Rotor-Gene® ScreenClust HRM® Software

For highly effective high-resolution melting analysis

Rotor-Gene ScreenClust HRM Software is a powerful tool for analysis of high-resolution melting (HRM) data from the Rotor-Gene Q or Rotor-Gene 6000 cycler. By grouping samples into clusters, Rotor-Gene ScreenClust HRM Software enables applications such as genotyping and mutation screening.

Rotor-Gene ScreenClust HRM Software provides:

- Innovative mathematical approach to HRM analysis
- Highly accurate identification of genotypes in supervised mode
- Automatic detection of new mutations in unsupervised mode
- Robust statistics for classifying and interpreting HRM data
- Minimal effort and standardized processes for data interpretation

Sequence variation analysis using HRM technology

HRM is an innovative technique that characterizes double-stranded PCR products based on their melting (dissociation) behavior as they transition from double-stranded DNA (dsDNA) to single-stranded DNA (ssDNA) with increasing temperature. First, the target sequence is amplified by PCR to a high copy number. Next, high-precision melting of PCR products enables discrimination of samples according to sequence, length, GC content, or strand complementarity, down to single base-pair changes. No prior sequence information is needed, enabling detection of previously unknown and even complex sequence variations in a simple and straightforward way.

Reliable HRM analysis requires a suitable HRM instrument, chemistry, and data analysis software. The Rotor-Gene Q cycler has a unique rotary design that provides outstanding thermal and optical performance, making it ideal for use in HRM analysis. The Type-it® HRM PCR Kit provides optimized chemistry for accurate resolution of sequence variations and unambiguous allelic discrimination. Rotor-Gene ScreenClust HRM Software enables reliable interpretation of data.

Powerful data analysis for reliable results

HRM data analysis discriminates between genotypes by comparing the position and shape of melting curves of different samples. Melting curves of heterozygotes and homozygotes differ in their shapes and melting points (T_m). In standard HRM software packages, variations in melting curve shape and position compared to a control are used to differentiate between samples. This method can cause unreliable, difficult-to-interpret results, and time-consuming manual data interpretation may be necessary. In contrast, Rotor-Gene ScreenClust HRM Software uses innovative mathematical algorithms to characterize samples and group them into clusters. These methods enable identification of even difficult class IV A/T SNPs which can have differences in melting temperatures as low as 0.1°C (Figure 1).

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Standardized workflow, minimal effort

Rotor-Gene ScreenClust HRM Software analyzes HRM data in 4 steps:

- Normalization
- Generation of a residual plot
- Principal component analysis
- Clustering

The software guides the user through all the steps, giving information about any choices that can be made at each step.

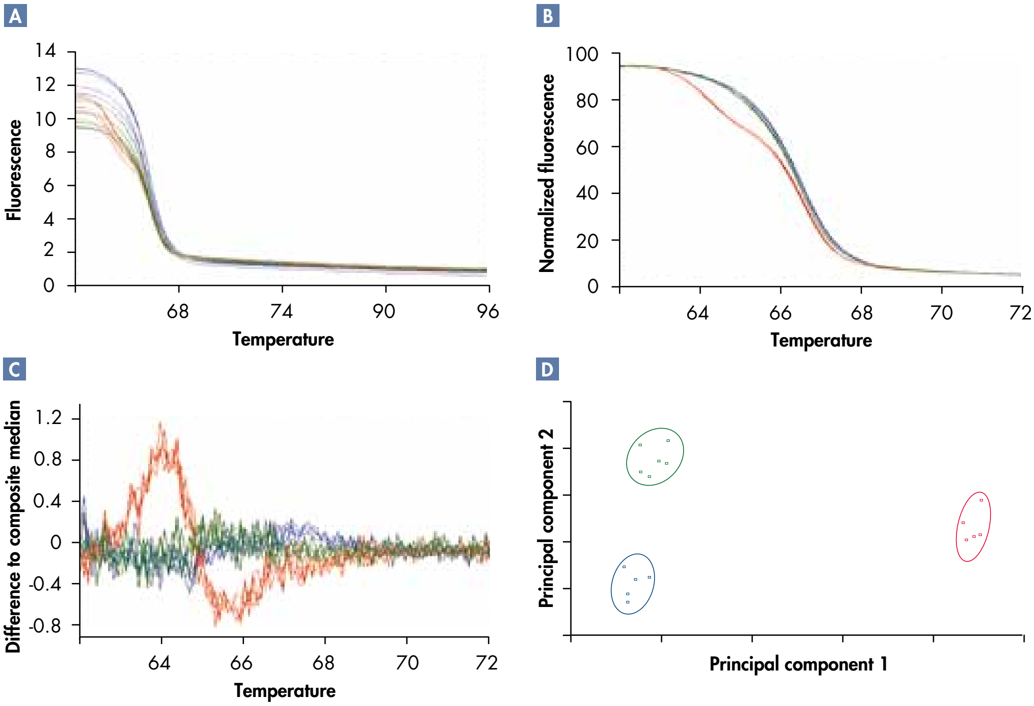

Figure 1. Powerful data analysis enables identification of a class IV SNP. A human A/T SNP in the AHRR7 gene was analyzed using genomic DNA from wild-type (blue), homozygous mutant (green), and heterozygous (red) samples. Experiments were performed using the Type-it HRM PCR Kit and a Rotor-Gene Q cycler with a HRM channel. Data analysis was performed with the unsupervised mode of Rotor-Gene ScreenClust HRM Software. A/T polymorphisms (class IV SNPs) are most difficult to discriminate due to minute differences between homozygote alleles (in this example, less than 0.1°C). **A** HRM raw data, **B** the normalized melting curve, **C** the residual plot, and **D** the cluster plot are shown. All pseudo-unknowns were correctly clustered according to genotype.

HRM performed on the Rotor-Gene cycler produces raw data (*.rex files) that can be further analyzed using Rotor-Gene ScreenClust HRM Software (Figure 1A). In the first step in analysis, raw data are normalized by applying curve scaling to a line of best fit so that the highest fluorescence value is equal to 100 and the lowest is equal to zero (Figure 1B). Next, the curves are differentiated and a composite median curve is constructed using the median fluorescence of all samples. The melt traces for each sample are subtracted from this composite median curve to draw a residual plot (Figure 1C). The individual sample characteristics are extracted by principal component analysis from the residual plot. Principal component analysis is a well-established method of data analysis. However, Rotor-Gene ScreenClust HRM Software is the first software application to apply principal component analysis to HRM data. Principal component analysis highlights similarities and differences in the data and is used to create a cluster plot in supervised or unsupervised mode (Figure 1D).

Accurate genotyping or mutation discovery

Rotor-Gene ScreenClust HRM Software performs clustering (grouping) of data according to allele in either supervised or unsupervised mode. Supervised mode is often used for SNP genotyping, where the genotypes are known. In supervised mode, the user assigns one or more control samples for each cluster and the software classifies (autocalls) all unknown samples to clusters according to their characteristics.

The unsupervised mode is used to find new mutations in the data when there is no prior knowledge or only partial knowledge of the genotypes present in the samples. In unsupervised mode, the software calculates the optimum number of clusters by itself. This feature is an excellent tool for the discovery of new polymorphisms.

The result of analysis in both modes is displayed as an easy-to-interpret cluster plot (Figure 1D). Statistical probabilities and typicalities are provided to allow easy comparison of results from different experiments. All data and graphs can be conveniently exported in various formats such as JPG, PDF, CSV, or XLS file formats and are summarized in an experimental report.

Wide range of applications

HRM analysis using Rotor-Gene ScreenClust HRM Software provides enormous potential for a wide range of applications. As well as SNP genotyping, mutation scanning or detection experiments can especially benefit from the power of this technology. In the example shown in Figure 2, various gene mutations resulting from insertions/deletions in the EGFR gene exon 19 were analyzed. Rotor-Gene ScreenClust HRM Software accurately separated multiple close and partially overlapping melt profiles by successfully extracting data characteristics into the 3 first principal components. All 6 pseudo-unknowns and the wild-type sample were correctly identified.

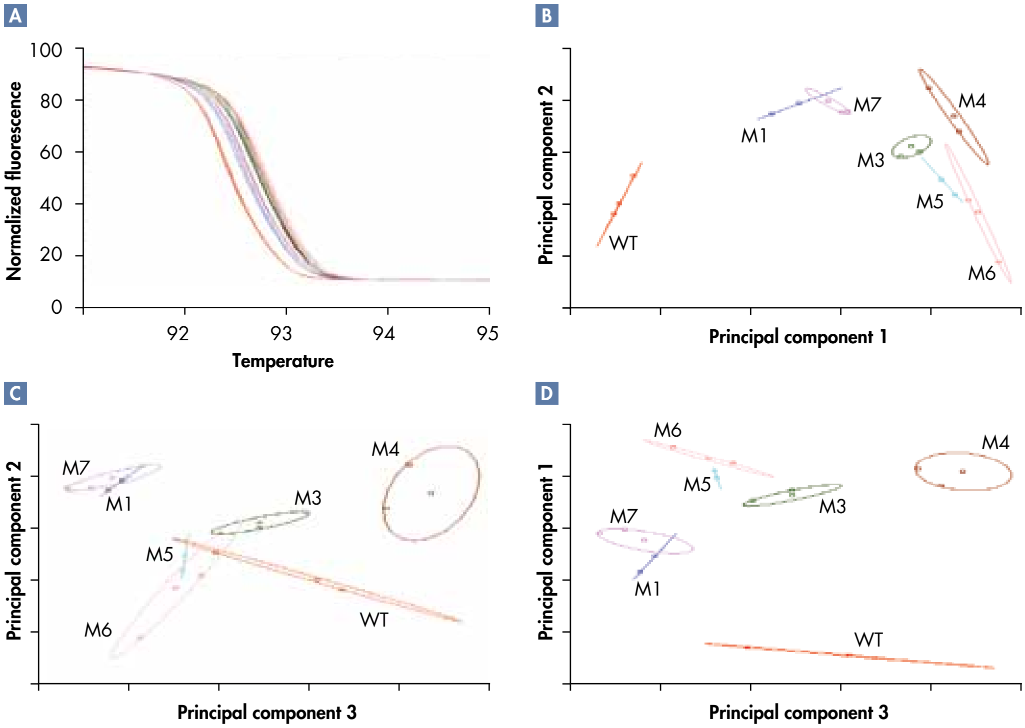

Figure 2. Successful mutation detection. Gene mutations (insertions/deletions) in EGFR exon 19 were analyzed using Rotor-Gene ScreenClust HRM Software in supervised mode. Experiments were performed using the Type-it HRM PCR Kit, a Rotor-Gene Q cycler with a HRM channel, and plasmid DNA templates. **A** A normalized melt plot shows very similar curve shape with only minute differences in melting points, making genotyping challenging. **B**–**D** Cluster plots using 3 principal components show the correct assignment of 6 different mutations and the wild type sample: **M1**: c.2235_2249del15, **M3**: c.2237_2252del16insT, c.2237_2238ins18, **M4**: c.2237_2238ins18, **M5**: c.2239_2248del10insC, **M6**: c.2240_2254del15, **M7**: c.2240_2257del18.

Ordering Information

Product	Contents	Cat. no.
Rotor-Gene ScreenClust HRM Software	Software CD, user guide	9020147
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	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	Inquire
Type-it HRM PCR Kit (100)	For 100 x 25 µl reactions: 2x HRM PCR Master Mix and RNase-free water	206542
Type-it HRM PCR Kit (400)	For 400 x 25 µl reactions: 2x HRM PCR Master Mix and RNase free water	206544

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Find out more about innovative HRM analysis at www.qiagen.com/HRM!

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