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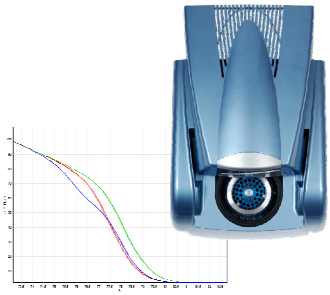
PRODUCT INFORMATION SHEET

HRM™

high-resolution melt



DNA melting (dissociation) analysis is widely used for the characterization of DNA amplification products, typically using SYBR® Green I fluorescent dye[1].

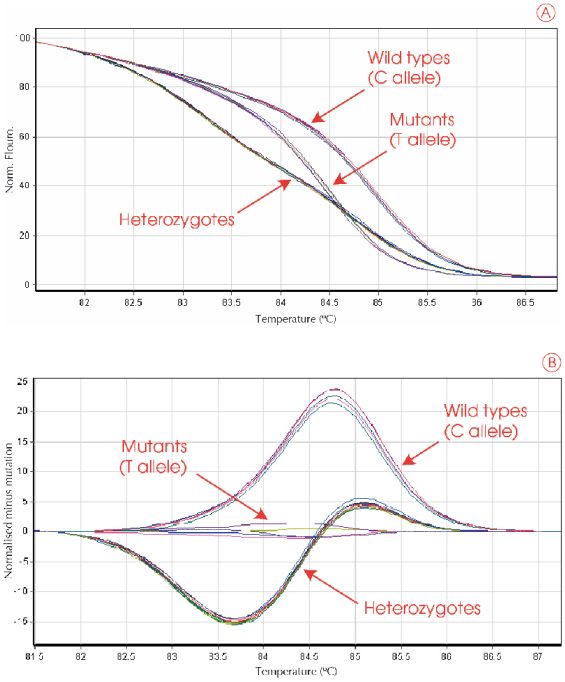
High Resolution Melt (HRM™) is a recent enhancement to traditional melting analyses that significantly increases the detail and information that can be captured. The introduction of HRM has renewed interest in the utility of DNA melting for a wide range of uses, including:

- Mutation discovery
- Screening for loss of heterozygosity
- DNA fingerprinting
- SNP genotyping
- Characterization of haplotype blocks
- DNA methylation analysis
- DNA mapping
- Species identification
- Determining the ratio of somatic acquired mutations
- HLA compatibility typing
- Identification of candidate predisposition genes
- Association studies (comparing cases and controls)
- Determining allele prevalence within a population

With HRM, these and other applications are done using low-cost generic dyes where previously custom labeled probes such as TaqMan® or fluorescence resonance energy transfer (FRET) probes were required (see Figure). HRM is thus a simpler and much more cost-effective way to characterize DNA samples according to their sequence, GC content, length or strand complimentarity.

Recently, HRM was the subject of a detailed and independent Technology Assessment report from the National Genetics Reference Laboratory (Wessex, UK). A wide range of sample types were tested, including examples of challenging G to C and A to T single base substitutions. The full report is now available for download[2].

HRM is made possible by advances in instrumentation and changes to the type of dye used. Currently, there are few instruments capable of true HRM[1]. The Rotor-Gene™ 6000 real-time rotary analyzer (Corbett Life Science) is one of these, and in addition, it is the only HRM instrument also capable of thermal cycling. Its rotary design provides the highest thermal and optical resolution yet achieved in a real-time system—critical factors for successful HRM.



SNP Genotyping by High Resolution Melt (HRM)
Discrimination of human ACTN3 (R577X) SNP genotypes (C to T substitution) using SYTO® 9 intercalation dye (no probes). Homozygous wild type, mutation and heterozygote samples are shown on a standard normalized melt plot (A) and a difference plot normalized to mutant samples (B). HRM analysis was done using a Rotor-Gene™ 6000 instrument (Corbett Life Science) and genotypes were automatically assigned by the Rotor-Gene software. The fragment was pre-amplified using a 40 cycle fast protocol (46 min. run time).

1. Herrman MG, Durtschi JD, Bromley KL, Wittwer CT, Voelkerding KV. Amplicon DNA melting analysis for mutation scanning and genotyping: cross-platform comparison of instruments and dyes. Clinical Chemistry 2006; 52:3, 494–503.

2. White H and Potts G. Mutation scanning by high resolution melt analysis. Evaluation of Rotor-Gene 6000 (Corbett Life Science), HR-1 and 384 well LightScanner (Idaho Technology). National Genetics Reference Laboratory (Wessex, 2006). (http://www.ngrl.org.uk/Wessex/downloads.htm)