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Research Article

A methodological approach for the construction of a radiation hybrid map of bovine chromosome 5

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Abstract

A bovine 5,000 rad WG-RH panel was used to construct an RH map of bovine chromosome 5 (BTA5). Twenty-one microsatellites and thirteen genes were scored in the panel using PAGE and radioactive labeling. Marker retention ranged from 8.9%-25.8% and averaged 17.8%. Pairwise locus analysis placed all markers in a single syntenic group with a LOD support of 4.0. At a LOD support of 8.0, a centromeric group of 23 syntenic markers was formed. Telomeric groups of 11 and 9 markers were assembled with a LOD support of 6.0 and 8.0, respectively. All markers were ordered by maximum likelihood methods using the program RHMAP. Only 13 markers were ordered with a LOD support of at least 3.0, while 25 and 29 markers were ordered with a support of at least 2.0 and 1.0, respectively. Total length of the comprehensive RH map was 435.9 $cR_{5,000}$, with an average marker separation of 12.8 $cR_{5,000}$. The largest gaps in the map were 55.0 and 30.4 $cR_{5,000}$ in length. The locus orders of markers common to both the RH map and the USDA-MARC linkage map were identical. The relationship between the RH and linkage maps was calculated to be 3.74 $cR_{5,000}$/cM.

Key words: RH map, bovine, gene mapping.

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Introduction

Radiation hybrid (RH) mapping is a modification of the somatic cell hybrid mapping technique that can resolve the location of genes to very small chromosomal segments. RH mapping utilizes variation between two species that are genetically more distant than those used in intra/interspecific crosses. In contrast to linkage mapping, RH mapping does not depend on the intraspecies polymorphism of markers or their segregation within a pedigree structure to map genes, but is based on the random retention of unselected donor genome fragments as insertions, translocations, and/or microchromosomes within hybrid cell lines.

Distance within a RH map is measured in centiRays (cR) and depends on the radiation dosage used to fragment the donor genome. A distance of one $cR_{5,000}$ between two markers, for example, corresponds to a 1% frequency of breakage between the markers within the hybrid cell line panel, after exposure of the donor genome to an X-radiation dosage of 5,000 rads (McCarthy *et al*., 1997). Because the frequency of breakage can be made high according to the radiation dosage applied, whole-genome RH mapping can produce high-resolution maps using a relatively small number of cell lines. For a linkage mapping study to obtain a similar resolution, a very large mapping population would be required.

The whole-genome radiation hybrid method was created by irradiating human cells and fusing them to mouse cells (Walter *et al*., 1994). Panels of human RHs provided a useful tool for high-resolution mapping of the human genome, being effectively utilized to integrate linkage and physical maps, to anchor or order large insert contigs, and to construct EST maps that currently contain more than 30,000 human genes (Deloukas *et al*., 1998).

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However, RH mapping has not been effectively used in other mammalian species, except for mouse (Schmitt *et al*., 1996; McCarthy *et al*., 1997), until a whole-genome radiation hybrid panel (RHA) was constructed by Womack *et al*. (1997) in cattle, using an X-radiation dosage of 5,000 rads. Recently, a high-dose radiation panel (12,000 rads, BovR12) has been constructed in cattle (Rexroad *et al*., 2000), and radiation panels also exist for rat (McCarthy *et al*., 2000), dog (Priat *et al*., 1998), cat (Murphy *et al*., 1999), pig (Yerle *et al*., 1998; Hawken *et al*., 1999), and zebrafish (Hukriede *et al*., 1999).

Individual RH maps of BTA1, BTA5, BTA7, BTA13, BTA15, BTA18, BTA19, BTA23, BTA27, BTA29, and BTAX have been published (Schläpfer *et al*., 1997; Band *et al*., 1998; Yang *et al*., 1998; Gu *et al*., 1999; Rexroad *et al*., 1999, 2000; Amarante *et al*., 2000; Band *et al*., 2000; Ozawa *et al*., 2000; Amaral *et al*., 2002; Ashwell *et al*., 2002; Goldammer *et al*., 2002). Additionally, a first generation whole-genome cattle RH map has also been published (Band *et al*. 2000; http://cagst.animal.uiuc.edu). Although this genome-wide RH map still contains gaps on many of the chromosomes, it represents a great advancement for the development of comparative maps, because it includes a large number of type I markers. However its utility will be subject to the establishment of the correct gene order in each map.

The objective of this study was to further develop the radiation hybrid map of BTA5 as a resource for gene mapping and cloning of economic trait loci on BTA5. For this purpose, 21 microsatellites and 13 genes located on BTA5 were scored in the bovine RHA panel. Fourteen of the markers reported here were not included in the map constructed by Band *et al*. (2000), including 5 genes that have not been mapped previously.

Materials and Methods

Scoring of markers

The 5,000-rad radiation hybrid panel (RHA, Womack *et al*., 1997), currently consisting of 90 individual cell lines, was used to develop a radiation hybrid map of BTA5. The map was constructed scoring eighteen microsatellites taken from the USDA Meat Animal Research Center (MARC, Kappes *et al*., 1997) linkage map, and three microsatellites derived from other maps. Primer information is available at http://SOL.MARC.USDA.GOV/, except for PZB2F (Senese *et al*., 1997), RM154 (Kossarek *et al*., 1996), and TEXAN15 (Burns *et al*., 1995). Additionally, 13 genes, with known human homologs, were scored on the panel (Table 1). The markers were cold-tested using the standard bovine cell line JEW38 DNA (50 ng) and the standard hamster cell line A23 DNA (50 ng), both used in the construction of the RHA panel.

To obtain a higher resolution for genotype identification, all of the genotyping was performed using "hot" PCR. For this, each forward primer was end-labeled with ^{32}P (NEN) using T_4 Polynucleotide Kinase (Promega, Madison, WI). All PCR was performed in a 30 μL volume containing panel DNA (50-100 ng), 200 μM dNTPs, 0.75 mM - 3 mM of magnesium chloride, 1X *Taq* reaction buffer, 1.0 U of *Taq* DNA polymerase, 0.1-0.7 μM ^{32}P-labeled forward and unlabeled reverse primers.

Most PCR products were run in 4.5 to 6.7% denaturing polyacrylamide gels containing 8 M urea, for 2.5 to 24 h at 15 to 50 W, depending on the size of the product. After electrophoresis, gels were blotted onto 3M filter paper, dried and exposed to X-ray film for 20 h to 10 days, depending on the radiation intensity of the PCR products as measured by a Geiger counter.

Due to the similarity in size of the amplified bovine and hamster PCR products or due to the presence of multiple bands, the loci CYP2D6, BM321, GAPD, MB, SP1 and TRA1 were run as single-strand conformational polymorphisms (SSCP) using 0.5-0.67X MDE gels (FMC Bioproducts). PCR products were electrophoresed at 5 W for 12 to 20 h, depending on the size of the products.

In addition to the genes reported here, we attempted to score the genes HOXC4 and PAH using previously published primers (Lyons *et al*., 1997), and LYZ, using primers designed from a publicly available bovine sequence (GenBank Accession No. M26242). However, these primers amplified hamster fragments of about the same size and, since the fragments were greater than 700 bp, it was not possible to use the SSCP approach to resolve the sequence identities, even though the bovine fragments were preferentially amplified, yielding stronger bands than the hamster bands when resolved in a polyacrylamide gel. The same situation occurred for MGF, however, an internal primer was designed from the partial sequence of the long fragment and, when used as the reverse primer, resulted in the amplification of species-specific fragments, with a bovine fragment of about 250 bp, and a larger mouse fragment.

Methodological approach

Most markers were run twice to detect ambiguous results. The autoradiographs were independently scored by two people, who assigned genotypes of present (+), absent (-) or ambiguous (?). Differences between scores were usually resolved by simultaneous rescoring of the autoradiographs. Unresolved differences were entered as ambiguous scores.

The RHMAP program package Version 3.0 (Boehnke *et al*., 1991; Lange *et al*., 1995) was used to analyze the data and to construct the RH map. This package assumes that: 1) radiation induced breakage occurs at random along the chromosome, with constant intensity and no interference; 2) different chromosomal fragments are retained independently in the radiation hybrids; and 3) retention probabilities are equal for all of the fragments.

Table 1 - Genes and primer information for the type I loci scored in the RH map of BTA5. Gene symbols are in accordance with the Human Gene Nomenclature database. Product sizes are approximate. The species and GenBank accession numbers of the sequences used for designing the primers used in this study are shown in the source column.

Symbol	Gene name	Annealing T °C	Product size bp	Primer Sequences	Source
COL2A1	Collagen, type II, alpha 1 chain	60	600	TGGTGGAGCAGCAAGAGC CCTTCTTGAGGTTGCCAGC	Lyons *et al.,* 1997
CYP2D6	Cytochrome P450 subfamily IID	60	600	CGGAGGATGGGCTGAAGGAA TCGGGGTGGCTGGGTCG	Bovine: X68481
GAPD	Glyceraldehyde-3-dehydrogenase	60	400	AAGGCAGAGAACGGGAAGC GGGGGCATCGGCAGAAGGT	Bovine: U85042
HMOX1	Heme oxydase	58	400	GGAGGAGGAGATTGAGCACAACA AGCGGTACAGCTGCTTGAACTTG	Jiang *et al.,* 1998
IFNG	Interferon gamma	50	300	TCCTGCCTACAATATTTGAATTT CATTGTATCATCAAGTGAAATAA	Lyons *et al.,* 1997
IGF1	Insulin-like growth factor 1	48	130	AGGAGGGAGTGCAGGAAACAAGAAC CAAGCCTGCTGAATGAATGTCTCTA	Bovine: M22044
IGFBP6	Insulin-like growth factor binding protein 6	60	450	GGAGAGAATCCCAAGGAGAG AGAAGCCCCTATGGTCACA	Human: M69055
MB	Myoglobin	60	100	GCCAAGCATCCTTCAGACTT GGAAGCCCAGCACCTTGTAC	Bovine: D00409
MGF	Mast cell growth factor (stem cell factor)	54	800, **250**	ATCCATTGATGCCTTCAAGG CTGTCATTCCTAAGGGAGCTG **CATGCCACTGATATTTGCTTTT**	Lyons *et al.,* 1997 **This study**
MYF5	Myogenic factor 5	55	250	GCCCACCCCTTGCCTCTCTG CGCCTTCCGCCGATCCAT	Bovine: M95684
SLC2A3	Solute carrier family 2 (glucose transporter protein), member 3	57	250	GATGAACCTGCTGGCCTTCATATC CTGGGCGATGAGGATGCC	Jiang *et al.,* 1998
SP1	Transcription factor SP1	50	250	CAGCAGGTGGAGAAGGAGAGAA GGGAACTCAGGGCAGGCAAAT	Human: J03133
TRA1	Tumor rejection antigen 1 (GP96)	57	300	TCCGTAAAACTCTGGACATGATC CCAGCCATGAAGTAGATTTTGTC	Jiang *et al.,* 1998

First, RH2PT was used to calculate, by maximum likelihood, the LOD score support for the pairwise association of markers. Groups of loci that showed linkage at odds of more than 1,000:1 were subsequently ordered by maximum likelihood, using the program RHMAXLIK. The options specifying an equal retention probability model and the machine-generated order for adding loci, rather than the user-generated order, were used.

Three approaches were used to determine the preliminary best order of loci to minimize computing time. First, the stepwise option was used for building locus orders including all the loci that showed linkage at a LOD score of 3.00. This option allows the determination of overall locus order, one locus at a time, but retains only those partial orders which are within a user-specified LOD score (SAVMAX) of the current best partial order (Boehnke *et al*., 1991). In general, this approach considers many fewer partial orders at the risk of missing the overall best order, but considerably reduces the required computing time. Larger values of SAVMAX increase the chance of identifying the best overall order, but imply a heavier computational burden.

The second employed approach utilized the branch and bound option, with groups of 14 markers at a time. The runs were initiated with the 14 most centromeric markers, with a moving frame of one marker towards the telomere for each subsequent analysis, and using the best locus order obtained in the stepwise approach. This method identifies early in the process a candidate order that is optimal, or nearly so, and eliminates solutions that are inferior either to the candidate order or to better orders subsequently encountered. However, the number of orders evaluated scales exponentially with the number of loci to be ordered, and is computationally prohibitive when all markers are simultaneously analyzed. With a large number of loci, the approach of partial runs with a sliding frame makes the analysis manageable.

The third approach was to analyze, with the stepwise option and no LOD support restriction, maps with all but one marker, deleting each marker one at a time, in order to identify problematic and/or the most influential markers for specific orders.

When the best order was obtained, the scoring data were rearranged according to the locus order, and a reevaluation of the scoring was performed. All of the scores which produced fragments containing only a single marker, as well as fragments containing the scores +-+, +?+ and ++?, were reexamined on the autoradiographs. The scoring of

the most influential hybrid cell lines for the best and next-to-the-best orders was also reexamined.

Results

Twenty-one microsatellite markers and 13 genes with known human homologs were scored on the bovine RHA panel. Here we report the assignment and ordering of 7 microsatellite markers and 7 genes not included in the RH map constructed by Band *et al*. (2000). Of these markers, only CSSM22, SLC2A3, and CYP2D6 have previously been scored in the RHA panel (Barendse *et al*., 2000), but a definitive order was not established. Mapping of the genes MGF, IGFBP6, TRA1, HMOX1, and GAPD is first reported in this study. These genes have well characterized human and mouse homologs that will expand the comparative maps among these species.

The percentage of radiation hybrid lines definitively typed (+ or -) ranged from 95.6 to 100%, with an average of 99.1% (Tables 2 and 3). Of the 90 hybrid lines in the RHA panel, 52.2% were informative for the scored markers, 45.6% retained none of the markers, and 2 hybrids retained all of the scored markers, although one had one untyped

Table 2 - Features of the RH map of BTA5. The percentage of lines typed is for the lines with + or - scores. Linkage groups formed in the 2-point analysis at different LOD supports are represented by bars. The position of the markers is according to the order in the comprehensive map, which represents the most likely marker order obtained with no LOD support restriction.

Marker	Percentage typed	Percentage retained	Linkage groups (LOD)			Comprehensive map position	LOD support
			4.00	6.00	8.00		
ILSTS042	98.9	22.5				0	>3.0
BM6026	98.9	21.3				4.1	<1.0
MYF5	97.8	20.5				19.7	>2.0
BP1	98.9	21.3				27.5	>2.0
MGF	95.6	19.8				43.1	>3.0
BL23	96.7	21.8				46.6	>2.0
IGFBP6	98.9	23.6				60.5	>3.0
BM321	98.9	22.5				64.4	>2.0
SP1	100.0	22.2				64.4	>2.0
CSSM34	97.8	19.3				67.7	>3.0
C0L2A1	97.8	20.5				67.7	>3.0
IFNG	100.0	17.8				94.8	>3.0
RM500	100.0	16.7				114.4	>3.0
BMS490	100.0	16.7				129.9	>1.0
ETH10	100.0	20.0				148.9	>3.0
RM154	100.0	16.7				167.9	>3.0
CSSM22	100.0	14.4				175.5	>3.0
IGF1	98.9	14.6				192.0	>2.0
TRA1	100.0	14.4				199.8	>3.0
HMOX1	100.0	14.4				216.3	>3.0
MB	100.0	15.6				220.1	>1.0
TEXAN15	100.0	17.8				227.5	<1.0
BM1819	98.9	16.9				227.5	<1.0
BMS1248	100.0	12.2				282.5	>2.0
BM8230	100.0	10.0				300.4	>2.0
BM315	100.0	8.9				325.1	<1.0
SLC2A3	95.6	10.5				330.9	>3.0
PZB2F	100.0	13.3				342.2	>1.0
JAB2	100.0	13.3				359.1	>2.0
GAPD	100.0	13.3				359.1	>2.0
MAF48	100.0	16.7				379.8	>2.0
ETH152	97.8	24.7				410.1	>1.0
CYP2D6	98.9	24.7				420.9	>2.0
BM8126	98.9	25.8				435.9	>1.0
Average	**99.1**	**17.8**				**12.8**	

Table 3 - Scoring data for the informative cell lines for the markers used in the construction of the RH map of BTA5. Loci are placed according to their most likely order determined by maximum likelihood methods. Hybrid cell lines for which all markers were absent are not shown. Boldface markers were scored by Barendse *et al*. (2000).

Line n.	ILST42	BM6026	MYF5	**SYT1**	BP1	MGF	BL23	**AGLA293**	IGFBP6	BM321	SP1	CSSM34	COL2A1	**LALBA**	IFNG	RM500	BMS490	ETH10	RM154	CSSM22	IGF1	TRA1	HMOX1	MB	TEXAN15	BM1819	BMS1248	BM8230	BM315	SLC2A3	PZB2F	**ETH2**	**CD9**	JAB2	GAPD	**TPI1**	MAF48	ETH152	CYP2D@	BM8126	**BZRP**
5	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
15	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	?	-	-	-	-	-	-	+	+	+	-	-
21	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	-	+	+	-	-	?	+	+	-	-	-	-	+	+	+	+	-
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27	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
28	-	-	-	-	-	-	-	+	+	+	+	+	+	-	+	-	-	-	-	-	?	-	-	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
34	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
38	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
40	-	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+
42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-
45	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	?	-	-	-	+	+	+	+	+	-	-	-
46	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
47	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+	-	+	+	+	-	-	-	-
51	+	+	+	+	+	+	+	+	+	+	+	?	?	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
52	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
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54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
56	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
58	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
59	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	?	-	-	-	-	-	-	+	+	+	+	+
62	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	+	-	-	-	-	-	-	-	-
65	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	?	+	-
68	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
69	+	?	-	-	?	-	-	-	-	?	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
70	+	+	+	-	+	?	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
72	-	-	-	-	+	?	+	-	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
74	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	?	-	+	+	+
75	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	+
76	+	+	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
79	+	+	?	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
80	+	+	+	-	+	+	+	+	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
81	+	+	+	+	+	+	?	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
82	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
83	-	-	-	-	-	+	+	+	+	+	+	?	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
87	?	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
89	-	-	+	+	+	?	?	-	-	-	-	-	?	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	
90	+	+	+	+	+	?	?	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?	+	+	+	

marker. Marker retention ranged from 8.9% for BM8230 to 25.8% for BM8126, with an average retention frequency of 17.8% (Table 2).

Using a likelihood ratio test, the hypothesis that retention frequencies were equal for each marker could not be rejected ($\chi^2_{33} = 41.5$; p 0.10). However, the average retention frequencies were 21.4% for the sub-centromeric (ILSTS042-COL2A1), 16.3% for the proximal interstitial (IFNG-HMOX1), 11.6% for the distal interstitial (BMS1248-GAPD), and 23.0% for the telomeric (ETH152-BM8126) chromosomal regions. Four pairs of markers possessed identical retention patterns in the panel (BM321-SP1, CSSM34-COL2A1, BM1819-TEXAN15 and JAB2-GAPD), making it impossible to determine the order of each of the markers in the pair.

Pairwise analysis placed all of the markers within a single linkage group at a LOD support of 4.0 (Table 2). At a LOD support of 6.0, two linkage groups were formed, a centromeric group containing 23 markers and a telomeric group containing 11 markers. The centromeric group remained associated when the LOD support was increased to 8.0, but only 9 of the telomeric markers remained associated.

Analysis of the data using the stepwise option of RHMAXLIK ordered all of the markers unambiguously, except for the four unresolved pairs. Greater values of SAVMAX (6.0 and 9.0) provided identical orders, suggesting that the detected order was optimal. The next best orders produced a change in marker order from BM315-SLC2A3-PZB2F to PZB2F-SLC2A3-BM315 or PZB2F-BM315-SLC2A3. All other marker orders produced Log_{10} likelihood values substantially different from these orders.

The comprehensive map showing the most likely marker order (Table 2, Figure 1b) spanned 435.9 $cR_{5,000}$, calculated using the stepwise option. This order requires 110 breaks among the markers and has a Log_{10} likelihood value of -195.5. The average distance between markers was 12.8 $cR_{5,000}$, and the major gaps were 55.0 $cR_{5,000}$ between HMOX1 and BMS1248, and 30.4 $cR_{5,000}$ between MAF48 and ETH152; no other gaps longer than 27.1 $cR_{5,000}$ were found. Thirteen markers were ordered with a LOD support of at least 3.00, while 25 and 29 markers were ordered with a LOD support of at least 2.00 and 1.00, respectively.

The approach of ordering the markers using the branch and bound option, for the analysis of partial maps of 14 markers at one time, revealed the same order, with some exceptions. Running the partial maps containing the markers in the 14 marker frame RM500-BM315 and the 5 subsequent frames to IGF1-MAF48 produced some changes in the marker order. The order of the segment TRA1-BM1819 was changed to TRA1-IGF1-TEXAN15-BM1819-MB-HMOX1 in all 6 frames. Additionally, the order of markers in the segment BMS1248-MAF48 was changed. This reflects the low retention frequencies of the markers in the interval from BM1819 to SLC2A3, which resulted in the formation of the two strongly supported centromeric and telomeric groups divided by this interval.

The approach of ordering the markers using the stepwise option and deleting one marker at a time led to similar conclusions. The elimination of the markers TRA1, HMOX1, TEXAN15 or BMS1248 produced the order TRA1-IGF1-TEXAN15-BM1819-MB-HMOX1, as previously detected. In addition, the elimination of BM315 and BM8126 inverted the order of the marker pairs PZB2F-SLC2A3 and CYP2D6-ETH152, respectively.

The retention data for the most likely order of markers indicated that there were 79 BTA5 fragments retained in the panel (Table 3). Fifteen (19.0%) fragments contained only one marker, and 13 (16.5%) contained two markers. Thirty-two (40.5%) of the fragments contained at least 5 markers. In this calculation of the number of fragments, the ambiguous data were reassigned to be + or - depending on position, according to the following rules: +?+ = +++, -?- = —-, and +?- = +—. The average size of the BTA5 fragments in the panel for this chromosome was estimated to be 1.7 Mb.

Discussion

The average marker retention frequency for BTA5 was much lower than has been observed for most chromosomes (Table 4), except for BTA2, BTA7 and BTA15 (BTAX was lower, but was also present in only one copy, since the library was developed from a male cell line). The average retention for the markers used in the previously published RH maps of BTA5 were similar (Band *et al*., 2000; Barendse *et al*., 2000; Ozawa *et al*., 2000).

The average retention for the more than 1000 markers scored by Band *et al*. (2000) in their bovine chromosome maps was higher (22.4%) than the average for BTA5. These authors found evidence for wide variation in marker retention rates from one chromosome to another, ranging from 14.4% for BTA27 to 34.3% for BTA28 (BTA19 which harbored the selectable marker possessed an average marker retention of 45.3%). There appears to be a tendency for the smaller chromosomes to have higher retention rates than the larger chromosomes. Wide variation in the average retention of markers has also been found among human chromosomes (Gyapay *et al*., 1996), ranging from 21.1 to 61.6%, with an average of 29.2%, murine chromosomes (Van Etten *et al*., 1999), ranging from 23.4 to 37.9, with an average of 30%, and canine chromosomes (Priat *et al*., 1998).

The variation found in retention rate among BTA5 markers was also found in the map of Band *et al*., showing the same patterns for the centromeric, interstitial and telomeric segments. A higher retention among centromeric markers was also reported for BTA23 (Band *et al*., 1998), while it was less evident for BTA13 (Schläpfer *et al*., 1997), and not detected for BTA1 (Rexroad *et al*., 1999),

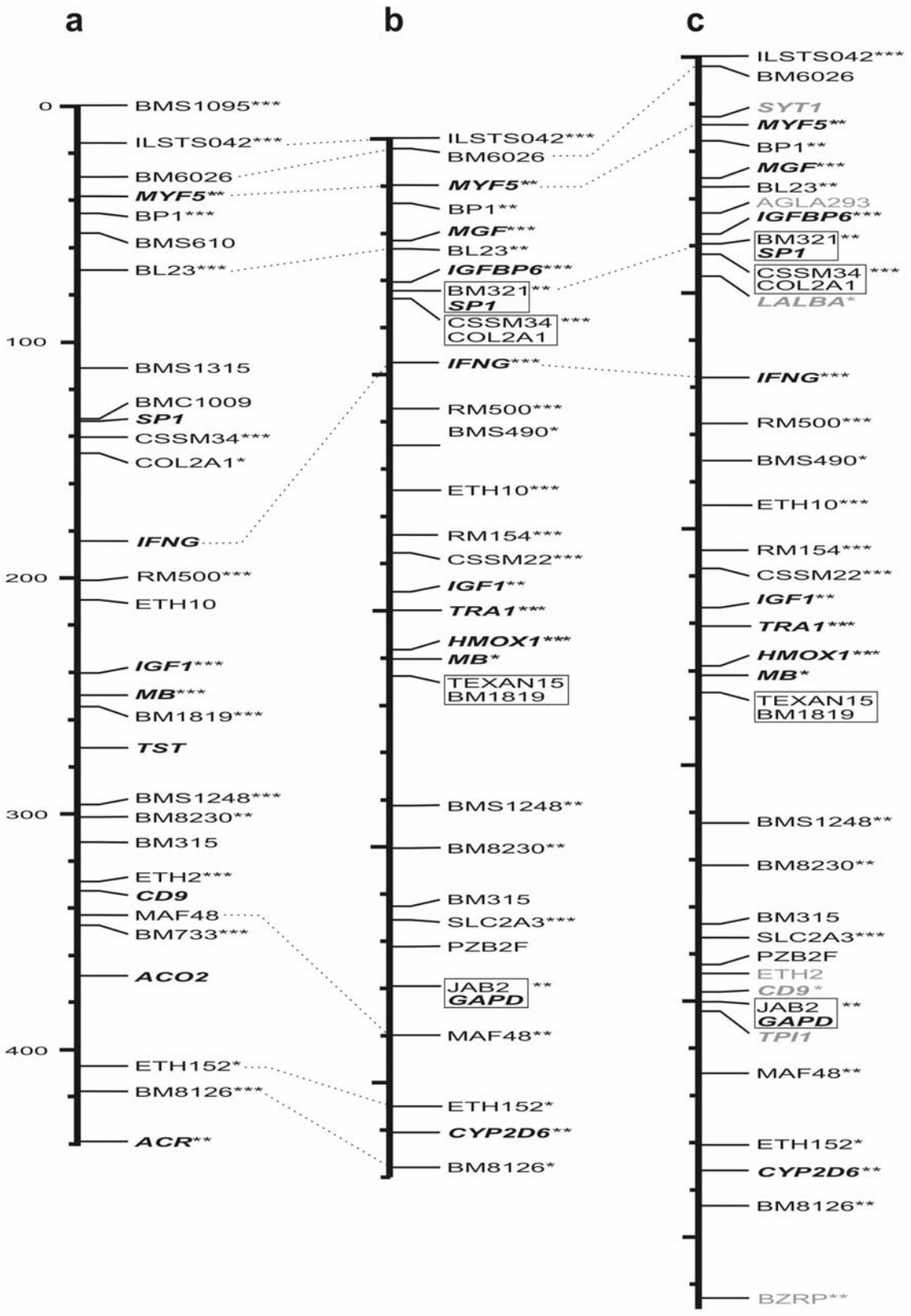

Figure 1 - Radiation hybrid map of BTA5. a) Updated version of the Band *et al*. (2000) map showing 30 of the 85 markers (http://cagst.animal. uiuc.edu). b) Comprehensive map showing the most likely order, using the program RHMAP 3.0. c) Comprehensive map including 7 of the makers scored by Barendse *et al*. (2000, shown in gray). Map increments are 20 $cR_{5,000}$. Boldfaced-italicized markers are near or within coding sequences. Markers with *, ** and *** were ordered with LOD supports of at least 1.0, 2.0 and 3.0, respectively. Dashed lines indicate regions with larger or smaller distances than the comprehensive map in "b".

Table 4 - Comparison of RH maps from several bovine chromosomes, constructed using the RHA panel.

Chromosome	Number of markers	Average retention %	RH map size[1]	Average interval	cR/cM[2] relationship	Kb/cR[3] relationship	References
1	41	26.9	1,383	33.7	9.73	127	Rexroad *et al*., 1999
1[4]	18	30.6	1,937 [5]	57.1	13.63	91	Rexroad *et al*., 2000
2	28	16.2	673	24.0	5.59	228	Wu, 1998
5	**34**	**17.8**	**436**	**12.8**	**3.74**	**333**	**This study**
5	85	18.3	439	5.2	3.41	306	Ozawa *et al*., 2000
7	34	14.5	962	28.3	7.16	130	Gu *et al*., 1999
13	27	26.4	650	24.1	8.18	143	Schläpfer *et al*., 1997
15	24	18.3	323	13.4	2.08	289	Amarante *et al*., 2000
18	103	24.0	1,666	16.2	20.47	47	Goldammer *et al*., 2002
19	29	48.0	824	28.4	8.76	93	Yang *et al*., 1998
23	24	27.0	255	10.6	3.75	246	Band *et al*., 1998
27	19	NR	418	22.0	6.38	131	Ashwell *et al*., 2002
29	11	35.9	147	13.3	1.30	407	Amarante *et al*., 2000
X[6]	56	17.3	681	14.2	4.90	253	Amaral *et al*., 2002
Genome-wide	1,087	22.4	9,330	8.6	3.21	322	Band *et al*., 2000
Average [7]	481	24.7	21,000 [8]	17.5	6.57	151	

[1]RH map sizes are for the comprehensive maps of each chromosome. [2]cR/cM relationships were calculated using the MARC linkage maps of each chromosome, using the segments limited by the common markers. [3]Kb/cR relationships were calculated with a 3,000 Mb estimated bovine genome size and the relative sizes of each chromosome from Popescu *et al.* (1996). [4]This chromosome was constructed using the BovR12 panel (12,000 rad). [5]The RH map size was estimated extrapolating from the region AGLA17-BMS4012. [6]The size, interval, cR/cM and Kb/cR relationships are underestimated because of the presence of 5 linkage groups which sum to 681 cR without estimating the size of the gaps. [7]The averages were calculated using all chromosomes information, except for the BovR12 panel. [8]Total map size estimated extrapolating from the coverage of the chromosomes presented here, except for the BovR12 panel and BTAX. NR: Not reported in the paper.

where a higher retention was observed for interstitial markers. An increase in retention frequency at the centromere has also been documented for many human chromosomes (James *et al*. 1994; Stewart *et al*., 1997), but not for murine chromosomes (McCarthy *et al*., 1997).

The stepwise option for building locus orders produced the marker order with the highest likelihood. Even though this approach may miss the global best order, this risk is greatly reduced when there is prior knowledge of order on many of the markers. In this case, many of the markers have previously been ordered in linkage maps, providing an approximate order for the initial map. However, when there is no prior knowledge of order, this approach should be used with caution, reevaluating the resulting orders against other approaches.

When using the branch and bound algorithm for 14 marker frames, most partial locus orders agreed with the order found for the previous frame. The consensus order of loci from this analysis was identical to the order obtained using the stepwise option. This approach was particularly valuable for identifying weaknesses in the map where establishing a robust marker order may be problematic. For example, we found that the order of markers in the segment IGF1-BM1819 was significantly affected by the more centromeric or telomeric markers, which oriented the segment. Similarly, the presence of ETH152 oriented the segment BMS1248-MAF48.

Using this approach, the segment IGF1-BM1819 could not be ordered unambiguously. The most important indicators of the most likely order were the Log_{10} likelihood value, the number of breaks and the map size. Order changes that substantially decreased the Log_{10} likelihood value, increased the number of breaks or the size of the map were indications of an incorrect locus order.

Analysis of the data using these approaches revealed two small chromosomal regions harboring the segments IGF1-BM1819 and BM315-PZB2F in which establishing marker order was problematic. These segments are located in the chromosome region possessing the lowest marker retention. However, since there was agreement among the marker orders generated under each of the approaches, the best marker order shown here (Table 2, Figure 1b) is thought to be the best order obtainable.

One possibility for solving the problems identified in this map could be the addition of markers that fall within these regions, especially those which have been included in other maps and for which there is a prior knowledge of correct ordering with respect to the other markers present. On the other hand, this approach may very well result in the production of sets of markers with identical retention patterns, due to the low marker retention frequencies in these

regions. Since there are few fragments present that contain the problematic region, a better approach would be to score these markers in another panel, such as the BovR12, or to use additional mapping techniques, such as linkage mapping, *in situ* hybridization and contig construction, to establish the correct marker order.

The RH map of BTA5 (Figure 1) revealed no difference in marker order with the MARC linkage map or the RH maps published by Ozawa *et al*. (2000) and Band *et al*. (2000). However, these previously published maps show some order differences with the linkage map, within the segments MYF5-BMS610 and BM2830-ETH152.

The size of the RH map of BTA5 is 8.7% larger than the map of Band *et al*. (2000). This could be due to the different software packages used to construct each map, which employ different algorithms to calculate marker order and distances. However, there might be another explanation, since there are regions of larger as well as smaller sizes for the markers common to our map (Figure 1). For example, the distance between ILSTS042 and BM6026 is larger in the Band *et al*. map than in ours, while the distance between BM6026 and MYF5 is smaller. Consequently, in their map, the regions BL23-IFNG and MAF48-ETH152 are larger, but the region IFNG-MAF48 is significantly smaller than in our map.

Even though the retention frequencies among the three BTA5 maps are similar, there are some significant differences, in particular, the average retention frequencies of the common markers are slightly higher than obtained here. Significant differences were seen between the Barendse *et al*. map and ours for the markers BM6026 (lower than our retention), CSSM22 (higher), ETH152 (higher) and CYP2D (lower), and between the Band *et al*. and our map for MYF5 (higher), IFNG (lower), BM8230 (higher) and ETH152 (higher). In addition, the markers CSSM34 and COL2A1 were not ordered in our map because they possessed identical retention patterns, however, they were ordered in the Band *et al*. map, at a distance of about 7.5 $cR_{5,000}$. Thus, it is very likely that the differences in marker distances among the maps can be attributed to differences in the scoring of the markers.

We considered the use of radioactively labeled PCR and polyacrylamide gel electrophoresis to be a very sensitive technique that could identify spurious bands that were close to, but not of the same size as, the target PCR amplification fragment, and resolve a weak radiographic signal that could not be detected by ethidium bromide staining. In this sense, the scoring performed with ethidium bromide staining would be more prone to errors than the method we used to score our markers.

The physical-linkage relationship between our RH and the MARC linkage map (Kappes *et al*., 1997) was estimated to be 3.81 $cR_{5,000}$/cM, calculated by dividing the RH map distance by the linkage map distance between the markers BM6026 and BM8126. Additionally, the estimated relationship between the RH map and the chromosome size of 134.4 Mb (based on a genome size of 3,000 Mb and the estimated chromosome size of 4.48% by Popescu *et al*., 1996) was estimated to be 333 kb/$cR_{5,000}$ (assuming an increase in size of 8.7% from the Band *et al*. map of 439 $cR_{5,000}$). The cR/cM relationship between the centromeric markers tended to be smaller than for the telomeric markers, but varied greatly. When analyzing the resolution of both the linkage and the radiation hybrid maps, no definitive conclusions could be made. For example, the markers MYF5 and BP1, as well as BMS1248 and BM8230, were not resolved in the MARC linkage map, but were resolved in the RH map presented here and in the Band *et al*. map; however, the markers TEXAN15 and BM1819, and JAB2 and GAPD were resolved in the TEXAN linkage map (Jeremy F. Taylor and Scott K. Davis, unpublished results), but not in our RH map. Similarly, the markers BM315 and BMS1658, and BM2830 and MAF48 were resolved in the MARC linkage map, but not in the Band *et al*. RH map.

The ordering in the Barendse *et al*. (2000) map differed for several of the markers included in our and in the Band *et al*. (2000) maps. There was not a high support for locus ordering in the Barendse *et al*. (2000) map, suggesting that their locus order is not correct. We utilized the Barendse *et al.* scoring data for the markers not scored here to determine their position within our map. For this purpose, each marker was sequentially added to the map, and the Log_{10} likelihood value, the number of breaks and map size were calculated (Table 5). Introducing the markers AGLA293, SYT1, LALBA and BZRP into our map produced major rearrangements, significantly increasing the number of breaks and the map length. Only introduction of the markers ETH2, CD9 and TPI1 produced modest decreases in the Log_{10} likelihood, increases in the number of breaks and also of map length with no major rearrangements in the locus order. An analysis of the map including all three markers produced rearrangements in the segment BM315-GAPD/JAB2. When all three markers were included, the order of the original markers was maintained, but the map size increased (11.7%, Figure 1).

Comparing the order of markers obtained here with that obtained by Barendse *et al*. (2000), we found three regions with differences: one where the markers BM6026, SYT1 and BP1 were included, another where ETH2, CD9 and TPI1 were included, and one involving the loci ETH152, CYP2D6 and BZRP. For the segment BM6026-BP1, the order presented here agrees with that obtained in the Band *et al.* (2000) RH map and the MARC linkage map (Kappes *et al*. 1997). It is worth mentioning that, even though SYT1 is believed to be close to MYF5 because of their proximity in the human genome (http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/maps.cgi), the scoring pattern obtained by Barendse *et al*. for this locus creates 4 double breaks in the fragments (cell lines 38, 68, 75 and 80; Table

Table 5 - Analysis of the maps produced by adding markers scored by Barendse *et al*. (2000). Only the markers not scored in this study were used for this analysis.

Loci added	Log likelihood	N. breaks	Map length	Order [1]
Comprehensive Map	-195.5	110	435.9	See Figure 1
AGLA293	-210.3	126	522.4	Cen-AGLA293-CSSM34 ILSTS042-IFNG
SYT1	-207.2	121	494.0	Cen-SYT1-BP1-MYF5-BM6026-ILSTS042-MGF
LALBA	-205.0	118	472.6	CSSM34-LALBA-IFNG
ETH2	-196.5	110	434.7	PZB2F-ETH2-JAB2/GAPD
CD9	-196.8	110	433.7	PZB2F-SLC2A3-BM315-CD9-JAB2/GAPD
TPI1	-198.7	112	446.2	PZB2F-JAB2/GAPD-TPI1-MAF48
BZRP	-207.5	120	476.3	BM8126-BZRP-Tel
ETH2, CD9, TPI1	-203.2	114	453.7	BM8230-ETH2-PZB2F-SLC2A3-BM315-CD9-JAB2/ GAPD-TPI1-MAF48
SYT1, AGLA293, LALBA, ETH2, CD9, TPI1, BZRP	-227.9	132	525.6	See Figure 1

[1]The markers not shown maintain the same order as in the comprehensive map.

3), which may explain the low support for ordering both here and in the Barendse *et al*. map. For the segment ETH2-TPI1, the order Cen-ETH2-CD9-Tel obtained here agrees with the order obtained in the Band *et al*. map. Additionally, the order CD9-GAPD-TPI1 obtained here agrees with the order for these genes in the short arm of human chromosome 12. Scoring for CD9 in cell line 49 and for TPI1 in cell line 41 (Table 3) also produces double breaks in the fragments. Finally, for the segment ETH152-BZRP, the order CYP2D6-BZRP-Tel agrees with the order for these genes in the long arm of human chromosome 22.

The size of the RH map of BTA5 was smaller than for other chromosomes which have a smaller physical size (Table 4). The radiation hybrid/genetic linkage relationship was similar to that reported by Band *et al*. (2000), but a lot smaller than for most of the previously published chromosomes, which range from 4.9 to 20.47 $cR_{5,000}$/cM. The relationships for BTA15, BTA23 and BTA29, however, were smaller. Similarly, the physical/radiation hybrid relationship for BTA5 was the largest of all the chromosomes, except for BTA29. The map size and the relationships cR/cM and kb/cR for BTA18 were markedly different from those of the other chromosomes. The estimated cR map size of the bovine genome was estimated to be about 21,000 $cR_{5,000}$, when using the data for BTA18, but only about 17,700 $cR_{5,000}$ when excluding these data. The cR/cM relationship also exhibited a significant decrease from 6.57 to 5.57 when the BTA18 data were excluded.

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