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Comparative mapping of human Chromosome 2 identifies segments of conserved synteny near the bovine *mh* locus

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Abstract. The ''double-muscling'' (*mh*) locus has been localized to an interval between the centromere and the microsatellite marker *TGLA44* on bovine Chromosome (Chr) 2 (BTA2). We identified segments of conserved synteny that correspond to this region of BTA2 by assigning large genomic clones containing bovine homologs of seven genes from the long arm of human Chr 2 (HSA2q). Polymorphic markers developed from these clones integrated the physical and linkage maps of BTA2 from 2q12 to 2q44 and extended genetic coverage towards the centromere. This comparative analysis suggests the *mh* locus resides on HSA2q near both the protein C and collagen type III alpha-1 genes. Overall, our data reveal a complex rearrangement of gene order between BTA2q12-44 and HSA2q14-37 that underscores the need to establish boundaries of conserved synteny when applying comparative mapping information to identify genes or traits of interest.

Introduction

The proficiency of cattle homozygous at the muscle hypertrophy locus (*mh*/*mh*) to convert feed into lean muscle and produce a higher quantity and quality of retail product per carcass (Hanset et al. 1987) makes this trait important for improving beef production efficiency. However, problems associated with the syndrome, such as reduced levels of stress tolerance, fertility, and calf viability, have hindered exploitation of this locus by ''classical'' genetic selection (Arthur 1995). Introgression of the *mh* allele through marker-assisted selection (MAS) into an alternative genetic background that neutralizes the negative aspects of the syndrome may be a more efficient method of utilizing the meat production potential of this allele.

Identification of the approximate position and construction of a detailed genetic map of the corresponding genomic region are prerequisites to either positional cloning or MAS of *mh.* A recent genome-wide linkage analysis of a Belgian Blue × Holstein herd segregating for heavy muscling suggested a microsatellite marker near the centromeric end of bovine Chromosome (Chr) 2 (BTA2); *TGLA44* was approximately 2 cM distal to the *mh* locus (Charlier et al. 1995). This information provides a starting point for map refinement of *mh.* However, current BTA2 linkage maps (Beever et al. 1994; Bishop et al. 1994; Ma et al. 1996; Barendse et al. 1994; 1997; Fisher et al. 1997), which include a total of 28 markers (6 of which represent known genes), are not sufficient for fine mapping of the *mh* locus.

Comparative mapping of positional candidate genes represents a potentially efficient way to increase marker density in the vicinity of quantitative trait loci (QTL). The overall conservation of synteny between species allows information from the gene-rich human map to be applied to low-resolution livestock maps. However, positional candidate genes can be properly selected for mapping only after identification of conserved syntenic segments, establishment of the boundaries of conservation, and evaluation of gene order changes within the candidate segment. Comparative maps available for BTA2 are based primarily on somatic cell panel studies and do not clearly identify regions of conserved synteny near the centromere. Recent Zoo-FISH data suggest that the majority of BTA2 (2q12-42) corresponds to human Chr 2q (HSA2q22-37), except for a small telomeric portion (2q44-46) that corresponds to HSA1p34-36 (Hayes 1995; Solinas-Toldo et al. 1995; Chowdhary et al. 1996). Comparison of mapped loci common to BTA2 and HSA2q suggests the existence of several differences in gene order within the conserved synteny that obscure identification of a specific HSA2q segment carrying the human homolog of *mh.* For example, the bovine assignments of inhibin beta B (INHBB; Goldhammer et al. 1995), ovine assignment of collagen type III alpha (*COL3A1;* Ansari et al. 1994), and RFLP analysis of *COL3A1* in cattle (Fisher et al. 1997) suggest that a segment of HSA2q from 2qcen-32.3 is inverted on BTA2. Assignment of interleukin 1 alpha (IL1A; O'Brien et al. 1993) and its receptor (IL1RA; Yoo et al. 1994) to BTA11 defines a break in conservation of synteny but does not precisely localize the position at which the translocation occurred during evolution. Accordingly, the segment of HSA2q that corresponds to the centromeric region of BTA2 needs to be clarified prior to selection of positional candidate genes for map refinement of *mh.*

Our objective was to apply comparative mapping to identify a region of HSA2q that corresponds to the previously reported location of *mh* on BTA2, and use this information to extend coverage towards the centromere. Seven genes located between HSA2q13-37 were selected to increase resolution of the BTA2 genetic and comparative maps. The results extend map coverage towards the centromere and indicate that BTA2 contains at least five syntenic segments of HSA2q. This analysis suggests that the human homolog of the *mh* locus could lie on either the segment containing protein C (PROC) or COL3A1.

Materials and methods

Primers. Bovine sequences were obtained from the GenBank database (Benson et al. 1993) and used to design primer pairs (Table 1) that specifically amplified exons of glucagon (*GCG*), *PROC,* transition protein 1 (*TNP1*), nebulin (*NEB*), and *COL3A1* (Lopez et al. 1983; Long et al. 1984; Kim et al. 1992; J. Killefer and M. Koohmaraie unpublished; W. Shang and J.D. Godkin unpublished, respectively). Primers that amplify a microsatellite (ms) associated with the natural resistance-associated macrophage protein (*NRAMP1*) locus in *sheep* (Pitel et al. 1996) were used to amplify the bovine analog (Table 1). The primers used to isolate the cosmid containing intestinal alkaline phosphatase (*ALPI*) were developed from a polymorphic ms within an intron of this gene (Table 1 and 2; Weissig et al. 1993). Amplification products for all loci were cloned and sequenced.

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Table 1. Primer pairs for amplification of bovine Type I loci.

Locus	Forward (Top) and Reverse (bottom) primer sequences (5′ → 3′)	Annealing temp. (°C)	Product size (bp)	Clone name	Clone type	Insert size (Kbp)	FISH assignment
ALPI	GTGGCCTGTGGAGAGAAGAG TTACTGAAGAGCTGGGTCTGC	56	181	BC43	Cosmid	ND[a]	2q44
COL3A1	CAGTGGGAATGTAAAGAAAGCC CCATCCTCCAGAACTGTGTATG	56	102	BY252/H12 BY282/B5	YAC YAC	1600–2200 1600–2200	2q12-13 (Chimeric) 2q12-13 & 12q15
GCG	TTCCCAGCTCCCCAGAC TCATCAACCACTGCACGAAG	56	142	BMC9002	Cosmid	ND	2q21-22
NRAMP1	GCCACGGGTGGGATGAGT TGAGCTAGGAGATAGCAGG	56	280	BMC9006	Cosmid	ND	2q43-44
NEB	GGCTATGAAGCCAGCAAGAC CTCCACATTCAAGGCATCG	56	150	BMC9001	Cosmid	ND	2q23-24
PROC	AGGTGGACCTGGACATCAAG AGCTTGCGCTCAGAGAGG	56	160	BMC9007	Cosmid	ND	2q12
TNP1	CCAGGTGCCCAACATAGG CCAATGCAGCTCAAGGCTAC	56	121	BY218/E10 BY279/E12	YAC YAC	1600-2200 825	2q42-43 (Chimeric) 2q42-43

[a] Not determined.

Table 2. Primer pairs for polymorphic markers associated with bovine Type I loci.

Locus[a]	Forward (top) and Reverse (bottom) primer sequences (5′ → 3′)	Annealing temp. (°C)	Allele (bp)	No. alleles (inf. meioses)	Two-point linkage analysis[b]		
			Min.-Max.		LOD	Rec. frac.	Marker
BMC9001 *(NEB)*	GAATGGATAAAGAAGATGTGGC AAATGGCATGAGTTTGTTCC	54	92–104	5 (179)	41.93	0.01	*FCB20*
BMC9002 *(GCG)*	GGCTACTTATGTGTATTACCTGAATG TCAACTCACTATTGTGTCCCC	50	118–176	4 (211)	38.39	0.04	*FCB20*
BMC9006 *(NRAMP1)*	CCTTCCAGAACTCCCTCTCC AAGGCAGCAAGACAGACAGG	60	156–162	4 (120)	31.01	0.00	*ETH152*
BMC9007 *(PROC)*	TCTGACCCCAGCTTCTGC CCACCAGGTCCCACTCTG	60	124–144	7 (260)	49.03	0.01	*TGLA44*
ALPI	GTGGCCTGTGGAGAGAAGAG TTACTGAAGAGCTGGGTCTGC	56	175–189	6 (152)	40.34	0.00	*BM2519*
TNP1[c]	AGACTTCCATCGCCCAGC AATGCAGCTCAAGGCTACCC	60	380, 540	2 (16)	4.52	0.00	*TEXAN-4, BM6444, BMS1866, AR028*
COL3A1-95[c]	CTGCCATTGCTGGTGTTG CCATCCTCCAGAACTGTGTATG	56	490, 1300	2 (18)	7.67 5.94	0.03 0.03	*ILSTS026*[d] *ILSTS026*
COL3A1-379[c]	ACTGGGGAAACGTGCATAAG CCATCCTCCAGAACTGTGTATG	56	240, 470	2 (18)	4.82	0.00	*ILSTS026*

[a] All loci listed are ms except *TNP1* and *COL3A1.* The gene associated with each ms is listed below the locus in parenthesis.
[b] Data presented only for the highest LOD score.
[c] Primers used to generate genotypes via PCR-RFLP.
[d] Two-point data for haplotype of *COL3A1-95* and *-379* (36 total informative meioses).

Isolation and analysis of large insert genomic probes for fluorescence in situ hybridization (FISH). Cosmid clones were isolated from a bovine pWE15 cosmid library (Stratagene, LaJolla, Calif.) by iterative screen/PCR based method with primer pairs listed in Table 1 (Heaton et al. 1997; T.P.L. Smith unpublished). Cosmid DNA was isolated by ionic exchange columns according to the manufacturer's protocol (Qiagen, Chatsworth, Calif.). Primer pairs for *TNP1, COL3A1,* and *TGLA44* (Georges and Massey 1992) were used to PCR screen DNA pools of the 22,944 clones in the USDA/MARC bovine YAC library (Smith et al. 1996). Bovine YAC and total yeast DNA were isolated with DNAzol (MRC Inc., Cincinnati, OH) from 50 mg of yeast cells according to the manufacturer's protocol. Metaphase chromosomes were prepared as reported (DiBerardino and Iannuzzi 1982). FISH analysis was performed essentially as described (Sonstegard et al. 1997).

Informative marker development. Microsatellites were identified and isolated from the cosmid clones used in FISH analyses of *NEB, GCG, NRAMP1,* and *PROC* as described (Alexander et al. 1996). Primer pairs developed from ms sequence (Table 2) were tested for polymorphism as described (Sonstegard et al. 1997). *TNP1* and *COL3A1* were genotyped via PCR-RFLP. The primers for *TNP1* amplified a 640-bp product (Table 2), and polymorphism was detected by *Rsa*I restriction enzyme digest. The primers for *COL3A1-95* and *COL3A1-379* amplified a 1600- and 750-bp product, respectively (Table 2). Polymorphism was detected for both products by *Alu*I restriction enzyme digest.

Linkage analysis. Marker genotypes were generated from the MARC reference population, independently scored, verified, and entered into an interactive database (Bishop et al. 1994; Keele et al. 1994). Unlikely double recombinants were evaluated, and genotypes reamplified when necessary. The markers, *COL3A1-95* and *COL3A1-379,* were treated as a haplotype for multipoint linkage analysis. However, both markers linked separately to BTA2 (LOD > 4.5) after two-point analysis (Table 2). The linkage map was constructed as described (Kappes et al. in press) with Cri-Map version 2.4 (Green et al. 1990).

Results

We first determined the approximate physical location of *TGLA44,* a microsatellite marker previously identified to be approximately 2 cM distal to the *mh* locus on BTA2 (Charlier et al. 1995), to provide a reference point for physical mapping. The YAC clone containing *TGLA44, BY321/E4,* was isolated and assigned to 2q12 (Fig. 1), establishing that the BTA2 linkage map extends close to the centromere (Fig. 2), and suggesting that *mh* is located in the interval 2q11-12.

We next determined whether a simple inversion event during the course of evolution could explain differences in gene order between HSA2q and BTA2. This model suggests genes that lie in the interval containing *COL3A1* and inhibin alpha (*INHA*) (HSA2q33-35) may map to BTA2q11-12 (Fig. 3). The possibility was tested by mapping *TNP1* (HSA2q34), *NRAMP1* (HSA2q35), and *ALPI* (HSA2q37.1). *COL3A1* was also included, since local-

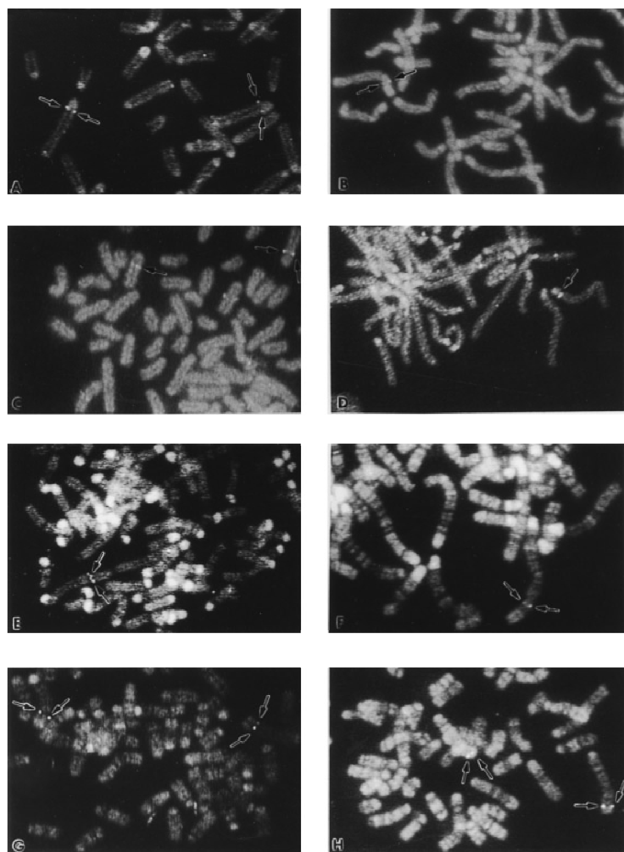

Fig. 1. FISH analysis of Chr 2 loci. A panel of eight bovine metaphase preparations showing FITC hybridization signals of selected loci to bovine Chr 2. Black arrowheads point to the location of the detected fluorescence signal of each probe on BTA2. The panel displays the following: A) *BY321/E4* (*TGLA44*) probe to 2q12, B) *BMC9007* (*PROC*) to 2q12, C) *BY282/B5* (*COL3A1*) to 2q12-13, D) *BY252/H12* (*COL3A1*) to 2q12-13, E) *BY218/E10* (*TNP1*) to 2q42-43, F) *BY279/E12* (*TNP1*) to 2q42-43, G) *BMC9006* (*NRAMP1*) to 2q43-44, and H) *BC43* (*ALPI*) to 2q44. FISH analysis of the *BMC9001* (*NEB*) and *BMC9002* (*GCG*) is presented elsewhere (Lopez-Corrales et al. 1997).

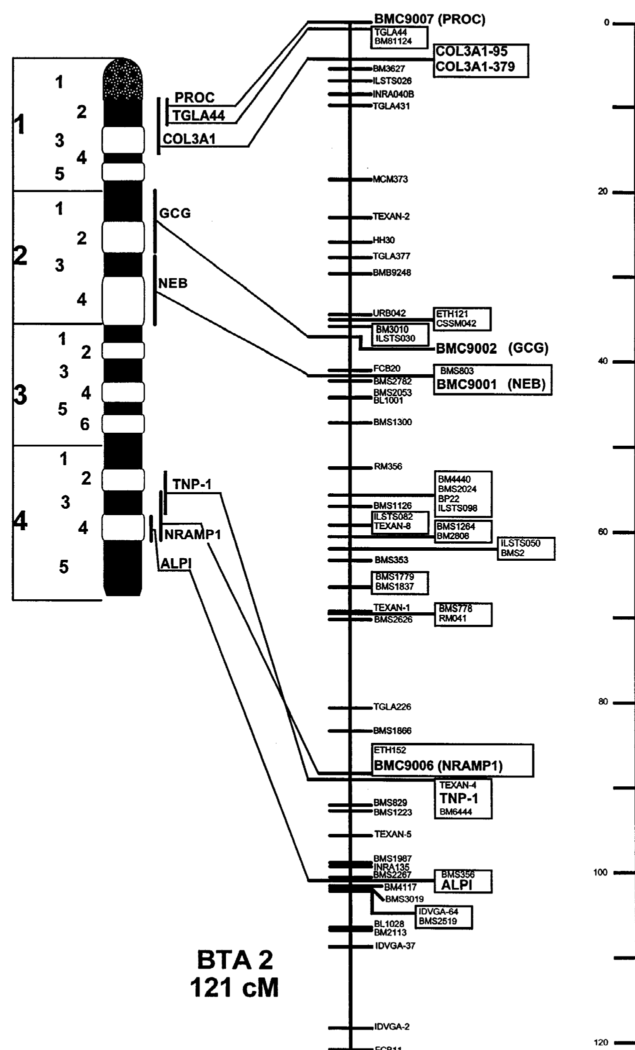

Fig. 2. Comparison of physical and genetic maps of bovine Chr 2. An R-banded ideogram of BTA2 (left) is next to a sex-averaged linkage map of BTA2 (121 cM, avg. interval 1.8 cM). A scale of genetic distance (cM) is next to the linkage map (far right). Loci assigned by FISH are indicated by vertical bars next to the ideogram. Markers developed for this study are in larger bold print.

ization of this locus to ovine Chr 2q12-21 (Ansari et al. 1994) suggested it might overlap with our assignment of *TGLA44.* An alternative to the simple inversion model suggests the human segment most likely to correspond to the proposed position of *mh* lies between *COL3A1* and *IL1RA.* In order to test this possibility, three genes located on HSA2q13-31 were physically mapped: NEB (2q24.1-24.2), GCG (2q23), and PROC (2q13-21).

Primers capable of amplifying genomic DNA of these seven genes were developed from cDNA sequence, tested, and used to screen a cosmid library to allow physical mapping. Five of the genes (*GCG, NEB, NRAMP1, ALPI, PROC*) were detected, and individual cosmid clones were isolated via iterative PCR screening (Table 1). Since *COL3A1* and *TNP1* were not detected in this cosmid library, three independent YACs carrying each target gene sequence were isolated from the USDA/MARC bovine YAC library (data not shown). All probes were assigned by FISH (Table 1 and Fig. 1), and physical assignment of YACs was confirmed with two independent probes.

Assignment of these seven loci negated the simple inversion model, and indicated that four smaller conserved syntenic segments from HSA2q are contained on BTA2 (Fig. 3). Assignment of TNP1, NRAMP1, and ALPI confirmed conservation of HSA2q34-37 (segment I; see Fig. 3) on BTA2 from 2q42-44, and correlated with previous assignment of *TNP1* and *NRAMP1* to BTA2 by somatic cell panel (Heriz et al. 1994; Feng et al. 1996, respectively) and *NRAMP1* in sheep to OAA2q41-42 by FISH (Pitel et al. 1995). A second segment of conserved synteny on BTA2 (2q21-24) extends from *GCG* to *NEB* and corresponds to HSA2q23-24.2 (segment III). These results and the assignment of *COL3A1* reveal a relatively complex rearrangement of gene order during evolution in either the bovine or human lineage. Together with the assignment of *PROC* to 2q12, the data suggest the presence of two small conserved HSA2q segments near the centromere of BTA2 (segments II and IV). The physical localization of these segments was indistinguishable from that of *TGLA44,* such that the resolution of the assignments is not sufficient to unambiguously define which chromosomal segment of conserved synteny from HSA2q lies closest to the BTA2 centromere.

Genetic markers were developed from these large insert clones (Table 2) in order to more accurately determine the location and orientation of HSA2q segments on the BTA2 comparative map. Genotypic data were merged with an existing BTA2 linkage group containing 62 informative markers (Kappes et al. 1997). The relative positions of all seven loci were consistent with their physical assignments (Fig. 2), confirming that BTA2 contains at least five HSA2q segments of conserved synteny (Fig. 3). The BTA2 seg-

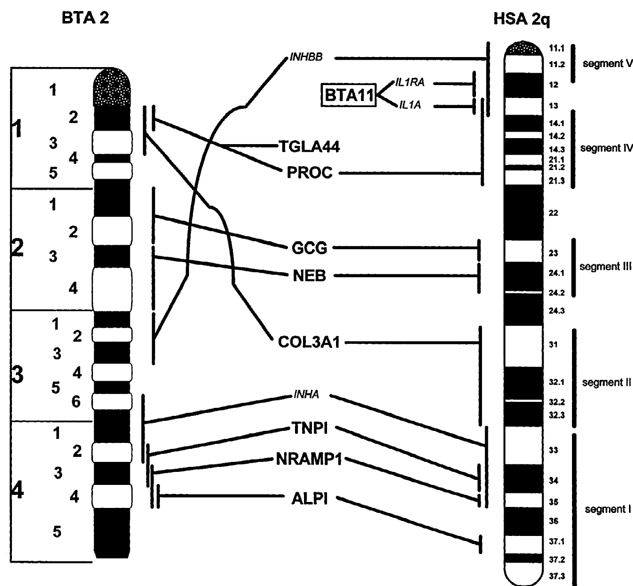

Fig. 3. Comparative map of bovine Chr 2 and human Chr 2. An R-banded ideogram of BTA2 (left) is opposite an ideogram of HSA2q (right). The FISH assignments are indicated by vertical bars next to each ideogram. Loci mapped in this study are in larger bold print. Assignments reported elsewhere (*italics*) include *IL1A* (O'Brien et al. 1993), *IL1RA* (Yoo et al. 1994), *INHA* (Brunner et al. 1995), and *INHBB* (Goldhammer et al. 1995). The vertical bars to the right of the human ideogram represent regions of segment conservation on the bovine map (e.g., segment I). Human mapping information was obtained from the human Chr 2 workshop (Spurr et al. 1996).

ments 2q21-24 and 2q42-44 corresponding to HSA2q23-24.2 and 2q34-37 appear conserved with respect to gene order and segment orientation to the centromere.

The most striking result was the placement of informative markers for *PROC* (*BMC9007*) and *COL3A1* (*COL3A1-95* and *COL3A1-379*), which positions the syntenic segments of HSA2q13-21 and HSA2q31-32 with respect to the centromere (Fig. 2). Linkage of BMC9007 extends the BTA2 linkage map an estimated 0.8 cM towards the centromere (Fig. 2) from *TGLA44* and places the segment of conserved synteny corresponding to HSA2q13-21 adjacent to the centromere. *COL3A1*-95 and *COL3A1*-379 were estimated to be 4.4 cM distal to BMC9007. Together, these results suggest the two segments of conserved synteny containing *PROC* and *COL3A1* respectively are contained within a small interval near the presumed location of the *mh* locus. Orientation of gene order within the *PROC* and *COL3A1*-containing segments will require additional comparative information.

Discussion

We have developed physical and genetic markers for bovine genes, whose human homologs were mapped to apply comparative mapping to BTA2 in the vicinity of *mh.* The addition of seven gene-associated markers more than doubled the number of linked genes on BTA2 and significantly improved the comparative map. Our results suggest HSA2q is divided into six smaller segments within the bovine genome, five of which lie on BTA2 and one on BTA11 (Fig. 3). The five segments of HSA2q are ordered 2q13-21 (segment IV), 2q31-32 (segment II), 2q23-24.2 (segment III), 2qcen-13 (segment V; Goldhammer et al. 1995), and 2q33-37 (segment I) from the centromere of BTA2, respectively. Conservation of synteny and gene order between cattle, humans, and mice is apparent for some of these fragments. For example, the bovine segment 2q42-44 that orders *TNP1, NRAMP1,* and *ALPI* is conserved on the human and mouse maps (Spurr et al. 1996). The estimated distance between *TNP1* and *ALPI* is 11.9 cM in cattle and 13.3 cM in mice (Mouse Chromosome 1 Committee, 1997). Similarly, the physical mapping of *GCG* and *NEB* to BTA2q21-22 and 2q23-24, respectively (Lopez-Corrales et al. 1997) corresponds to that in humans (Fig. 3), and the estimated genetic distance in cattle (4.6 cM) parallels that on mouse Chr 2 (6.0 cM; Mouse Chromosome 2 Committee, 1997). The resolution of the comparative map does not define the size or gene orientation for all the HSA2q segments that lie on BTA2, but future efforts should elucidate these characteristics.

The centromeric extension of the BTA2 map produced by the placement of *PROC* provides a starting point for fine mapping of *mh.* Two specific segments of the gene-rich HSA2q map can now be used to identify positional candidate genes for *mh.* Development of genetic markers from these candidates will enhance placement of an informative marker on the proximal flank of *mh* and increase overall marker density near the BTA2 centromere. Comparative mapping of some of the genes currently assigned on HSA2q between *PROC* and *IL1RA,* and near *COL3A1* (Morton et al. 1992), will orient the portion of these conserved segments that lie on BTA2 and refine the syntenic boundaries between these two segments and the HSA2q12-13 segment that maps to BTA 11.

The genotypes generated from the USDA/MARC F1 bull resource population (*mh*/+ founding sires; R.T. Stone personal communication) with markers developed in this mapping study and those generated from positional candidate genes and YACs containing *PROC, BMC9007, TGLA44, BM81124,* and *COL3A1* will allow more accurate resolution of the *mh* locus on BTA2. These markers also ensure flexibility in informative marker selection for MAS of *mh* in extant populations. A preliminary linkage analysis within this population lacked sufficient resolution to identify which syntenic segment of HSA2q near *mh* contains this trait (E. Casas personal communication). Eventually, the overall increase in marker density and informative meioses for the centromeric region of BTA2 will enhance efforts towards identification of the *mh* gene.

The complexity of gene order rearrangement that we observe within relatively short spans of overall conserved synteny for BTA2 underscores a difficulty inherent in using human map data to alleviate the lack of Type I markers on livestock maps. Despite the observed complexity, our results represent a significant step in the process of making the *mh* locus amenable to MAS and in identifying the actual gene.

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