# Rotor-Gene® Q — Pure Detection

## Now with even more applications!


QIAGEN®

Sample & Assay Technologies


# The Rotor-Gene Q — for your success

Quantitative, real-time PCR is a precision science that places high demands on the instrument, chemistry, and software. High thermal and optical uniformity, short equilibration times, and fast ramping rates are critical for precise and rapid quantitative analysis. Sensitivity, speed, and specificity are also highly dependent on the performance of the DNA polymerase and reaction components.

QIAGEN's real-time PCR cycler, the Rotor-Gene Q, combines multiple optimized design features to provide the outstanding performance and reliable results that your research demands. Together with optimized QIAGEN® kits for real-time PCR, the Rotor-Gene Q enables streamlined analysis for a wide range of applications.

**Benefits of the Rotor-Gene Q:**

- Outstanding thermal and optical performance due to rotary format
- An unmatched optical range spanning UV to infrared wavelengths
- Various state-of-the-art analyses supported by user-friendly software
- Low maintenance and maximum convenience due to robust design
- High performance in multiple applications with QIAGEN kits

**Comprehensive application range**

The Rotor-Gene Q combined with optimized QIAGEN kits addresses a wide range of real-time PCR and high-resolution melting (HRM™) applications:

- Gene expression analysis
- Pathogen detection
- DNA methylation analysis
- Genotyping
- Mutation analysis
- miRNA research

For details of applications, see page 8.

Visit www.qiagen.com/PCR-applications and navigate your way through the Rotor-Gene Q Virtual World. Experience more than 20 instrument and software animations!


# Unique rotary design for outstanding performance

The unique centrifugal rotary design of the Rotor-Gene Q makes it the most precise and versatile real-time PCR cycler currently available (Figure 1). Each tube spins in a chamber of moving air, keeping all samples at precisely the same temperature during rapid thermal cycling. Detection is similarly uniform. When each tube aligns with the detection optics, the sample is illuminated and the fluorescent signal is rapidly collected from a single, short optical pathway. This thermal and optical uniformity results in sensitive, precise, and fast real-time PCR analysis (Figure 2). It also eliminates sample-to-sample variation and edge effects. These are unavoidable in traditional block-based instruments due to temperature gradients across the block and multiple, complex optical pathways.

**The rotary design delivers:**

- Well-to-well temperature variation below ±0.02°C
- Uniform detection eliminating the need for ROX reference dye
- Fast ramping and negligible equilibration times for short run times
- Complete confidence in your results!

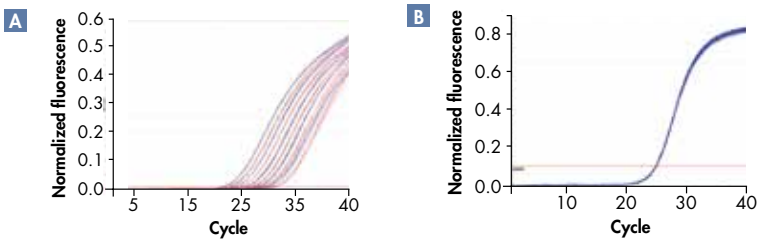



**Figure 2. Precise real-time PCR analysis.** A Twofold dilutions of human genomic DNA from 30 ng (10,000 copies) to 0.06 ng (20 copies) were used as template in real-time PCR. Five replicate reactions were run for each dilution using a self-designed TaqMan® assay for IL1R2 and the Rotor-Gene Probe PCR Kit on the Rotor-Gene Q. The average difference in the $C_T$ values between all dilutions was 1.07 cycles. B Human genomic DNA was used as template in 72 replicate real-time PCRs using a self-designed TaqMan assay for BCL2 on the Rotor-Gene Q without ROX normalization. The average $C_T$ value was 24.94 with a standard deviation of only 0.05, equivalent to a CV of 0.2%.

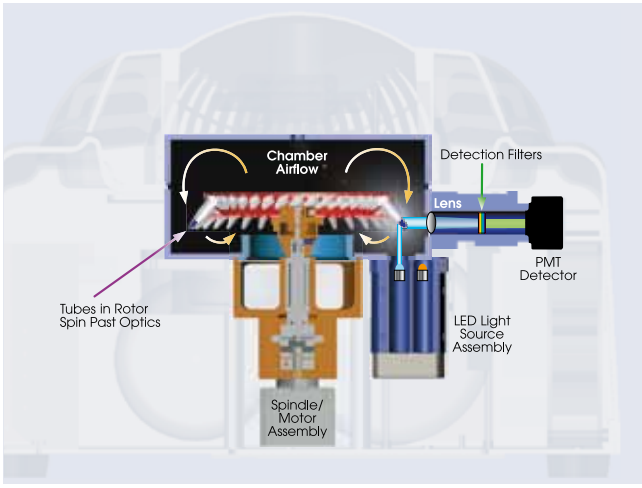



**Figure 1. Cross-section of the Rotor-Gene Q.** Heating/cooling is achieved by rapid airflow in the reaction chamber. Tubes spin past the excitation/detection optics every 150 milliseconds enabling high-speed data capture. Up to 6 separate LED light sources can be used in combination with 6 different detection filters and a highly sensitive photomultiplier detector.

Placing a 72-Well Rotor into the Rotor-Gene Q.

## Unrivaled optical range enables multiple applications

Whether your assay is based on intercalating dyes such as SYBR® Green, probes such as hydrolysis (TaqMan), hybridization (FRET), or Scorpion probes, the Rotor-Gene Q meets your requirements. With up to 6 channels spanning UV to infrared wavelengths, the cycler delivers the widest optical range currently available (Table 1), and is highly suited for all multiplex applications. In addition, the software allows you to create new excitation/detection wavelength combinations, which means that the Rotor-Gene Q is compatible with dyes you may use in the future.

**Table 1. Channels for optical detection**

| Channel | Excitation (nm) | Detection (nm) | Examples of fluorophores detected |
|---|---|---|---|
| Blue | 365±20 | 460±20 | Marina Blue®, Edans, Bothell Blue, Alexa Fluor® 350, AMCA-X |
| Green | 470±10 | 510±5 | FAM™, SYBR Green I, Fluorescein, EvaGreen®, Alexa Fluor 488 |
| Yellow | 530±5 | 557±5 | JOE™, VIC®, HEX, TET™, MAX™, 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±5 | 660±10 | Cy5, Quasar® 670, LightCycler®, Red640, Alexa Fluor 633 |
| Crimson | 680±5 | 712 high pass | Quasar 705, LightCycler Red705, Alexa Fluor 680 |
| HRM | 460±20 | 510±5 | SYBR Green I, SYTO®9, LC Green®, LC Green Plus+, EvaGreen |

Multiple PCR tube and Rotor-Disc formats.

## Flexible formats match your workflows

The Rotor-Gene Q supports multiple PCR tube formats to suit a range of needs. Changing the format, by simply switching the snap-fit metal rotor that holds the tubes, takes just seconds.

As well as tubes, Rotor-Disc™ rotors are available, which offer accelerated setup and higher throughput. Rotor-Discs are circular plates of vertically-oriented reaction wells. The Rotor-Disc 100 is the equivalent of a 96-well plate with an additional 4 reference wells. These extra wells can be conveniently used for more reactions or additional controls. Rotor-Discs can be quickly and easily sealed with plastic film using a Rotor-Disc Heat Sealer.

You can perform manual reaction setup, or take advantage of QIAGEN's automated solutions for reaction setup. The QIAgility® is cost-effective and delivers rapid, high-precision PCR setup, while the QIAsymphony® AS is ideal for laboratories performing routine PCR tests on a day-to-day basis Both instruments perform automated reaction setup in Rotor-Gene formats, allow direct transfer of sample lists, and are supplied with verified protocols for real-time PCR master mixes.

## Minimum maintenance, maximum convenience

The Rotor-Gene Q is engineered to reduce the need for maintenance and to maximize ease of use. This saves time and costs and allows you to focus on your research, not on keeping the cycler up and running. For example, highly stable excitation source LEDs are provided with a life-long guarantee, eliminating the need for frequent and time-consuming lamp replacement.

**Features of the Rotor-Gene Q:**

- Few moving parts and short, fixed optical path increase robustness
- No need for optical alignment, calibration, or lamp replacement
- Small, light instrument is easily transportable for flexibility in your lab

## Easy routine verification

Laboratories may often want to verify thermal accuracy. For most cyclers, this requires interaction with a service engineer. With the Rotor-Gene Q, this is not necessary. Instead, the easy-to-use, cost-effective Rotor-Disc OTV (Optical Temperature Verification) Kit automates accuracy testing. The kit includes a specialized Rotor-Disc filled with temperature-sensitive liquid crystals and dedicated analysis software. The full procedure takes less than 30 minutes.

## Reliable support for your peace of mind

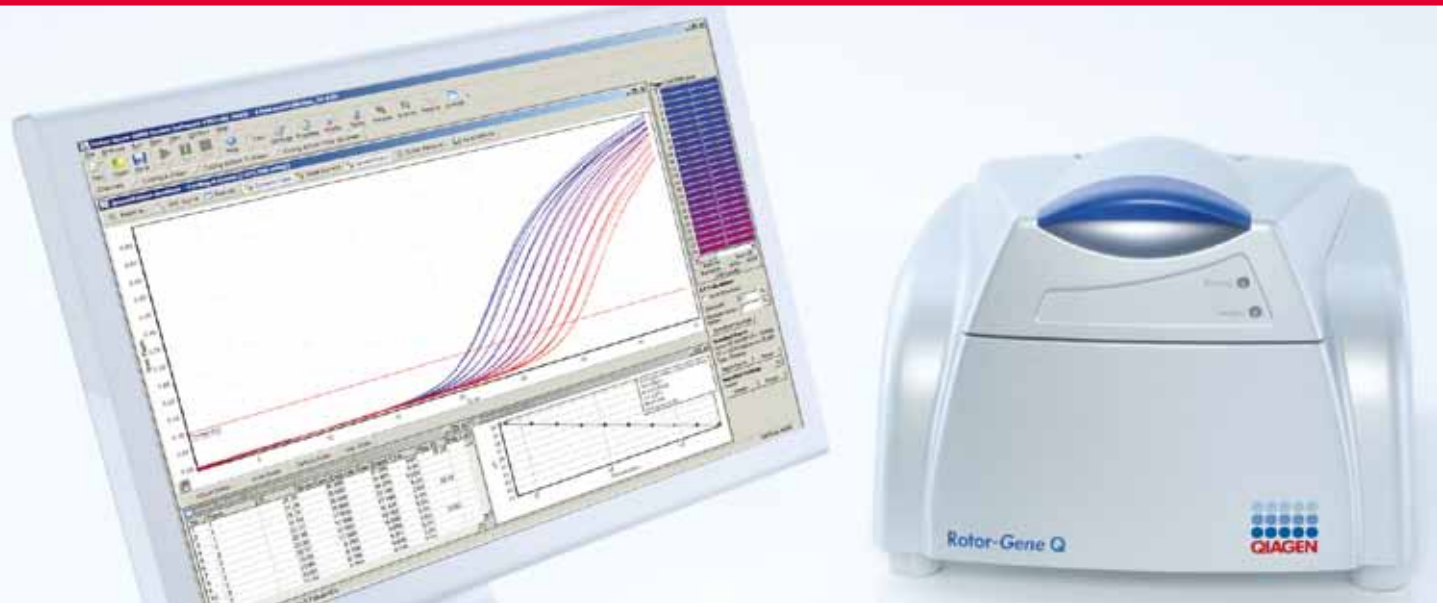
In the unlikely event of any service issues with your Rotor-Gene Q, QIAGEN Instrument Service provides comprehensive support services to ensure the continued success of your PCR applications. QIAGEN Instrument Service offers a wide range of flexible Service Support products, giving you peace of mind and letting you enjoy complete coverage and cost control. Our Application Services and Training Programs give you the freedom and flexibility to adapt your system to specific or changing research needs. With ISO 9001/ISO 13485 certification and an international team of highly qualified and experienced Support Specialists, we deliver the high-quality service that you deserve and that your applications demand.

## Specifications of the Rotor-Gene Q

View the outstanding technical features of the Rotor-Gene Q (Table 2).

**Table 2. Rotor-Gene Q specifications**

| | |
|---|---|
| Dimensions and weight | Width 370 mm (14.6 in.) x Depth 420 mm (16.5 in.) x Height 286 mm (11.3 in.) |
| | Depth (door open): 538 mm (21.2 in.) |
| | Weight: 12.5 kg (27.6 lb) |
| Thermal performance | Temperature uniformity: ±0.02°C (Standard deviation) |
| | Temperature accuracy: ±0.5°C |
| | Temperature resolution: ±0.02°C |
| | Temperature range: 35 – 99°C |
| Optical system | Up to 6 separate channels (365–680 nm excitation, 460–750 nm detection) |
| | Fixed optical path, separate high-power excitation LEDs and emission filters per channel |
| | Highly sensitive photomultiplier (PMT) detector with gain setting (sensitivity control) |
| | Dynamic range: 10 orders of magnitude (assay dependent) |
| Rotor and well configurations | Rotor-Disc 100: 30 µl x 100 wells, 15–25 µl recommended reaction volume |
| | Rotor-Disc 72: 0.1 ml x 72 wells, 20–25 µl recommended reaction volume |
| | Strip Tubes 0.1 ml: 0.1 ml x 72 wells, 10–50 µl recommended reaction volume, strips of 4 tubes and caps |
| | PCR Tubes 0.2 ml: 0.2 ml x 36 wells, 20–50 µl recommended reaction volume, individual tubes with caps |
| Typical run time | 40 cycles in 45–60 minutes with QIAGEN Rotor-Gene Kits (detection method dependent) |
| Electrical requirements | 100–240 V AC, 50/60 Hz; 560 VA (peak) |
| Warranty | 1 year on instrument; lifetime guarantee on excitation LEDs |




# Software enables quantification and enhances data security

The comprehensive Rotor-Gene Q software package supports all current state-of-the-art real-time analysis procedures with basic to advanced algorithms. This provides complete freedom to analyze your valuable experimental data and increases the reliability of your results. Data security is assured and process steps are trackable from starting the run to exporting the results.

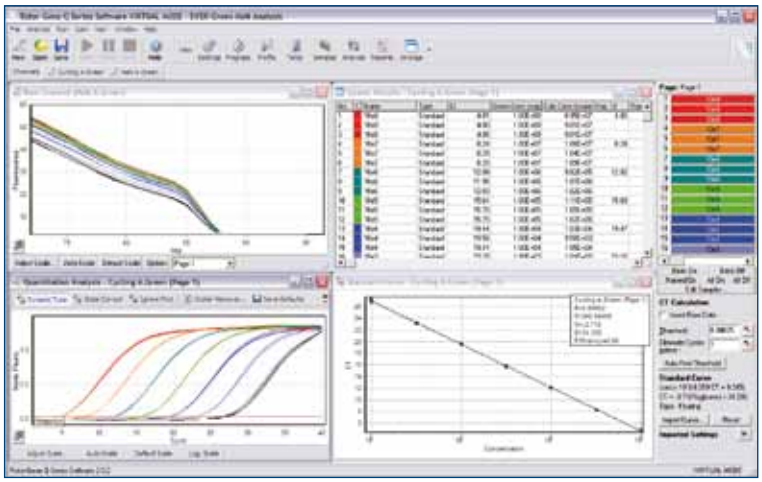
**Analysis procedures supported by Rotor-Gene Q software**

- Absolute quantification
  - Standard curve quantification
- relative quantification
  - 2 standard curves
  - $\Delta\Delta C_T$ relative quantification
  - Comparative quantification
  - Export to LinRegPCR (assumption-free analysis)
  - REST (Relative Expression Software Tool)
- Analysis of genetic differences
  - Allelic discrimination
  - Scatter plot analysis
  - Melt analysis
  - HRM analysis
  - Rotor-Gene Screen Clust HRM Software†
- Other analysis tools
  - End-point analysis
  - Concentration analysis

* REST employs differing PCR efficiencies and multiple reference genes for normalization, available free of charge at www.qiagen.com/rest † Software available separately

**The easy-to-use software provides:**

- Unlimited user licenses and individual user management
- A digital signature for every result file
- Audit trails to track changes made to experiment files
- Various result reports and export functions
- Raw data export for validation purposes



## Expand your research with HRM

High-resolution melting analysis (HRM) is a closed-tube, post-PCR analysis that has raised enormous scientific interest. HRM characterizes double-stranded PCR products based on their dissociation (melting) behavior. It is similar to classical melting curve analysis, but provides far more information for a wider range of applications. PCR products can be discriminated according to sequence, length, GC content, or strand complementarity, down to single base-pair changes. Previously unknown and even complex sequence variations can be readily detected and characterized in a robust and straightforward way. The Rotor-Gene Q is ideal for HRM applications due to its outstanding temperature and fluorescence precision for every well.

**The HRM option for the Rotor-Gene Q features:**

- A specially tuned high-intensity optical HRM channel
- Thermal resolution down to 0.02°C
- High data acquisition rates
- Comprehensive HRM software

HRM on the Rotor-Gene Q is capable of deciphering minute genetic differences such as difficult A/T class IV SNPs with melting point differences below 0.2°C (Figure 3 and 4).

**Harness the power of HRM for applications such as:**

- Genotyping
- Pathogen typing
- Gene scanning
- Quantitative methylation analysis

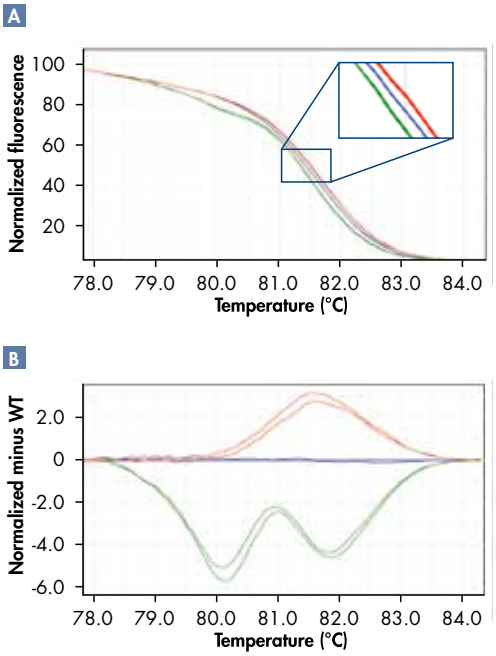



**Figure 3. Successful genotyping of an A/T Class IV SNP.** Typing the SNP (rs2270938) in the human GYS1 gene using the Type-it HRM PCR Kit results in highly reproducible and accurate results. The normalized melting curve and difference plot show successful and reliable discrimination of all 3 genotypes (Blue: wild-type; Green: heterozygous; Red: mutant). A normalized melt curve. B difference plot normalized to wild type samples.

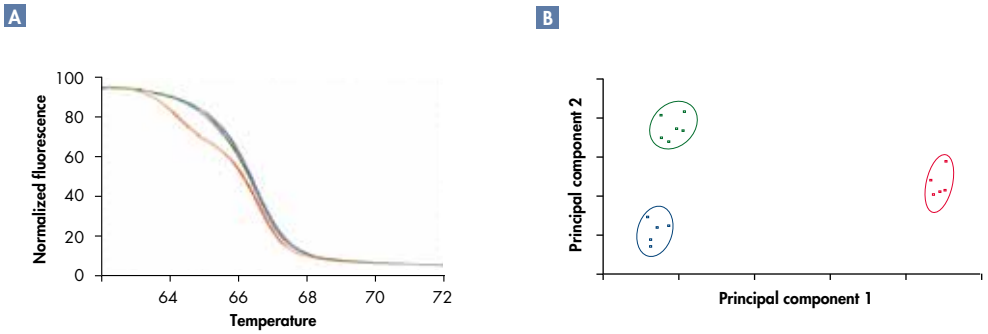



**Figure 4. HRM Analysis of a class IV SNP with less than 0.1°C difference between homozygote alleles.** A human A/T SNP in the AHRR gene was analyzed using genomic DNA from wild-type (blue), homozygous mutant (green), and heterozygous (red) samples. Experiments were performed using the Type-it HRM PCR Kit, Rotor-Gene Q HRM instrument, and Rotor-Gene ScreenClust HRM Software. A Normalized melting curves: homozygote curves are almost identical. B ScreenClust HRM cluster plot: all pseudo-unknowns including homozygous alleles were unambiguously clustered according to the correct genotype.

# Optimized reagents for all your applications

A range of QIAGEN kits for the Rotor-Gene Q enables reliable quantification in your real-time PCR applications without the need for optimization of reaction and cycling conditions (Table 3).

## Features and benefits of QIAGEN's kits for real-time PCR

Highly specific amplification is assured through a balanced combination of $NH4^+$ and $K^+$ ions that minimizes nonspecific primer annealing. Fast cycling without compromising performance is achieved using Q-Bond, a proprietary PCR additive that enables cycler run times of as low as 45 minutes. For multiplex PCR applications, synthetic Factor MP allows different amplicons in the same reaction to all be amplified with the same high efficiency. Factor MP, an innovative PCR additive, increases the local concentration of primers at the template and stabilizes specifically bound primers, allowing efficient primer extension by DNA polymerase.

**Table 3. Pure detection for all your applications**

| Application | Detection method | Procedure | QIAGEN kit | Data |
|---|---|---|---|---|
| Gene expression analysis | SYBR Green | PCR and 2-step RT-PCR | Rotor-Gene SYBR Green PCR Kit | Table 4 |
| | SYBR Green | 1-step RT-PCR | Rotor-Gene SYBR Green RT-PCR Kit | Fig. 5 |
| | SYBR Green | 2-step RT-PCR array | Pathway-Focused RT² Profiler PCR Arrays | Fig. 9 |
| | Probe (singleplex) | PCR and 2-step RT-PCR | Rotor-Gene Probe PCR Kit | Fig. 2 |
| | Probe (singleplex) | 1-step RT-PCR | Rotor-Gene Probe RT-PCR Kit | |
| | Probe (multiplex) | PCR and 2-step RT-PCR | Rotor-Gene Multiplex PCR Kit | Fig. 7 |
| | Probe (multiplex) | 1-step RT-PCR | Rotor-Gene Multiplex RT-PCR Kit | Fig. 8 |
| | Probe (duplex) | 2-step RT-PCR and 1-step RT-PCR | QuantiFast Probe Assays and Kits | Fig. 6 |
| miRNA detection | SYBR Green | Two-step RT-PCR | miScript SYBR Green PCR Kit | Fig. 10 |
| Pathogen detection | Probe (multiplex) | PCR, 2-step RT-PCR, and 1-step RT-PCR | QuantiTect® Virus +ROX Vial Kit | |
| | Probe (duplex) | PCR or 1-step RT-PCR incl. universal internal control | QuantiFast Pathogen +IC Kits | Fig. 11 |
| Food safety testing | Probe | Real-time PCR assays | *mericon*™ food testing portfolio | Fig. 12 |
| Pathogen typing | EvaGreen | HRM | Type-it HRM PCR Kit | Fig. 13 |
| Genotyping | Probe | PCR | Type-it Fast SNP Probe PCR Kit | Fig. 14 |
| | EvaGreen | HRM | Type-it HRM PCR Kit | Fig. 15, 3 |
| Methylation analysis | Probe | PCR | EpiTect® MethyLight PCR Kit | Table 5 |
| | EvaGreen | HRM | EpiTect HRM PCR Kit | Fig. 16 |

## Gene expression analysis

The combination of Rotor-Gene SYBR Green Kits, QuantiTect Primer Assays, and the Rotor-Gene Q provides a complete, ready-to-run solution for gene expression analysis. QuantiTect Primer Assays are predesigned primer sets for any gene from human, mouse, rat, and many other species. Assays can be easily ordered online at the GeneGlobe® Web portal (www.qiagen.com/GeneGlobe). When the assays are used together with Rotor-Gene SYBR Green Kits, highly sensitive quantification of specific PCR products is achieved without the need for optimization (Figure 5 and Table 4).

**Table 4. Superior performance in RT-PCR with SYBR Green**

| | QIAGEN | | Supplier $A_{II}$ | |
|---|---|---|---|---|
| | $C_T$ | Mean deviation | $C_T$ | Mean deviation |
| BAX (BCL2-associated X protein) | 24.84 | 0.05 | 29.57 | 0.46 |
| BCL2 (apoptosis gene) | 26.96 | 0.05 | 32.83 | 0.29 |
| MYC (proto-oncogene) | 28.42 | 0.21 | 35.26 | 0.72 |
| β-Actin (housekeeping gene) | 20.24 | 0.03 | 24.39 | 0.12 |

Human leukocyte cDNA (1 ng) was used as template in SYBR Green-based real-time two-step RT-PCR. Triplicate reactions were run using QuantiTect Primer Assays for 4 different targets: BAX, BCL2, MYC, and β-Actin. The Rotor-Gene Q and Rotor-Gene SYBR Green PCR Kit provided highly sensitive detection, indicated by lower $C_T$ values and lower mean deviations compared to an instrument and kit from Supplier $A_{II}$.

For gene expression analysis using probe detection, QuantiFast Probe Assays, containing a premixed primer pair and dual-labeled probe (TaqMan-based probe), can be used in combination with dedicated master mix kits on the Rotor-Gene Q for fast and sensitive quantification. QuantiFast Probe Assays are designed using a proprietary algorithm to enable amplification and detection of RNA and cDNA targets less than 100 bp in size with high efficiency and reliability. They are highly suited for use with degraded starting material such as formalin-fixed, paraffin-embedded (FFPE) analytes. (Figure 6).

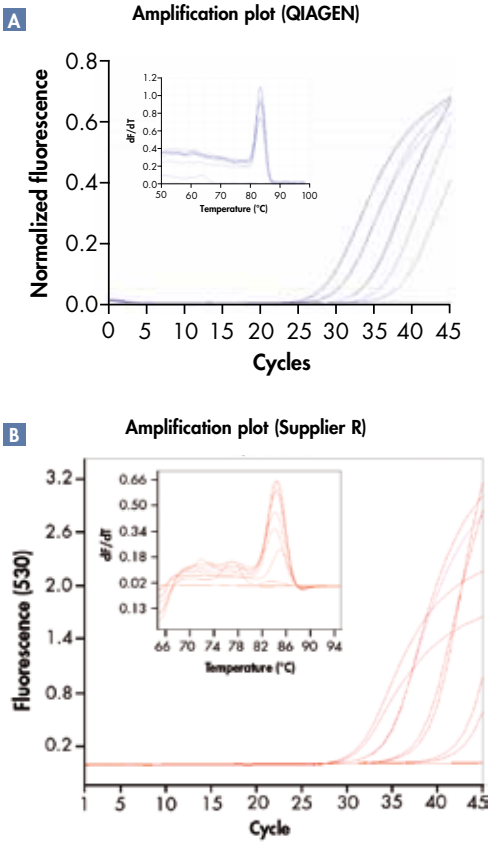



**Figure 5. Specific and sensitive detection using SYBR Green.** Tenfold dilutions of human leukocyte RNA (100 ng to 10 pg) were used as template in SYBR Green-based real-time one-step RT-PCR. Duplicate reactions were run using the QuantiTect Primer Assay for BCL2 (B-cell CLL/lymphoma 2). A The Rotor-Gene Q and Rotor-Gene SYBR Green RT-PCR Kit provided sensitive detection from 10 pg RNA and amplification of specific PCR product (melting curve shown in inset). B In contrast, an instrument and kit from Supplier R provided detection only after optimization of $Mg^{2+}$ concentration. However, the limit of detection was 100 pg RNA and coamplification of nonspecific products was observed (melting curve shown in inset).

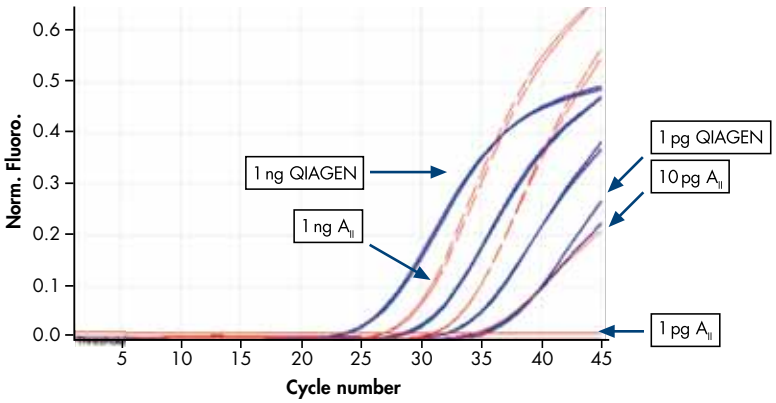



**Figure 6. Highly efficient real-time RT-PCR detection using RNA from FFPE samples.** Total RNA (1 ng, 100 pg, 10 pg, and 1 pg) was purified from a breast tissue FFPE sample using the RNeasy FFPE Kit. Transcripts of the human MUC1 gene were amplified and detected on the Rotor-Gene Q cycler using the QuantiFast Probe RT-PCR Plus Kit and the QuantiFast Probe Assay (blue curves) or a predesigned assay from Supplier $A_{II}$ (red curves). Results from the QuantiFast Probe Assay showed lower $C_T$ values and higher sensitivity compared to results using the assay from Supplier $A_{II}$. As low as 1 pg template was detectable using the QuantiFast Probe Assay (1 pg was undetectable using the assay from Supplier $A_{II}$).

The ultimate solution for fast and reliable gene expression analysis is provided by the combination of the Rotor-Gene Multiplex PCR and RT-PCR Kits and the Rotor-Gene Q. Up to 4 cDNA targets can be simultaneously and rapidly quantified in the same tube, increasing throughput and saving precious sample material (Figures 7 and 8). Genes with different expression levels are all amplified in the same tube with the same high efficiency, enabling reliable relative quantification of gene expression.

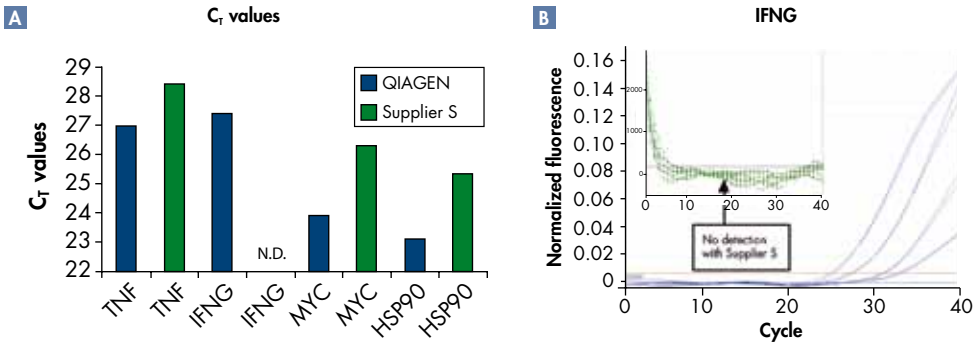



**Figure 7. Reliable multiplex analysis.** Tenfold dilutions of human leukocyte cDNA (10 ng to 10 pg) were used as template in 4-plex, real-time PCR. Reactions were run in triplicate using either the Rotor-Gene Q and Rotor-Gene Multiplex PCR Kit or an instrument and kit from Supplier S. Target genes: TNF (tumor necrosis factor), IFNG (interferon, gamma), MYC (v-myc myelocytomatosis viral oncogene homolog [avian]), HSP90AA1 (heat shock protein 90 kDa alpha [cytosolic], class A member 1). A $C_T$ values obtained for all 4 targets (instrument and kit from Supplier S did not successfully detect IFNG; **N.D.**). Lower $C_T$ values on the Rotor-Gene Q demonstrate detection with greater sensitivity. B Amplification plots for IFNG (plots for Supplier S in inset).

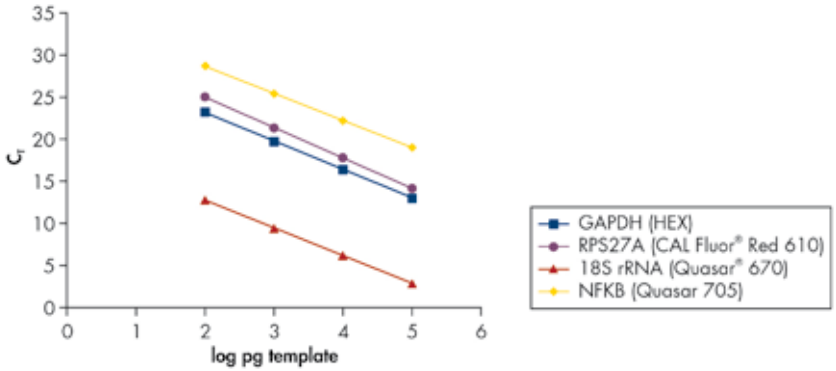



**Figure 8. Highly efficient 4-plex analysis.** 4-plex, real-time one-step RT-PCR was performed using the Rotor-Gene Multiplex RT-PCR Kit and self-designed TaqMan assays for the indicated targets. Reactions were run on the Rotor-Gene Q using 100, 10, 1, or 0.1 ng RNA from HeLa cells. The plots of $C_T$ value versus log template amount were parallel, indicating all 4 targets were amplified with the same high efficiency. GAPDH: glyceraldehyde-3-phosphate dehydrogenase; RPS27A: ribosomal protein S27a; NFKB: nuclear factor of kappa light polypeptide gene enhancer in B-cells.

## Pathway- and disease-focused PCR arrays

Pathway-focused RT² Profiler PCR Arrays are exeptionally reliable tools for analyzing the expression of a focused panel of genes. Each 100-well Rotor-Disc PCR Array includes SYBR Green-optimized (laboratory-tested) primer assays for a thoroughly researched panel of relevant, pathway- or disease-focused genes on the Rotor-Gene Q. RT² profiler PCR Arrays can also be customized to contain a panel of genes tailored to your specific research interests. Our high-quality primer design and master mix formulation enable the RT² profiler PCR Array to amplify 96 different gene-specific products simultaneously under uniform cycling conditions. This combination provides the RT² profiler PCR Array with the specificity and the high amplification efficiencies required for accurate real-time SYBR Green results (see Figure 9).* The simplicity of RT² profiler PCR Arrays makes them accessible for routine use in every research laboratory.

New developments in PCR array technology enable optimal performance of RT² Profiler PCR Arrays using RNA prepared from regular samples (0.1–5 µg RNA), FFPE samples, and small samples (1–100 ng RNA). See the PCR Array Protocol Guide to learn more.

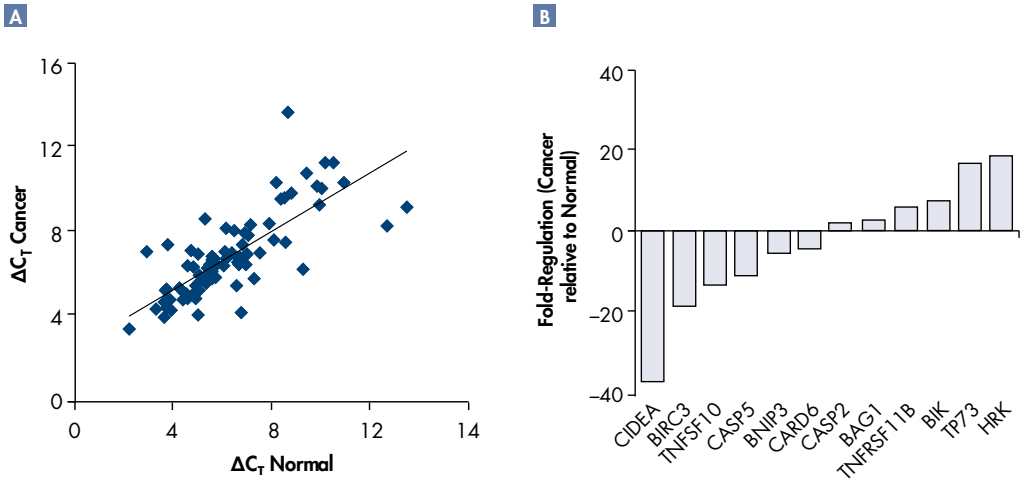



**Figure 9. Gene expression profiling of normal and cancerous colon tissue.** Using the Human Apoptosis RT² Profiler PCR Array, gene expression analysis of both normal (adult ascending colon) and cancer (adult colon adenocarcinoma) colon was performed using the Rotor-Gene Q. A $\Delta C_T$ values (data normalized to the geometric mean of 5 housekeeping genes) were plotted for both the normal and cancer tissue. B The fold-changes for selected targets that underwent significant (greater than 2-fold) changes in expression (cancer relative to normal) were plotted. Altogether, a total of 36 genes had a differential expression pattern in the cancer versus normal tissue, with 30 genes showing down-regulation and 6 genes showing up-regulation. Collectively, these data demonstrate the power of RT² Profiler PCR Arrays performed on the Rotor-Gene Q to profile differential gene expression patterns in cancer versus normal tissue.

* Data analysis software for pathway-focused RT² Profiler PCR Arrays is available free of charge at www.sabiosciences.com/pcarraydataanalysis.php

## miRNA detection

miRNAs play an important role in many diverse biological processes. Dysregulation of miRNA expression is associated with several cancers and other diseases. For this exciting, emerging research area, QIAGEN offers the miScript PCR System, a three-component system that covers all steps of conversion of miRNA, mRNA, and other noncoding RNAs into cDNA and their subsequent detection of miRNAs by SYBR Green-based real-time PCR. Fast and easy reverse transcription using the miScript Reverse Transcription Kit is followed by sensitive and specific miRNA detection using the miScript SYBR Green PCR Kit and a miScript Primer Assay on the Rotor-Gene Q. Using appropriate assays, it is possible to detect either mature miRNA or precursor miRNA (Figure 10). For more information on the miScript PCR System, visit www.qiagen.com/miRNA.

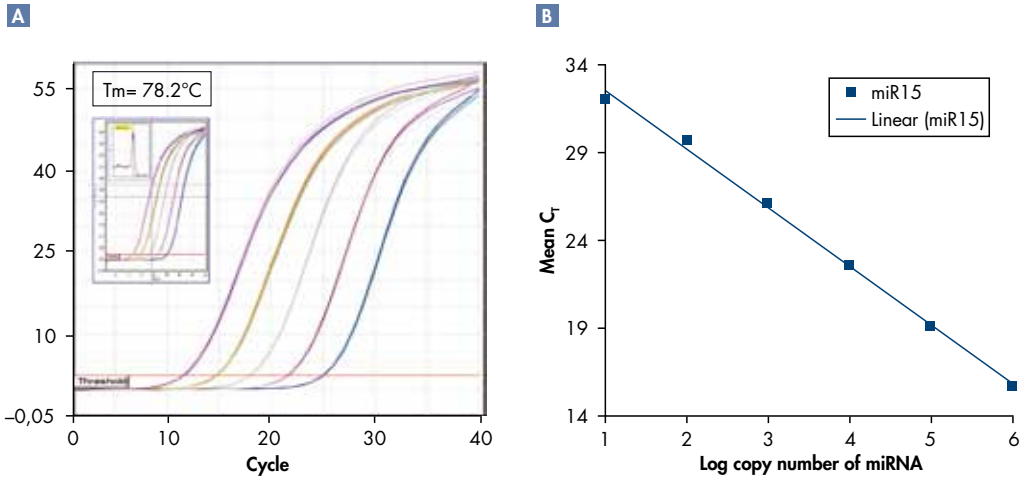



**Figure 10. Quantification of miR-15 and RNU6B using the miScript PCR System on the Rotor-Gene Q.** A Sensitive, specific quantification for a wide range of template amounts. The miScript Reverse Transcription Kit was used to generate cDNA from HeLa S3 cells. A range of cDNA amounts from 1 pg to 10 ng was used in real-time PCR with the miScript PCR System and a miScript Primer Assay for RNU6B. Real-time PCR was performed on the Rotor-Gene Q cycler. The amplification plot shows sensitive, accurate quantification from low to high cDNA template amounts. The melting curve (inset) shows a single peak indicating specific detection. B Accurate detection of as low as 10 miRNA copies. Synthetic miR-15 was used to generate cDNA using the miScript Reverse Transcription Kit. A range of amounts from 10 copies to $10^6$ copies of this cDNA was used in real-time PCR using the miScript PCR System. Real-time PCR was performed on the Rotor-Gene Q cycler. The resultant $C_T$ values decreased linearly with increasing miRNA copy number, indicating sensitive detection from a wide range of template amounts. The resultant $C_T$ values were plotted against the log copy numbers, showing sensitive and linear detection from a wide range of template amounts.

## Pathogen detection and food safety testing

Regardless of the pathogen or the material being examined, standard pathogen testing typically begins with a screening assay to simply assess presence or absence of a target. If results from the screening assay indicate presence of pathogens, further typing of the pathogen may be beneficial. Real-time PCR is the method of choice for accurate and reliable detection of pathogens and QIAGEN technologies and assays greatly facilitate this screening process. To streamline detection workflows, QIAGEN offers verified, ready-to-use assays as well as QuantiTect Virus Kits and QuantiFast Pathogen +IC RT-PCR Kits that enable multiplex qPCR for the detection of multiple targets in a single run.

### Highly sensitive detection of viral RNA and/or DNA

The combination of the QuantiTect Virus +ROX Vial Kit and the Rotor-Gene Q provides highly sensitive and rapid detection of up to 4 viral DNA and RNA targets simultaneously. A concentrated master mix allows larger volumes of template to be added to assays, lowering the limit of detection. Viral nucleic acid targets can be individually detected in singleplex assays or detected together with internal controls in multiplex assays.

QuantiFast Pathogen +IC Kits show the same performance as QuantiTect Virus Kits, but are also delivered with a universal internal control to enable high process safety through correct interpretation of negative results e.g., by detecting PCR inhibition. In combination with the Rotor-Gene Q, the QuantiFast Pathogen +IC Kits enable simultaneous detection of viral RNA or DNA targets plus internal control over a wide linear range without loss of sensitivity when duplexing (Figure 11).

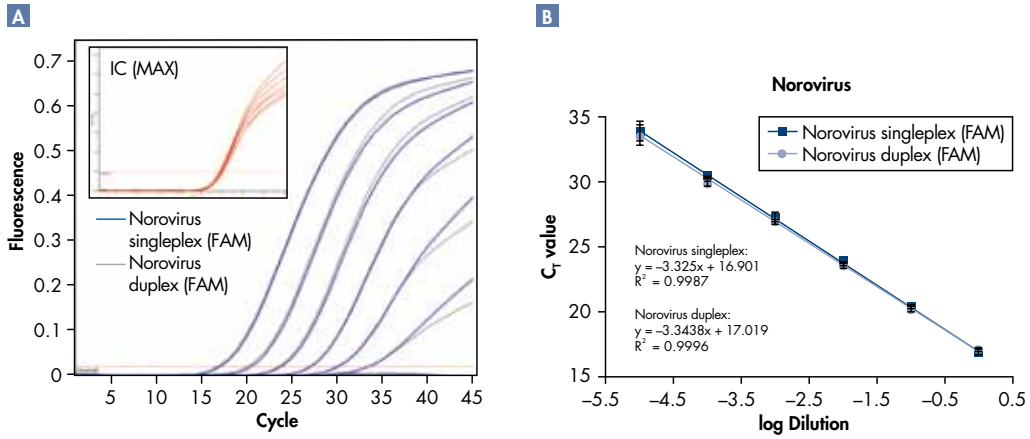



**Figure 11. Sensitive and precise detection of Norovirus on the Rotor-Gene Q.** A Norovirus transcript was serially diluted and detected by either singleplex real-time RT-PCR or by duplex real-time RT-PCR in parallel with the QIAGEN international control. Real-time RT-PCR was carried out using the QuantiFast Pathogen +IC RT-PCR Kit on the Rotor-Gene Q without any PCR optimization. The duplex reactions contained a fixed amount of IC template. Each dilution was analyzed in triplicate, one replicate per dilution is shown. B A 6-log range of both Norovirus RNA singleplex detection and Norovirus/IC duplex detection shows high precision and linearity. Error bars each represent ±1 SD of 3 real-time RT-PCR replicates.

### Rapid identification of animal pathogens with the Rotor-Gene Q

QIAGEN provides everything you need for pathogen identification and genotyping — reagents, enzymes, and instruments. *cador* real-time PCR reagents are developed for sensitive identification of specific animal pathogens (viral and bacterial) using PCR on the Rotor-Gene Q. For example, *cador* TKP PCR Reagent contains reagents and enzymes for simultaneous multiplex amplification of highly conserved regions of *Taylorella equigenitalis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* genomes. The amplicons are detected in one tube by measuring the green, orange, and crimson fluorescence on a Rotor-Gene Q. An internal control is included in the same tube, to monitor DNA extraction and/or the presence of PCR inhibitors. QIAGEN is one of the few companies with a worldwide PCR license for animal testing, without additional licensing costs for the user.

## Complete workflow solutions for food safety testing

The *mericon*™ food testing portfolio is a complete system of sample preparation and assay kits that meet the increasing demands of food research and monitoring. The assays, with detection by real-time PCR, enable sensitive and accurate detection of a broad range of pathogens, genetically-modified organisms, and plant and animal matter in food, animal feed, or pharmaceutical products. The entire workflow for food safety testing using *mericon* sample preparation kits and *mericon* PCR Assays has been validated. *mericon* PCR Assays perform optimally on the Rotor-Gene Q (Figure 12).

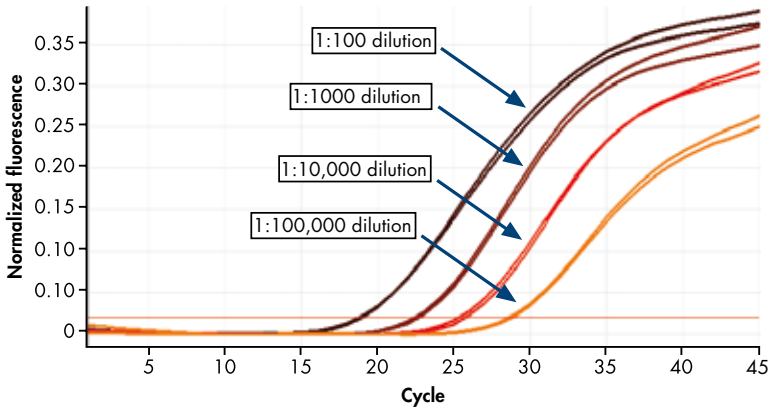



**Figure 12. Highly sensitive pathogen detection, even in difficult food matrices such as peanut butter.** Peanut butter was homogenized in buffered peptone water, spiked with < 5 cfu of Salmonella, and enriched for 20 h at 37°C. DNA was extracted from serially diluted samples of the enrichment culture using the *mericon* DNA Bacteria Kit and then tested with the *mericon* Salmonella Kit on the Rotor-Gene Q. Although the original inoculation was small, Salmonella was still reliably detected at a dilution factor of 1:100,000.

## Beyond pathogen detection — pathogen typing using HRM technology

In pathogen research, categorization of various pathogen subtypes is often required. This pathogen typing enables identification of different strains, for instance drug-resistant mutations or highly pathogenic variants. The resolution and flexibility of HRM in combination with the Type-it HRM PCR Kit on the Rotor-Gene Q, makes it the method of choice for pathogen typing in many application areas (Figure 13).

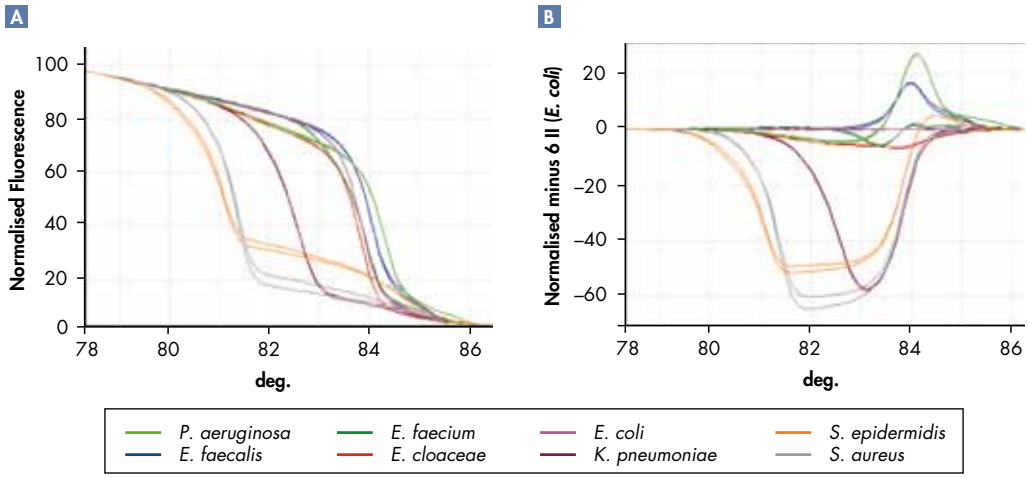



**Figure 13. Pathogen typing using robust HRM detection.** HRM primers were designed to recognize and differentiate the 16S ribosomal DNA of 8 different bacterial strains. Microbial DNA (10 pg) from each strain was used as template and amplified using the Type-it HRM PCR Kit. PCR products were sequenced to confirm the results and the robustness of the HRM detection method for this application.

## Genotyping

The analysis of differences in DNA between individuals has become increasingly important in all areas of biological and medical research. The term "genotyping" is used as a synonym for a wide range of applications associated with human, animal, plant, microbial, or viral samples. QIAGEN has developed highly specific and accurate PCR solutions and versatile detection methods to address the requirements of genotyping studies for accuracy, speed, reliability, and standardization.

### PCR-based genotyping analysis

The Type-it Fast SNP Probe PCR Kit provides accurate SNP genotyping using the Rotor-Gene Q, even for difficult templates or SNPs and low template amounts. Outstanding separation and tight allele clustering, together with the allelic discrimination or scatter plot analysis functions of the Rotor-Gene Q software, ensure high call rates and accurate, reproducible, and reliable genotyping results (Figure 14). The kit is functionally validated with commercially available SNP genotyping assays and is compatible with TaqMan MGB probes as well as custom assays consisting of TaqMan MGB, TaqMan, or other dual-labeled probes.

### Accurate genotyping with the Type-it HRM PCR Kit

The optimized Type-it HRM PCR Kit ensures accurate resolution of sequence variations and is an unmatched tool for unambiguous allelic discrimination using HRM technology. Together with the Rotor-Gene Q, the Type-it HRM PCR Kit requires no optimization in the development of new HRM assays. Due to the unique master mix chemistry and optimized HRM buffer, specific amplification products and reliable results are consistently ensured, even when analyzing Class IV SNPs (Figures 3 and 4). Unlike HRM kits from other suppliers, the unique features of the Type-it HRM PCR Kit enable amplification of the most challenging subtle sequence differences, even in difficult gene mutations in cancer-related genes (Figure 15).

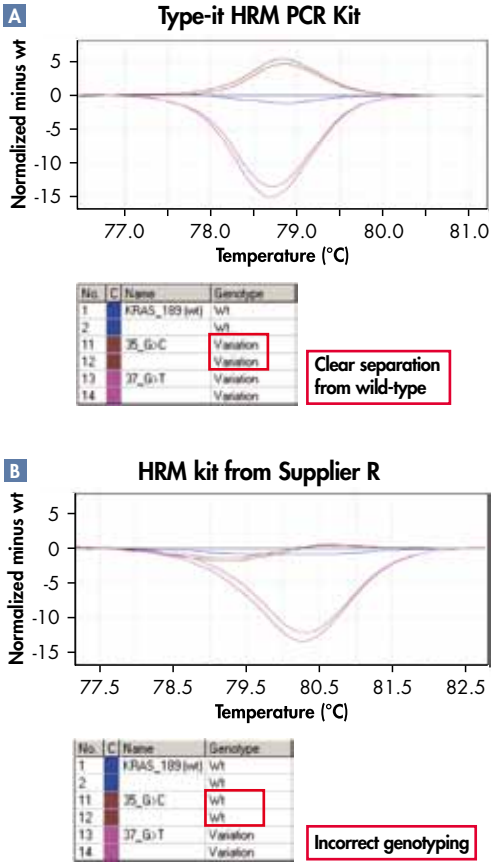



**Figure 15. Successful typing of gene mutations in the human KRAS gene using the Type-it HRM PCR Kit.** A Difference plot showing reliable discrimination between the wild-type sequence (blue), the c.35 G>C mutation (brown), and the c.37G>T mutation (pink). Reproducible discrimination with high confidence was obtained without the need for optimization. B The c.35 G>C mutation could not be resolved from the wild-type even after extensive optimization of $Mg^{2+}$ concentration and cycling parameters when using the HRM master mix from Supplier R. The mutation affects the amino acid in position 12, resulting in p.G12A and in position 13, resulting in p.G13C.

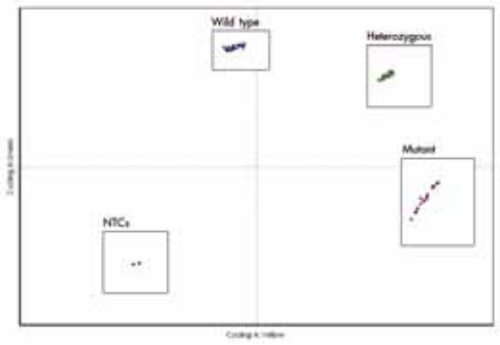



**Figure 14. Reliable SNP genotyping even with low template amounts.** Allelic discrimination plot analysis was performed with a panel of 70 different genomic DNAs (1 ng each) using the Type-it Fast SNP Probe PCR Kit. PCR was performed on the Rotor-Gene Q with a TaqMan SNP genotyping assay for rs 951134 and two no template controls (NTCs). **Black**: NTCs. **Green**: heterozygous samples. **Red**: homozygous for VIC fluorescence (A allele). Even using low amounts of template, tight clustering and reliable genotyping results were observed.

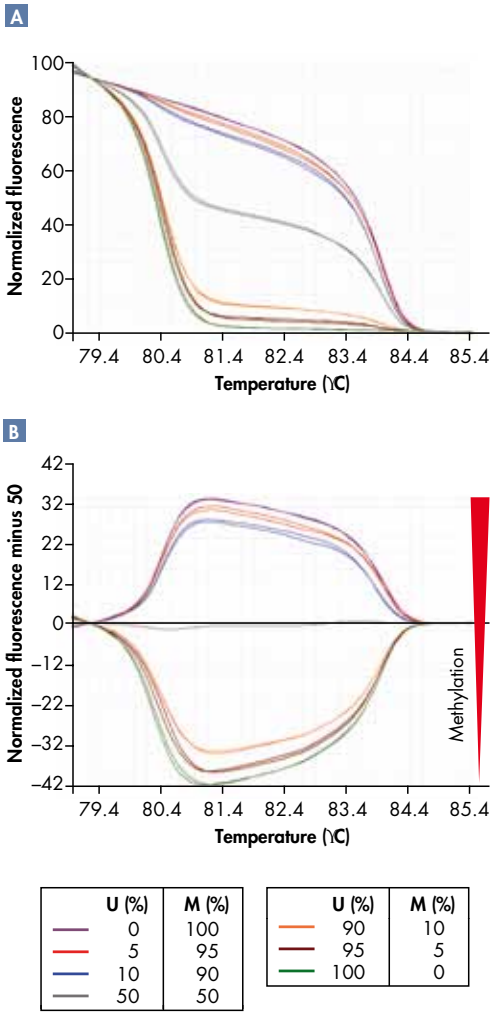



**Figure 16. Highly sensitive results — detection of even low percentages of methylated DNA.** Mixtures of methylated and unmethylated DNA of varying ratios were used as template. A CpG island from the promoter region of the APC gene (adenomatosis polyposis coli) was amplified and the degree of methylation was determined by HRM methylation analysis on the Rotor-Gene Q using the EpiTect HRM PCR Kit. A A standard normalized melt curve and B a difference plot normalized to the 50% methylated sample are shown.

## DNA methylation analysis

### EpiTect HRM PCR Kit — for reliable screening of changes in methylation status by HRM analysis

The EpiTect HRM PCR Kit uses high-resolution melting (HRM) technology for fast screening and accurate detection of changes in the CpG methylation status of bisulfite-converted DNA.

### The EpiTect HRM PCR Kit offers:

- Highly specific PCR amplification without the need for optimization
- Distinct melting curves due to EvaGreen fluorescent dye
- Convenient master mix format and optimized protocol
- Fast and easy development of new HRM methylation assays

For CpG methylation analysis by HRM, the DNA has to undergo complete bisulfite conversion before the melting behavior of DNA can be measured by HRM analysis. Unknown samples can be compared to a standard control based on their melting characteristics. The sensitivity ensured by the EpiTect HRM PCR Kit together with the Rotor-Gene Q means that even low amounts of methylated DNA can be detected (Figure 16).

### EpiTect MethyLight PCR Kits — for quantitative, real-time probe-based PCR analysis of methylation status

Once methylation sites have been identified using HRM, probe-based techniques can be used for sensitive quantification of CpG sites. Using the EpiTect MethyLight PCR Kit with the Rotor-Gene Q allows sensitive and reliable analysis of methylation status using TaqMan or other dual-labeled probes. Highly accurate quantitative methylation analysis is achieved when the kit is used together with probe-based Methylation assays (such as EpiTect MethyLight Assays). EpiTect MethyLight Assays consist of PCR primers and 2 probes — one methylation-specific, the other nonmethylation-specific — which can be used in a single reaction to simultaneously detect methylated and unmethylated sites (Table 5).

**Table 5. Sensitive detection of small changes in methylation**

| Defined experimental methylation degree | Mean$C_{T(CG)}$ value (FAM probe) | Mean $C_{T(TG)}$ value (VIC probe) | Calculated methylation degree in % per sample |
|---|---|---|---|
| 0% methylation | 45 | 30.37 | 0.004 |
| 10% methylation | 34.62 | 30.72 | 6.278 |
| 50% methylation | 31.27 | 31.67 | 56.887 |
| 90% methylation | 30.52 | 33.43 | 88.258 |
| 100% methylation | 30.05 | 45 | 99.997 |

## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| Rotor-Gene Q 2plex | Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories, 1-year warranty on parts and labor, optional installation and training | Inquire |
| Rotor-Gene Q 2plex HRM | Real-time PCR cycler and HRM instrument with 2 channels (green, yellow) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, optional installation and training | Inquire |
| Rotor-Gene Q 5plex | Real-time PCR cycler with 5 channels (green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor, optional installation and training | Inquire |
| Rotor-Gene Q 5plex HRM | Real-time PCR cycler and HRM instrument with 5 channels (green, yellow, orange, red, crimson) plus HRM channel, laptop computer, software, accessories, 1-year warranty on parts and labor, optional installation and training | Inquire |
| Rotor-Gene Q 6plex | Real-time PCR cycler with six channels (blue, green, yellow, orange, red, crimson), laptop computer, software, accessories, 1-year warranty on parts and labor, optional installation and training | Inquire |
| Warranty PLUS 1 Basic, Rotor-Gene Q | 2-year warranty, all labor, travel, and parts | 9241779 |
| Rotor-Disc 100 Starter Kit | Kit includes: 2 Rotor-Disc 100 packs, Rotor-Disc Heat Sealer, Rotor-Disc Heat Sealing Film, Rotor-Disc 100 Rotor and Locking Ring, Rotor-Disc 100 Loading Block, Rotor-Disc Pipetting Aid | Inquire |
| Rotor-Disc 100 (30) | Pack of 30 individually wrapped discs for 3000 reactions | 981311 |
| Rotor-Disc 72 Starter Kit | Kit includes: 3 Rotor-Disc 72 packs, Rotor-Disc Heat Sealer, Rotor-Disc Heat Sealing Film, Rotor-Disc 72 Rotor and Locking Ring, Rotor-Disc 72 Loading Block, Rotor-Disc Pipetting Aid | Inquire |
| Rotor-Gene Probe PCR Kit (400)* | For 400 x 25 µl reactions: 2x Master Mix, RNase-Free Water | 204374 |
| Rotor-Gene Probe RT-PCR Kit (400) | For 400 x 25 µl reactions: 2x Master Mix, RT Mix, RNase-Free Water | 204574 |
| QuantiFast Probe Assay (400)* | For 400 x 25 µl reactions: dual-labeled, probe-based, predesigned 20x lyophilized assays; includes master mix and reagents for real-time one-step or two-step RT-PCR | Varies |
| Rotor-Gene Multiplex PCR Kit (400)* | For 400 x 25 µl reactions: 2x Master Mix, RNase-Free Water | 204774 |
| Rotor-Gene Multiplex RT-PCR Kit (400)* | For 400 x 25 µl reactions: 3 x 1.7 ml 2x Rotor-Gene Multiplex RT-PCR Master Mix, 100 µl Rotor-Gene RT Mix, 2 x 2 ml RNase-Free Water | 204974 |
| QuantiTect Primer Assay (200)* | For 200 x 50 µl reactions: 10x QuantiTect Primer Assay (lyophilized) | Varies |

* Various kit sizes available; please inquire.

## Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QuantiTect Reverse Transcription Kit (50)* | For 50 x 20 µl reactions: Buffers, Quantiscript® Reverse Transcriptase, RT Primer Mix, RNase-Free Water | 205311 |
| miScript SYBR Green PCR Kit (200)*† | For 200 x 50 µl reactions: 2x Master Mix, Universal Primer, RNase-Free Water | 218073 |
| QuantiTect Virus +ROX Vial Kit (200)* | For 200 x 50 µl reactions: 5x Master Mix, RT Mix, RNase-Free Water, Nucleic Acid Dilution Buffer | 211033 |
| QuantiFast Pathogen PCR +IC Kit (400)* | For 400 x 25 µl reactions: Master Mix, lyophilized Internal Control Assay, lyophilized Internal Control DNA, ROX Dye Solution, High-ROX Dye Solution, RNase-Free Water, Nucleic Acid Dilution Buffer, Buffer TE | 211354 |
| QuantiFast Pathogen RT-PCR +IC Kit (400)* | For 400 x 25 µl reactions: Master Mix, RT Mix, lyophilized Internal Control Assay, lyophilized Internal Control RNA, ROX Dye Solution, High-ROX Dye Solution, RNase-Free Water, Nucleic Acid Dilution Buffer, Buffer TE | 211454 |
| Type-it Fast SNP Probe PCR Kit (800)* | For 800 x 25 µl reactions: 2x Master Mix, RNase-Free Water, Q-Solution® | 206045 |
| EpiTect MethyLight PCR Kit (200)* | For 200 x 50 µl reactions: 2x Master Mix, RNase-Free Water | 59436 |
| EpiTect HRM PCR Kit (100) | For 100 x 25 µl reactions: 2x Master Mix, RNase-Free Water | 59445 |
| Type-it HRM PCR Kit (100) | For 100 x 25 µl reactions: 2x Master Mix, RNase-Free Water | 206542 |
| Pathway-Powered RT² Profiler PCR Arrays | PCR Array includes SYBR Green-optimized primer assays for a panel of relevant pathway- or disease-focused genes | 330231 |
| Custom RT² Profiler PCR Arrays | Custom PCR Arrays containing any set of human, mouse, rat, dog, rhesus macaque, or fruit fly genes | 330131 |
| RT² SYBR Green ROX FAST Mastermix | For 200 x 20 µl reactions: 2x SYBR Green ROX FAST qPCR Mastermix, HotStart DNA Taq Polymerase, dNTP mix, dyes | 330520 |

* Various kit sizes available; please inquire. † The kit is part of the miScript PCR System; for details, visit www.qiagen.com/miRNA.

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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