

Interpretation Aid MC006 Lactase Persistence Genotyping 2

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Melt patterns and T_m per SNP detected by MC006

Each MC006 probe targets a genomic region containing multiple SNPs, including variants associated with Lactase Persistence (LP) as well as variants not associated with LP. This interpretation aid is meant to provide example peak patterns for a number of these SNPs, in addition to those included in the IFU. This aid is not exhaustive as positive samples are not available for each SNP present within the probe target regions.

Each SNP under the probe changes the melting temperature (T_m) of the peak as well as the shape to a certain extent. SNPs that alter the T_m in a significant way can be easily identified. For SNPs that cause only subtle changes or produce peak patterns highly similar to those of other SNPs/WT sequence, detection should be confirmed by MC005 or other means.

MC006 FAM probe (Region 1)

At the start of interpretation of results it is important to identify samples displaying a homozygous WT peak pattern (figure 1).

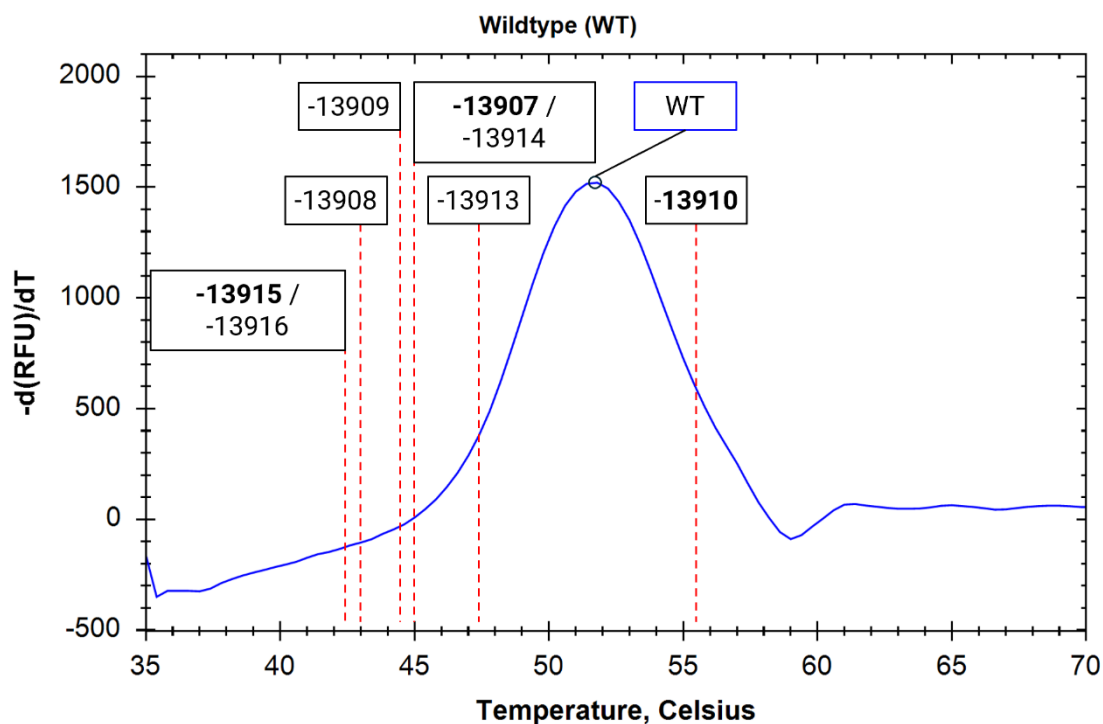


Figure 1. This graph shows the peak pattern of a homozygous WT sample in blue (T_m is ~51.5°C). The red dotted lines indicate the T_m of the SNPs mentioned in the box attached to the dotted line. SNPs in bold are associated with LP. The figure includes the T_m values for all SNPs for which positive samples were available.

Next, when WT samples are identified, samples positive for SNPs associated with LP should be identified. For the MC006 region 1 (FAM) probe these are -13907, -13910 and -13915.

-13910

The T_m of -13910 is distinct from the WT and other SNPs and can therefore be detected by MC006 alone (figure 2).

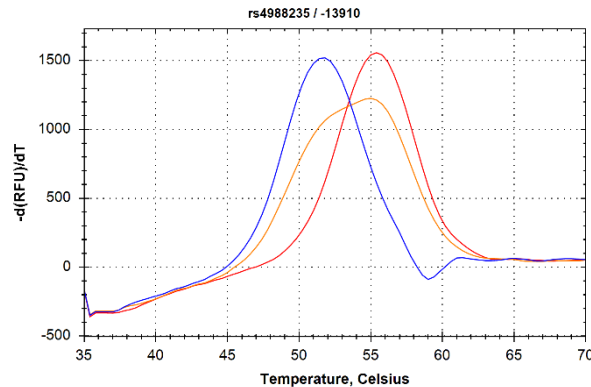


Figure 2. This figure shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs4988235 (-13910) (red) and a heterozygous sample (orange) as detected with MC006. The WT peak in this region has a T_m of ~51.5°C. The SNP peak has a T_m of ~55.5°C.

-13915

The T_m of -13915 is distinct from the WT (figure 3); however it is less distinguishable from other SNPs. -13915 cannot be confirmed using MC005 as this SNP is not covered by this product. Confirmation through other means is recommended.

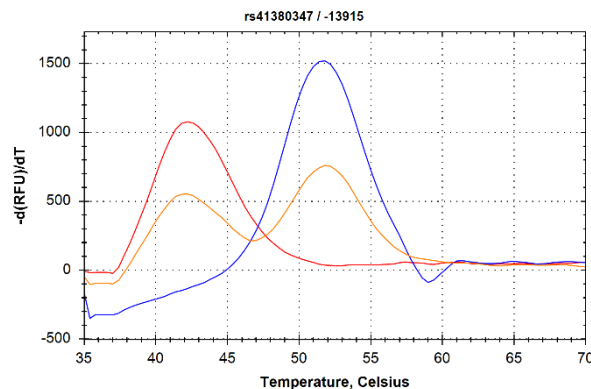


Figure 3. This figure shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs41380347 (-13915) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of ~51.5°C. The SNP peak has a T_m of ~42.5°C.

-13907

The T_m of -13907 is similar to the T_m of other, non-LP associated SNPs, -13909 and -13914. Conclusive detection of -13907 is possible when MC006 results are used to confirm MC005 results.

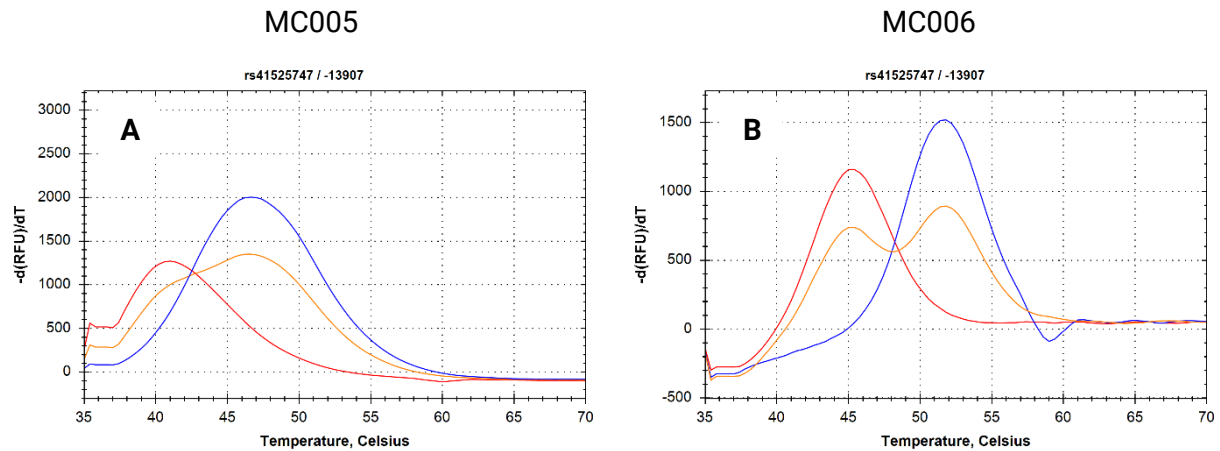


Figure 4. This figure shows the peak pattern for SNP -13907 as detected with both MC005 (figure 4A) and MC006 (figure 4B). **Figure 4A** shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs41525747 (-13907) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of ~47°C. The SNP peak has a T_m of ~41°C. **Figure 4B** shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs41525747 (-13907) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of ~51.5°C. The SNP peak has a T_m of ~45°C. When these peak patterns are observed by MC005 and MC006 respectively, detection of -13907 is confirmed.

Other SNPs

When no peak patterns for WT, -13910, -13915 or -13907 are found, a SNP not associated with LP could be present in the sample. Example peak patterns for SNPs -13908, -13909, -13913, -13914 and -13916 can be found in figure 5 below.

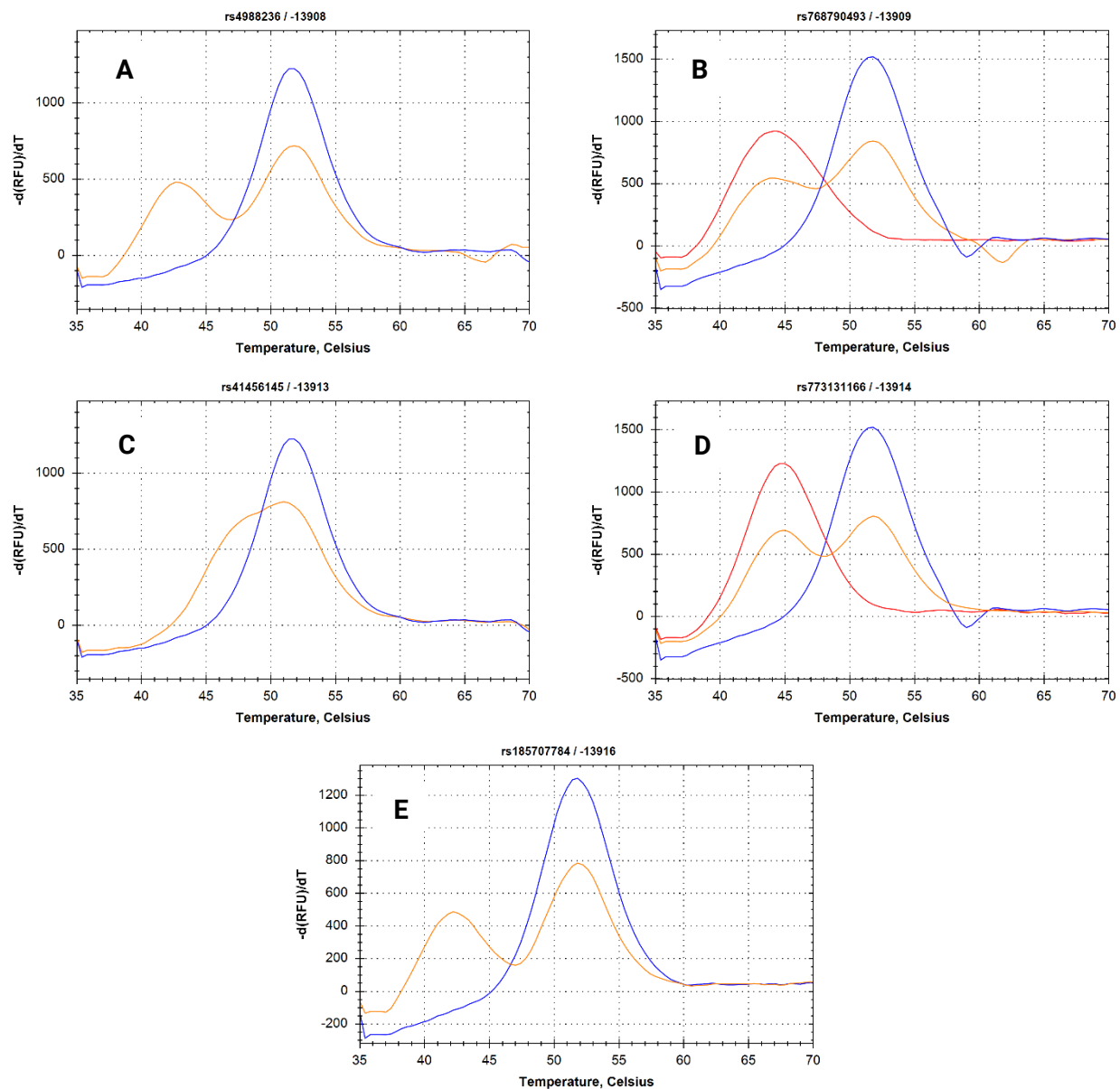


Figure 5. This figure shows the peak pattern for SNPs -13908, -13909, -13913, -13914 and -13916 as detected by MC006. The WT peak in this region has a T_m of $\sim 47^\circ\text{C}$.

Figure 5A shows the peak pattern of a homozygous wildtype sample (blue) and a heterozygous sample for SNP rs4988236 (-13908) (orange). A homozygous sample was not available. The SNP peak has a T_m of $\sim 43^\circ\text{C}$.

Figure 5B shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs768790493 (-13909) (red) and a heterozygous sample (orange). The SNP peak has a T_m of $\sim 44.5^\circ\text{C}$.

Figure 5C shows the peak pattern of a homozygous wildtype sample (blue) and a heterozygous sample for SNP rs41456145 (-13913) (orange). A homozygous sample was not available. The SNP peak has a T_m of $\sim 47.5^\circ\text{C}$.

Figure 5D shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs773131166 (-13914) (red) and a heterozygous sample (orange). The SNP peak has a T_m of $\sim 45^\circ\text{C}$.

Figure 5E shows the peak pattern of a homozygous wildtype sample (blue) and a heterozygous sample for SNP rs185707784 (-13916) (orange). A homozygous sample was not available. The SNP peak has a T_m of $\sim 42.5^\circ\text{C}$.

MC006 ROX probe (Region 2)

At the start of interpretation of results it is important to identify samples showing a homozygous WT peak pattern (figure 6).

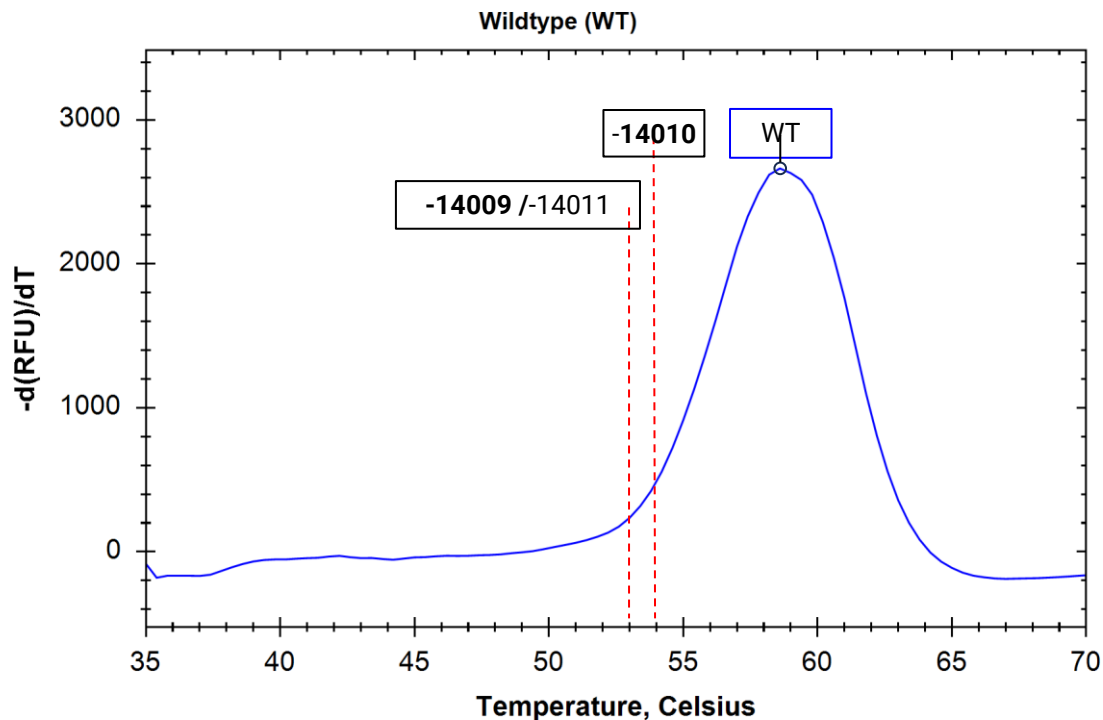


Figure 6. This graph shows the peak pattern of a homozygous WT sample in blue (T_m is $\sim 58.5^\circ\text{C}$). The red dotted lines indicate the T_m of the SNPs mentioned in the box attached to the dotted line. SNPs in bold are associated with LP. The figure shows the T_m for all SNPs for which positive samples were available. No positive sample was available for SNP -13992, -14000 and -14002. The T_m for these SNPs are therefore unknown.

Next, when WT samples are identified, samples positive for SNPs associated with LP should be identified. For the MC006 region 2 (ROX) probe these are -14009 and -14010.

-14010

The T_m of -14010 is distinct from the WT, but due to the proximity to the T_m of other SNPs in the region it is recommended to use MC006 results only to confirm MC005 results (figure 7).

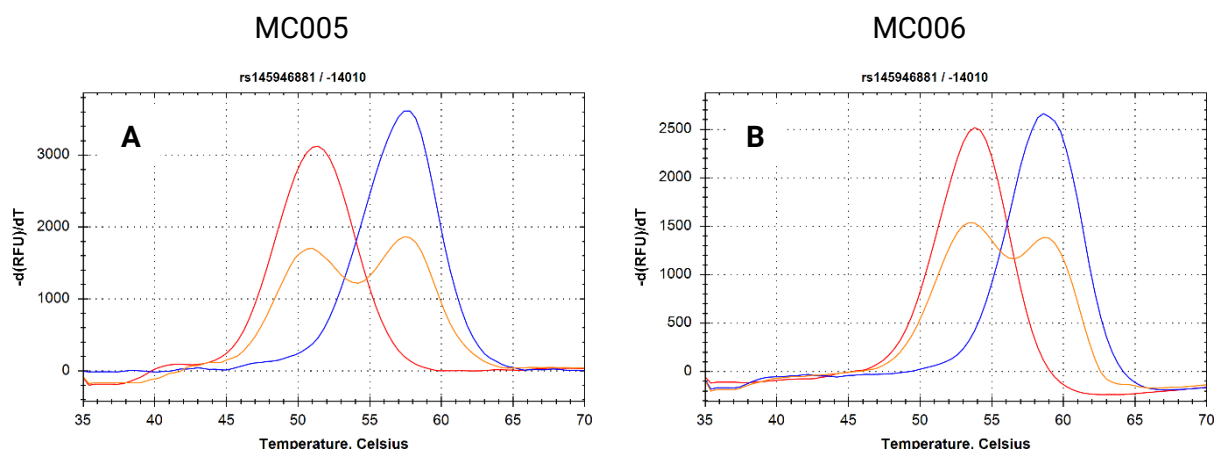


Figure 7. This figure shows the peak pattern for SNP -14010 as detected with both MC005 (figure 7A) and MC006 (figure 7B). **Figure 7A** shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs145946881 (-14010) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of $\sim 57.5^\circ\text{C}$. The SNP peak has a T_m of $\sim 51^\circ\text{C}$. **Figure 7B** shows the peak pattern of a homozygous wildtype sample

(blue), a homozygous sample for SNP rs145946881 (-14010) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of $\sim 58.5^\circ\text{C}$. The SNP peak has a T_m of $\sim 54^\circ\text{C}$. When these peak patterns are observed by MC005 and MC006 respectively, detection of -14010 is confirmed.

-14009

For SNP -14009 it is necessary to co-detect it using both MC006 and MC005. When the combined result of MC005 and MC006 is positive for the -14009 SNP, detection of this SNP is confirmed.

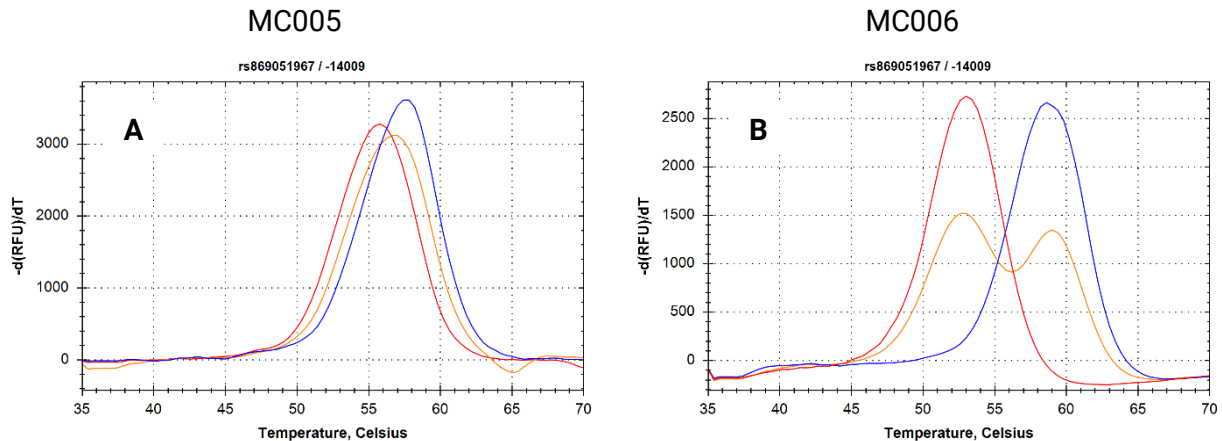


Figure 8. This figure shows the peak pattern for SNP -14009 as detected with both MC005 (figure 8A) and MC006 (figure 8B). **Figure 8A** shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs869051967 (-14009) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of $\sim 57.5^\circ\text{C}$. The SNP peak has a T_m of $\sim 55.5^\circ\text{C}$. **Figure 8B** the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs869051967 (-14009) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of $\sim 58.5^\circ\text{C}$. The SNP peak has a T_m of $\sim 53^\circ\text{C}$. When these peak patterns are observed by MC005 and MC006 respectively, detection of -14009 is confirmed.

Other SNPs

When no peak patterns for WT, -14010 or -14009 are found, a SNP weakly associated with LP could be present in the sample. Example peak patterns for SNP -14011 can be found in figure 9 below.

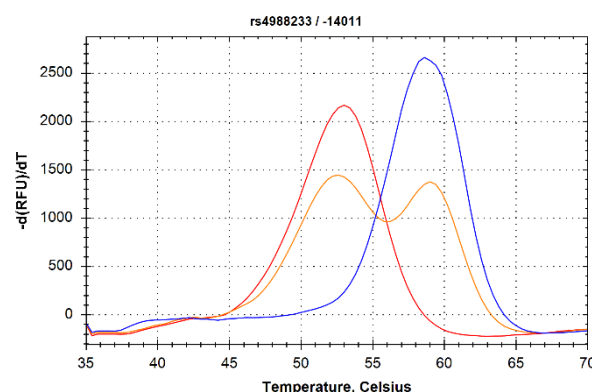


Figure 9. This figure shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs4988233 (-14011) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of $\sim 58.5^\circ\text{C}$. The SNP peak has a T_m of $\sim 53^\circ\text{C}$.

MC006 Q-fragment ROX probe (Region 2)

In case too little input DNA is used, a quantity fragment (Q-fragment) peak will be observed. In MC006, this Q-fragment peak has a T_m of $\sim 45.5^\circ\text{C}$. Reactions in which the Q-fragment peak is higher than any other peak should be regarded as failed due to an insufficient amount of sample DNA. Examples of the Q-fragment can be found in figure 10 below.

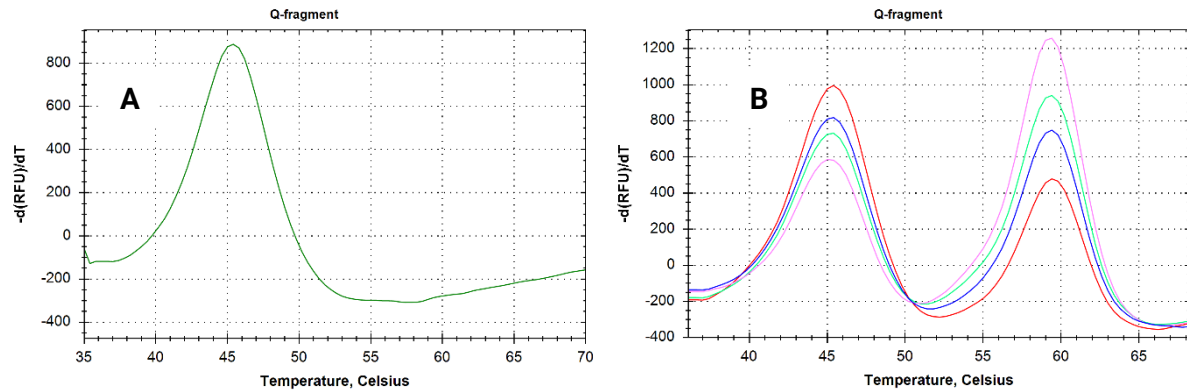


Figure 10. This figure shows the peak pattern of the Q-fragment in MC006 region 2 (ROX probe). **Figure 10A** shows the peak pattern for a reaction without input DNA. A Q-fragment peak has a T_m of $\sim 45.5^\circ\text{C}$. **Figure 10B** shows a dilution series with decreasing amounts of input DNA (pink = ~ 0.5 ng, green = ~ 0.3 ng, blue = ~ 0.25 ng and red = ~ 0.125 ng). A decrease in input DNA (WT peak at $\sim 58.5^\circ\text{C}$) results in an increase in height of the Q-fragment peak (peak at $\sim 45.5^\circ\text{C}$).

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MC005		SNP	MC006	
ROX - Region 1	FAM - Region 2		FAM - Region 1	ROX - Region 2
Peak at 47°C		Wildtype (hom) Confirmation possible with MC005	Yes: Only 1 peak at 51.5°C No: Peak at 55.5°C	Start → Only 1 peak at 58.5°C No → Peak at 54°C No → Peak at 53°C (Co-detection) No → Peak at other Tm
Peak at 57.5°C	-13910 present Confirmation possible with MC005	Yes: Peak at 55.5°C No: Peak at 45°C		
Peak at 41°C	-13907 possibly present Primary detection with MC005 is strongly recommended	Yes: Peak at 42.5°C No: (Path continues down)		
	-13915 present Confirmation possible with positive sample	No		
	Wildtype (hom) Confirmation possible with MC005	Yes: Only 1 peak at 58.5°C		
	Peak at 57.5°C	-14010 possibly present Primary detection with MC005 is strongly recommended	Yes: Peak at 54°C	
	Peak at 51°C	-14009 possibly present Co-detection with MC005 required	Yes: Peak at 53°C (Co-detection)	
	Peak at 55.5°C (Co-detection)	Other SNP present.	Yes: Peak at other Tm	
			No: Peak at other Tm	Yes: Peak at other Tm

Detection:
 The assay can sufficiently distinguish the SNP from all other SNPs in the region.

Confirmation:
 The assay can confirm the result obtained with the other assay but cannot sufficiently distinguish this SNP from all other SNPs in the region when used on its own.

Co-detection:
 The combined results of both assays are required to sufficiently distinguish this SNP from all other SNPs in the region.

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