

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). Numerous labs have cooperated in mapping DNA-based polymorphic markers by genotyping samples from the same two international reference crosses, the Compton population (Bumstead and Palyga, *Genomics* 13, 690-697, 1992), and the East Lansing population (Crittenden *et al.*, *Poultry Science* 72, 334-348, 1993). This map has been enhanced by genotyping of a third cross, the Wageningen population, by Martien Groenen and colleagues (Groenen *et al.*, *Genomics* 49, 265-274, 1998). A consensus map based on all three map populations has been published (Groenen *et al.*, *Genome Res.* 10:137-147, 2000). Updates bring the number of markers on the consensus map to 2204, placed into 51 linkage groups, covering nearly 4000 cM (International Chicken Genome Sequencing Consortium, *Nature* 432:695-716, 2004). The East Lansing map has expanded to 1276 markers on 42 linkage groups (other evidence places E46 on GGA2 and E66 on GGA5, but there is not enough statistical support in our map alone to establish these linkages). This map includes 326 mapped genes. 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 future high resolution linkage mapping experiments (International Chicken Polymorphism Map Consortium, *Nature* 432:717-722, 2004).

PROGRESS TOWARD OBJECTIVE 2. Physical maps and map integration.

A library of over 115,000 BACs (~15X; Lee *et al.*, *Animal Genetics* 34:151-152, 2003) was generated and 65,000 of these were fingerprinted at Texas A&M, leading to a first generation physical map (Ren *et al.*, *Genome Research* 13:2754-2758, 2003). P. de Jong (Childrens Hospital of Oakland Research Institute, CHORI) generated a ~10X BAC library (CHORI-261) with extra large inserts using DNA we provided from the same bird used for the Texas A&M BACs and for sequence analysis. CHORI also prepared a large insert turkey BAC library (CHORI-260). The Washington U. Genome Sequencing Center (WUGSC) was provided copies of the Texas A&M and CHORI-261 chicken BAC libraries and fingerprinted over 150,000, generating over 130,000 useable BAC fingerprints. These were employed to generate a second generation BAC contig map comprised of 260 contigs, 226 of which have been anchored to the

genetic linkage/chromosome map (Wallis et al., *Nature* 432:761-764, 2004). Several labs participated in integrating the BAC contigs and sequence with the linkage map, primarily using overgo hybridization (Romanov et al., *Cytogenetics and Genome Res.*, 102:277-281, 2003). This research generated over 7800 BAC assignments to over 900 distinct markers or genes. Recently, similar efforts applied to the turkey CHORI-260 library have generated over 2400 BAC assignments for 176 markers/genes.

Boardman et al. (*Current Biology* 12:1965-19-69, 2002) announced the sequencing of over 300,000 chicken ESTs from a wide variety of tissues and developmental stages. A joint project between the U. of Manchester and the Sanger Institute (Jane Rogers) sequenced full length chicken cDNA clones using both UMIST and other libraries. A world-wide consortium of investigators report 19,626 finished cDNAs and 485,337 ESTs (Hubbard et al., *Genome Research* advance Epub, Dec. 8, 2004). NCBI's dbEST (<http://www.ncbi.nlm.nih.gov/dbEST/>) presently lists 531,351 chicken ESTs. 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. 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 is being constructed (e.g., Morisson et al., *Mamm. Genome* 15:732-739, 2004).

The Washington U. Genome Sequencing Center (WUGSC) has completed 6.6X sequencing of the chicken genome (primarily whole genome shotgun) and the first assembly of the draft chicken sequence was released on March 1, 2004. The initial analysis and annotation of the sequence was recently published (International Chicken Genome Sequencing Consortium, *Nature* 432:695-716, 2004). In addition to the companion physical map and SNP papers mentioned above, the January, 2005 issue of *Genome Research* will be devoted to companion chicken sequence analysis papers (*Genome Research* advance Epub, Dec. 8, 2004).

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/). See also the WUGSC chicken site at <http://genome.wustl.edu/projects/chicken/>. SNP data can be accessed at <http://chicken.genomics.org.cn/index.jsp> or the UCSC or Ensembl browsers. 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 BAC map can also be obtained by ftp at <http://genome.wustl.edu/projects/chicken/>. 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 is constructed in the comparative mapping Arkdb format. Arkdb was primarily developed at the Roslin Institute. ChickGBASE is available in the Arkdb format at <http://www.thearkdb.org/browser?species=chicken>. A mirror site for the poultry database has been mounted 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. **WWW Homepage:** We maintain a

WWW homepage (<http://poultry.mph.msu.edu>) for the Poultry Genome 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.

Primer Kits: Several kits of microsatellite primer pairs have been made available for free distribution. The first of these is the **Population Tester Kit**. This 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 (thanks in part to a generous donation from Hy-Line International). One or more kits have now been provided over 120 different labs, worldwide. Two **Chicken Gene Primer Pair** kits have been made available containing a total of 300 primer pairs to sequenced chicken genes for use in EST mapping and expression analysis.

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 now available.

Chicken Microarrays: A 13K chicken spotted cDNA glass slide array is now available from the Array Facility at the Fred Hutchinson Cancer Research Center, FHCRC. 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 small number (12) of free test arrays available to NAGRP members, in hopes of getting as many users involved as possible. A limited number of additional array sets remain available. 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 WWW Homepage, electronically on the angenmap email discussion group and via hard copy to scientists worldwide.

Meetings: Over 1800 scientists attended the joint Plant and Animal Genome XII meeting held last January, held jointly with the annual NAGRP meeting. Coordination funds helped support attendance at PAG-XII and will do so again for the upcoming PAG-XIII in January, 2005. The Chicken Genome: New Tools and Concepts meeting was held at the Stowers Institute for Medical Research in Kansas City, April 30-May 2. Additional presentations were made at the Int'l Symposium on Avian Endocrinology, PSA Annual Mtg. Ancillary Scientists Symposium and USDA Animal Genomics Workshop, Washington, DC, in 2004.

PLANS FOR THE FUTURE.

OBJECTIVE 1. High resolution poultry genome maps.

Efforts will be made to improve overall map marker density as a result of the genome sequence. QTL analysis should expand at selected locations that have generated appropriate populations and families. The potential use of linkage disequilibrium approaches in chicken mapping requires additional studies and, possibly, new techniques. New primer panels based either on SNPs and/or microsatellites may be developed, depending on demand expressed.

OBJECTIVE 2. Physical maps and map integration.

Additional efforts are underway to fill gaps between contigs, improve map resolution and integrate these physical maps with existing linkage maps. Further annotation of the sequence will be essential, as numerous gaps have been identified. Discussions continue about the possibility of completing a “pre-finishing” stage of sequencing that will resolve some of the missing genes, sequence gaps and, possibly, assembly errors. 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.

OBJECTIVE 3: Database and other map resources.

The availability of the draft sequence has induced much greater interest at NCBI, Ensembl, and UCSC in supporting chicken genome information. Efforts need to be expanded to work with these sites and update maps as new data appear. Efforts also need to be made to assist bioinformatic support for the use of transcriptional profiling, so that data from different labs becomes comparable. Bioinformatic support for chicken proteomics needs to be explored. 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. Affymetrix chicken chips have recently come on the market, and we look forward to seeing them employed in transcriptional profiling for the chicken.

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