Beginners Guide to High Resolution Melt (HRM) analysis

Why HRM?

High Resolution Melt or HRM analysis as it will be referred to herein is a hugely powerful technique for the detection of mutations, polymorphisms and epigenetic differences in double stranded DNA samples.

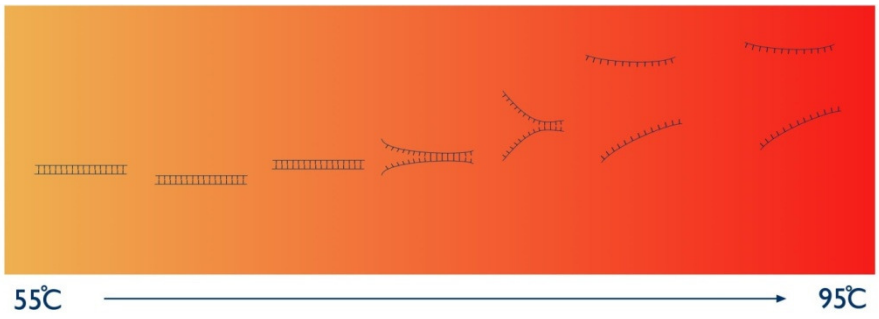
It has huge advantages over other genotyping technologies. Namely:

- It is massively **cost effective** vs. other genotyping technologies such as sequencing and Taqman SNP typing. This makes it ideal for large scale genotyping projects
- It is **fast and powerful** thus able to accurately genotype huge numbers of samples in rapid time
- It is **simple**. With a good quality HRM assay powerful genotyping can be performed by non-geneticists in any laboratory with access to an HRM capable real-time PCR machine.

How does it work?

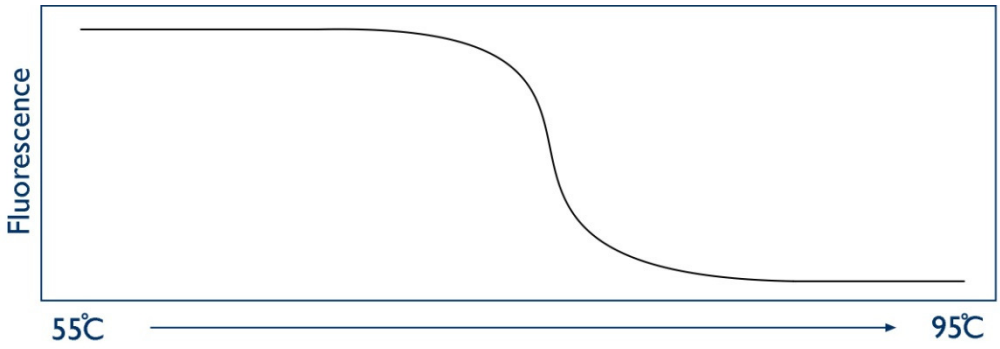
HRM analysis is performed on double stranded DNA samples. Typically the user will use the real-time polymerase chain reaction prior to HRM analysis to amplify the DNA region in which their mutation of interest lies. (If you are new to real-time PCR take a look at the "Beginners guide to real-time PCR" on the PrimerDesign website.). Essentially the real-time PCR process turns a tiny amount of your region of DNA of interest in to a large amount so you have enough to be worth analysing. Now in the tube there are billions of copies of your region of DNA of interest. This region that is amplified is known as the amplicon.

After the PCR process the HRM analysis begins. The process is simply a precise warming of the amplicon DNA from around 50˚C up to around 95˚C. At some point during this process the melting temperature of the amplicon is reached and the two strands of DNA "melt" apart.



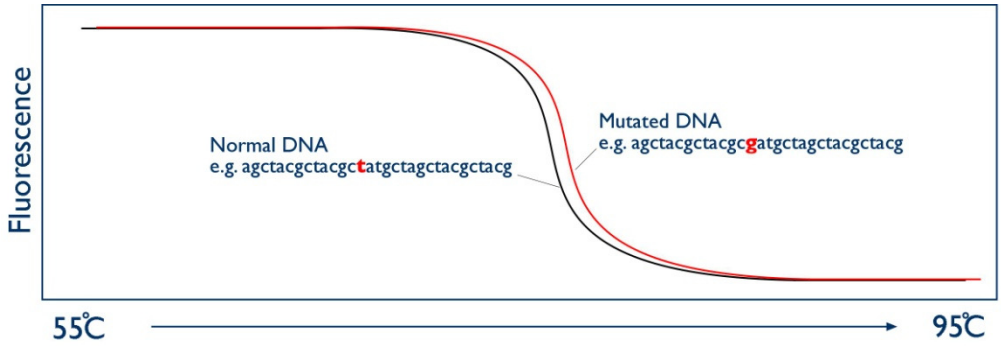
The secret of HRM is to monitor this process happening in real-time. This is achieved by using a fluorescent dye. The dyes that are used for HRM are known as intercalating dyes and have a unique property; they bind specifically to double-stranded DNA and when they are bound they fluoresce brightly. In the absence of double stranded DNA they have nothing to bind to and they only fluoresce at a low level.

So at the beginning of the HRM analysis there is a high level of fluorescence in the sample because of the billions of copies of the amplicon. But as the sample is heated up and the two strands of the DNA melt apart there is no longer any double stranded DNA present and thus fluorescence is reduced. The HRM machine has a camera that watches this process by measuring the fluorescence. The machine then simply plots this data as a graph known as a melt curve showing the level of fluorescence vs the temperature:



Spot the difference

The temperature that the amplicon melts and the two DNA strands come apart is an entirely predictable process. It is dependant on the sequence of the DNA bases. If you are comparing two samples from two different people they should give exactly the same shaped melt curve. However if one of the people has a mutation in the DNA region you have amplified then this will alter the temperature at which the DNA strands melt apart. So now the two melt curves appear different. The difference may only be tiny, perhaps a fraction of a degree, but because the HRM machine has the ability to monitor this process in "high resolution" it is possible to accurately document these changes and therefore identify if a mutation is present or not.



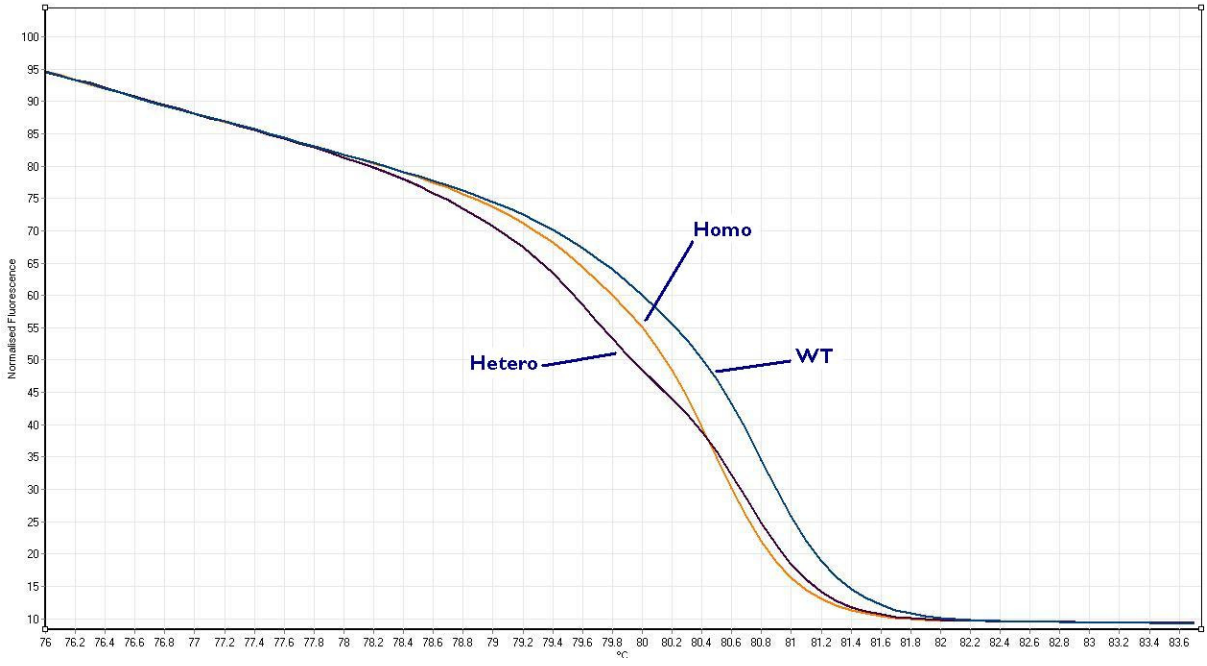
Wild type, heterozygote or homozygote?

Things become slightly more complicated than this because organisms contain two copies of each gene, known as the two alleles. So, if a sample is taken from a patient and amplified using PCR both copies of the region of DNA (alleles) of interest are amplified. So if we are looking for mutation there are now three possibilities:

1. Neither allele contains a mutation
2. One or other allele contains a mutation
3. Both alleles contain a mutation.

These three scenarios are known as "Wild –type", "Heterozygote" or "homozygote" respectively. Each gives a melt curve that is slightly different. With a high quality HRM assay it is possible to distinguish between all three of these scenarios.

This is some real data looking for a mutation in a gene associated with breast cancer. There are three different patients here. You can clearly see that one of the patients has the Wild type (WT) genotype, one is Homozygous (homo) and the other has the Heterozygous (hetero) genotype.



Everything you need for HRM analysis

PrimerDesign can provide all of the kits, reagents and expertise that you require for an HRM project.

Custom designed High Resolution Melt analysis assays

Each assay is individually designed by one of expert team. The assay is then synthesised and the assay is validated in our laboratory to ensure perfect specificity. The assay is then shipped and guaranteed to work with no further optimisation.

Precision High Resolution Melt MasterMix

A complete real-time PCR MasterMix for high resolution melt analysis supplied at an exceptionally low price. The mix is premixed with an HRM specific intercalating dye for sharp, reproducible results.

Gene scanning High Resolution Melt kits

If you wish to scan a whole gene for the presence of novel SNPs or mutations we can provide a series of assays that span the entire length of your sequence. As always every assay is individually prevalidated to ensure you get great data.

Visit www.highresolutionmelt.co.uk for full details