HIGH RESOLUTION PHYSICAL AND COMPARATIVE MAPS OF HORSE
CHROMOSOMES 14 (ECA14) AND 21 (ECA21)

A Thesis

by

GLENDA GOH

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2005

Major Subject: Genetics
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ABSTRACT

High Resolution Physical and Comparative Maps of Horse Chromosomes 14 (ECA14) and 21 (ECA21). (May 2005)

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In order to identify genes or markers responsible for economically important traits in the horse, the development of high resolution gene maps of individual equine chromosomes is essential. We herein report the construction of high resolution physically ordered radiation hybrid (RH) and comparative maps for horse chromosomes 14 and 21 (ECA14 and ECA21). These chromosomes predominantly share correspondence with human chromosome 5 (HSA5), though a small region on the proximal part of ECA21 corresponds to a ~5Mb region from the short arm of HSA19. The map for ECA14 consists of 128 markers (83 Type I and 45 Type II) and spans a total of 1828cR. Compared to this, the map of ECA21 is made up of 90 markers (64 Type I and 26 Type II), that segregate into two linkage groups spanning 278 and 760cR each. A total of 218 markers provide on average one marker every 0.9Mb along the length of the two equine chromosomes. This represents a 5-fold improvement over the previous maps. Of greater significance is the ~8-fold increase in the density of Type I loci that provide a comprehensive and finely aligned map for the two chromosomes in relation to homologues in a range of evolutionarily distantly related species, viz., human, chimpanzee, mouse, rat, dog, cattle, pig, cat and chicken. The orientation and alignment
of the linkage groups was strengthened by 28 new FISH localizations, of which 27 are gene-specific (22 from HSA5 and 5 from HSA19). Comparative analysis between the horse and human reveals that the order of genes on HSA5 is remarkably well conserved in the horse, with an evolutionary break/fusion point that could be correlated to a ~2Mb region between 68.5 – 70.9Mb positions on HSA5. Among the species analyzed to date, the HSA5 and 19p neighboring segment combination is unique to Perissodactyls and Cetartiodactyls, but, in the Perissodactyls, the portion of HSA5 that corresponds to this combination is HSA5p – q13, while in the Cetartiodactyls, it is HSA5q13 – qter. This leads us to postulate that this neighboring segment combination arose as separate events during the divergence of Perissodactyls and Cetartiodactyls from a common ancestor.
DEDICATION

This thesis is dedicated to the memory of Peter Kim Hwa Goh (1944 – 1996), who instilled in me the drive and determination to pursue my goals.

To my mother and sister and without whose continuous support and encouragement this may not have been possible, and to Sam who offered me unconditional love, patience and understanding through this endeavour.
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INTRODUCTION

The horse belongs to the order Perissodactyla, the odd-toed ungulates, which contains 16 species divided into three families, Equidae (horses, zebras, and asses), Rhinocerotidae (rhinoceroses), and Tapiridae (tapirs) (Prothero and Schoch 1989). Perissodactyls are strict herbivores (browsers and gazers) and are adapted for running. The family Equidae is made up of eight living species of the genus Equus. These include Equus asinus (African wild ass), Equus kiang (Tibetan wild ass), Equus burchellii (Burchell’s zebra or Plains zebra), Equus grevyi (Grevy’s Zebra), Equus zebra (Mountain zebra), Equus onager (Onager), Equus caballus (Domestic horse), and Equus przewalskii (Przewalski’s wild horse) (http://www.ultimateungulate.com/Perissodactyla.html).

The horse is believed to have been domesticated about 5000 – 6000 years ago in the Eurasian grassland steppe, in the vicinity of the current Ukraine, Kazakhstan, and Mongolia for meat, riding or traction (Olsen 1996, Clutton-Brock 1999, Levine 2002). Today, horses serve multiple roles including transport, livestock, companionship, meat, sports and recreation. With about 6.9 million horses in the United States of America alone, the horse industry has a notable influence on the economy of this country. In 1996, the horse industry had a total impact of $112.1 billion on the U.S. Gross Domestic Product (GDP), while the horse racing industry alone had a total impact of approximately $34.03 billion (http://www.horsecouncil.org/statistics.htm). Efforts are

This thesis follows the style and format of the journal Mammalian Genome.
being made to update the National Economic Impact of the Horse Industry in the United States of America (http://www.horsecouncil.org/releases.htm). Given the importance of the horse industry, it is not surprising that the health, welfare and performance of horses are of primary concern to equine practitioners and breeders worldwide. In order to understand and identify the genes that are responsible for health, disease resistance, reproduction and performance in the horse, the development of a detailed horse gene map is essential.

Early horse breeders selected individuals based on phenotypic appearance without any information regarding their genetic status, or genotype. A prime example of how this selection method can be detrimental is well documented in the case study of the spread of hyperkalemic periodic paralysis (HYPP) in Quarter Horses. This muscular disease is caused by a point mutation in the sodium channel (α subunit) (SCN4A) gene, and individuals carrying this mutation are predisposed to potassium-induced attacks of skeletal muscle paralysis (Fontaine et al. 1990, Ptacek et al. 1991, Rudolph et al. 1992a, Rudolph et al. 1992b). HYPP is inherited as an autosomal co-dominant trait where the homozygous individual is more severely affected than the heterozygous individual (Naylor 1994, Naylor et al. 1999).

The origin of this mutation in Quarter Horses can be traced back to a single horse, Impressive, which was preferentially selected and bred for its musculature (Naylor 1997). The frequency of this allele has been calculated at 0.02 (Bowling et al. 1996)
which means that approximately 2% of all Quarter Horses carry this mutation. With more than 3.14 million Quarter Horses registered with the American Quarter Horse Association, (AQHA; http://www.aqha.com/association/who/statistics.html), the total number of affected individuals is estimated at almost 63 000 worldwide.

In humans, mutations in the SCN4A gene are associated with several disorders including hyperkalemic periodic paralysis (HyperPP), hypokalemic periodic paralysis (HypoPP), paramyotonia congenita (PC), and potassium aggravated myotonias (PAM) (http://www3.ncbi.nlm.nih.gov/entrez/dispomim.cgi?id=170500) (Ashcroft 2000). The SCN4A gene maps to human chromosome 17q23 – 25, and is closely linked to the human growth hormone gene. In the horse, Rudolph et al. studied the sodium channel gene in horses affected by HYPP and found that the same gene that causes HYPP in humans causes the same disorder in horses (Rudolph et al. 1992a, Rudolph et al. 1992b). This gene has been mapped to horse chromosome 11 (ECA11) using comparative Zoo-FISH analysis (Raudsepp et al. 1996), synteny mapping (Caetano et al. 1999b) and radiation hybrid mapping techniques (Chowdhary et al. 2003). DNA tests are now available to identify the genotype of horses and to identify horses homozygous for HYPP, carriers of the mutant gene, as well as normal unaffected horses.

The American Quarter Horse Association (AQHA) currently requires that “foals born in 1998 and later and tracing to Impressive will have a statement placed on their Certificates of Registration that recommends testing for the condition unless test results
indicating the foal is negative (N/N) are on file with AQHA” (http://www.aqha.com/association/registration/hypp.html). This is a positive step taken by the AQHA which will significantly decrease the number of horses affected by HYPP. Horse breeders now have access to the carrier status of a particular horse, and are able to conduct informative breeding choices and prevent the spread of the mutant $SCN4A$ allele in the population.

Other genes that have been found to be responsible for valuable traits associated with equine biology, health and performance include genes responsible for severe combined immunodeficiency (SCID) (Wiler et al. 1995, Shin et al. 1997b, a), overo lethal white foal syndrome (Metallinos et al. 1998, Santschi et al. 1998, Yang et al. 1998), a putative horse homolog of congenital aniridia (Ewart et al. 2000), and base coat color genes (Singleton and Bond 1966, Andersson and Sandberg 1982, Marklund et al. 1996, Santschi et al. 1998, Locke et al. 2001, Rieder et al. 2001, Brooks et al. 2002, Henner et al. 2002, Swinburne et al. 2002, Mariat et al. 2003, Terry et al. 2004). The genes that cause diseases or different phenotypes in horses were identified by using comparative gene mapping, as well as linkage, synteny and radiation hybrid mapping.

Therefore, in order to select and breed horses for performance, conformation and reproduction while avoiding hereditary health problems, a better knowledge of the horse genome and genes controlling desirable traits as well as diseases is of utmost importance. The development of a high resolution gene map would be a very important
step to help identify genes that are responsible for valuable traits in the horse including performance, health, and reproduction. In addition, a high density gene map will also contribute to a better understanding of mammalian evolution through comparative mapping between the horse and other species. This thesis focuses on the development of a high resolution physical map of horse chromosomes 14 (ECA14) and 21 (ECA21), as well as a comparative map between these two horse chromosome with their human counterpart, human chromosome 5 (HSA5).

**History of Gene Mapping**

In 1909, Thomas Hunt Morgan discovered that the Y chromosome in *Drosophila* is responsible for the sex of the fruit fly, and that genes are located in a linear fashion on chromosomes. He also describes the theory of linkage which is the theory that genes that are located close together on a chromosome are more likely to be inherited together, and that crossing over during gamete formation leads to greater variability in the offspring. The first gene map of a chromosome was created in 1913 by Alfred Henry Sturtevant, who was Morgan’s student, of the *Drosophila* X chromosome and was made up of 6 genes. Today, the distance between two markers on a linkage map is known as a centiMorgan (cM) which is defined as the distance between two markers that would recombine once in every hundred matings (i.e. a 1% recombination frequency).

The discovery of recombinant DNA technology, sequencing, and polymerase chain reaction (PCR) in the 1970s and 1980s accelerated the expansion in knowledge and
expertise of scientists around the world. These discoveries eventually led to the initiation of the effort to sequence all human DNA, otherwise known as the Human Genome Project, in 1989. The initial draft sequence of the human genome was reported in 2001 and represents an important landmark in genetics (Lander et al. 2001, Venter et al. 2001). Efforts are still being undertaken to convert this draft into a genome sequence with high accuracy and nearly complete coverage (International Human Genome Sequencing Consortium 2004). Concurrently, the sequence and analysis of individual human chromosomes are being reported, notably human chromosomes 5 (HSA5) (Schmutz et al. 2004), 6 (HSA6) (Mungall et al. 2003), 7 (HSA7) (Hillier et al. 2003, Scherer et al. 2003), 9 (HSA9) (Humphray et al. 2004), 10 (HSA10) (Deloukas et al. 2004), 13 (HSA13) (Dunham et al. 2004), 14 (HSA14) (Heilig et al. 2003), 16 (HSA16) (Martin et al. 2004), 19 (HSA19) (Grimwood et al. 2004), 20 (HSA20) (Deloukas et al. 2001), 21 (HSA21) (Hattori et al. 2000), and 22 (HSA22) (Dunham et al. 1999, Tapper et al. 2001).

To date, the genomes of 249 organisms have been published, including 21 archael genomes, 196 bacterial and 32 eukaryal (http://www.genomesonline.org/). In addition, there are also currently 1000 genome projects that are focusing on the mapping or sequencing of genomes, of which 537 are prokaryotic and 463 are eukaryotic organisms (http://www.genomesonline.org/). More specifically, the genome sequence of vertebrates such as the chimpanzee (Pennisi 2003, Watanabe et al. 2004), mouse (Waterston et al. 2002), rat (Gibbs et al. 2004), dog (Kirkness et al. 2003), chicken (Hillier et al. 2004,
Wallis et al. 2004), frog (http://genome.jgi-psf.org/Xentr3/Xentr3.home.html), zebrafish (http://www.sanger.ac.uk/Projects/D_rerio/), and pufferfish (Aparicio et al. 2002) were recently completed and the data has been made publicly available to researchers worldwide. This has provided an essential source of information in understanding genome organization, changes in genome evolution, identification of unknown genes and functional inference. Using this resource, many gene maps have been built in a variety of species for which sequence information is sparse, including the horse, pig, sheep, goat, cat, and others.

These maps have been generated using several different techniques including genetic linkage, cytogenetic, somatic cell hybrid, and radiation hybrid mapping techniques. The methods of map generation are described below. Two classes of loci are used for construction of gene maps, namely Type I and Type II markers. Type I markers are coding sequences such as specific genes and expressed sequence tags (ESTs), which show conservation among distantly related mammalian species, and are useful as landmarks for comparing genomes of different species (Jiang et al. 1998, Lyons et al. 1999, Jiang et al. 2001). Type II markers are more abundant and include microsatellites, random amplified polymorphic DNA (RAPD) markers, and anonymous coding sequences. These markers are invaluable for mapping within a pedigree but less useful for comparative purposes.
The Significance/Application of Genome Mapping in Mammals

The purpose of gene mapping is to construct a comprehensive map of the entire genome of the species of interest, and to use it firstly, as a resource for locating the genetic determinants of hereditable characteristics, behaviors, and phenotypes and secondly, as a template for resolving and interpreting patterns of evolving genome organization in its ancestry (O'Brien et al. 1999).

The availability of these gene maps have helped in the study of genes that are responsible for hereditary disorders, breed specific quantitative trait loci (QTLs), and traits of economic importance in several different livestock species. In pigs, several studies have been performed to identify QTLs associated with meat quality, growth and fat deposition (Varona et al. 2002, Roehe et al. 2003, Rothschild 2003, Sato et al. 2003, Gaboreanu et al. 2004, Rothschild 2004, Thomsen et al. 2004, Nii et al. 2005), mapping of several inherited disorders including immotile short tail sperm defect (Sironen et al.
2002), Campus syndrome (Tammen et al. 1999), as well as melanoma susceptibility (Geffrotn et al. 2004). In cattle, these maps have been helpful in the search for disease associated genes such as bovine chondrodysplastic dwarfism (Yoneda et al. 1999, Takeda et al. 2002, Takeda and Sugimoto 2003), the identification of the mutation responsible for bovine leukocyte adhesion deficiency (BLAD) (Nagahata 2004) and spinal muscular atrophy (Eggen et al. 1998, Pietrowski et al. 1998, Strasswimmer et al. 1999, Iannuzzi et al. 2003a, Medugorac et al. 2003). The identification of QTLs in cattle have also progressed with the availability of dense gene maps for example in the search for QTLs associated with milk production and composition, mastitis resistance, fertility, and twinning (Komisarek and Dorynek 2002, Boichard et al. 2003, Rupp and Boichard 2003, Ashwell et al. 2004, Khatkar et al. 2004, Kuhn et al. 2004, Olsen et al. 2004, Schrooten et al. 2004). Efforts are currently underway to identify QTLs associated with wool quality, reproduction, milk production, growth, callipyge, muscle development, bone density and carcass quality in sheep (Cockett et al. 1999a, Campbell et al. 2003, Kim et al. 2004, Walling et al. 2004, Barillet et al. 2005, Cockett et al. 2005, Elsen 2005, Notter and Cockett 2005, Purvis and Franklin 2005). Also, the gene that may be responsible for Spider Lamb Syndrome or ovine hereditary chondrodysplasia, has recently been mapped to the telomeric end of sheep chromosome 6 (OAR6) (Cockett et al. 1999b).
Finally, the gene maps produced may eventually answer some questions about genome organization in different mammalian species and genome evolution. Several patterns of chromosome conservation and disruption during mammalian evolution have led to the identification of evolutionarily conserved breakpoints as well as species specific breakpoints during mammalian diversification (Grewal et al. 1998, de Pontbriand et al. 2002, Farber et al. 2003, Murphy et al. 2003, Wimmer et al. 2005). Attempts have been undertaken to reconstruct the ancestral karyotype of eutherian mammals based on observations from comparative chromosome hybridization (Chowdhary et al. 1998, Murphy et al. 2001, Fronicke et al. 2003, Richard et al. 2003, Yang et al. 2003a, Svartman et al. 2004, Wienberg 2004, Froenicke 2005). High resolution comparative gene maps can be used to further identify any minor inversions or chromosomal rearrangements that cannot be identified using comparative chromosome hybridization (Crooijmans et al. 2001, Nilsson et al. 2001, Volik et al. 2003).

**Methods in Genome Mapping**

There are two broad categories of maps, namely genetic maps and physical maps. Genetic maps are produced by calculating recombination frequencies, which is obtained by a technique called linkage mapping which will be discussed below. Physical maps, on the other hand, describe the actual physical location of a marker on a chromosome. Several methods used to construct physical maps include somatic cell hybrid, radiation hybrid, *in situ* hybridization, pulse field gel electrophoresis (PFGE), contig mapping,
optical mapping and sequencing. These maps can be integrated to form a comprehensive genome map which can be used for comparative assessment with other species.

*Genetic Linkage Mapping*

Mendel’s Law of Independent Assortment states that during gamete formation, segregating pairs of alleles assort independently of one another. Therefore, according to this law, all possible combinations of gametes will be formed at equal frequency. However, when two genes or markers are located close together on the same chromosome, this law no longer applies. This phenomenon is known as genetic linkage. Two genes or markers that are on the same chromosome but are not located close together may still assort independently (in this case, the markers are syntenic but not linked).

Map distance between loci is calculated based on the recombination frequency between two loci. The percentage of recombinants may be between 1% and 50%. If the recombination frequency is 50%, the loci in question are considered unlinked (although they may be syntenic). The lower the percent of recombinants for a pair of loci, the closer the two loci are on a chromosome. In a linkage map, the distance between two markers is measured in centiMorgans (cM), named after the pioneer of linkage mapping, Thomas Hunt Morgan. CentiMorgans are calculated from observed percent recombination, where one cM is equal to a recombination frequency of 1%. Linkage map distances, however, are not indicative of the actual physical distance between two
markers on a chromosome. Female (homogametic) mammals often have a higher recombination frequency compared to their male (heterogametic) counterparts, except in the fat tailed dunnart, *Sminthopsis crassicaudata* (Bennet et al. 1986), gray, short-tailed opossum, *Monodelphis domestica* (Samollow et al. 2004) and tammar wallaby (*Macropus eugenii*) (Zenger et al. 2002). This observation of reduced recombination in female marupials is of great interest because it is in contrast to the female recombination rates observed in eutherian mammals.

The most important materials needed for successful linkage mapping are the availability and access to pedigree material and polymorphic markers (e.g. microsatellites). Linkage mapping is performed by following the segregation of alleles in full-sib or half-sib families to identify if one allele co-segregates with another allele at another locus. Polymorphic markers are crucial to determine the origin of an allele (paternal or maternal) in an offspring because of the ability to differentiate between alleles based on the number of repeats they possess or any SNPs that may be present. The most common polymorphic markers used for linkage mapping in mammals are microsatellite markers or simple sequence repeats (SSRs), which are stretches of DNA characterized by varying numbers of highly repeated di-, tri-, or tetrnucleotide motifs.

Microsatellite markers are genotyped on reference families and data can then be analyzed using several different programs including CRI-MAP (Green 1988, 1992), LINKAGE (Lathrop et al. 1984, Lathrop and Lalouel 1988) and FASTLINK
(Cottingham et al. 1993, Schaffer et al. 1994). All three programs analyze genotyping data from pedigrees and determine linkage between markers, assign linkage groups and calculate the distance between markers in centiMorgans.

**Somatic Cell Hybrid (SCH) Mapping**

Barski et al. first described the successful fusion of somatic cells *in vitro* using two mouse cancer cell lines that originally derived from a single mouse fibroblast cell (Barski et al. 1961). They found that when the two cell lines were grown together in culture, fusion between the two cell lines occurred and produced hybrid cells with chromosome number roughly equal to the sum of the two original cell lines (Barski et al. 1961). It was also observed that after some time in culture, there was approximately a 10% random loss of some chromosomes (Barski et al. 1961). This technique was taken one step further by Boris Ephrussi and Mary Weiss in 1965, when they reported the successful construction of mononucleate interspecific somatic hybrids by fusing mouse and rat cell lines (Ephrussi and Weiss 1965). In 1967, Mary Weiss and Howard Green reported a human-mouse hybrid cell line containing a full complement of the mouse genome and a greatly reduced complement of the human chromosomes (Weiss and Green 1967). It is also with great foresight that these authors predicted the future use of these hybrid cell lines when they proposed that “study of clones containing a small number of human chromosomes should permit the localization of other human genes” (Weiss and Green 1967).
Somatic cell hybrids are cell culture lines that contain the entire genome of the host cell as well as one (or more) chromosomes from another species (Scaletta et al. 1967, Weiss and Green 1967). For reasons that are not entirely understood, hybrids formed between two different species will preferentially eliminate chromosomes from one of its parental lines (Weiss and Green 1967), while intraspecific hybrids retain more than 90% of the sum of the parental chromosomes (Barski et al. 1961, Engel et al. 1969).

The hybrid cell lines are produced by mixing cells from two different species in the presence of a fusing agent (e.g. Sendai virus or polyethylene glycol) (Gordon 1975). The development of the HAT selection system greatly facilitated the selection of hybrid cell lines (Szybalska and Szybalski 1962, Littlefield 1964, Szybalski 1992). The host cell line is mutant for a specific function (e.g. HPRT\(^-\) or TK\(^-\)) and by using selective medium (HAT medium – hypoxanthine, aminoptherin and thymidine), only hybrid cells will survive because they contain chromosome(s) from another species that complements or rescues that function (Littlefield 1964). Cytogenetic techniques such as karyotyping are then used to identify the foreign chromosome(s) that have been integrated into the host nuclei (Barski et al. 1961, Ephrussi and Weiss 1965, Weiss and Green 1967, Engel et al. 1969).

Somatic cell hybrid mapping is also known as synteny mapping because markers located on the same chromosome are said to be syntenic (meaning “on the same string” in Greek). The presence or absence of markers in a somatic cell hybrid (SCH) panel is
analyzed using enzymatic assays or PCR on a cell line. Each panel consists of a large number of cell lines (20 to 80) and the distribution of two markers will indicate whether the genes occur on the same or different chromosomes (Bailey and Binns 1998). If two genes are present on the same chromosome, the distribution observed among the cell lines will be similar. However, if they are on different chromosomes, the distribution will appear unrelated (Bailey and Binns 1998). Several advantages of this mapping technique include the fact that it does not require microsatellite markers or extensive pedigree material for analysis, unlike linkage analysis. However, this method can only identify syntenic markers but cannot physically order nor predict the distance between markers. The usefulness of synteny mapping is further enhanced when performed in conjunction with in situ hybridization which can be used to physically anchor syntenic groups to individual chromosomes.

Radiation Hybrid Mapping

Radiation hybrid mapping was first suggested as a method for producing gene maps by Goss and Harris in 1975 (Goss 1976, Goss and Harris 1977a, b). Today, it is an important tool for rapid construction of physically ordered whole genome maps in a variety of animals including the horse (Chowdhary and Bailey 2003, Chowdhary et al. 2003), cattle (Everts-van der Wind et al. 2004), pig (Rothschild 2003, 2004), sheep (Cockett 2003), dog (Greer et al. 2003), cat (Menotti-Raymond et al. 2003a, Menotti-Raymond et al. 2003b), mouse (Flaherty and Herron 1998), and rat (Bihoreau et al. 2001). In 1990, Cox et al. demonstrated that a hamster-human somatic cell hybrid
containing only one human chromosome could be used as a donor in hybrid formation, and that the selectable marker could lie in the hamster portion of the irradiated donor genome (Cox et al. 1990). In this method, high dose of X-rays is used to disrupt the genome of interest into several fragments and subsequently fused to a host cell and the hybrids are then grown in culture to form the radiation hybrid panel. The further apart two markers are on a chromosome, the more likely the X-rays will break the chromosome between them, placing the markers on two separate chromosomal fragments (Cox et al. 1990).

By analyzing the frequency of co-segregation between markers, it is possible to statistically calculate the distance as well as the order of markers on a chromosome. Distance between markers is expressed in centiRays (cR), where one centiRay corresponds to the 1% frequency of chromosomal breakage between the markers after exposure to a particular X-ray dose. The intensity of the X-ray dose used depends on the degree of resolution required, where a high dose of X-rays will cause more breaks within genome and result in smaller fragments being retained by the host cells, and thus increasing the resolution of the map (Walter and Goodfellow 1993).

One advantage of radiation hybrid mapping technique is the fact any marker that can be amplified by PCR can be used to generate a RH map. Mapping of microsatellite markers on the radiation hybrid panel will provide a useful tool to incorporate the linkage map with the comparative map. Furthermore, pedigree material is not required when
performing RH mapping, and the only material needed is a RH panel and markers which
can be amplified by PCR, regardless of polymorphic status. Also, RH mapping produces
physically ordered high resolution maps of any genome and when used in conjunction
with FISH, these maps can be anchored to specific chromosomes or chromosome bands.
Several softwares are available RHMAP (Boehnke et al. 1996), MultiMap (Matise et al.
1993, 1994), RHMAPPER (Stein et al. 1995, Slonim et al. 1997), MapManager QT and
QTX (Manly and Olson 1999, Manly et al. 2001), CONCORDE (Combinatorial
Optimization and Networked Combinatorial Optimization Research and Development
Environinent) (Agarwala et al. 2000), and Carthagene (Schiex and Gaspin 1997, de
Givry et al. 2004). Some programs try to determine the order of markers that minimizes
the number of obligate chromosome breaks (OCB) (e.g. RHMAP), while others compare
the likelihood of several locus orders (e.g. MultiMap, RHMAPPER) (Hitte et al. 2003).
The CONCORDE program on the other hand, is described as an improved option to
compute maps, resulting in marker orders with higher maximum likelihood estimates
(MLE) and lower OCB values (Agarwala et al. 2000, Hitte et al. 2003).

In situ Hybridization

*In situ* hybridization is a very important tool in gene mapping because when used in
conjunction with linkage and radiation hybrid mapping (see above), it helps localize,
anchor and orient linkage or syntenic groups to specific chromosomes. This technique
was initially discovered in 1969 by several groups who found that it is possible to
hybridize labeled nucleic acid probes to chromosomes on a glass slide without disrupting
the general morphology of the chromosome (Gall and Pardue 1969, John et al. 1969, Pardue and Gall 1969). Early *in situ* hybridization was performed using radioactively labeled probes, but advances in fluorescent labels have allowed investigators to visualize hybridization signals on chromosomes using fluorescent microscopy. The technique of performing *in situ* hybridization using fluorescent probes is referred to as *Fluorescent In Situ* Hybridization or FISH.

The probe is a specific strand of DNA that is labeled radioactively or fluorescently to allow detection by autoradiography or under a fluorescent microscope. A variety of probes can be used for FISH, total genomic DNA, repetitive sequences, and unique sequences (Trask and Pinkel 1990, Brandriff et al. 1991, Trask 1991). Probes can be labeled using several different methods, such as Nick Translation, Random Priming and PCR (Trask 1991). When performing ISH, probes can be labeled using radioisotopes such as $^{32}\text{P}$ or $^{35}\text{S}$, while for FISH, there are two ways to label probes; direct labeling or indirect labeling. Direct labeling method uses fluorochromes (e.g. fluorescein or rhodamine) which are bound directly to the probe and allows for direct detection of the probe. Indirect labeling takes advantage of other molecules (e.g. digoxigenin, biotin) that are attached to nucleotides and subsequently detected by a secondary molecule (e.g. antidigoxigenin, streptavidin) that is conjugated to a fluorochrome (Bauman et al. 1980, Trask 1991).
The target DNA is a chromosomal spread from the species of interest that is fixed onto a microscope slide. FISH can be used on metaphase spreads as well as interphase chromatin where the chromosomes are less dense compared to metaphase chromosomes. This provides a high-resolution alternative to metaphase FISH to determine the relative order of DNA sequences separated by as little as 1Mb (Lawrence et al. 1990, Trask et al. 1991). The use of multi-color FISH combined with interphase FISH has enabled investigators to resolve the order between several markers or DNA regions on a chromosome (Lawrence et al. 1990, Trask et al. 1991). High resolution Fiber FISH can also be used to further define a particular region of interest on a chromosome and markers or DNA segments can be resolved to a higher degree (Heng and Shi 1997, Heng and Tsui 1998).

The probe and target are denatured and allowed to anneal, where the probe will hybridize to its complementary sequence on the chromosome. In order to reduce unspecific hybridization, unlabeled genomic DNA or highly repetitive DNA, which acts to block dispersed repetitive elements present in the genome, is added to the hybridization mix (Cremer et al. 1988, Pinkel et al. 1988). Unbound probe and blocking agents are washed off and the probe bound to a specific complementary sequence on a chromosome can then be visualized using a fluorescent microscope.
Comparative Mapping

There are approximately 4600 to 4800 mammalian species living today comprising approximately 28 orders, including the primitive egg-laying mammals (Monotremata), 7 marsupials orders and 20 placental (eutherian) orders, that are believed to have evolved from an earlier mammalian ancestor (Wilson and Reeder 1993, O'Brien et al. 1999). Chromosome number in mammals range from $2n = 6/7$ (female and male, respectively) in the Indian muntjac to $2n = 102$ in the South American desert rodent (Wurster and Benirschke 1970, Contreras et al. 1990). The genome size in mammals range from ~1.7pg in *Miniopterus schreibersi*, the bent-winged bat, to ~6.3pg in *Proechymis* spp., the echimyid rodents, with an average of ~3.5pg in all mammals (http://www.genomesize.com/summary.htm) (Gregory 2005).

In general, comparative mapping is the mapping of the same DNA sequences in two or more species. Comparative gene mapping is performed based on the observation that genes that are closely linked in one species tend to be closely linked in other species, while loosely linked genes in one species tend to be unlinked in related species (Nadeau and Sankoff 1998). Nadeau and Taylor observed lengths of autosomal segments that were conserved during evolution when comparing linkage maps available for human and mouse (Nadeau and Taylor 1984). The ability to transfer information obtained from species with dense sequence information such as humans, mouse, and rats to different species with less sequence information such as cattle, horses, sheep, deer and others is one of the biggest advantages of comparative gene mapping (O'Brien et al. 1993,
Andersson et al. 1996). The knowledge obtained from comparative gene mapping will help identify genes that are responsible for health, reproduction, traits of economic and agricultural importance and disease in a variety of species as well as shed some light on genome evolution (Andersson et al. 1996).

The use of interspecies chromosome painting (also known as Zoo-FISH) has helped identify evolutionary conserved chromosomes, chromosome arms, and segments by fluorescently labeling DNA from individually flow-sorted chromosomes from one species followed by hybridization to metaphase spreads of chromosomes from another species (Wienberg et al. 1990, Scherthan et al. 1994, Chowdhary et al. 1998, Richard et al. 2003). However, this method is not sensitive enough to reveal segments that are ~5Mb or smaller. This method can be improved upon with reciprocal chromosome painting between two species to ensure that small unpainted regions are not overlooked in either species (Graves 1998).

**The Equine Genome**

*Genome Structure and Size*

The average size of the mammalian genome appears to be conserved at approximately ~3.4 Gb (http://www.genomesize.com/summary.htm) (Gregory 2005). The current human genome sequence accounts for approximately 2.85 billion nucleotides and covers approximately 99% of the euchromatic genome (Venter et al. 2001, International Human Genome Sequencing Consortium 2004) while the mouse and rat genomes are
approximately 2.5Gb (Waterston et al. 2002) and 2.75Gb (Gibbs et al. 2004), respectively. The size of the horse has genome has been estimated at 3.15pg, where 1pg is equal to 1 Gigabase (Gb) (http://www.genomesize.com/units.htm).

The genome of all eukaryotes is packaged into linear chromosomes made up of proteins (most of which are histones) and DNA. There are two essential features of all eukaryotic chromosomes, centromeres and telomeres. Centromeres are condensed regions on a chromosome that are responsible for segregation of the replicated chromosome during mitosis and meiosis. Telomeres, on the other hand, are the physical ends of linear eukaryotic chromosomes that are required for replication and stability of the chromosome. There are essentially four broad types of chromosomes, based on the position of the centromere; meta-, submeta- and acro- and telocentric chromosomes.

*The Karyotype*

A karyotype is a visual representation of chromosomes of a single cell, arranged in pairs based on their banding pattern and size according to a standard classification. The diploid chromosome number for the horse is $2n = 64$, and consists of 13 pairs of metacentric/ submetacentric and 18 pairs of autosomes, as well as a large submetacentric X and a small acrocentric Y chromosome (Bowling et al. 1997). The standard karyotype for the horse was defined by the International System for Cytogenetic Nomenclature of the Domestic Horse (ISCNH) in 1997.
The chromosome number in various equids varies from \(2n = 32\) in the Hartmann's zebra (*Equus zebra hartmannae*) to \(2n = 66\) for Przewalski's horses (*Equus przewalskii*).

Despite the degree of difference in chromosome number between equids, various equine species can be induced to hybridize under artificial conditions. Most hybrids, however, are sterile probably due to chromosome instability during meiosis. The most common hybrid is the mule, which is produced by crossing a male donkey with a female horse. Mules have 63 chromosomes, compared to 62 for donkeys and 64 for horses.

**Current Status of Horse Genome Mapping**

The development of dense gene maps in the horse has proceeded at a rapid pace since its inception in 1995 during the First International Equine Gene Mapping Workshop held in Lexington, Kentucky (http://www.uky.edu/Ag/Horsemapper/Workshop/first.html). Five main methods are used to produce a gene map in the horse, genetic linkage mapping, somatic cell hybrid mapping, cytogenetic mapping and radiation hybrid mapping and comparative mapping. Comparative mapping incorporates information from all four mapping methods and enables researchers in equine genomics to study the evolution of equine chromosomes with respect to other animals (Chowdhary and Bailey 2003).

Currently, the most updated horse genome database is maintained at the Institut National de la Recherche Agronomique (INRA) in France (http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/main.pl?BASE=horse). The HorseMap database currently has a total of 2353 loci of which 2078 markers are reported to have been assigned using
at least one of the mapping methods mentioned above (http://locus.jouy.inra.fr/cgi-bin/lgbc/mapping/common/summary.operl?BASE=horse – 20/01/05 Freeze).

Linkage Map

There are currently three major family resources that have contributed to the linkage maps developed in the horse;

i) the Uppsala half-sib family (Lindgren et al. 1998) which provided the first autosomal male linkage map of the horse genome by segregation analysis of 140 genetic markers within eight half-sib families with 263 offspring. This map spanned a total distance of 679cM with 25 linkage groups, 22 of which could be assigned physically to 18 different chromosomes (Lindgren et al. 1998).

ii) the International Horse Reference family Panel (IHRFP) where a total of 161 markers were tested on 12 paternal half-sib families with 263 offspring in the first generation linkage map (Guerin et al. 1999). This map identified 29 linkage groups spanning 936cM and covered 26 out of 31 autosomes (Guerin et al. 1999). A second generation linkage map was generated by mapping 310 markers on 13 paternal half-sib families with 503 half-sibling offspring (Guerin et al. 2003). This map was an improvement over the first generation map because it identified 34 linkage groups and represented all 31 autosomes, and spanned 1151cM (Guerin et al. 2003).
iii) the Animal Health Trust (AHT) 3-generation full-sibling family, comprising 61 individuals in the F2 generation was used to map 353 microsatellite markers and 6 biallelic markers (Swinburne et al. 2000a). This was the first linkage map to cover all 31 autosomes as well as the X chromosome, with 37 linkage groups that spanned a total of 1780cM.

A total of 462 markers were typed on at least one of the four linkage maps, with approximately one-third of markers that are shared between any two maps (Lindgren et al. 1998, Guerin et al. 1999, Swinburne et al. 2000a, Guerin et al. 2003). In general, there is good agreement among all maps for location and order of markers (Chowdhary and Bailey 2003). Recent efforts to construct a consensus linkage map incorporating genotype data from the four previous linkage maps as well as 359 new microsatellites mapped on IHRFP resulted in a linkage map consisting of 768 markers distributed throughout the equine genome and spans 3826cM in 31 linkage groups, and is the most dense linkage map in the horse to date (Penedo et al. 2005).

*Syntenic Map*

There are five separate reports describing the generation of somatic cell hybrid (SCH) panels in the horse. The first panel was developed in 1992 by Lear et al. using horse x mouse cell hybrids (Lear et al. 1992). A subsequent study reported the construction of a 35 horse-mouse hybridoma cell line on which 10 genes were mapped and significant association was found between 8 of the genes analyzed that formed three separate
synteny groups and two loci were independent (Richards et al. 1992, Williams et al. 1993). In 1995, Bailey et al. developed a panel of 88 horse x mouse heterohybridoma cells as well as the mapping of 29 loci, including 23 microsatellite markers, 3 DNA markers for specific genes and 3 markers identified using biochemical techniques (Bailey et al. 1995). They identified five synteny groups made up of between 2 – 4 markers each (Bailey et al. 1995). This panel was used in the linkage study in the identification of primary candidate gene for equine combined immunodeficiency disease (CID) where DNA-PK was found to be tightly linked to microsatellite markers HTG4 and HTG8 (Bailey et al. 1997).

Another horse x mouse heterohybridoma panel was constructed by fusing mouse myeloma cells with horse lymphocytes and consisted of 60 cell lines (Raney et al. 1998). A total of 62 markers (9 Type I, 53 Type II microsatellite) were tested on this panel which resulted in a total of 13 syntenic groups made up of 37 of the markers, of which 10 groups were consistent with groups recognized in earlier studies (Raney et al. 1998). Adding on to that study, an additional 48 universal mammalian sequence tagged sites were mapped on this horse x mouse SCH panel, bringing the total number of markers to 57 Type I and 55 Type II markers (Terry et al. 1999).

The last panel was constructed in University of California Davis (UC Davis) by Shiue et al. by fusing pSV2new transformed primary horse fibroblasts to either RAG or LMTk− mouse cells followed by G418 antibiotic selection (Shiue et al. 1999). This SCH panel
consisted of 108 cell lines on which a total of 240 genetic markers (58 random amplified polymorphic DNA (RAPD) markers and 182 microsatellite) (Shiue et al. 1999). Thirty three syntenic groups were defined, comprised of 2 to 26 markers per group, of which 22 groups were assigned to horse chromosomes while 11 were provisionally assigned to the remaining chromosomes (Shiue et al. 1999). Following this study, an additional 18 Type I genes were mapped onto this panel and were assigned to previously established syntenic groups, while FISH and Zoo-FISH data helped confirm the physical assignment of 12 syntenic groups to the respective horse chromosomes (Caetano et al. 1999b).

Comparative anchor tagged sequences (CATS) which are universal PCR primers designed to anneal to regions of Type I loci that show high sequence conservation in different species of mammals were then tested on the UC Davis SCH panel (Caetano et al. 1999a, Lyons et al. 1999). A total of 21 CATS for genes located on human chromosome 5 (HSA5) were chosen, and 8 of these produced horse specific molecular markers that were used for syntenic mapping (Caetano et al. 1999a). These eight CATS mapped to two syntenic groups, UCD14 and UCD21, which were later assigned to ECA14 and ECA21, respectively, based on FISH and previous comparative chromosome painting (Caetano et al. 1999a). An effort was taken to develop a comparative gene map of the horse genome using 127 Type I loci which were mapped onto the UC Davis SCH panel. Of the 127 Type I loci used, 26 were from previous mappings (Caetano et al. 1999a, Caetano et al. 1999b), 68 were new markers from previously published primers for mammalian Type I loci (Venta et al. 1996, Caetano et
al. 1999c, Lyons et al. 1999), and 32 Type I loci mapped by other groups working on the
characterization of the horse gene map (Caetano et al. 1999c). This study confirmed the
assignment of 24 equine synteny groups to their respective chromosomes, and
provisionally assigned nine synteny groups to chromosomes (Caetano et al. 1999c).
Another study mapped 23 markers (9 Type I, 13 Type II, and one pseudogene) to the
horse X chromosome as well as 3 Type I markers to the Y chromosome, which allowed
the assignment of synteny groups UCD32 and UCD33 to the X and Y chromosomes,
respectively (Shiue et al. 2000). Finally, an additional 4 genes were mapped onto the UC
Davis SCH panel (Lindgren et al. 2001a).

A total of 394 markers have reportedly been mapped in the horse using the SCH panel
constructed at UC Davis (Caetano et al. 1999a, Caetano et al. 1999b, Caetano et al.
2003). This panel has been the most significant contributor to the gene mapping effort in
the horse to date (Chowdhary and Bailey 2003). Genes and microsatellites mapped by
SCH analysis in conjunction with FISH has led to the assignment of syntenic groups to
all equine chromosomes (Caetano et al. 1999a, Caetano et al. 1999b, Caetano et al.
from this map contributed also to the generation of a comparative map between the horse
and other species (Caetano et al. 1999c).
Cytogenetic Map

The first gene mapped using in situ hybridization was for the equine leucocyte antigen (ELA) which was mapped using a cloned DNA sequence from a class I gene of the porcine major histocompatibility complex (MHC) as well as a cDNA clone representing a human MHC class I gene to ECA20q14-q22 (Ansari et al. 1988, Makinen et al. 1989).

Since then, several other genes, including glucosephosphate isomerase (GPI), 6-phosphogluconate dehydrogenase (PGD), and calcium release channel (CRC) were also localized on the equine genome using in situ hybridization (Harbitz et al. 1990, Chowdhary et al. 1992, Gu et al. 1992). GPI and CRC were mapped to ECA10pter by in situ hybridization of a porcine cDNA (Harbitz et al. 1990, Chowdhary et al. 1992), while PGD was localized on ECA2 (Gu et al. 1992).

The first gene mapped in the horse using fluorescent in situ hybridization (FISH) was the alpha globin gene (HBA) which was localized to ECA13q (Oakenfull et al. 1993). The FISH localization of 18S rDNA using a mouse probe and complement component 3 (C3) using a human probe resulted in the identification of 6 chromosomes to which 18S rDNA hybridized, confirming the reported number of equine nucleolus organizer regions (NORs), while the exact chromosome location for C3 probe could not be confirmed (Millon et al. 1993). Since then, significant effort has been taken to expand the cytogenetic map in the horse.
In 1994, the first equine satellite-type DNA sequence was localized onto the equine genome using a molecularly cloned portion of satellite-type DNA that consisted mainly of 221-bp tandem repeats and represented 3.7 – 11% of the equine genome (Sakagami et al. 1994). This study reported the presence of this satellite-type DNA preferentially localized on the centromeric regions of all chromosomes except ECA2, ECA9 and ECA11 (Sakagami et al. 1994). The following year, the same group reported the localization of a microsatellite marker ECA-3 from a horse cosmid library to ECA2p1.3 – 4 using FISH (Tozaki et al. 1995).

Several years later, three studies were published reporting the localization of two genes (IGF2 and DNA-PK) and 36 microsatellite markers derived from horse plasmid and cosmid clones (Bailey et al. 1997, Godard et al. 1997, Raudsepp et al. 1997). DNA-PK was identified as a candidate gene for equine combined immunodeficiency disease (CID) based on linkage analysis using a stallion and 19 of its offspring diagnosed with CID, six clinically normal offspring and the dams of eight of the foals, and localized to ECA9p12 (Bailey et al. 1997). This report represented the first application of whole genome scanning to localize a gene defect in horse that may be responsible for a disease condition (Bailey et al. 1997). Meanwhile, the gene for insulin like growth factor II (IGF2) was localized to ECA12q13, and represents the first imprinted gene mapped to the equine genome (Raudsepp et al. 1997).
The following year, five reports were published assigning 19 Type I and 8 Type II markers to specific horse chromosomes (Godard et al. 1998, Lear et al. 1998a, Lear et al. 1998b, Lear et al. 1998c, Tozaki et al. 1998). Godard et al. also reported the construction of a horse BAC library consisting of ~ 40,000 clones and representing a 1.5 genome coverage (Godard et al. 1998). In 1999, five Type I markers from an equine genomic library and two microsatellites from two horse BAC clones were FISH mapped (Godard et al. 1999, Lear et al. 1999b, Raudsepp et al. 1999).

In the year 2000, 50 markers (49 Type I and 1 Type II) were cytogenetically localized onto individual horse chromosomes (Godard et al. 2000, Lear et al. 2000). More specifically, Godard et al. localized 44 new coding sequences using horse as well as goat BAC clones distributed over 21 equine chromosomes (Godard et al. 2000). This report significantly improved the cytogenetic map of the horse by doubling the number of mapped Type I markers from 35 to 79, distributed over 28 out of 31 chromosomes except ECA19, ECA27 and ECA30 (Godard et al. 2000). The progress did not end here, as four groups reported FISH mapping a total of 95 Type I markers and 19 Type II markers (Lear et al. 2001, Lindgren et al. 2001a, Lindgren et al. 2001b, Mariat et al. 2001, Raudsepp et al. 2001). This brings the total number of cytogenetically mapped markers to 174 Type I and 67 Type II markers (241 in total). In the following year, another 151 markers (142 Type I and 9 Type II) were FISH mapped to the equine genome (Chowdhary et al. 2002, Hanzawa et al. 2002, Milenkovic et al. 2002, Raudsepp et al. 2002), making the total number of cytogenetically mapped markers on the equine
genome to 392. All 392 markers were localized to specific horse chromosomes and covered all 31 autosomes, and the X and Y chromosomes.

The year 2003 was a landmark year for equine genomics with the construction of the First Generation Radiation Hybrid Map in the horse which integrated synteny, linkage as well as the cytogenetic map to physically align and anchor linkage groups (Chowdhary et al. 2003). This study reported 69 new FISH assignments (33 Type I and 36 Type II) that were merged with 191 other FISH mapped markers (Chowdhary et al. 2002, Milenkovic et al. 2002, Raudsepp et al. 2002) which were used as ‘anchors’ and physically aligned 88 out of 101 RH groups (Chowdhary et al. 2003). Finally, another five reports on FISH mapping of an additional 101 markers (97 Type I and 4 Type II) on four separate chromosomes (ECA17 [13 Type I and 4 Type II], ECA22 [33 Type I], ECAX [29 Type I] and ECAY [22 Type I]) (Lee et al. 2004, Raudsepp et al. 2004a, Raudsepp et al. 2004b, Gustafson-Seabury et al. 2005) were recently published. Therefore, more than 550 markers have been cytogenetically mapped to the horse genome, and can be used as anchors for linkage groups as well as to align and orient maps that are built using other methods such as, linkage analysis, somatic cell hybrid analysis and radiation hybrid mapping.
**Radiation Hybrid Map**

The radiation hybrid (RH) effort in the horse had a relatively late start compared to other species such as the human (Cox et al. 1990, Burmeister et al. 1991), mouse (Ollmann et al. 1992), rat (Watanabe et al. 1999), cattle (Womack et al. 1997), pig (Yerle et al. 1998), dog (Priat et al. 1998), and cat (Murphy et al. 1999). Despite the late start, much progress has been achieved in the horse radiation hybrid mapping project.

The first whole-genome radiation hybrid panel in the horse was constructed using horse embryonic endothelia primary lung cells (male) and an established hamster fibroblast cell line A23 and irradiating the donor cells (horse cells) with 3000 rads of X-rays (Kiguwa et al. 2000). A total of ~160 hybrids were generated from which 94 clones were chosen at random and in order to ensure that these clones contained horse DNA, they were subjected to PCR and/or FISH (Kiguwa et al. 2000). In order to assess the usefulness of the panel, RH maps were produced for two horse chromosomes, ECA1 (39 Type II markers) and ECA10 (15 Type II markers) (Kiguwa et al. 2000). The map generated using this panel was then compared to genetic linkage maps and it was concluded that this panel was able to produce an accurate genome map which was in good agreement with the genetic and physical maps for these two chromosomes (Kiguwa et al. 2000).
The second whole genome radiation hybrid panel was constructed at Texas A&M University and is the most extensively used to produce comprehensive radiation hybrid and comparative maps for the equine genome (Chowdhary et al. 2002, Chowdhary and Bailey 2003). This panel was constructed by fusing normal diploid fibroblast culture cells from a male Arabian horse (JEW66) and Chinese hamster TK− (thymidine kinase deficient) fibroblast cell line A23 and irradiating the donor cell line (JEW66) with a cobalt source delivered 185 rad/min for a total dose of 5000 rad (Chowdhary et al. 2002). A total of 168 hybrid cell lines were obtained after two irradiation-fusion experiments (116 from the first and 52 from the second experiment) (Chowdhary et al. 2002). All 168 hybrid cell lines were analyzed with eight markers from horse chromosome 11 (ECA11), as this chromosome is expected to contain the TK gene, for which the Chinese hamster cell line was deficient (Chowdhary et al. 2002). Any hybrids that did not show a positive amplification for any of the markers were subsequently discarded, resulting in the exclusion of 30 hybrid cell lines (Chowdhary et al. 2002). From the remaining 138 cell lines, 93 were chosen for the final panel and the remaining 45 cell lines were reserved for any future need for inclusion in the panel (Chowdhary et al. 2002).

This panel was also assessed to ensure overall representation of the genome by typing 30 random cell lines with 64 pairs of primers for markers located on different chromosomes (two markers per chromosome) (Chowdhary et al. 2002). Using this method, Chowdhary et al. demonstrated that all the chromosomes were represented in this panel (Chowdhary et al. 2002).
et al. 2002). Furthermore, FISH analysis was also performed on 12 hybrid cell lines with horse genomic DNA as well as BAC DNA from ECA11 (Chowdhary et al. 2002).

Using this panel to show proof of principle, Chowdhary et al. developed a comprehensive radiation hybrid and comparative map of ECA11 made up of 24 markers (12 Type I and 12 Type II) (Chowdhary et al. 2002). This map spanned 4 linkage groups with a total distance of 346.5 centiRays (cR5000) and covered almost the entire length of the horse chromosome 11 (Chowdhary et al. 2002). For the first time, this map integrates available linkage, cytogenetic and comparative map information to provide a comprehensive map of ECA11.

The next map to be published using the 5000 rad radiation hybrid panel was a comprehensive and comparative map for the X chromosome in the horse (ECAX) (Raudsepp et al. 2002). This map was made up of 34 markers (16 Type I and 18 Type II) and spanned a total of 676 cR5000 covering almost the entire length of ECAX (Raudsepp et al. 2002). Furthermore, six new FISH assignments were also reported in this study, bringing the total number of FISH mapped markers on ECAX to 17, and collectively provided physical anchor points for the RH map (Raudsepp et al. 2002). This RH map of ECAX resolved the physical order of tightly linked markers on the linkage map, determined the order of loci in the SCH map, as well as determined the order of markers assigned to the same chromosomal band (Raudsepp et al. 2002). Several gaps were
identified in this map where more markers are required to improve the resolution and coverage on this chromosome (Raudsepp et al. 2002).

In order to address this problem, a high resolution radiation hybrid map of ECAX was subsequently published which included a total of 175 markers (139 Type I and 36 Type II), expanding the RH map for ECAX by more than four-fold (Raudsepp et al. 2004a). Gene specific markers were selected in a step-wise fashion from human and mouse X chromosome sequence templates (Raudsepp et al. 2004a). This map was divided into six RH linkage groups with an estimated effective map size of ~1600 cR5000, providing the densest and most uniformly distributed map of the X chromosome in the horse to date with markers distributed at an average of 880 kb intervals (Raudsepp et al. 2004a).

The First Generation Whole Genome Radiation Hybrid Map in the horse was reported in 2003, and consisted of 730 markers (258 Type I and 472 Type II) (Chowdhary et al. 2003). The markers are clustered in 101 RH groups distributed all over the equine autosomes and the X chromosome, with the highest number of markers on ECA1 (the largest chromosome) and the lowest number of markers on ECA28 (one of the smaller chromosomes) (Chowdhary et al. 2003). The total estimated size of the RH map in this study is 14, 587 cR5000 for all the chromosomes (except the Y), and provides a coverage of approximately one marker every 4 Mb (~19cR5000) of the equine genome (Chowdhary et al. 2003). Additionally, 69 markers were localized by FISH in this study, and including previously FISH mapped markers, a total of 253 anchors (118 Type I and 135
Type II) were used to align 88 of the 101 RH groups to individual chromosomes (Chowdhary et al. 2003). This study marks the first whole genome radiation hybrid map in the horse, and incorporates ~340 genetic linkage markers, 395 markers mapped by somatic cell hybrid analysis and >400 markers localized cytogenetically to produce the most comprehensive map of the horse genome to date (Chowdhary et al. 2003).

The development of a detailed radiation hybrid and comparative map for horse chromosome 17 (ECA17) was reported in 2003, comprising 75 markers (56 Type I and 19 Type II) organized into 6 RH linkage groups spanning a total of 824.5 cR,5000 and covers almost the entire length of the chromosome (Lee et al. 2004). Assuming the size of ECA17 is ~102 Mb, this map provided a coverage of approximately 1 marker every 1.4 Mb and is among the most comprehensive RH map for a whole chromosome constructed in the horse (Lee et al. 2004).

Following this, a radiation hybrid map of the horse Y chromosome was published comprising 8 genes and 15 sequence-tagged site (STS) markers spanning a total of 88 cR (Raudsepp et al. 2004b). These genes were located on the euchromatic region of the Y chromosome comprising ~15 Mb of the total 45 – 40 Mb size, and resides in the distal one-third of the long arm where the pseudoautosomal region (PAR) is located terminally (Raudsepp et al. 2004b).
Finally, a high resolution radiation hybrid map of horse chromosome 22 was recently constructed comprising 83 markers (52 Type I and 31 Type II) (Gustafson-Seabury et al. 2005). These markers were mapped to a single RH linkage group and are distributed evenly along the length of the chromosome, and spanned a total of 831 cR_5000_ (Gustafson-Seabury et al. 2005). Assuming the size of ECA22 is ~64Mb, this map provides a coverage of one marker every 770kb or 10cR intervals, which represents an almost four fold increase in the number of mapped markers compared to the previously published map for ECA22 with 21 loci (Chowdhary et al. 2003, Gustafson-Seabury et al. 2005).

Comparative Map

The first application of comparative gene mapping in the horse was in the identification of the causative gene for hyperkalemic periodic paralysis (HYPP) (Fontaine et al. 1990, Ptacek et al. 1991, Rudolph et al. 1992a). With the identification of the gene and the mutation that caused HYPP in humans, researchers working in the horse intuitively used linkage analysis of polymorphisms in the horse homologue of the human muscle sodium channel gene (SCN4A) (Ptacek et al. 1991, Rudolph et al. 1992a). This search proved successful when it was found that the sodium channel was tightly linked to HYPP, indicating that they are homologous disorders (Rudolph et al. 1992a). This gene was subsequently mapped to ECA11 using comparative Zoo-FISH analysis (Raudsepp et al. 1996), synteny mapping (Caetano et al. 1999b) and radiation hybrid mapping techniques (Chowdhary et al. 2003).
Similarly, the gene responsible for severe combined immunodeficiency disease (SCID) in horses was also identified based on a homologous disorder in humans and mice (Blunt et al. 1995, Kirchgessner et al. 1995, Wiler et al. 1995, Shin et al. 1997b, a). This gene was subsequently mapped to ECA9p12 by linkage analysis, somatic cell hybrid mapping as well as FISH (Bailey et al. 1997).

Several genes have previously been mapped to horse chromosomes using in situ hybridization or its fluorescent counterpart (Ansari et al. 1988, Makinen et al. 1989, Harbitz et al. 1990, Chowdhary et al. 1992, Gu et al. 1992, Millon et al. 1993, Oakenfull et al. 1993). These genes provided a brief glimpse at the comparative map between the human and horse (Chowdhary et al. 1992), but the number of markers is not sufficiently dense to deduce an overall conclusion regarding the conservation of chromosomal segments or gene order.

The first comparative map for the horse was reported in 1996 using cross species chromosome painting or Zoo-FISH to identify regions of homology between the human and horse genome (Raudsepp et al. 1996). Human chromosome specific libraries (CSLs) were individually applied to equine metaphase chromosomes using the FISH technique, which resulted in the painting of 43 conserved chromosomal segments (Raudsepp et al. 1996). Some autosomes could not be painted using any of the CSLs, namely ECA6p, 12, 13p, 27 and 31 probably due to weak hybridization signals rather than the absence of
similarity with the human genome (Raudsepp et al. 1996). This study found that eight human chromosomes painted only one segment each in horses, while 11 painted two separate segments each, three human chromosomes showed homeology with three separate horse chromosomes and finally, HSA12 was the only chromosome that painted four separate blocks in the equine genome (Raudsepp et al. 1996). Using this resource, it was possible to assign several syntenic/linkage groups to their specific equine chromosomes (Raudsepp et al. 1996).

More recently, Yang et al. further refined the comparative Zoo-FISH map for the horse by performing reciprocal Zoo-FISH analysis on human, horse, donkey and mule chromosomes (Yang et al. 2004). Using this method, a total of 60 conserved segments were found between the human and horse genomes, and was in general agreement with the previously published Zoo-FISH map (Raudsepp et al. 1996, Yang et al. 2004). This study further identified several segments that were not previously detected, allowing for more precise comparison between the human and horse genomes (Yang et al. 2004).

With the availability of the Zoo-FISH comparative genome map, the reasonable successive step would be to map and chromosomally assign a set of ~300 ‘anchor loci’ representing conserved genes which are evenly spread on human and mouse chromosomes as suggested by O’Brien et al. (O’Brien et al. 1993). However, linkage mapping of these ‘anchor loci’ would be more time consuming as it would rely on the identification of polymorphisms in the loci before they can be added to the map, while
somatic cell hybrid mapping would be a more feasible way of mapping these anchor loci (Marti and Binns 1998).

In line with this proposal, a report was published in 1999 announcing the mapping of 18 equine Type I genes by somatic cell hybrid analysis (Caetano et al. 1999b). The 18 horse genes were assigned to previously established synteny groups, and comparative mapping analysis confirmed the physical assignment of 12 synteny groups to the respective horse chromosomes (Caetano et al. 1999b). This study also presented a comparative analysis of the 18 Type I markers with the genomes of 4 other mammalian species, namely human, cow, pig and mouse (Caetano et al. 1999b).

Zoo-FISH was performed with microdissected arm specific paints for HSA2, 5, 6, 16 and 19 to delineate regions of the equine genome which is homologous to individual arms of these chromosomes (Chaudhary et al. 1998). The results obtained in this study were in agreement with the previously published human-horse Zoo-FISH map (Raudsepp et al. 1996) and lends support to the accuracy of the whole genome Zoo-FISH map. However, this study was able to identify that the potential breakpoints for two human chromosomes that are conserved as two separate blocks in the horse were not located at the centromere (c.f. conserved synteny of individual chromosomal arms) (Chaudhary et al. 1998). For HSA2, the author found that the breakpoint was located on HSA2q13 while on HSA5, the breakpoint was on HSA5q13.
Comparative anchor tagged sequences (CATS) from human chromosome 5 (HSA5) were used to generate maps of horse chromosomes 14 and 21, which have been found to be homologous to HSA5 based on earlier Zoo-FISH studies (Raudsepp et al. 1996, Caetano et al. 1999a). Eight horse specific markers were mapped to UCD14 and UCD21 which led to the assignment of UCD14 to ECA14 and UCD21 to ECA21 (Caetano et al. 1999a). This study also corroborates the finding by Chaudhary et al. that the evolutionary breakpoint for HSA5 may be located on HSA5q13, by suggesting that the proximal region of ECA21 contains material homologous to the proximal region of HSA5q (Chaudhary et al. 1998, Caetano et al. 1999a).

An effort was undertaken in 1999 to construct a whole genome comparative gene map of the horse using somatic cell hybrid analysis (Caetano et al. 1999c). A total of 127 loci were assembled, based on the new assignment of 68 equine Type I loci in this study as well as other previously published and unpublished assignments of Type I loci mapped on the SCH panel (Caetano et al. 1999a, Caetano et al. 1999b, Caetano et al. 1999c). This combined data allowed the authors to confirm the assignment of 24 equine synteny groups to their respective chromosomes, and to provisionally assign nine synteny groups using data obtained by Zoo-FISH studies (Raudsepp et al. 1996, Caetano et al. 1999c). Furthermore, synteny data suggested that ECA6p contains material orthologous to HSA2 based genes mapped to synteny group UCD-D which contained genes from human chromosomes 2 and 12, and comparative Zoo-FISH showed that ECA6q is homologous
to HSA12 and was unable to show any specific hybridization onto ECA6p (Raudsepp et al. 1996, Caetano et al. 1999c).

Another study sought to determine if the conservation of sex chromosome linked genes among placental mammals could be extended to the horse genome by mapping 13 Type I loci using SCH mapping (Shiue et al. 2000). Nine Type I loci and one pseudogene were mapped to ECAX while three type I markers were mapped to ECAY (Shiue et al. 2000). The order of the genes on both the horse X and Y chromosomes were the same as were compared with the reported order for human X and Y chromosomes (Shiue et al. 2000).

In 1999, comparative mapping of horse chromosome 3 with human and donkey homologues proved a success using equine cDNA clones, as well as equine and porcine BAC clones to identify homologous segments in all three species (Raudsepp et al. 1999). This was done by first using microdissected ECA3 painting probes on donkey metaphase chromosomes followed by painting of donkey chromosomes with human chromosomes 4 and 16q specific paints, which were previously shown to be homologous to ECA3q and 3p respectively, to identify homologous segment(s) in the donkey (Raudsepp et al. 1999). Finally, localization of 6 cDNA and BAC probes were confirmed equine metaphase spreads by FISH, followed by hybridization of the same probes onto donkey metaphase chromosomes (Raudsepp et al. 1999). The findings of this study revealed the
feasibility of FISH mapping of BAC probes across evolutionarily diverged species (Raudsepp et al. 1999).

This study was further extended by the localization of 13 loci by FISH in 2001 for comparative analysis between horse, donkey and human genomes (Raudsepp et al. 2001). A total of 32 loci (including 19 that were previously localized) were used for this comparative study, comprising 22 Type I markers (19 equine and 3 bovine BACs), 8 equine sequence tagged site (STS) markers and 2 equine microsatellite markers (Lear et al. 1998a, Godard et al. 2000, Mariat et al. 2001, Raudsepp et al. 2001, Chowdhary et al. 2002). Comparative analysis using this data was performed between the horse and donkey genomes and revealed a relatively high degree of karyotype conservation between the horse and donkey (Raudsepp et al. 2001).

The construction of two whole genome radiation hybrid panels in the horse (Kiguwa et al. 2000, Chowdhary et al. 2002), opened the doors to large scale mapping of genes on the equine genome (Chowdhary et al. 2002, Raudsepp et al. 2002, Chowdhary et al. 2003, Lee et al. 2004, Raudsepp et al. 2004a, Raudsepp et al. 2004b, Gustafson-Seabury et al. 2005). The first radiation hybrid and comparative map was generated for ECA11, which is homologous to HSA17 and MMU11 (Chowdhary et al. 2002). All the genes mapped to this chromosome (except HEST19) are located on HSA17 which is in agreement with Zoo-FISH results (Raudsepp et al. 1996). An overall conservation of gene order was observed between ECA11 and HSA17 with minor intrasegmental
rearrangements, and when compared to data available on seven mouse loci, a broad conservation of gene order was also evident (Chowdhary et al. 2002). The human and mouse homologues have a reverse orientation compared to the order of genes on ECA11, and the additional mapping of more gene-specific markers may reveal any additional intrachromosomal rearrangements present (Chowdhary et al. 2002).

The radiation hybrid and comparative map for ECAX was used to construct a comparative map between the human and mouse X chromosomes consisting of 16 Type I loci (Raudsepp et al. 2002). The gene order observed in the horse was found to be almost the same as that observed in humans with one difference in the relative order of two markers (ALAS2 and RBM3), which may be resolved with the addition of more markers to generate a denser RH and comparative map (Raudsepp et al. 2002).

This led to the development of a high resolution radiation hybrid map of the equine X chromosome made up of 175 markers (139 Type I and 36 Type II) (Raudsepp et al. 2004a). When comparing the gene order of the 139 Type I markers mapped on the horse with the human, Raudsepp et al. found that the relative location of the centromere and order of loci is exceptionally conserved from Xpter to Xqter with minor exceptions involving four to five interruptions on the short arm (Raudsepp et al. 2004a). This study also identified 13 blocks/clusters of loci which demonstrated conserved order across the horse, human and mouse, allowing the authors to deduce that these clusters may
potentially represent the most conserved X chromosome regions of the ancestor common
to horse, human and mouse.

The generation of the First Generation Whole Genome radiation hybrid map in the horse
provided an insight into the whole genome organization of horse compared to human
and mouse (Chowdhary et al. 2003). A total of 447 Type I loci were used to construct a
comparative map between the horse, human and mouse by including data from
previously published synteny and cytogenetic maps (Chowdhary et al. 2003). Using the
comparative location of the 447 Type I loci, the authors identified a total of 44
conserved syntenies (two or more pairs of homologous genes located on the same
chromosome regardless of order) (Nadeau and Sankoff 1998), between the horse and
human genomes, while a total of 71 conserved syntenies were observed between the
horse and mouse genomes (Chowdhary et al. 2003). Within the 44 horse-human
conserved syntenies, 84 segments of conserved linkage (maximally contiguous
chromosomal region with identical gene content and order) (Nadeau and Sankoff 1998)
that included 87% of the compared loci were observed where the gene order was
conserved between the two segments (Chowdhary et al. 2003). Extending this
observation to the horse-mouse genomes, 71 conserved syntenies were split into 80
conserved linkages consisting of 66% of the compared loci (Chowdhary et al. 2003).
Finally, comparison of the gene order for all 447 Type I loci across the horse, human and
mouse genomes revealed 85 clusters of genes that were highly conserved across all three
species lines (Chowdhary et al. 2003).
In the pursuit of constructing high resolution radiation hybrid and comparative maps in the horse, a 1.4Mb interval RH map was developed for ECA17 (Lee et al. 2004). ECA17 has been found to be homologous to HSA13 and appears to be highly conserved in a number of mammalian species (Chowdhary et al. 1998, Chowdhary and Raudsepp 2001, Lee et al. 2004). The comparative map for ECA17 was constructed using 56 Type I loci which were mapped on the 5000rad radiation hybrid panel (Lee et al. 2004). ECA17 showed a one-to-one homology with HSA13, as previously reported (Raudsepp et al. 1996, Chowdhary et al. 2003, Lee et al. 2004) and the authors also identified an intrachromosomal rearrangement where ~11 Mb of HSA13 (between 39.6 – 51.0 Mb) is inverted on ECA17 (Lee et al. 2004). HSA13 is conserved on five separate blocks in the mouse genome, namely MMU1, 3, 5, 8 and 14 (Carver and Stubbs 1997, Scalzi and Hozier 1998). This study found that the intrasegmental order of the murine loci is similar to that found in the horse for all conserved chromosomal segments, which is largely evident on MMU14 where the gene order of 24 comparative loci spanning a large segment (~40Mb) is conserved in the among the three species studied (Lee et al. 2004).

Next, a physical map of the Y chromosome was published comparing the order of genes on the equine Y chromosome with that of the human, mouse, rat, pig, cattle, cat, dog and wallaby Y chromosomes (Raudsepp et al. 2004b). This map was constructed using a combination of FISH methods (metaphase, interphase and fiber FISH) as well as STS content mapping and radiation hybrid mapping to accurately align the loci (Raudsepp et
al. 2004b). This study revealed that the equine Y chromosome most closely resembles the porcine counterpart (Raudsepp et al. 2004b).

Finally, a high resolution comparative map was constructed for ECA22 with a total of 52 gene specific loci mapped by radiation hybrid (Gustafson-Seabury et al. 2005). The order of genes were compared with the genomes of a variety of animals including human, mouse, rat, dog, chicken, cattle, pig, cat, zebrafish and medaka (Gustafson-Seabury et al. 2005). The density of Type I loci on this chromosome was increased by more than seven-fold, and greatly increased the power of comparative mapping for this chromosome (Gustafson-Seabury et al. 2005). Comparison of the gene map between the horse and human shows that the proximal half of ECA22 corresponds to HSA20p while the distal half corresponds to HSA20q (Gustafson-Seabury et al. 2005). The authors also identified a putative breakage/fusion point compared to the human homologue at the ECA22q15 region (Gustafson-Seabury et al. 2005). The order of genes between HSA20 and ECA22 is conserved except for an inversion in the genes from HSA20p on ECA22, while the order of genes between the ECA22 and dog chromosome 24 (CFA24) is almost identical (Gustafson-Seabury et al. 2005). This observation has led the authors to infer that ECA22 and CFA24 are ancestral among placental mammals.
Limitations and Drawbacks of Current Equine Genome Map

Assuming the size of the horse genome is approximately 3,000 Mb as in other mammals (http://hanuman.math.berkeley.edu/~cdewey/genomes/#vertebrates), the First Generation Whole Genome Radiation Hybrid map provides coverage of approximately one marker every 4Mb. This coverage is distributed among 101 RH groups, with an estimated genome coverage of approximately 90% (Chowdhary et al. 2003). The current resolution of the equine genome map is insufficient for efficient identification of genes associated with disease, fertility, or disease resistance.

Recently, high resolution radiation hybrid maps were developed for several chromosomes, namely ECA17, 22, X and Y (Lee et al. 2004, Raudsepp et al. 2004a, Raudsepp et al. 2004b, Gustafson-Seabury et al. 2005). This leaves the remaining 29 equine chromosomes for which comprehensive maps are not available that can be used for any practical purposes such as the search for genes of economic importance, health, reproduction and/or disease resistance.

More specifically, the limitations of the current maps available for the equine genome are:

- Low density of Type I and Type II markers
- Markers not distributed uniformly over the entire genome
- ‘Gaps’ present in certain under-represented regions in current maps need closing
- Insufficient number of Type I markers FISH mapped to the genome
• Uneven distribution of available FISH mapped markers
• Inability to integrate available synteny, linkage, cytogenetic markers into a
general consensus map
• Insufficient number of Type I loci mapped which can be used for comparative
mapping between the horse and other species

**Proposed Solutions**

To address the limitations and drawbacks of the current map, several steps need to be
taken to expand on the current map to aid in the discovery of genes associated with
disease phenotype or other traits of interest in the horse. The aim of the international
equine genomics community is to produce a high-resolution map of the horse genome
with one gene-specific marker every megabase and to have one microsatellite marker
every 3 – 5 Mb (http://www.tard.state.tx.us/index.php?mode=Listing&rl_id=1033) (Goh
et al. 2004). This map will also incorporate data from cytogenetic, synteny, linkage and
radiation hybrid mapping techniques and the evenly distributed microsatellite markers
can be used in an eventual genome scan panel for traits of interest in the horse. This map
will eventually be useful in the construction of a dense and comprehensive comparative
map between the horse, human, mouse, rat and other animals.
In order to achieve this aim, a focused effort is currently being undertaken to generate a high resolution physically ordered map of the equine genome by:

a) Generation of new gene specific markers using EST sequences available from databases (29, 254 equine ESTs, http://www.ncbi.nlm.nih.gov/dbEST/), as well as sequencing of 10,000 EST sequences from a skeletal muscle cDNA library at Texas A&M University,

b) Developing equine gene specific primer pairs using comparative methods from regions identified in the genome where more Type I loci are required,

c) PCR screening of BAC libraries (TAMU and CHORI–241) to isolate BACs containing genes or microsatellites for FISH mapping, with particular focus on regions with no physically localized markers,

d) Screening of microsatellite enriched whole genome libraries and BACs for microsatellite markers, with emphasis on using BACs from regions with low microsatellite density identified from the First Generation Whole Genome Radiation Hybrid Map (RH-I),

e) Radiation hybrid mapping of microsatellite markers present on linkage maps which are linked to regions of low coverage on RH-I,

f) Construction of a high resolution radiation hybrid map with the equine gene specific markers and microsatellite markers described above, and integration of synteny, genetic linkage as well as cytogenetic maps, and
g) Generation of comprehensive comparative map by comparing all the gene specific loci mapped and comparing it to genomes of other species including human, mouse, rat, dog, cat, cattle, pig, and other animals.

The final map will thus be made up of evenly distributed Type I and Type II markers that will ultimately provide a comprehensive map of the equine genome and will integrate all available radiation hybrid, synteny, cytogenetic and linkage information. The most important application of such a map would be its usefulness in the search for genes and markers associated with equine genetic disorders, disease resistance, reproduction, fertility, performance and other traits of interest. Secondly, the map can also be used for genome and chromosome evolutionary studies between the horse, human, mouse, rat and other species.

There are currently 4 high resolution maps available for 2 autosomes and the X and Y sex chromosomes in the horse (Lee et al. 2004, Raudsepp et al. 2004a, Raudsepp et al. 2004b, Gustafson-Seabury et al. 2005). This represents only a small fraction (12%) of the 31 autosomes and two sex chromosomes in the equine genome, with another 29 chromosomes remaining for which only the First Generation RH maps are available (Chowdhary et al. 2003). To address this issue, the aim of this project was to focus on two equine chromosomes, ECA14 and ECA21, which have been found to be homologous to HSA5 based on comparative Zoo-FISH and microdissected arm specific
paints as well as previous radiation hybrid and synteny maps (Raudsepp et al. 1996, Chaudhary et al. 1998, Caetano et al. 1999a, Chowdhary et al. 2003)

**Current Status of ECA14 and ECA21 – Homologues of HSA5**

*Human Chromosome 5 (HSA5)*

Human chromosome 5 is approximately 181 Megabases (Mb) which accounts for approximately 6% of the human genome (Ensemble, http://www.ensembl.org/Homo_sapiens/mapview?chr=5). It also has one of the lowest gene densities among all the human chromosomes; between 923 (Schmutz et al. 2004) and 1317 gene loci (http://www.ensembl.org/Homo_sapiens/mapview?chr=5), which is an average of approximately 5 – 7 genes every Megabase of HSA5. To date, 98 human disorders/diseases have been associated with genes on HSA5 including spinal muscular atrophy (*SMN1*), muscular dystrophy (*LGMD1A, LGMD1, TTID*), basal cell carcinoma (*RASA1, GAP*), colorectal cancer (*APC, GS, FPC*), endometrial cancer (*MSH3*), corneal dystrophy (*TGFBI, CSD2, CDGG1, CSD*), autosomal dominant deafness (*POU4F3, BRN3C*), Laron dwarfism (*GHR*), acute premyelocytic leukemia (*NPM1*), salt-resistant hypertension (*NPR3, ANPRC*), diastrophic dysplasia (*SLC26A2*), as well as Cri-du-chat syndrome, which is caused by a deletion of the short arm of chromosome 5 resulting in partial aneusomy (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM). A list of the 98 diseases/disorders which have been associated with genes on HSA5 is provided in Appendix I.
Human chromosome 5 shows conservation either as a single chromosome (or arm/segment of a chromosome) or as two major blocks on different chromosomes in a large variety of mammals (Chowdhary et al. 1998, Richard et al. 2003). Human chromosome 5 has been shown to be homologous to horse chromosomes 14 (ECA14) and 21 (ECA21) (Raudsepp et al. 1996, Chaudhary et al. 1998, Caetano et al. 1999a, Chowdhary et al. 2003). Current data shows that ECA14 is homologous to HSA5q13–qter while ECA21 corresponds to HSA5p and HSA5q13, and that the evolutionary breakpoint lies on HSA5q13 band (Chaudhary et al. 1998, Caetano et al. 1999a, Chowdhary et al. 2003). This finding is of significant interest because it shows that the evolutionary breakpoint does not lie at the centromere but on the q arm proximal to the centromere.

*Horse Chromosomes 14 (ECA14) and 21 (ECA21)*

The latest linkage map for ECA14 is made up of 31 markers and integrates markers from the previously published linkage maps in the horse and an additional 13 new microsatellite markers (Lindgren et al. 1998, Guerin et al. 1999, Swinburne et al. 2000a, Guerin et al. 2003, Penedo et al. 2005). This new linkage map spanned ~170 centiMorgans (cM) and was anchored and aligned using 6 FISH mapped markers (Penedo et al. 2005). The synteny map for ECA14, on the other hand, contains 11 markers (5 Type I and 6 Type II), and all 6 microsatellite markers on the synteny map are also mapped on the linkage map to the same chromosome (Lindgren et al. 1998, Caetano et al. 1999a, Caetano et al. 1999b, Caetano et al. 1999c, Guerin et al. 1999, Shiue et al. 2000, Swinburne et al. 2000a, Chowdhary et al. 2003, Guerin et al. 2003, Penedo et al. 2005).

The First Generation Radiation Hybrid map for ECA14 has a total of 22 markers (10 Type I and 12 Type II), organized into four linkage groups spanning 323.1cR (Chowdhary et al. 2003). The 21 loci (15 Type I and 6 Type II) that have been FISH mapped to this chromosome were used to anchor, align and orient the radiation hybrid linkage groups (Chowdhary et al. 2003). Additionally, of the 31 markers mapped on the linkage map, 9 are included in the RH map, while 7 of the 11 markers mapped by SCH analysis can be found on the RH map and 4 of the markers have been mapped using all three methods (Chowdhary et al. 2003).
Sixteen Type I loci that were FISH and/or RH mapped were used to construct a comparative map between the horse, human and mouse homologues (Chowdhary et al. 2003). The overall coverage provided for this chromosome by the RH map is one marker every 5.5Mb. When considering Type I markers alone, the coverage on this map drops to one gene specific marker every 12 Mb, while the coverage of Type II markers is approximately one microsatellite marker every 10 Mb (Chowdhary et al. 2003).

Gaps are present on this chromosome where there are no markers either FISH or RH mapped to these regions (See Figure 1a). For example, the region between UM032 and SOD2ps (ECA14q15 – 14q22), is devoid of any FISH anchored RH markers. The region between CAMK4 and CSPG2 (ECA14q24 – 14q26) would require additional markers localized by FISH to achieve a cytogenetic map with markers evenly distributed along the length of ECA14. Additionally, more markers will be required to be RH mapped in the linkage group containing markers SOD2ps and CAMK4. Finally, ECA14q16 – 2.3 does not have any markers mapped by RH and a targeted approach is needed identify more gene specific markers in this region.
Figure 1a – Ideogram of ECA14 from First Generation Radiation Hybrid map.
G-banded ideogram of ECA14 showing available synteny, linkage and comparative information in relation to human and mouse gene maps (Chowdhary et al. 2003). To the left of the ideogram, all new (red; this study) and available synteny data are shown. Alphabetically arranged Type I (proximal group) and Type II (distal group) loci are separated by a gap. To the right of the ideogram, all new (red; this study) and available FISH data are shown. The vertical rounded bars in the RH map represent RH groups (RHMAP 2pt; lod ≥7) that are cytogenetically aligned with anchor markers. CentiRay (cR) distances are shown to the left of the bars, and the deduced order of mapped loci is to the right. Framework markers are depicted in bold italics. Regional homology of ECA14 and ECA21 (based on mapped Type I loci) with the human genome is shown (colored vertical bars). Next to the bars, an ordered assembly of all RH (bold italics) and FISH-mapped Type I loci in the horse is shown, along with the Genome Browser location of human and mouse orthologs in the respective draft sequences (http://genome.ucsc.edu; version June 2002 in human and February 2002 in mouse). Orthologs showing conserved locus order compared to the derived order of equine genes are grouped in boxes demonstrating the degree of gene order conservation (conserved linkages) in human and mouse compared to that seen in the horse. Mouse chromosome number is shown in bold before the sequence location of each locus. Yellow-shaded horizontal regions represent loci that have preserved gene order (or are clustered together) in horse, human, and mouse. These clusters signify core ancestral segments.

ECA21 is also an acrocentric chromosome and is estimated to contain approximately 81Mb of DNA and currently has a total of 18 markers mapped by genetic linkage analysis (Lindgren et al. 1998, Guerin et al. 1999, Swinburne et al. 2000a, Guerin et al. 2003, Penedo et al. 2005), 11 markers mapped by somatic cell hybrid analysis (Caetano et al. 1999a, Caetano et al. 1999b, Caetano et al. 1999c, Shiue et al. 2000, Chowdhary et al. 2003), 18 markers mapped by radiation hybrid analysis (Chowdhary et al. 2003), and 11 markers mapped by FISH (Godard et al. 1997, Kakoi et al. 1999, Godard et al. 2000, Lindgren et al. 2001b, Milenkovic et al. 2002, Chowdhary et al. 2003).

The latest linkage map for ECA21 is made up of 18 markers and integrates markers from the previously published linkage maps in the horse and an addition 7 new microsatellite markers (Lindgren et al. 1998, Guerin et al. 1999, Swinburne et al. 2000a, Guerin et al. 2003, Penedo et al. 2005). This new linkage map spanned ~80 cM and was anchored and aligned using 4 FISH mapped markers (Penedo et al. 2005). The synteny map for ECA21, on the other hand, contains 11 markers (4 Type I and 7 Type II), and all 7 microsatellite markers on the synteny map are also mapped on the linkage map to the same chromosome (Lindgren et al. 1998, Caetano et al. 1999a, Caetano et al. 1999b, Caetano et al. 1999c, Guerin et al. 1999, Shiue et al. 2000, Swinburne et al. 2000a, Chowdhary et al. 2003, Guerin et al. 2003, Penedo et al. 2005).
The First Generation Radiation Hybrid map for ECA21 has a total of 18 markers (7 Type I and 11 Type II), organized into three linkage groups spanning 306.8cR (Chowdhary et al. 2003). The 11 loci (7 Type I and 4 Type II) were used to anchor, align and orient the radiation hybrid linkage groups (Chowdhary et al. 2003). Additionally, of the 18 markers mapped on the linkage map, 9 of them are included in the RH map, all of the markers mapped by SCH analysis can be found on the RH map, and 7 of the markers have been mapped using all three methods (Chowdhary et al. 2003).

Nine Type I loci that were FISH and/or RH were used to construct a comparative map between the horse, human and mouse homologues (Chowdhary et al. 2003). The overall coverage on this chromosome is one marker every 4.5Mb. When considering Type I markers alone, the coverage on this map drops to one gene specific marker every 12 Mb, while the coverage of Type II markers is approximately one microsatellite marker every 7 Mb (Chowdhary et al. 2003).

Gaps are present on this chromosome where more markers are required in order to provide a clear picture on the organization of genes on this chromosome (See Figure 1b). For example, ECA21q12 is devoid of any FISH or RH mapped markers, and the terminal part of ECA21 (ECA21q18 – 19.3) is also devoid of any FISH mapped markers except for BASP1. This RH linkage group consists of 3 markers (1 Type I and 2 Type II), and only BASP1 provides an anchor for this group, thus not allowing for the proper
orientation of the RH linkage group unless more markers are FISH mapped to this region.

Figure 1b – Ideogram of ECA21 from First Generation Radiation Hybrid map. G-banded ideogram of ECA21 showing available synteny, linkage and comparative information in relation to human and mouse gene maps (Chowdhary et al. 2003). To the left of the ideogram, all new (red; this study) and available synteny data are shown. Alphabetically arranged Type I (proximal group) and Type II (distal group) loci are separated by a gap. To the right of the ideogram, all new (red; this study) and available FISH data are shown. The vertical rounded bars in the RH map represent RH groups (RHMAP 2pt; lod ≥7) that are cytogenetically aligned with anchor markers. CentiRay (cR) distances are shown to the left of the bars, and the deduced order of mapped loci is to the right. Framework markers are depicted in bold italics. Regional homology of ECA14 and ECA21 (based on mapped Type I loci) with the human genome is shown (colored vertical bars). Next to the bars, an ordered assembly of all RH (bold italics) and FISH-mapped Type I loci in the horse is shown, along with the Genome Browser location of human and mouse orthologs in the respective draft sequences (http://genome.ucsc.edu; version June 2002 in human and February 2002 in mouse). Orthologs showing conserved locus order compared to the derived order of equine genes are grouped in boxes demonstrating the degree of gene order conservation (conserved linkages) in human and mouse compared to that seen in the horse. Mouse chromosome number is shown in bold before the sequence location of each locus. Yellow-shaded horizontal regions represent loci that have preserved gene order (or are clustered together) in horse, human, and mouse. These clusters signify core ancestral segments.

In summary, the genetic maps of both ECA14 and ECA21 are not sufficiently dense to support a comprehensive comparative study with HSA5. More gene specific markers need to be mapped to both these chromosomes in order to achieve the goal of generating a high resolution physical and comparative map of the horse genome, with one marker at every megabase interval. This will be achieved by designing primers for gene specific markers on HSA5 at every megabase intervals, resulting in the addition of ~120 Type I loci to the maps of ECA14 and ECA21, collectively. The increase in Type I marker density would allow for the construction of a comprehensive comparative map between ECA14 and 21 with their human, mouse, rat, dog, cat, cattle, pig and chicken homologues. Furthermore, microsatellite markers will be obtained from our collaborators at the University of Minnesota as well as the Laboratory of Racing Chemistry in Japan.

This comprehensive radiation hybrid and comparative map generated using this method will eventually aid in the search for genes or markers that are responsible for health, performance, and fertility in the horse. This map can also be used for evolutionary studies to compare the order of genes in the horse and human and other mammals and to identify any rearrangements that may have occurred during the course of evolution from a common ancestor.
AIMS AND OBJECTIVES

The aim of this study is to generate high resolution, physically ordered gene maps of ECA14 and ECA21. This goal will be achieved through the following specific objectives:

• Develop a 1Mb resolution integrated radiation hybrid (RH) map of the two equine chromosomes using
  o HSA5 gene specific markers and
  o newly developed microsatellite markers

• Align the RH map to the specific chromosomal regions by FISH mapping of selected markers

• Develop a high resolution comparative map of the two horse chromosomes with relation to different mammals and/or vertebrates
MATERIALS AND METHODS

Marker Selection and Primer Design

Genes from HSA5 were identified from the human genome sequence data available from National Center for Biotechnology Information build 35 (http://www.ncbi.nlm.nih.gov/genome/guide/human/) as well as Ensembl v21.34d.1 (http://www.ensembl.org/Homo_sapiens/). The genes were selected at ~1Mb intervals, beginning at 0Mb (distal top of the short arm) and ending at 181Mb (distal end of the long arm). PCR primers were designed by using multiple alignments (http://www.ddbj.nig.ac.jp/search/clustalw-e.html) of human, mouse, rat, pig, cow, or other mammalian sequences that are available from NCBI and other resources (NCBI; http://www.ncbi.nlm.nih.gov/BLAST/ and UCSC; http://genome.ucsc.edu/). The alignments were used to design heterologous primers for PCR amplification of horse DNA in a hamster DNA background, as previously described (Jiang et al. 1998, Jiang et al. 2001, Lee et al. 2004, Raudsepp et al. 2004a). Primers were designed in a single large exon whenever possible, or in two adjacent exons flanking an intron of less than 1kb. Sequence homology was chosen such that there is a 100% sequence identity among human, cattle, pig, etc. orthologues, but with one to three mismatches with the rodent (mouse, rat) sequences, preferably in the 3’ end of the primer. Primers were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).
**Microsatellite Marker Generation**

Microsatellite markers were generated using previously described methods (Mickelson et al. 2003, Mickelson et al. 2004, Wagner et al. 2004a, Wagner et al. 2004b, Wagner et al. 2004c). In brief, horse genomic DNA was digested with the restriction enzyme *MboI*, size selected by gel electrophoresis for fragments between 200 and 1200 bp, and ligated into the *BamHI* site of the M13 phage vector. Clones containing potential microsatellites were identified by screening the library with $^{32}$P 5’-end labeled oligo [dCA]$_{16}$ and oligo [dGT]$_{16}$ probes. DNA from positive plaques was then isolated and the inserts were sequenced using an ABI3100 automated sequencer. Primer pairs for PCR amplification of the markers were developed using the PRIMER program (Version 0.5) (S.E. Lincoln, M.J. Daly, E.S. Lander, unpublished; http://www.es.embnet.org/Doc/primer/).

Alternatively, another source of microsatellite markers were isolated from a microsatellite enriched library prepared as described previously (Tozaki et al. 2000, Tozaki et al. 2001, Tozaki et al. 2004). Briefly, horse genomic DNA prepared from one stallion was digested with restriction enzyme *Sau3A1* and compatible oligonucleotide adapters were ligated to the restricted fragments. After ligation, PCR was performed using the adapter sequences to obtain source DNA for a streptavidin-biotin capture method. A biotinylated oligo-(CA)$_{10}$ probe was added to the PCR product and the mixture denatured to make target microsatellites accessible to the probe. The probe was then hybridized to the denatured PCR product and then a nucleotide substrate-biased
polymerase reaction was performed using dCTP and dATP as a substrate. dCTP and dATP are incorporated only to repeat regions.

The mixtures were then incubated with streptavidin-coated magnetic beads, and the magnetic bead complexes were captured by a magnet. Single stranded DNA containing microsatellites was released from the beads, and double stranded DNA was subsequently prepared by PCR using the adapter sequences. Finally, the double stranded DNA enriched for (CA)n repeats was inserted into a T-vector (Promega, WI). These recombinants are transformed into competent XL-I Blue MRF’ *Eschericia coli* cells by electroporation (Bio-Rad, Tokyo, Japan). The resulting clones are sequenced using an automatated DNA sequencer (ABI Prism 377; Applied Biosystems, CA).

**Primer Optimization**

Primers obtained were initially diluted to a stock concentration of 100 μM and stored at −20°C, and working dilutions were then prepared by diluting the primer stock by a factor of 10 to obtain a final concentration of 10 μM and stored at 4°C. PCR reactions were then carried out in a 96-well Touchgene Gradient thermal cycler (Techne Inc., NJ) or DNA Engine Dyad (MJ Research Inc., MA) on 50 ng each of horse and hamster genomic DNA with 3 pmol of each primer, 0.25 Units JumpStart™ REDTaq™ DNA Polymerase (Sigma-Aldrich, MO), and 10X RedTaq PCR Reaction Buffer which contains 100mM Tris-HCl pH 8.3 at 25°C, 500mM KCl, 15mM MgCl₂, and 0.01% gelatin (Sigma-Aldrich, MO), in a final reaction volume of 10 μl. The PCR reactions
were pre-heated at 94°C for 30 seconds, and then cycled at 94°C for 30 seconds, 58°C for 30 seconds, 72°C for 30 seconds, for 30 cycles; 72°C for 5 minutes and held at 12°C.

PCR products were resolved on 2% agarose gels with 25µg/ml of ethidium bromide for visualization under UV. If the PCR product resolved as a single prominent band using horse genomic DNA and no PCR product was observed with the hamster control, no further optimization was required, and the primer pairs were ready to be typed on the radiation hybrid panel. However, if the PCR reaction produced multiple bands or a faint band in the hamster control, the primer pairs were further optimized by increasing the annealing temperature (to 60°C or higher) and/or MgCl₂ concentration in order to increase the specificity of the PCR reaction. On the other hand, if a faint band is observed in the horse genomic DNA PCR reaction, the annealing temperature was decreased (to 55°C or lower; or Touch Down PCR) in order to decrease the stringency of the PCR reaction. All primer pairs were individually optimized using horse and hamster genomic DNA and only primer pairs that provided horse-specific amplification products were retained for further analysis on the radiation hybrid cell panel.

**Radiation Hybrid Cell Panel Typing**

The 5000rad horse x hamster radiation hybrid panel was constructed at Texas A&M University (Chowdhary et al. 2002). Briefly, equine donor cells (JEW66) were obtained from a normal diploid fibroblast culture from an Arabian male horse named Sonny. The cells were irradiated at room temperature using a cobalt 60 source delivered at 185
rad/min for a total dose of 5000 rad. These cells were then used for fusion with recipient Chinese hamster TK− (thymidine kinase deficient) fibroblast cell line A23.

PCR typing on the 5000-rad horse x hamster RH panel of 93 hybrid cell lines and data analysis were performed as described (Chowdhary et al. 2002, Chowdhary et al. 2003). Briefly, all markers were typed on 96-well or 384-well polypropylene PCR plates on Touchgene Gradient (Techne Inc., NJ) or DNA Engine Dyad (MJ Research Inc., MA) thermal cyclers. Cycling conditions for each primer pair used was based on the optimization experiments. All PCR reactions were performed in duplicate to avoid any ambiguity and PCR products were resolved on 2% agarose gels with 25µg/ml of ethidium bromide for visualization under UV, and scored manually.

**Sequencing of PCR Products**

Equine PCR amplification products obtained with heterologous (not equine specific) primers sequenced to confirm their identity. In order to sequence gene specific markers, PCR reactions were run for each marker in 100 µl volume reactions and subsequently run on 2% agarose gels with 25µg/ml of ethidium bromide. Bands were excised from the gel and PCR products were purified using QIAquick PCR Purification Kit (Qiagen, CA) according to the manufacturer’s protocol. One microliter of the purified PCR products was then run on 1% agarose gels with 25 µg/ml of ethidium bromide to ensure that purified DNA was present, as well as to estimate the concentration of DNA available.
The concentration of the purified DNA was also measured by spectrophotometer analysis at 260 nm wavelength.

Sequencing reactions are then set up using 3 ng of DNA per 1 µl reaction per 100 bp (e.g. if PCR product is 200 bp, use 60 ng per 10 µl sequencing reaction). 10 µl reactions are set up using 2 µl of BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, CA), 0.5 µl of 5% DMSO and 5 µM of either forward or reverse primer. The sequencing reactions were pre-heated at 94°C for 3 minutes, and then cycled at 94°C for 30 seconds, 50°C for 20 seconds, 60°C for 4 minutes, for 35 cycles; 60°C for 10 minutes and held at 12°C in Touchgene Gradient (Techne Inc., NJ) or DNA Engine Dyad (MJ Research Inc., MA) thermal cyclers.

All sequencing reactions were purified prior to running on a sequencing gel, to remove unused primers, dNTPs, ddNTPs, Taq DNA Polymerase, and template DNA. Purifications were performed using Sephadex G-50 columns (SPIN-50; Biomax Inc., MD) according to manufacturer’s protocols. After purified sequencing products were collected, they were dried down in a Vacufuge (Eppendorf, NY) and stored at −20°C until they were run on an automated DNA sequencer, ABI377 (Applied Biosystems, CA).

The identities of the sequences were confirmed through BLAST (NCBI; http://www.ncbi.nlm.nih.gov/BLAST/) and BLAT (UCSC; http://genome.ucsc.edu/cgi-
bin/hgBlat) searches, as described (Lee et al. 2004). Sequence homology at \( e \) value of \( 10^{-30} \) or better for BLAST and/or 85% for BLAT was considered acceptable to confirm the identity of equine PCR products in relation to known genes in human and other species. Any sequences that did not meet the above criteria were excluded from further analysis.

**Radiation Hybrid Analysis**

RH typing data were initially analyzed against the markers from the First Generation Radiation Hybrid Map in the horse using RHMAPPER version 1-22 (Stein et al. 1995, Slonim et al. 1997) at LOD 12 or greater to initially identify two point linkage between markers (http://equine.cvm.tamu.edu/cgi-bin/ ecarhmapper.cgi). All markers identified as belonging on ECA14 or ECA21 were clustered and maintained in a database specific for each chromosome. The RH typing data for each individual chromosome was then used to compute radiation hybrid maps.

RH maps were computed using the a combination of the software packages rh_tsp_map (Agarwala et al. 2000), Qsopt (http://www.isye.gatech.edu/~wcook/qsopt), and CONCORDE (http://www.mathematik.uni-bielefeld.de/ documenta/Welcome-eng.html) (Applegate et al. 1998). The maps were constructed similarly to the cat maps in Menotti-Raymond et al. (Menotti-Raymond et al. 2003b). However, we also took advantage of the new Qsopt package and recent enhancements to rh_tsp_map. The rh_tsp_map package performed well in an independent comparison (Hitte et al. 2003). Pairwise LOD
scores were computed and inter-marker distances were estimated. Linkage groups were identified with pairwise LOD score thresholds of 7.0 for ECA14 and ECA21.

Ben-Dor and Chor showed that one is unlikely to correctly order dozens of markers with a panel size of 92, so a subset of markers in each linkage group was selected for creating a robust map (Ben-Dor and Chor 1997). The program frame_markers eliminated from initial consideration markers that had too many 2 entries and one marker out of each pair of markers that were too close to each other. These markers were candidates for getting placed relative to the robust map at a later stage. For this study, the intergenic markers were preferentially retained over microsatellite markers to obtain more useful comparative maps. Robust maps were created by finding a globally optimum order for an automatic translation of the RH mapping problem to the well-studied traveling salesman problem (TSP), and then using the CONCORDE package (Applegate et al. 1998) linked together with Qsopt to solve the TSP instances to guaranteed optimality, as described earlier (Agarwala et al. 2000). These robust maps for a subset of markers were called "MLE-consensus maps" (instead of the more generally used term “framework map”) because they are the optimal for order for three definitions of MLE that differ in how the 2 entries are treated. The robustness of the maps were tested using the map_eval (LOD ≥0.25) and flips programs, comparing the best order to the second best with a threshold of LOD ≥0.25 and a window size of 8.
The placement program was then used to find for each marker dropped by frame_markers its best placement relative to the MLE-consensus map. Placed markers with vectors identical to MLE-consensus markers were set aside at this stage. Markers with non-identical vectors that could not be placed with a LOD > 0.1 were dropped. Following this, maps for multiple linkage groups on the same horse chromosome were concatenated using intermarker distances estimated earlier. The order and orientation of linkage groups on a chromosome was determined by FISH data and available genetic maps (Swinburne et al. 2000a, Milenkovic et al. 2002, Chowdhary et al. 2003, Guerin et al. 2003, Penedo et al. 2005).

Finally, in order to generate cR positions, a restricted TSP instance was constructed and solved in which 1) the MLE-consensus markers were required to stay in the same order and 2) the placed markers were required to lie in the preferred interval between MLE-consensus markers or between the extreme MLE-consensus marker and the telomere. The only flexibility in these reduced TSP instances occurred when multiple placed markers are assigned to the same interval by the placement program. Although as many markers as possible were placed with cR positions, only the markers on the MLE-consensus map were used for comparison of marker/gene order with other mammalian genomes.
**BAC Library Screening**

CHORI–241 equine BAC library was screened for ECA14 and ECA21 markers using hierarchical PCR screening of superpools, platepools and row and column pools to isolate BAC clones (Goh et al. 2003). Plate pools and superpools for 190 out of 192 plates from segment I of the CHORI–241 Equine BAC library were generated. Plate pools were constructed by growing each 384 well plate in 4 x 96 deep well growth boxes. DNA was isolated from 4 x 96 well plates and half was used for plate pools and half for superpools. This generated a total of 24 superpools (8x384 well plates each) and 190 plate pools for PCR screening. Row and column pools were then generated by growing each individual plate in 96 deep well growth boxes and retaining half the volume of each well for row and column pools, resulting in 16 rows (A – P) and 24 columns (1 – 24) per plate.

PCR screenings were first performed on BAC superpools using primer pairs from markers selected at regular intervals along the chromosomes, as well as from gaps previously identified in the cytogenetic map for ECA14 and ECA21. PCR reactions from BAC superpool screenings were screened for positives by running the PCR product on 2% agarose gels with 25µg/ml of ethidium bromide for visualisation under UV. Each positive represented a particular platepool containing the BAC with the marker of interest. Platepools were then screened by PCR to identify the exact plate in which the BAC was located. After the BAC was isolated to a specific plate, row and column pools from that plate were then screened by PCR for the BAC containing the marker of
interest. PCR reactions were then run on 2% agarose gels with 25µg/ml of ethidium bromide for visualisation under UV and positive row and columns were identified which represent the coordinates for individual clones to be picked from glycerol stocks which are kept at –80°C.

Individual clones were picked from 384 well plates using the coordinates obtained after screening superpool, platepool and row and column pools of the CHORI–241 Equine BAC library. Clones are picked from frozen glycerol stocks using sterile toothpicks into 2ml tubes containing 1.5 ml of 2-YT broth (Invitrogen, CA) with 15 µg/ml of chloramphenicol (diluted in ethanol). Clones were grown at 37°C with constant shaking for 7 hours, after which 500 µl of cell suspension was removed, while the remaining 1 ml was stored at 4°C. The 500 µl of cell suspension was spun at 13, 500 rpm for 5 minutes to obtain a cell pellet. The supernatant was removed and cell pellet was resuspended in 50 – 200 µl of 1X TE Buffer (depending on the size of the cell pellet). A PCR reaction was then performed on the cell lysate to confirm that the clone selected contains the BAC carrying the marker/gene of interest. PCR reactions were then run on 2% agarose gels with 25µg/ml of ethidium bromide for visualisation under UV.

Clones that provide a clear positive band from the above step were then streaked (from the 1 ml cell suspension previously stored at 4°C) onto an LB agar plates (Invitrogen, CA) containing 15 µg/ml of chloramphenicol (diluted in ethanol). Plates were incubated at 37°C overnight to allow for growth of the BAC clones. The following day, 2 colonies
from each plate are picked with sterile toothpicks and transferred into 2 ml tubes containing 1.6ml 2-YT broth (Invitrogen, CA) with 15 µg/ml of chloramphenicol (diluted in ethanol). Once again, clones were grown at 37°C with constant skaking for 7 hours, after which 500 µl of cell suspension was removed, while the remaining 0.9 ml was stored at 4°C. The 500 µl of cell suspension was used for PCR check as described above.

After electrophoresis gels had confirmed that the BAC clones selected contained the marker of interest, BACs were grown overnight in 100 ml Erlenmeyer Flasks in 50 ml of 2-YT broth (Invitrogen, CA) with 15 µg/ml of chloramphenicol (diluted in ethanol) at 37°C using 50 µl of the 0.9 ml of cell suspension that was stored at 4°C previously. Glycerol stocks of each individual BAC clone was also prepared by mixing 600 µl of the remaining cell suspension with 600 µl 2-YT:glycerol solution, and stored at −80°C for future use.

**BAC DNA Isolation**

Overnight cultures of single colony BACs grown in 50 ml of 2-YT broth (Invitrogen, CA) and 15 µg/ml of chloramphenicol (diluted in ethanol) were transferred into 50ml tubes and spun at 6 000 rpm for 20 minutes at 4°C. The supernatant was discarded and cell pellet was then resuspended in 10 ml of cold P1 with RNase A (Qiagen, CA) using a blood transfer broad tip pipet, followed by 10 ml of freshly aliquoted P2 solution (Qiagen, CA), mixed gently by inversion and placed on ice for no more than 3 minutes.
Finally, 10 ml of cold P3 (Qiagen, CA) was added and mixed by inversion and left on ice for 15 minutes.

The resulting mix was then centrifuged at 6 000 rpm for 20 minutes at 4°C, and the clear supernatant transferred to a clean tube by filtering. Equal volume of Isopropanol was then added to the supernatant and mixed by inversion, and left to stand at room temperature for several minutes and then centrifuged at 8 000 rpm for 30 minutes at 4°C. The supernatant was then discarded, leaving a small white pellet at the bottom of the tube which was then resuspended in 500 µl 1X TE Buffer, and transferred to a 1.7ml microcentrifuge tube. Fifty microliters of RNase A/T1 mix (Fermentas, MD) was then added to the resuspended pellet and incubated at 37°C for 1hour (or more).

Five hundred microliters or equal volume of PCI (Phenol-Chloroform-Isoamylalcohol 25:24:1) or Tris-saturated Phenol (Roche Applied Science, IN) was added to the supernatant and RNAse/T1, and mixed by inversion and centrifuged at 13 000 rpm for 2 minutes at room temperature. The aqueous upper layer was then transferred to a clean microcentrifuge tube and the PCI step was repeated. Next, 500 µl of 10X TE saturated Chloroform (EMD Chemicals Inc., NJ) was added to the aqueous upper layer, and mixed by inversion and centrifuged at 13 000 rpm for 2 minutes at room temperature. The aqueous upper layer was then transferred to a clean microcentrifuge tube and the chloroform step was repeated.
Finally, the aqueous layer was transferred to a clean microcentrifuge tube and 2.5 volumes of cold 100% EtOH and 0.05 volumes of 5M NaCl was added. This mixture was mixed by inversion until white DNA fibers were visibly seen precipitating out of solution and left at −80°C or −20°C for 30 minutes. The tubes were then centrifuged at 13 000 rpm for 10 minutes at room temperature to pellet the DNA, which was washed twice in 400 µl of 70% EtOH (room temperature) and spun down at 13 000 rpm for 30 seconds. The pellet was allowed to dry and resuspended in 100 – 300 µl distilled water. The concentration and quality of the DNA was checked by running 1 µl on a 1% agarose gel with 25µg/ml of ethidium bromide for visualization under UV as well as spectrophotometer analysis.

**Fluorescent in situ Hybridization (FISH) of BAC DNA**

BAC DNA labeling, *in situ* hybridization, signal detection, microscopy, and image analysis were carried out as described (Raudsepp et al. 1999, Chowdhary et al. 2003). Briefly, 1 µg of each probe was labeled with biotin or digoxigenin using the BioNick Labeling (Invitrogen, CA) or DIG-Nick Translation Mix (Roche Applied Science, IN) according to manufacturer’s protocol. The nick translation product was purified through Sephadex G-50 columns (SPIN-50; Biomax Inc., MD) to remove unincorporated nucleotides. The size and quantity of the labeled probe was checked by running 5 µl on a 1% agarose gel with 25µg/ml of ethidium bromide for visualization under UV.
The purified nick translated probe was vacuum dried together with 10 – 40X excess unlabeled horse genomic DNA in a Vacufuge (Eppendorf, NY). The probe and competitor DNA mix was then dissolved in 3 µl of distilled water and added to 7 µl hybridization mix containing 70% formamide, 14% dextran sulphate, and 3 X SSC, in a 3:7 ratio (probe DNA:hybridization mix). Probe mix was then denatured at 70°C for 10 minutes and pre-annealed for 20 minutes at 37°C to allow repetitive sequences from the probe and genomic DNA to renature and hybridize to one another in order to reduce unspecific hybridization of repeats to chromosomal DNA.

In order to prepare metaphase spreads of horse chromosomes, 1 ml of buffy coat from horse peripheral blood was grown in culture with 9 ml of growth medium containing 30% Fetal calf serum (FCS), 70% RPMI medium, 1.4% antibiotics (Penicillin and Streptomycin), and 2% pokeweed mitogen. Cultures were grown for 72 hours at 37°C and mixed twice a day. Towards the 68th to 72nd hour of growth, 100 µl of ethidium bromide (1 mg/ml) was added to the growth culture and incubated for another hour at 37°C. At the end of the hour, 100 µl of colcemid (10 µg/ml) was added to the culture and further incubated for 1 – 1.5 hours at 37°C.

In order to harvest the cells, the cultures were centrifuged at 1000 rpm for 10 minutes, and the supernatant was discarded. The cells were then mixed gently with a Pasteur pipette, and mixed with 10 – 15 ml of pre-warmed (~37°C) 0.075M KCl and further incubated at 37°C for 35 – 40 minutes. The cells were then centrifuged at 1000 rpm for
10 minutes, and the supernatant was discarded, and the cells were gently mixed with a Pasteur pipette. Ice-cold freshly made fixative (Methanol: Acetic Acid; 3: 1) was added to the cells drop by drop and the cells were then gently mixed and centrifuged at 1000 rpm for 10 minutes and the supernatant was discarded. This fixative step was repeated four more times and the final cell suspension was dropped with a pipette onto a clean ice cold glass slide and allowed to air dry.

The labeled pre-annealed probes were then hybridized to denatured (at 70°C in 70% formamide (FA) and 2XSSC) horse metaphase chromosome spreads. Two to four microliters of pre-annealed probe was applied to the chromosome slide, and covered with a 4 X 4 mm coverslip. The edges of the coverslip were then sealed with rubber cement and the slide was placed in a moist chamber and incubated at 37°C for 24 hours or longer. The rubber cement was then removed, and the slide was rinsed in 2 X SSC to remove the coverslips. The slide was then washed three times with 50% formamide, 2 X SSC at 40 - 45°C at room temperature for 5 minutes each, and three times in 4 X SSC, 0.05% Tween 20 at room temperature for 2 minutes each.

For the first detection step, biotin labeled probes were detected using 5ng/µl of fluorescein isothicyanate (FITC) conjugated avidin (Avidin-FITC) (Vector Laboratories Inc., CA) in 200 µl PNM buffer containing 5% dry milk in 0.1M 0.1M Na₂HPO₄ X 2H₂O; pH 8.0 was added to the slide, and covered with a coverslip and incubated for 30 minutes at 37°C. Digoxigenin labeled probes were detected using anti-gidoxygenin-
rhodamine (Boehringer Mannheim, CT). The slide was then washed three times in 4 X SSC, 0.05% Tween 20 at room temperature for 2 minutes each, and once in 4 X SSC for 2 minutes at room temperature. In the second detection step, 5ng/µl of Biotinylated Anti-avidin (Vector Laboratories Inc., CA) was then applied to the slide and covered with a coverslip, and incubated for 30 minutes at 37°C. The slide was once again washed three times in 4 X SSC, 0.05% Tween 20 at room temperature for 2 minutes each, and once in 4 X SSC for 2 minutes at room temperature. The third detection step was the same as the first with Avidin-FITC (Vector Laboratories Inc., CA). Finally, the slide was washed three times in 4 X SSC, 0.05% Tween 20 at room temperature for 2 minutes each, and once in 4 X SSC for 2 minutes at room temperature.

The chromosomes were counterstained with 4’6-diamidino-2’-phenylindole dihydrochloride (DAPI), which produces a G-band like pattern. Hybridization results were examined under an Axioplan 2 fluorescent microscope (Zeiss, NY) using Cytovision/Genus application software version 2.7 (Applied Imaging, CA). All metaphases (30 – 60) within the 4 X 4 mm area were examined, and at least 10 were photographed. Additionally, closely positioned or overlapping markers in the RH map were cohybridized in differently labeled pairs or triplets on metaphase and interphase chromatin to refine the relative physical order.
Comparative Mapping

In order to obtain a refined comparative among horse, human, chimpanzee, dog, mouse, rat and chicken chromosomes that are homologous to HSA5, the precise sequence location of the human, chimpanzee, dog, mouse, rat and chicken orthologs for all physically aligned equine genes from the available genomic finished or draft sequences from the University of California Santa Cruz website (UCSC; http://genome.ucsc.edu/).

For the human, the May 2004 release was used to identify locations of human orthologs, while the November 2003, July 2004, May 2004, June 2003 and February 2004 releases were used for the chimpanzee, dog, mouse, rat and chicken ortholog locations, respectively. Information for cattle, pig, and cat were obtained from published RH and/or physical maps for each species obtained through literature searches as well as the ArkDB databases (http://www.thearkdb.org/) (Hu et al. 2001). Finally, comparative Zoo-FISH data for 42 species belonging to 8 orders were analyzed to identify the conservation of human chromosome 5 as well as 19p.
RESULTS

Primer Design from Orthologous Gene Specific Markers

Primer pairs were designed for genes selected at approximately every Megabase interval along the length of human chromosome 5. A total of 170 gene-specific primer pairs were designed for equine orthologs of HSA5 genes. Individual optimizations of the primer pairs generated 130 (~76% success rate) horse-specific amplifications in the presence of control hamster DNA and were retained for further analysis. Forty pairs of primers were excluded due to weak amplification of horse DNA, multiple PCR products or amplification products of equal sizes for both horse and hamster.

RH Typing and Sequencing

A total of 186 markers (130 gene-specific and 56 microsatellites) were RH genotyped in duplicate on the 5000rad horse x hamster RH panel, as previously described (Chowdhary et al. 2002, Raudsepp et al. 2002, Chowdhary et al. 2003, Lee et al. 2004, Raudsepp et al. 2004a, Raudsepp et al. 2004b, Gustafson-Seabury et al. 2005). Direct sequencing of the equine PCR amplification products obtained with heterologous (not equine specific) primers were sequenced to confirm their identity. This led to the exclusion of 4 primer pairs because BLAST/BLAT of the sequences did not correspond to the expected genes.

The RH genotyping data from 182 markers (126 gene-specific and 56 newly generated microsatellites) were analyzed against markers previously mapped in the First Generation Radiation Hybrid Map (RH-I) (http://equine.cvm.tamu.edu/cgi-
bin/ecarhmapper.cgi) (Chowdhary et al. 2003). This preliminary analysis showed that all loci were linked to either ECA14 or ECA21 at a LOD score threshold of 12 or higher. Therefore, a total of 126 equine orthologs for human chromosome 5 genes were successfully designed, which represents a ~74% (126/170) success rate in using the multiple alignment technique to develop equine orthologs for HSA5 genes.

**Generation of Composite RH Map**

The data for the 182 markers was added to previously published data for ECA14 (10 Type I, 12 Type II) and ECA21 (7 Type I, 11 Type II) (Chowdhary et al. 2003) which brought the total number of markers on the two chromosomes to 222 markers. Of these, 137 markers (88 Type I and 49 Type II) were linked to ECA14, and 85 markers (55 Type I and 30 Type II) were linked to ECA21. A ~5 – 6 Mb region from human chromosome 19p13.11 – p13.12 which maps proximal to the centromere of ECA21 was found to map to ECA21, and 18 gene specific markers from this region were RH genotyped and added to the data set for ECA21. This brought the total number of markers mapped to both ECA14 and ECA21 to 240 loci (ECA14: - 137 markers, 88 Type I and 49 Type II; ECA21: - 103 markers, 73 Type I and 30 Type II).

The distribution of the markers on the two chromosomes was further verified by pairwise LOD score of the entire data-set. Detailed information about all markers mapped to ECA14 and ECA21 by radiation hybrid mapping to date is listed in Appendix II and III, respectively. The tables include marker name, brief description, horse
cytogenetic location (where available), primer sequences, PCR product size, PCR conditions, references, and horse and human accession numbers (where available).

The outcome of the analysis of all markers leading to the development of RH maps for ECA14 and ECA21 is provided below.

**ECA14**: Analysis of the 137 markers assigned to ECA14 resulted in the markers segregating into one linkage group (LOD score threshold 7 or greater) comprising 128 markers along the entire length of the chromosome. The remaining 9 markers (5 Type I and 4 Type II) were deleted from further analysis because they segregated as singletons during analysis and could not be placed with a high degree of confidence. It is noteworthy that 65 of the 128 markers were in the MLE-concensus map and were therefore ordered with high degree of confidence. The remaining loci were placed around these markers from the MLE concensus map using the maximum likelihood estimates calculations. The total length of the map for ECA14 is 1827.76 centirays (cR) and covers the almost the entire length of both chromosomes with no significant gaps between markers (See Figure 2a)

**ECA21**: Analysis of the 103 markers on ECA21 clustered the markers into 2 linkage groups at LOD threshold of 7 or greater. The proximal group comprises of 22 markers (10 with distinct RH vectors in the MLE-concensus map) and corresponds primarily to HSA19 and spans 278.07 cR. The distal group is made up of 66 markers corresponding
to HSA5 and spans 760.02 cR. The remaining thirteen markers were deleted from further analysis because they segregated as singletons during analysis and could not be placed with a high degree of confidence. These include 4 Type I markers from HSA5, 5 from HSA19 and 4 microsatellites. A total of 37 markers were in the MLE-concensus map and were therefore ordered with a high degree of confidence while the remaining loci were placed around these markers from the MLE concensus map using the maximum likelihood estimates calculations.

A summary of the number and type of markers mapped to ECA14 and ECA21 that have been RH and/or FISH mapped, as well as number of comparative loci is presented in Table 1. Additionally, a table summarizing the coverage and fold-improvement of the maps previously published for both chromosomes compared to the current map is also presented in Table 2. Briefly, the overall coverage on both these chromosomes has been improved by almost six-fold compared to the previously published map (Chowdhary et al. 2003), while the coverage for Type I markers was dramatically improved by more than eight-fold. The number of microsatellite markers was increased from 23 to 71, representing a 3-fold improvement over the maps published in the First Generation Radiation Hybrid Map for both these chromosomes (Chowdhary et al. 2003).
Table 1. Summary of Mapped Markers on ECA14 and ECA21.

<table>
<thead>
<tr>
<th>Horse chromosome</th>
<th>RH mapped loci</th>
<th>Type I</th>
<th>Type II</th>
<th>FISH mapped loci</th>
<th>Comparative loci (RH+FISH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On map</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total New</td>
<td>Total New</td>
<td></td>
<td>Total New</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HSA19 loci</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECA14</td>
<td>128 107</td>
<td>83 73</td>
<td>0</td>
<td>45 34</td>
<td>37 (31) 29 16 (16) 88 72</td>
</tr>
<tr>
<td>ECA21</td>
<td>90 72</td>
<td>64 57</td>
<td>13</td>
<td>26 15</td>
<td>24 (19) 20 12 (11) 65 56</td>
</tr>
<tr>
<td>TOTAL</td>
<td>218 179</td>
<td>147 130</td>
<td>13</td>
<td>71 49</td>
<td>61 (50) 49 28 (27) 153 128</td>
</tr>
</tbody>
</table>

Table 2. Summary of Coverage and Fold Improvement on ECA14 and ECA21.

<table>
<thead>
<tr>
<th>Horse chromosome</th>
<th>All RH Mapped Loci</th>
<th>Type I markers</th>
<th>Type II markers</th>
<th>FISH mapped loci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coverage (1 Marker per x Mb)</td>
<td>Fold Improvement</td>
<td>Coverage (1 Marker per x Mb)</td>
<td>Fold Improvement</td>
</tr>
<tr>
<td></td>
<td>Old New</td>
<td>Old New</td>
<td>Old New</td>
<td>Old New</td>
</tr>
<tr>
<td>ECA14</td>
<td>5.5 0.9</td>
<td>6.1</td>
<td>12 1.5</td>
<td>8.0</td>
</tr>
<tr>
<td>ECA21</td>
<td>4.5 0.9</td>
<td>5.0</td>
<td>11.6 1.3</td>
<td>8.9</td>
</tr>
<tr>
<td>OVERALL</td>
<td>5.0 0.9</td>
<td>5.6</td>
<td>11.8 1.4</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Retention frequency of the 128 markers on ECA14 ranged from 9.78% (BNIP1 and TKY1053) to 26.09% (FLJ36090), with an average of 17.16% (Figure 2a). On ECA21, the retention frequency of the 90 markers ranged from 7.61% (FLJ13611 and TKY806) to 27.17% (UBA52), with an average of 15.01% (Figure 2b). The overall retention frequencies of markers on both ECA14 and ECA21 did not show any bias or peaks in any regions in the chromosomes.

![Figure 2a – Bar Chart of Retention Frequency of Markers Mapped to ECA14.](image-url)
Screening BAC Libraries and FISH Mapping

Twenty eight additional BAC clones (27 gene specific and 1 microsatellite) were localized to horse chromosomes 14 and 21 by FISH on metaphase spreads and interphase chromatin using single, or two-color hybridizations (Figure 3). Of these, 16 gene specific loci were mapped to ECA14, and 11 to ECA21 of which 5 were from the \(\sim 5 - 6\) Mb region of homology between HSA19 and ECA21. Additionally, one microsatellite locus was also FISH mapped to ECA21. All loci mapped to the expected chromosomal location based on the new RH map and previous FISH localizations. These new localizations increase the total number of cytogenetically mapped loci on ECA14 and ECA21 to 37 and 24, respectively, almost doubling the number of FISH mapped
markers on both chromosomes (see Table 1 and 2). The physical order of closely located markers was delineated using two color FISH on metaphase spreads and/or interphase chromatin (Figure 3).

**Figure 3 Partial Horse Metaphase and Interphase Spreads of Horse Chromosomes.**
Figures show (arrows) single and double color FISH results for selected loci on ECA14 and ECA21. a: CRSP9 – ECA14q15-q16, b: HK3 (red) and RARS (green) – ECA14q 13 and ECA14q14, respectively, c: MADH5 (red), KIF3A (green), and CHSY2 (red) – ECA14q21, ECA14q21, and ECA14q22.1, respectively, d: CCNB1 – ECA21q13-q14, e: SDHA (red) and DNAH5 (green) – ECA21q19.2 – q19.3 and ECA21q18, f: GMIP (green), CCNB1 (red), and ADAMTS6 (green) - ECA21q13-q14, ECA21q13-q14, and ECA21q14, respectively.
Fourty nine out of the 61 loci localized to ECA14 and ECA21 by FISH were used to anchor, orient and accurately align the radiation hybrid linkage groups to individual chromosomes (see Table 1). A total of 29 FISH mapped loci were used to anchor the linkage group for ECA14, while 20 loci localized by FISH were used to anchor the two linkage groups for ECA21. In general, the FISH locations of markers were in agreement with the order of loci in the RH groups. Of the 27 genes FISH mapped in this study, 22 represent equine orthologs for HSA5 genes, while the remaining 5 represent equine orthologs for HSA19 genes.

**Comparative Mapping**

A comparative map of ECA14 and ECA21 was developed using precise sequence locations of human, chimpanzee, mouse, rat, dog and chicken orthologs for all gene specific loci from the most updated draft sequence data as of October 2004 (Human – Build May 2004; chimpanzee – Build November 2003; mouse – Build May 2004; rat – Build June 2003; dog – Build July 2004; chicken – Build February 2004; http://genome.ucsc.edu/cgi-bin/hgGateway). Comparative map information for corresponding loci in the cattle, pig and cat were obtained from published information and databases (NCBI; http://www.ncbi.nlm.nih.gov/Genomes/index.html, ArkDB; http://iowa.thearkdb.org/).
**ECA14:**

Eighty eight orthologous loci were used to construct the comparative map for ECA14, of which 81 were mapped by RH analysis and 7 loci were ‘placed’ based on previous FISH data and their locations in relation to adjacent loci. Two RH mapped gene specific loci on ECA14, (LOC153195 and SOD2ps) could not be added to the map because their comparative information is not available (See Figure 4a).

Maximally contiguous chromosomal regions with similar gene order in relation to the derived gene order in horse are clustered together in boxes (conserved linkages) (Chowdhary et al. 2003). A regional disagreement of order within ~2 Mb is overlooked presuming deviations attributed to statistical analysis for the development of the horse map. In cattle, pig and cat, only comparative data for mapped equine genes were used.
Figure 4a – High Resolution Radiation Hybrid and Comparative Map of the Horse Chromosome 14 (ECA14)

To the left of the RH maps is the diagrammatic representation (ideogram) of G-banded ECA14. On the far left, regional homology of the horse chromosome (based on mapped Type I loci) with the human chromosome is shown (colored vertical bars). The human chromosome number and megabase correspondence is shown to the left of the colored vertical bar. Just to the right of the ideogram is FISH localizations, 16 newly mapped markers on ECA14 (shown in bold and with an asterisk on the side). FISH mapped markers with a “?” on the side indicates questionable FISH localizations (see text below for explanation). FISH mapped loci that were used as anchors for the RH linkage groups are connected by lines to their respective positions on the RH map. One linkage group is present, is depicted by a vertical white bar with hatchings on the left at every 50 cR intervals. Listed to the right of the bars are mapped equine loci. MLE consensus markers are shaded yellow, markers placed with LOD score threshold of 3 or greater are shown in bold. Markers placed with a LOD score threshold of less than 0.5 are italicized; and were not used for reliable comparative analysis. Brackets and vertical lines are shown to the right and left of RH mapped markers indicate totally linked markers that have the same position on the RH map. Markers shown in red on the RH map were added to the comparative map based on FISH mapping data only. Next to the RH map are the sequence locations in Megabases for human (HSA), chimpanzee (PTR), mouse (MMU), rat (RNO), dog (CFA), and chicken (GGA) orthologs of the mapped horse genes (http://genome.ucsc.edu/cgi-bin/hgGateway - information for all species available at this website). Maximally contiguous chromosomal regions with similar gene order in relation to the derived gene order in the horse are clustered together in boxes (black vertical boxes). Conserved gene order with 3 or more loci across mammals is represented by rectangular light green shade, while conserved synteny with 2 loci across mammals is represented by rectangular tan shade. Conserved segments across vertebrates containing 3 or more loci are shown by rectangles with solid red margins, while conserved segments with two loci across vertebrates are shown with rectangles with dashed red margins. A comparative status is also provided in cattle (BTA), pig (SSC), and cat (FCA) for orthologs with available mapping information.
**ECA21:**

A total of 65 orthologous loci were used to construct a comparative map for ECA21, of which 62 were mapped by RH analysis and an additional 3 loci were ‘placed’ based on previous FISH data and their locations in relation to adjacent loci. Two RH mapped gene specific loci on ECA21, (HESTG14 and LOC134146) could not be added to the map because their comparative information is not available (See Figure 4b).

Maximally contiguous chromosomal regions with similar gene order in relation to the derived gene order in horse are clustered together in boxes (conserved linkages) (Chowdhary et al. 2003). A regional disagreement of order within ~2 Mb is overlooked presuming deviations attributed to statistical analysis for the development of the horse map. In cattle, pig and cat, only comparative data for mapped equine genes were used.
Figure 4b – High Resolution Radiation Hybrid and Comparative Map of the Horse Chromosome 21 (ECA21)

To the left of the RH map is the diagrammatic representation (ideogram) of G-banded ECA21. On the far left, regional homology of the horse chromosome (based on mapped Type I loci) with the human chromosomes is shown (colored vertical bars). The human chromosome numbers and megabase correspondence is shown to the left of the colored vertical bar. Just to the right of the ideogram is FISH localizations, 12 newly mapped markers on ECA21 (shown in bold and with an asterisk on the side). FISH mapped loci that were used as anchors for the RH linkage groups are connected by lines to their respective positions on the RH map. Two linkage groups are present on ECA21, and are depicted by vertical white bars with hatchings on the left at every 50 cR intervals. Listed to the right of the bars are mapped equine loci. MLE consensus markers are shaded yellow, markers placed with LOD score threshold of 3 or greater are shown in bold. Markers placed with a LOD score threshold of less than 0.5 are italicized; and were not used for reliable comparative analysis. Brackets and vertical lines are shown to the right and left of RH mapped markers indicate totally linked markers that have the same position on the RH map. Markers shown in red on the RH map were added to the comparative map based on FISH mapping data only. Next to the RH map are the sequence locations in Megabases for human (HSA), chimpanzee (PTR), mouse (MMU), rat (RNO), dog (CFA), and chicken (GGA) orthologs of the mapped horse genes (http://genome.ucsc.edu/cgi-bin/hgGateway - information for all species available at this website). Maximally contiguous chromosomal regions with similar gene order in relation to the derived gene order in the horse are clustered together in boxes (black vertical boxes). Conserved gene order with 3 or more loci across mammals is represented by rectangular light green shade, while conserved synteny with 2 loci across mammals is represented by rectangular tan shade. Conserved segments across vertebrates containing 3 or more loci are shown by rectangles with solid red margins, while conserved segments with two loci across vertebrates are shown with rectangles with dashed red margins. A comparative status is also provided in cattle (BTA), pig (SSC), and cat (FCA) for orthologs with available mapping information.
DISCUSSION

Radiation Hybrid Mapping

This study provides the most comprehensive physically ordered map for horse chromosomes 14 and 21 (ECA14 and ECA21). The high resolution maps for the equine genomic regions corresponding to HSA5 generated in this study confirm and refine earlier observations regarding synteny conservation between human chromosome 5 (HSA5) and equine chromosomes 14 and 21 (Raudsepp et al. 1996, Chaudhary et al. 1998, Chowdhary et al. 1998, Caetano et al. 1999a, Caetano et al. 1999c, Chowdhary et al. 2003). These results also provide gene mapping evidence for the recently detected Zoo-FISH correspondence between a small segment of HSA19 and horse chromosome 21 found by reciprocal Zoo-FISH analysis (Yang et al. 2004).

Mapping a total of 181 new markers (179 by RH analysis and 2 exclusively by FISH) to ECA14 and ECA21 increased the overall density of mapped markers on both these chromosomes by over 5-fold compared to the previously published map (Chowdhary et al. 2003). The number of gene specific markers increased from 17 to 147, representing more than an 8-fold improvement, while the number of microsatellite markers increased from 23 to 71, representing a 3-fold improvement over the First Generation Radiation Hybrid Map (Chowdhary et al. 2003). These improvements are also clearly reflected in a considerable enhancement in the resolution of the maps for the two chromosomes. The estimated size for ECA14 and ECA21 is approximately 120 and 81 Mb, respectively. The 218 markers on the two chromosomes indicate a distribution equivalent to 1 marker
every 0.9 Mb, which is consistent with more than a 5-fold improvement over the previous resolution of 1 marker/5.0 Mb (Chowdhary et al. 2003). In terms of RH map length, the two chromosomes have approximately one marker every 13 cR.

The success rate of designing primers using sequence information for genes from HSA5 is approximately 74%. Out of a total of 170 primer pairs designed in this study, 126 provided horse specific amplification in the presence of a hamster DNA background. The lack of success with the 26% of the primers led to some inconsistency (e.g. terminal region of ECA21) in otherwise uniform and fine alignment of the two equine chromosomes with the entire length of HSA5.

The additional 28 new FISH assignments reported in this study (16 on ECA14 and 12 on ECA21) result in almost doubling the number of earlier cytogenetically mapped markers and brings the total on the two chromosomes to 37 and 24, respectively (Milenkovic et al. 2002, Chowdhary et al. 2003). Of the 11 new markers that were FISH mapped to ECA21 in this study, 5 originate from human chromosome 19 (HSA19) and are located on the proximal part of the q arm of ECA21 (q12 – q14). The order of cytogenetically
mapped markers on both these chromosomes essentially corroborates the physical order of markers on the RH map. The markers also serve as excellent physical anchor points to align and orient different groups along the length of both chromosomes.

The three FISH-RH mapping discrepancies observed by us include INSL3, SLC6A3 and RASA1. INSL3 was previously assigned to ECA7q17 – p18 by Milenkovic et al. who performed FISH using a horse BAC containing this gene (Milenkovic et al. 2002). New primers were designed by us for this gene, and the amplification product was sequenced to confirm the identity of the amplified product. Subsequent FISH mapping with a new BAC clone showed that INSL3 unambiguously mapped to the proximal part of ECA21. Furthermore, previous FISH assignment of SLC6A3 (Milenkovic et al. 2002) and RASA1 (Godard et al. 2000) using goat BACs on ECA14 are not in agreement with the current RH map. Based on fine comparative mapping information generated in this study between HSA5 and ECA14/ECA21, we predict that SLC6A3 will most likely map to the distal end of ECA21q, and RASA1 to the distal part of ECA14q.

**Comparative Map**

The addition of 130 new gene specific markers in this study – 117 equine orthologs of HSA5 genes and 13 equine orthologs of HSA19 genes – represents a substantial increase from mere 18 gene specific markers previously mapped on ECA14 and ECA21 (Chowdhary et al. 2003). On average, one Type I marker is mapped every 1.37 Mb of both chromosomes, which is comparable to recently published high-resolution maps for
ECA17 (Lee et al. 2004), ECAX (Raudsepp et al. 2004a), ECA22 (Gustafson-Seabury et al. 2005) and homologues of HSA19 (Brinkmeyer et al. 2005).

The results help to more accurately define the known correspondence between HSA5 and ECA14/ECA21 (Raudsepp et al. 1996, Chaudhary et al. 1998, Chowdhary et al. 1998, Caetano et al. 1999a, Yang et al. 2004) by delineating the homology in terms of megabase position of loci in the human genome sequence. The results show that the distal three-quarter of ECA21 corresponds to the short arm and part of the long arm of HSA5 (0 – 68.5 Mb; pter – 5q13) while ECA14 corresponds to the rest of the long arm of the human chromosome (70.9 – 180.2 Mb; q13 – qter). Consequently the evolutionary break/fusion point falls not at the centromere, but in the proximal region of the long arm of HSA5, somewhere between 68.5 – 70.9 Mb position on the HSA5 sequence map.

This considerably refines the previous observation using microdissected arm specific paints of HSA5 where ECA14 corresponds to HSA15q13 – qter and ECA21 corresponds to HSA5p and proximal part of 5q, narrowing the evolutionary break/fusion point to the HSA6q13 band (Chaudhary et al. 1998).

Recently, Yang et al. reported the presence of a small segment corresponding to HSA19 on the proximal one-quarter of ECA21 (Yang et al. 2004). Mapping of 13 gene specific markers from HSA19 to this region for the first time provides mapping evidence indicating the precise region of homology with the human chromosome (~15 – 20 Mb sequence region of HSA19) (Brinkmeyer et al. 2005). The order of genes in this region
is largely conserved between the horse (ECA21), human (HSA19), chimpanzee (PTR20), mouse (MMU8), rat (RNO16), dog (CFA20), and chicken (GGA28). It is noteworthy that among rodents, AKAP8 mapped to different chromosomes in mouse (MMU17) and rat (RNO7), suggesting an evolutionary breakpoint different from canines, perrisodatyls or primates.

**Human:** Comparison of the gene order between HSA5 with the order generated for ECA14/ECA21 showed that despite minor differences in regions with densely mapped markers, there is an overall conserved linkage observed between the two species. On ECA14, the gene order is inverted with respect to the HSA5 with the proximal region of horse chromosome 14 corresponding to the terminal region of the q arm of HSA5 (~180.2Mb) and vice versa. Thus, the telomeric region of ECA14 is at the putative evolutionary break/fusion point on HSA5, while the telomeric region on HSA5q is centromeric in the horse. A similar inverted arrangement is also observed between HSA5 and ECA21, leading to difference in centromere/telomere position of orthologs in the two species.

**Chimpanzee:** Comparison of gene order for HSA5 homologues between the horse (ECA14/ECA21) and chimpanzee chromosome 4 (PTR4) led to identification of four distinct blocks. Two of the blocks on ECA14 correspond to (in order) ~188 – 104 Mb and ~19 – 45 Mb, while the two blocks on ECA21 correspond to ~47 – 100 Mb and 18 – 0 Mb segments of PTR4. The arrangement of loci is unique to chimpanzee because other
mammalian species hitherto sequenced show blocks similar to those identified in the horse-human comparison. It is known that there is a ~80Mb inversion and additional rearrangements between HSA5 and PTR4 involving the segment corresponding to HSA5p14 – 5q15 (Schmutz et al. 2004). Such inversions events are suggested to contribute to the process of speciation by preventing fertile mating between hybridizing taxa (Noor et al. 2001). Part of this inversion event lies on the distal end of ECA14q between PCSK1 and MCCC2 spanning a region of approximately 26 Mb, while the remaining is found on ECA21q12 – q17 between CCNB1 and CDH12 which covers approximately 52 Mb.

**Rodents:** Comparison of the gene order on ECA14 and ECA21 with the sequence data in the rodents presents a familiar situation of considerably rearranged rat and mouse genomes in relation to other mammals. Consequently, a number of mouse/rat chromosomes correspond to the two equine chromosomes (and hence also HSA5; Figure 3a and 3b), of which chromosomes 11, 13, 15, and 18 are most conspicuous in mouse and chromosomes 2, 10, 17 and 18 in rat. Of considerable interest is the fact that on ECA21, almost the entire segment corresponding to HSA5 (a ~60 Mb stretch from 9.1Mb – 68.5 Mb) shows conserved linkage between the horse, human (HSA5) and rat (RNO2), but not the mouse. In the mouse, this region is conserved in two separate chromosomes, MMU13 and MMU15. Schmutz et al. found 142 chromosomal rearrangements which range in size from 200kb to 17Mb between the mouse genome sequence and human chromosome 5 sequence (Schmutz et al. 2004). We identify
several breakpoints which are shared between the mouse and rat genomes, as well as regions of synteny where the gene order is conserved between the horse and rodents. On ECA14, we found at least 11 breakpoints that are shared between the rodents but are not present in horse, humans, and chimpanzee genomes.

**Carnivores:** Six separate dog chromosomes (CFA2, CFA3, CFA4, CFA11, CFA20, and CFA34) correspond to ECA14 and ECA21. Of these, all except CFA20 correspond to human chromosome 5, which is in contrast (and perhaps expected) with the cat where HSA5 equates to a single chromosome on chromosome A1. The syntenic blocks in dog essentially show conserved gene order within individual blocks compared to horse/human. This also applies to the ~5 Mb segment on the proximal part of ECA21 that shares homology with FCA20/HSA19.

**Chicken:** Next, comparison of available gene order in chicken with the order obtained in the horse shows that a considerable part of ECA14/ECA21 (HSA5) primarily corresponds to the chicken Z chromosome (GGAZ), followed by GGA2 and GGA13. Rearrangements are evident within the chicken conserved syntenic blocks when compared with corresponding segments in mammals. The proximal segment of ECA21 that equates to part of GGA28/HSA19, however, shows conserved linkage.

Interestingly, part of human chromosome 5 is homologous to the chicken sex chromosome, Z (and W). The comparative maps for ECA14 and ECA21 indicate that the
distal ~11 Mb region of the short arm of chicken Z chromosome shares homology with ECA14 and ECA21. Chicken chromosome Z has been found to be largely syntenic with human chromosome 9, but it also carries segments that map to human chromosomes 5, 8, and 18 (Nanda et al. 1999, Nanda et al. 2000), which is further evidence for an independent origin of mammalian and avian sex chromosomes since birds diverged from mammals approximately 300 – 350 million years ago (Kumar and Hedges 1998, Blair Hedges and Kumar 2003, Schmutz et al. 2004).

**Cattle:** Though draft sequence data is presently not available in cattle, we used published gene mapping and comparative human information to relate to the observed order of loci in the horse. It is evident that the bovine homologues of HSA5 (BTA7 and BTA20) show a rearranged organization of human loci compared to that seen in the horse (Zhang and Womack 1992, Gao and Womack 1997b, a, Amarante et al. 2000, Band et al. 2000, Hu et al. 2001, Stone et al. 2002, Gautier et al. 2003, Hayes et al. 2003, Everts-van der Wind et al. 2004). For example, loci mapped on ECA14 form part of two inverted segments with likely conserved linkage on BTA7 and two segments on BTA20. One of the latter segments also corresponds to part of ECA21.

It is worth noting that the second generation radiation hybrid map in the cattle (Everts-van der Wind et al. 2004) did not distinctively demonstrate previously identified homology between HSA5 and BTA10 (Zhang and Womack 1992, Gautier et al. 2003,
Hayes et al. 2003). This could in part be attributed to lack of sufficient representation of ESTs from this region. Nevertheless, our comparative analysis shows that two of the genes assigned to BTA10 are mapped to the terminal part of ECA14q indicating correspondence between the two chromosomes. It is therefore likely that the ~74 \textit{(HMGCR)} – 79 Mb \textit{(THBS4)} region of HSA5 shares homology with BTA10, in all likelihood with the region next to centromere (based on mapping data for the two loci) (Zhang and Womack 1992, Gautier et al. 2003, Hayes et al. 2003). This observation gets support from the mapping of \textit{ARSB} locus to the proximal part of the RH group on BTA10 (Everts-van der Wind et al. 2004). Interestingly, the human sequence position of the locus is 78.1 Mb which fits nicely with our predictions. Our comparative analysis thus provides interesting clues on segmental homology between HSA5 and BTA10 by defining, for the first time, the regions to a reasonable extent.

on HSA5 while SSC16 with \(~0 – 70\) Mb and \(~150 – 180\) Mb. Although comparative mapping data for HSA19p loci mapped to the proximal part of ECA21 is not available, pig-human bidirectional chromosome painting indirectly indicates that it shares correspondence with SSC2q11 – q21 (Fronicke and Wienberg 2001).

**Comparative Organization of HSA5 Homologues**

Human chromosome 5 (HSA5) has been proposed to have evolved as a single chromosome since the common ancestor of the eutherian mammals (about 100 million years ago) and was probably acrocentric (Chowdhary et al. 1998, Haig 1999, Murphy et al. 2001, Fronicke et al. 2003, Richard et al. 2003, Yang et al. 2003a, Svartman et al. 2004, Wienberg 2004, Froenicke 2005). In this study, we analyzed the conservation of human chromosome 5 and 19p in 42 mammalian species belonging to 8 orders. Figure 5 represents a summary of these findings.


Primates, Afrotherians, rodents (except murids), Carnivores (except canids) show a one to one homology with a HSA5, while Perissodactyls, Cetartiodactyls (except cattle and goat), Lagomorphs, Insectivores, Chiropters, display conservation as two major blocks on different chromosomes (Chowdhary et al. 1998, Schibler et al. 1998, Richard et al. 2003, Chaves et al. 2004, Froenicke 2005) (See Figure 5). Meanwhile, human chromosome 5 is conserved in several fragments (at least 5) in the murids and canids, as well as in the chicken.
Figure 5 – Summary of Comparative Zoo-FISH Information Showing Conservation of Human Chromosome 5 and 19p in 8 Mammalian Orders.
CONCLUSION

Salient findings of this study are as follows:

- A comprehensive physically ordered high resolution map of horse chromosomes 13 and 21 (ECA14 and ECA21) was generated in this study. These two chromosomes primarily correspond to human chromosome 5 (HSA5), though ECA21 shares homology also with part of HSA19p.

- A total of 179 new markers (130 gene specific and 49 microsatellites) were mapped in this study. This led to an increase in the number of mapped markers on both these chromosomes from 40 to 218 – a 5 – 6 fold increase in density.

- The density of gene specific markers is increased by almost 9-fold.

- The resolution of the map is 1 marker/ 0.9 Mb along the length of the two chromosomes.

- Comparative mapping between the horse and human reveals that the order of genes on HSA5 is remarkably well conserved in the horse, with no major rearrangements between the chromosomes.

- The evolutionary fusion/break point on human chromosome 5 in relation to horse chromosomes is narrowed down to a ~2 Mb region between 68.5 – 70.9 Mb positions (i.e. between markers \textit{CCNB1} and \textit{MCCC2}).

- Segments of conserved synteny and gene order between ECA14/ECA21 and homologous segments in a range of mammals are identified. This led to the identification of ancestral segments among compared mammals/vertebrates.
- HSA5p – 5q13 was found to be associated with a ~5 Mb region from human chromosome 19p on horse chromosome 21 as a neighboring segment. Interestingly, this combination of human chromosome 5 and 19p is also observed in the order Cetartiodactyla but the segment of HSA5 that is associated with 19p in this order is from HSA5q13 – qter. This neighboring segment combination between HSA19p and HSA5 has not been found in any animals studied to date and appears to be unique to Perissodactyls and Cetartiodactyls. The observation that two different segments of HSA5 are involved in these two orders suggest that this combination occurred as separate events and were not part of the same event.

Finally, this map will be a resource for precise physical assignment of genes responsible for traits of interest within these two chromosomes such as genes responsible for diseases, fertility, performance and health. The density of the map will help to quickly localize an analyzed condition to a specific narrow genomic region and considerably facilitate the search for genes of interest on both these chromosomes. Once extended to the entire genome, such maps will play a key role in improved localization of specific regions to allow for fine mapping and identification of genes responsible for economically important traits in horses. This map may also facilitate the annotation of sequences when the genome of the horse is eventually sequenced.
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**APPENDIX I**

**LIST OF DISEASE GENES ON HUMAN CHROMOSOME 5**

<table>
<thead>
<tr>
<th>Location</th>
<th>Symbol</th>
<th>Title</th>
<th>MIM #</th>
<th>Disorder</th>
</tr>
</thead>
<tbody>
<tr>
<td>5p15.3-p15.2</td>
<td>MTRR</td>
<td>Methionine synthase reductase</td>
<td>602568</td>
<td>Homocystinuria-megaloblastic anemia, cbl E type, 236270 (3)</td>
</tr>
<tr>
<td>5p15.2</td>
<td>CTNND2, NPRAP</td>
<td>Catenin, delta-2</td>
<td>604275</td>
<td>Mental retardation in cri-du-chat syndrome, 123450 (2)</td>
</tr>
<tr>
<td>5p15.2-p14.1</td>
<td>ANKH, HANK, ANK, CMDJ, CCAL2, CPPDD</td>
<td>Ank, mouse, homolog of</td>
<td>605145</td>
<td>Craniometaphyseal dysplasia, 123000 (3); Chondrocalcinosis 2,118600 (3)</td>
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<tr>
<td>5p15</td>
<td>SDHA, SDH2, SDHF</td>
<td>Succinate dehydrogenase complex, subunit A, flavoprotein</td>
<td>600857</td>
<td>Mitochondrial respiratory chain complex II deficiency, 252011 (3)</td>
</tr>
<tr>
<td>5p15</td>
<td>SLC6A19, HND</td>
<td>Solute carrier family 6 (neurotransmitter transporter), member 19</td>
<td>608893</td>
<td>Hartnup disorder, 234500 (3)</td>
</tr>
<tr>
<td>5p15-p14</td>
<td>DNAH5, HL1, PCD, CILD3</td>
<td>Dynein, axonemal, heavy chain 5</td>
<td>603335</td>
<td>Ciliary dyskinesia, primary, 3 608644 (3); Kartagener syndrome, 244400 (3)</td>
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<tr>
<td>5p14-p12</td>
<td>NPR3, ANPRC</td>
<td>Natriuretic peptide receptor C</td>
<td>108962</td>
<td>?Hypertension, salt-resistant (1)</td>
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<tr>
<td>5p13.3-p13.2</td>
<td>BDA1B</td>
<td>Brachydactyly, type A1, locus B</td>
<td>607004</td>
<td>Brachydactyly, type A1, 112500 (2)</td>
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<tr>
<td>5p13.2-q11.1</td>
<td>AMACR</td>
<td>Alpha-methylacyl-CoA racemase</td>
<td>604489</td>
<td>Alpha-methylacyl-CoA racemase deficiency (3)</td>
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<tr>
<td>5p13.1</td>
<td>LIFR, STWS, SWS, SJS2</td>
<td>Leukemia inhibitory factor receptor</td>
<td>151443</td>
<td>Stuve-Wiedemann syndrome/Schwartz-Jampel type 2 syndrome, 601559 (3)</td>
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<tr>
<td>5p13.1</td>
<td>NIPBL, CDLS</td>
<td>Nipped-B-like (delangin)</td>
<td>608667</td>
<td>Cornelia de Lange syndrome, 122470 (3)</td>
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<tr>
<td>5p13</td>
<td>SCOT, OXCT</td>
<td>Succinyl CoA:3-oxoacid CoA transferase</td>
<td>245050</td>
<td>Ketoacidosis due to SCOT deficiency (3)</td>
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<tr>
<td>5p13-p12</td>
<td>GHR</td>
<td>Growth hormone receptor</td>
<td>600946</td>
<td>Laron dwarfism, 262500 (3); Short stature, idiopathic (3); Short stature, autosomal dominant, with normal serum growth hormone binding protein (3)</td>
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<tr>
<td>5p</td>
<td>MATP, AIM1</td>
<td>Membrane-associated transporter protein</td>
<td>606202</td>
<td>Oculocutaneous albinism, type IV, 606574 (3)</td>
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<tr>
<td>5q</td>
<td>MPD2</td>
<td>Myopathy, distal 2</td>
<td>606070</td>
<td>Myopathy, distal, 606070 (2)</td>
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<td>Location</td>
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<td>MIM #</td>
<td>Disorder</td>
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<tr>
<td>5q</td>
<td>PROP1</td>
<td>Prophet of Pit1, paired-like homeodomain transcription factor</td>
<td>601538</td>
<td>Pituitary hormone deficiency, combined, 601538 (3)</td>
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<tr>
<td>5q11</td>
<td>MOCS2, MPTS</td>
<td>Molybdenum cofactor synthesis-2</td>
<td>603708</td>
<td>Molybdenum cofactor deficiency, type B, 252150 (3)</td>
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<tr>
<td>5q11-q12</td>
<td>MSH3</td>
<td>mutS, E. coli, homolog of, 3</td>
<td>600887</td>
<td>Endometrial carcinoma (3)</td>
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<tr>
<td>5q11.1</td>
<td>NDUFS4, AQDQ</td>
<td>NADH dehydrogenase (ubiquinone) Fe-S protein 4, 18kD (NADH-coenzyme Q reductase)</td>
<td>602694</td>
<td>Leigh syndrome, 256000 (3); Mitochondrial complex I deficiency, 252010 (3)</td>
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<tr>
<td>5q11.2</td>
<td>FST, FS</td>
<td>Follistatin</td>
<td>136470</td>
<td>Polycystic ovary syndrome, 184700 (2)</td>
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<td>5q11.2</td>
<td>KFS</td>
<td>Klippel-Feil syndrome</td>
<td>214300</td>
<td>?Klippel-Feil syndrome (2)</td>
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<tr>
<td>5q12</td>
<td>PDE4D, DPDE3, STRK1</td>
<td>Phosphodiesterase-4D, cAMP-specific (dunce, Drosophila, homolog of, phosphodiesterase-E3)</td>
<td>600129</td>
<td>(Stroke, susceptibility to, 1), 606799 (3)</td>
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<td>5q12-q13</td>
<td>MCCC2, MCCB</td>
<td>3-Methylcrotonyl-CoA carboxylase 2</td>
<td>210210</td>
<td>3-Methylcrotonylglycinuria II (3)</td>
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<td>5q12.2-q12.3</td>
<td>DMGDH, DMGDHD</td>
<td>Dimethylglycine dehydrogenase</td>
<td>605849</td>
<td>Dimethylglycine dehydrogenase deficiency, 605850 (3)</td>
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<td>5q12.2-q13.3</td>
<td>SMN1, SMA1, SMA2, SMA3, SMA4</td>
<td>Survival of motor neuron 1, telomeric</td>
<td>600354</td>
<td>Spinal muscular atrophy-1, 253300 (3); Spinal muscular atrophy-2, 253550 (3); Spinal muscular atrophy-3, 253400 (3); Spinal muscular atrophy-4, 271150 (3)</td>
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<td>5q13</td>
<td>HEXB</td>
<td>Hexosaminidase B, beta polypeptide</td>
<td>606873</td>
<td>Sandhoff disease, infantile, juvenile, and adult forms, 268800 (3); Spinal muscular atrophy, juvenile (3)</td>
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<td>5q13-q14</td>
<td>AAT2, FAA2</td>
<td>Aortic aneurysm, familial thoracic 2</td>
<td>607087</td>
<td>Aortic aneurysm, familial thoracic 2 (2)</td>
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<td>5q13-q14</td>
<td>WGN1, ERVR</td>
<td>Wagner syndrome (erosive vitreoretinopathy)</td>
<td>143200</td>
<td>Wagner syndrome (2); Erosive vitreoretinopathy (2)</td>
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<tr>
<td>5q13-q22</td>
<td>CMAL</td>
<td>Capillary malformations, hereditary</td>
<td>163000</td>
<td>Capillary malformations, hereditary (2)</td>
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<td>5q13-q22</td>
<td>GINGF2, GGF2, HGF2</td>
<td>Fibromatosis, gingival, 2</td>
<td>605544</td>
<td>Fibromatosis, gingival, 2, 135300 (2)</td>
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<td>5q13.3</td>
<td>RASA1, GAP, CMAVM, PKWS</td>
<td>RAS p21 protein activator 1 (GTPase activating protein)</td>
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<td>Basal cell carcinoma (3); Parkes Weber syndrome, 608355 (3); Capillary malformation-arteriovenous malformation, 608354 (3)</td>
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<tr>
<td>Location</td>
<td>Symbol</td>
<td>Title</td>
<td>MIM</td>
<td>Disorder</td>
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<td>5q13.3</td>
<td>VG5Q, HUS84971, FLJ10283</td>
<td>VG5Q gene</td>
<td>608464</td>
<td>Klippel-Trenaunay syndrome, 149000 (3)</td>
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<td>5q14</td>
<td>MASS1, VLGR1, KIAA0686, FEB4, USH2C</td>
<td>Monogenic, audiogenic seizure susceptibility 1, mouse, homolog of (very large G protein-coupled receptor 1)</td>
<td>602851</td>
<td>Convulsions, familial febrile, 4, 604352 (3); Usher syndrome, type II C, 605472 (3)</td>
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<td>5q15-q21</td>
<td>PCSK1, NEC1, PC1, PC3</td>
<td>Proprotein convertase subtilisin/kexin type 1</td>
<td>162150</td>
<td>Obesity with impaired prohormone processing, 600955 (3)</td>
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<td>5q2</td>
<td>HSD17B4</td>
<td>Hydroxysteroid (17-beta) dehydrogenase 4</td>
<td>601860</td>
<td>D-bifunctional protein deficiency, 261515 (3)</td>
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<tr>
<td>5q21</td>
<td>MCC</td>
<td>Mutated in colorectal cancers</td>
<td>159350</td>
<td>Colorectal cancer (3)</td>
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<tr>
<td>5q21-q22</td>
<td>APC, GS, FPC</td>
<td>Adenomatous polyposis coli</td>
<td>175100</td>
<td>Gardner syndrome (3); Adenomatous polyposis coli (3); Colorectal cancer (3); Desmoid disease, hereditary, 135290 (3); Turcot syndrome, 276300 (3); Adenomatous polyposis coli, attenuated (3); Gastric cancer, 137215 (3); Adenoma, periampullary (3)</td>
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<td>5q23</td>
<td>ADAMTS2, NPI</td>
<td>A disintegrin-like and metalloproteinase with thrombospondin type 1 motif, 2 (procollagen I N-proteinase)</td>
<td>604539</td>
<td>Ehlers-Danlos syndrome, type VII C, 225410 (3)</td>
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<td>5q23</td>
<td>DTR, DTS, HBEGF, HEGFL</td>
<td>Diphtheria toxin receptor (heparin-binding EGF-like growth factor)</td>
<td>126150</td>
<td>(Diphtheria, susceptibility to) (1)</td>
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<tr>
<td>5q23-q31</td>
<td>FBN2, CCA</td>
<td>Fibrillin-2</td>
<td>121050</td>
<td>Contractural arachnodactyly, congenital (3)</td>
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<tr>
<td>5q23-q31</td>
<td>ITGA2, CD49B, BR</td>
<td>Integrin, alpha-2 (CD49B; alpha-2 subunit of VLA-2 receptor; platelet antigen Br)</td>
<td>192974</td>
<td>Neonatal alloimmune thrombocytopenia (2); ?Glycoprotein la deficiency (2)</td>
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<td>5q23.1-q23.3</td>
<td>SNCAIP</td>
<td>Synuclein-alpha-interacting protein (synphilin 1)</td>
<td>603779</td>
<td>Parkinson disease, 168600 (3)</td>
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<td>5q23.3-q31.2</td>
<td>LOX</td>
<td>Lysyl oxidase</td>
<td>153455</td>
<td>Cutis laxa, recessive, type I, 219100 (1)</td>
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<td>5q31</td>
<td>ADLD</td>
<td>Leukodystrophy, adult-onset, autosomal dominant</td>
<td>169500</td>
<td>Leukodystrophy, adult-onset, autosomal dominant (2)</td>
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<td>5q31</td>
<td>DIAPH1, DFNA1, LFHL1</td>
<td>Diaphanous, Drosophila, homolog of, 1</td>
<td>602121</td>
<td>Deafness, autosomal dominant 1, 124900 (3)</td>
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<tr>
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<td>MIM #</td>
<td>Disorder</td>
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<td>5q31</td>
<td>FACL6, ACS2</td>
<td>Fatty-acid-Coenzyme A ligase, long-chain 6 (long-chain acyl-CoA synthetase 2)</td>
<td>604443</td>
<td>Myelodysplastic syndrome (3); Myelogenous leukemia, acute (3)</td>
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<td>5q31</td>
<td>GRAF</td>
<td>GTPase regulator associated with the focal adhesion kinase pp125</td>
<td>605370</td>
<td>Leukemia, juvenile myelomonocytic, 607785 (3)</td>
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<td>IBD5</td>
<td>Inflammatory bowel disease-5</td>
<td>606348</td>
<td>(Inflammatory bowel disease-5), 266600 (2)</td>
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<td>5q31</td>
<td>IL13, ALRH</td>
<td>Interleukin-13</td>
<td>147683</td>
<td>(Asthma, susceptibility to), 600807 (3); (Allergic rhinitis, susceptibility to), 607154 (3)</td>
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<td>5q31</td>
<td>MSS</td>
<td>Marinese-Sjogren syndrome</td>
<td>248800</td>
<td>Marinese-Sjogren syndrome (2)</td>
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<td>5q31</td>
<td>NR3C1, GCR, GRL</td>
<td>Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)</td>
<td>138040</td>
<td>Cortisol resistance (3)</td>
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<td>PDB4</td>
<td>Paget disease of bone 4</td>
<td>606263</td>
<td>Paget disease of bone, 602080 (2)</td>
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<td>POU4F3, BRN3C</td>
<td>POU domain, class 4, transcription factor-3</td>
<td>602460</td>
<td>Deafness, autosomal dominant 15, 602459 (3)</td>
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<td>5q31</td>
<td>TGFBI, CSD2, CDGG1, CSD, BIGH3, CDG2</td>
<td>Transforming growth factor, beta-induced, 68kD</td>
<td>601692</td>
<td>Corneal dystrophy, Groenouw type I, 121900 (3); Corneal dystrophy, lattice type I, 122200 (3); Corneal dystrophy, Reis-Bucklers type, 608470 (3); Corneal dystrophy, Avellino type, 607541 (3); Corneal dystrophy, lattice type IIIA, 608471 (3); Corneal dystrophy, Thiel-Behnke type, 602082 (3)</td>
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<td>TTID, MYOT</td>
<td>Myotilin (titin immunoglobulin domain protein)</td>
<td>604103</td>
<td>Muscular dystrophy, limb-girdle, type 1A, 159000 (3)</td>
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<td>5q31-q32</td>
<td>PDGFRB, PDGFR</td>
<td>Platelet-derived growth factor receptor, beta polypeptide</td>
<td>173410</td>
<td>Myeloproliferative disorder with eosinophilia, 131440 (3); Myelomonocytic leukemia, chronic (3)</td>
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<td>AITD2</td>
<td>Autoimmune thyroid disease, susceptibility to, 2</td>
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<td>(Autoimmune thyroid disease, susceptibility to, 2) (2)</td>
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<td>ATOD6</td>
<td>Dermatitis, atopic, 6</td>
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<td>Dermatitis, atopic, 603165 (2)</td>
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<td>EOS</td>
<td>Eosinophilia, familial</td>
<td>131400</td>
<td>Eosinophilia, familial (2)</td>
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<td>Disorder</td>
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<td>5q31-q33</td>
<td>HCl, HEMC</td>
<td>Hemangioma, capillary infantile</td>
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<td>Hemangioma, capillary infantile</td>
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<td>5q31-q33</td>
<td>PFBI</td>
<td>Plasmodium falciparum blood infection levels</td>
<td>248310</td>
<td>(Plasmodium falciparum parasitemia, intensity of)</td>
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<td>5q31-q33</td>
<td>PPP2R2B</td>
<td>Protein phosphatase 2, regulatory subunit B, beta</td>
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<td>Spinocerebellar ataxia 12, 604326</td>
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<td>SM1</td>
<td>Schistosoma mansoni infection, susceptibility/resistance to</td>
<td>181460</td>
<td>(Schistosoma mansoni infection, susceptibility/resistance to)</td>
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<td>SCGB3A2, UGRP1</td>
<td>Secretoglobin, family 3A, member 2 (uteroglobin-related protein 1)</td>
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<td>(Asthma, susceptibility to), 600807</td>
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<td>IRF1, MAR</td>
<td>Interferon regulatory factor-1</td>
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<td>Macrocytic anemia, refractory, of 5q- syndrome, 153550; Myelodysplastic syndrome, preleukemic; Myelogenous leukemia, acute; Gastric cancer, 137215; Nonsmall cell lung cancer</td>
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<td>LIM domain protein ril</td>
<td>603422</td>
<td>(Osteoporosis, susceptibility to), 166710</td>
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<td>Sar1a, S. cerevisiae, homolog 2</td>
<td>607690</td>
<td>Anderson disease, 607689; Chylomicron retention disease, 246700; Chylomicron retention disease with Marinesco-Sjogren syndrome, 607692</td>
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<td>5q31.1-q33.1</td>
<td>GABRG2, GEFS3, CAE2, ECA2</td>
<td>Gamma-aminobutyric acid (GABA) A receptor, gamma-2</td>
<td>137164</td>
<td>Epilepsy, generalized, with febrile seizures plus, 604233; Epilepsy, childhood absence, 607681; Myoclonic epilepsy, severe, of infancy, 607208</td>
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<td>5q31.1-q33.1</td>
<td>IL12B, NKS F2</td>
<td>Interleukin-12B (natural killer cell stimulatory factor-2, cytotoxic lymphocyte maturation factor-2, p40)</td>
<td>161561</td>
<td>BCG and salmonella infection, disseminated, 209950; (Asthma,susceptibility to), 600807</td>
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<td>EPD, PDE</td>
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<td>PDE6A, PDEA</td>
<td>Phosphodiesterase-6A, cGMP-specific, rod, alpha</td>
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<td>GLRA1, STHE</td>
<td>Glycine receptor, alpha-1 polypeptide</td>
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<td>SPINK1, PSTI, PCTT, TATI</td>
<td>Serine protease inhibitor, Kazal type I (pancreatic secretory trypsin inhibitor)</td>
<td>167790</td>
<td>Pancreatitis, hereditary, 167800 (3); (Fibrocalculous pancreatic diabetes, susceptibility to) (3); Tropical calcific pancreatitis, 608189 (3)</td>
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<td>SLC26A2, DTD, DTDST, D5S1708, EDM4</td>
<td>Solute carrier family 26 (sulfate transporter), member 2 (diastrophic dysplasia sulfate transporter)</td>
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<td>Diastrophic dysplasia, 222600 (3); Atelosteogenesis II, 256050 (3); Achondrogenesis Ib, 600972 (3); Epiphysial dysplasia, multiple, 226900 (3); Diastrophic dysplasia, broad bone-plateyspondylar variant (3)</td>
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<td>TCOF1, MFD1</td>
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<td>Treacher Collins mandibulofacial dysostosis, 154500 (3)</td>
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<td>SGCD, SGD, LGMD2F, CMD1L</td>
<td>Sarcoglycan, delta (35kD dystrophin-associated glycoprotein)</td>
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<td>Muscular dystrophy, limb-girdle, type 2F, 601287 (3); Cardiomyopathy, dilated, 1L, 606685 (3)</td>
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<td>Coagulation factor XII (Hageman factor)</td>
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<td>SLC22A5, OCTN2, CDSP, SCD</td>
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<td>Colony-stimulating factor-1 receptor, oncogene FMS (McDonough feline sarcoma)</td>
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<td>5q34</td>
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<td>Potassium large conductance calcium-activated channel, subfamily M, beta member 1</td>
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<td>(Hypertension, diastolic, resistance to), 608622 (3)</td>
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<td>NKX2E, CSX</td>
<td>NK2 transcription factor, Drosophila, homolog of, E</td>
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<td>msh, Drosophila, homeo box homolog of, 2</td>
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<td>NPM1</td>
<td>Nucleophosmin 1 (nucleolar phosphoprotein B23, numatrin)</td>
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<td>Sequestosome 1</td>
<td>601530</td>
<td>Paget disease of bone, 602080 (3)</td>
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<td>FGFR4</td>
<td>Fibroblast growth factor receptor-4</td>
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<td>Cancer progression/metastasis) (3)</td>
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<td>B4GALT7, XGALT1, XGPT1</td>
<td>Xylosylprotein 4-beta-galactosyltransferase, polypeptide 7</td>
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<td>Ehlers-Danlos syndrome, progeroid form, 130070 (3)</td>
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<td>FLT4, VEGFR3, PCL</td>
<td>fms-related tyrosine kinase-4 (vascular endothelial growth factor receptor 3)</td>
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<td>Chr.5</td>
<td>AP3B1, ADTB3A, HPS2</td>
<td>Adaptor-related protein complex 3, beta 1 subunit (adaptin, beta-3a)</td>
<td>603401</td>
<td>Hermansky-Pudlak syndrome, 608233 (3)</td>
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<td>CAQ5</td>
<td>Circulating adiponectin QTL on chromosome 5</td>
<td>606770</td>
<td>Circulating adiponectin QTL) (2)</td>
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<td>Chr.5</td>
<td>CKN1</td>
<td>Cockayne syndrome 1, classical</td>
<td>216400</td>
<td>Cockayne syndrome-1 (3)</td>
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## APPENDIX II

DETAILED INFORMATION ON ALL MARKERS MAPPED TO ECA14

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<thead>
<tr>
<th>Marker</th>
<th>Name</th>
<th>Horse</th>
<th>Forward Primer (5’ – 3’)</th>
<th>PCR Product Size</th>
<th>Ta (°C)</th>
<th>MgCl2 (mM)</th>
<th>References</th>
<th>Accession Number</th>
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<tr>
<td>ADRB2</td>
<td>Adrenergic, beta-2-, receptor, surface</td>
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<td>BCL2/adenovirus E1B 19kDa interacting protein 1</td>
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<td>C1q and tumor necrosis factor related protein 2</td>
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<td>C5orf7</td>
<td>Jumonji domain containing 1B</td>
<td>14q21</td>
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<td>Caetano et al. 1999a</td>
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<td>F: TTCTTTAAGCTGCTCAGAG G R: TCCAGCAATCTAGCAGTT</td>
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<td>HEXB</td>
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<td>See Chowdhary et al. 2003 AF115752 AF115753</td>
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<td>HK3</td>
<td>Hexokinase 3 (white cell)</td>
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<td>F: AGTGGGGCGTCTTCTAGTGAT R: CACCGTCCACCCAGGTACA</td>
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<td>5-hydroxytryptamine (serotonin) receptor 4</td>
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<td>F: GAACATGCCACCCAGATC R: AACCCTAGTATGCACAG</td>
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<td>F: TCTTAGGCTCTGGCAAAAACCT R: TGGAAACGAGGTTGAGAA</td>
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<td>See Chowdhary et al. 2003</td>
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<td>Lectin, mannose-binding 2</td>
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<td>F: ATGACACCTTCCTGGCTGCTG  R: AGGCCAACAGTACTAGGCCG</td>
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<td>137</td>
<td>58</td>
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<td>See Chowdhary et al. 2003</td>
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<td>From Australia</td>
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| RGNEF  | Rho-guanine nucleotide exchange factor | | F: TAATGCACAAACAGAGATGCATC  
R: AGAAAGAATGCTGGTGATT | 156 | 50 | 1.5 | | XM_376405 | |
| SEMA6A | Semaphorin 6A1 | | F: TGAGATGACACACATCGGT  
R: GCTCGAGACGAAATGGTACA | 186 | 50 | 1.5 | | NM_020796 | |
| SIAT8D | Sialyltransferase 8D (alpha-2, 8-polyosialyltransferase) | 14q25 | F: AGTTTGGCTGAGATGGGA  
R: CTCGCTTGGACATGAAAGCA | 172 | 58 | 1.5 | | NM_005668 | |
| SMAP-5 | Golgi membrane protein SB140 | 14q21 | F: GGAATCATTCCTCAGCTGG  
R: TTAGGGAAGACACTCGGTAT | 139 | 58 | 1.5 | | NM_030799 | |
| SNCAIP | Synuclein, alpha interacting protein (synphilin) | | F: ACAGCTGATGCAAGGTCAC  
R: GCTTTCTGCGCTGGCATAC | 145 | 58 | 1.5 | | NM_005460 | |
| SOD2ps | | 14q22.3-q23 | F: GACAAACCTGAGCCCCAAT  
R: CTTATTGAAGCCGAGCCAAC | 148 | 58 | 2.0 | See Chowdhary et al. 2003 | |
| SOX30 | SRY (sex determining region Y)-box 30 isoform a | | F: GTTGTGGGCAAGGATCCAC  
R: AGTTTGGCCACTCTAACCCC | 100 | 62 | 2.0 | | AY008814 | NM_178424 |
| SPARC | Secreted protein, acidic, cysteine-rich (osteonectin) | 14q15-q16 | F: ACCCCTAGGCTCCATTT  
R: CTGGGAGCAATACCTAA | 199 | 58 | 2.0 | See Chowdhary et al. 2003 | AF115756 | NM_003118 |
| SV2C | Synaptic vesicle protein 2C | | F: CAAGACGGGATGTCAGATTACC  
R: GAAACAGCTGATCCCCGAA | 634 | 58 | 1.5 | | XM_043493 | |
| TAF7 | TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 55kDa | 14q21 | F: AGATGCTTGTCTCCACAGTT  
R: CTGGTCATGTTCTAGTGATCA | 377 | 58 | 1.5 | | NM_005642 | |
| THBS4 | Thrombospondin 4 | | F: ACATCGACAGTACCCCGAC  
R: GGCAATCTGTCTGCAATTCT | 342 | TD 60 | 1.5 | | NM_003248 | |
| TKY1053 | Microsatellite | 14 | F: ATACTGCGCTTTAGCTCACAG  
R: ATCCACCCAGAGTTAATGG | 92 | 58 | 2.0 | Tozaki et al. 2004 | AB104271 |
| TKY1145 | Microsatellite | | F: TGGCATCCCACATAAACACAG  
R: ACTGATGTCAGACAGGGAG | 257 | 58 | 0. | Tozaki et al. (in preparation) | AB104363 | |
| TKY1151 | Microsatellite | | F: TCAGGCAATTCCTCCTTTGG  
R: TGCCCTAACATTTGAGTG | 259 | 58 | 0. | Tozaki et al. (in preparation) | AB104369 | |
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<td>Tozaki et al. 2004</td>
<td>AB103656</td>
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<td>Tozaki et al. 2004</td>
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APPENDIX III

DETAILED INFORMATION ON ALL MARKERS MAPPED TO ECA21

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<td>ADAMTS6</td>
<td>A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 6</td>
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| ERBB2IP | v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian) interacting protein | F: CCACAGTCTGCACCTCAAAATA  
R: TGATTTTCTGTGCCTGTAGCTTAG | 164 | 58 | 1.5 | | | NM_018695 |
| FBXL7 | F-box and leucine-rich repeat protein 7 | F: CCAAGTACTGCAGGCAAGCTG  
R: CAAGACTTGAGGCTAGGCG | 199 | 58 | 1.5 | | | NM_012304 |
| FBXO4 | F-box protein 4 | F: CCCTTCTGATGATGGCTGTC  
R: CATCAATCGGCTGAGGCG | 176 | 58 | 1.5 | | | NM_012176 |
| FKBP8 | FK506 binding protein 8, 38kDa | F: AACTCCTAGC TTCGCGCAT  
R: GCCGTGATGTTGTCAGGCTG | 642 | 60 | 1.5 | | | NM_012181 |
| FLJ11193 | Hypothetical protein FLJ11193 | F: TATACCGGGCAATGGCTCAG  
R: ACGTGTCGCTTGCATATT | 109 | 55 | 1.5 | | | NM_018356 |
| FLJ13611 | Hypothetical protein FLJ13611 | F: GGACACATTAAGGGAGTA  
R: CATATCTGAGGATGGCCTCC | 1309 | 58 | 1.5 | | | NM_024941 |
| FLJ14054 | Hypothetical protein FLJ14054  | F: GGGAGAAATTGGAATCGCAG  
R: GCCCTTATTTCGTCGATA | 103 | 50 | 4.0 | See Chowdhary et al. 2003 | | NM_024563 |
| FLJ21308 | Hypothetical protein FLJ21308 | F: TCCTGCAGCGCTAATTACGTA  
R: TCTTTGCGACATGATGACGTA | 998 | 58 | 1.5 | | | NM_024615 |
| FLJ35954 | Hypothetical protein FLJ35954 | F: TACCTCTAACGGGCCTGAG  
R: GAGAGAAAAGCTATCATACAA | 253 | 58 | 1.5 | | | NM_152622 |
| FLJ39155 | Hypothetical protein FLJ39155 | F: TCCTGTCGCACTACACAGG  
R: CTGGTGTACTGGCTTCCC | 146 | 58 | 1.5 | | | NM_152403 |
| FST | Follistatin | F: TGCCCTGACATGAGCTGAG  
R: ATCGCGAATGCTTTACTCC | 124 | 58 | 1.5 | | | NM_006350 |
| GDF1 | Growth differentiation factor 1 | F: CTCAGGTCCTGTAGCCA  
R: TGTACGGAACCAGGAGG | 124 | 58 | 1.5 | | | NM_001492 |
| GDNF | Glial cell derived neurotrophic factor | F: TTTTCAAGGTCACGCAAGGCG  
R: GGTCGTCTCAAAAGGCGATG | 141 | 55 | 1.5 | | | NM_000514 |
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<td>Growth hormone receptor</td>
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<td>Sema domain, seven thrombospondin repeats (type 1 and type I-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5A</td>
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VITA

CONTACT INFORMATION

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M.S., Genetics, Texas A&M University, College Station, Texas
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Graduation Date: December 2000

RESEARCH INTERESTS

Gene Mapping
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Gene and Genome Sequencing
Molecular Biology

PUBLICATIONS

