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Sensitivity and specificity of high-resolution melting analysis in screening unknown SNPs and genotyping a known mutation*

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Abstarc :

High-resolution melting analysis (HRMA) was used to screen potential SNPs in the exons of chicken CAPN1 (?-calpain/large subunit) gene. A total of 312 DNA samples from Beijing-you chickens were used for detection. Twelve pairs of primer were designed to amplify twelve different exons and SNPs were detected in five of them. HRMA was also compared with PCR-SSCP analysis for genotyping of a known SNP site in the chicken adipocyte fatty acid binding protein gene (A-FABP). Amplicons of 275-bp fragment, bracketing the polymorphic site, were grouped by PCR-SSCP into three genotypes designated as CC, TT and CT. Small amplicons (56 bp) within the 275-bp fragments were designed to maximize the Tm difference between homozygotes and to genotype all possible three genotypes after a single melting analysis successfully. Results from different methods were cross-validated and sequencing results from randomly selected heterozygotes and homozygotes confirmed the specificity of HRM technique. The full consistency proved that HRMA was a useful tool for rapid, close-tube genotyping of polymorphic sites. It has great potential for SNPs detection and scanning especially on a large scale.

Key Word :
A-FABP, CAPN1, chicken, HRMA, SSCP analysis

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