Differentiation

Determination of genetic polymorphisms of the amplified pathogen sequences allows typing of pathogens or detection of mutations associated with genetic diseases.

- The melting curve of amplification product (upper curves in the melting graph) indicates the temperature at which the probes separate from the amplification product.
- The peak of the negative first derivative (lower curves in the melting graph) of the melting curve identifies the temperature at which maximum probe separation occurs = melting point ($T_m$).
- Single base mismatches reduce the melting point by ~2°C and allow for reliable detection and typing of mutations.

![Melting curve](image)

**Melting point determination of the amplification products of Babesia gibsoni and Babesia canis.** The Babesia gibsoni/canis real-time PCR assay amplifies the target sequence of both species, but the sequences to which the green fluorescent donor probe attaches differ by 2 bases between B. gibsoni and B. canis. These differences result in a Tm of 64.2°C for B. gibsoni and of 60.0 °C for B. canis and allow clear identification of each species by melting curve analysis in the same PCR.

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