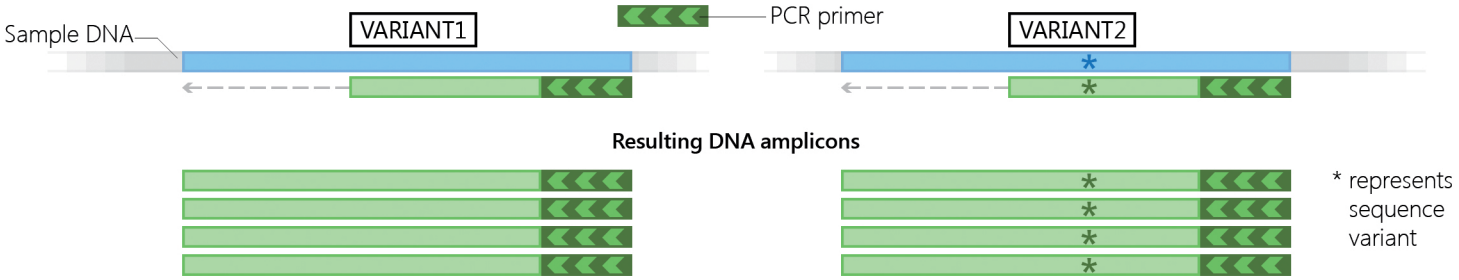
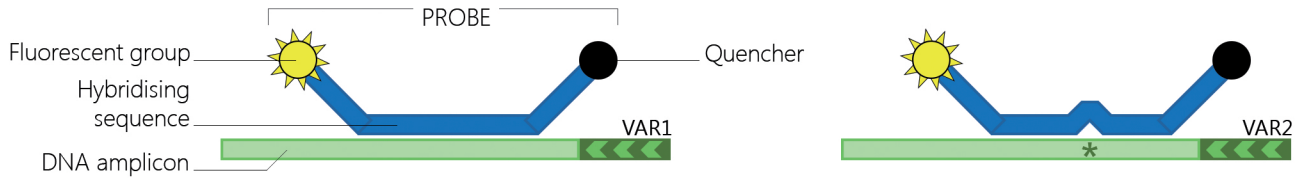
SALSA® MELT ASSAY

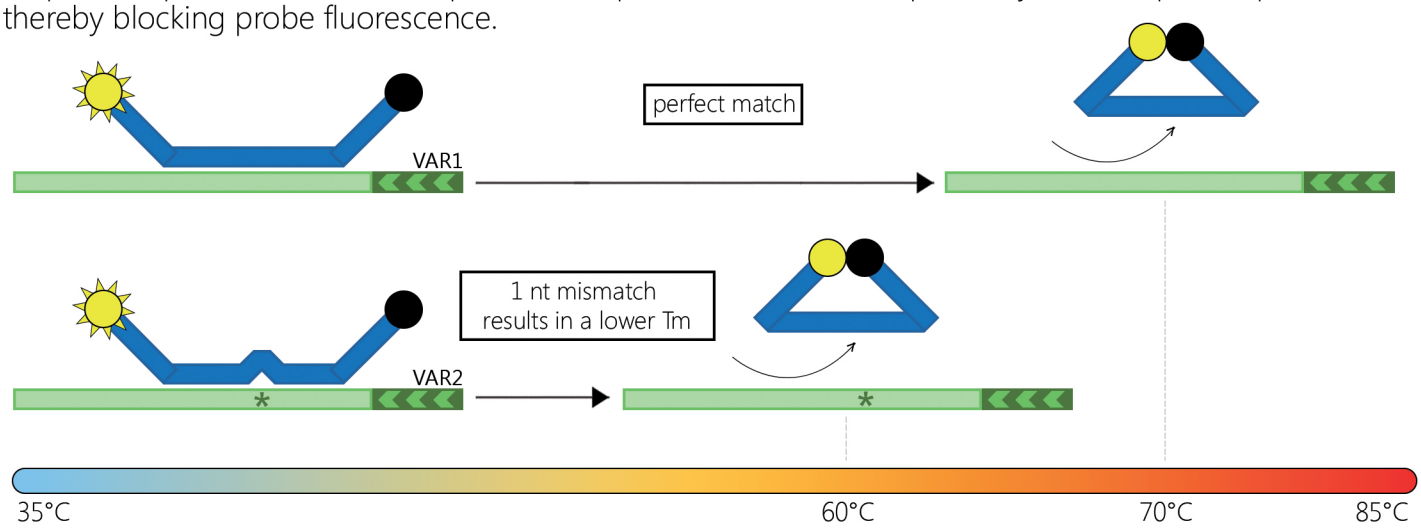
1. PCR amplification: The sequence of two genetic variants is amplified using a single PCR primer pair, with one primer in excess (asymmetric PCR).



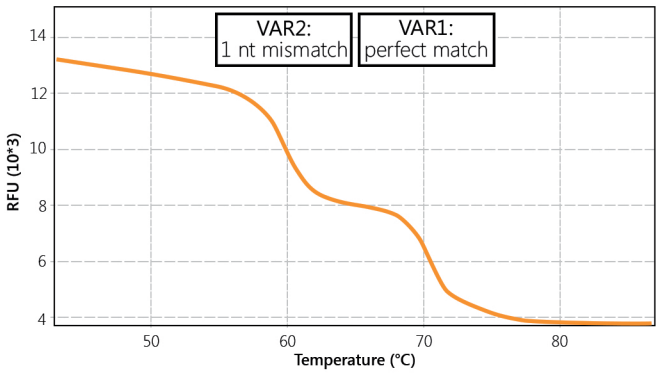
2. Hybridisation: A fluorescently labelled probe binds to an amplicon, separating the fluorophore from its quencher, resulting in fluorescence emittance.



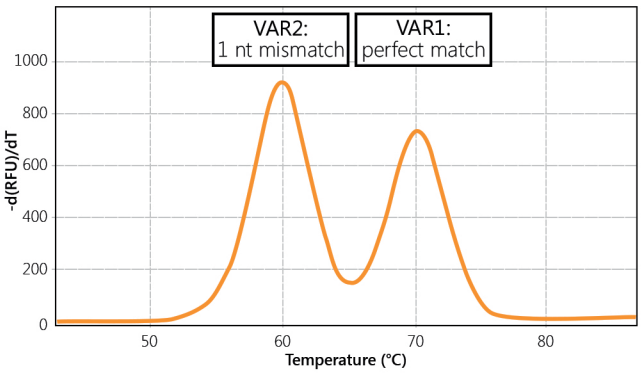
3. Heating: The probe-amplicon hybrid mixture is slowly heated, resulting in dissociation of the probe from the amplicon. Upon dissociation, the probe fluorophore comes in close proximity with the probe quencher, thereby blocking probe fluorescence.



4. Analysis: Probe-amplicon dissociation is visualised by the rapid loss of fluorescence at given temperatures.



3A. Fluorescence vs. temperature
This plot shows the fluorescence vs. temperature of a sample with amplicons whose sequence is identical to the probe, and amplicons containing a mismatch. As the temperature increases, more probe molecules are dissociated from the amplicons and the fluorescence decreases.



3B. Probe-amplicon melting temperature
Shown above is the first derivative (-d(RFU)/dT) of the curve shown in 3A. The graph above shows a peak at the temperatures with the most rapid fluorescence changes. These temperatures are the Tm values for the probe-amplicon hybrids. The graph above displays a heterozygous genotype.

D-MC-g-002