applied biosystems™
by life technologies™

MeltDoctor™ High Resolution Melt Reagents

Part of the Applied Biosystems HRM complete solution

Introduction

High Resolution Melting (HRM) analysis is a post-PCR analysis method used to identify variation in nucleic acid sequences. The method is based on detecting small differences in PCR melting (dissociation) curves. It is enabled by high-brightness, dsDNA-binding dyes used in conjunction with real-time PCR instrumentation that has precise temperature ramp control and advanced data capture capabilities. Data are analyzed and manipulated using software designed specifically for HRM analysis.

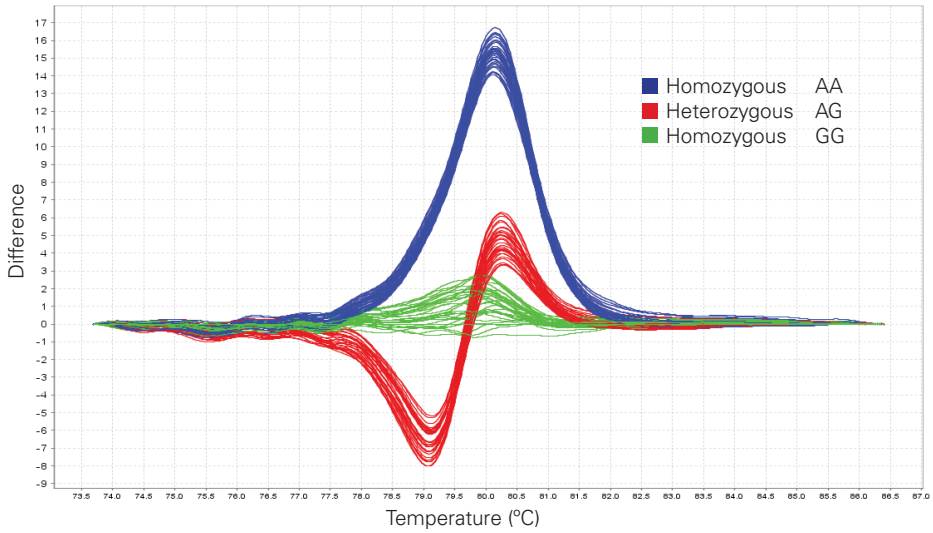

Figure 1. The MeltDoctor™ Workflow

Benefits

Scan for mutations with greater confidence	Avoid spending time optimizing your experiments	Scan genomic DNA for mutations without the complexity and expense of other methods
High resolution—sharp, clean melt curves for unambiguous discrimination between wild-type and variant sequences	All-inclusive formulation—MeltDoctor™ HRM Master Mix eliminates need to optimize Mg2+, dye, or other components for each target	One instrument—perform PCR and variant identification using your real-time PCR instrument; no need for HPLC or gels
Accurate results—very low false negative rate	Flexible formulation—MeltDoctor™ HRM Reagent Kit provides individual components for rapid optimization of HRM mix when more flexibility is needed	Low reagent consumption and waste—one mix used for PCR and HRM; no need for additional reagents, solvents, or gels
Reproducible results—minimal lot-to-lot, run-to-run, and well-to-well variation	Comprehensive coverage—reagents have been tested across a wide range of variant types including all categories of SNP mutations, insertions, deletions, and bisulfite-converted DNA	Low risk of contamination—closed-tube format and UDG compatibility minimize risk of amplicon contamination
High specificity—minimal primer-dimer and nonspecific amplification—tested across a wide range of targets, sequence contexts, and PCR primer pairs		Integrated workflow—HRM integrates seamlessly into downstream analysis using Applied Biosystems® Capillary Electrophoresis (CE) Sequencing Systems

Comprehensive HRM Product Offering

Applied Biosystems HRM products—including instrumentation, software, and reagents—provide the highest-resolution melt analysis available today. When used with the Applied Biosystems® ViiA™ 7, 7900HT Fast, 7500 Fast, StepOnePlus™, or StepOne™ Real-Time PCR Systems and HRM Software, MeltDoctor™ HRM reagents offer a comprehensive range of products to produce best-in-class accuracy and reproducibility in your HRM experiments.

MeltDoctor™ HRM Master Mix

The MeltDoctor™ HRM Master Mix contains all components needed for HRM-PCR (excluding template and primers). It is formulated for superior HRM performance across a wide range of genomic targets. Unlike some mixes available from other providers, the MeltDoctor™ HRM Master Mix does not require additional mixing prior to use, and was developed and optimized solely for HRM applications. Components of the master mix, provided in a convenient 2X mix, include:

- AmpliTaq Gold® 360 DNA Polymerase, a highly purified DNA polymerase that provides hot-start performance, minimizing nonspecific product formation and enabling reactions to be set up at room temperature
- MeltDoctor™ HRM Dye, a stabilized form of the fluorescent SYTO® 9 double-stranded nucleic acid stain developed by Molecular Probes. The MeltDoctor™ HRM Dye possesses significant optical and chemical properties important for high-performance HRM, including:
  - Low background fluorescence
  - High brightness in the presence of double-stranded DNA
  - Minimal temperature shift of DNA melting due to dye binding
  - Thermal stability to tolerate PCR cycling conditions
  - No inhibition of DNA polymerase activity, resulting in high PCR efficiency
- A dNTP blend including dUTP, which minimizes carryover contamination by allowing amplicon degradation by uracil DNA glycosylase (UDG) in subsequent PCR reactions
- Magnesium salts and other buffer components, precisely formulated to obtain optimal HRM results

MeltDoctor™ HRM Reagent Kit

For flexibility in reagent formulation, the MeltDoctor™ HRM Reagent Kit includes all PCR components and the MeltDoctor™ HRM Dye required for HRM analysis individually:

- AmpliTaq Gold® 360 DNA Polymerase which, when combined with AmpliTaq Gold® 360 Buffer and 360 GC Enhancer, amplifies a vast range of DNA sequence contexts. AmpliTaq Gold® 360 DNA Polymerase delivers 360° coverage for a full range of targets.
- GeneAmp® dNTP Blend
- MeltDoctor™ HRM Dye, a stabilized form of the fluorescent SYTO® 9 double-stranded nucleic acid stain developed by Molecular Probes

MeltDoctor™ HRM Positive Control Kit

The MeltDoctor™ HRM Positive Control Kit provides components to demonstrate and troubleshoot HRM analysis. The kit consists of forward and reverse primers and three DNA templates representing alleles AA, AB, and BB.

MeltDoctor™ HRM Calibration Plates

Ready-to-use MeltDoctor™ HRM Calibration Plates, containing all the components required for pure dye and HRM calibration, reduce the complexity of getting started with HRM experimentation. Provided in 96-well and 384-well formats, the plates provide the thermal and optical calibration required for accurate and reproducible discrimination between melt curves.

HRM Application Focus: Mutation Scanning

HRM analysis can be used to scan for mutations in target genes for the identification of variant samples prior to sequencing analysis (Figure 2). As a mutation scanning technique, HRM offers significant advantages over conventional methods such as Denaturing High-Performance Liquid Chromatography (DHPLC) and Denaturing Gradient Gel Electrophoresis (DGGE). Specifically, the advantages of HRM for mutation scanning include:

- Low reagent consumption, with little waste: HRM requires only a 20 µL PCR reaction for analysis of each sample, eliminating the need for HPLC solvents or DGGE gels
- Simple, fast workflow: no additional instrumentation is required after PCR amplification. A high-resolution melt step can be simply added to the end of the PCR profile for immediate analysis
- Fast optimization: unlike DHPLC, thermal optimization is not required
- Low sample consumption: following HRM analysis, the PCR product can be used directly in a Sanger sequencing reaction

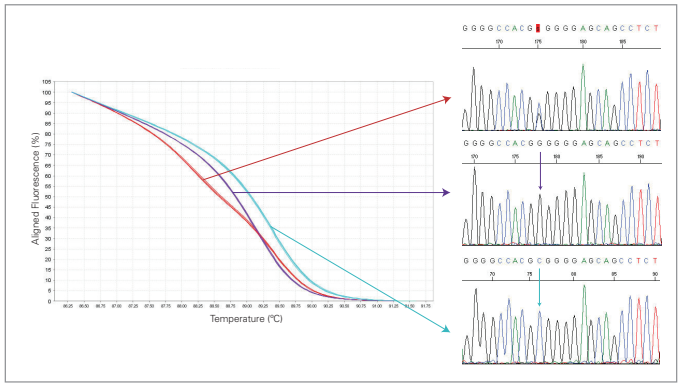

Figure 2. Mutation Scanning Using the Applied Biosystems HRM Workflow. Genomic DNA samples from three cell lines (HeLa, Raji, and Jurkat) were analyzed using HRM, followed by DNA sequencing. Primers were designed to amplify 152 bp of exon4 of the p53 tumor suppressor. Three genotypes are clearly distinguishable in the aligned HRM profile (left), and they were called accurately by the analysis software. Following HRM, the genotype of each sample (GC, GG, CC) was identified by sequencing (right). HRM results were generated using MeltDoctor™ HRM Master Mix on an Applied Biosystems® 7500 Fast Real-Time PCR Instrument and analyzed using Applied Biosystems® HRM Software v2.0. Sequencing results were obtained by dilution of the HRM PCR product into a sequencing reaction using the BigDye® Terminator v1.1 Cycle Sequencing Kit, and run on an Applied Biosystems® 3130 Capillary Electrophoresis sequencing platform.

High Resolution for Accurate and Reproducible Results

The Applied Biosystems family of integrated HRM products has been optically, thermally, and chemically optimized to provide the highest-resolution melt curves available today. MeltDoctor™ HRM Reagents, when used with Applied Biosystems HRM instrumentation and software, minimize variation among technical replicates, while maximizing the difference between the melting profiles of different alleles. The superior resolution of the Applied Biosystems HRM reagent set and platform ensures that the smallest sequence variations between samples are easily detected (Figure 3).

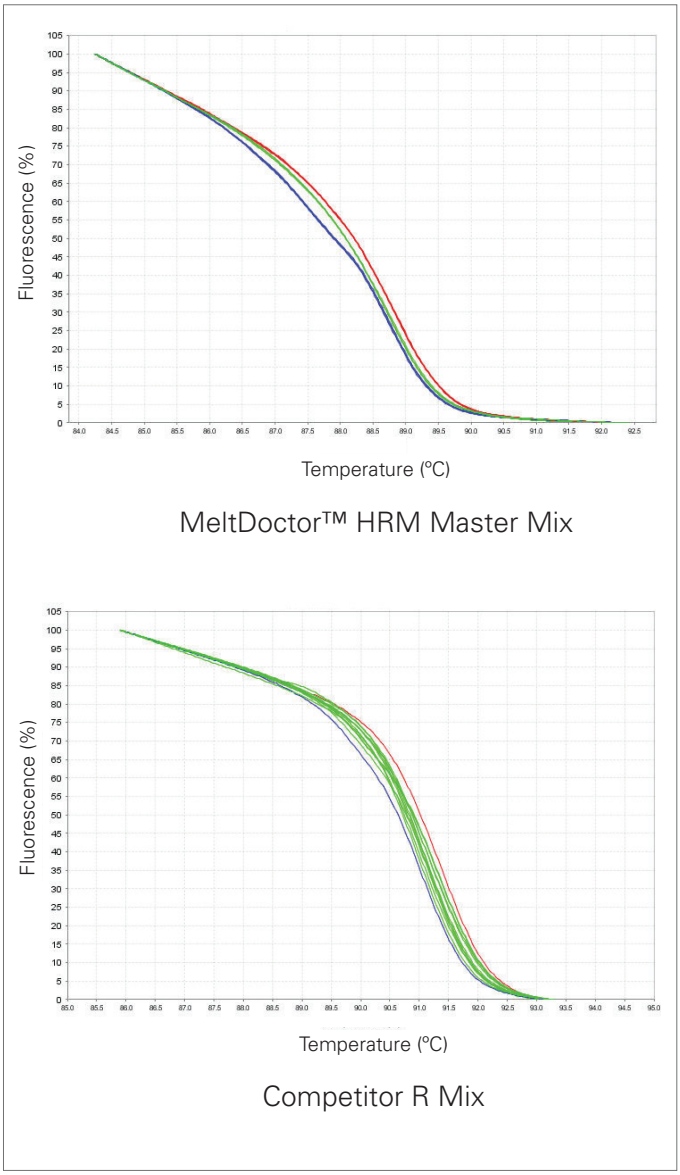

Figure 3. Detection of an A/T (Class 4) SNP in a 118 bp Amplicon with High (68%) GC Content. Using MeltDoctor™ HRM Master Mix (left), the melt curves for the three alleles are clearly resolved, resulting in unambiguous discrimination between genotypes. The mix from Competitor R (right) produces significant variation in melt curve profiles, even among technical replicates. Experiments were performed on an Applied Biosystems® 7500 Fast Real-Time PCR System. A concentration of 2.0 mM $MgCl_2$ was used for the mix from Competitor R. HRM calibration was performed separately for each mix using the MeltDoctor™ Calibration Standard.

Conclusion

MeltDoctor™ HRM reagents from Applied Biosystems offer a comprehensive range of products to produce best-in-class accuracy and best-in-class resolution in your HRM experiments. These reagents, when used in combination with Applied Biosystems HRM-compatible real-time PCR instruments and HRM Analysis Software, provide the highest resolution melt analysis commercially available today.

HRM analysis can be used for several applications including mutation scanning, genotyping and methylation. The Applied Biosystems HRM Guide provides detailed recommendations for designing and executing HRM experiments. To download the HRM Guide or to learn more about HRM applications, **go to www.appliedbiosystems.com/hrm.**

Performance Comparison

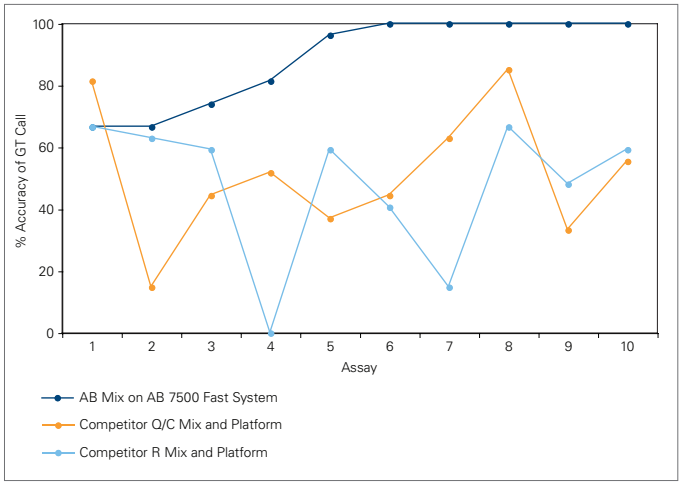

Figure 4. Applied Biosystems HRM Complete Solution Provides Greater Accuracy than Complete Solutions Provided by Other Vendors. Genotyping results using Applied Biosystems instruments, software and reagents were compared to results obtained from instrument-software-reagent solutions provided by other vendors. The accuracy of each call was confirmed by comparison to genotypes obtained using TaqMan® SNP assays and Sanger sequencing.

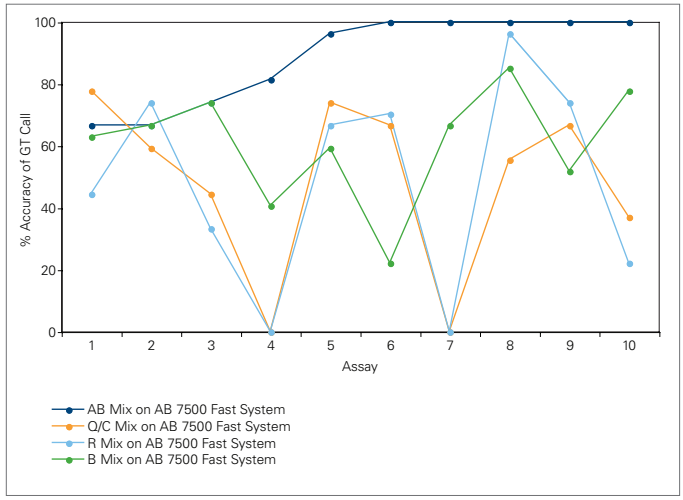

Figure 5. On an Applied Biosystems Real-Time PCR System, MeltDoctor™ HRM Master Mix Provides Greater Accuracy Than HRM Reagents Provided by Other Vendors. The genotyping accuracy of MeltDoctor™ HRM Master Mix was compared to the accuracy obtained using other mixes. The comparison was performed across a panel of 10 assays using the Applied Biosystems® 7500 Fast Real-Time PCR System and HRM software. The accuracy of each call was confirmed by comparison to genotypes obtained using TaqMan® SNP assays and Sanger sequencing.

Description	Reactions*	Quantity	Part Number
MeltDoctor™ Reagents			
MeltDoctor™ HRM Master Mix	500	5 mL	4415440
	2,500	5 x 5 mL	4415452
	5,000	10 x 5 mL	4415450
	5,000	50 mL	4409535
MeltDoctor™ HRM Positive Control Kit	450	1 kit	4410126
MeltDoctor™ HRM Reagent Kit		1 kit	4425557
MeltDoctor™ HRM Calibration Plate		1 384-well plate	4425559
		1 Fast 96-well plate	4425618
MeltDoctor™ HRM Calibration Standard		1 tube, 1 mL	4425562
Real-Time PCR Instruments			
ViiA™ 7 Real-Time PCR System with 384-Well Block		1 unit	4453536
ViiA™ 7 Real-Time PCR System with TaqMan® Array Block		1 unit	4453537
ViiA™ 7 Real-Time PCR System with 96-Well Fast Block		1 unit	4453535
ViiA™ 7 Real-Time PCR System with 96-Well Block		1 unit	4453534
7900HT Fast Real-Time PCR System with 384-Well Block Module		1 unit	4329001
7900HT Fast Real-Time PCR System with 384-Well Block Module and Automation Accessory		1 unit	4329002
7900HT Fast Real-Time PCR System with Fast 96-Well Block Module		1 unit	4351405
7900HT Fast Real-Time PCR System with Standard 96-Well Block Module		1 unit	4329003
7500 Fast Real-Time PCR System with Notebook Computer		1 unit	4351106
7500 Fast Real-Time PCR System with Tower Computer		1 unit	4351107
StepOnePlus™ Real-Time PCR System with Laptop Computer		1 unit	4376598
StepOnePlus™ Real-Time PCR System with Tower Computer		1 unit	4376599
StepOne™ Real-Time PCR System with Laptop Computer		1 unit	4376373
StepOne™ Real-Time PCR System with Tower Computer		1 unit	4376374
Software			
ViiA™ 7 Software HRM Module		1 software	4453724
– for use with ViiA™ 7 Real-Time PCR System			
HRM Analysis Software v2.0		1 software	4397808
– for use with data collected from 7900HT Fast Real-Time PCR System			
High Resolution Melt Software v3.0		1 software	4461357
– for use with data collected from StepOne™, StepOnePlus™, and 7500 Fast Real-Time PCR Systems			

* Assumes 20 µL reactions

Life Technologies offers a breadth of products DNA | RNA | protein | cell culture | instruments

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