

U.S. Poultry Species Coordination Activities
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Overview: Coordination of Poultry Genome Mapping under the National Animal Genome Research Program (NAGRP) is a joint effort of Michigan State University (MSU) and the USDA, ARS, Avian Disease and Oncology Laboratory (ADOL). CSREES support is allocated via NRSP-8. The NAGRP is made up of the membership of the Animal Genome Technical Committee, including the Poultry Species Subcommittee.

FACILITIES AND PERSONNEL: Jerry Dodgson, Department of Microbiology & Molecular Genetics, MSU, serves as Coordinator with Hans Cheng of ADOL as Co-Coordinator. Both MSU and ADOL provide facilities and support.

OBJECTIVES: 1. Develop high resolution comparative genome maps aligned across species that link agricultural animal maps to those of the human and mouse genomes, 2. Increase the marker density of existing linkage maps used in QTL mapping and integrate them with physical maps of animal chromosomes, and 3. Expand and enhance internationally shared species genome databases and provide other common resources that facilitate genome mapping.

PROGRESS TOWARD OBJECTIVE 1. High resolution poultry genome maps.

The Reference Linkage Map(s). The genetic linkage map of the chicken has provided a framework for numerous QTL and other mapping experiments and a platform on which genome sequences have been assembled and linked to chromosomes. Updates brought the number of markers on the consensus map to over 2200, covering nearly 4000 cM (International Chicken Genome Sequencing Consortium, *Nature* 432:695-716, 2004). In connection with the genome sequence, the Beijing Genomics Institute randomly sequenced 0.25X, each, of a broiler, layer and Silkie genome, generating 2.8 million potential SNPs for high resolution linkage mapping experiments (International Chicken Polymorphism Map Consortium, *Nature* 432:717-722, 2004). In work supported by a consortium of industry, NRI Tools & Reagent grant, ARS and NRSP-8 funding, Illumina Corp was contracted to obtain ~3000 SNP genotypes, each, from ~5300 birds (about half the birds and data are or will be in the public domain). About 88% (2733) of the SNP assays worked and almost all of the submitted DNAs were successfully typed. Since members of the East Lansing and Wageningen reference linkage families were included among the panel, these data will greatly enhance the chicken linkage map, more than doubling the number of markers. The data are also being used to enhance the second build of the draft genome sequence. One of the corporate partners, Aviagen, has recently contracted with Illumina to genotype an additional ~3000 SNP and will use the combined data as part of their breeding designs. A parallel effort by EU scientists is genotyping approximately 13,000 SNPs in a variety of birds, including a Red Junglefowl (RJF) x White Leghorn F2 cross, similar to the East Lansing reference backcross. These data should provide a parallel high density linkage map.

PROGRESS TOWARD OBJECTIVE 2. Physical maps and map integration.

BAC libraries, prepared in part with NRSP-8 and NRI Tools & Reagent funding, were fingerprinted extensively and integrated with linkage and gene maps (mostly using the overgo mapping technique). These data were employed to generate a second generation BAC contig map comprised of 260 contigs, 226 of which were anchored to the genetic

linkage/chromosome map (Wallis et al., *Nature* 432:761-764, 2004). The BAC contig physical map is being updated in parallel with the second build of the chicken genome sequence that will be made public in late 2005. Similar efforts applied to the turkey CHORI-260 library have begun in hopes of generating a BAC contig physical map of the turkey genome and a comparative chicken-turkey map.

NCBI's dbEST (<http://www.ncbi.nlm.nih.gov/dbEST/>) presently lists 578,445 chicken ESTs. These have been critical in a variety of gene discovery efforts, especially in annotating the genome sequence. A joint project between the U. of Manchester and the Sanger Institute sequenced full length chicken cDNA clones. A world-wide consortium of investigators (Hubbard et al., *Genome Research* 15:174-183, 2005) described 19,626 finished cDNAs and 485,337 ESTs. The full length cDNAs begin to uncover alternative splicing products and were of particular value in annotating the genome. Array development will be reported below.

Masabanda et al. (*Genetics* 166:1367-1373, 2004) generated a molecular cytogenetic analysis of the chicken, including identification of all microchromosomes, either by chromosome paints or BAC FISH probes. Similar results have been obtained in the lab of M. Delany (personal communication). Radiation hybrid (RH) panels have been constructed by Vignal and colleagues at INRA (Morisson et al., *Genet. Sel. Evol.* 34:521-533, 2002), and a framework RH map has been constructed (e.g., Morisson et al., *Genet Sel Evol.* 37:229-251, 2005 and references therein). RH map data are being used to improve the new genome sequence build.

The first assembly of the draft 6.6X chicken sequence was released on March 1, 2004. The initial analysis and annotation of the sequence was published late last year (International Chicken Genome Sequencing Consortium, *Nature* 432:695-716, 2004). In addition to the companion physical map and SNP papers mentioned above, much of the January, 2005 issue of *Genome Research* was devoted to companion chicken sequence analysis papers. Additional sequence data, physical, RH and SNP data are being used to assemble a second, improved "build" of the chicken genome. This is expected to be released to the browsers (see below) in late 2005. In addition, the National Human Genome Research Institute has approved funding that will allow additional directed sequencing to bring the chicken genome to a "finished" state (W. Warren, personal communication). This will likely be done in 2006.

PROGRESS TOWARD OBJECTIVE 3: Database and other map resources.

Sequence and Map: The sequence, along with a variety of options and tools, can be accessed at three different browsers: the UCSC Chicken Genome BrowserGateway, (<http://genome.ucsc.edu/cgi-bin/hgGateway?org=Chicken&db=0&hgsid=30948908>); the NCBI Chicken Genome Resources, (<http://www.ncbi.nlm.nih.gov/genome/guide/chicken/>); and the EBI's Ensembl Chicken Genome Browser, (http://www.ensembl.org/Gallus_gallus/). The ChickFPC browser at <http://www.bioinformatics.nl/gbrowse/cgi-bin/gbrowse/ChickFPC> allows for various searches of the BAC contig map. Similarly, BAC locations denoted by BAC end sequences can be found on other sequence browsers noted above. The SNP data generated by the Beijing Genomics Institute (described above) can be accessed on the UCSC or Ensembl browsers, but more extensive descriptions (including QTL information) are available at the BGI site at <http://chicken.genomics.org.cn/index.jsp>.

ChickGBASE: The latest version of ChickGBASE in the comparative mapping Arkdb format is housed at the Roslin Institute at <http://www.thearkdb.org/browser?species=chicken>. It is mirrored at the Iowa State database site, <http://www.genome.iastate.edu/>. James Reecy at Iowa State has taken over direction of all bioinformatics efforts for the NAGRP, including chicken. **US Poultry Genome Homepage:** We maintain a homepage for the NRSP-8 U.S.

Poultry Genome project (<http://poultry.mph.msu.edu>) that provides a variety of genome mapping resources, including the latest EL maps and mapping data, an updated list of published microsatellites, descriptions of available resources, the latest cytogenetic map, and access to a host of other information relating to both genetic and physical maps.

Reference Panel DNA: DNA from the East Lansing international reference population has been sent to many laboratories throughout the world. As noted above (Objective 1), this panel continues to be of widespread value to mapping and sequencing projects.

Primer Kits: The **Population Tester Kit** is one of the microsatellite primer pair kits that have been made freely available. It contains 9 primer pairs which define microsatellites with high polymorphic information content (numerous alleles widely distributed in several populations). A version of a framework primer kit (with 147 well-spaced microsatellite marker primer pairs) called the "**Comprehensive Mapping Kit #7**" is still available. One or more kits have now been provided to over 130 different labs, worldwide.

Physical Mapping Resources: At least three public BAC libraries for chicken and one for turkey are now available, as described above. Filter arrays of BAC clones are being distributed for both the Texas A&M and CHORI-261 chicken BAC libraries. Filter arrays of the turkey CHORI-260 library are also available.

Chicken Microarrays: A 13K chicken spotted cDNA glass slide array remains available from the Array Facility at the Fred Hutchinson Cancer Research Center, FHCRC (Burnside et al., *BMC Genomics* 6:13, 2005). This is the result of combined efforts of FHCRC (Jeff Delrow and Paul Neiman), the U. of Delaware (Joan Burnside), GSF, Munich (Jean-Marie Buerstedde) and the Roslin Institute (Dave Burt) with partial support from Coordination funds. FHCRC arrays are available at \$150 per array. NAGRP Coordination funds have been used to make a some free test arrays available to NAGRP members, in hopes of getting as many users involved as possible. A technical report describing details of the construction and use of the arrays and the source of the cDNAs spotted can be downloaded from <ftp://milano.fhcrc.org/ArrayLab/chicken13k/tech.report/>. Affymetrix, Inc. is now marketing their GeneChip® Chicken Genome Array. Their chip measures levels of 32,773 chicken transcripts and 684 chicken viral transcripts from 17 different avian viruses. See <http://www.affymetrix.com/products/arrays/specific/chicken.affx> for more information.

Newsletter: The Poultry Genome Newsletter is published quarterly and is distributed through our Homepage, electronically on the angenmap email discussion group and via hard copy to scientists worldwide.

Meetings: Over 2000 scientists attended the joint Plant and Animal Genome XIII meeting held last January, held jointly with the annual NAGRP meeting. Coordination funds helped support attendance at PAG-XIII and will do so again for the upcoming PAG-XIV in January, 2006. The (3rd) Workshop on Chicken Genomics and Development was held at Cold Spring Harbor Lab, May 8-11, 2005 and will be held there again next May.

PLANS FOR THE FUTURE.

OBJECTIVE 1. High resolution poultry genome maps.

Resolution will improve as a result of SNP genotyping as described above. QTL analysis should expand at selected locations that have generated appropriate populations and families. Coordination-supported SNP genotyping may assist members in designing appropriate

matings and genotyping strategies. Industries have already begun to apply high throughput mapping data in modifying their breeding strategies. The data may also be of use with regard to biosecurity and food safety issues. The potential use of linkage disequilibrium or other association approaches in chicken mapping requires further study and, possibly, new techniques. New primer panels based either on SNPs and/or microsatellites may be developed, depending on the demand expressed.

OBJECTIVE 2. Physical maps and map integration.

The second build of the chicken genome should be made public shortly. Finishing sequence work will begin next year, after which another, significantly improved, build(s) will be generated. Detailed physical mapping of the turkey has begun and will continue, along with comparative turkey-chicken mapping and additional linkage map analysis in the turkey. New, massively parallel, low cost sequencing technology may open up additional options for both public and private poultry geneticists.

OBJECTIVE 3: Database and other map resources.

The availability of the draft sequence has generated invaluable support at NCBI, Ensembl, and UCSC, and among the general bioinformatics community, for chicken genomics. Efforts also need to be made to enhance bioinformatic support for the use of transcriptional profiling, so that data from different labs becomes comparable. Bioinformatic support for chicken proteomics needs to be expanded. Some of this is being done via specific bioinformatics grant programs at the NRI or from NRSP-8 funding via the Bioinformatics Coordinator. The Poultry Genome Newsletter and homepage information will be continue to be distributed and enhanced. We also will continue to distribute reference panel DNAs, microsatellite primer panels, BAC library resources (library, clones, filter sets) and Type I gene-based primers. Chicken microarrays are now available through the Fred Hutchinson Cancer Research Center Genome Facility with initial development support from coordination and other funds. Initial sets of free arrays will continue to be available to committee members, as supplies allow. Coordination support assisted numerous member labs in obtaining initial SNP data this past year, and we hope that future opportunities of this sort may occur.

(Prepared 11/18/05)