

Analyzing Genetic Differences

New tools and technologies — Supplement



Discover QIAGEN's state-of-the-art solutions for genotyping

Genotyping encompasses a range of applications used to analyze genetic differences between individuals or cells. QIAGEN recognizes the most common challenges in genotyping studies and has developed advanced solutions to ensure experimental success.

We now expand our genotyping product range with innovative tools and technologies that include:

- A complete solution for genotyping using High-Resolution Melting (HRM[®]) technology
- Versatile detection platforms using Pyrosequencing[®]

From typing of disease or cancer loci to biomarker discovery and pathogen detection, ensure success at the first attempt and trust QIAGEN for all your genotyping requirements.



Sample & Assay Technologies

HRM technology — the fast, sensitive, and cost-effective approach to genotyping

HRM characterizes double-stranded PCR products based on their melting behavior as they transition from double-stranded DNA to single-stranded DNA with increasing temperature. PCR products can be easily discriminated according to sequence, length, GC content, or strand complementarity, down to single base pair changes. Understanding the genetic differences associated with human disease or cancer as well as linking them to biomarkers, such as SNPs or deletions, is crucial for the prognosis and future treatment of genetic disorders. QIAGEN's HRM hardware, software, and kits deliver the most specific, sensitive, and accurate results, without time-consuming optimization, and outperform all other available solutions.

PCR-based genotyping analysis and versatile detection platforms

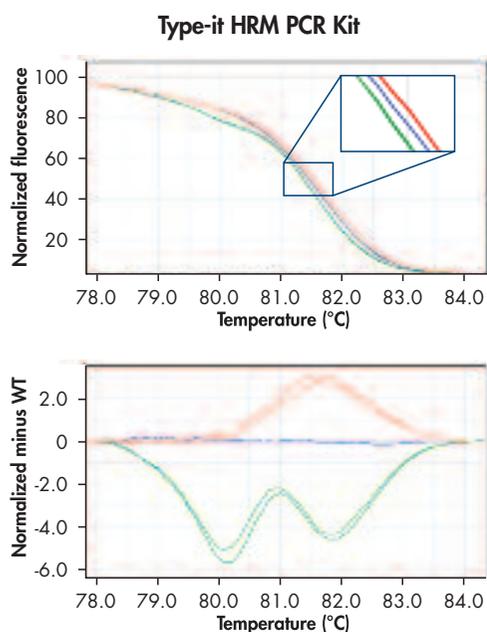


Figure 1. Successful genotyping of an A/T Class IV SNP using the Type-it HRM PCR Kit. Typing the SNP (rs2270938) in the human GYS1 gene using the Type-it HRM PCR Kit results in highly reproducible and accurate results. The normalized melting curve and difference plot show successful and reliable discrimination of all 3 genotypes (wild-type, heterozygote, and mutant) of a Class IV SNP. Blue: wild-type; Green: heterozygous; Red: mutant. Analysis was performed on the Rotor-Gene Q 5plex HRM instrument.

Table 1. Typical HRM-based genotyping applications

Dedicated application	Description
Detection of SNPs	Class I-IV (most difficult and rarest)
Mutation detection	Deletions, insertions, translocations, point mutations
Mutation scanning	Screening of unknown samples for new mutations
Pathogen typing/detection	Screening for microbiology research

Accurate genotyping with the Type-it® HRM PCR Kit

The latest addition to the dedicated genotyping product line, Type-it, is the Type-it HRM PCR Kit. The optimized kit ensures accurate resolution of sequence variations and is the preeminent tool for unambiguous allelic discrimination using HRM technology. The kit enables successful analysis of genomic loci that are difficult to amplify and is compatible with all real-time instruments suitable for HRM analysis, such as the Rotor-Gene Q, the LightCycler® 480, and the Applied Biosystems® 7500 and 7900 Fast Systems. Novel EvaGreen® fluorescent dye contained in the master mix ensures distinct melting curves. In contrast to kits from other suppliers, 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 (Figure 1).

Outstanding real-time results with the Rotor-Gene Q

QIAGEN's real-time PCR cycler, the Rotor-Gene Q (Figure 2), combines multiple optimized design features to provide the consistent performance and reliable results that your research demands. The rotary design of the Rotor-Gene Q and its outstanding thermal and optical performance are highly suited to HRM. The Rotor-Gene Q is the only real-time cycler currently capable of accurately deciphering the most difficult Class IV SNPs by HRM. The HRM option for the Rotor-Gene Q includes a specially tuned high-intensity optical HRM channel and a thermal resolution of 0.02°C, as well as powerful new HRM analysis software, ensuring straightforward and successful results.



Figure 2. Rotor-Gene Q.

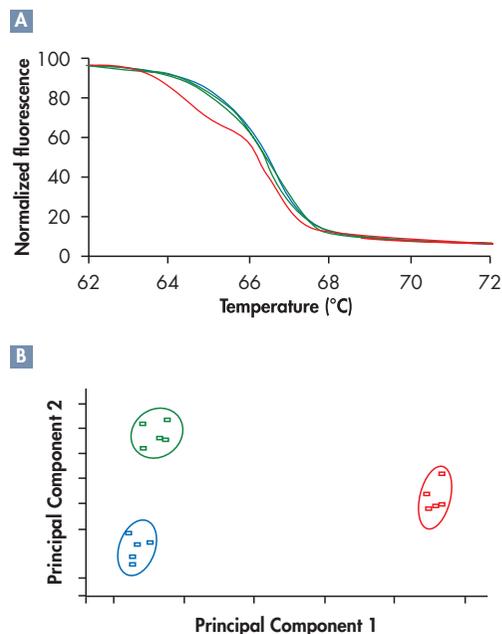
Efficient data analysis with Rotor-Gene ScreenClust HRM Software

Rotor-Gene ScreenClust HRM Software uses an innovative mathematical approach and advanced statistical methods to differentiate between different alleles in an HRM experiment (Figure 3). By grouping samples into clusters, Rotor-Gene ScreenClust HRM Software enables applications such as genotyping and mutation scanning. Analysis with the software is rapid, highly specific, and sensitive, giving you data you can trust and faster results. The powerful software exceeds the analytical performance of all other currently available HRM software packages.

A winning combination for HRM-based genotyping

The Type-it HRM PCR Kit, together with the Rotor-Gene Q and Rotor-Gene ScreenClust HRM Software, provides a complete solution for reliable and accurate HRM-based genotyping. Our products ensure highly specific amplification combined with maximum thermal resolution and straightforward software analysis and outperform other currently available HRM solutions.

Figure 3. Accurate identification of difficult A/T Class IV SNPs. The AHRR7 gene was analyzed with the Type-it HRM PCR Kit on the Rotor-Gene Q. Rotor-Gene ScreenClust HRM Software correctly identified wild-type (blue), homozygous mutant (green), and heterozygous (red) alleles. The difference between homozygote alleles in this experiment was less than 0.1°C. **A** A normalized melting curve. **B** A principal component plot showing the samples grouped into clusters.



Detection using PyroMark systems



Figure 4. PyroMark Q24 with PyroMark Q24 Vacuum Workstation.

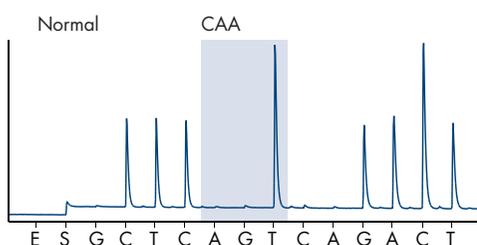


Figure 5. Results from mutation analysis of codon 61 using PyroMark Q24 KRAS v2.0 test and PyroMark Q24. Analysis of codon 61 is set up as a reverse assay, which means that CAA is read as TTG. The Pyrogram® trace shows a sample with a normal genotype. Light blue areas indicate the variable position.

Pyrosequencing — the definitive detection platform for genetic analysis

Pyrosequencing ensures ultimate precision and accuracy when performing highly sensitive mutational analysis or when quantifying alleles in mixed cell populations. Data are presented in a sequence context which serves as a built-in quality control of the results. Pyrosequencing may also be performed after an HRM experiment. This combines fast and effective mutational screening by HRM with detailed sequence analysis and verification by Pyrosequencing (Figure 5).

PyroMark® Q24

The PyroMark Q24 uses proven Pyrosequencing technology for mutational analysis of 1–24 samples in as little as 15 minutes. The system consists of the PyroMark Q24 instrument, the PyroMark Q24 Vacuum Workstation for preparation of single-stranded DNA, reagents and controls, as well as PyroMark Q24 Software for analysis (Figure 4).

PyroMark Q96 MD

For higher throughput needs, we offer the PyroMark Q96 MD, which is highly suitable for real-time, sequence-based detection of SNPs and mutations, and allele frequencies. It enables analysis of up to 96 samples. The PyroMark Q96 MD instrument in combination with the PyroMark Q96 Vacuum Workstation, application software, reagents, and controls provides a complete solution for Pyrosequencing analysis.

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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