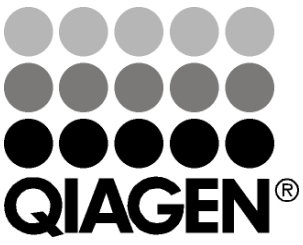
Second Edition **July 2011**

Rotor-Gene® Multiplex Handbook

Rotor-Gene Multiplex PCR Kit

Rotor-Gene Multiplex RT-PCR Kit

For fast multiplex real-time PCR, two-step RT-PCR, and one-step RT-PCR using sequence-specific probes on Rotor-Gene cyclers



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Contents

Kit Contents

Rotor-Gene Multiplex PCR Kit	**(80)**	**(400)**
Catalog no.	**204772**	**204774**
Number of 25 µl reactions*	**80**	**400**
2x Rotor-Gene Multiplex PCR Master Mix, containing: ■ HotStarTaq® *Plus* DNA Polymerase ■ Rotor-Gene Multiplex PCR Buffer ■ dNTP mix (dATP, dCTP, dGTP, dTTP)	1 ml	3 x 1.7 ml
RNase-Free Water	2 ml	2 x 2 ml
Handbook	1	1

Rotor-Gene Multiplex RT-PCR Kit	**(80)**	**(400)**
Catalog no.	**204972**	**204974**
Number of 25 µl reactions*	**80**	**400**
2x Rotor-Gene Multiplex RT-PCR Master Mix, containing: ■ HotStarTaq *Plus* DNA Polymerase ■ Rotor-Gene Multiplex RT-PCR Buffer ■ dNTP mix (dATP, dCTP, dGTP, dTTP)	1 ml	3 x 1.7 ml
Rotor-Gene RT Mix, a mixture of the QIAGEN® products: ■ Omniscript® Reverse Transcriptase ■ Sensiscript® Reverse Transcriptase	20 µl	100 µl
RNase-Free Water	2 ml	2 x 2 ml
Handbook	1	1

* Reaction setup can be automated on the QIAgility™ (www.qiagen.com/goto/QIAgility). However, the number of reactions will be 10–20% lower than indicated to guarantee process safety and optimal performance.

Shipping and Storage

Rotor-Gene Multiplex Kits are shipped on dry ice. The kits should be stored immediately upon receipt at –20°C in a constant-temperature freezer 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 Multiplex Master Mixes can also be stored protected from light at 2–8°C for up to 1 month without showing any reduction in performance.

Product Use Limitations

Rotor-Gene Multiplex 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.

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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 Multiplex 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 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

Component	Test
2x Rotor-Gene Multiplex PCR or RT-PCR Master Mix*	PCR sensitivity and reproducibility assay: Sensitivity and reproducibility in real-time PCR or RT-PCR are tested in parallel reactions containing variable amounts of nucleic acid templates.
HotStarTaq *Plus* DNA Polymerase†	Efficiency and reproducibility in PCR are tested. Functional absence of RNases,‡ exonucleases, and endonucleases is tested.
Rotor-Gene Multiplex PCR or RT-PCR Buffer†	Conductivity and pH are tested.
RNase-free water	Conductivity, pH, and RNase activities are tested.
Rotor-Gene RT Mix	Efficiency of cDNA synthesis and functional absence of RNases, exonucleases, and endonucleases are tested.

* See quality-control label inside the kit box or on the kit envelope for lot-specific values.

† Included in 2x Rotor-Gene Multiplex PCR Master Mix and 2x Rotor-Gene Multiplex RT-PCR Master Mix.

‡ Absence of RNases tested in 2x Rotor-Gene Multiplex RT-PCR Master Mix only.

Product Description

Component	Description
HotStarTaq *Plus* DNA Polymerase*	HotStarTaq *Plus* DNA Polymerase is a modified form of a recombinant 94 kDa DNA polymerase, originally isolated from *Thermus aquaticus*. The enzyme 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 Multiplex PCR or RT-PCR Buffer*	Novel buffer for fast and highly sensitive quantification of DNA, cDNA, or RNA targets in multiplex format; includes Factor MP to facilitate multiplex PCR
dNTP mix*	Contains dATP, dCTP, dGTP, and dTTP of ultrapure quality
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 *E. coli*.

* Included in 2x Rotor-Gene Multiplex PCR Master Mix and 2x Rotor-Gene Multiplex RT-PCR Master Mix.

† Supplied with the Rotor-Gene Multiplex RT-PCR Kit.

Introduction

Rotor-Gene Multiplex Kits provide rapid real-time quantification of genomic DNA, cDNA, or RNA targets on the Rotor-Gene Q in a multiplex format. The kits are also compatible with the Rotor-Gene 3000 and Rotor-Gene 6000. Depending on the cycler configuration, up to 4 targets can be quantified simultaneously in the same tube. Two kit formats are available:

- **Rotor-Gene Multiplex 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 39).
- **Rotor-Gene Multiplex 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.

High specificity and sensitivity in multiplex PCR and RT-PCR are achieved without any time-consuming optimization steps through the use of the hot-start enzyme HotStarTaq *Plus* DNA Polymerase together with a specialized 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 TaqMan® probes on 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 Multiplex Master Mixes

The components of 2x Rotor-Gene Multiplex PCR Master Mix include HotStarTaq *Plus* DNA Polymerase and Rotor-Gene Multiplex PCR Buffer (see descriptions below). 2x Rotor-Gene Multiplex RT-PCR Master Mix contains HotStarTaq *Plus* DNA Polymerase and Rotor-Gene Multiplex RT-PCR Buffer (see descriptions below). The optimized master mixes ensure that the PCR products in a multiplex reaction are amplified with the same efficiency and sensitivity as the PCR products in corresponding singleplex reactions.

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 Multiplex Buffers

Rotor-Gene Multiplex 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 Multiplex PCR Buffer is specifically developed for fast-cycling, multiplex, real-time PCR using sequence-specific probes. Rotor-Gene Multiplex RT-PCR Buffer is specifically developed for fast-cycling, multiplex, real-time one-step RT-PCR using sequence-specific probes. 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/probe annealing to a few seconds. This allows a combined annealing/extension step of only 15 seconds in multiplex PCR. In addition, the unique composition of the buffers supports the melting behavior of DNA, enabling short denaturation and annealing/extension times.

Rotor-Gene Multiplex PCR Buffer is based on the unique QIAGEN PCR buffer system. Rotor-Gene Multiplex RT-PCR Buffer is based on the unique QIAGEN OneStep RT-PCR buffer system. The buffers contain a balanced combination of KCl and $(NH_4)_2SO_4$, 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 $MgCl_2$ concentration, so optimization by titration of Mg^{2+} is not required. The buffers also contain Factor MP, which facilitates multiplex PCR. This synthetic factor increases the local concentration of primers and probes at the DNA template and stabilizes specifically bound primers and probes, allowing efficient

annealing and extension. The combination of these various components of Rotor-Gene Multiplex Buffers prevents multiple amplification reactions from affecting each other.

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

Use of 2x Rotor-Gene Multiplex 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 Multiplex 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® 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 39.

Sequence-specific probes

Rotor-Gene Multiplex Kits can be used with all types of probe. This handbook contains optimized protocols for use with TaqMan probes, a major type of sequence-specific probe used in quantitative, real-time PCR (see below). For

more details on sequence-specific probes, and their design and handling, see the appendix, page 36.

TaqMan probes

TaqMan probes are sequence-specific oligonucleotides with a fluorophore and a quencher moiety attached (Figure 1). The fluorophore is at the 5' end of the probe, and the quencher moiety is usually located at the 3' end or internally. During the extension phase of PCR, the probe is cleaved by the 5'→3' exonuclease activity of *Taq* DNA polymerase, separating the fluorophore and the quencher moiety. This results in detectable fluorescence that is proportional to the amount of accumulated PCR product.

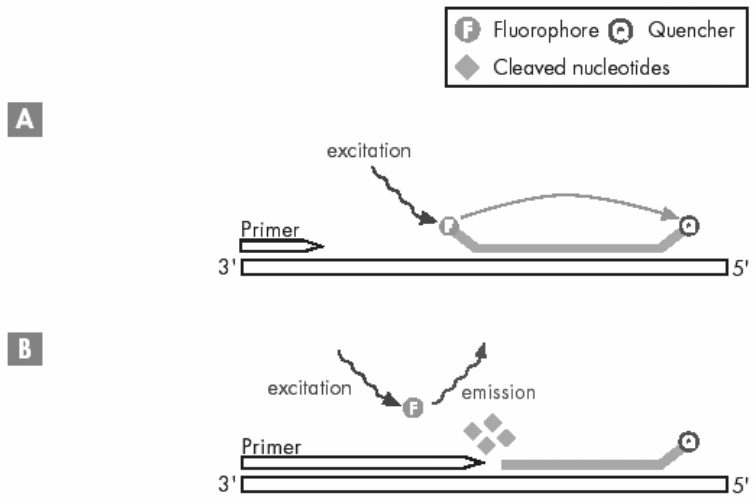

Figure 1. Principle of TaqMan probes in quantitative, real-time PCR. A Both the TaqMan probe and the PCR primers anneal to the target sequence during the PCR annealing step. The proximity of the quencher to the fluorophore strongly reduces the fluorescence emitted by the fluorophore. B During the PCR extension step, *Taq* DNA polymerase extends the primer. When the enzyme reaches the TaqMan probe, its 5'→3' exonuclease activity cleaves the fluorophore from the probe. The fluorescent signal from the free fluorophore is measured. This signal is proportional to the amount of accumulated PCR product.

Using the correct protocol

This handbook contains 2 protocols:

- Multiplex, real-time PCR and two-step RT-PCR (page 21)
- Multiplex, real-time one-step RT-PCR (page 26)

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

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.

- Primers and probes from an established oligonucleotide manufacturer. Primers should be of standard quality, and probes should be HPLC purified. Lyophilized primers and probes should be dissolved in TE buffer to provide a stock solution of 100 µM; concentration should be checked by spectrophotometry (for details, see appendix, page 36). Primer and probe stock solutions should be stored in aliquots at –20°C. Probe stock solutions should be protected from exposure to light.
- Nuclease-free (RNase/DNase-free) consumables. Special care should be taken to avoid nuclease contamination of PCR reagents and consumables.
- Rotor-Gene cycler
- PCR tubes or Rotor-Disc® for the Rotor-Gene cycler
- Optional: Trizma® base and EDTA for preparing TE buffer for storing primers and probes (see appendix, page 36). Use RNase/DNase-free water and plastic consumables to prepare TE buffer.
- Optional: QIAgility® for rapid, high-precision automated PCR setup; for details, visit www.qiagen.com/goto/QIAgility. Please note that the number of reactions will be 10–20% lower than indicated on the kit packaging to guarantee process safety and optimal performance.

Important Notes

Guidelines for effective multiplex assays

Rotor-Gene Multiplex Kits work with most existing probe systems that have been designed using standard design methods. However, for optimal performance of a probe system in quantitative, multiplex, real-time PCR, some considerations need to be made, including the choice of a compatible combination of reporter dyes (i.e., the fluorophores on the probes) and the quality of the primers and probes. Please read the following guidelines before starting.

- Check the functionality of each set of primers and probe in individual assays before combining the different sets in a multiplex assay.
- Choose compatible reporter dyes and quenchers. For details, see "Suitable combinations of reporter dyes", page 15.
- PCR products should be as short as possible, ideally 60–150 bp. For details, see the appendix, page 36.
- Always use the same algorithm or software to design the primers and probes. For optimal results, only combine assays that have been designed using the same parameters (e.g., similar melting points [T_m]). For details, see the appendix, page 36.
- Check the concentration and integrity of primers and probes before starting. For details, see the appendix, page 36.
- Check the user manual supplied with your Rotor-Gene cycler for **correct setup of the Rotor-Gene cycler for multiplex analysis** (e.g., setting up detection of multiple dyes from the same tube). Be sure to use the correct dye channel for each reporter dye used.
- Always start with the **cycling conditions specified in the protocols** on pages 21 and 26.
- Optimal analysis settings (e.g., threshold values and background settings) for each reporter dye channel are a prerequisite for accurate quantification data. For details, check the user manual supplied with your Rotor-Gene cycler.
- Perform appropriate controls for evaluating the performance of your multiplex assays (e.g., amplifying each target individually and comparing the results with those for the multiplex assay).

Suitable combinations of reporter dyes

Multiplex, real-time PCR requires the simultaneous detection of different fluorescent reporter dyes (Table 1). For accurate detection, the fluorescence spectra of the dyes should be well separated or exhibit only minimal overlap. **Please read the recommendations on the next few pages before starting**.

Table 1. Dyes commonly used in multiplex, real-time PCR on Rotor-Gene cyclers

Channel*	Excitation maximum (nm)	Emission maximum (nm)	Suitable dyes
Blue	365±20	460±15	Biosearch Blue®, Marina Blue®, Edans, Bothell Blue, Alexa Fluor® 350
Green	470±10	510±5	FAM™, Alexa Fluor 488
Yellow	530±5	555±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, Alexa Fluor 633
Crimson	680±5	712 long pass	Quasar 705, Alexa Fluor 680

* Channels correspond to those of the Rotor-Gene Q and Rotor-Gene 6000.

General recommendations

- Before starting, choose suitable combinations of reporter dyes and quenchers that are compatible with multiplex analysis using the detection optics of your Rotor-Gene cycler. Order the probes from an established oligonucleotide manufacturer.
- For optimal results, follow the recommended combinations of dyes shown in Table 2 (page 17) and Table 3 (page 19).

- For duplex analysis, the use of nonfluorescent quenchers (e.g., Black Hole Quencher® [BHQ®] on TaqMan probes) is preferred over fluorescent quenchers (e.g., TAMRA™ fluorescent dye). TAMRA quencher can be used in duplex analysis if the 2 reporter dyes are 6-FAM dye and HEX, JOE, or VIC dye.
- **For triplex and 4-plex analyses, use nonfluorescent quenchers.** For certain combinations of reporter dyes, triplex and 4-plex analyses may only be possible with nonfluorescent quenchers.

Recommendations for the Rotor-Gene Q and Rotor-Gene 6000

The Rotor-Gene Q and Rotor-Gene 6000 have up to 6 detection channels and use a separate high-power LED as an excitation source for each channel. This provides flexibility when selecting reporter dyes for multiplex assays. However, care must be taken to select suitable combinations of reporter dyes and channels that exhibit minimal crosstalk. Suitable combinations of reporter dyes for multiplex assays using the Rotor-Gene Q and Rotor-Gene 6000 are given in Table 2 (page 17).

- The Rotor-Gene Q and Rotor-Gene 6000 have 5 preset channels that provide the best choice for multiplex assays: Green, Yellow, Orange, Red and Crimson. Each channel detects reporter dyes that emit light at a particular wavelength.
- Check that each selected reporter dye is compatible with one of the dye channels installed on the instrument. Ensure that each reporter dye is detected by a different channel.
- Refer to the user manual supplied with your Rotor-Gene cycler for information on setting up additional dye channels and correctly setting up the instrument for multiplex analysis.

Table 2. Suitable combinations of reporter dyes for the Rotor-Gene Q and Rotor-Gene 6000

Type of assay	Green channel*	Yellow channel*	Orange channel	Red channel	Crimson channel
Duplex	6-FAM	HEX VIC Yakima Yellow			
Duplex	6-FAM		ROX		
Duplex	6-FAM			Cy5	
Duplex	6-FAM				Quasar 705
Triplex	6-FAM	HEX VIC Yakima Yellow	ROX		
Triplex	6-FAM	HEX VIC Yakima Yellow			Quasar 705
Triplex	6-FAM		ROX		Quasar 705
4-plex	6-FAM	HEX VIC Yakima Yellow	ROX		Quasar 705
4-plex	6-FAM	HEX VIC Yakima Yellow		Cy5	Quasar 705

* Use the green channel and the yellow channel to detect the least abundant target and the second least abundant target, respectively.

Recommendations for the Rotor-Gene 3000

The Rotor-Gene 3000 uses a 4-channel light source and a detection filter wheel with 6 detection filters. This provides flexibility when selecting reporter dyes for multiplex assays. However, care must be taken to select suitable combinations of reporter dyes and filters that exhibit minimal crosstalk. Suitable combinations of reporter dyes for multiplex assays using the Rotor-Gene 3000 are given in Table 3 (page 19).

- The Rotor-Gene 3000 has 4 preset channels that provide the best choice for multiplex assays. Each channel detects a particular reporter dye (FAM, JOE, ROX, or Cy5) and is named after the dye it detects. Other reporter dyes with similar spectra can also be detected by these channels, and do not require calibration.
- The detection filter wheel contains three 10 nm band-pass filters (only signals within a certain wavelength band can pass through) and 3 high-pass filters (only signals with a wavelength above a certain limit can pass through). With regard to the 4 preset channels, the FAM, JOE, and ROX channels each use a 10 nm band-pass filter, while the Cy5 channel uses a high-pass filter. The band-pass filters are named after the wavelength they let through followed by the unit “nm”: 510 nm, 555 nm, and 610 nm. The high-pass filter is named after its wavelength limit followed by “hp”: 665 hp. There are 2 additional high-pass filters (610 hp and 585 hp), which can be used to detect dyes that cannot be detected using the preset channels. However, their use may be limited in multiplex assays.
- Check that each selected reporter dye is compatible with one of the detection channels installed on the instrument. Ensure that each reporter dye is detected by a different channel.
- Refer to the Rotor-Gene 3000 software manual for additional information on setting up detection channels and correctly setting up the instrument for multiplex analysis.

Table 3. Suitable combinations of reporter dyes for the Rotor-Gene 3000

Type of assay	Channel 1 (470/510)*†	Channel 2 (530/555)*†	Channel 3 (585/610)*†	Channel 4 (625/665)*†
Duplex	6-FAM	HEX JOE VIC		
Duplex	6-FAM		Texas Red ROX	
Duplex	6-FAM			Cy5
Triplex	6-FAM	HEX JOE VIC	Texas Red ROX	
Triplex	6-FAM	HEX JOE VIC		Cy5
Triplex	6-FAM		ROX‡ Cy3.5‡	Cy5
4-plex	6-FAM	HEX JOE VIC	ROX‡	Cy5

* The numbers in parentheses indicate the wavelengths of the excitation light source and the detection filter.

† Use channel 1, channel 2, and channels 3 and 4 to detect the least abundant target, the second least abundant target, and the 2 most abundant targets, respectively.

‡ We do not recommend using channel 3 to detect Texas Red dye if channel 4 is also being used. This is because Texas Red dye is partly detected by channel 4.

Controls

No template control (NTC)

All quantification experiments should include an NTC, containing all the components of the reaction except for the template. This enables detection of contamination.

No RT control

All RT-PCR experiments should include a negative control to test for contaminating DNA. However, detection of this contamination can be eliminated by using primers or probes that avoid amplification and detection of genomic DNA sequences. If it is not possible to use such primers or probes, DNA contamination can be detected by performing a control reaction in which no reverse transcription is possible. The control “no RT reaction” contains all components including template RNA, except for the reverse transcriptase. Reverse transcription therefore cannot take place. When an aliquot of this control is used as a template in PCR, the only template available would be contaminating DNA.

Alternatively, DNA in the sample can be removed by digestion with DNase before RT-PCR amplification.

Positive control

In some cases it may be necessary to include a positive control, containing a known concentration or copy number of template. Positive controls can be absolute standards or known positive samples.

Absolute standards include commercially available standards and in-lab standards, such as a plasmid containing cloned sequences. Absolute standards are used at a known copy number and provide quantitative information.

A positive sample is usually a substitute for an absolute standard and is used only to test for presence or absence of the target.

Protocol: Multiplex, 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

- Always start with the **cycling conditions** and **primer concentrations** specified in this protocol.
- We strongly recommend testing the performance of primer–probe sets in individual assays before combining them in a multiplex assay.
- Read "Guidelines for effective multiplex assays", page 14. Check whether your Rotor-Gene cycler is compatible with the chosen combination of reporter dyes.
- If using an already established multiplex real-time PCR assay, use the previously established primer and probe concentrations in combination with the cycling conditions specified in this protocol. It is not necessary to determine primer limiting concentrations again.
- **Optimal analysis settings are a prerequisite for accurate quantification data.** For data analysis, you should always adjust the analysis settings (e.g., threshold values and background settings) for analysis of every reporter dye channel in every run. For details, refer to the user manual supplied with your Rotor-Gene cycler.

Things to do before starting

- For ease of use, we recommend preparing for each of your targets a 20x primer–probe mix containing target-specific primers and probe. A 20x primer–probe mix for multiplex PCR consists of 10 µM forward primer, 10 µM reverse primer, and 4 µM probe in TE buffer.

Procedure

1. **Thaw 2x Rotor-Gene Multiplex PCR Master Mix, template DNA or cDNA, primer and probe solutions, and RNase-free water. Mix the individual solutions, and place them on ice.**
2. **Prepare a reaction mix according to Table 4.**

   Note: We strongly recommend starting with the optimized Mg^{2+} concentration provided by 2x Rotor-Gene Multiplex PCR Master Mix.

   Note: Due to the hot start, it is not necessary to keep samples on ice during reaction setup nor while programming the Rotor-Gene cycler.

Table 4. Reaction setup

Component	Volume/reaction	Final concentration
2x Rotor-Gene Multiplex PCR Master Mix	12.5 µl	1x
20x primer–probe mix 1*	1.25 µl	0.5 µM forward primer 1† 0.5 µM reverse primer 1† 0.2 µM probe 1‡
20x primer–probe mix 2*	1.25 µl	0.5 µM forward primer 2† 0.5 µM reverse primer 2† 0.2 µM probe 2‡
Only for triplex and 4-plex PCR: 20x primer–probe mix 3*	1.25 µl	0.5 µM forward primer 3† 0.5 µM reverse primer 3† 0.2 µM probe 3‡
Only for 4-plex PCR: 20x primer–probe mix 4*	1.25 µl	0.5 µM forward primer 4† 0.5 µM reverse primer 4† 0.2 µM probe 4‡
RNase-free water	Variable	–
Template DNA or cDNA (added at step 4)	Variable	≤100 ng/reaction
Total reaction volume	**25 µl***	**–**

* A 20x primer–probe mix for multiplex PCR consists of 10 µM forward primer, 10 µM reverse primer, and 4 µM probe in TE buffer.

† A final primer concentration of 0.5 µM is optimal. Before adapting primer concentration, check the concentration of your primer solutions.

‡ A final probe concentration of 0.2 µM gives satisfactory results in most cases. Depending on the synthesis quality and purification method used, the optimal concentration may be between 0.1 µM and 0.4 µM.

3. **Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes.**
4. **Add template DNA or cDNA (≤100 ng) to the individual PCR tubes.**

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

5. **Program the Rotor-Gene cycler according to Table 5 (below) and Figures 2 and 3 (pages 24 and 25).**

   Note: Check the user manual supplied with your Rotor-Gene cycler for correct instrument setup for multiplex analysis (e.g., setting up detection of multiple dyes from the same tube). Be sure to activate the detector for each reporter dye used.

Table 5. 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
2-step cycling			**Important: Optimal performance is only assured using these cycling conditions**
Denaturation	**15 s**	**95°C**	
Annealing/extension	**15 s**	**60°C**	Combined annealing/ extension step with fluorescence data collection
Number of cycles	40–45		The number of cycles depends on the amount of template DNA or cDNA and the expression level of the target gene

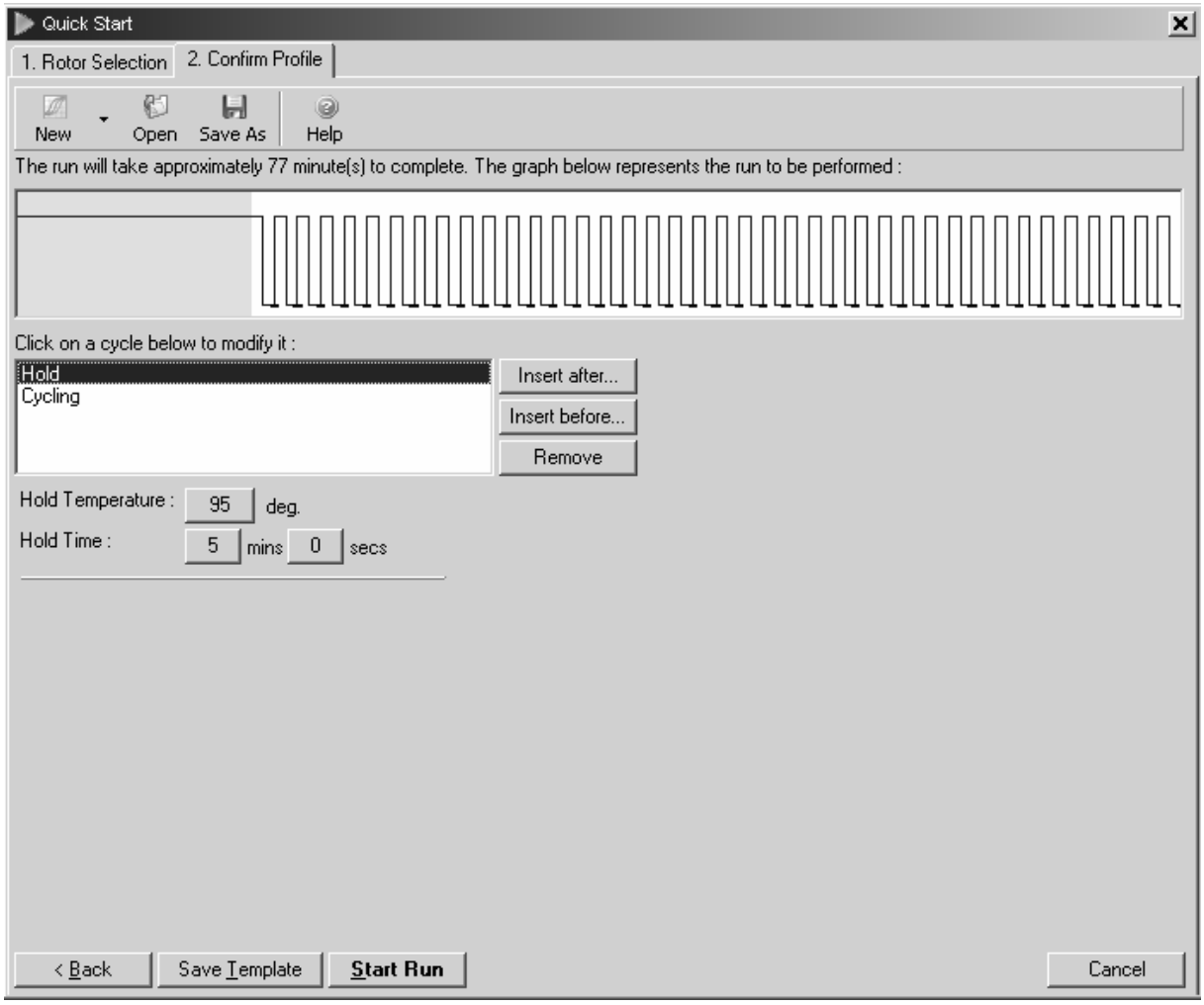

Figure 2. PCR initial activation step. PCR requires an initial incubation at 95°C for 5 min to activate HotStarTaq *Plus* DNA Polymerase.

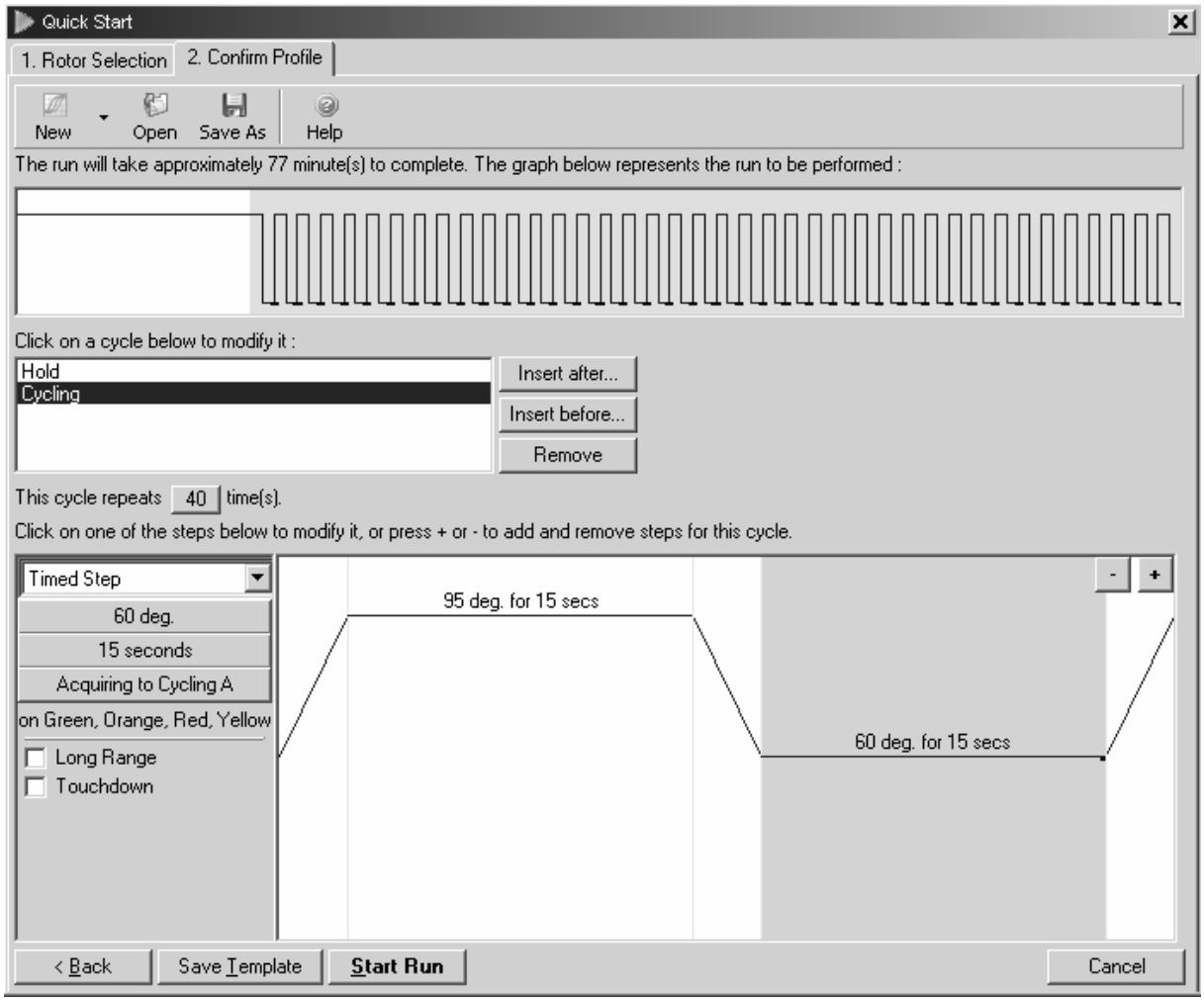

Figure 3. Two-step cycling. PCR requires 40–45 cycles. Each cycle is comprised of 2 steps: 95°C for 15 s (denaturation step) and 60°C for 15 s (annealing/extension step).

6. **Place the PCR tubes in the Rotor-Gene cycler, and start the cycling program.**
7. **Perform data analysis.**

   Before performing data analysis, select each channel and optimize the analysis settings (e.g., threshold values). Optimal analysis settings are a prerequisite for accurate quantification data.

Protocol: Multiplex, 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

- Always start with the **cycling conditions** and **primer concentrations** specified in this protocol.
- We strongly recommend testing the performance of primer–probe sets in individual assays before combining them in a multiplex assay.
- Read "Guidelines for effective multiplex assays", page 14. Check whether your Rotor-Gene cycler is compatible with the chosen combination of reporter dyes.
- If using an already established multiplex real-time RT-PCR assay, use the previously established primer and probe concentrations in combination with the cycling conditions specified in this protocol. It is not necessary to determine primer limiting concentrations again.
- **Optimal analysis settings are a prerequisite for accurate quantification data.** For data analysis, you should always adjust the analysis settings (e.g., threshold values and background settings) for analysis of every reporter dye channel in every run. For details, refer to the user manual supplied with your Rotor-Gene cycler.

Things to do before starting

- For ease of use, we recommend preparing for each of your targets a 20x primer–probe mix containing target-specific primers and probe. A 20x primer–probe mix for multiplex RT-PCR consists of 8 µM forward primer, 8 µM reverse primer, and 4 µM probe in TE buffer.

Procedure

1. **Thaw 2x Rotor-Gene Multiplex RT-PCR Master Mix, template RNA, primer and probe solutions, and RNase-free water. Mix the individual solutions, and place them on ice. Rotor-Gene RT Mix should be taken from –20°C immediately before use, always kept on ice, and returned to storage at –20°C immediately after use.**

2. **Prepare a reaction mix according to Table 6.**

   Keep samples on ice while preparing the reaction mix.

   Note: We strongly recommend starting with the optimized Mg^{2+} concentration provided by 2x Rotor-Gene Multiplex RT-PCR Master Mix.

Table 6. Reaction setup

Component	Volume/reaction	Final concentration
2x Rotor-Gene Multiplex RT-PCR Master Mix	12.5 µl	1x
20x primer–probe mix 1*	1.25 µl	0.4 µM forward primer 1† 0.4 µM reverse primer 1† 0.2 µM probe 1‡
20x primer–probe mix 2*	1.25 µl	0.4 µM forward primer 2† 0.4 µM reverse primer 2† 0.2 µM probe 2‡
Only for triplex and 4-plex PCR: 20x primer–probe mix 3*	1.25 µl	0.4 µM forward primer 3† 0.4 µM reverse primer 3† 0.2 µM probe 3‡
Only for 4-plex PCR: 20x primer–probe mix 4*	1.25 µl	0.4 µM forward primer 4† 0.4 µM reverse primer 4† 0.2 µM probe 4‡
Rotor-Gene RT Mix	0.25 µl	–
RNase-free water	Variable	–
Template RNA (added at step 4)	Variable	≤100 ng/reaction
Total reaction volume	**25 µl***	**–**

* A 20x primer–probe mix for multiplex RT-PCR consists of 8 µM forward primer, 8 µM reverse primer, and 4 µM probe in TE buffer.

† A final primer concentration of 0.4 µM is optimal. Before adapting primer concentration, check the concentration of your primer solutions.

‡ A final probe concentration of 0.2 µM gives satisfactory results in most cases. Depending on the synthesis quality and purification method used, the optimal concentration may be between 0.1 µM and 0.4 µM.

3. **Mix the reaction mix thoroughly, and dispense appropriate volumes into PCR tubes.**
4. **Add template RNA (≤100 ng) to the individual PCR tubes.**
5. **Program the Rotor-Gene cycler according to Table 7 (below) and Figures 4–6 (pages 29–31).**

   Note: Check the user manual supplied with your Rotor-Gene cycler for correct instrument setup for multiplex analysis (e.g., setting up detection of multiple dyes from the same tube). Be sure to activate the detector for each reporter dye used.

Table 7. Cycling conditions

Step	Time	Temperature	Additional comments
Reverse transcription	**15 min**	**50°C**	
PCR initial activation step	**5 min**	**95°C**	HotStarTaq *Plus* DNA Polymerase is activated by this heating step
2-step cycling			**Important: Optimal performance is only assured using these cycling conditions**
Denaturation	**15 s**	**95°C**	
Annealing/extension	**15 s**	**60°C**	Combined annealing/ extension step with fluorescence data collection
Number of cycles	40–45		The number of cycles depends on the amount of template RNA and the expression level of the target gene

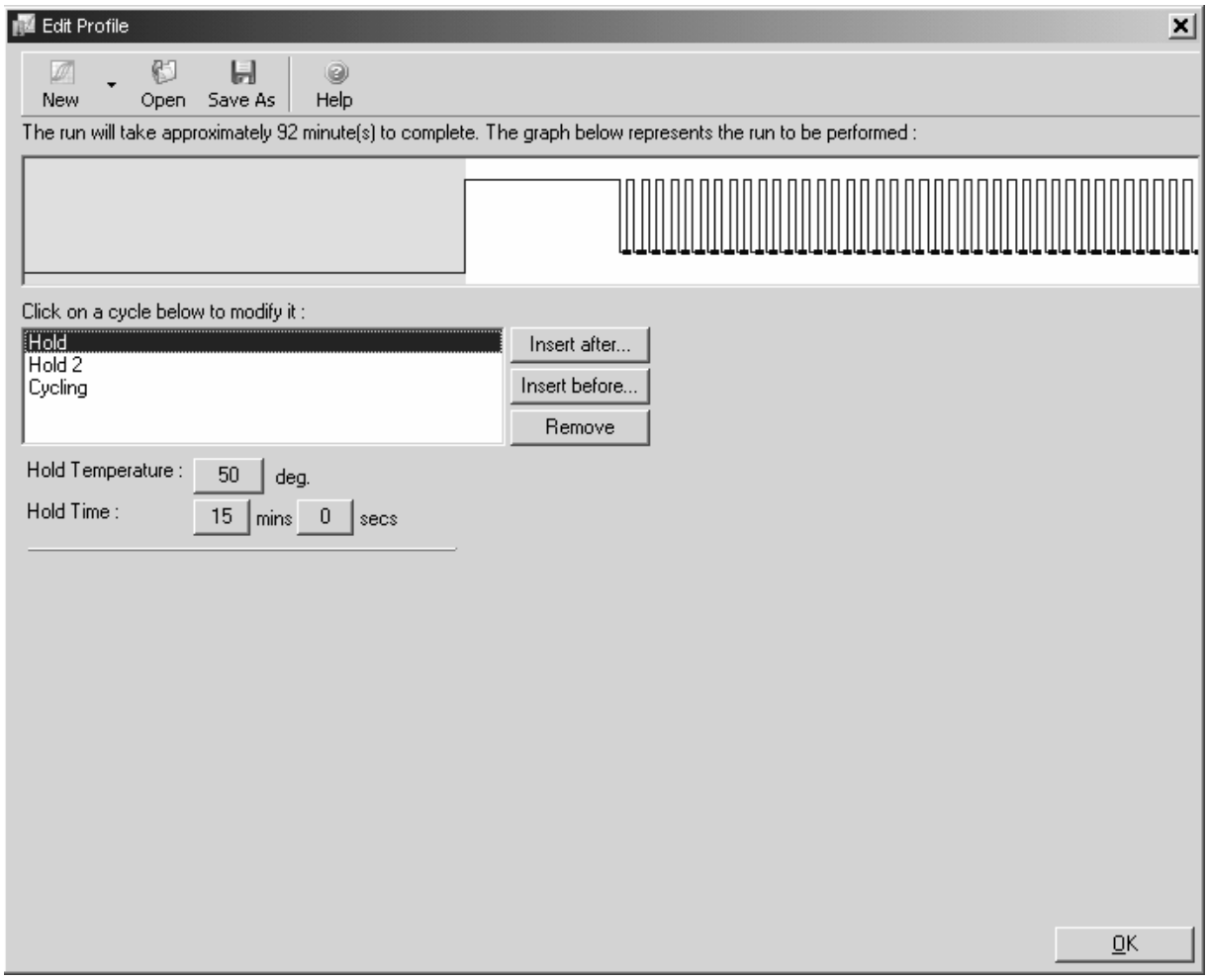

Figure 4. Reverse transcription. Before starting PCR, reverse transcription must be carried out. Reactions are incubated at 50°C for 15 min.

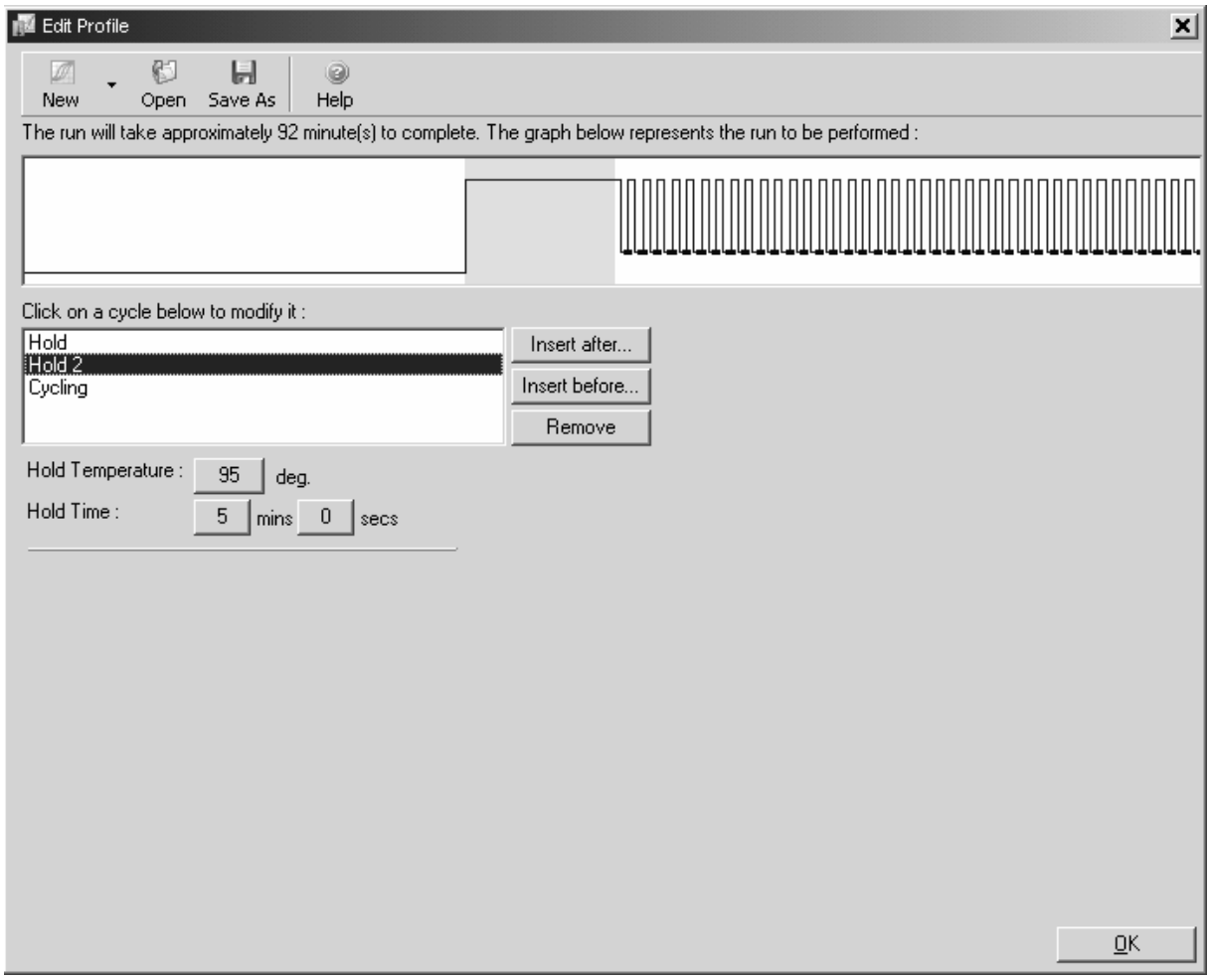

Figure 5. PCR initial activation step. After reverse transcription is completed, PCR can be carried out. PCR requires an initial incubation at 95°C for 5 min to activate HotStarTaq *Plus* DNA Polymerase.

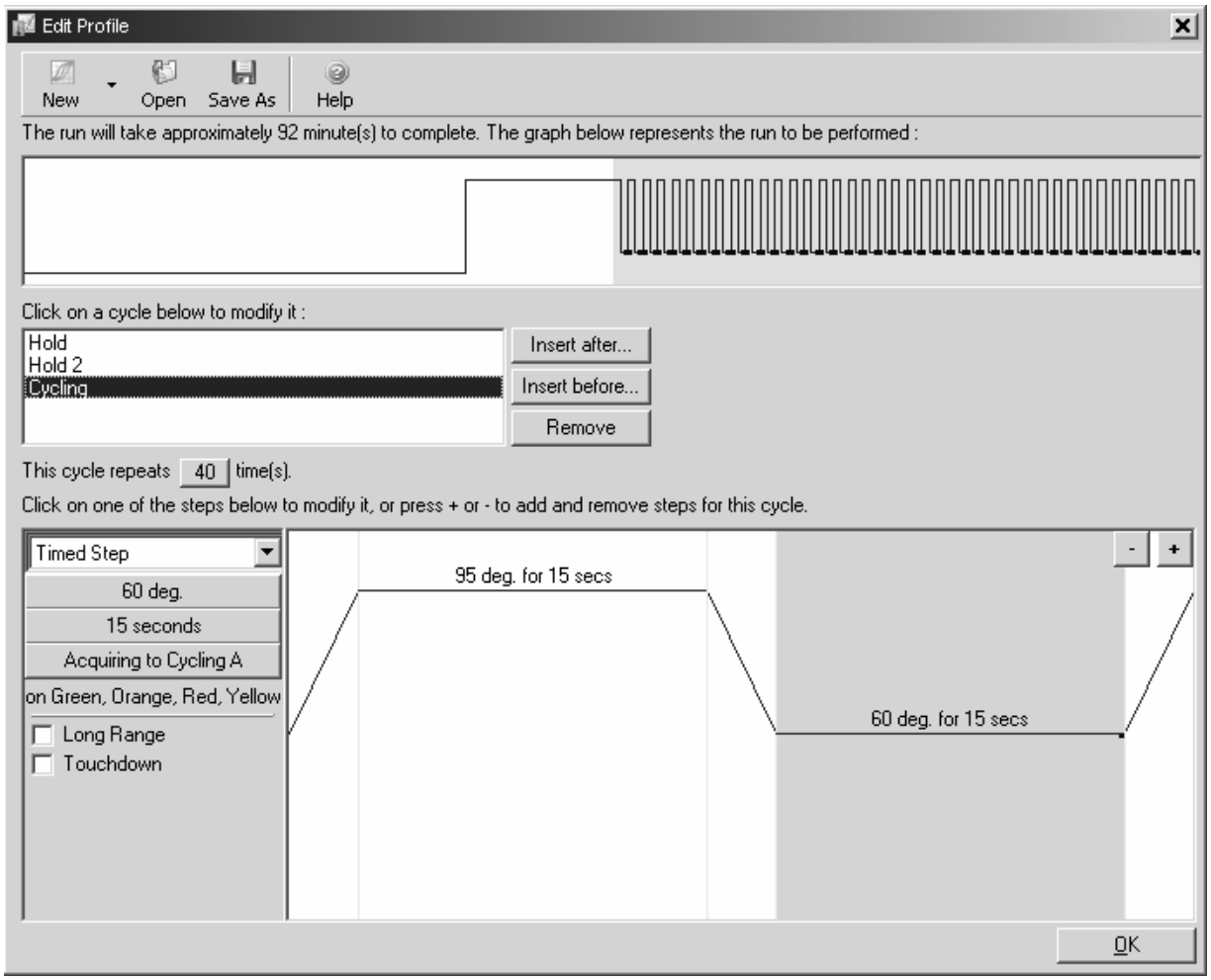

Figure 6. Two-step cycling. PCR requires 40–45 cycles. Each cycle is comprised of 2 steps: 95°C for 15 s (denaturation step) and 60°C for 15 s (annealing/extension step).

6. **Place the PCR tubes in the Rotor-Gene cycler, and start the cycling program.**
7. **Perform data analysis.**

   Before performing data analysis, select each channel and optimize the analysis settings (e.g., threshold values). Optimal analysis settings are a prerequisite for accurate quantification data.

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
No signal, or one or more signals detected late in PCR	
a) Wrong cycling conditions	Always start with the optimized cycling conditions specified in the protocols. Be sure that the cycling conditions include the initial step for activation of HotStarTaq *Plus* DNA Polymerase (95°C for 5 min), and the specified times for denaturation and annealing/extension. If performing one-step RT-PCR, be sure that the cycling conditions include the RT step (50°C for 15 min) prior to the HotStarTaq *Plus* DNA Polymerase activation step.
b) HotStarTaq *Plus* DNA Polymerase not activated	Ensure that the cycling program includes the HotStarTaq *Plus* DNA Polymerase activation step (5 min at 95°C) as described in the protocols.
c) Pipetting error or missing reagent	Check the concentrations and storage conditions of the reagents, including primers, probes, and template nucleic acid. See the appendix, page 36, for details on evaluating the concentration of primers and probes. Repeat the assay.
d) Wrong or no detection step	Ensure that fluorescence detection takes place during the combined annealing/extension step when using TaqMan probes.

	Comments and suggestions
e) Primer or probe concentration not optimal	In most cases, a primer concentration of 0.5 µM (for PCR and two-step RT-PCR) or 0.4 µM (for one-step RT-PCR) gives satisfactory results. Depending on the real-time PCR assay, results may be improved by adjusting primer concentration within the range of 0.3–0.6 µM. In most cases, a probe concentration of 0.2 µM gives satisfactory results. Depending on the quality of your probe, results may be improved by adjusting probe concentration within the range of 0.1–0.4 µM. In particular, when using neighboring channels, reducing the probe concentration to 0.1 µM might limit potential crosstalk. Check the concentrations of primers and probes by spectrophotometry (see appendix, page 36). If using commercial probe-based assays (e.g., TaqMan Gene Expression Assays), the final concentration in reactions should be 1x, as recommended by the supplier.
f) Mg^{2+} concentration not optimal	While the Mg^{2+} concentration in 2x Rotor-Gene Multiplex Master Mixes has been optimized, results may be improved by increasing the final Mg^{2+} concentration by 0.5–1 mM.
g) Problems with starting template	Check the concentration, storage conditions, and quality of the starting nucleic acids. If necessary, make new serial dilutions of template nucleic acid from the stock solutions. Repeat the assay using the new dilutions. Ensure that all reagents, buffers, and solutions used for purification and dilution of template nucleic acids are free of nucleases.
h) 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.
i) Insufficient number of cycles	Increase the number of cycles.

	Comments and suggestions
j) Probe design not optimal	If the amplification reaction itself was successful (this can be checked by gel electrophoresis analysis of the PCR products), there may be a problem with the probe. Review the probe design guidelines (see appendix, page 36).
k) Wrong dye channel chosen	Ensure that the correct dye channel is activated. Check whether the chosen combination of reporter dyes is compatible with the selected dye channels.
l) Fluorescence crosstalk	Check that the reporter dyes used in your assay are suitable for multiplex analysis on your Rotor-Gene cycler. Run appropriate controls to estimate potential crosstalk effects.

Differences in C_T values or in PCR efficiencies between a multiplex assay and the corresponding singleplex assays

a) Wrong cycling conditions	Always start with the optimized cycling conditions specified in the protocols. Be sure that the cycling conditions include the initial step for activation of HotStarTaq *Plus* DNA Polymerase (95°C for 5 min), and the specified times for denaturation and annealing/extension. If performing one-step RT-PCR, be sure that the cycling conditions include the RT step (50°C for 15 min) prior to the HotStarTaq *Plus* DNA Polymerase activation step.
b) Analysis settings (e.g., threshold settings) not optimal	Check the analysis settings for each reporter dye. Repeat analysis using optimal settings for each reporter dye.
c) Partial spectral overlap of reporter dyes	Since multiplex assays use multiple probes, each with a fluorescent dye, the increased fluorescent background may affect the shape of the amplification plots obtained on Rotor-Gene cyclers. This may lead to differences in C_T values of up to 5% between the multiplex assay and the corresponding singleplex assays; this can usually be avoided by using optimal threshold settings. Reducing the probe concentration to 0.1 µM might improve results.

	Comments and suggestions
No linearity in ratio of C_T value to log of the template amount	
a) Template amount too high	When signals are coming up at very early C_T values, adjust the background settings accordingly.
b) Template amount too low	Increase template amount if possible. Note that detection of very low starting copy numbers may not be in the linear range of a standard curve.
Increased fluorescence or C_T value for "No Template" control	
a) Contamination of reagents	Discard all the components of the multiplex assay (e.g., master mix, primers, and probes). Repeat the multiplex assay using new components.
b) Minimal probe degradation, leading to sliding increase in fluorescence	Check the amplification plots, and adjust the threshold settings.

Appendix: Assay Design and Handling Primers and Probes

Important factors for success in quantitative, multiplex, real-time PCR include the design of optimal primer pairs and probes, the use of appropriate primer and probe concentrations, and the correct storage of primers and probes.

Assay design

Guidelines for the optimal design of primers and probes are given below. It is particularly important to minimize nonspecific annealing of primers and probes. This can be achieved through careful assay design.

T_m of primers for TaqMan assays

- Use specialized design software (e.g., Beacon Designer) to design primers and probes.
- T_m of all primers should be 58–62°C and within 2°C of each other.
- T_m of probes should be 8–10°C higher than the T_m of the primers.
- Avoid a guanidine at the 5' end of probes, next to the reporter, since this causes quenching.
- Avoid runs of 4 or more of the same nucleotide, especially of guanidine.
- Choose the binding strand so that the probe has more C than G bases.
- All assays should be designed using the same settings to ensure that they will work optimally under the same cycling conditions (60°C annealing/extension).

Primer sequence

- Length: 18–30 nucleotides.
- GC content: 30–70%.
- Always check the specificity of primers by performing a BLAST® search (www.ncbi.nlm.nih.gov/blast). Ensure that primer sequences are unique for your template sequence.
- Check that primers and probes are not complementary to each other.
- Try to avoid highly repetitive sequences.
- Avoid complementarity of 2 or 3 bases at the 3' ends of primer pairs to minimize primer–dimer formation.
- Avoid mismatches between the 3' end of primers and the template sequence.
- Avoid runs of 3 or more Gs and/or Cs at the 3' end.

- Avoid complementary sequences within a primer sequence and between the primer pair.

Product size

Ensure that the length of PCR products is 60–150 bp. Some longer amplicons may amplify efficiently in multiplex PCR, with minimal optimization.

Handling and storing primers and probes

Guidelines for handling and storing primers and probes are given below. For optimal results, we recommend only combining primers of comparable quality.

Storage buffer

Lyophilized primers and probes should be dissolved in a small volume of low-salt buffer to give a concentrated stock solution (e.g., 100 µM). We recommend using TE buffer (10 mM Tris·Cl, 1 mM EDTA, pH 8.0) for standard primers and probes labeled with most fluorescent dyes.

However, probes labeled with fluorescent dyes such as Cy3, Cy3.5, Cy5, and Cy5.5 should be stored in TE buffer, pH 7.0, since they tend to degrade at higher pH.

Storage

Primers should be stored in sterile, nuclease-free TE buffer in small aliquots at –20°C. Standard primers are stable under these conditions for at least 1 year. Fluorescently labeled probes are usually stable under these conditions for at least 6–9 months. Repeated freeze–thaw cycles should be avoided, since they may lead to degradation.

For easy and reproducible handling of primer–probe sets used in multiplex assays, we recommend preparing 20x primer–probe mixes, each containing 2 primers and 1 probe for a particular target at the suggested concentrations (see protocols).

Dissolving primers and probes

Before opening a tube containing lyophilized primer or probe, centrifuge the tube briefly to collect all material at the bottom of the tube. To dissolve the primer or the probe, add the required volume of sterile, nuclease-free TE buffer, mix, and leave for 20 minutes to allow the primer or probe to completely dissolve. Mix again and determine the concentration by spectrophotometry as described below.

We do not recommend dissolving primers and probes in water. They are less stable in water than in TE buffer and some may not dissolve easily in water.

Concentration

Spectrophotometric conversion for primers and probes:

1 A_{260} unit = 20–30 µg/ml

To check primer concentration, the molar extinction coefficient (ε_{260}) can be used:

A_{260} = ε_{260} x molar concentration of primer or probe

If the ε_{260} value is not given on the data sheet supplied with the primers or probes, it can be calculated from the primer sequence using the following formula:

ε_{260} = 0.89 x [(A x 15,480) + (C x 7340) + (G x 11,760) + (T x 8850)]

Example

Concentration of diluted primer: 1 µM = 1 x 10^{-6} M

Primer length: 24 nucleotides with 6 each of A, C, G, and T bases

Calculation of expected A_{260}: 0.89 x [(6 x 15,480) + (6 x 7340) + (6 x 11,760) + (6 x 8850)] x (1 x 10^{-6}) = 0.232

The measured A_{260} should be within ±30% of the theoretical value. If the measured A_{260} is very different to the theoretical value, we recommend recalculating the concentration of the primers or probes, or having the primers or probes resynthesized.

For probes, the fluorescent dye does not significantly affect the A_{260} value.

Primer and probe quality

The quality of 18–30mers can be checked on a 15% denaturing polyacrylamide gel;* a single band should be seen. Please contact QIAGEN Technical Services or your local distributor for a protocol.

Probe quality

The quality of the fluorescent label and the purity of TaqMan probes can be determined by comparing fluorescence before and after DNase digestion. Incubate probes with or without 5 units DNase* at 37°C for 1 hour. A significant difference in fluorescence following DNase treatment should be detectable.

* 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.

Ordering Information

Product	Contents	Cat. no.
Rotor-Gene Multiplex PCR Kit (80)	For 80 x 25 µl reactions: 1 ml 2x Master Mix, 2 ml RNase-Free Water	204772
Rotor-Gene Multiplex PCR Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204774
Rotor-Gene Multiplex RT-PCR Kit (80)	For 80 x 25 µl reactions: 1 ml 2x Master Mix, 20 µl RT Mix, 2 ml RNase-Free Water	204972
Rotor-Gene Multiplex RT-PCR Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204974
Accessories		
QuantiTect Reverse Transcription Kit — for fast cDNA synthesis for sensitive real-time two-step RT-PCR		
QuantiTect Reverse Transcription Kit (50)*	For 50 x 20 µl reactions: gDNA Wipeout Buffer, Quantiscript® Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water	205311
FastLane Cell cDNA Kit — for high-speed preparation of cDNA without RNA purification for use in real-time RT-PCR		
FastLane Cell cDNA Kit (50)	Buffer FCW, Buffer FCP, and components for 50 x 20 µl reverse-transcription reactions (gDNA Wipeout Buffer, Quantiscript Reverse Transcriptase, Quantiscript RT Buffer, RT Primer Mix, and RNase-Free Water)	215011
Rotor-Gene SYBR® Green PCR Kit — for fast real-time PCR and two-step RT-PCR using SYBR Green I on Rotor-Gene cyclers		
Rotor-Gene SYBR Green PCR Kit (80)	For 80 x 25 µl reactions: 1 ml 2x Master Mix, 2 ml RNase-Free Water	204072
Rotor-Gene SYBR Green PCR Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204074

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

Product	Contents	Cat. no.
Rotor-Gene SYBR Green RT-PCR Kit — for fast real-time one-step RT-PCR using SYBR Green I on Rotor-Gene cyclers		
Rotor-Gene SYBR Green RT-PCR Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204174
Rotor-Gene Probe PCR Kit — for fast real-time PCR and two-step RT-PCR using sequence-specific probes on Rotor-Gene cyclers		
Rotor-Gene Probe PCR Kit (80)	For 80 x 25 µl reactions: 1 ml 2x Master Mix, 2 ml RNase-Free Water	204372
Rotor-Gene Probe PCR Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 2 x 2 ml RNase-Free Water	204374
Rotor-Gene Probe RT-PCR Kit — for fast real-time one-step RT-PCR using sequence-specific probes on Rotor-Gene cyclers		
Rotor-Gene Probe RT-PCR Kit (400)	For 400 x 25 µl reactions: 3 x 1.7 ml 2x Master Mix, 100 µl RT Mix, 2 x 2 ml RNase-Free Water	204574

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.

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Notes

Notes

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