

January 2014

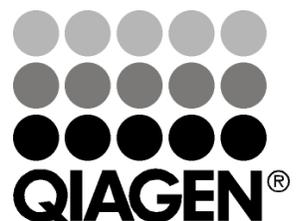
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# **Rotor-Gene<sup>®</sup> SYBR<sup>®</sup> Green Handbook**

Rotor-Gene SYBR Green PCR Kit

Rotor-Gene SYBR Green RT-PCR Kit

For fast real-time PCR, two-step RT-PCR, and  
one-step RT-PCR using SYBR Green I on  
Rotor-Gene cyclers



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**Sample & Assay Technologies**

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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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- microRNA research and RNAi
- Automation of sample and assay technologies

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## Kit Contents

<b>Rotor-Gene SYBR Green PCR Kit</b>	<b>(400)</b>
<b>Catalog no.</b>	<b>204074</b>
<b>Number of 25 <math>\mu</math>l reactions</b>	<b>400</b>
2x Rotor-Gene SYBR Green PCR Master Mix, containing:	3 x 1.7 ml
■ HotStarTaq <sup>®</sup> <i>Plus</i> DNA Polymerase	
■ Rotor-Gene SYBR Green PCR Buffer	
■ dNTP mix (dATP, dCTP, dGTP, dTTP)	
RNase-Free Water	2 x 2 ml
Handbook	1

<b>Rotor-Gene SYBR Green RT-PCR Kit</b>	<b>(400)</b>
<b>Catalog no.</b>	<b>204174</b>
<b>Number of 25 <math>\mu</math>l reactions</b>	<b>400</b>
2x Rotor-Gene SYBR Green RT-PCR Master Mix, containing:	3 x 1.7 ml
■ HotStarTaq <i>Plus</i> DNA Polymerase	
■ Rotor-Gene SYBR Green RT-PCR Buffer	
■ dNTP mix (dATP, dCTP, dGTP, dTTP)	
Rotor-Gene RT Mix, a mixture of the QIAGEN <sup>®</sup> products:	100 $\mu$ l
■ Omniscript <sup>®</sup> Reverse Transcriptase	
■ Sensiscript <sup>®</sup> Reverse Transcriptase	
RNase-Free Water	2 x 2 ml
Handbook	1

## Shipping and Storage

Rotor-Gene SYBR Green Kits are shipped on dry ice. The kits should be stored immediately upon receipt at  $-15^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  and protected from light. When the kits are stored under these conditions and handled correctly, performance is guaranteed until the expiration date (see the quality-control label inside the kit box or on the kit envelope). 2x Rotor-Gene SYBR Green Master Mixes can also be stored protected from light at  $2-8^{\circ}\text{C}$  for up to 1 month without showing any reduction in performance.

## Product Use Limitations

Rotor-Gene SYBR Green Kits are intended for molecular biology applications. These products are 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](http://www.qiagen.com)).

## Technical Assistance

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 Rotor-Gene SYBR Green Kits 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](http://www.qiagen.com/Support) or call one of the QIAGEN

Technical Service Departments or local distributors (see back cover or visit [www.qiagen.com](http://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 safety data sheets (SDSs). These are available online in convenient and compact PDF format at [www.qiagen.com/safety](http://www.qiagen.com/safety) where you can find, view, and print the SDS for each QIAGEN kit and kit component.

### 24-hour emergency information

Chemical emergency or accident assistance is available 24 hours a day from:  
CHEMTREC

**USA & Canada** ■ Tel: 1-800-424-9300

**Outside USA & Canada** ■ Tel: +1-703-527-3887 (collect calls accepted)

## Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each component of Rotor-Gene SYBR Green PCR Kits and Rotor-Gene SYBR Green RT-PCR Kits is tested against predetermined specifications to ensure consistent product quality. See the quality-control label inside the kit box or on the kit envelope for lot-specific values.

## Product Description

Component	Description
HotStarTaq <i>Plus</i> DNA Polymerase*	HotStarTaq <i>Plus</i> DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from <i>Thermus aquaticus</i> . HotStarTaq <i>Plus</i> DNA Polymerase is provided in an inactive state and has no enzymatic activity at ambient temperature. The enzyme is activated by a 5-minute, 95°C incubation step.
Rotor-Gene SYBR Green PCR or RT-PCR Buffer*	Contains Tris·Cl, KCl, (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , MgCl <sub>2</sub> ; also contains Q-Bond® and other additives that enable fast cycling on Rotor-Gene cyclers
dNTP mix*	Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality
Fluorescent dyes*	SYBR Green I
RNase-free water	Ultrapure quality, PCR-grade
Rotor-Gene RT Mix†	Contains an optimized mixture of the QIAGEN products Omniscript Reverse Transcriptase and Sensiscript Reverse Transcriptase, which are recombinant heterodimeric enzymes expressed in <i>E. coli</i> .

\* Included in 2x Rotor-Gene SYBR Green PCR Master Mix and 2x Rotor-Gene SYBR Green RT-PCR Master Mix.

† Supplied with the Rotor-Gene SYBR Green RT-PCR Kit.

## Introduction

Rotor-Gene SYBR Green Kits provide rapid real-time quantification of genomic DNA, cDNA, or RNA targets on the Rotor-Gene Q in an easy-to-handle format. The kits are also compatible with the Rotor-Gene 3000 and Rotor-Gene 6000. Two kit formats are available:

- **Rotor-Gene SYBR Green PCR Kit:** The kit can be used in real-time PCR of genomic DNA targets, and also in real-time two-step RT-PCR of RNA targets following reverse transcription with, for example, the QuantiTect® Reverse Transcription Kit (see ordering information, page 28).
- **Rotor-Gene SYBR Green RT-PCR Kit:** The kit can be used in real-time one-step RT-PCR of RNA targets, with reverse transcription and PCR taking place sequentially in the same tube.

The fluorescent dye SYBR Green I in the master mix enables the analysis of many different targets without having to synthesize target-specific labeled probes. High specificity and sensitivity in PCR and RT-PCR are achieved by the use of the hot-start enzyme HotStarTaq *Plus* DNA Polymerase together with a specialized fast PCR or RT-PCR buffer. For one-step RT-PCR, the optimized Omniscript and Sensiscript blend for the reverse-transcription step further enhances sensitivity. Short cycling steps without loss of PCR sensitivity and efficiency are enabled by Q-Bond, a patent-pending additive in the PCR and RT-PCR buffers.

The kits have been optimized for use with Rotor-Gene real-time PCR cyclers, which employ a unique centrifugal rotary design. PCR tubes are placed into a rotor which spins tubes past the same excitation light source and the same 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.

## 2x Rotor-Gene SYBR Green Master Mixes

The components of 2x Rotor-Gene SYBR Green PCR Master Mix include HotStarTaq *Plus* DNA Polymerase, Rotor-Gene SYBR Green PCR Buffer, and SYBR Green I (see descriptions below).

2x Rotor-Gene SYBR Green RT-PCR Master Mix contains HotStarTaq *Plus* DNA Polymerase, Rotor-Gene SYBR Green RT-PCR Buffer, and SYBR Green I (see descriptions below).

## HotStarTaq *Plus* DNA Polymerase

HotStarTaq *Plus* DNA Polymerase is a modified form of QIAGEN *Taq* DNA Polymerase. It is provided in an inactive state and has no enzymatic activity at ambient temperature.

In real-time PCR and two-step RT-PCR, this inactivity of HotStarTaq *Plus* DNA Polymerase prevents the formation of misprimed products and primer–dimers during reaction setup and the first denaturation step, leading to high PCR specificity and accurate quantification. The enzyme is activated at the start of a reaction by a 5-minute, 95°C incubation step. The hot start enables reactions to be set up rapidly and conveniently at room temperature.

In real-time one-step RT-PCR, HotStarTaq *Plus* DNA Polymerase remains completely inactive during the reverse-transcription reaction and does not interfere with it. This prevents formation of misprimed RT-PCR products and primer–dimers during reaction setup, reverse transcription, and the first denaturation step. The enzyme is activated after the reverse-transcription step by a 5-minute, 95°C incubation step. The hot start also inactivates the reverse transcriptases, ensuring temporal separation of reverse transcription and PCR, and allowing both steps to be performed sequentially in a single tube.

For all reactions, the concentration of HotStarTaq *Plus* DNA Polymerase in the master mixes is optimized to allow short extension times in the combined annealing/extension step of each PCR cycle.

## Rotor-Gene SYBR Green Buffers

Rotor-Gene SYBR Green Buffers are specially optimized to support the Rotor-Gene cyclers' fast-cycling capabilities, which are based on the cyclers' unique centrifugal rotary design. Rotor-Gene SYBR Green PCR Buffer is specifically designed for fast-cycling, real-time PCR using SYBR Green I. Rotor-Gene SYBR Green RT-PCR Buffer is specifically designed for fast-cycling, real-time one-step RT-PCR using SYBR Green I. A novel additive in the buffers, Q-Bond, allows short cycling times on Rotor-Gene cyclers. Q-Bond increases the affinity of *Taq* DNA polymerases for short single-stranded DNA, reducing the time required for primer annealing to a few seconds. This allows a combined annealing/extension step of only 10 seconds. In addition, the unique composition of the buffers support the melting behavior of DNA, enabling short denaturation and annealing/extension times.

Rotor-Gene SYBR Green PCR Buffer is based on the unique QIAGEN PCR buffer system. Rotor-Gene SYBR Green RT-PCR Buffer is based on the unique QIAGEN OneStep RT-PCR buffer system. The buffers contain a balanced combination of KCl and  $(\text{NH}_4)_2\text{SO}_4$ , which promotes a high ratio of specific to nonspecific primer binding during the annealing step of each PCR cycle. This creates stringent primer annealing conditions, leading to increased PCR specificity.

When using these buffers, primer annealing is only marginally influenced by the  $\text{MgCl}_2$  concentration, so optimization by titration of  $\text{Mg}^{2+}$  is not required.

## **SYBR Green I**

2x Rotor-Gene SYBR Green Master Mixes contain an optimized concentration of the fluorescent dye SYBR Green I. SYBR Green I binds all double-stranded DNA molecules, emitting a fluorescent signal on binding. 2x Rotor-Gene SYBR Green Master Mixes can be stored at  $-15^\circ\text{C}$  to  $-30^\circ\text{C}$  without loss of SYBR Green I fluorescence activity. The excitation and emission maxima of SYBR Green I are at 494 nm and 521 nm, respectively, which are compatible with use on Rotor-Gene cyclers.

## **cDNA synthesis for real-time one-step RT-PCR**

Use of 2x Rotor-Gene SYBR Green RT-PCR Master Mix together with Rotor-Gene RT Mix allows both reverse transcription and PCR to take place in a single tube. All reagents required for both reactions are added at the beginning, so there is no need to open the tube once the reverse-transcription reaction has been started.

Rotor-Gene RT Mix contains an optimized Omniscript and Sensiscript blend. Both enzymes exhibit a high affinity for RNA, facilitating transcription through secondary structures that may inhibit other reverse transcriptases. Omniscript is designed for reverse transcription of RNA amounts greater than 50 ng, and Sensiscript is optimized for use with very small amounts of RNA (<50 ng). This enzyme combination provides highly efficient and sensitive reverse transcription over a wide range of RNA template amounts.

## **cDNA synthesis for real-time two-step RT-PCR**

If quantifying cDNA targets with the Rotor-Gene SYBR Green PCR Kit, RNA must first be reverse transcribed into cDNA. A portion of the reverse-transcription reaction is then transferred to another tube where real-time PCR takes place. This entire process is known as real-time two-step RT-PCR, since reverse transcription and real-time PCR are carried out in separate tubes.

For reverse transcription, we recommend using the QuantiTect Reverse Transcription Kit. The kit provides a fast and convenient procedure, requiring only 20 minutes to synthesize first-strand cDNA and eliminate genomic DNA contamination. An optimized mix of oligo-dT and random primers enables cDNA synthesis from all regions of RNA transcripts, even from 5' regions of very long mRNA transcripts. cDNA yields are high, allowing sensitive detection of even low-abundance transcripts in real-time two-step RT-PCR. An alternative to the QuantiTect Reverse Transcription Kit is the FastLane<sup>®</sup> Cell cDNA Kit, which allows cDNA to be prepared directly from cultured cells without RNA purification. The FastLane Cell cDNA Kit is useful for experiments where

archiving of purified RNA is not required. For ordering information for these 2 kits, see page 28.

## **Validated, ready-to-use primer sets**

If performing real-time two-step or one-step RT-PCR, we recommend using QuantiTect Primer Assays. These are ready-to-use primer sets that are guaranteed to provide specific and sensitive quantification when used in combination with Rotor-Gene SYBR Green Kits. Where possible, primer sets are designed to specifically amplify cDNA sequences derived from mRNA transcripts. This prevents amplification of contaminating genomic DNA, which would then lead to inaccurate quantification. QuantiTect Primer Assays are easily ordered online at [www.qiagen.com/GeneGlobe](http://www.qiagen.com/GeneGlobe) and are available genomewide for human, mouse, rat, and many other species.

## **Using the correct protocol**

This handbook contains 2 protocols. The first protocol (page 12) is for real-time PCR and two-step RT-PCR. The second protocol (page 18) is for real-time one-step RT-PCR.

For background information on real-time PCR, please refer to “Guidelines for real-time PCR and RT-PCR” at [www.qiagen.com/resources/info](http://www.qiagen.com/resources/info), which contains guidelines on template preparation, primer design, controls, data analysis, and other topics.

## Protocol: Real-Time PCR and Two-Step RT-PCR

This protocol can be used with the Rotor-Gene Q, Rotor-Gene 3000, or Rotor-Gene 6000.

### Important points before starting

- The Rotor-Gene SYBR Green PCR Kit has been developed for use in a **two-step cycling** protocol, with a denaturation step at 95°C and a combined annealing/extension step at 60°C. This protocol will also work for primers with a  $T_m$  well below 60°C.
- For the highest efficiency in real-time PCR using SYBR Green I, targets should ideally be 60–200 bp in length.
- The PCR must start with an **initial incubation step of 5 minutes at 95°C** to activate HotStarTaq *Plus* DNA Polymerase.
- We recommend a final reaction volume of 25  $\mu$ l.
- **Always start with the  $Mg^{2+}$  concentration as provided** in 2x Rotor-Gene SYBR Green PCR Master Mix.
- If using QuantiTect Primer Assays, the final concentration in the reaction should be 1x. Also, follow the cycling protocol in Table 2.

### Procedure

1. **Thaw 2x Rotor-Gene SYBR Green PCR Master Mix, template DNA or cDNA, primers, and RNase-free water. Mix the individual solutions.**
2. **Prepare a reaction mix according to Table 1 (page 13).**

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:** We strongly recommend starting with the  $Mg^{2+}$  concentration as provided in 2x Rotor-Gene SYBR Green PCR Master Mix.

**Table 1. Reaction setup**

<b>Component</b>	<b>Volume/reaction</b>	<b>Final concentration</b>
2x Rotor-Gene SYBR Green PCR Master Mix	12.5 $\mu$ l	1x
Primer A*	Variable	1 $\mu$ M
Primer B*	Variable	1 $\mu$ M
Template DNA or cDNA (added at step 4)	Variable	$\leq$ 100 ng/ reaction
RNase-free water	Variable	
<b>Total reaction volume</b>	<b>25 <math>\mu</math>l</b>	

\* If using QuantiTect Primer Assays, the final concentration in the reaction should be 1x.

**3. Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes.**

**4. Add template DNA or cDNA ( $\leq$ 100 ng/reaction) to the individual PCR tubes containing the reaction mix.**

For two-step RT-PCR, the volume of the cDNA added (from the undiluted RT reaction) should not exceed 10% of the final PCR volume.

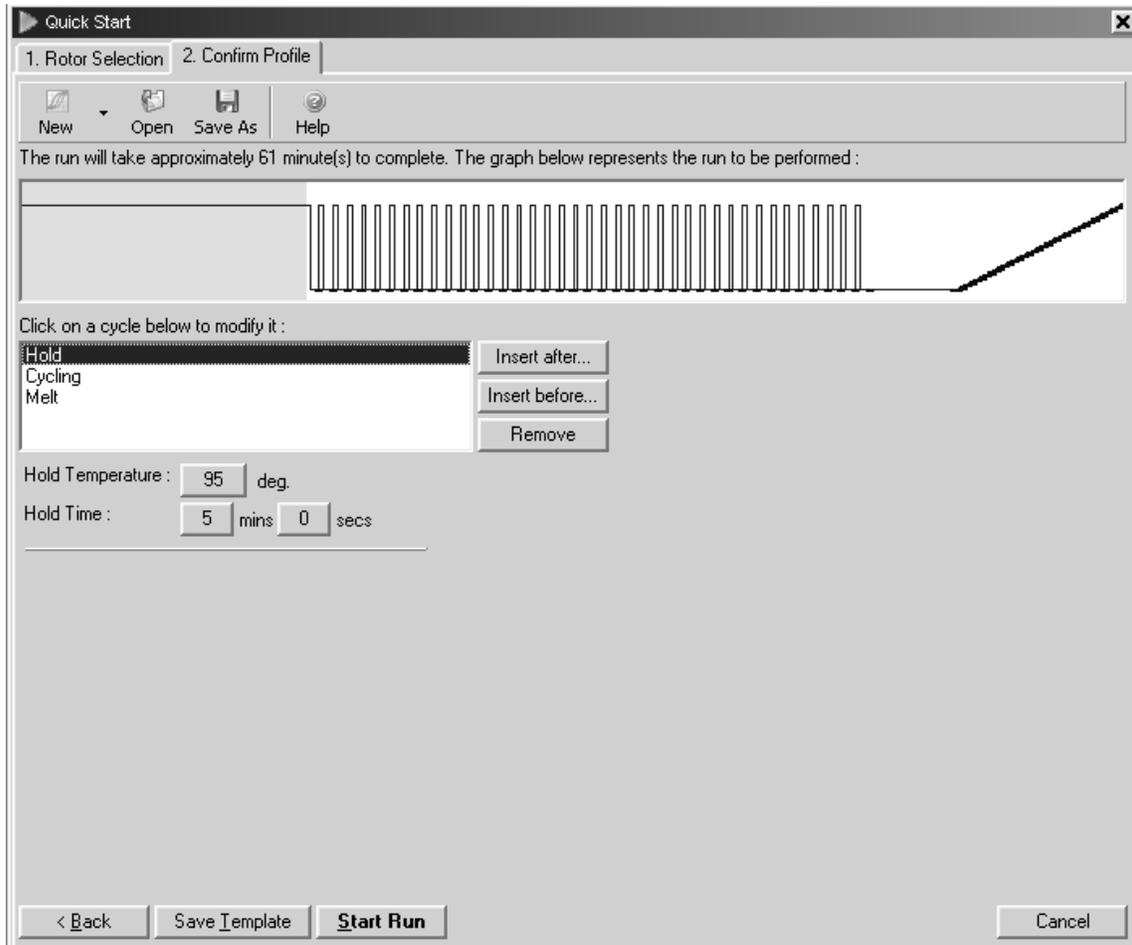
**5. Program the Rotor-Gene cyclers according to the program outlined in Table 2 (page 14) and Figures 1 and 2 (pages 15 and 16).**

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

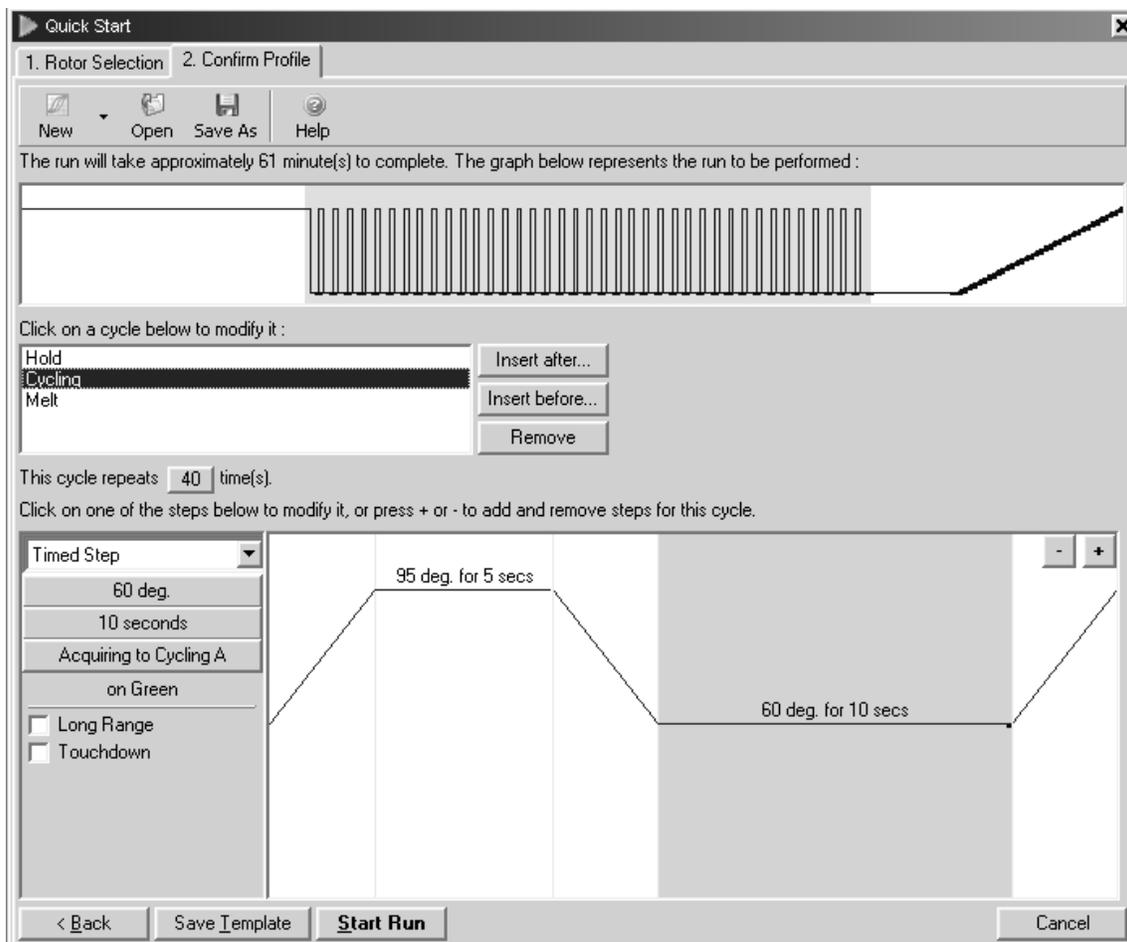
**Table 2. Cycling conditions**

<b>Step</b>	<b>Time</b>	<b>Temperature</b>	<b>Additional comments</b>
<b>PCR initial activation step</b>	5 min	95°C	HotStarTaq <i>Plus</i> DNA Polymerase is activated by this heating step
<b>Two-step cycling</b>			
Denaturation	5 s	95°C	
Combined annealing/ extension	10 s	60°C*	Perform fluorescence data collection
Number of cycles	35–40		The number of cycles depends on the amount of template DNA

\* This temperature should also be used for QuantiTect Primer Assays and for all primer sets with a  $T_m$  well below 60°C.



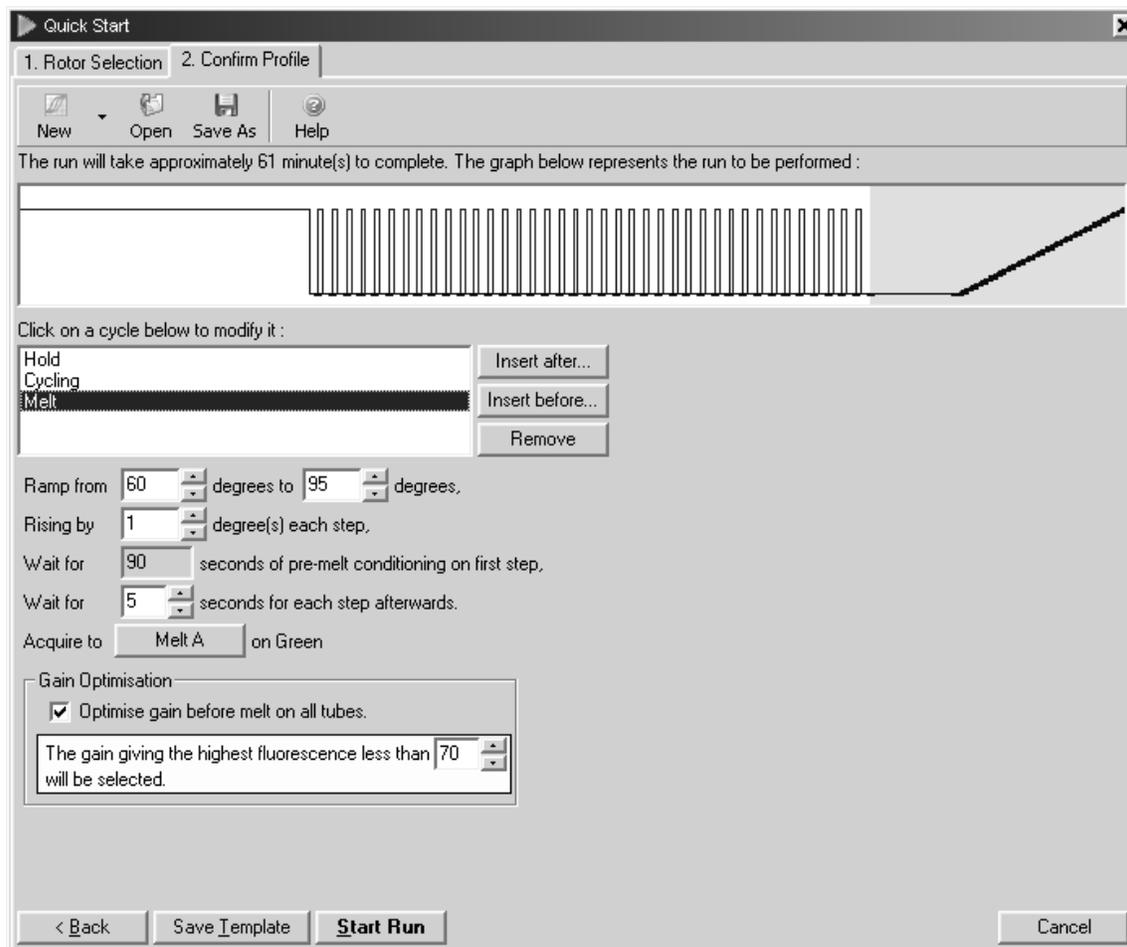
**Figure 1. PCR initial activation step.** PCR requires an initial incubation at 95°C for 5 min to activate HotStarTaq *Plus* DNA Polymerase.



**Figure 2. Two-step cycling.** PCR requires 35–40 cycles. Each cycle is comprised of 2 steps: 95°C for 5 s (denaturation step) and 60°C for 10 s (annealing/extension step).

6. **Place the PCR tubes in the Rotor-Gene cycler, and start the cycling program.**
7. **Optional: Perform melting curve analysis of the PCR product(s) to verify their specificity and identity (see Figure 3, page 17).**

Melting curve analysis is an analysis step built into the software of the Rotor-Gene cycler. Please follow instructions provided in the user manual supplied with your Rotor-Gene cycler.



**Figure 3. Melting curve analysis.** After PCR is completed, melting curve analysis can be performed to check the specificity of the reaction.

## 8. Optional: Check the specificity of the PCR product(s) by agarose gel electrophoresis.

## Protocol: Real-Time One-Step RT-PCR

This protocol can be used with the Rotor-Gene Q, Rotor-Gene 3000, or Rotor-Gene 6000.

### Important points before starting

- The Rotor-Gene SYBR Green RT-PCR Kit has been developed for use in a **two-step cycling** protocol, with a denaturation step at 95°C and a combined annealing/extension step at 60°C. This protocol will also work for primers with a  $T_m$  well below 60°C.
- For the highest efficiency in real-time RT-PCR using SYBR Green I, targets should ideally be 60–200 bp in length.
- After reverse transcription, the PCR step of the RT-PCR must start with an **initial incubation step of 5 minutes at 95°C** to activate HotStarTaq *Plus* DNA Polymerase.
- Set up all reactions on ice to avoid premature cDNA synthesis.
- We recommend a final reaction volume of 25  $\mu$ l.
- **Always start with the  $Mg^{2+}$  concentration as provided** in 2x Rotor-Gene SYBR Green RT-PCR Master Mix.
- If using QuantiTect Primer Assays, the final concentration in the reaction should be 1x. Also, follow the cycling protocol in Table 4.

### Procedure

1. **Thaw 2x Rotor-Gene SYBR Green RT-PCR Master Mix, template RNA, primers, and RNase-free water. Mix the individual solutions, and place them on ice. Rotor-Gene RT Mix should be taken from –15°C to –30°C immediately before use, always kept on ice, and returned to storage at –15°C to –30°C immediately after use.**
2. **Prepare a reaction mix according to Table 3 (page 19).**

Keep samples on ice while preparing the reaction mix.

**Note:** We strongly recommend starting with the  $Mg^{2+}$  concentration as provided in 2x Rotor-Gene SYBR Green RT-PCR Master Mix.

**Table 3. Reaction setup**

<b>Component</b>	<b>Volume/reaction</b>	<b>Final concentration</b>
2x Rotor-Gene SYBR Green RT-PCR Master Mix	12.5 $\mu$ l	1x
Primer A*	Variable	1 $\mu$ M
Primer B*	Variable	1 $\mu$ M
Rotor-Gene RT Mix	0.25 $\mu$ l	
Template RNA (added at step 4)	Variable	$\leq$ 100 ng/reaction
RNase-free water	Variable	
<b>Total reaction volume</b>	<b>25 <math>\mu</math>l</b>	

\* If using QuantiTect Primer Assays, the final concentration in the reaction should be 1x.

**3. Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes.**

Keep the PCR tubes on ice.

**4. Add template RNA ( $\leq$ 100 ng/reaction) to the individual PCR tubes containing the reaction mix.**

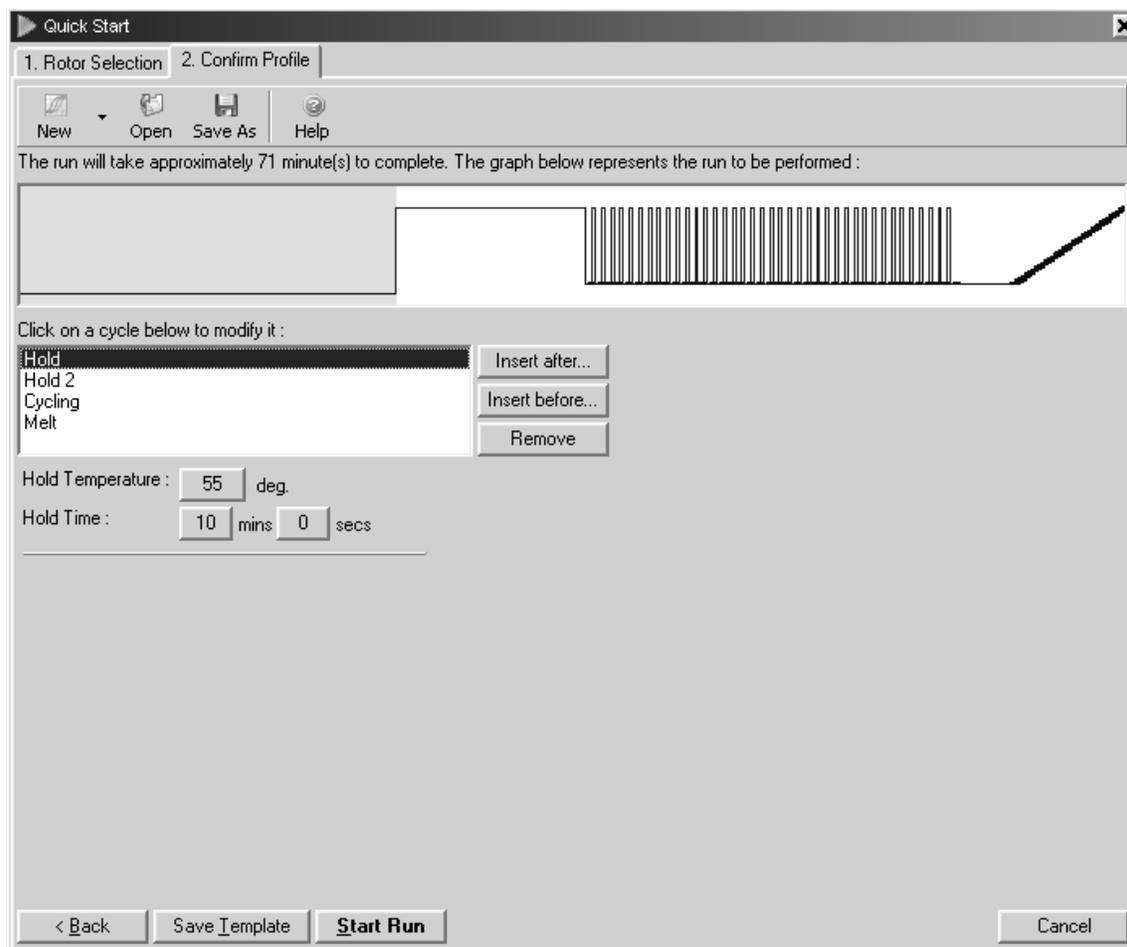
**5. Program the Rotor-Gene cycler according to the program outlined in Table 4 (page 20) and Figures 4–6 (pages 21–23).**

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

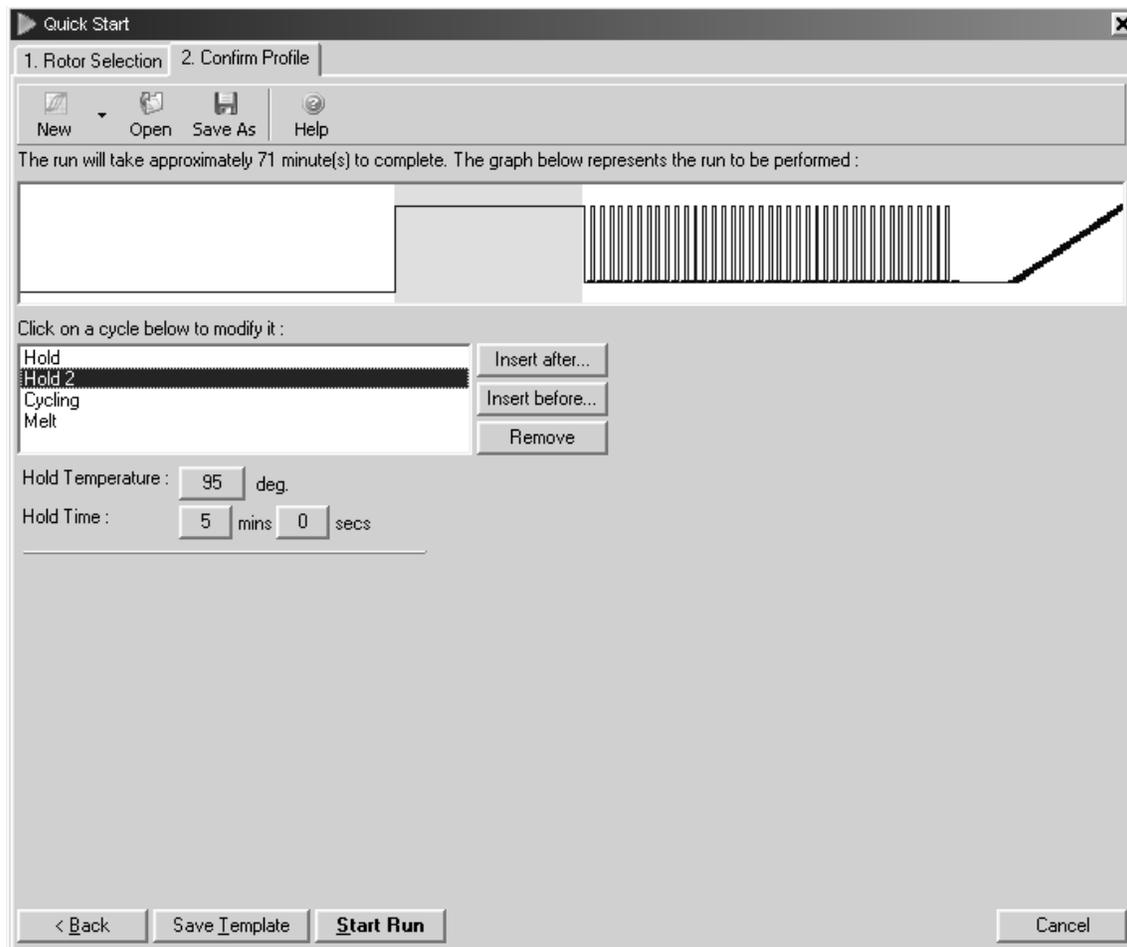
**Table 4. Cycling conditions**

<b>Step</b>	<b>Time</b>	<b>Temperature</b>	<b>Additional comments</b>
<b>Reverse transcription</b>	10 min	55°C	
<b>PCR initial activation step</b>	5 min	95°C	HotStarTaq <i>Plus</i> DNA Polymerase is activated by this heating step
<b>Two-step cycling</b>			
Denaturation	5 s	95°C	
Combined annealing/ extension	10 s	60°C*	Perform fluorescence data collection
Number of cycles	35–40		The number of cycles depends on the amount of template RNA

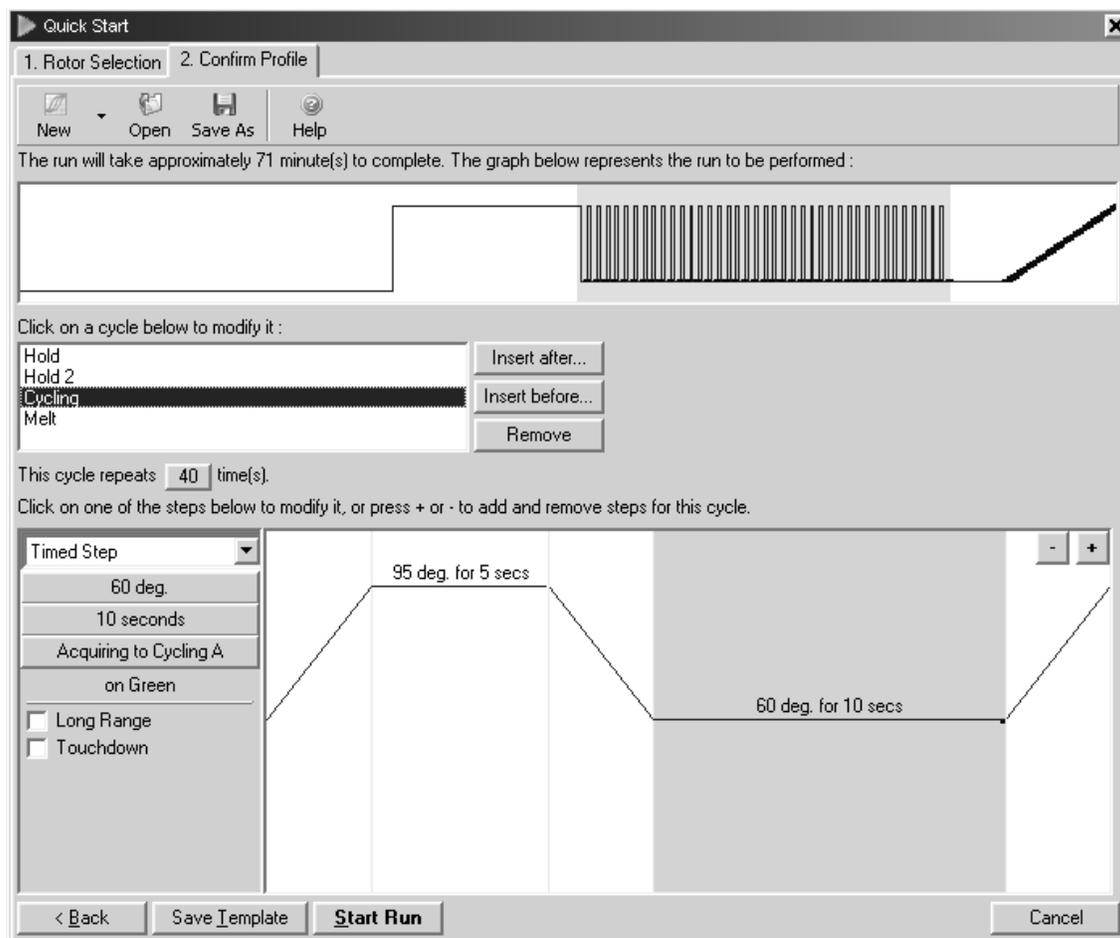
\* This temperature should also be used for QuantiTect Primer Assays and for all primer sets with a  $T_m$  well below 60°C.



**Figure 4. Reverse transcription.** Before starting PCR, reverse transcription must be carried out. Reactions are incubated at 55°C for 10 min.



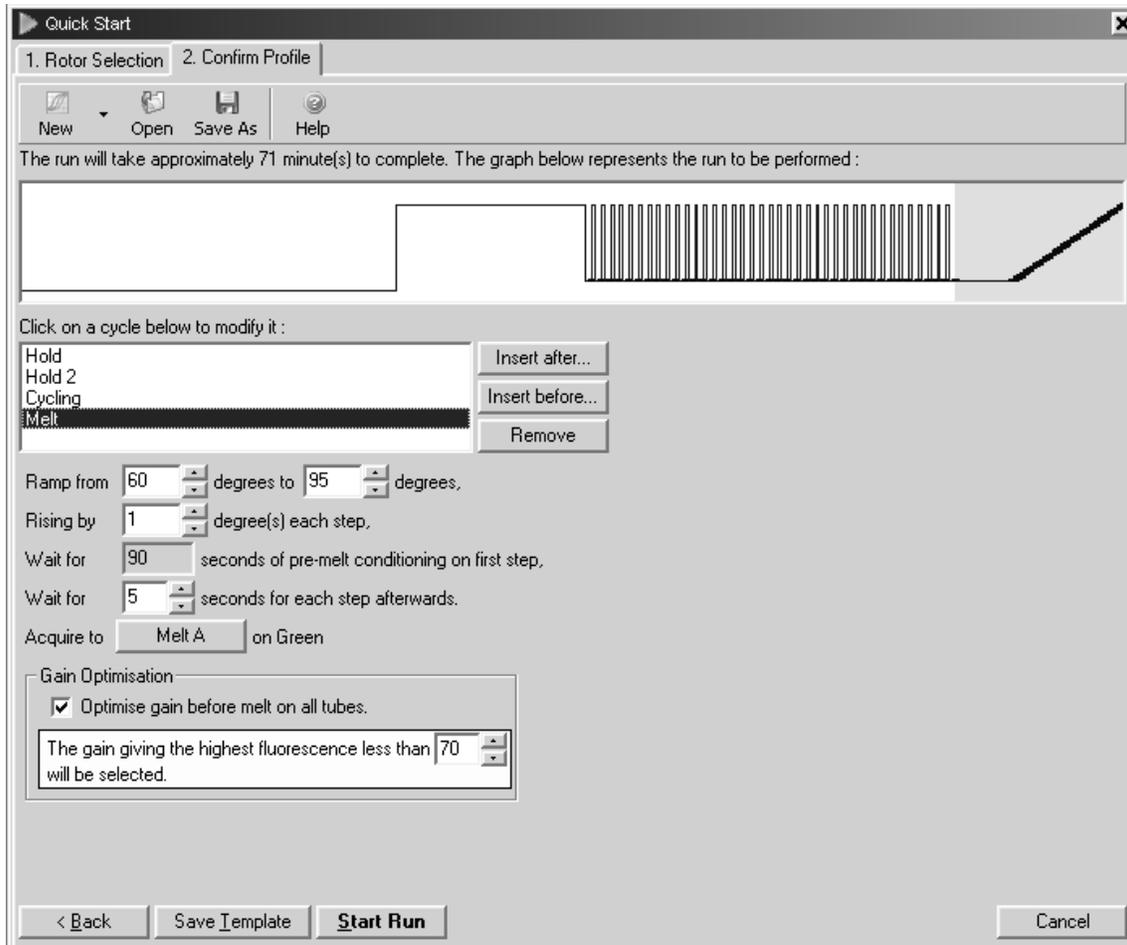
**Figure 5. PCR initial activation step.** After reverse transcription is completed, PCR can be carried out. PCR requires an initial incubation at 95°C for 5 min.



**Figure 6. Two-step cycling.** PCR requires 35–40 cycles. Each cycle is comprised of 2 steps: 95°C for 5 s (denaturation step) and 60°C for 10 s (annealing/extension step).

- 6. Place the PCR tubes in the Rotor-Gene cycler, and start the cycling program.**
- 7. Optional: Perform melting curve analysis of the RT-PCR product(s) to verify their specificity and identity (see Figure 7, page 24).**

Melting curve analysis is an analysis step built into the software of the Rotor-Gene cycler. Please follow instructions provided in the user manual supplied with your Rotor-Gene cycler.



**Figure 7. Melting curve analysis.** After PCR is completed, melting curve analysis can be performed to check the specificity of the reaction.

**8. Optional: Check the specificity of the RT-PCR product(s) by agarose gel electrophoresis.**

## 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](http://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](http://www.qiagen.com)).

### Comments and suggestions

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#### **No product, or product detected late in PCR, or only primer-dimers detected**

- |  |   |
|--|---|
| a) PCR annealing/extension time too short              | Use the recommended annealing/extension time of 10 s.   |
| b) Mg <sup>2+</sup> concentration adjusted             | Do not adjust the Mg <sup>2+</sup> concentration in 2x Rotor-Gene SYBR Green Master Mixes.  |
| c) HotStarTaq <i>Plus</i> DNA Polymerase not activated | Ensure that the cycling program includes the HotStarTaq <i>Plus</i> DNA Polymerase activation step (5 min at 95°C) as described in the protocols.   |
| d) Pipetting error or missing reagent                  | Check the concentrations and storage conditions of the reagents, including primers and template nucleic acid.* Repeat the PCR.  |
| e) Wrong or no detection step                          | Ensure that fluorescence detection takes place during the combined annealing/extension step.  |
| f) Primer concentration not optimal                    | Use each primer at a concentration of 1 μM, as described in the protocol. If using a 10x QuantiTect Primer Assay, the final concentration in the reaction should be 1x.<br><br>Check the concentrations of primers by spectrophotometry.* |
| g) Reaction volume too high                            | We recommend a final reaction volume of 25 μl.  |

\* For details, refer to "Guidelines for real-time PCR and RT-PCR" at [www.qiagen.com/resources/info](http://www.qiagen.com/resources/info).

## Comments and suggestions

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- |  |  |
|--|--|
| h) Problems with starting template   | Check the concentration, storage conditions, and quality of the starting template.*<br><br>If necessary, make new serial dilutions of template nucleic acid from the stock solutions. Repeat the PCR using the new dilutions.  |
| i) Insufficient amount of starting template                                | Increase the amount of template, if possible. Ensure that sufficient copies of the target nucleic acids are present in your sample.  |
| j) Insufficient number of cycles   | Increase the number of cycles in steps of 5 cycles.  |
| k) PCR product too long  | For optimal results, PCR products should be between 60 and 200 bp. PCR products should not exceed 300 bp.  |
| l) Primer design not optimal   | Check for PCR products by melting curve analysis* or gel electrophoresis. If no specific PCR products are detected, review the primer design guidelines.* Alternatively, use QuantiTect Primer Assays, which are predesigned primer sets for real-time RT-PCR (see ordering information, page 28). |
| m) No detection activated  | Check that fluorescence detection was activated in the cycling program.  |
| n) Primers degraded  | Check for possible degradation of primers on a denaturing polyacrylamide gel.  |
| o) <b>Two-step RT-PCR only:</b> Volumes of RT reaction added were too high | High volumes of RT reaction added to the PCR may reduce amplification efficiency and the linearity of the reaction. Generally, the volume of undiluted RT reaction added should not exceed 10% of the final PCR volume.  |
| p) <b>One-step RT-PCR only:</b> RT step not performed                      | Ensure that the cycling program includes the RT step (10 min at 55°C) as described in the protocol.  |
| q) Wrong dye channel chosen  | Ensure that the correct dye channel is activated for SYBR Green I.   |

\* For details, refer to "Guidelines for real-time PCR and RT-PCR" at [www.qiagen.com/resources/info](http://www.qiagen.com/resources/info).

## Comments and suggestions

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### Primer–dimers and/or nonspecific PCR products

- |   |  |
|---|--|
| a) Mg <sup>2+</sup> concentration adjusted      | Do not adjust the Mg <sup>2+</sup> concentration in 2x Rotor-Gene SYBR Green Master Mixes.   |
| b) Primer design not optimal                    | Check for PCR products by melting curve analysis* or gel electrophoresis. If no specific PCR products are detected, review the primer design guidelines.* Alternatively, use QuantiTect Primer Assays, which are predesigned primer sets for real-time RT-PCR (see ordering information, page 28).   |
| c) PCR product too long                         | For optimal results, PCR products should be between 60 and 200 bp. PCR products should not exceed 300 bp.  |
| d) Primers degraded                             | Check for possible degradation of primers on a denaturing polyacrylamide gel.  |
| e) Contamination of RNA sample with genomic DNA | Design primers that span exon–exon boundaries, so that only cDNA targets can be amplified and detected. Alternatively, use QuantiTect Primer Assays, which are predesigned primer sets that avoid amplification of genomic DNA where possible (see ordering information, page 28).<br><br>Alternatively, treat the RNA sample with DNase to digest the contaminating genomic DNA. If carrying out real-time two-step RT-PCR, perform reverse transcription with the QuantiTect Reverse Transcription Kit, which provides cDNA synthesis with integrated genomic DNA removal. |

\* For details, refer to “Guidelines for real-time PCR and RT-PCR” at [www.qiagen.com/resources/info](http://www.qiagen.com/resources/info).

## Ordering Information

Product	Contents	Cat. no.
Rotor-Gene SYBR Green PCR Kit (400)	For 400 x 25 $\mu$ l reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204074
Rotor-Gene SYBR Green RT-PCR Kit (400)	For 400 x 25 $\mu$ l reactions: 3 x 1.7 ml 2x Master Mix, 100 $\mu$ l RT Mix, 2 x 2 ml RNase-Free Water	204174
<b>Accessories</b>		
<b>QuantiTect Reverse Transcription Kit — for fast cDNA synthesis for sensitive real-time two-step RT-PCR</b>		
QuantiTect Reverse Transcription Kit (50)*	For 50 x 20 $\mu$ l reactions: gDNA Wipeout Buffer, Quantiscript <sup>®</sup> Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water	205311
<b>FastLane Cell cDNA Kit — for high-speed preparation of cDNA without RNA purification for use in real-time RT-PCR</b>		
FastLane Cell cDNA Kit (50)	Buffer FCW, Buffer FCP, and components for 50 x 20 $\mu$ l reverse-transcription reactions (gDNA Wipeout Buffer, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water)	215011
<b>QuantiTect Primer Assays — for use in real-time RT-PCR with SYBR Green detection (search for and order assays at <a href="http://www.qiagen.com/GeneGlobe">www.qiagen.com/GeneGlobe</a>)</b>		
QuantiTect Primer Assay (200)	For 200 x 50 $\mu$ l reactions or 400 x 25 $\mu$ l reactions: primer set supplied in single tube <sup>†</sup>	Varies
<b>Related products</b>		
<b>Rotor-Gene Probe PCR Kit — for fast real-time PCR and two-step RT-PCR using sequence-specific probes on Rotor-Gene cyclers</b>		
Rotor-Gene Probe PCR Kit (400)	For 400 x 25 $\mu$ l reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204374

\* Trial-size kit and larger kit available; please inquire.

† Primer set is a lyophilized mix of forward and reverse primers.

Product	Contents	Cat. no.
<b>Rotor-Gene Probe RT-PCR Kit — for fast real-time one-step RT-PCR using sequence-specific probes on Rotor-Gene cyclers</b>		
Rotor-Gene Probe RT-PCR Kit (400)	For 400 x 25 $\mu$ l reactions: 3 x 1.7 ml 2x Master Mix, 100 $\mu$ l RT Mix, 2 x 2 ml RNase-Free Water	204574
<b>Rotor-Gene Multiplex PCR Kit — for fast multiplex real-time PCR and two-step RT-PCR on Rotor-Gene cyclers</b>		
Rotor-Gene Multiplex PCR Kit (80)	For 80 x 25 $\mu$ l reactions: 1 ml 2x Master Mix, 2 ml RNase-Free Water	204772
Rotor-Gene Multiplex PCR Kit (400)	For 400 x 25 $\mu$ l reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204774

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## Notes

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