

# Synteny mapping of five human chromosome 7 genes on bovine chromosomes 4 and 21

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**Abstract.** Five genes on human chromosome 7 (HSA 7) were assigned to bovine chromosome 21 (BTA 21) and 4 (BTA 4) using a bovine-rodent somatic hybrid cell panel. These five genes were alpha-I subunit of adenylate cyclase-inhibiting G-protein (GNAI1), alpha/beta preprotachykinin (TAC1), reelin (RELN), c-AMP dependant protein kinase type II beta regulatory chain (PRKAR2B) and apolipoprotein A1 regulatory

protein 1 (TFCOUP2). Four genes mapped to BTA 4 (GNAI1, TAC1, RELN, PRKAR2B) while one gene mapped to BTA 21 (TFCOUP2). This study confirms the synteny conservation between HSA 7 and BTA 4, finely maps the breakpoints of conserved synteny on HSA 7 and defines a new synteny conservation between HSA 7 and BTA 21.

A complete and accurate comparative map of high resolution is necessary for the implementation of comparative positional candidate mapping. However, human chromosomes can be homologous to more than one bovine chromosome (Chowdhary et al., 1996; Hayes, 1995; Solinas-Toldo et al., 1995). Recent studies have shown even more complex situations where several interrupted regions of a human chromosome are conserved on one bovine chromosome (Aleyasin et al., 1997; Beever et al., 1997; Sonstegard et al., 1997). This observed complexity underscores the difficulty in using human gene maps in a comparative positional gene approach.

The accurate definition of boundaries of conserved synteny is therefore vital to the use of comparative gene maps in cattle. The objective of our study is to accurately define the bound-

aries of human chromosome 7 q arm conserved segments. Two segments of HSA7 have been shown to be homologous to BTA 4 (<http://bos.cvm.tamu.edu>; Chowdhary et al., 1996; Hayes, 1995; Solinas-Toldo et al., 1995) according to the Texas nomenclature (Popescu et al., 1996). We report the assignment of five genes that lie on HSA 7; alpha-I subunit of adenylate cyclase-inhibiting G-protein (GNAI1), alpha/beta preprotachykinin (TAC1), reelin (RELN), c-AMP dependent protein kinase type II beta regulatory chain (PRKAR2B) and apolipoprotein A1 regulatory protein 1 (TFCOUP2) to BTA 4 and 21 and refine the boundaries of conserved synteny between BTA 4, 21, and 25 with HSA 7.

## Materials and methods

### DNA extractions

Bovine genomic DNA and cell line DNA were prepared as described previously (Li et al., 1992).

### Primer sequences

Primers for GNAI1, TAC1 and PRKAR2B were designed from published bovine cDNA sequences (Luo et al., 1990; Nawa et al., 1983; Nukada et al., 1986) and are located in the 3' untranslated region (3'UTR) of the respective cDNA. Primer sequences are as follows: GNAI1-F (5'-GCAAAG-TGAACAGCATTCCA-3') and GNAI1-R (5'-AAGGAAAAATCCCCA-AATG-3'), TAC1-F (5'-AATTTCTCCCCAAAGCACAG-3') and TAC1-R (5'-TGAAACATGCTGCAGGGATA-3'), PRKAR2B-F (5'-TATTGGAGC-

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AAGACCTGTAGTGA-3'), and PRKAR2B-R (5'-AAACCTGAAATC-ACAGAAAATGC-3'). The PCR product sizes are 217 bp for GNAI1, 220 bp for TAC1 and 100 bp for PRKAR2B. Primers for RELN and TFCOUP2 were designed from the consensus coding sequences computed (Higgins et al., 1992) using RELN human (DeSilva et al., 1997) and mouse (D'Arcangelo et al., 1995) or TFCOUP2 human (Ladias et al., 1991), mouse (Jonk et al., 1994), rat (unpublished, GenBank accession number AF003944), and chicken (Lutz et al., 1994) cDNA sequences. Primer sequences are as follows: RELN-F (5'-TCTTTTCTGTGCACCCAC-3') and RELN-R (5'-CTG-GTTACCAAACCTGGGTGTC-3'), TFCOUP2-F (ACCTCAGATGCC-TGTGGTC) and TFCOUP2-R (CCTACCAAACGGACGAAAAA). After sequencing of the PCR fragments, new primers specific to the bovine sequences of RELN and TFCOUP2 were designed and used subsequently to assign these genes. Their sequences are as follow: RELNBOV-F (5'-GAG-ATGGCATCCCAGACATT-3'), RELNBOV-R (5'-AAGACGAGAGCC-TGGAAGT-3'), TFCOUP2BOV-F (5'-CCTCAGATGCCTGTGGTTC-3'), and TFCOUP2BOV-R (5'-CAAATCGTGTGGTGTGGT-3'). The PCR product sizes are 117 bp for TFCOUP2 and 67 bp for RELN.

#### PCR amplifications

The polymerase chain reactions were performed using 50 ng of bovine, rodent or hybrid cell genomic DNA in a buffer containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.001% gelatin (w/v), 1.5 mM MgCl<sub>2</sub>, 0.5 unit of *Taq* polymerase (Promega, Madison, Wis.), 300 μM dNTPs, and 2 μM of forward and reverse primers. A touchdown PCR protocol was used: 2 min at 94°C, then 20 cycles for 30 s at 94°C, 30 s at 65°C and decreasing 0.5°C every cycle, 30 s at 72°C, followed by 20 cycles for 30 s at 94°C, 30 s at 55°C, 30 s at 72°C. A final extension step at 72°C for 10 min was performed. The reaction products were electrophoresed on 3% agarose gels.

#### Cloning and sequencing

PCR fragments were cloned in the plasmid pCR 2.1 using the TA cloning kit (Invitrogen) and sequenced using the Sequenase DNA sequencing kit (Amersham/USB).

#### Assignment of genes to bovine chromosome

PCR amplifications were conducted on 31 cell lines from a bovine-rodent somatic cell hybrid panel previously described (Womack and Moll, 1986). The rodent DNA that is the somatic cell background did not yield a fragment with any of the primer pairs. Only one fragment was amplified from the bovine DNA for all primer pairs used. For testing synteny, the correlation coefficients proposed by Chevalet and Corpet (1986) were calculated. When the number of cell lines is 30, the correlation coefficient must be higher than 0.87 to declare synteny, for an error level of 0.003. The power of this test is 0.79 (Chevalet and Corpet, 1986).

## Results and discussion

PCR fragments were sequenced to verify the specificity of the reactions. The RELN PCR fragment translated sequence is 100% identical to the human protein. TAC1, PRKAR2B and GNAI1 PCR fragment sequences are 100% identical to the bovine cDNA 3' UTR sequences. The bovine TFCOUP2 PCR fragment translated sequence is 97% identical to the human apolipoprotein A1 regulatory protein 1 (TFCOUP2). Since human TFCOUP2 protein is 85% identical to the human coup transcription factor I (Genbank accession number P10589), there was a possibility that the bovine PCR fragment represented a homolog of human coup transcription factor I. However, the bovine PCR fragment translated sequence is only 89% identical with the human coup transcription factor I protein and therefore the PCR fragment corresponds most likely to the bovine apolipoprotein A1 regulatory protein 1 gene (TFCOUP2).

Correlation analysis (Chevalet and Corpet, 1986) assigned RELN, GNAI1, TAC1, and PRKAR2B to cattle chromosome 4 while TFCOUP2 is assigned to cattle chromosome 21 (Table 1). The amplification profiles in the somatic cell hybrid panel of the three reference markers for chromosome 4 (MAF50, BM6458, RM188) used to compute the correlation scores are different. This may be explained by chromosome breaks that resulted in the retention of one reference marker in a given cell line while another marker was not retained. This variation resulted in different correlation scores between the genes reported in this manuscript and MAF50, BM6458, and RM188. Based on the higher correlation between PRKAR2B and MAF50 (0.916) or BM6458 (0.906) compared to the correlation between PRKAR2B and RM188 (0.590), it is reasonable to predict that PRKAR2B is positioned closer to MAF50 (47.4 cM) and BM6458 (68.3 cM) than to RM188 (24.7 cM). The correlation value between RELN and MAF50 (1.00) is higher than between RELN and BM6558 (0.829) or RM188 (0.645). Therefore, it is plausible to expect RELN to be closer to MAF50. TFCOUP2 has only a correlation value of 0.809 with the BTA 21 marker ETH131 (32.3 cM) when compared with a correlation of 1.00 with CSSM18 (81.5 cM) and consequently can be expected to be closer to CSSM18.

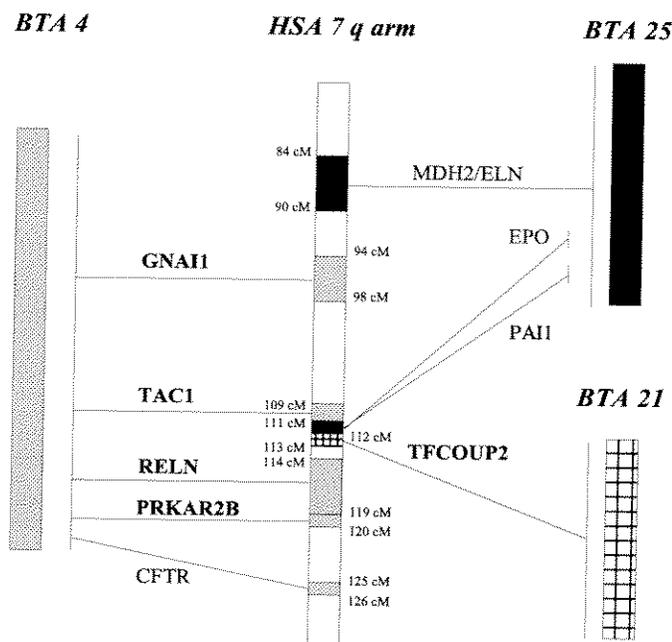
Databases were searched for the location on HSA 7 of genes previously mapped to bovine chromosomes. Our results, combined with the already published data, allow us to refine the

**Table 1.** Correlation analysis of HSA 7 genes with bovine chromosomes

Chromosome	TAC1*	RELN	TFCOUP2
1	0.408	0.408	0.184
2	0.446	0.487	0.247
3	0.022	0.043	0.008
4	<b>0.916</b>	<b>1.000</b>	0.508
5	0.067	0.000	0.075
6	0.595	0.615	0.464
7	0.000	0.000	0.000
8	0.079	0.302	0.242
9	0.323	0.395	0.389
10	0.000	0.057	0.100
11	0.046	0.087	0.223
12	0.244	0.333	0.057
13	0.078	0.200	0.098
14	0.323	0.395	0.508
15	0.279	0.373	0.000
16	0.398	0.480	0.309
17	0.204	0.288	0.344
18	0.077	0.034	0.058
19	0.239	0.129	0.221
20	0.321	0.271	0.383
21	0.832	0.713	<b>1.000</b>
22	0.406	0.333	0.318
23	0.204	0.257	0.438
24	0.071	0.167	0.380
25	0.000	0.000	0.181
26	0.000	0.099	0.048
27	0.000	0.057	0.423
28	0.164	0.223	0.167
29	0.000	0.000	0.042
X	0.046	0.137	0.000

\* GNAI1 and PRKAR2B display the same correlation values as TAC1.

boundaries of the conserved segments of HSA 7 (Fig. 1, Table 2). The most centromeric fragment contains the malate dehydrogenase (MDH2) and elastin (ELN) genes and ends between ELN and GNAI1 (4–14 cM interval, transcript map; <http://www.ncbi.nlm.nih.gov/SCIENCE96/>). The next conserved segment starts between TAC1 and the erythropoietin gene (EPO) (2–4 cM interval, YAC map; <http://www.nhgri.nih.gov/DIR/GTB/CHR7/>) and ends between the plasminogen activator inhibitor 1 (PAI1) gene and TFCOUP2 (1 cM interval, transcript map). Interestingly, EPO and PAI1 map on HSA 7 to the same 1 cM interval (Table 2) while they are separated by at least 7 cM on BTA 25 (<http://spinal.tag.csiro.au>). Consequently, there could be genes on BTA 25 located between EPO and PAI1 that do not map between these two genes on HSA 7. Alternatively, the larger genetic distance in cattle could also be due to a recombination hot spot between the two genes. The following segment is conserved on BTA 21 and to our knowledge, it is the first time a conservation between HSA 7 and BTA 21 is described. This region is small (1–2 cM) and therefore would not be detected by ZOO-FISH experiments (Hayes, 1995). The telomeric boundary of this fragment is between the TFCOUP2 and RELN (1–7 cM interval, transcript map). The most telomeric homology fragment to BTA 4 also contains PRKAR2B and extends at least up to the cystic fibrosis transmembrane conductance regulator gene (CFTR; Wallis et al., 1994).



**Fig. 1.** Conserved synteny between human chromosome 7 (HSA 7) and bovine chromosomes 4 (BTA 4), 21 (BTA 21), and 25 (BTA 25). The genes mapped in this study are in bold type.

**Table 2.** Comparative localization of genes in human and bovine ordered by relative position on HSA 7. Breakpoints in conserved synteny are identified by a line

Bovine chromosome	Gene symbol	Gene name	Human location			
			FISH	NIH transcript map <sup>c</sup>	NHGRI YAC Contig <sup>d</sup>	Closest polymorphic markers in NHGRI YAC contig <sup>e</sup>
25 <sup>a</sup>	MDH2	Malate dehydrogenase	7q11→q22 <sup>a</sup>	84–90 cM	NA	NA
25 <sup>a</sup>	ELN	Elastin	7q11.2 <sup>a</sup>	84–90 cM	NA	NA
4 <sup>f</sup>	GNAI1	Alpha 1 subunit of adenylate cyclase-inhibiting G-protein	NA	94–98 cM	NA	NA
4 <sup>f</sup>	TAC1	Alph-beta preprotachykinin	7q21→q22 <sup>b</sup>	109–111 cM	J	125–126 cM
25 <sup>a</sup>	EPO	Erythropoietin	7q22 <sup>a</sup>	111–112 cM	K	128–129 cM
25 <sup>a</sup>	PAI1	Plasminogen activator inhibitor 1	7q22 <sup>a</sup>	111–112 cM	L	129 cM
21 <sup>f</sup>	TFCOUP2	Apolipoprotein A1 regulatory protein 1	NA	112–113 cM	NA	NA
4 <sup>f</sup>	RELN	Reelin	NA	114–119 cM	N	131–132 cM
4 <sup>f</sup>	PRKAR2B	c-AMP dependant protein kinase type II beta regulatory chain	NA	119–120 cM	N	138–139 cM
4 <sup>a</sup>	CFTR	Cystic fibrosis transmembrane conductance regulator	7q31 <sup>a</sup>	125–126 cM	O	144 cM

<sup>a</sup> <http://bos.cvm.tamu.edu/htmls/Bov25.html>

<sup>b</sup> <http://www.ncbi.nlm.nih.gov/Entrez/Genome/org.html>

<sup>c</sup> <http://www.ncbi.nlm.nih.gov/SCIENCE96/>

<sup>d</sup> <http://www.nhgri.nih.gov/DIR/GTB/CHR7/>

<sup>e</sup> Interval is defined by the closest AFM markers centromeric and telomeric of the gene.

<sup>f</sup> This study.

The identification of the above listed boundaries through the mapping of HSA 7 genes does not preclude the existence of additional regions of conserved synteny with BTA 4, 21, 25 or other bovine chromosomes. Just as this work identifies a novel conserved fragment between HSA 7 and BTA 21, the addition of bovine genes to physical and genetic maps will undoubtedly define more, previously unknown regions of conservation. Un-

til a more complete comparative map is established, use of the comparative candidate gene approach to quantitative traits loci (QTL) identification will be severely limited.

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