AccuMelt™ HRM — High Resolution Melt Analysis SuperMix

ACCURATELY CHARACTERIZE GENETIC VARIATIONS FROM NOVEL MUTATIONS TO RARE POLYMORPHISMS AND MORE

AccuMelt HRM SuperMix maximizes differences in melt temperature and curve shape to allow discrimination of DNA sequence differences amongst different samples. AccuMelt is suitable for a broad range of applications including SNP analysis, mutation scanning, transgene analysis, species identification and DNA methylation analysis.

Quanta BIOSCIENCES™

FEATURES AND BENEFITS

- **See sequence differences clearly — robust amplification ensures sufficient yield of products to generate discrete melt curves**
- **Accurate genotype calling — comparable or better performance than TaqMan Genotyping**
- **Work with rare or precious samples — large range of template inputs possible**
- **Specificity — works with lower Mg2+ concentration than other systems thus enhancing assay accuracy**

AccuMelt HRM SuperMix

AccuMelt HRM SuperMix is a ready-to-use 2X concentrated hot-start PCR mix containing SYTO 9™ green fluorescent DNA-binding dye. AccuStart Taq DNA Polymerase allows for room-temperature reaction assembly and storage at +4°C for 6 months.

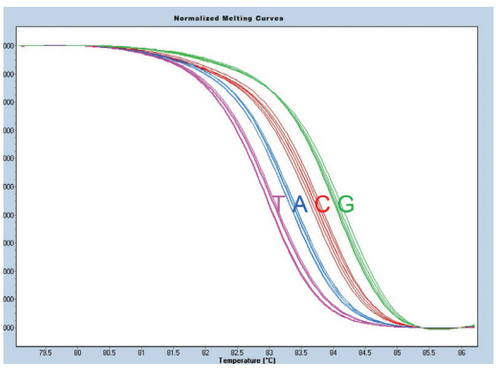

Fig.1 High resolution melting analysis of a model SNP system with a single A,C,G,or T variant base. AccuMelt HRM SuperMix readily resolves each genotype and Tm differences are easily visualized in normalized melting curve plots (Roche, Lightcycler 480).

Superior Resolution of Genotypes

SNP Genotyping is a useful application for HRM and illustrates the capabilities of AccuMelt HRM SuperMix. Genotypes are readily identified based on unique melting profiles depending on a sample's sequence (Figure 1). Furthermore, AccuMelt HRM SuperMix gives superior resolution of difficult genotypes when compared to the leading competitor's mix based on greater Tm differences observed for A→T transversions (Figure 2).

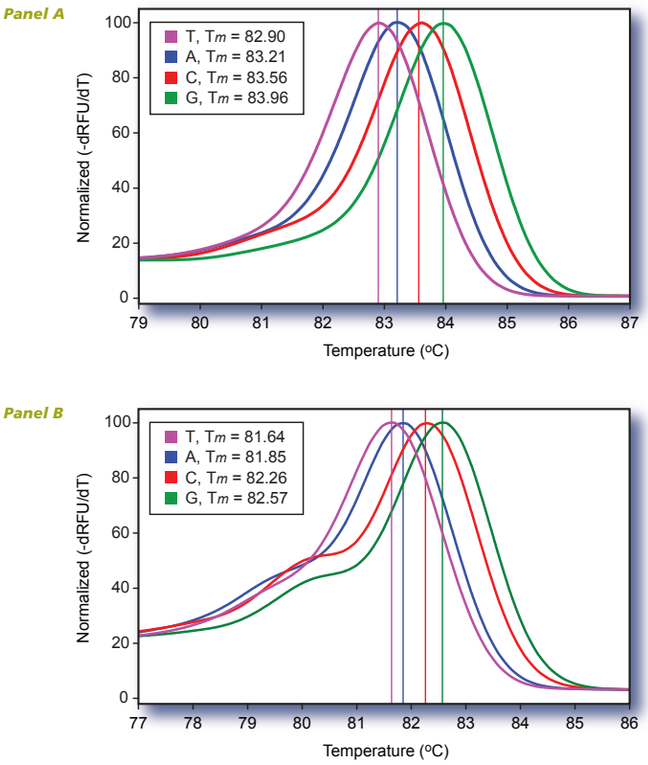

Fig.2 Effect of T,A,C, or G variant base on Tm in a model HRM SNP system with either AccuMelt HRM SuperMix (Panel A), or a competitor's SYTO 9 dye master mix (Panel B). Plots of averaged melt peaks normalized to maximum signal for each system.

COMPARISON TO TAQMAN GENOTYPING

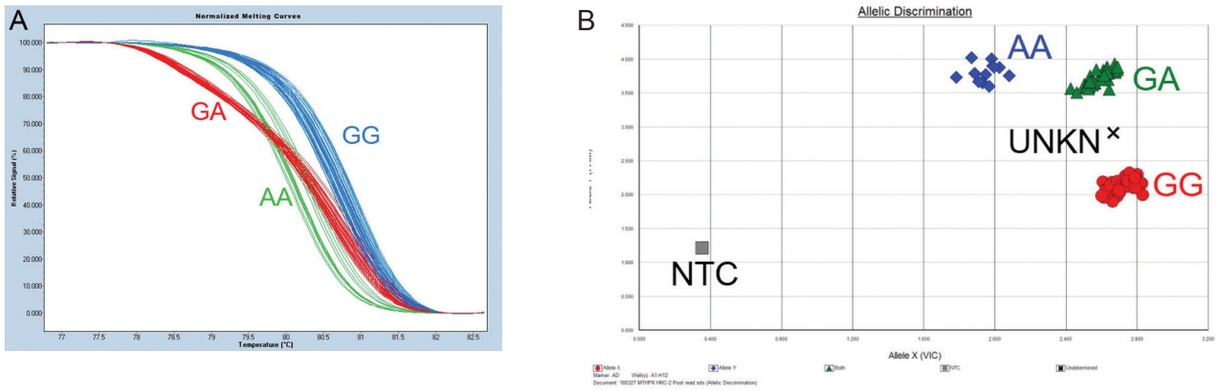

Fig.3 Accuracy of HRM genotyping with AccuMelt HRM SuperMix was evaluated by comparison to TaqMan detection of the G>A rs1801133 SNP in the MTHFR gene. Panel A) HRM normalized melting curves; Panel B) TaqMan allelic discrimination plots. TaqMan failed to resolve Sample D3 (labeled as "UNKN") which was typed as a heterozygote by HRM.

For procedural details please visit our website www.quantabio.com/hrm.

COMPARISON TO TAQMAN GENOTYPING

TaqMan Genotyping has been used successfully in SNP analysis and other allelic discrimination applications. This widely adopted standard in genotyping was used as a benchmark to assess the utility of HRM with our SuperMix. AccuMelt HRM was determined to be just as effective as TaqMan Genotyping in SNP analysis and was even able to call the genotype for a difficult sample which the TaqMan assay could not resolve (see Figure 3).

ROBUST AMPLIFICATION

Consistent robust amplification is critical to accuracy in HRM analysis. AccuMelt HRM SuperMix will drive all PCR amplifications to plateau regardless of the quantity of template input (Figure 4). This ensures accurate results regardless of the quantity of DNA available.

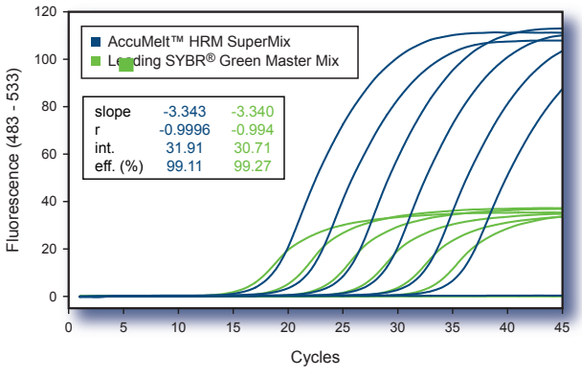

Fig.4 High yield, high efficiency PCR with AccuMelt HRM SuperMix. Real-time PCR of GAPDH was amplified from log-fold serial dilutions of qScript(tm) synthesized cDNA from HeLa cell total RNA (10 ng to 0.1 pg) was carried out with either a leading SYBR Green Master Mix or AccuMelt HRM SuperMix using the following cycling conditions: 95°C, 20s; followed by 45 cycles of: 95°C, 3s; 60°C, 20s. Averaged plots for quadruplicate reactions for each input quantity are shown.

PRODUCT	Quanta Cat. No.	Pack Size
AccuMelt HRM SuperMix	95103-250	250 X 20 ul rxns
	95103-012	1250 X 20 ul rxns
	95103-05K	5000 X 20 ul rxns

Quanta BIOSCIENCES™

www.quantabio.com