

QUANTITATION OF CANINE DNA BY REAL-TIME PCR USING THE CANINE MELANOCORTIN 1 RECEPTOR GENE

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Animal forensic laboratories face the same challenges as human laboratories when analyzing trace DNA samples. Low template samples exhibit increased allelic dropout and peak imbalance. Additionally, we frequently encounter mixtures between species, especially in instances of dog attacks on people or other animals. When analyzing evidence from a fatal dog mauling, there are usually large amounts of human blood and tissue intermingled with canine saliva and hairs. Effective analysis of such evidence necessitates accurate quantitation. Real-time or quantitative PCR (qPCR) is rapidly becoming the method of choice for quantitating forensic DNA samples. Compared to spectrophotometric and blot hybridization techniques, Real-time PCR has been demonstrated to be highly sensitive, discriminating, and reproducible. Since it determines the concentration of target DNA by quantitating amplicons throughout PCR cycling, it facilitates an a priori determination of the success of applications utilizing PCR. Because MC1R is involved in hair and fur coloring, it is a gene of interest for a number of species and has been extensively characterized. The MC1R gene is a seven-pass transmembrane G-protein-coupled receptor that is transcribed from a single exon. It is involved in the determination of hair color by promoting the production of eumelanin which causes the black or brown coloration of hair and fur. Our goal was to exploit the available knowledge of this gene to design a suite of real-time assays for quantitating the DNA of a variety of species beginning with canids.