Quantification of allele specific expression (ASE) for two alleles of the HTR2B gene in different broiler chickens and different tissues

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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. The HTR2B gene has shown some association with hypertension in broilers (1, 2) and has been the focus of further investigations.

Purified RNAs were subjected to reverse-transcriptase PCR for a region of exon 3, containing a missense SNP (G/A) that distinguishes the two major alleles in our chicken lines. Chickens heterozygous for the two alleles were selected for analysis of ASE based on these two SNPs (3). It was very important to have very low levels of background "noise" to discern the relative expression (trace height) of the two alleles.

Six representative sequence histograms are presented in Figure 1. It is important to note that for optimal results, the following was identified to be crucial:

1) The PCRs were 30 µl.
2) Post-PCR sample was transferred to a clean 1.5-ml microfuge using the RapidTip® and passed over the RapidTip resin 5 times.

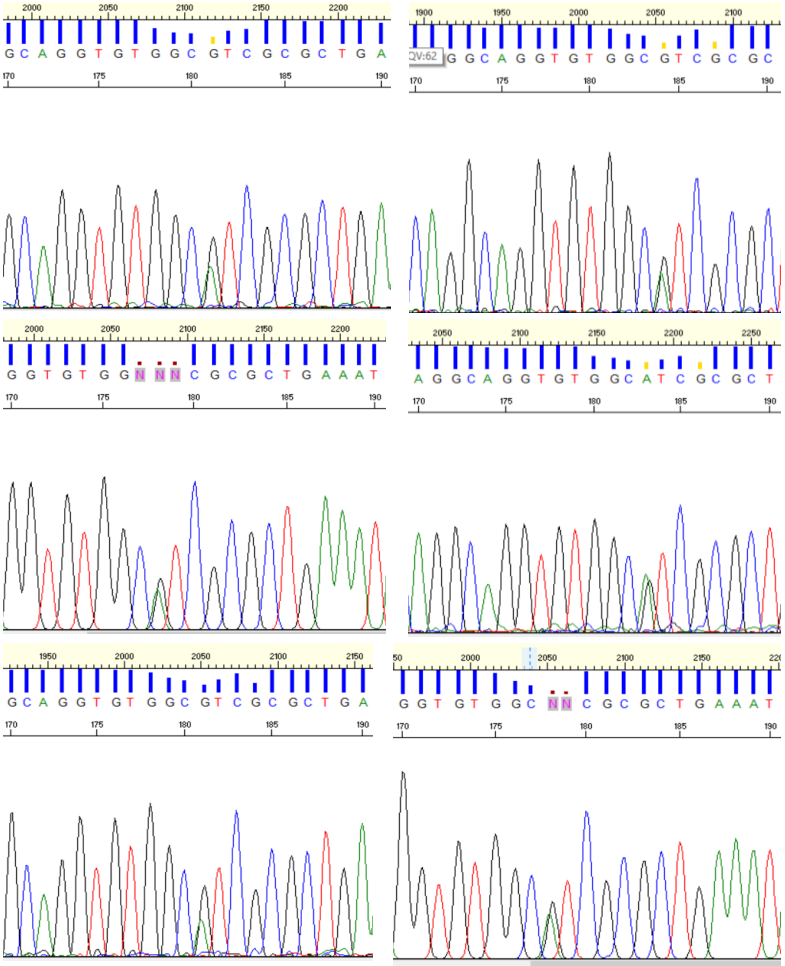

Figure 1. Representative sequence traces for 6 different RNA samples subjected to Reverse-Transcriptase PCR to amplify a region of the chicken HTR2B mRNA. The sequence traces were viewed using Sequence Scanner v1.0 (Applied Biosystems).

References:

1) Krishnamoorthy S, C Smith, A Al-Rubaye, RF Wideman, GF Erf, NB Anthony and DD Rhoads. (2014) A QTL for Ascites on Chromosome 9 in Broiler Chicken Lines. Poultry Science 93:307–317
2) Alzahrani K. 2013. Genetic analysis of the promoter region of the serotonin receptor 5HTR2B and its contribution to pulmonary hypertension syndrome in broiler-type chickens. MS Thesis, University of Arkansas.
3) Alzahrani K, S Krishna, P Greenburg, NB Anthony, NB, and Rhoads, DD. 2018. SNP Analysis of HTR2B Gene and Detection of Allele Expression Imbalance Effect on Ascites Syndrome in Broilers. (Manuscript in Preparation)