DETECTION OF QTLS IN ANGUS BEEF CATTLE ON CHROMOSOMES 2 AND 11 AFFECTING GROWTH AND CARCASS TRAITS

by

M'heni Ben Abdallah

A Dissertation submitted to the faculty of the

DEPARTMENT OF ANIMAL SCIENCES

In partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

In the Graduate College

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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by M'heni Ben Abdallah

entitled DETECTION OF QTLS IN ANGUS BEEF CATTLE ON CHROMOSOMES 2 AND 11 AFFECTING GROWTH AND CARCASS TRAITS

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

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Ralph L. Price, PhD	Date 8/2/99
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Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

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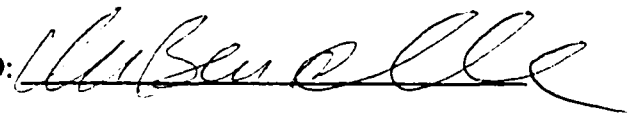
SIGNED: 

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ABSTRACT

Mapping quantitative trait loci (QTL) using genetic markers is the first step in implementing marker assisted selection programs. Microsatellite loci and polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) were used as genetic markers for QTL affecting carcass traits: hot carcass weight (HCW), 12th rib fat thickness (FT), kidney, pelvic and heart fat (KPH) percentage, marbling score (MB), quality grade (QG), ribye area (REA) and yield grade (YG) and growth traits: weaning weight (WW) and yearling weight (YW).

Phenotypic data were collected on seven Angus half-sib family groups with an average of 28 progeny per sire. DNA extracted from ear tissue samples was used evaluate nine microsatellites covering a 40 centimorgan (cM) region on chromosome 2, and thirteen microsatellite markers and one PCR-RFLP spanning 38 cM on chromosome 11. Each sire was heterozygous for an average of 4 markers for each chromosome. Data were analyzed by family with the interval-mapping program, ANIMAP.

The results revealed that the region on chromosome 2 flanked by the microsatellite loci *BM2808* and *RM041* were strongly associated with KPH percentage (total LOD =5.81) with an effect of about 1.5 phenotypic standard deviation in one family. Markers *BMS2872* (46 cM), *BMS2024* (55 cM), and *BMS1126* (56.3 cM) on chromosome 2 tend to be associated with REA, HCW, and YW (total LOD of 2.8, 3.75

and 2.5). Regions nearby *BMS989* (85.4 cM), *BMS2208* (113.2 cM), and *HEL 13* (114.5 cM) on chromosome 11 indicate linkage associated with KPH, FT and MB respectively (total LODs of 3.56, 3.1, and 2.54). All these marker-linked QTLs had an estimated effect more than .75 phenotypic standard deviation.

These findings show that there are several chromosome regions associated with carcass traits in beef and demonstrate the power of genetic linkage analysis to detect these regions even in small families.

CHAPTER ONE- INTRODUCTION

Traditionally, livestock improvement programs have utilized selection based on phenotypic evaluation of animals and their relatives. However, there are biological constraints which limited genetic gain that can be achieved using phenotypic information only. The trait milk yield in dairy cattle is sex-limited, because it can be measured only on one sex. Carcass traits may be termed age-limited, since for the most part they can only be measured after the animals have been slaughtered. Other traits such as disease resistance are difficult or expensive to measure on a routine basis. Furthermore, by using only phenotypic information it is not possible to explain any of the genetic variation within family until progeny information becomes available. Genetic markers can potentially ameliorate problems with traditional selection methods because they can be measured at any time, in both sexes. Genetic markers mark chromosomal regions (and sometimes individual genes), and so can follow the inheritance of these regions from parent to offspring. Thus, if chromosomal regions which contain genes of value were known, markers may be used to identify animals that have inherited favorable alleles of a gene and hence have the best genotype, whether or not phenotypic records or progeny information are available.

Most traits of importance in animal breeding are of a quantitative nature, thus the phenotypes have a continuous distribution, usually described by a normal distribution.

This variations reflects the collective action of multiple loci and environmental effects that make the identification of genetically superior animal a difficult task (Visscher et al., 1998). The identification of quantitative trait loci (QTL) can contribute to the improvement of a breeding design by increasing the accuracy and intensity of selection. Whole genome, with medium to high density marker maps are now available for all major livestock species making linkage analysis for QTL a valid approach for initial genetic localization of the corresponding genes (Kappes et al., 1997). Most notable is the application of the polymerase chain reaction (PCR) to the identification of size differences between loci containing simple sequence repeats (microsatellites).

Consumers of beef desire a lean, yet palatable, product. The beef industry responded by trimming excess fat from the carcass (Smith et al., 1992), but a more efficient approach would be to reduce excess subcutaneous fat and increase lean production, while maintaining the eating quality of beef (Pariacote, et al. 1998). Furthermore, the American Angus Association declared that the long term problem of cattle producers is the shortage of high quality beef and consumer's dissatisfaction with so much of the beef produced today. The same problem was identified by the 1995 National Beef Quality Audit (Boleman et al., 1997), and the 2000 and Beyond Task Force conducted by Webb Technical Group Inc. Consequently, the American Angus Association established a Plan for the years 2000 and beyond which recommends the two important goals for commercial beef cattle producers: a) 95% percent of calves grading USDA choice and prime, and b) all cattle with yield grade 3 or leaner. Manipulating

carcass and fat composition via dietary means are often challenging and controversial, and changes tend to be minor (Saintz, 1997). Based on the above information, and for value-based marketing to be a success, making fundamental genetic changes in the cow herd to reduce external fat while maintaining quality is a primary goal.

Selection using DNA-based marker technology holds promise for achieving changes in carcass quality through marker-assisted selection programs (Haley and Visscher, 1998). The first step in implementing such programs is detection of molecular markers for quantitative trait loci (QTL). The power to detect QTL within a pure breed is poor because linkage disequilibrium between markers and QTLs created within purebred families is minimal. With a two allele systems where the frequency of the alleles is .5 (maximizing the additive variance), the probability that a sire is heterozygous for the QTL is only .5. Coupled with homozygosity of the markers, the probability to detect significant QTL effects is limited within purebred populations. However, selection of elite families will probably continue within pure breeds, thus detection and/or evaluation of QTL needs to move into the populations where it will be used. The purpose of this study was to investigate whether QTL that influence carcass characteristics are segregating on chromosomes 2 and 11 in seven paternal half-sib purebred Angus families.

CHAPTER TWO- LITERATURE REVIEW

1. Value Based Marketing of Beef

Historically, beef cattle production in this country has been haphazard. Clearly, the beef cattle industry has not always understood consumer needs, or given them high priority. Thus, in the past beef producer did not consistently deliver to the wanted product consumer. Consequently, the beef industry has initiated programs to evaluate product quality and to measure consumer satisfaction with beef. The most obvious concerns about quality are: 1) excess external fat, excessive seam fat, and overall cutability; and 2) enhancing taste by assuring sufficient intramuscular fat (marbling). In fact, in 1991, the amount of "lost opportunity" per steer and heifer as attributed to total waste was $219.25 (Smith et al., 1992). Since the time of National Beef Quality Audit-1991(NBQA-1991; Smith et al., 1992) the beef industry has progressed in some area (e.g., reduction of excess fat) but has failed in other areas (e.g., reduction in marbling) as reported on the National Beef Quality Audit-1995 (NBQA-1995; Boleman et al., 1997).

Knowing that the genetic correlations between marbling score and fat thickness at the 12th rib is nearly zero (-0.05), more research efforts that include genetic linkage mapping are necessary in order to provide the basis for systematic approach to dissecting single and multigenic economically important traits and therefore making permanent genetic changes in beef cattle to reduce fat while maintaining eating quality, thus insuring

product improvement and total cost reductions.

2. Physiology of Fat Distribution

Growth in adipose tissue comprises both hyperplastic and hypertrophic growth. Hyperplasia includes the processes of proliferation and differentiation of fibroblast-like mesenchymal cells into adipocytes. In the adipocytes, hypertrophy involves the accumulation of intracellular lipid. Postnatal fat accretion occurs primarily through hypertrophy of existing adipocytes, with smaller contribution of hyperplasia. Intramuscular fat accretion, on the other hand, is due to the appearance of new adipocytes as well as an increase in their lipid content. In cattle there are four kinds of fat: intermuscular, intramuscular, internal (visceral, caul, and kidneys), and subcutaneous. In beef cattle, fat deposition follows three systematic steps. In the first phase, fat is deposited around the viscera and kidneys, and within the caul (over the stomach) and mesenteries. In the second phase, it is deposited subcutaneously and intermuscularly. In the third phase fat is deposited as intramuscular fat.

Intramuscular adipose tissue appears as a delicate pattern of wavy lines in the muscle; hence its common name, marbling. Much of the characteristic flavor associated with beef originated from carbonyl compounds concentrated in the adipose tissue. A choice grade carcasses usually containing 28-30% fat. Thus, in the US market, beef carcasses containing less than about 28-30% fat are heavily discounted by packers, because this is the fat content that roughly corresponds to the small marbling score (choice quality grade). On the other hand, this amount of fat usually results in reduced

meat yields due to large trim losses, particularly of visceral and subcutaneous fat. Therefore, our market contains conflicting objectives for the production of fat, with intramuscular fat commanding a price premium, and other depots mainly subcutaneous, intermuscular and viscera incurring penalties.

Manipulation of total body or carcass fat is possible using exogenous hormones, nutrition or genetics. Adipose cell size appears to be regulated nutritionally, but changes tend to be minor (Sainz, 1997). In contrast, genetic effects on fat distribution can be large, and seem to be due to differences in fat cell number among depots (Paschal et al., 1995). Therefore, genetic manipulation through breed, selection, or more direct means will be required to achieve substantial change in fat distribution.

3. Beef Quality Grades

Voluntary grading systems have been developed and implemented for the purpose of segmenting carcass and/or meat products into smaller, more homogenous groups based on factors which predict important marketing and consumers acceptance of meat products. US congress passed the grading act in 1924, but it was not until 1927 that it was used for field operation (Hale, 1994). This act created two grades, a quality grade comprised of marbling score and maturity, and yield grade based on the yield of boneless retail cuts determined by carcass weight, fat and muscling. A carcass may be identified with a quality grade, yield grade, both or neither.

Bullock, steers, heifers, and cow carcasses are assigned quality grades based on subjective evaluation of three characteristics, maturity, marbling (intramuscular fat), and

muscle firmness, texture and color. Animal maturity has been related to beef tenderness. As animals become older, the amount of connective tissue in the muscle increases, and collagen within the muscle becomes less soluble. This maturity is determined mainly by evaluating the size, shape, and ossification of the bones and cartilage of the carcass. This is judged specifically by the amount of ossification in the cartilaginous buttons of the split thoracic vertebrae.

USDA recognizes five maturity groups, designed A, B, C, D, and E. Any carcass with the "A" maturity group is designated only as "A- maturity", without consideration to advanced maturity within that maturity group. However, the situation is different for carcass in the maturity group B, C, D, and E. Subdivisions of the maturity groups are made by percentage unit position within the group. This will vary from B^{00} for the very youngest carcass to B^{100} to the very oldest carcass within that group.

3.1. Marbling Scores

Beef carcasses containing high levels of evenly distributed intramuscular fat are eligible for higher quality grade (USDA, 1989). A carcass with a higher quality grade would be expected to produce meat with more desirable palatability (juiciness, tenderness, and flavor) than the meat from carcasses of lower quality grades. Savel and Cross (1988) developed the concept of beef acceptability based on the relationship between chemical lipid (intramuscular fat) in the lean of muscle tissue and the overall palatability of the cooked muscle. When fat content is less than 3%, the palatability declines below an acceptable level. Intramuscular fat enhances juiciness by increasing

the water-holding capacity of meat. High level of marbling also increases beef tenderness and reduces the variation in tenderness of cooked beef (Smith et al., 1987). Lipid is deposited in the perivascular cells interspaced within perimysium muscle connective tissue. As fat deposition increases, the strength of connective tissue decreases and the subsequent meat is more tender (Miller, 1994).

Marbling is evaluated by utilizing the amount of intramuscular fat within the *Longissimus dorsi* muscle at the 12th rib. There are 10 degrees of marbling: Abundant, Moderately Abundant, Slightly Abundant, Moderate, Modest, Small, Slight, Traces, Practically Devoided, and Devoid. Marbling and maturity are considered togther using an official USDA Beef Quality Chart.

Except for carcasses in the "A" maturity group, the marbling required increases with increasing maturity within a quality grade. The minimum amount of marbling required for the "A" maturity carcasses to receive the USDA choice grade is small. The American Angus Association recommends that the target marbling score for carcass will be small^{0} or higher. According to the 1995- National Beef Quality Audit, the mean of USDA quality grade for the carcasses evaluated was high select (Boleman et al., 1997). Heritability of marbling score has been estimated at a range between 0.26 to 0.73 by Wilson et al. (1993). On the other hand, Marshall (1994) reported an estimate ranged from 0.23 to 0.47, and averaging 0.35 for the heritability of marbling. The moderate to large heritability estimates for marbling suggests that low genetic potential for this measure of marbling can be remedied by selection within.

4. Beef Yield Grades

The yield grade (YG) of beef carcass is determined by considering four characteristics: (1) the amount of external fat or fat thickness (FT), (2) the amount of kidney pelvic and heart fat (KPH) in percentage, (3) the area of the ribeye muscle (REA), and (4) the hot carcass weight (HCW). The yield grade is determined by mathematical equation, and is expressed as a whole number, any fractional part of the grade is dropped. The yield is determined on the basis of the following equation:

$$YG = 2.5 + (2.5 \times \text{adjusted FT, inches}) + (0.20 \times \%\text{KPH fat}) + (0.0038 \times \text{HCW, pounds}) - (0.32 \times \text{REA, square inches})$$

A carcass of yield grade 1 usually has only thin layer of external fat over the rib, loin, rump, and clod. This carcass has also a slight deposits of fat in the flanks and cod or udder. Muscles are usually visible through the fat in many areas of the carcass. However a carcass of yield grade 4 or 5 is completely covered with fat. This carcass has a small ribeye and large deposits of kidney, pelvic, and heart fat exceeding 5% of its carcass weight.

4.1. Hot carcass weight

Hot carcass weight is the greatest determinant of carcass value at constant age at slaughter. Breeding strategies to increase hot carcass weight will increase the genetic potential of the area of *longissimus* muscle of the 12th rib and also tend to increase the fat thickness over the 12th rib and percentage of kidney pelvic and heart fat. Moreover, a

slight decrease in marbling score is not unusual (Pariacote, 1998). Furthermore, as the carcass weight increases the percentage of retail cut decreases (Koch, et al., 1982). The American Angus Association recommends that the target weight range for carcasses between 295 and 385 kg. Heritability of carcass weight has been estimated at 0.31 from Angus field records.

4.2. Ribeye area

The area of the ribeye is measured where the *longissimus* muscle is exposed by ribbing. Cross-sectional area of the *Longissimus dorsi* muscle at the 12th rib has been shown to account for significant amount of variation in percent retail product at a constant carcass weight (Lorenzen et al., 1993). There is high positive genetic correlation (.72) between 12 rib ribeye area and pounds of total retail product. The American Angus Association recommends that the target range for ribeye area will be between 77 and 90 sq cm.

4.3. Fat thickness

The amount of subcutaneous fat on a carcass is measured over the *longissimus* muscle at the 12th rib. The 12th rib fat thickness heritability has been estimated to be 0.26 from Angus data (Wilson et al., 1993). Yield grade was significantly correlated genetically with fat thickness (0.67) (Pariacote et al., 1998). On the other hand the genetic correlation between *longissimus* muscle area and 12th rib fat thickness is very close to zero. Fat thickness and marbling are negatively genetically correlated (-0.13), (Wilson et al., 1993). The American Angus Association recommends that the target 12th rib fat

thickness will be of 1 cm or less.

4.4. Kidney, pelvic, and heart fat

Kidney, pelvic, and heart fat includes the kidney knob surrounding fat, the lumbar and pelvic fat in the loin and the round, and the heart fat in the chuck and brisket area. The amounts of these fats are expressed as percent of the carcass weight to determine the kidney, pelvic, and heart fat percentage. For Shorthorn beef cattle, kidney, pelvic, and heart fat is slightly positively correlated with marbling (0.1) and moderately negatively correlated with fat thickness (-0.21), Pariacote et al., 1998). Table 2.1. summarizes heritability estimates and genetic and phenotypic correlations for carcass traits.

5. Genome Structure

At the molecular level, chromosomes are composed of DNA and proteins. The DNA carries the genetic information. Proteins catalyze replication, recombination, and segregation of the chromosome. The main part of the chromosome comprises coding sequences (exons), intragenic (introns) and intergenic non-coding sequences, regulatory sequences, tandem repeats, and other dispersed repeats. In addition chromosomes contain telomeres at the ends and centromeres generally are more centrally located (Liu, 1998).

5.1. Introns and exons

Introns were initially discovered in the chicken ovalbumin and, in rabbit and mouse β-globin genes (Breatnach et al., 1977; Jeffreys and Flavell, 1977). This was demonstrated by cloning the cDNA of these genes to investigate possible differences in the structure of the gene in expressing and non-expressing cells.

Genomic DNA was treated with restriction enzymes that did not hydrolyze the cDNA. Southern blot hybridizations of genomic restriction fragments generated by the enzymes revealed several bands in the hybridized blots. The data demonstrated existence of insertion sites in the middle of the protein coding sequences. Moreover, these insertions were present in both expressing and non-expressing cells. The gene insertions that are not translated into protein were named introns and the sequences that are translated were termed exons.

Introns are transcribed to RNA, but not translated into proteins, which means that these sequences are not phenotypically expressed. Genes that are related by evolution have exons of similar size, however the introns may vary in length leading to variation in the length of the gene. As opposed to exons, introns are not related to other sequences in the genome although they contain the majority of dispersed, highly repetitive sequences. Some genes are constructed by patching together copies of individual exons recruiting from different genes. This pattern is known as exon shuffling. Introns are usually larger than exons, specially in higher eukaryotes (Primrose, 1995).

5.2. The mammalian genome and noncoding repetitive DNA sequences

An amazing feature of the eukaryote genomic organization is that the coding sequences only constitute a minor part of the heredity material e.g. 5-10% in mammals (Hochgeschwender and Brennan, 1991). The remaining and dominating share of DNA represent non-coding sequences for which functional roles have not been demonstrated. These non-coding sequences consist of two groups: the single copy DNA and the

repetitive DNA (Figure 2.1; Ellgren, 1993).

Repetitive DNA can be separated from the bulk genomic DNA by density gradient centrifugation. In cesium chloride (CsCl) centrifugation, they appeared as "satellite" bands. Subsequently it was shown that the satellite fractions consists of repetitive DNA in which the GC/AT ratio in the basic repeat unit significantly deviates from that in the bulk DNA i.e. the GC content of DNA molecule governs its buoyant density (Tautz, 1993). Renaturation kinetics shows that these satellites consist of highly repetitive DNA, usually of millions of tandem repeat of relatively short sequence pattern (Britten and Kohne, 1968). Moreover, these repetitive units showed a variation within species and the repetitive pattern often differs between species (Brutlag 1980). Two different types of repeats have been determined: those arranged in a direct tandem fashion and those that are interspersed in the genome.

5.2.1. Interspersed repetitive DNA

In mammals, two majors groups can be distinguished; short interspersed elements (SINES) and long interspersed elements (LINES). LINES have arisen through the reverse flow of genetic information called retroposition (Rogers, 1985). There is a single abundant LINE family, called *KpnI* in humans (Singer, 1982). There are about 10,000-100,000 total copies per genome, 6-7 kb in length. SINEs, on the other hand it may exist in several gene families within the mammalian genome. *Alu* family and B1 families are predominating in primates and rodents, respectively. Each of these two families have copy numbers in excess of 500,000 constituting 3 to 10% of the genome (Rogers, 1985).

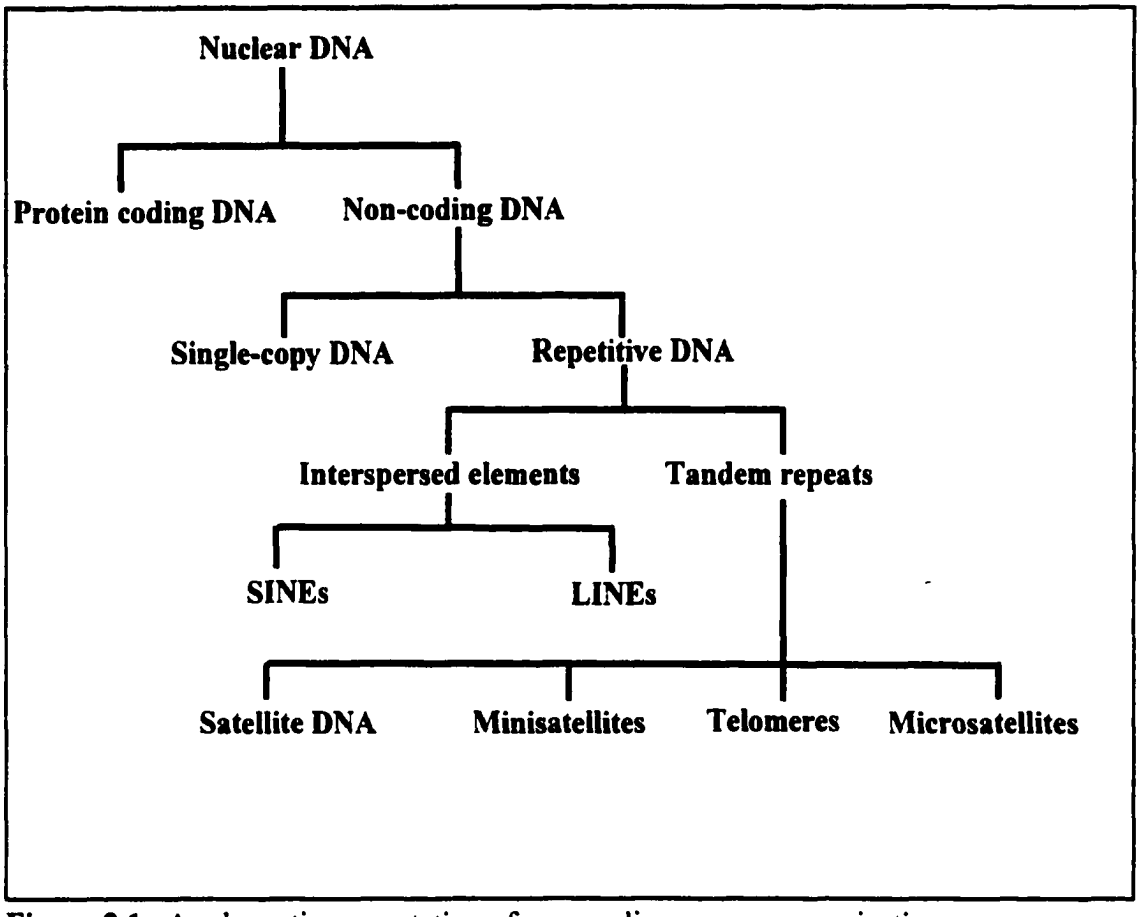


Figure 2.1. A schematic presentation of mammalian genome organization

B1 repeat is 130 base pairs (bp) monomer sequences (Karayev et al., 1982). *Alu* is a dimer formed by two head-to-tail oriented 130-160 bp monomers (Schmid and Jelineck 1982). LINEs are transcribed by RNA polymerase II, and SINEs are transcribed by RNA polymerase III (Weiner et al., 1986).

5.2.2. Tandemly repeated Noncoding DNA

The tandem repeats are broadly referred to as satellite types of DNA as stated previously. This designation was derived from the original DNA preparations obtained in CsCl centrifugation. These localized arrays of nucleotide repeats are further classified according to the size of the repeat unit and or the repeat region as satellite DNA (very large arrays of typically long, complex repeat units), minisatellites (large arrays of long repeat units), and microsatellites (small tandem arrays of very short repeat units).

5.2.2.1. Satellite DNA

Satellite DNA is used to describe DNA classes which have a degree of repetition of 10^3-10^7 at each locus. The satellite DNA is characterized by few number of loci; at most one or two per chromosome for a given type of repeat unit. These elements are typically organized as large clusters, having up to 10^8 base pairs. Satellite DNA is usually located in the heterochromatic regions of the chromosomes, mainly near centromeres and telomeres (Tautz, 1993). There is no evidence for satellite DNA function, however, their strong association with heterochromatin indirectly suggests a possible role in mediating various heterochromatin functions like chromosome pairing, segregation and recombination (Blackburn and Szostak, 1984).

5.2.2.2. Minisatellites

A second class of tandem repeats is minisatellite DNA. Minisatellites are distinctly different from microsatellites in that each cluster has a moderate degree of repetition. Additionally, the length of the repeat units is more restricted and they are dispersed throughout the genome, but often clustered in telomeric regions. These elements are composed of 10 to 100 bp that in some cases may be repeated up to thousands of times. Minisatellites constitute many thousands of loci, but each locus shows a distinctive repeat unit. Minisatellites are also known as variable number tandem repeat VNTR (Royle et al., 1988). The minisatellites exist in several families within mammalian genome, members of each family showing moderate to significant mutual sequence homology (Ellegren, 1993).

5.2.2.3. Telomeres

Telomeres are the natural end of the chromosome. In most eukaryotes, this class of tandem repetitive DNA is made of repetition of the motif TTAGGG (Moyzis et al., 1988). In human, the length of telomeric regions can vary from 10 to 15 kb (Hastie and Allshire, 1989). Telomerase is the enzyme responsible for replication of the repeat units of the telomere. The length of the telomeric region decreases with age and telomere shortening may be directly correlated with cellular senescence (Counter et al., 1992).

5.2.2.4. Microsatellites

Microsatellites are simple small arrays of tandem repeated sequences that can be mono-, di-, tri-, and tetranucleotides, microsatellites were initially identified in eukaryotic

genome as stretches of TG alternating sequences with varying length (Hamada et al., 1982). Subsequent reports showed that CA/GT repeats are also frequently present at many thousand loci in mammalian genomes (Weber, 1990a; Litt and Luty, 1989).

At any one locus, the numbers of copies of the repeat may vary from five to about a hundred. Microsatellites are more or less randomly scattered around the genome of numerous species. However, they are not uniformly spaced along chromosomes, being underepresented at the subtelomeric regions (Weissenbach, 1993). One common simple sequence repeats, for example GT repeats. This sequences forms a left handed helix, called Z-DNA which is created preferentially in tracts of alternating purine-pyrimidine nucleotides as GT repeats itself (Hamda et al.,1982). In mammalian cells runs of (A.T)*n* are very common and can account for 0.3% of the genome. In contrast, runs of (G.C)*n* are much rarer.

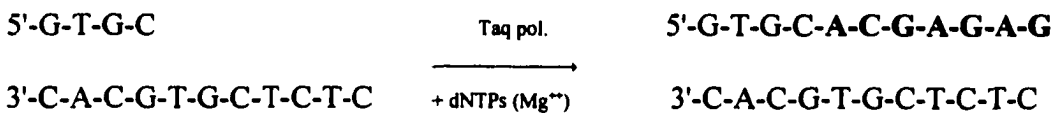
The origin and function of repetitive sequences is not clear at present. The initial occurrence of a short repeat could be due to chance as the probability of a (CA)*n* repeat is 0.004. Short repeats could have emerged as mutations from the poly (A)*n* sequences at the 3' end of adjacent *alu* sequences (Beckmann and Weber, 1992). Additionally, the discerning domination of (CA)*n* repeats can be attributed to the methylation of C residues at 5'G-C3'. Methylated C residues can be deaminated, producing a transition of C to T. This process can lead to increase abundance of 5'G-T3' units and their complementary 5'C-A3' units (Livenson and Gutman, 1987). Additionally, microsatellites may have arisen by slippage mechanisms which suggested that they should be polymorphic

(Levinson and Gutman, 1987). New microsatellites can be cloned directly from total genomic DNA or DNA enriched for certain chromosomes. Genomic libraries can be assembled and then screened for microsatellites by hybridization with simple repetitive oligonuleotide probes, and the positive clones sequenced (Cornall et al., 1991). Computers programs are available to assist primer design from surrounding sequences (Lowe et al., 1990).

6. The Polymerase Chain Reaction (PCR)

The polymerase chain reaction technique, which was first reported in 1985, enables a segment of DNA or RNA sequences from a given template to be selectively *in vitro* amplified to a thousand or even a million fold (Saiki *et* al.,, 1985). In addition to the template, two primers (typically: oligodeoxynuclotides of 11-20 nucleotides in length) are needed, whose sequence must be complementary to the template DNA at the 5' region of the positive and negative strand. These primers hybridize to opposite strands of the target sequence by making use of complimentary base pairing of DNA bases, adenine pairs with thymine and guanine pairs with cytosine. PCR is an enzymatic reaction catalyzed by DNA polymerase (*Taq polymerase*) in the presence of dNTPs as substrate with the aid of magnesium ions (Mg^{++}). The sequence between the two regions complementary to the primers is doubled by repeating the cycle which involve essentially 3 steps: 1-template denaturation; 2- primer annealing; 3- primer extension. These 3 steps occur at different but defined temperature and time intervals (Saiki, et al., 1988). Temperature denaturation occurs at a temperature greater than the melting temperature of

the DNA (94°C). Denaturation separates template DNA into single strands allowing subsequent primer annealing. Primer annealing occurs at lower temperature which is typically 50 to 70°C. The higher the temperature the annealing, the more specific the annealing is, and the extent of annealing of mismatched primer to template is reduced. However, as primer annealing temperature increases, there is an associated decrease in the sensitivity of detection of the DNA being amplified. Temperature of annealing is an important factor in optimization PCR. The final step of PCR is primer extension. Extension involves the synthesis of the DNA strand complementary to the template. As new DNA strands are synthesis at an intermediate temperature optimal of DNA polymerase:



Thus, a double stranded DNA copy of the original target sequence results at the end of the first cycle. The doubling time is equal to the time required to complete each cycle of annealing and extension. Since the extension products are also complementary to the primers, the new synthesized sequence is available as template in the next cycle; therefore, theoretically, a twofold increase of template is achieved in each step. In theory, 30 cycles should yield an amplification of specific sequence by a factors of $2^{30} \approx 1 \times 10^9$, as long as substrate or enzyme is not limiting. These illustrate the obvious capability of PCR technique to generate unlimited quantities of material from minute and otherwise

unanalyzable DNA samples.

7. The Requirement for Linkage Mapping

7.1. Polymorphic genetic markers

In addition to pedigrees in which the relationships are known, polymorphic genetic loci are an important key component of genetic (or linkage) mapping. Genetic markers are loci where alternative alleles can be identified and used to follow the inheritance of a section of chromosome in a mapping study. Genetic markers need to be stable, informative or polymorphic, and heritable (Liu, 1998). Three types of genetic markers have been used in genomic analysis: morphological markers (Thoday, 1961), protein based markers (Edwards et al., 1987) and DNA based markers. Morphological characters (the phenotypes) are reliable indicators of specific genes and are useful as genetic markers on chromosomes. However, many mapping population are needed to obtain reasonable numbers of morphological markers. A commonly used type of protein marker is the isozyme marker. Before DNA markers were discovered, isozymes were extensively used in plants and animals (Liu, 1998). Protein variation can be due to post translational modification and it can not be used as genetic markers. DNA markers are more commonly used at the present time, giving the most effective genetic markers to trace the segregating of QTLs. A saturated map of genetic markers is necessary however to identify QTL with a high power. The different types of DNA genetic markers are outlined in the following.

7.1.1. Restriction fragment length polymorphism (RFLP)

RFLP was one of the first strategies developed to detect DNA polymorphisms. This approach is based on the use of specific gene regions of interest as molecular probes of restriction endonuclease digested DNA. The variants detected are in the form of restriction fragment length polymorphisms (Lander and Botstein, 1989). Briefly, genomic DNA is isolated, and digested with restriction endonuclease. These enzymes recognize specific short sequences of DNA (e.g., the enzyme EcoRI recognizes the sequences GAATTC) and cleave the DNA at every occurrence of that sequence. Mutations of as little as a single base-pair substitution can appear to establish new sites or eliminate old ones. The digested DNA fragments are separated by size in gel matrix by means of electrophoresing the samples through an agarose or polyacrylamide gel. The double stranded DNA is then made single-stranded and transferred (Southern blotted) to filter, and probed with clones from the genomic region of interest.

Restriction maps are constructed based on different sized fragments. Comparison of maps for a sample of gene isolates provides estimates of DNA sequence variation polymorphism among the sample alleles. With the arrival of PCR based technology, RFLP can be detected with PCR products, rather than Southern blotting, creating a rapid analysis tool. The PCR product is digested with the appropriate restriction enzyme and electrophoretic separation determines the presence or the absence of the restriction site.

7.1.2. Single-stranded conformation polymorphism (SSCP)

Single-stranded conformation polymorphisms are based on the fact that the

mobility of single-stranded nucleic acid molecules electrophoresed under non-denaturing conditions is determined by both their fragment length and their secondary structure (Orita et al., 1989, Hayashi, 1991). PCR-amplified DNA, usually 100-200 bp long, is denatured and then cooled rapidly to form single-stranded annealing. As the molecule migrates through a nondenaturing gel, it reanneals with itself, folding into a hairpin structure. The conformation and mobility of this structure depend on the sequence of DNA residues. The sensitivity of the method allows detection of single nucleotide differences in a fragment composed of several hundred nucleotides (Hoelzel, 1996). Thus, subtle differences between individuals including point mutation can be revealed with this technique.

7.1.3. Random amplified polymorphic DNA (RAPD)

Random amplified polymorphic DNA, also known as arbitrarily primed PCR, allows the detection of polymorphism without prior knowledge of nucleotide sequence. RAPD analysis uses just one short primer (usually 10-12 bp) of arbitrary nucleotide sequence that anneal in the first few cycles at low stringency. This will ensure the generation of products by allowing the oligonucleotide to anneal by chance on each template strand over a size range that can be amplified by PCR (Welsh et al., 1995). The subsequent PCR cycles are carried out, under standard high stringency conditions to amplify initial products. When the interceding DNA varies in VNTR, the amplified fragment will show length variation when separated on gels. In addition, polymorphisms can occur as a result of a single base mutation that may interfere with the annealing of the

primer in some individuals (Newton and Graham, 1997). This will result in the presence or absence of same size band. RAPD creates large numbers of bands which generate complex fingerprints that exhibit differences between different DNA templates. Such differences can be utilized in ways largely similar to the uses of RFLP (Deragon et al. 1992). Although RAPD have been used extensively to characterize plant genomes, its use has been limited in animals.

7.1.4. Amplified fragment length polymorphism (AFLP)

The AFLP technique is based on the selective PCR amplification of the restriction fragment from a total restriction enzyme digest of genomic DNA (Deragon et al., 1992). First genomic DNA is digested with restriction enzymes, usually a rare cutter and frequent cutter, such as *EcoRI* and *MseI*. Subsequently, oligonucleotide adapters are ligated to the restriction fragments. PCR amplification of specific product is conducted using primers designed from both the restriction enzyme recognition sequence and the adapter sequences. Polyacrylamide sequencing gels are employed to ensure adequate resolution of the products (Vos et al., 1995). AFLP analysis can be used as a technique of pooling phenotypically similar samples and to identify markers that are linked to specific traits. The advantages of AFLP technology include the capability to develop thousands of polymorphic markers without any cloning and sequencing steps. The limitation of AFLP include the extend of detection of both alleles at any given locus, thus sometimes the markers can be treated as dominant/recessive and are less informative for linkage analysis than codominant markers in which both alleles can be scored (Archibald and Haley,

1998).

7.1.5. Microsatellite markers

Microsatellites represent optimal markers because they are polymorphic within populations, highly abundant and evenly dispersed throughout the genome. Microsatellites are inherited in a codominant fashion, and are fast and easy to type (Rohrer et al., 1994, Wu and Tanksley, 1993). (GT)*n* repeats frequently exhibit length polymorphisms (Weber and May, 1989). Most often, alleles of different sizes vary in length by multiples of two bases, due to the presence of variable number of the (GT)*n* repeat. The degree of polymorphism for mammalian microsatellites is positively correlated with the average number of repeat units (Ellegren et al., 1992c).

The informativeness of microsatellites is determined by the Polymorphic Information Content (PIC) which is defined by the following equation:

$$PIC = 1 - (\sum_{i=1}^{n} p_i^2) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

Where p_i and p_j are the population frequencies for the ith and the jth alleles.
The PIC of dinucleotide microsatellites increases with the increasing average number of repeats, PIC, values ranging from 0 for 10 or fewer repeats to 0.8 for 24 or more repeats (Weber, 1990b). The human genome is estimated to contain approximately 12000 $(CA)_n$ mirosatellites with PIC > 0.5.

7.1.5.1. Detection of genetic polymorphisms with microsatellite DNAs

Bell et al., (1982) published the first reports of polymorphisms at tandemly

repetitive loci. Few years later Jeffreyes and coworkers (1985) identified families of hypervariable minisatellite regions related to polymorphisms in the human genome. Consensus probe that cross-hybridizes to several highly polymorphic minisatellites has made individual genomic fingerprinting possible. Locus specific analysis of individual minisatellites or VNTRs have also contributed to gene mapping (Nakamura et al., 1987).

Sequence analysis of simple repetitive DNA regions from different human individuals indicated that microsatellites, like minisatellites, may show genetic polymorphism due to varying number of iterated residues. Spritz (1981) observed (ATTTT)*n* repeats with n=4, 5 or 6 in human β globin alleles. Subsequently, (CA)*n* and (AT)*n* repeats were shown between the β globin and δ globin genes with the number of repeats varying from 7 to 17 (Semenza et al., 1984; Chebloune et al., 1984; Savatier et al., 1988). At that time, microsatellite polymorphisms could only be detected by laborious cloning and sequencing procedures; thus, their importance for genome analysis appeared insignificant. However, the introduction of PCR (Saiki et al., 1988) dramatically changed the effort required and led to amplification of these microsatellite loci throughout both the human and the bovine genome. Microsatellite analyses use standard high-stringency annealing conditions leading to more specific and reproducible banding patterns. By designing synthetic oligonucleotides flanking a microsatellite, a locus-specific amplification of the repeat region can be primed known as sequence tagged repeat (STR). The length of the amplified fragment will vary according to the number of repeated residues (Figure 2.2, from Diehl, 1996).

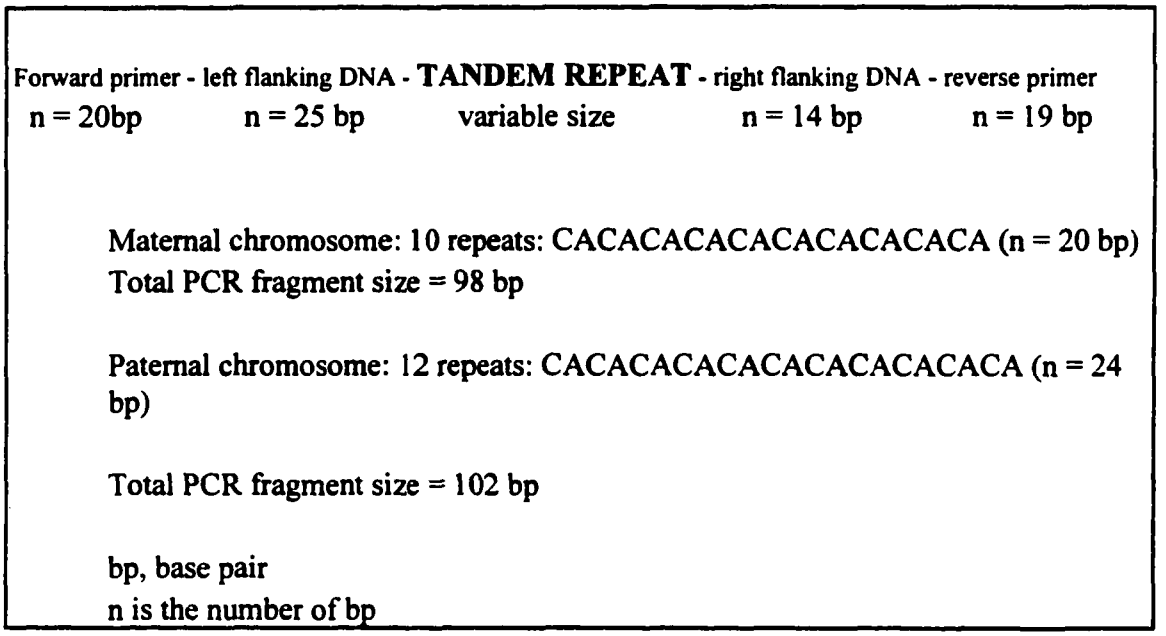


Figure 2.2. Schematic representation of a hypothetical microsatellite marker locus based on variation in a dinucleotide (CA) tandem repeat. The illustrated 10 dinucleotide repeats and, following PCR amplification, produces a fragment of 98 bp (20 bp + 25 bp + 20 bp + 14 bp + 19 bp). The individual's paternal chromosome produces a fragment that is 4 bp larger (102 bp) owing to the presence of additional repeats (12 dinucleotide repeats), from Diehl, 1996, after modifications.

Microsatellite polymorphisms can be visualized by electrophoresis of the amplification products, and detection by staining, autoradiography, or fluorescence (Dietrich et al., 1994).

By separating the PCR products on DNA sequencing or polyacrylamide gels, and by using modifications of fluorescent detection DNA sequencing machines, it is possible to confirm the polymorphic nature of several microsatellite loci and show that they follow Mendelian segregation in families (Litt, 1991; Hearne et al., 1992). Thus, PCR-based microsatellite scoring has become the marker of choice in genome projects (Beckman and Soller, 1990).

The high degree of polymorphism is crucial for linkage mapping by maximizing the number of informative meiosis in each pedigree. Besides in humans and rodents, polymorphic microsatellites have been documented in cattle (Fries et al., 1990), pigs (Johansson et al., 1992a), sheep (Crawford et al., 1992), horses (Ellergren et al., 1992), birds (Ellegren, 1992) and fishes (Goff et al., 1992).

7.1.5.2. PCR primers for microsatellite amplifications

The guidelines for designing primers for standard PCR amplification (Saiki et al., 1988) are also applicable for microsatellites amplification. The primers are designed to anneal to the upstream and downstream nucleotides from the repeat ensuring unique amplification. Precautions need to be taken against primer complementarity, secondary structure and homology to *Alu* repeat sequences which are often near microsatellites (Litt, 1991). The primers should be between 20 and 30 nucleotides in length with base compositions in the range of 35-55% GC. In addition, the primer should not be situated in areas containing simple sequence repeats. It is advantageous to place the primers as close to the microsatellite repeats as possible, minimizing the size of the allele fragments, promoting efficient amplification, improving subsequent electrophoresis resolution of the PCR products, and allowing scoring to be performed more quickly (Koreth et al., 1997; Newton and Graham, 1997).

7.1.5.3. Detection and scoring of microsatellite markers

Amplified microsatellites may be scored by agarose gel electrophoresis and ethidium bromide staining if alleles differ considerably (>45bp, 4% agarose). For most

repeats, however, acrylamide gels are required to detect 1-2 base differences. It is important to note a problem common to polyacrylamide gels detection methods, specially with dinucleotide repeats, is the presence of additional ('stutter') bands in addition to the microsatellite band, differing by 1 or 2 bp, making the scoring difficult.

The most reliable and unambiguous approach to typing microsatellites is to probe the PCR products with a specific repeat oligonucleotide, so that only the specific sequence-containing products are detectable (Cohen et al., 1992). However, this process is tedious and time consuming and required mainly for reaction optimization which is aggravated with the presence of 'stutter' bands beside the microsatellite bands (Koreth et al., 1996).

Radioactive methods

Radiolabeling has traditionally been the standard for the detection and quantification of PCR-amplified mirosatellites, either by internal labeling (direct incorporation of a single labeled deoxynucleoides triphosphate like [α-^{32}]dCTP during thermal cycle) or end labeling (incorporation of single 5' [δ-^{32}P]dATP end-labeled primer in the PCR reaction). Internal labeling method is easy to perform and more sensitive, however the end-labelled approach minimizes additional bands and produces cleaner results. The products are resolved on sequencing gels, fixed, dried, and autoradiographed (Naidoo and Chetty, 1998)

Non-radioactive methods

a. Ethidium bromide. Resolution of products on acrylamide gels, either non-

denaturing (Bethwaite et al., 1995) or denaturing (Eckert et al., 1994) gels followed by ethidium bromide staining is the simplest means of visualization. Subjective quantification and low sensitivity (require > 10 ηg of double-stranded DNA) are the drawback of the ethidium bromide staining (Sambrook et al., 1989)

b. Silver staining. Greater detection sensitivity is obtained in native gels with silver staining as compared to ethidium bromide staining (Todd et al., 1991). The disadvantages include problems with variable background and non-linear deposition of silver. Furthermore, Koreth et al. (1996) reported that in a non-denaturing polyacrylamide format, band intensity on silver staining does not correlate well with radioactive RFLP quantitation as opposed to ethidium bromide staining.

Non-denaturing Polyacrylamide gel

To separate PCR product differing in only a few bp in length (one dinucleotide repeat (CA)); 6 to 10% acrylamide gel can be used. Whereas non-denaturing gels work very well and in some case separation my be carried out more efficiently (Kwiatkowski Jr. et al., 1991) unusual extra bands appear when microsatellites are separated on this type of gels.

Denaturing polyacrylamide gel

Denaturing 6% polyacrylamide gel/7M urea sequencing gel can be used to separate alleles. PCR products are normally visualized by autoradiography. It is preferable to use end-labeled primers rather than including radioactive nucleotide in the PCR (Knowles et al., 1992). Denaturing gel are more expensive, time consuming and

might prove technically more difficult than non-denaturing gels.

Fluorescence

The fluorescence label is quantitative for the amplification reaction, thus alleles can be distinguished by their relative intensities. In a multi-plexing spectrum four fluorescent dyes are used to label PCR primers for high-sensitivity laser detection of four different microsatellites (Ziegle et al., 1992). In this procedure, fluorescent phosphoramitides are connected to the 5' end of the primers in the PCR assay. The labels used include FAM (blue), JOE (green), TAMRA (yellow), and ROX (red). Internal labeling in the PCR, can also be used with fluorescently labeled dNTPs (R110, blue; R6G, green; TAMRA, yellow). The PCR products, fluorescently labeled, are separated on a polyacrylamide gel and detected when excited to fluoresce by a laser. Computer analysis of the output signal detected by a photomultiplier or charge couple device (CCD) camera. offers considerable advantages in terms of sizing accuracy. Allele sizes may be determined accurately and consistently by the inclusion of fluorescent size standard in each sample (Koreth et al., 1996). Simultaneous amplification of different loci (multiplex PCR) has been greatly enhanced by the use of fluorescent labeling technology (Edwards et al., 1991). Fluorescence based technology is at least as accurate as standard radiolabelling techniques and it is recommended to be used in high-resolution genomic analysis (Schwengel et al., 1994)

7.1.6. New marker technology: single nucleotide polymorphisms (SNPs)

Technological advances in molecular genetics have been a driving force in the rapid

progress of the human, animal, and plant genome projects and modern genetics. The discovery of microsatellite markers was considered a major advances in genetics markers because they are more abundant, have fairly higher polymorphisms rates, and can be easily typed as compared to RFLP. However, the requirements to separate the loci on gels and to distinguish several alleles make it hard to fully automate the genotyping process. Recent attention has focused on the use of single nucleotide polymorphisms (SNPs) as genetic markers. SNPs are major contributors to genetics variation, comprising 80% of all known polymorphisms, and their density in the human genome is estimated at more than 1 per 1000 base pairs, or more than 3 million in the genome (Kruglyak, 1997). SNPs have only two alleles and consequently are less informative than microsatellite markers, but they are more frequent and mutationally more stable to offset their low polymorphisms rates and making them suitable for association studies. In addition because SNP are biallelic genotyping them requires only a plus/minus assay rather than a length measurement, making them more amenable to automation (Chee et al., 1996). Several methods are currently been developed for easy, large-scale SNP genotyping. Some of these methods include dynamic allele-specific hybridization, microplate array diagonal gel electrophoresis, oligonucleotide -specific ligation, and whitehead/affymetrix SNP chips. All these technique require target amplification of each SNP by PCR. Mass spectrometry in combination with invasive cleavage might eventually eliminate the need for PCR. In humans, the first commercial SNP chip is expected to contain 2,000 SNP loci with an average density of 2-3 cM, which should be superior in information to

current microsatellite-marker sets (Kruglyak, 1997).

7.2. Pedigree for linkage mapping

7.2.1. Experimental crosses

There are two main types of crosses employed in linkage studies: backcrosses and intercrosses. Initially, two different pure inbred lines are crossed to generate F_1 offspring. F_1 animals are heterozygous for those loci that are fixed for different alleles in the pure inbred parental lines. In a backcross design, the F_1 progeny are crossed to either or both of the pure inbred parental lines. In an intercross design, the F_1 animals are crossed to each other to generate F_2 individuals. However, in livestock linkage studies, and especially for pig and cattle experiments where these types of pedigrees are used, the animals are not pure inbred lines or genetically uniform. Thus, the cross may be, in effect, an intercross for some loci and backcross for others (Otsu et al. 1991). For examples, Andersson et al. (1994) used backcross population strategies to map genes responsible for a multitude of phenotypic differences observed between wild boar and domestic pigs.

The most commonly used methods for detection of QTL in such crosses using the collected marker genotype and phenotype of interest are interval mapping (multipoint approach) in which the position of hypothetical QTL is moved through a fixed map (Georges, 1998). Maximum likelihood methods (Lander and Botstein, 1989), multiple regression (Haley et al.,, 1994) or non-parametric rank-based tests (Kruglyak and Lander, 1995) are the different computation methods used to determine evidence in favor of the

presence of the QTL. The advantages of the backcross approach include high fixation of QTL alleles and increased likelihood of marker heterozygosity; important QTL allele substitution affects in the F_2 or backcross generation (because most of F_1 animals are heterozygous for the same QTL alleles).

The disadvantages of the experimental cross approach include high cost and long duration of the experiment; the loci explaining differences between highly divergent lines may not contribute to the genetic variation that exist within-strain variation present in commercial populations such as elite commercial strains (Georges, 1998).

7.2.2. Outbred pedigrees

In outbred pedigrees, markers are followed in closely related populations. Some of the outbred pedigrees include the half-sib families, mixture of random and self matings, multi-generation pedigrees (Liu, 1998). In livestock, the most common outbred pedigree is the half-sib families. For example using semen of one bull to inseminate many female animals are typically half-sib families. Genetic variation within a half-sib family is a resource to search for genes controlling traits of interest. Half-sib family pedigrees are used to map QTL that are underlying the genetic variation observed for a trait of interest in a commercial population. The identification of these QTL is therefore expected to directly allow for more efficient MAS. On the other hand, elite populations are subjected to intensive selection pressure which is expected to nearly fix alleles with major effects, leaving only modest allele substitution effects to be mapped. Thus, in half-sib families genetic polymorphisms will generally be lower and therefore marker

heterozygosity will be reduced as compared to experimental cross. Furthermore, phenotypic data, collected under field conditions, is often variable in quality (Georges, 1998).

The QTL mapping methods for paternal half-sib families are similar to those used in experimental crosses. Interval mapping techniques are used to estimate the effects of hypothetical QTL alleles on the traits of interest using maximum likelihood methods, multiple regression or non-parametric rank-based strategies (e.g. Georges et al., 1995; Baret et al., 1998; and Coppieters et al., 1998).

8. Genomic Analysis with DNA Markers

Molecular biology has been booming ever since Watson and Crick solved the structure of DNA. Continuous technical and theoretical innovations like cracking the code, sequence determination of DNA and protein molecules, development of recombinant DNA technology and PCR have the provided the scientific community with a means and challenge to analyze the genome by its smallest constituents. In 1988, the National Institute of Health (NIH) and the Department of Energy (DOE), coordinated their efforts in the human genome project. The objective of the project was a complete genetic and physical map of each human chromosome as well as determination of the nucleotide sequence of the entire human genome (Cantor, 1990; Watson, 1990; Jordan, 1992; Robinson, 1992). Sequencing and mapping the human genome has the potential capability of directly translating information for clinical purposes either for diagnostic or, ultimately, for therapies (Kolberg, 1992). When RFLPs were first identified in human, it

was thought that these might serve as an abundant source of genetics markers and form the basis of the whole genome marker (Bostein et al., 1980). It is, however, the development of PCR in combination with the discovery of microsatellites as abundant source of polymorphic markers (Weber and May, 1989; and Litt and Luty, 1989) that boosted the construction of DNA marker maps in wide variety of mammalian species in the last five years. Information accumulated from the human genome project has also been used in comparative analysis of other species. Livestock species are of particular interest because of their economical and agriculture significance. Various farm animal genome projects have been published. By constructing linkage maps of the genome, these projects can address the genetic basis of, for instance, meat quality, growth characteristics and disease susceptibility (Rudolph et al., 1992; Shuster et al., 1992; Georges et al., 1993b; Womack, 1992; Georges, 1998).

9. Mapping Genes and Genome

Mapping genes and the creation of genetic maps is a fundamental part of the current science of genetics. This is because much of the genetics is concerned with the understanding and manipulation of the inheritance of particular traits. For plants and animals of agronomic importance this means selective breeding and the identification of those offspring with the desired combination of characteristics. In the case where the traits are associated with particular genes the task is relatively easy. Unfortunately there are many times more traits than identified genes and so geneticists use marker genes (there are about 50,000 to 100,000 genes in the bovine genome but only few are known).

These are genes which can be easily identified and which are genetically linked to a gene for the traits of interest (Primrose, 1995). In order to map the unknown gene influencing a trait by genetic linkage, a panel of markers are observed for co-segregation with the trait at meiosis. If statistically adequate evidence of such cosegregation is observed, it is concluded that the trait gene must be located on the same chromosome region as the marker genes (Diehl, 1996).

The size of the mammalian genome is about 5×10^9 bp (3×10^9 for humans and bovine), and the fact that many plants and animals have a much higher size of the genome is a reflection of the presence of large amounts of repetitive DNA. In all eukaryotes there are 7000-20000 genes, except for the vertebrates which have 50000-100000 genes (Bird, 1995).

9.1. Genetic linkage maps

Solomon and Bodmer (1979) and Boststein et al. (1980) first proposed the use of DNA polymorphisms as markers for linkage mapping. In order to locate and subsequently clone and sequence genes of economic importance, the first efforts have been devoted to developing a linkage map based on the construction of maps of anonymous polymorphic DNA markers, the number of which far exceed those expressed genes on a number of species. Ultimately, however, the linkage map will become saturated with coding genes through the combination of physical and genetic linkage mapping efforts.

A genetic linkage map is based on of the frequency of coinheritance, such

distances are loosely related to the physical distances separating the genes for these traits on the chromosome.

Crossover refers as the exchange of genetic material between homologous chromosomes during meiosis (Klein, 1996). Long chromosomes tend to experience multiple crossovers. The degree of crossing over between any two loci on single chromosome is proportionate to the distance (d) between them. Thus, the larger the distance between two loci, the greater the chance that there will be one or several crossovers between them. Consequently, the distance between the two loci is defined as the average number of crossovers on a chromatid between these loci. For loci located near each other, the chance that multiple crossovers will occurs between them is small. Recombination fraction may then be interpreted as map distance. The unit of measurement is the Morgan (M), with one morgan (M) equal to the probability of a crossover event of 100% (King and Stansfield, 1990). A crossover value of 10% is a decimorgan (dM); 1% is centimorgan or 1 map unit.

9.2. Mapping functions

A function or equation that relates map distance to the recombination fraction is called a mapping function (Ott, 1991). The simple mapping function is the Morgan's map function where the $\theta = d$ and therefore assumes complete interference (only one recombination event has taken place between two loci). In this case each chromosome, would have the same genetic length of 50 cM. Morgan's function appear to be adequate for genes that recombine with frequencies less than 5% (King and Stansfield, 1990). In

the case where genes far apart from each other, multiple crossovers may occur; consequently, the recombination fraction can not generally be taken as a measure of genetic distance.

Under the assumption that recombination events are independent i.e, absence of interference (I), Haldane's mapping function (Haldane, 1919) relates recombination fraction θ to mapping distance d in (M):

$$d = -\tfrac{1}{2}\ln(1 - 2\theta)$$

or

$$\theta = \tfrac{1}{2}[1 - e^{(-2d)}]$$

The drawback of Haldane's function is that it does not account for interference. In many species however, crossovers do not occur independently of each other: one crossover tends to reduce the chance that another crossover will occur nearby, a phenomena known as positive interference. Other mapping functions have been derived to account for interference including the Kosambi's mapping function which assume that interference is a function of the recombination fraction (Kosambi, 1944):

$$I = (1 - 2\theta)$$

The transformation of Haldane function by adding the interference yield the Kosambi's

mapping function where:

$$d = \tfrac{1}{4}\ln\left[\frac{(1 + 2\theta)}{(1 - 2\theta)}\right]$$

Given the map distance (d) it is also possible to calculate the recombination fraction (θ) by using the equation:

$$\theta = \tfrac{1}{2}\left[\frac{(e^{4d} - 1)}{(e^{4d} + 1)}\right]$$

For more than 3 loci, Kosambi's mapping function might not be feasible because it does not provide valid gamete probabilities. Haldane and Kosambi's mapping functions are used predominately in animal linkage studies.

9.3. Linkage analysis

DNA technology, and the ability to detect allelic forms of DNA sequences has revolutionized genetic mapping. Loci reside on the chromosome, of which each bovine cell contains 30 pairs (29 autosomal pairs and two sex chromosomes). Each parent has contributed one chromosome to such a pair in an individual. Recombination can be defined as the generation in progeny of combinations of genes that were not present together in their parent due to, either independent assortment of nonhomologous chromosomes during meiosis or crossing over (breakage and exchange of parts) of homologous chromosomes during meiosis and mitosis (Snustad, 1996). Linkage

describes the relationship between of the genes to be inherited together as a result of their location on the same chromosome. It is measured by percent recombination between loci. Genes that are far apart may assort independently despite the fact they reside in the same chromosomes. Although they show no direct linkage, each can be linked to genes that lie between them, and so genetic maps can be extended beyond the limit of 50% recombination. The theory of recombinant fraction is the basis for development of genetics maps, although the nature of genetic markers may have changed from a phenotypic variant to DNA variant markers such microsatellites or RFLPs (Archibald and Haley, 1998). Linkage between loci is tested by pedigree analysis where a least one parent is heterozygous at both loci. The most widely used method is the maximum likelihood method estimating the ratio of the likelihood of an observed pattern of segregation of a trait and a marker locus in a set of families under the hypothesis of linkage (H_1) to the likelihood under the null hypothesis (H_0), that the trait, and the marker loci are unlinked (Diehl, 1996). The value of recombination fraction (θ) maximizing the likelihood of the data is determined and the H_0 hypothesis is rejected to favor linkage at the corresponding recombination rate if lodscore is greater than a predetermined level (Georges et al. 1995). A lodscore of 3 or greater is a stringent threshold (odds of linkage of 1000:1) corresponding to a type I error of approximately 5%. Linkage at the corresponding θ is excluded when lodscore is inferior to -2, while the lodscore is considered inconclusive for intermediate LOD values ($-2 < LOD < 3$) requiring additional data (Georges, 1998). In analyzing data particularly with complex pedigrees, the

calculation of likelihood ratio can get rather complicated. Fortunately there are several widely available computer programs that can be used for this task, including, LINKAGE (Lathrop and Lalouel, 1988) and its variants, CRI-MAP (Green et al., 1990) and ANIMAP (Georges et al., 1995).

9.4. QTL mapping

Linkage analysis can be used to map QTLs underlying production traits in livestock. One approach consists of whole genome scan to identify regions of chromosomes with effects on the traits of interest. The problem with this approach is that it demands the typing of a target population for markers covering the entire genome. In addition to the genotyping load that this entails, the large number of statistical tests require a stringent significance threshold to avoid false positive results being reported (Lander and Kruglyak, 1995). An alternative approach to performing an entire scan of the genome is to focus on candidate genes and to look for associations between polymorphisms within these genes and performance of the trait under study. The advantage of this approach is that less genotyping is required; however, unless luck play a big role, it is unlikely that a polymorphism identified within the gene will directly identify performance traits. A good example of success of candidate gene approach is the identification of associations between the oestrogen receptor locus (ESR) and litter size in swine (Rothschild et al., 1996).

9.5. Linkage disequilibrium and marker-associated quantitative effects

In population genetics, at two or more loci, linkage is tested as the departure of gametic

frequencies from expectation under allelic independence among the loci. Determination of linkage between marker loci and QTL will depend on the presence of linkage disequilibrium between alleles at the marker locus and alleles at the QTL. This disequilibrium will create marker-associated quantitative effects that can be detected by statistical analysis (Soller, 1991). Crossing population that differ in alleles frequencies at marker loci and/or QTL can generate linkage disequilibrium. In a population there are other processes that can produce linkage disequilibrium. These processes are selection, migration, mutation, and random effects in a small population. On the other hand, meiotic recombination reduces linkage disequilibrium. The degree of disequilibrium in a finite population is primarily function of the effective population size (Ne) and the proportion of recombination (θ) between marker loci and QTL. Noticeable disequilibrium is expected when $\theta \leq 1/Ne$ (Hill and Robertson, 1968). So if the recombination fraction is below 0.05 some degree of linkage disequilibrium is expected for marker-QTL associations (Soller, 1991).

9.6. Power and precision of QTL mapping

The power of a QTL-detection experiment, defined as the probability of detecting QTL at a given level of statistical significance, depends upon the strength of the QTL and the number of progeny used in the analysis (Manly and Olson, 1999). Strong QTL explaining over 20% of the trait variance can be detected with a power greater than 80% even with small sizes. However, weak QTLs (explaining 1% or less of the trait variance) require at least a thousand progeny to detect them with high power (Lynch and Walsh, 1998).

Moderate QTL can be detected with reasonable sample size, but not necessarily at high power. The consequences of detecting QTLs with samples sizes of low power, include limited number of QTL detections, and those that are detected will be overestimated (Beavis, 1994). The number of progeny require to detect a QTL is proportional to the variance of nongenitic contribution and inversely proportional to square of the effect of the QTL (Lynch and Walsh, 1998). Most of the measurements were performed in controlled crosses such as F_2 and backcross, and these crosses usually have higher linkage disequilibrium than outbred designs. Georges (1998) stated that 10 to 20 individuals can be sufficient to map single gene trait by linkage analysis, however, with mapping genes underlying complex traits, substantially larger experiments are necessary (count of hundreds for experimental cross designs, count of thousands for outbred design) to reveal the modest contribution of QTL to the overall traits variance. The power of a test for marker-QTL linkage based on the effects associated with the marker genotype will be generally greater that 50% (Soller, 1991). The power to detect linkage increases with decreasing recombination fraction; power also increase with increasing heterozygosity H. Thus, low heterozygosity may be compensated for by high marker density and vice versa (Ott, 1996). Type I error, usually denoted by α, is the probability the H_0 will be rejected when it is true. This false positive occurs if a linkage between two genes is declared when they are truly unlinked. Genome-wide Type 1 error of 5% is commonly accepted as the level of significance (LOD=3). With type I error probability of 0.001, only strong QTLs, or in large crosses, the strongest moderate QTLs would expected to achieve this level of

significance.

9.7. Physical or chromosome mapping

Physical mapping relies on assigning cloned pieces of DNA to a particular location on a chromosome. Sometimes a small deletion in a chromosome produces a particular phenotype, indicating the location of a gene (Bolsover et al., 1997). Physical gene mapping has provided vital information about the organization of the genome at the individual chromosome level. This mapping approach complements linkage maps each providing unique pieces of information (Chowdhary, 1998).

Techniques for physical mapping have improved rapidly in the past several years. Early techniques includes traditional cytogenetic methods such as somatic hybridization (Kao, 1983) and chromosomal *in situ* hybridization (Chowdhary et al., 1989). Radiation hybrid mapping had a rebirth in the 1990s when it was used for the construction of high resolution maps (Gyapay et al., 1996). Fluorescent *in situ* hybridization (FISH) is the non-radioactive version of *in situ* hybridization. A bovine yeast artificial chromosome (YAC) library system of the bovine genome was constructed to allow the transition from genetic mapping information to physical localization of the genes using genomic DNA from a male Japanese black cattle (Wagyu) for DNA source. This library incorporated 20,000 clones with an average insert of 500 kb (Takeda et al. 1996). Clones from the yeast artificial chromosome (YAC) library have mapped specific chromosomal locations using fluorescent *in situ* hybridization (Chowdhary, 1998). Chromosome microdissection creates a fine physical map that generates scraped DNA libraries that can be used to

screen genomic libraries for isolating region-specific BAC/YAC clones, which can then be used for high resolution mapping in that region (Guan et al., 1994)

9.8. Livestock genetic linkage maps

Livestock genetic linkage maps have been developed to assist in identifying regions of the genome that influence economically important traits. Most traits of interest in cattle are quantitative in nature and relate to production efficiency, reproduction, or carcass characteristics. The continuous phenotypic distribution observed in most quantitative traits reflects the joint action of multiple loci and environmental influences that increase the difficulty in identifying genetically superior animals. Identification of these genes known quantitative trait loci (QTL) can improve selection accuracy and intensity.

Linkage maps with low, medium and high marker density have been published for cattle (Barendse et al., 1994; Bishop et al., 1994; Ma et al., 1996; Barendse et al., 1997; Kappes et al., 1997), swine (Ellergren et al., 1994; Rohrer et al., 1994; Archibald et al., 1995; Rohrer et al., 1996), sheep (Crawfold et al., 1995; deGortari et al., 1998) and goats (Vaiman et al., 1996). These maps have been used to identify loci in cattle: horn development (Georges et al., 1993a), Weaver Syndrome (Georges et al., 1993b), milk production (Georges et al., 1995), roan coat color (Charlier at al., 1996), muscle hypertrophy (Charlier et al., 1995); sheep: fecundity (Montgomery et al., 1994) and muscle hypertrophy (Cockett et al., 1994); and pigs: fat and growth traits (Andersson et al., 1994). Most of these loci have been mapped to 10 cM intervals or they extend beyond

the end of the linkage group (Kappes et al., 1997).

The high resolution (average interval 2.5 cM) bovine linkage map with 1250 polymorphic loci covering 2990 cM that has been published by Kappes et al. (1997) contains 627 new markers and 623 previously linked markers, providing a basis for integrating the four published bovine maps (Barendse et al., 1994; Bishop et al., 1994; Ma et al., 1996; Barendse et al., 1997). This map increases the power of detecting QTL and improves the potential resolution of mapped QTL by providing sufficient number of recombinant meiosis. This map will contribute to implementation of marker assisted selection (MAS) mating schemes. The second-generation map of the sheep genome (deGortari et al., 1998) represents considerable improvement in resolution over the first generation (average interval 6.5 cM as compared to higher than 10 cM of previous map reported by Crawfold et al. (1995). The overall genetic length of the sheep genome is similar to the second-generation map of the bovine genome (Kappes et al., 1997).

9.8.1. Summary of livestock QTL mapping

With the availability of marker maps in most livestock species, systematic genome scans are increasingly being undertaken for the purpose of locating genes or QTLs of interest by linkage analysis. In the past few years, QTL mapping in pigs and dairy cattle received more attention than QTL mapping in beef cattle. Currently some beef carcass and growth trait projects are under review (Stone et al., 1999).

9.8.1.1. Gene/marker-linked QTL in dairy cattle

Georges et al. (1995) published the first whole genome scan to map QTL affecting

milk production in elite Holstein Friesian dairy cattle. A total of 1,518 sires, with progeny tests based on the milking performance of more than 150,000 daughters, were genotyped for 159 microsatellites markers that cover 2/3 of the bovine genome. Regions on chromosomes 1, 6, 9, 10 and 20 showed very strong evidence for the presence of QTL controlling milk production or components. The confirmation of QTL on chromosome 20 that affecting milk yield and composition has been reported by Arranz et al. (1998).

Kühn et al. (1996) used a granddaughter design with 310 sons from 5 German Holstein Friesian families to search for QTL for milk production traits on chromosome 6. All sons were genotyped at each of 14 loci spanning the entire chromosome. The results indicated that two QTL with different effects are found on chromosome 6. At locus *ILSTS97* a group of sons with allele A had significantly superior milk yield compared to sons with allele B (no difference in milk fat and milk protein). Another family displayed significantly higher milk, milk fat and milk protein yield of sons with allele A compared to B at locus *TGLA37*. The authors stated that these two QTL are probably not within the casein locus on chromosome 6.

Evidence of QTL with effects on milk yield and composition on chromosome 9 was reported by Vilkki et al. (1997). Eleven half-sib families were genotyped for 6 microsatellite markers. Data analysis found evidence for QTL segregating on chromosome 9 that affecting milk yield and protein percentage.

Another whole genome scan was undertaken comprising 1158 progeny-tested Holstein- Friesian bulls to map QTL influencing milk yield and composition (Coppieters,

1998b). A QTL with major effect on fat and protein percentage as well as milk yield was identified on the centromeric end of bovine chromosome 14.

In an effort to understand underlying genetic variation of the traits number of stillbirths and calving difficulties in dairy cattle, Grupe et al. (1998) used 12 paternal half-sib families comprising a total of 498 sons in granddaughter design experiment. A total of nine autosomal microsatellites were genotyped. Paternal components of stillbirth and calving difficulty were significant in 4 of the 8 families analyzed for the marker CYP21 suggesting the presence of QTL responsible for both of these traits on chromosome 23.

Ashwell et al. (1997) reported a potential QTL for somatic cell score, fat yield, fat percentage, protein yield and protein percentage in seven large half-sib US Holstein families. Out of the 16 markers used for genotyping, three markers (BM203, BM4505 and BM2078) were associated with significant effects for different traits. In another study they found that the somatic cell score QTL was located near the microsatellite marker 513, located on chromosome 23 in an existing Holstein population (Ashwell et al., 1996).

Vukasinovic et al. (1999) reported that the growth hormone locus and QTL affecting protein percentage in Holstein cattle are tightly linked. A total of 553 Holstein bulls were typed by PCR-RFLP for at least one of the three bovine growth hormone (bGH) loci designated: GH427, GH891, and GH441. The estimated effect of an allele substitution at the QTL ranged from 0.57 for fat content to 1.6 for protein yield.

A PCR-RFLP was used to genotype for A and B alleles of bovine growth hormone in order to evaluate the association of these allelic variant with milk production

traits among the Canadian Holstein AI bulls (Sabour and Lin, 1996). The allele A codes for amino acid leucine at position 127 in contrast to valine coded by allele B. A total of 160 unrelated Holstein bulls and was genotyped for the A and B alleles. The results indicated that the B allele is preferred for increased milk production traits, particularly protein.

Moody et al. (1996) compared 3 populations of Hereford cattle to determine differences in allele frequency and genetic variation. These authors found significant difference at 6 of 7 polymorphisms genotyped. In one family (Miles City Line 1 Herefords) it was found that substitution of a B allele for an A allele of the kappa-casein gene accounted for 15 and 8% of the variability in expected progeny differences for birth weight 180-d gain from birth to weaning, respectively. It was concluded that kappa-casein may be useful marker for these traits in MAS.

9.8.1.2 Gene/marker-linked QTL in beef cattle

Beever et al. (1990) published the first report on QTLs influencing growth and carcass traits of beef cattle. A half-sib family (n=146) with a sire that was heterozygous for six polymorphic marker loci (BoLA-A (class I major histocompatibility complex), B, C, and F blood group systems, serum transferrin (Tf), and Vitamin D binding protein (Gc) was used in the study. These authors localized QTLs influencing 205 day and 365 day weight, preweaning average daily gain and subcutaneous fat thickness to the region of chromosome 12 marked by the erythrocyte antigen B locus.

Six microsatellites on chromosome 2 were used to determine the presence or

absence of the *mh* allele as well as confirm the location of loci affecting carcass traits using 2 half-sib families (n=246, and n=209) (Casas et al. 1998). Individuals inheriting one allele had leaner, more heavily muscled carcasses compared with those inheriting the alternative allele.

A primary genome scan of 238 microsatellites on 185 progeny form a *Bos indicus* × *Bos taurus* sire mated to *Bos taurus* cows was used to map QTL influencing carcass and growth traits (Stone et al., 1999). F-statistic profiles computed at 1-cM intervals for each chromosome by regression analysis were used to find significant QTLs. They reported evidence for a QTL affecting rib bone and dressing percentage on chromosome 5. Other putative QTLs for retail product yield on chromosome 2 and chromosome 13, rib eye area on chromosome 14, and birth weight on chromosome 1 were detected at or just below the threshold of significance.

A 2-yr study was conducted to determine the effect of sire marbling expected progeny difference (EPD) on marbling score, palatability, and carcass fatness of progeny (Gwartney et al., 1996). Steer carcasses from the high marbling EPD group, adjusted to the mean number of days on feed, had significantly more marbling ($P < 0.01$) and less subcutaneous fat in the ½ carcass and the hindquarter ($P < 0.10$) than their low marbling EPD counterparts. Furthermore, steers from the high marbling EPD groups showed a higher rate of marbling deposition compared to low marbling EPD groups. Similar relationships of a greater magnitude were found for heifers. It was concluded that it is possible, using existing genetic resources, to maintain marbling score and reduce carcass

fatness without compromising the eating quality of beef.

9.8.1.3. Other gene/marker-linked QTL in bovine

Fifteen paternal half-sib families with a total of 168 calves segregating for horned/polled were tested with eight microsatellites to study genetic markers linked to polled locus in cattle (Schmutz et al., 1996). The results of this study showed that each of *TGLA49* and *BM6438* was linked to polled at 0.1 cM, *BMS1928* was linked at 3 cM, *BM8139* at 9 cM, *BMS574* at 21 cM, and *BMS711* at 22 cM. In addition, comparative mapping showed that the sheep keratin-associated protein 8 (*KAP8*) marker was linked to polled at 11 cM. On the other hand, these authors did not find any linkage between human microsatellites and the polled locus.

Harlizius et al. (1997) demonstrated close genetic linkage between a set of bovine chromosome 1 markers and the polled locus in four half-sib families representing two European cattle breeds: Simmental and Pinzgauer. The microsatellites INRA212 and the gene for keratin-associated protein 8 (*KAP8*) showed significant linkage with polled gene at recombination rate $\theta = 0$ (LOD= 6.92), and recombination rate $\theta = 0.033$ (LOD= 6.52) respectively.

A granddaughters design was used to search for quantitative trait loci for ovulation rate in cattle (Blattman et al., 1996). The results of this study indicated ovulation rate QTL on chromosome 7 and 23. Marker UWCA20 on chromosome 7 was associated with one phenotypic standard deviation and accounted for approximately 10% of the phenotypic variation. Marker CYP21 on chromosome 23 was associated with an

effect about ½ phenotypic standard deviation and accounted for approximately 4% of the phenotypic variation (Blattman et al., 1996).

Muscular hypertrophy in the Belgian Blue breed is caused by a major gene of partially recessive effect and markers linked to muscular hypertrophy locus have been described (Charlier et al., 1995; Smith et al., 1997). Dunner et al. (1996) found that the doubling phenotype within in Asturiana de los Valles breed, consistent with the involvement of the same locus located on chromosome 2 as in Belgian Blue breed. Furthermore, the position of the muscular hypertrophy locus coincided in both breed populations. Evidence for linkage disequilibrium between the muscular hypertrophy locus and the closest marker (TGLA44) was identified in both breeds.

Table 2.1. Literature summaries of heritability (on diagonal) and genetic (below diagonal) and phenotypic (above diagonal) correlations of carcass trait parameters (From Wilson et al., 1993; Marshall, 1994; Pariacote et al., 1998)

Trait	Range	HCW	FT	KPH	MB	REA	YG	WW	YW
HCW	Low	.31	.19	.11	.08	.37	.20	.59	—
	High	.68	.42	.31	.28	.58	.20	.68	—
	Average	.41	.32	.15	.16	.48	.20	.63	—
FT	Low	-.22	.24	.20	.12	-.16	.78	.12	—
	High	.95	.68	.20	.38	.04	.78	.31	—
	Average	.27	.44	.20	.24	-.09	.78	.21	—
KPH	Low	-.30	-.21	.37	.10	-.05	.37	—	—
	High	.21	-.21	.45	.16	.19	.37	—	—
	Average	-.05	-.21	.41	.13	.07	.37	—	—
MB	Low	-.33	-.13	.10	.23	-.08	.22	-.05	—
	High	.64	.73	.59	.88	.19	.22	.16	—
	Average	.12	.35	.35	.40	.03	.22	.08	—
REA	Low	.02	-.44	-.31	-1.34	.01	-.61	.23	—
	High	.8	.03	.36	.57	.97	-.61	.38	—
	Average	.51	-.16	.03	-.14	.44	-.61	0.30	—
YG	Low	-.37	.67	.22	.26	-.85	.18	—	—
	High	-.11	.67	.22	.26	-.85	.63	—	—
	Average	-.24	.67	.22	.26	-.85	.38	—	—
WW	Low	.48	.04	.33	-.02	.16	—	.17	—
	High	1.11	.49	.33	.81	.72	—	.17	—
	Average	.81	.59	.33	.40	.45	—	.17	—
YW	Low	.98	—	.25	.47	.71	—	—	.42
	High	.98	—	.25	.47	.71	—	—	.42
	Average	.98	—	.25	.47	.71	—	—	.42

CHAPTER THREE - MATERIALS AND METHODS

1. Phenotypic Data

Animals used in this study were part of a progeny testing program developed by 21st Century Genetics in Shawno, Wisconsin. Angus bulls in their program were bred by artificial insemination to a crossbred commercial cows located in South Dakota. All calves were born in a two-year period and raised under similar conditions. Calves were weaned at approximately 7 months, then backgrounded before entering the feedlot. Cattle were fed standard feedlot diets and slaughtered at 450 to 470 days of age. Over 800 ear-tissue samples of the calves sired by seven bulls (A through G) were collected on the farm and sent to the University of Arizona for further evaluation.

The following traits were taken from Angus Herd Improvement Record: weaning weight (WW), yearling weight (YW), hot carcass weight (HCW), adjusted 12th rib fat thickness (FT), kidney pelvic and heart fat percentage (KPH%), intramuscular fat or marbling score (MB) quality grade (QG), *longissimus* muscle or ribeye area (REA), and yield grade (YG). These traits are used by the American Angus Association for their Certified Angus Beef (CAB) carcass specification program. Approximately half of the original animals were slaughtered through the Certified Angus Beef Program where data were recorded.

1.1. Weaning and yearling weights

Two growth traits were measured: WW and YW. WW reflects the preweaning

growth rate of the calf. Weights were adjusted to the 205 days and for age of the dam according the Angus Herd Improvement Record.

YW or adjusted 365 day weight reflects the weight of a calf at a year of age (after it has been on feed for 160 days after weaning). YW was computed as follows:

Adjusted YW = (160×average daily gain from weaning to yearling) + adjusted WW

1.2. Carcass traits

HCW weight was obtained at slaughter stage before the final carcass washing and shrouding occurred. Carcass were adjusted to 480 days of age at slaughter according to the American Angus Association linear carcass adjustment factors (refer to www.angus.org/sireeval/carcass.htm).

The beef carcass was ribbed between the 12 and 13 ribs to assess QG and yield grade of the carcass. Quality grade is assigned by evaluating a number of factors (marbling, maturity, color, texture, firmness), which give consumers some indication of the palatability (tenderness, juiciness and flavor) of the meat. YG is an estimation of the expected yield of boneless, closely trimmed, retail cuts from the round, loin, rib and chuck. Ribbing exposes the ribeye, (*longissimus muscle*) so that marbling, fat thickness and ribeye area can be determined.

The amount of external fat on a carcass is measured over the ribeye muscle. FT was determined by measuring the depth of the fat perpendicular to the outside of the cross-sectioned ribeye muscle at the 12^{th} rib. In addition, this measurement was performed at a point ¾ the length of the ribeye muscle from the end of its chine bone.

The 12th rib FT measurement may be adjusted to reflect uneven amounts of fat in other areas of the carcass.

The amount of KPH fat percentage was evaluated subjectively. The weight of KPH was estimated for each side and it is expressed as a percentage of the HCW. KPH will range from 0.5% to 6% and averages 3%.

Marbling is interspersed fat within the muscle which aids in enhancing eating quality of meat. A beef carcass containing a high level marbling are eligible for higher quality grade (USDA, 1989). Marbling was evaluated by utilizing the amount of intramuscular fat within the *longissimus dorsi* muscle at the 12th rib. There are 10 degrees of marbling: abundant, moderately abundant, slightly abundant, moderate, modest, small, slight, traces, practically devoided, and devoid. Each degree of marbling was subdivided into percentage increments of 10% from 0 to 90% and was written as a number following a decimal point in complying with the American Angus Association. For the sake of ease of entering and analysis of data, MB was multiplied by 100. For example MB of score of 5.20 (small 20) was entered as 520.

QG is the relationship between marbling and maturity, with adjustment for inferior color, texture and firmness of lean. American Angus Association recognizes five QGs: prime, choice, select, standard, and utility (Table 3.1). In order to analyze the data, numerical values were assigned for each grade, starting from the utility to the prime grade as follow: 1, 2, and 3 for utility; 4, 5 and 6 for standard; 7,8 and 9 for the select; 10, 11 and 12 for choice; and 13,14, and 15 for the prime grade.

Yield grade is a numerical value from 1 to 5 based upon the yield of boneless closely trimmed, retail cuts from the round, loin, rib, and chuck. These four wholesale cuts constitute 75% of the weight, but about 90% of the value. Yield grade was calculated as follow:

YG = 2.5 + (2.5× adjusted FT at 12^{th} rib, inches) + (0.0038× HCW, pound) + (0.2×percentage KPH)-(0.32× REA, square inches).

The REA was determined by measuring the area (in square cm) using a grid or planimeter.

2. DNA Extraction

Sire DNA was extracted from semen samples and progeny DNA was extracted from ear-tissue samples. To extract DNA from semen, a defrosted semen straw was placed into a microcentrifuge tube, mixed with 1 ml of dH_2O, vortexed for 3-5 seconds, and centrifuged at 7000 rpm for 5 min or until a compact pellet formed. The supernatant was discarded by aspiration or drying, leaving a pellet as dry as possible. The pellet was resuspended into 500 µl buffer (10 mM EDTA, 10 mM Tris.HCl, pH 8, 50mM NaCl, 2% SDS), 7.5 µl of 20 mg/ml Proteinase K, and 20 µl DTT (1M in 10 mM sodium acetate, pH 5.2), and digested for 2 to 3 hours at 37°C. The tube was gently inverted to obtain a homogenous solution. A one volume of 5M NaCl was added to the solution. The tube was centrifuged at 12,000 g for 1 min. After transferring the aqueous phase into new microcentrifuge a tube, the DNA was precipitated by adding 2 volume of cold 100% ethanol. Finally the pellet was washed with 70% ethanol and dried using a speed

vacuum. Then the pellet was redissolved in dH_2O. The concentration of DNA was assayed by spectrophotometer in order to arrive to a final stock concentration of 250 ng/ul.

To extract DNA from ear tissue, one gram of tissue was collected at the ranch, frozen, and sent to the lab. The sample was cleaned, denuded of hair, sliced with a razor blade and placed in liquid nitrogen. After the frozen tissue was crushed thoroughly, it was transferred to 15 ml centrifuge tube, and 2-3 ml of digestion buffer (0.05M EDTA, 0.01M Tris.HCl, pH 8, 0.4M NaCl, 2% SDS), 10 µl of 50 µg/µl proteinase K, and 50 µl trypsin (100 µg/µl), and digested overnight or until the tissue was dissolved at 60°C. After tissue was dissolved, 2 µl RNase A (50 µg/µl) was added and the solution was digested at 50-60°C for 1 h. One third of volume of 5M NaCl was mixed with the digested sample to extract the DNA. At this point, the remaining steps were the same as for the semen samples.

The concentration of DNA on the extracted samples was measured with a spectrophotometer (Hitachi U-2000 spectrophotometer). The machine was turned on at least 30 min before start to warmup. The spectrophotometer was calibrated before each use using 50 µl HPLC quality grade water. The cuvette was cleaned with distilled water and detergent, if necessary. Care was taken to avoid any fingerprints on side of the cuvette. Three calibrations were made. These included system baseline, wavelength, and user baseline. DNA samples was measured by mixing 5 µl of sample with 50 µl of water. The absorption of sample was measured at different wavelength to assess purity and

concentration of DNA. Absorption at 260 nm is quantitative for relatively pure nucleic acids. If the reading at 260 nm is higher than one, then the sample needs further dilution. Absorption at 280 nm is quantitative for proteins. So, the ratio of absorption at 260 and 280 nm was used as an indicator for nucleic acid purity. Ratios of 1.8 to 1.9 and 1.9 to 2.0 indicate highly purified preparations of DNA and RNA, respectively. Moreover, two other absorptions were also used to gain more information about sample purity and contamination. Absorbance at 325 nm indicates particulate in the solution or dirty cuvette. Absorbance at 230 nm reflects contamination of the samples by peptide bonds or aromatic moieties such as protein and phenol.

Absorbance at 260 of 1.0 indicates 50 μg/ml of double stranded DNA, the double stranded DNA concentration in the sample was calculated as follows:

$$[\text{DNA } \mu g/\mu l] = \frac{(\text{reading@260} \times 50)(\text{total volume (ml)})}{\text{sample volume}(\mu l)}$$

3. Molecular Markers

Markers were selected to evaluate a 40 cM region of chromsome 2 and 30 cM region of chromsome 11 for associations with growth and carcass traits. The mirosatellites used in this study are mapped on the USDA Meat Animal Research Center (MARC) Genome map database (Kappes et al., 1997). For each microsatellite, the forward and the reverse sequence of the oligonucleotide primers were synthesized (Genosys Biotechnologies, Inc. The Woodlands, TX) and resuspended in HPLC grade water to a final concentration of 20 µM.

PCR-RFLP was used to determine β-lactoglobulin genotype (LGB locus), the only non-microsatellite marker. This marker is located on chromosome 11 at 108.7 cM with two allele variants A and B. The primers used require an annealing temperature of 55°C and 1.5 mM of Magnesium (Table 3.3.). The characteristics of the informative markers on chromosome 2 and chromosome 11 are summarized in Table 3.2. and Table 3.3. respectively.

3.1. PCR preparation of markers

Based on 12.5 μl reaction, 12 μl of master mix (MM) was prepared. Three controls were used in this experiment; a reagent control which contained all components of the reaction except template DNA; a negative DNA control which did not contain target sequences and a positive control of DNA containing target sequences that were known to anneal to the primers. The MM contained water, 10X *Taq* polymerase buffer magnesium free, dNTP 200 μmM each (dATP, dGTP, dCTP, dTTP) primers (20 pmol/ul) $MgCl_2$ (1to 2 mM) and *Taq* DNA polymerase 2.5 units/100 ul reaction (Promega). The MM was vortexed before distributing it in each PCR tube (0.5 ml). Template DNA (100 ng) was added to each MM, then each tube was vortexed gently. The content of each tube was overlaid by mineral oil to avoid evaporation of the sample. After spinning at 4,000×g, samples were amplified, using the Perkin-Elmer 480 thermocycler, for 30 cycles; 95 for 3 min, 52 to 60°C for 1 min (depending on the primers), and 72°C for 1 min, followed by 29 cycles at 94°C for 1 min, 52 to 60°C for 1 min (depending on the primers), and 72 °C for 1 min with final extension of 10 min at

72°C.

4. Detection of PCR Products

Molecules of linear double-stranded DNA migrate through gel matrices at rate that are inversely proportional to the $\log_{10}$ of the number of base pairs. Larger molecules migrate more slowly than smaller molecules. Verification of the success of PCR (about 20% of the samples) was checked on 1.5% gel containing 0.5 to 1μg/ml ethidium bromide, a fluorescent dye that intercalates between stacked base pairs. The amplified DNA fragment can then be visualized and photographed under UV light.

The procedure consisted of placing a comb (8-24 well) in a gel caster and making sure that the comb did not touch the bottom of the caster by using a spacer to set the gap. For the mini-gel, 35 ml of 1XTE buffer was transferred into 125-ml flask. Then 0.5 g of agarose was transferred into the flask containing the buffer. The agarose was dissolved in the microwave oven. From time to time, the buffer was swirled to dissolve the agarose completely. Ethidium bromide was added just before the agarose dissolved completely. The agarose was then cooled to approximately 55°C and poured into the gel caster for 15 to 20 min until solidified. Five μl of PCR product was mixed with 1μl of 6X of desired loading dye (xylene cyanol or bromophenol blue) depending on the size of the amplified product, and slowly loaded into the well. A DNA of known size was loaded into a well on the right side of the gel to determine or confirm the size of the amplified PCR samples. The gel was carried over at 75 volts for about 20 min or until the loading dyes migrated the appropriate distance through the gel. The DNA bands were visualized under

the UV light using the Alpha Innotech transilluminator. The gel picture was taken using a digital imaging system, the AlphaImager2000 (Alpha Innotech, San Leandro, California). This system consists of a high-performance CCD camera attached to a digital computer as well as AlphaEAse image processing and analysis software. The gel pictures can either be printed using special thermal paper or saved and forwarded to the appropriate graphic software.

4.1. Preparation and loading of polyacrylamide gels

Polyacrylamide gels have the power to separate molecules of DNA whose lengths differ by as little as 1 bp. The polyacrylamide gel apparatus in the laboratory consists of vertical gel boxes where two glass plates are separated by two spacers at each side parallel to the edges so that the poured gel is 1mm thick and 20 cm long. Agarose was used to seal the edges. Thirty ml of 8 to 10% acrylamide solution was prepared from a stock of 40% then 200 µl of 10% ammonium persulfate was added. After the solution was deaerated (avoid air bubbles when pouring), 20 µl of TEMED (N,N,N',N'-tetramethylethylenediamine) was added and the solution was drawn into 30 ml serological pipette and expelled carefully into the space between the glass plates at the top. Immediately after the space was filled, the 28-well comb was carefully inserted and the acrylamide polymerized for at least 1 hour at room temperature. After polymerization, the comb was removed, and immediately the wells were rinsed with dH_2O and the gel was submerged into the electrophoresis tank containing 10 l 1X TBE buffer. This tank was equipped with constant temperature immersion circulator,

refrigerated circulating bath, and varistaltic circulating pump. The dye and DNA samples were loaded into the wells using special long gel loading pipet tips (size 1-200 μL) as quickly as possible. A 6× blue and orange marker dye was loaded into the last well on the right. Electrophoresis was conducted at constant 300 volts and safe operating limits using digital series 90-power supply (Fisher, Pittsburg, PA). The current was stopped when the marker dyes migrated 90 mm from the bottom of the well. The gel was removed and submerged in staining solution (0.5 μg/ml ethidium bromide in 1X TBE) for 30 min. Visualization of DNA bands and photographs are similar as previously discussed for agarose.

Nondenaturing polyacrylamide gels were used to determine sire heterozygosity and progeny genotypes for microsatellites. Polymorphism for the number of repeats in the tandem array results in PCR products of varying lengths (more than one band different by few bp) which are scored as allelic polymorphisms.

5. PCR Optimization for Microsatellites

PCR were optimized for each microsatellite used in this study in order to achieve an adequate reproducibility of the banding patterns. Banding patterns were affected by primer, template, and Mg^{2+}concentration, as well as of annealing temperature, with the effect of the latter two factors most pronounced. For example, the microsatellite *BMS655* was poorly amplified at 1 mM of Mg^{2+}, but at 1.5 mM, the reaction was optimized with significantly more product produced. Higher annealing temperature, close to the melting temperature (Tm) of the primers, produced more specific binding of the primers to the

complementary microsatellite repeats and eliminated spurious bands due to mismatch priming (the cross-annealing of primers to non-target sequences), a common problem in PCR amplification of microsatellites. Weising and colleagues (1995) found that primer and magnesium concentration as well as the annealing temperature had a considerable influence on the quality of banding patterns of microsatellite primed PCR. Koreth et al. (1996) recommended that the annealing temperature for microsatellite PCR should be as high as possible, even higher than the T_m of the primers, to avoid mispriming. A technique called 'hot start' PCR can be useful for PCR with primers lacking specificity because of different T_m of the forward and reverse primer or poor quality template (degraded). In the procedure, $MgCl_2$ is omitting from the PCR reaction mixture; then added only after the mixture has reached a temperature of 85°C.

6. PCR-RFLP Genotyping

The β-lactoglobulin genetic variants were typed using PCR-RFLP techniques. Specific primers were used to amplify 247-bp fragment of the β-lactoglobulin gene (89 base pair of exon IV and 158 bases of the intron IV). The PCR product was digested with 25 units of HaeIII. The digested PCR product was run on 4% TBE/EthBr agarose gels and the DNA bands were visualized under UV light. The PCR-RFLP marker for β-lactoglobulin gene was encoded as two allele system 1 and 2 which correspond to the two allele variant A and B. This setting yielded 3 possible genotypes 11, 12 and 22.

7. Microsatellite Genotyping Strategy

Each microsatellite genotype was tested on every sire to assess heterozygosity. If

a sire was heterozygous for a marker, then the microsatellite was amplified for each progeny to determine their genotypes. Sire heterozygosity and genotype determination were interpreted by visual examination of a digital image of the acrylamide gel. For convenience, all microsatellite markers were coded as a three allele system: alleles 1 and 2 corresponding the two alleles of the founder sire, whereas all other alleles encountered corresponding to the alleles of the dams were pooled as allele group 3. This arrangement resulted in 5 possible genotypes: 11, 12, 13, 22, and 23. Genotypes were independently scored twice for each marker and discrepancies identified. When genotype differences could not be resolved between two independent scorers after reamplification and rescoring of the PCR product, data were excluded from the analysis.

Genotypes were entered in spreadsheet software where the gene frequencies of alleles 1, 2 and 3 were calculated within each family. Marker allele frequencies, required for the likelihood computation were determined from the progeny for each pedigree as follows:

$$P_1 = \frac{(1 - P_3)n_{11}}{(n_{11} + n_{22})}$$

$$P_2 = \frac{(1 - P_3)n_{22}}{(n_{11} + n_{22})}$$

$$P_3 = \frac{(n_{13} + n_{23})}{n+2}$$

with n_{xy} being the number of daughters in the pedigree with genotype "xy", n the total number of daughters in the pedigree and P_i the frequency of marker allele i.

8. Data Collection and Analyses

Each of the seven half-sib families were analyzed independently for each trait by chromosome. Within a pedigree, the linkage groups were sequentially tested for the presence of linked QTL affecting each trait.

All marker linkage analyses were performed using the interval mapping program ANIMAP (Georges, 1995). ANIMAP consists of 4 separate programs, LODTABLE, MAKEMAP, MAPQTL, and CHECKPED designed to perform genetic linkage analysis among half-sib pedigrees.

MAPQTL was used to estimate the probability that a quantitative trait was segregating with markers using phenotypic information provided. Probabilities are estimated for 2 cM intervals along the chromosome, testing a hypothesis that a QTL is segregating with genotypic marker data within a founder sire's offspring, versus a null hypothesis that no QTL is segregating with the genetic marker data. The program, ANIMAP uses LOD scores as the test statistic (Morton, 1955). The LOD score corresponds to the $\log_{10}$ of a likelihood ratio: likelihood of the genotypic data under the alternative hypothesis (H_1) of linkage between the two loci at given recombination rate ($\theta<0.5$), divided by the likelihood of the genotypic data under the null hypothesis (H_0) of independent assortment of both loci ($\theta = 0.5$) (Lander & Botstein, 1989), so the likelihood ratio (LR) statistic (Lathrop et al., 1991) is:

$$LR = \frac{[\theta^r(1-\theta)^{n-r}]}{0.5^n}$$

where r is the number of recombinants, and n is the numbers of informative meiosis, and θ is the recombination frequency. LOD score between 2.0 and 3.0 was considered suggestive of linkage (Botstein at al., 1980).

The ANIMAP programs assumes that phenotypes are approximately normally distributed and that the QTL effect is additive. Three input files are required to run MAPQTL: a pedigree file, containing the pedigree structure and genotype information with the founder sire at the head of the pedigree, a data file containing information specific to the loci and to the analysis, and a phenotype file containing the phenotypes for the traits to be analyzed. The program commences analysis 10-cM outside the first marker, and selects steps of 2 cM through the map until it attains a position of 10 cM outside the last marker locus and estimates LR at each location. The program also estimates the effect size of the QTL and the variance of the segregating populations. All of the above results are furnished on the output file default named mapQTL.out. LOD scores can be added together for independent families provided that they are at the same location (Archibald and Haley, 1998).

Table 3.1. Association of USDA QG system, amount of marbling and subsequent numerical score

Quality Grade	Amount of Marbling	Numerical Score
Prime +	Abundant	10.0 - 10.9
Prime	Moderately abundant	9.0 - 9.9
Prime -	Slightly abundant	8.0 - 8.9
Choice +	Moderate	7.0 - 7.9
Choice	Modest	6.0 - 6.9
Choice-	Small	5.0 - 5.9
Select	Slight	4.0 - 4.9
Standard	Traces	3.0 - 3.9
Standard	Practically devoid	2.0 - 2.9
Utility	Devoid	1.0 - 1.9

Table 3.2. Characteristics of the informative markers on chromosome 2.

Name	MP[a] (cM)	AT[b] (°C)	Mg[c] (mM)	Size[d] (base pairs)	Alleles[d]
BMS2782	41.7	56	1.5	181 - 191	5
RM356	51.9	56	1.5	113 - 127	5
BMS2024	55	54	2	97 - 123	9
BMS1126	56.3	56	1.5	122 - 158	13
ILSTS082	58.6	56	2	104 - 116	7
BM2808	59.9	56	1.5	149 - 170	6
RM041	69	56	1.5	74 - 94	7
BMS2626	69.7	56	1.5	168 - 176	5
TGLA226	80	56	1.5	129 - 151	12

[a]Linkage map position
[b]Annealing temperature of the reverse and the forward primer of the microsatellite or the gene segment to be amplified.
[c]Magnesium concentration
[d]USDA-MARC genome database information (Kappes et al, 1997)

Table 3.3. Characteristics of the informative markers on chromosome 11.

Name	MP[a] (cM)	AT[b] (°C)	Mg[c] (mM)	Size[d] (Base pairs)	Alleles[d]
BMS989	85.4	56	2	90 - 114	11
BM746	89.5	54	1.5	146 - 152	4
BMS607	105.3	58	1.5	133 - 169	14
RM051	105.3	60	1	100 - 112	4
ILSTS028	105.3	58	2	130 - 160	8
ILSTS045	106.4	56	1	172 - 200	8
BMS352	107	58	1.5	90 - 108	6
BMS655	108.7	55	1.5	131 - 157	7
LGB	108.7	55	1.5	247	2
BMS2208	113.1	56	2	109 - 127	8
HEL13	114.5	50	2	177 - 197	8

[a]Linkage map position
[b]Annealing temperature of the reverse and the forward primer of the microsatellite or the gene segment to be amplified.
[c]Magnesium concentration
[d]USDA-MARC genome database information (Kappes et al, 1997)

CHAPTER FOUR- RESULTS

1. Marker Informativeness

All markers were tested for polymorphism in seven sires. *BMS1350*, *RM379*, and *BM6491* (chromosome 11) were monomorphic in all sires. The markers *ILSTS028* (chromosome 11), *RM051* (chromosome 11) *BMS2208* (chromosome 11), *RM356* (chromosome 2), *BMS2626* (chromosome 2), and *TGLA226* (chromosome 2) were polymorphic only with sire B, sire G, sire E, sire A and sire C respectively. The remaining markers were informative in at least two families. Table 4.1 summarize sire heterozygosity for markers located on chromosome 2 and chromosome 11. Data from marker BMS352 (chromosome 11) for sire A were excluded from the analysis due to the difficulty in genotyping the alleles.

2. QTL Mapping

The linkage groups were sequentially tested for the presence of linked QTL affecting each trait independently for each sire group using all the informative markers. The LOD scores and estimated effect size of the QTLs were obtained.

2.1. QTL for FT, KPH, MB, QG, REA, and YG on chromosome 2

LOD scores, estimated effect sizes, family phenotypic means and standard deviations by family for FT are reported in Table 4.2. LOD score never reached a significant threshold for rejecting the null hypothesis of no QTL segregating for FT with

the markers tested on chromosome 2. The total LOD score was 1.69. However, in family sire E, a LOD score of 1.02 was obtained at *BM2808* map position, suggesting that there is some indication of effects on backfat thickness. The size of the effect within this family was 0.74 in phenotypic standard deviation units (SDP).

Results in Table 4.3, revealed a strong evidence of QTL with a major impact on KPH percentage. The LOD score was maximized in the interval flanked by *BM2808* and *RM041* and approached a LOD score of 5 at 64 cM in sire C family group. For this sire group, the maximum likelihood estimates of the QTL effect was 1.45 SDP. The mean of KPH from offspring that inherited marker allele 1 was 2.35 (n=14) and marker allele 2 was 1.81 (n=13). The offspring heterozygous for sire alleles (1,2) and therefore uninformative had a KPH mean of 2.15 (n=10).

The results did not indicate any significant QTL for MB segregating with markers in the 40 cM region of chromosome 2. The total LOD scores summed over the 7 families were only 1.13. None of the sire group yielded LOD score of at least 1 (Table 4.4).

For QG trait, the results followed a similar trend as for MB trait, although the total LOD score approached 2. Again, none of the families yielded LOD score of a least 1 (Table 4.5). These results were not surprising, since QG was determined mostly by MB score.

A QTL associated with REA may be segregating on chromosome 2 within these families (Table 4.6). The location of the QTL was estimated at 46 cM flanked by *BMS2872-BMS2024*, midway between the markers. In sire group D family, a LOD score

of 1.75 was reached at 46 cM. At this position, the maximum likelihood estimate of effect size was 1.71 in SDP units. For sire group D family, marker *BMS2024* (55 cM) is the closest informative marker to the position where the LOD score is the highest. At this position, the pedigree information of the sire D indicated that 9 progeny inherited allele 1 from their sire and had a phenotypic mean of 75.1 cm^2 for REA. Five individuals inherited the allele 2 from their sire and their phenotypic mean was 86.1 cm^2 of REA. Only one offspring had a genotype the same as the sire.

The microsatellite marker *RM041* may be linked to genes influencing YG. A LOD score of 1.32 was calculated in sire C family group. Although the summed LOD score did not quite reach the statistical significance, the *RM041*-linked QTL had an effect of 0.9 SDP (Table 4.7).

The formula used to compute the yield grade includes HCW, FT, REA, and KPH (see materials and methods section), but HCW and FT influence YG more than KPH and REA . This partially explains why the YG was not as significant as the KPH percentage QTL in the family group sire C. None of the markers used on chromosome 2 were significant for FT and HCW traits within the sire C family.

2.2. QTL for FT, KPH, MB, QG, REA, and YG on chromosome 11

The results of the QTL interval analysis for FT are presented on Table 4.8. A total LOD value of 3.1 was reached at the optimum location of 113.2 cM (*BMS2208*). At that location, the sire group D family had the highest LOD score of 2.28. For that family, the likelihood estimate of FT-QTL effect was 1.79 SDP at 113.2 cM. Sire D was not

heterozygous for the marker *BMS2208,* however, he was heterozygous for the *LGB* locus located at 4.5 cM away from BMS2208 (108.7 cM). The mean of progeny inheriting the A alleles were 0.91 (n=1) and the mean of progeny inheriting the B alleles were 1.6 (n=4). The mean of FT of heterozygous progeny for sire allele AB was 1.04 cm (n=10).

There was evidence for a QTL for KPH on chromosome 11 located in the region near the marker BMS989 (85.4 cM), the LOD score was 3.56. Two families showed LOD scores greater than 1: the sire E had a LOD score of 2.0 and the sire G had a LOD score of 1.4. (Table 4.9).

The estimated effects for the KPH in the sire E and sire G families were 1.11 and 0.86 SDP, respectively. For sire group E, the mean of KPH for individuals inheriting the allele 1 from their sire (n=14), and allele 2 from their sire (n=11) were 2.89 and 2.14, respectively. The heterozygous individuals (inherited allele 1 and 2 from their sire) had a KPH mean of 2.43 (n=7). For the sire group G the mean of KPH for individuals inheriting allele 1 of their sire (n=10), allele 2 of their sire (n=16), and both allele 1 and 2 of their sire (heterozygous individuals, n=13) were 2.4, 2, and 2.19, respectively.

A QTL influencing MB trait may be located near the marker *HEL13* (total LOD = 2.54). Sire group E resulted in LOD score of 1.31, the largest among the 7 families used in the analysis.(Table 4.10). The maximum likelihood estimate of the intramuscular fat effect for the family Sire E was 0.86 SDP. The mean MB score of offspring inheriting marker allele 1 was 630 (modest[30]) comprising 2 animals, and marker allele 2 was 537 (small[30]) with a total of 17 individuals. The heterozygous progeny for sire alleles (1,2)

was of 630 (modest[30]) constituting 13 individuals.

Maximum LOD scores, the estimated effect size, with the phenotypic means, and standard deviation for QG are presented in Table 4.11. The optimum map position for the highest significance was 114.5 cM at the marker *HEL13*, which is the location of the QTL associated with MB trait previously. The total summed LOD scores over all the families were 2.82; however none of the families reached a LOD of 1 although for the sire group E the LOD score approached 1 (0.91). The overall QTL mapping results for QG were similar to the MB trait. These findings were also true for QTL mapping on chromosome 2 as discussed above.

Linkage analysis did not detect QTL for REA on chromosome 11 (Table 4.12). The total LOD scores were very low (0.50) excluding the presence of any region within the 35 cM region of chromosome 11 studied that could be segregating for REA in the 7 sire groups used.

For YG, the total LOD score obtained was 2.39, suggesting a QTL may be segregating that influences the components of yield grade (Table 4.13). The results of the QTL mapping for YG showed the same trends as for FT. For both traits, the sire group D family showed a LOD score over 2, close to marker HEL13 (114.5 cM). The likelihood estimate of YG effect at 114.5 cM and calculated on SDP units was 1.70. Since the YG is influenced by multiple traits, FT traits seems to be responsible for the effects seen for YG.

2.3. QTL for weights (HCW, WW and YW) on chromosome 2

Maximum LOD scores and their effect sizes for HCW trait analyses with markers on chromosome 2 are presented in Table 4.14. A QTL for HCW may be segregating (total LOD=3.75) in the region near *BMS2024* (55 cM). The sire group B had a LOD score of 1.61. The likelihood estimate of HCW effects were 0.96 SDP. Offspring of sire B that inherited the sire's allele 1 (n=12) had a mean of 338.5 kg, heterozygous progeny (n=8) had a mean of 336.8 kg, and progeny inheriting the sire's allele 2 (n=12) had a mean of 310 kg.

For the family of sire G, although the LOD score did not quite reach the value of 1, the likelihood estimate of the magnitude of the HCW effects was larger than 0.5 SDP proposing that there is also some indication of effects on HCW on chromosome 2 in the sire G family. The HCW is influenced by the percentage of fat in the carcass, and a QTL for KPH percentage was found in the region between *BM2808* and *RM041* on chromosome 2. This region is not far from the location where mapping QTL for HCW yielded the highest LOD score implying that fat percentage may also be responsible for significance detected for HCW.

Markers on chromosome 2 were not associated with QTL for WW. However the sire G family showed a noticeable effect (0.65 SDP) in the region near the marker *BMS1126* on chromosome 2 (Table 4.15). The WW reflects the weight of the animal at weaning which is influenced by the body composition including fat. Once again, it seems that on chromosome 2 and at approximately the same region as for KPH, and CW (60

cM), a QTL segregating for fat may be present.

Marker *BMS1126* (56.3 cM) on chromosome 2 was linked with a QTL for YW (Table 4.16) . The total LOD score was 2.50 and sire group G had a LOD score of 1.46, with maximum likelihood estimate of yearly weight effects of 0.90 SDP.

2.4. QTL for weights (HCW, WW, WY) on chromosome 11

Results in Table 4.17 indicated none of the markers was significantly linked to QTL associated with HCW in the 35 cM region of chromosome 11. The total LOD scores did not reach the value of 2 and none of the family yielded a LOD score of 1.

Concerning the WW traits, data were similar as for the HCW, the total LOD score was not high enough to reject the null hypothesis of no QTL segregating with the markers tested on chromosome 11 (Table 4.18). The total LOD score summed over all the 7 families was 1.7. Additionally, none of the families had a LOD score of 1.

Regarding the YW traits, the results did not illustrated any significant QTL segregating with the markers tested in the 35 cM region of chromosome 11 covered (Table 4.19). Among the 7 families tested, the sire group D family presented some indications of effects on YG. The likelihood estimate of YG effect was 1.11 SDP. But this effect did not reach statistical significance (LOD=0.74).

Table 4.1. Sire heterozygosity for markers on chromosome 2 and 11

Sire	no. of progeny	Heterozygous markers	
		Chromosome 2	**Chromosome 11**
Sire A	14	*BMS2872, BMS2024, BMS1126, RM041, and BMS2626*	*BMS989, BM746, BMS607, ILSTS045, BMS352, BMS655, and LGB*
Sire B	32	*BMS2872, ILSTS082, and RM041*	*ILSTS028, ILSTS045, and BMS352*
Sire C	37	*BMS1126, BM2808, RM041, and TGLA226*	*BM746, RM051, ILSTS045, and BMS655*
Sire D	15	*BMS2024, and BMS1126*	*BMS989, ILSTS045, and LGB*
Sire E	32	*BMS2872, RM356, BMS2024, BMS1126 BM2808, and RM041*	*BMS989, BMS607, ILSTS045, BMS655, LGB, and HEL13*
Sire F	25	*BMS2872, BMS2024, ILSTS082, BM2808, and RM041*	*BMS607, BMS655, and HEL13*
Sire G	40	*BMS2024, BMS1126, BM2808, and RM041*	*BMS989, BMS2208, and HEL13*

Table 4.2. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for fat thickness (FT, cm) on chromosome 2

Location: chromosome 2 near *BM2808* at 59.9 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire B	0.47	0.19	1.13	0.33	0.59
Sire C	0.14	0.10	1.77	0.40	0.26
Sire D	0.05	0.01	1.18	0.38	0.26
Sire E	1.02	0.23	1.61	0.30	0.74
Sire F	0.01	0.02	1.64	0.22	0.11
Sire G	0.00	0.01	1.70	0.37	0.01
Total	1.69	AVG[e] a = 0.11		AVG a/SD= 0.54	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.3. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for kidney pelvic and heart fat percentage (KPH,%) on chromosome 2

Location: chromosome 2, flanked by *BM2808* and *RM041*, at 64 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.07	0.18	2.38	0.55	0.32
Sire B	0.08	0.12	2.11	0.53	0.22
Sire C	4.97	0.71	2.11	0.49	1.45
Sire D	0.29	0.32	2.37	0.48	0.66
Sire E	0.19	0.26	2.53	0.68	0.38
Sire F	0.18	0.19	2.13	0.47	0.39
Sire G	0.05	0.06	2.17	0.42	0.15
Total	5.81	AVG[e]a = 0.26		AVG SDP= 0.51	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.4. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for marbling score (MB) on chromosome 2

Location: chromosome 2 near *RM041*, at 69 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.41	41.0	535.4 small^{30}	57.7	0.71
Sire B	0.01	6.2	583.8 small^{80}	90.1	0.07
Sire C	0.29	40.3	631.5 modest^{30}	104.1	0.39
Sire D	0.01	10.1	534.7 small^{30}	107.8	0.09
Sire E	0.29	34.5	605.6 modest^{0}	85.3	0.40
Sire F	0.11	36.1	623.8 modest^{20}	103.9	0.35
Sire G	0.01	3.4	555.4 small^{50}	98.9	0.03
Total	1.12	AVG[e] a = 24.5		AVG SDP= 0.29	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.5. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for quality grade (QG) on chromosome 2

Location: chromosome 2 near *RM041*, at 69 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.53	0.64	11.85 (choice+)	0.80	0.80
Sire B	0.39	0.45	12.31 (choice+)	0.97	0.46
Sire C	0.48	0.60	12.89 (choice+)	1.22	0.49
Sire D	0.01	0.22	11.67 (choice+)	1.50	0.15
Sire E	0.30	0.44	12.50 (choice+)	1.08	0.41
Sire F	0.20	0.54	12.79 (choice+)	1.14	0.47
Sire G	0.01	0.05	11.87 (choice+)	1.42	0.03
Total	1.92	AVG[e] a = 0.42		AVG SDP= 0.40	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.6. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for ribeye area (REA, cm^2) on chromosome 2

Location: chromosome 2, 4.3 cM from *BMS2872* at 46 cM [a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.42	8.28	72.26	11.15	0.74
Sire B	0.27	3.15	73.80	7.29	0.43
Sire C	0.01	0.58	80.46	11.15	0.05
Sire D	1.75	12.84	78.76	7.51	1.71
Sire E	0.13	2.44	80.13	6.41	0.38
Sire F	0.21	3.09	77.05	7.18	0.43
Sire G	0.01	0.81	78.20	6.89	0.12
Total	2.8	AVG[e] a = 4.46		AVG SDP= 0.55	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.7. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for yield grade (YG) on chromosome 2

Location: chromosome 2 near *RM041*, at 69 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire B	0.03	0.05	3.14	0.32	0.16
Sire C	1.32	0.72	3.51	0.80	0.90
Sire E	0.01	0.04	3.45	0.43	0.09
Sire F	0.43	0.25	3.59	0.34	0.73
Sire G	0.04	0.07	3.46	0.54	0.07
Total	1.83	AVG[e] a = 0.23		AVG SDP= 0.39	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.8. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for fat thickness (FT, cm) on chromosome 11

Location: chromosome 11 near *BMS2208*, at 113.2 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire B	0.01	0.01	1.13	0.33	0.03
Sire C	0.04	0.06	1.77	0.40	0.15
Sire D	2.28	0.68	1.18	0.38	1.79
Sire E	0.33	0.14	1.61	0.30	0.47
Sire F	0.01	0.01	1.63	0.22	0.05
Sire G	0.43	0.18	1.70	0.37	0.49
Total	3.1	AVG[e] a = 0.18		AVG SDP= 0.5	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.9. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for kidney pelvic and heart fat percentage (KPH,%) on chromosome 11

Location: chromosome 11 near *BMS989*, at 85.4 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.13	0.22	2.36	0.53	0.40
Sire C	0.02	0.06	2.11	0.49	0.13
Sire D	0.01	0.05	2.37	0.48	0.11
Sire E	2.00	0.76	2.53	0.68	1.11
Sire G	1.40	0.36	2.17	0.42	0.86
Total	3.56	AVG[e] a = 0.29		AVG SDP= 0.52	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.10. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for marbling score (MB) on chromosome 11

Location: chromosome 11 near *HEL13*, at 114.5 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.12	40.0	552.1 (small^{50})	83.7	0.48
Sire B	0.01	4.6	583.8 (small^{80})	90.1	0.05
Sire C	0.01	7.9	631.4 (modest^{30})	104.1	0.08
Sire D	0.42	80.5	534.7 (small^{30})	107.8	0.75
Sire E	1.31	73.4	605.6 (modest^{0})	85.3	0.86
Sire F	0.41	56.4	620.4 (modest^{20})	103.1	0.55
Sire G	0.26	34.5	555.4 (small^{50})	98.8	0.35
Total	2.54	AVG[e] a = 42.5		AVG SDP= 0.45	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.11. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for quality grade (QG) on chromosome 11

Location: chromosome 11 near *HEL13*, at 114.5 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.32	0.71	12.00 (choice+)	0.96	0.74
Sire B	0.08	0.25	12.31 (choice+)	0.97	0.25
Sire C	0.06	0.24	12.89 (choice+)	1.22	0.20
Sire D	0.77	1.49	11.67 (choice+)	1.50	0.99
Sire E	0.91	0.78	12.50 (choice+)	1.08	0.72
Sire F	0.27	0.51	12.76 (choice+)	1.13	0.45
Sire G	0.41	0.62	11.87 (choice+)	1.42	0.43
Total	2.82	AVG[e] a = 0.66		AVG SDP= 0.54	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.12. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for ribeye area (REA, cm^2) on chromosome 11

Location: chromosome 11 near *BM746* at 89.6 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.37	7.31	71.43	11.15	0.65
Sire C	0.03	1.54	80.46	11.15	0.14
Sire D	0.02	1.36	78.76	7.51	0.18
Sire E	0.00	0.10	80.13	6.41	0.02
Sire G	0.08	2.03	78.20	6.89	0.3
Total	0.5	AVG[e] a = 2.47		AVG SDP= 0.26	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.13. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for yield grade (YG) on chromosome 11

Location: chromosome 11 near *HEL13*, at 114.5 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire B	0.04	0.06	3.14	0.32	0.19
Sire C	0.01	0.06	3.51	0.80	0.08
Sire D	2.02	0.63	3.20	0.37	1.70
Sire E	0.06	0.08	3.45	0.43	0.18
Sire F	0.10	0.10	3.60	0.34	0.29
Sire G	0.16	0.15	3.46	0.54	0.30
Total	2.39	AVG[a] a = 0.18		AVG SDP= 0.46	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.14. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for hot carcass weight (HCW, kg) on chromosome 2

Location: chromosome 2 near *BMS2024*, at 55 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.32	18.7	308.8	28.9	0.65
Sire B	1.61	23.3	327.6	24.1	0.96
Sire C	0.40	11.0	335.8	24.3	0.45
Sire D	0.02	5.1	323.3	32.2	0.16
Sire E	0.26	10.8	339.2	28.6	0.38
Sire F	0.29	12.1	337.8	26.4	0.46
Sire G	0.85	17.7	330.0	29.0	0.61
Total	3.75	AVG[e] a = 14.1		AVG SDP = 0.52	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.15. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for weaning weight (WW, kg) on chromosome 2

Location: chromosome 2 near *BMS1126*, at 56.3 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.01	1.4	235.5	34.7	0.04
Sire B	0.01	1.5	240.2	32.9	0.05
Sire C	0.01	1.6	250.0	20.3	0.08
Sire D	0.06	6.20	234.5	24.5	0.25
Sire E	0.25	13.8	251.9	33.3	0.41
Sire F	0.10	4.8	241.5	16.7	0.29
Sire G	0.73	18.1	255.1	27.8	0.65
Total	1.17	AVG[e] a = 6.8		AVG SDP= 0.25	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.16. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for yearling weight (YW, kg) on chromosome 2

Location: chromosome 2 near *BMS1126*, at 56.3 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.44	51.8	394.9	70.1	0.74
Sire B	0.03	5.6	434.6	37.9	0.15
Sire C	0.01	1.1	463.4	46.5	0.02
Sire D	0.02	6.4	411.5	49.3	0.13
Sire E	0.22	22.6	476.0	58.3	0.39
Sire F	0.33	16.8	459.9	31.2	0.54
Sire G	1.46	56.6	454.9	63.2	0.90
Total	2.5	AVG[e] a = 23.0		AVG SDP= 0.41	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.17. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for hot carcass weight (HCW, kg) on chromosome 11

Location: chromosome 11 near *RM051*, at 105.3 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.51	21.4	307.5	28.2	0.76
Sire B	0.37	11.1	327.6	21.1	0.46
Sire C	0.03	3.1	335.8	24.3	0.13
Sire D	0.68	30.0	323.3	32.2	0.93
Sire E	0.10	7.0	339.2	28.6	0.24
Sire F	0.02	3.0	337.8	25.9	0.11
Sire G	0.05	6.0	330.0	29.0	0.21
Total	1.76	AVG[e] a = 11.7		AVG SDP = 0.41	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.18. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for weaning weight (WW, kg) on chromosome 11

Location: chromosome 11 near *RM051*, at 105.3 cM[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.41	28.7	228.7	42.0	0.68
Sire B	0.16	11.1	240.2	32.9	0.34
Sire C	0.68	31.4	241.6	49.8	0.63
Sire D	0.01	1.5	234.5	24.5	0.06
Sire E	0.14	10.4	251.9	33.3	0.31
Sire F	0.15	5.8	241.5	16.7	0.35
Sire G	0.15	31.4	247.9	54.5	0.58
Total	1.70	AVG[e] a = 6.8		AVG SDP= 0.25	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

Table 4.19. Summary of LOD scores, QTL association with marker, and estimated effect sizes with phenotypic means and standard deviations for yearling weight (YW, kg) on chromosome 11

Location: chromosome 11, 5.5 cM from *BM746* at 95 cM of MP[a]					
Sire	**LOD**	**a[b]**	**Mean**	**SD[c]**	**SDP[d]**
Sire A	0.14	31.7	388.3	71.7	0.44
sire B	0.00	0.6	434.6	37.9	0.01
Sire C	0.09	28.0	446.8	98.7	0.28
Sire D	0.74	54.9	411.5	49.3	1.11
Sire E	0.02	6.2	476.0	58.3	0.11
Sire F	0.00	0.5	459.9	31.2	0.01
Sire G	0.18	51.7	441.3	105.1	0.49
Total	1.16	AVG[e] a = 24.8		AVG SDP= 0.35	

[a]linkage map position
[b]estimated effect size
[c]phenotypic standard deviation
[d]estimated effect size expressed in standard deviation units.
[e]average

CHAPTER FIVE- DISCUSSION

The reasoning behind the choice of growth and carcass traits for the study is three-fold: 1) these traits are important in achieving the demand for the high quality meats with best efficiency of production; 2) these traits are moderately to highly heritable (Arnold et al., 1991); 3) carcass traits cannot be measured in live animals.

Heritability of a trait is a function of the magnitude of the effects of genes controlling the trait and the number and frequency of the alleles in the population (Falconer and Mackay, 1996). Consequently, if a trait has a high heritability and genetic variation is controlled by a small number of genes, these genes will have relatively large effects and the QTLs underlying these effects can be detected and mapped with the aid of the genetic markers. When genetic variation is controlled by a large numbers of genes, each with a small effect, it is also possible to detect the QTL underlying these effects. However, larger numbers of markers and family sizes are needed to reduce the type I error rate.

All the marker-linked QTLs detected in this study had an estimated effect more than .75 phenotypic standard deviation. However, the families used in this study had a relatively small sizes (15-40). For QTL with moderate effects, there are two consequences of detecting QTLs with sample size of low power. First, not all QTL will be detected. Second, the strength of those that are detected will be most likely overestimated (Manly and Olson, 1999) . For example Lynch and Walch (1998) stated

that QTL detected in an experiment with 10% power will be overestimated an average of fourfold. Thus, in this study, it is most likely the QTL effects detected were overestimated, unless it is a major gene.

1. QTLs on Chromosome 2

In the present study, there was evidence for QTL on chromosome 2 linked to molecular markers that accounted for a significant fraction of the genetic variation in Angus beef fat as measured by KPH percentage. The chromosomal regions flanked by the microsatellite loci *BM2808* and *RM041* were strongly associated with KPH percentage within one family. This 64 cM map position-linked QTL had an effect of about 1.5 SDP. The same chromosomal region appeared to affect the quantitative trait of YG (0.90 SDP) within the same family with a LOD score of 1.34. Within about 5 cM form the QTL for KPH percentage, the marker *BM2808* showed 0.74 SDP effect (LOD=1.02) on the 12^{th} rib FT within the sire E family, enforcing the possibility for the existence of a QTL segregating for fat deposition within the 10 cM chromosomal region bounded by the markers *BM2808*, and *RM041* or within close proximity.

The region near the marker *BMS2024* (55 cM) on chromosome 2 showed a notable association with HCW within one family. The effect of this QTL was about one SDP. Although the effect of this QTL is considered large, it did not quite reach the statistical significance in the sire group B alone. However, by summing the LOD scores obtained from the other families, the total LOD score obtained exceeds a pre-determined threshold, so the presence of QTL is inferred. The HCW is an indication of growth

reflected by the muscle, bone and fat weight. For the *BMS2024*-linked HCW, it is not apparent which components of the carcass weight were affected. The chromosomal regions near the marker *BMS2024* is approximately 10 cM downstream from the chromosomal location of QTL for KPH percentage. It is possible that *BMS2024*-associated HCW effect is governed in part by the amount of internal fat in the carcass.

Marker *BMS2872* (46 cM) indicated 1.71 SDP effect on REA trait within one family. HCW is highly positively correlated with the REA and moderately correlated with KPH, FT, and YG in the study of Pariacote et al. (1998) using shorthorn beef cattle. However, Koch et al. (1978) reported that in Hereford, HCW is not genetically correlated to longissimus muscle (r=.02) but extremely highly positively correlated to fat trim weight (r=.90). In Angus cattle, HCW is moderately genetically correlated with REA and FT (Wilson et al., 1993). The region near *BMS1126* (56.3 cM) on chromosome 2 was associated with large effect with the growth trait YW within the sire G sire group. The *BMS1126* linked YW effect was about one SDP. This growth linked QTL did not reach the significance level for WW although the estimated effect was .66 SDP for the same family. Although after weaning, intermuscular, subcutaneous and abdominal fat, and kidney fat have completed their hyperplastic development, they still grow hypertrophically by filling existing adipocytes (Cianzio et al., 1985). In beef carcasses, intermuscular fat is generally the largest fat depot, followed by subcutaneous, internal, and then intramuscular fat (Sainz, 1997). The sire D family group did not show any significant effects on growth traits even though it had significant QTL for REA at

approximately 10 cM downstream from the markers *BMS1126*. Thus, the region near the marker BMS1126 (56.3 cM) is very close to the one associated with carcass weight (1.3 cM upstream), suggesting that a QTL related to fat accretion is at least in part responsible for the weight effect reflected by HCW, YW and to a lesser degree by WW. In beef cattle, intramuscular fat (marbling) is deposited last and continues to recruit adipocytes as well as filling existing cells with lipid; while other earlier developing fat depots (i.e., subcutaneous, intermuscular, kidney and mesenteric fat), have completed their hyperplastic development. The families used in this study had a average quality grade of choice 50, thus intramuscular fat accretion has occurred liberally in these animals; however, there is no evidence of QTL for marbling with the markers on chromosome 2 used in this study.

The USDA Animal Research Center evaluated QTL affecting carcass and growth traits in cattle (Stone, et al. 1999). A significant QTL for retail product yield (RPYD) was detected on chromosome 2 near the marker *URB042* at about 35 cM. Other traits significant at the suggestive level (determined by F-statistics values 10.7 and 17.23) were rib muscle, MB at nearby location of the RPYD trait. Also, at the suggestive level REA, rib fat, fat trim yield, fat weight, and FT were also significant. The significant association between chromosome 2 markers and KPH, REA, and HCW at 64 cM, 46cM, and 55 cM from the beginning of chromosome 2 linkage group respectively, thus QTLs for REA, and KPH found in this study may be the same as the QTL for RPYD which is at 35 cM in the study of Stone et al. (1999) but unrelated to the double muscling, *mh*, locus which is

at 4 cM (Smith et al., 1997). Although the LOD score was maximized at 64 cM of map position for QTL for KPH, it was also significant around the 35 cM position of the QTL for RPDY trait. REA and KPH are component traits of RPDY, thus it is conceivable that the QTL reported by Stone et al. (1999) for RPDY may be the same as reported by REA and KPH in this study.

2. QTLs on Chromosome 11

Markers used in this study were located around the β-lactoglobulin locus (LGB) which was mapped at the telomeric region of the chromosome 11 located at 108.7 cM of map position (Jamieson et al., 1987). Markers on chromosome 11 were associated with QTL for FT, KPH, MB, and YG, primarily within two sire groups.

The region near *BMS2208* had an association with FT in one family of 1.75 SDP. The The same effect was also associated with the chromosomal region near the marker *HEL13* on chromosome 11 for YG within the same family. The markers *BMS2208* and *HEL13* are very closely linked (1.3 cM apart); thus it is very likely that the effect on FT and YG are caused by a single QTL associated with fat accretion. The formula used to compute the yield grade includes HCW, FT, REA, and KPH, with FT and HCW being the most influential traits, and KPH and REA being the least. The genetics correlation between YG and FT supports the hypothesis that a QTL may be segregating for chromosome 11 influencing both traits; however, a small number of animals were genotyped in this family (n = 15) increasing the probabilities of type I error.

Another QTL for FT was identified by Beever et al. (1990) using a paternal half-

sib family several year ago. However, this QTL was located a region of chromosome 12 marked by erythrocyte antigen B locus and influencing also growth traits (WW, and YW). These were the first results with beef cattle demonstrating significant associations between genetic markers and quantitative traits in a half-sib family. Use of DNA markers such microsatellites around the erythrocytes antigen B locus as alternative to isozymes that were used in the study could be interesting. This is because genes that encode isozymes are limited and only a small portion of the genome can be effectively marked (Du, 1998).

The QTL for KPH percentage near the marker *BMS989* on chromosome 11 (85.4 cM) had an effect higher than one SDP. These results were quite similar to those found by Stone et al. (1999) who reported a suggestive linkage of a QTL for KPH at 90 cM on chromosome 11. The most telomeric marker of chromosome 11, *HEL13* was associated with a QTL for marbling in one family with a maximum likelihood estimate of the phenotypic effect calculated in SDP unit was 0.86. The QTL near the marker *BMS989* and the QTL near the marker *HEL13* for KPH and MB respectively are two different QTL segregating within the same sire group. *BMS989* and *HEL13* were separated by greater than 30 cM and, as a consequence, they would be considered unlinked. However, at the map position of the *BMS989*, a LOD score of 1.3 was obtained for the MB trait (data not shown) suggesting that the marker *BMS989* was also associated with detected effect of MB in sire group E family. There is a low genetic correlation (r_g) between MB and KPH (r_g=0.10), this may be explain why, the QTL for KPH is more significant than the QTL

for MB.

3. Summary, Implications, and Future Work Needed

The objective of this study was to identify regions on chromosomes 2 and 11 that provide evidence for the presence of genes affecting carcass traits. An extremely high LOD score threshold must be adopted to avoid false positives. For the promulgation of a QTL, a stringent LOD score of 3 must be chosen to reduce type I error, especially when large numbers of markers were used in the analysis (Churchill and Doerge, 1994). Archibald and Haley (1998) reported that in linkage analysis, false positive QTL increase as the numbers of loci used in the analysis increase, but also the significance threshold increases until you exceed the number of 100 markers. Paterson et al. (1988) stated that in the tomato genome and by using RFLP markers, a threshold of 2.4 gives a probability of less than 5% than even a single false positive will occur anywhere in the genome. However, Georges et al. (1995) reported that a very high LOD score threshold has the disadvantage that it reduces the power of the design and results in overestimation of the QTL effects when using the maximum likelihood method. In this study, for the significant QTL detected, only one family yielded a LOD score of higher than 3 by itself. The other QTLs reached the threshold of significance by totaling the LOD score over all the families and usually there is at least one family that had a LOD score greater than one. Furthermore, in this study, we found that sometimes the LOD score decrease considerably as markers were added in the analysis.

It is possible to increase the efficiency by performing an across-pedigree,

considering all half-sib pedigrees jointly. Given the limited sample size (small families) in this study and the varying marker informativeness, it is expected, that some markers are less significant and consequently, these experiments have a low power to detect the postulated QTL. Components that influence the power of QTL detection include, the size of the QTL effect to be detected, the type I error, and experimental design and number of animals used. Increased accuracy of the analysis and significance of the results could be obtained with more progeny animals per sire family and additional markers if necessary. Analysis of additional data will also be required to draw conclusions about some QTL that indicated large effect but did not quite reach LOD score threshold. With small sample sizes, only QTL with large effects can be detected, so very large sample sizes are required to detect QTL with moderate to smaller effects.

As a conclusion to this study, the magnitude of the observed effects for the significant traits suggests that these identified chromosomal location of the carcass loci may be used in practical animal breeding by MAS specially when traditional selection is not effective. However, the data used in this study had a little power to detect QTL given the number of progeny per sire and the lack of linkage disequilibrium likely to be found within a breed. Thus, the interesting associations revealed in this study between chromosome 2 markers and KPH, REA and HCW and between chromosome 11 markers and FT, KPH, and MB can be further validated with larger elite family as well as other breeds.

More mapping efforts yet to be undertaken in the future so that each region of the

bovine genome would be given its appropriate weight in beef breeding programs by MAS. These mapping efforts will also serve as the starting steps toward cloning of the loci and the subsequent identification of the mutation.

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