BNITM
Bernhard Nocht Institute for Tropical Medicine

STANDARD OPERATING PROCEDURE

Title	Rotor-Gene® Q: programming, starting a run and analyzing data
Short title	Rotor-Gene® Q

SOP ID	B.09g
SOP type	Device SOP
Version number	V01
Effective date	16/10/2020

Written by:

Name/Title	Signature	Date
Sophie Duraffour, PhD		14/09/2020

Reviewed by:

Name/Title	Signature	Date
Ndapewa Ithete, PhD		08/10/2020
Christine Jacobsen, PhD		14/10/2020

Approved by:

Name/Title	Signature	Date
Stephan Günther, Prof.		16/10/2020

Uncontrolled when printed or photocopied without dated QA stamped "copy" and signature.

Table of Contents

1 Purpose

This Standard Operating Procedure (SOP) describes how to use the real-time polymerase chain reaction (PCR) platform Rotor-Gene® Q from Qiagen, run samples and perform data analysis. The Rotor-Gene® Q instrument is designed to perform real-time and end-point thermal cycling using PCR and high-resolution melting analysis (HRM) in molecular biology applications as well as for other applications such as concentration measurement, protein analysis, and enzyme kinetics.

2 Scope

This SOP applies to all laboratory staff involved in performing real-time polymerase chain reaction.

3 Abbreviations and definitions

Abbreviations and definitions included in alphabetical order

3.1 Abbreviations

BNITM	Bernhard Nocht Institute for Tropical Medicine
Ct	Cycle threshold
HRM	High-resolution melting analysis
IC	Internal control
ID	Identifier
INC	Inconclusive
IREP	Internal repeat
Lab ID	Laboratory identifier
N/A	Not applicable
NEG	Negative
NEG EX CTRL	Negative extraction control
NEG PCR CTRL	Negative PCR control
OTV	Optical Temperature Verification
PCR	Polymerase chain reaction
POS	Positive
POS PCR CTRL	Positive PCR control
SOP	Standard Operating Procedure
UPS	Uninterruptible power supply
VA	Volt ampere
VIR	Department of Virology

3.2 Definitions

OTV	OTV is a method that verifies the in-tube temperature in a Rotor-Gene®Q. Validation of in-tube temperature can be an important procedure in certified laboratories. OTV is performed using a Rotor-Disc OTV Kit.
Replicates	repetitions of the same sample, where amplification of a single sample template preparation is performed multiple wells (usually 3) in a PCR run.
SOP	A detailed, written instruction to archive uniformity of the performance of a specific function.
INC	An inconclusive sample: send a fresh sample to the laboratory

4 Responsibilities

Role/Title	Responsibilities/Tasks
Head of Laboratory	Release valid version of the SOP for use in the laboratory.
Quality assurance manager or other designated senior laboratory staff	Co-validation of results, troubleshooting and results reporting.
Trained laboratory personnel	Analysis and validation of results, as well as troubleshooting.

5 General description

Refer to the Rotor-Gene® Q manual for detailed description of the equipment.

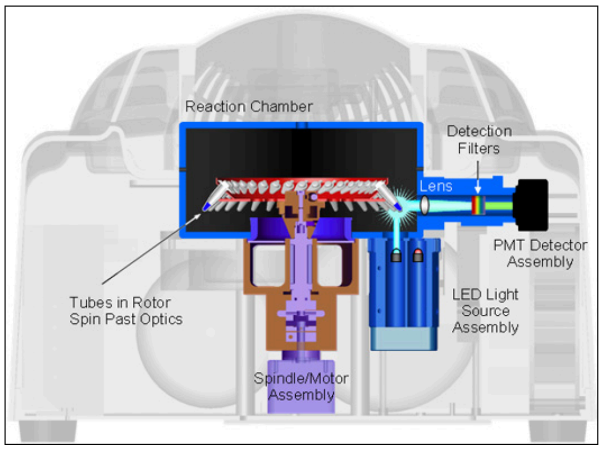


Illustration of the optical system.

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

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

Samples spin continuously 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.

With a choice of up to 6 excitation sources and 6 detection filters combined with a short, fixed optical path, the Rotor-Gene®Q 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™.

6 Device requirements

Rotor-Gene®Q instruments must be placed away from direct sunlight, away from heat sources, and away from sources of vibration and electrical interference. Refer to the user manual for more information about humidity and temperature requirements.

- The installation site should be free of excessive drafts, excessive moisture, excessive dust, and not subject to large temperature fluctuations
- Ensure that the workbench is dry, clean, and has additional space for accessories
- It is extremely important that the Rotor-Gene®Q instrument is placed on a stable surface, which is level and vibration free. The instrument must be placed within approximately 1.5 m of a properly grounded (earthed) AC power outlet.

6.1 Power requirements

The Rotor-Gene®Q operates at: 100–240 V AC, 50/60 Hz; 560 VA (peak).
Make sure that the voltage rating of the Rotor-Gene®Q is compatible with the AC voltage available at the installation site. Main supply voltage fluctuations cannot exceed 10% of nominal supply voltages.

6.2 Grounding requirements

To protect operating personnel, QIAGEN recommends that the Rotor-Gene®Q 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.

6.3 Installation of AC power cord

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

6.4 PC requirements

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

Description	Minimum requirement
Operating system	Microsoft® Windows® XP Professional edition (32 bit); Microsoft Windows 7 Professional edition (32 bit or 64 bit)*
Processor	Intel® Core™ 2 Duo T5500 1.66 GHz or better
Main memory	1 GB RAM
Hard disk space	10 GB HDD
Graphics	Adapter and screen with al least 1200 x 800 pixels
Interface	RS-232 serial port or USB port

* Microsoft Windows XP or Windows 7 Professional edition is required to run the Rotor-Gene®Q software with security features (see Section 7.9). Security features are not available if the Home edition of Windows XP or Windows 7 is used.

7 Material and equipment

7.1 General

- Rotor-Gene® Q
- Laptop connected to the Rotor-Gene® Q on which the Rotor-Gene® Q software is installed
- 36- or 72-well rotor (the 36 has red colour; 72 is blue)
- 36- or 72-well rotor locking ring
- Rotor-Gene® PCR tubes of 0.2 mL or strip tubes and caps of 0.1 mL
- Installation guide
- Compact disc (software)
- Compact disc (user manuals)
- Metallic loading block 36 for 0.2 mL tubes or 72 for 0.1 mL tubes
- Rotor holder (dismantled for safe transport)
- USB and RS-232 serial cable
- International power cable set
- Disposable gloves

7.2 Maintenance

- Rotor-Disc™ for OTV
- Absolute ethanol or isopropanol
- Cotton bud
- 50 ml flat bottom centrifuge tube
- Cover for the device and the computer

7.3 Storage

- Room temperature less than 28°C
- Rotor-Disc™ for OTV must be protected from sunlight and lengthy exposure to artificial light must be kept to a minimum.

8 Safety

When operating the Rotor-Gene® Q, always wear disposable gown and gloves.
Refer to the Rotor-Gene® Q manual for detailed safety instructions.

9 Procedural description

9.1 Start-up process

9.1.1 Functional check

Make sure you have access to UPS to ensure uninterrupted power supply during the run and make sure the device and laptop are connected to it. Switch on the laptop, then switch on the Rotor-Gene® using the switch on the back of the device. A blue light appears on the device. Open the Rotor-Gene®Q Series software by double clicking the icon on the desktop, the software is launched.

9.1.2 Calibration

N/A

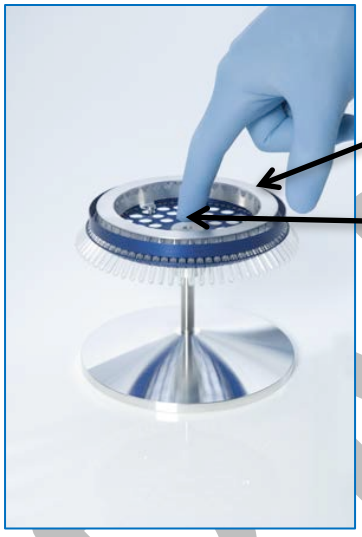
9.1.3 Validation

N/A

9.2 Place the tubes in the rotor

Once the PCR reaction set up is complete, the PCR tubes must be added to the Rotorgene according the procedures described here. The 72-well rotor is given as example in the picture below, but a similar procedure should be followed when the 36-well rotor is used.

- Make sure that PCR tubes are completely closed before placing them in the 72-well rotor, in the desired order starting from position '1' of the rotor and continue clockwise direction.
- Each position in the rotor must contain a tube: fill all positions in the rotor to ensure even airflow to every tube. To do this, keep a set of empty capped tubes available that can be used to fill any unused positions. Note: Filling the wells is important to achieve maximum temperature uniformity.
- Insert the 72-well rotor locking ring (grey) onto the 72-well rotor by pushing the locating pin through the outer holes of the rotor. The locking ring ensures that caps remain on tubes during a run (see picture below).

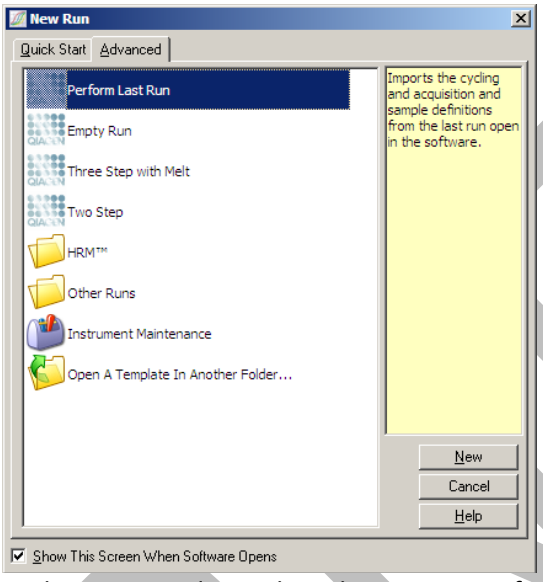


Insert the assembly into the Rotor-Gene® chamber by clicking into place using the locating pin on the rotor hub. To remove, simply push down on the rotor hub to release and pull out.

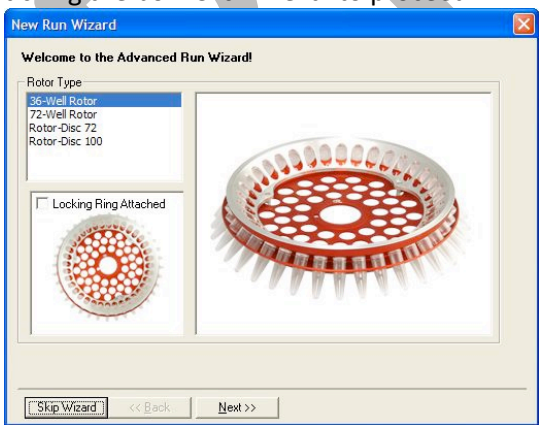
Close the lid by gently moving it forward and set up the run profile using the Rotor-Gene®Q Series Software.

9.3 Starting a run

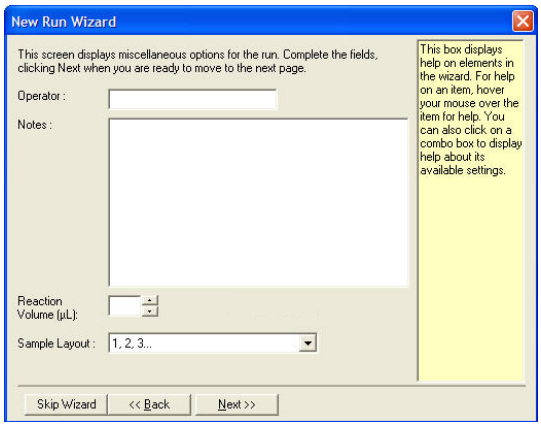
- Make sure you have access to a UPS to ensure uninterrupted power supply during the run and make sure the device and laptop are connected to it.
- Switch on the laptop, then switch on the Rotor-Gene® using the button on the back of the device. A blue light appears on the device. Open the Rotor-Gene®Q Series software by double clicking the icon on the desktop, to launch the software.
- When starting the software, you can choose between the Quick Start Wizard or the Advanced Wizard. Choose the Advanced Wizard and choose the appropriate template for the run by double-clicking on it in the list (e.g. altona LASV 2.0). To define a new template, see section below.



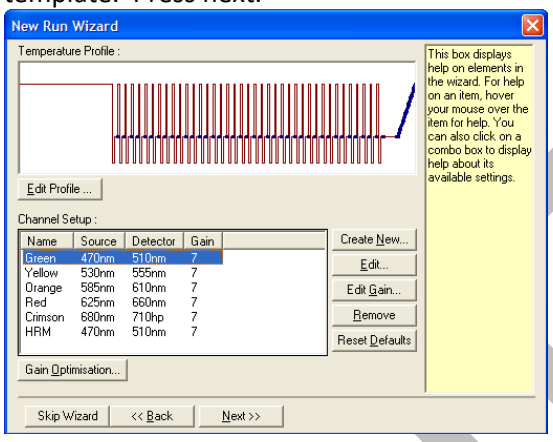
In the next window select the rotor type from the list and confirm that the locking is attached by ticking the box. Click “next” to proceed.



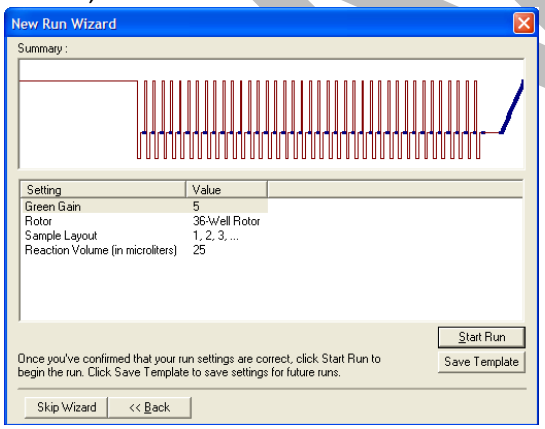
In the next window choose the reaction volume and enter the Operator’s name, then click next.



The next window shows the Temperature Profile and Channel Setup as programmed in the chosen template. Press next.



The next window summarizes all run information. Check the parameters and, when everything is correct, click “Start Run”.



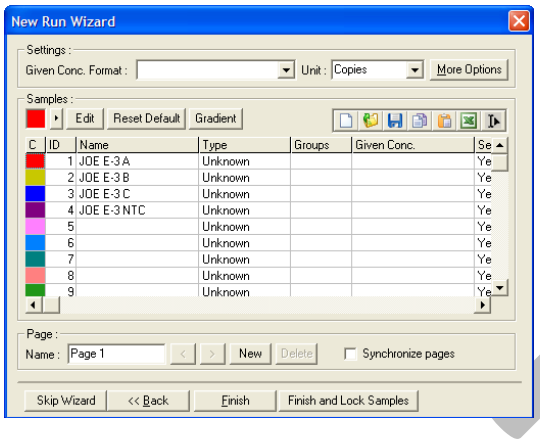
You will be prompted for a file name. Save the file in the “Rotor-Gene® Runs”-folder. Enter a unique filename. For example, a file name may be:
YYYYYMMDD_SITE_STUDYTITLE_AssayX_A–B, of which

- **YYYYMMDD**: for the date with YYYY for the year, MM for the month and DD for the day
- **SITE**: unique name of the site
- **STUDYTITLE**: unique study title

- **AssayX**: Altona_2.0_GPC or Altona_2.0_L
- **A-B**: range of sample ID with A being the lowest sample ID and B the highest sample ID

File names are included in the study-specific SOPs.

Press “Save”.
The run will start after saving the run file directly. The following pop-up window appears prompting to name the samples:



Enter the sample names while the run is in progress and click “Finish” to proceed to the run monitoring.

Note: The functionality of this window is identical to the “Edit samples” window. The information on the sample can be also updated at a later stage. Therefore, you can start the run without entering the sample ID information. Sample information may be entered during the run or after the run has finished.

9.4 Run monitoring

The run progress can be followed in real-time. On the right side of the software window, the sample(s) of interest can be selected to view their amplification plot(s). You can also see the thermal profile progress and the remaining run time.

9.5 General data analysis and validation of results

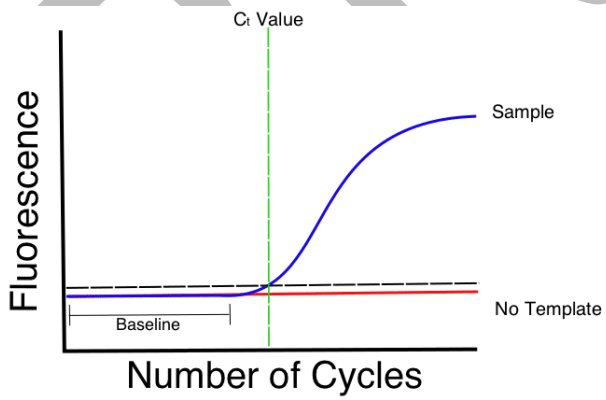
Once the run is complete, click on “Analysis” in the toolbar and the following window will appear:

- In this window the user can start new analyses and view existing ones. The method of analysis is selected using the tabs - choose “Quantitation”.
- In the “Quantitation” tab the different channels are displayed. For example, to analyze data acquired in the FAM™-channel, choose “Cycling A. Green”; for the JOE™-channel, select “Cycling A. Yellow” etc.
- Double-click on the channel(s) of interest in the list according to your PCR protocol. This will prompt the associated analysis windows as “Quantitation analysis” window (amplification plots) and the “Quantitation results” window (Ct-values) to open. The logarithmic scale of the amplification plot is displayed.

Tip: organizing the workspace for the analysis
Each time a new analysis is performed by double clicking on one channel, the windows are automatically arranged by the software to fit those already displayed 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.

9.5.1 Select the threshold

- Click on the graph of interest (e.g. Cycling A. Green) and select ‘Linear scale’ at the bottom of the window to switch the display from logarithmic scale to linear scale.
- On the right side of the screen, a threshold value can be entered. The threshold can also be manually adjusted by moving the line with the mouse on the amplification plot. Choose the threshold level so that it is placed in the middle of the sigmoidal amplification curve (see doted black line on below figure).
- To enable the comparison of PCR results across runs using the same PCR kit, the threshold value set should be identical in all run analyses.
- When a kit is being used for the first time, multiple runs should be performed to evaluate the fluctuations of the background fluorescence and to determine the best threshold average value. Refer to kit related SOPs.



It may be that certain analysis options need to be chosen to ‘flatten’ the amplification curves and/or adjust the background fluorescence (i.e. baseline) plot. Each PCR kit will have its specific analysis parameters. The analysis parameters options include the ‘Dynamic tube’, and ‘Slope correct’. See the

note below about the 'Slope correct' option. Those options are in the tabs at the top in the amplification plot(s) window (e.g. 'Cycling A. Green').

Note: Slope correction
The background fluorescence of a sample should ideally remain constant before amplification. However, sometimes the background 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 to determine the noise level instead of an average and 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 (point at which the amplification curve crosses the threshold, known as the cycle threshold or Ct value).

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.

Repeat the same process for the other channels. The threshold values and analysis parameters must be set for each channel and may be different between the channels.

Once the analysis parameters have been determined as well as the threshold value, the corresponding Ct values can be found in a table under 'Quantitation results'.

The Ct value is defined as the number of cycles needed for the fluorescence to cross the threshold (fluorescence emitted above the threshold, see figure above).

9.5.2 Validity of results

Validation of results and decision algorithm, are PCR-kit specific. Described below, are the general procedures that must be followed to confirm the validity of the results. Briefly, a 4-step analysis must be performed.

9.5.2.1 Step 1. Overall set-up and labeling

- Wear gloves before opening the device, remove the rotor and place it on the rotor holder.
- Check the volume levels in each tube, they must be identical
    - If not, troubleshoot (e.g. RNA forgotten? Pipetting errors?); write down anything unusual in the corresponding form and report to the quality assurance manager.
- Remove the locking ring
- Ensure that the tubes are positioned in the rotor according to the experiment layout and to the sample ID list entered in the computer
    - If not, troubleshoot, write down in the corresponding form and report to the quality assurance manager.
- Proceed to step 2.

9.5.2.2 Step 2. Run level

Check the results of the positive- (POS PCR CTRL) and negative PCR controls (NEG PCR CTRL) to infer on the validity of the run.

- A run is reported as either valid or invalid
- Positive and negative PCR controls have pre-defined ranges of Ct values and must be similar across runs:
    - The POS PCR CTRL must be positive, the amplification curve follows the expected shape (fluorescence height) and Ct value range (check handbook of the PCR kit in use) in the target channel. If applicable, an internal control (IC) must depict similar properties (fluorescence height and Ct value range) in the control channel.
    - The NEG PCR CTRL must be negative, no amplification curve in the target channel and, if applicable, the IC shows the correct Ct value range and fluorescence height in the control channel in the control channel.
- If the positive and negative PCR controls are as expected, the run is valid
- If the positive and negative PCR controls are not as expected, the run is invalid
    - Decide on a troubleshooting procedure with the quality assurance manager
- If the run is valid proceed to step 3
- If the run is invalid, troubleshoot with the quality assurance manager to determine what happened
    - The run likely needs repeated.

9.5.2.3 Step 3. Batch level

Check the negative extraction control (NEG EX CTRL) to verify the validity of the batch of samples (one negative extraction is processed along with samples during preparation for PCR; one NEG EX CTRL per batch)

- The NEG EX CTRL must be negative, no amplification curve in the target channel and, if applicable, the IC shows the correct Ct value range and fluorescence height in the control channel
- If the NEG EX CTRL is as expected the batch of samples is valid and the samples can be analyzed one by one
- If the batch is valid proceed to step 4
- If the batch is invalid, troubleshoot with the quality assurance manager to determine what happened:
    - The batch may need to be repeated in a new PCR run, or the sample(s) may need to be re-inactivated and re-extracted to repeat the PCR.

9.5.2.4 Step 4. Sample level

- Follow analysis and validation algorithm described in the PCR kit-specific SOPs.
- Refer to the corresponding PCR worksheet to report Ct values and final results based on kit-specific algorithm SOPs. Report the intensity of the amplification curve, which can be reported as either “high” (H) or “low” (L). “L” is defined as a fluorescence intensity level less than half of the fluorescence level of the positive PCR control.
- A sample can be positive (POS), negative (NEG), inconclusive (INC) or internal repeat (INREP). An internal repeat is reported on the form as intermediate result when the result is inconclusive but can be repeated internally to troubleshoot it; a INREP result will not be

reported as the final result. Once the INREP is performed, the sample should be classified as POS, NEG or INC.

Critical eye about the shape of the amplification curves:

- Check the shape of the amplification curve, it should be sigmoid. You can sometimes observe that the baseline crosses the threshold with a straight angle, this is not an amplification curve.
- Open the Rotor-Gene® and verify whether the volume in the tube is correct, if the tube is well positioned in the rotor and/or tube is closed (compared with the others). Alternatively, a dust particle at the bottom of the tube could be the reason for such a curve.
- The PCR needs to be repeated for that sample and troubleshooting may therefore be required. The algorithm used for the validation of a run is described in the PCR-kit-specific SOPs.

9.5.3 Result's reporting and documentation

A sample is reported as:

- Positive (POS)
- Negative (NEG)
- Inconclusive (INC): it means a fresh sample must be requested as it is impossible to get a final POS or NEG result from the current sample

A "four eyes" principle for result's reporting is applied with the designated senior lab staff or quality assurance manager to co-validate all PCR results.

9.6 Saving results and switching off

To close the run and switch off the device:

- The run is automatically saved from the moment it was started
- To save the analysis parameters, click on save
    - If this is not done, the analysis parameters will not be saved
- Close the software
- Switch off the device
    - Do not switch off the instrument before closing the software
- Turn off the computer
- Cover the computer and the device

9.7 Analysis in virtual mode

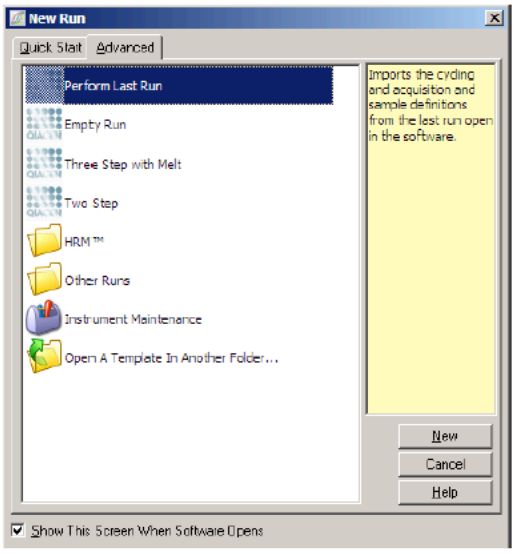
It is possible to analyze or re-analyze results in virtual mode which means that the computer is not connected to the instrument (the instrument is off).

- Launch the Rotor-Gene® Q Series software by double clicking the icon on the desktop, the software is launched
- Choose "run in virtual mode"
- Open the run to analyze
- Any new analysis parameters will overwrite the run file if you click on save!
    - It is recommended to not do that as the file will be saved with the day of use. If it is a historical file, it will not be possible to organize the files by date of run performed.
- Switch off as described in 9.6

9.8 Create a cycling profile

Creating a cycling profile is detailed in the Rotor-Gene® user manual. Briefly, follow the procedure until 9.3 where you access the “Advanced wizard” tab. Click on this tab, it will allow the operator to configure a cycling profile.

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:

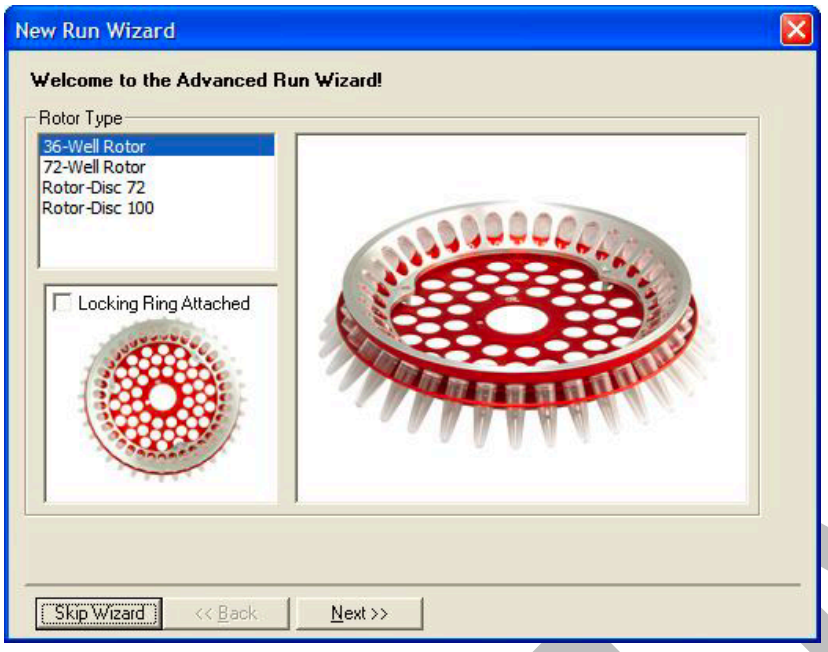


Perform Last Run: it imports the cycling, acquisition, and sample definitions from the last run open in the software.
Empty Run: this is an empty run which allows the user to define all parameters of the profile. This option is thus chosen to create a cycling profile.
Three Step with Melt: this is a three-step cycling profile and a melt curve with data acquisition in 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.

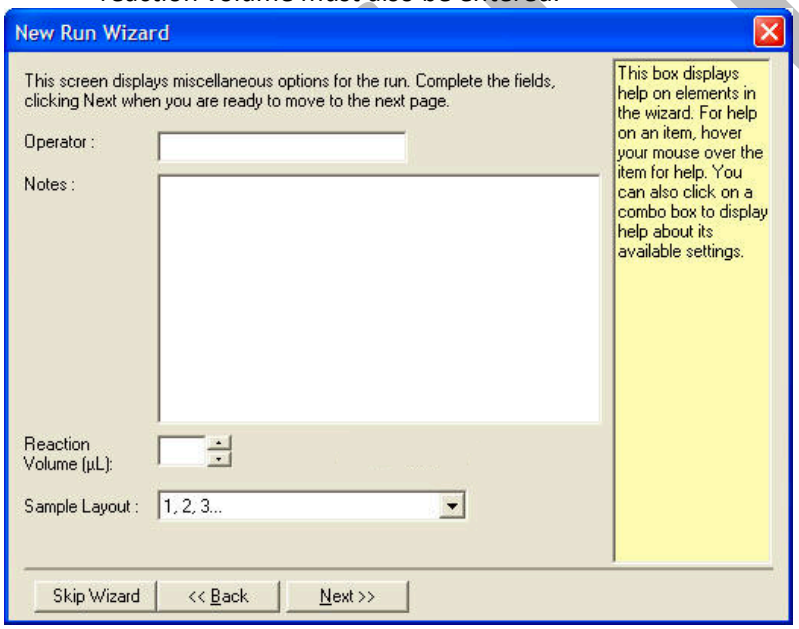
Note: User-defined templates can be added to the template list by copying or saving *.ret files to C:\Program Files\Rotor-Gene®Q Software\Templates\. After copying a file to this path, the template will appear as an icon in the list.

A new cycling profile can be created as follows:

- Choose “Empty Run”
- In the next window, select the rotor type from the list.

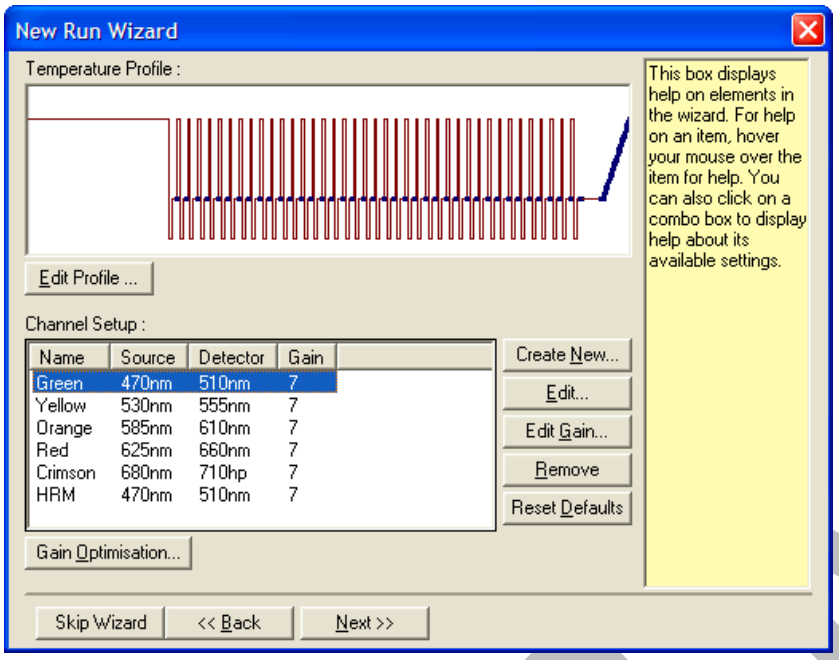


- Check the “Locking Ring Attached” checkbox
- Click “Next”
- In the next window, the operator’s name and notes about the run can be entered. The reaction volume must also be entered.

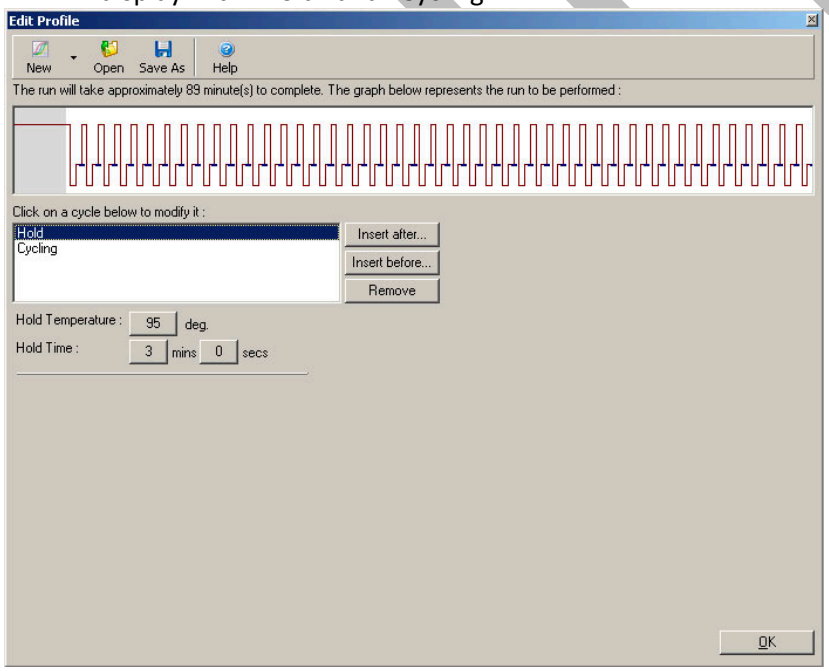


If the 72-Well Rotor was selected in window 1, three “Sample Layout” options are available in the drop-down menu. “1, 2, 3...” is the default option. Most users select this option. “1A, 1B, 1C...” should be selected when samples were loaded in adjacent 0.1 ml strip tubes using a multichannel pipette with 8 channels. The “A1, A2, A3...” layout may be selected if appropriate.

- Click “Next”



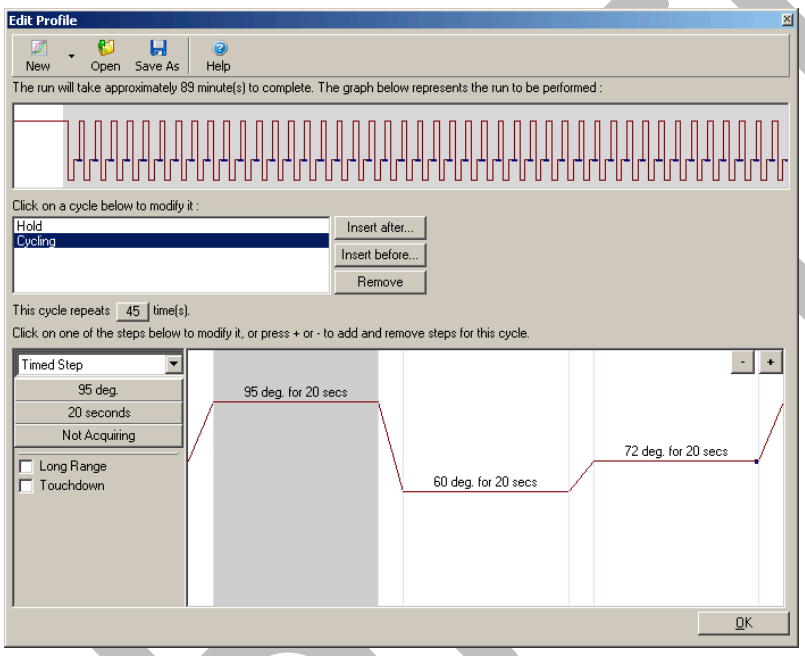
- In this window, the “Temperature Profile” and “Channel Setup” can be modified.
- Click on the “Edit Profile...” button to set-up cycling conditions and to select acquisition channels.
- The initial profile shown is based on the template selected (here, “Empty Run”). The profile is displayed graphically. The list of the segments of the profile appears below the graphical display with “Hold” and “Cycling”.



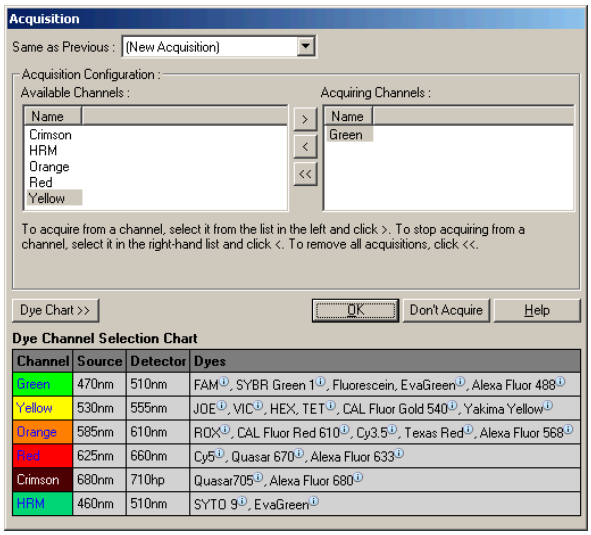
- Each stage of the profile can be edited by clicking on the appropriate area of the graphical display or on the name in the list, and then change the settings which appear.
    - **Insert after...**: This allows addition of a new cycle after the selected cycle.

- **Insert before...**: This allows addition of a new cycle before the selected cycle.
- **Remove**: This removes the selected cycle from the profile

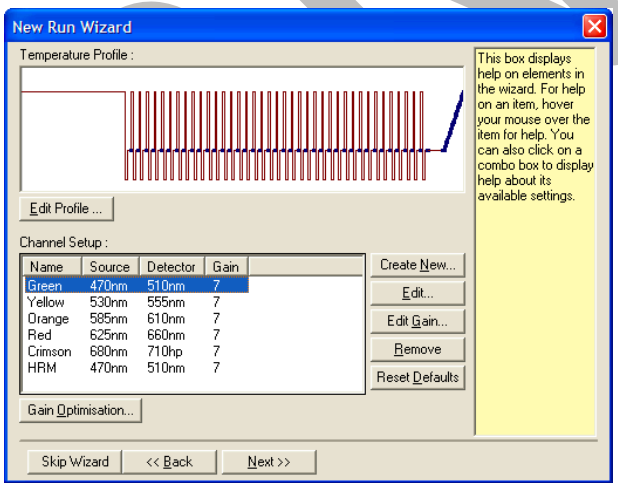
- A “Hold” instructs the device to remain at the designated temperature for a set time. To change the temperature, click on the “Hold Temperature” button and type or use the slide bar to select the desired temperature. To change the duration of the Hold, click on the “Hold Time”, “mins”, and “secs” buttons.
- “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.



- Acquisition: data can be acquired in any channel at any cycling step. To set a channel to acquire data, click on the “Not Acquiring” button at the correct cycling step (if a channel has already been set to acquire at this step, then the acquiring channels are listed here).
- The “Acquisition” window appears:



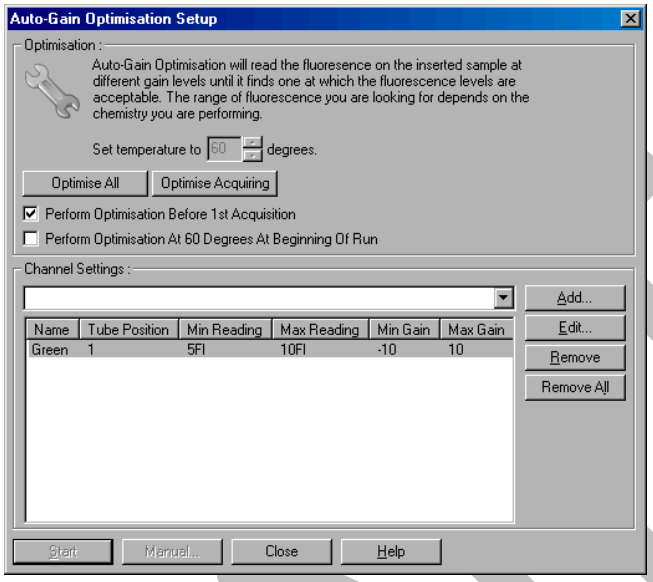
- To set a channel to acquire, select the channel and move it from the “Available Channels” list to the “Acquiring Channels” list using the “>” button. To remove a selected channel from the “Acquiring Channels” list, use the “<” button. The button “<<” removes all the channels from the “Acquiring Channels” 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.
- Once channels have been selected, click “OK”
- The “New Run Wizard” window with the selected cycling conditions is prompted:



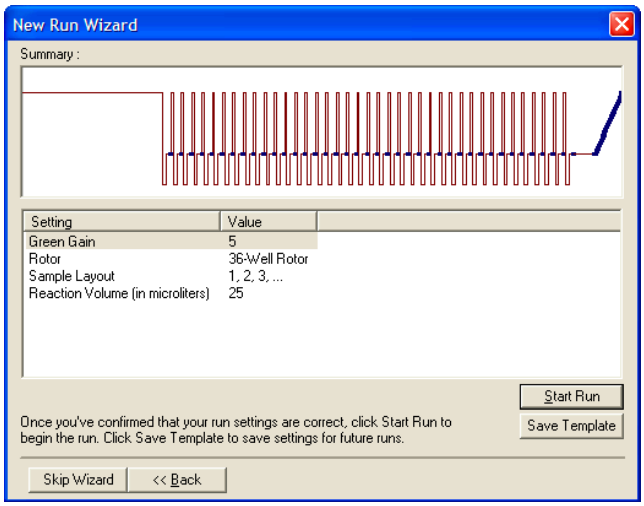
- Gain Optimisation: when setting up a new run, it is helpful to use the “Gain Optimisation” function. This allows you to optimize the gain to a setting that will provide the desired range of starting fluorescence at a set temperature (usually the temperature at which data

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).

- Auto-Gain Optimisation is recommended. If another option is chosen, refer to the Rotor-Gene®Q manual.
- Click on the “Gain Optimisation…” button
- Choose “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.



- In the “Channel Settings” drop-down menu, choose the channel(s) of interest and click “Add”.
    - We recommend always performing the gain optimization on the tube displaying all the fluorescence expected from the kit. For example, a positive PCR control tube will for sure depict the expected fluorescence of the target and thus is always set-up at position “1” of each run as position “1”, as indicated in the above picture, is the tube used for gain optimization.
- Keep the automatic parameters with the above default values for “Min Reading”, “Max Reading”, “Min Gain” and “Max Gain”.
- Click “Close”
- A summary window of the run is prompted



- Check the parameters and if they are correct, click “Save Template”. You will be prompted for a file name so that the run settings are saved for future runs.
    - Save with a unique name as stipulated in the study manual.

10 Waste management

After a PCR run, wear gloves and dispose the used consumables and plasticware which may contain hazardous chemicals and infectious materials in designated waste bags. Waste bag must be sealed when full, collected and disposed of properly according to local safety regulations.

11 Maintenance

11.1 Internal

11.1.1 Daily

- Cover – not recorded

Cover the Rotor-Gene® overnight or when not in use for longer periods of time, to prevent any pollution with dust or other particles.

- Cleaning the Rotor-Gene® – not recorded

When used regularly, the rotor may not click in properly on the ring. This is because the ring connection, both in the machine and on the rotor holder, becomes dirty. To clean it, dunk a cotton swab into isopropanol or absolute ethanol, dry it a bit on a tissue so that it is not completely soaked, and carefully clean the ring connection parts. Move the ring up and down several times so that the ethanol-based solution cleans the side beads. The lenses of the Rotor-Gene® can also be cleaned with this cotton swab dunked in isopropanol. Do not use any other chemicals to clean the Rotor-Gene®Q.

11.1.2 Monthly

- OTV-Kit - recorded

To verify the thermal accuracy or the Rotor-Gene® cycler, run the OTV Kit every two months. For detailed information please refer to the Rotor-Disc™ OTV Handbook and associated SOP.

11.2 External

N/A

12 Troubleshooting

In the event of troubleshooting, refer to the Rotor-Gene® user manual. If it cannot be solved using the procedures described in the troubleshooting section of the manual, the log archive file can be sent to Qiagen technical services (check your in-country contact support).

Note: The software keeps an unmodified record of each run, along with diagnostic information, in its log archive repository. 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.

Troubleshooting linked to run interpretation is addressed in PCR-kit-specific-SOPs.

13 References

N/A

14 Associated Documents

14.1 Tools associated with this SOP

Device SOP:
OTV Kit (optical temperature verification)

Form OTV runs

14.2 Other documents associated with this SOP

Rotor-Gene® Q manual
Rotor-Disc™ OTV Handbook

15 Document History

Review date	Version number	Brief description of changes
N/A	V01	First release