Gene sequencing validation for select commercial turkey genes

Gene expression analysis via polymerase chain reaction (PCR) is a common tool used to determine, using molecular techniques, the physiological basis underlying experiments with animal tissues. Some genes are "turned on" and others silenced in response to different treatments and PCR allows us to study individual genes. Gene expression is determined by amplifying a section of the target gene using specific “primers” which are short pieces of DNA that are unique to the target gene. Primers can bind to the gene base sequence and act as a guide for a polymerase enzyme that is responsible for the amplification. Primers are specific to the gene of interest so a successful PCR protocol requires an accurate base sequence for the gene(s) of interest. This poses a challenge when working with tissues from a species that do not have a fully sequenced genome, such as the turkey.

Our goal was to sequence select turkey genes to be used in an experiment to evaluate the effect of probiotics on intestinal physiology. Our genes of interest were *MUC2*, a component of the small intestinal mucous layer and *TFF2*, a protein vital in the maintenance of the intestinal barrier. We also sequenced two housekeeping genes, *RPS13* and *TBP*, which are involved in basic cell maintenance and are expressed consistently so as to standardize the expression data for the experimental target genes. The primers for the target turkey genes were based chicken gene sequences using purified turkey DNA as a template. The product of a PCR assay is an amplified portion of the target gene called and amplicon. If the primers chosen are sufficiently complimentary, the result is an amplicon with a predicted approximate size. The primers designed for the selected turkey genes resulted in amplicons within the expected size range.

Once we had successfully designed primers for our four genes of interest, a commercial bacterial cloning system was used to sequence the amplicons. Each amplicon was placed into separate bacterial DNA plasmids which are small circular DNA strands that bacteria can carry in addition to their individual genomes. A plasmid acts like a train, carrying genetic material from one bacteria to another. The plasmid and amplicon were joined, the combined plasmid+amplicon was inserted into a bacterial strain that is engineered to take up plasmid DNA. Once the bacteria had incorporated a plasmid, cultures of the bacteria+plasmid+amplicon were grown to increase the quantity of the plasmid + amplicon. The plasmid+amplicon combinations were extracted from the bacterial cells and the isolated plasmids were sent to the Ohio State University Plant Genomic Lab for sequencing. The commercially available plasmids have a known sequence so the genomic lab could determine the amplicon sequence.

The determined sequences for each of the four genes were compared to the original chicken sequences to determine if differences existed between the chicken and turkey sequences. Differences were observed within the *MUC2* and *RPS13* primer regions so new primers were designed based on the determined amplicon sequences from the turkey DNA. The new primers

proved to be a 100% match to the newly sequenced turkey genes thus we had successfully sequenced sections from four target genes in turkeys that can be used in future gene expression experiments.

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Publication

Degenerative primer design and gene sequencing validation for select turkey genes.
Hutsko SL, Lilburn MS, Wick M
Poult Sci. 2016 Jun 1