Manipulation of primer affinity improves high-resolution melting accuracy for imprinted genes


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ABSTRACT. High-resolution melting (HRM) is considered an inexpensive, rapid, and attractive methodology for methylation analysis. In the application of the polymerase chain reaction (PCR) to methylation analysis, amplification efficiencies are biased towards unmethylated, rather than methylated, templates: a phenomenon known as PCR bias. To overcome PCR bias, primers that include CpG site(s) and are fully complementary to the methylated sequence have been proposed. However, genes mapped within imprinted regions usually present higher methylation levels, and an unusual PCR bias towards the methylated template can therefore arise. The manipulation of primer affinity attempts to overcome this problem. We attempted to show that mismatches at the primer’s methylated binding sites increase the area between the 50 and 100% methylation plots on the melting curves, and may increase HRM accuracy for samples that have high methylation levels. Sets of primers for imprinted genes that included CpG sites at their binding sequences were designed, and were complementary to methylated or unmethylated templates. Primers fully complementary
to methylated templates produced a very small area between the 50 and 100% methylation plots. When using primers that were fully complementary to the unmethylated sequence, we were able to increase the area between the 50 and 100% methylation plots. Therefore, when samples are highly methylated, such as targets in genes mapped in imprinted regions, we propose that primers should favor amplification of the rarest, unmethylated sequence. Primers may be designed to include one CpG at its binding site and be fully complementary to the unmethylated template.

**Key words:** Imprinted genes; Methylation; High-resolution melting; Epigenetics; Polymerase chain reaction