dialog box will automatically pop up (see below). Select the user by changing the “User Name”. If no user has been defined, you can proceed with “guest”. Click on the "Log In" button to start the software.



Login

Enter your user name and password to log in.

User Name: guest

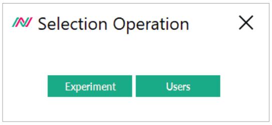
Password:

Log In
Exit Application

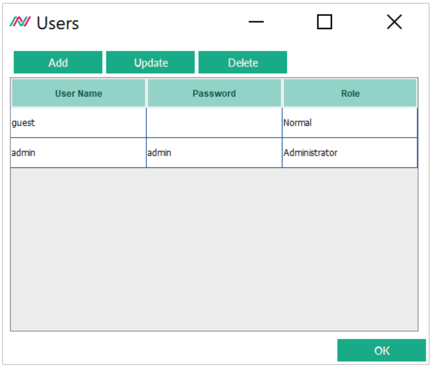
4. Adding a user

Log in as Administrator. Select “admin” in the User Name and enter the Administrator password. The default password is ***admin*** (Please let the administrator of the instrument change the password as soon as possible).

Select “Users” in the pop-up window.

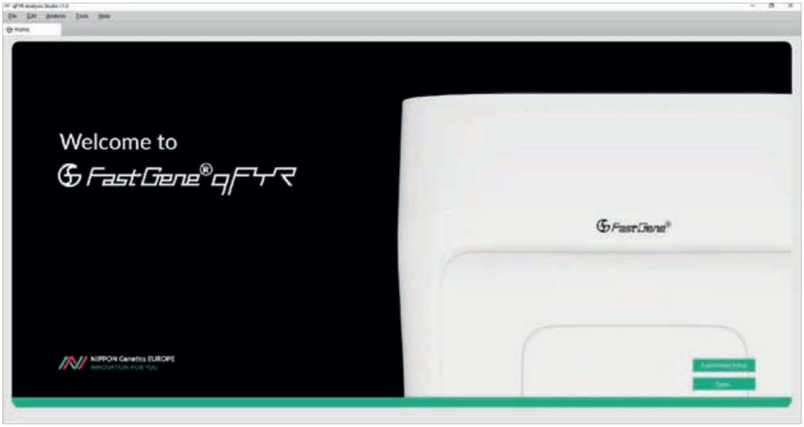

Selection Operation
Experiment
Users

The next window will let you add, edit (“Update”), or delete users.


Users
Add
Update
Delete
OK

| User Name | Password | Role |
|---|---|---|
| guest | | Normal |
| admin | admin | Administrator |

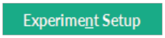
5. Startup interface will be shown:

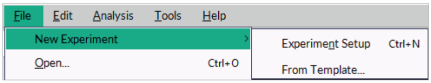

Welcome to
FastGene® qFYR
FastGene®
NIPPON Genetics EUROPE

## 4.2 Create New Experiment

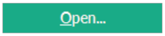
The Startup interface is used to create a new experiment, run, or open previously saved templates to create a new experiment.

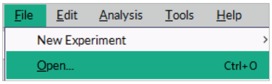
### 4.2.1 New Experiment or Loading existing files


Experiment Setup


File
Edit
Analysis
Tools
Help
New Experiment
Open...
Ctrl+O
Experiment Setup
Ctrl+N
From Template...

1. Click the "Experiment Setup" button at the bottom right of the startup interface or select "New Experiment" from the menu bar to start a new experiment setup.
2. Click "From template" in the menu bar to create a new experiment by loading a previously saved experiment template.
3. Click the "Open" button at the bottom right of the startup interface or select "Open" from the menu bar to open previously saved experiment or template files.




File
Edit
Analysis
Tools
Help
New Experiment
Open...
Ctrl+O

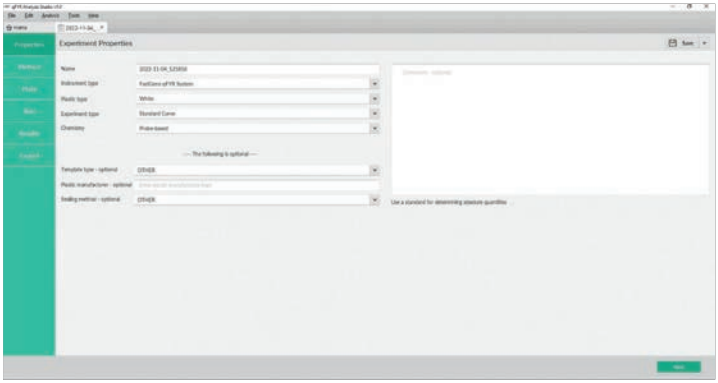
The file types are as follows:

a) “*.qpt” file is a template file for experiment operation and contains information about operating conditions and reaction plate settings.

b) “*.qps” file is a complete raw data file, including operating conditions, reaction plate settings and experimental results (see Chapter 5).

## 4.3 Experiment Properties

The "Experiment Properties" interface is used to define the relevant properties of the experiment according to the actual experiment. This includes the name and type of the experiment, as well as the selection of the reaction tube and PCR reagent.



Experiment Properties

Plastic type: White

Experiment type: Standard Curve

Chemistry: Probe-based

— The following is optional —

Template type - optional: OTHER

Plastic manufacturer - optional

Sealing method - optional: OTHER

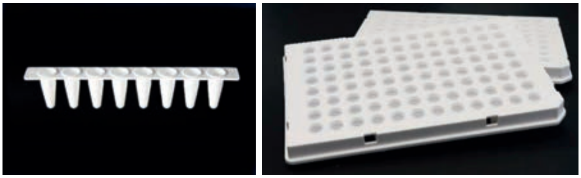
Use a standard for determining absolute quantities

### 4.3.1 Name

The default setting for the experiment name is the current time and date, which can be changed as needed. Special characters are not supported.

### 4.3.2 Plastic Type

The instrument is compatible with the common fluorescent quantitative reaction tube types, including 0.1 mL PCR single tube, 8-well strip, and 96-well plates. White tube or transparent tube (including frosted tubes) can be selected according to the reaction tube material. Reading of the wells will be adjusted according to the selected reaction tube type. Compared with transparent tubes, white tubes can reduce the loss of optical signal and crosstalk. Therefore, we recommend to use white opaque reaction tubes and plates to obtain better experimental data.



### 4.3.3 Experiment type

Select the experiment type according to the actual purpose of the experiment. The software will associate the subsequent experiment settings according to the selected experiment type. For the experiment type, please refer to the following table:

| Type of experiment | Explanation |
|---|---|
| **Standard Curve (Absolute quantification)** | Absolute quantification based on a standard curve is a real-time quantitative PCR analysis method suitable for researchers who need to determine the actual concentration or copy number of target genes. For absolute quantification, a template solution with a known concentration needs to be serially diluted several times. The obtained data is used to generate a standard curve. The standard curve is plotted with each concentration and corresponding Cq value. The Cq value of the unknown sample is then compared with this standard curve to determine its quantity. |
| **Relative Standard Curve or Comparative Cq (ΔΔCq) (Relative quantification)** | Relative quantification is suitable for most gene expression studies. It can analyze the up-regulation or down-regulation of target gene expression levels in control samples and one or more experimental samples. With this technique, there is no need to accurately determine the copy number, but to focus on the fold change compared to the control. The ΔΔCq method is a very common method of relative quantitative analysis. It compares the results of experimental samples including controls (such as untreated or wild-type samples) and normalization targets (such as housekeeping gene expression). Using this method, the Cq of the target gene in the same two samples can be compared based on the Cq of the normalization gene in the test sample and the control sample. The resulting ΔΔCq value can be used to determine the fold difference in expression. |
| **Melt curve or High Resolution Melt** | The melt curve experiment is used to determine the melting temperature (Tm) of the PCR product detected by an intercalating dye, such as SYBR™ Green dye. Gene sequences with different base compositions or lengths have different Tm, so different reaction products can be determined according to the Tm value, including non-specific product.<br>During a high-resolution melt analysis, the change in fluorescence is detected at very small temperature increase steps, allowing the identification of SNPs and mutations. For the detection of unknown or new mutations, saturated DNA combined with dyes is usually used to genotyping by high resolution melting curve. |
| **Genotyping** | Real-time fluorescence quantitative PCR can provide different methods for genotyping analysis. For the detection of known mutations, the most commonly used basic method is the end point genotyping using enzyme cleavage hydrolysis probe, and the more advanced method is the fusion curve genotyping using hybrid probe. The method can be easily improved and applied to the detection of various other SNP. |
| **Presence or Absence** | Protocol using a probe-based assay to control the presence or the absence of a target. |

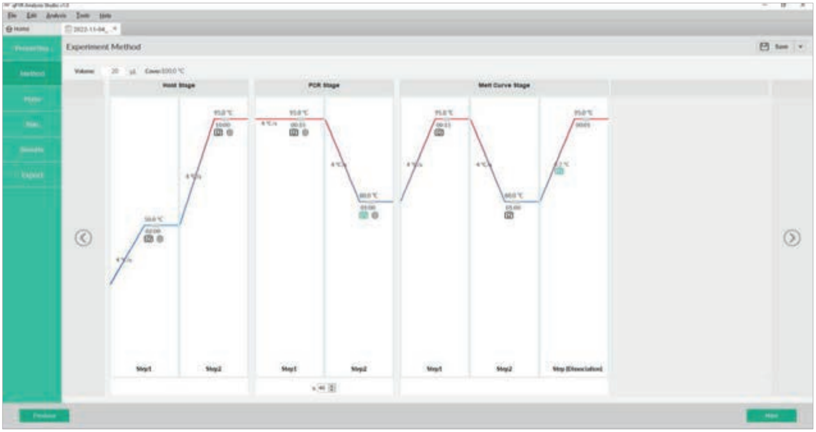
### 4.3.4 Chemistry

The instrument is compatible with most of the fluorescent quantitative PCR reagent consumables in the market. You can choose the probe method (TaqMan™ reagent) or the dye method (SYBR™ Green reagent) to carry out subsequent experiments according to the specific experimental design. For the comparison of the probe method and the dye method, please refer to the following table:

| Methods | Advantages | Disadvantage | Scope of application |
|---|---|---|---|
| **Dye-based Method** | Widely used<br>Sensitive<br>Convenience<br>Cheap | Difficult-to-design primers<br>Non-specific binding | Suitable for quantitative analysis of various target genes in scientific research, research of gene expression, research of transgenic animals |
| **Probe-based Method** | High specificity<br>Good repeatability | Expensive<br>Only suitable for specific targets | Pathogen detection, disease resistance gene research,<br>Drug efficacy assessment, diagnosis of genetic diseases |

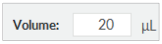
## 4.4 Experiment Method

The "Method" interface is used to set the specific parameters of a quantitative PCR, including reaction volume, stage, step, temperature, time, and cycle number, etc. The above parameters can be carried out according to specific experimental needs and corresponding reaction reagent requirements.


Experiment Method
Volume: 20 µL Cover:100.0 °C
Hold Stage
PCR Stage
Melt Curve Stage
50.0 °C
02:00
95.0 °C
60.0 °C
Step1
Step2
Step (Dissociation)
Save

### 4.4.1 Reaction volume

The default volume is 20 µL. Volumes starting from 1, up to 50 µL are supported.



**Volume:** 20 µL

### 4.4.2 Thermal lid temperature

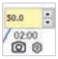
Thermal lid temperature is fixed to 100°C and cannot be changed.

**Cover:**100.0 °C

### 4.4.3 Basic setting

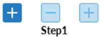
1. The values of time, temperature, heating rate (melting curve mode) can be set by entering a value in the value area or clicking the arrows ⬍. Temperature can additionally be changed by clicking and dragging ━ in the temperature curve.

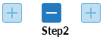
   *Note: The minimum value of the time setting is 00:01 minutes.*



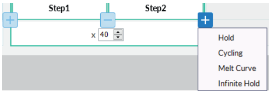
*The temperature is selected*

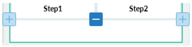
2. Light up the camera icon 📷 to select the data collection point
3. The camera is turned on by default to collect data after the extension is completed
4. If you need to add a reaction step in a certain stage of PCR, hover the mouse to the bottom left or right step area and click "+" to add a step before or after the current step. The "-" button removes the selected step.



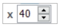


5. If you need to add a reaction stage of the PCR, hover the mouse to the bottom left or right stage area and click "+" to add a stage before or after the current stage and select the type of stage (Hold, Cycling, Melt Curve, Infinite Hold) that should be added. The "-" button removes the selected stage.

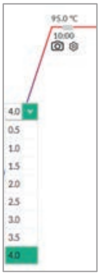

Step1
Step2
x 40
Hold
Cycling
Melt Curve
Infinite Hold


Step1
Step2

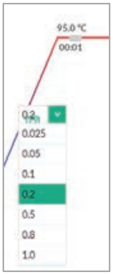
6. The number of cycles can be changed by entering a value in the value field or clicking the arrows. The standard number of cycles is 40.



7. The ramp rate can be set in the drop-down list at the beginning of each step. Ramp rate is given at temperature change within the sample volume and can be set between 0.5 and 4 C/s.

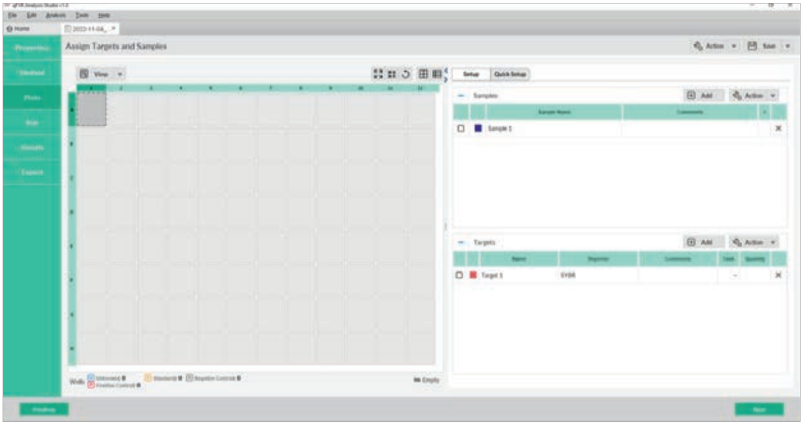

95.0 ℃
10:00
4.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0

8. The temperature interval can be set in the drop-down list on the camera icon during a melt curve analysis in which the fluorescence signal is measured. The smaller the value, the more data points are collected. Standard value is 0.2 °C/detection step.


95.0 ℃
00:01
0.2
0.025
0.05
0.1
0.2
0.5
0.8
1.0

## 4.5 Plate Setup

The “Plate Setup” interface is used to set the properties of the reaction wells, including assigning samples, test targets, reaction well tasks and other properties to each reaction well. This will make the identification of the sample and the subsequent analysis possible.

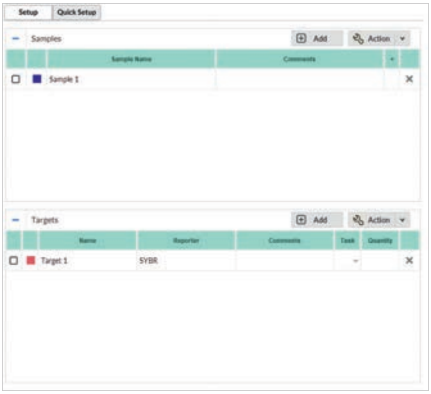

Assign Targets and Samples
Action
Save
View
Setup
Quick Setup
Samples
Add
Action
Sample 1
Targets
Target 1
SYBR

*Note: The plate setup can be performed before, during and after the experiment.*

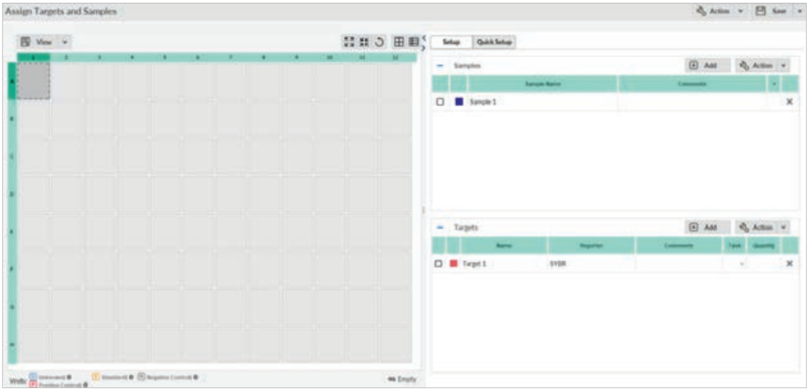
### 4.5.1 Setup

Define and assign well properties in the “Setup” window of the plate setup:

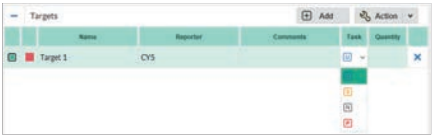
1. Click ⊞ Add, to add samples and target items


Setup
Quick Setup
Samples
Add
Action
Sample Name
Comments
Sample 1
Targets
Add
Action
Name
Reporter
Comments
Task
Quantity
Target 1
SYBR

2. Click on the right × to remove samples and targets items
3. In ⊞ (well plate view), select the reaction well according to the corresponding well position of the actual reaction tube


Assign Targets and Samples
Action
Save
View
Setup
Quick Setup
Samples
Add
Action
Sample Name
Comments
Sample 1
Targets
Add
Action
Name
Reporter
Comments
Task
Quantity
Target 1
SYBR
Wells
Empty

4. Assign the sample (nucleic acid source) and the target item (gene of interest) to the selected well position and click the text box of each field to name the sample and target gene for actual test.
5. When you enter a new sample or target item in the "Setup" window, the software will automatically fill the Reporter with default values (FAM/SYBR) and quenching groups (NFQ-MGB) and assign tasks (unknown). Change the dye or probe used accordingly. After the setting is completed, check the corresponding samples and target to assign the set attributes to the corresponding well positions selected previously.
6. Assign tasks in the window of the "Plate Setup" tab:


Targets
Add
Action
Name
Reporter
Comments
Task
Quantity
Target 1
CY5

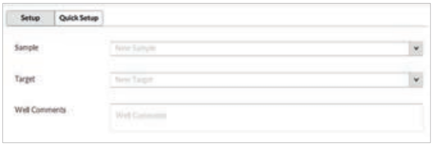
7. Select the reaction well in ⊞ (well plate view).
8. In the inspection item, select the check box of the inspection item, and then select the task from the drop-down list.

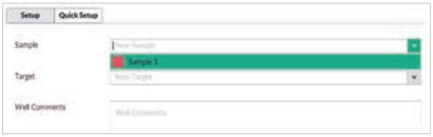
| Reaction type | Duty |
|---|---|
| Unknown (sample to be tested) | U |
| Negative control | N |
| Positive control | P |
| Standard | S |
| Homozygous alleles 1/1 | $^{1}{}_{1}$ |
| Homozygous alleles 2/2 | $^{2}{}_{2}$ |
| Heterozygous alleles 1/2 | $^{1}{}_{2}$ |
| Positive Control - IPC | I |
| Blocked IPC control | $\cancel{I}$ |

After the above settings are completed, assign the corresponding samples and targets items to the corresponding selected wells.

### 4.5.2 Quick Setup

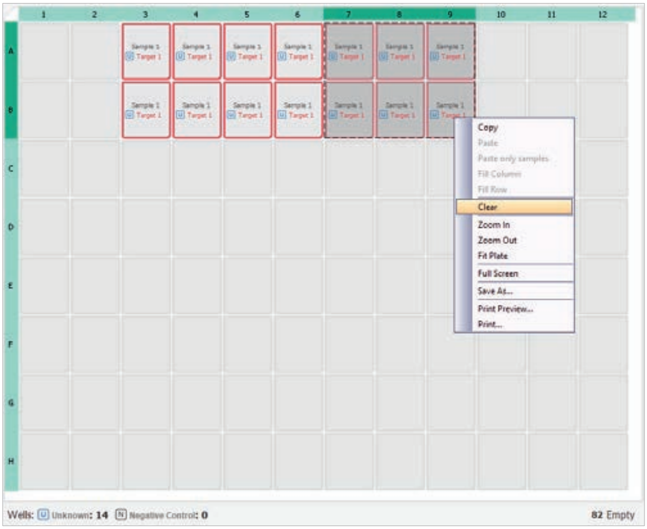
"Quick Setup" is a function module for quick and easy setup of samples and targets. After selecting the well position to be set, you can click the sample text box and target text box to manually enter the name. If settings have been made in the “Setup”, you can select the options defined in the “Setup” through the drop-down menu to assign attributes to the corresponding well positions.


Setup
Quick Setup
Sample
New Sample
Target
New Target
Well Comments
Well Comments


Setup
Quick Setup
Sample
New Sample
Sample 1
Target
New Target
Well Comments
Well Comments

### 4.5.3 Remove Wells from the Reaction

Select the wells to be removed, click the right mouse button, and select “Clear”. For multiple wells, drag select these wells, then click the right mouse button to select “Clear”.


1
2
3
4
5
6
7
8
9
10
11
12
A
B
C
D
E
F
G
H
Sample 1
U Target 1
Copy
Paste
Paste only samples
Fill Column
Fill Row
Clear
Zoom In
Zoom Out
Fit Plate
Full Screen
Save As...
Print Preview...
Print...

Wells: U Unknown: 14 N Negative Control: 0 82 Empty

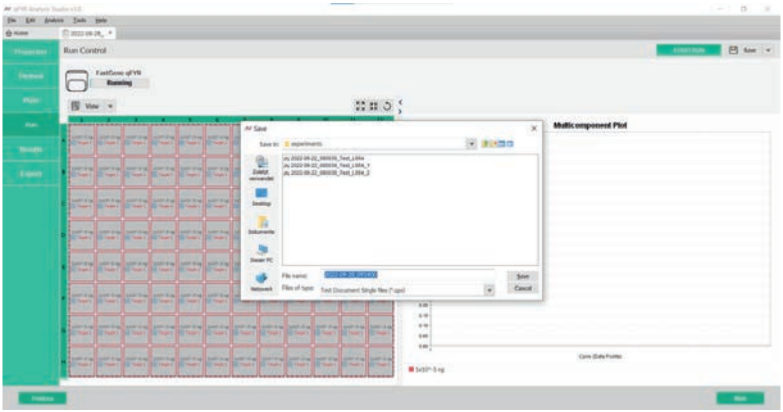
## 4.6 Run an Experiment

### 4.6.1 Insert a Plate or Reaction tubes

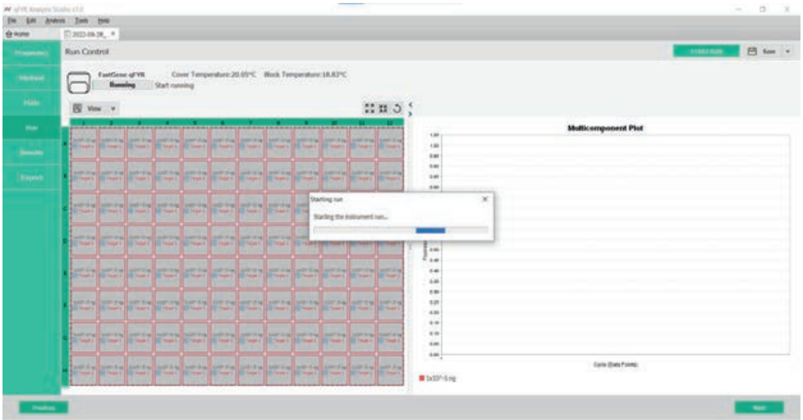
1. Press the access button on the top of the instrument.
2. The instrument´s heating block will be ejected.
3. Load the reaction tube or plate onto the 96-well block.
   *Note: When the drawer is closed, the heating lid will apply the appropriate pressure to securely place the tube band in the module.*
4. Press the access button again to close the heating block.
5. After the operation is completed, press the access button to eject the heating block, remove the reaction tube or plate from the heating block, and then close the door by pressing the access button.
   *Watch out! Risk of personal injury. During the operation of the instrument, the heating block can reach a temperature of 100 ° C. Allow it to cool down to room temperature before processing.*

### 4.6.2 Run the Experimental Program

1. In the "Run" tab of the desktop software, click "Start Run". After clicking "Start Run", a save dialog box appears, select the data file storage location and name.


Run Control
FastGene qFYR
Running
View
Multicomponent Plot
Save
Save in:
experiments
Desktop
Dokumente
Network
File name:
Files of type:
Save
Cancel

2. After saving, the instrument starts to run the program, and the real-time heating lid temperature and reaction plate temperature will be displayed in the upper left corner of the running interface.



Run Control

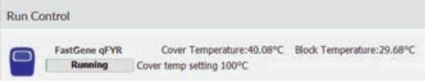
FastGene qFYR Cover Temperature:20.05°C Block Temperature:18.83°C

Running Start running

Starting run

Starting the instrument run...

File
Edit
Analysis
Tools
Help
Home
View
Multicomponent Plot
Fluorescence
Cycle (Data Points)

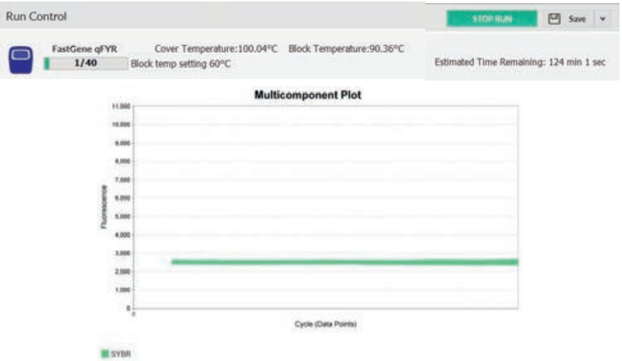


Run Control

FastGene qFYR Cover Temperature:40.08°C Block Temperature:29.68°C

Running Cover temp setting 100°C

3. When the temperature of the heating lid reaches 100 ° C, the real-time heating lid´s and reaction plate´s temperature, and the cycle number of the reaction will be displayed in the upper left corner of the running interface.



Run Control

FastGene qFYR Cover Temperature:100.04°C Block Temperature:90.36°C

1/40 Block temp setting 60°C

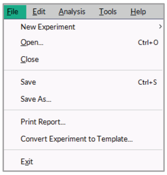
Estimated Time Remaining: 124 min 1 sec

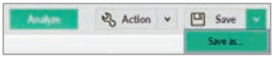
STOP RUN
Save
Multicomponent Plot
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
Fluorescence
Cycle (Data Points)
SYBR

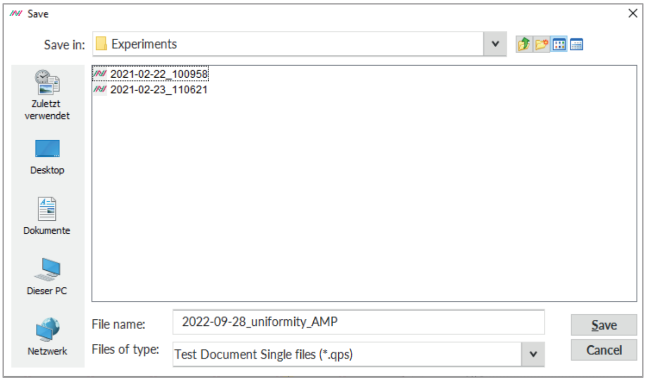
***Note:*** *In the initial reaction cycles, the original data map is most of the background or baseline. When the amplification reaction enters the exponential growth phase, the curve will rise.*

## 4.7 Save Experiment

Click the "File" option in the menu bar, choose “Save” or “Save as” to save the original experimental data. At each step of the experiment process, you can choose to save the experiment in the upper right corner of the interface. The extension of the experiment file saved before running is .qpt (experiment running template). The extension of the experiment file saved after the operation is completed, is .qps (original data file), which contains all the original operation data and analysis results.


File
Edit
Analysis
Tools
Help
New Experiment
Open...
Ctrl+O
Close
Save
Ctrl+S
Save As...
Print Report...
Convert Experiment to Template...
Exit


Analyze
Action
Save
Save as...


Save
Zuletzt verwendet
Desktop
Dokumente
Dieser PC
Netzwerk
Save
Cancel

Save in: Experiments

2021-02-22_100958
2021-02-23_110621

File name: 2022-09-28_uniformity_AMP

Files of type: Test Document Single files (*.qps)

# Chapter 5 Operation of Different Experiment Types

FastGene® qFYR Real-Time PCR System experiments are divided into seven steps:

1. Create an experiment
2. Set Experimental Properties
3. Set the operating conditions
4. Set the samples and targets
5. Start the experiment
6. View and analyze the results
7. Export the experimental results

Please see Chapter 4 for how to create, run, analyze, and review an experiment. The next chapter explains how to operate the different types of experiments within the software.

## 5.1 Absolute Quantification (Standard Curve) Experiment

Absolute quantification is a real-time fluorescence quantitative PCR analysis method suitable for researchers who need to determine the actual copy number of targets. To carry out absolute quantification, the target template solution with known concentration should be diluted several times continuously, amplified by real-time fluorescence quantitative PCR, and a standard curve is generated by using the obtained data. A standard curve is drawn with the concentration of each target and the corresponding Cq value. Cq values of unknown samples are then compared with this standard curve to determine their concentration or copy number.

The standard curve method is used for absolute quantification, and the template standard with known concentration or quantity is required, which means that the template should be accurately quantified before the curve is generated. Quantitative accuracy is directly related to the quality of the standard curve. The template used to generate the standard curve and the method used to quantify the template are the basis of the experiment. The accuracy of continuous dilution is very important, and the RT and PCR efficiency of target template and continuous dilution of actual samples are very important.

Although you may need uniform pure templates for initial copy number determination and standard curve generation, it is best to use target templates as similar as possible to experimental samples. Since steps such as nucleic acid extraction and reverse transcription can affect the dynamic range of the reaction, the same processing steps as the experimental samples should be adopted.

In the standard curve experiment:

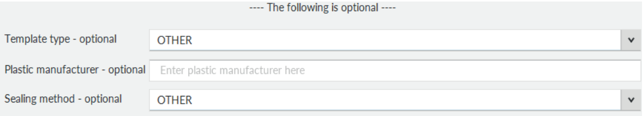
- Software generates standard curves using data from standard dilution series.
- Software uses standard curves to calculate the number of unknown samples.

Type of response:

- Many samples can be analyzed in standard curve experiments, but each sample needs its own standard curve.
- The following table lists the types of sample settings involved in the standard curve experiment:

| Type Setting | Example Description |
|---|---|
| Standard | contains a known number of target samples |
| Unknown | sample to be tested |
| No template control (NTC) | water or buffer replacement template, no target amplification should occur in NTC hole |

The accuracy of quantitative experiments increases with the increase of repeated reactions (usually containing at least three repeats). For accurate detection, at least five dilution points in a wide range of standard quantities should be prepared. The range of the standard quantity can be adjusted according to the expected level of presence of the gene.



---- The following is optional ----

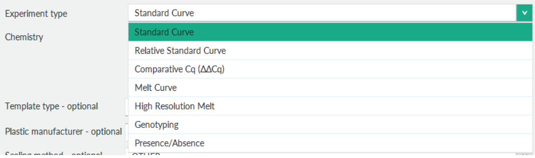
Template type - optional: OTHER

Plastic manufacturer - optional: Enter plastic manufacturer here

Sealing method - optional: OTHER

### 5.1.1 Setup of a Standard curve

1. Select experiment type “Standard Curve”.



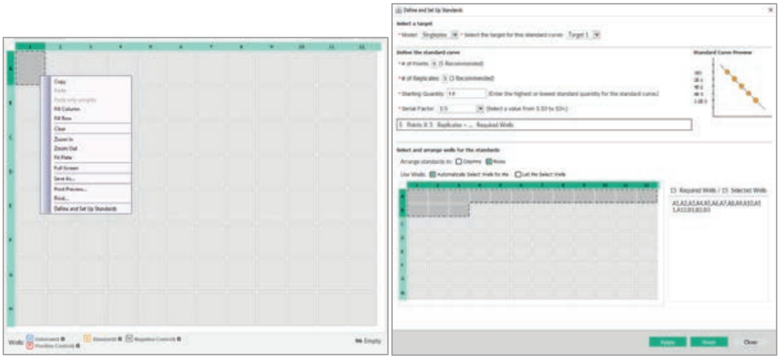
Experiment type: Standard Curve

Chemistry
Standard Curve
Relative Standard Curve
Comparative Cq (ΔΔCq)
Melt Curve
Template type - optional
High Resolution Melt
Plastic manufacturer - optional
Genotyping
Presence/Absence

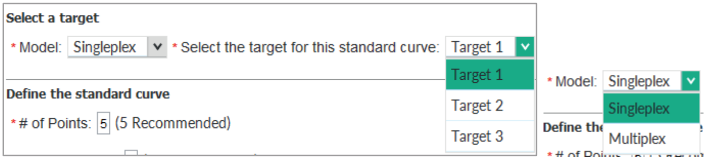
2. Choose the used Plastic type and Chemistry.
3. Add name and optional information.
4. After the Method setting is completed, enter the Plate section, or click Next in the lower right corner of the page to enter the well plate setting interface.

   ***Note**: The plate setup can be set before, during, or after the reaction operation.*

5. Right-click in any area of the plate view and select "Define and Set Up standards".



6. Select the target, which should be used for generation of the standard curve.



**Select a target**

* Model: Singleplex * Select the target for this standard curve: Target 1

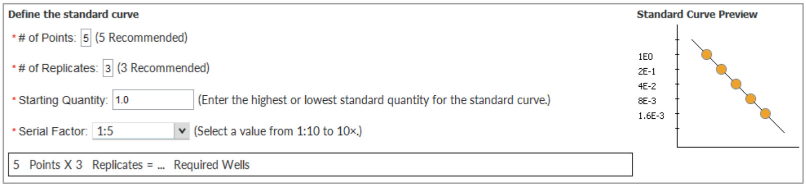
**Define the standard curve**

* # of Points: 5 (5 Recommended)

* Model: Singleplex

***Note:*** *Singleplex means that there is only one gene to be tested in each reaction well, and Multiplex means that there are two or more genes to be tested in each well.*

7. Set the number of points and replicates, dilution factor and starting quantity according to your experiment.



**Define the standard curve**

* # of Points: 5 (5 Recommended)

* # of Replicates: 3 (3 Recommended)

* Starting Quantity: 1.0 (Enter the highest or lowest standard quantity for the standard curve.)

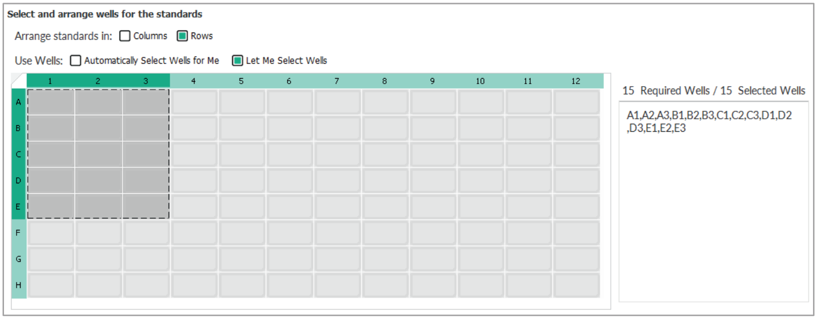
* Serial Factor: 1:5 (Select a value from 1:10 to 10×.)

5 Points X 3 Replicates = ... Required Wells

The parameters of the standard curve can be set according to the specific experimental requirements and conditions:

- Standard concentration dilution gradient: recommended at least five different dilutions.
- Three replicates per dilution are recommended.
- Starting Quantity: Enter the maximum or minimum quantity value of the standard, excluding units (units containing copies, copies/µL or ng/µL) and the dilution factor.

8. Select and arrange standard wells.



**Select and arrange wells for the standards**

Arrange standards in: ☐ Columns ☑ Rows

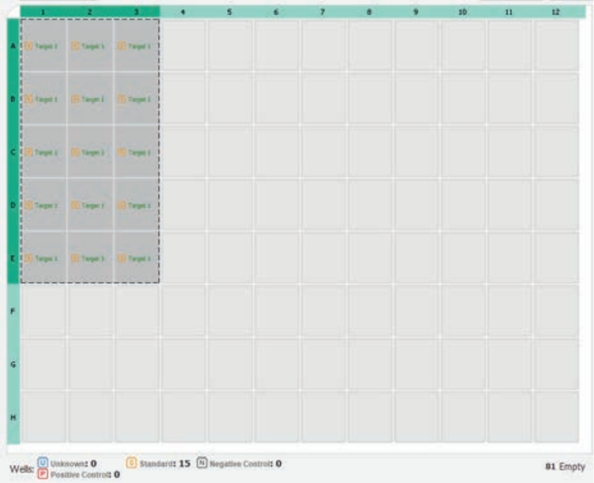
Use Wells: ☐ Automatically Select Wells for Me ☑ Let Me Select Wells

15 Required Wells / 15 Selected Wells

A1,A2,A3,B1,B2,B3,C1,C2,C3,D1,D2,D3,E1,E2,E3

- The software can automatically select the reaction well by columns or rows or the operator can select their own reaction well according to the need and arrange the well position of the standard product.
- After the standard well position distribution is set, the software will arrange the standard well position according to the above setting, and display the corresponding well position arrangement and the number of wells.

9. The software will automatically apply the above settings to the plate setup, and automatically define the above well position as the standard property (as shown below), so that the plate setup of the standard is completed.

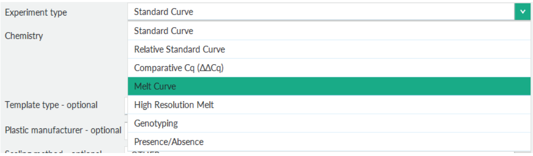


Wells: U Unknown: 0 P Positive Control: 0 S Standard: 15 N Negative Control: 0 81 Empty

## 5.2 Experiment of Melting Curve

### 5.2.1 Setup of Melt Curve Analysis

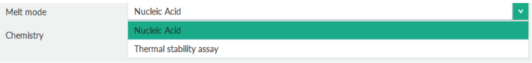
1. Select experiment type “Melt Curve”.


Experiment type
Standard Curve
Chemistry
Standard Curve
Relative Standard Curve
Comparative Cq (ΔΔCq)
Melt Curve
Template type - optional
High Resolution Melt
Plastic manufacturer - optional
Genotyping
Presence/Absence

2. Choose the used Plastic type and Chemistry.
3. Add name and optional information.
4. After the Method setting is completed, enter the Plate section, or click Next in the lower right corner of the page to enter the well plate setting interface.

   ***Note**: The plate setup can be set before, during, or after the reaction operation.*

5. Melting Mode:
   a. For analysis of nucleic acids, select “Nucleic Acid” here
   b. For analysis of protein thermostability, select “Thermal stability assay” here


Melt mode
Nucleic Acid
Chemistry
Nucleic Acid
Thermal stability assay

## 5.3 Relative Quantification Experiment（ΔΔCq）

Relative quantification is suitable for most gene expression studies and can analyze the up- or down-regulation of target gene expression levels in calibrated (normal) samples and one or more experimental samples. Using this technology does not require accurate determination of copy number but focuses on multiple changes compared with calibration samples. ΔΔCq method is a very common method of relative quantitative analysis, which can be used to compare the results of experimental samples, including calibrators (such as untreated or wild-type samples) and standard products (such as housekeeping gene expression), which can be used. Using this method, the target gene in the same two samples can be compared according to the Cq of the standard (normal) gene in the test sample and the calibration sample Cq, and the ΔΔCq value obtained can be used to determine the multiple difference of expression.

Relative quantitative experiments are often used to:

*Compare gene expression levels in different tissues.

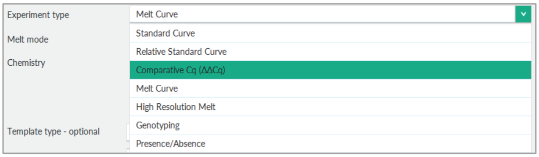
*Compare the expression level samples of genes in treated and untreated samples.

*Compare the expression levels of genes of interest in different genetic backgrounds.

*Analysis of the conditions of gene expression over time under specific treatment.

### 5.3.1 Setup of comparative Cq (ΔΔCq) Analysis

1. Select experiment type “Comparative Cq (ΔΔCq)”.


Experiment type
Melt mode
Chemistry
Template type - optional
Melt Curve
Standard Curve
Relative Standard Curve
Comparative Cq (ΔΔCq)
Melt Curve
High Resolution Melt
Genotyping
Presence/Absence

2. Choose the used Plastic type and Chemistry.
3. Add name and optional information.
4. After the Method setting is completed, enter the Plate section, or click Next in the lower right corner of the page to enter the well plate setting interface.

   ***Note**: The plate setup can be set before, during, or after the reaction operation.*

## 5.4 Genotyping Experiments

In the study of gene variation, in order to obtain a large number of characteristic markers, a large number of individuals must be genotyped. The reasons for the differences in individual genomes at different levels include single nucleotide polymorphisms (SNP) and DNA covalent modification. more than 90% of the gene sequence variants that cause individual differences belong to single nucleotide polymorphisms (SNP). SNPs mainly refer to DNA sequence polymorphisms caused by mutations at the nucleotide level of the genome frequency of at least one allele in the population is not less than 1%, including conversion of single base, transversion and insertion and deletion of single base: in addition, changes in DNA covalent bond modifications, such as DNA methylation.

Real-time fluorescence quantitative PCR can provide different methods for genotyping analysis. For the detection of known mutations, the most commonly used basic method is the end point genotyping using enzyme cleavage hydrolysis probe, and the more advanced method is the fusion curve genotyping using hybrid probe. For the detection of unknown or new mutations, saturated DNA combined with dyes is usually used to genotyping by high resolution melting curve. In this chapter we will introduce the method of allele analysis of known mutation sites using the TaqMan$^{TM}$ probe endpoint method. The method can be easily improved and applied to the detection of various other SNP.

Common genotyping applications:

*Genetic identification of genetic diseases.

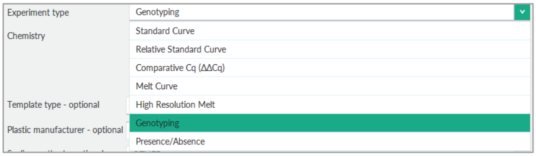
* Identification of tumor susceptibility genes and hotspot mutations.

*Identification of pathogen gene mutations and drug resistance.

*Animal and plant forensics, breeding research.

### 5.4.1 Set Up of a Genotyping analysis

1. Select experiment type “Genotyping”.



Experiment type: Genotyping

2. Choose the used Plastic type and Chemistry.

   ***Note**: Genotyping experiments can only be performed using probe-based chemistry.*

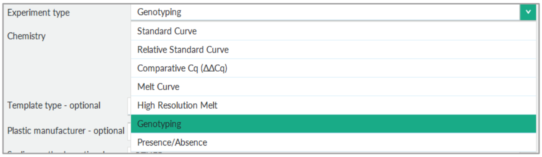
3. Add name and optional information.

4. After the Method setting is completed, enter the Plate section, or click Next in the lower right corner of the page to enter the well plate setting interface.

**Note**: *The plate setup can be set before, during, or after the reaction operation.*

### 5.4.2 Set Up of a High-Resolution Melt Analysis (HRM)

1. Select experiment type "High-Resolution Melt".


Experiment type
Genotyping
Chemistry
Template type - optional
Plastic manufacturer - optional
Standard Curve
Relative Standard Curve
Comparative Cq (ΔΔCq)
Melt Curve
High Resolution Melt
Genotyping
Presence/Absence

2. Choose the used Plastic type and Chemistry.

   **Note**: *Genotyping experiments can only be performed using dye-based chemistry. Please use an HRM-specific saturating dye.*

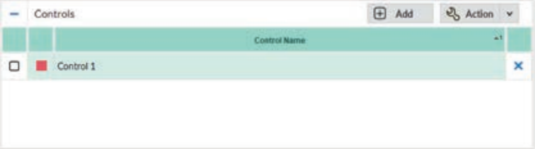
3. Add name and optional information.

4. After the Method setting is completed, enter the Plate section, or click Next in the lower right corner of the page to enter the well plate setting interface.

   **Note**: *The plate setup can be set before, during, or after the reaction operation.*

5. For HRM experiments controls can be defined during the plate setup (optional).

   Click ⊞ Add to add controls and select wells to assign controls similar to assigning samples and targets


Controls
Add
Action
Control Name
Control 1

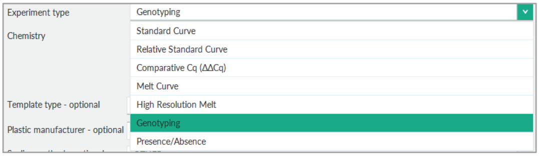
## 5.5 Presence / Absence Experiments

Presence / Absence experiments are endpoint experiments that can be performed to determine whether a target is present or absent in a sample. This type of experiment can be used for the detection of pathogens.

Presence / Absence experiments can only be performed using a probe-based assay and data is collected at the end. The amount of accumulated target is quantified for each sample. For quantification, the normalized intensity of the reporter dye (Rn) is used and displayed.

### 5.5.1 Set Up of a Presence / Absence analysis

1. Select experiment type "Presence/Absence".



Experiment type: Genotyping
Chemistry
Template type - optional
Plastic manufacturer - optional

Standard Curve
Relative Standard Curve
Comparative Cq (ΔΔCq)
Melt Curve
High Resolution Melt
Genotyping
Presence/Absence

2. Choose the used Plastic type and Chemistry.

   ***Note***: *Presence/Absence experiments can only be performed using probe-based chemistry.*
3. Add name and optional information.
4. After the Method setting is completed, enter the Plate section, or click Next in the lower right corner of the page to enter the well plate setting interface.

   ***Note***: *The plate setup can be set before, during, or after the reaction operation.*
5. For Presence / Absence experiments four types of tasks can be applied to the plate setup: U Unknown-IPC (well contains unknown sample and IPC (internal positive control)), I positive control (well contains a positive control and IPC), N NC (negative control)-IPC (well contains no-template control and IPC) and ☒NC-blocked IPC (well contains no-template control and IPC blocking agent).

# Chapter 6 Result Analysis and Data Export

This chapter explains the data overview, analysis, and export.

## 6.1 Result Tab

### 6.1.1 Buttons in Toolbar

| Button | Name | Function |
|---|---|---|
| ⤢ | zoom in | Zoom in for plate overview and curves |
| ⤡ | zoom out | Zoom out for plate overview and curves |
| ↻ | Return | return to last step |
| ⊞ | Plate view | Plate overview for results |
| ▤ | List | Experiment results by list |
| 🖨 | Send to printer | Print current window image file |
| ⎘ | Copy | Copy current window as image file |
| 💾 | Save as file | Save current window as file<br>(.jpg, .svg, .png, .emf, .pdf) |
| ⚙ | Plot Properties | Plot properties set up |
| ▭ | Hide plot result | Hide plot result |
| ▤ | Display plot result | Display plot result |
| 🔍 | Show plot settings | Show plot settings |

### 6.1.2 Other Toolbars

| Toolbar | Name | Function |
|---|---|---|
| Analyze | Analyze | Click to analysis result after changing plate or plot settings |
| Action ⌄ | Action | Click to select sample or target |
| Save ⌄ | Save | Save current results as .qps file |
| View ⌄ | View | View current results by plate or list format |
| Group by ⌄ | Group by | Click to show results by different groups |
| ‹<br>› | Expand/Shrink | Expand/Shrink plate or image view |

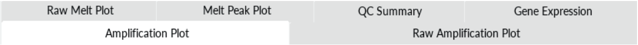
## 6.2 Result Overview

FastGene® qFYR Analysis Studio will analyze experiment results by default settings after an experiment is finished, and show amplification plot in the Result tab.

***Attention:*** *After omitting wells or changing any settings, please click* Analyze *to obtain new results.*

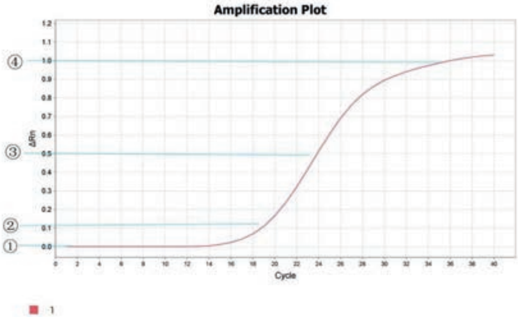
### 6.2.1 Amplification plot

1. Enter the Amplification plot tab to view the amplification curve.


Raw Melt Plot
Melt Peak Plot
QC Summary
Gene Expression
Amplification Plot
Raw Amplification Plot

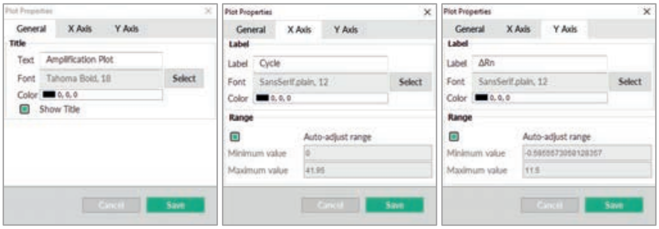
2. Overview amplification curve and evaluate curve shape. A typical amplification curve includes four different parts:

①Baseline stage ②Exponential stage ③Linear stage ④Plateau stage



### 6.3.1 Plot Properties

Select [icon], open the Plot Properties settings. In the "General"," X Axis"," Y Axis" tab, you can adjust the title, name, font, color, range and much more, as shown below. Select "Save" if any adjustment is made. The adjusted curve will be shown after saving.


Plot Properties
General
X Axis
Y Axis
Select
Cancel
Save

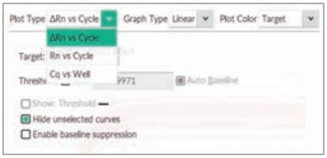
Title
Text: Amplification Plot
Font: Tahoma Bold, 18
Color: 0, 0, 0
☑ Show Title

Label
Label: Cycle
Font: SansSerif.plain, 12
Color: 0, 0, 0
Range
☑ Auto-adjust range
Minimum value: 0
Maximum value: 41.95

Label
Label: ΔRn
Font: SansSerif.plain, 12
Color: 0, 0, 0
Range
☑ Auto-adjust range
Minimum value: -0.5856573058128357
Maximum value: 11.5

### 6.2.2 Plot Settings

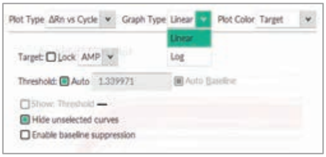
1. Select to enter the Plotting Settings and to adjust plot type, graph type, threshold etc.


Plot Type: ΔRn vs Cycle
Graph Type: Linear
Plot Color: Target

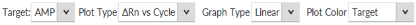
2. Use the drop-down menu to choose the Plot Type. The default setting is “Rn vs cycle”, but “△Rn vs cycle”, “Cq vs well” are available as well.

| Plot Type | Description | Purpose |
|---|---|---|
| Rn vs cycle | Rn value is the normalized fluorescence value. | Recognize and checking unnormal amplification well. |
| Cq vs well | Cq value is the cycle number which fluorescence signal achieved threshold. | Recognize the unnormal amplification well. |

3. Graph Type can be changed from “Linear” to “Log” by using the drop-down list. The default setting is “Linear” graph.

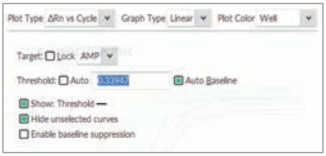

Plot Type: ΔRn vs Cycle
Graph Type: Linear
Plot Color: Target
Target: ☐ Lock AMP
Threshold: ☑ Auto 1.339971
☑ Auto Baseline
☐ Show: Threshold
☑ Hide unselected curves
☐ Enable baseline suppression

***Note:*** *Plot settings can also be adjusted using the tool bar above the graph.*


Target: AMP
Plot Type: ΔRn vs Cycle
Graph Type: Linear
Plot Color: Target

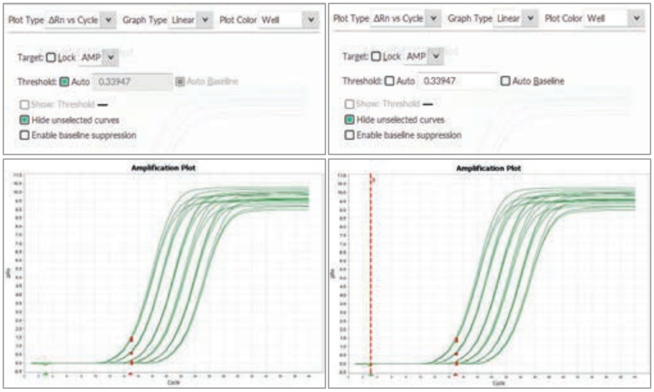
### 6.2.3 Threshold Settings

The software will calculate the threshold value by an automatic model. However, it can still be adjusted by unchecking “Auto” and changing the value. Additionally, the threshold line in the graph can be dragged. The new Cq value will be automatically calculated and displayed after the threshold value is adjusted. The threshold line will not be displayed if “Show Threshold” is not selected.


Plot Type: ΔRn vs Cycle
Graph Type: Linear
Plot Color: Well
Target: ☐ Lock AMP
Threshold: ☐ Auto 0.33947
☑ Auto Baseline
☑ Show: Threshold
☑ Hide unselected curves
☐ Enable baseline suppression

### 6.2.4 Baseline Settings

The software automatically sets the baseline in the first few cycles of the reaction, usually withing 3 to 15 cycles. If you need to set the baseline manually, remove the automatic threshold check, you can drag and drop the baseline (green triangle) directly on the curve and change it within the 1st to 15th cycle. In case of an early Cq value, it is suggested that the baseline should be set in the straight area of the curve, and when the baseline setting range is too wide, fluorescence can be detected in the 10th cycle, which may cause a curve drop and Cq delay.

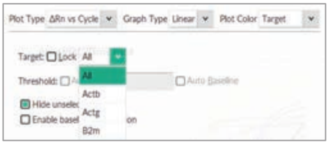

Plot Type ΔRn vs Cycle   Graph Type Linear   Plot Color Well
Target: ☐ Lock AMP
Threshold: ☑ Auto 0.33947 ☑ Auto Baseline
☐ Show Threshold
☑ Hide unselected curves
☐ Enable baseline suppression

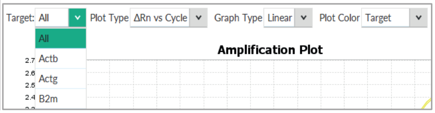
Plot Type ΔRn vs Cycle   Graph Type Linear   Plot Color Well
Target: ☐ Lock AMP
Threshold: ☐ Auto 0.33947 ☐ Auto Baseline
☐ Show Threshold
☑ Hide unselected curves
☐ Enable baseline suppression

***Note:*** *Please do not forget to click* Analyze *after a baseline setting is changed.*

### 6.2.5 Target Settings

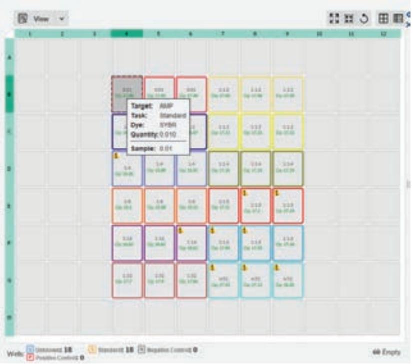
You can select different targets, or all targets to be shown:


Plot Type ΔRn vs Cycle   Graph Type Linear   Plot Color Target
Target: ☐ Lock All


Target: All   Plot Type ΔRn vs Cycle   Graph Type Linear   Plot Color Target

### 6.2.6 Results as Reaction Board

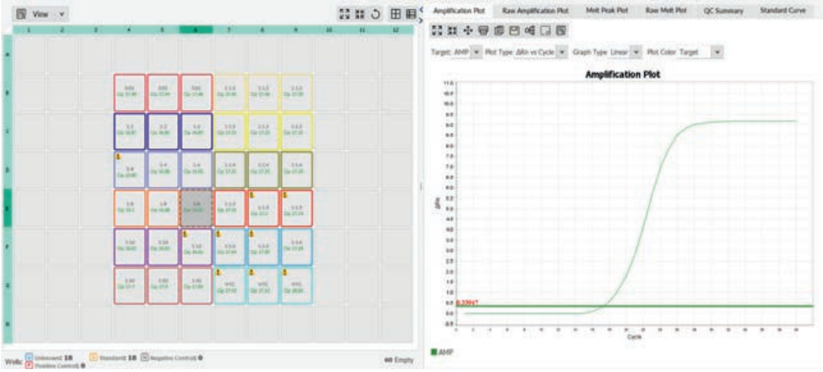
To view the results in plate format, click ⊞.


View

Target: AMP
Task: Standard
Dye: SYBR
Quantity: 0.010
Sample: 0.01

Wells
Unknown 18
Standard 18
60 Empty

In the plate view, the information of each well (incl. target, task, dye, sample) will be showed while the mouse is moved to and paused on each well. E.g., information of well B4 as in figure above.


View
Amplification Plot
Raw Amplification Plot
Melt Peak Plot
Raw Melt Plot
QC Summary
Standard Curve
Amplification Plot
ΔRn
Cycle
AMP
Wells
Unknown 18
Standard 18
60 Empty

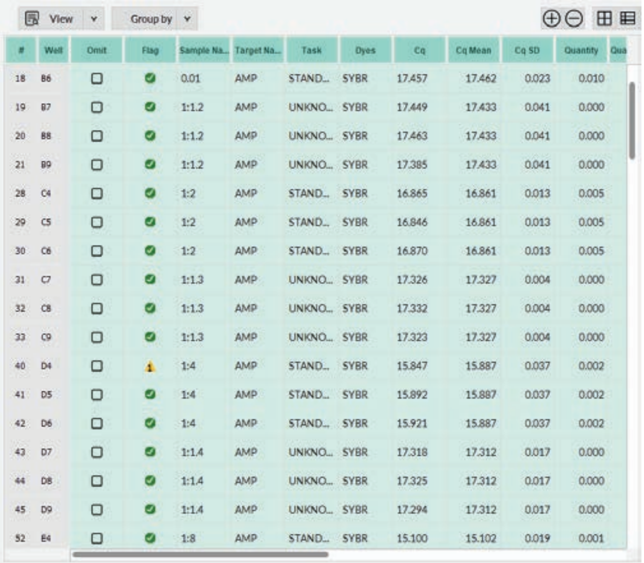
Target: AMP
Plot Type: ΔRn vs Cycle
Graph Type: Linear
Plot Color: Target

If a different well is selected, the amplification plot will be shown accordingly. E.g., the amplification curve of well E6 is shown after selecting well E6 in the plate format, as in the figure above.

Click buttons of Zoom in ⛶, Zoom out ⛶, Return to original size ↻, to change the plate layout.

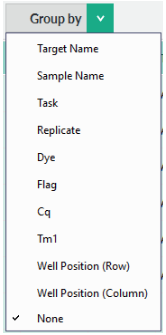
### 6.2.7 Results as List

To display the results as list, click ▤.
All the experiment information is listed, including well, sample, target, task, Cq, Cq mean, Cq SD etc.



| # | Well | Omit | Flag | Sample Na... | Target Na... | Task | Dyes | Cq | Cq Mean | Cq SD | Quantity | Qua |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | B6 | ☐ | ✓ | 0.01 | AMP | STAND... | SYBR | 17.457 | 17.462 | 0.023 | 0.010 | |
| 19 | B7 | ☐ | ✓ | 1:1.2 | AMP | UNKNO... | SYBR | 17.449 | 17.433 | 0.041 | 0.000 | |
| 20 | B8 | ☐ | ✓ | 1:1.2 | AMP | UNKNO... | SYBR | 17.463 | 17.433 | 0.041 | 0.000 | |
| 21 | B9 | ☐ | ✓ | 1:1.2 | AMP | UNKNO... | SYBR | 17.385 | 17.433 | 0.041 | 0.000 | |
| 28 | C4 | ☐ | ✓ | 1:2 | AMP | STAND... | SYBR | 16.865 | 16.861 | 0.013 | 0.005 | |
| 29 | C5 | ☐ | ✓ | 1:2 | AMP | STAND... | SYBR | 16.846 | 16.861 | 0.013 | 0.005 | |
| 30 | C6 | ☐ | ✓ | 1:2 | AMP | STAND... | SYBR | 16.870 | 16.861 | 0.013 | 0.005 | |
| 31 | C7 | ☐ | ✓ | 1:1.3 | AMP | UNKNO... | SYBR | 17.326 | 17.327 | 0.004 | 0.000 | |
| 32 | C8 | ☐ | ✓ | 1:1.3 | AMP | UNKNO... | SYBR | 17.332 | 17.327 | 0.004 | 0.000 | |
| 33 | C9 | ☐ | ✓ | 1:1.3 | AMP | UNKNO... | SYBR | 17.323 | 17.327 | 0.004 | 0.000 | |
| 40 | D4 | ☐ | ⚠ | 1:4 | AMP | STAND... | SYBR | 15.847 | 15.887 | 0.037 | 0.002 | |
| 41 | D5 | ☐ | ✓ | 1:4 | AMP | STAND... | SYBR | 15.892 | 15.887 | 0.037 | 0.002 | |
| 42 | D6 | ☐ | ✓ | 1:4 | AMP | STAND... | SYBR | 15.921 | 15.887 | 0.037 | 0.002 | |
| 43 | D7 | ☐ | ✓ | 1:1.4 | AMP | UNKNO... | SYBR | 17.318 | 17.312 | 0.017 | 0.000 | |
| 44 | D8 | ☐ | ✓ | 1:1.4 | AMP | UNKNO... | SYBR | 17.325 | 17.312 | 0.017 | 0.000 | |
| 45 | D9 | ☐ | ✓ | 1:1.4 | AMP | UNKNO... | SYBR | 17.294 | 17.312 | 0.017 | 0.000 | |
| 52 | E4 | ☐ | ✓ | 1:8 | AMP | STAND... | SYBR | 15.100 | 15.102 | 0.019 | 0.001 | |

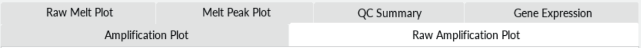
The list can be grouped by the following:

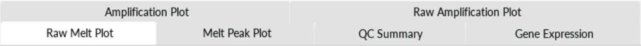


**Please note that sections 6.3.1 to 6.2.7 can be applied to all subsequent plot types.**

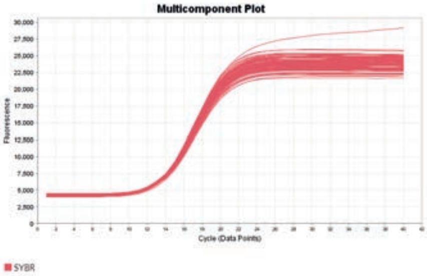
## 6.2.8 Raw Amplification Plot and Raw Melt Peak Plot

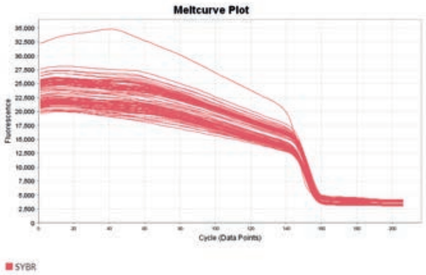
Raw plots present the real time fluorescence signal change. The cycle number is shown on the X-Axis while the fluorescence signal value is plotted on the Y-Axis.


Raw Melt Plot
Melt Peak Plot
QC Summary
Gene Expression
Amplification Plot
Raw Amplification Plot


Amplification Plot
Raw Amplification Plot
Raw Melt Plot
Melt Peak Plot
QC Summary
Gene Expression

By entering the corresponding tabs multicomponent plots for the amplification stage or for the melt curve step will be shown.

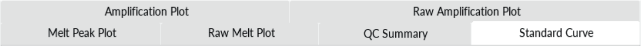




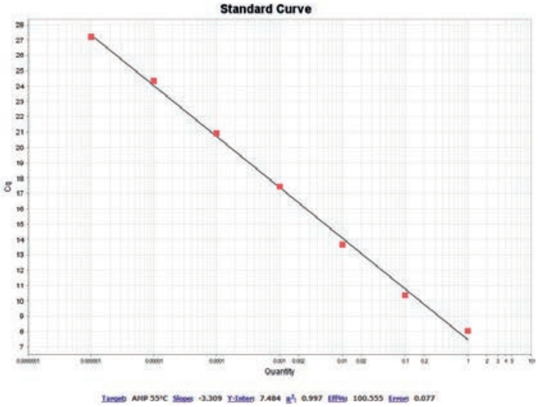
### 6.2.9 Standard Curve

Standard curves can be used to verify the amplification efficiency of comparative Cq experiments or to perform an absolute quantitative PCR experiment.

1. Click Standard Curve


Amplification Plot
Raw Amplification Plot
Melt Peak Plot
Raw Melt Plot
QC Summary
Standard Curve

2. View standard



Target AMP 55°C Slope -3.309 Y-Inter 7.484 $R^2$ 0.997 Eff% 100.555 Error 0.077

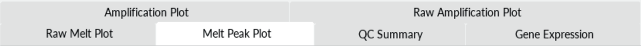
***Note***: *The amplification efficiency (Eff%), slope, Y-Intercept, R2 and Error will be shown below the graph. A slope of -3.2~-3.5, a* $R^2$*>0.99 and an Efficiency of 90 to 110% are indicators of a reliable experiment.*

3. The default mode shows red dots as standard samples, while blue and green are unknown samples.

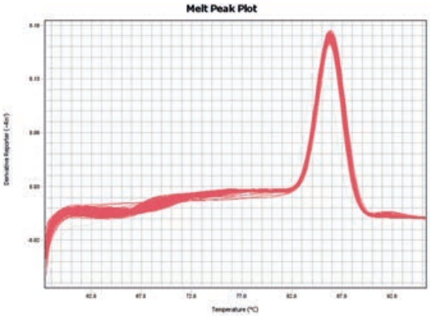
### 6.2.10 Melt Curve

The melt curve shows the reduction in fluorescence caused by the denaturation of the DNA double helix during the final slow heating step (melt curve analysis).

1. Enter Melt curve plot in the menu.

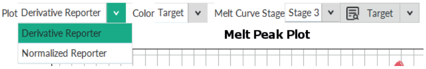

Amplification Plot
Raw Amplification Plot
Raw Melt Plot
Melt Peak Plot
QC Summary
Gene Expression

2. View derivative of the melt curve fluorescence plot



***Note:** Melt curve plot is applied to check the specificity of PCR reaction and products: Two or more peaks are indicators of an unspecific amplification or of multiple amplicons.*

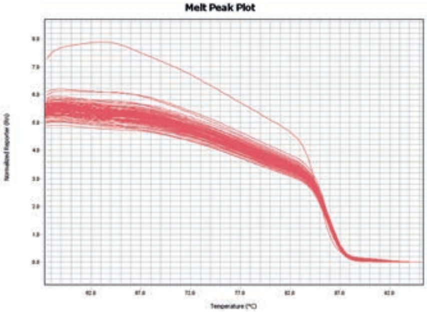
3. Change the plot configurations



Plot Derivative Reporter
Color Target
Melt Curve Stage Stage 3
Target

Derivative Reporter
Normalized Reporter
Melt Peak Plot

***Note:** The "Derivative reporter" plot displays the first derivative of fluorescence signal change according to the temperature. The T$_m$ (Temperature of melt, the temperature of 50% of dsDNA unwinding to ssDNA) could be used to detect unspecific amplification and primer dimer.*

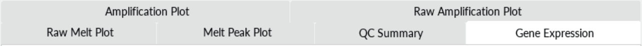


*The "Normalized reporter plot" displays the fluorescence signal value decreasing as temperature rises.*

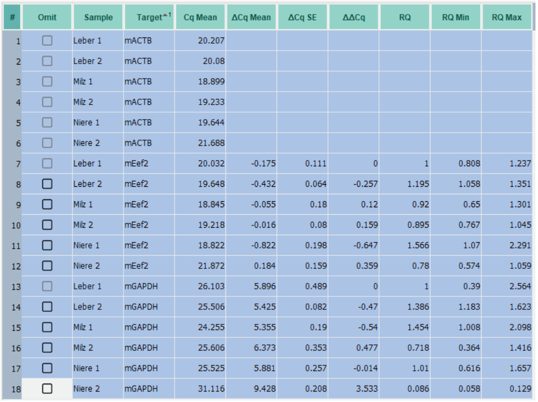
### 6.2.11 Gene Expression Map

Gene expression maps can be used to view the results of relative quantitation calculations and the gene expression profile.

1. Click Gene Expression

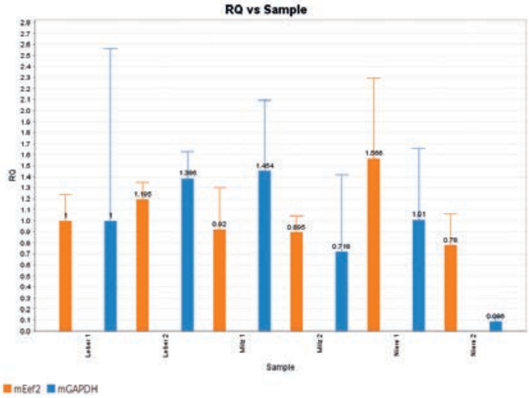


2. All sample information is shown in the selected table



| # | Omit | Sample | Target▲1 | Cq Mean | ΔCq Mean | ΔCq SE | ΔΔCq | RQ | RQ Min | RQ Max |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ☐ | Leber 1 | mACTB | 20.207 | | | | | | |
| 2 | ☐ | Leber 2 | mACTB | 20.08 | | | | | | |
| 3 | ☐ | Milz 1 | mACTB | 18.899 | | | | | | |
| 4 | ☐ | Milz 2 | mACTB | 19.233 | | | | | | |
| 5 | ☐ | Niere 1 | mACTB | 19.644 | | | | | | |
| 6 | ☐ | Niere 2 | mACTB | 21.688 | | | | | | |
| 7 | ☐ | Leber 1 | mEef2 | 20.032 | -0.175 | 0.111 | 0 | 1 | 0.808 | 1.237 |
| 8 | ☐ | Leber 2 | mEef2 | 19.648 | -0.432 | 0.064 | -0.257 | 1.195 | 1.058 | 1.351 |
| 9 | ☐ | Milz 1 | mEef2 | 18.845 | -0.055 | 0.18 | 0.12 | 0.92 | 0.65 | 1.301 |
| 10 | ☐ | Milz 2 | mEef2 | 19.218 | -0.016 | 0.08 | 0.159 | 0.895 | 0.767 | 1.045 |
| 11 | ☐ | Niere 1 | mEef2 | 18.822 | -0.822 | 0.198 | -0.647 | 1.566 | 1.07 | 2.291 |
| 12 | ☐ | Niere 2 | mEef2 | 21.872 | 0.184 | 0.159 | 0.359 | 0.78 | 0.574 | 1.059 |
| 13 | ☐ | Leber 1 | mGAPDH | 26.103 | 5.896 | 0.489 | 0 | 1 | 0.39 | 2.564 |
| 14 | ☐ | Leber 2 | mGAPDH | 25.506 | 5.425 | 0.082 | -0.47 | 1.386 | 1.183 | 1.623 |
| 15 | ☐ | Milz 1 | mGAPDH | 24.255 | 5.355 | 0.19 | -0.54 | 1.454 | 1.008 | 2.098 |
| 16 | ☐ | Milz 2 | mGAPDH | 25.606 | 6.373 | 0.353 | 0.477 | 0.718 | 0.364 | 1.416 |
| 17 | ☐ | Niere 1 | mGAPDH | 25.525 | 5.881 | 0.257 | -0.014 | 1.01 | 0.616 | 1.657 |
| 18 | ☐ | Niere 2 | mGAPDH | 31.116 | 9.428 | 0.208 | 3.533 | 0.086 | 0.058 | 0.129 |

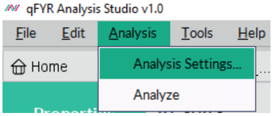
3. Check the gene expression map:



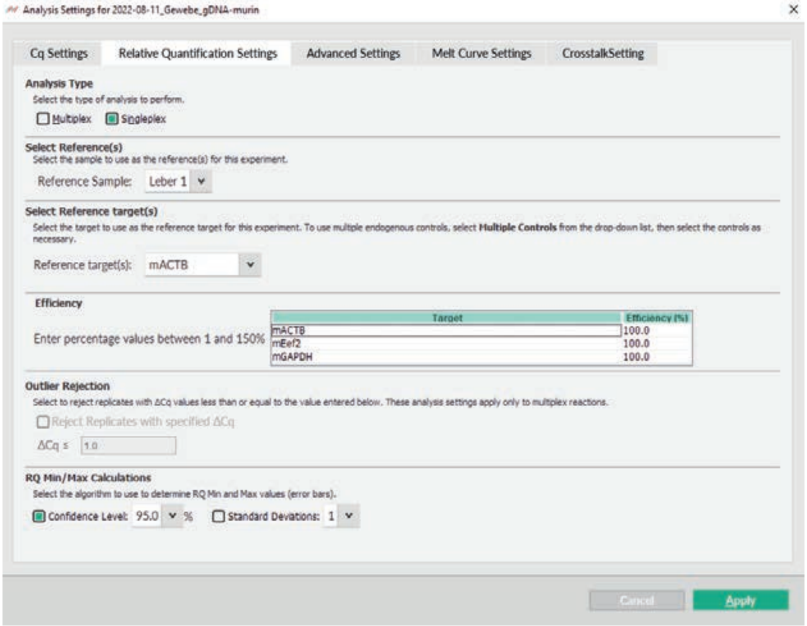
**Note:** *There are two plot types available: RQ vs Target (relative quantitation (RQ) values are grouped by target), and RQ vs Sample (relative quantitation (RQ) values are grouped by sample).*

### 6.2.12 Analysis Settings

General Analysis settings can be changed by entering the Analysis settings in the menu toolbar:


qFYR Analysis Studio v1.0
File
Edit
Analysis
Tools
Help
Home
Analysis Settings...
Analyze

Enter the tab Relative Quantification Settings to edit settings used for relative quantification of data, such as References, Efficiency of used primers, Outlier Rejection and RQ Min/Max calculations.


Analysis Settings for 2022-08-11_Gewebe_gDNA-murin
Cq Settings
Relative Quantification Settings
Advanced Settings
Melt Curve Settings
CrosstalkSetting
Cancel
Apply

**Analysis Type**

Select the type of analysis to perform.

☐ Multiplex ☑ Singleplex

**Select Reference(s)**

Select the sample to use as the reference(s) for this experiment.

Reference Sample: Leber 1

**Select Reference target(s)**

Select the target to use as the reference target for this experiment. To use multiple endogenous controls, select **Multiple Controls** from the drop-down list, then select the controls as necessary.

Reference target(s): mACTB

**Efficiency**

Enter percentage values between 1 and 150%

| Target | Efficiency (%) |
|---|---|
| mACTB | 100.0 |
| mEef2 | 100.0 |
| mGAPDH | 100.0 |

**Outlier Rejection**

Select to reject replicates with ΔCq values less than or equal to the value entered below. These analysis settings apply only to multiplex reactions.

☐ Reject Replicates with specified ΔCq

ΔCq ≤ 1.0

**RQ Min/Max Calculations**

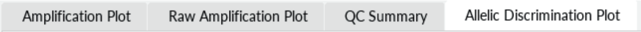
Select the algorithm to use to determine RQ Min and Max values (error bars).

☑ Confidence Level: 95.0 % ☐ Standard Deviations: 1

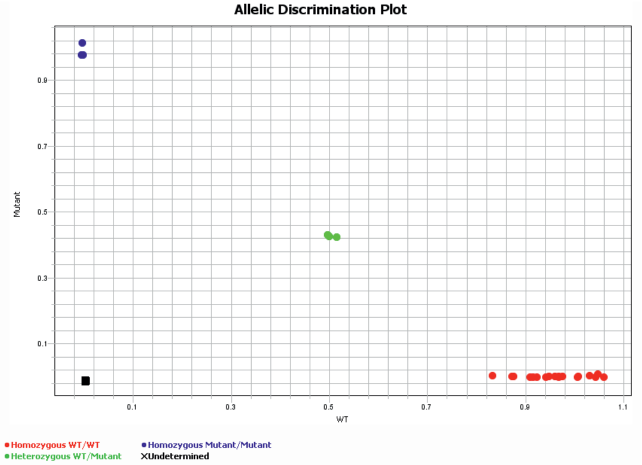
### 6.2.13 Genotyping Maps

The allelic discrimination plot clusters the normalized reporter fluorescence (Rn) of the allele-specific probes used in the SNP assay for the three possible genotypes (Allele 1 homozygous, Allele 2 homozygous, and Allele 1/2 heterozygous) as well as for negative controls (undetermined)

1. Click the allele identification map.


Amplification Plot
Raw Amplification Plot
QC Summary
Allelic Discrimination Plot

2. Check the allele identification map.

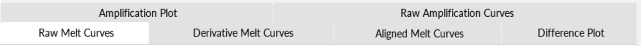


3. The allele identification map, the colors of different samples and the allele types represented by each color will be displayed.

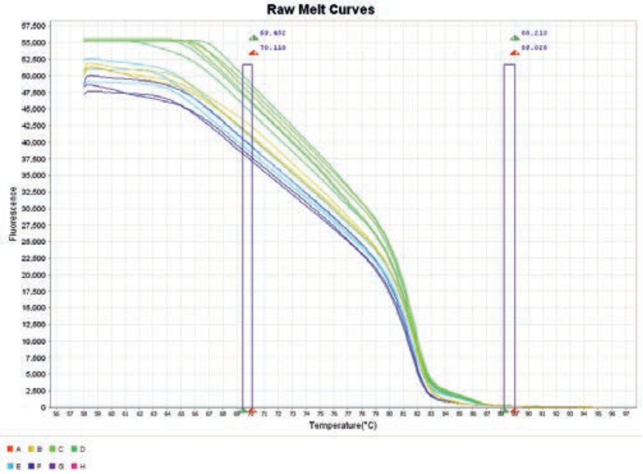
### 6.2.14 Raw Melt Curves of the High-Resolution Melt analysis

The melt profile of a high-Resolution Melt analysis (HRM) reflects the mix of amplicons present in the sample. Variations in sample characteristics like GC content, length, sequence, and heterozygosity are defining the melt curve characteristics of each amplicon.

1. Click the Raw Melt curves tab.


Amplification Plot
Raw Amplification Curves
Raw Melt Curves
Derivative Melt Curves
Aligned Melt Curves
Difference Plot

2. Check the raw melt curves.



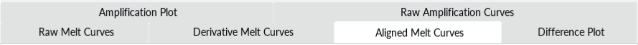
3. The software automatically defines the pre- and post-melt regions by pairs of vertical bars before and after the active melt region. If you need to set the regions manually, you can drag and drop the vertical bars of each region directly on the curve. The area between the pair of bars to the left of the active melt region is used to designate 100% fluorescence, where all amplicons are double-stranded. Respectively, the area between the bars to the right of the active melt region is used to determine 0% fluorescence, where all amplicons are single-stranded.

***Note:*** *Please do not forget to click* Analyze *after melt-region setting is changed.*

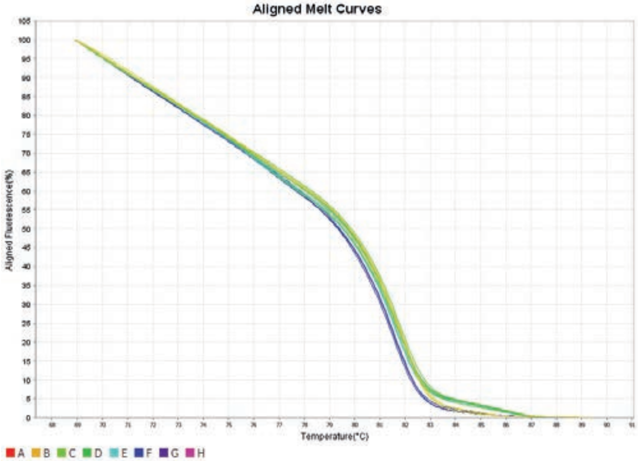
### 6.2.15 Aligned Melt Curves of the High-Resolution Melt analysis

During HRM two types of melt curves can be observed. On the one hand, melt curves that differ from each other in melting temperature ($T_m$) of the amplicon, but are similar in curve shape. Usually, this kind of curve is generated by homozygous variant samples. On the other hand, melt curves that differ in shape from homozygote melt curves usually indicate heterozygous samples due to the presence of base-pairing mismatches (heteroduplexes) present in the PCR product mix.

1. Click the Aligned Melt curves tab


Amplification Plot
Raw Amplification Curves
Raw Melt Curves
Derivative Melt Curves
Aligned Melt Curves
Difference Plot

2. Check the raw melt curves

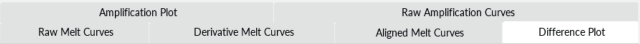


***Note***: *A scaled view of the data is displayed in the aligned plot. Sequence variants with differences in their melt curve (e.g., homozygous vs. heterozygous variants) can be easily discriminated.*

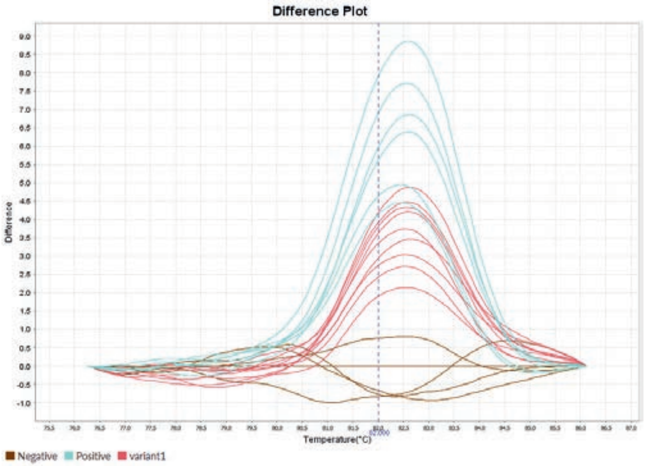
### 6.2.16 Difference plot of the High-Resolution Melt analysis

The difference plot is a tool to easily visualize small differences in melt curves of HRM analysis. Curves displayed here are generated by the differences between samples and a single reference.

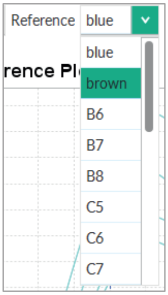
1. Click the Difference Plot tab.


Amplification Plot
Raw Amplification Curves
Raw Melt Curves
Derivative Melt Curves
Aligned Melt Curves
Difference Plot

2. Check the difference plot.

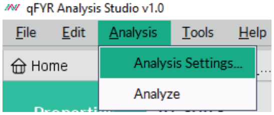


3. References can be changed by using the drop-down menu and can be either pre-defined controls or single wells.

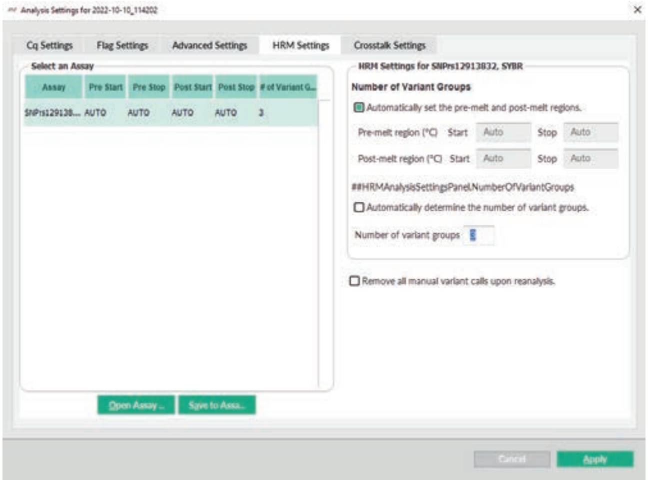

Reference
blue
blue
rence Pl
brown
B6
B7
B8
C5
C6
C7

4. The default number of variants in the software pre-set is three. If the actual number of variants is less, any slight difference among the replicates may lead the replicates of one

sample to be divided into different variation groups, even if they have similar curve characteristics. Default settings can be changed by opening the analysis settings in the menu toolbar.


qFYR Analysis Studio v1.0
File
Edit
Analysis
Tools
Help
Home
Analysis Settings...
Analyze

In the tab “HRM Settings” uncheck “Automatically determine the number of variant groups” and enter the desired number. Confirm by clicking Apply.


Analysis Settings for 2022-10-10_114202
Cq Settings
Flag Settings
Advanced Settings
HRM Settings
Crosstalk Settings
Open Assay ...
Save to Assa...
Cancel
Apply

Select an Assay

| Assay | Pre Start | Pre Stop | Post Start | Post Stop | # of Variant G... |
|---|---|---|---|---|---|
| SNPrs129138... | AUTO | AUTO | AUTO | AUTO | 3 |

HRM Settings for SNPrs12913832, SYBR

Number of Variant Groups

☑ Automatically set the pre-melt and post-melt regions.

Pre-melt region (°C) Start Auto Stop Auto

Post-melt region (°C) Start Auto Stop Auto

##HRMAnalysisSettingsPanel.NumberOfVariantGroups

☐ Automatically determine the number of variant groups.

Number of variant groups 3

☐ Remove all manual variant calls upon reanalysis.

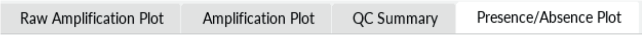
Settings can be saved as default for future experiments using this SNP assay by clicking Save to Assa... or opened from previous experiments by clicking Open Assay ... .

***Note:*** *Please do not forget to click* Analyze *after HRM setting is changed.*

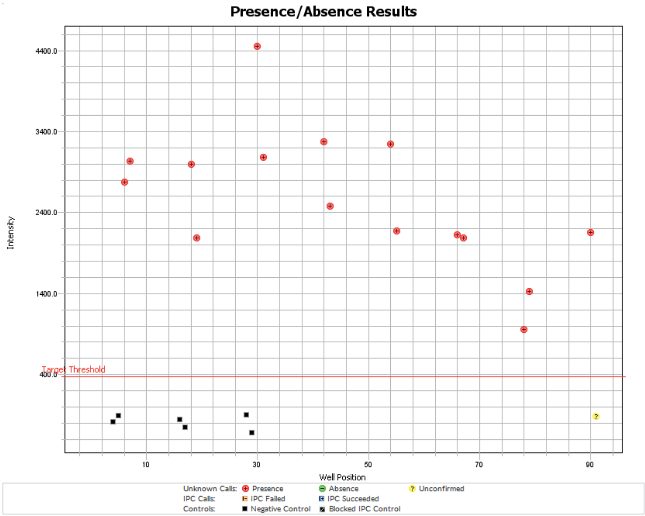
### 6.2.17 Presence/Absence Plot

The Presence/Absence plot display the fluorescence intensity measured in each well.

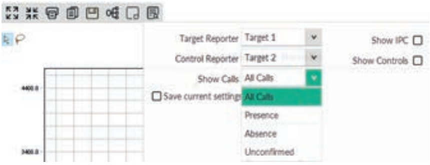
1. Enter the Presence/Absence plot tab.


Raw Amplification Plot
Amplification Plot
QC Summary
Presence/Absence Plot

2. Check the Presence/Absence results.



3. There are 4 types of plot views that can be chosen in the Plot settings: “All calls” – all calls are displayed, “Presence” – only samples where the target is present are displayed, “Absence” - only samples where the target is absent are displayed, and “Unconfirmed”, only unconfirmed samples are displayed. Additionally, you can choose to display fluorescence intensity of the IPC target (“Show IPC”) as well as fluorescence intensity of the IPC target in Negative Control-IPC wells and Negative Control-Blocked IPC wells (“Show Controls”).


Target Reporter: Target 1
Control Reporter: Target 2
Show Calls: All Calls
☐ Save current settings
Show IPC ☐
Show Controls ☐
All Calls
Presence
Absence
Unconfirmed
4400.0
3400.0

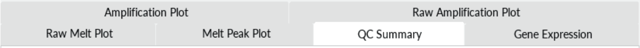
4. The software is calculating two types of thresholds for the Presence/Absence plot that are used for the call:
   - The “IPC Threshold” is calculated from Negative Control- Blocked IPC reactions.
   - The “Target Threshold” is calculated from Negative Control- IPC reactions.

- Targets are called regarding their fluorescence intensity:
    - Call is present if fluorescence intensity is higher than the target threshold,
    - Call is absent if fluorescence intensity is lower than the target threshold, and fluorescence intensity of the IPC is higher than the IPC threshold,
    - Call is unconfirmed if fluorescence intensity is lower than the target threshold, and fluorescence intensity of the IPC is lower than the IPC threshold.

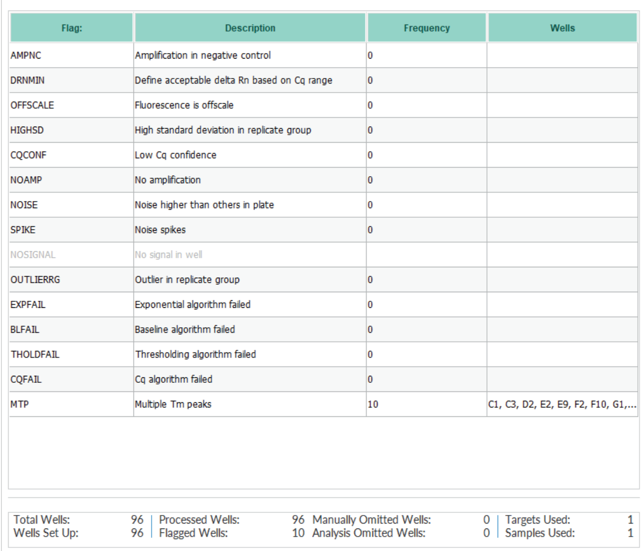
### 6.2.18 QC Summary

The QC Summary screen displays a list of defined experiment flags, including the flag frequency and location of flagged wells for the experiment.

1. Enter the QC Summary tab.


Amplification Plot
Raw Amplification Plot
Raw Melt Plot
Melt Peak Plot
QC Summary
Gene Expression

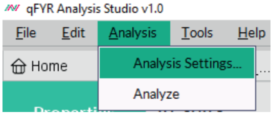
2. Check the quality of results.

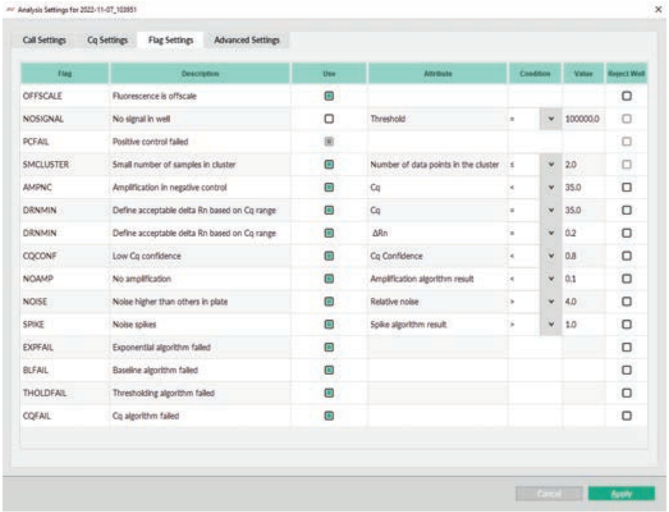


| Flag: | Description | Frequency | Wells |
|---|---|---|---|
| AMPNC | Amplification in negative control | 0 | |
| DRNMIN | Define acceptable delta Rn based on Cq range | 0 | |
| OFFSCALE | Fluorescence is offscale | 0 | |
| HIGHSD | High standard deviation in replicate group | 0 | |
| CQCONF | Low Cq confidence | 0 | |
| NOAMP | No amplification | 0 | |
| NOISE | Noise higher than others in plate | 0 | |
| SPIKE | Noise spikes | 0 | |
| NOSIGNAL | No signal in well | | |
| OUTLIERRG | Outlier in replicate group | 0 | |
| EXPFAIL | Exponential algorithm failed | 0 | |
| BLFAIL | Baseline algorithm failed | 0 | |
| THOLDFAIL | Thresholding algorithm failed | 0 | |
| CQFAIL | Cq algorithm failed | 0 | |
| MTP | Multiple Tm peaks | 10 | C1, C3, D2, E2, E9, F2, F10, G1,... |

Total Wells: 96 | Processed Wells: 96 Manually Omitted Wells: 0 | Targets Used: 1
Wells Set Up: 96 | Flagged Wells: 10 Analysis Omitted Wells: 0 | Samples Used: 1

***Note:*** *For some experiment types QC settings can be changed by entering the analysis settings in the menu toolbar:*


qFYR Analysis Studio v1.0
File
Edit
Analysis
Tools
Help
Home
Analysis Settings...
Analyze


Analysis Settings for 2022-11-07_103951
Call Settings
Cq Settings
Flag Settings
Advanced Settings
Cancel
Apply

| Flag | Description | Use | Attribute | Condition | Value | Reject Well |
|---|---|---|---|---|---|---|
| OFFSCALE | Fluorescence is offscale | ☑ | | | | ☐ |
| NOSIGNAL | No signal in well | ☐ | Threshold | = | 100000.0 | ☐ |
| PCFAIL | Positive control failed | ☒ | | | | ☐ |
| SMCLUSTER | Small number of samples in cluster | ☑ | Number of data points in the cluster | ≤ | 2.0 | ☐ |
| AMPNC | Amplification in negative control | ☑ | Cq | < | 35.0 | ☐ |
| DRNMIN | Define acceptable delta Rn based on Cq range | ☑ | Cq | = | 35.0 | ☐ |
| DRNMIN | Define acceptable delta Rn based on Cq range | ☑ | ΔRn | = | 0.2 | ☐ |
| CQCONF | Low Cq confidence | ☑ | Cq Confidence | < | 0.8 | ☐ |
| NOAMP | No amplification | ☑ | Amplification algorithm result | < | 0.1 | ☐ |
| NOISE | Noise higher than others in plate | ☑ | Relative noise | > | 4.0 | ☐ |
| SPIKE | Noise spikes | ☑ | Spike algorithm result | > | 1.0 | ☐ |
| EXPFAIL | Exponential algorithm failed | ☑ | | | | ☐ |
| BLFAIL | Baseline algorithm failed | ☑ | | | | ☐ |
| THOLDFAIL | Thresholding algorithm failed | ☑ | | | | ☐ |
| CQFAIL | Cq algorithm failed | ☑ | | | | ☐ |

*Confirm by clicking* Apply *and click* Analyze *after Flag setting is changed.*

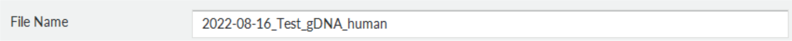
## 6.3 Data Export

### 6.3.1 Toolbars in Export Tab

| Toolbars | Name | Function |
|---|---|---|
| Export | Export | Export data |
| Save | Save | Save as .qps file |

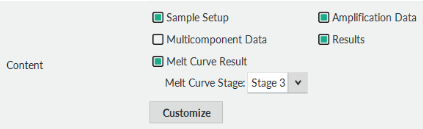
### 6.3.2 Data Export

1. File name: The default file name is the name defined in “Plot properties” or could be changed to customized name. English characters are supported, but not special characters.



File Name 2022-08-16_Test_gDNA_human

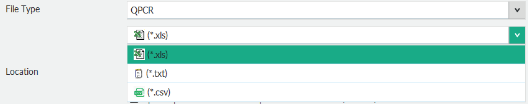
2. Export content: Include sample setup, Amplification data, Multicomponent data, Results, Melt Curve Result. The contents can be selected by box checking.



Content
☑ Sample Setup ☑ Amplification Data
☐ Multicomponent Data ☑ Results
☑ Melt Curve Result
Melt Curve Stage: Stage 3

3. The default option is “Unify above content into one file”, which will be included as different sheets in one excel file. To export single files, choose “Split above content items into individual files”.

4. File type: Select QPCR and .xsl, .txt or .csv.

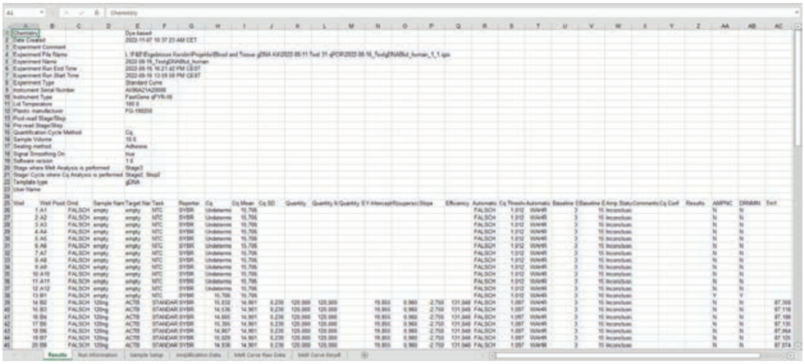


File Type QPCR
(*.xls)

5. File Location: The default file location is desktop, which can be changed to other file location by click Browse... .

6. File Export: The experiment result will be saved and opened in Excel automatically after clicking Export .

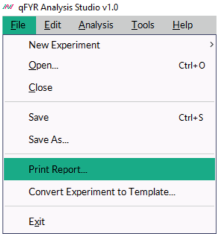
7. The export is shown in Excel as below.



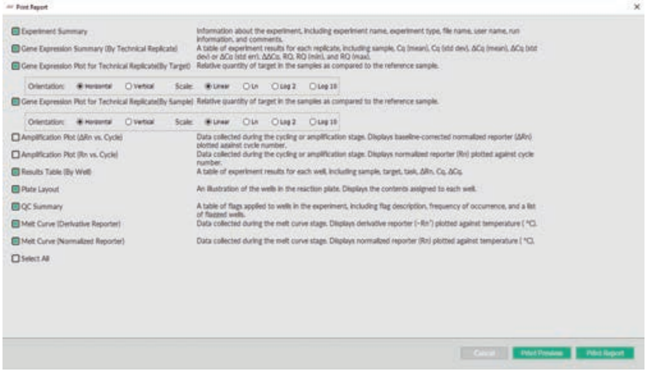
### 6.3.3 Printed report

Next to data export, a report of the complete data for an experiment, including graphs, can be saved, and printed.

1. In the result section, select the wells that should be reported
2. Access the main menu toolbar, enter the “File” drop down and click “Print Report”



3. A pop-up menu will open to select the data and graphs to be reported


Print Report

☑ Experiment Summary — Information about the experiment, including experiment name, experiment type, file name, user name, run information, and comments.

☑ Gene Expression Summary (By Technical Replicate) — A table of experiment results for each replicate, including sample, Cq (mean), Cq (std dev), ΔCq (mean), ΔCq (std dev) or ΔCq (std err), ΔΔCq, RQ, RQ (min), and RQ (max).

☑ Gene Expression Plot for Technical Replicate(By Target) — Relative quantity of target in the samples as compared to the reference sample.

Orientation: ● Horizontal ○ Vertical Scale: ● Linear ○ Ln ○ Log 2 ○ Log 10

☑ Gene Expression Plot for Technical Replicate(By Sample) — Relative quantity of target in the samples as compared to the reference sample.

Orientation: ● Horizontal ○ Vertical Scale: ● Linear ○ Ln ○ Log 2 ○ Log 10

☐ Amplification Plot (ΔRn vs. Cycle) — Data collected during the cycling or amplification stage. Displays baseline-corrected normalized reporter (ΔRn) plotted against cycle number.

☐ Amplification Plot (Rn vs. Cycle) — Data collected during the cycling or amplification stage. Displays normalized reporter (Rn) plotted against cycle number.

☑ Results Table (By Well) — A table of experiment results for each well, including sample, target, task, ΔRn, Cq, ΔCq.

☑ Plate Layout — An illustration of the wells in the reaction plate. Displays the contents assigned to each well.

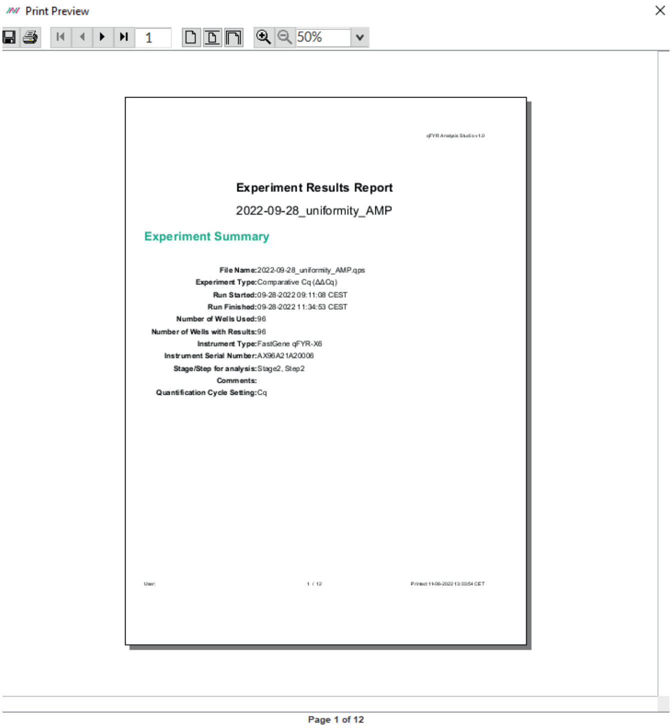
☑ QC Summary — A table of flags applied to wells in the experiment, including flag description, frequency of occurrence, and a list of flagged wells.

☑ Melt Curve (Derivative Reporter) — Data collected during the melt curve stage. Displays derivative reporter (-Rn') plotted against temperature ( °C).

☑ Melt Curve (Normalized Reporter) — Data collected during the melt curve stage. Displays normalized reporter (Rn) plotted against temperature ( °C).

☐ Select All

4. Report can either be directly send to a connected printer by clicking Print Report or previewed and saved by clicking Print Preview.
5. “Print Preview” will open a new pop up and document can be reviewed and saved or printed. File format for saving is *.pdf and file can be password-secured.



qFYR Analysis Studio v1.0

Experiment Results Report

2022-09-28_uniformity_AMP

Experiment Summary

**File Name:**2022-09-28_uniformity_AMP.qps
**Experiment Type:**Comparative Cq (ΔΔCq)
**Run Started:**09-28-2022 09:11:08 CEST
**Run Finished:**09-28-2022 11:34:53 CEST
**Number of Wells Used:**96
**Number of Wells with Results:**96
**Instrument Type:**FastGene qFYR-X6
**Instrument Serial Number:**AX96A21A20006
**Stage/Step for analysis:**Stage2, Step2
**Comments:**
**Quantification Cycle Setting:**Cq

User: 1 / 12 Printed: 11-08-2022 13:03:54 CET

Page 1 of 12

# Chapter 7 Instruments Maintenance

This instrument does not need a lot of maintenance in case of routine use. If this instrument is used for a long time, it is necessary to clean and maintain the instrument regularly to ensure normal instrument operation. Before cleaning the instrument, please read this chapter carefully. A correct maintenance and cleaning help to prolong the instrument's service life.

If you need to replace any instrument parts during the maintenance, please contact us for professional technical confirmation, and only replace the parts provided by our original factory or our official license agency.

## 7.1 Instrument Cleaning

Do not clean or maintain the instrument during electrification or operation.

Do not pour water or other solutions into the sample bin, sample module or any instrument component. When the instrument is electrified, the flow of liquid may lead to electric shock!

70% medical disinfectant alcohol is recommended for instrument cleaning and disinfectant. However, ethanol is a volatile liquid. Exposure can irritate the eyes, skin, and respiratory tract, and can lead to central nervous system dysfunction and liver damage. When cleaning with ethanol, please wear suitable protective goggles, clothing, and gloves.

**Note**: If the instrument user has any questions about the compatibility of disinfectants or cleaning solution with the materials contained in the equipment parts, please consult the manufacturer or official license agency.

During operation, the sample module may have high temperature. Before cleaning or maintaining, wait for the temperature to drop to room temperature to avoid scalding.

**Biological Hazards**

Please treat and operate all samples as potential biological hazards. If liquid samples overflow or splatter, please immediately take appropriate disinfectants for disinfection, to avoid contamination or damage to laboratory personnel.

Do not deal with and operate any potential biological hazards without any safety precautions.

### 7.1.1 Cleaning the Instrument

Turn off the instrument and unplug the power cord.
Use a damp soft cloth to clean the instrument shell, use a mild cleaner to clean if necessary.

**Note**: Do not spray the cleaner directly on the instrument, which may cause the failure of the electronic instrument.

Please avoid using strong acid and alkali, more than 75% concentrated alcohol, organic solvents or strong detergent and other cleaning disinfectants to clean the instrument without our company's permission, it will destroy the surface coating.

### 7.1.2 Clean Sample-tray and Reaction-holes

Please close the instrument and open the sample bin.
Use damp soft cloth to clean the sample tray, use mild detergent to clean it if necessary.
Please clean the reaction hole regularly, to ensure that the consumable test tube is in full contact with the reaction hole and the heat conduction is perfect.
Use cotton swabs dipped in mild detergent to clean the reaction hole.

Please close the heat cover after sample tray and reaction hole are completely dry. The reaction module with detergent on the heating surface may damage the instrument.

Do not spray the cleaner directly on the instrument, which may cause the failure of the electronic instrument.

## 7.2 Instrument maintenance

### 7.2.1 Keep Air-Circulation

The instrument must be positioned with a possibility of free air circulation to ensure accuracy of the target temperature. Please check the placement area regularly to ensure a free air circulation, and no items that can affect the air flow.

### 7.2.2 Keep Stable Electricity Supply

A stable power supply is required for the normal operation, please check it regularly to ensure that the supply voltage is consistent with the voltage requirement (±10% deviation is allowed). Ensure that the power outlet rated load is not less than the instrument requirement.

### 7.2.3 Keep Clean

Sample tray, reaction modules and optical components contaminations may interfere with thermal cycling and data collection. Please refer to the following instructions to avoid contamination of this instrument:

Make sure the outside of the plates or tubes is clean before adding them to the instrument.

Please clean the reaction module and inner cover regularly, to prevent accumulation of dirt, biological hazards, or fluorescent solvents.

Please confirm the seal of the tubes or plates before running the reaction.

Do not put open reaction consumables or damaged lids into the sample tray. The reagent may leak out and cover the hot-lid surface, sample tray and reaction hole.

Do not run PCR reactions containing volatile reagents, which may lead to contamination in the hot-lid, sample tray and reaction hole.

When this instrument is not used for a long time, please unplug and cover with soft cloth or dustproof bag to prevent dust from entering.

### 7.2.4 Waste Disposal

After each experiment, there is a large number of amplified products in the test tube, which should be treated as soon as possible according to relevant regulations, to avoid contamination of laboratories and instruments.

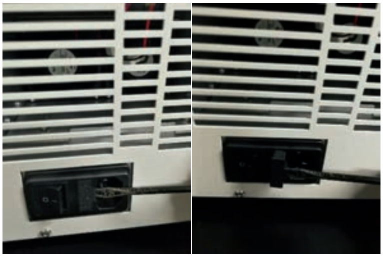
Do not open the test tube cap after removing from the reaction module! The high concentration of nucleic acid might pollute the laboratory.

### 7.2.5 Fuse Replacement

When the circuit fails or is abnormal, current increase or rising of the current may damage important components. If the fuse is installed correctly, the fuse will break when the current rises to a certain height and heat, to protect the instrument or the user.

FastGene® qFYR Real-Time PCR System are equipped with two 10 A. When the fuse is damaged, the user can replace the fuse as follows:

- Cut off the mainframe power supply and unplug the power cord
- Open the fuse box lid with a screwdriver
- Check the inner fuse tube. If the middle is disconnected (two or one of them), it means that the fuse is broken
- Replace the new fuse tube (fuse type Φ5×20 mm –T10AH250VP)
- Install the replaced fuse seat under the socket.
- **Note**: Power must be turned off and removed before replacing fuse.



### 7.2.6 Overheating Protection

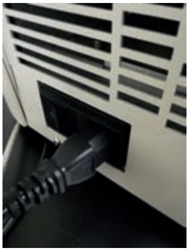
The instrument heating system is equipped with overheating protection. When the heating system fails and the temperature value exceeds the upper limit of the allowable range, the protection will automatically disconnect, and cannot be restored. At this time, the heating system cannot continue to rise and cool normally. After the heating system broke down, user should stop using the instrument, contact the manufacturer for maintenance in time.

# Chapter 8 Troubleshooting

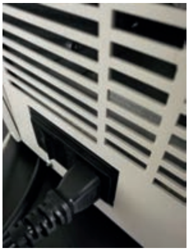
a) **Observation:** Switch on the instrument, but no reaction, and power light off.

**Possible cause:** Inconsistent connection with power cable

**Recommended action:** Switch off the power on the rear of instrument, re-plug power cable.



Inconsistent plug

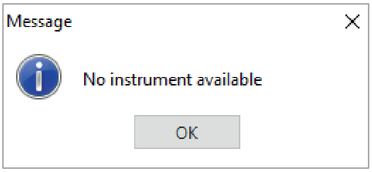


correct plug

b) **Observation:** Shows “No Available Instrument” after click “Start Run”.

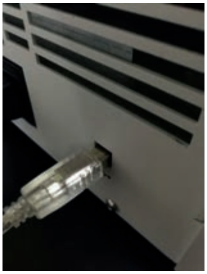
**Possible cause:** Inconsistent connection between instrument and computer.

**Recommended action:** Unplug and plug the USB data cable again, re-start qFYR Analyzer Studio.

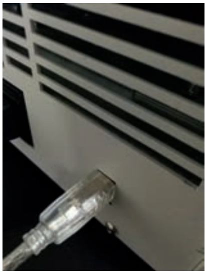


Message

No instrument available



Inconsistent data cable



correct connection

c) **Observation:** There is no fluorescence signal appearing on "Multicomponent plot", while the sound of scanning can be heard.

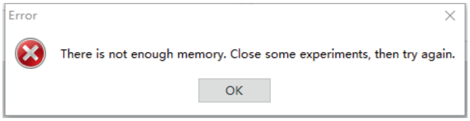
**Possible cause:** The "sample" and "target item" columns on the "well-plate setup" interface are not filled in. The fluorescence signal is displayed before filled in both. If the user does not fill in "sample" or "target item ", the instrument will collect and store the fluorescence signal of all channels in 96 wells in the experimental file, but not display them on the experimental interface. It can be displayed after the user completes the" well plate setting "information.

**Recommended action:** Return to Plate Tab, complete the "Sample" and "Target" information.

d) **Observation:** Error notice of "Not enough memory, please close some experiment".

**Possible cause:** Too many experiment files are opened.

**Recommended action:** Closed some inactive experiments, the error notice will be closed. It is recommended No more than six experiment files are opened simultaneously.



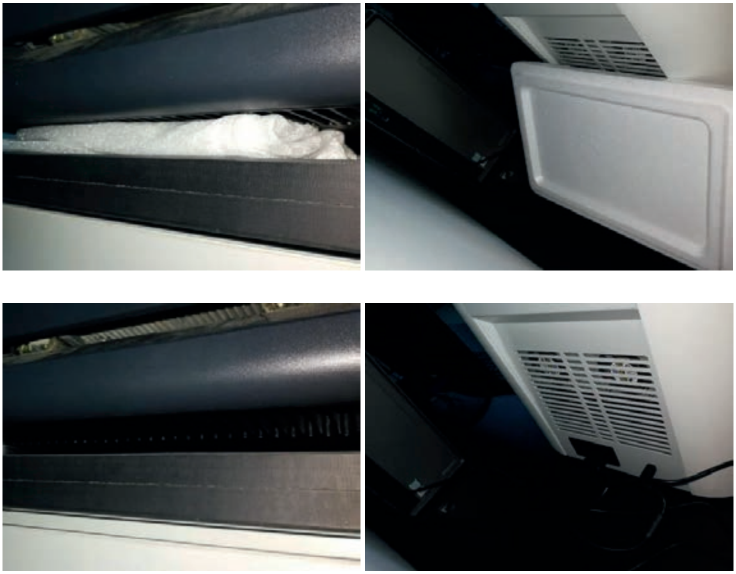
Error

There is not enough memory. Close some experiments, then try again.

e) **Observation:** The ramp rate slowed down obviously.

**Possible cause:** The outlet of airflow was blocked.

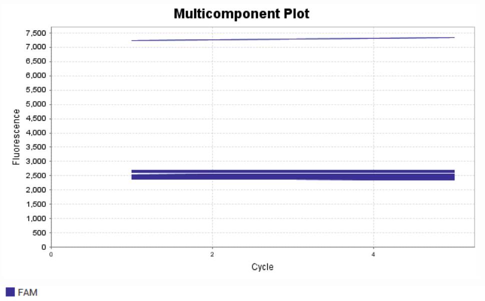
**Recommended action:** Clean the tamper on inlet and/or outlet of airflow.



f) **Observation:** While scanning empty block, background signal of some wells is much higher than average.

**Possible cause:** There are contamination in these wells.

**Recommended action:** Clean the contamination, please refer to Section 5.3 Monthly maintenance.



g) **Observation:** Reaction reagent been evaporated

**Possible cause:** The tubes or seal on plate are not closed correctly.

**Recommended action:** Select better air-tight consumables and restart experiment.

**Note: If the user cannot judge and troubleshoot the above, contact our company directly.**

**If the following circumstances occur, the power supply should be cut off immediately, contact the supplier or our company, we will assign qualified maintenance personnel to handle:**

- Liquid flows inside the instrument.
- Instruments be rained or watered.
- The instrument has any abnormal sound or smell.
- Damage to the shell caused by instrument drop down.
- Instrument function changes obviously.

**NIPPON Genetics EUROPE GmbH**

Production Permit Number:

Product Registration Certificate No./ Product Technical Requirements No.:

Production Enterprise: NIPPON Genetics EUROPE GmbH

Address: Mariaweilerstraße 28-30 | 52349 | Düren |Germany

Tel: +49 2421 554960
Web: www.nippongenetics.eu

LUBIO SCIENCE

## Your distributor in Switzerland

LubioScience GmbH
Baumackerstrasse 24
8050 Zürich
+41 (0)41 417 02 80

info@lubio.ch
www.lubio.ch

Referenznr.

NIPPON Genetics EUROPE GmbH

---

+49 2421 554960
+49 2421 55496 11

info@nippongenetics.de
www.nippongenetics.eu