March 2012

Rotor-Gene® Type-it® HRM® Discovery Handbook

For demonstration and evaluation of successful HRM-based genotyping analysis on the Rotor-Gene Q

Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents **4**
Storage **4**
Intended Use **4**
Safety Information **5**
Quality Control **5**
Introduction **6**
Principle and procedure 6
Class IV SNP assay 7
Deletion assay 8
Description of protocols 9
Equipment and Reagents to Be Supplied by User **10**
Protocols
■ **1: Manual Reaction Setup** **11**
■ **2: Automated Reaction Setup Using the QIAgility** **14**
■ **3: PCR and HRM analysis on the Rotor-Gene Q** **16**
Troubleshooting Guide **22**
Ordering Information **25**

Kit Contents

Rotor-Gene Type-it HRM Discovery Kit	
Catalog no.	**206541**
Number of 25 µl reactions	**56***
2x HRM PCR Master Mix	1.3 ml
10 µM Class IV SNP Primer Mix†	0.1 ml
10 µM Deletion Primer Mix†	0.1 ml
Class IV SNP Standards 1–3‡	1 tube each (yellow caps)
Class IV SNP Unknown Samples 4–5‡	1 tube each (yellow caps)
Deletion Standards 6–9‡	1 tube each (red caps)
Deletion Unknown Samples 10–12‡	1 tube each (red caps)
RNase-Free Water	1.9 ml
Protocol leaflet	1

* Kit contains template DNA, HRM PCR master mix, and primer mixes for 56 reactions; for use with a 72-well rotor.

† Contains a mix of forward and reverse primers (10 µM each).

‡ All standards and unknown samples for the SNP and deletion assays provide 1 x 10^5 copies of target DNA per reaction.

Storage

The Rotor-Gene Type-it HRM Discovery Kit is shipped on dry ice. The kit should be stored immediately upon receipt at –20°C in a constant-temperature freezer and protected from light. When the kit is stored under these conditions and handled correctly, performance is guaranteed until the expiration date (see the Quality Control label inside the kit box). The 2x HRM PCR Master Mix can also be stored at 2–8°C (protected from light exposure) for up to 2 months without showing any reduction in performance.

When stored under these conditions and handled correctly, the kit can be kept for at least 18 months from date of receipt without reduction in performance.

Intended Use

The Rotor-Gene Type-it HRM Discovery Kit is intended for molecular biology applications. This kit is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of Rotor-Gene Type-it HRM Discovery Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

High-resolution melting (HRM) analysis is an innovative technique that is based on the 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.

Before performing HRM analysis, the target sequence must be amplified to a high copy number in the presence of the dsDNA-binding fluorescent dye, EvaGreen®. The dye does not interact with ssDNA but actively binds to dsDNA and fluoresces brightly when bound. Change in fluorescence can be used to measure the increase in DNA concentration during PCR and to directly measure thermally-induced DNA melting during HRM.

To perform high-resolution melting analysis, the temperature is increased from a lower to a higher temperature. The fluorescence of EvaGreen is measured continuously as the temperature is increased, and is plotted against the temperature. EvaGreen fluoresces as long as it is bound to dsDNA. Due to the amplification procedure before HRM analysis, fluorescence is high at the start of the HRM analysis. Upon melting of dsDNA, EvaGreen is released and the fluorescence is reduced to a background level.

HRM is easier and more cost-effective than probe-based genotyping assays, and unlike conventional methods, it is a closed-tube system that reduces the potential for contamination with post-PCR products.

The Rotor-Gene Type-it HRM Discovery Kit has been developed to demonstrate the high performance of Type-it HRM chemistry in combination with the advanced thermal and optical properties of Rotor-Gene Q, for detection of gene mutations and SNPs. With the Rotor-Gene Type-it HRM Discovery Kit, the reliability and reproducibility of HRM analysis with Rotor-Gene technologies is thoroughly evaluated.*

Manual pipetting steps can be avoided by using the QIAgility®, a compact bench-top instrument that provides rapid, high-precision PCR setup. Errors introduced with manual reaction setup can be minimized and may be eliminated. The QIAgility perfectly complements the Rotor-Gene Q and Rotor-Gene Kits, enabling easy dispensing of liquids into tubes, strip tubes, and Rotor-Discs®.

To learn more, visit www.qiagen.com/goto/Rotor-GeneQ.

Principle and procedure

The Rotor-Gene Type-it HRM Discovery Kit enables detection of a 9 bp deletion and a class IV (A to T transversion) SNP via high-resolution melting analysis. The kit includes everything required to successfully perform analysis of these

* The kit can also be used with the Rotor-Gene 6000.

typical, but challenging genotyping assays using the supplied standards (3 for SNP assay and 4 for the deletion assay) and the supplied unknown samples (2 for the SNP assay and 3 for the deletion assay).

The master mix provided with the Rotor-Gene Type-it HRM Discovery Kit contains the novel double-stranded DNA-binding fluorescent dye, EvaGreen, and includes an optimized HRM buffer and HotStarTaq® *Plus* DNA Polymerase. Together, these components eliminate nonspecific amplification and provide reliable results. Q-Solution®, a PCR additive included in the master mix, ensures specific amplification of difficult genomic loci, leading to successful results.

The Rotor-Gene Type-it HRM Discovery Kit provides two types of HRM assays: a class IV SNP transversion mutation assay (Figure 1) and a 9 bp deletion assay (Figure 2).

Note: The overall number of reactions that can be performed using the Rotor-Gene Type-it HRM Discovery Kit is 56:

5 x 4 reactions for the class IV SNP assay

4 no template controls for the class IV SNP assay

7 x 4 reactions for the deletion assay

4 no template controls for the deletion assay

All reactions are run simultaneously on the Rotor-Gene Q.

Class IV SNP assay

Each reaction consists of:

- DNA template (standards and unknown samples)
- 2x HRM PCR Master Mix
- Primer mix specific for the class IV SNP assay
- RNase-free water

Three standards, representing different genotypes (wild-type, mutant, and heterozygous), are used to determine the genotypes of two unknown samples. The standard and unknown sample templates are provided at a concentration of 1×10^5 copies per reaction. All templates are processed in quadruplicates — 5 x 4 reactions for the class IV SNP assay, plus 4 no template controls. In total, 24 reactions are analyzed to evaluate the class IV SNP assay. The expected result is shown in Figure 1.

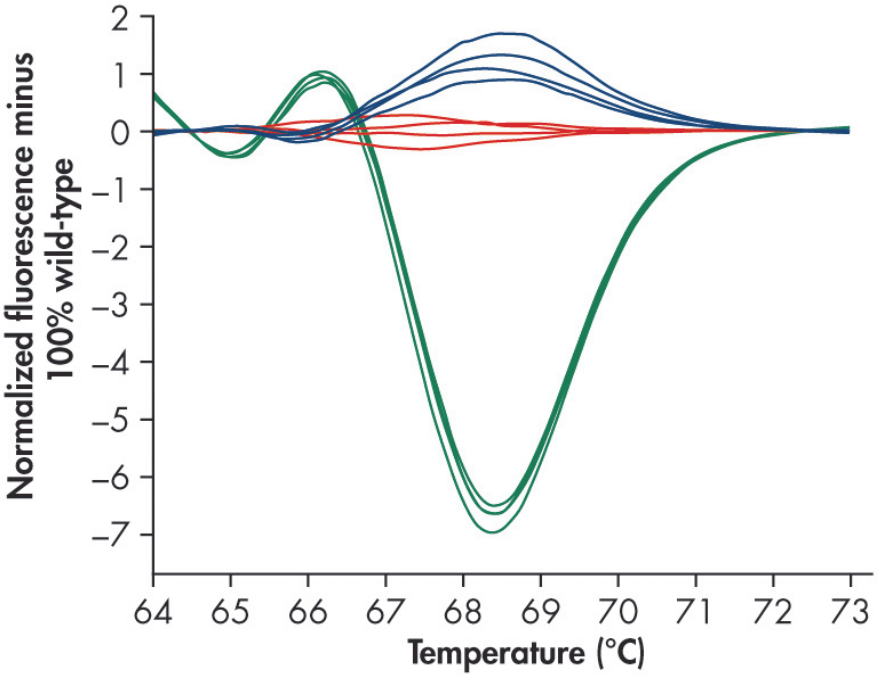

Figure 1. Class IV SNP transversion mutation assay. Red: WT (sample 1); **blue**: mutant (sample 2); **green**: heterozygous (sample 3).

Deletion assay

Each reaction consists of:

- DNA template (standards and unknown samples)
- 2x HRM PCR Master Mix
- Primer mix specific for the deletion assay
- RNase-free water

Four standards, representing different genotypes (wild-type, 100% mutant, 50% mutant, and 20% mutant) are used to determine the genotypes of three unknown samples. The standard and unknown sample templates are provided at a concentration of 1 x 10^5 copies per reaction. All templates are processed in quadruplicates — 7 x 4 reactions for the deletion assay, plus 4 no template controls. In total, 32 reactions are analyzed to evaluate the deletion assay. The expected result is shown in Figure 2.

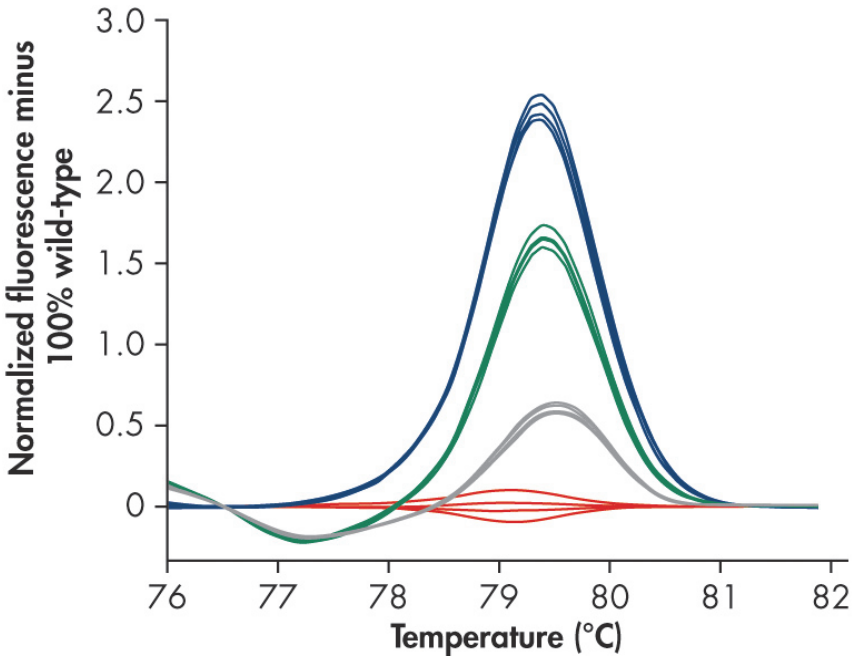

Figure 2. The 9 bp deletion assay. Red: WT (sample 6); **blue**: 100% mutant (sample 7); **green**: 50% mutant (sample 8); **grey**: 20% mutant (sample 9).

Description of protocols

This handbook contains 3 protocols.

- Protocol 1: Manual Reaction Setup (page 11)
- Protocol 2: Automated Reaction Setup Using the QIAgility (page 14)
- Protocol 3: PCR and HRM analysis on the Rotor-Gene Q (page 16)

Follow either the protocol for manual reaction setup (page 11) or the protocol for automated reaction setup using the QIAgility (page 14), and then proceed to the protocol for PCR and HRM analysis on the Rotor-Gene Q (page 16).

Equipment and Reagents to Be Supplied by User

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

For manual reaction setup

- Strip tubes and caps, 0.1 ml (cat. no. 981103); 18 strips are required
- Micro tube 1.5 ml Safety Cap (cat. no. 72.690 www.sarstedt.com)

For automated reaction setup using the QIAgility

- Adapter, 72 x 0.1 ml Strip Tubes (cat. no. 9018917)
- 50 µl Conductive Filtered Tips (cat. no. 990512); at least 64 tips are required
- 200 µl Conductive Filtered Tips (cat. no. 990522); at least 8 tips are required
- Tip Receptacle Box (cat. no. 990550)
- Strip Tubes and Caps, 0.1 ml (cat. no. 981103); 18 strips are required
- Micro tube 1.5 ml Safety Cap (cat. no. 72.690; see www.sarstedt.com)

For PCR and HRM analysis on the Rotor-Gene Q

- 72-Well Rotor (cat. no. 9018903)
- Locking Ring 72-Well Rotor (cat. no. 9018904)

Protocol 1: Manual Reaction Setup

This protocol describes how to set up PCR reactions manually. After reaction setup, proceed to "Protocol 3: PCR and HRM analysis on the Rotor-Gene Q" on page 16 to perform PCR and HRM analysis on the Rotor-Gene Q.

Important points before starting

- Always use the cycling conditions specified in this protocol.
- Yellow-capped tubes contain templates for the class IV SNP assay and red-capped tubes contain templates for the deletion assay.

Procedure

1. **Thaw the 2x HRM PCR Master Mix, standards, unknown samples, primer mixes, and RNase-free water.**

   Note: To avoid localized concentrations of salt and to ensure homogeneity, mix all solutions well before use by vortexing and briefly centrifuging them.

2. **Prepare a reaction mix according to Table 1 (class IV SNP assay) or Table 3 (deletion assay).**

   Due to the hot start, it is not necessary to keep samples on ice during reaction setup or while programming the Rotor-Gene cycler.

   Note: See Table 1 to set up the class IV SNP assay. In this experiment, 24 reactions of 25 µl volume each will be run for the class IV SNP assay (quadruplicate reactions for 3 standards, 2 unknown samples, and one NTC). As some reaction mix may be lost during pipetting, 25 µl volumes of reaction mix for 26 reactions should be prepared.

   Note: See Table 3 to set up the deletion assay. In this experiment, 32 reactions of 25 µl volume each will be run for the deletion assay (quadruplicate reactions for 4 standards, 3 unknown samples, and one NTC). As some reaction mix may be lost during pipetting, 25 µl volumes of reaction mix for 34 reactions should be prepared.

3. **Mix the reaction thoroughly, and dispense 20 µl volumes into PCR tubes.**

   Use six 0.1 ml strip tubes and caps if analyzing the SNP and eight 0.1 strip tubes and caps if analyzing the deletion.

   Note: Each strip contains 4 tubes.

4. **Add 5 µl DNA (standards or unknown samples) to each PCR tube. For the no template control (NTC), add RNase-free water instead.**

   IMPORTANT: When analyzing the class IV SNP assay, follow the pipetting scheme outlined in Table 2. When analyzing the deletion assay, follow the pipetting scheme outlined in Table 4.

5. **After reaction setup, proceed to 'Protocol 3: PCR and HRM analysis on the Rotor-Gene Q' (page 16).**

Table 1. Manual reaction setup for the class IV SNP assay

Component	Volume/25 µl reaction	Volume/520 µl reaction*	Final concentration
2x HRM PCR Master Mix	12.5 µl	325 µl	1x
Class IV SNP Primer Mix	1.75 µl	45.5 µl	0.7 µM
RNase-free water	5.75 µl	149.5 µl	–
DNA (unknown samples or standards; added at step 4)	5 µl	–	1 x 10^5 copies

* A 520 µl reaction mix is equivalent to 26 reactions comprising 25 µl each.

Table 2. Recommended pipetting scheme for the class IV SNP assay

Reaction number	Template
1–4	Standard: Class IV SNP Template DNA WT (Tube 1)
5–8	Standard: Class IV SNP Template DNA Mut (Tube 2)
9–12	Standard: Class IV SNP Template DNA Het (Tube 3)
13–16	Unknown: Class IV SNP Template DNA UK1 (Tube 4)
17–20	Unknown: Class IV SNP Template DNA UK2 (Tube 5)
21–24	NTC†

† For the NTC, add 5 µl RNase-free water to 20 µl reaction mix.

Table 3. Manual reaction setup for the deletion assay

Component	Volume/25 µl reaction	Volume/680 µl reaction*	Final concentration
2x HRM PCR Master Mix	12.5 µl	425 µl	1x
Deletion Primer Mix	1.75 µl	59.5 µl	0.7 µM
RNase-free water	5.75 µl	195.5 µl	–
DNA (unknown samples or standards; added at step 4)	5 µl	–	1 x 10^5 copies

* A 680 µl reaction mix is equivalent to 34 reactions comprising 25 µl each.

Table 4. Recommended pipetting scheme for the deletion assay

Reaction number	Template
25–28	Standard: Deletion Template DNA WT (Tube 6)
29–32	Standard: Deletion Template DNA Mut (Tube 7)
33–36	Standard: Deletion Template DNA 50% Mut (Tube 8)
37–40	Standard: Deletion Template DNA 20% Mut (Tube 9)
41–44	Unknown: Deletion Template DNA UK1 (Tube 10)
45–48	Unknown: Deletion Template DNA UK2 (Tube 11)
49–52	Unknown: Deletion Template DNA UK3 (Tube 12)
53–56	NTC†

† For the NTC, add 5 µl RNase-free water to 20 µl reaction mix.

Protocol 2: Automated Reaction Setup Using the QIAgility

This protocol describes how to automate setup of 56 PCR reactions using the QIAgility, which takes about 23 minutes. After reaction setup, proceed to "Protocol 3: PCR and HRM analysis on the Rotor-Gene Q" on page 16 to perform PCR and HRM analysis on the Rotor-Gene Q.

Important points before starting

- Always use the cycling conditions specified in this protocol.
- Yellow-capped tubes contain templates for the class IV SNP assay and red-capped tubes contain templates for the deletion assay.
- Please refer to the *QIAgility User Manual* before operating the QIAgility.

Procedure

1. **Thaw the 2x HRM PCR Master Mix, standards, unknown samples, primer mixes, and RNase-free water.**

   To avoid localized concentrations of salt and to ensure homogeneity, mix all reagents well before use by vortexing and briefly centrifuging them.

2. **Double click on the "QIAgility" icon on the desktop to start the QIAgility Software.**

   Note: Before starting the software, ensure that the instrument hood is closed and that the QIAgility is switched on.

3. **Click on the "Protocols" tab to display a list of Q Protocols. Click on "Rotor-Gene Type-it HRM Discovery Kit" to select it, and then click on the "Open" button. Alternatively, double-click on "Rotor-Gene Type-it HRM PCR Discovery Kit" to open it directly.**

   Note: Q Protocols have to be installed in the QIAgility software before use. Find out how to at:
   http://www.qiagen.com/products/QIAgility.aspx#Tabs=t5
   Guidelines for installing Q Protocols - English (PDF)

4. **A description of the Q Protocol will appear. Review the description and then click on the "Close" button.**

5. **Select "Wizards/Generate report" to view the pre-run report. Open the instrument hood, and prepare the worktable as described in the report.**

   Note: If using consumables other than those specified in the pre-run report, the Q Protocol may need to be adjusted accordingly to prevent errors in reaction setup. Ensure that the correct number of tips is selected.

6. **Close the instrument hood, and select "Control/Start". Click "Cancel" when asked to save the file. The pre-run "Checklist" dialog box will appear.**

   Note: Ensure that the tip receptacle box has sufficient space to accommodate additional used tips that will be produced from the run. It is recommended to empty the tip receptacle box before each run.

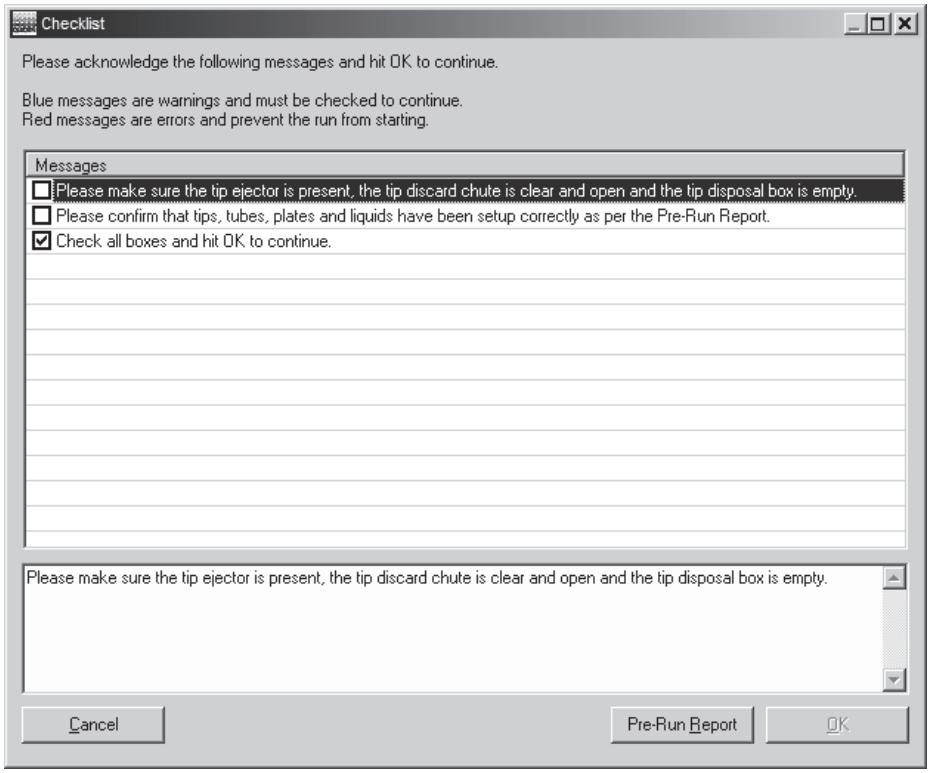

Figure 3. Pre-run checklist.

7. **If the run has been set up correctly, the checklist will not list any warnings or errors other than those listed above. If errors are listed, user intervention is required before the run can be started. Select the boxes next to the warnings to continue (Figure 3).**
8. **If the worktable is correctly set up, click on the "OK" button to start the run.**

   Note: The location of the pipetting head will be highlighted on the software worktable in real time, and a summary of the progress of the run will be displayed in the right-hand pane. Reaction setup will be completed in 23 min (if the tip reuse option is set to 8 times).
9. **After reaction setup, proceed to 'Protocol 3: PCR and HRM analysis on the Rotor-Gene Q' (page 16).**

Protocol 3: PCR and HRM analysis on the Rotor-Gene Q

Before starting this protocol, set up reactions manually (see "Protocol 1: Manual Reaction Setup" on page 11) or using the QIAgility (see "Protocol 2: Automated Reaction Setup Using the QIAgility" on page 14)

Important point before starting

- Please refer to the *Rotor-Gene Q User Manual* before operating the Rotor-Gene Q.

Procedure

1. **Close the PCR tubes containing the reactions. Place the 24 tubes for the SNP assay and the 32 tubes for the deletion assay in the 72-well rotor in the Rotor-Gene cycler, and attach the locking ring.**

   Note: Empty positions in the 72-well rotor should be filled with empty PCR tubes.

2. **Open the ready-made protocol file "Rotor-Gene Type-it HRM Discovery Kit.ret" by double-clicking on it. Alternatively, open the Rotor-Gene Q software and select the "HRM" folder in the advanced tab of the wizard. Choose the protocol folder entitled "HRM with Pre-Amplification" and click on "New" (Figure 4).**

   Note: The ready-made protocol file can be downloaded from www.qiagen.com.

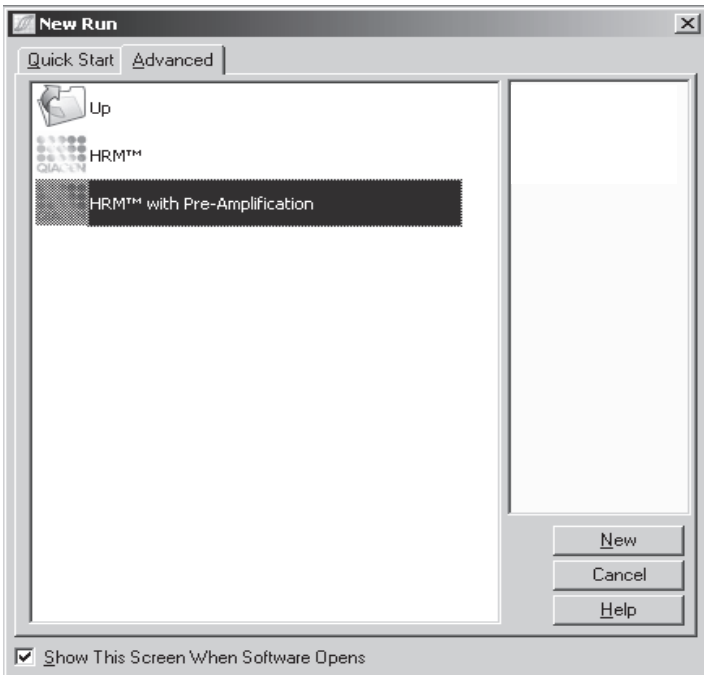

Figure 4. Choosing the protocol file.

3. **Select "72-Well Rotor", and confirm that you have attached the locking ring by checking the box. Click "Next" to continue (Figure 5).**

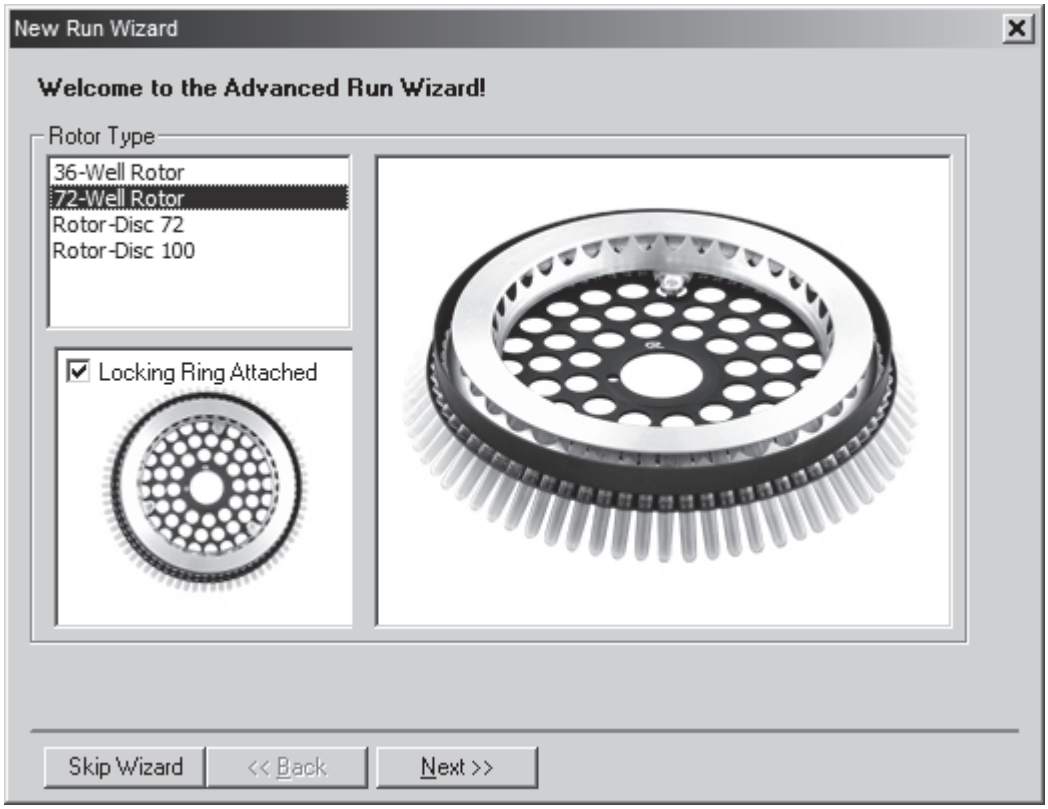

Figure 5. Selecting the rotor.

4. **Ensure that the reaction volume is set to 25 µl. Click "Next" to continue (Figure 6).**

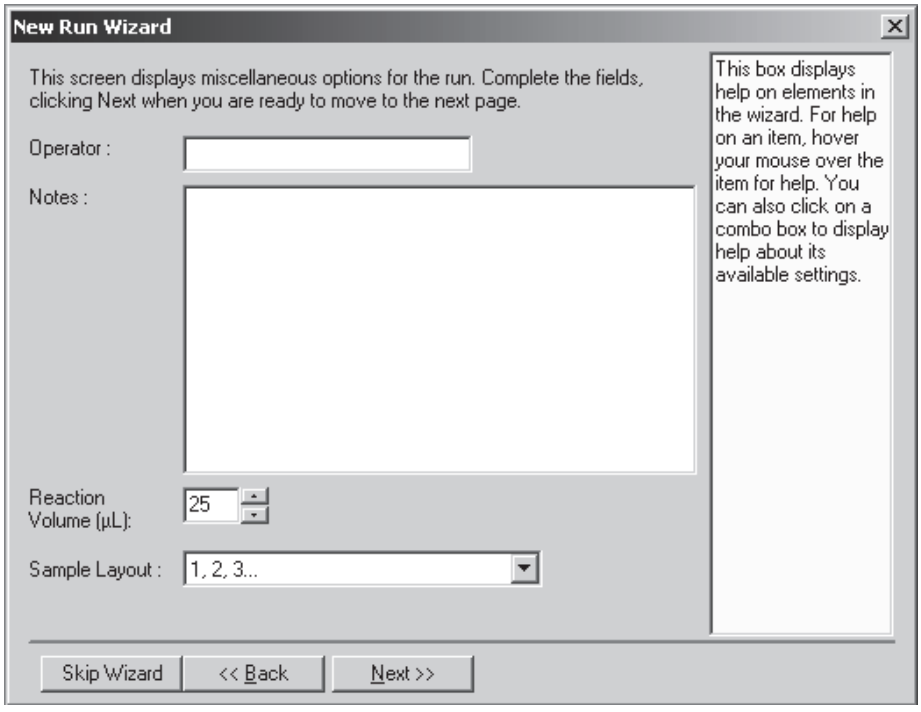

Figure 6. Selecting the reaction volume.

5. **Click on "Edit Profile", and program the Rotor-Gene Q according to the program outlined in Table 5 and Figures 7–9 (pages 20–21).**

   Note: Data acquisition should be performed during the combined annealing/extension step.

6. **Click "Next" to confirm the temperature profile and channel setup, and check the summary to ensure all parameters are correct. Start the Rotor-Gene Q by clicking "Start run". You will be prompted to enter a file name and to save the run file.**
7. **After the run has started, click on "Finish".**

   Note: Windows displaying raw data, temperature, and progress profile will appear.

Table 5. Cycling protocol for use with the Rotor-Gene Q

Step	Time	Temperature	Additional comments
Initial activation step	5 min	95°C	HotStarTaq *Plus* DNA Polymerase is activated by this heating step
2-step cycling:			
■ Denaturation:	10 s	95°C	
■ Annealing/extension:	30 s	55°C	Perform fluorescence data collection using the "Green" channel with auto-gain optimization.
Number of cycles:			45
HRM	2 s	60–85°C; increments of 0.1°C **T_m of SNP assay**: ~68°C Minimal recommended melting range: 63–73°C **T_m of deletion assay**: ~79°C Minimal recommended melting range: 74–84°C	Optimize gain before melt on all tubes (the gain giving the highest fluorescence less than 95 will be selected).

Note: Overall time for PCR and HRM is as follows:

PCR cycling: 81 min

HRM (60–85°C) for both the class IV SNP assay and the deletion assay: 44 min

HRM (63–73°C) for only the class IV SNP assay: 19 min

HRM (74–84°C) for only the deletion assay: 19 min

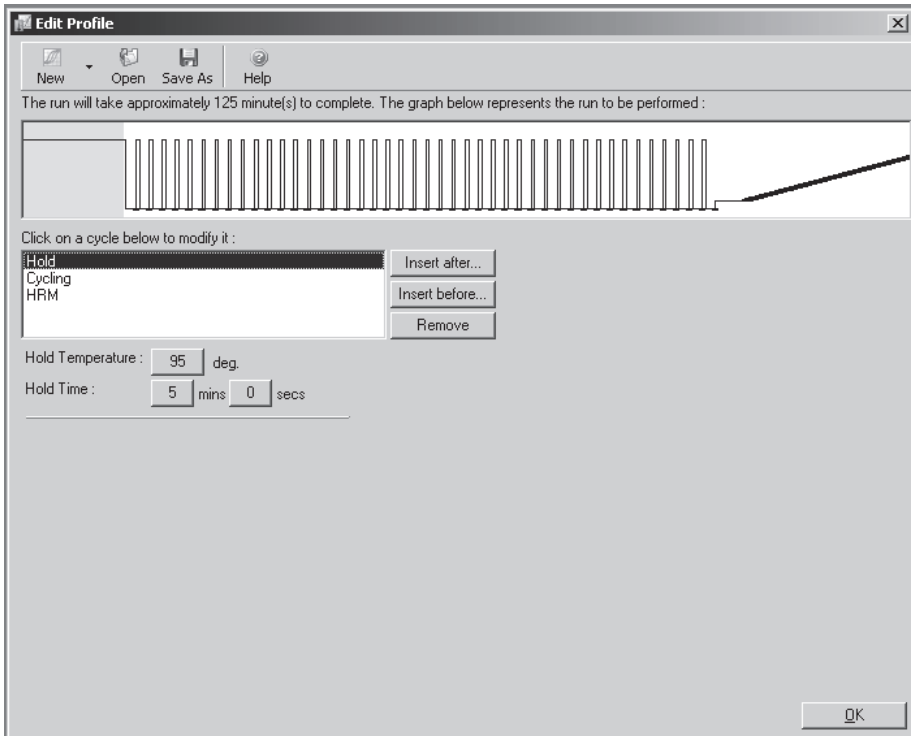

Figure 7. Initial activation step. PCR requires an initial incubation at 95°C for 5 min to activate HotStarTaq *Plus* DNA Polymerase.

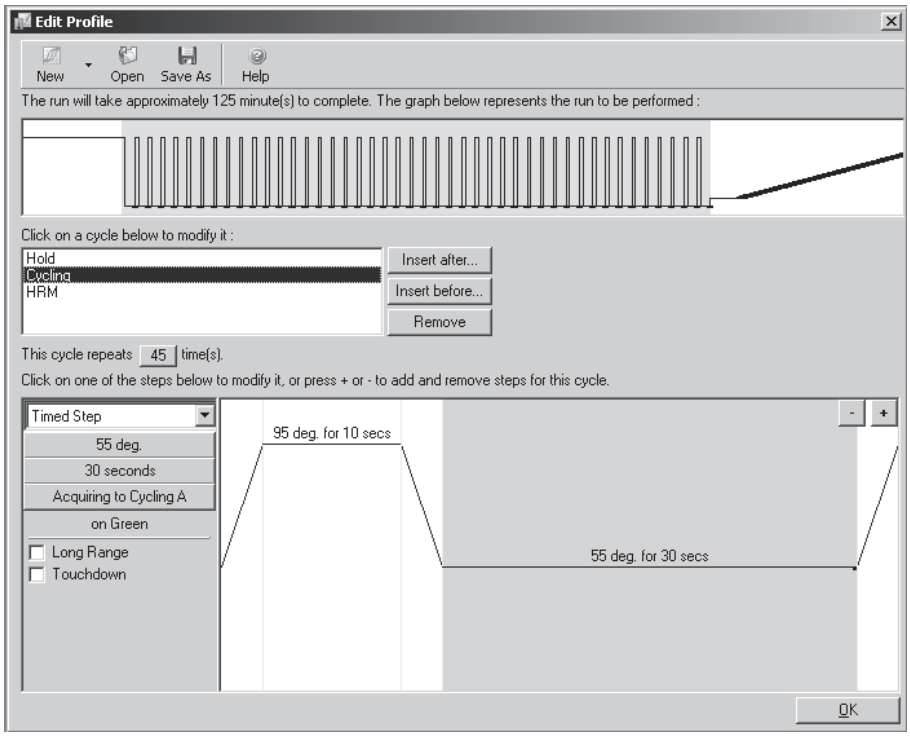

Figure 8. Two-step cycling. PCR requires 45 cycles. Each cycle is comprised of 2 steps: 95°C for 10 s (denaturation step) and 55°C for 30 s (annealing/extension step).

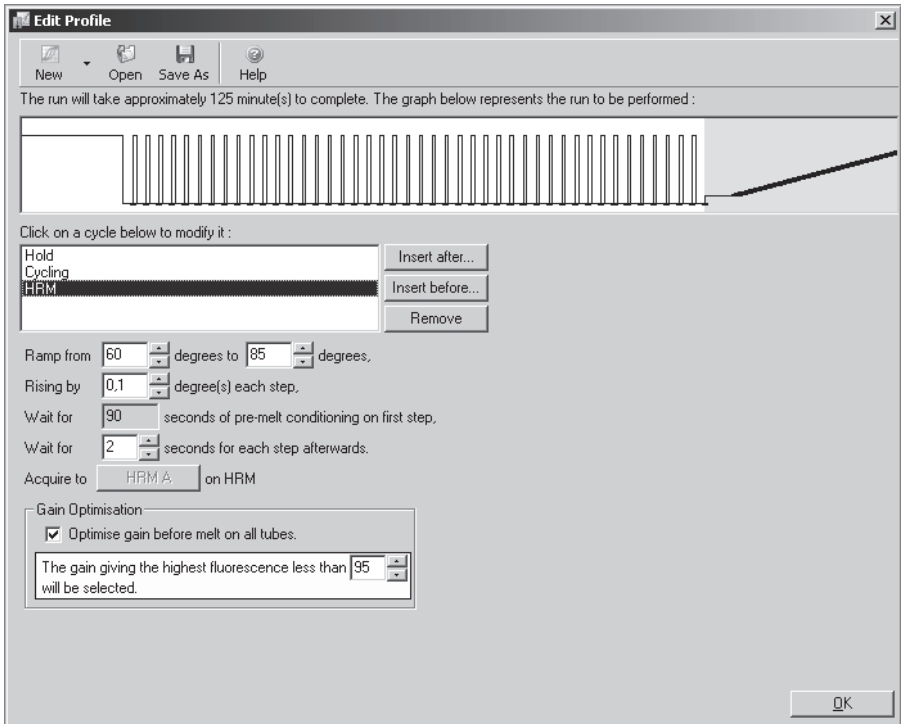

Figure 9. HRM analysis. After PCR is completed, HRM analysis can be performed, ramp from 60°C to 85°C, rising by 0.1°C in each step. Ensure that the "Optimize gain before melt on all tubes" check box is checked. Approve by clicking "OK".

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and suggestions
QIAgility:	
The QIAgility stops	
a) Wrong allocation of tips and/or adaptors.	Place all tips and/or adaptors in the correct position.
b) Liquid level not found	The QIAgility tries to resolve the error automatically (if liquid should be dispensed, the QIAgility goes as low as possible and dispenses. If liquid should be absorbed, the user has to resolve the error).
Variability in signal (C_T and/or Rn in HRM) between replicates	
a) Incorrect tube filling by the QIAgility	Repeat the entire run.
b) Tube contents not mixed properly by centrifugation after thawing	Repeat the entire run and centrifuge all the tubes.
No PCR results and/or wrong T_m	
Primer tubes placed at the wrong position or switched	Repeat the entire run with all tubes at the correct positions.
Incorrect genotyping in the HRM analysis	
One or more template tubes placed at incorrect positions in the adaptor	Repeat the entire run with all tubes at the correct positions.

Rotor-Gene Q:

PCR and HRM data have been generated but do not match the expected results shown in Figures 1–2

Wrong orientation of the tubes in the Rotor-Disc (due to mixing up of tubes or not starting at position 1)	If possible, bring all values to the correct order during post-HRM analysis.

Unexpected fluorescence signals in HRM (extremely high or low)

Gain normalization in the HRM step is not activated	Repeat only the HRM analysis with the same samples, and also include a premelting step of 95°C for 20 s and 45°C for 20 s.

No PCR data collected but HRM curve looks OK

PCR signals acquired with the HRM channel	If PCR results are required, repeat the entire run and use the green channel for PCR cycling. If PCR results are not required, it is possible to use the HRM curve generated from the original run.

HRM analysis is not possible

Only a classical melt curve is obtained instead of an HRM plot	Melt the same samples again, this time using the HRM function, and make sure to use the HRM channel. Also include a premelting step of 95°C for 20 s and 45°C for 20 s.

No data collected

Incorrect programming of the cycler	When programming the cycler, the wrong plastic format was selected in the protocol setup and/or no acquiring point was set. Repeat the entire run.

Variability in the signal (C_T and/or Rn in HRM) between replicates

Liquid evaporated because tubes may not be correctly closed	Repeat the entire run and be sure to close the tubes correctly.

Unexpected fluorescence peaks and/or C_T values

a) Wrong cycling protocol	Repeat the run with the correct cycling protocol.
b) The room temperature is incorrect (too high)	Repeat the run with a lower room temperature.

Amplification plot looks wavy

a) Room temperature not constant	Repeat the run in a room with constant temperature.
b) Air-flow around the Rotor-Gene Q is obstructed	Ensure that there is nothing obstructing the air flow around the cycler. Air-flow obstruction can lead to overheating of the instrument.

Ordering Information

Product	Contents	Cat. no.
Rotor-Gene Type-it HRM Discovery Kit	For 56 reactions: 2x HRM PCR Master Mix, Primer Mixes, Standards, Unknown Samples, RNase-Free Water	206541
Accessories for the Rotor-Gene Q		
Strip Tubes and Caps, 0.1 ml (250)	250 strips of 4 tubes and caps for 1000 reactions	981103
Accessories for the QIAgility		
Adapter, 72 x 0.1 ml Strip Tubes	For holding 72 x 0.1 ml Strip Tubes; tubes are secured with a locking mechanism	9018917
50 µl Conductive Filtered Tips	Box of 5; Carbon-impregnated conductive tips (960 tips) for use with liquid-level sensing; tips contain high-set filters; for use with Adapter, Tip Rack Holder (cat. no. 9018949)	990512
200 µl Conductive Filtered Tips	Box of 5; Carbon-impregnated conductive tips (960 tips) for use with liquid-level sensing; tips contain high-set filters; for use with Adapter, Tip Rack Holder (cat. no. 9018949)	990522
Tip Receptacle Box	Box of 10; waste collection box to fit tip ejector chute; fold-up design	990550
Related products		
Type-it HRM PCR Kit (100)	For 100 x 25 µl reactions: 2x HRM PCR Master Mix (containing HotStarTaq *Plus* DNA Polymerase EvaGreen dye, Q-Solution, dNTPs, and optimized concentration of $MgCl_2$) and RNase-Free Water	206542

Product	Contents	Cat. no.
Type-it HRM PCR Kit (400)	For 400 x 25 µl reactions: 2x HRM PCR Master Mix (containing HotStarTaq *Plus* DNA Polymerase EvaGreen dye, Q-Solution, dNTPs, and optimized concentration of $MgCl_2$) and RNase-Free Water	206544
Type-it HRM PCR Kit (2000)	For 2000 x 25 µl reactions: 2x HRM PCR Master Mix (containing HotStarTaq *Plus* DNA Polymerase EvaGreen dye, Q-Solution, dNTPs, and optimized concentration of $MgCl_2$) and RNase-Free Water	206546

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

www.qiagen.com

Australia ■ Orders 1-800-243-800 ■ Fax 03-9840-9888 ■ Technical 1-800-243-066

Austria ■ Orders 0800-28-10-10 ■ Fax 0800-28-10-19 ■ Technical 0800-28-10-11

Belgium ■ Orders 0800-79612 ■ Fax 0800-79611 ■ Technical 0800-79556

Brazil ■ Orders 0800-557779 ■ Fax 55-11-5079-4001 ■ Technical 0800-557779

Canada ■ Orders 800-572-9613 ■ Fax 800-713-5951 ■ Technical 800-DNA-PREP (800-362-7737)

China ■ Orders 86-21-3865-3865 ■ Fax 86-21-3865-3965 ■ Technical 800-988-0325

Denmark ■ Orders 80-885945 ■ Fax 80-885944 ■ Technical 80-885942

Finland ■ Orders 0800-914416 ■ Fax 0800-914415 ■ Technical 0800-914413

France ■ Orders 01-60-920-926 ■ Fax 01-60-920-925 ■ Technical 01-60-920-930 ■ Offers 01-60-920-928

Germany ■ Orders 02103-29-12000 ■ Fax 02103-29-22000 ■ Technical 02103-29-12400

Hong Kong ■ Orders 800 933 965 ■ Fax 800 930 439 ■ Technical 800 930 425

Ireland ■ Orders 1800 555 049 ■ Fax 1800 555 048 ■ Technical 1800 555 061

Italy ■ Orders 800-789-544 ■ Fax 02-334304-826 ■ Technical 800-787980

Japan ■ Telephone 03-6890-7300 ■ Fax 03-5547-0818 ■ Technical 03-6890-7300

Korea (South) ■ Orders 080-000-7146 ■ Fax 02-2626-5703 ■ Technical 080-000-7145

Luxembourg ■ Orders 8002-2076 ■ Fax 8002-2073 ■ Technical 8002-2067

Mexico ■ Orders 01-800-7742-639 ■ Fax 01-800-1122-330 ■ Technical 01-800-7742-436

The Netherlands ■ Orders 0800-0229592 ■ Fax 0800-0229593 ■ Technical 0800-0229602

Norway ■ Orders 800-18859 ■ Fax 800-18817 ■ Technical 800-18712

Singapore ■ Orders 1800-742-4362 ■ Fax 65-6854-8184 ■ Technical 1800-742-4368

Spain ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

Sweden ■ Orders 020-790282 ■ Fax 020-790582 ■ Technical 020-798328

Switzerland ■ Orders 055-254-22-11 ■ Fax 055-254-22-13 ■ Technical 055-254-22-12

UK ■ Orders 01293-422-911 ■ Fax 01293-422-922 ■ Technical 01293-422-999

USA ■ Orders 800-426-8157 ■ Fax 800-718-2056 ■ Technical 800-DNA-PREP (800-362-7737)

1071370 03/2012

Sample & Assay Technologies