# High-Resolution Melt with the xxpress® qPCR Thermal Cycler

A high-resolution melt (HRM) is a powerful, post-PCR analysis technique, used to identify the DNA products present. This method has a wide variety of uses from determining the specificity of a PCR reaction to detecting mutations and polymorphisms. It is also useful for checking that the PCR reaction was specific, and the correct DNA has been amplified. The xxpress® qPCR thermal cycler is reliable, sensitive and ultra-fast, making it the perfect system for performing HRMs with confidence.

## 1. High-Resolution Melt

A HRM is performed after PCR amplification. The technique characterizes amplified DNA targets according to the temperature of their transition from double-stranded DNA (dsDNA) to single-stranded DNA (ssDNA) when heated.

First, PCR is performed with an intercalating dye present. These dyes have the unique property of only binding to dsDNA. Once incorporated into the newly synthesised amplicon, the dye fluoresces brightly.

During a HRM the amplicon is heated. Once its specific melting point (Tm) is reached, the DNA denatures and becomes ssDNA. This transition is monitored by the decrease in fluorescence that occurs as the dye dissociates. Third generation, saturating dyes, like Eva Green, are best because they do not re-associate with the DNA.

A particular DNA fragment will have a precise melting profile. HRM curves are specific and sensitive enough to detect sequence variations as small as a single base.


Figure 1. The xxpress® qPCR thermal cycler is reliable, sensitive and ultrafast, making it the perfect system for performing HRMs with confidence.

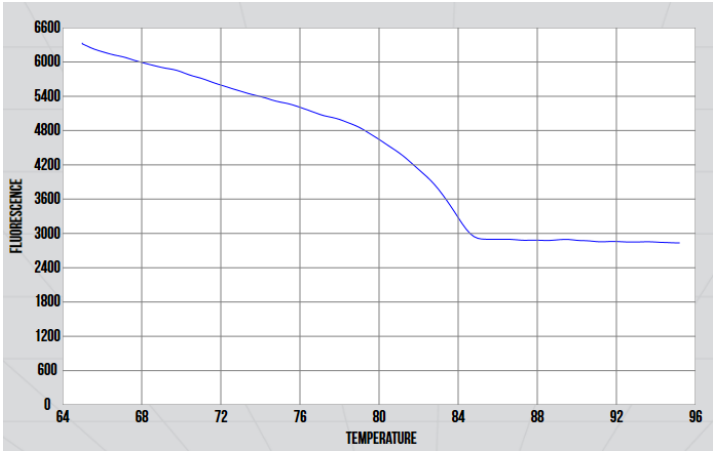
## 2. Protocol

Reaction mixture: 10µL Precision Melt Supermix (Bio-Rad), 3µL nuclease free water, 1µL forward 18srRNA primer (10µM), 1µL reverse 18srRNA primer (10µM), 5µL human genomic DNA (10ng/µL). 20µL reaction mixture pipetted into a well of a 24 well xxplate™ SBS.

HRM thermal protocol: following ultra-fast PCR, perform the high-resolution melt from 65°C - 95°C taking 5 measurements per second in optical channel 1.

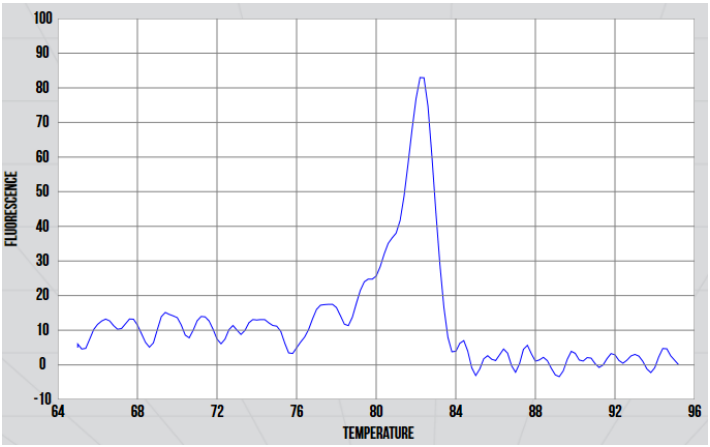
## 3. Results

The xxpress® qPCR thermal cycler performs quick, simple, HRMs and displays the data in an intuitive melt graph, as shown in graph 1 below.




Graph 1. HRM melt curve produced by the xxpress® qPCR thermal cycler displaying the results from the detailed HRM.

To clearly visualise the Tm values as peaks, the xxpress® can produce a first derivative plot of the data, see graph 2 below. There is a choice of export formats too, .csv or RDML.




Graph 2. First derivative plot graph produced by the xxpress® qPCR thermal cycler.

## 4. Conclusions

HRM is a powerful, post-PCR analysis technique with a wide variety of uses. Performing HRM on the xxpress® qPCR thermal cycler produces reliable, intuitive results in just a short time, increasing throughput. Users can confidently determine whether the qPCR reaction was specific, and that the correct DNA was amplified.

## 5. References


- Bustin, S., et al. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry*. **55**(4), 611–622.
- Gundry, C., Vandersteen, J., Reed, G., Pryor, R., Chen, J. and Wittwer, C. (2003). Amplicon Melting Analysis with Labeled Primers: A Closed-Tube Method for Differentiating Homozygotes and Heterozygotes. *Clinical Chemistry*. **49**(3) 396-406.
- Reed, G., Kent, J. and Wittwer, C. (2007). High-resolution DNA melting analysis for simple and efficient molecular diagnostics . *Pharmacogenomics*. **8**(6), 597-608.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