September 2018

Rotor-Gene® Q MDx User Manual (US)

US Version

For use with Rotor-Gene Q Software version 2.3.4 or higher

IVD

MAT   1114362EN

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QIAGEN®

Sample & Assay Technologies

Contents

Contents

Contents

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1 Safety Information

Before using the Rotor-Gene Q MDx, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

The following types of safety information appear throughout this manual:

WARNING ⚠	The term WARNING is used to inform you about situations that could result in **personal injury** to you or other persons. Details about these circumstances are given in a box like this one.

CAUTION ⚠	The term CAUTION is used to inform you about situations that could result in **damage to the instrument** or other equipment. Details about these circumstances are given in a box like this one.

The advice given in this manual is intended to supplement, not supersede, the normal safety requirements prevailing in the user's country.

1.1   Proper use

WARNING/ CAUTION ⚠	**Risk of personal injury and material damage** [W1] Improper use of the Rotor-Gene Q MDx may cause personal injuries or damage to the instrument. The Rotor-Gene Q MDx must only be operated by qualified personnel who have been appropriately trained. Servicing of the Rotor-Gene Q MDx must only be performed by QIAGEN Field Service Specialists.

WARNING/ CAUTION ⚠	**Risk of personal injury and material damage** [W2] Rotor-Gene Q MDx is a heavy instrument. To avoid personal injury or damage to the instrument, take care when lifting.

WARNING/ CAUTION ⚠	**Risk of personal injury and material damage** [W3] Do not attempt to move the Rotor-Gene Q MDx during operation.

CAUTION ⚠	**Damage to the instrument** [C1] Avoid spilling water or chemicals onto the Rotor-Gene Q MDx. Damage caused by water or chemical spillage will void your warranty.

Note: In case of emergency, switch off the Rotor-Gene Q MDx at the power switch at the back of the instrument and unplug the power cord from the power outlet.

WARNING/ CAUTION	**Risk of personal injury and material damage** [W4]
⚠	Do not try to open the lid during an experiment, or while the Rotor-Gene Q MDx is spinning. Otherwise, if you overcome the lid lock and reach inside, you risk contact with parts that are hot, electrically live, or moving at high speed, and you may injure yourself and damage the instrument.

WARNING/ CAUTION	**Risk of personal injury and material damage** [W5]
⚠	If you need to stop an experiment quickly, turn off the power to the instrument, then open the lid. Let the chamber cool before reaching inside. Otherwise you risk injury by touching parts that are hot.

WARNING/ CAUTION	**Risk of personal injury and material damage** [W6]
⚠	If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

WARNING/ CAUTION	**Risk of personal injury and material damage** [W7]
⚠	Loose paper underneath the Rotor-Gene Q MDx interferes with instrument cooling. It is recommended that the area beneath the instrument is kept free of clutter.

CAUTION	**Damage to the instrument** [C2]
⚠	Always use a locking ring on the rotor. This stops caps from coming off tubes during an experiment. If caps come off during an experiment, they may damage the chamber.

CAUTION	Damage to the instrument	[C3]
⚠️	Visually inspect and make sure the rotor is not damaged or deformed before each run.	

If you touch the Rotor-Gene Q MDx during an experiment, while you are charged with static electricity, in severe cases the Rotor-Gene Q MDx may reset. However, the software will restart the Rotor-Gene Q MDx and continue the experiment.

1.2 Electrical safety

Disconnect the line power cord from the power outlet before servicing.

WARNING	Electrical hazard	[W8]
⚡	Any interruption of the protective conductor (earth/ground lead) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous.	
	Intentional interruption is prohibited.	
	Lethal voltages inside the instrument	
	When the instrument is connected to line power, terminals may be live, and opening covers or removing parts is likely to expose live parts.	

To ensure satisfactory and safe operation of the Rotor-Gene Q MDx, follow the advice below:

■ The line power cord must be connected to a line power outlet that has a protective conductor (earth/ground).
■ Do not adjust or replace internal parts of the instrument.
■ Do not operate the instrument with any covers or parts removed.
■ If liquid has spilled inside the instrument, switch off the instrument, disconnect it from the power outlet, and contact QIAGEN Technical Services.

If the instrument becomes electrically unsafe, prevent other personnel from operating it, and contact QIAGEN Technical Services; the instrument may be electrically unsafe when:

■ It or the line power cord appears to be damaged.
■ It has been stored under unfavorable conditions for a prolonged period.
■ It has been subjected to severe transport stresses.

WARNING	Electrical hazard	[W9]
	The instrument has an electrical compliance label which indicates the voltage and frequency of the power supply as well as fuse ratings. The equipment should only be operated under these conditions.	

1.3 Environment

Operating conditions

WARNING	Explosive atmosphere	[W10]
	The Rotor-Gene Q MDx is not designed for use in an explosive atmosphere.	

CAUTION	Damage to the instrument	[C4]
	Direct sunlight may bleach parts of the instrument and cause damage to plastic parts.	
	The Rotor-Gene Q MDx must be located out of direct sunlight.	

1.4   Biological safety

Specimens and reagents containing materials from biological sources should be treated as potentially infectious. Use safe laboratory procedures as outlined in publications such as *Biosafety in Microbiological and Biomedical Laboratories*, HHS (www.cdc.gov/od/ohs/biosfty/biosfty.htm).

Samples

Samples may contain infectious agents. You should be aware of the health hazard presented by such agents and should use, store and dispose of such samples according to the required safety regulations.

WARNING	**Samples containing infectious agents** [W11]
⚠	Some samples used with this instrument may contain infectious agents. Handle such samples with the greatest of care and in accordance with the required safety regulations.
	Always wear safety glasses, 2 pairs of gloves, and a lab coat.
	The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are suitably trained and not exposed to hazardous levels of infectious agents as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH,† or COSHH‡ documents.
	Venting for fumes and disposal of wastes must be in accordance with all national, state and local health and safety regulations and laws.

1.5 Chemicals

WARNING	**Hazardous chemicals** [W12]
⚠️	Some chemicals used with this instrument may be hazardous or may become hazardous after completion of the protocol run.
	Always wear safety glasses, gloves, and a lab coat.
	The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA,* ACGIH,† or COSHH‡ documents.
	Venting for fumes and disposal of wastes must be in accordance with all national, state and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

† ACGIH: American Conference of Government Industrial Hygienists (United States of America).

‡ COSHH: Control of Substances Hazardous to Health (United Kingdom).

Toxic fumes

If working with volatile solvents or toxic substances, you must provide an efficient laboratory ventilation system to remove vapors that may be produced.

1.6 Waste disposal

Used consumables and plasticware may contain hazardous chemicals or infectious agents. Such wastes must be collected and disposed of properly according to local safety regulations.

1.7 Mechanical hazards

The lid of the Rotor-Gene Q MDx must remain closed during operation of the instrument.

WARNING	Moving parts [W13]
⚠	To avoid contact with moving parts during operation of the Rotor-Gene Q MDx, the instrument must be operated with the lid closed.

WARNING/ CAUTION	Risk of personal injury and material damage [W14]
⚠	Open and close the lid of the Rotor-Gene Q MDx carefully to avoid trapping fingers or clothing.

CAUTION	Damage to the instrument [C5]
⚠	Make sure that the rotor and locking ring are installed correctly. If the rotor or locking ring show signs of mechanical damage or corrosion, do not use the Rotor-Gene Q MDx; contact QIAGEN Technical Services.

CAUTION	Damage to the instrument [C6]
⚠	When the Rotor-Gene Q MDx is started immediately after delivery in cold climates, mechanical parts can stall. Allow the instrument to acclimatize to room temperature for at least an hour before turning the instrument on.

WARNING	Moving parts [W15]
⚠	In case of breakdown caused by power failure, remove the power cord and wait 10 minutes before attempting to manually open the lid.

WARNING	Risk of overheating [W16]
⚠️	To ensure proper ventilation, maintain a minimum clearance of 10 cm at the sides and rear of the Rotor-Gene Q MDx.
	Slits and openings that ensure the ventilation of the Rotor-Gene Q MDx must not be covered.

1.8 Heat hazard

WARNING	Hot surface [W17]
⚠️	The Rotor-Gene Q MDx chamber can reach temperatures above 120°C (248°F). Avoid touching it when it is hot.

WARNING	Hot surface [W18]
⚠️	When pausing a run, the Rotor-Gene Q MDx will not be cooled completely to room temperature. Exercise caution before handling the rotor or any tubes in the instrument.

1.9 Maintenance

Perform the maintenance as described in Section 9, page 9-1. QIAGEN charges for repairs that are required due to incorrect maintenance.

WARNING/ CAUTION	Risk of personal injury and material damage [W19]
⚠️	Only perform maintenance that is specifically described in this user manual.

WARNING	Risk of fire [W20]
⚠️	When cleaning the Rotor-Gene Q MDx with alcohol-based disinfectant, leave the Rotor-Gene Q MDx lid open to allow flammable vapors to disperse.
	Only clean the Rotor-Gene Q MDx when the chamber has cooled down.

WARNING/ CAUTION	Risk of electric shock [W21]
⚡	Do not disassemble the Rotor-Gene Q MDx instrument.

CAUTION	Damage to the instrument housing [C7]
⚠️	Never clean the instrument housing with alcohol or alcohol-based solutions. Alcohol will damage the housing. To clean the housing, use distilled water only.

1.10 Symbols on the Rotor-Gene Q MDx

Symbol	Location	Description
⚠️	Near the sample chamber, visible when lid is open	Heat hazard — the temperature of the chamber can reach temperatures above 120°C (248°F)
📖ℹ️	Back of the instrument	Consult instructions for use

Symbol	Location	Description
$C\epsilon$	Type plate on the back of the instrument	CE marking for European Conformity
IVD	Type plate on the back of the instrument	In vitro diagnostic medical device
(CSA) c US	Type plate on the back of the instrument	CSA listing mark for Canada and the USA
(manufacturer symbol)	Type plate on the back of the instrument	Legal manufacturer
(WEEE symbol)	Type plate on the back of the instrument	WEEE mark for Europe
FCC	Type plate on the back of the instrument	FCC mark of the United States Federal Communications Commission
(RCM symbol)	Type plate on the back of the instrument	RCM mark for Australia (supplier identification N17965)
(25)	Type plate on the back of the instrument	RoHS mark for China (the restriction of the use of certain hazardous substances in electrical and electronic equipment)

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2 Introduction

Thank you for choosing the Rotor-Gene Q MDx (cat. no. 9002035). We are confident it will become an integral part of your laboratory.

Before using the Rotor-Gene Q MDx, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

2.1 General information

2.1.1 Technical assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Services departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the Rotor-Gene Q MDx or QIAGEN products in general, do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, call the QIAGEN Technical Services department (see back cover) or local distributors.

For up-to-date information about the Rotor-Gene Q MDx, visit www.qiagen.com/products/rotor-geneqmdx.aspx.

2.1.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services.

2.1.3 Version management

This document is the *Rotor-Gene Q MDx User Manual*, version 2.0, revision R2 for Rotor-Gene Q MDx instruments using Rotor-Gene Q software version 2.3.4, or higher.

2.2 Intended use of the Rotor-Gene Q MDx

The Rotor-Gene Q MDx instrument is designed to perform real-time thermal cycling, detection and/or quantification using the polymerase chain reaction (PCR) in clinical applications.

The Rotor-Gene Q MDx instrument is intended to be used only in combination with QIAGEN kits indicated for use with Rotor-Gene Q instruments, for applications described in the respective QIAGEN kit handbooks.

If the Rotor-Gene Q MDx instrument is used with kits other than QIAGEN kits, it is the user's responsibility to validate the performance of such product combination for any particular application.

The Rotor-Gene Q MDx instrument is intended for in vitro diagnostic use.

The Rotor-Gene Q MDx instrument is intended for use by professional users, such as technicians and physicians trained in molecular biological techniques and the operation of the Rotor-Gene Q MDx instrument.

3 General Description

The Rotor-Gene Q MDx is an innovative instrument that enables high-precision real-time PCR and is highly suited for in vitro diagnostic applications, in combination with QIAGEN IVD marked kits.

The powerful and user-friendly software provides simplicity for beginners as well as an open experimental platform for advanced users.

Air vents on lid
Lid handle
Rotor chamber
Instrument status lights
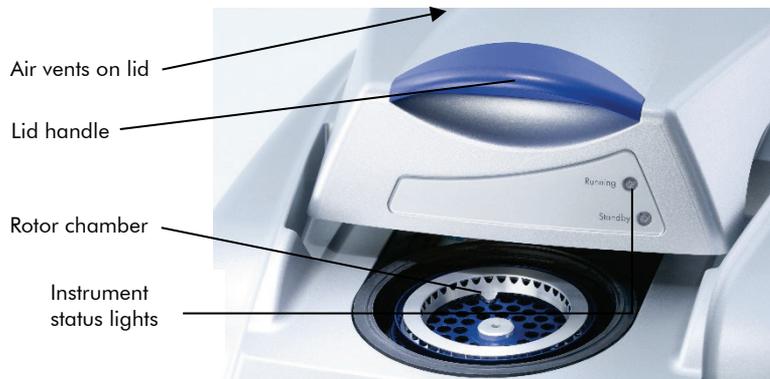
3.1 Thermal performance

The Rotor-Gene Q MDx uses a sophisticated heating and cooling design to achieve optimal reaction conditions. The unique rotary format ensures optimal thermal and optical uniformity between samples which is critical for precise and reliable analysis.

Samples spin continually at 400 rpm during a run. Centrifugation prevents condensation and removes air bubbles, but does not pellet DNA. In addition, samples do not need to be spun down prior to a run.

Samples are heated and cooled in a low-mass–air oven. Heating is achieved by a nickel-chrome element in the lid. The chamber is cooled by venting the air out through the top of the chamber while ambient air is blown up through the base.

Heating
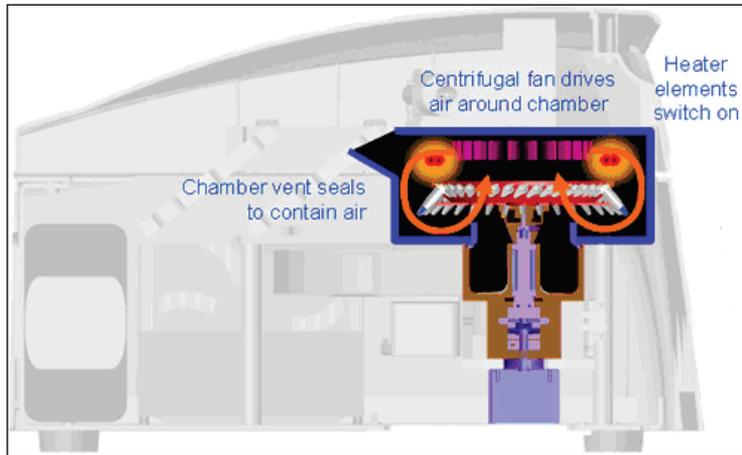
Cooling
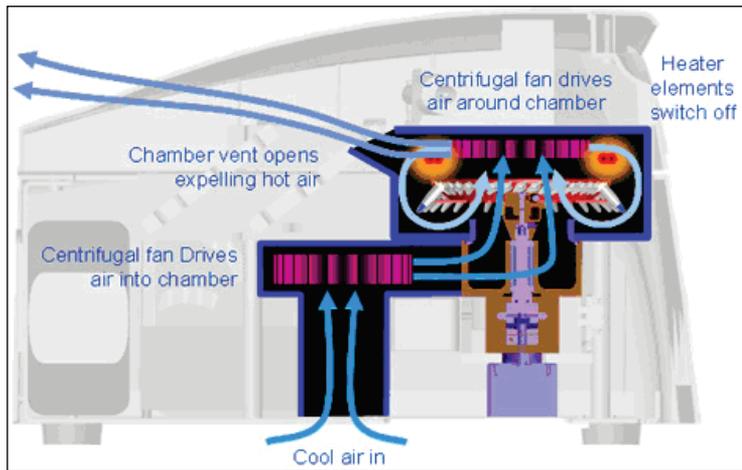
Illustration of the heating and cooling system.

3.2   Optical system

With a choice of up to 6 excitation sources and 6 detection filters combined with a short, fixed optical path, the Rotor-Gene Q MDx can be used for multiplex reactions, ensuring minimum fluorescence variability between samples and eliminating the need for calibration or compensation.

Samples are excited from the bottom of the chamber by a light-emitting diode. Energy is transmitted through the thin walls at the base of the tube. Emitted fluorescence passes through emission filters on the side of the chamber and is then collected by a photomultiplier. The fixed optical path ensures consistent excitation for every sample, which means that there is no need to use a passive internal reference dye such as ROX™.
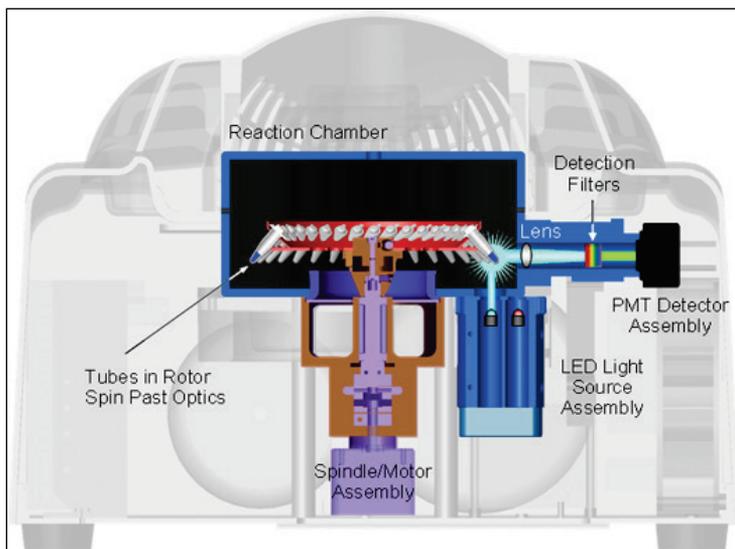
Illustration of the optical system.

Available channels

Channel	Excitation (nm)	Detection (nm)	Examples of fluorophores detected
Blue	365±20	460±20	Marina Blue®, Edans Bothell Blue, Alexa Fluor® 350, AMCA-X, ATTO 390
Green	470±10	510±5	FAM®, SYBR® Green I, Fluorescein, EvaGreen®, Alexa Fluor 488
Yellow	530±5	557±5	JOE™, VIC®, HEX™, TET™, CAL Fluor® Gold 540, Yakima Yellow®
Orange	585±5	610±5	ROX, CAL Fluor Red 610, Cy®3.5, Texas Red®, Alexa Fluor 568
Red	625±10	660±10	Cy5, Quasar® 670, LightCycler® Red640, Alexa Fluor 633
Crimson	680±5	712 high pass	Quasar 705, LightCycler Red705, Alexa Fluor 680
High resolution melt (HRM)	460±20	510±5	SYBR Green I, SYTO®9, LC Green®, LC Green Plus+, EvaGreen

Note: QIAGEN kits indicated for use with the Rotor-Gene Q MDx instruments are optimized with respect to certain dye combinations. Please refer to the corresponding kit handbooks for more information.

4 Installation Procedures

4.1 Site requirements

The Rotor-Gene Q MDx instruments must be located out of direct sunlight, away from heat sources and away from sources of vibration and electrical interference. Refer to Appendix A for the operating conditions (temperature and humidity). The installation site should be free of excessive drafts, excessive moisture, excessive dust and not subject to large temperature fluctuations.

Refer to Appendix A for the weight and dimensions of Rotor-Gene Q MDx instruments. Ensure that the workbench is dry, clean and has additional space for accessories. For further information about required specifications of the workbench, contact QIAGEN Technical Services.

Note: It is extremely important that the Rotor-Gene Q MDx instrument is placed on a stable surface that is level and vibration-free. Refer to operating conditions – see Appendix A.

The Rotor-Gene Q MDx instrument must be placed within approximately 1.5 m (59 in.) of a properly grounded (earthed) AC power outlet.

WARNING	**Explosive atmosphere** [W10]
	The Rotor-Gene Q MDx instrument is not designed for use in an explosive atmosphere.

WARNING	**Risk of overheating**	[W16]
⚠️	To ensure proper ventilation, maintain a minimum clearance of 10 cm (3.94 in.) at the rear of the Rotor-Gene Q MDx instrument.	
	Slits and openings that ensure the ventilation of the Rotor-Gene Q MDx instrument must not be covered.	

4.2　AC power connection

Power requirements

The Rotor-Gene Q MDx operates at:

■　100–240 V AC at 50–60Hz, 520 VA (peak)

Make sure that the voltage rating of the Rotor-Gene Q MDx is compatible with the AC voltage available at the installation site. Mains supply voltage fluctuations are not to exceed 10% of nominal supply voltages.

Grounding requirements

To protect operating personnel, QIAGEN recommends that the Rotor-Gene Q MDx be correctly grounded (earthed). The instrument is equipped with a 3-conductor AC power cord that, when connected to an appropriate AC power outlet, grounds (earths) the instrument. To preserve this protection feature, do not operate the instrument from an AC power outlet that has no ground (earth) connection.

Installation of AC power cord

Connect one end of the AC power cord to the socket located at the rear of the Rotor-Gene Q MDx instrument, and connect the other end to the AC power outlet.

4.3   PC requirements

The laptop computer, optionally supplied with the Rotor-Gene Q MDx, fulfills the requirements of the Rotor-Gene Q software detailed in the following table:

PC system requirements

Description	Minimum requirement
Operating system	Microsoft® Windows® 7 Professional edition (32 and 64 bit)* (Service Pack 1)
	Microsoft Windows 10 Professional edition (64 bit)
Processor†	Intel® Core™ 2 Duo 1.66 GHz or better
Main memory†	Minimum 1 GB RAM
Hard disk space†	Minimum 10 GB HDD
Graphics	Adapter and screen with at least 1200 x 800 pixels
Ports†	USB port or RS-232 serial port
DVD-ROM drive	1
Pointing device	Touchpad or mouse or equivalent is required
Bluetooth®	Must be switched off
PDF viewer, or similar	Must be installed; not part of the software installation packages
Power options	Never turn off hard disks, hibernate or go to standby

* Microsoft Windows 10 or Windows 7 Professional edition is required to run the Rotor-Gene Q software with security features (see "Access Protection", page **Error! Bookmark not defined.**). Security features are not available if the Home edition of Windows 10 or Windows 7 is used.

† When using Rotor-Gene AssayManager® version 1.0 or 2.1 software, the following minimum PC requirements are different: Intel Core i3-380M processor, 4 GB RAM main memory, 250 GB hard disk space, USB port required.

4.4 Configuration for Windows 7 security

The laptop computers that are provided by QIAGEN for use with your Rotor-Gene Q MDx instrument have Microsoft Windows 7 pre-installed and are configured with a standard (non-administrative) Windows user account and with an administrator account. In routine usage of the system, the standard account shall be used, because Rotor-Gene Q software and the Rotor-Gene AssayManager version 1.0 or 2.1 is designed to run without administrator rights. The administrator account – the one with the red desktop background – shall only be used to install the Rotor-Gene Q or the Rotor-Gene AssayManager version 1.0 or 2.1 software and an antivirus software (please see section on "Antivirus software", page 4-14). Using the administrator account is indicated by a red desktop background. Please make sure that you always log in as standard user for routine use.

Q1a#g3n!A6 is the default password of the administrator account. Please change the administrator password after first login. Please make sure that the password is secure and does not get lost. There is no password for the operator account.

If your configuration is different and no non-administrative account is contained, system administrators shall set up an additional standard Windows user account to prevent access to critical system areas, such as Program Files, Windows directory (e.g. access to installation or uninstallation functionality, including applications, operating system components, date/time settings, Windows updates, firewall, user rights & roles, antivirus activation) or performance-relevant settings like power saving.

To create a standard user account in Windows 7, please follow these steps described in section "Creating a new user account":

Open the Windows control panel via the Start menu and select "User Accounts" and then "Manage Accounts".

1. Choose "Create a new account".
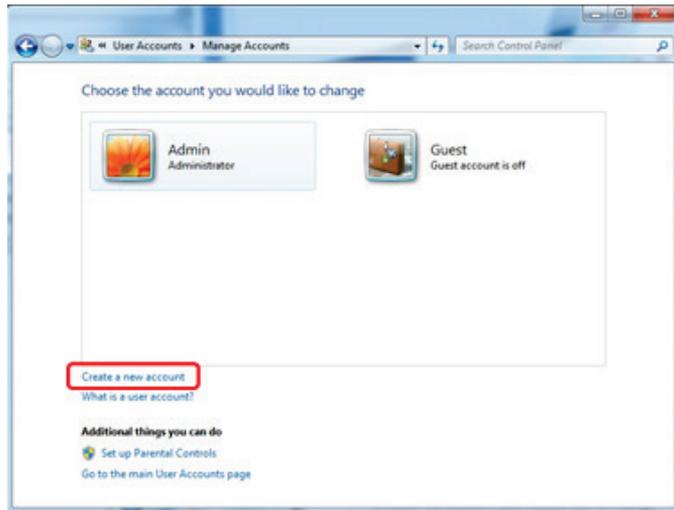
2. Name the account and select "Standard User" as the account type.
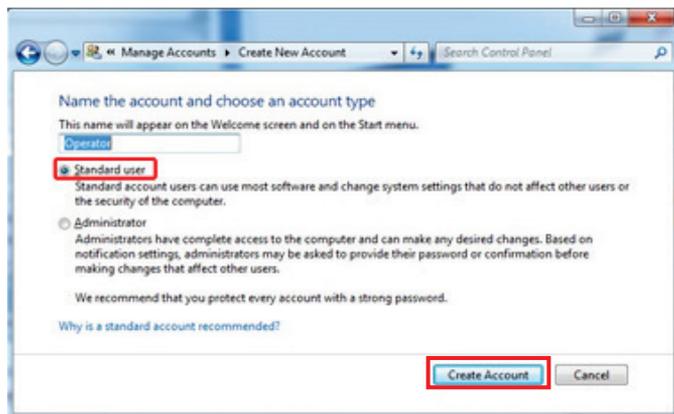
3. Click "Create Account".

4.5 Unpacking the Rotor-Gene Q MDx

The Rotor-Gene Q MDx is delivered with all the necessary components for setting up and running the instrument. The box also contains a list of all the components provided.

Note: Check this list for completeness to ensure that all the components are present.

Note: Check that the instrument and delivered accessories are free from transport damage before installation.

The accessories box sits on top of the foam packing. The accessories box contains:

■ Installation guide (English; translations available on the CD with manuals)
■ CD (software)
■ CD (manuals)
■ Loading Block 96 x 0.2 ml Tubes
■ Loading Block 72 x 0.1 ml Tubes
■ Rotor Holder (dismantled for safe transport)
■ 36-Well Rotor (this rotor is red in color)
■ 36-Well Rotor Locking Ring

The following items are packed on each side of the foam packing:

■ USB and RS-232 serial cable
■ International power cable set
■ PCR Tubes, 0.2 ml (1000)
■ Strip Tubes and Caps, 0.1 ml (1000)

Once all these components have been removed from the box, remove the foam packing on top of the Rotor-Gene Q MDx. Carefully remove the Rotor-Gene Q MDx from the box and unwrap the plastic cover. Open the lid by sliding it towards the back to access the reaction chamber.

The following items are already installed inside the Rotor-Gene Q MDx:

■ 72-Well Rotor (this rotor is blue in color)

■ 72-Well Rotor Locking Ring

A laptop computer may be included in the packaging, depending on your order details.

4.6 Accessories

Rotor-Discs and accessories can be ordered separately for use with the Rotor-Gene Q MDx. For more details, see Appendix C.

4.7 Hardware installation

Once the Rotor-Gene Q MDx has been unpacked, proceed with installation as described below.

CAUTION	Damage to the instrument [C6]
⚠	When the Rotor-Gene Q MDx is started immediately after delivery in cold climates, mechanical parts can stall. Allow the instrument to acclimatize to room temperature for at least an hour before turning the instrument on.

Proceed as follows:

1. Place the Rotor-Gene Q MDx on a level surface.

2. Ensure that there is sufficient space behind the instrument for the lid to open fully.

3. Ensure that the power switch at the back of the instrument can be reached easily.

4. Do not obstruct the back of the instrument. Ensure that the power cord can be easily detached if required, to disconnect power to the instrument.

5. Connect the USB cable or RS-232 serial cable, which was supplied with the instrument, to a USB or communications port on the back of the computer.

6. Connect the USB or RS-232 serial cable to the back of the Rotor-Gene Q MDx.

7. And then connect the Rotor-Gene Q MDx to the power supply. Connect one end of the AC power cord to the socket located at the rear of the Rotor-Gene Q MDx and the other end to the AC power outlet.

On/off switch

Power supply port

Type plate including serial number

Cooling fan   Serial port   USB port
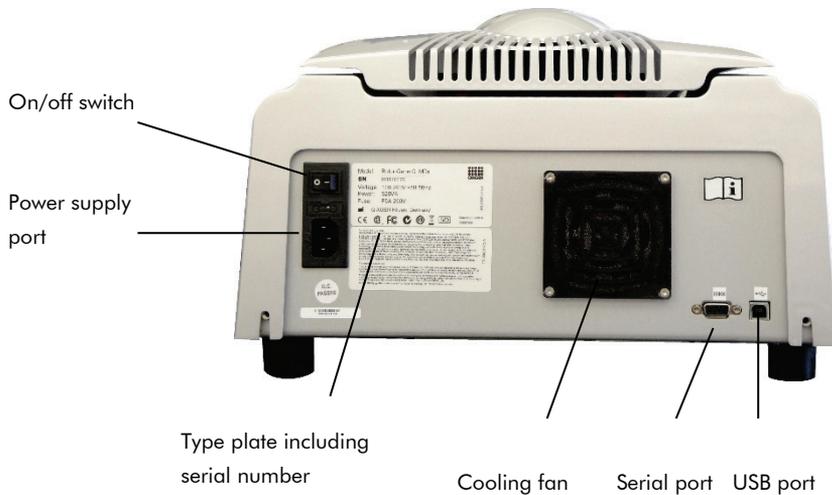
Note: Only connect the Rotor-Gene Q MDx to the computer with the USB and serial cables delivered with the instrument. Do not use other cables.

4.8   Software installation

1. To install the Rotor-Gene Q software, insert the CD (software) delivered with the instrument into the CD drive of the computer.

2. Select "Install Operating Software" in the window that appears.

    Note: Please refer to the *Rotor-Gene Q Installation Guide* provided with the instrument for easy installation and to guide you through the next steps of software installation.
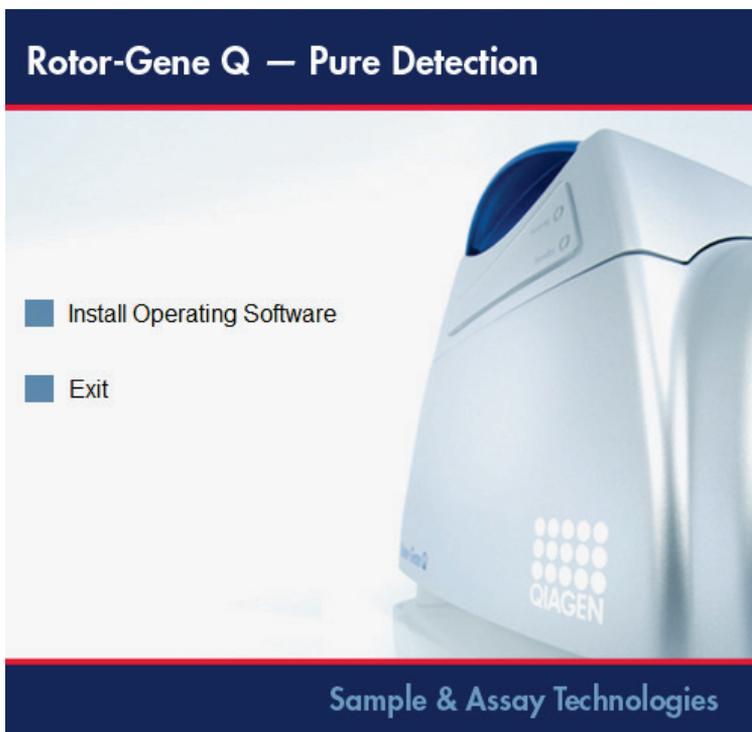
3. Once the software has been installed, a desktop icon will be created automatically.

4. Switch on the Rotor-Gene Q MDx by moving the switch, located at the back on the left-hand side, to the "I" position. A blue "Standby" light on the front of the Rotor-Gene Q MDx indicates that the instrument is ready for use.
Note: When starting connected to a computer for the first time, the Rotor-Gene Q MDx will be recognized by the operating system and a number of messages will appear. Please refer to the *Rotor-Gene Q Installation Guide* provided with the instrument (CD and printed edition) for guidance.

5. Double-click the "Rotor-Gene Q Series Software" desktop icon to initiate the software.

6. A "Welcome" window appears the first time the software is started, but does not appear for subsequent software upgrades.
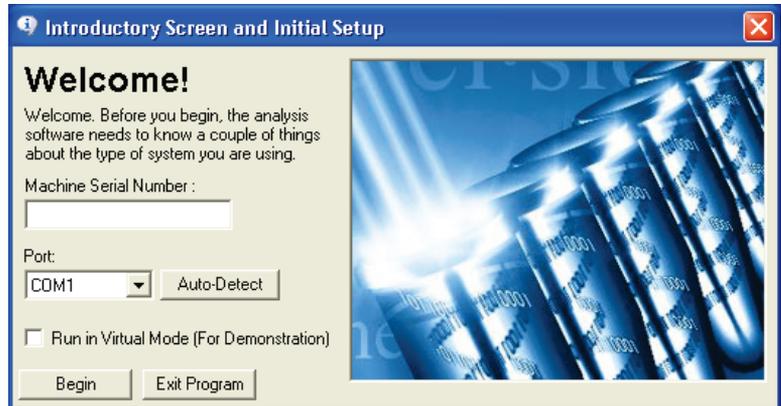
Machine Serial Number:	Type in the serial number (7 digits), which can be found on the back of the Rotor-Gene Q MDx.
Port:	Choose either USB or serial cable. Select the appropriate communications port or click the "Auto-Detect" button.
Auto-Detect:	When using this option, the corresponding USB or serial port will be detected automatically and displayed in the "Port" drop-down list.

Run in Virtual Mode (for demonstration):	Checking this box allows installation of the Rotor-Gene Q software on a computer that is not connected to a Rotor-Gene Q MDx. The software is fully functional and can simulate runs.
	Note: If this box is checked and a Rotor-Gene Q MDx is connected to the computer, the following message appears before the run starts: "You are about to run in Virtual mode". To perform a real run, the setup must be changed in the "Setup" window (see Section 7.5.4).
Begin:	When all the information has been entered, click "Begin". Wait until initialization is finished, which may take a few seconds. If virtual mode was chosen the following message appears:
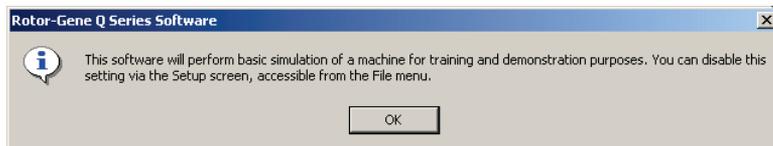
	If the "Run in Virtual Mode" box is unchecked, the software initializes and opens automatically.
Exit Program:	Clicking on this button exits the program.

4.9   Software version

To find out your version number, click "Help" then "About This Software…".
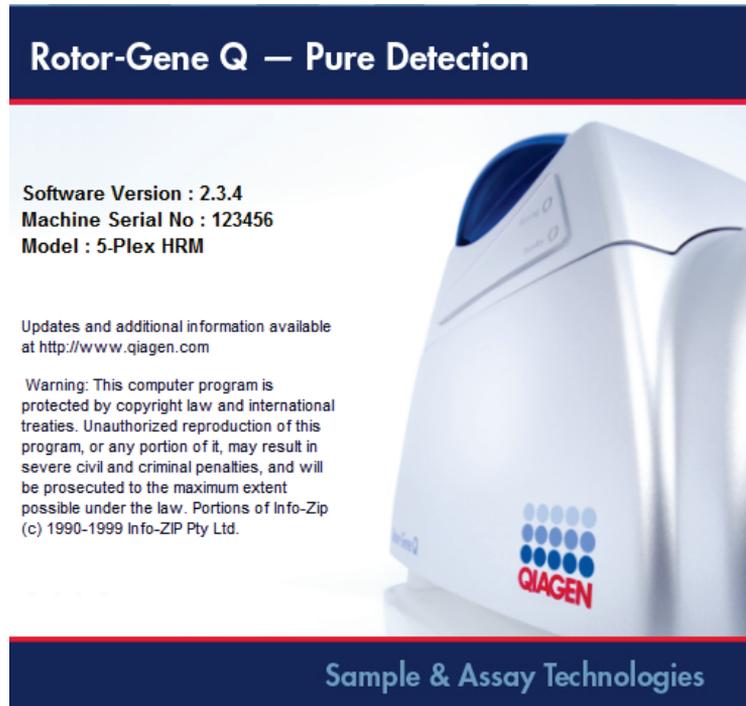
This window displays general information about the software, including the version of the software and the serial number and model of the instrument.

The software may be freely copied for use within an organization that owns a Rotor-Gene Q MDx. The software may not be copied and distributed to others outside the organization.

4.10 Additional software on computers connected to Rotor-Gene Q MDx instruments

Rotor-Gene Q software manages time-critical processes during the PCR run and the data acquisition process. For this reason, it is important to ensure that no other processes use significant system resources and thus slow down the Rotor-Gene Q software. It is particularly important to pay attention to the points listed below.

System administrators are advised to consider any impact that a modification to the system may have on the resources before implementing it.

4.10.1 Antivirus software

QIAGEN is aware of the threat that computer viruses pose to any computer that exchanges data with other computers. Rotor-Gene AssayManager versions 1.0 and 2.1 are expected to be primarily installed in environments where local policies are in place to minimize this threat. However, QIAGEN recommends the use of an antivirus software in any case. The selection and installation of an appropriate virus scanning tool is in the customer's responsibility. However, QIAGEN has validated the Rotor-Gene Q software and Rotor-Gene AssayManager versions 1.0 or 2.1 with the QIAGEN laptop in combination with the following two antivirus software to show compatibility:

- Symantec® Endpoint Protection V12.1.6
- Microsoft Security Essentials V4.10.2091

Please refer to the product page on QIAGEN.com for the latest versions of antivirus software that have been validated in combination with the Rotor-Gene Q software and Rotor-Gene AssayManager version 1.0 or 2.1.

1 **Note**: After installation of "Microsoft Security Essentials", you should check Windows updates are deactivated since the installation might activate this setting (please read chapter "Operating system updates").

If an antivirus software is selected, make sure that it can be configured in a way that the database folder path can be excluded from the scan. Otherwise, there is the risk of database connection errors. Since Rotor-Gene AssayManager version 1.0 and 2.1 create new database archives dynamically, it is required to exclude the folder path to the files and not single files. We do not recommend the use of antivirus software where only single files can be excluded, e.g. McAfee Antivirus Plus V16.0.5. If the computer is used in an environment without network access, please also make sure that the antivirus software supports offline updates.

To get consistent results after installation of an antivirus software, a system administrator should ensure the following:

■ As explained above, the database folder path of the Rotor-Gene AssayManager 1.0 and 2.1 (C:\Program Files\Microsoft SQL Server\ MSSQL10_50.RGAMINSTANCE\MSSQL\DATA) needs to be excluded from file scans.
■ Updates to the virus database are not performed when the Rotor-Gene AssayManager 1.0 or 2.1 is in use.
■ All full or partial scans of the hard drive are disabled during real-time PCR data acquisition. Otherwise there is a risk of adverse impact on the performance of the instrument.

Please read the manual of your selected antivirus software for configuration details.

4.10.2   Firewall and networks

The Rotor-Gene Q software can run either on computers without network access, or can run in a network environment, if a remote database server is used. For networked operation, the firewall on the laptop computer provided by QIAGEN is configured in a way that inbound traffic is blocked for all ports, except those ones required to establish a network connection.

Please note that blocking incoming connections does not affect responses to requests triggered by the user. Outgoing

connections are allowed as this may be required for retrieving updates.

If your configuration is different, QIAGEN recommends to configure the firewall in the same way as described above. To this end, a system administrator must login and perform the following steps:

1. Open the "Control Panel" and select "Windows Firewall".
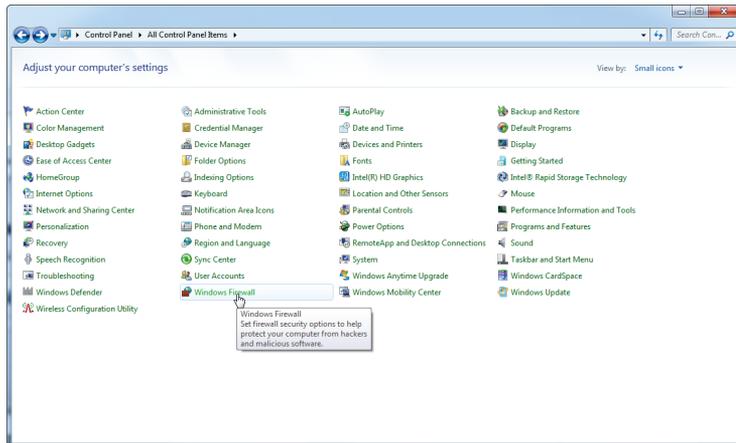
2. Select "Use recommended settings".
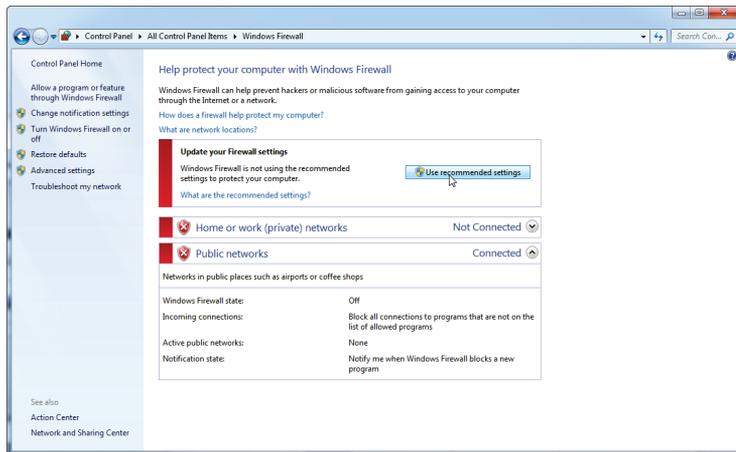
3. Check that the following settings are active:
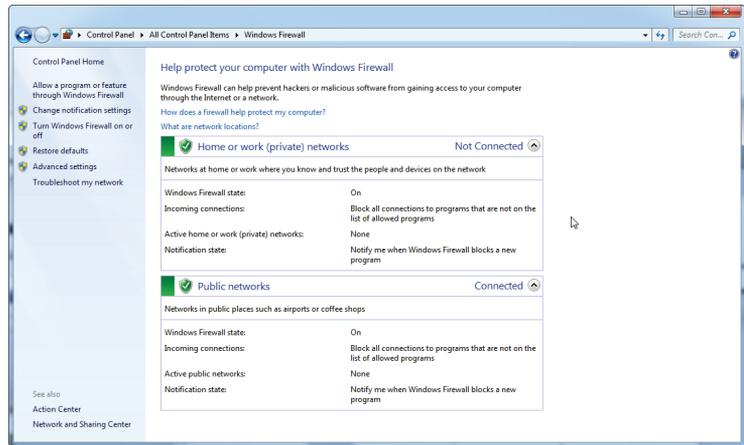
For security and reliability reasons cable-based network access shall be used instead of Wi-Fi. The laptop computers that are provided by QIAGEN have a disabled Wi-Fi adapter. If your configuration is different, a system administrator must disable the Wi-Fi adapter manually; this can be done through the following steps:

1. Open the "Control Panel" and select "Network and Sharing Center".
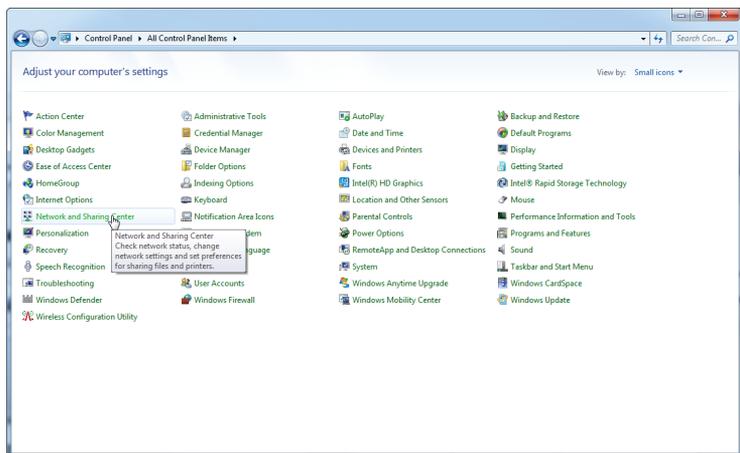
2. Select "Change adapter settings".
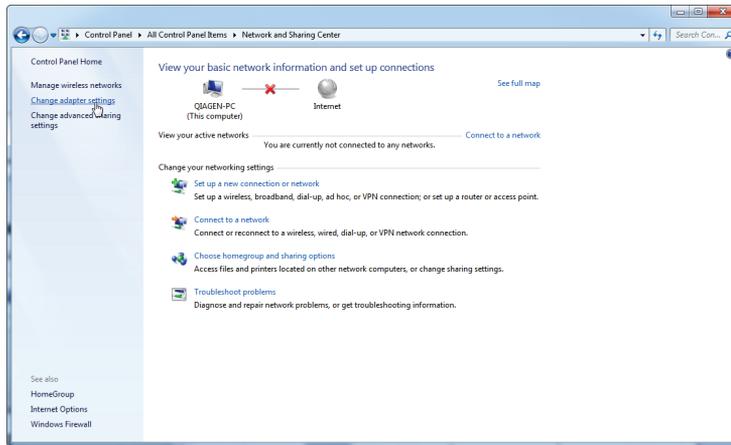
3. Hover over "Wireless Network Connection", press the right mouse button and select "Disable" from the context menu.
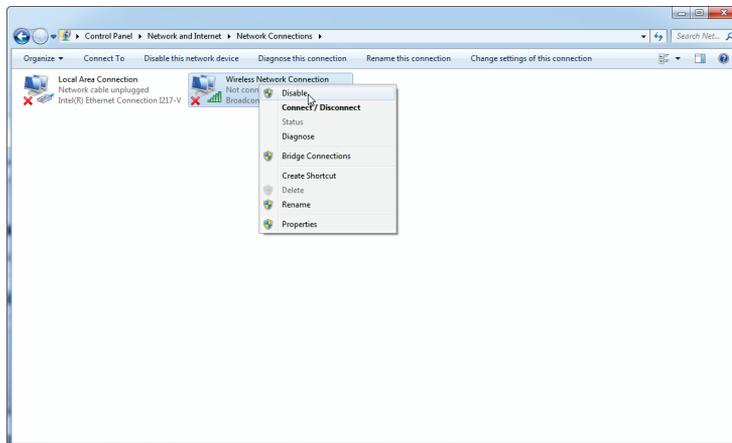
4. Check that the Wireless Network Connection is disabled.
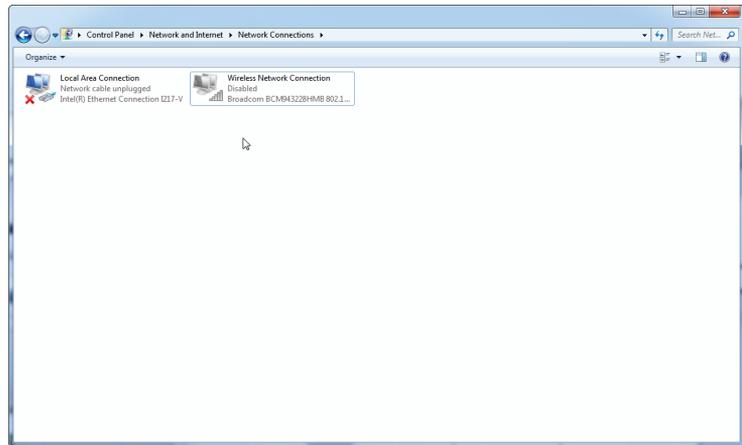
4.10.3   System tools

Many system tools may use significant system resources even without any user interaction. Typical examples of such tools are:

■   File indexing, which is performed as a background task by many contemporary office applications
■   Disk defragmentation, which often also employs a background task
■   Any software that checks for updates on the internet
■   Remote monitoring and management tools

Please be aware that due to the dynamic nature of the IT world, this list may not be complete and tools may be released that are not known at the time of writing. It is important that system administrators take care that such a tool is not active during a PCR run.

4.10.4 Operating system updates

The laptop computers provided by QIAGEN is configured in a way that automatic updates of the operating system are disabled. If your configuration is different, a system administrator must disable any automatic update process of the operating system which can be done by the following steps:

1. Open the "Control Panel" and select "Windows Update".
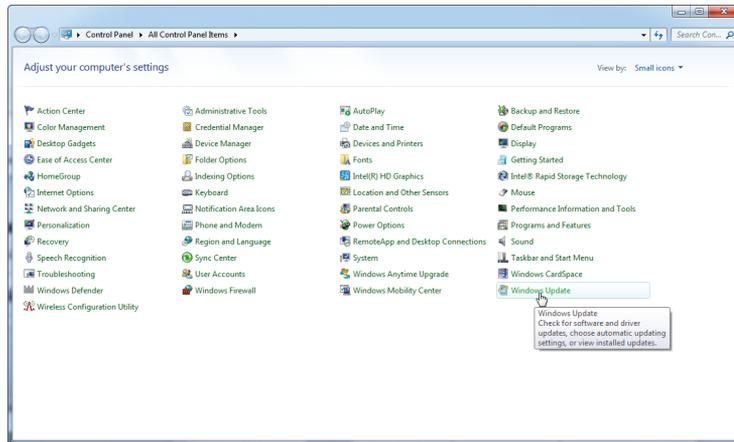
2. Select "Change settings".
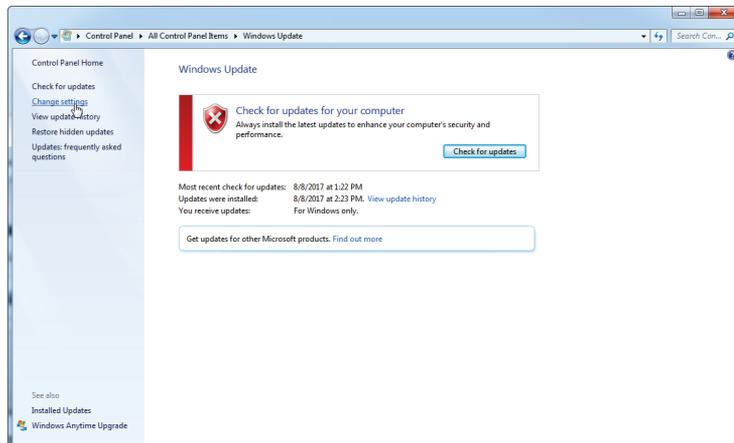
3. Select "Never check for updates".
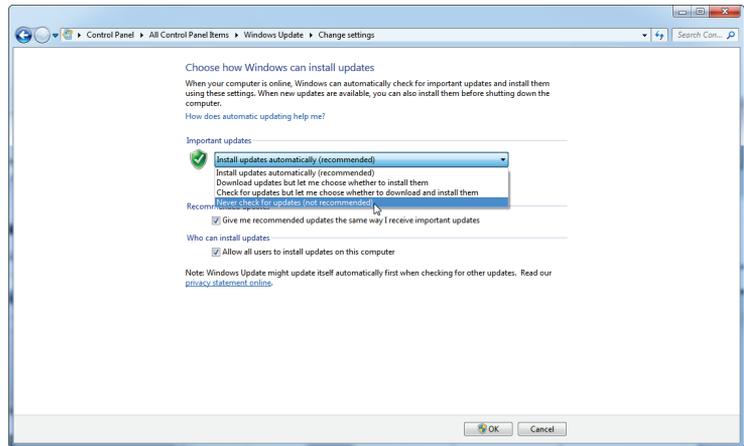
4. Check that the "Important updates" option "Never check for updates" is active.
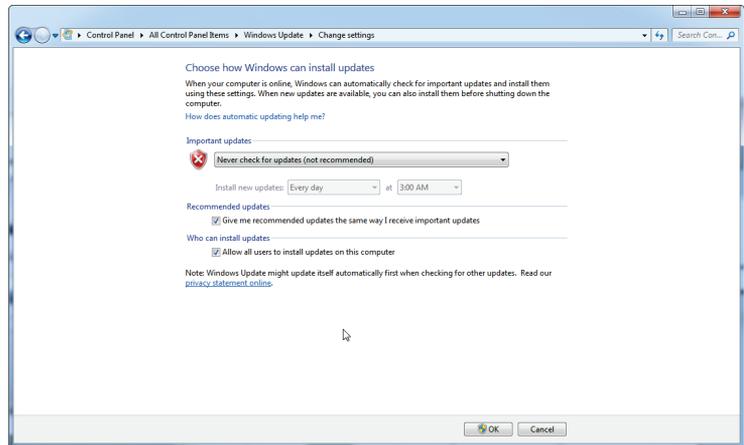
QIAGEN designed the QIAGEN System Updater to facilitate Windows updates in a controlled manner. The QIAGEN System Updater is only for use with your QIAGEN laptop computer, in conjunction with Microsoft Windows 7 or Microsoft Windows 10.

The QIAGEN System Updater will only allow Windows updates after they have been tested and verified with QIAGEN software. Windows security updates provided by the QIAGEN System Updater will ensure that your system is performing as intended.

The QIAGEN System Updater and the corresponding instructions for use can be downloaded from www.qiagen.com. The instructions for use will provide a guide for the installation and usage of the QIAGEN System Updater.

4.11 Updating software

Software updates are available from the QIAGEN website at www.qiagen.com/products/rotor-geneqmdx.aspx, which can be accessed also from the "Help" menu in the software. To download the software, it is necessary to register online.

5 Operating Procedures — Hardware

This section describes operation of the Rotor-Gene Q MDx.

5.1 Rotor types

First, select which tube type and rotor to use. There are 4 rotors available to accommodate different tube types.

Note: 36-Well Rotor and 72-Well Rotor are delivered with the instrument. The Rotor-Disc® Rotors are accessories.

IMPORTANT: Use identical tubes in a run. Do not mix different tube types or tubes from different manufacturers, because this will affect optical uniformity. We recommend use of tubes from QIAGEN, which are specially designed for use with the Rotor-Gene Q MDx (see Appendix C). Tubes from alternative manufacturers may autofluoresce, which could affect the reliability of results. In addition, tubes from alternative manufacturers can vary in length and thickness, resulting in misalignment of the optical path of the Rotor-Gene Q MDx and the reaction in the tube. QIAGEN reserves the right to refuse technical support for problems induced by non–QIAGEN-certified plastic materials on the Rotor-Gene Q MDx instrument.

IMPORTANT: Any use of non–QIAGEN-certified plastic materials on the Rotor-Gene Q MDx may void your instrument warranty.

CAUTION	Damage to the instrument	[C3]
⚠️	Visually inspect and make sure the rotor is not damaged or deformed before each run.	

36-Well Rotor

The 36-Well Rotor is red in color. The 36-Well Rotor and 36-Well Rotor Locking Ring enable the use of 0.2 ml tubes. The tubes do not need to have optically clear caps because the Rotor-Gene Q MDx reads fluorescence from the bottom of the tube rather than from the top. Domed capped tubes can also be used.

72-Well Rotor

The 72-Well Rotor is blue in color. The 72-Well Rotor and 72-Well Rotor Locking Ring are used with Strip Tubes and Caps, 0.1 ml, which can be used for volumes as low as 20 μl. The caps provide a safe and reliable seal.

Rotor-Disc 72 Rotor

The Rotor-Disc 72 Rotor is dark gray in color. The Rotor-Disc 72 Rotor and Rotor-Disc 72 Locking Ring enable use of the Rotor-Disc 72. The Rotor-Disc 72 is a disc with 72 wells for high-throughput use. To seal the Rotor-Disc 72, a clear polymer film is applied to the top and heat sealed. The film is quick and easy to apply, and it prevents contamination by providing a strong, durable and tamper-proof seal. For more information on the Rotor-Disc 72, see Section 5.1.

Rotor-Disc 100 Rotor

The Rotor-Disc 100 Rotor is gold in color. The Rotor-Disc 100 Rotor and Rotor-Disc 100 Locking Ring enable use of the Rotor-Disc 100. The Rotor-Disc 100 is a disc with 100 wells for high-throughput use. The Rotor-Disc 100 is the rotary equivalent of a 96-well plate but with an additional 4 reference wells. It enables integration of the Rotor-Gene Q MDx with 96-well laboratory workflows. The extra wells can be conveniently used for more samples, additional control reactions or orientation reactions, without occupying any of the standard 96-well positions.

For seamless 96-well workflow compatibility, Rotor-Disc 100 wells use 96-well plate labeling conventions, i.e., A1–A12 through to H1–H12. The additional 4 reference wells are labeled R1–R4. For more information on the Rotor-Disc 100, see Section 5.1.

Rotor specifications

Rotor type	Well capacity	Sample no.	Tube type	Recommended reaction volume
36-Well Rotor	200 μl	36	PCR Tubes, 0.2 ml	20–50 μl
72-Well Rotor	100 μl	72	Strip Tubes and Caps, 0.1 ml	20–50 μl
Rotor-Disc 72 Rotor	100 μl	72	Rotor-Disc 72	20–25 μl
Rotor-Disc 100 Rotor	30 μl	100	Rotor-Disc 100	15–20 μl

Note: The 36-Well Rotor and 72-Well Rotor for the Rotor-Gene Q MDx are not to be used on Rotor-Gene 3000 instruments, due to optical alignment incompatibilities. Please continue to use the older 36-position and 72-position rotors with Rotor-Gene 3000 instruments.

5.2 Reaction setup

IMPORTANT: Adequate controls should be used in each run to ensure reliable results.

Reactions can be prepared using the Loading Block 96 x 0.2 ml Tubes (for PCR Tubes, 0.2 ml), the Loading Block 72 x 0.1 ml Tubes (for Strip Tubes and Caps, 0.1 ml set up with a single-channel pipet), the Loading Block 72 x 0.1 ml Multi-channel (for Strip Tubes and Caps, 0.1 ml set up with a multichannel pipet), the Rotor-Disc 72 Loading Block (for the Rotor-Disc 72) or the Rotor-Disc 100 Loading Block (for the Rotor-Disc 100). All blocks are made of aluminum and can be precooled.

The Loading Block 72 x 0.1 ml Tubes (pictured) holds 18 Strip Tubes as well as up to eight 0.5 ml tubes, which can be used to prepare master mix, and up to sixteen 0.2 ml tubes, which can be used to set up standard curves. The procedure below describes reaction setup using the 72-Well Rotor. The same procedure can be used for reaction setup using the 36-Well Rotor and appropriate accessories.

1. Place the Strip Tubes into the Loading Block and aliquot the reaction components.

2. Place the Caps securely on the Strip Tubes and visually inspect to confirm a tight seal.

3. Insert the Strip Tubes into the 72-Well Rotor, ensuring that each tube sits correctly in place in the correct orientation. Samples will not be optimally aligned over the detection system if not placed correctly in the rotor. This could result in a reduction in acquired fluorescence signal and detection sensitivity. A Rotor Holder that enables easy tube loading is provided with the instrument.
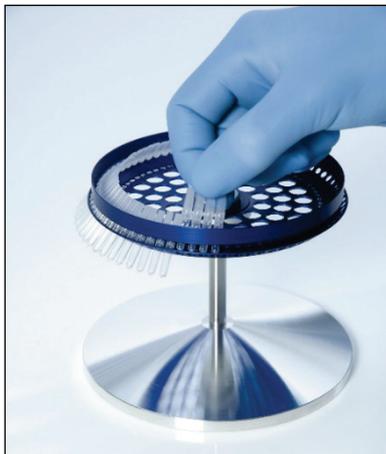
IMPORTANT: To achieve maximum temperature uniformity, each position in the rotor must contain a tube. Filling all positions in the rotor ensures even airflow to every tube. Keep a set of empty capped tubes available that can be used to fill any unused positions.

4. Insert the 72-Well Rotor Locking Ring onto the 72-Well Rotor by pushing the 3 locating pins through the outer holes of the rotor.

The Locking Ring ensures that caps remain on tubes during a run.

5. Insert the assembly into the Rotor-Gene Q MDx chamber by clicking into place using the locating pin on the rotor hub. To remove, simply push down on the rotor hub to release and pull out.

6. Close the lid and set up the run profile using the Rotor-Gene Q software.**Rotor-Disc setup**

IMPORTANT: Please read the Product Sheet supplied with the Rotor-Disc Heat Sealer before beginning this procedure.

1. Switch on the Rotor-Disc Heat Sealer using the switch located on the back, at the left-hand side.

   A red "Power" light turns on. It takes the Rotor-Disc Heat Sealer approximately 10 minutes to reach operating temperature, upon which time, a green "Ready" light turns on.

2. Choose permanent or removable seal.

   Note: Once the Rotor-Disc Heat Sealer is ready, it is safe to keep it running.

3. Insert the Rotor-Disc into the Rotor-Disc Loading Block using the Position 1 tab on the Rotor-Disc and the tube guide holes on the Rotor-Disc Loading Block.

Note: The Position 1 tab is a small square tab that protrudes on the inside of the circle of the Rotor-Disc. It fits into a corresponding cutout in the Rotor-Disc loading block.

4. Set up reactions in the Rotor-Disc by manual pipetting or using an automated liquid-handling system.

5. Take one sheet of Rotor-Disc Heat Sealing Film and remove the smaller circular cutout in its center by slightly folding the film in half without creasing, pinching the central piece and carefully tearing it out.

6. Place the film over the Rotor-Disc by sliding the circular part of the loading block through the hole you made in the film. Ensure that the "SIDE UP" wording is positioned on the rectangular protrusion of the Rotor-Disc Loading Block so that no part of the film hangs outside the perimeter of the loading block.

7. Slide the assembly into the Rotor-Disc Heat Sealer using the guide rails on the side of the Rotor-Disc Loading Block. Ensure that the Rotor-Disc Loading Block is pushed in completely.

8. To activate the sealing mechanism, first press down on the blue anodized bar at the top of the Heat Sealer, and then push back the black catch.

9. When the sealing mechanism has lowered, an orange "Sealing" light turns on. If the Rotor-Disc Loading Block is not in the correct position, a warning beep sounds.

10. When sealing is finished, a continuous beep will sound and the orange "Sealing" light starts blinking. Press down on the blue anodized bar to raise and release the sealing mechanism back to its original position.

    IMPORTANT: Do not continue sealing for any longer than indicated by the beep, or the Rotor-Disc may get deformed.

    Note: To warn if you accidentally fail to release the locking mechanism, the blinking orange "Sealing" light will stop blinking and stay steadily on instead, and the continuous beep will change to an intermittent sound.

11. Slide the Rotor-Disc Loading Block out of the Rotor-Disc Heat Sealer. Allow the film to cool for approximately 10 seconds. Remove the excess sealing film by pushing it down to detach. Do not pull the excess film upwards.

12. Remove the Rotor-Disc from the Rotor-Disc Loading Block.

13. Load the Rotor-Disc into the rotor, using the Position 1 tab as a guide to the correct orientation.This page intentionally left blank

6 Operating Procedures — Software

New runs can be set up using the Quick Start wizard or the Advanced wizard, which appear when the software is started up. The Quick Start wizard is designed to allow the user to start the run as rapidly as possible. The Advanced wizard enables more options, such as configuration of Gain Optimization and volume settings. For convenience, the wizards have a number of templates with default cycling conditions and acquisition channels. To change the wizard type, select the appropriate tab at the top of the "New Run" window.

6.1 Quick Start wizard

The Quick Start wizard allows the user to start the run as rapidly as possible. The user can select a template (i.e., a file with preconfigured parameters that can be used as is or modified before running) from a set of commonly used templates, and then enter the minimum required parameters to get started. The Quick Start wizard assumes that the reaction volume is 25 μl. For other reaction volumes, use the Advanced wizard (see Section 6.2).

As a first step, select the desired template for the run by double-clicking on the template from the list in the "New Run" window.
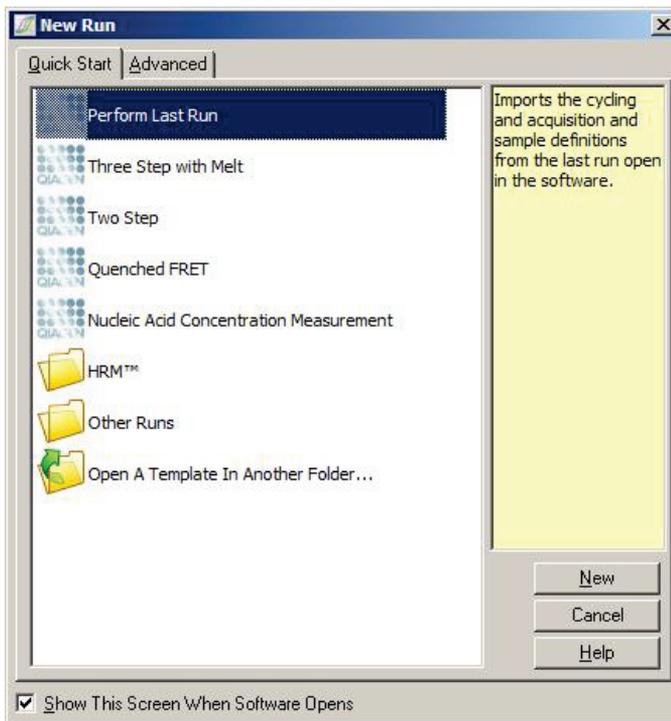
Perform Last Run: "Perform Last Run" uses the cycling, acquisition and sample definitions from the last run that is open in the software.

Three Step with Melt: This is a three-step cycling profile and a melt curve with data acquisition on the green channel.

Two Step: This is a two-step cycling profile with data acquired on green, yellow, orange and red channels.

Quenched FRET: This is a three-step cycling profile and a melt curve. Unlike Three Step with Melt, acquisition is at the end of the anneal step.

Nucleic Acid Concentration Measurement: This is a default template for measuring nucleic acid concentration using intercalating dyes.

HRM:	This folder contains high resolution melt profiles.
Other Runs:	This folder contains additional profiles.

The cycling and acquisition profiles for all templates can be altered using the wizard.

Note: User-defined templates can be added to the template list in the Quick Start wizard by copying or saving ***.ret** files to **C:\Program Files\Rotor-Gene Q Software\Templates\Quick Start Templates**. After copying a file to this path, the template will appear as an icon in the list. If you would like custom icons for your templates, create an ***.ico** image with the same file name as the template.

Subfolders can be created to group-related templates. This allows organization of templates, which could be convenient if, for example, several users are using the same instrument.

6.1.1   Rotor selection

In the next window, select the rotor type from the list.

Check the "Locking Ring Attached" checkbox, and then click "Next".
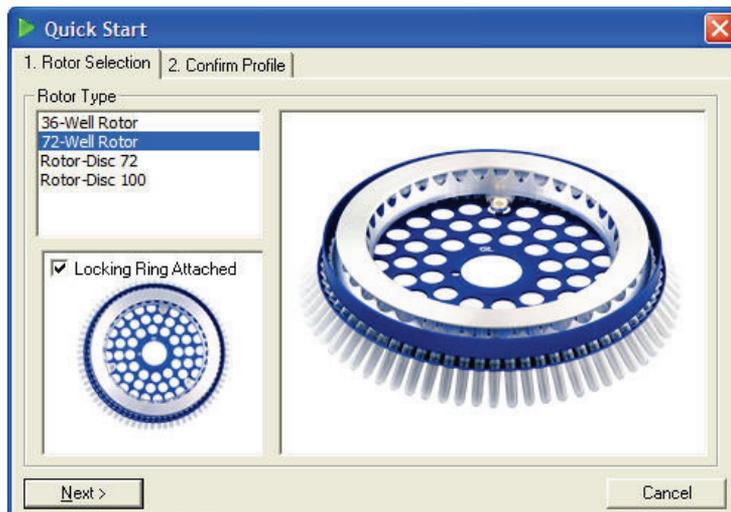
6.1.2 Confirm profile

The cycling conditions and acquisition channels of the template chosen are imported. These can be altered using the "Edit Profile" window (see Section 6.2.4).

To initiate a run, click the "Start Run" button. It is also possible to save the template before starting the run by clicking on "Save Template".
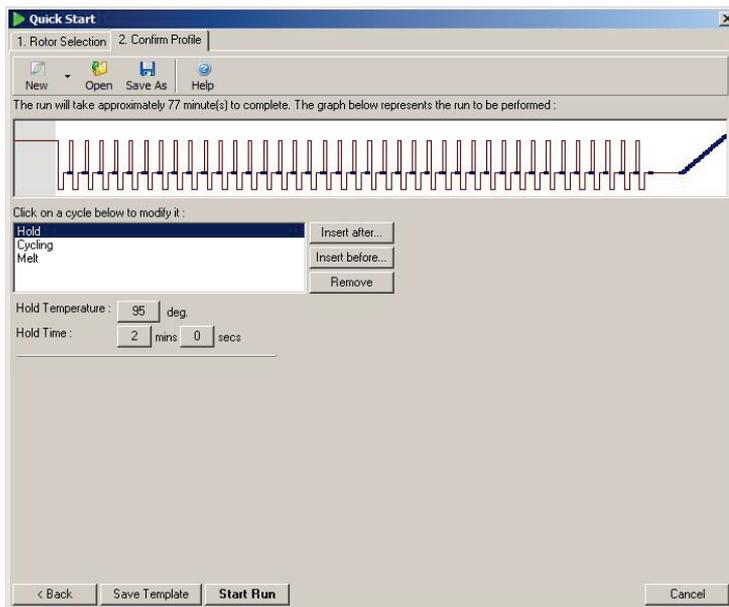
6.1.3 Save run

After you click the "Start Run" button, the "Save As" window should appear. The run can be saved in the user's desired location. The run is given a file name that consists of the template used and the date of the run. A serial number (1, 2, etc.) is also included in the file name to allow automatic naming of numerous runs that use the same template on the same day.
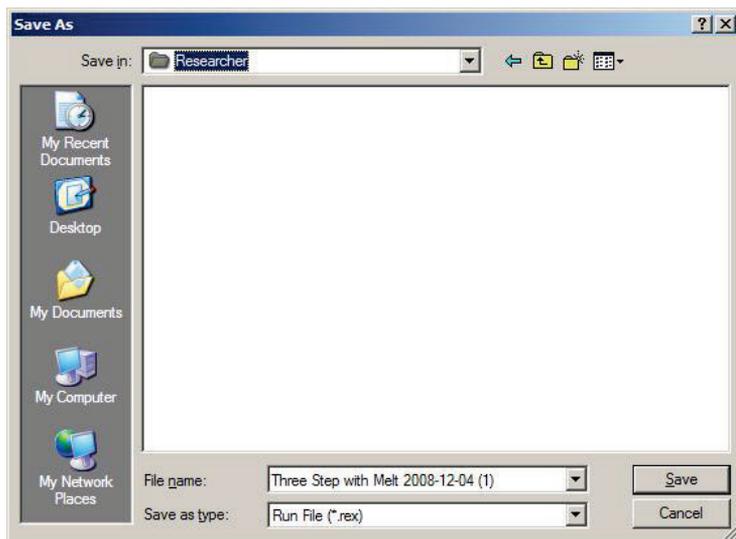
6.1.4　　　Sample setup

Once the run has started, the "Edit Samples" window allows samples to be defined and described.
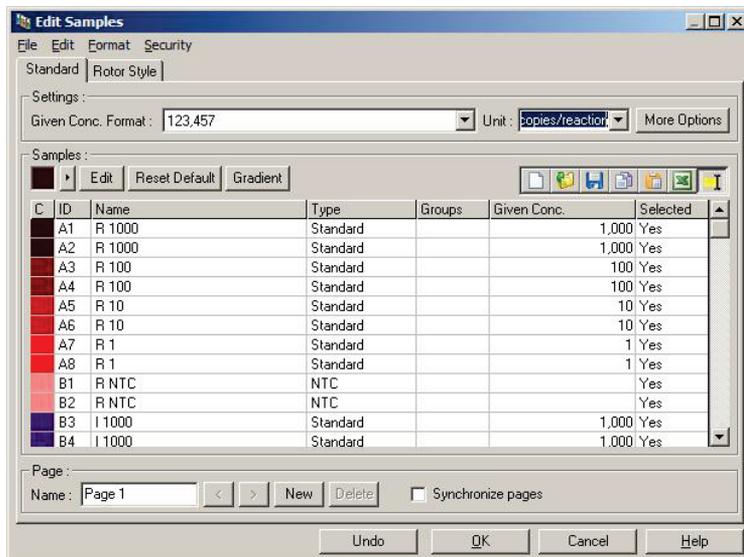
The "Edit Samples" window appears after the run has started so that the user can use this time to enter sample names. If

the sample names are entered very quickly during the run (e.g., using a bar code scanner), it can lead to transposed letters within the sample names. Therefore, it is recommended to avoid using a bar code scanner and, if applicable, enter the sample names after the run has finished. For information about setting up sample definitions in the "Edit Samples" window, see Section 7.8.4.

6.2 Advanced wizard

The Advanced wizard enables options that are not available in the Quick Start wizard, such as configuration of gain optimization.

To use the Advanced wizard, select a template by double-clicking the template name from the list under the "Advanced" tab of the "New Run" window.
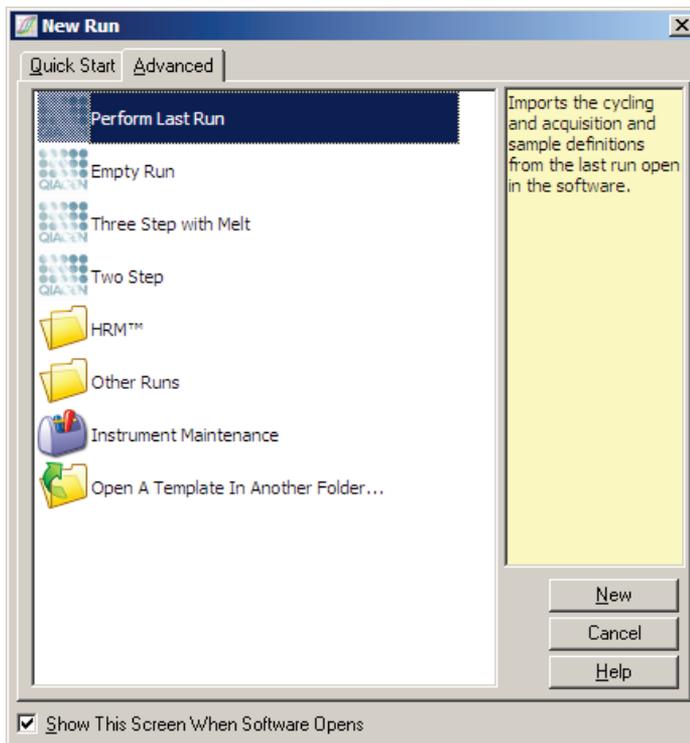
Template options provided in this window are similar to those provided when using the Quick Start wizard (Section 6.1).

Perform Last Run: "Perform Last Run" imports the cycling, acquisition and sample definitions from the last run that is open in the software.

Empty Run: This is an empty run, which allows the user to define all parameters of the profile.

Three Step with Melt: This is a three-step cycling profile and a melt curve with data acquisition on the green channel.

Two Step: This is a two-step cycling profile with data acquisition on the green channel only, to speed up the run.

HRM: This folder contains 2 high resolution melt profiles.

Other Runs: This folder contains additional profiles.

Instrument Maintenance: This contains the template used during Optical Temperature Verification (OTV). For more information, see Section 10. This template is locked to ensure the profile will always operate correctly.

Note: User-defined templates can be added to the template list by copying or saving ***.ret** files to **C:\Program Files\Rotor-Gene Q Software\Templates**. After copying a file to this path, the template will appear as an icon in the list.

6.2.1   New Run Wizard window 1

In the next window, select the rotor type from the list.

Check the "Locking Ring Attached" checkbox and click "Next" to proceed.
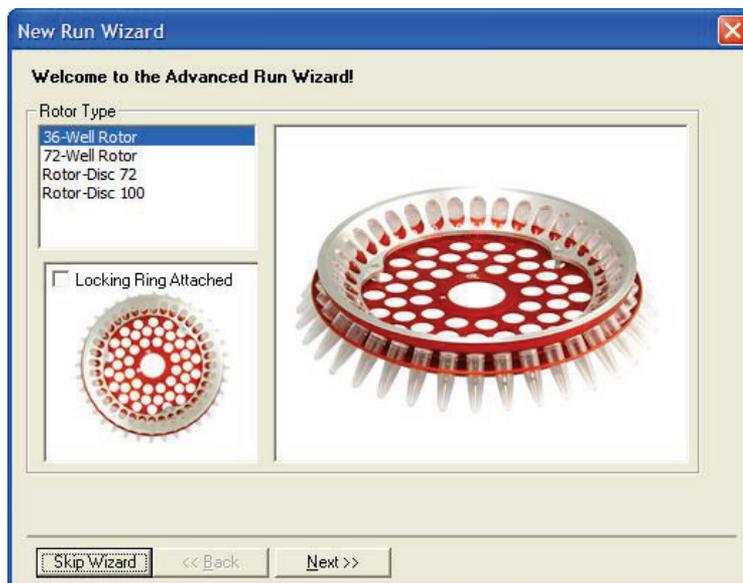
## 6.2.2   New Run Wizard window 2

In the next window, the user's name and notes about the run can be entered. The reaction volume must also be entered.

If the 72-Well Rotor was selected in window 1, three "Sample Layout" options are available in the drop-down menu:

■ "1, 2, 3…" is the default option. Most users select this option.

■ "1A, 1B, 1C…" should be selected when samples were loaded in adjacent 0.1 ml Strip Tubes using a multichannel pipet with 8 channels.

■ The "A1, A2, A3…" layout may be selected if appropriate.
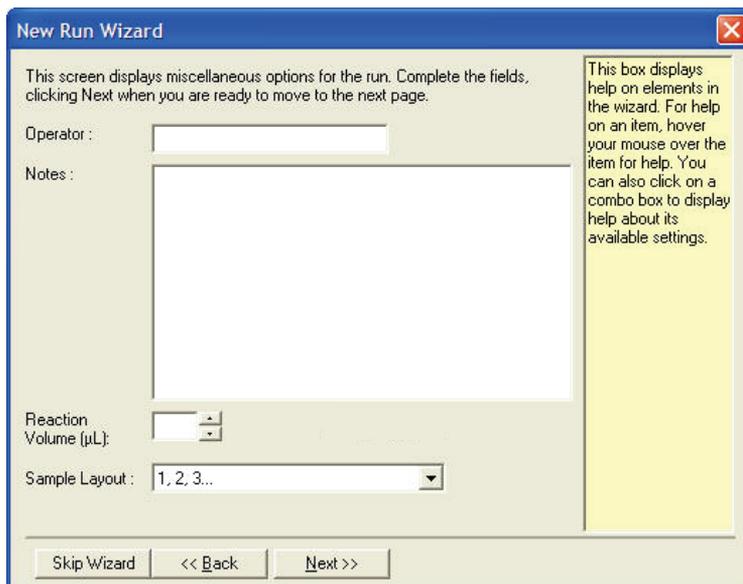
6.2.3 New Run Wizard window 3

In this window, "Temperature Profile" and "Channel Setup" can be modified. If the "Edit Profile…" button is clicked, the "Edit Profile" window appears, enabling alteration of cycling conditions and selection of acquisition channels (Section 6.2.4).

After setting up the profile, click the "Gain Optimisation…" button to bring up the "Auto-Gain Optimisation Setup" window (see page 6-23).
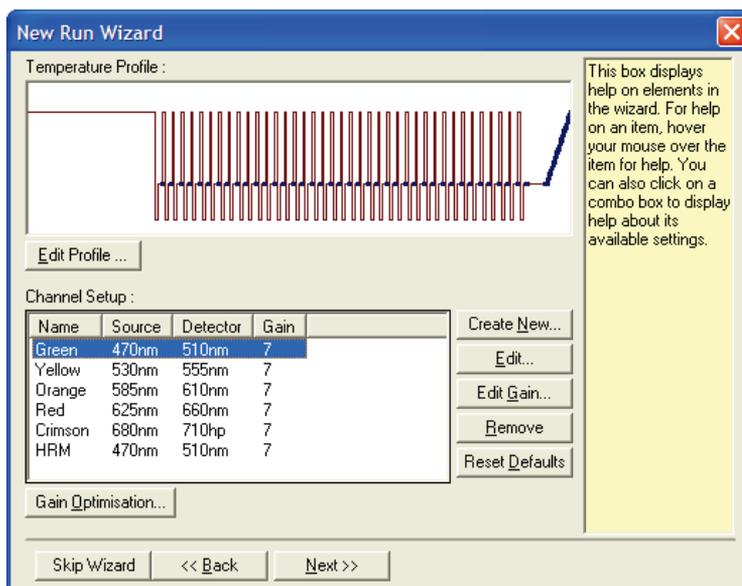
6.2.4 Edit Profile

The "Edit Profile" window allows the cycling conditions and acquisition channels to be specified. The initial profile shown is based on the template selected when setting up the run (see page 6-1). The profile is displayed graphically. The list of the segments of the profile appears below the graphical display. This list can include Hold (page 6-11), Cycling (page 6-12), Melt (page 6-15) or HRM if the instrument has a HRM channel (page 6-16).

Each stage of the profile can be edited by clicking on the appropriate area of the graphical display or on the name in the list, and then changing the settings that appear.

Insert after...:   This allows addition of a new cycle after the selected cycle.

Insert before...:   This allows addition of a new cycle before the selected cycle.

Remove: This removes the selected cycle from the profile.

Hold

A Hold instructs the Rotor-Gene Q MDx to remain at the designated temperature for a set time. To change the temperature, click on the "Hold Temperature" button and type or use the slide bar to select the desired temperature. To change the duration of the Hold, click the Hold Time "mins" and "secs" buttons.
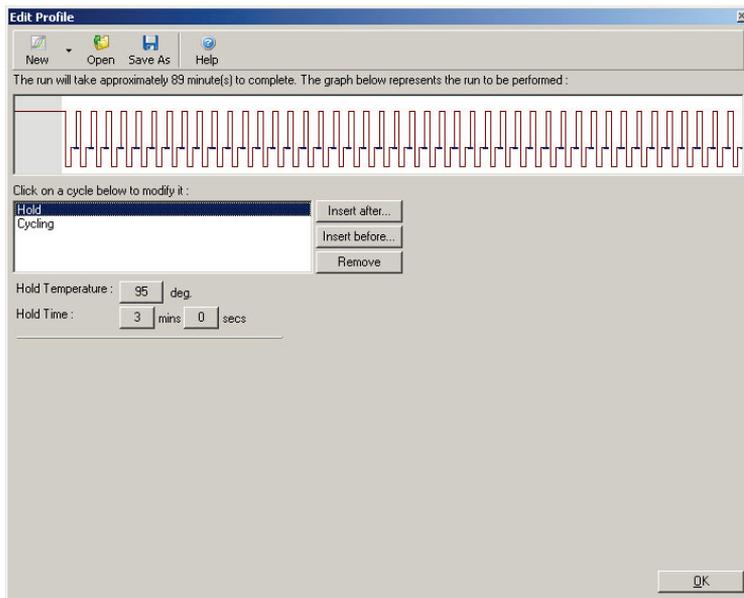
If performing Optical Denature Cycling, a Hold can be used as a calibration step. In this case, a calibration melt is performed before the Hold. By default, this is configured for the first Hold in the run, but this may be changed if required.
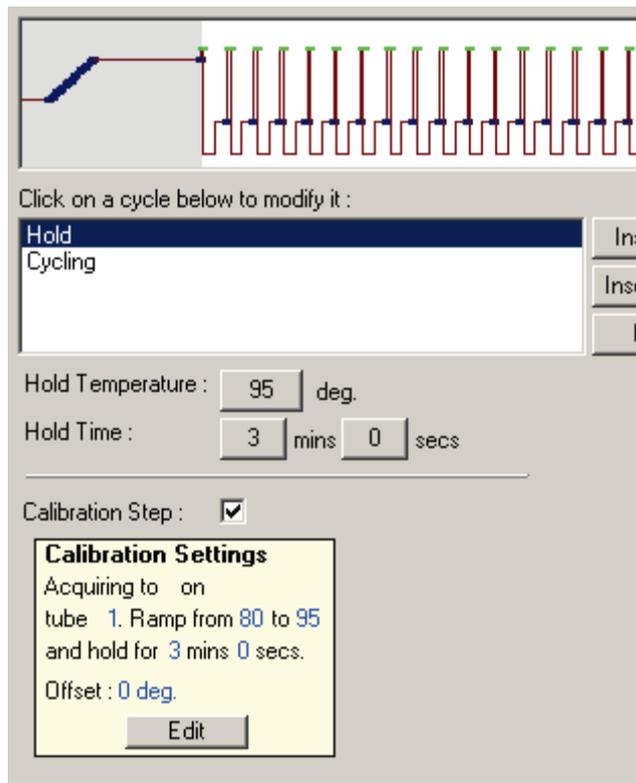
For more information about Optical Denature Cycling, see page 6-16.

Cycling

Cycling repeats the user-defined temperature and time steps a specified number of times. The number of repeats is set using the "This cycle repeats X time(s)" button.

A single cycle is displayed graphically (as shown in the screenshot below). Each step of the cycle can be altered. The temperature can be changed by dragging the temperature line in the graph up or down. The duration of the step can be changed by dragging the temperature boundary in the graph left or right. Alternatively, click on the step and use the temperature and time buttons to the left of the graph.

Steps can be added or removed from the cycle using the "−" and "+" buttons at the top right of the graph.
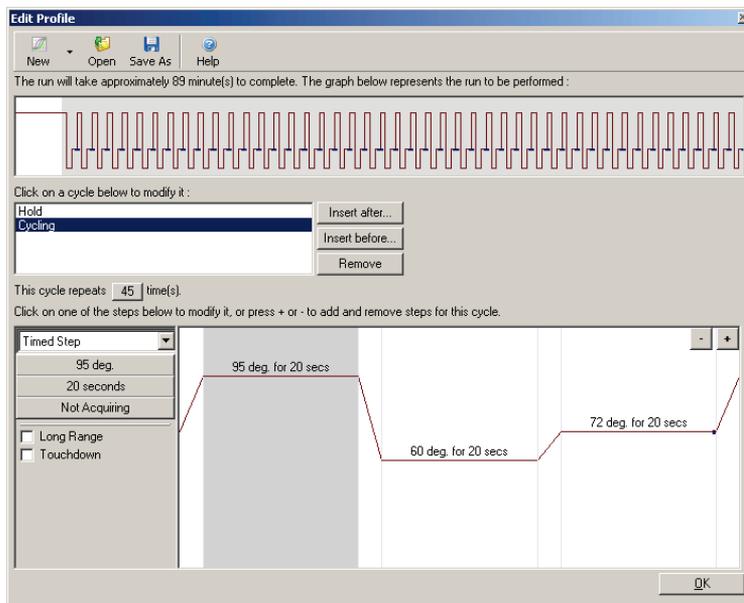
Long Range: Checking this box increases the hold time of the selected step by one second with each new cycle.

Touchdown: Checking this box decreases the temperature by a specified number of degrees for a specified number of initial cycles. This is then shown in the display.

Acquisition

Data can be acquired on any channel at any cycling step. To set a channel to acquire data, click on the "Not Acquiring" button. (If a channel has already been set to acquire at this step, then the acquiring channels are listed here.)

After clicking the "Not Acquiring" button, the "Acquisition" window appears.
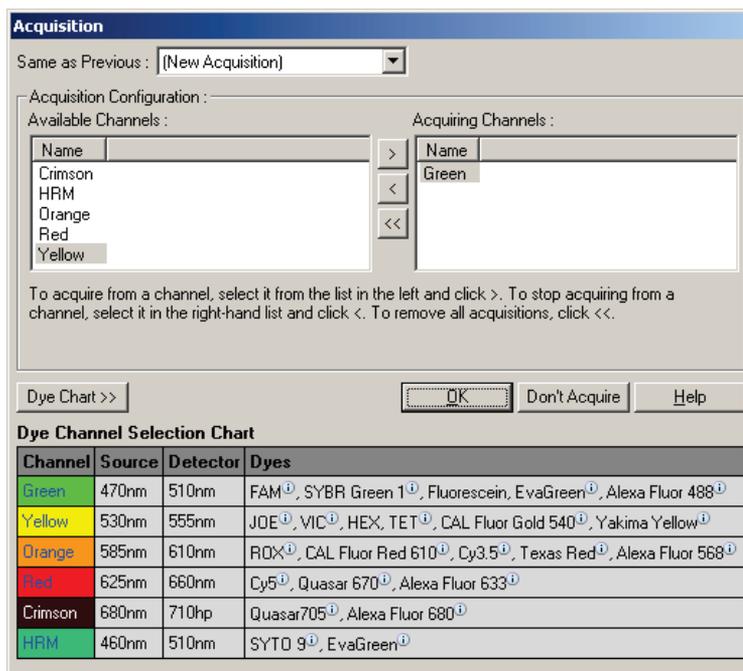
To set a channel to acquire, select the channel and move it from the "Available Channels" list to the "Acquiring Channels" list using the ▶ button. To remove a selected

channel from the "Acquiring Channels" list, use the ⟨ button. The ⟨⟨ button removes all the channels from the "Acquiring Channels" list. Clicking the "Don't Acquire" button also removes all acquisitions from the step.

If more than one cycling sequence is included in the profile, the acquired data can be appended to the data acquired from the earlier cycling. Use the "Same as Previous" drop-down menu to select the cycling step to which the data should be appended.

The Dye Channel Selection Chart helps the user to decide which channel is appropriate for dye they intend to use. The dyes shown in the table are those that are commonly used and do not indicate the limits of the instrument.

The acquisition options described above also apply to "Melt" steps, except that it is not possible to append acquisition data using the "Same as Previous" menu.

Melt and hybridisation

A Melt is a ramp between two temperatures, from a lower to a higher temperature. The permitted temperature range is 35–99°C.

To set up a Melt, specify the start temperature, the end temperature, the temperature increments, the length of time to hold at the first acquisition temperature before the ramp is initiated, the time each increment is to be held for and the acquisition channels.

A ramp will be generated between the two temperatures. If the start temperature is higher than the end temperature, the name of the step will change to "Hybridisation". The "Acquiring To" option, set to Melt A in the screenshot below, can be changed by clicking the button. The "Acquisition" window will appear and the channels can be selected.

When running a standard melt, the temperature is increased by increments of 1°C, waiting for 5 seconds before each acquisition. The Rotor-Gene Q MDx can be configured to perform melts in 0.02°C increments. The minimum hold time between temperature steps varies depending on the number of degrees between each step.

High Resolution Melt

High resolution melt (HRM) analysis characterizes double-stranded DNA samples based on their dissociation (melting) behavior. It is similar to classical melting curve analysis but provides far more information for a wider range of applications. Samples can be discriminated according to sequence, length, GC content, or strand complementarity, down to single base-pair changes.

HRM analysis can only be performed on instruments that have HRM hardware and software installed. Data is acquired using specialized HRM sources and detectors. HRM analysis also includes the option to perform Gain Optimisation just before the Melt begins. After performing HRM, the data can be analyzed with HRM analysis software (Section 11).

Optical Denature Cycling

Optical Denature Cycling is an exciting technique, available on the Rotor-Gene Q MDx, which performs real-time melt analysis to determine the melt peak of a reference sample. This indicates PCR product denaturation with higher precision than setting a particular denature temperature for a hold time. To perform this technique, simply place a reference tube of PCR product in tube position 1 of the rotor. The

reference tube must also contain a detection chemistry that enables detection of strand dissociation.

When heating to the initial denature temperature, a melt is performed on the green channel from 80°C to 95°C, by default. The parameters of this initial melt can be adjusted by the user. From this data, a melt curve is generated and automatically analyzed.
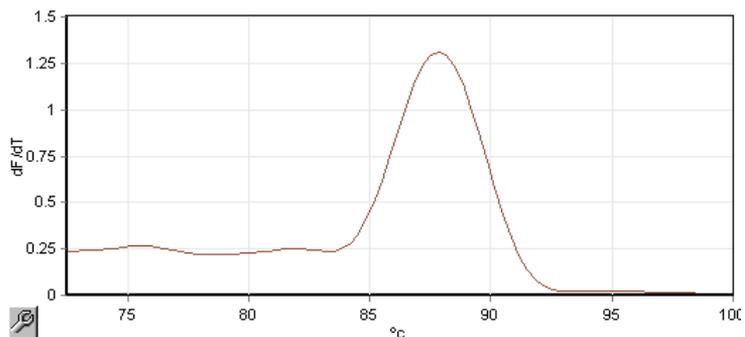
The melt peak is referenced back to the raw data to obtain a denature threshold. Then, every Optical Denature Cycling step, the instrument is heated as quickly as possible, and data is acquired continually. Once the reference tube has reached the denature threshold fluorescence level, the instrument is immediately cooled and proceeds to the next programmed step in the cycle. A peak is not calculated while cycling. Instead, the fluorescence level is referenced to the melt peak, and this designates the denature threshold.

In the following graph, the raw fluorescence readings and the first derivative have been overlaid. This shows the correspondence between the denature threshold and the melt peak obtained during the calibration.
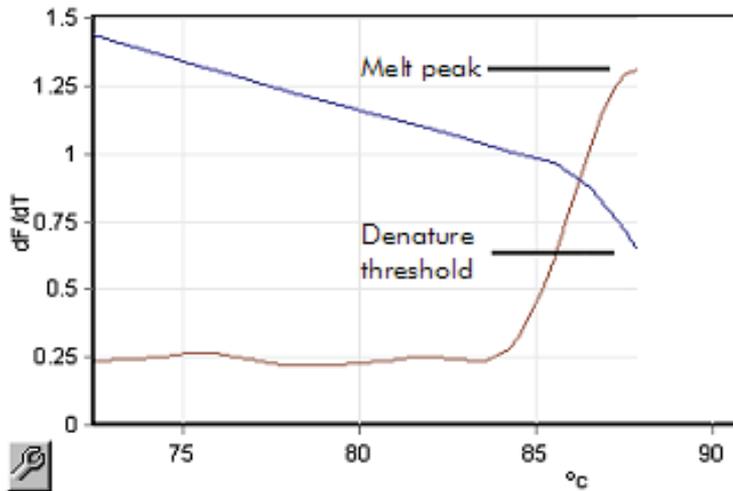
To perform Optical Denature Cycling, you will need:

■ A preamplified PCR product to place in position 1 of the rotor. This sample should contain the same PCR product as the samples of interest and a detection chemistry for monitoring PCR product dissociation.

■ An optical denature profile. A new profile can be created or an existing profile can be edited (see details below).

An Optical Denature Cycle appears almost identical to other cycles. The principal differences are the melt step automatically inserted at the beginning of the profile and the sharp profile of the denature step during cycling. The Optical Denature Cycle does not require defined hold times, because the dissociation of the product is monitored at each cycle.

To perform this technique, the following information about the run is required:

■ The initial denaturation temperature (this is the same temperature as the Denature step in a standard cycling profile)

■ The tube position of the PCR sample that will produce a melt curve on the green channel

■ A defined Optical Denature Cycling profile

Create a new Optical Denature Cycle as follows.

1. Open the "Edit Profile" window. Then click on "New". In the window that appears, click the "Insert after" button and select "New Cycling" from the menu. Select one of the temperature steps by clicking on the graph. In the drop-down menu, change "Timed Step" to "Optical Denature". A default profile containing a Denature step and an Optical Denature Cycle step will appear.
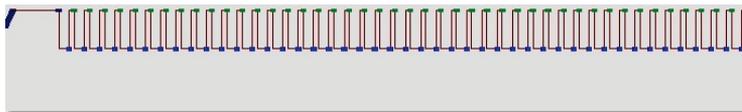
   The ramped region at the beginning of the run represents the calibration process. The green dots represent the acquisitions taken each cycle during heating. The blue dots represent the acquisition at the end of the anneal step at 60°C. Note that while the profile shows each step with the same denature temperature, this may not be the case. If the sample requires a slightly longer time to melt towards the end of the run, the optical denature process waits for the melt according to the fluorescent data and not according to time. For this reason, the temperature trace may vary for each cycle.

2. Click on the first half of the graph with the Optical Denature symbol ◿. The "Calibration Settings" information appears on the left of the screen.
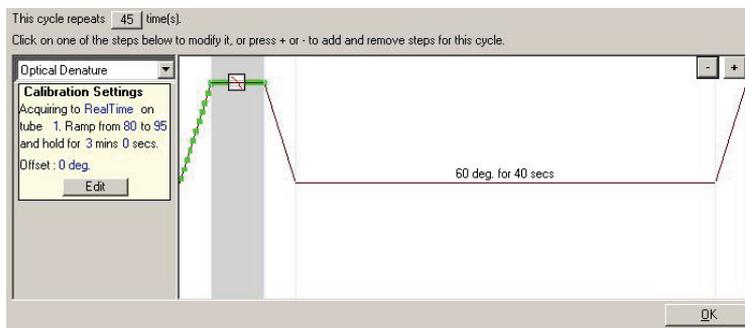
3. The "Calibration Settings" information is usually correct. To modify it, if necessary, click "Edit". The "Calibration Settings" window appears.
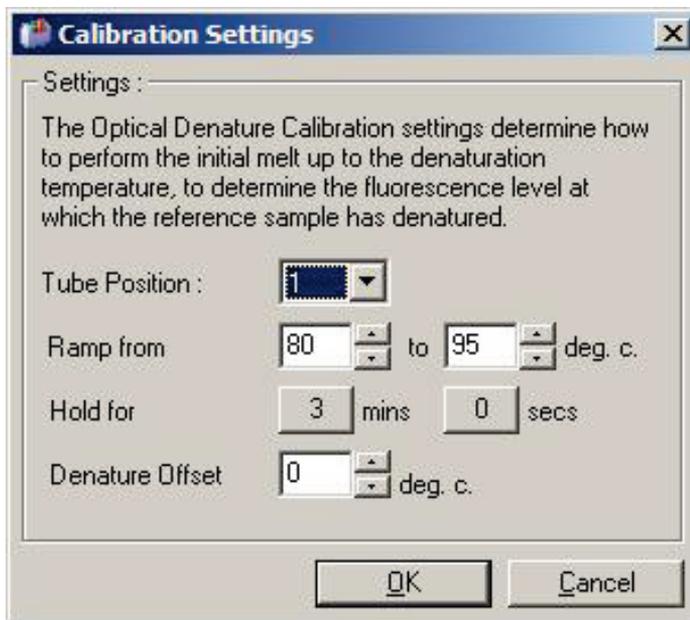
4. Ensure that:

   ■ The tube indicated in "Tube Position" contains a PCR product that will show a melt peak on the green channel.

   ■ The final ramp temperature will not burn the sample but will be high enough to allow it to melt.

   ■ The hold time is sufficient to denature the sample.

   ■ The denature offset is set appropriately. The default of 0°C is appropriate for most melts. Melts with very sharp transitions may require a denature offset of −0.5°C to −2°C, as determined by the user, to ensure that melt transition is detected.

You can also define a Denature step by introducing a new Hold step. Click on "Insert before" and select "New Hold at Temperature" from the menu. The calibration settings will appear.
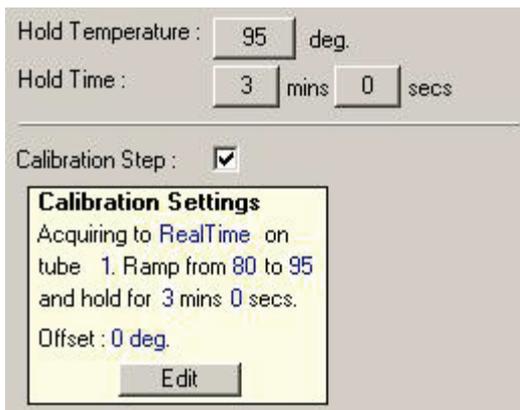
The calibration settings are synchronized with the denature settings, so a change in the hold time in the Denature step will automatically update the calibration hold time. This is because the calibration process and denaturation are equivalent in Optical Denature Cycling.

Changing an existing step to use Optical Denature Cycling

To change an existing Denature step in a cycling sequence, select the cycle in the list in the "Edit Profile" window. Then, select the Denature step by clicking on it in the display.
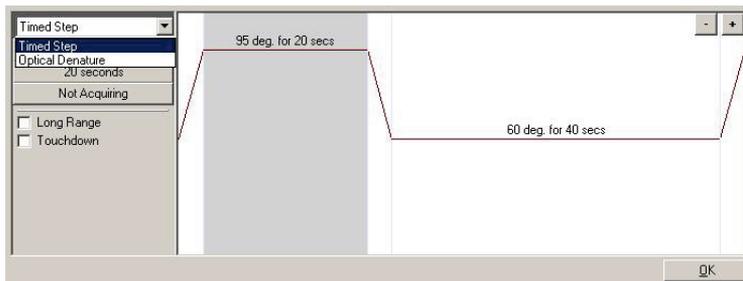
Click on the drop-down menu and select "Optical Denature". The temperature and hold time are removed, and the "Optical Denature" icon is displayed.

Gain Optimisation

When setting up a new run, it is helpful to use the "Gain Optimisation" function. This allows you to optimize the gain

to a setting that will provide the desired range of starting fluorescence at a set temperature (usually the temperature at which data acquisition occurs) in each of the channels being acquired. The aim of Gain Optimisation is to ensure that all data is collected within the dynamic range of the detector. If the gain is too low, the signal will be lost in background noise. If it is too high, all signal will be lost off scale (saturated).

The gain range for each channel is −10 to 10, where −10 is the least sensitive and 10 is the most sensitive.

When running reactions for the first time, we recommend preparing a test sample containing all the reaction components. Place the test sample in the Rotor-Gene Q MDx and use Gain Optimisation to determine the best gain setting. If the gain chosen by Gain Optimisation results in a poor signal, then the "Target Sample Range" should be increased. If it results in a signal that is saturated, then the "Target Sample Range" should be decreased.

To perform Gain Optimisation, click on the "Gain Optimisation…" button in New Run Wizard window 3 (see Section 6.2.3).
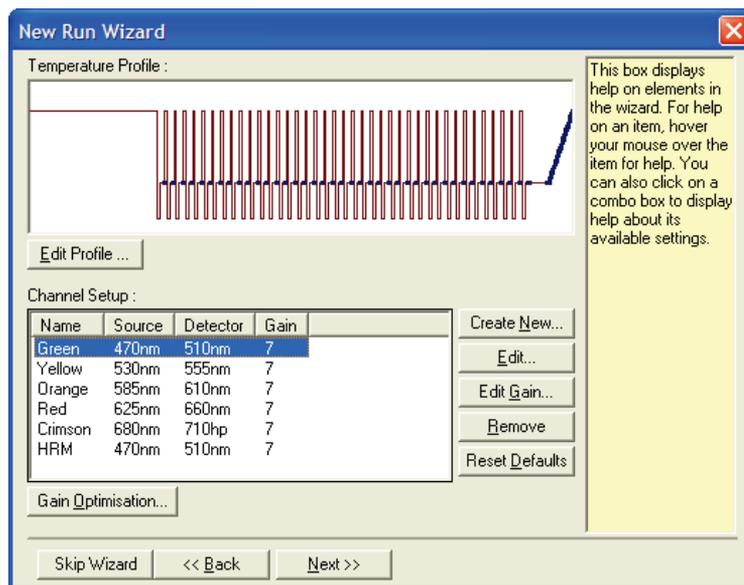
The "Auto-Gain Optimisation Setup" window appears. This window enables optimization by automatically adjusting the gain settings until the readings for all selected channels fall within or below a certain threshold.
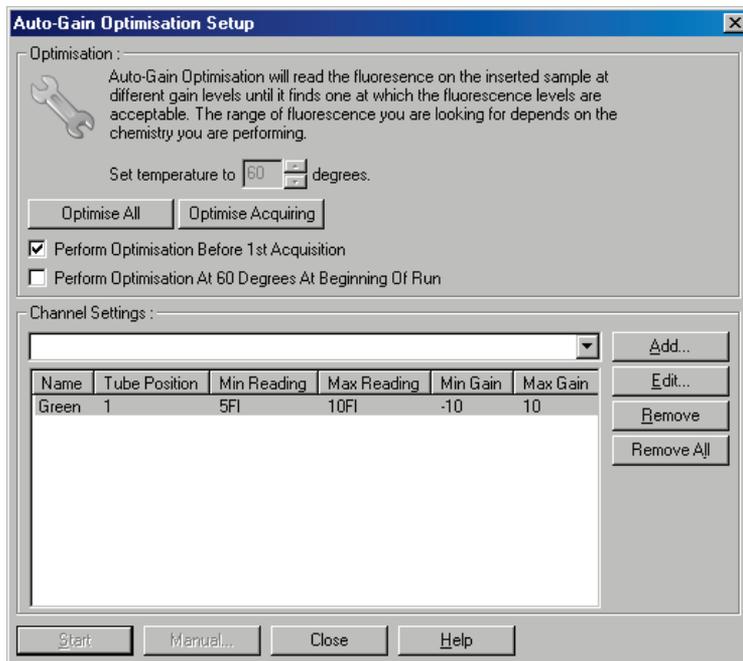
Set temperature to:	Before reading, the Rotor-Gene Q MDx will be heated or cooled to match the specified temperature. By default, this is set as the acquisition temperature.
Optimise All/Optimise Acquiring:	"Optimise All" will attempt to optimize all channels known by the software. "Optimise Acquiring" will only optimize the channels that are used in the thermal profile defined in the run (cycling and melt).
Perform Optimisation Before 1st Acquisition:	Check this box to perform Gain Optimisation at the first cycle in which data acquisition occurs. This is recommended for Auto-Gain Optimisation.

Perform Optimisation At [x] Degrees At Beginning of Run:	Check this box to perform Gain Optimisation just before starting the run. The Rotor-Gene Q MDx is heated to the specified temperature, Gain Optimisation is performed, and then cycling begins on the first step, usually a Denature step. This option may be chosen if Gain Optimisation during the run would result in too much time spent on the initial step. Usually "Perform Optimisation Before 1st Acquisition" is preferred because Gain Optimisation is performed as close as possible to the run conditions.
Channel Settings:	This drop-down menu allows channels to be added. Choose the channel of interest and click "Add".
Edit...:	This opens a window in which the "Target Sample Range" can be set. The "Target Sample Range" is the range of initial fluorescence that should be set for the sample in the specified tube. Auto-Gain Optimisation reads each channel using gain settings in the range specified by "Acceptable Gain Range". It chooses the first gain setting that results in a fluorescence reading within the "Target Sample Range". In the example shown, Auto-Gain Optimisation searches for a gain setting between −10 and 10 that gives a reading between 5 and 10 fluorescence in tube 1. In general, for intercalating dyes, a "Target Sample Range" of 1–3 fluorescence is appropriate, while a range of 5–10 fluorescence is more suitable for probe chemistries.

Auto-Gain Optimisation Channel Settings

Channel Settings :

Channel : Green   Tube Position : 1

Target Sample Range : 5   Fl up to 10   Fl.

Acceptable Gain Range: -10   to   10

OK   Cancel   Help
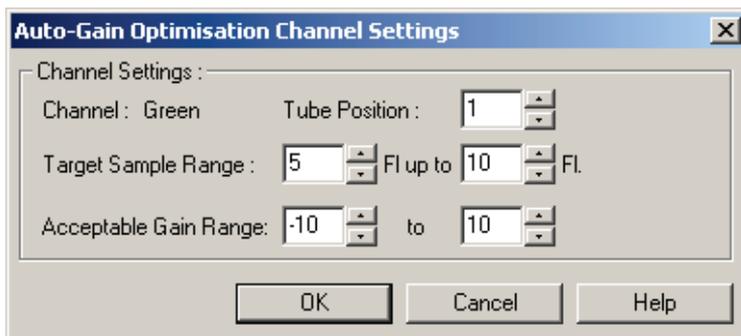
Remove/ Remove All:	"Remove" removes the highlighted channel. "Remove All" removes all channels.
Start:	"Start" begins Gain Optimisation. A gain is chosen which results in fluorescence signal levels within the specified range. If fluorescence falls outside the specified range, the gain is set to give the closest match possible.
Manual:	This opens the "Manual Gain Adjustment" window (see page 6-26).
Changing Gain During a Run:	If the gain at the beginning of the run was too high or too low, it can be changed within the first ten cycles. A vertical line appears where the gain has been changed. The cycles before the change are excluded from the analysis.

Note: Gain Optimisation may choose a setting that does not fall within the specified range. This can be due to changes in fluorescence after the first Hold step. However, the result of Gain Optimisation provides a good indication of the fluorescence level on which the run will be started.

Manual Gain Adjustment

To perform Manual Gain Adjustment, click on "Manual…" in the "Auto-Gain Optimisation Setup" window. The "Manual Gain Adjustment" window appears. This window displays the fluorescent readings at any given temperature in real-time. It is used when the background of a sample is unknown and therefore the gain must be determined to ensure the sample signal is sufficient for detection.
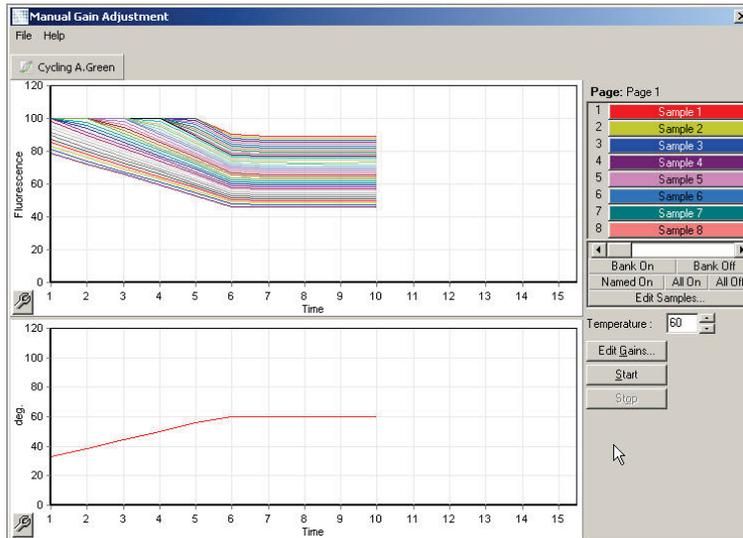
By default, all samples are shown in the display. Samples can be removed from or added to the display using the toggler on the right. The toggler consists of colored cells, each of which corresponds to a sample in the display. Samples with a brightly colored cell are displayed, while samples with a faded cell are not displayed. Samples can be switched on or off by clicking on the cell or by dragging the mouse pointer across several cells at time.

We recommend performing Manual Gain Adjustment as follows:

1. Adjust the temperature in the "Manual Gain Adjustment" window to the acquisition temperature required for the run.

   Note: The temperature will not be adjusted while the Rotor-Gene Q MDx is operating. Restart the Rotor-Gene Q MDx to apply changes made to the temperature.

2. Click on "Start". The run begins. The Rotor-Gene Q MDx temperature is adjusted to the temperature specified in the window. The graphs in the window start to display data.

3. Wait for the temperature to stabilize.

4. Note the end point fluorescence reading.

5. If the fluorescence reading is not at the required level, click on "Edit Gains…" and then edit as required. This process may not be instantaneous, because the Rotor-Gene Q MDx takes approximately 4 seconds to acquire each point in each channel, and during this time the user interface is deactivated.

6. Repeat the process until the fluorescence is at the desired level.

7. Click on "Stop". If the run is still acquiring data when the "Stop" button is clicked, the Rotor-Gene Q MDx finishes acquiring first, and then stops. This process can take up to 5 seconds for each acquiring channel.

6.2.5 New Run Wizard window 4

This window summarizes the run. Check the parameters, and then if they are correct, click "Start Run". You will be prompted for a file name. You can also save the run settings as a template for future runs by using the "Save Template" button.
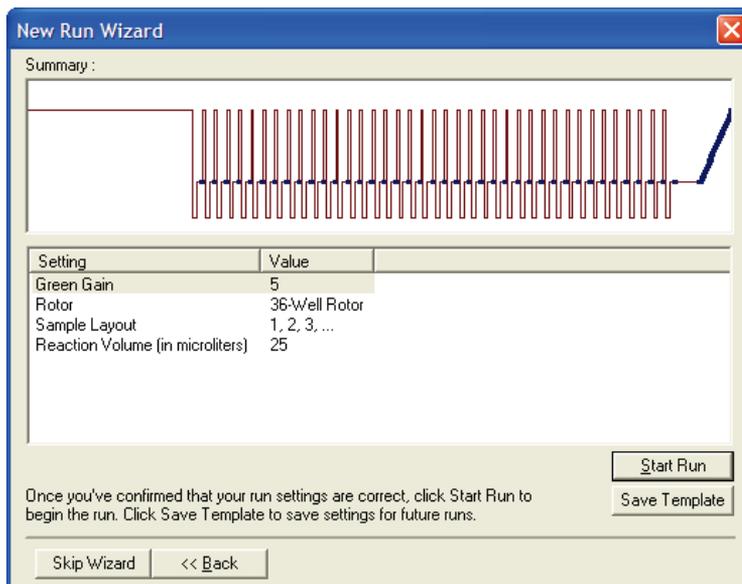
6.2.6 New Run Wizard window 5

Enter sample types and descriptions in this window while the run is in progress. The functionality of this window is identical to the "Edit Samples" window (page 7-71). Sample information may also be entered after the run has finished.

The "Finish and Lock Samples button" closes the screen and prevents the sample names from being modified. For more

information about this and other security features, see
"Access Protection for Rotor-Gene Q Software" (page 7-81).
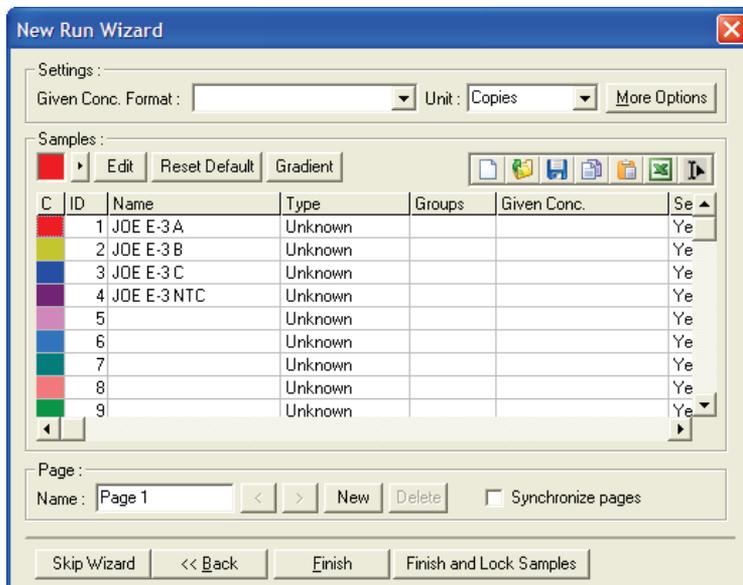
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7 Analysis User Interface

Note: This chapter describes the Rotor-Gene Q software user interface.

7.1 Workspace

The workspace is the backdrop of the main window. In this area, raw data plots and analysis results can be opened. If several windows are opened simultaneously, they can be organized by clicking the "Arrange" button on the toolbar. There are several window arrangement options available that can be selected by clicking on the down arrow next to the "Arrange" button.
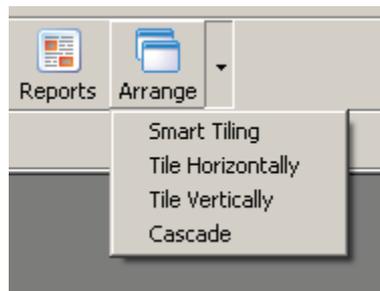
7.2 Toolbar

These buttons are shortcuts to frequently used operations. These operations can also be accessed from the drop-down menus.

7.3 View raw channels

Click on these buttons to view the raw (unanalyzed) data from particular channels in the run.
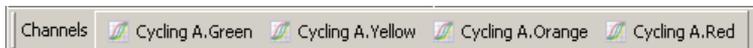
When viewing this data, a number of options are available to change the data presentation. The raw data may also be transformed to facilitate different types of analysis.

Adjust Scale: To select "Adjust Scale", right-click on the appropriate window. "Adjust Scale" brings up a window in which a scale can be specified.
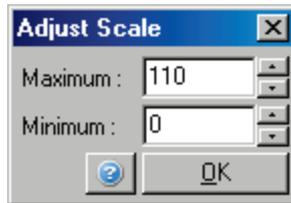
Autoscale: "Autoscale" attempts to fit the scale to the maximum and minimum readings of the data.

Default Scale: "Default Scale" resets the scale to display from 0 to 100 fluorescence units.

Spanner/wrench icon: See Section 8.5 for more information.
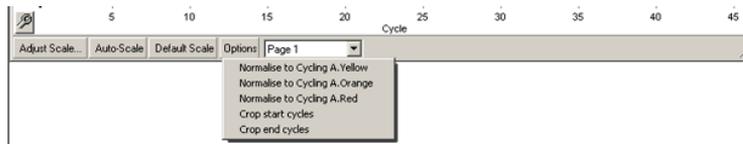
Options: This displays the drop-down menu shown above, which provides options for transformation of the raw data.

Normalise to ...: This takes amplification data and normalizes it to data from a passive reference dye (e.g., ROX) that was acquired from another channel.

Crop start cycles: This creates a new channel data set in which some start cycles have been removed. This is useful if large jumps are observed in the initial cycles, which can occur when using certain chemistries.

Crop end cycles: This creates a new channel data set in which some end cycles have been removed.

Page 1: This indicates the page that is currently selected to display the raw data plots. The "Edit Sample" window allows the creation of multiple sample definitions. For example, data can be viewed with varying line thickness, sample definitions and other display options. This is particularly useful if relative quantitation is performed in a single channel, because the user can easily switch the view between the gene of interest and housekeeper samples by defining two sample pages.

7.4 Toggling samples

At the right-hand side of the main window is a toggler, which includes a sample legend. This consists of colored cells, each of which corresponds to a sample in the display. The toggler is used to control which samples can be seen in the display. Samples with a brightly colored cell are displayed, while samples with a faded cell are not displayed. Samples can be switched on or off by clicking on the cell or by dragging the mouse pointer across several cells at time. The "Bank On" and "Bank Off" buttons hide or display, respectively, all samples currently visible in the list. The scroll bar can be used to display the next group of samples.

Note: The number of displayed samples is dynamic and depends on the space available in the window.

Clicking "Named On" shows only those samples that have been given a name. This is a quick way to show only relevant samples. Clicking "All On" or "All Off" displays all or none, respectively, of the samples in the rotor. Pressing "Edit Samples…" opens the "Edit Samples" window, where sample names, types and standard concentrations can be edited (see Section 7.8.4).

The toggler is shown below. The additional options are displayed by right-clicking on the toggler.
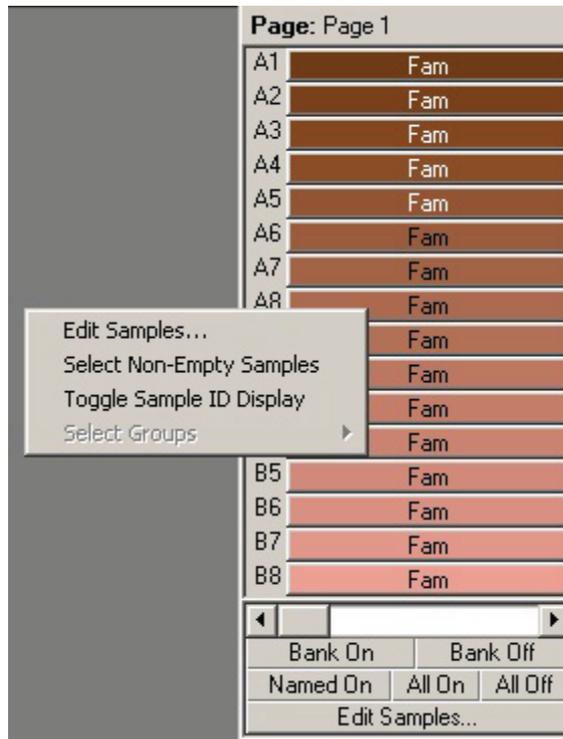
Page: This label at the top of the toggler indicates the sample page that is displayed. Pages allow varied independent analyses from one channel data set. For example, you can run two standard curves in the green channel and generate independent reports. More information on setting up sample pages is available in Section 7.8.4.

Toggle Sample ID Display: If a 72-Well Rotor is used, samples are shown in the format A1 to A8, B1 to B8, etc. The "Toggle Sample ID Display" option allows the user to switch to a numerical sample order (1 to 72).

Select Non-Empty Samples: This option deselects any samples that have a "Type" specified as "None" in the "Edit Samples" window. This ensures that only samples relevant for the analysis are displayed.

Select Groups: If you have defined groups, this feature will toggle (switch on/off) the display of the samples in the groups. Groups are arbitrary collections of samples that allow advanced reporting of statistical results. For example, groups of treated and untreated patient samples can be defined. Groups can be set up in the "Edit Samples" window.

7.5   File menu

7.5.1   New

After selecting "File" and then "New", the "New Run" window appears. This window provides commonly used templates organized under "Quick Start" and "Advanced" tabs. Once

the template is selected, the wizard guides you through the run setup and allows modification of settings and profiles.
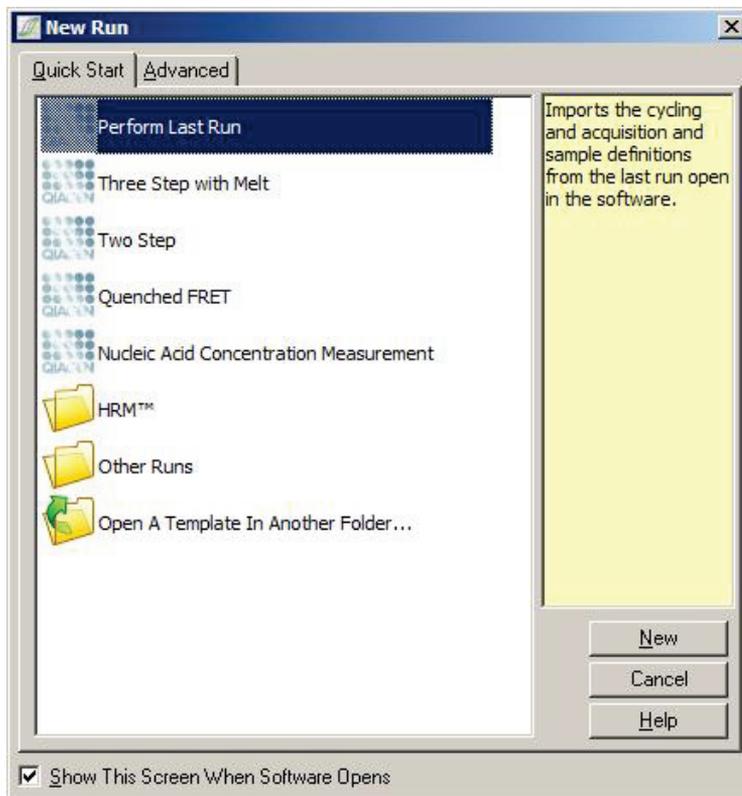
For information about the templates provided, see Section 6.1 and Section 6.2.

New Run

New…:	This initiates the run setup using the selected template.
Cancel:	This closes this window.
Help:	This opens the online help.
Show This Screen When Software Opens:	If this box is checked, the "New Run" window is displayed when the software is started.

7.5.2　　Open and Save

Open...:　　　　This opens a previously saved Rotor-Gene Q run file (***.rex**) or Rotor-Gene Q run archive (***.rea** file).

Open Recent...:　　This displays the last four files that have been opened or saved.

Save:　　　　　This saves any changes that have been made into a run file.
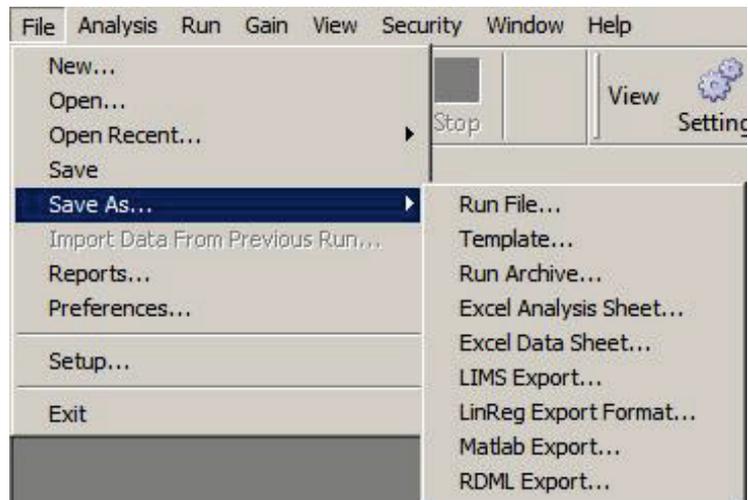
Save As...:　　　Use this function to save the run file or data in various formats. The options are listed below.

Run File...:　　　This saves a copy of the file. The user can change the name and the save location. This is the default format.

Template...:　　　This saves the profile setup and associated settings but not the run data. The template can be used to initiate future runs.

Run Archive...:　　This saves in a more compact file format. Save files in this format before they are e-mailed. This reduces the time required to send the file and ensures that files are not corrupted by e-mail clients.

LIMS Export:	This saves the analysis in LIMS-compatible formats according to the requirements of the user. Please contact QIAGEN Technical Services for more information.
Excel Data Sheet…:	This exports all the raw channels to an Excel® sheet. Only the selected samples are exported.
Excel Analysis Sheet…:	This exports all the analyses in the current run into a single Excel sheet.
LinReg Export Format…:	This exports all raw channel data into a format that can be read by LinReg (an efficiency analysis tool). See "Exporting To LinReg", page 7-8, for more details.
Matlab Export…:	This exports the data into a format that can be read by the scientific package MATLAB® (or its open-source equivalent, Octave). This may be useful for methods research.
RDML Export:	This provides for an RDML v1.1–compliant file export. The RDML export file created is a ZIP compressed XML format file, with an ***.rdml** file extension and is compliant with the RDML schema document (http://www.rdml.org/RDML_v1_1_PR.xsd) provided on the website http://www.rdml.org/files.php.

Exporting To LinReg

LinReg is a tool developed by Ruijter et al.* The LinReg tool is available from http://LinRegPCR.nl.

Rotor-Gene Q software allows the user to export raw data in a format that can then be imported by the LinReg tool for analysis:

* Ruijter, J.M. et al. (2009) Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res. **37**, e45.

1. Open the Rotor-Gene Q run file containing the raw data.

2. Export the data to the LinReg export format by selecting "Save As…" and then "LinReg Export Format…".

3. Microsoft Excel automatically displays the exported raw data.

4. Start up the LinReg tool.

   The tool asks you to select the cell range where the raw data are located. The tool can only analyze one raw channel at a time, so an appropriate region of the Excel sheet should be selected.

7.5.3   Reports

After selecting "Reports", the "Report Browser" window appears. If the data has already been analyzed, the report of that analysis can be displayed from the "Report Browser" window. Several report types are offered with varying degrees of detail.
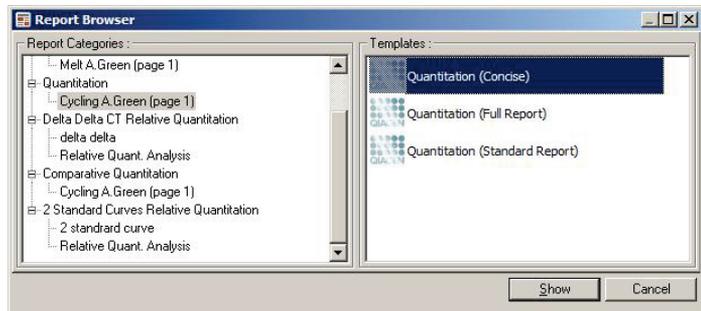
7.5.4 Setup

The initial setup of the Rotor-Gene Q MDx should be completed during installation. However, this option allows you to change the Rotor-Gene Q MDx connection setup if you wish to do so after installation.
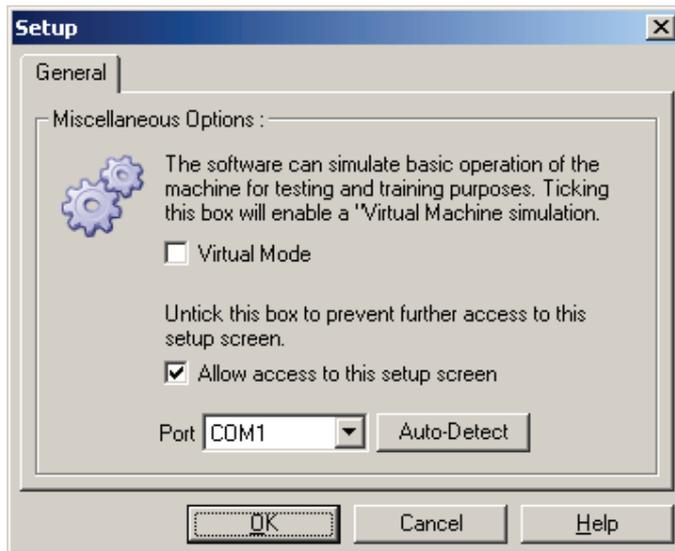
Virtual Mode: Select this option if the software will be used without a connected Rotor-Gene Q MDx. The software retains all functions. This mode is useful for demonstration purposes, data analysis and setting up templates.

Allow access to this setup screen: If this option is not checked during setup, this window can no longer be accessed. This security measure prevents users from altering the settings. To reestablish access, contact your distributor.

Port: Select the correct communication port to enable communications between the computer and the Rotor-Gene Q MDx.

Auto-Detect: If you are unsure which port to select, click "Auto-Detect" to search for all available ports.

7.6   Analysis menu

7.6.1   Analysis

After clicking "Analysis", the "Analysis" window appears. This window allows the creation of new analyses and the display of existing analyses. The method of analysis is selected using the tabs. A list of the channels that can be analyzed using the selected method is shown. Multiple assays that are run in the same channel can be analyzed independently if they have been set up as separate pages in the "Edit Samples" window. Pages that have already been analyzed have a green checkmark next to them. This means that threshold and normalization settings have been saved for this analysis. To view or analyze a channel, double-click on it. The specific analysis window appears.
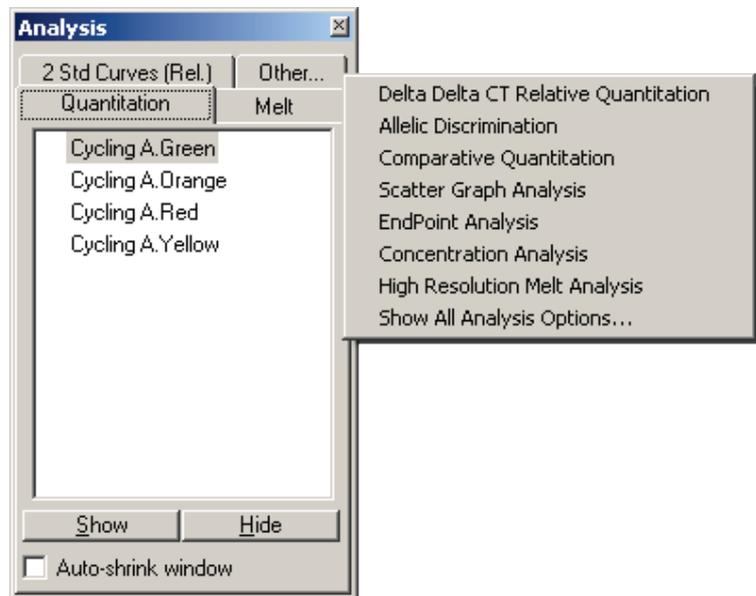
Auto-shrink window: Selecting "Auto-shrink window" shrinks the window when it is not in use. Moving the cursor over the window enlarges the window again.

Organizing the workspace

Each time a new analysis is started, its windows are arranged to fit in with those already on the screen. If many windows are displayed, this can be cumbersome. Close the windows you do not require, then click "Arrange" on the toolbar. The windows are automatically arranged according to the "Smart Tiling" method. Alternatively, select another arrangement method by clicking the arrow next to the "Arrange" button. Right-clicking on the name of an analysis also provides additional options.
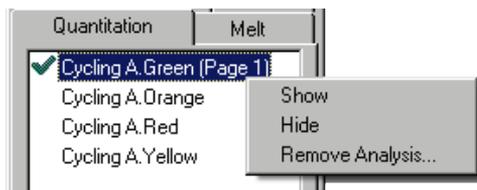
Show: This displays the selected analysis.

Hide: This hides the selected analysis.

Remove Analysis…:   This removes the selected analysis completely. This means that any normalization settings or melt bins set up in the analysis will be lost.

7.6.2   Quantitation

Select the "Quantitation" tab in the "Analysis" window, and then double-click on the channel name, or select the channel and then press the "Show" button to open the channel of interest. Three windows appear: the main screen, the standard curve and the results.

Reports

Reports: "Reports" opens the "Report Browser"
                window, where a report of the current
                analysis can be generated. There are three
                options: standard report, full report and
                concise report. Double-click on the desired
                option to open the report in the "Preview"
                window.

                After the report has been generated, the
                buttons at the top of the "Preview" window
                can be used to print, save or e-mail the
                report, or export it to Word.

Standard Curve

Std. Curve: This button opens the "Standard Curve"
                window. By default, this window is opened
                when an analysis is opened. If you close
                the window, it can be reopened using this
                command.
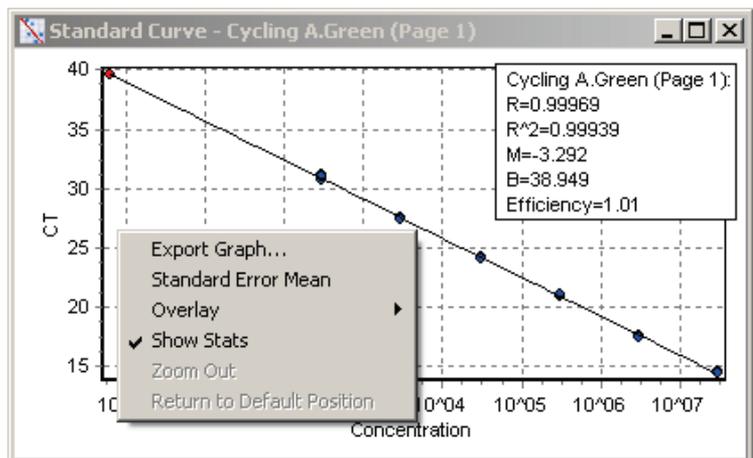
	The values on the standard curve are recalculated dynamically as the threshold level is varied by clicking and dragging the threshold line in the main window.
	Blue dots on the curve represent the samples that have been defined as standards, and red dots represent the unknown sample data points.
	Note: If redefining standards to recalculate the standard curve, toggling the standard sample visibility to off using the toggler on the right of the screen will remove it from the standard curve calculation. Removing standards from the graph to increase the $R\wedge 2$ value is not scientifically valid. A failed standard is an indication that the samples may also have failed, and so it should be included in the results.
Efficiency:	This is the reaction efficiency of the run. This value is discussed in more detail on page 7-26.
$R\wedge 2$ value (correlation coefficient):	The $R\wedge 2$ value, or R^2 value, is the percentage of the data that is consistent with the hypothesis that the standards form a standard curve. If the R^2 value is low, then the standards do not easily fit onto a line of best fit. This means that the results (i.e., the calculated concentrations) may not be reliable. A good R^2 value is approximately 0.999.
	Note: It is possible to achieve a high $R\wedge 2$ value with a poor standard curve if an insufficient number of standards have been run. The $R\wedge 2$ value improves as the number of standards decreases. For a more accurate indication of the reliability of the results, use the confidence intervals on the calculated concentrations as a guide.

R value (square root of correlation coefficient):	The R value is the square root of the R^2 value. In general, the R^2 value is more useful for determining correlation.
M and B:	The slope (M) and the intercept (B) of the standard curve are automatically calculated using the formula y = Mx + B, and they are shown in the "Standard Curve" window.
Export Graph...:	Right-clicking on the standard curve displays the option to export the graph (see Section 8.4).
Overlay:	When multiple quantitation runs have been performed in the same run, it is possible to overlay the standard curves in the same window. This is useful for graphically viewing the difference between different thresholds. This feature is shown in the screenshot below.
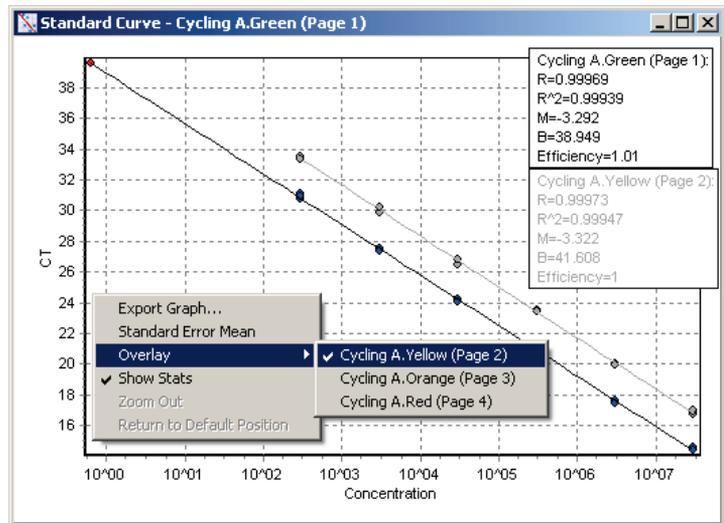
Standard curve calculation

Two versions of the equation used to relate C_T values and concentrations are "conc = ... *C_T + ... " and "C_T = ... ". In

publications, the "C_T = ... " formula is most commonly used. The standard curve can be either "Floating" or "Fixed". If "Floating", an optimal equation for the standard curve is calculated each time the threshold is moved in the main window. If "Fixed", the equation does not change, because it has been imported from another run.

Standard Curve
conc= 10^(-0.304*CT + 11.832)
CT = -3.292*log(conc) + 38.949
Type : Floating
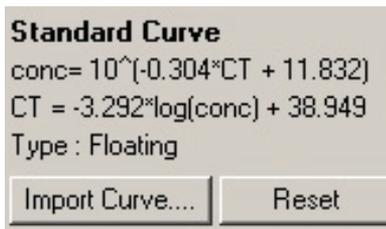
| Import Curve.... | Reset |

Import Curve

Importing a standard curve allows estimation of concentrations when a standard curve is not available in a particular run and the reaction efficiency has not varied between two runs. Curves can be imported from another channel or from another run by clicking on "Import Curve".

It is possible to adjust the standard curve if required. Adjusting the standard curve means that only the efficiency of the source standard curve is imported into the current run. Whether or not the standard curve should be adjusted depends on the chemistry used.

To adjust the standard curve, use a reference in the new run with a known concentration. Define a reference by setting the sample type to "Standard" and entering a concentration value in the "Edit Samples" window. Multiple copies of the same reference can be entered to improve accuracy. Note that it is not possible to define more than one reference concentration or standard. For example, it is possible to have three replicate references of 1000 copies, but it is not possible to have one reference of 1000 copies and another with 100 copies in the same run.

Once the standard curve has been imported, the standard curve type changes to "Fixed". Click "Reset" to change the standard curve type back to "Floating".

A screenshot of the "Import Standard Curve" window is shown below.
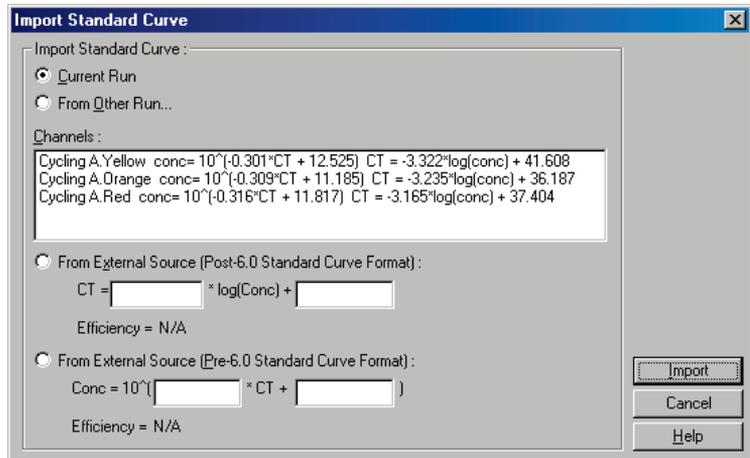
Using this window, a standard curve can be imported from another channel analyzed in the current run, or from another run.

Current Run:	When this option is selected, quantitation analyses on other channels from this run are listed with the corresponding standard curves.
From Other Run…:	Selecting this option brings up a dialog from which a run file can be selected to open. If any quantitation analysis has been performed for the run, standard curves are listed for each channel analyzed. **Note**: The quantitation analysis settings must have been saved in the run file.
Channels:	This lists the analyzed channels and their standard curve formulas.
From External Source:	In this area, M and B values can be entered directly. This is useful in cases where the values are from an external source, such as an Excel spreadsheet.

C$_T$ Calculation

Invert raw data: Some chemistries produce a fluorescent signal that decreases exponentially instead of increasing. It is possible to analyze these data using "Quantitation", but the "Invert Raw Data" checkbox should be checked. For all other quantitation analyses, this option should remain unchecked.

☐ Invert Raw Data

C$_T$ Calculation: The C$_T$ value is the cycle number at the point where the amplification curve crosses a threshold of detection. By setting a threshold line and calculating the intersection with each of the curves, the C$_T$ value for each sample is established.

Threshold: To set the threshold, click on the icon (a grid with a red arrow), and then click and hold on the graph and drag the line to the desired level. Or enter a log value. Alternatively, "Auto-Find Threshold" can be used to automatically determine the threshold. When setting a threshold manually, it should be set in the exponential phase of the run, significantly above the background level to avoid noise and below the onset of signal plateau in later cycles.

CT Calculation

☐ Flip sign of normalised data

Threshold :   0.0353

Eliminate Cycles before :   1
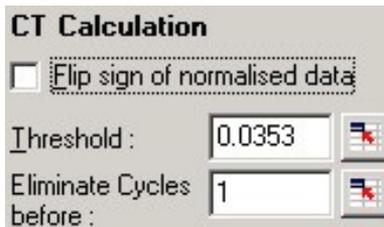
Eliminate Cycles before:

To set, click on the icon (a grid with a red arrow), and then click and hold on the graph and drag the line to the right. This eliminates the threshold for low cycle numbers.

Note: This is useful when there is noise during the initial cycles, due for instance to sample mixing effects.

Auto-Find Threshold:

This function scans the selected region of the graph to find a threshold setting that delivers optimal estimates of given concentrations. The selected region can be changed by entering new upper and lower bounds in the text boxes that appear.

For most analyses, the default upper and lower bounds are suitable. The range of threshold levels is scanned to obtain the best fit of the standard curve based on the samples that have been defined as standards (i.e., where the R value is closest to 1.0).

Auto-Find Threshold

Results

This opens the "Quantitation Results" window. By default, this window is opened when an analysis is opened. If it has been closed, it can be reopened using this command.

In the "Quantitation Results" window, the results from the run are summarized in a table. Right-clicking and selecting "Export to Excel" exports the table to Excel. Excel opens automatically. To copy the data into an existing spreadsheet, choose the "Copy" option instead, open the spreadsheet, and then select "Paste".

The "Quantitation Results" window includes the following columns:

Analysis: The current data set (acquiring channel and sample page)

No.:   The sample number

Color: The defined individual sample graph color

Type: The defined sample type

Ct: The determined C_T value

Ct Comment:	An automatic annotation of the C_T determination, if C_T values are excluded. The following flags are possible:
	NEG (Multi Ct): The threshold crosses the fluorescence curve at least two times (double intersect). An unambiguous C_T value cannot be determined
	NEG (NTC): The overall fluorescence increase does not fulfill the conditions defined in "NTC threshold" function of the "Outlier Removal" menu (see page 7-26). For example, a fluorescence curve intersects with the given threshold, but the minor overall slope increase suggests a non-template control, and a C_T value is not given
	NEG (R.Eff): The overall fluorescence increase does not fulfill the conditions defined in "Reaction efficiency threshold" function of the "Outlier Removal" menu (see page 7-26). Samples that do not have a certain reaction efficiency are excluded, and the C_T value is not given. This flag is only shown if the corresponding function is enabled
%Var:	The percentage variation between the calculated and the known concentration. %Var=Abs(Calculated/Given–1)
Rep. Ct:	The average C_T of all samples with the same name as this sample
Rep. Ct Std. Dev.:	The standard deviation of the C_T value of all samples with the same name as this sample
Rep. Ct 95% C.I.:	A C_T range that, statistically, accounts for 95% of the variation in the C_T value. This is a conservative statistical measure, which can be used as a quality measure. This range can be narrowed by running more replicates or by having less variation in the replicates

Rep. Calc. Conc: The calculated concentration for all the samples with the same name.

Note: This is not the simple average of the calculated concentrations. It is the geometric mean, which is a mathematically more appropriate average due to the exponential nature of real-time amplification

Rep. Calc. Conc. 95% C.I.: A range of concentrations that accounts for 95% of the variation in the individual sample as well as the linear regression model on which it is based. An interpretation of this measure is that it is the range of concentrations that could be expected 95% of the time if this run were performed repeatedly with the same amount of variation. This is a conservative estimate, and the range can be quite large due to the variation inherent in any real-time analysis. This range can be large if standards are run with concentrations different from the unknown samples, if a small number of replicates are used, or if there is significant variation.

IMPORTANT: The variations reported by this measure are inherent to the exponential process of real-time amplification and are not due to the Rotor-Gene Q MDx. Similar tests performed on block-based cyclers would yield greater variation due to the lower temperature uniformity of block-based systems. To compare cyclers, if desired, we recommend comparing the standard deviation of the C_T value.

 Note: More detailed information on confidence intervals is available in Appendix B.

 Note: Except for Color, Name, Ct and Ct Comment, each column can be displayed or hidden by right-clicking on the window and then selecting or deselecting the column name.

For increased convenience, the "AutoStat" feature automatically calculates the average, standard deviation, and minimum and maximum values of samples of interest. Select the results of interest by dragging with the left mouse button; the values will displayed in a table on the right of the screen.

In this screenshot, the concentrations of several samples are analyzed.

IMPORTANT: The "AutoStat" feature is context-aware. This means that, where possible, it only generates information that is useful.

For example:

- It is not possible to obtain a 95% confidence interval from a set of selected calculated concentrations, because the regression model must also be taken into account.
- The "Orders of Magnitude" standard deviation is reported for calculated concentrations rather than an absolute value. This is a percentage variation. For example, a value of 1.07537 represents a 7.54% variation $(278,974 – 322,611)=[(300,000/1.07537) – (300,000*1.07537)]$. Reporting an absolute value does not make sense for a standard curve. The value could be reported at the lowest concentration to create a perceived low error (± 3 copies) or at the high concentration ($\pm 3,000,000$ copies). For this reason, the "Orders of Magnitude" standard deviation is reported.
- For calculated concentrations, the geometric mean is used instead of the arithmetic mean. This accounts for the exponential nature of real-time PCR. For example, in the case of twofold dilutions with 1, 2, 8 and 16 copies, the average should be 4 copies, because it is the middle of the dilution series. However the arithmetic mean is 6.75. The geometric mean is $(1*2*8*16)^{(1/4)}=4$ copies.

Dynamic tube normalization

The "Dynamic Tube" option is selected by default and is used to determine the average background of each sample just before amplification commences.

Standard normalization simply takes the first five cycles and uses these as an indicator of the background level of each sample. All data points for the sample are then divided by this value to normalize the data. This can be inaccurate, because for some samples the background level over the first five cycles may not be indicative of the background level just prior to amplification. In contrast, dynamic tube normalization uses the second derivative of each sample

trace to determine a takeoff point for each sample. The background level is then averaged from cycle 1 up to this takeoff cycle number for each sample. This gives the most precise quantitation results.

Note that for some data sets, background fluorescence is not consistent during the cycles before amplification commences. In these cases, it may be necessary to deselect dynamic tube normalization by clicking on "Dynamic Tube" because it could result in less precise quantitation.

Noise slope correction

The background fluorescence of a sample should ideally remain constant before amplification. However, sometimes the fluorescence shows a gradual increase or decrease over several cycles due to the chemistry used. This produces a skewed noise level. Noise slope correction uses a line of best fit instead of an average to determine the noise level, and then it normalizes to that line. Selecting this option by clicking the "Slope Correct" button can improve data from replicates if sample baselines are noticeably sloped. Noise slope correction improves the data when raw data backgrounds are observed to slope upward or downward before the takeoff point (C_T).

Where the slope is not steady or the initial cycles of the baseline show a significant increase or decrease of the signal compared to the rest of the curve, Noise Slope Correction can lead to some undesired effects, such as negative control curves crossing the threshold, due to approximation of the baseline as a line-of-best-fit and normalizing the raw data accordingly. As a consequence, this function does not always improve the quality of the data and should be used only if the raw data curves show a steady slope.

Takeoff point adjustment

The takeoff point adjustment algorithm can be used to define a minimum length of the baseline utilized for normalization. To apply the takeoff point adjustment, two parameters have to be defined. If a takeoff point is calculated by the "Dynamic Tube" that is lower than the first parameter, then the second

parameter is used as the takeoff point. The takeoff point adjustment can only be used in conjunction with "Dynamic Tube" normalization.

Ignore First

The fluorescence signal from the first few cycles in a run might not be representative of the rest of the run. For this reason, better results may be achieved if the first few cycles are ignored. Up to 10 cycles can be ignored. However, if the first cycles look similar to subsequent cycles, better results will be achieved by deselecting "Ignore First", because the normalization algorithm will have more data to work with.

Outlier Removal

To distinguish between minor changes in fluorescence and genuine reactions in no template controls (NTCs), two measures are provided: "NTC Threshold" and "Reaction Efficiency Threshold". "NTC Threshold" is recommended for most applications. The approach used should be validated.
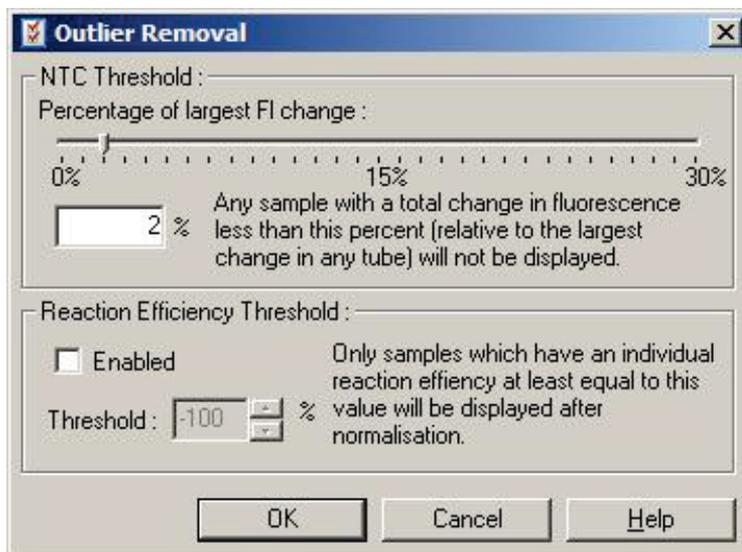
NTC Threshold: This allows samples or NTCs that have a slight drift upwards to be excluded from analysis. All samples with a change below the "NTC Threshold" will not be reported, and a flag "NEG (NTC)" will be shown in the "CT Comment" column.

The percentage is relative to the largest maximum change found in any tube. For example, if one sample began at a background of 2 fluorescence and increased to 47 fluorescence, then 45 fluorescence represents 100%. An "NTC Threshold" of 10% would consider any sample less than 4.5 fluorescence as noise.

Reaction Efficiency Threshold: The "Reaction Efficiency Threshold" is an alternative method of excluding noise from analysis. This normalizing algorithm uses the reaction efficiency estimation techniques used in comparative quantitation (see Section 7.6.6). All samples that do not have a reaction efficiency of at least this level are excluded, and a flag "NEG (R.Eff)" will be shown in the "CT Comment" column.

A level of 0% indicates that during the exponential phase, no reaction took place. 100% indicates that a completely efficient reaction took place during the exponential phase. Negative percentages indicate that during the exponential phase, the fluorescent signal declined.

Current research is not conclusive on the precise levels of efficiency needed to distinguish genuine reactions from contamination and other effects. For this reason, we recommend using this feature conservatively, with the assumption that any

sample with a genuine reaction will have some visible exponential phase with some increase in fluorescence. Setting this value higher than 0% will exclude some samples with inefficient but perceptible increase in fluorescence, whereas setting below 0% will display samples that decreased in fluorescence during the exponential phase, which should clearly be excluded.

Note: If a value is excluded due to activation of either of these techniques, a corresponding C_T value in the "Quantitation Results" window will not be displayed. Simultaneously a flag indicating the exclusion will be shown in the "Ct Comment" column. It is therefore important to make sure the "Ct Comment" column is displayed at all times.

In the image below, samples 7, 8 and 9 were excluded due to the "Reaction Efficiency Threshold".
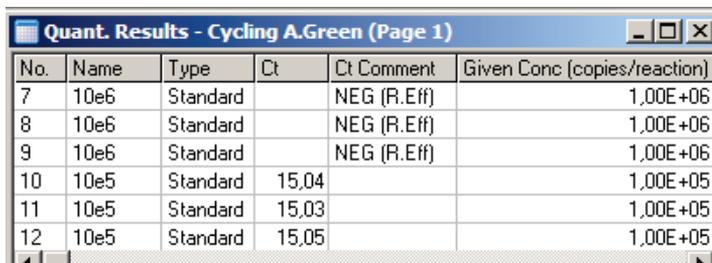
No.	Name	Type	Ct	Ct Comment	Given Conc (copies/reaction)
7	10e6	Standard		NEG (R.Eff)	1,00E+06
8	10e6	Standard		NEG (R.Eff)	1,00E+06
9	10e6	Standard		NEG (R.Eff)	1,00E+06
10	10e5	Standard	15,04		1,00E+05
11	10e5	Standard	15,03		1,00E+05
12	10e5	Standard	15,05		1,00E+05

Quant. Results - Cycling A.Green (Page 1)

Slope, amplification, reaction efficiency

The slope (M) of a reaction (shown in the "Standard Curve" window) can be used to determine the exponential amplification and efficiency of a reaction using these calculations:

Exponential amplification = $10^{(-1/M)}$

Reaction efficiency = $[10^{(-1/M)}] - 1$

Optimal values for M, exponential amplification and reaction efficiency are −3.322, 2 and 1, respectively. The reaction efficiency is displayed in the report (in full and standard reports, see page 7-13) and in the "Standard Curve" window.

The slope is calculated as the change in C_T divided by the change in log input (e.g., copy number). A 100% efficient amplification means a doubling of amplification product in each cycle, thus resulting in an M value of -3.322, an amplification factor of 2 and a reaction efficiency of 1.

Given an M value of -3.322, the calculations are as follows:

Exponential amplification: $10^{(-1/-3.322)} = 2$

Reaction efficiency: $[10^{(-1/-3.322)}] - 1 = 1$

As an alternative example: an M value of 3.8 means that the reaction has an exponential amplification of approximately 1.83 and a reaction efficiency of 0.83 (or 83%).

Offset

In a formula describing the relationship between two variables, the offset is expressed with the letter B (y = Mx + B). The offset is also sometimes referred to as the intercept. B represents the C_T for a given concentration of 1 unit. By substituting 1 into the concentration formula, as shown below,

$C_T = \log(1) * M + B$

$C_T = 0 * M + B$

… the result is $C_T = B$.

The intercept can change from run to run and is a less stable measurement than the gradient. For this reason, the gradient is analyzed more frequently than the intercept.

Main window

The main window displays the amplification plots on a log scale.

Clicking "Linear Scale" at the bottom of the window changes the scale from the log scale to the linear scale, and vice versa. Changing between these scales only alters the display of the graphs, not the calculations. This can be verified with the pinpointer tool by right-clicking on the graph and selecting "Show pinpointer". Using a log scale, smaller

values are more visible on the graph, whereas a linear scale facilitates a view of the entire reaction.

Note: Amplification plots update in real time, because the Rotor-Gene Q MDx is actively acquiring data during a run. This real-time monitoring of data enables the user to see results as soon as the curves show exponential growth. Preliminary conclusions can be drawn and decisions made for the next run.

Quantitation analysis templates

Quantitation analysis templates allow the user to export normalization and threshold settings into a single ***.qut** file. This file can be imported and reapplied in other experiments. See Section 8.1 for more details.

7.6.3   Two standard curve

Relative gene expression analysis using a normalizing gene can be performed using the two standard curve method.

The method requires a standard curve for each gene. The concentration for each gene is quantified according to its standard curve. The expression of the gene of interest is then normalized with the normalizing gene (often a housekeeping gene).

It is important that the standards and replicate samples are designated correctly during sample setup (see Section 6.1.4). In particular, corresponding samples must have the same name in each analysis. In a multiplex reaction, where the tube positions of the gene of interest and the normalizing gene are the same, one set of sample definitions is sufficient. If performing relative analysis with a normalizing gene using a single channel (i.e., reactions are run in separate tubes using the same fluorophore), then two sample pages should be created. The first should label the tube positions with sample names for the gene of interest, with the other

positions left unnamed. The second should label the
positions used for the normalizing gene. The software will
then match samples across the two analyses based on their
names.

Expression analysis using the two standard curve method

Data can first be analyzed for each gene using quantitation
analysis. Otherwise, the results for each gene will
automatically be determined using the "Autofind Threshold"
tool.

1. From the "Analysis" window, select the "2 Std Curves
    (Rel.)" tab. Click "New Analysis...".

2. Enter a name for the analysis.
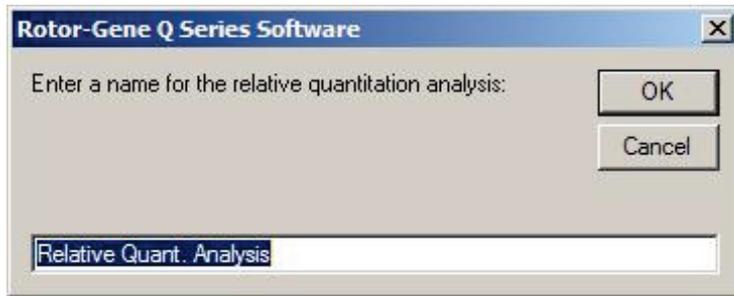
3. Designate the pages used for normalizing gene analysis and gene of interest analysis. For example, clicking on "Gene of Interest Standard Curve" brings up the "Selection of Gene of Interest Standard..." window. Select the page where the gene of interest was quantitated. Repeat the procedure for the normalizing gene. Optionally, a calibrator can be defined. If this option is selected, the calibrator is assigned a value of 1, and all other sample concentrations are calculated relative to this sample.
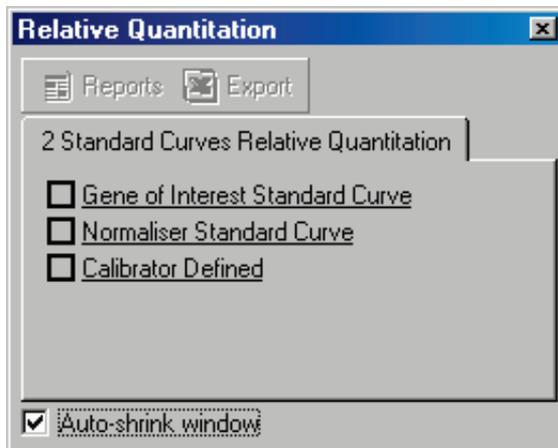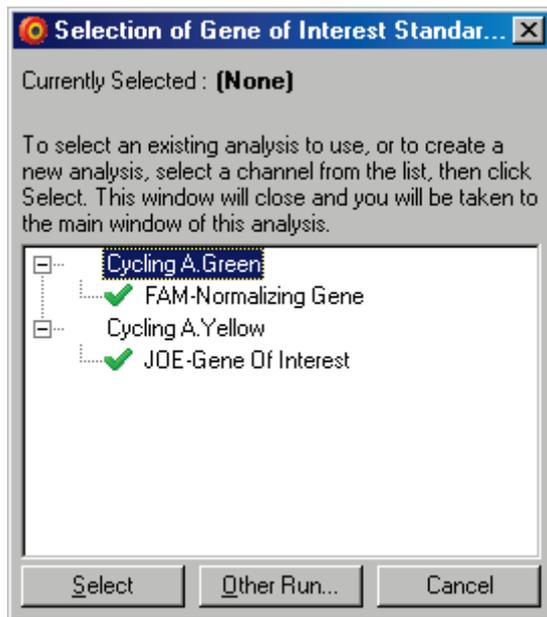
After completing the selections, the options will be checked with a check mark as shown below.
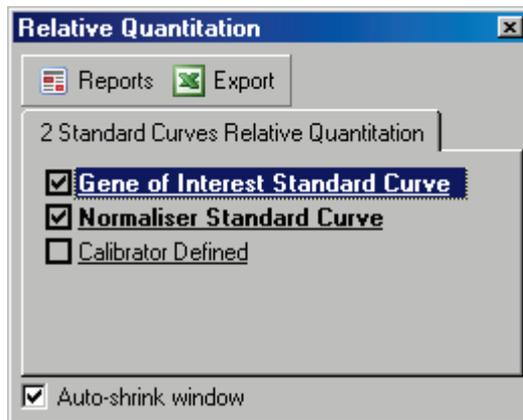
4. Click the "Reports" button to display the "Report Browser". Select the analysis with the correct name from the list. Click the "Show" button to display the relative quantitation report.

Cick the "Export" button to export the results to a new Excel spreadsheet. If a calibrator is included, the results are calculated relative to the calibrator sample, which is assigned a value of 1.
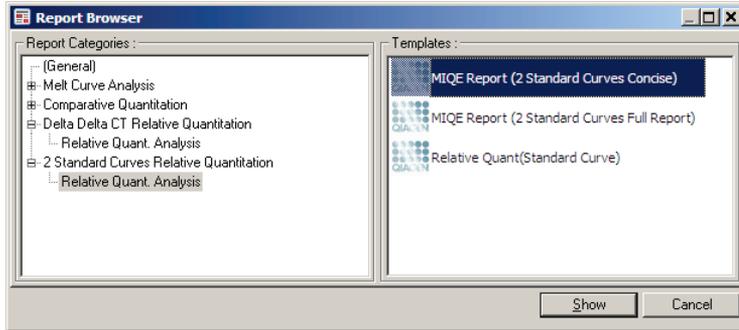
5. The concentrations – as read from the standard curves for the gene of interest (GOI Conc.) and the normalizing gene (Norm. Conc.), as well as the relative concentration (Relative Conc.) – are displayed. The results can be saved as a Word file.
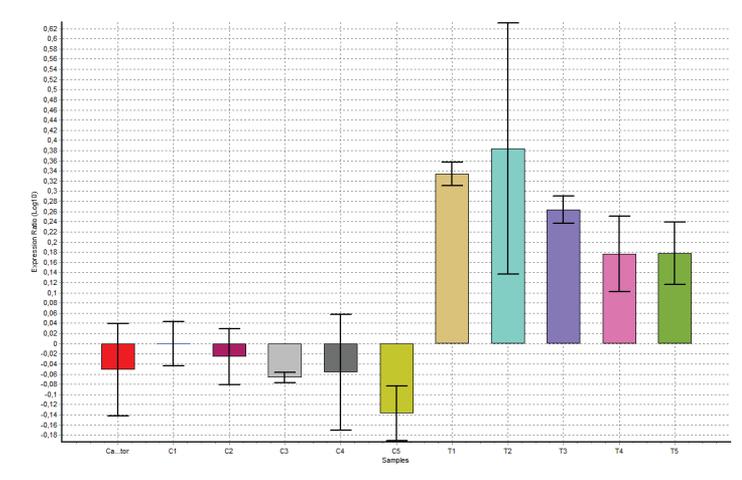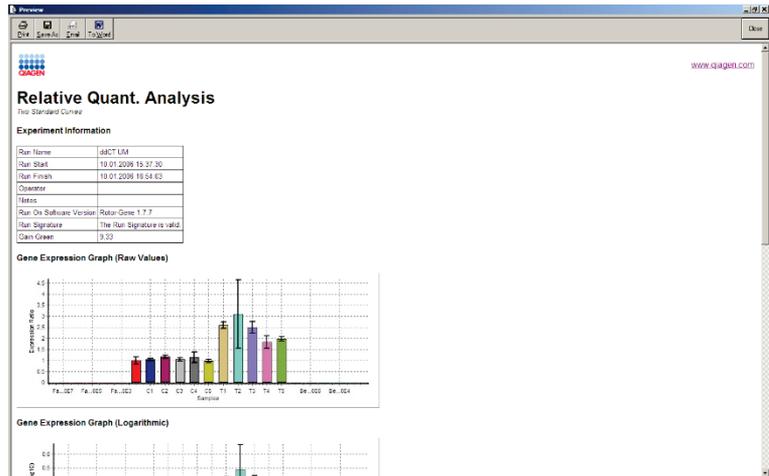
6. The Rel Min and Rel Max values are generated by calculating the standard deviation of the quotient from the standard deviations of the GOI and Normalizer using the following formula:

$$cv_{relconc} = \sqrt{cv_{GOI}^2 + cv_{Norm}^2}$$

where:

$$cv = \frac{s}{\overline{X}} = \frac{stddev}{meanvalue}$$

7.6.4   Delta delta C_T relative quantitation

The delta delta C_T method enables relative gene expression analysis. It is described by Livak and Schmittgen (2001)[*].

This method does not require standard curves to be included in each run. Each sample is first normalized for the amount of template added by comparison with the normalizing gene. These normalized values are further normalized relative to a

* Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{\wedge}$[–delta delta C(T)] method. Methods **25**, 402–408.

calibrator treatment. The calibrator could be, for example, wild-type, untreated control or time-zero samples.

It is essential that the amplification efficiencies of the gene of interest and the normalizing gene are identical and that this is validated according to the guidelines of Livak and Schmittgen.

It is essential that the sample names are defined correctly in the "Edit Samples" window, with the same samples labeled identically in each composite quantitation analysis.

1. Analyze the data using "Quantitation". It is not necessary to run a standard curve once validation has been performed.

2. Click on the "Other" tab in the "Analysis" window to make a menu appear. Select "Delta Delta C_T Relative Quantitation", and then select "New Analysis".
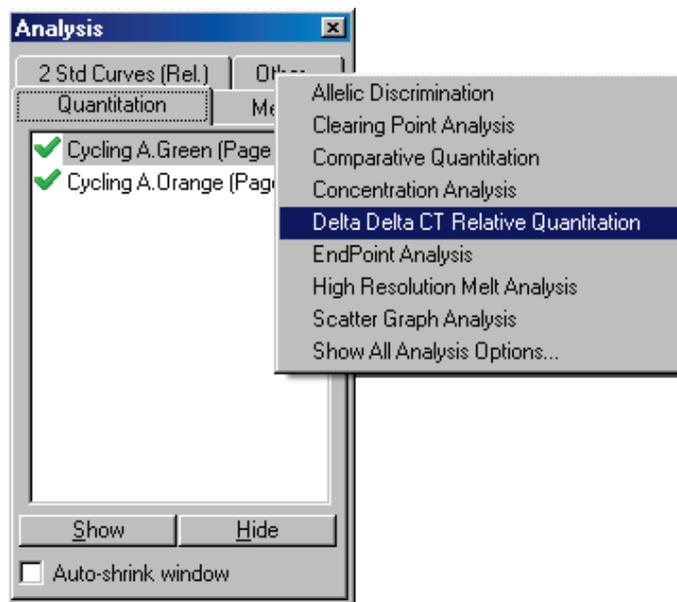
3. Enter a name for the analysis.
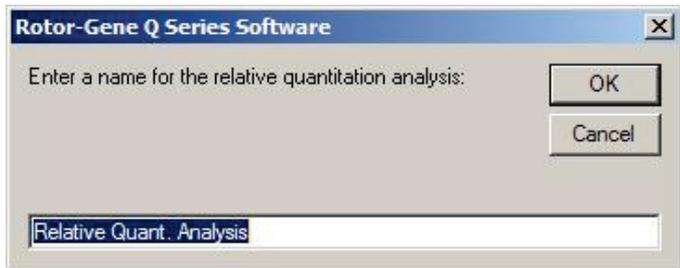
4. "Validation Run Performed" must be checked to proceed with the analysis. Define the pages where the gene of interest and normalizing gene have been analyzed.
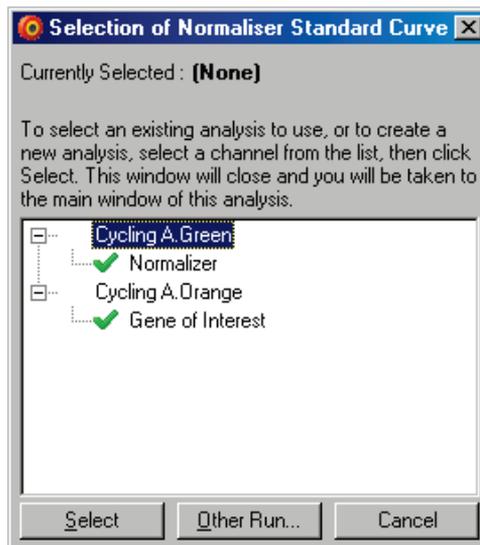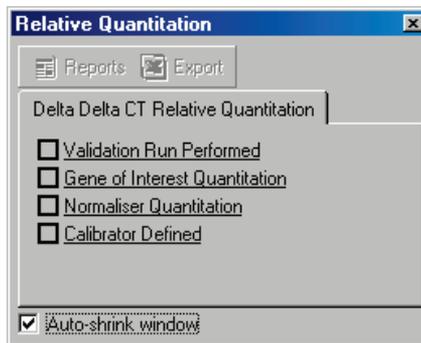
5. Click the "Reports" button to display the "Report Browser" window. Select the analysis with the correct name from the list. Click the "Show" button to display the relative quantitation report.

The "Export" button exports the results to a new Excel spreadsheet. If a calibrator is included, the results are relative to the calibrator sample, which has a value of 1.
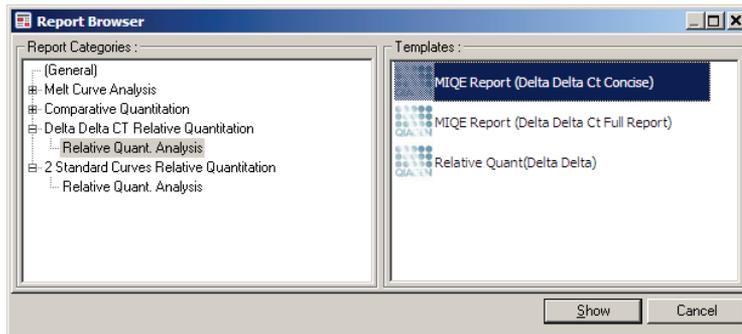
An example of results from this analysis is shown below. The C_T values for the gene of interest (GOI CT), C_T values for the normalizing gene (Norm. CT), Delta C_T, Delta Delta C_T and relative concentration (Relative Conc.) are displayed. The expression is relative to the calibrator sample, which is assigned a relative expression of 1.

For further information on the derivation of the Rel Min and Rel Max calculations, refer to Litvak and Schmittgen (2001).[*]

* Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2^[−delta delta C(T)] method. Methods **25**, 402–408.

C	Replicate Name	GOI CT	Norm. CT	Delta CT	Delta Delta CT	Relative Conc.	Rel Min	Rel Max	Calibrator
	Dilution 8		28.37						
	Dilution 7	37.61	28.39	9.22	4.40	0.04728	0.04128	0.05414	
	Dilution 6	35.72	28.28	7.44	2.62	0.16228	0.14904	0.17669	
	Dilution 5	35.04	28.24	6.80	1.98	0.25292	0.11715	0.54605	
	Dilution 4	32.94	28.12	4.82	0.00	1.00000	0.69432	1.44025	Yes
	Dilution 3	31.66	28.23	3.43	-1.38	2.60825	2.16257	3.14579	
	Dilution 2	30.05	28.02	2.03	-2.79	6.92153	6.49040	7.38130	
	Dilution 1	28.61	27.92	0.69	-4.12	17.41896	16.47839	18.41322	
	QS 0,1 IU/µl		28.11						
	0,316 IU/µl	37.62	28.10	9.51	4.70	0.03857	0.03633	0.04094	
	1 IU/µl	36.84	28.15	8.69	3.88	0.06805	0.04415	0.10489	
	3,16 IU/µl	34.45	28.05	6.40	1.59	0.33305	0.28206	0.39325	
	QS4	32.67	28.29	4.38	-0.43	1.34925	1.09820	1.65770	
	QS3	30.07	27.98	2.09	-2.73	6.61982	6.18888	7.08076	
	QS2	26.88	27.64	-0.76	-5.57	47.61474	45.02202	50.35677	
	QS1	24.07	27.10	-3.03	-7.85	230.60440	208.45384	255.10870	

7.6.5 Melt curve analysis

Melt curve analysis analyzes the derivative of the raw data after smoothing. This analysis is commonly used for genotyping and allelic discrimination. Peaks in the curve are grouped into bins, and all peaks below the threshold are discarded. Bins can then be mapped to genotypes using the "Genotypes" command.

After a run has finished, for some chemistries a melt step can be added to visualize the dissociation kinetics of the amplified products. The temperature is increased at a linear rate, and the fluorescence of each sample is recorded. A typical melt curve analysis is shown below.

Peak Calling

☑ Flip sign of dF/dT

Threshold : `0.30821`

Temperature
Threshold : `55`

Peak Bins

Name	Value	Width	
Bin A	80.42	4	
Bin B	88.55	4	

| New | Edit | Remove |

Imported Settings
<none>

| Import... | Export... |

Flip sign of
dF/dT:

Before defining peaks, ensure that the
dF/dT sign is correct for the data set to give
positive peaks.

Defining peaks:

In melt curve analysis, peaks can be
defined and reported using different
methods. One is to automatically call all
the peaks for each sample. The other is to
assign peaks to bins, which is useful for
genotyping.

Bins define the area where peaks are
expected to occur. The melt curve analysis
software clusters peaks into bin groups,
based on actual peak values in the curve.
Bins can be edited if required.

Any peak that is within the defined range of
the bin will be assigned to the bin. If there
are two bins close together, then the peak
will be assigned to the closest bin.

Note: The bins should not be visually
positioned to estimate peak positions. Set
the bins in the approximate area of
interest, and then use the actual reported
values in the results table for a more
accurate result.

Peak Bins:	To define a bin, click the "New Bin" button, and then click and hold on the graph to define the center of the bin. To add another bin, repeat the process. Use the "Remove" button to delete bins.
Threshold:	To set the threshold (y axis), click the [icon] icon, and then click and hold on the graph and drag the threshold line to the desired level.
Temperature Threshold:	To set a temperature threshold (x axis), click the [icon] icon, and then click and hold on the graph and drag the threshold line to the right. This eliminates the threshold line for the lower temperatures. **Note**: This is useful when there is noise in the signal at low temperatures.

Reports

This opens the "Report Browser", where a report can be chosen to preview. A report can be generated based upon the currently selected channel, or a multichannel genotyping report can be generated.

Results

This displays the "Melt Curve Results" window, which shows sample peaks.

Genotypes

Click "Genotypes…" and select the genotypes, as shown below.
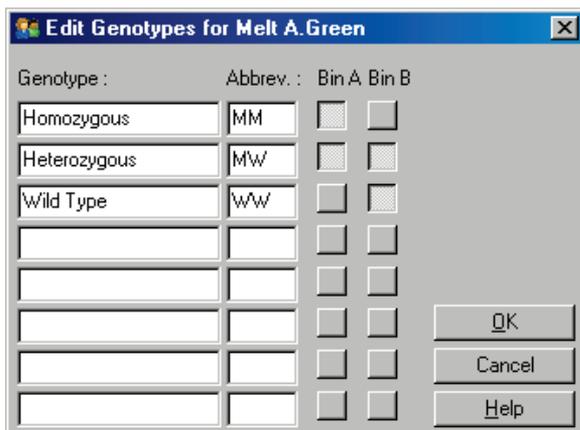
This window allows genotypes to be assigned to the incidence of peaks in bins. The default genotype configuration is shown in the screenshot, with heterozygous samples having two peaks: homozygous samples, a peak in the first bin, and wild type samples, a peak in the second bin. An abbreviation can be typed into the field next to the name of each genotype. This is used when printing multichannel genotyping reports, so that all results from multiple channels can be easily read.

For multiplex analysis, genotypes must be set up in each channel. If, for instance, a dual-channel quenched FRET analysis is run, where a wild type and a heterozygous genotype are expected in each channel, the bin parameters must be set up for each channel. The results will then be given in a multiplex report.

Melt analysis templates

Melt analysis templates allow the user to export normalization, threshold, genotype and bin settings into a single ***.met** file. This file can be imported and reapplied in other experiments. See Section 8.1 for more details.

7.6.6   Comparative quantitation

Comparative quantitation compares the relative expression of samples to a control sample in a run when a standard curve is not available. This is frequently used in microarray analysis. Warton et al. (2004)[*] provide an example of this technique.

1. To perform the analysis, select "Other" and then "Comparative quantitation" in the "Analysis" window. Double-click on the channel to analyze.

2. Choose a control sample using the drop-down menu on the right-hand side of the screen below the toggler.

3. The results are automatically calculated and displayed in the "Comparative Quantitation Results" window below the graph.

The first columns of the "Comparative Quantitation Results" window show the sample number and name. The "Takeoff" column gives the takeoff point of the sample. The second derivative of the amplification plot produces peaks corresponding to the maximum rate of fluorescence increase in the reaction. The takeoff point is defined as the cycle at which the second derivative is at 20% of the maximum level and indicates the end of the noise and the transition to the exponential phase.

This graph shows a second derivative of an amplification plot, showing the relative positions of the second derivative peak and the takeoff point.

[*] Warton, K., Foster, N.C., Gold, W.A., and Stanley, K.K. (2004) A novel gene family induced by acute inflammation in endothelial cells. Gene **342**, 85–95.
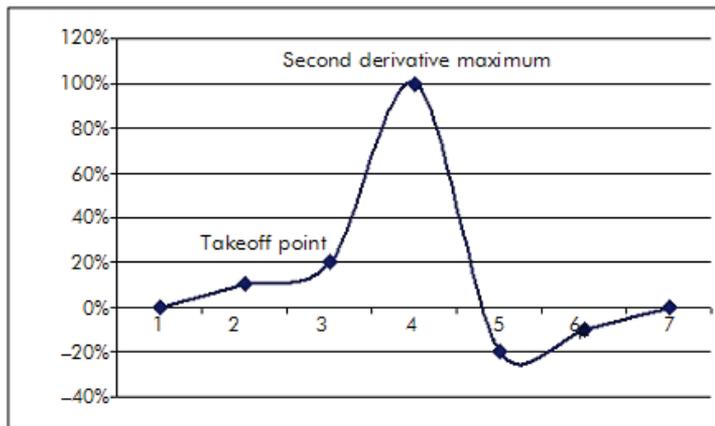
The "Amplification" column provides the efficiency of the sample. A 100% efficient reaction would result in an amplification value of 2 for every sample, which means that the amplicon has doubled in every cycle. In the raw data, the signal should be doubling in the exponential phase. For example, if the signal was 50 fluorescence units at cycle 12 and then 51 fluorescence units at cycle 13, it should increase to 53 fluorescence units at cycle 14. All of the amplification values for each sample are averaged to produce the amplification value that is shown on the right of the screen under the toggler. The greater the variation between the estimated amplification values of each sample, the larger the confidence interval will be (indicated by the value after the ± sign). The confidence interval, for a large sample number (N), gives a 68.3% probability that the true amplification of the samples lies within this range (1 standard deviation). By doubling the ± interval, a 95.4% confidence interval for a large N is achieved.

Calibrator Replicate

As in the delta delta C_T method, a calibrator sample is required, and measurements are relative to this calibrator sample. Replicates of the calibrator can be analyzed because, if multiple sample positions have the same name, the average of the takeoff points of these samples will be

used. To use this feature correctly, ensure that replicates have identical names.

Calibrator Replicate

[1] 3X10^8

Results
Average Amplification 1.78+-0.04
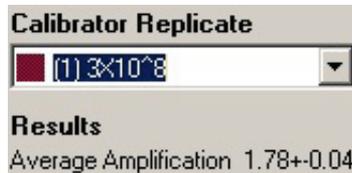
The average amplification is used to calculate expression. For example, a sample with a low amplification value will take longer to reach a certain absolute copy number than a sample with a higher amplification value. The "Rep. Conc." column of the "Comparative Quantitation Results" window provides the relative concentration. The relative concentration of each sample compared with the calibrator sample is calculated based on the takeoff point and the reaction efficiency. This is expressed in scientific notation.

Note: The value displayed in "Average Amplification" to the right of the ± represents the standard deviation of the average amplification after removal of outlier amplification values. If this value is large, then there may be a large error in the overall calculated concentration values.

Relative concentrations are calculated by the software as follows:

1. The takeoff point of each sample is calculated by looking at the second derivative peak.

2. The average increase in raw data four cycles after the takeoff is calculated. This is the amplification value for the sample.

3. Outlier amplifications are removed to account for noise in background fluorescence.

4. The remaining amplifications are averaged. This is the average amplification.

5. The average takeoff point is calculated for each calibrator replicate.

6. The relative concentration for a sample is calculated as Amplification^(Calibrator takeoff − Sample takeoff).

7. The result is displayed in scientific notation in the "Rep. Conc." column of the "Comparative Quantitation Results" window.

7.6.7 Allelic discrimination

Allelic discrimination uses real-time kinetic data from two or more channels to genotype samples. To perform this analysis, select "Other" and then "Allelic Discrimination" in the "Analysis" window. When performing allelic discrimination, it is not sufficient to double-click on one channel to analyze, because this analysis is performed using multiple channels simultaneously. To perform this analysis, either hold down CTRL and click to highlight each channel you wish to analyze, or drag the mouse pointer over these channels. Once the desired channels have been highlighted, click "Show". The list will update to show all the channels on one line, with a checkmark beside them. This indicates that they will all be used in one analysis. To remove one or more of these channels, right click on the analysis and select "Remove Analysis…". Those channels can then be included in another allelic discrimination analysis. A channel can only be used in one analysis at a time.

Reports: This opens the "Allelic Discrimination Analysis" report for preview.

Results: This displays the "Allelic Discrimination Results" window. This window is opened by default when the analysis is first displayed.

Normalization options: A variety of options are available to optimize raw data normalization:

■   Dynamic Tube (dynamic tube normalization)

■   Slope Correct (noise slope correction)

■   Ignore First x cycles (correction for noise in initial cycles)

■   Takeoff point adjustment

For more details, see page 7-24.

Discrimination Threshold: Enter values in these text boxes to position the discrimination threshold. All curves passing this threshold are considered to be genotyping samples. Click on the icon to the right of each text box, and then drag the threshold on the graph to set these values visually.

Genotypes: This opens the "Genotyping" window, which is used to define which genotype is detected in each channel. This window enables genotypes to be assigned to channels for allelic discrimination analysis.

In the example below, a sample is heterozygous if readings in channels Cycling A.Green and Cycling A.Yellow cross the threshold.

Genotype	Reacting Channels	
Wild Type	Cycling A.Green	
Heterozygous	Cycling A.Green	Cycling A.Yellow
Mutant		Cycling A.Yellow

OK   Cancel   Help
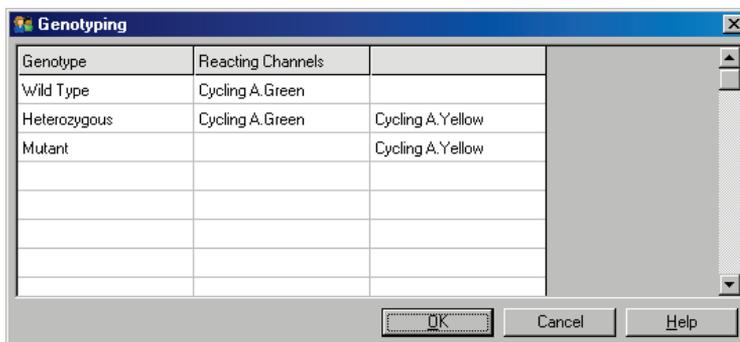
Allelic analysis templates: Allelic analysis templates enable export of normalization, threshold and genotype settings into a single ***.alt** file. This file can be imported and reapplied in other experiments. See Section 8.1 for more details.

Imported Settings
<none>
Import...   Export...

7.6.8 Scatter graph analysis

Scatter graph analysis enables genotyping based on the relative expression of amplification plots across two channels. Unlike allelic discrimination, genotype is decided based on regions defined from the scatter graph rather than from a single threshold. To perform this analysis, select

"Other" and then "Scatter Graph Analysis" in the "Analysis" window.

When performing scatter graph analysis, it is not sufficient to double-click on one channel to analyze, because this analysis is performed using two channels simultaneously. To perform this analysis, either hold down SHIFT and click to highlight the channels that you want to analyze, or drag the mouse pointer over the channels. Once the desired channels have been highlighted, click "Show".

The list will update to show all the channels on one line, with a checkmark next to them. This indicates that they will all be used in one analysis. To remove one or more of these channels, right-click on the analysis and select "Remove Analysis...". Those channels can then be included in another scatter graph analysis. A channel can only be used in one analysis at a time.

Reports:	This opens the "Scatter Analysis" report for preview.
Results:	This displays the "Scatter Analysis Results" window. The genotype for each sample is determined by the regions defined by the user on the scatter graph.
Normalization options:	A variety of options are available to optimize the way in which the raw data plots are normalized:

- Dynamic Tube (dynamic tube normalization)
- Slope Correct (noise slope correction)
- Ignore First x cycles (correction for noise in initial cycles)
- Takeoff point adjustment

For more details, see page 7-24.

Genotypes...:   This opens the "Genotyping" window, which is used to define which genotype is detected in each channel. In this window, genotypes can be assigned based on the channels in which a sample reacts. The channels selected will be used to label the corners of the scatter graph and will guide the user to the general area of the scatter graph in which regions should be defined.
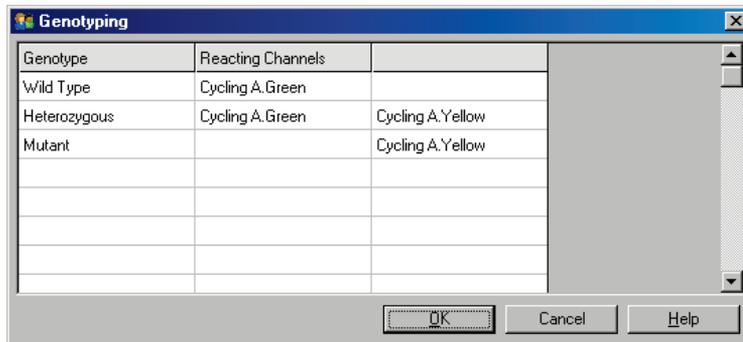
Scatter Graph: The scatter graph displays the relative expression of the two selected channels. The display is normalized to account for different fold increases in each channel and log transformed to accentuate the differences in expression between samples.

To perform genotyping, the user defines regions by clicking and dragging a selection on the graph. The selection can then be labeled based on the genotypes configured in the "Genotyping" window.
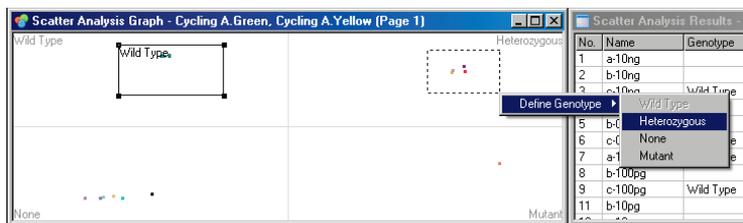
Scatter graph analysis templates:

Scatter graph analysis templates enable genotype and region settings to be exported into a single ***.sct** file. This file can be imported and reapplied in other experiments. See Section 8.1 for more details.

Imported Settings

<none>

[Import...]   [Export...]

7.6.9   EndPoint analysis

EndPoint analysis allows discrimination between amplified and unamplified samples at the end of a run. Results are qualitative (positive/negative), not quantitative.

EndPoint analysis is shown in the screenshot below.
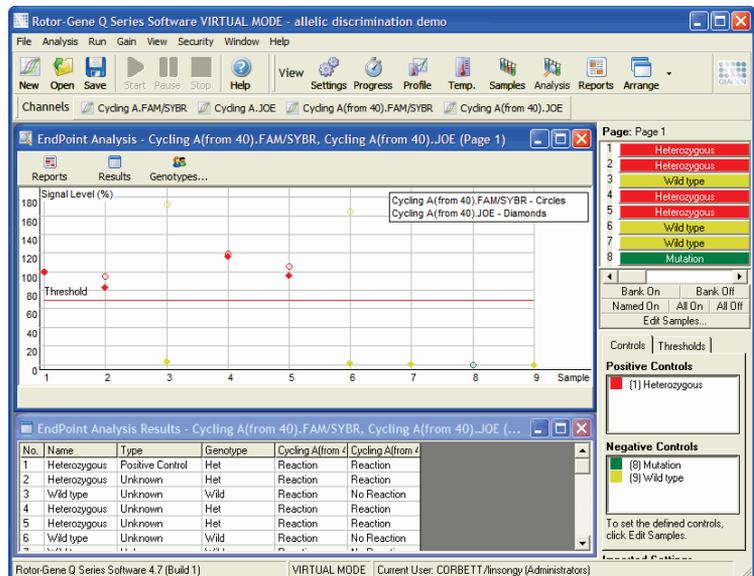
EndPoint analysis is similar to allelic discrimination in that the results are qualitative and that names can be assigned to certain permutations of reactions over different channels. However, in EndPoint analysis, only a single reading is

available, in contrast to allelic discrimination, which uses a cycle-by-cycle reading for each sample. This means that the user must identify positive and negative controls to facilitate the analysis. For the raw data, signal levels are normalized relative to the known positive and negative controls for each channel. The user then selects a percentage signal level as the threshold.

Terms used in EndPoint analysis

Some terms used in EndPoint analysis are explained below.

Positive control: This is a sample which is known to amplify.

Negative control: This is a sample which is known not to amplify. This represents the typical background signal.

Threshold: The threshold is a signal level above which a sample is said to be positive (amplified). This setting must be adjusted by the user for each run.

Signal level: A percentage of fluorescent signal, normalized so that the highest signal of the positive controls is 100% and the lowest signal of the negative controls is 0%.

Genotype: An interpretation of different permutations of reactions on different channels. For example, the genotype "heterozygous" could be assigned to samples that reacted in both channels green and yellow. The genotype can also be used to report the results of reactions with internal controls. For example, results could be reported as "inhibited", "positive" or "negative", depending on whether a reaction was seen in certain channels or not.

Profile configuration
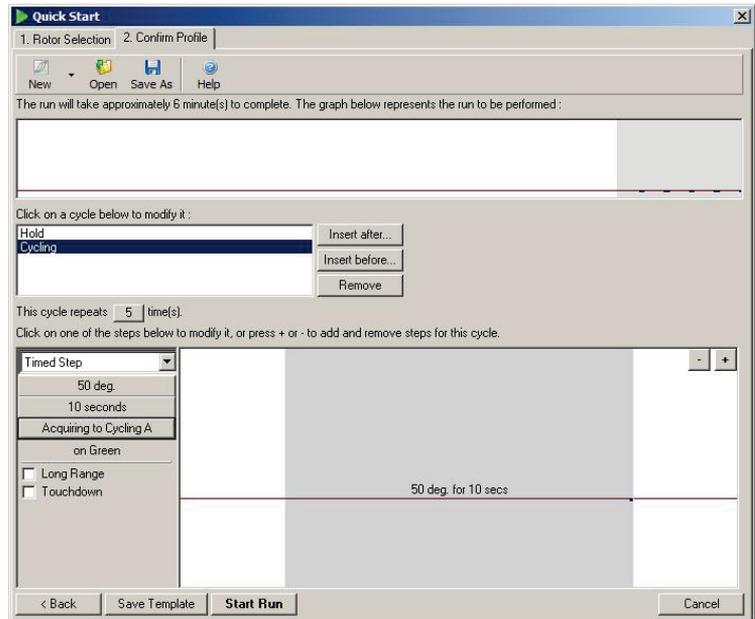
To perform EndPoint analysis, perform a profile with a hold at 50°C for several minutes, and then a cycling step with 1 step (50°C for 10 seconds), acquiring on the required channel. Set the number of repeats to 5, as shown above. These times are a guide only and may vary for your particular application. The more repeats in the profile, the more information will be available to perform the analysis. The analysis will automatically average all the readings to achieve a single value for each sample. There is no specific number of repeats required. Unless a very high level of accuracy is required, five repeats are usually sufficient.

Analysis

EndPoint analysis can be performed on a number of channels simultaneously. To create a new analysis, click on the "EndPoint" tab, select the channels by dragging over them with the mouse pointer, and then click "Show".

Define controls

When an EndPoint analysis is opened for the first time, the following message will be displayed if positive and negative controls have not been defined:
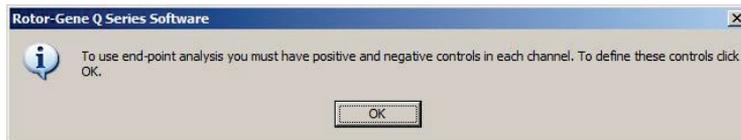
Click "OK". The "Select Positive and Negative Controls" window appears, allowing positive and negative controls to be defined. To define a sample in the "Name" column as a positive or negative control, click the cell beside it (in the "Type" column) and select its relevant control type from the drop-down menu that appears.

Note: Also check the "Selected" column (far right of the window). If the sample's corresponding cell says "No", the sample will not be analyzed. Click the cell to change this.
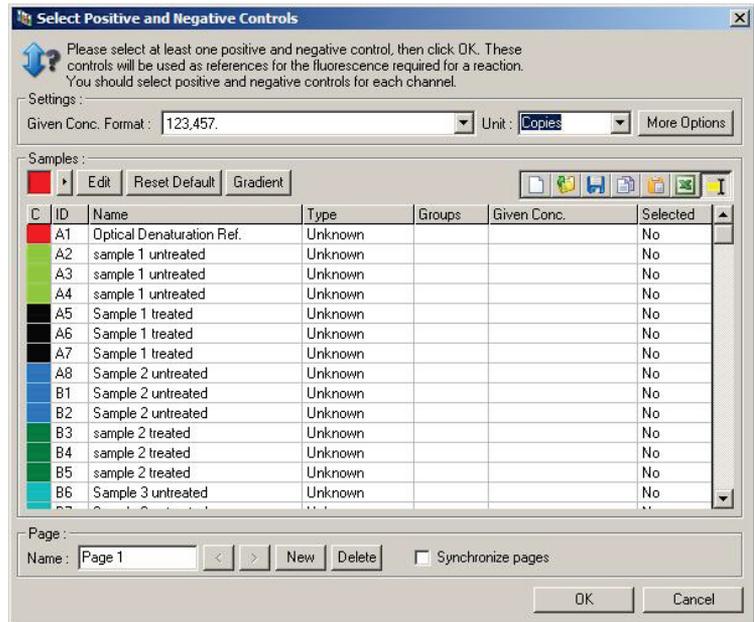
This screen functions in the same way as the "Edit Samples" window (Section 6.1.4).

Normalization

Normalization of EndPoint analysis data scales all signal levels to within the range of 0–100%. At least one positive and one negative control must be selected, or more if analyzing multiple channels and the standards are not multiplexed. More than one positive and one negative control should be run if there is a risk that a positive control may not amplify.

1. For each channel, all the positive controls are analyzed, and the one with the highest fluorescence is set to be 100%. This means that if duplicate controls are run, a positive control can fail without affecting the run.

2. All the negative controls are analyzed, and the one with the lowest fluorescence level is set to be 0%.

3. The raw fluorescence values of the remaining samples are scaled relative to the highest positive control and the lowest negative control.

For example:

Sample	Type	Fluorescence
1	Positive control	56.3
2	Positive control	53.0
3	Negative control	4.5
4	Negative control	4.3
5	Sample	48.1
6	Sample	6.4

This run was a success, as the two positive and two negative controls are close together and are outside the fluorescence values of the samples.

The normalized values are:

Sample	Type	Expression (%)
1	Positive control	100.0
2	Positive control	93.7
3	Negative control	0.4
4	Negative control	0.0
5	Sample	84.2
6	Sample	4.0

Sample 1 was the positive control with the highest fluorescence, so it was set to 100%. The other positive control was slightly lower. Sample 4, the lowest negative control was set to 0%. It is now clear that sample 5 has probably amplified, whereas sample 6 has probably not amplified.

Note: Depending on the positive and negative controls selected, it is possible to achieve expression levels greater

than 100% or less than 0%. A result greater than 100% can be interpreted to mean that the sample is more highly expressed than the positive controls. A result less than 0% can be interpreted to mean it is less likely that the sample amplified than that the negative controls amplified. Since this analysis is qualitative, such results are not a concern.

If the negative controls result in higher fluorescence than the positive controls, the samples have been incorrectly set up and the following message appears:
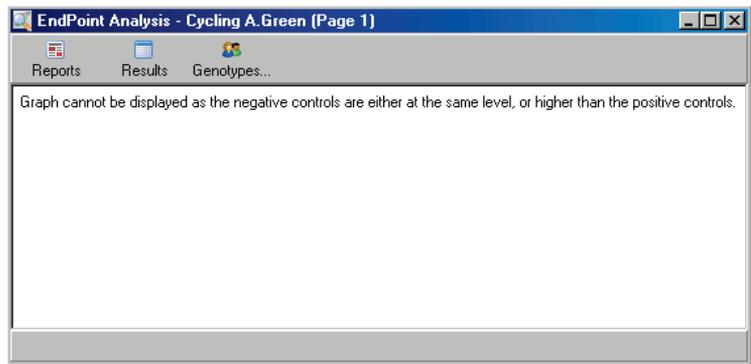
Normalization in multiple channels

It is possible to analyze signal data over multiple channels, but the sample setup is more complex. EndPoint analysis assumes that multiplexing is performed, and so each tube can only have a single tube position. It is not currently possible to analyze a setup in which a sample position is a positive control for one channel and a negative control for another.

Although only one sample definition per tube position is given in the "Edit Samples" window, normalization occurs independently for each channel.

If a tube position is a positive control for at least one channel, it should be specified as a positive control in the "Type" column of the "Edit Samples" window. Otherwise, its type should be "Sample". This also applies for negative controls.

For example, if a sample is a positive control in the green channel but not in the yellow channel, the sample should still be defined as a positive control. Since the highest positive control in each channel is used, if there is least one positive control in the yellow channel that amplifies, the definition of the sample as a control for the green channel is ignored.

Threshold

The threshold is used to determine the percentage expression required for a reaction in each channel. Once the positive and negative controls have been defined, all channels will be normalized to the same 0–100% scale. For this reason, only one threshold is needed, even when analyzing multiple channels.

Click and drag the threshold line to an area between 0 and 100. The threshold should not be too close to samples on either side of the line, because this indicates that the run was not conclusive. If the difference between a sample being defined as amplified or not amplified is just a few percent, this means that if the reaction were repeated, the sample could appear on the other side of the threshold.

Genotypes

This option opens the "Genotyping" window, which is used to define which genotype is detected in each channel.

This window allows genotypes to be assigned to channels. In the example above, a sample is heterozygous if readings in channels Cycling A.Green and Cycling A.Yellow cross the threshold.

EndPoint analysis templates

EndPoint analysis templates allow the user to export genotype and threshold settings into a single ***.ent** file. This file can be imported and reapplied in other experiments. See Section 8.1 for more details.

7.6.10   Concentration analysis

Concentration analysis allows the Rotor-Gene Q MDx to be used to measure DNA concentrations or obtain fluorometer readings.

The screenshot below shows this analysis.
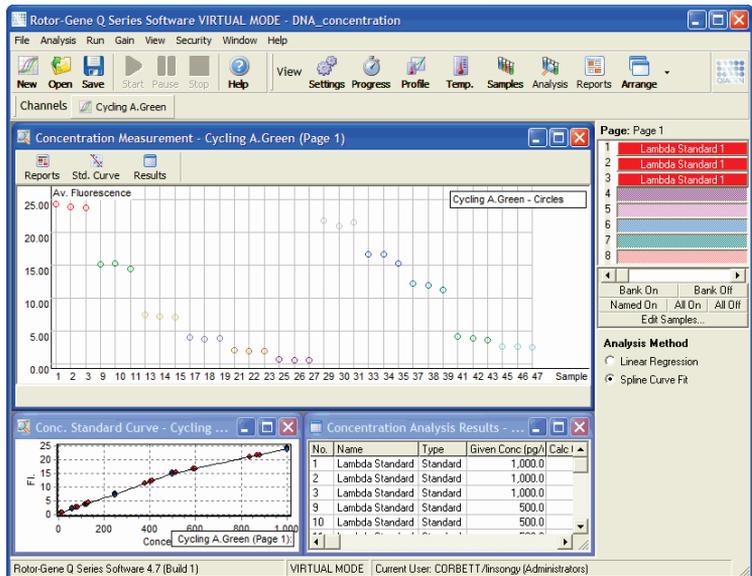
Preparing a run

To perform concentration analysis, first prepare fluorescent standards and samples, ideally in triplicate.

Preparation of standards

A standard curve is used to determine the concentration of DNA from each sample measured.

The DNA used for the standard curve should be a similar type of DNA as in the samples being measured. The concentration of at least one DNA sample should be determined using ultraviolet spectrophotometry, and this sample should be used as the standard. A minimum of three standards (with replicates) should be used. Importantly, DNA standards used in fluorescence detection are only linear within the range of 1–100 ng/µl. Within this range, if the concentration of DNA is halved, so is the fluorescent reading. The confidence intervals for any concentration outside this range are very broad due to nonlinearity of the chemistry.

Type of DNA measured

Differences have been observed in the measurement of various forms of DNA (e.g., genomic DNA compared to plasmid DNA). Therefore, only similar DNA types should be measured together, and the use of plasmid DNA as a standard should be avoided when measuring genomic DNA.

Run setup

To set up the run, select "Nucleic Acid Concentration Measurement" from the Quick Start wizard.
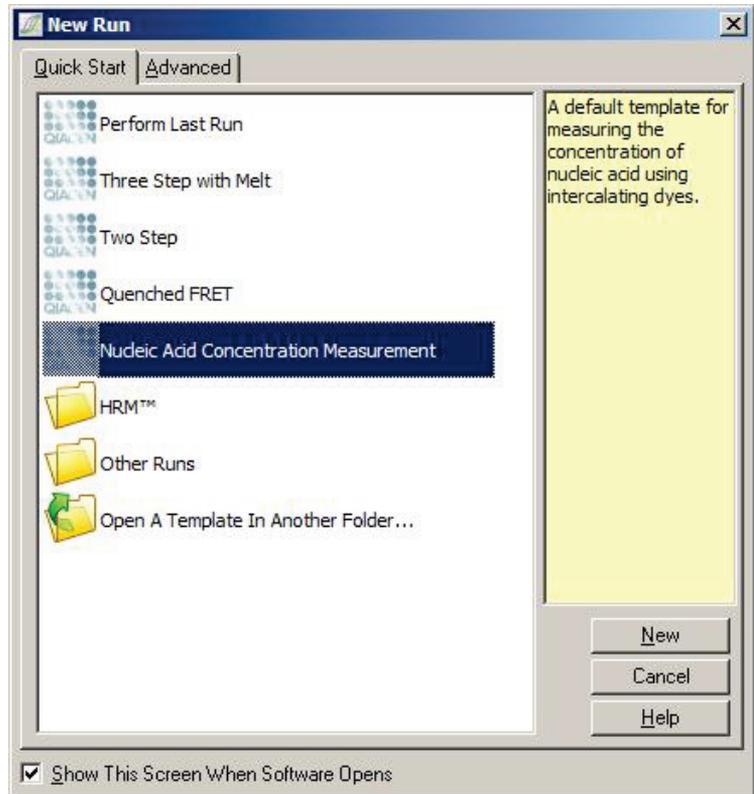
Note: Ensure that a positive control, such as a high concentration standard, is run in tube position 1. Without a positive control, the software will be unable to optimize gain settings for maximum sensitivity. You will be prompted for this before each run.

Analysis

Concentration analysis operates by relating the fluorescence level to a concentration value. Two analysis models are available. The optimal analysis to choose depends on the chemistry and application.

"Linear Regression" analyzes data by assuming a linear relationship and estimating unknown values on the basis of a generated linear model. It determines measurement error by examining the deviation of the readings from a linear model. If concentration readings are linear, this is the most suitable analysis because it provides statistical analysis of variation (ANOVA) to the user.

"Spline Curve Fit" assumes only that concentration values increase with fluorescence. While this approach makes estimates of nonlinear data more accurate, it cannot provide ANOVA, because it does not assume a linear model.

7.6.11   High resolution melt analysis

High resolution melt (HRM) analysis characterizes samples based on sequence length, GC content and complementarity. HRM analysis is used in genotyping applications, such as analysis of gene mutations or single nucleotide polymorphisms (SNPs), and in epigenetics applications for analysis of DNA methylation status. HRM analysis provides accurate results and savings on probe and label costs, compared to other methods.

To perform the analysis, select "Other" and then "High Resolution Melt Analysis" in the "Analysis" window. Double-click on the channel to analyze. The melt curves from the raw channel are normalized by averaging all starting and ending fluorescence values and then forcing the end points of each sample to be the same as the average.
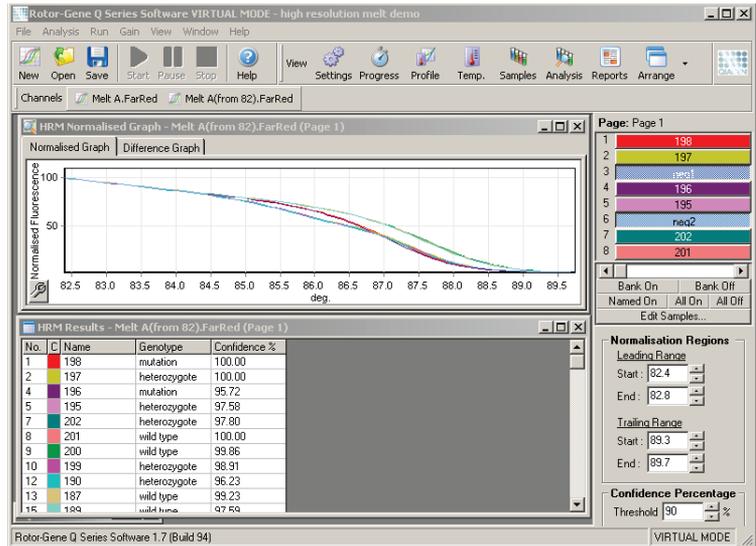
Autocalling of samples is achieved by clicking "Genotypes". Enter the name of the genotype, followed by the sample number, which is used as a positive control to call unknown samples automatically.
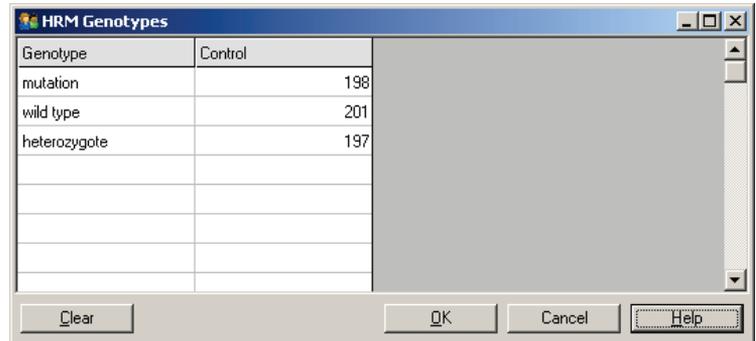
For more details on HRM analysis, see Section 11.

7.7 Run menu

7.7.1 Start Run

This option starts the defined temperature profile with the current gain settings. Before the run starts, the "Profile Run

Confirmation" window appears. A graphical representation of the temperature profile is displayed along with the gain settings for each channel.

7.7.2 Pause Run

This option enables a run to be paused and resumed. Pausing and resuming can seriously affect the results of a run. For this reason, a marker in the data will indicate that the run was paused and how long the pause was. A message is also placed in the "Messages" tab of the "Run Settings" window (see Section 7.8.1).

WARNING/ CAUTION ⚠️	**Hot surface** [W18] When pausing a run, the Rotor-Gene Q MDx will not be cooled completely to room temperature. Exercise caution before handling the rotor or any tubes in the instrument.

7.7.3 Stop Run

If this option is selected, a prompt will appear, asking for confirmation that the run should be stopped.

7.8 View menu

7.8.1 Run Settings

GeneralThis window allows the setup of run information, run file name, analysis date, operator and any associated notes.

The window contains all information, except for the profile, that is required to configure a run. After a run has finished, the following information is displayed in this window: cycler used, gain settings, number of channels and time of start and finish.
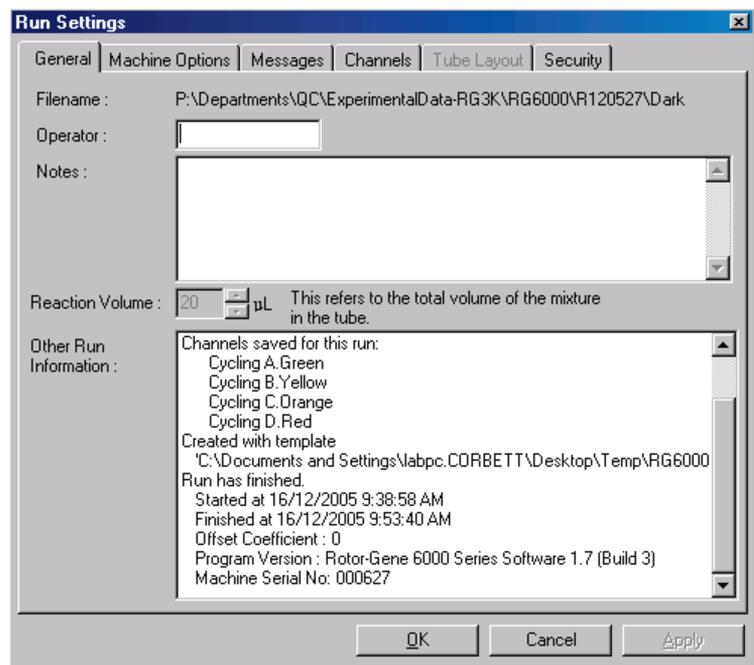
Machine Options

This tab displays settings for the configuration of the Rotor-Gene Q MDx.
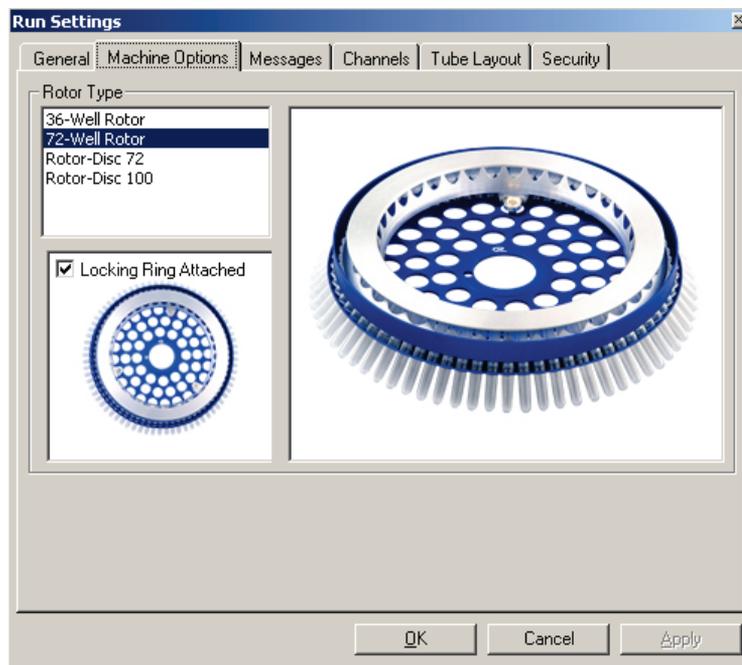
The rotor selected in "Rotor Type" should match the one that is currently installed in the Rotor-Gene Q MDx. If opening an existing run, this setting will reflect the rotor that was installed in the cycler at that time.

Messages

This tab displays messages that indicate if the user has made changes, such as pausing the cycler or skipping cycles, during a run. It also displays warnings received during the run. This tab should be checked if results are not as expected.

Channels

If configuring a new run, the "Channels" tab displays the current configuration of the available channels. If viewing an existing run, the information displayed represents the

configuration of the channels when the run was performed. If a run corrupts the channel settings, default channels can be restored by clicking "Reset Defaults".

Name	Source	Detector	Gain
Green	470nm	510nm	10
Yellow	530nm	555nm	8
Orange	585nm	610nm	10
Red	625nm	660nm	9
Crimson	680nm	710hp	7
HRM	460nm	510nm	7
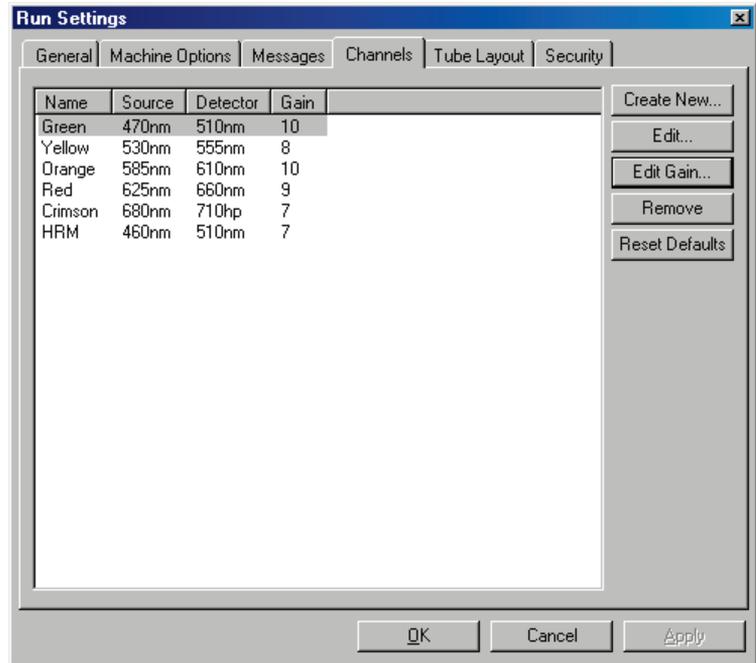
Name: This is the name of the channel.

Source: This specifies the excitation wavelength of the source LED.

Detector: This specifies the detection wavelength and filter type (nm=band pass, hp=high pass).

Gain: This specifies the gain for that particular channel.

Create New…: This feature enables creation of new channels. Clicking "Create New…" opens a window that asks for a new name, source and detection filter. The filters can be chosen using the drop-down menu next to each window.

Channels: Green, yellow, orange and red channels are standard configurations for 4-channel multiplex detection.

Tube Layout

If using a 72-Well Rotor, samples can be arranged to closely match the labeling on a 9 x 8 block. By default, the "Tube Layout" tab allows samples to be labeled sequentially (i.e., 1, 2, 3, …). This means that samples are labeled consecutively, in the order in which they are placed in the Rotor-Gene Q MDx. Alternatively, samples can be labeled 1A, 1B, 1C, etc. This option can be useful if samples were set up with a multichannel pipet.

Security

The "Security" tab displays information about the run signature. The run signature is an irreversible key that is regenerated every time the file is changed. If any section of the ***.rex** file is modified outside the software, the signature and the file will no longer match. Checking the signature allows confirmation that the raw data was not modified outside the application, that the profile has not been tampered with and that the temperature graph is valid. The signature also protects against corruption such as file-system errors.

Note: If ***.rex** files are e-mailed, the encryption process can invalidate the signature. To avoid this, zip the file before e-mailing.
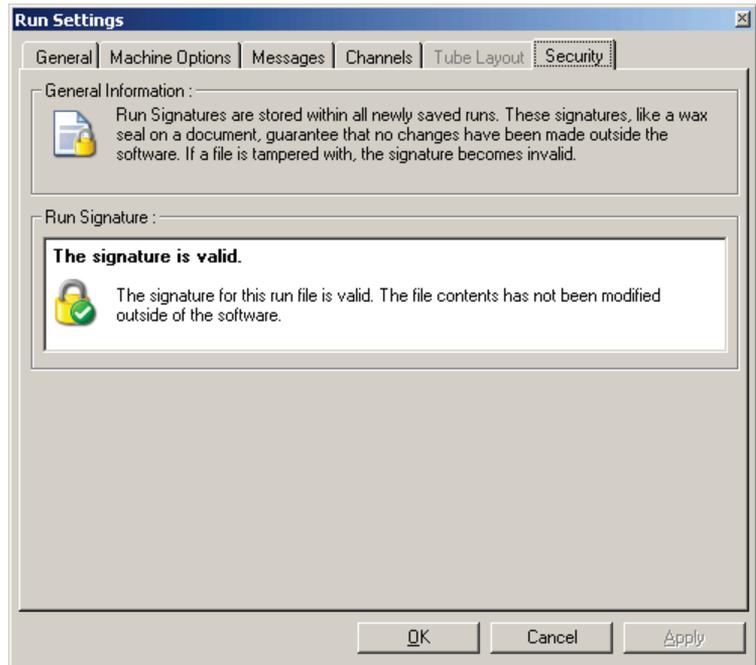
7.8.2   Temperature Graph

Select "Temperature Graph" from the "View" menu, or click on the "Temp." button to bring up the "Temperature" window. The graph displays the course of the set temperatures during cycling. It does not reflect a real-time temperature measurement. As the run proceeds, the "Set", "Actual" and "Hold" times are shown for each step of the program. For an existing run file, the "Temperature" window shows the temperature history during the run. The vertical scale represents temperature, and the horizontal scale represents time. Use the scrollbar to scroll backward and forward through the "Temperature" window.
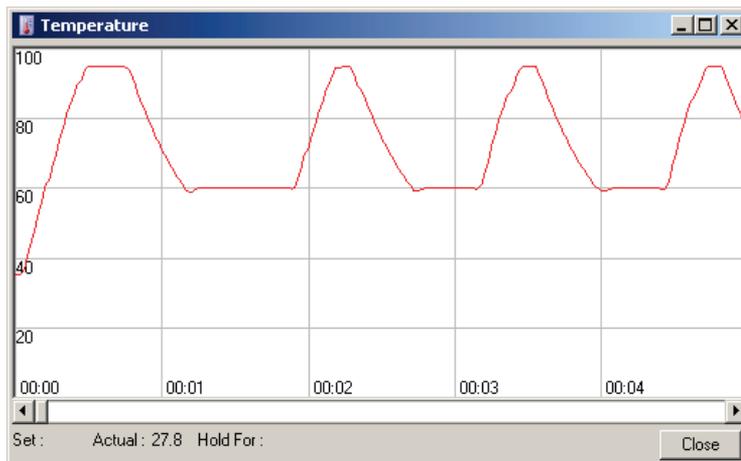
7.8.3   Profile Progress

Select "Profile Progress" from the "View" menu, or click on the "Progress" button to bring up the "Profile Progress" window. This window shows a graphical representation of the thermal profile associated with the run. When performing a run, the shaded portion of the window indicates the number of cycles that have been completed. There is also an estimate of how many minutes the run will take to finish.
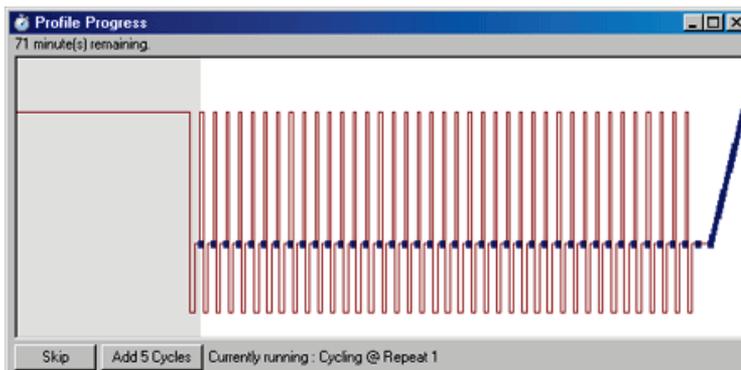
Skip: "Skip" allows any steps of the profile to be skipped.

Add 5 Cycles: "Add 5 Cycles" adds five repeats to the current cycling step.

7.8.4 Edit Samples

Edit Samples

File Edit Format Security

Standard | Rotor Style |

Settings :
Given Conc. Format : 123,457   Unit : copies/reaction   More Options

Samples :
Edit | Reset Default | Gradient

C	ID	Name	Type	Groups	Given Conc.	Selected
	A1	R 1000	Standard		1,000	Yes
	A2	R 1000	Standard		1,000	Yes
	A3	R 100	Standard		100	Yes
	A4	R 100	Standard		100	Yes
	A5	R 10	Standard		10	Yes
	A6	R 10	Standard		10	Yes
	A7	R 1	Standard		1	Yes
	A8	R 1	Standard		1	Yes
	B1	R NTC	NTC			Yes
	B2	R NTC	NTC			Yes
	B3	I 1000	Standard		1,000	Yes
	B4	I 1000	Standard		1,000	Yes

Page :
Name : Page 1   < >   New   Delete   □ Synchronize pages

Undo   OK   Cancel   Help

Click on the "Samples" button to bring up the "Edit Samples" window. The "Edit Samples" window can also be accessed by right-clicking on the sample list at the right of the screen. This window has identical functionality with the "Edit Samples" window in the wizards, except that the toolbar functions are also available in the File and Edit menus.

Four menus appear at the top of the window: File, Edit, Format and Security. The File menu is used to create a new (blank) "Edit Samples" window, to open an existing sample template or to save sample names as a template for future use. The extension of these template files is ***.smp**. The Edit menu allows rows to be copied and pasted. The Format menu changes the contents of the ID column from "A1, A2, etc." to "1, 2, etc." The Security menu enables the sample definitions to be locked.

Note: If the sample names are entered very fast during the run (e.g., using a bar code scanner) it can lead to transposed letters within the sample names. Therefore, it is recommended to avoid using a bar code scanner and, if applicable, enter the sample names after the run has finished.

This drop-down menu is used to choose a suitable format for the concentration display. Concentrations are automatically formatted according to the currently selected location.

This drop-down menu sets the units of measurement for the assay.

Button	Importance
Line style:	To access this feature, click on the right-arrow button next to the Edit button. The style of the line can be modified to improve readability of graphs on black and white printers. Certain lines can be emphasized by modifying their style.
Edit	Pressing "Edit" opens the color selector. Multiple rows can be selected when assigning a color to tubes.
Reset Default	Click "Reset Default" to reset all selected color cells back to their default color values.
Gradient	"Gradient" allows a gradient to be chosen from the first to the last selected color. Several gradients can be defined in a sample setup.
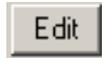
Button   Importance

The "New" icon clears the "Edit Samples" window in preparation for data entry.

The "Open" icon brings up a dialog box in which a Rotor-Gene Q MDx file can be selected to import.

Note: The number of samples in the open window and the file being imported must match.

The "Save" icon brings up a dialog box that prompts for a file name and folder in which to save a copy of the current sample definitions.

The "Copy" icon copies the selected cells.

The "Paste" icon pastes cells that had been selected with the Copy command into the currently selected position on the grid.

The "Excel" icon brings up a dialog box that prompts for a file name and folder in which to save the sample information. After pressing "Save", the Excel file is opened automatically.

The "Append/Overwrite" icon changes the editing of cells in the "Edit Samples" window. If "Overwrite" is selected, existing data is overwritten when editing. If "Append" is selected, new data is added to the end of existing data when editing.

Button   Importance

Sample   Samples can be defined as one of several types,
Types: listed in the following table:

Sample type	Description
None	No sample in that position
NTC	No template control
Negative Control	Negative control
Positive Control	Positive control
Unknown	Unknown sample to be analyzed
Standard	Standard values are used to construct a standard curve to calculate unknown sample concentrations
Calibrator (RQ)	A calibrator is assigned a value of 1, and all other sample concentrations are calculated relative to this sample

Page: This function allows the user to have
               different sample definitions, and also
               separate experiments, in the same run. This
               is useful for the analysis of different
               products in different channels. Use the
               arrow buttons to move between the sample
               pages. Use the "New" and "Delete" buttons
               to create and delete pages. It is possible to
               have multiple sample definitions for the
               same channel, to run multiple standard
               curves without multiplexing. Simply define
               the samples of interest and their related
               standard curves on separate pages. The
               single channel can then be analyzed with
               each set of definitions independently.
               Sample pages can be labeled "Page 1",
               "Page 2", etc., or they can be given any

Button   Importance

name (e.g., "Housekeeper"). This name will
appear in reports.

When viewing the raw data, the sample
definitions used to display the data can be
selected using the drop-down menu next to
the "Options" button:
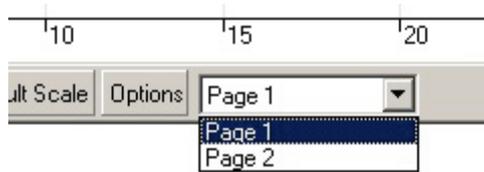
The sample page to use when performing
an analysis can be selected in the
"Analysis" window (see Section 7.6.1).
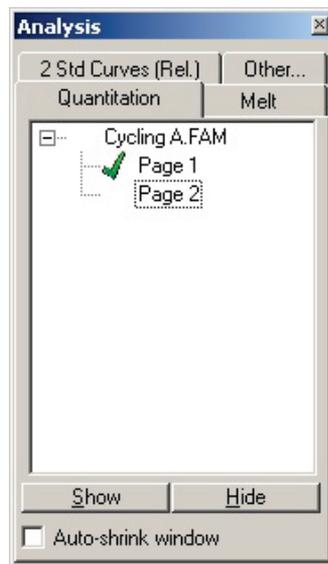
Given Conc.: This shows the concentration for each of the standards. The units can be defined as a decimal or log number. If the standards are a dilution series, it is only necessary to type the first two standards. By pressing ENTER, the program automatically adds the next logical dilution in the series.

Line style: The style of the line can be modified to improve readability of graphs on black and white printers. Certain lines can be emphasized by modifying their style. To access this feature, click on the right-arrow button next to the "Edit" button.

The toolbar will show the default style, "Solid". This can be changed to "Dashed", "Dotted", "Hairline", "Thin" or "Thick". When finished, click the left-arrow button to return to the Edit, Reset Default and Gradient view.

Multiple row entry: If the same information needs to be entered in several rows at once, select all the rows, and then begin to type. The information will be entered into each row. This also works for selecting sample types, choosing colors or entering concentrations.

Sample type hotkey:	To quickly select a sample type, enter the first letter of its name. For example, to set five samples to be "No template" controls, select them in the "Sample type" column, and then press N for NTC. All samples will be converted to NTC.
Save it, reuse it:	A complete sample description can be saved as a sample file (***.smp**) and loaded into future runs with the same sample configuration.

Rotor Style

This tab in the "Edit Samples" window provides an alternative way of entering sample names. Select replicates by clicking and dragging the mouse pointer over the rotor image. The list on the right of the window will update. The sample name can be typed in, and this will set the same name for every item in the current selection. The software recognizes these wells as replicates.
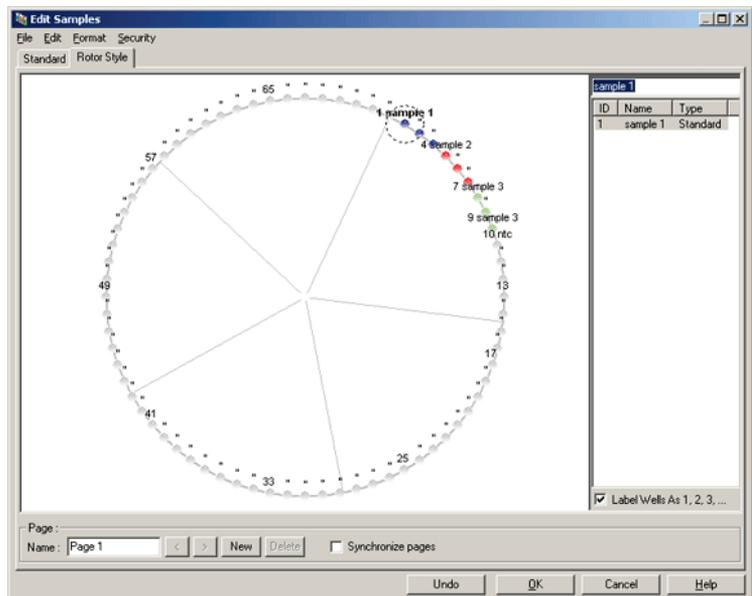
The "Rotor Style" tab provides a cutdown version of the "Standard" tab and is designed for users who want to setup sample names and colors quickly. It is not possible to define some settings, such as whether the sample represents a standard or the known concentration of each standard, in this tab. If these need to be defined, the standard tab should be used.

Sample Page Suitability

To access the "Sample Page Suitability" window, click on "More Options" in the "Edit Samples" window, and then click on "Define Suitabilities". The "Sample Page Suitability" window allows users to match sample pages to channels. For example, the sample page for the gene of interest may apply to the green channel, and the sample page for the housekeeper gene may apply to the yellow channel. In this example, setting up sample page suitability reduces the number of analysis options available to just include those relevant for the particular assay.

The "Sample Page Suitability" window is shown below.
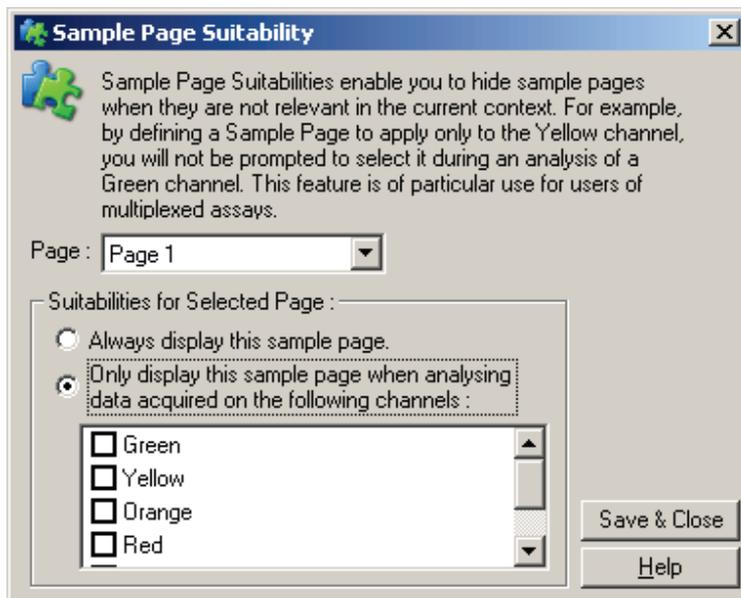
Note: When setting up an assay, create all of the sample pages and sample page suitabilities, and then save them as a template. This reduces the amount of setup required for each run.

Groups

Sample groups allow statistics to be calculated for an arbitrary collection of samples. Unlike replicates, which must have identical names, samples can have any name, can be positioned anywhere in the rotor and can belong to multiple groups.

1. To define a group, type the full name of the group next to a sample, and then press ENTER.
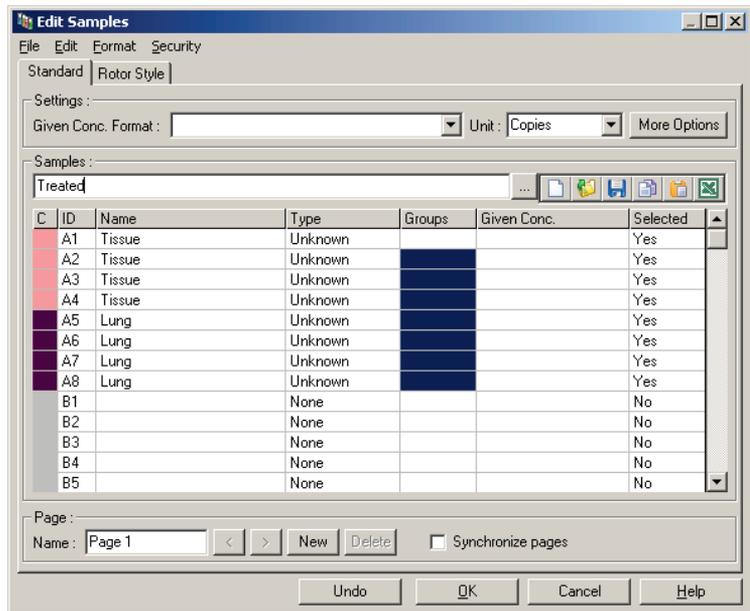
2.   The "Edit Group" window appears.
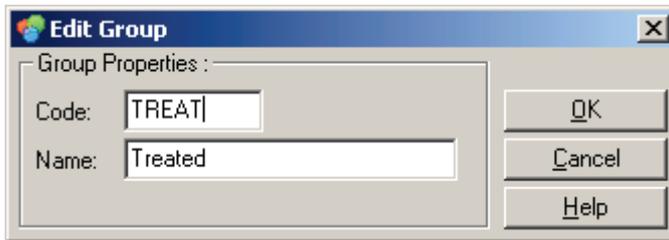
3.   Define a suitable abbreviation, and then click "OK". The abbreviation can now be used to set up groups. Aggregate results, such as average value and 95% confidence intervals, are calculated automatically for groups in any analysis.

7.8.5   Display Options

The display options menu is shown below.

Show at Most 2 Analysis Windows:	If this option is checked, a maximum of two analysis windows are shown at once. If multiple windows are opened, readability may be affected. Checking this option closes the first analysis window and replaces it with the last opened window. If the option is unchecked, more than two analysis windows can be displayed.
Show at Most 6 Windows:	To improve readability, the software removes unused windows when new windows are opened. This option is enabled by default, because it keeps the Rotor-Gene Q software screen clear. If it is necessary to see more than six windows at once, uncheck this option.
Reset All "Don't Show This Message Again" Dialogs:	If this is selected, the software will redisplay all dialog boxes where the "Do not display this message again" checkbox was checked. These include messages about suspicious settings that may have previously been set not to display again. This may be useful for a new user who is unfamiliar with the Rotor-Gene Q MDx or the Rotor-Gene Q software.

7.9   Access Protection for Rotor-Gene Q Software

Note: This chapter describes access protection for Rotor-Gene Q software. See the "Rotor-Gene AssayManager 1.0 Core Application User Manual (US) IVD" in Part II of Volume 2 of the *QIAsymphony RGQ MDx (US) User Manual* or *Rotor-Gene AssayManager v2.1 MDx* core application user manual for information about the relevant Rotor-Gene AssayManager software.

The Rotor-Gene Q software includes features that enable it to operate securely. When correctly configured, the Rotor-Gene Q software can ensure the following:

■ Access to the Rotor-Gene Q MDx or the analysis software is restricted to user groups
■ Modifications to run files are logged
■ Unauthorized modifications are detected (signatures)
■ Templates used to perform runs are logged
■ Sample names are protected

Integration with Windows security

To provide a strong level of accountability, Rotor-Gene Q software does not manage security internally. Accounts, groups and passwords are all managed using the Windows built-in security model (Windows Security). Integration allows the same password that provides access to network files and programs to control Rotor-Gene Q software access, leading to less administration. In larger organizations, for example, network administrators can easily remove access to ex-users due to the centralized security model.

For this reason, setting up the Rotor-Gene Q software securely primarily involves configuration of the Windows security roles according to best practices.

Prerequisites

To use security, you must be running Windows 10 or Windows 7 Professional edition. The security features cannot be used with Windows 10 or Windows 7 Home edition, because the Home editions do not have the fine-grained access model used by the software. The software must be installed with the "Force authentication through Windows domain" option.

Note: The Security menu will not appear if you are logged into a Linux Samba domain. You must have either a local logon or a Windows server to use the security features.

7.9.1   Configuration for Windows 7

This section describes how to set up the system to run Rotor-Gene Q software securely.

To use the security features, the software must be installed with the "Force authentication through Windows domain" option. This queries the Windows domain for your access level and credentials and is essential for providing the accountability and security features.

Running as administrator

Many users run their computers as an administrator, with no password. While this is convenient, it makes it impossible to determine who is using the computer. This eliminates accountability and prevents many Rotor-Gene Q software security measures from activating. When running as an administrator, all the software features are enabled. Therefore running as an administrator ensures that users who do not need security features can access all the software features.

Creating a new user account

Create user accounts for each user of the software. For each user, repeat the steps below until all accounts have been created.

1. To create a new user, select "Start > Control Panel > Administrative Tools > Computer Management" and then navigate to "Local Users and Groups" on the left side.

2. In the window that appears, select the "Users" folder. Right-click on the "Users" folder and select "New User".

3. Enter a username and password. By default, users will be created with normal access privileges. This means they can run software but not install new programs or change system settings.

4. Click "Create". You can now log on as this user.

Assigning roles to each user

You should now assign roles to each user. Access is divided into the following areas:

■ Rotor-Gene Q Operator — can perform runs but cannot generate reports or perform analysis

■ Rotor-Gene Q Analyst — can analyze run data and generate reports but cannot perform new runs

■ Rotor-Gene Q Operator and Analyst — has the capabilities of both roles

■ Administrator — can unlock sample names and perform all operations of Analysts and Operators

■ None — access to the software is denied

To assign roles:

1. Log in to Windows as an administrator, or use the "Rotor-Gene Q Software Login" icon to open the software and log in.
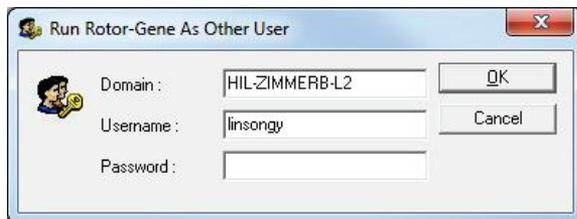
Note: To create the RG Groups with the Rotor-Gene Q software it is necessary to run the software with administrator rights. This is done by right-clicking on the desktop icon and choosing "Run as administrator" in the context menu.

2. Once the software is open, click on the "Security" menu. The first time the "Security" menu is accessed, Rotor-Gene Q software configures a number of system groups that will control access to the software.
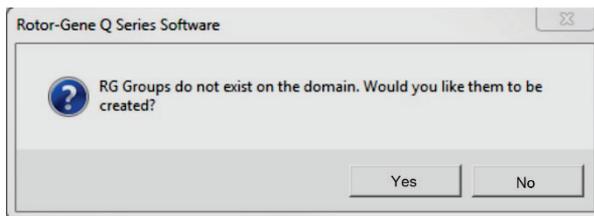
3. Click "Yes". The "User Admin" window appears. In the top panel, all the users of the computer are displayed. Some accounts are used by the system, and so they will be unfamiliar. The bottom pane shows the groups assigned to the user.
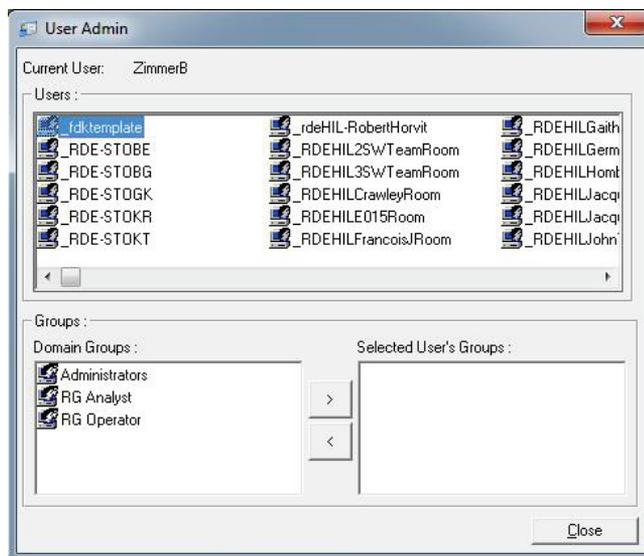
4. To assign a group to a user, select the user's name from the list. The bottom panel will update. If the user has no groups, they cannot launch the software. In the example below, we assign the user "linsongy" to the RG Analyst group by selecting the group on the left-hand side, then clicking the ">" button. Groups can be removed by selecting them, then clicking the "<" button.
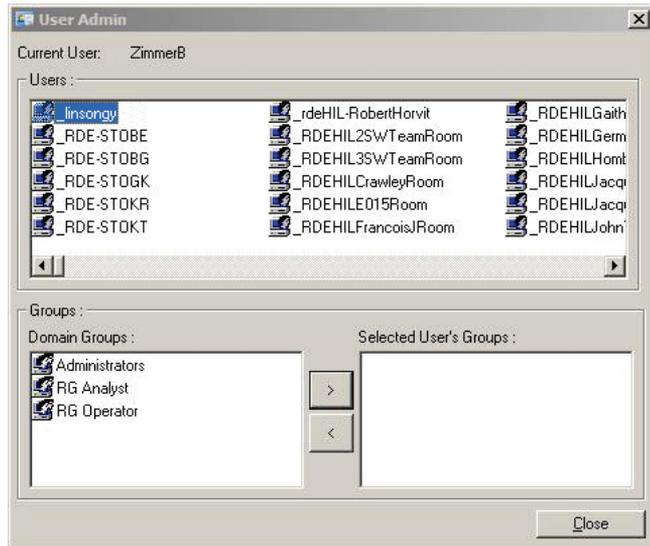
5. Now log in as this user. As an RG Analyst, the Run menu and "Profile" button are unavailable. However, existing files can be opened and analyzed, as shown in the screenshot below. The status bar indicates that the user "linsongy" is an RG Analyst.
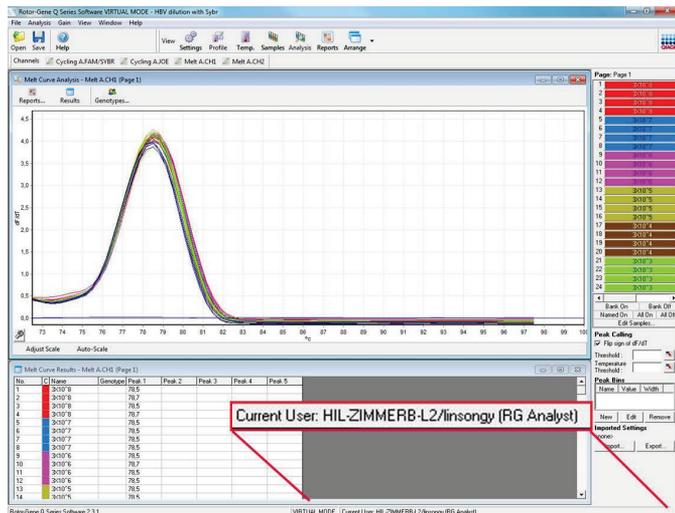
6. By logging in as an administrator again, RG Operator rights can be assigned to "linsongy" and the RG Analyst

rights can be removed again. Then, the software needs to be launched again. This time, the Analysis menu and "Reports" button are missing, and the Run menu is enabled. The status bar indicates that the user "linsongy" belongs to the RG Operator group.
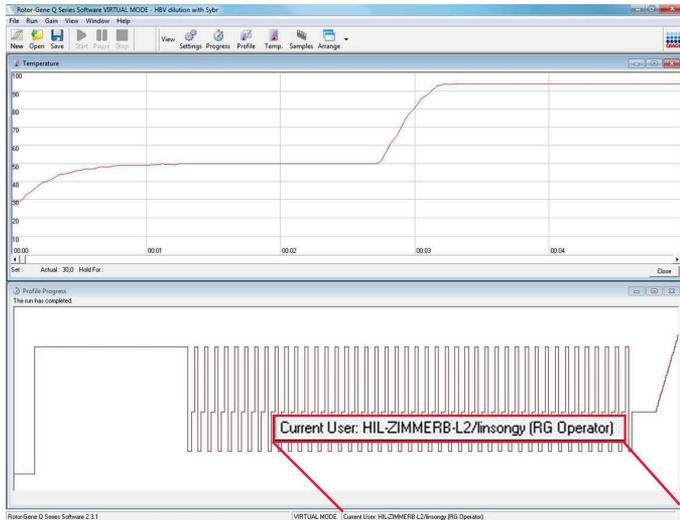
7. If you log in as administrator and remove all groups from the user "linsongy", the following message will appear when "linsongy" opens the software:

7.9.2 Configuration for Windows 10

This section describes how to set up the system to run Rotor-Gene Q software securely.

To use the security features, the software must be installed with the "Force authentication through Windows domain" option. This queries the Windows domain for your access

level and credentials and is essential for providing the accountability and security features.

Running as administrator

Many users run their computers as an administrator, with no password. While this is convenient, it makes it impossible to determine who is using the computer. This eliminates accountability and prevents many Rotor-Gene Q software security measures from activating. When running as an administrator, all the software features are enabled. Therefore, running as an administrator ensures that users who do not need security features can access all the software features.

Creating a new user account

Create a user account for each user of the software by repeating the steps below until all accounts have been created:

1. To create a new user, select "Start", enter "Computer Management", press Enter and navigate to "Local Users and Groups" on the left side.

2. In the window that appears, select the "Users" folder. Right-click on right-hand window and select "New User".

3. Enter a username and password. By default, users will be created with normal access privileges. This means they

can run software but not install new programs or change system settings.

4. Click "Create". You can now log on as this user.

Assigning roles to each user

You should now assign roles to each user. Access is divided into the following areas:

■ Rotor-Gene Q Operator — can perform runs but cannot generate reports or perform analysis
■ Rotor-Gene Q Analyst — can analyze run data and generate reports but cannot perform new runs
■ Rotor-Gene Q Operator and Analyst — has the capabilities of both roles
■ Administrator — can unlock sample names and perform all operations of Analysts and Operators
■ None — access to the software is denied

Note: In Microsoft Windows 10, it is not possible to create user groups with the Rotor-Gene Q software. The domain administrator needs to work within the domain to create groups and assign users to specific groups. Run menu is enabled. The status bar indicates that the user "linsongy" belongs to the RG Operator group.

7.9.3 Running multiple users on the same computer

To use the Rotor-Gene Q software with multiple users, create a user account that does not have access to the Rotor-Gene Q software. Log into Windows using this account, so that users cannot anonymously access the Rotor-Gene Q MDx software.

1. Using the "Rotor-Gene Q Software Login" icon, users can open their user account in the Rotor-Gene Q software.

2. Enter the username and password (mandatory) in the box that appears.
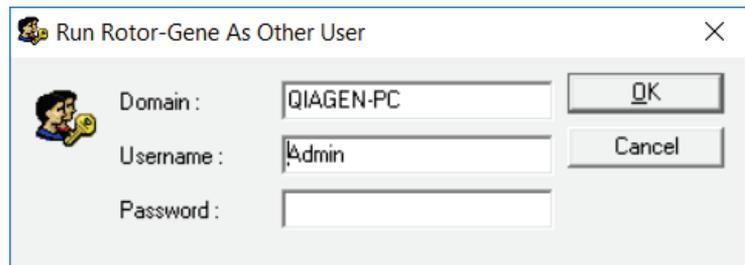
3. The domain is either the computer you are logging into or the name of your local network, together with the hostname. Consult your network administrator if unsure which domain to enter in this field.

   Note: After logging in, all of the user files will be available for that user. Each user can save files in their own area. This ensures a high level of security.

Note: Each user should log out after their run has been completed, to prevent other users from performing a run in their name.

7.9.4 Audit trails

Every time a file is saved by a user, the user's details are recorded in "Run Settings" under the "Messages" tab as Security Audit Trail Summary and Security Audit Trail Detail.
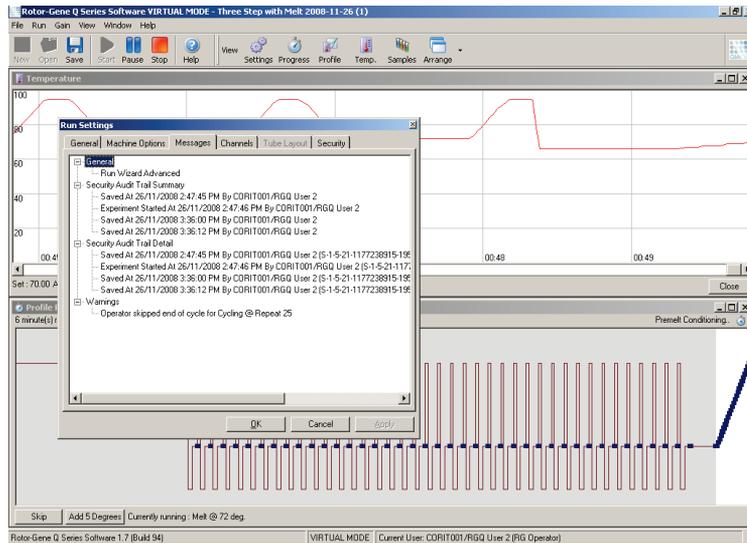
This can be used to monitor who has modified the contents of a file. The Security Audit Trail Detail contains more detail, such as the unique identifier of the user. This identifier is important to prevent users from creating an account with the same name on another computer and thereby impersonating another user. With this identifier, although user names can still be the same, the account IDs will be different.

The identifier for the account CORIT001/RGQ User 2, S-1-5-21-1177238915-195, is shown in the details.
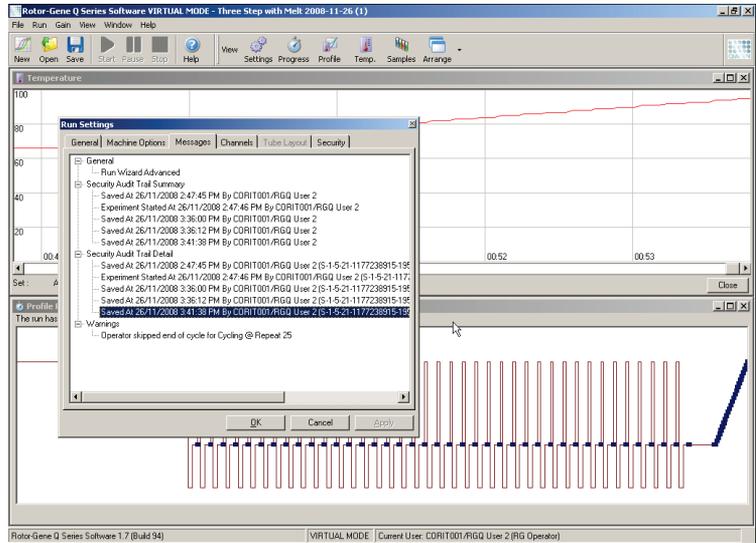
7.9.5   Run Signatures

The audit trail is stored in the Rotor-Gene Q run file. To avoid any unwanted modification of these files, they should be kept in a safe location accessible only by designated Windows accounts. However, if files are stored in a shared area, Run Signatures provide extra security. The screenshot shows the "Security" tab in Run Settings for a file with a Run Signature.
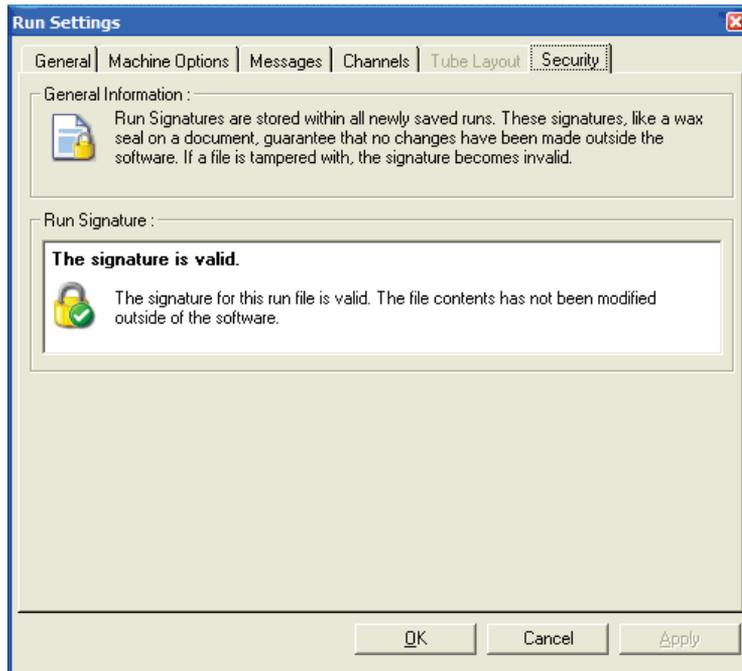
The Run Signature is a long series of characters that is generated every time the file is saved; it is linked to the contents of the file. For example, the signature for this file is 517587770f3e2172ef9cc9bd0c36c081. If the file is opened in Notepad and an edit is made (e.g., the run date is changed to three days earlier), the following message appears when the file is reopened:
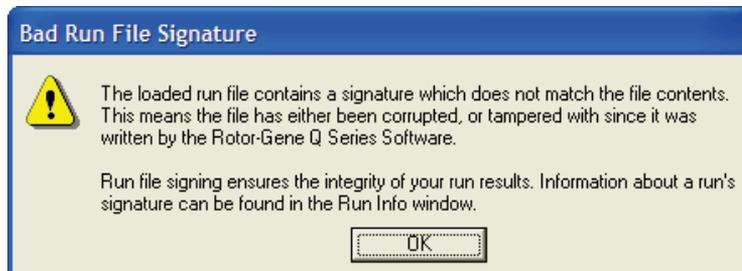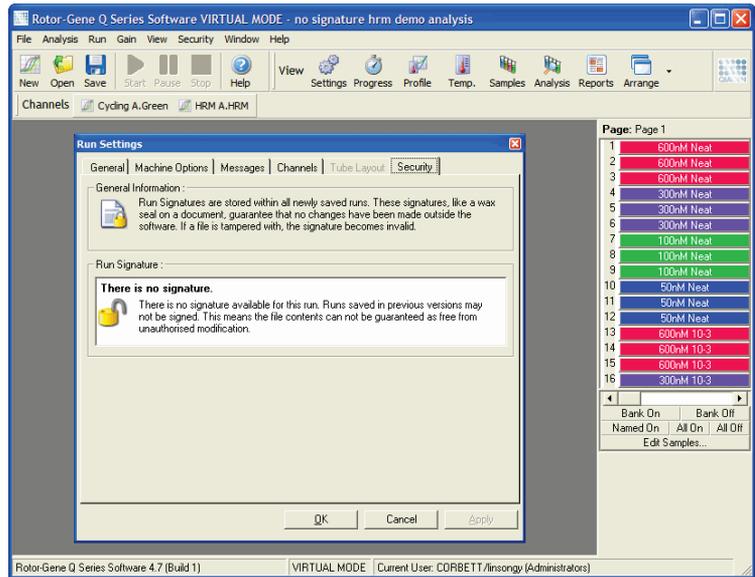
Note: If files are e-mailed, the encryption process can invalidate the signature. To avoid this, zip the file before e-mailing.

7.9.6 Sample locking

It is important to ensure that sample names are not accidentally or intentionally changed once a user has started a run. For this reason, the Rotor-Gene Q software provides sample locking. Sample names can be locked by any user but can only be unlocked by an administrator. For users who run their computers in administrator mode, this option is of limited value. To use this option, the computer must be configured securely, as described in the previous sections.

Note: If you wish to lock samples, do not run the software as an administrator. Create an account with RG Operator and RG Analyst groups, and keep the administrator password secret. Users will then require authorization from the administrator to unlock files.

When using the Advanced wizard, samples can by locked before starting a run by clicking "Finish and Lock Samples".
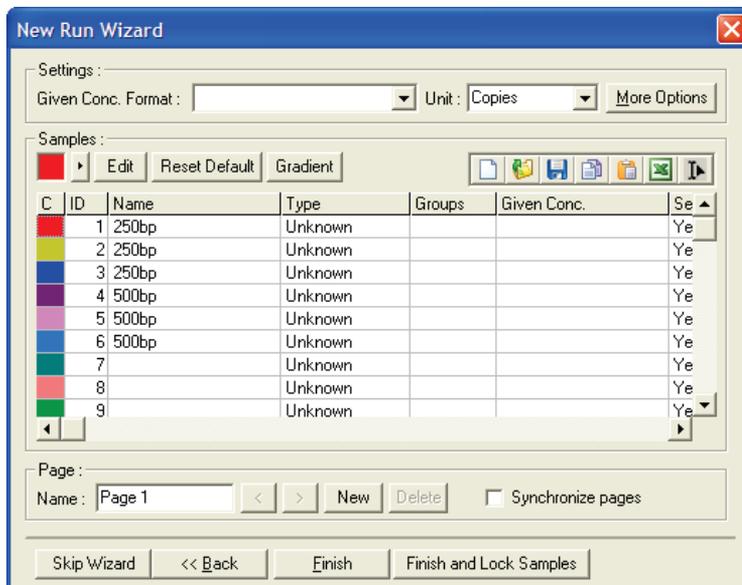
The following warning will appear. Click "Yes" to confirm.
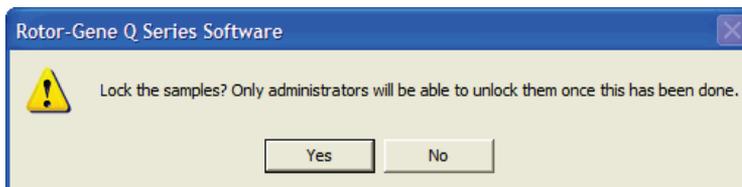
Once the samples are locked, it will not be possible to edit samples in the "Edit Samples" window.
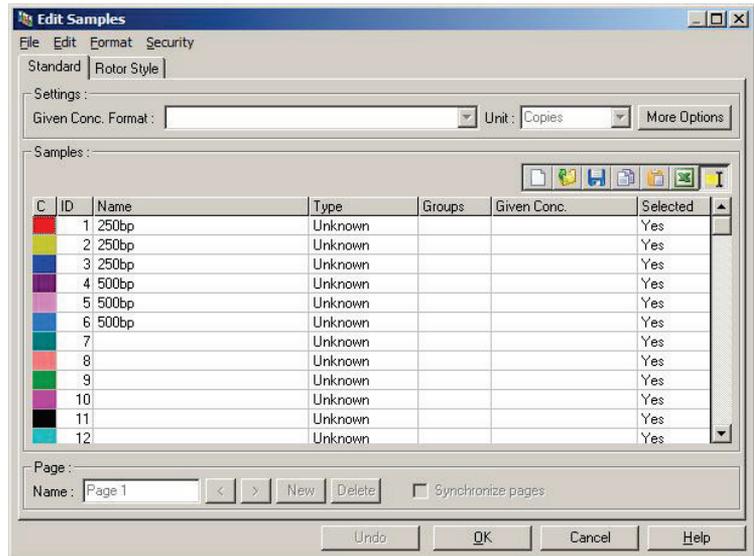
Samples can also be locked and unlocked in the "Edit Samples" window. However, only an administrator can unlock samples once they have been locked.
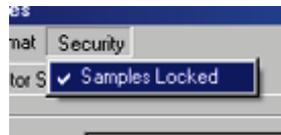
Any unauthorized change to the file will invalidate the Run Signature.

7.9.7 Locked templates

It is currently not possible for the user to create read-only template files using the Rotor-Gene Q software. However, if desired, it can be specified as a requirement that all runs should be performed using a specific template file. In order to ensure read-only access to this template, it should be stored on a network drive where users cannot modify data. Users can still run and modify their own profiles, while the template on a network drive such as this is protected. In order to track which template has been used, the Rotor-Gene Q software stores the name of the template file that was run. This information can be accessed by clicking the "Settings"

button, which then enables the "Run Settings" window to be displayed. The template information is stored in "Other Run Information".

Other Run
Information :

```
Run file has a valid signature.
Template was signed.
Using 72-well carousel.
Channels saved for this run:
Created with template
  'C:\Dual Labeled Probes.ret'
Run is currently in progress.
  Started at 16/02/2006 11:16:36 AM
```
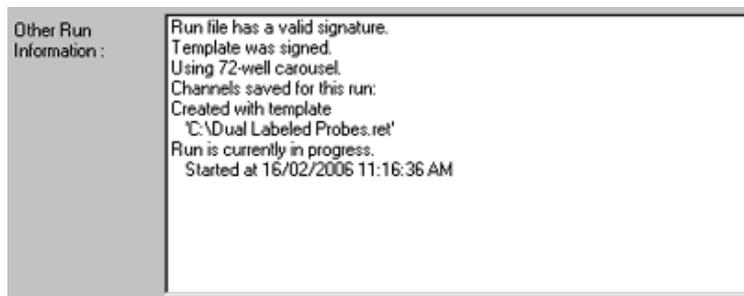
7.10   Gain menu

Click on the Gain menu to view the gain settings for the current run. This sets the gain of the specified channel before a run. Gain settings are retained from the last run. These can be modified if the run has not yet started or is still in the initial cycles. Use the up/down arrows next to each text field to modify the fields. Then click "OK".

The gain can be changed during the initial cycles. A red line will be drawn in the appropriate channel, showing where the gain was changed. Cycles before the gain change will be excluded from analysis.

Gain Settings

Green	10
Yellow	5
Orange	5
Red	5
Crimson	7
HRM	7

OK
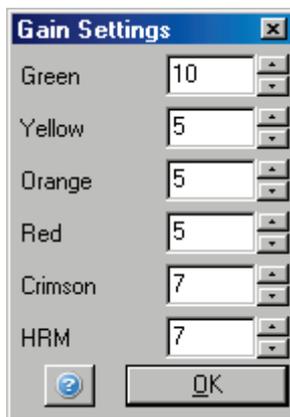
7.11   Window menu

This menu enables the windows to be tiled vertically or horizontally or arranged in a cascade. To access further options, click the arrow on the right of the "Arrange" button.

7.12   Help function

When using the Help button or Help menu, the following drop-down menu will open:
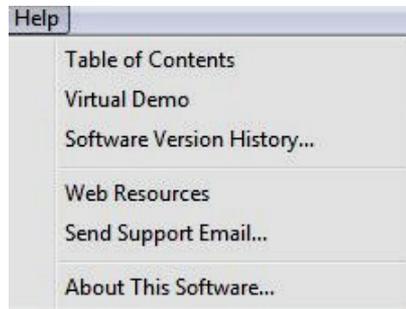
Table of Contents:	This provides access to the Help function.
Virtual Demo:	This links to a QIAGEN Web page with an interactive demonstration of the software.
Software Version History…:	This gives a brief overview of new features added since the previously installed software release.
Web Resources:	This opens a QIAGEN Web page in a new browser window, with valuable latest information on Rotor-Gene Q MDx instruments and corresponding reagents.
About This Software…:	This provides information about the connected machine, the serial number of the Rotor-Gene Q MDx and the software version.

7.12.1   Send Support E-Mail

The Send Support Email option in the Help menu allows you to send to QIAGEN a support-request e-mail that includes all relevant information from a run.

The "Save As" option will save all the information to a file that you can copy into a disk or across a network, in case you do not have access to e-mail on the computer running the Rotor-Gene Q MDx.

 If you use the support e-mail function on the laptop computer provided optionally with the Rotor-Gene Q MDx (country dependent) for the first time, you have to configure your e-mail settings.

Configure the e-mail settings

Click the option "Send Support Email…". The following window will open:

1. Type in your name and click "Next". The "Internet E-mail Address" window will open.
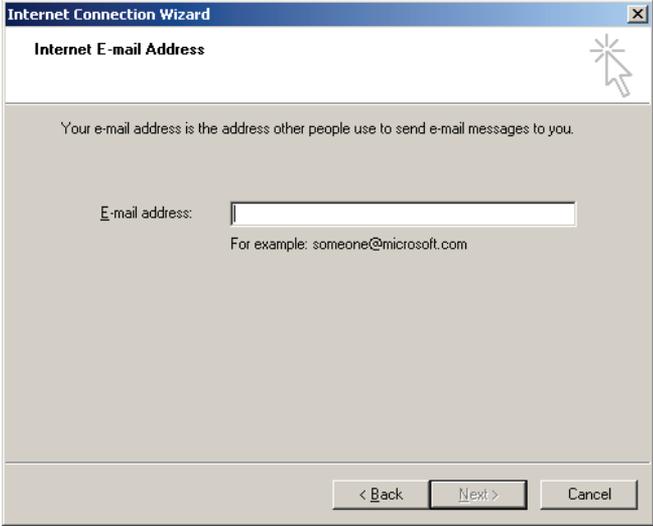
```
Internet Connection Wizard                                        ×

  Internet E-mail Address                                       ☀

  Your e-mail address is the address other people use to send e-mail messages to you.


      E-mail address:        [                                      ]
                             For example: someone@microsoft.com






                                        < Back      Next >      Cancel
```

2. Type in your e-mail address and press "Next". The "E-mail Server Names" window will open.
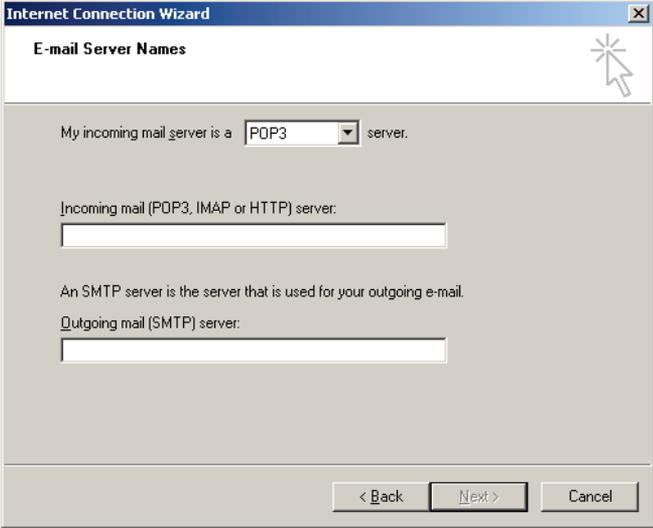
```
Internet Connection Wizard                                        ×

  E-mail Server Names                                           ☀

  My incoming mail server is a   [POP3      ▼]  server.


  Incoming mail (POP3, IMAP or HTTP) server:
  [                                             ]


  An SMTP server is the server that is used for your outgoing e-mail.
  Outgoing mail (SMTP) server:
  [                                             ]




                                        < Back      Next >      Cancel
```

3. Select the type of mail server for incoming mails, and specify the server names for incoming and outgoing

e-mails. Then press "Next". The window "Internet Mail Logon" will open.

4. Enter your e-mail account name and password, if your server uses secure password authentication. Then click "Next". The "Congratulations" window will open.
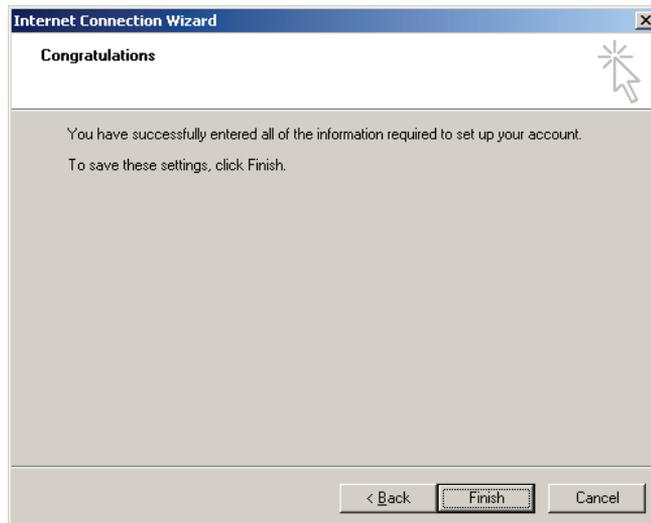
5. Confirm with "Finish" to complete the e-mail account setup.

Setting up in Outlook

1. Open "Outlook Express" from the Start menu (Start > All programs > Outlook Express).

2. Select "Tools", and then "Options". The window below appears.
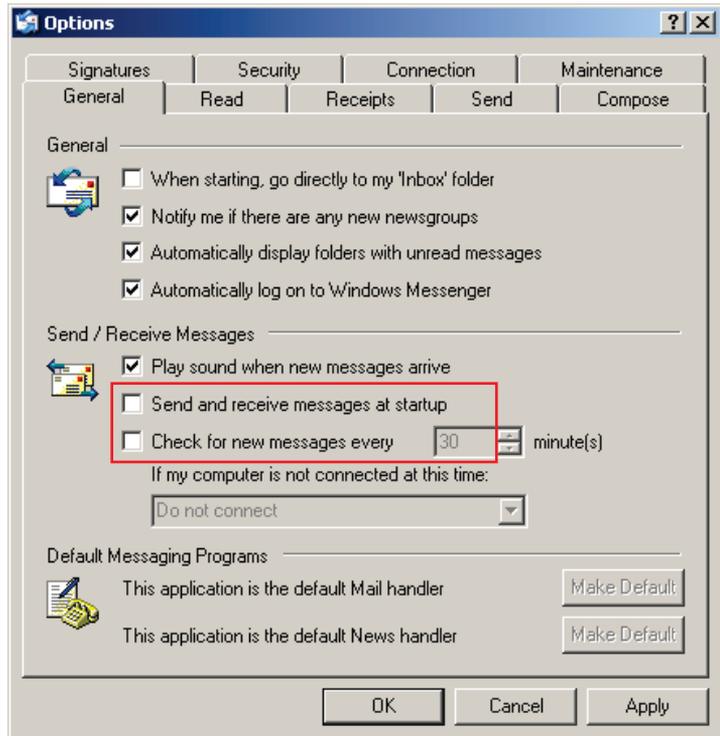
Important: To avoid any retrieval of e-mails during PCR runs, disable the default entries in the "Send/Receive Messages" screen.

3. Disable "Send and receive messages at startup".

4. Disable "Check for new messages every x minute(s)".

5. Confirm changes with "OK".

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8 Additional Functions

8.1 Analysis templates

Some analyses require the user to define thresholds, normalization settings and genotype settings. Often these settings are reused frequently in multiple experiments.

Analysis templates enable the user to save and reuse these settings. This reduces the effort of reentering settings and reduces the risk of error.

Quantitation, Melt, Allelic discrimination, Scatter graph analysis and EndPoint analysis support analysis templates. These analyses allow the user to export a template that is unique to the analysis (e.g., Quantitation analysis allows export and import of ***.qut** files that contain quantitation settings).

After an analysis template has been imported or exported, the file name of the template is displayed for future reference.

8.2 Opening a second run

While performing a run, it is possible to open and analyze runs that were performed earlier. Several functions, such as "New" or "Start Run" buttons, are not activated in the second window. A new run can be started from the first window once the first run has finished.

8.3 Scaling options

To access "Adjust Scale", click on "Adjust Scale…" at the bottom of the main window, or right-click on the graph and select "Adjust Scale…" on the menu that appears. A scale can be manually entered in the window that appears.
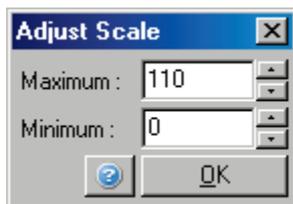
To access "Auto-Scale", click on "Auto-Scale…" at the bottom of the main window, or right-click on the graph and select "Auto-Scale…" on the menu that appears. "Auto-Scale" attempts to fit the scale to the maximum and minimum readings in the data.

To access "Default Scale", click "Default Scale…" at the bottom of the main window, or right-click on the graph and select "Default Scale…" on the menu that appears. "Default Scale" resets the scale to display from 0 to100 fluorescence units.

8.4 Exporting graphs

Picture export

The following steps describe how to save an image:

1. Right-click on the image and select "Export" from the menu that appears.

2. The "Export Dialog" window appears. Select the desired format from the "Format" list.
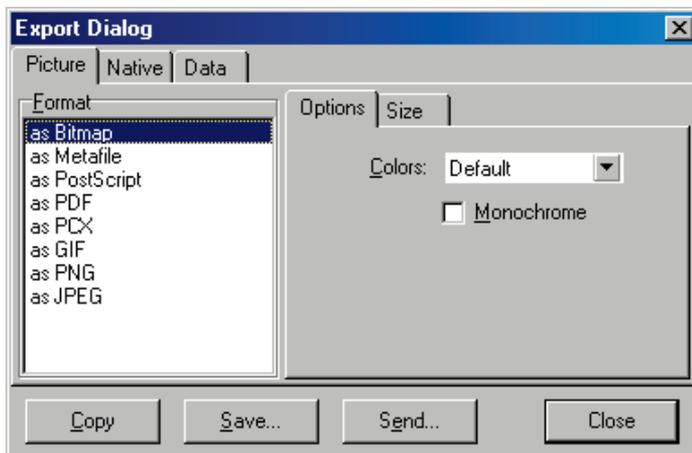
3. Select the "Size" tab and specify the desired size.
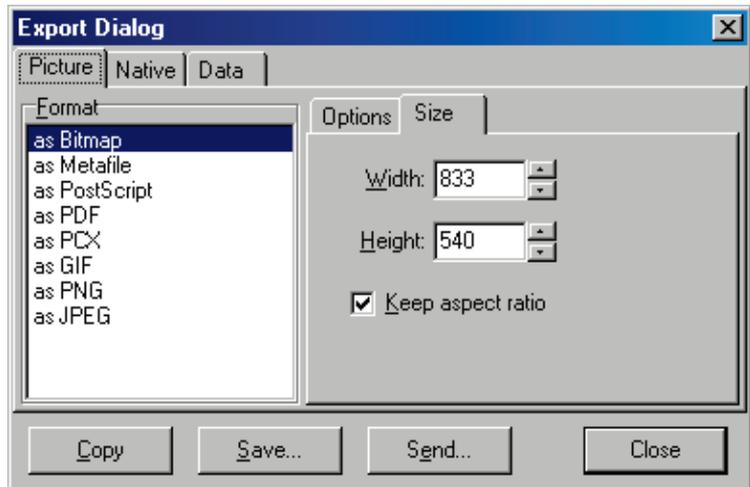
4. Check the "Keep aspect ratio" checkbox to keep the image in the correct proportion when adjusting its size.

5. Click "Save", and then select a file name and location for the file in the dialog box that appears.

If a higher resolution image is required, we recommend either increasing the size of the image until it meets your requirements or saving the graph as a metafile (***.emf**, ***.wmf**). This is a vector-based format that can be opened in software such as Adobe® Illustrator®, allowing the user to create an image of any resolution.

Native format export

Graphs in the Rotor-Gene Q software use the third party TeeChart® component developed by Steema software. To save a graph in native format, select the "Native" tab in the "Export Dialog" window (see previous screenshot), and then click "Save". Native format is the standard TeeChart file format. This allows the user to use TeeChart Office from Steema software. TeeChart Office is available as freeware and is installed as part of the Rotor-Gene Q software package. To access the software, click on the TeeChart icon on the desktop.

TeeChart Office enables manipulation of exported graphs, including changing colors of curves, adding annotations, changing fonts and adjusting data points.
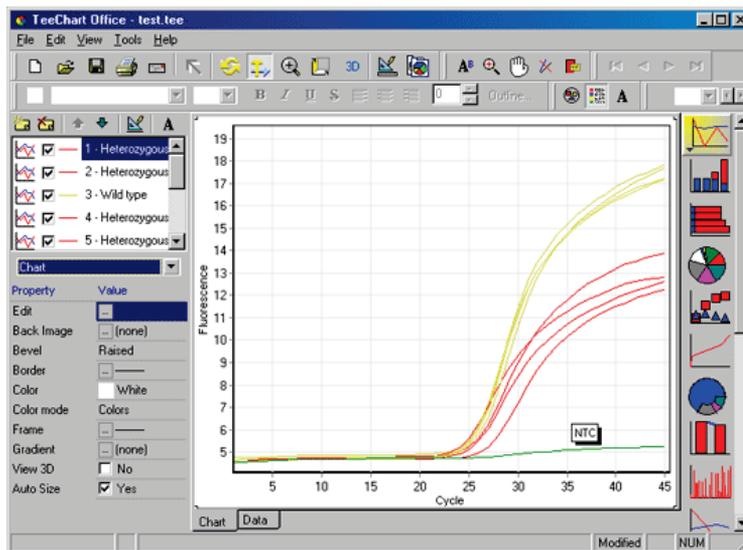
Data export

To export data in various formats, select the "Data" tab in the "Export Dialog" window. The exported file contains the raw data points used in the graph.
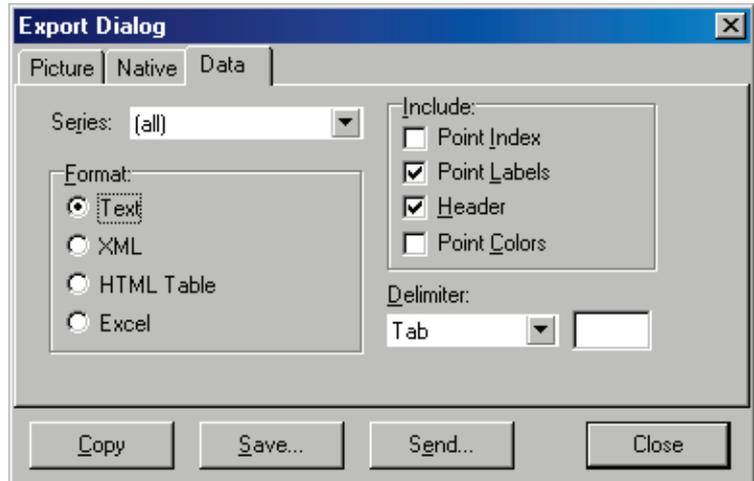
Exporting raw data and analysis data can also be performed by selecting "Save As" under the "File" menu (see Section 7.5).

8.5 Spanner/wrench icon

The spanner/wrench icon appears at the bottom left of the main window. Clicking on the spanner/wrench icon enables several options. These options can also be accessed by right-clicking on the graph.

Adjust Scale, Autoscale…, Revert to Default Scale:	See Section 8.3.
Export…:	This saves the graph in a variety of formats (see Section 8.4).
Copy Chart to Clipboard:	This copies the graph image to the clipboard.
Edit Chart in TeeChart Office…:	This opens the graph directly in TeeChart Office for editing (see Section 8.4).
Print…:	This prints the graph.
Digital Filter…:	This modifies the currently selected digital filter on the graph. The digital filter smoothes data using a sliding window of points.
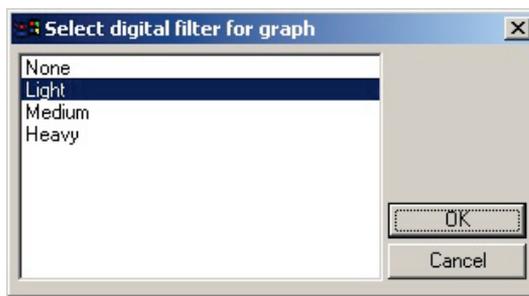
Show Pinpointer: This opens a window that displays the exact coordinates of the mouse pointer position.

Grouping: This visually groups samples that have identical names. This can be useful on full rotor runs. Selecting this option does not affect calculated values.

8.6 Selected area options

An area of a graph can be selected by clicking and holding the left mouse button and dragging the mouse pointer. The following options appear:

Select Only These Samples
Deselect These Samples
Zoom

Cancel

Select Only These Samples: Samples outside the selected area are deselected.

Deselect These Samples: All samples in the selected area are deselected.

Zoom: This zooms in on the selected area of the graph. Click the "Default Scale" button to zoom out.

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9 Maintenance Procedures

Maintaining the working performance of the Rotor-Gene Q MDx is easy. Optical performance is maintained by ensuring that the lenses, located at both the emission and detection source, are clean. This is achieved by gently wiping a cotton tip applicator, moistened with ethanol or isopropanol,* over the lenses.

Note: Clean the lenses at least once a month, depending on usage. Wipe the rotor chamber at the same time.

Keep the work bench area clean and free from dust and sheets of paper. The air inlet of the Rotor-Gene Q MDx is at the bottom and loose material such as paper or dust may compromise performance.

To avoid dust build up, keep the lid of the Rotor-Gene Q MDx closed when the instrument is not in use.

If the rotor chamber becomes contaminated, it can be cleaned by wiping the surfaces with a lint-free cloth dampened (but not dripping) with a 0.1% (v/v) bleach solution.* Wipe the chamber with a lint-free cloth dampened with PCR-grade water to remove traces of bleach.

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* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

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10 Optical Temperature Verification

Optical Temperature Verification (OTV) is a method that verifies the in-tube temperature in a Rotor-Gene Q MDx. Validation of in-tube temperature can be an important procedure in certified laboratories. OTV is performed using a Rotor-Disc OTV Kit (see Appendix C).

In the following sections, only a short introduction to the OTV principle is given. Performance of the OTV procedure is explained in the Rotor-Gene Q MDx software. For a more detailed description of the OTV procedure, including a troubleshooting guide, please refer to the *Rotor-Disc OTV Handbook*.

10.1 OTV principle

OTV uses the optical properties of three thermochromatic liquid crystals (TLC)* as absolute temperature references. When heated, TLCs change from opaque to transparent at very precise temperatures (50°C, 75 °C and 90 °C). TLCs do not themselves fluoresce. Therefore, it is necessary to cover the excitation source with a fluorescent insert so that the TLC transition points can be detected by the Rotor-Gene Q MDx optical system. TLCs that are below their transition temperature are opaque and reflect light. Some of the reflected light scatters towards the detector, increasing fluorescence. When the in-tube temperature reaches the TLC transition point, the TLC becomes transparent and light passes through the sample rather than being reflected toward the detector, resulting in a decrease in fluorescence. The change in fluorescence is used to determine the precise transition temperature of each TLC. The transition temperature is compared with the temperature reported by the factory calibration file for the OTV Rotor-Disc to verify

---

* When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier.

whether the Rotor-Gene Q MDx is within temperature specification.

10.2   Rotor-Disc OTV Kit components

The following components are required to run an OTV:

- A Rotor-Disc OTV Kit, which includes:
    - Sealed Rotor-Disc 72 OTV Rotor (contains TLCs)
    - Fluorescent scatter plate insert (Rotor-Gene 3000 instrument or Rotor-Gene Q/6000 instruments)
    - A CD that contains the following files: OTV Rotor serial number and expiry date file (***.txt**); OTV test template file (***.ret**); Product Sheet (***.pdf**); factory calibration file (***.rex**)
    - Product Sheet
- Rotor-Gene Series Software Version 1.7 or above, which contains the easy-to-use OTV Rotor wizard
- Rotor-Disc 72 Rotor
- Rotor-Disc 72 Locking Ring

10.3   Running an OTV

1. Place the fluorescent insert over the emission lens in the bottom of the Rotor-Gene Q MDx chamber.

2. Place the OTV Rotor-Disc into a Rotor-Disc 72 Rotor. Secure using a Rotor-Disc 72 Locking Ring. Place the assembly into the Rotor-Gene Q MDx and click into place. Close the Rotor-Gene Q MDx lid.

3. Access the Advanced wizard by selecting the "Advanced" tab in the "New Run" window. In the Advanced wizard, click on "Instrument maintenance" and then "OTV". The wizard prompts for the OTV serial number. This number can be read from the label on the OTV Rotor-Disc or can be imported from the CD by clicking on "Browse" and choosing the **.otv** file provided on the CD. Once the number is entered, click "Start".
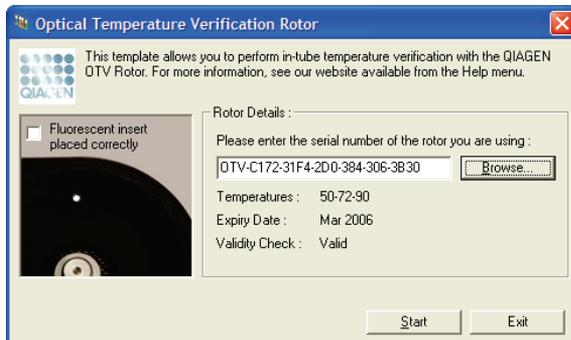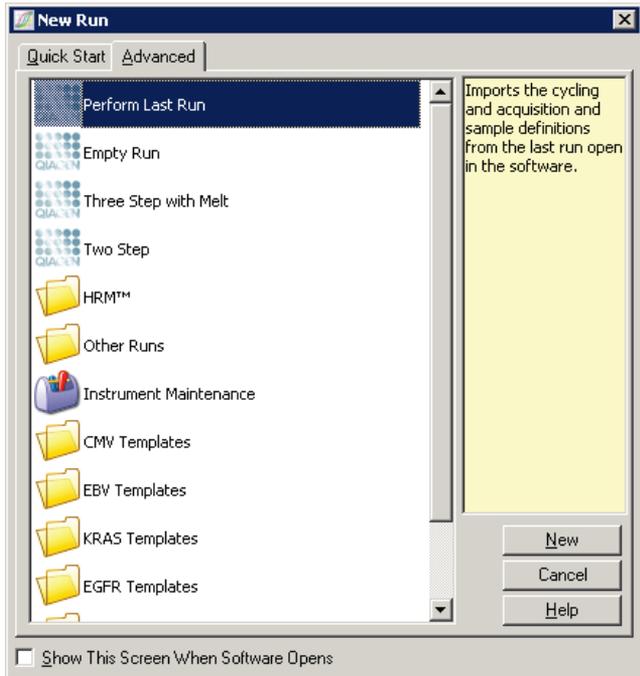
4. The software then prompts for a file name for the run. Then the run begins.

5. The run performs a series of melts that determine the thermal characteristics of the Rotor-Gene Q MDx.
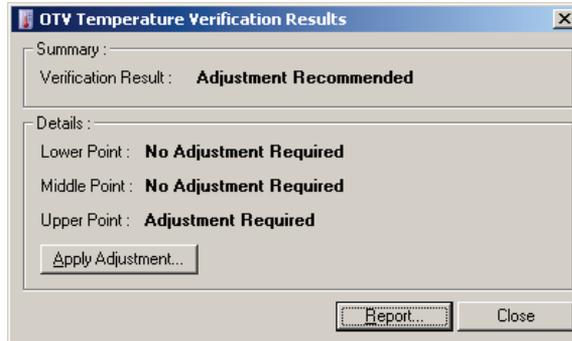
6. When the run is finished, the software indicates whether the Rotor-Gene Q MDx is within specification.

7. If adjustment is required, the user should click "Apply Adjustment". This prompts the user to perform a verification run. After the verification run is complete, no adjustment should be required. If further adjustment is required, contact your distributor.

8. When the Rotor-Gene Q MDx is within specification, a report of the run can be reviewed and printed.

11   High Resolution Melt Analysis

High resolution melt (HRM) analysis is an innovative technique that is based on analysis of DNA melting. HRM characterizes DNA samples according to their dissociation behavior as they transition from double-stranded DNA (dsDNA) to single-stranded DNA (ssDNA) with increasing temperature (see Figure below). An HRM instrument collects fluorescent signals with extremely high optical and thermal precision, creating many application possibilities.
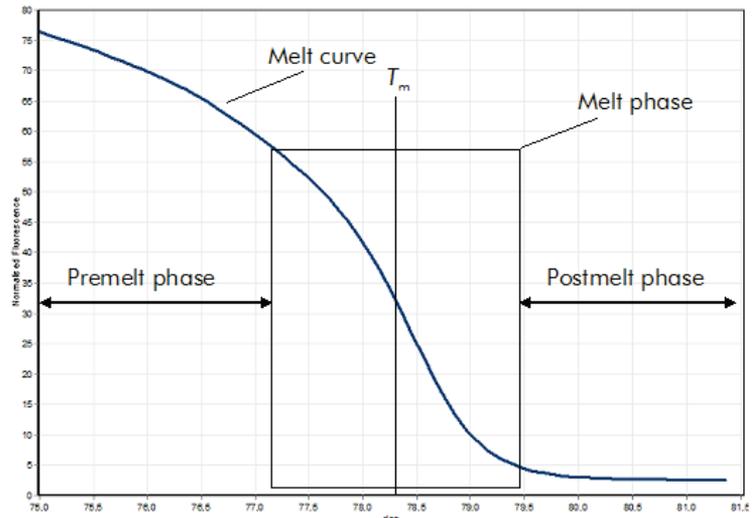
A typical HRM plot. The melt curve plots the transition from the high fluorescence of the initial premelt phase, through the decrease in fluorescence of the melt phase, to the basal level of fluorescence at the postmelt phase. Fluorescence decreases as DNA intercalating dye is released from dsDNA as it melts into single strands. The midpoint of the melt phase, at which the rate of change in fluorescence is greatest, defines the melting temperature (T_m) of the DNA under analysis.

Before performing HRM analysis, the target sequence must be amplified to a high copy number. This is usually performed by PCR in the presence of a dsDNA intercalating fluorescent dye. The dye does not interact with ssDNA but actively intercalates with dsDNA and fluoresces brightly when intercalated. Change in fluorescence can be used to

measure the increase in DNA concentration during PCR and then to directly measure thermally induced DNA melting by HRM. During HRM, fluorescence is initially high because the sample starts as dsDNA. Fluorescence decreases as the temperature is raised and DNA dissociates into single strands. The observed melting behavior is characteristic of a particular DNA sample.

Using HRM, the Rotor-Gene Q MDx can characterize samples based on sequence length, GC content and DNA sequence complementarity. HRM can be used in genotyping applications, such as analysis of insertions/deletions or single nucleotide polymorphisms (SNPs), or in screening for unknown genetic mutations. It can also be used in epigenetics applications for the detection and analysis of DNA methylation status. It can also be used to quantitatively detect a small proportion of variant DNA in a background of wild-type sequence at sensitivities approaching 5%. This can be used, for example, to study somatically acquired mutations or changes in the methylation state of CpG islands.

HRM on the Rotor-Gene Q MDx facilitates multiple applications, including:

- Identification of candidate predisposition genes
- Association studies (comparing cases and controls, genotype to phenotype)
- Determination of allele prevalence within a population or subgroup
- SNP screening and validation
- Screening for loss of heterozygosity
- DNA fingerprinting
- Characterization of haplotype blocks
- DNA methylation analysis
- DNA mapping
- Species identification
- Mutation discovery
- Determining the ratio of somatic acquired mutations
- HLA typing

HRM is easier and more cost-effective than probe-based genotyping assays, and unlike conventional methods, it is a closed-tube system that prevents contamination with PCR products. Results are comparable to conventional methods such as SSCP, DHPLC, RFLP and DNA sequencing.

11.1 Instrumentation

The Rotor-Gene Q MDx provides these demanding, real-time and thermo-optical capabilities required for HRM:

- High-intensity illumination
- Highly sensitive optical detection
- Fast data acquisition
- Finely controlled sample temperature
- Minimal sample-to-sample thermal and optical variation

11.2 Chemistry

QIAGEN offers the Type-it® HRM PCR Kit for analysis of SNPs and mutations using HRM and the EpiTect® HRM PCR Kit for methylation analysis. Both kits contain the third-generation, intercalating fluorescent dye EvaGreen. The kits combine optimized HRM buffer and HotStarTaq® *Plus* DNA Polymerase to avoid unspecific amplification products and provide reliable results.

Note: All QIAGEN HRM kits and reagents are indicated for use with Rotor-Gene Q instruments only for the applications described in the respective QIAGEN Kit handbooks.

11.3 SNP genotyping example

In the example shown, the Type-it HRM PCR Kit was used in HRM analysis to differentiate between homozygous wild type, homozygous mutant and heterozygous forms of the human SNP rs60031276. For technical details, consult the *Type-it HRM PCR Handbook*.

A

B

C

No.	C	Name	Genotype	Confidence %
22		AA Human SNP rs60031276	homo AA	100,00
23		unknown	homo AA	99,49
24		unknown	homo AA	99,76
28		AG Human SNP rs60031276	hetero AG	100,00
29		unknown	hetero AG	99,49
30		unknown	hetero AG	98,47
34		GG Human SNP rs60031276	homo GG	100,00
35		unknown	homo GG	98,80
36		unknown	homo GG	99,53
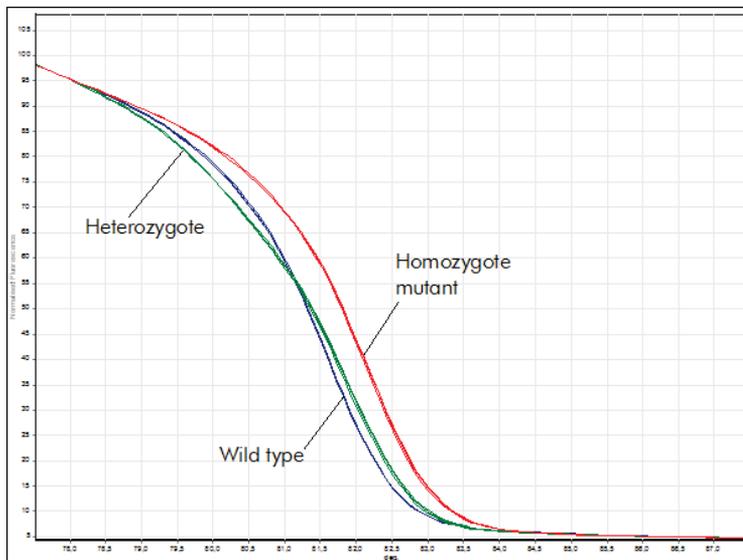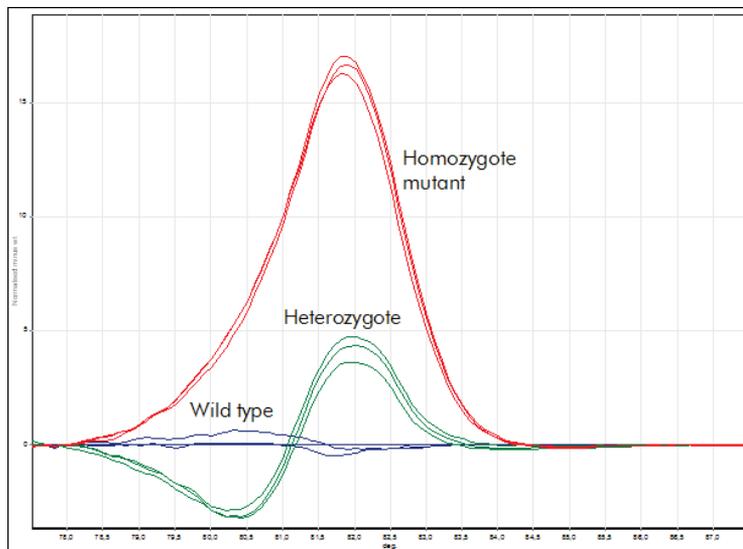
HRM Results - HRM A.HRM (Page 1)

SNP genotyping by HRM. Human SNP rs60031276 (A to G substitution) in the PPP1R14B gene (protein phosphatase 1, regulatory [inhibitor] subunit 14B) was analyzed on the Rotor-Gene Q using 10 ng genomic DNA of different genotypes and the Type-it HRM Kit. Homozygous wild type (AA), homozygous mutant (GG) and heterozygous (AG) samples are shown on A a standard normalized melt curve and B a difference plot normalized to wild type samples. C Genotypes for the unknown samples were assigned by the Rotor-Gene Q software.

11.4 Methylation analysis example

In the example shown, the EpiTect HRM PCR Kit was used in HRM analysis to discriminate various ratios of methylated and unmethylated DNA. For technical details, consult the *EpiTect HRM PCR Handbook*.
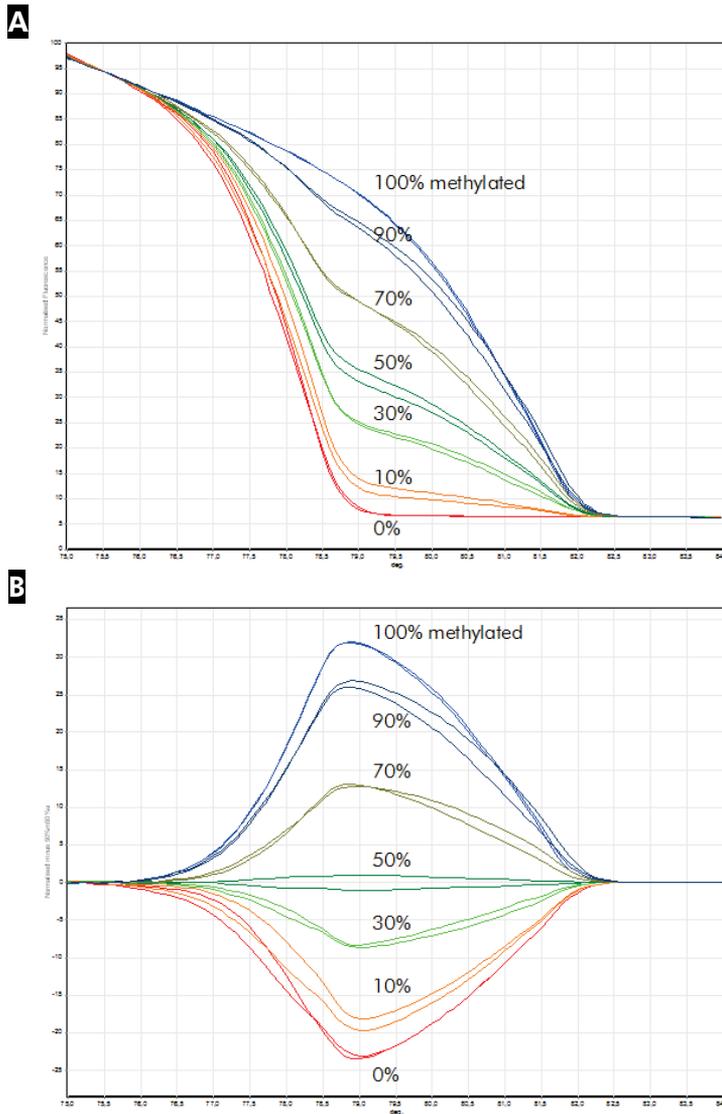
Quantitative methylation analysis by HRM. Various ratios of methylated and unmethylated DNA-APC (adenomatosis polyposis coli) were analyzed and discriminated by HRM methylation analysis on the Rotor-Gene Q using the EpiTect HRM Kit. **A** A standard normalized melt curve and **B** a difference plot normalized to the 50% methylated sample are shown.

11.5   Guidelines for successful HRM analysis

The success of HRM analysis depends largely on the particular sequence under investigation. Certain sequence motifs, such as hairpin loops or other secondary structures, localized regions of unusually high or low GC content, or repeat sequences can all affect the outcome. In addition, use of standardized kits and optimized protocols from QIAGEN can overcome many of the potential challenges listed. Some simple guidelines to help ensure success are detailed below.

Analyze small DNA fragments

Analyze fragments no greater than about 250 bp. Larger products can be analyzed successfully but usually provide lower resolution. This is because, for example, a single base variation has a greater effect on the melting behavior of a 100 bp amplicon than on a 500 bp amplicon.

Ensure that PCR contains only specific product

Samples contaminated with post-PCR artifacts such as primer-dimers or nonspecific products can make HRM results difficult to interpret. Kits from QIAGEN for HRM analysis ensure maximum specificity with no need for optimization.

Use sufficient preamplification template

Analysis of real-time PCR data can be very useful when troubleshooting HRM analyses. Amplification plots should have a C_T (threshold cycle) of less than or equal to 30 cycles. Products that amplify later than this (due to low starting template amount or template degradation) typically produce variable HRM results due to PCR artifacts.

Normalize template concentration

The amount of template added to the reaction should be consistent. Normalize the starting concentrations so that all amplification plots are within 3 C_T values of each other. This ensures input concentrations are within a 10-fold range.

Check for aberrant amplification plots

Prior to running HRM, examine amplification plot data carefully for an abnormal amplification plot shape. Plots with a log-linear phase that is jagged, not steep, or that reaches a low signal plateau compared to other reactions can indicate poor amplification or a fluorescence signal that is too low (e.g., this could occur if primer concentration was too low). Poor reactions can be caused by reaction inhibitors or incorrect reaction setup. HRM data from such samples can either be inconclusive or of low resolution. To avoid unreliable results, we recommend QIAGEN kits for sample preparation and HRM analysis.

Keep postamplification sample concentrations similar

The concentration of a DNA fragment affects its melting temperature (T_m). For this reason, sample DNA concentrations must be kept as similar as possible. When analyzing PCR products, ensure that every reaction has amplified to the plateau phase. At plateau, all reactions will have amplified to a similar extent irrespective of their starting amount. Note, however, that poor reactions may not reach plateau with the same amplified quantity – due, for instance, to inconsistent assay setup (e.g., the primer concentration was too low).

Ensure sample-to-sample uniformity

All samples must be of equal volume and should contain the same concentration of dye. DNA melting behavior is affected by salts in the reaction mix, so it is important that the concentration of buffer, Mg and other salts are as uniform as possible in all samples. Similarly, use only identical reaction tubes from the same manufacturer, to avoid variations due to plastic thickness and autofluorescence properties.

Allow sufficient data collection for premelt and postmelt phases

Capture HRM data points over approximately a 10°C range, centered around the observed T_m (see figure on page 11-1). This provides sufficient baseline data points for effective

curve normalization and will result in more reproducible replicates and easier data interpretation.

11.6 Sample preparation

Sample degradation should be avoided during purification and storage. Avoid excessive amounts of inhibitors such as those from ethanol carryover. To improve HRM results, we recommended keeping consistent the amount of template used between samples. Spectrophotometric analysis for determining DNA concentration and purity is recommended. We recommend QIAGEN kits for sample preparation.

Note: At 260 nm, one absorbance unit is equal to 50 μg/ml DNA. Pure DNA will provide a 260–280 nm ratio of 1.8.

11.7 Software setup

1. Open a new run file by selecting "New…" from the File menu. In the Advanced wizard, select "HRM".
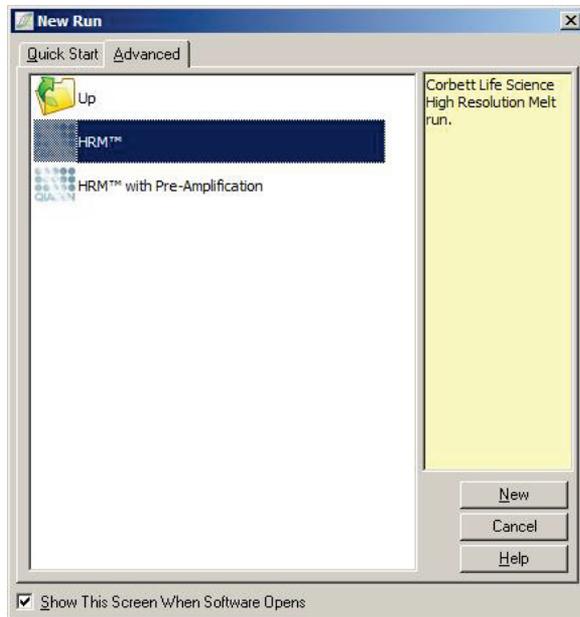
2. Set the rotor type (in this example, the 72-Well Rotor is used). Ensure that the locking ring is in place and the "Locking Ring Attached" checkbox is checked before proceeding to the next step.
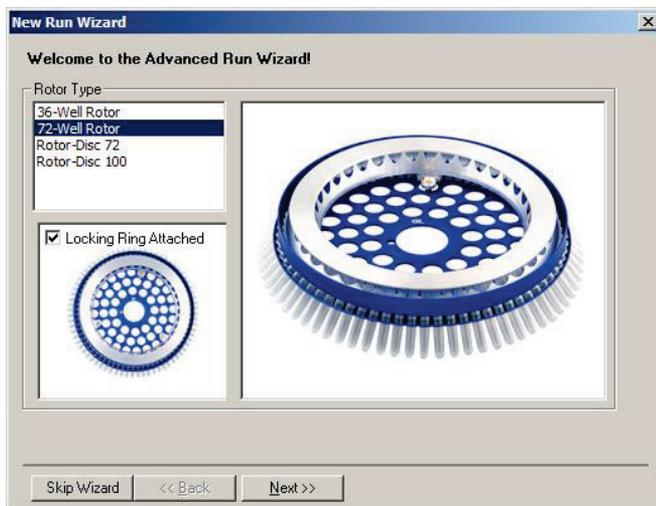
3. Set the run details. Enter the operator name (optional) and add any notes about the experiment (optional). Select the reaction volume (required) and sample layout desired.
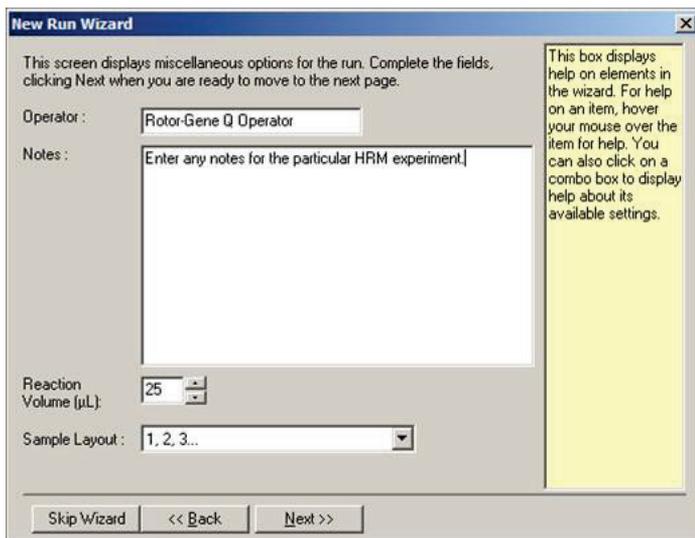
4. Click on the "Edit Profile…" button to modify the times and temperatures of the reaction.
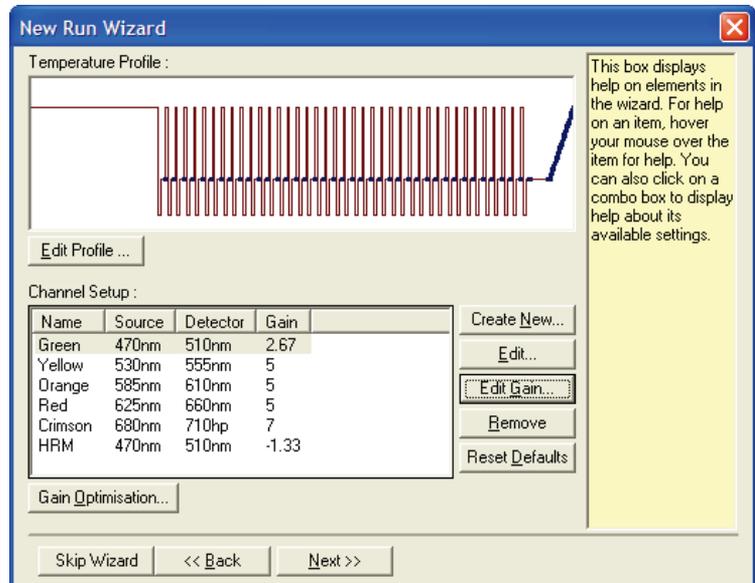
5. Set an appropriate initial hold time. This time depends on the type of DNA polymerase used. The Type-it HRM PCR Kit and the EpiTect HRM PCR Kit require a 5-minute activation time. The default activation time is 10 minutes.
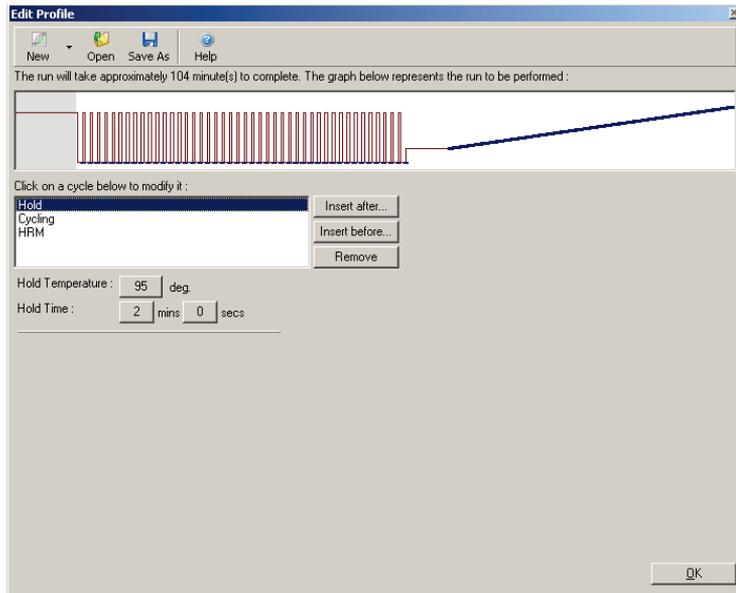
6. Modify the cycling to suit the amplicon.
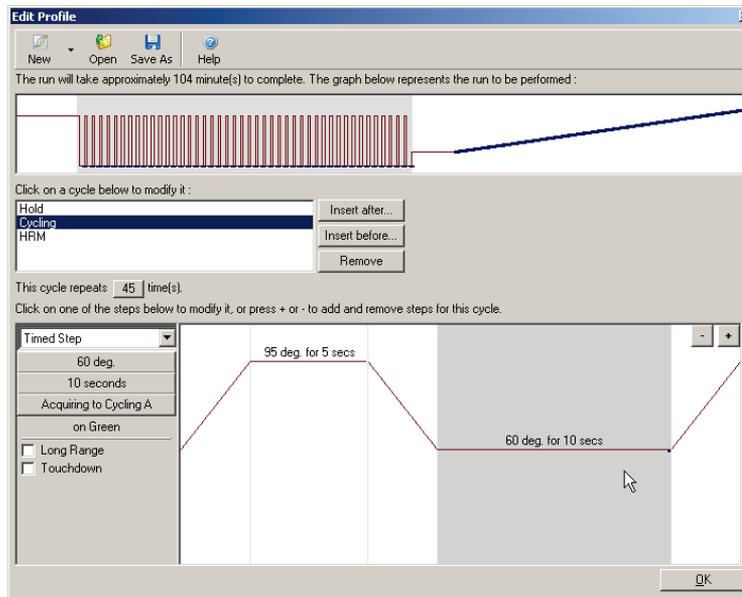
7. Ensure fluorescence data will be acquired. Acquire data to the green channel at the end of the anneal step.

8. Set HRM run conditions. Modify the conditions to suit the amplicon. For the first set of experiments, allow for a wide melt domain. Use the theoretical T_m as a guide to a suitable range. Once you have determined where the product will melt, reduce the melt domain to no greater than 10°C. Ensure that the start of the melt occurs 5°C prior to the first melt transition. The default ramp is set to 0.1°C, with a hold of 2 seconds at each step. The minimum ramp transition is 0.05°C, with a second hold at each step. Data is automatically acquired by the HRM channel. Automatic Gain Optimisation is performed by default. The software will search for the optimal gain setting so that the highest fluorescence value reported is

no greater than 70 units on a scale of 100. Note that this can be increased to a maximum of 100.
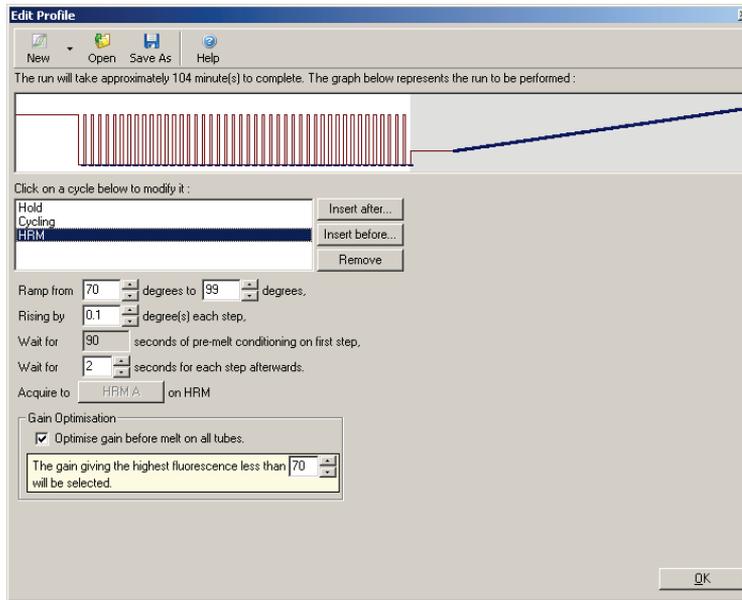
9. Optional: Set Auto-Gain Optimisation. This applies to the real-time amplification step only and is set for the green channel. Click the "Optimize Acquiring" button (to optimize only those channels used by a run). Optimization is best performed just before the first acquisition step, so check the "Perform Optimization Before First Acquisition" checkbox. The recommended background fluorescence range for intercalating dyes is between 1 and 3 fluorescence units. To change this setting, click the channel name to select it in the list, and then click the "Edit" button.
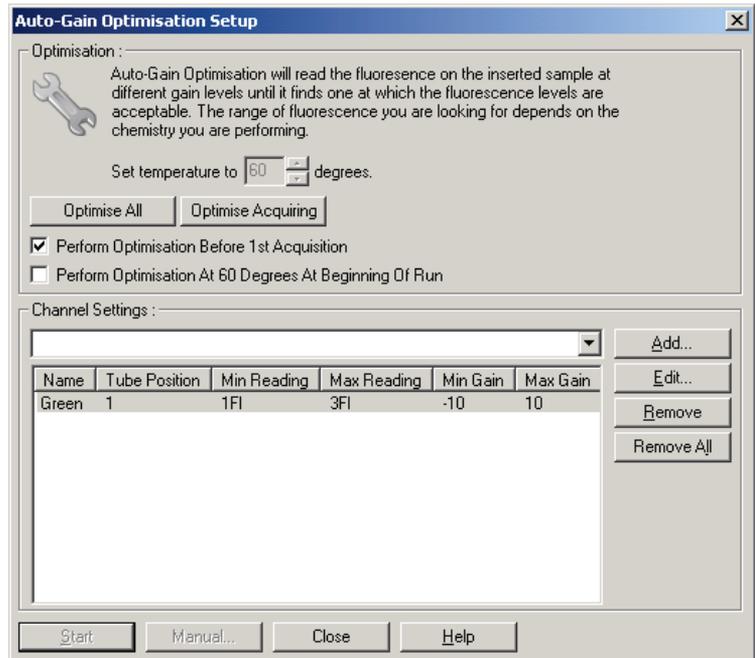
10. Start the run by clicking "Start Run", and save the run file to your computer.
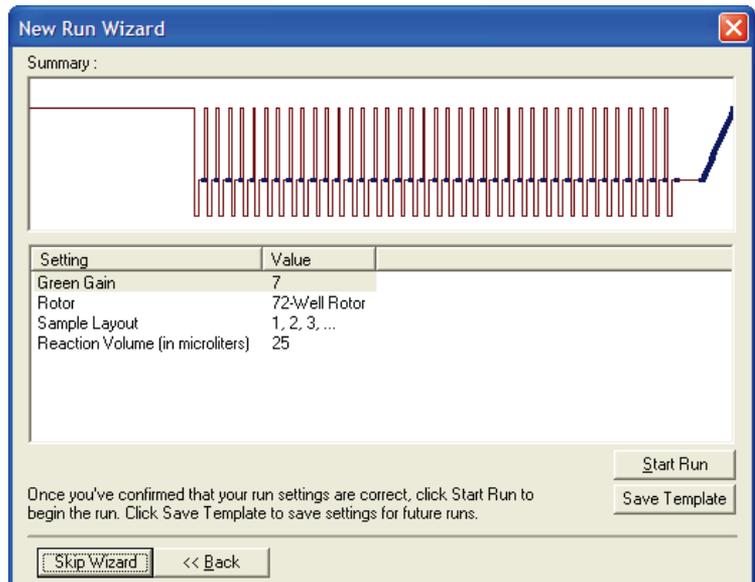
11. Edit the sample names (optional). Sample names can be edited during or after a run.
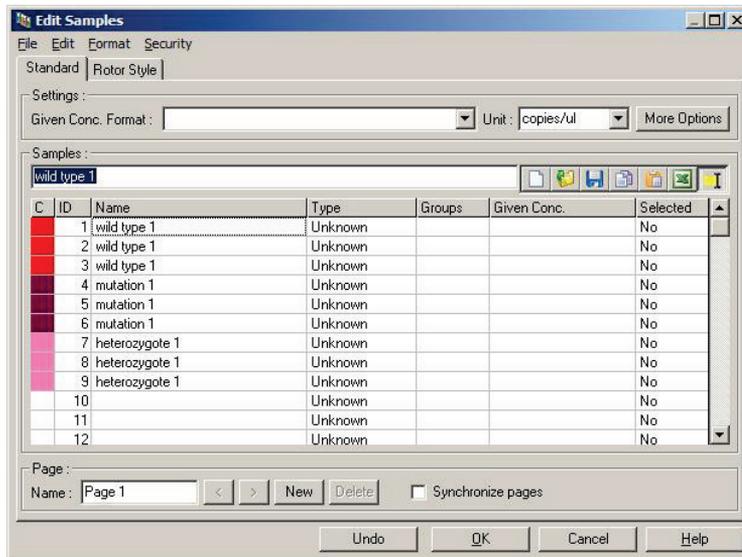
11.8   Real-time PCR data analysis

Analysis of the real-time PCR data prior to HRM data analysis is advantageous. Real-time PCR data can highlight poorly performing assays. Identifying these outliers and filtering them out of the subsequent HRM analysis will greatly improve the overall effectiveness of HRM analysis, since analyzing poor-quality PCR product will result in poor HRM results. We recommend analyzing quantitative real-time PCR data as follows:

1. Analyze the real-time data using the "Quantitation" option from the "Analysis" window. If any C_T values are 30 or higher, the corresponding reactions are considered to have amplified too late. These samples must be analyzed with suspicion or removed from the analysis as outliers. Late amplification is usually due to a too small amount of starting template and/or high levels of sample degradation.

2. Assess the end-point fluorescence level. If end point fluorescence in any of the amplification plots is low compared with the majority of plots in the data set, omit those samples from the analysis even if their C_T value is less than 30. Low end-point fluorescence can indicate an incorrect dye amount, incorrect levels of reaction components (such as primers) or the action of inhibitors.

3. Use the "Comparative Quantitation" option from the "Analysis" window to obtain the reaction efficiency of each sample. If the efficiency is not similar to other reactions in the experiment or is less than approximately 1.4, omit the reaction as an outlier.
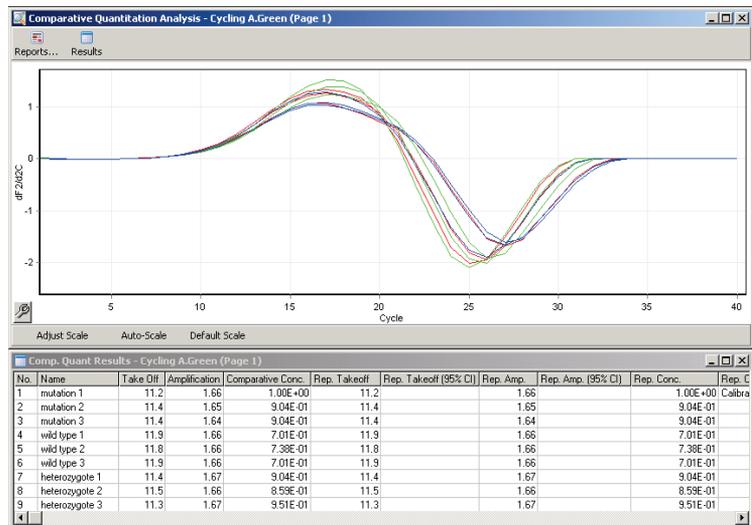
Comparative quantitation results. The reaction efficiency is shown in the "Amplification" column as a score out of 2 (2 = 100% efficiency).

> **Note**: If you suspect the presence of primer-dimers or nonspecific products, assess reactions by drawing a derivative plot using the "Melt" option from the "Analysis" window. Ensure that there is a single peak, indicative of a single product. If possible, run a gel to check that there is a single amplification product. If there is more than one product, the reaction should be repeated or reoptimized.

11.9   HRM data analysis

HRM analysis enables both visual- and auto-calling of genotypes. Results can be viewed as either a normalized melt plot or a difference plot. Normalized curves provide the basic representation of the different genotypes, based on curve shifting (for homozygotes) and curve shape change (for heterozygotes).

Difference plots are an aid to visual interpretation. They plot the difference in fluorescence of a sample to a selected control at each temperature transition. Difference plots provide an alternative view of the differences between melt curve transitions.

Note: First derivative melt curve analysis (as used by the standard "Melt" option in the "Analysis" window) is considered inappropriate for HRM analysis. This is because any derivation of the data adds artificial noise and makes data interpretation more difficult.

The following steps describe the analysis of HRM results using Rotor-Gene Q software:

1. Select the "HRM" option from the "Analysis" window.
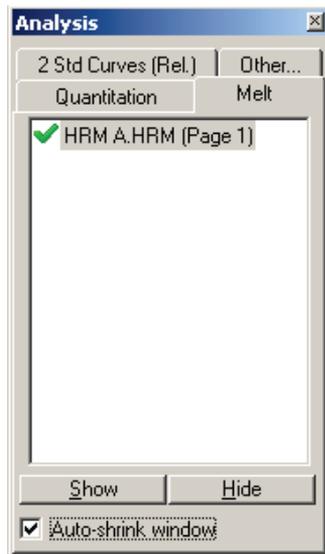
2. Windows appear showing the raw data, the normalized graph and the results. The raw data window allows adjustment of the regions of normalization. Normalization allows all the curves to be compared with the same starting and ending fluorescent signal levels to aid interpretation and analysis. Two cursors per region are provided, defaulted to the ends of the curve. The data points within the regions are used to normalize fluorescence (the y axis only) for the start (Region 1) and end (Region 2) of the melt plot. Data outside the set regions are ignored. Adjust the regions to encompass representative baseline data for the premelt and postmelt phases. Widening the regions (by clicking and dragging) allows the software to adjust for the slope of the baseline. To ensure curves normalize effectively, avoid widening the normalization regions into the melt phase.
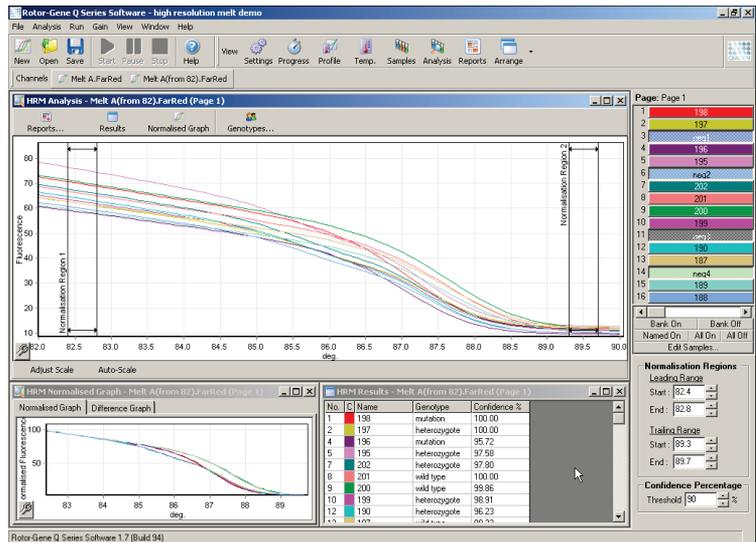
Note: We recommend that cursors be moved only if you wish to avoid areas of the melt curve. Movement of the cursors toward the melt phase transitions can affect subtraction plots and confidence percentages.

3. The "Normalised Graph" window displays the normalized melt curves. Samples can also be viewed as a difference plot against one of the controls.
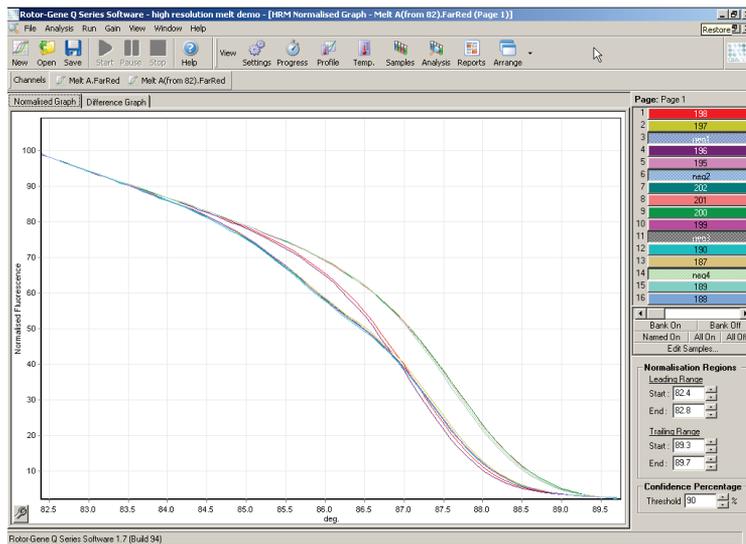
4. Click on the "Genotypes…" button to define the genotypes. Input each genotype category name, and select a representative sample for each from the sample list.
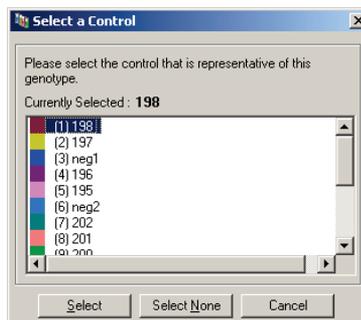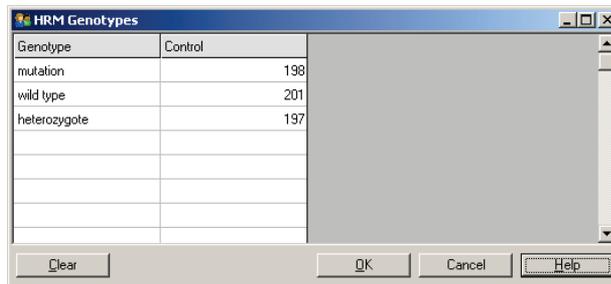
5. View the difference plot by selecting the "Difference Graph" tab. Then, using the drop-down menu at the top of the window, select the genotype against which you wish to compare all the other samples. In the example shown, all samples are plotted, subtracted from an average plot of all samples labeled "Mutation 1".
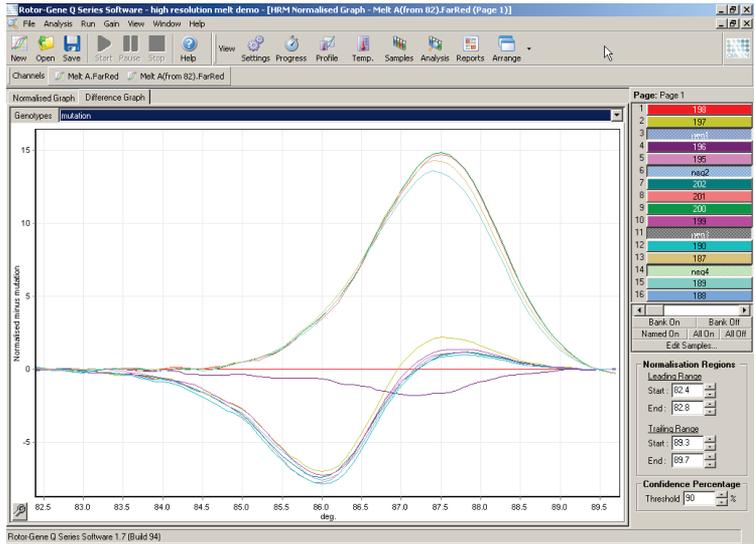
6. Genotypes will be called automatically by the software in the "Results" window. A confidence value is provided as an integrity check of autocalled results. The threshold value, above which autocalls are made, can be edited. Samples that fall below the set threshold will be marked as a variation for closer investigation or retesting.

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12 Troubleshooting

12.1 Log Archives

The software keeps an unmodified record of each run, along with diagnostic information, in its Log Archive repository. By using the Help, Send Support Email option, you can send an e-mail along with all the necessary diagnostic information to QIAGEN Technical Service (see Section 7-100).

To save disk space, only Log Archives of the 60 most recent runs are stored. Older run Log Archives will be overwritten as new run Log Archives are created.

12.2 HRM troubleshooting

	Comments and suggestions
Unable to run HRM	
Rotor-Gene Q MDx model is not HRM equipped	Contact your local QIAGEN representative.
No HRM data obtained	
Incorrect setup	Check filter settings.
	Check if the rotor type is correct.
	Check if the correct reagents have been used.
	Check if the reaction was set up correctly.
	Run a positive control experiment (i.e., an assay that is known to provide results).

	Comments and suggestions
Plots look jagged	
Poor or no amplification	Check if the correct protocols and reagents have been used. We recommend QIAGEN kits for HRM analysis.
	Check if the reaction was set up correctly.
	Check the cycling conditions.
	Check the starting quality and quantity of the template. We recommend QIAGEN kits for sample preparation.
Amplification or melt plots are saturated	
Gain set too high	Use Auto-Gain Optimisation (see page 6-21).
Confidence percentages have changed	
Normalization regions were moved by clicking and dragging	Only move normalization regions if it is necessary to avoid parts of the melt curve.
Outliers are present in the data	
Inconsistent reaction setup	Check if the correct reagents have been used.
Inhibitors present in sample	Check that the tubes used are uniform.
Too little or degraded template	Check that the same master mix was used for all samples.
	Check the starting quality and quantity of the template.

12.3   General instrument errors

	Comments and suggestions
Can't open the serial port<COMPORT>	This error occurs on software startup if the software cannot communicate with the instrument via the configured COM port. This is commonly caused by faulty cables, loose cables, faulty serial ports, faulty USB ports, a USB driver problem, or a USB-to-serial converter driver problem.
	Reconnect or replace the cable. Reinstall the appropriate drivers. Start the software in "Virtual Mode" and select "Setup/Auto-Detect button" from the "File" menu to reset the configured COM port.
Chamber lid open	
Could not continue run; the chamber lid was opened during a run. Please reset the machine and restart the software.	This error occurs when the software has detected the lid is open in the middle of a run.
	Reset the machine and restart the software.
Chamber lid open	
The instrument chamber lid is open. Please close the lid and then click Continue.	This error occurs when the user tries to start a run while the instrument lid is open.
	Close the lid of the instrument chamber and then click "Continue".

Comments and suggestions

Communication corrupted

This error occurs when the data received from the instrument do not conform to the expected pattern.

Further investigations are required by a QIAGEN Field Service Specialist to diagnose the problem with the instrument.

Please contact your distributor or QIAGEN Technical Services.

Communication out of sequence

Instrument has received data from the machine that is out of sequence.

This error occurs when the data received from the instrument are not in the correct order.

Further investigations are required by a QIAGEN Field Service Specialist to diagnose the problem with the instrument.

Please contact your distributor or QIAGEN Technical Services.

Communication protocol error

A communication protocol error occurred with this run.

This error occurs when the communication protocol configured in the firmware is not the same as the expected protocol.

Further investigations are required by a QIAGEN Field Service Specialist to diagnose the problem with the communication protocol or the instrument.

Comments and suggestions

**Detector motor jam,
stopped machine**

This error can occur when the Rotor-Gene Q MDx is started immediately after delivery in cold climates.

In this case, allow the instrument to acclimatize to room temperature for at least an hour before turning the instrument on.

If the error persists, please contact your distributor or QIAGEN Technical Services.

Fatal hardware malfunction

The instrument detected that there was a fatal hardware malfunction. Do not attempt to re-use the machine until the machine has been serviced by your distributor.

This error occurs when the software has detected a fatal hardware malfunction and has activated a safe-protection procedure to turn off the machine.

Turn off the instrument immediately and contact your distributor or QIAGEN Technical Services.

Machine error

This run was stopped as machine errors occurred that could not be recovered from. Please contact your distributor if this occurs again, attaching a support archive file.

This error occurs when the software has detected errors on the machine that could not be recovered from. The software has stopped the run.

Try another run. If the problem persists, contact your distributor or QIAGEN Technical Services and attach a support archive file.

Comments and suggestions

Machine unplugged

The instrument is not responding and failed with the message <ERROR MESSAGE >. This is an unrecoverable failure, please reset the instrument and restart the software.

This error occurs if the instrument does not communicate with the software after a defined timeout interval. It is often caused by an instrument fault or by excessive activity from the PC, which causes a packet to be lost.

Common software-related causes include processor-intensive tasks, such as antivirus resident protection or antivirus scheduled scans, wireless cards, or infrared cards.

Disable or uninstall the relevant processor-intensive software/task.

Reset the instrument and restart the software.

Please contact your distributor or QIAGEN Technical Services if the problem persists.

Machine unplugged

The instrument is not connected to your computer on <PORT NAME>. Reconnect the serial cable to the back of the computer and then click Continue.

This error occurs when the serial or USB communication to the instrument is lost.

Reconnect the serial or USB cable to the back of the computer and then click the "Continue" button.

Comments and suggestions

Object variable or with block variable not set

This error occurs on software startup if the default experiment template file has become corrupt. This may happen if the software/computer is shut down without exiting correctly, for example, during a power outage.

Delete the file **C:\Program Files\Rotor-Gene Q Software\Templates\normal.ret** and then restart the software.

Rotor speed failure

Time out while setting the rotor speed.

This error occurs when the software has attempted to set the rotor speed and failed to set the target speed within a time-out period.

Further investigations are required by a QIAGEN Field Service Specialist to diagnose the problem with the instrument.

Please contact your distributor or QIAGEN Technical Services.

Serial port in use

The serial port is currently being used by another application. Close any applications such as communications or synchronization software and then retry.

This error occurs when the software tries to connect to the machine on the configured COM port when the port is being used by another software.

Close any applications such as communications or synchronization software and then retry.

Comments and suggestions

Shutdown timeout

The instrument has exceeded the expected time to shutdown. Please reset the machine, and reset the software.

This error occurs when the software has issued shutdown command to shut down the instrument and the machine keeps sending data back after an expected grace period of time.

Reset the machine and restart the software.

Temperature protection activated

The instrument detected that the chamber temperature increased above a safe level. It has therefore entered a self-protection mode. Please turn off the instrument and contact your distributor if the problem persists.

This error occurs when the software has detected the chamber temperature has increased to above a safe level and hence activated a safe-protection procedure.

Turn off the instrument immediately and contact your distributor or QIAGEN Technical Services.

Thermistor is open

The instrument detected that the thermistor is open, and so to prevent damage to the machine, it has been turned off. Please contact your distributor if this occurs again.

This error occurs when the software has detected that the thermistor is open and therefore cannot read the temperature; the software has then activated a safe-protection procedure to turn off the machine.

Turn off the instrument immediately and contact your distributor or QIAGEN Technical Services.

Comments and suggestions

Unrecoverable errors occurred

This run was stopped as machine errors occurred that could not be recovered from. Please contact your distributor if this occurs again, attaching a support archive file.

This error occurs in the middle of the run after the software has made all possible attempts to recover and failed.

Further investigations are required by a QIAGEN Field Service Specialist to diagnose the problem with the instrument.

Please contact your distributor or QIAGEN Technical Services.

12.4 Rotor-Gene Q Software messages

The following is a list of use, warnings and other messages that may appear in the Rotor-Gene Software during hardware and software operation. Any part of the message that is variable, e.g., characteristic error descriptions, are given in brackets (e.g., "< ERROR DESCRIPTION >").

Message text

General messages

1   A raw channel already exists for this page. If you would like to recreate this page, you must first delete the raw channel via the "Options" button, and then try again.

2   A serious problem has occurred which requires shutting down the software. After you click "OK", your current work will be saved, and the machine will be turned off, if possible. If this problem persists, please contact your distributor.

3   Cannot delete this page. There must always be at least one sample page.

	Message text
4	Can't connect to instrument on serial port \<COMPORT\>. Check the machine is correctly plugged into the back of the computer, then retry
5	Can't open the serial port \<COMPORT\> to connect to the instrument. Check you do not have any communications software open, then retry.
6	Could not save to run because some data on the form was invalid. Please check your entries then try again.
7	Couldn't save file. Confirm the disk has enough space and that it is free of errors.
8	E-mail application could not be started. Confirm that it has been correctly installed on your computer.
9	Encountered an error during run: \<ERROR DESCRIPTION\>. The run will continue, and a message will be logged in the messages tab of Run Info.
10	Instrument was not detected. Please ensure you have correctly connected the instrument, and that the instrument is turned on.
11	Logging is currently disabled due to a previous error. Archived logs cannot be viewed until the software has been restarted.
12	Not all samples could be normalised as the fluorescent level was too low.
13	Only runs performed with the same rotor as the current run may be imported.
14	Please note that log files for the current run will not be available until it has completed.
15	Please type valid number of times to repeat. It should be more than 0.

Message text

16	Problem encountered while updating log data. Logging has been disabled, but will be renabled on the next run.
17	Run file signing ensures the integrity of your run results. Information about a run's signature can be found in the Run Info window.
18	Sample ID is locked. Cannot paste over locked samples.
19	TeeChart Office has not been installed on this computer. Please re-install the Rotor-Gene software.
20	The COM port configured for the instrument is not selected. You must select a COM port.
21	The loaded run file contains a signature which does not match the file contents. This means the file has either been corrupted, or tampered with since it was written by the Rotor-Gene software.
22	The loaded run file has no signature. The contents of this file cannot be guaranteed.
23	The Machine serial number is not valid. Serial numbers must be at least 6 digits long.
24	The machine will now be be cooled to <TEMPERATURE> degrees. The chamber and surfaces will still be very hot when opening the machine. Please exercise due caution and wear protective gloves if touching any of the surfaces or tubes.
25	The regional settings for your computer are conflicting. Ensure your currency and numeric decimal placeholders are matching.

	Message text
26	The serial number entered in the welcome screen <SERIAL NUMBER1> does not match the serial number stored in the attached machine <SERIAL NUMBER2>. The computer's serial number has now been updated to match the connected machine.
27	There was a problem communicating with the communication board. You should reboot the computer and then retry.
28	There was a timeout attempting to talk to the instrument. Check it is correctly plugged in.
29	This feature cannot be used in virtual mode.
30	This profile file was created in a more recent version of the Rotor-Gene software. Certain aspects may not load correctly.
31	This run file was created in a more recent version of the Rotor-Gene software. Certain aspects of the run may not load correctly.
32	This sample file was created in a more recent version of the Rotor-Gene software. Certain aspects may not load correctly.
33	This software will perform basic simulation of a machine for training and demonstration purposes. You can disable this setting via the Setup screen, accessible from the File menu.
34	This template was created in a more recent version of the Rotor-Gene software. Certain aspects of the template may not load correctly.
35	Unable to load this sample file as tube layouts do not match. Load these samples before starting the run.

Message text

36	Unable to open communications with the machine because another application is already using <COMPORT>. Check you do not have any applications running that use the same serial port, then retry.
37	Unrecoverable errors were encountered while attempting to load the file. The file was not loaded.
38	You cannot stop the program while the run is in progress.
39	You have insufficient rights to use the software. Please contact the domain administrator to set up groups.
40	You must have performed a quantitation analysis to export samples.
41	You must select a COM port before continuing.
42	Your run could not be saved to its default location. On the following window, select an alternative location to save your run.
43	Your settings have been saved. Click OK to close the software.
44	You must select a rotor before continuing.
45	You cannot start the run until you tick the checkbox to confirm that the locking ring has been attached.

Autogain adjustment messages

46	Manual gain adjustment uses the channels you have defined in your profile. As you have not defined any acquisition points in your profile, you cannot perform manual gain adjustment.

	Message text
47	The temperature you entered was not saved because it was outside the range of the machine. Enter a valid temperature.

Editor messages

48	Please enter a valid group code. Group codes must be a maximum of 5 characters, and contain no spaces or commas.
49	Please enter a valid group name. Group names cannot contain commas or be empty.

Optical denature calibration messages

50	Unable to set as optical denature point due to calibration failure. Please enter a valid number of seconds to hold. It should be a positive value.
51	A melt peak could not be detected during Optical Denature Calibration. This may be because the incorrect tube was selected for calibration, or that an inappropriate chemistry was used for this sample. A timed step profile was run instead.

OTV messages

52	You must enter a valid OTV serial number to perform the run.
53	This temperature verification file has been corrupted. Please uninstall and re-install the Rotor-Gene software to correct this error.
54	This run file is not correctly signed. Results cannot be displayed.

Message text

55	You cannot start until you tick the checkbox to confirm that the fluorescent insert has been placed correctly.
56	This rotor has expired. Please contact your distributor to obtain a replacement.

Security menu messages

57	Could not open the Windows user/group manager.
58	Could not create groups.
59	Cannot modify access of inbuilt accounts.

Analysis menu

60	You have only selected one channel for analysis. To select multiple channels, drag a rectangle around the channels you wish to display in the analysis selection window.
61	You have selected multiple channels for analysis. This analysis technique only allows single channels to be analysed.

Concentration measurement messages

62	Concentration Measurement performs auto-gain optimisation on the first rotor position. Ensure you have your highest concentration standard in the first rotor position.

End-point analysis messages

63	To use end-point analysis you must have positive and negative controls in each channel. To define these controls click OK.
64	You have not defined any positive controls. You must define positive controls for each channel you are analysing.

	Message text
65	You have not defined any negative controls. You must define negative controls for each channel you are analysing.
66	You have not defined any NTC controls. You must define NTC controls for each group.

HRM analysis messages

67	Genotype <GENOTYPE NAME> does not have a control defined.
68	Duplicate genotype combinations are not allowed.
69	High resolution melts are not supported on this instrument. Please contact your distributor for more information.

Melt analysis messages

70	The genotypes can not be defined until bins have been placed. Please define all bins and then try again.
71	You must enter an abbreviation for <GENOTYPE NAME> genotype.

Scatter plot analysis messages

72	Scatter plot analysis requires exactly 2 channels to be selected. To select multiple channels, drag a rectangle around the channels you wish to display in the analysis selection window, or click while holding the SHIFT key on each channel.

Quantitation analysis messages

73	The auto-find threshold feature requires that you have defined at least 2 selected standards. To set this up, right-click on the sample list and select "Edit Samples…"

13   Glossary

Term	Description
Acquisition	Acquisition is the collection of fluorescent data. Each acquisition (set of fluorescent data) from a channel is displayed in the software as unanalyzed data in a "Raw channel" window. This data can be analyzed using the options in the "Analysis" menu.
Bins	In a melt analysis, bins are set to define a region where a melt peak is expected to occur. Genotypes can be defined based on the presence of peaks in certain bins or in combinations of bins.
CE-IVD	Compliance with the in vitro diagnostic medical device European Directive 98/79/EC.
Channel	A channel consists of a light emitting diode (LED) with an excitation filter paired with an emission filter. The LED and excitation filter excite samples at a given wavelength. Fluorescence emitted by samples is passed through the emission filter, before being detected by a photomultiplier.
Gain	The Rotor-Gene Q MDx uses a photomultiplier to collect fluorescence photons and convert them to electronic signals. The gain is a setting that determines the sensitivity of the photomultiplier. If the gain is set too high, the signal is oversaturated. If the gain is set too low, it is not possible to differentiate signal from background noise.
Gain Optimisation	Gain Optimisation is a process that dynamically adjusts the gain setting, allowing an appropriate setting to be selected which results in optimal signal detection.

Term	Description
Loading Block	Loading Blocks are aluminum blocks available in different formats which are used to hold tubes or Rotor-Discs during reaction setup. Rotor-Disc Loading Blocks are also used with the Rotor-Disc Heat Sealer to heat seal Rotor-Discs.
Locking Ring	Locking Rings are metal rings that fit onto the rotor to prevent tubes and caps from coming loose during operation of the Rotor-Gene Q MDx. Loose caps and tubes could cause damage to the instrument.
Rotor	The metal rotor holds tubes or Rotor-Discs in the Rotor-Gene Q MDx. It enables samples to spin in the instrument chamber and ensures that samples are correctly aligned with the optical system. The rotor is secured with a Locking Ring.
Rotor-Disc	Rotor-Discs are circular plates of vertically oriented reaction wells. Rotor-Disc formats for 72 and 100 reactions are available. Rotor-Discs are sealed using Rotor-Disc Heat Sealing Film and the Rotor-Disc Heat Sealer.

Appendix A

Technical data

QIAGEN reserves the right to change specifications at any time.

Environmental conditions

Operating conditions

Power	100–240 V AC, 50–60Hz, 520 VA (peak)
	Power consumption 60 VA (standby)
	Mains supply voltage fluctuations are not to exceed 10% of the nominal supply voltages.
Fuse	F5A 250 V fuse
Heat dissipation/ thermal load	Average: 0.183 kW (632 BTU/hour)
	Peak: 0.458 kW (1578 BTU/hour)
Overvoltage category	II
Air temperature	18–30°C (64–86°F)
Relative humidity	10–75% (noncondensing)
Altitude	Up to 2000 m (6500 ft.)
Place of operation	For indoor use only
Pollution level	2
Environmental class	3K2 (IEC 60721-3-3)
	3M2 (IEC 60721-3-3)

Transportation conditions

Air temperature	−25°C to 60°C (−13°F to 140°F) in manufacturer's package
Relative humidity	Max. 75% (noncondensing)
Environmental class	2K2 (IEC 60721-3-2)

Storage conditions

Air temperature	15–30°C (59–86°F) in manufacturer's package
Relative humidity	Max. 75% (noncondensing)
Environmental class	1K2 (IEC 60721-3-1)

Mechanical data and hardware features

Dimensions	Width: 370 mm (14.6 in.) Height: 286 mm (11.3 in.) Depth (without cables): 420 mm (16.5 in.) Depth (door open): 538 mm (21.2 in.)
Weight	12.5 kg (27.6 lb.) standard configuration
Capacity	Up to 100 samples per run using a Rotor-Disc 100
Software	Rotor-Gene Q software (version 2.3.4 or higher)

Thermal specifications

Description	Specification
Temperature range	35–99°C (95–210.2°F) (50–99°C [122–210.2°F] for cycling applications)
Temperature accuracy	±0.5°C (calibrated using Rotor-Disc OTV procedure)
Temperature resolution	±0.02°C (smallest programmable increment)
Temperature uniformity	±0.02°C

Optical specifications

Description	Specification
Excitation sources	High energy light-emitting diodes
Detector	Photomultiplier
Acquisition time	4 s

FCC Declaration

The ''United States Federal Communications Commission'' (USFCC) (in 47 CRF 15. 105) declared that the users of this product must be informed of the following facts and circumstances.

''This device complies with part 15 of the FCC: Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.''

"This Class B digital apparatus complies with Canadian ICES-0003.''

The following statement applies to the products covered in this manual, unless otherwise specified herein. The statement for other products will appear in the accompanying documentation.

Note: This equipment has been tested and found to comply with the limits for a Class B digital device, pursuant to Part 15 of the FCC Rules and meets all requirements of the Canadian

Interference-Causing Equipment Standard ICES-003 for digital apparatus. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that the interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

■ Reorient or relocate the receiving antenna

■ Increase the separation between the equipment and receiver

■ Connect the equipment into an outlet on a circuit different from that to which the receiver is connected

Consult the dealer or an experienced radio/T.V. technician for help.

QIAGEN GmbH Germany is not responsible for any radio television interference caused by unauthorized modifications of this equipment or the substitution or attachment of connection cables and equipment other than those specified by QIAGEN GmbH, Germany. The correction of interference caused by such unauthorized modification, substitution or attachment will be the responsibility of the user.

Declaration of Conformity

Name and address of the legal manufacturer

QIAGEN GmbH

QIAGEN Strasse 1

40724 Hilden

Germany

An up-to-date Declaration of Conformity can be requested from QIAGEN Technical Services.

Waste Electrical and Electronic Equipment (WEEE)

This section provides information about disposal of waste electrical and electronic equipment by users

The crossed-out wheeled bin symbol (see below) indicates that this product must not be disposed of with other waste; it must be taken to an approved treatment facility or to a designated collection point for recycling, according to local laws and regulations.

The separate collection and recycling of waste electronic equipment at the time of disposal helps to conserve natural resources and ensures that the product is recycled in a manner that protects human health and the environment.

Recycling can be provided by QIAGEN upon request at additional cost. In the European Union, in accordance with the specific WEEE recycling requirements and where a replacement product is being supplied by QIAGEN, free recycling of its WEEE-marked electronic equipment is provided.

To recycle electronic equipment, contact your local QIAGEN sales office for the required return form. Once the form is submitted, you will be contacted by QIAGEN either to request follow-up information for scheduling collection of the electronic waste or to provide you with an individual quote.

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Appendix B

This appendix describes the mathematical techniques used in more detail.

Quantitation

Calculated concentrations are obtained from a simple linear regression model, with the known values the log concentrations (x) and the experimental values the C_T values (y).

The log concentrations and C_T values of the standards are used to construct a model in the form:

y = Mx + B

Confidence intervals for calculated concentrations

We use the following confidence interval $100(1- \alpha)\%$ for an estimate of a new observation x0 from the standard curve:

$$\frac{Y_0 - \hat{\beta}_0}{\hat{\beta}_1} \pm \frac{S}{\hat{\beta}_1}(1 + \frac{1}{n} + \frac{(x_0 - \bar{x})^2}{S_{xx}})^{\frac{1}{2}} t_{n-2,\alpha/2}$$

This is the confidence interval for the concentration of a single unknown.

Suppose now we have k further observations at x = x0 and we denote their average by $\bar{Y}_0$. Then,

$$\bar{Y}_0 \sim N(\beta_0 + \beta_1 x_0, \frac{\sigma^2}{k})$$

and arguments similar to above give

$$\frac{Y_0 - \hat{\beta}_0}{\hat{\beta}_1} \pm \frac{S}{\hat{\beta}_1}(\frac{1}{k} + \frac{1}{n} + \frac{(x_0 - \bar{x})^2}{S_{xx}})^{\frac{1}{2}} t_{n-2,\alpha/2}$$

This formula determines how confidence intervals for concentrations of replicate unknowns are determined.

For estimation of standards, a tighter confidence interval can be obtained:

$$\frac{Y_0 - \hat{\beta}_0}{\hat{\beta}_1} \pm \frac{S}{\hat{\beta}_1}\left(\frac{1}{n} + \frac{(x_0 - \bar{x})^2}{S_{xx}}\right)^{\frac{1}{2}} t_{n-2,\alpha/2}$$

The implication of this formula is that adding replicates to an standard individual concentration reduces the width of the interval for all estimates, as n is increased. Adding a large number of replicates to an unknown reduces its uncertainty to that of a single standard. The extra replicates reduce the uncertainty due to the unknown not forming part of the linear model.

Confidence intervals for C_T values

We assume that error in replicate C_T values is linear and normally distributed.

We therefore use the One-Sample t Confidence Interval. Let μ be the mean value for a replicate's C_T values

$(x_0 \ldots x_{n-1})$. Then, a 100(1– α)% confidence interval for a C_T value μ is:

$$\left(\bar{x} - t_{\alpha/2,n-1} \cdot \frac{s}{\sqrt{n}},\ \bar{x} + t_{\alpha/2,n-1} \cdot \frac{s}{\sqrt{n}}\right)$$

We would like to thank Peter Cook from the Mathematics department of the University of NSW, Sydney, Australia, whose help was invaluable in verifying the mathematical approaches used.

Appendix C

Rotor-Gene Q MDx products, accessories and consumables

Product	Contents	Cat. no.
Rotor-Gene Q MDx 2plex	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire
Rotor-Gene Q MDx 2plex HRM	Real-time PCR cycler and High Resolution Melt analyzer with 2 channels (green, yellow), plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire
Rotor-Gene Q MDx 5plex	Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire
Rotor-Gene Q MDx 5plex HRM	Real-time PCR cycler and High Resolution Melt analyzer with 5 channels (green, yellow, orange, red, crimson), plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire
Rotor-Gene Q MDx 6plex	Real-time PCR cycler with 6 channels (blue, green, yellow, orange, red, crimson), including laptop computer, software, accessories, 1-year warranty on parts and labor	Inquire

Product	Contents	Cat. no.
Accessories		
Rotor-Disc 100 Starter Kit	Kit includes: 2 Rotor-Disc 100 packs, Rotor-Disc Heat Sealer, Rotor-Disc Heat Sealing Film, Rotor-Disc 100 Rotor and Locking Ring, Rotor-Disc 100 Loading Block, Rotor-Disc Pipetting Aid	Inquire
Rotor-Disc 100 (30)	30 individually wrapped discs for 3000 reactions	981311
Rotor-Disc 100 (300)	10 x 30 individually wrapped discs for 30,000 reactions	981313
Rotor-Disc 100 Rotor	For holding Rotor-Disc 100 discs in the Rotor-Gene Q MDx; requires Rotor-Disc 100 Locking Ring	9018895
Rotor-Disc 100 Locking Ring	For locking a Rotor-Disc 100 in the Rotor-Disc 100 Rotor	9018896
Rotor-Disc 100 Loading Block	Aluminum block for manual and automated reaction setup in Rotor-Disc 100 discs	9018909
Rotor-Disc Pipetting Aid	Aid for marking well during manual reaction setup on a Rotor-Disc Loading Block	9018897
Rotor-Disc Heat Sealer	Heat sealing instrument for use with Rotor-Discs; requires Rotor-Disc 72 or 100 Loading Block	9018898
Rotor-Disc Heat Sealing Film (60)	60 films for sealing Rotor-Disc 100 or Rotor-Disc 72 discs	981601

Product	Contents	Cat. no.
Rotor-Disc Heat Sealing Film (600)	10 x 60 films for sealing Rotor-Disc 100 or Rotor-Disc 72 discs	981604
Rotor-Disc 72 Starter Kit	Kit includes: 3 Rotor-Disc 72 packs, Rotor-Disc Heat Sealer, Rotor-Disc Heat Sealing Film, Rotor-Disc 72 Rotor and Locking Ring, Rotor-Disc 72 Loading Block, Rotor-Disc Pipetting Aid	Inquire
Rotor-Disc 72 (24)	24 individually wrapped discs for 1728 reactions	981301
Rotor-Disc 72 (240)	10 x 24 individually wrapped discs for 17,280 reactions	981303
Rotor-Disc 72 Rotor	For holding Rotor-Disc 72 discs in the Rotor-Gene Q MDx; requires Rotor-Disc 72 Locking Ring	9018899
Rotor-Disc 72 Locking Ring	For locking a Rotor-Disc 72 in the Rotor-Disc 72 Rotor	9018900
Rotor-Disc 72 Loading Block	Aluminum block for manual and automated reaction setup in Rotor-Disc 72 discs	9018910
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Strip Tubes and Caps, 0.1 ml (2500)	10 x 250 strips of 4 tubes and caps for 10,000 reactions	981106
72-Well Rotor	For holding Strip Tubes and Caps, 0.1 ml; requires Locking Ring 72-Well Rotor	9018903

Product	Contents	Cat. no.
Locking Ring 72-Well Rotor	For locking Strip Tubes and Caps, 0.1 ml, in the 72-Well Rotor	9018904
Loading Block 72 x 0.1 ml Tubes	Aluminum block for manual reaction setup with a single-channel pipet in 72 x 0.1 ml tubes	9018901
Loading Block 72 x 0.1 ml Multi-channel	Aluminum block for reaction setup with multichannel pipets in 72 x 0.1 ml tubes	9018902
PCR Tubes, 0.2 ml (1000)	1000 thin-walled tubes for 1000 reactions	981005
PCR Tubes, 0.2 ml (10000)	10 x 1000 thin-walled tubes for 10,000 reactions	981008
36-Well Rotor	For holding PCR Tubes, 0.2 ml; requires 36-Well Rotor Locking Ring	9018907
36-Well Rotor Locking Ring	For locking PCR Tubes, 0.2 ml, in the 36-Well Rotor	9018906
Loading Block 96 x 0.2 ml Tubes	Aluminum block for manual reaction setup in a standard 8 x 12 array using 96 x 0.2 ml tubes	9018905
Rotor-Disc OTV Kit	Kit for optical temperature verification of Rotor-Gene systems, includes a Rotor-Disc preloaded with thermochromatic liquid crystals, fluorescent inserts, CD with calibration files; requires Rotor-Disc 72 Rotor and Locking Ring or Rotor-Disc 72 Starter Kit	981400

Product	Contents	Cat. no.
Rotor Holder	Metal free-standing holder for assembling tubes and Rotor-Discs into rotors	9018908

For an up-to-date list of QIAGEN kits indicated for use with the Rotor-Gene Q MDx, please refer to www.qiagen.com/products/rotor-geneqmdx.aspx.

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Appendix D

Liability clause

QIAGEN shall be released from all obligations under its warranty in the event repairs or modifications are made by persons other than its own personnel, except in cases where the Company has given its written consent to perform such repairs or modifications.

All materials replaced under this warranty will be warranted only for the duration of the original warranty period, and in no case beyond the original expiration date of original warranty unless authorized in writing by an officer of the Company. Read-out devices, interfacing devices and associated software will be warranted only for the period offered by the original manufacturer of these products. Representations and warranties made by any person, including representatives of QIAGEN, which are inconsistent or in conflict with the conditions in this warranty shall not be binding upon the Company unless produced in writing and approved by an officer of QIAGEN.

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Index

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Revision History

Revision no.	Description of change
R6 09/2018	This version has been updated to include Windows 10 as an operating system.

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Sample & Assay Technologies