INVESTIGATING SCUR CANDIDATE GENES IN *BOS TAURUS* CATTLE

A Thesis submitted to the
College of Graduate and Postdoctoral Studies in
Partial Fulfillment of the Requirements for the
Degree of Master of Science in the
Department of Animal and Poultry Science
University of Saskatchewan
Saskatoon, SK

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ABSTRACT

Scurs are loose horns that are inherited in a sex-influenced manner and appear in cattle that are heterozygous (*Pp*) for the polled mutation. Beef producers find them undesirable, but they are difficult to eradicate because of a complex inheritance. The aims of this study were: 1) to confirm the polled/horned genotype in scurred families from a Canadian beef research herd (CBRH), scurred cattle families from producers, and feedlot steers using the Celtic poll test (P_C) and Friesen poll test (P_F), and 2) to identify new candidate genes near microsatellite *BMS2142* on BTA19. Through PCR amplification, the P_C genotype was confirmed in the phenotyped CBRH, Simmental and Blonde D'Aquitaine (BA) scurred families, and in 625 feedlot steers. One hundred and forty nine scurred animals (out of 692) had one P_C allele. PCR amplification revealed that the P_F allele was present in four polled steers that were horned using the P_C test. Five scur candidate genes (*CTDNEP1*, *FGF11*, *SOX15*, *SHBG*, and *DHRS7C*) were chosen based on position and function on BTA19. To identify SNPs segregating with scurs, 16 animals were chosen from the P_C genotyped feedlot steers, 8 *Pp* scurred steers and 8 *Pp* polled steers. Two SNP's found in *CTDNEP1* and *DHRS7C* were examined in the CBRH and BA with PCR-RFLP using *BseRI* and *AciI*, respectively, but did not segregate with scurs. Multipoint analysis calculated by CRI-MAP 2.5.4, determined that there was significant linkage of the *scur* locus to two microsatellites on BTA19 (*BMS2142* LOD=5.42; *IDVGA42* LOD=3.47). In conclusion, this study's fine mapping of the scur locus has increased the LOD scores of surrounding loci and was linked to two microsatellites on BTA19. Also, to identify scurs the animals should be carefully phenotyped and genotyped for P_C, using the P_F for inconsistent results in beef breeds.

ACKNOWLEDGEMENTS

I would like to acknowledge the University of Saskatchewan Devolved Scholarship, the J.M. Bell Postgraduate Scholarship, the C. Paul and Marianne M. Zielke Postgraduate Bursary in Agriculture, and the John Spencer Middleton & Jack Spencer Gordon Middleton Graduate Bursary for funding support. Thank you to my supervisor, Dr. Mika Asai-Coakwell, for helping me through this process and being a sounding board for all of my ideas. I would like to thank my committee members Dr. Fiona Buchanan, Dr. John Campbell, Dr. Bart Lardner for the streamlining of the thesis process and additional ideas for my project. Thanks to Dr. Ryan Brook for being the graduate chair on my committee and Dr. Bill Roesler for agreeing to be my external committee member. I wish to extend a big thank you to Dr. Matthew Links for helping us run the CRI-MAP software and teaching us computer language. Finally, I would like to thank my family, and especially my mother, for encouraging me through this process and finding my research fascinating.

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LIST OF ABBREVIATIONS

BA	Blonde d'Aquitaine family
bp	base pair
BTA(x)	*Bos taurus* autosomal chromosome (chromosomal number)
CB	Charolais bull
CBRH	Canadian Beef Research Herd
dpc	days post coitus
dpf	days post fertilization
EMT	epithelial to mesenchymal transmission
FPCP	French Polled Charolais Program
GWAS	Genome Wide Association Study
Kb	Kilo base pairs (x 1,000)
LFCE	Livestock and Forage Centre of Excellence steers from 2019
lincRNA	long intervening non-coding RNA
LOD	Logarithm of odds
MAF	Minor allele frequency
Mb	Mega base pairs (x 1,000,000)
P_C	Celtic polled mutation
P_F	Friesen polled mutation
P_G	Guarani polled mutation
PIS	Polled Intersex Syndrome
P_M	Mongolian polled mutation

PMS	Polled and Multisystemic Syndrome
SM	Simmental family
SNP	Single nucleotide polymorphism
T2SS	Type 2 Scurs Syndrome
USF	U of S feedlot steers from 2003
wt	wild type

1.0 INTRODUCTION

Scurs are loose horn-like appendages that are rough in appearance and vary in size from a small scab scur to horn-like (Gowen 1918; White and Ibsen 1936; Asai 2001; Asai et al. 2004). They only appear in cattle that are heterozygous polled, having one poll allele (*P*) and one horn allele (*p*) (Asai et al. 2004; Wiedemar et al. 2014). Archeologists have found *Bos taurus* skeletons from 3500 BC, that appear to be buried in a religious fashion with the horns buried separately from the rest of the skeleton (Kyselý 2010). Since there were no markings on the skull to indicate dehorning, they believe that these are loose horns or scurs, indicating that this is the oldest recorded scur (Kyselý 2010).

Horns in wild populations of ruminants were used for protection against predators and for establishing dominance to determine mating rights (Duijvesteijn et al. 2018). Through the rise of domestication, horns were no longer necessary on cattle, whereby animals were protected by their owners. Because of the inherent risk of horns, before 2016, cattle with horns were penalized when sold to feedlots in Alberta and Saskatchewan, and continue to be penalized in British Columbia with a fine of $10 CDN per head (Briere 2016; CIDC 2019). In order to protect themselves and other animals, producers will disbud/dehorn the cattle by various mechanical means, including caustic paste, hot iron, knife, obstetrical wire, or a gouger (Prayaga 2007). To improve the welfare of the cattle it has been recommended that horned cattle should be bred to polled cattle (Goonewardene and Hand 1991). This provides a natural way of dehorning since the polled mutation is dominant. As a result of this recommended breeding program to naturally dehorn cattle, scurs have become more common in the beef cattle industry.

Scurs are often ignored when small and are classified as horns if they are longer than five cm (Beef Cattle Research Council 2018). This is an economic loss for producers, since cattle with horns are penalized in auction marts (Goonewardene and Hand 1991). In British Columbia, any protrusion on the head that is greater than five cm are fined, which would include long scurs (CIDC 2019). Slaughter houses also penalize cattle with protrusions on their heads, because of the additional processing that is required (Beef Cattle Research Council 2018).

By understanding the inheritance of scurs, it may be possible to gradually remove cattle with scurs or that carry the scur allele from the herd. However, since scurs mainly affect the appearance of cattle and do not interfere with any other production traits, the removal of these animals could be detrimental to the beef industry because of the reduction of the gene pool. Therefore, identifying the scur mutation and creating a simple DNA test to eradicate scurs in the beef industry is crucial to improve breeding programs and avoid penalties when selling cattle.

2.0 LITERATURE REVIEW

2.1 Poll, Horn and Scur Inheritance

Poll/horn inheritance has been studied for over a century in *Bos taurus* cattle. The terms polled and poll refer to the cattle with no horns. These cattle may have an increased peak at the top of their heads (the poll), or it may be rounded. Horned or horns refers to cattle that have an outer keratin sheath and a bony core that is ossified to the skull. The terms scurred or scurs refers to cattle that have bony protrusions not attached to the skull, covered with keratin sheaths. In the original studies of poll and horn inheritance, it was thought that there was a simple Mendelian relationship between these two phenotypes, where the polled phenotype was dominant over horns (Spillman 1905; Lloyd-Jones and Evvard 1916). Subsequently White and Ibsen (1936) defined four genes that have been accepted as the main influencers of the poll/horn inheritance. The genes are poll (*P*), horn (*H*), scur (*Sc*), and African horn (*Ha*), with the polled, horned and scurred phenotypes shown in Figure 2.1. Since the *African horn* gene has not been mapped to any chromosome and occurs only in *Bos indicus* cattle (Prayaga, 2007), it will not be discussed further.

2.1.1 *Poll* Locus

The polled phenotype is defined as the absence of horns (Figure 2.1a), with the *poll* locus dominant over the *horn* locus and can exist as homozygous (*PP*) or heterozygous (*Pp*) (White and Ibsen 1936). The *poll* locus was initially linked to two microsatellite markers, *GMPOLL-1*

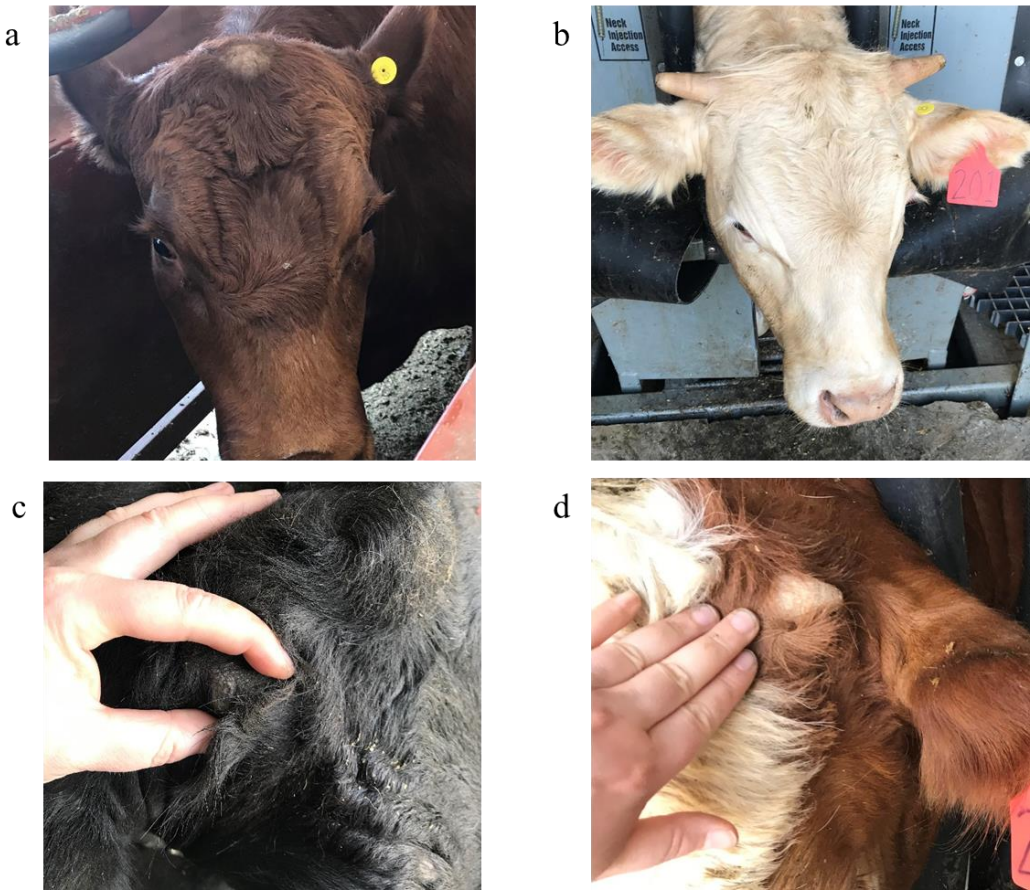

Figure 2.1 Head phenotypes of cattle. a. Polled; b. Horned; c. Flat scab/button scur; d. Horn-like scur.

and *GMPOLL-2*, that were assigned to *Bos taurus* autosomal chromosome 1 (BTA1) (Georges et al. 1993). Schmutz et al. (1995) further refined the location of the poll locus, by mapping it approximately 0 cM to the centromere on BTA1 through linkage to microsatellite markers *TGLA49* and *BM6438*.

The first DNA-based diagnostic test for the polled mutation was called TRU-POLLED, but could only be used on Charolais, Gelbvieh, Hereford, Limousin, Saler, and Simmental cattle (Prayaga 2007). The author stated that 10-15% of the animals tested were expected to have inconclusive results, which is problematic to the breeding programs of cattle breeders. Prayaga

(2007) noted that the test could not be used to determine scurs and may not be correct in populations that were influenced by *Bos indicus* genetics.

Only within the past decade has progress been made on identifying the *poll* locus. In two independent studies using *Bos taurus* breeds, two different mutations were found that cause the polled phenotype on BTA1 (Medugorac et al. 2012; Wiedemar et al. 2014). The mutations can be classified by the ancestry of the cattle: breeds with a Celtic background (Angus, Galloway, Simmental, etc.) and a Friesen background (Holstein-Friesen). The Celtic polled mutation (P_C) is comprised of a 212 bp duplication and a 10 bp deletion (P_{202ID}), while the Friesen polled mutation (P_F) is comprised of a 260 kb haplotype with five variants P_{5ID}, P_{80kbID}, $P_{G165445A}$, $P_{C1655463T}$, and $P_{G1768587A}$ (Medugorac et al. 2012). Wiedemar et al. (2014) reported similar results where the P_C mutation was a 208 bp duplication with a 6 bp deletion (P_{202ID}), and the P_F mutation had 34 variants that were perfectly associated with the polled phenotype i.e. in linkage disequilibrium. The Friesen haplotype further refined to the 80 kb insertion-deletion (P_{80kbID}) when recombination eliminated the other variants (Rothammer et al. 2014). The variants for P_F and P_C are not found in any known coding sequence (Medugorac et al. 2012; Allais-Bonnet et al. 2013; Wiedemar et al. 2014). Shared ancestry is noted throughout the *Bos taurus* breeds, considering that there are instances where a Celtic-ancestry animal will have the Friesen mutation and vice versa. Wiedemar et al. (2014) found that 5% of the polled Limousin and Charolais cattle in the study had one Friesen allele, while 3.5% of polled Holsteins had one Celtic allele.

2.1.2 *Horn* Locus

The presence of horns is the natural state or wild type for cattle (Shrode and Lush 1947). Horns consist of pneumatized bony core that is ossified to the skull covered with a keratinized sheath (Figure 2.1b) (Allais-Bonnet et al. 2013; Wiedemar et al. 2014). The *horn* locus is assumed to be always present in all cattle in its homozygous state (*HH*) and is responsible for the growth of horns (White and Ibsen 1936; Williams and Williams 1952). It is also believed to be an essential part of the genetic complex that distinguishes cattle from other species (Shrode and Lush 1947). Since the *horn* locus is assumed to be the same in all cattle, Shrode and Lush (1947) advised that the inclusion of this locus when stating the genotype of cattle is redundant and does not need to be specified. Because the poll mutation is dominant over the *horn* locus, the recessive genotype (*pp*), or the absence of the polled mutation, is now how the genotype for horns is indicated. The location of the locus or mutation causing horns is still unknown.

2.1.3 *Scur* Locus

Scurs are corneous growths that appear in the horn bud area, but are not firmly attached to the skull (White and Ibsen 1936). There are many different sizes of scurs, from crusts or scabs to large horn-like formations (Figure 2.1c and d; Asai et al. 2004; Wiedemar et al. 2014). The *scur* locus was mapped to *Bos taurus* chromosome 19 (BTA19) by Asai et al. (2004) through linkage mapping using a mixed breed cattle embryo transfer herd (Schmutz et al. 2001). Capitan et al. (2009) disagreed with Asai et al. (2004) on the location, since they did not find the *scur* locus on BTA19 in the French Polled Charolais Program (FPCP) cattle using microsatellite genotyping and linkage mapping. The cattle that were used in this study were all half or full-sibs, sired from bulls in the FPCP using artificial insemination. Seventeen animals displayed a

phenotype similar to scurs, but were genotyped as horned, and the mutation in these animals was mapped to BTA4 in a later publication (Capitan et al. 2011). Although they did not find the causative mutation for scurs, Tetens et al. (2015) found association of the scur trait to one SNP on BTA19 through a genome wide association study (GWAS) using 150 scurred Simmental cattle.

The inheritance of scurs is complex, whereby scurs only grow when the animal is heterozygous for the polled mutation, vary in size, are sex-influenced, and grow later in life (Long and Gregory 1978; Asai et al. 2004; Wiedemar et al. 2014). When cattle are homozygous polled (*PP*) or horned (*pp*) the scur will not grow, even when the animal has two scur alleles (Table 2.1). Only in cattle that are heterozygous polled (*Pp*), will scurs appear, regardless if the polled mutation is of the Celtic or Friesen variety (Asai et al. 2004; Wiedemar et al. 2014).

The distribution of scurred animals between the sexes indicate that scurs are sex-influenced because of the higher proportion of males with scurs than females. White and Ibsen (1936) first suggested that *Pp* males need at least one scur allele while females need two to show the phenotype. This inheritance theory was substantiated by Long and Gregory (1978), Asai et al. (2004) and Wiedemar et al. (2014). Long and Gregory (1978) reported that scurs will present when the animal is *PPScSc* (for male or female), but with the discovery of the polled mutation, no *PP* scurred animals have been found (Asai et al. 2004; Wiedemar et al. 2014; Tetens et al. 2015). Some researchers also believe that the *scur* locus may have genetic heterozygosity, where more than one mutation could control the growth of scurs (Capitan et al. 2009; Tetens et al. 2015). Presently without breeding trials, cattle that are genetic carriers for the scur allele are unknown, unless the producer keeps excellent records of which cattle have scurs and their parentage in the herd (Asai et al. 2004).

Table 2.1 Interaction of scur and poll genotypes.

Poll Genotype	Scur Genotype	Male Phenotype	Female Phenotype
PP	*ScSc*	Smooth polled	Smooth polled
PP	*Scsc*	Smooth polled	Smooth polled
PP	*scsc*	Smooth polled	Smooth polled
Pp	*ScSc*	Scurs	Scurs
Pp	*Scsc*	Scurs	Smooth polled
Pp	*scsc*	Smooth polled	Smooth polled
pp	*ScSc*	Horns	Horns
pp	*Scsc*	Horns	Horns
pp	*scsc*	Horns	Horns

Another factor that makes the appearance of scurs difficult to predict, is the delayed appearance of the scur. In males, scurs can appear anywhere from 4 months of age to a year, while females may develop scurs even later, at 18 months of age (Spire et al. 1981; Mariasegaram et al. 2010; Capitan et al. 2011). This affects the record keeping of producers, because at birth the calf will appear to be smooth polled. In Mariasegaram et al.'s (2010) study with Brahman calves, to accurately run comparison models on the RNA samples that were taken when the calves were 1-2 weeks old, the calves heads were examined for a year to accurately identify the head phenotype. Therefore, to monitor the development of scurs, heads must be felt at weaning and after, until approximately 18 months of age, in order to be certain that the animal is in fact smooth polled.

2.2 Horn Development

Since horns are solidly attached to the skull, it was once assumed that they grew from the skull itself , however, an anatomical study of horns and scurs by Dove (1935) found that horns are ossified to the skull approximately 2 months after birth. By transplanting undifferentiated tissues from the site that will develop into horns (horn bud) to the forehead region on one-week-old calves and goat kids, horns grew from this aberrant location. These experiments revealed that the horn's keratin sheath, or spike, developed from the ectoderm and mesoderm from the horn bud site, not the skull (Dove 1935). More recent studies have been conducted to examine horn growth using transcription profiling (Mariasegaram et al. 2010), histological analysis (Allais-Bonnet et al. 2013), SNP genotyping, and quantitative Real-Time PCR of fetal tissue (Allais-Bonnet et al. 2013; Wiedemar et al. 2014).

Mariasegaram et al. (2010) took tissue samples from the horn bud site on Brahman calves at 1 to 2 weeks of age, and phenotyped for polled, scurred, and horned at one year of age. A comparison of gene expression between the phenotyped animals revealed 573 genes that were differentially expressed when comparing the phenotypes in three categories: polled vs horned, polled vs scurred, and horned vs scurred (Mariasegaram et al. 2010). Through functional clustering analysis, it was found that between polled vs horned calves the differing genes corresponded to the cytoskeleton, extracellular region, epidermal development, cell communication, intercellular junctions, intermediate filaments, and striated muscle contraction. When comparing polled vs scurred calves they found that the differences were between genes that corresponded to skeletal development, ECM-receptor interaction, intermediate filament cytoskeleton, and fibrillary collagen (Mariasegaram et al. 2010). It was determined through hierarchical clustering analysis that each head phenotype had differing gene expression

signatures, indicating that the genes activated in the horn bud site were distinct between each phenotype (Mariasegaram et al. 2010).

After the discovery of the P_C and P_F mutations, researchers could study differences in fetal horn growth since horn buds begin developing *in utero*. At 90 days post-fertilization (dpf), Allais-Bonnet et al. (2013) found that the horn growth area in *Bos taurus* fetuses exhibited differences in skin development, with no anatomical differences of the forehead skin between P_Cp and horned (*pp*) genotypes. The histological evidence indicated that *pp* fetuses, have clusters of dermal cells that show glandular/ductal differentiation, supernumerary layers of vacuolated keratinocytes (for the keratin sheath), and an absence of hair follicle germs in comparison to P_Cp fetuses (Capitan et al. 2012). It was also noted that there was no evidence of osteoblast differentiation for the bony core at this time, but suggested that the dermal ossification would occur later in development or after birth (Capitan et al. 2012).

Variations in gene expression between polled and horned fetuses (Capitan et al. 2011, 2012; Allais-Bonnet et al. 2013; Wiedemar et al. 2014), and one week old calves (Mariasegaram et al. 2010) were reported, along with the differences in the skin development of the horn bud site. The differentially expressed genes were dependent on the age and the phenotype of the fetus or calf (Table 2.2) (Allais-Bonnet et al., 2013; Capitan et al., 2012, 2011; Mariasegaram et al., 2010; Wiedemar et al., 2014). These studies found multiple genes involved in horn development and growth. The most compelling candidates were *relaxin family peptide receptor 2* (*RXFP2*), *forkhead box L2* (*FOXL2*) and two long intervening non-coding RNAs (lincRNA) (Allais-Bonnet et al. 2013; Wiedemar et al. 2014). *RXFP2* and *FOXL2* were of particular interest as they may be involved in horn development in sheep and goats, respectively (Pailhoux et al. 2001; Johnston et al. 2011). The two lincRNAs, on BTA1 between the Celtic and Friesian polled

Table 2.2 Comparing gene expression in polled vs horned phenotypes in calves and fetuses.

Gene	Chromosome	Observation	Age of animal	Author
FOXL2	BTA1	Lower expression	90 dpf fetus; 70-175 dpf fetuses	Allais-Bonnet et al. 2013 Wiedemar et al. 2014
LincRNA#1	BTA1	Higher expression; not detectable	90 dpf fetus; 70-175 dpf fetuses	Allais-Bonnet et al. 2013 Wiedemar et al. 2014
LincRNA#2	BTA1	Not detectable; lower expression	90 dpf fetus; 70-175 dpf fetuses	Allais-Bonnet et al. 2013 Wiedemar et al. 2014
OLIG1	BTA1	Lower expression; not found in younger fetuses	70-175 dpf fetuses	Wiedemar et al. 2014
OLIG2	BTA1	No difference in expression; expression decreased with age of fetus	90 dpf fetus; 70-175 dpf fetuses	Allais-Bonnet et al. 2013 Wiedemar et al. 2014
ZEB2	BTA2	Polled and Multisystemic Syndrome; No difference in expression;	90 dpf fetus	Capitan et al. 2012 Allais-Bonnet et al. 2013
TWIST1	BTA4	Type 2 Scurs Syndrome; No difference in expression	90 dpf fetus	Capitan et al. 2011 Allais-Bonnet et al. 2013
RXFP2	BTA12	Lower expression	90 dpf fetus	Allais-Bonnet et al. 2013 Wiedemar et al. 2014
DHRS7C	BTA19	Higher expression	1-2 week old calves	Mariasegaram et al. 2010
DSC1	BTA24	Higher expression	1-2 week old calves	Mariasegaram et al. 2010
DSG1	BTA24	Higher expression	1-2 week old calves	Mariasegaram et al. 2010

mutations, do not overlap any protein coding regions, but may play a role in the development of horns as lincRNA have the ability to regulate transcription in a locus- and allele-specific manner (Allais-Bonnet et al. 2013; Wiedemar et al. 2014). Allais-Bonnet et al. (2013) observed a higher expression of lincRNA#1 (*LOC100848368*) in *Pcp* fetus skin from the horn bud area (horn buds) compared to the frontal skin. They also observed a trend for increased expression of lincRNA#1 in the horn buds of *Pcp* fetuses than *pp* fetuses. However, Wiedemar et al. (2014) did not detect the lincRNA#1 in their fetuses. They reported a lincRNA that overlapped 4.7 kb with the 3' region of Allais-Bonnet et al.'s (2013) 74 kb lincRNA#2, which was under-expressed in polled fetuses regardless of the location of the tissue.

In multiple studies, it was thought that horn development used a process called epithelial to mesenchymal transmission (EMT), where new mesenchymal tissue is locally generated from the epithelial cells, by having the cellular junctions disassociated, allowing the loss of intercellular adhesion (Dove 1935; Mariasegaram et al. 2010; Capitan et al. 2012; Allais-Bonnet et al. 2013). Research that discovered disruption (Capitan et al. 2012) and mutation (Capitan et al. 2011) in horn growth, reported that the deletion in whole or part of the genes *ZEB2* and *TWIST1*, which are the most likely causes of the syndromes, are also master regulators of the EMT process. These genes were differentially expresses in *pp* fetuses at 70 dpf but not after 90 (Allais-Bonnet et al. 2013). This suggests that EMT has an early role in horn development and stops after 90 dpf. In horned *Bos indicus* calves, there was a four times reduction of RNA expression of E-cadherin, a protein involved in encouraging homotypic interactions between cells, compared to polled calves (Mariasegaram et al. 2010). This indicates that EMT may occur after birth and could play a role in horn growth.

These studies have provided a strong foundation to understanding horn ontogenesis, but since scurs do not grow until after birth it is unknown which P_Cp fetuses are smooth polled or scurred, which may impact the gene expression data and skew the results. By including P_Cp fetuses, without knowing whether the fetus was smooth polled or scurred, there may be gene expression that is specifically related to scur development since each phenotype is genetically different (Mariasegaram et al. 2010). Because of the inclusion of these unknown phenotypes, the results from Mariasegaram et al. (2010) were not the same as Allais-Bonnet et al. (2013) or Wiedemar et al. (2014). As well, Allais-Bonnet et al. (2013) and Wiedemar et al. (2014) were investigating genes only on BTA1 that were related to the polled mutation, while Mariasegaram et al. (2010) compared genes between the three phenotypes across the entire genome. Another factor that causes difficulty when studying genes that control the phenotypes is using cattle that display similar characteristics to known phenotypes (polled and scurred) but are categorically different. Examples of this are of two syndromes found in French Charolais cattle that resulted in a mutation or disruption of horn growth (Capitan et al. 2011, 2012). However, these syndromes offer unique insights into horn ontogenesis and the genes involved during development.

2.2.1 Type 2 Scurs Syndrome

A study by Capitan et al. (2009) observed cattle belonging to the FPCP to determine the location of the *scur* locus. They suggested that in the French Charolais breed the inheritance pattern for scurs was autosomal recessive and found that their linkage mapping did not concur with Asai et al.'s (2004) mapping of the *scur* locus to BTA19. When determining the phenotype of these animals, Capitan et al. (2009) examined all animals twice, between 4-6 and 9-18 months of age, and defined scurs as any corneous growth that were attached loosely. By conducting a

genome-wide scan of 323 individuals using the Illumina Bovine SNP50 chip, they performed haplotype reconstruction for the polled phenotype on BTA1, and classified the polled and scurred animals in two groups according to the haplotype.

Further investigation revealed that the FPCP herd included cattle with an anomalous scur type that was not similar in phenotype nor inheritance pattern with the typical scur (Capitan et al. 2011). These related animals could be traced back to the same sire for a maximum of six generations and did not segregate according to the previously stated mode of inheritance for scurs (Table 2.1). These cattle were horned (*pp*) since they did not have either of the polled haplotypes, P_C or P_F (Capitan et al. 2011). Because the characteristics of these animals were different from the normal scur, Capitan et al. (2011) named this type of horn defect as type 2 scurs syndrome (T2SS). The physical examination of these affected animals determined that the size of the type 2 scur in affected females could be scab-like to 15 cm long, while in affected males they were usually longer than 10 cm and less mobile (Capitan et al. 2011). In both sexes, the terminal end of the type 2 scur was identified by irregular keratin sheets, compared to the smooth keratin sheets in normal horns. Another feature of this syndrome is the mild to pronounced acrocephaly and a ridge-shaped bone deposition found on the interfrontal suture. The size of this bone deposit was negatively correlated to the size of the type 2 scur.

In a genome wide scan using multipoint linkage analysis, linkage (LOD = 7.2) was discovered between T2SS and BTA4, with the 95% confidence interval spanning a 1.7 Mb distance covering six different genes (Capitan et al. 2011). The gene *TWIST1* was identified as the most likely cause of this syndrome, based on its regulation of multiple processes including cranial suture patterning and fusion. Capitan et al. (2011) sequenced the entire *TWIST1* gene in two affected females and an unaffected male, and found a 10 bp duplication in exon 1. To

confirm the mutation's association with T2SS, 17 affected and 20 non-affected animals from the founder population, plus an additional 48 unaffected animals had the *TWIST1* exon 1 sequenced. The author's confirmed that the affected animals were heterozygous for the mutation, while the unaffected animals were homozygous for the wild type. The mutation produced by *TWIST1 c.148-157dup* on BTA4 is predicted to cause a frameshift which would inactivate the gene (Capitan et al. 2011). The *TWIST1* mutation was also identified as embryonic lethal to the fetuses that have inherited both copies of the mutation, since after genotyping 32 offspring from affected parents, no homozygous affected offspring was found. Because of the simplicity of the mutation causing type 2 scurs, this mutation can be used to study horn ontogenesis (Capitan et al. 2011).

The only known record of this syndrome is in the French Charolais cattle that were a part of the FPCP. Neither the Canadian herd (Asai et al. 2004) nor the German Simmental herd (Tetens et al. 2015) mapped the *scur* locus to chromosome 4, which suggests that this gene is not related to the scurs found in most cattle. In summary, T2SS cattle are genotyped as *pp*, have a bony ridge along the frontal stuture that is negatively correlated to the size of the type 2 scur, have a 10 bp duplication in *TWIST1*, and are embryonic lethal when homozygous. Based on this evidence, cattle with T2SS are not scurred and Capitan et al. (2009) should remove these animals from their data set. While T2SS gives insight to horn development, this mutation should not be used when studying the interactions between the poll mutation and the *scur* locus.

2.2.2 Polled and Multisystemic Syndrome

Capitan et al. (2012) discovered another unusual case of interrupted horn growth in the French Charolais breed. From horned parents, a Charolais bull (CB) was born with abnormal horns that were small horny scabs. Thought to be polled, CB was bred to horned cows resulting

in 60 horned offspring and 16 polled offspring, of which 14 were female. Apart from complete horn agenesis, polled offspring from CB displayed additional phenotypic abnormalities including facial dimorphism with frontal bossing and a narrow muzzle, variable neurological disorders, postnatal growth retardation, chronic diarrhea, congenital heart defects, male embryonic lethality and female reproductive anomalies. The reproductive tract was examined in the two surviving females at the time of the study, with the observation that they had a normal reproductive system, but with very small ovaries, pale vulvar vestibular mucosa, no cervical mucosa, and low progesterone concentrations indicating non-cyclicity. When one of the females died, the necropsy revealed that the female had premature ovarian failure, which explained why the female did not show signs of estrus. Because of the numerous syndromes that these animals had, the condition was called Polled and Multisystemic Syndrome (PMS) (Capitan et al. 2012).

To determine the cause of PMS, DNA was collected from CB, 19 unaffected offspring, and three affected daughters and their dams, which were then genotyped with the Illumina bovine 50K SNP chip (Capitan et al. 2012). There were numerous Mendelian errors in the affected animals on BTA2. Through haplotype reconstruction, it was discovered that the unaffected progeny had received one of two haplotypes from CB, while the affected daughters were hemizygous, with only the maternal haplotype. To identify the genomic region involved in PMS, the three affected heifers and CB were genotyped with the Illumina Bovine HD SNP chip (700K SNPs) and one heifer was completely genome sequenced. They discovered a 3.7 Mb deletion and a 4 bp insertion in BTA2 that contained *ZEB2, GTDC1*, and the last exon of *ARHGAP15* (Capitan et al. 2012).

As CB was largely unaffected by the symptoms, it was determined through DNA sequencing that he was mosaic for the 3.7 Mb deletion. The non-Mendelian ratio of polled

offspring revealed that the deletion caused specific lethality in male progeny. To determine the main cause of PMS, the functions of the deleted genes were examined and it was found that *ZEB2* was the main candidate for PMS as there are many similarities between it and the Mowat-Wilson Syndrome in humans (Capitan et al. 2012).

Since this is a rare case, and the affected progeny are out of production, there is an extremely low possibility that PMS could be in the Canadian beef herd. Samples from PMS cattle should not be used when studying gene expression differences between the three phenotypes, because they are genotyped as *pp* and have a 3.7 Mb deletion on BTA2. More research is required to determine how EMT effects scurs, and the role that *ZEB2* would have in regards to being a master regulator of the EMT process.

2.3 Poll/Horn in Other Ruminants

Through domestication, wild bovid species that have horns were bred for the polled phenotype. Due to the similarity in the horn function and appearance, inheritance of poll, horn and scur may be assumed to be similar across horned animals, however, closer study revealed differences in the inheritance of these phenotypes. In recent years, the advancement of technology in genetic research has made it possible to conduct research on genes that could be involved in horn growth.

2.3.1 Sheep

In domesticated sheep (*Ovis aries*), the mode of inheritance for horns differs across breeds (Ibsen and Cox 1940; Lühken et al. 2016b). In some breeds all sheep are horned or polled, like Dorset and Suffolk, respectively, while in other breeds the inheritance of horns is sex-influenced, with males horned and females polled, such as Rambouillet (Ibsen and Cox 1940).

The occurrence of scurs, knobs, and horn pits has been noted in sheep and complicate inheritance as well (Ibsen and Cox 1940; Duijvesteijn et al. 2018). Castrated rams have been observed to have halted or reduced horn development, which complicates phenotyping scurs (Duijvesteijn et al. 2018). Another factor that complicates understanding the mode of inheritance of horns in sheep is that the mutation that causes the polycerate phenotype (multiple horns) was found on *Ovis aries* chromosome 2 (OAR2) rather than on OAR10 which is associated with polledness in single horn sheep (Ren et al. 2016).

Horn data have been documented in Soay sheep, a feral population located on the island of Hirta in the St. Kilda archipelago since 1985 (Johnston et al. 2010). In this breed, male sheep may be horned or scurred, while females may be horned, scurred or polled. The inheritance model for this breed has three genotypes: $Ho^{+}Ho^{+}$ (horned: male and female), $Ho^{+}Ho^{P}$ (horned: male, scurred: female), and $Ho^{P}Ho^{P}$ (scurred: male, polled: female). Ho^{+} is the wild-type allele that gives the normal horn phenotype, and the Ho^{P} allele allows the males to have scurs and the females to be polled, though there are some sheep that do not fit this model (Johnston et al. 2011). In these studies, Johnston et al. (2010, 2011) hypothesized that there is an antagonistic selection between the sexes since they observed that scurred males and polled females have reduced fitness in comparison to the other head phenotypes, and that scurred females are more desirable for breeding as they winter well and produce heavier lambs (Robinson et al. 2006).

Johnston et al. (2011) mapped *Horns* on OAR10 in the sheep genome and identified the gene *relaxin/insulin-like family peptide receptor 2* (*RXFP2*) as a candidate for the variation in horn size in male sheep. A 1.78 kb insertion in the 3'UTR of *RXFP2* was associated with polledness in sheep breeds that are either completely horned or completely polled (Wiedemar and Drögemüller 2015). Currently, two SNPs that are linked with the insertion are being utilized

by the Australian Sheep Cooperative Research Centre, but the prediction accuracy is not 100% (Duijvesteijn et al. 2018). The two suggested reasons that explain this are that the predictive SNP used is not in full linkage disequilibrium or that the penetrance is incomplete (Duijvesteijn et al. 2018). Furthermore, it was found that the 1.78 kb insertion did not wholly segregate in sheep that are crosses of polled and horned breeds or in breeds with sex-influenced horns, demonstrating that the 3' UTR insertion in *RXFP2* is not the only factor of polledness in sheep (Lühken et al., 2016). Lühken et al. (2016) suggested that future studies for the horn gene should be conducted in breeds that have sex-influenced horns and to examine the interaction with the insertion in *RXFP2*. Johnston et al. (2011) also noted that *RXFP2* was not homologous between sheep and cattle, and concluded that horn morphology is controlled by different genes between the species.

In contrast to sheep breeds with only two horns, breeds that carry the polycerate phenotype may grow multiple horns. Through GWAS, a 132 Mb genomic region on OAR2 was identified to be the location for the *polycerate* locus in sheep breeds with multiple horns (He et al. 2016; Kijas et al. 2016; Ren et al. 2016; Greyvenstein et al. 2016). Further GWAS between polycerate sheep and two horned sheep, found that the SNP rs399639314 on OAR2, segregated for the polycerate phenotype (He et al. 2018b). A gene ontology protein analysis comparing sheep with multiple scurs to multi-horned and two horned sheep revealed that the highest categories for differentially expressed proteins were involved in biological and cell adhesion processes, extracellular matrix and structure organization processes, and single-multicellular and multicellular organism processes (He et al. 2018a). A KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis on the same data set revealed 12 pathways that were significant with the top five pathways being involved with PI3K-Akt signalling pathway, dilated cardiomyopathy,

protein digestion and absorption, focal adhesion pathway, and EMC-receptor interaction (He et al. 2018a).

Currently, the mutations that indicate polledness and polycerate in sheep are located on two different chromosomes, OAR2 and OAR10, showing the complexity of the relationship between the different phenotypes in sheep (Wiedemar and Drögemüller 2015; He et al. 2018b). Interestingly, the data of He et al. (2018a) and Mariasegaram et al. (2010) both show that the EMC-receptor pathway is significant in the development of scurs.

2.3.2 Goats

The head phenotypes in goats (*Capra aegagrus hircus*) are polled and horned. Unlike cattle, where scurs are loose and are inherited separately from horns, in goats, scurs are thought to be regrowth's of the true horn after disbudding has occurred. There is no research on whether these appendages are truly horns grown from live horn cells that were missed when disbudding occurred, but when fully grown, the scurs on goats do not move. Based on the present knowledge, a more appropriate term for scurs in goats would be deformed horns.

In goats, the polled allele is dominant to horns, but is associated with a recessive intersex allele (OMIA, 2019). When goats are homozygous polled, males (XY) are typically sterile, while females (XX) are regularly intersex, which is unwanted by breeders. These animals are labelled as having polled intersex syndrome (PIS) and present normal male or female features, but all PIS goats contain testicular-like structures but no ovarian structures (Vaiman et al. 1997). Another feature of PIS goats are that the karyotype of all animals are XX, even those that are male in appearance. A 11.7 kb deletion on chromosome 1, is the cause of this syndrome and induces the transcriptional silencing of genes *forkhead box L2* (*FOXL2*), *PIS-regulated transcript 1*

(*PISRT1*), *promoter FOXL2 inverse complementary* (*PFOXic*), and *PISRT2* (Pailhoux et al. 2001; Pannetier et al. 2005, 2012). Pailhoux et al. (2001) found that at 36 days post coitus (dpc), *PISRT1* was observed to be of the ovarian type, but by 40 dpc it switched to a testicular type in PIS embryos. In wild type male goats, the expression of *PISRT1* increased at 70 dpc to birth, and remained highly expressed in the testicles throughout adulthood. Similarly, *FOXL2* increased in wild type male fetuses at 70 dpc to birth, but instead disappeared in adulthood (Pailhoux et al. 2001). *FOXL2* is the only protein-coding gene, while the other three genes correspond to long noncoding RNAs that may be involved in regulating *FOXL2* (Elzaiat et al. 2014). *FOXL2* was determined to be an antitestis gene rather than a female-promoting gene in goats, which explained why sex-reversal occurred when the 11.7 kb deletion was homozygous (Elzaiat et al. 2014).

2.3.3 *Bos indicus*

Similar to *Bos taurus* breeds, *Bos indicus* breeds have three head phenotypes: polled, scurred and horned. Because of crossbreeding with *Bos taurus* cattle as early as 1492, the polled mutation is of taurine origin in many *Bos indicus* breeds, such as the South African Bonsmara and Drakensberger breeds and the South American Nellore breed. Producers are therefore able to utilize the P_C mutation for identification of the polled genotype (Grobler et al. 2018; Utsunomiya et al. 2019). Scurs were recorded in these breeds and followed the same inheritance pattern of *Bos taurus* scurs. However, indigenous South African and Sanga breeds show inconclusive results with the P_C test. Recently, a novel 110 kp duplication on chromosome 1 in the same area as P_C and P_F was discovered in 11 hornless Nellore bulls from South America, while the other known polled mutations were not detected (Utsunomiya et al. 2019). The authors proposed that

this new mutation is to be called P_G for Guarani which is the name of the aquifer under the region. Even though this mutation was discovered in a *Bos indicus* breed, Utsunomiya et al. (2019) believe the mutation to be of taurine origin, because the Nellore breed has been bred to *Bos taurus* breeds since the 19th and early 20th centuries. The P_G mutation should be tested across other *Bos indicus* breeds to determine if it is a true polled mutation for *Bos indicus* or if it is breed specific.

2.3.4 Mongolian Yak

Introgressive hybridization of *Bos taurus* breeds have also occurred in Mongolian yaks (*Bos grunniens*) (Medugorac et al. 2017). Crossbreeding of yaks and cattle yield sterile males and females with low fertility, so these animals are usually bred for meat. However, by breeding back these crossbred females to male yaks, there is now an average of 1.31% cattle genes in the yak genome (Medugorac et al. 2017). Medugorac et al. (2017) discovered a novel polled mutation (P_M, Mongolian) on yak chromosome 1, consisting of a 219 bp duplication-insertion (P_{219ID}) in addition to another 7 bp deletion and 6 bp insertion (P_{1ID}) located 621 bp upstream. In the P_{219ID} mutation there is an eleven base pair sequence that is conserved in the *Bovidae* family and corresponds to the P_F mutation (Medugorac et al. 2017).

2.4 Scurs in the Beef Industry

Scurs are a problem in the beef industry because of the complexity of inheritance and the downgrading of animals at slaughter (Asai et al. 2004; Beef Cattle Research Council 2018). In personal communications with purebred producers, they have stated that scurred bulls will sell for less, even if the bulls have other excellent traits, such as low birth weights and high weaning

weights. These bulls will usually be sold to the commercial producers, and will continue to transmit the scur trait.

Since scurs develop later, this could be an additional cost for commercial producers as they must use pain control if they choose to dehorn/descur animals over 6 months of age with noticeable horn/scur growth (CCA-NFACC 2013). Feedlot producers will offer less for intact horned cattle, as dehorning steers in the feedlot will cause stress and reduce the average daily gain over 106 days by 4.3% (Goonewardene and Hand 1991).

In the 2016/17 National Beef Quality Audit, approximately 1% of the cattle carcasses that went through Canadian slaughter plants were examined (Beef Cattle Research Council 2018). In the audit, it was perceived that the economic losses from bruising and horns was $5.55 million ($1.90/hd) and $176, 086 ($0.06/hd), respectively. The bruising damage was caused by transportation, rough cattle handling, horns, and poorly designed facilities. Because of the many different ways that bruising may occur it is unknown the exact economic loss that has ensued from horns due to bruising. The economic losses from horns is stated to be due to head condemnations and the extra labour of removing the horns in the packing plant (Beef Cattle Research Council 2018). During the audit, the percentage of horns was recorded. The sampled cattle phenotypes from feedlots were distributed as follows: hornless (polled and dehorned), 90.8%; scurs (a horn that is less than 2”), 1.6%; stubs (horns between 2”-4”), tipped horns (4”) and full horns, 2.6%. However, these measurements are not an accurate gauge for the incidence of scurs in the Canadian beef population, as scab/button scurs and descurred animals would have been classified as hornless. Also, scurs have been observed to grow larger than 2” (Asai 2001), so measuring the length of the appendage is not a true indicator for scurs.

The solution for horned cattle in feedlots and slaughter plants is to breed with polled bulls (Goonewardene et al. 1999; CCA-NFACC 2013). However, this will increase the occurrences of scurs in the beef industry since the *scur* locus has not yet been discovered. Until the locus is discovered and a DNA test developed for the identification of the scur genotype, scurs will be present in herds that crossbreed horned and polled cattle.

2.5 Candidate Genes for the *Scur* Locus

Asai et al. (2004) reported through linkage mapping, the *scur* locus is linked to the microsatellite *BMS2142* (LOD = 4.21) on BTA19. Genes that are proximal and distal to this microsatellite based on the cow assembly UMD3.1 (Ensembl release 94) were identified, with emphasis on the function of the gene. The function of the genes to be selected had to be involved in pathways related to bone growth, steroid transfer, or embryogenesis. The five candidate genes chosen were all located within an area that is 2 Mb proximal and 500 kb distal of *BMS2142* (Figure 2.2; Table 2.3; Zerbino et al. 2018). It is important to note that the majority of research conducted on these genes has been in human and mouse models, which is significant since these models do not grow horns. Since the horn and scur gene pathways are still uncertain, functional studies in horned and scurred cattle are needed to further our knowledge of these pathways.

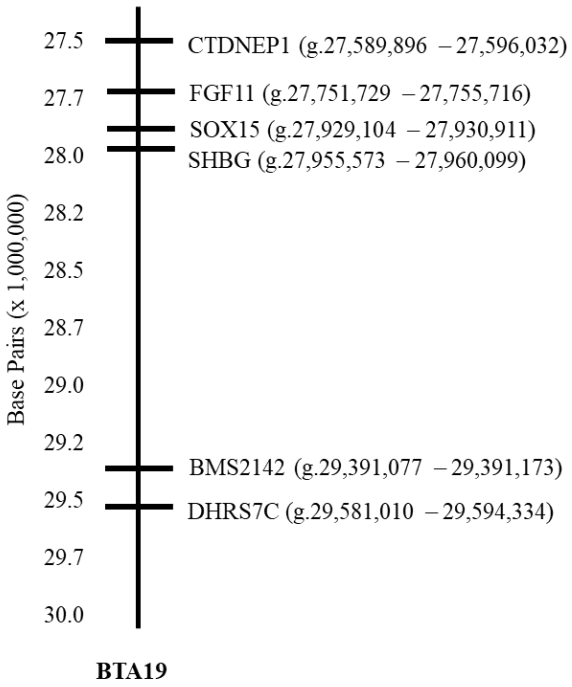

Figure 2.2 Pictorial representation of scur candidate genes in relation to microsatellite *BMS2142* on BTA19. Gene locations are taken from Ensembl release 94 with the assembly UMD3.1.

Table 2.3 Scur candidate genes. All information was taken from Ensembl release 94 (2018). Gene size is inclusive of the 5' UTR, 3' UTR, exons, and intron. Coding exons refer to the DNA sequence that is translated into amino acids (aa) for the protein.

Gene Name	Gene Size (bp)	Number of Coding Exons	Protein Size (aa)	Transcript ID
CTDNEP1	6,136	8	244	ENSBTAG00000019443.5
FGF11	3,987	5	225	ENSBTAT00000025622.4
SOX15	1,807	2	233	ENSBTAT00000007184.3
SHBG	4,526	8	401	ENSBTAT00000005537.5
DHRS7C	13,324	5	259	ENSBTAT00000044540.1

2.5.1 *CTDNEP1*

C-Terminal domain nuclear envelope phosphatase 1 (*CTDNEP1*), also known as *Dullard*, encodes a protein serine/ threonine phosphatase and is a family member of phosphatases that dephosphorylate target substrates (Satow et al. 2006). *CTDNEP1* participates

in a crucial part of nephron maintenance after birth through bone morphogenic protein regulation (Sakaguchi et al. 2013), triacylglycerol synthesis in the liver, and in multiple pathways involving bone growth (Naderi et al. 2017). In one bone growth pathway, *CTDNEP1* regulates endochondral ossification, which is an important developmental process in the growth of bones through suppression of transforming growth factor-β (TGF-β) (Hayata et al. 2015). *CTDNEP1* is required during embryogenesis, since it was shown that there were no viable *CTDNEP1* null mouse embryos past day 11 and the mouse embryos showed poor body development (Tanaka et al. 2013). The involvement of *CTDNEP1* in embryogenesis and bone development may suggest that if there was a mutation of this gene, it could explain the delayed development of scurs and how they are not attached to the skull.

2.5.2 *FGF11*

Fibroblast growth factor 11 (*FGF11*) has been documented to be present in tissues when there is limited or no oxygen (hypoxic areas), such as tumors, to stimulate capillary-like endothelial tube formation associated with angiogenesis (Yang et al., 2015). Since *FGF11* is functional in hypoxic areas, it stimulates osteoclast-mediated resorption of bone similar to the condition of rheumatoid arthritis (Knowles 2017). A mutation in *FGF11* may explain why scurs are not ossified to the skull, since RNA expression is upregulated in the horn bud tissue in horned animals in comparison to polled animals (Mariasegaram et al. 2010). The fibroblast growth factor family also is involved in biological functions such as wound healing and repair, cell differentiation, growth, embryonic development and metabolic regulation (Beenken and Mohammadi 2009). Since the focus of *FGF11* research has been on therapeutic treatments for tumors (Yang et al. 2015), more functional studies on bone morphogenesis processes are needed.

2.5.3 *SOX15*

Sex-determining region Y (SRY) box 15 (*SOX15*) is a member of the SOX gene family, which is involved in cell development and differentiation (Koopman et al. 2004; Thu et al. 2013). *SOX15* is involved in embryonic stem cell development (Maruyama et al. 2005), skeletal muscle regeneration (Lee et al. 2004), and may be involved in gonad development (Sarraj et al. 2003). It is also a candidate for tumor suppression in pancreatic cancer (Thu et al. 2013), as *SOX15* has been shown to repress Wnt signaling (Thu et al. 2013; Moradi et al. 2017). One of the key signaling pathways, the Wnt signaling pathway regulates many genes that are involved in cellular differentiation, proliferation, and survival (Moradi et al. 2017). Because of its involvement in gonad development, a mutation in *SOX15* may influence the disproportionate growth of scurs in male cattle comparted to female cattle.

2.5.4 *SHBG*

Sex hormone binding globulin (*SHBG*) is responsible for transporting and regulating the access of steroids to their target tissues (Westphal 1986). In *Bubalus bubalis*, buffalo bulls, from Egypt, it was found that there are three genetic variants that may be related to the amount of testosterone produced (Naeem et al. 2018). Genotype 1 (KY653957) had two amino acid substitutions, but was not associated with any differences in testosterone to other buffalo bulls the same age. Genotype 2 (KY653958) also had two amino acid substitutions but is associated with a decrease in testosterone concentration. With three amino acid substitutions, genotype 3 (KY653959) is associated with an increase of testosterone concentration (Naeem et al. 2018). From a study on the presence of natural hormones in cattle, it was found that cows had testosterone levels that ranged from 0.02 to 0.76 µg/L while the testosterone levels ranged from

1.56 to 16.2 µg/L in bulls (Woźniak et al. 2016). Because testosterone concentration is higher in males, it was considered that a mutation of *SHBG* might influence the development of scurs, since the growth of scurs is more prevalent and grows faster in males.

2.5.5 *DHRS7C*

Dehydrogenase/reductase member 7C (*DHRS7C*) is a member of the short-chain dehydrogenase/reductase superfamily, where many members have an important role in the conversion of steroids and retinoids in their inactive or active forms (Štambergová et al. 2016). *DHRS7C* plays a role in the distribution of Ca^{2+} in the endoplasmic and sarcoplasmic reticulum of skeletal muscle cells (Arai et al. 2017) and a subfamily protein may be involved in the pathway for retinol dehydrogenase (Ruiz et al. 2018). In two week old Brahman calves, that were phenotyped regularly for a year, it was found that the *DHRS7C* mRNA expression from skin samples taken from the horn area were the greatest in the polled calves, then the scurred calves with the horned calves having the lowest expression (Mariasegaram et al. 2010). Since *DHRS7C* expression is different for each head phenotype, it was an intriguing candidate gene for scurs.

2.6 Hypothesis

This study hypothesizes that the *scur* locus is located on bovine chromosome 19 (BTA19) between microsatellite *CSSME070* and *BP20*.

2.7 Objectives

The objectives of this study are to (i) confirm the horned/polled genotype in previously phenotyped cattle, (ii) identify novel genes that may be involved in scur development for candidate genes, (iii) identify novel SNP's that correspond with the scur phenotype, (iv) perform PCR-RFLP on identified SNP's in scurred cattle families to remap the *scur* locus, and (v) develop a DNA-based test for scurs.

3.0 INVESTIGATING SCUR CANDIDATE GENES IN *BOS TAURUS* CATTLE

3.1 Introduction

In cattle, there are three main phenotypes for the animal's head condition: polled, horned, or scurred. In past studies, the genotype for the head condition was determined through breeding trials but unexpected phenotypes of the offspring cast doubt on the parents genotype (Gowen 1918; White and Ibsen 1936; Blackwell and Knox 1958; Long and Gregory 1978). With this uncertainty and the growing awareness and regulations for pain mitigation during dehorning, it was crucial to identify a reliable genetic test that would enable producers to make informed breeding decisions for the head condition (Goonewardene and Hand 1991). The polled mutation was mapped near the centromere of BTA1, with the microsatellite markers *TGLA49* and *BM6438* linked to the *poll* locus (Schmutz et al. 1995). Recently researchers identified two different polled mutations that enable producers to confidently and accurately genotype beef and dairy cattle for the polled phenotype (Medugorac et al. 2012; Wiedemar et al. 2014; Rothammer et al. 2014). The Celtic polled mutation (P_C) is associated with beef breeds, which has a 212 bp duplication and a 10 bp deletion (Medugorac et al. 2012); the other mutation is associated with breeds from Friesen ancestry, or dairy breeds, which has an 80 kb duplication (Rothammer et al. 2014). With the discovery of the polled mutations, researchers and producers are now able to determine polled genotypes in *Bos taurus* cattle without breeding trials and can correlate the head condition to the genotype (Tetens et al. 2015).

Scurs are corneous growths in cattle, similar to horns in location and structure but are not ossified to the skull, enabling the scur to move (Dove 1935). They are masked by the homozygous polled mutation, only appearing when the animal is heterozygous polled, and are sex-influenced, with males requiring only one copy of the scur allele to produce scurs while females require two copies (Long and Gregory 1978; Asai et al. 2004). In addition to these complexities, there is delayed growth for scurs, where males start to grow scurs from 4 months of age to a year, while females mainly begin to develop scurs after one year (Spire et al. 1981). Therefore, when identifying the scur phenotype, the animals must be observed for at least one year to obtain the correct phenotype (Long and Gregory 1978; Mariasegaram et al. 2010). Since the *scur* locus or mutation has yet to be discovered, the scur genotype for the animal is uncertain without using breeding trials (Long and Gregory 1978; Asai et al. 2004; Tetens et al. 2015).

The *scur* locus bas been confirmed to be on BTA19 through linkage mapping and GWAS testing (Asai et al. 2004; Tetens et al. 2015). Nonetheless these studies did not agree on the location of the *scur* locus on BTA19. Since there is uncertainty of the location for the scur locus on BTA19, our goal was to compare the recorded phenotypes of the animals used in Asai et al.'s (2004) study with the polled genotype using the polled Celtic mutation from Medugorac et al. (2012) and identify candidate genes for the scur locus near microsatellite *BMS2142*.

3.2 Materials and Methods

3.2.1 Animals

All animals used for this study were cared for under the terms of the Canadian Council on Animal Care (Canadian Council on Animal Care 1993). DNA samples from cattle that were previously phenotyped for the head condition included five families: a purebred Blonde

D’Aquitaine (BA) and purebred Simmental (SM) family with scurred offspring (Figure 3.1; Asai et al. 2004), and three embryo transfer families segregating for scurs in a Canadian beef research herd (CBRH; Figure 3.2; Schmutz et al. 2001). There were also two feedlot populations: 207 University of Saskatchewan feedlot steers that were previously phenotyped in 2003 (USF) and 418 feedlot steers from the Livestock and Forage Center of Excellence (LFCE 2019). To determine the polled, scurred, or horned status, heads were palpated in the horn region. DNA was previously extracted from the samples of the USF steers, while blood samples were collected from the tail vein and tail hairs obtained when no blood could be collected in the LFCE herd.

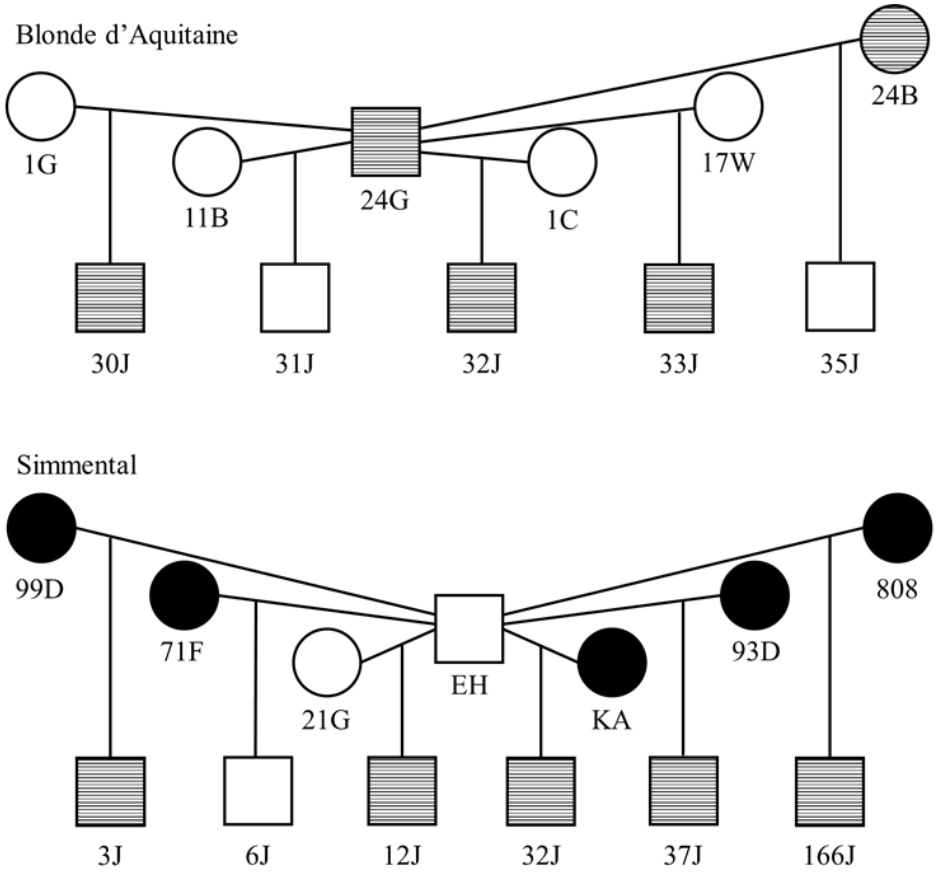

Figure 3.1 Beef cattle family pedigrees with phenotype. Adapted from Asai et al. (2004). Square= male; circle= female; white= polled; shaded= scurred; black= horned.

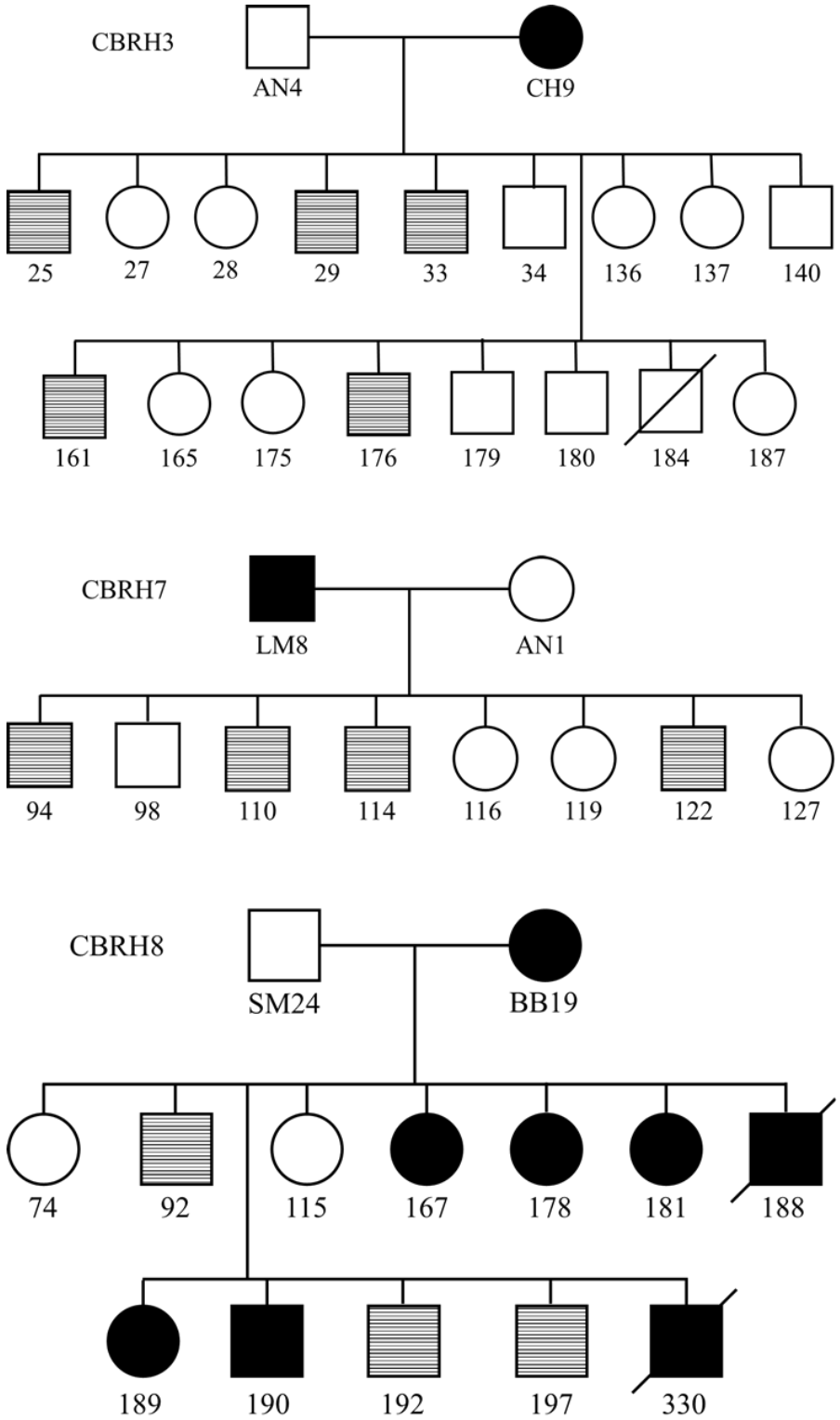

Figure 3.2 Embryo transfer family pedigrees from the Canadian Beef Research Herd (CBRH) with phenotype. Adapted from Asai et al. (2004). Square= male; circle= female; white= polled; shaded= scurred; black= horned.

3.2.2 DNA Extraction

DNA was extracted from blood samples by lysing the red blood cells and using a DNeasy Blood and Tissue Extraction kit (Qiagen). In a 1.5 ml Eppendorf tube, 300 ul of whole blood was lysed in 900 µl of cold red blood cell lysing solution (150 mM NH4Cl, 10 mM KCl, and 0.1 mM EDTA). The blood was incubated for 1 minute on ice, then centrifuged for 20 seconds at 16000 x g. The supernatant was poured off with the remaining white blood cells in a pellet. These steps were then repeated. The pellets were re-suspended in 100 µl of remaining supernatant. The concentrated white blood cells were then used as whole blood in the DNeasy kit where we followed the manufacturer's instructions.

To extract DNA from the tail hair, a base and acid solution method was used. Solution A (base) contained 200 mM NaOH, and Solution B (acid) contained 200 mM HCl plus 10 mM TrisHCl (pH8.5). The hair roots were placed into a 600 µl tube, then 75 µl of Solution A was added. The tubes were centrifuged at maximum speed for 30 seconds, then heated to 97˚C for 15 minutes. Next, 75 µl of Solution B was added and the tubes were vortexed for 15 seconds. The extracted DNA was stored at 4˚C and -80˚C for short and long term storage, respectively.

The extracted DNA from the blood and tail hairs were then nanodropped to determine each samples purity and concentration. Working aliquots for PCR were made with DNA concentrations of 50-100 ng/µl. All aliquots were stored at 4˚C and -20˚C for short and long-term storage, respectively.

3.2.3 Primers

All primers were designed using the UMD 3.1 cow assembly from Ensembl release 94 with the primer sets being constructed using Primer3 version 4.0.0. (Untergasser et al. 2012). The PCR optimization protocol was as follows: amplification conditions were 95˚C for 5 min,

followed by 35 cycles of 95˚C for 30 sec, annealing temperatures of 55-61˚C for 30 s (Appendix A), and extension at 72˚C for 40 s and a final extension of 72˚C for 10 min. Amplicon DHRS7C A (Appendix A) was further optimized with annealing temperatures of 59.3-63˚C as the gel bands were non-specific in the first optimization. Electrophoresis was performed on the PCR product in a 1% agarose gel at 100V for 1.5 hours.

3.2.4 Polled Testing

All animals in this study were genotyped for P_C. The primers used were from Medugorac et al. (2012) with a PCR annealing temperature of 58˚C and the other parameters as described previously. The SM family, scurred feedlot steers, and steers that were phenotyped polled but P_C genotyped *pp*, were also tested for the presence of P_F, with primers from Wiedemar et al. (2014). An additional control primer pair (FGF11 B, Appendix A) was multiplexed with the P_F primers with an annealing temperature of 58˚C, since the P_F only indicates the presence of the polled mutation. Electrophoresis was performed on the PCR products in a 1% agarose gel at 100V for 20 minutes to 1.5 hours depending on the size of the agarose gel.

3.2.5 DNA Sequencing

For the SNP discovery population, 16 animals were chosen from the heterozygous polled USF steers based on the phenotype (8 *Pp* scurred and 8 *Pp* smooth polled (Appendix B)) and on the P_C band appearance from the P_C test. PCR products were Sanger sequenced using forward primers for all amplicons and reverse sequencing was completed on amplicons CTDNEP1 A, CTDNEP1 E, CTDNEP1 F, FGF11 A, FGF11 B, FGF11 D, SHBG B, SHBG E, SHBG F, SOX15 A, SOX15 B, and SOX15 C (Appendix A) at Plateforme de séquençage et de génotypage des génomes, Quebec City, QC. Sequences were analysed on Sequencher 5.4.6

(GeneCodes, Ann Arbor, USA). The minor allele frequency (MAF) for the SNPs in the discovery population, CBRH 3, CBRH7, CBRH 8, and BA were calculated by counting the number of the two different alleles, then dividing with the total number of alleles in the group.

3.2.6 PCR-RFLP

Restriction fragment length polymorphism (RFLP) was performed on amplicons CTDNEP1 C and DHRS7C G to identify the genotypes of the SNPs in CBRH 3, CBRH 7, CBRH 8, and BA. The primers and annealing temperatures used for these SNPs can be found in Table 3.1. This was followed by digestion of the PCR product with restriction endonucleases *BseRI* and *AciI* (New England Biolabs, ON, Canada), for CDTNEP1 C and DHRS7C G, respectively, following the manufacturers protocols. The fragment sizes of CTDNEP1 C that resulted from the digestion were 443 bp and 211 bp for the *G* allele, and 657 bp for the *A* allele. The DHRS7C G fragment sizes were 195 bp and 62 bp for the *C* allele, and 164 bp, 31 bp, and 62 bp for the *G* allele. Electrophoresis was performed on the digested PCR product in a 3% agarose gel at 100V for 40 minutes to 2 hours, depending on the size of the cleaved product.

Table 3.1 PCR-RFLP forward and reverse primers, amplicon size, and annealing temperature.

Amplicon	**Forward primer (5'-3')**	**Reverse Primer (5'-3')**	**Amplicon Size (bp)**	**Annealing Temp (˚C)**
CTDNEP1 C	cctgaaggtctacactggaatc	gcttgcatcctgacctactg	657	60
DHRS7C G	atttggggattagcacgcag	cctggtagatgaagaggagacc	257	65

3.2.7 CRI-MAP

CRI-MAP 2.507 is a software program that allows the user to determine multilocus linkage maps and to calculate the logarithm of the odds (LOD) score between two loci (Green et al. 1990; Maddox et al. 2015). The families of CRBH 3, CRBH 7, CRBH 8, and BA were used for determining the placement of the *scur* locus within the known order of 20 microsatellites and genes, and LOD scores. The SNP data that was collected from the two PCR-RFLPs were added to the 18 microsatellites and genes previously examined (Asai 2001, Asai et al. 2004). The predetermined order of the 20 microsatellites and genes used were established by their location on BTA19 in the assembly UMD3.1 (Ensembl release 94), which was: *BM6000, X82661, HEL10, BMS745, UW27, TGLA51, BMS1920, CSSME070, ALOX12, CTDNEP1, SHBG, BMS2142, DHRS7C, BP20, IDVGA46, BMS2389, MFAP4, CSSM65, ETH3,* and *BMC1013*. The command 'all' was used to determine where the *scur* locus fit best by inserting it into each possible position in the ordered microsatellites and genes. A log likelihood score, which is the logarithmic transformation of the likelihood function, is calculated for each possible placement of all loci creating a negative parametric value. CRIMAP 2.502 then used the log likelihood function to measure the relative support of one parametric value against another, where the lowest log likelihood score will be the order that is most likely to be the correct loci order. To determine the distance between the *scur* locus and the other microsatellites and genes, the command 'twopoint' was used. This command calculates the probability of linkage between the two selected loci and calculates the LOD scores across theta (θ).

3.2.8 Logarithm of Odds Score

Logarithm of odds (LOD) score was used to calculate the parametric linkage between the *scur* locus and the ordered loci. For a given set of pedigree genetic data, LOD may be calculated based on the ratio of two different probabilities, where the null hypothesis, L_{H0}, is the probability of no linkage ($\theta = 0.5$) and the alternate hypothesis, L_{HA}, is the probability of linkage ($\theta < 0.5$), where theta (θ) is the recombinant fraction. The maximum LOD score is obtained by calculating different values of θ.

$$LOD\ score = \log_{10}\left(\frac{L_{HA}}{L_{H0}}\right)$$

Two loci are significantly linked, when the LOD score is greater than 3.3 ($P = 4.9 \times 10^{-5}$), indicating that the loci are 1000 times more likely to be linked than not linked (Nyholt 2000). Suggestive linkage is indicated by a LOD score greater than 1.86 ($P = 1.7 \times 10^{-3}$), while areas of potential interest have a LOD score greater than 0.58 (P = 0.05) (Nyholt 2000).

3.3 Results

3.3.1 Scurred animals from CBRH, SM and BA

We were able to determine that all of the scurred animals from the CBRH families and BA used in Asai's (2001) study were *Pp* polled. However, four male offspring from the SM family that were phenotyped as scurred by the owner were genotyped as horned (*pp*), not the expected *Pp* (Figure 3.3). By removing the SM family from the original recombination data set, it was observed that the non-recombination area for the scur trait shifted from *BMS2142* (g.29278750) towards the centromere (or proximally) to *CSSME070* (g.27068573) on BTA19, as observed by the green line in Figure 3.3. The distal boundary, *BP20* (g.29958329), remained the same.

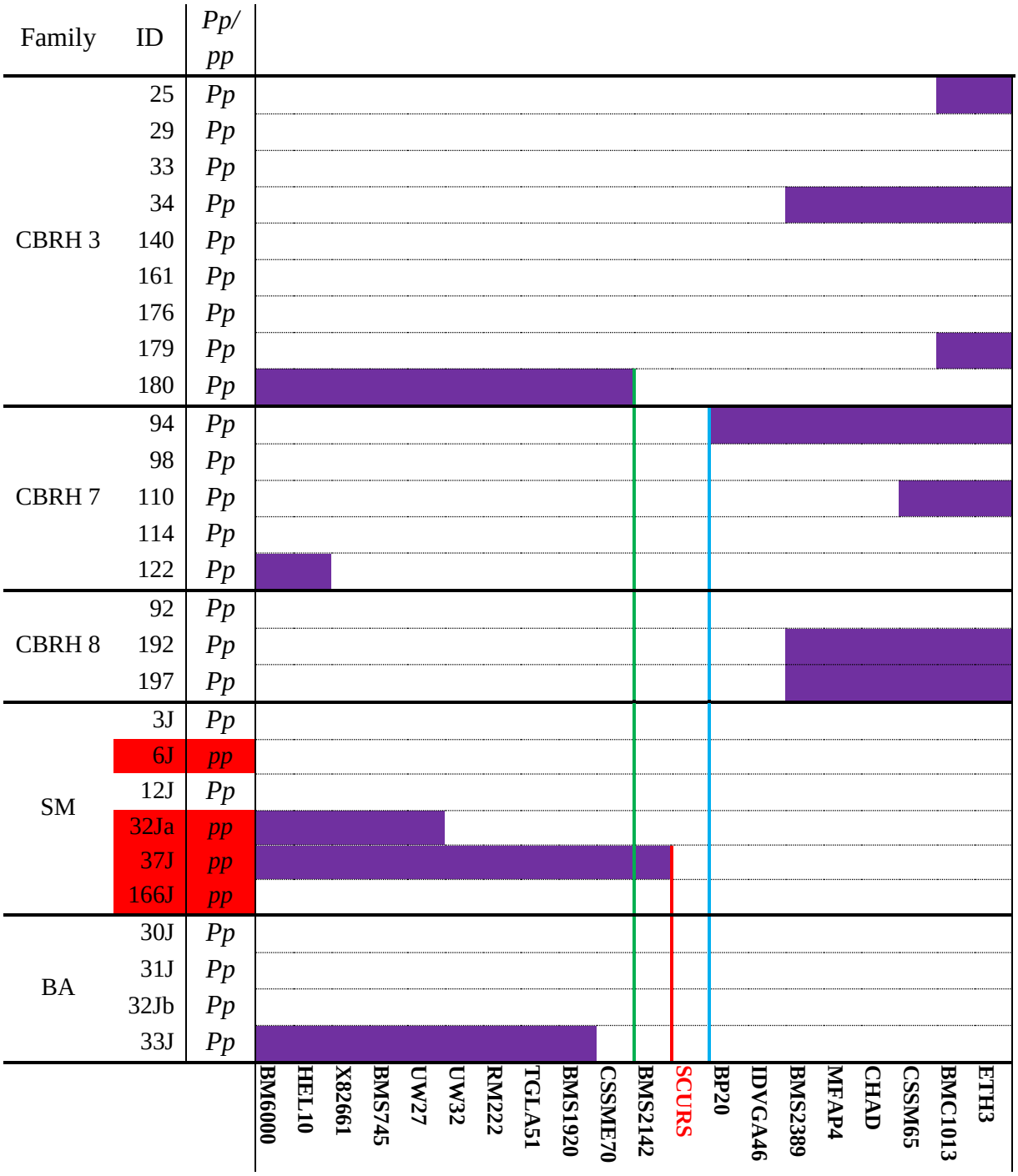

Figure 3.3 Original recombination data from Asai (2001) and P_C/P_F polled genotypes. Red boxes indicate the horned animals that shift the original non-recombinant region (blue line to red line) to the new non-recombinant region (blue line to green line) where the scur mutation may be located. Purple=recombinant; white=non-recombinant; CBRH=Canadian Beef Research Herd; SM=Simmental; BA=Blonde D'Aquitaine.

3.3.2 P_C and P_F Results

The P_C genotype of the USF and LFCE steers were compared to the phenotype of the head condition that was reported (Figures 3.4). The percentage of steers that were phenotyped as polled are 41.5% and 72.0%, scurred are 25.6% and 20.8%, and horned are 32.9% and 6.9% for USF and LFCE steers, respectively. The frequency of P_C genotypes are *PP*, 16.9% and 40.4%; *Pp*, 45.4% and 49.5%; and *pp*, 37.7% and 9.8% for USF and LFCE steers, respectively. As was expected, *PP* was only found when the animal was phenotyped as polled, but *Pp* and *pp* genotypes were found in all of the phenotypes. The presence of the P_F mutation was examined in 140 steers that were phenotyped as scurred and the 12 steers that were phenotyped as polled but were *pp* for the P_C mutation (Appendix B and C). The P_F mutation was found in four of the 12 phenotyped polled steers, two from each herd, but was not found in the scurred steers. In both herds, the frequency of the P_F mutation is 0.6%.

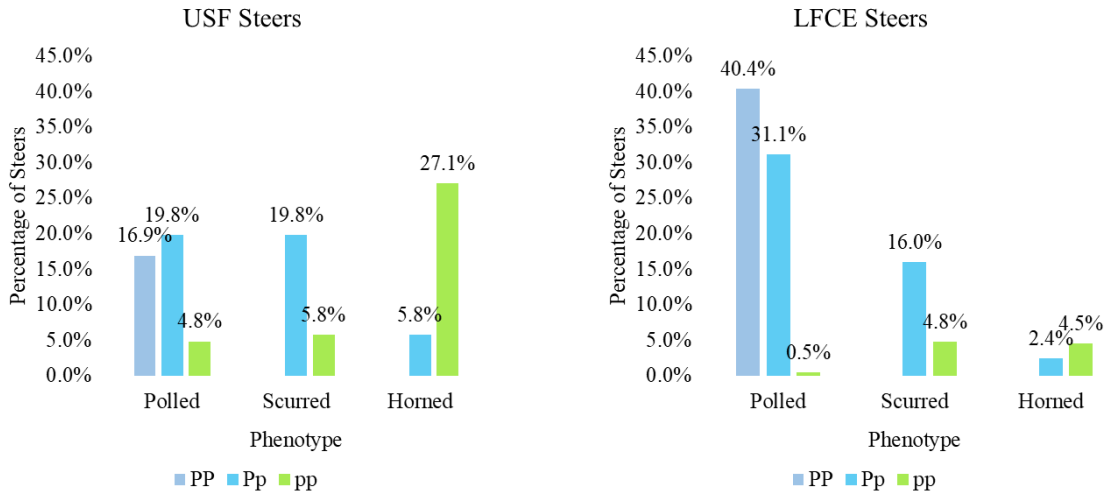

Figure 3.4 Comparison of the head phenotype from the USF (n=207) and LFCE (n=418) steers and their P_C genotype.

Incorrect phenotyping was classified as the occurrence of the polled genotype not being consistent with the reported phenotype of the animal (*pp*-polled, *pp*-scurred, or *Pp*-horned). The occurrence of steers being incorrectly phenotyped in the USF and LFCE herds was 15.4% and 7.1%, respectively, with a total of 62 steers that were incorrectly phenotyped from both herds.

3.3.3 SNP Discovery population

Twelve variants were identified after sequencing the 5' UTR, 3' UTR, and exons of candidate genes *CTDNEP1*, *FGF11*, *SHBG*, *SOX15*, and *DHRS7C* (Table 3.2). No variants were found in the amplicons of *FGF11* in the discovery population. Figures 3.5 and 3.6 show the heterozygosity of SNPs *DHRS7Cc.-6-13C>G* and *CTDNEP1c.462G>A*, respectively. The minor allele frequency of these two SNPs are found in Table 3.3.

Table 3.2 SNP variants found in scur candidate genes.

SNP	Location	Amino Acid Change	Reference Number
CTDNEP1c.289-71G>A	Intron 3		rs445629898
CTDNEP1 c.361-22C>G	Intron 4		rs41904291
CTDNEP1c.462G>A	Exon 5	Arg>Arg	rs209808631
*CTDNEP1c.*538_*539del*	3' UTR		rs135231783
DHRS7C c.-6-13C>G	Intron 1		rs209052501
SHBG c.1057+19G>T	Intron 8		rs41904585
SHBG c.1057+96A>C	Intron 8		rs379245197
SHBG c.1058-30C>T	Intron 8		rs132806166
SOX15 c.219A>G	Exon 1	Lys>Lys	rs41904550
SOX15 c.437C>T	Exon1	Pro>Leu	rs380351003
SOX15 c.567G>A	Exon 2	Pro>Pro	rs110109386
*SOX15 c.*177T>A*	3' UTR		rs41904553

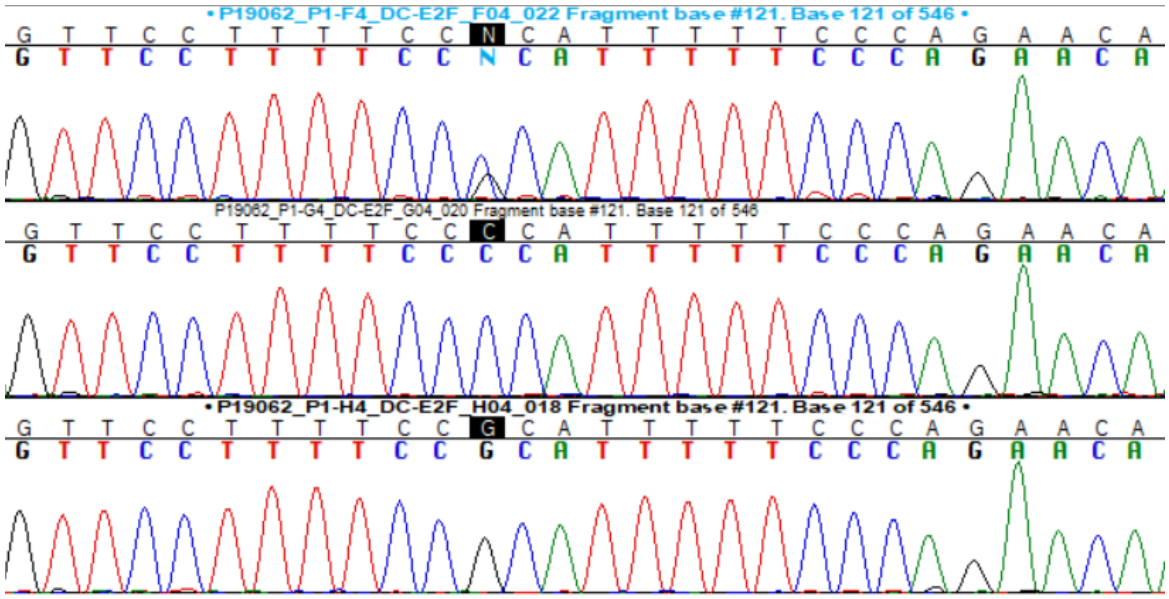

Figure 3.5 Chromatogram for *DHRS7C c.-6-13C>G* of three SNP discovery animals. Top: *CG*, middle: *CC*, bottom: *GG*.

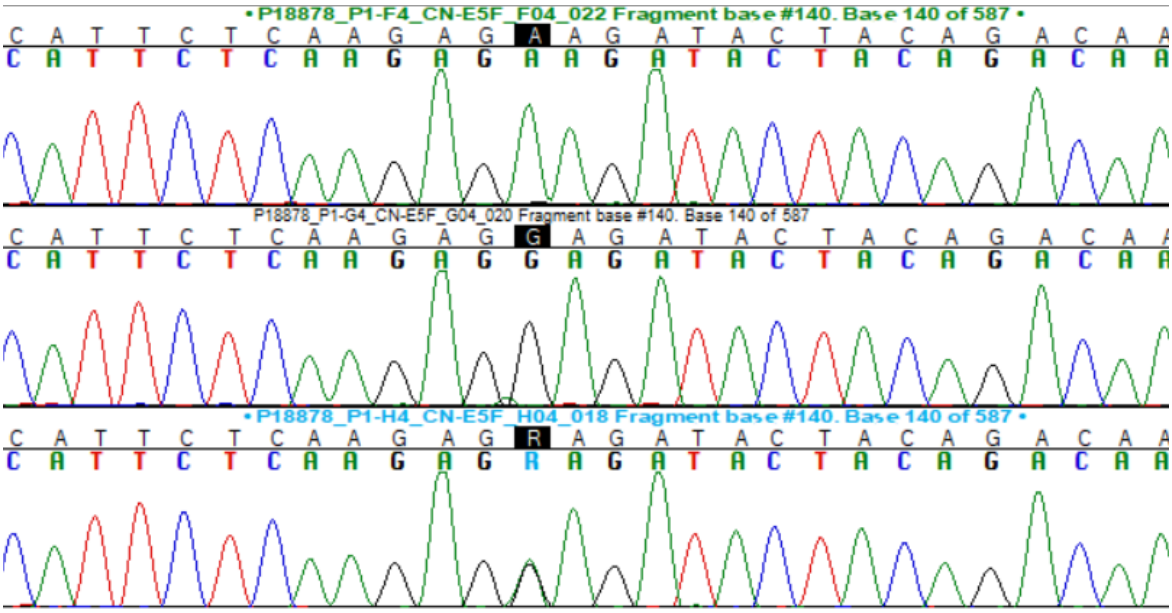

Figure 3.6 Chromatogram for *CTDNEP1c.462G>A* of 3 SNP discovery animals. Top: *AA*, middle: *GG*, bottom: *GA*.

Table 3.3 Minor allele frequency of selected SNPs.

SNP	Location	Allele Count	Allele Frequency	Allele Count	Allele Frequency
		Discovery population		*CBRH and BA*	
CTDNEP1c.462 A>G	Exon 5	G= 26	G= 0.81	G= 39	G= 0.54
		A= 6	A= 0.19	A= 33	A= 0.46
DHRS7C c.-6-13C>G	Intron 1	C= 21	C= 0.66	C= 53	C= 0.76
		G= 11	G= 0.34	G= 17	G= 0.24

3.3.4 PCR-RFLP of scurred families

The parents and male offspring of CBRH3, CBRH7, CBRH8, and BA were genotyped for the two SNPs, *CTDNEP1c.462G>A* and *DHRS7Cc.-6-13C>G*. The minor allele frequency for the CBRH and BA families can be found in Table 3.3. There was no segregation of alleles for the scurred trait in the pedigrees, as is observed in Figures 3.7-3.10.

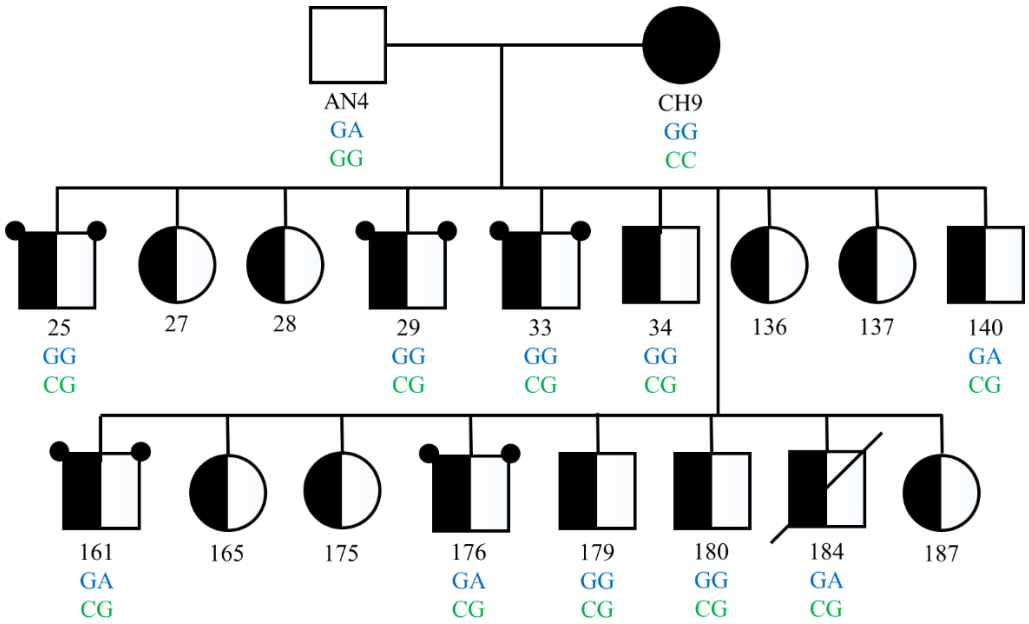

Figure 3.7 Pedigree of CBRH 3 with SNPs from PCR-RFLP. *CTDNEP1* SNP is blue, *DHRS7C* SNP is green. Males are represented by squares and females are represented by circles. White indicates animal is *PP*, black indicates animal is *pp*, and half white/half black indicates animal is *Pp*. Small black circles at top of shape indicates animal is scurred.

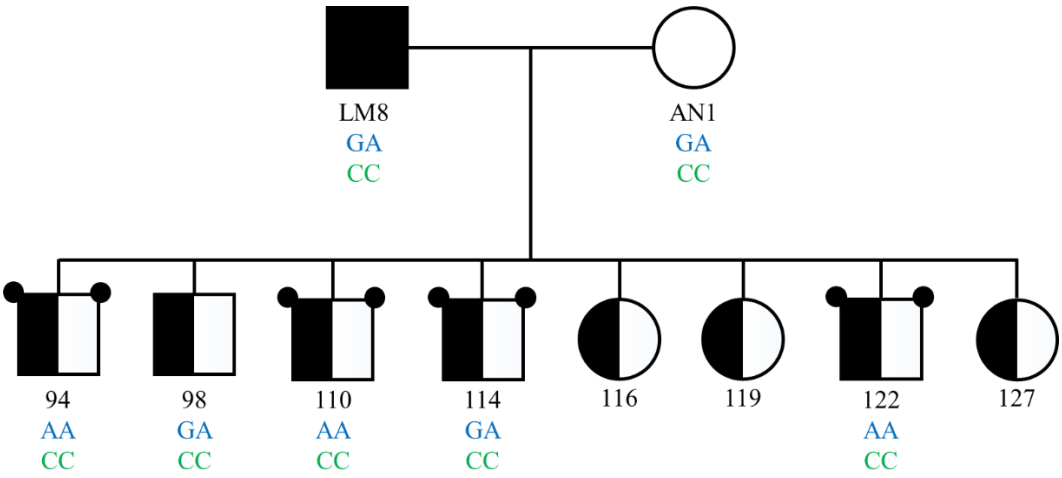

Figure 3.8 Pedigree of CBRH 7 with SNPs from PCR-RFLP. *CTDNEP1* SNP is blue, *DHRS7C* SNP is green. Males are represented by squares and females are represented by circles. White indicates animal is *PP*, black indicates animal is *pp*, and half white/half black indicates animal is *Pp*. Small black circles at top of shape indicates animal is scurred.

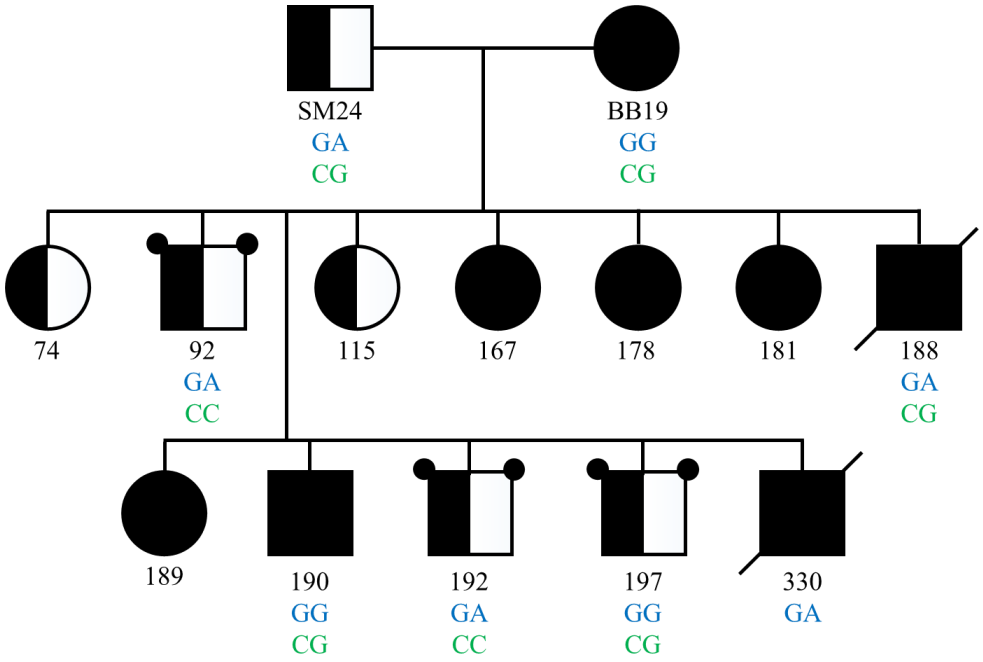

Figure 3.9 Pedigree of CBRH 8 with SNPs from PCR-RFLP. *CTDNEP1* SNP is blue, *DHRS7C* SNP is green. Males are represented by squares and females are represented by circles. White indicates animal is *PP*, black indicates animal is *pp*, and half white/half black indicates animal is *Pp*. Small black circles at top of shape indicates animal is scurred.

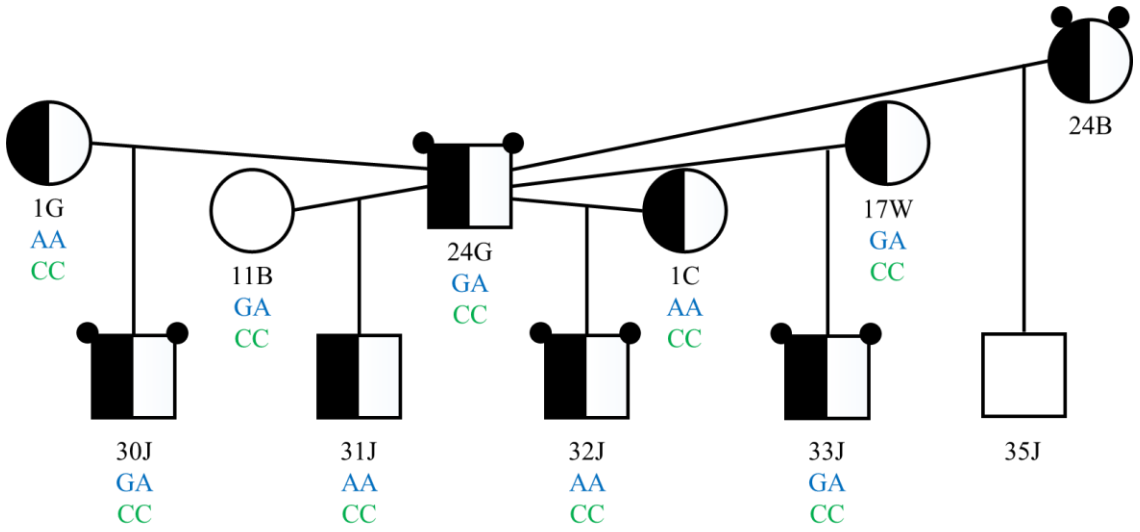

Figure 3.10 Pedigree of BA with SNPs from PCR-RFLP. *CTDNEP1* SNP is blue, *DHRS7C* SNP is green. Males are represented by squares and females are represented by circles. White indicates animal is *PP*, black indicates animal is *pp*, and half white/half black indicates animal is *Pp*. Small black circles at top of shape indicates animal is scurred.

3.3.5 The *Scur* Locus

Through examination of the *CTDNEP1* and *DHRS7C* SNPs, it was concluded that the recombination events were undetermined. A new recombination figure was created (Figure 3.11), revealing that the current scur non-recombinant boundaries are between microsatellites *CSSME070* and *BP20*. The CRI-MAP 'all' command assigned the placement of the *scur* locus between candidate gene *DHRS7C* and microsatellite *BP20* with a log likelihood score of -92.78, indicating that there is high confidence in this placement (Figure 3.12). By comparing the *scur* locus to the 20 ordered loci, using the 'two-point' command in CRI-MAP it was determined that two had LOD scores greater than 3 and four suggested linkage (Table 3.4). The highest LOD score was 5.42 with *BMS2142* (θ=0.00), and the second was 3.47 with *IDVGA46* (θ=0.06). Microsatellites *BMS745*, *BMS1920*, *CSSME070*, and *BMS2389* all approach significance with LOD scores ranging from 2.44 to 2.92.

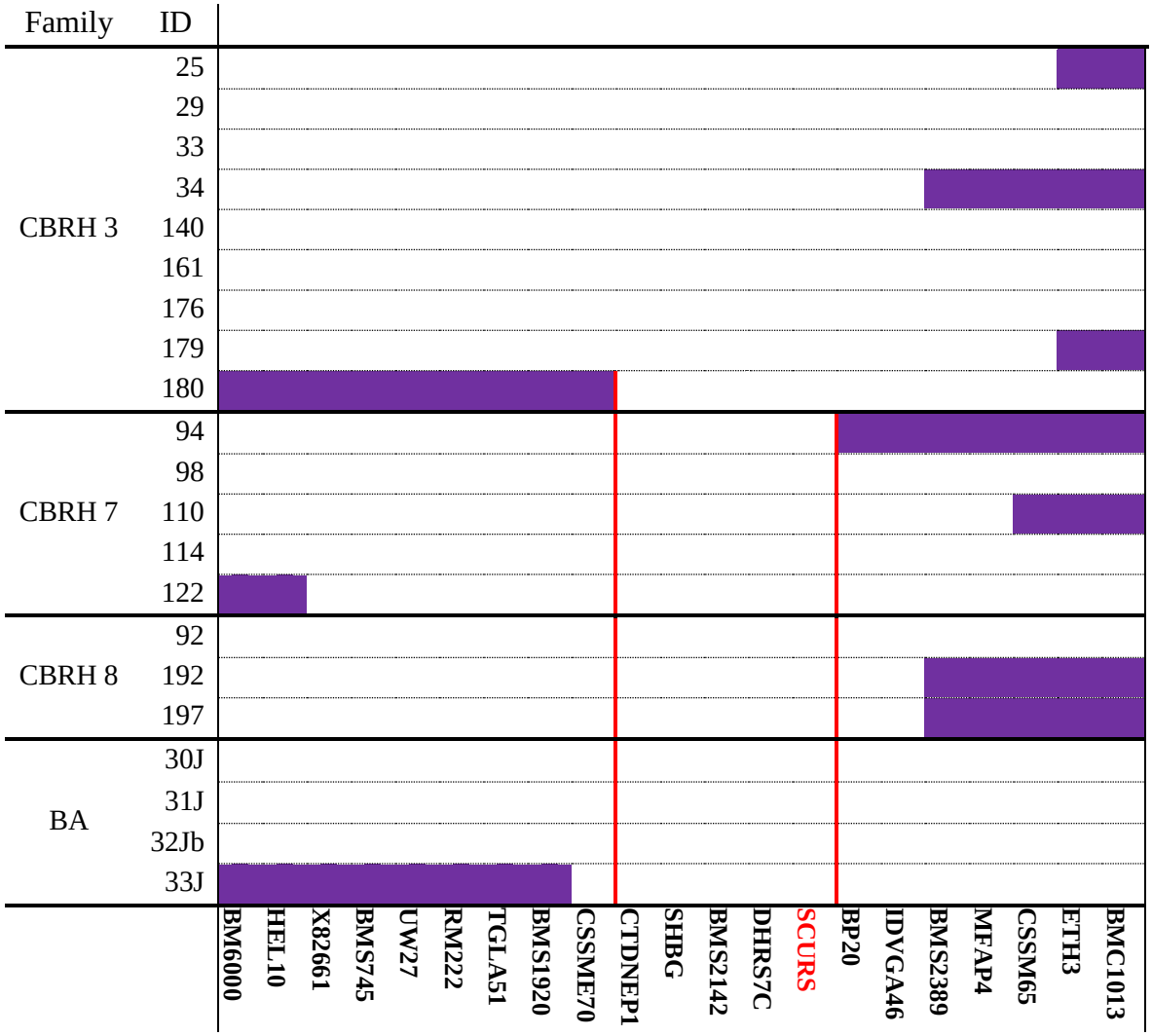

Figure 3.11 Recombination data with updated information from candidate genes. The red lines signify the boarders of the non-recombinant boundaries for the scur trait. Purple= recombinant; white= non-recombinant; CBRH= Canadian Beef Research Herd; BA= Blonde d'Aquitaine.

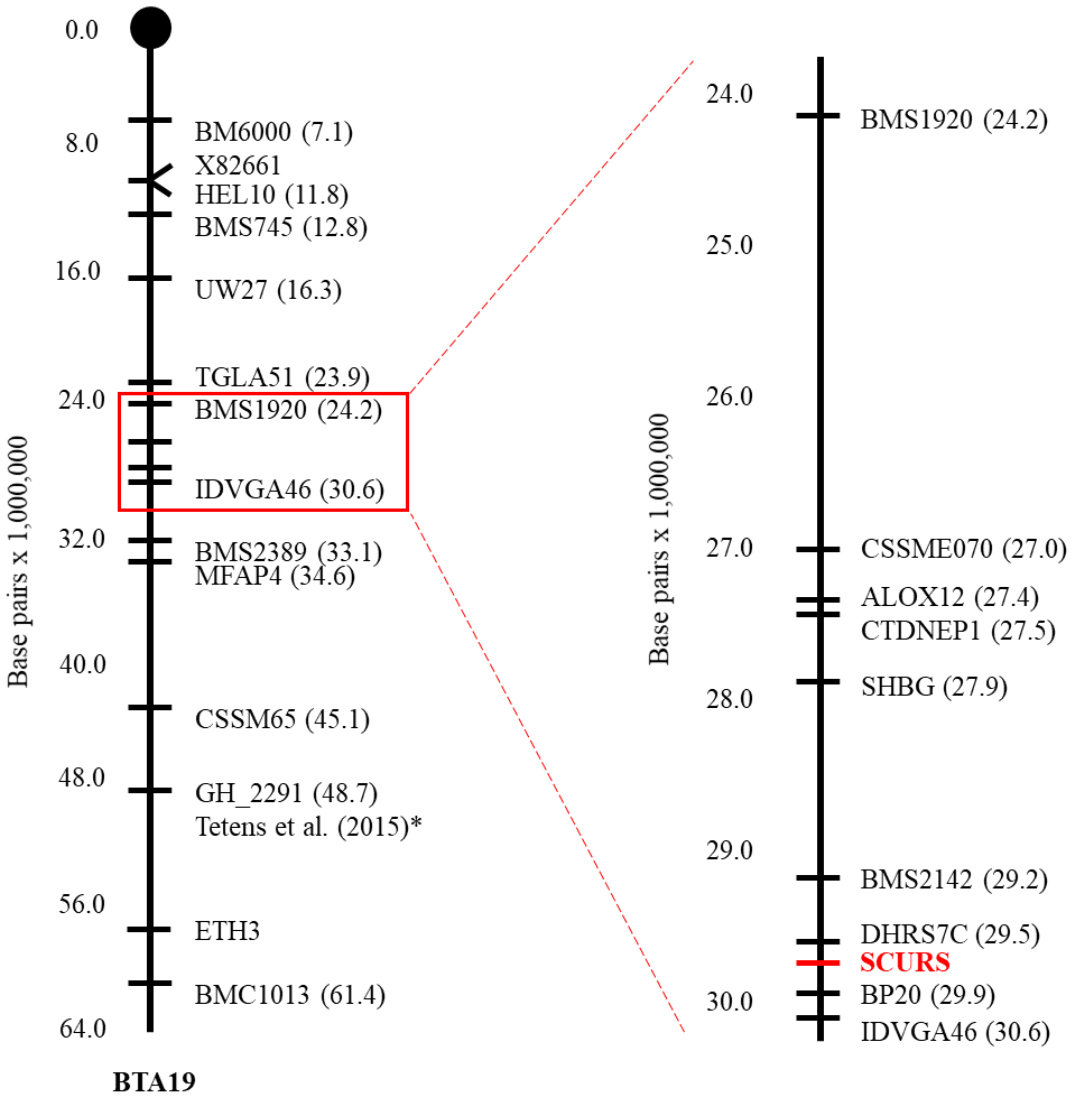

Figure 3.12 Diagram of BTA19 and the new *scur* locus position based on CRI-MAP 2.507. Numbers in brackets signify the position on BTA19 in base pairs x 1,000,000 as determined by assembly UMD3.1 in Ensembl release 94. Red box indicates enlarged area. *rs109191047

Table 3.4 CRI-MAP 2.507 'two-point' results comparing the 20 ordered loci with the scur locus. Red boxes indicate significant LOD scores (<3.3), green boxes indicate LOD scores approaching significance (<1.86).

Locus ID	LOD Score	Rec. Frac. (θ)[1]	cM											
			0.001	0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50
BM6000	0.42	0.200	-1.50	-0.51	0.12	0.32	0.40	0.42	0.40	0.36	0.30	0.22	0.12	0.00
HEL10	1.10	0.125	-0.59	0.38	0.95	1.09	1.09	1.03	0.93	0.80	0.64	0.46	0.24	0.00
X82261	0.90	0.000	0.90	0.89	0.84	0.77	0.69	0.61	0.53	0.44	0.34	0.24	0.12	0.00
BMS745	2.71	0.000	2.71	2.67	2.51	2.30	2.07	1.84	1.58	1.32	1.03	0.71	0.37	0.00
UW27	1.46	0.188	-4.19	-1.24	0.62	1.22	1.43	1.46	1.39	1.23	1.02	0.74	0.40	0.00
TGLA51	1.35	0.111	-0.29	0.67	1.23	1.34	1.32	1.24	1.11	0.95	0.76	0.54	0.29	0.00
BMS1920	2.71	0.000	2.71	2.67	2.51	2.30	2.07	1.84	1.58	1.32	1.03	0.71	0.37	0.00
CSSME070	2.92	0.670	1.51	2.45	2.90	2.87	2.70	2.46	2.16	1.82	1.44	1.01	0.53	0.00
ALOX12	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CTDNEP1	1.20	0.000	1.20	1.19	1.12	1.02	0.92	0.82	0.70	0.58	0.46	0.32	0.17	0.00
SHBG	1.72	0.143	-1.79	0.16	1.35	1.67	1.72	1.65	1.51	1.31	1.06	0.76	0.41	0.00
BMS2142	5.42	0.000	5.41	5.34	5.02	4.59	4.15	3.67	3.17	2.63	2.05	1.43	0.75	0.00
DHRS7C	0.60	0.000	0.60	0.59	0.56	0.51	0.46	0.41	0.35	0.29	0.23	0.16	0.08	0.00
BP20	0.42	0.200	-1.50	-0.51	0.12	0.32	0.40	0.42	0.40	0.36	0.30	0.22	0.12	0.00
IDVGA46	3.47	0.059	2.11	3.05	3.46	3.39	3.16	2.87	2.52	2.12	1.67	1.17	0.62	0.00
BMS2389	2.44	0.118	-0.89	1.05	2.18	2.43	2.41	2.27	2.04	1.75	1.40	0.99	0.53	0.00
MFAP4	1.20	0.000	1.20	1.19	1.12	1.02	0.92	0.82	1.70	0.58	0.46	0.32	0.17	0.00
CSSM65	1.09	0.235	-6.89	-2.94	-0.38	0.52	0.90	1.06	1.09	1.01	0.86	0.64	0.36	0.00
ETH3	0.64	0.294	-9.89	-4.93	-1.65	-0.43	0.15	0.46	0.61	0.64	0.59	0.47	0.27	0.00
BMC1013	0.25	0.357	-10.79	-5.82	-2.49	-1.20	-0.54	-0.15	0.08	0.21	0.25	0.23	0.14	0.00

[1] Rec. Frac.= Recombinant Fraction

3.4 Discussion

3.4.1 Phenotyping

Scurs are corneous growths that are loose in the horn region and appear when cattle are *Pp* for the Celtic or Friesen polled mutation (Wiedemar et al. 2014). Yet, Capitan et al. (2009) stated that scurs do occur when cattle are either *PP* or *Pp* when the scur genotype is homozygous that is, it is not sex-influenced. The mode of inheritance that Capitan et al. (2009) proposed does not correspond with our findings. After phenotyping and genotyping 140 scurred steers from the LFCE and USF for both of the polled mutations, we determined that there were no *PP* scurred cattle. This finding is in agreement with Wiedemar et al. (2014) and Tetens et al. (2015), as both groups phenotyped and genotyped the cattle for the polled mutations. However, after P_C genotyping the 140 steers, we determined that erroneous phenotyping did occur between horned and scurred cattle.

Incorrect phenotyping mainly occurred in this study when cattle were dehorned or descurred. The frequency of the USF and LFCE steers being incorrectly phenotyped for each head category are polled 4% and 0%, scurred 5.8% and 4.8%, and horned 5.8% and 2.4%, respectively (Figure 3.4). Incorrect phenotyping could be based on the quality of horn removal and the age of the animal. If the animal is dehorned properly before the horn bud is ossified to the skull at 2 months of age, then its head may grow similarly to that of a naturally polled animal (Spire et al. 1981). If there is any portion of the horn bud left intact or the horn bud ossified to the skull and was not totally removed, horn regrowth is possible. Erroneous phenotyping may also result if the movability of a scur and horn scar tissue from dehorning are similar when palpating cattle heads.

To avoid incorrectly phenotyping the head condition in cattle, two factors must be considered. First, phenotyping should be completed at frequent intervals or once later on in their development to determine which cattle have scurs. Since scurs have a delayed growth pattern, it is important that the animals are phenotyped when they are between four and six months of age if male and females twelve months or older (Long and Gregory 1978; Asai et al. 2004; Capitan et al. 2009; Mariasegaram et al. 2010). If the phenotyping is completed before four months, scurs may be missed and the animals will be improperly phenotyped as polled instead of scurred. The distinction between polled and scurred is important since gene expression may vary with each head condition (Mariasegaram et al. 2010). The second way to avoid incorrect phenotypes is to feel each animals' head. This is crucial for the identification of scab or button scurs because if heads are not physically felt, these types of scurs may be missed. For example, in Figure 3.13, the steer has a round poll and has no noticeable scurs, so by visual observation this animal would be phenotyped as polled. However, when palpating the horn area and moving the hair, scab scurs are noticeable. In *Bos indicus* cattle, Grobler et al. (2018) noted that incorrect phenotyping occurred on farms as well, mainly between scurs and horns, but some polled animals were also incorrectly phenotyped.

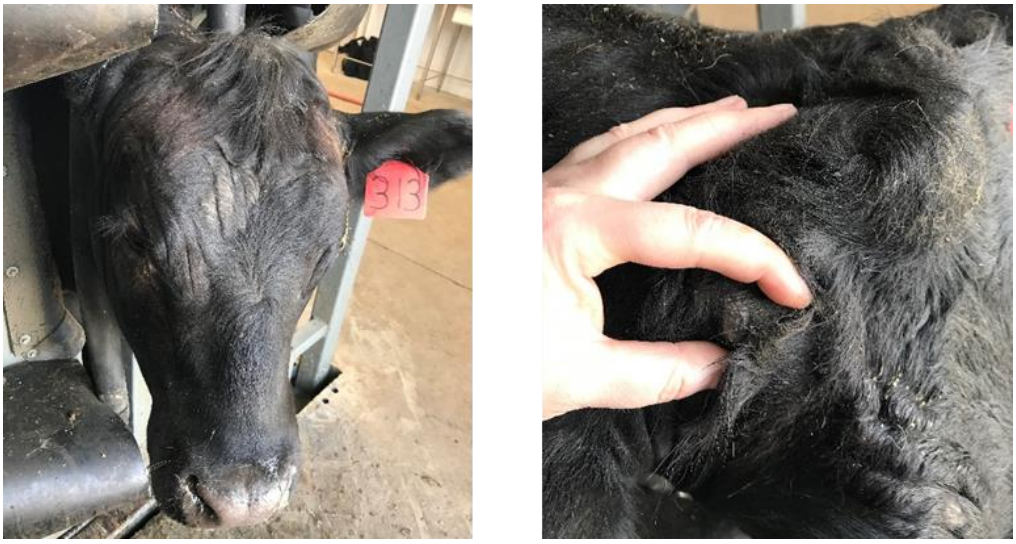

Figure 3.13 Scurred steer with round poll. Left: Frontal view of steer, cannot see scurs. Right: Scab scur hidden under hair.

3.4.2 Scurs versus Horns

In cattle, the genetic pathways that regulate the growth of horns and scurs are still unknown. Research has shown that genetically scurs and horns are different from each other, since the gene expression in scurred cattle are more closely related to the gene expression in polled cattle (Mariasegaram et al. 2010). However, some producers may call any horn regrowth after dehorning scurs, similar to goats. This classification of horn regrowth is detrimental to the investigation for the scur mutation, as it causes confusion and erroneous results in data (Asai et al. 2004), as was observed in the SM family that was removed from the current study.

Inclusion of cattle that have T2SS may have also obscured the research for the scur mutation, because these animals are still classified as scurred even though they have a horned (*pp*) genotype. Normal scurs and type 2 scurs are similar in their shape, size, and movability, but the differences between the two phenotypes illuminate that these are distinct mutations. The *TWIST1* 10 bp duplication causes an extra bone deposit along the interfrontal suture to form and

shows evidence that when homozygous for the mutation there is embryonic lethality (Capitan et al. 2011). There is no evidence of scurs affecting embryo growth or additional bone growth in cattle, suggesting that the mutation or pathway that controls the growth of scurs is in an area that does not affect growth, similar to the polled mutations (Medugorac et al. 2012; Wiedemar et al. 2014). In Capitan et al.'s (2011) study, there was no genetic comparison of cattle with T2SS and scurs, even though they stated that the type 2 scur and normal scurs were similar. Instead, they only genetically compared T2SS animals to horned animals. The T2SS mutation discovery may aid us in finding the mutation for the more common scur.

It is unlikely that the scurred phenotype is caused by a large deletion, like PMS and PIS in goats. The deletions in these two syndromes caused sex reversals (Pailhoux et al. 2001), reproductive anomalies, growth retardation, neurological disorders and other symptoms (Capitan et al. 2012), while there is no evidence that scurs cause any impairments to cattle growth. Also, the effects that are caused by the deletions in the genome in PMS and PIS are observed at birth, while it is unknown which cattle will grow scurs because of the delayed growth pattern.

When examining cattle for scurs, care must be taken that the animals are properly phenotyped and genotyped for both polled mutations. Current research suggests that only animals that are heterozygous polled with a keratinous growth should be classified as scurred (Asai et al. 2004; Wiedemar et al. 2014). Research should be conducted to determine if the regrowth after dehorning is truly the horn growing from live horn cells, like goats, or if the cattle that have a *Sc* allele are growing scurs. Based on transcriptomic analysis that was conducted on *Bos indicus* calves, 93 out of 302 genes were differentially expressed only between horned and scurred calves, and 21 differentially expressed genes were the same when comparing the scurred phenotype to horned and polled animals (Mariasegaram et al. 2010). It would be interesting to

determine if the removal of the horn bud encourages the scur to grow, which may lie dormant under the horn, and grow according to the growth habits and inheritance patterns of normal scurs, however the *scur* locus must be identified first.

3.4.3 Epistasis and Scurs

Since Bateson (1909) first used the term epistasis to describe a masking effect observed in pea flower color, numerous definitions and terminology have been used for epistasis, which have contributed to the confusion on how to properly define the term (Cordell 2002). The most basic definition of epistasis is the interaction between genes. Numerous terms describe the interaction of genes classified as epistasis, such as masking, dominant suppression, duplicate gene action, and modifier genes (Miko 2008). When more than two loci are involved in these epistatic relationships, the mechanics of how the genes will interact can be complicated by multiloci and multiway interactions between some or all of the loci (Cordell 2002). When two genes independently affect different characteristics, such as flower color and plant height, Mendel's phenotypic ratio of 9:3:3:1 is to be expected. But when two genes affect a single characteristic, resulting ratios depend on the type of interaction, such as 9 purple:7 white pea flowers for masking effects or 15 yellow:1 white wheat kernel for duplicate gene action (Miko 2008).

The explanation of the masking epistasis interactions between polled (*P*), horned (*H*), and scurred (*Sc*) were first theorized by White and Ibsen (1936). *P* is completely epistatic to *H*, and can be described as being dominant to *H*. Between *H* and *Sc*, *H* is epistatic to *Sc*, so all cattle will be horned, and in the third interaction of *Sc* and *P* there is a sex influenced interaction, where *Sc* is epistatic to *Pp* always in males and only when *ScSc* in females (White and Ibsen 1936). The

epistatic relationship between *P* and *H* is confirmed by the identification of the polled mutations that have shown when cattle have *PP* or *Pp* they are polled and with the absence of the mutation (*pp*) the cattle are horned (Medugorac et al. 2012; Wiedemar et al. 2014). Since the *scur* locus has not yet been identified, White and Ibsen's (1936) theory of epistasis cannot yet be confirmed, though this pattern of inheritance still holds true (Asai et al. 2004; Wiedemar et al. 2014; Tetens et al. 2015). The epistatic relationship between *H* and *Sc* has not yet been tested.

To classify the type of epistasis seen in scur inheritance is challenging because of the multiple interactions between at least three loci in addition to the influence of sex hormones. Because the interaction of these loci causes three phenotypes to occur, codominance or incomplete dominance could be possible terms to describe the interaction. Codominance is defined as when two different alleles for the same characteristic are simultaneously expressed in the heterozygote, and incomplete dominance is when the heterozygote has a phenotype that is closer to one of the homozygous phenotypes. Scurred animals have bony protrusions covered in a keratinous sheath that grows from the horn bud region like horns, but is not ossified to the skull, showing a polled characteristic, which could be termed as intermediate between the two homozygous phenotypes. If the scur phenotype truly showed codominance or incomplete dominance, all of the *Pp* offspring would be scurred, regardless of the scur allele, with an offspring phenotypic ratio of 1 polled: 2 scurred: 1 horned. Because the offspring phenotypic ratio for a mating between a bull and cow that both have a genotype of *PpScsc* is 6 polled: 6 scurred: 4 horned for male offspring, and 10 polled: 2 scurred: 4 horned for female offspring (Figure 3.14), the terms codominance and incomplete dominance cannot be used. With the observations of White and Ibsen (1936) on epistasis and the phenotypic ratio of offspring from heterozygous parents, it could be stated that this is a sex influenced multiway interaction.

	PSc	***pSc***	***Psc***	***psc***
PSc	*PPScSc*	*PpScSc*	*PPScsc*	*PpScsc*
pSc	*PpScSc*	*ppScSc*	*PpScsc*	*ppScsc*
Psc	*PPScsc*	*PpScsc*	*PPscsc*	*Ppscsc*
psc	*PpScsc*	*ppScsc*	*Ppscsc*	*ppscsc*

Figure 3.14 Punnet square for the mating of a *PpScSc* bull to a *PpScsc* cow. Blue = smooth polled; Green= scurred; Orange= male scurred, female polled; yellow=horned.

A proposed theory for the loci interactions and epistasis reactions are built upon the known facts of the polled mutation and on hypotheses of other researchers. Six assumptions of this theory are: (i) the *horn* locus produces a product that enables horn growth (horn product), not the horn itself; (ii) the polled mutation will only affect the horn product, with no other effect on other functions of the unknown *horn* locus; (iii) the *poll* locus will have no interaction with the *scur* locus; (iv) the *scur* locus produces a scur product that interacts with the horn product; (v) hormone production is consistent in animals, regardless of genotype and will be considered to be testosterone because of the known imbalance between males and females with the scur phenotype (Woźniak et al. 2016); and (vi) the effect of the dominant scur allele will be increased through hormone interaction.

When there is no *P*, the physical horn is able to grow from the horn product that is naturally produced from the *horn* locus. The scur product will still interact with the horn product, but will have no effect because the horn is already developed. Therefore, the scur will not grow, regardless of the scur genotype (Figure 3.15a-c). When the polled mutation is in homozygous

form (*PP*), it will block the production of the horn product preventing horn growth. The scur product will then have no horn product to interact with and no scur growth will occur, no matter of the scur genotype (Figure 3.15d-f). Finally, when the polled mutation is *Pp*, the horn will not grow but some horn product will still be produced. The scur products produced from *ScSc* animals will interact with hormones and the horn product to enable scurs to grow, overcoming the polled effect on the horn production (Figure 3.15g). When the scur genotype is *Scsc*, the effects of the hormones will be greater in male cattle than female cattle. In males there will be enough production of the scur product with the amount of hormones available to overcome the polled effect, while female scur product production will not be able to overcome the polled effect leaving these animals polled (Figure 3.15h). When there is no scur product produced, the polled effect will remain and the horn product produced will not interact with other products so the animal will remain polled regardless of sex (Figure 3.15i).

Currently, the relationship between the scurred and horned phenotypes assumes that *H* is epistatic to *Sc* (White and Ibsen 1936). It could be theorized that the *scur* locus is present in horned cattle and the scur product is merely lying dormant. Because the horn bud is present at birth, horns will preferentially grow over scurs. When horned calves are dehorned at birth, it is expected that no regrowth will occur because the horn bud is destroyed. But when keratin sheaths appear during weaning (approximately 6 months), these sheaths may be scurs or horn regrowth. The possible scur growth may be similar to *Pp* growth in that it is sex influenced, and requires the interaction of the scur product with the remaining horn product. Because the normal horn is not present, scurs may grow instead (Figure 3.16). However, this is dependent on how well the animal was dehorned.

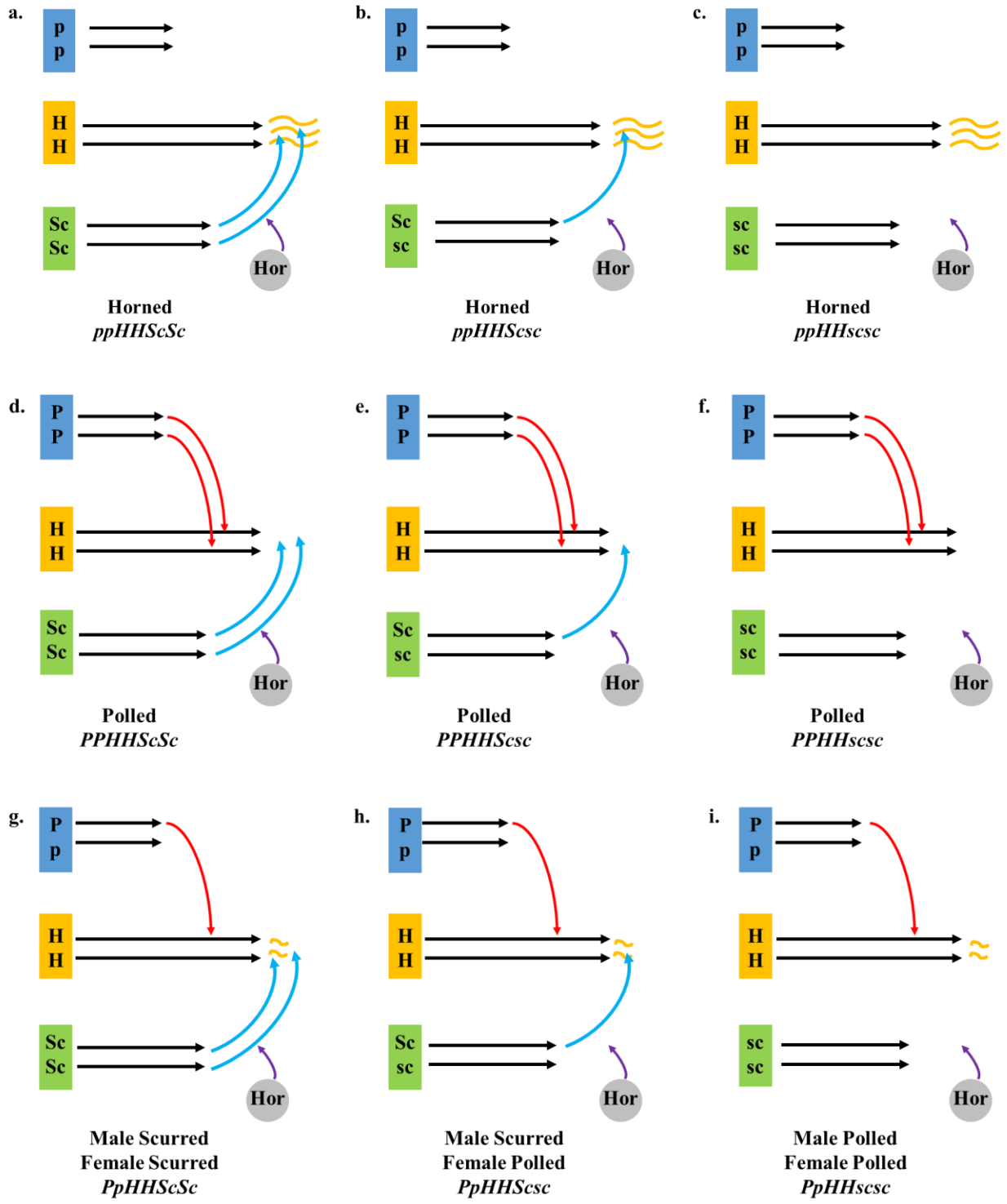

Figure 3.15 Diagram of possible interactions with the polled mutation (*P*), *horn* locus (*H*) and *scur* locus (*Sc*). a-c. No *P*, so *H* activates horn production, masking *Sc*; d-f. The polled mutation blocks horn product, preventing horn and scur growth; g. *Pp* interferes with horn product production, but does not fully stop it, enabling the *ScSc* to interact with the horn product while

the hormone boosts the effectiveness of the scur product; h. *Pp* interferes with horn product production, but does not fully stop it, enabling the *Scsc* to interact with the horn product while the hormone boosts the effectiveness of the scur product in males, but has no effect in females; i. *Pp* interferes with horn product production, but does not fully stop it, however, no scur product is being produced, so both sexes will be polled. Phenotype of animal is listed below diagram, with genotype in italics. Hor = hormone.

To support this theory, there were two instances while phenotyping the LFCE feedlot steers when the keratin sheath was pulled from the skin due to physical force from the animal between the keratin sheath and the metal chute. These two steers were genotyped *pp* with the P_C mutation and did not have a P_F allele (Appendix C). Other steers that were phenotyped as scurred and genotyped as *pp* also give weight to this theory, because of the loose keratin sheath (Appendix C). Discovery of the scur mutation will enable researchers and producers to accurately identify these sheaths as scurs or horn regrowth.

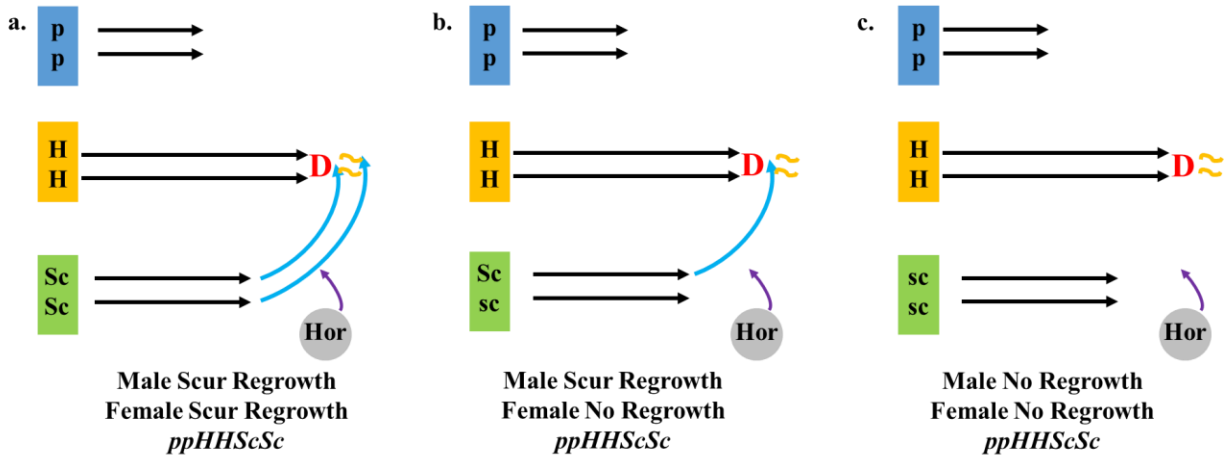

Figure 3.16 Diagram of possible interactions between the *horn* locus (*H*) and the *scur* locus (*Sc*) for dehorned (D) cattle at weaning age. a. Calf is dehorned, enabling *ScSc* to interact with remaining horn product and grow scurs. b. Calf is dehorned, enabling *Scsc* to interact with remaining horn product in males and grow scurs, while females will have no regrowth. c. Calf is dehorned, but there is no scur product produced because animal is *scsc*, so no regrowth will occur for either sex. There is no interaction with the polled mutation since all animals are *pp*. Phenotype of animal is listed below diagram, with genotype in italics. Hor = hormone.

3.4.4 The *Scur* Locus

To identify cattle with scurs before they are grown, the mutation for them must be identified. The *scur* locus was mapped to BTA19, through linkage mapping (Asai et al. 2004) and GWAS (Tetens et al. 2015). Since the two sites of interest are 19.5 million bp from each other (Figure 3.12), the focus of this study remained on the genes surrounding microsatellite *BMS2142* that was linked to the *scur* locus with a LOD score of 4.21 (Asai et al. 2004). The chosen candidate genes that were proximal to *BMS2142* were, *CTDNEP1*, *SHBG*, *FGF11*, and *SOX15*, while the distal candidate gene was *DHRS7C*. The functions of these genes and their gene families, in bone development, hormone transfer, embryogenesis, and gonad development, encouraged us to analyze the exons for mutations in the DNA sequence. The SNPs that had a MAF greater than 20% were used as possible indicators for the scur mutation. *CTDNEP1 c.462G>A* and *DHRS7Cc.-6-13C>G*, had frequencies of 19% and 34%, respectively, in the SNP discovery population (Table 3.3). Even though the *CTDNEP1* SNP had a MAF lower than 20%, it was still used because all three genotypes (GG, GA, AA) were observed (Figure 3.6). When we examined the frequencies in the CBRH and BA families, the MAF changed to 46% and 24%, respectively, indicating that there was increased variability in the *CTDNEP1c.462G>A* compared to *DHRS7Cc.-6-13C>G* (Table 3.4).

When examining the BA and CBRH pedigrees (Appendix D), it was noted that the dams were the scur carriers in the CBRH families, and the sire and dam 24B were the scur carriers in the BA family. Since none of the parents in CBRH had scurs, by studying the other CBRH breeding pairs (Asai 2001) it was possible to determine the scur carriers (Appendix D) (Asai 2001). In CBRH 3, the sire AN4, was also bred to polled and horned cows in families 2 and 10, respectively, with no scurred offspring in these pairings. The dam, CH9, was not paired with any

other males, so is considered to be the scur carrier in CBRH 3. In family CBRH 7, the dam, AN1, was the scur carrier because of recombination data (Asai 2001). The sire of CBRH 8, SM24, was genotyped as *Pp* and did not have scurs, so according to the scur mode of inheritance (Table 2.1) this bull was not a scur carrier, indicating that the dam, BB19, was the carrier. Only one parent was assumed to be a carrier (Scsc), since not all of the male offspring were scurred, and there were no scurred females. The sire and dam 24B in the BA family both had scurs, making them obligate carriers. There was not enough information on the other dams to determine if any of them carried scurs as well (Asai 2001).

When determining which allele to follow for scurs from the scur carrier, it was important to be consistent. For example, in CBRH 3 for *CTDNEP1c.462G>A* the dam's genotype is *GG* and the sire's genotype is *GA* (Figure 3.7). For the offspring, we could determine which allele was inherited from the sire because he is heterozygous. However, it was unclear which allele was inherited from the dam since she is homozygous, and it was not possible to know with certainty which *G* allele was inherited by the scurred and polled offspring. In the same way, *DHRS7Cc.-6-13C>G* was homozygous for the sire and dam of CBRH 3, so we could not determine with certainty which allele was inherited. Therefore, from the PCR-RFLP data that was collected, the two SNPs were uninformative for recombination events in the five families. Recombination occurs during meiosis when two aligned homologous chromosomes from the dam and sire exchange pieces of DNA when the arms of the chromosomes overlap and temporarily fuse, causing a crossover. A recombination map can provide an overview of the chromosome of interest and offer a pictorial representation of where recombination events may have occurred. The area of non-recombination indicates the most probable location of the trait that is being investigated. Therefore, the animals used must be correctly phenotyped and genotyped for the

trait, in this case scurs. Previously, Asai's (2001) recombination map included animals that were phenotyped as heterozygous polled and scurred. However when the DNA from the animals in that study were P_C and P_F genotyped, three offspring in the Simmental family were horned (*pp*), eliminating this family from the recombination map. With these animals removed, the boundaries of non-recombination were shifted from *BMS2142* and *BP20* to *CSSME070* and *BP20*. When observing the results from the PCR-RFLP tests, there was no segregation of either SNP genotype in relation to the scur phenotype within or between the families (Figure 3.7-3.10). Also, using the two SNPs as a indicative haplotype was not possible, as the haplotypes with the *CTDNEP1/DHRS7C* SNPs (*GG/CG, GA/CG, AA/CC, GA/CC*) were present for both scurred and non-scurred cattle.

Through CRIMAP 2.502 software, we combined genotyping data from this study and the previous by Asai et al. 2004. To increase the reliability of the 'all' command, the known loci were ordered according to genomic sequencing from the assembly UMD3.1 (Ensembl release 94). The inserted locus, *scur*, was positioned distal to *BMS2142* (LOD = 5.42, theta = 0.000) and proximal to *IDVGA46* (LOD = 3.47, theta = 0.059) (Table 3.4). The *scur* locus was mapped to the same location on BTA19 from Asai et al. (2004), with the two added SNPs and removal of the SM family increasing the LOD scores to be significant for both flanking microsatellites.

The investigation for scurs is hampered by both known and unknown complications. The known complications are incorrectly phenotyped animals, the delayed growth pattern for scurs, and the sex-influenced inheritance of scurs. The first unknown complication that may influence the ability to locate the scurs mutation is that scurs may have genetic heterozygosity, similar to the polled mutations. This heterozygosity is reflected in the different locations on BTA19 that Tetens et al. (2015) and this study presented for the scur mutation. The other unknown

complication is the possibility of the scur mutation being located in intronic sequence, which was not fully sequenced in this study. Since scurs do not have any negative effects to the growth or reproductive performance of the cattle, small deletions that cause a frameshift in a gene (Capitan et al. 2011) or large deletions that remove several genes (Capitan et al. 2012) are not likely candidates for the scur mutation.

3.5 Conclusion

In this study, we were able to confirm the horned/polled genotype in previously phenotyped cattle using the P_C and P_F tests, and removed the SM family from Asai's (2001) recombination and mapping data. With the removal of erroneous samples, the recombination boundaries shifted from the previously reported region on BTA19 of *BMS2142* and *BP20*, to microsatellites *CSSME070* and *BP20*. This increased the non-recombination area, where the *scur* locus may be found, from 1.4 Mb to 3.6 Mb. In this genomic area, candidate genes *CTDNEP1, FGF11, SHBG, SOX15,* and *DHRS7C* were chosen based on gene functions that could be related to scur growth, bone development, steroid transfer and embryogenesis. Twelve genetic variants were found in these candidate genes, though none of them segregated with the scur trait. PCR-RFLP was conducted on SNPs *CTDNEP1 c.462A>G* and *DHRS7C c.-6-13C>G*, in the families CBRH 3, CBRH 7, CBRH 8 and BA, and the recombination events for these SNPs were undetermined. The addition of the PCR-RFLP data to previously genotyped microsatellites and genes (Asai et al. 2004), enabled us determine the placement of the *scur* locus to be between *DHRS7C* and *BP20* with CRI-MAP. CRI-MAP also calculated the LOD scores of the 20 microsatellites and genes to the *scur* locus, with *BMS2142* and *IDVGA46* increasing from Asai et

al.'s (2004) study. No causative mutations for the scur trait were determined in this study, therefore a DNA test for scurs was not developed.

3.6 Future Studies

Future studies for the scur mutation should include a whole genome study, focusing on the DNA sequence of BTA19. Male cattle that are phenotyped as scurred and polled and genotyped as *Pp* should be selected in order to remove all scur carriers from this study (Table 2.1). Sequence comparison between the different phenotypes would enable the researcher to determine any mutations that could segregate with the scur trait.

When the causative mutation for scurs is found, studies to determine which hormone, if any, result in the sex-influenced scur growth. If there is a hormonal interaction, determining the hormone effect in bulls, steers, and cows will be a possible approach to illuminate how a hormonal interaction with genes could cause sex-influenced growth patterns.

Identification of the scur mutation would also enable an investigation of whether regrowth in dehorned animals is a scur or a malformed horn. This study would start with calves of mixed phenotypes, which would be separated into two groups, control and dehorned. With continual phenotyping of the calves throughout the first year, it would be possible to determine what regrowth, if any, is a scur or horn.

Finally, a survey of scurs in Canadian beef cattle should be done, to determine the prevalence of the scur trait. Within this survey, an economic study should also be done to determine what the cost of scurs are to producers. This study could observe the cost differences between commercial and purebred producers and how scurs affect the final sale price of cattle.

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5.0 APPENDIX

Appendix A: Scur candidate gene forward and reverse primers, amplicon size, and annealing temperature used in PCR.

Amplicon	Forward Primer (5'-3')	Reverse Primer (5'-3')	Amplicon Size (bp)	Annealing Temp (˚C)
CTDNEP1 A	cctttgggcggtaaaatggc	ccgaaaacccttgatcaccg	700	58
CTDNEP1 B	tgcttcctatggttagtgggg	ttcctcccagaactgccttc	664	59
CTDNEP1 C	cctgaaggtctacactggaatc	gcttgcatcctgacctactg	657	59
CTDNEP1 D	cacccttttctctcttgaaccc	atctggcccttgtgtccatc	667	59
CTDNEP1 E	ttcaccgctgatgttcgttc	acagcaggctcttctctctc	745	59
DHRS7C A	ctactagagaggcacccagg	attcctaagagcagcagggg	526	63
DHRS7C B	atttggggattagcacgcag	atggcctctcatccttcagg	606	61
DHRS7C C	atggaggaggggctattcac	ctgggtcctctgagtctgac	513	61
DHRS7C D	gttactgttgccttggtccc	ggtttgcaggggttgaagac	744	61
DHRS7C E	acttgcgtgcattggagaag	cgtcagggttaagaatgcagg	663	61
DHRS7C F	acaatcatgaacagcagccc	aatctgtcccgggtatgtcg	727	61
FGF11 A	agcgggcttctctggg	gagttcctggcctcaacctc	339	59
FGF11 B	ttctctcctctgattccgcc	caagagctggagggataggg	601	59
FGF11 C	ggctcccttctagtccagtg	tcaataccctcccatgtggc	324	59
FGF11 D	ggagcctattcagagccctc	agaagtgatcagccaggacc	401	59
FGF11 E	gaccctcagactcttaggcc	tgaagtcaggggtccatctg	428	59
SHBG A	cagcttgcagaacgggtatg	catccctttctccctcaccc	374	58
SHBG B	ctctgcaggtaggcttggag	gaggagctgatggagagagg	701	59
SHBG C	gaactcctcctccctcaacc	cactctggacctgtcacctg	589	59
SHBG D	ggtgacaggtccagagtgag	tccccaccctgtttattccc	619	59
SHBG E	aggggaataaacagggtggg	aggtcatttgcttgctgtgg	656	59
SHBG F	aggccaagacaagagagctg	aagctcctccccactttttc	588	59
SHBG G	ggatctgcccctcatcttg	cacagcaccgagaggacag	721	59
SOX15 A	gctgagacctggtgagagag	gccgtttgaccttctccaag	581	59
SOX15 B	aggagagggcgtgtagaaac	caggggcacaagtttctgtc	625	59
SOX15 C	gtccagatagccagggatgg	aagaatgactcaggcagggc	684	59

Appendix B: Individual steer head phenotypes and P_C and P_F genotypes from the University of Saskatchewan feedlot (USF) in 2003. The P_C genotype is indicated by letters: A = *PP*, B = *Pp*, C = *pp*. The P_F genotype indicates the presence of an allele: YES = present, NO = not present.

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
03-095	Scurred	B	NO	
03-096	Scurred	B	NO	
03-099	Scurred	B	NO	SNP discovery population
03-100	Scurred	B	NO	
03-136	Polled	B		SNP discovery population
03-137	Polled	B		SNP discovery population
03-138	Polled	C	NO	
03-139	Horned	C		Horned?
03-140	Horned	A		
03-141	Polled	B		SNP discovery population
03-143	Polled	C	NO	
03-144	Scurred	C	NO	
03-145	Horned	C		
03-146	Scurred	B	NO	
03-147	Horned	C		
03-148	Polled	B		SNP discovery population
03-150	Polled	A		
03-151	Polled	B		
03-152	Polled	B		
03-153	Scurred	B	NO	
03-154	Scurred	C	NO	
03-155	Horned	C		
03-156	Horned	C		
03-157	Polled	B		Polled? SNP discovery population
03-158	Polled	A		
03-159	Polled	A		
03-160	Polled	A		
03-161	Polled	A		
03-163	Horned	C		
03-164	Horned	C		
03-165	Scurred	B	NO	SNP discovery population
03-166	Horned	C		
03-170	Polled	B		
03-171	Scurred	B	NO	
03-172	Horned	B		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
03-173	Scurred	B	NO	
03-174	Horned	C		
03-176	Scurred	B	NO	SNP discovery population
03-178	Polled	B		
03-179	Horned	C		
03-180	Horned	C		
03-181	Polled	B		
03-182	Polled	B		
03-184	Scurred	B	NO	
03-185	Horned	C		
03-186	Polled	B		
03-187	Scurred	C	NO	
03-188	Polled	A		
03-190	Horned	C		
03-191	Horned	C		
03-192	Scurred	B	NO	
03-193	Polled	B		SNP discovery population
03-194	Scurred	B	NO	SNP discovery population
03-195	Polled	A		
03-196	Polled	C	YES	
03-197	Horned	C		
03-198	Polled	A		
03-199	Polled	A		
03-200	Scurred	B	NO	SNP discovery population
03-201	Horned	C		
03-203	Polled	A		Polled???
03-204	Horned	B		Dehorned?
03-205	Polled	B		SNP discovery population
03-207	Polled	A		
03-208	Horned	B		Dehorned?
03-209	Polled	A		
03-210	Scurred	B	NO	
03-212	Horned	C		
03-213	Polled	C	NO	Polled???
03-215	Horned	B		Dehorned?
03-217	Polled	B		
03-218	Polled	B		
03-219	Polled	C	NO	
03-220	Horned	C		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
03-221	Horned	C		
03-222	Polled	A		
03-223	Polled	B		
03-224	Polled	C	NO	Polled???
03-225	Polled	A		
03-226	Horned	B		Dehorned?
03-227	Scurred	B	NO	
03-228	Horned	C		
03-229	Horned	C		
03-255	Horned	C		
03-256	Polled	B		
03-257	Polled	B		
03-258	Horned	C		Dehorned
03-259	Scurred	B	NO	
03-260	Polled	A		
03-261	Polled	A		
03-262	Polled	A		
03-263	Polled	A		
03-264	Polled	A		
03-265	Horned	C		
03-266	Horned	C		
03-267	Scurred	B	NO	
03-268	Polled	A		
03-269	Horned	C		Dehorned?
03-270	Polled	B		
03-271	Scurred	B	NO	
03-272	Polled	A		
03-273	Polled	A		
03-274	Horned	C		
03-275	Polled	B		
03-276	Polled	A		
03-277	Scurred	C	NO	
03-278	Polled	B		
03-279	Polled	B		
03-280	Horned	C		
03-281	Polled	A		
03-282	Polled	A		
03-283	Scurred	B	NO	
03-284	Polled	A		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
03-285	Polled	B		SNP discovery population
03-286	Polled	B		
03-287	Polled	A		
03-288	Polled	B		
03-289	Polled	A		
03-290	Polled	B		
03-291	Polled	B		
03-292	Polled	A		
03-295	Polled	A		
03-296	Scurred	B	NO	SNP discovery population
03-299	Polled	C	NO	
03-300	Polled	A		
03-301	Scurred	C	NO	
03-302	Polled	B		
03-303	Scurred	B	NO	SNP discovery population
03-304	Polled	B		
03-305	Polled	A		
03-306	Polled	B		
03-307	Scurred	B	NO	
03-308	Polled	A		
03-309	Horned	C		
03-310	Horned	B		Dehorned?
03-312	Scurred	B	NO	
03-313	Polled	B		
03-314	Horned	B		Dehorned?
03-316	Polled	B		
03-317	Polled	B		
03-318	Polled	B		
03-319	Horned	C		
03-320	Horned	C		
03-321	Polled	B		
03-322	Polled	B		
03-323	Scurred	C	NO	
03-325	Horned	B		
03-326	Scurred	C	NO	
03-327	Polled	B		
03-328	Horned	C		
03-329	Polled	B		
03-330	Horned	C		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
03-333	Scurred	B	NO	
03-334	Scurred	C	NO	
03-335	Polled	C	YES	
03-336	Scurred	B	NO	
03-337	Scurred	C	NO	
03-338	Polled	B		
03-339	Scurred	C	NO	
03-340	Polled	A		
03-341	Polled	B		
03-343	Polled	C	NO	
03-344	Polled	B		
03-346	Horned	C		
03-347	Polled	A		
03-350	Scurred	B	NO	SNP discovery population
03-366	Scurred	B	NO	
03-368	Horned	C		
03-369	Horned	C		
03-378	Scurred	B	NO	
03-381	Horned	C		
03-382	Scurred	B	NO	
03-384	Scurred	C	NO	
03-389	Scurred	B	NO	
03-390	Scurred	B	NO	
03-392	Scurred	C	NO	
03-394	Scurred	B	NO	
03-398	Horned	C		
03-400	Horned	C		Dehorned
03-401	Horned	C		
03-412	Horned	B		
03-419	Horned	C		
03-420	Scurred	B	NO	
03-423	Scurred	B	NO	
03-426	Scurred	C	NO	
03-428	Scurred	C	NO	
03-433	Scurred	B	NO	
03-435	Scurred	C	NO	
03-438	Horned	C		
03-439	Scurred	B	NO	
03-440	Scurred	C	NO	

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
03-442	Scurred	C	NO	
03-444	Horned	C		
03-445	Horned	C		
03-446	Horned	C		
03-447	Scurred	C	NO	
03-449	Horned	C		
03-450	Horned	C		
03-451	Horned	C		
03-455	Horned	C		
03-464	Scurred	C	NO	
03-465	Horned	C		
03-467	Scurred	C	NO	
03-468	Horned	B		
03-469	Scurred	B	NO	
03-470	Scurred	C	NO	
03-476	Horned	C		
03-480	Horned	B		
03-482	Scurred	B	NO	
03-488	Horned	C		
03-494	Scurred	B	NO	
03-495	Scurred	C	NO	
03-496	Scurred	B	NO	
03-497	Scurred	B	NO	
03-498	Horned	B		
03-501	Horned	C		
03-502	Horned	C		
03-503	Horned	C		
03-504	Scurred	C	NO	
03-505	Horned	C		Dehorned

Appendix C: Individual steer head phenotypes and P_C and P_F genotypes from the Livestock and Forage Center of Excellence (LFCE) in 2019. The P_C genotype is indicated by letters: A = *PP*, B = *Pp*, C = *pp*. The P_F genotype indicates the presence of an allele: YES = present, NO = not present.

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-001	Horned	C		tipped
19-002	Polled	A		
19-003	Scurred	B	NO	scab
19-004	Polled	B		
19-005	Polled	A		
19-006	Polled	A		
19-007	Polled	B		
19-008	Polled	A		
19-009	Polled	B		
19-010	Polled	A		
19-011	Polled	A		
19-012	Polled	B		
19-013	Polled	A		
19-014	Polled	A		
19-015	Polled	A		
19-016	Horned	B		dehorned
19-017	Scurred	C	NO	12.5cm and 7cm
19-018	Scurred	B	NO	2.5cm
19-019	Polled	A		
19-020	Polled	A		
19-021	Polled	A		
19-022	Polled	B		
19-023	Polled	A		
19-024	Polled	A		
19-025	Polled	B		
19-026	Polled	A		
19-027	Scurred	B	NO	0.5cm
19-028	Scurred	B	NO	2.5cm
19-029	Horned	B		dehorned
19-030	Scurred	B	NO	1.0cm
19-031	Horned	B		dehorned?
19-032	Polled	A		
19-033	Scurred	B	NO	4cm and 3.5cm
19-034	Polled	A		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-035	Polled	A		
19-036	Scurred	B	NO	11cm and 7cm bleeding from scur b/c chute
19-037	Polled	B		
19-038	Polled	A		flat
19-039	Scurred	B	NO	1.5cm
19-040	Scurred	B	NO	1.0cm
19-041	Polled	B		
19-042	Polled	B		
19-043	Scurred	B	NO	1cm
19-044	Scurred	B	NO	1.0cm and 2.0cm
19-045	Polled	B		
19-046	Horned	C		4.5cm and 9.0cm tipped/bad dehorn job
19-047	Polled	A		
19-048	Polled	B		
19-049	Scurred	B	NO	0.25cm/button
19-050	Polled	A		
19-051	Scurred	B	NO	1.0cm
19-052	Horned	C		10cm and 13cm
19-053	Scurred	B	NO	0.25/button
19-054	Polled	A		
19-055	Polled	A		
19-056	Polled	A		flat
19-057	Polled	B		flat
19-058	Polled	B		flat
19-059	Polled	B		
19-060	Polled	A		
19-061	Polled	A		
19-062	Polled	B		
19-063	Polled	A		
19-064	Polled	A		
19-065	Scurred	B	NO	scab
19-066	Scurred	B	NO	scab
19-067	Polled	A		
19-068	Polled	A		wart that feels like button in horn area
19-069	Polled	B		
19-070	Polled	A		flat
19-071	Polled	B		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-072	Polled	A		
19-073	Polled	A		
19-074	Scurred	C	NO	descurred long vertical line
19-075	Scurred	B	NO	1cm
19-076	Horned	B		dehorned
19-077	Polled	A		scab?
19-078	Polled	A		
19-079	Polled	A		
19-080	Polled	A		
19-081	Scurred	B	NO	1.0cm
19-082	Scurred	C	NO	5cm, previously descurred
19-083	Polled	B		wart near
19-084	Scurred	C	NO	descurred, long vertical line
19-085	Polled	A		
19-086	Polled	A		
19-087	Scurred	B	NO	2.0cm
19-088	Polled	A		
19-089	Polled	A		
19-090	Polled	A		
19-091	Polled	B		flat/dehorned? NOT dehorned, just a flat poll
19-092	Scurred	B	NO	1.5cm
19-093	Scurred	B	NO	scab
19-094	Polled	B		
19-095	Scurred	B	NO	button
19-096	Scurred	B	NO	2.0 3.0 cm
19-097	Horned	C		tipped
19-098	Polled	A		
19-099	Polled	A		
19-100	Polled	A		
19-101	Horned	B		dehorned
19-102	Polled	A		
19-103	Polled	B		
19-104	Polled	B		
19-105	Polled	A		
19-106	Polled	A		
19-107	Polled	B		
19-108	Polled	B		
19-109	Polled	B		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-110	Polled	B		
19-111	Scurred	C	NO	descurred
19-112	Polled	A		
19-113	Polled	B		
19-114	Polled	A		
19-115	Polled	A		
19-116	Polled	B		flat
19-117	Polled	B		
19-118	Polled	A		
19-119	Horned	C		tipped
19-120	Polled	A		
19-121	Horned	C		7.0 8.0 cm black angus
19-122	Polled	A		flat
19-123	Scurred	B	NO	button
19-124	Polled	B		
19-125	Scurred	B	NO	scab
19-126	Polled	A		
19-127	Polled	A		
19-128	Polled	A		
19-129	Polled	B		
19-130	Polled	A		
19-131	Horned	C		16cm
19-132	Polled	A		
19-133	Scurred	B	NO	scab
19-134	Scurred	B	NO	scab
19-135	Polled	B		
19-136	Scurred	B	NO	??
19-137	Horned	C		21cm
19-138	Polled	B		
19-139	Polled	B		
19-140	Scurred	C	NO	5cm
19-141	Polled	A		
19-142	Polled	A		
19-143	Polled	A		
19-144	Polled	B		
19-145	Polled	A		
19-146	Polled	A		
19-147	Polled	A		
19-148	Polled	A		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-149	Polled	A		
19-150	Polled	B		
19-151	Polled	B		
19-152	Polled	B		
19-153	Horned	C		20cm
19-154	Polled	A		
19-155	Polled	A		
19-156	Polled	A		
19-157	Polled	A		wart near horn site
19-158	Polled	A		flat
19-159	Polled	A		
19-160	Scurred	C	NO	5.0 1.5cm
19-161	Polled	A		
19-162	Polled	B		
19-163	Polled	A		flat
19-164	Polled	B		flat
19-165	Scurred	B	NO	4.0cm
19-166	Polled	A		
19-167	Polled	A		
19-168	Scurred	C	NO	button
19-169	Polled	B		
19-170	Polled	B		flat
19-171	Scurred	B	NO	4.0cm
19-172	Polled	A		
19-173	Polled	A		
19-174	Polled	A		
19-175	Polled	A		
19-176	Horned	C		tipped
19-177	Scurred	C	NO	tight
19-178	Polled	A		
19-179	Polled	A		
19-180	Polled	A		
19-181	Polled	A		
19-182	Scurred	B	NO	
19-183	Polled	A		
19-184	Polled	B		flat
19-185	Polled	A		
19-186	Polled	A		
19-187	Polled	A		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-188	Polled	A		
19-189	Scurred	B	NO	5.0cm
19-190	Polled	A		
19-191	Polled	B		
19-192	Scurred	C	NO	
19-193	Polled	B		
19-194	Scurred	B	NO	button
19-195	Polled	B		
19-196	Horned	B		dehorned
19-197	Polled	B		
19-198	Polled	A		
19-199	Horned	B		dehorned
19-200	Polled	B		
19-201	Polled	A		
19-202	Polled	A		
19-203	Scurred	C	NO	5.0cm
19-204	Polled	B		
19-205	Horned	C		dehorned/tipped
19-206	Horned	C		10cm
19-207	Polled	A		
19-208	Polled	B		
19-209	Horned	C		
19-210	Polled	A		
19-211	Polled	A		
19-212	Polled	B		
19-213	Polled	B		flat
19-214	Polled	B		
19-215	Polled	A		
19-216	Polled	A		
19-217	Scurred	B	NO	scab
19-218	Polled	A		
19-219	Horned	C		tipped
19-220	Polled	B		flat
19-221	Polled	A		
19-222	Polled	A		
19-223	Scurred	B	NO	scab?
19-224	Polled	B		
19-225	Polled	A		
19-226	Polled	A		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-227	Polled	B		
19-228	Polled	A		
19-229	Polled	B		flat
19-230	Polled	B		
19-231	Polled	A		
19-232	Polled	B		
19-233	Polled	B		
19-234	Polled	B		
19-235	Polled	A		
19-236	Polled	B		
19-237	Polled	B		
19-238	Polled	A		
19-239	Polled	A		
19-240	Polled	B		
19-241	Horned	C		
19-242	Polled	A		
19-243	Polled	A		
19-244	Polled	C	YES	
19-245	Scurred	C	NO	scur torn off in chute
19-246	Polled	A		
19-247	Scurred	B	NO	2.5cm
19-248	Horned	C		
19-249	Scurred	B	NO	scab
19-250	Polled	B		
19-251	Horned	C		
19-252	Polled	A		
19-253	Polled	B		
19-254	Polled	B		
19-255	Polled	A		
19-256	Polled	C	YES	
19-257	Scurred	B	NO	1.0cm
19-258	Scurred	B	NO	7.0cm
19-259	Polled	A		
19-260	Scurred	B	NO	10.0cm
19-261	Polled	A		
19-262	Polled	A		
19-263	Polled	B		
19-264	Polled	A		
19-265	Polled	B		flat

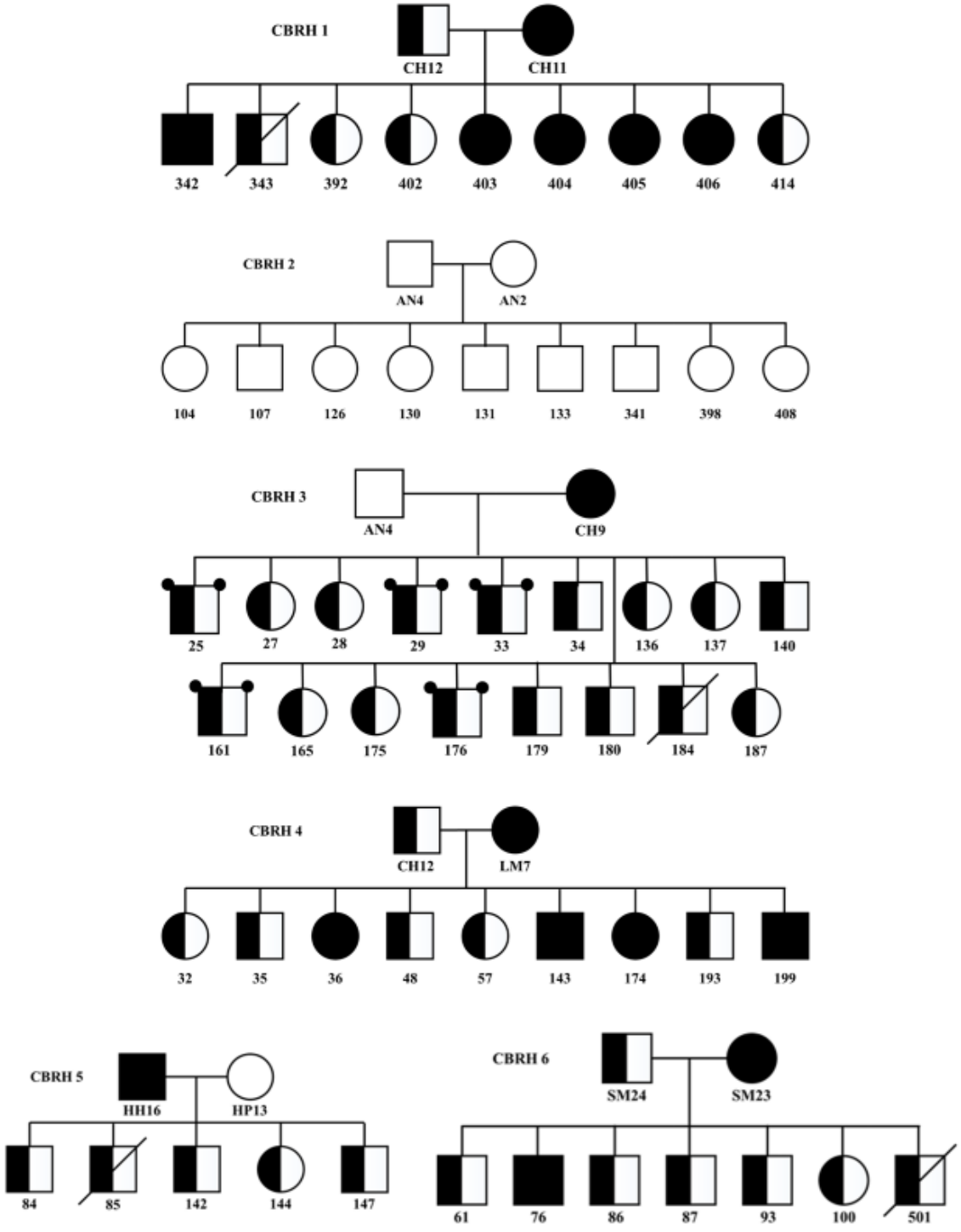
Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-266	Polled	B		
19-267	Polled	A		flat
19-268	Polled	A		
19-269	Polled	B		
19-270	Polled	A		
19-271	Polled	A		
19-272	Polled	A		
19-273	Polled	A		
19-274	Polled	B		
19-275	Polled	A		
19-276	Polled	A		
19-277	Polled	B		
19-278	Scurred	B	NO	2.5cm
19-279	Polled	A		
19-280	Scurred	B	NO	2.5cm
19-281	Scurred	B	NO	scab
19-282	Polled	B		
19-283	Horned	C		20.0cm
19-284	Polled	A		
19-285	Scurred	B	NO	scab
19-286	Polled	B		
19-287	Polled	B		
19-288	Polled	A		
19-289	Polled	B		
19-290	Polled	B		
19-291	Polled	A		flat
19-292	Scurred	B	NO	scab
19-293	Scurred	C	NO	scab
19-294	Polled	A		
19-295	Polled	A		
19-296	Polled	B		flat
19-297	Polled	B		
19-298	Polled	A		
19-299	Polled	B		
19-300	Scurred	C	NO	scab/dehorned?
19-301	Polled	B		flat
19-302	Polled	B		
19-303	Polled	B		
19-304	Polled	B		

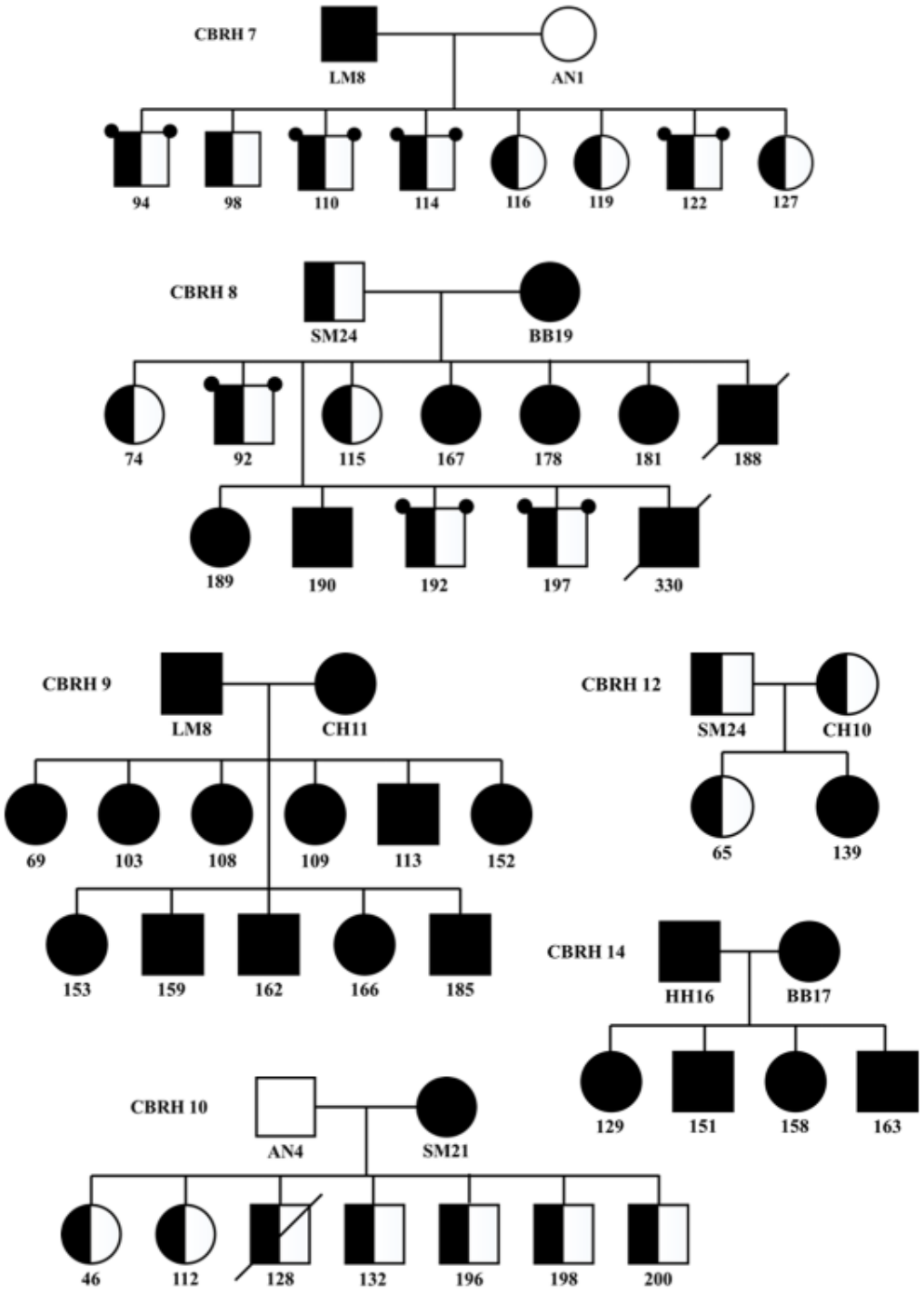
Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-305	Polled	A		
19-306	Polled	A		flat
19-307	Scurred	B	NO	2.5cm
19-308	Polled	B		
19-309	Polled	A		
19-310	Horned	B		dehorned/polled flat?
19-311	Polled	B		
19-312	Polled	B		
19-313	Polled	A		
19-314	Polled	B		
19-315	Polled	A		
19-316	Polled	A		flat
19-317	Polled	A		
19-318	Polled	A		
19-319	Polled	A		
19-320	Horned	B		dehorned
19-321	Polled	A		
19-322	Polled	B		
19-323	Scurred	B	NO	2.5cm
19-324	Polled	B		
19-325	Polled	B		
19-326	Polled	B		
19-327	Polled	A		
19-328	Scurred	B	NO	1.0cm
19-329	Polled	B		
19-330	Scurred	B	NO	2.5cm
19-331	Polled	A		
19-332	Polled	A		
19-333	Polled	A		
19-334	Polled	A		
19-335	Polled	B		flat
19-336	Polled	B		
19-337	Polled	A		
19-338	Polled	B		
19-339	Scurred	B	NO	2.5cm
19-340	Polled	A		
19-341	Polled	B		
19-342	Scurred	B	NO	button
19-343	Scurred	C	NO	scur off in chute

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-344	Polled	A		
19-345	Polled	B		
19-346	Polled	B		flat
19-347	Polled	A		
19-348	Polled	A		
19-349	Polled	B		
19-350	Scurred	B	NO	button
19-351	Polled	B		
19-352	Scurred	B	NO	5.0 2.5cm
19-353	Polled	B		
19-354	Polled	B		
19-355	Polled	B		
19-356	Scurred	B	NO	2.0cm
19-357	Polled	B		
19-358	Polled	A		
19-359	Scurred	B	NO	7.0 9.0cm
19-360	Polled	A		
19-361	Polled	B		
19-362	Scurred	C	NO	one side tight, other side loose, bad dehorn??
19-363	Polled	A		
19-364	Polled	B		
19-365	Polled	A		
19-366	Scurred	C	NO	12cm
19-367	Polled	A		
19-368	Horned	B		dehorned
19-369	Polled	A		
19-370	Polled	A		
19-371	Scurred	C	NO	button
19-372	Polled	B		
19-373	Polled	A		
19-374	Polled	A		
19-375	Scurred	B	NO	10cm
19-376	Polled	B		
19-377	Scurred	B	NO	button
19-378	Scurred	C	NO	scab
19-379	Scurred	B	NO	descurred
19-380	Scurred	B	NO	7.5cm
19-381	Polled	B		

Lab ID	Phenotype	P_C Genotype	P_F Genotype	Comments
19-382	Polled	B		
19-383	Polled	A		
19-384	Polled	B		
19-385	Scurred	B	NO	1.0cm
19-386	Polled	B		
19-387	Polled	B		
19-388	Polled	B		
19-389	Polled	B		
19-390	Polled	B		
19-391	Polled	B		
19-392	Polled	B		
19-393	Polled	B		
19-394	Scurred	B	NO	
19-395	Scurred	C	NO	10cm tight 5.0cm loose
19-396	Scurred	B	NO	2.5cm
19-397	Scurred	B	NO	3.0cm
19-398	Polled	B		
19-399	Polled	B		
19-400	Polled	B		flat
19-401	Polled	B		
19-402	Polled	B		
19-403	Scurred	B	NO	scab
19-404	Polled	B		
19-404	Scurred	B	NO	button, no blood, tail hair
19-405	Polled	B		
19-406	Scurred	B	NO	2.5cm
19-407	Polled	B		
19-408	Polled	B		
19-409	Polled	A		tail hair
19-410	Polled	A		tail hair
19-411	Polled	A		tail hair
19-412	Polled	A		tail hair
19-413	Polled	A		tail hair
19-414	Polled	A		tail hair
19-415	Scurred	B	NO	one scur coming off, tail hair, keratin sheath peeling, button
19-416	Horned	C		20.0cm, tail hair
19-417	Polled	A		tail hair
19-418	Scurred	B	NO	button, tail hair

Appendix D: Canadian beef research herd (CBRH). Adapted from Asai (2001). Males are represented by squares and females are represented by circles. White indicates animal is *PP*, black indicates animal is *pp*, and half white/half black indicates animal is *Pp*. Small black circles at top of shape indicates animal is scurred.




CBRH 7
LM8
AN1
94
98
110
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122
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BB19
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92
115
167
178
181
188
189
190
192
197
330
CBRH 9
LM8
CH11
69
103
108
109
113
152
153
159
162
166
185
CBRH 12
SM24
CH10
65
139
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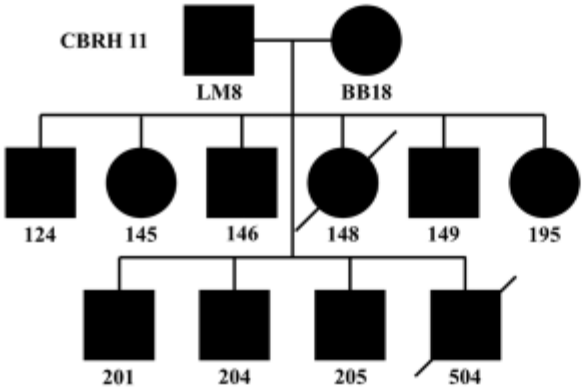
SM21
46
112
128
132
196
198
200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LM8
BB18
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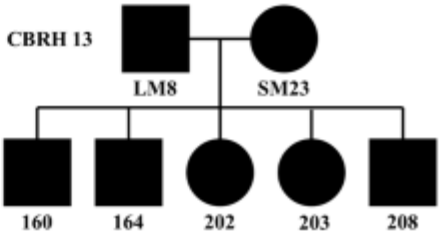
145
146
148
149
195
201
204
205
504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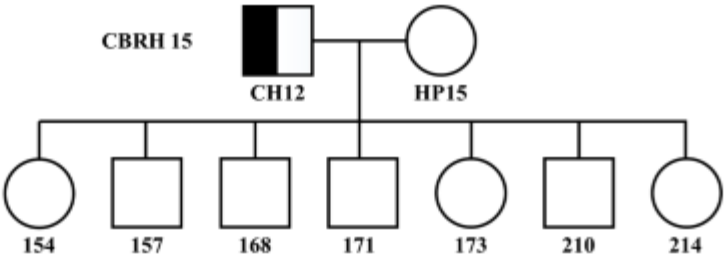
LM8
SM23
160
164
202
203
208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CH12
HP15
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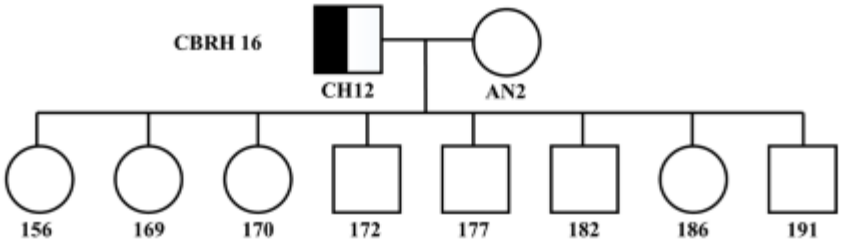
157
168
171
173
210
214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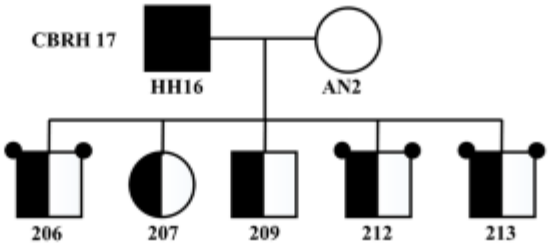
CH12
AN2
156
169
170
172
177
182
186
191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AN2
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207
209
212
213