METHYLDETECT
DNA METHYLATION ASSAYS

Technical Guide: What Is DNA Melting?

Introduction

When a double-stranded DNA helix splits into single strands, it is referred to as DNA melting. This can be achieved simply by heating the double-stranded DNA.

The temperature at which the strands separate depends on the number of hydrogen bonds holding the complementary strands together:

- There are **two** hydrogen bonds between adenine (A) and thymine (T).
- There are **three** hydrogen bonds between guanine (G) and cytosine (C).

Therefore, in principle, the more G and C bases a DNA sequence contains, the higher the temperature required to melt it.

How to Detect Differences in Melting Temperature of PCR Products?

The most common method to determine the melting temperature of a PCR product is to subject it to a temperature gradient in the presence of an **intercalating dye** (a chemical that only emits light when bound to double-stranded DNA).

The process works as follows:

- The PCR product is mixed with the dye, and the fluorescence emitted by the mix is monitored as the sample is slowly heated.
- The outcome is a curve displaying the fluorescence changes over the temperature range, commonly referred to as a **melting profile** (Figure 1).

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Reading the Melting Profile

Analyzing the melting curve involves three key phases:

- **Beginning of the experiment (Figure 1 – A):** The temperature is low, and all DNA is double-stranded. Consequently, we observe high levels of fluorescence.

- **During heating (Figure 1 – B):** Fluorescence remains high until the temperature reaches the point where hydrogen bonds break and the amount of double-stranded DNA drastically decreases. This results in a **sharp drop in detected fluorescence**.

- **End of the experiment (Figure 1 – C):** At high temperatures, no double-stranded PCR product remains, and fluorescence levels drop close to zero.
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Because the temperature at which this sharp drop occurs depends on the number of hydrogen bonds, the melting temperature is unique and specific to the analyzed fragment. This analysis can be performed directly on standard RT-qPCR instruments, as most of them feature built-in modules for post-PCR melting analyses

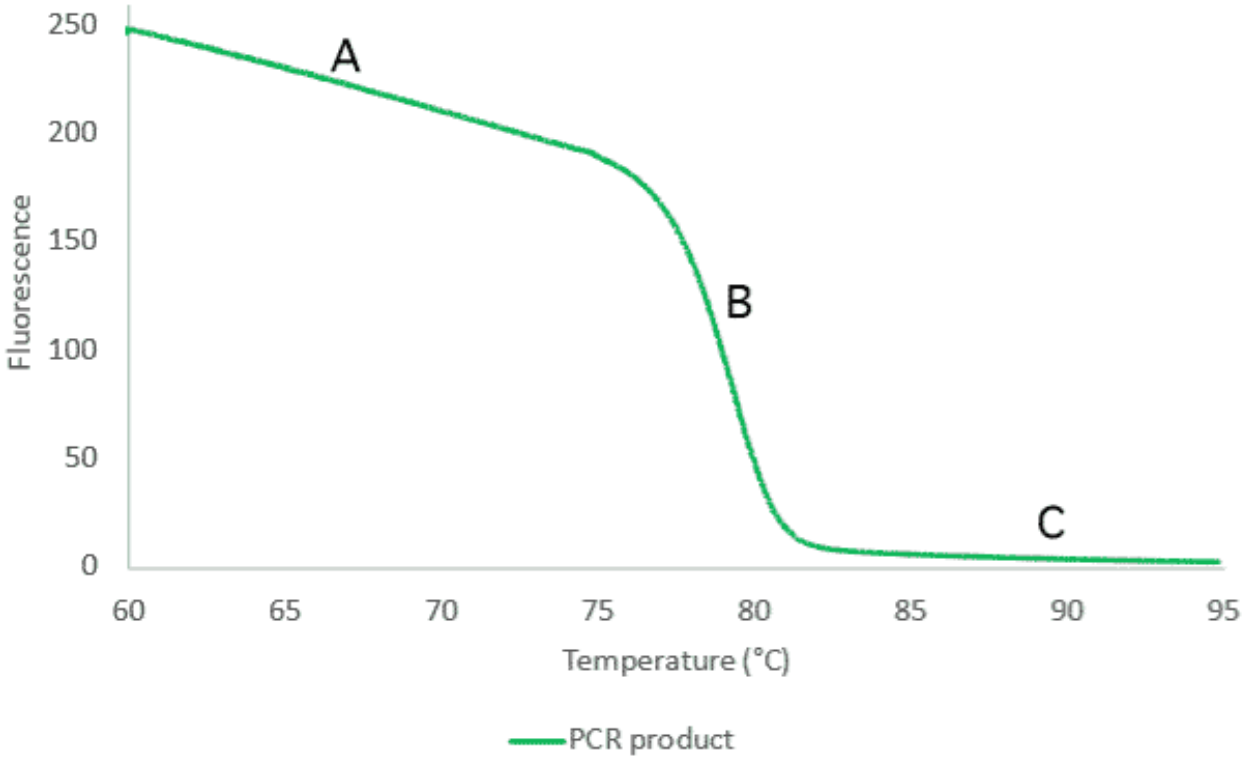

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Figure 1: Representative melting curve profile illustrating (A) the beginning of the experiment with high fluorescence, (B) the transition phase during heating with a sharp drop in fluorescence, and (C) the end of the experiment where fluorescence levels drop close to zero.

Assay Support & Applications

MethylDetect provides pre-validated, ready-to-use assays and master mixes designed for standard qPCR instruments, facilitating high-resolution melting and methylation analysis.

For technical assistance or to request a quote, contact our team at info@methyldetect.com or visit www.methyldetect.com.

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