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Markers on Bovine Chromosome 20 Associated with Carcass Quality and Composition Traits and Incidence of Contracting Infectious Bovine Keratoconjunctivitis

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MARKERS ON BOVINE CHROMOSOME 20 ASSOCIATED WITH CARCASS QUALITY AND COMPOSITION TRAITS AND INCIDENCE OF CONTRACTING INFECTIOUS BOVINE KERATOCONJUNCTIVITIS

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The objective of this study was to use single nucleotide polymorphisms (SNP) located on bovine chromosome 20 to fine map a previously identified QTL associated with the incidence of infectious bovine keratoconjunctivitis (IBK). Crossbred steers (GPE 7; n = 539) derived from sires of 7 Bos taurus breeds and having veterinary records related to IBK were used to test the association of a total of 105 SNP located under the most relevant region of the QTL. Five SNP were significantly associated with IBK ($P < 0.05$), as animals inheriting differing genotypes from individual SNP exhibited significantly different incidence rates of IBK. The population also had numerous other phenotypes, supporting evaluation of association of the 105 markers with carcass traits to identify potential antagonistic effects of implementing a marker-assisted selection program for IBK susceptibility. An association of 2 SNP for marbling and tenderness was identified, along with 3 SNP associated with the percentage of carcasses classified as choice. Four SNP were significantly associated with fat yield, 2 SNP with longissimus muscle area, and 2 additional SNP with dressing percentage. The association of these markers indicates that the evaluated QTL region may, in fact, harbor the causative mutations responsible for the variation observed in IBK susceptibility and carcass quality and composition traits. Thus, further evaluation of SNP in this region is necessary in order to identify mutations accounting for the largest degree of variation for IBK and carcass traits.

Keywords: Beef cattle; Carcass traits; Health; Pinkeye; Selection

INTRODUCTION

Although selection for disease resistance is an attractive alternative to prevent or reduce monetary losses associated with disease, undesirable genetic relationships may exist. In dairy cattle, selection for increased milk yield has been associated with

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decreased levels of fertility.[1] The phenotypic and genotypic associations between health and production traits have only recently been evaluated.[2] However, prior to incorporating health traits into selection indices, it is important to understand the antagonistic relationships that may exist between health and production traits.

Infectious bovine keratoconjunctivitis (IBK) is an economically relevant disease in cattle. Initial genetic studies indicate IBK has a genetic component, although heritability estimates are generally low, ranging from 0.00 to 0.28.[3] A QTL has been reported on bovine chromosome 20 that may harbor genes associated with the probability of contracting IBK.[4,5]

MATERIALS AND METHODS

A total of 539 F_1 crossbred steers of *Bos taurus* decent (Angus, Hereford, Gelbvieh, Simmental, Charolais, Limousin, and Red Angus) comprising Cycle 7 of the Germplasm Evaluation project (GPE 7);[6] and were used to assess the association of SNP on BTA20 with IBK and carcass traits. The population was born in 1999 and 2000, with steers harvested in 2000 and 2001. Approximately 10% of the population was treated for pinkeye ($n = 57$). Carcass traits included hot carcass weight (kg), adjusted fat thickness (cm), longissimus muscle area (cm), yield grade, percentage of animals classified as choice, dressing percentage, estimated kidney, pelvic, and heart fat, marbling score, meat tenderness, and retail, fat, and bone yield.

Calves were monitored daily by a staff veterinarian, as well as the beef cattle research technicians. Diagnosis was determined by physical examination after expression of symptoms was noted. Records included unilateral and bilateral frequency, but severity of infection was not recorded. Infection was recorded as a binary trait with animals that were treated for pinkeye coded as 1 and unaffected animals as 0. Animals that presented symptoms of IBK at least once in their lifetimes were coded as treated.

Single nucleotide polymorphism markers utilized in the current study have been previously reported.[7] Single nucleotide polymorphisms selected on chromosome 20 (supplementary Tables 1a–1d) were within a 10 megabase (Mb) region, either 5Mb upstream or downstream from the highest *F*-statistic of the IBK QTL region.[4] Genotyping was performed using a mass spectrometry-based analysis of the extension products on a MassArray system as suggested by Sequenom Inc. (San Diego, CA).

The GPE 7 population was analyzed using the Mixed Model procedure of SAS (SAS Inst., Cary, NC) with pinkeye or fat related carcass traits treated as dependent variables. The model included fixed effects of sire line, dam line, the interaction between sire line and dam line, year of birth, slaughter group within year, and BTA20 marker genotype. Weaning age was included as a linear covariate. Sire was included in the model as a random effect nested within sire line. Statistical analyses were conducted using similar methods reported in previous studies.[8]

RESULTS

Five SNP were significantly associated with incidence of IBK (Table 1). Animals inheriting the minor allele genotype or the heterozygous genotype for marker BFGL-NGS-107368 were similar in their levels of IBK incidence, but had

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Table 1 Levels of significance, numbers of animals from each genotype, least square means, and S.E. for markers significantly associated with IBK

Marker	Allele*	Minor** allele genotype frequency	Het** frequency	Major** allele genotype frequency	Total	*P*-value	Minor allele genotype mean***	Het genotype mean***	Major allele genotype mean***
BFGL-NGS-107368	C/T	6	81	262	349	0.001	0.25 ± 0.10^{a}	0.12 ± 0.03^{a}	0.02 ± 0.02^{b}
BTA-51496-no-rs	G/A	107	241	117	465	0.008	0.003 ± 0.02^{a}	0.06 ± 0.02^{b}	0.1 ± 0.02^{b}
BFGL-NGS-92754	G/A	103	193	193	489	0.02	$0.11 \pm 0.02^{a,b}$	0.03 ± 0.02^{a}	0.06 ± 0.02^{b}
rs17870710_R1	A/G	5	118	368	491	0.04	$0.0 \pm 0.1^{a,b}$	0.01 ± 0.02^{a}	0.07 ± 0.01^{b}
BTB-01950117	A/G		47	458	515	0.04		0.0 ± 0.03^{a}	0.06 ± 0.01^{b}

[a,b]Genotypes with differing superscripts indicate a significant difference of IBK incidence $P < 0.05$ within row.

*Representation of the minor allele is located on the left.

**Number of animals inheriting each genotype.

***Genotype means were calculated based on the frequency of IBK incidence within each genotype group based on binary trait information (0 = unaffected, 1 = affected).

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Table 2 Carcass traits, levels of significance, number of animals from each genotype, least square means, and S.E. for markers significantly associated with carcass composition and quality traits, by genotype

Trait*	Marker	Allele**	Minor*** allele genotype frequency	Het*** genotype frequency	Major allele*** genotype frequency	*P*-value	Total	Minor allele genotype mean	Het mean	Major allele genotype mean
Choice, %	BTB-00772611	G/A		62	327	0.02	389		0.58 ± 0.06^{a}	0.73 ± 0.03^{b}
Choice, %	BTA-51461-no-rs	G/A	112	153	116	0.02	381	0.82 ± 0.04^{a}	0.67 ± 0.04^{b}	0.69 ± 0.04^{b}
Choice, %	BTB-00775721	C/T	31	152	252	0.04	435	0.86 ± 0.08^{a}	0.71 ± 0.04^{ab}	0.65 ± 0.03^{b}
Marble****	BTA-51461-no-rs	G/A	112	153	116	0.01	381	550.71 ± 6.72^{a}	527.00 ± 5.67^{b}	533.32 ± 6.51^{b}
Dress, %	rs42819483_K	G/T	39	176	271	0.002	486	61.60 ± 0.22^{a}	61.26 ± 0.11^{a}	60.92 ± 0.09^{b}
Dress, %	Hapmap29398-BTA-134941	T/A	47	183	115	0.03	345	61.34 ± 0.21^{a}	60.89 ± 0.12^{b}	61.24 ± 0.14^{b}
Fat YD, %	rs42819483_K	G/T	39	176	271	0.008	486	$24.36 \pm 0.57^{a,b}$	25.44 ± 0.29^{a}	24.40 ± 0.25^{b}
Fat YD, %	BFGL-NGS-47410	C/G	61	196	254	0.02	511	25.96 ± 0.48^{a}	$25.04 \pm 0.29^{a,b}$	24.51 ± 0.25^{b}
Fat YD, %	BTB-00772611	G/A		62	327	0.02	389		23.75 ± 0.50^{a}	25.06 ± 0.24^{b}
Fat YD, %	BTB-00774515	T/C	60	204	160	0.02	424	25.97 ± 0.47^{a}	24.29 ± 0.28^{b}	24.81 ± 0.32^{b}
Ribeye, cm^2	BTB-00770178	G/A		157	381	0.03	538		12.97 ± 0.1^{a}	13.22 ± 0.07^{b}
Ribeye, cm^2	BFGL-NGS-76487	T/C	11	56	447	0.04	514	$13.71 \pm 0.36^{a,b}$	13.38 ± 0.17^{a}	13.06 ± 0.07^{b}
Shear, kg	rs17870710_R1	T/C	85	200	125	0.05	410	4.10 ± 0.09^{a}	4.24 ± 0.06^{b}	4.39 ± 0.08^{b}

[a,b]Means with differing superscripts indicate a significant difference of $P < 0.05$ within row.

*Choice = percentage of animals classified as choice, Dress = Dressing percentage, Fat YD = fat yield percentage, Ribeye = Rib eye area, Shear = Warner Bratzler shear force measurement.

**Representation of the minor allele is located on the left.

***Number of animals within each genotype.

****Marbling: 400 = slight, 500 = small, 600 = modest.

a significantly ($P < 0.05$) higher incidence of IBK than animals that inherited the major allele genotype. Animals that were homozygous for the minor allele genotype for markers BTA-51496-no-rs and BTB-01950117 had significantly different ($P < 0.05$) levels of IBK incidence than animals that inherited either the heterozygous genotype or the homozygous major allele genotype who were similar in their levels of IBK incidence. Furthermore, animals inheriting the homozygous minor allele genotypes from markers BFGL-NGA-92754 and rs17870710 were not significantly different in their levels of IBK incidence from animals inheriting the heterozygous or major allele genotypes. However, animals inheriting the heterozygous genotypes from these 2 markers had significantly lower rates of infection with IBK than animals inheriting the major allele genotype.

Ten unique SNP were significantly associated with carcass quality and composition, and 3 SNP were significantly associated with multiple traits (Table 2). The traits identified as being significantly associated with SNP included percentage of animals classified as choice, marbling score, dressing percent, fat yield LM area, and shear force measurements.

Animals inheriting the homozygous minor allele genotype from 7 markers had higher levels of performance for multiple carcass traits. Animals that were homozygous for the minor allele genotype had a higher percentage of animals classified as choice (BTA-51461-no-rs BTB-00775721), had a higher degree of marbling (BTA-51461-no-rs), higher dressing percentage (rs42819483_K, Hapmap29398-BTA-134941), higher fat yields (BFGL-NGS-47410, BTB-00774515), and lower shear force measurements (rs17870710_R1), indicating more tender meat (Table 2). Animals that were heterozygous for 4 of these markers (BTA-51461-no-rs, rs17870710_R1, BTB-00774515, Hapmap29398-BTA-134941) had lower levels of performance than the animals that inherited the homozygous minor allele genotypes but were similar to animals that had inherited the homozygous major allele genotype (Table 2). Animals inheriting the heterozygous genotype for 4 markers (BTB-00775721, rs42819483_K, BFGL-NGS-47410, BFGL-NGS-76487) had performance levels that were similar to their homozygous minor allele counterparts (Table 2). However, inheritance of the major allele genotype resulted in higher levels of performance in some instances. Inheritance of the major allele resulted in an increase of performance for the following traits, percentage of animals classified as choice, fat yield and LM area. However, the 2 SNP (BTB-00772611 and BTB-00770178, respectively) that accounted for this affect lacked individuals that inherited the minor allele genotype.

DISCUSSION

The importance of evaluating marker associations for multiple traits in putative QTL regions is necessary as previously observed,[9] that intensive selection for individual markers or traits can be antagonistic to other important traits. Furthermore, it was important that carcass trait association studies were conducted as previous studies[10,11] have identified QTL associated with carcass yield and quality traits located near or in the current region of interest. The hypothesis that markers in a proposed animal health QTL region could be significantly associated with other unrelated traits was further validated in the current study with the discovery of

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multiple markers associated with both IBK and fat related carcass traits. The concept that a single marker could be associated with more than a single trait was also observed. When evaluating the marker BTA-51461-no-rs, a significant association was reported for Marbling and a trend observed for IBK.

Subsequently, it could be concluded from the data presented herein that selection for alleles associated with IBK may, in fact, affect carcass traits as these markers may be closely linked. However, the advent of large SNP libraries creates a dilemma for this and future SNP association studies. Presently, there are >2,000 reported SNP residing in the presently evaluated QTL region utilized in this study. Identification of specific markers that are in coding regions or residing on candidates of known physiological function would allow future studies a more accurate methodology to conduct association analyses in this QTL region. The validation of the currently presented SNP in other populations and a greater number of SNP overall in this region is necessary to properly understand the physiological effects and properly select animals for increased disease resistance without detriment to performance. The ultimate goal of this type of research is to generate information about molecular markers that could be utilized in marker assisted selection programs. The identification of the causative mutations accounting for the largest amount of variability for these traits would not only allow for increased accuracy of selection but would also allow for focused genotyping of markers essential for selection of a specific trait. Thus, continued evaluation of this region and the markers contained within must be further evaluated prior to being implemented into a marker assisted selection program.

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Supplementary Table 1a Primers and extension probes utilized for amplification and subsequent visualization of genotypes on bovine chromosome 20

SNP	dbSNP	Forward sequence	Reverse Sequence	Extension Sequence
BFGL-NGS-112214	rs-112214	ACGTTGGATGCCATGTGGC-CAACAGTAAAC	ACGTTGGATGTCTCCAACAT-GACTGGTAGG	CTGGTTCCTGCTGCT
BFGL-NGS-47410	rs-47410	ACGTTGGATGGTAAGGCAAT-CACCTGCAAG	ACGTTGGATGACCCTT-GAAAACTTTTCTG	GCTGTGCAGATATGTGG-TATA
BFGL-NGS-76487	rs-76487	ACGTTGGATGGAGACATG-TATTGACGTTAG	ACGTTGGATGCCTGATGG-TAAGCTCTTTGC	CAGAGACATGTATTGACGT-TAGCTTTAT
BTA-51461-no-rs	rs-1638165	ACGTTGGATGGTCCTGTAGA-TAATCTGTCC	ACGTTGGATGGCTAGGA-TAATAAAGTTGCTG	TCCATGACTCAAAG-TATCTTTA
BTB-00770093	rs-7700093	ACGTTGGATGTTGTGCCGA-TAGAACTGTGC	ACGTTGGATGTCTCTG-CAACCTTAAGGTGG	GGGAAGGGAACTTGCT-CTTCTCG
BTB-00770178	rs-41929440	ACGTTGGATGTCCTTTCCCC-TAACTTCCTC	ACGTTGGATGGTTCTTTC-TAATTTTGACTCC	GCGCCTTGGGAAAAATGTA-CAAAAACA
BTB-00771394	rs-41930165	ACGTTGGATGCTGCCCA-CAAAGAGAAACTG	ACGTTGGATGA-GACCTGGCTGCCTAAATTG	GAACTCCAGGGAGCGT-GACTT
BTB-00861450	rs-42025094	ACGTTGGATGTTTCT-TAATTGGGAGGAAGG	ACGTTGGATGTTAGCAAGA-TATCCTAGAC	CGGGGAGGAAGGCTGAAA
BTB-01752678	rs-42862871	ACGTTGGATGGAAATGAG-GAATGTGGCTGG	ACGTTGGATGTCTGAGCAT-CAATCAGTGGG	GGTTGCTGGACTAACTCTT-TAAAAA
BTB-01863103	rs-42972488	ACGTTGGATGATGGCATGTT-CAGGGTGATG	ACGTTGGATGTAATGGCAT-TAGAATTCTC	GGGTGATGGTAGAACATAC
BTB-01863159	rs-42973344	ACGTTGGATGCTGGATTT-TAAATCATCTGAC	ACGTTGGATGATA-CATCCTCTTGTCTAGGG	CATCTGACATAAAAAGCT-CCTAC
BTB-01950117	rs43059001	ACGTTGGATGTCTTTGGAG-GAGCTTAAGTG	ACGTTGGATGCATCACT-CATGGTGCCTTTC	GGCGTTGGAGGAGCTTAA-GTGAACTCAT
Hapmap28557-BTA-136980	rs-136980	ACGTTGGATGACTGAGTCCA-GAACATCACC	ACGTTGGATGGGGAGATT-TAAGGAAAGAGG	TCACCCCCATCTGGC
BFGL-NGS-107368	rs-107368	ACGTTGGATGGGTAGG-GAGTTTATAATTAC	ACGTTGGATGTGACAAGA-CATTTCTTAGC	GGTGGAATGTGTTTTTTG-GAAA
BTB-00482504	rs-43687072	ACGTTGGATGAAACTTTCA-CAGCGCCACAG	ACGTTGGATGTGGGACAA-TATTTTGAGGTG	AAACATCTTATCTTCTTTT-TAGATTA

(*Continued*)

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Supplementary Table 1a Continued

SNP	dbSNP	Forward sequence	Reverse Sequence	Extension Sequence
BTB-00772611	rs-41933638	ACGTTGGATGGATCAAATA-TACACAGCTTC	ACGTTGGATGGCTGG-GAAATGACTCTCAAC	TCCAGATACACAGCTTCTG-TAAGAG
BTB-00774485	rs-41935294	ACGTTGGATGAAGAG-GAAACCCAACAACCG	ACGTTGGATGCCAGCTGA-GACTTCAACTTG	GCATCTTCTTCCCAGC
BTB-00774515	rs-41935324	ACGTTGGATGTCCTT-GAATTCCTGTAGGTG	ACGTTGGATGGTGGAAG-CAAAACAAAGCAG	TCCTGTAGGTGTCAGGA
BTB-00775400	rs-41936607	ACGTTGGATGCT-GAAGGCCCCTTACCTTAC	ACGTTGGATGCTTATTT-TAACTGAATGAAG	CTGGGTGAAATTCTCTGT
BTB-00775501	rs-41935508	ACGTTGGATGCATGGCCAAT-CATTTTGCAG	ACGTTGGATGCTAAGCTCT-GAGAACCAAAG	TCCCATCATTTTGCAGAT-GGGCTA
BTB-01085374	rs-42269361	ACGTTGGATGGATC-TATGTTGTGTCTATAGG	ACGTTGGATGTCCCT-CATCTTTTCTTTAC	TTAAGTCTTTCATAGA-TAGGGTA
BTB-01085509	rs-42240696	ACGTTGGATGTAGCT-GATTGTCAGGACAGG	ACGTTGGATGGGGTGA-GAAAAGTTATGGAG	CACAGCTGAGTTGCTA
BTB-01237744	rs-41654603	ACGTTGGATGGG-GACTTTTTTTTCCTTTGTC	ACGTTGGATGATGCTGGT-GAGCTGAACAAC	CCTCCTTTGTCTTATGAA-CTGT
BTB-01341977	rs-42463259	ACGTTGGATGCCAGCCA-CATGCCTTCTTTA	ACGTTGGATGTTACTCTGCT-GAGGGAAGTC	CCTGAATATAAACTGGAA-GGG
Hapmap29398-BTA-134941	rs-134941	ACGTTGGATGTTGCA-GATGTTCTGACAGGC	ACGTTGGATGAGCTTTCTTG-GAGCTAAGTC	TCCAGGCACAATCTATTAA-TATTTTACC
Hapmap34681-BES4_Contig350_166	rs-43708742	ACGTTGGATGCTGTGGTTT-TAGTCTGGAGG	ACGTTGGATGTTTGC-CAACTCTGGCCAAAC	AGGAATGCTTTTTATTCTT-GCT

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Supplementary Table 1b Primers and extension probes utilized for amplification and subsequent visualization of genotypes on bovine chromosome 20

SNP	dbSNP	Forward sequence	Reverse Sequence	Extension Sequence
BTB-01478441	rs-42597165	ACGTTGGATGATCCAGCTA-CATTCCCACTC	ACGTTGGATGCTTCTG-TGCGTCTTCACAGC	CCCACTCTCTAGCCT
BTA-51496-no-rs	rs-41582834	ACGTTGGATGTTCCCATCT-GAGCCTCGTTC	ACGTTGGATGGTGGGTT-GGCTGTGAATTTC	GCCACCTCCACTTTG
BTB-00473041	rs-43682486	ACGTTGGATGTTGGGTTT-GCCCCTGTGAG	ACGTTGGATGAGTACCT-GGTGCCGCCAGAA	GTGGTGCAGCTGCTT
BFGL-NGS-92754		ACGTTGGATGGAAG-CAGGTCGGAACCAAC	ACGTTGGATGTCTCCGCC-GAGAGACTGAG	ACCGCTGCTAGTTCAT
BTB-01947182	rs-43059066	ACGTTGGATGTGGTG-GAGCGGCTAATGAG	ACGTTGGATGCTTGGG-GAACCATCGGATCT	ATAATGAGCTCCGCCT
BTA-118245-no-rs	rs41664902	ACGTTGGATGCTAGGACG-CAAGTCATAACC	ACGTTGGATGATGGTG-TAAATGTCAACAGC	ACTTTCCCCTTCAACCA
BTA-51433-no-rs	rs41638151	ACGTTGGATGTCTCCATC-TAGGGACTGAAG	ACGTTGGATGGAACCTC-CACACTGTGTTAC	TGGCAGGTGGATTCTTC
BTB-00772587	rs-41932814	ACGTTGGATGGGGCATTA-CAGTGACTGGAT	ACGTTGGATGAAT-GAGCTGTCTGTGTCCTG	TCATGTCCATTTCCAGAT
BTB-00772677	rs-41933703	ACGTTGGATGCTAAACCC-CCTTCTTGGTTG	ACGTTGGATGAATC-CAGCCTCCCTTCCTTC	GGTCCCTGGTAGCCATAG
BTA-09617-rs29025775	rs-29025775	ACGTTGGATGTGAAAAAG-TAGAGGAACGGG	ACGTTGGATGATTACT-GAGCCAGCAGCAAG	CCTAACGGGGAGGAAGTA
BTB-00775721	rs-41936727	ACGTTGGATGGACTTG-TAGCTACTGGGAAG	ACGTTGGATGAGCC-CATTGCCCATTGTTTC	TCCCGATAGCAGGTAGATA
BTA-01066-rs29012033	rs-29012033	ACGTTGGATGAGGTTCA-CACTCCGCTATTC	ACGTTGGATGCAAA-GACTGCTTTCTAGCCC	TGCGCCGCTATTCCACCTTT
BTB-01749826	rs-42862631	ACGTTGGATGATGATT-GAAGGCAGACGAGG	ACGTTGGATGTACATATG-GAGGAAGGTGGG	TGCATTAACTTGATCCTCTT
ANKRA-RS541933917	rs541933917	ACGTTGGATGTTGAGCCA-GAGTTTCCGCC	ACGTTGGATGAAGTCT-CGTTCTGTCGCCAC	GGGTAGCCGCGCTGG-GAGTT

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Supplementary Table 1b Continued

SNP	dbSNP	Forward sequence	Reverse Sequence	Extension Sequence
BTA-51440-no-rs	rs-41565393	ACGTTGGATGCGACTGA-GAACACAGAATAC	ACGTTGGATGTCTATAAG-GATATAGGGGAG	GAAGGACCTATAG-GAAGTTTA
BTB-00771308	rs-41929279	ACGTTGGATGCCAG-TAATTTCTTACAATCAC	ACGTTGGATGCACTA-GAGGGAGCTCATTTC	TTTCTGAGACACA-CAAAATTTC
BTB-02048187	rs-43148862	ACGTTGGATGCCAG-CAAAAGGCACAGAAAG	ACGTTGGATGCAGGATC-CATAAACCTCCAC	AGAGCAGACAAC-CACCGTCTGT
BFGL-NGS-78615		ACGTTGGATGTCAAGGAGC-TAAGGATATGC	ACGTTGGATGGAAAGGCA-GAAAAGGCCAAG	GTAAGGATATGCTCCAGT-GAAA
BTB-00773802	rs-41936416	ACGTTGGATGACCCCGCT-CCTTCTTTTTTC	ACGTTGGATGTCCTGG-GATCCTTGGATTTC	GGGGTGCGGGG-TGCTCTGAGTC
BTB-00473193	rs-43675538	ACGTTGGATGAGAAAGT-TACCGTAAGTAGC	ACGTTGGATGCTTTAT-GAGCAACGGGAAGG	GAGGTACCGTAAGTAGC-TAGGGT
BTB-00771346	rs-41929317	ACGTTGGATGCCAGA-CAGTTTCCAAAAGGC	ACGTTGGATGCCATGTA-CAAGGAAGGCATC	CCTGCTGCATTCCTGTAA-GAACAA
BTB-00770119	rs-41938670	ACGTTGGATGCA-GATCTCTCCCTGTACATC	ACGTTGGATGACTTTT-GAAGCATACCCCCG	CTTGTGACCATTATCAG-GAAAAAT
BTA-89290-no-rs	rs-41660214	ACGTTGGATGACCTCATT-GAAAAGTTCTT	ACGTTGGATGAAAATCCC-GCATCCCATGTG	AGTTCTTTTTTTTAAGA-TATTGGG
BTB-01237795	rs-41652554	ACGTTGGATGGCGTTGTTT-CACTCTTACCC	ACGTTGGATGTATGA-GAAACCTGCTCAGTC	CCCCCCCATTGACACTTT-GACCGAA
BTB-01085475	rs-42240662	ACGTTGGATGCTTAGAG-GAAATCTGTTAG	ACGTTGGATGTCTGCT-GATCAGCCAAAAC	TAGAGGAAATCTGTTA-GAAACATAG
BTB-00774711	rs-41936620	ACGTTGGATGCCCTTTG-GAACAATGATCCC	ACGTTGGATGTGTAACT-CACTCTGCCCAAG	CTGTCACAATGATCCCTGC-TATTAGA

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Supplementary Table 1c Primers and extension probes utilized for amplification and subsequent visualization of genotypes on bovine chromosome 20

SNP	dbSNP	Forward sequence	Reverse Sequence	Extension Sequence
BTA-89292-no-rs	rs-41602924	ACGTTGGATGTCGCACA-GACTATTGTACAC	ACGTTGGATGCCATAT-GAATATAAAGAATTAC	CCACTAGCAAATGAGTAA-CATAATAA
BTA-118088-no-rs	rs-41619540	ACGTTGGATGCCTCAAAT-GACAATGATGCG	ACGTTGGATGATGGGA-CACCTCATTCCTTG	AAAGGATGCGGTAT-TATCTGTGACAA
BTB-00774932	rs-41938540	ACGTTGGATGCCTG-CTTTTCTTACCTCCAG	ACGTTGGATGTCAAG-GAAGCCTCAAATCGG	CTTGTCTTACCTCCA-GATTCTGCCATA
BTB-00473367	rs-43676704	ACGTTGGATGAGAT-GAAAAGGTTGGGCCAG	ACGTTGGATGTGACAGC-TAAAGGTCATGGG	AAGGGTGGGCCA-GAGGGTGTTCAAACC
BTB-00772564	rs-41932791	ACGTTGGATGGCCTGAC-TATCAAAAAGTGC	ACGTTGGATGGCGAT-GACTTCAACTGACAC	CCCCCCTATCAAA-AAGTGCTTTAGGAAC
BTA-05712-rs29019870	rs29019870	ACGTTGGATGGAATGGA-TAAAGATGTGCTG	ACGTTGGATGGTTGTTG-CAAAAGGCAGCAT	ATCCAGATGTGCTGTATA-CATATACAAA
BTB-02002539	rs-43104842	ACGTTGGATGAAGAT-GTGCCCCTGGTATAG	ACGTTGGATGACTTCT-CATCCATGGTCCTG	TATATAGCAGAATT-CACTGCAGATAGTA
BTB-00773933	rs-41938147	ACGTTGGATGGAACATT-CATAATGGAGGAC	ACGTTGGATGGGGAAA-AACTCAAATAGCAAG	GGATTGTGATAAATGG-TAGCTAAGTCAT
rs17870710_R2	rs17870710_R2	ACGTTGGATGATGCCA-CATGAAGATGTGGT	ACGTTGGATGCAGGGCG-CATAATATGGCAG	TACAAGCTGGCCTCA
rs17872710_Y	rs17872710_Y	ACGTTGGATGTCCAG-CAACTATTGTTTGGG	ACGTTGGATGCCTA-CAAAGTCTAGAAATGAG	GTTTGGGTGGGTGAA
rs41933914_S	rs41933914_S	ACGTTGGATGACCAAGTG-GAAATAGAATGC	ACGTTGGATGGGCT-TACCCGGTCAATTTTG	GCTTATCTTGGCACCA
rs41933866_S	rs41933866_S	ACGTTGGATGGGAATTCG-GAGAAGGCAATG	ACGTTGGATGCGTC-CATGGGATTTTCTAGG	GGCAATGGCATCCTACT
rs42819488_Y	rs42819488_Y	ACGTTGGATGGAGCT-CAATGAGTTTTCACC	ACGTTGGATGTAGGTGCT-GAGAAGGTTCTG	GGGACACATCCATGTGA
rs17870347_R1	rs17870347_R1	ACGTTGGATGCAAACAA-TAGTTGCTGGAGG	ACGTTGGATGGAGA-CAGGGCAACTATCAAG	GTTGCTGGAGGATAAGT
rs41933902_S	rs41933902_S	ACGTTGGATGGCCTGGA-GAATTACATGGAC	ACGTTGGATGCTCACT-CAGTGGTATCTGAC	TAGGAGCCTGGGTCCACG

(*Continued*)

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Supplementary Table 1c Continued

SNP	dbSNP	Forward sequence	Reverse Sequence	Extension Sequence
rs41933911_M	rs41933911_M	ACGTTGGATGACG-GAGTCGCAAAAAGAGTG	ACGTTGGATGAGAGG-CAGTAAGCCTTAACG	AAAAAGAGTGGGACAGGA
rs41933907_Y	rs41933907_Y	ACGTTGGATGCTGGGCT-GATCCTTCTTCTG	ACGTTGGATGGCAGTTA-CAGTCTAAGTGGC	CTTCTTCTGCACTGGAGTC
rs41933871_K	rs41933871_K	ACGTTGGATGAGTTCCCT-GAGTCATAGAGC	ACGTTGGATGTTTCAAAG-GACTTCCCTGGC	GCAAATTCCCAGTGG-GAAG
rs17871543_Y1	rs17871543_Y1	ACGTTGGATGTAGGT-CAGGTAACGTTCTGG	ACGTTGGATGAGGAA-GACCTTCAGATGACC	TACTAACACAAACGTC-TACT
rs41933867_W	rs41933867_W	ACGTTGGATGCACTTCC-TTCTCCAATGCAG	ACGTTGGATGCTGG-TAGGCTGCAGTCCAT	CCAGGAAAGTGAAAAGT-GAA
rs41933915_R	rs41933915_R	ACGTTGGATGGAAT-CACCTCAACCAGTCAG	ACGTTGGATGCCTGTTAT-TAGCACCCACTG	CCCCTCGGCCCTAAC-GAAATA
rs41933863_R	rs41933863_R	ACGTTGGATGCTGGCTG-GAGATACAAAAAC	ACGTTGGATGTCTACTT-CAGAATGTAAGG	ACTGTACATATA-CATTGTCTG
rs42819485_R	rs42819485_R	ACGTTGGATGACGGCAAT-GACATTAAAGGC	ACGTTGGATGTGAGA-GACCCTCCAAATTTC	TGACATTAAAGGCATT-GAACC
rs41933906_Y	rs41933906_Y	ACGTTGGATGGTATCAT-GAGGGCTGTGAAC	ACGTTGGATGACCAGG-GAATTCCCCAAAAC	GGATGGCTGTGAACCA-CATGAT
rs41933904_S	rs41933904_S	ACGTTGGATGGCTAAT-TAGCCAGAGCTAGG	ACGTTGGATGAACTTTA-GAAGAACTGAGG	CTTTACTCTCAAGGC-TAGTTTTT
rs41933870_M	rs41933870_M	ACGTTGGATGGCAGTCC-CAAAGGTTGAAAC	ACGTTGGATGTTACCAAA-GAGGCACCCAAC	GGCACAAAGGTTGAAAC-TAGACC

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Supplementary Table 1d Primers and extension probes utilized for amplification and subsequent visualization of genotypes on bovine chromosome 20

SNP	dbSNP	Forward sequence	Reverse Sequence	Extension Sequence
rs41933908_R	rs41933908_R	ACGTTGGATGATAGTCACT-GAGACAGGAAG	ACGTTGGATGACAACT-GAGCCACTGGAAAG	GCTGAGACAGGAAGAAAA-TAAAT
rs17870711_Y	rs17870711_Y	ACGTTGGATGCCCAAAACA-GAAAAATCCAG	ACGTTGGATGGGGAGATAA-GATTACAATGC	AAACATCCAGTAACTTGAA-CAACT
rs41933916_S	rs41933916_S	ACGTTGGATGAAT-GATGCCTCACAGGCGAC	ACGTTGGATGACAGAACGA-GACTTCCCTCC	GGACGGTGTCCCGGCGTCC-CAGGA
rs42819484_R	rs42819484_R	ACGTTGGATGGAGACTG-TATCCCCAACTTG	ACGTTGGATGCAGCTCTGG-TATTGTGCTTG	CCCCACTTGTTTCC-CAAATGGCTAT
rs42819487_W	rs42819487_W	ACGTTGGATGTCCTTAG-GAATCAAGGGCAG	ACGTTGGATGGAGCC-TGGGTTGTAACATTC	TTAAGAATCAAGGGCAGA-TAATTCA
rs17871567_Y	rs17871567_Y	ACGTTGGATGCATGAAT-GACTACACTTGCC	ACGTTGGATGGATG-TAATGGCAGAGGATCA	ACTAAACTTGCCTATTTAAC-TAGATC
rs41933913_R	rs41933913_R	ACGTTGGATGCACAGT-GAAGTAAAAGTTGGC	ACGTTGGATGGGATTGTTA-TAAAAATCTCGG	CAGCTAGCCCCTGTTTA-CAGTTTTAA
rs41933903_Y	rs41933903_Y	ACGTTGGATGTTG-CAGCTGTCAGAATTGCC	ACGTTGGATGGCCTAA-CATTTGCAAATGTC	TGCCTTAAACATATATGAGT-CAATTCTC
rs17871560_R	rs17871560_R	ACGTTGGATGCCCCGCAGA-CAATGTATATG	ACGTTGGATGTTTTT-GATCCTCTGCCATT	GTATCTCCAGCCAGC
rs42819483_K	rs42819483_K	ACGTTGGATGGGAGT-TATTTGTACCATGGTG	ACGTTGGATGCTTACCAT-CAAACTGACTTC	ACCATGGTGGCATTG
rs41933918_R	rs41933918_R	ACGTTGGATGTCGCAC-GAAGGTGACGTCAG	ACGTTGGATGTCCTCCTC-CACCATAGCCC	TGTGACGCAGGGAGGG
rs17870710_R1	rs17870710_R1	ACGTTGGATGTTCCCT-GAGGCCCTCATGTA	ACGTTGGATGAAAGCAAAG-CAGCAAGGACG	AGCCCCAGCACCCCGCA
rs41933869_M	rs41933869_M	ACGTTGGATGTGGCCAT-GACTTCCTGATTG	ACGTTGGATGAATGGAAG-GAAAAGCTGAAG	TTTCAACCTTTGGGACT
rs41933910_R	rs41933910_R	ACGTTGGATGGGTTTCT-CAAGCGAGAATAC	ACGTTGGATGAATGCAGGA-GATGTGGGTTC	AATACTGGAATGGGTTGC
rs17871544_Y	rs17871544_Y	ACGTTGGATGTAGGTCAGG-TAACGTTCTGG	ACGTTGGATGAGGAA-GACCTTCAGATGACC	CGTCTACTCCTGGATGCTC

(*Continued*)

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Supplementary Table 1d Continued

SNP	dbSNP	Forward sequence	Reverse Sequence	Extension Sequence
rs17872710_R2	rs17872710_R2	ACGTTGGATGAAACT-TAGTCTATCACTGG	ACGTTGGATGG-GACTCTTCTTCAGTTCCAA	AGTCTATCACTGGCTTTAA
rs17872710_R1	rs17872710_R1	ACGTTGGATGGAGACAGGG-CAACTATCAAG	ACGTTGGATGCAAACAA-TAGTTGCTGGAGG	CAGATCTGTAAATAGGTGTG
rs41933862_Y	rs41933862_Y	ACGTTGGATGTTCT-GAGTCCGGTGTTCTAC	ACGTTGGATGGTACA-TAGCTGAGTTCAAAC	CCCGTTACG-GACTGTCCTGTA
rs17870709_R2	rs17870709_R2	ACGTTGGATGCAGGGCGCA-TAATATGGCAG	ACGTTGGATGATGCCACAT-GAAGATGTGGT	CAGTGGCAGCTCAC-GATTCTC
rs17870348_Y	rs17870348_Y	ACGTTGGATGGGGTAAAAT-CAACTGATTCC	ACGTTGGATGCAGCAAC-TATTGTTTGGGTG	AAATGAGTTTGTTACTGT-TACT
rs41933901_R	rs41933901_R	ACGTTGGATGCAGCTGGT-GAACAGATAAAG	ACGTTGGATGAAACATGA-GATGATGGTAG	AAAAAGTTTCACATG-TATCTGTA
rs41933865_R	rs41933865_R	ACGTTGGATGTGTTTGCCTG-CAGCTTTTTC	ACGTTGGATGGGCAAAAG-TACTGGAGTAGG	TAAGATTATTAATG-GAATTCGGA
rs41933861_R	rs41933861_R	ACGTTGGATGCTTG-GACTTTCTATAATATG	ACGTTGGATGTG-TAAAACTGGGAGGATCAC	CCCCCATGCTAAGGGCTAT-CATTA
rs41933912_M	rs41933912_M	ACGTTGGATGACGGAGTCG-CAAAAAGAGTG	ACGTTGGATGAGAGGCAG-TAAGCCTTAACG	CCGTCATTAGGGACGAAA-CAAAAC
rs41257757_K	rs41257757_K	ACGTTGGATGTGCTAA-CACTTGATCTGAAC	ACGTTGGATGGTTCCTGAG-TAGTGAAAGGC	GGGGTGATCTGAA-CAAAAACTGAT
rs41933909_S	rs41933909_S	ACGTTGGATGCTGCATTGG-CAGGCGAATTT	ACGTTGGATGATAGTCACT-GAGACAGGAAG	GCCTTGGCAGGC-GAATTTTTTACAA
rs41933864_R	rs41933864_R	ACGTTGGATGGAGT-CACTGGTTTATAAAAA	ACGTTGGATGCATGCCA-CAAAACACTCAC	TGATGGTTTTTTTTTTTAA-TAAATGT