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THE USE OF A WHOLE GENOME SCAN TO FIND A GENETIC MARKER FOR DEGENERATIVE SUSPENSORY LIGAMENT DESMITIS IN THE PERUVIAN PASO HORSE

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ABSTRACT OF THESIS

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The Graduate School

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2005

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DEGENERATIVE SUSPENSORY LIGAMENT DESMITIS
IN THE PERUVIAN PASO HORSE

ABSTRACT OF THESIS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of
Science in the College of Agriculture at the University of Kentucky

By Diane I. Strong

Sadieville, Kentucky

Director: Dr. E. Gus Cothran, Department of Veterinary Science

Lexington, Kentucky

2005

ABSTRACT OF THESIS

THE USE OF A WHOLE GENOME SCAN TO FIND A GENETIC MARKER FOR
DEGENERATIVE SUSPENSORY LIGAMENT DESMITIS
IN THE PERUVIAN PASO HORSE

Degenerative suspensory ligament desmitis (DSLD) is a debilitating disease of connective tissues seen in many breeds but has become prevalent in the Peruvian Paso horse. DSLD is believed to be a genetic disorder caused by one primary founder and most likely has a recessive mode of inheritance although a dominant or co-dominant mode of inheritance has not been ruled out.

A genome scan using 259 microsatellite markers was used to test for linkage disequilibrium between one or more markers and DSLD. Two groups of Peruvian Paso horses were selected from one population including the US and Canada. The only difference between the two groups of horses besides the size of the two groups was the presence of DSLD in the affected group and the absence of DSLD in the unaffected group. It was assumed that differences seen between the two groups in homozygosity and or common allele frequency could be an indication of linkage to DSLD.

As a connective tissue disorder, there were a large number of candidate genes for DSLD to consider, yet no identical human or animal model exists. The genome scan identified five chromosomal regions where statistically significant differences were seen between affected and unaffected sample populations that could be indications of linkage to DSLD. Those chromosomes were: ECA 6, 7, 11, 14, and 26.

Sequencing of a portion of the G domain in the Chondroitin Sulfate Proteoglycan 2 (CSPG2) gene has mostly ruled out that segment of chromosome 14 as having linkage to DSLD. Further research needs to be conducted in the regions of ECA 6,7,11 and 26 where statistically significant differences were seen between the affected and unaffected groups, especially on ECA 6 and 11 since possible candidate genes are located in those regions based on the human comparative map.

Key Words: Degenerative Suspensory Ligament Desmitis, Peruvian Paso, whole genome scan, connective tissue diseases, linkage disequilibrium

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(Director of Dissertation)

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(Director of Graduate Studies)

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This thesis is dedicated to my Mother who believed I could do it, my father whom never thought I would do it, my husband who stuck by me while I did it and my daughter who happened along the way.

ACKNOWLEDGEMENTS

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Chapter One

Introduction and Background

"It is easier to cull undesirable traits immediately than to deal with them in future generations." This has been the Peruvian Paso Horse breeders' philosophy for over 400 years. The Peruvian Paso is a superb animal that has lived up to its legacy of being a well-bred horse. However, Degenerative Suspensory Ligament Desmitis (DSLD) has slipped through the fingers of Peruvian Paso breeders the world over, and become a serious problem for the breed as a whole. Though actual numbers are impossible to calculate do to a lack of reporting and denial of the problem, DSLD has been reported by veterinarians to occur at an extremely high frequency in the Peruvian Paso. On some farms, it has been reported as high as 40% (anonymous due to confidentiality requirements) but this may not be the case breed wide. From this information and from pedigree data, it is believed to be a genetic disease. The primary reason that DSLD occurs so frequently in the Peruvian Paso is due to the late onset of the disease. Horses present with DSLD, on average, between 4 and 10 years of age (Mero and Scarlett, 2005) and quite frequently as late as 20 years of age. These animals have reached or even passed their prime reproductive years by the time they are showing signs of disease, and thus already have passed on the debilitating defect to the next generation of horses. This emphasizes the need to identify a genetic marker for DSLD and to develop a genetic test to detect the disease prior to an animal entering the breeding population.

Because DSLD is seen at such a high frequency in the Peruvian Paso breed, it is believed to be a genetic disorder. Pedigrees reviewed by Dr. D. P. Sponenberg suggest that DSLD has a recessive mode of inheritance (personal communication). The purpose of this

research was to find a genetic marker for DSLD. The method used was a whole genome scan.

The History of DSLD

  The Peruvian Paso is not the only breed known to suffer from DSLD. It is also seen in Arabians, Quarter Horses, Thoroughbreds, the Saddle bred, and in National Show Horses. However, the disease is believed to be specifically different in the Peruvian Paso horse and Peruvian Paso crosses. Unlike other breeds, what is seen with DSLD in the Peruvian Paso is not the result of overt trauma or high speeds (Pryor, Pool, and Wheat, 1981). In addition, unlike all other breeds, the Peruvian Paso does not return to pasture soundness but rather the disease progresses to the point of requiring euthanization (Young, 1993).

  Until recent years, DSLD was a term used loosely when diagnosing older broodmares or heavily trained horses of various breeds presenting with swollen, hard and painful fetlocks or those individuals that have sustained prior suspensory ligament injuries. In the late 1970's and early 1980's DSLD was being seen more and more frequently around the country, especially in the Peruvian Paso breed. It is unknown whether it is coincidence that the incidence of DSLD increased around the same time the Peruvian Paso was introduced into the United States.

  In 1981, Pryor, Pool and Wheat of the University of California at Davis were the first to report DSLD in the Peruvian Paso. In their unpublished paper titled: "Clinical and pathological characterization of suspensory apparatus failure in Peruvian Paso horses", the authors discuss clinical and pathological findings of 17 Peruvian Paso horses examined at UC Davis Veterinary Teaching Hospital between January 1969 and March 1984. All 18 of the Peruvian Paso horses in the study had what the authors characterize as suspensory

apparatus failure (SAF). The horses ranged in age from 7 months to 18 years and consisted of 8 females, 7 males and 2 geldings. In the study, there were two sets of dams and daughters and one set of sire and son. The authors point out the difference between SAF in the Peruvian Paso and SAF associated with trauma and high speeds. "SAF in the Peruvian Paso horses appears to be a degenerative problem that shows no association with overt trauma or high speeds." The second author of that paper states that the possibility of a collagen defect is most interesting. He then goes on to note that there are a number of connective tissue disorders seen in other species including humans. Heritable connective tissue disorders in humans include Ehlers-Danlos syndrome (EDS), Marfan syndrome, Epidermolysis bullosa (EB), and Osteogenesis imperfecta (OI). Of these, Ehlers-Danlos syndrome and Marfan syndrome are the most similar to DSLD as they involve similar connective tissue defects.

The consequences of human selective breeding of domestic animals have been seen throughout the world in cats, dogs, horses, and zoo animals. In canids, deafness in Dalmatians is just one example of a genetic disease that has become prevalent as a direct result of poor breeding practices (Wood et al., 2004). One might speculate that the limited number of Peruvian Paso horses in the country gave rise to more line breeding and lower genetic diversity. This is due to fewer stallions and family lines to choose from, and therefore a smaller gene pool being available to mare owners. Americans are known to hold good bloodlines with high regard, and frequently line breed in order to increase the amount of "good blood" within the line. This is not a practice that supports genetic diversity. When this practice is combined with a small gene pool containing a genetic disease, the results can be detrimental.

The Biology and Pathology of DSLD

DSLD affects connective tissues of the cardiovascular system, nuchal ligaments, suspensory ligaments, patellar ligaments, deep digital flexor tendons, superficial digital flexor tendons and the sclera of the eye. In the horse, it is especially noticeable in the suspensory ligament (Halper et al., 2005). Along with the superficial and deep digital flexor tendon, the suspensory ligament is a large powerful tendon, which provides support for the lower legs and maintains the elevation of the fetlock and the angles of the hooves with the upper limb (Siegal 1996).

The suspensory ligament begins at the back of the most distal row of carpal bones (at the back of the knee) and at the back part of the upper metatarsus on the forelimb. On the hind limb, most of the attachments originate at the metatarsus but there are some attachments to the distal row of tarsal bones. The suspensory ligament divides into a branch (medial and lateral branches) just before reaching the fetlock joint where it wraps around the outside surface of the two proximal sesamoid bones (Giffin and Gore1989). From the sesamoid bones the suspensories continue distally from the fetlock as lateral and medial extensor branches which then insert into the Common Digital Extensor tendon at the front of the pastern between the fetlock and the foot (©2000-2002 Veterinary Professional Services Ltd.).
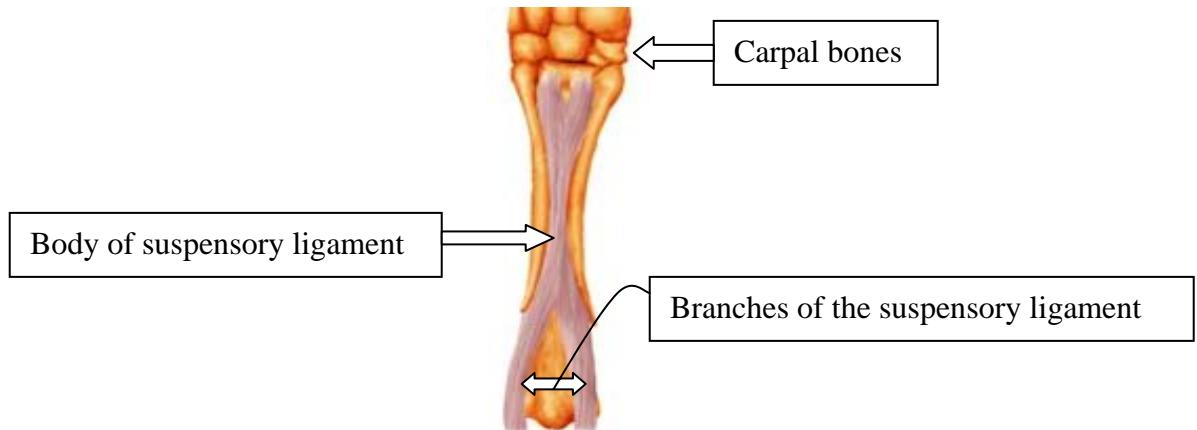
Figure 1.1 Structure of the suspensory ligament

Figure 1.2 Example of mare with DSLD in forelimbs. (Compliments of www.dsld.org)

Gross physical changes are usually, but not always present in a horse with DSLD. Often there will be a thickening along the backside of the cannon bone that travels down to the pastern area. Do to a lack of integrity in the suspensory ligament, the fetlock joint looses the spring action created by the sesamoid bones. Symptoms of DSLD include obscure recurrent lameness, overt lameness, marked swelling around the fetlocks, a sinking of the fetlock toward the ground, back pain and excessive lying down with a reluctance to move (Mero and Pool, 2002). Back pain occurs as a result of the horse shifting its weight off the affected limbs causing an abnormal posture.

In the rear limbs, a dropping of the fetlock can occur giving a post-legged appearance. The post-legged conformation is a direct result of DSLD. Horses that naturally have a post-legged conformation are not any more prone to developing DSLD than a horse without the post-legged conformation (Bennet, 1994)

Figure 1.3 Post-legged appearance caused by DSLD (compliments of www.dsld.org)
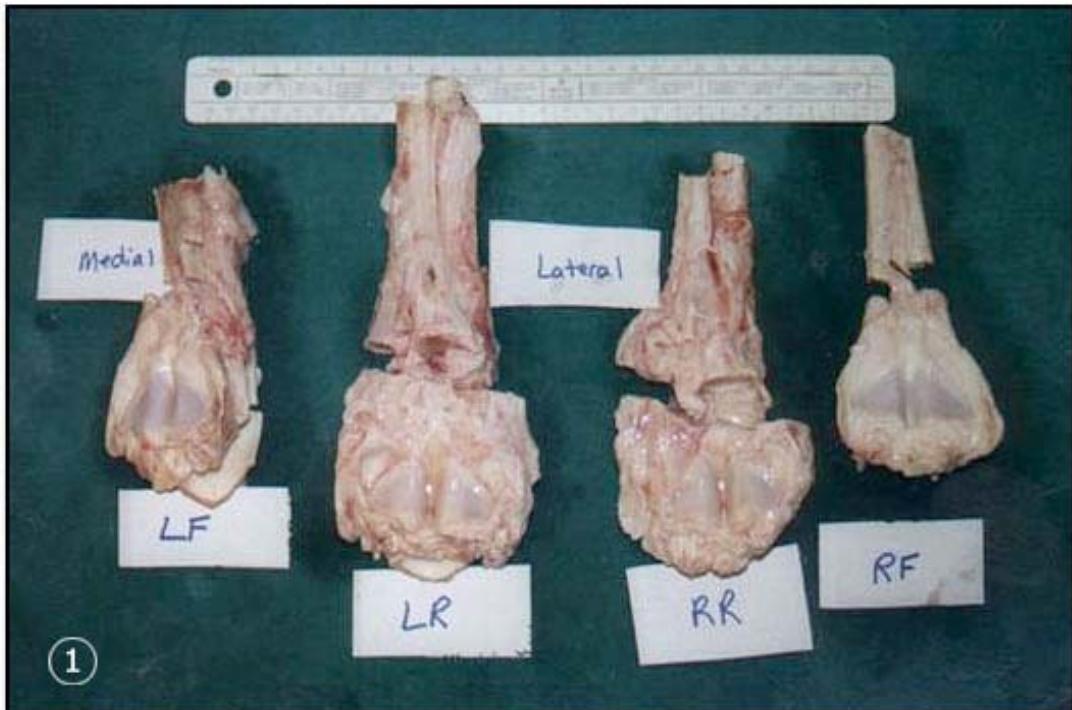
Figure 1.4 Post mortem DSLD photos: left front, left rear, right front. Note the right front is least affected and most normal looking, but was confirmed DSLD under microscope (compliments of www.dsld.org).

DSLD causes degeneration and swelling in the collagen cells of the suspensory ligaments and suspensory branches. This creates a loss of architecture within the collagen bundles. Instead of being replaced with collagen, damaged collagen is replaced with chondrocytes that produce cartilage and bone. This ultimately results in an abnormally large suspensory ligament made up of disorganized scar tissue that is mechanically useless (Mero and Pool, 2002).

The following figures are from work done by Dr. Jaroslava Halper of the Department of Pathology at the University of Georgia, Athens, GA. Dr. Halper is one of the top experts in the world dealing with tendon and ligament analysis. Her research has provided this study

with candidate genes involving proteoglycans. Dr. Halper has proposed Equine Systemic Proteoglycan Accumulation (ESPA) as a more descriptive name of the condition rather than DSLD. She has confirmed the presence of proteoglycan in lesions of the connective tissues of the cardiovascular system, nuchal ligaments, suspensory ligaments, patellar ligament, deep digital flexor tendon, superficial digital flexor tendon, and in the sclera of the eye in DSLD affected horses. In addition, she was able to demonstrate by electron microscopy, increased cell permeability in tendons and smooth muscle cells of the media of the aorta. Gel chromatography revealed the presence of additional proteoglycan in fractions of connective tissues from affected but not control extracts (Halper et al., 2005). Overall, Halper was able to prove for the first time that DSLD is a disease that affected all connective tissues of the body, not just the suspensory ligaments. This is important when examining possible candidate genes.
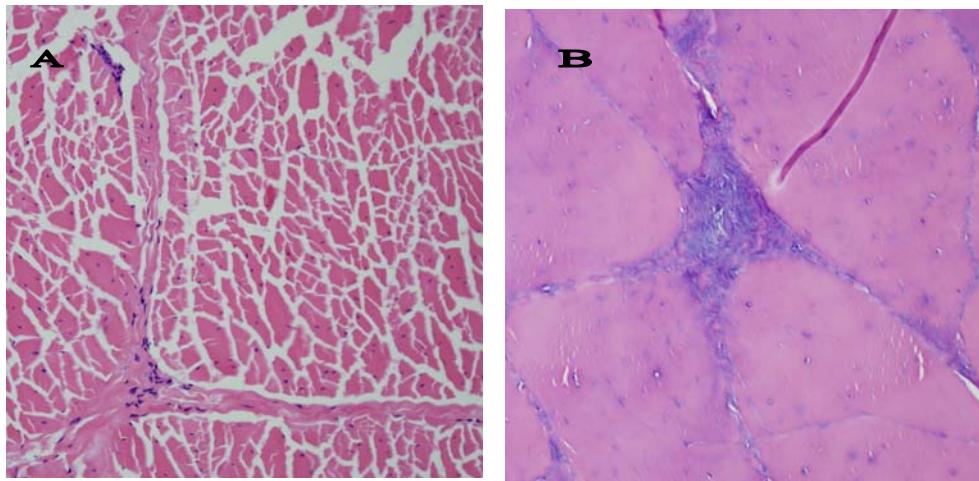
Figure 1.5 Comparison of normal (A) and ESPA-affected /DSLD (B) tendons.

A. Only thin septa separate bundles of collagen and elastic fibers in a normal tendon. Hematoxylin & eosin, magnification x 200. B. A section of ESPA-affected tendon reveals PG (proteoglycan) deposits between collagen fibers and in septa. Hematoxylin & eosin, magnification x 200 (compliments of Halper et al., 2005).

History of the Peruvian Paso

The Peruvian Paso is a breed renowned for its smooth gait, long stride, and ability to cover great amounts of ground in short time. The history of the breed is confusing and controversial. Depending on the resource referenced, the breed is a hot blood or a warm blood, of Friesian decent or not of Friesian decent. Fortunately, for the sake of science, the breed's most recent history is the most likely culprit when it comes to DSLD. For the purpose of thoroughness, the following is a brief history of the breed starting at the arrival of their ancestors in Peru.

In their quest to conquer new territories, conquistadors brought the Spanish horse to the American continent. Christopher Columbus brought the first Spanish horse to the island of Santa Domingo to begin a breeding farm in 1493. Eventually the breeding practices extended to Central America where the numbers multiplied enough to support the needs of the mounted troops (Reusser, 1997).

Francisco Pizzaro brought the ancestors of the Peruvian Paso to Peru in 1532. (Hartley, 1991). After reaching the shores of Peru, the Spanish Horse (Spanish Jennet), a breed consisting of Galician (Celtic) horses of the North, Sorraia, and Barb of Morocco, was bred only to Andalusians with the propensity to amble (von Rust McCormick, 1997). The result was the breed known as "Caballo Peruano de Paso", the Peruvian Paso horse with the amazing four beat lateral gait.

It has only been about 35 years since the Peruvian Paso was imported to North America. There are approximately 20,000 registered Peruvian Paso horses in the United States with an average annual increase of around two to three percent (Castro, 2005, personal communication).

The Purpose of This Research:

The end goal of this research is to find a genetic marker that can be used as a means to determine with high dependability the likelihood that a specific Peruvian Paso is a DSLD carrier (heterozygote), a sufferer (homozygote) or none of the above. A reliable test for DSLD would equip breeders with the required knowledge to make informed decisions when choosing which animals to breed. Ultimately, should the breeders as a whole choose to do so, the disease could be eradicated from the registry entirely.

The research conducted for this thesis represents the initial stages toward the ultimate goal. The vast search for a genetic marker requires that one know the approximate chromosomal location of the problem. The goal of this research was to narrow that search and begin the process of looking for a marker. This research demonstrates the author's understanding of the required process, technical ability to carry out required experiments, and ability to analyze and interpret data.

Review of Genetic Literature

This research utilized a whole genome scan approach to test for linkage of one or more of microsatellite DNA markers to a possible DSLD gene. The research is dependent on the disease being genetic and caused by a single founder individual. Microsatellite DNA markers are used to test for linkage to the gene causing DSLD. With a recessive mode of inheritance, the expected results would be an excess of homozygosity in the affected individuals as compared to non-affected individuals. Excess homozygosity can be caused by four factors. 1) Null allele being present, skewing results. A null allele is one that is present but not detected doe to the absence of a gene product at the molecular level. Null alleles will not corrupt mapping efforts but can cause loss of information (Callen et al., 1993). Null

alleles can also cause misleading information when individuals are haplotyped for linkage disequilibrium testing (Callen et al., 1993). In the case of this study, a null will not mislead since data is compared to a second population that would also contain the null; 2) inbreeding may be common in the population, as in the Peruvian Paso population; 3) the locus is being selected for; and 4) a population substructure may lead to Walhunds' effect. Steps were taken in order to differentiate between these reasons for excess homozygosity. Comparing two groups of horses of the same breed, similar in age and work histories allows one to assume that differences seen between the two groups must be due to DSLD most of the time. The rest of the time can be due to statistical chance.

This method proved to be successful in the search for the gene causing equine severe combined immunodeficiency disease (SCID). Bailey et al. (1997) found evidence for linkage disequilibrium with the microsatellite DNA marker HTG8 to the primary candidate gene (DNA-PK) for SCID.

Lieto and Cothran (2003) mapped the Epitheliogenesis imperfecta (EI) locus to equine chromosome 8 in American Saddle bred horses. This was accomplished by comparing microsatellite marker information from affected and unaffected individuals. Pathological signs of EI were similar to a disease in humans known as Herlitz Junctional Epidermolysis Bullosa (HJEB) that is known to be caused by one of the three subunits of the laminin 5 proteins. Linkage disequilibrium (LD) between microsatellite markers suggested the putative location of ECA 8, the location of LAM α3.

Microsatellites were used as a tool in the genetic mapping of GBE1 and its association with glycogen storage disease IV in American Quarter horses (Ward et al., 2003). In that study, all 9 foals affected with glycogen storage disease IV were homozygous for allele 3 of GBEms1 as well as allele 3 of UMNe66 on ECA26. Although allele 3 was the

most common allele in affected and unaffected groups, Chi squared tests demonstrated the allele distributions for both GBE1 and UNMe66 to be significantly different between the affected foal and control populations (Ward et al., 2003).

Chapter Two

Mode of Inheritance Analysis

With an autosomal recessive gene, Mendelian inheritance predicts that one in four children of parents who are both heterozygous for a single gene mutation will be homozygous for that mutation. In humans, cystic fibrosis is a autosomal recessive disorder among Caucasians, affecting about 1/2500 newborns. Therefore, it can be calculated that the carrier frequency is about 4%. These numbers are relatively low considering it is the one of the most common autosomal recessive disorders among humans.
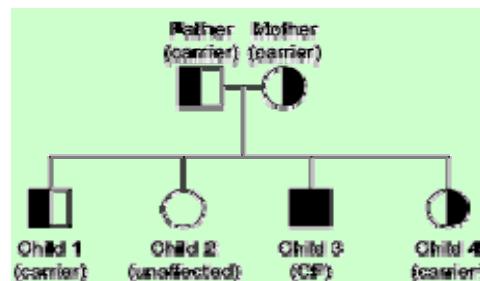
Figure 2.1 Diagram of how cystic fibrosis (any autosomal recessive disease) is inherited (hcd2.bupa.co.uk/.../ Cystic_fibrosis.html).

In horses, and especially in breeds with a small population size as in the Peruvian Paso, the same type of autosomal recessive mode of inheritance can produce disease in a population that approaches the numbers never seen with a human disease. There are 3 factors that collectively create the higher numbers. One, with a recessive disease, carriers are unknown. When one horse is bred to another, without a test, there is no way to know if either are carriers (unless they have produced a diseased foal in the past). Secondly, there is a small population that contains a few very prominent founders. If one of the prominent

founders was heterozygous for the recessive disease and he was bred to a large percent of the population then half of his progeny will be carriers. Finally, the biggest factor contributing to the situation is the fact that the breed is highly inbred. This creates a situation where the large percent of the population that are now carriers of the disease, are bred to each other. From these breedings, one in four will have the phenotype. Since DSLD often presents well into the teens, the DSLD diseased horses most likely have plenty of opportunity to pass on the disease gene to their offspring.

The exact percent of Peruvian Paso horses with DSLD is impossible to determine at this time, however occurrence is reported by veterinarians as being extremely high. Any numbers reported have been speculative. It is virtually impossible to determine what percent of the Peruvian Paso population has DSLD due to lack of reporting, ignorance about the disease or covering-up the problem. To produce a number of the incidence of the disease with what little information is available in breeding records accumulated in this study would be irresponsible and unscientific. To assume that because some farms have a frequency as high as forty percent (as given above) that this represents the entire breed also is irresponsible as farms will differ in the lineages of horses that are present. The determination of recessive mode of inheritance is based on pedigree analysis done by Dr. Sponenberg, and by considering the breed dynamics of the Peruvian Paso as discussed above. Figure 2.2 is an actual example of a typical Peruvian Paso pedigree. It shows multiple cases of inbreeding including mother/ son breedings. Figure 2.3 is an actual pedigree example of a DSLD positive Peruvian Paso. Sire A and Dam A are reported as showing no signs of DSLD. In a recessive mode of inheritance, this is typical. Both Sire A and Dam A are presumed to be carriers (heterozygous), thus explaining how their offspring was affected with DSLD. If however, Dam A or Sire A is actually affected, but has been not

been diagnosed as having DSLD, the disease could be dominant. This is an example of the complexity of determining the mode of in heritance in a disease where the reporting of the incidence has not been consistent. Thus, given the available information, the recessive mode is the most likely mode of inheritance, but dominant or codominant has not been ruled out.

Figure 2.2 Actual pedigree from a Peruvian Paso; this **amount of inbreeding is typical of the breed. Notice that Sire C is bred to his own mother on the topside fifth generation back.**
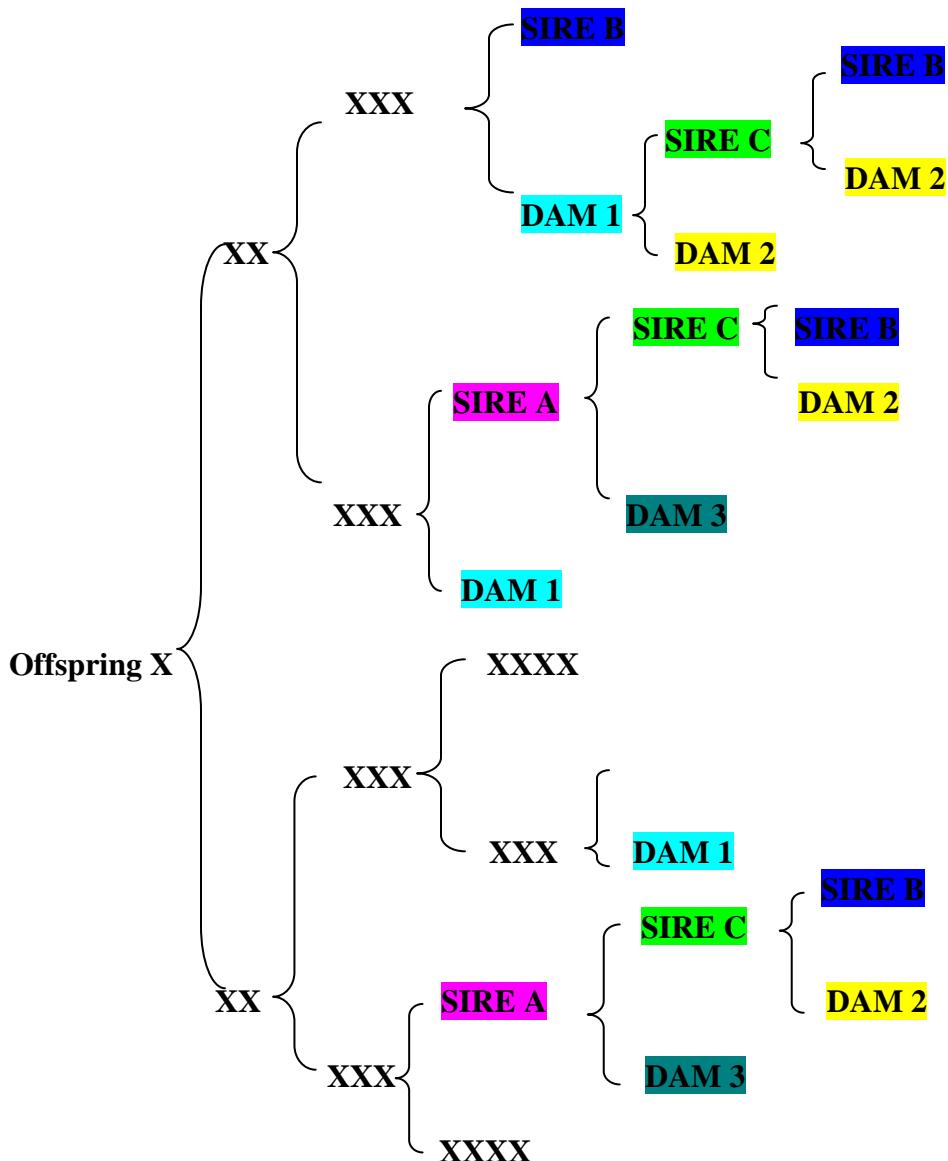
Figure 2.3 Pedigree example of DSLD positive Peruvian Paso

Horses highlighted in red are proven DSLD positive, half red and half blue are presumed to be carriers (heterozygous) for the offspring to be DSLD positive (homozygous).
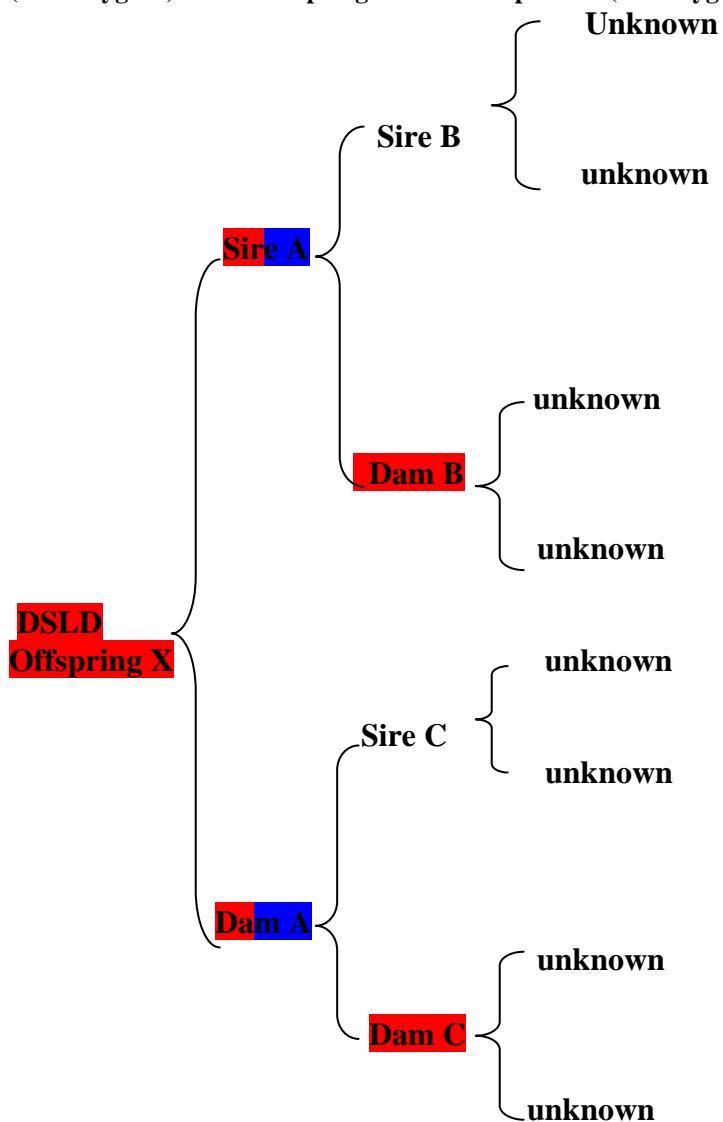
Chapter Three

Linkage Disequilibrium and the Genome Scan:

Homozygosity Analysis

Haplotype frequencies are said to be in Linkage Disequilibrium (LD) when observed frequencies of a sample population do not correspond to expected haplotype frequencies of a randomly chosen normal population. LD allows one to measure differences between populations by visualizing allele association at specific loci.

Microsatellites in the horse are dinucleotide repeats and are extremely useful as a genetic marker. The closer a genetic marker is to a particular gene the less likely that marker is to be separated during DNA repair, replication or recombination during meiosis. LD studies are used when a single recessive mutation is suspected in a closed breeding population such as is the case in DSLD and the Peruvian Paso horse.

All microsatellite results must be tested for deviations from the Hardy-Weinberg equilibrium which states that the genotype frequencies and gene frequencies of a large, randomly mating population remain constant provided immigration, mutation, and selection do not take place .Where the unaffected group did not follow Hardy-Weinberg equilibrium, data was considered suspect. This is because expected frequencies from a group that is not in Hardy-Weinberg equilibrium, (i.e., those frequencies are abnormal for one of various reasons) may be a result of non-random processes which invalidates the expectations these calculations are based upon.

Distances at which LD is seen can vary depending on the age of the mutation and the population size. With a newer, more recent mutation, recombination events have occurred between the mutation and the linked marker (Hastbacka et al., 1992). The Peruvian Paso horse breed is at most 500 years old. The population is considered small at around

20,000 and is derived from a relatively small number of founders. Information on the exact distance beyond which LD extends is not available for the horse, however in cattle it has been reported that considerable LD can extend for 20 cM (Farnir et al., 2000), and similar results have been reported in sheep, (McRae et al., 2002) and dogs (Hyun et. al. 2003). It is for these reasons that linkage disequilibrium should be seen at a relatively large distance from the mutated gene. When possible, for this study microsatellites were spaced no farther than 20 cM apart, and in regions where possible candidate genes were presumed to occur, they were spaced closer together. Anytime a microsatellite produced data suggestive of linkage, available microsatellites on either side were run on both affected and unaffected groups. This was done in order to see if the expected trend for adjacent microsatellites was observed. One would expect to see increasing linkage disequilibrium the closer one got to the mutation.

<u>Materials and Methods:</u>

Animals

        Whole blood for DNA extraction was collected from horses as whole blood by Dr. J. Mero. All horse subjects underwent a series of tests including history, physical, and lameness and ultrasound examinations in order to classify them as DSLD affected or DSLD unaffected. Efforts were made to find DSLD unaffected horses that were similar in age to those of the DSLD affected group. This was done to avoid inadvertently selecting for longevity genes. Thirty- eight DSLD affected Peruvian Paso horses ranging in age from 4 to 20 years (avg. age 11.8 years) and 32 DSLD unaffected Peruvian Paso horses ranging in age from 6 to 27 years (avg. age 12.2 years) were selected to be in the study. Initial, the unaffected horse group consisted of only 28 samples, later it was increased to 32 samples as more samples were provided. Horses used in this study were from approximately 20 different farms located in at least 10 different states in the United States of America.

DNA isolation:

        DNA isolation was performed using PUREGENE DNA PURIFICATION SYSTEM (Gentra Systems, Minneapolis, MN) following the manufacturers recommendations, briefly, 3 ml red blood cells were lysed using RBC lysis solution. The samples were then centrifuged and the white cell pellet collected. The pellet was lysed using Cell Lysis Solution and treated with RNase A Solution. Proteins were recovered using Protein Precipitation Solution and DNA precipitation was done using 100% isopropanol. The DNA was washed twice with 70% ethanol and allowed to dry. Finally, the DNA was hydrated

using DNA Hydration Solution. All samples were analyzed quantitatively and qualitatively using a Spectronic Genesys 5 Spectrometer (Milton Roy). The stock samples were stored at 4 °C and working dilutions were made for polymerase chain reaction (PCR) from the stock at a concentration of 25ng/ml for use in PCR.

Low Density Genome Scan

The primers for two hundred and sixty microsatellite markers listed in Table 3.1 were designed using published sequence data (Appendix ii). Each microsatellite was dye labeled with either 6-FAM, NED or VIC (created by Applied Biosystems, Foster City, California) and were run using standard protocol. Microsatellites were arranged into multiplexes ranging in size from 13 to 2 with some being run alone. Running protocol for each is summarized in Table 3.1. All PCR reactions were run using a MJ thermocycler PTC-100 (MJ Research, Watertown, MA). The program for all microsatellites was as follows: step 1 (initial denaturation) = 95 °C for 5 minutes, step 2 (denaturation) 95 °for 1 minute, step 3 (annealing) = as stated in table 1 for 30 seconds, step 4 (elongation) = 72 °C for 30 seconds, step 5 = go to step 2 twenty-nine times, step 6 (final elongation) = 74 °C for 30 minutes, step 7 (cooling) = 4°C forever.

Initially, only affected horses were tested against each microsatellite, and if homozygosity calculated from data was greater than 50%, then unaffected horses were tested. The 50% cut-off was chosen because it is unlikely that any locus with heterozygosity greater than 50% in the affected group would provide an indication of LD with a possible DSLD gene. Average heterozygosity of all loci typed in the unaffected group was 56%. After approximately 100 microsatellites were tested, the decision was made to run the remaining microsatellites on both affected and unaffected horses. This decision was made as

a precautionary measure to make data collected relevant should the disease prove not to have a recessive mode of inheritance. In addition, obtaining data from both groups allows for one to compare common allele frequencies, which is relevant regardless of the inheritance mode.

Table 3.1 Running protocol for all microsatellites

TABLE 3.1

PRIMER (ref)	LABEL	Size	Anneal Deg. C	Primer con.	Multiplex	ECA
1CA25 (36)	NED	201-207	58	0.2	P10-4PLX	1
1CA43 (36)	VIC	122-126	58	0.2	P10-4PLX	1
A14 (26)	VIC	220-248	58	0.8	COMBINE	2
A-17 (26)	6-FAM	102-118	58	0.15	7PLEX	26
AHT02 (35)	NED	96-110	58	0.2	P15	15
AHT07 (37)	VIC	121-133	58	0.25	P10-3PLX	25
AHT14 (38)	6-FAM	138-158	58	.25dil>1:1	P12_4PX2	16
AHT16 (38)	VIC	129-155	60	0.05	DIANMP4	15
AHT19 (38)	VIC	137-145	58	0.15	P-AHT19	7
AHT21 (38)	VIC	199-215	58	0.09	RINK6	1
AHT22 (38)	VIC	189-197	60	0.5	P15mx	3
AHT24 (37)	NED	197-199	58	0.25	P6-4PLEX	5
AHT25 (37)	NED	179-189	58	0.25	PAHT25	8
AHT30 (37)	NED	181-197	58	0.25	P6PLEXb	22
AHT32 (37)	NED	138-142	58	0.15/dil1: 1	p16_4px	24
AHT33 (37)	VIC	149-169	58	0.25	P6PLEXb	31
AHT34 (37)	NED	121-150	58	0.25	P6PLEXb	31
AHT36 (37)	VIC	137-155	58	0.3	P5-4PLEX	3
AHT37 (37)	VIC	206-218	58	0.25	P10-3PLX	16
AHT38 (37)	6-FAM	130-140	58	0.25	P6PLEXb	16
AHT41 (37)	VIC	243-265	58	0.25	P6PLEXb	19
AHT43 (37)	NED	172-200	58	0.25	P5-4PLEX	4
AHT51 (39)	6-FAM	146-181	58	0.25	P10-3PLX	25
AHT53 (39)	VIC	260-280	58	0.25	p16_4px	9
AHT60 (39)	VIC	182-308	58	.25dil>1:1	P12_4PX1	16
AHT86 (39)	6-FAM	195-216	58	0.2	P15_4px	10
AHT91 (39)	NED	108-118	58	.25dil>1:1	P12_4PX2	16
AHT97 (39)	6-FAM	151-165	60	0.025	P15mx	3

Table 3.1   **cont.**

ASB1 (4)	VIC	153-165	58	0.25	P7-5PLEX	13
ASB6 (4)	6-FAM	185-212	58	0.6	RINK2	10
ASB8 (4)	VIC	138-164	58	0.05	DIANMP1	1
ASB9 (4))	6-FAM	67-113	60	0.3	DMP	10
ASB13 (4)	6-FAM	125	58	0.25	P7-5PLEX	2
ASB14 (4)	6-FAM	118-136	58	0.05	COMBINE	8
ASB17 (4)	6-FAM	93-125	58	0.1	RINK	2
ASB18 (4)	6-FAM	196-213	58	0.15	P26	2
ASB22 (4)	NED	155-177	58	0.1	4PLEX	4
ASB35 (24)	VIC	220-240	58	0.25	P3PLEX	11
ASB37 (21)	VIC	132-146	58	0.04	RINK3	13
ASB41 (21)	NED	156-168	58	0.3	RINK3	1
B8 (26)	6-FAM	88-110	56	0.1	RINK3	15
COR002 (19)	VIC	235-243	58	0.05	RINK3	14
COR003 (19)	6-FAM	195-215	58	0.15	4PLEX	8
COR004 (19)	NED	297-319	63	0.05	P7	7
COR007 (19)	NED	163-177	58	0.04	COMBINE	17
COR008 (19)	VIC	251-277	58	0.08	DIANMP3	9
COR010 (19)	6-FAM	289-299	58	0.2	P6-4PLEX	6
COR013 (19)	VIC	248-254	58	0.25	P8-3PLX1	9
COR014 (19)	VIC	149-164	58	0.04	RINK3	15
COR016 (19)	6-FAM	187-203	58	0.12	MP12	22
COR017 (19)	VIC	241-267	58	0.15	10PLEX	27
COR018 (19)	NED	251-283	58	0.1		25
COR020 (19)	NED	162-178	58	0.25	DIANMP1	10
COR022 (30)	VIC	154-164	58	0.2	P12_4PX2	22
COR024 (30)	VIC	214-226	58	0.05	DIANMP2	24
COR026 (30)	NED	222-240	58	0.25	P5-4PLEX	2
COR027 (30)	VIC	231-255	56	0.12	RINK	29
COR028 (30)	VIC	227-260	60	0.25	P8-3PLX3	3
COR029 (30)	NED	222-228	58	0.15	P13_3PX	20

Table 3.1   cont.

COR030 (30)	VIC	233-241	58	0.3	P13DPLX	12
COR031 (30)	VIC	210-224	58	0.08	10PLEX	27
COR032(30)	NED	247-270	58	0.25	P6PLEXb	17
COR033(30)	VIC	222-254	58	0.2	RINK1	3
COR035 (30)	VIC	195-223	58	0.15	P8-3PLX2	2
COR037 (30)	NED	225-245	58	0.25	P11-2PLX	2
COR038 (30)	VIC	210-214	58	0.1	RINK2	31
COR039 (30)	NED	254	58	0.1	P10-4PLX	16
COR040 (30)	NED	282-300	58	0.25	10PLEX	27
COR043 (33)	NED	121-147	58	0.25	P8-3PLX1	2
COR048 (33)	NED	179-194	58	0.6	DIANMP3	10
COR049 (33)	6-FAM	190-206	58	0.25	PCOR049	2
COR051 (33)	VIC	290-292	58		P26	2
COR055 (33)	NED	240-270	58	0.1	8PLEX	23
COR056 (33)	6-FAM	194-220	58	0.2	COMBINE	8
COR058 (33)	VIC	218-244	58	0.1	4PLEX	12
COR059 (33)	VIC	265-277	58	0.2	P11-3PXB	1
COR061 (41)	6-FAM	197-227	58	0.1	7PLEX	
COR063 (41)	NED	103	65	0.1	P11-C63	1
COR065 (41)	NED	280-292	58	0.3	COMBINE	2
COR068 (41)	6-FAM	144-170	58	25	p16_1px	21
COR069 (41)	NED	273-287	58	0.13	5PLEX	13
COR070 (41)	NED	279-307	58	0.2	4PLEX	6
COR071 (41)	6-FAM	188-210	58	0.05	MP9	26
COR073 (41)	6-FAM	187-205	58	0.1	RINK1	21
COR080 (41)	6-FAM	198-206	58	0.2	P12_3PX	25
COR082 (40)	VIC	199-233	58	0.4	MP11	29
COR083 (40)	VIC	272	58	0.05	P6-3PLEX	10
COR084 (40)	VIC	180-196	58	.25* dilute	PCOR84_85	23
COR085 (40)	6-FAM	150-156	58	.25* dilute	PCOR84_85	10
COR089 (40)	NED	282-304	58	0.1	RINK	4

Table 3.1   **cont.**

COR092 (40)	6-FAM	191-203	58	0.1	8PLEX	19
COR094 (40)	6-FAM	283-293	58	0.25	P8-3PLX1	2
COR095 (40)	6-FAM	206-216	58	0.5	P6-COR95	7
COR096 (40)	NED	315-329	58	0.14	DIANMP3	18
COR100 (40)	VIC	212-230	56	0.15	RINK3	1
COR101 (37)	VIC	232-272	58	0.25	P9-6PLX	18
EB2E8 (35)	6-FAM	144-152	58	0.2	P26	26
HLM2 (46)	VIC	125-160	60	0.25	P8-3PLX3	11
HLM3 (46)	NED	123-131	58	0.2	P12_3PX2	18
HMS02 (1)	6-FAM	284-306	58	0.2	P10	10
HMS05 (1)	NED	102-108	58	0.25	P7-5PLEX	5
HMS15 (15)	VIC	207-245	58	0.2	DIANMP1	1
HMS18 (16)	NED	170	58	0.25	P7-5PLEX	30
HMS20 (16)	6-FAM	116-140	58	0.25	8PLEX	16
HMS25 (16)	6-FAM	124	58	0.25	P15_4px	17
HMS42 (15)	6-FAM	130-150	58	0.3	P13HMS42	20
HMS45 (16)	NED	181-201	58	.25dil>1:1	P12_4PX2	27
HMS46 (16)	VIC	131-151	58	.25dil>1:1	P12_4PX2	18
HMS47 (16)	6-FAM	203-215	58	0.17	RINK	22
HMS51 (25)	VIC	170	58	0.25	P11-3PXB	2
HTG05 (12)	VIC	75-101	58	0.1	p16_1px	20
HTG06 (12)	NED	84-102	58	0.2	P15	15
HTG07 (34)	VIC	120-130	58	0.2	P4	4
HTG08 (27)	6-FAM	185-197	56	0.4	MP13	9
HTG12 (27)	NED	111	60	.2 may need to dil. PCR 2:1	P-HTG12	1
HTG17 (23)	6-FAM	153	58	0.3	P9-6PLX	18
HTG18 (22)	VIC	166-172	58	0.25	P4PLEX2	14
HTG18 (22)	NED	168-170	60	0.3	P15mx	14
HTG21 (23)	VIC	131-143	58	0.08	RINK	22
HTG22 (22)	VIC	186-198	58	0.25	P5-2PLEX	4
HTG23 (22)	NED	188-198	58	0.15	p16_1px	19

Table 3.1   cont.

HTG27 (22)	6-FAM	141-171	58	0.25	P14_2	30
HTG28 (23)	NED	179-189	58	0.3	P9-6PLX	18
HTG30 (22)	6-FAM	228-248	58	0.25	P3PLEX	28
HTG31 (23)	VIC	152-182	58	0.3	P6-4PLEX	6
HTG32 (22)	NED	138-144	58	0.15	p16_2px	21
I-18 (26)	6-FAM	93-119	58	0.05	5PLEX	16
L12.2 (17)	VIC	136-156	58	0.05		29
L15.2 (17)	VIC	147-165	58	0.07	7PLEX	16
LEX004 (8)	NED	282-300	58	0.15	16PLX	5
LEX015 (9)	FAM	132-145	58	0.2	P6-3PLEX	7
LEX016 (9)	NED	171-191	58	.2/dil.pcr1:1	p16_3px	18
LEX018 (9)	NED	228-242	58	0.25	P13CPLX	29
LEX019 (9)	NED	157-161	58	0.25	P6-4PLEX	9
LEX020 (9)	6-FAM	198-222	58	0.12	RINK6	1
LEX022 (9)	6-FAM	110-124	58	0.12	10PLEX	X
LEX023 (9)	VIC	233-257	58	0.15	RINK4	8
LEX025 (10)	VIC	152-168	58	0.04	10PLEX	30
LEX034 (10)	NED	252-262	58	0.05	5PLEX	5
LEX036 (10)	NED	148-170	58	0.05	7PLEX	19
LEX037 (10)	6-FAM	189-197	58	0.25	P7-5PLEX	21
LEX038 (10)	6-FAM	133-145		0.05	P12_1PX	7
LEX042 (10)	6-FAM	210-227	58	0.25	p16_4px	24
LEX043 (10)	FAM	232-254	58	0.25	P4PLEX2	14
LEX046 (10)	VIC	115-127	60	0.15	P15mx	15
LEX047 (10)	VIC	237-257	58	0.25	P4PLEX2	14
LEX048 (10)	NED	160-190	58	0.25	P5PLEX	16
LEX052 (6)	VIC	208-214	58	0.05	RINK1	20
LEX054 (6)	NED	170-190	58	0.08	RINK4	18
LEX055 (6)	VIC	216-232	58	0.15	16PLX	17
LEX056 (6)	VIC	218-234	58	0.08	7PLEX	16
LEX058 (6)	VIC	224-236	58	0.25	P11-3PLX	1
LEX063 (6)	6-FAM	241-294	58	0.15	P12_3PX2	23

Table 3.1 **cont.**

LEX065 (7)	VIC	144-156	58	.25dil>1:1	P12_4PX1	6
LEX068 (7)	NED	162-174	58	0.5	DIANMP2	11
LEX069 (7)	NED	142-170	56	0.6	MP13	5
LEX070 (7)	6-FAM	233-260	60	0.25	P8-3PLX3	9
LEX071 (2)	6-FAM	192-211	58	0.12	16PLX	20
LEX073 (2)	NED	249-277	58	0.15	7PLEX	19
LEX074 (2)	NED	155-175	58	0.15	RINK1	24
LEX076 (2)	VIC	210-236	58	0.25	P5PLEX	17
NV007 (17)	VIC	230-236	58	0.25	P15_4px	10
NV18 (17)	VIC	119-161	58	0.06	4PLEX	10
NV43 (32)	NED	150-170	58	0.2	P3PLEX	25
NV54 (3)	VIC	172-186	58	0.1	P13CPLX	28
NV70 (3)	6-FAM	192-208	58	0.08	10PLEX	26
NV79 (3)	6-FAM	175-197	58	0.08	6PLEX	17
NV81 (3)	NED	166-168	58	0.15	P10-MPLX	6
NV82 (3)	VIC	133-147	58	0.01	COMBINE	6
NV90 (32)	NED	90-105	58	0.1	P13_3PX	11
NV100 (32)	6-FAM	197-217	58	0.1	RINK3	1
SGCV04 (16)	VIC	213	58	0.1	p16_2px	23
SGCV07 (16)	NED	131-155	58	0.2	P14_1	18
SGCV08 (16)	VIC	126-143	58	0.2	16PLX	12
SGCV10 (16)	NED	179-187	56	0.6	MP13	12
SGCV13 (16)	6-FAM	169-191	58	0.1	P14_3	11
SGCV14 (16)	6-FAM	188	58	0.25	P9-6PLEX	21
SGCV16 (16)	NED	154-194	58	0.12	10PLEX	21
SGCV23 (16)	VIC	221-233	58	0.6	MP13	4
SGCV24 (16)	VIC	125-141	56	0.5	MP13	11
SGCV28 (16)	NED	147-167	58	0.25	P12_3PX	7
SGCV32 (16)	6-FAM	139	60	0.1	P15_5px	8
SGCV33 (16)	6-FAM	110	65	0.1	P-SGCV33	3
TKY002 (34)	6-FAM	113-127	58	0.35	P5-3PLEX	1
TKY005 (18)	NED	106-126	58	0.25	P-TKY005	7

Table 3.1   **cont.**

TKY010 (18)	6-FAM	180-190	58	0.25	P8-3PLX2	11
TKY017 (18)	NED	120-140	58	0.3	P9-3PLX	18
TKY018 (18)	6-FAM	122-150	58	0.15	P9-3PLX	28
TKY19 (20)	NED	147-173	56	0.07	DMP	18
TKY021 (20)	VIC	117-132	58	0.2	P9-6PLEX	21
TKY024 (18)	6-FAM	158	60	0.15	P11-t24	2
TKY033 (18)	NED	86-106	60	0.13	P8-3PLX3	11
TKY034 (18)	VIC	126-172	58	0.15	P6-2PLEX	7
TKY106 (28)	NED	113-120	58	0.25	P5-3PLEX	1
TKY111 (28)	6-FAM	125-131	58	.25dil>1:1	P12_4PX1	6
TKY272 (42)	VIC	100-116	58	0.15	P6-3PLEX	7
TKY274 (42)	VIC	106-144	58	0.2	P9-3PLX	31
TKY279 (42)	6-FAM	119-133	58	0.25	P10-MPLX	16
TKY284 (42)	NED	159-175	58	0.2	P6-2PLEX	6
TKY285 (42)	6-FAM	164-188	58	0.25	P5PLEX	22
TKY310 (43)	NED	130-148	58	0.25	P4PLEX2	14
TKY319 (44)	NED	112-118	58	0.05	P13_2PLX	28
TKY335 (44)	6-FAM	251-259	58	0.12	P13CPLX	2
TKY412 (44)	VIC	234-248	60	0.2	P15_5px	6
TKY438 (44)	VIC	227-295	58	0.05	P14_1	14
TKY491 (44)	6-FAM	256-268	60	0.15	P15_5px	14
TKY497 (44)	6-FAM	288-300	58	0.2	P14_3	2
TKY515 (44)	NED	145-151	58	0.2	P14_3	28
TKY521 (44)	NED	217-223	60	0.2	P15_5px	5
TKY533 (44)	VIC	232-240	58	0.15	P14_3	9
TKY627 (44)	6-FAM	216-240	58	0.25	P14_2	9
TKY636 (44)	6-FAM	206-234	58	0.25	P13DPLX	14
TKY648 (44)	NED	286-306	58	0.25	P14_2	11
TKY692 (44)	6-FAM	130-156	58	.2/dil.pcr1:1	p16_3px	18
TKY749 (44)	VIC	227-247	58	0.25	P14_2	14
TKY798 (44)	6-FAM	221-255	58	0.1	P14_1	2
TKY842 (44)	NED	81-110	58	0.07	P14T842	2
TKY909 (44)	VIC	193-207	58	.2/dil.pcr1:1	p16_3px	18

Table 3.1 **cont.**

UCD136 (11)	6-FAM	153-163	58	0.25	P12_3PX	17
UCD380 (35)	NED	129-133	58	0.15	P11U380	2
UCD387 (35)	6-FAM	78-88	58	0.25	P5PLEX	18
UCD425 (11)	VIC	236-250	58	0.1	RINK2	28
UCD437 (11)	NED	167-193	58	0.1	MP12	3
UCD439 (35)	NED	122-152	58	0.2	P15_4px	11
UCD440 (35)	6-FAM	106-112	58	0.25	P11-3PXB	1
UCD465 (35)	6-FAM	200-206	58	0.2	P10-4PLX	6
UCD487 (11)	VIC	113-121	58	0.25	P5-3PLEX	1
UCD493 (35)	6-FAM	199	58	0.25	P10-MPLX	1
UCD497 (35)	VIC	107-125	58	0.1	P13_3PX	12
UM003 (29)	VIC	134-170	58	0.2	P5PLEX	28
UM004 (29)	VIC	113-122	58	0.2	P5-4PLEX	1
UM010 (29)	6-FAM	112-126	58	0.05	6PLEX	14
UM011 (29)	NED	167-187	58	0.1	8PLEX	20
UM015 (29)	NED	153-163	58	.25dil>1:1	P12_4PX1	6
UM030 (14)	VIC	127-143	60	0.25	P15_5px	13
UM034 (14)	NED	102-118	58	0.25	P8-3PLX2	8
UM037 (14)	VIC	107-118	58	0.1	P13um037	9
UM038 (14)	6-FAM	187-213	58	0.2	MP11	X
UM043 (14)	VIC	144-150	58	0.25	P10-MPLX	1
UMNeq050 (31)	6-FAM	121-131	58	0.05	p16_2px	18
UMNeq076 (31)	6-FAM	98-102	58	0.15	P11-3PLX	2
VHL047 (45)	VIC	134-150	58	0.05	5PLEX	13
VHL066 (45)	6-FAM	113-123	58	0.25	P5-2PLEX	5
VHL209 (45)	6-FAM	91-105	58	0.2	6PLEX	14
VIAS-H7 (35)	6-FAM	116-146	58	0.3	RINK2	7
VIAS-H21 (35)	NED	147-149	58	0.25	P9-6PLEX	31
VIAS-H34 (13)	VIC	144-160	58	0.9	DIANMP2	1

Statistical Analysis

Allele frequency was calculated using direct count. Observed homozygosity/heterozygosity was calculated using direct count. Expected and observed heterozygosities were calculated using the Windows 95 based program CERVUS (Marshell 1998) as was Hardy-Weinberg equilibrium test. Chi-Square was used to test heterozygosity differences between the affected and unaffected groups. With a recessive mode of inheritance model, a decrease in heterozygosity (excess in homozygosity) should be seen in the affected group compared to the unaffected group when the marker being tested (microsatellite) is near the DSLD gene. These differences in heterozygosities between the two groups were calculated using a FORTRAN program written by Dr. E. Gus Cothran for this analysis. A similar method was used to test for differences in homozygosity of the common allele between the two groups.

Because the samples tested in the two groups are presumed to differ from each other only in that individuals of the affected group should be homozygous for a DSLD gene, if markers were not linked to DSLD the proportion of heterozygous individuals in that group should be the same as that in the unaffected group. This was tested by calculating the Hardy-Weinberg expected proportion of heterozygosity based upon allele frequencies of the unaffected group then using that proportion to calculate the expected number of heterozygotes in the affected group. For a two-allele locus, the proportion would be $2pq$ and the expected number would be $2pq$ x the number of affected horses tested. This value was then compared to the observed number of heterozygous individuals in the affected group by Chi-Square analysis.

<u>Results:</u>

Table 3.2 is a summary of Chi-Square analysis for differences in heterozygosity between the affected and unaffected groups for each microsatellite listed in map order by chromosome along with distances in cM between each.

Note. Distances above top microsatellite and below bottom microsatellite are distances to the last mapped microsatellite on that chromosome. If this number is lacking, the last microsatellite was included in this study. * distance unknown and unable to estimate. ~ approximate distance given known mapped distances.

Table 3.2 Summary of Chi-Square analysis for differences in heterozygosity between the affected and unaffected groups for each microsatellite listed in order of chromosome along with distances in cM between each

	mSAT	heterozygosity affected	heterozygosity unaffected	distance cM Between mSats	chi square df=1
CHROM 1					
	VIASH034	0.8		45	
	ASB041	0.65		17.3	
	LEX020	0.92		8.6	
	NVHE100	0.75		35.6	
	COR100	0.75		~25	
	COR059	0.471	0.483	<5	2.02
	UCDE487	0.79	0.692	0	0.038
	TKY007	0.344	0.613	~4.6	0.198
	AHT021	0.82		~39.4	
	LEX058	0.63	0.621	58.3	0.445
	ASB008	0.61		9.8	
	1CA043	0.5	0.571	10.1	0.429
	TKY002	0.75	0.714	2.2	0.005
	1CA025	0.553	0.703	7.1	1.276
	UCD493	0.722	0.963	<7.6	1.174
	UM043	0.342	0.393	<7.6	0.03
	TKY106	0.74	0.679	<7.6	0.129
	UM004	0.68	0.609	<7.6	0.394
	HTG12	0.212	0.357	<7.6	0.803
	UCD440	0.645	0.69	12.6	0.004
	HMS015	0.74	0.815	8.8	1.575
	COR063	0	0	19.3	0

Table 3.2 continued

	mSAT	affected	unaffected	distance cM	chi square df=1
CHROM2				0	
	COR065	0.68	0.66		1.366
				~0	
	ASB18	0.76	0.46		0
				34.8	
	COR037	0.543	0.724		0.345
				58.1	
	TKY024	0	0		1.106
				<9.8	
	ASB13	0.676			
				<9.8	
	ASB17	0.842	0.893		
				5.7	
	HMS51	0.676	0.8		0.001
				8.6	
	UCD380	0.47	0.47		1.29
				<26.3	
	COR049	0.026	0.036		0
				<26.3	
	A-14	0.784	0.667		
				30.1	
	TKY335	0.69	0.66		0.009
				13.6	
	TKY497	0.68	0.61		0.066
				2.9	
	TKY798	0.57	0.53		0.031
				16.7	
	TKY842	0.78	0.66		0.051
				*	
	COR094	0.056	0.074		1.668
				*	
	UMNe76	0.625	0.613		0.017
				*	
	COR035	0	0		
				*	
	COR026	0.278	0.536		3.38
				*	
	COR043	0.405	0.3		2.249
				*	

Table 3.2	continued				
	mSAT	affected	unaffected	distance cM	chi square df=1
CHROM 3					
	AHT036	0.842	0.815		0.023
				18.3	
	COR028	0.842	0.643		0.064
				0.6	
	COR033	0.842			
				9.8	
	AHT022	0.23	0.22		0.985
				18	
	UCD437	0.838	0.63		0.399
				35.2	
	ASB23	0.823	0.929		
				*	
	SG33	0.79	0.73		1.747
				*	
	AHT097	0.54	0.78		1.694
CHROM 4					
	AHT043	0.95			
				~20	
	HMS06	0.65	0.84		0.737
				56.9	
	COR089	0.89			
				2.5	
	ASB022	0.58			
				<8.2	
	LEX033	0.84	0.6		5.232
				<8.2	
	HTG007	0.68	0.56		1.597
				42.2	
	HTG022	0.68	0.72		0.028
				9.1	
	SGCV023	0.76			

Table 3.2 **continued**

	mSAT	affected	unaffected	distance cM	chi square df=1
CHROM 5					
	AHT024	0.68			
				32.2	
	LEX004	0	0		
				<39.5	
	VHL066	0.47	0.54		0.11
				27.5	
	HMS005	0.59	0.48		1.392
				6.2	
	LEX069	0.61			
				14.7	
	LEX034	0.71			
				39.2	
CHROM 6					
	HTG31	0.8			
				<30.6	
	COR010	0.63			
				4.7	
	NV82	0.65	0.72		0.214
				11.1	
	LEX065	0.676	0.71		0.178
				12	
	UM015	0.636	0.621		0.083
				12	
	TKY111	0.176	0.258		0.123
				*	
	NV081	0.026	0		0.996
				15.9	
	COR070	0.76	0.79		0.11
				9.5	
	UCD465	0.342	0.5		1.485
				21.2	
	TKY412	0.74	0.8		0.051
				8.1	
	TKY284	0.61	0.81		1.31
				5.5	

Table 3.2	continued				
	mSAT	affected	unaffected	distance cM	chi square df=1
CHROM 7					
	VIASH7	0	0.04		1.466
				*	
	COR095	0.53			
				*	
				*	
	TKY272	0.5	0.36		0.304
				*	
	TKY005	0.45	0.32		2.089
				12.6	
	AHT019	0.368	0.5		2.9
				18.5	
	SGCV028	0.457	0.5		0.024
				*	
	TKY283	0.43	0.36	33.2	0.436
				*	
	LEX015	0.32	0.61		0.355
				*	
	TKY35	0.18	0.61		13.875
				*	
	TKY34	0.87			
				*	
CHROM 8					
	AHT005	0.71	0.679		0.402
				8.8	
	AHT025	0.5	0.37		0.018
				49.8	
	UM034	0.658	0.75		0
				12.3	
	LEX023	0.68			
				<9.4	
	ASB14	0.68	0.679		0.008
				<19.3	
	SGCV32	0.63	0.52		0.654
				5.6	
	COR003	0.82			
				41.5	
	COR056	0.76	0.756		0.573
				16.5	

Table 3.2	continued			
CHROM 9	mSAT	affected	unaffected	distance cM	chi square df=1
	HTG004	0.68	0.821		0.332
				34.5	
	HMS003	0.76	0.821		0.111
				5.2	
	COR008	0.82			
				<11	
	TKY627	0.8	0.63		1.068
				<11	
	TKY533	0.65	0.71		0.192
				<9.7	
	COR013	0.314	0.464		3.048
				<9.7	
	HTG008	0.52	0.704		1.155
				0	
	UM037	0.46	0.7		4.064
				~0	
	LEX070	0.63	0.77		0.534
	AHT53	0.56	0.48	69.1	0.088
	LEX019	0.45	0.556		0.088
CHROM 10				14.3	
	COR020	0.84			
				<20.9	
	COR048	0.73			
				12	
	NV018	0.83			
				5.8	
	ASB06	0.86	0.71		3.833
				12.8	
	NV007	0.26	0.1		9.443
				<48	
	COR083	0	0	48	
				<48	
	HMS2	0.79	0.692		0.428
				3.2	
	ASB9	0.64			
				*	
	COR085	0.37	0.43		0.032
	AHT86	0.66	0.59	*	0.393

Table 3.2	continued				
	mSAT	affected	unaffected	distance cM	chi square df=1
CHROM 11				9	
	UCDEQ439	0.54	0.45		0.078
				17.9	
	LEX068	0.66			
				14.9	
	SGCV024	0.85			
				11.5	
	SGCV013	0.59	0.72		0.113
				~.65	
	ASB35	0.03	0.36		9.164
				*	
	TKY033/32	0.5	0.893		0.891
				*	
	NV090	0.281	0.285		0.02
				*	
	TKY010	0.342	0.344		0.422
				*	
	TKY648	0.438	0.56		0.426
				22.4	
	HLM2	0	0.034		1.251
CHROM 12				14.3	
	SGCV010	0.85			
				13.4	
	SGCV008	0.56	0.91		2.012
				<19	
	COR030	0.66	0.59		0.072
				<19	
	COR058	0.87			
				23.4	
	UCDEQ497	0.43	0.53		0.097
CHROM 13					
	COR069	0.76			
				19.9	
	UM030	0.6	0.6		1.445
				3.9	
	ASB037	0.62			
				<3.8	
	AHT030	0.4	0.214		2.9
				<3.8	
	VHL047	0.63			
				46.8	
	ASB001	0.703			
				30	

Table 3.2	continued			
	mSAT	affected	unaffected	distance cM	chi square df=1
CHROM 14					
	LEX043	0.71	0.92		0.008
				63	
	UM010	0.86			
				6	
	VHL209	0.84			
				2.4	
	LEX047	0.29	0.26		0.687
				14.5	
	TKY310	1.0	0.89		1.301
				<30.9	
	HTG29	0.96			
				<30.9	
	AHT83	0.47	0.78		0.184
				<30.9	
	AHT88	0.51	0.5		0.491
				<30.9	
	HTG018	0.29	0.25		0.333
				24.2	
	LEX078	0.6	0.79		0.003
				0	
	TKY491	0.54	0.65		1.138
				6	
	TKY749	0.645	0.52		1.373
				6.2	
	TKY438	0.618	0.516		0.323
				1.1	
	COR002	0.35	0.75		2.877
				5.2	
	TKY636	0.882	0.852		0.088
				8.9	

Table 3.2	continued			
mSAT	affected	unaffected	distance cM	chi square df=1
CHROM 15			51.4	
B-8	0.61			
			24.1	
LEX046	0.79	0.9		0.182
			22.2	
ASB02	0.87	0.893		0.141
			15.2	
AHT016	0.77			
			9.4	
HTG006	0.44	0.607		0.242
			12.5	
AHT002	0.711	0.654		0.061
			11.6	
COR014	0.68	0.62		0.086
			13.2	
CHROM 16				
AHT037	0.34	0.42		0.886
			<14.7	
TKY279	0.81	0.77		0.001
			*	
HTG3	0.76	0.5		0.018
			26.7	
HMS20	0.77	1.0		0.043
			0	
AHT038	0.81			
			16	
LAM15.2	0.87			
			<53.1	
COR039	0	0		0
			<53.1	
AHT014	0	0		0
			<3.3	
LEX018	0.79	0.75		0.003
			<3.3	
LEX056	0.79			
			37.8	
I-18	0.58			
			*	
AHT60	0.94	0.8		0.138
			*	
AHT91	0.37	0.35		0.17

Table 3.2	continued mSAT	affected	unaffected	distance cM	chi square df=1
CHROM 17					
	COR007	0.78	0.78		0.165
				<32.2	
	LEX055	0.21	0.18		14.596
				*	
	NVHEQ79	0.7			
				<32.3	
	COR032	0.42	0.32		0.354
				20	
	HMS025	0.54	0.40		0.463
				12.3	
	LEX076	0.16	0.29		0.937
				~50	
CHROM 18					
	LEX054	0.71	0.31		24.717
				23.9	
	TKY19	0.62			
				4.4	
	UCD136	0.771	0.813		0.106
				<25	
	UMNEQ50	0.54	0.42		2.228
				<25	
	HMS46	0.273	0.71		5.37
				<2	
	TKY909	0.69	0.65		0.289
				1.6	
	SGCV07	0.47	0.448		0.003
				<10	
	TKY692	0.69	0.77		0.001
				8.6	
	HTG28	0.283	0.143		0.016
				14.41	
	COR096	0.632	0.71		0.283
				13.6	
	HTG17	0.5	0.645		2.229
				15.8	
	UCD387	0.61	0.75		0.001
				50.1	
	COR101	0			
				<14	
	TKY017	0.632	0.679	14	0.318
				<14	
	HLM3	0.571	0.613		1.034

Table 3.2	continued			
mSAT	affected	unaffected	distance cM	chi square df=1
CHROM 19			8.3	
AHT041	0.78			
			12.9	
HTG23	0.62	0.55		0.054
			26.4	
LEX036	0.63	0.59		0.017
			7.6	
LEX073	0.68			
			32.2	
COR092	0.89			
			19.6	
CHROM 20			11.6	
HTG005	0.71	0.66		0.692
			~19.6	
LEX052	0.63			
			~30	
UM011	0.66			
			~66	
LEX071	0.76	0.83		0.003
			<27.3	
HMS42	0.656	0.566		0.503
CHROM 21				
SGCV014	0.421	0.393		1.689
			0	
SGCV016	0.5	0.667		2.43
			2.1	
TKY021	0.816	0.75		0.233
			19.8	
HTG010	0.87	0.741		0.366
			3.8	
COR073	0.97			
			4.7	
COR068	0.68	0.6		0.37
			27.1	
HTG32	0.37	0.48		0.301
			8.6	
LEX037	0.568			
			14.2	

Table 3.2	continued				
	mSAT	affected	unaffected	distance cM	chi square df=1
CHROM 22					
				11.7	
	TKY285	0.87			
				12.2	
	COR022	0.657	0.613		0.294
				1.8	
	HTG21	0.89			
				22.8	
	COR016	0.65			
				33.7	
	HMS047	0.61	0.56		0.06
				11.1	
CHROM 23					
				2.4	
	COR055	0.86			
				28.7	
	LEX063	0.686	0.774		0.017
				<45.9	
	COR084	0.53	0.71	45.9	0.095
				<45.9	
	SGCV004	0.57	0.35		7.668
CHROM 24					
				23.3	
	LEX042	0.67	0.72		0.245
				2	
	AHT032	0.71	0.74		0.051
				6.8	
	AHT004	0.79	0.645		2.288
				~11.7	
	LEX074	0.82			
				11.7	
	COR061	0.79			
				26.6	
	COR024		0.66		

Table 3.2	continued			
	mSAT	affected	unaffected	distance cM	chi square df=1

CHROM 25

mSAT	affected	unaffected	distance cM	chi square df=1
			<1	
AHT051	0.791	0.607		0.009
			<1	
NVHEQ43	0.89	1		0.197
			10.8	
AHT007	0.667	0.581		0.018
			15.6	
COR018	0.68	0.643		0.065
			9.5	
COR080	0.057	0.088		8.843
			15.7	

CHROM 26

mSAT	affected	unaffected	distance cM	chi square df=1
			27.2	
NVHEQ70	0.61	0.69		0.363
			14.2	
A17	0.53	0.69		0.247
			3.4	
EB2E8	0.237	0.429		5.918
			3.4	
COR071	0.89			
			32.2	

CHROM 27

mSAT	affected	unaffected	distance cM	chi square df=1
COR021	0.71			
			20.9	
COR040	0.82			
			<7.2	
HMS45	0.613	0.6		0.386
			~68	
COR017	0.82			

Table 3.2	continued				
	mSAT	affected	unaffected	distance cM	chi square df=1
CHROM 28				2.7	
	NV54	0.711	0.32		8.12
				3.5	
	HTG030	0.162	0.393		7.398
				<.3	
	TKY018	0.842	0.893		0.232
				0.3	
	UM003	0.711			
				16.7	
	TKY319	0.777	0.742		1.093
				23.1	
	TKY515	0.5	0.8		1.824
				~32.1	
	UCD425	0.694	0.636		0.198
CHROM 29					
	LEX018	0.788	0.75		0.003
				30.3	
	COR082	0.74	0.75		0.228
				45.2	
	COR027	0.65			
				26	
	LAM12.2	0.74			
				13.2	
CHROM 30					
	LEX025	0.583	0.625		0.008
				<25.8	
	HTG27	0.58	0.565		0.201
				<25.8	
	HMS18	0.59			
				15.2	
	VHL20	0.882	0.893		0.314
				23	

Table 3.2   Cont.
CHROM 31

				13.9	
COR038	0.25	0.522			3.034
				4.6	
VIAS-H21	0.211	0.188			0.371
				~0	
AHT033	0.806				
				<33.2	
TKY274	0.816	0.626			0.163
				<33.2	
AHT034	0.757				

Results continued:

Following are Figures of heterozygosities of loci tested for all equine chromosomes in which at least 2 microsatellites were run on affected and unaffected horse groups. There was only one chromosome not shown (ECA27), in which only one microsatellite was run on both groups. The heterozygosities were the same in affected and unaffected groups for that microsatellite. For all other markers on ECA27, heterozygosity of the affected group was greater than 50%. As stated above, it is unlikely that any locus with heterozygosity greater than 50% in the affected group would provide an indication of linkage disequilibrium with a possible DSLD gene.

It should be pointed out that peaks and valleys on the plots of heterozygosity are unimportant in this representation and only differences between the crimson line, which represents the affected group, and the navy-blue line, which represents the unaffected group are important. Statistically different (Chi-Square p value of 0.05 or less) points are highlighted in red when heterozygosity values are lower in the affected individuals.

Heterozygosities are on a scale of 0 to 1, with 0 being completely homozygous for one allele and 1 being completely heterozygous. The generally acknowledged standard for accepting linkage disequilibrium is the more conservative $p < 0.001$ (Lieto, 2001), and those values are given more explanation directly following the figure. When the p-value is statistically significant with lowest heterozygosity in the unaffected, it is presumed due to chance based upon a recessive mode of inheritance and left un-highlighted, however, it is referred to in figure explanation.

In some cases, large differences in heterozygotes of affected and unaffected groups do not show statistical significance in the comparison of overall heterozygotes. In these

instances, a Chi-Square test for differences in common allele homozygosity of affected compared to unaffected was performed. Following Hardy-Weinberg expectation, the expected number of individuals homozygous for the common allele in the affected group is equal to the number of affected horses tested times the square of the common allele frequency of the unaffected group. A green asterisk is used to denote values which have a statistically significant difference in the figures, and an explanation follows in the text.

ECA1

	COR059	UCDE487	TKY007	LEX058	1CA043	TKY002	1CA025	UCD493	UM043	TKY106	UM004	HTG12	UCD440	HMS015	COR063
Affected	0.471	0.79	0.344	0.63	0.5	0.75	0.553	0.722	0.342	0.74	0.68	0.212	0.645	0.74	0
Unaffected	0.483	0.692	0.613	0.621	0.571	0.714	0.703	0.963	0.393	0.679	0.609	0.357	0.69	0.815	0

mSats
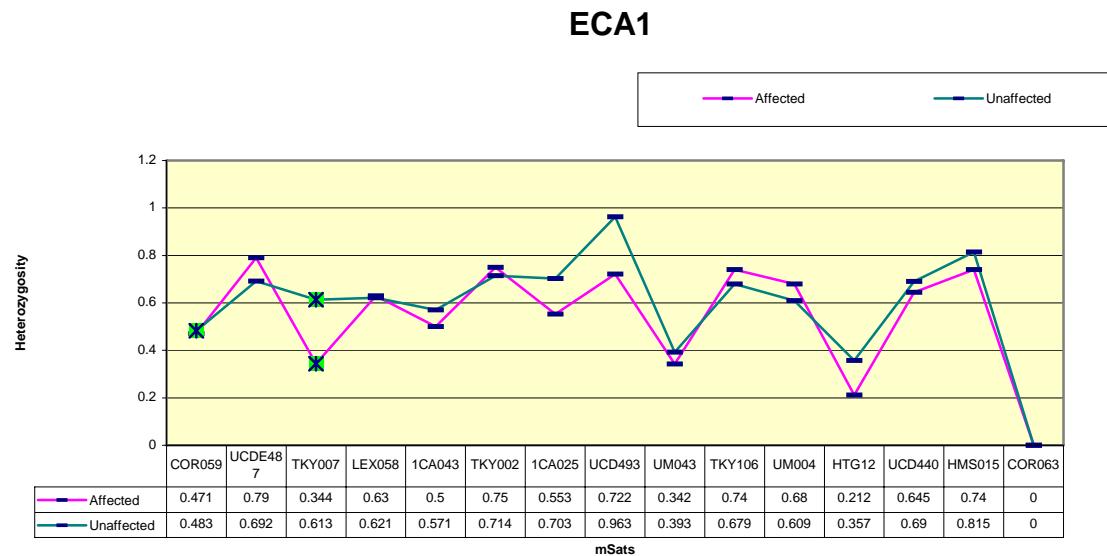
Figure 3.1 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome one. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text. *COR059 and TKY007 did show significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 8.22 (p=0.01) and 4.43 (p=0.05) respectively. However, the pattern of variation across all ECA1 loci does not give evidence for an association of any marker locus with DSLD.

ECA2

mSATS	COR0 65	ASB1 8	COR0 37	TKY02 4	ASB1 7	HMS5 1	UCD3 80	COR0 49	A-14	TKY33 5	TKY49 7	TKY84 2	COR0 94	UMNe 76	COR0 35	COR0 26	COR0 43
affected	0.68	0.76	0.543	0	0.842	0.676	0.47	0.026	0.784	0.69	0.68	0.78	0.056	0.625	0	0.278	0.405
unaffected	0.66	0.46	0.724	0	0.893	0.8	0.47	0.036	0.667	0.66	0.61	0.66	0.074	0.613	0	0.536	0.3
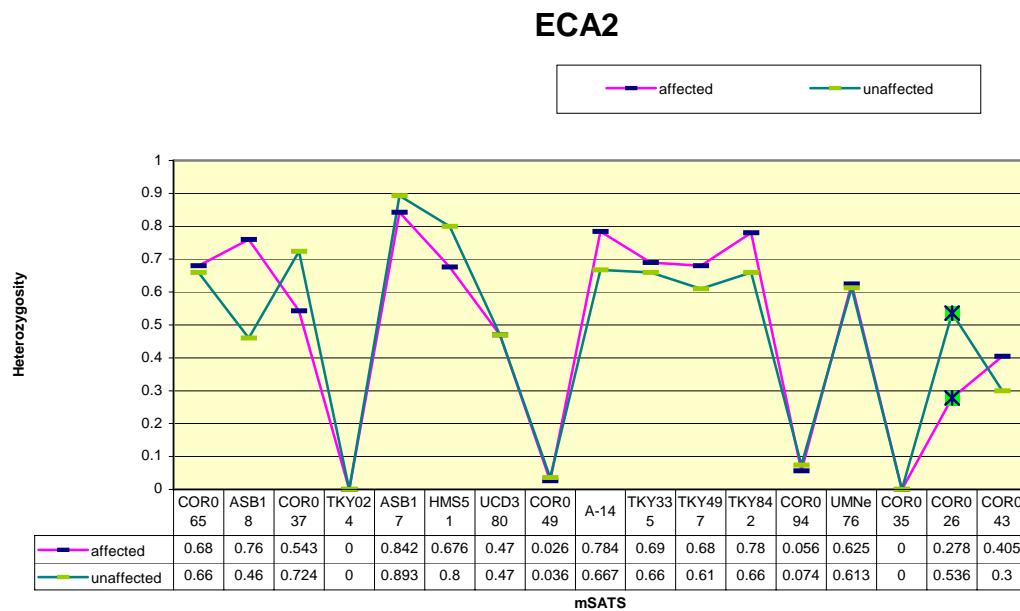
Figure 3.2 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome two. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text. *COR026 did show significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi- Square value of 9.12 (p<0.01). The pattern of variation across all ECA2 loci does not give evidence for an association of any marker locus with DSLD.

ECA3
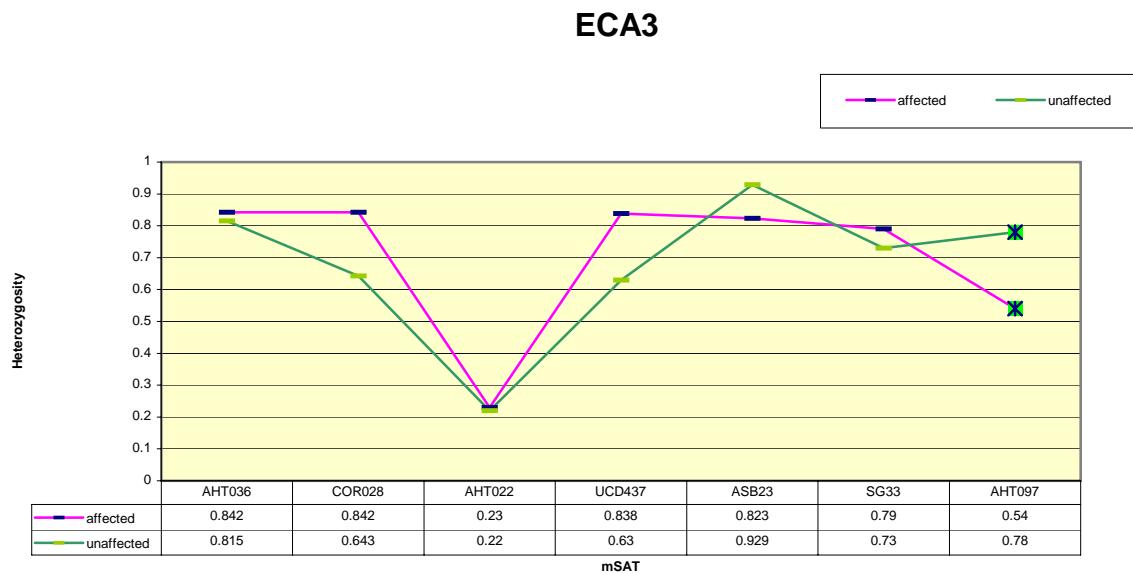
Figure 3.3 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome three. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text. *AHT097 did show significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 8.99 (p<0.01). SGCV33 does not support the trend expected in a marker for DSLD, however, the actual distance between the microsatellites is unknown, it has only been fish mapped, (Milenkovic et al., 2002). If the distance between the two is too far to show linkage (more than 20 cM) it is unlikely that SGCV33 and AHT97 will show linkage disequilibrium. AHT097 has a level of polymorphism larger than would be expected in a locus close to the DSLD gene based upon the recessive gene model.

ECA4

	LEX033	HTG007	HTG022
affected	0.84	0.68	0.68
unaffected	0.6	0.56	0.72
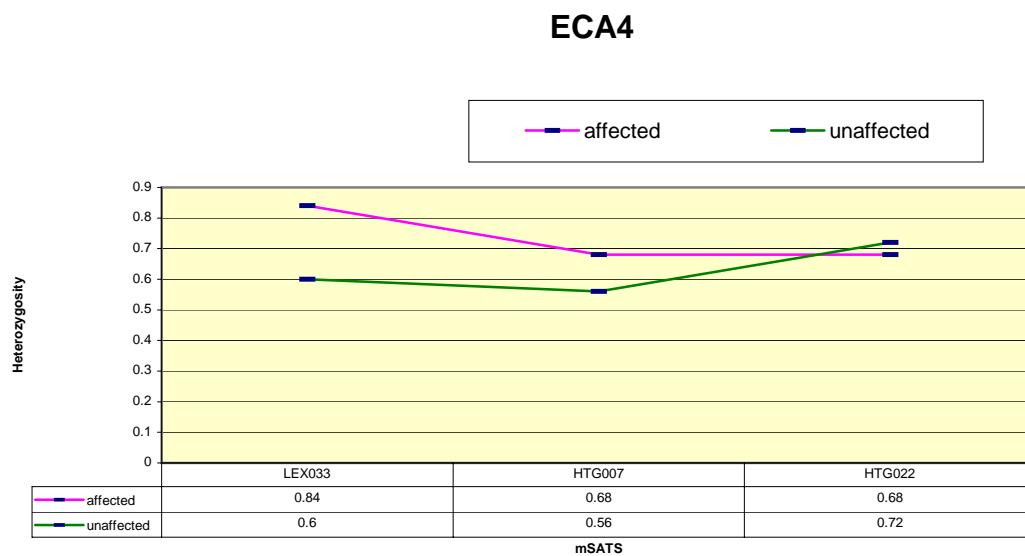
Figure 3.4   Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome four. No heterozygosity differences with lower heterozygosity in the affected are statistically significant for ECA4 as determined by Chi-Square test for heterozygosity. The Chi-Square value was 5.232 for LEX033 (p< 0.05) however, there is lower heterozygosity in the unaffected not the affected as would be expected based upon the recessive gene model.

ECA5

	LEX004	VHL066	HMS005
affected	0	0.47	0.47
unaffected	0	0.54	0.821
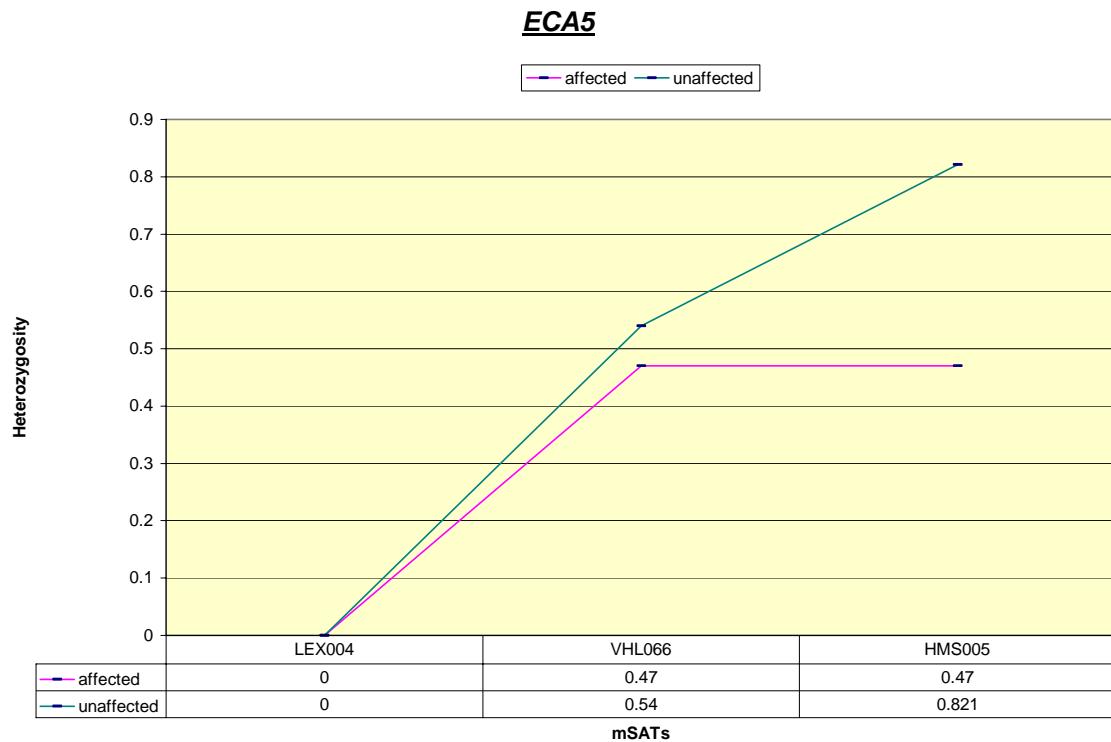
Figure 3.5 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome five. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.
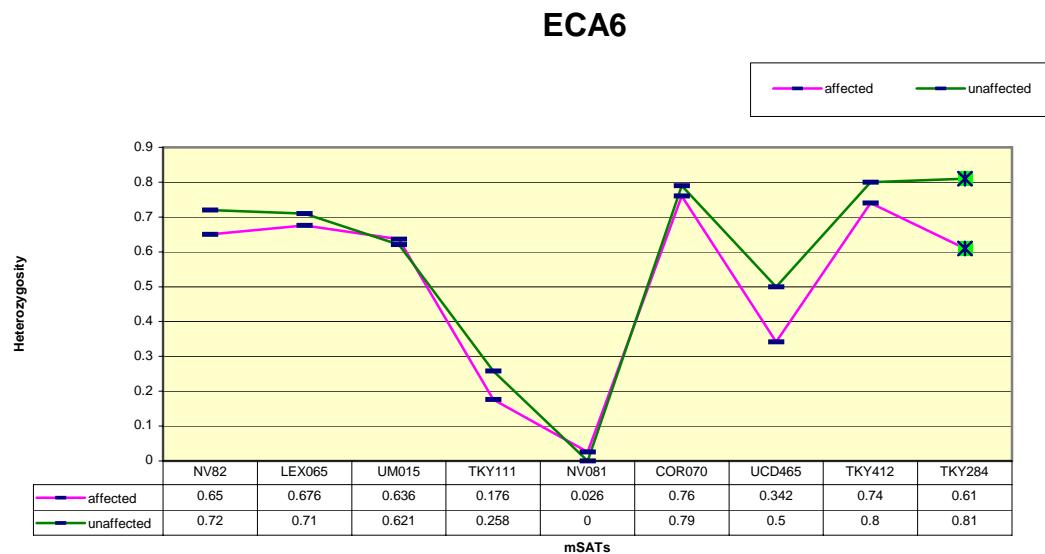
Figure 3.6 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome six. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text. *The common allele Chi-Square value was 17.128 (significant p< 0.001) for TKY284. TKY412 is 8.1 cM from TKY284 (Guerin et al., 2003) and it does not have a significant differences in heterozygosities. Because TKY284 is at the end of the chromosome, it is possible that a gene for DSLD could be telomeric of this marker locus, thus testing TKY952 may be warranted.

ECA7

mSATs	VIASH7	TKY272	TKY005	AHT019	SGCV028	TKY283	LEX015	TKY35
affected	0	0.5	0.45	0.368	0.457	0.43	0.32	0.18
unaffected	0.04	0.36	0.32	0.5	0.5	0.36	0.36	0.61
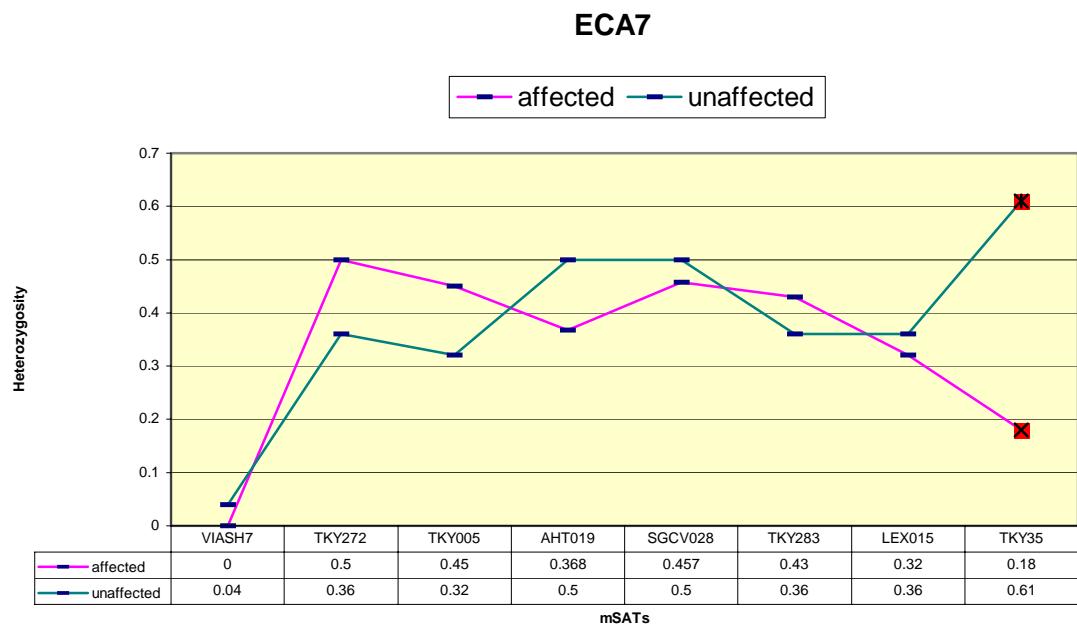
Figure 3.7 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome seven. *The Chi-Square value was 13.875 for TKY35 (significant p< 0.001). The distance from LEX015 to TKY35 in cM is unknown. According to the RH (1.0) map they are 37.1 cR from each other (Chowhardy et al., 2003). There is a possible candidate gene that could be telomeric to TKY35. FAA1 (AORTIC ANEURYSM, FAMILIAL THORACIC 1) is a locus that has been linked to human chromosome 11q23.2-24 in one family (Vaughan et al., 2001). Comparative mapping suggests TKY35 may be near FAA1. This is covered more in more detail in the discussion.

ECA8

mSats	AHT005	AHT025	UM034	ASB14	SGCV32	COR056
affected	0.71	0.5	0.658	0.68	0.63	0.76
unaffected	0.679	0.37	0.75	0.679	0.52	0.756
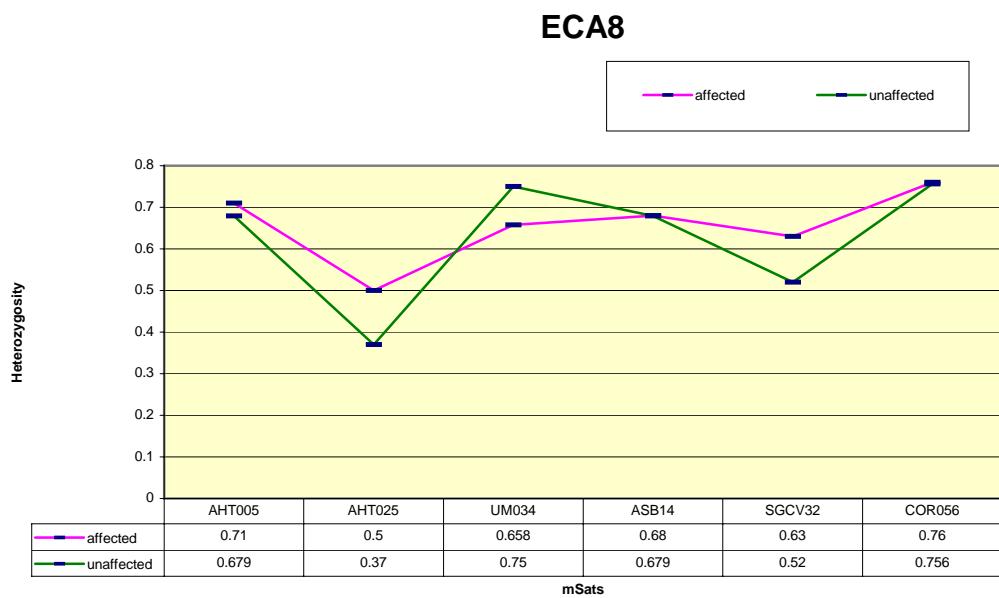
Figure 3.8   Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome eight. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.

ECA9

mSATs	HTG004	HMS003	TKY627	TKY533	COR013	HTG008	UM037	LEX070	AHT53	LEX019
affected	0.68	0.76	0.8	0.65	0.314	0.52	0.46	0.63	0.56	0.45
unaffected	0.821	0.821	0.63	0.71	0.464	0.704	0.7	0.77	0.48	0.556
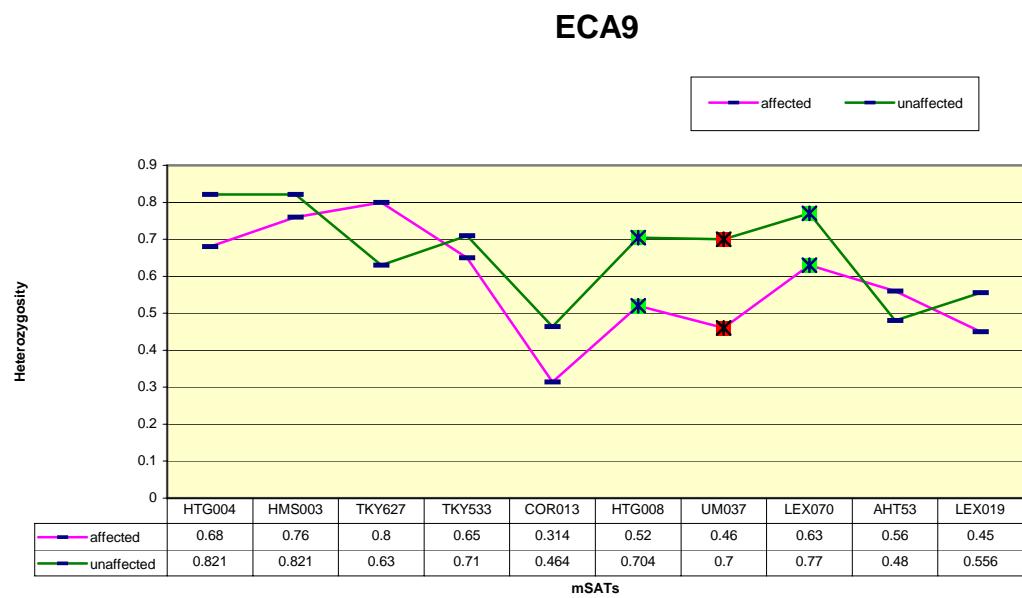
Figure 3.9   Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome nine. *The Chi-Square value was 4.064 for UM037 (p< 0.05). Despite the significant value, the result is uninformative because the locus is highly polymorphic in both affected and unaffected groups. One common homozygous allele is not shared by all or even most of affected individuals as would be expected in a recessive model. * LEX070 and HTG008 did show significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 25.71 (significant p<0.001) and 4.48 (p<0.05), respectively. LEX070 and HTG008 are also highly polymorphic, not what is expected when looking for linkage. The pattern of variation across all ECA9 loci does not give evidence for an association of any marker locus with DSLD.

ECA10

mSATs	ASB06	NV007	COR083	HMS2	COR085	AHT86
affected	0.86	0.26	0	0.79	0.37	0.66
unaffected	0.71	0.1	0	0.692	0.43	0.59
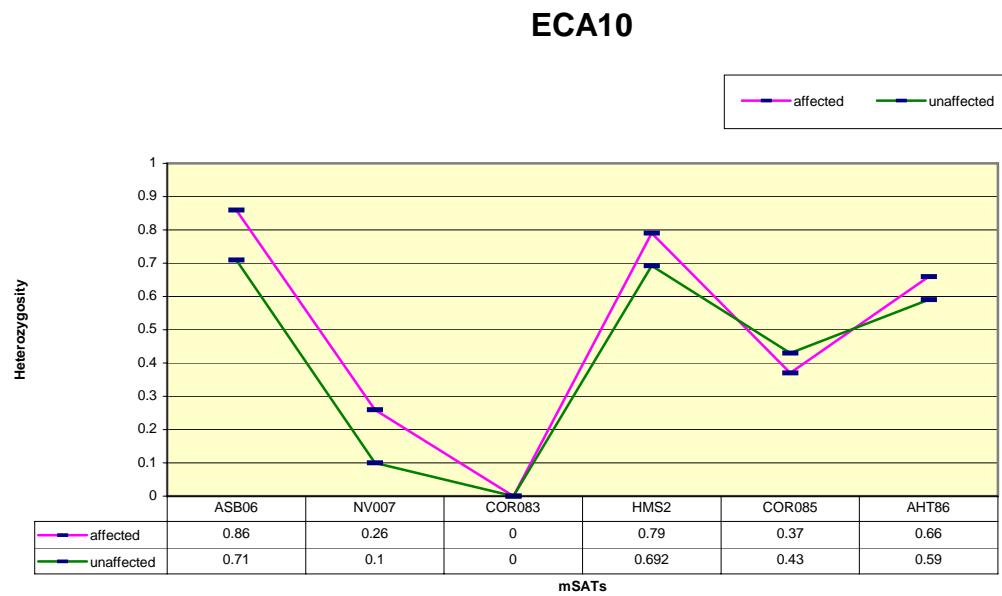
Figure 3.10 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 10. No heterozygosity differences are statistically significant for ECA10 as determined by Chi-Square test for heterozygosity.

ECA11

mSATs	UCDEQ439	SGCV013	ASB35	TKY033/32	NV090	TKY010	TKY648	HLM2
affected	0.54	0.59	0.03	0.5	0.281	0.342	0.438	0
unaffected	0.45	0.72	0.36	0.893	0.285	0.344	0.56	0.034
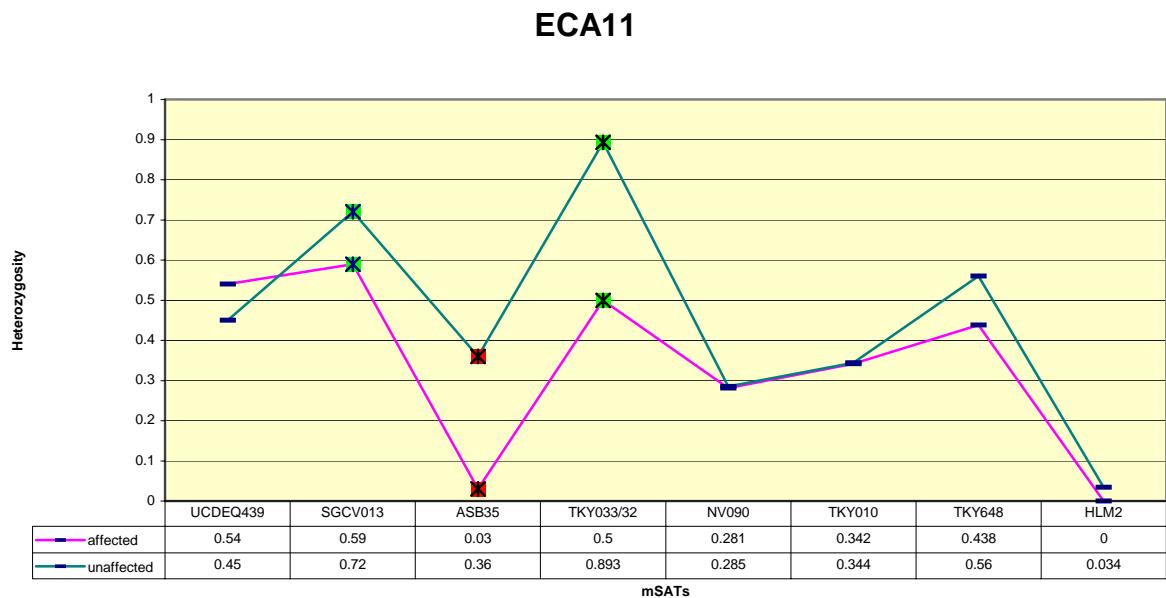
Figure 3.11 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 11. *The Chi-Square value was 9.164 for ASB35 (p< 0.01).   The microsatellites on either side follow the pattern expected for linkage. TKY033/32 did show significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 22.34 (significant p<0.001) and SGCV013 had common allele homozygosity of 14.54 (significant p<0.001). A possible candidate gene, COL1A1 has been RH mapped to the q arm of ECA11 (Chowdhary et al., 2003), with a location 13 cR from SGCV13 (telomeric) (Chowdhary et al., 2003). More will follow in discussion.

ECA12
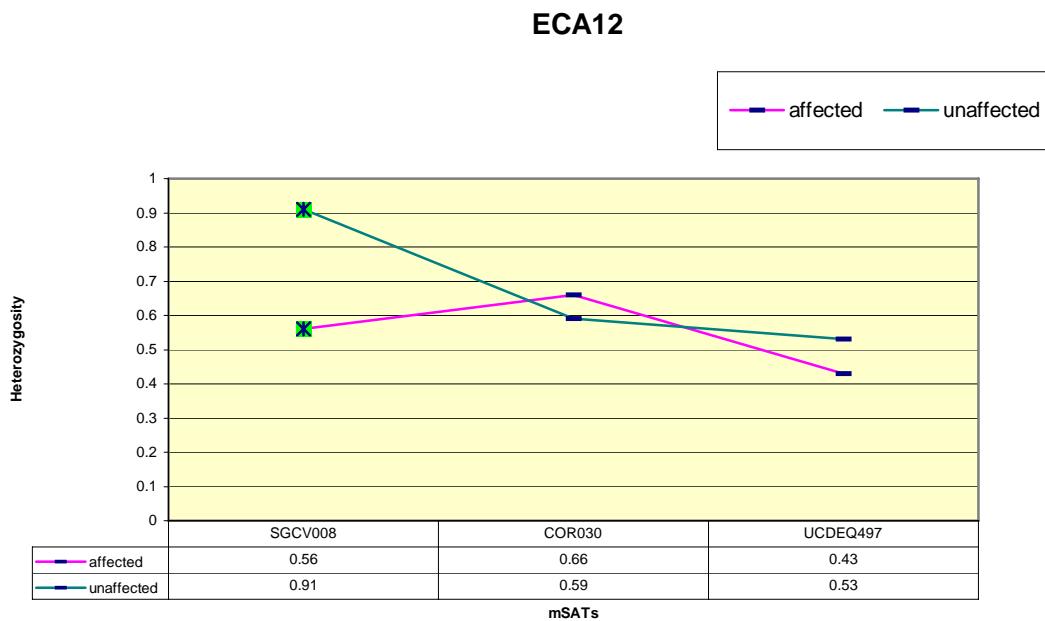
Figure 3.12 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 12. *SGCV008 did show significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square score of 20.90 (significant p<0.001). Data for other microsatellites on the chromosome do not provide additional support for these findings, however more microsatellites may need to be run on the telomeric side of SGCV008 to eliminate all possibilities.

ECA13

	UM030	AHT030
Affected	0.6	0.4
Unaffected	0.6	0.214

mSATs
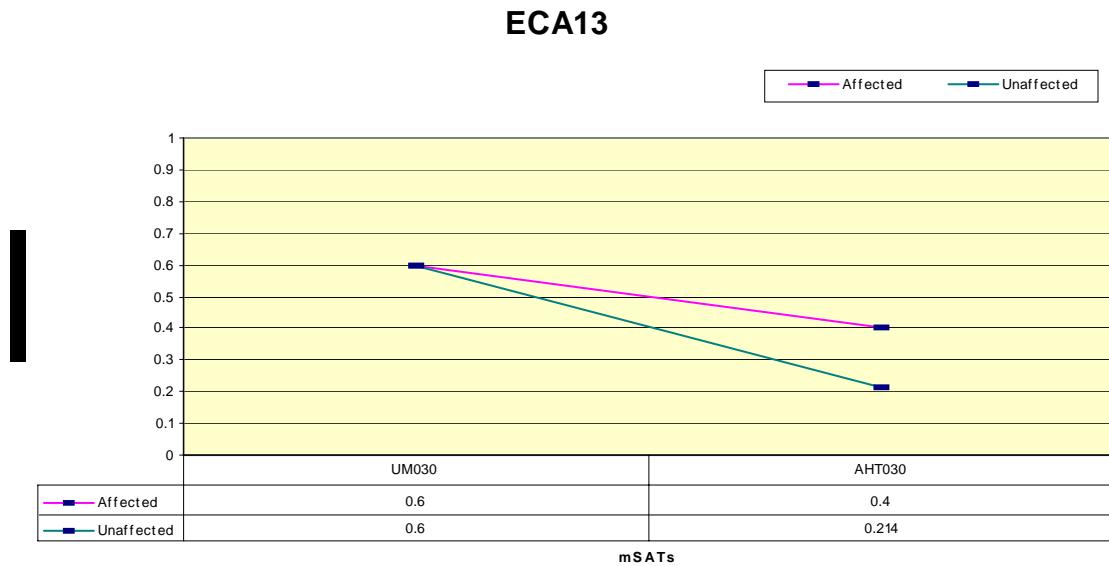
Figure 3.13 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 13. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.

ECA14

	SGCV008	COR030	UCDEQ497	LEX043	LEX047	TKY310	AHT83	AHT88	HTG018	LEX078	TKY491	TKY749	TKY438	COR002	TKY636
affected	0.56	0.66	0.43	0.71	0.29	1	0.47	0.51	0.29	0.6	0.54	0.645	0.618	0.35	0.882
unaffected	0.91	0.59	0.53	0.92	0.26	0.89	0.78	0.5	0.25	0.79	0.65	0.52	0.516	0.75	0.852

mSATS
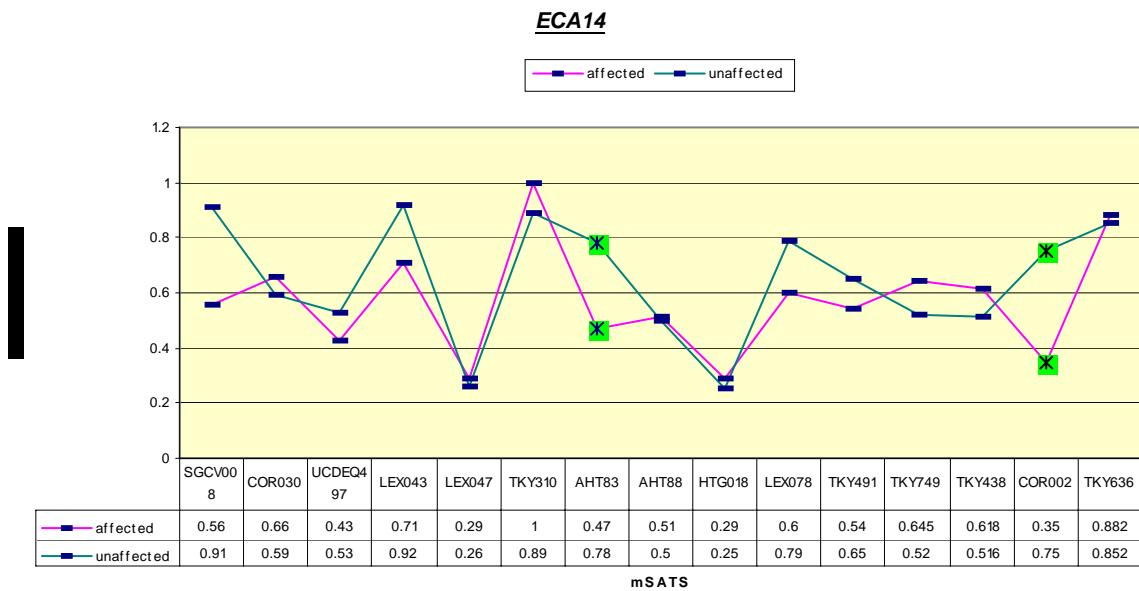
Figure 3.14 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 14. *AHT83 and *COR002 did show significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 4.52 (p<0.05) and 10.344 (p<0.01) respectively. At least 4 candidate genes exist on ECA14; however further testing does not support any of them. This is discussed in further detail in the discussion section.

ECA15

mSATs	LEX046	ASB02	HTG006	AHT002	COR014
affected	0.79	0.87	0.44	0.711	0.68
unaffected	0.9	0.893	0.607	0.654	0.62
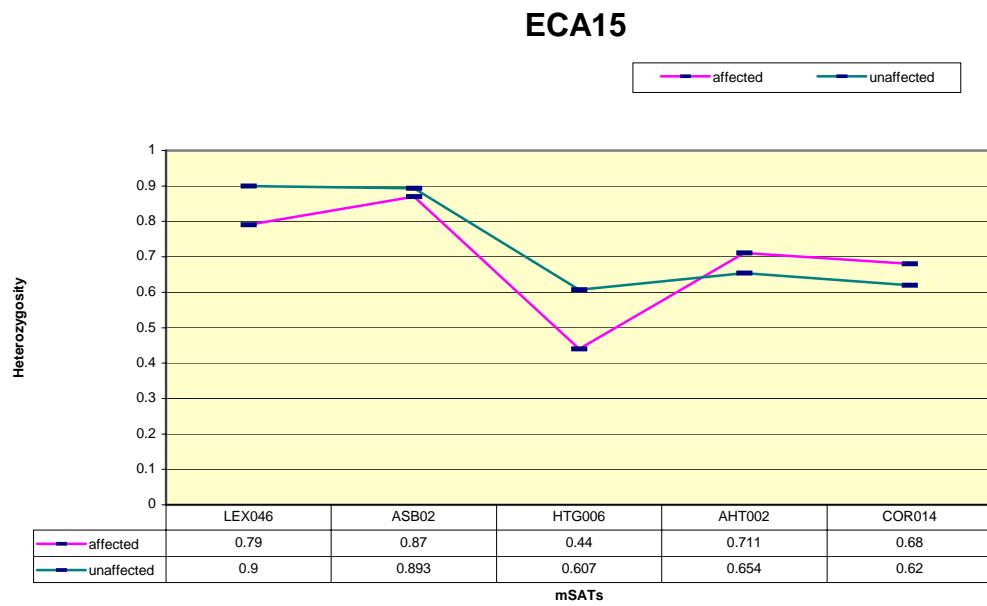
Figure 3.15 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 15. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.

ECA16

	AHT037	TKY279	HTG3	HMS20	COR039	AHT014	LEX018	AHT60	AHT91
affected	0.34	0.81	0.76	0.77	0	0	0.79	0.94	0.37
unaffected	0.42	0.77	0.5	1	0	0	0.75	0.8	0.35

mSATs
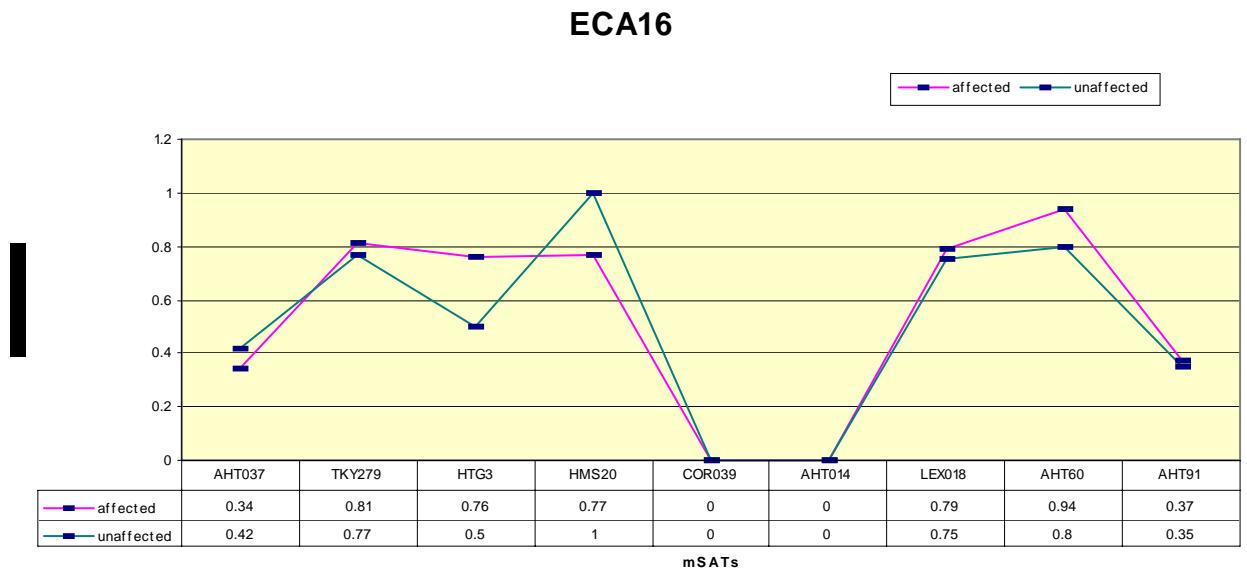
Figure 3.16 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 16. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.

ECA17

mSATs	COR007	LEX055	COR032	LEX076
Affected	0.78	0.21	0.42	0.16
Unaffected	0.78	0.18	0.32	0.29
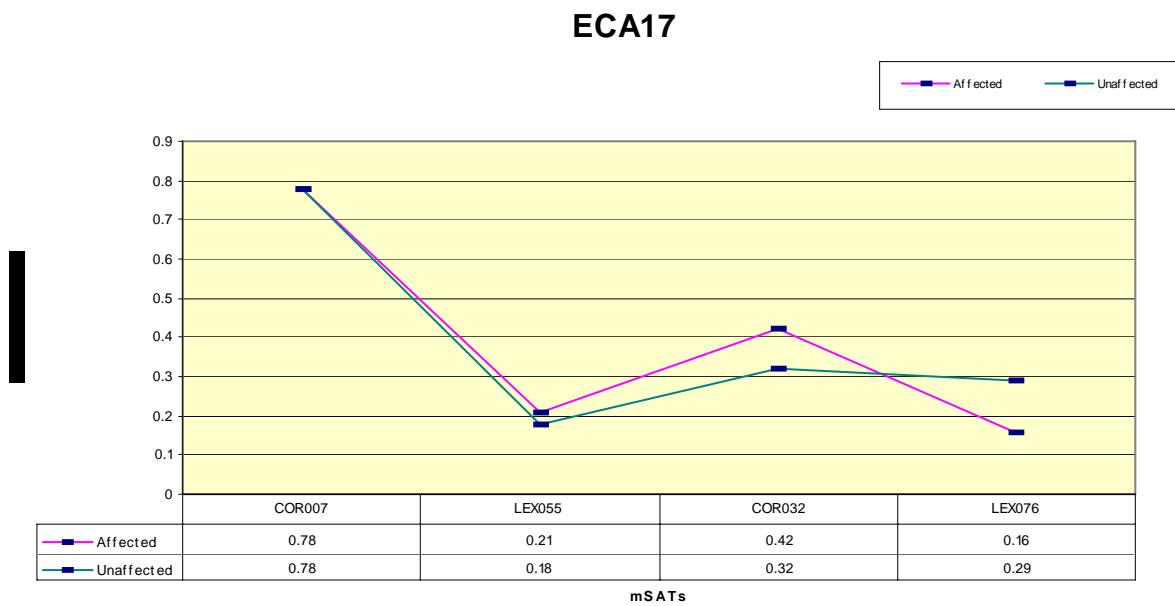
Figure 3.17 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 17. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text. LEX055 had a Chi-Square of 14.596 however, the unaffected group did not fall within Hardy Weinberg Equilibrium and therefore may not be informative.

ECA18

mSATs	LEX054	UCD136	UMNEQ50	HMS46	TKY909	SGCV07	TKY692	HTG28	COR096	HTG17	UCD387	COR101	TKY017	HLM3
Affected	0.71	0.771	0.54	0.273	0.69	0.47	0.69	0.283	0.632	0.5	0.61	0	0.632	0.571
Unaffected	0.31	0.813	0.42	0.71	0.65	0.448	0.77	0.143	0.71	0.645	0.75	0	0.679	0.613
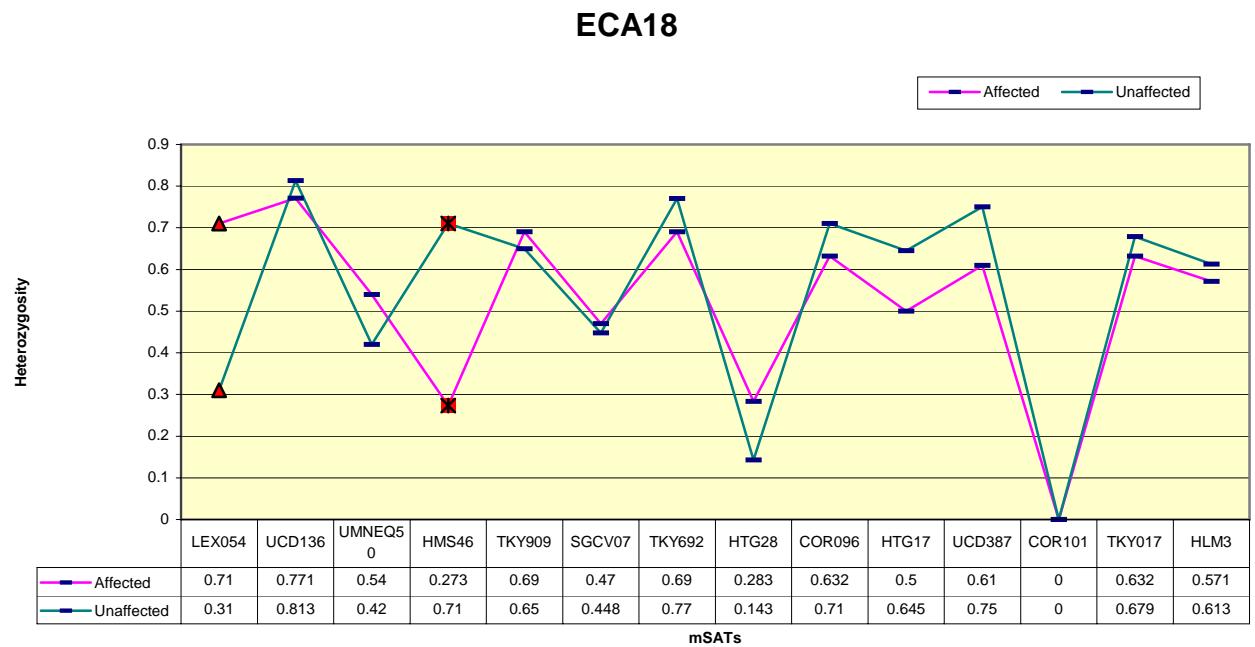
Figure 3.18a Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 18. *The Chi-Square value was 5.370 for HMS46 (p< 0.05). Despite the significant Chi-square, the pattern of variation across all ECA18 loci does not give evidence for an association of any marker locus with DSLD. The newly published locations are shown in ECA18b and reinforce the statement above.
^ Pattern inverts so unaffected total heterozygosity was less than affected.

ECA 18 alternative order
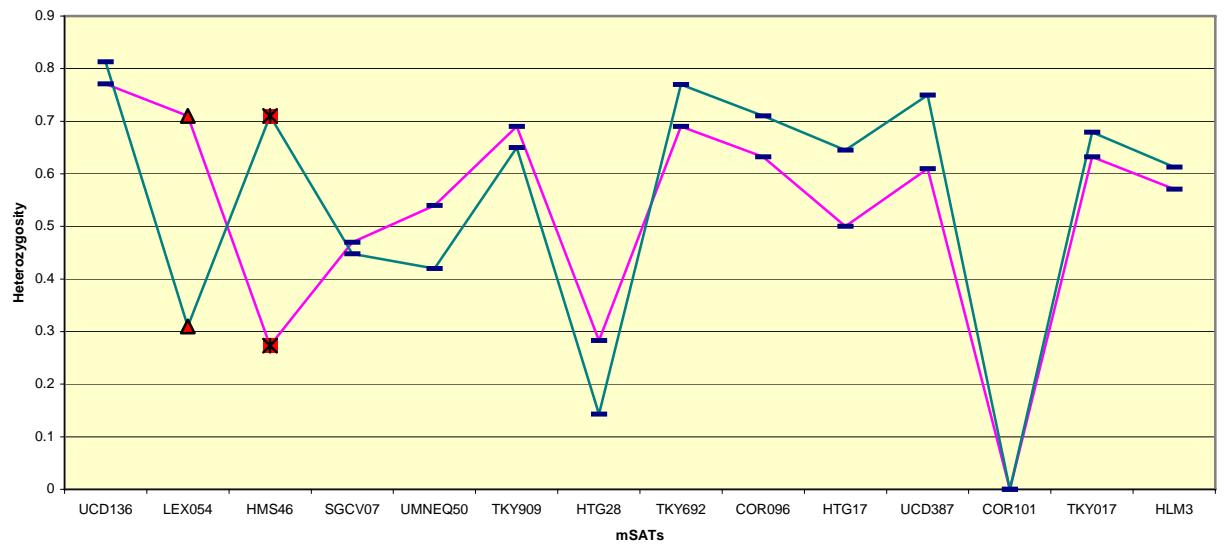
Figure 3.18b Alternative microsatellite order for equine chromosome 18, according to newly published data (Penedo et al., 2005).

ECA19

	Affected	Unaffected
	HTG23	LEX036
Affected	0.62	0.63
Unaffected	0.55	0.59

mSATs
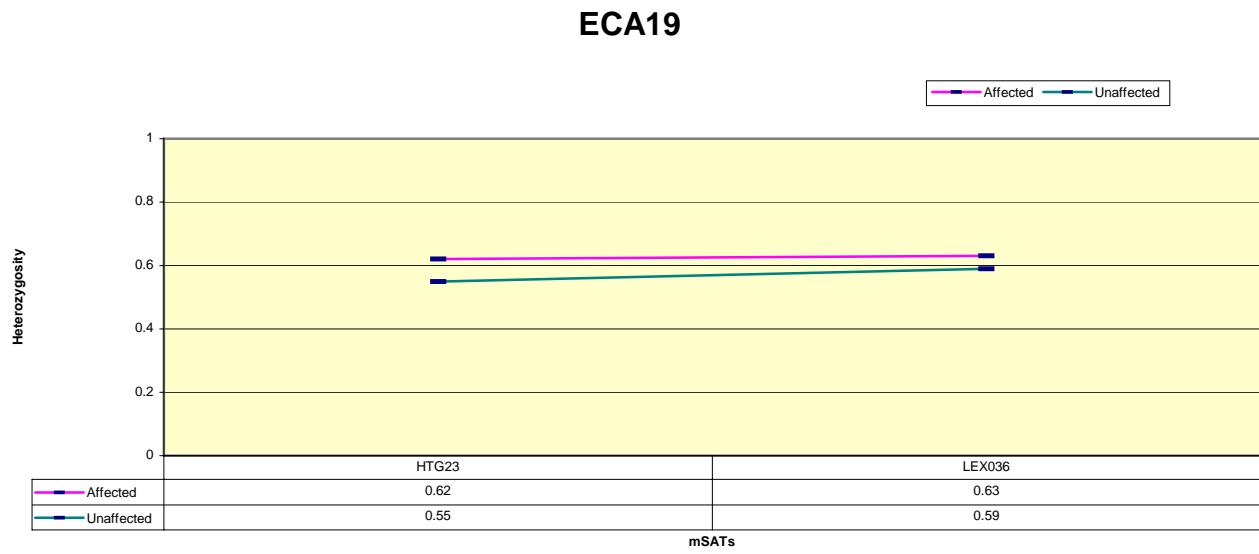
Figure 3.19   Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 19. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.

ECA20

	HTG005	LEX071	HMS42
Affected	0.71	0.76	0.656
Unaffected	0.66	0.83	0.566

mSATs
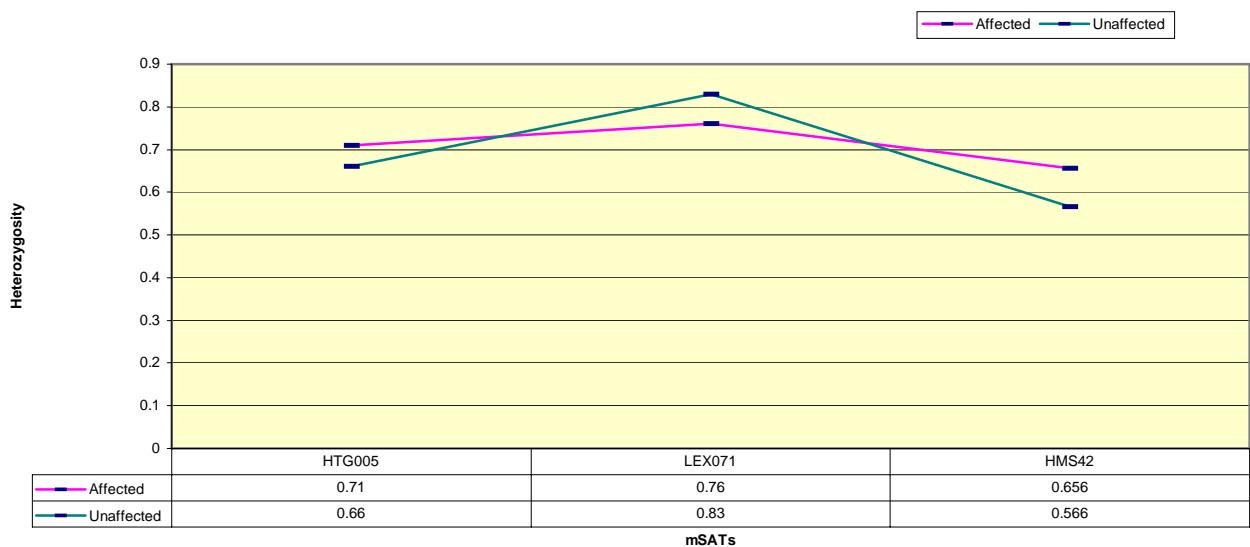
Figure 3.20 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 20. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.

ECA21

	SGCV014	SGCV016	TKY021	HTG010	COR068	HTG32
Affected	0.421	0.5	0.816	0.87	0.68	0.37
Unaffected	0.393	0.667	0.75	0.741	0.6	0.48

mSATs
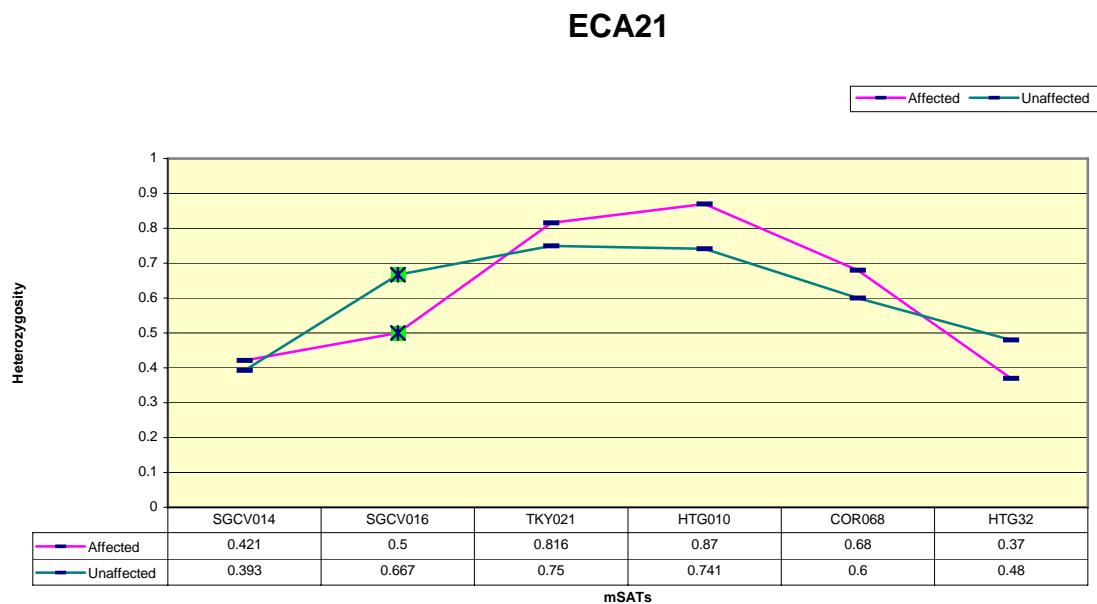
Figure 3.21 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 21. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text. *SGCV016 did show significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 6.8 (p<0.010) however the locus is highly polymorphic and lacks support by surrounding microsatellites (which are less than 5cM from SGCV016). This is not supportive of linkage.

ECA22
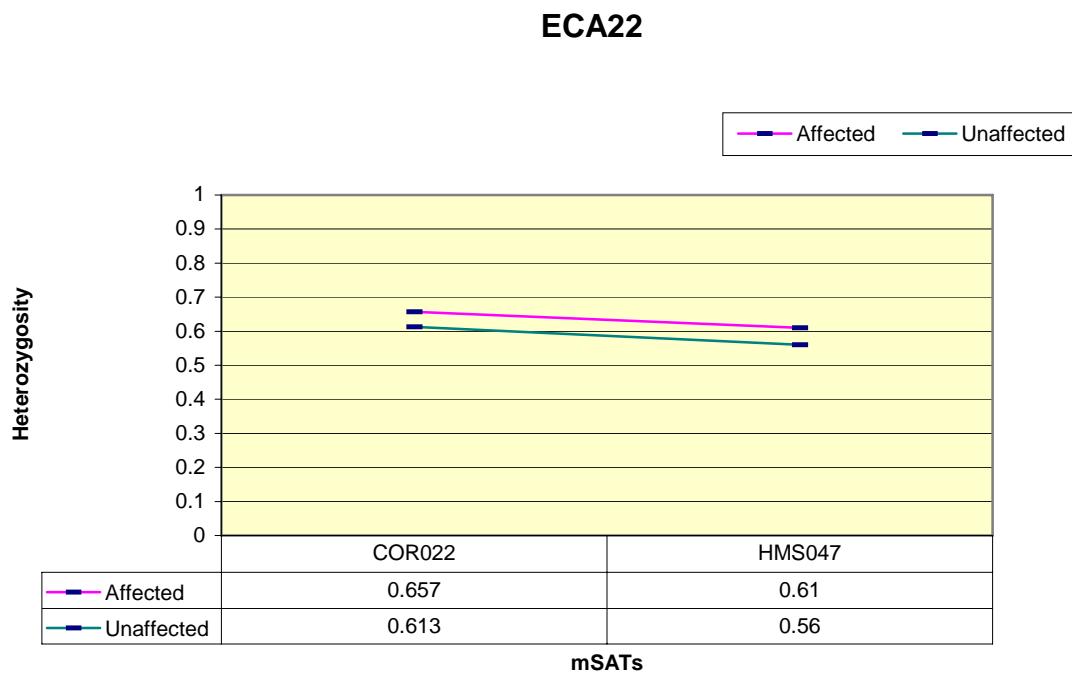
Figure 3.22   Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 22.   No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.
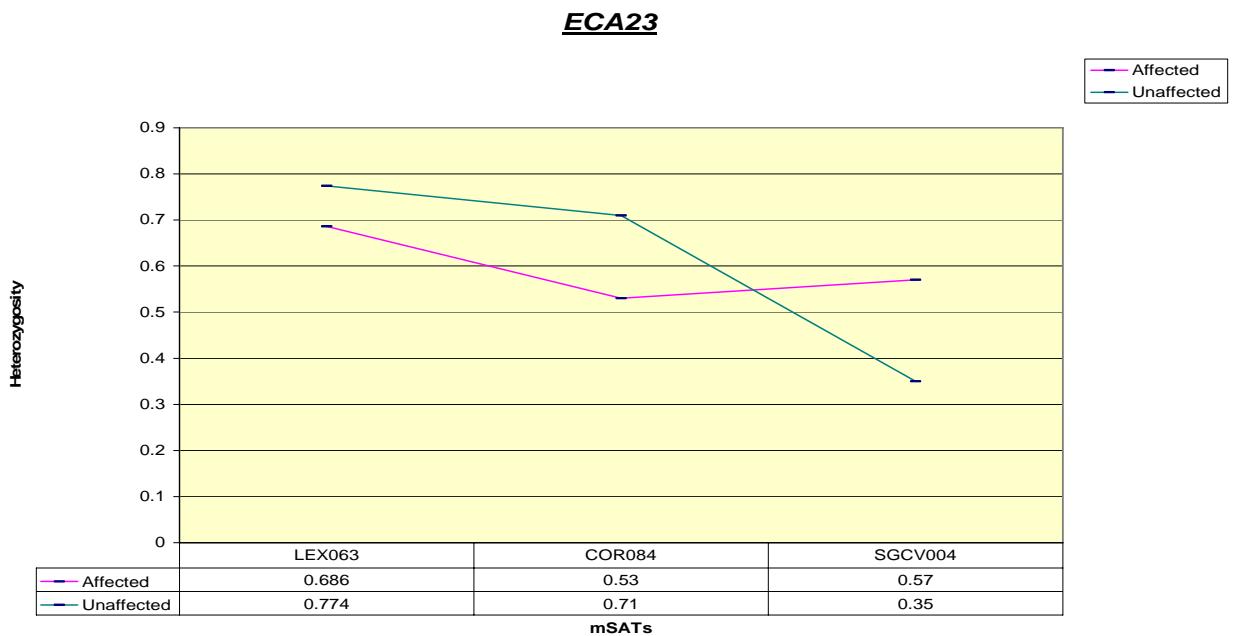
Figure 3.23 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 23. No heterozygosity differences (with lower heterozygosity in the affected) are statistically significant for ECA23 as determined by Chi-Square test for heterozygosity.

ECA24

	LEX042	AHT032	AHT004
Affected	0.67	0.71	0.79
Unaffected	0.72	0.74	0.645

mSATs
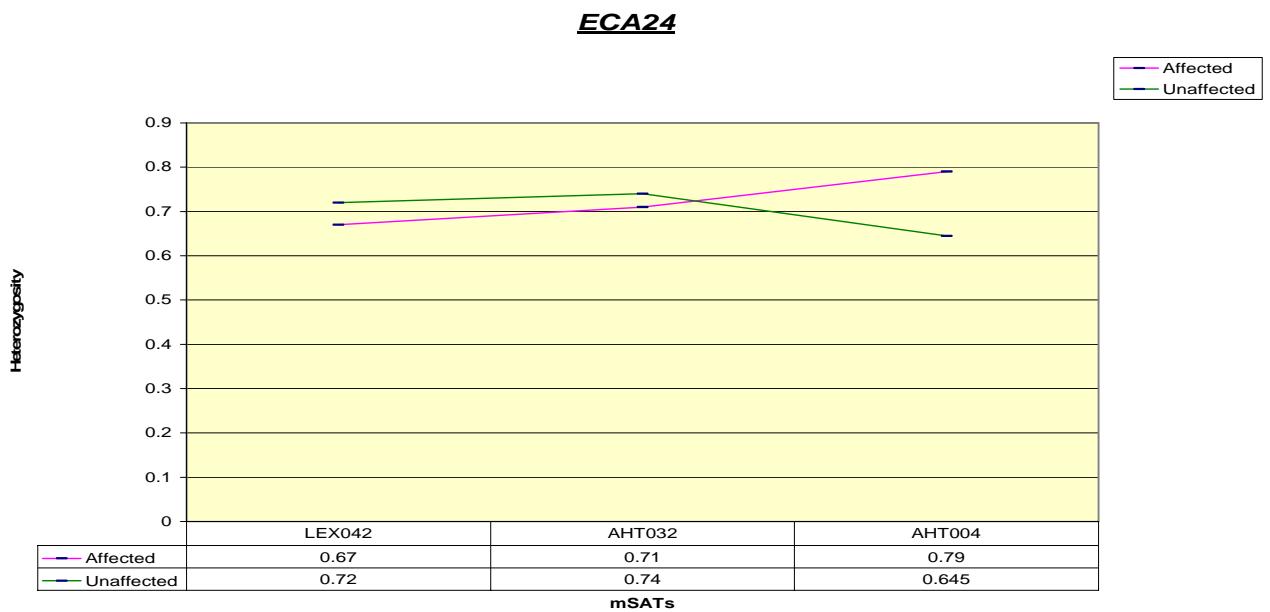
Figure 3.24 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 24. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text. LEX042 has a common allele Chi-Square value of 4.24 (p<0.05).

ECA25

| mSATs	AHT051	NVHEQ43	AHT007	COR018	COR080
Affected	0.791	0.89	0.667	0.68	0.057
Unaffected	0.607	1	0.581	0.643	0.088
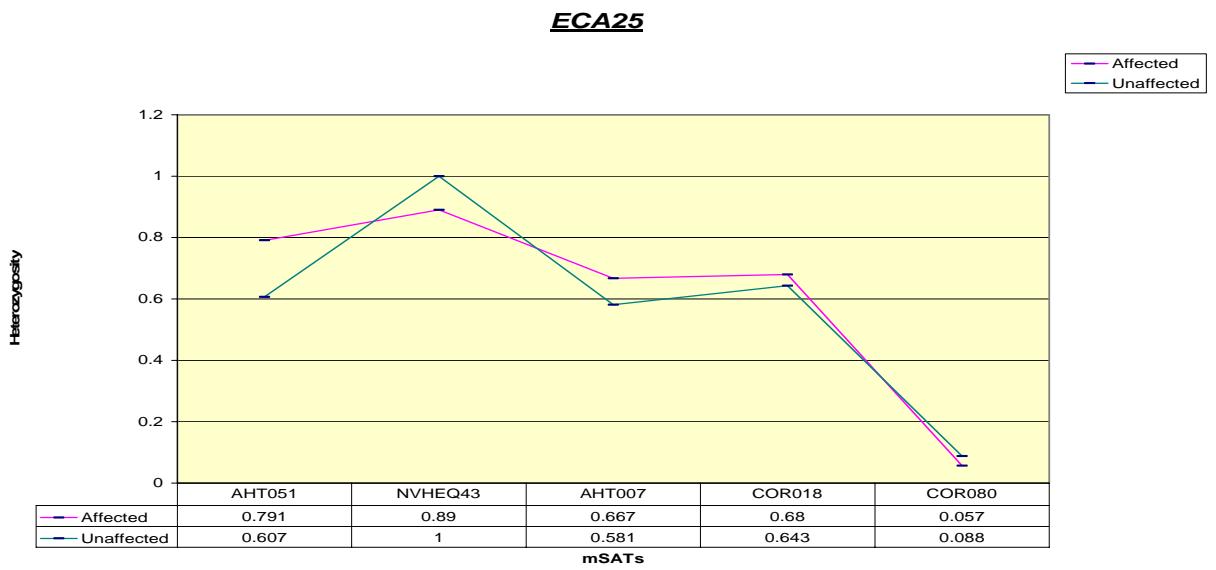
Figure 3.25 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 25. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.

ECA26
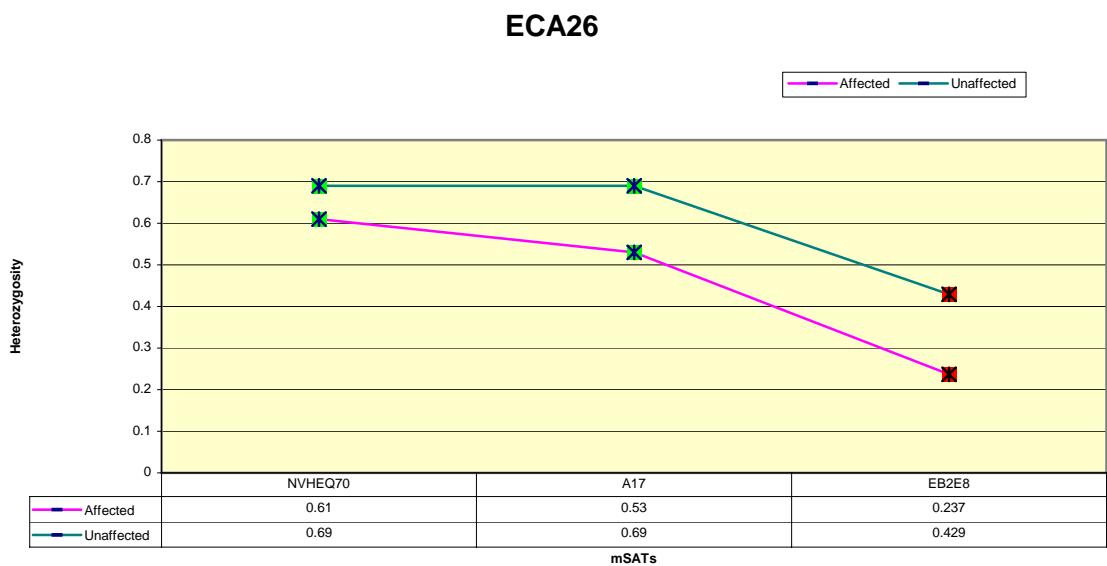
Figure 3.26 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 26. *The Chi-Square value was 5.918 for EB2E8 (p<0.05). *NVHEQ70 and A17 had common allele Chi-Square values of 6.36 and 4.45 (p<0.05) respectively. These three microsatellites fit the expected trend for the recessive gene model, however, a p-value of <0.001 would be expected in EB2E8 and possibly an increasing, yet still significant p-value the further the distance away. There is one candidate gene; COL6A1, based on the human genome (Tanaka et al. 2003), but its location is questionable. More in discussion.

NOTE: No Figure for ECA 27. Only one point is available and the point is the same in both populations. Not significant.

ECA28

	NV54	HTG030	TKY018	TKY319	TKY515	UCD425
Affected	0.711	0.162	0.842	0.777	0.5	0.694
Unaffected	0.32	0.393	0.893	0.742	0.8	0.636
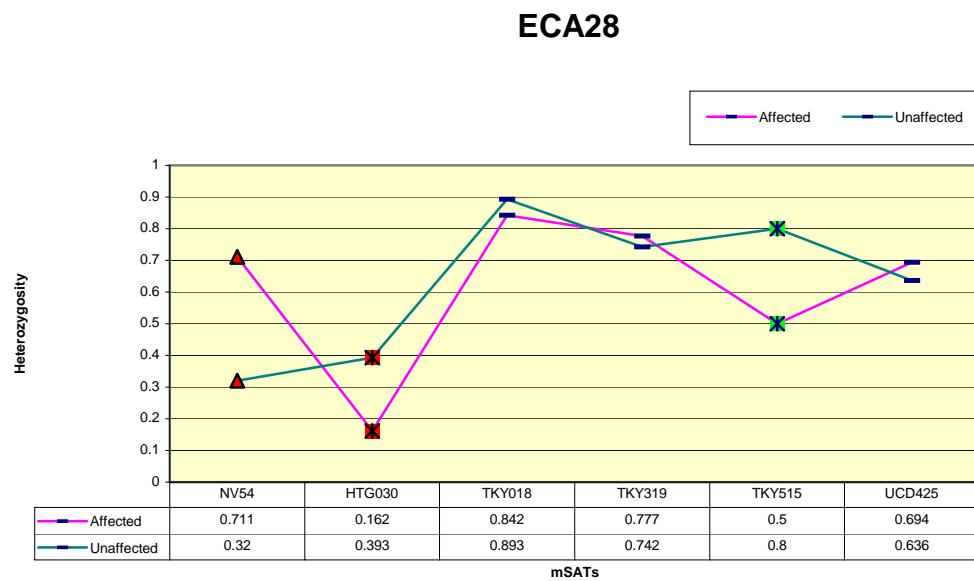
Figure 3.27 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 28. *A Chi-square value of 7.398 was reported for HTG030 (p<0.01). HTG030 is located just over 3cM from ^NV54 (Guérin et al., 2003) which is even more significant in the opposite direction (unaffected are more statistically significant homozygous). A microsatellite with such close proximity to HTG030 should show the same trend. This is not seen and HTG030 does not suggest linkage to DSLD. * TKY515 had a common allele Chi-Square value of 13.469 (significant p<0.001). The pattern of variation across all ECA28 loci does not give evidence for an association of any marker locus with DSLD, however, they are both over 20 cM from TKY515. More testing in the area may be advised now that more microsatellites in the area have become available.
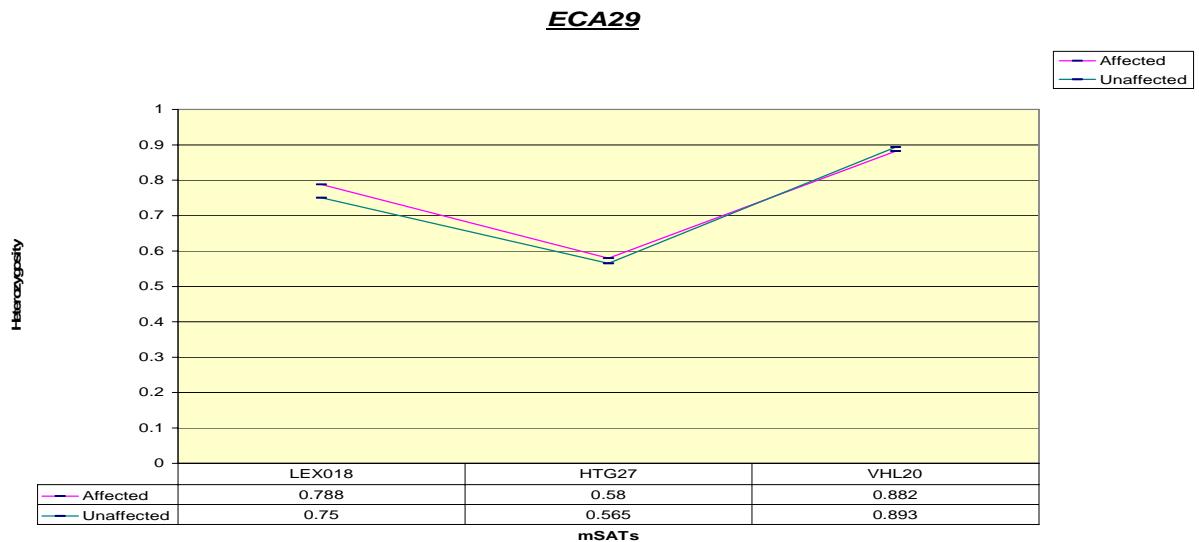
Figure 3.28 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 29. No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.
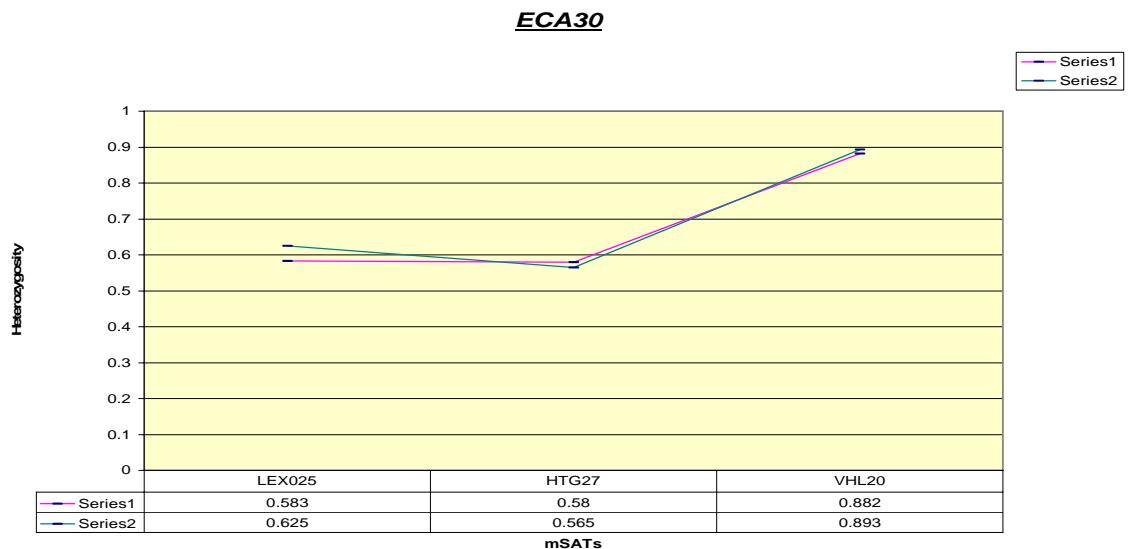
Figure 3.29   Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 30.   No statistically significant difference in total heterozygosity of the two groups was observed based upon Chi- Square test as described in the text.
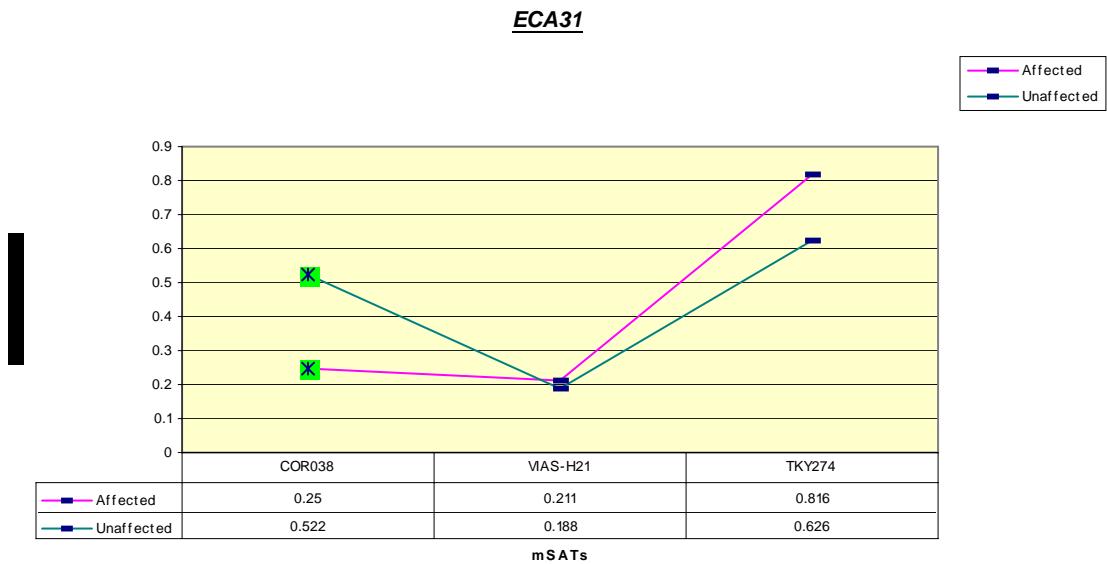
Figure 3.30 Total heterozygosity of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 31. No total heterozygosity differences are considered statistically significant for ECA31as determined by Chi-Square test for heterozygosity. COR038 did show significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 3.88 (p<0.05). COR038 is only 4.6 cM from VIAS-H21 (Guérin et al., 2003), which does not show any significant differences between the two groups.

Discussion

It is important to note that the absence of a candidate gene in a region does not indicate that one does not exist there. It simply means that there is no gene of known function that might be related to DSLD that has been mapped to that region. It is also important to note that in this study the statistical significance of heterozygosity differences for a particular locus was never dismissed based upon the lack of a candidate gene in the area.

Of the 260 microsatellites run representing all 31 autosomal chromosomes, 6 were found to have statistically significant (p<0.05) higher homozygosity in affected horses compared to unaffected horses as determined by Chi-Square analysis. There were 17 microsatellites that had statistically significant (p<0.05) differences in homozygosity of the common allele by Chi-Square. These 23 significant microsatellites were dispersed over 16 chromosomes: ECA 1, 2, 3, 6, 7, 9, 11, 12, 14, 18, 21, 22, 24, 26, 28, and 31. Of these 16 chromosomes ECA 2, 3, 18, 21, 24, and 31 lacked supporting evidence of linkage due to no neighboring microsatellites with excess homozygosity in the affected group and no clear candidate genes in the region based on the human genome. The remaining chromosomes will be discussed individually. The remaining 15 chromosomes did not have any statistically significant Chi-Square results and will be excluded from further discussion and those include: ECA 4, 5, 8, 10, 13, 15, 16, 17, 19, 20, 23, 25, 27, 29, and 30.

ECA1

ECA1 had two microsatellites COR059 AND TKY007 with a statistically significant excess of homozygosity in the affected group and showed significant differences between the affected and unaffected groups for homozygosity of the most common allele (of

the affected group) with a Chi-Square value of 8.22 (p=0.01) and 4.43 (p=0.05) respectively. Linkage mapping locates UCDEQ487 lays between COR059 and TKY007 (Guérin et al., 2003). UCDEQ487 did not have a significant Chi-Square for heterozygosity or common allele Chi-Square value. The three microsatellites fall within a span of 5 cM, a small enough distance that signs of linkage should be seen for UCDEQ487. Microsatellite COR100 linkage mapped less than 5 cM distal to the centromere from COR059 (Guérin et al., 2003), this microsatellite also lacks any homozygosity with statistical significance. Having two non-significant microsatellites mapped within 5 cM from COR059 and TKY007 makes linkage to a DSLD highly unlikely for this region based upon the recessive gene expectations.

<u>ECA6</u>

TKY284 located on the q arm of ECA 6 had a significant common allele Chi-Square value of 17.13 (p<0.001). TKY412 is located 8.1 cM centromeric to TKY284 (Guérin et al., 2003) and was not statistically significant. LEM Domain Containing 3, (LEMD3) known as (MAN1) in the horse is a candidate gene in this region based on the human map. LEMD3 (MAN1) has been RH mapped to this region of ECA6 (Chowdhary et al., 2003), and is responsible for Osteopoikilosis, (Buschke-Ollendorff syndrome) in humans (Lin et al., 2000) which is an autosomal dominant disorder (Melnick, 1959). It had been known to cause abnormalities in collagen and elastin fibers in the connective tissue of the skin. (Uitto et al., 1981, and Morrison et al., 1997). Very close to LEMD3 is Decorin (DCN), also known as Proteoglycan II. It is a distinct small proteoglycan found in connective tissue and has been associated with a lethal form of Marfan syndrome (Pulkkinen et al., 1990). Further investigation of this region of ECA6 may be warranted, possibly looking for SNP's in the

LEMD3 gene since it has been sequenced in the horse (Chowdhary et al., 2003). Since TKY412 did not have evidence of linkage it is unlikely that there is a DSLD gene in the region. However, TKY284 is near the end of the p arm and to rule out the possibility of a gene telomeric to the locus, it would be useful to type TKY952, which has been mapped between TKY284 and the end of the chromosome (Penedo et al., 2005) to rule out ECA6 entirely.

ECA7

The Chi-Square value for heterozygosity was 13.88 (p<0.001) for microsatellite TKY35 on ECA7. The distance from LEX015 (a statistically non-significant microsatellite) to TKY35 (in cM) is unknown. According to the RH (1.0) map they are 37.1 cR from each other (Chowdhary et al., 2003). The Chi-Square value suggests the possibility of the DSLD gene being telomeric to TKY35. ECA7 corresponds to the area of human chromosome HSA11 that contains Aortic Aneurysm, familial thoratic 1 (FAA1) (Chowdhary et al., 2003). FAA1 a locus that has been linked to HSA 11q23.2-24 in one family (Vaughan et al., 2001). Because FAA1 is associated with connective tissue disease of the aorta in humans and DSLD has been known to cause connective tissue lesions in hearts of some horses (Halper et al. 2005), it could be considered a candidate gene. While comparative mapping suggests TKY35 may be near FAA1 (Chowdhary et al., 2003), lack of agreement between the RH, Linkage and FISH maps make it difficult to decipher whether further testing is warranted at this time. It should be noted that FAA1 is a phenotype based locus and not an identified gene. Vaughan et al., (2001) screened genes in the area of the locus for mutations but none were found. FAA1 is merely a syndrome that has been mapped to a region on human chromosome eleven.

ECA9

Multiple microsatellites had heterozygosity differences that were statistically significant on ECA9.   The Chi-Square value was 4.064 for UM037 (p< 0.05). Despite the significant value, the results are not consistent with expectations of a marker linked to DSLD, as the locus is highly polymorphic in both affected and unaffected groups. One common allele is not shared by all or even most of affected individuals in UM037. When actual linkage disequilibrium is seen, most if not all of the homozygous alleles will be the same.   LEX070 and HTG008 did show significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 25.71 (significant p<0.001) and 4.48 (p<0.05) respectively. LEX070 and especially HTG008 were also highly polymorphic, not what is expected when looking for linkage disequilibrium. All three microsatellites map to the same region of ECA9 with no reported distance between them (Guérin et al., 2003). Overall this is not suggestive of linkage to a nearby gene. There were no clear-cut candidate genes located on ECA9 based upon comparison with the human genome (Chowdhary et al., 2003).

ECA11

ECA11 had three microsatellites with statistically significant Chi-Square values. The Chi-Square value was 9.164 for ASB35 (p< 0.01). The microsatellites on either side follow the pattern expected for linkage. TKY033/32 showed significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 22.34 (significant p<0.001) and SGCV013 had a Chi-Square of 14.54 (significant p<0.001). A potential candidate gene in this region, Collagen I,

alpha-1 polypeptide (COL1A1) has been mapped to ECA11q (Chowhardy et al., 2003), and RH mapped 13 cR telomeric to SGCV013 (Chowdhary et al., 2003). COL1A1 is responsible for the collagen of skin, tendon, and bone (Nuytinck et al., 2000). Abnormalities in COL1A1 cause Ehlers-Danlos syndrome, Gravis type (EDS I) (Nuytinck et al., 2000), and Arthrochalasia type (EDS VIIA) (Eyre et al., 1985), both of which are autosomal dominant connective tissue disorders seen in humans. Mutations in COL1A1 also cause Caffey syndrome or infantile cortical hyperostosis (Gensure et al., 2005) which has some clinical features similar to Ehlers-Danlos syndrome, but is also characterized by subperiosteal bone formation and localized inflammatory response (Halper et al., 2005). Sequencing portions of the COL1A1 gene would be the next step in investigating this region of the chromosome as there are no other microsatellites in the region that would be closer to the candidate gene.

Figure 3.31 Equine chromosome 11 RH map (Chowdhary et al., 2003) taken from Horse Genome Project website.
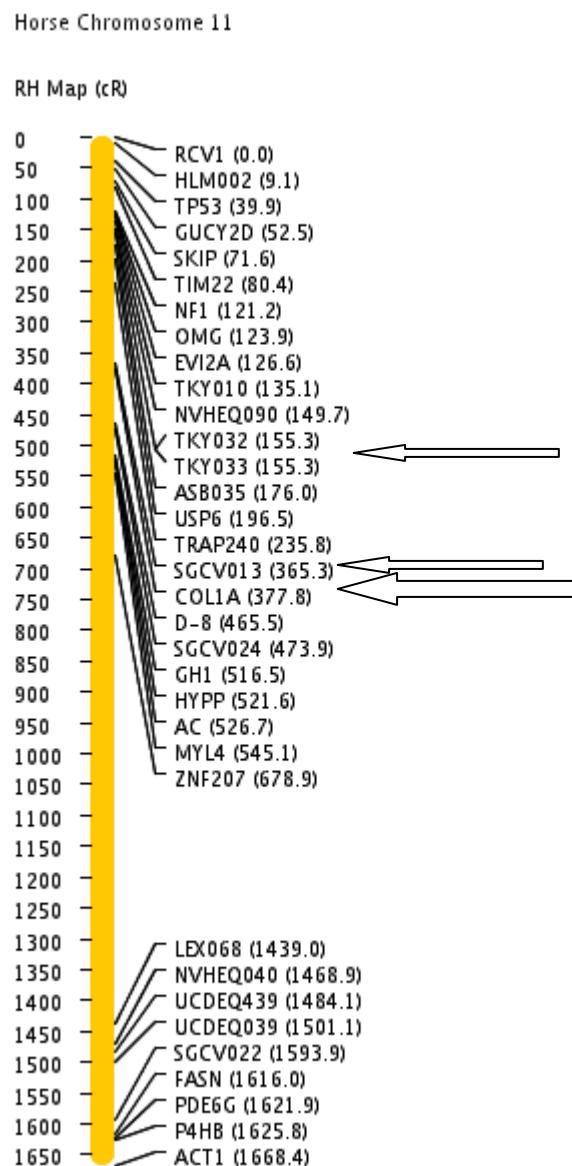
ECA12

On ECA12, SGCV008 had one microsatellite that had a statistically significant common allele difference Chi-Square value of 20.90 (p<0.001). SGCV008 has been RH mapped to 13.4 cM from SGCV010 (Chowdhary et al., 2003), which is only 15% homozygous and thus not suggestive of linkage. SGCV008 is less than 10 cM from COR030, which is not significant either. There are no clear candidate genes in the region.

ECA14

ECA14 had two microsatellites with statistically significant Chi-Square values: AHT83 and COR002 showed significant differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 4.52 (p<0.05) and 10.344 (p<0.01) respectively. Chromosome 14 was of particular interest because of the high number of candidate genes mapped to it. Pathology examinations of the tendons from DSLD affected horses showed deposits of what is believed to be proteoglycans obliterating what should be collagen tissue (Halper et al., 2005). The accumulation of proteoglycans would lead to disruption of normal connective tissue structure (Halper et al., 2005). This information is the basis for considering Chondroitin Sulfate Proteoglycan 2 (CSPG2) as a serious candidate gene for DSLD. CSPG2 is located at ECA14q26-q27, approximately 22.5 cM from AHT83 (Chowdhary et al., 2003). Although it is near maximum, at 22.5cM linkage disequilibrium would be seen (Khatkar et al., 2004), CSPG2 is a protein-coding gene related to Proteoglycan. Large chondroitin sulfate proteoglycans were first identified in hyaline cartilage, where they specifically interact with hyaluronan and form large supramolecular complexes. Together with other matrix

glycoproteins, they provide mechanical support and a fixed negative charge. Such molecules exist also in a variety of soft tissues where they may play additional physiologic roles (Kjellen and Lindahl, 1991). Proteoglycan is found in connective tissues, in fibrous, articular, and elastic cartilages, in the central and peripheral nervous system, in the epidermis, and in all three wall- layers of veins and elastic arteries (Bode-Lesniewska et al., 1996). Both AHT83 and COR002 had higher homozygosity in affected individuals, as compared to unaffected. AHT83 was 53% homozygous in affected individuals and 22% homozygous in unaffected individuals. COR002 was 65% homozygous in affected individuals and 25%homozygous in unaffected individuals. Because AHT83 has not been linkage mapped, the distance between AHT83 and COR002 in cM is unable to be determined. However, it can be determined that they are less than 62.9 cM from each other based on linkage mapping from COR002 to the closest mapped microsatellite to AHT83 on the side farthest from COR002 (Penedo et al., 2005) as compared to RH map (Chowdhary et al., 2003), which shows the two as being 96.2 cR apart. The microsatellites mapped between AHT83 and COR002 (AHT88, HTG018, LEX078, TKY491, TKY749, and TKY438) tested on both affected and unaffected groups did not have statistically significant differences in total heterozygosity based on Chi-Square test nor did they have significant differences between the affected and unaffected groups for homozygosity of the most common allele (of affected group). It is not likely that both AHT83 and COR002 are linked to DSLD since microsatellites between them do not support the pattern of variation that would be seen with linkage to a gene.

Other candidate genes have been mapped to ECA14 or are expected to be there based on the human chromosome 5 map. A Disintegrin-like and Metalloproteinase With Thrombospondin Type 1 Motif, 2, (ADAMTS2), is mapped to HSA 5q23, and causes the

connective tissue disorder Ehlers-Danlos syndrome, dermatosparaxis type, (EDS VIIC). ADAMTS2 has not yet been mapped to the horse, but based upon comparative mapping should be in the region of ECA14. Cartilage Link Protein 1, (CRTL1) is mapped to ECA14q27. CRTL1 stabilizes aggregates of aggrecan and hyaluronan, giving cartilage its tensile strength and elasticity (Watanabe, and Yamada, 1999). Because of its close proximity to COR002, is was considered as a candidate gene. Other candidate genes on ECA14 include Xylosylprotein 4-Beta-Galactosyltransferase, Polypeptide 7, (4GALT7) the gene responsible for Ehlers-Danlos syndrome, progeroid form, and A Disintegrin-like and Metalloproteinase with Thrombospondin Type 1 Motif, 6, (ADAMTS6), a gene partly responsible for tissue architecture. Despite the number of genes related to connective tissue and proteoglycans expected to be on ECA14q, and the two microsatellites with homozygosity excess in the affected group, the bulk of evidence from the loci tested does not support the presence of the gene for DSLD on this chromosome.

ECA26

All three microsatellites from ECA26 tested on affected and unaffected groups had statistically significant differences in heterozygosity. The Chi-Square value was 5.918 for EB2E8 ($p < 0.05$). NVHEQ70 and A17 had common allele Chi-Square values of 6.36 and 4.45 ($p < 0.05$) respectively. COL6A1 is a possible candidate gene in this region, it maps to human chromosome 21q22-q23 (Tanaka et al. 2003). Comparative mapping of human chromosome 21 ties this genomic region to ECA26. COL6A1 is associated with Ossification of the Posterior Longitudinal Spinal Ligaments, (OPLL) in humans. Twy ('tiptoe' walking Yoshimura) mice are a naturally occurring mutant of OPLL (Yamazaki et al., 1991). Okawa et al.,(1998), observed that heterotopic ossification occurs not only in the spinal ligaments

but also in the joint capsules, tendon enthesis, chondral tissues, and peripheral ligaments. The human COL6A1 is positioned centromeric to Crystalline, Alpha-A, (CRYAA) and V-ETS Avian Erythroblastosis Virus E26 Oncogene Homolog 2, (ETS2), both of which have been RH mapped to ECA26 (Milenkovic et al., 2002), in the region where EB2E8 is. This suggests COL6A1 is a possible candidate gene, and may warrant further investigation.

ECA28

ECA28 had two microsatellites with statistically significant heterozygosities. A Chi-square value of 7.398 was calculated for HTG030 heterozygosities (p<0.01). HTG030 is located just over 3 cM from NV54, in which the unaffected were more homozygous than the affected which is opposite from what would be expected based upon a recessive model. A microsatellite that maps with such close proximity to HTG030 should be following the expected trend if it is near a marker for DSLD. This is not seen, thus HTG030 does not appear to be a likely marker for linkage disequilibrium. TKY515 had a common allele Chi-Square value of 13.469 (significant p<0.001). The pattern of variation across all ECA28 loci does not give evidence for an association of any marker locus with DSLD. However, they are both over 20 cM from TKY515. More testing in this area may be advised now that microsatellites, which map closer to TKY515 such as TKY872, are available. There are no clear-cut candidate genes in this region based upon comparison with the human genome (Chowdhary et al., 2003).

ECA31

No total heterozygosity differences are considered statistically significant for ECA31as determined by Chi-Square test for heterozygosity. COR038 did show significant

differences between the affected and unaffected groups for homozygosity of the most common allele (of the affected group) with a Chi-Square value of 3.88 (p<0.05). COR038 is only 4.6 cM from VIAS-H21 (Guérin et al., 2003), which does not show any significant differences between the two groups. This is not supportive of linkage to a marker for DSLD.

Chapter Four

Sequencing Chondroitin Sulfate Proteoglycan 2 (CSPG2)

Results of pathological examination of the tendons of DSLD affected horses in a study led by Jaroslava Halper, MD, PhD Associate Professor in the Department of Pathology of the Collage of Veterinary Medicine University of Georgia, showed accumulated deposits of what she believed to be proteoglycans obliterating what should be collagen tissue (Halper et al., 2005). Dr. Halper states that the accumulation of proteoglycans would lead to disruption of normal connective tissue structure. In her paper, Halper discusses various human disease models for DSLD and mutations in the genes that cause disease in humans. She states "As far as we know none of theses human hereditary disorders is known to be caused by a defect in proteoglycan biochemistry or genetics, thus making it different from ESPA [her proposed new title for DSLD]." This information is the basis for Chondroitin Sulfate Proteoglycan 2, (CSPG2) being considered as a serious candidate gene for DSLD.

CSPG2 was RH mapped ECA14q26-q27, and 22.5 cR from AHT83 (Chowdhary et al., 2003). The marker AHT083 had a homozygous common allele seen more often in the affected group than the unaffected with a Chi-Square value of 4.52 (p<0.05). Although it is not linkage mapped and distance in cM is not known, it is very likely that linkage disequilibrium should be seen (Khatkar et al., 2004). CSPG2 is a protein-coding gene. Proteoglycan is found in connective tissues, in fibrous, articular, and elastic cartilages, in the central and peripheral nervous system, in the epidermis, and in all three wall- layers of veins and elastic arteries (Bode-Lesniewska et al., 1996).

<u>Materials and Methods</u>

In order to find additional markers to test whether CSPG2 or a closely linked gene could be associated with DSLD, primer sequences were chosen based on the human CSPG2 gene sequence which in its entirety is over 124,400 bp in length, since the candidate gene has not yet been sequenced in the horse. The primers were designed using Oligo Primer Analysis Software version 5.0 (Wojciech and Piotr Rychlik 1989-1995). A primer, which amplified a region beginning near the end of Exon 10 and the beginning of Exon 11 in the G3 domain of the CSPG2 gene, was chosen.

DNA from affected and unaffected horses were run using the Expand Long Template PCR System (Roche Diagnostics GmbH, Mannheim, Germany) since the piece being amplified was approximately 2500 bp (based on human sequence (Soma et al., 2005) and known size of human intron). The sequencing reaction contained the primer at a final concentration of 5pMol. PCR protocol per reaction consisted of 29μl Sigma water, 7μl dNTP's, 2μl forward primer, 2μl reverse primer, 5μ of the #2 buffer (10X concentration with 27.5 mM MgCl2), 0.75 μl of 15uM MgCl2, and 0.75 μl Expand Long Template Enzyme mix. The reaction mixture was added to 2μl DNA and run in a MJ thermocycler PTC-200 (MJ research, Watertown, MA)

The Expand program protocol was run in a MJ thermocycler PTC-200 (MJ research, Watertown, MA) which consisted of: step1 (initial denaturation) =93 °C for 7 minutes, step2 (denaturation) =93 °C for 10 seconds, step3 (annealing) = 62 °C for 30 seconds, step 4 (elongation) 68 °C for 3.5 minutes, step5= go to step2 nine times, step6 (denaturation) = 93 °C for 10 seconds, step 7 (annealing) = 62 °C for 30 seconds, step 8 (elongation) 68 °C for 3 minutes plus 20 seconds per cycle elongation for each successive cycle, step 9 = go to step 6 19 times, step 10 (final elongation) = 68 °C for 7 minutes, step 11 (cooling) = 4 °C forever.

The sequencing reaction mixture consisted of 7 µl Sigma water, 2 µl of primer, 8µl Big Dye reaction mixture (polymerase, dNTPs, fluorescent ddNTP's and buffer) and 4 µl of PCR reaction mixture that had undergone treatment with ExoSAP-IT (USB Corporation, Cleveland, OH). The sequencing reactions were done in an MJ thermocycler PTC-200 (MJ Research, Watertown, MA) for 25 cycles with the following cycling parameters, 98 °C for 15 seconds, 50 °C for 5 seconds, 60 °C for 4 minutes. One-second ramping times between steps was used. The sequencing reactions were purified (excess DyeDeoxy terminators removed) using Centricep Columns (Princeton Separations, Inc, Adelphia, NJ).

Sequences were aligned and analyzed using AlignX a component of Vector NTI Advance 9.1.0 (Invitrogen Corp. 2004)

Results of Sequencing

A section of DNA was sequenced which originated at the distal end of exon 10 of CSPG2 and extended for approximately 600 base pairs into the intron between exon 10 and exon 11, the reverse of the same sequencing amplification started in exon 11 of CSPG2 approximately 600 bp and into the same intron. The forward and the reverse did not overlap At 157bp (consensus sequence) a single nucleotide polymorphism (SNP) was discovered. The sequence with the SNP is in an intron and is as follows;

AAAGAGGGTCTTGCTTTTCCAGAAG[A/G]TATAGAGGGGGGCAGTTTATGTTGT

At 362 bp a deletion was discovered within an intron. The results of typing for both the SNP and the deletion is reported for seven affected and six unaffected horses where (-) is delete (Table 4.1).

Account number	157 SNP	362 T DELETE
Affected		
A3-001	A	-/-
A3-002	A	+/-
A3-003	A	+/-
A3-004	A	+/-
A3-005	G/A	+/+
A3-006	A	+/+
A3-008	A	+/-
Unaffected		
03-001	A	+/+
03-002	G/A	+/+
03-004	A	+/-
03-005	A	+/-
03-0028	A	-/-
03-032	A	+/-

Table 4.1.   The results of both the SNP and the deletion is reported on seven affected and 6 unaffected horses where (-) is delete.

Discussion:

The results of typing for 157 SNP was not supportive of linkage to CSPG2 as the A to G/A was seen in one of seven affected and one of six unaffected individuals. The frequency of the SNP was the same for both groups. The 362 T deletion was homozygous for the deletion in one of each group, heterozygous for the deletion in four of the affected and three of the unaffected and was not present in two affected and two unaffected. The frequency is the same for both affected and unaffected groups with the presence or absence of the deletion not coinciding with either group. This is not supportive of linkage of the CSPG2 gene to DSLD. If either the SNP or the T deletion was supportive of linkage to CSPG2 one would expect to see the A to G in all affected animals and the T deletion either homozygous in all affected animals or not present in all affected animals. This is not the case as they occur equally in each group. Since both the SNP and the deletion marker occur in the DNA sequence and are thus closer to the suspected region on the mutation in CSPG2 than any microsatellite marker tested in the area, they would have to show stronger evidence of linkage than a microsatellite marker if linkage were present (unless the SNP or T delete is newer than the DSLD gene mutation which is not likely). Given the sequencing results, CSPG2 is not likely the gene responsible for DSLD.

Chapter Five

Dominant or Other Mode of Inheritance

Introduction

After completing the genome scan and sequencing, new DSLD samples were submitted to the project that consisted of family groups. Complete pedigrees including known occurrence of DSLD in each generation were included in the submission. Upon the examination of these pedigrees the mode of inheritance became less clear, and the decision was made to analyze existing data as if DSLD had a dominant or co-dominant mode of inheritance.

In addition to the reason above, many of the human-based disease models for DSLD, including Marfans syndrome and most forms of Ehlers-Danlos Syndrome, have a dominant mode of inheritance. The information derived from statistical analysis based on a dominant mode of inheritance would ultimately benefit recessive mode results by enforcing statistical data derived from that analysis.

Materials and methods

The materials and methods are the same as chapter three, the only thing that is different is the way the data was statistically analyzed.

Statistical analysis

In a dominant mode of inheritance, since only one copy of the diseased gene is required for the phenotype, it is expected that all affected individuals would share at least one allele in common when the marker (microsatellite) being tested is near the disease gene.

It is for this reason, statistical analysis of the data compared the common allele for each microsatellite between the affected and unaffected groups to see if the common allele occurred more frequently in the affected group than the unaffected group than would be expected by chance. A students' t-Test was used to calculate the significance of the frequency of occurrence of the most common allele in the two groups. For each microsatellite tested on both affected and unaffected groups the common allele was chosen. The common allele was designated as the allele with the highest frequency in the affected group since this is the group that should have all individuals with at least one copy of the DSLD gene. For each microsatellite, each horse was assigned either 0,1 or 2, depending on how many times the common allele occurred in the horse. From this point the normal t-Test was performed using windows based SAS v9.

<u>Results:</u>

Table 5.1 is a summary of t-Test analysis for each microsatellite listed in order by chromosome along with distances in cM (when available) between each, and common allele frequency in affected and unaffected groups.

Note: Distances above top microsatellite and below bottom microsatellite are distances to the last mapped microsatellite on that chromosome. It this number is lacking, the last microsatellite was included in this study. * distance unknown and unable to estimate. ~ approximate distance given known mapped distances.

Table 5.1 Summary of Student t-Test analysis of common allele for each microsatellite listed in order by chromosome along with distances in cM between each.

Table 5.1

	mSAT	distance cM	affected com.freq.	unaffected com.freq.	t-test
CHROM 1		45			
	VIASH034		0.2429		
		17.3			
	ASB041		0.4189		
		8.6			
	LEX020		0.2368		
		35.6			
	NVHE100		0.2361		
		~25			
	COR100		0.3438		
		<5			
	COR059		0.7059	0.4828	p<0.01
		0			
	UCDE487		0.3158	0.3269	p<0.8
		~4.6			
	TKY007		0.8281	0.6452	p<0.01
		~39.4			
	AHT021		0.3553		
		58.3			
	LEX058		0.4444	0.3966	p<0.6
		9.8			
	ASB008		0.4605		
		10.1			
	1CA043		0.5132	0.4844	p<0.7
		2.2			
	TKY002		0.3889	0.3125	p<0.2
		7.1			
	1CA025		0.6184	0.45	p<0.04
		<7.6			
	UCD493		0.2222	0.0161	p<0.2
		<7.6			
	UM043		0.6711	0.7656	p<0.3
		<7.6			
	TKY106		0.4737	0.2656	p<0.004
		<7.6			
	UM004		0.3919	0.4783	
		<7.6			
	HTG12		0.8919	0.8281	p<0.2
		12.6			
	UCD440		0.4355	0.4828	p<0.6
		8.8			
	HMS015		0.4474	0.4038	p<0.4
		19.3			
	COR063		1	1	

Table 5.1	continued				
	mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM2		0			
	COR065		0.5135	0.1613	p<0.000008
		~0			
	ASB18		0.2763	0.3958	p<0.2
		34.8			
	COR037		0.3714	0.3448	p<0.7
		58.1			
	TKY024		1	0.9844	p<0.3
		<9.8			
	ASB13		0.3784		p<0.9
		<9.8			
	ASB17		0.3553		
		5.7			
	HMS51		0.4118	0.4167	p<0.9
		8.6			
	UCD380		0.5972	0.4167	p<0.04
		<26.3			
	COR049		0.9868	0.9821	p<0.8
		<26.3			
	A-14		0.4189		
		30.1			
	TKY335		0.3571	0.3704	p<0.8
		13.6			
	TKY497		0.4412	0.3833	p<0.5
		2.9			
	TKY798		0.6	0.625	p<0.7
		16.7			
	TKY842		0.3594	0.3448	p<0.8
		*			
	COR094		0.973	0.931	p<0.9
		*			
	UMNe76		0.5	0.339	p<0.06
		*			
	COR035		1	1	
		*			
	COR026		0.7917	0.6429	p<0.07
		*			
	COR043		0.7703	0.8	p<0.6
		*			

Table 5.1   **continued**

	mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM 3					
	AHT036		0.171	0.167	p<0.03
		18.3			
	COR028		0.2895	0.2813	p<0.9
		0.6			
	COR033		0.3421		
		9.8			
	AHT022		0.7714	0.8103	p<0.6
		18			
	UCD437		0.3421	0.1481	p<0.01
		35.2			
	ASB23				
		*			
	SG33		0.5132	0.5577	p<0.5
		*			
	AHT097		0.6143	0.4219	p<0.02
CHROM 4					
	AHT043		0.3553		
		~20			
	HMS06		0.5588	0.2903	p<0.001
		56.9			
	COR089		0.3947		
		2.5			
	ASB022		0.6447		
		<8.2			
	LEX033		0.3421	0.569	p<0.008
		<8.2			
	HTG007		0.5789	0.6481	p<0.3
		42.2			
	HTG022		0.5132	0.4194	
		9.1			
	SGCV023		0.3485		

Table 5.1	continued				
	mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM 5					
	AHT024		0.5263		
		32.2			
	LEX004		1	1	
		<39.5	*		
	VHL066		0.6447	0.625	p<0.5
		27.5	*		
	HMS005		0.5789	0.3571	p<0.009
		6.2			
	LEX069		0.4242		
		14.7			
	LEX034		0.3289		
		39.2			
CHROM 6					
	HTG31		0.2714		
		<30.6			
	COR010		0.4737		
		4.7			
	NV82		0.3784	0.2031	p<0.02
		11.1			
	LEX065		0.2857	0.2581	p<0.7
		12			
	UM015		0.3824	0.4138	p<0.7
		12			
	TKY111		0.8714	0.871	p<0.9
		*			
	NV081		0.9868	1	p<0.3
		15.9			
	COR070		0.2838	0.2885	p<0.3
		9.5			
	UCD465		0.7895	0.6833	p<0.1
		21.2			
	TKY412		0.4429	0.4	p<0.6
		7.1			
	TKY284		0.6184	0.3387	p<0.0009

Table 5.1   **continued**

mSAT	distance cM	Affected com.freq.	unaffected com.freq.	
CHROM 7				
VIASH7		0.9444	0.9792	p<0.4
	*			
COR095		0.3289		
	*			
COR004		0.5769		
	*			
TKY272		0.0526	0.0536	p<0.4
	*			
TKY005		0.7237	0.8036	p<0.2
	12.6			
AHT019		0.5132	0.4219	p<0.3
	18.5			
SGCV028		0.6571	0.7031	p<0.5
	*			
TKY283	33.2	0.7432	0.7857	p<0.5
	*			
LEX015		0.8158	0.75	p<0.3
	*			
TKY35		0.9079	0.6964	p<0.0002
	*			
TKY34		0.3553		
	*			
CHROM 8				
AHT005		0.3676	0.2742	p<0.2
	8.8			
AHT025		0.5526	0.6667	p<0.2
	49.8			
UM034		0.5	0.5469	p<0.5
	12.3			
LEX023		0.2237		
	<9.4			
ASB14		0.3784	0.3281	p<0.5
	<19.3			
SGCV32		0.5143	0.3871	p<0.1
	5.6			
COR003		0.3816		
	41.5			
COR056		0.1892	0.1563	p<0.6
	16.5			

Table 5.1	continued				
	mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM 9					
	HTG004		0.4559	0.3103	p<0.1
		34.5			
	HMS003		0.3553	0.3036	p<0.5
		5.2			
	COR008		0.3553		
		<11			
	TKY627		0.2833	0.5556	p<0.009
		<11			
	TKY533		0.3676	0.4107	p<0.6
		<9.7			
	COR013		0.5286	0.65	p<0.2
		<9.7			
	HTG008		0.3788	0.2143	p<0.06
		0			
	UM037		0.3857	0.3833	p<0.9
		~0			
	LEX070		0.3684	0.2258	p<0.09
	AHT53	69.1	0.6912	0.569	p<0.1
	LEX019		0.75	0.7037	p<0.5
CHROM 10		14.3			
	COR020		0.3108		
		<20.9			
	COR048		0.2692		
		12			
	NV018		0.3143		
		5.8			
	ASB06		0.5714	0.6042	p<0.5
		12.8			
	NV007		0.7714	0.95	p<0.7
		<48			
	COR083	48	1	1	
		<48			
	HMS2		0.395	0.16	p<0.003
		3.2			
	ASB9		0.4394		
		*			
	COR085		0.629	0.6786	p<0.2
	AHT86	*	0.3824	0.2778	p<0.3

Table 5.1	continued mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM 11		9			
	UCDEQ439		0.6029	0.569	p<0.7
		17.9			
	LEX068		0.2647		
		14.9			
	SGCV024		0.2656		
		11.5			
	SGCV013		0.5968	0.3393	p<0.001
		~.65			
	ASB35		0.9286	0.8214	p<0.9
		*			
	TKY033/32		0.4143	0.2969	p<0.3
		*			
	NV090		0.8529	0.8103	p<0.4
		*			
	TKY010		0.7838	0.8281	p<0.5
		*			
	TKY648		0.6129	0.48	p<0.1
		22.4			
	HLM2		1	0.9828	p<0.3
CHROM 12		14.3			
	SGCV010		0.303		
		13.9			
	SGCV008		0.4844	0.2857	p<0.06
		<19			
	COR030		0.4483	0.3889	p<0.5
		<19			
	COR058		0.1974		
		23.4			
	UCDEQ497		0.6857	0.6667	p<0.8
CHROM 13					
	COR069		0.3026		
		19.9			
	UM030		0.5143	0.3226	p<0.05
		3.9			
	ASB037		0.5658		
		<3.8			
	AHT030		0.7763	0.8571	p<0.2
		<3.8			
	VHL047		0.5		
		46.8			
	ASB001		0.3784		
		30			

Table 5.1   **continued**

	mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM 14					
	LEX043		0.4868	0.3654	p<0.1
		63			
	UM010		0.4054		
		6			
	VHL209		0.3784		
		2.4			
	LEX047		0.7895	0.8704	p<0.2
		14.5			
	TKY310		0.2895	0.1667	p<0.06
		<30.9			
	HTG29		0.3529		
		<30.9			
	AHT83		0.6944	0.537	p<0.08
		<30.9			
	AHT88		0.5	0.5	1
		<30.9			
	HTG018		0.8571	0.875	p<0.7
		24.2			
	LEX078		0.5714	0.5536	p<0.8
		0			
	TKY491		0.4857	0.4355	p<0.5
		6			
	TKY749		0.6129	0.54	p<0.3
		6.2			
	TKY438		0.6029	0.4844	p<0.1
		1.1			
	COR002		0.8194	0.5833	p<0.002
		5.2			
	TKY636		0.3429	0.2857	p<0.3
		8.9			
CHROM 15		51.4			
	B-8		0.5789		
		24.1			
	LEX046		0.4412	0.4355	p<0.9
		22.2			
	ASB02		0.2237	0.2656	p<0.5
		15.2			
	AHT016		0.3289		
		9.4			
	HTG006		0.7105	0.6607	p<0.5
		12.5			
	AHT002		0.4605	0.3462	p<0.2
		11.6			
	COR014		0.5946	0.569	p<0.7
		13.2			

Table 5.1	continued				
	mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM 16					
	AHT037		0.7571	0.7571	p<0.7
		<14.7			
	TKY279		0.3378	0.3378	p<0.4
		*			
	HTG3		0.5263	0.5536	p<0.7
		26.7			
	HMS20		0.3286	0.3286	p<0.3
		0			
	AHT038		0.3056		
		16			
	LAM15.2		0.2895		
		<53.1			
	COR039		1	1	0
		<53.1			
	AHT014		1	1	0
		<3.3			
	LEX018		0.4091	0.3571	p<0.5
		<3.3			
	LEX056		0.2632		
		37.8			
	I-18		0.3421		
		*			
	AHT60		0.2	0.2	p<0.3
		*			
	AHT91		0.8	0.8065	p<0.9
CHROM 17					
	COR007		0.3649	0.1739	p<0.1
		<32.2			
	LEX055		0.3947	0.1304	p<0.01
		*			
	NVHEQ79		0.5		
		<32.3			
	COR032		0.7368	0.7857	p<0.5
		20			
	HMS025		0.5	0.6333	p<0.1
		12.3			
	LEX076		0.8684	0.8704	p<0.6
		~50			

Table 5.1	mSAT	Distance: cM	affected com.freq.	unaffected com.freq.	
CHROM 18					
	LEX054		0.5132	0.8438	p<0.000006
		23.9			
	TKY19		0.5833		
		4.4			
	UCD136		0.4571	0.4219	p<0.6
		<25			
	UMNEQ50		0.3714	0.3226	p<0.5
		<25			
	HMS46		0.8571	0.6452	p<0.0008
		<2			
	TKY909		0.5714	0.5645	p<0.9
		1.6			
	SGCV07		0.7206	0.7069	p<0.8
		<10			
	TKY692		0.3333	0.45	p<0.1
		8.6			
	HTG28		0.6447	0.8438	p<0.03
		14.41			
	COR096		0.421	0.4583	p<0.6
		13.6			
	HTG17		0.3684	0.3548	p<0.8
		15.8			
	UCD387		0.421	0.429	p<0.9
		50.1			
COR101			1	1	
		<14			
	TKY017	14	0.4868	0.5469	p<0.4
		<14			
	HLM3		0.4571	0.4355	p<0.8
CHROM 19		8.3			
	AHT041		0.3194		
		12.9			
	HTG23		0.5294	0.4828	p<0.6
		26.4			
	LEX036		0.6447	0.5469	p<0.2
		7.6			
	LEX073		0.5		
		32.2			
	COR092		0.4143		
		19.6			

Table 5.1	continued				
	mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM 20		11.6			
	HTG005		0.4706	0.6034	p<0.1
		~19.6			
	LEX052		0.3553		
		~30			
	UM011		0.3382		
		~66			
	LEX071		0.2368	0.0652	p<0.004
		<27.3			
	HMS42		0.4839	0.5893	p<0.2
CHROM 21					
	SGCV014		0.7632	0.8125	p<0.4
		0			
	SGCV016		0.5395	0.3889	p<0.1
		2.1			
	TKY021		0.4342	0.1875	p<0.001
		19.8			
	HTG010		0.2237	0.3167	p<0.2
		3.8			
	COR073		0.4211		
		4.7			
	COR068		0.4118	0.3036	p<0.1
		27.1			
	HTG32		0.7857	0.7258	p<0.3
		8.6			
	LEX037				
		14.2	0.5946		
CHROM 22					
		11.7			
	TKY285		0.2763		
		12.2			
	COR022		0.4286	0.5806	p<0.06
		1.8			
	HTG21		0.2297		
		22.8			
	COR016		0.4324		
		33.7			
	HMS047		0.5526	0.1719	p<0.00002
		11.1			

Table 5.1	continued mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM 23		2.4			
	COR055		0.2273		
		28.7			
	LEX063		0.4143	0.4516	p<0.6
		<45.9			
	COR084	45.9	0.6053	0.5714	p<0.6
		<45.9			
	SGCV004		0.7	0.8226	p<0.06
CHROM 24		23.3			
	LEX042		0.5758	0.3448	p<0.006
		2			
	AHT032		0.5286	0.4032	p<0.09
		6.8			
	AHT004		0.3971	0.6129	p<0.01
		~11.7			
	LEX074		0.25		
		11.7			
	COR061		0.3289		
		26.6			
	COR024		0.4143		
CHROM 25		<1			
	AHT051		0.25	0.1207	p<0.09
		<1			
	NVHEQ43		0.2703	0.22	p<0.3
		10.8			
	AHT007		0.3889	0.5161	p<0.1
		15.6			
	COR018		0.4211	0.3438	p<0.3
		9.5			
	COR080		0.7714	0.7813	p<0.9
		15.7			
CHROM 26		27.2			
	NVHEQ70		0.5395	0.371	p<0.05
		14.2			
	A17		0.5921	0.4839	p<0.2
		3.4			
	EB2E8		0.8553	0.6552	p<0.03
		3.4			
	COR071		0.4605		
		32.2			

Table 5.1 **continued**

	mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM 27					
	COR021		0.3947		
		20.9			
	COR040		0.2632		
		<7.2			
	HMS45		0.6364	0.3667	p<0.004
		~68			
	COR017		0.3684		
CHROM 28		2.7			
	NV54		0.6571	0.8103	p<0.03
		3.5			
	HTG030		0.6757	0.7143	p<0.6
		<.3			
	TKY018		0.25	0.3281	p<0.2
		0.3			
	UM003		0.4342		
		16.7			
	TKY319		0.4571	0.4194	p<0.6
		23.1			
	TKY515		0.6324	0.4	p<0.06
		~32.1			
	UCD425		0.5139	0.5682	p<0.5
CHROM 29					
	LEX018		0.4091	0.3571	p<0.5
		30.3			
	COR082		0.2368	0.25	p<0.8
		45.2			
	COR027				
		26	0.4189		
	LAM12.2				
		13.2	0.3056		

Table 5.1	continued				
	mSAT	distance cM	affected com.freq.	unaffected com.freq.	
CHROM 30					
	LEX025		0.6667	0.5833	p<0.3
		<25.8			
	HTG27		0.6316	0.5	p<0.1
		<25.8			
	HMS18		0.527		
		15.2			
	VHL20		0.2206	0.258	p<0.9
		23			
CHROM 31		13.9			
	COR038		0.875	0.7174	p<0.02
		4.6			
	VIAS-H21		0.8947	0.9063	p<0.8
		~0			
	AHT033		0.527		
		<33.2			
	TKY274		0.2368	0.3906	p<0.4
		<33.2			
	AHT034		0.3919		

<u>Results continued:</u>

Figures 5.1 through 5.31 depict common allele frequencies of the affected (crimson) and unaffected (Navy blue) horse groups. The Student t-Test was performed to determine whether differences in allele occurrence in individual horses between the groups were statistically significant. The Student t-Test takes into account the variability of the groups when calculating differences in the mean.

If the mode of inheritance of DSLD is dominant, all affected individuals should have at least one copy of the mutated allele. Any marker locus in linkage disequilibrium with DSLD should show a pattern where nearly all affected individuals have a common allele but this same pattern would not necessarily be seen in the unaffected group. Evidence of linkage disequilibrium in the following analysis would present as a significant difference in the frequency/occurrence of the common allele of the affected group as compared to the unaffected group. One would expect to see one common allele in every affected individual providing the microsatellite is close enough to the causative gene to act as a marker. The common allele can also be seen in the unaffected group but would not be at as high a frequency.

In the following figures * depicts $p < 0.05$, * depicts $p < 0.010$, * depicts $p < 0.001$ and ^ depicts an area where the common allele occurred in the unaffected horses at a significantly higher level and will be highlighted in the appropriate color.
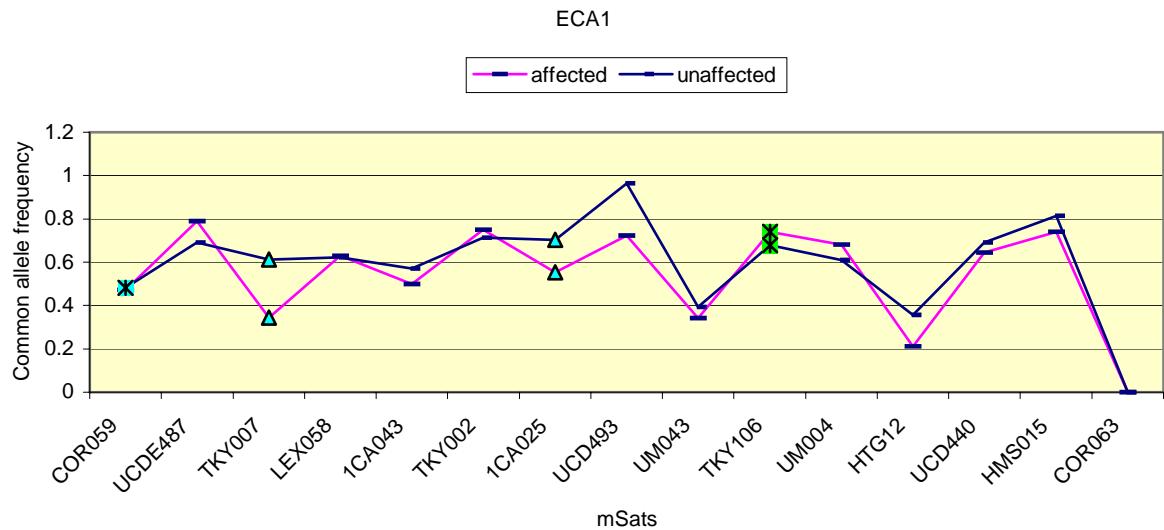
Figure 5.1 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 1. COR059, 1CA25 and TKY106 had common allele t-Test p values of 0.01, 0.04, and 0.004 respectively.
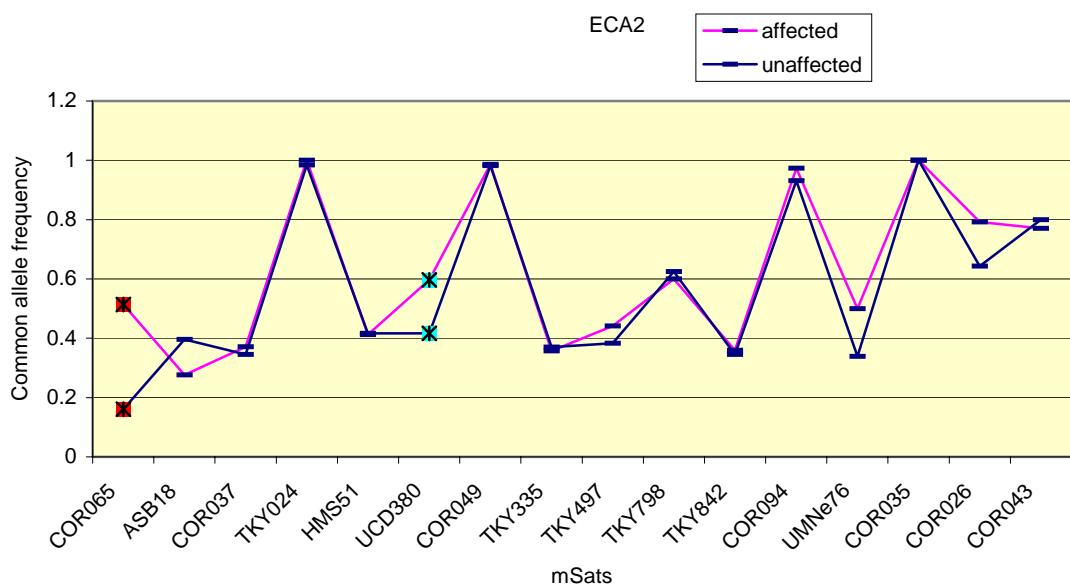
Figure 5.2 Common allele frequencies of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 2. COR065 and UCD380 had common allele t-Test p values of 0.000008 and 0.04 respectively.
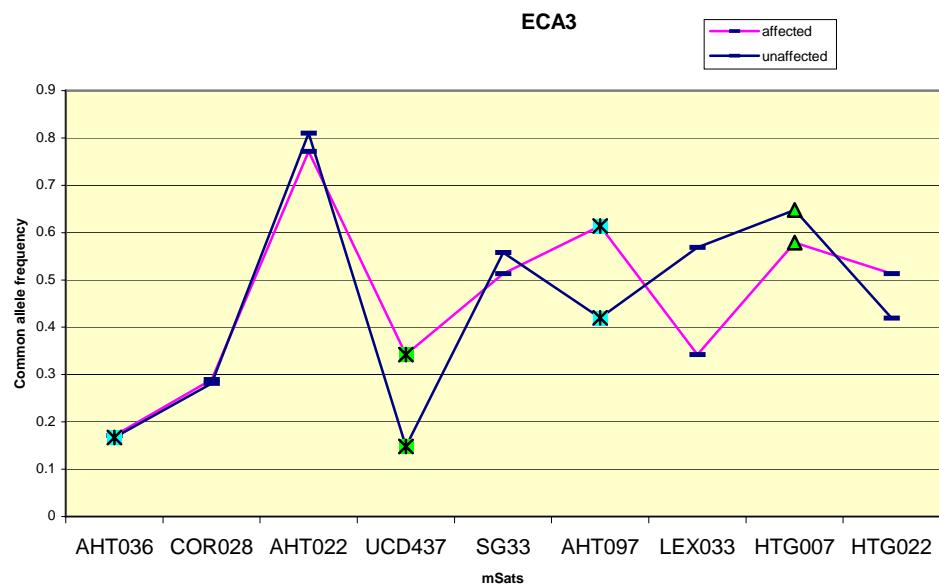
Figure 5.3. Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 3. Common allele t-Test p values for AHT036, UCD437, AHT097, and HMS06 were 0.03, 0.01, 0.02, and 0.001 respectively.
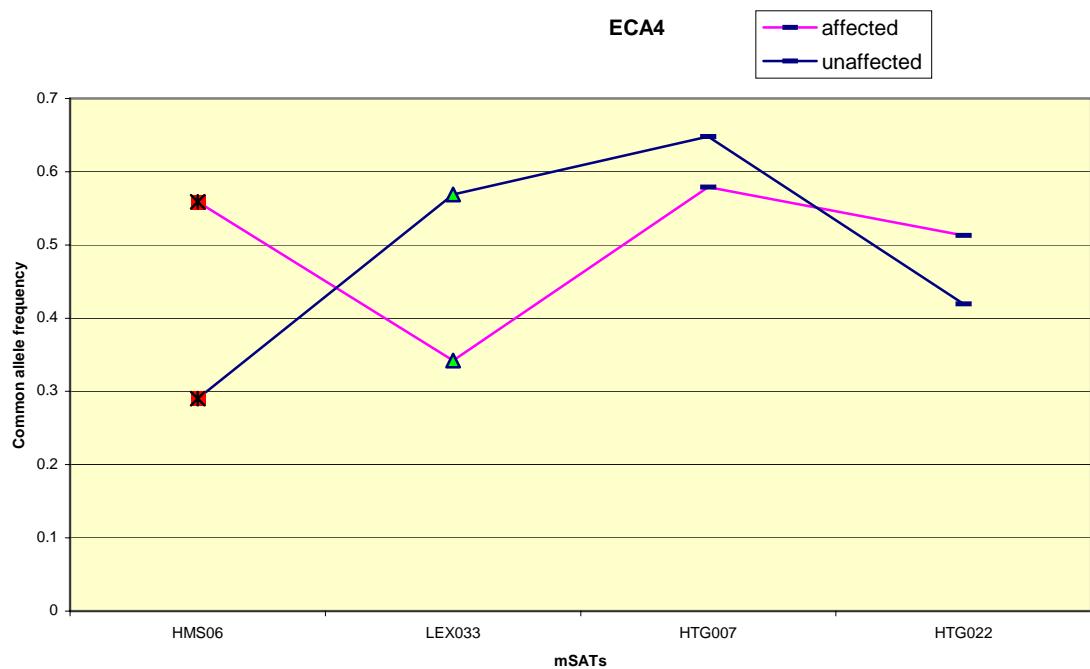
Figure 5.4 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 4. Common allele t-Test p value for HMS06 was 0.001.
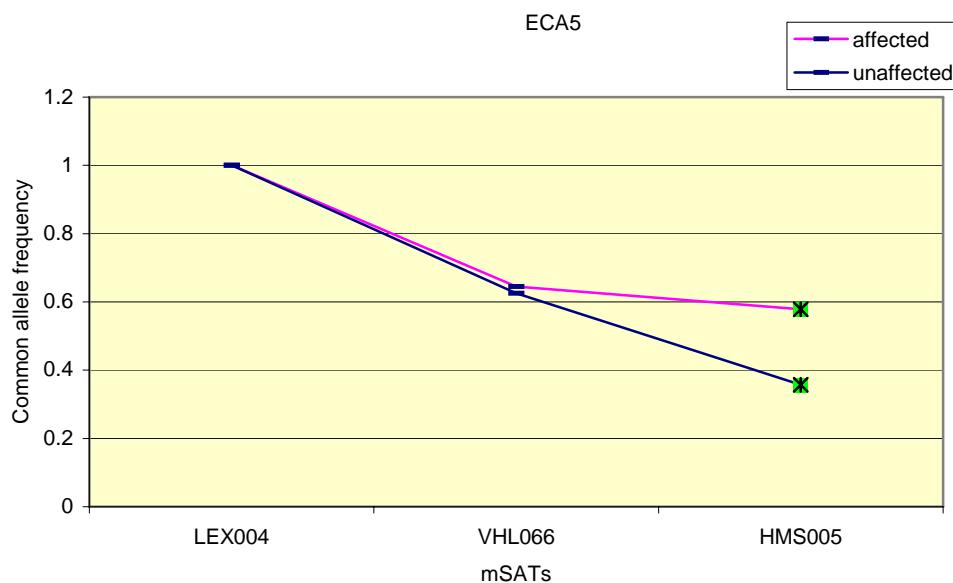
Figure 5.5 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 5. Common allele t-Test p value for HMS005 was 0.009.
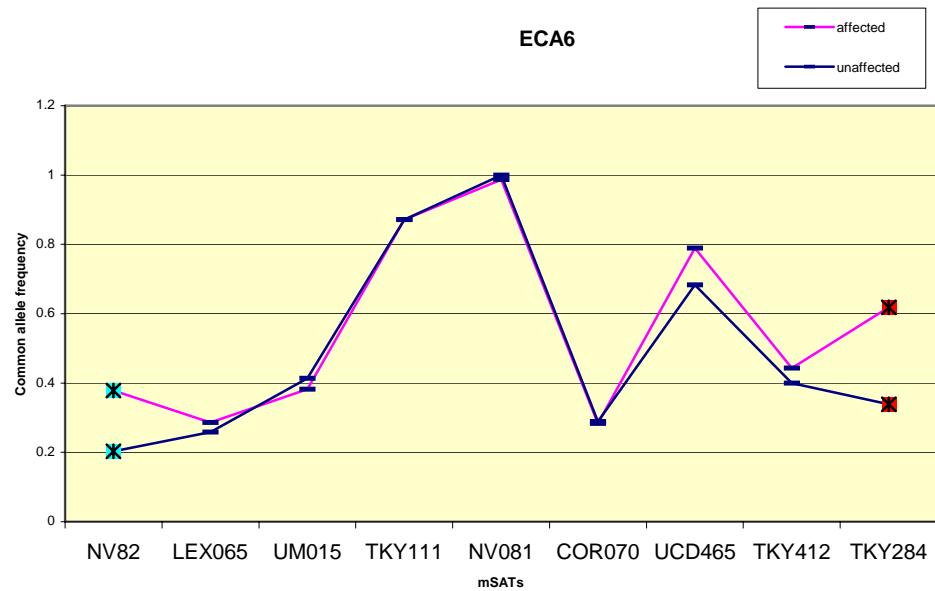
Figure 5.6 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 6. Common allele t-Test p value for NV82 and TKY284 were 0.02 and 0.0009 respectively. TKY284 is near 2 candidate genes (LEMD3 and DCN) and may be suggestive of LD, more to follow in discussion.
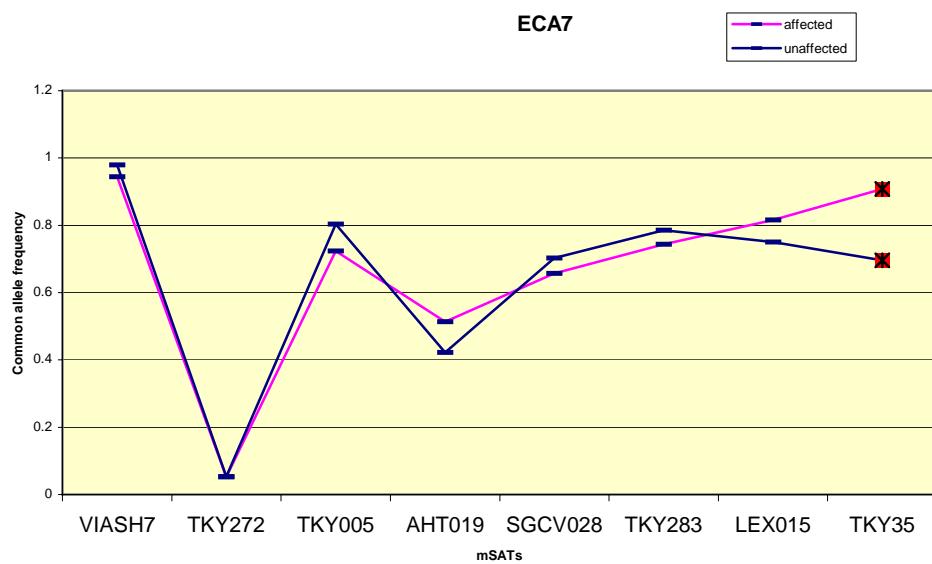
Figure 5.7 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 7. Common allele t-Test p value for TKY35 was 0.0002. Only two alleles were present at this locus making TKT35 more likely to be support for linkage to DSLD as a recessive mode of inheritance.

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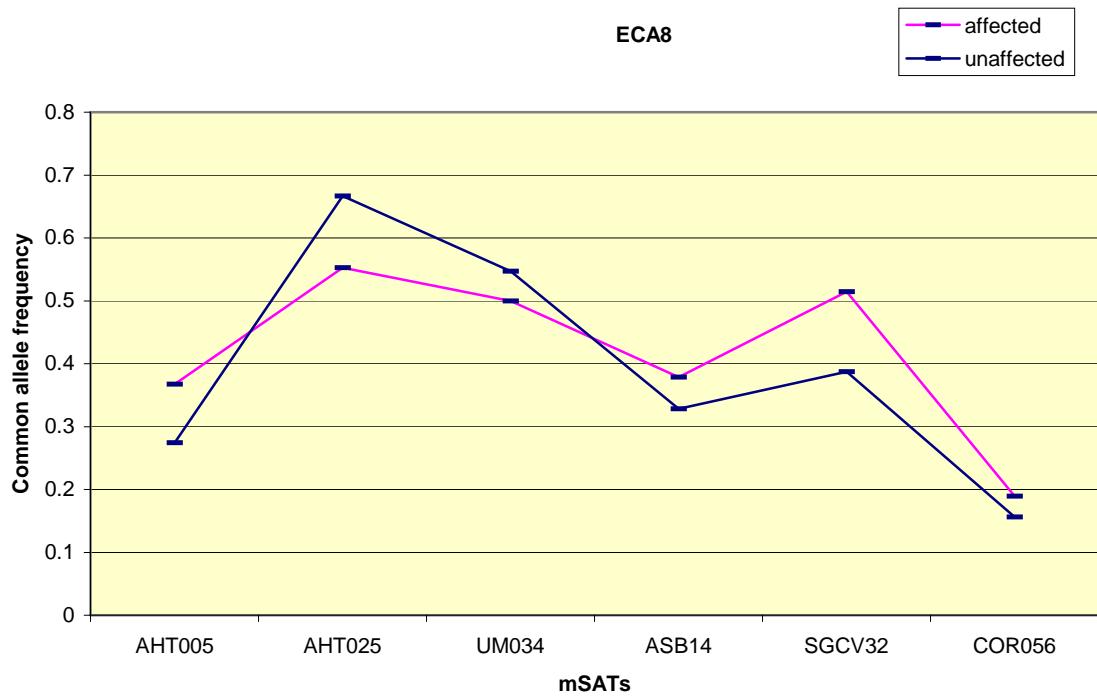
Figure 5.8 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 5. There were no significant common allele t-Test p values for ECA8.
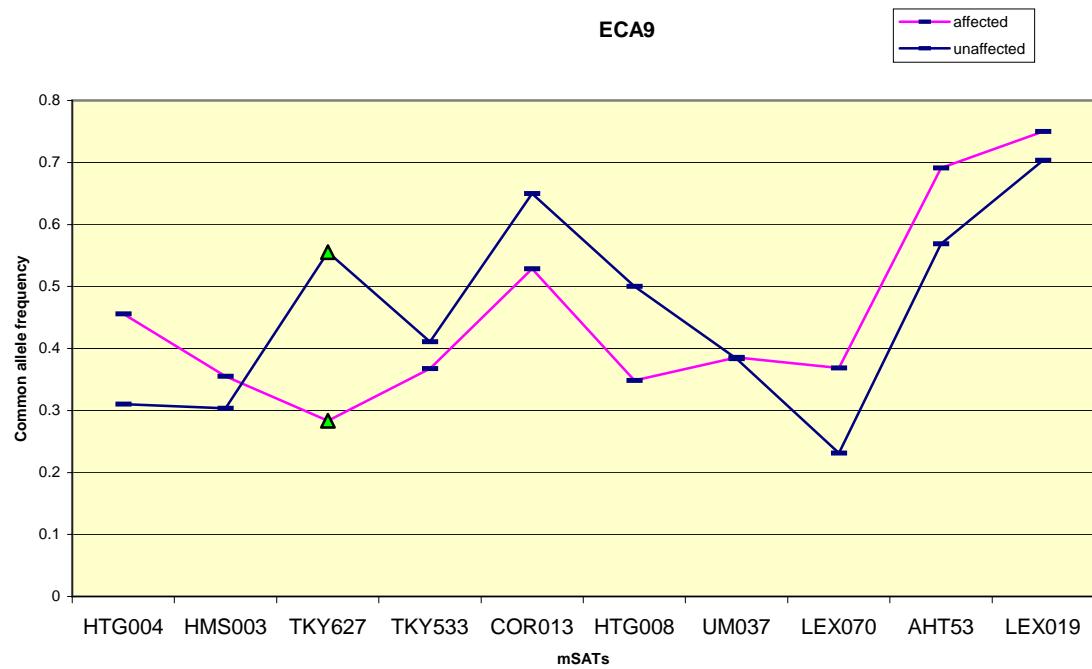
Figure 5.9 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 9. There were no significant common allele t-Test p values for ECA9.
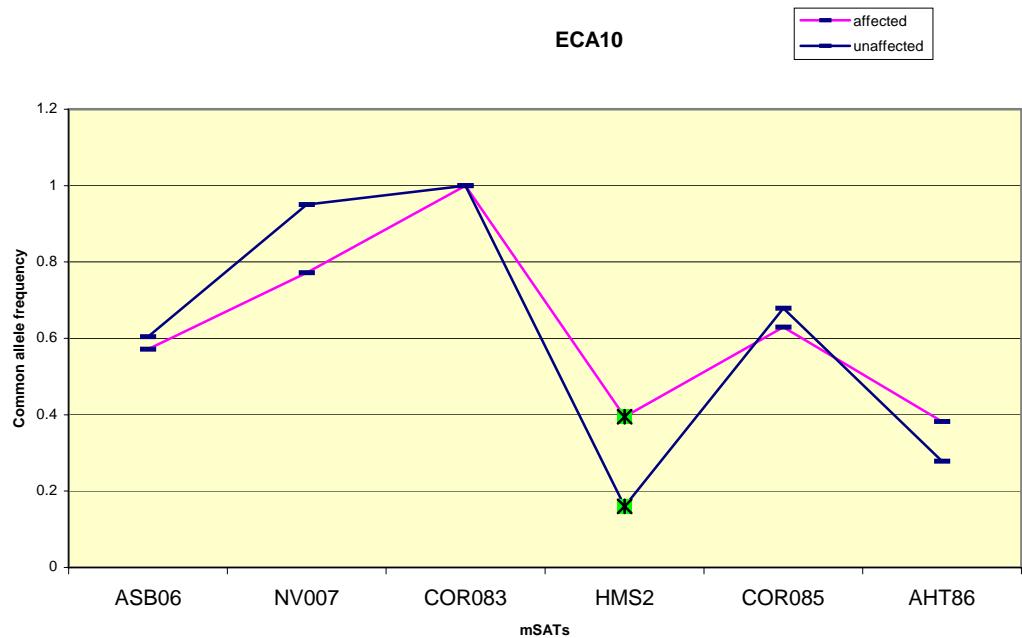
Figure 5.10 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 10. Common allele t-Test p value for HMS2 was 0.003.
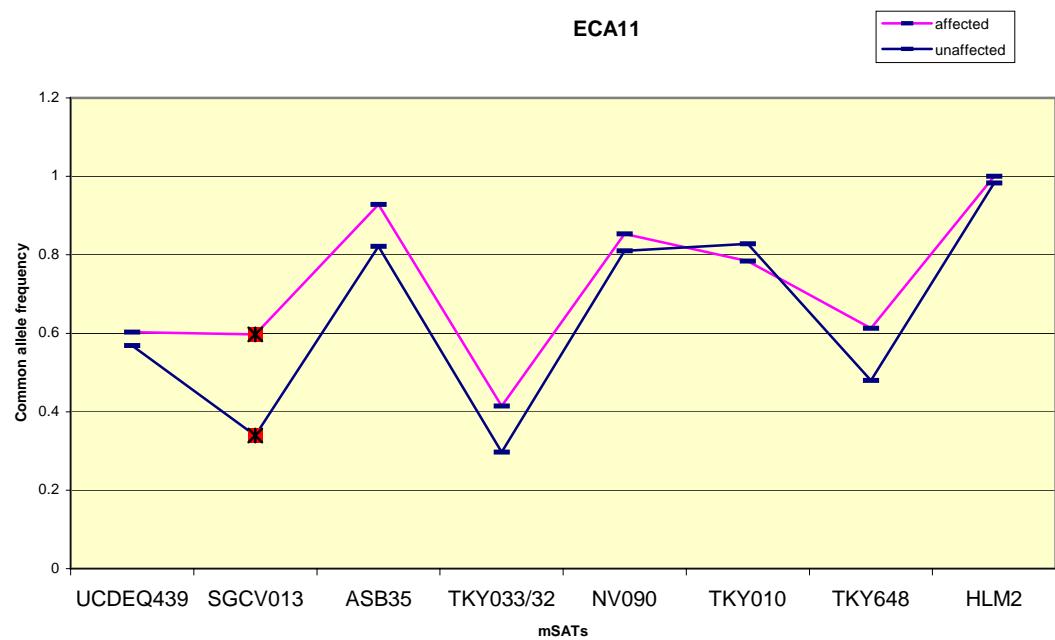
Figure 5.11 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 11. Common allele t-Test p value for SGCV013 was 0.001. SGCV13 is linkage mapped 13 cR from COL1A1 (Chowdhary et al., 2003) and variation of neighboring microsatellites fit the pattern expected for linkage with DSLD, more to follow in discussion.
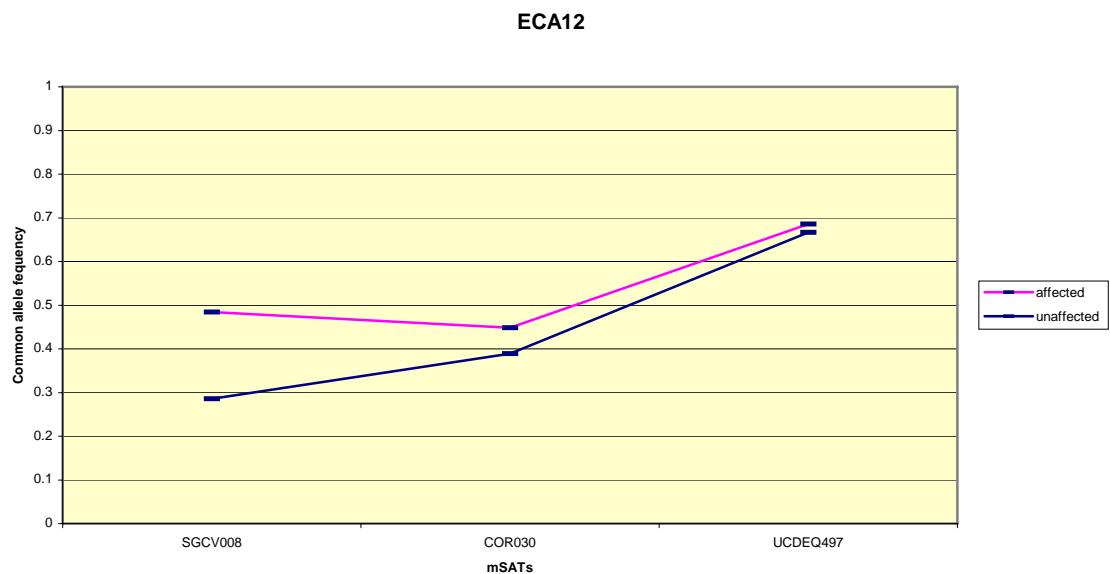
ECA12

Figure 5.12 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 10. The was no significant common allele t-Test p values for ECA12.
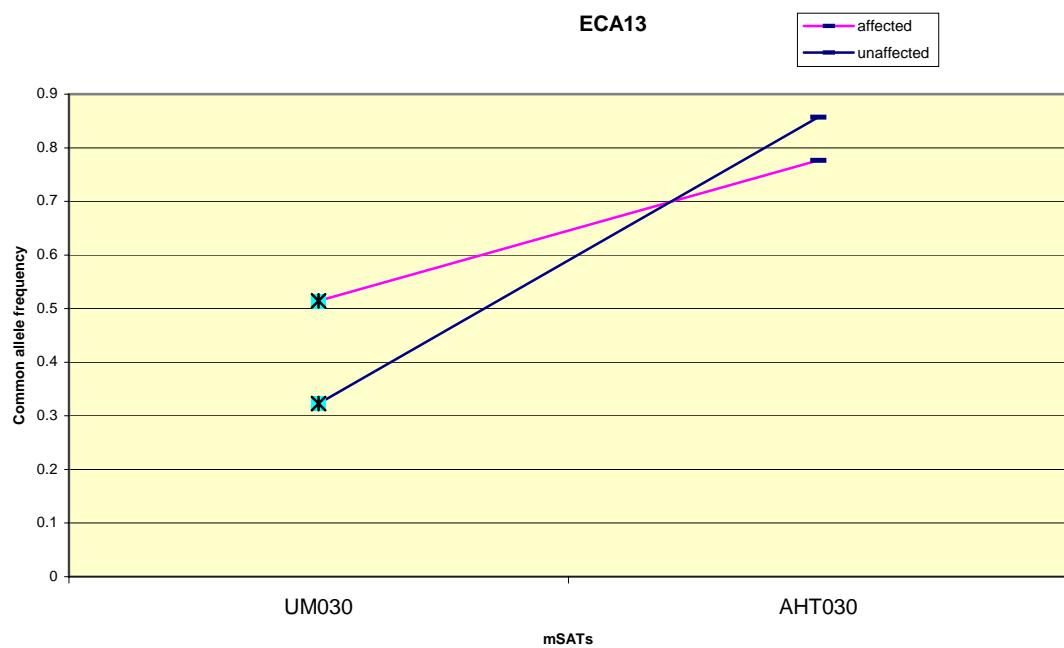
Figure 5.13 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 13. Common allele t-Test p value for UM030 was 0.05.
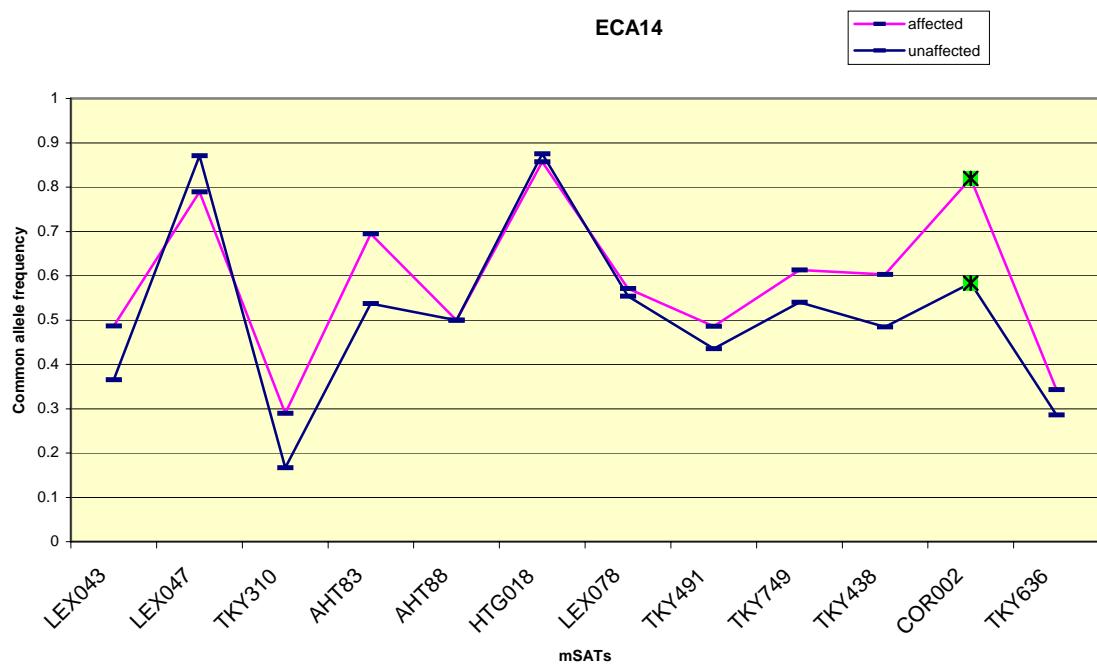
Figure 5.14 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 14. Common allele t-Test p value for COR002 was 0.003. COR002 results follow expectations more suggestive of recessive mode of inheritance, more to follow in discussion.
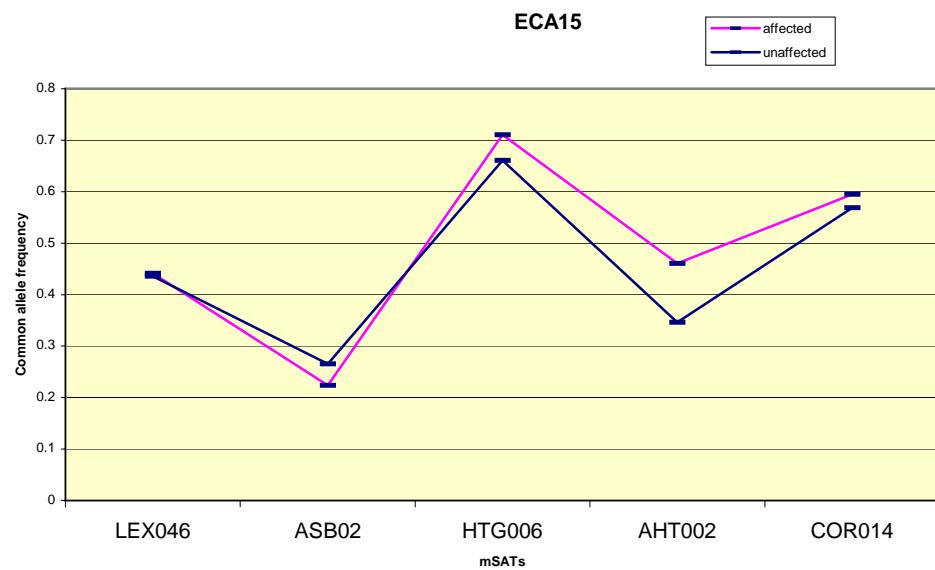
Figure 5.15 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 15. There were no significant common allele t-Test p value for ECA15.
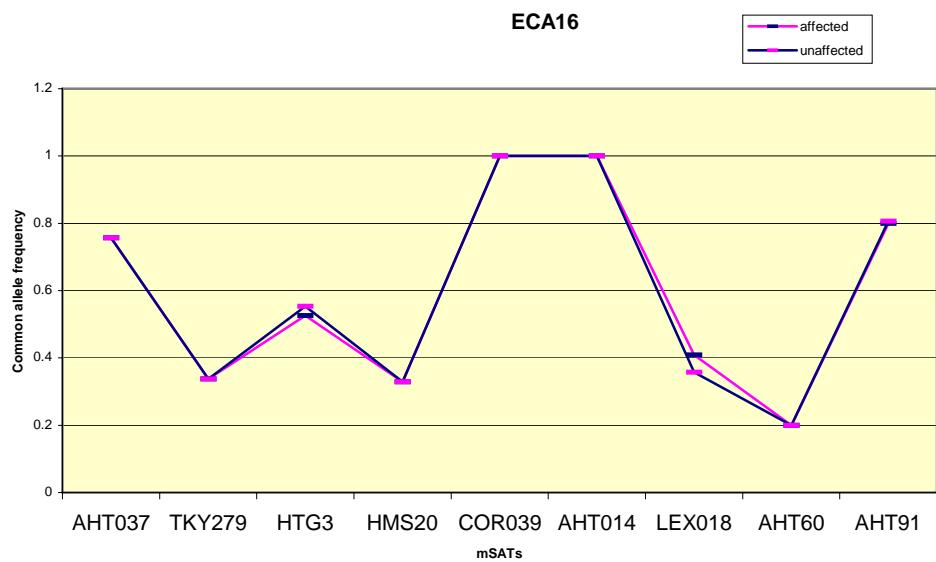
Figure 5.16 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 16. There were no significant common allele t-Test p values for ECA16.
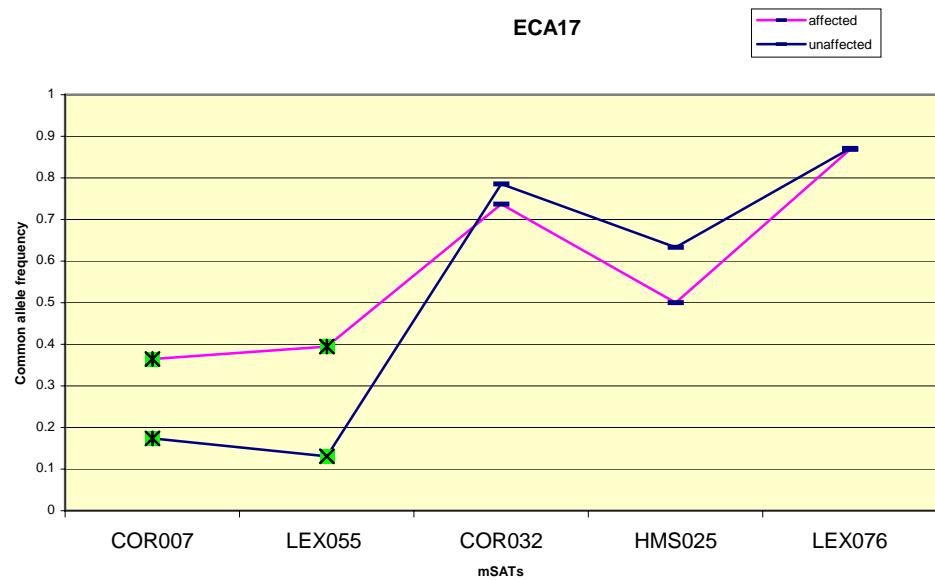
Figure 5.17 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 17. Common allele t-Test p value for COR007 and LEX055 were both 0.01.
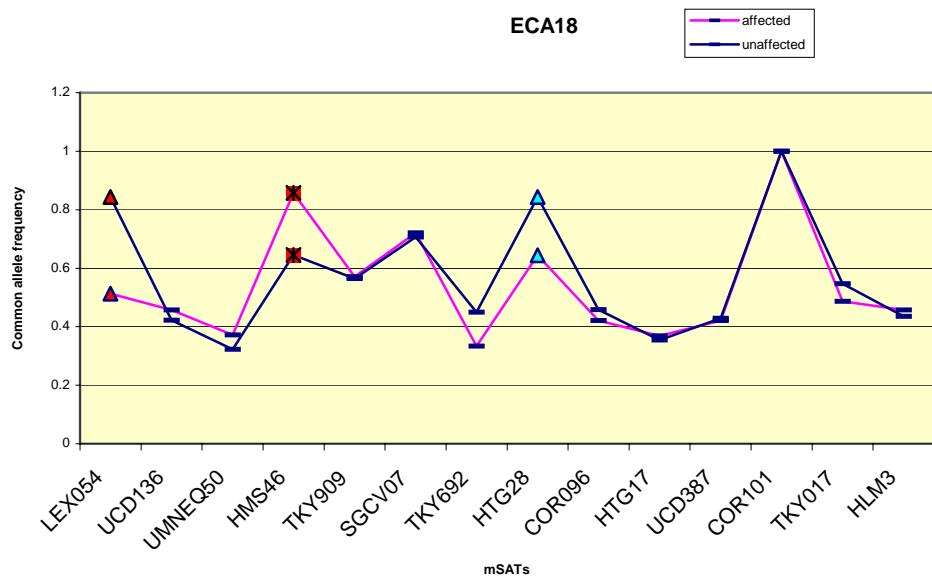
Figure 5.18 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 18. Common allele t-Test p value for HMS46 was 0.0008. HMS46 results follow expectations more suggestive of recessive mode of inheritance, more to follow in discussion.
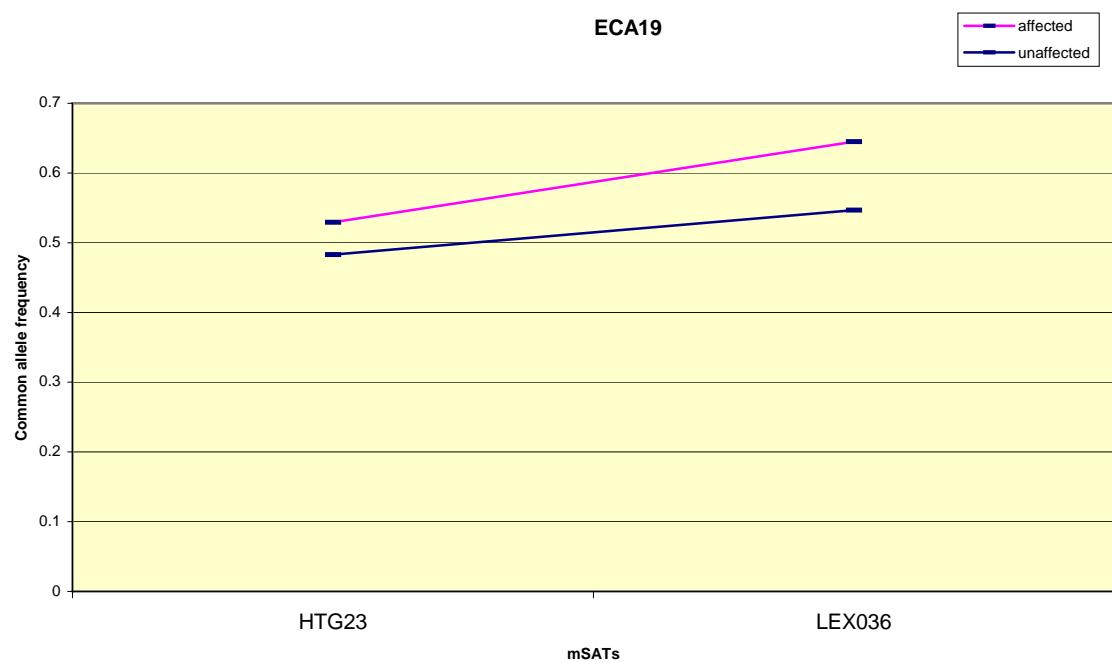
Figure 5.19 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 18. There were no significant common allele t-Test p value for ECA19.
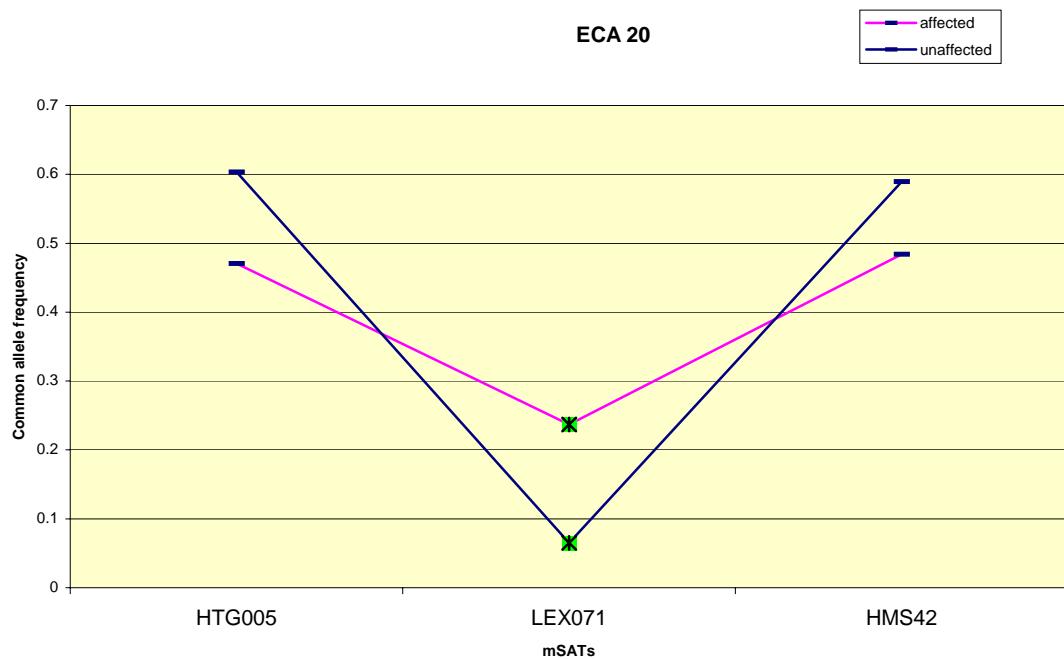
Figure 5.20 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 20. Common allele t-Test p value for LEX071 was 0.004.
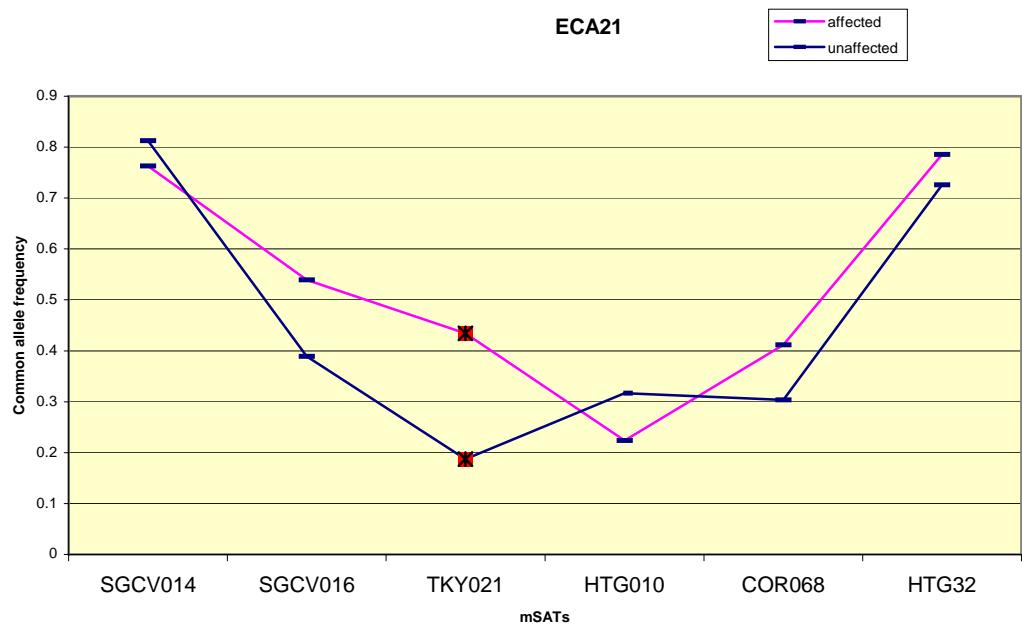
Figure 5.21 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 21. Common allele t-Test p value for TKY021 was 0.001. The common allele for this locus is not seen in 10 of the affected horses and neighboring microsatellites do not follow expectations of linkage. More will follow in discussion.
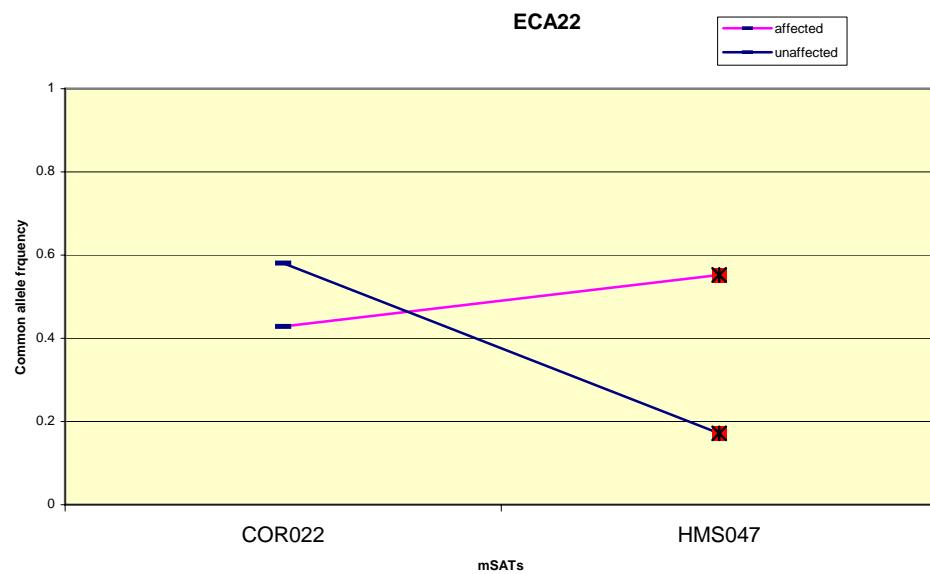
Figure 5.22 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 22. Common allele t-Test p value for HMS047 was 0.00002. More testing may be warranted for ECA22, more will follow in discussion.
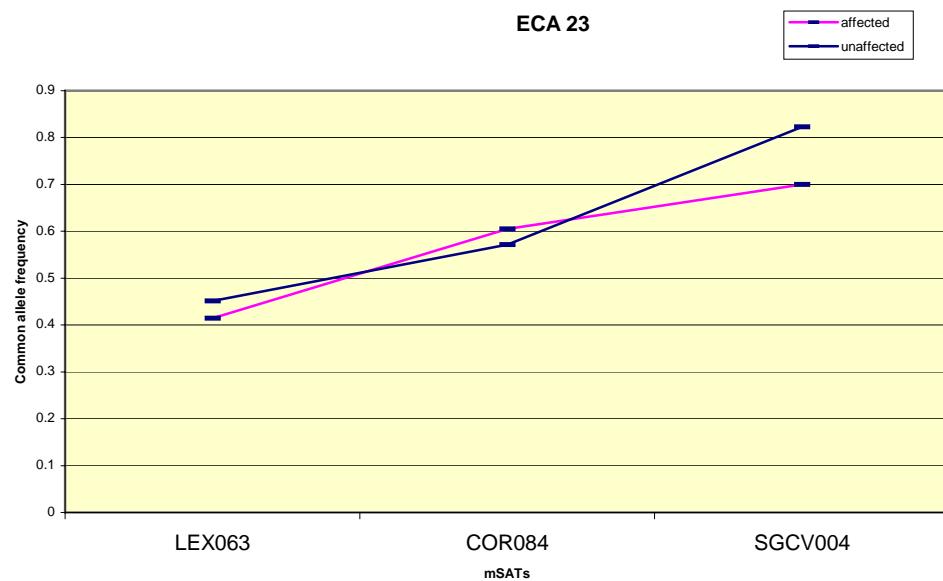
Figure 5.23 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 23. There were no significant common allele t-Test p values for ECA 23.
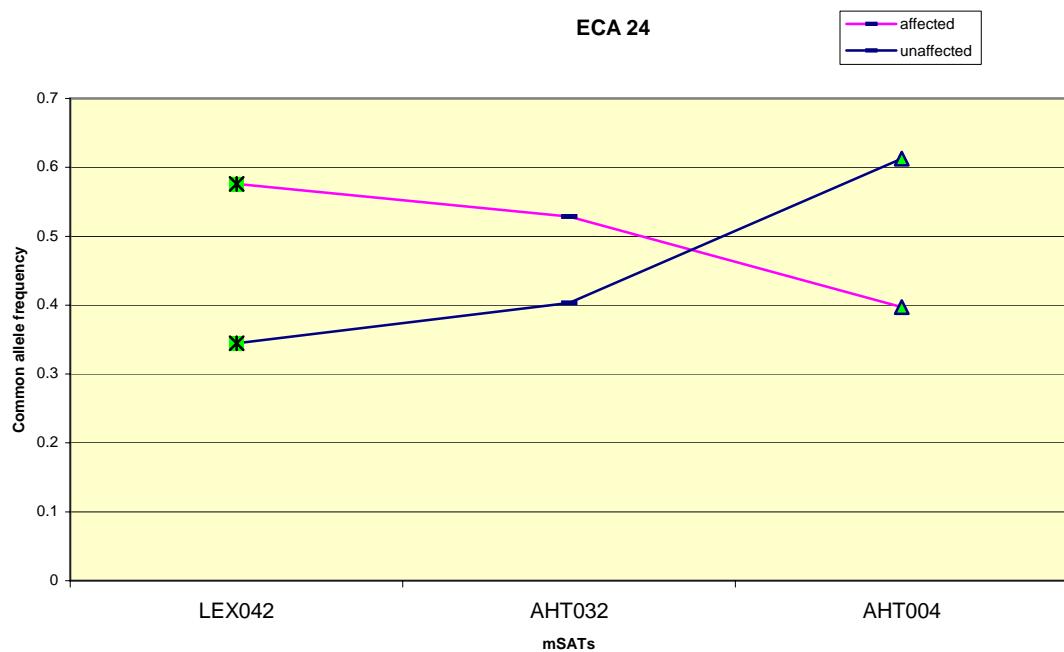
Figure 5.24 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 24. Common allele t-Test p value for LEX042 was 0.006.
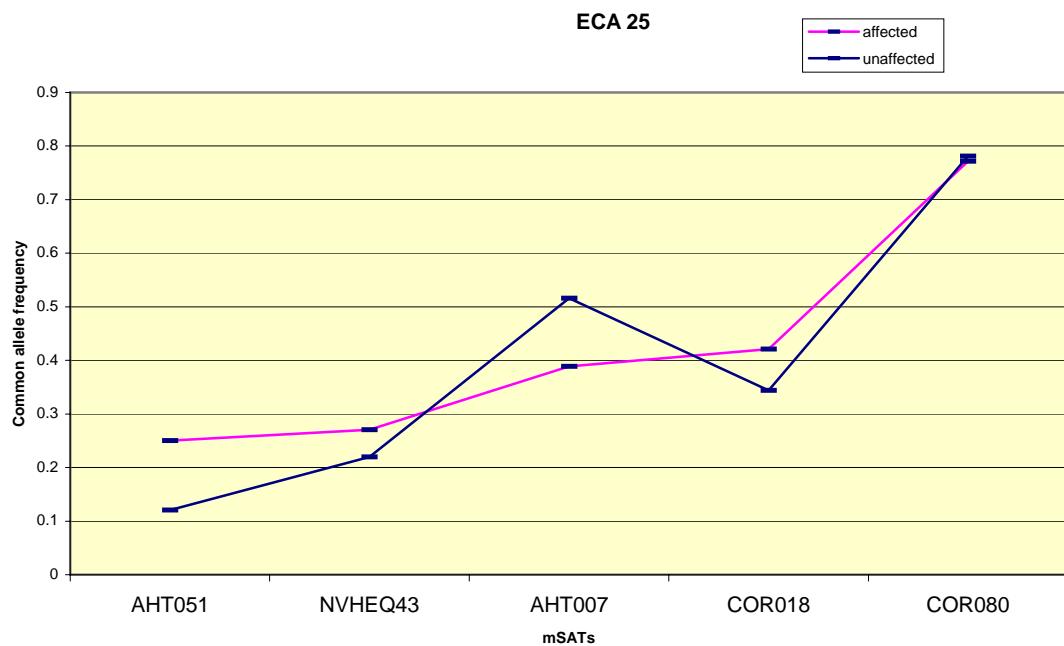
Figure 5.25 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 25. There were no significant common allele t-Test p values for ECA25.
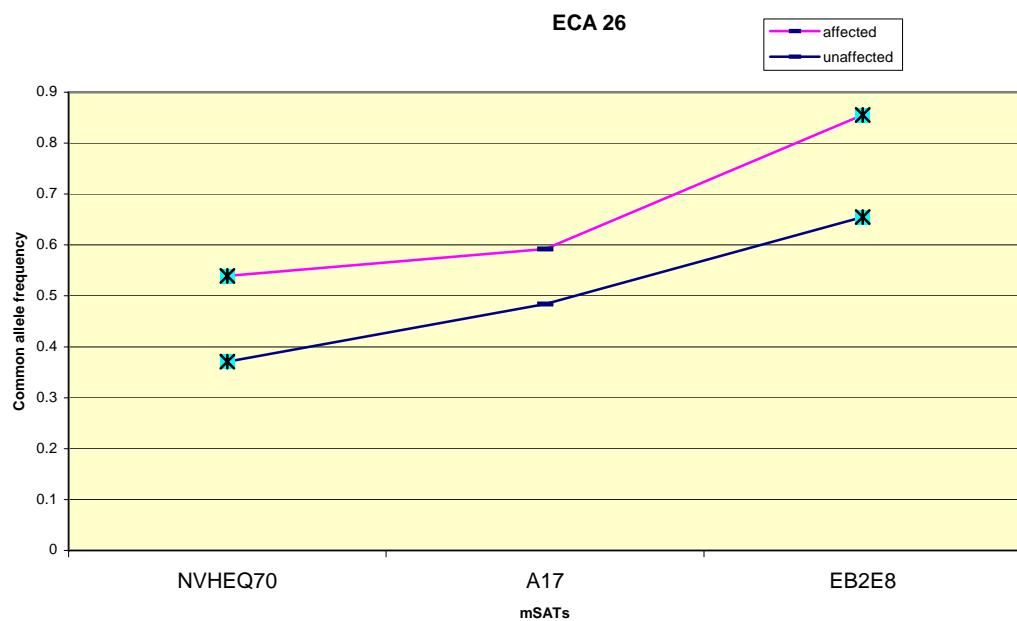
Figure 5.26 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 26. Common allele t-Test p value for NVHEQ70 and EB2E8 were 0.05 and 0.03 respectively.

ECA27
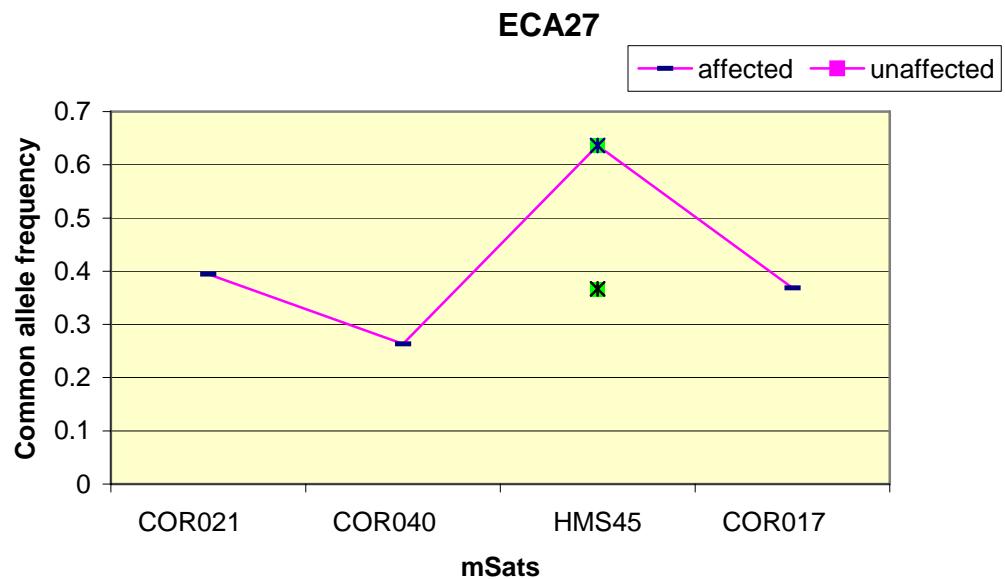
Figure 5.27 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 27. Common allele t-Test p value for HMS45 was 0.004. A common allele did not occure in all or most of affected in the remaining microsatellites as would be expected in a dominant mode of inheritance, thus unaffected were not tested on those microsatellites. There is no other support for linkage to a DSLD gene in this region.
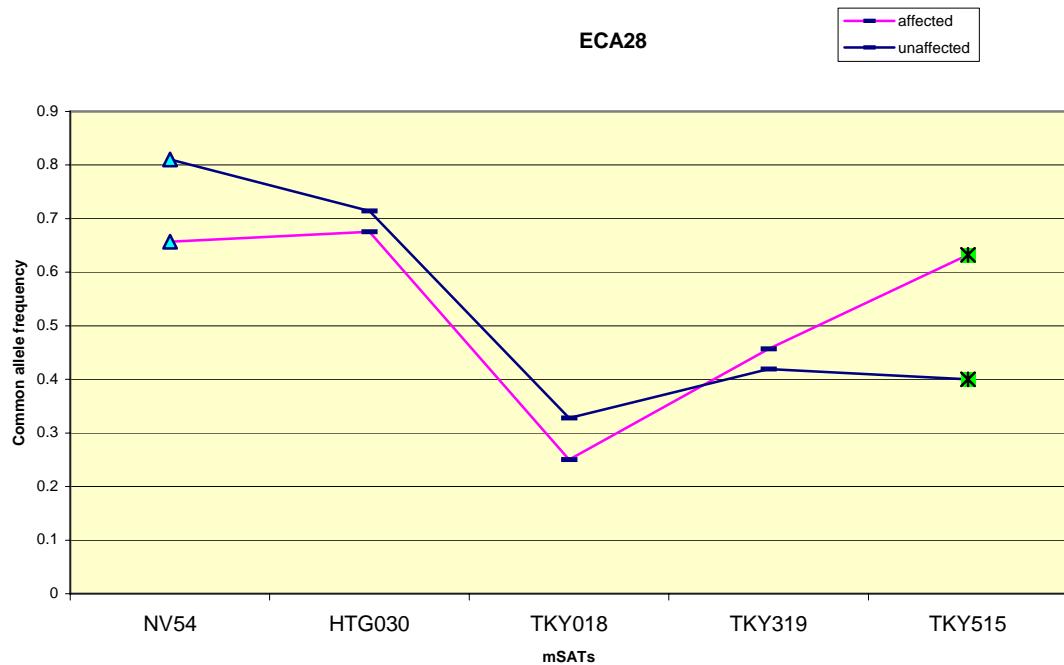
Figure 5.28 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 28. Common allele t-Test p value for TKY515 was 0.006.
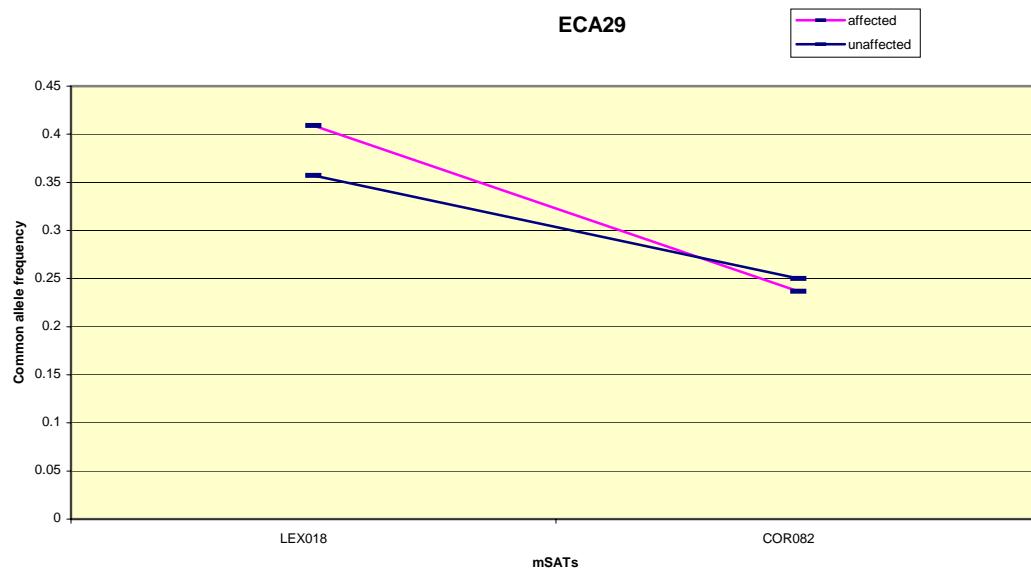
Figure 5.29 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 29. There were no significant common allele t-Test p values for ECA29.
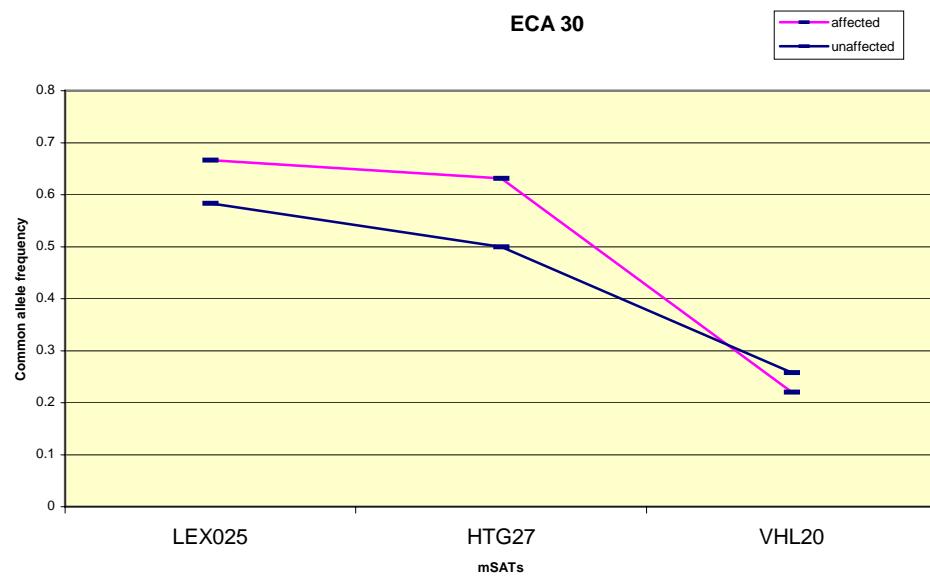
Figure 5.30 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 30. There were no significant common allele t-Test p values for ECA30.
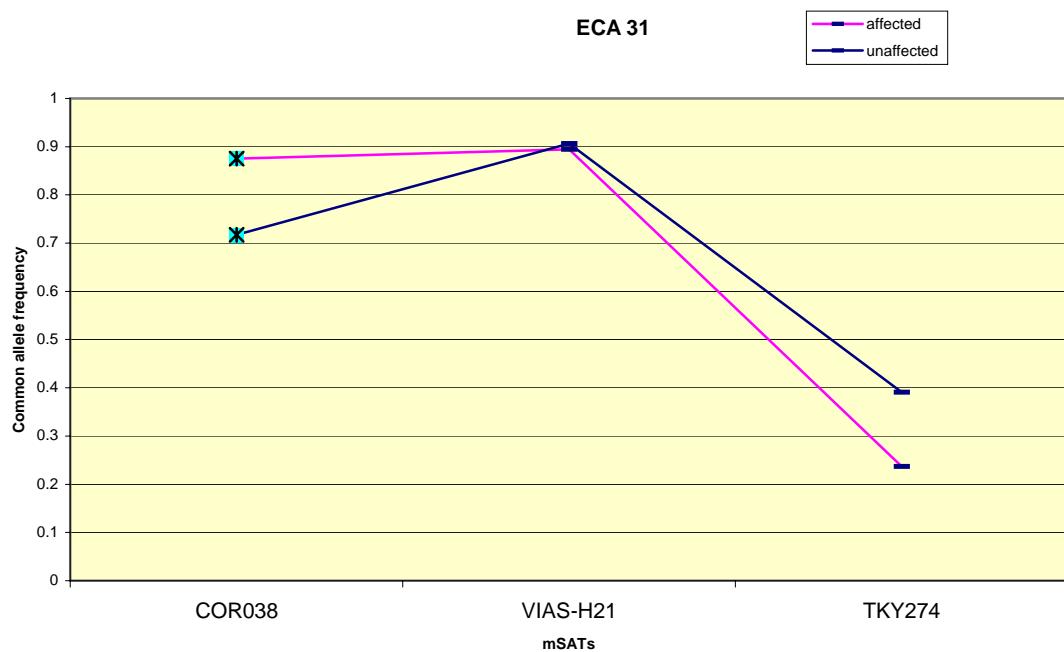
Figure 5.31 Common allele frequency of affected and unaffected horse groups of microsatellite loci mapped to equine chromosome 31. Common allele t-Test p value for COR038 was 0.02. The common allele is seen in all but one unaffected individual. The significance is due to excess homozygosity in affected.

Discussion

It is important to note that the absence of a candidate gene in a region does not indicate that one does not exist there. It simply means that there is no gene of known function that might be related to DSLD that has been mapped to that region . It is also important to note that the statistical significance of a particular locus was never dismissed based upon the lack of a candidate gene in the area.

Of the 31 chromosomes, eleven had no statistically significant common allele t-Test values: ECA8, 9, 12, 15, 16, 17, 19, 23, 25, 29, and 30. There were 20 chromosomes with significant common allele t-Test values: ECA1-7, 10, 11, 13, 14, 18, 20-22, 24, 26-28, and 31.

ECA1 (1CA25), ECA3 (AHT097), ECA4, 5, 26, and 28 had microsatellites with significant common allele T-Test values however, in each case the marker lacked a common allele shared by all affected individuals. The significant t-Test was due to common allele homozygotes in the affected individuals, which ultimately increased the frequency.

ECA3 (AHT036,UCD437), ECA6 (NV82), ECA10 (HMS2), ECA13 (UM030), and ECA20 (LEX071), had microsatellite markers with a significant common allele excess in affected individuals, however the frequency at which the common allele occurred was too low to be consistent with the expectations of a marker associated with a DSLD gene. This occurs when there are a high number of allele possibilities for that marker locus and they all occur at a moderate rate, (i.e.: the most common allele is still only occurring 27% of the time).

COR059 on ECA1 had significant differences (p<0.01) between the common allele occurrence in affected and unaffected horses. The allele occurred in all but one of the affected animals and all but 10 of the unaffected animals. It is located 140.2 cR from the

most telomeric marker mapped to the p arm of ECA1 (Chowdhary et al., 2003). There were five microsatellites between COR059 and the end of ECA1 tested only in affected individuals (VIAS-H34, ASB41, LEX020, NV100, and COR100). None of the affected shared a common allele in any of these microsatellites making the likelihood of a DSLD gene in that region highly unlikely based a dominant gene model. COR059 is linkage mapped to the same point as UCD487 (Guérin et al. 2003) which does not have a common allele shared between affected individuals as would be expected if COR059 was linked to a DSLD gene in the region. TKY106 had a significant ($p<0.004$) excess of the common allele in the affected as apposed to the unaffected group, however the common allele did not occur in eight of the affected. HTG12, UM004 and UCDE440 are all mapped 0 cM from TKY106 (Guerin et al. 2003) and none of them showed significant allele frequency differences. Most likely this is not evidence of linkage to a DSLD gene in the region given a dominant mode of inheritance.

TKY284 located on ECA6 had a significant ($p< 0.0009$) excess of the common allele in affected horses compared to unaffected horses. The common allele occurred in 85% of the affected and only 55% of the unaffected individuals. TKY284 is linkage-mapped 7.1cM from TKY412 (Guérin et al. 2003) and showed no significant differences. The human syntenic relationship with this region of ECA6 is HSA12 (Milenkovic et al., 2002). There is a candidate gene in this region based on the human. As discussed in chapter three, LEMD3 (MAN1) has been RH mapped to this region of ECA6 (Chowdhary et al.,2003) and is responsible for Osteopoikilosis; (Buschke-Ollendorff syndrome) in humans (Lin et al., 2000) and is an autosomal dominant disorder (Melnick, 1959). It had been known to cause abnormalities in collagen, elastin fibers in the connective tissue of the skin. (Uitto et al., 1981, and Morrison et al., 1997). Very close to LEMD3 is DCN (Decorin), a gene also

known as Proteoglycan II. This gene codes for a distinct small proteoglycan found in connective tissue and has been associated with a lethal form of Marfan syndrome (Pulkkinen et al., 1990). Further investigation of this region of ECA 6 may be warranted, possibly looking for SNP's in the LEMD3 gene since it is sequenced in the horse.

TKY35 on ECA7 has a significant ($p< 0.0002$) excess of the common allele in the affected individuals, however, there were only 2 alleles seen. The most common allele occurred in every horse, affected and unaffected, making the high value more informative as support for linkage to a gene with a recessive mode of inheritance.

SGCV013 on ECA11 had a significant ($p<0.001$) excess of the common allele in the affected compared to the unaffected. The common allele is seen in all but 5 of the affected and is seen in all but 11 of the unaffected. As discussed in chapter three, SGCV013 is 13 cR from the possible candidate gene COL1A1 (Chowhardy et al., 2003). COL1A1 is the gene responsible for the collagen of skin, tendon, and bone and is the gene responsible for Ehlers-Danlos syndrome, Gravis type (EDS I) (Nuytinck et al., 2000), and Arthrochalasia type (EDS VIIA) (Eyre et al., 1985) both of which are autosomal dominant connective tissue disorder seen in humans. Microsatellites centromeric to SGCV013 have common allele frequencies in the affected group that are consistent with expectation of SGCV013 as a marker for a DSLD gene.

COR002 located on ECA14 had a significant ($p<0.003$) excess of the common allele in affected individuals, however the common allele is seen in all but one unaffected individuals also. There was an excess of homozygosity in the affected individuals, which gave the significant T-Test value. This could be supportive of linkage under the recessive mode of inheritance model.

HMS46 located on ECA18 had a significant (p<0.0008) excess of the common allele in affected individuals, however the common allele is seen in all of the unaffected individuals also. There was an excess of homozygosity in the affected individuals, which gave the significant t-Test value. This could be supportive of linkage under the recessive mode of inheritance model.

TKY021 on ECA21 had a significant (p< 0.001) excess of the common allele in affected individuals. Although the common allele is not seen in all of the affected individuals, (missing from 10), there is a definite excess in the affected group. The nearest microsatellite, SGCV14, is linkage mapped to the same point (Guérin et al., 2003) and does not show any excess in any allele among the affected group. Therefore, it is unlikely that this is linked to a DSLD gene in the region with a dominant mode of inheritance model.

HMS047 located on ECA22 has a significant (p<0.00002) excess of the common allele in affected individuals, however, the common allele is not seen in all affected individuals. The common allele occurred only 28% of the unaffected while it occurred in 82% of the affected. No apparent candidate genes are in the region based on human synteny, however it may be beneficial to test a microsatellite that is closer to HMS047 such as TKY572 and AHT031.

Chromosome 24 lacked supporting evidence of linkage due to no closely neighboring microsatellites with a common allele shared between affected individuals .

HMS45 located on ECA27 had a significant (p<0.004) excess of the common allele in the affected group than the unaffected. No other microsatellite data on ECA27 were run on the unaffected because none of them had a common allele shared by the affected. Affected frequencies are shown on Figure 5.27 to show that no frequency was high enough to meet the expectations of a marker near a DSLD gene under a dominant mode of

inheritance. There are no apparent candidate genes on ECA27 however it may be advised to run microsatellite TKY294 before eliminating ECA27 entirely.

COR038 located on ECA31 had a significant (p<0.02) excess of the common allele in affected individuals, however the common allele is seen in all but one unaffected individuals also. There was an excess of homozygosity in the affected individuals, which gave the significant t-Test value. This is could be supportive of linkage under the recessive mode of inheritance model.

Overall, there were no areas where there was supportive evidence for linkage to a DSLD gene under the expectations of a dominant mode of inheritance. In some instances, significant t-Test values were supportive of a recessive mode of inheritance. Testing another microsatellite near TKY284 on ECA6 may be useful to rule out the possibility of linkage to the region of LEMD3 and DCN, however given the presumed close proximity of TKY412 (7.1 cM), it is not needed unless this distance proves to be too distance to show linkage.

Chapter Six

Summary and Conclusions

The Peruvian Paso horse is a superb animal with many attributes ranging from its smooth gait, to its excellent disposition, to its ease of training and handling. It is these attributes that have made the Peruvian Paso so popular. Many a Peruvian Paso the world over have won the dedicated hearts of their owners. The Peruvian Paso is overall a highly inbred breed. DSLD has become prevalent in the Peruvian Paso population, likely due to one very popular founder with a genetic mutation. Given the information collected by veterinarians and scientists, it is clear that DSLD is a genetic disease most likely with a simple recessive mode of inheritance, although there is a possibility of another mode of inheritance. This research was conducted with Peruvian Paso owners in mind, so they may never be forced to say goodbye to another Peruvian friend before their time.

Summary

This research project has narrowed the search for the gene causing DSLD by eliminating all but six chromosomal regions of equine genome. Three chromosomal regions are of particular interest, one of which was studied further by sequencing portions of CSPG2 gene and by comparing a SNP and a deletion found within CSPG2.

A genome scan using 259 microsatellite markers was used to test for linkage disequilibrium between one or more markers and DSLD. Two groups of Peruvian Paso horses were selected from one population including the US and Canada. The only difference between the two groups of horses besides the size of the groups was the presence of DSLD in the affected and the absence of DSLD in the unaffected animals. It was assumed that

differences seen between the two groups in homozygosity and/or common allele frequency could be an indicator of linkage to DSLD.

Conclusion

As a connective tissue disorder involving collagen, proteoglycan, decorin, biglycan, and aggrecan (Halper et al., 2005) there were numerous candidate genes to consider for DSLD, yet no identical human or animal disease model is known. In many instances in this work, it is stated that there was no clear candidate gene in the chromosomal region of the horse based on comparison with the human genome (Chowdhary et al., 2003). It is important to note that the absence of a candidate gene in a region does not indicate that one does not exist. It simply means that there is no gene of known function that might be relative to DSLD has been mapped to that region. It is also important to note that the statistical significance of differences between the two groups for a particular locus was never dismissed based upon the lack of a candidate gene in the area. All statistically significant loci were considered and dismissed as likely markers only when they lacked any supporting evidence from neighboring microsatellites. Even then, they were only dismissed when neighboring microsatellites were close enough to meet the expectations of linkage disequilibrium in the mode of inheritance being analyzed. It should also be noted that the criteria for considering a gene as a candidate was extremely broad, and any gene with any known relationship to disorders of connective tissue or those tissues related to connective tissues (collagen, proteoglycan, decorin, biglycan, and aggrecan) and those tissues related to tendons and ligaments were considered.

In some instances, more microsatellites need to be tested to completely rule out a specific chromosomal region. Those regions which should be tested are as follows: 1) The

region on ECA6 located between TKY284 and the end of the q arm; 2) the region on ECA22 around HMS047; 3) the region around HMS45 on ECA27 and the region on ECA28 near TKY515.

ECA4, 5, 8, 10, 13, 15, 16, 17, 19, 20, 23, 25, 27, 29, and 30 were excluded from the discussion for the recessive mode of inheritance to a lack of any statistically significant differences in homozygosity between the affected and unaffected groups. ECA8, 9, 12, 15, 16, 17, 19, 23, 25, 29, and 30 were excluded from the discussion of the dominant mode of inheritance due to lack of a statistically significant differences between common allele occurrence in the affected as compared to unaffected groups.

Statistical evidence for linkage disequilibrium is not the only criteria used when deciding whether a region of a chromosome is worthy of further investigation. Other evidence of linkage to support statistical evidence is needed. Neighboring microsatellite data should also be significant however possibly to a lesser degree, or at least follow the pattern expected in linkage disequilibrium as a recessive mode of inheritance or a dominant mode of inheritance depending on the mode being analyzed. In some instances, despite statistical significance, the actual data was not suggestive of linkage. The following chromosomes containing regions with statistically significant differences between the affected and unaffected groups were ruled out for various reasons: ECA1, 2, 3, 18, 21, 22, 24, 28, and 31. Further explanation can be found in the applicable chromosomal figure or in the discussion following the results of the recessive mode of inheritance in chapter three or dominant mode of inheritance in the chapter five.

The genome scan identified 5 chromosomes with statistically significant differences between the affected and unaffected sample populations based on the criteria above: ECA6, 7, 11, 14 and 26.

ECA6 contains a region of chromosome that was statistically different given either mode of inheritance. It is the only situation where the results do not weigh more heavily toward one mode of inheritance. TKY284 located on ECA6q had a significant excess of homozygosity in the affected individuals compared to unaffected (p<0.001) supporting linkage based upon a recessive mode of inheritance. TKY284 had a significant (p< 0.0009) excess of the common allele in affected horses compared to unaffected horses supporting linkage based upon a dominant mode of inheritance. The common allele occurred in 85% of the affected and only 55% of the unaffected individuals. TKY412 is located 8.1 cM centromeric to TKY284 (Guérin et al., 2003). TKY412 was not statistically significant for either differences in homozygosity or common allele. As discussed in chapter three, LEMD3 (MAN1) and DCN are candidate genes in this area based on human comparison mapping (Chowdhary et al., 2003). Investigating this region of ECA6 may be warranted. Looking for SNP's in LEMD3 or utilizing BAC clones to find additional microsatellites in the area would be useful for linking DSLD to a marker if there is one in the area. However, TKY284 is near the end of the p arm and to rule out the possibility of a gene at the very end, it would be useful to type TKY952, which is mapped, between TKY284 and the end of the chromosome (Penedo et al., 2005).

TKY35 located on ECA7, had a statistically significant excess of homozygosity in the affected as opposed to the unaffected individuals. TKY35 also had a common allele that occurred more frequently in the affected than in the unaffected at a statistically significant level. However, the differences in allelic frequency is compatible with the simple recessive mode of inheritance since the locus only had two alleles and the common allele occurred in every horse. As discussed in chapter three, TKY35 is positioned very near where a proposed gene for Familial Aortic Aneurysm 1 (FAA1) in man is located based on human genome

map (Chowdhary et al., 2003). FAA1 is a dominant disorder seen in one human family, it was linked by 2point lod-score to human chromosome 11q23.2-24, but no mutation in surrounding genes were identified(Vaughan et al. 2001). FAA1 cannot yet be mapped in the horse as no gene has been identified but genes located on either side of FAA1's position on the human map have been mapped in the horse. These genes are Ferredoxin-1 (FDX1) located on human chromosome 11 at q22 (Morel et al., 1988) and, Apolipoprotein A-IV (APOA4) located on human 11 at q23. FAA1 falls in sequence; mapping to HSA 11 at q23.2-24. Human chromosome 11 has been comparative mapped to Equine 7 (Milenkovic et al., 2002) and APOA4 and FDX1 fall above and below, respectively where TKY035 is on ECA7. The distance between TKY035 and LEX015 in cM is unknown, however according to the RH map (Chowdhary et al., 2003) they are 37.1 cR apart. Variability at LEX015 does not fit the expectation of linkage disequilibrium to DSLD, however, it may be too distant from TKY035 to indicate that. There is not likely to be a gene for DSLD in this area, especially if the DSLD gene is telomeric to TKY035.

ECA11 has three microsatellites with statistically significant higher homozygosity seen in the affected as compared to the unaffected. ASB35 had a statistically significant excess of homozygosity in the affected group compared to the unaffected (p< 0.01). The microsatellites on either side follow the pattern expected for linkage. TKY033/32 showed significantly more homozygosity in the affected group than the unaffected groups (p<0.001) and SGCV013 also had a statistically significant excess homozygosity in the affected group compared to unaffected group (p<0.001). As discussed in chapter three, COL1A1 is an excellent candidate gene in this region of ECA11. Mutations in this gene are responsible for connective tissue disorders in humans including (EDS I) (Nuytinck et al., 2000), (EDS VIIA) (Eyre et al., 1985), and Caffey syndrome (Gensure et al., 2005). Further investigation of the

region using methodologies such as bacterial artificial chromosome clones or sequencing portions of the COL1A1 gene to look for SNP's to compare between the affected and unaffected groups would be the next step for this region of the chromosome as there are no other microsatellites mapped in this region that would be closer to the candidate gene. If a statistical difference is seen in the SNP's between the two groups they could be used as a potential marker and eventually lead to the mapping of the actual DSLD gene.

In depth investigation of ECA14q took place in this study in part as a result of pathological examination of the tendons of DSLD affected horses in a study led by Jaroslava Halper, MD. PhD Associate Professor in the Department of Pathology of the Collage of Veterinary Medicine University of Georgia, showed accumulated deposits of what she reported as an unidentified proteoglycan obliterating what should be collagen tissue. Dr. Halper states that the accumulation of proteoglycans would lead to disruption of normal connective tissue structure (Halper et al., 2005). This information was the basis for CSPG2 being considered as a serious candidate gene for DSLD.

Located on ECA14q, the microsatellite AHT83 was 53% homozygous in affected individuals and 22% homozygous in unaffected individuals. The candidate gene Chondroitin Sulfate Proteoglycan 2 (CSPG2) is located at ECA14q26-q27, approximately 22.5 cM from AHT83. As discussed in chapter four, CSPG2 is a protein coding gene and an aggregating proteoglycan. Large chondroitin sulfate proteoglycans were first identified in hyaline cartilage, where they specifically interact with hyaluronan and form large supramolecular complexes. Together with other matrix glycoproteins, they provide mechanical support and a fixed negative charge. Such molecules exist also in a variety of soft tissues where they may play additional physiologic roles (Kjellen and Lindahl, 1991). Proteoglycan is found in connective tissues, in fibrous, articular, and elastic cartilages, in the

central and peripheral nervous system, in the epidermis, and in all three wall- layers of veins and elastic arteries (Bode-Lesniewska et al., 1996).

In this study, an intronic portion of the G domain of CSPG2 was sequenced in seven affected and six unaffected horses. One SNP at position 157 on the consensus sequence and one deletion at position 362 on the consensus sequence were found and compared between the two populations. Neither the SNP nor the deletion showed a correlation between affected or unaffected and ultimately did not support linkage of DSLD to the CSPG2 gene. Since both the SNP and the deletion marker occur in the DNA sequence and are thus closer to the suspected region for a mutation in CSPG2 than any microsatellite marker tested in the area, they would have to show stronger evidence of linkage than a microsatellite marker if linkage were present (unless the SNP or T delete is newer than the DSLD gene mutation). Given the sequencing results, CSPG2 is not likely the gene responsible for DSLD.

Both AHT83 and COR002 (located on ECA14q) had higher homozygosity in affected individuals, as compared to unaffected. AHT83 was 53% homozygous in affected individuals and 22% homozygous in unaffected individuals. COR002 was 65% homozygous in affected individuals and 25% homozygous in unaffected individuals. AHT83 and COR002 are less than 62.9 cM from each other based on linkage mapping from COR002 to the closest mapped microsatellite to AHT83 on the side farthest from COR002 (Penedo et al., 2005) as compared to RH map (Chowdhary et al., 2003), which shows the two as being 96.2 cR apart. The microsatellites mapped between AHT83 and COR002 (AHT88, HTG018, LEX078, TKY491, TKY749, and TKY438) that were run on both affected and unaffected groups did not have statistically significant differences in total heterozygosity based on Chi-Square test nor did they have significant differences between the affected and unaffected groups for homozygosity of the most common allele (of affected group). It is not likely that both

AHT83 and COR002 are related to DSLD since microsatellites between them do not support the pattern of variation that would be seen with linkage to a gene. As discussed in chapter three, multiple candidate genes have been mapped to ECA14q or are expected to be there based on the human chromosome 5 map, however there is no good evidence to support linkage of any of them to the DSLD gene.

All three microsatellites from equine chromosome 26 tested on affected and unaffected groups had statistically significant excess in homozygosity seen in the affected group. The Chi-Square value was 5.918 for EB2E8 ($p < 0.05$). NVHEQ70 and A17 had homozygosity of the common allele Chi-Square values of 6.36 and 4.45 ($p < 0.05$) respectively. As discussed in chapter three, COL6A1 is a possible candidate gene and mutations in it are associated to OPLL, a disease in humans involving the ossification of spinal ligaments. Due to the location of COL6A1 based on human comparison mapping (Chowdhary et al., 2003), it is a good candidate gene, and warrants further investigation.

Following all of the leads produced by the results of the genome scan is not with in the scope of this study. There is more work that needs to be done on DSLD, beginning with testing the above-mentioned microsatellites, and perusing the regions on chromosomes 6, 7, 11, 14 and 26. Given the statistical information and the presence of candidate genes, ECA6 and ECA11 should be the first chromosomes to continue with.

The possibility of a protector gene (A gene that suppresses the phenotypic expression of another gene, especially of a mutant gene) also should be considered, given the statistically significant homozygosity seen in the unaffected horses on ECA18 (LEX54), ECA28 (NV54) and the statistically significant increase in common allele frequency seen in the unaffected group on ECA1 (TKY007), ECA4 (LEX33), ECA9 (TKY627), ECA18 (HTG28), and ECA24 (AHT004). Protector genes have been seen in association with

multiple sclerosis (Madigand et al., 1982) and with ankylosing spondylitis (Cipriani et al., 2003) which is (according to the National Ankylosing Spondylitis Society) painful, progressive, rheumatic disease that affects the spine but it can also affect other joints, tendons and ligaments. Other areas, such as the eyes, lungs, bowel and heart can also be involved.

Subgroup Genomic Scan

Because DSLD is difficult to diagnose and its onset can be well into the second decade of life, concerns of the integrity of the unaffected group were always present in the background. It is quite possible and somewhat expected that a small percentage of the unaffected group will ultimately be diagnosed with DSLD despite the expertise of the veterinarian making the diagnosis of unaffected. Regardless of this possibility, differences between the two groups will be seen and the results of statistical significance just as powerful. However, in order to reduce the possibility of analyzing misdiagnosed samples, 10 horses from the affected population with severe DSLD in all 4 legs and 10 horses from the unaffected population that were either older than 20 years or had a history of heavy work with no signs of DSLD were compared using the same statistical analysis. This was done to increase the likelihood of differences between the two populations in hopes of unveiling new areas of interest or reinforcing the current areas. No new information was derived from this side study and the results of the data are not included in this study.

Further Research Recommendations

As stated previously, the microsatellites mentioned above, which are located in areas where there are differences between the affected and unaffected groups, should be tested to

exclude or include those regions. The COL1A1 gene should be considered for further investigation using methodologies such as BACs or sequencing to look for SNPs or a marker to show further evidence in that region if it exists. Since lesions have been found in the connective tissue of the heart in DSLD horses (Halper et al., 2005), it would also be advised to go to great lengths to get necropsies done on Peruvian Paso horses that die suddenly of unknown reasons to see if aortic aneurysm is the cause. The collection of family information for further pedigree analysis and possible family studies should be continued. It may also be useful to get updated information on horses used in this research to make sure none of the unaffected horses became affected with DSLD after submission of the samples. If an excess of those horses did change their status, the research data may need to be revisited.

Why were there no markers found?

With such a large number of microsatellites run in this study, why was no association of DSLD with any marker found? There are a couple of possible answers, the most likely being that no microsatellite was close enough to the DSLD gene to show linkage disequilibrium. If the estimation of 20cM as a reasonable distance to observe linkage disequilibrium (that is 20 million base pairs) is too distant, then linkage may have been missed. In addition, it is possible that the gene for DSLD occurs in a region of the horse genome where no marker has been mapped. At the time this study was undertaken, there were still significant gaps in the horse gene map. Another alternative is that DSLD could have an autoimmune component to it as seen with human disorders such as Rheumatoid Arthritis and Scleroderma, both of which are diseased that occur at varying ages. However,

no inflammatory response was seen in the histology of affected horses as would be expected with an autoimmune disease (Halper 2005, personal communication). Finally, as with Ehlers-Danlos disease, DSLD may be a group of disorders caused by different mutations in different genes. In this case, the different disorders would have to be recognized and separated before a whole genome scan approach could be utilized. This would not appear to be a very likely possibility due to the high degree of relationship among all members of the breed and the apparently recent origin of DSLD.

Appendix I List of Microsatellites and results for affected and unaffected groups by chromosome.

ECA1

ACCNO	VIASH34	ASB41	LEX020	NV100	COR100	COR059	UCDEQ487	TKY007	AHT021	ASB08	LEX058
A3-001	144/146	156	204/218	201	220/224	277/285	149/151	160	207/215	140/148	230/236
A3-002	156/158	156	200/218	203/219	218/224	277/287	147	160	215/221	164	230/240
A3-003	148	164	214/216	203/207	224/228	277/289	151/157	160	221/223	148	228/230
A3-004	144/156	156	216/218	207	218/228	277	147	160	213	156/164	230/240
A3-005	148/156	164/166	200/216	201/209	218/220	277	149/157	160	219	150/164	240
A3-006	148/154	164/166	212/218	197/217	222/228	277	155/157	154/160	219/223	158/164	
A3-007	158	156/164	200/214	197/217	212/224	277/289	147/157	148/160	215/219	148	240
A3-008	148	162/164	214/216	207	218	277	147	160	219	148/156	
A3-009	148/156	156/164	214/220	197/207	218/224	277/287	147/153	160	211/219	148	230/240
A3-010	144/154	156/164	200/216	201/209	218/224	277	147/149	154/160	215/219	148	230/234
A3-011		162/164	204/216	197/209	224	277	147/157	160	219/223	156	230
A3-012	156/158	156/162	216/218	207/217	218/224	277	147/149	160	207/221	164	238/240
A3-013	156/158		204/214			277	149/157	160	219/223	148/164	230/234
A3-014	146/156	156/164	214/220	203/215		277	157	160	221/225	148	230
A3-015	156/158	162	200/216	201/209		277	153	160	219/221	148/154	230/236
A3-016	148/150	164	214/218	201/209	218	277/287	147/149	146/160	207/223	148	
A3-017	146/154	162/164	216/218	201/217	218/224	289	147/149	146/160	211/219	148/150	230/232
A3-018	148/158	162/164	216	215/217	214/222	277/287	149/155	160	223	148/156	236/240
A3-019	146/156	162/164	210/218	215/217	212/228	277/287	147/157	160	217	156	240
A3-020	144/154	164/166	214/218	197/217	218/224	277/287	149/155	154/160	207/219	148/156	240
A3-021	146/156	156	200/220	215	220/224	277/287	143/157		211/223	164	
A3-022	148	164/166	204/216	201/203	224/228	277/287	147/151		219	156	
A3-023	154/158	162	214/216	205/207		277	147/157		219	150/156	
A3-024	158	162/164	214/216	203/207	218/224	277/289	149/153	154/160	211/219	148	230/236
A3-025	156/158	162/166	200/216	197/203	218		147/157		215/219	150/156	
A3-026	148/154	164	200/204	203/207	218	277	147	160	215/219	148/164	230
A3-027	144/156	156	218	197/217	224	277/289	149	154/160	211/219	148	230
A3-028	148	156/164	200/216	197/217	222/224	277	149/155	154/160	219/223	150/164	230/238
A3-029	148/158	164	214	217	212/228	277/289	149	160	219/223	148/156	230/240
A3-030	148/156	156/166	214/220	217	214/224	287	149/151	160	207/211	148/164	230/232
A3-031	154/158	164/166	200/220	207	218		147/155		211/215	148/156	
A3-032	146/154	164	200/220	205/207	218/224	277	147/149	154/160	215/225	148/156	234/240
A3-033	154/158	164	200/214	207/217	218/228	277/287	151/153	160	211/215	148/150	230/238
A3-034		164/166	200/216	201			149/155		219/221	148	
A3-035		156/166	218/220		224		147/149	160	211/219	148/150	
A3-036	156	156/166	200/220	201/219		277	147/157	160	215/221	148/164	
A3-037	154/158	162/164	216/218	207	218/222	277/287	147/151	160	215/219	148/164	230
A3-038	148/156	156/164	214/220	197/207	224/228	277	149/157	154/160	207/219	148/156	240
homozygosity	**20%**	**35%**	**8%**	**25%**	**25%**	**53%**	**21%**	**66%**	**18%**	**39%**	**37%**

TKY002	ICA43	ICA25	TKY106	UCDEQ493	UM043	HTG12	UM004	UCDEQ440	HMS15	COR063
119/121	130	215	142	233/241	158	119	127/129	116/118	221/243	144
119/123	130/132	215	138	215	160	119	119/123	112	221/227	144
123	132	213/215	138/140	215/241	158/160	119/123	119/123	112/116	233/243	144
121/123	132	215	138	215/241	158/160	119	119/123	114/116	239/241	144
119/121	130/132	215	138	207/223	158/160	119	123/125	112/116	223/243	144
121/123	130/132	213/215	138/142	223	160	119	119/121	116	223	144
121/123	132	215	138/142	207/241	158	119	119/125	112/118	223/229	144
119/121	130/132	215	138/142		158	119	123	112/116	223/239	144
119/121	130	213/215	140/142	215/241	158/160	119	119/123	112/116	239/243	144
119/123	130/132	215	142	241	158	119	119/123	112	223/243	144
121	130	211/217	140/148	203/225	160	119	119/125	112/118	223	144
119/121	130/134	211/215	138/140	215/223	160	119	119/123	112	223/229	144
117/121	132	215	138/142	215/223	160	119	119/125	112	223/243	144
121/123	130/132	211	138/140	205	160	119	123/125		229/233	144
117/121	130/134	213/215	138/142	213/229	160	119	121		223/229	144
	130	213/215	138/140	213/229	160		123	116	239	144
123	130	213/215	138/140	205/223	160	119/127		112/116	223	144
121/123	130/132	211/215	138/140	207/217	158/160	119	125	116/118	223/239	144
111/121	132	215	138/142	207/215	158/160	119	119/123	114/116	229/239	144
117/121	132	213/217	140/142	207	160	119/123	119/123	114/116	243	144
123	130/132	215	138/142	207/223	160	119/123	119/121	116/118	223/243	144
121	132	215	138	241	158	119	121/123		223/233	144
121	130/132	211/215	138/140	203/241	158/160	119	121/123	112/116	223/233	144
119/123	130	211/215	142	241	158	119	123	112/116	223/239	144
113/123	132	213/215	142	207/233	158/160	119	125		229/239	
119-123	130/132	215	138/142	215/241	158/160	119	119/123	112/114	223/241	144
119-123	130	213/215	138/142	223/241	158/160	119	123	112	223/241	144
117/123	132	211/215	138/142	209/213	160	119/123	119/121	112/116	223/243	144
117/121	132	211/215	138/140	209	158/160	119	123/125	118	223/239	144
117/123	130/132	211/213	138/142	213/223	160	119/123	119/123	112/114	223/241	144
121/123	130/132	211/215	138/140	203/215	160	119	119/123		223	
119/123	132/134	215	138/142	205/241	158/160	119	119	112	223	144
123	130/132	211/213	138/140	223	160	119	119	112/114	229/239	144
	130/132	213/215	140/142		160	119/121	119/121		223	
123	130	213/215	138/140	205/223	160	119/127	123		223	144
119/123	130/132	215	138	215/223	160	119	119/125	114/116	241/243	144
123	130/132	213	138	223	160	119	123	112	221	144
119/121	132	215	138/142	233/241	158/160	119	121/123	116	223/239	144
					1					
31%	**50%**	**45%**	**26%**	**28%**	**66%**	**79%**	**30%**	**35%**	**26%**	**100%**

ACCNO	VIASH34	ASB41	LEX020	NV100	COR100	COR059	ucdeq487	TKY007	AHT021	ASB08	LEX058
03-001						277/287		148/160			230
03-002								160			
03-003								160			
03-004						277/287		160			230/240
03-005						285		150/160			230/232
03-006						289		160			230/234
03-007						277/287		154/160			230/238
03-008						287		146/160			240
03-009						277		154/160			228/230
03-010						277/287		160			230/240
03-011						287/289		160			240
03-012						277/287		160			230/240
03-013						277/287		160			234/240
03-014						277		154/160			230/234
03-015						277		148/160			230
03-016						283/287		160			240
03-017						277/287		146/160			230/234
03-018						277/285		148/170			230
03-019											
03-020						287/289		148/160			228/240
03-021						277		160			230/236
03-022						277/287		160			240
03-023						277		154/160			230
03-024						277		154/160			230
03-025						277/287		154/160			236/238
03-026						285/289		148/160			234/236
03-027						277		160			234/236
03-028						277		154/160			230/238
03-032						287		148/154			234/238
03-033						287		144/150			230
03-034						289		148/160			232/240
03-035						277		154/160			240
homozygosity						**52%**		**39%**			**38%**

TKY002	ICA43	ICA25	TKY106	UCDEQ493	UM043	HTG12	UM004	UCDEQ440	HMS15	COR063
	130/132	215/217		217/225	160	119	119/123	116	211/231	144
	132	211/215	138/142	213/241	158/160	119/123	121		211/221	144
	130/132	213/215		215/241	158/160	119	119/121		229/231	144
	130	213/215	138	215/223	160	119	119/123	112	195/211	144
	130/132	213/215		207	158/160	119	123/125	112/114	211/217	144
	132	211/213	138/142	205/207	160	119	123	114/116	217/227	144
	130	213/215	138	207/217	160	119	123	112/116	211	144
	130/132	213/215	138/140	205/207	158/160	119/121	119/125	112/118	211	144
	130/132	211/217	142/142	207/213	160	119/123	119/123	112/114	221/227	144
	134/136	209		205/207	160	119/121	119/123	112/114	217	144
	132	215	140/140	233/241	158/160	119	119/123	112	217/227	144
	130/132	215/217		223/225	160	119	119/123	112/114	211/227	144
	130	213/217	140/142	223/241	158/160	119/123	119/123	112	211/217	144
	132	211	140/142	209/233	158/160	119	123	114/118	211/221	144
	130/132	215		207	158	119	123	112/118	211/231	144
	132/134	215	138	205/245	158/160	119	119/123	112	211/227	144
	130/132	215/217	140/142	223/233	160	119/123	119/125	112/116	217	144
	130/132	213	138/142	209/223	160	119	123	112/116	211/217	144
	132/134	211/215		213/223	160	119/123			211	144
	130/132	215		215/233	158/160	119		112/116	195/217	144
	130/134	211/215	140/142	205/241	158/160	119	119	112/114		144
	130/132	215/217		215/225	160	119	119	112		144
	132	211/215		213/241	160	119/123	119/123	114/116	217/221	144
	132				160	119		114/116	211/231	144
	130/132	213/215	138	217/225	160	119	119/121	114/116	211/231	144
	130	211		213/241	158/160	119/123	123	114/116	227/231	144
	130	215/217		217/225	160	119		116	211/231	144
	130	213/215		205/217	160	119/121		112/116	211/227	144
	130/134	211/215		223/233	158/160	119/123		112/116		144
	130/132			225/241	158/160	119		112		144
	132	215/217		209/225	160	119		112/114		144
	132	215/217		225/241	158/160	119		112		144
	44%	**27%**	**69%**	**6%**	**56%**	**56%**	**46%**	**31%**	**25%**	**100%**

ECA2

ACCNO	COR065	ASB18	COR037	TKY024	ASB17	HMS51	UCDEQ380	COR049	A14	ASB13	TKY335
A3-001	284/292	197/201	254/254	158	99/103	180/182	141	200	230	141	255/265
A3-002	284	199/203	250/252	158	99	172	139	200	232/246	131	257/265
A3-003	280/284	197/211	252	158	99/113	172/182	139	200	230/232	137/141	261/265
A3-004	284	197/211	250/252	158	99	172/182	137/139	200	232/246	131/137	263/265
A3-005	284	197	238/250	158	113/115	182	139	200	230/232	137/141	261/263
A3-006	284/292	203/211	256	158	105/123	170/182	139	200	230/244	131/135	257/263
A3-007	280/284	197/203	256	158	99/123	170	137/141	200	230/232	131/137	257/265
A3-008	280	201	254/254	158	113/125	170/182	141	200	232	137	265
A3-009	284	201	250/252	158	103/111	170/182	139	200	232	137	263
A3-010	280/284	197	252/256	158	99	172	139	200	232	137	253/265
A3-011	284/292	197/203	250/256	158	99/103	172/182	139	200	224/246	131	257/261
A3-012	284	197/203	250/256	158	99/115	172	137/139	200	232/244	135/135	257/261
A3-013	280/284	197/211	250	158	103/117	172	139/141	200	230/232	135/141	261/265
A3-014	280/284	197/203	250/252	158	117/123	172/182	137/141	200	220/232	131/137	261/265
A3-015	280	193/197	250	158	99/113	172/182	139	200	230/232	131/141	261/263
A3-016	280/292	197/203	256	158	99/107	172/182	139	200	232	137	265
A3-017	284	203	250/252	158	99/115	172/182	137/141	200	222/244	131/135	265
A3-018	280/292	203	250/256	158	105/125	170/182	137/141	200	230/232	137/141	263/265
A3-019	280/284	203	250/256	158	113/123	182	137/141	200	230	137/141	265
A3-020	284/292	211	250/256	158	105/113	182	139/141	200	230/244	131	261/265
A3-021	284/292	193/201	252	158	99/115	172/182	139	200	230/244	131/135	257/265
A3-022	284/292	201/207	252	158	99/113	172/182	139	200/202	232	131/137	257/263
A3-023		207/213	252	158	115/121	180/182	139/141	200		135/141	263
A3-024	284/292	197/203	250/252	158	115/125	172/182	139	200	230/232	131/137	263
A3-025	280	201/207		158	107/115		139	200	230/232	131/137	
A3-026	284	199/203	250/252	158	99	172	139	200	224/232	131/137	263
A3-027	280/284	197/199	250/252	158	99	172/182	137/139	200	232/246	131/137	257/263
A3-028	284	197/203	252	158	99/115	172/182	139	200	220/232	131/137	257/265
A3-029	284/292	199/203	250	158	117/123	170/172	139/141	200	230/232	137/141	257/263
A3-030	284/292	199/203	250/252	158	99/125	170	137/139	200	244/246	131/135	253/263
A3-031	280/284	197/199		158	105/121			200	224/232	131/141	
A3-032	284/288	197/211	250	158	103/125	182/184	137/141	200	224/232	131/141	261/261
A3-033	290/292	203	252/256	158	99/115	182	139/141	200	224/244		257/263
A3-034	280/284	197/207		158	107/117			200	224/230	131	
A3-035	284/292	199/203	252	158	99/113		137/141	200	222/246	131	265
A3-036	276/284	197/211	250/252	158	99/103	172/182	139	200	230/246	131/141	261/265
A3-037	280/284	199/201	252	158	99/115	170/172	139/141	200	232/246	131/137	263/267
A3-038	280	199/201	250/256	158	99/125	172/184	139/141	200	232	137	265
homozygosity	**34%**	**24%**	**45%**	**100%**	**13%**	**39%**	**53%**	**97%**	**24%**	**32%**	**34%**

TKY497	TKY798	TKY842	COR094	UMNEQ76	COR035	COR026	COR043
284/288	247/253	95/109	293	106	215	234	139
280/288	253	95	293	106	215	234	139/141
282/284	247/253	99/103	293	106	215		139/141
284/288	253	95/109	293	106/108	215	234	139/141
284	253	103/109	293	108/110	215	234	139
284/286	253	95/99	293	106/110	215		139
286	227/253	103/109	293	106/108	215	234	139/141
284/288	253	95/99	293	108	215	234	139
284/288	247/253	99/109	293	108	215	234	139
284/288	247/253	99/103	293	106/108	215	234	139
282/284	249/253	103	293	106/108	215	234	139
280/284	253	95	293	106/108	215	234	139
284/288	247	95	293/295	106/108	215	234/236	139
282/286	253	103	293	106/108	215	234	139/141
284	247/249	95/103	293	108	215	234	139
280/284	253	95/111	293	108	215	234	139
284/288	253	95/99	293/295	108	215	234/236	139/141
282/284	249/253	99/109	**/**	108/110	215	234/236	139/141
284	247/253	95/99	293	106/108	215	234/236	139/141
284/288	247/253	95/99	293	106	215	236	139/141
286/288	247/249	103/111	293		215	234	139
	247/249		293		215	234/236	139
284	247/249	99/103	293		215	234/236	139
284	247/253	99/109	293	108	215	234	139
			293		215	234/236	139
284/288	247/253		293	106/108	215	234	139
284/288	249/253		293	108	215	236/238	139/141
288	249/253	95/103	293	106/108	215	234/236	139/141
284	247/253	95/103	293	106/108	215	234/236	139
284/288	247/253	109/113	293	106/108	215	234	139/141
			293		215	234	139
288/290	249/253	95	293	106/110	215	234	139/141
288	247	99/107	293	106/108	215	234	139
					215	234	
288	253	95/99	293	106/108	215	234	139/141
284/288	253	95/103	293	106	215	234	139/141
288	253	95	293	106/108	215	236	139/141
286/288	253	103/109	293	108/110	215	234	139/141
39%	**43%**	**34%**	**92%**	**47%**	**100%**	**74%**	**55%**

ACCNO	COR065	ASB18	COR037	TKY024	ASB17	HMS51	UCDEQ380	COR049	A14	ASB13	TKY335
03-001		211	250/252	158		170/182	139/141	200			257/265
03-002	276	203	252/256	158		**/**	139/141	200			257/263
03-003	282/290	197/201	**/**	158		172/182	139	200			**/**
03-004	276/294	199/199	250/252	158		170/172	139/141	200			261/265
03-005	282/290	203	250	158		170/182	137/139	200			**/**
03-006	278/290	203	250/252	158		172/182	139/141	200			263/265
03-007	282/290	203	250/256	158		172/182	137/139	200			257/261
03-008	280	201/211	252/256	158		172/182	137/139	200			257/265
03-009	276	203	252/256	158		170/172	137/139	200			263/265
03-010	280	197/211	256	158		172/182	139/141	200			263
03-011	280	207/211	252/256	158		172/182	141	200			263/265
03-012	282/290	201	250	158		172	137/139	200			263/265
03-013	282/284	201/207	250/252	158		172/182	137/139	200			263/265
03-014	278/282	203	250/256	158		170/182	141	200			261/265
03-015	280	203	252/256	158		170/172	139/141	200			263/265
03-016	284/292	197/207	252/256	158		172/182	141	200			261/265
03-017	276/282	199/201	252/256	158		172/182	139	200			265
03-018	278/282	197/211	250/252	158		182	139/141	200			
03-019	280/290	201/211	**/**	158		**/**	**/**	200			
03-020		197	250/252	158		170/182	141	200			261/263
03-021	282/290	199/211	252/256	158/160		172/182	141	200			265
03-022	282/290	203	250	158		170/172	139/141	200			257/261
03-023	284/292	203	250/256	158		170/182	137/141	200/204			261/265
03-024	278/282	199/203	**/**	158		170/182	**/**	200			
03-025	286/294		252/256	158		172	139	200			257/265
03-026	278/292		252	158		170/182	137/139	200			265
03-027	284		250	158		182	139/141	200			257
03-028	280/284		250/252	158		182	137/139	200			257
03-032	280/284		252/256	158		180/182	137/139				261/263
03-033	280/292		236	158		182/184	137/137				257/261
03-034	280/284		250	158		168/170	137/137				263/265
03-035	280/284		252/252	158		170	137/139				257
	280/284										
homozygosity	34%	24%	45%	100%		39%	53%	97%			34%

TKY497	TKY798	TKY842	COR094	UMNEQ76	COR035	COR026	COR043
280/286	249/253	95/109	293/301	106/110	215	234	139
284/288	247/249	**/**	293	106/110	215	234/236	**/**
290/290	253	**/**	293	106	215	234/236	**/**
288	253	95/103	293	106	215	234/236	139/141
286	253	99/103	293	106/108	215	234	139/141
284	253	103	293	106/108	215	234	139
284/288	247/253	95/103	293	106/108	215	234/236	141/141
288	227/253	103/109	293	106/108	215	234	139
286/288	249	99/111	293	106/110	215	234/236	139/141
284/288	249/253	95/99	293	106/108	215	234	139
284/288	253	103	293	108	215	234/236	139
284/288	253	95/103	293	108/110	215	234	139
284/288	247/253	101/103	293	106/108	215	234/236	
284/288	247/253	103/109	293/301	106	215	234/236	
284/288	253	99/103	293	106/108	215	234	
288/290	253	95	293	106	215	234	139/141
284/288	247/253	95	293	106/108	215	234/236	139
284/288	247/253	95	293/295	106	215	236/238	139
	247/253		293		215	234/236	139/141
284/288	247/253	99/109	293	106/108	215	234/236	
288	253	95/99	293	106/108	215	234/236	
280/288	253	95/103	293	106	215	234	
284/288	247	99/103	**/**	106/110	215	234	139
	249/253	95/99	293	106/110	215	236	139
284	249/253	95/99	293	88/106	215	236	139
284/288	247/251	103/109	293	106/108	215	232/234	
280/288	249/253	95	293	88/106	215	234	
288	249/253	95	293	88/106	215	232/234	
284	247/253	109/111	293	108	215		
284	253	95/113	293/295	106/108	215		139
284/288	253	99		108	215		139
284/288	249/253	103	293	108	215		139/141
39%	**47%**	**34%**	**92%**	**47%**	**100%**	**74%**	**55%**

ECA3

ACCNO	AHT036	COR028	COR033	SGCV33	AHT022	UCDEQ437	AHT097
A3-001	141/147	239	238/252	114	203	177/187	157
A3-002	147/157	247/253	240	118/120	197/203	177	171
A3-003	145/147	241/247	242/248	118/120	203	181/187	157
A3-004	145/157	239/247	238/240	118/120	203	187	157/171
A3-005	145/147	241/249	242/244	118/120	197/203	181/187	167/171
A3-006	143/157	243/247	242/248	120/122	197/203	177/189	157/171
A3-007	145/151	241/249	242/244	118/120	203	179/181	157/169
A3-008	151	239/243	242/244	120/122	203	177/181	153
A3-009	143	245/249	242/244	118/120	203	179/181	157/173
A3-010	145/149	233/237	244/252	114/120	203	177	157
A3-011	143/147	241/249	244/252	114/120	203	177/181	157/171
A3-012	147/157	241/249	242	120/122	197/203	177/187	167/171
A3-013	145	241/245	242	118/120	203	181/187	157/171
A3-014	145/149	239/247	240/248	118/120	203	187/189	157/173
A3-015	143/147	247	240/248	120/122	203	181/187	157/173
A3-016	151	241	244	118/120	203	177/179	157/171
A3-017	149/155	239/241	244/248	120	197	177/181	157
A3-018	147	239/245	242/252	114/118	203	177/187	157
A3-019	149/155	239/247	242/248	118/120	203	181/187	157/171
A3-020	153	247/253	242/248	114/120	197	177/189	165/171
A3-021	145/157	241/247	244/248	120/122	203/205	177	157
A3-022	145/151	241/247	244/252	118/120	203	177	157
A3-023	143/151	241/245	242/244	120	203	177/179	157
A3-024	149/151	241	244/248	118/120	203	181/187	157
A3-025	143/147	239/241	248/252	118/120		179/181	
A3-026	143/151	241/245	242/244	118/120	203	179/187	157/171
A3-027	147/157	245/247	242/252	118/120	197/203	177/181	157
A3-028	143/147	247	242	118/120	203	179/181	157
A3-029	151/155	247/249	242/248	118/120	203	181	157/171
A3-030	143/157	239/249	242/248	118/120	197	177/179	157
A3-031	149/153	0.6/245	242	120		177/181	
A3-032	145/153	239/245	242/248	118/120	197/203	179/181	171/173
A3-033	143/151	241/245	244/248	118/120	203	179/181	157
A3-034	143/151	239/245	242/248	118/120		177/187	
A3-035	155/157	239/241	244/248	120	197	177/181	157/165
A3-036	145/157	241/247	242/248	120	197/203	167/177	157/171
A3-037	147/149	241	244/248	118	203	177/187	157
A3-038	145/149	241/247	242/244	118	203	177/181	157/173
homozygosity	**16%**	**16%**	**16%**	**21%**	**77%**	**16%**	**46%**

174

ACCNO	AHT036	COR028	COR033	SGCV33	AHT022	167/179	AHT097
03-001	145/151	147/153		120/122	203	179/181	165/171
03-002	143/151	243/249		118/120	203	177/181	157
03-003	143/157	249		118/120		187	157/171
03-004	147/157	249/253		120	203	187/189	171
03-005	145/151	241/243		120	203	179	157/171
03-006	145/151	243		120	203	181	157/173
03-007	143	243/249		120	203/205	181	157/173
03-008	151	239/241		120/122	203	179/187	157/173
03-009	149/151	241/247			203	167/177	157/165
03-010	147/149	249/253		120/122	203	179/189	157/165
03-011	145/151	241/247		114/118	203	181/187	165/171
03-012	143/149	237/241		120/122	203	177/179	157/173
03-013	145/149	249		114/120	203	177/181	167/171
03-014	143/151	241		120/122	203	181/187	157/169
03-015	151/153	243			203	177/179	171/173
03-016	145	249/253		114/118	203	177	157
03-017	151/155	241/247		114/118	203	177/181	165/171
03-018	145/155	241/247		120	203/205	181/187	171/173
03-019	151/153	241		118/120		187	157
03-020	147/151	239/241		118/120	203	181/187	157/171
03-021	149/153	239/274		120/122	197		157/167
03-022	147	249/253		120	203	179/187	171
03-023	145/151	239/241		120/122	203/205	181	157/165
03-024		243		114/120		181	157/165
03-025	151/155	241		114/118	203	181/189	157/171
03-026	149	237		120	197/203	181	157
03-027	147/151	241/249		118/120	203/205	181	165/169
03-028	143/151	249		118/120	205		157/169
03-032		241/249			203		167/171
03-033		241/249			197/203		157/165
03-034		247/249			203		157/173
03-035		247			203/205		157
		241/243					
homozygosity	**19%**	**34%**		**27%**	**78%**	**47%**	**22%**

ECA4

ACCNO	AHT043	HMS06	COR089	ASB22	LEX033	HTG07	HTG22	SGCV23
A3-001	178/186	159/169	284/296	161/167	205/219	126/128	190/194	203/225
A3-002	186/188	161/169	284/294	173	205/219	120	190/194	205
A3-003	186/188	161/169	292/294	159/179	207/217	128	194	203/205
A3-004	178/184	169	284/294	173	205/219	120/128	190/194	205
A3-005	184/196	161/163	284/294	173	205/219	120/128	190	203/217
A3-006	184	169	284/294	159	215/217	128	190	203
A3-007	184/198	161/169	292/294	173	205/215	120/128	188/190	203/233
A3-008	174/184	167	292/296	173	203/205	120/128	190	203/205
A3-009	184/196	169	286/294	173	213/215	124/128	190/194	
A3-010	184/186	159/169	284/294	159/173	205/215	126/128	190	225/231
A3-011	186/192	159/169	294/296	159/173	215/217	128	190/196	221/223
A3-012	186/190	163/169	284/296	173/179	205/207	120/128	190/196	221/223
A3-013	184/190	163/167	294/296	159/173	215/217	128	190/196	205/225
A3-014	184/190		284/294	173	205	128	190/192	217/233
A3-015	186/198	161/169	296/298	167/173	205/207	126/128	190/194	205/233
A3-016	184/188	167/169	284/294	159/173	205/217	126/128	190	203
A3-017	186/190		294	173	203/207	128/130	190/196	205
A3-018	188/190	159	294/296	169/173	203/215	120/128	190	235
A3-019	184/194		292/296	173	205/215	120/128	190	213/233
A3-020	184	169	282/292	159/173	205/215	120/128	190	
A3-021	176/184		282/294	173	205/215	120/128	188/190	
A3-022	184/186	161/169	292	159/173	207/217	120/128	192/194	205
A3-023	184/190	163/167	284/288	161/173	205/217	126/128	190/196	205/217
A3-024	184/186	159/169	284/294	173	205	120/126	194/196	221/229
A3-025	186/196	159/163	294	159/173	205/217	126/128	190/194	
A3-026	186/188	159/169	284/296	171/173	205/213	128	194	205
A3-027	184/186	169	292/294	173	205/215	120/128	194/196	205/213
A3-028	186/196	169	282/286	161/173	205/217	120/130	192/194	205/221
A3-029	184/186	163/169	286/290	161/173	215/217	124/128	190/196	219/225
A3-030	184/186	167/169	294/296	173	205/215	120/128	192/194	205/213
A3-031	184/186	169	294/296	159/173	215/217	120/128	190/194	217/231
A3-032	182/184	163/169	292/294	159/173	207/217	128	188/190	
A3-033	184/188	159/169	282/294	171/173	213/215	128	196	203/205
A3-034	182/184	169	286/294	169/171	205/213	126	190	217/231
A3-035	184/188	169	294	173	203/207	128	190/196	205/225
A3-036	188/194	163/169	284/294	169/173	207/211	126/128	190/196	205/217
A3-037	188/196	169	288/294	159/173	203/207	120/128	190/196	205/213
A3-038	182/184	167/169	284/294	173	205	128	190/194	205/213
homozygosity	**5%**	**35%**	**11%**	**42%**	**8%**	**32%**	**32%**	**24%**

ACCNO	AHT043	HMS06	COR089	ASB22	LEX033	HTG07	HTG22	SGCV23
03-001		167/169			205/217	128/130	192	
03-002		161/163			207/217	120/128	196	
03-003		163/167			207/217	128	194	
03-004		161/169			205	120	190/194	
03-005		159/161			203/215	128	194/196	
03-006		159/167			217	126/128	190	
03-007		163			205/207	126/128	190/194	
03-008		167/169			205	128	190/196	
03-009		163/169			205/207	120	194	
03-010		163/169			205	126/128	190/196	
03-011		167/169			205/207	128	188/190	
03-012		163/169			215/217	124/128	196	
03-013		159/161			205	128	190/196	
03-014		163/169			205/207	126/128	190/196	
03-015		159/169			215/217	124/128	190/194	
03-016		163/167			205/213	128	188/192	
03-017		161/163			205	128	190/196	
03-018		169				120/128	190/194	
03-019		159/167			205	128	190/194	
03-020		159/163			205	128	190/194	
03-021		167/169			205/215	120/128	190/194	
03-022		161/169			205/215		188/190	
03-023		163			205/217	120/128	190/194	
03-024		161/169			205/207	120/128	190/194	
03-025		161/163			203/205	120/128	192/196	
03-026		169			205/217	126/128	190/196	
03-027		159/167			205	128/130	190	
03-028		159/163			205	128	190	
03-032		167/169			203/205			
03-033		161/169					190/194	
03-034		163			205		190/196	
03-035							190/194	
homozygosity		16%			40%	44%	28%	

ECA5

ACCNO	AHT24	LEX004	VHL66	HMS05	LEX069	LEX034
A3-001	205	294	112	104	142	256
A3-002	203/205	294	112/144	104/110	142/154	254/260
A3-003	205/207	294	110/112	104	160	254/260
A3-004	205/207	294	108/110	110	142/154	256/258
A3-005	205	294	110/112	104/110	142/160	260/262
A3-006	205/207	294	112	104	160	254/260
A3-007	205/207	294	112	104/110	142/170	258/260
A3-008	203/205	294	112	104/110	142/154	254/260
A3-009	203/207	294	110/114	104		254/262
A3-010	205/207	294	112	104	142	256/258
A3-011	205/207	294	112/114	104	160	254/260
A3-012	203/205	294	112/114	110	160	258
A3-013	207	294	112	104	142/160	258
A3-014	205/207	294	110/112	104/110	160/170	254/262
A3-015	205	294	112	110	142	254/260
A3-016	205	294	112	104	142/154	254/260
A3-017	205/207	294	112	110	142/160	256/258
A3-018	203/205	294	112	104/106	160	258
A3-019	205/207	294	110/112	104/106	154/160	254/260
A3-020	203/207	294	112	104		254/260
A3-021	205/207	294	112	104		254/260
A3-022	205	294	110/112	104/110	142/160	260/262
A3-023	205	294	112	106	160	254/260
A3-024	207	294	112/114	104/110	160	254
A3-025	205/207	294	110/112	104		254/260
A3-026	205/207	294	110/112	104/106	142/160	258
A3-027	205/207	294	110/112	104/110	142/170	260
A3-028	207	294	112	104	160	258
A3-029	205/207	294	110/110	104/110	142/154	254/260
A3-030	205	294	110	110	142/170	254/260
A3-031	203/205	294	110	104	142/160	254/262
A3-032	205/207	294	112	104		254/260
A3-033	207	294	112	104/106	160	254/260
A3-034	203/205	294	110	106/110	142	254/260
A3-035	205/207	294	110/112	104/110	142/156	260/262
A3-036	205/207	294	112/114	104/110	142/160	258
A3-037	205	294	110/112	104/110	142/160	258
A3-038	205/207	294	112	104/110	154/170	260
homozygosity	**32%**	**100%**	**53%**	**53%**	**39%**	**29%**

ACCNO	AHT24	LEX004	VHL66	HMS05	LEX069	LEX034
03-001		294	110	104		
03-002		294	112	104/106		
03-003		294	112/114	106/110		
03-004		294	110/112	104/110		
03-005		294	112	104/110		
03-006		294	112	110		
03-007		294	112	106/110		
03-008		294	110/112	106/110		
03-009		294	110/112	104/106		
03-010		294	112/114	104/106		
03-011		294	112	104/110		
03-012		294	110	106/110		
03-013		294	110/112	104/110		
03-014		294	110/112	106/110		
03-015		294	110/112	104		
03-016		294	112	104		
03-017		294	112	104/110		
03-018		294	112/114	104/110		
03-019		294	110/112	104		
03-020		294	110	104/110		
03-021		294	110/112	104/110		
03-022		294	112/114	104/110		
03-023		294	110/112	104/110		
03-024			110/112	104/110		
03-025			110/112	104/110		
03-026			112	104/106		
03-027			112	104/110		
03-028			112	104/106		
03-032						
03-033						
03-034						
03-035						
homozygosity		100%	46%	18%		

ECA6

ACCNO	HTG31	COR010	LEX065	NV82	UM015	TKY111	NVHEQ81	UCDEQ465	COR070	COR070	TKY412	TKY284
A3-001	150/156	307	154/158	139	320/322	133/137	172	198/208	297	297	226/230	169/177
A3-002	148/154	297	154/158	135	316/320	137	172	206	281/299	281/299	228/230	175/179
A3-003	156	307/309	154/166	137/139	316/320	137	172	206/210	281/303	281/303	226	175/181
A3-004	154/158	299/307	154/158	137/139	316/318	137/139	172	206	281/305	281/305	228/230	175
A3-005		299	158	133/137	320/322	137	172	206	281/307	281/307	228/230	175
A3-006	148/156	307	158	137	322	137	172	206/210	295/307	295/307	230/232	175/177
A3-007	156/158	297/299	164	137	320/322	137/139	172	206	281/303	281/303	222/228	175/179
A3-008	154/158	297	154/158	135/137	314/316	137	172	206	297/303	297/303	228/230	175
A3-009	148/150	299/307	154/160	137	316/322	137	172	206	299/305	299/305	224/228	171/175
A3-010		299/307	162/166	139	320/322	137	172	206	297/303	297/303	224/228	171/175
A3-011	148/156	307/309	160	135/139	314	137	172/178	206/208	299/307	299/307	226/228	175
A3-012	154	299/307	154/166	135/139	314/320	137	172	206	281	281	228/230	175
A3-013	148/150	299	154/166	135/137	314/322	137	172	206/210	295/299	295/299	230/234	175/181
A3-014	150/156	299/307	162/166	139	316	137	172	206	299/307	299/307	230/236	175/181
A3-015	150/158	307	166	137/141	316/320	137	172	206/208	305/307	305/307	230/234	175/177
A3-016	150/154	299/307	154	133/137	318	137/139	172	206	297/299	297/299	228/230	169/175
A3-017	158	299/307	166	133/139	316/318	137	172	206	281	281	230	171/175
A3-018	150/154	299/307	154/166	137/139	316/318	137/139	172	206/208	303	303	224/230	175/179
A3-019	148/158	299/307	160/166	139	316	137	172	206	285/297	285/297	222/230	175/179
A3-020	148/154	307	158	133/139	314/320	137	172	206/210	295/307	295/307	222/232	177/179
A3-021	154/158	307/309	158	137/139		137	172	206	281/297	281/297	228/230	175/179
A3-022	148/150	299/307	158/160	133/135	322	137	172	206	281	281	226/228	175
A3-023	148/154	299/307	158/166		316/320	137	172	206	281	281	230	175
A3-024	148/156	307	166	133/137	316/324	137	172	208	299/305	299/305	222/228	169
A3-025	150/154	307/309		139			172	206/208	299/307	299/307		169/175
A3-026	148/158	307	154/160	137/147	316	137	172	206	301/305	301/305	226/230	175
A3-027	154/158	299/307	154/166	137/139	316/318	137/139	172	206	281/305	281/305	230	175
A3-028	150/156	299	154/166	133/137	318/324	137/139	172	206	281	281	230	175
A3-029	158	299/309	160/166	137	316/322	137	172	206	281/297	281/297	222	175
A3-030	148/154	299/301	154	135/137	316/324	137	172	206	297/305	297/305	230/236	169/175
A3-031	148/158	307		139			172	206	279	279		171/179
A3-032	154/158	299/307	154	139	316/322	137	172	206/212	295	295	228/230	177/179
A3-033	158	307	158/160	137	316/320	137	172	206/210	281/303	281/303	228	169/175
A3-034		307/309		137/139			172	206	305/307	305/307		169/175
A3-035	148/158	299	158/166	133/135	316/324	137	172	206/208	303/307	303/307	222/230	175
A3-036	158	299/307	154/166	137/139	316	137	172	206	281/307	281/307	230	175
A3-037	148/154	299/301	158/166	135/137	316	137	172	206	281/295	281/295	230/232	175
A3-038	154	299/307	154/166	137/139	318	139	172	206/210	299/303	299/303	230	169/175
homozygosity	**20%**	**37%**	**34%**	**35%**	**29%**	**80%**	**97%**	**66%**	**24%**	**24%**	**26%**	**39%**

ACCNO	HTG31	COR010	LEX065	NV82	UM015	TKY111	NVHEQ81	UCDEQ465	281/297	281/297	TKY412	TKY284
03-001			154/162	133/139	316/318	137	172	206	299/305	299/305	230	169/175
03-002			154/158	137/139	316	137/139	172	206/208			228/230	169/177
03-003				135/137	318/324	137	172	206	299/305	299/305	228/230	173/175
03-004			154/166	135/139	316/320	137	172	206	297/307	297/307	226/230	169/175
03-005			154/166	137/139	322/324	137	172	206/208	285/299	285/299	222/228	173/175
03-006			158/166	139		137	172	206	303/305	303/305	230	169/173
03-007			158/160	135/137	318/322	137	172	208	297/303	297/303	228	169/175
03-008			154	139	316/320	137/139	172	206/212	281/297	281/297	226/230	177/181
03-009			158/162	135/137	316	137	172	206/208	281	281	230	169/175
03-010			158	133/135	316/322	137	172		281/299	281/299	226/230	173/175
03-011			158	139	320	137	172	206/210	305/307	305/307	222/226	175/179
03-012			158/160	137/139	318	137	172	206/208	281/307	281/307	228/238	169
03-013			154/158	135/139	316	137	172	206/208	299/305	299/305		175
03-014			158/166	133/137	316	137	172	208/210	285/295	285/295	226/230	175/181
03-015			154/162	133/147	316/324	137	172	206/210	281/297	281/297	230/232	173/177
03-016			154/158	135/139	318	137/139	172	206/208	281	281	222/226	179/181
03-017			158	133/139	316/320	137	172	206/210	297/301	297/301	222/226	175/179
03-018			166	137/139	316/320	137/139	172	208/210	297/307	297/307	226/232	169
03-019			166	137/139	322/324	137/139	172	206	297/307	297/307		169/175
03-020			166	139	320/322	137/139	172	206	281	281	226/230	169/175
03-021			154/166	139	314/316	137	172	206	301	301	228/230	169/175
03-022			154/158	135	314/320	137	172	206	281/307	281/307	226/228	169/179
03-023			154/162	137/139			172	206/208			228/230	173/175
03-024			154/158	135/139	318	137	172		285/297	285/297	228/230	175
03-025			154/158	135/147	316	137	172	206	281/303	281/303	230	173/179
03-026			158/166	133/139	320/324	137/139	172	206	281/297	281/297	228/230	175
03-027			158/162	133/135	316	137	172	206	281/305	281/305	228/230	169/179
03-028			154/158	135/139	318/320	137	172	206/208			228/230	169/179
03-032			154/158	137/139		137	172	206/208			228	
03-033			158/166	139	316/322	133/137	172	206			228/230	169/183
03-034			166	137	316	137	172	206			228/230	175/183
03-035			158	135	314/316	137	172	206			226/228	169/177
homozygosity			**29%**	**28%**	**38%**	**74%**	**100%**	**50%**	**25%**	**25%**	**20%**	**19%**

ECA7

ACCNO	TKY34	TKY35	LEX015	TKY283	TKY005	TKY272	COR004	COR095	SGCV28	AHT019	VIASH7
A3-001	138/172	122	153	203	116	122	317	222	169	153	
A3-002	126/172	120/122	153	203/205	108/116	86/122		218/222	167/171	153	
A3-003	130/132	120/122	143/153	203	116	122	317	222	169	153	132
A3-004	126/168	122	153	203/207	108/116	86	311	218	167/169	153	132
A3-005	130	122	153	203	116	86/122	311	220	169	151	132
A3-006	130/146	120/122	153	203	108/116	122	317	220/224	169	151/153	132
A3-007	130/168	120/122	153	203	116	86/122	311	218	169	153	132
A3-008	130/168	122	143/153	203	116	122	317	220/224	159/169	151/153	132
A3-009	130/150	122	153/155	203/207	108/116	86/108	311	218/224	167/169	153	132
A3-010	130/160	122	143/153	203/207	108/116	118/122	317	222/232	159/169	151/153	132
A3-011	130/156	122	153	203	116	86/122	317	218	159/169	151	132
A3-012	130/172	122	153	203	116	122	317	218	169/171	149/155	132
A3-013	130/162	122	143	203	108/116	86/122	307	208/222	169	151/153	134
A3-014	130/164	122	153	203	116	122	317	222/226	169	151/153	134
A3-015	130	122	153	203	116	86/122	311	216/218	169	153	132
A3-016	142/168	120/122	153	203/205	108/116	122	317	222	169	151	132
A3-017	126/130	122	153	205	108	86		222	169/171	151	132
A3-018	126/142	122	143/153	203/205	108/116	122	317	224	171	153	132
A3-019	130/132	122	143/153	203/207	108/116	108/122	317	218/224	161/167	151	132
A3-020	138/142	122	143/153	203	116	122		218	169	151/153	132
A3-021	130/148	120/122	153	203	116	122		216/218	169/171	151	132
A3-022	130/166	122	143/153	203	116	122	317	218/222	169	151/153	132
A3-023	124/130	122	143/153	203	116	122	317	222	159/169	151	132
A3-024	166/168	122	153	203	116	122		222	169	151/153	132
A3-025	130	122	153	203/207	108/116	86	307	224		151	132
A3-026	126/166	122	153	203/205	108/116	86/122		222	169	151/153	132
A3-027	126/132	122	153	203/207	108/116	86/122		218	167	151/153	132
A3-028	130	122	143/153	203	116	86/122	307	218/226	169	151	132
A3-029	130/132	122	143/153	203	116	86/122		220/224	159/169	153	132
A3-030	130/132	122	153	203	116	122	317	218/222	165/171	151	132
A3-031	132/142	120/122	143/153	203/205	108/116	122	317	222/226		151	132
A3-032	126/132	122	153	203/205	116	122		218/226	167/169	153	132
A3-033	130/138	122	153	201/203	108/116	108/122	309	216/218	169	151/153	132
A3-034	126/132	122	153		116/118	86/122		222/226		151/153	132
A3-035	126/132	122	153	201/207	108	86/108	309	216/218	167/171	153	132
A3-036	126/172	122	153	203/205	108/116	86/122		222/226	169	151/153	132
A3-037	126	122	153	203/205	108/116	86/122		222	169	151	132
A3-038	130/132	122	153	203	116	86/122	309/311	218	167/169	151	132
homozygosity	13%	82%	68%	57%	55%	50%	96%	47%	54%	63%	100%

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ACCNO	TKY34	TKY35	LEX015	TKY283	TKY005	TKY272	COR004	COR095	SGCV28	AHT019	VIASH7
03-001		120/122	143/153	201/203	108/116	86/122			169	151	132
03-002		122	153	203	116	122			169/171	155	132
03-003		120/122	153	203	116	122			169/171	151/153	132
03-004		120/122	153	203/207	108/116	86/122			167/171	153/155	132
03-005		120/122	153	203	116	122			169	153/155	132
03-006		120/122	153	203	116	86/122			169	151/153	132
03-007		120/122	153	203	116	122			169	151	132
03-008		120/122	143/153	203/207	108/116	86/122			167/169	151/153	132
03-009		120/122	143/153	203/205	108/116	86/122			167/169	153	132
03-010		120/122	153	203	116	86/122			169/171	151/153	132
03-011		122	143/153	203	116	122			159/169	151/153	132
03-012		122	143/153	203	116	122			169	153	132
03-013		122	153	201/203	108/116	108			169	151/153	132
03-014		120/122	143/153	203	116	122			169	151/153	132
03-015		122	153	203/207	108/116	122			169	153/155	132
03-016		120/122	143	203	116	122			159	151	132
03-017		120/122	153	201/203	108/116	108/122			159/169	153	132
03-018		120/122	153	203	116	118/122			169	153	132
03-019		120/122	143/153	203/205	108/116	122			169	153	132
03-020		120/122	153	203	116	122			169	151/153	132
03-021		122	153	203	116	86/122			169	153	132
03-022		122	153	203	116	122			169/171	151/155	132
03-023		122	143/153	201/205	108	86			167/169	153	132
03-024		122	143/153	201/205	108/116	86			167/169	153	132/136
03-025		122	143	203	116	122			159/169	151/153	
03-026		120/122	143/153	203	116	122			169/171	151/153	
03-027		122	153	203	116	86/122			169	151/153	
03-028		120/122	153	203	116	122			159/169	151/153	
03-032									159/169	151	
03-033									159/169	151	
03-034									169	151	
03-035									169	151	
homozygosity		39%	64%	64%	68%	64%			50%	50%	96%

ECA8

ACCNO	AHT005	AHT025	UM034	LEX023	ASB14	SGCV32	COR003	COR056
A3-001	140	187	122/138	245	128	143/145	195/199	208/220
A3-002	134/142	183/185	122	247/257	128/134	133/145	195/209	212
A3-003	138/146	183	120/138	235/249	128/130	143/145	199/209	194/220
A3-004	132/142	183	138/140	245/257	128/132	133/143	199/209	212/214
A3-005	138/142	183/185	120/138	247/257	128	143/145	209	194/204
A3-006	140	183/185	120	245/257	128/134	145	195/199	208/220
A3-007	142/146	183/185	122/140	243/247	132	143	199	216
A3-008	138/142	183	122/138	249/257	132	143/145	201/209	202/214
A3-009	134/146	183	120/136	243/257	130/132	143/145	199	206/220
A3-010	134	183/187	122	235/249	132	143/145	207/209	212
A3-011	134/140	183/185	122	243/257	128/132	145	195/199	202/208
A3-012	142/146	183	122/138	247	120/128	145	195/211	212
A3-013	134/142	183	120/122	249	132	143/145	199/201	206/220
A3-014		183/187	122	243/257	128/134	145	207/209	206/214
A3-015	138/140	183/185	120/140	249	122/128	143	195/199	216/220
A3-016	140	179/183	120/122	235/257	128/132	143/145	201/209	202/208
A3-017		179/185	122/136	255	128/134	143	207/209	206/216
A3-018	142	183/185	138/140	245/255	128/132	143/145	209/213	214/220
A3-019		183	122	233/241	128/132	143/145	199/209	206/220
A3-020	132/140	183/185	122	235/255	128/130	145	199/207	208
A3-021		183/185	136/138	255	128	145	195/209	206
A3-022	142/146	183	122/140	255	126/134	145	199/209	202/212
A3-023	132/138	183/185	122	245/249		143/145	209	
A3-024	138	183	118/144	255	128	143/145	199/201	198/202
A3-025	134/140	187	122/138	247/255	128/132		209	194/214
A3-026	134/140	183/185	122/140	245/255	128/130	143/145	199/209	220
A3-027	134/140	185	122	255	128/134	143/145	207	198/206
A3-028	142	183	138	237/247	122/128	145	199/209	214
A3-029	138/140	183/185	122	243/257	134	143/145	199/209	208/220
A3-030	140/142	183/185	120/122	235/257	128/134	143/145	207/211	198/220
A3-031	140	185	122	247	122/132		209/211	202/214
A3-032	134/140	185/187	122/136	257	132	133/145	209/211	208
A3-033	140/142	183	120/140	235/245	130/132	143	199/209	206/220
A3-034	134/140	183	122/140	245/257	132		199/209	202/220
A3-035	140	179/185	122/136	257	128/134	143	207/209	206/216
A3-036	138/140	185	122/138	247/257	126/134	133/143	209	204/212
A3-037	140	185	122/140	245/249	132	133/145	201/209	206/212
A3-038	138/140	183	122	247/249	128/132	143/145	199/207	198/216
homozygosity	**29%**	**50%**	**34%**	**32%**	**32%**	**37%**	**18%**	**24%**

ACCNO	AHT005	AHT025	UM034	LEX023	ASB14	SGCV32	COR003	COR056
03-001	132/138	183	118/122		132	143		208/216
03-002	138/142	183	122/138		128/134	143		216/220
03-003	140	185/187	122/138		128/132	143		204/212
03-004	134/140	185	122/140		132/134	143		206/212
03-005	140/142	183	122		128	143		202/212
03-006	138/142	183/185	122/136		128/132	143/145		194/214
03-007	138	183/185	120/122		128	143		194/204
03-008	140	183/185	120/140		128/132	145		204/220
03-009	142	183	120/122		128	145		206
03-010	140/142	183	122/140		128/132	143		216
03-011	134/142	183/187	136/140		122/128	143		212/220
03-012	144	183	118/136		132	143/145		206/208
03-013	134/140	183	118/122		132			206/220
03-014	142/146	183	122/140		122/134	145		206/208
03-015	138/140	179/183	120/122		128/132	143/145		220
03-016	134/142	183	122		134	133/143		208/212
03-017	140/142	183	122/140		122/128	133/143		212/220
03-018	140/146	183	122/138		132/134	143/145		208
03-019	132/138	183	122/136		132/134	145		206/220
03-020	134	185/187	122/140		128/132	143/145		208/214
03-021	132/140	185	122		128	143/145		202/214
03-022	134	185	122/140		132	143/145		208/212
03-023	140/142	183	120/122		120/132	143/145		206/220
03-024	134		122		128/132	143/145		206
03-025	140/142	183/185	122		122/128	133/145		208/220
03-026	140/146	183/185	136/138		132/134	145		214
03-027	132/138	183/185	122		130/132	143/145		208/216
03-028	134	183	122		122/132	143/145		194/208
03-032	132/140		122		132	143/145		194/202
03-033	134		118/122		128/132	143		202/214
03-034	140/142		120/140		128/134	143/145		212/214
03-035			120/122		132	143		194/216
homozygosity	32%	63%	25%		34%	48%		19%

ECA9

ACCNO	HTG4	HMS03	COR008	TKY627	TKY533	COR013	HTG08	LEX070	UM037	AHT53	LEX019
A3-001	131/139	160/166	261	212/224	218/222	260/264	185	255	116/122	272/274	169/173
A3-002	131/133	166/168	273/275	212/226	216/220	260	189/195	245/247	124	272	169
A3-003	133	166/170	257	216	220/222	260/262	185/195	247/255	116/122	266/274	169/171
A3-004	133/137	164/170	257/261	216/220	222	262	195	245/247	124	272	169/173
A3-005	133	164/166	255/261	214/216	220/224	264	189/195	245/247	116/124	272/274	169
A3-006	133	166/170	261	220/222	220/224	262	185/197	245/247	116/124	272/276	169
A3-007	133/137	160/170	261/263	216/220	222	260	185/189	237/245	116	272/276	165/169
A3-008	129/131	170	257/261	216/222	222/224	260/262	195	245/247	118	266/272	169/171
A3-009	131/133	160/170	257/263	222	222	260		245/257	116/118	266/272	169/171
A3-010	137/139	166/170	257/261	216	222	260	189	235/247	120/124	272	169/171
A3-011	129/135	170	257/261	216/222	216/218	264	185/195	245/257	122	266/272	169
A3-012	129/131	166/170	261/275	222/224	220/224	262	185/195	247/249	124	272	169
A3-013	133	152/166	261		218		185/195	247	118/122	266/272	165/169
A3-014		164	255/263	212/232	222/224	260	195	247/249	116/124	272	169
A3-015	135/139	166	255/271	212/214	218/224	260/262	185/195	239/245	118/124	266/272	167/169
A3-016	129/133	170	261	222/224	224	262	195	249	118	272	169
A3-017		162/168	263/275	212/222	216/220	260	185/189	239/249	124	266/272	169
A3-018	133/135	160/166	257/261	222/226	222		185/197	247	116	272	169/173
A3-019		160/170	251/257	216/222	218/222	260/262	195	249/255	116	272	169
A3-020	131/137	166	261/273		220/222	260		247/249	116/118	272/276	169
A3-021		170	251/261	218/222	220/224	262		241/247	124	266/272	169
A3-022	133	168/170	257/263			260	185	247/255	122/124		169/171
A3-023	129/133	168/170	263		218	260	185	247	118/122	266/272	167/169
A3-024	131/133	166/170	257/261	216	218/222	260	185	247	116/122	266/274	169
A3-025	129/139	166	257/261			262		249			165/169
A3-026	133	152/170	261/273		218/224	260/262	195	249	116	266/272	169
A3-027	129/133	166/170	257/273	216	222	260/262	195	249	118/124	272	169/173
A3-028	129	170	261	222	224	262	197	249	124	272/274	169
A3-029	133	164/170	255/261	216/222	222	260	185/197	249/255	124	272	169
A3-030	133	166/170	255/257	212/216	222	260/262	185/197	249/255	124	272	173
A3-031	129	164/166	255/257			258	185/189	241/249			169/173
A3-032	131/133	160/164	255/263	218/232	218/224	258/260		249	118	272	165/169
A3-033	133	152/170	257/273	218/222	218/224	260	195	249	116	266/272	169
A3-034	131/139	164	255				189/195	247/249			165/169
A3-035	133/137	162/170	257/263	216/222	220/222	262	185/197	247/249	124	272/274	169
A3-036	133/141	164/168	261/275	212/214	216/220	260	189/195	249	116/124	272	169
A3-037	133/137	166/168	257/275	212/226	216/220	260/262	189	249	122	272	169
A3-038	137/139	166	261	212/220	220/222	260/262	185	247/255	122/124	272	169
homozygosity	**32%**	**29%**	**24%**	**20%**	**35%**	**69%**	**48%**	**37%**	**54%**	**44%**	**55%**

ACCNO	HTG4	HMS03	COR008	TKY627	TKY533	COR013	HTG08	LEX070	UM037	AHT53	LEX019
03-001	131/139	152/170		220/222	218/224	264	185/189	239/247	124	266	165/169
03-002	131/133	166/170		214/216	222	260	185	247/255	116/122	272/274	169
03-003	133	168/170				260	185/189	247/249	110		169/171
03-004	133/135	164/170		216	218/222	260/262	185/197	249/255	116/124	272	169
03-005	131/133	164/166		216		260/266	189/195	247/249		272/274	169/173
03-006	131/139	166/170		216	218/222	260	185	251/257	118	268/272	169
03-007	133/135	162/170		222/226	218/220	260/262	185/195	251	124	272	167/169
03-008	131/141	164/166		220/226	220/224	260/264	185/195	247/255	124	272/274	169
03-009	129/133	164/170		216/222	218/224	260/262	185/189	249/255	118/118	272	169/171
03-010	131/135	152/164		216	218	260	185	247	118/122	272	165/169
03-011	131/133	160/166		216	222	260	185/195	247/249	118/124	272	169
03-012	131	166/170		216/220	222	260	185/195	241/247	116/124	274	169
03-013	133	170		216	222	260	185/194	247/255	118/126	272/274	167/169
03-014	133	166/170		216/220	216/218	258/262	189/195	249/255	118/124	274/276	167/169
03-015	131/133	164/166			222	260	185		116/124	262/274	169
03-016	129/139	166		216	222	260	191/195	247/249	118/124	266/272	169
03-017	131/135	166		216	220/222	260/262	185/195	249/255	118/124	272	165/171
03-018	131/141	166		216	220/222	260/266	185/191	241/247	123	272	161/169
03-019	129/133	164/166				260/262	189/197	247/249			169
03-020	131/135	164/170		220/226	222/224	260/262	185/195	247/249	116/118	272/274	165/169
03-021	133/135	164/170		216/226	218/222	260	185/195	241/249	122	266/272	169
03-022	131/135	160/166		216/226	218/220	260/262	185/195	247/255	116/124	272	169
03-023	131/133	152/170		216/222	218/224	260	189/197	249	120/124	272	169
03-024	129/139	152/158				260		247	110/124		
03-025	131/135	166/170		216/228	218/222	260/264	185	247	124	266/274	167/169
03-026	131/141	166/168		212/218	218/224	264	189	247/249	118/124	274	167/169
03-027	129/133	164/170			218/224	262/264	185	247/251	118/122	266/272	169/171
03-028	131/135			222/226	218	260	185	251	118/124	272	167/169
03-032	133/135	164/170		216/226	218/222	260/262	189/195	241/247	118/124	266	
03-033				216	216/222	260/262		247/255	116/124	272/274	
03-034				220/222	216/222			247/255	116/124	272/274	
03-035				216/224	216/222			247	116	272	
homozygosity	**17%**	**14%**		**37%**	**29%**	**50%**	**29%**	**23%**	**30%**	**52%**	**44%**

ECA10

ACCNO	COR020	COR048	ASB06	NVHEQ018	NV007	COR083	ASB09	HMS02	COR085	AHT86
A3-001	162	184		129/131	232	282	101/107	286/306	160/162	215
A3-002	168/174	184/196		159	232	282	97/105	292/298	160	215
A3-003	164/170	176/180	188/202	131/159	232	282	97/105	292/294	160	213/215
A3-004	174/178	180/182	188/198	131/133	232	282	101	292/304	162	213/215
A3-005	162/170	184/190	188/202	119/133	232	282		290/292	160/162	217
A3-006	162/170	180/184	188/202	159	232	282	101	286/292	160	199/217
A3-007	170	176/182	188/202	119/133	232/234	282	99/105	286/304	160	211/215
A3-008	168		188/198	119/121	232/236	282		292	160	213/215
A3-009	172	182	188/202	131	232	282	105	294	160	215
A3-010	168/174	184/196	188/202	131/159	232	282	101/105	284/294	162/166	211/217
A3-011	170/174	190	188/202	121/133	230/232	282	105	292	160/162	215/217
A3-012	168/174		188/202	133/159	232	282	99/105	284/298	160	215
A3-013	162/168		188/202	119/133	232	282		292	160	199/215
A3-014	168/170	176	188/202	119/131	232/236	282	105/107	292/304	160	213
A3-015	162/170		188	119/133	232	282	101	304	160/162	211/213
A3-016	168		188/202	139/159	232	282	99/101	292/304	160	211/213
A3-017	172/178	176/180	188	119/131	232/234	282	105	292/298	160	215
A3-018	164/170		188/202	131/159	230/236	282	101/105	284/292	160/162	211/213
A3-019	170/174	176	188/202	129/131	232	282	101/105	284/304	160	215
A3-020	168/170	180/184	188/202	131/159	232/236	282	101/105	292/294	160	211/217
A3-021	162/170	176/196	188/198	131/133	232	282	105	294/304	160/162	199/219
A3-022	168/170		188/202	119/131	232	282	97/105	292/294	160	215
A3-023			188/202	137/159	232	282	101	292/304	162	211/217
A3-024	162/164	176/184	188/202	129/131	232	282		304	160	213/217
A3-025	162/170	184	188/202	129		282	103/107	292/304	160/162	
A3-026	170/172		188/202	129/135	232	282	107/111	284	160/162	211/215
A3-027	174/178		188/202	131	232	282	101/105	292/298	160	213/215
A3-028	168/170	182/190	188	133/159	232	282	101/105	292/304	160/162	211/217
A3-029	164/170	190/196	188/202	119/131	232	282	101/105	292/304	160	211
A3-030	170/178		188/202	131	232	282	101/105	292	160/162	211/213
A3-031	170/172	176/182	188/202	131/133		282	105	292/294	162	
A3-032	170/172	190	188/202	133	232	282	105/107	290/292	160/162	211/213
A3-033	170	184/190		119/135	232	282	105/111	284/294	162	211/217
A3-034	168/174		188/202	131/137		282		286/292	162/166	
A3-035	172/174	176/184	188	119/159	232/236	282	101/105	292/298	160	213/215
A3-036	162/174	184/190	188/202	133/159	232	282	105	292/298	162	215/217
A3-037	162/174	184/190	188	119/159	230/232	282	105	292/298	160/166	215
A3-038	170/174	182/196	188/198	119/131	232	282	101	304	160	213
homozygosity	16%	27%	14%	18%	74%	100%	36%	24%	63%	34%

ACCNO	COR020	COR048	ASB06	NVHEQ018	NV007	COR083	ASB09	HMS02	COR085	AHT86
03-001			188		232	282		286/292	160	215
03-002			188		232	282		286/294	162	209/215
03-003			188			282		286/294	162	215
03-004			188/190		232	282		292	160	213
03-005			188/198		232/236	282		286	160/162	213/217
03-006			188/204			282		292/306	162/166	
03-007			188/190		232	282		286/290	160	199/211
03-008			188/198		232	282		286	164/166	
03-009			188		232	282		294/306	160	213
03-010			188/198		232	282		286/304	160/166	215/217
03-011			188/198		232	282		286/288	160/162	199/211
03-012			188/204		232	282		286	160/162	211/215
03-013			188/204		232	282		294	160/162	211/217
03-014			206		232	282		304	160	215/217
03-015			188/202		232	282		286/294	160/162	211
03-016			188		232	282		288/292	160	211/213
03-017			188/206		232	282		294	160/162	211/217
03-018			188		232	282			160	
03-019			188/198		232	282		300/304	160/162	
03-020			188/198		232	282			160/162	217
03-021			188/190		230/232	282		292/304	160/162	215
03-022			188/190		232	282		294	160	215
03-023			188/202		232	282		286/304	160	213/215
03-024			188/202		232	282			160	
03-025					232	282		288/294	160	211/217
03-026					232	282		290/292	160	217
03-027					232	282		288/292	160	217
03-028					232	282		288/304	160	211/217
03-032					232					213/215
03-033					232					213/217
03-034					232/236					211/215
03-035					232					213
										217
homozygosity			**29%**		**90%**	**100%**		**32%**	**57%**	**41%**

ECA11

ACCNO	UCDEQ439	LEX068	SGCV24	SGCV13	ASB35	TKY033_32	NVHEQ90	TKY010	TKY648	HLM2
A3-001	214/216	164/170	125/139	171/189	224	96	106/108	186	284/290	131
A3-002	214/216	162/170	127/137	171/181	226	100	108	184	288	131
A3-003	216	164	125/131	171/189	226	92/94	108	184	290	131
A3-004	216	166/172	125/133	171/183	226	96	108	184	290	131
A3-005	216/218	166/172	125/141	171/183	226	96	108	184	290	131
A3-006	216	168/170	123/137	171/189	226	94/100	108	184	288	131
A3-007	212/218	164	125/139	183/189	226	92/94	106/108	182/186	290	131
A3-008	216/218	164/170	125/139		224	92/94	108	182/184	290	131
A3-009	212/216	162/164		171/189	226	94/100	108	184	288/290	131
A3-010	216	166/168	125/139	171/183	226	96	108	184	290	131
A3-011	216/218		133/137	171	226	100	108	184	288	131
A3-012	216/218	162/164	133/137		226	94/100	106/108	184	288	131
A3-013	216	166/174	141		226	96	108	184		131
A3-014	216/218	164	133/139	171/183	226	94/96	104/108	184	288/290	131
A3-015	216	166	131/137	171	226	96/100	108	184	288/290	131
A3-016	216/218	170	125/133	171/183	226	94/100	108	182/184	290	131
A3-017	216/218	162/172	133	171	226	96	108	184	290	131
A3-018	218	164	125/139	183/189	226	96	108	182/184	288/290	131
A3-019	216/218	164	127/131	171/183	226	94	108	184/186	288/290	131
A3-020	216	162		171/189	226	94/100	108	184	288/290	131
A3-021	216	164/170		171	226	94/100	106/108	184/186	290	131
A3-022	216/218	164/170	121/131		226	92/94	106/108	184/186		131
A3-023	216	162/170	135/141		226	94	108	184/186		131
A3-024	216	162/172	127/141	189	226	96	106/108	184/186	288/290	131
A3-025		170/174		181	226	94/100		184/186		131
A3-026	216	162	123/137	171	226	100	106/108	184	288/290	131
A3-027	218	172/174	123/133	171	226	96	108	184	290	131
A3-028	216/218	166/172	125/141	183/189	226	94	106/108	184	288/290	131
A3-029	216	162	125/133	171	226	94/100	108	184	288/290	131
A3-030	218	168/172	133	171	226	96	108	184/186	290	131
A3-031		164/166	125/141	171	226	96/100		184		131
A3-032	216/218	164/166		171/189	226	94/100	108	184	288	131
A3-033	218	170	125/141	171/189	226/228	92/94	106/108	184/186	288/290	131
A3-034			125		226	94		184/186		131
A3-035	216/218		125/133	171	226	96	108	184	288/290	131
A3-036	216/218	166/172	125/133	171/183	226	94/96	108	184	290	131
A3-037	216/218	162	133	171		96/100	108	184	288/290	131
A3-038	216/218	168/170	131/141	171/189	226	96	108	184	290	131
homozygosity	**46%**	**34%**	**15%**	**41%**	**97%**	**50%**	**71%**	**66%**	**56%**	**100%**

ACCNO	UCDEQ439	LEX068	SGCV24	SGCV13	ASB35	TKY033_32	NVHEQ90	TKY010	TKY648	HLM2
03-001	216			171/181	226	96/100	108	184	288/290	131
03-002	210/218			171/185	226	96/100	108	184	288/290	131/155
03-003	216/218				226	94/96	108	184		
03-004	216			171/181	226/228	96/100	108	184	288	131
03-005	216			171/189	226	94/96		184		131
03-006				171/189	226/234	94/96	108	184	288/290	131
03-007	216/218			171/189	226	94/100	108	184	288	131
03-008	214/216			189	224/226	94/96	106/108	184	288/290	131
03-009	214/216			171	226	94/96	108	184/186	288/290	131
03-010	216			181/189	226/228	94/96	108	184/186	290	131
03-011	212/216			189	226	94/96	106/108	184	290	131
03-012	216			171/18.	224/226	94/96	108	182/184	288/290	131
03-013	216			171/189	226	94/96	108	184/186	288/290	131
03-014	214/216			171/183	226/234	94/96	108	184	290	131
03-015	216			171/189	226	94/100	106/108	184		
03-016	216			189	226	94/96		184/186	288/290	131
03-017	216/218			171/189	226	94/100	106/108	184/186	290	131
03-018	216/218			171/189	226	94/100	106/108	184/186		131
03-019	216/218				226/232	94		184		131
03-020				171/181	226	94/100	108	184	288	131
03-021	214/216			189	226	94/96	108	184	288/290	131
03-022	218			181/183	226/228	94/100	108	182/184	288/290	131
03-023	212/216			171/189	226	94	106/108	184/186	288/290	131
03-024					226	94/96	108	184/186		
03-025	214			171/189	226/232	94/96	108	184/186	290	131
03-026	218			171/189	226/228	94/100	106/108	184	288	131
03-027	218			181/183	226	94/100	106/108	184		131
03-028	218			183/189	226	94	106/108	184	288	131
03-032	216			181		94/100	108	184	286/288	131
03-033	216			171/189		94/96	108	184	290	131
03-034	216			189		94/96	106	184	288/290	131
03-035	212/218			189		94/100	108	184	286/288	131
							108	186	288/290	131
homozygosity	**55%**			**28%**	**64%**	**9%**	**69%**	**66%**	**44%**	**97%**

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ECA12

ACCNO	SGCV10	UCDEQ497	SGCV08	COR030	COR058
A3-001	185/187	113	132/140	247	222/224
A3-002	181/189	113	128/138	243/251	222
A3-003	185/189	113/115	128	243/245	214/216
A3-004	181/187	113	128	243	222/236
A3-005	181/189	113		247/249	222/234
A3-006	185/187	113	136/144	243/251	224/232
A3-007	187/189	113	128/138	243/245	222
A3-008	187/189	113	128/138	245	228/240
A3-009		115	132/138	243/245	218/222
A3-010	185/187	113/115	128/142	247/251	230/232
A3-011	181/185	113/115		245/249	228/234
A3-012	183/187	113	128	243/247	222/224
A3-013	181/189	113/117		243	216
A3-014	189	113/115	128/134	245/249	214/230
A3-015	189	113	138	243/249	228/230
A3-016	185/187	113			228
A3-017	181	113/117	128	243	222/228
A3-018	181	113	138	243/247	228/232
A3-019	181/189	115	128	247	216/222
A3-020		113	132/136		222/224
A3-021		113/115	128/142	243/247	216/222
A3-022	187/189	113/115	128/138		214/226
A3-023	187	113/117		245/249	228
A3-024	183/187	115/117	138/144	243	228/230
A3-025			128/138		234/240
A3-026	185/189	113/115	138	233/243	228/234
A3-027	181/187	113	128	243	232/234
A3-028	185/187	113/115	138/140	243/245	220/228
A3-029	185/189	113/115	128		220/226
A3-030	185/187	113	128	243	232/234
A3-031	187/189		128/138		216/226
A3-032		115	128	245	216/230
A3-033	185/187	113/115	138	243/245	228/236
A3-034	187/189				232/240
A3-035	181/185	113/117	128/138		216/236
A3-036	181/189	113	128	243/245	222/232
A3-037	181/187	113	128/132	243/245	222/236
A3-038	187/189	113	132/138		216/230
homozygosity	**15%**	**57%**	**44%**	**34%**	**13%**

ACCNO	SGCV10	UCDEQ497	SGCV08	COR030	COR058
03-001		113	132/138	243/247	
03-002		113		233/247	
03-003		113/117	126/128		
03-004		113/115	128/132	243/251	
03-005			128/138		
03-006		113/115	128/134	243	
03-007		113	138	247	
03-008		113	128/140	243/245	
03-009		115	128/138	243/245	
03-010		113	136/138	245	
03-011		113	136/140	245	
03-012		113/117	128/132	243/247	
03-013		115	136/140	245	
03-014		113/115	136/138	243/245	
03-015		113		243	
03-016			128/136	243/245	
03-017		113	128/136	243/245	
03-018		113	136/138		
03-019		113	128		
03-020		113/115	132/138		
03-021		113/115	138/140	245/251	
03-022		113/115	132/138	247/251	
03-023		113/115	128/138	243	
03-024		113/115		243	
03-025		113		243/245	
03-026		113/115		243/247	
03-027		113		247	
03-028		113/115		247	
03-032		113/115		243/247	
03-033		113/115		245	
03-034		113/115		243/245	
03-035		113/115		243/245	
homozygosity		**47%**	**10%**	**41%**	

ECA13

ACCNO	COR069	UM030	VHL047	AHT30	ASB37	ASB01
A3-001	275/285	135/151	134/148	183/191	138	161/169
A3-002	273/291	145	134	189/191	138	161/171
A3-003	283/291	135	134/148	191	138	167/171
A3-004	277/285	135/145	134/148	191	132/138	161/167
A3-005	279	137	134/148	189/191	138/142	171
A3-006	285	145	134/148	191	138/146	167
A3-007	283/287	135/145	146/148	189/191	132/138	161/171
A3-008	275/285	143/145	134	191/195	144/146	169/175
A3-009	277/285	151	134/146	191/195	134/146	167/171
A3-010	277/285	145	134	191	132/138	167/175
A3-011	283/285	145/151	134	189/191	132/138	167/169
A3-012	273/279	137/145	134/148	191	138/142	161/171
A3-013	285	139/147	134	191/195	138	167
A3-014	283/285	135/145	134	191	134/146	167
A3-015	285	139/145	134/146	191/195	132/138	161
A3-016	277/285	135/145	148	191	138	167
A3-017	283/291	145/151	134/144	191	132/138	161/169
A3-018	277/285	135/145	148	191	132	167/175
A3-019	277/279	145	134/148	191	138/146	167/171
A3-020	277/285	137/145	134/148	191	132/134	167
A3-021	277/283	145/151	134/146	191/195	144	161
A3-022	279/291	137	134	195	134/138	167/171
A3-023	285	145	134/148	191/195	142/146	161/167
A3-024	279/285	145/151	134/146	191	138/142	161/167
A3-025	277/279		134/148	191	138	161/167
A3-026	279/291	145/151	134	191	138	161/167
A3-027	279	145	134	191	134/138	161
A3-028	279	145	134	191/195	134/146	167/175
A3-029	279/287	145/151	134/146	191/195	138	171/175
A3-030	279	145	134/146	191	138/146	161
A3-031	283		144/148	191	138/146	161/167
A3-032	285/287	137	146/148	191	138	161/167
A3-033	277/279	145	134/148	191	132/142	
A3-034	277/279		134/146	191	138	161/167
A3-035	279/283	145/151	134/144	191/195	138	161
A3-036	285/291	135/145	134	191/195	132	161/167
A3-037	279/285	135/145	134/148	191	132/146	161/175
A3-038	283/285	149/151	146	191		161/167
homozygosity	**24%**	**40%**	**37%**	**61%**	**38%**	**30%**

ACCNO	COR069	UM030	VHL047	AHT30	ASB37	ASB01
ACCNO						
03-001		145		191		
03-002		135/137		191/195		
03-003		135/145		191/195		
03-004		145		189/191		
03-005		135/149		191		
03-006		143		191		
03-007		145		191		
03-008		135/139		191		
03-009		135		191		
03-010		145		191/195		
03-011		135/151		191		
03-012		145		191		
03-013				189/191		
03-014		139/145		191		
03-015		137/145		191		
03-016		151		191/195		
03-017		145/151		191		
03-018		135		191		
03-019		135/137		191		
03-020		135/137		191		
03-021		135/137		191		
03-022		145		189		
03-023		135/139		191		
03-024		135/139		191		
03-025		143/151		191		
03-026		137/137		191		
03-027		145		191		
03-028		145		191		
03-032		149/151				
03-033		135				
03-034		135/151				
03-035		151				
homozygosity		**40%**		**79%**		

ECA14

ACCNO	UM010	VHL209	LEX047	TKY310	HTG29	LEX043	AHT83	AHT88	HTG018	LEX078	TKY491
A3-001	112/126	97/101	249/253	134/148		246/250	106	307	168		242/246
A3-002	118/126	91/97	253	130/142	116/126	250/252	106/116	311/313	168		244
A3-003	116/126	95/97	253	134/144	128	246	106/116	311	168	162	244/246
A3-004	114/126	91/95	253	134/144	118/130	246/250	106/116	311/313	168	162	244
A3-005	114/126	99/103	249/253	148/150	116/120	246/250	106	307	168/170	162	246
A3-006	118/126	91/97	251/253	134/150	116/120	246	116		168	162	246
A3-007	126	95/97	253	142/148	116	246	106	311	168	162	244/246
A3-008	116/126	97	253	144/150	116	246/250	106	311	168/170	162	244/246
A3-009	114/116	91/97	253	134/140	120/128	246	116	311	168	164	246
A3-010	114/116	99/101	253	138/144	116/126	232/250	106	307/311	168/170	164/166	246/254
A3-011	116/126	91/99	249	130/150	116/120	244	106/116	311	168	162	244/246
A3-012	116/126	91/99	255	142/148	120/126	250/252	106	313	168	162/164	244
A3-013	126	101/103	253	144/148	116/120	246/248	106/116	311/313	168	162/164	244/246
A3-014	116/124	91/95	249/253	134/142	102/116	248	106	311	168	166	244
A3-015	116/126	97/103	253	134/142	118/120	246/248	106	307/311	168/170	162/164	246
A3-016	114/118	95/99	253	144/150	116/118	248/250	106/116	307/313	168/170	162	244/246
A3-017	114/126	91/97	249/253	134/144	116/118	246/250	106/116	311/313	168	162	244
A3-018	114/126	95/97	249/253	134/140	120/126	246/248	106	307	168	162/166	244/246
A3-019	114/116	91	253	130/150	120/126	246/248	104/106	307	168	162/164	244/246
A3-020	120/126	95/97	251/253	142/150	120/126	246/250	106/116	307	168/170	164/166	248/254
A3-021	116/126	95/97	253	134/144	126/128	246/248	104/106		168	162/164	242/246
A3-022	116	97	253	130/144	116/118	248/252	106/116	311/313	168	162/164	246
A3-023	114/126	99/103	253	130/134	128	246	106	307/313	168	164	246/254
A3-024	116/124	97	249/253	134/148	116/120	246	104/106	311	168/170	162/164	244/246
A3-025	114/116	95/97	253	134/140	116/128	246	106	311		162/164	
A3-026	114/126	95/97	253	134/148	116/128	246/250	106/116	311/313	168	162/164	244
A3-027	118/126	97/101	253	134/148	118/130	246/250	104/106	307/313	168	162/164	244/246
A3-028	126	97	249/253	132/134	128	246	106	311	168/170	164	246/256
A3-029	114/118	95/97	253	134/148	120	246/250	106/116	311/313	168	162/164	244
A3-030	118/126	99/101	253	134/148	118/128	246	106	313	168	162/164	244
A3-031	120/126	97/101	249/253	134/144	116/130	246/252	106/116	311		162/166	
A3-032	114/118	95/99	253	144/148		246/248	106/116	311/313	168	162/164	242/244
A3-033	114/116	91/95	249/251	134/146	116/126	248/250	106	311/313	168	162/166	244
A3-034	118/126	97/101	253	130/142		232/250	106	311/313			
A3-035	114/126	95/99	253	130/134	116	246/250	106	311/313	168	162/164	244/256
A3-036	118/126	97	253	132/134	116	246/250	106	311/313	168/170	162	244/246
A3-037	126	91/97	253	130/148		246/250	106		168	162/166	244
A3-038			253	144/148	116/130	248/250	106/116	307/311	168/170	162	244
homozygosity	**14%**	**16%**	**71%**	**0%**	**24%**	**29%**	**53%**	**49%**	**71%**	**43%**	**46%**

TKY438	TKY749	COR002	TKY636
265/281	235	237	208/216
281/283	233/235	237/241	198/200
281/283	233/235	237/241	198/200
283	233	237	198/208
273/283	233/235	237/239	198/208
283	233/235	237	224
273/281	235	237/241	198/210
275/285	235	237/239	200/210
283	235	237	224
281/283	233/235	237	200/224
273/283		237	198/212
281/283	233/235	237	196/198
283		237	216/224
281/283	233/235	237/239	200
265/283	235	237/241	198/208
281/283	235	237	198/200
283	233/235	239/241	208
275/281	233/235	237	198/200
265/283	235	237	198/208
265/283	235	237	208/224
283	233/235	237/239	198/208
		237/241	
281/283		237/239	196/210
281	233/235	237	198/212
		237	
265/283	233/235	237	198/208
283	233/235	237	198/200
265/283	235	237	198/210
283	233/235	237	198/210
283	233/235	237/241	198/200
		237	198
283	233/235	237	198/200
281/283	233/235	237	208/216
		237	
265/283	233/235	237/241	198/208
283	233	237	208/212
283	233/235	237	198/200
283/285	233/235		198/200
38%	**35%**	**65%**	**19%**

ACCNO	UM010	VHL209	LEX047	TKY310	HTG29	LEX043	AHT83	AHT88	HTG018	LEX078	TKY491
03-001			253	142/148		246/248	106/116	307	168	162/164	244/246
03-002			253	130/134		248/250	106	311/313	168	162/166	246/250
03-003			253	134/134		248/250	106/116	311/313	168	162	246
03-004			253	134/142		246/252	106/108	311/313	168	162/164	244/256
03-005			253	134/140		246/250	106/108	313	168	164	244
03-006			253	134/148		246/252	106/116	311/313	168	162	244
03-007			253	130/134		248/250	106/108	307	168	162/164	246
03-008			253	144/148		246/248	106	313	168	162/164	244
03-009			253	148/150		246/248	116	311	168	162	244
03-010			249/253	142/144		246	106/116	311	168	162/166	244/246
03-011			253	142/144			104/106	307/311	168/170	162/164	246/256
03-012			249/253	144/148		248/250	106/116	311/313	168	162/164	246/248
03-013			249/253	142/142		246/250	104/106	307/313	168/170	162/164	
03-014			253	134/150		246/248	106	311	168	162/166	236/244
03-015			249/253	130/148		232/246	106/116	311	168/170	162/164	246/256
03-016			253	130/142		246/250	104/106	307/311	168	162	246
03-017			253	142/142		246/250	104/116	307/311	168/170	162/166	246/252
03-018			249/253	134/144		246/248	106/116	307/311	168	162/164	244/248
03-019			253	142/148		232/248	106/120	311	168	162/164	246/250
03-020			253	140/142		246/252	106	311	168/170	162/164	246/256
03-021			253	130/144		248	106/116	307/313	168	162	242/244
03-022			253	142/148		246/252	106/116	311	168	162/164	244/256
03-023			253	138/148		246/250	106/116	311/313	168	162/164	244
03-024							106/116	311/313	168	162/164	246
03-025			249/253	146/148		246/248	106	307/311	168	162/164	244/256
03-026			253	142/148		248/250	106/116	307	168/170	160/162	244/246
03-027			253	130/148		246/250	116/120	311	168	162/166	244
03-028			249/253	130/146		248/250		307	168	164/166	244/246
03-032									168/170		242/244
03-033									166/168		244
03-034									168		244/254
03-035									168		244/246
homozygosity			**74%**	**0%**		**8%**	**22%**	**50%**	**75%**	**21%**	**35%**

TKY438	TKY749	COR002	TKY636
273/275	233/235	237/241	210/212
283	233/235	239/241	208/210
285		237	
281/283	233/235	237/241	200/208
283/285		237/241	
283	233/235	237	198/208
283	235	237/239	200/208
281	235	237/241	196/200
283	233	235/237	198
283	233/235	237/239	210
265/281	233/235	237/241	198/210
265/283	235	237/241	218/224
283	235	237	208/210
275/281	233/235	239/241	200/208
265/275		237	200
281/283	233	237/241	198/210
281/283	233	237/241	208/210
277/285		237/239	
283/285		237/239	
281/283	235	237/241	
281/283	233/235	237/239	208/224
281/283	233/235	237/241	198/200
283	233/235	237	198/224
285		237	198/224
265/283	235		198/208
283	233		198/208
285			198/224
283	235		200/224
273/283	233/235		198/216
281	233/235		198
275/283	233		200/210
275/283	233/235		198/210
			198/224
44%	**48%**	**25%**	**15%**

ECA15

ACCNO	B8	LEX046	ASB02	AHT16	HTG006	COR014	AHT002
A3-001	98/104	127/137	248/254	143	96/100	158	106
A3-002	96/104	129/137	246/254	137/151	100	158/166	104/108
A3-003	100/102	129/137	246/252	131	100	158	96/108
A3-004	100/102	127/129	244/254	137/153	100	156	104/106
A3-005	96/102	137	236/252	139/153	90/100	158/168	104/106
A3-006	96	133/137	252	151	100	158	106
A3-007	102	127/137	244/252	137/143	96/100	158	106
A3-008	102		244	131/137	100	166/170	106
A3-009	102	129/137	248/254	137/151	88/100	158/164	104/108
A3-010	88/102	131/137	240/250	137	100	158/160	96/108
A3-011	88/102	129/135	248/254	131/143	84/90	158/162	104/106
A3-012	96/102	127/129	246/252	137/143	84/100	158/166	106/108
A3-013	96/102	129/135	248/252	143/151	100		108/110
A3-014	88/102	127/135	248/252	131/137	90/102	158/162	108
A3-015	102	129/137	236/246	137/143	88/100	158	106
A3-016	102	129/137	244/254	131/137	100	158/168	106/108
A3-017	102	129	252/254	153	100	158/160	96/104
A3-018	88/102	129/135	244/252	139/143	100	166/170	104/106
A3-019	96/102	133/135	246	131/137	88/100	158	106
A3-020	96	127/137	240/252	131/137	90/102	158/160	104/106
A3-021	96/102	129	248/252	131/137	100	158/160	104/108
A3-022	102	129	244/254	131	100	158/160	96/104
A3-023	88/96	129/135	244/246	135/143	84/100	158/160	96/106
A3-024	96/110	129/137	248/252	139/153	90/100	160/170	106
A3-025	102		244/246	131/151	100	158/170	106/108
A3-026	102	129	252/254	139/153	96/100	158/160	104/106
A3-027	102	129/137	244/254	137/153	100	158	104/108
A3-028	102/104	129/137	244/248	131/137	84/96	158	106
A3-029	96/102	129	240/252	137/153	100	158/164	104/106
A3-030	102/110	133/137	248/254	137/153	84/100	158/160	104/106
A3-031	102/104		248/252	137/139	100	158	108
A3-032	102	129	248/254	131/151	100	158	104/106
A3-033	104/110	131/133	240/246	137	100	158/160	104/106
A3-034	102		246/248	137/153	100	158/170	106/108
A3-035	102/104	129/137	252/254	151	100	158/160	96/104
A3-036	96/102	129/137	236	137	88/100	158/168	106/108
A3-037	102/104	129/137	236/246	137/139	88/100	158	104/106
A3-038	102	129/137	248/254	131/139	84/100	154/158	106
Homozygosity	**39%**	**21%**	**11%**	**23%**	**55%**	**32%**	**29%**

ACCNO	B8	LEX046	ASB02	AHT16	HTG006	COR014	AHT002
						158/160	
03-001		129/137	236/244		100	158	104/106
03-002		127/129	252/254		84/100		106/108
03-003		133/137	236/252		84/100	158	106/108
03-004		129/137	236/246		88/100	158	106/108
03-005		129/133	224/254		84/90	158/160	106
03-006		125/135	252		100	158	104/106
03-007		129/135	248/254		90/100	158/162	106/108
03-008		135/137	240/254		84/100	158/166	106
03-009		125/129	250/252		100	158/160	108
03-010		129/133	252/254		100	158/164	104/108
03-011		129/133	246/252		100	158/160	106/108
03-012		129/137	240/244		84/100	158/168	106/108
03-013		129/135	248/252		90/100	158/164	106
03-014		129/135	240/246		100	158/160	104/106
03-015		129/135	236/246		100	164/166	104/108
03-016		129/137	240/252		96/100	158	104/106
03-017		129/137	248/252		96/100	160/166	104
03-018		129/137	248/254		100/102		96/106
03-019		129/135	252		100/102	158/160	108
03-020		129/133	246/252		100	158	
03-021		129	252		100	158/166	104/108
03-022		129/133	246/248		100	166/172	108
03-023		125/137	248/250		100		108
03-024			248/250		90/100	160/166	
03-025		125/135	236/244		90/100	158	104/108
03-026		135	240/246		100/102	158	104
03-027		129/133	248/254		84/90	158/164	106/108
03-028		129/135	248/254		90/100	158	96/108
03-032		127/129	248/254			158/168	
03-033		129/133	246/252			158/170	
03-034		129	246/248			168/170	
03-035		129/133	246/252				
Homozygosity		**10%**	**10%**		**39%**	**38%**	**35%**

ECA16

ACCNO	AHT037	TKY279	HTG3	HMS20	COR039	AHT014	LEX018	AHT038	L15-2	LEX056	I18
A3-001	214	125/137	117/123	128/144	264	146	248/252		161/169	224/232	109
A3-002	214	133/137	117/119	132/134	264	146	248/250	140	155/165	224/228	117
A3-003	214/216	135	115/117	128/132	264	146	248	138/140	161/165	224/228	113/117
A3-004	214	125/135	117/125	134	264	146	236/248	140/142	161/165	228/230	107/117
A3-005	214	133/139	121		264	146	246/248	138/142	161/165	222/232	117
A3-006	214	131/133	123/125	134	264	146	248	140	165	218/230	113/117
A3-007	214	131/139	117	134/144	264	146	238/242	140/142	155/163	224	113/115
A3-008		135	117/123	132/140	264	146	246/248	142	155/161	224/234	109/115
A3-009	214	125/131	117/125	132/140	264	146	242/250	142/144	155/169	218/228	107/117
A3-010	214	131/135	115/117	128/134	264	146	248	138/142	155/161	228/234	109
A3-011	214	131	117/123	134/144	264	146	242/246	138/142	155/161	218/222	113/117
A3-012	214	125/133	117/119	132	264	146	236/248	140/144	161/165	224/228	117
A3-013	214	125/135	121/123	130/138	264	146	236/246	140/142	155/157	218/222	113
A3-014	214	131/137	117	130/132	264	146	242/250	138/144	161/163	222/230	109
A3-015	214/216	131/135	117/121	132/140	264	146	248	138/144	153/165	222	109
A3-016			117	128/132	264	146	246	140/142	161/163	228/234	107
A3-017	214/226	135/139	117/123	132/140	264	146	248/250	138/144	159/165	224/230	109/119
A3-018		131/135	115/117	134/144	264	146	248/250	142	155/161	224	117
A3-019	214/226	131/135	117/125	134	264	146	246/248	138/146	155/157	228	109/113
A3-020	214	125/131	121/123		264	146	242/248	140	153/165	232	115/117
A3-021	214	125/131	117/125	136/144	264	146	246/250	138/142	155/165	224/228	109/115
A3-022	214	135/139	115/123	128/134	264	146		138/140	161/165	228/230	109/115
A3-023	226	135	117		264	146	248	138/140	155/165	218/222	113
A3-024	214	125/131	115/117	130/132	264	146	240/248	140/144	157/159	228	113/117
A3-025	214	131/137	117/123	134	264			140/142	159/163	224/228	109
A3-026	214	131/135	117/119	140/144	264	146	246/248	142/144	155/159	224	109/117
A3-027	214/226	125/135	117/125	134	264	146	236/248	138/144	159/161	224/230	109/117
A3-028	214	131/133	117	134	264	146	246	140/148	157	218/228	113
A3-029	214/226	135	115/117	128	264		242/246	140/142	155/165	232	115/117
A3-030	216/226	135	117/125	134/144	264	146	242/248	142/144	155/159	224/228	115/117
A3-031	214	137/139	117/125	130/132	264			138/142	163	222/230	117
A3-032	214/226	131/135	117	128/132	264	146	236/242		155/157	222/230	107/115
A3-033	214/216	125/131	117	130/134	264	146	250/252	138/150	155/165	224/228	109/113
A3-034	214	131/139	115/117	134/140	264			140/142	163/165	218/228	109/117
A3-035	214/226	135/139	115/117	128/134	264	146	248/250	138/140	165	228/230	107/117
A3-036	214/226	133/139	119/121	130/140	264	146	238/248	138/140	165	222/228	117
A3-037	226	135	117	132/144	264	146		140/142	155/165	218/224	107/117
A3-038	214/226	131/135	117/123	130/134	264	146	246/248	142	161/165	222/234	107
homozygosity	**66%**	**19%**	**24%**	**23%**	**100%**	**100%**	**21%**	**17%**	**13%**	**21%**	**42%**

AHT60	AHT91
302/314	124
209/308	124
290/292	124/126
310/312	124
294/310	124
302/316	124
290/304	124/126
292/302	124
292/300	124
292/312	124
292/300	124
290/292	124/126
302/314	124
290/292	124
312/314	124/126
292/312	118/124
310/312	124
292/302	124
302/312	124/126
292/314	124
	124
	124
292/302	124
302/316	124/126
292/312	124
292/312	124/126
290/302	124
290/292	126/134
292/302	124/126
300/312	124/126
312/312	124
310/312	124
310	124
292	124/126
292/312	124/126
6%	**63%**

ACCNO	AHT037	TKY279	HTG3	HMS20	COR039	AHT014	LEX018	AHT038	L15-2	LEX056	I18
03-001	214	125	117	132/138	264	146	242/248				
03-002	212/214	135/139	119/123	140/144	264	146	236/248				
03-003	214	137/139	117/121	134/140	264	146					
03-004	214	133/137	117/119	134/140	264	146	248/252				
03-005	214	131/139	119/121	132/144	264	146					
03-006	216/226	131/135	117	130/132	264	146	236/242				
03-007	214/216	125/131	115/123	132/134	264	146	248				
03-008	214	135/137	115/115	134/134	264	146	236/248				
03-009	214/218	135/139	117/121	134/144	264	146	246/248				
03-010	214	139	121/123	130/134	264	146	246				
03-011	214/226	131/135	117	134/140	264	146	238/246				
03-012	214	131/133	117/121	134/140	264	146	248				
03-013	216/226	135	117	130/132	264	146	238/246				
03-014	214/226	131/135	117	130/144	264	146	242/246				
03-015	214	131	117/123	130/144	264	146	246				
03-016	214	133/135	117/121	132/132	264	146	238/248				
03-017	214	125/131	117	132/134	264	146	246/248				
03-018	216	135	117	132/132	264	146					
03-019	214	125/135	115/117	132/134	264		240				
03-020	214	125	125	134/140	264	146	246/248				
03-021	214/226	131/135	117	134/134	264	146					
03-022	214	137/139	117	132/134	264	146	248/250				
03-023	214/218	125/139	117	134/144	264	146	246/252				
03-024			117/123	134/134	264	146	240/242				
03-025	214/226	125/135	123/125	134/140	264	146	242/248				
03-026	214	125/137	117	134/134	264	146	248				
03-027	214	125/135	115/117	134/138	264	146	248				
03-028	214/216	125/135	115/115	132/134	264	146	238/248				
03-032	210/214	133/139			264	146	248/250				
03-033	214	139/141			264	146	246/250				
03-034	214/226	125/135			264	146	242/246				
03-035	214	131			264	146	242/246				
homozygosity	58%	23%	50%	0%	100%	100%	25%				

AHT60	AHT91
290/314	124/126
	124
	124/130
292	124/126
290/314	124/126
308/312	124/126
294/304	124
312	124
290	124
304/310	124
290/312	124/126
302	124/126
300/302	124
292/310	124
292/300	124
300/304	124
290/310	124/126
310/312	124
294/304	
292/302	124
292/308	124
292	124
294/314	124/134
304/316	126/136
302/314	124/134
302/316	124
302/312	124
290/302	124
292/304	124
292/302	124
302/312	124
292	124
20%	**65%**

ECA17

ACCNO	COR007	LEX076	NVHEQ79	LEX055	COR032	HMS25
A3-001	175/179	226	179/189	218	257	132
A3-002	175/185	226	179/191	218	257/259	130
A3-003	165/179	218/228	179	232	257/265	132
A3-004	165/175	228	179	224	257	132
A3-005	179/181	228	179/193	218	257	130/132
A3-006	165/175	228	189/193	222	257	130
A3-007	165/185	228	179/189	224/232	257	130/132
A3-008	179/181	228	175/189	224/232	257/265	130/132
A3-009	165/175	228	189/191	216/224	259	130
A3-010	165	228	179/189	218/224	257	130/132
A3-011	175/181	228	177/189	224	257/261	130
A3-012	175/179	228	179/191	222	257	130/132
A3-013	175/179	228	179	220/226	257/261	132/134
A3-014	179	228	179/189	218	257	132
A3-015	165/179	228	189	222	257/261	132/134
A3-016	165/179	228	179	224	257/259	132
A3-017	165/181	228	179	218/224	257/259	130
A3-018	165	228	179	226	257/259	130/134
A3-019	179/185	218/228	179	224	257	130
A3-020	179/181	228	179/189	218	257	130/132
A3-021	165/185	228	175/179	218	257	130
A3-022	179/181	218/228	177/189	224	257	130/132
A3-023		228	189/191	220/232	257/263	130/132
A3-024	165/179	228	179/189	224	257/261	130/134
A3-025	165/181	228	179	222	257	
A3-026	165	228	179/189	222/224	257	132
A3-027	165/179	228/230	179/191	224	257/259	130/132
A3-028	165/175	228	187/189	224	257/259	130/132
A3-029	165/179	228	179/189	232	257/263	130/132
A3-030	179	228/230	189/195	224	257/259	130
A3-031	165	218/228	177/179	218	257/259	
A3-032	165/185	228	179/195	224	257	130
A3-033	165/179	228	175/179	222	257	130/134
A3-034	181	228	179	222	257	
A3-035	165/181	228	179	224	257	130/132
A3-036	165/181	228	177/179	218	257	130/132
A3-037	185	228	175/191	218	259	130/132
A3-038	165/175	228		224	257	132
homozygosity	**22%**	**84%**	**30%**	**79%**	**58%**	**46%**

ACCNO	COR007	LEX076	NVHEQ79	LEX055	COR032	HMS25
03-001	175/185	228/230		220	257	130/132
03-002	179/181	228		218	257/261	130/132
03-003	165/179	218/228		218	257	132
03-004	179/185	228		222	257	132
03-005	175	228		222	257	130
03-006	179	228		218	257	
03-007	165/179	228		218	257	130
03-008	179	228		218	257/259	130
03-009	165/179	218/228		218	257	130/132
03-010	179	228		222	257	130/132
03-011	179/185	228		228	257	130
03-012	165/175	228		218/224	257/259	132
03-013	175/185	228		228	257	130/132
03-014	179	228		224	257/261	130
03-015	165/181	228		218	257	130
03-016	179/185	228		218/230	257/263	130/132
03-017	179/185	228		224/232	257/263	130/132
03-018	175/181	228		216/220	259/261	130/132
03-019	165/179	228		218	257	130/132
03-020	181/185	228/230		220/230	257/259	130/132
03-021	165/179	228		224	257	130
03-022	175/179	228		222	257	130/132
03-023	165/179	218/228		218	257	130
03-024		218/228			257	
03-025		228/230			257	132
03-026		228			259	130
03-027		228/230			257/259	130
03-028		228			257	130
03-032						130
03-033						130/132
03-034						132
03-035						130
homozygosity	22%	71%		78%	68%	60%

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ECA18

ACCNO	TKY19	UCDEQ136	LEX054	UMNEQ050	HMS46	TKY909	SGCV07	TKY692	HTG28	COR096	HTG17
A3-001	161/167	121	178/184	131/133	139	197/205	147/151	118/144	173	319/325	137
A3-002	167	123/125	184	137	139	203/205	147	138/144	173	323	141/147
A3-003	153/161	119/123	178/184	131/133	139	203/205	143/145	138/144	169/173	321	141
A3-004	161/167	119/125	172/182	131/133	139	205	147	138/144	171/173	321/323	141/147
A3-005	159/167	119/123	184	131/135	139	205/209	147	138	173	321	139
A3-006	159	119	184/190	131	139	205	147	138	169	321/323	139
A3-007	159/167	119/123	172/184	131	139	205	143/147	118/138	173/177	319	139/141
A3-008	167	121/123	182/184	133/135	137/139	197/203	143/151	144	173	319/325	137
A3-009	153/163	121/123	172/184	131	139	205	147	144	171/173	323	139
A3-010	167	119/123	172/182	131/135	139	205/207	145/147	118/144	171/173	315/323	141/145
A3-011	153/167	121/123	180/184	137	139/143	203/205	147	134/140	169/171	321/323	139
A3-012	161/167	119/125	184	137	139	197/203	147/151	138	169/173	323	139/147
A3-013	167	119/125	184	131/133	137/139	199/205	147/151	118/144	173	319/325	139
A3-014	153/167	121/123	184/194	133	139	197/205	147/151	138/140	173	321/325	139/143
A3-015	153	119/123	184/188	139	135/139	205	147	138	173	321/323	137/139
A3-016	163/167	119/121	182/184	131/135	137/139	203/205	143/147	138/144	173	321	137
A3-017	159/167	119	172/188	139	135/139	205	147	118/138	169/173	319/323	139/141
A3-018	153/167	119/121	172	131/133	139	205	147	118/144	173	323	141
A3-019	130/142	117/119	178/184	139	139	203/205	145/147	138	173	317/325	139/141
A3-020	161/167	121/123	184/188	137	139	199/205	147	138/144	173	315/321	139
A3-021	159/167	119/123	184/194	131/133	139	197/205	147/151	118	173	315/323	141
A3-022	167/169	119/121	172/180	133	139	197/203		118/138	173	323	137/141
A3-023	165/167	121/123	184	131/133	139	197/203	147/151	134/144	173	315/325	147
A3-024	167	121	184	133/135	137/141	211	145/147	118/144	171	321/325	139/141
A3-025	163/167		184/190					118/138	171	325	137/141
A3-026	167	119/123	184	133/135	139	205	147	138/144	173	323/325	141/147
A3-027	161/167	119	172/184	131/135	139	205	147	118/144	173	323	141
A3-028	167	119	172/184	131/133	139	205	147	134	173	323	141
A3-029	162/163	119/121	182/184	137	139/141	199/205	147	118/144	173	321/323	139/141
A3-030	167	119	184	133/135	137/139	205/211	145/147		171	323/325	139/141
A3-031	153/167		172/194					138/140	171/173	323/325	139
A3-032	161	119	184	131/133	139	203/205	145/151	118/134	173	321/323	141/145
A3-033	167	119/123	184/186	133/135	139	203/205	147		171	321/325	141
A3-034			172/184					118/144	171	321/325	141
A3-035	163/167	119/125	172/184	131	139	199/205	147/151	144	173	319/323	147
A3-036	161/167	119/121	172/184	131	139	205/209	147	138/144	171	321/323	139/147
A3-037	167	119/123	172	131	139	205/207	147	138/144	171	323	137/147
A3-038	167	119/123	172/182	131/133	139	203/205	147	134	171/173	321/323	137/141
homozygosity	38%	23%	29%	46%	74%	31%	53%	31%	74%	34%	50%

UCD387	COR101	TKY017	UCDEQ387	HLM3
84/86	182	130/134	84/86	129/137
84/86	182	128/132	84/86	125
86	182	128	86	135/137
84/88	182	128/132	84/88	129
84/88	182	128/132	84/88	129
86/90	182	132/134	86/90	135
86	182	132	86	131/137
84/86	182	128/132	84/86	125/129
84/86	182	128/134	84/86	129/135
84/88	182	132	84/88	129
86	182	128/132	86	131/135
84/86	182	128/132	84/86	125/137
84	182	132	84	129
84/88	182	132	84/88	129/137
86/88	182	128/132	86/88	135/137
86	182	128	86	125
84	182	128/132	84	127/129
86	182	128/132	86	131/137
86	182	128/132	86	135
84/86	182	132/134	84/86	129/135
84/88	182	134	84/88	129
84/86	182	128/132	84/86	129/135
84	182	128/132	84	129
84/88	182	128/132	84/88	129
84	182	132	84	
84/86	182	128/132	84/86	125/137
84/88	182	128/132	84/88	129
84	182	128	84	129
86/88	182	132	86/88	129/137
86/88	182	132	86/88	129
84/88	182	132/134	84/88	
86	182	128/132	86	135/137
86	182	128/134	86	135/137
84	182	132	84	
84/86	182	128/132	84/86	125/135
84	182	128	84	129
84/88	182	128/132	84/88	125/129
86/88	182	128	86/88	125/129
39%	**100%**	**37%**	**39%**	**43%**

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ACCNO	TKY19	UCDEQ136	LEX054	UMNEQ050	HMS46	TKY909	SGCV07	TKY692	HTG28	COR096	HTG17
03-001		121/123	184	131/133	135/139	203/211	145/147	138/144	173	315/323	141
03-002		121/125	184	137	139/143	203/205	147	138/140	171	323	139
03-003		119	172/184	131/137	139/141	205/211	147		173	323	141/147
03-004		119	172/184	131/135	139/141	205	147	118/138	173	321/323	141
03-005		119/121	184	131/133	139	203/205	147	118	173	319/323	139
03-006		119/123	184	133	139/143	205	147	118/144	171	323	
03-007		119/125	184/188	139	135/139	203	147	138	173	323	139/141
03-008		119/125	184	131/133	139/143	203/205	145/147	138/144	173	319	137/139
03-009		117/119	184	133	139	205/211	143/145	138/144	173	319/321	137
03-010		117/119	184	137	135/139	203/205	145/147	118	173	319/321	137/139
03-011		119/123	184	137	139/143	203/205	147	138/144	173	319/323	137/141
03-012		119	184	135	139	199/205	145/147	138/144	173	319/321	137/141
03-013		123/125	184	137	139/141	205	147	134/141	173	321/323	137/141
03-014		119/121	178/184	131	137/139	203/205	145/147	138/144	169/173	319/323	139/141
03-015		119/121	184	133	139	199/205	147	138	173	323	139/143
03-016		119/123	182/184	137	139/141	203/205	147/151	134/140	173	319/323	141
03-017		119/125	182/184	137	139/143	211	147	138	173	317/323	141/145
03-018		117/121	184	131/135	139/141		147	118/134	173	315/325	139/141
03-019		117/121	184			197/205			173	315/323	139
03-020		119/121	184	131/133	139	197/205	151	138/144	171/173	319/325	137/139
03-021		123/125	184/194	137	139	203/211	147/151	138/144	173	323/325	141/145
03-022		119/123	184	135	139/141	205	147	118/138	173	321/323	141
03-023		119/121	184	131/133	139	205	143/151	138/144	173	319/323	137/139
03-024		119/121	184	131/135	135/139	205/211		138/144	173	325	137/139
03-025		121	184	131/135	135/139	199/205	145/147	138/144	173		139/141
03-026		119/121	182/184	133/135	137/139	203/211	147	138	171/173		141/147
03-027		123	184	131/133	135/139	205/211	145/147	138/144	173		139/141
03-028		123/125	184	131	135/139	205	145/147	138/144	171/173		139
03-032		119/121	184/188	139	135/139	205	147	118/138	169		137/139
03-033		119	172/184	131	139	205	147	118/144	173		139/147
03-034		119/121	184	131	139	205/211		138	173		139
03-035		117/119	184	131/135	139/141	205	147/151	118/144	173		141
homozygosity		**19%**	**69%**	**58%**	**29%**	**35%**	**55%**	**23%**	**88%**	**29%**	**35%**

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UCD387	COR101	TKY017	UCDEQ387	HLM3
84/86	182	132	84/86	135/137
86	182	132	86	131/135
84/86	182	128	84/86	
84/86	182	128/132	84/86	125/129
84/86	182	128/134	84/86	125/129
86	182	128/132	86	135
86/88	182	132	86/88	135
84/86	182	128/132	84/86	125/129
84/88	182	128/132	84/88	129/135
84/86	182	132	84/86	125/135
84	182	128/134	84	129
84/86	182	128/134	84/86	129/137
84	182	128/132	84	129
84/86	182	132	84/86	131/137
86/88	182	132/134	86/88	129/137
84/88	182	128/132	84/88	129
84/86	182	128	84/86	129/135
86/88	182	128/132	86/88	129/137
84/86	182	128/132	84/86	129/137
84/86	182	132	84/86	129/131
84/86	182	128/132	84/86	125/137
86	182	132	86	125/131
88	182	128/132	88	129
84	182	128/132	84	129
84/86	182	128/132	84/86	129/137
84/86	182	132/134	84/86	135
84/86	182	128/132	84/86	135
84/86	182	128/132	84/86	135
	182	128/132		129/137
	182	132		129
	182	132/134		129
	182	128/132		127/129
25%	**100%**	**31%**	**25%**	**39%**

ECA19

ACCNO	AHT041	HTG23	LEX036	LEX073	COR092
A3-001	257/263	190/194	150	253	195/199
A3-002	251/259	190	150/154	251/253	195/197
A3-003	257/261	190/194	150	253	201/203
A3-004	251/259	190/196	150/170	251/269	195/197
A3-005	257/261	190/196	150	253/261	
A3-006	257	192/194	150	263	195/197
A3-007	257/261	190/196	150/164	253/267	195/201
A3-008	261	190	168/170	267	191/195
A3-009	257/261	190	150/170	251/267	195/201
A3-010	251/257	194	150	253	195/197
A3-011	259/263	190	150/170	253/267	195/197
A3-012	257/259	190/194	150/154	253/261	191/195
A3-013	259/263	196	170	261/269	191/201
A3-014	257	194	150/170	253/263	191/197
A3-015	261	190/196	150	253/261	195/201
A3-016	261	190	150	253/261	195/197
A3-017	251/259	190/196	150	251/267	195/197
A3-018	257/259	190/194	150/164	253/267	197/201
A3-019	255/257	190/196	150	253/267	193/195
A3-020	257/263	192/196	150/154	253/263	
A3-021	251/255	194/198	150/164	253/263	191/203
A3-022	257/261		150/168	253/261	197/199
A3-023	257/259	194/196	150	253/261	
A3-024	261	190	150	253	197/203
A3-025	253/257		150	253	195/197
A3-026	259/261	190/196	150/170	253	195/201
A3-027	251/259	190/196	150/170	251/269	195/197
A3-028	261	190	150/170	253	195/197
A3-029	255/261	190	150/170	253/269	195/197
A3-030	257/259	192/196	150/170	261/269	195
A3-031	257/259		150/160	253	195
A3-032		190	150	253/263	195/197
A3-033	257/261	184/190	150/170	253/261	195/201
A3-034	257		150/168	253	191/197
A3-035	251/257	190/192	150/168	253/267	193/197
A3-036		190/194	150/168	253/261	193/197
A3-037	261/263	190	150/170	251	195
A3-038	259/261	190/196	150/154	251/253	195
homozygosity	**22%**	**38%**	**37%**	**32%**	**11%**

ACCNO	AHT041	HTG23	LEX036	LEX073	COR092
03-001		194	154		
03-002		194	170		
03-003			150/168		
03-004		190	150		
03-005		190/198	150/170		
03-006		194/196	170		
03-007		190/194	150		
03-008		190/196	154/170		
03-009		190/194	150		
03-010		190	158/164		
03-011		190	150		
03-012		192/196	150/170		
03-013		190/196	150/164		
03-014		194/196	164/164		
03-015		190	150		
03-016		190/194	150/170		
03-017		190/194	150		
03-018		190	150/168		
03-019			150		
03-020		190/194	150/154		
03-021		190/194	150		
03-022		190	150/154		
03-023		190	150/168		
03-024			150/154		
03-025		194/196	150/168		
03-026		190/194	150		
03-027		194	150/154		
03-028		194	150/168		
03-032		190	158/170		
03-033		190	150/170		
03-034		188/196	150		
03-035		196/198	150/170		
homozygosity		**45%**	**41%**		

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ECA20

ACCNO	HTG5	LEX052	UM011	LEX071	HMS42
A3-001	91/99	210	175/177	196/200	120/142
A3-002	93/101	210	171/181	200/210	120/142
A3-003	93	212	171/183	192/202	
A3-004	95/99	210/212	171/181	202/210	120/140
A3-005	95/101	208/210		198/202	120/142
A3-006	91/95	208/210	171	196	120
A3-007	93/99	210/212	181	196/202	120
A3-008	95	208/212	175	196/208	120
A3-009	95	208/212	183	196/202	120
A3-010	91/93	206/212	167/181	198/202	120/134
A3-011	95/99	208	183	192/202	142
A3-012	93/99	210	171/181	196/210	120/142
A3-013	93/99	210/212		202/210	120/142
A3-014	93/99	208/210	183	196	120/134
A3-015	95/101	210/212	181	198/210	120/142
A3-016	91/93	208/212	171/183	198	120
A3-017	95/101	212	183	200/210	
A3-018	95/99	210	175/183	202/210	120/134
A3-019	95	212	171/183	198	120/134
A3-020	95/101	208		196	134
A3-021	95	208	183	202/210	
A3-022		212	167/171	198	
A3-023	95	208/210		196/202	120
A3-024	95	208/210	175/181	192/198	120/142
A3-025		206/208	175/183	198/210	
A3-026	95	210	171/183	208/210	134/140
A3-027	99/101	208/212	183	202/210	120/140
A3-028	93/95	208/212	167/183	198/202	120/134
A3-029	93/95	208/212	169/175	202	120/142
A3-030	95/101	210/212	175/183	192/210	120/140
A3-031		210	167/171	200/202	
A3-032	93/99	206/212	171/175	198/208	120/134
A3-033	89/95	208/210	181	192/206	134/142
A3-034		206/210	167/175	198/202	
A3-035	95/101	210/212	177/183	200/210	142
A3-036	95	210	167/171	202/210	140/142
A3-037	95/101	208/212	181/183	210	120/140
A3-038	95	208/212	175/183	196	134
homozygosity	**29%**	**37%**	**32%**	**24%**	**32%**

ACCNO	HTG5	LEX052	UM011	LEX071	HMS42
03-001	91/101			198/200	120/134
03-002	93/95			192/196	120/134
03-003				208/210	
03-004	95/101			210	140/142
03-005	95/99			196/208	
03-006	95/99			196/200	120
03-007	93/95			196/198	120/142
03-008	89/95			196/200	120
03-009	95			196	120
03-010	95			196/200	120/142
03-011	95			196/200	120
03-012	95/99			196	120
03-013	95/99			198/200	120
03-014	95/101			196/202	120/134
03-015	95			202/210	142
03-016	93/95			196/210	120/142
03-017	93/95			198/210	136/142
03-018	93/95			196/200	120
03-019				192/210	
03-020	93/101			200/210	134/142
03-021	95			196	120/134
03-022	93/101			202/210	134/142
03-023	95			196/200	120
03-024					
03-025	91/95				120/142
03-026	95				120/134
03-027	95				120/134
03-028	95				120
03-032	91/95				120/142
03-033	89/93				120/134
03-034	95				120/142
03-035	93/95				120
homozygosity	**34%**			**17%**	**39%**

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ECA21

ACCNO	TKY021	SGCV14	SGCV16	HTG10	COR073	COR068	HTG32	LEX037
A3-001	134	194	154	105	195/203	160/166	138/140	195/199
A3-002	138	194/196	158/194	109/111	197/203	160/166	138	199
A3-003	134/140	194/196	158/194	103/109	195/197	160/166	138/140	195/199
A3-004	138/140	196	158/194	99	197/203	156/162	138	199
A3-005	126/134	194/196	154	103/111	199/201	156	138	193/195
A3-006	126/138	192/196	158/164	97/105	197/203	156/160	138/140	197
A3-007	134/140	196	164/194	109/111	197/203	162	138/142	199
A3-008	126/140	194/196	158	101	195/203	156/162	138	199
A3-009	140	196	158	105/109	197/199	156	138/140	195/199
A3-010	134/140	196	194	101/109	197/203	162/164	138/142	199/201
A3-011	126/134	196	158	103/111	197/203	162	138/142	195/199
A3-012	134/138	196/200	194	103/109	195/199	160/166	138	195/199
A3-013	138/140	194/196	194	97/105	197/203	156/166	138	199/201
A3-014	126/140	194/196	158/194	109/111	199/203	156/16	138	195
A3-015	126/140	196	154/194	101/109	197/203	156/164	138/142	195/199
A3-016	134/140	196	158	101/111	197/203	156/160	138	195/199
A3-017	134/140	196/198	194	105/109	195/203	166	138	199
A3-018	138/140	196	194	101/105	197/203	164/166	142	199
A3-019	126/140	194/196	158/194	101/109	197/203	156/162	138	195/199
A3-020	138/140	196	154/194	97/111	195/197	156	138	197
A3-021	126/140	194/196	158/194	99/105	195/197	156	138/140	199/201
A3-022	126/140	194/196	158	103/109	197/201		138/140	195/199
A3-023	126/134	196/200	154/194	97/109	197/203	156/162	138/142	199
A3-024	126/140	196	194	99/101	197/199	156/160	138	197/199
A3-025	140	196	158/194	101/111	197/203			199/201
A3-026	134/140	196	194	101/109	195/197	162/166	138	199
A3-027	138/140	196	194	99/101	197	156	138	199
A3-028	140	196	194	99/111	197/203	156/162	138/140	195/199
A3-029	126/140	196	194	101/111	197/203	162	138	199
A3-030	140	196	194	101/105	197/203	156/166	138	199
A3-031	136/140	196	154/194	101/109	197/203			195/197
A3-032	134/140	196	154/194	99/111	197/203	160/162	138	195/197
A3-033	126/138	196	154/194	99/109	197/203	156/160	138	
A3-034	126/138	196	154/164	99/111	201/203			199/201
A3-035	138/140	196/198	194	99/101	197/199	156	138	199
A3-036	134/138	194/196	154/194	105/109	197/199	162/166	138/142	199
A3-037	138/140	194/196	158/194	99	197/205	156/162	138	195/199
A3-038	140	196	158/194	101	197/203	156	138	195/199
homozygosity	**18%**	**58%**	**50%**	**13%**	**3%**	**32%**	**63%**	**43%**

ACCNO	TKY021	SGCV14	SGCV16	HTG10	COR073	COR068	HTG32	LEX037
03-001	132/138	196	164/194	101/105		162/166	138	
03-002	134/138	194/196	158/194	99		156	138	
03-003	134/138	194/196	154/194	109			138/140	
03-004	138	194/196	185/194	109/111		160/166	138	
03-005	126/138	196	162/194	97/99		156/164	138	
03-006	126/134	194/196	158/162	101/105		156/166	138/140	
03-007	126/138	196	154/194	97/101		156	138	
03-008	126/140	196	154/158	99/101		160/162	138/140	
03-009	126/134	196	158/194	101/105		162/166	138	
03-010	126/134	194/196	158	97/105		156/164	138	
03-011	134	196	194	99		162	138	
03-012	126/138	196	154/158	101/111		162	138	
03-013	126/138	196	154/194	99/101		162/166	138/140	
03-014	126/140	196	158	99/101		160/162	138/140	
03-015	126/140	196	194	105/111		156	138/144	
03-016	126	196	158/162	111		156/158	138/140	
03-017	126	196	158	101/111		156/162	138/140	
03-018	140	196	158/194	101/105		160/164	138/144	
03-019	126/140	196	154	101				
03-020	126/140	196	158	101/113		93/101	138/140	
03-021	134/138	196	194	99/109		95	138	
03-022	138/140	194/196	158/194	111		93/101	138	
03-023	134	194/196	154/194	101/105		95	138	
03-024	134	196					138/140	
03-025	126/134	194/196	154/162	103/105		91/95	138/140	
03-026	134/140	194/196	154/194	99/105		95	138	
03-027	132/134	194/196	194	101/111		95	138	
03-028	126/134	194/196	154/194	101/111		95	138	
03-032	126	196		101		91/95	138/142	
03-033	126	196		103/113		89/93	138/140	
03-034	138/140	194/196				95	138/140	
03-035	140	196		101/111		93/95	142	
homozygosity	31%	63%	33%	23%		40%	52%	

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ECA22

ACCNO	TKY285	COR022	HTG21	COR016	HMS47
A3-001	172	274	139/143	188/206	205/209
A3-002	174/182	272	133/141	190/200	209
A3-003	170/178	270/272	139/145	190/200	209/215
A3-004	174/182	270/274	139/143	200	205/209
A3-005	172/178	270	137	188	209/215
A3-006	174/182	272/274	135/143	190	205/215
A3-007	174/178	270/272	133/139	178/200	209/215
A3-008	178/182	272/274	139/141		203/209
A3-009	174/182	272/274	139/143	200	209/215
A3-010	168/178	272/274	139/141	180/206	205
A3-011	174/178	270/272	133/139	190/200	197/205
A3-012	174/178	272/274	133/137	190/200	197/215
A3-013	172/182	274	137/141	190/200	205/215
A3-014	168/182	272/274	141/143	180/200	205
A3-015	178/180	272/274	135/137	200	205/209
A3-016	174/182	272/274	133/141	200/206	209
A3-017	168/178	270/272	131	200	209
A3-018	164/170	272/274	141/143	200	209
A3-019	164/182	270	135/137	190/202	209/215
A3-020	172/180	274	137	190/206	203/209
A3-021	174/178	272/274	139/143	190/200	209
A3-022	170/174	270/272	143/145	200	205/215
A3-023	172/178	274	137/139	190/200	209
A3-024	178	270/272	139/145	190/206	205/209
A3-025	168		139/141	180	205/209
A3-026	172/178	272	141/145	190/200	209
A3-027	178/182	272/274	139/141	200	205/209
A3-028	178/182	270/272	135/143	190/200	209
A3-029	178	272	131/137	190/200	205/209
A3-030	172/182	274	137/143	200	205/209
A3-031	170/180			180/190	197/205
A3-032	178/180	272/274	139/141	180/190	205/209
A3-033	172/178	272	139/145	190	209
A3-034	164/180		139/145	188	209
A3-035	172/174	270/272	137	190/200	209
A3-036	174	270	139/141	190/200	205/215
A3-037	178/182	270/272	133/141	190/200	209
A3-038	168/180	270/272	141/145	188/206	209
homozygosity	**13%**	**34%**	**11%**	**35%**	**39%**

ACCNO	TKY285	COR022	HTG21	COR016	HMS47
03-001		270			205/209
03-002		272/274			205/215
03-003					209/215
03-004		272			197/207
03-005		272			215
03-006		272/274			205/209
03-007		270/274			205
03-008		270/272			205
03-009		272			197/205
03-010		272			205/209
03-011		272/274			205/215
03-012		270/274			205
03-013		272			209
03-014		272			205/209
03-015		270/272			205/215
03-016		270/272			205/207
03-017		272			205/215
03-018		266/272			205
03-019		272			205
03-020		270/274			205
03-021		274			197/205
03-022		270/272			197
03-023		272			205
03-024		272			197/205
03-025		272/274			209
03-026		272/274			209/215
03-027		272/274			205
03-028		270/272			205
03-032		268/272			205
03-033		270/272			205/215
03-034		270/272			197/207
03-035		270/272			205/209
homozygosity		39%			44%

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ECA23

ACCNO	COR055	LEX063	COR084	SGCV004
A3-001	258/274	250	202	217/221
A3-002	258/274	244/250	202	221
A3-003	242/258	240/252	200/202	221
A3-004	258/262	244/250	202/204	221
A3-005		244	202/204	221/223
A3-006	262/268	240/250	204	217/221
A3-007	240/274	240/250	204	221
A3-008	262	240	202	215/221
A3-009	258/274	250	200/204	221/223
A3-010	266/268	250	202	215/221
A3-011	258/268	244/250	202	215/219
A3-012	266/274	240/250	202	221
A3-013		250	202	215/221
A3-014	258/268	246/250	200/202	217/221
A3-015		240/244	202/204	221/223
A3-016	262/274	240	200/202	221
A3-017	258/268	240/244	202	215/221
A3-018	242/262	240/250	202/204	221
A3-019	240/256	240/250	202	217/221
A3-020		244/246	200/202	221
A3-021	262/268	240/250	202	221/223
A3-022	262/266	240/244	202/204	221/223
A3-023		240	202/204	221
A3-024	266/268	240/246	202	221
A3-025	262/266		200/204	
A3-026	258	244/250	202/204	215/221
A3-027	262/268	244/250	202/204	221
A3-028	268/274	250	200/202	221/223
A3-029	242	240/246	202	215/221
A3-030	262/268	240/250	204	221
A3-031	242/262		200/202	
A3-032	242/268	250	202	221
A3-033	258/268	246/250	204	215/221
A3-034	262/274		200/202	
A3-035	262/268	240/244	202	215/221
A3-036	258/266	250	202/204	221
A3-037	242/268	244/248	202/204	221/223
A3-038	262/268	240/250	202/204	221
homozygosity	**9%**	**31%**	**47%**	**43%**

ACCNO	COR055	LEX063	COR084	SGCV004
03-001		240/244	200/204	221
03-002		240/250	202	221/223
03-003			202	221
03-004		250	202/204	221
03-005		248/250	202/204	221
03-006		230/244	202/204	221
03-007		244/248	202	221/223
03-008		240/244	200/204	221
03-009		250	202/204	215/221
03-010		244/246	200/204	221
03-011		244/250	202/204	221
03-012		240/250	200/204	221
03-013		250/258	202/204	221
03-014		250	200/202	215/221
03-015		240/244	200/202	221
03-016		240/250	202	221
03-017		240/250	202	221
03-018		240/250	200/202	215/221
03-019		240/250	202	
03-020		250	202/204	217/221
03-021		240/246	202	221/223
03-022		250	202	221
03-023		240/250	202/204	215/221
03-024		240/250	200/202	221
03-025		240/250	200/202	221
03-026		240/248	202/204	221
03-027		240/248	202/204	217/221
03-028		248/258	200/202	221
03-032		244/250		215/221
03-033		250		221
03-034		240/250		215/221
03-035		250		221
homozygosity		**23%**	**29%**	**65%**

ECA24

ACCNO	LEX042	AHT32	AHT4	COR061	LEX074	COR024
A3-001	219	149	148/154	209	163/173	218
A3-002	225/227	145/147	162/164	209	169	216/226
A3-003	223/225	147/151	162	211/217	163/173	222/226
A3-004	225	147/149	150/162	209/219	167/171	222/226
A3-005	225	151	148/152	211/215	163/169	214/226
A3-006	225/227	147	162	209/211	167/177	226
A3-007	225/227	147/151	148/162	209/211	167/169	226
A3-008	225	147/151	148/162	211/221	167/175	226
A3-009	227	147/151	148/150	203	173	222
A3-010	225/227	147	148/162	203/207	167/177	214/224
A3-011	219/225	147	156/164	211/217	173/175	
A3-012	225	147/179	152/164	209/211	163/169	214/216
A3-013	225/227	147/151	156/162	203/209	167	216/224
A3-014	219/225	149/151		209/221	165/167	226
A3-015	225	147/149	152/160	209/219	167/173	214
A3-016	219/225	147/149	162/164	201/219	171/173	222/226
A3-017	225/227	147		209/211	163/169	222/226
A3-018	219/225	147/149	156/162	209/211	165/175	214/226
A3-019	225/227	147/151		209/219	169/171	226
A3-020		147/149	148/152	215/219	173	216/222
A3-021	225/229	147/149		209/219	163/177	216/222
A3-022		147/149	162	209/211	163/177	222/226
A3-023	219/225	147/151	148/162	209/211	165	222/226
A3-024	225	147/151	148/162	211	163	222/226
A3-025			156/162	211/217	163/169	222/226
A3-026	219/225	147/149	162	203/219	167/171	226
A3-027	225/231	147	154/164	211/221	167/169	222
A3-028	225/227	147/149	148/152	203/209	163/173	214/222
A3-029	219/225	147	162	209	163/167	214/222
A3-030	225/227	147/151	162/164	211	159/169	222/226
A3-031			162	209/219	165/171	216/226
A3-032	225	147/151	145/158	207/221	167/177	218
A3-033	219	149	162	203	167	218/226
A3-034			148/156	203/217	169/173	
A3-035	225/227	147	148/164	209	163/167	
A3-036	225	149/151	150/152	209/215	167/169	216/226
A3-037	225/231	147/149	150/164	209/211	167/169	216/222
A3-038	219/225	147/149	152/162	203/221	167/173	226
homozygosity	**33%**	**29%**	**21%**	**21%**	**18%**	**34%**

ACCNO	LEX042	AHT32	AHT4	COR061	LEX074	COR024
03-001	225/229	147/151	148/162			
03-002	219/231	147/149	162			
03-003		147/149	148/152			
03-004	225/229	147/149	150/162			
03-005	219/229	149/151	148/152			
03-006	219/231	147/151	160/162			
03-007	227	147/151	162			
03-008	225/231	151	148/160			
03-009	219/225	147	162			
03-010	219/223	147/151	162			
03-011	219/227	147	148/162			
03-012	219/227	147/149	162			
03-013	219/225	147/151	148/162			
03-014		147/151	160/162			
03-015	219/229	149	152/154			
03-016	219	147/149	162			
03-017	219	147/149	150/162			
03-018	225	151	154/162			
03-019			162			
03-020	225/229	147/151	158/162			
03-021	225/231	147/149	150/162			
03-022	227/229	147	162			
03-023	219	147/149	150/162			
03-024	225	147/149	162			
03-025	225	145/147	150/162			
03-026	225	145/147	154/162			
03-027	225/231	151	162			
03-028	225/227	151	158/162			
03-032	219/225	149/151	150/162			
03-033	219/225	149/151	162			
03-034	219/225	149/151	150/162			
03-035	219/227	145/147				
homozygosity	**28%**	**26%**	**35%**			

ECA25

ACCNO	AHT051	NVHEQ043	AHT007	COR018	COR080
A3-001	164/172	156/158	131	271/281	212
A3-002	178/180	156/164	135/141	259	212
A3-003	162/164	156/158	131/133	261/275	212
A3-004	154/180	160/164	131/135	261	212
A3-005	166/180	152/156	131	261/275	210
A3-006	154/166	152/160	131	261	212
A3-007	164	156/158	131/133	271/275	210
A3-008		156/160	129/131	261/283	212
A3-009	162/166	152/156	141	259	212
A3-010	154/162	156/160	127/131	271/283	212
A3-011	180	160/164	131/135	261	212
A3-012	166/180	156/160	133/141	261/271	212
A3-013	154/164	156/160	127/137	261/273	212
A3-014	172	158/160	133/139	261/271	210
A3-015	162	152	127/131	261/273	212
A3-016		156/158		261/273	212
A3-017	162/180	152/164	127/131	261	212
A3-018		152		271	212
A3-019	166	152/160	131	261/273	212
A3-020	154/180	158/160	137	273	208
A3-021	154/166	152/160	141	273	212
A3-022	180/184	156/164	129/135	273/283	212
A3-023		156	129	273/283	212
A3-024	162	152	131	273	212
A3-025	154/162	156/160	127/131	261/271	
A3-026	166/180	152/164	131/135	261/273	212
A3-027	162/180	156/164	131/135	261	212
A3-028	166/180	152/164	135/141	271/275	212
A3-029	154/166	156/160	137	261/279	210/212
A3-030	166/180	152/164	131/135	261/271	212
A3-031	162/186	152/156	131	261	
A3-032	166/180	160/164	127/135	261/273	208/212
A3-033	154/166	156/160	141	261/273	208
A3-034	154/162	152/160	127/131	261/273	
A3-035	162/180	152/164	131/135	261/273	212
A3-036	180	156/164	131/135	261/275	212
A3-037	162/166		135/141	261/273	210
A3-038	166/176	152/158	131/137	261/271	210
homozygosity	**21%**	**11%**	**33%**	**32%**	**94%**

ACCNO	AHT051	NVHEQ043	AHT007	COR018	COR080
03-001	166	151/161	127/131	261/273	210/212
03-002		151/165	133/135	261/273	210
03-003	180/184		135/139	273/275	212
03-004	180	155/165	135/141	261	212
03-005	164/172	153/157	133	273	210
03-006	162	151/155	131	261	212
03-007	154/162	151/163	131/133	271/275	210
03-008	176	157/159	131	273/283	210/212
03-009	166	151/161	131/135	273	212
03-010	162/166	151/161	131	273	212
03-011	166	151/155	137/139	261/273	212
03-012	154/164	157/163	131/139	261	212
03-013	162	151/155	127/131	261	212
03-014	164	153/155	131/141	273/285	208/212
03-015	162	151/155	131	273/283	212
03-016	154/166	157/161	137/141	261/273	208/212
03-017	162/164	155/157	131/133	261/283	208/212
03-018	180	159/161	131/141	261	212
03-019	162/164	151/157	131	261/273	212
03-020	162/180	151/155	141	273	212
03-021	162	151/155	135	261	212
03-022	166/180	155/161	127/141	261/273	212
03-023	154/166		131	273/283	212
03-024		155/163		261/273	212
03-025		151/163	131	261/273	212
03-026	154/162	157/161	131	271/273	210
03-027	166/174	157/159	131/141	261/271	212
03-028	154/174	151/161	131/133	271/283	212
03-032	154/162		131	273	212
03-033	154/162		131	273	212
03-034	162/172		131/133	273	210/212
03-035	162		131/133	273	212
homozygosity	**41%**	**0%**	**42%**	**44%**	**81%**

ECA26

ACCNO	NVHEQ070	A17	EB2E8	COR071
A3-001	196/200	112/116	148	202/210
A3-002	192/196	112/114	148	198
A3-003	196/202	112	144/148	200/208
A3-004	196	108/112	148	198/210
A3-005	192/196	110/112	148	198/210
A3-006	196/206	112	148	198/202
A3-007	200/206	112/114	148	198/210
A3-008	192/196	114	148	198/202
A3-009	196/206	108/114	148	198/206
A3-010	192/196	112/114	148	198/210
A3-011	196/206	108/110	148	188/206
A3-012	192	112/114	144/148	188/198
A3-013	192	112	148	198/208
A3-014	196	118	144/148	188/210
A3-015	192	112	148	198/210
A3-016	196	108/112	148	198/206
A3-017	196	108/112	148	198/210
A3-018	196/206	108/112	148	198/210
A3-019	196	108/112	148	198/206
A3-020	196/206	112	144/148	198/202
A3-021	192/196	106	144/148	188/202
A3-022	196	112	148	200/208
A3-023	196/206	108/114	148	198/206
A3-024	192/206	112	148	188/198
A3-025	192/206	112	144/148	198/208
A3-026	196	112/114	144/148	188/198
A3-027	192/196	112	148	198/210
A3-028	206	112	148	198/202
A3-029	192/200	108/112	144	206/208
A3-030	192/196	112	148/152	198/208
A3-031	196/206	114/118	148	188/198
A3-032	192/196	112	148	198/210
A3-033	196	112	144/148	198/202
A3-034	192/196	112/114	148	198/208
A3-035	196	112	148	198/210
A3-036	196	108/116	148	198
A3-037	196	110/112	148	198
A3-038	196/206	112	148	198
homozygosity	**39%**	**47%**	**76%**	**11%**

ACCNO	NVHEQ070	A17	EB2E8	COR071
03-001	192/196	112	144/148	
03-002	196/200	112/118	144	
03-003	196	108/114	148/152	
03-004	196	108/116	146/148	
03-005	192/204	112/118	144	
03-006	196/206	112	148	
03-007	192	112	148	
03-008	196	112	148	
03-009	206	108	148	
03-010	196/206	112/114	144/148	
03-011	192/200	106/112	144/148	
03-012	192/206	112	148	
03-013	192/196	106/112	144/148	
03-014	196	106/112	144/152	
03-015	196/206	112/114	148	
03-016	192/196	106/112	144/152	
03-017	192/196	106/108	144/148	
03-018	196/204	112/116	148	
03-019	**/**	112	148	
03-020	192/200	112/114	144/148	
03-021	196/206	108/112	148	
03-022	192/196	114/116	148	
03-023	196/206	108/112	148/152	
03-024	196/206	108/112	148	
03-025	196/206	108/112	148	
03-026	200	112	146/148	
03-027	200/202	106/114	148	
03-028	192/202	106/112	148	
03-032	206			
03-033	206	118		
03-034	196/206	112/114		
03-035	206	108/118		
homozygosity	**31%**	**31%**	**57%**	

ECA27

ACCNO	COR021	COR040	HMS45	COR017
A3-001	218	294/296	199	257
A3-002	216/222	292/294	199/205	257
A3-003	222	292	199/203	257/265
A3-004	216/218	292/294	197/199	253/263
A3-005	216/222	294	199	257/265
A3-006	218	284	199	257/265
A3-007	216/220	290	199	253/265
A3-008	216/218	296/298	197/199	253/265
A3-009	218	288/296	197/199	257
A3-010	220/222	290/296	199/201	257/263
A3-011	216/218	288/292	197/199	253/257
A3-012	210/222	292/294	197/205	257/265
A3-013	218/222	286/294	199	253
A3-014	216/218	296/300	199	257/265
A3-015	216/218	292/294	199	257/265
A3-016	218	292/298	197/199	265
A3-017	210/216	294/300	197/199	263/265
A3-018	210/216	292/294	197/199	263/265
A3-019	218	300	197/199	257/265
A3-020	210/218	286/300	199/205	253/265
A3-021	210/218	284/296	197/199	257/265
A3-022	216/222	290		253/257
A3-023	220/222	292/294	199	255/257
A3-024	210/218	290/294	197	255/265
A3-025	216/218	292/294		257
A3-026	216/218	294/296	197/199	259/265
A3-027	216/218	292/294	197/199	253/263
A3-028	218	292/298	199	253/265
A3-029	216/222	296/298	199/203	257/265
A3-030	216/218	290/294	197/199	253/263
A3-031	216/222	296		257/265
A3-032	210/218	290/292	197/199	257/259
A3-033	222	288/290	197	259/265
A3-034	216/218	286/294		253/257
A3-035	216	294/296	199	257
A3-036	216	294/296	199	257/263
A3-037	216/218	286/294	199	253/257
A3-038	218	288/292		253/265
homozygosity	**29%**	**18%**	**42%**	**18%**

ACCNO	COR021	COR040	HMS45	COR017
03-001			197/199	
03-002			197/203	
03-003				
03-004			197/199	
03-005			197/203	
03-006			197/199	
03-007			199	
03-008			197	
03-009			197/203	
03-010			199	
03-011			197/205	
03-012			197/201	
03-013			197	
03-014			199	
03-015			197/199	
03-016			197/203	
03-017			197/205	
03-018			197/203	
03-019				
03-020			197	
03-021			199	
03-022			197/205	
03-023			197	
03-024			199	
03-025			199	
03-026			197/199	
03-027			197/201	
03-028			199/201	
03-032			199	
03-033			197/199	
03-034			197	
03-035			199/205	
homozygosity			**40%**	

ECA28

ACCNO	HTG30	UM003	NVHEQ54	TKY319	TKY515	TKY018	UCDEQ425
A3-001	232	160/164	188	120/126	129	132/140	
A3-002	232	160/164	178/188	120/126	129/133	128/140	
A3-003	238	160/164	188/190	120	135	128/142	250/252
A3-004	232/238	160	188	120/126	129	128/140	244/252
A3-005	244	158/164	178/188	120/126	129/133	130/140	244/252
A3-006	232	158/164	188	120/126	133	128/130	252
A3-007	232	164	188	120/126	129/135	140/142	244/248
A3-008	232	162/164	188/190	124/126	129/135	136/140	252
A3-009	238	160/164	188/190	120/124	129/133	136/140	250/252
A3-010	232	160	178/188	120/126	129/133	128/142	250/252
A3-011	232	164	188	126	129	128/140	252
A3-012	232	160/164	178/188	120/126	133/135	1470/142	252
A3-013	238	160	178/190	126	129	130/142	254
A3-014	232	158/164	178/188	120/126	129/135	130/134	250/252
A3-015	232	160/164	188/190	120/126	129	142	244/254
A3-016	232	160/164	178/188	120/124	129	130/142	244/252
A3-017	232	160	188	120/126	129	128/134	244/250
A3-018	232	160/162	188	120/124	129/133	128/132	252/254
A3-019	232	160	188	126	129	130/142	244
A3-020	232	158	188	120/126	129	130	244/252
A3-021	232	160/162	188/190	120/126	129/131	130/140	250/252
A3-022	232	160/164	188	120/126		140	252
A3-023	232/238	160	178/188	126	129	132/136	248/252
A3-024	238	160/164	188/190	120/126	129	128/142	248/252
A3-025	244	158/164				138/142	252/254
A3-026	232/244	158/162	178/188	124/126	135	128	250/254
A3-027	232	160	178/188	120	129/135	128/140	244/252
A3-028	244	160/164	188	120/126	129/135	130/142	252
A3-029	232/238	164	188	120	129/135	130/140	244/252
A3-030	232	160/162	188/190	120	129	128/140	244/252
A3-031	232	160/164				128/142	250/252
A3-032	232	160/162	190	124/126	129/133	130/140	238/252
A3-033	232/242	158/164	178/188	120/124	133/135	130/142	250
A3-034	244	158/162				128/142	250/252
A3-035	232	160/164	178/188	120/126	129/133	128	250/252
A3-036	232/244	158/160	178/188	120/126	129	128/143	244/252
A3-037		158/160	178/188	120/126	129/133	128/136	252
A3-038	238	160/164	188	120/126	129	142	252
homozygosity	**84%**	**29%**	**40%**	**23%**	**50%**	**16%**	**31%**

ACCNO	HTG30	UM003	NVHEQ54	TKY319	TKY515	TKY018	UCDEQ425
03-001	232		188	124/126	129/135	124/128	244/252
03-002	232/238		178/190	120/124	131/133	126/128	252/254
03-003	238/244			120/126	129/133	130/134	252
03-004	232		188	120	129/133	128/140	252
03-005	232/238		178/188	120	129/133	130/142	252
03-006	232		188	120	133/135	128/130	250/254
03-007	232/244		188	120	129/133	138/142	244/256
03-008	230/232		178/188	124/126	129/133	130/142	244/252
03-009	238		188/190	120/126	129/135	128/140	250/252
03-010	232/252		178/188	120/130	131/133	130/142	236/252
03-011	232/242		188	120/126	129	128/142	252
03-012	232		188	116/126	129	128/130	252/254
03-013	232/238		188	126	129/135	128/130	248/252
03-014	232		188	120/126	129/131	140	252
03-015	232/244		188	120/126	129/135	132/142	
03-016	232		188/190	126	133/135	130/140	244/254
03-017	232		188	124/126	135	140/142	244/252
03-018	232		188	124/126	133/135	130/132	244/248
03-019	232					128/130	236/256
03-020	232		188	120/126	129	128/132	252
03-021	232		188	120/126	129/135	128/140	244/252
03-022	232		178/188	120	133	128/140	252
03-023	232		188/190	120/126	129/133	128/140	252
03-024	234			120/126		128	
03-025	232		188	120/126	129/131	128+	
03-026	232		188	120/126	131/133	128/132	
03-027	232/244		188	120/124	129/133	128/130	
03-028	232/244		188	120/126	133	130/142	
03-032			188/190	124/126	129/133	128/130	
03-033			188	124/126	129/133	128/130	
03-034			178/188	120/126	129/135	140/142	
03-035			188	126	129/135	130/142	
homozygosity	**61%**		**66%**	**26%**	**20%**	**9%**	**36%**

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ECA29

ACCNO	LEX018	COR082	COR027	L122
A3-001	248/252	237	245/249	140/146
A3-002	248/250	231	239	140/150
A3-003	248	207/237	249	150
A3-004	236/248	231/233	239/247	150/152
A3-005	246/248	229/237	239	150/152
A3-006	248	233/237	239/247	140/146
A3-007	238/242	229/231	247/249	146/156
A3-008	246/248	229/237	247	140/152
A3-009	242/250	231/233	249	140/148
A3-010	248	229/237	239/249	146/156
A3-011	242/246	231/235	249	146/150
A3-012	236/248	231/237	239/249	146/150
A3-013	236/246	233	247/249	
A3-014	242/250	231/233	247	140/146
A3-015	248	233	239/247	146/150
A3-016	246	229	249/253	146
A3-017	248/250	231/235	239/249	140/156
A3-018	248/250	235	239/247	146
A3-019	246/248	235	245/249	146/150
A3-020	242/248	233	249	148/154
A3-021	246/250	229/237	249	146
A3-022		231/233	239/249	146/154
A3-023	248	207/237	247	
A3-024	240/248	235/237	247/249	140
A3-025		215/229	245/249	150/156
A3-026	246/248	229/233	231/239	150/156
A3-027	236/248	229/231	239/247	152/156
A3-028	246	229	249	150/156
A3-029	242/246	229/231	247/249	140/146
A3-030	242/248	231/233	239/249	146/156
A3-031		229/237		146
A3-032	236/242	229/237	247/249	140/146
A3-033	250/252	229	247/249	154
A3-034		215/237	249	154/156
A3-035	248/250	231/235	239/249	140/156
A3-036	238/248	231/233	239	150/152
A3-037		231/237	239/247	156
A3-038	246/248	229/233	247/249	146/152
homozygosity	**21%**	**26%**	**35%**	**22%**

ACCNO	LEX018	COR082	COR027	L122
03-001	242/248	233/237		
03-002	236/248	233		
03-003		207/233		
03-004	248/252	215/233		
03-005		207/233		
03-006	236/242	231/237		
03-007	248	233		
03-008	236/248	229/235		
03-009	246/248	229/237		
03-010	246	229/231		
03-011	238/246	231/233		
03-012	248	229/231		
03-013	238/246	231		
03-014	242/246	229/231		
03-015	246	229		
03-016	238/248	231		
03-017	246/248	207/233		
03-018		235/237		
03-019	240	229		
03-020	246/248	215/229		
03-021		231/237		
03-022	248/250	215/231		
03-023	246/252	229/237		
03-024	240/242	229/231		
03-025	242/248			
03-026	248			
03-027	248			
03-028	238/248			
03-032	248/250			
03-033	246/250			
03-034	242/246			
03-035	242/246			
homozygosity	**25%**	**44%**		

ECA30

ACCNO	LEX025	HTG27	HMS18	VHL20	AHT033	TKY274	COR038	VIASH21	AHT034
A3-001		145/149	176	89/95	167	120/122		255	142
A3-002		157	178	93/105	163/171	122/126		255	130/142
A3-003	166	145/157	176/178	89/97	167/173	122/140	216	255	130/146
A3-004	166	157	176/178	101/107	171	108/140	214/216	253/255	130/142
A3-005	164/166	157	176	95/107	165/167	122/140	214/216	255	146/148
A3-006	166/168	153/157	176/178	89	157/167	126/140	216	255	146
A3-007	166	145/155	176/180	89/107	159/167	118/126	216	253/255	142/146
A3-008	164/166	151/157	178	97/107	167/173	126/128	216	253/255	130/146
A3-009	166/168	155/157	176/178	97	167/173	126	216	255	146/148
A3-010	166	153/157	176/178	93/101	171/173	108/126	214/216	255	144/146
A3-011	164/166	155/157	176/178	95/107	159/173	118/126	216	253/255	130/146
A3-012	152/166	153/157	176/178	89/105	161/171	126/140	216	255	142/146
A3-013	152/164	157	176/178	105/107	159/167	118/140	216	255	130/146
A3-014	166	157	176		169/171	120/122	216	255	142
A3-015	166/168	155/157	176/178	89/101	161/173	140	214/216	253/255	142
A3-016	166/168	145/151	178	95/97	169/171	122/126	214/216	255	130/142
A3-017	166/168	157	178		155/171	118/140	216	255	142/146
A3-018	166	145/153	176/178	93/105	157/167	118/138	216	253/255	130/146
A3-019	166	157	178		161/167	118/122	216	253/255	142/148
A3-020	166/168	145/157	176	101/107	163/167	126/140	216	253/255	146
A3-021	166	157	178/180	89	161/173	126/140	216/222	255	146/152
A3-022	166/168	157	176/178		167/173	108	216	255	142/146
A3-023	166/168	157	178	89/101	167/173	140	216	255	146/150
A3-024	168	157	176/178	97/101	161	122/126	216	255	142
A3-025	166/168	157	176/178	89/107	167	122/126	216	255	146/150
A3-026	164/166	157	176/178	89/103	157/169	118/122	216	255	130/150
A3-027	166	157	176/178	89/95	161/171	122	216	255	142/146
A3-028	166/168	155/159	176/178	97/107	157/167	122/126	216	255	142/148
A3-029	166/168	145/157	176/178	89/97	161/167	122/138	216/222	255	142
A3-030	166	145/157	176/178	93	169/171	122/140	216	255	130/142
A3-031	166	145/157	176	99/105		108/126	216	255	130/142
A3-032	166/168	145/151	176	95/97		108/122	216	255	
A3-033	166	145/157		89/93	171/173	108/140	216	255	142/146
A3-034	152/166	145/157	178	97/107	167	122/126	216	255	142/146
A3-035	166	157	178	93/95	155/171	118/140	216	255	142
A3-036	166	157	176/178	93/107	155	140	216	255	142
A3-037	166/168	151/157	178	93/107	171	138	214/216	255	130/142
A3-038	166/168	151/157	176/178	97/105	161/171	122/126	214/216	255	130/146
homozygosity	**42%**	**42%**	**41%**	**17%**	**19%**	**18%**	**75%**	**79%**	**24%**

ACCNO	LEX025	HTG27	HMS18	VHL20	AHT033	TKY274	COR038	VIASH21	AHT034
03-001	166/168	145/157		89/107		118/122	216	253/255	
03-002	164/168			97/107		122	216	255	
03-003	162/166			89/93		122	216	255	
03-004	166/168	145/157		93/105		122	216	255	
03-005	164/166			89/107		122	214/216	253/255	
03-006	166/168	145/151		95/97		122	216/222	255	
03-007	166/168	151/157		89/105		118/140	216	255	
03-008	166	157		97/107		126	214/216	255	
03-009	166/168	149/157		89/107		122	214/216	255	
03-010	166	145		89		138/140	216/222	255	
03-011	166	157		89/107		122/138	216/222	255	
03-012	166/168	145/159		107		122/140	214/222	255	
03-013	166/168	157		89/105		118/126	216	255	
03-014	166	149/157		89/107		140	216	255	
03-015	162/166			93/97		122/140	214/216	255	
03-016	166	157		89/97		118/140	216	253/255	
03-017	166	157		89/107		118/138	216/222	253/255	
03-018	164/166			97/105		118/122	216	255	
03-019	162			97/101		122/126	214/216	255	
03-020	152/166	145/155		105/107		118/126	216	255	
03-021	166	157		93/95		122	214/216	253/255	
03-022	152/166	145/157		89/105		126	216	255	
03-023	168	157		107		120/122	214/216	255	
03-024	166/168			89/105		122/140		255	
03-025		145/157		89/107		122/138		255	
03-026				97/105		108/140		255	
03-027				97/105		118/140		255	
03-028		153/157		105/107		118		255	
03-032		145/157		93/95		126		255	
03-033		145		89/105		118/126		253/255	
03-034		145		107		108/122		255	
03-035		145/153				122/126		255	
homozygosity	**38%**	**43%**		**13%**		**38%**	**48%**	**81%**	

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ECA31

ACCNO	AHT33	TKY274	COR038	VIASH21	AHT34
A3-001	167	120/122		255	142
A3-002	163/171	122/126		255	130/142
A3-003	167/173	122/140	216	255	130/146
A3-004	171	108/140	214/216	253/255	130/142
A3-005	165/167	122/140	214/216	255	146/148
A3-006	157/167	126/140	216	255	146
A3-007	159/167	118/126	216	253/255	142/146
A3-008	167/173	126/128	216	253/255	130/146
A3-009	167/173	126	216	255	146/148
A3-010	171/173	108/126	214/216	255	144/146
A3-011	159/173	118/126	216	253/255	130/146
A3-012	161/171	126/140	216	255	142/146
A3-013	159/167	118/140	216	255	130/146
A3-014	169/171	120/122	216	255	142
A3-015	161/173	140	214/216	253/255	142
A3-016	169/171	122/126	214/216	255	130/142
A3-017	155/171	118/140	216	255	142/146
A3-018	157/169	118/138	216	253/255	130/146
A3-019	161/167	118/122	216	253/255	142/148
A3-020	163/167	126/140	216	253/255	146
A3-021	161/173	126/140	216/222	255	146/152
A3-022	167/173	108	216	255	142/146
A3-023	167/173	140	216	255	146/150
A3-024	161	122/126	216	255	142
A3-025	167	122/126	216	255	146/150
A3-026	157/169	118/122	216	255	130/150
A3-027	161/171	122	216	255	142/146
A3-028	157/167	122/126	216	255	142/148
A3-029	161/167	122/128	216/222	255	142
A3-030	169/171	122/140	216	255	130/142
A3-031		108/126	216	255	130/142
A3-032		108/122	216	255	
A3-033	171/173	108/140	216	255	142/146
A3-034	167	122/126	216	255	142/146
A3-035	155/171	118/140	216	255	142
A3-036	155	140	216	255	142
A3-037	171	138	214/216	255	130/142
A3-038	161/171	122/126	214/216	255	130/146
homozygosity	**19%**	**18%**	**75%**	**79%**	**24%**

ACCNO	AHT33	TKY274	COR038	VIASH21	AHT34
03-001		118/122	216	253/255	
03-002		122	216	255	
03-003		122	216	255	
03-004		122	216	255	
03-005		122	214/216	253/255	
03-006		122	216/222	255	
03-007		118/140	216	255	
03-008		126	214/216	255	
03-009		122	214/216	255	
03-010		138/140	216/222	255	
03-011		122/138	216/222	255	
03-012		122/140	214/222	255	
03-013		118/126	216	255	
03-014		140	216	255	
03-015		122/140	214/216	253/255	
03-016		118/140	216	253/255	
03-017		118/138	216/222	255	
03-018		118/122	216	255	
03-019		122/126	214/216	255	
03-020		118/126	216	253/255	
03-021		122	214/216	255	
03-022		126	216	255	
03-023		120/122	214/216	255	
03-024		122/140		255	
03-025		122/138		255	
03-026		108/140		255	
03-027		118/140		255	
03-028		118		255	
03-032		126		255	
03-033		118/126		253/255	
03-034		108/122		255	
03-035		122/126		255	
homozygosity		**38%**	**48%**	**81%**	

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Appendix II: List of microsatellites used in this research along with reference as listed in table 3.1.

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