Agilent's High Resolution Melt Solutions

Brilliant HRM Ultra-Fast Loci Master Mix

DATA SHEET

High Resolution Melt Application

High resolution melt analysis (HRM) is a quick method to monitor and record the melt profile of amplicons in a sample post PCR. HRM's high sensitivity enables detection of small differences in melting temperatures, for example, caused by single-base changes, nucleotide repeats, and small deletions in DNA.

Some of the more common applications of HRM include: genotype confirmation, mutation identification and screening, clone confirmation, and methylation analysis.

HRM is often used at hypervariable loci to find hard-to-detect base changes. HRM detects new or unknown mutations that can easily be missed by TaqMan assays. In contrast to TaqMan assays, HRM can detect **any mutation between two primers** in the assay. HRM can cost-effectively resolve small changes, but melting temperature control is crucial.

Brilliant HRM Ultra-Fast Loci Master Mix Advantages

- **Mix and Go.** $MgCl_2$ and dNTPs are included.

- **Better stability.** Stable after multiple freeze thaws, reducing wastage and increasing batch-to-batch reliability.

- **Fast-Start Taq.** Activation time for the proprietary mutant Fast-Start Taq polymerase is only three minutes.

- **EvaGreen Release-on-Demand based dye.** It is non-toxic and can be added at saturating concentrations— insuring minimal inhibition while preserving high sensitivity.

- **Platform independent.** Use with any HRM-capable thermocycler.

Agilent's Two High-Performance HRM Options:

1

Brilliant HRM Ultra-Fast Loci Master Mix
Faster HRM on any platform with total confidence

2

Complete AriaMx HRM Solution
The fastest way to confidently identify hard-to-detect genotypes

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Brilliant HRM Ultra-Fast Loci Master Mix

For the scientist seeking to "mix and go" faster and with greater confidence, Agilent now offers the Brilliant HRM Ultra-Fast Loci Master Mix. The Master Mix combines a mutant Fast-Start Taq polymerase, optimized $MgCl_2$, dNTPs, and an EvaGreen, release-on-demand dye to provide faster HRM with total confidence —even for difficult genotypes. It is validated for use on the AriaMx Real-time PCR instrument and third-party HRM-capable thermal cyclers.

To test the ability of the Brilliant HRM Master Mix to resolve difficult-to-detect changes, Agilent developed an HRM assay targeted at the Class IV SNP (T>A), Rs9939609 FTO. This assay was used to demonstrate the performance of the Master Mix on the Agilent AriaMx qPCR instrument and third-party HRM-capable intruments.

This SNP has been correlated with obesity in humans and the risk allele (A) is present in at least one copy in approximately 50% of the general population. The test amplicon was 142 bp and the T_m difference between the homozygotes was determined at only 0.13°C.

Figure 1 shows this SNP being resolved in the same assay, with the same cycling conditions, on three different instruments, using the software platform innate to each instrument. While some slight variations in resolving power were observed, all demonstrated unequivocal resolution of the hard-to-detect SNP.

A. Agilent AriaMx QPCR System
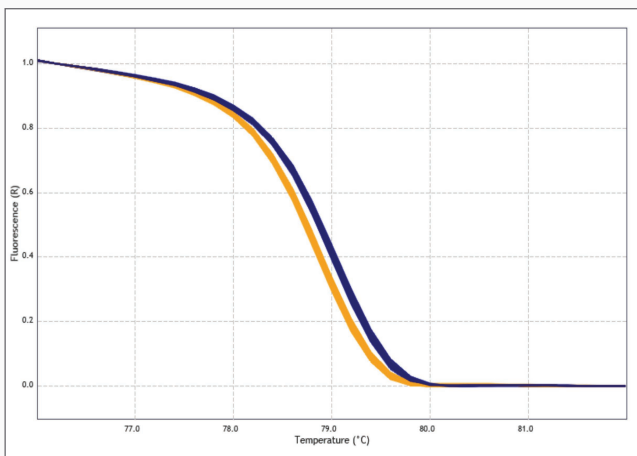
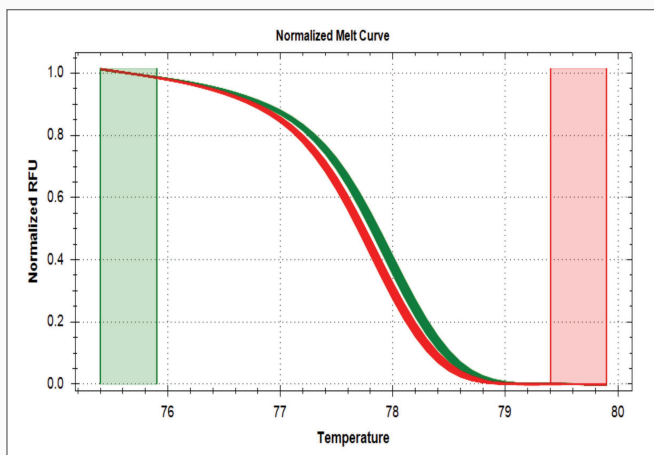

AB. Competitor AB
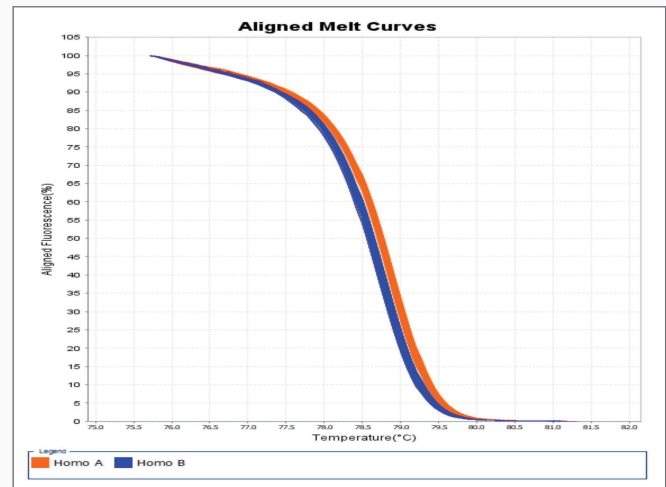
B. Competitor B

Figure 1. HRM of SNP Rs9939609 (FTO) using Brilliant HRM Master Mix on three different instruments.
Panel A: SNP identified using Brilliant HRM Master Mix on Agilent's AriaMx qPCR Instrument. **Panel B:** The same SNP identified using Brilliant HRM Master Mix on Competitor B qPCR Instrument. **Panel AB:** The same SNP identified using Brilliant HRM Master Mix on the Competitor AB qPCR Instrument.

Agilent's Complete HRM Solution

For researchers ready for a change in genotyping speed and confidence, Agilent offers a complete solution: an **HRM Master Mix**, an **instrument** and an **analysis software** for HRM analysis. Agilent's solution features precision thermocyling, fast scanning optics, novel HRM-specific algorithms, and the robust, high-quality Brilliant HRM Ultra-Fast Loci Master Mix. Now researchers can identify difficult-to-detect genotypes much faster than any other 96-well plate based solution, with greater ease and confidence.

Figure 2 compares the time needed to run the same HRM assay using the Brilliant HRM Ultra-Fast Loci Master Mix on three different instruments. The time differences observed are due to: 1) differences in instrument scanning speeds, 2) the number of measurements over the temperature profile, and 3) Taq polymerase's activation time.
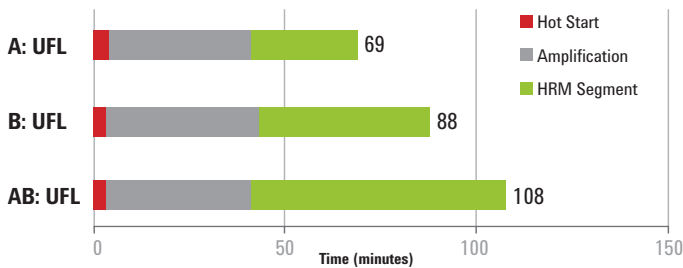

Figure 2. Agilent AriaMx qPCR Instrument is > 20% faster than competitor instruments. This graph shows the time required to identify the Class IV SNP Rs9939609 FTO with the Brilliant HRM Ultra-Fast Loci Master Mix (UFL) on three different qPCR instruments. The assay consisted of a hot start, 40 cycles of qPCR amplification and a high resolution melt segment. (**A:** AriaMx instrument with Brilliant HRM Master Mix; **B:** Competitor B qPCR instrument with Brilliant HRM Master Mix; **AB:** Competitor AB qPCR instrument with Brilliant HRM Master Mix).
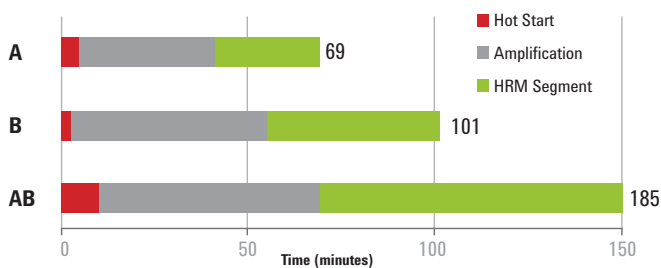

Figure 3. Agilent's Complete HRM Solution is > 30% faster than competing HRM solutions. This graph shows the time to identify the Class IV SNP Rs9939609 FTO on three competing qPCR instruments with manufacturer's respective HRM chemistries. Manufacturer's recommended HRM protocols were used. (**A:** Agilent AriaMx qPCR Instrument with Agilent Brilliant HRM Ultra-Fast Loci Master Mix **B:** Competitor B qPCR instrument and competitor B's HRM Master Mix; **AB:** Competitor AB qPCR instrument with competitor AB's HRM Master Mix).

Using Agilent's complete HRM solution, the time to correctly identify the most difficult-to-detect genotypes can be cut in half (Figure 3). The longest run time, consisting of 185 minutes, was on the AB instrument with the AB reagents. In contrast, on the Agilent AriaMx instrument with the Brilliant HRM Ultra-Fast Loci Master Mix, the same result was obtained in only 69 minutes.

Intuitive HRM Software

Agilent includes fully featured software for HRM analysis. It is intuitive and has several benefits over other software packages with respect to plate set up, analysis and reporting (Table 1). Moreover, unlike competing HRM software, Agilent HRM software comes included at no charge to the user with the AriaMx Real-Time PCR System.

Table 1. Comparison of Instrument Software for HRM Analysis.

FEATURE	Instrument		
	A (AriaMx)	B	AB
Set up replicates quickly using "smart-rules-based" software	🟢	🟡	🔴
Use files from a previously run experiment as a template	🟢	🔴	🔴
Use a highlighting feature to link plots to the results table for easier analysis	🟢	🟢	🔴
Hover over each plot to reveal key data (e.g. X,Y co-ordinates, replicate number, dye channel)	🟢	🔴	🔴

🟢 Yes   🔴 No   🟡 Partial

The Agilent platform is so much faster than the competition due to fast scanning and an algorithm to interpolate the maximum T_m peak value. Other platforms report the highest melt peak value observed during the run, requiring many more scans to obtain an equivalent answer.

Whether looking for a more trustworthy **HRM Master Mix** or a new, **Ultra-Fast Complete HRM Solution**, Agilent delivers total confidence.

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DELIVERING FASTER ANSWERS IS IN OUR GENES

The efficient workflow provided by Agilent's genomic portfolio gets you better answers, faster.

FOR MORE INFORMATION

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For Americas, call **800.227.9770** or for other regions, consult **www.agilent.com/genomics/contactus**

For a virtual demo, visit
www.agilent.com/genomics/ariamxvideos
For a live demo, call your Agilent sales representative.

ORDERING INFORMATION

Part Number	Description
5190-7827	Brilliant HRM Ultra-Fast Loci Master Mix
5190-7702	AriaMx HRM Calibration Kit
600536	Optional: Passive Reference Dye (Rox)
G8830A	AriaMx Real-Time PCR System

See Deeper. Reach Further.

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