IDENTIFICATION OF LOCI INTERACTING WITH MELANOCORTIN-1 RECEPTOR TO MODIFY BLACK COAT COLOR IN AN F_2 NELLORE-ANGUS POPULATION

A Thesis

by

LAUREN LORENE HULSMAN

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

MASTER OF SCIENCE

May 2010

Major Subject: Animal Breeding

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Approved by:

Chair of Committee,	Clare A. Gill
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Head of Department,	Gary Acuff

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ABSTRACT

Identification of Loci Interacting with Melanocortin-1 Receptor to Modify Black Coat Color in an F_2 Nellore-Angus Population. (May 2010)

Lauren Lorene Hulsman, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Clare A. Gill

In cattle, base color is attributed to activity at the melanocortin-1 receptor (MC1R), historically termed the extension locus, with alleles coding for black (E^D), red (e), and wild-type (E^+). These alleles, in most mammals, are presumed to follow the dominance model $E^D > E^+ > e$, although exceptions are often seen. In *Bos indicus* x *Bos taurus* F_2 cattle, E^DE^+ heterozygotes observed were discordant with the dominance series for the *MC1R* alleles and displayed various degrees of reddening on an otherwise predicted black background. The objective of this study was to identify loci modifying black coat color in these individuals. The hypothesis was that degree of reddening was a quantitative trait controlled by multiple genes of small effect. Reddening was classified utilizing photographs for 5 subjective scoring systems and analyzed by general linear model procedures of SAS with fixed effects of sex, sire, family nested within sire, season of photo, and spotted status. Residuals from these models were utilized for interval analyses to identify quantitative trait loci (QTL). Analyses of 19 bovine autosomal chromosomes, identified chromosome-wise suggestive ($P < 0.05$) and significant ($P < 0.01$) QTL on bovine chromosomes (BTA) 4, 5, 15, 18, 21, 27, and 29.

Unexpectedly, there was evidence of a major gene ($F = 67.88$) affecting reddening at 71 Mb of BTA 6 (based on build Btau4.0 of the bovine genome sequence) that accounted for 61.1% of the variation in reddening. This QTL coincided closely with a cluster of tyrosine kinase receptor genes (*PDGFRA*, *KIT* and *KDR*). Fitting SNP haplotypes for a 1 Mb region containing all 3 genes and centered on *KIT* accounted for all the variation attributed to this QTL. These data suggested that one of these 3 genes, or a gene in high linkage disequilibrium with them, was responsible for the majority of variation in degree of reddening. Two recombinants within this region identified *PDGFRA* as the strongest candidate gene. Functional analyses will be required to verify the role of *PDGFRA* and its interaction with *MC1R* to modify black coat color of *Bos indicus* influenced cattle.

To my family and friends

“The journey not the arrival matters.”

- T. S. Eliot

ACKNOWLEDGEMENTS

A person's life is touched by everyone they meet and interact with. In my graduate school endeavor, many people have touched my life, allowing me to grow and prosper in my graduate education. To these people, I wish to express my utmost appreciation and gratitude for their contribution to my success.

My advisor and committee chair, Dr. Clare Gill, has invested countless hours into teaching and mentoring me as I progressed through my classes and project. Her knowledge of both molecular and quantitative genetics has been an invaluable resource as I've furthered my education and branched into areas I was unfamiliar with. For all the experiences, opportunities, and knowledge you have provided, I will forever be grateful.

My committee members, Drs. Andy Herring and Jim Sanders, provided immeasurable discussions and guidance for not only my project but my educational goals in life. Dr. David Riley, my newest resource, has provided phenomenal impromptu discussions on SAS and quantitative genetics that were integral to my project's success. To these gentlemen, your contribution to my growth and success were vital. Thank you.

Colette Abbey, the Animal Breeding and Genetics Lab research associate, was always available for discussions and to teach. Her patience and willingness to teach as I started and furthered my education in this field were invaluable. I am exceedingly grateful for all your contributions to my education.

To everyone involved with the McGregor Genomics Herd Project, your hard work and involvement have allowed for unparalleled cattle research to occur here at

Texas A&M and for students, like myself, to gain experience and knowledge with cattle and in the field of genetics among others. Thank you.

Research cannot proceed without funding and I wish to express my greatest appreciation for the grant that provided the support for my project. This project was partially supported by National Research Initiative competitive grant no. 2008-35205-18767 from the USDA National Institute of Food and Agriculture Animal Genome Program, by Texas AgriLife Research and by the Moore Excellence Fund.

Last, but definitely not least, I must recognize and thank my family and dearest friends. Even in my toughest times during my graduate education, your support and encouragement helped me to stay on the right path for accomplishing my goals and continued education. For this, along with so many other opportunities and experiences you provided in my life, I want to express a wholehearted thank you. I love you all so much.

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CHAPTER I

INTRODUCTION

Coat color phenotypes historically have been used in breed recognition for livestock species, especially cattle. Marketing strategies, like the Certified Angus Beef (CAB) program that accepts cattle that are at least 51% black hided and exhibit Angus influence, have opened new venues for price discrimination based on coat color phenotypes, regardless of the animal's actual lineage. Availability of such premiums or discounts can have a direct impact on producers' breeding strategies.

Activity of the melanocortin-1 receptor (MC1R) is responsible for variation in coat color in most mammals, resulting in production of eumelanin (black to brown pigment) or phaeomelanin (red to yellow pigment) through the melanogenesis pathway. In cattle, base color is attributed to *MC1R*, historically termed the extension locus, with alleles coding for black (E^D), red (e), and wild-type (E^+) animals. These alleles are presumed to follow a dominance model, in which $E^D > E^+ > e$. The wild-type allele (E^+) produces a functional receptor that responds to both the α-melanocyte-stimulating hormone (α-MSH) ligand and its antagonist, agouti signaling protein (ASIP). The E^D allele, caused by a leucine to proline point mutation, is a constitutively active receptor that responds only to α-MSH, resulting in eumelanin production. The recessive allele (e) produces only phaeomelanin due to a frameshift that creates a prematurely terminated, non-functional receptor (Klungland et al., 1995).

This thesis follows the style of Journal of Animal Science.

In a large population of *Bos indicus* x *Bos taurus* cross cattle, we observed various degrees of reddening in E^DE^+ individuals that were expected to be black based on the dominance series for alleles at MC1R. This observation plus anecdotal evidence from other cattle crosses and studies in mice suggest that other loci are able to modify the expected black coat color. Based on this evidence, the hypothesis for this study was that the degree of reddening is a quantitative trait controlled by multiple genes of small effect. The objective of this study was to identify loci modifying black coat color in F_2 Nellore-Angus cattle heterozygous (E^DE^+) for *MC1R*.

CHAPTER II

LITERATURE REVIEW

Melanogenesis

Pigment cell progenitors are derived from the neural crest cell lineage and they are the only cell type to migrate along the dorso-lateral pathway during embryonic development. Melanoblast precursors of pigment cells are derived from neural crest precursors as early as embryonic day 8.5 in the mouse. Migration of melanoblasts occurs between the dermomytome and the ectoderm within the mesenchyme (Silver et al., 2006; Seo et al., 2007). Once melanoblasts reach the skin, they colonize the interfollicular space, hair follicle, and the dermis.

Melanoblasts differentiate into melanocytes at the onset of pigment production. The rate-limiting enzyme in melanin synthesis is tyrosinase (TYR). Tyrosinase activates melanin synthesis through the hydroxylation of tyrosine to dihydroxyphenylalanine (DOPA) and further oxidation of DOPA to DOPA quinone (Mason, 1948; reviewed by Prota, 1988; Seo et al., 2007; Cheli et al., 2009).

Melanocytes migrate not only to the skin and hair of mammals, but also to the iris and choroid of the eye, the inner ear, and internal organs (Bennett and Lamoreux, 2003; Silver et al., 2006). Within each melanocyte are melanosomes, which are specialized organelles for melanin synthesis. Once the melanosomes are formed and contain melanins, they are transported to the dendrite tips of the melanocytes and then transferred into keratinocytes of the hair and skin. Control of melanocyte differentiation continues into adulthood to allow for repopulation of melanocytes in hair follicles, where

a dopachrome tautomerase-positive melanocyte stem cell pool is housed within the follicle's bulge area (Silver et al., 2006).

Melanosomes of either eumelanin (black to brown pigment) or phaeomelanin (red to yellow pigment) can be found within melanocytes. Regulation of pigment switching from eumelanin to phaeomelanin and vice versa is primarily through the activity of melanocortin-1 receptor (MC1R) and its ligands α-melanocortin stimulating hormone (α-MSH) and agouti signaling protein (ASIP). Genes involved in pigment switching are probably involved in the reddening phenotype in cattle being investigated herein and, thus, will be the focus of this literature review.

Melanocortin-1 Receptor. Robbins et al. (1993) determined that the extension locus, responsible for the pigment switch between eumelanin and phaeomelanin in many mammals, was encoded by *MC1R* in the mouse. Melanocortin-1 receptor is a G-protein-coupled 7 transmembrane domain receptor located on the membrane of melanocytes. Synthesis of eumelanin is initiated by binding of α-MSH to the receptor, which activates adenylyl cyclase and increases synthesis of cyclic adenosine monophosphate (cAMP). This leads to an increase in the synthesis of TYR. This in turn causes a decrease in the level of intracellular cysteine and increases in the activity of TYR, dopachrome tautomerase (DCT) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA) and production of eumelanin (reviewed by Cone et al., 1996; Wolff, 2003).

When MC1R is not activated, either through the absence of bound α-MSH or antagonism by ASIP, TYR, tyrosinase related protein-1 (TYRP1), and DCT activities remain low, leaving naturally high levels of cysteine to combine with DOPA quinone.

The reaction between cysteine and DOPA quinone produces metabolites of cysteinylDOPA that are oxidized to the default pigment phaeomelanin via benzothiazine intermediates (Hearing and Tsukamoto, 1991; Silver et al., 2006; Seo et al., 2007).

In addition to the wild-type (E^{+}) allele, Robbins et al. (1993) characterized 4 variants of mouse *MC1R* that affected coat color: (1) recessive yellow (e) due to a frameshift mutation between the fourth and fifth transmembrane domains that results in premature termination of the protein; (2) tobacco darkening (E^{tob}) caused by a serine to leucine substitution at codon 69 of the first intracellular loop; (3) sombre ($E^{so\text{-}3J}$) due to a glutamic acid to lysine substitution at codon 92 of the second transmembrane domain; and (4) a second sombre (E^{so}) phenotype due to a leucine to proline substitution at codon 98 of the second transmembrane domain. Robbins et al. (1993) cloned the E^{+}, e, $E^{so\text{-}3J}$ and E^{tob} variants into a mammalian expression vector and transfected human kidney cells to test the responsiveness of transfectants to various concentrations of α-MSH. When compared to the normal dose-dependent expression response of the E^{+} allele, the e allele was shown to be unable to couple functionally to adenylyl cyclase, indicating that the truncated MC1R was inactive and could not bind the α-MSH ligand. $E^{so\text{-}3J}$ transfectants were unresponsive to α-MSH (1pM to 0.1μM), but on average the basal activity of adenylyl cyclase was 4 times more than in E^{+} cells, providing evidence of a dominant, constitutively active receptor. Although not tested, Robbins et al. (1993) assumed that the E^{so} allele would function in the same way. In contrast to $E^{so\text{-}3J}$ and E^{so} mutants, the E^{tob} allele is not constitutively expressed. Instead, Robbins et al. (1993) demonstrated

that E^{tob} produces a hyperactive receptor that has a much greater ability than E^{+} to stimulate adenylyl cyclase and increase intracellular cAMP in response to α-MSH.

Lu et al. (1998) expanded the work of Robbins et al. (1993) and characterized the activities of the mouse $E^{so\text{-}3J}$ (E92K) and E^{so} (L98P), fox E^{A} (C125R) and sheep E^{D} (D119N) alleles utilizing β-galactosidase assays as an indirect measure of cAMP. Elevated basal levels ranged from 30 to 90% of the maximal stimulation level, demonstrating that each of these dominant alleles of *MC1R* produces a receptor that is constitutively active. Like other G-protein linked receptors, this constitutive activity was predicted to be due to enhanced affinity for ligand caused by a disruption in electrostatic interactions that constrain the receptor. In contrast, Lu et al. (1998) showed that MC1R dominant mutants have lower affinity for α-MSH and proposed that the introduction of basic residues in an acidic domain causes receptor activation by ligand mimicry.

In cattle, the wild-type E^{+} allele of *MC1R* encodes a full-length protein of 317 amino acids that shares high sequence similarity with mouse MC1R. At least 5 other *MC1R* alleles have been reported in the literature (Klungland et al., 1995; Rouzard et al., 2000; Graphodatskaya et al., 2002). The E^{D} allele that causes dominant black coat color in cattle was identified as a leucine to proline substitution at codon 99 in the second transmembrane domain of MC1R (Klungland et al., 1995; Graphodatskaya et al., 2002). Although Lu et al. (1998) did not characterize the cattle E^{D} (L99P) allele pharmacologically, they expected it to be constitutively active like the mouse E^{so} allele because of the similar position, class, and charge of the amino acid change. Like in the species characterized by Lu et al. (1998), Graphodatskaya et al. (2002) showed the cattle

E^D allele was unresponsive to different α-MSH concentrations suggestive of constitutive activity. However, basal receptor activity relative to the E^+ allele was not reported.

The e allele that causes recessive red coat color in cattle is due to a frameshift mutation in the last transmembrane domain of *MC1R* that truncates the protein (Klungland et al., 1995; Joerg et al., 1996; Graphodatskaya et al., 2000; Klungland et al., 2000; Graphodatskaya et al., 2002). Similar to mouse, the truncated protein produced by the e allele in cattle was unresponsive to varying concentrations of α-MSH and is a non-functional receptor (Graphodatskaya et al., 2002).

The E^{d1} allele is caused by an alanine to tyrosine substitution at codon 223, whereas the E^{d2} allele (also called the E^1 allele by Rouzard et al., 2000) results from a duplication of 4 amino acids in the third intracellular loop of MC1R (Graphodatskaya et al., 2002). Both of these variants respond to stimulation with α-MSH in a dosage dependent manner. Graphodatskaya et al. (2002) also describe the e^f allele resulting from an isoleucine to threonine substitution at codon 297. The e^f allele had a delayed response to α-MSH requiring 10-fold higher concentrations of ligand to produce the same intracellular levels of cAMP as E^{d1} and E^{d2}.

Agouti Signaling Protein. The agouti locus that produces ASIP is one of the genetically most well characterized loci in mice (reviewed by Siracusa, 1991). Agouti signaling protein controls the amount and distribution of eumelanin and phaemelanin because it is the primary antagonist of MC1R. In mice that are homozygous for the wildtype A allele, the hair is banded black-yellow-black because of a switching mechanism that regulates whether eumelanin or phaeomelanin is produced by hair bulb

melanocytes. There are at least 25 *ASIP* alleles in mice, some of which were found to be spontaneous mutations and others that were generated by irradiation. Typically, *ASIP* alleles resulting in increased phaeomelanin production are dominant to alleles resulting in increased eumelanin.

Many pleiotropic effects also have been associated with dominant *ASIP* mutations related to obesity, diabetes, and development of neoplasms (Morgan et al., 1999; Dolinoy et al., 2006). For example, mice carrying agouti viable yellow (A^{vy}) vary in color from completely yellow to pseudoagouti and may become obese. Morgan et al. (1999) showed that this phenotypic variation was due to the epigenetic inheritance of the A^{vy} allele. Increased methylation of the maternal A^{vy} allele causes a more mottled, or agouti appearance. Morgan et al. (1999) and Dolinoy et al. (2006) showed that maternal effects or diet types also contribute to the methylation status of the A^{vy} allele and could ultimately affect the obesity of the mouse.

Lu et al. (1994) demonstrated that mouse ASIP acts as a high affinity competitive antagonist of MC1R by inhibiting α-MSH binding to the receptor. Willard et al. (1995) showed that the C-terminal region of ASIP is directly involved in binding to MC1R on the surface of melanocytes, thereby preventing α-MSH from binding to the receptor. Subsequently, Sakai et al. (1997) demonstrated that ASIP also acts as a negative antagonist of MC1R in the absence of α-MSH and can directly elicit changes in cell shape and in the number and type of melanosomes produced.

Although the agouti phenotype is observed in the fur of many mammals, it is not observed in humans. Lu et al. (1994) showed that mouse ASIP inhibited human MC1R

but at much higher concentrations than was required to inhibit mouse MC1R. Given that the agouti phenotype is not observed in humans, Lu et al. (1994) suggested that perhaps human ASIP is not a potent inhibitor of human MC1R in the hair follicle. Voisey and Van Daal (2002) showed that overexpression of ASIP in human melanoma cells has no effect on MC1R providing some support for this hypothesis.

Likewise, in cattle there is little evidence that ASIP has a direct role in the type of pigment switching seen in mice. Royo et al. (2005) investigated *ASIP* in 6 Spanish and 3 French cattle breeds with wild-type coloration and found no variation in coding sequence. Due to this, they hypothesized that *ASIP* does not affect coat color variation in cattle. Although no coding polymorphisms have been found, Girardot et al. (2005) determined that bovine *ASIP* has at least 3 different tissue-specific promoters. Girardot et al. (2006) reported that the Normande breed contained an insertion of a full-length long interspersed nuclear element between the skin specific promoter and first coding exon that may be associated with brindling.

Using an in vitro system in which cells expressing MC1R were co-cultured with cells expressing ASIP, Graphodatskaya et al. (2006) showed that bovine ASIP does act as an antagonist of MC1R and significantly reduces production of cAMP. This demonstrates that in vivo it is likely that bovine *ASIP* does produce a functional protein that is secreted from the cells surrounding melanocytes in the hair bulb and binds to MC1R on the melanocyte cell membrane to down-regulate its activity.

Other Genes or Ligands Involved in Pigment Switching

Competitive inhibition of the binding of α-MSH to MC1R by ASIP is the primary mechanism controlling the switch from production of eumelanin to phaeomelanin in mammalian skin and hair. However, results from a number of studies of skin and hair of various species provide evidence that there are either other genes involved in pigment switching or other ligands that are able to bind to MC1R.

In fox there are 5 distinct coat color phenotypes attributed to combinations of *MC1R* and *ASIP* alleles (Våge et al., 1997). In contrast to mouse, the presence of a single constitutively active MC1R receptor (E^A) is not sufficient to override the inhibition of eumelanin production by a single wild-type allele in ASIP, and foxes of this genotype have red pigmentation around the flanks, midsection, and neck. Våge et al. (1997) suggest that either ASIP functions as a negative antagonist in the fox or that there is a second target for ASIP on the melanocyte in addition to MC1R.

Klungland et al. (2000) reported that a sample of Egyptian river buffalo sequenced and genotyped for *MC1R* contained a relatively high frequency of the recessive e allele, although all of the animals were either black or brown in color. The presence of the e allele in these black or brown animals and red in E^D animals indicates that the MC1R variants reported to date do not explain all the pigment type switches.

Klungland and Våge (1999) described a red and white spotted Norwegian calf out of a red and white spotted cow that both genotyped E^De for *MC1R* and therefore would be expected to be black. Interestingly, the calf had a black patch over one half of its face that partially covered a white star on its forehead. No mutations in the coding

sequence of *MC1R* or *ASIP* were identified that could explain the difference between genotype and phenotype of this calf. Klungland and Våge (1999) hypothesized that the E^D allele was inactivated in the mother and partially reactivated in the offspring. This report indicates that other genes or possibly ligands other than α-MSH and ASIP could be contributing to the switch between eumelanin and phaeomelanin in cattle. Because the black pigment was able to invade the white forehead star, a gene altering melanocyte migration may be involved.

Recently, Candille et al. (2007) showed that dominant black coat color in dogs was due to mutations in a β-defensin gene, *CBD103*. The CBD103 protein is similar in structure to ASIP, is expressed 300-fold higher than ASIP in skin of dogs and binds with high affinity to MC1R. However, binding of CBD103 to the receptor does not alter intracellular concentrations of cAMP. The high abundance of this protein in the skin suggests that it competitively inhibits the binding of wild-type ASIP to MC1R, thereby preventing ASIP from antagonizing MC1R and enabling synthesis of eumelanin.

Conversely, CBD103 was recently proposed as the causative gene for the dominant variant red phenotype seen in Holstein cattle, in which cattle carrying at least one E^D allele were red instead of black (Dreger and Schmutz, 2009). However, no functional data were presented in support of association between CBD103 and the variant red phenotype.

Most studies related to *MC1R* and *ASIP* in cattle have been conducted in breeds of *Bos taurus* origin. There are very few descriptions in the literature of loci affecting coat color in *Bos indicus* and *Bos indicus* influenced cattle. The recessive gray or silver

phenotype, for example, is prevelant in *Bos indicus* breeds. In Nellore and Guzarat cattle, Rhoad (1936) showed the extent of the non-pigmented tips of hairs resulted in varying degrees of gray. For example, wide bands result in the white coloration seen in Nellore. In mice, the silver phenotype is caused by a single base insertion in *SILV* and is attributed to premature death of pigment cells during the hair cycle (Kwon et al., 1995).

Finally, a population of *Bos indicus* x *Bos taurus* crossed cattle currently being researched at Texas A&M University display a phenotype discordant to their *MC1R* genotype. The observed reddening phenotype acts across several families and is especially visible in the $E^{D}E^{+}$ heterozygotes of these F_2 Nellore-Angus crossed cattle, where various degrees of red pigmentation are present on an otherwise expected black background. Therefore, it is of interest to investigate these animals in order to gain a better understanding of genes involved in the pigment type switch in *Bos indicus* influenced cattle.

CHAPTER III

MATERIALS AND METHODS

Cattle Population

The Texas A&M McGregor Genomics Cycle 1 Population consists of 14 full-sib F_2 families founded by Nellore grandsires and Angus granddams. Five F_1 sires were mated to 13 F_1 dams to produce full-sib families by multiple ovulation and embryo transfer. Semen quality for one of the F_1 bulls (2855) was inadequate, so use of the bull was discontinued and the 2 progeny produced by that sire were excluded from all analyses. The remaining 4 F_1 sires were also mated to F_1 and F_2 Brahman-Angus, Nellore-Angus and Brahman-Hereford cows to produce paternal half-sib families through natural service. These natural service calves were produced in multiple sire pastures and required DNA testing to determine paternity. A total of 480 F_2 and 266 natural service calves were produced from 2003 to 2007.

Calves were photographed at birth, steers were photographed in the feeding pens and females were photographed shortly after each calving. Blood samples were previously collected on all live-born animals in the population, and DNA was extracted and used to genotype the melanocortin-1 receptor (*MC1R*). Samples were also run on the BovSNP50 assay (Illumina Inc., San Diego, CA) that produced genotypes for 52,875 SNP. All procedures involving animals were approved by the Texas A&M Institutional Care and Use Committee; AUP 2002-116, 2005-147 and 2008-234.

Variation in reddening was noticeably observed within the E^DE^+ F_2 and natural service half-sib cattle, and therefore this subset of the population (n = 329) was

investigated for this phenotype. Because cattle tend to change coat color after their first shedding, it was important to only evaluate the cattle at a weaning age or older to obtain the best score for the reddening phenotype. Photographs previously taken either in the feedlot or pasture at weaning age or older were found for 215 of the 239 E^DE^+ F_2 cattle. This included one of the progeny of bull 2855, which was subsequently excluded, as stated above. Of the 24 E^DE^+ F_2 offspring not utilized in this study, 17 either died at birth or shortly after and 7 died before weaning. The 214 E^DE^+ F_2 cattle evaluated for the reddening phenotype were 4 bulls, 115 steers, and 95 females.

In addition to the F_2 cattle, 84 of the 90 E^DE^+ natural service half-sib cattle (42 steers and 42 females) had photographs available at weaning age or older. The other 6 E^DE^+ natural service calves were sold shortly after weaning and were not utilized in this study. Although the E^DE^+ natural service half-sib cattle were evaluated using the scoring systems described below, none were included in the statistical or interval analyses.

Investigation of Melanocortin-1 Receptor (MC1R)

The Texas A&M McGregor Genomics Cycle 1 Population was initially genotyped for *MC1R* utilizing the E3 (GTGCCTGGAGGTGTCCATC) and E4 (GAAGTTCTTGAAGATGCAGCC) primers designed by Klungland et al. (1995). These primers amplified a 739 base pair fragment of *MC1R* encompassing the SNP T296C (E^+ to E^D allele) and the 310/311 G deletion (e allele). To determine if a novel mutation in *MC1R* was contributing to the reddening phenotype, the MC1-RF2 (ACGATGCCTGCACTTGGCTCCCAG) and MC1-RR1 (CCTCACCAGGAG

CACTGCAGCAC) primers from Graphodatskaya et al. (2000) were used to characterize the entire coding sequence of *MC1R* in a subset of the Cycle 1 Population (n = 31) that varied in their degree of reddening and their *MC1R* genotype. Of the 24 F_2 sequenced with the MC1-RF2 primer, 2 were E^DE^D, 20 were E^DE^+, and 2 were E^+E^+. The 7 individuals from the natural service, paternal half-sib families consisted of 2 E^DE^+, 1 E^+E^+, 1 E^+e, and 3 E^De. Straightbred *Bos indicus* and *Bos taurus* cattle and F_1 *Bos indicus* x *Bos taurus* cross cattle were also sequenced to further characterize discovered SNP (Table 1).

Table 1. Breed and initial genotypes of samples used to sequence *MC1R*

		MC1R				
Breed	**n[1]**	**E^D E^D**	**E^D E^+**	**E^+ E^+**	**E^+ e**	**E^D e**
Bos indicus						
Nellore	3	0	0	3	0	0
Brahman	8	0	0	8	0	0
Gir	5	0	0	4	1	0
Bos taurus						
Angus	2	0	1	0	0	1
Bos indicus x Bos taurus						
F_1 Angus-Brahman	1	0	1	0	0	0
F_1 Nellore-Angus	8	0	7	1	0	0
F_2 Nellore-Angus	24	2	20	2	0	0
Nellore-Angus sired[2]	7	0	2	1	1	3

[1]n = number of animals sequenced for each breed or cross
[2] Nellore-Angus sired natural service calves out of F_1 and F_2 Brahman-Angus, Nellore-Angus or Brahman-Hereford cows

Phenotype Scoring System

The degree of reddening was classified from photographs utilizing 5 numeric, subjective scoring systems. The subjective scoring systems were Majority Black, Red

Present, Degree of Black – Simple, Degree of Black – Complex, and Degree of Darkness (Figure 1 and Table 2). Each of the 214 $E^{D}E^{+}$ F_2 embryo transfer and the 84 natural service half-sib animals with a photograph available was simultaneously evaluated for each scoring system.

Table 2. Subjective scoring systems utilized to evaluate the reddening phenotype

Scoring System[1]	Description	Mechanism
Majority Black	Yes or No question on whether the majority or all of the animal's pigmented areas contained black pigmentation.	No – 1; Yes - 2
Red Present	Yes or No question on whether there was any noticeable red pigmentation on the animal's pigmented areas.	Yes - 1; No - 2
Degree of Black - Simple	A simple grouping based on the amount of black pigmentation present on the animal's pigmented areas.	1 to 3 scale: 1 – Light, 2 – Fair to Moderate, 3 – Abundant to Solid
Degree of Black - Complex	Detailed groupings based on the overall amount of black pigmentation observed on the animal's pigmented areas. These scores were assigned by breaking the simple groupings further down.	1 to 9 scale: Degree of Black - Simple group 1 separated into 1 - 3; Degree of Black - simple group 2 separated into 4 - 5; Degree of Black - Simple group 3 separated into 7 – 9 (Figure 1)
Degree of Darkness	A grouping based on the overall shade of the animal's pigmented areas that include both black and red pigmentation.	1 to 4 scale: 1 – Light, 2 – Moderate, 3 – Dark, 4 – Extremely Dark

[1]For all systems, a score of 1 is the reddest or lightest phenotype.

Bos indicus breeds and *Bos indicus* influenced cattle are known to darken during the fall and winter although the characteristic is not well recorded in literature. To avoid bias, each photograph was scored by itself and during the scoring process no

Figure 1. Degree of black – complex scoring system. Examples of $E^{D}E^{+}$ F_2 Nellore-Angus cattle that scored a 1 through 9, respectively, starting at the top left and proceeding left to right down the rows.

comparisons were made between photos. Some photographs used were taken in the spring/summer while others were taken in the fall/winter. Photographs were classified as "S" for spring/summer (May to October), or "W" for fall/winter (November to April). Date of the digital image was used to assign the season for 127 photographs of the 210 $E^{D}E^{+}$ F_2 embryo transfer cattle, whereas 83 photographs which did not possess a digital date stamp were provided a season assignment through evaluation of the photograph.

Statistical Analysis of Phenotype Scores

General linear model procedures of SAS (SAS Institute Inc., Cary, NC) were used to evaluate fixed effects, including sire, family nested within sire, sex, birth year season, year born, season of photograph and spotted status for all scoring systems. Least square means were generated for significant effects of each scoring system. Residuals from these models were used for interval analysis.

Interval Analysis and Candidate Gene Identification

Map and SNP genotyping files were assembled for each autosome using coordinates from the Btau4.0 build of the bovine genome sequence. Together with residuals generated for the 5 scoring systems, these files were used for interval analysis with GRID*QTL* software (Seaton et al., 2006) to identify QTL involved in modifying black coat color. For each chromosome, the additive and dominance model was invoked at a 1 cM step permutated chromosome-wide with 5,000 iterations. It was assumed that every 1 Mb on the Btau4.0 build was equivalent to 1 cM.

In order to identify candidate genes for the reddening phenotype, the coordinates of identified QTL were used to identify genes concordant with the QTL region based on the Btau4.0 sequence assembly and genome maps at the National Center for Biotechnology Information (NCBI; www.ncbi.nlm.nih.gov). Genes within a 2 Mb region surrounding the QTL position were investigated based on available information in the NCBI database and literature searches. Because information in the NCBI database for cattle genes is limited, information on functional homologs in mouse was investigated as well.

After identifying the most likely position of chromosome-wise suggestive ($P < 0.05$) or significant ($P < 0.01$) QTL, SNP haplotypes spanning a 1Mb region and centered on a candidate gene were recovered. Single nucleotide polymorphisms that fell within this 1 Mb region that had a minor allele frequency greater than 0.05 and a completion rate greater than 90% were phased with fastPHASE software (Scheet and Stephens, 2006). By tracking the resolved haplotypes through the three-generation pedigree, a fully phased breed-of-origin (Angus (AA), Angus-Nellore (AN), Nellore-Angus (NA) or Nellore (NN), where breed of sire is listed first) was assigned for the candidate gene for each individual. This breed-of-origin assignment was included in the general linear model in SAS to generate new residuals for interval analysis. The proportion of phenotypic variation accounted for by the candidate genes was calculated following Darvasi and Soller (1997).

To allow for the case where haplotypes were present in both the Nellore and Angus founders and, therefore, the candidate gene was segregating in both populations,

each unique haplotype observed in the F_1 Nellore-Angus parents was assigned a number. As for the assignment of breed-of-origin, these haplotypes were tracked through the three-generation pedigree to phase the genotypes in the F_2 progeny with the haplotype inherited from the sire listed first. Any animal that had a recombination event within the 1 Mb region was labeled “rec” to identify it. These phased genotypes were included in the general linear model in SAS to generate new residuals for interval analysis.

CHAPTER IV

RESULTS AND DISCUSSION

Investigation of Melanocortin-1 Receptor

Except for the dam of family 74 (640H), which was E^+e for *MC1R,* all of the parents of the F_2 embryo transfer families (70 to 84), were E^DE^+ with the E^+ allele inherited from the Nellore founders. In the natural service half-sib families (95 to 98), the e allele was observed in calves out of both Brahman-Angus and Brahman-Hereford dams. The population frequencies for the E^D, E^+ and e alleles of *MC1R* were 0.454, 0.481, and 0.065, respectively (Table 3). The embryo transfer population did not deviate from Hardy-Weinberg proportions for *MC1R*, whereas the natural service population did deviate ($P < 0.05$). This was expected because the sires did not carry the e allele and, therefore, homozygosity of the e allele could not occur. In addition, both Brahman-Hereford and Brahman-Angus cows were utilized and the frequency of e allele in these dams would be expected to be different. This difference in subpopulation structure and reduction in heterozygosity is known as the Wahlund effect.

One heifer (8402) was originally genotyped and analyzed as E^DE^+ but upon resequencing was shown to be E^DE^D. After removing this female from the dataset, there were 238 embryo transfer calves genotyped as E^DE^+ and 90 natural service calves.

Two previously unreported SNP (C583T and T663C) were identified in the *MC1R* coding sequence of the 22 E^DE^+ animals selected for sequencing because of variation in their degree of reddening. The C583T SNP was a non-synonymous substitution and caused an amino acid change from leucine to phenylalanine. These 2

SNP were in complete disequilibrium with the T296C (E^+ to E^D allele) SNP in these animals and were, therefore, not associated with degree of reddening.

Table 3. Frequency of *MC1R* genotypes in the McGregor Genomics Cycle 1 Population

		***MC1R* Genotype**[1]				
Family	**n**[2]	**E^+E^+**	**E^DE^+**	**E^DE^D**	**E^+e**	**E^De**
70	35	0.257	0.514	0.229	-	-
71	69	0.275	0.435	0.290	-	-
72	46	0.304	0.435	0.261	-	-
73	10	0.400	0.500	0.100	-	-
74	8	0.500	0.125	0	0.125	0.250
75	43	0.116	0.581	0.302	-	-
76	10	0.200	0.500	0.300	-	-
77	42	0.190	0.524	0.286	-	-
79	2	0	0.500	0.500	-	-
80	69	0.188	0.551	0.261	-	-
81	62	0.242	0.484	0.274	-	-
82	15	0.400	0.333	0.267	-	-
83	38	0.316	0.579	0.105	-	-
84	30	0.267	0.533	0.200	-	-
95	70	0.271	0.300	0.100	0.129	0.200
96	123	0.179	0.358	0.114	0.146	0.203
97	56	0.214	0.357	0.071	0.071	0.286
98	17	0.235	0.294	0	0.235	0.235
Total	745	0.236	0.440	0.193	0.048	0.082

[1]Genotype is based on T296C and 310/311 deletion. Genotypes not possible for each family are designated as "-".
[2]n = number of animals in each family for which DNA was available regardless of photograph availability at weaning age or older.

Characterization of the *MC1R* coding sequence in additional straightbred *Bos indicus*, *Bos taurus* and *Bos indicus* x *Bos taurus* cross cattle demonstrated that the T and C alleles at positions 583 and 663, respectively, were of *Bos indicus* origin and were in phase with the *Bos indicus* derived E^+ allele (Table 4 and Appendix Table A-1). Because the MC1-RF2 forward sequencing primer was used and the new SNP were

located downstream of the previous mutations used to genotype *MC1R*, any animal heterozygous for the e allele, a frameshift mutation (Table 1), could not be scored.

Unexpectedly, one of the F_1 Nellore-Angus bulls (410P) used as sire for the Cycle 2 population genotyped as E^+E^+. His Angus dam (002E) was genotyped as E^DE^+, but was homozygous for the C and T alleles of C583T and T663C, respectively. Although the e allele is present at low frequencies in Angus cattle, the presence of the E^+ allele in Angus cattle is actually considered a genetic defect or factor by the American Angus Association and is therefore not typically seen. This dam, although considered Angus, does not have a registered pedigree. Regardless, one of the E^+ alleles carried by 410P was of *Bos indicus* origin and heterozygosity for C583T and T663C is consistent with the T and C alleles, respectively, being derived from *Bos indicus*.

Table 4. Presence of *MC1R* haplotypes in straightbred and crossbred cattle selected for sequencing

T296C-C583T-T663C Haplotypes[1]	**n**	**Breeds or crosses with haplotype**
TTC	67	Brahman, Gir, Nellore, F_1 Angus-Brahman, F_1 Nellore-Angus, F_2 Nellore-Angus, and Nellore-Angus sired
CCT	33	Angus, F_1 Angus-Brahman, F_1 Nellore-Angus, F_2 Nellore-Angus, and Nellore-Angus sired
TCT[2]	2	Angus and F_1 Nellore-Angus

[1]T296C causes E^+ allele change to E^D allele.
[2]Haplotype observed twice due to related individuals (identical by descent).

Phenotype Scoring System

Simple means for each scoring system were generated separately for F_2 and natural service half-sib animals and are reported in Tables 5 and 6, respectively. Individual scores for all F_2 and natural service half-sib animals are available in Appendix A.

These calculations include animal 8402 that was later found to be E^DE^D. Simple means and standard deviations were recalculated without 8402 and did not substantially differ from previously generated means and standard deviations. Therefore inclusion of this one individual in the analyses is not expected to significantly affect the results reported.

Table 5. Means and standard deviations for E^DE^+ F_2 Nellore-Angus cattle for each scoring system

Scoring System	Mean	SD
Majority black	1.794	0.405
Red present	1.304	0.461
Degree of black - simple	2.579	0.726
Degree of black - complex	6.921	2.367
Degree of darkness	3.360	0.773

Table 6. Means and standard deviations for E^DE^+ natural service, Nellore-Angus sired, half-sib cattle for each scoring system

Scoring System	Mean	SD
Majority black	1.869	0.339
Red present	1.310	0.465
Degree of black - simple	2.643	0.688
Degree of black - complex	7.250	2.167
Degree of darkness	3.440	0.782

Statistical Analysis of Phenotype Scores

Sire, family nested within sire, sex, birth year season, year born, season of photograph and spotting status were all evaluated for their effects on the reddening phenotype as measured by the various scoring systems (Table 7). The final model for each scoring system included sire, family nested within sire, sex, season of photograph, and spotting status.

Table 7. *P*-values for fixed effects for each scoring system

	Scoring system				
Fixed effect[1]	**Majority black**	**Red present**	**Degree of black - simple**	**Degree of black - complex**	**Degree of darkness**
Sex	0.087	0.037	0.022	0.014	0.005
Sire	0.179	0.027	0.077	0.024	0.036
Family (sire)	0.021	0.221	0.005	0.001	< 0.001
Birth year season	0.953	0.232	0.920	0.827	0.426
Season of photograph	0.007	0.837	0.002	0.005	0.008
Spotted status	0.006	0.041	0.006	0.001	0.001

[1]Removal of birth year season changes *P*-values for all other effects.

Because bulls tend to be darker than steers (Gilmore et al., 1961) and there were 4 bulls in the dataset that ranged from 7 to 9 for Degree of Black - Complex, these were excluded from subsequent analyses. Neither birth year season nor year born contributed to variation in reddening ($P > 0.25$), and were therefore both excluded from the final model for each scoring system. Season of photograph was a significant effect for each scoring system except Red Present. Interactions among sex, sire, and family nested within sire were evaluated and were not significant.

Table 8. Frequency of spotting in E^DE^+ F_2 Nellore-Angus cattle

Degree of Black - Complex Score	Non-Spotted	Spotted	Frequency of Spotting
1	6	2	0.25
2	10	2	0.17
3	5	5	0.50
4	5	3	0.38
5	8	5	0.38
6	6	3	0.33
7	17	8	0.32
8	55	10	0.15
9	55	5	0.08
Total:	167	43	0.20

Table 9. Types of spotting seen in E^DE^+ F_2 Nellore-Angus cattle evaluated

Degree of Black - Complex Score	n[2]	Type of Spotting[1]			
		Body	Face	Udder/Cod	Underline
1	2	0	2	1	1
2	2	0	2	0	0
3	5	4	4	3	4
4	3	0	2	2	2
5	5	0	4	4	3
6	3	1	2	2	1
7	8	5	5	7	4
8	10	2	5	7	2
9	5	1	3	3	3

[1]Animal was evaluated for each type of spotting and may be represented in one or more types.
[2]n = number of spotted E^DE^+ cattle in the Degree of Black - Complex group.

In addition to season of photograph, an association of the reddening phenotype with spotting was observed (Tables 8 and 9). Because spotting was included in the model, it is expected that some variation in reddening attributed to the families expressing spotting is being removed. Offspring of sire 437J were redder ($P < 0.05$) for Degree of Black – Simple and Degree of Black – Complex and were lighter ($P < 0.05$) for Degree of Darkness than all other sires (Table 10). It should be noted, however, that

437J sired Family 74, which produced only one individual that genotyped E^DE^+. This individual (7403), which shared 437J with 3 other families, had a high degree of reddening (Degree of Black – Complex score of 2) and was moderate in color (Degree of Darkness score of 2). Therefore inclusion of this individual's family could be driving the statistical difference between 437J and the other sires. In addition, offspring of 297J had less red pigmentation than 437J and 551G. For Red Present, the least squares means for offspring of sire 432H were not statistically different from any of the sires, but tended ($P < 0.1$) to show less red pigmentation.

Table 10. Least squares means and standard errors by sire for red present, degree of black – simple, degree of black – complex, and degree of darkness using E^DE^+ F_2 Nellore-Angus cattle

Sire	Red present[1]	Degree of black – simple[2]	Degree of black – complex[3]	Degree of darkness[4]
297J	1.457 ± 0.075[a]	2.715 ± 0.114[a]	7.536 ± 0.361[a]	3.595 ± 0.114[a]
432H	1.375 ± 0.113[a,b]	2.596 ± 0.171[a]	6.989 ± 0.541[a]	3.425 ± 0.171[a]
437J	1.107 ± 0.121[b]	2.076 ± 0.183[b]	5.194 ± 0.579[b]	2.870 ± 0.183[b]
551G	1.213 ± 0.079[b]	2.499 ± 0.119[a]	6.660 ± 0.378[a]	3.322 ± 0.120[a]

[1]Ranges from 1 to 2, where 1 has red present and 2 does not have red present.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[4]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

In addition to sire, there was a significant difference between females and steers for Red Present, Degree of Black – Simple, Degree of Black – Complex, and Degree of Darkness (Table 11). Steers had more black pigmentation and were darker than females, indicating a difference between sexes that may be separate from the reddening phenotype.

Not all breeds of cattle display this characteristic difference between sexes, but this characteristic blackish pattern where bulls tend to be blacker than cows has often been reported in dairy breeds. Ibsen (1933) discussed the inheritance of the dominant "black spotting" trait in Jersey and Ayrshire cattle, which provide a canvas to see differences between males and females, termed M and L modifiers. The actual cause behind this is difference, whether predominately hormonal or genetic, is still uncertain, but provides basis for why this effect was expected to be relevant, and therefore included in the model.

Table 11. Least squares means and standard errors by sex for red present, degree of black – simple, degree of black – complex, and degree of darkness using $E^{D}E^{+}$ F_2 Nellore-Angus cattle

Sex	Red present[1]	Degree of black – simple[2]	Degree of black – complex[3]	Degree of darkness[4]
Female (F)	1.217 ± 0.062^{a}	2.357 ± 0.094^{a}	6.189 ± 0.299^{a}	3.145 ± 0.095^{a}
Steer (S)	1.359 ± 0.066^{b}	2.586 ± 0.099^{b}	7.000 ± 0.313^{b}	3.461 ± 0.099^{b}

[1]Ranges from 1 to 2, where 1 has red present and 2 does not have red present.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[4]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Table 12. Least squares means and standard errors by family nested within sire for majority black, degree of black – simple, degree of black – complex, and degree of darkness using E^DE^+ F_2 Nellore-Angus cattle

Sire	Family	n[1]	Majority black[2]	Degree of black – simple[3]	Degree of black – complex[4]	Degree of darkness[5]
297J	70	15	1.740 ± 0.099	2.560 ± 0.174	7.084 ± 0.551	3.537 ± 0.174
	71	24	1.967 ± 0.080	2.871 ± 0.140	7.988 ± 0.444	3.653 ± 0.140
432H	72	20	1.600 ± 0.085	2.310 ± 0.149	5.817 ± 0.473	2.909 ± 0.150
	73	4	1.933 ± 0.194	2.901 ± 0.339	8.101 ± 1.074	3.870 ± 0.340
	82	5	1.782 ± 0.195	2.576 ± 0.341	7.049 ± 1.08	3.497 ± 0.342
437J	74	1	1.022 ± 0.383	1.105 ± 0.670	2.290 ± 2.124	2.125 ± 0.672
	75	21	1.973 ± 0.086	2.896 ± 0.151	7.921 ± 0.478	3.700 ± 0.151
	81	28	1.702 ± 0.079	2.352 ± 0.138	5.919 ± 0.438	3.017 ± 0.139
	83	19	1.521 ± 0.094	1.952 ± 0.164	4.647 ± 0.521	2.638 ± 0.165
551G	76	4	1.909 ± 0.194	2.844 ± 0.339	7.648 ± 1.074	3.791 ± 0.340
	77	21	1.657 ± 0.088	2.380 ± 0.154	6.382 ± 0.487	3.202 ± 0.154
	80	35	1.671 ± 0.073	2.387 ± 0.127	6.141 ± 0.402	3.016 ± 0.127
	84	17	1.677 ± 0.103	2.384 ± 0.179	6.469 ± 0.569	3.280 ± 0.180

[1]n = number of E^DE^+ animals in each family.
[2]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[3]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[4]Ranges from light (1) to solid (9) amount of black pigmentation present.
[5]Ranges from light (1) to extremely dark (4).

Table 13. Least squares means and standard errors by season of photograph for majority black, degree of black – simple, degree of black – complex, and degree of darkness using $E^{D}E^{+}$ F_2 Nellore-Angus cattle

Season of photograph	Majority black[1]	Degree of black – simple[2]	Degree of black – complex[3]	Degree of darkness[4]
Spring/Summer (S)	1.642 ± 0.053[a]	2.293 ± 0.093[a]	6.028 ± 0.295[a]	3.107 ± 0.093[a]
Fall/Winter (W)	1.812 ± 0.058[b]	2.650 ± 0.101[b]	7.161 ± 0.319[b]	3.499 ± 0.101[b]

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[4]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Family nested within sire was significant for all scoring systems except Red Present (Table 12). The least squares means for Family 74, which possessed only individual 7403, were numerically redder and lighter than the other families. Photographs taken in the spring/summer showed animals that were redder and lighter than animals with photographs taken in the fall/winter (Table 13). Likewise, animals that were spotted were redder and lighter than non-spotted animals (Table 14).

Interval Analysis

Interval analysis was conducted for 19 autosomes (Appendix C) because the remaining 10 autosomes produced runtime errors, the cause of which has yet to be identified. One possibility is incorrect assembly of the sequence (and therefore order of SNP) on these autosomes resulting in artificial inflation of the number of crossovers. To test this, coordinates for SNP on the University of Maryland assembly (UMD3) of the bovine genome sequence were used instead of Btau4.0 coordinates and also caused runtime errors for these autosomes. A second possibility is inclusion of too many consecutive markers that are heterozygous in all offspring of specific families because the parents were homozygous for alternate alleles of the SNP. This could be tested by filtering subsets of markers with this property and will be completed in future work.

Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide QTL were identified on BTA 4, 5, 6, 15, 18, 21, 27, and 29 for the different scoring systems (Tables 15-19). Surprisingly, evidence of a major gene modifying black coat color in these animals was found in the interval from 71 to 72 Mb region on BTA 6. This QTL

Table 14. Least squares means and standard errors by spotted status for all scoring systems using E^DE^+ F_2 Nellore-Angus cattle

Spotted status	Majority black[1]	Red present[2]	Degree of black - simple[3]	Degree of black - complex[4]	Degree of darkness[5]
No (1)	1.837 ± 0.043[a]	1.377 ± 0.050[a]	2.660 ± 0.075[a]	7.277 ± 0.237[a]	3.532 ± 0.075[a]
Yes (2)	1.617 ± 0.075[b]	1.199 ± 0.087[b]	2.283 ± 0.131[b]	5.913 ± 0.416[b]	3.075 ± 0.132[b]

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from 1 to 2, where 1 has red present and 2 does not have red present
[3]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[4]Ranges from light (1) to solid (9) amount of black pigmentation present.
[5]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Table 15. Locations, test statistics and size of effects of QTL for majority black detected using E^DE^+ F_2 Nellore-Angus cattle

			Test statistics			Effects		
BTA	Position (Mb)	Flanking markers	F	LRT	LOD	Mean ± SE	Additive[a] ± SE	Dominance[b] ± SE
4*	58	BTA-16397-no-rs - BTB-01403737	7.84	15.11	3.281	-0.1012 ± 0.0390	-0.0853 ± 0.3902	0.1727 ± 0.0525
6**	72	BTA-76705-no-rs - Hapmap23983-BTC-070420	58.28	92.29	20.04	-0.1154 ± 0.0287	-0.2499 ± 0.0287	0.2504 ± 0.0413
21*	12	ARS-BFGL-NGS-109184 - Hapmap47554-BTA-26517	8.22	15.81	3.434	-0.0698 ± 0.0469	0.1535 ± 0.0416	0.1099 ± 0.0626

[a]QTL genotypic value of Nellore homozygote such that 2a = NN - AA.
[b]NA heterozygote deviation from QTL homozygote midpoint such that d = NA - 0.5(NN + AA).
*Suggestive Chromosome-wide ($P < 0.05$).
**Significant Chromosome-wide ($P < 0.01$).

Table 16. Locations, test statistics and size of effects of QTL for red present detected using E^DE^+ F_2 Nellore-Angus cattle

BTA	Position (Mb)	Flanking markers	Test statistics			Effects		
			F	LRT	LOD	Mean ± SE	Additive[a] ± SE	Dominance[b] ± SE
5**	31	Hapmap39353-BTA-73120 - ARS-BFGL-NGS-119788	9.41	18.01	3.91	-0.0162 ± 0.0411	-0.1750 ± 0.0411	0.0423 ± 0.0597
6**	71	ARS-BFGL-NGS-93633 - Hapmap24750-BTC-042016	11.58	21.95	4.766	0.0264 ± 0.0397	-0.1907 ± 0.0397	-0.0317 ± 0.0561
15*	11	Hapmap51391-BTA-112776 - ARS-BFGL-NGS-25540	6.94	13.42	2.915	-0.0644 ± 0.0478	-0.1652 ± 0.0472	0.1129 ± 0.0686
18*	7	BTB-00698737 - ARS-BFGL-NGS-2048	6.88	13.31	2.891	0.1592 ± 0.0770	0.2278 ± 0.0768	-0.1900 ± 0.0860
27*	32	ARS-BFGL-NGS-116607 - BTA-23660-no-rs	5.95	11.56	2.51	0.0952 ± 0.0453	0.1033 ± 0.0446	-0.1826 ± 0.0697
29*	18	ARS-BFGL-NGS-112818 - ARS-BFGL-NGS-47918	7.29	14.09	3.06	0.1501 ± 0.0511	-0.0787 ± 0.0483	-0.2851 ± 0.0813

[a]QTL genotypic value of Nellore homozygote such that 2a = NN - AA.
[b]NA heterozygote deviation from QTL homozygote midpoint such that d = NA - 0.5(NN + AA).
*Suggestive Chromosome-wide ($P < 0.05$).
**Significant Chromosome-wide ($P < 0.01$).

Table 17. Locations, test statistics and size of effects of QTL for degree of black - simple detected using E^DE^+ F_2 Nellore-Angus cattle

BTA	Position (Mb)	Flanking markers	Test statistics			Effects		
			F	LRT	LOD	Mean ± SE	Additive[a] ± SE	Dominance[b] ± SE
4**	58	BTA-16397-no-rs - BTB-01403737	11.11	21.1	4.582	-0.2013 ± 0.0672	-0.1868 ± 0.0671	0.3424 ± 0.0904
5*	110	Hapmap3063-BTA-15439 - UA-IFASA-8960	7.55	14.56	3.162	0.1624 ± 0.0614	-0.0027 ± 0.0609	-0.3914 ± 0.1015
6**	72	BTA-76705-no-rs - Hapmap23983-BTC-070420	60.28	94.82	20.59	-0.2027 ± 0.0498	-0.4428 ± 0.0498	0.4382 ± 0.0718
21*	12	ARS-BFGL-NGS-109184 - Hapmap47554-BTA-26517	7.67	14.79	3.211	-0.1173 ± 0.0747	0.2604 ± 0.0728	0.1827 ± 0.1096

[a]QTL genotypic value of Nellore homozygote such that 2a = NN - AA.
[b]NA heterozygote deviation from QTL homozygote midpoint such that d = NA - 0.5(NN + AA).
*Suggestive Chromosome-wide ($P < 0.05$).
**Significant Chromosome-wide ($P < 0.01$).

Table 18. Locations, test statistics and size of effects of QTL for degree of black - complex detected using E^DE^+ F_2 Nellore-Angus cattle

BTA	Position (Mb)	Flanking markers	Test statistics			Effects		
			F	LRT	LOD	Mean ± SE	Additive[a] ± SE	Dominance[b] ± SE
4**	58	BTA-16397-no-rs - BTB-01403737	10.45	19.89	4.32	-0.5968 ± 0.2138	-0.6120 ± 0.2137	1.0196 ± 0.2878
5*	110	Hapmap3063-BTA-15439 - UA-IFASA-8960	7.83	15.09	3.276	0.5359 ± 0.1947	-0.0610 ± 0.1930	-1.2715 ± 0.3218
6**	71	ARS-BFGL-NGS-93633 - Hapmap24750-BTC-042016	67.88	104.2	22.62	-0.5121 ± 0.1558	-1.5816 ± 0.1558	1.1363 ± 0.2202
21*	12	ARS-BFGL-NGS-109184 - Hapmap47554-BTA-26517	6.95	13.44	2.919	-0.3495 ± 0.2379	-0.7907 ± 0.2319	0.5488 ± 0.3492

[a]QTL genotypic value of Nellore homozygote such that 2a = NN - AA.
[b]NA heterozygote deviation from QTL homozygote midpoint such that d = NA - 0.5(NN + AA).
*Suggestive Chromosome-wide ($P < 0.05$).
**Significant Chromosome-wide ($P < 0.01$).

Table 19. Locations, test statistics and size of effects of QTL for degree of darkness detected using E^DE^+ F_2 Nellore-Angus cattle

BTA	Position (Mb)	Flanking markers	Test statistics			Effects		
			F	LRT	LOD	Mean ± SE	Additive[a] ± SE	Dominance[b] ± SE
4*	59	Hapmap23995-BTA142201 - ARS-BFGL-NGS-105821	7.99	15.38	3.34	-0.1653 ± 0.0657	-0.1597 ± 0.0656	0.2939 ± 0.0931
5*	110	Hapmap3063-BTA-15439 - UA-IFASA-8960	7.19	13.89	3.016	0.1579 ± 0.0617	-0.0045 ± 0.0611	-0.3839 ± 0.1019
6**	71	ARS-BFGL-NGS-93633 - Hapmap24750-BTC-042016	44.86	74.42	16.16	-0.1268 ± 0.0529	-0.4490 ± 0.0529	0.2798 ± 0.0748
21*	49	ARS-BFGL-NGS-117837 - BTB-00481797	6.36	12.33	2.678	0.0718 ± 0.0612	0.1905 ± 0.0612	-0.1564 ± 0.0885

[a]QTL genotypic value of Nellore homozygote such that 2a = NN - AA.
[b]NA heterozygote deviation from QTL homozygote midpoint such that d = NA - 0.5(NN + AA).
*Suggestive Chromosome-wide ($P < 0.05$).
**Significant Chromosome-wide ($P < 0.01$).

was significant for all scoring systems with F-statistics ranging from 11.58 for Red Present to 67.88 for Degree of Black – Complex. This QTL accounted for 61.1% of the phenotypic variation in Degree of Black – Complex in E^DE^+ animals (Table 20) assuming that 2 QTL (Q and q) alleles were present in the population at equal frequencies. This assumption is unlikely to be valid in an outbred population. In addition, the large genetic variance due to the QTL (1.574) may be due to an upward bias in the estimation of QTL effects and, therefore, the proportion of the variance accounted for by the QTL may be over-estimated (Utz et al., 2000).

Table 20. Proportion of variance explained by the QTL on BTA 6 for all scoring systems using E^DE^+ F_2 Nellore-Angus cattle

Scoring system	Additive effect estimate (a)	Dominance effect estimate (d)	Genetic variance (g)[1]	QTL variance (v)[2]
Majority Black	-0.250	0.250	0.047	0.045
Red Present	-0.191	-0.032	0.018	0.018
Degree of Black - Simple	-0.443	0.438	0.146	0.127
Degree of Black - Complex	-1.582	1.136	1.574	0.611
Degree of Darkness	-0.449	0.280	0.120	0.107

[1]Calculation of genetic variance due to QTL (g) is based on the equation for F_2 animals where $g = (2a^2 + d^2)/4$ and assumes $p = q = 0.5$ (Darvasi and Soller, 1997).
[2]Proportion of variance explained by QTL (v) $= g/(1 + g)$ (Darvasi and Soller, 1997).

Identification of Candidate Genes for QTL of Reddening

In addition to the major gene on BTA6, which will be discussed in subsequent sections, 7 other QTL were shown to contribute to variation in the degree of reddening utilizing various scoring systems. There were no obvious candidate genes for the QTL on BTA 5, 15, 18, 21, or 27. Interferon-related developmental regulator 1 (*IFRD1*) is a candidate gene on BTA 4 for Majority Black, Degree of Black – Simple, Degree of

Black – Complex, and Degree of Darkness QTL. The protein product of this gene, also known as TIS7 in mice, has been shown to inhibit osteopontin expression through lymphoid enhancer binding protein-1 (LEF-1) and to down-regulate β-catenin/T-cell factor-4 (TCF-4) transcriptional activity (Vietor et al., 2005). Components of the wingless/INT-related (WNT)/β-catenin pathway play a major role in the initial induction and expansion of neural crest cells into pigment cells (reviewed by Silver et al., 2006). Over-expression of the dominant, negative form of LEF-1, which lacks the β-catenin binding domain, and T-cell factors family (*LEF-1/TCF*) significantly reduces microphthalmia-associated transcription factor (*MITF*) expression, the master regulatory gene for melanogenesis (Yasumoto et al., 2002; Silver et al., 2006).

In addition to *IFRD1*, growth factor receptor-bound-associated binding protein 2 (*GAB2*) is a candidate gene for the QTL identified on BTA 29 for the Red Present scoring system. Growth factor receptor-bound-associated binding protein 2 is shown in the chronic myeloid leukemia pathway (KEGG 05220) to be upsteam of RAS, RAF, and MEK, which are components of the MAPK signaling pathway. These 3 genes, as part of the MAPK signaling pathway, are also shown in the melanogenesis pathway (KEGG 04916) to be influenced by v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) and its ligand (KITLG). v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog, a tyrosine kinase receptor, is necessary for pigment cell survival and migration. Expression of KIT coincides closely with MITF expression in both pre-migratory and migratory cells, and upregulation of KIT past normal expression levels requires MITF (Bennett and Lamoreux, 2003). v-kit Hardy-Zuckerman 4 feline sarcoma

viral oncogene homolog influences pigment cell survival and migration throughout development by the complementary expression pattern of its ligand, as well as through other roles in hemopoiesis and gametogenesis (Broxmeyer et al., 1991; Besmer et al., 1993; Silver et al., 2006).

Investigation of a QTL on BTA 6 with a Major Effect on Reddening

The QTL on BTA6 with a major effect on reddening coincides with a cluster of tyrosine kinase receptors including platelet-derived growth factor receptor alpha polypeptide (*PDGFRA*), *KIT*, and kinase insert domain receptor (*KDR*). As previously mentioned, *KIT* has a known role in melanogenesis and is associated with spotting in cattle (Grosz and MacNeil, 1999; Reinsch et al., 1999). Due to this, breed of origin of a 1 Mb region spanning these 3 genes and centered on *KIT* (referred to hereafter as the *KIT* region) was assigned for 206 of the 210 F_2 E^DE^+ Nellore-Angus cattle. Genotyping errors precluded assignment for the remaining 4 individuals (7212, 7540, 7542, and 8401). For all scoring systems, incorporating breed of origin of this region into the statistical model was significant ($P < 0.05$) as was the interaction of breed of origin with sire. Least squares means and standard errors for *KIT* region breed of origin and sire by *KIT* region breed of origin interaction are reported in Tables 21 and 22. No re-ranking of least squares means occurred for the other significant effects in the model (Appendix B).

Table 21. Least squares means and standard errors by *KIT* region breed of origin for all scoring systems using E^DE^+ F_2 Nellore-Angus cattle

Breed of origin[1]	Majority black[2]	Red present[3]	Degree of black – simple[4]	Degree of black – complex[5]	Degree of darkness[6]
AA	1.990 ± 0.055[a]	1.573 ± 0.089[a]	2.940 ± 0.098[a]	8.371 ± 0.292[a]	3.886 ± 0.108[a]
AN	1.960 ± 0.050[a]	1.372 ± 0.082[b]	2.887 ± 0.090[a]	7.941 ± 0.269[a,b]	3.671 ± 0.099[a,b]
NA	1.989 ± 0.056[a]	1.270 ± 0.092[b,c]	2.885 ± 0.100[a]	7.580 ± 0.300[b]	3.617 ± 0.111[b]
NN	1.258 ± 0.050[b]	1.103 ± 0.081[c]	1.685 ± 0.089[b]	4.080 ± 0.265[c]	2.604 ± 0.098[c]

[1] Based on haplotypes for 1 Mb region containing *PDGFRA*, *KIT* and *KDR* where N = Nellore and A = Angus.
[2]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[3]Ranges from 1 to 2, where 1 has red present and 2 does not have red present
[4]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[5]Ranges from light (1) to solid (9) amount of black pigmentation present.
[6]Ranges from light (1) to extremely dark (4).
[a,b,c] Within a column, means without a common superscript differ ($P < 0.05$).

Table 22. Least squares means and standard errors for the interaction of sire and *KIT* region breed of origin for majority black, degree of black - simple, and degree of black - complex using E^DE^+ F_2 Nellore-Angus cattle

Sire	Breed of origin[1]	Majority black[2]	Degree of black – simple[3]	Degree of black – complex[4]
297J	AA	2.001 ± 0.097[d,e,f,g,h]	3.015 ± 0.173[a,b,c,d]	8.772 ± 0.516[a,b,c]
	AN	1.975 ± 0.084[c,d,e,f,g,h]	2.963 ± 0.149[a,b,c,d]	8.506 ± 0.445[a,b,c,d]
	NA	2.059 ± 0.075[e,f,g,h]	3.111 ± 0.134[a,b,c]	8.455 ± 0.400[a,b,c,d]
	NN	1.635 ± 0.100[b,c,d,e]	2.155 ± 0.179[d,e,f,g,h]	5.806 ± 0.535[c,d,e,f]
432H	AA	2.102 ± 0.104[e,f,g,h]	3.112 ± 0.187[a,b,c,d]	8.883 ± 0.557[a,b,c]
	AN	2.008 ± 0.113[c,d,e,f,g,h]	2.988 ± 0.202[a,b,c,d,e]	8.254 ± 0.602[a,b,c,d]
	NA	2.115 ± 0.148[d,e,f,g,h]	3.008 ± 0.265[a,b,c,d,e]	7.401 ± 0.791[a,b,c,d,e,f]
	NN	1.007 ± 0.126[a]	1.627 ± 0.225[g,h,i]	4.116 ± 0.670[g]
437J	AA	1.840 ± 0.105[b,c,d,e,f,g,h]	2.621 ± 0.187[a,c,d,e,f,g]	7.295 ± 0.559[b,c,d,e]
	AN	1.790 ± 0.099[b,c,d,e,f,g]	2.492 ± 0.178[c,d,e,f,g]	6.370 ± 0.530[c,d,e,f]
	NA	1.751 ± 0.087[b,c,d,e,f]	2.388 ± 0.156[d,e,f,g]	6.154 ± 0.465[c,e,f]
	NN	1.248 ± 0.087[a]	1.549 ± 0.156[h,i]	3.432 ± 0.466[g]
551G	AA	2.019 ± 0.075[d,e,f,g,h]	3.012 ± 0.134[a,b,c,d]	8.536 ± 0.401[a,b,c,d]
	AN	2.070 ± 0.074[e,f,g,h]	3.106 ± 0.132[a,b,c]	8.635 ± 0.395[a,b,c]
	NA	2.031 ± 0.067[e,f,g,h]	3.031 ± 0.120[a,b,c,d]	8.311 ± 0.357[a,b,c,d]
	NN	1.140 ± 0.079[a]	1.409 ± 0.141[h,i]	2.968 ± 0.422[g]

[1] Based on haplotypes for 1 Mb region containing *PDGFRA*, *KIT* and *KDR* where N = Nellore and A = Angus.
[2]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[3]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[4]Ranges from light (1) to solid (9) amount of black pigmentation present.
[a,b,c] Within a column, means without a common superscript differ ($P < 0.05$).

For all scoring systems, AA homozygotes for *KIT* were different ($P < 0.05$) from NN homozygotes, where red pigmentation appeared to be recessive and was associated with the Nellore allele. In addition, NA and AN heterozygotes were never statistically different from each other but NA were numerically lower for each trait. Consequently, NA heterozygotes were different from AA homozygotes for Red Present, Degree of Black – Complex, and Degree of Darkness and AN heterozygotes were only different from AA homozygotes for Red Present. For 3 of the sires (432H, 437J, and 551G) the NN homozygotes had less black pigmentation from the alternate genotypes (AN, NA, and AA) for Majority Black, Degree of Black – Simple, and Degree of Black – Complex.

In addition to fitting breed of origin of the KIT region in the models for the various scoring system, the effect of fitting specific haplotype combinations was investigated as well. Modeling haplotypes instead of breed of origin allows for the situation where the same alleles for these 3 tyrosine kinase receptors may be present in both *Bos indicus* and *Bos taurus*. Because the phased genotypes for the KIT region were based on the parents of the F_2 individuals, neither sire nor family nested within sire was included in these models to avoid confounding. Fixed effects for this model were sex, season of photograph, spotted status, and phased *KIT* genotype for all scoring systems. For each of the factors included in previous models, there was no re-ranking of the least squares means (Appendix B).

Table 23. Least squares means and standard errors by phased genotypes for *KIT* region for all scoring systems using $E^{D}E^{+}$ F2 Nellore-Angus cattle

Phased genotype for the *KIT* region[1,2]	Breed of origin[1]	Majority black[3]	Red present[4]	Degree of black - simple[5]	Degree of black - complex[6]	Degree of darkness[7]
2_2	AA	2.126 ± 0.183	1.985 ± 0.301	3.252 ± 0.327	9.726 ± 0.982	4.206 ± 0.359
2_5	AN	2.096 ± 0.150	1.705 ± 0.247	3.164 ± 0.268	9.138 ± 0.806	4.105 ± 0.295
2_14	AN	2.086 ± 0.099	1.719 ± 0.163	3.168 ± 0.177	9.060 ± 0.532	3.992 ± 0.195
2_15	AA	2.073 ± 0.106	1.665 ± 0.174	3.138 ± 0.189	9.059 ± 0.568	3.934 ± 0.208
5_17	NA	2.111 ± 0.119	1.450 ± 0.196	3.201 ± 0.213	9.000 ± 0.639	3.955 ± 0.234
2_6	AA	2.022 ± 0.183	1.979 ± 0.302	2.976 ± 0.328	8.831 ± 0.985	3.857 ± 0.360
13_2	NA	2.029 ± 0.146	1.642 ± 0.241	3.052 ± 0.262	8.795 ± 0.786	4.025 ± 0.288
6_1	AN	2.062 ± 0.070	1.543 ± 0.115	3.094 ± 0.125	8.785 ± 0.375	3.957 ± 0.137
5_12	NA	2.091 ± 0.150	1.316 ± 0.247	3.160 ± 0.268	8.761 ± 0.804	4.089 ± 0.294
6_8	AA	2.061 ± 0.183	1.536 ± 0.301	3.071 ± 0.327	8.673 ± 0.981	3.989 ± 0.359
1_6	NA	2.062 ± 0.106	1.674 ± 0.174	3.108 ± 0.189	8.633 ± 0.567	3.898 ± 0.207
6_4	AA	2.074 ± 0.063	1.481 ± 0.104	3.072 ± 0.113	8.604 ± 0.338	3.768 ± 0.124
6_17	AA	2.101 ± 0.184	1.093 ± 0.303	3.167 ± 0.329	8.516 ± 0.988	3.621 ± 0.362
1_15	NA	2.077 ± 0.099	1.155 ± 0.162	3.142 ± 0.176	8.410 ± 0.530	3.961 ± 0.194
6_3	AN	2.063 ± 0.118	1.381 ± 0.194	3.087 ± 0.211	8.389 ± 0.634	3.597 ± 0.232
6rec_4	AA[8]	2.086 ± 0.256	0.927 ± 0.421	3.157 ± 0.458	8.384 ± 1.374	3.074 ± 0.503
5_4	NA	2.051 ± 0.102	1.409 ± 0.168	3.055 ± 0.182	8.372 ± 0.548	3.671 ± 0.200
6_16	AA	1.892 ± 0.118	1.594 ± 0.194	2.761 ± 0.211	8.036 ± 0.633	3.693 ± 0.232
3_8	NA	2.022 ± 0.256	0.979 ± 0.421	2.976 ± 0.458	7.831 ± 1.374	3.857 ± 0.503
2_7	AN	1.978 ± 0.133	1.271 ± 0.219	2.774 ± 0.238	7.669 ± 0.714	3.643 ± 0.261
2_9	AN	2.054 ± 0.182	0.953 ± 0.300	3.067 ± 0.326	7.607 ± 0.979	3.465 ± 0.358
2_4	AA	1.965 ± 0.087	1.396 ± 0.143	2.788 ± 0.156	7.602 ± 0.467	3.701 ± 0.171
2_3	AN	2.003 ± 0.092	0.984 ± 0.152	2.888 ± 0.165	7.515 ± 0.495	3.278 ± 0.181
5_16	NA	1.908 ± 0.110	1.102 ± 0.182	2.794 ± 0.197	7.458 ± 0.592	3.371 ± 0.217
6_7	AN	1.960 ± 0.180	0.943 ± 0.296	2.905 ± 0.321	7.158 ± 0.965	3.368 ± 0.353
1_14	NN	1.957 ± 0.128	1.286 ± 0.211	2.421 ± 0.229	6.808 ± 0.687	2.963 ± 0.251
3_4	NA	2.118 ± 0.150	1.021 ± 0.247	2.891 ± 0.269	6.656 ± 0.807	3.177 ± 0.295
7_4	NA	1.905 ± 0.083	1.098 ± 0.137	2.600 ± 0.149	6.575 ± 0.447	3.075 ± 0.164
7_13	NN	1.627 ± 0.087	1.091 ± 0.143	2.293 ± 0.155	5.729 ± 0.467	2.973 ± 0.171
7_3	NN	1.396 ± 0.090	1.107 ± 0.148	1.914 ± 0.161	4.596 ± 0.483	2.893 ± 0.177
1_5	NN	1.237 ± 0.150	0.962 ± 0.247	1.836 ± 0.268	4.528 ± 0.806	3.228 ± 0.295
2rec_6	AN[9]	1.166 ± 0.257	1.042 ± 0.423	1.348 ± 0.459	4.068 ± 1.379	2.338 ± 0.505

Table 23. Continued

Phased genotype for the *KIT* region[1,2]	Breed of origin[1]	Majority black[3]	Red present[4]	Degree of black - simple[5]	Degree of black - complex[6]	Degree of darkness[7]
3_7	NN	0.979 ± 0.091	0.996 ± 0.150	1.488 ± 0.163	3.374 ± 0.491	2.164 ± 0.180
7_11	NA	1.166 ± 0.257	1.042 ± 0.423	1.348 ± 0.459	3.068 ± 1.379	2.338 ± 0.505
5_1	NN	1.060 ± 0.097	1.002 ± 0.160	1.265 ± 0.174	2.931 ± 0.521	2.393 ± 0.191
5_3	NN	1.193 ± 0.093	0.987 ± 0.153	1.495 ± 0.166	2.821 ± 0.499	2.192 ± 0.183
7_9	NN	1.246 ± 0.114	0.998 ± 0.187	1.306 ± 0.204	2.724 ± 0.611	1.911 ± 0.224

[1] Haplotype pairs are in order of inheritance from sire followed by dam, where N = Nellore and A = Angus.
[2] "rec" indicates that a recombination event occurred within the 1 Mb region surrounding the *KIT* locus, all breed of origin assignments are based on genotype at the *KIT* locus.
[3]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[4]Ranges from 1 to 2, where 1 has red present and 2 does not have red present
[5]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[6]Ranges from light (1) to solid (9) amount of black pigmentation present.
[7]Ranges from light (1) to extremely dark (4).
[8] Haplotype inherited from sire was Angus (6) until the recombination event occurred after *KIT* locus where it changed to Nellore (5).
[9] Haplotype inherited from sire was Nellore (1) until the recombination event occurred before *KIT* locus where it changed to Angus (2).

Least square means and standard errors for the phased genotypes of the *KIT* region are reported in Table 23 and are sorted by Degree of Black – Complex from largest (most black) to smallest (most red). Within this 1 Mb interval, 2 recombinants were identified: 7014 (2rec_6) and 8051 (6rec_4). In individual 8051, the recombination event occurred after the *KIT* locus, which was of Angus origin and before *KDR,* which was of Nellore origin. The least squares means for 8051 clustered with others of Angus origin thereby eliminating *KDR* as a candidate for the reddening effect. Conversely, the recombination event in 7014 occurred before the *KIT* locus such that the region encompassing *PDGFRA* was of Nellore origin whereas the region encompassing *KIT* and *KDR* was of Angus origin. The least squares means for 7014 clustered with those of Nellore origin thereby precluding both *KIT* and *KDR* as candidates for reddening. Breed of origin assignment was repeated for the 1 Mb region centered on *PDGFRA*. No

additional recombinants were identified. Therefore, based on these data *PDGFRA* appears to be the causal gene for the reddening phenotype.

Platelet Derived Growth Factor Receptor Alpha

Platelet derived growth factor receptor alpha is a member of the platelet-derived growth factors (PDGF) that consist of 2 receptors (α and β) and 4 ligands (A-D). The function and cellular responses have been extensively studied in the mouse with the use of genetic manipulation during development. To function as a tyrosine kinase receptor, the PDGF-receptor must first bind a ligand, then dimerize with another ligand-bound PDGF-receptor (reviewed by Hoch and Soraino, 2003). Signal transduction pathways including the Ras-mitogen activated protein kinase (Ras-MAPK), phosphatidylinositol 3-kinase (PI3K), and phospholipase Cγ pathways can be activated by both PDGF-receptors.

In relation to pigmentation, roles of PDGFRA are known in mouse hair follicle development (Karlsson et al., 1999) and possibly in melanocyte migration (Soriano, 1997) although differentiating the role of PDGFRA from KIT has been difficult. Based on the functions described in the mouse (reviewed by Hoch and Soriano, 2003), PDGFRA could be influencing the MAPK signaling pathway in melanogenesis and therefore influencing the expression of MITF. This would be similar to the function of KIT, which can affect the complex cascade of events that MITF regulates. Furthermore, PDGFRA can activate protein kinase C (PKC) expression and influence Ca^{2+} levels, thereby directly impacting melanin synthesis in melanogenesis. Future work should

include gene expression analyses for both *PDGFRA* and *KIT* utilizing skin biopsies of F_2 Nellore-Angus cattle to further characterize the roles of these genes in the reddening phenotype. In addition, breed of origin assignment could be expanded by continuously sliding the 1 Mb regions to the left of PDGFRA until the breed of origin and least squares means are no longer concordant. This would define a genomic region that contains the causative gene.

Interval Analysis with KIT Region Factored into Model

Both breed of origin and phased genotypes for the *KIT* region accounted for the variation attributed to the QTL on BTA 6 for all scoring systems (Figures 2-6). No KIT region haplotypes were shared between the Nellore and Angus founders. Because the phased genotypes did not provide any additional information, residuals generated by the inclusion of breed of origin in the model were utilized for subsequent interval analyses. Interestingly, once the variation due to this QTL on BTA 6 was removed, a second QTL for Degree of Black – Simple was identified on BTA 6 at 17 Mb (Figure 4). Retinal pigment epithelium derived rhodopsin homolog (*RRH*) is located at 16.9 Mb on BTA 6 based on Btau4.0. The murine homolog of this gene (*Rrh*) has G-protein coupled receptor activity, photo receptor activity, and signal transducer activity.

Retinal pigment epithelium derived rhodopsin homolog, also known as peropsin in human and mouse, is localized in the microvilli of retinal pigment epithelium, but the true function and ligands of *RRH* are still unclear (Sun et al., 1997). Although visual pigment traits are not always inherited with skin or hair pigmentation traits, it is

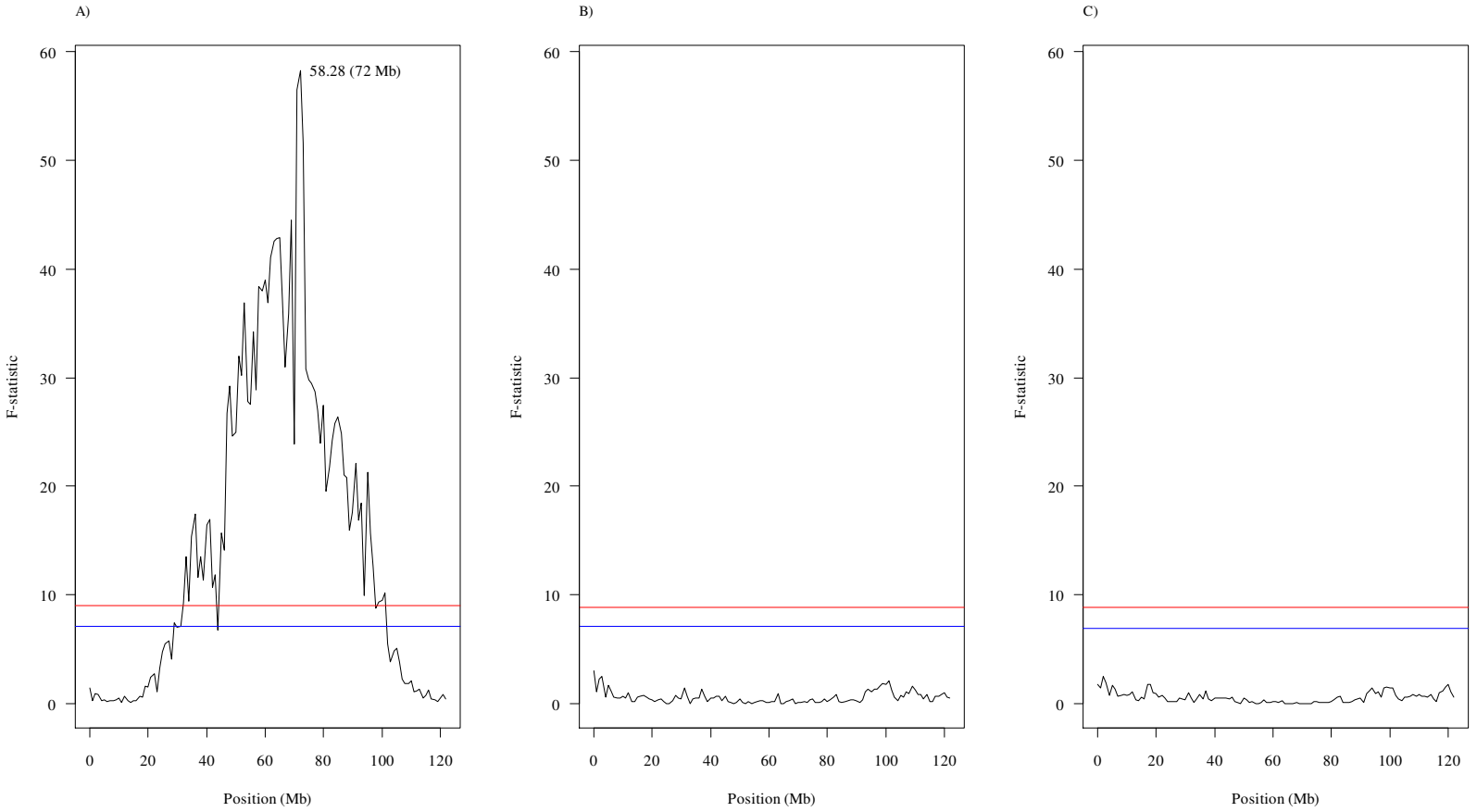


Figure 2. Interval analysis on BTA 6 for majority black. (A) Original model, (B) model including *KIT* region breed of origin and (C) phased genotypes. Suggestive and significant chromosome-wide levels are represented by blue and red lines, respectively.

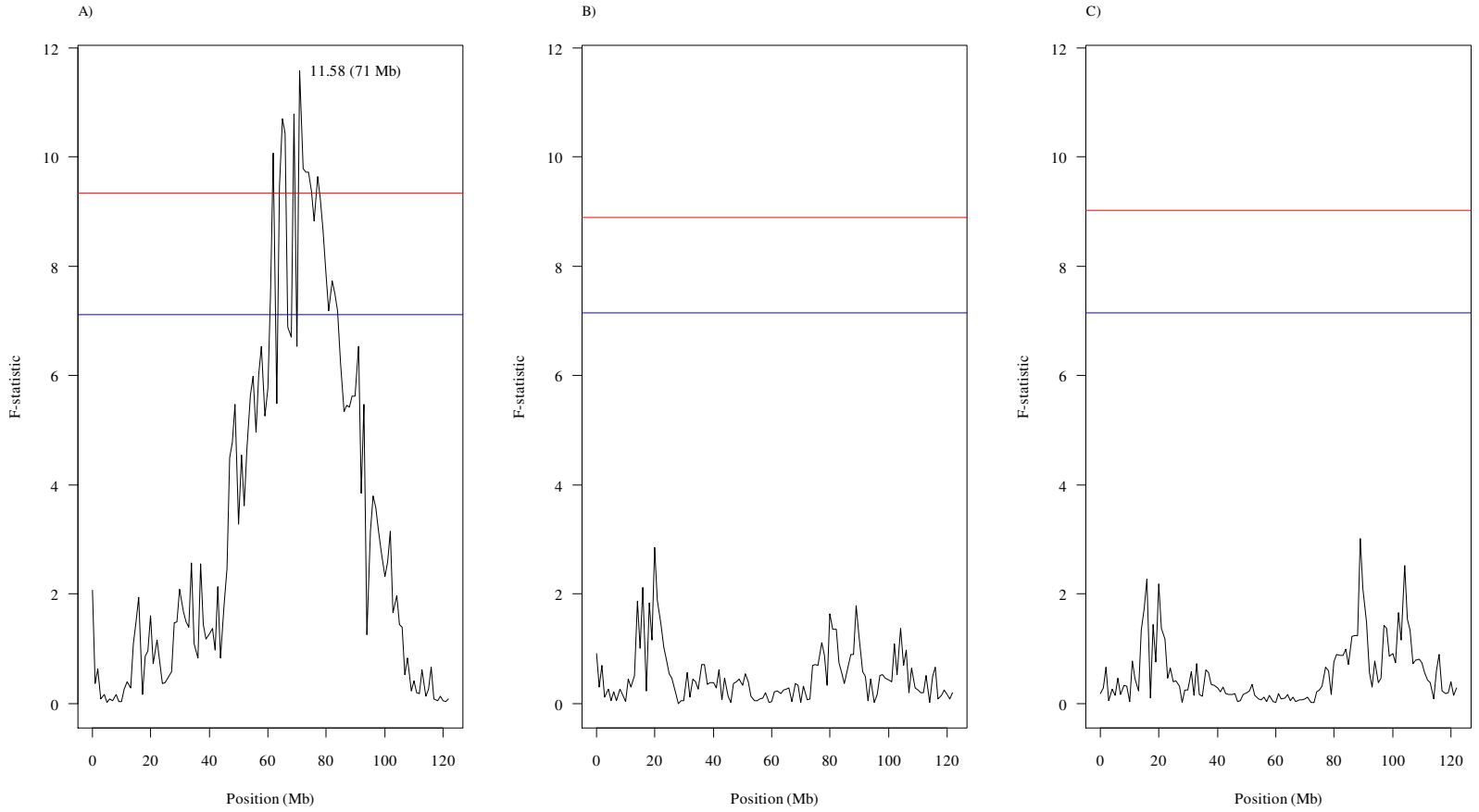


Figure 3. Interval analysis on BTA 6 for red present. (A) Original model, (B) model including *KIT* region breed of origin, and (C) phased genotypes. Suggestive and significant chromosome-wide levels are represented by blue and red lines, respectively.

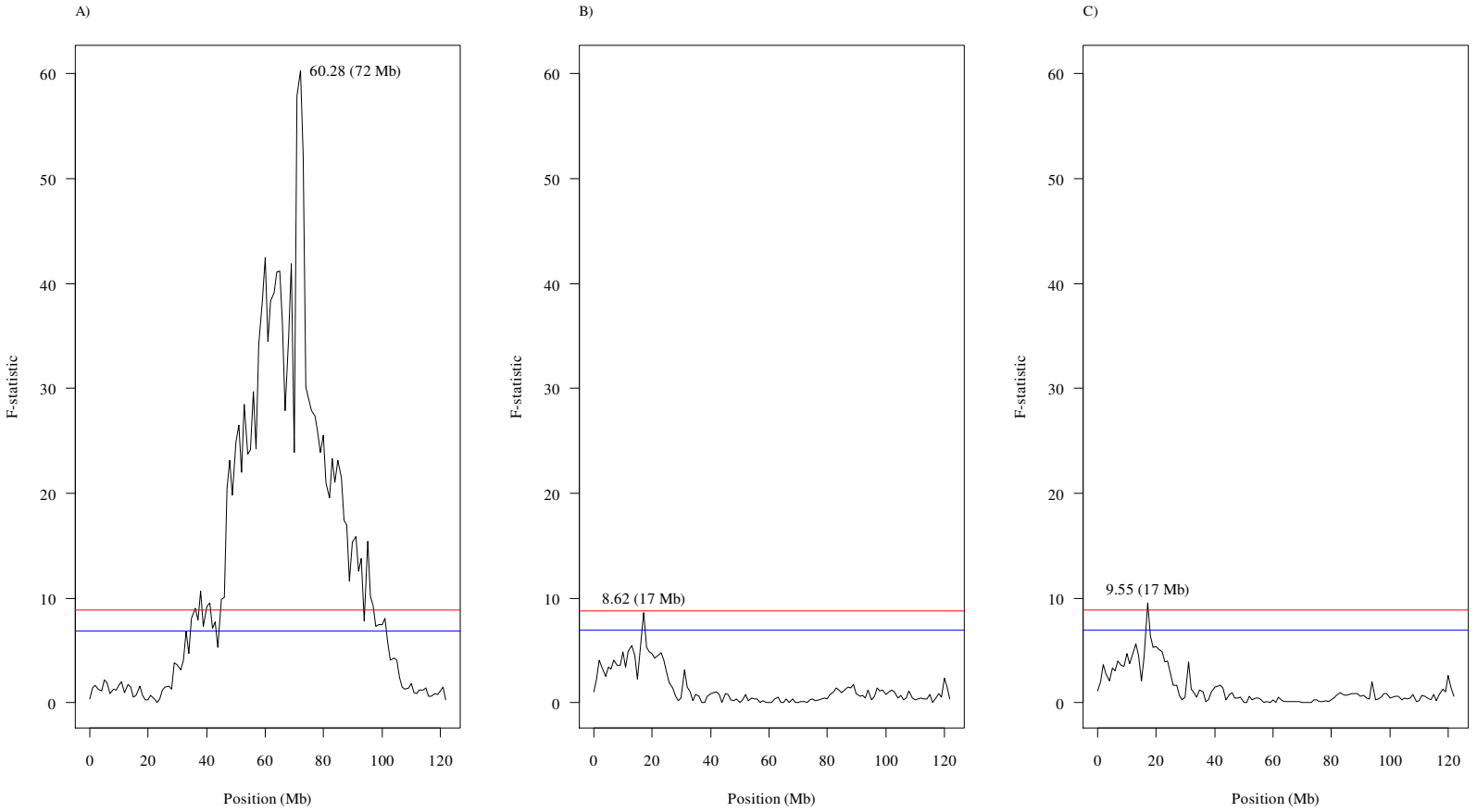


Figure 4. Interval analysis on BTA 6 for degree of black - simple. (A) Original model, (B) model including *KIT* region breed of origin, and (C) phased genotypes. Suggestive and significant chromosome-wide levels are represented by blue and red lines, respectively.

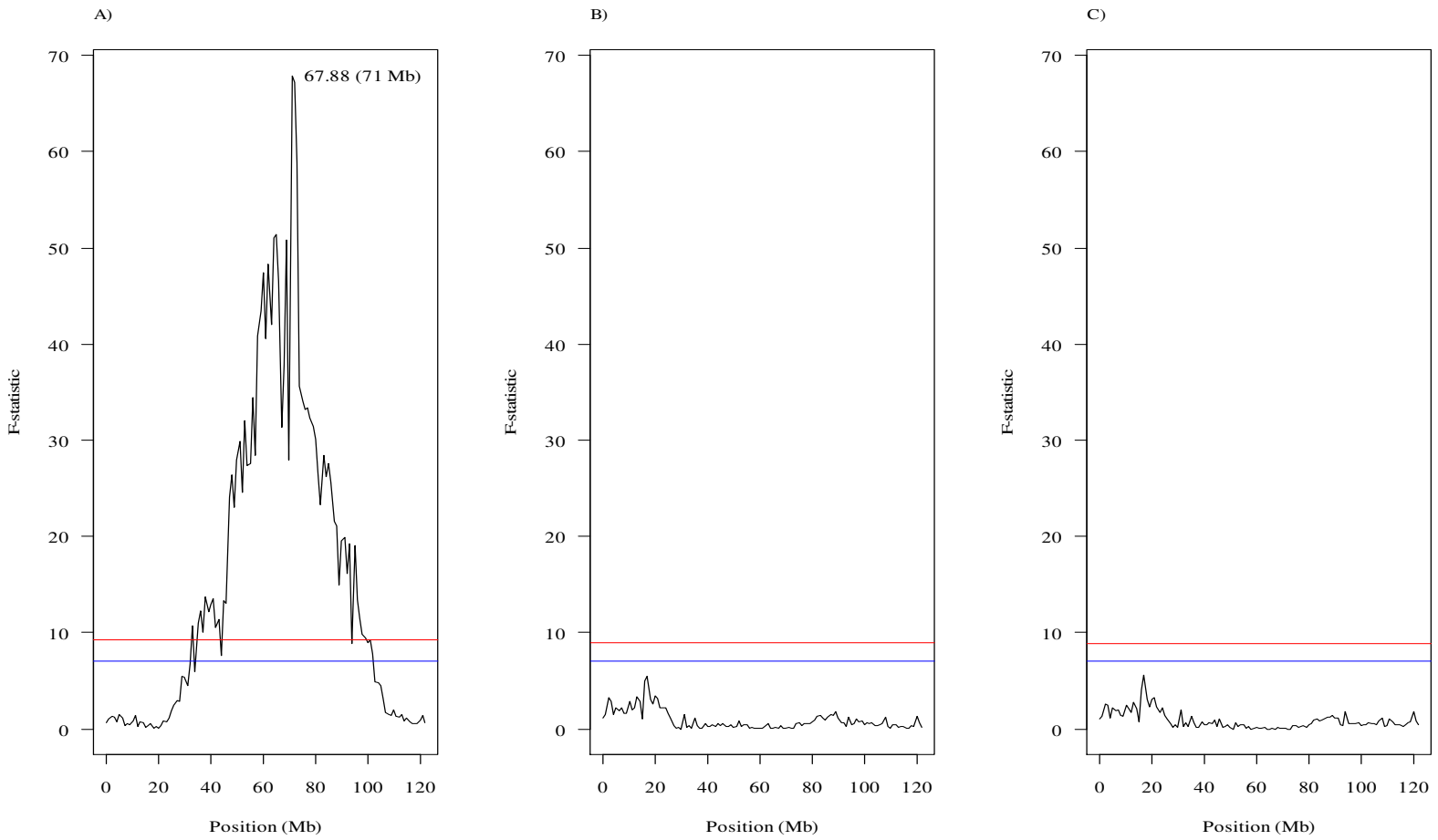


Figure 5. Interval analysis on BTA 6 for degree of black - complex. (A) Original model, (B) model including *KIT* region breed of origin, and (C) phased genotypes. Suggestive and significant chromosome-wide levels are represented by blue and red lines, respectively.

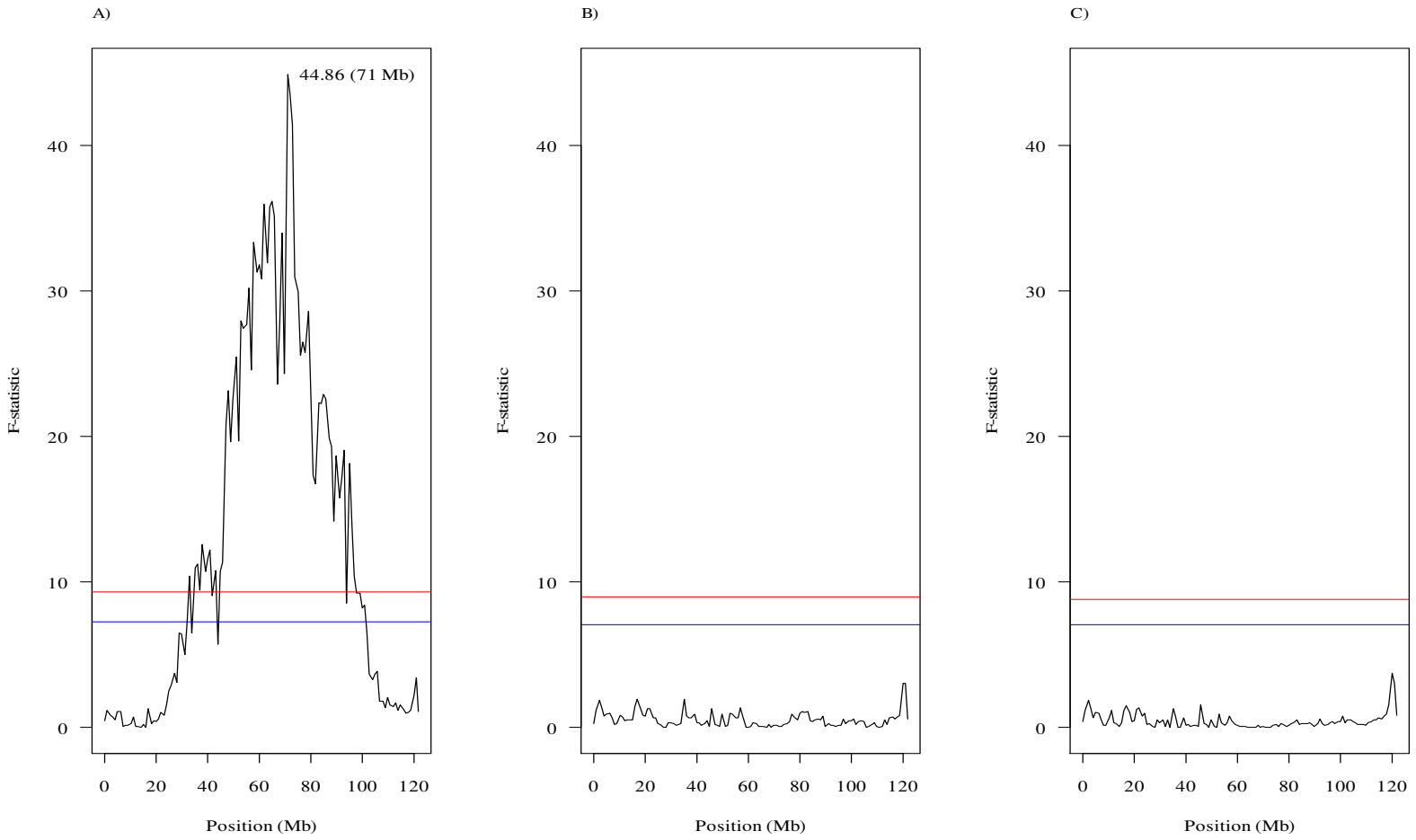


Figure 6. Interval analysis on BTA 6 for degree of darkness. (A) Original model, (B) model including *KIT* region breed of origin, and (C) phased genotypes. Suggestive and significant chromosome-wide levels are represented by blue and red lines, respectively.

Table 24. Locations, test statistics and size of effects of QTL for majority black identified with *KIT* region breed of origin in the model

BTA	Position (Mb)	Flanking markers	Test statistics			Effects		
			F	LRT	LOD	Mean ± SE	Additive[a] ± SE	Dominance[b] ± SE
16*	72	BTA-39939-no-rs - ARS-BFGL-NGS-113782	6.7	12.98	2.818	0.0576 ± 0.0226	0.0015 ± 0.0226	-0.1312 ± 0.0358

[a]QTL genotypic value of Nellore homozygote such that 2a = NN - AA.
[b]NA heterozygote deviation from QTL homozygote midpoint such that d = NA - 0.5(NN + AA).
*Suggestive Chromosome-wide ($P < 0.05$).
**Significant Chromosome-wide ($P < 0.01$).

Table 25. Locations, test statistics and size of effects of QTL for red present identified with *KIT* region breed of origin in the model

BTA	Position (Mb)	Flanking markers	Test statistics			Effects		
			F	LRT	LOD	Mean ± SE	Additive[a] ± SE	Dominance[b] ± SE
4*	18	Hapmap40227-BTA-85393 - BTA-71588-no-rs	7.05	13.63	2.959	0.0425 ± 0.0370	0.1227 ± 0.0369	-0.0929 ± 0.0554
5*	30	ARS-BFGL-NGS-115922 - ARS-BFGL-NGS-119788	8.75	16.78	3.644	-0.0262 ± 0.0382	-0.1542 ± 0.0382	0.0543 ± 0.0551
27*	32	ARS-BFGL-NGS-116607 - BTA-23660-no-rs	6.05	11.75	2.551	0.0848 ± 0.0420	0.0969 ± 0.0413	-0.1692 ± 0.0647

[a]QTL genotypic value of Nellore homozygote such that 2a = NN - AA.
[b]NA heterozygote deviation from QTL homozygote midpoint such that d = NA - 0.5(NN + AA).
*Suggestive Chromosome-wide ($P < 0.05$).
**Significant Chromosome-wide ($P < 0.01$).

Table 26. Locations, test statistics and size of effects of QTL for degree of black - simple identified with *KIT* region breed of origin in the model

BTA	Position (Mb)	Flanking markers	Test statistics			Effects		
			F	LRT	LOD	Mean ± SE	Additive[a] ± SE	Dominance[b] ± SE
4*	53	BTA-16657-no-rs - BTB-00185894	7.77	14.97	3.251	-0.1439 ± 0.0468	-0.0096 ± 0.0455	0.2879 ± 0.0734
6*	17	ARS-BFGL-NGS-104977 - ARS-BFGL-NGS-41348	8.62	16.54	3.592	-0.0439 ± 0.0470	0.1787 ± 0.0466	0.0919 ± 0.0669

[a]QTL genotypic value of Nellore homozygote such that 2a = NN - AA.
[b]NA heterozygote deviation from QTL homozygote midpoint such that d = NA - 0.5(NN + AA).
*Suggestive Chromosome-wide ($P < 0.05$).
**Significant Chromosome-wide ($P < 0.01$).

Table 27. Locations, test statistics and size of effects of QTL for degree of black - complex identified with *KIT* region breed of origin in the model

BTA	Position (Mb)	Flanking markers	Test statistics			Effects		
			F	LRT	LOD	Mean ± SE	Additive[a] ± SE	Dominance[b] ± SE
4*	53	BTA-16657-no-rs - BTB-00185894	7.79	15	3.258	-0.4330 ± 0.1396	0.0412 ± 0.1358	0.8643 ± 0.2191

[a]QTL genotypic value of Nellore homozygote such that 2a = NN - AA.
[b]NA heterozygote deviation from QTL homozygote midpoint such that d = NA - 0.5(NN + AA).
*Suggestive Chromosome-wide ($P < 0.05$).
**Significant Chromosome-wide ($P < 0.01$).

interesting that a gene suspected of being involved in visual pigmentation is found at the location of the QTL for Degree of Black – Simple.

Except for the QTL on BTA 27, fitting the *KIT* region accounted for the variation attributed to the other previously identified QTL, which is suggestive of epistasis among these QTL. Previously unidentified QTL were also identified on other chromosomes when breed of origin of *KIT* was included in the model for the various scoring systems for reddening (Tables 24-27 and Appendix C).

Investigation of Non-spotted F_2 E^DE^+ Nellore-Angus Cattle

Spotting, or the absence of pigmentation, is attributed to death of melanoblasts or the failure of melanocyte migration, division or differentiation (Herlyn et al., 2000; Bennett and Lamoreux, 2003; Silver et al., 2006). Because an association of the reddening phenotype was observed with spotting in these cattle and *KIT* is known to be causative of spotting in some species, an additional analysis was conducted utilizing only non-spotted F_2 E^DE^+ Nellore-Angus cattle to verify the QTL being detected on BTA 6 was for reddening and not spotting. The statistical model consisted of sex, sire, family nested within sire, and season of photograph. Least squares means and standard errors are reported in Appendix B. Residuals generated from this analysis identified the same QTL region as previously found for all scoring systems on BTA 6. Likewise, incorporation of breed of origin of the *KIT* region breed of origin into the model accounted for the variation attributed to the QTL (Figure 7 and Appendix C) and revealed the second QTL at 17 Mb for Degree of Black – Simple (Figure 8).

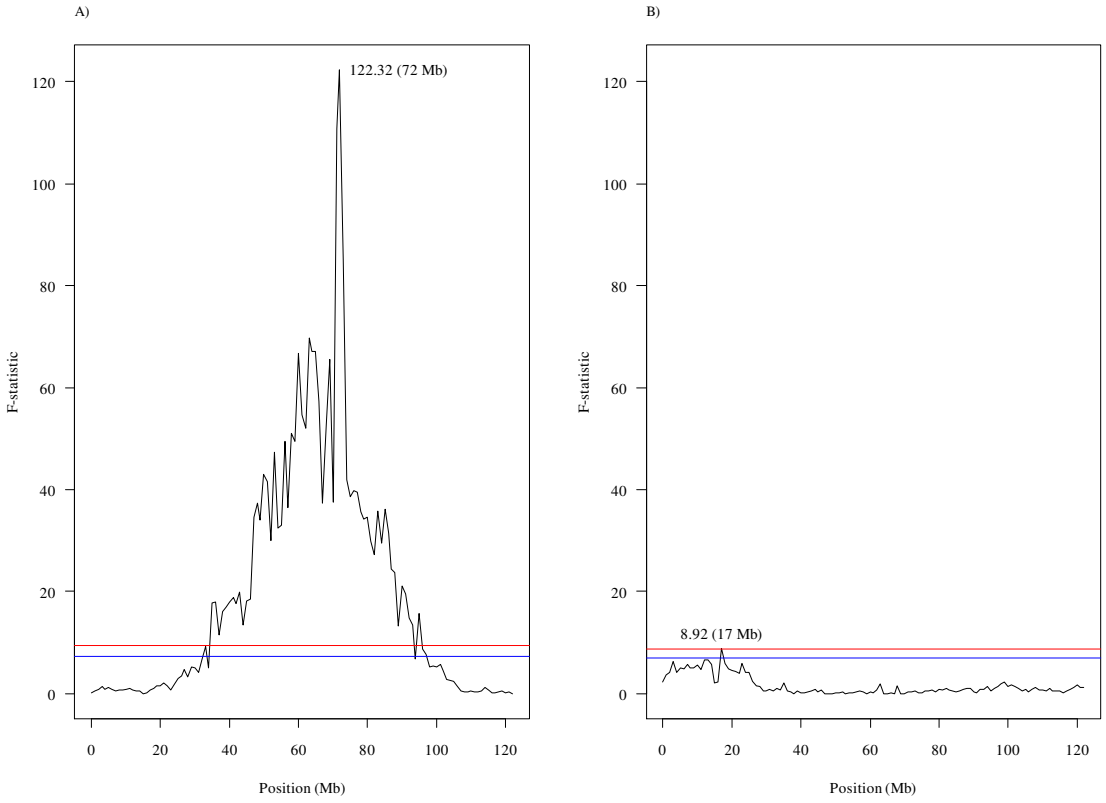


Figure 7. Interval analysis on BTA 6 for degree of black – simple utilizing only non-spotted $E^{D}E^{+}$ F_2 Nellore-Angus cattle. (A) Original model and (B) including *KIT* region breed of origin. Suggestive and significant chromosome-wide levels are represented by blue and red lines, respectively.

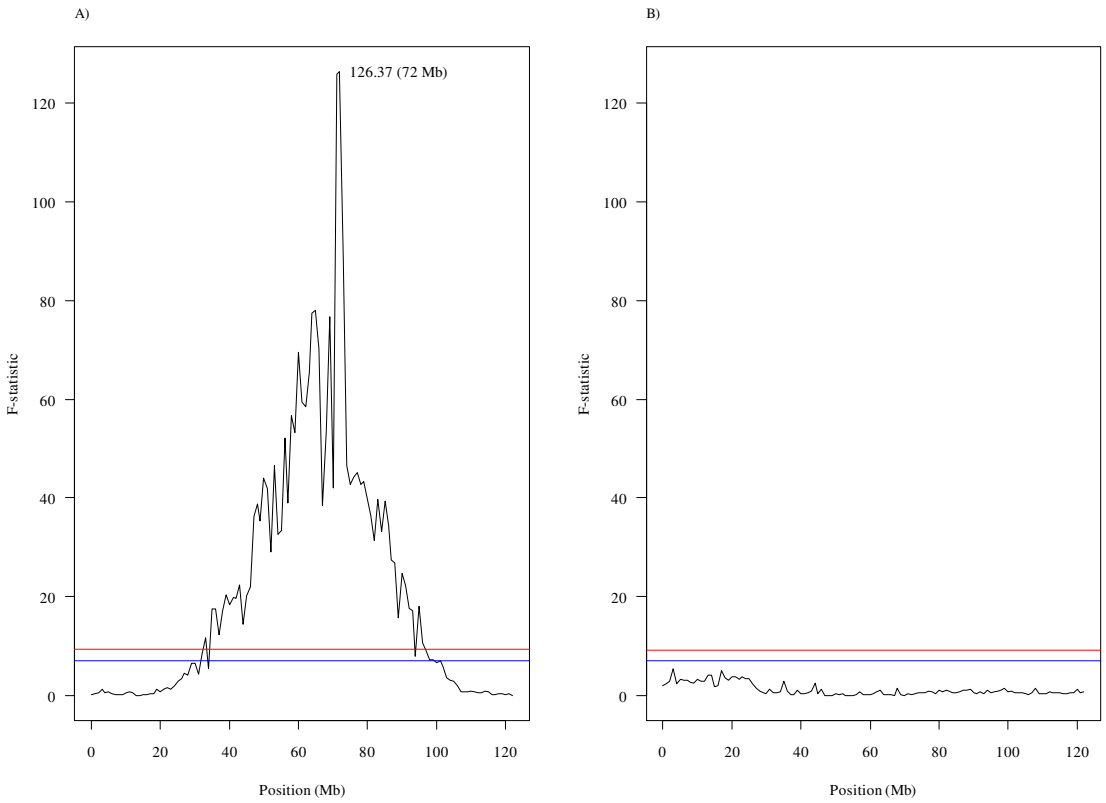


Figure 8. Interval analysis on BTA 6 for degree of black – complex utilizing only non-spotted $E^{D}E^{+}$ F_2 Nellore-Angus cattle. (A) Original model and (B) including *KIT* region breed of origin. Suggestive and significant chromosome-wide levels are represented by blue and red lines, respectively.

CHAPTER V

CONCLUSION

Suggestive and significant QTL at the chromosome-wide level were identified on BTA 4, 5, 15, 18, 21, 27, and 29 for the 5 scorings systems developed to investigate the reddening phenotype and there was evidence of a major gene on BTA 6 at 71 to 72 Mb. Based on the Degree of Black – Complex scoring system, which provides the greatest resolution for the reddening phenotype, 61.1% of the phenotypic variation is accounted for by this QTL. The most likely position of the QTL coincides with a cluster of tyrosine kinase receptors including *PDGFRA*, *KIT*, and *KDR*. Fitting breed of origin of the 1 Mb region containing these 3 genes in the model to produce residuals for mapping accounted for the variation attributed to the QTL. Therefore, one of these 3 genes, or a gene in high linkage disequilibrium with these genes, is responsible for the majority of the variation in reddening. Two recombinants within the F_2 E^DE^+ Nellore-Angus cattle identified *PDGFRA* as the strongest candidate gene for the reddening phenotype. Functional analyses will be required to verify the role of *PDGFRA* and its interaction with *MC1R* to modify black coat color of *Bos indicus* influenced cattle.

LITERATURE CITED

Bennett, D. and M. Lamoreux. 2003. The coat colour loci of mice – a genetic century. Pigment Cell Res. 16: 333-344.

Besmer, P., K. Manova, R. Duttlinger, E. J. Huang, A. Packer, C. Gyssler, and R. F.Bachvarova. 1993. The kit-ligand (steel factor) and its receptor c-kit/W: pleiotropic roles in gametogenesis and melanogenesis. Development Suppl. 1993: 125-137.

Broxmeyer, H. E., R. Maze, K. Miyazawa, C. Carow, P.C. Hendrie, S. Cooper, G. Hangoc, S. Vadhan-Raj, and L. Lu. 1991. The kit receptor and its ligand, steel factor, as regulators of hemopoiesis. Cancer Cells 3: 480-487.

Candille, S. I., C. B. Kaelin, B. M. Cattanach, B. Yu, D. A. Thompson, M. A. Nix, J. A. Kerns, S. M. Schmutz, G. L. Millhauser, and G. S. Barsh. 2007. A β-defensin mutation causes black coat color in domestic dogs. Science 318: 1418-1423.

Cheli, Y., M. Ohanna, R. Ballotti, and C. Bertolotto. 2009. 15-year quest in search for MITF target genes. Pigment Cell Melanoma Res. 23: 27-40.

Cone, R. D., D. Lu, S. Koppula, D. I. Vage, H. Klungland, B. Boston, W. Chen, D. N. Orth, C. Pouton and R. A Kesterson. 1996. The melanocortin receptors: agonists, antagonists, and the hormonal control of pigmentation. Recent Prog. Horm. Res. 51: 287-318.

Darvasi, A. and M. Soller. 1997. A simple method to calculate resolving power and confidence interval of QTL map location. Behav. Genet. 27: 125-132.

Dolinoy, D. C., J. R. Weidman, R. A. Waterland, and R. L. Jirtle. 2006. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. Environ. Health Perspect. 114: 567-572.

Dreger, D. and S. Schmutz. 2009. The variant red coat colour phenotype of Holstein cattle maps to BTA27. Anim. Genet. 41: 109-112.

Gilmore, L. O., N. S. Fechheimer, and C. S. Baldwin. 1961. Inheritance of black hair patterns in cattle lacking the extension factor for black (E). IV. Partitioning phenotypes by castration. Ohio J. Sci. 61: 273-277.

Girardot, M., J. Martin, S. Guibert, H. Levéziel, R. Julien, and A. Oulmouden. 2005. Widespread expression of the bovine Agouti gene results from at least three alternative promoters. Pigment Cell Res. 18: 34-41.

Girardot, M., S. Guibert, M.-P. Laforet, Y. Gallard, H. Larroque, and A. Oulmouden. 2006. The insertion of a full-length *Bos taurus* LINE element is responsible for a transcriptional deregulation of the Normande Agouti gene. Pigment Cell Res. 19: 346-355.

Graphodatskaya, D., H. Joerg, and G. Strazinger. 2000. Polymorphism in the MSHR gene of different cattle breeds. Veterinarni Medicina-UZPI (Czech Republic). 10-11: 290-295.

Graphodatskaya, D., H. Joerg, and G. Stranzinger. 2002. Molecular and pharmacological characterization of the MSH-r alleles in Swiss cattle breeds. J. Recept. Signal Transduct. Res. 22: 421-430.

Graphodatskaya, D., H. Joerg, M. Asai-Coakwell, F. Janett, and G. Stranzinger. 2006. Expression and function of agouti signaling protein in cattle. Anim. Sci. J. 77: 33-41.

Grosz, M. D. and M. D. MacNeil. 1999. The "Spotted" locus maps to bovine chromosome 6 in a Hereford-cross population. J. Hered. 90: 233-236.

Hearing, V., and K. Tsukamoto. 1991. Enzymatic control of pigmentation in mammals. FASEB J. 5: 2902-2909.

Herlyn, M., C. Berking, G. Li, and K. Satyamoorthy. 2000. Lessons from melanocyte development for understanding the biological events in naevus and melanoma formation. Melanoma Res. 10: 303-312.

Hoch, R. V. and P. Soriano. 2003. Roles of PDGF in animal development. Development 130: 4769-4784.

Ibsen, H. L. 1933 Cattle inheritance. I. Color. Genetics 18: 441-480.

Joerg, H., H. R. Fries, E. Meijerink, and G. F. Stranzinger. 1996. Red coat color in Holstein cattle is associated with a deletion in the *MSHR* gene. Mamm. Gen. 7: 317-318.

Karlsson, L., C. Bondjers, and C. Betsholtz. 1999. Roles for PDGF-A and sonic hedgehog in development of mesenchymal components of the hair follicle. Development 126: 2611-2621.

Klungland, H. and D. I. Våge. 1999. Presence of the dominant extension allele E^D in red mosaic cattle. Pigment Cell Res. 12: 391-393.

Klungland, H., H. G. Olsen, M. S. Hassanane, K. Mahrous, and D. I. Våge. 2000. Coat colour genes in diversity studies. J. Anim. Breed. Genet. 117: 217-224.

Klungland, H., D. I. Våge, L. Gomez-Raya, S. Adalsteinsson, and S. Lien. 1995. The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. Mamm. Gen. 6: 636-639.

Kwon, B. S., R. Halaban, S. Ponnazhagan, K. Kim, C. Chintamaneni, D. Bennett, and R. T. Pickard. 1995. Mouse silver mutation is caused by a single base insertion in the putative cytoplasmic domain of Pmel 17. Nucl. Acids Res. 23: 154-158.

Lu, D., D. Willard, I. R. Patel, S. Kadwell, L. Overton, T. Kost, M. Luther, W. Chen, R. P. Woychik. W. O. Wilkson, and R. D. Cone. 1994. Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. Nature 371: 799-802.

Lu, D., D. I. Våge, and R. D. Cone. 1998. A ligand-mimetic model for constitutive activation of the melanocortin-1 receptor. Molecular Endocrinology 12: 592-604.

Mason, H. S. 1948. The chemistry of melanin. III. Mechanism of the oxidation of dihydroxyphenylalanine by tryosinase. J. Biol. Chem. 172: 83-99.

Morgan, H. D., H. G. E. Sutherland, D. I. K. Martin, and E. Whitelaw. 1999. Epigenetic inheritance at the agouti locus in the mouse. Nat. Genet. 23: 314-318.

Prota, G. 1988. Progress in the chemistry of melanins and related metabolites. Med. Res. Rev. 8: 525-556.

Reinsch, N., H. Thomsen, N. Xu, M. Brink, C. Looft, E. Kalm, G. A. Brockmann, S. Grupe, C. Kühn, M. Schwerin, B. Leyhe, S. Hiendleder, G. Erhardt, I. Medjugorac, I. Russ, M. Förster, R. Reents, and G. Averdunk. 1999. A QTL for the degree of spotting in cattle shows synteny with the *KIT* locus on chromosome 6. J. Hered. 90: 629-634.

Rhoad, A. O. 1936. The silver gray color in Indian cattle. J. Hered. 27: 113-118.

Robbins, L. S., J. H. Nadeau, K. R. Johnson, M. A. Kelly, L. Roselli-Rehfuss, E. Baack, K. G. Mountjoy, and R. D. Cone. 1993. Pigmentation phenotypes of variant extension locus alleles result from point mutations that alter MSH receptor function. Cell 72: 827-834.

Rouzard, F., J. Martin, P. F. Gallet, D. Delourme, V. Goulemot-Leger, Y. Amigues, F. Menissier, H. Leveziel, R. Julein, A. Oulmouden. 2000. A first genotyping assay of French cattle breeds based on a new allele of the extension gene coding the melanocortin-1 receptor (Mc1r). Genet. Sel. Evol. 32: 511-520.

Royo, L. J., I. Alvarez, I. Fernandez, J. J. Arranz, E. Gomez, and F. Goyache. 2005. The coding sequence of the ASIP gene is identical in nine wild-type coloured cattle breeds. J. Anim. Breed. Gen. 122: 357-360.

Sakai, C., M. Ollmann, T. Kobayashi, Z. Abdel-Malek, J. Muller, W. D. Vieira, G. Imokawa, G. S. Barsh, and V. J. Hearing. 1997. Modulation of murine melanocyte function in vitro by agouti signal protein. EMBO J. 16: 3544-3552.

Scheet, P. and M. Stephens. 2006. A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. Am. J. Hum. Genet. 78: 629-644.

Seaton G., J. Hernandez, J. A. Grunchec, I. White, J. Allen, D. J. De Koning, W. Wei, D. Berry, C. Haley, S. Knott. 2006. GridQTL: A grid portal for QTL mapping of compute intensive datasets. Proceedings of the 8th World Congress on Genetics Applied to Livestock Production, August 13-18, 2006. Belo Horizonte, Brazil.

Seo, K., T. R. Mohanty, T. Choi, and I. Hwang. 2007. Biology of epidermal and hair pigmentation in cattle: a mini review. Vet. Dermatol. 18: 392-400.

Silver, D. L., L. Hou, and W. J. Pavan. 2006. The genetic regulation of pigment cell development. Page 155 in Advances in experimental medicine biology. J.-P. Saint-Jeannet, ed. Landes Bioscience and Springer Science+Business Media, Boston, MA.

Siracusa, L. D. 1991. Genomic organization and molecular genetics of the Agouti locus in the mouse. Ann. N. Y. Acad. Sci. 642: 419-430.

Soriano, P. 1997. The PDGFα receptor is required for neural crest cell development and for normal patterning of the somites. Development 124: 2691-2700.

Sun, H., D. J. Gilbert, N. G. Copeland, N. A. Jenkins, and J. Nathans. 1997. Peropsin, a novel visual pigment-like protein located in the apical microvilli of the retinal pigment epithelium. Proc. Natl. Acad. Sci. U. S. A. 94: 9893-9898.

Utz, H. F., A. E. Melchinger, and C. C. Schön. 2000. Bias and sampling error of the estimated proportion of genotypic variance explained by quantitative trait loci determined from experimental data in maize using cross validation and validation with independent samples. Genetics 154: 1839-1849.

Våge, D. I., D. Lu, H. Klungland, S. Lien, S. Adelsteinsson, and R. D. Cone. 1997. A non-epistatic interaction of *agouti* and *extension* in the fox, Vulpes vuples. Nat. Genet. 15: 311-315.

Vietor, I., R. Kurzbauer, G. Brosch, and L. A. Huber. 2005. TIS7 regulation of the beta-catenin/Tcf-4 target gene osteopontin (OPN) is histone deacetylase-dependent. J. Biol. Chem. 280: 39795-39801.

Voisey, J. and A. Van Daal. 2002. Agouti: From mouse to man, from skin to fat. Pigment Cell Res. 15: 10-18.

Willard, D. H., W. Bodnar, C. Harris, L. Kiefer, J. S. Nichols, S. Blanchard, C. Hoffman, M. Moyer, W. Burkhart, J. Weiel, M. A. Luther, W. O. Wilkison, and W. J. Rocque. 1995. Agouti structure and function: characterization of a potent α-melanocyte stimulating hormone receptor antagonist. Biochemistry 34: 12341-12346.

Wolff, G. L. 2003. Regulation of yellow pigment formation in mice: a historical perspective. Pigment Cell Res. 16: 2-15.

Yasumoto, K. K. Takeda, H. Saito, K. Watanabe, K. Takahashi, and S. Shibahara. 2002. Microphthalmia-associated transcription factor interacts with LEF-1, a mediator of Wnt signaling. EMBO J. 21(11): 2703-2714.

APPENDIX A

Investigation of Melanocortin-1 Receptor

Appendix Table A-1: Initial and novel *MC1R* genotypes in straightbred and crossbred cattle

Breed	Animal ID	Extension Genotype	C583T[1]	T663C
Bos indicus				
Brahman	1/8	$E^+ E^+$	T/T	C/C
	5/6	$E^+ E^+$	T/T	C/C
	358568	$E^+ E^+$	T/T	C/C
	459237	$E^+ E^+$	T/T	C/C
	506341	$E^+ E^+$	T/T	C/C
	670695	$E^+ E^+$	T/T	C/C
	958/9	$E^+ E^+$	T/T	C/C
	978/0	$E^+ E^+$	T/T	C/C
Gir	GIR14	$E^+ E^+$	T/T	C/C
	GIR16	$E^+ E^+$	T/T	C/C
	GIR18	$E^+ E^+$	T/T	C/C
	GIR20	$E^+ E^+$	T/T	C/C
	GIR21	$E^+ E^+$	T/T	C/C
Nellore	034/0	$E^+ E^+$	T/T	C/C
	172/9	$E^+ E^+$	T/T	C/C
	VASUVEDA	$E^+ E^+$	T/T	C/C
Bos taurus				
Angus	002E	$E^D E^+$	C/C	T/T
Bos indicus x Bos taurus				
F_1 Angus-Brahman	32T	$E^D E^+$	C/T	C/T
F_1 Nellore-Angus	410P	$E^+ E^+$	C/T	C/T
	429H	$E^D E^+$	C/T	C/T
	432H	$E^D E^+$	C/T	C/T
	511G	$E^D E^+$	C/T	C/T
	551G	$E^D E^+$	C/T	C/T

Appendix Table A-1: Continued

Breed	Animal ID	Extension Genotype	C583T[1]	T663C
F_1 Nellore-Angus	637H	$E^D E^+$	C/T	C/T
	664J	$E^D E^+$	C/T	C/T
	732H	$E^D E^+$	C/T	C/T
F_2 Nellore-Angus	7026	$E^D E^D$	C/C	T/T
	7103	$E^D E^D$	C/C	T/T
	7144	$E^D E^+$	C/T	C/T
	7202	$E^D E^+$	C/T	C/T
	7205	$E^D E^+$	C/T	C/T
	7216	$E^D E^+$	C/T	C/T
	7218	$E^D E^+$	C/T	C/T
	7226	$E^+ E^+$	T/T	C/C
	7234	$E^D E^+$	C/T	C/T
	7235	$E^+ E^+$	T/T	C/C
	7242	$E^D E^+$	C/T	C/T
	7244	$E^D E^+$	C/T	C/T
	7245	$E^D E^+$	C/T	C/T
	7246	$E^D E^+$	C/T	C/T
	8001	$E^D E^+$	C/T	C/T
	8010	$E^D E^+$	C/T	C/T
	8021	$E^D E^+$	C/T	C/T
	8026	$E^D E^+$	C/T	C/T
	8027	$E^D E^+$	C/T	C/T
	8035	$E^D E^+$	C/T	C/T
	8036	$E^D E^+$	C/T	C/T
	8067	$E^D E^+$	C/T	C/T
Nellore-Angus X F_2 Brahman Hereford	9519	$E^+ E^+$	T/T	C/C
F_2 Nellore-Angus	9605	$E^D E^+$	C/T	C/T
Nellore-Angus X F_2 Brahman Hereford	9804	$E^D E^+$	C/T	C/T

[1]A non-synonymous SNP that causes a leucine to phenylalanine amino acid change

Phenotype Scoring Results

Appendix Table A-2: Phenotypic scores for F_2 and natural service, half-sib E^DE^+ Nellore-Angus cattle on all scoring systems

Animal ID	Sex	Majority Black	Red Present	Degree of Black - Simple	Degree of Black - Complex	Degree of Darkness
7001	F	2	1	3	8	4
7004	S	2	2	3	9	4
7005	S	2	2	3	9	4
7006	F	2	2	3	8	4
7007	S	2	2	3	9	4
7011	S	2	1	3	7	4
7012	F	2	2	3	9	4
7013	F	2	1	3	8	4
7014	F	1	1	1	3	2
7019	S	2	1	3	7	3
7021	S	2	2	3	9	4
7024	S	1	1	1	3	3
7025	F	2	2	3	9	4
7030	F	1	1	2	5	3
7031	S	2	2	3	9	4
7102	S	2	2	3	9	4
7105	S	2	1	3	8	4
7107	F	2	1	3	8	3
7112	S	2	2	3	9	4
7114	F	2	1	3	7	3
7115	S	2	1	3	8	4
7117	S	2	2	3	9	4
7120	F	2	1	3	8	4
7121	F	2	2	3	9	4
7123	S	2	1	3	8	4
7124	F	2	1	3	8	4
7129	F	2	1	3	8	4
7133	F	2	1	2	6	2
7135	S	2	2	3	9	4
7140	F	2	2	3	9	4
7141	F	2	1	3	8	4
7145	F	2	1	3	7	3

Appendix Table A-2: Continued

Animal ID	Sex	Majority Black	Red Present	Degree of Black - Simple	Degree of Black - Complex	Degree of Darkness
7147	F	2	1	2	6	3
7148	F	2	2	3	9	4
7149	S	2	2	3	9	4
7155	S	2	2	3	9	4
7156	F	2	2	3	9	4
7164	F	2	1	3	7	3
7168	S	2	2	3	9	4
7201	F	1	1	1	3	2
7202	S	2	1	2	6	3
7205	F	1	1	1	2	2
7210	F	1	1	1	3	2
7211	F	1	1	2	4	3
7212	F	2	1	3	8	3
7216	S	2	1	3	7	3
7218	F	1	1	2	5	2
7220	F	2	1	3	8	3
7221	F	2	1	2	4	3
7228	S	2	1	3	8	4
7229	S	2	1	3	8	4
7230	S	1	1	2	4	3
7233	S	2	1	3	8	4
7234	S	2	1	3	7	3
7239	S	1	1	2	5	2
7242	S	1	1	1	1	1
7244	F	2	2	3	9	4
7245	F	2	1	3	7	3
7246	F	2	2	3	9	4
7302	S	2	1	3	8	4
7304	F	2	2	3	8	4
7307	F	2	2	3	9	4
7308	S	2	2	3	9	4
7403	F	1	1	1	2	2
7504	S	2	1	3	8	4
7505	F	2	1	3	7	3
7508	F	2	2	3	7	3
7509	S	2	2	3	9	4
7511	S	1	1	1	3	2

Appendix Table A-2: Continued

Animal ID	Sex	Majority Black	Red Present	Degree of Black - Simple	Degree of Black - Complex	Degree of Darkness
7512	F	2	1	3	8	4
7513	F	2	1	3	7	3
7515	F	2	1	3	7	3
7516	F	2	2	3	9	4
7517	F	2	2	3	9	4
7518	S	1	1	2	4	3
7520	F	2	2	3	9	4
7523	S	2	2	3	9	4
7525	F	2	1	2	6	3
7526	S	2	1	3	7	3
7531	S	2	1	3	8	4
7532	F	1	1	1	3	3
7536	F	2	1	3	8	3
7540	F	2	1	3	8	4
7542	F	2	1	3	8	4
7543	F	2	1	2	6	3
7601	S	2	1	3	8	4
7605	F	2	1	3	8	4
7606	S	2	1	3	8	4
7609	S	2	2	3	9	4
7703	F	2	2	3	9	4
7707	S	2	2	3	9	4
7709	S	2	2	3	9	4
7712	S	2	1	3	8	3
7713	F	1	1	1	3	3
7714	F	1	1	1	3	2
7715	S	2	1	3	8	4
7716	S	2	2	3	9	4
7717	S	2	1	3	7	3
7718	F	1	1	1	2	2
7720	F	1	1	1	1	2
7721	F	2	1	3	8	4
7722	S	2	2	3	9	4
7724	S	2	2	3	9	4
7726	S	1	1	2	5	2
7729	S	2	1	2	6	3
7730	S	2	1	3	8	4

Appendix Table A-2: Continued

Animal ID	Sex	Majority Black	Red Present	Degree of Black - Simple	Degree of Black - Complex	Degree of Darkness
7731	S	1	1	2	5	3
7735	F	2	1	3	8	4
7738	S	2	1	3	8	4
7742	S	2	1	3	8	3
7902	S	2	2	3	9	4
8001	S	1	1	2	4	2
8002	F	2	1	3	7	3
8005	S	2	2	3	9	4
8007	S	2	2	3	9	4
8008	S	2	1	3	8	3
8009	F	2	1	3	8	3
8010	S	2	1	2	5	3
8011	F	1	1	1	1	1
8012	F	2	1	3	8	4
8013	S	2	2	3	9	4
8015	S	1	1	1	1	2
8021	F	1	1	1	1	2
8023	F	2	1	3	8	3
8024	S	2	1	3	8	4
8025	S	2	1	3	8	3
8026	S	2	2	3	9	4
8027	F	1	1	1	2	2
8028	F	2	1	3	8	4
8030	S	1	1	1	2	2
8031	F	2	1	3	8	4
8035	S	2	2	3	9	4
8036	S	2	2	3	9	4
8037	S	2	1	3	8	3
8040	S	2	2	3	9	4
8042	S	2	2	3	9	4
8044	S	2	1	3	8	4
8045	S	2	1	3	8	4
8051	S	2	1	3	8	3
8052	S	2	2	3	9	4
8053	S	2	1	3	7	3
8056	S	1	1	2	4	3
8057	S	2	2	3	9	4

Appendix Table A-2: Continued

Animal ID	Sex	Majority Black	Red Present	Degree of Black - Simple	Degree of Black - Complex	Degree of Darkness
8067	S	2	1	3	7	3
8069	F	2	2	3	9	4
8070	S	2	2	3	9	4
8107	F	2	1	2	5	3
8108	S	2	1	3	8	3
8109	F	2	1	3	8	4
8111	S	2	2	3	9	4
8114	F	2	1	3	7	3
8115	S	1	1	1	2	3
8117	S	1	1	1	2	3
8122	S	2	1	2	5	3
8124	S	2	2	3	9	4
8127	F	2	1	2	6	3
8128	S	2	1	3	7	3
8130	S	2	1	3	8	4
8134	S	2	1	3	8	4
8138	F	1	1	2	4	2
8140	S	2	1	3	7	3
8141	S	2	1	3	7	3
8142	S	2	1	3	8	3
8144	S	1	1	1	1	2
8145	F	2	1	3	7	3
8148	S	2	2	3	9	4
8150	S	2	1	3	8	4
8154	B	2	1	3	8	4
8159	S	2	1	2	6	3
8162	F	2	1	2	5	3
8163	F	1	1	2	5	2
8164	S	2	2	3	9	4
8165	F	2	1	3	8	4
8167	F	1	1	2	5	2
8204	S	2	1	3	8	4
8210	F	2	2	3	9	4
8212	S	2	2	3	9	4
8213	B	2	2	3	9	4
8215	S	2	1	3	8	4
8303	S	1	1	1	2	2

Appendix Table A-2: Continued

Animal ID	Sex	Majority Black	Red Present	Degree of Black - Simple	Degree of Black - Complex	Degree of Darkness
8304	S	2	1	2	6	3
8305	S	2	1	3	8	4
8306	S	2	2	3	9	4
8308	S	2	1	3	7	3
8310	F	1	1	2	4	3
8315	S	2	1	3	7	3
8316	F	1	1	1	1	2
8320	F	1	1	1	2	2
8321	F	2	1	2	5	2
8322	F	1	1	1	2	1
8326	S	2	2	3	9	4
8327	F	2	1	3	8	4
8332	B	2	2	3	9	4
8333	S	2	1	3	8	4
8334	S	2	1	3	7	3
8337	S	1	1	1	1	2
8338	F	2	1	2	5	3
8339	F	1	1	1	3	2
8401	F	2	1	3	8	4
8402	F	2	1	3	8	3
8404	S	2	2	3	9	4
8406	F	1	1	1	2	2
8411	F	2	1	3	8	4
8414	S	2	2	3	9	4
8416	F	2	1	3	8	3
8417	F	2	2	3	9	4
8418	S	2	2	3	9	4
8419	S	1	1	1	3	3
8420	S	2	1	3	8	4
8422	F	2	1	3	8	4
8425	F	2	2	3	9	4
8426	F	2	2	3	9	4
8427	S	1	1	1	2	2
8428	B	2	1	3	7	3
8429	S	2	1	3	8	4
9506	S	2	1	3	8	3
9510	S	2	2	3	9	4

Appendix Table A-2: Continued

Animal ID	Sex	Majority Black	Red Present	Degree of Black - Simple	Degree of Black - Complex	Degree of Darkness
9511	S	2	2	3	9	4
9515	S	2	1	3	8	3
9516	F	2	1	2	5	3
9520	F	2	2	3	9	4
9521	S	2	2	3	9	4
9525	S	2	2	3	9	4
9526	F	1	1	2	4	2
9533	S	2	2	3	9	4
9537	F	2	2	3	9	4
9544	F	1	1	1	1	2
9545	F	2	2	3	9	4
9547	S	2	1	3	8	4
9548	S	2	1	3	8	4
9550	S	2	1	2	6	3
9556	S	2	1	3	8	4
9557	S	2	2	3	9	4
9558	F	2	1	2	6	2
9566	F	2	2	3	9	4
9605	S	1	1	1	2	2
9615	F	2	1	3	8	3
9618	S	2	1	2	6	3
9623	S	2	1	3	8	4
9629	F	2	2	3	9	4
9630	F	2	2	3	9	4
9632	S	2	1	3	8	4
9636	F	2	1	3	8	4
9639	F	2	1	3	7	3
9642	F	2	1	2	6	3
9648	S	2	2	3	9	4
9654	F	2	2	3	9	4
9657	F	2	1	3	8	4
9664	F	1	1	1	2	2
9665	F	2	1	3	8	4
9666	F	2	1	3	8	3
9674	S	2	1	3	8	4
9676	S	1	1	1	1	1
9678	F	2	1	3	8	4

Appendix Table A-2: Continued

Animal ID	Sex	Majority Black	Red Present	Degree of Black - Simple	Degree of Black - Complex	Degree of Darkness
9679	F	2	1	3	8	3
9680	F	1	1	1	2	2
9682	S	2	1	3	8	4
9688	S	2	1	3	8	4
9690	F	1	1	1	3	2
9691	F	2	2	3	9	4
9692	F	2	2	3	9	4
9693	S	2	1	3	7	3
9697	S	2	1	3	8	4
9698	S	2	1	3	8	4
9705	S	2	1	3	7	3
9706	S	2	1	3	8	4
9710	F	2	1	2	5	3
9713	S	2	1	3	8	4
9717	S	1	1	1	2	3
9718	S	2	2	3	9	4
9719	S	2	1	2	6	3
9720	S	2	1	3	8	4
9721	S	2	1	2	6	3
9722	F	2	1	3	7	3
9724	F	2	1	3	7	3
9728	F	2	2	3	9	4
9732	F	2	2	3	9	4
9733	S	2	1	3	8	4
9735	F	2	2	3	9	4
9736	S	2	1	3	8	4
9741	F	2	2	3	9	4
9749	F	2	1	3	7	3
9752	S	2	2	3	9	4
9753	S	2	1	2	6	3
9755	F	2	2	3	9	4
9804	F	2	1	3	8	3
9807	F	2	2	3	9	4
9810	S	2	1	3	7	3
9813	F	2	1	3	8	4
9816	S	2	1	3	8	4
96100	S	1	1	1	3	2

Appendix Table A-2: Continued

Animal ID	Sex	Majority Black	Red Present	Degree of Black - Simple	Degree of Black - Complex	Degree of Darkness
96103	S	2	2	3	9	4
96107	F	2	1	3	8	3
96110	F	2	2	3	9	4
96114	F	1	1	1	3	2
96116	S	2	1	3	8	4
96117	S	1	1	1	3	1
96118	F	2	1	3	8	4
96119	F	2	1	3	8	4

APPENDIX B

Least Squares Means and Standard Errors for KIT Region Breed of Origin

Appendix Table B-1. Least squares means and standard errors by sire for majority black, degree of black - simple, degree of black - complex, and degree of darkness with *KIT* region included in model using E^DE^+ F_2 Nellore-Angus cattle

Sire	Majority black[1]	Degree of black – simple[2]	Degree of black – complex[3]	Degree of darkness[4]
297J	1.917 ± 0.046[a]	2.811 ± 0.082[a]	7.885 ± 0.243[a]	3.698 ± 0.090[a]
432H	1.808 ± 0.073[a,b]	2.684 ± 0.130[a]	7.164 ± 0.387[a,b]	3.503 ± 0.143[a]
437J	1.657 ± 0.073[b]	2.263 ± 0.131[b]	5.812 ± 0.391[c]	3.080 ± 0.144[b]
551G	1.815 ± 0.050[a,b]	2.640 ± 0.090[a]	7.112 ± 0.269[b]	3.497 ± 0.099[a]

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[4]Ranges from light (1) to extremely dark (4).
[a,b,c] Within a column, means without a common superscript differ ($P < 0.05$).

Appendix Table B-2. Least squares means and standard errors by sex for majority black, degree of black - simple, degree of black - complex, and degree of darkness with *KIT* region included in model using E^DE^+ F_2 Nellore-Angus cattle

Sex	Majority black[1]	Degree of black – simple[2]	Degree of black – complex[3]	Degree of darkness[4]
Female (F)	1.760 ± 0.039[a]	2.496 ± 0.069[a]	6.638 ± 0.207[a]	3.296 ± 0.076[a]
Steer (S)	1.839 ± 0.042[b]	2.702 ± 0.075[b]	7.348 ± 0.223[b]	3.593 ± 0.082[b]

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[4]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Appendix Table B-3. Least squares means and standard errors by season of photograph for majority black, degree of black - simple, degree of black - complex and degree of darkness with *KIT* region included in model using E^DE^+ F_2 Nellore-Angus cattle

Season of photograph	Majority black[1]	Degree of black – simple[2]	Degree of black – complex[3]	Degree of darkness[4]
Spring/Summer (S)	1.750 ± 0.039[a]	2.480 ± 0.069[a]	6.642 ± 0.206[a]	6.642 ± 0.206[a]
Fall/Winter (W)	1.849 ± 0.042[b]	2.718 ± 0.075[b]	7.344 ± 0.225[b]	7.344 ± 0.225[b]

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[4]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Appendix Table B-4. Least squares means and standard errors by family nested within sire for majority black, degree of black - simple, degree of black - complex, and degree of darkness with *KIT* region included in model using E^DE^+ F_2 Nellore-Angus cattle

Sire	Family	Majority black[1]	Degree of black – simple[2]	Degree of black – complex[3]	Degree of darkness[4]
297J	70	1.808 ± 0.070	2.666 ± 0.124	7.509 ± 0.371	3.654 ± 0.137
	71	2.026 ± 0.055	2.956 ± 0.098	8.260 ± 0.294	3.743 ± 0.108
432H	72	1.792 ± 0.067	2.566 ± 0.120	6.488 ± 0.359	3.146 ± 0.132
	73	1.888 ± 0.156	2.876 ± 0.278	7.826 ± 0.829	3.847 ± 0.306
	82	1.744 ± 0.136	2.610 ± 0.243	7.177 ± 0.724	3.514 ± 0.267
437J	74	1.081 ± 0.267	1.248 ± 0.478	2.763 ± 1.425	2.37 ± 0.526
	75	1.984 ± 0.064	2.910 ± 0.115	8.045 ± 0.342	3.702 ± 0.126
	81	1.877 ± 0.058	2.657 ± 0.104	6.940 ± 0.309	3.365 ± 0.114
	83	1.686 ± 0.067	2.236 ± 0.119	5.503 ± 0.355	2.880 ± 0.131
551G	76	1.866 ± 0.138	2.749 ± 0.246	7.366 ± 0.733	3.793 ± 0.270
	77	1.730 ± 0.061	2.502 ± 0.110	6.751 ± 0.328	3.342 ± 0.121
	80	1.840 ± 0.054	2.678 ± 0.097	7.030 ± 0.288	3.308 ± 0.106
	84	1.824 ± 0.075	2.629 ± 0.134	7.303 ± 0.400	3.544 ± 0.148

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[4]Ranges from light (1) to extremely dark (4).

Table B-5. Least squares means and standard errors by spotted status for majority black, degree of black - simple, and degree of black - complex with *KIT* region included in model using F_2 E^DE^+ Nellore-Angus cattle

Spotted status	Majority black[1]	Degree of black – simple[2]	Degree of black – complex[3]
No (1)	1.713 ± 0.032[a]	2.448 ± 0.057[a]	6.596 ± 0.170[a]
Yes (2)	1.886 ± 0.060[b]	2.751 ± 0.108[b]	7.391 ± 0.322[b]

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Least Squares Means and Standard Errors for Phased Genotypes of KIT Region

Appendix Table B-6. Least squares means and standard errors by sex for majority black, degree of black - simple, degree of black - complex, and degree of darkness with phased genotypes for the *KIT* region included in the model using E^DE^+ F_2 Nellore-Angus cattle

Sex	Majority black[1]	Degree of black – simple[2]	Degree of black – complex[3]	Degree of darkness[4]
Female (F)	1.801 ± 0.037[a]	2.559 ± 0.065[a]	6.790 ± 0.197[a]	3.266 ± 0.072[a]
Steer (S)	1.881 ± 0.038[b]	2.750 ± 0.067[b]	7.475 ± 0.202[b]	3.530 ± 0.074[b]

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[4]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Appendix Table B-7. Least squares means and standard errors by season of photograph for degree of black - simple, degree of black - complex, and degree of darkness with phased genotypes for the *KIT* region included in the model using E^DE^+ F_2 Nellore-Angus cattle

Season of photograph	Degree of black – simple[1]	Degree of black – complex[2]	Degree of darkness[3]
Spring/Summer (S)	2.564 ± 0.063[a]	6.856 ± 0.189[a]	3.289 ± 0.069[a]
Fall/Winter (W)	2.745 ± 0.069[b]	7.409 ± 0.208[b]	3.506 ± 0.076[b]

[1]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[2]Ranges from light (1) to solid (9) amount of black pigmentation present.
[3]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Appendix Table B-8. Least squares means and standard errors by spotted status for majority black, degree of black - simple, and degree of black - complex with phased genotypes for the *KIT* region included in the model using E^DE^+ F_2 Nellore-Angus cattle

Spotted status	Majority black[1]	Degree of black – simple[2]	Degree of black – complex[3]
No (1)	1.747 ± 0.027[a]	2.493 ± 0.049[a]	6.683 ± 0.147[a]
Yes (2)	1.934 ± 0.059[b]	2.817 ± 0.106[b]	7.582 ± 0.319[b]

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Least Squares Means and Standard Errors for Non-spotted E^DE^+ F_2 Nellore-Angus Cattle

Appendix Table B-9. Least squares means and standard error by sex for all scoring systems using only non-spotted E^DE^+ F_2 Nellore-Angus cattle

Sex	Majority black[1]	Red present[2]	Degree of black - simple[3]	Degree of black - complex[4]	Degree of darkness[5]
Female (F)	1.757 ± 0.051[a]	1.303 ± 0.066[a]	2.521 ± 0.093[a]	6.789 ± 0.300[a]	3.374 ± 0.094[a]
Steer (S)	1.924 ± 0.049[b]	1.465 ± 0.063[b]	2.811 ± 0.088[b]	7.839 ± 0.286[b]	3.724 ± 0.090[b]

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from 1 to 2, where 1 has red present and 2 does not have red present
[3]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[4]Ranges from light (1) to solid (9) amount of black pigmentation present.
[5]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Appendix Table B-10. Least squares means and standard error by sire for red present, degree of black - complex, and degree of darkness using only non-spotted E^DE^+ F_2 Nellore-Angus cattle

Sire	Red present[1]	Degree of black - complex[2]	Degree of darkness[3]
297J	1.569 ± 0.087[a]	8.202 ± 0.398[a]	3.805 ± 0.125[a]
432H	1.462 ± 0.116[a,b]	7.765 ± 0.530[a]	3.707 ± 0.167[a]
437J	1.202 ± 0.130[b]	5.956 ± 0.595[b]	3.156 ± 0.188[b]
551G	1.303 ± 0.073[b]	7.332 ± 0.334[a]	3.529 ± 0.105[a,b]

[1]Ranges from 1 to 2, where 1 has red present and 2 does not have red present
[2]Ranges from light (1) to solid (9) amount of black pigmentation present.
[3]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

Appendix Table B-11. Least squares means and standard error by season of photograph for majority black, degree of black - simple, degree of black - complex, and degree of darkness using only non-spotted E^DE^+ F_2 Nellore-Angus cattle

Season of photograph	Majority black[1]	Degree of black - simple[2]	Degree of black - complex[3]	Degree of darkness[4]
Spring/Summer (S)	1.756 ± 0.048[a]	2.493 ± 0.087[a]	6.722 ± 0.282[a]	3.338 ± 0.089[a]
Fall/Winter (W)	1.925 ± 0.053[b]	2.839 ± 0.095[b]	7.905 ± 0.307[b]	3.760 ± 0.097[b]

[1]Ranges from 1 to 2, where 1 does not have a majority of black pigmentation and 2 does.
[2]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[3]Ranges from light (1) to solid (9) amount of black pigmentation present.
[4]Ranges from light (1) to extremely dark (4).
[a,b] Within a column, means without a common superscript differ ($P < 0.05$).

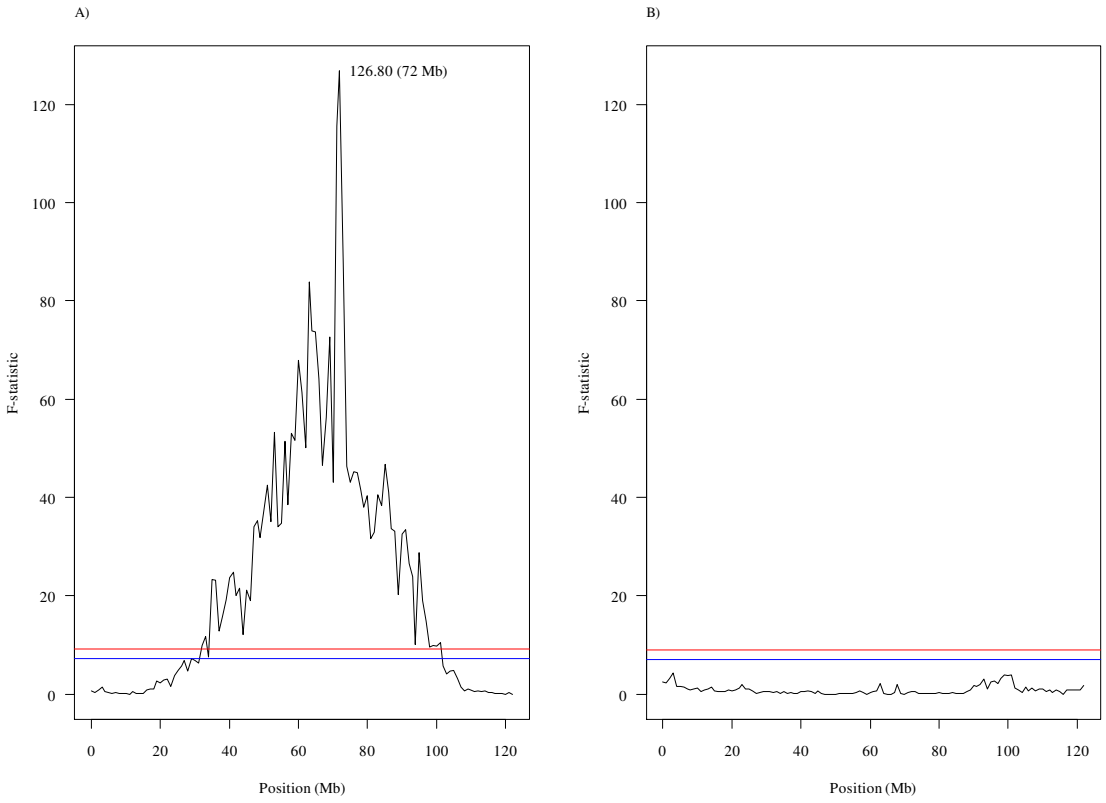
Appendix Table B-12. Least squares means and standard error by family nested within sire for degree of black - simple, degree of black - complex, and degree of darkness using only non-spotted E^DE^+ F_2 Nellore-Angus cattle

Sire	Family	Degree of black – simple[1]	Degree of black – complex[2]	Degree of darkness[3]
297J	70	2.758 ± 0.194	7.882 ± 0.629	3.654 ± 0.198
	71	2.999 ± 0.152	8.523 ± 0.492	3.955 ± 0.155
432H	72	2.636 ± 0.186	6.853 ± 0.602	3.314 ± 0.190
	73	3.086 ± 0.322	8.796 ± 1.044	4.106 ± 0.329
	82	2.755 ± 0.327	7.646 ± 1.060	3.701 ± 0.334
437J	74	1.318 ± 0.648	3.117 ± 2.096	2.386 ± 0.661
	75	3.173 ± 0.268	9.092 ± 0.867	4.211 ± 0.273
	81	2.465 ± 0.132	6.308 ± 0.429	3.134 ± 0.135
	83	2.151 ± 0.161	5.306 ± 0.522	2.894 ± 0.164
551G	76	3.014 ± 0.323	8.283 ± 1.047	4.018 ± 0.330
	77	2.607 ± 0.156	7.194 ± 0.504	3.386 ± 0.159
	80	2.534 ± 0.115	6.696 ± 0.373	3.209 ± 0.118
	84	2.578 ± 0.162	7.156 ± 0.524	3.505 ± 0.165

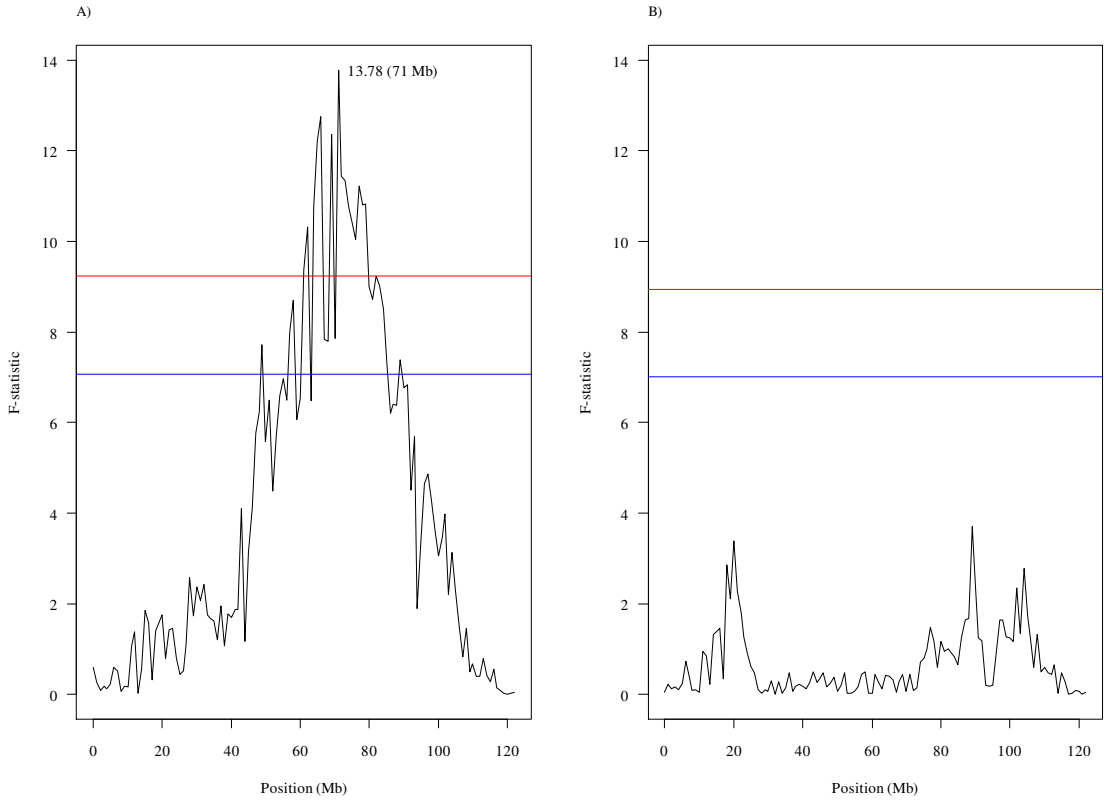
[1]Ranges from light (1) to abundant or solid (3) amount of black pigmentation present.
[2]Ranges from light (1) to solid (9) amount of black pigmentation present.
[3]Ranges from light (1) to extremely dark (4).

APPENDIX C

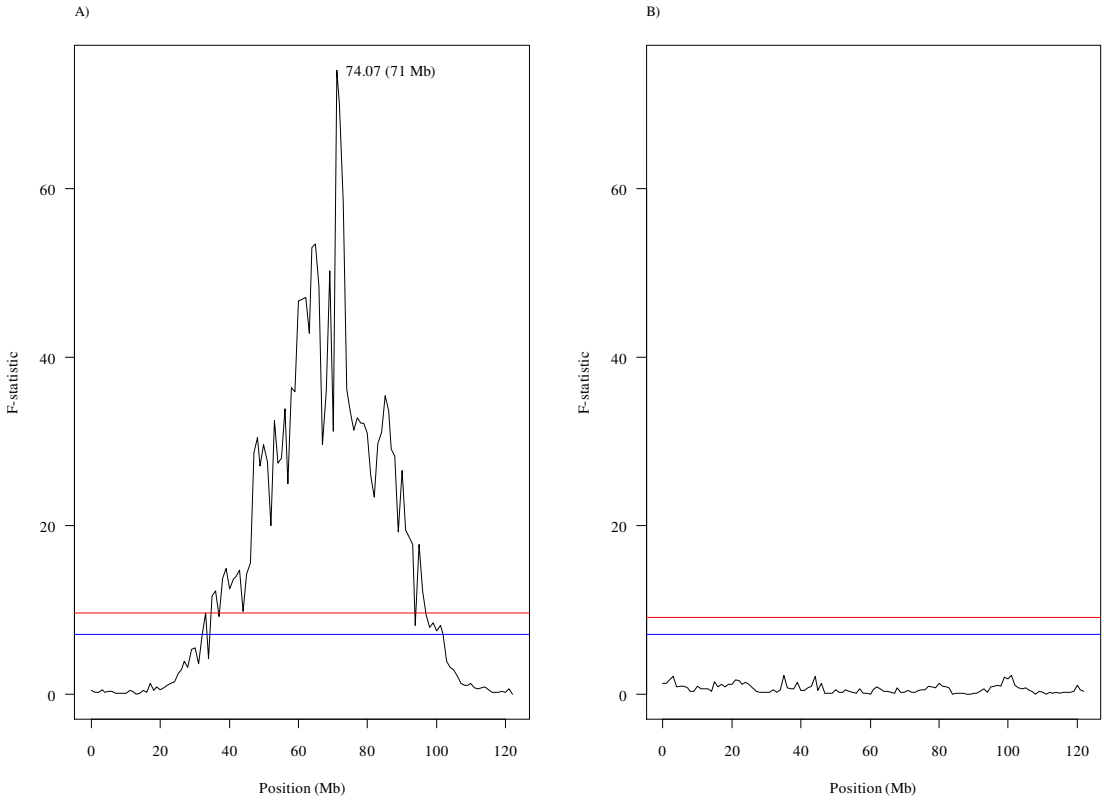
Interval Analysis of Non-spotted E^DE^+ F_2 Nellore-Angus Cattle



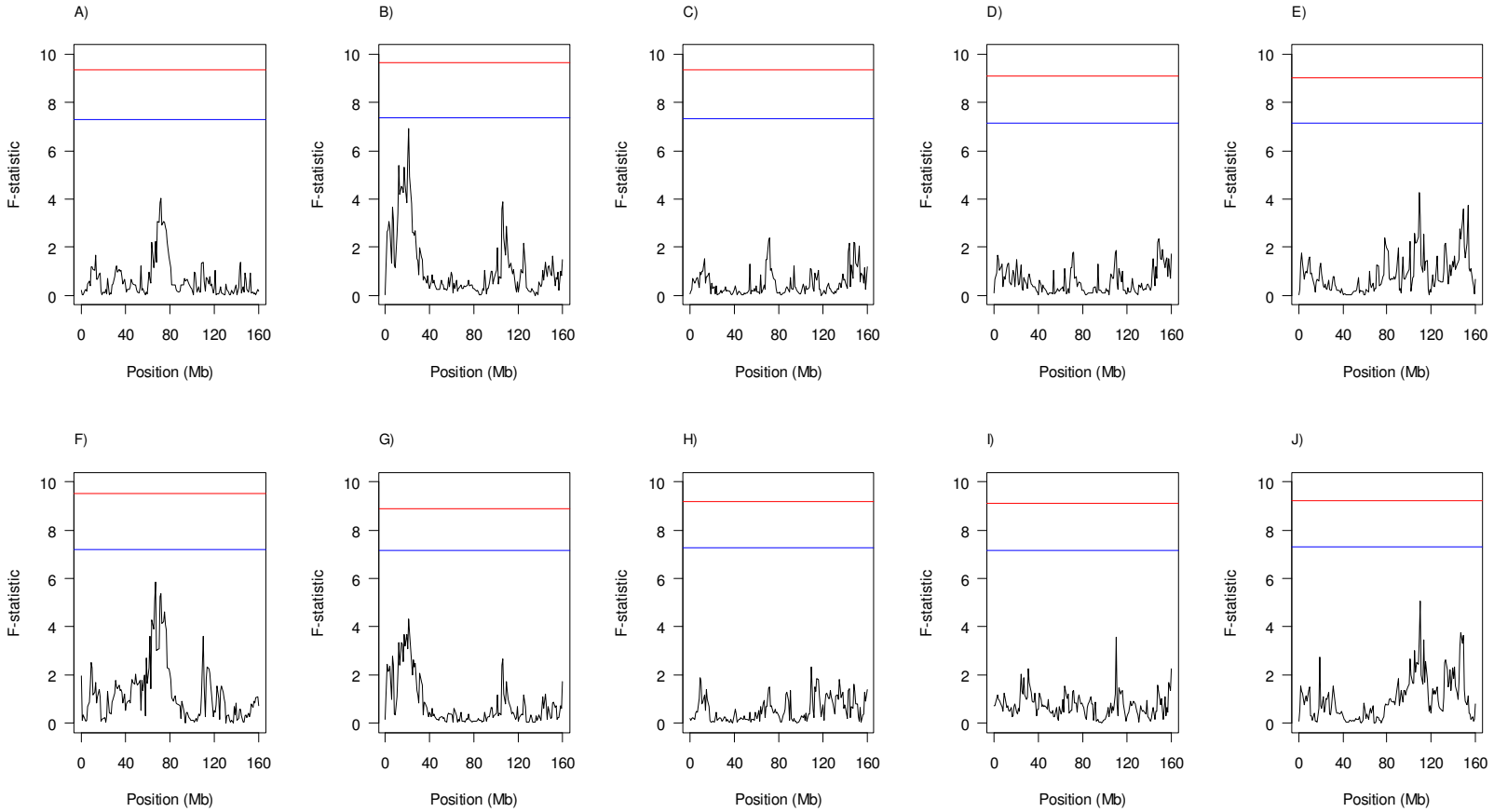
Appendix Figure C-1. Interval analysis on BTA 6 for majority black utilizing only non-spotted E^DE^+ F_2 Nellore-Angus cattle. (A) Original model and (B) including *KIT* region breed of origin. Suggestive and significant chromosome-wide levels are represented by blue and red lines, respectively.



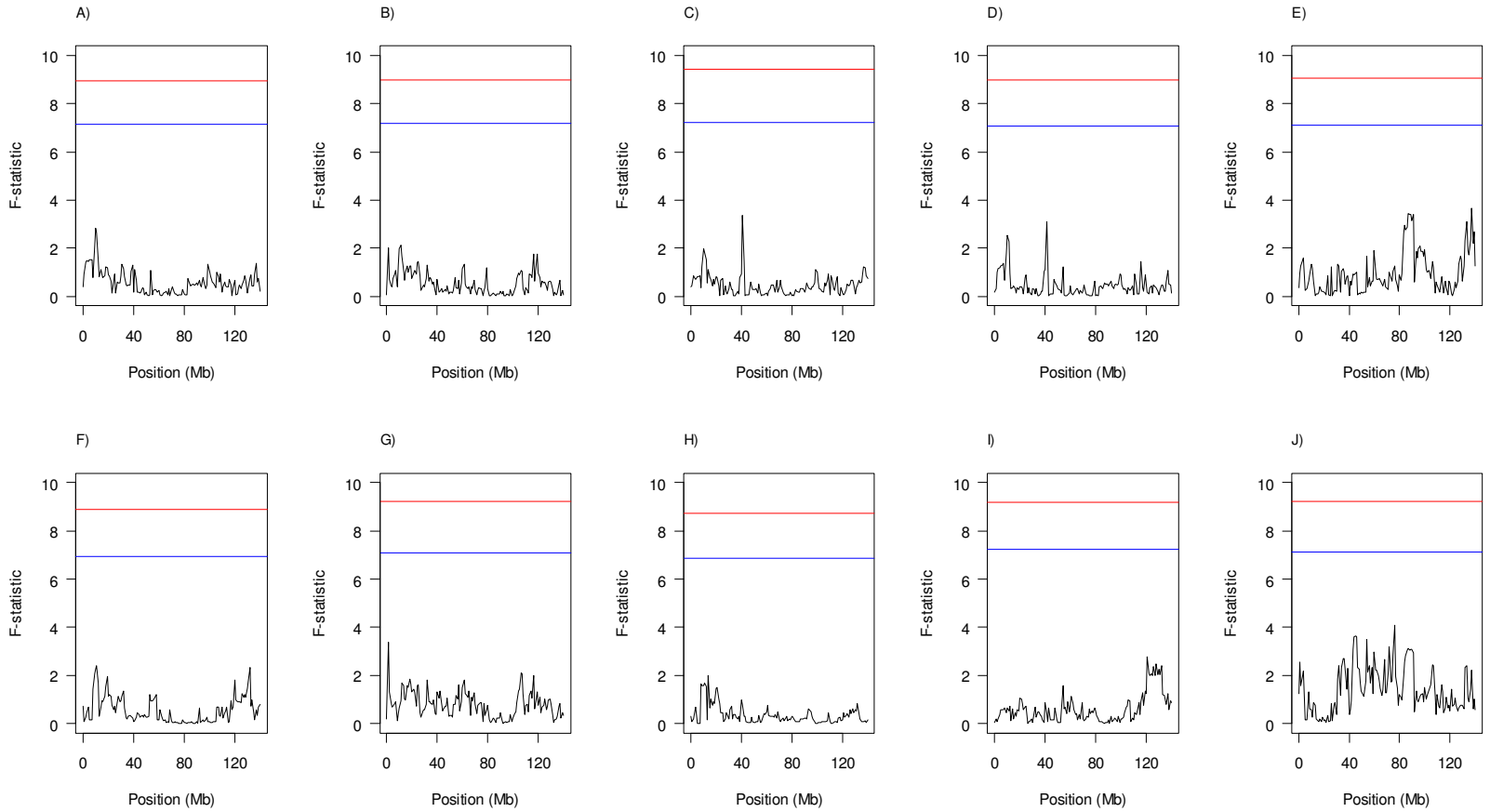
Appendix Figure C-2. Interval analysis on BTA 6 for red present utilizing only non-spotted $E^{D}E^{+}$ F_2 Nellore-Angus cattle. (A) Original model and (B) including *KIT* region breed of origin. Suggestive and significant chromosome-wide levels are represented by blue and red lines, respectively.



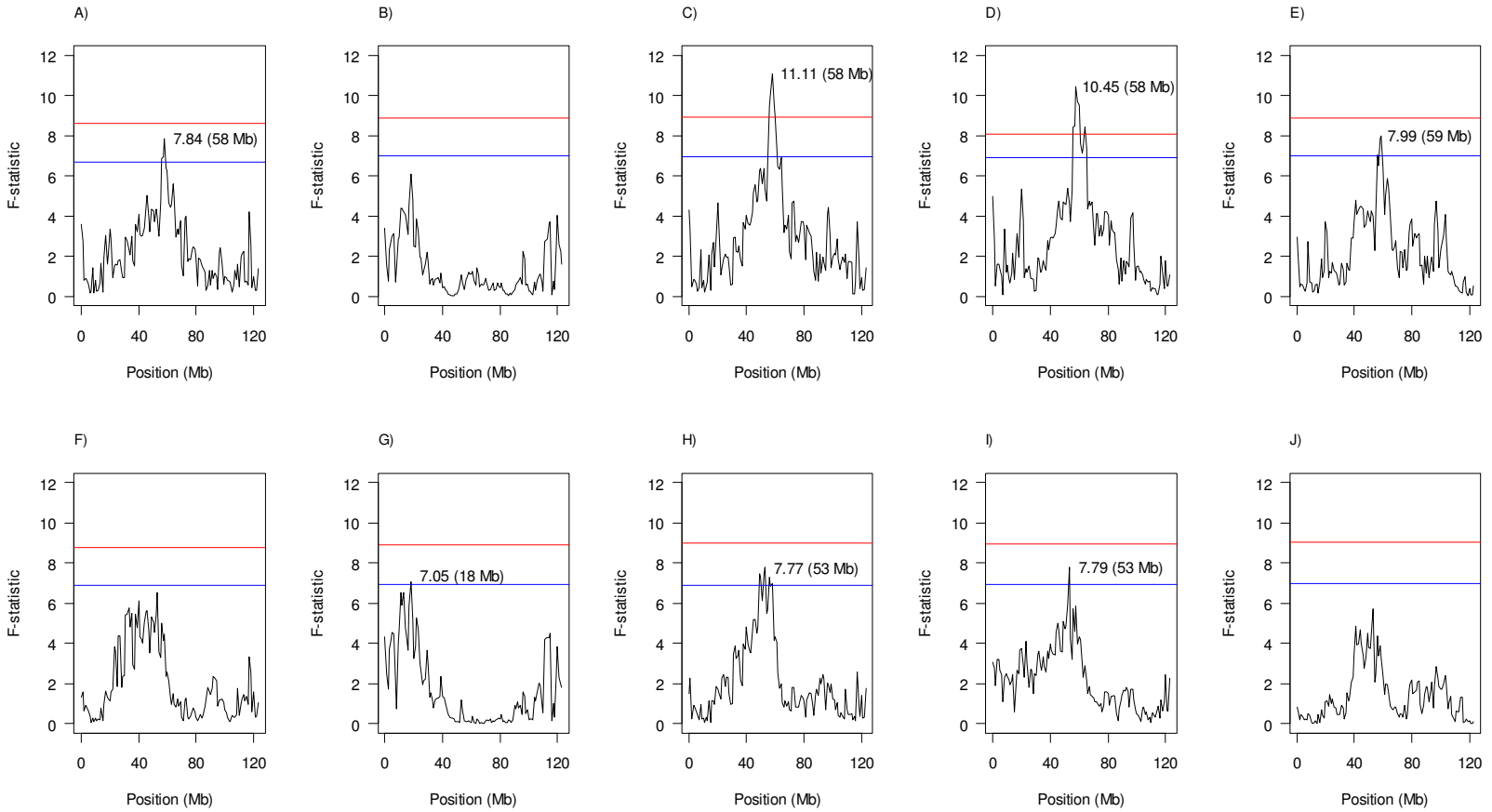
Appendix Figure C-3. Interval analysis on BTA 6 for degree of darkness utilizing only non-spotted $E^{D}E^{+}$ F_2 Nellore-Angus cattle. (A) Original model and (B) including *KIT* region breed of origin. Suggestive and significant chromosome-wide levels are represented by blue and red lines, respectively.



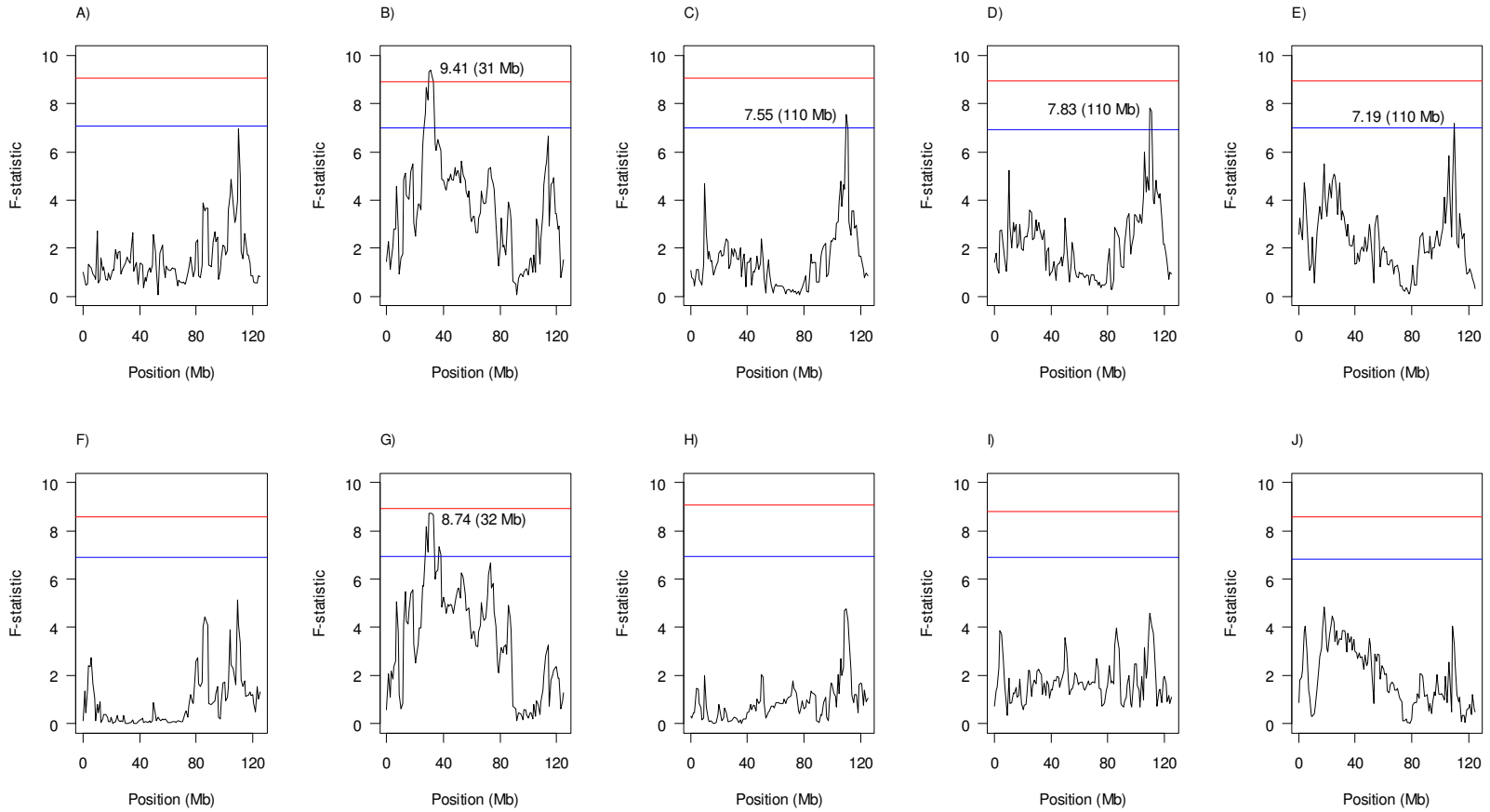
Appendix Figure C-4. Interval analysis for BTA 1. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



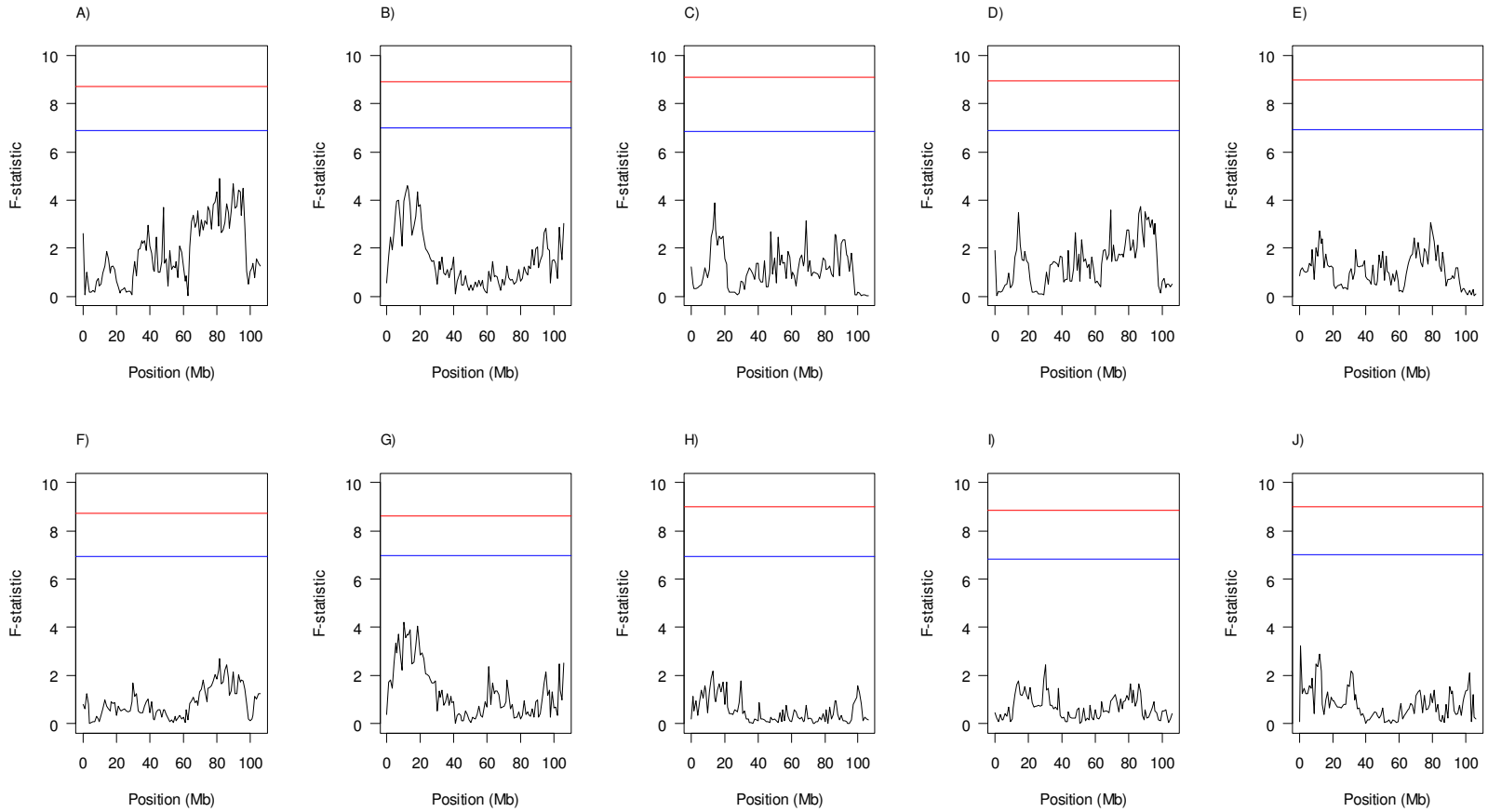
Appendix Figure C-5. Interval analysis for BTA 2. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



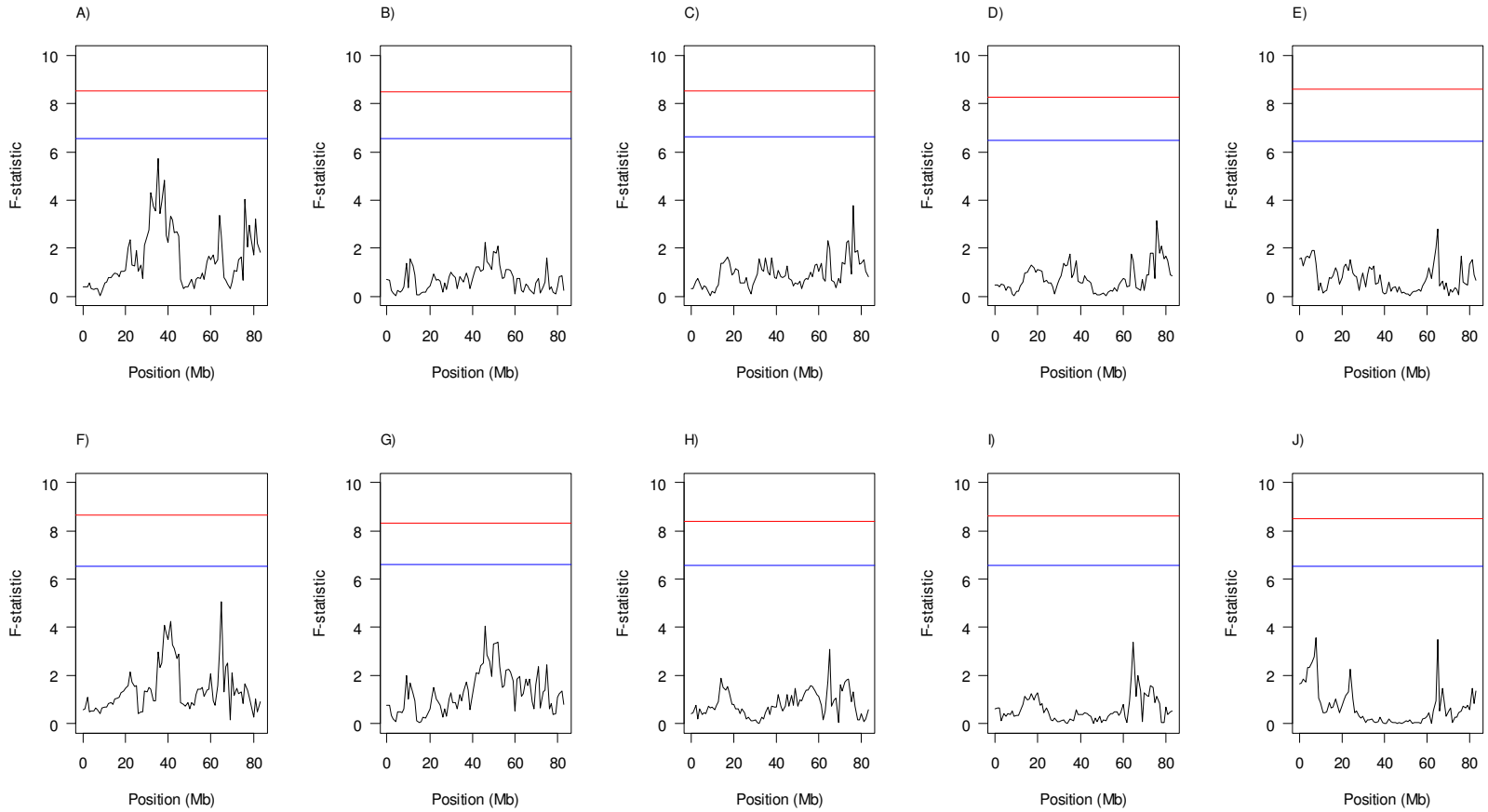
Appendix Figure C-6. Interval analysis for BTA 4. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



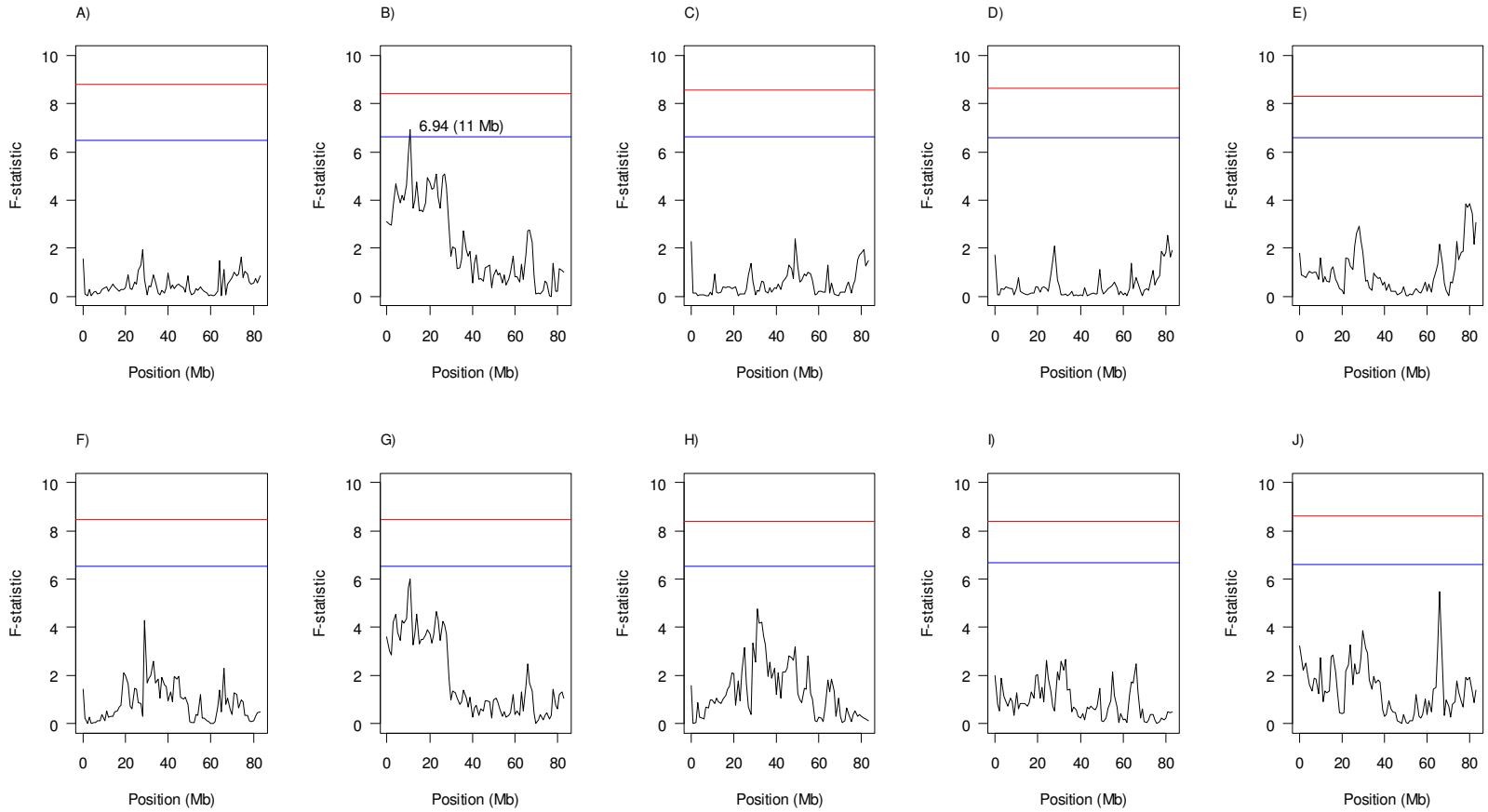
Appendix Figure C-7. Interval analysis for BTA 5. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



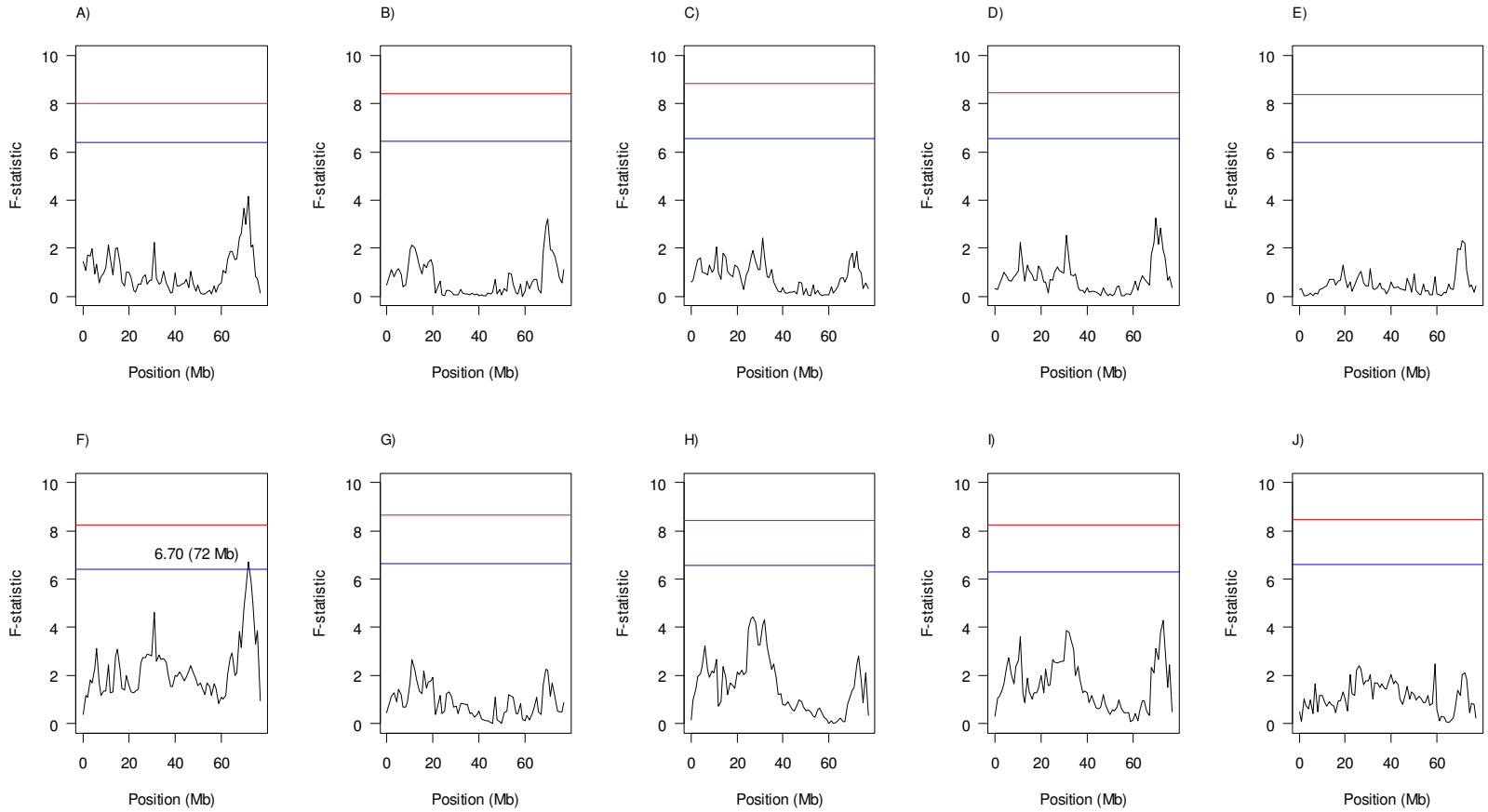
Appendix Figure C-8. Interval analysis for BTA 10. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



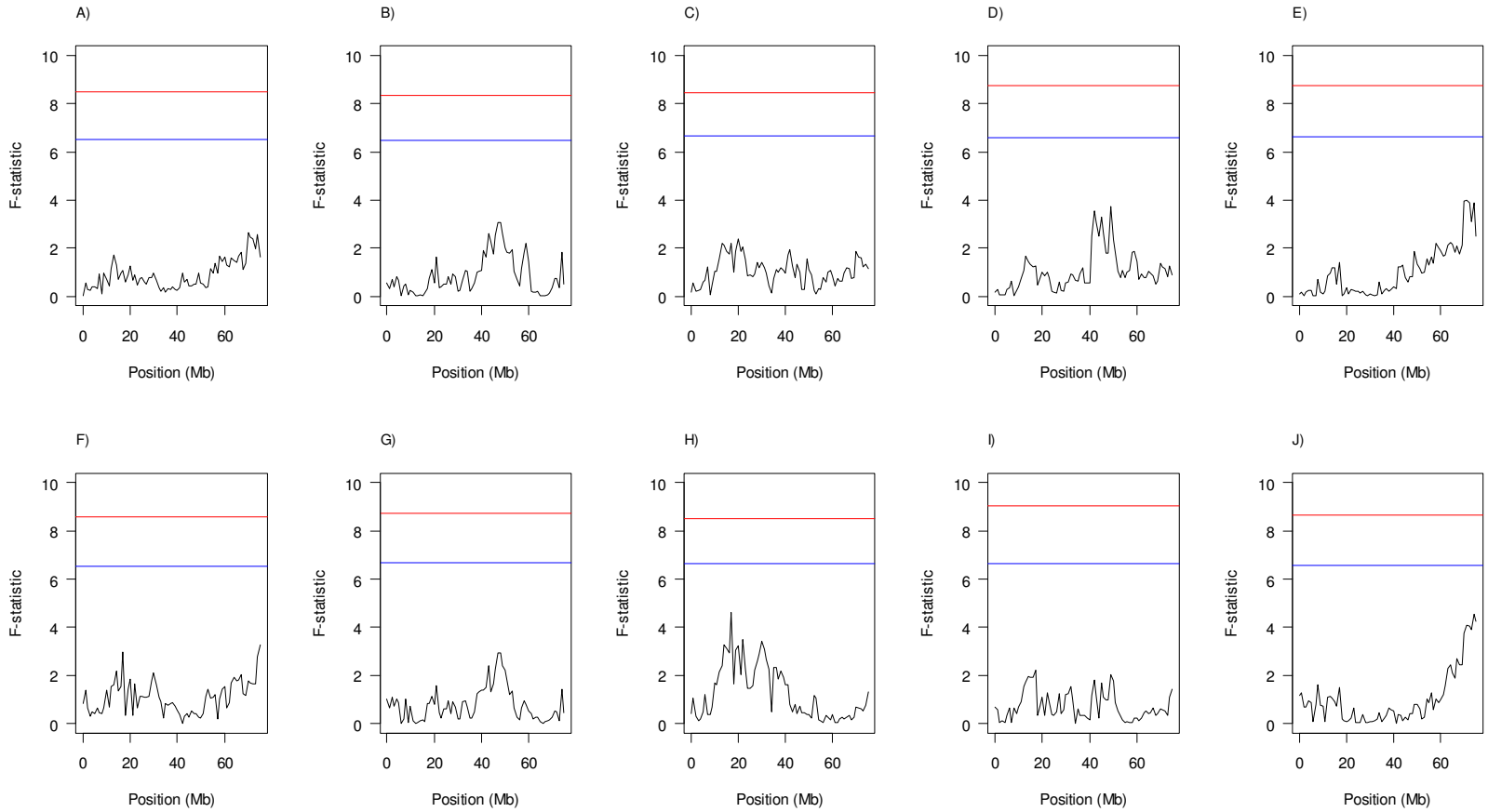
Appendix Figure C-9. Interval analysis for BTA 13. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



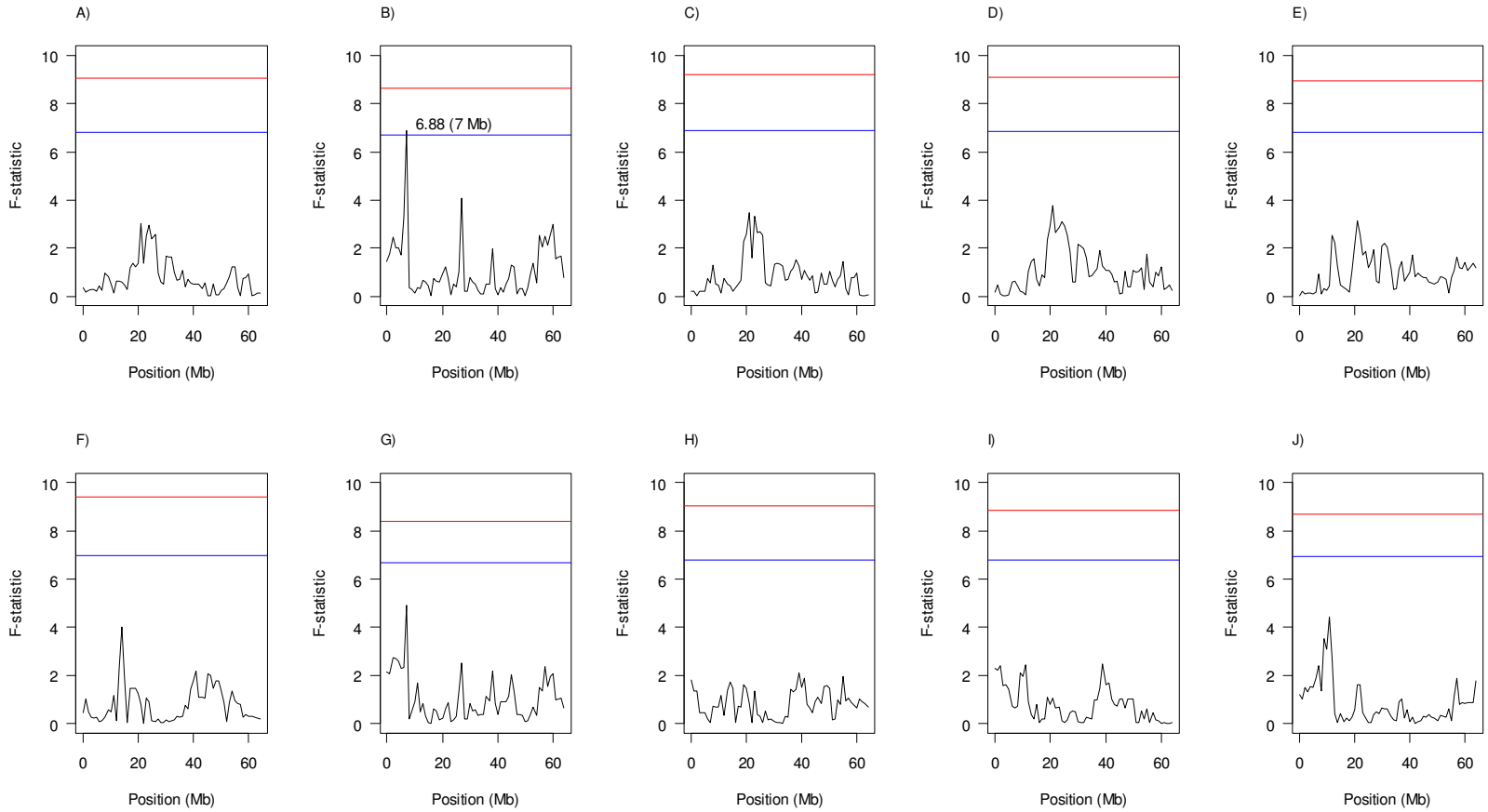
Appendix Figure C-10. Interval analysis for BTA 15. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



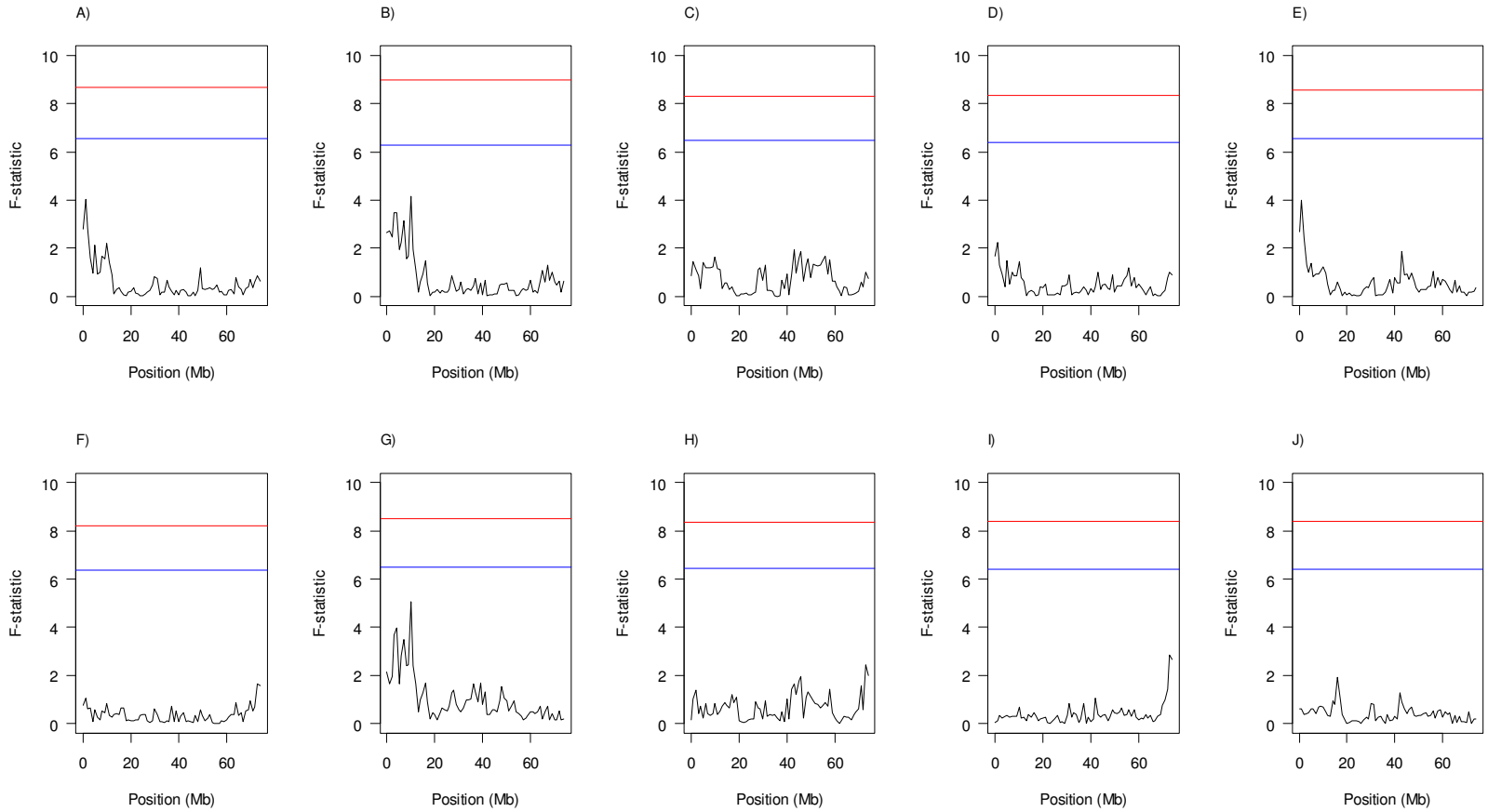
Appendix Figure C-11. Interval analysis for BTA 16. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



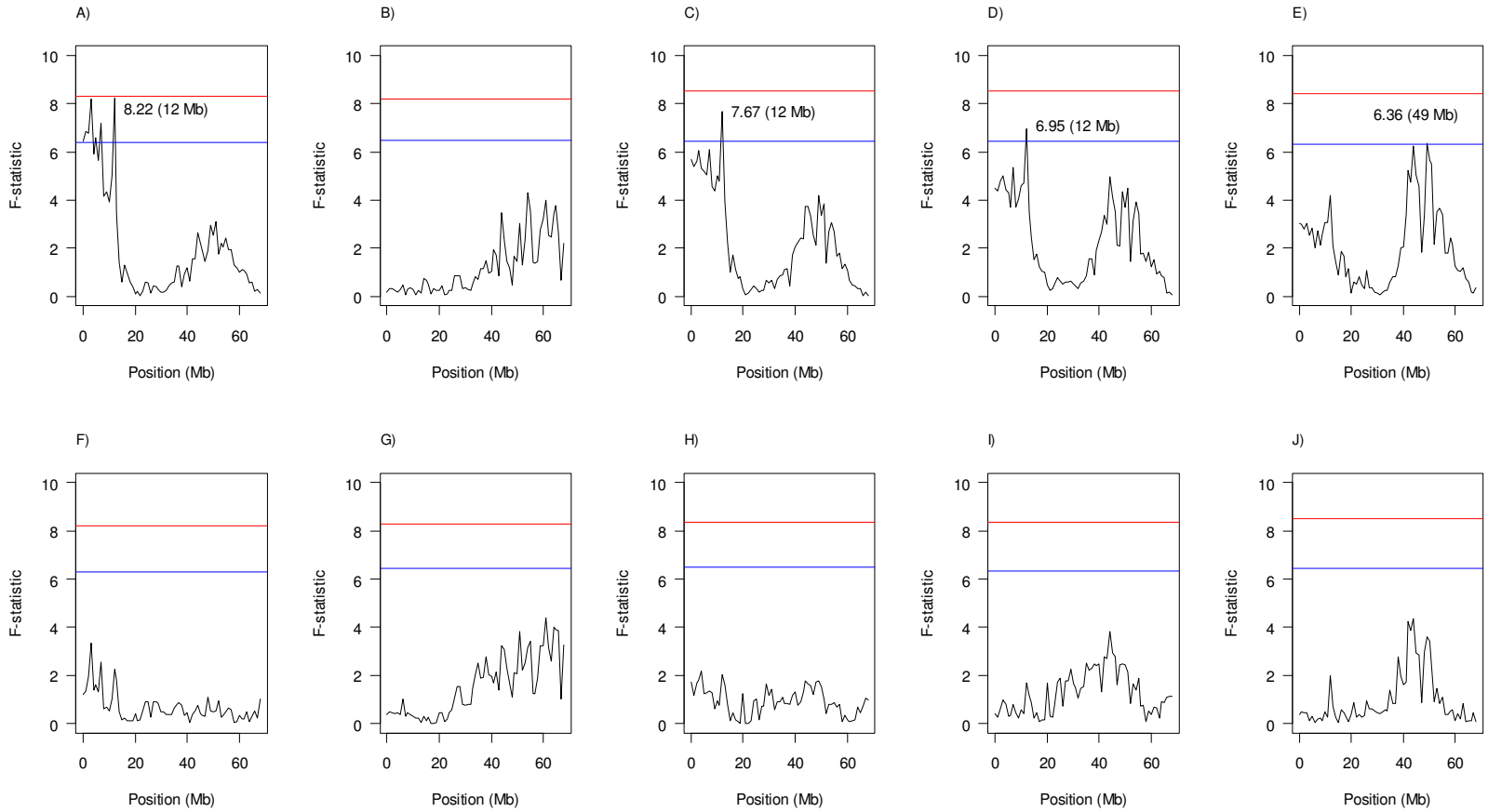
Appendix Figure C-12. Interval analysis for BTA 17. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



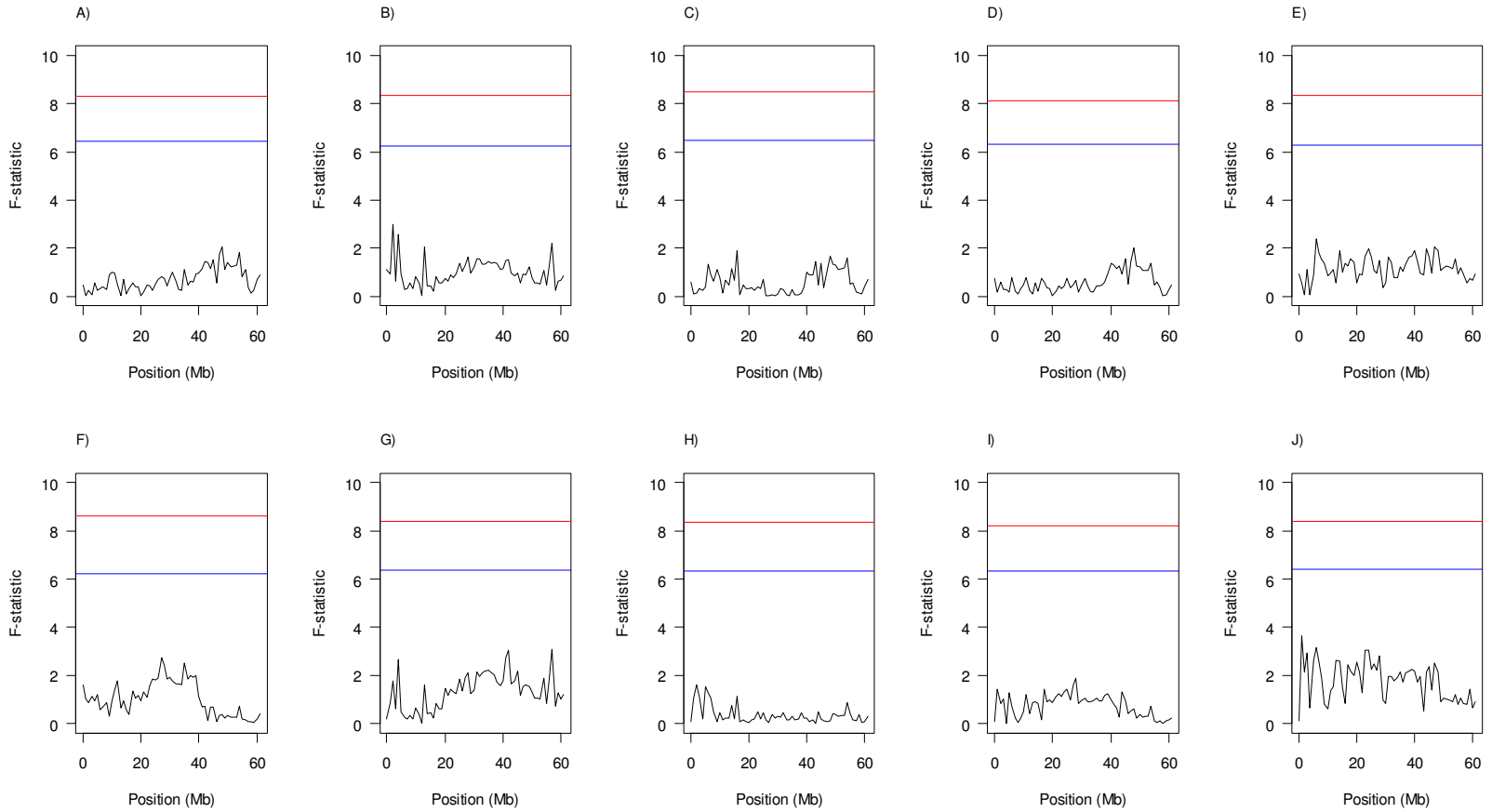
Appendix Figure C-13. Interval analysis for BTA 18. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



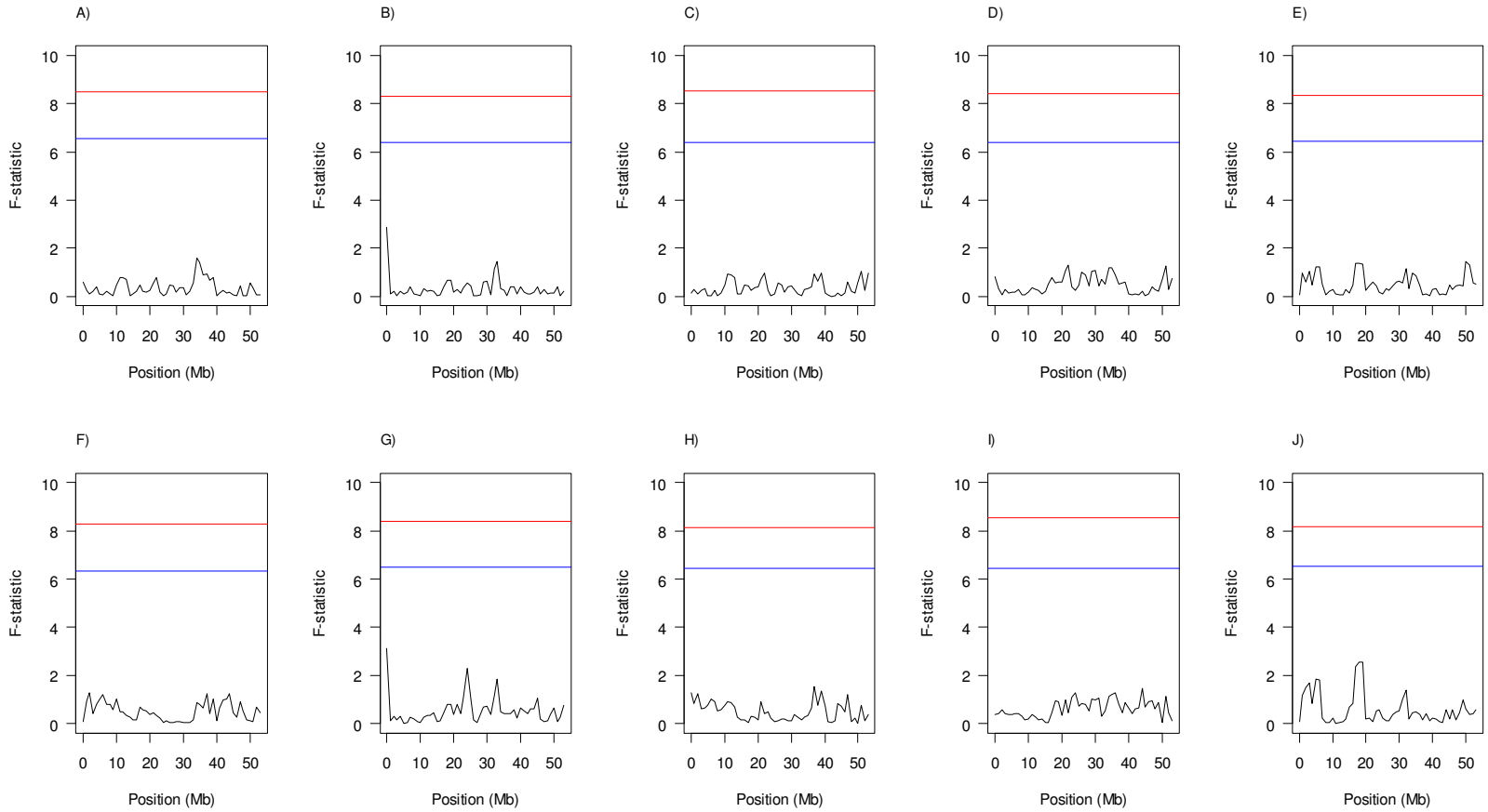
Appendix Figure C-14. Interval analysis for BTA 20. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



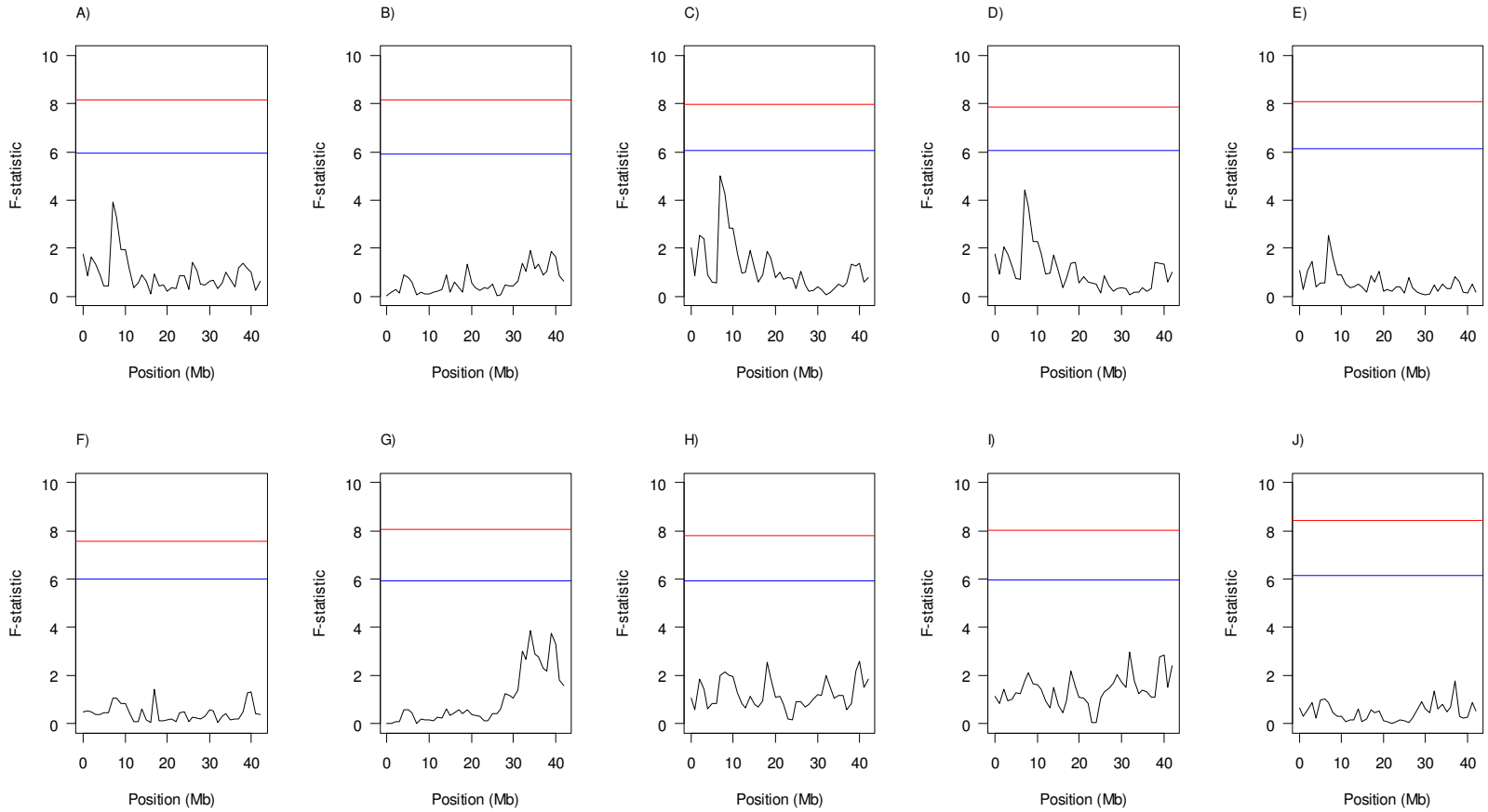
Appendix Figure C-15. Interval analysis for BTA 21. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



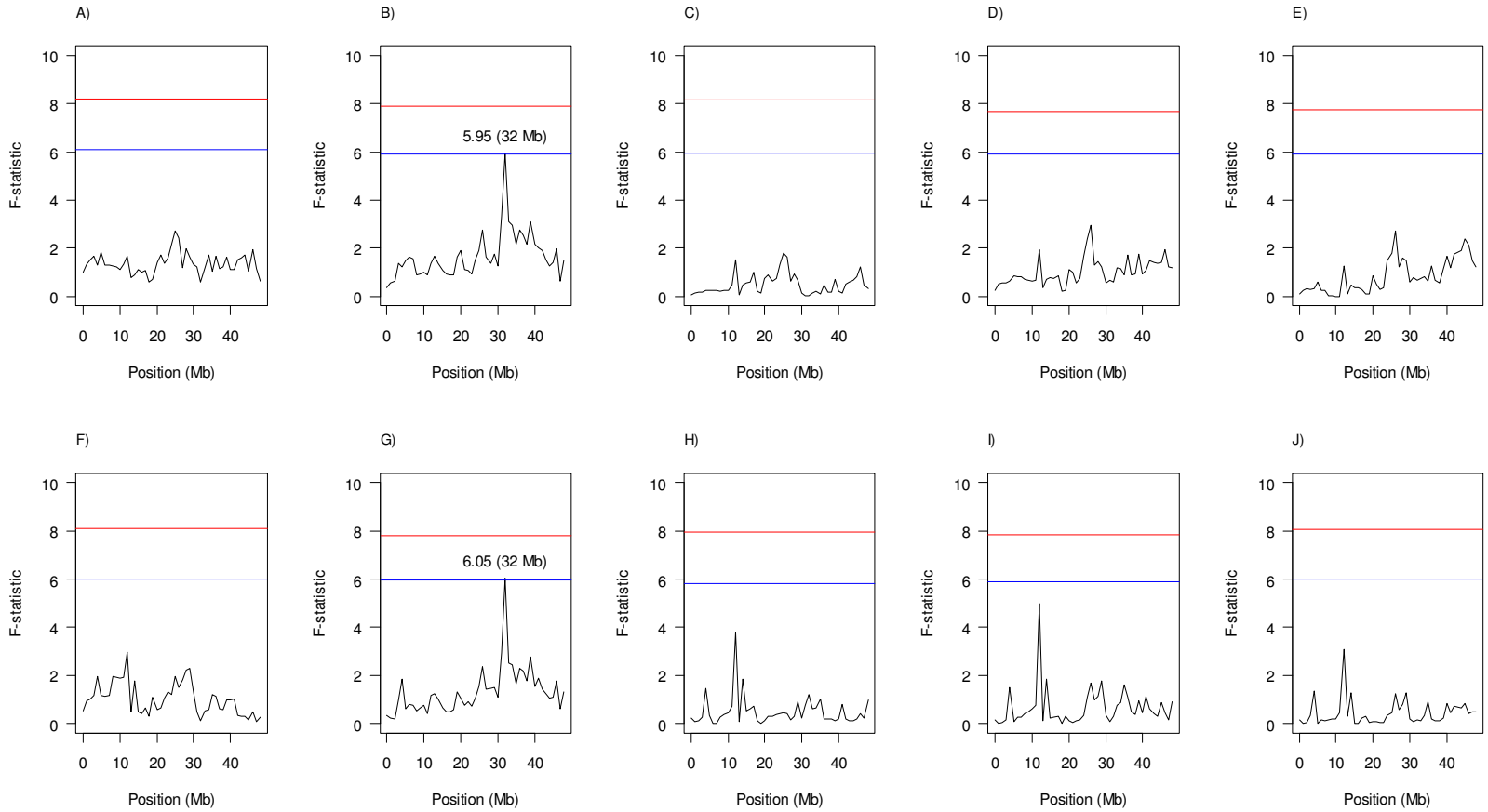
Appendix Figure C-16. Interval analysis for BTA 22. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



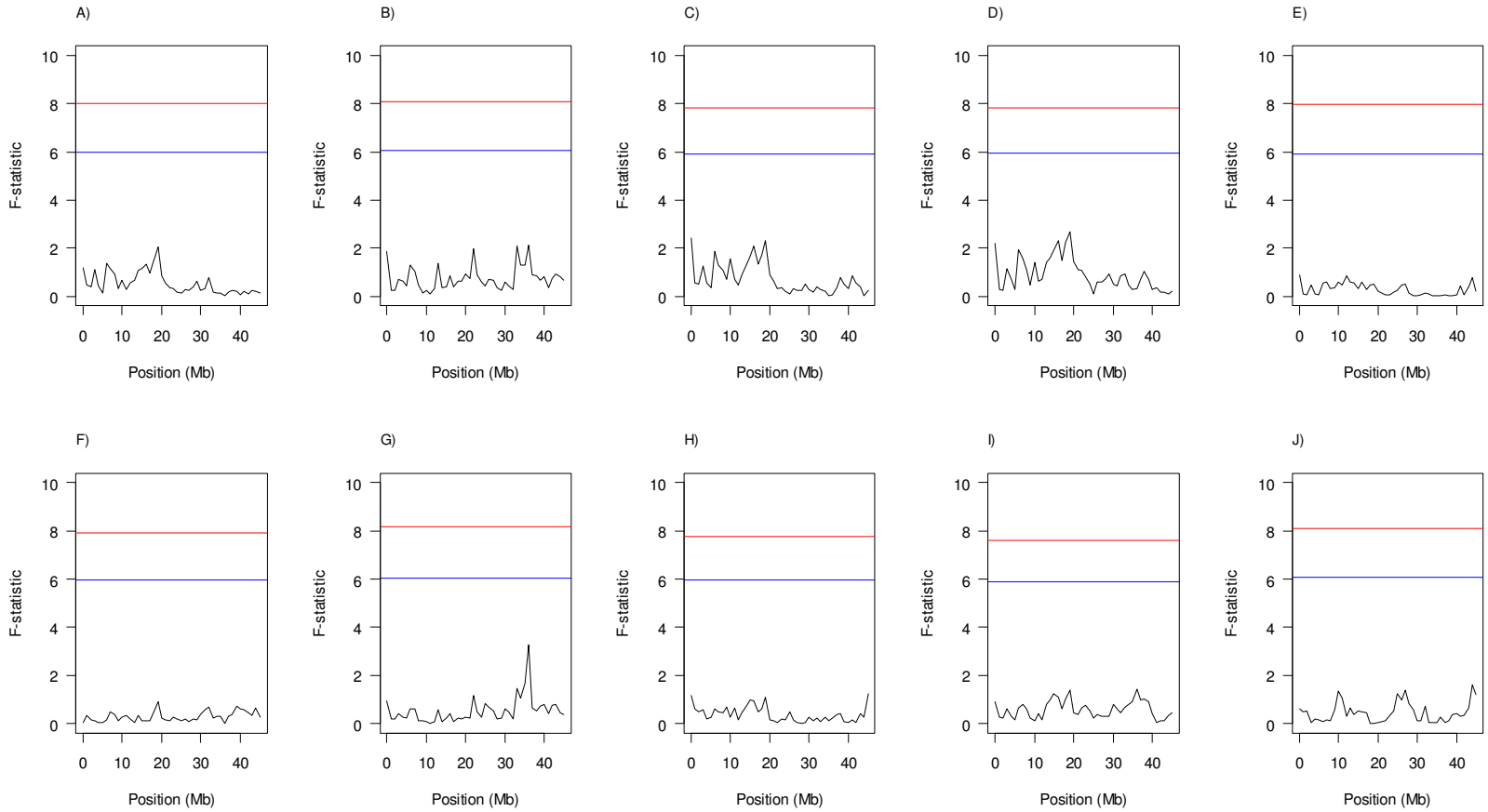
Appendix Figure C-17. Interval analysis for BTA 23. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



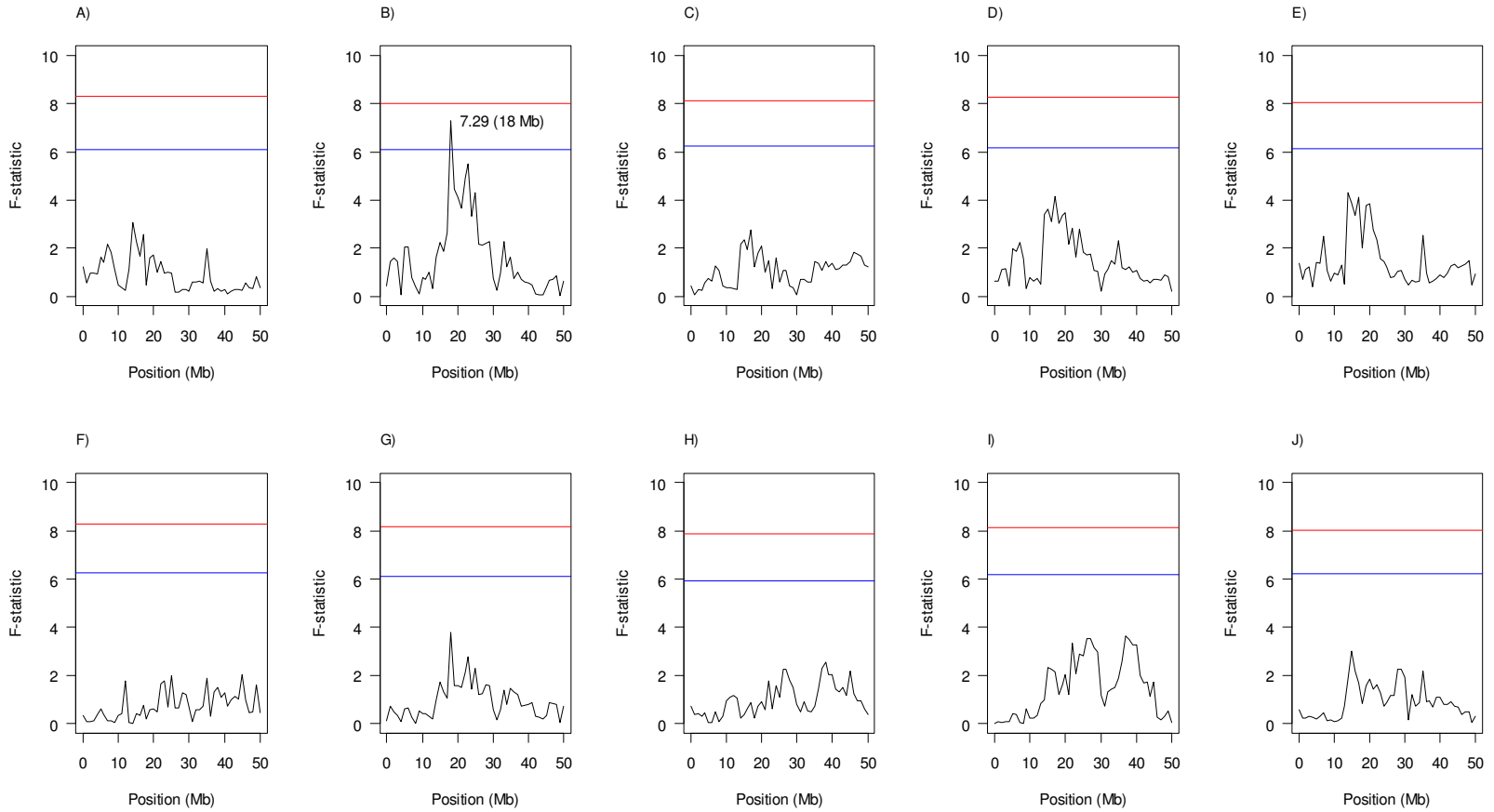
Appendix Figure C-18. Interval analysis for BTA 25. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



Appendix Figure C-19. Interval analysis for BTA 27. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



Appendix Figure C-20. Interval analysis for BTA 28. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.



Appendix Figure C-21. Interval analysis for BTA 29. Residuals generated using the original model (A-E) and including *KIT* region breed of origin for majority black (A, F), red present (B, G), degree of black – simple (C, H), degree of black – complex (D, I) and degree of darkness (E, J), respectively. Suggestive ($P < 0.05$) and significant ($P < 0.01$) chromosome-wide levels are blue and red lines, respectively.

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M.S., Animal Breeding, Texas A&M University, 2010

Research & Work Experience:

Animal Breeding & Genetics Lab, Texas A&M University, College Station, TX
Student Worker, January 2010 – Present

- Continued computational research and database record maintenance.

Graduate Research Assistant, January 2008 – December 2009

- Molecular research: DNA extraction, PCR, and sequencing for investigation of genes involved in cattle temperament.
- Computational research: database record maintenance, linkage map building for microsatellite markers, interval analysis for QTL, and statistical analyses.

Leadership Experience:

Graduate Student Council, Spring 2008 – Present

- Animal Science Departmental Primary Representative, Spring 2008, Fall 2009 – Present
- Vice President of Information, 2008 – 2009 Academic Year
- Member of Texas A&M University: Dining Service Advisory Council, Fall 2008 - Present, Code Maroon Operations Committee, 2008 – 2009 Academic Year, and Spring 2009 Student Led Award for Teaching Excellence selection committee

Animal Science Graduate Student Association, Spring 2008 – Present

- Treasurer, Fall 2009 – Present
- Representative-at-Large, Fall 2008 – Spring 2009
- Member of Professional Development Committee, Fall 2008 – Spring 2009

Honors & Awards:

- 2009 Buck Weirus Spirit Award Recipient
- Member of the Texas A&M University Chapter of Gamma Sigma Delta