

High Resolution Melt (HRM) analysis is a powerful technique for the analysis of mutations, polymorphisms and epigenetic differences in double stranded DNA (dsDNA) samples. HRM is a post PCR analysis whereby the temperature is increased in small increments, in the presence of a 3rd generation, saturating dsDNA binding dye. As the DNA dissociates (or melts) the dye is released and fluorescence reduces. Fluorescence is measured at regular temperature points to create a melt profile. This profile is sufficiently sensitive to discriminate class I to IV mutations as well as CpG methylation differences.

## Features

- 3rd generation saturating dye SyGreen 2
- Novel hot start for improved sensitivity
- Compatible on all real-time PCR platforms
- Standard and fast cycling conditions

# Applications

- Accurate SNP genotyping
- Gene scanning
- CpG methylation analysis
- Efficient specific amplification from GC rich and AT rich sequences



### Figure 1. Class IV SNP

Three samples of human genomic DNA were amplified using qPCRBIO HRM Mix with primers specific for a fragment of beta-globulin gene. The primers flanked a class IV single nucleotide polymorphism (17A/T). After amplification the products were subjected to HRM analysis. The 3 traces show clear allele calling of a class IV SNP. The black trace is for homozygous A, the purple trace homozygous T and the green trace heterozygous green.





### qPCRBIO HRM Mix

qPCRBIO HRM Mix uses the novel SyGreen 2 dye, a 3rd generation, saturating dsDNA binding dye. Unlike other dsDNA binding dyes, saturating dyes can be used at saturating concentrations without inhibiting PCR.

Proprietary small molecular inhibitor technology prevents formation of primer dimer and non-specific products leading to improved reaction sensitivity and specificity. Combining the latest advancements in polymerase technology and advanced buffer chemistry we offer market leading performance with minimal or no optimisation. High throughput screening has resulted in a buffer system that allows efficient amplification from GC rich and AT rich templates, under fast and standard cycling conditions.



### Figure 2. Class I SNP

Three samples of human genomic DNA were amplified using qPCRBIO HRM Mix with primers specific for a fragment of Factor V gene. The primers flanked a class I single nucleotide polymorphism (1691G/A). After amplification the products were subjected to HRM analysis. The 3 traces show clear allele calling of a class I SNP. The black trace is for homozygous A, the purple trace homozygous T and the green trace heterozygous green.



#### Figure 3. Class II SNP

Three samples of human genomic DNA were amplified using qPCRBIO HRM Mix with primers specific for a fragment of MTHFR gene. The primers flanked a class II single nucleotide polymorphism (1298A/C). After amplification the products were subjected to HRM analysis. The 3 traces show clear allele calling of a class II SNP. The black trace is for homozygous A, the purple trace homozygous T and the green trace heterozygous green.



### Figure 4. Class III SNP

Three samples of human genomic DNA were amplified using qPCRBIO HRM Mix with primers specific for a fragment of HFE gene. The primers flanked a class III single nucleotide polymorphism (187C/G). After amplification the products were subjected to HRM analysis. The 3 traces show clear allele calling of a class III SNP. The blue trace is for homozygous A, the red trace homozygous T and the green trace heterozygous green.

Catalogue Number	Product Name	Pack size	Presentation
PB20.31-01	qPCRBIO HRM Mix	100 x 20µl rxns	1 x 1ml
PB20.31-05		500 x 20µl rxns	5 x 1ml
PB20.31-20		2000 x 20µl rxns	20 x 1ml

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