

## A radiation hybrid framework map of bovine chromosome 13

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**In this paper we present a 5000-rad radiation hybrid framework map of bovine chromosome 13 (BTA13) containing 13 loci, including five conserved genes and eight polymorphic microsatellites. All framework markers are ordered with odds greater than 1000:1. Furthermore, we present a comprehensive map of BTA13 integrating 11 genes and 16 microsatellites. The proposed order is in general agreement with the recently published medium-density linkage maps. A model of five blocks of genes with conserved order between human, mouse and cattle is presented.**

**Key words:** cattle, framework map, gene mapping, radiation hybrid, whole genome

### Introduction

Radiation hybrids (RHs) were first developed by Goss & Harris (1975). This somatic cell hybrid technique has since been applied extensively in human genome mapping (e.g. Walter *et al.* 1994, Dib *et al.* 1996). The principal advantage of RHs lies in the fact that polymorphic as well as non-polymorphic markers can be mapped, and therefore conserved anchor loci and other type I loci (O'Brien *et al.* 1993) can be integrated with existing meiotic maps composed of highly polymorphic microsatellites (for review see Walter & Goodfellow 1993).

The genome mapping effort is not limited to human and mouse. Tremendous progress has been made in recent years, for example, in mapping the bovine genome. Second-generation, medium-density bovine linkage maps are now published (Barendse *et al.* 1997, Kappes *et al.* 1997). In these maps 1600 loci, most of which are microsatellites, cover more than 95% of the bovine genome. The average interval size between two markers has been reduced to less than 2.5 centiMorgans (cM). However, although 160 DNA markers that are located in or near a coding sequence are presented in the map by Barendse *et al.* (1997), a major disadvantage of the genetic linkage maps is still their lack of a sufficient number of type I markers.

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For the mapping of economic trait loci (ETL) to bovine chromosome regions and ultimately the identification and cloning of the actual gene(s) responsible for the trait of interest, comparative gene mapping relative to the human and mouse genome plays an increasingly important role owing to the existence of high-resolution gene maps in the two later species. Although conservation of synteny between cattle and human, and to a slightly lesser extent cattle and mice, is extensive (Womack & Moll 1986, Hayes 1995, Solinas-Toldo *et al.* 1995, Chowdhary *et al.* 1996), gene order is not consistently conserved. However, the extrapolation of data from map-rich genomes in a 'positional candidate cloning' (Collins 1995) approach to cattle will require the establishment of the linear order of comparative anchor loci and the definition of the boundaries of conserved gene order. RH mapping is a potentially powerful tool for building high-resolution ordered comparative maps of the bovine genome integrated with extensive bovine linkage maps.

A 5000-rad bovine whole-genome radiation hybrid (WG-RH) panel has recently been developed in our lab (Womack *et al.* 1997). The present study made use of this panel and was focused on bovine chromosome 13 (BTA13). Somatic cell hybrid mapping (Womack & Moll 1986) and Zoo fluorescence *in situ* hybridization (FISH) studies (Hayes 1995, Solinas-Toldo *et al.* 1995, Chowdhary *et al.* 1996) have shown BTA13 to be homologous to parts of the human chromosomes 10 and 20 (HSA10 and HSA20), which in turn are, among others, represented on mouse chromosome 2 (MMU2). Our goal was the development of an ordered comparative map of anchor loci (O'Brien *et al.* 1993) and additional type I marker loci, previously assigned to BTA13, by analysis of RHs. Based on our findings we attempted to describe possible blocks of chromosomal rearrangements in cattle as compared with the human and mouse gene orders. The degree of concordance between the bovine WG-RH map and published meiotic maps was determined by integrating these type I loci with existing BTA13 linkage maps.

### Material and methods

#### Construction and characterization of the WG-RH panel

The construction of this cattle-hamster WG-RH panel is described elsewhere (Womack *et al.* 1997). Briefly, JEW 38, a bovine fibroblast cell line was irradiated with 5000 rad

(185 rad/min) at room temperature using a  $^{60}\text{Co}$  source and subsequently polyethylene glycol (PEG) fused to A23, a thymidine kinase-deficient ( $\text{TK}^-$ ) hamster recipient cell line, which allows selection of hybrids using hypoxanthine-aminopterin-thymidine (HAT) medium. Rapid selection against bovine cells was facilitated with ouabain.

A total of 101 HAT-resistant WG-RH clones were picked and transferred to tissue culture flasks and harvested at confluence for DNA extraction. DNA of the first 74 hybrids was tested for the presence of bovine chromosome fragments using the bovine short interspersed nuclear element (SINE) sequence (Lenestra *et al.* 1993) derived primer BOVA (5' CAG AGT CGG ACA CGA CTG AA). Bovine DNA was amplified in 69 hybrids (data not shown). All 101 hybrids were then tested using a bovine-specific primer pair for the thymidine kinase (*TK*) gene (Y. Yang, unpublished data). As A23 is deficient in the *TK* gene, the hybrid cells need the bovine gene substitution in order to survive in the HAT-selective medium. All but one hybrid (RH34.0) amplified the bovine-specific *TK* fragment. However, this cell line clearly showed several fragments amplified with the BOVA primer. We assumed that RH34.0 is an A23  $\text{TK}^+$  revertant that contains a bovine chromosome fragment. RH34.0 was therefore excluded from further analysis. DNA of the remaining 100 RHs was subsequently typed for all 27 BTA13 loci.

#### Loci

The 11 type I loci previously assigned to BTA13 were: arginine-vasopressin, neurophysin II (*AVP*) and prepro-oxytocin, neurophysin I (*OXT*, Dietz *et al.* 1992); casein kinase 2, alpha 1 polypeptide (*CSNK2A1*, Gao *et al.* 1997); guanidine nucleotide binding protein, alpha subunit (*GNAS1*, Gao & Womack 1993); haemopoietic cell kinase (*HCK*) and prion protein (*PRNP*, Womack *et al.* 1991); interleukin 2 receptor alpha (*IL2RA*) and vimentin (*VIM*, Threadgill & Womack 1991); superoxide dismutase-1, soluble like (*SOD1L*, Gallagher *et al.* 1992); thrombomodulin (*THBD*) and phosphatidylinositol-specific phospholipase C (*PLC-II*, Schlöpfer *et al.* 1997a). As many BTA13 microsatellite markers as possible were included in the

analysis as they became available, which led to a total of 16 microsatellites (Table 1) selected from published maps.

#### PCR primers

MacVector Version 4.1.4 Sequence Analysis Software was used for the primer design. PCR primers for the type I loci were designed either directly from published bovine sequences or based on information of conserved exon sequences from human or mouse homologous genes (Table 2). The primers for *CSNK2A1* have previously been published (Gao 1996). Microsatellite primers were selected from published BTA13 linkage maps (Table 1).

#### PCR conditions

PCR was performed in a Perkin Elmer GeneAmp System 9600 thermocycler. Standard PCR conditions were: 10 mM Tris-HCl, 1.5 mM  $\text{MgCl}_2$ , 50 mM KCl, pH 8.3 (20°C), 100  $\mu\text{M}$  each dNTP, 0.3  $\mu\text{M}$  each primer, 50 ng genomic DNA, and 0.5 units of *Taq* polymerase (Boehringer Mannheim, Indianapolis, IN, USA) in a total reaction volume of 10  $\mu\text{l}$ . The standard PCR protocol consisted of a 3-min denaturation step at 95°C, followed by 36–40 cycles of 30 s at 95°C, 15–30 s at the respective annealing temperature (Tables 1 & 2), and 30–60 s extension at 72°C. A final extension was performed for 5 min at 72°C. The appropriate annealing temperature was optimized in preliminary experiments to yield specific amplification of the bovine product in a hamster background. A touchdown protocol (Don *et al.* 1991) was implemented for several microsatellite markers (Table 1) to further increase the specificity of the PCR. Amplification products were electrophoresed on 2% agarose or 4% NuSieve 3:1 (FMC, Rockland, ME, USA) gels containing ethidium bromide and then photographed. Each of the 27 markers was analyzed at least twice for the presence or absence of a bovine-specific product. Additional typings were performed in case of ambiguous findings.

#### Statistical analysis and generation of RH maps

The data obtained were of the three categories 'present', 'absent', or 'not typed'. The RHMAP statistical software

**Table 1.** Microsatellites used in RH analysis of BTA13

Symbol	GenBank access #	Reference	Annealing temp.	Fragment size in bp
ETH7/D13S1	Z22746	Barendse <i>et al.</i> (1994)	68	~380
TGLA23/D13S2	–	Bishop <i>et al.</i> (1994)	65 → 58*	~110
TGLA6/D13S3	–	Ma <i>et al.</i> (1996)	60	~170
CSSM30/D13S5	–	Barendse <i>et al.</i> (1994)	66 → 64*	~155
HUJ616/D13S6	M88250	Barendse <i>et al.</i> (1994)	65 → 60*	~125
AGLA232/D13S10	–	Bishop <i>et al.</i> (1994)	68 → 64*	~180
BL42/D13S11	–	Bishop <i>et al.</i> (1994)	68 → 65*	~230
BMC1222/D13S13	G19106	Bishop <i>et al.</i> (1994)	60	~285
JAB3/D13S16	X98437	Barendse <i>et al.</i> (1997)	60	~230
ILSTS86/D13S17	L37238	Ma <i>et al.</i> (1996)	58	~190
URB07/D13S22	–	Ma <i>et al.</i> (1996)	63	~190
URB58/D13S24	–	Ma <i>et al.</i> (1996)	63	~165
TGLA381/D13S26	–	Kappes <i>et al.</i> (1997)	66 → 62*	~180
UWCA25/D13S29	L35541	Barendse <i>et al.</i> (1997)	58	~110
MILSTS77/D13S32	L37274	Ma <i>et al.</i> (1996)	60	~190
ILSTS59/D13S33	L37266	Ma <i>et al.</i> (1996)	64	~180

\*Touchdown procedure (Don *et al.* 1991).

– no GenBank access # available.

**Table 2.** Primers for type I loci used in RH analysis of *BTA13*

Symbol	GenBank access #	Primer sequence	Reference	Annealing temp.	Fragment size in bp
IL2RA	U24226	F'GCTGATGTGGAGGTTCTTCGTATTC R'TCGGTGATGACTGAGGCAAAG	Schläpfer <i>et al.</i> This paper	65 → 60*	~225
VIM	L13263	F'GCCAGATTCAAGAACAGCATG R'CATTGTTGCGGTTAGCAGCTTC	This paper	61	~700
CSNK2A1	M93665		Gao (1996)	60	~2000
AVP	X00503	F'GCCACGTCACCTTCAGAGGGAAC R'TCAGCTCCAGGTCGGACATG	Schläpfer <i>et al.</i> This paper	61	~225
OXT	X58474	F'TCCACAGCACTTACCACCCCTAAC R'CAGTTCATTGGCATGGCTGGTC	Schläpfer <i>et al.</i> This paper	59	~335
PRNP	D26150	F'GGACCTAATTCCTCATCCCC R'GGAAGACAGATGCTTCGGCTTG	Schläpfer <i>et al.</i> This paper	58	~725
THBD	M14657		Schläpfer <i>et al.</i> (1997a)	60	~180
HCK	M16591	F'GCTGCCAACATCTTGGTCTCTG R'AGCTGTCCACTTGATGGGGAAC	Schläpfer <i>et al.</i> This paper	60	~2000
PLC-II	Y00301		Schläpfer <i>et al.</i> (1997a)	60	~200
GNAS1	M21142	F'TGACTGCCATCATCTTCGTGG R'CAGCAAGGACTTTCTCAGCGAG	Schläpfer <i>et al.</i> This paper	59	~335
SOD1L	M81129	F'TCTACTTGGTTGGGGCGTCTCTAG R'CAAAGTATGGACGTGGAATCC	Schläpfer <i>et al.</i> This paper	56	~350

\*Touchdown procedure (Don *et al.* 1991).

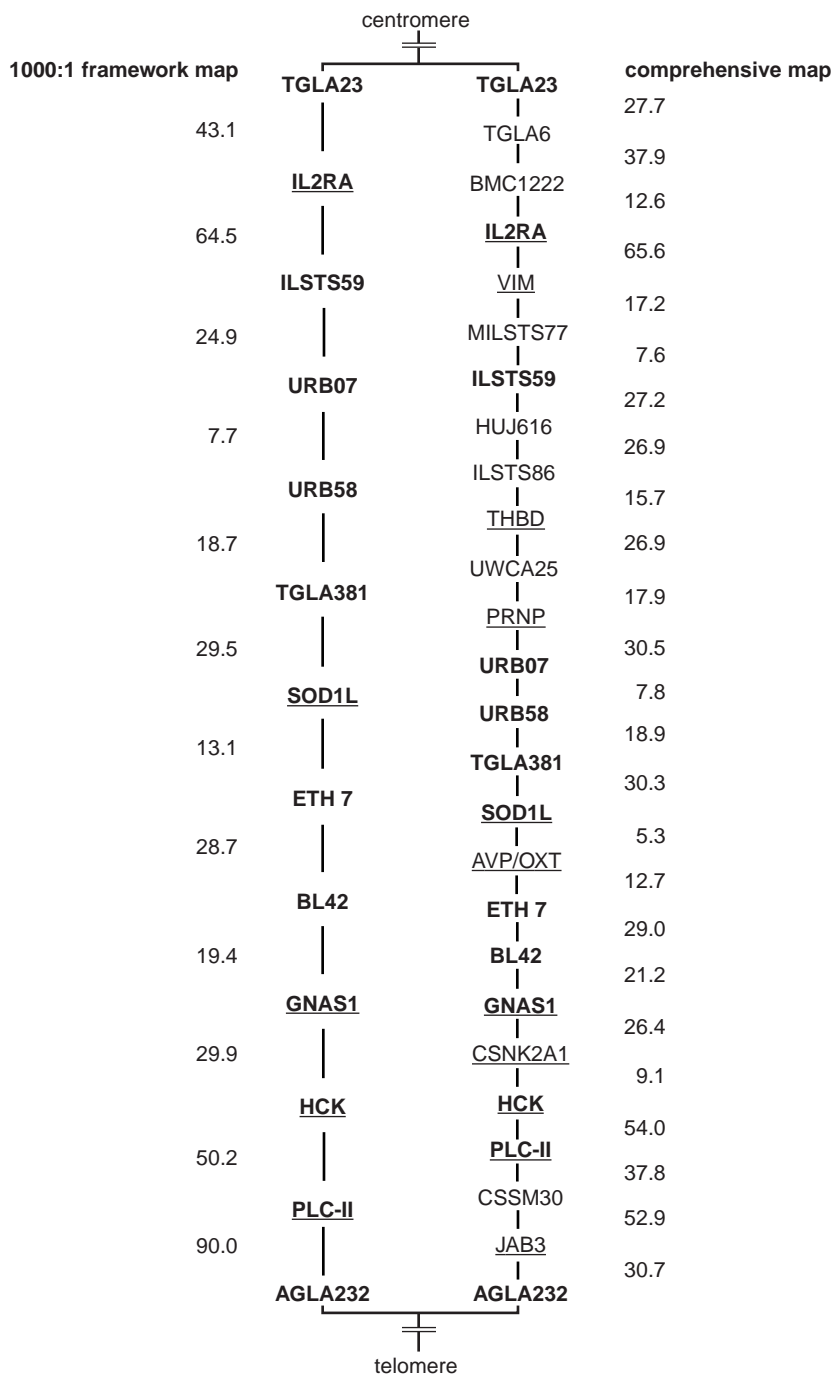
program version 2.01 and 3.00, consisting of the three fortran 77 programs RH2PT, RHMINBRK and RHMAXLINK (Boehnke *et al.* 1991, Lange *et al.* 1995), was used for data analysis.

RH2PT was first used to estimate marker retention frequencies. Two-point distance estimates were later used as references for the construction of the comprehensive marker map. Linkage groups were identified in which loci are linked on the basis of two-point lod scores of at least 2.0, 3.0, 4.0, 6.0 and 8.0. Finally, the program was used to identify markers with identical retention patterns. RHMINBRK was run with different subsets of loci in the stepwise ordering strategy. SAVMAX was increased until no change was observed in the respective locus orders. Results of the RHMINBRK analysis were again used as reference in developing the comprehensive map. Because of the large number of loci, RHMAXLINK was used in the stepwise ordering strategy with a SAVMAX setting of 9.00. The data were analyzed under both equal retention and centromeric retention models. The centromeric retention model resulted in locus orders that were in better agreement with the RHMINBRK computations and was used for further analyses. In a first step, all loci were used to develop a framework map of loci ordered with a relative likelihood of at least 1000:1 (ADDMIN 3.00). To add framework markers to both ends of the map, the markers were divided into two subsets and a framework marker in the central portion of the map was fixed as an end locus. RHMAXLINK was rerun with ADDMIN 3.00 with the respective subset towards the centromeric and the telomeric end of the map. All loci ordered with this level of support were then forced in this order (NFORCE in LNAMEA) and the program rerun to add loci with a level of support of at least 100:1 (ADDMIN 2.00). The additional loci ordered in this manner were added to the above forced order and the program run again with ADDMIN 0.00. The result of this last step, together with information derived from RH2PT as well as RHMINBRK analysis, led to the most probable comprehensive map.

## Results

Of the 100 hybrids analyzed, 22 were negative and three were positive for all loci tested. The average retention frequency was 26.4% with the lowest for *THBD* (16.5%) and the highest for *AGLA232* (41.4%). Seventy-five unique retention patterns for the 27 loci tested were observed. *AVP* and *OXT* were never discordant and were therefore treated as one locus (*AVP/OXT*). All 27 markers were part of one linkage group at lod score levels of 2.0, 3.0, 4.0, and 6.0. A lod score threshold of 8.0 resulted in four linkage groups, the first consisting of *AVP/OXT*, *HCK*, *PRNP*, *VIM*, *THBD*, *GNAS1*, *CSNK2A1*, *SOD1L*, *MILSTS77*, *BL42*, *TGLA381*, *ILSTS86*, *ILSTS59*, *URB07*, *URB58*, *ETH7*, *UWCA25*, and *HUJ616*. The second linkage group was composed of *IL2RA*, *TGLA23*, *TGLA6*, and *BMC1222*. *CSSM30* and *PLC-II* were in the third linkage group and *AGLA232* and *JAB3* in linkage group four.

A framework map with 13 loci ordered with odds greater than 1000:1 was developed. This framework map consists of eight microsatellites and five type I loci (Figure 1). The linear order of loci in this framework map is: *centromere* – *TGLA23* – *IL2RA* – *ILSTS59* – *URB07* – *URB58* – *TGLA381* – *SOD1L* – *ETH7* – *BL42* – *GNAS1* – *HCK* – *PLC-II* – *AGLA232*. The most probable comprehensive map is also given in Figure 1. However, in the comprehensive map, it was not possible to order *BMC1222*, *VIM*, *MILSTS77*, *HUJ616*, *ILSTS86*, *THBD*, *UWCA25* and *PRNP* relative to the framework markers *IL2RA* and *ILSTS59* and within the framework markers *TGLA23* and *URB07* with odds greater than 10:1. Four possible orders of the chromosome segment in question are given in Table 3.



**Figure 1.** BTA13 framework map with 13 loci, all ordered with odds greater than 1000:1 (left), and a comprehensive map with 27 loci based on results from MAXLINK, RHMINBRK and RH2PT analysis (right). Framework markers are bold, type I loci are underlined. The comprehensive map spans a total distance of 650 cR<sub>5000</sub>.

## Discussion

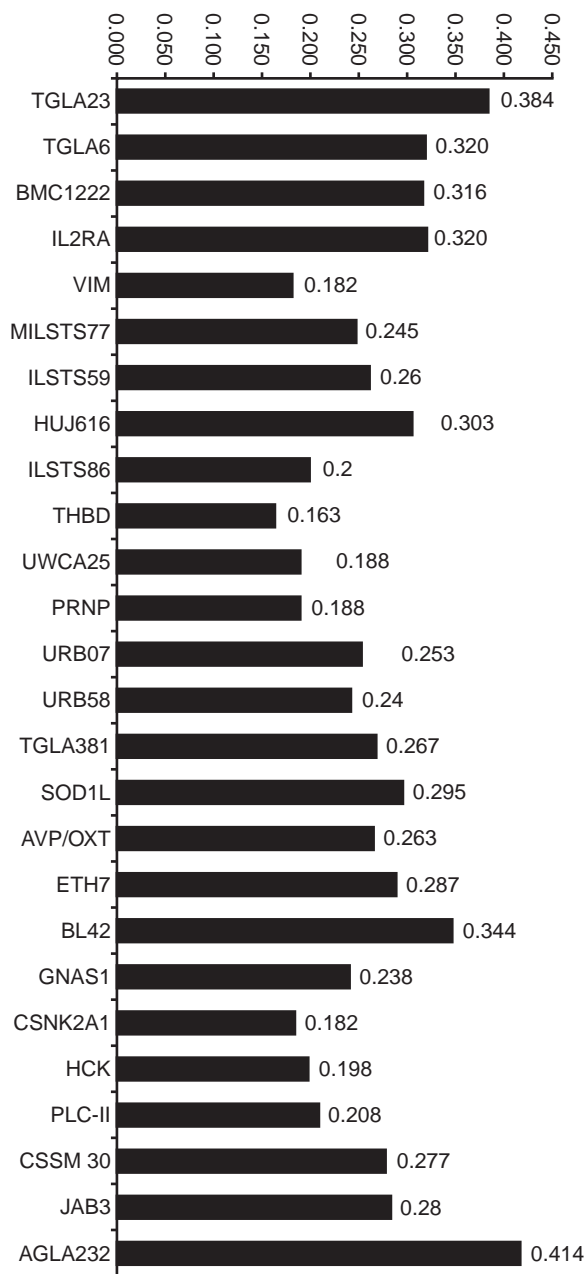
We have constructed a 5000-rad WG-RH mapping panel consisting of 100 hybrids to provide a tool for the development of framework maps for each of the 30 bovine chromosomes. It was possible to develop a framework map of BTA13 consisting of 13 loci, all

ordered with odds greater than 1000:1, as well as the most probable comprehensive map consisting of 27 loci.

An average marker retention frequency of 26.4% was found for the 27 BTA13 loci tested. The analysis of such a panel provides sufficient data to find the true marker

**Table 3.** Maximum likelihood locus orders for 13 most centromeric comprehensive map loci

Rank	Relative likelihood	Obligate breaks	Locus order
1	1.0	107	TGLA23-TGLA6-BMC1222-IL2RA-VIM-MILSTS77-ILSTS59-HUJ616-ILSTS86-THBD-UWCA25-PRNP-URB7
2	1.5	107	TGLA23-TGLA6-IL2RA-BMC1222-HUJ616-ILSTS59-MILSTS77-VIM-ILSTS86-THBD-UWCA25-PRNP-URB7
3	1.6	107	TGLA23-TGLA6-IL2RA-BMC1222-VIM-MILSTS77-ILSTS59-HUJ616-ILSTS86-THBD-UWCA25-PRNP-URB7
4	6.7	111	TGLA23-TGLA6-BMC1222-IL2RA-PRNP-UWCA25-THBD-ILSTS86-VIM-MILSTS77-ILSTS59-HUJ616-URB7



**Figure 2.** Distribution of retention frequencies for 27 loci over BTA13 under the best comprehensive radiation hybrid map locus order.

order of a set of loci (Barrett 1992). Our findings are in agreement with results reported by Walter *et al.* (1994), which found a retention frequency of 23.7% for HSA14-specific markers in a human-hamster WG-RH panel. Loci located at both the centromeric and the telomeric region of the BTA13 show higher retention frequencies (*TGLA23* = 38.8%, *AGLA232* = 41.4%) as compared with markers in the central portion (*URB58* = 24.0%) of the map. A 'centromere effect' in RH mapping has been

described in human and mouse and is probably due to selection for centromeres that confer stability on chromosomal fragments (Ceccherini *et al.* 1990, Sefton *et al.* 1992). An increase in the retention frequency for markers near the telomere has also been noted (Ceccherini *et al.* 1990, Lawrence *et al.* 1991). Figure 2 illustrates the distribution of the retention frequencies over the chromosome segment covered, arranged in the most probable comprehensive map locus order.

Based on the multipoint distance estimates for the framework map, the loci in the central part of the chromosome segment covered are relatively evenly distributed, whereas toward the two ends of the map (*ILSTS59* – *IL2RA* – *TGLA23* and *HCK* – *PLC-II* – *AGLA232*) the distances tend to increase. The uneven distribution of the selected loci along the chromosome becomes obvious at the centromeric third of the comprehensive map. The five loci *HUIJ616*, *ILSTS86*, *THBD*, *UWCA25*, and *PRNP* have their most probable comprehensive map position between the two framework markers *ILSTS59* and *URB07*. For the former five loci and the additional markers *VIM*, *MILSTS77*, and *BMC1222* we found four possible orders with odds smaller than 10:1 in our comprehensive map (Table 3). The low level of support reveals a limited resolving power of the 5000-rad panel and is probably due to the clustering of loci in a distinct area on the chromosome, as sets of markers with considerable interval variation are generally more difficult to map than markers that are evenly spaced (Jones 1996).

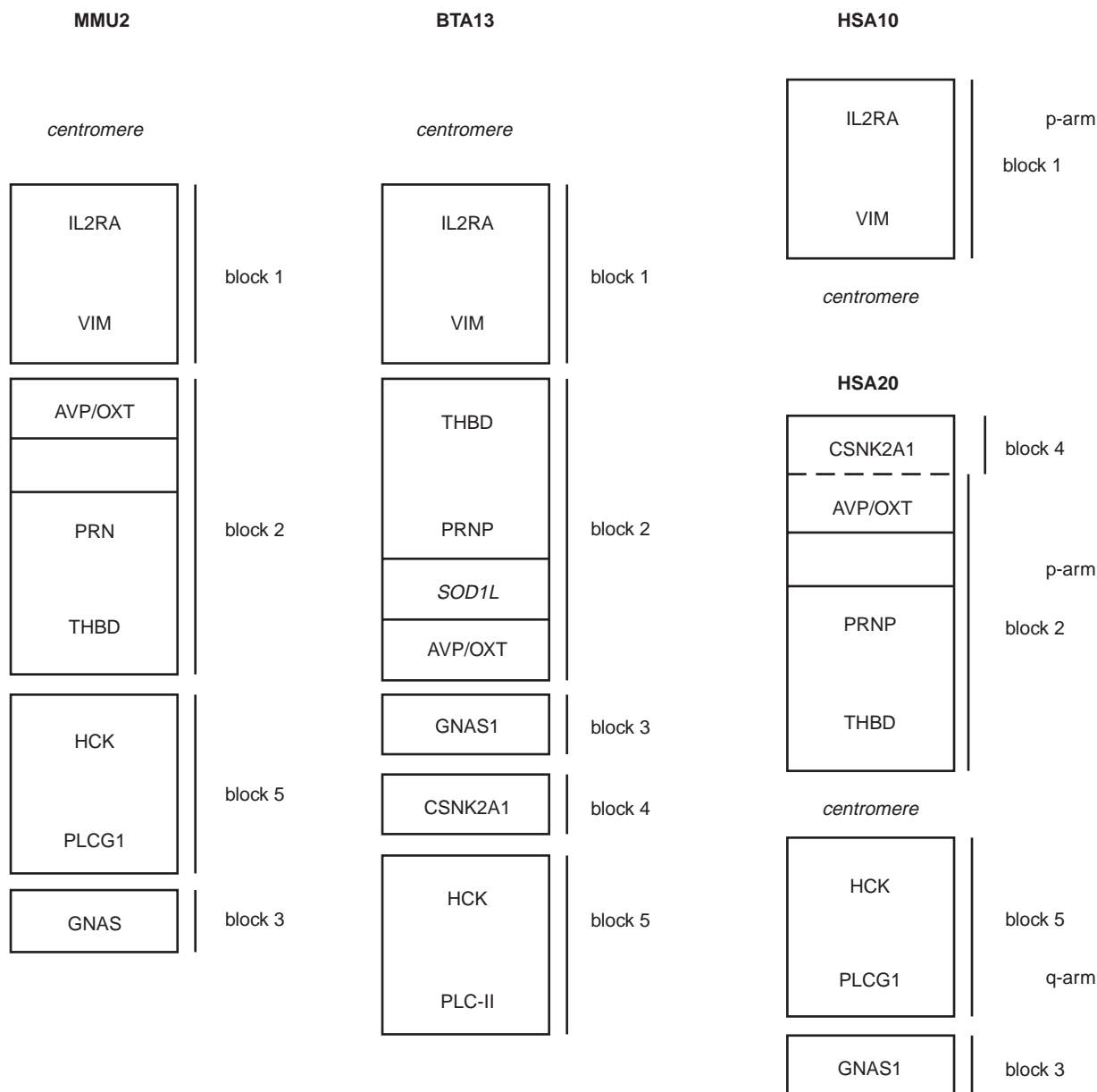
The framework map indicates a distance of 24.9 centiRays (cR) between *ILSTS59* and *URB07*. Under the most probable locus order of the comprehensive map this distance inflates to 145.1 cR. Inflation of the map length by adding markers to a map is also known in recombination-based linkage mapping, partially because of an increase in genotyping errors in marker-rich regions (Kappes *et al.* 1997). Typing errors reduce the ability to order loci and inflate estimates of interlocus distances (Lunetta *et al.* 1995). We found the main typing error problem in RH mapping to be the scoring of false-negative results in cases where only a bovine-specific PCR product was amplified. To reduce the risk of introducing typing errors, duplicate typing of every marker was performed before statistical analysis, as this results in a better probability of identifying the true order as one of the best orders (Lunetta *et al.* 1995). In some cases, mainly in the analysis of microsatellites, ambiguous results were observed between the two typings (data not shown). For those markers the typing was repeated until reproducible results were received, or the marker was called 'not typed' for a certain RH cell line.

The total length of the comprehensive BTA13 RH map between the markers *TGLA23* and *AGLA232* is 650 cR<sub>5000</sub>. Barendse *et al.* (1997) found the two markers separated by 130 cM (5 cR<sub>5000</sub>/cM) and Kappes *et al.* (1997) indicate an interval of 79.5 cM (8.2cR<sub>5000</sub>/cM). These data differ from the human-hamster WG-RH

map (Walter *et al.* 1994) where 1 cM is equivalent to 10 cR<sub>3000</sub>. Assuming, that 1 cM approximately equals 1 Mb, the cR/cM ratios found in chromosome-specific human-hamster RH maps vary from 18 cR<sub>9000</sub>/cM (Richard *et al.* 1991) to 33 cR<sub>6500</sub>/cM (Warrington *et al.* 1992). Our lower estimates might be due to an inaccurate estimation of either the length of the genetic maps or the RH map, and/or variation in chromosome and chromosome region-specific sensitivity to irradiation. Depending on the relative location of the microsatellites on BTA13, the cR<sub>5000</sub>/cM ratios, compared with the map by Kappes *et al.* (1997), varied from 3.3 for the interval *TGLA23* – *BMC1222* and 18.7 for *ILSTS86* – *TGLA381*. Chromosome region-specific cR/cM ratios have also been described in human (Raeymaekers *et al.* 1995). However, to allow an accurate comparison of distances derived from RH analysis with linkage mapping results, centiRays have to be transformed into physical distances by methods such as pulsed-field gel electrophoresis (PFGE) and yeast artificial chromosome (YAC) or bacterial artificial chromosome (BAC) cloning (Lawrence *et al.* 1991).

The microsatellite marker order of the present comprehensive RH map is in good agreement with the linkage map by Kappes *et al.* (1997). Compared with the linkage map by Barendse *et al.* (1997) there are two disagreements: (1) our map separates the microsatellite *MILSTS77* and the *VIM* gene by 17.2 cR, whereas the meiotic map treats the two loci as one marker, and (2) at the telomeric end of our comprehensive map the locus order is *JAB3* – *AGLA232* – *telomere* whereas the map by Barendse *et al.* (1997) has an inverted order for the former two loci. The telomeric part of the comprehensive RH map of BTA13 is in concordance with findings of a study that used clones of a bovine bacterial artificial chromosome (BAC) library (Cai *et al.* 1995) as probes to locate cytogenetically eight loci in this region of *BTA13* (Schläpfer *et al.* 1997b). Our findings disagree with those of the male bovine linkage map (Ma *et al.* 1996), in that the order of marker pairs at both ends of the two maps is inverted. The study identifies *TGLA6* as being telomeric to *BMC1222*, which marks the most centromeric locus. A serious discrepancy is the order of *centromere* – *URB58* – *URB07*, as these two loci are, in the order *centromere* – *URB07* – *URB58*, in the RH framework map. However, the fact, that the two reversed marker pairs occur at the ends of the linkage map probably accounts for flipovers often seen in those locations.

Based on the analysis of our data set, we did not find the HSA20 homologous segment on BTA13 located centromeric to the HSA10 homologs *IL2RA* and *VIM* (Figure 3), as reported by three Zoo-FISH studies (Hayes 1995, Solinas-Toldo *et al.* 1995, Chowdhary *et al.* 1996). This is striking because loci representing both the p-arm (*THBD*, *PRNP*, *GNAS1*, *HCK* and *PLC-II*) and the q-arm (*CSNK2A1*, *AVP* and *OXT*) of HSA20 were incorporated in this study. As the three studies used human chromosome-specific DNA libraries as probes to



**Figure 3.** Five possible blocks of chromosomal rearrangement in cattle as compared with the human and mouse gene orders. The bovine *PLC-II* gene is homologous to the human *PLCG1* locus and bovine *PRNP* corresponds to mouse *PRN*. No homologous gene to cattle *SOD1L* is known in man or mouse. *CSNK2A1* is not yet assigned to a mouse chromosome.

hybridize to bovine chromosomes, the specific loci representing this HSA20 homologous segment on BTA13 are not known. Additional HSA20 comparative loci will have to be mapped on BTA13 to identify the HSA20 homologous loci of the centromeric region. Our findings, however, confirm a HSA20 segment telomeric to the two HSA10 loci, containing all of the remaining markers tested.

A goal of the present study was to describe blocks of conserved gene order between the bovine and human

chromosome segments. All but two loci targeted in this study are represented on MMU2. To our knowledge, *CSNK2A1* has not yet been assigned to MMU2 and no homolog to the bovine *SOD1L* locus has been identified yet in human or mouse. As *AVP/OXT* were treated as one single locus during the RH analysis, our considerations involve only nine loci, two *HSA10* and seven *HSA20* homologs, or eight MMU2 homologous loci. Because *CSNK2A1/AVP/OXT* are merely cytogenetically mapped to HSA20p13, the linear gene order of the

three loci is not known in human. The most parsimonious explanation would make *CSNK2A1* the most telomeric of the three loci in human. Under this assumption we can define five conserved blocks between cattle and man (Figure 3). Block one consists of the HSA10 homologs *IL2RA* and *VIM*. All loci in the remaining four blocks are HSA20 homologs. Block two consists of *THBD*, *PRNP*, *AVP/OXT*, blocks three and four contain *GNAS1* and *CSNK2A1*, respectively, and *HCK* and *PLC-II* make up block five. However, if the assumption of *CSNK2A1* being the most telomeric of *AVP/OXT/CSNK2A1* in man is not correct, an additional block has to be postulated. Irrespective of this later point, it becomes evident that, although synteny is conserved between HSA10/HSA20 and BTA13, gene order within the homologous segments has been significantly altered by intrachromosomal rearrangements. This is also true compared with the gene order on MMU2. Similar results have been noted by Johansson *et al.* (1995), who found significant intrachromosomal rearrangements in conserved synteny groups of human and pig.

Our findings of five blocks of conserved locus order in cattle as compared with human and mouse homologous regions represent a basis for the subsequent high-resolution mapping necessary to enable the exploitation of the vast amount of information available from the map-rich human and mouse species for the 'comparative positional candidate cloning' (Womack & Kata 1995) of ETLs. Because of the limited number of type I loci in this study, we consider these data as preliminary, and additional comparative genes have to be incorporated into the blocks in order to verify our findings. Blocks three and four await centromeric and telomeric expansion to define their end points and respective orientation on BTA13. Human homologous genes, that lie within the loci defining blocks one, two and three, have to be targeted and added to BTA13 to exclude possible subdivisions of these blocks.

Finally, the search for the loci representing the centromeric HSA20 homologs on BTA13, as found in ZOO-FISH studies, has to be emphasized. As RH analysis is an additive approach, promising new markers can be incorporated into an existing map as their sequences become available for PCR primer design and amplification. This will be especially true when the second WG-RH panel, featuring a dosage of 12000 rad, which is currently being constructed in our lab, becomes available. This panel should allow the true marker order in marker-rich chromosomal regions to be resolved, clarify existing disagreements with other published maps and extending the comprehensive map presented.

However, this study demonstrates that WG-RHs are a powerful tool for integrating non-polymorphic, comparative loci with existing genetic maps. Although the resolution of the panel is limited if markers are clustered within specific chromosome regions, the 5000-rad dosage has been shown to result in sufficient resolution power to construct a BTA13 framework map.

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