Mammalian Genome 13, 320–326 (2002).
DOI: 10.1007/s00335-001-2133-8

Mammalian Genome
Incorporating Mouse Genome

A radiation hybrid map for the bovine Y Chromosome

Wan-Sheng Liu,[1] Paola Mariani,[2] Craig W. Beattie,[3] Leeson J. Alexander,[2] F. Abel Ponce de León[1]

[1]Department of Animal Science, College of Agricultural, Food, and Environmental Sciences, University of Minnesota, 305 Haecker Hall, 1364 Eckles Avenue, St. Paul, Minnesota 55108, USA
[2]Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Minnesota, 1971 Commonwealth Avenue, St. Paul, Minnesota 55108, USA
[3]Department of Animal Biotechnology, College of Agriculture, Biotechnology and Natural Resources, University of Nevada, Reno, Nevada 89557, USA

Received: 4 October 2001 / Accepted: 1 March 2002

Abstract. Screening a bovine Y Chromosome-specific DNA library resulted in 34 new microsatellites, six of which mapped to the pseudoautosomal region (PAR), and 28 localized to the Y-specific region. These microsatellites, together with 23 markers previously mapped to the bovine Y Chr, were scored on a 7000-rad cattle–hamster radiation hybrid (RH) panel. Retention frequency of individual markers ranged from 18.5% to 76.5% with an average of 48.4%. Markers with high retention frequency (>55%) were found to exist in multiple copies on the Y Chr. Thirteen markers were placed on the PAR RH map with the *AmelY* gene proximal to the pseudoautosomal boundary and 46 markers, including *Sry* and *Tspy* gene, on the Y-specific region of the RH map. The microsatellites developed and mapped in this work will be useful for comparative mapping of cattle, sheep, and goat, studying the origin, evolution, and migration of *bovidae* species and provide an initial platform to develop a high-resolution map of the Y Chr and positional cloning of Y-specific genes.

Introduction

The mammalian Y Chr is composed of two distinct regions, the pseudoautosomal region (PAR), a small portion that remains homologous to the X Chr, and the Y-specific region, which differs from all nuclear chromosomes. These two regions have contrasting genetic properties. The PAR pairs and recombines with the X at meiosis, while the Y-specific region does not. The non-recombining region, essentially comprised of repetitive sequences, constitutes about 95% of the Y Chr. The absence of recombination makes genetic mapping of the Y-specific region impossible, and the complexity of the repetitive sequences makes physical mapping of the Y-specific region difficult. Hence, there is no Y Chr map available so far for most of the mammalian species except human and mouse.

For many years, it was assumed that the Y Chr was a wasteland carrying no genetic information apart from the sex-determining gene *SRY* (sex-determining region on Y Chr). However, in addition to the 10 genes and the 5′ region of an 11th mapped on the PAR region (Graves et al. 1998), there are apparently more than 30 genes or gene families residing in the non-recombining region of the human Y Chr (Lahn and Page 1997). These genes play important roles not only in sex determination, but also in spermatogenesis, male fertility, and growth control (Ogata and Matsuo 1993; Lahn and Page 1997; Vogt et al. 1997; Kirsch et al. 2000). Recently the human genome-wide sequencing project has revealed that there are at least 104 genes localized on the human Y Chr (Venter et al. 2001).

There are now over 3600 genetic markers mapped in the bovine genome (Barendse et al. 1994, 1997; Bishop et al. 1994; Georges et al. 1995; Ma et al. 1996; Kappes et al. 1997). Of these, ~1200 markers are type I and 2400 are type II (*http://locus.jouy.inra.fr/cgi-bin/bovmap/intro.pl*). Over 37,000 singletons out of 170,536 bovine expressed sequence tags (ESTs) have been recently reported to the TIGR Cattle Gene Index (*http://www.tigr.org*), and 768 genes, which have human orthologs with mapping data, have been placed in an ordered cattle–human comparative map (Band et al. 2000). Unfortunately, the current linkage, physical, or comparative maps do not provide a comprehensive coverage of the bovine Y Chr (BTAY), since only 35 Y Chr markers, including 6 genes and 29 DNA fragments, have been reported in the bovine genome database (*http://locus.jouy.inra.fr/cgi-bin/bovmap/intro.pl*). Moreover, only five microsatellite markers (MS) have been mapped within the Y-Chr PAR region (Barendse et al. 1997; Kappes et al. 1997), leaving the Y Chr map incomplete.

RH mapping has proven to be a valuable technique for genome analysis (Gross and Harris 1975; Cox et al. 1990). This is a somatic cell hybrid technique that provides a direct method for ordering loci at a resolution not easily obtained by other mapping methods and is well suited for the construction of high-resolution, long-range contiguous maps of chromosomes. As such, RH mapping offers the best approach to develop a contiguous map of the BTAY non-recombining region. Two bovine whole genome RH (WG-RH) panels at 5000 and 12,000 rads (Womack et al. 1997; Rexroad et al. 2000) have been used to construct RH maps of the whole genome (Womack et al. 1997; Band et al. 2000), as well as several individual bovine chromosomes (Yang et al. 1998; Gu et al. 1999; Rexroad and Womack 1999; Rexroad et al. 1999; Amarante et al. 2000; Barendse et al. 2000), respectively.

Here, we report the construction of an RH map for BTAY. In order to increase the marker density of BTAY and construct a comprehensive Y Chr map, we first developed 34 new MS and 5 sequence-tagged sites (STSs) from a bovine Y Chr-specific library that was constructed by chromosome microdissection and microcloning (FA Ponce de León, C Carpio, unpublished data). The newly developed MS and STSs, together with 23 known Y-Chr markers, were genotyped on a 7000-rad bovine WG-RH panel (P Mariani, Y Sugimoto, CW Beattie, unpublished data) constructed recently in our laboratories. The resulting map contains 13 markers located in the PAR and 46 in the Y-specific region.

Present address for P. Mariani: C.E.R.S.A., Palazzo L.I.T.A., Via F.lli Cervi 93, 20090 Segrate (MI), Italy.

Correspondence to: F.A. Ponce de León; E-mail: apl@umn.edu

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF483744-AF483776).

Materials and methods

Library screening and microsatellite isolation. The BTAY microdissected library was prepared as described (Ponce de León et al. 1996) and screened for MS on the basis of a procedure described by Ambady et al. (1997) with some modifications. Briefly, a total of 6.6×10^4 phage clones (approximately 1× BTAY chromosome equivalent) were subjected to two rounds of screening with γ-^{32}P ATP end-labeled $(AC)_{12}$, phagemids rescued by in vitro excision and phagemid DNA purified by using the Wizard mini prep kit (Promega). MS-positive phagemids were amplified by PCR with T3/T7 flanking primer sites. To eliminate clones that contained repetitive sequences, the PCR-amplified inserts were dot-blotted on nylon membranes (Nytran®N, Schleicher and Schuell) and hybridized with ^{32}P dCTP-labeled probes of male and female bovine genomic DNA. Clones devoid of repetitive sequences were subjected to another round of PCR screening to identify clones containing MS sequences having enough flanking sequences to develop primer pairs for genotyping. Each clone was amplified with two primer combinations—T3/$(CA)_{12}$ and T7/$(CA)_{12}$. The size of the amplified product was compared with the T3/T7 amplified product, and the approximate position of the MS within the clone was determined. Selected clones were sequenced and searched for sequence similarity against sequences in GenBank. Primer pairs were designed for unique clones by using the PRIMER 2.0 software (Scientific and Educational Soft Ware, State Line, PA). Prior to use for RH mapping, all new MS were tested by PCR with male and female bovine genomic DNA as the template to optimize PCR conditions for RH mapping.

Known Y Chr markers. Thirty-five known BTAY markers were selected from the literature or sequence data available in public databases (GenBank, INRA Bovmap Datebase and Bovine ArkDB-US Node). All markers were verified by PCR amplification of bovine and hamster genomic DNA prior to use for RH mapping. Twenty-three markers, including three genes—*Sry, AmelY* (amelogenin, Y-related), and *Tspy* (testis-specific protein, Y-linked)—were finally chosen for genotyping the RH panel.

RH typing. A 7000-rad cattle–hamster WG-RH panel comprising 92 hybrids was used for RH mapping. Sixty-two markers were typed in duplicate. PCR was performed in a 96-well Techne Cyclogene thermocycler. Each reaction contained 20 ng of hybrid DNA, 1× buffer (TaKaRa, Biomedicals), 3 pmol of each primer, and 0.5 U TaKaRa Ex *Taq* DNA polymerase in a total volume of 20 μl. The amplification cycle included denaturation at 94°C for 3 min, followed by 35 cycles at 94°C for 30 s, 52°~64°C for 30 s, 72°C for 45 s, and a final extension step at 72°C for 5 min. Controls consisted of male hamster genomic DNA, male and female bovine genomic DNA, and a reaction containing no DNA. PCR products were electrophoresed in 2% agarose gels. Markers were scored as present, absent, or ambiguous.

RH map construction. Marker retention data were analyzed by the program RHMAP, version 3.0 (Boehnke et al. 1991; Lange et al. 1995). We assumed random breakage along the chromosome and equiprobable retention of fragments. The RH map was constructed in two steps. (I) Two-point linkage was computed by using the RH2PT program of the RHMAP 3.0 software with options of the haploid and diploid model. (II) Multi-point analyses and ordering of markers were performed with the RHMAXLIK program of the RHMAP 3.0 (Boehnke et al. 1991; Lange et al. 1995) at LOD scores ≥4.0 and ≥6.0.

Results

Characterization of isolated BTAY DNA sequences. Approximately 6.6×10^4 plaques from the BTAY-specific library were screened with end-labeled $(CA)_{12}$ oligos to identify clones carrying CA/GT MS repeats. In total, 284 MS-positive plaques were isolated and amplified by PCR with T3/T7 primers to determine insert size. Clone insert size ranged from 500 bp to 2.0 kb (Ave., 1.1 kb). To avoid sequencing clones containing non-microsatellite repetitive sequences, PCR products amplified from all positive clones were dot blotted and screened with radiolabeled bovine genomic DNA. This eliminated 69 clones. Prior to sequencing, the remaining 215 clones that were not positive for repetitive sequences were taken through another round of elimination process by T3/$(CA)_{12}$ and T7/$(CA)_{12}$ PCR to determine the approximate location of the MS sequence along the length of the insert. This analysis eliminated six clones containing an MS sequence too close to either the 5′ or 3′ ends.

Table 1. Classification of the BTAY sequences and their homologies.

Set and Group	Number of Clones	Homologous Sequences	Map Position	Accession Numbers
Set I a	11	BT, *TSPY*	Y (all over Y)	emb/X74028
		BTMS, INRA182	?	emb/X73935
		BT, Y repeat	Y	gb/M74507
I b	16	BTMS, IGA50	Yq	emb/X85067
		BTMS, IGA61S1	?	emb/X85076
I c	7	BTMS, AT08 (INRA057)	Y (DYS4)	emb/X71501
I d	6	BTMS4237	?	emb/Y07735
		BTMS, INRAB (INRA182)	Y	emb/X73935
I e	4	BTMS, IGA50FF	?	emb/X89422
I f	4	BTMS, AT48 (INRA113)	?	emb/X71541
I g	1	BOVRSY	Y centromere	gb/M26067
		BTMS, AT53 (INRA124)	(DYS6)	emb/X71546
I h	2	BTMS, INRBB (INRA141)	?	emb/X74168
I i	9	Ovis aries, Y repeat (OY1)	Y	gb/U30305
		Ovis aries, Y repeat (OY11.1)	Y	gb/U30307
I j	7	BT, SINE (Bov-t1, Bov2)	?	Many
Set II	51	Novel	Y	—

Since preliminary sequencing results indicated high clone redundancy for two sequences—the bovine *Tspy* gene (Matthews and Reed 1992; Vogel et al. 1997a, 1997b) and a newly identified repetitive sequence labeled clone UMN0920—the original dot blots containing the 284 clones were screened with *Tspy* and UMN0920 probes. In total, 79 clones (27.8%) were positive for *Tspy* and 52 clones (18.3%) for UMN0920. These paralogous clones differed in the number of AC/GT repeats and hence were derived from different regions of the Y Chr. Finally, 118 unique and/or low-repeat sequence clones were selected for sequencing. After BLAST search (NCBI) against sequence databases, we classified the sequences into two major sets. Set I contained 67 clones identical to previously reported sequences and was distributed in ten groups. Set II contained 51 clones of newly identified sequences distributed in 20 groups (Table 1). A group was defined by sequences that shared MS flanking sequence homology, but varying numbers of CA/GT repeats. In Set I, 75% of the sequences were localized in the bovine or ovine Y Chr, confirming the quality of our BTAY-specific DNA library. The remaining 25% of the sequences have not, as yet, been localized.

To address the question of whether different copies of the *Tspy*-MS show polymorphism, nine clones, randomly picked out of 79 found to hybridize to *Tspy,* were sequenced. Sequences flanking the MS were 99% similar, while the MS itself varied in CA repeats with several C→T and A→G transitions. At least six different variant CA repeat sequences were identified for the nine sequenced clones. Similarly, 12 of 52 clones that hybridized to the UMN0920 yielded seven different variant CA repeat sequences. Sequences of ten clones homologous to a previously reported marker IGA50 (Table 1, I b) also showed seven variants. In addition, several newly identified MS, i.e., *UMN0304, UMN2404, UMN2405, UMN2102, UMN2707* and *UMN1201,* gave different numbers of variant sequences. A common feature of these clones is that they have identical flanking sequences, but differ in the numbers of CA/GT repeat units.

Microsatellite development and mapping. In total, 45 primer pairs were designed and MS characterized by PCR amplification of male and female bovine genomic DNA. PCR amplification of an MS from male and female genomic DNA was indicative that the

MS was located in the PAR. If an MS amplified from male DNA only, it would be considered to be Y-specific. Six of the 45 new MS mapped to the PAR, and 28 to the Y-specific region (Table 2). Eleven of these MS amplified nonspecific bands, were difficult to map, and were excluded.

After analyzing the PCR products amplified from a single male and female animal, we found that 13 MS (*UMN0920, Tspy-MS, UMN0304, UMN2713, UMN2404, UMN2908, UMN0103, UMN0307, UMN1605, UMN2706, UMN1201, UMN2405, UMN2102*) amplified multiple bands or a smear (results not shown), consistent with multiple copies present on the Y Chr. These MS all localized to the Y-specific region (Table 2).

An RH map for BTAY. A panel of 92 cattle–hamster WG-RHs was typed for 62 markers, including 3 genes, 49 MS, and 10 STSs (Table 2). Retention frequency (RF) of individual markers ranged from 18.5% (*BL22A*) to 76.5% (*UMN0311*) with an average of 48.4% (Table 2). The retention frequencies of the pseudoautosomal markers were within the same range as the whole genome in the RH panel (15~50%). However, the Y-specific markers varied widely in their RFs and could be divided into three groups: (A) RF < 48%, (B) RF > 48% to RF < 55%, and (C) RF > 55%. Sequence analysis showed that group A markers were unique or low-copy sequences. Group B markers were partially homologous to the bovine Y centromeric sequence (M26067). Group C markers with extremely high retention frequency were those found to have multiple copies on the Y Chr, as described earlier.

Pairwise analysis with the RH2PT program (Boehnke et al. 1991) found that all typed markers except *UMN0803, BL22A, HEL26* and *UMN2908* tended to fall into one linkage group at a two-point (2pt) LOD score ≥4.0. However, when the LOD score was increased to ≥6.0, eight different linkage groups were identified, five of which mapped to the PAR. The other three linkage groups localized to the Y-specific region (Fig. 1). An initial RH map was constructed by using RHMAXLIK (Lange et al. 1995) and markers linked at 2pt LOD ≥6.0, while the order of linkage groups in the RH map was based on the information at 2pt LOD ≥4.0 (Fig. 1).

Thirteen markers were placed in the PAR of BTAY RH map. The largest linkage group in the PAR contained eight markers (*IOZARA1489, INRA030, UMN0929, XBM451, UMN0108, MAF45, TGLA325*, and *UMN2008*) with *UMN2008* proximal to the pseudoautosomal boundary region (PBR). The *AmelY* gene mapped near the PBR in the PAR (Fig. 1).

Forty-six markers were placed in the Y-specific region with marker *PBRF1R1A* near the PBR. Markers *BM861, INRA189,* and *BC1.2,* previously assigned to the Y Chr either by FISH or by genetic linkage analysis (Popescu et al. 1988; Schwerin et al. 1992; Kappes et al. 1997), were localized to the Y-specific region. Three markers designed from the high-mobility group (HMG) box, downstream region of HMG box, and 3′ UTR region of the bovine *Sry* gene were used in the present study and co-localized in one linkage group at 2pt LOD ≥6. Since our RH2PT pairwise analysis data demonstrated a similar linkage association between markers *Sry* and either *UMN3008 or UMN0103,* the *Sry* gene could not be localized to one specific location on the Y-RH map. Two possible locations for the *Sry* in the RH map are shown in Fig. 1. Group B Y-specific markers *UMN0910, UMN1203, UMN0301, INRA124,* and *INRA057* are centromere-related sequences located close to each other in the RH map. Group C Y-specific markers are multiple copies or repetitive sequences difficult to fine map. These markers are presented as a separate group in Fig. 1.

Discussion

We have developed and localized 34 new MS by screening a BTAY microdissected library, doubling the marker density for BTAY. This confirms that PCR-generated small insert chromosome (or chromosome region)-specific libraries are an efficient approach for developing type II genetic markers (Ponce de León et al. 1996; Ambady et al. 1997; Sonstegard et al. 1997a, 1997b).

As in most of the mammalian species, the bovine Y Chr is the smallest Chr. The PAR localizes on the distal region of Yp (Fig. 1) and is homologous to the Xq4 region (Ponce de León et al. 1996). The major portion of BTAY is composed of Y-specific sequences rich in repetitive sequences, making the development and mapping of MS from the Y-specific library technically different from libraries constructed from either the X Chr or autosomes in two important aspects. First, screening the Y-specific library with an oligo CA/GT probe usually results in more positive clones than expected, owing to an abundance of CA/GT repeats within the Y-specific repetitive sequences. Second, a Y Chr marker can be easily pre-mapped to either the PAR or the Y-specific region by PCR amplification of male and female bovine genomic DNA before applying any other more sophisticated analysis.

Our sequencing and RH mapping results indicated that many BTAY MS have multiple copies on the Y-specific region (Fig. 1). The most abundant MS—*Tspy-MS, UMN0920*, and *BTIGA50* (*UMN2102*)—were not composed solely of highly repetitive sequences as shown by dot-blot hybridization. Since metaphases from only one bull were used for the Y Chr microdissection and library construction, we conclude that the different variants found for a given MS represent different loci on the Y Chr. It has been reported that the bovine *Tspy* gene family has over 1200 copies (Matthews and Reed 1992). FISH analyses demonstrated that *Tspy*-related sequences appear to be spread over most of the Y Chr (Vogel et al. 1997a, 1997b), and the BTIGA50 probe paints the entire Yq (Mezzelani et al. 1995). In human, apart from the TSPY gene family, several other male-specific expressed gene families, such as deleted azoospermia (DAZ), RNA binding motif (RBM), and basic protein Y (BPY1 and BPY2), exist in multiple copies and clusters on the Y-specific region (Lahn and Page 1997; Gläser et al. 1998). It is likely that some of the Y-specific multicopy MS found in this study behave and are distributed in the same way as Y-specific gene families. The complexity of these loci has made physical mapping of the Y-specific region extremely difficult.

The RH panel allowed ordering of markers within the BTAY-specific region and provided the opportunity to order 62 markers on BTAY. Thirteen markers were assigned to the PAR including five MS (*HEL26, INRA030, XBM451, MAF45,* and *TGLA325*) that were mapped previously on the PAR RH maps of BTAX (Band et al. 2000; Moore et al. 2001) and on the PAR genetic linkage maps (Kappes et al. 1997; Barendse et al. 1997; Sonstegard et al. 2001). The most probable order for these five common markers determined with the 7000-rad RH panel agrees with the PAR RH map of BTAX, where the 5000-rad RH panel (BOVRH5) was used (Band et al. 2000; Moore et al. 2001). It also agrees in general with the genetic linkage maps, although markers *XBM451* and *INRA030, HEL26* and *INRA030* switched their respective positions when compared with the genetic maps of Kappes et al. (1997) (Fig. 1) and Barendse et al. (1997) respectively.

In the current BTAY RH map, the *AmelY* gene mapped closer to the PBR in the PAR, in agreement with the localization of *AmelX* to the PAR on the BTAX RH map (Band et al. 2000), confirming the PAR location of the *Amel* gene in cattle. In human, AMELX and AMELY are located in the X- and Y-specific regions very proximal to the PAR, respectively. AMELX and AMELY are active, but with a low transcription rate for AMELY (Lau et al. 1989; Graves 1995, 1998). In mice, the *Amel* gene is unique to the X Chr (Lau et al. 1989; Chapman et al. 1991), while in marsupials and monotremes, it is autosomal (Watson et al. 1992). It is clear that the *Amel* gene varies in its map position among species and is exempt from "Ohno's law" of X-chromosome conservation (Ohno 1967). This exception can be explained by the "addition-attrition"

Table 2. Summary of characterized markers on the bovine Y Chromosome.

Name of Marker	Primer Sequences [5′-3′; forward (above) and reverse (below)]	Annealing Temp. (°C)	Retention Frequency (%)	Chromosome Assignment[a]	Reference
UMN0108	GATCCATCCACATTGCTCCA CCAAGCGTCCATCAATTTAC	61	41.1	PAR	This paper
UMN0803	GATCCACATCCCCCTCAC CTGCTTCTCTTGTCCGCTAA	58	21.2	PAR	This paper
UMN2008	CAAGCATATCAGTGGCCTGG GCTGCAAGGAAACTATTTCA	61	43.5	PAR	This paper
UMN2908	GGACTGAAGCGAGTTAGCAC CACATCCCTGCTCACACACG	60	31.9	PAR	This paper
UMN0905	ATCAACCGTGGTAGCTCTAA CTAGAATGTAAACCAGCTGC	61	20.9	PAR	This paper
UMN0929	ACCAGCTGATACACAAGTGC GGTCAGAGAATGAAACAGAG	61	39.3	PAR	This paper
INRA030	ATGCAAATCTGCTACATCACCTAT CCCAACTCTCACATCCAGAT	55	40.2	PAR	Ciampolini et al. 1993
MAF45	ATTGACACTTCATAAGTTAACAATGG CAGACACAACTGAGCAACTAGCGC	58	42.9	PA	Swarbrick et al. 1992
TGLA325 (DXYS3)	GGGCACTTTACTCTCTGAACAAATC GCTGACAGTCTATTTCCAGAAGGTA	56	47.7	PAR	Georges and Massey 1992
XMB451	GTATCCATCCACAGGGTTGC TATGTAGGGACAGCCCATCC	61	38.1	PAR	Sonstegard et al. 1997a
AMELY (SE47/48)	CAGCCAAACCTCCCTCTGC CCCGCTTGGTCTTGTCTGTTGC	57	37.5	PAR	Ennis & Gallagher 1994
HEL26 (DXS29)	GCTTCTCTAAACAGGTGCCTT AGGCTACAGTCCATGGGATT	60	33.7	PAR	Vilkki et al. 1995
IOZARA1489 (DXYS4)	ATGCACCGAACACGGAATGG TGCGGCAGCGTCAAAATGAC	63	26.1	PAR	Martin-Burriel et al. 1996
XBM31	GATCCAACGGATGTTAGCAA GCCACACAGTCAAATGAATCA	58	29.3	X-specific	Ponce de leon et al. 1996
BL22	CTGCTCCCCTGACTGCCC TTTCTTCCTTTATCTGTTCAGTTGG	64	18.5 (A)[b] 27.2 (B)[b]	X-specific	Smith et al. 1997
UMN0307	GATACAGCTGAGTGACTAAC GTGCAGACATCTGAGCTGTG	58	37	Y-specific	This paper
UMN1203	AACCAGTTGCGCACTCACCA AGGCGACTTGTTCACAAGGT	55	53.9	Y-specific	This paper
UMN1514	CTTCCTGAGAGTGTTCCAGT TATTCACAAGGCCTCTGGAC	52	66.7	Y-specific	This paper
UMN2303	TACTTGCTTGAGACTTACTG TGTGAACACATCTGATTCTG	55	65.1	Y-specific	This paper
UMN3008	TTGTGGAGGACTATTCATGG TCTGGACTCGACAGGACACC	52	47.8	Y-specific	This paper
UMN0103	ACACAGAGTATTCACCTGAG ATTTACCTGGGTCAAAGCAC	60	37	Y-specific	This paper
UMN0301	GCCTTGGCTAGTGCGCAACC CAAAACTGTTGCACTGTTTC	55	57.1	Y-specific	This paper
UMN0304	TGATATTCACAAGGCCGCTG GGCTGTGGTATACTATGGAG	55	39.6	Y-specific	This paper
UMN0311	GTTGAGGTCTCTTGCATCTG CCAACAATGCTTCATCCTTC	55	76.5	Y-specific	This paper
UMN0406	GTTGAGGACTCTTGCATCTG TGCTTCATCCTTCATTCCAC	61	71.1	Y-specific	This paper
UMN0504	AGGCCATCTGCATAGTGAAG TGCTGGACTGCTCATCTCTG	61	35.2	Y-specific	This paper
UMN0705 (TSPY-MS)	TATAGCTGAGACACGTGAGT CTCCTCCAGACCTGTGTATG	64	69	Y-specific	This paper
UMN0920	GTTGAGGACTCTTGCATCTG CACAGGCCTAGAAGATTGAG	61	69.5	Y-specific	This paper
UMN1113	ACAGCACTTCTTAACAAAGC TAGCCACACATCATGTTC	58	71.6	Y-specific	This paper
UMN1201	TGCTTCATCCTTCATTCCAC TTGTTGAGGACTCTTGCATC	58	72.6	Y-specific	This paper
UMN1307	GAGTTGATATCTTGTGGCAG ACATGTCAAGGTCACACACC	60	75	Y-specific	This paper
UMN1605	CCACACTGAACTGCCTAACT AACTATCTGCTAAGGATTGG	55	74.4	Y-specific	This paper
UMN2102 (BTIGA50)	TCACCTTCCTTGTGTCATCTG GAACAAGACCGCATTGCCA	58	70.9	Y-specific	This paper
UMN2404	GGTACAATTGAAAATATG TGTACCTACACTGATATGTT	52	56.3	Y-specific	This paper
UMN2405 (BTMS4237)	CCTGCCATCCATTGTGAAGA CTGCTTACCTGGTCAGGATT	55	71.9	Y-specific	This paper
UMN2611	CCAGACTTGTGTATGCTGAG CTAAAAATAACTGACATGGG	61	64.8	Y-specific	This paper
UMN2706	TTGTTGAGGACTCTTGCATC CCACATATCAGGCAAAGTCAT	61	73	Y-specific	This paper
UMN2713	GTACCTACACTAATATGTTCA CCAAAGAAAGTTCAGGTACA	64	57.5	Y-specific	This paper

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Table 2. *Continued.*

Name of Marker	Primer Sequences [5′-3′; forward (above) and reverse (below)]	Annealing Temp. (°C)	Retention Frequency (%)	Chromosome Assignment[a]	Reference
UMN2905	CAGATAAGAATGCGAAAGTC GCAAAGTGTCAGCTTAAACC	52	60.9 (A) 72.5 (B)	Y-specific	This paper
UMN0907	CTGTTGATACTTTCTTCCTG CTGATGGACATCTGATATTC	55	70.5 (A) 68.5 (B)	Y-specific	This paper
UMN0910	AACCAGATTCTTTGTAGCCA CCTGTTTCCTAGAGACTGTG	58	48.9	Y-specific	This paper
BM861 (DYS8)	TTGAGCCACCTGGAAAGC CAAGCGGTTGGTTCAGATG	56	28.9	Y-specific	Bishop et al. 1994
INRA008 (DYS3)	GAGCCTGTGTGTGTATACAC GGCACTTTCCTCTCCTGTCGCG	58	67.9	Y-specific	Vaiman et al. 1994
INRA057 (DYS4)	CCTAGCGACTGTCCAAGCG CACGGCTGAGAATTCAAAC	58	50	Y-specific	Vaiman et al. 1994
INRA062 (DYS5)	TGTGCAGCACCTTGTCTCC ACATGCATGTGCTTGTGTCG	56	72	Y-specific	Vaiman et al. 1994
INRA124 (DYS6)	GATCTTTGCAACTGGTTTG AGGACACAGGTCTGACAATG	56	52.8	Y-specific	Vaiman et al. 1994
INRA189	TTTTGTTTCCCGTGCTGAG GAACCTCGTCTCCTTGTAGCC	58	27.2	Y-specific	Kappes et al. 1997
TSPY	CCTTGTTCATCCCGACAGAC GTGAGAACACATCCCAGCCT	58	70.5	Y-specific	Jakubiczka et al. 1993
BC1.2 (DYZ6)	ATCAGTGCAGGGACCGAGATG AAGCAGCCGATAAACACTCCTT	56	41.8	Y-specific	Schwerin et al. 1992
BRY.1 (DYZ7)	GGATCCGAGACACAGAACAGGCTGC TTGATCAAGCTAATCCATCCATCCTAT	60	67	Y-specific	Schwerin et al. 1992
BOV97M (DYS2)	GATCACTATACATACACCACTC TGATAAAAAGGCTATGCTAC	55	69.4	Y-specific	Miller & Koopman 1990
PBRF1R1	GGTAAACACAACTCAAGTGC CTTAACTGAATTAGGAGTGG	53	21.6 (A) 37 (B)	Y-specific	This paper
PBRF1R2	GGTAAACACAACTCAAGTGC ATGATAGAGGACGATATGGT	55	28.3	Y-specific	This paper
PBRR1-0907R	CTTAACTGAATTAGGAGTGG CTGATGGACATCTGATATTC	56	48.9 (A) 50 (B)	Y-specific	This paper
Sry-HMG	CCATGAACGCCTTCATTGTG GCTCTCCGACGAGGTCGATA	56	31.8	Y-specific	Kato et al. 1995
Sry-R2801	CCATGAACGCCTTCATTGTG CATATCATACAGTTTCACCATGGAC	56	31.1	Y-specific	Kato et al. 1995
Sry 3′UTR	TGGTCCTCTGTTAATCAGTTCT TGGATGTTATTAATCGCCTC	55	27.8	Y-specific	Kato et al. 1995

[a] Determined by genotyping a male and a female animal. PAR; pseudoautosomal region; Y-specific: Y-specific ration; X-specific: X-specific region.
[b] Primer pairs amplified two DNA fragments, A and B, with different retention frequency in the RH panel.

theory, since the *Amel* gene is a relic of an ancestral PAR (Graves 1995; Graves et al. 1998). Localization of the bovine *Amel* gene to the PAR near the PBR on BTAY by RH mapping not only provides evidence for the addition-attrition theory, but also demonstrates the differences in map position of this gene among cattle, human, and mouse.

In the BTAX genetic linkage maps, a group of closely linked markers (*XBM31, TGLA325, HEL26* and *BL22*) mapped to the PAR near the PBR (Ponce de León et al. 1996; Kappes et al. 1997; Sonstegard et al. 2001). However, *XBM31* maps to different positions. Kappes et al. (1997) mapped *XBM31* to the PAR, while Ponce de León et al. (1996) mapped it to the X-specific region. In the recently published consensus linkage map of the bovine sex chromosomes, *XBM31* maps to the X- and Y-specific regions (Sonstegard et al. 2001). In order to verify whether *XBM31* is an X-Y shared marker on the PAR or an X-unique marker, we ran PCR for these markers on the original DNA cocktails obtained from microdissected BTAX and BTAY from which the X and Y Chr-specific libraries were prepared. PCR results showed that the *XBM31* primer amplified from the BTAX, BTAXq, male and female genomic DNA, but not from the BTAY DNA and its library, suggestive that *XBM31* is X-unique. Conversely, *TGLA325* and *HEL26* primers amplified from all DNA samples used, confirming the PAR localization of these markers. The BL22 primer amplified two major bands (BL22A and BL22B) with a background smear from the bovine genomic DNA, smear-like bands from the BTAX, BTAXq, and BTAY specific-DNA, indicating that *BL22* is a multicopy MS and thus is difficult to map. Therefore, we conclude that *XBM31* is X-unique, whereas *TGLA325* and *HEL26* are pseudoautosomal. This conclusion is also supported by the fact that *XBM31* was placed into an X-specific linkage group proximal to the PAR in the BTAX RH map (Band et al. 2000).

The low density of single-copy markers adjacent to the *Sry* gene precluded precise mapping of *Sry* on BTAY. Our data suggest two possible positions for the *Sry:* one on the Yp near the PBR, and the other in the distal region on the Yq. Although the *Sry* gene has been previously mapped by FISH to the distal region of the Yq in cattle, sheep, and goat (Cui et al. 1995, 1996), a recent FISH study showed that the ovine *Sry* is localized to the sheep Y Chr short arm at Yp11-12 (Gill et al. 1999). Comparative mapping between cattle and sheep has revealed that two MS, *INRA030* and *MAF45,* map to the PAR in both species by linkage analysis. Combining linkage data with *Sry* FISH analysis assigns the MS and the *Sry* gene to the sheep Yp (Sheep ArkDB at *http://bos.cvm.tamu.edu/sheeparkdb.html*). Under the assumption that the *bovidae* genomes are highly conserved, we would expect the bovine *Sry* gene to be assigned to BTAYp. Our work indicated that this is possible. However, it further requires FISH analysis of the *Sry* gene based on a BAC clone, and/or increasing the marker density of the *Sry* region in the RH map to confirm this assignment.

In summary, the markers developed for this work enabled us to construct a BTAY RH map. Further characterization of these MS should provide useful information for comparative mapping of cattle, sheep, and goat. It should also aid in the study of the origin, evolution, and migration of *bovidae* species through haplotype

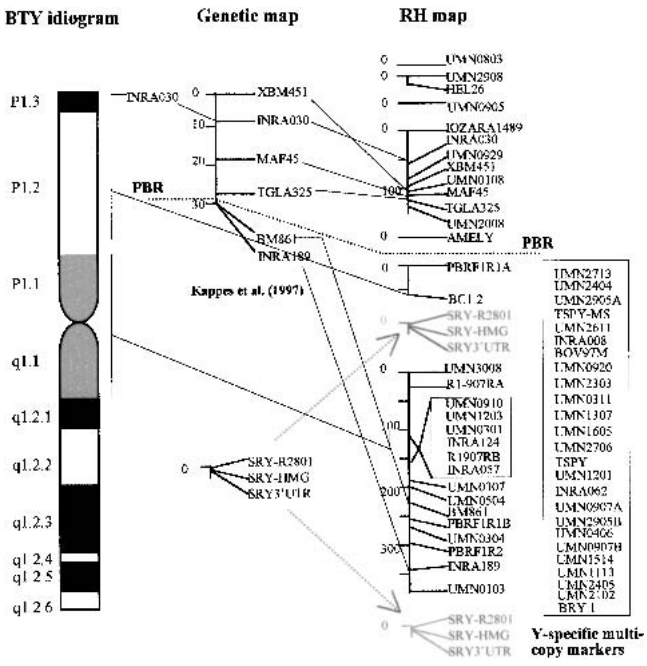

Fig. 1. RH map of the bovine Y Chr. An idiogram of the G-banded Y Chr (left); a genetic linkage map for the PAR (middle); and the RH map of the Y (right). The PBR dotted line indicates the presumptive pseudoautosomal boundary. The two possible locations of the *Sry* markers are indicated in grey. Markers in the box of the RH map are centromeric, and in the far right box are Y-specific multicopy markers, which could not be mapped on the RH map.

analysis of BTAY. This map will be useful for positional cloning of Y-specific genes and identification of Y-Chr QTLs.

Acknowledgments. We thank Dr. Sakthikumar Ambady, Mr. Yasuhiro Uno, and Ms. Paula Ramos for valuable input and Mr. Jack Otis and Mr. Donald Swiatek for technical assistance. This work was supported by a grant from USDA, NRICGP (No. 96-35205-3757) to F.A. Ponce de León.

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