

Validation of an exonuclease genotyping assay for a pair of SNPs within the chicken FNDC9 gene

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APPLICATION NOTE

INTRODUCTION

The chicken was the first among farm animals to have its genome sequenced and the chicken remains a key organism in genetic research. Pulmonary hypertension may affect chickens and is similar to the disease in humans. Therefore, the chicken can be used as a medical model for pulmonary hypertension in humans. Through genome resequencing we have mapped regions showing association with ascites (1). Genotyping of additional samples to confirm the regions depends on highly reliable exonuclease assays.

Quantitative PCR (qPCR) exonuclease assays were performed on multiple DNAs. Each 20 µl qPCR mixture contained forward and reverse primers, and three different quenched fluorescent probes. Post-PCR fluorescent signal patterns were evaluated, and selected PCR products were then purified for

sequence verification. The PCR products were diluted with 10 µl of 10 mM Tris, 0.1 mM EDTA, pH 7.5, and then collected in a RapidTip®. The PCR products were transferred to a clean 1.5-ml microfuge tube, and pipetted up and down over the RapidTip resin 5 times in the new tube. For the post-exonuclease assay cleanup, it was important to remove all of the primers and probes including the digested probes, containing the fluorescent tags and quenchers. The cleaned samples were submitted for capillary sequencing and then the traces evaluated to confirm the SNPs in the product to correlate with the qPCR signals (Figure 1). There are two C/T SNPs that are four bases apart. The sequence histograms allowed us to determine how the qPCR signal was correlated with particular genotypes.

References:

- 1) Whole genome resequencing identifies the CPQ gene as a determinant of ascites syndrome in broilers. Dey S., A. Parveen, K.J. Tarrant, T. Licknack, B.C. Kong, N.B. Anthony, D.D. Rhoads. 2018. Whole Genome Resequencing Identifies the CPQ Gene as a Determinant of Ascites Syndrome in Broilers. PLOS One 13(1): e0189544. <https://doi.org/10.1371/journal.pone.0189544>

