Annual Report for NC1170
Period Covered: January 1, 2014 to December 31, 2014
Prepared by: Douglas Rhoads

Institutional Stations (Institutional Abbreviation: Members)
Beckman Research Institute at the City of Hope (**COH**: M. Miller)
Cornell University (**CU**: P. Johnson)
Iowa State University (**IA**: S. Lamont, J. Dekkers)
Michigan State University (**MI**: J. Dodgson)
Mississippi State University (**MS**: C. McDaniel, M. Edelmann; B. Nanduri)
North Carolina State University (**NC**: C. Ashwell, J. Petitte)
Oregon State University (**OR**: D. Froman)
Pennsylvania State University (**PA**: A. Johnston, R. Ramachandran)
Purdue University (**IN**: W. Muir)
University of Arizona (**AZ**: F. McCarthy, S. Burgess)
University of Arkansas (**AR**: W. Kuenzel, B. Kong, D. Rhoads)
University of California, Davis (**CA**: M. Delany, H. Zhou)
University of Delaware (**DE**: B. Abasht)
University of Georgia (**GA**: S. Aggrey)
University of Maryland (**MD**: T. Porter, J. Song)
University of Minnesota (**MN**: K. Reed)
University of Tennessee-Knoxville (**TN**: A. Saxton, B. Voy)
Virginia Tech (**VA**: Eric Wong, E. Smith)
University of Wisconsin (**WI**: G. Guilherme)
USDA-ARS-Avian Disease and Oncology Lab (**ADOL**: H. Cheng, H. Zhang).

Administration

Executive Director- Jeff Jacobsen, Michigan State University
Lakshmi Matukumalli- NIFA Representative
Christina Hamilton- System Administration.

NC1170 Business Meeting 2015

Attendees: B. Abasht (DE), H. Cheng, H Zhang (ADOL), M. Delaney, H Zhou (CA), P Johnson (CU), BW Kong, DD Rhoads (AR), S. Lamont (IA), F McCarthy (AZ), B Muir (IN), K. Reed (MN), J. Song (MD), E. Wong (VA)

Administrators: S. Lamont (Admin Advisor)

Guests: Annett Weigend (Bund Germany), J Fulton (Hy-Line), G Strasburg (MI), C Stephens (CU), C. Schmidt (DE)

1. Poultry Workshop PAG:

a. Attendance: Saturday AM=85; Sunday AM= 90; peak attendance >120
b. Representatives of 12 stations attended at some point during the weekend
c. One survey showed 1 or more representatives from 22 other institutions including the poultry industry, U.S. government, the United Kingdom, European Union, and China
d. Guest Speakers: Doris Bachtrog (UC Berkeley); Steffen Weigend (Bund, Germany), Deepali Vasoya (Roslin, UK); Ignacy Misztal (UGA); C. Titus Brown (UC Davis); T Y Kim (UC Davis Jorgenson Travel award winner)
e. Seven graduate students gave short presentations about their posters.
f. Updates: J Reecy on NRSP8 bioinformatics; W. Warren on chicken genome reference updates
g. 17 NC1170 members presented research updates

1. Business meeting Sunday January 11, 2015. 11:20 AM in San Diego, CA, Sunset Room, Town and Country Convention Center:
   a. Minutes from 2014 approved as circulated
   b. Next meeting of NC1170 will be coordinated with PAG at Town and Country in January 2016.
   c. USDA Administrative Advisor: Sue Lamont
      i. Project is going well.
      ii. New members from Penn State, UT Knoxville, Cornell, Mississippi State.
   d. Lakshmi Matukumalli (reported by Douglas Rhoads):
      i. RFP for 2015 not yet available except for RFP on undergraduate, graduate and postdoctoral fellows. When RFP is available all will be notified.
   e. NRSP8 Poultry co-Coordinators: Delaney and Cheng
      i. Funding is secure
      ii. Members need to put in requests for pilot studies or small projects
      iii. Some partial travel money for students/postdocs to attend PAG, but members need to request.
2. NC1170 Leadership for coming year: Douglas Rhoads leader, Benham Abasht secretary
   a. NIFA meeting proposal: Rhoads and McCarthy
      i. There was discussion about needs, strategies, and descriptions.
      ii. A new proposal will be submitted.
3. Other Business
   a. NONE
4. Adjourned.

Accomplishments by Objective

This document summarizes by objective the major accomplishments achieved by the NC1170 Multistate Research Project covering 2014. Achievements included the application of next generation technologies in sequencing to poultry genomes and numerous contributions to advances in bioinformatics, annotation, and transcriptomics as applied to a variety of fundamental disciplines in poultry biology. Our work is conducted under the auspices of many collaborative arrangements with stakeholders involved in the allied poultry industries for the purpose of improving poultry production. The scholarly publications resulting from this project (see appendix) includes over 80 articles in peer-reviewed journals and books/chapters. Special note should be made of the large number of publications involving collaboration between members of this project as well as scholars from around the world. During the

past two years this project has added new participants from Cornell University, Mississippi State University, Pennsylvania State University, and University of Tennessee-Knoxville, to a total membership of 33 participants.

Objective 1: Create and share data and technology to enhance the development and application of genomics and systems biology in poultry.

ADOL- Efforts to improve our understanding of the complex trait of genetic resistance to Marek's disease (MD) and use this information to improve commercial poultry breeding via genomic selection; MD is an agronomically-important disease of chickens characterized by T cell lymphomas induced by the Marek's disease virus (MDV), a highly oncogenic alphaherpesvirus. A second goal was to demonstrate of the power and utility of the method to identify specific genes impacting disease and genetic resistance. Previously, work identified 4,528 SNPs in 3,718 genes that exhibit ASE in response to MDV infection using F_1 progeny from experimental inbred White Leghorn layer lines that differ greatly in MD genetic resistance; lines 6 and 7 are MD resistant and susceptible, respectively. The data show that these genes with cis-regulatory elements account for the majority of the genetic variation between two experimental White Leghorn (egg laying) lines and verify our genomic predictions by creating lines differentiated for the identified cis-regulatory elements that demonstrate associated changes in MD resistance. To address whether genes with SNPs exhibiting ASE in response to MDV infection are associated with MD genetic resistance, a line 6x7 F_6 MD resource population was generated and genotyped with a custom 9K SNP array that included 1,824 ASE SNPs; the remaining 7,064 "random" SNPs were equally spaced throughout the chicken genome to provide genome-wide coverage of which 2,821 were placed in genes as controls. Analysis using the GS3 package (Legarra et al., 2013) found that ASE SNPs account for more than 83% of the genetic variance in MD resistance. Further analyses comparing effects of ASE SNPs to random SNPs, both within and between coding regions of genes, indicated that on a relative basis, effects associated with ASE SNPs, which are always within coding regions, were 15.2% above average, whereas effects within and between coding regions of random genes were 6.9% and 8.2% below average, respectively, and consequently exhibit all the highest genome-wide associations. This result supports the importance of transcriptional regulation on genetic resistance to MD, and also supports the infinitesimal model concept of a polygenic trait comprised of many genes each with a small effect (Hill, 2014). To validate the association of ASE SNPs with MD genetic resistance, 200 F_7 generation roosters were genotyped, best linear unbiased prediction (BLUP) estimated breeding values (EBVs) based on SNPs and pedigree calculated, and roosters were bidirectionally selected based on the SNP EBVs. The top 30 and bottom 30 ranked roosters were each reciprocally mated to 6 random F_7 hens, and the resulting progeny tested for MD incidence. As a result, after only one generation of selection, there was greater than 22% difference in MD incidence after bidirectional selection based on the ASE SNPs, which is in line with that predicted based on the genetic variance accounted for by the ASE SNPs and the selection differentials.

AR- in collaboration with Oklahoma State University sequenced, assembled, and deposited into NCBI, the genome for a species of Staphylococcus the AR group has found associated with lameness (bacterial

chondronecrosis and osteomyelitis) in broilers. The project investigated the etiology of bacterial infection of the leg joints, and the time course associated with bacterial septicemia.

AZ in collaboration with MS supported functional modeling in agricultural organisms through AgBase (http://www.agbase.msstate.edu/) to facilitate modeling of functional genomics data and structural and functional annotation of agriculturally important animal, plant, microbe and parasite genomes. This currently (21 November 2014) provides 1,808,007 Gene Ontology (GO) annotations for 383,527 gene products across 77 species, including agriculturally important species and their pathogens. This information includes GO annotations for 54,446 chicken and 2,958 turkey gene products, respectively. In collaboration with DE to extend manual biocuration efforts to additional species (including turkey, sheep and salmon) through text mining of published literature and identify (a) papers likely to contain GO terms for agricultural species; and (b) additional 'informative terms' (iTerms) or functional information described in the literature. iTerms were linked to functional terms from other, existing ontologies (such as the cell ontology and anatomy ontologies), creating an ontology mapping files for this literature. AZ began biocuration of host-pathogen interaction data to support network modeling of animal health data sets. Via the Host-Pathogen Interaction Database (HPIDb) we submitted 1,829 molecular interactions to the EMBL European Bioinformatics Institute (EBI) IntAct database. With collaborators at Mississippi State we will continue to develop the HPIDb, adding interaction data and developing tools to support interaction prediction. As part of the USDA funded iAnimal Project we also worked with the iPlant Collaborative to develop functional analysis pipelines within iPlant. During 2014 we added tools to support (a) functional annotation of large scale transcript data and (b) GO and patwhays enrichment analyses of differentially expressed data. AZ participated in the 2014 the Chicken Gene Nomenclature Committee (CGNC) to update data reporting to support the use of this data by both NCBI and Ensembl. This provides standardized nomenclature for 16,422 genes and this data is now routinely distributed to both NCBI Entrez and Ensembl. To facilitate future nomenclature efforts, including sharing data with other vertebrate species (such as turkey), AZ is developing a chicken reference gene set. The chicken reference gene set will be the focus of future annotation efforts and will facilitate comparative analysis with other species. The initial cattle gene nomenclature was provided by the Bovine Genome Database and was updated to assign standardized gene nomenclature for 9,910 *Bos taurus* genes (http://www.animalgenome.org/genetics_glossaries/bovgene). Work with HGNC will support the development and use of standardized gene nomenclature for livestock species. AZ contributed to Chickspress for developing a tissue specific compendium of gene expression for chicken gene products. Tissues were collected from 15 different adult male and female red jungle fowl for mRNA sequencing, small RNA sequencing and proteomic analysis. Results were compared to both NCBI and Ensembl gene models and both tissue expression and sex-specific expression is also analyzed. Our initial mRNA analysis identified 66,309 transcripts, of which 35% differ from current gene models provided by NCBI or Ensembl; most represent alternate transcripts from predicted gene models. We also identified 16,496 miRNAs from these same tissues and during 2014 we completed target prediction for the 15,291 novel miRNAs in this data set. Likewise, we used proteomics to identify 16,003 NCBI and Ensembl proteins form these same tissues as well as 77,418 peptides that are not supported by current chicken protein sequences, most commonly representing exons not represented in current NCBI and Ensembl gene models. All data from this project is publicly available at sequence and proteomics repositories and may

be viewed on the Chickspress genome browser (http://geneatlas.arl.arizona.edu/) that allows researchers to visualize genome wide, tissue specific expression. During 2014 we completed a searchable database for this data that allows researchers to do expression queries based upon the tissue, gene product type and both NCBI and Ensembl accessions. Taken together these results provide a comprehensive gene expression analysis of adult chicken, including tissue and sex specific expression and allele specific variation. AZ also collected data from Carl Schmidt (DE) and Parker Antin, (AZ) to compile on a genome browser so that users may see tissue/development stage specific expression. AZ used the Comparative Genomic (CoGe) Platform to support analysis of the 47 bird genomes sequenced by the BGI and Genome 10K projects (approx. 50 genomes), as well as the existing bird genomes (chicken, turkey, zebra finch, parrot). The work focused on building capacity for researchers to display their own experimental, quantitative data tracks using this resource. This development will enable poultry researchers to do comparative analyses and to share their data and results with other researchers

CA- The ASBAMC web portal is fully functional and available at http://asbamc.org. The complete computational pipeline and the web-based reporting software has been installed and is up and running at UC Davis. RNA-seq data processing and analysis functionality has been integrated in the pipeline and AMVIZmanager. P value and false discovery rate features have been implemented in the system. A key functionality added now enables the user to compare analysis between two different reports, such as comparing two different mutant strain host responses. The tool can automatically generate heat-maps showing the time course differences between pathways activation or gene expressions. An outreach and education training workshop for animal systems biology analysis and modeling was held on November 2014 at department of animal science, University of California, Davis. The ASBAMC has sent the RFA early 2014 and received many applications and started to analyze selected 4 biological projects from University of Delaware (C. Schmidt and L. Cogburn), Penn State University (W. Liu), UC Davis (T. Berger). A total of 12 different projects have been selected and data have been processed and analyzed and more than 100 reports have been generated. CA continued functional analysis of chicken IRF7 in response to dsRNA analog poly (I:C) by integrating overexpression and knock-down assays. In order to develop new antiviral strategy against more virulent pathogens emerged from the birds, a better understanding of antiviral host response mechanism is essential. Type I interferon (IFN) is the first line of antiviral response and interferon regulatory factors (IRFs) are the master modulators of IFN signaling and it has been suggested that IRF7 plays an important role in the regulation of host immune response to Avian influenza viruses (AIV) infection in chickens. The objective of this study was to identify candidate genes and pathways associated with IRF7 regulation at the transcriptome level as a first step towards elucidating the underlying cellular mechanism of IRF7 modulation in antiviral response in chickens. The IRF7 over-expression and knock-down DF-1 cell lines were established and stimulated by various pathogen-associated molecular patterns (PAMPs). Significant IRF7 and type I IFN expression changes were observed between the control and IRF7 over-expression or knock-down cell lines that were exposed to double strand RNA (dsRNA) analog Poly(I:C). The cDNA libraries were prepared from the Poly(I:C) and mock-exposed cell lines samples for the RNA-seq analysis. Integrative analysis using *in vitro* over-expression, knock-down identified potential novel interferon stimulated genes and immune signal transducers that IRF7 may regulate in host response to dsRNA related viral infection. The results

suggest that chicken IRF7 may solely be responsible for type I IFN regulation and likely to have conserved functional activity in antiviral response with its mammalian counterpart despite of its lower homology with its mammals. Potential biomarkers and therapeutic target revealed in the study warranty its further investigation. CA sought to investigate whether Marek's disease vaccines exhibit viral integration into the chicken host genome. Week old chicks were exposed to 5,000 pfu SB-1, HVT or Rispens and spleen cells were examined (1, 4, 7, 14, 21 dpi) using our standard cytogenetic profiling assay (using an MDV-BAC clone as a hybridization probe to interrogate if the vaccines integrate into the host genome). In brief, three of four previously observed virus-host interaction phenotypes were found in the spleen cell populations following vaccine challenge: (1) null (no evidence of virus), (2) associated (evidence for vaccine DNA signals within the nucleus and surrounding the chromosomes, interpreted to represent cells in the lytic/replicating phase of the virus life cycle), (3) associated plus integrated (as described in (2) and including vaccine DNA-signals integrated into the chromosomes at the telomeres). A 4th phenotype lacking in these vaccine trials is the "integrated-only" phenotype. This is interesting as this result was also observed when non-oncogenic meq-deleted virus was used as the challenge. This 4th phenotype (integrated only) is interpreted to be the phenotype for the 'latent" status cells, which are presumed to be the targets for oncogenic transformation. Thus, oncogenic, vaccine, and non-oncogenic modified strain all do show integration, but the latter two virus types lack a significant phenotype observed with oncogenic strains, i.e., non oncogenic strains lack the cell type wherein the virus is no longer replicating episomally *and is only present* in a chromosomally-integrated form. New work was initiated using USDA-ARS ADOL lines 6 (resistant) and 7 (susceptible) birds infected with vvMDV (e.g., Md5) or vaccine (HVT or Rispens) or vaccinated/then challenged (and at two different ages). Samples of spleen were collected 1, 4, 7, 14 or 21 dpi or dpc or dpi/c. Spleens were processed for cytogenetic analysis. We are just now starting to run our FISH experiments. The total number of samples involved (individual birds) is 281. The purpose of this study is to explore genetic resistance/susceptibility genotype differences (if they exist) when it comes to host-virus genome interactions. Further we hope to establish if vaccine challenge interrupts the virulent MHC integration profile, which could impact either persistence of the virus (ability to re-express) and/or oncogenesis and tumor progression. CA continued to characterize developmental mutants. Talpid-2. A collaborative venture involving NC1170 members with a group of mouse and human-focused researchers having expertise in human and murine developmental biology. Through this work the autosomal recessive lethal chicken talpid-2 mutation (which has the phenotype of cleft palate and limb malformations) and its role in craniofacial development via a ciliopathy has been determined. The mutation results in a malfunction of cilia, tiny hairlike structures on the surface of cells present in the affected region. Cilia play a vital role in passing along signals during development. The gene encoding the ciliary protein C2CD3 (encoded on GGA 1 in the candidate region identified by mapping and further assessed for variation by sequencing) was found to harbor a 19 bp deletion in talpid-2 that produces a premature stop codon and thus a truncated protein, and is the likely causal allele for the phenotype. Wingless-2. We are re-initiating studies of wg-2 for a PhD dissertation which had been previously narrowed to a candidate region less than 300 Kb on GGA 12 with several interesting candidate genes. We are continuing with fine-mapping (several generations have passed, so we hope the region may have narrowed slightly), conducting re-sequencing to confirm capture array sequencing work and improve coverage, and expression analysis via RNASeq.

COH- **Verification of YF BAC clone sequences**. In an effort to create sequence data for the *MHC-Y* region that might be used to enhance the application of genomics to poultry selection they isolated and sequenced a number of Red Jungle Fowl BAC clones for *MHC-Y*. Assembly of *MHC-Y* sequences for the BAC clones was completed with Sanger sequencing by T. Shiina and K. Hosomichi at Tokai U. Re-sequencing of the BAC clones representing two of the three *MHC-Y* contigs used Illumina high throughput methods. The new sequences were assembled using the earlier Sanger sequences as templates. Correspondence was excellent verifying the high accuracy of the original assemblies. There were only eight differences (single and double nucleotide differences) for the first BAC clone of 212,253 bp. The Sanger sequence of the second smaller (139,017 bp) BAC clone was fully supported by Illumina data and no corrections needed, indicating these two contigs are correct. Further experiments are underway to test the hypothesis that the *MHC-Y* haplotype is actually an assemblage of three discrete segments as suggested earlier by Solinac et al 2010. **Structural studies of YF1*7.1.** With the aim of understanding how different YF molecules might function in antigen presentation, the structure of the well-expressed YF1*7.1 molecule was determined. An unidentified non-peptidic ligand, possibly cetrimonium (a cationic surfactant), was observed in the binding groove in the crystal structure. To begin to define natural ligands that bind within the YF1*7.1 binding groove we are folding recombinant YF1*7.1 and β2-microglobulin in the presence of candidate ligands including cetrimonium as the positive control analyzing the results by mass spectrometry and in structural studies.

MN- **Improve the turkey genome sequence.** Turkey genome build 5.0 has been submitted to NCBI for public release. Annotation of the new build is to be complete by the NCBI annotation group and will utilize RNAseq data to improve gene predictions. In addition, a Maker annotation of this assembly, that utilized a comprehensive RNAseq dataset, has been completed by Mark Yandell's group at the University of Utah. **Quantify expression of genes in the developmental transcriptome.** Collaborated with VA to compile a developmental transcriptome of the turkey. Seventeen tissue types were collected from birds of both sexes at day of hatch, weeks 1-4 and at 24 weeks of age. Over 700 individual libraries that were subjected to deep RNA sequencing (~10M 101 bp reads/library). Sequence data has been assembled and analysis of differential expression is ongoing.

MS- Proteomics were applied to identify and quantify new chicken deubiquitinases and serine hydrolases. Chicken deubiqutinating enzymes were labeled by an active-site probe, followed by quantitative proteomic analysis to detect down-regulation of UCH-L3 and up-regulation of UCH-L5 in HD11 chicken macrophages infected with *Salmonella enterica* Typhimurium. Reduced function of chicken UCH-L5 was associated with a decreased number of bacteria surviving in macrophages following infection. Moreover, decrease of UCH-L5 activity or protein amount led to a decrease in inflammasome activity. These data point towards an important role for UCH-L5 in inflammasome activation during *Salmonella* infection of chicken cells. This data also was used to characterize and clone novel deubiqutinases in chicken. On the basis of gel-based activity-based protein profiling, at least 8 different serine hydrolases were detected in the HD-11 cell line, one of which can be confidently identified as monoacylglycerol lipase (MAGL), due to its selective inhibition by JZL-184 (a potent small molecule inhibitor of MAGL). MAGL is the canonical enzyme responsible for the degradation of 2-AG in cells and tissues. Although no differences were observed in the profiles of serine hydrolases after 2-h infection,

prolonged infection of HD-11 cells (18 h) appeared to downregulate and upregulate several serine hydrolases activities in the chicken macrophages. Initial biochemical experiments have already demonstrated that preincubation of intact living HD-11 cells with JZL-184 caused an increase in the concentration of 2-AG in culture medium following cellular stimulation with ionomycin. (Ionomycin is a compound that causes an elevation in cytosolic calcium levels, which subsequently leads to enhanced rates of 2-AG biosynthesis). This effect is due to the blockade of MAGL activity by JZL-184 and the reduced ability of MAGL to hydrolyze and catabolize 2-AG.

MI- **Chicken genome sequence analysis and annotation.** Completed the analysis of NGS sequence of several chicken lines, particularly developmental mutants: stumpy, diplopodia3, diplopodia4, talpid2, limbless and eudiplopodia. Candidate genes and SNP were identified and are being pursued further. A likely candidate gene for talpid2 has been identified as the C2CD3 gene and a likely causal allele, a 19 bp indel, has been found in talpid2 C2CD3. Examining the latest chicken genome sequence assembly (galGal4) and exploring approaches to fill gaps and obtain missing segments of the assembly (particularly on microchromosomes). Optical map data have recently been obtained for the UCD001 reference bird (#256) and Moleculo sequence analysis is in progress. These efforts parallel work at the Wash. U. Genome Center that is doing targeted gap filling and PacBio sequencing. In addition, a full manual annotation of the transcripts in the latest galGal4 reference sequence assembly is currently in progress.

Objective 2: Facilitate the creation and sharing of poultry research populations and the collection and analysis of relevant new phenotypes including those produced by gene transfer.

ADOL- Maintains a large number of chicken lines that are characterized for a number of traits, especially those associated with viral diseases, and maintained under specific pathogen free (SPF) conditions. Besides providing unique genetic resources to our location, ~1500 embryos or chicks are supplied yearly to academic institutions or companies in the United States. The lines and maintenance are briefly summarized below. ADOL maintains 39 chicken lines with special genetic characteristics for tumor or viral susceptibility that also differ remarkably for immunological and physiological traits. All but 3 (C, N and P) were developed at the ADOL over the last 67 years. These include 4 of the world's most highly inbred lines (6_3, 7_1, 7_2, and $15I_{5,}$), all of which are well defined for avian leukosis virus (ALV) receptor genes, endogenous virus loci (EV), and resistance to MD. Two of the lines are outbred, 2 of which are highly utilized worldwide for ALV analyses (0 and 15B1). Four congenic lines exist for analysis of EV genes; 3 (0.44-TVBS1-EV21, 0.44-TVBS3-EV21, and RFS) were developed from line 0 and 1 (100B) from line 7_2. Eight congenic lines exist for analysis of the influence of the MHC (*B* haplotype) on resistance to tumor diseases, immune responses or vaccinal immunity; 7 (15.6-*2*, 15.7-*2*, 15.15I-*5*, 15.C-*12*, 15.P-*13*, 15.P-*19*, and 15.N-*21*) were developed from line $15I_5$, and 1 (15.N-*21*) from line 0. Lines 6_3 and 7_2 differ markedly for MD resistance and immune function traits, as well as ALV and EV genes, but have the same B haplotype. Nineteen recombinant congenic strains (RCS) are under development to identify non-MHC genes that influence traits differing between lines 6_3 and 7_2. ADOL also developed one transgenic chicken line (0.ALV6) that is very beneficial for analysis of ALV. ADOL lines are routinely tested by blood-typing using 40 antisera either to ensure purity or to maintain heterozygosity (EV21, 100B, and O.P-*13*) during annual line reproduction. The breeders are unique in that they are maintained in a quarantined

state and, on the basis of frequent serologic tests for 11 pathogens, are considered free of infection from common poultry pathogens.

AZ- Continued work on the chicken anatomy ontology focusing on (1) linking adult chicken anatomy terms with the Uberon ontology (of generic anatomical terms) and (2) adding developmental terms provided by Professor Burt's group at the Roslin Institute. Currently the chicken anatomy ontology contains 14,627 terms, cross-referenced with the Uberon ontology (and other related anatomy ontologies). This ontology will be required for the Functional Annotation of Animal Genomes (FAANG) to begin in 2015.

IA- Iowa State University Chicken Genetic Resource Populations. Maintains 13 unique chicken research lines [including highly inbred, MHC- congenic, closed populations; and advanced intercross lines (AIL)] that serve as resources for identifying genes and QTL of economic importance. All adult breeders are housed in individual cages and matings done by artificial insemination to ensure pedigree accuracy. All MHC-defined lines are blood-typed to assure MHC serologic haplotype. The continued production of two AILs (now at generation F23) facilitates fine-mapping of QTL with the goal of identifying genomic regions and candidates genes controlling important phenotypes. Journal papers based on studies using these lines have been published in 2014 on copy-number variation, avian influenza response, NK- lysin, heat-stress response, adiposity and gene annotation. These genetic lines formed a discovery platform for research on the genomics heat resistance in a USDA-AFRI-NIFA project with C Schmidt of U Del, C Ashwell of NCSU, M. Persia of VT, and M Rothschild of ISU; and a USAID project on genomics of resistance to Newcastle disease virus and heat with H Zhou, D. Bunn and Rodrigo Gallardo of UC-Davis, and C Schmidt at UDEL, and African partners. In addition to studies conducted at Iowa State University, genetic material (chicks, fertile eggs, blood, tissues, DNA or RNA) has been shared with many cooperating investigators to expand studies on the chicken genome. Active collaborations utilizing ISU chicken genetic lines include the international Avian RNA-seq consortium, H Zhou of UC-Davis (copy-number variation, avian influenza response, NDV and heat- stress response), J Womack of Texas A&M (NK-lysin), A Clark of Cornell University (imprinting), R Coulombe of Utah State (aflatoxin sensitivity), B Abasht (imprinting), B Voy of UTenn-Knoxville (adiposity), C Keeler of University of Delaware (microarray analysis of host response to *Salmonella*), and H Lillehoj of USDA-ARS (response to *Eimeria* and other pathogens)

Objective 3: Elucidate genetic mechanisms that underlie economic traits and develop new methods to apply that knowledge to poultry breeding practices.

ADOL- Marek's disease (MD) is an important neoplastic disease of chickens caused by the Marek's disease virus (MDV), an oncogenic alphaherpesvirus. In the most recent study, ADOL chicken lines 6 (MD resistant) and 7 (MD susceptible) were screened for immune response and core-gut flora, and analyzed to understand differences between these bird lines. Birds from both lines were sampled at 14, 21, 28, 35, and 42 days post infection (dpi) for splenic tissue and cecal samples, which were used to estimate immune function and core-gut flora, respectively. The immune profile in both resistant and susceptible birds reveled a similar significant rise in CD4 and CD8 α/β cell populations at 21 and 28 dpi ($P < 0.005$). However, CD8 α/α profiles were different between the chicken lines with the resistant birds showing a

pronounced response in both control and MD infection (P<0.005), when compared to the susceptible birds. Microbial analysis using 16S rRNA-V6 region revealed at the phyla level the majority of sequences were assigned to Firmicutes (>93%) with Tenericutes, Proteobacteria, and Actinobacteria constituting the remaining fraction. Post-hoc analysis using Tukey-Kramer test at the genera level showed OTU level differences (P<0.05) between resistant and susceptible birds under control and MDV infection. To relate these core-gut flora differences to gene function, communities were evaluated using PICRUSt. Results indicate the differences in functional profile between resistant and susceptible birds indicating MD influences has influence on the core-gut microbiome which, in turn, affects gut function and health. Global miRNA profiling was conducted in MD resistant and susceptible chickens in response to MD vaccination. The profiling was accomplished by deep sequencing of small RNA samples extracted from spleen samples of the ADOL MD resistant line 6_3 and susceptible line 7_2 chickens 5 days post MD vaccine inoculation. Over 14.6 to 27.9 million pass-filter reads of small RNAs were used in the analyses to profile miRNA expression for each of six treatment groups, which are control, HVT, and CVI988/Rispens for each of the two lines of chickens. For the lines of 6_3 and 7_2, 418 and 411 miRNAs in the control groups, 372 and 286 miRNAs in the HVT groups, 400 and 359 miRNAs in the CVI988/Rispens groups were identified, respectively. In comparison to its control group, HVT-induced up-regulated expression for 19 and down-regulated expression for 39 miRNAs (Log_2 Fold Change > 1.5) in the line 6_3; Rispens-induced up-regulated expression for 15 and down-regulated expression for 2 miRNAs in the line 6_3. In contrast, HVT-induced up-regulated expression for 17 miRNAs and induced no miRNA with significantly down-regulated expression in the line 7_2; Rispens-induced up-regulated expression for 13 and down-regulated expression for 44 miRNAs in the line 7_2. Further analysis in target genes for the differentially expression miRNAs, relatively large numbers of unique target genes were predicted for different comparison groups. Highly differentially expressed miRNAs (Log_2 FC > 2) identified between the HVT and Control groups were predicted with 861 target genes in line 6_3, and 1,152 target genes in line 7_2. Of which only 170 target genes were in common between the lines 6_3 and 7_2. The highly differentially expressed miRNAs identified between the Rispens and Control groups were predicted with 1,297 target genes in line 6_3, and 2,179 target genes in line 7_2. Of which only 526 target genes were in common between the lines 6_3 and 7_2. It is evident that HVT, and CVI988/Rispens induced differentially expressed expression of miRNAs, which target a relatively large number of functional genes in chickens, suggesting that miRNAs play important roles in response to MD vaccination, and likely involved in modulation of vaccine protective efficacy through regulation of target gene expression at post transcriptional level. It is anticipated that the final results of this study will provide important insights on host genetics and epigenetics roles underlying vaccine immunity and protective efficacy, and be highly beneficial to poultry and vaccine industries.

AR- **Male fertility.** In collaboration with OR continues to focus on the anatomy, genetics and physiology underlying male gonadal development and mechanisms negatively impacting male fertility traits in the chicken. Two GWAS in two research populations demonstrated a high impact of epistasis, however, the Z chromosome is still critical. Primordial germ cell lines from high and low mobility research lines were established for RNAseq work and embryo transplantation. Those data along with past genetic, anatomical and physiological data will be used to elucidate a basic mechanism responsible for contributing to increased male fertility. **Genetics of ascites.** In

collaboration with other faculty at AR, focused on polymorphisms of the 5HTR2B gene promoter and female susceptibility. A locus on GgaZ was associated with male susceptibility. Now performing marker assisted selection using these two loci for ascites and production traits. **Research line genome sequencing**. AR continued genome-wide SNP analysis for genetically selected chicken lines with other faculty at AR. Poultry lines being investigated include: 1) ascites resistant (RES) vs. susceptible (SUS) lines plus parental Relaxed (REL) population; 2) Arkansas Rous sarcoma Progressor (AP) vs. Regressors (AR) lines; 3) high vs low muscle quality (HMQ vs. LMQ) lines plus parental Random (RAN) population; 4) autoimmune disorder susceptible lines; and 5) broiler phenotypes expressing differences in feed efficiency. A pathway analysis tool, various potential genetic markers showing amino acid changes and potential roles in phenotype development were identified. **Avian Vasotocin receptors.** The avian vasotocin 4 receptor (VT4R) is homologous to the mammalian vasopressin 1a receptor (V1aR). The avianV1aR has been shown to occur in corticotropes of the anterior pituitary gland, cells that are involved in the neuroendocrine stress response of birds and mammals resulting in the release of the stress hormone corticosterone. This year the avian V1aR was examined in the brain. The V1aR was found in not only neurons but glia. In glia, the receptor was found in all 10 circumventricular organs (CVOs) that are specialized brain areas usually lacking a blood-brain barrier and highly vascular. AR investigated genes that responded to a transfer of birds from a short photoperiod to a long photoperiod. Three brain regions containing 4 loci proposed to house deep-brain photoreceptors (DBPs) showed significantly increased gene expression for three photopigments following the placement of birds in the long photoperiod. DBPs are specialized neurons proposed to sense long-day photoperiods and activate the reproductive system of birds. Zhou and Kuenzel investigated individual neurons that responded directly to photostimulation. Neurons were found in the septal region that showed clear depolarization following direct photostimulation. The overall goal of these studies is to identify neurons, brain regions and genes that respond to light treatments as it is well known that birds transferred to long photoperiods show rapid reproductive development in a number of avian species, particularly poultry.

AZ- provided training, outreach and support for poultry researchers via AgBase to ensure that they are able to better leverage their functional genomics data to understand key economic traits for poultry. During 2014 researchers from the US, Canada, Japan, India, China, France, Germany, Portugal, Brazil, the Russian Federation, and an additional 113 countries accessed AgBase. We provided direct support for researchers studying crow, zebra finch, *Botrytis cinerea* (a grape pathogen), pear, Reniform, *Coregonus clupeaformis* (lake whitefish), Japanese quail, *Psophocarpus tetragonolobus* (Winged Bean), *Ciona intestinalis* (sea squirt) and soil metatranscriptome data. During 2014 AgBase had 23,311 visitors, including researchers from 48 US states (who made up 19% of the total researchers using AgBase).

CA- **Feed the Future Innovation Lab for Genomics to Improve Poultry.** Partnered with IA, DE, Sokoine University of Agriculture -Tanzania, and the University of Ghana within a USAID funded project. The five-year research program applies advanced genetics and genomics approaches to sustainably enhance innate resistance to Newcastle disease virus (NDV) and heat stress in chickens to improve production. This project directly addresses the President's FY2012 "Feed the Future" initiative. We are investigating two stressors (biotic: NDV and abiotic: heat stress). Birds of two genetically distinct and

highly inbred lines (Fayoumi and Leghorn), and Hy-Line Brown were either exposed to NDV only (Iowa State) or NDV and heat stress (UCD). Measures of body temperature, blood gas parameters, NDV titers from tears, and antibody response in serum were taken on the live birds, and tissues were collected for transcriptome analysis. Three ecotypes each in Ghana and Tanzania will be exposed to NDV. DNA isolated from Hy-Line Brown and Africa ecotype birds will be genotyped using chicken 600K SNP for GWAS. CA maintained 100 birds from two inbred lines (50 birds per Fayoumi and Leghorn) were used. At 14 days of age (one week before NDV challenge) the heat-stress treatment will begin. To mimic parts of the world that are subject to relatively continuous high heat and humidity, the heat-stress rooms were continuously at 35 C with 60-70% relative humidity until day 31. Phenotypic measurements using iSTAT were collected at four stages: (1) at day 13 before the temperature increase ("pre-heat"), (2) at day 14 ("acute heat"), (3) at day 20 before the NDV challenge ("chronic heat before pathogen challenge") and (4) at day 23 ("chronic heat after pathogen challenge"). The cloacal temperatures were measured, too. Virus titers from tears were measured at 2 and 6 days post-infection (dpi). Antibody response to NDV was measured at 10dpi. There were significant differences between Fayoumi and Leghorn birds in virus titers, antibody response to NDV infection and heat stress (Fayoumi is resistant and Leghorn is susceptible to both stressors). Tissues such as liver, lung, spleen will be used for RNA-seq next year.

DE- RNA-seq Gene Expression Study of Breast Muscle from Broiler Chickens with High and Low Feed Efficiencies. High-throughput RNA sequencing was performed on 23 breast muscle samples from commercial broiler chickens with extreme high (n=10) and low (n=13) FEs. An average of 34 million paired-end reads (75 bp) were produced for each sample, 80% of which were properly mapped to the chicken reference genome (Ensembl Galgal4). Differential expression analysis identified 1,059 genes (FDR < 0.05) that significantly divergently expressed in breast muscles between the high- and low-FE chickens. Gene function analysis revealed that genes involved in muscle remodeling, inflammatory response and free radical scavenging were mostly up-regulated in the high-FE birds. Additionally, growth hormone and IGFs/PI3K/AKT signaling pathways were enriched in differentially expressed genes, which might contribute to the high breast muscle yield in high-FE birds and partly explain the FE advantage of high-FE chickens. This study provides novel insights into transcriptional differences in breast muscles between high- and low-FE broiler chickens. Our results show that feed efficiency is associated with breast muscle growth in these birds; furthermore, some physiological changes, e.g., inflammatory response and oxidative stress, may occur in the breast muscles of the high-FE chickens, which may be of concern for continued selection for both of these traits together in modern broiler chickens. **RNA-seq Analysis of Abdominal Fat from Modern Commercial Broiler Chickens with High and Low Feed Efficiency.** mRNA-seq analysis was carried out on the total RNA of abdominal fat from 10 HFE and 12 LFE commercial broiler chickens. In total, 1.48 billion of 75-base sequence reads were generated. On average, 11,565 genes were expressed (>5 reads/gene/sample) in the abdominal fat tissue, of which 286 genes were differentially expressed (DE) at q < 0.05 and fold change > 1.3 between HFE and LFE chickens. Expression levels from RNA-seq were validated with the NanoString nCounter analysis system. Functional analysis showed the DE genes were significantly (p < 0.01) enriched in lipid metabolism. Specifically, the LFE chickens had higher expression of lipid synthesis genes and lower expression of

lipid “removal” genes. The divergent expression of lipid metabolism genes represents the major difference between HFE and LFE chickens in abdominal fat gene expression. Our study contributes toward understanding the biological basis of FE and gene expressions of chicken adipose tissue. The DE genes and upstream regulators identified in the present study are of potential importance for finding genetic markers of FE and adiposity in broiler chickens. **RNA-Seq Analysis of Wooden Breast Disease: Characterizing a Novel Myopathy in Commercial Chickens through Differential Gene Expression.** Complementary DNA (cDNA) libraries were constructed for 4 affected and 4 unaffected breast muscle samples from a line of commercial broiler chickens. After paired-end sequencing of samples using an Illumina HiSeq 2000, we used TopHat to align the resulting sequence reads to the chicken reference genome. Using Cufflink to determine significant changes in gene transcript expression between each group, our results indicate over 1000 genes differentially expressed between affected and unaffected birds. Furthermore, a characteristic gene expression profile emerged for the disorder through the use of Ingenuity Pathways Analysis (IPA®). By comparing the resultant gene list to previously reported histologic lesions for the myopathy, we have found convincing evidence to support the existence of fiber-type switching, localized hypoxia, oxidative stress, and increased intracellular calcium as characteristic features of Wooden Breast Disease in commercial broilers. **Linkage Disequilibrium in Crossbreds and Pure Lines Chickens.** A 60K SNP panel, was used to estimate LD and haplotype structure in crossbreds and their component pure lines populations (one male and two female lines) and calculate the persistence of LD between these populations. The average level of LD (measured by r2) between adjacent SNPs across all chicken autosomes studied here ranged from 0.34 to 0.40 in the pure lines but was only 0.24 in crossbreds, with 28.4% of adjacent SNP pairs having an r2 > 0.3. Compared with pure lines, crossbreds consistently showed a lower level of LD, smaller haploblock sizes and lower haplotype homozygosity on macro-, intermediate- and micro-chromosomes. Furthermore, we found high correlations of LD between markers at short distances (0–10 kb) between crossbreds and pure lines (0.83–0.94). Results from this study suggest that using crossbreds instead of pure lines can be advantageous for high-resolution QTL (quantitative trait loci) mapping in GWA studies and for achieving high persistence of accuracy of genomic breeding values over generations in genomic selection. These results also provide useful information for designing and implementation of GWA studies and genomic selection using crossbreds.

GA- Association of the transsulfuration pathway in feather follicle and subcutaneous tissue development. There have been several conflicting reports on the relationship of cysteine and growth, fatness and feather pecking. Feathers are primarily methionine and cysteine, and it has been postulated that methionine and/or cysteine deficiency is related to feather pecking. However, because homocysteine is at the intersection of the re-methylation and transsulfuration pathways, it is difficult to conduct a cysteine study independent of methionine. Gelatin was substituted for soybean meal to create a cysteine deficiency diet to test the involvement of cysteine in feather follicle development and fatness. The effect of the transsulfuration pathway was limited by maintaining dietary methionine levels at 95% of requirement. The data suggest that cysteine is associated with feather follicle development, subcutaneous tissue development, feather growth and also abnormal fatness. **Genetics of skeletal integrity in meat-type chickens.** Genetic selection in

meat-type chickens has led to improvement of growth rate, feed efficiency and meat yield. Improvement in production performance has resulted in metabolic disorders such as leg problems and development of the skeleton has been compromised. The genetic association among leg problems, bone quality traits and growth rate has not been conclusively characterized. A random mating broiler population was developed to analyze the genetic basis of leg problems such as tibial dychondroplasia (TD) and varus-valgus deformities (VVD), and several bone quality traits. The leg problems and the bone quality traits studied showed an additive genetic component but the genetic associations of TD and VVD with growth rate and bone quality traits were weak. The results also suggested that genetic selection for growth rate impacted negatively some traits that are related with the integrity of leg bones.

IA- **SNP discovery and genomic architecture of highly inbred Leghorn and Fayoumi chicken populations assessed using pooled-sample whole genome resequencing data.** Analyses of sequence and structural variants of Leghorn and Fayoumi line were used to define the genetic differences of the two breeds. Downstream FST analysis and subsequent gene set enrichment analysis elucidated major differences between the two lines. The genes with high FST values for both breeds were used to identify enriched gene ontology terms. Over-enriched GO annotations were uncovered for functions indicative of breed- related traits of pathogen resistance and reproductive ability. Variant analysis of the lines elucidated GO functions indicative of breed-predominant phenotypes related to genomic variation in the lines, showing a possible link between the genetic variants and breed traits. **Variation in African chicken ecotypes assessed using a 600k SNP chip.** The goal of this study is to characterize the genomic diversity and identify selection signatures in indigenous chickens from hot climates to identify regions that may confer advantages to their natural environment. We genotyped 196 birds using the Axiom ® 600k Chicken Genotyping Array. Ecotypes were designated based upon sampling location within Uganda or Rwanda. Kuroilers, which were developed in India and imported to Africa, were also sampled in Uganda. Quality control filtering left 505,965 SNPs with a genotyping rate of 0.997054 and minor allele frequency (MAF) < 0.02 for analysis. Examination of population stratification was performed to better understand the population structure of the sampled animals and to facilitate more accurate downstream analysis of linkage disequilibrium and haplotypes related to possible selection signatures within or between populations. A Multi-Dimensional Scale (MDS) clustering analysis was carried out that grouped the ecotypes into 10 clusters based on an identity by state (IBS) distance matrix. Additional phylogenetic analysis also revealed stratification of the populations, evidenced by cladograms with short branch lengths that are indicative of subpopulations of mixed ancestry. Results showed more admixture among the areas sampled within a country than between countries. The Rwandan-sampled birds had the highest level of inbreeding and number of homozygosity runs among the ecotypes. This analysis provides insight into population structures and genomic diversity of the sampled populations. The amount of population stratification seen in all samples may be due to a combination of trade between areas, natural or artificial selection of birds on productivity or survival instead of breed characteristics, farming practices and trade across ecotype locations. With the understanding that the sampling populations are admixed and stratified we are better prepared to examine the ecotypes' adaptations to their environment. Further analyses is ongoing into the haplotype structure

of the populations to identify selection signatures for loci that may be related to the sampling environment. **Metagenomic analyses of distinct chicken lines and impact of dietary fiber.** This study evaluated the effects of dietary fiber diet on cecal short chain fatty acid (SCFA) concentration and microbiota of commercial broiler and layer chicks. Broiler and layer chicks were randomly assigned to the high and low fiber diets with 11 replicates of 8 chicks each. One cecum each from three chicks was collected from each replicate: one cecum underwent SCFA concentration analysis and the other two ceca underwent bacterial DNA isolation for terminal restriction fragment length polymorphism (TRFLP) and metagenomics analyses. There were higher concentrations of acetic acid and propionic acid in broilers compared to layers. Higher dietary fiber resulted in a reduction of butyric acid. There were interactions between genetic line and dietary fiber for acetic acid and total SCFA. TRFLP analysis showed that cecal microbiota varied due to diet and to line. Metagenomics analyses identified differences in the relative abundance of Helicobacter pullorum and Megamonas hypermegale and the genera Enterobacteriaceae, Campylobacter, Faecalibacterium, and Bacteroides in different treatment groups. The high fiber diet induced different physiological responses from genetically obese (broiler) versus lean (Leghorn) chicken lines. Analysis of the cecal content suggests that gut microbiome may be associated with the differences observed. **Genome-wide association study of blood chemistry in response to heat in a highly advanced intercross chicken line.** The objective of this study is to identify genomic regions associated with resistance to heat stress in chickens. A highly advanced intercross line (AIL) was created by crossing a single outbred broiler (meat-type chicken) male with very highly inbred Fayoumi females. The Fayoumi breed is indigenous to Egypt and resistant to many pathogens and heat stress, while the broiler line represents selection for rapid and efficient accretion of muscle. Phenotypes were measured on F18 and F19 AIL generations. From d 22 to d 28 post hatch, birds were subjected to a daily, cyclical heat cycle of 35 C for 7 hr per day, then returned to 25 C for the remainder of each day. Three major phenotyping phases were: pre-heat (before heating), acute heat (mid-point of first daily heat cycle) and chronic heat (midpoint of last daily heat cycle). Blood chemistry values were assayed for: BE, Glu, HB, HCO3, Hct, iCa, K, Na, PCO2, pH, PO2, sO2, TCO2. A total of 480 individual birds (468 AIL, 6 Fayoumis, 6 broilers) were genotyped using a 600K chicken SNP array (Affymetrix). All phenotypic traits and their changes in response to heat are being used for a genome-wide association study (GWAS) using GenSel software, assessing 1Mb windows. This study will give insight into genomic regions associated with resistance to heat in chickens, thus facilitating genomic selection for improved response under heat stress. **Parameter Estimates of Average Daily Feed Consumption and Association with CCKAR Genotypes in White and Brown Egg-Type Laying Hens.** There is an evidence for a QTL affecting body size and thus potentially feed intake at 78 Mb on chromosome 4 (www.animalgenome.org), which contains the candidate gene CCKAR. One SNP within and a SNP close to the CCKAR were investigated for association with Average Feed Consumption (AFC) from pedigree lines of brown (BL) and white (WL) egg-type laying chickens. Genotypes were collected on sires and AFC on their daughters in two consecutive weeks. High genetic correlation between week1 and week2 AFC (rG>0.87) were estimated. Heritabilities of weekly WL ranged from 0.23 to 0.29. In WL, the effects of CCKAR SNPs were highly significant for week1 and average AFC but only suggestively significant in week2. Generation by genotype interaction was also significant (p>0.001). For the BL the genotype effect was not significant. **The Effect of Nested vs Factorial Mating on Response to Pedigree and Genomic**

Selection. Objective was to investigate the effect of nested vs factorial mating on genetic gain and inbreeding (F) in 10 generations (G) of selection. Phenotypes and marker genotype were simulated following 850 generations of random mating in a population of size 500 (G0-800) and 100 (G801-850) to generate linkage disequilibrium between loci. The trait was controlled by 500 QTL randomly distributed across 20 chromosomes (30 Morgans), and genomic selection was based 50,000 randomly-distributed SNPs. QTL effects were from a Normal distribution and scaled to simulate a heritability of 0.1, 0.3, or 0.5. The QTL and marker loci were chosen to have minor allele frequency ≥ 0.01. Pedigree (BLUP) or genomic (GenSel-BayesB) selection started in G851. Three mating designs were compared; the first was nested mating each with 90 sires mated to 6 dams (90 mating groups); the second and third were partial factorial designs with random mating of 5 sires with 30 dams in each of 18 groups or 10 sires and 60 dams in each of 9 groups. In the designs, full- and half-sib matings were avoided and in the factorial designs a dam was allowed to mate with more than 1 sire. Each generation, 1,350 male progeny without records and 2,700 female progeny with records were simulated. Based on EBV, the top 90 males and 540 females were selected to be the parents of the next generation. The average EBV, true BV, accuracy and pedigree-based F were computed from 100 replicates for each mating design (Table). Results were consistent for the three simulated levels of heritability. After 10 generations of pedigree or genomic selection, F was significantly higher ($P < 0.05$) for the nested than for the factorial designs. The mating systems had no significant effect on response to either pedigree or genomic selection, but genomic selection had greater response and less inbreeding than pedigree selection. **A class of Bayesian methods to combine large numbers of genotyped and non-genotyped animals for whole genome analyses.** To obtain predictions that are not biased by selection, the conditional mean of the breeding values must be computed given the data that were used for selection. When single nucleotide polymorphism (SNP) effects have a normal distribution, it can be argued that single-step best linear unbiased prediction (SS-BLUP) yields a conditional mean of the breeding values. Obtaining SS-BLUP, however, requires computing the inverse of the dense matrix G of genomic relationships, which will become infeasible as the number of genotyped animals increases. Also, computing G requires the frequencies of SNP alleles in the founders, which are not available in most situations. Furthermore, SS-BLUP is expected to perform poorly relative to variable selection models such as BayesB and BayesC as marker densities increase. A strategy is presented for Bayesian regression models (SSBR) that combines all available data from genotyped and non- genotyped animals, as in SS-BLUP, but accommodates a wider class of models. Our strategy uses imputed marker covariates for animals that are not genotyped, together with an appropriate residual genetic effect to accommodate deviations between true and imputed genotypes. Under normality, one formulation of SSBR yields results identical to SS-BLUP, but does not require computing G or its inverse and provides richer inferences. At present, Bayesian regression analyses are used with a few thousand genotyped individuals. However, when SSBR is applied to all animals in a breeding program, there will be a 100 to 200-fold increase in the number of animals and an associated 100 to 200-fold increase in computing time. Parallel computing strategies can be used to reduce computing time. In one such strategy, a 58-fold speedup was achieved using 120 cores. In SSBR and SS-BLUP, phenotype, genotype and pedigree information are combined in a single-step. Unlike SS-BLUP, SSBR is not limited to normally distributed marker effects; it can be used when marker effects have a t distribution, as in BayesA, or mixture distributions, as in

BayesB or BayesC π. Furthermore, it has the advantage that matrix inversion is not required. We have investigated parallel computing to speedup SSBR analyses so they can be used for routine applications.

IN- used allele-specific expression (ASE) to find specific genes and genetic markers conferring Marek's disease (MD) resistance in poultry. A 1,000 F6 MD resource population was analyzed using a mixed model analysis with SNPs and/or pedigree effects treated as random. In the F7 generation, roosters were genotyped, BLUP EBVs calculated, and selected roosters progeny tested for MD resistance. This identified 4,528 ASE SNPs putatively associated with MD genetic resistance. Correlation of EBVs with progeny test performance demonstrated the accuracy of selection was 61% higher for selection based on ASE SNPs compared to pedigree. Thus these ASE SNPs are functionally linked to causative polymorphisms that alter transcriptional levels in genes that manifest the changes in disease incidence.

MD- Designed an in-depth exploratory study that will significantly extend current research in genetics by addressing for the first time the potential influence of Marek's Disease Virus (MDV) and infectious laryngotracheitis virus (ILTV) exposure on epigenetic regulation as it relates to MD and ILT incidences and disease risk. MSB-1 derived from chicken Marek's disease (MD) lymphomas is an MDV-transformed CD4+ T-cell line for MD study. To capture the regulatory elements specific to MSB1 cells and explore the molecular mechanisms of T- cell transformation caused by MDV in MD, they developed high-quality of DHSs map and gene expression profile for functional analysis in MSB1 cell line. DHSs distribution varied between chromosomes and they preferred to enrich in the gene-rich chromosomes. DHSs enrichments appeared to be scarce on regions abundant in CpG islands. DHSs tended to enrich on high expressed genes throughout whole gene regions while DHSs did not show significant changes for low and silent expressed genes. Furthermore, the correlation of DHSs with lincRNAs expression was also calculated and it implied that enhancer-associated lincRNAs probably originated from enhancer-like regions of DHSs. Together, the results indicated that DNase I HS sites highly correlate with active genes expression in MSB1 cells, suggesting DHSs can be considered as markers to identify the *cis*-regulatory elements associated with chicken Marek's disease. Infectious laryngotracheitis is an acute, contagious, upper respiratory disease of chickens caused by gallid herpes virus 1. Due to mortality rates that can reach up to 70% depending on the virulence of the virus, the disease is of great economic importance to the poultry industry. In this study, 15-d-old specific pathogen-free White Leghorn chickens were used to perform transcriptome analysis of chicken trachea immunized with infectious laryngotracheitis virus vaccine. Myosin and several collagen-related genes were downregulated in the immunized group, suggesting that normal function and structure may be compromised. In addition, we identified some cytokine receptors and several immune genes, such as Granzyme A (GZMA), CD4 molecule (CD4), CD8a molecule (CD8A), and CD8b molecule (CD8B), that were upregulated upon vaccination. The gene ontology analysis shows that genes included in the biological process cluster were related to antigen processing and presentation, positive regulation of immune system processes, T cell selection, and positive regulation of T cell activation. In conclusion, chicken embryo origin vaccine activation of the major histocompatibility complex 1 and 2 pathways provides insight for evaluation and design of infectious laryngotracheitis vaccines.

MN- **Genomics to increase aflatoxin resistance in turkeys.** To investigate the response to aflatoxin exposure used RNA-Seq approaches to characterize the transcriptome level changes in the liver, intestine and spleen of birds exposed to AFB1 Analyed data collected from the livers and spleens of domestic birds exposed to AFB1 with and w/o probiotic treatment. Results of the liver data have been published (Monson et al., 2014) and a second manuscript is in review (Monson et al). A new AFB1 challenge of wild and domestic turkeys has been conducted at our collaborating institution (Utah State University, RA Coulombe) and RNA-seq data are currently being collected. Completed a laboratory experiment using and in ovo assay for AFB1 exposure in the turkey. This experiment examines the effects of early exposure on domestic and wild turkeys to investigate gene expression differences related to aflatoxin exposure. Liver and spleen tissues have been sequenced and RNA-seq data are in analysis. This experimental approach provides a direct method for side-by-side comparison of exposure at a high risk stage of development. Currently conducting comparative sequencing of GSTA genes in domestic, heritage and wild turkeys in an effort to identify sequences responsible for gene silencing. The information obtained from these experiments can be used in the development of future in-depth studies of AFB1 responses. **Influence of thermal challenge on turkey muscle development and meat quality.** This project in collaboration with Michigan State University and Ohio State University seeks to quantify climate change impacts on poultry breast muscle growth and development, morphological structure, intramuscular fat deposition, and protein functionality to develop appropriate strategies to mitigate the undesirable changes in meat quality. The overall goal is to define the molecular mechanisms by which heat stress affects breast muscle development in poultry. The hypothesis is that heat stress alters expression of genes affecting the growth and development of muscle and calcium signaling in muscle cells, leads to greater fat deposition, detrimental changes in muscle structure, and reduced protein functionality. Current work is to determine changes in transcriptional profiles of thermally challenged and non-challenged satellite cells by deep transcriptome RNA sequence analysis.

OR- Previous work outlined how the quantitative trait sperm mobility helps define semen quality in the chicken. The present work sought a genotype underlying low phenotype. Two complementary experimental approaches were used. The first tested the likelihood that phenotype was subject to environmental variables. This possibility was tested by feeding 6-N-propyl-2-thiouracil (PTU) to male chicks and then measuring sperm mobility after sexual maturity. Whereas body weight of treated, sexually mature roosters was 9% less than that of controls ($P = 0.0063$), sperm mobility was not affected ($P = 0.3764$). Testis weight did not differ between treatments when expressed as a percentage of body weight ($P = 0.4457$). Consequently, it seemed likely that a genotype could be associated with the low sperm mobility phenotype. This possibility was tested as follows. A reciprocal cross was made using sires and dams from lines of chickens selected for low or high sperm mobility ($n = 75$ breeding pairs per cross). The F1 phenotype distribution was normal and intermediate to the two skewed distributions observed for the P generation. Moreover, phenotype did not differ due to cross ($P = 0.2822$). A second reciprocal cross was made with F1 sires and dams ($n = 115$ breeding pairs per cross). The F2 phenotype distribution was comparable in shape and location to the F1 distribution. The phenotype of 48 roosters within each tail of the F2 distribution, i.e. those whose phenotype was a standard deviation below or above the population mean, was confirmed by nested ANOVA ($P < 0.001$).

In addition, no difference was observed among roosters within tails (P < 0.8441). A genome wide association study (GWAS) was performed with 32 roosters selected randomly from each subpopulation. GWAS was performed by DNA Landmarks with a 60k chip. Monomorphic loci, those with minor allele frequencies < 5% and those that deviated from Hardy Weinberg expectations (P < 0.05) were deleted prior to data analysis. Chi-square values were log-transformed and then plotted as averages over a sliding window of 10 single nucleotide polymorphisms. This analysis identified quantitative trait loci on the following chromosomes independent of any founder effect: 1, 8, 19, 27 and the Z chromosome.

VA- I**ntestinal response to coccidiosis**. The site of invasion and lesions in the intestine is species-specific, for example E. acervulina affects the duodenum, E. maxima the jejunum, and E. tenella the ceca. Lesions in the intestinal mucosa cause reduced feed efficiency and body weight gain. The growth reduction may be due to changes in expression of digestive enzymes and nutrient transporters in the intestine. This study compared the expression of digestive enzymes, nutrient transporters and an antimicrobial peptide in broilers challenged with either E. acervulina, E. maxima or E. tenella. The genes examined included digestive enzymes (APN and SI), peptide and amino acid transporters (PepT1, ASCT1, bo,+AT/rBAT, B0AT, CAT1, CAT2, EAAT3, LAT1, y+LAT1 and y+LAT2), sugar transporters (GLUT1, GLUT2, GLUT5 and SGLT1), zinc transporter (ZnT1) and an antimicrobial peptide (LEAP2). Duodenum, jejunum, ileum and ceca were collected seven days post challenge. E. acervulina challenge resulted in downregulation of various nutrient transporters or LEAP2 in the duodenum and ceca, but not the jejunum or ileum. E. maxima challenge produced both downregulation and upregulation of nutrient transporters and LEAP2 in all three segments of the small intestine and ceca. E. tenella challenge resulted in the downregulation and upregulation of nutrient transporters and LEAP2 in the jejunum, ileum and ceca, but not the duodenum. At the respective target tissue, E. acervulina, E. maxima and E. tenella infection caused common downregulation of APN, bo,+AT, rBAT, EAAT3, SI, GLUT2, GLUT5, ZnT1 and LEAP2. The downregulation of nutrient transporters would result in a decrease in the efficiency of protein and polysaccharide digestion and uptake, which may partially explain the weight loss during infection. The downregulation of the host defense peptide LEAP2 may be a general mechanism that Eimeria utilizes to propagate the infection. **Transport functions in the yolk sak.** The YS is an extra-embryonic tissue that surrounds the yolk, which digests and transports nutrients during incubation of the avian embryo. Understanding the function and development of the YS may lead to enhanced nutrient uptake and optimized embryo development. The objective of this project was to perform a transcriptome analysis of the YS during late embryonic development on embryonic (e) days 13, 15, 17, 19, and 21. Functional annotation as well as histological analysis revealed that two main cell types of the YS were analyzed: epithelial cells and erythropoietic cells. There was up regulation of genes involved in lipid transport and metabolism between e13 and e19. Genes associated with cytoskeletal structure were down regulated between e17 and e21, supporting histological evidence of degradation of YS epithelial cells towards hatch. Expression patterns of hemoglobin synthesis genes indicated a high erythropoietic capacity of the YS between e13 and e15. The YS also produced high levels of genes coding for plasma carrier proteins normally produced by the liver. Expression of the sodium glucose transporter (SGLT1) and the anionic amino acid transporter (EAAT3) increased from e13 to e19. During the final week of chick embryonic development, the YS acts as a multifunctional organ that plays the

role of several organs that have not yet reached their full functional capacity. The YS acts as the intestine in digestion and transport of nutrients, the liver in production of plasma carrier proteins and the bone marrow in the synthesis of blood cells.

WI- **Effect of allele frequencies, effect sizes and number of markers on prediction of quantitative traits in chickens.** The objective was to assess goodness of fit and predictive ability of subsets of single nucleotide polymorphism (SNP) markers constructed based on minor allele frequency (MAF), effect sizes and varying marker density. Target traits were body weight (BW), ultrasound measurement of breast muscle (BM) and hen house egg production (HHP) in broiler chickens. We used a 600 K Affymetrix platform with 1352 birds genotyped. The prediction method was genomic best linear unbiased prediction (GBLUP) with 354 564 single nucleotide polymorphisms (SNPs) used to derive a genomic relationship matrix (G). Predictive ability was assessed as the correlation between predicted genomic values and corrected phenotypes from a threefold cross-validation. Predictive ability was 0.27 ± 0.002 for BW, 0.33 ± 0.001 for BM and 0.20 ± 0.002 for HHP. For the three traits studied, predictive ability decreased when SNPs with a higher MAF were used to construct G. Selection of the 20% SNPs with the largest absolute effect sizes induced a predictive ability equal to that from fitting all markers together. When density of markers increased from 5 K to 20 K, predictive ability enhanced slightly. These results provide evidence that designing a low-density chip using low-frequency markers with large effect sizes may be useful for commercial usage. **Dissection of additive genetic variability for quantitative traits in chickens using SNP markers.** The aim of this study was to separate marked additive genetic variability for three quantitative traits in chickens into components associated with classes of minor allele frequency (MAF), individual chromosomes and marker density using the genomewide complex trait analysis (GCTA) approach. Data were from 1351 chickens measured for body weight (BW), ultrasound of breast muscle (BM) and hen house egg production (HHP), each bird with 354 364 SNP genotypes. Estimates of variance components show that SNPs on commercially available genotyping chips marked a large amount of genetic variability for all three traits. The estimated proportion of total variation tagged by all autosomal SNPs was 0.30 (SE 0.04) for BW, 0.33 (SE 0.04) for BM, and 0.19 (SE 0.05) for HHP. We found that a substantial proportion of this variation was explained by low frequency variants (MAF <0.20) for BW and BM, and variants with MAF 0.10–0.30 for HHP. The marked genetic variance explained by each chromosome was linearly related to its length (R2 = 0.60) for BW and BM. However, for HHP, there was no linear relationship between estimates of variance and length of the chromosome (R2 = 0.01). Our results suggest that the contribution of SNPs to marked additive genetic variability is dependent on the allele frequency spectrum. For the sample of birds analyzed, it was found that increasing marker density beyond 100K SNPs did not capture additional additive genetic variance.

Brief Impact Statements

1. Variation in cis-regulatory elements is the major mechanism that accounts for the majority of variation in MD genetic resistance and, most likely, other complex traits.
2. Genomic selection is more powerful when using SNPs associated with allele-specific expression.
3. There is an association of specific gut microbes with immune response to MDV challenge suggesting a possible role in MD genetic resistance.

4. Profiling of miRNAs and miRNA expression in response to MD vaccination is the first step to understand part of the host genetics/epigenetics influence over vaccine efficacy. This is of critical importance in vaccine development and usage with consideration of the host genetic background.
5. The genetics underlying male gonadal development and genetic mechanisms negatively impact male fertility traits in the chicken. Elucidation of the underlying anatomy, genetics and physiology that influence male fertility will lead to a fundamental understanding of sperm mobility and its inter-relationship with selection for production.
6. The genetics of ascites is directed at a fundamental understanding of the genetics and physiology of ascites and how selection can be augmented to reduce susceptibility.
7. Identification of genetic diversities representing special phenotypes in populations of commercial and experimental chicken lines are expected to find direct application as biomarkers for the selection of newly hatched chicks in the commercial agricultural setting, as well as, in the diagnosis of human genetic disorders.
8. The neuroendocrine regulation of stress and vasotocin receptors are getting closer to finding the actual set or sets of neurons responsible for sensing long-day photoperiods that result in the activation of the neuroendocrine reproductive system.
9. Identification of genes that are associated with resistance to heat stress and Newcastle disease virus
10. Identification of potential genes that are associated with avian influenza virus infection.
11. Development of in vitro overexpression and knock-down assay for gene functional study.
12. Telomere/telomerase dysregulation and Marek's Disease virus (MDV). MDV is a major cause of mortality leading to substantial economic losses to the poultry industry. Interestingly, the oncogenic MDV genome (which is circular and has no need for a telomere-maintenance system) contains two copies of the chicken telomerase RNA gene as well as several sets of telomere repeats. We hypothesize the MDV is utilizing aspects of the telomere-telomerase system to integrate into the chicken genome at the site of telomeres, and that this contributes to aspects of the disease state – specifically latency, persistence, oncogenesis.
13. The developmental genetic mutations studied are common to poultry and a cause of sporadic embryo mortality, and are similar to a number of common human congenital malformations (affecting limb, heart, craniofacial features). The chicken provides a versatile model to contribute to our understanding of genes and genetic mechanisms important to skeletal, limb and organ development.
14. Fundamental annotation information (both structural and functional) underpins functional modeling of genomic data sets. This enables poultry researchers to more accurately identify genes involved in the systems they are studying and translate long lists generated by functional genomics into a biological model that they can use to improve poultry production.
15. The *MHC-Y* region is potentially valuable in contributing genetic differences in immune responses to microbial infections and may help to define which *YF* alleles are most beneficial and the role of YF class I-like genes in chicken immune responses.
16. The identification of ASE SNPs will be of great value to fine map QTLs to genes, understanding genetic architecture, and designing SNP chips for improving traits with low heritability or traits for which direct measurement is not possible, such as disease resistance.

17. Potential epigenetic markers and regulatory elements located near genes associated with responses to viral infection suggest the possibility of applying the epigenetic biomarker to diagnose and prevent poultry disease and infectious.
18. Feed efficiency is associated with breast muscle growth in some broiler chicken populations. Inflammatory response and oxidative stress, may occur in the breast muscle of the high-FE chickens, which may be of concern for continued selection for both of these traits together in modern broiler chickens.
19. Low Feed Efficiency chickens had higher expression of lipid synthesis genes and lower expression of lipid "removal" genes in the abdominal fat tissue. The divergent expression of lipid metabolism genes represents the major difference between HFE and LFE chickens in abdominal fat gene expression.
20. Fiber-type switching, localized hypoxia, oxidative stress, and increased intracellular calcium as characteristic features of Wooden Breast Disease in commercial broilers.
21. Crossbreds instead of pure lines were shown to be advantageous for high-resolution QTL (quantitative trait loci) mapping in GWA studies and for achieving high persistence of accuracy of genomic breeding values over generations in genomic selection.
22. A greater understanding of the innate immune system and how the endogenous cannabinoid and proteasome system interfaces with it is crucial for development of next-generation antimicrobials that Salmonella cannot evade. More generally, this basic knowledge could also be translated to other host-pathogen interactions that plague other Mississippi agroindustries, such as aquaculture.
23. Characterization of novel serine hydrolases and deubiquitinating enzymes in chicken is an important goal of our project. Characterization of chicken enzymes by a chemical proteomics platform can then be used by other researchers to study these enzymes in other contexts, including chicken development and other avian diseases.
24. Application of techniques of molecular genetics and genomics to analysis of variation in structure, function and gene expression within the chicken genome has identified genes, pathways and genomic regions associated with important biological traits in chickens.
25. Genetic variation has been characterized in commercial research lines, research lines and indigenous lines of chickens.
26. Single-step Bayesian methods for large-scale genomic prediction using genotyped and non-genotyped animals were developed. Alternate mating strategies for use with traditional and genomic selection programs were evaluated.
27. Increasing innate resistance to AFB1 in turkeys will result in numerous health benefits. Transformational improvements in AFB1 resistance require a multidisciplinary approach to identify protective alleles with potential to reduce disease. Genetic markers to improve AFB1-resistance have a potentially high commercial value and positive economic impact to industry, owing to improvements in health and well-being, productivity, and a safer product for consumers.
28. Temperature variations due to climate change threaten the quality of poultry muscle as a healthy, high quality food product. Identification of molecular mechanisms associated with altered muscle development will result in development of mitigation strategies based on improved genetic

selection, nutritional intervention, and other strategies to improve poultry muscle food quality and quantity.

29. NGS sequence analysis is now feasible for numerous individual chicken lines. For single gene traits, this provides a short list of possible candidate SNP that may include the causal allele. For complex traits, this provides data for genome-wide association analysis. These efforts are compromised by the incomplete nature of the reference genome sequence and the annotation that links it with genes and transcripts. New methodologies will substantially improve the reference sequence and better annotation will make that reference more useful to all users.
30. The genetic contributions to sperm mobility phenotype was confirmed and in doing so shows the potential for using genotype to identify males at hatch that will be subfertile at sexual maturity.
31. A common set of nutrient transporters and an antimicrobial peptide (LEAP-2) were downregulated following challenge with E. acervulina, E. maxima, and E. tenella. The downregulation of nutrient transporters would partly explain the weight loss and the suppression of the host defense peptide LEAP2 may be a general mechanism for propagating an Eimeria infection.
32. During the final week of chick embryonic development, the YS plays the role of several organs that have not yet reached their full functional capacity. The YS is a multifunctional organ that acts as the intestine in digestion and transport of nutrients, the liver in production of plasma carrier proteins and the bone marrow in the synthesis of blood cells.
33. Analyses suggest that contribution of SNPs to marked additive genetic variability is dependent on the allele frequency spectrum. Increasing marker density beyond 100K SNPs did not capture additional additive genetic variance.
34. Analyses of SNP based selection provide evidence that designing a low-density chip using low-frequency markers with large effect sizes may be useful for commercial usage.
35. Cysteine deficiency was found to be is associated with: thinning of the epidermis and dermis, thickening of the hypodermis, reduction in feather weight and size, and increased abdominal fatness.
36. Leg problems and bone quality have low heritability. Genetic association between leg problems and growth is weak. Management strategies would ameliorate leg problems better than genetic solutions.

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Effect of Eimeria infection on expression of nutrient transporters in the intestine of chickens, John Lee Pratt Animal Nutrition Program (Virginia Tech), 2013-2015, $73,200, co-PI Wong

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Genomic Characterization of a Bacterial Species Contributing to Lameness and Bacteremia in Poultry. Arkansas Biosciences Institute; 7/13-6/14; $11,600; PI, Rhoads.

Genomics for improving animal production. USDA NIFA National Need Training Grant 2014-38420-21796 1/2014-12/2018, $238,000 PI: H. Zhou, J. Murray, P. Ross.

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Genotype-based selection for ascites susceptible and resistant broilers. USDA Animal Health; 7/14-8/15; $11,000; coPI, Rhoads.

Heat shock proteins, AMPK and cell bioenergetics in muscle and liver cells. Arkansas Biosciences Institute; 7/12-6/15; $135,101; PI, Bottje; coPI, Kong

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Identification, characterization, and validation of genetic mutations incurred during in vitro attenuation of Marek's disease virus. USDA, AFRI, award no. 2009-01659

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Improving food security in Africa by enhancing resistance to disease and heat in chickens; Feed the future innovation lab for genomics to improve poultry, USAID AID-OAA-A-13-00080. 9/2013-9/2018, $6,000,000, PI: H. Zhou, D. Bunn, S. J. Lamont, J. Dekkers et al.

Improving the chicken genome assembly and annotation. USDA-NIFA grant 2013-67015-21357,:, Period: 01/01/14-12/31/15, $500,000 PI: Warren, co-PI: Dodgson, Cheng, and Brown

Influence of thermal challenge on turkey muscle development and meat quality. USDA-NIFA-AFRI. 2013-2016, $975,000 co-PD: Reed, Strasburg and Velleman.

Isolation and evaluation of functional probiotic strains to improve gut integrity in chickens. Arkansas

Biosciences Institute; 7/14-6/15; $49,421; PI, Kwon; coPI, Kong

Knowledge representation resources for agricultural researchers. USDA AFRI Competitive grant MIS-391110: 2011-2014.

Molecular and metabolomics mechanisms underlying Met isomers and their analogues in broilers. EVONIK Industries AG. PI: Aggrey

Molecular signatures and mechanistic modeling for improving feed efficiency in broilers. USDA-AFRI; 2/14-1/16; $274,498; PI, Bottje; coPI, Kong

Multi-State Project, Comparative aflatoxin response in wild vs. domestic turkey: Development of an in ovo assay. USDA-Minnesota Agricultural Experiment Station. 2013-2014, $24,414 co-PI Reed.

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Physiological and biochemical aspects of Met isomers and their analogue in broilers. Evonik Industries , 2013-2014, $79,368 co-PI Wong

Strategies to enhance de novo biosynthesis of methionine in organic chicken. USDA-NIFA. PI: Aggrey, et al.

Susceptibility to Bacteraemia and Lameness in Broilers. Cobb Vantress; 9/13-8/14; $100,014.90; coPI, Rhoads

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US Poultry Genome Coordinators Funds for resequencing Hatch and Animal Health funds to Iowa State University

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Validation of identified stress receptor blockers in an avian biomedical model of stress and functional role of the VT4R. Arkansas Bioscience Institute; 7/2013 to 6/2014. $50,000; PI: W.J. Kuenzel, Co-PI: S.W. Kang.

Whole-genome sequencing for genetically selected chicken lines important for both biomedical models and agriculture in Arkansas. Arkansas Biosciences Institute; 7/11-6/14; $150,000; PI, Kong.