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Limitation of high-resolution melting curve analysis for genotyping simple sequence repeats in sheep

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ABSTRACT. Variation in microsatellite or simple sequence repeat (SSR) loci has, until recently, relied heavily on the use of gel-based methods that can be both time consuming and difficult to genotype. Non gel-based systems are therefore important to increase simplicity and improve turn-around time without compromising assay sensitivity and accuracy. In this report, we assessed the latest of the non-gel-based methods, high-resolution melting (HRM) curve analysis. HRM is a technique that monitors exactly the decreasing fluorescence of intercalating dye in the process of dissociation of double-stranded DNA. The measurement immediately follows polymerase chain reaction in a one-step, closed-tube method. Four SSR loci of different complexity in sheep, namely MAF209, MCM140, CB226, and SRCRSP5, were assessed using the LightScanners System with LC Greens PLUS DNA binding dye. In order to improve the accuracy of genotyping,

we applied internal oligo nucleotide calibrators while performing HRM. DNA polymorphisms were previously identified using capillary electrophoresis analysis (CE). The result showed that CE detected more genotypes than HRM in the same loci regardless of the level of polymorphism at the SSR loci. We demonstrate current limitations of the HRM method for the analysis of SSR loci.

Key words: Simple sequence repeat; High-resolution melting; Genotyping; Internal oligonucleotide calibrators; Limitations

