Functionally Validate Enhancers in the Chicken Genome using MPRAs

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Enhancers are sequences that bind transcription factors, which help regulate tissue-specific gene expression in development, disease, and other biological processes. The Functional Annotation of Animal Genomes (FAANG) consortium has made great progress in the identification and annotation of enhancers in the genomes of many farm animal species including chicken. While an important first step, the FAANG annotated enhancers may have low concordance with their *bona fide* functional activities, thus, experimental validation of functional activity is essential. In this study, we validated two splenic-specific enhancers (Proly 4-Hydroxylase Subunit Beta and Migration and Invasion Inhibitory Protein) using the standard luciferase reporter assay in the DF-1 chicken cell line. We are currently developing high-throughput, massively parallel reporter assays (MPRAs) in DF1 cells to more efficiently functionally validate annotated chicken enhancers. A typical MPRA library involves the synthesis of ~2,000 putative enhancers, each 300 bp in length, with unique 15-bp reporter barcodes. Our first two sets of candidate enhancers will include (1) predicted strong enhancers associated with high expression genes in DF-1 cells, and (2) predicted strong enhancers with allele specific expression genes related to genetic resistance to Marek's disease. A positive control and a negative control sets will also be included. The development of this high throughput enhancer validation system will enable the genome-wide discovery and functional characterization of enhancers in the chicken genome, which could provide a growing knowledge base for the systematic exploration of their role in chicken biology and disease susceptibility.