

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. 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 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 greatly enhanced the chicken linkage map, more than doubling the number of markers, and were critical in the second build of the genome sequence (see below). A parallel effort by EU scientists genotyped 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. During the past year, coordination funds contributed to another Illumina Corp. SNP typing consortium initiated by NRSP-8 members. This project could only have occurred with the re-use of the SNP genotyping reagents developed by the first Illumina consortium. Together, the rapid expansion of SNP data will provide a high density linkage map and should aid numerous on-going attempts to identify the causative genetic changes involved in many chicken QTL (see <http://www.animalgenome.org/QTLdb/chicken.html>.)

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, most of which have been anchored to the genetic linkage/chromosome map (Wallis et al., *Nature* 432:761-764, 2004). The BAC contig physical map was updated in parallel with the second build of the chicken genome sequence (see below). Similar efforts applied to the turkey CHORI-260 library are underway 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 almost 600,000 chicken ESTs. These have been critical in a variety of gene discovery efforts, especially in annotating the genome sequence and in array development to be reported below.

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. Additional sequence data, physical, RH and SNP data were used to assemble a second, improved "build" of the chicken genome, released in May, 2006. The second build moved a large portion of the previously unplaced sequence contigs into specific chromosomal locations and enhanced the general contiguity and accuracy of the sequence assembly. 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 soon.

PROGRESS TOWARD OBJECTIVE 3: Database and other map resources.

Sequence, Map and QTL: 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 are available at the BGI site at <http://chicken.genomics.org.cn/index.jsp>. A recent survey of chicken QTL was developed (Abasht et al., *Poultry Science* 85:2079-2096, 2006) and is made available through the NRSP-8 Bioinformatics team at <http://www.animalgenome.org/QTLdb/chicken.html>.

ChickGBASE: The latest version of ChickGBASE developed by the Roslin Institute is at <http://www.thearkdb.org/arkdb/do/getChromosomeDetails;jsessionid=B8A6A5EA698B84AF80EE99BE7530B04E?accession=ARKSPC00000004>. **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, descriptions of available resources, the latest cytogenetic map, and access to a host of other information relating to both genetic and physical maps, including our newsletter archive. **Newsletter:** The Poultry Genome Newsletter is published quarterly and is distributed through our Homepage, electronically on the angenmap email discussion group and via direct email to scientists worldwide.

Reference Panel DNA: DNA from the East Lansing international reference population has been sent to many laboratories throughout the world.

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. 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: Recently, coordination funds were used to provide samples of a new **44,000 element long oligonucleotide chicken array** made by **Agilent Corp.** to several NRSP-8 participants. Results from the use of these arrays should appear next year. **Operon Biotechnologies Inc.** distributes a *Gallus gallus* (chicken) Roslin/ARK CoRe Array V1.0 ready-to-spot oligonucleotide set that contains 20,673 long oligo probes for creating microarrays. The oligo probes were designed by **ARK-Genomics** using Ensembl gene transcripts, the BBSRC fully sequenced cDNA set and the DT40 full length sequencing set. See http://www.operon.com/arrays/oligosets_chicken.php. A chicken whole genome long oligo array is also available (~\$150 each) at the **U. of Arizona**, www.grl.steelecenter.arizona.edu and can also be purchased on a custom basis from **Nimblegen**, www.nimblegen.com. A 13K chicken spotted cDNA glass slide array remains available from the Array Facility at the **Fred Hutchinson Cancer Research Center**. See **Burnside et al.**, *BMC Genomics* 6:13 (2005) (<http://www.biomedcentral.com/bmcgenomics>) for more details. 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. NAGRP Coordination funds have been used to make a some free test arrays available to NAGRP members. **Affymetrix, Inc.** is marketing the GeneChip® Chicken Genome Array that measures levels of 32,773 chicken transcripts and 684 chicken viral transcripts. See <http://www.affymetrix.com/products/arrays/specific/chicken.affx>.

Meetings: Over 2000 scientists attended the joint Plant and Animal Genome XIV meeting held last January, held jointly with the annual NAGRP meeting. Coordination funds helped support attendance at PAG-XIV and will do so again for the upcoming PAG-XV in January, 2007. The 2006 Cold Spring Harbor Chicken Genomics & Development Workshop (fourth in the series) was held at Cold Spring Harbor Lab last May. Next year, this meeting will be held in Barcelona, Spain, see www.chicknet.es.

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 will continue to 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.

OBJECTIVE 2. Physical maps and map integration.

The second build of the chicken genome has appeared. Finishing sequence work will begin soon, 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 should open up additional options for both public and private poultry geneticists for genome sequencing different lines and species.

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 was expanded this year via CSREES grant support to AgBase at Mississippi State (<http://www.agbase.msstate.edu/>) as well as in Europe. 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 primers, BAC library resources (library, clones, filter sets) and to assist in microarray studies and SNP genotyping studies. These functional genomics resources have been our major emphasis since the genome sequence appeared two years ago.

(Prepared 12/05/06)