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Comparative Mapping of the Chicken Genome

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Steven P. Suchyta

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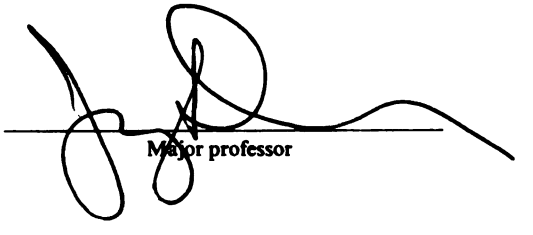

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COMPARATIVE MAPPING OF THE CHICKEN GENOME

By

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ABSTRACT

COMPARATIVE MAPPING OF THE CHICKEN GENOME

By

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Comparative mapping has been performed between the chicken and human genomes in regions corresponding to human chromosomes 1, 4, and 9, along with several other smaller areas of conserved synteny. These regions were initially chosen because of their relevance to previously identified Marek's disease (MD) resistance quantitative trait loci (QTL) (Vallejo *et al.* 1998, Yonash *et al.* 1999). Segments of chicken orthologues of mapped human genes were PCR-amplified from parental DNA of the East Lansing Backcross (BC) reference population, and the two parental alleles were sequenced. Single nucleotide polymorphisms (SNP) differences were then used to design allele-specific PCR primers with which to genotype the mapping panel; 52 BC progeny. Inheritance data were analyzed and the map location of the chicken orthologues were determined. Statistical analysis, based on the theoretical treatment of Nadeau and Taylor (1984), was performed using the region specific comparative map data to derive an estimate of the genome-wide conservation of gene order between avian (chicken) and mammalian (human) genomes. The average length of a conserved segment was calculated to be 38 $\pm$ 9 centimorgans (cM), approximately 1% of the present estimate of the total

genome. This corresponds to a rate of .13 $\pm$ 0.04 reciprocal translocations per million years of evolution, a rate substantially less than found for some intra-mammalian genomes, suggesting an unusual level of evolutionary stability exists among avian genomes. A significant portion of human chromosome 9 was shown to correspond to a portion of the chicken Z sex chromosome, thereby providing some insight into the evolution of ZW-type chromosomal sex determination in birds.

In addition to the comparative map, the initial steps to building a physical map of the chicken genome were begun. Recently, through collaboration with the Texas A&M BAC Center, a 5-fold BAC library of the chicken genome has been generated. This is comprised of approximately 38,000 clones with an average insert size of 150 kb. The BAC library is composed of chromosomal DNA from a Jungle Fowl (JF) female parent of the reference population. Because of the relative marker density, MD QTL, and number and positions of conserved markers between humans and chickens, microchromsome E41 was chosen to begin the physical mapping project. The BAC library has been spotted on 20 nylon membrane filters and these were screened using radio-labeled probes derived from six markers on E41. Ten positive BAC clones have been identified from four of the six markers tested.

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KEY TO ABBREVIATIONS

AFLP: Amplified Fragment Length Polymorphism

Amp: ampicillin

BAC: Bacterial Artificial Chromosome

BC: Backcross

BLAST: Basic Local Alignment Search Tool

c-chr: Chicken Chromosome

cDNA: Complementary DNA

CHEF: Clamped Homogeneous Electric Fields

cM: Centimorgans

DNA: Deoxyribonucleic Acid

EL: East Lansing

EST: Expressed Sequence Tagged Site

FISH: Fluorescent *in situ* Hybridization

Gb: Gigabase

h-chr: Human Chromosome

INRA: Institut National de la Recherche Agronomique

JF: Jungle Fowl

kb: Kilobase

Mb: Megabase

LOD: $\log_{10}$ of Odds

MD: Marek's Disease

Mya: Million Years Ago

NCBI: National Center for Biotechnology Information

NOR: Nucleolar Organizer Region

OMIM: Online Mendelian Inheritance in Man

PASA: PCR Amplification of Specific Alleles

PCR: Polymerase Chain Reaction

PFGE: Pulse Field Gel Electrophoresis

PPSCG: Primer Pairs to Sequenced Chicken Genes

QTL: Quantitative Trait Loci

RAPD: Random Amplified Polymorphic DNA

RFLP: Restriction Fragment Length Polymorphism

RH: Radiation Hybrid

SNP: Single Nucleotide Polymorphism

Tm: Melting Temperature

UDEL: University of Delaware

UMSTS: Universal Mammalian Sequence Tagged Sites

WL: White Leghorn

YAC: Yeast Artificial Chromosome

ZOO-FISH: Interspecies Chromosome Painting

Chapter 1: INTRODUCTION AND LITERATURE REVIEW

Comparative Mapping: Terms and Techniques

Before giving a definition of comparative mapping it will be useful to review the terms and techniques associated with the construction and utilization of comparative maps. Comparative maps rely on the placement of homologous genes on the genome maps of two or more species. An important factor to consider when analyzing homologous genes between different species is whether the genes are orthologous or paralogous. Orthologous genes are homologous genes in different species that are descended from the same gene in the last common ancestor of the two species. In contrast to this, paralogous genes are homologous genes that are not descended from the same ancestral gene. Paralogous genes arise through gene duplication prior to the existence of the last common ancestral species. Thus, paralogues may diverge and change location within the genome at times both before and after the time of the last common ancestor, whereas orthologues can only do so after that time point. Thus, selection of orthologous genes will provide the most accurate and useful comparative map.

Two additional terms used to define the structure (similarities and differences) of a comparative map are conserved synteny and conserved segment. Originally, the term synteny was used to describe genes found on the same

chromosome, regardless if they were genetically localized or not (Renwick, 1971). With the continued use of somatic cell hybrids, there was a need to classify genes found on the same chromosome, but that could not be linked through recombination analysis. The term conserved synteny is now used in comparative mapping to describe the situation when two or more genes are syntenic (reside on a single chromosome) in different species, regardless of gene order or non-contiguous interspersed segments. The determination of synteny is through some type of genome mapping, such as linkage analysis or radiation hybrid (RH) mapping. A conserved segment between two species is a chromosome interval (defined by two or more genes) that shares the same gene order and has no non-contiguous interspersed gene segment. A comparative map is constructed using established conserved segments and syntenies.

Comparative maps are unique in that they rely on other types of genome maps for their construction. In order to maximize the information across species there has been an effort to produce homologous anchored reference loci. Two groups of reference loci have been developed, the comparative anchor tagged sequences (CATS) (Lyons *et al.* 1997) and the Universal Mammalian Sequence Tagged Sites (UMSTS)(Venta *et al.* 1996). The CATS primer set was optimized for the cat and the UMSTS set was optimized for the canine. These markers were developed by designing PCR primers based on conserved exon sequences from many

different mammalian species. The primers were designed through computer analysis of adjacent exonic sequences from over 20 mammalian species (Venta *et al.* 1996; Lyons *et al.* 1997). The exon sequences can be used to verify the PCR product and the intron is a potential source for sequence or length polymorphisms to be used for genome mapping. Primer sets for over 500 genes are available and approximately 75% should be successfully amplified in any mammalian species (Lyons *et al.* 1997). These tools will greatly aid in the construction of a reference comparative map that can be used across many mammalian species and there is now a comparative genome map between mice and humans based on 314 of the CATS anchor loci (Chen *et al.* 1999).

One of the most studied intra-mammalian comparative maps has been derived from the cat. Therefore, it will be useful to review its construction. The feline genome map was first developed using a rodent X cat somatic cell hybrid panel and fluorescent *in situ* hybridization (FISH) (O'Brien and Nash 1982; Yuhki and O' Brien 1988; Lopez *et al.* 1996; O'Brien *et al.* 1997). FISH mapping relies on fluorescently labeling a portion of the gene or marker of interest and hybridizing on metaphase chromosome spreads. Somatic cell hybrids can assign markers only to their respective chromosomes, and do not give information on gene order. An interspecies backcross (BC) population between the domestic cat and an Asian leopard cat has also been developed (Lyons *et al.* 1994). The CATS or UMSTS reference

loci could be mapped through linkage analysis of polymorphisms found in the PCR product (Lyons *et al.* 1997). There is a 4 cM limit to the resolution of this linkage map (Lyons *et al.* 1994).

In order to develop a high-resolution gene map in the cat, a RH panel was developed (Murphy *et al.* 1999). For a complete review of RH mapping, see McCarthy (1996). RH panels are made by irradiating a donor cell line (in this case, derived from the cat) with a lethal dose of X-rays or γ rays, the DNA fragments from the donor cell line are rescued by a recipient cell line (hamster cells were used for the feline RH panel). Using a selectable marker, the only post-fusion cells that will grow are those containing donor DNA. The hybrid colonies are picked individually and DNA is extracted. Genes or other markers are screened in the panel usually through PCR. The retention pattern of the markers for each hybrid is compared to determine linkage, and from this data, the map distances can be calculated. High-resolution RH maps have been successfully constructed for humans (Gyapay *et al.* 1996; Stewart *et al.* 1997) and mouse (McCarthy *et al.* 1997). The CATS or UMSTS markers are PCR primers and can be used for RH mapping as can any other sequence tagged site (STS).

The current feline-human comparative map was developed using the RH panel and FISH mapped genes placed on the feline genome (Lyons *et al.* 1997). An additional tool used to assess the amount of conservation between the two genomes

on a broader scale was interspecies chromosome painting (ZOO-FISH) (Lyons *et al.* 1997). The ZOO-FISH procedure first uses special PCR conditions to amplify flow sorted metaphase chromosomes and the amplified chromosome is fluorescently labeled and used for *in situ* hybridization on metaphase chromosomal spreads from distantly related species (O'Brien, 1993; Weinberg and Stanyon 1995; Rettenberger *et al.* 1995; Solinas-Toldo *et al.* 1995; Fronicke *et al.* 1996; Goureau 1996). The ZOO-FISH method can give a direct assessment of the amount of genome conservation between two species through visualization of the labeled metaphase chromosomes.

Unlike the feline genome map, the chicken genome map has been developed primarily through linkage analysis. There are currently three main reference families through which DNA-based sequence polymorphims have been placed: the Compton population (Bumstead and Palyga 1992), the East Lansing (EL) population (Crittenden *et al.* 1993,) and the Wageningen population (Groenen *et al.* 1998). A consensus map combining all three that contains 1889 markers (approximately 300 are genes) has been developed (Groenen *et al.* in press). The chicken-human comparative map data developed in this thesis was based on genes placed on the EL reference map, so a more detailed description of it will be useful. The EL population was constructed by first mating an inbred male UCD001 Red Jungle Fowl (JF) to an inbred UCD003 White Leghorn (WL) female and then 2 F1 male progeny were backcrossed to the WL line (Crittenden *et al.* 1993). This

interspecies cross maximizes the potential for sequence polymorphism and each marker is biallelic in the BC population. Four hundred animals were produced in the BC from which the panel of 52 BC birds normally used for the mapping panel are derived.

Comparative Mapping: Definition and Utilization

Comparative gene mapping is the comparison of the chromosomal arrangement of orthologous genes in the genomes of two or more species. Comparative gene mapping has been an essential tool in the genetic analysis of many species and has given insight into the evolution of genome organization. Among mammals, much of the power of comparative mapping relates to the extensive mapping and sequence information now available for human genes. To date, over 7,000 known genes and over 16,000 expressed sequence tagged sites (ESTs) have been mapped on the human genome (Adams *et al. 1995; Hudson et al. 1995; Schuler et al. 1996; DeLoukas et* al. 1998, Online Mendelian Inheritance in Man, OMIM, http://www.ncbi.nlm.gov/omim/, 2000). The other model mammalian species, the mouse, now has over 7,000 genes mapped on its genome (Copeland *et al.* 1993; Adams *et al.* 1995; Dietrich *et al.* 1996; Marra *et al.* 1999; Van Etten *et al.* 1999). All of this data is readily available through National Center for Biotechnology Information (NCBI) Genbank databases. Through the use of a framework comparative map

between a reference/model genome (e.g., mouse, human) and a genome of interest that has been less extensively studied, it is possible to infer the location of genes in the latter species that exist in the gaps between orthologous genes previously mapped in both species. Framework comparative maps between a number of related species (e.g., mammals, O'Brien *et al.* 1999) depend upon placing orthologous comparative anchor loci on two or more members of that group. Ideally, the same anchor loci are mapped in several member species, which allows integration of the respective maps and, potentially, an estimate of the pattern of chromosome rearrangements that explain the evolution of gene order within the species group.

Comparative mapping has been applied to the genomes of a variety of mammalian species (O'Brien *et al.* 1999). The mouse presents a special case in the development of its comparative map. The mouse genome has been far more extensively mapped than that of any other mammal, excepting humans. Additional interest derives from the putative unusual qualities of mouse chromosomes in an evolutionary sense (reviewed in Graves 1996). There is now a high-resolution comparative map between the mouse and human genomes, which provides great insight into chromosomal rearrangements that have occurred during the evolution of the mouse (Copeland *et al.* 1993; Debry and Seldin 1996; Carver and Stubbs 1997). Although there exist large regions with a high degree of conservation between the two genomes

this is the exception (e.g., on both species' chromosome 1 there is a >10Mb region with conserved gene content, spacing, and order, Oakley *et al.* 1992). Most syntenic segments contain numerous rearrangements. As maps have improved, several syntenic segments initially thought to be conserved intact are not truly contiguous. One example is the q arm of human chromosome 5 which contains a large segment initially thought to be completely conserved with a region on mouse chromosome 11, but which now has been shown to be interspersed with orthologous genes from mouse chromosomes 13, 18, and 17 (Carver and Stubbs 1997). At least four rearrangements in mouse chromosome 11 would be needed to account for this (Watkins-Chow *et al.* 1997). Extensive analysis of the mouse and human Major Histocompatibility Complex regions and T-Cell Receptor loci reveal that many deletions, duplications, and inversions exist between the two species (Weiss et al. 1984; Hood et al. 1993; Koop et al. 1992, 1994; Amadou et al. 1995). One of the advantages of comparing the mouse and humans genomes is the large amount of sequence information available for both (Januzzi *et al.* 1992; Koop et al. 1992, 1994; Lamerdin *et al.* 1995, 1996; Oeltjen *et al.* 1997) These studies compared the sequences from a diverse set of genes and flanking regions of the two genomes. Overall, it appears there is a general conservation of exons, introns, and intergenic sequences. Exonic sequences in the T-Cell Receptor gene region have a 66-79% similarity, whereas

intronic and intergenic sequences have approximately 66% similarity (Oeltjen *et al.* 1997). The mouse and human gene regions also had conservation in the sizes and order of the exons, introns, and intergenic areas (Januzzi *et al.*1992; Renucci *et al.* 1992; Koop *et al.* 1992, 1994; Lamerdin *et al.* 1995, 1996; Oeltjen *et al.* 1997). Thus, it appears the relative instability of the mouse genome is in the placement and order of genes on the chromosomes, while the sequence and organization of the genes themselves has remained stable. Thorough analysis of the comparative map between human and mouse gives rise to 180 conserved segments with lengths ranging from 1 to 10 cM (Copeland *et al.* 1993; Debry and Seldin 1996; O'Brien *et al.* 1999).

Although fewer data points are available for other mammals, comparative mapping across a wide range of mammals reveals that the mouse genome is the exception (with its large number of rearrangements), relative to that of the human. In other words, the rearrangements observed between the mouse and human genome have occurred primarily in the evolutionary line to the mouse, not to the human, from the last common ancestor of both species (O'Brien *et al.* 1999).

An example of this high degree of conservation can be found in the feline-human comparative map. Even though the initial construction of the feline-human comparative map relied on somatic cell hybrid panels and FISH mapping (O'Brien and Nash 1982, Yuhki and O'Brien 1988, Lopez *et al.* 1996) and contained only 105 homologous genes, it showed a

considerable amount of conservation between the two species (O'Brien *et al.* 1997). Now there are approximately 500 homologous markers mapped on the feline map (Yuhki and O'Brien 1988; Lopez *et al.* 1996; O'Brien *et al.* 1997; O'Brien *et al.* 1999), covering all 19 feline chromosomes. Many of these genes were mapped on the feline high resolution RH map (Murphy *et al.* 1999; O' Brien *et al.* 1999). There is extensive syntenic conservation with the human map across most of the chromosomes. Chromosome D1 in the feline is conserved completely with human chromosome 11 and there is complete X chromosome conservation. Comparative map data based on gene maps will have gaps unless there are thousands of homologous markers as in the human and mouse. In order to confirm the comparative map, ZOO-FISH analysis was performed using feline-human reciprocal hybridizations (O'Brien *et al.* 1997). ZOO-FISH painting physically covers 90% of the chromosomes. This allows for direct observation of the minimal number of translocation rearrangements between the two genomes, but the technique will miss translocation of small segments or internal rearrangements within a single chromosome. The ZOO-FISH method also confirms that the framework provided by the location of homologous markers on the genetic map is accurate. The majority of differences between the feline and human genomes appear to be the splitting and rejoining of chromosomes; with only two interspersed human chromosomal segments in the feline genome (O'Brien *et al.* 1997). The high resolution RH

map illustrated that there was also a high degree of gene order conservation for human chromosomes 12 and 22 with feline chromosomes B4 and D3 respectively (Murphy *et al.* 1999)

Very large segments of conserved synteny with the human genome have also been reported in other mammals such as dogs (Priat *et al.* 1998; Murphy *et al.* 1999; Neff *et al.* 1999,), cattle (Yoo *et al.* 1994; Hayes *et al.* 1995; Solinas-Tolda *et al.* 1995; Wienberg and Stanyon 1995; Chowdhary *et al.* 1996; Pirottin *et al.* 1999) and pigs (Rettenberger *et al.* 1995; Fronicke *et al.* 1996; Goureau *et al.* 1996; Marklund *et al.* 1996; Rohrer *et al.* 1996; INRA, http://www.toulouse.inra.fr/lgc/pig/compare/compare.htm). Unlike the feline map, the canine, pig, and bovine maps primarily have employed genetic mapping to build the comparative maps. These comparative maps have been confirmed on a larger scale and gene order appears to be conserved as well. As high-resolution maps are eventually made of these species, smaller rearrangements will likely appear, as was observed with the human-mouse high-resolution map (Carver and Stubbs 1997). This will pose a problem when the comparative map is used to locate potential candidate genes.

One of the uses of comparative maps is to find candidate genes based on the assumption of common inheritance of a complete interval flanked by two syntenic framework markers. An example exists on bovine chromosome 2, which was shown to contain the gene for muscular hypertrophy

(Charlier *et al.* 1995; Dunner *et al.* 1997). This region shares conserved synteny with human chromosome 2, and there are several potential candidate genes in this area (Sonstengard *et al.* 1997b). Refinement of the comparative map in this region in cattle revealed several cases of complex gene shuffling throughout (Sonstegard *et al.* 1998). Rearrangements in gene order may cause the initially identified candidate genes to be reevaluated. This may result in considerably more effort than anticipated in gene identification, as was the case with muscular hypertrophy. However, it should be noted that the gene responsible for muscular hypertrophy, myostatin, was identified through a comparative approach (Grobet *et al.* 1997). Comparative maps built using anchor loci will be a valuable tool in identifying potential candidate genes, but small rearrangements in gene order show that dense comparative maps will often be required to make confident predictions. At the moment, maps with this level of resolution are lacking for vertebrate species outside of mouse, rat, and human. In general, the wider the evolutionary difference between two species, the greater is the desired resolution of comparative maps used to infer candidate genes for traits.

Overall, it appears there is a great deal of genome conservation between mammalian species. Compared to the mouse and human genomes that can be divided into 180 conserved segments (O'Brien *et al.* 1999)(when gene order is

considered there are over 200 segments, Eppig and Nadeau 1995; Debry and Seldin 1996), all of the other species studied have a much higher level of conservation. Human and feline maps are divided into 32 conserved segments (O'Brien *et al.* 1997; O'Brien *et al.* 1999), human and bovine maps have 50 conserved segments (Rettenberger et al. 1995; Fronivke *et al.* 1996; Goureau *et al.* 1996; O'Brien *et al.* 1999), and human and porcine maps can be divided into 47 segments (Marklund *et al.* 1996; Rohrer *et al.* 1996; O'Brien *et al.* 1999). These do not take into consideration small changes that affect gene order, but it is clear the genome organization is very similar among a variety of mammalian species.

Chicken comparative mapping:

One of the most important non-mammalian species is the chicken. It is of great importance as an agricultural commodity and as a research tool. At first glance, it appeared that building a comparative map between chicken and any mammalian species might be difficult. The last common ancestor between avian and mammalian lines lived approximately 300-350 million years ago (Mya), so there have been 600-700 million years of separate evolutionary history (along both lines) for chromosomal rearrangements to occur between the chicken and, for example, the human genome. Applying the formula of Paterson et al. (1996) (based on

comparative plant genome maps and early data from mouse and human genomes) leads one to calculate the size of a segment of the genome with a 50% probability of not being rearranged between chicken and human to be about 1.7 cM. As the chicken genome is about 3500 cM, this would be equivalent to roughly 2000 chromosomal rearrangements between the two genomes. Several studies, including those described in this thesis, have demonstrated that this is a gross over-estimate.

Avian chromosomes have been conserved over a long period of time. Analysis of karyotypes of over 800 species of birds has shown that avian chromosome morphology (banding pattern) and number have been highly conserved for 150 million years (Rodionov 1996). This is similar to the case in turtles (Bickman 1981) and salamanders (Maxson and Wilson 1975), where chromosomes have remained relatively constant (at the cytogenetic level of analysis) for over 200 million years in some cases. The typical avian genome is comprised of eight to ten macrochromosomes and between 30 to 34 microchromosomes. The distinction is arbitrarily based on the size of the chromosome; there is no clear quantitative cut-off defining the boundaries between macro and microchromosomes. Generally macrochromosomes are between 2.5 to 6 μm in length and the microchromosomes are less than 2.5 μm long during mitosis (reviewed in Rodionov 1996, 1997). Avian macrochromosomes are probably generally homologous to turtle macrochromosomes (Takagi and Sasaki

1974; Stock and Mengden 1975), so there may be a similar evolutionary mechanism involved. The conservation of chromosomes over this long period may be due to a selection for high genomic homeostasis or a strong stabilizing selection for the ancestral chromosome number and morphology (Bickman 1981; Rodionov 1996).

The stability of avian chromosomes should greatly increase the effectiveness of comparative mapping between mammals and chickens by reducing the amount of change that has occurred since the last common ancestor. In addition, the formula derived by Paterson et al. (1996) was heavily weighted by a few comparisons (e.g., mouse/human) in which high levels of genome rearrangement have occurred. Although there are not enough data to make a definitive estimate among birds, recent broad analysis of mammalian genomes (O'Brien *et al.* 1999) suggests that genomes are often highly stable over long evolutionary time, but that particular lineages (e.g., the rodent lineage) go through periods of unusually rapid rearrangement. Fortunately, as will be described below, such bursts of chromosomal rearrangement may have been relatively rare in the lineages leading to both the chicken and human from their last common ancestor.

Using the data available at that time, Burt (1997) calculated that approximately one-third of the syntenic genes (genes on the same chromosome in this case) from the last common ancestor between human and chickens now have conserved synteny between the species. As discussed earlier,

there has been a high rate of chromosomal rearrangement in the mouse compared to other mammalian species, and only 40% of these original syntenic relationships remain between humans and mouse (Bengtsson *et al.* 1993). Only 18% of the original syntenic relationships remain for chickens and mice. The low percentage of conserved syntenies between chicken and mouse is heavily influenced by the high rate of rearrangements found in rodent species. The divergence time is approximately 70 million years between human and mouse (Graves 1996) and 300 million years between mammals and birds (Kumar and Hedges 1998). Considering the difference in divergence time between the species, it is interesting to note that the number of rearrangements predicted between the human and mouse genomes was similar to those predicted between the human and chicken genome.

There has been recent further progress into the construction of a chicken-mammalian comparative map. One successful approach used by our group and others has been to map chicken genes with known sequence information (Klein *et al.* 1996; Smith *et al.* 1997; Fridolfsson *et al.* 1998; Groenen *et al.* 1999). FISH analysis, Restriction Fragment Length Polymorphisms (RFLP), and polymorphic intergenic microsatellite sequences are common methods used for the chromosomal placement of chicken genes (Klein *et al.* 1996; Smith *et al.* 1997; Fridolfsson *et al.* 1998; Groenen *et al.* 1999). FISH mapping using the gene of interest as a fluorescent probe allows for visualization of the

chromosomal placement of the gene. RFLP analysis uses the gene as a probe to identify a polymorphism and to genetically map the gene through linkage analysis in a reference population. PCR primers are designed to cross polymorphic intergenic microsatellite sequences in order to genetically map the gene through linkage analysis. A technique successfully used by our group has been the use of PCR amplification of specific alleles (PASA) to genotype sequence polymorphisms identified in the EL reference map population. Using available sequence information from a gene, primers are designed to cross a less conserved region such as an intron or 3' untranslated region (UTR), and sequence information is obtained from both parental lines of the EL population (WL, JF). If a polymorphism is found, segregation of the JF allele in the BC mapping animals is determined through preferential amplification of the JF allele. A more detailed description of this technique is described in Chapter 2 of this thesis. Although a few genes have been successfully amplified using the CATS and UMSTS set of primers (Smith *et al.* 1997), we have experienced a relatively high failure rate and now rely almost entirely on chicken genes with known sequence information.

This initial work has shown that a robust chicken mammalian comparative map could be made. Several large regions with conserved synteny and regions with conserved segments were found. Some of the conserved regions extend over 50 cM on the chicken genetic map (Smith *et al.* 1997;

Groenen *et al.* 1999). For the purposes of this thesis, the focus was placed on the chicken-human comparative map. The mouse genome, as was discussed earlier, appears to be relatively unstable, which could limit its usefulness in a comparative map. Additionally, the human genome has by far the most comprehensive genome map. Although many regions of the chicken-human comparative map were added to in this thesis, we focused on a few select regions rather than seeking broad coverage. Since the comparative map of human chromosome 1 was the most complete, an attempt was made to fill in some of the gaps to identify the extent of the conservation. Our initial work had identified a large region conserved between human chromosome 4 and chicken chromosome 4, and an attempt was made to extend the chicken-human chromosome 4 map. Initial work by our group and others had identified a large region of conservation between human chromosome 9 and the Z chicken sex chromosome (Smith *et al.* 1997; Fridolfsonn *et al.* 1998; Nanda *et al.* 1999). The comparative map of the Z chromosome was extended in the hopes of elucidating some of the dynamics of the evolution of the avian sex chromosomes. By focusing on relatively few regions we hoped to get good coverage of these chromosomes, in an attempt to get a general idea of the number of chicken segments that would cover a human chromosome.

One of the goals of the work in this thesis was to add to the number of conserved segments between chickens and humans. The approach taken was to try to saturate the

coverage over entire human chromosomes using those chicken orthologues that had already been sequenced. The starting points were the conserved groups found in our initial work in Smith *et al.* (1997). Additionally, the statistical approach of Nadeau and Taylor (1984) used in the early stages of the mouse-human comparative map was used to make a genome-wide estimate of the total level of conservation of gene order between the avian and mammalian genomes.

Statistical approach:

In the early 1980s, far less map data existed for both the mouse and human genomes. In order to analyze the amount of genomic conservation between the two species, Nadeau and Taylor (1984) derived a method to estimate overall genome conservation from a limited data set of gene segment comparisons. They estimated the average length of a conserved segment between mouse and human genomes to be 8.1 ± 1.6 cM. This was based on 13 known conserved linkage groups (containing two or more genes) and 54 mapped single homologous markers. In 1993, Copeland *et al.* came to the same estimated conserved segment length of approximately 8 cM. This was based on over 140 conserved linkage groups, nearly covering both genomes. Thus, it appears that the Nadeau and Taylor (1984) model generated an accurate prediction of average genome conservation, despite the relatively poor level of map coverage at that time. In

comparing the human genome to those of most non-rodent mammals, direct observation techniques (chromosome banding patterns, ZOO-FISH), are most often used to estimate average conserved segment length (or estimated number of rearrangements), since there typically exist relatively few changes (O'Brien *et al.* 1999). However, until recently little effort has gone into comparative genome mapping between more distantly related species (e.g., birds and mammals) due to the greater challenge in identifying an adequate collection of orthologues and initial estimates that conserved segment lengths would be small (e.g., Paterson *et al.* 1996). Recently, Burt *et al.* (1999) looked at all of the available gene data on the chicken reference maps. By analyzing the total number of conserved segments between humans, mice and chickens, Burt *et al.* (1999) concluded that the organization the human genome is closer to that of the chicken genome than to the mouse genome. The work in this thesis will help to substantiate these findings as well as adding to the overall chicken-human comparative map.

Physical mapping:

Physical mapping is the construction of a genome map using large insert clones (e.g., Bacterial Artificial Chromosomes: BACs, Yeast Artificial Chromosomes: YACs) to ascertain the physical size of the chromosomes.

Additionally, these clones will serve as a source for a great deal of the sequence information in the genome. Physical mapping using large insert clone libraries has been applied successfully to a wide range of genomes (e.g., Hardy *et al.* 1986; Burmeister *et al.* 1988; Martin *et al.* 1993; Bent *et al.* 1994; Song *et al.* 1995; Van Houten *et al.* 1996; Mcdermid *et al.* 1996; Yoshimura et al. 1996; Lauer *et al.* 1997; DeLoukas *et al.* 1998). Initially, limits on resources available and the state of technology in general led most investigators to take a regional map-building approach focused on a single large genome segment (e.g., major histocompatibility locus, Abderrhim *et al.* 1994; Totaro *et al.* 1996) or chromosome (Chang *et al.* 1994; Kunz *et al.* 1994; Moir *et al.* 1994; Nagata *et al.* 1995; Smith *et al.* 1995; Soeda *et al.* 1995; Nagaraja *et al.* 1997). Cohen *et al.* (1993) were among the first to attempt the physical mapping of a large genome (human) all at once. This was based on fingerprint analysis of large human YAC clones. Fingerprinting is based on the analysis of banding patterns of large insert clones after cutting them with restriction enzymes. The digested clones are run on high-resolution polyacrylimide sequencing gels. The analysis is done with a computer and looks for common and overlapping bands (Zhang and Tao 1997; Chang et al. 1999; Tao *et al.* 1999). This approach, however, is complicated by the tendency of YAC inserts to rearrange and other difficulties in handling and mapping YAC clones. BAC clone inserts are generally smaller

(typically 100-300 kb) than observed in YAC libraries (up to about 1 Mb, on average), which means that many more clones must be analyzed to generate a complete map. However, BAC inserts are much more stable, and BAC DNA is comparatively easy to purify and fingerprint. Whole genome maps based on extensive BAC clone analysis have begun to appear (Marra *et al.* 1999; Mozo *et al.* 1999).

Recently, two chicken BAC clone libraries have been constructed at the Texas A&M BAC Center (Crooijmans *et al.* personal communication). The Crooijmans library consists of approximately 50,000 clones with an average insert size of 130 kb (about 5X coverage of the genome). The BAC library described in this thesis presently consists of about 38,000 clones with an average insert size of about 150 kb (ca. 5X coverage). Insert DNA fragments were derived from partial digestion with MboI and cloned into the BamHI site of pBeloBac11. Plans are underway to expand this library to about 80,000 clones including inserts derived by partial HindIII and EcoRI digests. Our BAC library has been constructed using DNA from a female of the inbred UCD001 JF line of chickens. Use of DNA from a UCD001 bird allows the possibility that dominant markers (e.g., AFLP and RAPD) previously identified in UCD001 birds may be applied in BAC analysis, if necessary.

Physical mapping: thesis focus

Originally, avian microchromosomes were considered genetically inert elements (Newcomer 1957; Ohno 1961; reviewed in Bitgood and Somes 1990). With continued study of the avian karyotype, it was found that there was a relatively constant number of these elements in most bird genomes. This led to the understanding that they were genuine chromosomes (Schmid 1962; Krishan 1964; Clement 1971). Additional studies showed that microchromosomes replicate, contain centromeres, and form meiotic bivalents (Kaelbling and Fechheimer 1983a, 1983b; Hutchison 1987; Bitgood and Shoffner 1990). Further study into their structure and recombination properties seem to indicate that microchromosomes may have some unique qualities.

There have been many studies on the composition of the microchromosomes. Data regarding the distribution of non-coding sequences in the chicken genome are of several types. C-banding studies have shown that heterochromatin is found on certain microchromosomes (Stefos and Arrighi 1974; Bulatova *et al.* 1977; Pollock and Fechheimer 1981; Belterman and De Boer 1984; Schmid and Guttenbach 1988; Rodionov *et al.* 1989), and clones showing a high proportion of repeated sequences have been isolated from microchromosomes (Matzke *et al.* 1992; Fillon *et al.* 1998). Therefore, there are non-coding regions found on microchromosomes. Additionally,

genetic markers based on non-coding repeat sequences such as microsatellites have been placed on microchromosomes (Cheng *et al.* 1995; Crooijamans *et al.* 1996). Primmer *et al.* (1997) demonstrated that, while microchromosomes contain microsatellite and other non-gene sequences, they appear to contain fewer than would be expected based on the genome content as a whole. They used Primed In Situ Labeling (Koch *et al.* 1989) with the $(CA)_{10}$ microsatellite on metaphase chicken chromosomes for this estimate.

Initial studies on chicken microchromosomes showed (by differential staining) that several microchromosomes are comprised of GC-rich R blocks (Rodionov 1985; Rodionov *et al.* 1989). FISH with probes enriched for CpG islands (CGIs) indicated that CGIs are enriched on chicken microchromosomes (McQueen *et al.* 1996). Increased acetylation of the amino-terminus of histone H4 is strongly correlated with the presence of genes (Turner 1993; Wade *et al.* 1997) Immunofluorescence with acetylated Histone H4 on metaphase spreads of chicken chromosomes, showed that the microchromosomes are enriched for acetylated Histone H4 (Mcqueen *et al.* 1998). Additionally, McQueen *et al.* (1998) demonstrated that microchromosomes replicate early in S phase, which is also associated with transcriptionally active DNA. By analyzing cosmids whose genomic origin was known, CGIs were approximately six times denser on microchromosomes (McQueen *et al.* 1998) McQueen *et al.* (1998) predicts that approximately 75% of chicken genes are located

on microchromosomes. Clark *et al.* (1999) sequenced 18 cosmids with known chromosomal origin and found an increase in gene density on microchromosome based cosmids, but their data was inconclusive for CGIs due to the small sample size. At present (Groenen *et al.* in press), there does not appear to be an unusually high density of genes located on microchromosomes, but since the choice of genes to map has not been random and since little is known of the physical length of microchromosomal DNA, this may not refute the McQueen *et al.* (1998) conclusion. Analysis of BACs comprising the physical map of a microchromosome on a sequence level should give some insight into its gene density as well.

Recombination rates on microchromosomes are also of interest. It was initially thought that crossover density in microchromosomes was less than macrochromosomes (Tegeldstrom and Ryttmann 1981; Slizinski 1964; Birshtein 1987), however, the opposite is now believed to occur. It is generally believed that chromosomes must have at least one or more cross-over events each (Carpenter 1994; Dutrillaux 1986; Kaback 1996) to insure proper meiotic segregation, and several studies have suggested that the microchromosomes also have about one chiasma per pair (Rahn and Solari 1986; Hutchinson 1987; Rodionov *et al.* 1992a, 1992b; Myakoshina and Rodionov 1994). Due to the small size of microchromosomes, if they indeed have at least one chiasma per meiosis, this would lead to unusually high recombination

frequencies per Mb of DNA. The macrochromosomes average about one crossover event per 30Mb (Rahn and Solari 1986; Rodionov *et al.* 1992a, 1992b; more recently, Groenen et al. in press, estimate the full length of the genome at 3800 cM, equivalent to 1 cM ≈ 32 Mb for a 1.2 Gb genome), and it has been estimated that microchromosomes should have one crossover event every 11-12Mb (Rodionov *et al.* 1992a). Thus, the ratio of genetic length to physical distance of microchromosomes should be about 3X that of macrochromosomes. The present consensus map (Groenen *et al.* in press) contains several linkage groups of length substantially below 100 cM (equivalent to one cross-over per chromosome per meiosis), but it is not known how completely any of these linkage groups covers the full length of DNA within the putative microchromosome they represent. In one case, chromosome 16, two small linkage groups are known to be on the same microchromosome separated by a recombination hot spot which is located at the nucleolar organizer region. Nor is the actual physical length of DNA represented by any particular linkage group/microchromosome known. Building a contiguous physical map across a microchromosome might shed some light on this question.

The large-scale project of building a genome-wide, BAC-based physical map of the chicken genome will be done through collaboration with the Texas A&M BAC center. The BAC research described in this thesis includes some preliminary characterization of the library and a test case use of the

library for regional physical mapping of linkage group E41. E41 has been identified as a microchromosome through FISH analysis (Sazanov, personal communication). Because of the small size of the microchromosomes (estimated at 1-10 Mb), it should be feasible to begin to construct a local physical map with relatively few (10-100) BAC inserts.

Some regional physical maps have been based on enriched libraries constructed with DNA from a single chromosome or chromosomal region (using flow sorting, microdissection, or somatic cell hybrid-based procedures). For the most part, these resources are not available, at present, for the chicken. The alternative approach of screening a full genome library with markers previously localized to the genetic linkage group in question has been employed (Figure 1, markers on E41). Restriction enzyme digestion patterns (fingerprints) of BAC inserts and cross-hybridization can be used to identify overlapping clones and build local clusters (called contigs) of such overlapping clones that contain the marker/gene used in screening the library. Given the present density of genetic markers in the chicken map (~2000 markers spanning 3,800 cM, Groenen et al. in press), rarely will it be the case that the contig containing one such marker will overlap with that containing the nearest available marker on the map. Gaps need to be filled either by increasing the density of useful genetic markers in the E41 genetic map and/or expanding contigs by "chromosome walking". Chromosome walking involves generation of new hybridization

probes from the ends of existing contigs (or isolated clones), followed by use of such probes to rescreen the BAC library. Each such "step" should extend the contig in question by about the length of a typical BAC insert (ca. 100-200 kb). The process can then be repeated to (slowly) fill in existing gaps. (Unless at least two genetic markers have already been placed relative to one another within a given contig, one must walk from both ends because the orientation of the contig to the genetic map is unknown.) In general, chromosome walking is too laborious for large-scale physical mapping, and it is mainly used to fill known gaps. Therefore, we have chosen to focus on a relatively densely mapped microchromosome to minimize the need for walking. The E41 test case will help to estimate the viability of such strategies for the chicken genome and our BAC library.

As noted above, it is most reasonable to choose a microchromosome with dense marker coverage as a test case for regional physical map building using BACs. Linkage group E41 has 21 markers covering approximately 70cM (Figure 1). This includes 7 genes and 13 microsatellite and AFLP markers, which are the types of markers most easily mapped to BACs. The decision to use microchromosome E41 was also based on the location of a Marek's Disease (MD) resistance Quantitative Trait Locus (QTL) on E41. MD is lymphproliferative disease that continues to be a significant health and financial problem for the poultry

industry (Purchase 1985). There is a continuing effort in the research community to improve the genetics of chickens to help combat this disease. One such approach has been to identify QTLs responsible for MD resistance, with the ultimate goal of finding the actual genes. Vallejo *et al.* (1998) and Yonash *et al.* (1999) did a genome wide scan for MD QTL, where a thorough description of the methods and results of the MD QTL analysis can be found. The E41 MD QTL specifically relates to differences in MDV viremia between similarly infected line 6 (resistant) and line 7 (susceptible) birds. Although actually locating the gene encoding this QTL is out of the scope of this research project, making a start on the E41 physical map might speed progress by others towards this ultimate goal. As will be described in Chapter 2, comparative mapping places several orthologues of known E41 genes to the end of human chromosome 9q. Detailed sequence analysis of this region in the human genome may also assist in suggesting candidate genes for this QTL-encoding chicken gene. Lines 6 and 7 were also shown to segregate MD QTL alleles found on chicken chromosomes four and eight, which was a factor in our choice to enhance the comparative chicken-human genome map covering these regions.

This thesis describes the construction of a chicken-human comparative genome map over several selected regions. Statistical analysis of the resulting data has been used to estimate the average conserved segment length between the

human and chicken genomes. Microchromosome E41, which is an integral part of the comparative map for human chromosome 9, was the starting point for a preliminary analysis of physical clones from a newly constructed BAC library.

Figure 1. Markers on chicken microchromosome **E41**.

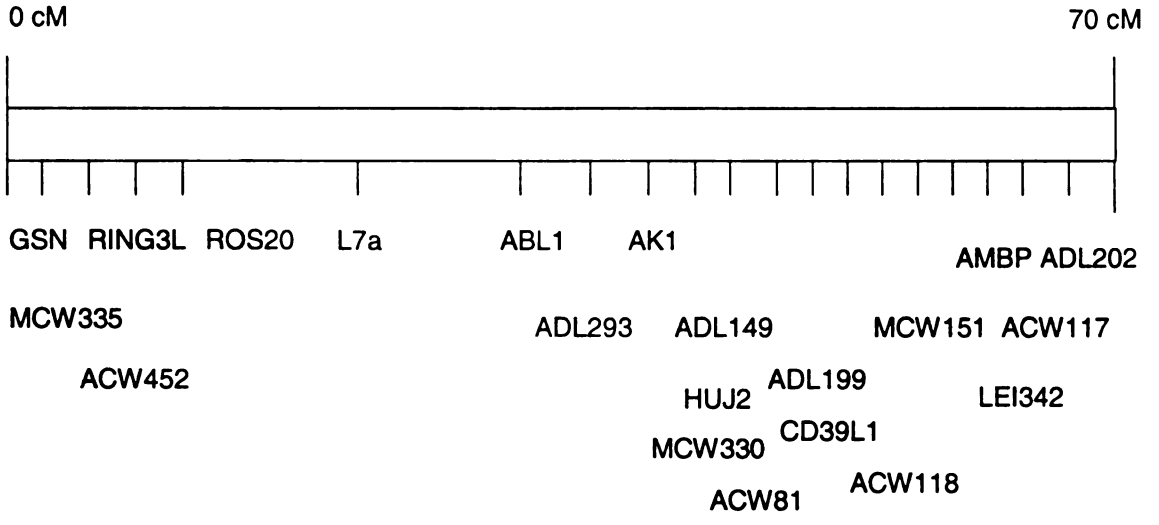


Figure 1. Markers on chicken microchromosome E41. Included are gene markers *GSN*, *RING3L*, *L7a*, *ABL1*, AK1, *CD39L1*, and *AMBP*, the rest of the markers are either microsatellite (10) or AFLP markers (4).

Chapter 2: Comparative Mapping of the Chicken Genome

INTRODUCTION

Recent work in our lab and others has shown that a robust avian-mammalian comparative map can be made (Smith *et al.* 1997; Groenen *et al.* 1999; O'Brien *et al.* 1999). Several large regions with conserved synteny and regions with conserved segments have been found. For the work in Chapter 2, the focus was placed on the human-chicken comparative map. The mouse genome appears to be relatively unstable (reviewed in Graves 1996; Carver and Stubbs 1997; O'Brien *et al.* 1999), which could limit its usefulness in an avian comparative map. Additionally, the human genome has by far the most comprehensive genome map. Although many regions of the chicken-human comparative map were added to in Chapter 2, we focused on a few select regions rather than seeking broad coverage. Since the comparative map of human chromosome (h-chr) 1 was the most complete, and an attempt was made to fill in some of the gaps to identify the extent of the conservation. Our initial work had identified a large region conserved between h-chr 4 and chicken chromosome (c-chr) 4, and an attempt was made to extend the chicken-human chromosome 4 map. Initial work by our group and others had identified a large region of conservation between h-chr 9 and the chicken Z sex chromosome (Smith *et al.* 1997; Fridolfsonn *et al.* 1998; Nanda *et al.* 1999). Two autosomal

sex-determining genes have recently been mapped to h-chr 9 and the c-chr Z (Nanda *et al.* 1999; Smith *et al.* 1999). In order to provide insight into the evolution of ZW-type chromosomal sex determination in birds, an effort was made to increase the comparative map between the chicken Z sex chromosome and human chromosomes 9. Additionally, c-chr 8, c-chr 4, and c-chr E41, which show a large degree of conservation with h-chr 1, h-chr 4, and h-chr 9 respectively, contain QTL for Marek's disease resistance in the chicken (Vallejo *et al.* 1999; Yonash *et al.* 1999). A comparative map in these areas may assist in identifying potential candidate genes for MD resistance. By focusing on relatively few regions, we hoped to get good coverage of these chromosomes. This was done in order to get a general idea of the number of chicken segments that would cover a human chromosome.

In the early 1980s, far less map data existed for both the mouse and human genomes. In order to analyze the amount of genomic conservation between the two species, Nadeau and Taylor (1984) derived a method to estimate overall genome conservation from a limited set of gene segment comparisons. When compared to data generated from a high-resolution human-mouse comparative map, their model generated an accurate prediction of average genome conservation (Copeland *et al.* 1993). Statistical analysis based on the work of Nadeau and Taylor (1984) was performed on the region specific comparative map data to derive an estimate of the

genome-wide conservation of gene order between chicken and humans.

MATERIALS AND METHODS

Determination of Orthologues:

Chicken cDNA sequences obtained either from National Center for Biological Information's Genbank database (NCBI: http://ncbi.nih.nlm.gov) or the University of Delaware (UDEL) cDNA library (Burnside and Morgan, http://udgenome.ags/chickenest/chick.htm) were compared to human gene sequences using the Basic Local Alignment Search Tool as provided by the NCBI web site (BLAST: http://www.ncbi.nlm.nih.gov/BLAST). Four main factors were used in determining the human orthologue to the chicken sequence. These are functional similarity, nucleotide (nt) sequence similarity, protein sequence similarity, and common chromosomal linkage relationships. Levels of nt identity were determined using the blastn program within BLAST and protein identity using the blastx program. Table 1 lists the *Gallus gallus* sequence and the percentage of nt and protein identities with the corresponding human genes. The comparison is made over the entire cDNA sequence. The nt identities range from 61%-94% and the protein identities range from 51%-99%. When there were multiple human genes that had high nucleotide and protein similarities, it was possible to distinguish the best candidate for the orthologue. For example, chicken skeletal muscle alpha-actinin cDNA (accession: X13874) has a nt identity of 80% and a predicted protein identity of 80%

with the human gene *ACTN3*. The two proteins also have a similar function. *ACTN2* has a nt identity of 83% and a protein identity of 95% (Table 1). *ACTN2* is linked to *ADPRT* on ch-chr 3 and *ACTN2* and *ADPRT* are closely linked on h-chr 1 (Figure 1), whereas *ACTN3* is located on h-chr 11. Thus, both sequence homology and linkage relationship supports the conclusion that the X13874 sequence is orthologous to human *ACTN2*. If there are two copies of the gene in humans, the nt identity was naturally very high for both copies with the respective chicken gene. This was the case with splicing factor arginine/serine-rich 2 (*SFSR2*). There is a copy of *SFSR2* on h-chr 4 and another on h-chr 17. *SFSR2* maps to linkage group E31 in chickens (Figure 9), along with two additional chromosome 17 syntenic loci, *FAS* and *H33B*. Therefore, the *SFSR2* found on E31 is mostly likely orthologous to the human gene on chromosome 17. All of the factors were taken into consideration when assigning chicken loci.

In some cases, the Unigene (Unigene: http://ncbi.nlm.nih.gov/UniGene) and Online Mendelian Inheritance in Man (OMIM: http://ncbi.nlm.nih.gov/omim) databases within Genbank were used to identify human genes in regions of interest followed by a search for an orthologous chicken cDNA in Genbank or the UDEL database. The Genbank searches were performed by using the human gene sequence and running a BLAST search against the *Gallus gallus* sequences in the database. The UDEL cDNA database has been BLASTed against

the entire Genbank database and positive genes are listed along with the corresponding percentage positive nts. The orthologous chicken sequence information was used to construct polymerase chain reaction (PCR) primers used to clone and sequence chicken genomic DNA from parental DNAs of our map population. For the Abelson murine leukemia viral oncogene homolog 1 (*ABL1*) chicken orthologue, primers from the Universal Mammalian Sequence Tagged Site (UMSTS) set (Venta *et al.* 1996) were used. Primers for the chicken gamma-carboxylglutamic acid protein, matrix (*MGP*) gene were from the Primer Pairs to Sequenced Chicken Genes (PPSCG) panel (appendix 2).

PCR Primer Design:

Where possible, PCR primers were chosen to amplify a large fraction of the 3' untranslated region (UTR) of the chicken gene of interest. When it was necessary to amplify predominantly coding regions, only PCR products larger than the predicted cDNA size were analyzed further, since these presumably include intron regions which are more likely to be polymorphic. Occasionally, when the available 3' UTR sequence was small, primers were designed to cover as much of the 3' UTR as possible and some coding region as well, in the hope that an intron would be included.

One problem that arose during the amplification of the gene products was that of product size. A nucleotide

polymorphism between the WL and JF parents is needed to genetically map the gene. The 3'UTR was chosen as the region to amplify in most of the genes. A nucleotide difference in a non-coding region may not have as great an effect as a difference would in a coding region and 3'UTRs should be less conserved over evolutionary time. Additionally, there are also fewer introns in 3' UTR. The product size between cDNA sequence and PCR products designed from the cDNA will more likely be the same. PCR products designed from within coding regions where there is no information about intron size or location can be problematic due to very large product size. An additional problem is that primers could be designed across intron boundaries. This can lead to the PCR product being too large to be cloned efficiently under normal conditions or no product at all. Both of the above problems were encountered where no 3' UTRs were available and primers were designed to cover coding regions. This was the case with the UDEL cDNA library where there are only partial cDNA sequences, occasionally the 3'UTR is just small in some genes, and in the PPSCG set of primers, which are designed within the coding region of the gene (Appendix 2). These large products led to a decrease in our success rate when these were the sources of our gene sequences.

PCR primers were designed with the PrimerSelect™ PCR Primer & Probe Design program within the Lasergene Biocomputing Software (DNASTAR Inc., Madison, WI) suite of

programs. Criteria used in the design included: similarity of melting temperature (Tm) between the two primers, predicted absence of primer dimers, and absence of hairpins. An attempt was made to keep primer size from 18 to 24 nt in length with around 50% GC content. In the hope that these primers could be used in multiplex PCR, the predicted Tm were all kept in the 55-60°C range. All primers were purchased from the Michigan State University MacroMolecular Structure Facility. All PCR and primer information is contained in Table 2.

Cloning and analysis of PCR products

PCR was performed using the conditions described in Table 3. The entire PCR reaction was run on 1% low melting temperature (LMT) agarose. When a single band was observed, the WL and JF bands were extracted and cloned into the TOPO-TA™ (Invitrogen Corporation, Carlsbad, CA) cloning vector using the Low-Melt Agarose Method for purification of PCR products as per the manufacturer's recommendations. Transformation into the One-Shot™ Chemically Competent cells (Invitrogen Corporation, Carlsbad, CA) was done according to the manufacturer's recommendations. The cells were plated on LB plus 50 µg/ml ampicillin (AMP) with 40 µl 40 mg/ml X-GAL per plate and incubated at 37°C for 16 to 18 h.

An additional test was performed on white colonies prior to sequencing to ensure they contained the product of

interest. Colonies were picked into 240 µl of LB plus AMP in individual wells of a flat bottomed 96 well plate (Cell Wells™, Corning Glass Works, Corning, NY). The plate was incubated at 37°C for 8-12 h. Four µl of the cells were placed into individual wells of a 96 well thin walled PCR plate (Thermowell™, Model M, Corning Glass Works, Corning, NY), covered by a drop of mineral oil, and heated to 94°C for five minutes to lyse the cells. The appropriate PCR mixture (23 µl) was added to the lysed cells and PCR was performed under the same conditions as for genomic PCR. The PCR reactions were then run on a 1% agarose gel to determine if they contained the same size insert as expected.

Plasmid DNA purification was done using the Qiaprep™ miniprep kit protocol (Qiagen Inc., Valencia, CA). Concentrations of purified plasmid DNAs were determined by fluorimetry (TKO Mini Fluorometer, Hoefer Scientific Instruments, San Francisco, CA). Three individual clones from both JF and WL genomic templates were sequenced using SP6 or M13 reverse primers and the T7 primer, using ABI 377 automated sequencers at either the Michigan State University Sequencing Facility or at the U.S.D.A. Avian Disease and Oncology Laboratory. Three clones were sequenced to insure that observed polymorphisms were unlikely to arise from PCR or sequencing errors.

Sequence analysis and genetic mapping:

BLAST analysis between cloned PCR products and chicken sequence data previously found in Genbank confirmed that the correct gene had been cloned. Greater than or equal to 99% identity in the known coding regions and 3'UTR was considered positive. Intron sequences were sometimes found in the cloned product that, of course, were absent from the earlier cDNA sequences, but the identity of introns could be confirmed by the presence of consensus intron boundary sequences (Keller and Noon 1984 Mariman *et al.* 1984). In order to control for sequencing errors, the alignment of the sequences from the cloned plasmids was performed using the Seqman™ Sequence Assembly and Contig Management program within the Lasergene Biocomputing Software (DNASTAR Inc., Madison, WI) suite of programs. The alignment of the sequences into contigs makes it possible to distinguish true SNPs and sequence differences due to errors in sequencing or PCR induced sequence errors. Alignment of successfully sequenced plasmid clones was done under the manufacturer's recommended parameters for contig assembly (Seqman™, DNASTAR Inc., Madison, WI). Alignment of the sequences into contigs allowed for the identification and placement of any SNP between WL and JF.

For mapping in the reference BC population, polymerase amplification of specific alleles (PASA) primers were designed based on the polymorphic nt alteration (usually a SNP) such that only the JF allele successfully amplified. PASA primers were designed to minimize the possibility of hairpin or dimer formation. If there were multiple JF vs. WL polymorphisms, the one giving rise to the predicted optimal allele-specific primer (ASP) was used. Either the forward or the reverse primer from the original PCR amplification was chosen as the other primer, based on best fit with the ASP. ASP were generally designed with the JF-specific nt at the 3' end and an additional mismatch to both the WL and JF sequence, three nt from the 3' end. As demonstrated by Okimoto and Dodgson (1996), the additional mismatch provides increased specificity and accuracy in genotyping. Occasionally, additional changes were made to adjust the Tm or to avoid predicted hairpins and/or dimer formation. In one case, *TNNT2*, there were multiple SNP available, and two opposing ASP were found to be necessary for genotype analysis (Table 2). All the PASA PCR primer information is provided in Table 2. PASA PCR genotyping was performed in duplicate on the 52 animals of the reference BC population (Crittenden *et al.* 1993). PCR products were run on 1% or 2% agarose gels and absence or presence of the JF allele was determined (Figure 1, Appendix 3). Segregation data were analyzed using MAPMANAGER version 2.6.5 (Manly, K., Roswell Park Institute, Buffalo, NY). The correct map positions were

determined using the following criteria: within the strain distribution patterns, the position with the least number of crossovers and with minimal double recombinants that generated the highest possible $\log_{10}$ of odds (LOD) score. In order to be considered linked to other markers the LOD score had to be greater the 3.0.

RESULTS

In order to generate a preliminary view of chromosomal evolution between birds and mammals, we chose to focus on a representative subset of the vertebrate genome, those genes contained on h-chr 1,4 and 9. These regions initially were targeted due to the fact that preliminary evidence suggested that they may contain QTL-encoding genes for resistance/susceptibility to Marek's Disease Virus as mapped by Vallejo *et al.* (1998) and Yonash *et al.* (1999). Subsequently we chose to map as many chicken orthologues as possible of the human genes already known to map to these regions. We believe that these observations can be extrapolated to derive conclusions about the overall comparative chicken-human genome map.

Figure 1 is a graphical representation of the comparative map of h-chr 1 and the corresponding segments of c-chr (or linkage groups, where a specific c-chr has yet to be identified). Table 4 lists the genes mapped in this study that provide comparative map coverage of h-chr 1. The source of the chicken cDNA sequences is also listed for each chicken gene (either Genbank or UDEL). As outlined in Materials and Methods, chicken gene sequence information was used to design PCR primers for amplification, cloning, and sequence analysis of selected gene segments from parental DNAs of the East Lansing reference mapping family (Crittenden *et al.* 1993). When sequence polymorphism was

observed between the WL(UCD003) and JF(UCD001) alleles, PCR-based assays were developed with which to genotype the standard reference gene mapping panel, thereby locating the chicken orthologue on the East Lansing reference map (http://poultry.mph.msu.edu) and the consensus chicken gene map (Groenen *et al.*, in press). The map position on the EL reference map is listed in Table 4 along with the human physical map position from OMIM (OMIM: http://ncbi.nlm.nih.gov/OMIM). The human genetic map information from Unigene (Unigene: http://ncbi.nlm.nih.gov/UniGene) tends to be more accurate than the physical map information (chromosomal placement is more precise). Because of this, there are a few discrepancies between the tables and figures. This was done when the physical map position covered a large range, such as *XPA*, the physical position is 9q22.3-q31, but the genetic map information more accurately places *XPA* near 9q22. Bold and underlined genes were mapped in the current study. Six segments of the chicken genome provide almost complete coverage of h-chr 1, with a few gaps not covered by corresponding chicken segments. Four chicken genome segments contain three or more genes whose orthologues map to h-chr 1. Two of these are linkage groups E54 (telomeric end of 1p) and E04 (1q31-q32.1). It is likely, but not certain that these linkage groups correspond to chicken microchromosomes. An internal segment of c-chr 3 appears to correspond to the telomeric end of 1q. C-chr 8 shows conservation to both the p and q arms of h-chr 1. *RPL5* has only been mapped on the

Compton reference population (Compton and Palyga 1992) and its precise location among the other markers is not known. Between these two conserved segments are two genes on h-chr 1 that map to a segment of c-chr 1 (*HSD3B*) and to E26 (*MCL1*), respectively. *HSD3B* is in a region of h-chr 1 for which we have no nearby marker information and *MCL1* is the only gene mapped to E26. Thus, further comparative mapping will be required to ascertain whether these two associations are part of large conserved segments, derive from small translocations (e.g., transposon-mediated rearrangements), or result from mistaken assignment of orthology. However, that the largest h-chr (approximately 300cM) appears to correspond to as few as 4-8 chicken genome segments is noteworthy, as is the fact that relative gene order is almost completely conserved (i.e., lack of evidence for inversions).

Figure 2 shows the location of chicken orthologues of genes on h-chr 9, with further information provided in Table 5. Conventions and methods used are as described above for Figure 1 and Table 4. In addition, one of these genes, the *ABL1* proto-oncogene, was amplified using UMSTS primers (Venta *et al.* 1996; Smith *et al.* 1997). Figure 2 demonstrates that much of h-chr 9 derives from segments that correspond to the chicken Z chromosome and the probable microchromosome E41, the latter corresponding to the telomeric end of human 9q. However, the chicken Z chromosome segment also contains at least four genes that do

not map to h-chr 9, and the human segment in question contains a single gene (*ALDH1*) which maps to the E18 linkage group. Again, further comparative map data will be required to elucidate the relevance of these single gene homologies. In addition, the h-chr 9-chicken Z chromosome segment exhibits two internal alterations in gene segment order (the *TPM2* gene and the *CTSL* to *XPA* segment). These could be due to inversions (intrachromosomal) within one large conserved segment or to independent translocations (interchromosomal) between the same pair of ancestral chromosomes. The independent origin of the avian sex chromosomes as opposed to their mammalian counterparts has been noted previously by others (Fridolffson *et al*. 1998; Nanda *et al*. 1999), and in some cases, rearrangements appear more common on sex chromosomes than autosomes. However, this trend is most striking on the sex chromosome that is mostly non-coding, i.e., the avian W and mammalian Y chromosomes. E41 is a microchromosome (Sazanov, personal communication). All seven genes mapped have the same gene order as on h-chr 9. It appears that most small linkage groups have been well conserved, for example E54 and E04 (Figure 1), although this is not always the case (E29, Figure 3; E52, Figure 4).

Table 6 and Figure 3 show the positions of chicken orthologues of genes on h-chr 4. A large section of c-chr 4 is conserved with the q arm of h-chr 4. Assuming that *EDNRA*, *SPP1*, *ALB-GC*, *PPAT*, and *NFKB1* are placed accurately, there appear to have been at least two inversions or three

independent translocation events in either the avian or mammalian line since the last common ancestral genome. The *FGFR3* gene at the distal end of h-chr 4p is also on c-chr 4, but this gene is quite distant from the segment previously described and is separated from it by at least two genes that map elsewhere in the chicken, so the synteny of *FGFR3* and the segment is likely to be fortuitous. Unfortunately, we have not been able to map chicken orthologues of genes at the most telomeric end of h-chr 4q.

In the early stages of this study and in the course of trying to extend or define conserved segments described above, several other genes were added to the overall chicken-human comparative map. These are summarized in Table 7 and Figures 4 through 9. Although we did not add more than one or two new genes to each of the relevant chromosomes or linkage groups, in several cases, our observations extended conserved segments observed by other laboratories (Fridolfsson *et al.* 1998, Nanda *et al.* 1999, Groenen *et al.* 1999).

Rate of Chromosomal Evolution:

Nadeau and Taylor (1984) calculated the expected lengths of conserved segments between the human and mouse genomes using thirteen homologous segments known at that time. As noted previously, the Nadeau and Taylor predictions in 1984 turned out to be surprisingly robust. Thus, we applied the Nadeau and Taylor theory to 19 conserved

segments between humans and chicken (Table 8). Table 8 lists the chromosomal location of the chicken genes and the corresponding location on the human genome. The majority of the conserved segments were found or added to in this study. Additional groups (such as *DNECL-CKB* and *CRYB-IGVPS-MIFL2*) were found by searching the chicken genome database in Arkdb-CHICK (http://www.ri.bbsrc.ak.uk/chickmap) for gene clusters that formed conserved segments with the human genome.

The mean of the expected segment lengths (mean m = 67) is transformed to account for segments lacking identified genes and conserved segments with single markers. The mean length would be biased toward longer segments since only those with two or more genes are included. The complete mathematical transformation is discussed in Nadeau and Taylor (1984). Their final equation is:

$E(x') = (L^2D + 3L)/(LD+1)$

where E(x') is the mean of the transformed lengths (67.4), and D is the total number of mapped homologous loci (~150 consensus map) (Groenen *et al.* in press) divided by the genome size (3,800 cM, Groenen et al. in press). The mean length of conserved segments between humans and chicken (using the data from Table 8) is 38 ± 9 cM.

The rate of chromosomal evolution between humans and chickens can also be calculated based on the model of Nadeau and Taylor (1984). This first step is to calculate the number of disruptions that have accumulated during the

evolutionary divergence of chickens and humans. The formula of Nadeau and Taylor (1984) is:

$R = (G/L) - N_0$

R is the number of disruptions, G is the genome length and N_0 is the total number of haploid chromosomes in the last common ancestor. The true N_0 is not known; therefore, the lower haploid number of the compared species (23) was used (O'Brien *et al.* 1999). (Reasonable values of N_0 have little effect on our final conclusions.) Using the value of L as 38, $R = 77 \pm 24$. The average rate of reciprocal disruption is R divided by twice the estimated time to the last common ancestor (300 myr, Kumar and Hedges 1998) to account for disruptions in both species or about 0.13 ± 0.04 disruptions per myr.

Discussion

Comparative map:

One of the goals of this project was to test whether it would be feasible to build an avian-mammalian comparative genome map. Our initial results and those of others (Klein *et al.* 1996; Smith *et al.* 1997; Fridolfsson *et al.* 1998; Groenen *et al.* 1999) showed that there were surprisingly large conserved segments between the human and chicken genomes. While a complete comparative map for these two species was beyond the scope of the present project, a more limited analysis focusing on human chromosomes 1, 4 and 9 was performed. Our results suggest that there will typically be between four to eight chicken segments per human chromosome, so the long-term goal of a complete comparative map between chicken and mammalian genomes is feasible. Two preliminary genome-wide comparative maps, based on some of the data reported herein plus that available from other labs, have recently been described (Burt *et al.* 1999; Groenen *et al.* in press). There is now general agreement that the chicken genome can be even more closely aligned with the human genome than can that of the mouse (Burt *et al.* 1997; Burt *et al.* 1999; O'Brien *et al.* 1999; Groenen *et al.* in press).

The level of similarity between the human and chicken genomes is especially remarkable, given the fact that the

former contains almost three times as much DNA as the latter. As can be seen in Figures 1 and 2, as yet there is no evidence for large, chromosome-sized segments of human DNA that contain no obvious chicken orthologues. If this is confirmed in more detailed comparative maps, one must conclude that the "excess" human DNA is mostly interspersed. Indeed, based on anecdotal evidence, it was observed long ago that chicken gene families tended to be more closely packed, and have smaller introns and fewer pseudogenes than their mammalian counterparts (Dodgson *et al.* 1979). Thus, it seems likely that a very large number of small deletions from the mammalian genome and/or insertions into the chicken genome have occurred during their separate evolution without significantly affecting the larger scale gene order. Thus, while at the level of DNA sequence the smallest evolutionarily conserved segments between the human and chicken genomes are likely to be rather small (probably on the order of a typical exon or about 1 kb), at the level of gene order, the average conserved segment appears to be 30-40 cM (ca. 10 Mb of chicken DNA and 30 Mb of human DNA). Thus, the mechanisms by which small deletion/insertion events occur (replication errors, transposable elements, unequal recombination, etc.) must be very distinct from large scale chromosomal rearrangements. A similar situation exists for several plant genome comparisons, for example, corn vs. rice (Gale and Devos 1998).

Microchromosomes:

One problem in assembling maps of the chicken genome has been the fact that chicken microchromosomes are not cytologically distinct (other than chromosome 16 which contains the NOR). However, with improved genetic maps (Groenen *et al.* in press) and preliminary fluorescent in situ hybridization experiments (Fillon et al. 1998), there has been some progress in categorizing microchromosomes. Identification of 16 chicken microchromosomes by molecular markers using two-color fluorescence *in situ* hybridization (FISH). Fillon *et al.* (1998) confirm that most of the undefined linkage groups in the EL reference map correspond to microchromosomes. Many presumptive microchromosomes, e.g., E41, appear to be conserved as a single block in the human genome. However, most of them do not contain enough cross-mapped genes to be confident of this conclusion. On the face of it, it is not surprising that microchromosomal segments survive intact, given that many of them may not be much larger than the average conserved segment length of 38 cM. On the other hand, microchromosomes have been proposed to be rich in both genes and recombination events compared to the autosomes (Rodionov 1996, 1997; Primmer *et al.* 1997; Sazanov *et al.* 1996; Fillon 1998). It remains unclear as to how one might reconcile differential gene density between micro and macrochromosomes with a high level of conservation of gene order with the human genome, where, to the best of

our knowledge, no such gene density distinction exists. Perhaps the density of internal insertion/deletion events discussed above (which generally appear to have little effect on gene order), may have been substantially different in genome segments which are microchromosomal in chickens vs. macrochromosomal.

Microchromosome E41 is of special interest and will be discussed further in chapter 3. It contains a suggestive QTL for MDV viremia levels (marker ADL0149 has a LOD = 2.5 with the QTL; Vallejo *et al.* 1998; Yonash *et al.* 1999). The Major Histocompatibility Complex (MHC, called the B complex in chickens) of genes on chromosome 16 is known to play an important role in MD infection and severity of disease (Bacon 1987). The Ring3-Like gene, which has been mapped to E41, is found near the qter end of h-chr 9 in band 34. Ring3 is a gene in the MHC class II region on chromosome 6, but there has been a second similar copy mapped to 9q34 (Thorpe *et al.* 1996). Based on its high protein and nucleotide similarity and its conserved linkage, it is highly probable that *RING3L* is on E41 and it was so designated in Figure 2. Several other MHC-related genes have also been mapped near *RING3L* on h-chr 9q, including Proteasome Subunit, Beta-Type, 7, *PSMB7*; Pre-B-Cell Leukemia Transcription Factor 3, *PBX3*; and Homolog of Drosophila Notch 1, *NOTCH1*. It seems likely that a similar group of the chicken orthologues of these genes will be found on E41, and they could serve as potential candidate genes for the MDV viremia-encoding QTL

allele(s). This is a preliminary, but illustrative, example of how the comparative human-chicken genome map can aid in the search for genes encoding chicken traits of interest.

Relevance of the Nadeau and Taylor Model to the Chicken-Human Comparative Map:

The original estimate of mouse vs. human average conserved segment length made by Nadeau and Taylor (1984) was 8.1 cM. Copeland *et al.* (1993) later calculated the average to be 8.8 cM, and O'Brien *et al.* (1999) estimates 8.1 cM in a review of several published reports. Thus, at least in the case of mouse vs. human, the model appears very robust. Still, there are many assumptions made in the model that need consideration. The first is that synteny between two markers in both species is presumptive evidence for conserved linkage. Evidence from many species (reviewed in Nadeau and Sankoff 1998 and O'Brien *et al.* 1999) generally supports this assumption, at least within mammals. The number of apparent conserved segments with several common markers, often in the same order (Figures 1-9; Burt *et al.* 1999; Groenen *et al.* in press), also supports the validity of the assumption when comparing chicken and human genomes, although probable exceptions (e.g., *FGFR3*, Figure 3) exist at low frequency. Second, the model assumes that chromosomal rearrangements fixed during evolution are randomly distributed throughout the genome. Although it is

well known that recombination rate is not uniform, this assumption is probably adequate for the calculation of mean conserved segment length at the level of resolution of presently available data. The model also assumes that orthologous markers are randomly distributed throughout the two genomes of interest. This assumption is important because the initial calculation of the expected value of r (r= the actual length in cM of the conserved segment, m= the expected value of r) is determined by calculating the expected range of a random sample taken from a uniform distribution. In this case, the random sample will be the mapped markers from the chicken map. An account is made for the bias toward long segments by assuming the frequency of segments containing two or more markers will follow a truncated Poisson distribution. A plot of the normalized cumulative distributions of the frequency of increasing adjusted segment sizes is illustrated in Figure 10. Included are curves for L = 5, 20, 30, 40, 56, and 75, as well as the cumulative distribution of the transformed segment lengths from this study. It appears that for the larger segment sizes the model fits quite well, (L > 50 cM) with the best fit around L = 40 cM, as calculated above. The smaller transformed segment lengths do not follow the same curve, tending to be smaller than would be expected. There could be several reasons for this, both technical and biological.

Technical errors could include sampling error (less than complete coverage and non-random selection of some

markers), errors in assessment of orthology or errors in the genetic map itself. Non-random marker placement could lead to an increase in the number of segments relatively small in size. In the current study, an attempt was made to cover certain human chromosomes but not to focus on a small area of interest, but this may not be true for all markers used in the analysis. In an attempt to increase the number of markers and to increase the density of the comparative map in a certain chromosomal area containing a gene of interest (such as a QTL), genes mapped by others may have focused on a narrow chromosomal region.

Although the limited sequence analysis of many chicken gene family members could create possible mistakes in assigning orthologous genes, most gene family members which show high sequence homology tend to be closely linked in the genome, in which case such an error would have no impact on the comparative map. Mapping errors are more likely in the chicken map, most of which is based on only 52 meioses. These would be most likely to alter the internal gene order within a conserved gene segment, thereby leading to a mistaken estimate of an inversion event. If a gene has been erroneously included as part of a conserved segment, this would lead to overestimation in the size of the conserved segment.

There are also possible biological explanations for the higher than expected proportion of short segments. First, it has been proposed that both recombination rate and gene

density on microchromosomes are abnormally high (Rodionov 1996, 1997; Primmer *et al*. 1997; reviewed in Fillon 1998). Although neither of these assertions has yet been proven by physical genome mapping or sequencing, either or both phenomena could contribute to the biphasic distribution seen in Figure 10. Second, chromosome rearrangements presumably involve multiple mechanisms, for example, intrachromosomal inversions, interchromosomal translocations, movement of internal segments via flanking transposable elements, etc. It seems unlikely that these different mechanisms would produce similar spectra of segment sizes. The effect of diversity in recombinational mechanism may be more apparent in the distant comparison of avian vs. mammalian genomes than it was in comparing mouse and human genomes.

Estimated Rate of Autosome Evolution:

Application of the Nadeau and Taylor (1984) model led us to estimate the average chromosomal evolution rate that separates the chicken and human genomes to be 0.13 ± 0.04 disruptions per myr. It has become increasingly clear that chromosomal evolution rate varies considerably in different evolutionary lines ranging from about 0.01 to >2.0 disruptions per myr (e.g., Bickham 1981; Nadeau and Taylor 1984; Paterson *et al*. 1996; O'Brien *et al*. 1999). It should be pointed out that the low end of this range (in turtle species, Bickham 1981) was based on karyotypic analysis of

banded chromosomes only and is likely an underestimate. Our estimate of 0.13 disruption/myr is similar to the estimates of O'Brien *et al.* (1999) for the most stable mammalian genomes (e.g., human, feline) relative to the common ancestral mammalian genome. This suggests that a similar rate of chromosomal evolution has been maintained in the lines leading to both the human and chicken genome from their last common ancestor. As noted by Rodionov (1996), karyotype analysis suggests a high level of genome stability within birds in general and thus, by extrapolation, within the line leading to modern chickens from the common mammalian-avian ancestor. Our comparative genetic mapping results confirm this conclusion.

Sex Chromosome Evolution:

In birds, the heterogametic sex is the female (ZW)and the homogametic sex is the male (ZZ). Very little is known about ZW sex determination in birds. Figure 2 demonstrates that a surprising number of chicken orthologues of genes on h-chr 9 were mapped to the Z chromosome. Previously, a few chromosome 9 genes had been mapped to the Z chromosome by our group and others (Smith *et al.* 1997; Fridolsson *et al.* 1998; Nanda *et al.* 1999), but the extent of conservation was unknown. The current theory of mammalian and avian sex chromosome evolution maintains that the respective sex chromosomes evolved independently from different autosomes

within the two evolutionary lines (Ohno 1966; Watson *et al.* 1991; Reed and Graves 1993; reviewed in Marin and Baker 1998). The genes mapped on the Z chromosome and chromosome 4 appear to fit this model (Figures 2 and 6).

As is expected, sex-controlling genes are found on avian sex chromosomes and sex reversal has been reported for different triploid arrangements in chickens (reviewed in Thorne and Sheldon 1992). The sex-determining gene SRY has been mapped in humans to the human Y chromosome (Sinclair *et al.* 1990). Sex reversal phenotypes can arise from chromosomal abnormalities on several autosomes as well as on the sex chromosomes in mammals (reviewed in Wachel 1987; reviewed in Reed and Graves 1993). One case of particular interest is XY chromosomal males that have a female phenotype and which exhibit a 9pter deletion (Raymond *et al.* 1998; Fleijter *et al.* 1998; Guioli *et al.* 1998). The phenotypes associated with this abnormality range from ambiguous genitalia to complete gonadal dysgenesis. The human genes *DMRT1* and *DMRT2* have been mapped to the minimal region contained in the deletion (Raymond *et al.* 1998, 1999). These genes were isolated due to their homology to the male regulatory genes *doublesex* in *Drosophila* and *mab-3* in *Caenorhabditis elegans*. Genetic analysis in the humans has shown that *DMRT1* and/or *DMRT2* may operate in a dose-dependent fashion in the male sex-determination pathway (Raymond *et al.* 1999). Recently the chicken gene *DMRT1*

has been mapped through FISH to the chicken Z chromosome at the p21 position (Nanda et al. 1999) Additionally, chickens have been shown to have gonadal specific expression of *DMRT1* (as does the mouse) (Smith *et al.* 1999). Two genes in the 9pter region (*VLDLR* and *TYRP1*) were mapped to the Z chromosome (Figure 2). The *DMRT1* and *DMRT2* genes lie within the microsatellite markers D9S129 and D9S143 on the pter region of h-chr9 segment (the interval is 1.9cM) (Raymond *et al.* 1998, 1999; Fleijter *et al.* 1998; Guioli *et al.* 1998). *VLDLR* is near the p telomere of chromosome 9 within the interval defined by D9S129 and D9S143 and *TYRP1* is about 25 cM down from *VLDLR*. The farthest *VLDLR* could be from *DMRT1* and *DMRT2* in humans would be 4.2 cM. Based on the formula from Nadeau and Taylor (1984) for calculating the probability of linkage based on the estimated mean conserved length (Probability = $e^{x/L}$, where x = 4.2 cM and L = 37.5 cM), there is a 90% probability that these loci are this closely linked to VLDLR on the Z chromosome. Therefore, it appears that this entire ancient sex-determining region has remained as a conserved segment between humans and birds.

Table 1.

Gallus gallus sequence:	Genbank accession or UDEL cDNA #:	Human loci:	Nucleotide identities*:	Protein identities**:
collapsin response mediator protein CRMP-62	U17277	CRMP1	79%	97%
PR264	X62446	SFRS2	83%	99%
endothelin type A receptor	AF040634	EDNRA	87%	80%
trans Golgi network protease furin	Z68093	PACE	84%	81%
Caspase-1	AF031351	CASP1	61%	49%
villin	J03781	VIL	84%	71%
NF-kappaB p50 precursor	M86930	NFKB1	85%	71%
preproalbumin	X60688	ALB	91%	61%
n-calpain-1 large subunit	D38028	CAPN1	71%	80%
poly(ADP-ribose) polymerase	X52690	ADPRT	79%	79%
tyrosine kinase	M35195	FGFR3	82%	82%
alpha-tubulin	V00388	TUBAL1	85%	98%
stem cell factor	D13516	MGF	90%	51%
homogenin	AF042795	GSN	83%	79%
ABL proto-oncogene	U66284	ABL1	87%	98%
aldehyde dehydrogenase	X58869	ALDH	81%	91%

Table 1. Cont.

Gallus gallus sequence:	Genbank accession or UDEL cDNA #:	Human loci:	nucleotide identities*:	protein identities**:
tyrosinase-related protein-1 precursor	AF003631	TYRP1	82%	82%
skeletal muscle alpha-actinin	X13874	ACTN2	83%	95%
axonin-1	X63101	TAX1	82%	75%
glutamine synthetase	S45408	GLUL	79%	88%
troponin T form I	M10013	TNNT2	83%	77%
prostaglandin G/H synthase	M64990	PTGS2	81%	82%
xpacch	D31896	XPA	81%	72%
cytosolic phospholipase A2	U10329	PLA2G4	80%	83%
lysyl hydroxylase	M59183	PLOD	80%	77%
trkB	X74109	NTRK2	85%	77%
pepsinogen	D00215	CTSE	87%	62%
smooth-muscle alpha-tropomyosin	K02446	TPM2	87%	95%
RPK-2	D14460	TGFBR1	85%	92%
glutamine phosphoribosylpyrophosphate amidotransferase	M60069	PPAT	80%	83%
VLDL/vitellogenin receptor	X80207	VLDLR	83%	83%
matrix GLA protein	Y13903	MGP	71%	61%

Table 1. Cont.

Gallus gallus sequence:	Genbnk accession or UDEL cDNA #.	Human loci:	nucleotide identities*:	protein identities**:
UDEL cDNA	pk0033.h4	RING3L	83%	81%
UDEL cDNA	pk0061.c12	JAK1	79%	89%
UDEL cDNA	pk0012.d1	UBE2A	89%	99%
UDEL cDNA	pk0006.b2	CTSL	79%	71%
UDEL cDNA	pk0031.e6	MCL1	83%	61%
UDEL cDNA	pk0049.f6	GC	85%	66%

Table 1. *Gallus gallus* gene sequences and the percentage nt and protein identity with the corresponding human gene.
*Percentage nucleotide identity obtained through a blastn comparison.
** Percentage protein identity obtained through a blastx comparison.

Table 2.

Genes Mapped:
Primer and PCR Information

Janus Kinase 1 (JAK1)	product size: 800bp	
	annealing temperature: 59°C	
	upper primer:	lower primer:
	5' TCG AAA AAG TGA ACT CCT GAC AAC 3'	5' GAT TCG CTC CAC GCA TTC TT 3'
JF specific - PASA	product size: 140bp	
	annealing temperature: 57°C	
	primer: use with lower primer	
	5' TGG ACA AAT ACT TCG GCT ACA 3'	
Ubiquitin-Conjugating Enzyme E2A (UBE2A)	product size: ~1kb	
	annealing temperature: 59°C	
	upper primer:	lower primer:
	5' ATC CAA ATA AGC CAC CTA CTG 3'	5' CAA CAA TCA CGC CAA CTC T 3'
JF specific - PASA	product size: 250bp	
	annealing temperature: 57°C	
	primer: use with upper primer	
	5' TTC TGC CCC CTT ACT AAA C 3'	

Table 2. Cont.

Gamma-Carboxyglutamic Acid Protein, Matrix (MGP)	product size: >2kb	
	annealing temperature: 59°C	
	upper primer:	lower primer:
	5' TGC GTG CTC TCA TCG TCC T 3'	5' CTC CTC CCA AAA TAG TGC CTG TAA 3'
JF specific - PASA	product size: 170bp annealing temperature: 57°C primer: use with lower primer 5' CAT AGA CAG ATA TTT AAG ATA CCA 3'	
Troponin T 2 (TNNT2)	product size: 500bp	
	annealing temperature: 59°C	
	upper primer:	lower primer:
	5' AAC GGA GCG GGA GAA GAA GAA AAA 3'	5' ATG TGG GGG TGT GGA GAT GAG AAT 3'
JF specific- PASA	product size: 80bp annealing temperature: 57°C	
	upper primer:	lower primer:
	5' GGC TCT GCT GCC TCC CCA ACG 3'	5' GCT GAG CAC CTG CCC ACC ACA 3'

Table 2. Cont.

Very Low Density Lipoprotein Receptor (VLDR)	product size: 900bp annealing temperature: 59^{0}C upper primer: 5' GCT TGG GCT GTT CTT CCT ATC T 3'	lower primer: 5' TAT CAT CCC CGT AAG TGT AAA AC 3'
JF specific- PASA	product size: 360bp annealing temperature: 57°C primer: use with upper primer 5' AAA GTC ACT TGG CAG GTC TTC G 3'	
Gelsolin (GSN)	product size: ~1.5kb annealing temperature: 59°C upper primer: 5' GGA GCT CGC CCA GTA CAG GTT TC 3'	lower primer: 5' GGG CAT CTT TTC CAA TCC ATA CA 3'
JF specific - PASA	product size: 210bp annealing tmperature: 57°C primer: use with lower primer 5' AAG CTT CCT GTC ATC ACC ACT A 3'	

Table 2. Cont.

Ring3-Like Gene (RING3L)	product size: ~1kb	
	annealing temperature: 59°C	
	upper primer:	lower primer:
	5' TAG TTA TGT TCC AGG CGT TTC TTG 3'	5' CAT CAG TTT GCT TGG CCT TTC TAC 3'
JF specific- PASA	product size: 220bp annealing temperature: 57°C primer: use with lower primer 5' ATC TCT CCA GCT CTG AAA AAC GAT 3'	
Collapsin Response Mediator Protein 1 (CRMP1)	product size: 2kb	
	annealing temperature: 59°C	
	upper primer:	lower primer:
	5' AAT CAC CAT CGC AAC CAA ACC AA 3'	5' CCC CGC AGG ACA GCA GTG AGT 3'
JF specific - PASA	product size: 300bp annealing temperature: 59°C primer: use with lower primer 5' TTG CTG CTC CAT GCT TTT ACC AGT 3'	

Table 2. Cont.

Transforming Growth Factor-Beta Receptor, Type 1 (TGFBR1)	product size: 500bp	
	annealing temperature: 52°C	
	upper primer:	lower primer:
	5' CAG AGT GGC GTG TTA AGA AGG TT 3'	5' TCC CCA CTA CTG AAT GAG GTC 3'
JF specific- PASA	product size: 80bp annealing temperature: 51°C primer: use with lower primer 5' TGT TGG AGT ATG CTT TGC GAG 3'	
Splicing Factor, Arginine/Serine-Rich, 2 (SFRS2)	product size: 500bp	
	annealing temperature: 59°C	
	upper primer:	lower primer:
	5' CTA CGG GAG CAG CGG TTA CG 3'	5' TGG AGA CAG ACG AGG ACT TTG ACT 3'
JF specific- PASA	product size: 180bp annealing temperature: 57°C primer: use with upper primer 5' GCT AAG GCT GCT GGG GAG AG 3'	

Table 2. Cont.

Tyrosinase-Related Protein 1 (TYRP1)	product size: 335bp annealing temperature: 59°C upper primer: 5' AAT ACA ACA TGG TGC CTT TCT 3' lower primer: 5' TGC CAT CTC TTC ATA CGA CA 3'
JF specific- PASA	product size: 250bp annealing temperature: 57°C primer: use with upper primer 5' GAA GAC TAG AAG AGC AAA CAC 3'
Endothelin Receptor, Type A (EDNRA)	product size: ~1kb annealing temperature: 59°C upper primer: 5' TAC CAC AAT CTT CTT ACC CGA CTG 3' lower primer: 5' GGC ACT GGC ATT TTG ACC TT 3'
JF specific - PASA	product size: 150bp annealing temperature: 57°C primer: use with lower primer 5' AA CCC ATC AGA AAA ATC TAT TAT 3'

Table 2. Cont.

Paired Basic Amino Acid Cleaving Enzyme (PACE)	product size: 400bp annealing temperature: 59°C upper primer: 5' GGA GGG CCC TTC GGA GTC G 3'	lower primer: 5' CCA GTC AGG GCA ACA CCA ACA AG 3'
JF specific- PASA	product size: 200bp annealing temperature: 57°C primer: use with upper primer 5' GAG GGG AGC CCA GAA TGA CG 3'	
Tropomyosin 2 (TPM2)	product size ~1.5kb annealing temperature: 59°C upper primer: 5' TGA ACC GCC GCA TCC AG 3'	lower primer: 5' GCG CTC CAG CTC TCC CTC AAG 3'
JF specific - PASA	product size: 150bp annealing temperature: 57°C primer: use with upper primer 5' GGA TGG TGA CTC CAT CAG AAG 3'	

Table 2. Cont.

Aldehyde Dehydrigenase 1 (ALDH1)	product size: 1kb	
	annealing temperature: 59°C	
	upper primer:	lower primer:
	5' CTT AGC AGC AGC AGT TTT TA 3'	5' AAG GCC ATA TTC TCC CAG TT 3'
JF specific - PASA	product size: 250bp annealing temperature: 57°C primer: use with lower primer 5' TCA GGG TAT ACT GCT ATC AC 3'	
Fibroblast Growth Factor Receptor 3 (FGFR3)	product size: 450bp	
	annealing temperature: 59°C	
	upper primer:	lower primer:
	5' CCG CTT GGT GAG GGC TGT TTT 3'	5' GCC CTG AGG TAT TCC CGC AAG TT 3'
JF specific - PASA	product size: 150bp annealing temperature: 55°C upper primer: 5' TTT TCT CAT AAG TTT ACA ATC ACG 3'	

Table 2. Cont.

Xeroderma Pigmentosum, Complemtation Group A (XPA)	product szie: 550bp	
	annealing temperature: 59°C	
	upper primer: 5' CAT GAA TAC GGA CCA GAA GAA AAT 3'	lower primer: 5' GAA ACC TCC CTC CAT CAA GT 3'
JF specific - PASA	product size: 200bp annealing temperature: 55°C primer: use with upper primer 5' GGT AAA CTT CCC TCC AG 3'	
Cathepsin L (CTSL)	product size: 450bp annealing temperature: 60°C	
	upper primer: 5' TGA TGA ATG GCT ATA AAC ACA AGA 3'	lower primer: 5' AGC CCA GCA AGA GCC ACA C 3'
JF specific - PASA	product size: 200bp annealing temperature: 57°C primer: use with upper primer 5' GAG GTA CTG AAT TTT ACT AAT CG 3'	

Table 2. Cont.

Prostaglandin-Endoperoxide Synthase 2 (PTGS2)	product size: 1.3kb	
	annealing temperature: 60°C	
	upper primer:	lower primer:
	5' GGT TGC CCT AGA TTC CTT TA 3'	5' AGT TCC CCA GCT GAG TTT AT 3'
JF specific - PASA	product size: 400bp annealing temperature: 57°C primer: use with lower primer 5' AAT TGG GAT GCT CTA CTA A 3'	
Tubulin, Alpha-Like, 1 (TUBAL1)	product size: 695bp	
	annealing temperature: 60°C	
	upper primer:	lower primer:
	5' ACT GCG CTT CGA TGG GGC TCT GA 3'	5' CGG GGG TGG GGT GGG GGA TAA 3'
JF specific - PASA	product size: 350bp annealing temperature: 57°C primer: use with upper primer 5' GAT GCC CAC CTT GAA ACC ACT T 3'	

Table 2. Cont.

Abelson Murine Leukemia Viral Oncogene Homolog 1 (ABL1)	product size: 600bp annealing temperature: 60°C upper primer: 5' GAG GAC ACC ATG GAG GTG GA 3'	lower primer: 5' GTG GAT GAA GAA GTT CTT CTT CTC 3'
JF specific - PASA	product size: 400bp annealing temperature: 55°C primer: use with upper primer 5' AAT TAT TAG GTA AGT GAT AAA TAG CG 3'	
Phospholipase A2, Group IV (PLA2G4)	product size: 625bp annealing temperature: 60°C upper primer: 5' GCA AGG CCA AGT GAT TCC AGT C 3'	lower primer: 5' AGT TGT GCA CAG CCC TTT ATT TCA 3'
JF specific - PASA	product size: 78bp annealing temperature: 55°C primer: use with lower primer 5' GCT TCA AGA AAC TGA TTC TTT T 3'	

Table 2. Cont.

Caspase 1, Apoptosis-Related Cysteine Protease (CASP1)	product size: 450bp annealing temperature: 60°C upper primer: 5' GCC AGC GCC ATC TTC ATT G 3'	lower primer: 5' GCC CTT CGC TCA TCT CCT CTA 3'
JF specific - PASA	product size: 400 bp annealing temperature: 57°C primer: use with lower primer 5' GCC CAG GCC CAA AGA CAC TCA A 3'	
Villin (VIL)	product size: 755bp annealing temperature: 60°C upper primer: 5' CTG CAG CGG GGA TGA GCG TGA GA 3'	lower primer: 5' AGG GCA AGT TGG CAA GGC AGA GC 3'
JF specific - PASA	product size: 200bp annealing temperature: 57°C primer: use with lower primer 5' TGA TGT GAC CTT GTC CCG CC 3'	

Table 2. Cont.

Transiently-Expressed Axonal Glycoprotein (TAX1)	product size: 600bp	
	annealing temperature: 60°C	
	upper primer:	lower primer:
	5' CTG AAG GGA GGA AGA AAG AA CA 3'	5' GCA TGG CAG CTG ATA CAA ACA 3'
JF specific - PASA	product size: 200bp annealing temperature: 57°C primer: use with lower primer 5' CTC TAA GGA GCG ATG GCA C 3'	
Actinin, Alpha 2 (ACTN2)	product size: >1kb	
	annealing temperature: 60°C	
	upper primer:	lower primer:
	5' AGA GAA ACA GCA GAT ACA GAC ACG 3'	GGA CAG ACA ACC TAA AAC CAA CA 3'
JF specific - PASA	product size: 132bp annealing temperature: 57°C primer: use with upper primer 5' CTG CAA GTAA AGG GGG C 3'	

Table 2. Cont.

ADP-Ribosyltransferase (ADPRT)	product size: >2kb annealing temperature: 60°C upper primer: 5' AGT CAG CGT TAC AAG CCA TTA 3'	lower primer: 5' GTT TCA GCA GGT ACT TCA GAT T 3'
JF specific - PASA	product size: 200bp annealing temperature: 57°C primer: use with lower primer 5' GCT TGA AAT GTT AGG ACT CCA 3'	
Calpain 1, (CAPN1)	product size: 800bp annealing temperature: 60°C upper primer: 5' ACC ATG TAC GCC TAA CCC CAG AGC 3'	lower primer: 5' CCA GGC CAA GGC ATA CCC AGA C 3'
JF specific - PASA	product size: 232bp annealing temperature: 57°C primer: use with upper primer 5' CTG TTG AAA GTA AAT GTC CAG G 3'	

Table 2. Cont.

Albumin (ALB)	product size: 1kb annealing temperature: 60°C	
	upper primer: 5' CAT GGC GAG GCA GAC TTC C 3'	lower primer: 5' GGG CTT GCG TTT AAT GAG GTT G 3'
JF specific - PASA	product size: 78bp annealing temperature: 57°C primer: use with upper primer 5' GTA CTC CCA AGG CAG GCT 3'	
Lysyl Hydroxylase (PLOD)	product size: 800bp annealing temperature: 60°C	
	upper primer: 5' CCG CAG TTT AAG GGG AGC ATT CAT 3'	lower primer: 5' GCA GTG GCG GGC AGA GGA 3'
JF specific - PASA	product size: 220bp annealing temperature: 57°C primer: use with lower primer 5' CTC TGA GGG CTC TTT GCG T 3'	

Table 2. Cont.

Cathepsin E (CTSE)	product size: >2kb annealing temperature: 60°C upper primer: 5' ACC CCT GCT GAA CAC CCT GGA CAT 3'	lower primer: 5' AGG CCT CTT GCT GCT CTG AAA AAC 3'
Jf specific - PASA	product size: 350bp annealing temperature: 57°C primer: use with upper primer 5' CCG GTG TCG AAG ACC ACT GC 3'	
Glutamine Synthetase (GLUL)	product size: 600bp annealing temperature: 60°C upper primer: 5' GTG CTC CCC GTA CCC CTA AAC TTC 3'	lower primer: 5' GAG ATC GCC TGA CTT CCA ATG A 3'
JF specific - PASA	product size: 250bp annealing temperature: 57°C primer: use with lower primer 5' CCG ACT TCC CCT TAT TTG AT 3'	

Table 2. Cont.

Nuclear Factor Kappa-B P105 Subunit (NFKB1)	product size: 800bp	
	annealing temperature: 60°C	
	upper primer:	lower primer:
	5' CGT GTG ACA GCG GCG TAG AGA C 3'	5' TGA AGG GAA CAG CCA GAA ACC ATC 3'
JF specific - PASA	product size: 300bp annealing temperature: 57°C primer: use with upper primer 5' AGG AAG TGA GGT TGA GGA TTT 3'	

Group-Specific Component (Vitamin D Binding Protein (GC)	product size: >2kb	
	annealing temperature: 60°C	
	upper primer:	lower primer:
	5' GTA GCA ACT CAC GCC GAA CAC C 3'	5' GAT GGG CAG GGA AAG GGG AGT C 3'
JF specific - PASA	product size: 450bp annealing temperature: 57°C primer: use with lower primer 5' AAT GAA GAG CTT ACC ACA CAC GCA 3'	

Table 2. Cont.

Neurotrophic Tyrosine Kinase, Receptor, Type 2 (NTRK2)	product size:	
	upper primer:	lower primer:
	5' GAT GTC TGG AGC CTG GGA GTT GTA 3'	5' TTT AAT GGA GTT CAG CGG CAG TTG 3'
JF specific - PASA	product size: 170bp annealing temperature: 57°C primer: use with lower primer 5' GGA TGT TGG CTA CGG GAA CCT AAT 3'	
Mast Cell Growth factor (MGF)	product size: >2kb	
	annealing temperature: 59°C	
	upper primer:	lower primer:
	5' ATG GCA TGT TTA GCT TTT GAT A 3'	5' TGC CTC TTT GTT ACT GTT ACT GCT 3'
JF specific - PASA	product size: 220bp annealing temperature: 57°C primer: use with upper primer 5' CTA TGT TAA CAG AGT GTA GTG 3'	

Table 2. Cont.

Myeloid Cell Leukemia 1 (MCL1)	product size: 129bp annealing temperature: 60°C upper primer: 5' TCG GAA ACT CAC GCC GAA CAC C 3'	lower primer: 5' GCA ACA AAG GCA CCA AAT G 3'
JF specific - PASA	product size: 90bp annealing temperature: 57°C primer: use with lower primer 5' GTG TGA GGT GGC TGC TGA C 3'	
Phosphoribosylpyrophosphate Amidotransferase (PPAT)	product size: 992kb annealing temperature: 60°C upper primer: 5' CTT GCC CTG AAT GTG AGA TA 3'	lower primer: 5' AAG ATG GGG AAG GAA AAA G 3'
JF specific - PASA	product size: 440bp annealing temperature: 57°C primer: use with lower primer 5' TTT TTC GCC TTC CAG ATT GC 3'	

Table 3.

PCR conditions:

25ul Reaction:		PCR cycle:
10X PCR Buffer		94°C 2 min. 30 sec.
1.5mM $MgCL_2$		94°C 30 sec.
.2mM dNTPs		55°C-60°C 1 min. 30 sec.
.2uM each primer		72°C 2 min.
1U Taq Polymerase		cycle 30 times
30 ng genomic DNA (WL or JF)		72°C 10 min..
		4°C

Table 4.

Genes Mapped:
Human Chromosome 1

	Source*:	Region Amplified**:	Chicken Map Position:	Human Map Position
Lysyl Hydroxylase; **PLOD**	Genbank cDNA	3' UTR	Chromosome E54, 59.6	1p36.3-p36.2
Janus Kinase 1; **JAK1**	U.Del. cDNA	Within coding region	Chromosome 8, 0.0	1p31.3
Myeloid Cell Leukemia 1; **MCL1**	U.Del. cDNA	Within coding region	Chromosome E26, 0.0	1q21
Phospholipase A2, Group IV; **PLA2G4**	Genbank cDNA	3' End including UTR	Chromosome 8, 50.5	1q25
Prostaglandin-Endoperoxide Synthase 2; **PTGS2**	Genbank cDNA	3' UTR	Chromosome 8, 50.5	1q25.2-q25.3
Glutamine Synthetase; **GLUL**	Genbank cDNA	3' End including UTR	Chromosome 8, 82.4	1q31
Cathepsin E; **CTSE**	Genbank cDNA	3' End including UTR	Chromosome E04, 17.7	1q31
Troponin T2, Cardiac; **TNNT2**	Genbank cDNA	3' End including UTR	Chromosome E04, 15.7	1q32
Transiently-Expressed Axonal Glycoprotein; **TAX1**	Genbank cDNA	3' UTR	Chromosome E04, 9.6	1q32.1
ADP-Ribosyltransferase; **ADPRT**	Genbank cDNA	Within coding region	Chromosome 3, 75.6	1q42
Actinin, Alpha 2; **ACTN2**	Genbank cDNA	3' End including UTR	Chromosome 3, 132.2	1q42-q43

Table 4. Genes mapped that are orthologous to genes on human chromosome 1. *Source: cDNA source for the chicken genes; Genbank: Genbank database at N.C.B.I., U.Del.: University of Delaware cDNA library. **Region amplified: region of genomic DNA sequenced; 3'UTR: 3' untranslated region, 3' End including UTR: 3' coding region and some or all of the 3'UTR, Within coding region: strictly coding region.

Table 5. Genes mapped that are orthologous to genes on human chromosome 9. *Source: cDNA or primer source for the chicken genes; Genbank: Genbank database at N.C.B.I., U.Del.: University of Delaware cDNA library, UMSTS: Universal Mammalian Sequence Tagged Sites. **Region amplified: region of genomic DNA sequenced; 3'UTR: 3' untranslated region, 3' End including UTR: 3'coding region and some or all of the 3'UTR, Within coding region: strictly coding region.

Table 5.

Genes Mapped:
Human
Chromosome 9

	Source:	Region Amplified:	Chicken Map Position:	Human Map Position:
Very Low Density Lipoprotein Receptor; **VLDLR**	Genbank cDNA	Within coding region	Chromosome Z, 92.3	9p24
Tyrosinase-Related Protein 1; **TYRP1**	Genbank cDNA	3' End including UTR	Chromsome Z, 102.3	9p23
Tropomyosin 2; **TPM2**	Genbank cDNA	Within coding region	Chromsome Z, 5.8	9p13.2-13.1
Aldehyde Dehydrogenase 1; **ALDH1**	Genbank cDNA	Within coding region	Chromosome E18, 10.0	9q21
Cathepsin L; **CTSL**	U. Del. cDNA	Within coding region	Chromosome Z, 113.1	9q21-q22
Neurotrophic Tyrosine Kinase, Receptor, Type 2; **NTRK2**	Genbank cDNA	3' End including UTR	Chromosome Z, 115.5	9q22.1
Xenoderma Pigmentosu Group A Complenting Protein; **XPA**	Genbank cDNA	3' End including UTR	Chromosome Z, 175.1	9q22.3-q31
Transformin Growth Factor-Beta Receptor, Type I; **TGFBR1**	Genbank cDNA	3' UTR	Chromosome 2, 153.8	9q21-22
Abelson Murine Leukemia Viral Oncogene Homolog 1; **ABL1**	UMSTS	Within coding region	Chromosome E41, 30.8	9q34.1
Gelsolin; **GSN**	Genbank cDNA	Within coding region	Chromosome E41, 16.0	9q34
Ring3-Like Gene; **RING3L**	U.Del. cDNA	Within coding region	Chromosome E41, 13.5	9q34

Figure 5. Legend on facing page.

Table 6.

Genes Mapped:
Human Chromsome 4

	Source*:	Region Amplified**:	Chicken Map Position:	Human Map position:
Fibroblast Growth Factor Receptor 3; **FGFR3**	Genbank cDNA	Within coding region	Chromosome 4, 3.8	4p16.3
Collapsin Response Mediator Protein 1; **CRMP1**	Genbank cDNA	Within coding region	Chromosome E38, 0.0	4p15-16.1
Phosphoribosylpyrophosphate Amidotranferase; **PPAT**	Genbank cDNA	3' UTR	Chromosome 4, 173.4	4q12-13
Albumin; **ALB**	Genbank cDNA	3' End including UTR	Chromosome 4, 132.3	4q11-q13
Group-Specific Component (Vitamin D Binding Protein); **GC**	U.Del. cDNA	Within coding region	Chromosome 4,132.3	4q12
Nuclear Factor Kappa-B P105 Subunit; **NFKB1**	Genbank cDNA	3' UTR	Chromosome 4, 165.7	4q23-q24
Endothelin Receptor, Type A; **EDNRA**	Genbank cDNA	3' UTR	Chromosome 4, 108.7	4q27-28

Table 6. Genes mapped that are orthologous to genes on human chromosome 4. *Source: cDNA source for the chicken genes; Genbank: Genbank database at N.C.B.I., U.Del.: University of Delaware cDNA library. **Region amplified: region of genomic DNA sequenced: 3'UTR: 3' untranslated region, 3' End including UTR: 3' coding region and some or all of the 3'UTR, Within coding region: strictly coding region.

Table 7.

Genes Mapped:
Human Chromosomes
11, 12, and Others

	Source*:	Region Amplified**:	Chicken Map Position:	Human Map Position:
Calpain 1; **CAPN1**	Genbank cDNA	3' UTR	Chromosome 5, 76.8	Chr.11
Caspase 1, Apoptosis-Related Cysteine Protease; **CASP1**	Genbank cDNA	5' UTR	Chromosome E52, 43.1	11q22.2-q22.3
Gamma-Carboxyglutamic Acid Protein, Matrix; **MGP**	Genbank cDNA	Within coding region***	Chromosome 1, 151.8	12p12.3-13.1
Mast Cell Growth Factor; **MGF**	Genbank cDNA	Within coding region	Chromosome 1, 143.2	12q22
Tubulin, Alpha-Like, 1; **TUBAL1**	Genbank cDNA	3' End including UTR	Chromosome E22, 20.4	Chr.12
Ubiquitin-Conjugating Enzyme E2A; **UBE2A**	U. Del. cDNA	Within coding region	Chromosome 4, 81.0	Xq24-25
Villin; **VIL**	Genbank cDNA	3' End including UTR	Chromosome 7, 73.1	2q35-q36
Paired Basic Amino Acid Cleaving Enzyme; **PACE**	Genbank cDNA	3' UTR	Chromosome E29, 6.3	15q25-26
Spicing Factor, Arginine/Serine-Rich, 2; **SFRS2**	Genbank cDNA	Within coding region	Chromsome E31, 0.0	17q24

Table 7. Genes mapped that are orthologous to genes on human chromosome 11, 12, X, 2, 15, and 17. *Source: cDNA source for the chicken genes; Genbank: Genbank database at N.C.B.I., U.Del.: University of Delaware cDNA library. **Region amplified: region of genomic DNA sequenced: 3'UTR: 3' untranslated region, 5' UTR: 5' untranslated region, 3' End including UTR: 3' coding region and some or all of the 3'UTR, Within coding region: strictly coding region. ***Within coding region: primers from the Primer Pairs to Sequenced Chicken Genes set.

Table 8.

Gene Combination	Length of Segment, cM		Chromosome	
	r*	m**	chicken	human
SFRS, H33B, and FAS	9.8	19.6	E31	17q
PACE, IGF1R, and B2M	98.1	196.2	E29	15q
SPP1, ALB, GC, and PPAT	35.3	58.8	4	4q
RPL37A, VIL, CD28, and EEF1B	44.3	73.8	7	2q
CDC2L1, AGRN, ENOL, PLOD, and SLC2A1	71.2	106.8	E54	1p
JAK1 and GGTB3	27.4	82.2	8	1p
PLA2G4, PTGS2, and GLUL	31.8	63.6	8	1q
TAX1, TNNT2, and CTSE	8	16	E04	1q
ADPRT, TGFB2, and ACTN2	65.1	130.2	3	1q
RING3L, GSN, L7a, ABL1, AK1, CD39, and AMBP	80.9	107.9	E41	9q
VLDLR and TYRP1	7.7	23.1	Z	9p
CTSL and NTRK2	2.1	6.3	Z	9q
ALDOB and XPA	26.6	79.8	Z	9q
GAPD and LDHB	17.3	51.9	1	12p
PGK1 and UBE2A	9.6	28.8	4	X
WNT11 and FUCTIV	29.6	88.8	1	11q
MPR1, PLN, ME1, and GSTA2	80.2	133.7	3	6q-6p
DNECL and CKB	1.9	5.7	5	14q
CRYB, IGVPS, and MIFL2	4	8	E18	22q

Table 8. Genetic lengths of conserved segments between chickens and humans. *r: genetic distance between outermost markers in the group based on the EL reference map. **m: expected value of the length of the conserved segment based on the treatment of Nadeau and Taylor (1984). Mean of r=34.3, standard deviation (SD)=30.3, mean of m=67.4, SD=52.1.

Figure 1.

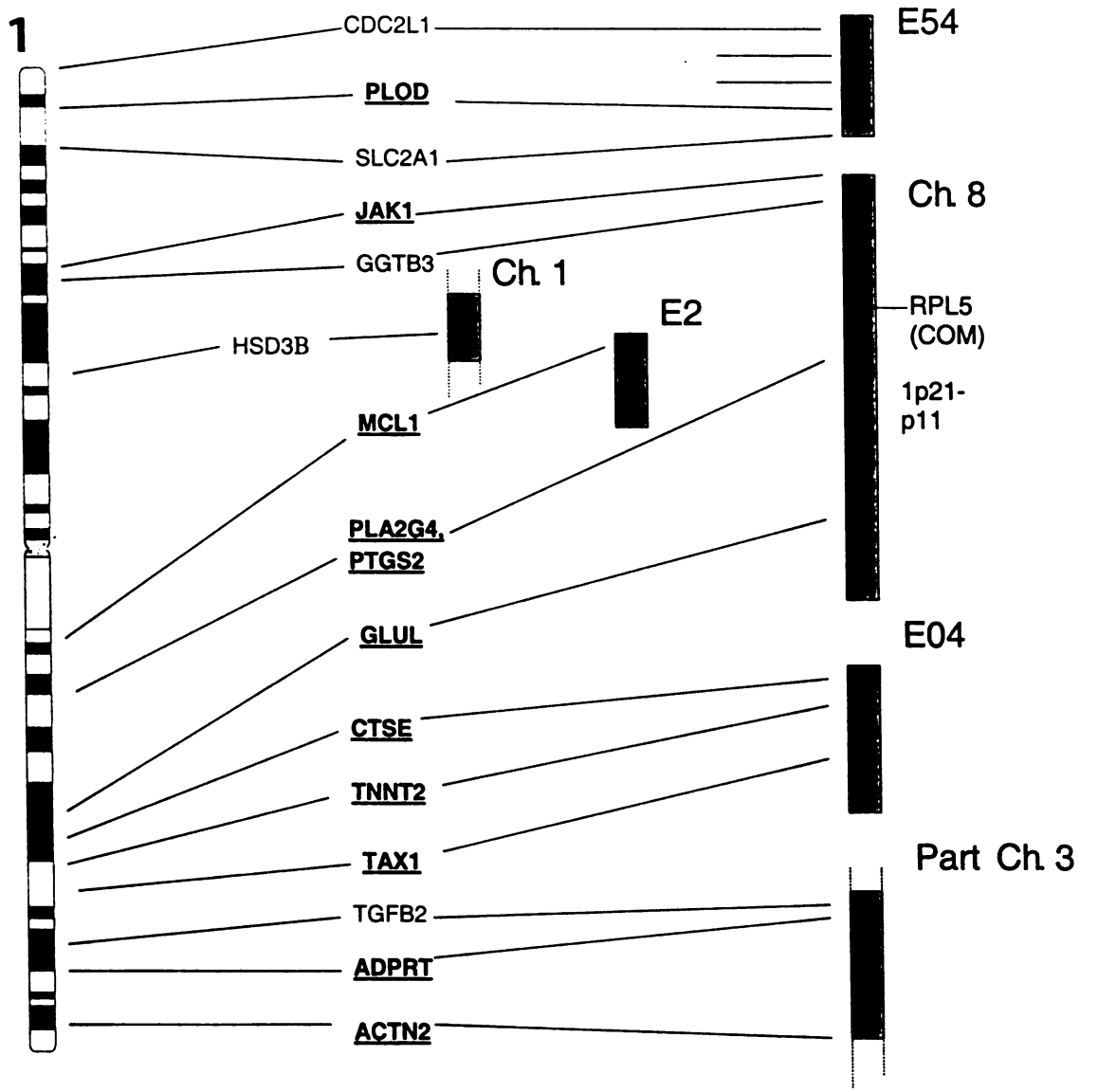


Figure 1. Syntenic groups mapped to human chromosome 1 and chicken chromosomes E54, 8, 1, E26, E04, and 3. The human physical map is compared to the chicken genetic map. Genes mapped in the current study are in bold and underlined. Dotted lines on the ends of c-chr represent the ends of the conserved segment. If all genes currently on the map of a c-chr are found on the same syntenic group, these are bordered by closed ends. *AGRN* and *ENO1* are found on the pter end of h-chr 1. *RPL5* is mapped on chromosome 8 on the Compton chicken genetic map (Compton and Palyga, 1992)

Figure 2.

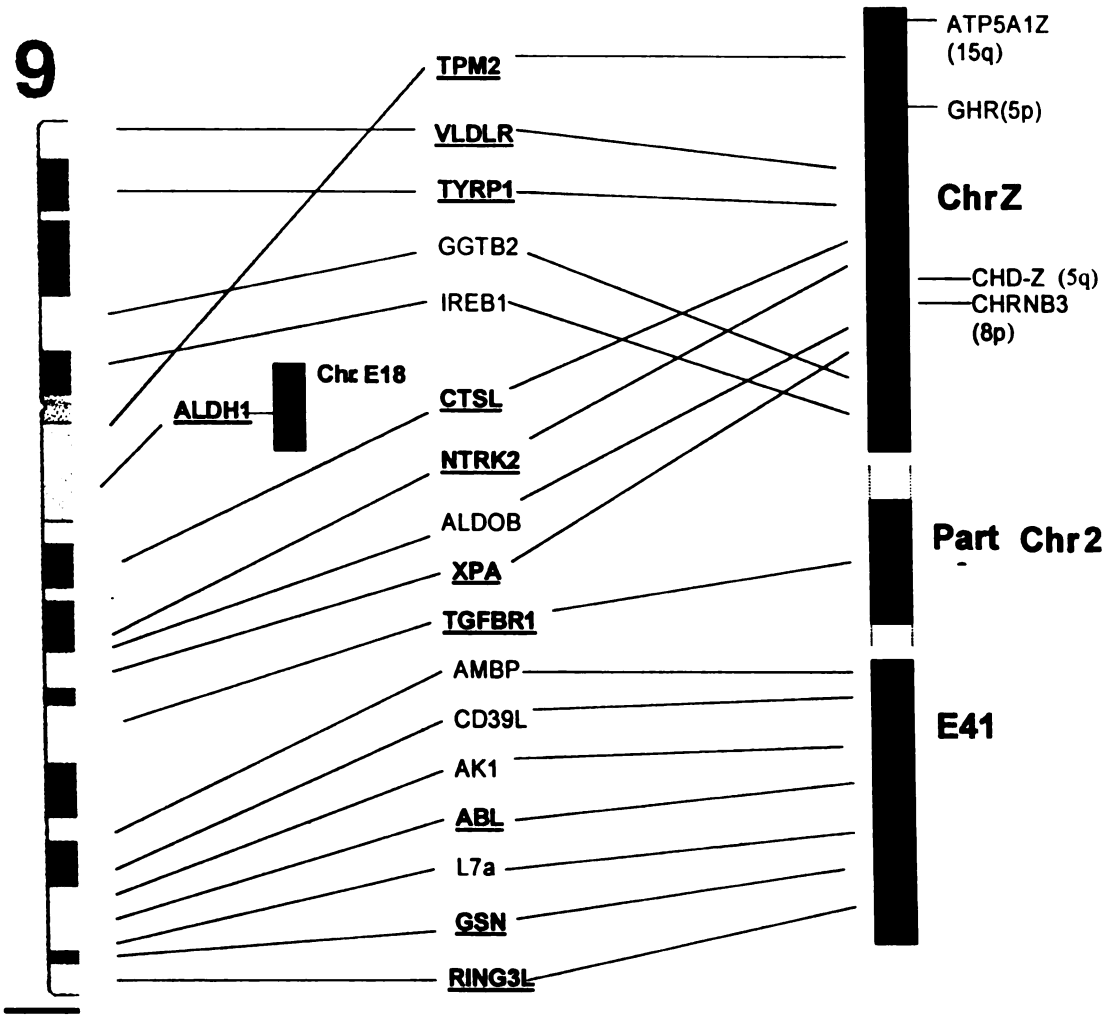


Figure 2. Syntenic groups mapped to human chromosome 9 and chicken chromosomes Z, E18, 1, 2, and E41. The human physical map is compared to the chicken genetic map. Genes mapped in the current study are in bold and underlined. Dotted lines on the ends of c-chr represent the ends of the conserved segment. If all genes currently on the map of a

Figure 3.

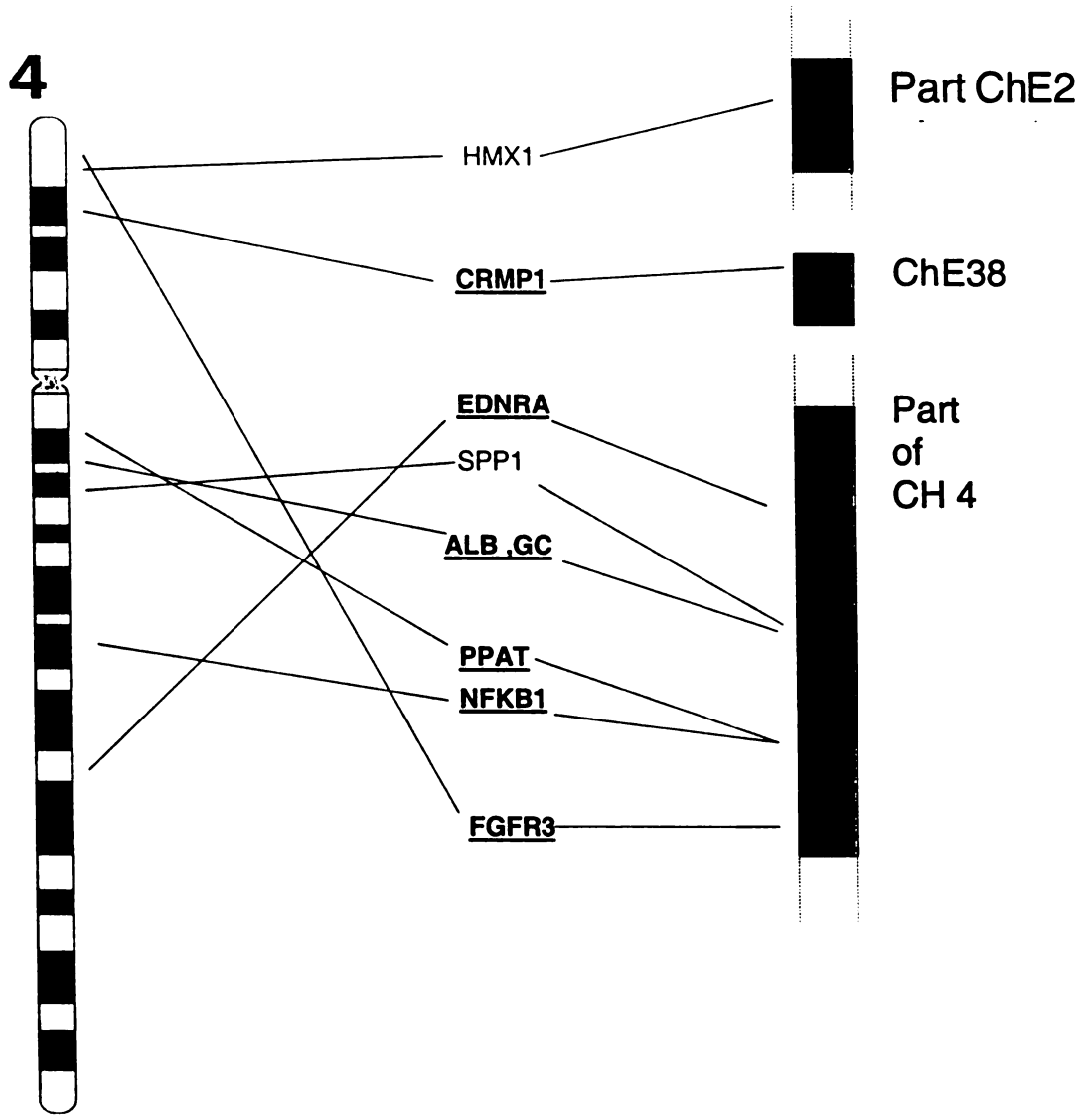


Figure 3. Syntenic groups mapped to human chromosome 4 and chicken chromosomes E29, E38, and 4. The human physical map is compared to the chicken genetic map. Genes mapped in the current study are in bold and underlined. Dotted lines on the ends of c-chr represent the ends of the conserved segment. If all genes currently on the map of a c-chr are found on the same syntenic group, these are bordered by closed ends.

Figure 4.

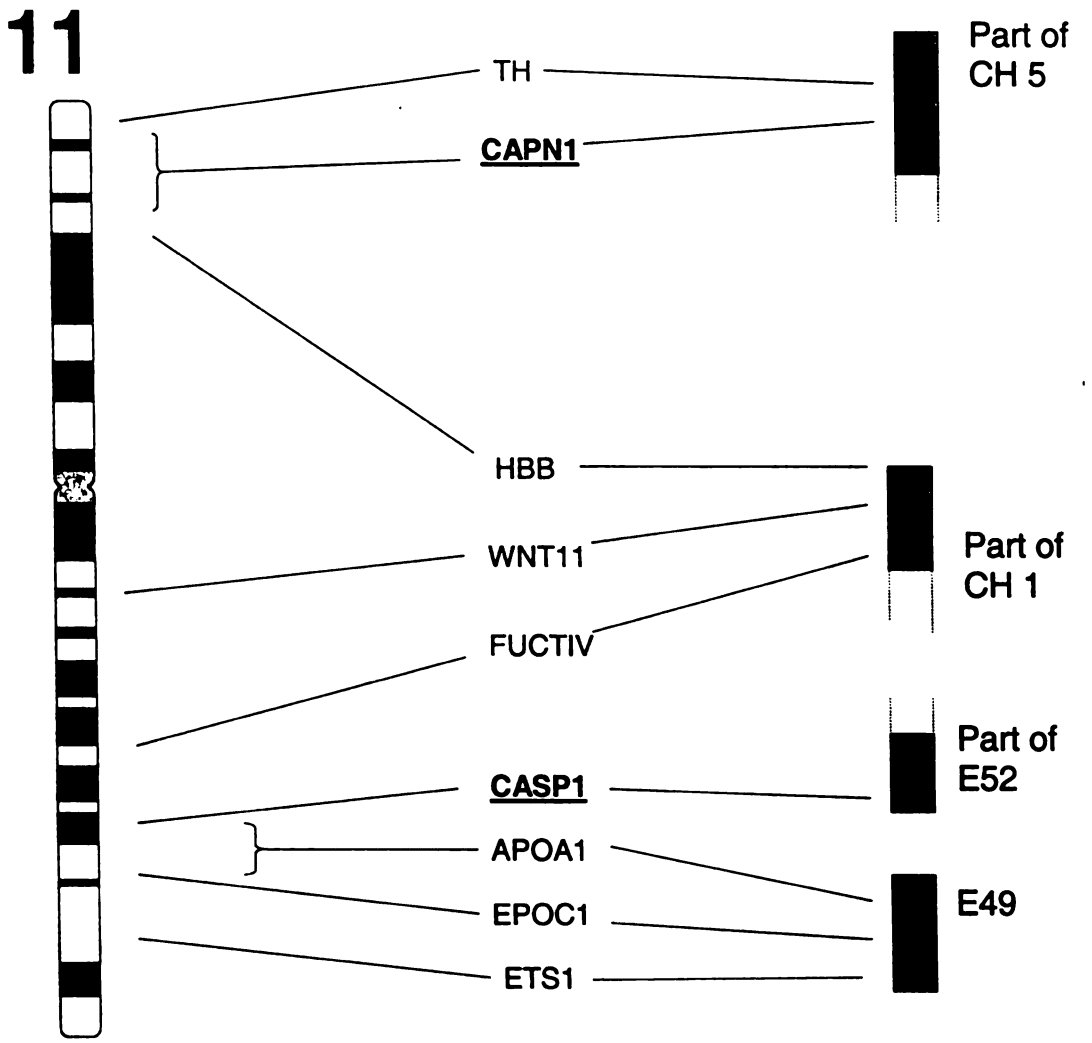


Figure 4. Syntenic groups mapped to human chromosome 11 and chicken chromosomes 5, 1, E52, and E49. The human physical map is compared to the chicken genetic map. Genes mapped in the current study are in bold and underlined. Dotted lines on the ends of c-chr represent the ends of the conserved segment. If all genes currently on the map of a c-chr are found on the same syntenic group, these are bordered by closed ends.

Figure 5.

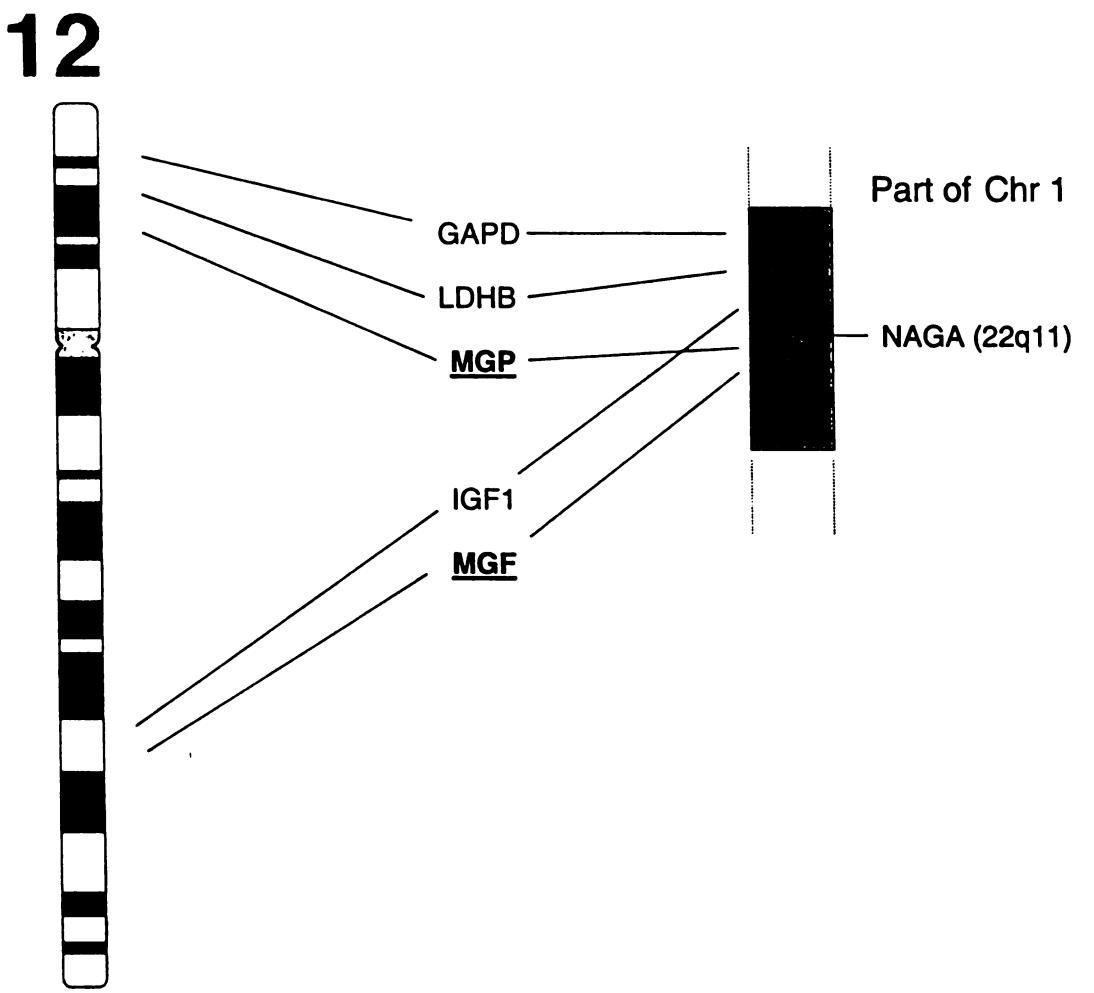


Figure 5. Syntenic groups mapped to human chromosome 12 and chicken chromosome 1. The human physical map is compared to the chicken genetic map. Genes mapped in the current study are in bold and underlined. Dotted lines on the ends of c-chr represent the ends of the conserved segment. If all genes currently on the map of a c-chr are found on the same syntenic group, these are bordered by closed ends.

Figure 6.

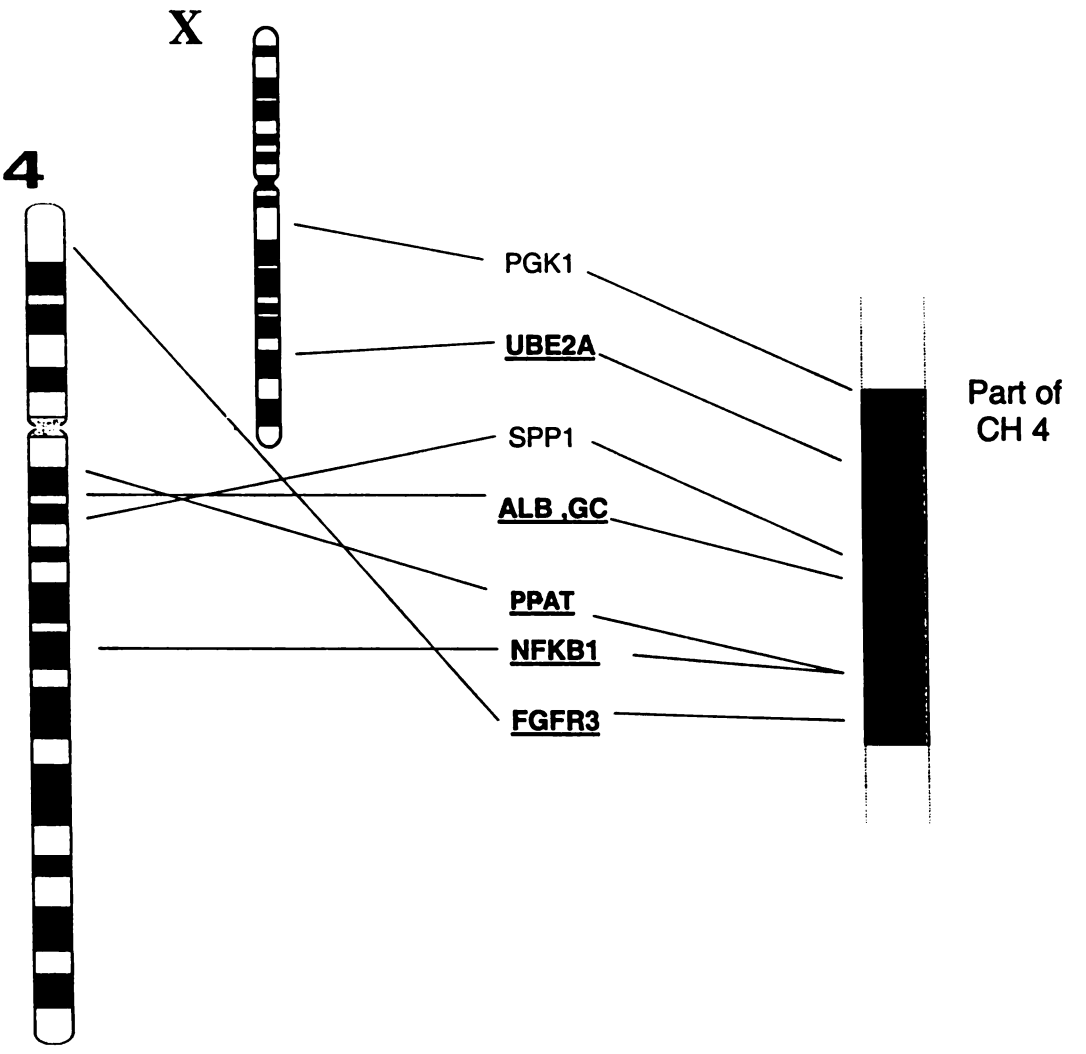


Figure 6. Syntenic groups mapped to human chromosome X and chicken chromosome 4. The human physical map is compared to the chicken genetic map. Genes mapped in the current study are in bold and underlined. Dotted lines on the ends of c-chr represent the ends of the conserved segment. If all genes currently on the map of a c-chr are found on the same syntenic group, these are bordered by closed ends.

Figure 7.

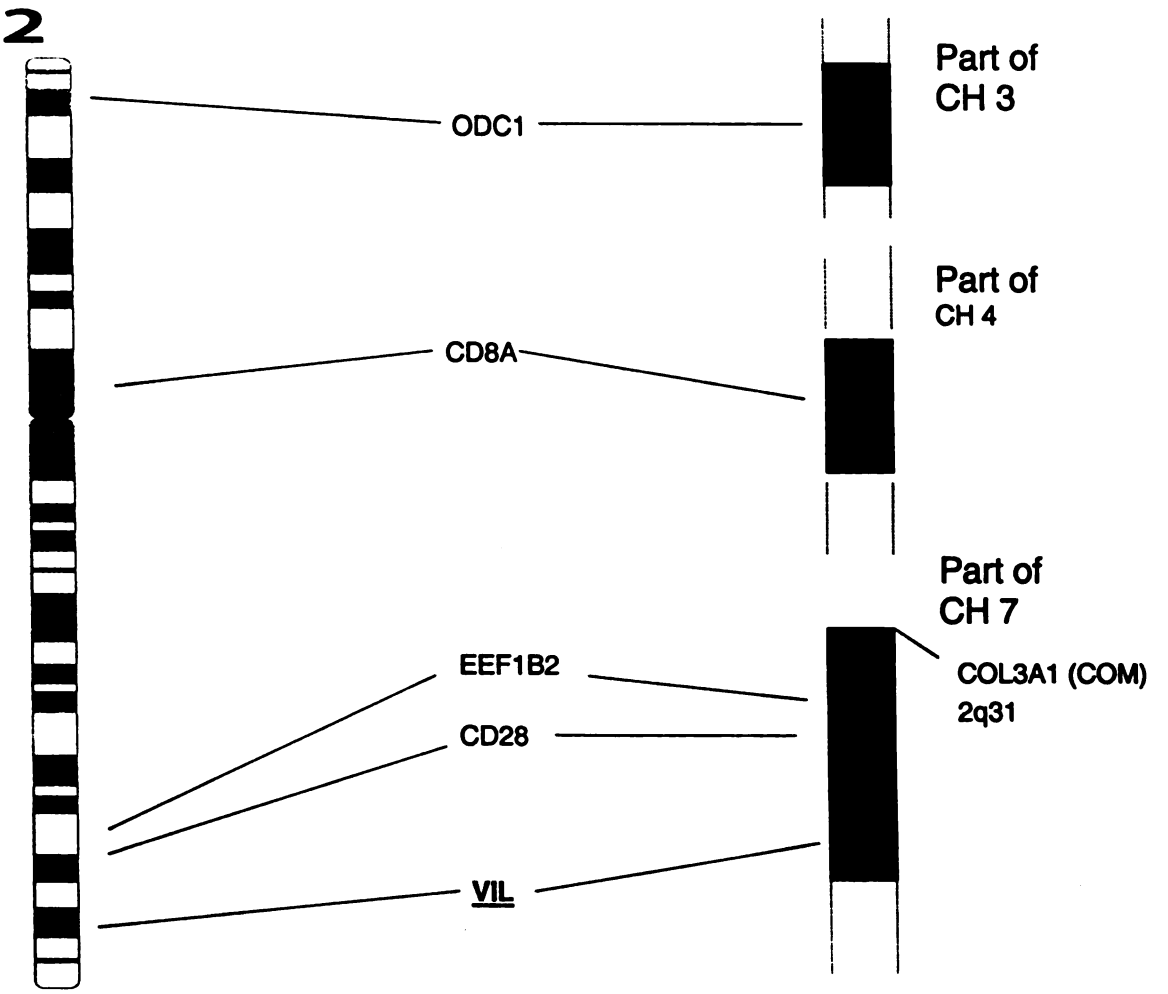


Figure 7. Syntenic groups mapped to human chromosome 2 and chicken chromosomes 3, 4, and 7. The human physical map is compared to the chicken genetic map. Genes mapped in the current study are in bold and underlined. Dotted lines on the ends of c-chr represent the ends of the conserved segment. If all genes currently on the map of a c-chr are found on the same syntenic group, these are bordered bv closed ends.

Figure 8.

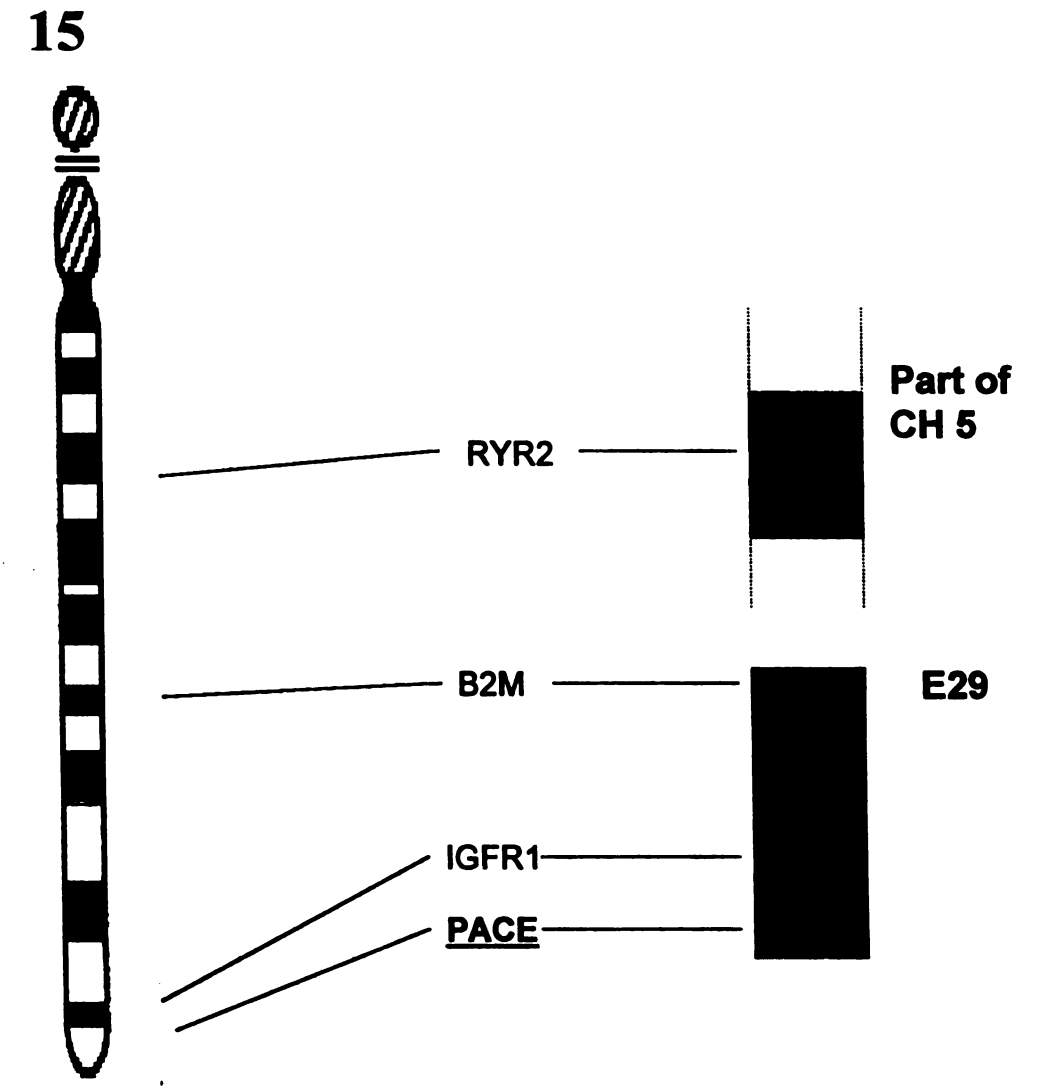


Figure 8. Syntenic groups mapped to human chromosome 15 and chicken chromosomes 5 and E29. The human physical map is compared to the chicken genetic map. Genes mapped in the current study are in bold and underlined. Dotted lines on the ends of c-chr represent the ends of the conserved segment. If all genes currently on the map of a c-chr are found on the same syntenic group, these are bordered by closed ends.

Figure 9.

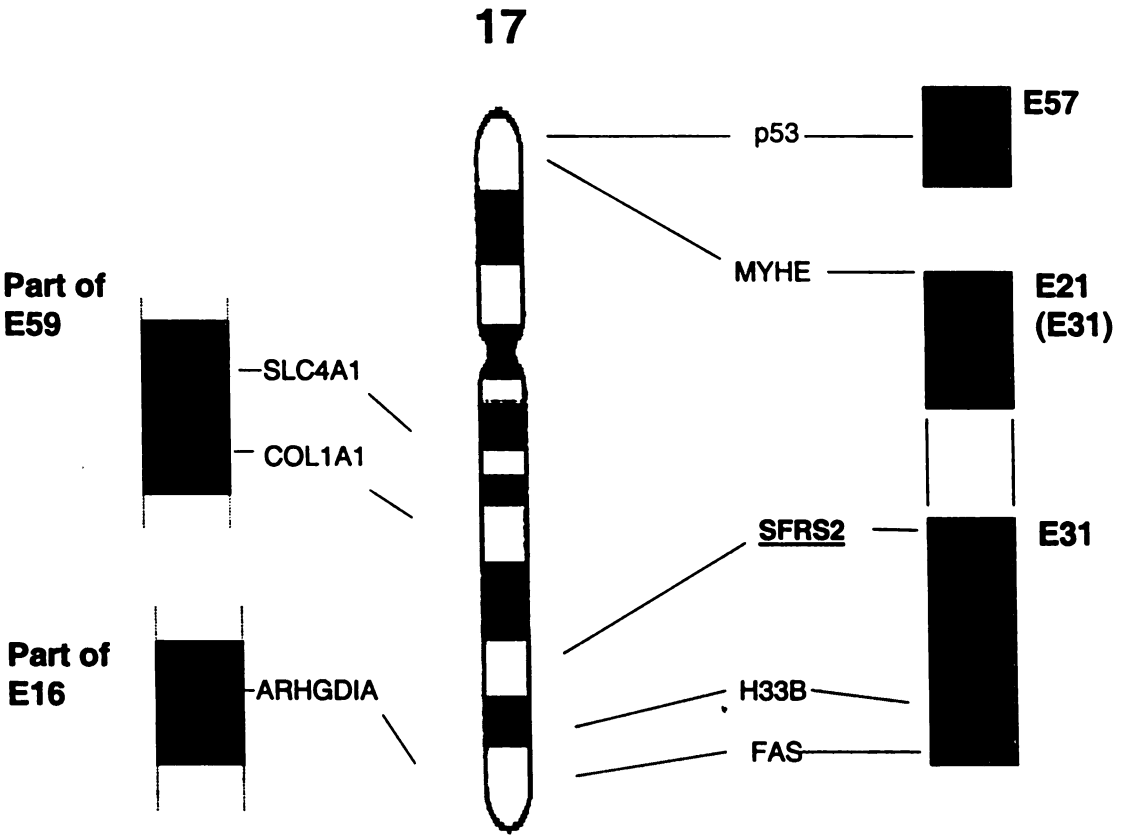


Figure 9. Syntenic groups mapped to human chromosome 17 and chicken chromosomes E57, E21, E31, E59, and E16. The human physical map is compared to the chicken genetic map. Genes mapped in the current study are in bold and underlined. Dotted lines on the ends of c-chr represent the ends of the conserved segment. If all genes currently on the map of a c-chr are found on the same syntenic group, these are bordered by closed ends.

Figure 10.

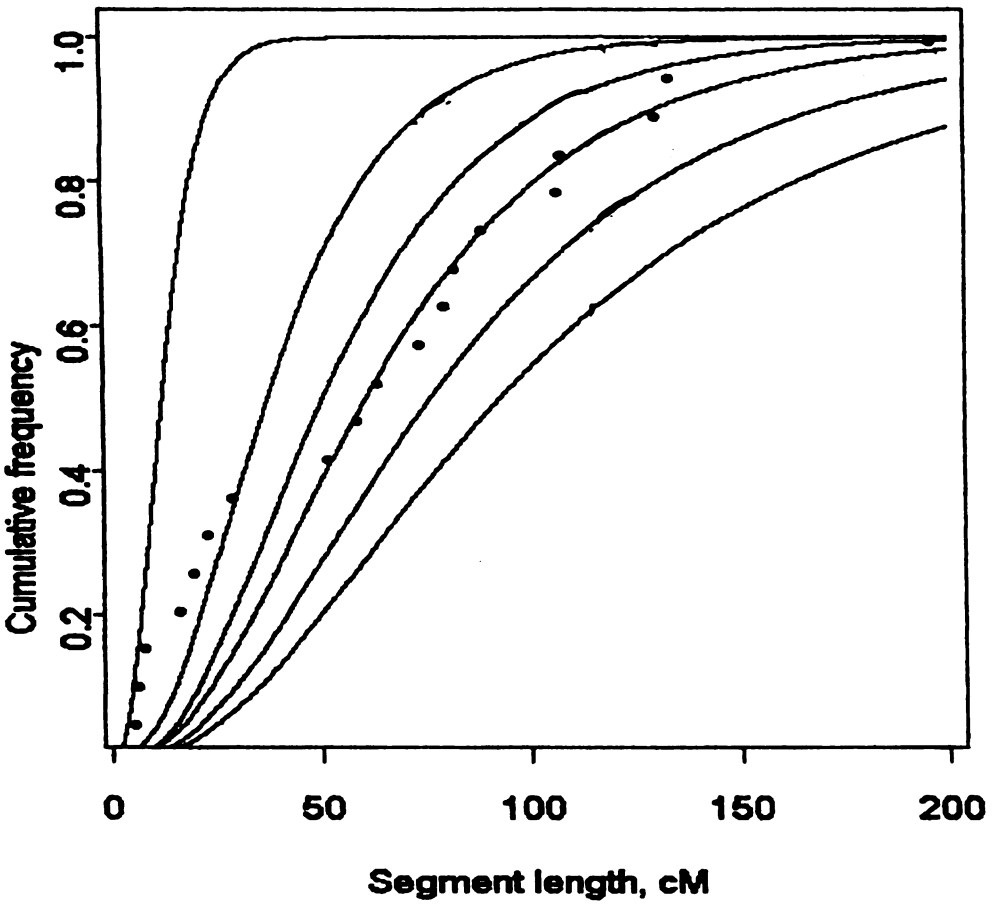


Figure 10. Curves illustrating expected cumulative frequency distributions of segments containing two or more markers at different values of L.
The circles represent the cumulative distribution of adjusted segment lengths used in this study.

Chapter 3: Physical Mapping of Chicken Microchromosome E41

Introduction:

The typical avian karyotype is composed of 8 macrochromosomes, plus the Z and W sex chromosomes, and around 30 microchromosomes. Although the microchromosomes vary in size, they are not large enough, nor do they have a banding pattern distinct enough to distinguish between them. Thus, the term "microchromosome" is somewhat arbitrary. There are many questions concerning avian microchromosomal physical and genetic structure. It has been hypothesized that microchromosomes may have an increased rate of recombination compared to macrochromosomes since it is thought that at least one chiasmata is required per microchromosome, regardless of size, to ensure proper meiotic segregation (Rodionov *et al.* 1992; Rodionov 1998). Microchromosomes have reduced levels of non-coding sequences such as microsatellites and initial studies suggest they may also be gene-rich (Sazanov *et al.* 1996; Rodionov *et al.* 1996; Primmer *et al.* 1997; McQueen *et al.* 1996, 1998, Clark *et al.* 1999). All of these theories remain unproven in the absence of a detailed physical map of any avian chromosome.

Recently, through collaboration with the Texas A&M Bacterial Artificial Chromosome (BAC) Center, a 5-fold BAC library of the chicken genome has been generated through the insertion of partial BamHI DNA fragments into pBeloBac11

(Figure 1). This is comprised of approximately 38,000 clones with an average insert size of 150 kb. The DNA source used is a UCD001 female Red Jungle Fowl. This is the same line as the non-recurrent parent of the East Lansing (EL) Reference Backcross family (Crittenden *et al.* 1993) which allows for identification of dominant JF markers such as AFLP (Knorr *et al.* 1999) within the library. BAC libraries have been used extensively in the generation of physical contig maps (Marra *et al.* 1999; Mozo *et al.* 1999), and we have begun to develop such a map for the chicken genome in a continuing collaboration with the BAC Center. As a test of the feasibility of such an approach, we have made initial steps into the generation of a contig map for the E41 microchromosome which are described below.

E41 is one of the most densely mapped microchromosomes (Groenen et al. in press) and several known genes are among the mapped markers (Smith *et al.* 1997; Chapter 2 of this thesis). Interestingly, all of the genes mapped on E41 are syntenic with telomeric portion of the q arm of human chromosome 9 (Chapter 2, Figure 2). The overall map of E41 contains 20 markers across approximately 70 cM (Chapter 1, Figure 1). Additionally, a quantitative trait locus (QTL) for a differential response in viremia to Marek's Disease Virus in line 6 and 7 chickens has been mapped to E41 (Vallejo *et al.* 1998; Yonash *et al.* 1999). These factors led to the decision to begin testing the newly constructed BAC library using genetic markers on E41. Our long-term goal is

to construct a complete physical contig across E41, which will allow for, among other things, comparison of its physical and genetic sizes.

Materials and Methods:

BAC library screening:

Six markers were chosen from linkage group E41 to screen the BAC library: *RING3L* (Ring3-Like Gene), *AK1* (Adenylate Kinase 1), *L7a* (Ribosomal Protein L7a), *ABL1* (Abelson proto-oncogene 1), *GSN* (Gelsolin), and microsatellite marker *ROS0020*. Our group had mapped *RING3L*, *AK1*, *ABL1*, *GSN*, and *L7a*, so the primers for these markers were available and had been tested. We wanted to test microsatellite markers for probing the BAC library and *ROS0020* is positioned between *GSN* and *L7a* on the genetic map (Chapter 1, Figure 1). All primer and PCR information including *ROS0020* are available on the chicken genome mapping web site (http://poultry.mph.msu.edu/).

PCR products from the markers were cloned into the TOPO-TA™ cloning vector (Invitrogen Corporation, Carlsbad Ca.). Plasmid isolation of positive clones was done using the Qiaprep™ miniprep kit protocol (Qiagen Inc., Valencia, CA). pBeloBac11 contains λ cos and LACZ gene sequences, therefore insert DNA to be used as a probe must first be extracted from any vector that contains these sequences (such as TOPO-TA™). Several restriction enzyme combinations based on the TOPO-TA™ vector-cloning site were tested to produce the largest useful insert (Figure 2). Insert DNA was

isolated using the Qiaex II™ gel purification kit protocol (Qiagen Inc., Valencia, CA). The BAC library has been spotted in duplicate onto Hybond-N+ (Amersham Pharmacia Biotech, Piscataway, NJ) nylon membrane filters. We employed a 30,000 clone sublibrary spotted on 20 filters. Prior to hybridization, the filters were prehybridized with 0.263 M Na_2HPO_4, 2% SDS, 1% BSA, 1 mM EDTA, and 200 µg/ml denatured salmon sperm DNA (HYB solution). Ten filters were prehybridized with 20 mls of HYB solution. Prehybridization was carried out at 65°C for 16 to 18 h with constant rotation. Approximately 25 ng of the purified fragments were radiolabeled with [^{32}P]-dCTP using the Prime-It II™ Primer Labeling Kit (Stratagene Cloning Systems, La Jolle, CA). All six denatured labeled probe reactions were added to the filters along with 10 ml fresh HYB solution and hybridization was carried out for 48 h at 65°C. Following hybridization, the filters were washed four times with 0.5X SSC and 0.1% SDS, 0.5-1 h each, at 65°C with gentle agitation. Autoradiography was carried out using Kodak Bio-Max™ (Eastman Kodak Company, Rochester, NY) film exposed at -70°C for 48 h.

BAC DNA purification:

Several protocols for isolating BAC DNA were tested including one from the PACBAC Resource Center at the Roswell Park Cancer Center Institute, Buffalo, NY

(http://bacpac.med.buffalo.edu/framebpmini.htm), the PSICLONE™ BAC DNA Kit (Princeton Separations, Adelphia, NJ), and the protocol for BAC Clone Analysis from the Texas A&M BAC Center (http://hbz.tamu.edu/bacindex.html). All of the procedures are similar, except that the PSICLONE™ procedure uses a filter column. In our lab, the protocol from the Texas A&M BAC Center produced the greatest amount of high quality BAC mini-prep DNA. All further analysis was performed using DNA isolated using that procedure.

BAC Insert Size Analysis

Miniprep BAC DNA (1-2 μg) was digested overnight at 37° C. Digested DNA was run in 1% agarose on a CHEF-DR™ II, Pulsed Field Electrophoresis (PFGE) System (Bio-Rad Laboratories, Richmond, CA) with a 5 s initial pulse time, 15 s final pulse time, 6 V/cm, for 16 h. 1X TAE buffer was continuously circulated over the gel and cooled to 14° C using the Model 1000 Mini Chiller (Bio-Rad Laboratories, Richmond, CA).

Results and Discussion:

Figure 3 demonstrates autoradiographic exposures of two of the filters after hybridization. There were several strong positive as well as many weakly positive signals throughout. The double spotting helps distinguish between background spots and likely positive signals.

Since the probes are all single copy PCR based markers, the putative positive clones were confirmed by PCR. Miniprep BAC DNA from strong positives and weak positives were used as the template in PCR reactions with all six primer pairs for the respective markers. The six PCR reactions were run on 3% Metaphor agarose gels along with a positive control templated by JF genomic DNA. Figure 4 shows a Metaphor gel with two of the positively identified markers. BAC 74/P21 amplified with *ABL1* primers is in lane 7 (JF genomic DNA positive control with *ABL1* primers is in lane 8) and BAC 23/J8 with *GSN* primers is in lane 10 (positive control is in lane 16). As is evident from the PCR reactions with BAC 74/P21 (lanes 3, 5, and 9), there was a problem with contamination, possibly from *E. coli* chromosomal DNA, which led to faint bands showing up in multiple lanes. This was a common problem and the PCR reactions were performed several times to confirm the identification. Positive identification was only given when there was at least one test PCR reaction with no background. Even with the occasional background

problem, after several trials it was clear which clones were positive for the markers. Figure 5 is a 1% agarose gel (additional trials were occasionally run on 1% agarose) with 4 putative positive BACs amplified with the six different primer sets (no JF genomic was run on these gels). On this gel there are no background bands and BAC 75/K22 is positive for *ABL1* (lane 12) and BAC 90/B4 is positive for *AK1* (lane 19). BAC 95/C11 (lanes 2-7) and BAC 71/I1 (lanes 20-25) are negative for the six primers tested. This is a clear example of two positives and two negative clones without background. Through this approach, we were able to identify 10 positive BACs representing four of the markers (Table 1). All of the positively identified BACs initially had strong positive signals on the filters, suggesting that the weakly hybridizing spots were due to background hybridization.

There are several possible reasons for the failure to isolate BACs corresponding to two of the markers tested. In this preliminary screen no effort was made to insure that all probes were of similar specific activity, so if a probe happened to be of low specific activity, it might have been obscured by the background of a more radioactive probe. Another possibility is that these markers are underrepresented in the BAC library. The sample screened was theoretically about 4X in coverage, but our lab and others have often detected only one (or no) positives to a particular probe. Regions of the genome very rich or very poor in BamHI sites could have been lost or depleted in the

library construction process. Microsatellite-based probes such as *ROS0020* may be particularly problematic, especially when the original clone is not available, but only the PCR-amplified region. Amplified microsatellite fragments are often designed to be fairly small (for high resolution of alleles on sequencing gels) and, by definition, they contain repetitive DNA sequences that could hybridize widely in the genome. (The actual simple sequence repeat is often found embedded in other repetitive sequences, as well.) We are presently screening the BAC library again with *RING3L* and *ROS0020*, to eliminate the likelihood of low quality probes and will attempt to use poly d(GT)-d(CA) as a competitor to minimize background repeat hybridization. Once the BAC library is expanded with HindIII and EcoRI partial digest inserts, we will also screen this more representative library.

Twenty-eight BACs that gave weak and strong positives on the filters were tested in the above manner. Although only 10 were confirmed as positives, all 28 were digested with NotI (1U per ug) (New England Biolabs, Beverly, MA) in order to test the average BAC insert size (Figure 6). There are several points to note. Gel 1 contains twenty-eight BAC clones isolated using the PSICLONE™ or Roswell method of BAC DNA isolation. This gel exhibits considerable smearing and several BACs do not show up at all. Gel 2 contains the same BACs (except 74/P21) isolated using the Texas A&M BAC Center protocol, and there appears to be less shearing and all BACs

were successfully detected. The 7.4kb band seen in most of the lanes is the pBeloBac11 vector. The average insert size is approximately 150 kb, consistent with previous estimates (H. Zhang, personal communication), with several larger BACs over 200kb. Lanes 11, 13, 15, and 17 of Gel 2 all contain a unique band that is smaller than the vector. These four BACs are 28/C12, 25/D13, 90/B4, and 42/N21, the four positive for *AK1*. These four also share additional larger bands. These shared fragments, especially the common, unusually small NotI fragment, suggest that the four BAC inserts overlap, as might be expected, since they were positive for the same probe. Together the four clones form an initial contig in the *AK1* gene region. As expected, it appears that the BAC clones for *GSN* share common bands, as do the BAC clones for *ABL1* (Figure 6). This suggests that all or most BAC clones isolated and confirmed by PCR do indeed contain the gene of interest and not some partially homologous sequence from elsewhere in the genome.

In order to confirm the overlapping nature of the BACs, a HindIII fingerprint digest was performed on each BAC DNA. The HindIII recognition site is AAGCTT, and it would be expected to produce more bands than a NotI digest. (In 50% GC, random sequence DNA there is about one HindIII site per 4 kb of DNA or around 40 in a 160 kb insert, whereas there would be one NotI site per 65 kb or 2-3 per 160 kb insert.) Figure 7 shows the HindIII digested BAC clones run on a 1% agarose gel (not PFGE). The first four lanes are the BACs

positive for *AK1*, lanes 6 through 8 are the *ABL1* clones, and lanes 9 and 10 are the *GSN* clones. Although, as expected, there are many bands in each lane, it is clear there are common bands among the putative overlapping clones.

As noted above, NotI (recognition site: GCGGCCGC) would be expected to cut random sequence, 50%-GC DNA approximately every 65 kb. However, it cuts most eukaryotic DNA much less frequently, since CpG dinucleotides are unusually rare and the NotI recognition site contains two CpG sequences. In an initial test of the BAC library by the Texas A&M BAC center, 56 random BAC clones were digested with NotI (unpublished results). These BACs were cut on average 1 to 2 times, and rarely three or more (averaging about one NotI cut per 100 kb). The E41 BACs isolated in the current study appear to be cut significantly more frequently by NotI, usually three or more cleavages per insert (Figure 6). This may reflect that these BACs all were isolated on the basis on the gene they contain, and gene sequences, especially promoters, are known to be comparatively rich in GC and especially in CpG dinucleotides (so-called CpG "islands", McQueen et al. 1996, 1998). Another possibility is that since these BACs derive from E41, a microchromosome, and since microchromosomes are known to be GC-rich and rich in CpG dinucleotides (McQueen et al. 1996, 1998), these sequence biases are reflected in the resulting BACs. Obviously considerably more work will need to be done to confirm this speculation.

We have isolated ten BAC clones from our UCD001 JF BAC library. This is an important first step in our long-term goal of building a physical map of the chicken genome. Along with a whole genome approach, we will continue to focus on the microchromosome E41. Figure 8 is a graphical representation of the EL genetic map alongside the BAC clones isolated to date. On-going experiments are aimed at reducing the gaps, especially between *AK1* and *ABL1*, by chromosome walking experiments, as well as screening the library with the rest of the available E41 gene and microsatellite markers. Improved hybridization screening methods and/or PCR-based screening may be required for some of these markers. In addition, the project can benefit from on-going efforts to expand our BAC library and from the use of another chicken BAC library that is now available (Crooijmans and Groenen, personal communication).

Figure 1.

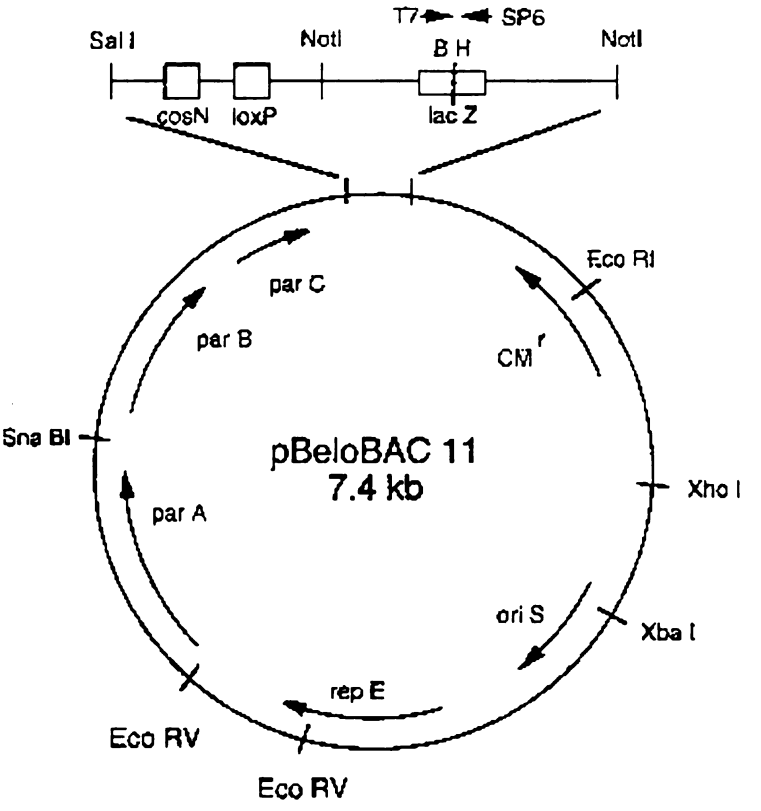


Figure 1. pBeloBac11 large insert cloning vector. B: BamH1 cloning site, H: HindIII cloning site.

Figure 2.

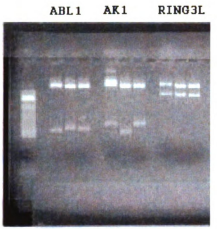


Figure 2. Restriction enzyme testing for three of the gene markers, *ABL1*, *AK1*, and *RING3L*; run on a 1% agarose gel. From right to left the enzyme combinations for each are EcorI, NotI and KpnI, NotI and SpeI. The first lane is a 100bp lambda ladder. In this case any of the three enzyme combinations extracted the entire insert from the TOPO-TA vector for *ABL1* and *RING3L*. The *AK1* insert must have an internal KpnI site, since there are two bands in that column. In the case of *AK1*, either EcorI or NotI and SpeI would be used for the large preparation of the insert.

Figure 3.

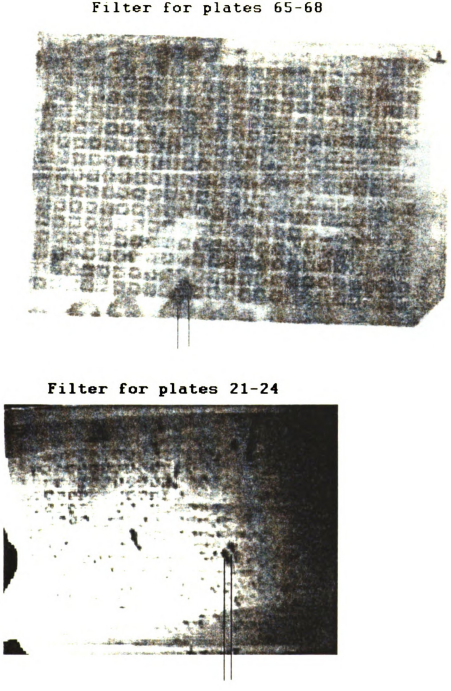


Figure 3. Autoradiographs of the filters for plates 65-68 and 21-24. Lines point to the positive signals (in duplicate) for 67/P10 (*ABL1* probe) and 23/J18 (*GSN* probe) respectively.

Figure 4.

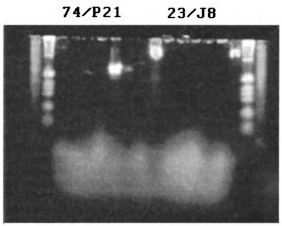


Figure 4. 3% Metaphor agarose gel of BAC clones 74/P21 and 23/J8 after PCR with the 6 sets of primers. Lane 1 and 18: 100bp lambda ladder. Lanes 2 and 17: 1kb lambda ladder. The order of primers for 74/P21: *GSN*, *ROS20*, *L7a*, *RING3L*, *ABL*, JF genomic DNA with *ABL1*, and *AK1*. The order of primers for 23/J8: *GSN*, *ROS20*, *L7A*, *RING3L*, *ABL*, JF genomic DNA with *GSN*.

Figure 5.

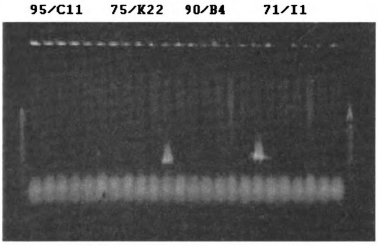
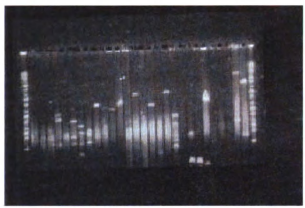


Figure 5. 1% agarose gel of BAC clones 95/C11, 75/K22, 90/B4, and 71/I1. Lanes 1 and 25: 100bp lambda ladder. The order of primers for all: *GSN*, *ROS20*, *L7a*, *RING3L*, *ABL1*, and *AK1*. 75/K22 is positive for ABL1 and 90/B4 is positive for *AK1*. 95/C11 and 71/I1 gave no amplified product for all six primer sets.

Figure 6.

Gel 1



Gel 2

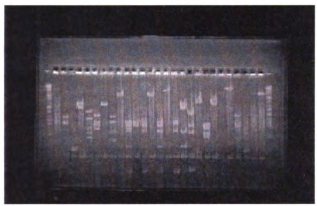


Figure 6. CHEF gels for the BAC clones tested in the study. CHEF gel conditions: 1% agarose, 5 second initial pulse, 15 second final pulse, 6 Volts/cm, 16 hours, 15°C. End lanes on both gels MidRange PFG Marker I (New England BioLabs, Beverly, MA). The Midrange Marker ranges from 15kb to 291kb. Positive clone from the present study on Gel 1: Lane 5- 74/P21. Positive clones from the present study on Gel 2: Lane 3- 23/J18, Lane 7- 22/I10, Lane 9- 98/F13, Lane 11- 28/C12, Lane 13- 25/D13, Lane 15- 90/B4, Lane17- 42/N21, Lane 18- 75/K22, Lane 19- 67/P10

Figure 7.

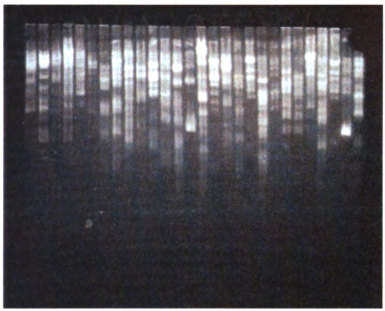


Figure 7. 1% agarose gel of BAC clones tested in this study. All BACs were digested with HindII at 37°C for 16 hours. Positive clones: Lane 1: 90/B4, Lane 2: 25/D13, Lane 3: 42/N21, Lane 6: 75/K22, Lane 7: 67/P10, Lane 8: 74/P21, Lane 9: 23/J18, Lane 22/I10, and Lane 11: 98/F13. The additional lanes are from BAC clones that gave a weakly positive signal on the filters.

Figure 8.

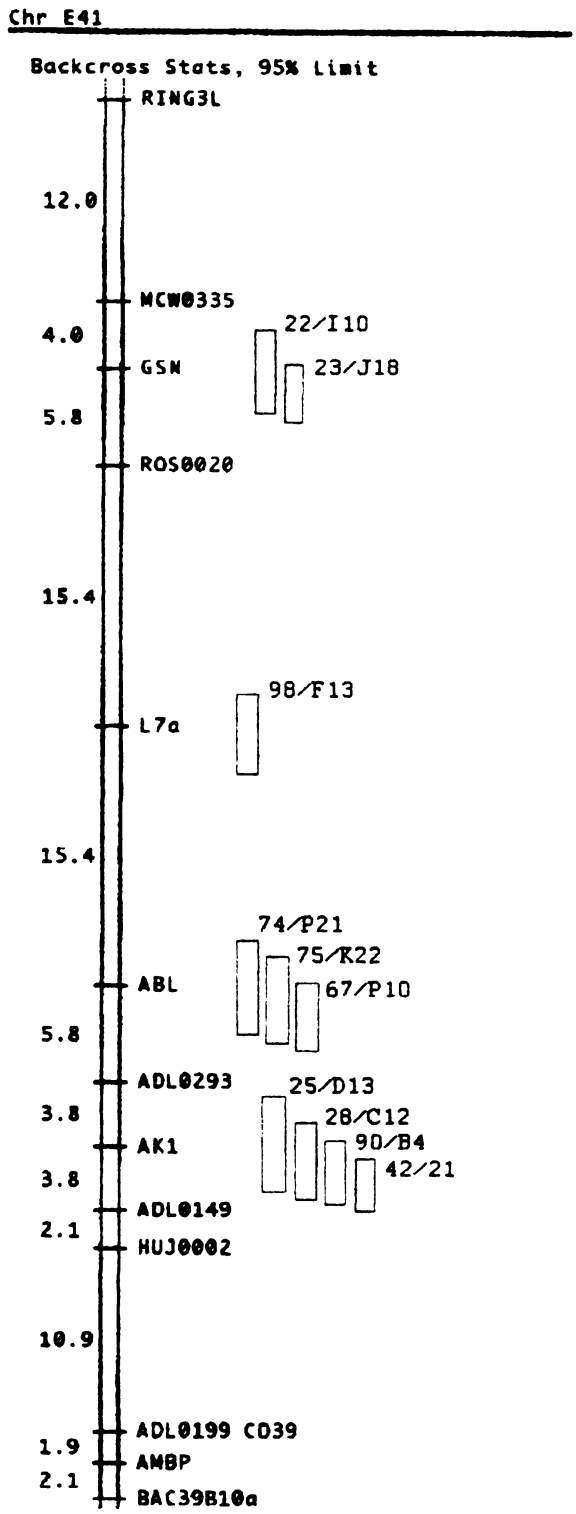


Figure 8. Chromosome E41 (EL reference map) and BAC clones identified in the current study.

Table 1.

Marker:	BAC ID:	Insert Size:
Gelsolin; **GSN**	22/I10	160kb
Gelsolin; **GSN**	23/J10	150kb
Ribosomal Protein L7a; **L7a**	98/F13	140kb
Abelson Murine Leukemia Viral Oncogene Homolog 1; **ABL1**	74/P21	200kb
Abelson Murine Leukemia Viral Oncogene Homolog 1; **ABL1**	75/K22	110kb
Abelson Murine Leukemia Viral Oncogene Homolog 1; **ABL1**	67/P10	140kb
Adenylate Kinase 1; **AK1**	25/D13	250kb
Adenylate Kinase 1; **AK1**	28/C12	200kb
Adenylate Kinase 1; **AK1**	90/B4	200kb
Adenylate Kinase 1; **AK1**	42/N21	100kb

Table 1. Identification of BAC clones on chromosome E41. BAC ID: Plate location of BAC clone. Insert size: approximate insert size based on NotI digest of the clone.

SUMMARY

The work in this thesis began the process of building a comparative map between avians (chickens) and mammals (humans). The comparative map data provided coverage for most of human chromosome 1, human chromosome 9, and human chromosome 4, while other regions were also added to. The regional comparative map data was used to produce an estimate on the mean conserved lengths of segments between humans and chickens (38 ±9 cM) and to estimate the rate of chromosomal evolution between humans and chickens (0.13 ± 0.04). This is a rate considerably less than for humans and mouse, but comparable to other intra-mammalian comparisons (e.g., cat, cattle, pig). The comparative map will be an invaluable tool for identification of potential candidate genes. The comparative map data for human chromosome 9, provides some insight into chicken ZW-type sex chromosome evolution. Two genes mapped in this thesis, *TYRP1* and *VLDLR*, suggest that an ancient sex-determining region has been conserved between mammals and birds. The comparative map for chicken chromosome E41, gave rise to MHC type genes in an area where a QTL for Marek's disease lies. MHC genes are known to play a role in Marek's disease susceptibility and severity of disease. *RING3L* is a MHC gene and was mapped in this thesis to microchromosome E41. *RING3L* on human

chromosome 9, is closely linked to several additional MHC-related genes. Due to the amount of conservation between microchromosome E41 and human chromosome 9, there is a high probability that this group of MHC-related genes are also on E41. This illustrates that the comparative map can already be used to identify potential candidate genes.

Additionally, the building of a regional physical map on microchromosome E41 was begun. Six markers from E41 were tested, two based on genes mapped in this thesis. A total of 10 BACs were isolated covering 4 of the markers tested. This was an initial screening of our newly constructed BAC library and the BACs isolated had an average insert size of 150 kb. These BACs were isolated from microchromosome E41 and appear to have an unusually high GC content. It has been suggested that microchromosomes are gene and GC-rich. Although this was a preliminary test, analysis of several isolated BAC clones, appear to support this theory. The regional physical map will eventually lead to a better understanding of the mechanisms and make-up of microchromosomes. Markers generated in the building of the comparative map were used and will continued to be used to build a genome-wide physical map. The ultimate goal of the physical map will be to align the genetic and physical maps, and to provide sequence data for the chicken genome.

APPENDIX 1: Lack of Polymorphisms in Several Chicken Genes

Introduction:

One of the reasons the East Lansing (EL) reference map has been successful is the genetic diversity between the two inbred lines used to produce the Backcross (BC) mapping population. Previous studies have shown that there is approximately a 1% difference between UCD001 Jungle Fowl (JF) and UCD003 White Leghorn (WL) (Okimoto and Dodgson 1996; Okimoto *et al.* 1997). By using two inbred lines, selected to be as different from one another as possible, Crittenden et al. (1993) hoped to optimize one's ability to identify sequence polymorphisms and to insure that all markers were strictly bi-allelic. Furthermore, a BC mating design was used to facilitate mapping of dominant fingerprint-type markers from the JF genome.

Despite the average 1% sequence difference observed between the UCD001 and UCD003 genomes (Okimoto and Dodgson 1996; Okimoto *et al.* 1997), sequenced blocks longer than 1 kb have been observed with no detectable polymorphisms. As part of the comparative map generation described elsewhere in this thesis, we have identified several other long stretches of DNA, both coding and non-coding, that were not polymorphic. Genes that could be important in filling in gaps on the comparative map were analyzed in detail through sequencing and Restriction Length Fragment Polymorphism

(RFLP) analysis between WL and JF. Although RFLP analysis only samples a small percentage of the flanking genome (those which contain the restriction sites for which we probed), it can efficiently sample large regions of DNA that flank a cloned gene of interest. Our data confirm that near certain genes, sequence conservation between UCD001 and UCD003 appears to extend across relatively large regions of DNA.

Materials and Methods:

Amplification and sequence analysis of gene fragments:

These techniques are described in detail in Chapter 2 of this thesis.

RFLP analysis:

Five ug of both WL and JF genomic DNA were digested with 10 different six-base cutters. Six-base cutters were used because the fragment size should be large enough to extend out of the gene itself. The genomic digests were run on 1% agarose gel at 30 volts for 16 to 18 hours. The DNA was then transferred to Zetabind^{tm} nylon filters (CUNO, Life Sciences Division, Meridan, CT) by the method of Southern (Southern 1975).

Insert DNAs of the cloned 3'UTR or coding regions were used as probes. 25 ng of the purified insert was labeled with [^{32}P]-dCTP, using the Prime-It II^{tm} Labeling Kit (Stratagene, La Jolla, CA). The filters were pre-hybridized overnight with constant rotation at 65°C in 10 ml of 0.263 M Na_2HPO_4, 1% SDS, 1% BSA, 1mM EDTA. Hybridization was carried out at 65°C overnight with constant rotation. Filters were washed three times at 65°C in 0.1 X SSC, 0.1% SDS, for 30 min each with gentle agitation. Autoradiography was carried out at -70°C for 48 h, using Kodak Bio-Max$^{t.m}$ (Eastman Kodak Company, Rochester, NY) film.

Results and Discussion:

Table 1 lists the genes for which sequence analysis failed to detect polymorphisms. The bulk of the sequence data is from 3'UTR and introns. A 302 bp fragment from the Gelsolin gene, containing protein coding sequence and about 100bp of 3'UTR had no polymorphisms between WL and JF. A larger fragment containing over 1 kb of intron yielded four polymorphisms, and the gene was subsequently mapped to linkage group E41 on the EL reference map. The majority of the sequenced regions cover over 800 bp of non-protein coding sequence.

The genes *IRF2*, *TXN* and *KIT* map to 4q12, 4q35.1, and 9q31, respectively, on the human genome. Placement of these genes on the chicken map would fill in gaps in the chicken-human comparative map. RFLP analysis was performed using probes for these three genes in an attempt to identify polymorphisms that could be used to map the genes. However, no RFLP were detected for any of the genes upon surveying 10 enzymes with 6 bp restriction sites. Figure 1 shows the autoradiography for the *KIT* RFLP analysis.

Our results (Table 1) suggest that the observed sequence diversity between UCD001 and UCD003 is not randomly distributed across the genome. For example, based on 1% sequence diversity, randomly distributed, the predicted probability of a 800 bp non-polymorphic region would be

0.03% (the Poisson 0 term = e^{-8}). Although it is difficult to assess statistical significance because our choice of genes and respective regions within genes to be sequenced was not random, it seems unlikely on the face of it that we would have obtained the results of Table 1 on this basis. The RFLP analysis samples fewer base pairs of sequence (12 bp per enzyme tested or 120 bp per observable fragment generated), but it does detect large insertion/deletion events over many kb of flanking DNA. Our limited RFLP results suggest that those genes lacking sequence polymorphism within the gene may be closely related, if not identical, in UCD001 and UCD003.

Based on our limited results to date and those of previous members of this lab (Okimoto and Dodgson 1996; Okimoto *et al.* 1997; Levin, unpublished results), we conclude that the UCD001 and UCD003 genomes show substantial linkage disequilibrium. The most likely explanation is that the UCD001 genome is not purely of Red Jungle Fowl origin. Inadvertent contamination of the line may have occurred by modern chickens (most likely, White Leghorn). This may have occurred in the early stages of developing the UCD001 line. Wild JF are difficult to breed in captivity, so WL traits/genes that were initially rare in the flock may have been highly selected. Furthermore, the inbreeding process itself and the likely narrow origins of the UCD001 and UCD003 lines would tend to promote linkage disequilibrium. The high level of interfertility observed between UCD001 and

UCD003 (Crittenden *et al.* 1993) also suggests that the two genomes have very few major chromosomal rearrangements with respect to one another, and that they are likely more similar to one another than might otherwise have been expected. Thus, the observed non-random distribution of polymorphism between the two lines is not surprising.

Table 1.

Non-polymorphic gene sequences:

Gene:	Genbank accession:	Region(s) sequenced:
CYSTEINE- AND GLYCINE-RICH PROTEIN 1; CSRP1	X73831	~300bp 3'UTR
PARATHYROID HORMONE-LIKE HORMONE; PTHLH	X52131	~800bp 3'UTR
CONTACTIN 1; CNTN1	X14877	~600bp 3'UTR
ANTI-MULLERIAN HORMONE; AMH	U61754	~800bp 3'UTR
NATRIURETIC PEPTIDE PRECURSOR A; NPPA	U.Del. cDNA	~1kb coding region (700bp: 1 intron)
GELSOLIN; GSN	AF042795	300bp coding region (100bp: 1 intron)
PHOSPHODIESTERASE 6C, cGMP-SPECIFIC, CONE, ALPHA PRIME; PDE6C	L29233	~800bp 3'UTR, 800bp coding region (500bp: 1 intron)
INTERFERON REGULATORY FACTOR 2; IRF2	X95478	~1kb 3'UTR
THIORODEXIN; TXN	J03882	~2kb coding region (1.5 kb: 3 introns)
V-KIT HARDY-ZUCKERMAN 4 FELINE SARCOMA VIRAL ONCOGENE HOMOLOG; KIT	D13225	~1.5kb 3'UTR, 1kb coding region (700bp: 2 introns)

Table 1. Non-polymorphic sequence data for genes listed. U.Del. cDNA: cDNA sequence from the University of Delaware cDNA library (Burnside and Morgan, http://udgenome.ags/chickenest/chick.htm). Genbank accessions are fron the National Center for Biotechnolgy Information Genbank database.3'UTR: 3' untranslated region.

Figure 1.

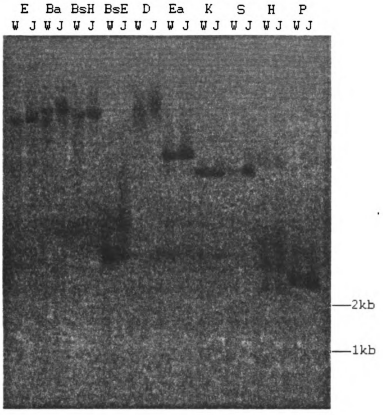


Figure 1. Filter with White Leghorn (W) and Jungle Fowl (J) genomic digests. Filter was probed a dctP^{32} labeled *KIT* 1.5kb 3'UTR fragment. Restriction enzymes: E= Ecor1, Ba= BamH1, BsH= BspH1, BsE= BspE1, D= Dra1, Ea= Eag1, K= Kpn1, S= Ssp1, H= Hind111, and P= Pst1.

APPENDIX 2: Primer Pairs to Sequenced Chicken Genes

A set of 300 PCR primers pairs, designed to amplify previously sequenced chicken genes has been developed. The cDNA sequences for these genes were taken from the National Center for Biological Information Genbank database. Primer pairs were designed using the PrimerSelect^{tm} PCR Primer & Probe Design program within the Lasergene Biocomputing Software (DNASTAR Inc., Madison, WI) Primers were optimized to have similar melting temperatures (T_m) and to minimize any propensity to contain hairpin loops or to generate primer-dimers during amplification.

Among other possible uses, this collection primarily is designed to be used in Reverse-Transcription PCR (RT-PCR). RT-PCR is most successful when the primers are within the coding region, away from the 3' end. The 3'end of genes may include untranslated sequences, which may produce secondary structures that can interfere with primer annealing. Therefore, all of the primers in this set amplify from within the coding region of the gene. The sequences of the 300 primer pairs are listed in Table 1, along with the gene name, locus symbol, and the RT-PCR product size.

The majority of chicken gene sequences now available are from cDNA clones, so cDNA sequences were used for all genes within the panel to maintain consistency. The region amplified by any given primer pair may include one or more introns which could interfere with successful amplification

from genomic DNA templates. However, at least several of the primer pairs may also be successful using chromosomal DNA templates, especially if conditions are optimized for long PCR product amplification (Cheng et al. 1994; Barnes, 1994). As an example, the primers for Matrix GLA protein (*MGP*, Table 1) have been used to amplify Jungle Fowl and While Leghorn genomic DNA. The RT-PCR product size is 298bp (Table 1) and the genomic product is over 2kb, due to intervening sequences. The genomic product was confirmed by sequence analysis to be *MGP*. *MGP* was mapped on the East Lansing Reference map to chromosome 1, position 151.8.

These primers, and subsequent gene primer panels yet to be synthesized, are being provided free of charge to interested users as part of the USDA-CSREES National Animal Genome Research Program Poultry Coordination effort. They are designed to be useful in analyzing transcription levels by RT-PCR, generating probe DNAs for microarrays, and cloning and sequencing portions of candidate genes (either from cDNA or genomic DNA) in hopes of locating a useful polymorphism for genetic linkage analysis (such as demonstrated for the *MGP* gene above).

Table 1. **Primer Pairs to Sequenced Chicken Genes**

Locus Symbol:	Gene Name:	Genbank ID:	Product size:	Primer 1:	Primer 2:
AANAT	Arylalkylamine N-Acetyltransferase	1781379	546	GCAGGGCCCCCGCAACTC	GCATGGCCCCGCACCTC
ACAC	Acetyl-CoA Carboxylase	2170499	645	GCGGGCACGGCAGGTTCTCATT	TCATCATCCACGTCCCCATCAGTT
ACTN1	Actinin, Alpha-1	517084	622	CAAGGAGGGGCTGCTGCTGTGGTG	GAAGGCGGGCCGGTTGCTCA
ACVR2A	Activin A Receptor 2A	505347	553	ACAAGGTTGCTGGCTGGATGACA	AATGCTGGTGCCTCGCTTCTCTG
ADHF	Alcohol Dehydrogenase F	2326999	582	AGGCTATGGGGCTGCTGTCA	ATCACGGTGCGAATGCTTTTG
ADPRT	ADP-Ribosyltransferase	1638784	709	GACATGGCCCTGAACTCCTTTGAT	GGCCCCGACCCCACTGC
AGTR1	Angiotensin Receptor 1	1763531	589	CTGGCTCCTTGCTGGTGTGG	GGGCAAGCGTATATTTTCTGGTG
AK1	Adenylate Kinase 1	222785	656	GATGGCAAACTCCTGGGGGTGGTG	CTCGCGAGGGTAGCCGTCAATG
AKT1	Serine-Threonine Protein Kinase, Oncogene AKT1	2745888	603	CCGGACGGTATTATGCTATGAA	ACAAAGTGCGTGGAAATCTAATCT
APE	Aminopeptidase Ey	2766186	592	GCCGCCCCGCAGCCATTG	TGCAGCCCCTCCTTGAACACATCT
APH	Aminopeptidase H	1850771	576	GCCCGTCACCAACCAGAAGAACTC	TAGAACTGCACCGGTGTCATAGGA
ASCL4	Achaete-Scute Complex (Drosophila) Homolog-Like 4	1905985	282	GTGGCCCGGCGCAACGAACG	GGAACAGGGCGAGGCGGAGGAATA

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
ATOH5	Atonal Homolog 5	2760442	322	AGAAGTGG ATCAGGCT GTGTTGTG	TTTGTCCCG TATAATGGT GGTAGC
AVR	Avidin	450255	651	GCCCGCCA CTGACTCC TTCTTCTT	GATGAGGG GAGTGGGG TCGTGAGC
B2M	Beta-2-Microglobulin	757849	325	AGGCGGCG GCGGTGGT G	TGCGGCTCC TTCAGGGTC TCGT
BBC1	Breast Basic Conserved Gene 1	516683	460	CCCATCCG GCCCATCG TGA	CGTCCTGCT CCGCCGCTT CTTTG
BKJ	Beta-Keratin Related Protein	2209150	215	TGCGATCC AGCCCCCA CCAG	TGCCACGTC CCAGAGTCC CACAG
BMP1	Bone Morphogenetic Protein 1	2852122	536	GGACACCG CGGCAGGA AGGAGT	GGGGGCCC GGGGGACC AGTAG
BMPR2	Type 2 Receptor for Bone Morphogenetic Proteins	2351082	641	GCGCCCAG GTGAGGAA GATAAT	TCAGCGGC GTAGTGGAC AT
CASP1	Caspase 1	2642240	489	CGGGACGG AGCTGAAG TGGAC	GGAGACAGT ATCAGGCGT GGAAGA
CDC37	Cell Division Cycle 37	2655421	653	CAGGCCCG CGTGGAGA GGATGGA	TGGTCGGC GGTCTTGAT TTTGGTG
CDC42	Cell Division Cycle 42	1127799	403	GTTGTGGG TGATGGTG CTGTTGGT	CCGTCTCTG GAGTTATGG GCTTCT
CDH10	Cadherin 10	1841295	649	CATCCACC TCCGCATT CCTG	AAACTGTGG GGCATTGTC ATTA
CDH4	Cadherin 4	222854	676	AGCGCCGT ACTTCCCAA CAAACCA	TGCAGCAGC CACCGCCCC AATAG

Table 1. Cont.

Locus:	Gene Name:	ID#	Size:	Primer 1:	Primer 2:
CDH6B	Cadherin 6B	867998	556	TCGGTTCC CCCAGAGC AC	CAATGTTTC CCGGTCAA GAGTTTT
CENPC	Centromeric Protein C	2749772	520	AATCGCAC CATCATCAC CTTCTCC	ATCCTCCC TTGGCATC ACCCTTCT
CFRA	CFR-Associated Protein	2737970	587	GGTTGCCA TTGCCTGT CA	CCTGCCCG TGGTAAAG TCC
CHOR	Chordin	2826738	594	GCCGAGCC GTGTGCGT TTCA	CTGCGGCG GGCGGTAA TGGTG
CHRND	Cholinergic Receptor, Nicotinic, Delta	211060	665	GGCGGTGT CTGTGTCC CAACTG	AGCCCGTC CTGCCCCT ACTCA
CHRNG	Cholinergic Receptor, Nicotinic, Gamma	211061	628	CCAGCCCC GCACATAA CTCATCC	TTCCCCATC CCCTTGCA TCACTTA
CL	C-Type Lectin	1142649	709	GCGGCTGT GGTTCTGG GGTCCTT	ACGGCGCC GGTTTGAT GTTCC
CNBP	Cellular Nucleic Acid Binding Protein	2232216	402	GGCCGTGG TCGTGGGA TGAG	GCCGCAGC GATAGCAG TTGAC
CNP2	Cyclic Nucleotide Phosphodiesterase 2	2760607	625	AGGCCGGC CAGGTGTT CTTG	CCCCGTTT GTTGGTGC TCTGTGTA
COCH5B 2	Cochlear 5b2	2293561	522	TGGGCTTC ATCTTCTCA G	GGCCACTA TACCAGCTT CTTCTA
COL1A2	Collagen, Type 1, Alpha 2	2587064	150	GAGACAAA GGGCCACA GGGAGAA	AGTCAGCC GCTTTAGAT GGAT
COL6A1	Collagen, Type 6, Alpha-1 Chain	576463	678	TACTTCCG CTGTGACC GCTTCCT	TTTGTCGC CCTTCATTC CTTGGTA

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
COL9A3	Collagen, Type 9, Alpha-3 Chain	211040	688	AGCCGGGC CCTTCTGGT TTG	CCTTTGGG CCCTCTGA ATCCTT
COLL5	Collapsin 5	2522205	534	AGCAAAGTG AATGGCGGT CTGTA	TTGTCTGC GGAAGCG TCTCT
CRES	Crescent	2226371	652	CTGCTGCTG CGCCTGCTG CTGTG	GCCTGCCT GGGATTTC GGTTCTGA
CRYAB	Crystallin, Alpha-B	1143827	416	GTTGACACC GAGCCGTAT CTTTG	GCTCAGG GACGTCG CTTTGTTT C
CRYBA4	Crystallin, Beta-A4	695157	404	TTCCAGGGG CAGCAGTTC GTGTTG	TGCTGGAC CCTGCGG ATGGACTG C
CSNK1A L	Casein Kinase 1 Alpha L	2828155	495	ACGGGGAG GAAGTTGCT GTGA	TGTACGGT ATGTGTTG CCTTGTCC
CTNNB	Catenin Beta	2511455	555	ACTGCGTGA ACAAGGTGC TATC	GGGCGGT ATCCAAGA GGTTC
CYP11A	Cytochrome P450, Subfamily 11A	1906770	578	CCTACGGCG TGCTCCTCA AGACAG	CACGCCG CCCGCCAT CATC
DAD1	Defender Against Cell Death 1	2149246	343	ACGGCGGG TTCGGGTGT GG	CGACGAG ATGCAGGA TGGTGTTG G
DNMT1	DNA Methyltransferase 1	1374774	739	CAGCGGTG CCGTGAAGC CCATCTA	GCCGCTG CCCCCAAA CTTCACCA T
ELN	Elastin	2169751	278	TCGGGGTG CAGCCTGGT CGTAAGC	TGCGCAG CCAACTCC ACCTCTAA A
ENO	Enolase	2842530	581	ATGTGGGTG ACGAGGGA GGATTT	GCAGGGG GCACCAGT CTTTAT

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
EPHA5	Ephrin Receptor EphA5	555617	602	ATTGCCCTG GTCTCTGTG CGTGTG	AGGCCGGT TTGCTGGG GGAGGTA
ETK	Eph-like Tyrosine Kinase	2462301	558	CAATGGGG GAAGAAATG ATGTG	GCGGTGGC TGTGGCTT CTTCT
FASN	Fatty Acid Synthase	1842199	430	CGCTCCGCC GGGGTGAA CGAC	GGGGGAGG GGACGGAG GAGGAGAA
FECH	Ferrochelatase	2323274	536	GAGCCAGAA ACGCGGAAA CCTAAA	TCGGCAAA GCACTGGA TGAGCA
FGF10	Fibroblast Growth Factor 10	2911145	506	CCTTTTCCC ACCTGCCTT GTTG	CATTTGCCT TCCATTGTG CTTCC
FSHR	Follicle-Stimulating Hormine Receptor	1827499	632	TCGGGCCTG TTGTTTTGG ATA	CCGGCTTT TGGTCTGG ATA
FTF	Fetoprotein Transcription Factor	2541857	543	GCCTTGCCT CCCACAGAC TATGAC	TTGCCCGG TAACCAGA AGGATG
FUT1	Fucosyltransferase 1	1657998	649	CCCGAGGG CGAGGTGA CG	GGGGGCCG AGGACAAC AGG
FZD7	Frizzled 7	2655275	570	CGGCGGCG CATCACGAG	CCAGGAAG CGGTAGCC CAAGTAGG
GBX2	Gastrulation and Brain Specific 2	2554936	516	CCCCCGGC CACTTCGTT CTACACC	GCGCGGGG CCGTTCTC GTC
GCG	Glucagon	2171808	390	CAGCTGGCA AAATCCTCT TCA	CTTCCTCGT CCATTCACT AACCA
GFRA1	Glial Cell Line-Derived Neuro-trophic Factor Receptor Alpha 1	2213802	597	AGGGGCAT GAAGAAGGA GAAAAAC	AATGAGCC CCGAGTAA GCGAGGAG

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
GIC	Gicerin	1009246	663	AGCTTCCCG TCCCCCAAC ATCACC	CCTGCCCC TCGCGTCC CTCCATT
GLI3	Gli-Kruppel Family Member 3	1932736	776	AGCCGAAAA CGAGCCCTG TCTATC	AACGTGCA CTTGTGGG GCTTCTCT
GUCA1A	Guanylate Cyclase Activator 1A	1839476	472	GGCCAGCTC ACCCTCTAT	GGTTCTTC CCATCGTT CTG
H2B	Histone H2B	2696697	487	CTCCTAATT TGCATACCG CCTCTA	TAATCCGC ACCGCTCT ACTTG
HD1	Histone Deacetlyase 1	2829213	617	AAGGCGAAC GCGGAGGA GAT	GACCCGC ACTGCAGG ACAACT
HD2	Histone Deacetylase 2	2791685	550	GGGCGGTA AAATTGAAC AGACAGC	TATCCACC TCCTCCTA ACATCAGC
HD3	Histone Deacetylase 3	2791687	668	CAACAACAT GCAGGGCTT CACCAA	ACCCGCCT CCTCCCAG CACCAGTA
HGF	Hepatocyte Growth Factor	1419543	574	GACCATGCG TTTGATCTG TTTGAA	GCCCCTG GATGCATG TTGTTGTC
HMG1	High-Mobility Group Protein 1	391635	533	TGTCTGCCT ATGCCTTCT TTGTGC	ATCCTCCT CCTCCTCT TCGTCCTC
HMG14	High-Mobility Group Protein 14	1160514	103	TCCGCCAAA CCTGTGCCT GAC	GCCCCTTT TTCCCCTT TGATTGAG
HOX7	Homeo Box 7	464146	538	CGATGGGC GGCGAGGA GGAG	TTGGCGC GGCGGTT CTGGA
HOXA2	Homeo Box A2	415799	706	CCATCGCTT GCTGAGTGC CTGACA	GAAACGC GTAGCCCT CCCTCTCC A

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
HPER1	Histone Phosphatase of the Endoplasmic Reticulum	2795771	641	GGGCGGCT CCTTCGGCA CCAA	CCGGCAG ACACAGG GCACTCG
HRY1	Hairy 1	2674153	494	CAAGCTGGA GAAGGCGG ACATC	CGTGGCG TTGGCGTA GAGTG
HSD17B 1	17-Beta-Hydroxysteroid Dehydrogenase 1	1944048	545	ATTCGGCAC CGCACGCA CCATTCA	ACGGCTG CTCCTCGG CGGCTTCA C
HSD17B 4	17-Beta Hydroxysteroid Dehydrogenase 4	2315980	523	GAAGGCCG AAAGTATAA CATCCAC	ACATTCCC ACACCAAG AGCATACA
HSPE1	Heat Shock Protein 10	2623878	268	AAATTCCTT CCCCTGTT	GAATGTCA CCGTCTCT AAA
IAP1	Inhibitor of Apoptosis 1	2656126	622	TTGGCTATT TCAGTGGCT CTTTTT	AACCTGGC CTGAACTT GACTTACA
IBSP	Integrin-Binding Sialoprotein	600155	471	GCCACTGCC TCCGCCTTC TC	CTCAGCTG CCACGGT GTTGTTCC
ICH1	Cysteine Protease ICH-1	1490877	570	CCCTAATGC CTTTTCAGC CTTCT	TTGCCATC AGTGCCAT AAACC
IGF1R	Insulin-Like Growth Factor 1, Receptor	2808532	888	TGGCCTGCC GCAATTACT ACTACG	AGCCGCA CGCATCTT GTCCAT
IL2	Interleukin 2 Precursor	2645805	328	ACTGATCTT TGGCTGTAT TTC	ACTTCCGG TGTGATTT AGAC
IREB1	IRE-Binding Protein 1	473700	664	TGGGCACTG ACTCGCACA CC	TCAAAGTC GCAGCCCT CAAAGTTA
IRK1	Inward Rectifier Potassium Channel	2460311	550	ACGATCGGC TATGGCTTC AGGTG	GGTGGCC CCAGAGG ATTTCATTT

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
ITGA1	Integrin, Alpha 1	2582829	560	CAGACGCTA CTTCAGTGC CTAACG	TTCGCCAT CAGTCACA ATAACCAT
JAK	Janus Tyrosine Kinase	2645986	527	GCACGGGC CCCTGGACC TCTACCT	TGTCGCG GATGATGG CACGGAA GC
JNK2A1	c-Jun N-terminal Kinase2 Alpha 1	1816447	590	TTGGGATAA ACGTTGCTG TAAAG	GGGCGTT CCTAGTTG CTCA
KCNMA1	Potassium Channel, Calcium Acitvated, Large Conductance, Subfamily M, Alpha Member 1	1907288	684	GCCATTACG AGCCAGCAA CTTTCA	TGGGGCG TGCTGTAA CCTCCTCT C
KS5	KS5 Protein	2827449	504	AATAGAGCC CAACTTCAG CAAAAC	AGTATCCC CAACAAAA GCATCAAA
LAMB2	Laminin Beta 2-Like Chain	2708706	548	CCCCCGCG CCGCATTGA CG	TCGCACAC GGCCCCG CTGGTATT C
LAP18	Leukemia-Associated Phosphoprotein p18	63796	358	GCGGATTGC CTCGGTCAT	TGCCTCAC GGTTCTCT TTGTTAG
LEP	Leptin	2406649	353	GACACCAAA ACCCTCATC AAG	CTCAAAGC CACCACCT CTGT
LFNG	Lunatic Fringe	2183042	601	GCCGCCAG CCGAGGAC ATCAC	CTTCACGC CCAGCAC GGACTCG
LHR	LH Receptor	2662292	600	GACTGCCGC CTCTGGATA AAT	TGTAGTAC TGCCCGCT TGTCTGAG
LIMH	LIM Homeodomain	2340818	547	ACATGCGCT GCCTGAAGT GCT	TTGCTGGG GTATTGCT GGTCTCTA
LUM	Lumican (Keratan Sulfate Proteoglycan)	2570518	544	TAAGGCTGG CTAGAAACA AAAT	TCCAAGCG AAGATAAC GAA

Table 1. Cont

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
MAFB	Musculoaponeurotic Fibrosarcoma Oncogene Family Protein B	516723	717	TCAGCCCCA CCGAACAGA AG	ACATGAAG AACTCGGG GGAAGAC G
MAFF	Musculoaponeurotic Fibrosarcoma Oncogene Family Protein F	439705	368	CGCTGCTGT CGGATGAGG A	GTTGGCAC CAGACTTG ACGAT
MAFK	Musculoaponeurotic Fibrosarcoma Oncogene Family Protein K	439707	401	ATGCCCCAG TGCTGAGCG ATGATG	TGAGAACG GCACGGA ACTGGATG A
MAFL	L-MAF, bZIP Transcription Factor	2645968	466	AACTTGACC CCGGAGGAT GCTGTG	CCCGCGC CGCCAACT TCTCGTA
MC1R	Melanocortin 1 Receptor	1065994	584	GGCCGCCAT CCTCAAGAA CAG	GCCCCCA GCAGATGA AGAAGACT C
MC2R	Melanocortin 2 Receptor	2696657	649	GGTCGTGGT GCCAGAGGA AGT	GGCCCAA CAGCAAAG GAAGAC
MCT3	Monocarboxylate Transporter 3	2198806	595	CATCGGGCT GGTCCTACTT AT	TGTTCTGG CAGCCTTG ATTGAC
MFH1	Mesenchyme Forkhead 1	2072323	785	AACCCGCCG CCCCCAAGG AC	GCTGCAC GCCGCGC TGTAACC
MGP	Matrix GLA Protein	2598420	298	TGCGTGCTC TCATCGTCCT T	CTCCTCCC AAAATAGT GCCTGTAA
MMP115	115-kDa Melanosomal Matrix Protein	1655466	678	GGGACGGCG CGGCAGAAC GACT	GACGGTG GGGCTGC GGGAGAT GAG

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
MP	M-Protein	222832	533	ACGGGAAG CTAACCATA AAAACTG	AAATATTGC CCTCCTCAT CCACAC
MSTN	Myostatin	2623569	526	CATGCCACA ACCGAGAC GATTAT	TCAGCGGG TAGCGACA ACAT
MUARP1	Mu-Adaptin-Related Protein	1929344	773	CGGGAGGG GCGGCACTT CGTC	CTCCCCGC CGGCTCCC ACTCCA
MYBPC3	Myosin Binding Protein C, Cardiac	1110448	586	GTGGTGGCT GGGAACAAA CTGAG	GCCGGGAC ATGCCAATA GA
MYF6	Myogenic Factor 6	222834	617	AACCGGCTC CTATTTCTTC TACTT	AGGCCGAC GACTCCAC CAT
MYLK	Myosin-Light-Ploypeptide Kinase	992992	671	AGAAGCCCC CTGCAGAGA ATGG	GGGAGTAG CTGCTTTTG GAGGAGT
NEL	Nel Gene	1483183	514	CACGCTTTG CCTTCTCCT CT	GGGCTTCT CCACAACT CTTTCATA
NEU	Neuropilin	10600870	525	AGCCCCATC ATTTACTCG CAGAA	CCAGCAGG CACAGTAC AGGACAA
NFKB2	Nuclear Factor Kappa-B, Subunit 2	755083	411	CGCCCTTGC ACCTCGCCA TCATCC	CGCCGTTC ACATCCGC ACCCTTCC
NKH1	Hyperglycinemia, Isolated Nonketotic, Type1	222820	763	GCCGCGGC ACGATGACT	GGAGCTGC CCAGGACA ACA
NKH2	Hyperglycinemia, Isolated Nonketotic, Type 2	222867	521	CATGGAGG GCAGAGCA GCAGAACT	GGGGCCCC ACCGATGT CAGC
NPPA	Natriuretic Peptide Precursor A	2170460	303	CAGCCCAG CAGAGCCAA CC	GCCGAAGC AGCCAGAA TC

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
NRTRA	Neurturin Receptor Alpha	2213804	627	GGGCTGGC CGAAGGAG AAGAGTT	GCAGGAGC ACCAGGGC GAGATAGT
OAZ	Ornithine Decarboxylase Antizyme	2317775	577	CCCTGCAGC GGATACTCA AC	TGGGGACA AGGGGATG C
OPOML	Opioid-Binding Protein, Cell Adhesion Molecule-Like	2897596	411	GATGGCCG CACTCCTCC TCTT	GCCAGCCG CTTGTCGT CTTT
PAD	Peptidylarginine Deiminase	2897752	580	GCTGGGCC GCATCCTCA TTGG	TGCCCGCA CCCGCTCC TC
PARA	Paranemin	2828800	679	AGCGCCTG GAGTAGCAT CTTTG	CAGCCCCT CCTCGGTG AACT
PAX6	Paired Box 6	2576236	660	CCCAGGGC GATCGGAG GTAGTAAG	GATGGGGA TGTGGCTG GGAGTGTT
PAX7	Paired Box 7	2576238	510	GCGCCCACT GCCCAACCA CATC	CTGCGGCG CTGCTTCCT CTTCAAA
PC2	Protocadherin 2	2196557	557	GCTGTACCC CCTCCCGAA CTCCAC	AACCACCC CGCACGGC ATCAACAT
PG	Pepsinogen	2760810	646	GCACCCCAC CGCAGGACT TCACT	GCCCCGAC TGCGCTTT GGATG
PHOX	Paired-Related Homeobox	222850	382	CCCGGCCG GAGCTTGTT GGAGTC	TGGGTCTT GGAGCTGG GCGAGGTA
PI3K	Phosphoinositide 3-Kinase Catalytic Subunit	2245505	571	TAAAGGCCG GAAGGGTG CTAA	CATTGCTTG CTCTGGCT TGATT
POU1F1	Pou Domain, Class 1, Transcription Factor 1	2842418	122	AGGAAGCG CAGAACCAC CATA	TTCTCAAGA TTAAGCCC CTCAGC

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
PPP2C	Phosphatase 2A Catalytic Subunit	517097	467	GAGCGCATT ACAATACTGA GAGGA	TTGGGCG CACTGAAA ATGG
PRG1	Proteoglycan 1	222846	501	CACGCTACA ACCGAATCA GGA	GCAAACG GGGCAGA CACATA
PRL	Prolactin	2170496	641	TTTCTGGCG GTTCTTCTGG TCTC	TGGATTAG GCGGCAC TTCAAAA
PRLR	Prolactin Receptor	222848	535	GGTCCCAAT TCCTGCTACT TCAA	GATGCCCT CCGTCTAA ACCAG
PRNP	Prion Protein	212610	521	CAGGCTGGG GTCAAGGCT ACAAC	GGTGGTG AGGAGGA GGAGGAG GAC
PROA	Pro-Apototic Protein	2599491	542	ACGGCGAAG AGTAAGGCG GCTAAG	AGAAAGGC GGCAAGG GAGGAAAC A
PSAP	Prosaposin	2077897	588	AGACTGCATT CGGCTGGTT ACTGA	ACTGGCTG CTGTGGAG GCTTGTT
PST	Polysialyltransferase	2749959	535	AGCGGGTGT GGAAAAGAG ATTGAT	TGTGAGG GCTGGCAT TAGAAAAG T
PTPN1	Protein-Tyrosine Phosphatase, Nonreceptor Type, 1	2058554	733	GGCCAAACA TCCCAGAAA	TGCCCCCT CAATAACA GC
PTPRA	Protein-Tyrosine Phosphatase, Receptor Type, Alpha	475901	881	ACACCGTCT GCCCTGCTT ACTGC	AGGCGCT GAGGCTTC TTGTTCGT
RAB5	Rabaptin-5	2329852	517	GCGGACGCA ACTTATGGA	AGATTTCT GCGTGTTG AGGAC

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
RAPSYN	Acetylcholine Receptor-Associated Protein	2257843	682	CGCGTTTT GGGTTGCC TCATCACT	AATCGTACC GGGGAAAG GCTGTCT
RDS1	Peripherin 1	2642233	571	CTCCGGAA GCGAAGCG AAGTGAT	TGTAGTGAG CCGAGTTGT TGGTGA
RGDC	RGD-CAP/beta ig-h3	2257600	643	CGCTGGGC TGACGATG GAG	GGTACCCG GCCTGTTCA AGTTCT
RHO	Rhodopsin	222856	507	CATCATGG GGGTCGCG TTCTCCT	CCATGCGG GTCACTTCC TTCTC
RPL30	Ribosomal Protein L30	397823	570	GTGGCCGC AAAGAAGA CG	CAGGGCATG CACTAATAC ACG
RPL37A	Ribosomal Protein L37A	222865	511	CCGGCTGC GTTTTGTCT CCTCAC	CCTCCCCTG GGTATGCTG TATGG
RPL5	Ribosomal Protein L5	222858	594	ACGTCCCT GCCGCCCC ATCC	GCACCACAT AAGCCAAAG CCATCC
RPL7A	Ribosomal Protein L7A	457652	499	CACCTGCC CCTGCTGT AGTCAAG	CTCAAAACC GTTCCCAAT CCACAC
RPS4	Ribosomal Protein S4	402295	572	CCGCGGCC CGAAGAAG C	CGATCCGGC CCAAGTTAG CAC
RREB1	Ras-Responsive Element Binding Protein	2772826	613	GCGGCGAG GATCTGAA GCATTAC	GCACCAAAG CCCAGAGGA CACT
RSN	Restin	2338713	694	TCTGTGGC TGGAGTTC GCTATTT	TACGGCTCG GCTTGCTGC TTAC
SCF	Stem Cell Factor	391648	459	AGCCTGCC TAATCACTG TTGG	TGCCTCTTT GTTACTGTT ACTGCT

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
SCYC1	Lymphotactin Precursor	2827881	205	CTCCACGCC ACAGTTCTC	TGATACCAT TTGCAGTG ATAA
SERC1	C-Serrate 1	1236280	685	GCAACACTG GCCCCGATA AATACC	AAGCACTG GGCACCGT TCTGG
SF1	Steroidogenic Factor 1	2541859	576	AACCCCGCC GCCCTGACA CCT	CTGCGCCG CCACTGCT GACA
SIAT8	Sialyltransferase 8	1763266	594	CCCTCGGC GTCTTCGTC CTCTG	GGCCCGTC CCCGTCTT CATTG
SMP1	Smooth Muscle Prot. Phosphatase Type 1-Binding Subunit	2198741	547	CAGGCTCCG GGGCTCGC ACTC	GTACGGGG CTTGGGGC TCTGAATG
SNF2L2	Sucrose Nonfermenting, Yeast, Homolog-Like 2	996019	783	GGCCAAACC CCAGATATG AGTGTC	CAGGCGTC TATCTTTCT TTTGGTC
SOD	Superoxide Dismutase	1142717	322	AAGGCCGT GTGCGTGAT GAAGG	TGCAGTGT GGTCCGGT AAGAGAAA
SOX2	SRY-Box 2	849043	469	AATGGCCCA GGAGAACC CGAAGAT	CTGCGAGC TGGTCATG GAGTTGTA
SRC1	Neural SRC Interacting Protein	2582523	454	ACCCCGTTA CTTCCGCAG CATCTT	GAACAGGC AGGTCTTG AGGCAGTC
STX1B	Syntaxin 1B	2564017	502	GGGCCTCAA CCGCTCCTC	CACCCCAA GAACAACG ACGAAAAT
SULT	Sulfotransferase	2687359	513	TTTGAAGCC AGAAGTGAT GATGTC	TCCCAGGG TTTGATTCT CTTTTAG
TAD	Thymocyte Activation and Developmental Protein	2665789	546	GCACGCCGT TCAGAAGTA AGATG	CAGGGATG TGGTGAGC AGAGGTA

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
TBP1	TATA-Binding Protein 1	1183016	667	ACAGCTTG CCGCCCTA CG	ACATCACAG CTGCCCACC AT
TCRG	T-Cell Receptor Gamma Chain Vg1-Jg2	2707426	489	GGCACCCT GAGAAGAA TG	TAAATCCCAG TAGGCACAG TAGTA
TENP	Transiently Express in Neural Precursors	2599571	568	CACCAGG GAGGCAG AAAGCAAG TC	TGAGCCCGC CCCAATGTG AAC
TFAP2	Transcription Factor AP-2	2289947	561	GGTCTTCG GCGGGGT GGTGA	AAGCCGTGC GAGATGAGG TTGAAG
TFT	T Brachyury	2529385	695	TCGGCGC CCACTGGA TGAAGG	CGCCGGGGT GATGGTGCT GTTACT
TGM2	Transglutaminase 2	2148921	736	GCCGCTAC CGCCTGAC ACTG	AGCGCTTGC CACCCATCG TATCC
THRB2	Thyroid Hormone Receptor Beta 2	63822	82	ATGGACAT GGCCCTG AATC	ATGGCGACT GCACTTGAG AAAA
TIMP2	Tissue Inhibitor of Metalloproteinase 2	2352472	291	TCGGCGAA GGAGGTG GATT	CCGCTGGTT GAGGCTCTT CTTCT
TJP	Tight Junction Protein	464148	614	TCGCCATG GCCGTGCT GTGCTTCC	CTGGTCGCC CCGGCTGCT GTAGGT
TMP E3-16	Putative Transmembrane Protein E3-16	2425049	568	ACGCCAAG GAGCCGG AGGATGT	CCACGGCAG AGGCGGTAA ATAAAG
TNNC1	Troponin C, Slow	222844	414	AGGCGGC GGTTGAGC AGTTG	TGTTTTTGTC GCCATCTTTC ATCA
TNNT	Troponin T (variant)	2921774	548	GCCTTGAT TGACAGCC ACTTT	CAGCGCCCG CCAACCTT

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
TOM1B	Tom1B Protein, v-Myb Target Gene	1915893	530	GACGGATC CTTGCGG GGTGAG	AAGGTGACG GGGTGGAG AATGGA
TOP1	Topoisomerase 1	1786131	765	AAAATGGG CATGTTGA AGAGACG	GTTGGCACG GTTATAGGA AAGGAT
TOP2B	Topoismerase (DNA) II beta	2463528	549	GTGAATGC CGCTGACA ATAAG	ATCTACGTA ACTGCGAAA TCCAT
TRP1	Tyrosinase-Related Protein 1	2828811	667	TGGCCCAT ACGCTTCTT CAAC	GGATGGGA CCGCCTTC AGT
TSC22	Transforming Growth Factor Beta Stimulated Clone 22	1722682	352	TGTAGACC GGCGGCAA TGGAT	CGGAGGAT GGCGGGGA ACC
TSHB	Thyrotropin Beta-Subunit	2660744	297	CGTGGAGA AGCGGGAG TGTG	TGTGGCTT GGTGCAGT AGTTTGTC
TYR	Tyrosinase	1655468	687	CCGCCCTG GGATGGAG AT	TGGGCTGA GTAAATTAG GGTTGGT
UBA52	Ubiquitin/Ribosomal Protein Fusion Product	1763014	289	TTACGGGG AAGACCAT CAC	TTCAGCAC GGCAAGTT TA
UBP41	Ubiquitin Specific Protease 41	2736063	522	CCGCGGGC CAATGCTG AC	GGTGGTGC CCGAGTGG TTAGAGAC
VDR	Vitamin D Receptor	2245698	676	GCTGAAGC GCTGCGTG GACATTGG	TCCGGCTT GGGTGACA TCGCTGAC
VEGF	Vascular Endothelial Growth Factor	2897813	447	CTGGCGGC GCTGCTCT ATCTGC	CCGGCCTT TCTTGCGC TTTCTCTT
VMO1	Vitelline Membrane Outer Layer Protein 1	487905	499	ACTCATCCT GCTCTTCTT TTTCTA	GAGCGCTG TATCATCAC GA

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
VTG1	Vitellogenin 1	2160471	624	GGAGGCTG GGATTGGA GGTATTT	TATGCTGG CCGACTTG AGAACTGT
WNT11	Wingless 11	505349	629	AGGCGGCA CGGGAAGT CATA	AGGCGAGC TCTGCAGG TAAATCA
WNT14	Wingless 14	2623870	578	GGCCGAGA CCCTGATG GA	TCTTGGGC GGGGAAAT GTC
WNT4	Wingless 4	505351	570	TGCGCTCC TTGCTGCT CATCATCC	CCTTCCGC CCCGCCTC ATTGTTAT
XDA	Xanthine Dehydrogenase	507879	775	GGGGCAGT TGGTGGAA GATACAGT	TACGGGTC GGCCAGTT TTGAA
XPA	Xenoderma Pigmentosu Group A Complementing Protein	505066	493	GCTGGCGG CCCGACCC TACCC	TCTGCTGT CACGGCGC TGTTCCTT
YES1	Yamaguchi Sarcoma Oncogene	939872	646	CCGGCAAT AAAGGGAT CAGCAGTT	GTTTCACC GTGGGACA TACAGTTG
ABP1	Zipcode-Binding Protein 1	2570920	532	GAAGGGGC CACCATCA GGAACATT	AGACGCTG CTGGGAGG AGGAGGTA
ZNF5	Zinc Finger Protein 5	1399186	726	ATTGGGGA ACCTAACG ATACC	GCAGGAAC CGCAGACA AAA
ZOV3	Ig-Superfamily Protein (ZOV3 Gene Product)	840669	577	GGGCCATC CAATTGAC CATCTC	CTGCTTTCC ACACCATT GCTTTCT
ZPC	Sperm Receptor Ligand	1694685	576	CAACGCTG CTGACCTG ACTCTGG	CGTGGCCG GGGTGTGA TGAAG
ADOR2B	Adenosine Receptor 2B	2145431	662	GGCAAGCG GGCGAGAG GAC	GAGGCAGC CGGAGCGT TCAC

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
AKR	Homeodomain Protein AKR	857681	617	TCGGGCAAA CGGAGGAG ACG	GGTGGGGT TGGAGGAG GAGTGTTA
BF1	Brain Factor 1	1546781	716	CGCCGCGG CCGAAGAAG AGGAC	AGGGCATG GGGTGGCT GGGGTAGG
BF2	Brain factor 2	1546783	859	CGTCGGCG CTGGCTGAA GAGA	AAGAGGGC GGAGTGGG GGTGGTAG
CAM2AB	Calcium/Calmodulin-Dependent Kinase 2, Alpha-B	3668370	968	CTGCAACCG CTTCACCGA GGAGTA	TCACGCCG TCATTCTTC TTGTTGC
CAM2B	Calcium/Calmodulin-Dependent Kinase 2, Beta	3668372	1041	GACGGGCG GAGAGCTGT TTGAGGA	GTGAGCCC CGGGTCGC AGATTTTC
CBX1	Chromobox Protein 1	3649782	343	GAGGAGGA GGAGTATGT GGTGGAG	CCCCGCTG GAATCTGT GG
CBX2	Chromobox Protein 2	3649784	277	AAACAGATG GTGCGAAAA GAAAAT	CATGAATG CCAAGTTA GTCGTT
CBX3	Chromobox Protein 3	3649786	1179	CTTCGCCCG CCGCTCCAA CAT	GGCTGCTG CGGGGGCT CTACG
CCNC	Cyclin C	1118027	612	TCCAGGCTT TAGGTGAAC ATCTTA	TAGCCATCT CTTTCCTCT CATCAA
CDA	Cytidine Deaminase	3746538	789	GGGCTGCA GGCTGGGA CACG	AGGGGACC GGCTGGGG ATGG
CO6	Putatitive Calcium-Activated Potassium Channel Regulatory Subunit	3341750	502	TGACTGCAC AGAAGCGA GGAGAG	GTGGGCCA AAGGAAAG TGAAGAG
CREB2	Cyclic AMP Response Element-Binding Protein 2	3757574	574	TTTTTATGCA CTGCCCCTG GATGT	GAAACGGG CCTGGAAC TGGAACTA

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
DCT	Dopachrome Tautomerase	3510493	881	CCCGGGCA GAGGCACA GTTC	TCTTGGCC TTCGTTGG AGCAGTC
E2F1	E2F Transcritpion Factor 1	944827	756	CCGGCAGA GGGGCAAA GT	GCGGCGAC AGGCTCAC G
ECH	Erythroid Cell-Derived CNC Family Transcritpion Factor	1037159	1022	CCAGCTCAG CGCGTTCAG TC	GGGCCAGC AGGAGGGT CTTT
EDNRA	Endothelin Receptor, Type A	2961104	995	CCTTGTATT TGCGAGTTT CTTCAC	TCTGCCGG GATCTCTTT CATTAT
EK10	Eph-Related Tyrosine Kinase 10	312201	689	GCGGCCCG GGGACGTTC AAATC	TCCGTCCA GCCGCACC GAGTTCTT
EK6	Eph-Related Tyrosine Kinase 6	312901	814	AACGGGGAT GGGGAGTG GATGG	GCCGGGCC GTGTTGGT CTGA
EK7	Eph-Related Tyrosine Kinase 7	3122058	870	GTGGGTGG GCTTCTTCT CTGC	CCTCCACG GCTTTAATC ACATCTT
EK8	Eph-Related Tyrosine Kinase 8	312216	826	AGCAGGAG GCGCAGCA AATACAGT	GTGGCAAC CGATACCC TTCCTCAA
EPH9	Ephrin Receptor 9	758788	756	AAGTAAGTG TCCGGGATG ATAAGG	TGTGGGCA GGGCAGAG AAG
ER81	ER81 Protein	3869359	1253	CCGCGTGG GAGAAACTG TAATGAG	AGTAAGGG GCGCTGGT TGTCTGG
ETS1	Erythroblastosis Virus E2 Oncogene	63382	776	ACCCCCAGC AGCAAGGAA ATGATG	GGCAGGGC GGCGGGGT AGT
EYK	Eyk Proto-Oncogene	438522	761	GGGAGAGG GGGAGTTCG GGTCAGT	ACGTCGGT CGGTCAGC AGGTTCAG

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
FGFR2	Fiborblast Growth Factor Receptor 2	63085	722	GGGCGCCC TATTGGACA CA	CATGCAGG CGATCAGG AAGACC
FKH1	Forkhead 1	3341440	717	CCGGGCTTC AGCGTGGA CAACATC	GCTGCCGG GAACGCCA TCTGACA
FOS	V-Fos FBJ Murine Osteosarcoma Viral Oncogene Homolog	62891	485	CTCGGTCGC CCCCTCCCA GAAC	GCGGCGCC TCGGTCATT AGC
FYN	Fyn Oncogene Related to Src, Fgr, Yes	62861	748	TCTTTTTGA GGCGCTTTA TGACT	GGGCCTCC TAGACACC ACAG
G22P1	Thyroid Autoantigen, 70-KD	3374508	1123	GGGGCGGG ACAGCTTGA TTTTCT	AGTGTTCC GGCGGGCG ATGTAT
GATA1	Gata-Binding Protein 1	212628	640	GGCTCCCCC ACTCCGTTC C	GGGGGCGC CGCTTTTTA CC
GATA2	Gata-Binding Protein 2	3650486	357	GTGCGGTTG GGGGCGGT GTGG	ACGGGGGC AGAAGGGT GGGAGGAA
GATA4	Gata-Binding Protein 4	511479	743	GCCCGTGTC ACCTCGCTT CTCCTT	TGGGGCGC ATTTCCTCA GTGGTC
GATA5	Gata-Binding Protein 5	511481	735	TGGACGGC CGGACACTT TGAGAGC	GAGCGCCA GGGCACAC CACGAGTC
GATA6	Gata-Binding Protein 6	511483	655	TCCGCGCCC AGCTCTCCC GTCTAC	TGGTGGTG GTGGTGTG GCAGTTGG
GJB3	Gap Junction Protein, Beta-3	3746661	615	TTCCGTATC ATGATCCTG GTTGTG	CTCATGGTT GGGGTGGT GTTTCTG
HLXB9	Homeo Box Gene HB9	3777536	686	CCGCGCAC CGACAGCC CCTCTC	CGCCTCCC GCCGCCTT TCTCC

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
HOM	Homogenin	3688783	1085	AAGGGCAG GAGAACAGT CAGAGCA	TCCCGAGATG TTCCACCCTT GTAA
HOXB1	Homeo Box B1	2979618	163	AGGAGAAG CTGCGAGA GGTG	CGGGCCCGG GTAAGGTA
HRAS	V-Ha-Ras Harvey Rat Sarcoma Viral Oncogene Homolog	63506	500	TGGTAGTGG GAGCTGGA GGTGTCG	TGGGTTCAGC TTGCGCAGTT TATG
IBRF	Initiation Binding Receptor F	984121	716	CCTCCAAAC CCAACCCTG TCTTCA	CGCCTGCGTT GTCTGGATGG TC
IRF2	Interferon Regulatory Factor 2	1524050	838	TGGGTGGG ATGTTGAAA AAGATGC	GCTGCTGCTA GTGGAGGCT GTGG
JTAP1	v-Jun Transformation Associated Target Protein	1017830	640	CCCCCGCG GCCGTGGAT TG	TTGGGGAAG CTGGCGAGG TTGG
JUN	V-Jun Sarcoma Virus 17 Oncogene	212221	587	ACAAGAACG CCGACATCC TCACCT	GGACGCCGC AATTCTGTTT CTCAT
JUND	Oncogene Jun-D	62927	782	CCGGCGGC AGCATGATG AAGAAGG	GCGGAGCAG GCTGGCGGT GGAG
LAMP1	Lysosome-Associated Membrane Protein 1	212253	805	TGTGCCCAT AGCCCCTTG TCCTGT	CCGCCCGCC CATCCCCCTT AC
LEF1	Lymphoid Enhancer-Binding Factor 1	3258664	1041	CTGCGCCAC CGACGAGAT GAT	CTGGGGCCT GTACCTGATG CTGAT
LRF	Leukemia/Lymohoma Related Factor	3599512	766	GGGGCGGA GGCGGAAG ACGAC	CGGCCGCCC CTCCTGCTAC ATT
MAP	Microtubule-Associated Protein	3002800	574	CTGCATCGC CCGCTTTTG GAACC	GCCCGCCTC ATCGCCCCTC TG

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
MAX	Max Protein	414723	770	GGGGGCTG TGGTGATG GGACTCTC	CAGGGCG TTGTGGT GGGCTCG TT
MIM1	Myeloid Protein 1	212341	878	GCAGGCGC TACAGATCT TATTTAC	GGAGGGC AGTGAGG GGTGAG
MYB	Myeloblastosis Viral Oncogene	558575	772	CACTCCGC CTGCTATC CTA	ACACGCA TTCAGTTT CTTCTTA
NEURO D	Neurogenic Differentiation	3094019	665	GCGCGGCC CCAAGAAG AAGAAGAT	CGGCGGG GTAGTGC ATGGTGA AGG
NFIA	Nuclear Factor I/A	63661	954	CCTGCAAG CCCGAAAG AGAAAATA	GAAGGCG AGGGACT GCTGAAA CC
NFIC	Nuclear Factor I/C	63677	1029	ACGAGGAG CGGGCGGT GAAGGA	GGATGGC CGTGTGG GGGAAAT AGG
NFKB1	Nuclear Factor Kappa-B, Subunit 1	2130627	845	GACGACGG CGCGGCTC AACCA	CGCACCC CGCTGTC CTGTCCA TTC
NFM	Nuclear Transcription Factor M	296511	646	GCAGCGGC GGCGGCAA GAAGC	CAGCGGG GCGAGGA AGCGAGC AG
NFYB	Nuclear Transcription Factor Y, Beta	63690	439	CCACGACG GATGCTTCT CAGTTAG	GTTCCCC CAATTCC CTTTTCTC C
NOG	Noggin, Mouse, Homolog of	3695028	525	AGCACCCG GACCCTAT CTTTGACC	AGCACTT GCACTCC GCGATGA TGG
NURR1	Nuclear Receptor-Related 1	683561	262	GGAGGGCC CTGCAAAAT GAAGAG	CCGTGGG GCCAGCA GAGGT
OCT6	Octamer Binding	3172416	641	CCGCGAGG	CCTCGAA

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
OKRT	Otokeratin	3746659	924	GTCCCAG GGGTCAG CCAGGTG	GTCCCC GTGTTTC CCAGCA GTG
PEA3	Polyomavirus Enhancer Activator 3	3869361	508	AGCGCCC CATGTCTG AGC	CGTTCCC CTGCCAC CTTCTG
PITX1	Paired-Like Homeodomain Transcription Factor 1	3236449	548	ACGACCC GGCGAAG AAGAAGAA GC	ATAGGGA CAAGCG GGCGAG GACAT
PITX2	Paired-Like Homeodomain Transcription Factor 2	3335642	782	CGCCTGG GAGCCGG GAATAATA AG	GCCGAG TTGAGGG AGGGGTT GC
POMC	Proopiomelanocortin	3869132	313	CAGCAGC GGAGGGC ACAAAA	GCCTTCC TCTTCCT CCTCTTC TTC
POU2F1	Pou Domain, Class 2, Transcription Factor 1	212466	1103	GCAGGGG CAGCAGG GTCTCC	GTAATGC GGCTGCT GCTGCTG TTT
PRH	Proline Rich Homeobox	297086	608	GGCGTCG GCGTCCCT CTGTA	CCTTCCG CCTCCTC CTTTTTG GTG
PS1	Processed Pseudogene Related to the Ras Oncogene Superfamily	63334	440	ACGCGGC CGGCAAAA CCAC	CCATGTC AGCCCTT CGTAGAG TCC
REL	Oncogene Rel	63922	829	AAGGGGC ATGCGTTT CAG	TTGCCTT TTTGCTT TGTTACC ATA
REM1	Rem 1 Protein	529655	322	GCTGCGC CCTGAAGT GCT	CCCGTTG CCATCCA GGTC
SDHB	Succinate Dehydrogenase Complex, Subunit B, Iron Sulfur Protein	3851611	637	ATGTGGGC CTATGGTA CTTGATGC	AGCGGC TGCCTTC TCTTTGT

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
SIM1	Single-Minded, Drosophila, Homolog of, 1	1173853	186	CGGACTAG GCGGGAGA AAGAAAAC	GCTGGTG CGGCTGG AGTGG
SLUG	Neural Crest Transcription Factor	495237	678	TCATACCG CAGCCAGA GAT	CTACGCA GCAGCCA GATTC
SOX1	Sry-Box 1	2947024	848	GCTGGGCG CCGAGTGG AAGGTGAT	CCCCCGT GCTGGCG CTCTGGT AGT
SOX11	Sry-Box 11	2982741	691	GCCTGGGC AAGCGGTG GAAAATG	CTGCCGG CCGACGA GGTGGAG ATG
SOX3	Sry-Box 3	2947026	539	CGGGGCCG ATTGGAAG C	TCTGCGA GTGCGAG GTGATGG
SOX9	Sry-Box 9	2982739	735	CATCTCCC CCAACGCC ATCTTCAA	CCGGCGG CGTGGCT GTAGTAG GAG
SPI1	Spleen Focus Forming Virus Proviral Integration Oncogene	2369862	709	CCTCATTCC CCCTCCCT CTG	CCCCCTT CCCATCA CCTCA
T	T Brachyury	2529385	755	TCGGCGCC CACTGGAT GAAGG	CACCGGA GAGCCAC GCAGGAA CT
TAL1	T-Cell Acute Lymphocytic Leukemia 1	62844	657	GCCACGAG CGAGCCCG ACAGC	GCCCCTT TGGTTTC CTTCCTC CTC
TBX2	T-Box Transcription Factor 2	3236441	379	CGGGTGAG CGGCCTGG ACAAGAA	GGTAGGC GGTGACG GCGATGA AGT
TBX3	T-Box Transcription Factor 3	3236443	303	ATAAAAGA GGCACGGA GATGGT	CGTGCTT GTCGGAG ATGTTG
TBX4	T-Box Transcription Factor 4	3236445	499	AAGCAGGC AGGAGGAT GTTT	AGGTCGC TGTCGTC ACTTC

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
TBX5	T-Box Transcription Factor 5	3236447	445	ACGAGGTG GGGACGGA GATG	GAGGTAACA GCGATGAAG GCAGTC
TBX6L	T-BoxTranscription Factor 6L	1806623	539	GCCCCAGCT CCCTTGTCG	CATGGCTGC TGTTCTGCT CTGA
TBXT	T-Box Transcription Factor T	1806621	601	TCCCCTTTG GCAGAGATT CA	GTGGGGAG CCTGTGGAG AGTG
TCF15	Transcription Factor 15	3413459	569	GGCCGGGT CCCCACTGC TGCTC	CGGGGCGG GTCTCCAAC ACG
TCF4	Transcription Factor 4	63356	800	ATCACCATC GCCGCTTAC AGG	ACATCCGGC CGAGTTCTT TGA
TEAD1B	Tea Domain Family Member 1B	1256008	926	GGGAGGGG CGGGAAGAT GG	TGCGCTGCT GTATTGACT GCTGAC
TFAP2B	Transcription Factor AP2 Beta	3309576	816	GGTACGGC GGCCAGAT GTCC	GTGAGGGC GGCGCAGAT AGC
THRB	Thyroid Hormone Receptor, Beta	63820	455	TGGCATGGC AACAGATTT	CGGGGTCAT AGCGAACT
UBP46	Ubiquitin Specific Protease 46	3800759	860	CAGAGATAC GCCCCACG CTTTGTT	TCTCGGGGC TTTCTGCTG TTCTTG
UBP52	Ubiquitin Specific Protease 52	3800761	760	TTCGGGGCT GCACACGTC GGATAG	CTTGGGGAT GGGCAGGG AGAGGTC
UBP66	Ubiquitin Specific Protease 66	3800763	1108	ATGCCGGG CTCCCTGCT GGTCT	GGGCCGGG TACATGCGT GAGGAT
WH1	Winged Helix Protein 1	1766072	631	GACGGGGC GAAATACAG CGAGGAC	CGTAGCGAA GCCGGGCA GGAAGG

Table 1. Cont.

Locus:	Gene Name:	ID#:	Size:	Primer 1:	Primer 2:
WH2	Winged Helix Protein 2	1766074	788	AACCCGCCGC CCCCAAGGAC	GCTGCACG CCGCGCTG TAACC
WH3	Winged Helix Protein 3	1766076	636	GCTGCCGCTG CCGCTGGACG AG	AGAGCGGC GGGGTGCG GGTAGG
WT1	Wilms Tumor	987062	655	GAGCGCTTTC ACCGTCCACTT CT	GGGGCGTT TTTCATTTG TCTCACT
YRK	Yes Related Kinase	63895	695	GCAGGCGCAC AGCAGCATCA CAG	TGCCCGGC TTCAGCGT CTTCACT
ZFP161	Zinc Finger Protein 161	1399186	726	ATTGGGGAAC CTAACGATACC	GCAGGAAC CGCAGACA AAA

Comparative Mapping of the Chicken Genome Using the East Lansing Reference Population

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ABSTRACT The annotation of known genes on linkage maps provides an informative framework for synteny mapping. In comparative gene mapping, conserved synteny is broadly defined as groups of two or more linked markers that are also linked in two or more species. Although many anonymous markers have been placed on the chicken genome map, locating known genes will augment the number of conserved syntenic groups and consolidate linkage groups. In this report, 21 additional genes have been assigned to linkage groups or chromosomes; five syntenic groups were identified. Ultimately, conserved syntenic groups may help to pinpoint important quantitative trait loci.

(*Key words*: synteny, polymerase chain reaction, comparative mapping, linkage, genes)

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INTRODUCTION

In the assembly of linkage maps, functional genes (Type I) (O'Brien, 1993) and anonymous polymorphic DNA markers (Type II), have served as markers in mapping the chicken genome (Burt *et al.*, 1995; Cheng *et al.*, 1995; Crooijmans *et al.*, 1996). Because chickens diverged from mammals about 300 million yr ago (Hedges, 1994), Type I markers have also revealed conserved linkage associations among other species. In view of the considerable amount of research associated with expressed genes, they are especially informative candidates for conserved synteny mapping. Using anchor loci, syntenic comparisons may provide clues to the location and orientation of orthologous genes. Functional genes are also useful as probes in fluorescent *in situ* hybridizations (FISH), in the physical mapping of genes, and in the assignment of linkage groups to specific chromosomes.

Currently, about 41 linkage groups and more than 617 loci have been placed on the East Lansing (EL) reference map; 101 loci represent known genes. An objective of this research is to annotate the genetic map of the chicken to facilitate marker-assisted selection of economically important traits. This report, therefore, extends earlier mapping data (Smith *et al.*, 1996).

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MATERIALS AND METHODS

Candidate Genes and PCR Primers

Chicken genes were selected based on the availability of sequences in the GenBank database. GenBank accession numbers and sequences were obtained through the Entrez[2] retrieval system at the National Center for Biological Information. Primers were selected using the OLIGO[3] primer analysis program. To determine conserved synteny, priority was given to cognate genes that were mapped in the human or mouse. Exon-based primers (18-mer) that amplified across introns were selected because there is a greater likelihood that base substitutions would be found in introns rather than in the more conserved exons. Alternatively, primers were based on sequences in the 3′ untranslated region (UTR) of complementary DNA (cDNA). Two chicken genes were mapped based on primer sequences from nonavian sources.

Linkage Analysis

Segregation data on inheritance of the Jungle Fowl (JF) allele of 52 male meioses was entered into the EL database using MAP MANAGER[4] version 2.6. Genes with the least number of crossovers between adjacent loci and minimal double recombinants were located in strain distribution patterns. Only genes with $\log_{10}$ of odds (LOD) scores greater than 3.0 were considered to be linked to other markers. Markers were deposited in the EL genome

TABLE 1. Primers used to amplify target regions in functional genes

Gene	Forward/reverse 5′-3′	Accession number	References
Nicotinic acetylcholine receptor	TCCGACATTGTTCTGTT/ CCCTTCAAAACGTCCATC	X83739	Clemencia-Hernandez *et al.*, 1995
Retinoblastoma oncogene	TGATGGAAACGATACCTC/ TGGCTTCAATCAGTAACG	X7228	Boehmelt *et al.*, 1994
G protein coupled purinoreceptor	AAAACAACAGGTTCACCA/ CGAGTCAAAACAGACATC	L06109	Kaplan *et al.*, 1993
Hydroxy steroid dehydrogenase	AGAGGTACACCAGCCAGT/ TGCTGAATGAGTTGGGTA	D43762	Nakabayshi *et al.*, 1995
N-acetyl galactosaminidase	TCCCAAAGCACTGCTCAT/ GGTCACTGGGAACACTCC	L18754	Davis *et al.*, 1993
Beta-globin	TCAATGTGGCCGAATGTG/ AGCATCCCCAAAAGGAGGT	V00409	Dolan *et al.*, 1983
Wingless-related MMTV int site	GTGCTGCTATGTCACCTG/ ATGGGAAAGATTTTGGAA	D31901	Tanda *et al.*, 1995
Abelson viral oncogene homolog[1]	GAGGACACCATGGAGGTGGA/ GTGGATGAAGAAGTTCTTCTTCTC	M14752	Shtivelman *et al.*, 1986
Alpha microglobulin[1]	ATGGTCGCAGACCTGCCG/ AGTAGAACTTGTTGCCGTTGCC	X54818	Vetr and Gebhard, 1990
Glucose transporter 1	GAGCCAGAGCGACAAGAC/ TGCTGAATCTATCGGCTT	L07300	Wagstaff *et al.*, 1995
Cell division cycle 2 protein kinase	CGTTGTCTCCGTTTCTCT/ CGGTGTCTCCGTTTCTCT	U16344	Li *et al.*, 1995
EnolaseA	GTTTTGCTGGAAGGAACT/ GCTCCAAACACTGAAGAA	D37900	Tanaka *et al.*, 1995
Glutathione-S-transferase	TGCTAATGTGAGGAAAAT/ TAAAAAGGGAGGGAAGAG	L15387	Liu *et al.*, 1993
Vimentin	TGCTTCTTTGAACCTGAGAG/ GTGTCCTCTTCGAGTGAGTG	J02759	Zehner *et al.*, 1987
cGMP phosphodiesterase-alpha	GAAAATCCAAAACATGTA/ TTTTTCTGGACAGTATGC	L29233	Semple-Rowland and Green, 1994
Beta B1 crystalline	GCGCAGTGTCATCGTCAG/ ATCTCCCCACGCATGTTG	U09951	Duncan *et al.*, 1995
Beta-2-microglobulin	GCTTGCAGCCTTTAGGAG/ TAAGCCGAGGTGGGATTA	Z48921	Riegert *et al.*, 1996
Osteopontin	AAAGCTGCCAGGAAGGTG/ GGCGTCATCCTCAATGAG	U01844	Rafidl *et al.*, 1994
Ryanodine receptor 3	GTATGAGGATCAACTCGG/ GCCTCTGATGTTCAAGTT	X95267	Ottini *et al.*, 1996
Ribosomal Protein L37a	TAGTGCTTTTTGGTATGG/ GAAATGCTAATGTCTCCA	D14167	Machida *et al.*, 1993
Ski-novel overexpressed N	GAGGACCCTATACCTTTTGA/ ATGTTTTGTTCTTCCAGCAT	S78406	Givol *et al.*, 1995

[1]Denotes primers derived from nonavian sequences.

database (Chick GBASE[5]) and will be integrated into a unified map with markers from the Compton reference population (Burt *et al.*, 1995, Crittenden *et al.*, 1995).

RESULTS AND DISCUSSION

The JF × White Leghorn (WL) backcross (BC) EL reference population (Crittenden *et al.*, 1993) and methods used to determine the segregation of the JF-specific allele were previously described (Smith *et al.*, 1996). Briefly, introns or 3′ UTR were amplified using PCR. Sequence analyses of cloned PCR products from the JF and WL parents of the reference population were conducted to identify base substitutions in either parent. When nucleotide substitutions were found, segregation of the nonredundant JF allele was typed through preferential amplification of the JF allele from DNA of BC progeny of the EL family.

References to nucleotide sequences and primers used to amplify introns or 3′ UTR of candidate genes are listed in Table 1. The initial PCR products were between 250 and 650 bp in size. Products for hydroxysteroid dehydrogenase (*HSD3B*) and β-crystalline (*CRYBB1*), however, had 2 and 11 bp differences between WL and JF, respectively. After electrophoretic separation, the differences in size enabled detection of the JF allele. For the other genes, base substitutions were found and primers mismatched at the 3′ terminus with respect to the WL allele were designed for preferential amplification of the JF allele. Although transitions occurred more frequently, mismatched primers based on transversions were preferred because they are less prone to false priming.

Type I candidate genes that were mapped are listed in Table 2. Their location and the position of other genes

[5]http://www.poultry.mph.msu.edu/.

TABLE 2. Comparative location of chicken, human, and mouse genes[1]

Gene	Symbol	Chicken	Human	Mouse
Lysosomal glycoprotein*	*LAMP1*	Ch1	13q.34	8
Retinoblastoma oncogene	*RB1*	Ch1	13q14.3	14
G protein purinoreceptor	*P2Y5*	Ch1	13q14.3	NM
Globin	*HBB*	Ch1	11p15	7
Wingless	*WNT11*	Ch1	NM	7
Abelson viral oncogene homologue	*ABL1*	E41	9q34	2
Adenylate kinase 1*	*AK1*	E41	9q34	2
Alpha microglobulin	*AMBP*	E41	9q32	4
Aldolase B*	*ALDOB*	EZ	9q22.3	4
Iron response element*	*IREBP*	EZ	9 & 15	4
Cell division cycle 2 protein kinase	CDC 2L1	E54	1p36	4
Enolase A	*ENO1*	E54	1p36	4
Glucose transporter 1	*SLC2A1*	UL	1p35	4
Ryanodine receptor 3	*RYR3*	E07	15q14	2
Beta-2-microglobulin	*B2M*	UL	15q21	2
Hydroxy steroid dehydrogenase	*HSD3B*	Ch1	1p13	3
Galactosaminidase	*NAGA*	Ch1	22q13	NM
Vimentin	*VIM*	Ch2	10p13	2
Glutathione-S-transferase	*GST.A2*	Ch3	6p12	9
Osteopontin	*SPP1*	Ch4	4q11	5
Creatine kinase B*	*CKB*	Ch5	14q32	12
Ribosomal protein-L37A	*L37a*	Ch7	NM	NM
Vitellogenin 2*	*VTG2*	Ch8	NM	NM
Phosphodiesterase	*PDEA*	E11	5q31	18
Beta crystalline	*CRYBB1*	E18	22q11	11
Ski novel overexpressed N	*SNON*	E36	NM	NM
Apolipoprotein A1*	*APOA1*	E49	11q23.3	9
Acetylcholine receptor	*CHRNB3*	EZ	8p11	NM

[1]Genes reported earlier (Smith *et al.*, 1996) are marked with an asterisk. UL, unlinked; NM, not mapped.

on the EL map enabled us to identify five novel conserved groups. On Chromosome (Ch) 1, *LAMP1*, the retinoblastoma susceptibility gene (*RB1*), and a G-protein coupled purinoreceptor (*P2Y5*) gene, exhibited conserved synteny with human Ch 13. We note that in human, *P2Y5* is in intron 17 of *RB1* (Webb *et al.*, 1996). Apparently, the *RB1-P2Y5* linkage has remained intact throughout evolution (LOD score 15).

Human orthologs to the *wnt* family have not been reported, but the chicken ortholog of the *Drosophila* segment polarity gene *wingless*, *Wnt-11*, is linked with β-globin on Ch 1; *HBB* and *Wnt-11* are linked on mouse Ch 7. In chicken, *Wnt-11* and *HBB* are about 15 cM apart, whereas they are about 2 cM apart in mouse. A representation of PCR product derived from preferential amplification of the JF allele of *HBB* among 15 BC progeny is shown in Figure 1.

Orthologs of the Abelson viral oncogene (*ABL1*), adenylate kinase 1 (*AK1*), and alpha microglobulin-bikunin precursor (*AMBP*) comprising 28.4 cM on chicken linkage group E41 are syntenic in human Ch 9 and mouse Ch 2. In mouse and human, however, *ABL1* and *AK1* are about 2 cM apart.

Z-Linked aldolase B (*ALDOB*) and the iron response element binding protein (*IREBP*) genes, purported to be a cytosolic isoform of aconitase, (Saitoh *et al.*, 1993), are linked to within 18 cM. These genes are also linked in human Ch 2 and mouse Ch 4.

Glucose transporter 1 (*SLC2A1*), p58 protein kinase associated with cell division cycle 2 (*CDC2-L1*), and enolase (*ENO1*) are syntenic in human and mouse. The latter two genes are provisionally placed in E54 (they are 26.9 cM apart, LOD score 2.5). In this context, we also note that *CDC2L1* and *ENO1* are telomeric on human Ch 1p.

Of the 38 pairs of chicken autosomes, about 30 pairs are classified as microchromosomal. Recently, CpG islands (CGI) were found to be highly concentrated on microchromosomes and *in situ* hybridizations with a CGI probe suggested that microchromosomal euchromatin is gene-rich (McQueen *et al.*, 1996). Although β-2-microglobulin (*B2M*) is, unlinked on the EL map, it was shown by FISH to be microchromosomal (Riegert *et al.*, 1996).

The ribosomal protein gene (*L37a*), was also mapped by FISH to chicken macrochromosome 7 (Nanda *et al.*, 1996). Although *L37a* was originally mapped to linkage group E02, it probably represents Ch 7. The FISH mapping supports the consensus map because the upper portion of E02 is Ch 7 (Bumstead and Cheng, unpublished data).

The other genes listed in Table 2 have not, at this point, been associated with a conserved syntenic group, but we note that vitellogenin and ostepontin are major components of avian egg yolk and shell membrane, respectively.

MISMATCH PRIMER PCR
Chicken β-Globin Gene

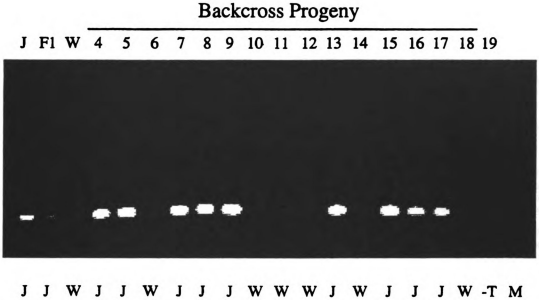

FIGURE 1. Preferential amplification of the 189 bp polymerase chain reaction (PCR) product of the Jungle Fowl allele of the *β*-globin gene using an 18-mer forward primer mismatched with respect to the 3′ terminus of the White Leghorn allele and the initial reverse primer (Table 1). The first three lanes represent aliquots of PCR reactions containing genomic DNA from the founder Jungle Fowl sire, the F_1 male, and a White Leghorn dam of the East Lansing backcross population, respectively. Lanes 4 to 18 represent F_2 progeny; –T represents an aliquot of a thermocycled mixture lacking DNA. Molecular size marker, *M* is a multiple of 123 bp.

With the relatively high incidence of polymorphisms in vertebrate genomes, selective PCR amplification of less conserved regions and preferential amplification of specific alleles provides a convenient and efficient approach to mapping cloned genes. Moreover, PCR requires little DNA and is amenable to large-scale testing, whereas restriction fragment polymorphisms require time-consuming Southern blot hybridizations that are fraught with technical difficulties.

Linkage mapping and FISH will collaterally characterize the numerous chicken microchromosomes that constitute about 25% of the chicken genome (McQueen *et al.*, 1996). In the case of *L37a* reported above, marker linkage supported the FISH assignment to Ch 7.

Apart from those discussed here, 19 other conserved syntenic groups have been found (Burt *et al.*, 1996). Although the repertoire of cloned chicken genes is limited, additional synteny may be revealed using comparative anchor tagged site primers (CATS) that are based on conserved exon sequences of mammalian species. In this context, we have successfully used non avian-based primers to amplify chicken *ABL1* and *AMBP*. Ultimately, a marker-rich map annotated with respect to orthologous mammalian genes will inform poultry geneticists on loci associated with economically important traits.

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REFERENCES

Boehmelt, G., E. Ulrich, R. Kurzbauer, G. Mellitzer, A. Bird, and M. Zehnke, 1994. Structure and expression of the

chicken retinoblastoma gene. Cell Growth Differ. 5: 221–230.

Burt, D. W., N. Bumstead, J. J. Bitgood, F. A. Ponce deLeon, and L. B. Crittenden, 1995. Chicken genome mapping: a new era in avian genetics. Trends Genet. 11:190–194.

Burt, D. W., C. T. Jones, D. R. Morrice, and I. R. Paton, 1996. Mapping the chicken genome—An aid to comparative studies. Page 105 *in*: XXVth International Conference on Animal Genetics. (Abstr.)

Cheng, H. H., I. Levin, R. L. Vallejo, H. Khatib, J. B. Dodgson, L. B. Crittenden, and J. Hillel, 1995. Development of a genetic map of the chicken with markers of high utility. Poultry Sci. 74:1855–1874.

Clemencia-Hernandez, M., L. Erkman, L. Matter-Sadzinski, T. Roztocil, M. Ballivet, and J. M. Matter, 1995. Characterization of the nicotinic acetylcholine receptor β3 gene. J. Biol. Chem. 270:3224–3233.

Crittenden, L. B., L. Provencher, L. Santangelo, I. Levin, H. Ablanalp, R. Briles, W. E. Briles, and J. Dodgson, 1993. Characterization of a Red Jungle Fowl Backcross reference population for molecular mapping of the chicken genome. Poultry Sci. 72:334–348.

Crittenden, L. B., J. Bitgood, and D. Burt, 1995. Genetic Nomenclature Guide Trends Genet. 11(Suppl.):33–34.

Crooijmans, R. P., P.A.M. van Oers, J. A. Strijk, J. J. van der Poel, and M.A.M. Groenen, 1996. Preliminary linkage map of the chicken (*Gallus domesticus*) genome based on microsatellite markers. Poultry Sci. 75:746–754.

Davis, M. O., D. J. Hata, D. Smith, and J. C. Walker, 1993. Cloning and sequence of a chicken alpha-N-acetylgalactosaminidase gene. Biochim. Biophys. Acta 1216:296–298.

Dolan, M., J. B. Dodgson, and J. D. Engel, 1983. Analysis of the adult chicken β-globin gene. J. Biol. Chem. 258:3983–3990.

Duncan, M. K., H. J. Roth, M. Thompson, M. Kantarow, and J. Piatigorsky, 1995. Chicken βB1 crystalline: gene sequence and evidence for functional conservation of promoter activity between chicken and mouse. Biochim. Biophys. Acta 1261:68–76.

Givol, I., P. L. Boyer, and S. H. Hughes, 1995. Isolation and characterization of the chicken c-*sno* gene. Gene 156: 271–276.

Hedges, S. B., 1994. Molecular evidence for the origin of birds. Proc. Natl. Acad. Sci. USA. 91:2621–2624.

Kaplan, M., D. I. Smith, and R. S. Sundick, 1993. Identification of a G-protein coupled receptor induced in activated T cells. J. Immunol. 151:628–638.

Li, H., J. Grenet, M. Valentine, J. M. Lahti, and V. Kidd, 1995. Structure and expression of chicken protein kinase PITSLRE-encoding genes. Gene 153:237–242.

Liu, L.-F., S.-H. Wu, and M. F. Tam, 1993. Nucleotide sequences of class alpha glutathione S-transferases from chicken liver. Biochim. Biophys. Acta 1216:332–334.

Machida, M., S. Toku, N. Kenmochi, and T. Tanaka, 1993. The structure of the gene encoding chicken ribosomal protein L37. Eur. J. Biochem. 213:77–80.

McQueen, H. A., J. Fantes, S. H. Cross, V. C. Clark, A. L. Archibald, and A. P. Bird, 1996. CpG islands of chicken are concentrated on microchromosomes. Nature Genet. 12: 321–323.

Nanda, N., T. Tanaka, and M. Schmid, 1996. The intron-containing ribosomal protein-encoding genes *L5*, *L7a* and *L37a* are unlinked in chicken. Gene 170:159–164.

Nakabayashi, O., O. Nomura, K. Nishimori, and S. Mizuno, 1995. The cDNA cloning and transient expression of a chicken gene encoding a 3β-hydroxysteroid dehydrogenase unique to major steroidogenic tissues. Gene 162:261–265.

O'Brien, S. J., J. E. Womack, L. A. Lyons, K. J. Moore, N. A. Jenkins, and N. G. Copeland, 1993. Anchored reference Loci for comparative mapping genome mapping in mammals. Nature Genet. 3:103–112.

Ottini, L., G. Marziali, A. Conti, A. Charlesworth, and V. Sorrentino, 1996. Alpha and β isoforms of ryanodine receptor from chicken skeletal muscle are the homologues of mammalian RYR1 and RYR3. Biochem. J. 315:207–216.

Rafidi, K., I. Simkina, E. Johnson, M. A. Moore, and L. C. Gerstenfeld, 1994. Characterization of the chicken osteopontin-encoding gene. Gene 140:163–169.

Riegert, P., R. A. Andersen, N. Bumstead, C. Döhring, M. Dominguez-Steglich, J. Engberg, J. Salomonsen, M. Schmid, J. Schwager, K. Skjodt, and J. Kaufman, 1996. The chicken β2-microglobulin gene is located on a non-major histocompatibility complex microchromosome: A small, G+C-rich gene with X and Y boxes in the promoter. Proc. Natl. Acad. Sci. USA 93:1242–1248.

Saitoh, Y., A. Ogawa, T. Hori, R. Kunita, and R. Kunita, 1993. Identification and localization of two genes on the chicken Z chromosome: implication of evolutionary conservation of the Z chromosome among avian species. Chromosome Res. 1:239–251.

Semple-Rowland, S. L., and D. A. Green, 1994. Molecular characterization of the alpha-subunit of cone photoreceptor cGMP phosphodiesterase in normal and *rd* chicken. Exp. Eye Res. 59:365–372.

Smith, E. J., H. H. Cheng, and R. L. Vallejo, 1996. Mapping functional chicken genes: an alternative approach. Poultry Sci. 75:642–647.

Shtivelman, E., B. Lifshitz, R. P. Gale, B. A. Roe, and E. Canaani, 1986. Alternative splicing of RNAs transcribed from the human *abl* gene and from the *bcr-abl* fused gene. Cell 47:277–284.

Tanaka, M., K. Maeda, and K. Nakashima, 1995. Chicken alpha-enolase but not β-enolase has a src-dependent tyrosine-phophorylation site: cDNA cloning and nucleotide sequence analysis. J. Biochem. 117:554–559.

Tanda, N., Y. Kawakami, T. Saito, S. Noji, and T. Nohno, 1995. Cloning and characterization of Wnt-4 and Wnt-11 cDNAs from chicken embryo. DNA Seq. 5:277–281.

Vetr, H., and W. Gebhard, 1990. Structure of the human alpha-1-microglobulin-bikunin gene. Hope-Seyler 371:1185–1196.

Wagstaff, P., H. Y. Kang, D. Mylott, P. J. Robbins, and M. White, 1995. Characterization of the avian GLUT1 glucose transporter: Differential regulation of GLUT1 and GLUT3 in chicken embryo fibroblasts. Mol. Biol. Cell 6:1575–1589.

Webb, T. E., M. G. Kaplan, and E. A. Barnard, 1996. Identification of 6h1 as a P2Y purinoreceptor: P2Y5. Biochim. Biophys. Res. Commun. 219:105–110.

Zehner, Z., Y. Li, B. A. Roe, B. M. Paterson, and C. M. Sax, 1987. The chicken vimentin gene. J. Biol. Chem. 262: 8112–8120.

References

Abderrahim, H., J.L. Sambucy, F. Iris, P. Ougen, A. Billaut, I.M. Chumakov, J. Dausset, D. Cohen, and D. LePaslier, 1994. Cloning the Human Major Histocompatibility Complex in YACs. Genomics 23:520-527.

Adams, M.D., A.R. Kerlavge, R.D. Fleischmann, R.A. Fuldner, C.J. Bult, N.H. Lee, E.F. Kirkness, k.G. Weinstock, J.D. Gocayne, O. White, 1995. Initial Assessment of Human Gene Diversity and Expression Patterns based Upon 83 Million Nucleotides of cDNA Sequence. Nature (Suppl. 6547S) 37, 3-174.

Amadou, C., M.T. Ribouchon, M.G. Mattei, N.A. Jenkins, D.J. Gilbert, N.G. Copeland, P.Avoustin, and P. Pontarotti, 1995. Localization of New Genes and Markers to the Distal Part of the Human Major Histocompatibility Complex (MHC) Region and Comparison with the Mouse: New Insights into the Evolution of Mammalian Genomes. Genomics 26:9-20.

Bacon, L.D., 1987. Influence of the Major Histocompatibility Complex on Disease Resistance and Productivity. Poultry Sci. 66:802-811.

Barnes, W, 1994. PCR Amplification of up to 35kb DNA With High Fidelity and High Yield From Lambda Bacteriophage Templates. Proc. Natl. Acad. Sci. 91:2216-2220.

Belterman, R.H.R, and L.E.M. De Boer, 1984. A Karyological Study of 55 Species of Birds, Including Karyotypes of 39 Species New to Cytology. Genetica 65:39-82.

Bengtsson, B.O., K. Klinga Levan, and G. Levan, 1993. Measuring Genome Reorganization from Synteny Data. Cyto. Cell Genet. 64:198-200.

Bent, A.F., B.N. Kunkel, D. Dahlbeck, K.L. Brown, R. Scmid, J. Hiraudel, J. Leung, and B.J. Staskawicz, 1994. RPS2 of Arabidopsis Thaliana: A Leucine-Rich Repeat Class of Plant Disease Genes. Science 265:1856-1860.

Bickham, J.W., 1981. Two-Hundred-Million-Year-Old-Chromosomes: Deceleration of the Rate of Karyotypic Evolution in Birds. Science 212:1291-1293.

Bird, A.P., 1987. CpG Islands as Gene Markers in the Vertebrate Nucleus. Trends Genet. 3:342.

Birshtein, V.Ya., 1987. Tsitogenetic and Molecular Aspects of Evolution of Vertebrates. Nauka, Moscow.

Bitgood, J.J., and R.G. Somes, 1990. Linkage Relationship and Gene Mapping. Poultry Breeding and Genetics, pp.469-405. Elsevier, Amsterdam.

Bitgood, J.J., and R.N. Schoffner, 1990. Cytology and Cytogenetics, Poultry Breeding and Genetics. Elesevier, Amsterdam.

Bulatova, N.Sh., 1977. Structure and Evolution of Avian Chromosomes, Cytogenetics of Hybrids, Mutations, and Evolution of the Karyotype, pp. 248-259. Novosibirsk, Nauka.

Bumstead, N., and J. Palyga, 1992. A Preliminary Linkage Map of the Chicken Genome. Genomics 13:690-697.

Burmeister, M.A, P. Monaco, E.F. Gillard, G.B. van Ommen, N.A. Affara, M.A. Fergusn-Smith, L.M. Kunkel, and H. Lehrach, 1988. A 10-Megabase Physical Map of Human Xp21, Including Duchene Muscular Dystrophy Gene. Genomics 2:189.

Burt, D.W., N. Bumstead, J.J. Bitgood, F.A. Ponce DeLeon, and L.B. Crittenden, 1995. Chicken Genome Mapping: A New Era in Avian Genetics. Trends Genet. 11:190-194.

Burt, D.W., 1997. Comparative Mapping with the Chicken - Clues to Our Ancestral Vertebrate Genome. Avian Molecular Cytogenetics Symposiumm Leicester, England. Abstract.

Burt, D.W., N. Bumstead, T. Burke, R. Fries, M. Groenen, M. Tixier-Boichard, and A. Vignal, 1997. Current Status of Poultry Genome Mapping - June 1997. In: Proceedings of the 12th AVIAGEN Symposium: Current Problems in Avian Genetics, Prague, Czech Republic, pp. 33-45.

Burt, D.W., C. Bruley, I.C. Dunn, C.T. Jones, A. Rmage, A.S. Law, D.R. Morrice, I.R. Paton, J. Smith, D. Windsor, A. Sazanov, R. Fries, and D. Waddington, 1999. The Dynamics of Chromosome Evolution in Birds and Mammals. Nature 402:411-413.

Carpenter, A.T.C, 1994. Chiasma Function. Cell 77:959-962.

Carver, E.A., and L. Stubbs, 1997. Zooming in on the Human-Mouse Comparative Map: Genome Conservation Re-examined on a High-Resolution Scale. Genome Res. 7:1123-1137.

Chang, Y.-L., Q. Tao, J. Wang, C. Scheuring, K. Meksem, and H.-B. Zhang, 1999. A Large Scale Plant Transformation- and Genome Sequence-Ready Physical Map of the Arabidopsis thaliana Genome. Proceedings of the Plant and Animal Genome VII Conference, p. 37 (abstract).

Chang, E., J. Luna, J. Giacalone, D. Uyar, G.A. Silverman, and U. Francke, 1994. Regional Localization of 56 New Human Chromosome 18-Specific Yeast Artificial Chromosomes. Cytogenet. and Cell Genet. 65:136-139.

Charlier, C., W. Coppieters, F. Farnir, L. Grobet, P.L. Leroy, C. Michaux, M. Mni, A. Schwers, P. Vanmanshoven, and R. Hanset, 1995. The mh Causing Double-Muscling in Cattle Maps to Bovine Chromosome 2. Mamm. Genome 6:788-792.

Chen, Z.-Q., J.A. Lautenberger, L.A. Lyons, L. McKenzie, and S.J. O'Brien, 1999. A Human Genome Map of Comparative Anchor Tagged Sequences. J. Hered. 90:477-484.

Cheng, S., C. Fockler, W. Barnes, and R. Higuchi, 1994. Effective Amplification of Long Targets From Cloned Inserts and Human Genomic DNA. Proc. Natl. Acad. Sci. 91:5695-5699.

Cheng, H.H., R.L. Vallejo, H. Khatib, J.B. Dodgson, L.B. Crittenden, and J. Hillel, 1995. Development of a Genetic Map of the Chicken with Markers of High Utility. Poultry Sci. 74:1855-1874.

Chowdharry, B.P., L. Fronicke, I. Gustavsson, and H. Scherthan, 1996. Comparative Analysis of the Cattle and Human Genomes: Detection of ZOO-FISH and Gene-Mapping Based Chromosomal Homologies. Mamm. Genome 7:297-300.

Clark, M.S., Edwards, Y.J.K., Y.J.K. Edwards, S.E. Meek, S. Smith, Y. Umrania, S. Warner, G. Williams, G. Elgar, 1999. Sequence Scanning Chicken Cosmids: A Methodology for Genome Screening. Gene 227:223-230.

Clement, W.M., 1971. DNA Replication Patterns in the Chromosomes of the Domestic Fowl. Cytologia 8:168-172.

Cohen, D., I. Chumakov, and J. Weissenbach, 1993. A First-Generation Physical Map of the Human Genome. Nature 366:698-701.

Copeland, N.G., N.A. Jenkins, D.J. Gilbert, J.T. Eppig, L.J. Maltais, W.F. Dietrich, A. Weaver, S.E. Lincoln, and R.G. Steen, 1993. A Genetic Linkage Map of the Mouse: Current Applications and Future Prospects. Science 262:57-66.

Crittenden, L.B., L. Provencher, I. Santangelo, H. Levin, H. Abplanalp. R.W. Briles, W.E. Briles, and J.B. Dodgson, 1993. Characterization of a Red Jungle Fowl by White Leghorn Backcross Reference Population for Molecular Mapping of the Chicken Genome. Poultry Sci. 72:334-348.

Crooijmans, R.P.M.A., J.J. van der Poel, and M.A.M. Groenen, 1994. Functional Genes Mapped on the Chicken Genome. Anim. Genet. 26:73-78.

Crooijmans, R.P.M.A., P.A.M. Van Oers, J.A. Strijk, J.J. Van Der Poel, and M.A.M. Groenen, 1996. Preliminary Linkage Map of the Chicken (Gallus domesticus) Genome Based on Microsatellite Markers: 77 New Markers Mapped. Poultry Sci. 75:746-754.

Debry, R.W., and M.F. Seldin, 1996. Human/Mouse Homology Relationships. Genomics 33:337-351.

Deloukas, P., G.D. Schuler, G. Gyapay, E.M Beasley, C. Soderlund, P. Rodriguez-Tome, L. Hui, T.C. Matise, K.B. McKusick, J.S. Beckmann, S. Bentolila, M.-T. Bihoreau, B.B. Birren, J. Browne, A. Butler, A.B. Castle, N. Chiannilkulchai, C. Clee, P.J.R. Day, A. Dehejia, T. Dibling, N. Drouot, S. Duprat, C. Fizames, S. Fox, S. Gelling, L. Green, P. Harrison, R. Hocking, E. Holloway, S. Hunt, S. Keil, P. Lejnzaad, C. Loiis-Dit-Sully, J. Ma, A. Mendis, J. Miller, J. Morissette, D. Mesulet, H.C. Nusbaum, A. Peck,S. rozen, D. Simon, D.K. Slonim, R. Staples, L.D. Stein, E.A. Stewart, M.A. Suchard, T. Thangarajah, N. Vega-Czarny, C. Webber, X. Wu, J. Hudson, C. Auffray, N. Nomura, J.M. Sikela, M.H. Polymeropoulos, M.R. James, E.S. Lander, T.J. Hudson, R.M. Myers, D.R. Cox, J. Weissenbachm M.S. Boguski, and D.R. Bentlry, 1998. A Physical Map of 30,000 Human Genes. Science 282:744-746.

Dietrich, W.F., J. Miller, R. Steen, M.A. Merchant, D. Damron-Boles, Z. Husain, R. Dredge, M.J. Daly, K.A. Ingalls, and T.J. O'Connor, 1996. A Comprehensive Genetic Map of the Mouse Genome. Nature 380:149-153.

Dodgson, J.B., J. Strommer, and J.D. Engel, 1979. Isolation of the Beta-Globin Gene and a Linked Embryonic Beta-Like Globin Gene From a Chicken DNA Recombinant Library. Cell 17:879-887.

Dunner, S., C. Charlier, F. Farnir, B. Brouwers, J. Canon, and M. Georges. 1997. Towards Interbreed IBD Fine Mapping of the mh Locus: Double-Muscling in the Asturiana de los Valles Breed Involves the Same Locus as in the Belgian Blue Cattle Breed. Mamm. Genome 8:430-435.

Dutrillaux, B., 1986. Le Role des Chromosomes dans L'Evolution; une Nouvelle Interpretation. Ann. Genet. 29:69-75.

Eppig, J.T., and J.H. Nadeau, 1995. Comparative Maps: The Mammalian Jigsaw Puzzle. Curr. Opin. Genet. Dev. 5:709-716.
Fillon, V., 1998. The Chicken as a Model to Study Microchromsomes in Birds: A Review. Genet. Sel. Evol., 30:209-219

Fillon, V., M. Morisson, R. Zoorob, C. Auffray, M. Douaire, J. Gellin, and A. Vignal, 1998. Identification of 16 Chicken Microchromosomes by Molecular Markers Using Two-Colour Fluorscence in situ Hybridization. Chromosome Res. 6:307-313.

Flejter, W.L., J. Fergestad, J. Gorski, T. Varvill, and S. Chandrasekharappa, 1998. A gene Involved in XY Sex Reversal is Located on Chromosome 9, Distal to Marker D9S1779. Am. J. Hum. Genet. 63:794-802.

Fridolfsson, A.-K., H. Cheng, N.G. Copeland, N.A. Jenkins, H.-C., Liu, T. Raudsepp, T. Woodage, B. Chowdhary, J. Halverson, and H. Ellegren, 1998. Evolution of the Avian Sex Chromosomes from and Ancestral Pair of Autosomes. Proc. Nat. Acad. Sci. 95:8147-8152.

Fronicke, L., B.P. Chowdhary, H. Scherthan, and I. Gustavsson, 1996. A Comparative Map of the Porcine and and Human Genomes Demonstrates ZOO-FISH and Gene Mapping-Based Chromosomal Homologies. Mamm. Genome 7:285.

Gale, M.D., and K.M. Devos, 1998. Comparative Genetics in the Grasses. Proc. Natl. Acad. Sci. 95:1971-1974.

Goureau, A., M. Yerle, A. Schmitz, J. Riquet, D. Milan, P. Pinton G. Frelat, and J. Gellin, 1996. Human and Porcine Correspondence of Chromosome Segments Using Bidirectional Chromosome Painting. Genomics 36:252-262.

Graves, J.A., 1996. Mammals That Break the Rules: Genetics of Marsupials and Monotremes. Annu. Rev. Genet. 30:233-260.

Grobet, L., L.J.R. Martin, D. Poncelet, D. Pirottin, B. Brouwers, J. Riquet, A. Schoeberlein, S. Dunner, F. Menissier, J. Massabanda, R. Fries, R. Hanset, and M. Georges, 1997. A Deletion in the Bovine Myostatin Gene Causes the Double-Muscled Phenotype in Cattle. Nature Genetics 17:71-74.

Groenen, M.A.M., and R.P.M.A. Crooijmans, Personal Communication. Department of Animal Breeding, Wageningen Institute of Animal Breeding, Wageningen Agricultural University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.

Groenen, M.A.M., R.P.M.A. Crooijmans, A. Veenendaal, H.H. Cheng, M. Siwek, and J.J. van der Poel, 1998. A Comprehensive Microsatellite Linkage Map of the Chicken Genome. Genome Res. 7:1162-1168.

Groenen, M.A.M., R.P.M.A. Crooijmans, R.J.M. Dijkhof, R. Acar, and J.J. van der Poel, 1999. Extending the Chicken-Human Comparative Map by Placing 15 Genes on the Chicken Linkage Map. Anim. Genet. 30:418-422.

Groenen, M.A.M, H.H. Cheng, N. Bumstead, B. Benkel, E. Briles, D.W. Burt, T. Burke, L.B. Crittenden, J. Dodgson, J. Hillel, S. Lamont, F.A. Ponce de Leon, H. Takahashi, and A. Vignal, 2000. A Consensus Linkage Map of the Chicken Genome. Anim. Genet., In Press.

Guioli, S., K. Schmitt, R. Critcher, M. Bouzyk, N.K. Spurr, T. Ogata, J.J. Hoo, L. Pinsky, G. Gimelli, L. Pasztor, and P.N. Goodfellow, 1998. Molecular Analysis of 9p Deletions Associated with XY Reversal: Refining the Localization of a sex-Determining Gene to the Tip of the Chromosome. Am. J. Hum. Genet. 63:905-908.

Gyapay, G., K. Schmitt, C. Fizames, H. Jones, N. Vega-Czarny, D. Spillett, D. Muselet, J.-.F Dib, C. Auffray, J. Morissette, J. Weissenbach, and P.N. Goodfellow, 1996. A Radiation Hybrid Map of the Huma Genome. Hum. Mol. Genet. 5:339-346.

Hardy, D.A., J.I. Bell, E.O. Long, T.Liindsten, and H.O. McDevitt, 1996. Mapping of the Class II Region of the Human Major Histocompatibility Complex by Pulsed Field Gel Electrophoresis. Nature 3323:453.

Hayes, H., 1995. Chromosome Painting With Human Chromosome-Specific DNA Libraries Reveals the Extent and Distribution of Conserved Segments in Bovine Chromosomes. Cytogenet. Cell Genet. 71:168.

Hood, L., B.F. Koop, L. Rowen, and K. Wang, 1993. Human and Mouse T-Cell-Receptor Loci: The Importance of Comparative Large-Scale DNA Sequence Analyses. Cold Spring Harbor Symp. Quant. Biol. 58:339-348.

Hudson, T.J., L.D. Stein, S.S. Gerety, J. Ma, A.B. Castle, J. Silva, D.K. Slonim, R. Baptista, L. Kruglyak, S.H. Xu, A.M.E. Colbert, C. Rosenberg, M.P. Reeve-Daly, S. Rozen, L. Hui, X. Wu, C. Vestergaard, K.M. Wilson, J.S. Bae, S. Maitra, S. Ganiatsas, C.A. Evans, M.M. DeAngelis, K.A. Ingalls, R.W. Nahf, L.T.Jr. Horton, M.O. Anderson, A.J. Collymore, W. Ye, V. Kouyoumjian, I.S. Zemsteva, J. Tam, R. Devine, D.F. Courtney, M.T. Renaud, H. Nguyen, T.J. O'Connor, C. Fizames, S. Faure, G. Gyapay, C. Dib, J. Morissette, J.B. Orlin, B.W. Birren, N. Goodman, J. Weissenbach, T.L. Hawkins, S. Foote, D.C. Page, and E.S. Lander, 1995. An STS-based Map of the Human Genome. Science 270:1945-1954.

Hutchison, N., 1987. Lampbrush Chromosomes of the Chicken, Gallus domesticus. J. Cell Biol. 105:1493-1500.

Januzzi, J.L., N. Arzolan, A. O'Connell, K. Aalto-Setala, and J.L. Breslow, 1992. Characterization of the Mouse Apolipoprotein Apoa-1/Apoc-3 Gene Locus: Genomic, mRNA, and Protein Sequence Comparisons to Other Species. Genomics 14:1081-1088.

Kaback, D.B., 1996. Chromosome-Size Dependent Control of Meiotic Recombination in Humans. Nat. Genet. 13:20-21.

Kaebling, M. and N.S. Fechheimer, 1983a. Synaptonemal Complexes and the Chromosome Complement of Domestic Fowl, Gallus Domesticus. Cytogenet. Cell Genet., 35:87-92.

Kaebling M., and F.S. Fechheimer, 1983b. Synaptonemal Analysis of Chromosome Rearrangements in Domestic Fowl, Gallus Domesticus. Cytogenet. Cell Genet. 36:567-572.

Keller, E.B., and W.A. Noon, 1984. Intron Splicing: a Conserved Internal Signal in Introns of Animals Pre-mRNAs. Proc. Natl. Acad. Sci. 81:7417-7420.

Klein, S., D.R. Morrice, H. Sang, L.B. Crittenden, and D.W. Burt, 1996. Genetic and Physical Mapping of the Chicken IGF1 Gene to Chromosome 1 and Conservation of Synteny with Other Vertebrate Genomes. J. Hered. 87:10-14.

Knorr, C., H.H. Cheng, and J.B. Dodgson, 1999. Application of AFLP Markers to Genome Mapping in Poultry. Anim. Genet. 30:28-35.

Koch, J.E., S. Kolvraa, K.B. Peterson, N. Gregersen, and L. Bolund, 1989. Oligonucleotide-Priming Methods for the Chromosome-Specific labelling of Alpha Satellite DNA in situ. Chromosoma 98:259-265.

Koop, B.F., R.K. Wilson, K. Wang, B. Vernooij, D. Zallwer, C.L. Kuo, D. Seto, M. Toda, and L. Hood, 1992. Organization, Structure, and Function of 95Kb of DNA Spanning the Murine T-Cell-Receptor C ALpha/C Delta Region. Genomics 13:1209-1230.

Koop, B.F., L. Rowen, K. Wang, C.L. Kuo, D. Seto, J.A. Lenstra, S. Howard, W. Shan, P. Deshpande, and L. Hood, 1994. The Human T-cell Receptor TCRAC/TCRDC (C α/C δ) Region: Organization, Sequence, and Evolution of 97.6Kb of DNA. Genomics 19:478-493.

Krishan, A., 1964. Microchromosomes in the Spermatogenesis of the Domestic Turkey. Exp. Cell Res. 33:1-7.

Kumar, S., and S.B. Hedges, 1998. A Molecular Timescale for Vertebrate Evolution. Nature 392:917-920.

Kunz, J., S.W. Scherer, I. Klawitz, S. Soder, Y.Z. Du, N. Speich, M. Kaiff-Suske, H.H. Heng, L.C. Tsui, and K.H. Grzeschik, 1994. Regional Localization of 725 Human Chromosome 7-Specific Yeast Artificial Chromosome Clones. Genomics 22:439-448.

Lamerdin, J.E., M.A. Montgomery, S.A. Stilwagon, L.K. Scheodecker, R.S. Tebbs, K.W. Brookman, L.H. Thompsom, and A.V. Carrano, 1995. Genomic Sequence Comparison of the Human and Mouse XRCC1 DNA Repair Gene Regions. Genomics, 25:547-554

Lamerdin, J.E., S.A. Stilwagon, M.H. Ramirez, L. Stubbs, and A.V. Carrano, 1996. Sequence Analysis of the ERCC2 Gene Regions in Human, Mouse, and Hamster Reveals Three Linked Genes. Genomics 34:399-409.

Larson, F., G. Gunderson, R. Lopez, and H. Prydz, 1992. CpG Islands as Gene Markers in the Human Genome. Genomics 13:1095.

Lauer, P., N.C. Meyer, C.E. Prass, S.M. Starnes, R.K. Wolff, and A. Gnirke, 1997. Clone-Contig and STS Maps of the Hereditary Hemochromatosis Region on Human Chromosome 6p21.3-p22. Genome Res. 7:457-470.

Lopez, J.V., S. Cevario, and S.J. O'Brien, 1996. Complete Nucletide Sequences of the Domestic Cat (Felis catus) Mitochondrial Genome and a Transposed mtDNA Tandem Repeat (Numt) in the Nuclear Genome. Genomics 33:229-246.

Lyons, L.A., M. Menotti-Raymond, and S.J. O'Brien, 1994. Comparative Genomics: The Next Generation. Anim. Biotechnol. 5:103-111.

Lyons, L.A., T.F. Laughlin, N.G. Copeland, N.A. Jenkins, J.E. Womack, and S.J. O'Brien, 1997. Comparative Anchor Tagged Sequences (CATS) for Integrative Mapping of Mammalina Genomes. Nat. Genet. 15:47-56.

Manly, K.F., 1993. A Macintosh Program for Storage and Analysis of Experimental Genetic Mapping Data. Mamm. Genome 4:303-313.

Mariman, E.C., P.T. Sillekens, R.J. vanBeekReinders, and W.J. vanVenrooij, 1984. A Model for the Excision of Introns 1 and 2 from Adenoviral Major Late Pre-Messenger RNAs. J. Mol. Biol. 178:47-62.

Marklund, L., M. Moller-Johansson, B. Hoyheim, W. Davies, M. Fredholm, R.K. Juneja, P. Mariani, W. Coppieters, H. Ellegren, and L. Andersonn, 1996. A Comprehensive Linkage Map of the Pig Based on a Wild Pig-Large White Intercross. Anim. Genet. 27:255-269.

Marra, M., T. Kucaba, M. sekhon, L. Hillier, R. Martienssen, A. Chinwalla, J. Crockett, J. Fedele, H. Grover, C. Gund, W.R. McCombie, K. McDonald, J. McPherson, N. Mudd, L. Parnell, J. Schein, R. Seim, P. Shelby, R. Waterson, and R. Wilson, 1999. Nat. Genet. 22:265-270.

Marin, I., and B.S. Baker, 1998. The Evolutionary Dynamics of Sex Determination. Science 281:1990-1994.

Martin, G.B., S.H. Brommonschenkel, J. Chunwongse, A. Frary, M.W. Ganal, R. Spivey, T. Wu, E.D. Earle, and S.D. Tanksley, 1993. Map-Based Cloning of Protein Kinase Gene Conferring Disease Resistance in Tomato. Science 262:1432-1436.

Matzke, A.J.M, F. Varga, P. Gruendler, I. Unfried, H. Berger, B. Mayr, and M.A. Matzke, 1992. Characterization pf a New Repetitive Sequence That is Enriched on Microchromosomes of Turkey. Chromosoma 102:9-14.

Maxson, L.R., and A.C. Wilson, 1979. Rates of Molecular and Chromosomal in Salamanders. Evolution 33:734-740.

McCarthy, L.C., 1996. Whole Genome Radiation Hybrid Mapping. Trends Genet. 12:491-493.

Mccarthy, L.C., J. Terrett, M.E. Davis, C.J. Knights, A.L. Smith, R. Critcher, K. Schmitt, J. Hudson, N.K. Spurr, and P.N. Goodfellow, 1997. A First-Generation Whole-Genome Radiation Hybrid Map Spanning the Mouse Genome. Genome Res. 7:1153-1161.

McDermid, H.E., K.E. McTaggart, M.A. Riazi, T.J. Hudson, M.L. Budarf, B.S. Emanuel, and C.J. Bell, 1996. Long-Range Mapping and Construction of a YAC Contig Within the Cat Eye Syndrome Critical Region. Genome Res. 6:1149-1159.

McQueen, H.A., J. Fantes, S.H. Cross, V.H. Clark, A.L. Archibald, and A.L. Bird, 1996. CpG Islands of Chickens are Concentrated on Microchromosomes. Nat. Genet. 12:321-324.

McQueen, H.A., G. Siriaco, and A.P. Bird, 1998. Chicken Microchromosomes are Hyperacetylated, Early Replicating, and Gene Rich. Genome Res. 8:621-630.

Moir, D.T., T.E. Dorman, J.C. Day, N.S. Ma, M.T. Wang, and J.I. Mao, 1994. Toward a Physical Map of Human Chromosome 10: Isolation of 183 YACs Representing 80 Loci and Regional Assignment of 94 YACs by Fluorescence in situ Hybridiztion. Genomics 22:1-22.

Mozo, T., K. Dewar, P. Dunn, J.R. Ecker, S. Fischer, S. Kloska, H. Lehrach, M. Marra, R. Martienssen, S. Meier-Ewert, and T. Altmann, 1999. A Complete BAC-Based Physical Map of the Aribidopsis thaliana genome. Nat. Genet. 22:271-275.

Murphy, W.J., M. Menotti-Raymond, L.A. Lyons, M.A. Thompson, and S.J. O'Brien, 1999. Development of a Feline Whole Genome Radiation Hybrid Panel and Comparative Mapping of Human Chromosome 12 and 22 Loci. Genomics 57:1.
Myakoshina, Y.A., and A.V. Rodionov, 1994. Meiotic Lampbrush Chromosomes in Turkey Meleagris gallopavo (Galliformes:Meleagridea). Genetika 30:649-656.

Nadeau, J.H., and B.A. Taylor, 1984. Lengths of Chromsomal Segments Conserved Since Divergence of Man and Mouse. Proc. Nat. Acad. Sci. 81:814-818.

Nadeau J.H., and D. Sankoff, 1998. Counting on Comparative Maps. Trends Genet. 14:495-501.

Nagaraja, R., S. MacMillan, J. Kere, C. Jones, S. Griffin, M. Schmatz, J. Terrell, M. Shomaker, C. Jermak, C. Hott, M. Masisi, S. Mumm, A. Srivastava, G. Pilia, T. Featherstone, R. Mazzarella, S. Kesterson, B. Mccauley, B. Railey, F. Burough, V. Nowotny, M. D'Urso, D. States, B. Brownstein, and D. Schlessinger, 1997. X Chromosome Map at 75-kb STS Resolution, Revealing Extremes of Recombination and GC Content. Genome Res. 7:210-222.

Nagata, T., E.H. Weiss, K. Abe, K. Kitagawa, A. Ando, Y. yara-Kikuti, M.P. Seldin, K. Ozato, H. Inoko, and M. Taketo, 1995. Physical Mapping of the Retinoid X Receptor in Mouse and Human. Immunogenetics 41:83-90.

Nanda, I., Z. Shan, M. Schart, D.W. Burt, M. Koehler, H. Nothwang, F. Grutzner, I.R. Paton, D. Windsor, I. Dunn, W. Grutzner, P. Staeheli, S. Mizuno, T. Haaf, and M. Schmid, 1999. 300 Million Years of Conserved Synteny, Between Chicken Z and Human Chromosome 9. Nat. Genet. 21:258-259.

Neff, M.W., K.W. Broman, C.S. Mellersh, K. Ray, G.M. Acland, G.D. Aguirre, J.S. Ziegle, E.A. Ostrander, and J. Rine, 1999. A Second-Generation Genetic Linkage Map of the Domestic Dog, Canis familiaris. Genetics 151:803.
Newcomer, E.H., 1957. The Mitotic Chromsomes of the Domestic Fowl. J. Hered. 48:227-234.

Oakley, R.J., M.L. Watson, and M.F. Seldin, 1992. Construction of a Physical Map on Mouse and Human Chromosome 1: Comparison of 13Mb of Mouse and 11Mb of Human DNA. Hum. Mol. Genet., 1:613-620.
O'Brien, S.J., and W.G. Nash, 1982. Genetic Mapping in Mammals: Chromosome Map of the Domestic Cat. Science 216:257-265.

O'Brien, S.J., 1993. Comparative Biology: The Genomics Generation. Curr. Biol. 3:395-397.

O'Brien, S.J., J. Wienberg, and L.A. Lyons, 1997. Comparative Genomics: Lessons From Cats. Trends Genet. 13:393-399.

O'Brien, S.J., M. Menotti-Raymond, W.J. Murphy, W.G. Nash, J. Wienberg, R. Stanyon, N.G. Copeland, N.A. Jenkins, J.E. Womack, and J.A.M. Graves, 1999. The Promise of Comparative Genomics in Mammals. Science 286:458-464.

Oeltjen, J.C., T.M. Malley, D.M. Muzney, W. Miller, R.A. Gibbs, and J.W. Belmont, 1997. Large-Scale Sequence Analysis of the Human and Murine Bruton's Tyrosine Kinase Loci Reveals Conserved Regulatory Domains. Genome Res. 7:315-329.

Ohno, S., 1966. Sex Chromosomes and Sex-Linked Genes. Springer-Verlag Berlin, Heidelberg, and New York.

Okimoto, R., and J.B. Dodgson, 1996. Improved PCR Amplification of Multiple Specific Alleles (PAMSA) Using Internally Mismatched Primers. Biotechniques 21:20-22,24,26.

Okimoto, R., H.H. Cheng, and J.B. Dodgson, 1997. Characteriztion of CR1 Repeat Random PCR Markers for Mapping the Chicken Genome. Anim. Genet. 28:139-145.

Paterson, A.H., T.-H., Lan K.P. Resichmann, C. Chang, Y.-R., Lin, M.D. Burow, S.P. Kowalski, C.S. Katsar, T.A. DelMonte, K.A. Feldman, K.F. Schertz, and J.F. Wendel, 1996. Toward a Unified Genetic Map of Higher Plants, Transcending the Monocot-Dicot Divergence. Nat. Genet. 14:380-382.

Pirottin, D., D. Poncelet, L. Grobet, L.J. Royo, B. Brouwers, J. Masabanda, H. Takeda, R. Fries, Y. Sugimoto, J.E. Womack, S. Dunner, amd M. Georges, 1999. High-Resolution, Human-Bovine Comparative Mapping Based on a Closed YAC Contig Spanning the Bovine MH Locus. Mamm. Genome 10:289-293.

Pollock, D.L., and N.S. Fechheimer, 1981. Variable C-Banding Patterns and a Proposed C-Band Karyotype in Gallus domesticus. Genetica 54:273-279.

Priat C., C. Hitte, F. Vignaux, C. Renier, Z. Jiang, S. Jouquand, A. Cheron, C. Andre, and F. Galibert, 1998. A Whole-Genome Radiation Hybrid Map of the Dog Genome. Genomics 54:361.

Primmer, C.R., T. Raudsepp, B.P. Chowdhary, A.P. Moller, and H. Ellegren, 1997. Low Frequency of Microsatellite in the Avain Genome. Genome Res. 7:471-482.

Purchase, H.G., 1985. Clinical Disease and its Economic Impact. In Marek's Disease, Scientific Basis and Methods of Control (ed. L.N. Payne) pp. 17-42. Martinus Nkjhoff Publishing, Boston.

Rahn, M.I., and A.J. Solari, 1986. Recombination Nodules in the Oocytes of the Chicken, Gallus domesticus. Cytogenet. Cell Genet. 43:187-193.

Raymond, C.S., C.E. Shamu, M.M. Shen, K.J. Seifert, B. Hirsch, J. Hodgkin, and D. Zarkower, 1998. Evidence for Evolutionary Conservation of Sex-Determing Genes. Nature 391:691-695.

Raymond, C.S., E.D. Parker, J.R. Kettlewell, L.G. Brown, D.C. Page, K. Kusz, J. Jaruzelska, Y. Reinberg, W.L. Flejter, V.J. Bardwell, B. Hirsch, and D. Zarkower, 1998. A Region of Human Chromosome 9q Required for Testis Development Contains Two Genes Related to Known Sexual Regulators. Hum. Mol. Genet. 8:989-996.

Reed, J.A., and K.C. Graves, 1993. Chapter 10. In Sex Chromosomes and Sex-Determining Genes. Harwood Academic Publishers, Switzerland.

Renwick, J.H., 1971. 4th International Congress of Human Genetics. Paris, France.

Renucci, A., V. Zappavigna, J. Zakany, J.C. Izpisua-Belmonte, K. Burki, and D. Duboule, 1992. Comparison of the Mouse and Human HOX-4 Complexes Defines Conserved Sequences Involved in the Regulation of HOX-4.4. EMBO J. 11:1459-1468.

Rettenberger, G., C. Klett, U. Zechner, J. Kunz, W. Vogel, and H. Hameister, 1995. Visualization of the Conservation of Synteny Between Humans and Pigs by Heterologous Chromosomal Painting. Genomics 26:372-378.

Rodionov, A.V., 1985. Genetic Activity of DNA from G and R Blocks of of Human Mitotic Chromosomes. Genetika 21:2057-2065.

Rodionov, A.V., L.A. Chelysheva, E.V. Kropotova, and E.R. Gaginskaya, 1989. Heterochromatic Chromosome Regions of Chickens and Japanese Quail in Mitosis and at the Lampbrush Stage. Tsitologia 31:867-873.

Rodionov, A.V., Y.U. Myakoshina, L.A. Chelysheva, and E.P. Gaginskaya, 1992a. Chiasmata on Lampbrush Chromosomes of Gallus gallus domesticus. Cytogenetic Investigations of Recombination Frequency and Linkage Group Length. Genetika 28:53-63.

Rodionov A.V., L.A. Chelysheva, I.V. Solovei, and Y.U. Myakoshina, 1992b. Chiasma Distribution in the Lampbrush Chromosomes of the Chicken Gallus gallus domesticus: Hot Spots of Recombination and Their Possible Role in the Proper Dysjunction of Homologous Chromosomes at the First Meiotic Division. Genetika 28:151-160.

Rodionov, A.V., 1996. Micro versus Macro: A review of Structure and Functions of Avian Micro and Macrochromosomes. Russian J. Genet. 5:517-527.

Rodionov, A.V., 1997. Evolution of Avian Chromosomes and Linkage Groups. Russian J. Genet. 6:605-617.

Rohrer, G.A., L.J. Alexander, Z. Hu, T.P. Smith, J.W. Keele, and C.W. Beattie, 1996. A Comprehensive Map of the Porcine Genome. Genome Res. 6:371-391.

Sazanov, A., Department of Animal Breeding, Technical University of Munich, Alte Akademie 12, 85350 Freising-Weihenstephan.

Sazanov, A., L.A. Alekseevich, A.L. Sazanova, and A.F. Smirnov, 1996. Mapping the Chicken Genome: Problems and Perspectives. Genetika 32:869-878.

Schmid, W., 1962. Replication Patterns of the Heterochromosomes in Gallus Domesticus. Cytogenetics 1:344-352.

Schmid, M., and M. Guttenbach, 1988. Evolutionary Diversity of Reverse (R) Fluorescent Chromosome Bands in Vertebrates. Chromosoma 97:101-114.

Schuler, G.D., M.S. Boguski, E.A. Stewart, L.D. Stein, G. Gyapay, K. Rice, R.E. White, P. Rodriguez, A. Aggarwal, E. Bajorek, S. Bentolila, B.W. Birren, A. Butler, A.B. Castle, N. Chiannikulchai, A. Chu, C. Clee, S. Cowles, P.J.R. Day, T. Dibling, N. Drouot, I. Dunham, S. Duprat, C. East, C. Edwards, J.B. Fan, N. Fang, C. Fizames, C. Garrett, L. Green, D. Hadley, M. Harris, P. Harrison, S, Brady, A. Hicks, E. Holloway, I. Hui, S. Hussein, C. Louis-Dit-Sully, J. Ma, A. MacGilvery, C. Mader, A. Maratukulam, T.C. Matise, K.B. McKusick, J. Morissette, A. Mungall, D. Muselet, H.C. Nusbaum, D.C. Page, A. Peck, S. Perkins, M. Piercy, F. Qin, J. Quackenbush, S. Ranby, T. Reif, S. Rozen, C. Sanders, X. She, J. Silva, D.K. Sloinim, C. Soderlund, W.L., Sun, P. Taber, T. Thangarajah, N. Vega-Czarny, D. Vollrath, S. Voyticky, T. Wilmer, X. Wu, M.D. Adams, C. Auffray, N.A.R. Walter, R. Brandon, A. Dehjia, P.N. Goodfellow, R. Houlgatte, J.R. Hudson Jr., S.E. Ide, K.R. Iorio, W.Y. Lee, N. Seki, T. Nagase, K. Schmitt, R. Berry, K. Swanson, R. Torres, J.C. Venter, J.M. Sikela, J.S. Beckmann, P. Deloukas, E.S. Lander, and T.J. Hudson, 1996. A Gene Map of the Human Genome, Science 274:540-546.

Sinclair, A.H., J.W. Foster, J.A. Spencer, D.C. Page, M. Palmer, P.N. Goodfellow, and J.A.M. Graves, 1990. Sequences Homologous to *ZFY*, a Candidate Human Sex-Determining Gene, are Autosomal in Marsupials. Nature 336:780-783.

Slizynski, B.M., 1964. Cytological Observations on a Duck Hybrid: Anas clypeata X Anas penelope. Genet. Res. Camb. 5:441-447.

Smith, T.P., G.A. Rohrer, L.J. Alexander, D.L. Troyer, K.R. Kirby-Dobbels, M.A. Janzen, D.L. Cornwell, C.F. Louis, L.B. Schook, and C.W. Beattie, 1995. Directed Integration of the Physical and Genetic Linkage Maps of Swine Chromsome 7 Reveals that the SLA Spans the Centromere. Genome Res. 5:259-271.

Smith, E.J., L.A. Lyons, H.H. Cheng, and S.P. Suchyta, 1997. Comparative Mapping of the Chicken Genome Using the East Lansing Reference Population. Poultry Sci. 76:743-747.

Smith, C.A., P.J. McClive, P.S. Western, K.J. Reed, and A.H. Sinclair, 1999. Conservation of a Sex-Determining Gene. Nature 402:601-602.

Soeda, E., D.X. Hou, K. Osoegawa, Y. Atsuchi, T. Yamagata, T. Shimokawa, H. Kishida, S. Okano, and I. Chumakov, 1995. Cosmind Assembly and Anchoring to Human Chromosome 21. Genomics 25:73-84.

Solinas-Toldo, S., C. Lengauer, and R. Fries, 1995. Comparative Genome Map of Human and Cattle. Genomics 27:486-496.

Song, W.Y., G.L. Wang, L.L. Chen, H.S. Kim, Y.P. Pi, T. Holsen, J. Gordnee, B. Wang, W.X. Zhai, L.H. Zhu, C. Faouquet, and P. Ronald, 1995. A Receptor Kinas-Like Protein Encoded by the Rice Disease Resistance Gene, Xa21. Science 270:1804-1806.

Sonstegard, T.S., N.L. Lopez-Corrales, S.M. Kappes, C.W. Beattie, and T.P. Smith, 1997. Comparative Mapping of the Bovine and Human Chromosome 2 Identifies Segments of Conserved Synteny and Increases Informative Marker Density Near the Bovine mh Locus. Mamm. Genome 8:751-755.

Sonstegard, T.S., S.M. Kappes, J.W. Keele, and T.P.L. Smith, 1998. Refinement of the Bovine Chromosome 2 Linkage Map Near the mh Locus Reveals Rearrangements Between the Bovine and Human Genomes. Anim. Genet. 29:341-347.

Southern, E.M.. 1975. Detection of Specific Sequences Among DNA Fragments Seperated by Gel Electrophoresis. J. Mol. Biol. 98:503.

Stallings, R.L., N.A. Doggett, D. Callen, S. Apostolou, L.Z. Chen, J.K. Nancarrow, S.A. Whitmore, P. Harris, H. Michison, and M. Breuning, 1992. Evaluation of a Cosmid Contig Physical Map of Human Chromosome 16. Genomics 13:1031-1039.

Stefos, A.D., and F.E. Arrighi, 1974. Repetitive DNA of Gallus domesticus and Its Cytological Localization. Exp. Cell. Res. 83:9-14.

Stewart, E.A., K.B. McKusick, A. Aggarwal, E. Bajorek, S. Brady, A. Chu, N. Fang, D. Hadley, M. Harris, S. Hussain, R. Lee, A. Maratukulam, K. O'Connor, S. Perkins, M. Piercy, F. Qin, T. Reif, C. Sanders, X. She, W.L. Sun, P. Tabar, S. Voticky, S. Cowles, J.B. Fan, D.R. Cox, et al., 1997. An STS-Based Radiation Hybrid Map of the Human Genome. Genome Res. 7:422-433.

Stock, A.D., and G.A. Mengden, 1975. Chromosome Banding Pattern Conservatism in Birds and Nonhomology of Chromosome Banding Patterns Between Birds, Turtles, Snakes, and Amphibians. Chromosoma 50:69-77.

Takagi, N. and M. Sasaki, 1974. A Phylogenetic Study of Bird Karyotypes. Chromosoma 46:91-120.

Tao, Q., Y.-L. Chang, J. Wang, H. Chen, M.N. Islam-Faridi, C. Scheuring, B. Wang, D.M. Stelly, and H.-B. Zhang, 1999. A Large-Scale Sequence-Ready Physical Map of the Rice Genome. Proceedings of the Plant and Animal Genome IV Conference, p. 101 (abstract).

Tegelstrom, H., and H. Ryttman, 1981. Chromosomes in Birds (Aves): Evolutionary Implications of Macro- and Microchromosome Numbers and Lengths. Hereditas 94:225-233.

Thorne, M. H., and B.L. Sheldon, 1992. Triploid Intersex and Chimeric Chickens: Usefel Models for Studies of Avian Sex Determination. Chapter 15. in Sex Chromosomes and Sex-Determining Genes. Harwood Academic Publishers, Switzerland.

Totaro, A., J.M. Rommens, A. Grifa, C. Lunardi, M. Carella, J.J. Huizenga, A. Roetto, C. Camaschella, G. DeSandre, and P. Gasparini, 1996. Hereditary Hemochromatosis: Generation of a Transcription Map within a Refined and Extended Map of the HLA Class I Region. Genomics 31:319-326.

Turner, B.M., 1993. Decoding the Nucleosome. Cell 75:5-8.

Vallejo, R.L., H. Liu, R.L. Witter, M.A.M. Groenen, J. Hillel, and H.H. Cheng, 1998. Genetic Mapping of Quantitative Trait Loci to Marek's Disease Virus Induced Tumors in F_2 Intercross Chickens. Genetics 148:349-360.

Van Etten, W.J, R.G. Steen, H. Nguyen, A.B. Castle, D.K. Slonim, B. Ge, C. Nusbaum, G.D. Schuler, E.S. Lander, and T.J. Hudson, 1999. Radiation Hybrid Map of the Mouse Genome. Nat. Genet. 22:384-387.

Van Houten, W., N. Kurata, Y. Umehara, T. Sasaki, and Y. Minobe, 1996. Generation of a YAC Comtig Encompassing the Extra Glume Gene, eg, in Rice. Genomics 39:1072-1076.

Venta, P.J., J.A. Brouillette, V. Yuzbasiyan-Gurkan, and G.J. Brewer, 1996. Gene-Specific Universal Mammalian Sequence-Tagged Sites: Application to the Canine Genome. Biochem. Genet. 34:321-341.

Wachtel, S.S., 1987. Evolutionary Mechanisms in Sex Determination. CRC Press Inc., Florida.

Wade, P.A., D. Pruss, and A.P. Wolfe, 1997. Histone Acetylation: Chromatin in Action. Trends Biochem. Sci. 4:128-132.

Watkins-Chow, D.E., M.S. Buckwalter, M.M. Newhouse, A.C. Lossie, M.L. Brinkmeier, and S.A. Camper, 1997. Genetic Mapping of 21 Genes on Mouse Chromosome 11 Reveals Disruptions in Linkage Conseervation with Human Chromosome 5. Genomics 40:114-122.

Watson, J.M., J.A. Spencer, A.D. Riggs, and J.A. Marshall Graves, 1991. Sex Chromosome Evolution: Platypus Gene Mapping Suggests that Part of the Human X Chromosome was Originally Autosomal. Proc. Nat. Acad. Sci., 88:11256-11260.

Wienberg, J., and R. Stanyon, 1995. Curr. Opin. Genet. Dev. 5:792-797.

Weiss, E.H., L. Golden, K. Fahrner, A.L. Mellor, J.J. Devlin, H, Bullman, H. Tiddens, H. Bid, and R.A. Flavell, 1984. Organization and Evolution of the Class I Gene Family in the Major Histocompatibility Complex of the C57BL/10 Mouse. Nature 310:650-655.

Yonash, N., L.D. Bacon, R.L. Witter, and H.H. Cheng, 1999. High Resolution Mapping and Identification of New Quantitative Trait Loci (QTL) Affecting Susceptibility to Marek's Disease. Anim. Genet. 30:126-135.

Yoo, J., R.T. Stone, S.M. Kappes, and C.W. Beattie, 1994. Linkage Analysis of Bovine Interleukin Receptor Types I and II (IL-1R I, II). Mamm. Genome 5:820-821.

Yoshida, K., M.P. Strathman, C.A. Mayeda, C.H. Martin, and M.J. Palazzolo, 1993. A Simple and Efficient Method for Constructing High Resolution Physical Maps. Nucleic Acids Res. 21:3553-3562.

Yoshimura, S., Y. Umehara, N. Kurata, Y. Nagamura, T. Sasaki, Y. Minobe, and N. Iwata, 1996. Identification of a YAC Clone Carrying the Xa-1 Allele, a Bacterial Blight Resistance Gene in Rice. Theor. Appl. Genet. 93:117-122.

Yuhki, N., and S.J. O'Brien, 1988. Molecular Characterization and Genetic Mapping of Class I and II MHC Genes of the Domestic Cat. Imunnogenetics 27:414-425.

Zhang, H.-B., and Q. Tao, 1997. A Simple, Economic and Universal Kit for Rapidly Fingerprinting Cloned DNA. Invention No.: TAMUS#1228 (International Patent in Pending).