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Fine Scale Mapping and Association Study of Economically Important Traits on Chromosomes 19 and 29 in Beef and Dairy Cattle

by

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Dedicated to my parents Mr. K.K. Prasad and Mrs. Sushila Prasad

ABSTRACT

The objective of this thesis was to construct radiation hybrid (RH) maps and estimate linkage disequilibrium (LD) using high density SNP markers on chromosomes 19 (BTA19) and 29 (BTA29) and use these as a tool to detect QTL in dairy and beef cattle. We have constructed RH maps of BTA19 and BTA29 consisting of 555 and 253 SNP markers respectively using a 12,000 rad whole genome RH panel. When aligned with the third draft of bovine genome sequence assembly, there was a significant internal rearrangement of the markers involving displacement, inversion and flips within the scaffolds with some scaffolds being misplaced in the genome assembly. Many of these mapped markers (370 and 186 SNP markers on BTA19 and 29 respectively) were further utilized to quantify the extent of LD using the square of the correlation coefficient (r^2) and to study the pattern of selection signatures in beef (Angus) and dairy (Holstein) breeds of *Bos taurus.* Along the chromosomes, patterns of LD were variable in both breeds and a minimum of 30,000 informative and evenly spaced markers would be required for whole genome association studies in cattle. In addition, chromosomal regions showing evidence of selection for economically important traits in Angus and Holstein were identified. Furthermore, the dense SNP markers were used to perform chromosome-wide scan to detect QTL for different economically important traits in beef and dairy cattle. Two approaches, single marker LD regression and Bayesian Monte Carlo Markov Chain, were used to map QTL. QTL for 10 and 5 traits in dairy cattle and for 2 and 1 trait in beef cattle on BTA19 and 29 respectively were detected

using both approaches of QTL mapping. The QTL detected in this study are a step towards the identification of positional candidate genes controlling these traits. In addition, we have detected several SNPs influencing economically important traits in both beef and dairy cattle. Some SNPs have been validated in an independent cattle population and has the potential of being utilized in the marker assisted selection of cattle.

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LIST OF ABBREVIATIONS

RH	Radiation Hybrid
LD	Linkage Disequilibrium
QTL	Quantitative Trait Loci
SNP	Single Nucleotide Polymorphism
BTA	*Bos taurus* Autosome
cM	Centi Morgans
cR	Centi Rays
EHH	Extended Haplotype Homozygosity
MAS	Marker Assisted Selection
LOD	Logarithm of the Odds
MCMC	Markov Chain Monte Carlo
HSA	*Homo sapiens* Autosome
BAC	Bacterial Artificial Chromosome
HSB	Homologous Synteny Block
BLAST	Basic Local Alignment Search Tool
Mb	Million Base Pairs
bp	Base Pairs
MAF	Minor Allele Frequency
GOLD	Graphical Overview of Linkage Disequilibrium
EBV	Estimated Breeding Value

1. Review of Literature

1.1. General Introduction

One of the primary goals of genomic research in agriculture is to genetically improve livestock species through selection of animals with desired traits. Most traits of economic importance in cattle are complex and quantitative in nature, such as backfat thickness, marbling score and milk yield. These traits are regulated by a combination of genes and environmental factors, which make it much more difficult to locate the genes controlling the trait of interest. Until recently, the genetic improvement of livestock species has been achieved using conventional breeding programs which are based on the statistical evaluation of breeding values estimated from the phenotypes of an individual animal and its relatives. However, some of the traits cannot be improved very efficiently using the conventional breeding program for reasons such as low heritability of the traits, difficulty or expense in collecting phenotypes, or phenotype collected later in life (Dekkers *et al*. 2004). The genetic progress of such traits can be achieved by selection using genetic markers (marker assisted selection; MAS). However, before the implementation of marker assisted selection, characterization of variants and their association with quantitative trait loci (QTL) in the cattle genome is essential.

A QTL is a chromosomal region that harbors a gene or genes influencing a quantitative trait. QTL mapping is of great interest in cattle breeding which aims at identifying genes affecting quantitative traits and then using existing variation in those genes to select for superior individuals. The bovine chromosomes 19 (BTA19) and 29 (BTA29) have been shown to be rich in a number of QTL of interest and thus are good candidates for mapping (MacNeil and Grosz 2002,

Bennewitz *et al.* 2003, Boichard *et al.* 2003, Casas *et al.* 2003, Hiendleder *et al.* 2003, Viitala *et al.* 2003, Kim *et al.* 2003, Li *et al.* 2004, Ashwell *et al.* 2005). Several mapping studies have been carried out previously to understand the genetic basis of several economically important traits (e.g. MacNeil and Grosz 2002, Bennewitz *et al.* 2003, Boichard *et al.* 2003, Casas *et al.* 2003, Hiendleder *et al.* 2003, Viitala *et al.* 2003, Kim *et al.* 2003, Li *et al.* 2004, Ashwell *et al.* 2005, Smaragdov *et al*. 2006, Kolbehdari *et al*. 2008). Most of these studies were carried out using microsatellite markers or a low density of single nucleotide polymorphism (SNP) markers which resulted in detection of QTLs with large confidence intervals. However, with the completion of the bovine genome sequence assembly a large number of SNP markers has become available making it possible to fine map the QTL regions and to perform association studies. An association between a genetic variation and a phenotype would suggest that either the variation at that locus is the causative mutation underlying the QTL or the variation is in linkage disequilibrium with the QTL. Detection of such polymorphisms is an important tool for marker assisted selection which will expedite genetic improvement of economically important traits. Further, QTL mapping would aid in positional candidate gene discovery thus allowing the study of molecular causes of existing variation.

1.2. Chromosome mapping

In order to fine map QTL, exact localization of the informative markers is required. A chromosome map can be defined as a linear order of genes or other

markers on the chromosome. A marker is a landmark on a chromosome, which can be an expressed region of the DNA (gene) (Type I marker) or a segment of the DNA with no coding function (Type II marker) but whose inheritance can be examined. The very first genetic map was published in 1913 by Alfred H. Sturtevant, who ordered six sex-linked factors on the Drosophila X-chromosome (Sturtevant 1913). This work laid the foundation for genetic mapping research. At that time, little was known about genes and chromosomes. Therefore, the study was carried out using easily observable discrete phenotypic characters such as wing shape and eye color. Later on, protein based markers were used (Briles and Briles 1982) which often lacked polymorphism and were also laborious due to technical limitations. These markers were generally clustered on a chromosome, so were not able to represent the whole genome. Later, as the recombinant DNA technology became available, Botstein *et al*. (1980) proposed restriction fragment length polymorphism (RFLP) as the first type of DNA marker. However, RFLP analysis requires large amounts of DNA which can create problems if the valuable DNA source is limited. Also, preparation and analysis of gels were laborious and expensive. In due course, several DNA polymorphism markers became available including single stranded conformation polymorphism (SSCP, Orita *et al*. 1989), randomly amplified polymorphic DNA (RAPD, Williams *et al*. 1990), amplified fragment length polymorphism (AFLP, Zabeau and Vos 1993) and microsatellite markers (Weber and May 1989). Microsatellites are short DNA segments consisting of repeat sequences such as CACACACA and are mostly located within introns or between genes (Li *et al.* 2002). They are known as excellent genetic

markers because of their high polymorphism. Band *et al.* (1997) states that the total number of $(TG)_n$ microsatellites in the bovine genome has been estimated to be between 15,000 and 44,000. This number is far less than the number of microsatellites estimated in a human or a mouse genome (Stallings *et al.* 1991, Stone *et al.* 1995). More recently, another DNA marker called single nucleotide polymorphism (SNP) has become available. A SNP is a single nucleotide variation in a DNA sequence, which occurs in coding as well as non-coding regions of the genome. SNPs are less informative than microsatellites because they are mostly biallelic, whereas microsatellites have many alleles (Vignal *et al.* 2002). However, SNPs occur more frequently than microsatellites and are present abundantly throughout the bovine genome (Snelling *et al.* 2005). The recently designed Infinium Bovine SNP50 Beadchip contains 54,074 SNPs with an average SNP spacing of 49.4 kb across the bovine genome (Settles *et al.* 2009). Moreover, large-scale SNP genotyping is relatively easy and cost-effective with a lower error rate (Kennedy *et al*. 2003), which makes them markers of choice. There are mainly two different ways of mapping markers on chromosomes, linkage and physical mapping.

1.2.1. Linkage mapping

Linkage mapping is based on linkage analysis and determined by how often two gene loci are inherited together. The closer two genes are, the more tightly they are linked and the more often they will be transmitted to the offspring together. In cattle, the first genetic linkage map was constructed (Barendse *et al*. 1994) by

genotyping 202 DNA polymorphisms in cattle families which comprised 295 individuals in full sibling pedigrees. In total, 171 loci were found linked to one other locus. The types of polymorphisms mapped in the study were microsatellites, restriction fragment length polymorphisms, single locus minisatellites and single strand conformation polymorphisms, and covered approximately 90% of the length of the bovine genome. Thereafter, a medium-density genetic linkage map of the cattle was constructed which covered more than 95% of the bovine genome (Barendse *et al*. 1997). In this study, 746 DNA polymorphisms were genotyped in cattle families comprising 347 individuals in full sibling pedigrees. The DNA polymorphisms genotyped in this study were dinucleotide microsatellites, single strand conformational polymorphisms, single locus DNA minisatellites and restriction fragment length polymorphisms. It was found that 703 loci were linked to one other locus. Another study reported a bovine linkage map constructed with 1236 polymorphic DNA markers and 14 erythrocyte antigens and serum proteins (Kappes *et al*. 1997). This map had 627 new markers and 623 previously linked markers, thereby providing a basis for integrating previously published bovine maps. These linkage maps provided a valuable resource for mapping QTL. However, more closely spaced markers were needed to fine map QTL. Thereafter, Shirakawa Institute of Animal Genetics in collaboration with United States Meat Animal Research Centre added 2277 microsatellite markers to the bovine genetic map (Ihara *et al*. 2004). But, because this map largely represented anonymous markers i.e. Type II markers, it provided limited information about genes underlying the QTL. Therefore, another study utilized bovine expressed sequence

tag (EST) and bacterial artificial chromosome (BAC) sequence data to develop 918 SNP markers to map genes on the bovine linkage map (Snelling *et al*. 2005). These SNPs further defined comparative relationships between the bovine linkage map and human and other model organism genome sequences.

1.2.2. Physical mapping

In contrast to linkage maps, a physical map displays distances between and within genes or specified markers regardless of their inheritance and defines absolute position of genes. Physical maps can be of three different types: cytogenetic maps, radiation hybrid (RH) maps and sequence maps. A cytogenetic map is the lowest resolution physical map, which is based on the characteristic banding patterns observed by light microscopy of stained chromosomes. Fluorescence in situ hybridization (FISH) is another cytogenetic method of physical mapping. This method involves hybridization of fluorescently labeled DNA probes to metaphase chromosomes and can be used to identify chromosomes, detect chromosomal abnormalities or determine the chromosomal location of specific DNA sequence (Trask 1991).

The second type of physical map is a radiation hybrid (RH) map which utilizes radiation rather than natural recombination to induce breaks between the markers. RH mapping consists of two stages: one is the experiment stage which is biological in nature and the other is the analysis stage which is mathematical in nature. In the experiment stage, the donor bovine cells, carrying a selectable marker thymidine kinase (TK), are lethally irradiated by X-rays to fragment the

chromosomes (Figure 1-1). These cells are then fused to a recipient hamster cell line, which is thymidine kinase deficient (TK-). The fused cells are then cultured in a media containing HAT (hypoxanthine, aminopterin, thymidine) to make sure that only hybrid hamster cells containing bovine chromosomal fragments will grow (Goss and Harris 1975, Walter *et al*. 1994). The resulting hybrid cells are then grown up to yield hybrid cell lines and a radiation hybrid panel consists of different hybrid cell lines (Slonim *et al.* 1997). The resolution of a radiation hybrid map depends on the radiation dosage. With increasing radiation dosage, the size of chromosome fragments after irradiation decreases and the resolution of the radiation hybrid panel increases. There are several whole genome radiation hybrid panels available for cattle including 3000 rad (Williams *et al*. 2002), 5000 rad (Womack *et al*. 1997), 7000 rad (Itoh *et al*. 2005) and 12,000 rad (Rexroad *et al*. 2000) panels of which the 12,000 rad panel has the highest resolution. The analysis stage of RH mapping consists of analysis of co-retention frequencies of markers on radiation-fragmented chromosomes in a panel of hybrid cell-lines. The closer two markers are, the smaller the chance that radiation would be able to induce a break between them. If that is the case, markers are said to be co-retained, that is the hybrid contains either both or neither of the markers. If the marker is retained by the hybrid, it is indicated by "1", otherwise "0". In case of ambiguous result, it is indicated by a "2". Thus, the data forms a matrix of 1, 0 and 2. Using this retention pattern in the matrix, markers are positioned on the chromosome (Slonim *et al.* 1997).

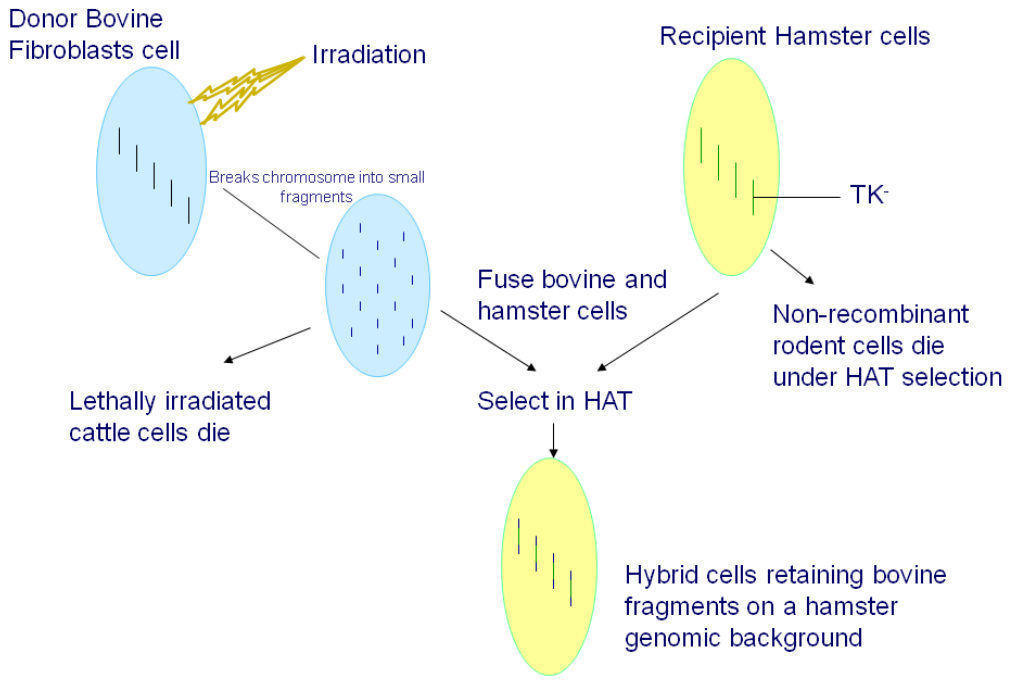

Figure 1-1. Schematic representation of construction of radiation hybrids (Modified from Jann 2005, personal communication)

Radiation hybrid maps have been successful in several species in contrast to classical linkage maps. First, RH maps have higher resolution than linkage maps. In linkage maps, resolution depends upon the number of informative meiosis in the pedigree analyzed. Resolution is affected if some of the markers are not informative in all the families of the pedigree analyzed. In RH mapping, control of irradiation dosage makes it possible to achieve a fine resolution. Secondly, a RH map can position both polymorphic as well as non-polymorphic markers (Cox *et al*. 1990). All types of single sequence tags (STS) and expressed sequence tags (EST) can be easily mapped which makes RH mapping a powerful tool to draw comparative maps (Foster *et al.* 1996, Drogemuller *et al.* 2002). Thirdly, unlike

linkage maps, RH maps do not require informative and large resource populations. This is especially useful in species, e.g. cattle, with long generation intervals. Besides this, RH mapping also facilitates the assembly of genome sequences (Weikard *et al*. 2006, Jann *et al*. 2006, Leroux *et al*. 2005). The conventional method was to type markers using PCR followed by gel electrophoresis, which was time-consuming. Recently, a high-throughput approach has been utilized to type large numbers of markers in a very short time (McKay *et al*. 2007a). In this study, the Illumina BeadStation 500G system was used and was shown to be a rapid and cost effective method to type markers.

The physical map that allows the most comprehensive information is the complete sequence map of the genome. Sequence maps show position of markers in base pairs. The genome of the first free-living organism, *Haemophilus influenza*, was sequenced in 1995 (Fleischmann *et al*. 1995) using the shotgun sequencing strategy, in which the entire genome was first fragmented and the random segments were sequenced and then assembled (i.e. put in order). The completion of this genome sequencing gave new directions to other genome sequencing projects. In 1996, the National Human Genome Research Institute funded pilot projects to find efficient approaches to completely sequence the human genome and tested the feasibility of large-scale sequencing. Thereafter, the first genome sequence of a multicellular organism, the roundworm *Caenorhabditis elegans*, was completed and released in December 1998 (*C. elegans* Sequencing Consortium 1998). The completion of this project provided insights on how the genomes of complex organisms function. In 2003, the

successful completion of the human genome project was announced and in 2004, the International Human Gene Sequencing Consortium published the finished or refined human gene sequence reducing the estimated number of genes from 35,000 to 20,000-25,000 (The International Human Genome Sequencing Consortium 2004). The assembly of bovine genome sequences was started in 2003 and has been carried out by Baylor College of Medicine's Human genome Sequencing Centre in Houston. The breed of the cattle used in sequencing project is Hereford, a beef breed. At the time this dissertation was carried out, three versions of the assembly had been released. The first draft of the assembly with 3X coverage was released in September 2004 (Btau_1.0), second draft with 6.2X coverage in June 2005 (Btau_2.0) and third draft with 7.1X coverage was released in August 2006 (Btau_3.1) (Liu *et al.* 2009). The bovine build 1.0 and 2.0 were assembled using only whole genome shotgun (WGS) reads from small insert clones and BAC end sequences (BES). The bovine build 3.1 was assembled using information from both WGS and BAC sequence. The source of DNA for the WGS libraries was from the Hereford cow L1 Dominette 01449 while for the BAC library DNA was Hereford bull L1 Domino 99375, the sire of the former animal. The genome sequence was reported for 29 autosomes and the X chromosome (Liu *et al.* 2009). A Previous study (e.g. Jann *et al*. 2006) has reported misaassignment of scaffolds and incorrectly assigned loci on many chromosomes, including bovine chromosomes 19 and 29, in the bovine build 2.0 utilizing whole genome radiation hybrid maps. Therefore, the building of radiation hybrid maps would provide an independent source of information to check the quality of bovine build

3.1. Later in October 2007, the latest assembly of the bovine genome was released (Btau_4.0). This draft did not relatively add new sequence data with respect to the earlier versions of the assembly, but incorporated different map information (including the information generated from this dissertation) to place the contigs and scaffolds in the genome which resulted in more accurate chromosome structures. Briefly, a contig is referred to as the contiguous blocks of sequence formed from overlapping sequencing reads. These ungapped contigs were then linked to each other using information from read pairs at the end of the clones to form scaffolds, which in turn were arranged along the chromosomes (Liu *et al.* 2009, George Weinstock, personal communication).

1.3. Linkage disequilibrium

Linkage disequilibrium (LD) maps are other important tools for investigating the genes underlying economically important traits in animal species. Linkage disequilibrium is the non-random association of alleles at different loci, but not necessarily on the same chromosome. This implies that if there were two alleles at two loci, certain combinations of alleles would occur at a higher frequency than expected. Let us consider two loci A and B with two alleles (A, a) and (B, b), respectively. The two loci are said to be in linkage disequilibrium if the chance of finding a B depends on the alleles in A. Linkage disequilibrium is not the same phenomenon as linkage which describes the association between two or more loci on a chromosome with limited recombination between them. Linkage focuses on a locus while linkage disequilibrium focuses on an allele. It is also important to note

that linkage measures co-segregation of markers in a pedigree, while linkage disequilibrium measures co-segregation in a population (Tillmar *et al*. 2008).

Quantifying the extent of LD is the essential first step to determine how many markers are required to perform whole genome association studies. In addition, patterns of LD aid in exploring the different evolutionary forces that may have generated LD in certain regions (Ardlie *et al.* 2002). Therefore, LD maps not only identify alleles that have undergone selection, but are also important for the design and application of association studies in cattle populations.

1.3.1. Measures of linkage disequilibrium

There are different measures of linkage disequilibrium including D, D' and r^2. The measure D or disequilibrium coefficient is the difference between the observed frequency of a haplotype and the frequency it would be expected to show if the alleles were segregating at random (Hill 1981). Consider two adjacent loci A and B, with two alleles at each locus (A, a) and (B, b). The observed frequency of haplotypes consisting of alleles A and B is denoted by P_{AB}. The expected frequency of haplotype, assuming independent assortment of alleles at both loci, is calculated as the product of allele frequency at each of the two loci denoted by P_AP_B, where P_A denotes frequency of allele A at first locus and P_B denotes frequency of allele B at second locus (Ardlie *et al*. 2002). Therefore D is calculated as:

$$D = P_{AB} - P_AP_B$$

However, linkage disequilibrium decays with time (t) and recombinational distance (r) according to the following formula:

$$D_t = (1-r)^t D_0$$

where D_0 is extent of disequilibrium at some starting point and D_t is extent of disequilibrium 't' generations later. Over time, recombination erodes linkage disequilibrium between alleles, which occurs more frequently between distantly located genes than between tightly linked genes. Therefore, D would be small between loci far apart from each other and would decrease with time as a result of recombination. Because of the dependence of D on allele frequencies, it has not been recommended to use for measuring and comparing the level of LD (Ardlie *et al*. 2002). The two most widely used measures of LD are absolute value of D' and r^2.

The absolute value of D' (also called Lewontin's D') is calculated by dividing D by its maximum possible value, given the allele frequencies at the two loci (Lewontin 1964).

$$D' = D/D_{max}$$

When D' equals 1, it suggests that the two loci are in complete LD and there has been no recombination between them. When D' is less than 1, it means that the two loci have been separated by recombination. When D' equals 0, it signifies no LD. One of the disadvantages of this measure is that it is upwardly biased in small samples for SNPs with common alleles and even more biased for SNPs with rare alleles. As a result, high D' values can be obtained even when the markers are in linkage equilibrium. Therefore, D' should be used to indicate if recombination has

occurred but it should not be used for measuring the extent of LD (Ardlie *et al*. 2002).

Another measure of linkage disequilibrium is the square of the correlation coefficient (r^2) between marker alleles. This measure, originally proposed by Hill and Robertson (1968), is less dependent on allele frequencies. It is calculated as D^2 divided by the product of the four allele frequencies at the two loci:

$$r^2 = \frac{D^2}{freq(A) * freq(a) * freq(B) * freq(b)}$$

When r^2 is equal to one for two markers, it shows complete linkage disequilibrium and one marker provides complete information about the other marker, making the other marker redundant (Ardlie *et al*. 2002). Early LD studies in cattle used the measure D', but r^2 has recently emerged as a measure of choice for comparing the extent of LD (Pritchard and Przeworski 2001, Weiss and Clark 2002). The decline of r^2 with distance determines how many markers are required in a genome scan to detect a QTL, which cannot be predicted by using D' (Hayes 2007). The measure r^2 shows much less inflation than D' when small samples are used (McRae *et al*. 2002, Weiss and Clark 2002).

1.3.2. Factors affecting linkage disequilibrium

Several factors influence linkage disequilibrium including genetic drift, mutation, gene conversion, recombination, age of alleles, admixture, hitchhiking, effective population size and selection (Ardlie *et al*. 2002).

Genetic drift is the change in the gene pool of a population every generation due to the random sampling of gametes during the production of offspring. The increased drift of a small, steady population will result in the loss of some haplotypes from the populations, thereby increasing LD (Terwilliger *et al*. 1998).

Linkage disequilibrium can be created by admixture, interbreeding between genetically differentiated populations, or by migration (gene flow). A mating system like inbreeding results in increases in haplotype sharing and thus increases in LD. Individuals in an inbred population share alleles that are identical by descent (IBD) that is the alleles can be traced back to an ancestor. Inbreeding results in the lowering of population diversity, thus increasing LD.

Another factor that affects the extent of linkage disequilibrium is variable recombination rates across the genome. The non-recombining regions of the genome will have strong LD while the recombination hot spots will correspond to the breakdown of linkage disequilibrium (Jeffreys *et al*. 2001). A gene conversion event, which is the non-reciprocal transfer of genetic information between homologous sequences has an effect similar to that of recombination and can break down LD (Frisse *et al*. 2001).

Variable mutation rates are another factor that influences linkage disequilibrium (Sunyaev *et al.* 2003, Ardlie *et al*. 2002). Some regions of the chromosome in the human genome have been reported to contain CpG dinucleotides which are known to mutate at a higher rate because cytosine is susceptible to deamination. Cytosines in CpG dinucleotides in most cases are

methylated and deamination of 5-methyl cytosine (5mC) produces thymidine. Deamination of unmethylated cytosine produces uracil (Fryxell and Moon 2005). A recently published paper by The Bovine Genome Sequencing and Analysis Consortium (2009) has reported the overall GC content in the cattle genome as 41.7%, similar to that of other mammals. It has been stated by Ardlie *et al*. (2002) that SNP located in the CpG islands may have higher mutation rates, therefore showing little or no LD with markers in close proximity even in the absence of any recombination.

Finite population size in livestock species is implicated to be a key cause of LD. Effective population size is defined as the number of individuals in a population having equal chances of contributing gametes to the next generation, which is generally smaller than absolute population size. Effective population size for most livestock species are relatively small, thus creating large amounts of linkage disequilibrium. In the recent past, the use of artificial insemination and a few elite sires have greatly reduced the effective population size of dairy cattle. Linkage disequilibrium at short distances is a function of effective population size many generations ago whereas LD at long distances reflects more recent population history (Hayes 2007).

Another factor which affects LD is natural selection. Natural selection affects LD in two ways- (1) hitchhiking effect, where an entire haplotype flanking a favored variant can be rapidly swept to high frequency or even fixation, thus inflating LD and (2) epistatic selection for combinations of alleles at two or more loci on the same chromosome (Cannon 1963, Parsch *et al*. 2001, Varrelli and

Eanes 2001, Ardlie *et al*. 2002, Wang *et al*. 2002). The effect of selection on the amount of LD averaged over the genome is little, as selection is localized around specific genes. Use of LD measures to detect selected areas of the genome is discussed in the next section 1.3.3.

1.3.3. Signatures of selection

Detection of signatures of selection is an important tool to identify potential genes that might underlie economically important traits and which will improve our ability to link genetic variants to the phenotype of interest. Linkage disequilibrium can be used to measure the association between a single allele at one locus with multiple loci at several distances. The characteristic feature of positive selection is that it results in a remarkable rise in allele frequency which occurs in such a short time that recombination is not able to break down the haplotype in which selection has occurred. Therefore, the signature of positive selection is an allele having a long range LD as well as high population frequency (Sabeti *et al*. 2002).

The multilocus measure of linkage disequilibrium is homozygosity (Sabatti and Risch 2002). Haplotype homozygosity (HH) measures variation at linked sites and is calculated as:

$$HH = \frac{\sum Pi^2 - 1/n}{1 - 1/n}$$

Where, Pi is the relative haplotype frequency and n is the sample size. To find out, how LD breaks down with increasing distance to a specified core region, HH is calculated in a stepwise manner for each haplotype (extended HH; EHH). The test for positive selection is to find a core haplotype with a combination of high

frequency and EHH in compared to other core haplotypes at that locus. The other core haplotypes serve as an internal control for one another at the same chromosomal region (Sabeti *et al*. 2002, Mueller and Andreoli 2004).

In humans, several studies have been carried out to study the selection signatures using EHH statistics (Sabeti *et al*. 2002, Miretti *et al*. 2005, Nash *et al*. 2005). Recently, detection of signatures of selection has been carried out on bovine chromosome 6 using dense SNP markers in Norwegian Red cattle (Hayes *et al*. 2008). Positive selection was detected using standardized integrated extended haplotype homozygosity (iHS) for each marker as suggested by Voight *et al*. (2006). Unstandardized iHS can be calculated as:

$$\text{Unstandardized iHS} = \ln(\text{iHHA}/\text{iHHD})$$

Where, iHHA is the integrated EHH calculated for the ancestor core allele and iHHD is the integrated EHH calculated for the derived core allele. Large negative values of unstandardized iHS indicate long haplotypes carrying the derived allele, while large positive values indicate long haplotypes carrying the ancestral allele. The unstandardized iHS is then adjusted to obtain a final statistic regardless of allele frequency at the core SNP because in neutral models, low frequency alleles are usually younger and are associated with longer haplotypes than higher frequency alleles (Voight *et al*. 2006).

1.3.4. Linkage disequilibrium in cattle

So far, several linkage disequilibrium studies have been performed in cattle. The first whole genome LD study was carried out in Dutch black and white dairy cattle

(Farnir *et al*. 2000). Two data sets were used to measure LD in this cattle population. The first data set comprised of a granddaughter design comprising of 949 bulls genotyped for 284 microsatellites resulting in a total of 276,048 genotypes. Genotypes for 581 maternal gametes were utilized to measure LD using Lewontin's (1964) normalized *D'* measure. The extent of LD was first estimated for syntenic marker pairs that are markers located on the same chromosome, where long range LD was observed. Results also showed highly significant gametic phase disequilibrium between non-syntenic loci. It was thought that the results may not be a true representative of the breed in general because gametes from elite cows that is from an active breeding population were used. Therefore a second dataset, consisting of 627 cows, assumed to be representative of the Dutch black-and-white general population were genotyped for eight microsatellite markers, located on different autosomes. In addition 175 of 627 cows were genotyped for another 19 markers, of which 16 were located on BTA14 and 3 on BTA6. For marker pairs that were less than 5 cM apart, D' averaged 46 % and it decayed to 24% on average for marker pairs at a distance of 30 cM or more. The departure from expectation was found to be very significant for both syntenic as well as non-syntenic markers. Therefore, the results confirmed that long-range LD and gametic associations between non-syntenic loci is a characteristic feature of Dutch black-and-white dairy cattle population. The long range LD observed is in contrast with the LD studies in human extending from 5kb to 4 Mb (Huttley *et al*. 1999, Pritchard and Przeworski 2001, Service *et al*. 2001). The high level of linkage disequilibrium observed was attributed to

random genetic drift and small effective population size, as low as 50, for Dutch black-and-white dairy cattle population. The reason for the small effective population size was explained by the widespread use of artificial insemination and intense selection for increased milk production. In Netherlands, 95% of the cows are bred by artificial insemination and 10 best bulls account for 40% of inseminations (Boichard 1996).

The second LD study in cattle was carried out by Vallejo *et al*. (2003), where the level of genetic diversity and extent of LD in the North American Holstein cattle population was carried out. Twenty-three elite Holstein bulls from US dairy industry were genotyped for 54 microsatellite loci spanning most of the bovine autosomal chromosomes. The animals chosen in the study were as unrelated as possible to include more independent and unique chromosomes. This has the promise to give a more global representation of the breed. It was found that the extent of LD observed for syntenic and non-syntenic marker pairs in the North American population was similar to that found in the Dutch dairy population (Farnir *et al*. 2000). Most of the observed LD in the US Holstein population was also explained by random genetic drift.

In the same year, Tenesa *et al*. (2003) estimated the extent of LD in the U.K. dairy cattle population. Fifty Holstein bulls were genotyped for 6 marker loci on BTA2 and 7 loci on BTA6. This study used statistical methods that do not require family information to infer population haplotype frequencies instead of family-based haplotyping methods. Marker pairs in synteny showed significant linkage disequilibrium extending to about 10 cM while non-syntenic markers did

not show significant linkage disequilibrium. Tenesa *et al*. (2003) attributed the difference in their results with Farnir *et al*. (2000) to two factors. The first factor was the relatedness among the samples. Relatedness between individuals can cause an increase in the level of LD, even between unlinked loci, due to larger identical by descent regions in related individuals. The second factor was the different sample sizes in the two studies which affected D'.

Thereafter, another study (Sandor *et al*. 2006) quantified the level of LD on the X chromosome in Holstein-Friesian dairy cattle. A granddaughter design comprising of 929 bulls were genotyped for 22 X-specific and 2 pseudoautosomal microsatellite markers. They also used phased genotypes available on the same dairy population for 202 autosomal microsatellites (Farnir *et al*. 2000). Pairwise LD was measured using r^2. The study compared the level of polymorphism and LD between X-linked and autosomal microsatellites in this dairy population. It was found that the microsatellites are as polymorphic on the X chromosome as on the autosomes. However, the level of LD between these markers is higher on the X chromosome than on the autosomes. Studies in humans have found genetic polymorphisms to be lower and higher LD for markers on the X chromosome (Dib *et al*. 1996). The lower level of polymorphism on the human X chromosome is thought to be due to higher genetic drift, lower female mutation rate than males and enhanced purifying selection due to male hemizygosity. The higher level of LD on the X chromosome in cattle was explained due to higher genetic drift and contributions from other undetermined factors.

In 2006, Odani *et al*. studied the degree of linkage disequilibrium for the first time in beef cattle. The study compared the level of LD between two breeds, Japanese Black and Japanese Brown beef cattle. Japanese Black cattle are known for its meat quality with prominent marbling while Japanese Brown cattle are characterized by larger mature size and faster growth rate than Japanese black. Linkage disequilibrium was measured using the parameter D' and significance of allelic associations were tested between syntenic and non-syntenic marker pairs. The Japanese black pedigree consisting of one sire and his 162 half-sib progeny was genotyped for 246 autosomal microsatellite loci, while a Japanese brown pedigree consisting of one sire and his 406 half-sib progeny were genotyped for 156 autosomal microsatellite loci. The study found high levels of LD among syntenic loci in both breeds, which ranged over several tens of cM. In general, significant LD was observed more frequently in Japanese Brown than in Japanese Black cattle. Linkage disequilibrium between non-syntenic loci was significant in Japanese Brown, while it was not found to be significant in Japanese Black. The study noted that this may be due to difference in sample size between the two breeds, as the P-values obtained from the test of significant departure from linkage equilibrium between loci depend largely on sample sizes. Therefore, even a weak LD could become statistically significant due to large samples.

Another study focused on BTA6 and estimated linkage disequilibrium in Holstein-Friesian cattle by genotyping a sample of 45 bulls for 15 closely-spaced microsatellites on two regions of the chromosome reported to harbor QTL for dairy traits (Khatkar *et al*. 2006a). LD was estimated using D' and the results

indicated high levels of LD (extending up to 18 Mb) on BTA6 in this Australian cattle population supporting previous studies of Farnir *et al*. (2000) and Vallejo *et al*. (2003). All of the above-mentioned previous studies were carried out using very informative microsatellite markers, but at a low marker density. In humans, extent of LD estimated using microsatellites is known to extend over longer distances compared to SNP based estimates of LD (Pritchard and Przeworski 2001). It would be interesting to see if such a pattern could be seen in cattle. With the completion of the bovine genome sequencing project, more and more SNP markers have become available, thereby increasing resolution of the bovine SNP map. In addition, their abundance throughout the genome (Snelling *et al*. 2005) and ease and low cost of large scale SNP genotyping (Hinds *et al*. 2005) have made SNPs the prime choice for mapping. Later on, Khatkar *et al*. (2006b) constructed a metric linkage disequilibrium map of BTA6 by genotyping 433 Australian dairy bulls for 220 SNP markers. The distance over which LD is likely to be useful for mapping was found to be 13.3 Mb, thus confirming extensive LD in Holstein-Friesian cattle. This estimate of 13.3 Mb calculated using SNP markers was found to be lower than the LD estimate based on low density microsatellite marker (18 Mb) on the same chromosome (Khatkar *et al*. 2006a).

More recently, McKay *et al*. (2007b) estimated linkage disequilibrium in eight breeds of cattle from the *Bos taurus* and *Bos indus* subspecies. Breeds from *Bos taurus* included Angus, Charolais, Dutch Black and White, Holstein, Japanese Black and Limousin, while breeds from *Bos indicus* included Brahman and Nellore. Approximately 2670 SNP markers across the bovine genome were used

to estimate pairwise r^2 values. The study found that LD extends up to 0.5 Mb in these eight breeds of cattle, which was in contrast with the long range LD found in previous studies (Farnir *et al*. 2000). This difference was attributed to differences in the measures used to report LD, D' and r^2. McKay *et al.* (2007b) found that the extent of LD was very similar within all the *Bos taurus* and *Bos indicus* breeds. However, *Bos indicus* breeds appear to have considerably lower levels of LD at short inter-marker distances than *Bos taurus*. This could be the result of effective population size or due to ascertainment bias. The majority of the SNP used in this study were previously identified as being variable within the *Bos taurus* genome, which could have resulted in ascertainment bias. This caused the minor allele frequencies of SNPs to be considerably lower in the *Bos indicus* breeds than in the *Bos taurus* breeds. It also resulted in the over-representation of common SNP within the *Bos taurus* genome. The study found that a minimum of 50,000 SNP markers would be required for whole genome association studies in cattle. However, the average r^2 values for BTA19 in McKay *et al*. (2007b) were not shown due to the presence of less than five informative locus pairs. Also, this study used 55 markers to estimate LD on BTA29. The time this dissertation was carried out there were no reports available on the extent of LD using high resolution SNP markers on BTA19 and 29. Later on, Sargolzaei *et al.* (2008) characterized the extent of LD in North American Holstein population using a total of 5,564 SNPs distributed across the bovine genome. The study found out that useful LD (measured as $r^2 > 0.3$) occurred at distances shorter than 100 kb and

suggested the use of a much denser SNP map for whole-genome fine mapping and genomic selection.

1.4. QTL Mapping

Most of the economically important traits are quantitative in nature which show a continuous range of phenotypes that cannot be easily classified into distinct categories. These traits are controlled by simultaneous segregation of many genes; each contributing a small amount to the value of the trait and following standard Mendelian rules of segregation. In addition, these traits are also influenced by environmental effects: for example measurement error, instrument limitations etc. Two models have been proposed to explain the genetic variation observed in such traits, the infinitesimal model and the finite loci model. The infinitesimal model assumes that such traits are controlled by an infinite number of loci each with infinitesimally small effect (Fischer 1918). However, a study by Ewing and Green (2000) found that there are only about 35,000 genes in the human genome suggesting that there must be some finite number of loci underlying the variation in quantitative traits. In 2004, the International Human Genome Sequencing Consortium reported that the human genome seems to encode only 20,000-25,000 protein-coding genes. Later on, the discovery of the effect of *Hal* gene on meat quality in pigs directed to a mixed model of inheritance of quantitative traits with many genes of small effect and a few genes with large effect (Hayes and Goddard 2001). The information from the hunt of these loci, underlying variation in quantitative traits, will be used to increase the accuracy of genetically superior

animals. There are several traits of economic importance in cattle. However, the traits studied in this thesis have been outlined in sections 1.4.1 and 1.4.2.

1.4.1. Carcass Merit Traits

Beef consumers expect lean, but tasty and juicy product. Therefore, carcass quality traits are of great importance to consumer satisfaction and ultimately determine the market value of the product. Carcass merit traits cannot be improved very efficiently using the conventional breeding program as the phenotype is only collected once the animal is slaughtered. Improvement of these traits can be carried out by selection using genetic markers via MAS. These traits have moderate to high heritability and consequently can be successfully selected in beef breeding programs. There are several carcass merit traits of interest such as backfat thickness, marbling score, ribeye area, carcass weight, yield grade, quality grade and lean meat yield. Backfat thickness is the subcutaneous fat thickness between twelfth and thirteen ribs. An excess of backfat is a waste. However, an optimum amount is important as it protects meat from chilling too quickly in the cooler and also enhances the tenderization process. Yield grade becomes less desirable as backfat thickness increases (University of California Cooperative Extension 2004). Marbling is the intramuscular fat or flecks of fat in the ribeye muscle, which makes the meat cut more tender and juicy. The more the marbling, the higher the quality grades will be, which results in increased consumer preferences. Ribeye area is the longissimus muscle measured between the 12^{th} and 13^{th} rib on the beef forequarter. It is the largest muscle in the body and gives an indication of overall

carcass muscling (Manitoba Agriculture, Food and Rural Initiatives 2008). Carcass weight is the hot or unchilled weight of carcass in pounds which is measured after removing hide, head, intestinal tract and internal organs. Yield grade measures the degree of fattening in carcass and labels a carcass as to the amount of red meat available, listed as Y1, Y2 and Y3 according to Canadian Beef Grading Agency. Yield grade 1 specifies the most meat and the least amount of fat, whereas, Yield grade 3 specifies carcasses with the most fat. Quality grade is determined by a composite evaluation of factors that affect the palatability of meat. Such factors include carcass maturity, firmness, texture, color of lean, amount and distribution of marbling within lean. The different quality grades in Canada are Canada Prime, Canada A, AA, AAA, B1, B2, B3, B4, D1, D2, D3, D4 and E. Lean meat yield is the yield reported by a grader as an estimation of the percentage of the carcass that is red meat (Canadian Beef Grading Agency 2008).

1.4.2. Dairy traits

In recent years, several tools have been used in the dairy industry to carry out selection such as artificial insemination and estimated breeding values (EBV) which has resulted in increased milk production and improved production systems. There are several traits of interests in the dairy industry: Milk production, functional and conformation traits. Milk production traits include milk production, fat yield, protein yield, fat percent, and protein % (Kolbehdari *et al.* 2009). Example of functional traits include somatic cell score count (SCS), herd life, persistency, daughter fertility, milking speed, milking temperament, calving ease

and maternal calving ease. Conformation traits include two types of traits, scorecard traits and descriptive traits. Examples of scorecard traits include conformation, mammary system, feet and legs, dairy strength and rump. Descriptive traits include angularity, bone quality, foot angle, heel depth, median suspensory, stature and udder texture (Kolbehdari *et al.* 2008).

1.4.3. Approaches to identify QTL

1.4.3.1. Candidate gene approach

In the candidate gene approach, a candidate gene with a potential role in the physiology of the trait is assumed to harbor the causative mutation for the variation of quantitative traits. A candidate gene can also be selected on the basis of the role of the gene in the physiology of a trait in another species. The candidate gene or parts of the gene is sequenced in some animals and any variation observed is then tested for association with the quantitative trait. There are two disadvantages of using this approach. First, there are several genes with potential roles in the physiology of the trait. Therefore, sequencing of large number of genes would have to be carried out and large numbers of association studies have to be performed. Second, the causative mutation may lie in a gene that would not have been considered as an apparent candidate for the trait of interest (Hayes 2007).

1.4.3.2. Genome scan approach

Another approach to identifying QTL is a genome scan approach which aims at identifying chromosomal regions associated with variation in the phenotypic traits. This approach assumes that the gene causing variation in the trait is unknown. Rather, it uses DNA markers to test for association between variations at the molecular level with the variation in the quantitative trait. If such an association is found, it implies that either the genetic variation at that locus affects the trait of interest or it is in linkage disequilibrium with the causative mutation (Mueller 2004).

Another strategy can be employed where both genome scan and candidate gene approach can be used to identify positional candidate genes. A chromosomal region associated with the phenotypic variation can be identified in a genome scan and then a candidate gene lying within that region with a possible role in the physiology of the trait can be studied as candidate gene. Such genes are called positional candidate genes. Following are the two main methods of mapping QTL.

1.4.3.2.1. Single marker association analysis

This method uses one marker at a time to test for association with a QTL and does not require knowledge of marker order or a linkage map. Single marker analysis compares the markers' genotypic means through a regression for the trait on coded marker genotypes by a t-test, an analysis of variance or a likelihood ratio test. A QTL is defined to be located near a marker for which the phenotypic values of the

trait differ significantly among their genotypic means (Liu *et al*. 1998). One of the disadvantages of using this method is that it is difficult to distinguish between the size of a QTL effect and its position and has less power if the markers are far apart. Therefore, exact location of the QTL cannot be estimated (Armidale Animal Breeding Summer Course 2003). Grapes *et al*. (2004) carried out a simulation study to compare the haplotype based or an identity by descent (IBD) model with single marker based regression methods to determine if haplotypes provide additional information for fine mapping QTL. The study found that when 10 markers were genotyped, the IBD based methods estimated the position of QTL more accurately than single marker regression methods. However, when 20 markers were genotyped, the mapping accuracy of regression based methods was comparable to or greater than IBD based methods. Therefore, it was concluded that genotyping of additional markers can make the single marker regression method much more robust to detect a QTL.

1.4.3.2.2. Interval mapping

Lander and Botstein (1989) first coined the term ‘interval mapping’ to describe mapping of a QTL between a pair of linked markers. There is much less confounding between QTL effect and its position than the single marker analysis (Armidale Animal Breeding Summer Course 2003). Interval mapping method estimates the parameters and unknown genotypes of the putative QTL by an expectation-maximization (EM) algorithm (Lander and Botstein 1989). The likely location of the QTL explained by LOD score, defined as the logarithm of the

likelihood ratio to the base ten, tests for the presence of a putative QTL at every locus (Satagopan *et al.* 1996).

Many economically important quantitative traits are affected by several genes with varying size of effects. Both single marker analysis and interval mapping have been modified to incorporate multiple QTL model in a step-wise fashion, where the fitting of a single-locus model is followed by examining the residuals to detect a second QTL and so on. However, step-wise fitting of models results in biased estimates of gene effect and often result in "ghost QTL", when actually no QTL exists (Knott and Haley 1992, Martinez and Curnow 1992, Satagopan *et al*. 1996). To address the issue of detecting multiple QTL simultaneously, more advanced methods such as Bayesian approaches have been developed. Bayesian methods sample from the joint posterior of the unknown parameters and missing data. Satagopan *et al*. (1996) suggests that Non-Bayesian methods, while calculating QTL confidence intervals, do not properly account for uncertainties in other parameters. Bayesian methods, however, do not completely overcome the issue but addresses such uncertainties. To identify multiple QTL and to estimate the size of their effect, Markov Chain Monte Carlo (MCMC) techniques are often used. MCMC utilizes a Bayesian approach to incorporate a multi-locus model rather than fitting one-locus at a time. In MCMC, the phenotypic trait is modeled as a linear function of additive and dominance effects of the unknown QTL genotypes (Satagopan *et al*. 1996). The location of QTL and their effects are obtained from the corresponding marginal posterior densities calculated by integrating the likelihood, rather than by optimizing the joint

likelihood surface as mentioned in Satagopan *et al*. (1996). This is accomplished by treating the unknown QTL genotypes and any missing marker genotypes as a supplement data and then incorporating these unknowns in the Markov Chain cycle along with the unknown parameters (Satagopan *et al*. 1996). The detection of QTL is explained by Bayes factor (posterior/prior ratio) instead of calculating the likelihood of the parameters (Kass and Raftery 1995). A Bayes factor of 3 or 2 $\log_e$ (BF) = 2.1 suggests significance of the presence of a QTL (Kass and Raftery 1995).

1.4.4. QTL studies on BTA19 and 29

The first study which utilized genetic markers to detect QTL was carried out by Sax *et al*. (1923). The study examined the association between morphological markers (seed coat pattern and pigmentation) and phenotypic trait (seed size differences) in *Phaseolus vulgaris*. Since then, several QTL mapping studies have been performed in cattle. The details of the QTL detected on BTA19 and 29 in previous studies have been shown in Table 1-1.

Table 1-1. Summary of QTL detected on BTA19 and 29.

BTA	Trait	QTL Location (cM)	Reference
19	Adjusted fat	45.92-73.23	Taylor *et al*. 1998
19	Ether extractable fat	45.92-73.23	Taylor *et al*. 1998
19	Ribeye muscle area	45.92-73.23	Taylor *et al*. 1998
19	Retail product yield	5.35-39.58	Casas *et al*. 2003
19	Yield grade	5.35-39.58	Casas *et al*. 2003
19	Backfat	4.8-15.9	Li *et al*. 2004
19	Backfat	39.4-46.5	Li *et al*. 2004
19	Backfat	65.7-99.5	Li *et al*. 2004
19	Ovulation rate	86.01-90.04	Kirkpatrick *et al*. 2000
19	Resistance to BSE	86.01-90.04	Zhang *et al*. 2004
19	Preweaning average daily gain	5.35-16.04	Kneeland *et al*. 2004
19	Average daily gain on feed	73.23-98.8	Taylor *et al*. 1998
19	Average daily gain on feed	51.34-52.19	Kneeland *et al*. 2004
19	Birth weight	73.23-98.8	Taylor *et al*. 1998
19	Milk fat	69.83	Shariflou *et al*. 2000
19	Milk fat	77.68-86.01	Bennewitz *et al*. 2003
19	Milk fat %	69.83-77.38	Viitala *et al*. 2003
19	Milk fat%	77.68	Boichard *et al*. 2003
19	Milk protein	69.83	Shariflou *et al*. 2000
19	Milk protein %	63.18	Lagziel *et al*. 1999
19	Milk yield	69.83	Shariflou *et al*. 2000
29	Milking speed	2.92-21.11	Hiendleder *et al*. 2003
29	Milking temperament	2.92-21.11	Hiendleder *et al*. 2003
29	Marbling score	6.8-24.48	MacNeil and Grosz 2002
29	Marbling score	40.16-62.53	MacNeil and Grosz 2002
29	Hot carcass weight	40.16-62.53	MacNeil and Grosz 2002
29	Meat tenderness	37.15-65.64	Casas *et al*. 2000
29	Meat tenderness	59.60-69.009	Smith *et al*. 2000
29	Birth weight	1.81-11.29	Alexander *et al*. 2007
29	Milk protein	21.11-62.53	Viitala *et al*. 2003
29	Milk yield	21.11-62.53	Viitala *et al*. 2003

1.5. Objectives

The goal of this thesis is to utilize the genetic variations on bovine chromosomes 19 and 29 as a tool to detect QTL in beef and dairy cattle. This goal would be accomplished by the construction of high resolution radiation hybrid (RH) maps and the estimation of linkage disequilibrium in beef and dairy cattle. The hypothesis is that there is extensive linkage disequilibrium and that there are QTL which affect several economically important traits on these chromosomes in beef and dairy cattle. The specific objectives of this thesis are:

1. Construction of high resolution radiation hybrid maps of BTA19 and 29. The RH maps are presented in Chapter 2 of the thesis.
2. Estimation of linkage disequilibrium and signatures of selection on BTA19 and 29 in beef and dairy cattle. Chapter 3 presents the findings of this study.
3. Detection of QTL for milk production, functional and conformation traits on BTA19 and 29 in Canadian Holstein cattle. A detailed analysis of QTL detected in this dairy population with validation of some of the markers in a larger size of dairy population is presented in Chapter 4.
4. Detection of QTL for carcass merit and fat metabolism traits on BTA19 and 29 in beef cattle. The QTL detected in this objective as well as validation of a subset of markers in an independent beef population are described in Chapter 5 of the thesis.

1.6. References

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2. High Resolution Radiation Hybrid Maps of Bovine Chromosomes 19 and 29: Comparison with the Bovine Genome Sequence Assembly

2.1. Background

Molecular genetic information of the major agricultural species, like cattle, is crucial in harnessing the benefit of genetic variation for economically important traits. The process of exploiting this information is greatly facilitated by the ordering of molecular markers along the chromosomes. High resolution RH mapping is a valuable approach to build maps, where both polymorphic as well as non-polymorphic markers can be included (Cox *et al*. 1990). Of the several whole genome radiation hybrid panels available for cattle (Williams *et al*. 2002, Womack *et al*. 1997, Itoh *et al*. 2005, Rexroad *et al*. 2000), the 12,000 rad whole genome RH (12K WG-RH) panel has been shown to have the highest mapping resolution (Schläpfer *et al*. 2002, Weikard *et al*. 2002, Liu *et al*. 2003, Weikard *et al*. 2006). Radiation hybrid maps also serve as one of the tools to facilitate the assembly of genome sequences (Weikard *et al*. 2006, Jann *et al*. 2006, Leroux *et al*. 2005). Direct comparison of an RH map with a genome assembly allows identification of inconsistencies between the optimal marker order, found using the RH data, and the marker order observed in the current genome assembly.

The bovine genome sequencing project, started in 2003, has released three different assemblies of the genome. The first preliminary assembly (Bovine build 1.0), produced with 3X coverage, was released in September 2004; the second assembly (Bovine build 2.0) with 6.2X coverage in June 2005; and the

third draft assembly (Bovine build 3.1) with 7.1X coverage in August 2006 (http://www.hgsc.bcm.tmc.edu/projects/bovine/). The third draft assembly was produced using a combination of whole genome shotgun reads and BAC end sequences (http://www.hgsc.bcm.tmc.edu/projects/bovine/). Previous comparisons of radiation hybrid mapping data with bovine genome sequence assembly (Bovine build 2.0) have shown large discrepancies on many chromosomes including BTA19 (156 mapped markers) and BTA29 (149 mapped markers) (Jann *et al*. 2006). These discrepancies and the fact that there have been many QTL identified on these chromosomes (Stone *et al*. 1999, Casas *et al*. 2001, MacNeil and Grosz 2002, Li *et al*. 2004), has prompted us to choose BTA19 and 29 as candidate chromosomes for high resolution mapping.

The traditional approach of RH mapping is to heuristically produce a so-called framework map, incorporating only a fraction of typed markers which are reliably ordered. However, a major disadvantage of building framework maps is that they position the remaining unplaced markers into bins of confidence, which may not be of true order. Instead, we have constructed high resolution maps of BTA19 and 29 using the comparative RH mapping approach recently introduced in CarthaGène (http://www.inra.fr/bia/T/ CarthaGène /, Schiex and Gaspin 1997, de Givry *et al*. 2005). This approach is based on a probabilistic Bayesian model integrating the usual RH probabilistic model with a probabilistic model of breakpoint occurrences with a reference order, typically obtained from the position of orthologous markers in a related sequenced genome (Faraut *et al*. 2007). In this probabilistic model, breakpoints induced by chromosomal

rearrangements are considered as rare events, following a Poisson law. Equivalently, we consider that genome assembly errors create rare spurious breakpoints between the RH map order and the current assembly order. Therefore, CarthaGène was used to produce a new RH map integrating the RH data with the current bovine genome assembly.

The objective of this study was to generate high resolution RH maps of BTA19 and 29, and to compare them with the current cattle genome sequence build. We also constructed cattle-human comparative maps of BTA19 and 29, which are known to be orthologous to human chromosome 17 (HSA17) and HSA11 respectively (Yang and Womack 1995, Amarante *et al*. 2000, Schibler *et al*. 2006). This comparative mapping information as well as the high resolution RH map provides an important independent source of information to improve the bovine genome sequence assembly.

2.2. Results and discussion

2.2.1. Genotyping of 12,000 rad panel and RH map construction

The bovine 12,000 rad panel was constructed to complement an existing 5000 rad panel and increase the mapping resolution (Womack *et al*. 1997, Rexroad *et al*. 2000). We used SNP markers for RH mapping because of their availability from the bovine genome sequencing project, their abundance throughout the genome (Snelling *et al*. 2005) and the ease and low cost of large scale SNP genotyping (Hinds *et al*. 2005). Correct SNP marker order is also essential for a variety of gene discovery approaches such as interval mapping or linkage

disequilibrium based methods. The SNP markers were chosen from the bovine build 2.0 and typed on the 12 K WG-RH panel using the Illumina BeadStation Genotyping System (Oliphant *et al*. 2002). This genotyping system produces reproducible and robust data due to its 30 fold redundancy at each locus. There is an average of 30 representatives of each bead type present on every array which allows for 30 independent genotypes of each SNP locus. Three positive (bovine genomic DNA) and three negative (rodent genomic DNA) controls were used in the experiment. All markers observed with even a small amount of amplification in any of the three negative controls were discarded. Also, any markers which did not exhibit clear cluster separation between positive and negative controls were discarded. The remaining markers were scored as described previously (McKay *et al*. 2007). A total of 66.7% (668 out of 1001) loci on BTA19 and 68.4% (366 out of 535) loci on BTA29 were successfully amplified and scored. Markers were selected from the bovine build 2.0 which had a significant number of SNPs misassigned to the wrong chromosomes. Hence, out of 668 and 366 successfully amplified loci on BTA19 and 29, we mapped 555 and 253 markers on BTA19 and BTA29, respectively. The details of the SNP markers mapped on BTA19 and 29 are provided in Table 2-1.

Table 2-1. NCBI IDs and position of SNP markers (in cR) mapped on BTA19 and 29

Chromosome	Markers	Position (cR)	NCBI Ids
19	BTA-25257	0	ss61478156
19	BTA-25119	25.9	ss61500417
19	BTA-46468	50.9	ss61483626
19	BTA-109954	77.7	ss61517100
19	BTA-86608	120.3	ss61493961
19	BTA-86613	124.5	ss61493966
19	BTA-86615	124.5	ss61493968
19	BTA-117829	134.6	ss61474593
19	BTA-117833	139.5	ss61474597
19	BTA-117835	143.3	ss61474599
19	BTA-87957	143.3	ss61563670
19	BTA-87958	145.3	ss61563671
19	BTA-22161	167.1	ss61528108
19	BTA-22160	172.5	ss61528107
19	BTA-22162	172.5	ss61500155
19	BTA-22155	174.3	ss61528102
19	SCAFFOLD210001_43773	188.5	ss38327778
19	BTA-05727	190.7	ss38327779
19	BTA-22149	190.7	ss61528098
19	BTA-22150	190.7	ss61528099
19	BTA-22153	190.7	ss61477401
19	BTA-08011	196.7	ss38330063
19	BTA-22143	198.5	ss61528092
19	BTA-96250	208	ss61505627
19	BTA-96256	208	ss61505629
19	BTA-22140	213.6	ss61528089
19	BTA-22142	217.1	ss61528091
19	BTA-28123	232.2	ss61531326
19	BTA-28126	232.2	ss61531329
19	BTA-28135	232.2	ss61531335
19	BTA-28131	235.8	ss61531334
19	BTA-02315	250.9	ss38324367
19	BTA-108967	258.1	ss61506376
19	BTA-108969	258.1	ss61506378
19	BTA-28111	272.2	ss61478864
19	BTA-28119	283.9	ss61531322
19	BTA-28104	287.7	ss61478862
19	BTA-28106	287.7	ss61531312
19	BTA-28107	287.7	ss61531313

19	BTA-28108	287.7	ss61531314
19	BTA-28112	287.7	ss61478865
19	BTA-28152	287.7	ss61531349
19	BTA-28153	287.7	ss61531350
19	BTA-28120	289.4	ss61531323
19	BTA-28121	289.4	ss61531324
19	BTA-28151	289.4	ss61531348
19	BTA-46442	293	ss61541098
19	BTA-46430	296.6	ss61541090
19	BTA-46432	303.6	ss61541092
19	BTA-46433	311.9	ss61541093
19	BTA-13349	325	ss38335401
19	BTA-46575	329.5	ss61541173
19	BTA-04223	341.1	ss38326275
19	BTA-44652	382.6	ss61467782
19	BTA-44665	382.6	ss61540135
19	BTA-44677	384.5	ss61540145
19	BTA-44716	390.5	ss61540166
19	BTA-44725	394.6	ss61467784
19	BTA-44761	405.7	ss61540207
19	SCAFFOLD226442_3035	411.1	ss38328701
19	BTA-06651	414	ss38328703
19	BTA-44787	414	ss61540228
19	BTA-44793	420.1	ss61483133
19	BTA-44815	423.7	ss61540241
19	BTA-44817	434.9	ss61540243
19	BTA-44865	434.9	ss61483148
19	BTA-44888	437.6	ss61540285
19	BTA-44889	440.2	ss61540286
19	BTA-44893	440.2	ss61540289
19	BTA-44927	447.4	ss61540307
19	BTA-44928	447.4	ss61540308
19	BTA-44930	447.4	ss61483164
19	BTA-44965	449.6	ss61540335
19	BTA-91865	456.2	ss61495411
19	BTA-45143	467.2	ss61540437
19	BTA-45487	479.6	ss61540586
19	BTA-45490	487.3	ss61540589
19	BTA-45492	489	ss61540591
19	BTA-45491	504	ss61540590
19	BTA-45669	517.6	ss61483394
19	BTA-45635	530.4	ss61540667
19	BTA-45631	532.3	ss61483384

19	BTA-45632	532.3	ss61483385
19	BTA-45636	532.3	ss61540668
19	BZ857409-C89KA	534	ss69357390
19	BTA-45584	536.1	ss61540643
19	BTA-45586	536.1	ss61483372
19	BTA-45574	542.7	ss61540637
19	CC531035-G564FA	546.1	ss69357391
19	BTA-11204	547.8	ss38333256
19	BTA-45159	570.5	ss61540445
19	BTA-45686	584.8	ss61540686
19	BTA-45689	588.6	ss61540689
19	BTA-45688	597.4	ss61540688
19	CC590090-C167FA	607.9	ss69357392
19	BZ886415-T167FG	618.4	ss69357393
19	BTA-45703	621.9	ss61483406
19	BTA-45726	648	ss61540711
19	BTA-45733	673.5	ss61540717
19	BTA-16243	681	ss61525107
19	CC498982-T72KC	689.6	ss69357395
19	CC498982-T89BC	689.6	ss69357394
19	CC498982-G89BA	691.3	ss69357396
19	BZ872308-T167FA	698.2	ss69357397
19	BTA-16709	715.2	ss61525327
19	SCAFFOLD23408_767	718.9	ss38328944
19	BTA-16718	724.4	ss61525329
19	BTA-104142	747	ss61471256
19	BTA-45810	769.1	ss61540749
19	BTA-46435	779.3	ss61541095
19	BTA-46436	779.3	ss61541096
19	BTA-46438	779.3	ss61508913
19	BTA-46440	784.8	ss61483612
19	BTA-13223	800.7	ss38335275
19	BTA-45982	800.7	ss61508872
19	BZ840034-A72KT	812.6	ss69357399
19	BZ840034-C72KT	812.6	ss69357398
19	BZ840034-A167FC	818.6	ss69357400
19	CC538776-CWR1752T	836.3	ss69357401
19	CC538776-G167FT	841	ss69357402
19	CC538776-TGR527C	845.7	ss69357403
19	BZ953217-CRM25KT	853.6	ss69357404
19	BTA-24942	863.3	ss61529599
19	BTA-24946	863.3	ss61529603
19	CC546172-T89BC	874	ss69357405

19	BTA-46447	878	ss61541103
19	CC507099-TGR527C	884.2	ss69357406
19	CC507099-A91DC	887.9	ss69357407
19	BTA-86490	898.2	ss61562878
19	BTA-86493	902.1	ss61562881
19	SCAFFOLD105007_21421	908.2	ss38322368
19	BTA-00316	910	ss38322368
19	BTA-86498	913.7	ss61493920
19	CC474822-GGR527C	917.4	ss69357408
19	CC767956-GRM25KC	922.9	ss69357409
19	BTA-93463	924.7	ss61566568
19	BZ914683-C93KT	926.5	ss69357410
19	CC509023-G167FA	933.8	ss69357414
19	CC518784-T89BG	933.8	ss69357416
19	CC574701-G89BA	933.8	ss69357411
19	CC574701-T167FC	933.8	ss69357413
19	CC574701-T89BC	933.8	ss69357415
19	CC574701-T91DC	933.8	ss69357412
19	BTA-93482	951.1	ss61566577
19	SCAFFOLD110615_4785	960	ss38322758
19	BTA-25637	979.3	ss61530009
19	CC571398-T89KC	988.5	ss69357417
19	BTA-46509	1015.9	ss61508916
19	BTA-97840	1022.6	ss61496935
19	BZ871466-CGR527T	1025.8	ss69357418
19	BZ924124-C69KG	1025.8	ss69357419
19	CC551636-GGR527C	1029	ss69357420
19	BTA-46474	1043.9	ss61541111
19	CC511666-T72KC	1055.7	ss69357421
19	CC519175-G89BA	1066	ss69357422
19	BTA-46456	1067.6	ss61483618
19	BTA-46449	1072.7	ss61541105
19	BZ859440-A89BC	1093.5	ss69357423
19	BZ859440-G89BA	1098.6	ss69357424
19	CC511143-A72K2G	1098.6	ss69357425
19	CC511143-G91DC	1098.6	ss69357426
19	BTA-46514	1107.3	ss61541141
19	BTA-46516	1109	ss61541143
19	SCAFFOLD276848_2797	1127.8	ss38331266
19	BTA-09214	1131.9	ss38331266
19	BTA-46564	1138.1	ss61541164
19	BTA-46552	1162.1	ss61508919
19	BTA-46543	1181.7	ss61541153

19	BTA-05909	1187	ss38327961
19	BTA-29947	1204.5	ss61532373
19	BTA-29943	1206.3	ss61532369
19	BTA-46527	1211.7	ss61502080
19	BTA-44521	1222.8	ss61467779
19	BTA-07806	1228.1	ss38329858
19	BTA-44536	1228.1	ss62520670
19	BZ838039-T89K2C	1228.1	ss69357427
19	CC532859-T93KC	1228.1	ss69357428
19	CC594171-C89BT	1228.1	ss69357429
19	BZ838039-A89K2G	1235.3	ss69357430
19	CC500064-A89K2G	1240.5	ss69357431
19	BTA-44540	1249.2	ss61540074
19	BTA-11922	1263.2	ss38333974
19	BTA-44552	1268.9	ss61540075
19	CC481382-C167FT	1275.8	ss69357432
19	BTA-44546	1279.7	ss61483081
19	BTA-44555	1279.7	ss61483089
19	BTA-44561	1287.6	ss61483090
19	BZ872811-C91DT	1289.9	ss69357433
19	BTA-44563	1308	ss61483092
19	BTA-44565	1323	ss61540078
19	BTA-44583	1340.9	ss61540092
19	BTA-44603	1368.4	ss61483099
19	BTA-44594	1379.5	ss61501960
19	BTA-44618	1396	ss61508803
19	BTA-44616	1399.3	ss61483106
19	BTA-13335	1401.1	ss38335387
19	BTA-44615	1401.1	ss61540107
19	BTA-44609	1418.6	ss61540104
19	BTA-44610	1422.2	ss61483103
19	BTA-44495	1447	ss61540055
19	BTA-44501	1447	ss61540061
19	BTA-20575	1460.3	ss61527391
19	BTA-46576	1471.4	ss61483648
19	BTA-46580	1471.4	ss61483651
19	BTA-46585	1471.4	ss61483654
19	BTA-46586	1471.4	ss61483655
19	BTA-46571	1473.1	ss61541169
19	BTA-15926	1480.1	ss61475707
19	BTA-44631	1489	ss61540116
19	BTA-44637	1494.4	ss61540119
19	BTA-44638	1497.9	ss61540120

19	BTA-44649	1511.2	ss61540123
19	BTA-44663	1530.9	ss61540133
19	BTA-44669	1548.3	ss61540139
19	BTA-18793	1579.1	ss61526405
19	BTA-07830	1592.8	ss38329882
19	BTA-04414	1594.6	ss38326466
19	BTA-118485	1594.6	ss61474762
19	BTA-44726	1617.1	ss61508809
19	BTA-44731	1624.6	ss61540179
19	BTA-44751	1632.1	ss61540197
19	BTA-44791	1688.2	ss61483132
19	BTA-44801	1703.3	ss61540235
19	BTA-01578	1727.9	ss38323630
19	BTA-44833	1735.2	ss61501974
19	BTA-44838	1747.8	ss61540255
19	BTA-44845	1752.7	ss61483141
19	BTA-115853	1769.8	ss61520292
19	BTA-11532	1779	ss38333584
19	BTA-44868	1779	ss61540275
19	BTA-07396	1810.1	ss38329448
19	BTA-108581	1823.8	ss61465737
19	BTA-44691	1855.1	ss61483117
19	BTA-44690	1863	ss61483116
19	BTA-44693	1866	ss61540153
19	BTA-44708	1887	ss61540159
19	BTA-08382	1901.4	ss38330434
19	BTA-105808	1903.6	ss61497989
19	BTA-98517	1924	ss61569222
19	BTA-20935	1968.2	ss61527570
19	BTA-44712	1978.5	ss61540162
19	BTA-14962	1990.5	ss38337014
19	BTA-44959	2015.2	ss61483169
19	BTA-44960	2017.1	ss61508819
19	BTA-44964	2025.2	ss61540334
19	BTA-44976	2030.8	ss61540343
19	BTA-44980	2045.7	ss61540347
19	BTA-44981	2045.7	ss61508821
19	BTA-44985	2057.7	ss61540350
19	BTA-44989	2057.7	ss61540354
19	BTA-01174	2067.6	ss38323226
19	BTA-44990	2067.6	ss61540355
19	BTA-44994	2075.5	ss61540357
19	BTA-104726	2087.8	ss61514424

19	BTA-45057	2120.4	ss61540395
19	BTA-67105	2135.2	ss61488938
19	BTA-45030	2158.2	ss61508824
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29	BTA-65113	1448.4	ss61488376
29	BTA-08389	1452.8	ss38330441
29	BTA-65124	1454.9	ss61551376
29	BTA-65147	1466.6	ss61551389
29	BTA-65151	1478.2	ss61488385

29	BTA-65154	1491	ss61488388
29	BTA-65153	1494	ss61488387
29	BTA-65157	1500.7	ss61551392
29	BTA-65162	1505.2	ss61468595
29	BTA-65165	1505.2	ss61551398
29	BTA-65166	1510.2	ss61551399
29	BTA-12811	1619.5	ss38334863
29	BTA-65224	1619.5	ss61488407
29	BTA-65220	1630.5	ss61509924
29	SCAFFOLD208955_20939	1644.7	ss38327767
29	BTA-65388	1653.9	ss61488451
29	BTA-65386	1676.4	ss61551522
29	BTA-85826	1680.3	ss61562525
29	BTA-85843	1682.3	ss61562541
29	BTA-85871	1682.3	ss61511148
29	BTA-85838	1693.4	ss61562536
29	BTA-85869	1693.4	ss61504910
29	BTA-85870	1693.4	ss61504911
29	BTA-65297	1700.3	ss61488425
29	BTA-65291	1708.8	ss61551476
29	BTA-03915	1714.2	ss38325967
29	BTA-65271	1714.2	ss61551459
29	BTA-65277	1714.2	ss61551463
29	BTA-65293	1714.2	ss61488421
29	BTA-65268	1716.3	ss61551456
29	BTA-65272	1716.3	ss61551460
29	BTA-65275	1716.3	ss61488419
29	BTA-65296	1716.3	ss61488424
29	BTA-65301	1716.3	ss61551481
29	BTA-65496	1716.3	ss61551577
29	BTA-65498	1716.3	ss61551579
29	BTA-65504	1716.3	ss61509946
29	BTA-65497	1718.6	ss61551578
29	BTA-106381	1739.1	ss61515245
29	BTA-106382	1748.4	ss61515246
29	BTA-106289	1752.5	ss61515192
29	BTA-106378	1752.5	ss61515242
29	BTA-65467	1756.6	ss61551562
29	BTA-90760	1763.1	ss61495107
29	BTA-90762	1769.8	ss61495109
29	BTA-90745	1774.4	ss61565086
29	BTA-90754	1776.6	ss61565091
29	BTA-90746	1778.8	ss61565087

29	BTA-90748	1778.8	ss61565089
29	BTA-65531	1792.7	ss61488495
29	BTA-65523	1804.3	ss61488489
29	BTA-65524	1804.3	ss61551597
29	BTA-65517	1808.9	ss61551594
29	BTA-65515	1815.7	ss61551592
29	BTA-65505	1832.8	ss61551582
29	BTA-22801	1835.1	ss61528438
29	BTA-22805	1835.1	ss61528442
29	BTA-10760	1837.4	ss38332812
29	BTA-65444	1844.6	ss61488475
29	BTA-65443	1849.9	ss61551550
29	BTA-65427	1869.9	ss61488470
29	BTA-65429	1879	ss61488472
29	BES2_Contig422_801	1891.5	ss66537751
29	BTA-65433	1898.5	ss61503444
29	BTA-74283	1911.4	ss61556182
29	BTA-65408	1936	ss61551538
29	BTA-65395	1956.6	ss61551525
29	SCAFFOLD125425_2197	1973	ss38323458
29	BTA-04535	2017.5	ss38326587
29	BTA-66492	2034.5	ss61488767
29	BTA-10766	2049.6	ss38332818
29	BTA-65574	2063.6	ss61551620
29	BTA-65570	2076.3	ss61488507
29	BTA-65564	2081.2	ss61488502
29	BTA-65568	2086.1	ss61488506
29	BTA-65555	2096.5	ss61509948
29	BTA-65554	2105	ss61509947
29	BTA-65658	2126.9	ss61551661
29	BTA-65662	2128.8	ss61551665
29	BTA-65717	2136.5	ss61551698
29	BTA-65713	2138.4	ss61551694
29	BTA-65699	2148.3	ss61551690
29	BTA-29794	2162	ss61479270
29	BTA-29792	2166.4	ss61479268
29	BTA-02252	2181.4	ss38324304
29	BTA-65681	2186	ss61488542
29	SCAFFOLD170015_32126	2195.5	ss38325791
29	BTA-73109	2214.7	ss61468970
29	BTA-65656	2242.8	ss61551659
29	BTA-65646	2250.8	ss61488537
29	BTA-65642	2253	ss61488533

29	BTA-07368	2261.6	ss38329420
29	BTA-99814	2263.8	ss61512056
29	BTA-102309	2376.9	ss61513209
29	BTA-65775	2390.2	ss61488551
29	BTA-65785	2395.9	ss61551730
29	BTA-65872	2404.9	ss61551768
29	BTA-65879	2441	ss61551771
29	BTA-106994	2446.7	ss61515558
29	BTA-106996	2446.7	ss61515560
29	BTA-65836	2497.7	ss61509959
29	BTA-65845	2497.7	ss61509968
29	SCAFFOLD115786_4123	2522.9	ss38323020
29	BTA-65853	2539.5	ss61509969
29	BTA-66030	2561.7	ss61551855
29	BTA-65950	2582	ss61503493
29	BTA-65947	2591.9	ss61551809
29	BTA-65943	2601.8	ss61551806
29	BTA-09465	2610.4	ss38331517
29	BTA-09466	2615.2	ss38331518
29	BTA-65938	2621.8	ss61488600
29	BTA-66057	2635.9	ss61551868
29	BTA-66045	2648.6	ss61488617
29	BTA-66150	2654.6	ss61488664
29	BTA-66333	2667.2	ss61488714
29	BTA-66126	2669.4	ss61488652
29	BTA-116993	2673.5	ss61474388
29	BTA-117001	2673.5	ss61466131
29	BTA-66071	2692.9	ss61551875
29	BTA-01521	2704	ss38323573
29	BTA-66095	2715.2	ss61488637
29	BTA-66099	2715.2	ss61503513
29	BTA-66106	2717.1	ss61551883
29	BTA-66122	2718.9	ss61488650
29	BTA-66154	2722.7	ss61488668
29	BTA-66215	2749.1	ss61488678
29	SCAFFOLD252706_2287	2810.1	ss38329944
29	BTA-14309	2867.9	ss38336361
29	BTA-44068	2884.1	ss61482957

RH maps were constructed using the comparative mapping approach of CarthaGène software (http://www.inra.fr/bia/T/ CarthaGène /, Schiex and Gaspin 1997, de Givry *et al*. 2005) which allows us to simultaneously exploit the RH data and the knowledge of a known related order. RH likelihood is sensitive to large scale ordering discrepancies, as produced by the assembly errors, but has difficulties to order closely related markers reliably. The assembly itself, despite possible assembly errors, is very informative at low scale (inside BACs). Because it exploits more data than pure RH mapping, it cannot be related to framework mapping. However, as shown earlier (Faraut *et al*. 2007), integrating these two types of information produces high resolution maps of better quality. In this case, it also pinpoints likely assembly errors.

On BTA19, we observed 455 different retention patterns, 390 unique retention patterns and 165 shared compatible retention patterns, out of 555 loci tested. The loci sharing compatible retention patterns suggest that they were so close that radiation could not induce any chromosomal break between them. The average retention frequency for all the mapped markers on BTA19 was 20.7% and varied from 2.8% for BTA-20935 to 87.7 % for BTA-45829 (Figure 2-1). The markers in the close vicinity of thymidine kinase gene on BTA19 reflected higher retention frequencies as this marker was used to select for hybrid cell lines (Womack *et al*. 1997). Similarly on BTA29 we observed 215 different retention patterns, 193 unique retention patterns and 60 shared compatible retention patterns, out of 253 loci tested. The average retention frequency for all the mapped markers on BTA29 was 15.02% and varied from 7.2 % for BTA-

70172 to 26.3% for BTA-09466 (Figure 2-2) with higher retention frequencies towards the telomeric end of the chromosome.

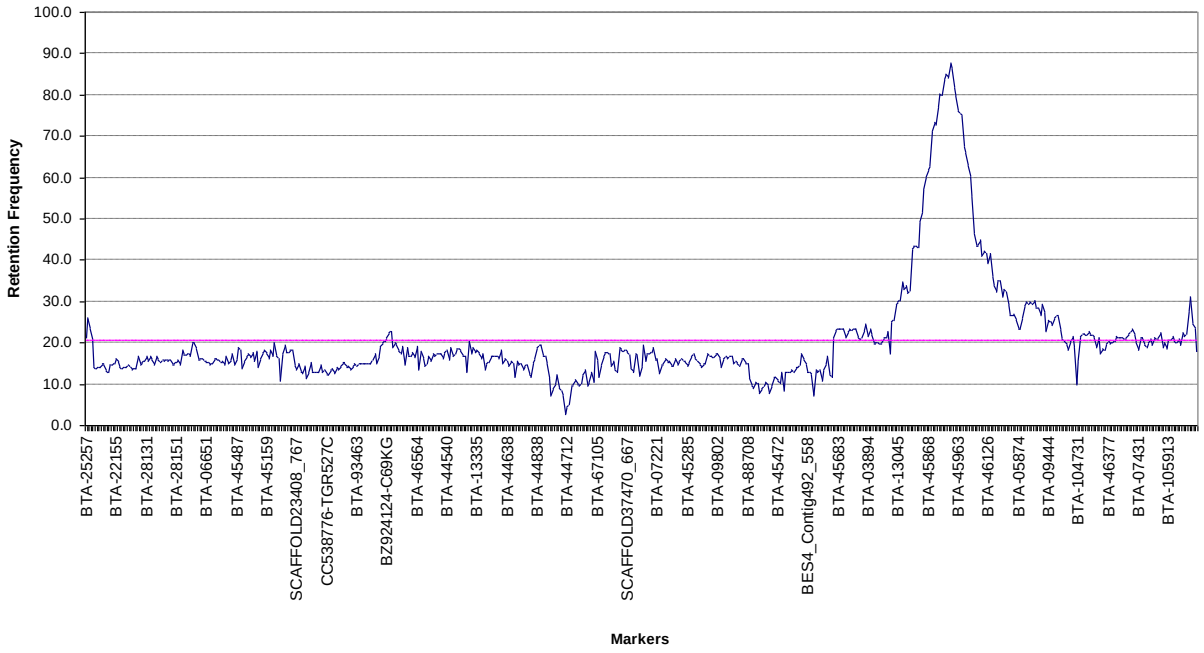

Figure 2-1. Retention frequencies for 555 markers on BTA19. Every seventeenth marker is shown on the X-axis and their corresponding retention frequencies on the Y-axis. The order of the markers in the graph corresponds to the order in the RH map. The left side of the horizontal axis represents centromere and right side represents telomere. The average retention frequency is shown by a pink colored line in the chart.

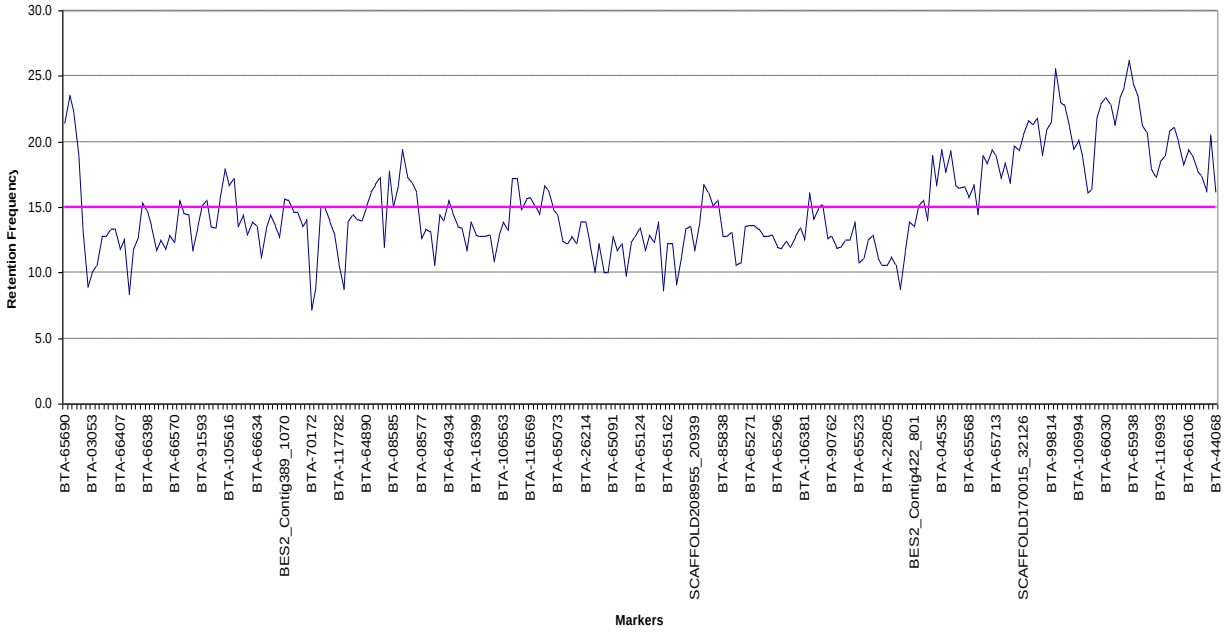

Figure 2-2. Retention frequencies for 253 markers on BTA29. Every sixth marker is shown on the X-axis and their corresponding retention frequencies on the Y-axis. The order of the markers in the graph corresponds to the order in the RH map. The left side of the horizontal axis represents centromere and right side represents telomere. The average retention frequency is shown by a pink coloured line in the chart.

Previous studies have reported that the pattern of retention frequencies varies markedly between chromosomes (Williams *et al*. 2002, Weikard *et al*. 2006). The total length of the RH maps of BTA19 and BTA29 extended to 4591.4 cR and 2884.1 cR, respectively. Additional information about the maps, including the average resolution, and the range and standard deviation of the marker distances, is provided in Table 2-2.

2.2.2. Comparison with the bovine build 3.1 sequences

We aligned our chromosomal maps with the bovine build 3.1 sequences for BTA19 and BTA29 and found an overall agreement of order of loci between the two maps, although a number of inconsistencies were observed. Out of the 555 markers mapped to the 12K map of BTA19, 524 markers were assigned to BTA19 by the bovine genome sequence assembly. For 16 loci, we could detect scaffolds, which were not assigned to any chromosome by the sequence assembly (See Table 2-3, indicated in bold). Fourteen loci did not show acceptable hits with the bovine genome sequence assembly. One hundred and four markers were found to be incongruous and twelve scaffolds were found to be misplaced. Five scaffolds were found to be transposed and six were found to be inverted. In total, seventy four markers within scaffolds were found to be misplaced. One marker, BTA-29943, was assigned bovine chromosome 10 by the sequence assembly (See Table 2-3, indicated in italics and in grey color). In addition, we observed a total of 8 gaps (more than 40 cR) on the BTA19 map (Figures 2-3 and 2-4).

Table 2-2. Summary statistics of the RH maps

Statistics	BTA19	BTA29
Markers typed on 12K RH Panel	1001	535
Markers successfully amplified	668	366
Markers mapped	555	253
Average retention frequency (%)	20.7	15.02
Markers with different retention patterns	455	215
Double markers	100	38
Total length (cR)	4591.4	2884.1
Bovine build 3.1 (bp)	63432577	44728515
Average resolution (Bovine build 3.1 (bp)/ Markers with different retention patterns)	1 locus/139 kb	1 locus/208 kb
Range of marker distances (cR)	0.9-56.2	1.8-134.8
Standard Deviation	8.870832	16.214068

Table 2-3. Assignment of markers in the bovine build 3.1 and corresponding scaffold information. Empty cells represent no acceptable hits of the loci when blasted with bovine genome sequence assembly.

Chromosome	Name	Btau 3.1_s_start (bp)	Scaffolds	Chromosome Assignment
19	BTA-25257	159987	NW_001493640.1	BTA19
19	BTA-25119			
19	BTA-46468	594380	NW_001493640.1	BTA19
19	BTA-109954		**NW_001502493.1**	**Unassigned**
19	BTA-86608		**NW_001501916.1**	**Unassigned**
19	BTA-86615	1673429	NW_001493643.1	BTA19
19	BTA-86613	1673261	NW_001493643.1	BTA19
19	BTA-117829	1815421	NW_001493643.1	BTA19
19	BTA-117833	1815848	NW_001493643.1	BTA19
19	BTA-117835	1816036	NW_001493643.1	BTA19
19	BTA-87957	1880727	NW_001493643.1	BTA19
19	BTA-87958	1880960	NW_001493643.1	BTA19
19	BTA-22161	2148369	NW_001493643.1	BTA19
19	BTA-22160	2159056	NW_001493643.1	BTA19
19	BTA-22162	2148488	NW_001493643.1	BTA19
19	BTA-22155	2159459	NW_001493643.1	BTA19
19	SCAFFOLD210001_43773	2385022	NW_001493643.1	BTA19
19	BTA-22153	2388842	NW_001493643.1	BTA19
19	BTA-22150	2446217	NW_001493643.1	BTA19
19	BTA-22149	2446034	NW_001493643.1	BTA19
19	BTA-05727	2385163	NW_001493643.1	BTA19
19	BTA-08011	2558999	NW_001493643.1	BTA19
19	BTA-22143	2560148	NW_001493643.1	BTA19
19	BTA-96256	2693562	NW_001493643.1	BTA19
19	BTA-96250	2693133	NW_001493643.1	BTA19
19	BTA-22140		**NW_001502587.1**	**Unassigned**
19	BTA-22142			
19	BTA-28126	2892590	NW_001493644.1	BTA19
19	BTA-28123	2892860	NW_001493644.1	BTA19
19	BTA-28135	2882099	NW_001493644.1	BTA19
19	BTA-28131	2889022	NW_001493644.1	BTA19
19	BTA-02315	3054038	NW_001493644.1	BTA19
19	BTA-108967	3084132	NW_001493644.1	BTA19
19	BTA-108969	3083810	NW_001493644.1	BTA19
19	BTA-28111	3155340	NW_001493644.1	BTA19
19	BTA-28119			
19	BTA-28107	3157312	NW_001493644.1	BTA19
19	BTA-28108	3157430	NW_001493644.1	BTA19
19	BTA-28153	3161383	NW_001493644.1	BTA19
19	BTA-28112	3155477	NW_001493644.1	BTA19
19	BTA-28104	3159191	NW_001493644.1	BTA19
19	BTA-28106	3157018	NW_001493644.1	BTA19
19	BTA-28152	3173234	NW_001493644.1	BTA19
19	BTA-28121	3137636	NW_001493644.1	BTA19
19	BTA-28151	3173372	NW_001493644.1	BTA19

19	BTA-28120			
19	BTA-46442	3304479	NW_001493644.1	BTA19
19	BTA-46430	3335659	NW_001493644.1	BTA19
19	BTA-46432	3336040	NW_001493644.1	BTA19
19	BTA-46433	3336269	NW_001493644.1	BTA19
19	BTA-13349	3612492	NW_001493644.1	BTA19
19	BTA-46575		**NW_001502008.1**	**Unassigned**
19	BTA-04223		**NW_001502892.1**	**Unassigned**
19	BTA-44665	4437781	NW_001493645.1	BTA19
19	BTA-44652	4392940	NW_001493645.1	BTA19
19	BTA-44677	4496823	NW_001493645.1	BTA19
19	BTA-44716	4607143	NW_001493645.1	BTA19
19	BTA-44725	4632356	NW_001493645.1	BTA19
19	BTA-44761	4713000	NW_001493645.1	BTA19
19	SCAFFOLD226442_3035	4765116	NW_001493645.1	BTA19
19	BTA-06651	4765081	NW_001493645.1	BTA19
19	BTA-44787	4765466	NW_001493645.1	BTA19
19	BTA-44793	4791088	NW_001493645.1	BTA19
19	BTA-44815	4836142	NW_001493645.1	BTA19
19	BTA-44865	4898966	NW_001493645.1	BTA19
19	BTA-44817	4840954	NW_001493645.1	BTA19
19	BTA-44888	4950633	NW_001493645.1	BTA19
19	BTA-44893	4955188	NW_001493645.1	BTA19
19	BTA-44889	4950776	NW_001493645.1	BTA19
19	BTA-44928	5047651	NW_001493645.1	BTA19
19	BTA-44930	5048765	NW_001493645.1	BTA19
19	BTA-44927	5048028	NW_001493645.1	BTA19
19	BTA-44965	5162772	NW_001493645.1	BTA19
19	BTA-91865	5270649	NW_001493645.1	BTA19
19	BTA-45143	7271040	NW_001493650.1	BTA19
19	BTA-45487	7227465	NW_001493650.1	BTA19
19	BTA-45490	7227099	NW_001493650.1	BTA19
19	BTA-45492	7223128	NW_001493650.1	BTA19
19	BTA-45491	7227000	NW_001493650.1	BTA19
19	BTA-45669	5577645	NW_001493648.1	BTA19
19	BTA-45635	5656693	NW_001493648.1	BTA19
19	BTA-45636	5653299	NW_001493648.1	BTA19
19	BTA-45631	5666813	NW_001493648.1	BTA19
19	BTA-45632	5667086	NW_001493648.1	BTA19
19	BZ857409-C89KA	5670667	NW_001493648.1	BTA19
19	BTA-45584	5779444	NW_001493648.1	BTA19
19	BTA-45586	5778246	NW_001493648.1	BTA19
19	BTA-45574	5846561	NW_001493648.1	BTA19
19	CC531035-G564FA	5865391	NW_001493648.1	BTA19
19	BTA-11204	5895385	NW_001493648.1	BTA19
19	BTA-45159	6031524	NW_001493648.1	BTA19
19	BTA-45686	6090492	NW_001493648.1	BTA19
19	BTA-45689	6094309	NW_001493648.1	BTA19
19	BTA-45688	6094357	NW_001493648.1	BTA19
19	CC590090-C167FA	6097197	NW_001493648.1	BTA19

19	BZ886415-T167FG	6099841	NW_001493648.1	BTA19
19	BTA-45703	6146724	NW_001493648.1	BTA19
19	BTA-45726	6202603	NW_001493648.1	BTA19
19	BTA-45733	6257000	NW_001493648.1	BTA19
19	BTA-16243	6312213	NW_001493648.1	BTA19
19	CC498982-T89BC	6412709	NW_001493648.1	BTA19
19	CC498982-T72KC	6412709	NW_001493648.1	BTA19
19	CC498982-G89BA	6412723	NW_001493648.1	BTA19
19	BZ872308-T167FA	6459990	NW_001493648.1	BTA19
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19	BTA-45982	10254002	NW_001493652.1	BTA19
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19	BZ840034-A72KT	9974496	NW_001493652.1	BTA19
19	BZ840034-A167FC	9974398	NW_001493652.1	BTA19
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19	CC538776-TGR527C	7554515	NW_001493651.1	BTA19
19	BZ953217-CRM25KT	7688635	NW_001493651.1	BTA19
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19	CC507099-A91DC	8131582	NW_001493651.1	BTA19
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19	BTA-86493	8444361	NW_001493651.1	BTA19
19	SCAFFOLD105007_21421	8486327	NW_001493651.1	BTA19
19	BTA-00316	8486477	NW_001493651.1	BTA19
19	BTA-86498	8486865	NW_001493651.1	BTA19
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19	CC767956-GRM25KC	8653685	NW_001493651.1	BTA19
19	BTA-93463	8663414	NW_001493651.1	BTA19
19	BZ914683-C93KT	8675895	NW_001493651.1	BTA19
19	CC574701-G89BA	8912030	NW_001493651.1	BTA19
19	CC574701-T91DC	8912098	NW_001493651.1	BTA19
19	CC574701-T167FC	8912030	NW_001493651.1	BTA19
19	CC509023-G167FA	8894214	NW_001493651.1	BTA19
19	CC574701-T89BC	8912065	NW_001493651.1	BTA19
19	CC518784-T89BG	8912935	NW_001493651.1	BTA19
19	BTA-93482	9227214	NW_001493651.1	BTA19
19	SCAFFOLD110615_4785	9300181	NW_001493651.1	BTA19

19	BTA-25637	9463692	NW_001493651.1	BTA19
19	CC571398-T89KC	9575655	NW_001493651.1	BTA19
19	BTA-46509	9757936	NW_001493651.1	BTA19
19	BTA-97840	11247598	NW_001493653.1	BTA19
19	BZ871466-CGR527T	11359802	NW_001493653.1	BTA19
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19	BTA-46474	11433573	NW_001493653.1	BTA19
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19	CC519175-G89BA	11843865	NW_001493653.1	BTA19
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19	BZ859440-G89BA	12168302	NW_001493653.1	BTA19
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29	BTA-65836	36780009	NW_001494538.1	BTA29
29	SCAFFOLD115786_4123	37089822	NW_001494538.1	BTA29
29	BTA-65853	37599641	NW_001494538.1	BTA29
29	BTA-66030	38343713	NW_001494538.1	BTA29
29	BTA-65950	38894436	NW_001494541.1	BTA29
29	BTA-65947	38967192	NW_001494541.1	BTA29
29	BTA-65943	39105363	NW_001494541.1	BTA29
29	BTA-09465	39238587	NW_001494541.1	BTA29
29	BTA-09466	39238774	NW_001494541.1	BTA29
29	BTA-65938	39286978	NW_001494541.1	BTA29
29	BTA-66057	39739339	NW_001494541.1	BTA29
29	BTA-66045	40105553	NW_001494544.1	BTA29
29	BTA-66150		*NW_001493372.1*	*BTA15*
29	BTA-66333	41083861	NW_001494546.1	BTA29
29	BTA-66126	41030087	NW_001494546.1	BTA29

29	BTA-117001	40841859	NW_001494545.1	BTA29
29	BTA-116993	40842109	NW_001494545.1	BTA29
29	BTA-66071	40392915	NW_001494545.1	BTA29
29	BTA-01521	41312565	NW_001494547.1	BTA29
29	BTA-66095	41561582	NW_001494547.1	BTA29
29	BTA-66099	41562560	NW_001494547.1	BTA29
29	BTA-66106	41637543	NW_001494547.1	BTA29
29	BTA-66122	41657341	NW_001494547.1	BTA29
29	BTA-66154	41737998	NW_001494547.1	BTA29
29	BTA-66215	42372421	NW_001494547.1	BTA29
29	SCAFFOLD252706_2287	43420807	NW_001494548.1	BTA29
29	BTA-14309	44728512	NW_001494551.1	BTA29
29	BTA-44068	44728515	NW_001494551.1	BTA29

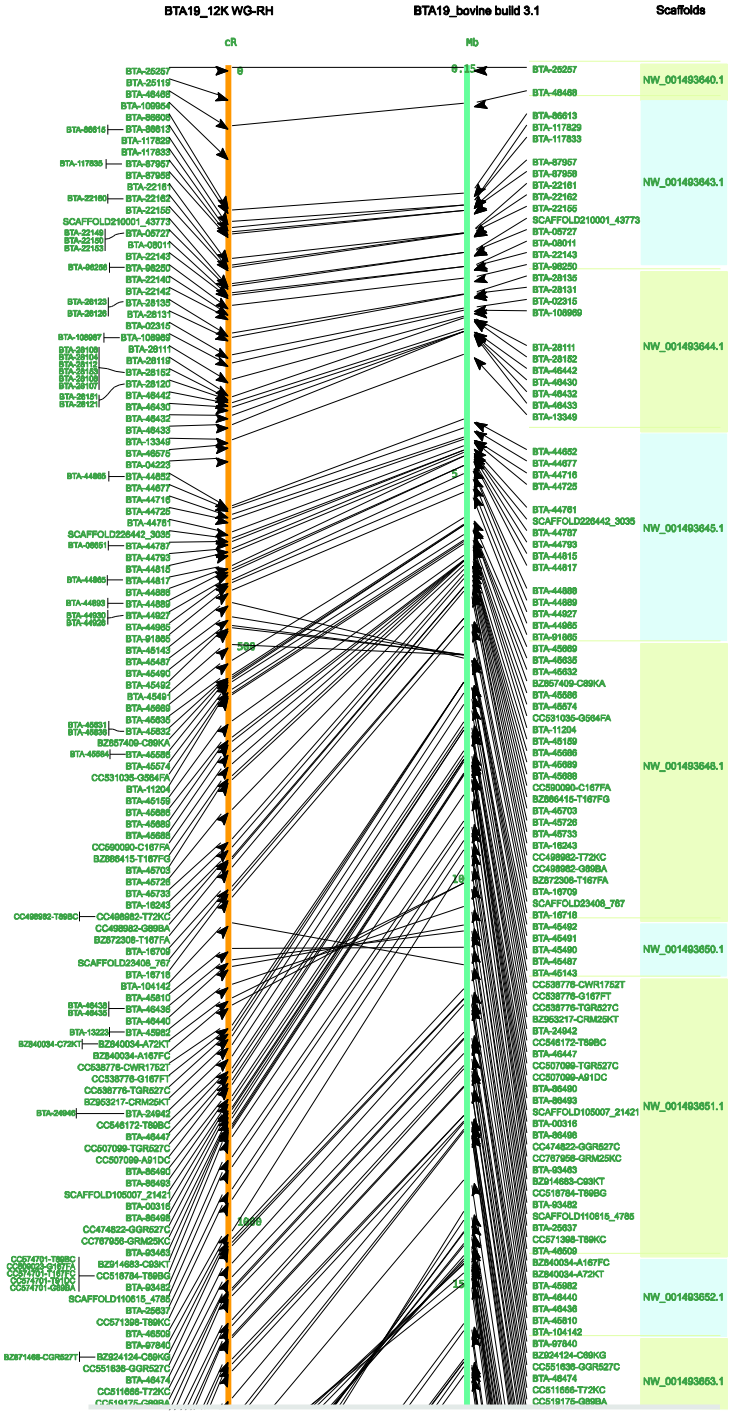

Figure 2-3. RH map of BTA19 (left) compared with the corresponding bovine build 3.1 (right). This figure shows the upper quartile, for the full image please see Figure 2-4. Lines between the maps connect markers in both maps. Distances of the RH map are scaled in (cR) CentiRays and on the bovine build 3.1 in (Mb) Mega base pairs. On the extreme right hand side, the coloured boxes represent scaffolds corresponding to each marker.

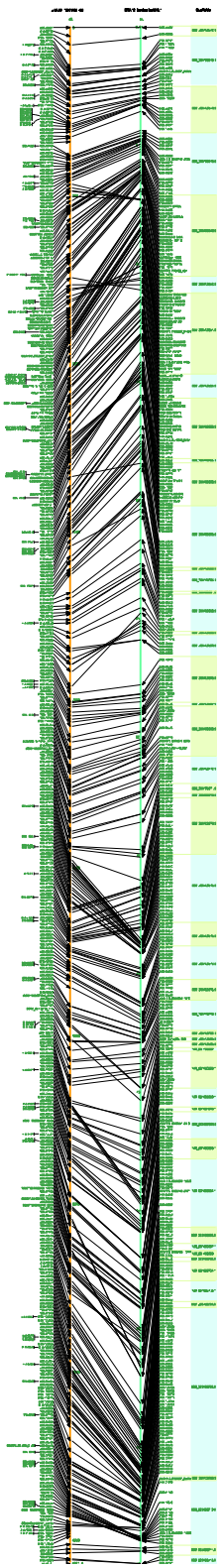

Figure 2-4. Full image of RH map of BTA19 compared with the corresponding bovine build 3.1 sequences

For BTA29, out of the 253 markers mapped, 215 markers were assigned to BTA29 by the bovine genome sequence assembly. Similarly, we could detect scaffolds for 25 loci, which were not assigned any chromosome by the sequence assembly (See Table 2-3, indicated in bold color). Twelve loci did not show any acceptable hits with the sequence assembly. Forty five markers were found to be incongruous and ten scaffolds were found to be misplaced. Four scaffolds were found to be transposed and three scaffolds were found to be inverted. One marker, BTA-66150, was assigned bovine chromosome 15 by the sequence assembly (See Table 2-3, indicated in italics and in grey color). In total, twenty five markers within scaffolds were found to be misplaced. Furthermore, we observed 5 gaps (more than 40 cR) on the BTA29 RH map (Figures 2-5 and 2-6).

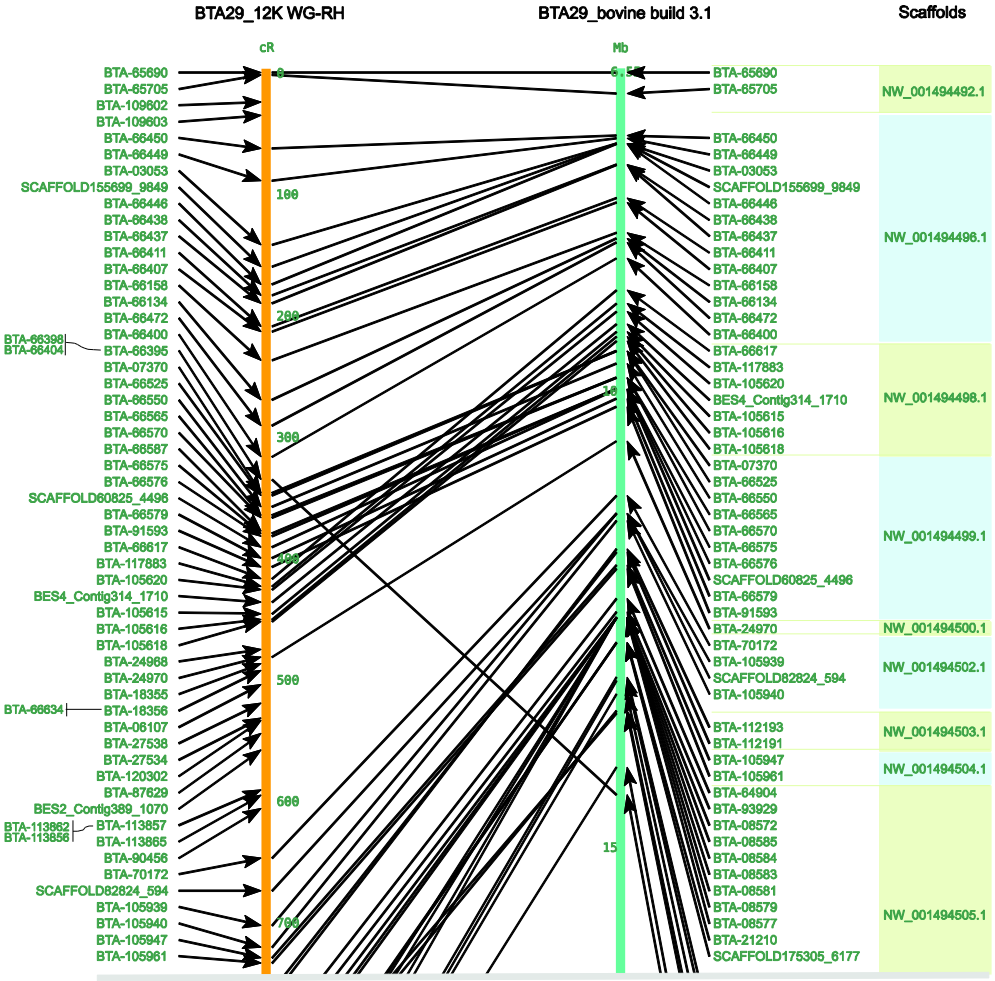

Figure 2-5. RH map of BTA29 (left) compared with the corresponding bovine build 3.1 (right). This figure shows the upper quartile, for the full image please see Figure 2-6. Lines between the maps connect markers in both maps. Distances of the RH map are scaled in (cR) CentiRays and on the bovine build 3.1 in (Mb) Mega base pairs. On the extreme right hand side, the coloured boxes represent scaffolds corresponding to each marker.

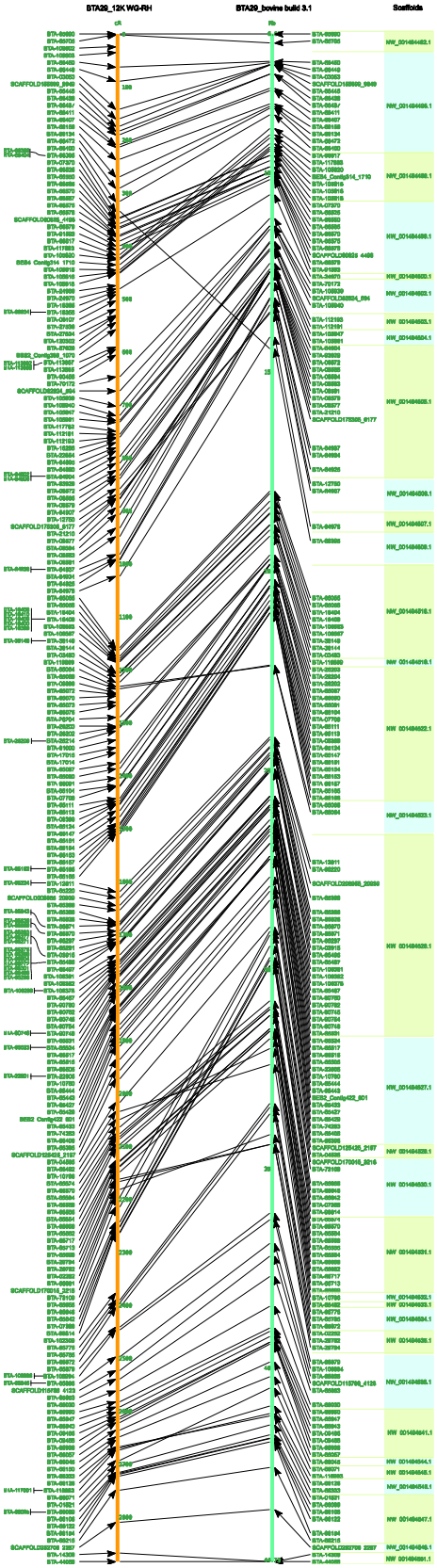

Figure 2-6. Full image of RH map of BTA29 compared with the corresponding bovine build 3.1 sequences

For comparison, we computed the loglikelihood and length of maps built according to the bovine genome sequence order. We re-evaluated maps under a pure diploid RH model using all markers that had a match on the bovine build 3.1 sequences. There were 524 markers that were in common with bovine build 3.1 sequences and RH map of BTA19. The map built according to the bovine build 3.1 sequence order has a log-10-likelihood of -5000.69 and extends up to 6083.9 cR, whereas the map built according to our RH map order has a log-10-likelihood of -4303.72 and extends up to 4508.4 cR. For BTA29, there were 215 markers that were common between RH map and bovine build 3.1 sequences. The map built according to the bovine build 3.1 sequence order has a log-10-likelihood of -2131.96 and extends up to 3822.5 cR, whereas the map built according to our RH map order has a log-10-likelihood of -1805.22 and extends up to 2763.7 cR. Thus based on the RH data, the map derived from the bovine genome sequence is much less likely than our RH map order with log10-likelihood ratio differences of -696 and -326 for BTA19 and BTA29 respectively.

2.2.3. Generation of the cattle-human comparative map

Excluding binned markers, four hundred and fourteen (BTA19) and one hundred and seventy-five (BTA29) markers having human orthologs (reference assembly build 36 version 2) were used for the construction of cattle-human comparative maps. We identified 60 homologous synteny blocks (HSBs, ≥2 markers) on BTA19 and 23 HSBs on BTA29 as shown in Figures 2-7 to 2-10 respectively

(See Table 2-4). Also, 149 breakpoints were identified between BTA19 and the corresponding segments in the HSA17, while 51 breakpoints were identified between BTA29 and HSA11. We compared our maps with the previous studies (Schibler *et al*. 2006, Everts-van der Wind *et al*. 2005). The details of the number of markers used in all the three studies, number of HSBs, their size range and their median is provided in Table 2-5. The HSBs identified in our study are more in number as well as smaller in size because of the high density of markers mapped on the chromosomes. In addition, several of the 555 and 253 SNP markers mapped on BTA19 and 29 respectively, did not produce hits on the bovine (31 markers on BTA19 and 38 markers on BTA29) and human (50 markers on BTA19 and 45 markers on BTA29) chromosome sequences at the given expectation threshold, and some (10 markers on BTA19 and 6 markers on BTA29) produced hits on other human chromosomes, thus resulting in a larger number of smaller HSBs than previously described. The coordinates of our HSBs overall were in agreement with those identified in both earlier studies. However, small discrepancies in the orientation of a few HSBs were observed. Nine of the previously identified HSBs on HSA17 and 4 on HSA11 (Everts-van der Wind *et al*. 2005) were split into 60 and 23 HSBs respectively, in our study. In the Schibler *et al*. study, 7 HSBs on HSA17 and 6 on HSA11 were split into 57 and 23 HSBs respectively. One of the HSBs on HSA17 (22.74-25.73 Mb) found in our study as well as in Everts-van der Wind *et al*. study, was not reported by Schibler *et al*. The synteny block from 0.2-2.9 Mb identified in both of the previous studies (Schibler *et al*. 2006, Everts-van der Wind *et al*. 2005) on

HSA11 is absent from our comparative map. We have only 2 markers in that region and they both show hits in the human genome at the same position of 0.95 Mb. Therefore, although we cannot define them as a synteny block, our data supports the presence of the synteny block on HSA11. One region from 129-132 Mb in HSA11 shows disagreement across all the three studies and needs further investigation. The reason for minor discrepancies with the previous studies may be attributed to the use of different radiation hybrid panel and the mapping approach used.

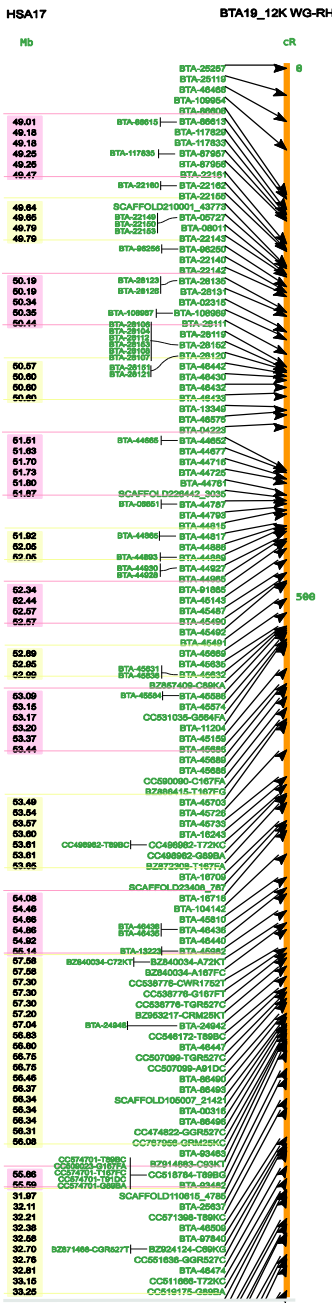

Figure 2-7. Cattle-human comparative map of BTA19 (right) and HSA17 (left). This figure shows the upper quartile, for the full image please see Figure 2-8. HSBs are coloured pink and yellow on HSA17 with the homologous sequence coordinates in the human genome (NCBI build 36) inside the HSBs.

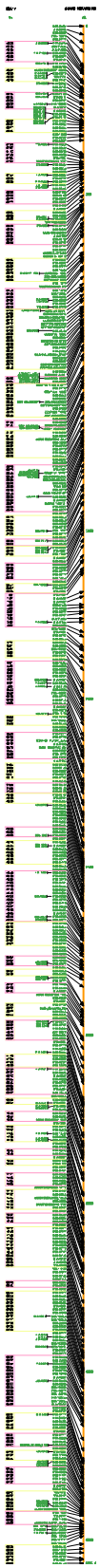

Figure 2-8. Full image of cattle-human comparative map of BTA19 and HSA17

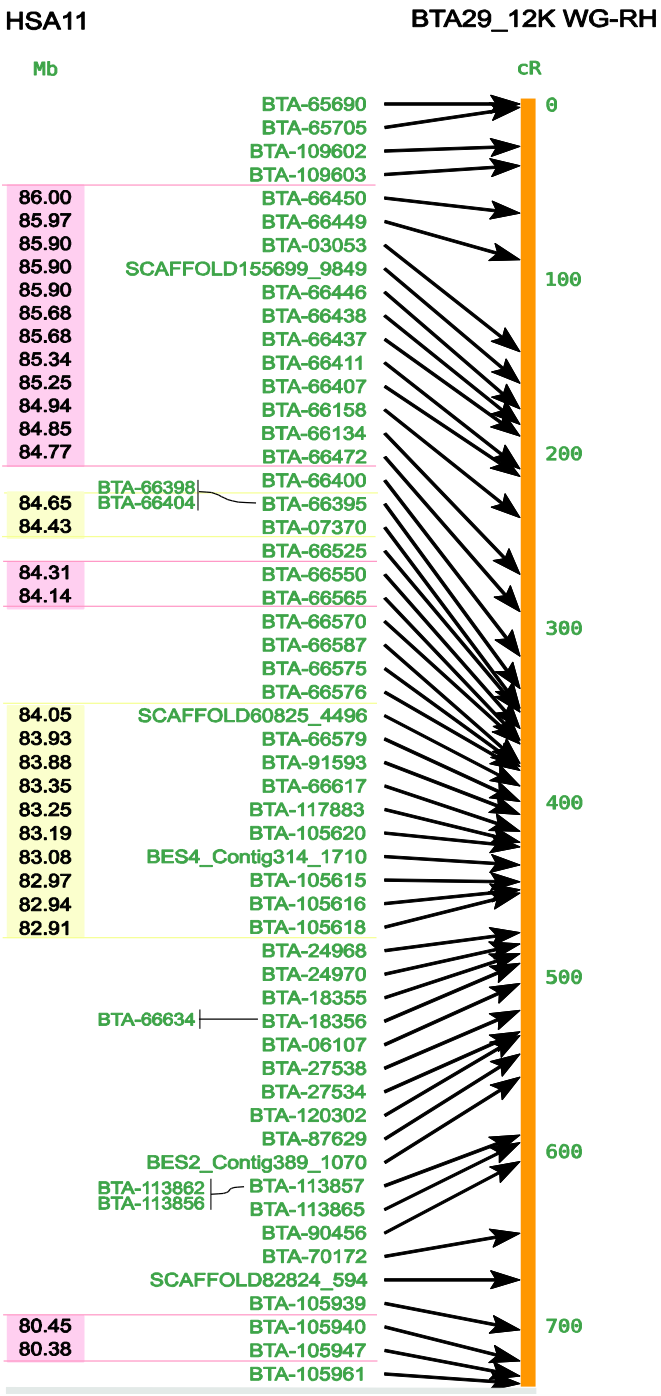

Figure 2-9. Cattle-human comparative map of BTA29 (right) and HSA11 (left). This figure shows the upper quartile, for the full image please see Figure 2-10. HSBs are coloured pink and yellow on HSA11 with the homologous sequence coordinates in the human genome (NCBI build 36) inside the HSBs.

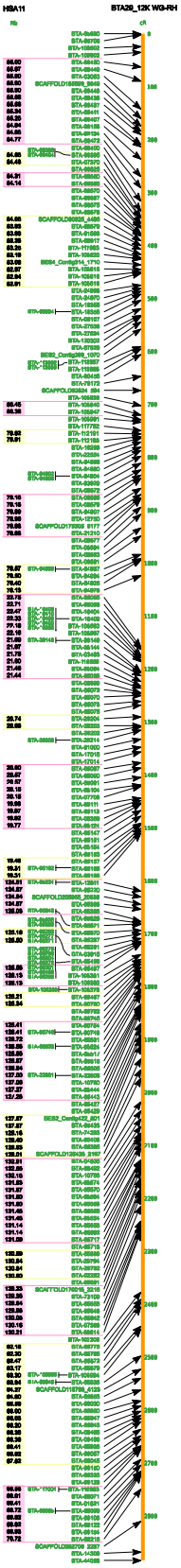

Figure 2-10. Full image of cattle-human comparative map of BTA29 and HSA11

Table 2-4. RH and human map coordinates for homologous synteny blocks for BTA19 and 29

BTA	Human Chromosome	RH start Coordinate(cR)	RH end Coordinate(cR)	HSA start Coordinate(Mbp)	HSA end Coordinate (Mbp)
19	17	124.5	167.1	49.01	49.47
19	17	188.5	198.5	49.64	49.79
19	17	232.2	272.2	50.19	50.44
19	17	293	311.9	50.57	50.60
19	17	382.6	411.1	51.51	51.87
19	17	434.9	440.2	51.92	52.05
19	17	456.2	487.3	52.34	52.57
19	17	517.6	532.3	52.89	52.99
19	17	536.1	584.8	53.09	53.44
19	17	621.9	698.2	53.49	53.65
19	17	724.4	800.7	54.08	55.14
19	17	812.6	922.9	57.58	56.08
19	17	933.8	951.1	55.86	55.59
19	17	960	1066	31.97	33.25
19	17	1072.7	1093.5	31.42	31.39
19	17	1107.3	1187	31.06	30.23
19	17	1211.7	1323	29.88	28.68
19	17	1368.4	1418.6	28.33	27.61
19	17	1447	1460.3	26.96	26.51
19	17	1471.4	1480.1	22.74	22.99
19	17	1497.9	1579.1	23.18	25.22
19	17	1594.6	1624.6	25.27	25.73
19	17	1632.1	1810.1	1.02	4.39
19	17	1887	1968.2	5.27	7.50
19	17	1990.5	2158.2	8.31	11.09
19	17	2187.7	2217	11.62	11.88
19	17	2256.1	2328	12.24	13.85
19	17	2358	2409.2	14.40	16.19
19	17	2446.4	2455	17.86	17.33
19	17	2476.8	2496.1	46.43	46.00
19	17	2539	2556.4	45.96	45.93
19	17	2576.9	2615.9	45.78	45.48
19	17	2633.6	2733.3	45.28	43.34
19	17	2748.5	2779.7	34.12	34.67
19	17	2813.5	2836.9	35.97	35.55
19	17	2848.6	2870.6	35.27	35.12
19	17	2944.2	2959.4	37.13	37.40
19	17	2987.2	3030.5	37.74	38.93
19	17	3086.9	3160.4	39.34	40.57
19	17	3226.4	3245.7	41.23	42.70
19	17	3258.7	3338.4	57.75	59.48
19	17	3351.2	3365.5	59.66	59.97
19	17	3382.8	3405.6	78.38	78.08
19	17	3435.8	3493.4	77.99	77.18
19	17	3527.6	3532.3	76.45	76.36

19	17	3560.2	3567	75.94	76.01
19	17	3615.4	3637.6	74.93	74.53
19	17	3648.6	3671.6	74.36	74.14
19	17	3702.3	3714.4	73.08	72.79
19	17	3722.3	3861.6	72.53	69.45
19	17	3905.3	3913.3	68.45	68.40
19	17	3947.5	4041.2	68.22	67.06
19	17	4089.8	4256.2	66.77	65.94
19	17	4293.3	4312.8	65.68	65.24
19	17	4326.2	4356.1	65.08	64.90
19	17	4367.8	4386.9	64.82	64.29
19	17	4398.2	4417.5	64.22	64.03
19	17	4430.7	4447.1	63.80	63.91
19	17	4450.3	4477.8	60.43	60.99
19	17	4532.6	4591.4	62.06	62.54
29	11	62.6	291	86.00	84.77
29	11	335	346.3	84.65	84.43
29	11	357.8	364.6	84.31	84.14
29	11	390.8	452.2	84.05	82.91
29	11	720.4	729	80.45	80.38
29	11	756.2	761.6	79.82	79.91
29	11	834	878.4	79.15	78.88
29	11	983.1	1023.8	78.57	78.13
29	11	1158.6	1236.2	22.75	21.44
29	11	1290.2	1307	20.74	20.86
29	11	1377.2	1454.9	20.60	19.77
29	11	1500.7	1510.2	19.46	19.31
29	11	1619.5	1676.4	124.61	125.03
29	11	1693.4	1700.3	125.16	125.50
29	11	1718.6	1748.4	125.58	126.13
29	11	1756.6	1763.1	126.21	126.34
29	11	1776.6	1849.9	126.41	127.28
29	11	1891.5	1973	127.67	129.01
29	11	2017.5	2136.5	132.91	131.09
29	11	2148.3	2181.4	130.89	130.60
29	11	2195.5	2263.8	129.23	130.21
29	11	2390.2	2648.6	62.18	67.62
29	11	2673.5	2749.1	68.53	70.72

Table 2-5. Comparison of the cattle-human comparative maps with previous studies

	Prasad *et al*. 2007		**Everts-van der wind *et al*. 2004**		**Schibler *et al*. 2006**	
	BTA19	**BTA29**	**BTA19**	**BTA29**	**BTA19**	**BTA29**
Total number of mapped markers	555	253	92	58	140	106
No. of HSB	60	23	9	5	7	7
Range of HSB sizes (Mb)	0.02-3.37	0.06-5.44	1.72-17.46	2.7-15.9	4.27-19.27	1.16-14.23
Median of HSB sizes (Mb)	0.44	0.44	5.29	8.5	10.56	4.35

2.3. Conclusion

We have built a high resolution RH map of bovine chromosomes 19 and 29 consisting of 555 and 253 SNP markers, respectively. Maps of both the chromosomes, when compared with the third draft of bovine genome sequence assembly, show that there is significant internal rearrangement of the markers involving displacement, inversion and flips within the scaffolds and some scaffolds were found to be misplaced by the third draft (bovine build 3.1) of the bovine genome assembly. Most of the scaffold changes suggested in this study have been incorporated in the fourth draft of bovine genome sequence assembly (Btau_4.0) which was released in October 2007. The RH maps reported here with an average resolution of 1 locus/139 kb and 1 locus/208 kb on BTA19 and BTA29 respectively, are useful for ordering SNP markers which can be used in future gene discovery investigations. Furthermore, they aid in the identification and rectification of potential errors in the current bovine genome sequence assembly.

2.4. Methods

2.4.1. Marker selection and genotyping of the RH panel

Sequence information for 1001 and 535 SNPs for BTA19 and BTA29, respectively, were obtained from public databases (http://www.ncbi.nlm.nih.gov/projects/SNP/, ftp://ftp.hgsc.bcm.tmc.edu/pub/data/Btaurus/snp). Out of 1001 SNPs, 68 SNPs

were identified from the clones of CHORI-240 library spanning QTL regions for backfat reported previously (Li *et al*. 2004, McKay *et al*. 2006). Oligonucleotides respective to the markers were designed at the Bovine Genomics Laboratory at the University of Alberta and the oligo pooled assays (OPA) were synthesized and assembled by Illumina Inc. (San Diego, CA). The markers were genotyped on the 12,000 rad RH panel using the Illumina BeadStation 500G genotyping system (Oliphant *et al*. 2002). Illumina GenCall Software was used to manually score the presence or absence of markers in 180 radiation hybrids as described previously (McKay *et al*. 2007).

2.4.2. Statistical analysis of RH results

The RH maps of the chromosomes were constructed using the CarthaGène software (http://www.inra.fr/bia/T/ CarthaGène /, Schiex and Gaspin 1997, de Givry *et al*. 2005). Pairs of markers with compatible retention patterns (double markers) were identified and each pair was merged into one marker to simplify the search for an optimal map. Initially, the loglikelihood under the haploid equal retention model was used to find the best marker order as advocated in (Lunetta *et al*. 1995). The bovine reference order files, which give the order of SNP markers in the bovine genome sequence assembly, were merged for the respective chromosomes using the *dsmergor* command. The traditional maximum multipoint likelihood criterion was replaced by the comparative mapping criterion using *dsbplambda* command, *lambda* set to 1. Then, the RH maps were built using the Lin-kernighan heuristic based commands: *lkh*, *lkhn*,

lkhl, *lkhd*, *lkhocb* and *lkhocbn*. These commands are based on the 2-point based simplified model proposed in (Ben-Dor *et al*. 2000) or on LOD, distance and obligate chromosome breaks respectively. Parameters "1 0" were used to evaluate all maps encountered using the full probabilistic model. The best loglikelihood map found was then used as the starting point for the *greedy* command, which tries to improve maps using a taboo search algorithm. The map was further tested using a *flips* algorithm, which checks all possible permutations in a sliding window of fixed size (size 7 was used), and a *polish* algorithm, which checks the reliability of map by successfully removing one marker from the initial map and trying to insert in all possible intervals. Final map distances were evaluated using the diploid equal retention model with an EM tolerance set to 10^{-5} (using *cgtolerance*).

2.4.3. Map comparison

Genomic sequence coordinates for SNPs were obtained by performing BLAST (Altschul *et al*. 1990) comparisons between SNP flanking sequences and the bovine build 3.1 sequences, using an expectation value threshold of 1e-50. Most SNPs could be unambiguously placed on the genomic assembly using this method. Coordinates of the putative orthologous SNP regions in humans were obtained by performing BLAST searches against the latest human genome assembly (reference assembly build 36 version 2). Whenever possible, the SNP flanking sequence used in the human comparison was extended (up to 20,000

bp) using the bovine genome assembly, since the existing 500 bp flanking sequence did not produce a significant BLAST hit in most cases. An expectation value threshold of 0.00001 was used for comparison with the bovine and human genome sequence, and homologous synteny blocks (HSBs) were identified according to the criteria defined elsewhere (Murphy *et al*. 2005). The maps were drawn using the CarthaGène software (http://www.inra.fr/bia/T/ CarthaGène /, Schiex and Gaspin 1997, de Givry *et al*. 2005).

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3. Linkage Disequilibrium and Signatures of Selection on Chromosomes 19 and 29 in Beef and Dairy Cattle

3.1. Introduction

Linkage disequilibrium (LD) is the non-random association of alleles at different loci. If two alleles at two different loci are in LD, combinations of alleles within haplotypes occur at frequencies that differ from that expected under the hypothesis of independence. An association between the genetic variation at a locus and a phenotype indicates that either the genetic variation at that locus directly affects the phenotype of interest or the locus is in LD with the causal mutation (Mueller 2004). The feasibility of association studies depend strongly on the extent of LD, which determines how many markers should be typed in a genome scan to detect a quantitative trait locus (QTL) using linkage disequilibrium.

The first whole-genome LD study in cattle, to quantify the extent and pattern of LD, was performed using 284 microsatellite markers sampled from 581 maternally inherited gametes in Dutch black and white dairy cattle, where high levels of LD extended over several tens of centimorgans (Farnir *et al.* 2000). Several subsequent studies have confirmed extensive LD in cattle (Vallejo *et al*. 2003, Tenesa *et al.* 2003, Odani *et al.* 2006, Khatkar *et al.* 2006a). Only recently, a study performed in a large mildly selected cattle population from Western Africa under an extensive breeding system has shown that LD extends over shorter distances than the previous studies from developed countries, which was explained by increasing selective pressure and/or by an

admixture process (Thevenon *et al.* 2007). All of these LD studies were performed using very informative microsatellite loci, but at a relatively low locus density. However, with the completion of the bovine genome sequencing project, it has become possible to estimate the extent of LD using dense SNP marker maps, thereby dramatically increasing resolution. In addition to their abundance in the genome (Snelling *et al.* 2005), SNP markers have low genotyping costs (Hinds *et al.* 2005). Khatkar *et al.* (2006b) reported a first-generation LD map of bovine chromosome 6 in Australian Holstein-Friesian cattle using SNP loci and estimated the extent of LD using D'. The distance over which LD is likely to be useful for association mapping was found to be 13.3 Mb confirming that the range of LD is extensive in Holstein-Friesian dairy cattle. McKay *et al.* (2007) generated LD maps for eight breeds of cattle from the *Bos taurus* and *Bos indicus* subspecies using 2670 SNP markers and observed that the extent of LD (estimated using r^2) available for association analysis does not exceed 500 kb. The differences in the extent of LD between McKay *et al.* (2007) and previous studies were attributed to the differences in measures used to report LD, which are specifically D′ versus r^2. D′ has been reported to overestimate the extent of LD (Ardlie *et al.* 2002, Ke *et al.* 2004) thus resulting in extensive LD at long intermarker distances in previous studies (Farnir *et al.* 2000, Vallejo *et al.* 2003, Tenesa *et al.* 2003, Odani *et al.* 2006, Khatkar *et al*. 2006a).

Here, we report a study of the extent of LD on chromosomes 19 and 29 and the pattern of selection signatures on these chromosomes in *Bos taurus* beef and dairy breeds (Angus and Holstein) using dense SNP markers. We have chosen

BTA19 and BTA29 as candidate chromosomes for mapping because QTL for several economically important traits have been identified on these chromosomes (Stone *et al.* 1999, Mosig *et al.* 2001, MacNeil and Grosz 2002, Casas *et al.* 2003, Li *et al.* 2004, Ashwell *et al.* 2005, Nkrumah *et al.* 2007). The information generated from this study, with a relatively large number of animals per breed compared to other studies, has important implications for the design and application of association studies in cattle populations as well as for selective breeding programs.

3.2. Materials and methods

3.2.1. Collection of DNA samples

DNA was collected from Angus (n = 126, US) and Holstein (n = 321, Semex Alliance, Canada) cattle. To maximize the genetic diversity within each sampled population, families were selected to span the diversity of each breed. Three-generation families were sampled so that chromosomes could be phased using linkage information. The general family structure consisted of a grandparent, parent and three or more progeny.

3.2.2. Marker selection and genotyping

A total of 1001 and 535 evenly spaced SNP markers for BTA19 and 29 were chosen from bovine sequence build 2.0 (ftp://ftp.hgsc.bcm.tmc.edu/pub/data/Btaurus/snp). The markers were genotyped within each population of beef and dairy animals using the Illumina BeadStation

500G genotyping system (Oliphant *et al.* 2002). However, only 555 and 253 SNP markers were used for the LD analysis. These loci had successfully been mapped on the high-resolution 12 000 rad radiation hybrid panel and were considered to be correctly ordered on both BTA19 and 29 (Prasad *et al.* 2007). Some loci did not amplify in the genotyped animals and those loci that were monomorphic or that had a minor allele frequency (MAF) <0.03 were removed from the study. After these filtering procedures, the LD analysis was performed using 370 and 367 markers on BTA19 and 186 and 179 markers on BTA29 for the Angus and Holstein populations respectively. The sequence and the NCBI IDs of the SNP used in the LD analysis are in Prasad *et al.* (2007). To test whether Holstein differ significantly from Angus in the distribution of MAF, the PROC FREQ procedure in SAS (v. 9.1; SAS, Inc.) was run using a two-way contingency table of loci against breeds.

3.2.3. Marker positions

Genomic sequence coordinates for SNPs were obtained by performing BLAST (Altschul *et al.* 1990) comparisons between SNP-flanking sequences and the 7.1X bovine genome assembly (Btau 3.1). The marker order and their corresponding genomic coordinates were corrected if they disagreed with the RH map order of Prasad *et al.* (2007). For each chromosome, a bp/cR conversion ratio was estimated by dividing the highest base-pair position by its corresponding cR position. The resultant ratios were 13816.49 and 15413.2 for BTA19 and 29 respectively. The relative positions of markers (in bp) were

estimated by multiplying the conversion ratio with the RH position. Markers that could not be separated by their RH positions were ordered according to their order in the bovine genome sequence assembly; RH mapping has difficulty ordering closely linked markers, although the sequence assembly is accurate at a fine scale. The list of SNPs used in the LD analyses and their inferred chromosomal positions in base pairs are in Table 3-1.

Table 3-1. List of the markers used in the LD analysis and their corresponding map positions.

BTA	Name	Btau3.1_Position (bp)	RH_position (cR)	Position used in LD analysis (bp)
19	BTA-25119	No acceptable hits	25.9	357847
19	BTA-46468	594380	50.9	703259
19	BTA-109954	No acceptable hits	77.7	1073541
19	BTA-86613	1673261	124.5	1720153
19	BTA-86615	1673429	124.5	1720321
19	BTA-117829	1815421	134.6	1859700
19	BTA-117833	1815848	139.5	1927400
19	BTA-117835	1816036	143.3	1979903
19	BTA-87957	1880727	143.3	2044594
19	BTA-87958	1880960	145.3	2072227
19	BTA-22161	2148369	167.1	2308735
19	BTA-22160	2159056	172.5	2393913
19	BTA-22155	2159459	174.3	2408214
19	BTA-22153	2388842	190.7	2634805
19	BTA-22149	2446034	190.7	2691997
19	BTA-22150	2446217	190.7	2692180
19	BTA-08011	2558999	196.7	2717704
19	BTA-22143	2560148	198.5	2742573
19	BTA-22140	No acceptable hits	213.6	2951202
19	BTA-22142	No acceptable hits	217.1	2999560
19	BTA-28135	2882099	232.2	3208189
19	BTA-28126	2892590	232.2	3218680
19	BTA-28123	2892860	232.2	3218950
19	BTA-28131	2889022	235.8	3257928
19	BTA-02315	3054038	250.9	3466557
19	BTA-108969	3083810	258.1	3566036
19	BTA-108967	3084132	258.1	3566358
19	BTA-28111	3155340	272.2	3760849
19	BTA-28119	No acceptable hits	283.9	3922502
19	BTA-28112	3155477	287.7	3975004
19	BTA-28106	3157018	287.7	3976545
19	BTA-28107	3157312	287.7	3976839
19	BTA-28108	3157430	287.7	3976957
19	BTA-28104	3159191	287.7	3978718
19	BTA-28153	3161383	287.7	3980910
19	BTA-28152	3173234	287.7	3992761
19	BTA-28120	No acceptable hits	289.4	3998492
19	BTA-28121	3137636	289.4	3999492
19	BTA-28151	3173372	289.4	4035228

19	BTA-46430	3335659	296.6	4097971
19	BTA-46432	3336040	303.6	4194686
19	BTA-46433	3336269	311.9	4309363
19	BTA-13349	3612492	325	4490359
19	BTA-46575	No acceptable hits	329.5	4552533
19	BTA-04223	No acceptable hits	341.1	4712805
19	BTA-44652	4392940	382.6	5286189
19	BTA-44665	4437781	382.6	5331030
19	BTA-44677	4496823	384.5	5357281
19	BTA-44716	4607143	390.5	5395339
19	BTA-44761	4713000	405.7	5605350
19	BTA-06651	4765081	414	5720027
19	BTA-44787	4765466	414	5720412
19	BTA-44793	4791088	420.1	5804307
19	BTA-44815	4836142	423.7	5854047
19	BTA-44817	4840954	434.9	6008792
19	BTA-44888	4950633	437.6	6104108
19	BTA-44889	4950776	440.2	6140031
19	BTA-44893	4955188	440.2	6144443
19	BTA-44928	5047651	447.4	6181498
19	BTA-44927	5048028	447.4	6181875
19	BTA-44930	5048765	447.4	6182612
19	BTA-44965	5162772	449.6	6211894
19	BTA-91865	5270649	456.2	6303083
19	BTA-45143	7271040	467.2	6455064
19	BTA-45487	7227465	479.6	6626389
19	BTA-45490	7227099	487.3	6732776
19	BTA-45492	7223128	489	6756264
19	BTA-45491	7227000	504	6963511
19	BTA-45669	5577645	517.6	7151415
19	BTA-45631	5666813	532.3	7354518
19	BTA-45586	5778246	536.1	7407020
19	BTA-45584	5779444	536.1	7408218
19	BTA-45574	5846561	542.7	7498209
19	BTA-11204	5895385	547.8	7568673
19	BTA-45159	6031524	570.5	7882308
19	BTA-45686	6090492	584.8	8079883
19	BTA-45689	6094309	588.6	8132386
19	BTA-45688	6094357	597.4	8253971
19	BTA-45703	6146724	621.9	8592475
19	BTA-45733	6257000	673.5	9305406
19	BTA-16709	6791742	715.2	9881554
19	BTA-16718	6881545	724.4	10008665

19	BTA-104142	10956484	747	10320918
19	BTA-45810	10747579	769.1	10626262
19	BTA-46438	10546183	779.3	10767191
19	BTA-46436	10550474	779.3	10771482
19	BTA-46435	10555950	779.3	10776958
19	BTA-46440	10478570	784.8	10843181
19	BTA-45982	10254002	800.7	11062864
19	BTA-13223	10277515	800.7	11086377
19	BTA-24942	7826776	863.3	11927776
19	BTA-24946	7830296	863.3	11931296
19	BTA-46447	8091343	878	12130878
19	BTA-86490	8380176	898.2	12409971
19	BTA-86493	8444361	902.1	12463856
19	BTA-00316	8486477	910	12573006
19	BTA-86498	8486865	913.7	12624127
19	BTA-93463	8663414	924.7	12776108
19	BTA-25637	9463692	979.3	13530489
19	BTA-46509	9757936	1015.9	14036172
19	BTA-97840	11247598	1022.6	14128743
19	BTA-46474	11433573	1043.9	14423034
19	BTA-46456	11853617	1067.6	14750485
19	BTA-46514	12421728	1107.3	15298999
19	BTA-09214	12775626	1131.9	15638885
19	BTA-46564	12838553	1138.1	15724547
19	BTA-46552	12859481	1162.1	16056143
19	BTA-46543	12936368	1181.7	16326946
19	BTA-05909	12963665	1187	16400174
19	BTA-29947	No acceptable hits	1204.5	16641962
19	BTA-46527	13542315	1211.7	16741441
19	BTA-44521	14791021	1222.8	16894804
19	BTA-07806	14692391	1228.1	17101885
19	BTA-44540	14522558	1249.2	17393413
19	BTA-11922	14498062	1263.2	17452990
19	BTA-44552	14453528	1268.9	17531744
19	BTA-44555	14309549	1279.7	17680962
19	BTA-44546	14460882	1279.7	17832295
19	BTA-44561	14180324	1287.6	17941446
19	BTA-44563	14030367	1308	18071969
19	BTA-44565	13927005	1323	18279216
19	BTA-44603	15275858	1368.4	18906485
19	BTA-44594	15359973	1379.5	19059848
19	BTA-44618	15704056	1396	19287820
19	BTA-44616	15732326	1399.3	19333414

19	BTA-13335	15739023	1401.1	19358284
19	BTA-44610	15895042	1422.2	19649812
19	BTA-44495	16561362	1447	20017797
19	BTA-20575	16925471	1460.3	20176220
19	BTA-46586	17123199	1471.4	20329583
19	BTA-46585	17125781	1471.4	20332165
19	BTA-46580	17179402	1471.4	20385786
19	BTA-46576	17183401	1471.4	20389785
19	BTA-46571	17289776	1473.1	20413273
19	BTA-15926	17426048	1480.1	20449787
19	BTA-44631	17544730	1489	20572754
19	BTA-44637	17598825	1494.4	20647363
19	BTA-44638	17602483	1497.9	20695720
19	BTA-44649	17803437	1511.2	20879480
19	BTA-44663	18306459	1530.9	21151665
19	BTA-44669	19992954	1548.3	21392071
19	BTA-07830	19271366	1592.8	22006905
19	BTA-118485	19244408	1594.6	22031775
19	BTA-04414	19361415	1594.6	22148782
19	BTA-44726	19578032	1617.1	22342646
19	BTA-44731	19737675	1624.6	22446270
19	BTA-44751	20261350	1632.1	22549893
19	BTA-44791	21257194	1688.2	23324998
19	BTA-44801	21642551	1703.3	23533627
19	BTA-01578	22221177	1727.9	23873513
19	BTA-44833	22422252	1735.2	23974373
19	BTA-44838	22520003	1747.8	24148461
19	BTA-44845	22530396	1752.7	24216162
19	BTA-115853	22857093	1769.8	24452424
19	BTA-11532	22994444	1779	24579536
19	BTA-44868	23062875	1779	24647967
19	BTA-07396	23642950	1810.1	25009229
19	BTA-108581	24048100	1823.8	25198514
19	BTA-44691	25378411	1855.1	25630971
19	BTA-44690	25378004	1863	25740121
19	BTA-44693	No acceptable hits	1866	25781570
19	BTA-98517	24572906	1924	26582927
19	BTA-20935	24268041	1968.2	27193616
19	BTA-44712	No acceptable hits	1978.5	27335925
19	BTA-14962	27237071	1990.5	27501723
19	BTA-44960	27613513	2017.1	27869242
19	BTA-44964	27762168	2025.2	27981156
19	BTA-44976	27919963	2030.8	28058528

19	BTA-44980	28189564	2045.7	28264394
19	BTA-44981	28207824	2045.7	28282654
19	BTA-44985	28293595	2057.7	28430191
19	BTA-44989	28299387	2057.7	28435983
19	BTA-44990	28303035	2067.6	28566975
19	BTA-01174	28376343	2067.6	28640283
19	BTA-44994	28396324	2075.5	28676125
19	BTA-104726	28456076	2087.8	28846068
19	BTA-67105	29137240	2135.2	29500969
19	BTA-45030	29180085	2158.2	29818749
19	BTA-45023	No acceptable hits	2180.9	30132383
19	BTA-13124	No acceptable hits	2182.6	30155871
19	BTA-45027	No acceptable hits	2182.6	30155871
19	BTA-29349	29493356	2187.7	30226335
19	BTA-106969	29630223	2211.8	30559313
19	BTA-45064	29835781	2217	30631158
19	BTA-45066	29999924	2224.4	30733400
19	BTA-45079	30126442	2231.3	30828734
19	BTA-20635	30064294	2256.1	31171383
19	BTA-45082	30242570	2268.3	31339944
19	BTA-11476	30576168	2282.1	31530612
19	BTA-05960	30794237	2294.6	31703318
19	BTA-17255	31126345	2320.3	32058402
19	BTA-11250	31636221	2354.3	32528162
19	BTA-97038	31641445	2358	32579283
19	BTA-45090	31880392	2378.3	32859758
19	BTA-45036	32554954	2401.7	33183064
19	BTA-45040	32558584	2409.2	33286688
19	BTA-45043	32893554	2423.2	33480119
19	BTA-45047	34013261	2446.4	33800661
19	BTA-45106	34198459	2448.1	33824149
19	BTA-45109	34336058	2455	33919483
19	BTA-45146	35290150	2476.8	34220682
19	BTA-07221	37703198	2488.3	34379572
19	BTA-45369	37840528	2498	34513592
19	BTA-45368	37840572	2498	34513636
19	BTA-45372	37840956	2506	34624124
19	BTA-45375	37841152	2510.4	34684916
19	BTA-45377	37846940	2517.8	34787159
19	BTA-45380	37856356	2525.3	34890782
19	BTA-45379	37856592	2530.7	34965391
19	BTA-45269	37893849	2539	35080068
19	BTA-11992	37891193	2556.4	35320475

19	BTA-45275	37937582	2556.4	35366864
19	BTA-45285	38036458	2576.9	35603713
19	BTA-45288	38061493	2586.1	35755529
19	BTA-45292	38071106	2587.9	35784807
19	BTA-45299	38255512	2597.8	35892478
19	BTA-45304	38305369	2610	36061039
19	BTA-45303	38305433	2612.8	36099725
19	BTA-45302	38305511	2615.9	36142556
19	BTA-45305	No acceptable hits	2619.9	36197822
19	BTA-45314	35539006	2630.1	36338750
19	BTA-45315	35541786	2630.1	36341530
19	BTA-45316	35541603	2633.6	36387108
19	BTA-45318	35649180	2637	36484440
19	BTA-09802	35728534	2644	36530800
19	BTA-45325	35965453	2672	36917661
19	BTA-05437	36271521	2690.7	37176030
19	BTA-45357	36426661	2697.3	37267218
19	BTA-45358	36426545	2699.4	37296233
19	BTA-45356	36426989	2699.4	37296677
19	BTA-45339	36701619	2709.4	37434398
19	BTA-45654	36909022	2715.3	37515915
19	BTA-45350	37204733	2722.6	37616776
19	BTA-45351	37205107	2724.4	37641645
19	BTA-45352	37252173	2729.8	37716254
19	BTA-88705	37321249	2733.3	37767801
19	BTA-45382	38873919	2748.5	37974623
19	BTA-45499	38945217	2755	38064430
19	BTA-45494	39087406	2761.5	38154237
19	BTA-45474	39242304	2772.7	38308982
19	BTA-04699	39335789	2779.7	38405697
19	BTA-45439	40294242	2795.1	38618471
19	BTA-45448	40305196	2803.3	38731766
19	BTA-45457	40473192	2813.5	38872695
19	BTA-45458	40473316	2819.8	38959739
19	BTA-45468	40815820	2836.9	39196000
19	BTA-45470	40884686	2836.9	39264866
19	BTA-45469	40875686	2840.8	39361533
19	BTA-45404	41160181	2870.6	39661616
19	BTA-57050	41395620	2925.4	40418760
19	BTA-57051	41395742	2925.4	40418882
19	BTA-57052	41395973	2925.4	40419113
19	BTA-57053	41396238	2927.2	40443630
19	BTA-55942	41647565	2944.2	40678510

19	BTA-55938	41647926	2950.7	40768317
19	BTA-56081	41842164	2959.4	40888521
19	BTA-45517	No acceptable hits	2974.6	41098531
19	BTA-45521	43831356	2982	41200773
19	BTA-45527	43835302	2987.2	41272619
19	BTA-03390	41925198	3028.6	41844622
19	BTA-45570	41959859	3030.5	41870873
19	BTA-99555	42351680	3086.9	42650123
19	BTA-99554	42351843	3086.9	42650286
19	BTA-45537	43162836	3151.8	43546813
19	BTA-45532	43365613	3160.4	43665635
19	BTA-45661	45198423	3207.6	44317773
19	BTA-45659	45093795	3214.8	44417252
19	BTA-45683	48428056	3221.8	44513967
19	BTA-45684	48423165	3223.3	44534692
19	BTA-45682	48434584	3223.3	44546111
19	BTA-45680	48436977	3226.4	44577523
19	BTA-45676	48528990	3228.7	44609301
19	BTA-02462	45729957	3245.7	44844182
19	BTA-93411	46177067	3258.7	45023796
19	BTA-93414	46180955	3258.7	45027684
19	BTA-45579	46206608	3261.8	45066627
19	BTA-45581	46496129	3275	45249005
19	BTA-45589	46607930	3279.9	45316706
19	BTA-45597	46814933	3284.7	45383025
19	BTA-45615	47303266	3313.6	45782321
19	BTA-45621	47361144	3319.8	45867984
19	BTA-03894	47669239	3338.4	46124970
19	BTA-103899	47734355	3346.3	46234120
19	BTA-45701	48130948	3365.5	46511232
19	BTA-45731	49034896	3372.5	46596113
19	BTA-45732	49077155	3372.5	46638372
19	BTA-45743	49322386	3379.4	46849705
19	BTA-45737	49442626	3382.8	46896681
19	BTA-45750	49549238	3387.6	46963001
19	BTA-13041	49751808	3432.4	47423720
19	BTA-45906	49754343	3435.8	47470696
19	BTA-45908	49775765	3447.9	47637876
19	BTA-13047	49792874	3449.6	47661364
19	BTA-13045	49793463	3449.6	47661953
19	BTA-45802	50817830	3490.8	48230603
19	BTA-45799	50817928	3493.4	48266526
19	BTA-45795	50821046	3493.4	48269644

19	BTA-45794	50821128	3493.4	48269726
19	BTA-45793	50822025	3496.1	48303831
19	BTA-45770	51407665	3527.6	48739050
19	BTA-45768	51450178	3532.3	48803988
19	BTA-05671	No acceptable hits	3537.3	48873070
19	BTA-91568	55295354	3567	49283420
19	BTA-45875	52236375	3614.4	49938321
19	BTA-45868	52241101	3615.4	49952138
19	BTA-45864	52296802	3619.4	50007404
19	BTA-45860	52533124	3634.2	50211888
19	BTA-45846	52711157	3648.6	50410845
19	BTA-00405	55700010	3655	50499271
19	BTA-04652	52871906	3664.7	50633291
19	BTA-45843	52879881	3671.6	50728625
19	BTA-45829	52921826	3676.1	50790799
19	BTA-45937	53717744	3702.3	51152791
19	BTA-03377	53837212	3710.1	51260560
19	BTA-45954	53958398	3714.4	51319970
19	BTA-45963	54067562	3719.6	51391816
19	BTA-45966	54247921	3724.8	51463662
19	BTA-45979	55147119	3746.7	51766243
19	BTA-07747	54813700	3757.6	51916843
19	BTA-46072	54631221	3771.7	52111655
19	BTA-46037	54290546	3785.8	52306468
19	BTA-46095	56834335	3814.7	52705764
19	BTA-46135	57321380	3837.5	53020780
19	BTA-46121	57514273	3849.1	53181052
19	BTA-46115	57601197	3851.9	53219738
19	BTA-111179	57747983	3871.1	53485014
19	BTA-46256	57887401	3876.4	53558242
19	BTA-46126	No acceptable hits	3886.1	53692262
19	BTA-01709	No acceptable hits	3889.7	53742001
19	BTA-46265	58766556	3964	54768566
19	BTA-46262	58895851	3969.4	54843175
19	BTA-46280	59045077	3977.6	54956471
19	BTA-46281	59052929	3981.7	55013118
19	BTA-46285	59187630	4001	55279776
19	BTA-46292	59377410	4011.5	55424850
19	BTA-46305	59453184	4014.4	55464917
19	BTA-109506	59487290	4017.4	55569770
19	BTA-05874	59610818	4023.6	55592029
19	BTA-77447	59684113	4030	55680455
19	BTA-46306	59453081	4050.1	55958166

19	BTA-46288	59361328	4051.5	55977509
19	BTA-46307	59452716	4051.5	56068897
19	BTA-46313	59462571	4051.5	56078752
19	BTA-46302	59450220	4052.8	56096714
19	BTA-109495	59528681	4057	56154743
19	BTA-109491	59552673	4058.4	56174086
19	BTA-77448	59683956	4061.3	56214154
19	BTA-03306	59922083	4070.1	56234496
19	BTA-46322	59950043	4089.8	56506681
19	BTA-09444	60031335	4104	56702875
19	BTA-84899	60090256	4109	56771957
19	BTA-84891	60159701	4109	56841402
19	BTA-84898	60090311	4112	56882852
19	BTA-84894	60152334	4116.4	56943644
19	BTA-46341	60271950	4132	57089737
19	BTA-46342	60271637	4136	57145003
19	BTA-46348	60310996	4147	57296984
19	BTA-104736	60528281	4164.1	57533246
19	BTA-104738	60528699	4167.1	57574695
19	BTA-104739	60528745	4171.1	57629961
19	BTA-104732	60619700	4224.4	58366380
19	BTA-93880	60795636	4285.3	59207805
19	BTA-46056	60849522	4293.3	59318337
19	BTA-46057	60849890	4293.3	59318705
19	BTA-07437	60862980	4294.8	59339061
19	BTA-46059	60879236	4296.4	59361168
19	BTA-46360	61206306	4312.8	59587758
19	BTA-46361	61297322	4319.5	59680329
19	BTA-46363	61356465	4326.2	59772899
19	BTA-46364	61366247	4338.5	59942842
19	BTA-05949	61366772	4341.9	59989818
19	BTA-46380	61525711	4367.8	60347665
19	BTA-46381	61526065	4367.8	60348019
19	BTA-05994	61807084	4383.7	60567347
19	BTA-46408	61840399	4388.5	60633666
19	BTA-46409	61840464	4388.5	60633731
19	BTA-46413	61843417	4388.5	60636684
19	BTA-46416	61865210	4391.7	60677879
19	BTA-46407	61840366	4398.2	60767686
19	BTA-46404	61840029	4399.8	60789793
19	BTA-21385	62425783	4407.8	60900325
19	BTA-21380	62416561	4410.1	60932103
19	BTA-07431	62452858	4419.2	61057833

19	BTA-21384	62425919	4424.1	61125533
19	BTA-21181	62359670	4430.7	61216722
19	BTA-29633	62489901	4437.3	61307911
19	BTA-29634	62489797	4440.7	61354887
19	BTA-07433	62452990	4443.9	61399100
19	BTA-07434	62453236	4443.9	61399346
19	BTA-29628	62485848	4443.9	61431958
19	BTA-29635	62489726	4443.9	61435836
19	BTA-12079	62296638	4450.3	61487525
19	BTA-21185	62065513	4467.2	61721024
19	BTA-01614	61960480	4474.4	61820503
19	BTA-105913	No acceptable hits	4482.8	61936561
19	BTA-105515	No acceptable hits	4488.6	62016697
19	BTA-105530	No acceptable hits	4500.7	62183877
19	BTA-105528	No acceptable hits	4508.8	62295790
19	BTA-13718	62877328	4547.4	62829107
19	BTA-46020	63437047	4577.4	63243601
19	BTA-46021	63436861	4579.1	63267089
19	BTA-46024	63432577	4591.4	63437032
29	BTA-65690	6551830	0	0
29	BTA-109603	No acceptable hits	35.3	544086
29	BTA-66450	7239703	62.6	964866
29	BTA-03053	7324685	141.9	2187133
29	BTA-66438	7553282	183.6	2829864
29	BTA-66437	7557431	190.2	2931591
29	BTA-66411	7917898	209	3221359
29	BTA-66407	7967828	213.4	3289177
29	BTA-66158	8298150	237.1	3654470
29	BTA-66134	8358370	269.5	4153857
29	BTA-66472	8403689	291	4485241
29	BTA-66400	8577397	316.4	4876736
29	BTA-66404	8576824	335	5163422
29	BTA-66395	14425067	335	5164422
29	BTA-07370	9587015	346.3	5337591
29	BTA-66525	9589878	348.5	5371500
29	BTA-66550	9725070	357.8	5514843
29	BTA-66565	9878783	364.6	5619653
29	BTA-66570	9880078	366.8	5653562
29	BTA-66587	No acceptable hits	377.8	5823107
29	BTA-66575	10001310	379.8	5853933
29	BTA-66576	10001422	381.9	5886301
29	BTA-66579	10109100	399.8	6162197
29	BTA-66617	8921274	416.8	6424222

29	BTA-117883	9067881	423.2	6522866
29	BTA-105620	9158277	425.7	6561399
29	BTA-105615	9374923	445.7	6869663
29	BTA-105616	9427564	450.3	6940564
29	BTA-105618	9477879	452.2	6969849
29	BTA-24968	No acceptable hits	474.9	7319729
29	BTA-24970	10570960	481.4	7419914
29	BTA-18356	No acceptable hits	492.3	7587918
29	BTA-66634	No acceptable hits	492.3	7588918
29	BTA-06107	No acceptable hits	503.9	7766711
29	BTA-27538	No acceptable hits	519.4	8005616
29	BTA-27534	No acceptable hits	531.6	8193657
29	BTA-120302	No acceptable hits	533.7	8226025
29	BTA-113857	No acceptable hits	590.9	9108660
29	BTA-113862	No acceptable hits	590.9	9109660
29	BTA-113865	No acceptable hits	595.1	9172395
29	BTA-90456	No acceptable hits	606	9340399
29	BTA-70172	11162025	647.1	9973882
29	BTA-105939	11355456	702.5	10827773
29	BTA-105940	11434395	720.4	11103669
29	BTA-105947	11918715	729	11236223
29	BTA-105961	11961509	733.3	11302500
29	BTA-117782	No acceptable hits	749.7	11555276
29	BTA-112191	11778368	756.2	11655462
29	BTA-112193	11745781	761.6	11738693
29	BTA-16286	No acceptable hits	763.7	11771061
29	BTA-22554	No acceptable hits	771.1	11885119
29	BTA-64906	12267097	797.9	12298192
29	BTA-64902	12331139	797.9	12362234
29	BTA-93929	12432106	810.9	12498564
29	BTA-08572	12491559	823.6	12694312
29	BTA-08585	12494591	834	12854609
29	BTA-08579	12494817	840.1	12948629
29	BTA-64907	13533785	858.7	13235315
29	BTA-12750	13506969	870.5	13417191
29	BTA-08577	12494944	894.2	13782483
29	BTA-08584	12494671	907.5	13987479
29	BTA-64938	13138674	983.1	15152717
29	BTA-64937	13142735	983.1	15156778
29	BTA-64934	13182085	991.6	15283729
29	BTA-64925	13329967	993.8	15317638
29	BTA-64976	14123339	1023.8	15780034
29	BTA-65055	17984263	1158.6	17857734

29	BTA-65056	18042011	1163.1	17927093
29	BTA-16404	18228382	1174.5	18102803
29	BTA-16399	18327908	1178.9	18170621
29	BTA-16409	18380865	1178.9	18223578
29	BTA-16410	18381007	1178.9	18223720
29	BTA-16408	18385172	1178.9	18227885
29	BTA-16406	18421151	1178.9	18263864
29	BTA-106563	18520840	1183.2	18330141
29	BTA-38148	18813901	1205.2	18575989
29	BTA-38149	18814122	1205.2	18576210
29	BTA-38144	18834618	1207.1	18605274
29	BTA-03493	18953172	1213.2	18699294
29	BTA-116569	19101039	1223.7	18861133
29	BTA-65064	22347626	1232	18989062
29	BTA-65068	22326401	1236.2	19053798
29	BTA-09899	No acceptable hits	1244.6	19183269
29	BTA-65072	No acceptable hits	1246.5	19212554
29	BTA-65070	No acceptable hits	1250	19266500
29	BTA-65073	No acceptable hits	1261.3	19440669
29	BTA-26204	19679349	1290.2	19886111
29	BTA-26203	19576768	1307	20145052
29	BTA-26202	19685207	1309.2	20178961
29	BTA-26209	No acceptable hits	1315.8	20280689
29	BTA-61000	No acceptable hits	1332.3	20535006
29	BTA-17015	No acceptable hits	1347.2	20764663
29	BTA-17014	No acceptable hits	1356.7	20911088
29	BTA-65087	19794934	1377.2	21227059
29	BTA-65091	19818653	1404.7	21650922
29	BTA-65104	20192322	1419	21871331
29	BTA-07708	20192592	1421.2	21905240
29	BTA-65111	20337107	1443.5	22248954
29	BTA-65113	20346560	1448.4	22324479
29	BTA-08389	20390911	1452.8	22392297
29	BTA-65147	20706613	1466.6	22604999
29	BTA-65151	20842012	1478.2	22783792
29	BTA-65154	20879747	1491	22981081
29	BTA-65153	20879798	1494	23027321
29	BTA-65157	20889230	1500.7	23130589
29	BTA-65162	20996680	1505.2	23199949
29	BTA-65165	21020932	1505.2	23224201
29	BTA-65224	24083463	1619.5	24961677
29	BTA-12811	24122252	1619.5	25000466
29	BTA-65220	24183498	1630.5	25131223

29	BTA-65388	24397290	1653.9	25491891
29	BTA-65386	24511854	1676.4	25838688
29	BTA-85826	24569830	1680.3	25898800
29	BTA-85843	24603206	1682.3	25929626
29	BTA-85871	24645632	1682.3	25972052
29	BTA-85838	24602780	1693.4	26100713
29	BTA-85869	24640245	1693.4	26138178
29	BTA-65297	24916205	1700.3	26207064
29	BTA-65291	No acceptable hits	1708.8	26338076
29	BTA-65277	No acceptable hits	1714.2	26421307
29	BTA-65293	24919792	1714.2	26422307
29	BTA-65271	25091644	1714.2	26594159
29	BTA-65301	24910888	1716.3	26626527
29	BTA-65296	24916160	1716.3	26631799
29	BTA-65498	24980684	1716.3	26696323
29	BTA-65275	25074152	1716.3	26789791
29	BTA-65272	25087569	1716.3	26803208
29	BTA-65268	25092063	1716.3	26807702
29	BTA-106381	25604485	1739.1	27159123
29	BTA-106382	25604798	1748.4	27302466
29	BTA-106378	25607225	1752.5	27365660
29	BTA-106289	25646652	1752.5	27405087
29	BTA-65467	25684513	1756.6	27468281
29	BTA-90762	25821044	1769.8	27671735
29	BTA-90745	25876637	1774.4	27742636
29	BTA-90754	25926348	1776.6	27776545
29	BTA-90746	25893154	1778.8	27810454
29	BTA-90748	25930615	1778.8	27847915
29	BTA-65531	26183895	1792.7	28062159
29	BTA-65524	26326158	1804.3	28240952
29	BTA-65517	26331428	1808.9	28311852
29	BTA-65515	26338295	1815.7	28416662
29	BTA-65505	26418334	1832.8	28680228
29	BTA-22805	26473417	1835.1	28715678
29	BTA-22801	26478459	1835.1	28720720
29	BTA-10760	26572851	1837.4	28756171
29	BTA-65444	26774637	1844.6	28867146
29	BTA-65427	27175084	1869.9	29257100
29	BTA-65433	27098654	1898.5	29261960
29	BTA-74283	27616714	1911.4	29460790
29	BTA-65408	27845359	1936	29839955
29	BTA-65395	27968227	1956.6	30157467
29	BTA-04535	28872454	2017.5	31096131

29	BTA-66492	32308046	2034.5	31358155
29	BTA-65574	30715403	2063.6	31806680
29	BTA-65570	30866005	2076.3	32002427
29	BTA-65564	30926928	2081.2	32077952
29	BTA-65568	30927204	2086.1	32153477
29	BTA-65555	31077355	2096.5	32313774
29	BTA-65658	31414716	2126.9	32782335
29	BTA-65662	31415195	2128.8	32811620
29	BTA-65717	31467606	2136.5	32930302
29	BTA-65713	31471238	2138.4	32959587
29	BTA-65699	31662505	2148.3	33112178
29	BTA-29794	34310798	2162	33323338
29	BTA-29792	34310594	2166.4	33391156
29	BTA-02252	34063621	2181.4	33622354
29	BTA-65681	No acceptable hits	2186	33693255
29	BTA-73109	29074886	2214.7	34135614
29	BTA-65656	29338358	2242.8	34568725
29	BTA-65646	29623983	2250.8	34692031
29	BTA-65642	29700474	2253	34725940
29	BTA-07368	29830428	2261.6	34858493
29	BTA-99814	29847555	2263.8	34892402
29	BTA-102309	No acceptable hits	2376.9	36635635
29	BTA-65775	33275674	2390.2	36840631
29	BTA-65785	33375735	2395.9	36928486
29	BTA-65879	36022288	2441	37623621
29	BTA-106996	36162872	2446.7	37711476
29	BTA-106994	36182954	2446.7	37731558
29	BTA-65836	36780009	2497.7	38497550
29	BTA-65853	37599641	2539.5	39141821
29	BTA-66030	38343713	2561.7	39483994
29	BTA-65943	39105363	2601.8	40102064
29	BTA-09465	39238587	2610.4	40234617
29	BTA-09466	39238774	2615.2	40308601
29	BTA-65938	39286978	2621.8	40410328
29	BTA-66057	39739339	2635.9	40627654
29	BTA-66045	40105553	2648.6	40823402
29	BTA-66333	41083861	2667.2	41110087
29	BTA-66126	41030087	2669.4	41143996
29	BTA-117001	40841859	2673.5	41207190
29	BTA-116993	40842109	2673.5	41207440
29	BTA-66071	40392915	2692.9	41506206
29	BTA-01521	41312565	2704	41677293
29	BTA-66095	41561582	2715.2	41849921

29	BTA-66106	41637543	2717.1	41879206
29	BTA-66122	41657341	2718.9	41906949
29	BTA-66154	41737998	2722.7	41965520
29	BTA-66215	42372421	2749.1	42372428

3.2.4. Estimation of phased haplotypes

We used GENOPROB v2.0 (Thallman *et al.* 2001a, 2001b) for data-quality checking and estimated phased haplotypes based on the pedigree and estimated recombination rates, which were set proportional to the physical distances among the loci. Both the pedigree and the marker locations (map) were used to estimate the segregation of alleles throughout the entire pedigree. By tracing closely linked markers through a multigenerational pedigree, linkage phase of the alleles was inferred. A set of five loci on BTA29 were chosen to illustrate this point, as shown in Table 3-2. Four progeny of sire 2672891 inherited alternate haplotypes from this sire (dark gray/in bold borders). Sire 2672891 inherited the dark grey haplotype from his maternal granddam. This represents only a small proportion of the markers on this chromosome; there were 22 markers centromeric of the first marker shown and an additional ~150 markers telomeric of this region. The combination of all pedigree and map information available allowed the accurate reconstruction of whole-chromosome-length haplotypes via linkage. GENOPROB estimates the probability that a genotype is correct (pGmx) and the order (phase) of the allele is correct (oGmx) conditional on the pedigree, locus order and map distances. For the LD analysis, we excluded all genotypes with pGmx≤0.95 but did not put any constraint on oGmx. The summary of average genotype and order probabilities for each breed is

shown in Table 3-3. As evident from Table 3-3, more than 90% of the genotypes have order (phase) probabilities >0.95.

Table 3-2. An example of a set of five loci on BTA29 to illustrate how linkage phase of the alleles was inferred using multigenerational pedigree in this study.

Generation	Animal	Sire	Dam	Marker Name	Mat	Pat
Great Grandsire	1775791	1180703	1348259	BTA-105620	1	1
				BTA-105615	1	1
				BTA-105616	3	3
				BTA-105618	3	2
				BTA-24968	2	1
Generation	**Animal**	**Sire**	**Dam**	**Marker Name**	**Mat**	**Pat**
Grandsire	2178378	1775791	2094766	BTA-105620	1	1
				BTA-105615	1	1
				BTA-105616	2	3
				BTA-105618	3	3
				BTA-24968	2	2
Generation	**Animal**	**Sire**	**Dam**	**Marker Name**	**Mat**	**Pat**
Sire	2672891	2178378	2672892	BTA-105620	3	1
				BTA-105615	2	1
				BTA-105616	3	2
				BTA-105618	2	3
				BTA-24968	1	2
Generation	**Animal**	**Sire**	**Dam**	**Marker Name**	**Mat**	**Pat**
Progeny	41276196	2672891	38271010	BTA-105620	3	1
				BTA-105615	2	1
				BTA-105616	3	2
				BTA-105618	2	3
				BTA-24968	1	2
Generation	**Animal**	**Sire**	**Dam**	**Marker Name**	**Mat**	**Pat**
Progeny	38580406	2672891	17160148	BTA-105620	3	1
				BTA-105615	1	1
				BTA-105616	2	2
				BTA-105618	3	3
				BTA-24968	2	2
Generation	**Animal**	**Sire**	**Dam**	**Marker Name**	**Mat**	**Pat**

Progeny	38362483	2672891	2413024	BTA-105620	1	3
				BTA-105615	2	2
				BTA-105616	3	3
				BTA-105618	2	2
				BTA-24968	1	1
Generation	**Animal**	**Sire**	**Dam**	**Marker Name**	**Mat**	**Pat**
Progeny	39020869	2672891	39020866	BTA-105620	1	3
				BTA-105615	1	2
				BTA-105616	3	3
				BTA-105618	2	2
				BTA-24968	1	1

Table 3-3. Summary of the proportion of genotypes in different ranges of probabilities that a genotype is correct (pGmx) and that the order or the phase of the allele is correct (oGmx) for Angus and Holstein

	pGmx		oGmx	
Range	**Holstein**	**Angus**	**Holstein**	**Angus**
<0.9000	0.0072	0.0174	0.0107	0.1140
0.9000-0.9500	0.0019	0.0027	0.0067	0.0279
0.9500-0.9900	0.0239	0.0104	0.0176	0.0567
0.9900-0.9990	0.3992	0.6831	0.0334	0.1679
≥0.999	0.5679	0.2864	0.8994	0.6336

3.2.5. Estimation of linkage disequilibrium

Linkage disequilibrium was measured as the square of the correlation coefficient (r^2) between marker alleles using GOLD (Hill and Robertson 1968, Abecasis and Cookson 2000). Only maternally inherited haplotypes were used to estimate LD in this study to avoid the over-representation of paternal haplotypes within the essentially all-male pedigrees. The r^2 values for all pairwise combinations of markers were binned according to the physical distances separating the markers. The average number of locus pairs within each intermarker distance bin for both breeds are shown in Table 3-4. The graphical representation of the patterns of LD along the chromosomes was generated using the GOLD package.

Table 3-4. Total number of locus pairs by inter-marker distances in Angus and Holstein averaged over BTA19 and 29

Intermarker distances	Holstein	Angus
5 kb	25	29.5
50 kb	71.5	80.5
100 kb	90.5	91.5
250 kb	232.5	244
500 kb	408.5	447.5
1 Mb	794.5	835.5
2 Mb	1473	1530
5 Mb	3933	4038.5
7 Mb	2463	2425.5
10 Mb	3424	3482.5
20 Mb	10160	10340
40 Mb	12880	13171
65 Mb	5590.5	6019

3.2.6. Estimation of signatures of selection

We computed allelic frequencies for those SNPs whose genotypes were scored in both breeds. There were 334 and 165 such markers on BTA19 and 29 respectively that were used for the LD study. For estimating signatures of selection, we also included markers that were fixed in one breed but that were still segregating in the other breed. There were an additional 21 and 10 such markers on BTA19 and BTA29 respectively that were included in this analysis. However, these markers had been excluded from the LD study because their MAF values were <0.03. Therefore, in total, the estimation of signatures of selection was carried out using 355 and 175 markers on BTA19 and BTA29 respectively. We also computed rolling average allele frequencies in both breeds (using the frequency of the allele with the lowest frequency averaged over both breeds) using a five-locus sliding window for both chromosomes and for each pair of averages; we subtracted the mean Angus allele frequency from that for Holstein. We plotted mean allele frequency differences against the location of the third locus within the five-locus window. To establish whether the allele frequency difference between the breeds differed significantly from zero and thus was putatively indicative of a selection signature, we performed 100,000 and 1,000,000 allele-frequency-against-locus permutation tests for BTA19 and BTA29 respectively to empirically identify the 5% significance level thresholds. To confirm the chromosomal regions identified using the sliding-window approach, we performed a chromosome-wide scan to detect regions showing evidence of selection using a Web-based tool to compute the extended haplotype homozygosity (EHH) statistic (Mueller and Andreoli 2004). First, the haplotypes

at the locus of interest (core haplotype) were identified and the decay of LD as a function of increasing distance from the core haplotype as measured by EHH was evaluated (Sabeti *et al.* 2002). The test for positive selection requires identification of a core haplotype with a combination of high frequency and high EHH, as compared to other core haplotypes at the locus (Sabeti *et al.* 2002). Again, only maternally inherited haplotypes were used for this analysis.

3.3. Results and discussion

The average MAF for SNPs on BTA19 was 0.27 for both Angus and Holstein, but was 0.25 and 0.27 respectively on BTA29. The distribution of MAFs for SNPs used in the LD analyses in both breeds is shown in Figure 3-1. It is evident from this figure that MAF distribution deviates from uniform and Holstein differs from Angus ($P < 0.001$) for its MAF distribution. The presence of a non-uniform distribution of SNP MAFs is due to the ascertainment bias in SNP discovery and does not represent the true distribution of SNP MAF in the genome, which is more appropriately modeled by a gamma distribution. Any difference between the MAF distributions probably reflects a breed of ascertainment effect (i.e., the SNPs were discovered because they were the most common SNPs on these chromosomes in Holstein) which would lead to an excess of high MAF SNPs in one breed and an excess of low MAF in the second. Therefore, the difference probably has no biological significance other than identifying the breed of SNP discovery. The distribution of MAF for SNPs used in the estimation of selection signatures using the five-locus-sliding-

window approach (results not shown) was not different than Figure 3-1, showing that the focus on these SNPs did not introduce an ascertainment bias.

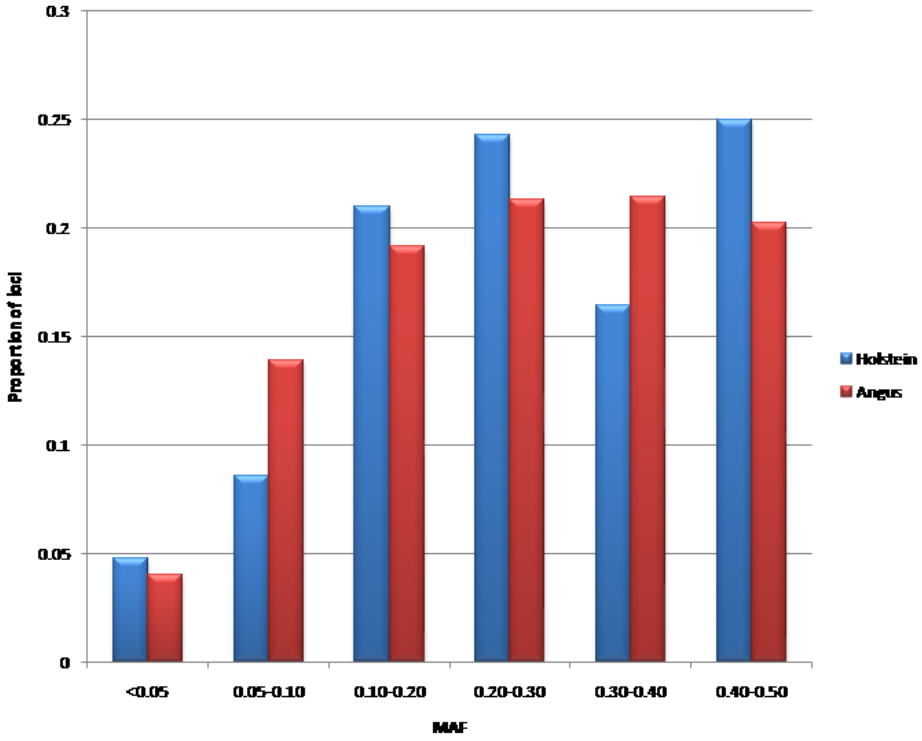

Figure 3-1. The distribution of minor allelic frequencies for the SNP markers (MAF > 0.03) used in the LD analysis on BTA19 and BTA29.

Details of the allelic frequencies for both breeds are provided in Table 3-5. After plotting the differences in rolling average allele frequencies between beef and dairy cattle against the third locus coordinate within a five-locus sliding window, we observed large fluctuations about the axis on both chromosomes (Figures 3-2 and 3-3). The allele frequency thresholds required to achieve statistical significance were found by performing permutation tests and were 0.27 and -0.25 on BTA19 and 0.19 and -0.21 on BTA29 respectively (shown by the red-colored lines in Figures 3-2 and 3-3). In total, we tested 351 and 171 sliding windows for BTA19 and 29 respectively and the number of chromosomal regions identified because differing between the breeds was greater than expected by chance. We found evidence of selection in five regions (6.18-7.35 Mb, 9.88-11.93 Mb, 14.75-17.10 Mb, 28.64-30.83 Mb and 57.15-59.68 Mb) in Holstein and three regions (4.00-5.40 Mb, 24-26 Mb and 60-61 Mb) in Angus on BTA19. On BTA29, there were three regions (11.77-15.15 Mb, 26.42-27.47 Mb and 33-34 Mb) in Holstein and (7.5-8.50 Mb, 18.75-19.45 Mb and 27.75-28.68 Mb) Angus with evidence of selection. Three QTL databases available online (http://bovineqtlv2.tamu.edu/index.html, http://www.animalgenome.org/QTLdb/cattle.html, http://www.vetsci.usyd.edu.au/reprogen/QTL_Map/) were used to identify the chromosomal coordinates of published QTL in beef and dairy cattle on BTA19 and 29. Markers within the reported QTL regions were aligned to the third draft of the bovine genome sequence assembly (Btau 3.1) to obtain the approximate position of these QTL in Mb, and these are reported in Table 3-6. We found

agreement between the regions with large allele frequency differences and those that had previously been identified to be harbouring beef or dairy QTL (Figures 3-2 and 3-3). Using this approach, we sought regions where Angus has been selected for alleles that have been selected against in Holstein. Such differences in allelic frequencies, however, may arise due to selection, drift or admixture. Although we cannot completely rule out the possibility of allele frequency differences due to drift or admixture, the finding that there is statistically significant agreement between chromosomal regions having large allele frequency differences with QTL regions provides independent evidence for selection over drift, which is a random process. Our approach does suffer from the fact that when markers are not equally spaced on the chromosome, the five-locus sliding window will not cover the same physical distance, which may affect the correlation between allele frequencies expected within each window and thus the range of breed differences. In addition, permutation tests may disrupt the correlation that is expected to exist between allelic frequencies at neighbouring loci as a result of selection. It is also important to note that the reported QTL peaks are generally quite broad and were reported from different resource populations, which may not have direct relevance to the populations studied here.

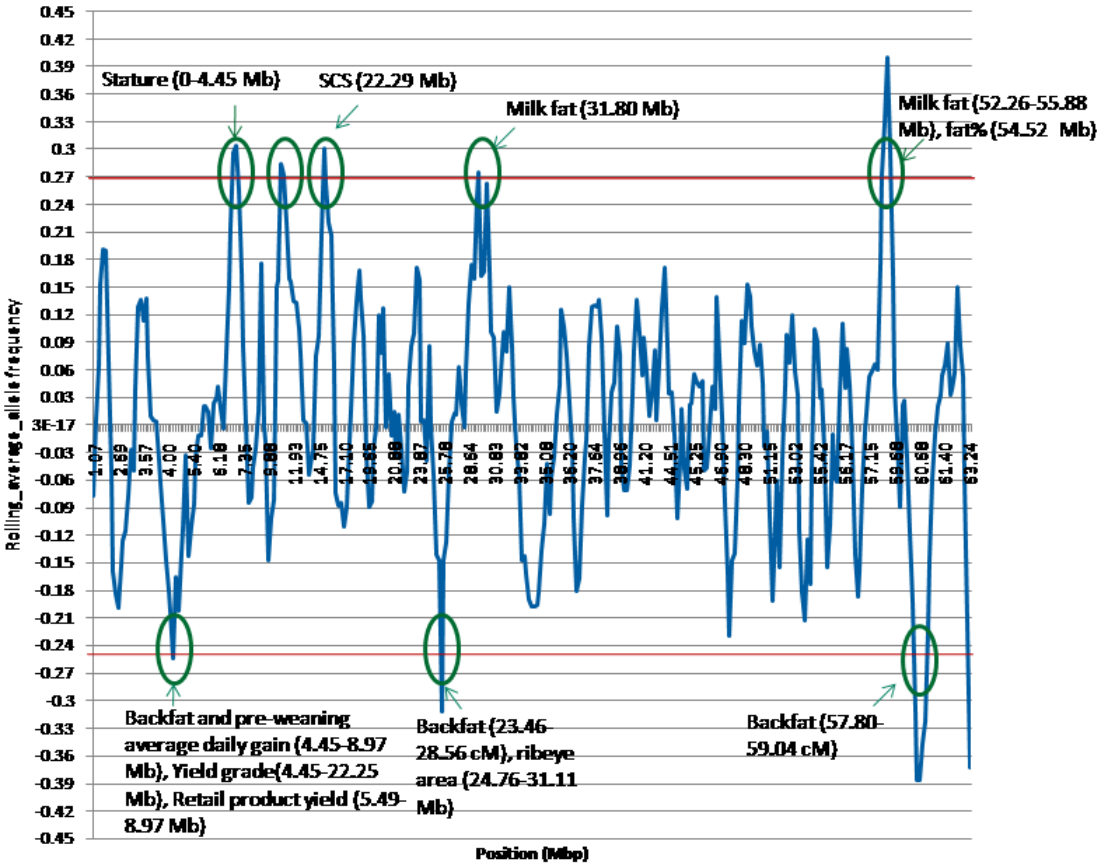

Figure 3-2. Rolling average allele frequency distribution of 355 SNP markers along BTA19 for beef and dairy cattle. The deviations above and below the axis show evidence of selection in dairy and beef cattle respectively with significant thresholds of 0.27 and -0.25 respectively shown by red lines.

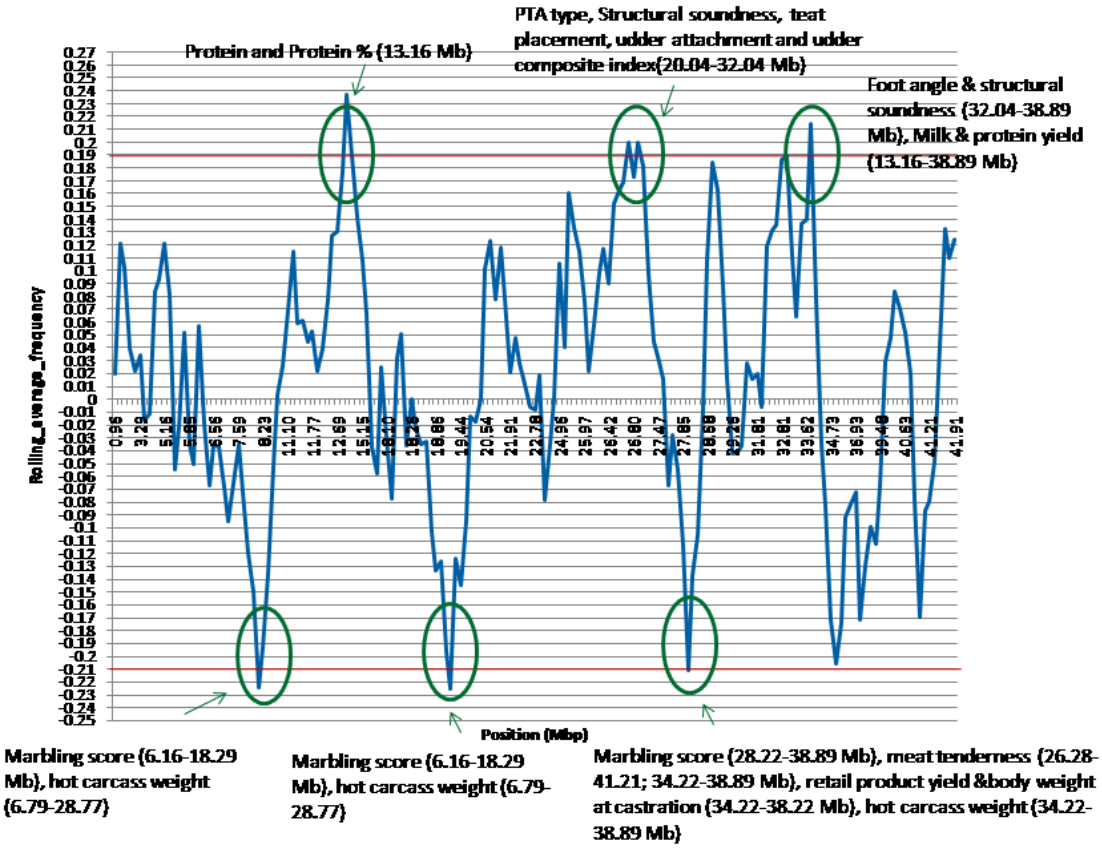

Figure 3-3. Rolling average allele frequency distribution of 175 SNP markers along BTA29 for beef and dairy cattle. The deviations above and below the axis show evidence of selection in dairy and beef cattle respectively with significant threshold of 0.19 and -0.21 respectively shown by red lines.

Table 3-5. List of the frequency of the alleles of 355 and 175 SNP markers on BTA19 and 29 respectively, common in both breeds of cattle, plotted in Figures 3-2 and 3-3

BTA	SNP_ID	Position (Mbp)	Allele	Frequency Holstein	Frequency Angus
19	BTA-25119	0.36	G	0.221183801	0.228
19	BTA-46468	0.70	C	0.335987261	0.226190476
19	BTA-109954	1.07	A	0.459627329	0.503968254
19	BTA-86613	1.72	G	0.00310559	0.220
19	BTA-86615	1.72	G	0.001557632	0.224
19	BTA-117829	1.86	A	0.462837838	0.058333333
19	BTA-117833	1.93	A	0.467391304	0.063492063
19	BTA-117835	1.98	A	0.467391304	0.067460317
19	BTA-87958	2.07	G	0.394409938	0.420634921
19	BTA-22161	2.31	G	0.327639752	0.555555556
19	BTA-22160	2.39	C	0.152647975	0.353174603
19	BTA-22155	2.41	G	0.153726708	0.353174603
19	BTA-22149	2.69	A	0.250778816	0.396825397
19	BTA-22150	2.69	C	0.250778816	0.396825397
19	BTA-22143	2.74	C	0.130434783	0.432539683
19	BTA-22140	2.95	G	0.471875	0.43495935
19	BTA-22142	3.00	A	0.456521739	0.528
19	BTA-28135	3.21	G	0.228571429	0.320512821
19	BTA-28126	3.22	A	0.386645963	0.340163934
19	BTA-28131	3.26	A	0.386292835	0.44047619
19	BTA-02315	3.47	A	0.037383178	0.1125
19	BTA-108969	3.57	A	0.641304348	0.272
19	BTA-108967	3.57	C	0.641744548	0.281746032
19	BTA-28111	3.76	G	0.413522013	0.330578512
19	BTA-28119	3.92	A	0.03894081	0.204
19	BTA-28112	3.98	C	0.411490683	0.365079365
19	BTA-28106	3.98	A	0.409937888	0.365079365
19	BTA-28107	3.98	A	0.409937888	0.365079365
19	BTA-28108	3.98	A	0.419254658	0.365079365
19	BTA-28104	3.98	C	0.038819876	0.206349206
19	BTA-28153	3.98	A	0.038819876	0.206349206
19	BTA-28152	3.99	A	0.290372671	0.370967742
19	BTA-28121	4.00	A	0.038819876	0.199186992
19	BTA-28151	4.04	C	0.040372671	0.2
19	BTA-46430	4.10	A	0.040372671	0.373015873
19	BTA-46432	4.19	G	0.0375	0.319148936
19	BTA-46433	4.31	C	0.040372671	0.373015873
19	BTA-46575	4.55	G	0.573208723	0.293650794

19	BTA-04223	4.71	G	0.254658385	0.599206349
19	BTA-44652	5.29	C	0.228971963	0.246031746
19	BTA-44665	5.33	A	0.305900621	0.448412698
19	BTA-44677	5.36	A	0.389751553	0.325396825
19	BTA-44716	5.40	A	0.23364486	0.507936508
19	BTA-44761	5.61	C	0.26552795	0.448
19	BTA-06651	5.72	A	0.141304348	0.035714286
19	BTA-44787	5.72	A	0.141304348	0.035714286
19	BTA-44793	5.80	G	0.5390625	0.352
19	BTA-44815	5.85	A	0.043478261	0.317460317
19	BTA-44888	6.10	A	0.361801242	0.384920635
19	BTA-44889	6.14	A	0.507788162	0.400793651
19	BTA-44893	6.14	A	0.479750779	0.4
19	BTA-44928	6.18	G	0.23136646	0.245967742
19	BTA-44927	6.18	C	0.23125	0.260330579
19	BTA-44930	6.18	G	0.433753943	0.424603175
19	BTA-44965	6.21	A	0.211838006	0.049180328
19	BTA-91865	6.30	A	0.082554517	0.120967742
19	BTA-45143	6.46	C	0.25931677	0.384
19	BTA-45487	6.63	A	0.41025641	0.036
19	BTA-45490	6.73	A	0.413522013	0.036
19	BTA-45492	6.76	G	0.534161491	0.044
19	BTA-45491	6.96	A	0.405660377	0.036
19	BTA-45669	7.15	A	0.273291925	0.362903226
19	BTA-45631	7.35	A	0.412772586	0.281746032
19	BTA-45586	7.41	A	0.204968944	0.277310924
19	BTA-45584	7.41	A	0.327639752	0.242063492
19	BTA-45574	7.50	A	0.06918239	0.122881356
19	BTA-11204	7.57	A	0.180124224	0.692
19	BTA-45686	8.08	A	0.536977492	0.373015873
19	BTA-45689	8.13	A	0.199376947	0.115079365
19	BTA-45688	8.25	G	0.552795031	0.361111111
19	BTA-45703	8.59	A	0.322429907	0.173387097
19	BTA-45733	9.31	C	0.49378882	0.202479339
19	BTA-16709	9.88	A	0.088509317	0.80952381
19	BTA-16718	10.01	G	0.07788162	0.084
19	BTA-45810	10.63	G	0.151090343	0.603174603
19	BTA-46438	10.77	A	0.637071651	0.254032258
19	BTA-46436	10.77	C	0.63836478	0.245901639
19	BTA-46435	10.78	G	0.685303514	0.25
19	BTA-46440	10.84	G	0.113354037	0.079365079
19	BTA-45982	11.06	C	0.375776398	0.194444444
19	BTA-13223	11.09	G	0.568322981	0.246031746

19	BTA-24942	11.93	A	0.211838006	0.08
19	BTA-24946	11.93	G	0.209375	0.08
19	BTA-46447	12.13	C	0.2734375	0.256
19	BTA-86490	12.41	A	0.102484472	0.023809524
19	BTA-86493	12.46	A	0.53894081	0.226190476
19	BTA-00316	12.57	A	0.105590062	0.119047619
19	BTA-86498	12.62	G	0.105590062	0.112
19	BTA-25637	13.53	A	0.244514107	0.591666667
19	BTA-46509	14.04	A	0.131987578	0.067460317
19	BTA-97840	14.13	G	0.35046729	0.317460317
19	BTA-46474	14.42	A	0.230529595	0.146825397
19	BTA-46456	14.75	A	0.366459627	0.338709677
19	BTA-46514	15.30	G	0.431677019	0.261904762
19	BTA-09214	15.64	A	0.524922118	0.356
19	BTA-46564	15.72	A	0.73125	0.140495868
19	BTA-46552	16.06	A	0.739130435	0.19047619
19	BTA-46543	16.33	G	0.293269231	0.448
19	BTA-05909	16.40	G	0.099688474	0.146825397
19	BTA-29947	16.64	A	0.416666667	0.317460317
19	BTA-46527	16.74	A	0.080745342	0.219512195
19	BTA-44521	16.89	C	0.25931677	0.390243902
19	BTA-07806	17.10	A	0.153726708	0.37398374
19	BTA-44540	17.39	G	0.077639752	0.108
19	BTA-44552	17.53	A	0.218068536	0.24796748
19	BTA-44546	17.83	C	0.031152648	0.064
19	BTA-44561	17.94	A	0.205607477	0.146341463
19	BTA-44563	18.07	G	0.327102804	0.216
19	BTA-44603	18.91	A	0.391509434	0.051587302
19	BTA-44594	19.06	A	0.312893082	0.111111111
19	BTA-44616	19.33	C	0.294392523	0.165322581
19	BTA-13335	19.36	A	0.319875776	0.409090909
19	BTA-44610	19.65	A	0.057632399	0.154471545
19	BTA-20575	20.18	C	0.282608696	0.591269841
19	BTA-46586	20.33	A	0.147975078	0.228
19	BTA-46580	20.39	C	0.529503106	0.369047619
19	BTA-46576	20.39	G	0.55362776	0.36440678
19	BTA-46571	20.41	C	0.121875	0.096774194
19	BTA-15926	20.45	C	0.476635514	0.168
19	BTA-44631	20.57	C	0.302795031	0.596
19	BTA-44637	20.65	C	0.624223602	0.218253968
19	BTA-44638	20.70	A	0.23447205	0.692
19	BTA-44649	20.88	A	0.4578125	0.14516129
19	BTA-44669	21.39	A	0.172897196	0.2

19	BTA-07830	22.01	A	0.408385093	0.572
19	BTA-118485	22.03	G	0.557632399	0.404
19	BTA-04414	22.15	G	0.232087227	0.445833333
19	BTA-44726	22.34	A	0.461180124	0.341269841
19	BTA-44731	22.45	G	0.116459627	0.37704918
19	BTA-44751	22.55	G	0.116352201	0.173553719
19	BTA-44791	23.32	A	0.709627329	0.079365079
19	BTA-44801	23.53	A	0.2046875	0.204
19	BTA-01578	23.87	A	0.413043478	0.224
19	BTA-44833	23.97	G	0.248417722	0.150793651
19	BTA-44838	24.15	A	0.361370717	0.491935484
19	BTA-44845	24.22	A	0.366459627	0.504
19	BTA-115853	24.45	G	0.118012422	0.111111111
19	BTA-44868	24.65	G	0.176012461	0.208
19	BTA-07396	25.01	G	0.795950156	0.071428571
19	BTA-108581	25.20	G	0.218944099	0.780487805
19	BTA-44691	25.63	G	0.031055901	0.444444444
19	BTA-44690	25.74	G	0.032608696	0.452
19	BTA-44693	25.78	A	0.324534161	0.392857143
19	BTA-98517	26.58	A	0.234375	0.328
19	BTA-44712	27.34	A	0.285046729	0.023809524
19	BTA-14962	27.50	A	0.288819876	0.607142857
19	BTA-44960	27.87	G	0.091614907	0.119047619
19	BTA-44964	27.98	A	0.306853583	0.107142857
19	BTA-44976	28.06	A	0.242990654	0.294354839
19	BTA-44985	28.43	G	0.367601246	0.112
19	BTA-44989	28.44	A	0.063862928	0.123015873
19	BTA-44990	28.57	T	0.261682243	0.492
19	BTA-01174	28.64	A	0.127725857	0.051587302
19	BTA-104726	28.85	A	0.403726708	0.161290323
19	BTA-67105	29.50	T	0.77484472	0.142857143
19	BTA-45030	29.82	A	0.535714286	0.380952381
19	BTA-45023	30.13	A	0.158878505	0.468253968
19	BTA-13124	30.16	A	0.55625	0.031746032
19	BTA-45027	30.16	G	0.406832298	0.027777778
19	BTA-29349	30.23	A	0.440993789	0.37398374
19	BTA-106969	30.56	A	0.224137931	0.052
19	BTA-45064	30.63	A	0.234177215	0.06147541
19	BTA-45079	30.83	A	0.091900312	0.043650794
19	BTA-20635	31.17	G	0.093167702	0.043650794
19	BTA-45082	31.34	C	0.104037267	0.068
19	BTA-05960	31.70	C	0.001552795	0.238095238
19	BTA-11250	32.53	A	0.527950311	0.253968254

19	BTA-97038	32.58	G	0.527950311	0.24796748
19	BTA-45090	32.86	G	0.47943038	0.321428571
19	BTA-45036	33.18	G	0.454402516	0.527777778
19	BTA-45040	33.29	A	0.543613707	0.432539683
19	BTA-45047	33.80	G	0.400311526	0.452
19	BTA-45106	33.82	G	0.382445141	0.368
19	BTA-45109	33.92	A	0.032608696	0.148
19	BTA-45146	34.22	A	0.194099379	0.424
19	BTA-07221	34.38	T	0.270186335	0.624
19	BTA-45368	34.51	A	0.057453416	0.087301587
19	BTA-45372	34.62	G	0.061708861	0.143442623
19	BTA-45375	34.68	C	0.152380952	0.408
19	BTA-45377	34.79	G	0.160377358	0.424603175
19	BTA-45380	34.89	A	0.1890625	0.543650794
19	BTA-45379	34.97	G	0.056074766	0.083333333
19	BTA-45269	35.08	C	0.212264151	0.221774194
19	BTA-11992	35.32	A	0.221183801	0.25
19	BTA-45275	35.37	G	0.338006231	0.456349206
19	BTA-45285	35.60	C	0.1890625	0.214285714
19	BTA-45288	35.76	A	0.161993769	0.46031746
19	BTA-45292	35.78	A	0.489130435	0.301587302
19	BTA-45299	35.89	G	0.405279503	0.325396825
19	BTA-45304	36.06	G	0.229813665	0.10483871
19	BTA-45303	36.10	A	0.229813665	0.107142857
19	BTA-45302	36.14	A	0.228971963	0.115079365
19	BTA-45305	36.20	A	0.198757764	0.099206349
19	BTA-45314	36.34	G	0.377329193	0.46
19	BTA-45315	36.34	T	0.326086957	0.463709677
19	BTA-45316	36.39	C	0.326086957	0.468
19	BTA-09802	36.53	G	0.366459627	0.609756098
19	BTA-45325	36.92	A	0.107142857	0.403225806
19	BTA-45358	37.30	A	0.242990654	0.262096774
19	BTA-45339	37.43	T	0.302884615	0.037190083
19	BTA-45654	37.52	G	0.2859375	0.166666667
19	BTA-45350	37.62	A	0.0578125	0.20661157
19	BTA-45351	37.64	A	0.450155763	0.234126984
19	BTA-45352	37.72	A	0.409677419	0.213709677
19	BTA-88705	37.77	G	0.540372671	0.265873016
19	BTA-45382	37.97	T	0.540625	0.436507937
19	BTA-45494	38.15	G	0.038819876	0.144
19	BTA-45474	38.31	A	0.4109375	0.408730159
19	BTA-04699	38.41	A	0.291277259	0.609756098
19	BTA-45439	38.62	C	0.124610592	0.297619048

19	BTA-45448	38.73	C	0.591900312	0.103174603
19	BTA-45457	38.87	C	0.277602524	0.097560976
19	BTA-45458	38.96	G	0.442546584	0.384920635
19	BTA-45470	39.26	G	0.099378882	0.111111111
19	BTA-45469	39.36	A	0.247648903	0.574380165
19	BTA-45404	39.66	C	0.037383178	0.156
19	BTA-57050	40.42	A	0.520440252	0.476190476
19	BTA-57051	40.42	A	0.52484472	0.468253968
19	BTA-57052	40.42	A	0.524922118	0.397540984
19	BTA-57053	40.44	G	0.52484472	0.468253968
19	BTA-56081	40.89	G	0.496884735	0.369047619
19	BTA-45517	41.10	A	0.619565217	0.304878049
19	BTA-45521	41.20	A	0.319875776	0.44214876
19	BTA-45527	41.27	C	0.319314642	0.423387097
19	BTA-03390	41.84	G	0.613207547	0.349206349
19	BTA-45570	41.87	G	0.447040498	0.504
19	BTA-99555	42.65	G	0.496865204	0.427419355
19	BTA-99554	42.65	G	0.506269592	0.488
19	BTA-45537	43.55	G	0.414596273	0.301587302
19	BTA-45532	43.67	A	0.334890966	0.448412698
19	BTA-45661	44.32	A	0.257009346	0.012
19	BTA-45659	44.42	A	0.672897196	0.300813008
19	BTA-45683	44.51	A	0.41588785	0.173387097
19	BTA-45684	44.53	A	0.118380062	0.4375
19	BTA-45682	44.55	A	0.035714286	0.403225806
19	BTA-45680	44.58	A	0.417445483	0.166666667
19	BTA-45676	44.61	G	0.51552795	0.386178862
19	BTA-45675	44.64	A	0.00931677	0.206349206
19	BTA-02462	44.84	A	0.412772586	0.428571429
19	BTA-93411	45.02	C	0.406832298	0.488
19	BTA-93414	45.03	C	0.406832298	0.484
19	BTA-45579	45.07	A	0.094720497	0.071428571
19	BTA-45581	45.25	G	0.6109375	0.349206349
19	BTA-45589	45.32	G	0.371118012	0.375
19	BTA-45597	45.38	G	0.421383648	0.348
19	BTA-45615	45.78	A	0.124223602	0.242063492
19	BTA-45621	45.87	G	0.496884735	0.5
19	BTA-03894	46.12	A	0.381987578	0.091269841
19	BTA-103899	46.23	G	0.196261682	0.686507937
19	BTA-45701	46.51	A	0.371473354	0.281746032
19	BTA-45731	46.60	A	0.3203125	0.23015873
19	BTA-45743	46.85	A	0.239875389	0.011904762
19	BTA-45737	46.90	A	0.310559006	0.142857143

19	BTA-45750	46.96	G	0.215625	0.095238095
19	BTA-13041	47.42	A	0.138198758	0.369918699
19	BTA-45908	47.64	A	0.138198758	0.388888889
19	BTA-13047	47.66	A	0.169254658	0.39516129
19	BTA-13045	47.66	A	0.169254658	0.396825397
19	BTA-45802	48.23	A	0.276397516	0.488
19	BTA-45799	48.27	G	0.495341615	0.317460317
19	BTA-45795	48.27	A	0.275700935	0.488
19	BTA-45794	48.27	G	0.495341615	0.317460317
19	BTA-45793	48.30	G	0.496884735	0.317460317
19	BTA-45770	48.74	G	0.4625	0.218253968
19	BTA-45768	48.80	A	0.214285714	0.158536585
19	BTA-05671	48.87	A	0.161490683	0.054166667
19	BTA-91568	49.28	A	0.270186335	0.16
19	BTA-45875	49.94	A	0.183229814	0.162698413
19	BTA-45868	49.95	G	0.2890625	0.182926829
19	BTA-45864	50.01	G	0.041925466	0.05952381
19	BTA-04652	50.63	G	0.369565217	0.152
19	BTA-45843	50.73	A	0.052795031	0.150793651
19	BTA-45937	51.15	A	0.107476636	0.432
19	BTA-03377	51.26	G	0.39184953	0.2
19	BTA-45954	51.32	A	0.084375	0.571428571
19	BTA-45963	51.39	C	0.211180124	0.448412698
19	BTA-45966	51.46	C	0.184952978	0.076
19	BTA-45979	51.77	A	0.23089172	0.108
19	BTA-07747	51.92	A	0.053125	0.334677419
19	BTA-46072	52.11	G	0.4328125	0.321428571
19	BTA-46037	52.31	G	0.208074534	0.055555556
19	BTA-46095	52.71	A	0.517080745	0.130952381
19	BTA-46135	53.02	G	0.440809969	0.467479675
19	BTA-46121	53.18	A	0.446708464	0.471774194
19	BTA-46115	53.22	A	0.200310559	0.392857143
19	BTA-46256	53.56	T	0.142857143	0.119047619
19	BTA-46126	53.69	C	0.056074766	0.406504065
19	BTA-01709	53.74	A	0.053291536	0.418032787
19	BTA-46262	54.84	A	0.273291925	0.451219512
19	BTA-46280	54.96	A	0.521806854	0.272727273
19	BTA-46281	55.01	G	0.151090343	0.373015873
19	BTA-46285	55.28	A	0.3140625	0.031746032
19	BTA-46292	55.42	C	0.503194888	0.112
19	BTA-46305	55.46	A	0.186335404	0.428571429
19	BTA-109506	55.57	A	0.232919255	0.290983607
19	BTA-05874	55.59	C	0.166149068	0.345238095

19	BTA-77447	55.68	A	0.31152648	0.366666667
19	BTA-46306	55.96	G	0.186335404	0.428
19	BTA-46307	56.07	A	0.211180124	0.223577236
19	BTA-46313	56.08	G	0.674454829	0.234126984
19	BTA-46302	56.10	A	0.23757764	0.646825397
19	BTA-109495	56.15	A	0.222741433	0.308
19	BTA-109491	56.17	A	0.23447205	0
19	BTA-77448	56.21	C	0.6828125	0.31147541
19	BTA-03306	56.23	A	0.3265625	0.233333333
19	BTA-46322	56.51	G	0.3734375	0.568
19	BTA-09444	56.70	A	0.159375	0.443089431
19	BTA-84899	56.77	G	0.130434783	0.264
19	BTA-84891	56.84	C	0.2609375	0.448412698
19	BTA-84898	56.88	A	0.130434783	0.264
19	BTA-84894	56.94	A	0.319875776	0.38
19	BTA-46341	57.09	A	0.236760125	0.064
19	BTA-46342	57.15	A	0.239130435	0.063492063
19	BTA-46348	57.30	C	0.350931677	0.337301587
19	BTA-104738	57.57	G	0.108695652	0.144
19	BTA-104739	57.63	G	0.108695652	0.144
19	BTA-104732	58.37	A	0.220496894	0.004
19	BTA-93880	59.21	C	0.437888199	0.292
19	BTA-46056	59.32	G	0.715838509	0.136
19	BTA-07437	59.34	G	0.577639752	0.119047619
19	BTA-46059	59.36	A	0.25310559	0
19	BTA-46360	59.59	G	0.625776398	0.05952381
19	BTA-46361	59.68	G	0.00621118	0.369047619
19	BTA-46363	59.77	A	0.104037267	0.206349206
19	BTA-46364	59.94	C	0.144409938	0.281746032
19	BTA-05949	59.99	G	0.458074534	0.5
19	BTA-46380	60.35	G	0.22826087	0.028
19	BTA-46381	60.35	T	0.235576923	0.044354839
19	BTA-05994	60.57	G	0.405279503	0.483870968
19	BTA-46408	60.63	A	0.013975155	0.464285714
19	BTA-46409	60.63	A	0.027950311	0.476
19	BTA-46413	60.64	G	0.01242236	0.19047619
19	BTA-46416	60.68	G	0.027950311	0.435483871
19	BTA-46407	60.77	A	0.013975155	0.463414634
19	BTA-46404	60.79	A	0.027950311	0.476190476
19	BTA-21385	60.90	G	0.143302181	0.392
19	BTA-21380	60.93	G	0.146417445	0.206349206
19	BTA-07431	61.06	G	0.422360248	0.332
19	BTA-21181	61.22	A	0.24378882	0.30952381

19	BTA-29633	61.31	C	0.351097179	0.357142857
19	BTA-29634	61.35	C	0.352484472	0.361788618
19	BTA-07433	61.40	C	0.422360248	0.325396825
19	BTA-07434	61.40	A	0.387850467	0.242063492
19	BTA-29628	61.43	G	0.420807453	0.376984127
19	BTA-29635	61.44	A	0.420560748	0.373015873
19	BTA-21185	61.72	G	0.150621118	0.035714286
19	BTA-01614	61.82	A	0.037267081	0.228
19	BTA-105913	61.94	A	0.213166144	0.035714286
19	BTA-105515	62.02	G	0.461180124	0.326612903
19	BTA-105530	62.18	G	0.681677019	0.166666667
19	BTA-105528	62.30	C	0.284161491	0.492063492
19	BTA-13718	62.83	A	0.145962733	0.5
19	BTA-46020	63.24	A	0.009404389	0.376
19	BTA-46021	63.27	G	0.140186916	0.8
19	BTA-46024	63.44	G	0.236024845	0.512195122
29	BTA-65690	0	G	0.229813665	0.301587302
29	BTA-109603	0.54	A	0.509345794	0.25
29	BTA-66450	0.96	G	0.26242236	0.170634921
29	BTA-03053	2.19	A	0.184782609	0.104
29	BTA-66438	2.83	A	0.057632399	0.321428571
29	BTA-66437	2.93	G	0.647975078	0.208
29	BTA-66411	3.22	G	0.520186335	0.357723577
29	BTA-66407	3.29	A	0.055900621	0.28
29	BTA-66134	4.15	A	0.238317757	0.242063492
29	BTA-66472	4.49	C	0.378504673	0.582644628
29	BTA-66400	4.88	A	0.27484472	0.087301587
29	BTA-66404	5.16	C	0.2734375	0.084
29	BTA-66395	5.16	G	0.450310559	0.2
29	BTA-07370	5.34	G	0.448757764	0.404
29	BTA-66525	5.37	A	0.26863354	0.336
29	BTA-66550	5.51	A	0.457680251	0.471311475
29	BTA-66587	5.82	A	0.186335404	0.676
29	BTA-66575	5.85	G	0.440993789	0.023809524
29	BTA-66576	5.89	G	0.442367601	0.028
29	BTA-66579	6.16	C	0.122670807	0.62601626
29	BTA-66617	6.42	A	0.143302181	0.2375
29	BTA-117883	6.52	G	0.439252336	0.390243902
29	BTA-105620	6.56	G	0.239130435	0.204
29	BTA-105615	6.87	C	0.2921875	0.115079365
29	BTA-105616	6.94	C	0.177018634	0.52
29	BTA-105618	6.97	G	0.180952381	0.286290323
29	BTA-24968	7.32	C	0.245341615	0.353174603

29	BTA-18356	7.59	G	0.291925466	0.388
29	BTA-66634	7.59	A	0.48757764	0.159836066
29	BTA-06107	7.77	G	0.330745342	0.524390244
29	BTA-27538	8.01	G	0.196875	0.552
29	BTA-27534	8.19	G	0.178571429	0.464285714
29	BTA-120302	8.23	A	0.2171875	0.463114754
29	BTA-113862	9.11	T	0.252365931	0.296747967
29	BTA-113865	9.17	A	0.249216301	0.2875
29	BTA-70172	9.97	C	0.465732087	0.531746032
29	BTA-105939	10.83	A	0.540372671	0.412698413
29	BTA-105940	11.10	T	0.478193146	0.444
29	BTA-105947	11.24	G	0.1609375	0.087301587
29	BTA-117782	11.56	G	0.312111801	0.134920635
29	BTA-112191	11.66	G	0.531446541	0.369047619
29	BTA-112193	11.74	G	0.343167702	0.495934959
29	BTA-16286	11.77	G	0.313084112	0.266666667
29	BTA-22554	11.89	A	0.144859813	0.154761905
29	BTA-64906	12.30	C	0.423676012	0.207317073
29	BTA-64902	12.36	A	0.150621118	0.142857143
29	BTA-93929	12.50	C	0.295031056	0.357142857
29	BTA-08572	12.69	G	0.475077882	0.238095238
29	BTA-08585	12.85	G	0.475	0.236
29	BTA-08579	12.95	A	0.468652038	0.236
29	BTA-08577	13.78	G	0.469648562	0.231707317
29	BTA-08584	13.99	G	0.476635514	0.236
29	BTA-64938	15.15	T	0.057632399	0.05952381
29	BTA-64937	15.16	C	0.059006211	0.05952381
29	BTA-64934	15.28	G	0.294392523	0.23015873
29	BTA-64925	15.32	G	0.073208723	0.044
29	BTA-65056	17.93	A	0.110248447	0.388888889
29	BTA-16404	18.10	A	0.190625	0.297619048
29	BTA-16399	18.17	A	0.517080745	0.099206349
29	BTA-16409	18.22	G	0.275700935	0.487704918
29	BTA-16410	18.22	T	0.2765625	0.487903226
29	BTA-16408	18.23	C	0.5734375	0.300813008
29	BTA-16406	18.26	A	0.094720497	0.107142857
29	BTA-38148	18.58	A	0.085403727	0.10483871
29	BTA-38149	18.58	A	0.083850932	0.111111111
29	BTA-38144	18.61	G	0.020186335	0.349206349
29	BTA-03493	18.70	G	0.24689441	0.036585366
29	BTA-116569	18.86	A	0.445652174	0.444444444
29	BTA-65064	18.99	G	0.132398754	0.503968254
29	BTA-65068	19.05	A	0.0703125	0.25

29	BTA-09899	19.18	A	0.065830721	0.361111111
29	BTA-65070	19.27	C	0.23447205	0.376
29	BTA-65073	19.44	G	0.232919255	0.375
29	BTA-26204	19.89	A	0.229813665	0.095238095
29	BTA-26203	20.15	A	0.035714286	0.318181818
29	BTA-26202	20.18	C	0.062111801	0.100806452
29	BTA-26209	20.28	G	0.363354037	0.103174603
29	BTA-61000	20.54	A	0.291277259	0.452380952
29	BTA-17015	20.76	C	0.278816199	0.055555556
29	BTA-17014	20.91	A	0.280564263	0.054621849
29	BTA-65087	21.23	A	0.24378882	0.174603175
29	BTA-65091	21.65	A	0.23447205	0.204918033
29	BTA-07708	21.91	G	0.326086957	0.284
29	BTA-65111	22.25	A	0.145962733	0.123015873
29	BTA-65113	22.32	A	0.084394904	0.146825397
29	BTA-08389	22.39	G	0.3109375	0.103174603
29	BTA-65147	22.60	G	0.190993789	0.261904762
29	BTA-65151	22.78	G	0.238244514	0.280487805
29	BTA-65153	23.03	A	0.216510903	0.28
29	BTA-65157	23.13	G	0.214953271	0.285714286
29	BTA-65162	23.20	G	0.358695652	0.015873016
29	BTA-65165	23.22	A	0.0984375	0.657258065
29	BTA-65224	24.96	A	0.275700935	0.099206349
29	BTA-12811	25.00	A	0.172360248	0.056
29	BTA-65220	25.13	G	0.552795031	0.102459016
29	BTA-65388	25.49	T	0.35046729	0.333333333
29	BTA-85843	25.93	A	0.22327044	0.178861789
29	BTA-85871	25.97	C	0.350931677	0.310483871
29	BTA-85838	26.10	A	0.219626168	0.195121951
29	BTA-65297	26.21	G	0.607142857	0.35483871
29	BTA-65291	26.34	A	0.071428571	0.325396825
29	BTA-65277	26.42	G	0.392523364	0.165322581
29	BTA-65293	26.42	G	0.596214511	0.353174603
29	BTA-65301	26.63	A	0.399068323	0.280487805
29	BTA-65296	26.63	A	0.404984424	0.286885246
29	BTA-65498	26.70	A	0.251552795	0.198412698
29	BTA-65275	26.79	G	0.389751553	0.108870968
29	BTA-65272	26.80	A	0.389751553	0.112903226
29	BTA-65268	26.81	A	0.3894081	0.119047619
29	BTA-106381	27.16	A	0.122670807	0.136
29	BTA-106382	27.30	G	0.316770186	0.133064516
29	BTA-106378	27.37	G	0.318322981	0.130081301
29	BTA-65467	27.47	A	0.146687697	0.286290323

29	BTA-90762	27.67	A	0.0609375	0.055555556
29	BTA-90745	27.74	T	0.121118012	0.19047619
29	BTA-90754	27.78	A	0.513975155	0.420634921
29	BTA-90746	27.81	A	0.147515528	0.373015873
29	BTA-90748	27.85	C	0.251552795	0.194444444
29	BTA-65531	28.06	C	0.172360248	0.304
29	BTA-65524	28.24	G	0.270186335	0.634920635
29	BTA-65517	28.31	A	0.045031056	0.436
29	BTA-65515	28.42	G	0.48757764	0.348
29	BTA-65505	28.68	A	0.414596273	0.193548387
29	BTA-22805	28.72	G	0.605919003	0.25
29	BTA-22801	28.72	A	0.444099379	0.23015873
29	BTA-10760	28.76	G	0.41588785	0.424
29	BTA-65444	28.87	A	0.414556962	0.383064516
29	BTA-65427	29.26	T	0.034161491	0.193548387
29	BTA-74283	29.46	A	0.420807453	0.416666667
29	BTA-65408	29.84	A	0.453416149	0.531746032
29	BTA-04535	31.10	A	0.419254658	0.424
29	BTA-66492	31.36	A	0.228125	0.170634921
29	BTA-65574	31.81	G	0.535825545	0.375
29	BTA-65564	32.08	A	0.420560748	0.48
29	BTA-65568	32.15	A	0.418495298	0.475609756
29	BTA-65555	32.31	G	0.387850467	0.52
29	BTA-65658	32.78	G	0.757763975	0.071428571
29	BTA-65662	32.81	C	0.25310559	0.031746032
29	BTA-65717	32.93	G	0.183229814	0.222222222
29	BTA-65713	32.96	A	0.456386293	0.261904762
29	BTA-65699	33.11	G	0.369565217	0.484
29	BTA-29794	33.32	G	0.298136646	0.003968254
29	BTA-02252	33.62	A	0.323987539	0.336
29	BTA-65681	33.69	A	0.331632653	0.008130081
29	BTA-73109	34.14	T	0.446540881	0.239669421
29	BTA-65656	34.57	G	0.549844237	0.28968254
29	BTA-65646	34.69	C	0	0.388
29	BTA-65642	34.73	A	0.195652174	0.76984127
29	BTA-07368	34.86	A	0.22741433	0.150793651
29	BTA-99814	34.89	A	0.246105919	0.48015873
29	BTA-102309	36.64	A	0.462616822	0.376984127
29	BTA-65775	36.84	C	0.200310559	0.427419355
29	BTA-65785	36.93	A	0.24068323	0.404
29	BTA-65879	37.62	A	0.364906832	0.238095238
29	BTA-106996	37.71	A	0.156832298	0.340163934
29	BTA-106994	37.73	G	0.172360248	0.587301587

29	BTA-65836	38.50	G	0.045031056	0.056
29	BTA-66030	39.48	A	0.198757764	0.216
29	BTA-65943	40.10	G	0.152173913	0.092
29	BTA-09465	40.23	G	0.163043478	0.119047619
29	BTA-09466	40.31	A	0.163043478	0.091269841
29	BTA-65938	40.41	A	0.163492063	0.083333333
29	BTA-66057	40.63	A	0.27258567	0.107142857
29	BTA-66045	40.82	G	0.429467085	0.43852459
29	BTA-66333	41.11	A	0.314641745	0.364
29	BTA-66126	41.14	A	0.257009346	0.348
29	BTA-117001	41.21	G	0.068965517	0.475806452
29	BTA-116993	41.21	C	0.001552795	0.297619048
29	BTA-66071	41.51	G	0.635514019	0.226190476
29	BTA-01521	41.68	A	0.148734177	0.162698413
29	BTA-66095	41.85	A	0.144859813	0.08
29	BTA-66106	41.88	A	0.184294872	0.142241379
29	BTA-66122	41.91	G	0.4390625	0.277777778
29	BTA-66154	41.97	A	0.426282051	0.132
29	BTA-66215	42.37	G	0.149068323	0.091269841

To address these issues, we utilized an extended haplotype homozygosity (EHH) approach, which detects selection by detecting the presence of long-range haplotypes that putatively harbour selected alleles within a population. The chromosome-wide scan detected three regions (44.417-44.514 Mb, 61.308-61.355 Mb and 62.017-62.184 Mb) in Holstein and one region (40.444-40.889 Mb) in Angus on BTA19 that showed evidence of selection. On BTA29, we found four regions (11.655-11.739 Mb, 29.840-31.096 Mb, 31.807-32.078 Mb and 33.693-34.136 Mb) in Holstein and one region (7.767-8.006 Mb) in Angus. In all of these regions identified using the EHH approach, we found a core haplotype with the highest frequency and highest EHH among other core haplotypes at those loci, indicating positive selection (Figures 3-4 to 3-12). By comparing these regions with the regions identified using the sliding-window approach, we found two regions (11.655-11.739 Mb and 33.693-34.136 Mb) in Holstein and one region (7.767-8.006 Mb) in Angus on BTA29 that were common in both approaches and showed evidence of selection (Table 3-6).

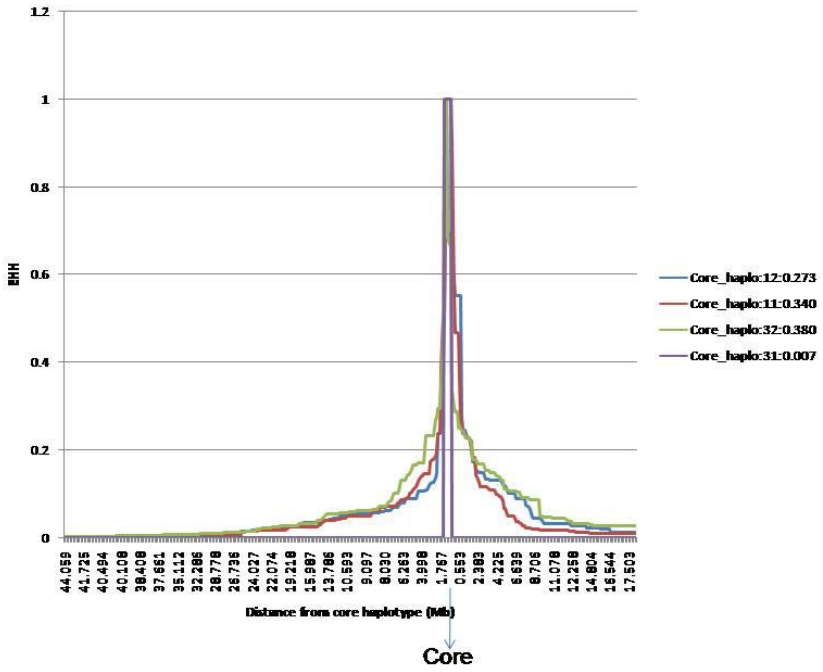

Figure 3-4. Decay of EHH as a function of distance in Holstein on BTA19. The chromosomal region of 44.417-44.514 Mb was considered as a candidate region, which was defined using markers BTA-45659 and BTA-45683. The sequence and population frequency of each core haplotype are shown after the colons, where A, C, G, and T are coded respectively as 1, 2, 3 and 4.

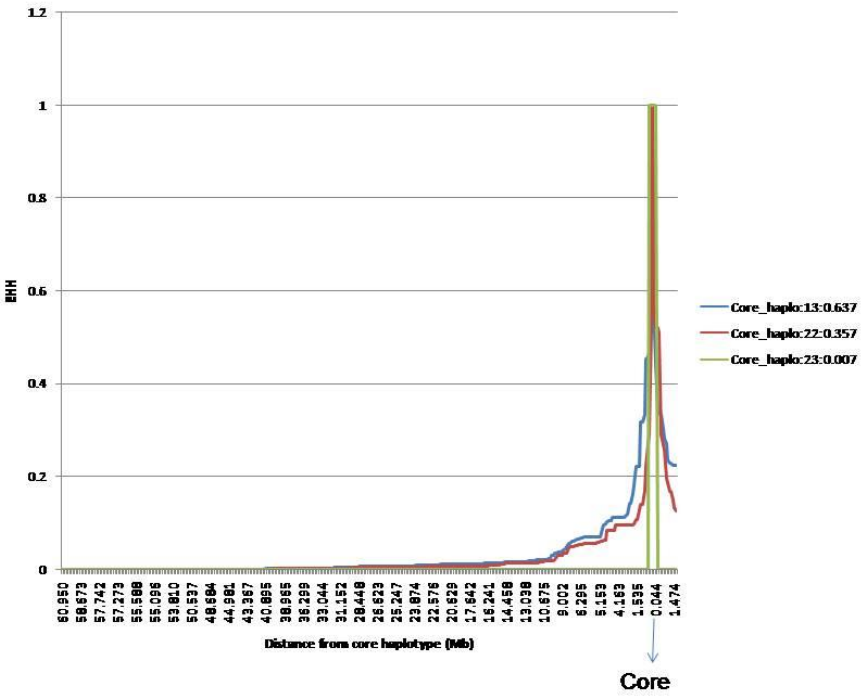

Figure 3-5. Decay of EHH as a function of distance in Holstein on BTA19. The chromosomal region of 61.308-61.355 Mb was considered as a candidate region, which was defined using markers BTA-29633 and BTA-29634. The sequence and population frequency of each core haplotype are shown after the colons, where A, C, G and T are coded respectively as 1, 2, 3 and 4.

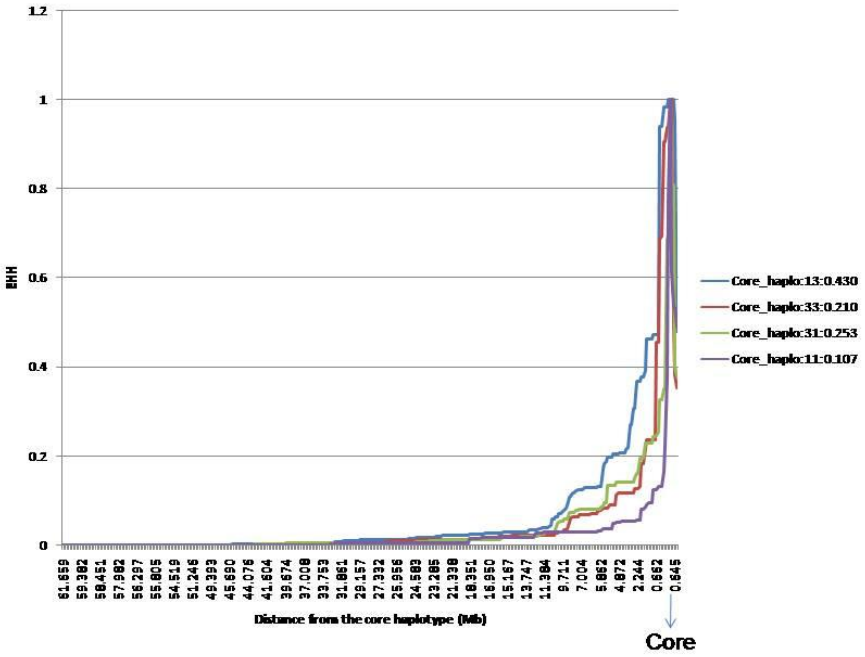

Figure 3-6. Decay of EHH as a function of distance in Holstein on BTA19. The chromosomal region of 62.017-62.184 Mb was considered as a candidate region, which was defined using markers BTA-45659 and BTA-45683. The sequence and population frequency of each core haplotype are shown after the colons, where A, C, G and T are coded respectively as 1, 2, 3 and 4.

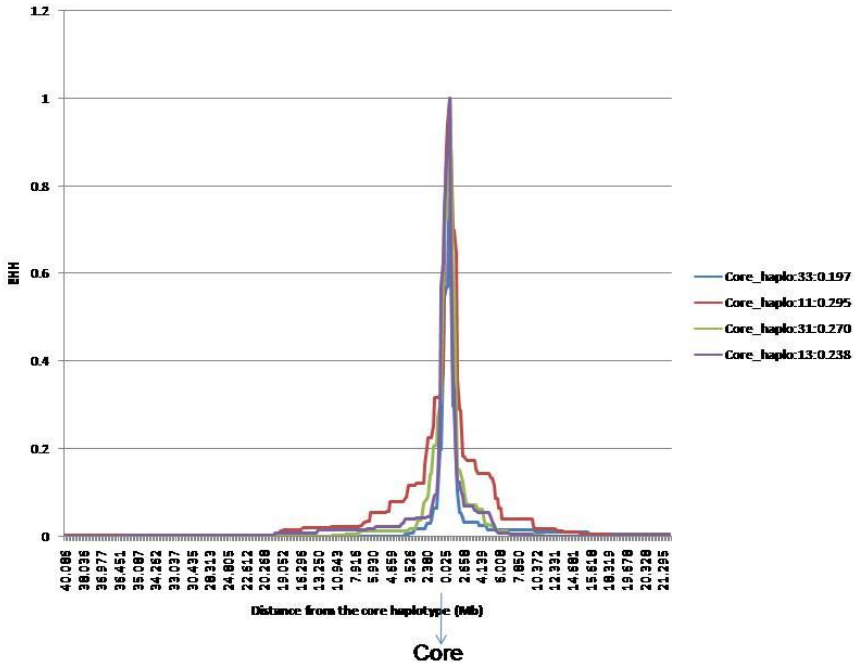

Figure 3-7. Decay of EHH as a function of distance in Angus on BTA19. The chromosomal region of 40.444-40.889 Mb was considered as a candidate region, which was defined using markers BTA-57053 and BTA-56081. The sequence and population frequency of each core haplotype are shown after the colons, where A, C, G and T are coded respectively as 1, 2, 3 and 4.

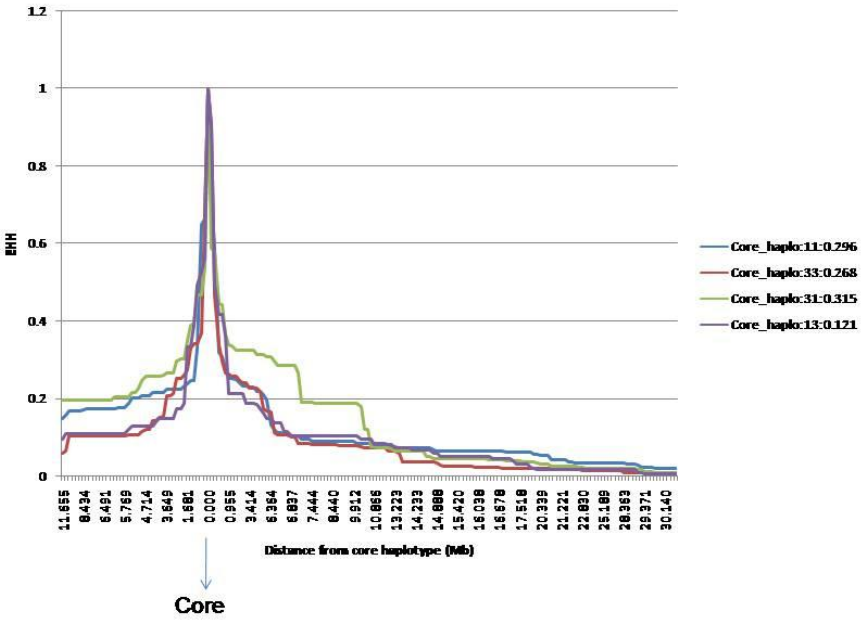

Figure 3-8. Decay of EHH as a function of distance in Holstein on BTA29. The chromosomal region of 11.655-11.739 Mb was considered as a candidate region, which was defined using markers BTA-112191 and BTA-112193. The sequence and population frequency of each core haplotype are shown after the colons, where A, C, G and T are coded respectively as 1, 2, 3 and 4.

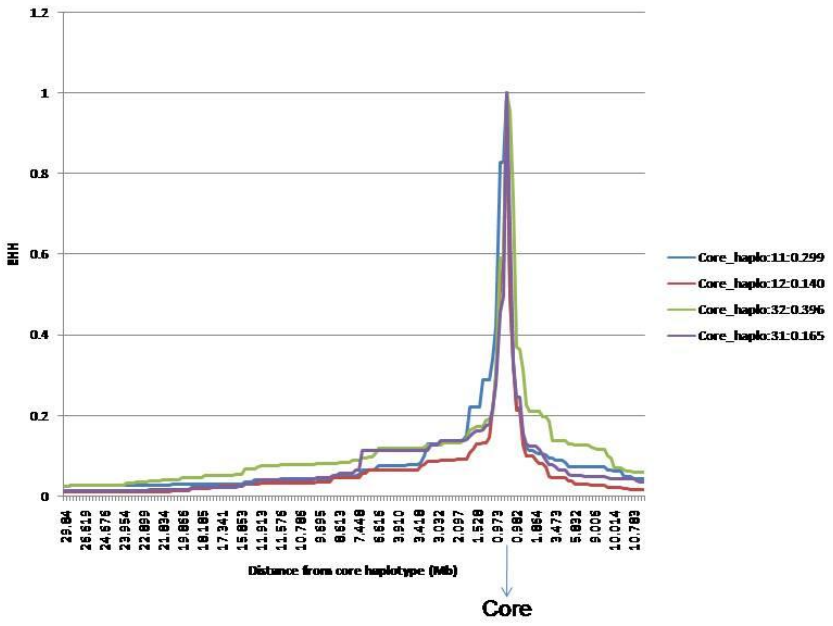

Figure 3-9. Decay of EHH as a function of distance in Holstein on BTA29. The chromosomal region of 29.840-31.096 Mb was considered as a candidate region, which was defined using markers BTA-65408 and BTA-04535. The sequence and population frequency of each core haplotype are shown after the colons, where A, C, G and T are coded respectively as 1, 2, 3 and 4.

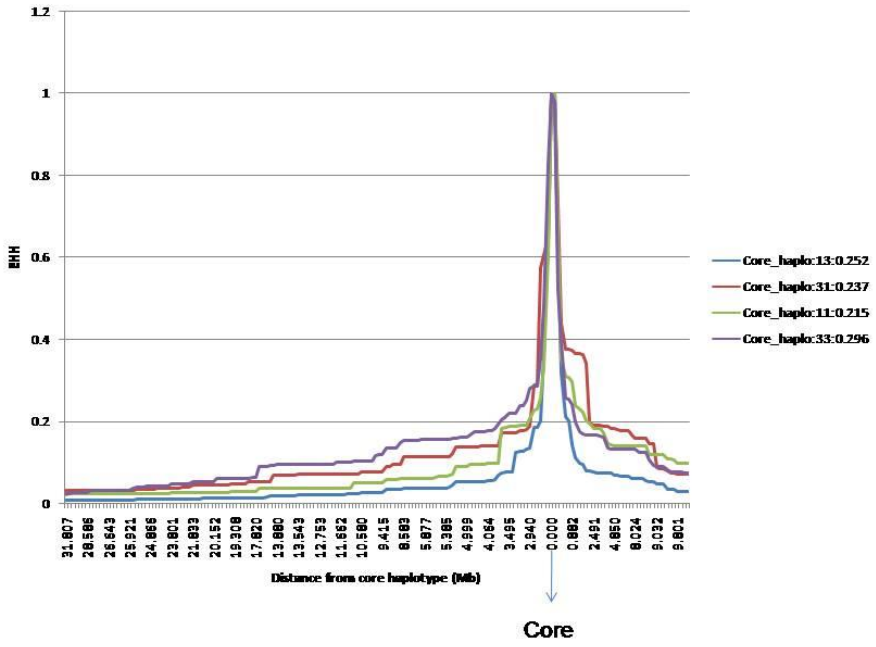

Figure 3-10. Decay of EHH as a function of distance in Holstein on BTA29. The chromosomal region of 31.807-32.078 Mb was considered as a candidate region, which was defined using markers BTA-65574 and BTA-65564. The sequence and population frequency of each core haplotype are shown after the colons, where A, C, G and T are coded respectively as 1, 2, 3 and 4.

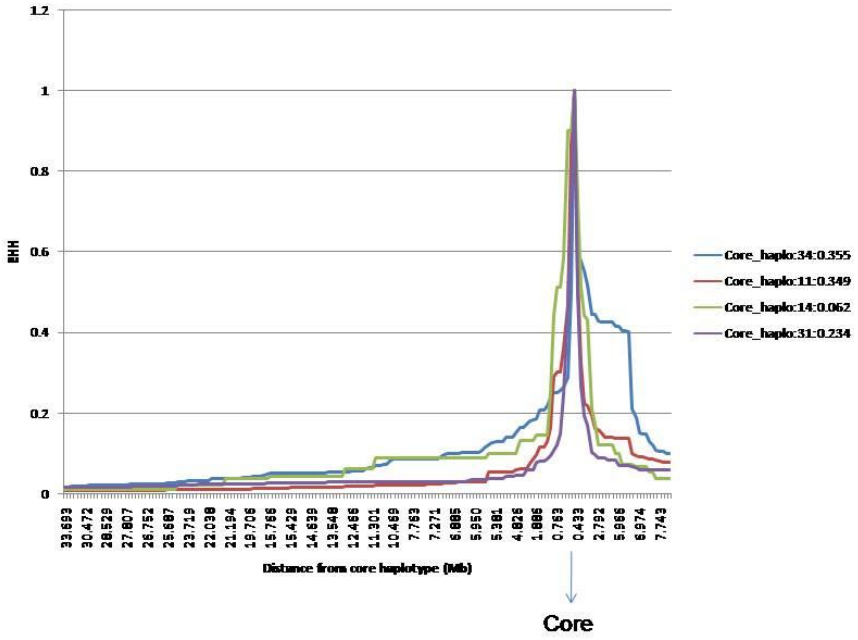

Figure 3-11. Decay of EHH as a function of distance in Holstein on BTA29. The chromosomal region of 33.693-34.136 Mb was considered as a candidate region, which was defined using markers BTA-65681and BTA-73109. The sequence and population frequency of each core haplotype are shown after the colons, where A, C, G and T are coded respectively as 1, 2, 3 and 4.

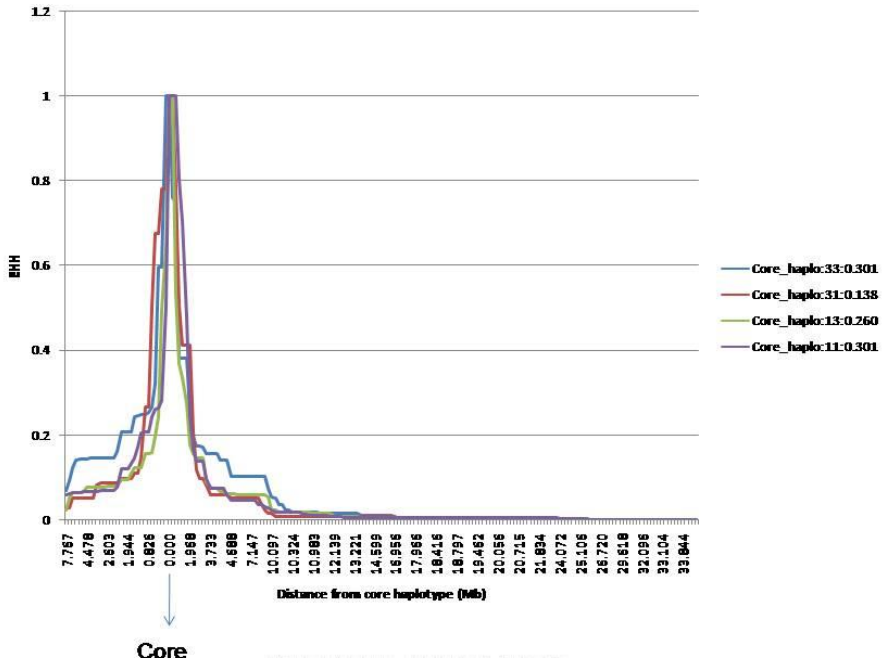

Figure 3-12. Decay of EHH as a function of distance in Angus on BTA29. The chromosomal region of 7.767-8.006 Mb was considered as a candidate region, which was defined using markers BTA-06107 and BTA-27538. The sequence and population frequency of each core haplotype are shown after the colons, where A, C, G and T are coded respectively as 1, 2, 3 and 4.

Table 3-6. Summary of the comparison of the chromosomal regions showing evidence of selection using five locus sliding window and EHH approach. The chromosomal regions, which showed evidence of selection in both approaches, are highlighted in grey colour

BTA	Method	Dairy/Beef	Selection signatures (Mb)	Trait	Position Btau3.1 (Mbp)	Reference
19	SW*	Dairy	6.18-7.35	Stature	0-4.45	Ashwell *et al.* 2005
			9.88-11.93	-	-	-
			14.75-17.10	SCS	22.29	Bennewitz *et al.* 2003
19	SW*	Dairy	28.64-30.83	Milk fat	31.80	Bennewitz *et al.* 2003
19	SW*	Dairy	57.15-59.68	Milk fat	52.26-55.88	Bennewitz *et al.* 2003
				Fat%	54.52	Boichard *et al.* 2003
19	SW*	Beef	4.00-5.40	Backfat	4.45-8.97	Li *et al.* 2004
				Pre-Weaning average daily gain	4.45-8.97	Kneeland *et al.* 2004
				Yield grade	4.45-22.25	Casas *et al.* 2003
				Retail product yield	5.49-8.97	Casas *et al.* 2003
19	SW*	Beef	24.00-26.00	Backfat	23.46-28.56	Li *et al.* 2004
				Ribeye area	24.76-31.11	Taylor *et al.* 1998
19	SW*	Beef	60-61	Backfat	57.80-59.04	Li *et al.* 2004
29	SW*	Dairy	11.77-15.15	Protein yield	13.16	Ashwell *et al.* 2004
				Protein%	13.16	Mosig *et al.* 2001
29	SW*	Dairy	26.42-27.47	PTA type	20.04-32.04	Ashwell *et al.* 2005
				Structural soundness	20.04-32.04	Ashwell *et al.* 2005
				Teat Placement	20.04-32.04	Ashwell *et al.* 2005
				Udder attachment	20.04-32.04	Ashwell *et al.* 2005
29	SW*	Dairy	33.00-34.00	Foot angle	32.04-38.89	Ashwell *et al.* 2005
				Structural soundness	32.04-38.89	Ashwell *et al.* 2005
				Milk yield	13.16-38.89	Viitala *et al.* 2003
				Protein yield	13.16-38.89	Viitala *et al.* 2003
29	SW*	Beef	7.5-8.50	Marbling Score	6.16-18.29	MacNeil and Grosz 2002
			18.75-19.45	Hot carcass weight	6.79-28.77	Kim *et al.* 2003
29	SW*	Beef	27.75-28.68	Marbling Score	28.22-38.89	MacNeil and Grosz 2002
				Meat tenderness	34.22-38.89	Casas *et al.* 2003
				Retail product yield	34.22-38.22	Casas *et al.* 2003
				Hot carcass weight	34.22-38.89	Casas *et al.* 2003
				Body weight at castration	34.22-38.22	Casas *et al.* 2004
19	EHH	Dairy	62.017-62.184	Milk fat	52.26-55.88	Bennewitz *et al.* 2003
19	EHH	Dairy	44.417-44.514	Milk yield	43.12	Shariflou *et al.* 2000
19	EHH	Dairy	61.308-61.355	Milk fat	52.26-55.88	Bennewitz *et al.* 2003
19	EHH	Beef	40.444-40.889	Adjusted fat	40.26-43.19	Kim *et al.* 2003
29	EHH	Dairy	11.655-11.739	Temperament	7.98-13.16	Hiendleder *et al.* 2003
				Milking speed and temperament	1.44-13.16	Hiendleder *et al.* 2003
				Protein	8.43-13.16	Ashwell *et al.* 2004
29	EHH	Dairy	29.840-31.096	Structural soundness	20.04-32.04	Ashwell *et al.* 2005
				Protein	13.16-38.89	Viitala *et al.* 2003
				Milk	13.16-38.89	Viitala *et al.* 2003
29	EHH	Dairy	31.807-32.078	Structural soundness	20.04-32.04	Ashwell *et al.* 2005
				Protein	13.16-38.89	Viitala *et al.* 2003
				Milk	13.16-38.89	Viitala *et al.* 2003
29	EHH	Dairy	33.693-34.136	Protein	13.16-38.89	Viitala *et al.* 2003
				Milk	13.16-38.89	Viitala *et al.* 2003
				Structural soundness	20.04-32.04	Ashwell *et al.* 2005

29	EHH	Beef	7.767-8.006	Marbling Score	6.16-18.29	MacNeil and Grosz 2002
				Hot carcass weight	6.79-28.77	Kim *et al.* 2003

*SW – Sliding Window Approach

Graphical representation of the patterns of LD shows regions of high and low LD across the chromosomes in both breeds. A clear difference in the pattern of LD is observed in Angus and Holstein (Figures 3-13 to 3-16). For instance, on BTA19 from 0-2.1 Mb, Holstein shows higher LD than Angus. On BTA29, we see moderate to high regions of LD in Holstein at regions 0.54-2.93 Mb and at 37.73-40.82 Mb, which are clearly absent in Angus. However, these regions of higher LD do not align with the regions that possess higher allele frequency differences (results not shown). Although these regions may be expected to show some correlation, the disparity may have arisen due to the use of different sets of SNPs. The linkage disequilibrium in these regions could have been generated by complex interactions between biological factors, such as recombination and mutation, and the population's evolutionary history (Mueller 2004). We observed long-range LD with LD dissipating to background levels at a locus separation of about 20 Mb on both chromosomes (Figure 3-17).

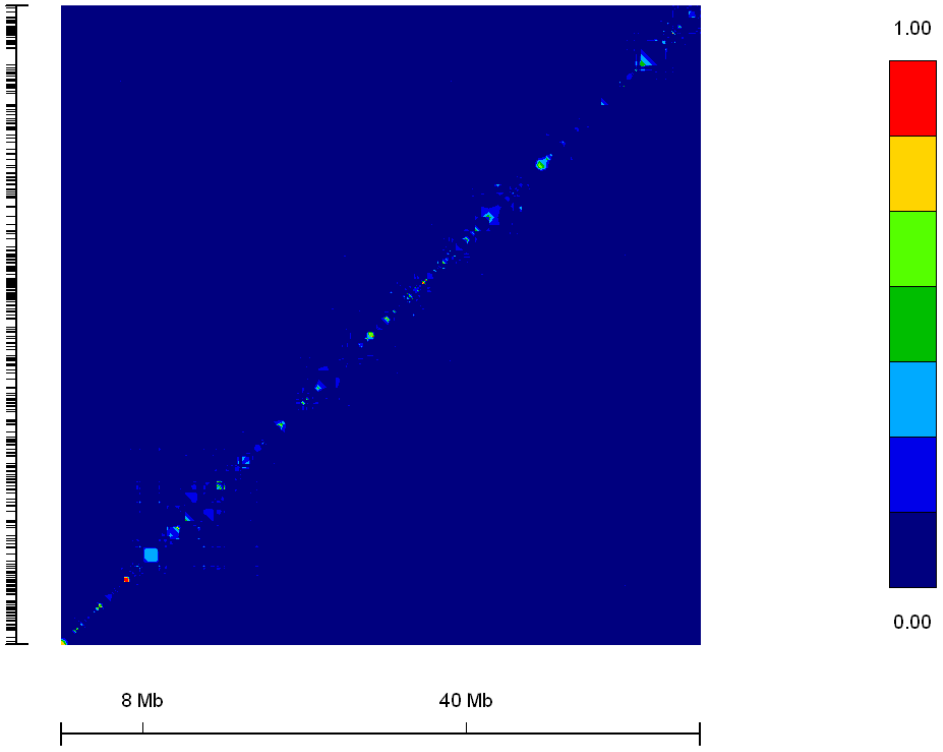

Figure 3-13. Pattern of LD estimated using 370 SNP markers on BTA19 in Angus. The horizontal and vertical axes are scaled according to the physical distance between markers. Red represents complete LD and blue represents zero LD for each marker pair.

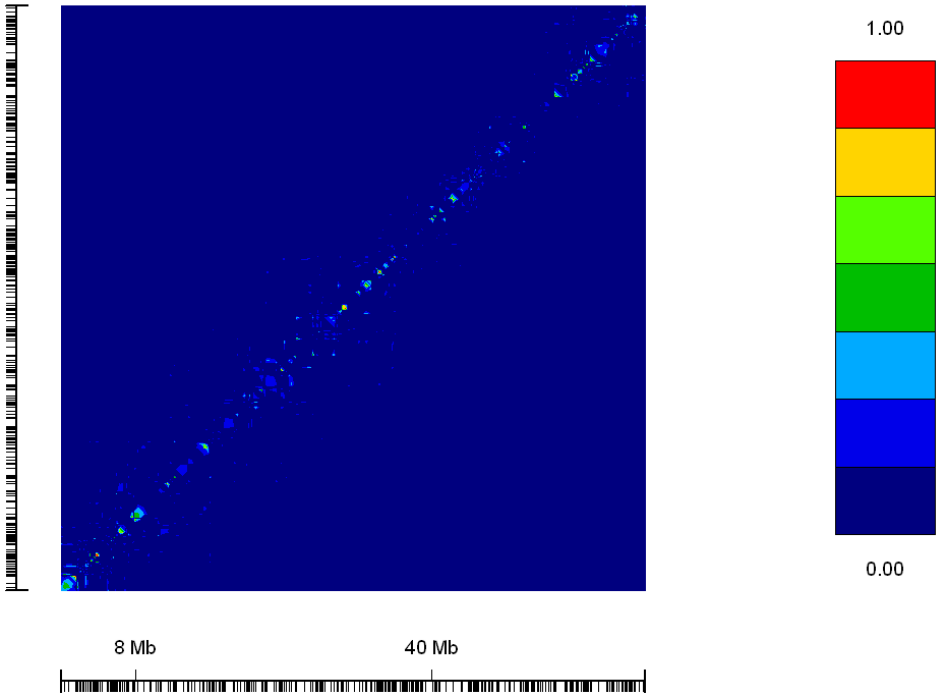

Figure 3-14. Pattern of LD estimated using 367 SNP markers on BTA19 in Holstein. The horizontal and vertical axes are scaled according to the physical distance between markers. Red represents complete LD and blue represents zero LD for each marker pair.

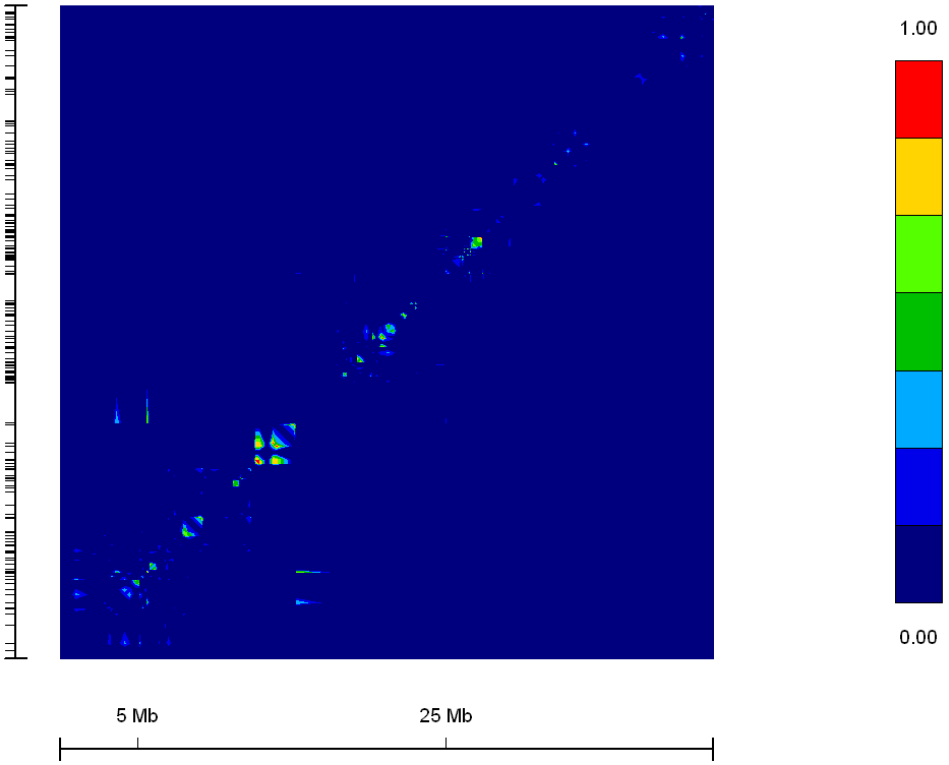

Figure 3-15. Pattern of LD estimated using 187 SNP markers on BTA29 in Angus. The horizontal and vertical axes are scaled according to the physical distance between markers. Red represent complete LD and blue represents zero LD for each marker pair.

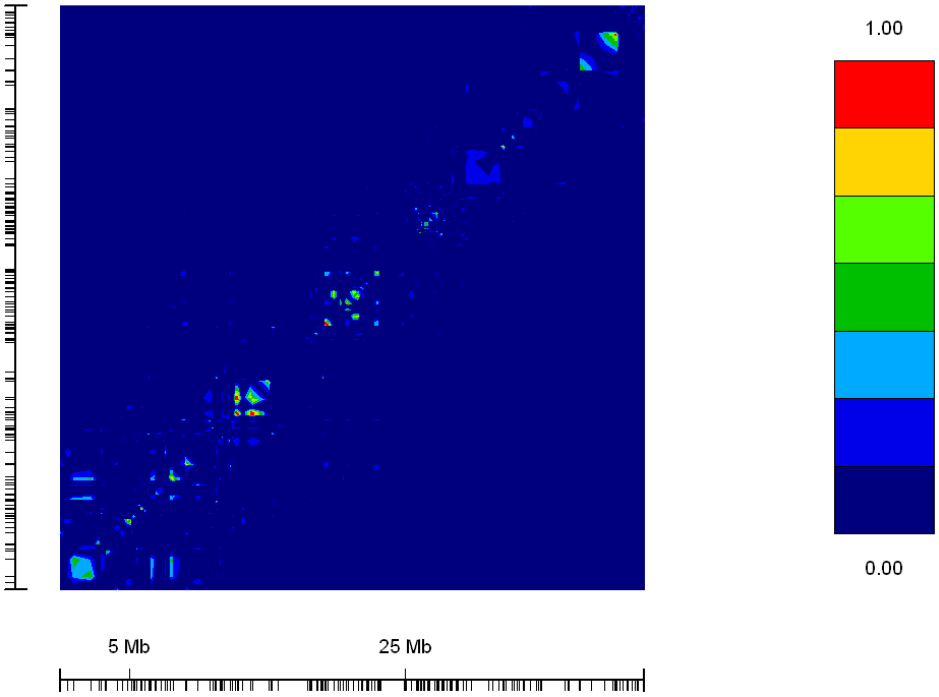

Figure 3-16. Pattern of LD estimated using 179 SNP markers on BTA29 in Holstein. The horizontal and vertical axes are scaled according to the physical distance between markers. Red represent complete LD and blue represents zero LD for each marker pair.

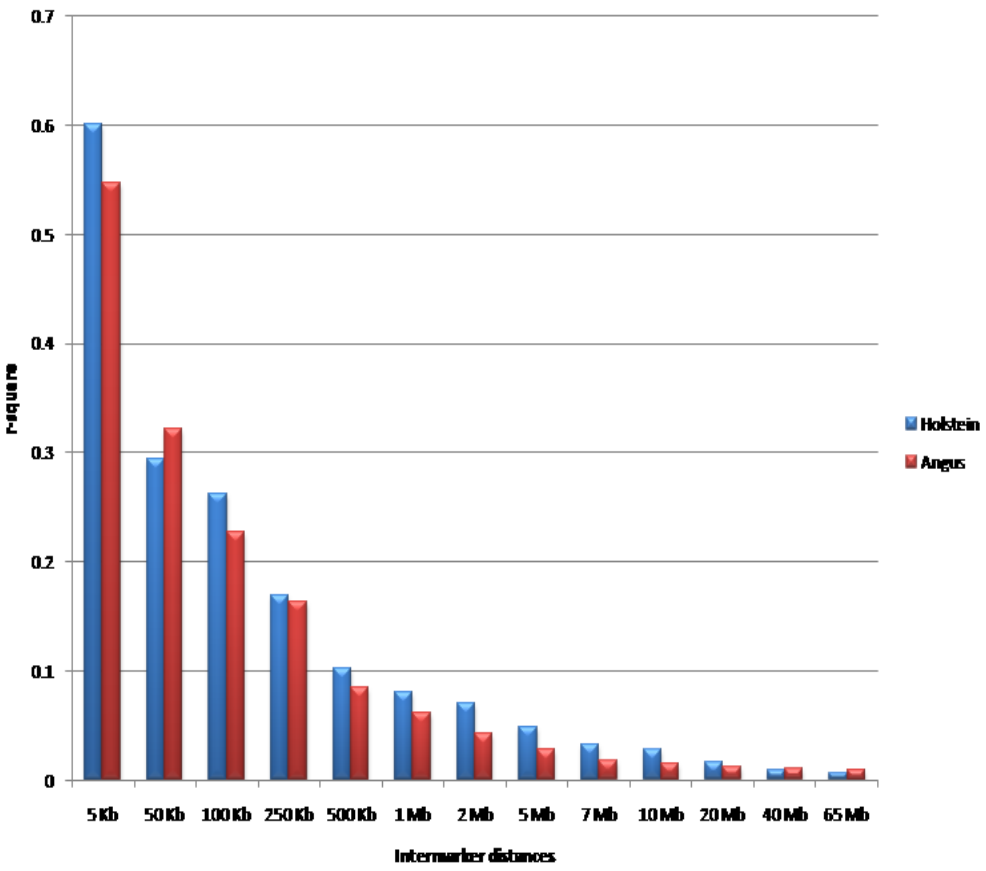

Figure 3-17. Decay of LD shown by the distribution of r^2 as a function of bins of intermarker distances averaged across both chromosomes.

We cannot make direct comparisons between our study and some of the previous LD studies in cattle (Farnir *et al.* 2000, Vallejo *et al.* 2003, Tenesa *et al.* 2003, Odani *et al.* 2006, Khatkar *et al*. 2006a) that used D′ as a measure of LD because we used r^2. We compared our study to that of McKay *et al.* (2007), where LD was also estimated using r^2 and found similar results for Angus and Holstein data. For example, at intermarker distances of 5 kb, 100 kb and 500 kb in Holstein, the r^2 values in our study were 0.6, 0.26 and 0.1, compared to 0.53, 0.23 and 0.1 in McKay *et al.* (2007). It is important to mention here that the SNPs used in our study and in the McKay *et al.* (2007) study were not the same. McKay *et al.* (2007) used approximately 2670 markers genome-wide, with 54 and 55 markers respectively for BTA19 and BTA29. However, the animals used by McKay *et al.* (2007) were included in our study. The average r^2 values for BTA19 in McKay *et al.* (2007) were not shown due to the presence of less than five informative locus pairs. However, with many more markers on these chromosomes and a larger sample size, our study demonstrates that LD persists over long inter-marker distances of up to 20 Mb. It is also important to note that the LD results from our study have come from only two chromosomes, which were not chosen at random, and from only two breeds. Therefore, the results from this study may not be representative of the genome as a whole or of all *Bos taurus* breeds. Our study shows that at a physical distance of 100 kb, the average r^2 value is 0.23-0.26. We can assume that any QTL we seek will be at most in the middle of the interval and therefore no more than 50 kb away from any marker. Hence, the average r^2 between these markers and a QTL located at the mid-

interval is approximately 0.3. This indicates that there should be an informative marker every 100 kb to achieve a moderate LD (r^2 values ≥0.2) for genome-wide association studies. Because the bovine genome is approximately 3 Gb, we would need a minimum of 30 000 evenly spaced and informative markers to perform a whole-genome association study, which agrees with McKay *et al.* (2007) but disagrees with Khatkar *et al.* (2007) and Gautier *et al.* (2007), who have suggested 75 000-100 000 and 300 000 SNPs, respectively capture most of the LD information within the different cattle breeds based on the identification of haplotype blocks and tag SNPs. Considering the fact that many SNPs may have low MAFs in certain breeds and with the goal of achieving an even spacing across the bovine genome, we concur with McKay *et al.* (2007) who suggested that a 50 000 SNP chip should be sufficient for whole genome association studies in *Bos taurus* cattle. The information generated from this study has important implications for the design and application of association studies in cattle populations.

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4. Detection of QTL for Milk Production, Functional and Conformation Traits on BTA19 and 29 in Canadian Holstein Bulls

4.1. Introduction

Marker assisted selection (MAS) is being considered an important advancement for successful breeding programs in the livestock industry. Since the first QTL mapping study in dairy cattle (Georges *et al*. 1995), several studies have been carried out to understand the genetic basis of the economically important traits such as milk production, reproduction, health and conformation (Kolbehdari *et al*. 2008, Smaragdov *et al*. 2006). Most of the previous studies were carried out using microsatellite markers or low density single nucleotide polymorphism (SNP) markers, which resulted in either detection of no QTL or QTL with large confidence intervals. However, with the completion of the bovine genome sequencing project large numbers of SNP markers have become available which have made possible the fine mapping of QTL and the performance of association studies. An association between a genetic variation and a phenotype suggests that either the variation affecting the phenotype is the causative mutation underlying the QTL or the variation is in linkage disequilibrium with the causative mutation. The polymorphisms associated with such traits are important tools for MAS, especially for traits where genetic improvement cannot be achieved using conventional breeding programs. The difficulty may arise for reasons such as low heritability of the traits, difficulty or expense in collecting the phenotypes, or phenotypes collected later in the life or for sex limited traits (Dekkers *et al*. 2004). However before the implementation of markers in MAS, it is essential to validate

the effect of those markers in an independent cattle population or with larger numbers of animals.

The objective of the present study was to perform a QTL scan on bovine chromosomes 19 (BTA19) and 29 (BTA29) for the milk production, functional and conformational traits in Canadian Holstein bulls using two statistical methods of analysis, single locus linkage disequilibrium regression model and Bayesian Monte Carlo Markov Chain. We have chosen BTA19 and 29 as candidate chromosomes for mapping as several QTL of interest have been found on these chromosomes previously (Kolbehdari *et al*. 2008, Boichard *et al*. 2003, Bennewitz *et al*. 2003, Shariflou *et al*. 2000, Schrooten *et al*. 2004). A subset of markers showing significant association with several traits in this study was further validated by increasing the sample size of this dairy population.

4.2. Materials and Methods

4.2.1. QTL Mapping

4.2.1.1. Animal Resource

Straws of semen were received for 322 Canadian Holstein bulls from Semex Alliance (Guelph, Ontario, Canada) and DNA was extracted by standard methods using proteinase K and phenol/chloroform. Briefly, 1 ml of 1XSTE buffer was added to the 250 µl of semen which was vortexed and centrifuged at 8,000 rpm for 2 minutes. The pellet was resuspended in 1 ml of 1XSTE buffer, vortexed and centrifuged again for 2 minutes. The pellet was resuspended in 700 µl of 1XSTE and vortexed well. To this solution, 70 µl of 20% SDS, 5 µl of 20 mg/ml

Proteinase K and 25 µl of 1M DTT were added. The samples were incubated at 56ºC for at least 2 hours of medium rotation. Following Proteinase K digestion, 700 µl of 25:24:1 phenol chloroform isoamyl alcohol (PCI) was added, mixed and centrifuged at 13,000 rpm for 5 minutes. The upper layer was transferred to a new tube and another step of PCI extraction was performed. When the interphase was clean, the upper layer was added to 600 µl of chloroform, mixed and then centrifuged at 13,000 rpm for 5 minutes. The upper layer was transferred to a fresh tube, to which 0.2 volumes of 10 M ammonium acetate and 2.0 volumes of 100% ethanol was added and centrifuged at 13,000 rpm for 5 minutes. The pellet was rinsed in 70% ethanol and dried. The DNA was dissolved in 100 µl of 10 mM Tris, 1mM EDTA and quantified using picogreen assays. The general family structure consisted of a grandparent, parent and three or more progeny. The EBV of the bulls for different traits was obtained from the National Genetic Evaluation Database maintained by the Canadian Dairy Network (Guelph, Ontario, Canada). The different traits analyzed in this study were milk production traits (milk yield, fat yield, fat percentage, protein yield, protein percentage), functional traits (somatic cell scores (SCS), calving ease, maternal calving ease, daughter fertility, herd life, persistency, milking speed, milking temperament) and conformation traits (angularity, bone quality, conformation, dairy strength, feet and leg, foot angle, heel depth, mammary system, rump, stature, median suspensory and udder texture). Functional traits represent those characters of an animal that increase efficiency not by higher productivity but by cutting down costs of input (Groen *et al.* 1997). Conformation traits show a strong linear relationship with longevity or

survival, a trait that influences profitability in dairy farm. Cows with high score for these traits were found to survive longer than cows with low scores. One of such traits is feet and legs, where cows with the intermediate score of 5 is considered to be optimum and cows with extremely straight legs or extremely curved legs are more likely to be culled (Canadian Dairy Network 2008). Another important descriptive type trait is median suspensory. Median suspensory is the most important part of suspensory system in cattle which provides proper attachments of the mammary gland to the body. More detailed information about these traits is provided at the Canadian Dairy Network (http://www.cdn.ca/articles.php). The mean, standard deviation and abbreviations of the traits studied are shown in Table 4-1.

Table 4-1. Summary of the traits analyzed in 322 Canadian Holstein bulls

Trait	Abbreviations	Units	Mean	Standard deviation (SD)
Milk yield	MY	kg	547.60	774.66
Fat yield	FY	kg	14.87	30.23
Protein yield	PY	kg	19.12	22.36
Fat percent	F%	%	-0.04	0.28
Protein percent	P%	%	0.01	0.12
Conformation	CN	score	2.93	5.50
Rump	RP	score	1.25	4.67
Mammary system	MS	score	2.78	5.96
Feet & legs	FL	score	1.51	4.94
Dairy strength	DS	score	1.69	5.14
Udder texture	UT	score	2.23	5.44
Median suspensory	MSU	score	2.08	5.38
Foot angle	FA	score	0.00	5.25
Heel depth	HD	score	0.48	5.18
Bone quality	BQ	score	1.58	5.00
Stature	ST	score	1.59	5.25
Angularity	ANG	score	2.22	5.15
Persistency	PS	score	66.89	2.93
Somatic cell score	SCS	score	3.02	0.29
Calving ease	CE	score	86.67	4.66
Maternal calving ease	MCE	score	86.12	5.18
Herd life	HL	score	3.03	0.21
Milking speed	MSP	score	85.45	4.17
Milking temperament	MT	score	89.52	3.63
Daughter fertility	DF	score	65.59	3.34

4.2.1.2. Marker selection and Genotyping

An oligo pool assay consisting of 1001 and 535 SNPs for BTA19 and BTA29, respectively, was assembled by Illumina Inc. (San Diego, CA) using sequence information obtained from the Baylor College of Medicine database for cattle (ftp://ftp.hgsc.bcm.tmc.edu/pub/data/Btaurus/snp). The Illumina BeadStation 500G system was used to genotype the markers on the panel of Canadian Holstein bulls (Oliphant *et al*. 2002). However, only 505 and 220 SNP markers on BTA19 and 29 respectively, which were mapped on the 12,000 rad radiation hybrid map of the chromosomes (Prasad *et al*. 2007) and considered to be correctly ordered, were used for this study. The sequences and the NCBI IDs of the markers used in this study are provided in Prasad *et al*. (2007). The positions of the markers used are described in Prasad *et al*. (2008). However, it is also important to note that we have used cM and Mbp interchangeably throughout the Chapters 3, 4 and 5 of this thesis. A local database consisting of over 1.8 million bovine SNP and about 30,000 genes from NCBI database (http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen) was developed at the Bovine Genomics Group at the University of Alberta. On querying the SNP markers against this database, we obtained information on each SNP regarding its map location and functional class, whether it is located in a gene or not and if it is located in a gene, then which part of the gene it is located (introns, exons, promoters, UTRs). For SNPs which were not located in any known gene, the nearest gene to the SNP was identified.

4.2.1.3. Statistical Analyses

Two statistical approaches were used in this study to map QTL on the chromosomes: linkage disequilibrium (LD) regression method and Bayesian model using Monte Carlo Markov Chain algorithm. Single locus LD regression model was used to test the association between SNPs and the economically important traits and to estimate the effects of the QTLs. This model is based on the assumption that the markers are in LD with the QTL and has been shown to have acceptable levels of accuracy and power for fine mapping QTL in previous studies (Grapes *et al*. 2004, Zhao *et al*. 2007). The allele substitution effect of each SNP was analyzed using ASREML package (Gilmour *et al.* 2006) with the following model as discussed in Kolbehdari *et al*. (2008):

$$\mathbf{y = Xb + Za + e}$$

where y = vector of trait EBV, b = vector of coefficients of the regression on recoded SNP genotypes, a = vector of additive genetic (polygene) effects treated as random effects, X = design matrix, Z = incidence matrix for animal polygenic effects, and e = vector of residual errors. There have been few approaches used previously to establish significance thresholds in the multiple testing including false discovery rate (FDR) and permutations tests (Benjamini and Hochberg 1995, Churchill and Doerge 1994). The FDR is a conservative approach when large numbers of markers are utilized in a QTL mapping scan. Permutation tests would be a reasonable approach to establish the significance threshold. Since it is very expensive computationally to run permutation tests for all the 725 markers used in the analysis, we ran 100,000 permutations for only 10 randomly

selected SNPs (with high MAFs) to determine an average significance threshold at $P \leq 0.01$ level. We also performed a t-test to determine if the threshold for the 10 markers at $P \leq 0.01$ levels is significantly different from each other.

The second method used in this study was a Bayesian model using a Monte Carlo Markov Chain (MCMC) method, to update the most likelihood position of putative QTL using the multiple marker genotypes, as implemented in LOKI (Heath *et al*. 1997). The quantitative trait is modeled by k diallelic QTLs, where for the ith QTL genotypes A_1A_1, A_1A_2 and A_2A_2 have effects a_i, d_i and $-a_i$, respectively. For the ith QTL, the additive (a_i) and dominance (d_i) genetic effects are represented together in the vector α_i. The following model was utilized for the trait y (n X 1; n animals):

$$y = X\beta + \sum_{i=1}^{k} Qi\,\alpha i + e$$

where, y is the phenotype, β is an (m X 1) vector of fixed effects, α_i is a (2 X 1) vector of effects for the ith QTL, e is an (n X 1) vector of normally distributed residual effects, k is the number of QTLs in the model, and X (n X m) and Q_i (n X 2) are incidence matrices for fixed and QTL effects, respectively. The position of QTL and their respective bayes factors were estimated using 100,000 iterations.

4.2.2. Validation of Markers

4.2.2.1. Animal Resource

Straws of semen were received from Canadian Holstein cattle (n=722) from Semex Alliance (Guelph, Ontario) and DNA was extracted using Proteinase K and

Phenol/Chloroform as mentioned in section 4.2.1.1. The Estimated Breeding Values (EBV) of the bulls was obtained from the Canadian Dairy Network. All the traits as mentioned in section 4.2.1 were analyzed in this study.

4.2.2.2. Selection of SNP markers

Out of 302 SNPs (on both chromosomes) showing association with different economically important traits in dairy cattle (n=322), 19 SNPs were chosen to validate their association in Canadian Holstein cattle (n=722). The SNPs which showed very significant P-values, or were significantly associated with more than one trait, or were associated with trait as well as located in the chromosomal region showing selection signatures (as discussed in Chapter 3), or associated with both dairy as well as beef traits (shown in Chapter 5 of the thesis) were selected for validation. Therefore in total, 21 SNPs were chosen to study their association with the traits in this dairy population. The details of the SNPs are shown in Table 4-2. The genotyping of animals were performed using the MassArray™Iplex Gold platform technology run on the Sequenom MassArray™ (Sequenom Inc., San Diego, California).

Table 4-2. List of SNPs selected for validation

SNP	BTA	Traits	F-test	*Estimate	P-value
BTA-21385	19	Stature	11.55	2.07E+00	0.0004
BTA-45537	19	Angularity	11.12	1.45	0.001
BTA-45537	19	Dairy Strength	11.07	1.46	0.001
BTA-01174	19	Foot Angle/Selection Signature	10.58	1.84	0.001
BTA-45733	19	Stature	17.05	1.61E+00	2.33E-05
BTA-44957	19	Calving Ease	15.65	1.90	4.74E-05
BTA-07830	19	Maternal Calving Ease/Beef Association	16.52	1.98	3.04E-05
BTA-44793	19	Milking Speed/Beef Association	12.20	1.24	0.00028
BTA-45690	19	Mammary System/Beef Association	10.90	1.91	0.001
BTA-45285	19	Somatic Cell Score	13.59	1.08E-01	0.00014
BTA-03377	19	Maternal Calving Ease	17.91	1.88	1.51E-05
BTA-118485	19	Maternal Calving Ease	16.41	1.98	3.22E-05
BTA-46348	19	Selection Signature	-	-	-
BTA-22801	29	Milk Yield	13.89	237.92	0.00012
BTA-65152	29	Protein %/Beef Association	12.76	3.37E-02	0.00021
BTA-65938	29	Milking Temperament	16.56	1.68	2.98E-05
BTA-08572	29	Rump/Selection Signature	10.53	1.38	0.001
BTA-65277	29	Dairy Strength/Selection Signature	10.69	1.44	0.001
BTA-26209	29	Dairy Strength/Beef Association	13.33	1.75	0.00016
BTA-26209	29	Stature/Beef Association	10.66	1.58E+00	0.001
BTA-105947	29	Daughter Fertility	11.53	1.14	0.0001
BTA-58630	29	Rump/Beef Association	11.29	1.38	0.001
BTA-29792	29	Selection Signature	-	-	-

*Estimate - Absolute value of allele substituition effect

4.2.2.3. Statistical Analysis

Single marker linkage disequilibrium regression model was used to test for the association between SNP and trait. The model as described in section 4.2.1.3 was used. Permutation tests on 10 markers were performed to establish a significant threshold at $P<0.05$.

4.3. Results and Discussion

4.3.1. QTL Mapping

We found the average heterozygosity for SNPs on BTA19 was 0.29 and on BTA29 was 0.31, with an average resolution of 1 locus/125 kb and 1 locus/203 kb, respectively. The first method of single marker regression model tested association between a SNP and the QTL for all the 25 traits (Table 4-1). By performing permutation tests we established a significant threshold for detecting false positive associations. We found that any association where the F-statistic is either 7 or in between 6.8-7.2, it is significant at P-value of 0.01. The t-test also explained that the threshold for 10 markers at $P=0.01$ level are not significantly different from each other.

The chromosome-wide scan for milk production (milk yield, fat yield, protein yield, fat %, protein % and somatic cell score), functional (persistency, calving ease, maternal calving ease, daughter fertility, herd life, milking speed and milking temperament), scorecard (overall conformation, mammary system, feet and legs, dairy strength and rump) and descriptive traits (angularity, foot angle, heel depth, stature, bone quality, median suspensory and udder texture) detected 302 SNP markers significantly associated with these traits. The details of the

SNPs including their positions, F-statistics, absolute value of allele substitution effects and P-values are provided in Table 4-3. QTL for all the 25 traits were detected on BTA19, while QTL for 20 traits were detected on BTA29 using this regression model. We found 73 SNP markers which were significantly associated with more than one trait in this dairy cattle population. The details of these markers are provided in Table 4-4. Once confirmed in an independent cattle population, these associations can be utilized in MAS schemes.

Table 4-3. Details of SNPs associated with several economically important traits on chromosomes 19 and 29

Trait	Markers	BTA	cM	F-test	*Estimate	P-value
ANG	BTA-45537	19	43.55	11.12	1.454	0.001
ANG	BTA-45829	19	50.79	7.67	1.604	0.006
ANG	BTA-46322	19	56.51	7.92	1.275	0.006
ANG	BTA-46364	19	59.94	8.13	1.757	0.005
ANG	BTA-65277	29	26.42	10.35	1.410	0.002
ANG	BTA-90745	29	27.74	9.51	1.953	0.002
ANG	BTA-90746	29	27.81	9.58	1.824	0.002
ANG	BTA-66057	29	40.63	12.07	1.582	0.0003
BQ	BZ871466-CGR527T	19	14.17	7.82	1.212	0.006
BQ	BZ924124-C69KG	19	14.18	8.43	1.246	0.004
BQ	CC551636-GGR527C	19	14.22	7.83	1.211	0.006
BQ	BTA-45843	19	50.73	9.51	2.654	0.002
BQ	BTA-03377	19	51.26	7.98	1.117	0.005
BQ	BTA-46126	19	53.69	9.79	2.398	0.002
BQ	BTA-01709	19	53.74	10.52	2.686	0.001
BQ	BTA-46265	19	54.77	7.59	1.340	0.007
BQ	BTA-77448	19	56.21	6.83	1.089	0.01
BQ	BTA-12079	19	61.49	8	1.686	0.005
BQ	BTA-113857	29	9.11	10.58	2.090	0.001
BQ	BTA-16399	29	18.17	11.39	1.416	0.00043
BQ	BTA-65699	29	33.11	7.95	1.197	0.006
CE	BTA-28152	19	3.99	12.84	1.682	0.0002
CE	BTA-45143	19	6.46	8.33	1.308	0.005
CE	BTA-46447	19	12.13	8.3	1.337	0.005
CE	BZ838039-T89K2C	19	16.97	7.59	1.998	0.007
CE	BZ838039-A89K2G	19	17.20	7.48	2.005	0.007
CE	BTA-46571	19	20.41	8.25	1.788	0.005
CE	BTA-07830	19	22.01	9.22	1.318	0.003
CE	BTA-44801	19	23.53	6.99	1.254	0.009
CE	BTA-44976	19	28.06	8.67	1.492	0.004
CE	BTA-56081	19	40.89	7.94	1.137	0.006
CE	BTA-45517	19	41.10	7.18	1.084	0.008
CE	BTA-45737	19	46.90	6.87	1.091	0.01
CE	BTA-45843	19	50.73	12.52	3.085	0.00024
CE	BTA-45954	19	51.32	8.79	2.056	0.004
CE	BTA-109491	19	56.17	8.11	1.421	0.005
CN	BTA-44815	19	5.85	9.56	3.199	0.002
CN	BTA-45631	19	7.35	8.32	1.250	0.005
CN	BTA-45689	19	8.13	12.54	1.875	0.0001
CN	BTA-45733	19	9.31	7.03	1.101	0.009

CN	BTA-46447	19	12.13	7.29	1.401	0.008
CN	BTA-44610	19	19.65	7.3	2.460	0.008
CN	BTA-01174	19	28.64	7.09	1.753	0.009
CN	BTA-05874	19	55.59	7.1	1.601	0.009
CN	BTA-77447	19	55.68	7.34	1.285	0.008
CN	BTA-77448	19	56.21	6.98	1.244	0.009
CN	BTA-21385	19	60.90	7.45	1.738	0.007
CN	BTA-12079	19	61.49	7.83	1.878	0.006
CN	BTA-03053	29	2.19	7.01	1.515	0.009
CN	BTA-66411	29	3.22	10.57	1.421	0.001
CN	BTA-06107	29	7.77	9.36	1.418	0.003
CN	BTA-113862	29	9.11	6.97	1.331	0.009
CN	BTA-65072	29	19.21	7.76	1.572	0.006
CN	BTA-26209	29	20.28	10.18	1.619	0.002
CN	BTA-12811	29	25.00	7.37	1.598	0.008
CN	BTA-65277	29	26.42	6.94	1.231	0.009
CN	BTA-106381	29	27.16	7.64	1.943	0.007
CN	BTA-66106	29	41.88	6.87	1.519	0.01
DF	CC519175-G89BA	19	14.73	7	0.848	0.009
DF	BTA-05874	19	55.59	8.79	1.038	0.004
DF	BTA-66450	29	0.96	9.19	0.951	0.003
DF	BTA-66407	29	3.29	7.72	1.638	0.006
DF	BTA-105947	29	11.24	11.53	1.138	0.0001
DF	BTA-112191	29	11.66	9.71	0.857	0.002
DF	BTA-65291	29	26.34	9.36	1.579	0.003
DF	BTA-90762	29	27.67	7.22	1.352	0.008
DS	CC474822-GGR527C	19	12.68	7.72	1.991	0.006
DS	BTA-11250	19	32.53	8.37	1.372	0.004
DS	BTA-97038	19	32.58	8.37	1.372	0.004
DS	BTA-45537	19	43.55	11.07	1.463	0.001
DS	BTA-45676	19	44.61	7.38	1.088	0.007
DS	BTA-05874	19	55.59	7.21	1.538	0.008
DS	BTA-77447	19	55.68	7.6	1.246	0.007
DS	BTA-21385	19	60.90	10.03	1.913	0.002
DS	BTA-21380	19	60.93	8.56	1.779	0.004
DS	BTA-06107	29	7.77	7.86	1.240	0.006
DS	BTA-26209	29	20.28	13.33	1.748	0.000156
DS	BTA-12811	29	25.00	10.19	1.779	0.002
DS	BTA-65277	29	26.42	10.69	1.442	0.001
DS	BTA-90745	29	27.74	9.2	1.935	0.003
DS	BTA-90746	29	27.81	8.59	1.743	0.004
DS	BTA-65444	29	28.87	8.97	1.178	0.003
F%	BTA-22161	19	2.31	6.92	0.067	0.009

F%	BTA-44665	19	5.33	7.35	0.062	0.008
F%	BZ840034-C72KT	19	11.23	9.01	0.079	0.003
F%	BZ840034-A72KT	19	11.23	7.81	0.074	0.006
F%	BTA-45457	19	38.87	9.14	0.073	0.003
F%	BTA-13047	19	47.66	7.43	0.077	0.007
F%	BTA-13045	19	47.66	7.43	0.077	0.007
F%	BTA-45802	19	48.23	7.82	0.064	0.006
F%	BTA-45795	19	48.27	7.24	0.061	0.008
F%	BTA-45770	19	48.74	10.1	0.066	0.002
FA	BTA-87957	19	2.04	7.53	1.707	0.007
FA	BTA-46575	19	4.55	7.7	1.121	0.006
FA	BTA-44815	19	5.85	13.06	3.233	0.00018
FA	BTA-16709	19	9.88	7.01	1.732	0.009
FA	BTA-01174	19	28.64	10.58	1.842	0.001
FA	BTA-45030	19	29.82	6.8	1.156	0.01
FA	BTA-45315	19	36.34	7.48	1.122	0.007
FA	BTA-45316	19	36.39	7.48	1.122	0.007
FA	BTA-46281	19	55.01	7.95	1.505	0.006
FA	BTA-24970	29	7.42	8.41	1.081	0.004
FA	BTA-65072	29	19.21	8.72	1.442	0.004
FA	BTA-65070	29	19.27	7.61	1.314	0.007
FA	BTA-65073	29	19.44	7.54	1.313	0.007
FA	BTA-65220	29	25.13	11.16	1.336	0.001
FA	BTA-65555	29	32.31	9.64	1.388	0.002
FL	BTA-25119	19	0.36	7.48	1.221	0.007
FL	BTA-109954	19	1.07	12.03	1.349	0.000308
FL	BTA-117829	19	1.86	8.22	0.925	0.005
FL	BTA-117833	19	1.93	8.23	0.972	0.005
FL	BTA-117835	19	1.98	8.23	0.972	0.005
FL	BTA-87957	19	2.04	7.14	1.618	0.008
FL	BTA-87958	19	2.07	7.29	1.018	0.008
FL	BTA-45669	19	7.15	9.17	1.231	0.003
FL	BTA-46126	19	53.69	9.06	2.192	0.003
FL	BTA-01709	19	53.74	10.51	2.531	0.002
FL	BTA-46313	19	56.08	10.24	1.246	0.002
FL	BTA-12079	19	61.49	9.32	1.730	0.003
FL	BTA-105913	19	61.94	10.81	1.444	0.001
FL	BTA-105528	19	62.30	7.05	1.032	0.009
FL	BTA-18356	29	7.59	6.97	1.153	0.009
FL	BTA-113857	29	9.11	12.8	2.231	0.000206
FL	BTA-112193	29	11.74	8.42	1.118	0.004
FL	BTA-65072	29	19.21	7.5	1.302	0.007
FL	BTA-65070	29	19.27	10.2	1.477	0.002

FL	BTA-65073	29	19.44	10.44	1.498	0.002
FL	BTA-17015	29	20.76	7.7	1.217	0.006
FL	BTA-17014	29	20.91	7.56	1.206	0.007
FL	BTA-65087	29	21.23	6.8	1.189	0.01
FL	BTA-65220	29	25.13	10.63	1.269	0.001
FY	BTA-44521	19	16.89	9.89	9.403	0.002
FY	BTA-44563	19	18.07	8.34	7.468	0.005
FY	BTA-44726	19	22.34	7.1	6.319	0.009
FY	BTA-26203	29	20.15	10.68	18.125	0.001
HD	BTA-45689	19	8.13	10.01	1.511	0.002
HD	BTA-16709	19	9.88	11.52	2.317	0.000403
HD	CC500064-A89K2G	19	17.27	8.82	1.347	0.004
HD	BTA-45352	19	37.72	7.24	1.032	0.008
HD	BTA-45661	19	44.32	7.01	1.203	0.009
HD	BTA-93411	19	45.02	10.1	1.301	0.002
HD	BTA-93414	19	45.03	10.1	1.301	0.002
HD	BTA-105913	19	61.94	7.39	1.289	0.007
HD	BTA-03493	29	18.70	8.22	1.347	0.005
HL	BTA-07806	19	17.10	7.68	0.066	0.006
HL	BTA-46576	19	20.39	9.79	0.052	0.002
HL	BTA-04414	19	22.15	8.94	0.057	0.003
HL	BTA-44801	19	23.53	8.11	0.056	0.005
HL	BTA-07396	19	25.01	12.14	0.073	0.000291
HL	BTA-44980	19	28.26	7.19	0.070	0.008
HL	BTA-44981	19	28.28	7.19	0.070	0.008
HL	BTA-45030	19	29.82	7.2	0.051	0.008
HL	BTA-03053	29	2.19	8.64	0.062	0.004
HL	BTA-66446	29	2.69	12.12	0.061	0.000294
HL	BTA-26203	29	20.15	10.43	0.128	0.002
MCE	BTA-46447	19	12.13	7	1.391	0.009
MCE	BTA-07806	19	17.10	10.09	1.989	0.002
MCE	BTA-44563	19	18.07	7.09	1.301	0.009
MCE	BTA-46580	19	20.39	13.77	1.647	0.000124
MCE	BTA-46576	19	20.39	16.49	1.766	3.09E-05
MCE	BTA-07830	19	22.01	16.52	1.977	3.04E-05
MCE	BTA-118485	19	22.03	16.41	1.978	3.22E-05
MCE	BTA-44833	19	23.97	9.25	1.410	0.003
MCE	BTA-45285	19	35.60	8.73	1.699	0.004
MCE	BTA-05671	19	48.87	13.19	2.125	0.000168
MCE	BTA-91568	19	49.28	7.7	1.481	0.006
MCE	BTA-45868	19	49.95	6.97	1.283	0.009
MCE	BTA-45843	19	50.73	12.85	3.476	0.0002
MCE	BTA-03377	19	51.26	17.91	1.880	1.51E-05

MCE	BTA-45954	19	51.32	6.88	2.046	0.01
MCE	BTA-45966	19	51.46	7.55	1.588	0.007
MCE	BTA-46305	19	55.46	6.97	1.510	0.009
MCE	BTA-46306	19	55.96	6.97	1.510	0.009
MS	BTA-45689	19	8.13	10.33	1.856	0.002
MS	BTA-45810	19	10.63	7.21	1.758	0.008
MS	BTA-46447	19	12.13	7.52	1.538	0.007
MS	BTA-46121	19	53.18	8.63	1.391	0.004
MS	BTA-66411	29	3.22	10.07	1.501	0.002
MS	BTA-113862	29	9.11	7.96	1.536	0.005
MS	BTA-65072	29	19.21	8.2	1.752	0.005
MS	BTA-106381	29	27.16	8.66	2.249	0.004
MSP	BTA-28126	19	3.22	7	0.956	0.009
MSP	BTA-28111	19	3.76	11.75	1.242	0.000357
MSP	BTA-28112	19	3.98	11.53	1.235	0.0004
MSP	BTA-28106	19	3.98	10.78	1.201	0.001
MSP	BTA-28107	19	3.98	10.78	1.201	0.001
MSP	BTA-28108	19	3.98	11.29	1.237	0.001
MSP	BTA-28120	19	4.00	10.62	1.193	0.001
MSP	BTA-44793	19	5.80	12.2	1.243	0.000282
MSP	BTA-44893	19	6.14	9.96	1.125	0.002
MSP	BTA-44965	19	6.21	8.53	1.344	0.004
MSP	BTA-05949	19	59.99	10.03	1.205	0.002
MSP	BTA-65275	29	26.79	7.76	1.074	0.006
MSP	BTA-65272	29	26.80	7.76	1.074	0.006
MSP	BTA-65268	29	26.81	7.61	1.065	0.007
MSP	BTA-66492	29	31.36	7.79	1.230	0.006
MSU	BTA-02315	19	3.47	7.11	2.796	0.009
MSU	BTA-44563	19	18.07	7.05	1.247	0.009
MSU	BTA-01174	19	28.64	7.41	1.697	0.007
MSU	BTA-13124	19	30.16	7.23	1.199	0.008
MSU	BTA-46115	19	53.22	7.75	1.483	0.006
MSU	BTA-05874	19	55.59	7.18	1.524	0.008
MSU	BTA-77447	19	55.68	11.04	1.485	0.001
MSU	BTA-77448	19	56.21	10.19	1.416	0.002
MSU	BTA-84894	19	56.94	12.46	1.662	0.000246
MSU	BTA-46348	19	57.30	7.9	1.205	0.006
MSU	BTA-104732	19	58.37	7.23	1.387	0.008
MSU	BTA-12079	19	61.49	9.24	1.926	0.003
MSU	BTA-105913	19	61.94	8.87	1.463	0.003
MSU	BTA-105530	19	62.18	8.2	1.261	0.005
MSU	BTA-06107	29	7.77	6.82	1.150	0.01
MSU	BTA-90746	29	27.81	9.53	1.826	0.002

MSU	BTA-65699	29	33.11	7.15	1.223	0.008
MSU	BTA-66071	29	41.51	7.17	1.145	0.008
MT	BTA-87958	19	2.07	8.46	0.857	0.004
MT	BTA-22140	19	2.95	11.67	0.988	0.000372
MT	BTA-28119	19	3.92	10.3	2.235	0.002
MT	BTA-28104	19	3.98	10.91	2.362	0.001
MT	BTA-28153	19	3.98	10.91	2.362	0.001
MT	BTA-28121	19	4.00	10.91	2.362	0.001
MT	BTA-28151	19	4.04	10.74	2.291	0.001
MT	BTA-04223	19	4.71	8.45	0.937	0.004
MT	BTA-06651	19	5.72	7.63	1.136	0.007
MT	BTA-44787	19	5.72	7.63	1.136	0.007
MT	BTA-44817	19	6.01	13.23	2.720	0.000165
MT	BTA-46514	19	15.30	8.85	0.919	0.003
MT	BTA-46543	19	16.33	7.51	0.824	0.007
MT	BTA-11922	19	17.45	7.37	1.197	0.007
MT	BTA-44555	19	17.68	7.37	1.197	0.007
MT	BTA-46580	19	20.39	7.71	0.826	0.006
MT	BTA-15926	19	20.45	7.46	0.789	0.007
MT	BTA-98517	19	26.58	11.34	1.238	0.000443
MT	BTA-44712	19	27.34	11.49	1.199	0.000409
MT	BTA-106969	19	30.56	8.25	1.105	0.005
MT	BTA-11250	19	32.53	10.81	1.069	0.001
MT	BTA-97038	19	32.58	10.81	1.069	0.001
MT	BTA-45275	19	35.37	10.86	1.025	0.001
MT	BTA-45299	19	35.89	8.54	0.854	0.004
MT	BTA-45304	19	36.06	15.9	1.410	4.17E-05
MT	BTA-45303	19	36.10	15.9	1.410	4.17E-05
MT	BTA-45302	19	36.14	15	1.379	0.000066
MT	BTA-45305	19	36.20	17.87	1.578	1.54E-05
MT	BTA-45352	19	37.72	9.2	0.847	0.003
MT	BTA-56081	19	40.89	10.77	0.960	0.001
MT	BTA-66525	29	5.37	7.34	0.899	0.008
MT	BTA-85871	29	25.97	7.81	0.874	0.006
MT	BTA-65642	29	34.73	7.42	1.025	0.007
MT	BTA-99814	29	34.89	8	0.968	0.005
MT	BTA-65785	29	36.93	8.12	0.972	0.005
MT	BTA-65879	29	37.62	14.79	1.206	7.35E-05
MT	BTA-66030	29	39.48	12.57	1.333	0.000232
MT	BTA-65943	29	40.10	16.6	1.744	2.92E-05
MT	BTA-09465	29	40.23	17.39	1.740	1.96E-05
MT	BTA-09466	29	40.31	17.39	1.740	1.96E-05
MT	BTA-65938	29	40.41	16.56	1.679	2.98E-05

MY	BTA-44726	19	22.34	7.28	166.915	0.008
MY	BTA-44985	19	28.43	7.55	175.755	0.007
MY	BTA-67105	19	29.50	7.26	216.733	0.008
MY	BTA-45082	19	31.34	7.05	278.960	0.009
MY	BTA-13041	19	47.42	7.16	230.230	0.008
MY	BTA-45908	19	47.64	7.16	230.230	0.008
MY	BTA-21385	19	60.90	7.72	239.574	0.006
MY	BTA-38148	29	18.58	10.99	387.625	0.001
MY	BTA-38149	29	18.58	10.35	386.699	0.002
MY	BTA-90745	29	27.74	6.92	239.796	0.009
MY	BTA-90746	29	27.81	9.79	264.358	0.002
MY	BTA-22801	29	28.72	13.89	237.923	0.000117
MY	BTA-65658	29	32.78	7.79	192.454	0.006
MY	BTA-07368	29	34.86	7.9	199.462	0.006
P%	BZ840034-C72KT	19	11.23	7.94	0.030	0.006
P%	BTA-46514	19	15.30	7.79	0.027	0.006
P%	BTA-44964	19	27.98	9.06	0.032	0.003
P%	BTA-45030	19	29.82	7.2	0.028	0.008
P%	BTA-45090	19	32.86	7.45	0.024	0.007
P%	BTA-45106	19	33.82	8.61	0.027	0.004
P%	BTA-57050	19	40.42	7.65	0.027	0.006
P%	BTA-22805	29	28.72	7.01	0.027	0.009
P%	BTA-65427	29	29.26	6.8	0.061	0.01
PS	BTA-45492	19	6.76	8.05	0.608	0.005
PS	BTA-46095	19	52.71	7.73	0.667	0.006
PS	BTA-65056	29	17.93	8.68	1.137	0.004
PS	BTA-07368	29	34.86	7.89	0.749	0.006
PY	BTA-44521	19	16.89	7.75	6.412	0.006
PY	BTA-44563	19	18.07	7.82	5.522	0.006
PY	BTA-44631	19	20.57	7.45	5.441	0.007
PY	BTA-44726	19	22.34	7.16	4.870	0.008
PY	BTA-38148	29	18.58	11.88	11.905	0.0003
PY	BTA-38149	29	18.58	10.8	11.676	0.001
PY	BTA-90746	29	27.81	8.69	7.357	0.004
PY	BTA-22801	29	28.72	8.38	5.487	0.004
PY	BTA-65658	29	32.78	8.76	5.993	0.004
PY	BTA-65662	29	32.81	8.14	5.668	0.005
PY	BTA-07368	29	34.86	10.28	6.683	0.002
PY	BTA-66057	29	40.63	7.03	5.137	0.009
RP	BTA-44731	19	22.45	6.92	1.646	0.009
RP	BTA-44980	19	28.26	8.63	1.824	0.004
RP	BTA-44981	19	28.28	8.63	1.824	0.004
RP	BTA-44990	19	28.57	8.97	1.312	0.003

RP	BTA-01174	19	28.64	7.06	1.544	0.009
RP	BTA-45339	19	37.43	7.53	1.055	0.007
RP	BTA-21385	19	60.90	8.26	1.611	0.005
RP	BTA-66411	29	3.22	10.09	1.225	0.002
RP	BTA-66407	29	3.29	8.75	2.675	0.004
RP	BTA-66400	29	4.88	7.22	1.238	0.008
RP	BTA-66404	29	5.16	7.61	1.259	0.007
RP	BTA-06107	29	7.77	11.33	1.373	0.001
RP	BTA-08572	29	12.69	10.53	1.375	0.001
RP	BTA-08585	29	12.85	8.65	1.235	0.004
RP	BTA-08579	29	12.95	7.8	1.131	0.006
RP	BTA-08584	29	13.99	9.32	1.303	0.003
RP	BTA-26203	29	20.15	6.87	2.491	0.01
SCS	BTA-44638	19	20.70	8.96	0.080	0.003
SCS	BTA-44669	19	21.39	8.84	0.089	0.003
SCS	BTA-44838	19	24.15	6.86	0.058	0.01
SCS	BTA-44845	19	24.22	7.33	0.059	0.008
SCS	BTA-115853	19	24.45	6.87	0.097	0.01
SCS	BTA-98517	19	26.58	6.83	0.074	0.01
SCS	BTA-44990	19	28.57	7.72	0.070	0.006
SCS	BTA-45082	19	31.34	7.53	0.108	0.007
SCS	BTA-45380	19	34.89	7.04	0.079	0.009
SCS	BTA-45352	19	37.72	7.64	0.060	0.007
SCS	BTA-66446	29	2.69	7.11	0.065	0.009
SCS	BTA-66575	29	5.85	6.99	0.058	0.009
SCS	BTA-66576	29	5.89	7.15	0.059	0.008
SCS	BTA-64907	29	13.24	7.11	0.082	0.009
SCS	BTA-17015	29	20.76	7.35	0.072	0.008
SCS	BTA-17014	29	20.91	7.29	0.071	0.008
ST	BTA-25119	19	0.36	7.61	1.403	0.007
ST	BTA-109954	19	1.07	10.98	1.482	0.001
ST	BTA-44716	19	5.40	9.16	1.482	0.003
ST	BTA-45631	19	7.35	8.58	1.211	0.004
ST	BTA-45733	19	9.31	17.05	1.606	2.33E-05
ST	BTA-09214	19	15.64	7.33	1.156	0.008
ST	BTA-44552	19	17.53	6.86	1.435	0.01
ST	BTA-44610	19	19.65	7.3	2.375	0.008
ST	BTA-44980	19	28.26	7.5	1.843	0.007
ST	BTA-44981	19	28.28	7.5	1.843	0.007
ST	BTA-04699	19	38.41	7.24	1.400	0.008
ST	BTA-45676	19	44.61	7.2	1.086	0.008
ST	BTA-05874	19	55.59	7.87	1.617	0.006
ST	BTA-21385	19	60.90	11.55	2.066	0.000396

ST	BTA-26209	29	20.28	10.66	1.579	0.001
ST	BTA-90746	29	27.81	7.7	1.664	0.006
UT	BTA-46432	19	4.19	6.8	2.506	0.01
UT	BTA-46447	19	12.13	7.01	1.381	0.009
UT	BTA-12079	19	61.49	11.54	2.257	0.000398
UT	BTA-105530	19	62.18	7.75	1.282	0.006
UT	BTA-117883	29	6.52	8.07	1.350	0.005
UT	BTA-06107	29	7.77	7.8	1.280	0.006
UT	BTA-38148	29	18.58	7.84	2.387	0.006
UT	BTA-38149	29	18.58	7.41	2.391	0.007
UT	BTA-85843	29	25.93	12.55	1.842	0.000234
UT	BTA-85838	29	26.10	11.62	1.769	0.000382

*Estimate – Absolute value of allele substituition effect

Table 4-4. Details of SNPs associated with more than one trait at P<0.01 using LD regression method

Trait	Markers	BTA	cM	F-test	*Estimate	P-value
Conformation	BTA-01174	19	28.64	7.09	1.75344	0.009
Rump	BTA-01174	19	28.64	7.06	1.54411	0.009
Foot Angle	BTA-01174	19	28.64	10.58	1.84209	0.001
Median Suspensory	BTA-01174	19	28.64	7.41	1.69655	0.007
Feet and Legs	BTA-01709	19	53.74	10.51	2.53143	0.002
Bone Quality	BTA-01709	19	53.74	10.52	2.68588	0.001
Herd Life	BTA-03053	29	2.19	8.64	0.061903	0.004
Conformation	BTA-03053	29	2.19	7.01	1.51502	0.009
Maternal calving Ease	BTA-03377	19	51.26	17.91	1.88005	1.51E-05
Bone Quality	BTA-03377	19	51.26	7.98	1.11705	0.005
Daughter Fertility	BTA-05874	19	55.59	8.79	1.03792	0.004
Conformation	BTA-05874	19	55.59	7.10	1.60092	0.009
Dairy Strength	BTA-05874	19	55.59	7.21	1.53813	0.008
Median Suspensory	BTA-05874	19	55.59	7.18	1.52442	0.008
Stature	BTA-05874	19	55.59	7.87	1.61682	0.006
Conformation	BTA-06107	29	7.77	9.36	1.4175	0.003
Dairy Strength	BTA-06107	29	7.77	7.86	1.24029	0.006
Rump	BTA-06107	29	7.77	11.33	1.37303	0.001
Median Suspensory	BTA-06107	29	7.77	6.82	1.14963	0.01
Udder Texture	BTA-06107	29	7.77	7.80	1.2797	0.006
Milk	BTA-07368	29	34.86	7.90	199.462	0.006
Protein	BTA-07368	29	34.86	10.28	6.68278	0.002
Persistency	BTA-07368	29	34.86	7.89	0.748914	0.006
Herd Life	BTA-07806	19	17.10	7.68	0.066132	0.006
Maternal calving Ease	BTA-07806	19	17.10	10.09	1.98927	0.002
Calving Ease	BTA-07830	19	22.01	9.22	1.31839	0.003
Maternal calving Ease	BTA-07830	19	22.01	16.52	1.97681	3.04E-05
Median Suspensory	BTA-105530	19	62.18	8.20	1.2605	0.005
Udder Texture	BTA-105530	19	62.18	7.75	1.2822	0.006
Feet and Legs	BTA-105913	19	61.94	10.81	1.44429	0.001
Heel Depth	BTA-105913	19	61.94	7.39	1.28871	0.007
Median Suspensory	BTA-105913	19	61.94	8.87	1.46331	0.003
Conformation	BTA-106381	29	27.16	7.64	1.94317	0.007
Mammary System	BTA-106381	29	27.16	8.66	2.24947	0.004
Feet and Legs	BTA-109954	19	1.07	12.03	1.3489	0.00030
Stature	BTA-109954	19	1.07	10.98	1.48238	0.001
Milking Temperament	BTA-11250	19	32.53	10.81	1.06905	0.001
Dairy Strength	BTA-11250	19	32.53	8.37	1.37219	0.004

Feet and Legs	BTA-113857	29	9.11	12.80	2.23078	0.00020
Bone Quality	BTA-113857	29	9.11	10.58	2.08998	0.001
Conformation	BTA-113862	29	9.11	6.97	1.33148	0.009
Mammary System	BTA-113862	29	9.11	7.96	1.53599	0.005
Conformation	BTA-12079	19	61.49	7.83	1.8782	0.006
Feet and Legs	BTA-12079	19	61.49	9.32	1.73008	0.003
Bone Quality	BTA-12079	19	61.49	8.00	1.68584	0.005
Median Suspensory	BTA-12079	19	61.49	9.24	1.92578	0.003
Udder Texture	BTA-12079	19	61.49	11.54	2.25716	0.00039
Conformation	BTA-12811	29	25.00	7.37	1.59775	0.008
Dairy Strength	BTA-12811	29	25.00	10.19	1.77911	0.002
Foot Angle	BTA-16709	19	9.88	7.01	1.73208	0.009
Heel Depth	BTA-16709	19	9.88	11.52	2.31688	0.0004
SCS	BTA-17014	29	20.91	7.29	7.14E-02	0.008
Feet and Legs	BTA-17014	29	20.91	7.56	1.2064	0.007
SCS	BTA-17015	29	20.76	7.35	7.17E-02	0.008
Feet and Legs	BTA-17015	29	20.76	7.70	1.21692	0.006
Milk	BTA-21385	19	60.90	7.72	239.574	0.006
Conformation	BTA-21385	19	60.90	7.45	1.73814	0.007
Dairy Strength	BTA-21385	19	60.90	10.03	1.9128	0.002
Rump	BTA-21385	19	60.90	8.26	1.61143	0.005
Stature	BTA-21385	19	60.90	11.55	2.06618	0.00039
Milk	BTA-22801	29	28.72	13.89	237.923	0.00011
Protein	BTA-22801	29	28.72	8.38	5.4873	0.004
Feet and Legs	BTA-25119	19	0.36	7.48	1.2212	0.007
Stature	BTA-25119	19	0.36	7.61	1.40265	0.007
Fat	BTA-26203	29	20.15	10.68	18.1246	0.001
Herd Life	BTA-26203	29	20.15	10.43	0.127948	0.002
Rump	BTA-26203	29	20.15	6.87	2.49079	0.01
Conformation	BTA-26209	29	20.28	10.18	1.61906	0.002
Dairy Strength	BTA-26209	29	20.28	13.33	1.74814	0.00015
Stature	BTA-26209	29	20.28	10.66	1.57937	0.001
Milk	BTA-38148	29	18.58	10.99	387.625	0.001
Protein	BTA-38148	29	18.58	11.88	11.9053	0.0003
Udder Texture	BTA-38148	29	18.58	7.84	2.38656	0.006
Milk	BTA-38149	29	18.58	10.35	386.699	0.002
Protein	BTA-38149	29	18.58	10.8	11.6755	0.001
Udder Texture	BTA-38149	29	18.58	7.41	2.39098	0.007
Fat	BTA-44521	19	16.89	9.89	9.40297	0.002
Protein	BTA-44521	19	16.89	7.75	6.41249	0.006
Fat	BTA-44563	19	18.07	8.34	7.46815	0.005
Protein	BTA-44563	19	18.07	7.82	5.52236	0.006

Maternal calving Ease	BTA-44563	19	18.07	7.09	1.30127	0.009
Median Suspensory	BTA-44563	19	18.07	7.05	1.24683	0.009
Conformation	BTA-44610	19	19.65	7.30	2.46002	0.008
Stature	BTA-44610	19	19.65	7.30	2.37539	0.008
Fat	BTA-44726	19	22.34	7.10	6.31879	0.009
Milk	BTA-44726	19	22.34	7.28	166.915	0.008
Protein	BTA-44726	19	22.34	7.16	4.86982	0.008
Calving Ease	BTA-44801	19	23.53	6.99	1.25423	0.009
Herd Life	BTA-44801	19	23.53	8.11	0.056108	0.005
Conformation	BTA-44815	19	5.85	9.56	3.19851	0.002
Foot Angle	BTA-44815	19	5.85	13.06	3.2325	0.00018
Herd Life	BTA-44980	19	28.26	7.19	0.069728	0.008
Rump	BTA-44980	19	28.26	8.63	1.8238	0.004
Stature	BTA-44980	19	28.26	7.50	1.84265	0.007
Herd Life	BTA-44981	19	28.28	7.19	0.069728	0.008
Rump	BTA-44981	19	28.28	8.63	1.8238	0.004
Stature	BTA-44981	19	28.28	7.50	1.84265	0.007
SCS	BTA-44990	19	28.57	7.72	7.03E-02	0.006
Rump	BTA-44990	19	28.57	8.97	1.31153	0.003
Protein%	BTA-45030	19	29.82	7.20	2.80E-02	0.008
Herd Life	BTA-45030	19	29.82	7.20	0.050777	0.008
Foot Angle	BTA-45030	19	29.82	6.80	1.15587	0.01
Milk	BTA-45082	19	31.34	7.05	278.96	0.009
SCS	BTA-45082	19	31.34	7.53	1.08E-01	0.007
SCS	BTA-45352	19	37.72	7.64	6.00E-02	0.007
Milking Temperament	BTA-45352	19	37.72	9.20	0.846709	0.003
Heel Depth	BTA-45352	19	37.72	7.24	1.03168	0.008
Dairy Strength	BTA-45537	19	43.55	11.07	1.46318	0.001
Angularity	BTA-45537	19	43.55	11.12	1.45402	0.001
Conformation	BTA-45631	19	7.35	8.32	1.24998	0.005
Stature	BTA-45631	19	7.35	8.58	1.21063	0.004
Dairy Strength	BTA-45676	19	44.61	7.38	1.08828	0.007
Stature	BTA-45676	19	44.61	7.20	1.08597	0.008
Conformation	BTA-45689	19	8.13	12.54	1.8752	0.0001
Mammary System	BTA-45689	19	8.13	10.33	1.85636	0.002
Heel Depth	BTA-45689	19	8.13	10.01	1.51054	0.002
Conformation	BTA-45733	19	9.31	7.03	1.10067	0.009
Stature	BTA-45733	19	9.31	17.05	1.6061	2.33E-05
Calving Ease	BTA-45843	19	50.73	12.52	3.08507	0.00024
Maternal calving Ease	BTA-45843	19	50.73	12.85	3.47577	0.0002
Bone Quality	BTA-45843	19	50.73	9.51	2.65351	0.002
Calving Ease	BTA-45954	19	51.32	8.79	2.05556	0.004

Maternal calving Ease	BTA-45954	19	51.32	6.88	2.04586	0.01
Feet and Legs	BTA-46126	19	53.69	9.06	2.19249	0.003
Bone Quality	BTA-46126	19	53.69	9.79	2.39819	0.002
Calving Ease	BTA-46447	19	12.13	8.3	1.33681	0.005
Maternal calving Ease	BTA-46447	19	12.13	7	1.39094	0.009
Conformation	BTA-46447	19	12.13	7.29	1.40088	0.008
Mammary System	BTA-46447	19	12.13	7.52	1.53817	0.007
Udder Texture	BTA-46447	19	12.13	7.01	1.38133	0.009
Protein%	BTA-46514	19	15.30	7.79	2.69E-02	0.006
Milking Temperament	BTA-46514	19	15.30	8.85	0.918835	0.003
Herd Life	BTA-46576	19	20.39	9.79	0.051555	0.002
Maternal calving Ease	BTA-46576	19	20.39	16.49	1.76633	3.09E-05
Maternal calving Ease	BTA-46580	19	20.39	13.77	1.64683	0.00012
Milking Temperament	BTA-46580	19	20.39	7.71	0.825746	0.006
Calving Ease	BTA-56081	19	40.89	7.94	1.13663	0.006
Milking Temperament	BTA-56081	19	40.89	10.77	0.960415	0.001
Feet and Legs	BTA-65070	29	19.27	10.2	1.47666	0.002
Foot Angle	BTA-65070	29	19.27	7.61	1.31442	0.007
Conformation	BTA-65072	29	19.21	7.76	1.57178	0.006
Feet and Legs	BTA-65072	29	19.21	7.5	1.30179	0.007
Mammary System	BTA-65072	29	19.21	8.2	1.75166	0.005
Foot Angle	BTA-65072	29	19.21	8.72	1.44151	0.004
Feet and Legs	BTA-65073	29	19.44	10.44	1.49809	0.002
Foot Angle	BTA-65073	29	19.44	7.54	1.31289	0.007
Feet and Legs	BTA-65220	29	25.13	10.63	1.26893	0.001
Foot Angle	BTA-65220	29	25.13	11.16	1.33574	0.001
Conformation	BTA-65277	29	26.42	6.94	1.23057	0.009
Dairy Strength	BTA-65277	29	26.42	10.69	1.44244	0.001
Angularity	BTA-65277	29	26.42	10.35	1.40959	0.002
Milk	BTA-65658	29	32.78	7.79	192.454	0.006
Protein	BTA-65658	29	32.78	8.76	5.99338	0.004
Bone Quality	BTA-65699	29	33.11	7.95	1.1967	0.006
Median Suspensory	BTA-65699	29	33.11	7.15	1.22254	0.008
Protein	BTA-66057	29	40.63	7.03	5.13718	0.009
Angularity	BTA-66057	29	40.63	12.07	1.58172	0.0003
Daughter Fertility	BTA-66407	29	3.29	7.72	1.63763	0.006
Rump	BTA-66407	29	3.29	8.75	2.67524	0.004
Conformation	BTA-66411	29	3.22	10.57	1.42123	0.001
Mammary System	BTA-66411	29	3.22	10.07	1.5014	0.002
Rump	BTA-66411	29	3.22	10.09	1.2248	0.002
SCS	BTA-66446	29	2.69	7.11	6.46E-02	0.009
Herd Life	BTA-66446	29	2.69	12.12	0.060726	0.00029

Conformation	BTA-77447	19	55.68	7.34	1.28547	0.008
Dairy Strength	BTA-77447	19	55.68	7.6	1.2459	0.007
Median Suspensory	BTA-77447	19	55.68	11.04	1.4853	0.001
Conformation	BTA-77448	19	56.21	6.98	1.24368	0.009
Bone Quality	BTA-77448	19	56.21	6.83	1.08899	0.01
Median Suspensory	BTA-77448	19	56.21	10.19	1.41635	0.002
Feet and Legs	BTA-87957	19	2.04	7.14	1.61821	0.008
Foot Angle	BTA-87957	19	2.04	7.53	1.70738	0.007
Milking Temperament	BTA-87958	19	2.07	8.46	0.857468	0.004
Feet and Legs	BTA-87958	19	2.07	7.29	1.01817	0.008
Milk	BTA-90745	29	27.74	6.92	239.796	0.009
Dairy Strength	BTA-90745	29	27.74	9.2	1.9353	0.003
Milk	BTA-90746	29	27.81	9.79	264.358	0.002
Protein	BTA-90746	29	27.81	8.69	7.35736	0.004
Dairy Strength	BTA-90746	29	27.81	8.59	1.74314	0.004
Angularity	BTA-90746	29	27.81	9.58	1.82397	0.002
Median Suspensory	BTA-90746	29	27.81	9.53	1.82644	0.002
Stature	BTA-90746	29	27.81	7.7	1.66402	0.006
Milking Temperament	BTA-97038	19	32.58	10.81	1.06905	0.001
Dairy Strength	BTA-97038	19	32.58	8.37	1.37219	0.004
SCS	BTA-98517	19	26.58	6.83	7.38E-02	0.01
Milking Temperament	BTA-98517	19	26.58	11.34	1.23803	0.00044
Fat%	BZ840034-C72KT	19	11.23	9.01	7.86E-02	0.003
Protein%	BZ840034-C72KT	19	11.23	7.94	3.04E-02	0.006

*Estimate – Absolute value of allele substituition effect

The second method of Bayesian MCMC by LOKI (version 2.4.5) performs linkage analysis by using oligogenic quantitative trait locus model. LOKI produced a test statistic called Bayes factor (posterior/prior ratio) which was calculated at every cM along the chromosomes 19 and 29. A Bayes factor of 3 or 2 $\log_e$ (BF) = 2.1 suggests significance (Kass and Raftery 1995) of the presence of a QTL. QTL for angularity, dairy strength, fat yield, fat%, maternal calving ease, milk yield, milking temperament, protein yield, protein %, rump and stature were detected on BTA19, while QTL for angularity, fat yield, mammary system, median suspensory, protein and protein% were detected on BTA29. The details are reported in Table 4-5.

On comparing the results of QTL mapping from two methods, we found QTL for 11 traits (milk yield, protein yield, fat yield, fat%, protein%, maternal calving ease, milking temperament, rump, stature, angularity and dairy strength) on BTA19 and 5 traits (fat yield, protein yield, angularity, mammary system and median suspensory) on BTA29 were in agreement in both analyses. The details of QTL identified from both methods are reported in Table 4-6 and the graphs showing results of traits confirmed using both methods are shown in Figures 4-1,4-2, 4-3, 4-4. The difference in the results obtained from the both methods of QTL mapping could be explained by the fact that single marker LD regression model treats each SNP as a separate regression whereas LOKI considers all the SNPs located on the chromosome simultaneously to calculate IBD at each position.

Table 4-5. Summary of QTL detected using LOKI

BTA	Trait	Confidence Interval (cM)	QTL Peak (cM)	Bayes Factor
19	Angularity	34	34	4.98
19	Angularity	32	32	3.14
19	Dairy strength	60-62	62	7.41
19	Dairy strength	45	45	3.00
19	Dairy strength	58	58	3.04
19	Dairy strength	29-30	29	4.09
19	Dairy strength	32	32	3.01
19	Fat yield	29-33	30	17.62
19	Fat yield	43-45	43	4.89
19	Fat yield	51	51	5.84
19	Fat yield	25-26	26	3.70
19	Fat%	8-13	11	12.78
19	Fat%	6	6	4.20
19	Maternal calving ease	29-34	31	35.93
19	Maternal calving ease	57-59	58	23.03
19	Maternal calving ease	16-18	18	8.26
19	Maternal calving ease	21	21	3.06
19	Maternal calving ease	23-24	23	8.18
19	Milk yield	33-36	36	13.63
19	Milk yield	43-44	43	5.54
19	Milk yield	51	51	8.10
19	Milk yield	27-28	27	6.60
19	Milk yield	11	11	4.30
19	Milk yield	22-23	22	5.28
19	Milking temperament	34	34	6.36
19	Protein yield	19-24	22	13.9
19	Protein yield	29	29	3.34
19	Protein yield	15	15	4.45
19	Protein yield	44-45	45	5.23
19	Protein yield	50	50	3.18
19	Protein%	19	19	3.20
19	Rump	35-36	36	9.15
19	Stature	1	1	3.20
19	Stature	11	11	4.08
29	Angularity	8-9	8	5.75
29	Fat yield	19	19	5.95
29	Mammary system	24-25	24	3.96
29	Median suspensory	33	33	3.77
29	Protein yield	20	20	3.86
29	Protein%	7	7	3.56

Table 4-6. List of the QTLs in agreement with regression and MCMC methods

		LOKI			Regression			
BTA	**Trait**	**QTL Position (cM)**	**Bayes factor**	**SNP**	**QTL Position (cM)**	**F-test**	***Estimate**	**P-value**
19	MY	33-36	13.63	BTA-45082	31.34	7.05	278.96	0.009
19	MY	43-44	5.54	BTA-13041	47.42	7.16	230.23	0.008
19	MY	27-28	6.60	BTA-44985	28.43	7.55	175.76	0.007
19	MY	22-23	5.28	BTA-44726	22.34	7.28	166.92	0.008
19	PY	19-24	13.90	BTA-44726	22.34	7.16	4.87	0.008
19	PY	15	4.45	BTA-44521	16.89	7.75	6.41	0.006
19	P%	19	3.20	BTA-46514	15.30	7.79	2.69E-02	0.006
19	FY	25-26	3.70	BTA-44726	22.34	7.10	6.32	0.009
19	F%	8-13	12.78	BZ840034-C72KT	11.22	9.01	7.86E-02	0.003
				BZ840034-A72KT	11.22	7.81	7.43E-02	0.006
19	F%	6	4.20	BTA-44665	5.33	7.35	6.24E-02	0.008
19	MCE	29-34	35.93	BTA-45285	35.60	8.73	1.70	0.004
19	MCE	57-59	23.03	BTA-46305	55.46	6.97	1.51	0.009
19	MCE	16-18	8.26	BTA-44563	18.07	7.09	1.30	0.009
				BTA-07806	17.10	10.09	1.99	0.002
19	MCE	21	3.06	BTA-07830	22.01	16.52	1.98	3.04E-05
				BTA-118485	22.03	16.41	1.98	3.22E-05
19	MCE	23-24	8.18	BTA-44833	23.97	9.25	1.41	0.003
19	MT	34	6.36	BTA-45275	35.37	10.86	1.02	0.001
				BTA-45299	35.89	8.54	0.85	0.004
19	RP	35-36	9.15	BTA-45339	37.43	7.53	1.05	0.007
19	ST	1	3.20	BTA-25119	0.36	7.61	1.40	0.007
				BTA-109954	1.07	10.98	1.48E+00	0.001
19	ST	11	4.08	BTA-104142	10.32	8.82	5.93	0.004
				BTA-45733	9.31	17.05	1.61E+00	2.33E-05
19	ANG	34	4.98	BTA-45109	33.92	6.76	2.78	0.01
19	DS	60-62	7.41	BTA-46416	60.68	10.17	3.90	0.002
				BTA-21385	60.90	10.03	1.91	0.002
				BTA-21380	60.93	8.56	1.78	0.004
19	DS	58	3.04	BTA-05874	55.59	7.21	1.54	0.008
				BTA-77447	55.68	7.60	1.25	0.007
19	DS	45	3.00	BTA-45676	44.61	7.38	1.09	0.007
19	DS	32	3.01	BTA-11250	32.53	8.37	1.37	0.004
				BTA-97038	32.58	8.37	1.37	0.004
29	ANG	8-9	5.75	BTA-66570	5.65	7.12	4.46	0.009
29	FY	19	5.95	BTA-26203	20.15	10.68	18.12	0.001
29	MS	24-25	3.96	BTA-106381	27.15	8.66	2.25	0.004
29	MSU	33	3.77	BTA-65699	33.11	7.15	1.22	0.008
29	PY	20	3.86	BTA-38148	18.58	11.88	11.91	0.0003
				BTA-38149	18.58	10.80	11.68	0.001

*Estimate – Absolute value of allele substituition effect

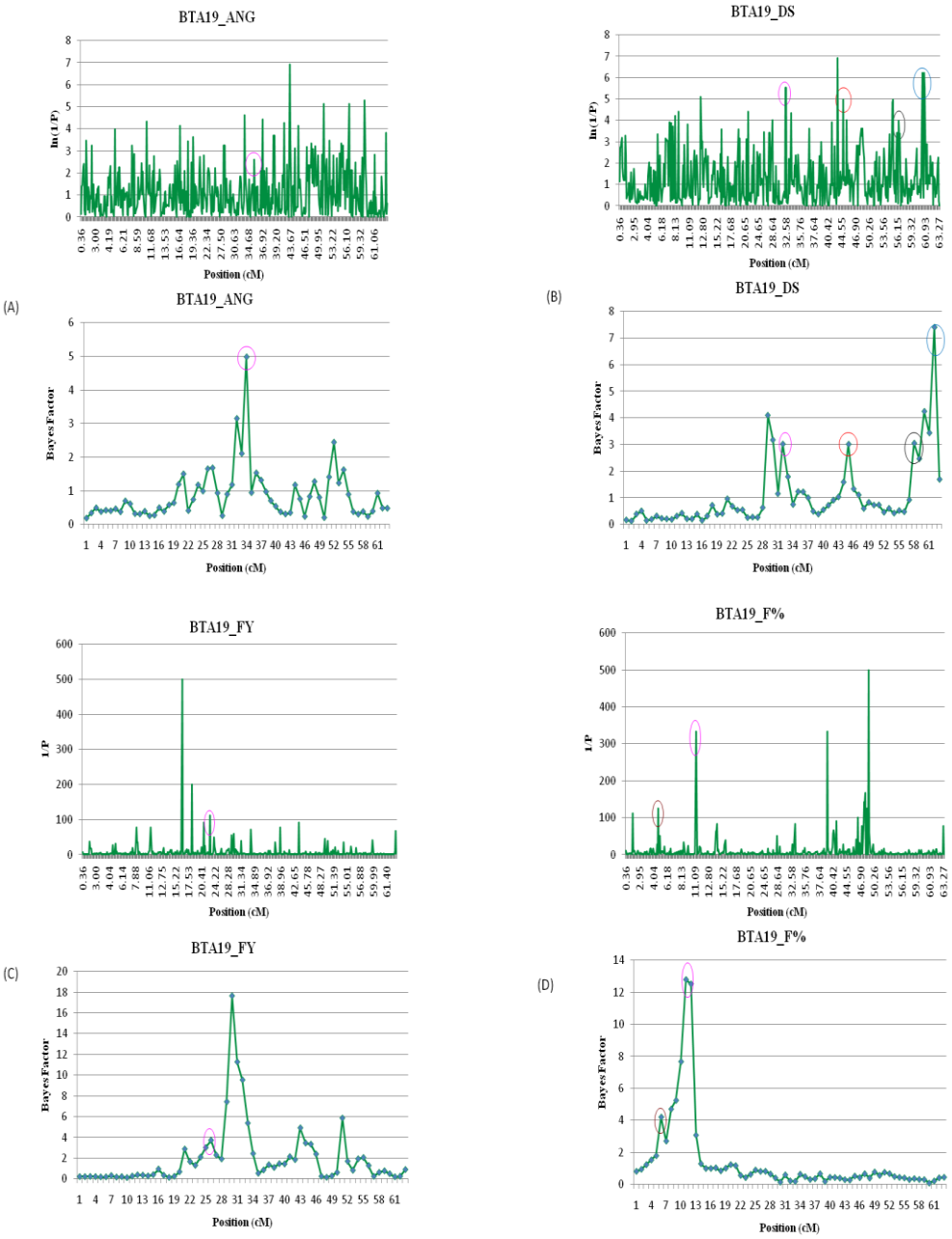

Figure 4-1. Graphs showing results of QTL mapping by LD regression and LOKI for angularity (A), dairy strength (B), fat yield (C) and fat% (D) traits along chromosome 19. Upper panel on each sections of A, B, C and D shows results by LD regression and the lower panel shows results by LOKI.

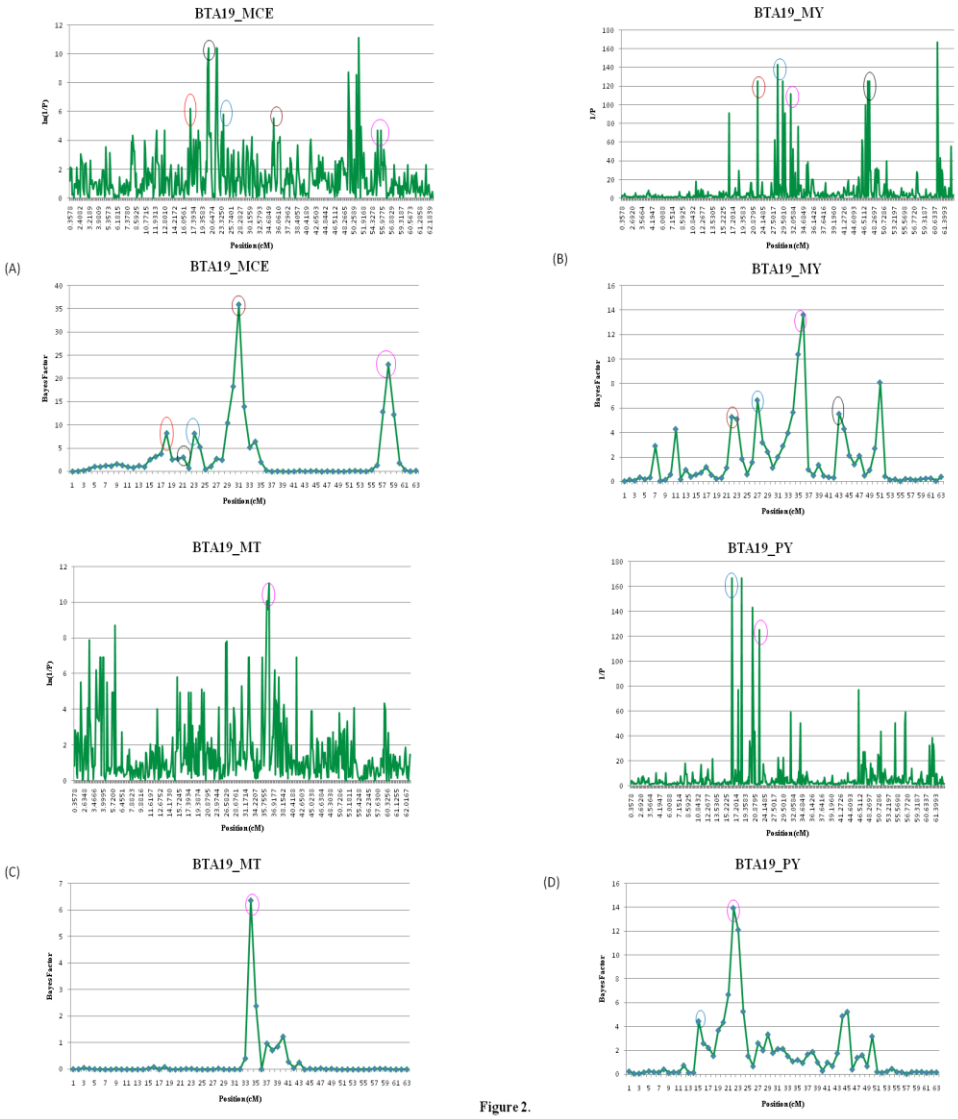

Figure 2.

Figure 4-2. Graphs showing results of QTL mapping by LD regression and LOKI for maternal calving ease (A), milk yield (B), milking temperament (C) and protein yield (D) traits along chromosome 19. Upper panel on each sections of A, B, C and D shows results by LD regression and the lower panel shows results by LOKI.

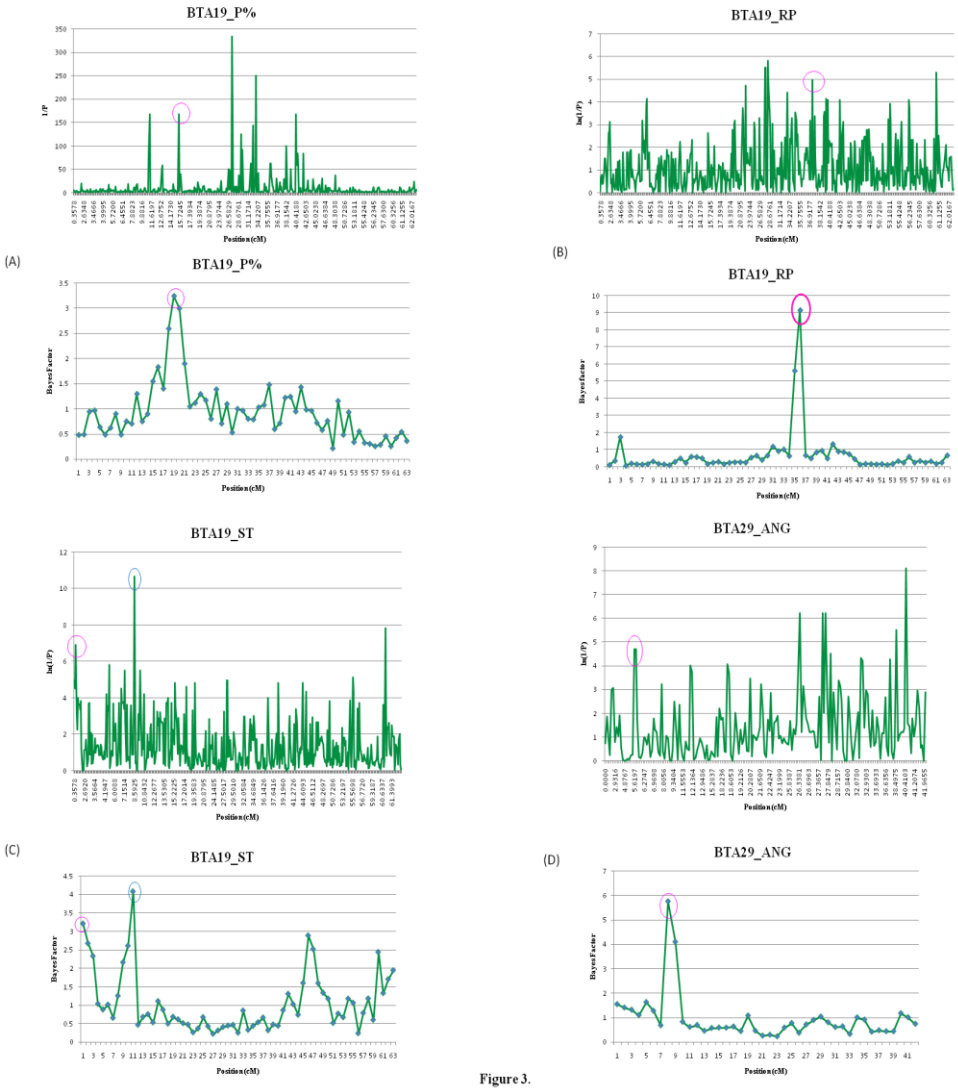

Figure 3.

Figure 4-3. Graphs showing results of QTL mapping by LD regression and LOKI for protein% (A), rump (B), stature (C) traits along chromosome 19 and for angularity (D) along chromosome 29. Upper panel on each sections of A, B, C and D shows results by LD regression and the lower panel shows results by LOKI.

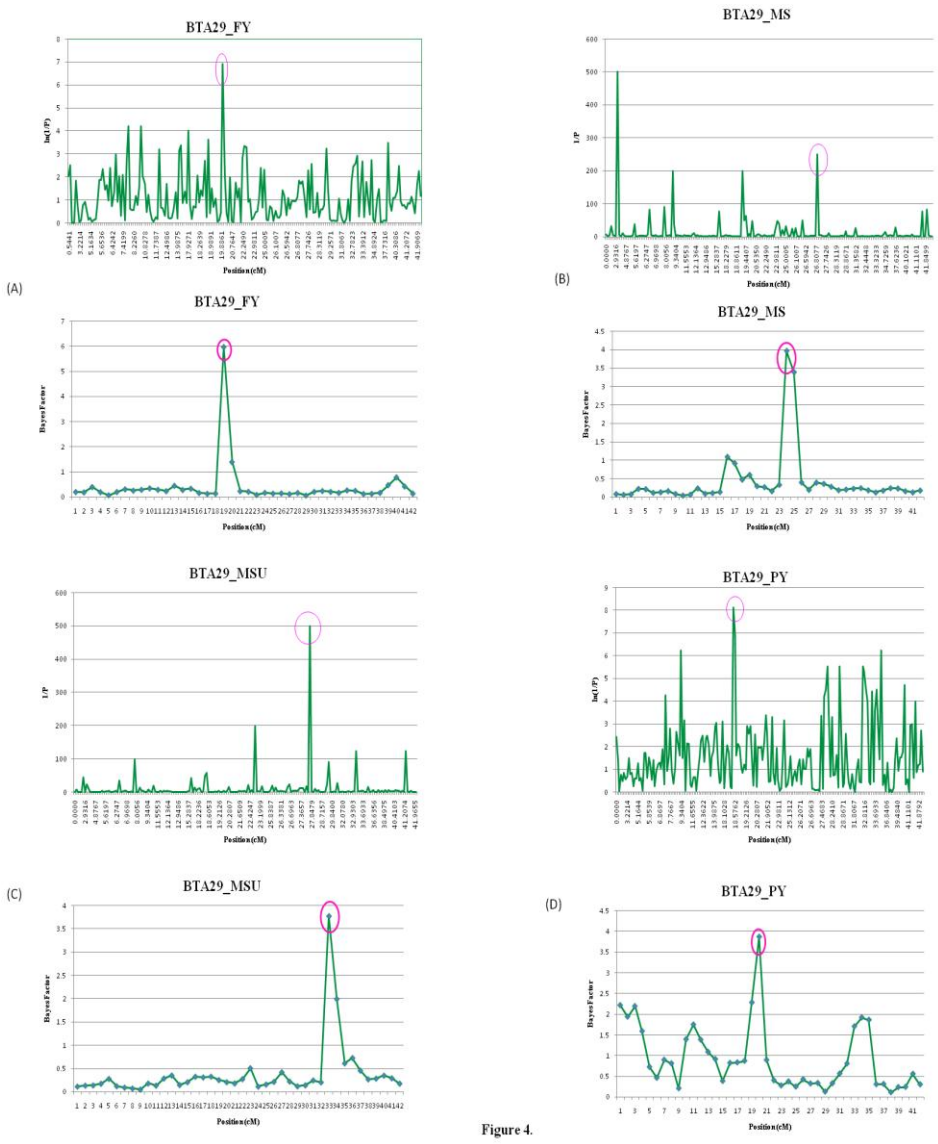

Figure 4.

Figure 4-4. Graphs showing results of QTL mapping by LD regression and LOKI for fat yield (A), mammary system (B), median suspensory (C) and protein yield (D) traits along chromosome 29. Upper panel on each sections of A, B, C and D shows results by LD regression and the lower panel shows results by LOKI.

We also looked to see if these QTL regions are in agreement with the chromosomal regions showing the evidence of signatures of selection in our previous study (Prasad *et al*. 2008). It is important to note that these animals are the ones on which we estimated the extent of linkage disequilibrium (LD) and studied the signatures of selection. Interestingly, almost all the chromosomal regions showing evidence of selection are in good agreement with the identified QTL (Table 4-7). The five regions on chromosome 19 which showed evidence of selection using the sliding window approach were in agreement with QTL for fat%, stature, protein yield, maternal calving ease, milk yield and dairy strength. Two of the chromosomal regions identified using the EHH approach on BTA19 were also in agreement with the QTL for dairy strength and milk yield identified in this dairy population. The three regions on chromosome 29 which showed selection signatures using the sliding window approach were in agreement with the QTL for angularity, mammary system and median suspensory identified in this dairy cattle population. The two chromosomal regions identified using the EHH approach on BTA29 were in agreement with QTL for angularity and median suspensory. It is important to note that these QTL are the ones which were detected using both statistical approaches of QTL mapping.

Table 4-7. Agreement between QTLs (identified using both LD regression and LOKI) and signatures of selection

BTA	Method	Selection signature (Mb)	Trait	QTL Position (cM)	Bayes Factor	Markers	cM	P-value
19	SW*	6.18-7.35	F%	6	4.20	BTA-44665	5.33	0.008
19	SW*	9.88-11.93	F%	8-13	12.70	BZ840034-C72KT	11.22	0.003
			ST	11	4.08	BTA-104142	10.32	0.004
						BTA-45733	9.31	2.33E-05
19	SW*	14.75-17.10	PY	15	4.45	BTA-44521	16.89	0.006
			MCE	16-18	8.26	BTA-07806	17.10	0.002
19	SW*	28.64-30.83	MY	27-28	6.60	BTA-44985	28.43	0.007
			MCE	29-34	35.9	BTA-45285	35.60	0.004
19	SW*	57.15-59.68	MCE	57-59	23.0	BTA-46305	55.46	0.009
			DS	58	3.04	BTA-05874	55.59	0.008
19	EHH	62.02-62.18	DS	60-62	7.41	BTA-21385	60.90	0.002
						BTA-21380	60.93	0.004
19	EHH	44.42-44.51	DS	45	3.00	BTA-45676	44.61	0.007
			MY	43-44	5.54	BTA-13041	47.42	0.008
19	EHH	61.31-61.36	DS	60-62	7.41	BTA-21385	60.90	0.002
						BTA-21380	60.93	0.004
29	SW*	11.77-15.15	ANG	8-9	5.75	BTA-66570	5.65	0.009
29	SW*	26.42-27.47	MS	24-25	3.96	BTA-106381	27.15	0.004
29	SW*	33.00-34.00	MSU	33	3.77	BTA-65699	33.11	0.008
29	EHH	11.65-11.74	ANG	8-9	5.75	BTA-66570	5.65	0.009
29	EHH	33.69-34.14	MSU	33	3.77	BTA-65699	33.11	0.008

SW*- Sliding Window approach

We looked at three QTL databases available online (http://www.animalgenome.org/QTLdb/cattle.html, http://www.vetsci.usyd.edu.au/reprogen/QTL_Map/, http://genomes.sapac.edu.au/bovineqtl/index.html) to find any QTL published previously on BTA19 and 29. The markers reported within the QTL regions using both statistical methods were aligned with the Btau_3.1 to get their approximate positions in Mb. We found that the QTL for milk yield on BTA19 at 43-44 cM reported in our study is in agreement with Shariflou *et al*. (2000), which reported a milk yield QTL at 43.12 cM on BTA19 in Australian Holstein-Friesian cattle. Another QTL for stature on BTA19 at 1 cM is in agreement with Ashwell *et al*. (2005), which reported a QTL for stature at 0-4.45 cM in Holstein-Friesian cattle. On BTA19, Kolbehdari *et al*. (2008) reported a QTL for dairy strength, angularity and milking temperament at 37.84 cM, which is very close to the QTL detected in our study which is 32 cM, 34 cM and 34 cM respectively. The slight difference in the position of QTL may be attributed to the fact that Kolbehdari *et al*. (2008) used the position of markers from the third draft of bovine genome sequence assembly (Btau_3.1) and we used the order of markers from the 12,000 rad RH map of chromosomes 19 and 29. It is possible that the QTL probably are the same in both studies. In total, we found that the QTL for five traits including milk yield, stature, dairy strength, angularity and milking temperament were in agreement with previous studies (Shariflou *et al*. 2000, Ashwell *et al*. 2005 and Kolbehdari *et al*. 2008) while the QTL for the other six traits on BTA19 including protein yield, fat yield, fat%, protein%, maternal calving ease, and rump in our study were

novel. Similarly on BTA29, a QTL for protein yield was reported by Viitala *et al*. (2003) in Finnish Ayrshire dairy cattle at 13.16-38.89 cM, which is in accordance with the protein yield QTL reported in our study at 18-20 cM. Also, the QTL for mammary system reported in our study on BTA29 at 24-27 cM is in accordance with a previous study of Ashwell *et al*. (2005) which reported a QTL for teat placement, udder attachment and udder composite index at 20.04-32.04 cM. Therefore, two QTL for protein yield and mammary system on BTA29 were in agreement with previous studies while three QTL were novel. Most of the QTL identified (using both statistical methods) in our study have been fine mapped to 1-2 cM wide distances and sets an important step for identification of positional candidate genes.

We further looked at some of the QTL detected by both methods in our study to find positional candidate genes based on their potential role in the physiology of the trait for future investigations. We looked at genes around the milk yield QTL found at 43-44 cM on BTA19. We found a thyroid hormone receptor, alpha (THRA) gene located at 41.63 cM as a potential candidate for milk production in cattle. Administration of thyroid hormones is known to increase milk production in dairy cows (Bhattacharjee and Vonderhaar 1984). Adjustments in metabolism of thyroid hormones during the transition from pregnancy to lactation seem to be very important in determining the metabolic priority for lactation (Capuco *et al*. 2008). Studies in rats (Jack *et al*. 1994) and cows (Pezzi *et al*. 2003) have shown that 5'- deiodinase activity, which enhances the biological activity of thyroid hormones, decreased in liver during the transition from

pregnancy to lactation while its activity increased in mammary tissue. The surgical removal of the thyroid gland and hormone replacement in mice showed that thyroid hormones are essential for galactopoietic response to prolactin and somatotropin and these galactopoietic hormones increased 5'-deiodinase activity specifically in mammary gland (Capuco *et al*. 2008, Capuco *et al*. 1999). We also looked for positional candidate genes for milk fat % QTL. This QTL was found to be located at 5-6 cM on BTA19. We suggest a gene called phosphatidylcholine transfer protein (PCTP) located at 5.35 cM as a potential candidate for this QTL. The function of this gene is lipid binding and is involved in the process of lipid transport (Roderick *et al.* 2002). Phosphatidylcholine is the most important phospholipid in milk. A study conducted by Long and Patton (1978) suggested that phosphatidylcholine synthesis regulates development of fat droplets in goat milk.

In addition, we looked for positional candidate genes for milk fat QTL located at 19-20.15 cM on BTA29. We propose a thyroid hormone responsive SPOT 14 (S14) gene located at 18.9 cM on BTA29 as a positional candidate gene for future investigation. S14 is a gene which codes for a nuclear protein closely associated with the regulation of fatty acid synthesis in lipogenic tissues (Cunningham *et al*. 1998). Knock-out of S14 gene in mice has resulted in lowering the level of lipogenesis, specifically in the production of medium chain fatty acids in the lactating mammary gland (LaFave *et al*. 2006). Another study by Harvatine and Bauman (2006) investigated the expression of S14 in the mammary tissue of lactating cows under two situations where milk fat synthesis was

reduced: diet induced milk fat depression and administration of *trans-10, cis-12* conjugated linoleic acid (CLA). The study revealed the role of S14 in the regulation of mammary synthesis of milk fat. We looked for another QTL for mammary system on BTA29 for positional candidate gene research. This QTL was found to be located at 24-25 cM by LOKI and 27.15 cM by LD regression method. We looked at the genes located in these regions and found a tumor susceptibility gene 101 (TSG101) located at 27.46 cM as a possible candidate for mammary system QTL. Wagner *et al*. (2003) reported mice with the conditional deletion of TSG101 and found out that this gene is essential for cell growth, proliferation and cell survival of embryonic and adult tissues. Mammary epithelial cells deficient of TSG101 showed a defect in cell cycle regulation and underwent increased cell death. Li *et al*. (1997) suggested that TSG101 is mutated at high frequency in human breast cancers and that defects in the gene happen during breast cancer tumorigenesis and/or progression. We suggest future investigation of these positional candidate genes for their potential role in the traits of interest.

4.3.2. Validation of Markers

Of the 25 traits analyzed for validation of 21 markers in the dairy population (n=722), we had convergence issues with 15 traits despite the scaling of the phenotype. Therefore, we are only reporting the results for 10 traits for which log likelihood was converged properly. The traits reported for this validation study are milk yield, milk protein, milk fat, stature, dairy strength, angularity, herd life, daughter fertility, milking temperament and calving ability. Of the 21 markers studied for association in this dairy population, 7 were found to be significantly

associated with different traits as shown in Table 4-8. Some of these associations were not detected in our previous study conducted with 322 Canadian Holstein bulls.

Table 4-8. List of SNPs showing association in the larger dairy population

SNP	BTA	Traits	F-test	Estimate	P-value
BTA-21385	19	Angularity	4.72	0.266274	0.031
BTA-21385	19	Milk Fat	3.81	1.37642	0.053 (suggestive)
BTA-45733	19	Milk Yield	6.59	26.4027	0.011
BTA-44793	19	Dairy Strength	4.45	0.238403	0.036
BTA-44793	19	Stature	3.94	0.224448	0.049
BTA-03377	19	Stature	6.23	0.33149	0.014
BTA-118485	19	Dairy Strength	5.45	0.230892	0.021
BTA-105947	29	Daughter Fertility	6.06	0.21862	0.015
BTA-105947	29	Protein	5.6	-0.92929	0.019
BTA-65152	29	Herd Life	4.19	-0.63738	0.042

It is important to note that out of 21 markers selected for validation study, 10 markers in the initial study showed association with traits for which we had convergence issues in this larger dairy population. As a result, we could not validate the effect of those 10 markers in this study. Only 1 out of 21 markers was validated in this dairy population. This marker, BTA-105947, is found to be associated with daughter fertility. This low success rate can be explained by three possible reasons. The first reason is recombination. The value of a marker and the effectiveness of marker assisted selection depend on how far the marker is located from the QTL of interest. If the marker is located very far from the QTL, the probability of inducing a break between them, by a crossover, is very high which can result in changes in linkage relationships. Therefore, for a certain period of time a marker may indicate the presence of one allele. However later on, the same marker will indicate the presence of a different allele. Consequently, a marker may mean one thing for closely related individuals in a population but an entirely different thing for another group (Bourdon 2000).

The second reason may be that there is an epistatic relationship among genes influencing the trait. Certain groups of relatives may share the same allele at one locus, but since they carry different alleles at other loci affecting the gene of interest, it may result in a different degree of expression of the gene or a completely different effect in one family than in another. Therefore, even if a marker is true or reliable in the sense that it always indicates the presence of one allele, it can become unreliable because it indicates a marker allele important for performance in one family but not in another. Consequently, results from one

family cannot be extended to other families even in the same breed of animals unless there is less epistatic effects and very close linkage to rule out recombination events (Bourdon 2000).

The third reason could arise through differences in performance caused by any one gene having too small of an effect to detect. These traits of interest are largely polygenic in nature which is controlled by many genes and no single gene has a predominant effect on the trait. Therefore, performance differences caused by a single gene may be too small and the noise caused by environment effects so large that it becomes difficult to identify the effect of a gene even with a saturated gene map and a large experimental population (Bourdon 2000).

4.4. Conclusion

Using high density SNP markers on bovine chromosomes 19 and 29 with an average resolution of 1 locus/125 kb and 1 locus/203 kb, respectively, we have identified, in total, 302 SNP markers associated with several economically important traits on both chromosomes in dairy cattle. We have detected 73 SNP markers which were significantly associated with more than one trait. A subset of markers (n=21) were selected to validate their effect in a larger dairy cattle population. We could only validate the effect of one marker in this population. More markers should be validated before their implementation in marker assisted selection. In addition, we have identified QTL for 11 and 5 traits on BTA19 and 29 respectively using both LD regression and Monte Carlo Markov Chain methods. QTL for five traits on BTA19 and two traits on BTA29 were in agreement with

previous studies, while rest of the six and three QTL on BTA19 and 29 respectively are novel. The QTL detected in our study is of particular interest to us, as they have been confirmed from both methods of QTL mapping in addition to being in agreement with the regions showing signatures of selection. We have suggested some positional candidate genes which should be investigated further for their potential role in the traits of interest.

4.5. References

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5. Detection of QTL for Traits of Carcass Merit on Bovine Chromosomes 19 and 29 in Beef Cattle

5.1. Introduction

One of the major goals towards better profitability in the beef industry is breeding of animals for optimal fat. Therefore, mapping of Quantitative Trait Loci (QTL) for fat metabolism and carcass merit traits are important positive steps towards achieving this goal. Several studies have been carried out in beef cattle where QTL related to fat traits have been reported (Stone *et al*. 1999, MacNeil and Grosz, 2002, Casas *et al*., 2003, Kim *et al*. 2003, Li *et al*. 2004). All of these studies were carried out using very informative microsatellite markers but at a low marker resolution. This resulted in detection of QTL with wide confidence intervals. However, with the completion of the bovine genome sequencing project (ftp://ftp.hgsc.bcm.tmc.edu/pub/data/Btaurus/snp), a wealth of information has become available. Several single nucleotide polymorphism (SNP) markers, abundant throughout the genome, have now become publicly available and their ease and relatively low cost of genotyping have made these the markers of primary choice for fine mapping QTL and association studies (Hinds *et al*. 2005).

Earlier reports suggested that bovine chromosomes 19 (BTA19) and 29 (BTA29) harbor QTL for several traits (MacNeil and Grosz 2002, Casas *et al*. 2003, Kim *et al*. 2003, Li *et al*. 2004, Ashwell *et al*. 2005, Taylor *et al*. 1998). However, the marker density used for the scans was very low and the QTL had wide confidence intervals. The objective of this study was to fine map QTL for fat metabolism and carcass merit traits on BTA19 and 29 using high density SNP

markers in beef cattle. We used two different statistical methods of analysis, single locus linkage disequilibrium (LD) regression (Grapes *et al.* 2004) and Bayesian Monte Carlo Markov Chain (LOKI) (Heath *et al*. 1997) methods to increase the confidence level of our results. We believe this fine mapping will assist as an important reference for positional candidate gene search. A subset of markers showing association with different traits in this beef cattle population were further validated in another beef cattle population at the University of Guelph, Ontario, Canada.

5.2. Materials and Methods

5.2.1. QTL Mapping

5.2.1.1. Animal Resource

A total of 451 hybrid cattle, of half-sib design, were used in this study. The animals were produced from a cross of Angus, Charolais or University of Alberta hybrid bulls and an experimental hybrid dam line. The dam line was produced from crosses among three composite cattle lines: Beef Synthetic 1 (BS1), composed of approximately 33% Angus and Charolais, 20% Galloway and rest composed of other breeds, Beef Synthetic 2 (BS2), composed of 60% Hereford and 40% other beef breeds and Dairy X Beef Synthetic (DBS), composed of 60% dairybreeds (Holstein, Brown Swiss or Simmental). Animals were tested for growth and feed efficiency at the University of Alberta Kinsella Research Station using GrowSafe automated feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). The details of the test procedures and data collection have been

described previously (Nkrumah *et al*. 2004). Briefly, the phenotype data was collected for about three years (November 2002-June 2005). All the animals used in this project were cared for according to the guidelines of the Canadian Council on Animal Care (CCAC 1993). There were six test groups in total, with two groups per year. When the tests started, the animals were of 252 days of age and weighed 353 kg. In the first year, test diet composed of 80.0% dry-rolled corn, 13.5% alfalfa hay pellet, 5% feedlot supplement (32% crude protein beef mineral supplement containing 440 mg/kg of monensin, trace minerals and vitamins) and 1.5% canola oil. In second and third year, test diet contained 64.5% barley grain, 20% oat grain, 9.0% alfalfa hay pellet, 5.0% beef feedlot supplement and 1.5% canola oil (Nkrumah *et al.* 2004). Different traits analyzed in this study were ultrasound backfat thickness, ultrasound marbling score, ultrasound rib eye area, slaughter weight, carcass weight, carcass average backfat, grade fat, carcass marbling score, carcass rib eye area, lean meat yield, yield grade, quality grade. The mean, standard deviation and abbreviations of the trait studied are shown in Table 5-1.

Table 5-1. Details and abbreviations of the traits analyzed in this study.

Traits	Abbreviations	Mean	Standard deviation
Ultrasound backfat thickness, mm	UBF	9.35	3.54
Ultrasound marbling score	UMAR	5.21	0.79
Ultrasound ribeye area, cm^2	UREA	83.35	10.67
Slaughter weight, kg	SLTWT	535.76	60.51
Carcass weight, kg	CARCWT	312.12	32.04
Carcass average backfat thickness, mm	CABF	12.32	4.30
Gradefat, mm	GRDFAT	10.84	4.34
Carcass marbling score	CMAR	2.51	0.54
Carcass ribeye area, cm^2	CREA	83.89	9.28
Lean meat yield, %	LMY	57.82	3.83
Yield grade	YGRADE	1.73	0.72
Quality grade	QGRADE	2.50	0.66

5.2.1.2. Marker selection and Genotyping

Oligonucleotides respective to the 1001 and 535 SNP markers specific for BTA19 and 29 were designed at the Bovine Genomics Laboratory at the University of Alberta and the oligo pooled assays (OPA) were synthesized and assembled by Illumina Inc. (San Diego, CA). Sequence information for these SNPs were obtained from the second draft (Btau_2.0) of the bovine genome sequence assembly. Out of 1001 SNPs, 68 SNPs were identified from the clones of CHORI-240 library (a bovine BAC library; www.chori.org/bacpac) spanning QTL regions for backfat reported previously (Li *et al*. 2004). The markers were used to genotype the panel of hybrid cattle population using the Illumina BeadStation 500G genotyping system according to the manufacturer's protocol (Olipant *et al*. 2002). However only 475 and 208 SNP markers were used for this study as these were successfully mapped on the 12,000 rad radiation hybrid (RH) maps of BTA19 and 29, respectively, and were considered to be correctly ordered (Prasad *et al.* 2007). The sequence and NCBI IDs of the markers used in this study are provided in Prasad *et al*. (2007). Genomic sequence coordinates for these SNPs were obtained by performing BLAST comparisons between 500 bp SNP flanking sequences and the bovine build 3.1 sequences, using an expectation value threshold of 1e-50. The order of the markers and their corresponding genomic coordinates were corrected if they disagreed with the RH map order of Prasad *et al*. (2007). Markers which could not be separated for their RH positions were ordered according to their order in the bovine genome sequence assembly (Btau_3.1) as RH mapping has difficulty ordering closely related markers, while

the sequence assembly is very informative at a fine scale. The marker positions were used as described in Prasad *et al*. (2008).

5.2.1.3. Statistical Analyses

Two statistical methods were used to map QTL on the chromosomes, linkage disequilibrium (LD) regression (Grapes *et al*. 2004) and Bayesian Monte Carlo Markov Chain (LOKI) (Heath *et al*. 1997) methods. The single locus LD regression model was used to test the association between SNPs and the traits of interest. This model is based on the theory that the markers are in LD with the QTL and has been shown to have an acceptable level of power and accuracy for fine mapping QTL in previous studies (Grapes *et al*. 2004, Zhao *et al*. 2007). The allele substitution effect of each SNP was analyzed with the following model using ASREML (Gilmour *et al*. 2006) package:

$$\mathbf{Y = Xb + Za + e}$$

Where y = vector of trait phenotype, b = vector of fixed effects (breed and batch as fixed effect and age as covariate), a = vector of additive genetic (polygene) effects treated as random effects, Z= incidence matrix for animal effects, X = design matrices, and e = vector of residual errors. There have been few methods reported to establish significance thresholds in multiple testing including false discovery rate (FDR) and permutation tests (Benjamini and Hochberg 1995, Churchill and Doerge 1994). FDR is a conservation approach when large numbers of markers are utilized. A permutation test is a good approach. However, it was very expensive computationally to run permutation tests for all the 683 markers used in the

analysis. Therefore, we selected 10 SNPs with high significance with the traits and high MAFs and ran 100,000 permutations for them to determine an average significant threshold at P=0.01 and P=0.05 levels. We also performed a t-test to determine if the threshold for the 10 markers at P=0.01 and 0.05 levels are significantly different from each other.

The second method used in this study was Bayesian model using Monte Carlo Markov Chain method (Heath *et al*. 1997) as implemented in LOKI. The quantitative trait is modeled by k diallelic QTLs, where for the ith QTL genotypes A1A1, A1A2 and A2A2 have effects ai, di and –ai, respectively. For the ith QTL, the additive (ai) and dominance (di) genetic effects are represented together in the vector αi. Following model was utilized for the trait y (n X 1; n animals):

$$y = X\beta + \sum_{i=1}^{k} \mathrm{Qi}\,\alpha\mathrm{i} + e$$

Where, y is the phenotype, μ is the overall mean, β is an (m X 1) vector of fixed effects and covariates, αi is a (2 X 1) vector of effects for the ith QTL, e is an (n X 1) vector of normally distributed residual effects, k is the number of QTLs in the model, X (n X m) and Qi (n X 2) are incidence matrices for fixed and QTL effects respectively. Breed (Angus, Charolais or Hybrid) and batch (test group nested within year, six levels) were used as fixed effects and age as a covariate. The position of QTL and their respective Bayes Factor was estimated using 50,000 iterations.

5.2.2. Validation of Markers

5.2.2.1. Animal Resource

Animal Resource from University of Guelph was utilized for the validation purpose. Briefly, these cattle came from three sources identified as Commercial, Elora and Rockwood respectively. The animals were crossbred with major contibuting breeds were Angus (AN), Charolais (CH), Limousin (LI) and Simmental (SI). The average composition of these four breeds were 0.46, 0.50, 0.50 and 0.50 for AN, CH, LI and SM, respectively for commercial cattle, 0.24, 0.36, 0.38 and 0.41 for Elora cattle and 0.51, 0.53, 0.59 and 0.41 for Rockwood cattle (Schenkel *et al*. 2005). The experimental procedure was approved by the Univesity of Guelph's Animal Care Committee and all the animals were taken care according to the guidelines of Canadian Council on Animal care (CCAC, 1993). Carcass measurement were available on 567 animals. Different carcass merit traits analysed for the validation are shown in Table 5-2.

Table 5-2. List of traits analyzed for the validation purpose

Trait	Description	Units
BodyFat	Weight of body fat trim (from rib section)	Kg
bodyfatofribwt	Weight of body fat trim as percentage of rib section	%
boneofribwt	Weight of bone as a percentage of whole rib section	%
Fat1	Fat depth (min fat in first quadrant)	mm
Fat2	Fat depth (min fat in second quadrant)	mm
Fat3	Fat depth (min fat in third quadrant)	mm
HCW	Hot carcass Weight	Kg
InterFat	Intermuscular fat trim	Kg
Lean	Lean weight	Kg
leanofribwt	Lean weight as percent of rib section	%
LM7D	Shear force of LD aged 7 days	Kg
Marbling	Marbling score	
REAcm	Rib eye area	CM^2
RibWeightkg	Rib weight	Kg
SubqFat	Subcutaneous fat trim	Kg
totalFatOfRib	Total fat from rib section	%
UGLeanYield	Estimated lean meat yield	%

5.2.2.2. Selection of SNP markers

Twenty four SNPs significantly associated with carcass merit traits in Kinsella beef population and one SNP located in the chromosomal region showing evidence of selection were selected to be validated in Guelph beef population as shown in Table 5-3. Out of twenty four above-mentioned SNPs, eleven of them were associated with more than one trait in the Kinsella population and nine of them were also located in chromosomal regions showing evidence of selection signatures as shown in Chapter 3 of this thesis. The genotyping of animals were performed using the MassArray™Iplex Gold platform technology run on the Sequenom MassArray™ (Sequenom Inc., San Diego, California).

Table 5-3. List of SNPs selected for validation

No.	SNP	BTA	Traits	F-test	Estimate	P-value
1	BTA-46390	19	Grade fat	8.35	0.888	0.004
	BTA-46390	19	Carcass average Backfat	7.71	0.852	0.006
2	BTA-11532	19	Carcass average Backfat	14.94	1.558	<0.001
	BTA-11532	19	Grade fat	10.83	1.331	0.001
	BTA-11532	19	Lean Meat yield	10.86	1.204	0.001
	BTA-11532	19	Signatures of selection	-	-	-
3	BTA-108581	19	Carcass average Backfat	11.11	1.036	0.001
	BTA-108581	19	Grade fat	9.79	0.976	0.002
	BTA-108581	19	Signatures of selection	-	-	-
4	BTA-44665	19	Carcass marbling	7.92	0.106	0.006
	BTA-44665	19	Signatures of selection	-	-	-
5	BTA-44868	19	Grade fat	7.83	0.893	0.006
	BTA-44868	19	Signatures of selection	-	-	-
6	BTA-45680	19	Lean Meat yield	8.93	0.799	0.003
	BTA-45680	19	Carcass weight	8.13	6.583	0.005
	BTA-45680	19	Carcass average Backfat	7.29	0.804	0.008
	BTA-45680	19	Grade fat	7.11	0.795	0.009
7	BTA-44980	19	Quality grade	12.97	0.251	<0.001
8	BTA-07830	19	Ultrasound marbling	8.14	0.144	0.005
9	BTA-44793	19	Carcass weight	8.63	6.963	0.004
10	BTA-45690	19	Ultrasound marbling	8.84	0.211	0.003
11	BTA-46408	19	Signatures of selection	-	-	-
12	BTA-65585	29	Ultrasound Backfat	11.07	0.862	0.001
	BTA-65585	29	Grade fat	10.31	1.259	0.002
	BTA-65585	29	Lean meat yield	9.74	1.101	0.002
	BTA-65585	29	Carcass average Backfat	10.42	1.268	0.002
	BTA-65585	29	Ultrasound marbling	8.70	0.181	0.004
13	BTA-27538	29	Lean Meat yield	9.31	0.799	0.003
	BTA-27538	29	Ultrasound Backfat	8.48	0.563	0.004
	BTA-27538	29	Yield Grade	7.95	0.141	0.006
	BTA-27538	29	Signatures of selection	-	-	-
14	BTA-27534	29	Ultrasound Backfat	7.14	0.521	0.008
	BTA-27534	29	Lean Meat yield	9.13	0.809	0.003
	BTA-27534	29	Signatures of selection	-	-	-
15	BTA-09899	29	Carcass rib eye area	7.72	2.058	0.006
	BTA-09899	29	Signatures of selection	-	-	-
16	BTA-65524	29	Ultrasound marbling	9.12	0.139	0.003
	BTA-65524	29	Signatures of selection	-	-	-
17	BTA-65515	29	Carcass marbling	9.41	0.112	0.003
	BTA-65515	29	Signatures of selection	-	-	-
18	BTA-66408	29	Carcass ribeye area	10.62	2.746	0.001
	BTA-66408	29	Yield grade	9.57	0.218	0.002
19	BTA-66477	29	Yield grade	9.63	0.155	0.002
	BTA-66477	29	Lean Meat yield	8.34	0.760	0.005
20	BTA-26209	29	Carcass average Backfat	9.81	1.031	0.002
	BTA-26209	29	Carcass weight	9.55	7.898	0.002

	BTA-26209	29	Grade fat	8.73	0.976	0.004
	BTA-26209	29	Lean Meat yield	8.67	0.875	0.004
	BTA-26209	29	Ultrasound Backfat	7.77	0.617	0.006
21	BTA-58630	29	Ultrasound Backfat	11.76	0.678	<0.001
22	BTA-65152	29	Ultrasound Backfat	11.27	0.649	0.001
23	BTA-65151	29	Ultrasound Backfat	12.75	0.715	<0.001
	BTA-65151	29	Ultrasound marbling	8.99	0.143	0.003
24	BTA-65153	29	Ultrasound ribeye area	11.72	1.932	<0.001
25	BTA-66122	29	Carcass marbling	19.45	0.209	<0.001

5.2.2.3. Statistical Analysis

Single marker regression was used to test the association between the traits and the SNPs selected for validation purpose. Allele substitution of each SNPs were calculated by fitting the following animal model in ASReml:

$$Y=Xb + Za + e$$

where, the fixed effects included contemporary group, age at end of test, breed and heterosis and the random effects included animals polygenic effects.

5.3. Results and Discussion

5.3.1. QTL Mapping

We found that the average heterozygosity for SNPs on BTA19 was 0.35 and on BTA29 was 0.34, with an average resolution of 1 locus/133 kb and 1 locus/215 kb, respectively. The first method of single marker regression model tested association between a SNP and the QTL for all the fat metabolism and carcass merit traits. By performing permutation tests we established a significance threshold for detecting false positive associations. An F-test value of 6.35-6.85 and 3.79-3.95 was found as a significance threshold at P=0.01 and 0.05 levels, respectively, determined using the permutation tests.

The chromosome-wide scan for all the twelve traits detected 201 and 118 SNP markers on BTA19 and BTA29 respectively, significantly associated ($P<0.05$) with these traits. Out of these markers, 49 and 55 of them on BTA19 and 29 respectively were associated with carcass traits at $P<0.01$. The details of the SNPs ($P<0.01$) including their position, F-statistics, allele substitution effects and P-value are provided in Table 5-4. Thirteen SNPs each on BTA19 and on BTA29 were significantly associated ($P<0.01$) with more than one trait in this beef cattle population. The details of these markers are provided in Table 5-5. Once confirmed in an independent cattle population, these associations can be utilized in marker assisted selection (MAS) schemes.

Table 5-4. Details of SNPs associated with carcass merit traits at P<0.01 level detected using LD regression method

Trait	SNP	BTA	Position (cM)	F-value	*Estimate	P-value
CABF	BTA-93482	19	13.14	6.82	1.00215	0.01
CABF	BTA-11532	19	24.58	14.94	1.55751	6.81E-05
CABF	BTA-108581	19	25.2	11.11	1.03584	0.001
CABF	BTA-45683	19	44.51	7.48	0.810897	0.007
CABF	BTA-45680	19	44.58	7.29	0.80401	0.008
CABF	BTA-46302	19	56.1	6.93	0.78923	0.009
CABF	BTA-26209	29	20.28	9.81	1.03086	0.002
CABF	BTA-17015	29	20.76	8.96	1.01892	0.003
CABF	BTA-17014	29	20.91	9.33	1.04231	0.003
CABF	BTA-65555	29	32.31	7.01	0.78739	0.009
CABF	BTA-01521	29	41.68	8.32	1.54735	0.005
CARCWT	BTA-44793	19	5.8	8.63	6.96281	0.004
CARCWT	BTA-45109	19	33.92	7.33	10.1112	0.008
CARCWT	BTA-24838	19	37.75	7.09	12.4342	0.009
CARCWT	BTA-45683	19	44.51	8.22	6.60852	0.005
CARCWT	BTA-45680	19	44.58	8.13	6.58254	0.005
CARCWT	BTA-109603	29	0.54	7.28	7.49571	0.008
CARCWT	BTA-26209	29	20.28	9.55	7.89753	0.002
CARCWT	BTA-17015	29	20.76	7.89	7.45273	0.006
CARCWT	BTA-17014	29	20.91	8.16	7.5787	0.005
CARCWT	BTA-65151	29	22.78	7.65	6.54528	0.006
CARCWT	BTA-65162	29	23.2	9.34	13.1293	0.003
CARCWT	BTA-65443	29	28.95	14.62	22.0529	8.03E-05
CMAR	BTA-44665	19	5.33	7.92	0.105884	0.006
CMAR	BTA-44669	19	21.39	7.14	0.12225	0.008
CMAR	BTA-45066	19	30.73	6.79	0.245937	0.01
CMAR	BTA-03390	19	41.84	6.36	0.090544	0.013
CMAR	BTA-46262	19	54.84	6.45	0.094782	0.012
CMAR	BTA-66617	29	6.42	6.77	0.111979	0.01
CMAR	BTA-16409	29	18.22	6.79	0.10713	0.01
CMAR	BTA-16410	29	18.22	7.47	0.10919	0.007
CMAR	BTA-65068	29	19.05	8.07	0.239566	0.005
CMAR	BTA-65515	29	28.42	9.41	0.1119	0.003
CMAR	BTA-65938	29	40.41	8.04	0.223807	0.005
CMAR	BTA-66122	29	41.91	19.45	0.20911	6.98E-06
CMAR	BTA-66215	29	42.37	7.8	0.16179	0.006
CREA	BTA-44594	19	19.06	6.97	2.29422	0.009
CREA	BTA-45030	19	29.82	7.09	1.99949	0.009
CREA	BTA-45109	19	33.92	7.26	2.6771	0.008
CREA	BTA-24838	19	37.75	12.13	4.28213	0.000292
CREA	BTA-45494	19	38.15	6.47	3.66324	0.012
CREA	BTA-105530	19	62.18	7.82	2.14949	0.006
CREA	BTA-66438	29	2.83	7.84	1.96596	0.006
CREA	BTA-66407	29	3.29	10.58	2.30865	0.001
CREA	BTA-66134	29	4.15	8.64	1.77042	0.004
CREA	BTA-66525	29	5.37	7.09	1.68886	0.009

CREA	BTA-66587	29	5.82	7.96	1.62453	0.005
CREA	BTA-113865	29	9.17	6.67	1.77652	0.011
CREA	BTA-09899	29	19.18	7.72	2.05774	0.006
CREA	BTA-106381	29	27.16	6.85	2.02363	0.01
CREA	BTA-106382	29	27.3	7.07	2.05798	0.009
CREA	BTA-74283	29	29.46	8.24	1.64889	0.005
FUBF	BTA-45027	19	30.16	6.86	0.734169	0.01
FUBF	BTA-45109	19	33.92	11.44	1.04865	0.00042
FUBF	BTA-45700	19	46.5	13.94	1.55213	0.000114
FUBF	BTA-27538	29	8.01	8.48	0.56278	0.004
FUBF	BTA-27534	29	8.19	7.14	0.52137	0.008
FUBF	BTA-117782	29	11.56	10.63	0.725639	0.001
FUBF	BTA-64904	29	12.32	6.88	1.29946	0.01
FUBF	BTA-26209	29	20.28	7.77	0.61675	0.006
FUBF	BTA-17015	29	20.76	6.88	0.601771	0.01
FUBF	BTA-17014	29	20.91	7.25	0.620311	0.008
FUBF	BTA-65151	29	22.78	12.75	0.71512	0.000211
FUBF	BTA-65166	29	23.28	6.39	1.08023	0.013
FUBF	BTA-85869	29	26.14	7.21	0.8026	0.008
FUBF	BTA-65836	29	38.5	6.55	0.77641	0.011
FUBF	BTA-01521	29	41.68	9.74	1.12472	0.002
FUMAR	BTA-45689	19	8.13	8.54	0.2086	0.004
FUMAR	BTA-07830	19	22.01	8.14	0.14415	0.005
FUMAR	BTA-44751	19	22.55	6.81	0.119316	0.01
FUMAR	BTA-45737	19	46.9	6.45	0.166313	0.012
FUMAR	BTA-46135	19	53.02	8.16	0.134486	0.005
FUMAR	BTA-84894	19	56.94	7.13	0.128029	0.008
FUMAR	BTA-46361	19	59.68	7.95	0.15504	0.006
FUMAR	BTA-105528	19	62.3	7.99	0.13787	0.005
FUMAR	BTA-65151	29	22.78	8.99	0.14308	0.003
FUMAR	BTA-65154	29	22.98	6.78	0.242688	0.01
FUMAR	BTA-65524	29	28.24	9.12	0.13939	0.003
FUMAR	BTA-106996	29	37.71	10.16	0.148961	0.002
FUMAR	BTA-106994	29	37.73	6.98	0.13806	0.009
FUMAR	BTA-66045	29	40.82	6.51	0.12152	0.012
FUREA	BTA-88705	19	37.77	6.71	1.39997	0.011
FUREA	BTA-46361	19	59.68	9.02	2.0063	0.003
FUREA	BTA-22554	29	11.89	7.59	1.61744	0.007
FUREA	BTA-38144	29	18.61	7.71	1.57286	0.006
FUREA	BTA-65153	29	23.03	11.72	1.93235	0.000362
FUREA	BTA-65157	29	23.13	10.97	1.88225	0.001
FUREA	BTA-65443	29	28.95	7.5	4.00861	0.007
GRDFAT	BTA-108967	19	3.57	8.57	0.952766	0.004
GRDFAT	BTA-108969	19	3.57	8.33	0.926906	0.005
GRDFAT	CC507099-TGR527C	19	12.22	9.16	0.84661	0.003
GRDFAT	CC767956-GRM25KC	19	12.75	8.34	1.34624	0.005
GRDFAT	BTA-93463	19	12.78	8.35	1.53007	0.004
GRDFAT	BTA-46509	19	14.04	6.7	1.45081	0.011
GRDFAT	BTA-44618	19	19.29	8.12	1.04079	0.005
GRDFAT	BTA-11532	19	24.58	10.83	1.33123	0.001

GRDFAT	BTA-44868	19	24.65	7.83	0.8932	0.006
GRDFAT	BTA-108581	19	25.2	9.79	0.976185	0.002
GRDFAT	BTA-45683	19	44.51	7.23	0.799786	0.008
GRDFAT	BTA-45680	19	44.58	7.11	0.795372	0.009
GRDFAT	BTA-26209	29	20.28	8.73	0.97637	0.004
GRDFAT	BTA-17015	29	20.76	7.43	0.932323	0.007
GRDFAT	BTA-17014	29	20.91	7.74	0.952719	0.006
GRDFAT	BTA-65166	29	23.28	6.6	1.63481	0.011
GRDFAT	BTA-85869	29	26.14	6.93	1.21436	0.009
GRDFAT	BTA-65555	29	32.31	7.97	0.84324	0.005
GRDFAT	BTA-01521	29	41.68	6.78	1.40325	0.01
LMY	CC767956-GRM25KC	19	12.75	8.92	1.25052	0.003
LMY	BTA-93482	19	13.14	7.85	0.965773	0.006
LMY	BTA-46509	19	14.04	7.33	1.35983	0.008
LMY	BTA-07806	19	17.1	7.14	0.77384	0.008
LMY	BTA-11532	19	24.58	10.86	1.20398	0.001
LMY	BTA-44868	19	24.65	6.65	0.738395	0.011
LMY	BTA-108581	19	25.2	6.83	0.73149	0.01
LMY	BTA-45683	19	44.51	9.06	0.80452	0.003
LMY	BTA-45680	19	44.58	8.93	0.79925	0.003
LMY	BTA-45846	19	50.41	6.82	1.40829	0.01
LMY	BTA-27538	29	8.01	9.31	0.799416	0.003
LMY	BTA-27534	29	8.19	9.13	0.80904	0.003
LMY	BTA-26209	29	20.28	8.67	0.874596	0.004
LMY	BTA-17015	29	20.76	8.04	0.87255	0.005
LMY	BTA-17014	29	20.91	8.28	0.88516	0.005
LMY	BTA-65395	29	30.16	6.44	1.01543	0.012
LMY	BTA-65658	29	32.78	6.51	0.975092	0.012
LMY	BTA-01521	29	41.68	9.33	1.46799	0.003
QGRADE	BTA-44561	19	17.94	6.71	0.12088	0.011
QGRADE	BTA-44980	19	28.26	12.97	0.25088	0.000188
QGRADE	BTA-44981	19	28.28	12.97	0.25044	0.000188
QGRADE	BTA-45288	19	35.76	7.84	0.13261	0.006
QGRADE	BTA-46348	19	57.3	9.97	0.14365	0.002
QGRADE	BTA-21385	19	60.9	6.51	0.23678	0.012
QGRADE	BTA-21384	19	61.13	6.4	0.23547	0.012
QGRADE	BTA-85843	29	25.93	6.81	0.14832	0.01
QGRADE	BTA-65517	29	28.31	6.67	0.135287	0.011
QGRADE	BTA-65555	29	32.31	7.8	0.13232	0.006
SLTWT	BTA-08011	19	2.72	6.97	14.7471	0.009
SLTWT	BTA-65443	29	28.95	7.62	27.16	0.007
YGRADE	CC767956-GRM25KC	19	12.75	14.57	0.301389	8.23E-05
YGRADE	BTA-93463	19	12.78	11.14	0.298712	0.001
YGRADE	BTA-93482	19	13.14	6.5	0.16824	0.012
YGRADE	BTA-46543	19	16.33	6.75	0.149376	0.01
YGRADE	BTA-45382	19	37.97	8.68	0.156507	0.004
YGRADE	BTA-45846	19	50.41	9.33	0.3119	0.003
YGRADE	BTA-66407	29	3.29	7.69	0.16458	0.006
YGRADE	BTA-66550	29	5.51	6.63	0.13065	0.011
YGRADE	BTA-66587	29	5.82	8.2	0.13765	0.005

YGRADE	BTA-66575	29	5.85	7.63	0.15004	0.007
YGRADE	BTA-66576	29	5.89	7.31	0.14747	0.008
YGRADE	BTA-91593	29	6.27	6.48	0.262303	0.012
YGRADE	BTA-27538	29	8.01	7.95	0.14066	0.006
YGRADE	BTA-65166	29	23.28	6.88	0.283952	0.01
YGRADE	BTA-65297	29	26.21	7.5	0.135346	0.007
YGRADE	BTA-65293	29	26.42	6.62	0.127707	0.011
YGRADE	BTA-65296	29	26.63	7.99	0.14236	0.005
YGRADE	BTA-65301	29	26.63	7.64	0.13963	0.007

*Estimate – Absolute value of allele substituition effect

Table 5-5. Details of SNPs associated (P<0.01) with more than one carcass merit traits detected using LD regression method

Trait	SNP	BTA	Position (cM)	F-value	*Estimate	P-value
CABF	BTA-108581	19	25.2	11.11	1.03584	0.001
GRDFAT	BTA-108581	19	25.2	9.79	0.976185	0.002
LMY	BTA-108581	19	25.2	6.83	0.73149	0.01
CABF	BTA-11532	19	24.58	14.94	1.55751	6.81E-05
GRDFAT	BTA-11532	19	24.58	10.83	1.33123	0.001
LMY	BTA-11532	19	24.58	10.86	1.20398	0.001
CARCWT	BTA-24838	19	37.75	7.09	12.4342	0.009
CREA	BTA-24838	19	37.75	12.13	4.28213	0.000292
GRDFAT	BTA-44868	19	24.65	7.83	0.8932	0.006
LMY	BTA-44868	19	24.65	6.65	0.738395	0.011
CARCWT	BTA-45109	19	33.92	7.33	10.1112	0.008
CREA	BTA-45109	19	33.92	7.26	2.6771	0.008
FUBF	BTA-45109	19	33.92	11.44	1.04865	0.00042
CABF	BTA-45680	19	44.58	7.29	0.80401	0.008
CARCWT	BTA-45680	19	44.58	8.13	6.58254	0.005
GRDFAT	BTA-45680	19	44.58	7.11	0.795372	0.009
LMY	BTA-45680	19	44.58	8.93	0.79925	0.003
CABF	BTA-45683	19	44.51	7.48	0.810897	0.007
CARCWT	BTA-45683	19	44.51	8.22	6.60852	0.005
GRDFAT	BTA-45683	19	44.51	7.23	0.799786	0.008
LMY	BTA-45683	19	44.51	9.06	-0.80452	0.003
LMY	BTA-45846	19	50.41	6.82	1.40829	0.01
YGRADE	BTA-45846	19	50.41	9.33	0.3119	0.003
FUMAR	BTA-46361	19	59.68	7.95	0.15504	0.006
FUREA	BTA-46361	19	59.68	9.02	2.0063	0.003
GRDFAT	BTA-46509	19	14.04	6.7	1.45081	0.011
LMY	BTA-46509	19	14.04	7.33	1.35983	0.008
GRDFAT	BTA-93463	19	12.78	8.35	1.53007	0.004
YGRADE	BTA-93463	19	12.78	11.14	0.298712	0.001
CABF	BTA-93482	19	13.14	6.82	1.00215	0.01
LMY	BTA-93482	19	13.14	7.85	0.965773	0.006
YGRADE	BTA-93482	19	13.14	6.5	0.16824	0.012
GRDFAT	CC767956-GRM25KC	19	12.75	8.34	1.34624	0.005
LMY	CC767956-GRM25KC	19	12.75	8.92	1.25052	0.003
YGRADE	CC767956-GRM25KC	19	12.75	14.57	0.301389	8.23E-05
CABF	BTA-01521	29	41.68	8.32	1.54735	0.005
FUBF	BTA-01521	29	41.68	9.74	1.12472	0.002
GRDFAT	BTA-01521	29	41.68	6.78	1.40325	0.01
LMY	BTA-01521	29	41.68	9.33	1.46799	0.003
CABF	BTA-17014	29	20.91	9.33	1.04231	0.003
CARCWT	BTA-17014	29	20.91	8.16	7.5787	0.005
FUBF	BTA-17014	29	20.91	7.25	0.620311	0.008
GRDFAT	BTA-17014	29	20.91	7.74	0.952719	0.006
LMY	BTA-17014	29	20.91	8.28	0.88516	0.005
CABF	BTA-17015	29	20.76	8.96	1.01892	0.003

CARCWT	BTA-17015	29	20.76	7.89	7.45273	0.006
FUBF	BTA-17015	29	20.76	6.88	0.601771	0.01
GRDFAT	BTA-17015	29	20.76	7.43	0.932323	0.007
LMY	BTA-17015	29	20.76	8.04	0.87255	0.005
CABF	BTA-26209	29	20.28	9.81	1.03086	0.002
CARCWT	BTA-26209	29	20.28	9.55	7.89753	0.002
FUBF	BTA-26209	29	20.28	7.77	0.61675	0.006
GRDFAT	BTA-26209	29	20.28	8.73	0.97637	0.004
LMY	BTA-26209	29	20.28	8.67	0.874596	0.004
FUBF	BTA-27534	29	8.19	7.14	0.52137	0.008
LMY	BTA-27534	29	8.19	9.13	0.80904	0.003
FUBF	BTA-27538	29	8.01	8.48	0.56278	0.004
LMY	BTA-27538	29	8.01	9.31	0.799416	0.003
YGRADE	BTA-27538	29	8.01	7.95	0.14066	0.006
CARCWT	BTA-65151	29	22.78	7.65	6.54528	0.006
FUBF	BTA-65151	29	22.78	12.75	0.71512	0.000211
FUMAR	BTA-65151	29	22.78	8.99	0.14308	0.003
FUBF	BTA-65166	29	23.28	6.39	1.08023	0.013
GRDFAT	BTA-65166	29	23.28	6.6	1.63481	0.011
YGRADE	BTA-65166	29	23.28	6.88	0.283952	0.01
CARCWT	BTA-65443	29	28.95	14.62	22.0529	8.03E-05
FUREA	BTA-65443	29	28.95	7.5	4.00861	0.007
SLTWT	BTA-65443	29	28.95	7.62	27.16	0.007
CABF	BTA-65555	29	32.31	7.01	0.78739	0.009
GRDFAT	BTA-65555	29	32.31	7.97	0.84324	0.005
QGRADE	BTA-65555	29	32.31	7.8	0.13232	0.006
CREA	BTA-66407	29	3.29	10.58	2.30865	0.001
YGRADE	BTA-66407	29	3.29	7.69	0.16458	0.006
CREA	BTA-66587	29	5.82	7.96	1.62453	0.005
YGRADE	BTA-66587	29	5.82	8.2	0.13765	0.005
FUBF	BTA-85869	29	26.14	7.21	0.8026	0.008
GRDFAT	BTA-85869	29	26.14	6.93	1.21436	0.009

*Estimate – Absolute value of allele substituition effect

The second method of MCMC analysis produced a test statistic called Bayes factor (posterior/prior ratio) at every cM along the chromosomes. A Bayes factor of 3 or 2 $\log_e(BF)=2.1$ suggests the significance of the presence of a QTL (Kass and Raftery 1995). We detected QTL for two traits on BTA19 and for one trait on BTA29 using this method as shown in Table 5-6.

Table 5-6. List of QTLs detected using LOKI

BTA	Trait	Location (cM)	Bayes factor
19	CREA	50	3.117527
19	CREA	55	3.207451
19	LMY	11	3.062982
19	LMY	18	3.168995
19	LMY	20-21	3.715808
19	LMY	25	3.125301
19	LMY	30	3.004228
19	LMY	46	3.194498
19	LMY	53	3.419924
19	LMY	61	3.03654
19	LMY	63	3.198494
29	GRDFAT	2-5	4.195691
29	GRDFAT	7	3.282101

On BTA19, 3 markers located between 56.34-57.57 cM showed association with CREA ($P<0.05$) using the regression model, thus confirming the QTL for CREA detected by LOKI at 55 cM. We also found the other QTL for CREA located at 50 cM detected by LOKI in agreement with regression method results as the marker, BTA-45979, located at 51.77 cM was associated with CREA ($P<0.05$).

All the QTLs for LMY, except the ones located at 61 and 63 cM, on BTA19 and GRDFAT on BTA29 detected by LOKI are also in agreement in LD regression analysis. The details of the QTL in agreement are shown in Table 5-7.

Table 5-7. List of QTLs in agreement with LD regression and MCMC methods

		LOKI		LD regression method			
BTA	**Trait**	**QTL position (cM)**	**Bayes Factor**	**SNP**	**QTL Position (cM)**	***Estimate**	**P-value**
19	CREA	50	3.117527	BTA-45979	51.77	1.70024	0.039
19	CREA	55	3.207451	BTA-46319	56.34	1.59087	0.04
				BTA-104739	57.63	1.66828	0.042
				BTA-104738	57.57	1.68978	0.039
19	LMY	11	3.062982	BTA-13223	11.09	0.56751	0.025
				BZ840034-A167FC	11.31	0.86896	0.026
19	LMY	18	3.168995	BTA-44603	18.91	0.77074	0.052
19	LMY	20-21	3.715808	BTA-44609	19.60	4.20315	0.001
				BTA-44954	19.06	0.78203	0.043
19	LMY	25	3.125301	BTA-108581	25.2	0.73149	0.01
				BTA-11532	24.58	1.20398	0.001
				BTA-44868	24.65	0.738395	0.011
19	LMY	30	3.004228	BTA-45030	29.82	0.738459	0.028
19	LMY	46	3.194498	BTA-45700	46.5	1.23843	0.026
				BTA-45701	46.51	0.62971	0.034
19	LMY	53	3.419924	BTA-45979	51.77	0.76267	0.034
				BTA-109506	55.57	0.692082	0.027
				BTA-05874	55.59	0.543944	0.051
29	GRDFAT	2-5	4.195691	BTA-66587	5.82	0.55137	0.053
29	GRDFAT	7	3.282101	BTA-27534	8.19	0.60387	0.047
				BTA-91593	6.27	1.27176	0.038
				BTA-27538	8.01	0.71737	0.016

*Estimate – Absolute value of allele substituition effect

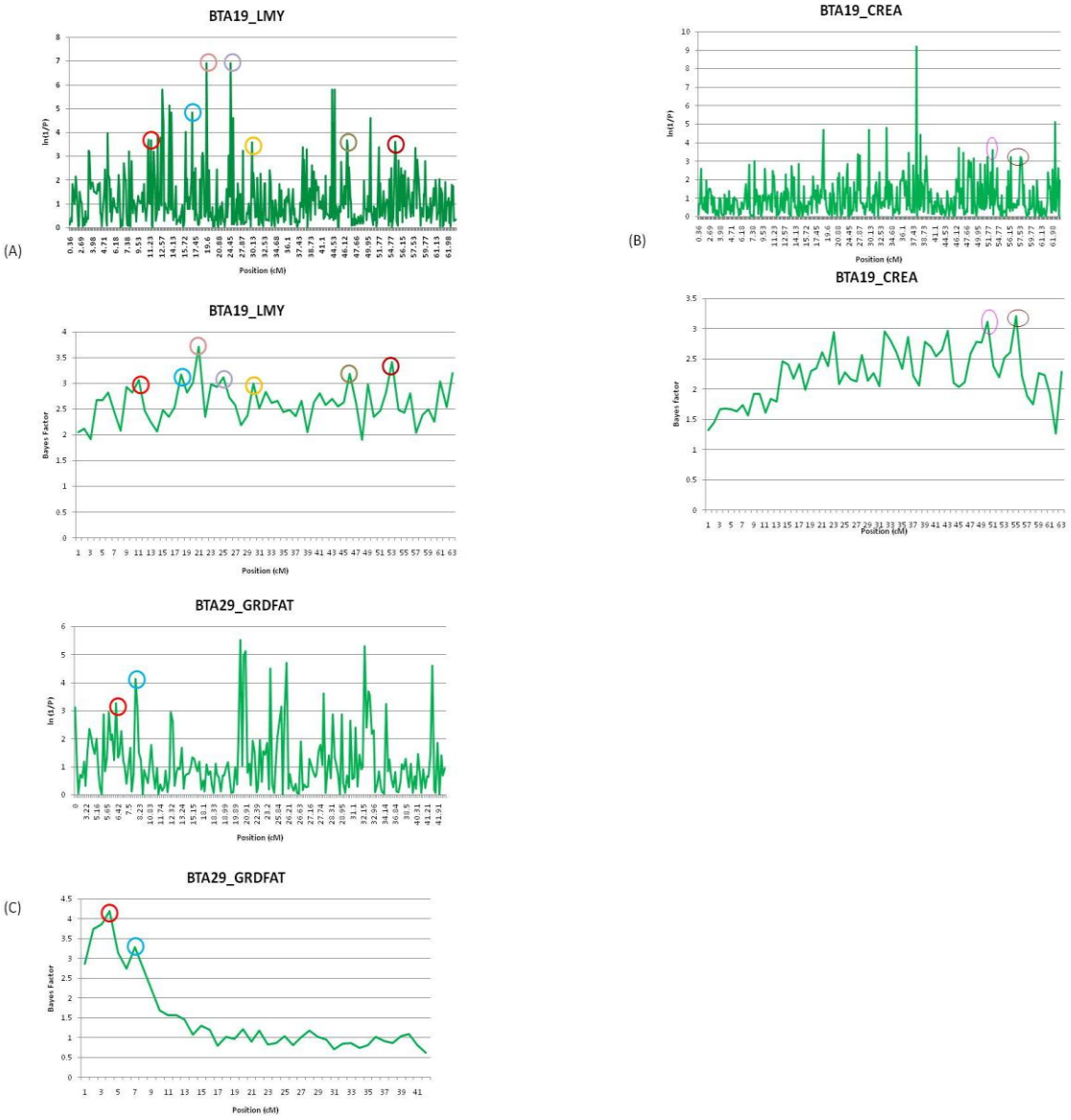

Figure 5-1. Graphs showing results of QTL mapping by LD regression and LOKI for LMY (A), CREA (B) along chromosome 19 and for GRDFAT (C) trait along chromosome 29. Upper panel on each sections of A, B and C shows results by LD regression and the lower panel shows results by LOKI.

We also looked if the QTL regions that we have found using both statistical methods in this study (as shown in Table 5-7) overlap with the chromosomal regions showing evidence of selection (as shown in Table 3-6 of Chapter 3). Four of the chromosomal regions showing evidence of selection in beef cattle were in agreement with the QTLs detected in this study (Table 5-8). However, it is also important to note that the beef population used to estimate signatures of selection were not the same as used in this QTL mapping study.

Table 5-8. Agreement between QTLs and signatures of selection

BTA	Method	Selection Signature (Mb)	Trait	QTL Position (cM)	Bayes Factor	SNP	QTL Position (cM)	P-value
19	Sliding Window	24-26	LMY	25	3.1253	BTA-108581	25.2	0.01
						BTA-11532	24.58	0.001
						BTA-44868	24.65	0.011
19	Sliding Window	60-61	CREA	55	3.2074	BTA-46319	56.34	0.04
						BTA-104739	57.63	0.042
						BTA-104738	57.57	0.039
19	EHH	40.44-40.88	LMY	46	3.1944	BTA-45700	46.5	0.026
						BTA-45701	46.51	0.034
29	Sliding Window	7.5-8.50	GRDFAT	7	3.2821	BTA-27534	8.19	0.047
						BTA-91593	6.27	0.038
						BTA-27538	8.01	0.016

We also looked at three QTL databases available online (http://www.animalgenome.org/QTLdb/cattle.html, http://www.vetsci.usyd.edu.au/reprogen/QTL_Map/, http://genomes.sapac.edu.au/bovineqtl/index.html) to find any QTL reported on BTA19 and 29 in the literature. The location of the markers located within the QTL region were aligned with Btau_3.1 or composite map, where available, to get its approximate position in Mb. We found that the QTL for carcass rib eye area

detected at 50 cM in our study (using both methods) is in agreement with a QTL for ribeye muscle area located at 27.61-52.12 cM (Taylor *et al*. 1998). The four QTL for lean meat yield detected in our study at 11, 18, 20-21 and 25 cM on BTA19 (using both methods) are in agreement with a QTL for retail product yield detected at 7.4-24.55 cM (Casas *et al*. 2003).

Afterwards, we looked for positional candidate genes to investigate their role in the carcass traits in cattle. Briefly, we looked for candidate genes located in the QTL regions with a possible role in the physiology of the trait. We suggest investigating a gene, tubulin folding cofactor D (TBCD), located at 51.23 cM on BTA19. TBCD is a centrosomal protein in the mammalian cells required for the organization of mitotic spindle and promotes the formation of α/β tubulin heterodimers (Cunningham and Kahn 2008). Both α- and β- tubulins are GTP-binding proteins and tubulin folding cofactors serve as GTPase-activating proteins (Tian *et al*. 1999). We have also found two SNP markers (BTA-45737 and BTA-45738) located in the intron of TBCD gene which showed significant association with ultrasound marbling score in this study. We suggest investigating another gene ATP synthase, H^+ transporting, mitochondrial F0 complex, subunit d (ATP5H) located at 58.01 cM on BTA19. ATP synthase uses an electrochemical gradient of protons across the inner membrane during oxidative phosphorylation and catalyzes ATP synthesis. This enzyme is composed of two complexes: the catalytic core, F1 and F0 which comprises the proton channel. The F0 complex has nine subunits (a, b, c, d, e, f, g, F6 and F8) and the gene ATP5H encodes the d subunit of the F0 complex (Aggeler *et al.* 2002). On BTA29, we suggest

investigating a gene called Adenylate kinase isoenzyme 2, mitochondrial (ATP-AMP transphosphorylase) located at 5.07 cM. This gene catalyzes the reversible transfer of the terminal phosphate group between ATP and AMP and is involved in the energy metabolism and nucleotide synthesis pathways (Walker and Dow 1982).

5.3.2. Validation of SNP Markers

Out of 25 markers selected for validation, 18 SNP markers were found to be associated with different traits in the University of Guelph population as shown in Table 5-9.

Table 5-9. List of SNP markers associated with carcass traits in University of Guelph population

SNP	Trait/U of G	F-statistic	P-value	Allele Substitution Effect
BTA-09899	Fat1	3.79	0.053	0.9482
BTA-108581	BodyFat	4.25	0.041	0.00666
BTA-108581	bodyfatofribwt	3.03	0.084	0.1076
BTA-11532	UGLeanYield	4.6	0.034	0.8238
BTA-26209	Fat2	4.07	0.045	1.044
BTA-26209	totalFatOfRib	3.19	0.076	0.6876
BTA-26209	Fat1	2.78	0.098	-1.074
BTA-27538	Fat3	3.89	0.063	.9404
BTA-27538	RibWeightkg	3.61	0.068	0.2692
BTA-27538	SubqFat	13.96	<.001	0.0816
BTA-44665	REAcm	5.06	0.026	1.644
BTA-44665	HCW	4.14	0.044	4.739
BTA-44665	UGLeanYield	3.22	0.075	0.3593
BTA-44793	bodyfatofribwt	4.23	0.041	0.1037
BTA-44793	SubqFat	3.63	0.059	0.01217
BTA-44793	UGLeanYield	3.04	0.083	0.3096
BTA-44793	BodyFat	2.81	0.095	0.00443
BTA-44868	Fat2	5.13	0.025	0.81
BTA-44980	totalFatOfRib	3.54	0.062	0.6343

BTA-44980	Fat1	3.08	0.081	1.017
BTA-44980	InterFat	2.9	0.091	0.02058
BTA-45680	Fat1	8.22	0.005	1.134
BTA-45690	leanofribwt	8.33	0.005	1.256
BTA-45690	BodyFat	2.82	0.095	0.007727
BTA-46408	Marbling	6.94	0.009	0.1079
BTA-46408	UGLeanYield	3.73	0.056	0.41
BTA-58630	Fat3	2.84	0.094	0.2958
BTA-58630	UGLeanYield	13.52	<.001	0.7264
BTA-65151	leanofribwt	2.96	0.087	0.5089
BTA-65152	bodyfatofribwt	6.16	0.014	0.1336
BTA-65152	BodyFat	4.77	0.03	0.006162
BTA-65153	bodyfatofribwt	6.58	0.011	0.1553
BTA-65153	Lean	3.77	0.054	0.04596
BTA-65153	BodyFat	3.59	0.06	0.00596
BTA-65153	RibWeightkg	3.36	0.068	0.06337
BTA-65153	leanofribwt	3.21	0.075	0.5363
BTA-65515	Marbling	5.11	0.025	0.08484
BTA-65585	RibWeightkg	9.87	0.002	0.1469
BTA-65585	Lean	8.62	0.004	0.09653
BTA-65585	BodyFat	4.49	0.036	0.00898
BTA-65585	REAcm	4.11	0.044	2.255
BTA-65585	HCW	12.68	<.001	12.06
BTA-66477	bodyfatofribwt	2.92	0.089	0.08934

In total, associations of 11 of the selected SNP markers were validated in this University of Guelph population. Details of those markers are provided in Table 5-10. These markers can serve as potential tools for marker assisted selection of beef cattle.

Table 5-10. Details of markers validated in Guelph beef population

		University of Guelph			Kinsella population		
No.	SNP	Trait	*Estimate	P-value	Trait	*Estimate	P-value
1	BTA-108581	BodyFat	0.0067	0.041	CABF	1.0358	0.001
	BTA-108581	bodyfatofribwt	0.1076	0.084	GRDFAT	0.9762	0.002
2	BTA-11532	UGLeanYield	0.8238	0.034	LMY	1.2040	0.001
3	BTA-26209	Fat2	1.044	0.045	CABF	1.0309	0.002
	BTA-26209	totalFatOfRib	0.6876	0.076	GRDFAT	0.9764	0.004
	BTA-26209	Fat1	1.0740	0.098	UBF	0.6168	0.006
4	BTA-27538	Fat3	0.9404	0.063	UBF	0.5628	0.004
	BTA-27538	SubqFat	0.0816	<.001			
5	BTA-44868	Fat2	0.8100	0.025	GRDFAT	0.8932	0.006
6	BTA-46408	Marbling	0.1079	0.009	signatures of selection	-	-
	BTA-46408	UGLeanYield	0.4100	0.056			
7	BTA-58630	Fat3	0.2958	0.094	UBF	0.6777	<.001
8	BTA-65152	bodyfatofribwt	0.1336	0.014	UBF	0.6485	0.001
	BTA-65152	BodyFat	0.0062	0.03			
9	BTA-65153	bodyfatofribwt	0.1553	0.011	UREA	1.9324	<.001
	BTA-65153	RibWeightkg	0.0634	0.068			
	BTA-65153	leanofribwt	0.5363	0.075			
10	BTA-65515	Marbling	0.0848	0.025	CAMR	0.1119	0.003
11	BTA-65585	Lean	0.09653	0.004	LMY	1.1015	0.002
	BTA-65585	BodyFat	0.0090	0.036	UBF	0.8621	0.001
					GRDFAT	1.2593	0.002
					CABF	1.2684	0.002

*Estimate – Allele substituition effect

5.4. CONCLUSION

The present chromosome-wide scan was conducted using high density SNP markers on BTA19 and 29, with an average resolution of 1 locus/133 kb and 1 locus/215 kb, respectively. We have identified, in total, 104 SNP markers associated ($P<0.01$) with fat metabolism and carcass merit traits on both chromosomes in beef cattle. In addition, we have detected 26 SNP markers which were significantly associated ($P<0.01$) with more than one carcass trait. A subset of markers (n=25) showing association in this beef population was selected for further validation in an independent University of Guelph beef population. Eleven of the twenty five markers showed association with the same or similar traits in Guelph beef population, thus validating the effect of those markers. These markers are potential tools for marker assisted selection in beef cattle. More number of markers should be validated in independent beef population before their implementation in marker assisted selection. Moreover, we have identified QTL for two traits, carcass rib eye area and lean meat yield, on BTA19 and for one trait, grade fat, on BTA29 using both regression and MCMC model. QTL for carcass rib eye area and lean meat yield were in agreement with previous studies while grade fat QTL on BTA29 seems to be novel. Some of the QTLs detected in this study are in agreement with four chromosomal regions showing evidence of selection. We suggest investigating some positional candidate genes for their potential role in the carcass traits in beef cattle.

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6. General Discussion

Several QTL have been reported previously on bovine chromosomes 19 (BTA19) and 29 (BTA29), but had wide confidence intervals (Li *et al*. 2004, Casas *et al*. 2003, Viitala *et al*. 2003, MacNeil and Grosz 2002). The goal of this thesis was to fine map QTL on these chromosomes using high density of SNP markers in both beef and dairy cattle. This was achieved by the construction of high resolution radiation hybrid (RH) maps and estimation of linkage disequilibrium in beef and dairy cattle.

We built RH maps of BTA19 and 29 consisting of 555 and 253 SNP markers respectively using the comparative mapping approach of Carthagene software (Schiex and Gaspin 1997, de Givry *et al*. 2005) which simultaneously utilizes RH data and knowledge of a known related order. The maps were then compared with the third draft of bovine genome sequence assembly (Btau_3.1). We found an overall agreement of order of markers among the two maps, however a number of inconsistencies were observed. Twelve scaffolds on BTA19 and ten on BTA29 were found to be misplaced in Btau_3.1. For comparison, we computed the log-likelihood and length of the maps using markers that were common between RH maps and the sequence assembly. We found out that the map derived from the bovine genome sequence data is much less likely than our RH map order data. We also constructed cattle-human comparative maps of these chromosomes which were mostly in concordance with previously published comparative maps (Schibler *et al*. 2006, Everts-van der wind *et al*. 2005). Minor discrepancies in the orientation of few homologous synteny blocks were observed which could be

explained by the use of different radiation hybrid panel and mapping approach used. The scaffold changes suggested from our RH maps were in agreement with another independent physical map of the bovine genome for these chromosomes (Snelling *et al*. 2007). Some of these scaffold changes have been incorporated in the fourth draft of bovine genome sequence assembly (Btau_4.0), which was released in October 2007. The RH maps reported in this thesis with an average resolution of 1 locus/139 kb and 1 locus/208 kb on BTA19 and 29 respectively are an important resource for positional candidate gene discovery.

The markers mapped on the RH maps were then utilized for the estimation of linkage disequilibrium and signatures of selection on chromosomes 19 and 29. The extent of LD was estimated using 370 and 186 SNP markers on BTA19 and 29 respectively using the square of the correlation coefficient (r^2) among alleles at pairs of loci. We found regions of high and low LD across the chromosomes in both breeds which could have been generated by complex interactions between biological factors, such as recombination and mutation, and the population's evolutionary history (Mueller 2004). We observed long range LD with LD dissipating to background levels at a locus separation of about 20 Mb on both chromosomes. We could not directly compare our results with the previous studies (Farnir *et al.* 2000, Vallejo *et al.* 2003, Tenesa *et al.* 2003, Odani *et al.* 2006, Khatkar *et al*. 2006) which used D' as a measure of LD because we used r^2. We compared our study to that of McKay *et al*. (2007) which also utilized r^2 and found similar results for Angus and Holstein. For example, at intermarker distances of 5 kb, 100 kb and 500 kb in Holstein, the r^2 values in our study were

0.6, 0.26 and 0.1, compared to 0.53, 0.23 and 0.1 in McKay *et al.* (2007). Nevertheless with many more markers on BTA19 and 29 and with much more sample size, we found that LD extends up to long intermarker distances up to 20 Mb. The result from this study is only derived from two chromosomes and two breeds; therefore it cannot be used as a representative of the whole genome and of all the *Bos taurus* breeds. At a physical distance of 100 kb, we found an average r-square value of 0.23-0.26. Assuming the size of bovine genome as 3 Gb, we would need a minimum of 30,000 evenly spaced and informative marker to perform whole genome association study in *Bos taurus* which is in concordance with McKay *et al*. (2007). However considering the fact that some of the SNPs may have low minor allele frequency in certain breeds, we concur with McKay *et al*. (2007) that a 50,000 SNP chip should be sufficient to perform whole genome association study. We also estimated signatures of selection using a novel five-locus sliding window approach using 355 and 175 markers on BTA19 and 29 respectively. On plotting the mean allele frequency differences against the location of the third locus within the five-locus window, we observed large fluctuations about the axis on both chromosomes. We found evidence of selection in five regions in Holstein and three regions in Angus on BTA19. On BTA29, there were three regions each in Holstein and Angus with evidence of selection. Almost all of these regions with high allele frequency differences were in agreement with the regions which had previously been identified to harbor beef or dairy QTL. However our sliding window approach does suffer from the fact that when markers are not equally spaced on the chromosome, the five-locus sliding window

will not cover the same physical distance which may affect the correlation between allele frequencies expected within each window and thus the range of breed differences. To confirm the chromosomal regions identified using the sliding window approach, we also performed a chromosome-wide scan to detect selection signatures using a web-based tool to compute extended haplotype homozygosity (EHH) statistic (Mueller and Andreoli 2004). The EHH approach detected three regions in Holstein and one region in Angus on BTA19 that showed evidence of selection. On BTA29, we found four regions in Holstein and one region in Angus. In all of these regions, we found a core haplotype with highest frequency and EHH among other core haplotypes, thus indicating positive selection at those loci. On comparing the regions identified using EHH and sliding window approach; we found two regions in Holstein and one region in Angus on BTA29 that were common between the two approaches. These regions showing signatures of selection may further be used to identify potential genes that might underlie QTL for economically important traits, thus improving our ability to link genetic variants to the phenotype of interest.

The SNP markers mapped on the 12,000 rad map of BTA19 and 29 were further utilized to perform a QTL scan for production, functional and conformational traits in Canadian Holstein bulls (n=322) using two statistical methods of analysis, single locus linkage disequilibrium regression model and Bayesian Monte Carlo Markov Chain (MCMC). Another way of fine mapping QTL could have been carried out using LD within a haplotype of closely linked markers. However, there have been studies in the literature where it has been

reported that single marker based LD regression method has similar or greater power than the haplotype based method (Grapes *et al.* 2004, Zhang *et al.* 2003, Zhao *et al.* 2007), especially when dense markers are used. Considering this information and the fact that single marker linkage disequilibrium regression method requires less computational time to detect QTL, we chose this method over haplotype based method. We have identified 302 SNP markers significantly associated with several traits, out of which 73 SNPs were associated with more than one trait ($P<0.01$). A subset of markers (n=21) were selected to validate their effect in a larger Canadian Holstein population (n=722). We could only validate the effect of one marker in this dairy population. We explained the reason of the low success rate of validation to three factors- recombination, epistatic relationship among genes influencing the trait and the polygenic nature of the traits where effect caused by any one gene might have too small effect to detect (Bourdon 2000). Moreover, the chromosome-wide scan detected QTL for 11 and 5 traits on BTA19 and 29 respectively using both regression and MCMC methods. We found that the QTL for 5 traits including milk yield, stature, dairy strength, angularity and milking temperament were in agreement with previous studies (Shariflou *et al*. 2000, Ashwell *et al*. 2005 and Kolbehdari *et al*. 2008) while the QTL for other six traits on BTA19 including protein yield, fat yield, fat%, protein%, maternal calving ease, and rump are novel. On BTA29, two QTL for milk protein yield and mammary system were in agreement with previous studies (Viitala *et al*. 2003, Ashwell *et al*. 2005) while three QTL for angularity, milk fat yield and median suspensory detected in our study are novel. We also looked if these QTL regions

were in agreement with the regions showing signatures of selection (as discussed in chapter 3). It was interesting to note that almost all the QTL detected in our study were in agreement with the chromosomal regions showing evidence of selection. The QTLs detected in the present study has set an important step for further positional candidate gene research. We have suggested four positional candidate gene for further investigation for their potential role in the different traits of interest- Thyroid hormone receptor, alpha (THRA), phosphatidylcholine transfer protein (PCTP), thyroid hormone responsive SPOT 14 (S14) and tumor susceptibility gene 101 (TSG101).

The SNP markers mapped on the RH maps were also used for the chromosome wide scan for detection of QTL for carcass traits in beef cattle (n=451). We again utilized LD regression and MCMC method to look for QTL. We have detected 49 and 55 SNP markers on BTA19 and 29 respectively which were associated ($P<0.01$) with carcass traits. There were thirteen SNPs each on both chromosomes which were associated with more than one carcass trait in this beef population. A subset of markers (n=25) associated with carcass traits in this beef population were selected to validate their effect in another independent beef population at University of Guelph. Associations of 11 SNPs were validated in the Guelph population. These markers have the potential to be utilized for marker assisted selection (MAS). We found QTL for two traits, carcass rib eye area and lean meat yield, on BTA19 and for one trait, grade fat, on BTA29 which were in agreement with both LD regression and MCMC methods. The QTL for carcass rib eye area detected at 50 cM in our study is in agreement with a QTL for rib eye

muscle area located at 27.61-52.12 cM (Taylor *et al*. 1998). The four QTL for lean meat yield detected in our study at 11, 18, 20-21 and 25 cM on BTA19 are in agreement with a QTL for retail product yield detected at 7.4-24.55 cM (Casas *et al*. 2003). We have suggested investigating three genes, tubulin folding cofactor D (TBCD), ATP synthase, subunit d (ATP5H) and Adenylate kinase isoenzyme 2, mitochondrial (ATP-AMP transphosphorylase) for their potential role in carcass traits.

6.1. Conclusion

The present study was carried out to fine map QTL for different economically important traits on BTA19 and 29 using high density of SNP markers in beef and dairy cattle. In order to fine map QTL, exact localization of informative markers is required. We built high resolution radiation hybrid (RH) maps of these chromosomes and then compared with the third draft of bovine genome sequence assembly. Several scaffolds were found to be incorrectly assigned by the assembly. These RH maps not only served as an important tool to rectify the assembly errors, but were also utilized for linkage disequilibrium and fine mapping studies. We found that moderate linkage disequilibrium ($r^2 \geq 0.2$) extends up to 100 kb on these chromosomes and that we would need a minimum of 30,000 evenly spaced and informative SNP markers to perform whole genome association studies in *Bos taurus* cattle. We have also identified some chromosomal regions showing evidence of selection. We have detected some QTL in both beef and dairy cattle and suggested some positional candidate genes for further investigation for their

role in the traits of interest. Several SNP markers have been identified in this thesis which is significantly associated with several traits in beef and dairy cattle. Some of the markers have been validated in an independent cattle population and many more needs to be validated. These markers have a potential for being utilized for the development of genetic tests that might determine the presence or absence of genes that control the desired trait and find greater utility through the marker assisted selection.

6.1. Future Prospects

The candidate genes suggested in this thesis should be further studied by sequencing them and any variation found should be tested for their potential role in the economically important traits of interest. Our linkage disequilibrium study suggested a same number of SNP chip as McKay *et al*. (2007). Recently, the Infinium BovineSNP50 BeadChip has been designed which features more than 54,000 SNPs. This BeadChip presents an average SNP spacing of 51.5 kb across the bovine genome and provides sufficient coverage to identify all the regions of interest in the cattle genome. Large number of animals has been already genotyped with this BeadChip. This high density of SNP markers would be utilized for whole genome association study in different breeds of cattle and would be a huge step towards discovery of new genes and QTL that affect beef and dairy cattle production traits. Lately structural variants involving larger segments of DNA have been reported in the human genome, with the most prevalent form as copy number variation (CNV) (Iafrate *et al*. 2004) which have been reported to be

involved in disease phenotypes (Somerville *et al*. 2005). In cattle, Liu *et al*. (2008) carried out the first study to detect CNVs in cattle and identified 25 high confidence CNVs from Holstein vs. Hereford comparisons on 16 cattle autosomes. More number of CNVs needs to be identified in the cattle genome, especially in the chromosomal regions harboring QTL, and their association with the traits of interest should be further examined.

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