July 2011

# Rotor-Gene® SYBR® Green PCR Demo Handbook

For evaluation of the performance of the

Rotor-Gene Q

QIAGEN®

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.

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- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

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# Contents

## Kit Contents

| **Rotor-Gene SYBR Green PCR Demo Kit** | **(80)** |
|---|---|
| **Catalog no.** | **204001** |
| **Number of 25 µl reactions** | **80** |
| 2x Rotor-Gene SYBR Green PCR Master Mix | 1.7 ml |
| 10x QuantiTect® Primer Assay for GPER* | 1 tube |
| Standards 1–5† (genomic DNA) | 1 tube each |
| Unknown Samples 1 and 2‡ (genomic DNA) | 1 tube each |
| Buffer TE | 1.9 ml |
| RNase-Free Water | 1.9 ml |
| Handbook | 1 |

* Contains a mix of lyophilized forward and reverse primers which must be reconstituted using Buffer TE, as described below in "Shipping and Storage".

† Five standards providing 2000, 1000, 500, 250, or 125 copies of target DNA per reaction.

‡ Two unknown samples providing 500 or 250 copies of target DNA per reaction.

## Shipping and Storage

The Rotor-Gene SYBR Green PCR Demo 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). 2x Rotor-Gene SYBR Green PCR Master Mix can also be stored protected from light at 2–8°C for up to 1 month without showing any reduction in performance.

10x QuantiTect Primer Assay can be stored either lyophilized or reconstituted at –20°C. To reconstitute the assay, briefly centrifuge the tube, add 1.1 ml Buffer TE, and mix by vortexing the tube 4–6 times; if necessary, gently warm the tube to help the primers dissolve. When stored under these conditions and handled correctly, the product can be kept for at least 18 months from date of receipt without reduction in performance.

## Product Use Limitations

The Rotor-Gene SYBR Green PCR Demo Kit is intended for molecular biology applications. This product 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. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

## Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department or distributor. We will credit your account or exchange the product — as you wish. Separate conditions apply to QIAGEN scientific instruments, service products, and to products shipped on dry ice. Please inquire for more information.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor (see back cover or visit www.qiagen.com).

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At QIAGEN, we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the Rotor-Gene SYBR Green PCR Demo Kit or QIAGEN products in general, please 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, please see our Technical Support Center at www.qiagen.com/Support or call one of the QIAGEN Technical Service Departments or local distributors (see back cover or visit www.qiagen.com).

# 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 SYBR Green PCR Demo Kit is tested against predetermined specifications to ensure consistent product quality.

# Introduction

High-precision in real-time PCR can be achieved using the combination of Rotor-Gene Kits and the Rotor-Gene Q real-time PCR cycler. The ready-to-use master mix supplied with Rotor-Gene Kits allows fast and reliable gene quantification without the need for reaction optimization, while the Rotor-Gene Q employs a unique centrifugal rotary design. PCR tubes are placed into a rotor which spins tubes past a single excitation light source and a single detector in a chamber of moving air. This means that there is minimal optical and temperature variation between tubes, enabling high precision in real-time PCR quantification. In addition, as the rotor spins continuously at 400 rpm, high-speed data acquisition is possible.

The Rotor-Gene SYBR Green PCR Demo Kit has been developed for use in demonstrating the high performance of Rotor-Gene Kits in combination with the Rotor-Gene Q. Using the kit, the reliability and reproducibility of gene quantification with Rotor-Gene technologies can be evaluated.*

Manual pipetting steps can be avoided by using the QIAgility®, a compact benchtop instrument that provides rapid, high-precision PCR setup. Mistakes in reaction setup due to human error are reduced and may be eliminated. The QIAgility perfectly complements the combination of the Rotor-Gene Q and Rotor-Gene Kits, enabling easy dispensing of liquids into tubes, strip tubes, and Rotor-Discs®.

## Principle and procedure

Using the Rotor-Gene SYBR Green PCR Demo Kit, SYBR Green-based real-time PCR is carried out to quantify different copy numbers of a genomic DNA target. Each reaction consists of:

- Human genomic DNA template of a defined copy number
- Rotor-Gene SYBR Green PCR Master Mix
- QuantiTect Primer Assay specific for the human G protein-coupled estrogen receptor 1 (GPER) gene

* The kit can also be used with the Rotor-Gene 6000 or Rotor-Gene 3000.

A standard curve is generated from the $C_T$ values obtained from a set of standards (2000, 1000, 500, 250, and 125 copies; each standard is analyzed in quadruplicate). The standard curve is then used to determine the copy number for 2 unknown samples (500 and 250 copies; 24 replicates* of each unknown sample are analyzed). In addition, 4 no template control (NTC) reactions are carried out. Thus, a total of 72 reactions are run at the same time on the Rotor-Gene Q.

## Description of protocols

This handbook contains 3 protocols. Follow either the protocol for manual reaction setup (Protocol 1, page 10) or the protocol for automated reaction setup using the QIAgility (Protocol 2, page 12). After reaction setup, proceed to the protocol for real-time PCR on the Rotor-Gene Q (Protocol 3, page 14).

* When performing manual reaction setup, a minimum of 4 replicates for each unknown sample can be set up instead.

# Equipment 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

### 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 81 tips are required
- 200 µl Conductive Filtered Tips (cat. no. 990522); at least 5 tips are required
- Tip Receptacle Box (cat. no. 990550)
- 5 ml Tube; Graduated, Flat-Base (cat. no. 990552)
- Strip Tubes and Caps, 0.1 ml (cat. no. 981103); 18 strips are required

### For real-time PCR 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 the reactions manually. After reaction setup, proceed to Protocol 3 on page 14 to carry out real-time PCR on the Rotor-Gene Q.

### Things to do before starting

- Thaw Buffer TE and mix by inverting the tube several times.
- Reconstitute the QuantiTect Primer Assay by briefly centrifuging the tube, adding 1.1 ml Buffer TE, and vortexing the tube 4–6 times. If necessary, gently warm the tube to help the primers dissolve.

### Procedure

1. **Thaw the 2x Rotor-Gene SYBR Green PCR Master Mix, standards, unknown samples, and RNase-free water. Mix well all solutions before use to avoid localized concentrations of salt.**
2. **Prepare a reaction mix according to Table 1.**

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

   In this experiment, thirty-two 25 µl reactions will be run (quadruplicate reactions for 5 standards, 2 unknown samples, and one NTC). A reaction mix for thirty-six 25 µl reactions should be prepared, as some reaction mix will be lost during pipetting.

**Table 1. Reaction setup**

| Component | Volume per 25 µl reaction | Volume per 900 µl reaction mix* | Final concentration |
|---|---|---|---|
| 2x Rotor-Gene SYBR Green PCR Master Mix | 12.5 µl | 450 µl | 1x |
| 10x QuantiTect Primer Assay | 2.5 µl | 90 µl | 1x |
| RNase-free water | 5 µl | 180 µl | – |
| Template DNA (added at step 4) | 5 µl | – | Varies |

* A 900 µl reaction mix is equivalent to thirty-six 25 µl reactions.

3. **Mix the reaction mix thoroughly, and dispense 20 µl into PCR tubes.**
   We recommend using eight 0.1 ml strip tubes and caps (each strip contains 4 tubes).
4. **Add 5 µl template DNA to the individual PCR tubes containing the reaction mix according to Table 2.**

**Table 2. Recommended pipetting scheme**

| Reaction number | Template | Copy number |
|---|---|---|
| 1–4 | Standard 1 | 2000 |
| 5–8 | Standard 2 | 1000 |
| 9–12 | Standard 3 | 500 |
| 13–16 | Standard 4 | 250 |
| 17–20 | Standard 5 | 125 |
| 21–24 | Unknown 1* | 500 |
| 25–28 | Unknown 2* | 250 |
| 29–32 | NTC† | – |

* This table shows the preparation of 4 replicates for each unknown sample. If desired, a maximum of 24 replicates for each unknown sample can be prepared instead. Empty positions in the 72-well rotor should be filled with empty PCR tubes.

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

5. **Proceed to Protocol 3 (page 14).**

# Protocol 2: Automated Reaction Setup Using the QIAgility

This protocol describes how to automate setup of 72 reactions using the QIAgility, which takes about 28 min. After reaction setup, proceed to Protocol 3 on page 14 to carry out real-time PCR on the Rotor-Gene Q. For more details about operating the QIAgility, refer to the *QIAgility User Manual*.

### Things to do before starting

- Thaw Buffer TE and mix by inverting the tube several times.
- Reconstitute the QuantiTect Primer Assay by briefly centrifuging the tube, adding 1.1 ml Buffer TE, and vortexing the tube 4–6 times. If necessary, gently warm the tube to help the primers dissolve.
- Thaw the 2x Rotor-Gene SYBR Green PCR Master Mix, standards, unknown samples, and RNase-free water. Mix well all solutions before use to avoid localized concentrations of salt.

### Procedure

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

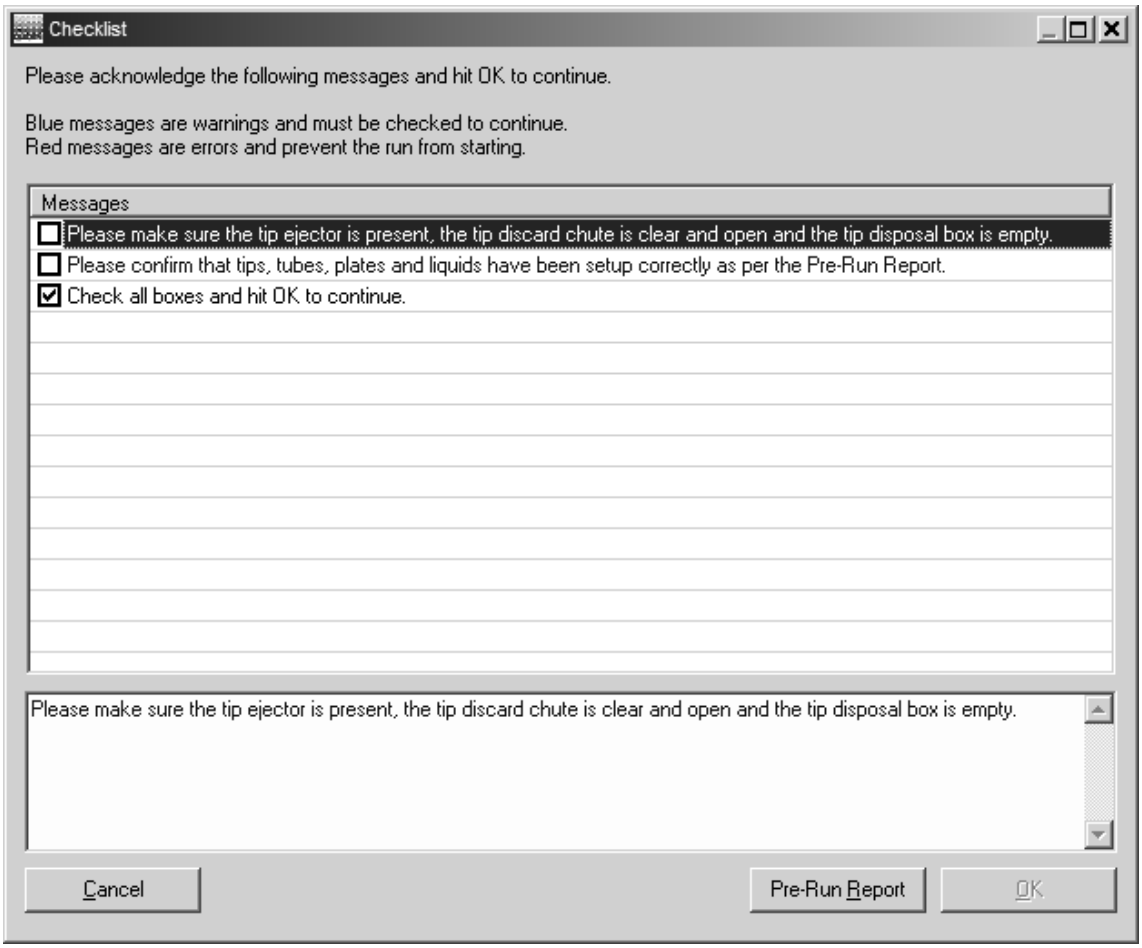
   **Note**: Before starting the software, ensure that the instrument hood is closed and that the QIAgility is switched on.

2. **Click on the "Protocols" tab to display a list of Q Protocols. Click on "Rotor-Gene SYBR Green PCR Demo Kit" to select it, and then click on the "Open" button. Alternatively, double-click on "Rotor-Gene SYBR Green PCR Demo Kit" to open it directly.**
3. **A description of the Q Protocol will appear. Review the description and then click on the "Close" button.**
4. **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.

5. **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.




6. **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.**
7. **If the worktable is correctly set up, click on the "OK" button to start the run.**

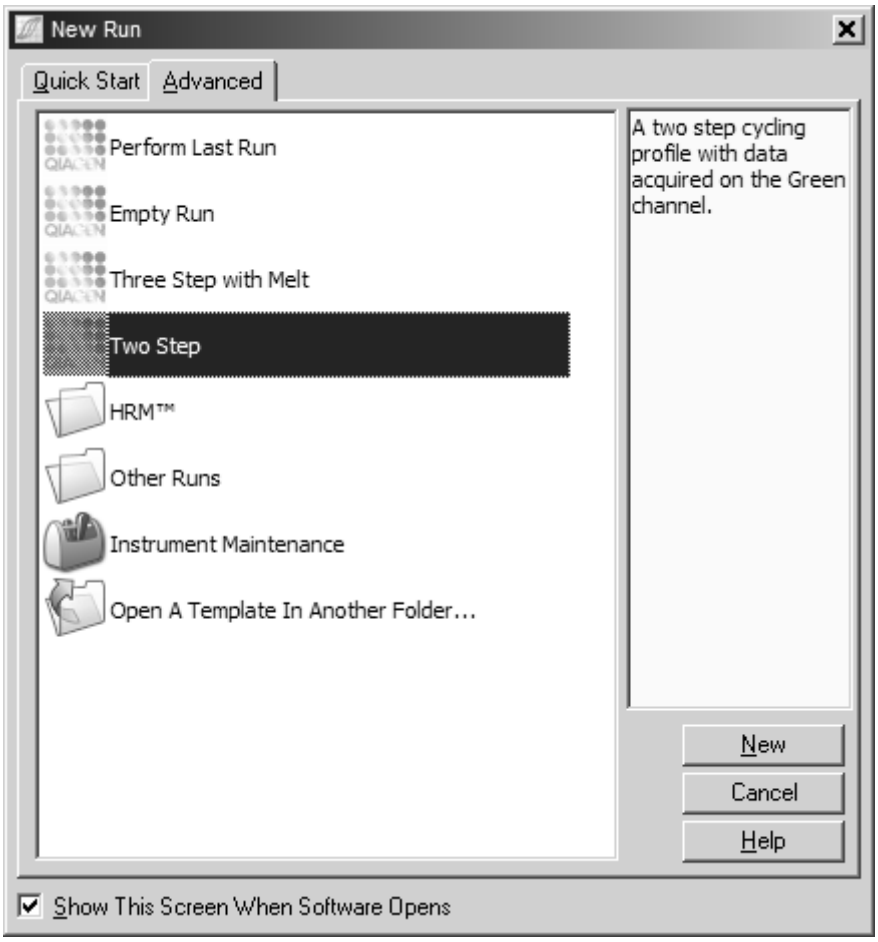
   The location of the pipetting head will be highlighted on the software worktable in real time, and a summary of the progress of the run will be displayed in the right-hand pane. Reaction setup will be completed in 28 min (if the tip reuse option is set to 8 times).
8. **Proceed to Protocol 3 (page 14).**

# Protocol 3: Demonstration of Real-Time PCR on the Rotor-Gene Q

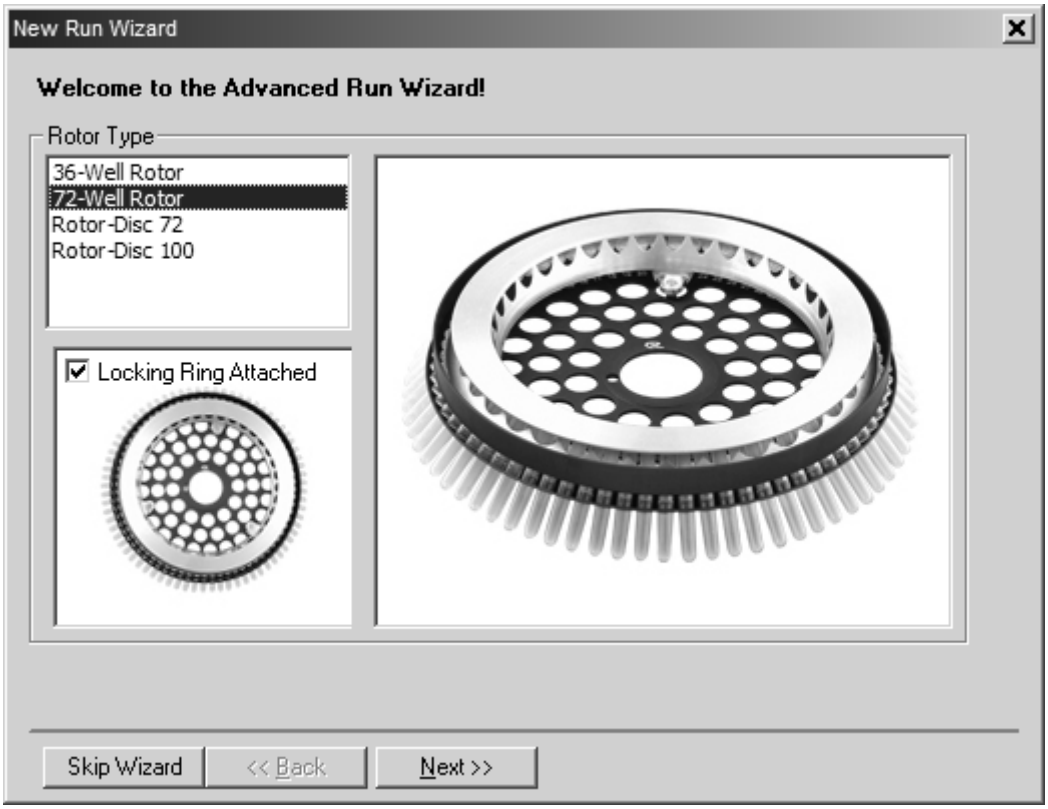
Before starting this protocol, set up the reactions by hand (Protocol 1, page 10) or by using the QIAgility (Protocol 2, page 12).

### Procedure

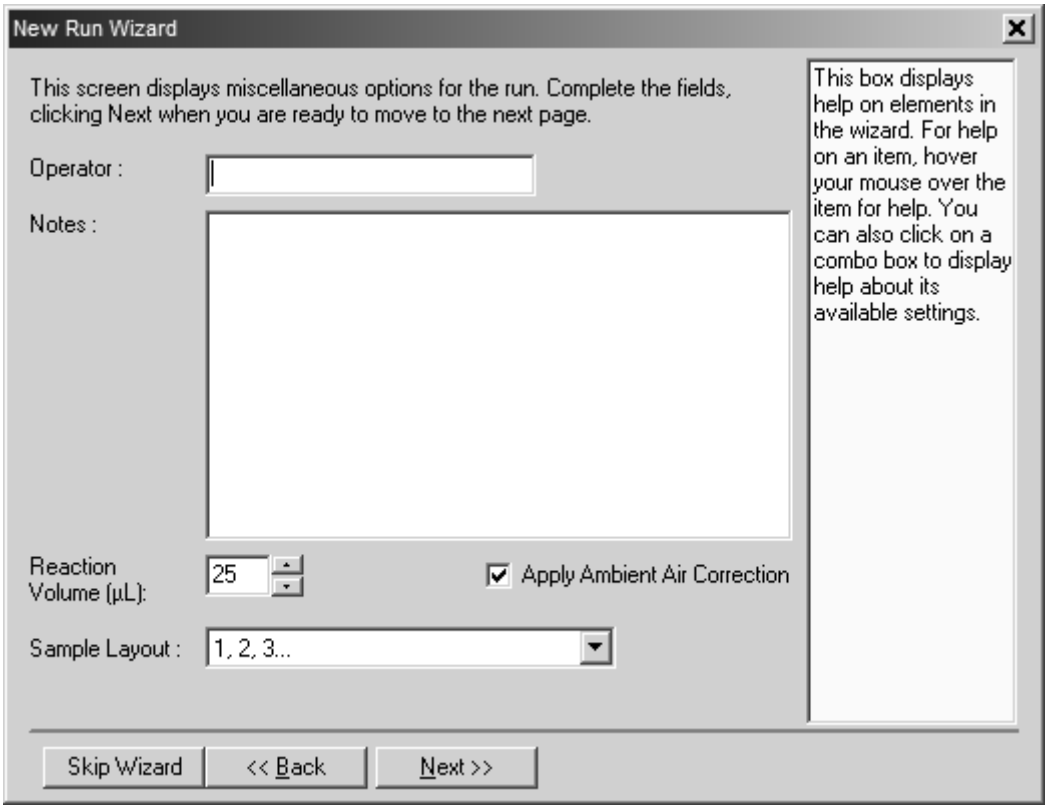
1. **Close the PCR tubes containing the reactions. Place the tubes in the 72-well rotor in the Rotor-Gene cycler, and attach the locking ring.**
   If manual reaction setup was carried out and there are fewer than 72 reactions, empty positions in the 72-well rotor should be filled with empty PCR tubes.
2. **Open the Rotor-Gene software, select in the Advanced wizard the "Two Step" cycling profile, and click on "New".**



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




4. **Make sure that the reaction volume is 25 µl and that the "Apply Ambient Air Correction" check box is checked. Click "Next" to continue.**



5. **Click on "Edit Profile", and program the Rotor-Gene cycler according to the program outlined in Table 3 and Figures 1–4 (pages 17–20).**
   Data acquisition should be performed during the combined annealing/extension step.

**Table 3. Cycling conditions**

| Step | Time | Temperature | Additional comments |
|---|---|---|---|
| **PCR initial activation step** | 5 min | 95°C | HotStarTaq® *Plus* DNA Polymerase is activated by this heating step |
| **Two-step cycling:** | | | |
| ■ Denaturation | 5 s | 95°C | |
| ■ Combined annealing/ extension | 10 s | 60°C | Perform fluorescence data collection using the Green channel with auto-gain optimization (see Figure 4, page 20) |
| ■ Number of cycles | 35 | | |
| **Optional: Melting curve analysis** | 90 s for first step; 5 s for subsequent steps | 65–95°C; increments of 1°C | After PCR is completed, melting curve analysis can be performed to check the specificity of the reaction |

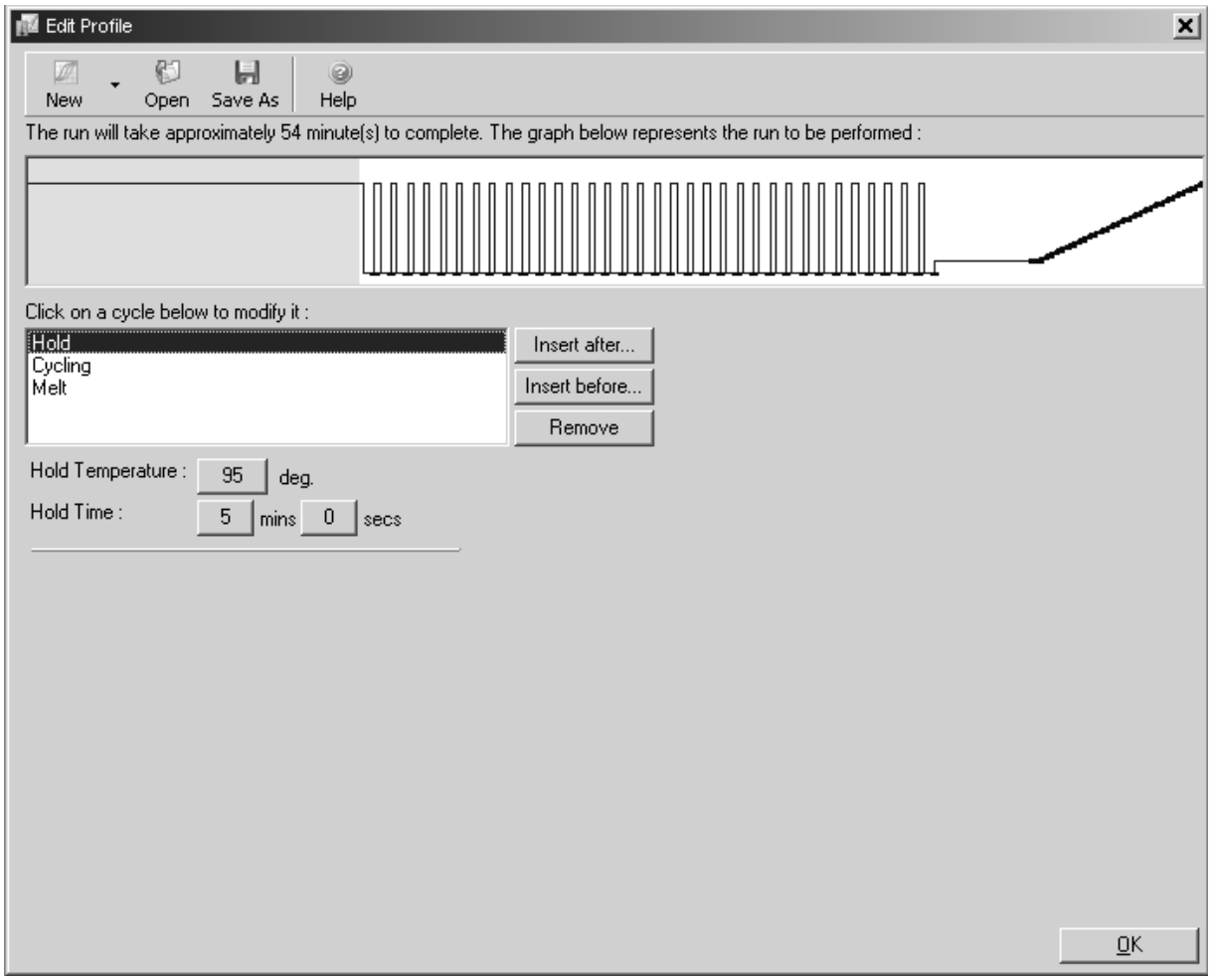



**Figure 1. PCR initial activation step.** PCR requires an initial incubation at 95°C for 5 min to activate HotStarTaq *Plus* DNA Polymerase.

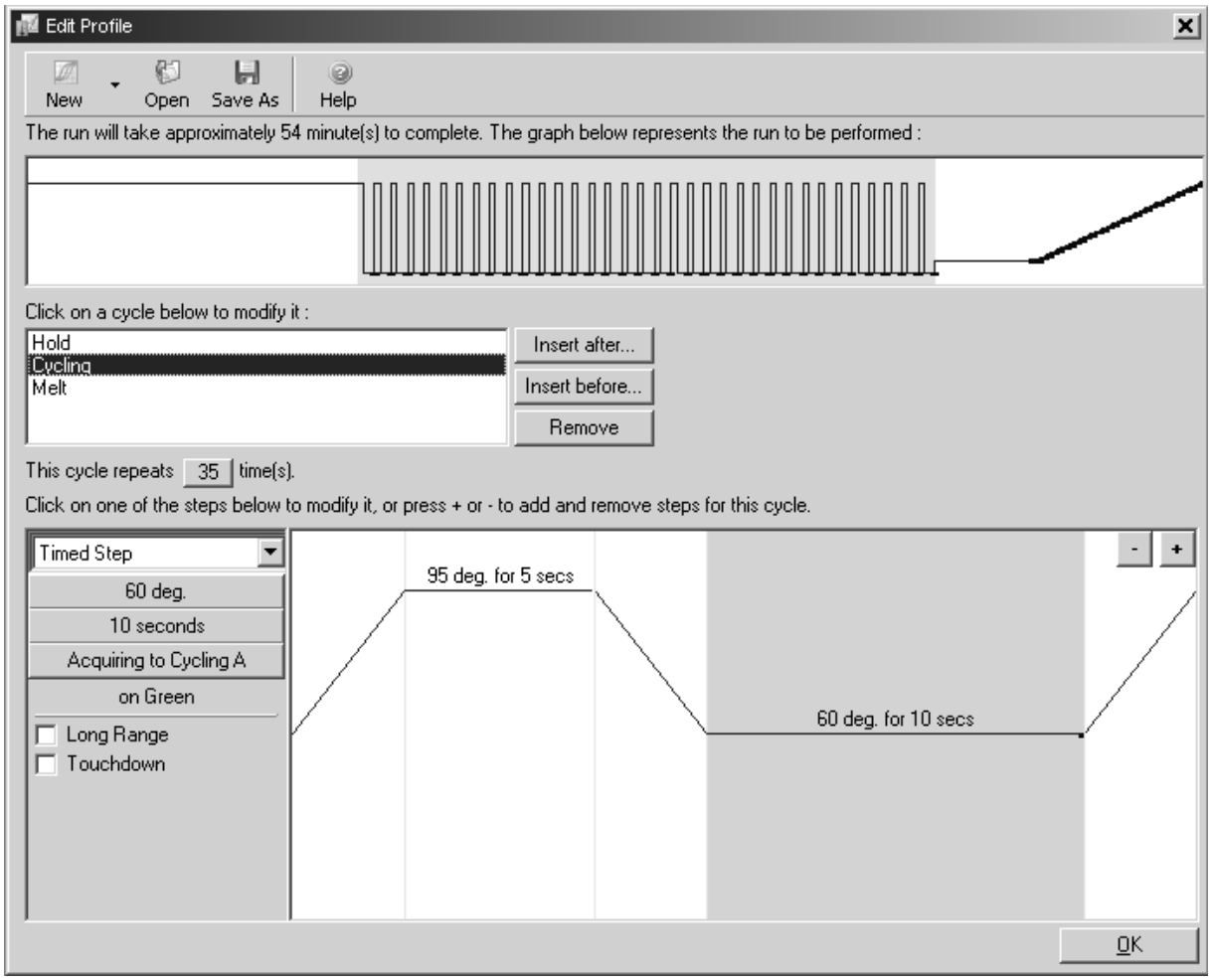



**Figure 2. Two-step cycling.** PCR requires 35 cycles. Each cycle is comprised of 2 steps: 95°C for 5 s (denaturation step) and 60°C for 10 s (annealing/extension step).

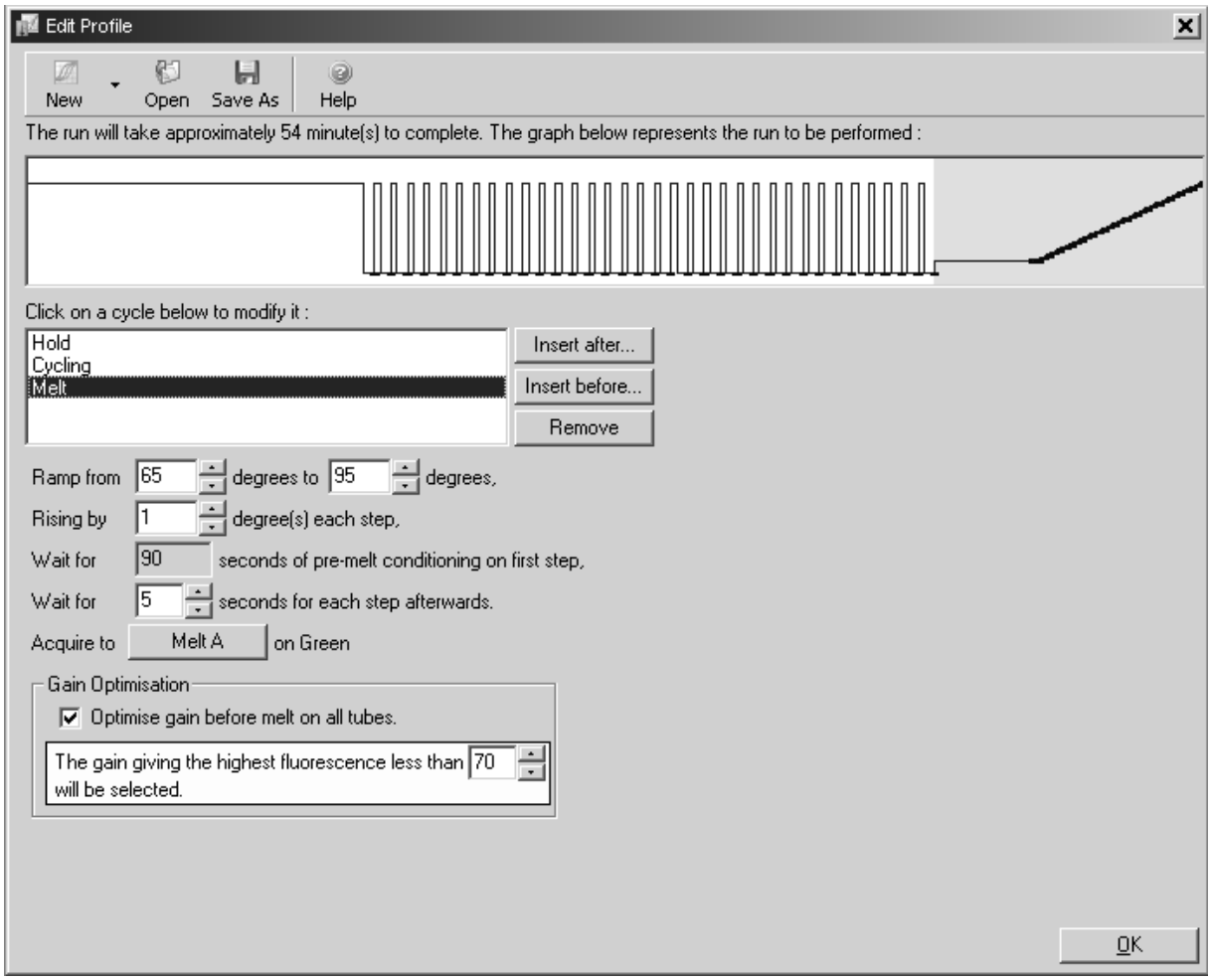
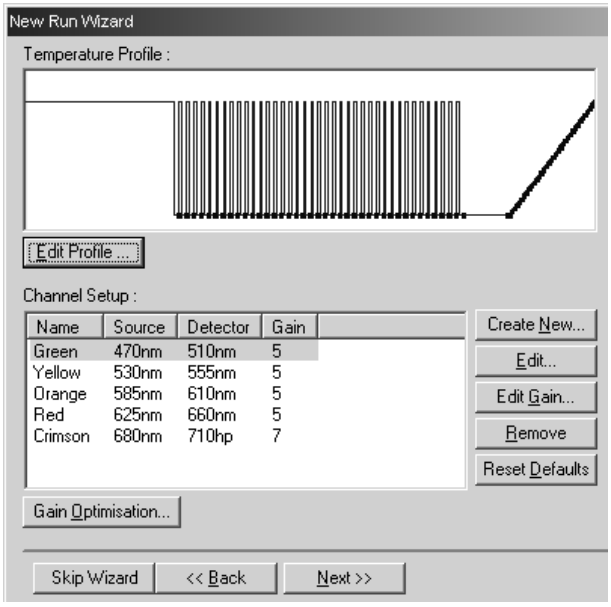
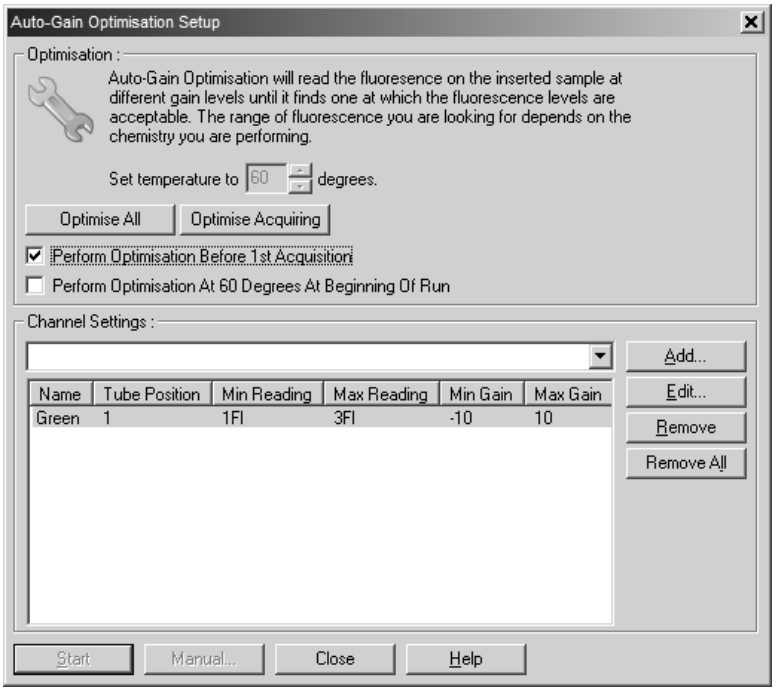



**Figure 3. Melting curve analysis.** After PCR is completed, melting curve analysis can be performed to check the specificity of the reaction. Ensure that the "Optimise gain before melt on all tubes" check box is checked.

A




B




C

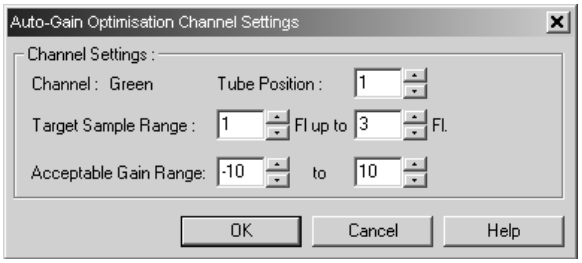
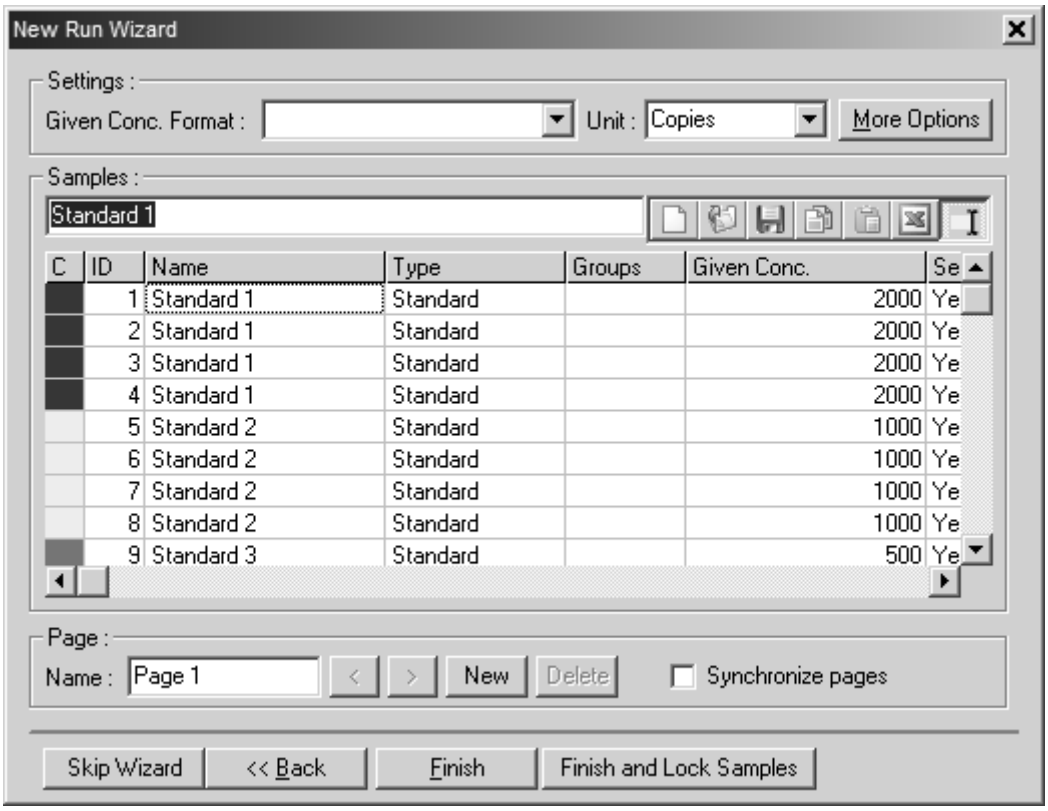



**Figure 4. Gain optimization for the Green channel.** A Select the Green channel, and click the "Gain Optimisation" button. B Click the "Optimise Acquiring" button. C In the pop-up box which appears, enter a "Target Sample Range" of 1 Fl up to 3 Fl. Then click "OK", check the "Perform Optimisation Before 1st Acquisition" box, and click "Close".

6. **Click "Next" to confirm the temperature profile and channel setup, and check in the summary if all parameters are correct. Then start the Rotor-Gene cycler 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, you can enter a name and description for each reaction while you wait for the run to end.**



# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| Rotor-Gene SYBR Green PCR Demo Kit (80) | For 80 reactions: 2x Rotor-Gene SYBR Green PCR Master Mix, 10x QuantiTect Primer Assay for GPER, Standards, Unknown Samples, Buffer TE, RNase-Free Water | 204001 |
| **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 |
| Strip Tubes and Caps, 0.1 ml (2500) | 10 x 250 strips of 4 tubes and caps for 10,000 reactions | 981106 |
| **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 | 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 | 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 |
| 5 ml Tube; Graduated, Flat-Base | Bag of 50; suitable for holding diluent and master mix on the instrument worktable; graduated, flat-base design with a tapered internal profile for minimum dead volume; screw cap included | 990552 |

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**To find out more about the QIAgility, Rotor-Gene Q, and Rotor-Gene Kits, visit www.qiagen.com/goto/Rotor-GeneQ**

## Notes

## Notes

## Notes

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**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

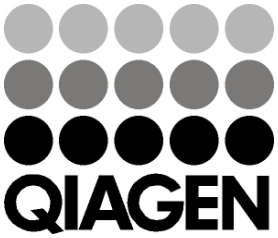
**Spain** ■ Orders 91-630-7050 ■ Fax 91-630-5145 ■ Technical 91-630-7050

**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)

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