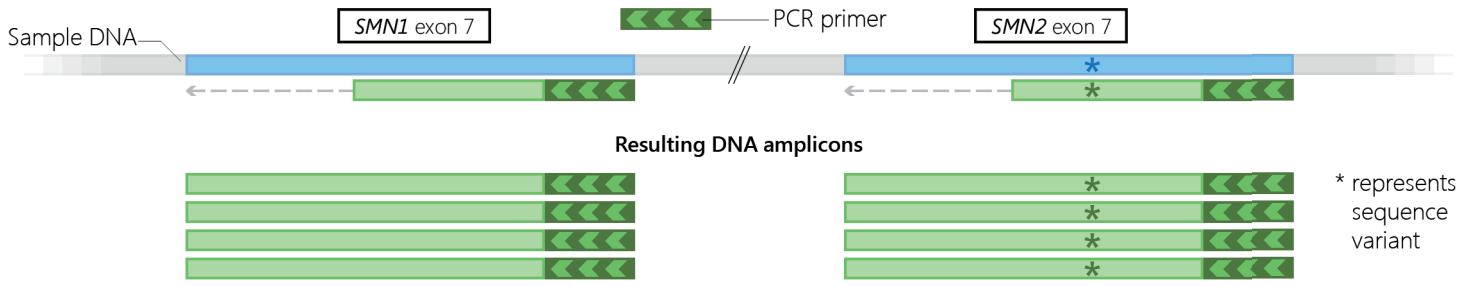
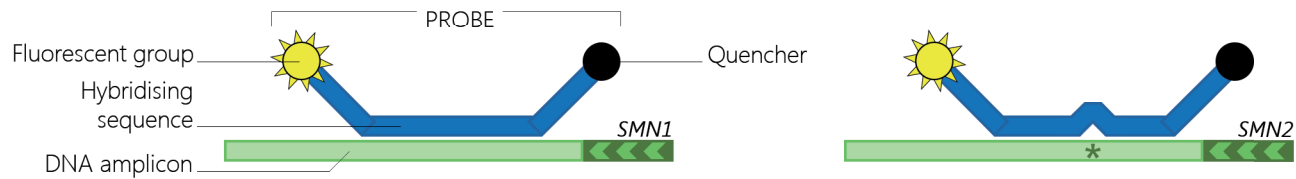


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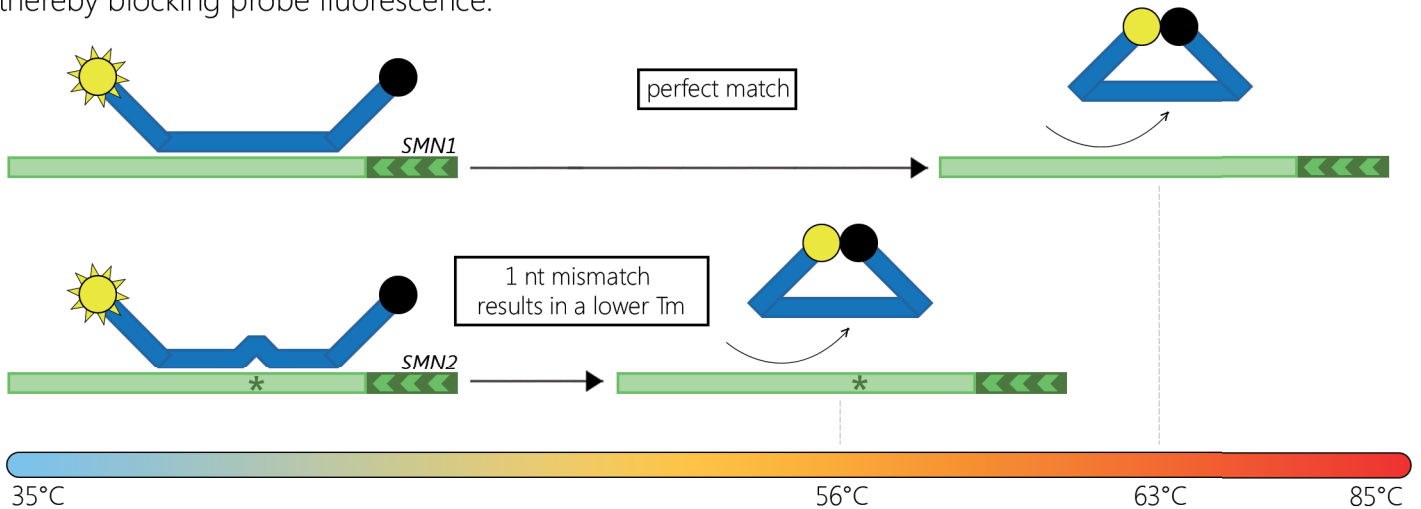
1. PCR amplification: The exon 7 sequence of *SMN1* and *SMN2* is amplified using a single PCR primer pair, with one primer in excess (asymmetric PCR).



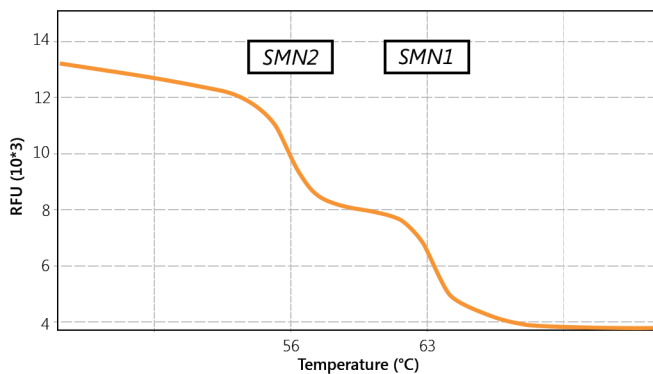
2. Hybridisation: A fluorescently labelled probe binds to an amplicon, separating the fluorophore from its quencher, resulting in fluorescence emission.



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.

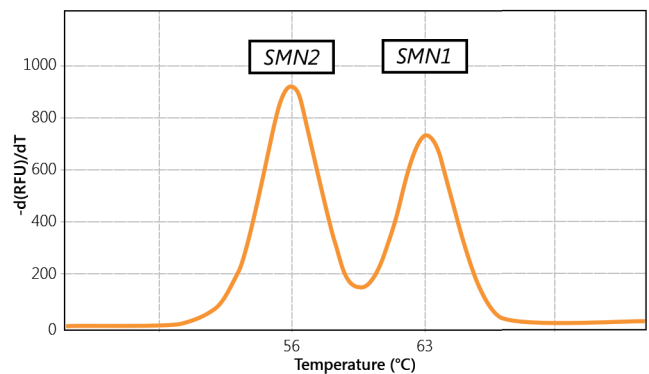


4. Analysis: Probe-amplicon dissociation is visualised by the rapid loss of fluorescence at given temperatures.



4A. Fluorescence vs. temperature

This plot shows the fluorescence vs. temperature of a sample with *SMN1* amplicons (sequence identical to the probe) and *SMN2* amplicons (containing a mismatch). As the temperature increases, more probe molecules are dissociated from the amplicons and the fluorescence decreases. The mismatched probe-amplicon hybrid (*SMN2*) dissociates first, the perfectly matched one (*SMN1*) last, at a higher temperature.



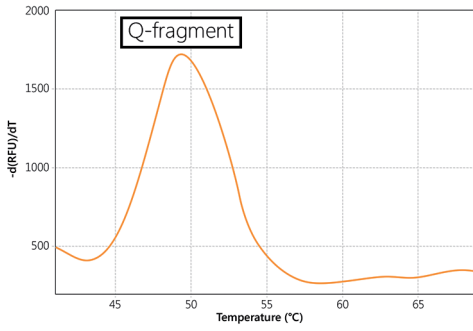
4B. Probe-amplicon melting temperature

Shown above is the first derivative ($-d(\text{RFU})/dT$) of the curve shown in 4A. The graph above shows a peak at the temperatures with the most rapid fluorescence changes. These temperatures are the T_m for the probe-amplicon hybrids: 56°C for *SMN2*, 63°C for *SMN1*.



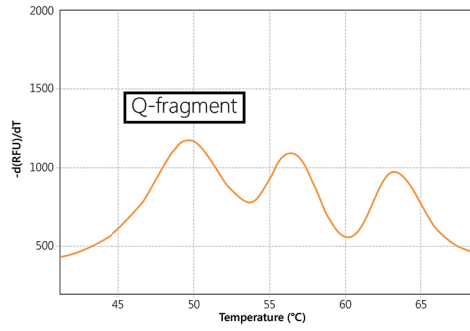
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5. Quality Control: Examine Q-fragment peak height (49°C).



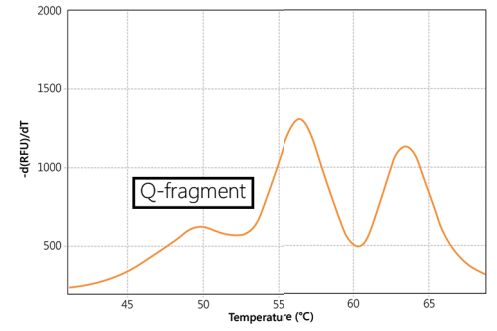
No DNA sample

The Q-fragment melt curve peak is the only peak present.



Sample with insufficient DNA

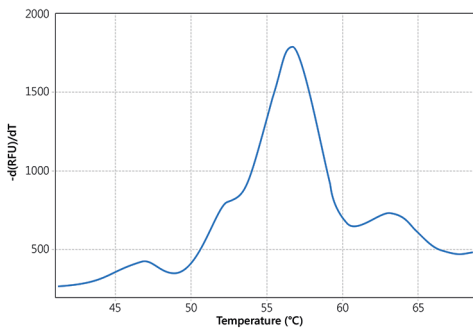
The Q-fragment melt curve peak is the highest of the three peaks present.



Sample with sufficient DNA

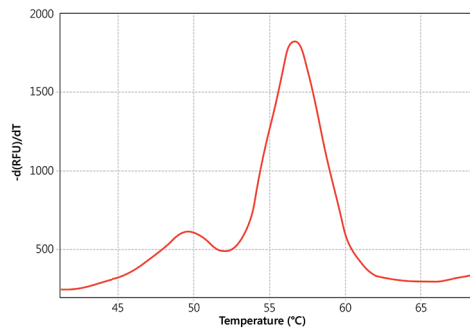
The Q-fragment melt curve peak is very low.

6. Typical MC002 results: Expected melt curve profiles for standard samples demonstrating *SMN1:SMN2* ratios.



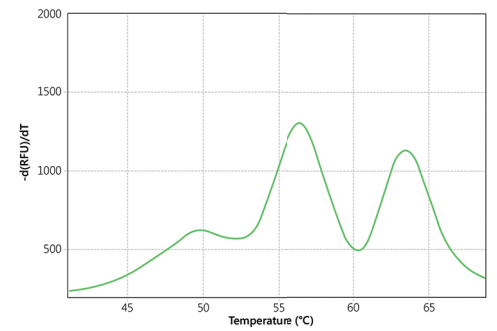
SD074 Threshold DNA sample

Melt curve profile with specific *SMN1* (63°C) and *SMN2* (56°C) peaks with known *SMN1:SMN2* ratio of 1:5.



SD075 Positive DNA sample

Melt curve profile showing an *SMN2* (56°C) peak and absence of the *SMN1* peak with known *SMN1:SMN2* ratio of 0:2, indicative of an SMA patient.



Sample DNA: unaffected individual

Melt curve profile from an unaffected individual with specific *SMN1* (63°C) and *SMN2* (56°C) peaks showing an *SMN1:SMN2* ratio of 1:1.