

Interpretation Aid MC005 Lactase Persistence Genotyping 1

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Melt patterns and T_m per SNP detected by MC005

Each MC005 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 MC006 or other means.

MC005 ROX probe (Region 1)

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

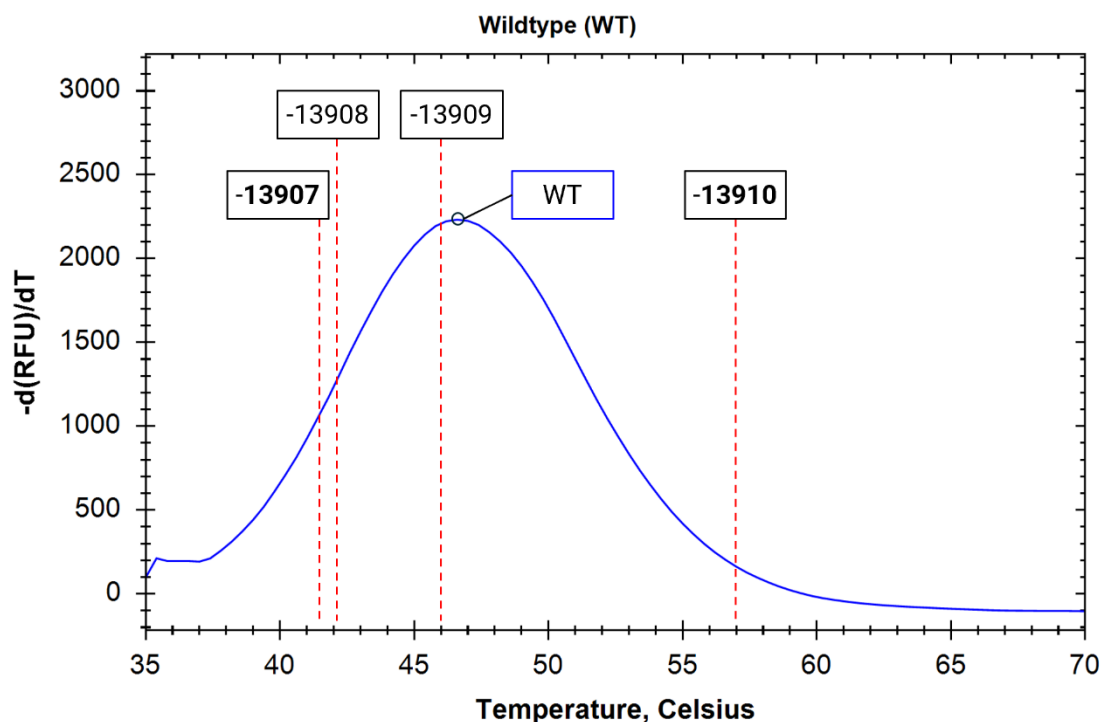


Figure 1. This graph shows the peak pattern of a homozygous WT sample in blue (T_m is ~47°C). The red dotted lines indicate the T_m of the SNPs mentioned in the box attached to the dotted line. SNPs in bold are associated with LP. The figure includes the T_m values for all SNPs for which positive samples were available. No positive sample was available for SNP -13898. The T_m for this SNP is therefore unknown.

Next, when WT samples are identified, samples positive for SNPs associated with LP should be identified. For the MC005 region 1 (ROX) probe, these are -13907 and -13910.

-13910

The T_m of -13910 is distinct from the WT and other SNPs and can therefore be detected by MC005 alone (figure 2).

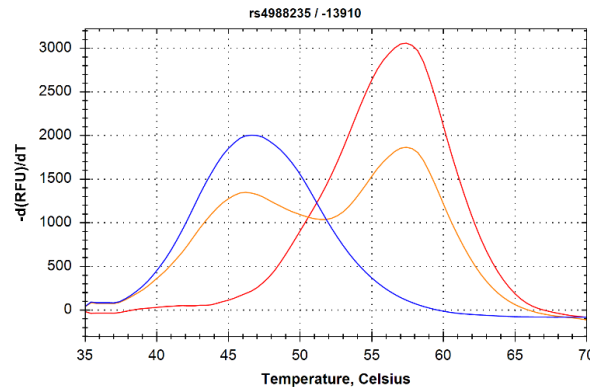


Figure 2. This figure shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs4988235 (-13910) (red) and a heterozygous sample (orange) as detected with MC005. The WT peak in this region has a T_m of ~47°C. The SNP peak has a T_m of ~57.5°C.

-13907

The T_m of -13907 is less distinct from the WT T_m and similar to the T_m of another, non-LP associated SNP, -13908. Conclusive detection of -13907 is possible by confirmation using MC006 (figure 3).

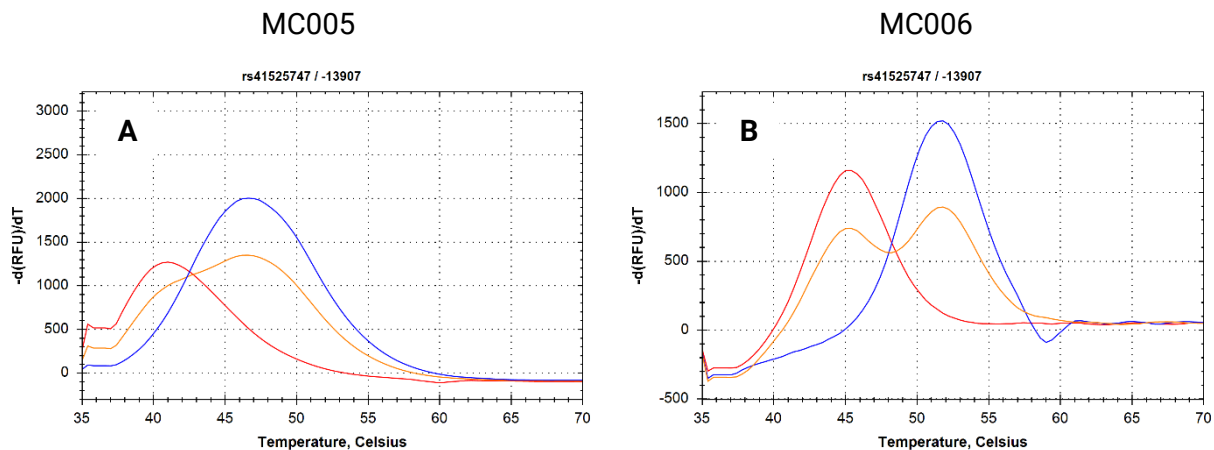


Figure 3. This figure shows the peak pattern for SNP -13907 as detected with both MC005 (figure 3A) and MC006 (figure 3B). **Figure 3A** shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs41525747 (-13907) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of ~47°C. The SNP peak has a T_m of ~41°C. **Figure 3B** 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 or -13907 are observed, a SNP not associated with LP could be present in the sample. Example peak patterns for SNPs -13908 and -13909 can be found in figure 4 below.

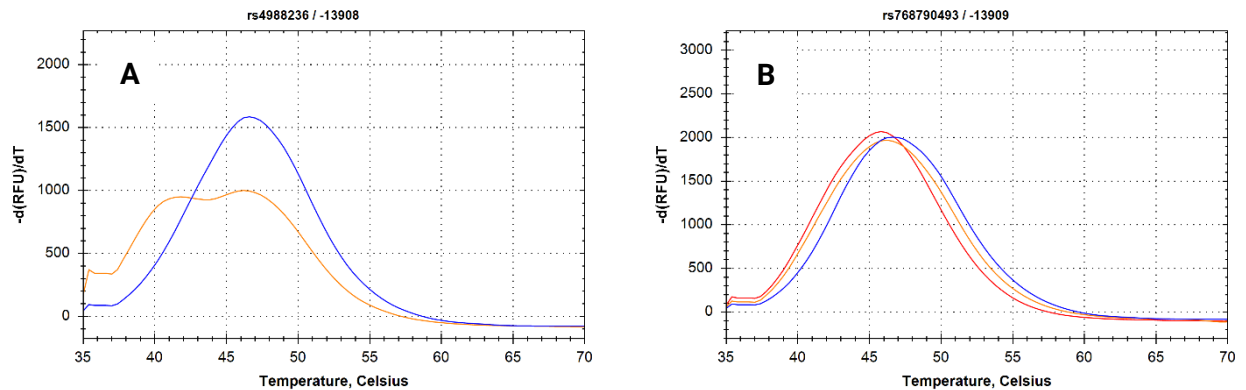


Figure 4. This figure shows the peak pattern for SNPs -13908 and -13909 as detected with MC005. **Figure 4A** shows the peak pattern of a homozygous wildtype sample (blue) and a heterozygous sample for SNP rs4988236 (-13908) (orange). A homozygous sample was not available. The WT peak in this region has a T_m of $\sim 47^\circ\text{C}$. The SNP peak has a T_m of $\sim 42^\circ\text{C}$. **Figure 4B** shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs768790493 (-13909) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of $\sim 47^\circ\text{C}$. The SNP peak has a T_m of $\sim 46^\circ\text{C}$.

MC005 FAM probe (Region 2)

At the start of interpretation of results, it is important to identify samples displaying a homozygous WT peak pattern (figure 5).

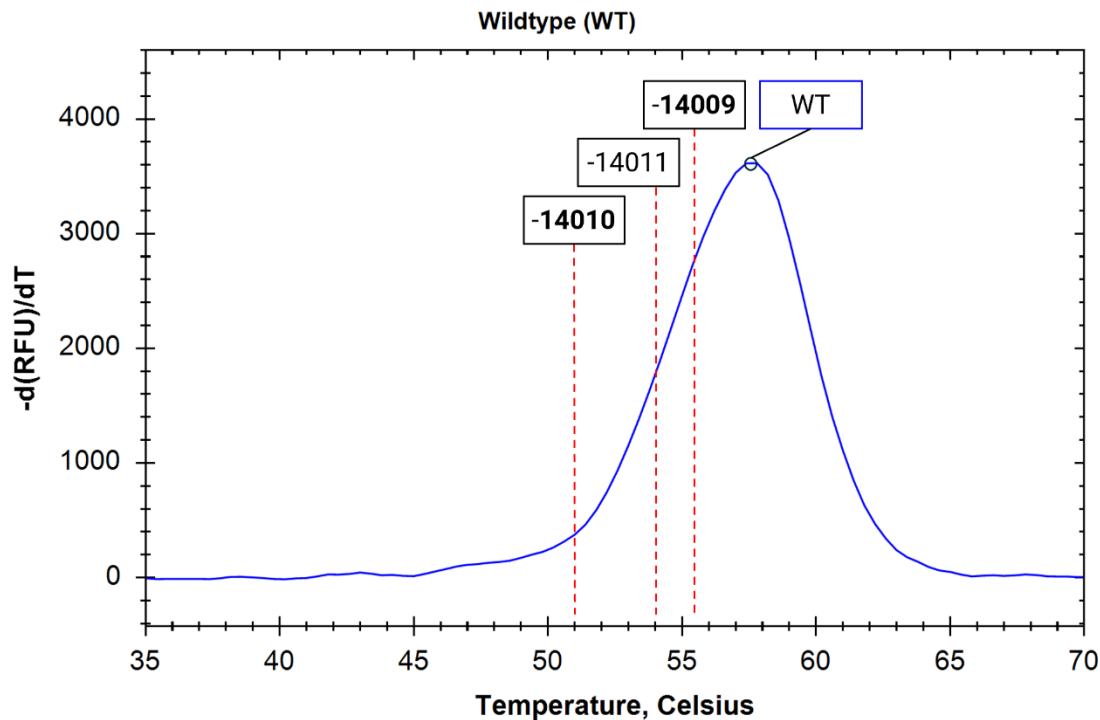


Figure 5. This graph shows the peak pattern of a homozygous WT sample in blue (T_m is $\sim 57.5^\circ\text{C}$). The red dotted lines indicate the T_m of the SNPs mentioned in the box attached to the dotted line. SNPs in bold are associated with LP. The figure shows the T_m for all SNPs for which positive samples were available. No positive sample was available for SNP -13992, -14000 and -14002. The T_m for these SNPs is therefore unknown.

Next, when WT samples are identified, samples positive for SNPs associated with LP should be identified. For the MC005 region 2 (FAM) probe these are -14009 and -14010.

-14010

Although the T_m of -14010 is distinct from the WT, due to the proximity to the T_m of other SNPs in the region it is recommended to confirm detection of this SNP with MC006 (figure 6).

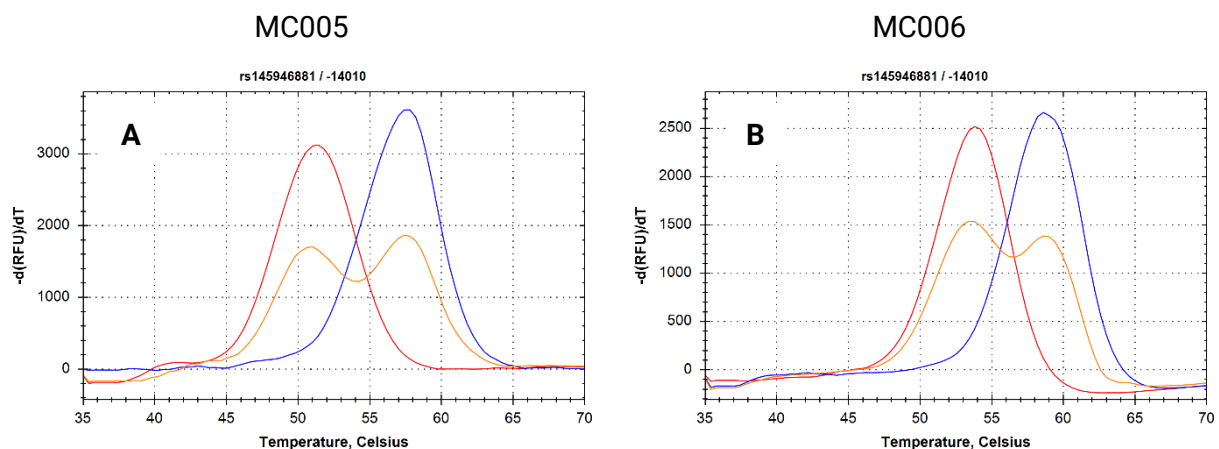


Figure 6. This figure shows the peak pattern for SNP -14010 as detected with both MC005 (figure 6A) and MC006 (figure 6B). **Figure 6A** shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs145946881 (-14010) (red) and a heterozygous sample (orange). The WT peak 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 6B** 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

The T_m of -14009 is less distinct from the WT. For this SNP it is therefore necessary to co-detect it using MC006. When the combined result of MC005 and MC006 is positive for the -14009 SNP, detection of this SNP is confirmed (figure 7).

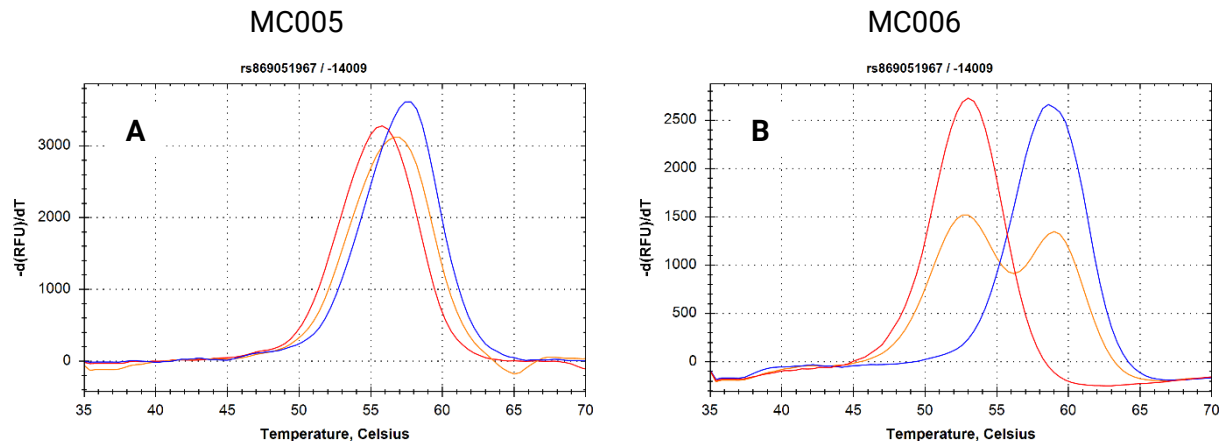


Figure 7. This figure shows the peak pattern for SNP -14009 as detected with both MC005 (figure 7A) and MC006 (figure 7B). **Figure 7A** shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs869051967 (-14009) (red) and a heterozygous sample (orange). The WT peak 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 7B** 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 $\sim 58.5^\circ\text{C}$. 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 8 below.

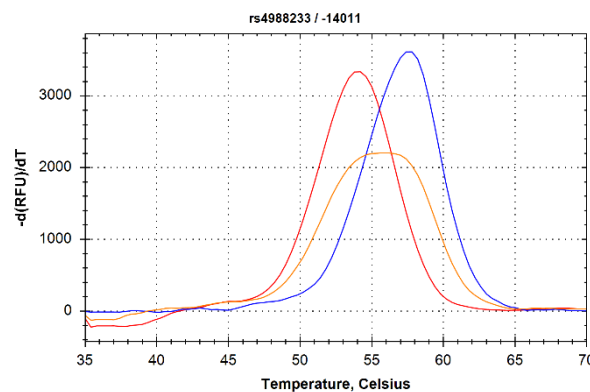


Figure 8. This figure shows the peak pattern of a homozygous wildtype sample (blue), a homozygous sample for SNP rs4988233 (-14011) (red) and a heterozygous sample (orange). The WT peak in this region has a T_m of $\sim 57.5^\circ\text{C}$. The SNP peak has a T_m of $\sim 54^\circ\text{C}$.

MC005 Q-fragment FAM probe (Region 2)

In case too little input DNA is used, a quantity fragment (Q-fragment) peak will be observed. In MC005, this Q-fragment peak has a T_m of $\sim 46^\circ\text{C}$. 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 9 below.

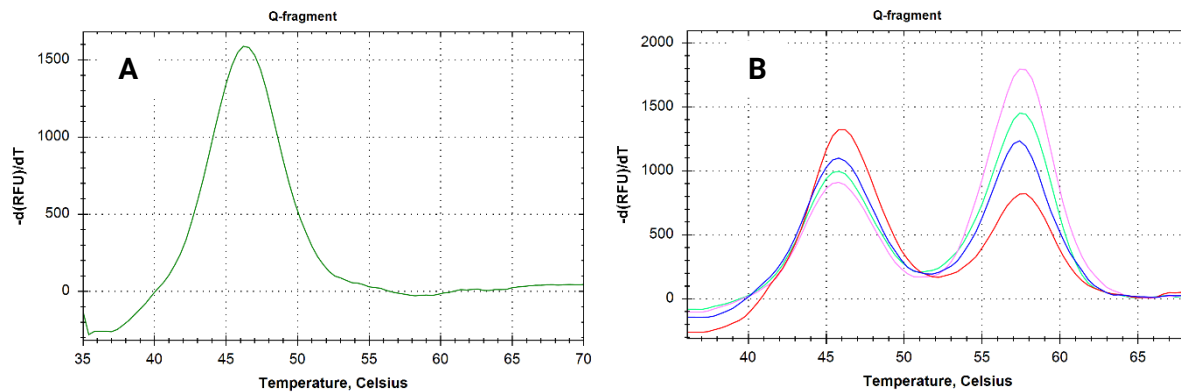


Figure 9. This figure shows the peak pattern of the Q-fragment in MC005 region 2 (FAM probe). **Figure 9A** shows the peak pattern for a reaction without input DNA. A Q-fragment peak has a T_m of $\sim 46^\circ\text{C}$. **Figure 9B** shows a dilution series with decreasing amounts of input DNA (pink = ~ 0.5 ng, green = ~ 0.3 ng, blue = ~ 0.25 ng and red = ~ 0.125 ng). A decrease in input DNA (WT peak at $\sim 57.5^\circ\text{C}$) results in an increase in height of the Q-fragment peak (peak at $\sim 46^\circ\text{C}$).

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Interpretation flowchart

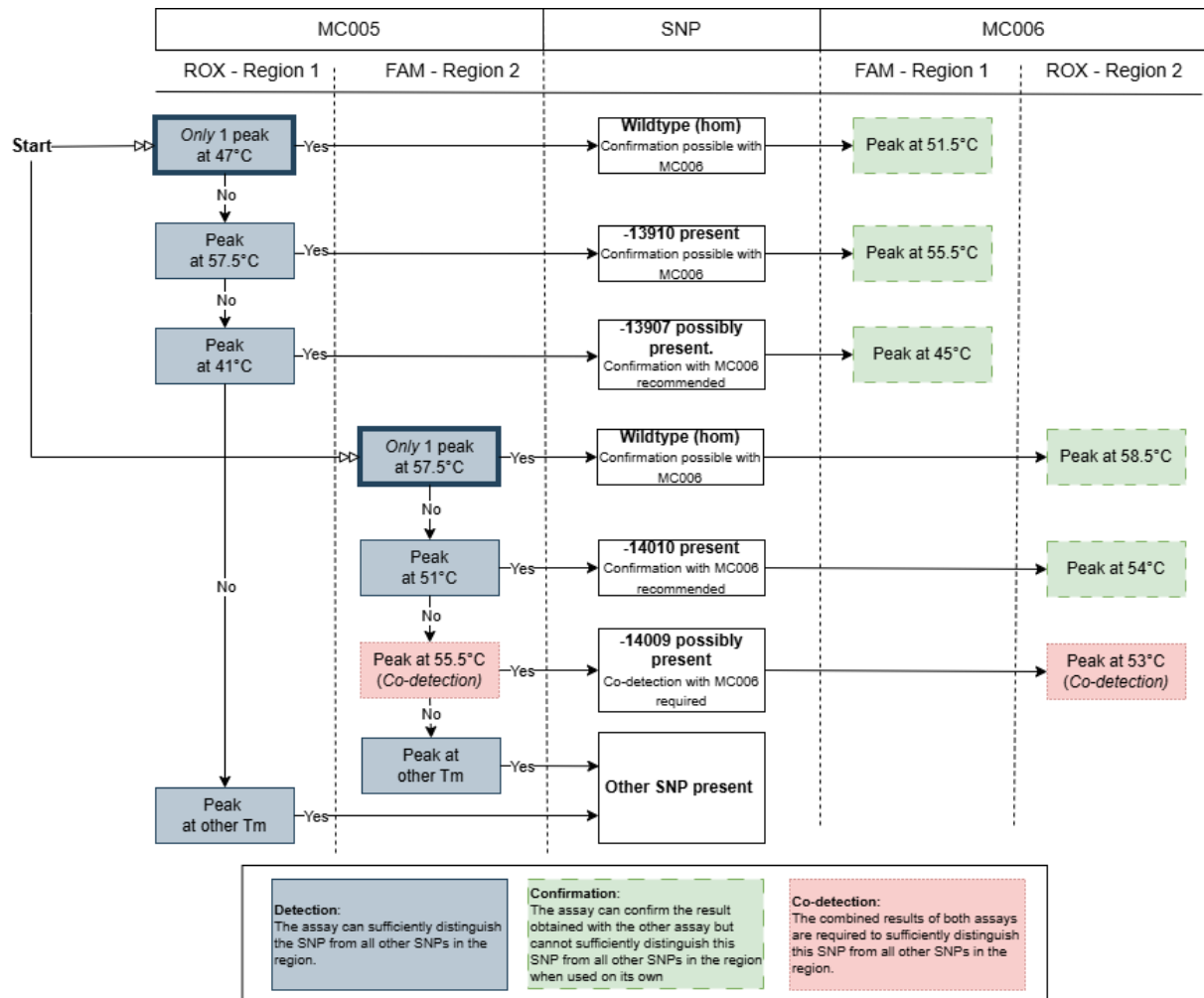


Figure 10. This figure displays an overview of possible outcomes when testing a sample with MC005 and the actions that may be necessary to confirm a potential finding. Use this flowchart in conjunction with the graphs displaying melt patterns and the T_m of SNPs in the section above to aid with the interpretation of your results.