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Short Communication

A 4 Mb high resolution BAC contig on bovine chromosome 1q12 and comparative analysis with human chromosome 21q22

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Abstract

The bovine RPCI-42 BAC library was screened to construct a sequence-ready ∼4 Mb single contig of 92 BAC clones on BTA 1q12. The contig covers the region between the genes *KRTAP8P1* and *CLIC6*. This genomic segment in cattle is of special interest as it contains the dominant gene responsible for the hornless or polled phenotype in cattle. The construction of the BAC contig was initiated by screening the bovine BAC library with heterologous cDNA probes derived from 12 human genes of the syntenic region on HSA 21q22. Contig building was facilitated by BAC end sequencing and chromosome walking. During the construction of the contig, 165 BAC end sequences and 109 single-copy STS markers were generated. For comparative mapping of 25 HSA 21q22 genes, genomic PCR primers were designed from bovine EST sequences and the gene-associated STSs mapped on the contig. Furthermore, bovine BAC end sequence comparisons against the human genome sequence revealed significant matches to HSA 21q22 and allowed the *in silico* mapping of two new genes in cattle. In total, 31 orthologues of human genes located on HSA 21q22 were directly mapped within the bovine BAC contig, of which 16 genes have been cloned and mapped for the first time in cattle. In contrast to the existing comparative bovine–human RH maps of this region, these results provide a better alignment and reveal a completely conserved gene order in this 4 Mb segment between cattle, human and mouse. The mapping of known polled linked BTA 1q12 microsatellite markers allowed the integration of the physical contig map with existing linkage maps of this region and also determined the exact order of these markers for the first time. Our physical map and transcript map may be useful for positional cloning of the putative polled gene in cattle. The nucleotide sequence data reported in this paper have been submitted to EMBL and have been assigned Accession Numbers AJ698510–AJ698674. **Copyright © 2005 John Wiley & Sons, Ltd.**

Keywords: **BTA 1; HSA 21; BAC; contig; comparative mapping; polled; cattle**

Introduction

A bovine physical map consisting of a contiguous assembly of overlapping BAC clones (contig) is considered a necessary prerequisite for the accurate assembly of whole genome shotgun sequences in the current efforts to obtain the bovine genome sequence (Gibbs *et al*., 2002). Although construction of preliminary genome-wide BAC contigs for cattle (*Bos taurus*) is in progress (Larkin *et al*., 2003; Schibler *et al*., 2004), there is a need to construct highly accurate physical maps of targeted regions to facilitate targeted sequencing and the discovery of species specific genes or quantitative trait loci (QTL) affecting economically important traits. Currently, successful positional cloning studies using detailed contig maps of specific cattle genome regions have been rare, e.g. the identification of the bovine *LIMBIN* gene causing dwarfism in Japanese brown cattle (Takeda *et al*., 2002;

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Takeda and Sugimoto, 2003) or the analysis of the bovine *DGAT1* gene as a functional candidate for milk yield and composition (Grisat *et al*., 2002, 2004; Winter *et al*., 2002, 2004).

In cattle, the hornless or polled phenotype is of special interest due to its economical importance in beef production. Hornless individuals are much safer to work with and they are less likely to injure themselves or other animals. The bovine polled phenotype shows a monogenic autosomal dominant inheritance and the still-unknown gene has been genetically mapped to the centromeric region of bovine chromosome (BTA) 1 (Georges *et al*., 1993; Schmutz *et al*., 1995; Harlizius *et al*., 1997). The first cattle–human comparative maps have been determined at low resolution by chromosome painting experiments and revealed that the proximal part of BTA 1 shows conserved synteny with human chromosome (HSA) 21 (Threadgill *et al*., 1991; Chowdhary *et al*., 1996). The recent expansion in the available number of bovine ESTs (Smith *et al*., 2001), in combination with sequence information of the nearly finished human genome project, provided the resources for detailed comparative maps. Subsequently, a medium-resolution bovine-human whole genome comparative map was generated by RH-mapping (Band *et al*., 2000). Additionally, different comparative RH maps of the centromeric BTA 1 region were constructed but revealed inconsistencies concerning the existence of chromosomal rearrangements between BTA 1q12 and HSA 21q22 (Rexroad *et al*., 1999, 2000; Drögemüller *et al*., 2002). Considering the difficulties with high-resolution RH mapping, a successful comparative positional cloning strategy of the polled gene should be complemented by a precise clone-based physical map of this region.

Herein we describe the construction of a BAC contig covering a ~4 Mb segment on BTA 1q12 and its comparative analysis with the syntenic region on HSA 21q22, which has previously been shown to contain the polled mutation. This genomic contig integrates a large number of genes and markers of physical, genetic, cytogenetic and RH maps of BTA 1q12. As a first step towards positional cloning of the polled gene in cattle, this high-resolution BAC contig map represents a valuable resource for future fine mapping and sequencing efforts.

Materials and methods

DNA library screening and chromosome walking

Library screenings with cDNA clones were performed as described (Drögemüller *et al*., 2002). PCR-amplified DNA fragments were labelled with ^{32}P and hybridized as probes on the high-density clone filters of the bovine genomic BAC library RPCI-42 (Warren *et al*., 2000) according to the RPCI protocol (**http://www.chori.org/bacpac/**). BAC DNA was prepared from 100 ml overnight cultures using the Qiagen Midi plasmid kit according to the modified protocol for BACs (Qiagen, Hilden, Germany). Insert sizes were determined as described (Martins-Wess and Leeb, 2003).

DNA sequence analysis

Isolated BAC DNA was sequenced with the thermosequenase kit (Amersham Biosciences, Freiburg, Germany) and a LICOR 4200L automated sequencer. BAC DNA was sequenced with IRD-labelled T7 and Sp6 sequencing primers. Sequence data were analysed with Sequencher 4.1.4 (GeneCodes, Ann Arbor, MI, USA). BLAST database searches were performed at NCBI (**http://www.ncbi.nlm.nih.gov/**) for human mRNA alignments against bovine EST entries and for the bovine–human comparison against the whole human genome sequence (build 34.3). Repetitive elements were identified with the RepeatMasker searching tool (**http://www.repeatmasker.org/**). Single-copy sequences were used to design primer pairs for the chromosome walking, using the program GeneFisher (**http://bibiserv.techfak.uni-bielefeld.de/genefisher/**).

Results

To construct a BAC contig of the bovine polled gene region we started to screen a bovine BAC library by hybridization of 12 different heterologous human IMAGE cDNA clones (Table 1). The physical localizations of six representative gene associated BAC clones were established by RH mapping and FISH on BTA 1q12 (Drögemüller *et al*., 2002) prior to the beginning of a chromosome walking strategy. Further sequence tagged

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Table 1. Human cDNA hybridization probes within the bovine BAC contig

Human gene symbol	IMAGE-ID	RZPD clone ID
TIAM1	3 197 030	IMAGp 998 O157814
SOD1	436 140	IMAGp 998 B131026
HUNK	768 063	IMAGp 998 H161890
C21orf108	25 729	IMAGp 998 G19138
C21orf59	124 398	IMAGp 998 E07121
SYNJ1	2 038 462	IMAGp 998 M235017
OLIG2	2 170 611	IMAGp 998 P045361
IL10RB	842 859	IMAGp 998 E042085
GART	2 901 218	IMAGp 998 J037162
SON	1 696 332	IMAGp 998 N134307
KCNE2	2 308 895	IMAGp 998 A245722
DSCR1	324 006	IMAGp 998 B07734

site (STS) probes that allowed the gradual joining of the individual emerging contigs into one large contig were generated from the BAC end sequences obtained from appropriate clones. Overlaps between clones were determined by STS content analysis. In total, 109 new STS markers were generated (Table 2). The complete BAC contig consisted of 92 clones (Figure 1). The physical mapping information derived from the contig assembly was refined by taking into account estimated BAC insert sizes from pulsed-field gels. The average insert size of the 92 BAC clones was 162 kb (range 30–200 kb). The entire contig spans approximately 4 Mb and can be covered with a minimal tiling path of 32 clones (Figure 1).

The clone-based physical map was anchored to the linkage and RH map of BTA 1 by STS content mapping of five previously described bovine microsatellites (*ARO9, ARO24, TGLA49, SOD1MICRO2, BM6438*) and two EST markers (EST0601, EST1413) (Figure 1). During construction of the bovine contig, primers were designed for 25 HSA 21q22 genes from corresponding bovine EST sequences (Table 3). PCR analysis of all 92 BAC clones with the gene-specific EST primer pairs revealed positive clones and the localization of these genes on the contig (Figure 1).

In total, 165 BAC end sequences with an average read length of 726 bp, totalling approximately 120 kb of genomic survey sequences, were generated. Thus, the BAC end sequences cover approximately 3% of the genomic region under study. The sequence information of these 165 BAC ends has been deposited in the EMBL nucleotide database under Accession Numbers AJ698510–AJ698674. Sequence alignments revealed eight pairs of identical BAC ends. The end sequences contain an average GC content of 44.3%, marginally exceeding the value of 41% that is generally accepted as the average GC content in mammalian genomes (Lander *et al.*, 2001). The GC content analysis further suggests that BTA 1q12 is indeed closely related to HSA 21q22, which has a GC content of 43.2% in the corresponding 4 Mb region. An analysis of repetitive sequences revealed that 39.1% of the BAC end sequences consisted of bovine repetitive DNA, mainly LINE (18.9%) and SINE (14.9%) elements; only 3.4% were of retroviral origin (LTRs) and 1.3% represented DNA transposons. In 56 cases, all or the majority of the BAC end sequences represented repetitive sequences and were therefore discarded for STS design. The repeat masked BAC end sequences were subjected to BLAST comparisons against the sequence of the human genome (build 34.3). The matches obtained confirmed the homology between the cloned chromosomal region in cattle with HSA 21q22. Significant and unique matches (e-value $<10^{-5}$) against human genomic sequences were observed for 38 (23%) bovine BAC end sequences. All but one of the 38 matches mapped to the expected location on HSA 21q22 (Table 4). All these BLAST matches corresponded well with the overall clone order in the bovine BAC contig and confirmed the correct assembly. In some cases the BLAST searches revealed the presence of genes within BAC end sequences and confirmed the previously obtained mapping results (Table 4). The *C21orf62* and *SFRS15* genes could be localized *in silico* by this approach on the contig for the first time (Figure 1). Only one single sequence (380C19-SP6) matched to a different human chromosome during the BLAST search. This unexpected BLAST result probably indicates a chimeric clone, as this BAC has been anchored in the contig by 4 STS markers and a gene specific bovine EST primer pair (Figure 1).

In total, the construction of this contig confirms the mapping of 15 previously mapped BTA 1 genes and provides 16 new chromosomal assignments of bovine orthologues to the human genes *SFRS15, C21orf45, C21orf108, C21orf63, C21orf59, C21orf66, C21orf62, IFNGR2, C21orf4, SON, MRPS6, C21orf82, C21orf51, KCNE1, DSCR1* and *CLIC6*. The gene order of the 31 assigned genes in the bovine BAC clone

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Table 2. Primer sequences of all used STS markers belonging to BAC end sequences of RPCI-42 clones

STS marker	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	T_M (°C)	PCR product (bp)
383K23-SP6	ATCTGAGCCACCAGAAAAGTC	GCATATGTCTTGGGAACATG	56	257
383K23-T7	CTTCTTTCCCAAGGACATAGTG	TTCCTGAGCACTCTCTTTATGG	58	184
394A5-SP6	ACTCAGAGGGCAATTGTAGAAG	GTTGCCAGCAGGGAATGG	58	593
386F4-T7	CCTGTCCCACCAGAAAGC	TGATGAACAGGGTAAGTTGG	55	252
352O20-SP6	TCCTGTTATATACCCCTCATGC	GAAGGGGGGAACAGTTATTGAG	59	337
44B5-T7	GGAGAATGGATACACAAGGTTC	CCTTCTCAAAAGGGGAAATACG	58	395
394A5-T7	CTCTCATGTTGGTAAGTGAGAG	CTTTGCTGTCCACTTGCAAC	57	415
292J15-T7	TCAGTGTGAATTTGCCCCATG	GTCATCTTTGGTGATCTCTC	54	308
234N12-T7	TCAATGGCCAAAGGATTCAC	GTAAACGGTAATGCCTTTCC	56	318
352O20-T7	ACTGACACTTTTCTGGGAGTAG	TCAATGGCCAAAGGATTCAC	57	390
506K17-SP6	AGGGTGTAAGTCTTCAGAAGAC	CTAAATCTTATCCAGGGCCTTG	58	265
506K17-T7	GATCCCCATGATGGTGCATC	CACAGAAGTTTTAGGTGGACAG	58	193
506K15-SP6	TTACCTAGGGGTGGTTTTTCAG	TATCCACATCACAGCCAAGATC	58	327
292J15-SP6	TCCCCTCAGCCTCCAGAAG	CACCAGGGAAGTTCTGGATC	58	259
311D23-T7	CAACCTACAATCGCATCATCC	GAGAACAGGTGAAGGGAGAG	57	420
320O18-T7	AATGTATCCTCCCTAAGGACAG	GGAGATAGAGAAAGCTTCTGAC	57	265
506K15-T7	CAGGAGGCTGTTAAACTTTGTG	ATAGTCCCCTTCTTCGATTACG	58	520
320O18-SP6	AATTCTTCAGGTGGAGAGTGTC	AAACCTGCCCCCTATCTAAGC	59	342
311D23-SP6	CCATCATTGAGGTCAGGGTTG	GCGGCAGCTATAACCACAAG	59	517
447G24-T7	GCTGGTTATTCTATCCCCTCTC	TTGTCTTGCCCAATGGTGAC	58	193
301M9-T7	CTGCCTTTTGCATAGGTGAAG	GGGGAAGGGGCTAATTTAGAC	58	414
26I16-T7	CCCTTTTTCCTCTCTGCCTTC	ACTAGCTGGGAAAAGACATTGC	59	517
180G7-T7	CTCAGGCTTTCCTTCTCCAA	GGACAGGAAGCTGACGTTT	59	161
196M18-T7	CCTTGTCTTCATCATCTGATCG	CCACCTTGCCTCCTCTCTC	58	275
374D19-T7	AGGAAGGGGTAAGACTCTTGTG	TTCTAGTTAGCCTGTACGTTGC	59	375
301M9-SP6	GACATGACTGAAGTGACTTAGC	GAGGAGGGAGGATACTAGAGAG	60	521
447G24-SP6	AAGCATCCCAAACTGTAAGC	AAAAGCCTAACTTGGGAAGG	56	415
199N3-T7	CCTAAATTCCTTTGGCTCTTCC	CCCTACCCTAGAGTGACCATG	59	267
266O23-SP6	AACAGCCAGGGGTTCTGAC	GGTTTCCTGAGAGGTCACATC	59	320
316N2-T7	GTCGGTAACACACGCACATC	ACCCTATGACATCATCTGTTGC	59	310
196M18-SP6	TCTGGTTGTACGTTGGTGATG	CTGTGCCCCTAACTAATAAAGC	58	245
292J17-SP6	CTCCAGAGAAGTTCTGTGTCTG	GAGGAATCTCGGGAGATTGC	60	200
420A17-SP6	CTTCTCGCCCACTCTATCTC	AGAGATTCAGTTCAGACGTCTG	57	326
46I17-SP6	AGAGTGCTGGCCAGATGTC	TCACCATGTTCTGCTTTGAC	57	520
553A8-SP6	TCGGTATCACACTTCTGTACAC	TCTCTCACTTTTCCTTCCTCTC	59	300
266O23-T7	ACATATACTGGCAGGCCTCTG	TGGAAGCCCTACTGGTCAAG	59	393
199N3-SP6	CAAGGTTAGTTTGGGAACAAGG	GTGATGCTGGCCACCAAC	58	518
213N17-T7	ACACTTCTACACTCTCACAAGG	CTTGTTAGTCTGACCCGTGAG	58	478
46I17-T7	TTGCAGGCAGTTTCCTTACTG	CCTTTAGGATGGACTGGTTGG	59	470
493P3-T7	ACTTGGAACCTAAGAGAGGATG	CCTGGGATGACTGAGAAGAG	57	520
553A8-T7	AATCAAGGTCTCCATGTGTAGC	TACGAGGTACAAACTCAGGTTC	59	217
320F13-T7	TTCAAATCTGTCCTCCACATCC	TGAGAATGCATCAGAGAGAGTG	59	185
68K7-T7	CATCAGTATCCTTTCAGCAACC	GGCAGAGAACAGGCATTCAG	58	225
249E18-T7	CCCCACAGCACTATTTCTTGG	TATAGCAGGAGGCATCAAAAGC	59	190
320F13-SP6	CCCATGGAATTTCCCTGCTAG	TGCATACCAGTCTGCAAGTTC	59	475
493P3-SP6	AGGAGTTAGTGACAGACACTTG	GAAGAGACTGGTGGACATCC	58	520
271E18-SP6	CGATGACTCACTTTGCTGTAC	GGAAACAGGAAATGAGGTTGC	58	184
470N12-SP6	CACAAAAGCAAGCAGTTCTCTC	TGTGTAGTGTACCATTGGCAAG	59	452
249E18-SP6	GGGTGAGTCCAGGGAGATG	AATGAGGGGCACAGCAGAG	60	206
217G23-SP6	TGGCCTTCCGTGTTTTCAC	TCACTGGTTTAGACTCAGTG	54	317
271E18-T7	GAAGCTGTCATCCTCTTTTTGG	AAAAGCGGCTAAGGGAACAC	59	500
569F23-SP6	TGACTTTGTTAGCTGGCTCAG	CTTCTGTTTCATTGTGGGTCTG	59	322
518G6-SP6	CCATCCCTGTCATGTTTTGG	TACAGGCACAGACTCATCAG	57	458
538E7-SP6	CTGTGGCCTACATAGTTTAGAG	GGTTTCATAGAGTCCCCTGATG	58	179
470N12-T7	AGGATCCTGCAAGCTGTGC	CTGACAGCAGGAACTTTTC	58	522
569F23-T7	CTGCCGGATCTGTGATTTGC	TTCTGCCTTTCCTCCTTTAACC	59	163

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Table 2. Continued

STS marker	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	T_M (°C)	PCR product (bp)
217G23-T7	GGAGGTTTTAGGAAAAGGATGC	ACTGCAGGTGAACTCTTTACC	57	230
161B10-SP6	CCGTCACTCCTTGTTTATCTTG	GTCAAGATTTTGTCAGCCCATC	59	188
518G6-T7	GAATTTGGGGGGAAGTGTAGC	CTCACTGCGGGATATTGATTCC	59	521
76J4-T7	CCCTGCAAGCAACAAAAGTG	TCCTCAATCCCACCCTCTTG	59	279
219G21-T7	ACAAGAAACAGAGTGCTTGG	TTTCACCAAACTCACCTAGC	56	310
76J4-SP6	TCAGGCTTAGGTGATCTCATCC	GGCAAAGTTCTCAATCCAAAGG	59	369
21K5-T7	AACGGTACAAGGAGAAAAGG	TGTCATAAATCCTGGGTCAC	55	464
554P19-SP6	CTTTACTCCTCGTAGCTGTC	TTCTGTGAGGGCAGAGTG	56	368
219G21-SP6	CTTAGAAGTGTGGCCGGTAG	GTGTTGATAAACTGACCCTCTG	58	418
351B8-SP6	CATGAATACTTAACACTACTG	CCTCTAATGTGGAATCCAG	52	147
52K19-T7	AAATGCAGGACAGAGAGAATCG	GTTATGTCCTAGGGCTGTGTC	59	279
554P19-T7	GGAATGGGTAGCTGTTCC	CCATAGAGTCCCAAAGAGTC	54	160
552B21-T7	TTCTTCAAAACCCACTCCTTCC	AAACTACACCCGGTCCTCTTC	59	244
52K19-SP6	ATCCCCAGCCAAGTGTAGTC	TGCCACTGACAGAATGAATCTG	59	180
487A22-T7	ACGCTTACTGAGGAAGGATGG	CAGGAAGGGGGACAGATACG	59	363
564N14-T7	CCTACAATGCTCTCAGCTGTC	GAGAAAGCTTGCTCATGTTGAC	60	474
241F8-SP6	ACAGACCTAAGTCTAGCTTG	TTCCCTGATGAAAGAGATGC	55	368
552B21-SP6	TCTATTACCTGGTTTCGGTTGG	GAAACTGAGCTCTTACTGCAG	59	143
332I5-SP6	GAGCAATGTAATATTGACTGG	ATTCCCCTCCCCAAATTTACC	58	184
79M3-T7	TATCTCCTTGAGGTTTGGAACG	GGGTCGTACACTGAATAAGTAG	58	311
564N14-SP6	AGTGACTAACACGCACGTTG	GCTAGTTCCTTGCCCTATTGTC	60	450
534N15-T7	ATCGGTGAACTTTCCTCATTCC	GCCAATATCACAGCCATTTCC	59	358
368A9-T7	GTTCCTATCAGCTCCGATTCG	GGCTATGGGTCTGGTAAATGG	59	410
543J10-T7	TCATTAGCACTGCCAGTTCTTC	CCAGGAGAGGGCAAATTCAAC	59	348
79M3-SP6	TAAACTAGCTGAGCAAGCCAAG	GCACATTAAAGTGGCTGGAAC	59	520
534N15-SP6	ATCACTGTTAGGTGACAGGTTG	ATGGTCACTGGTCCACACAG	59	419
79N19-SP6	TCTCTTCTCATCCCTGGGAAC	GGCACCTGGTATCTCTTATGC	59	273
204M10-SP6	GTTTACACCGTGGCTTTAGC	TTCATGCTGTTTGCTGAACC	58	452
218J7-SP6	AGAAATGGCCGTGATCTGTG	CATCAGCCTACAGAACATAACG	58	308
221H19-T7	GGTTGAGAGAAGAAGGGCTTG	CAGAGAAAGCAAAGCTGAGAAG	59	143
79N19-T7	AGGGATGACATAACCATAGG	CTTGCTGTTATGTCACAACG	54	115
204M10-T7	ATAACAGACCAGCGGGTGAC	TTCTACAAAGACCACAGCCATC	59	209
218J7-T7	TGCAGGTGGCTCTTCAGTATC	AGGTAGGGAGCCTGGATTGT	60	154
380B9-SP6	TTCGTAGTCTCTAAGGGAAG	CTCTTGGCCTTTATCAAGTC	53	313
420O24-T7	CACCTTCTTCCAAGGCTAGTG	AGGTTACTCTGTCTCTGAATGC	59	269
167I16-T7	CTCAGGGAATGATTCTTTCC	CCATCCAACCATCTCATC	55	253
51I7-SP6	AGAAGTCTAATAAGCTCTGCTGCAT	AACAAACGTTTCCCCTCTACA	59	100
182B8-T7	TTTTTCTAGTTCTGTGTATTC	ACCTCTCTTAAATGTAGAC	50	408
382D7-SP6	TGGCCTGTGACTAGTTTAGTTC	CATGGGTATGAAAACCACAGTG	58	159
420O24-SP6	GGTTGCTATAGCAGCCTCTC	GAATGCCCTAGACGTCCATC	58	367
167I16-SP6	CATCCCTGAAGGCTTTAGG	TCTTATTGAGCACCCACTG	55	397
182B8-SP6	CTTCTCCAGCGGATCTTC	GACGGAAGGTTTTGTTACC	54	517
80B9-T7	AAGCATCCCATCCCAGGAAC	TATCTGTCTCTCTGGGCATCC	59	521
132D12-SP6	TTAAGGATGAGGGGGTCTAGG	CTTCAGGGAAATGGGCTCTC	58	269
540F4-T7	GTTGGTAGAAAAAGCCACCATC	CCCCATAAGCAGCACTTCTC	59	310
31K20-T7	AGCTTCAGTTGAACCCAAGTAC	CTTCCAGTAGTTCACCAGACTG	59	422
543J23-SP6	ATTCTGAATTCAGGCCAACC	TGTTTACAGCAACAGCTGTC	56	199
80B9-SP6	AAGGGCACAGGAGATTTTCAAG	AAAGAGGCTGGGCTGAGATG	59	312
328M7-T7	AGGAATGGCAGGGAACTGAC	CACACATATAGCATGTGCTTGG	59	183
31K20-SP6	GTGTCAGTTCTGTGGGTTTCC	GAGGTCCAGGTCCTTCCTTC	59	268
494B13-SP6	GGTACATTGGAGTCTCTGACAC	TGATGGAGGCAAAACAGGTTC	59	281
543J23-T7	GGAGCAGTCTGCTATCAAAGG	GTCATATCAACAGAGTGCATGC	59	232
494B13-T7	TAGATAACAGAGGGTGGGGATG	TAACTCAGCTCTGATGTGGTAC	58	181

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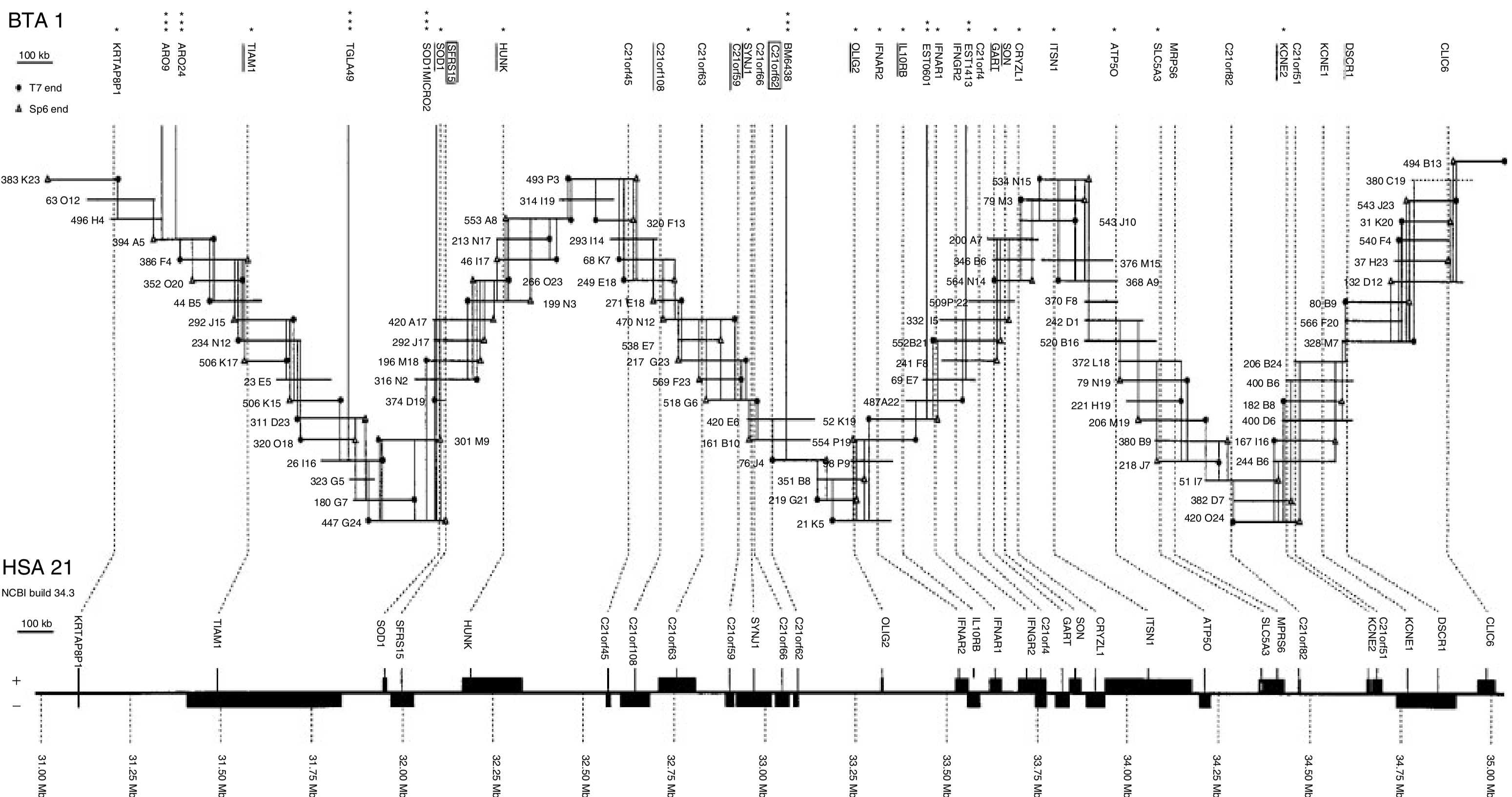

Figure 1. Physical map of the isolated bovine BAC contig on BTA 1q12. All mapped loci are indicated vertically at the top. Previously published BTA 1 mapping results are marked by one (genes), two (ESTs) or three (microsatellites) asterisks. Underlined gene markers were initially assigned by human cDNA hybridization probes. The two framed genes were localized on the contig *in silico*. RPCI-42 BAC clones are shown below the markers as continuous horizontal lines with their corresponding abbreviated clone names. A single chimeric BAC is shown by a dashed horizontal line. A minimal tiling path of 32 clones is indicated by thick lines. Bovine microsatellite, EST and STS markers are represented by vertical solid lines. Bovine markers that are associated to corresponding human genes are plotted by dotted vertical lines and linked to 31 genes on the 4 Mb sequence segment of HSA 21q22 (NCBI build 34.3) at the bottom. Comparative mapping of 31 gene-associated markers revealed a complete conservation of the gene order across the entire 4 Mb interval between *Bos taurus* and *Homo sapiens*

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Table 3. Gene-specific bovine EST primer sequences within the BAC contig

Human gene symbol	Bovine EST (Accession Nos)	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	T_M (°C)	PCR product (bp)
KRTAP8P1	X98351	TTGCTGAAATACCAGAGGCA	ATGACAAGAGTCATGAGCATGG	55	212
TIAM1	BE757612	GCACTGGAAAGCAAATTACC	AAAATCACCACACCTCACTC	55	509
SOD1	M81129	GTTTGGCCTGTGGTGTAATTGGAA	GGCCAAAATACAGAGATGAATGAA	60	275
C21orf45	BE668325	GGAAGATGTTTTGAAAGCC	GAATGTGGGCCTTGGAAC	60	101
C21orf63	BM107239	CTTGGTCATCAGAGTGTCATG	GTGTCCAAACCACTGTTCATC	60	277
C21orf59	BI537216	CGTCATCAAAGAATCAGAGG	CCCACGTAGTCTGAAAGCTTC	60	81
SYNJ1	BE752169	GGTCCTAGTCACTGGATG	GTGGGCATTAAGACTCAG	60	205
C21orf66	AW462169	GTGGGACCCCTTTTCTAC	CTGCATTCACCACTGAAGG	60	89
IFNAR2	AV666571	CCCTTCACCAACCCCTCTAC	TCCTCCCCAGGGAGAACAC	59	227
IFNAR1	X68443	AGAAGTTTTCGTCGTCCTTTG	TGATGGTGGTATTCAGGTTCTTC	55	290
IFNGR2	BF654282	CCCGTTCAGGAGTAACTTCA	GTTTCACAGCAAGATATGTTGC	57	117
C21orf4	BF043420	GTGCTCGAGTTTGGCTCTTC	CATAGGCACCAAAGAGAATCC	59	84
GART	BF039462	CAGTGAAGAGGGGTGACACTG	GCCATTTTCTCCAAGCCGTAC	60	122
SON	BF041673	GATAAATGGAAGCGCTTACCAG	CTGATTACAGGCAAGTGTTAC	58	515
CRYZL1	BG692873	GGACAGAAGCTGCTGGAACC	CTTGCTCCGTCCATTATCAG	59	109
ITSN1	AV605000	TCAAAAGAGCCTTAGAAG	GAAAATATCAATCTCCTG	60	108
ATP50	BM364861	GCCACCTGTTCAGATATATGGC	CTACTCGCAACAACTCCTTCTC	60	110
SLC5A3	BE664959	GTGGCCCTGTATTTTATCCTG	CCACCAAATCGCTTGGACAAG	60	321
MRPS6	BI775325	CTTGGTGGACTTTTATGCAC	GGACGATCCCTTCACATTC	60	137
C21orf82	CB444814	CAAGGCTGCAAATTCAGAGG	TAGTGTCTTGGCCTGGGTTC	60	183
KCNE2	BG938225	CAGGACGGAAATATGCCAAC	GATTTCACCGTGCTCACCAG	60	234
C21orf51	BM433498	CTGCTGCTGTATATCTTGGC	GCTTCCTCTTCTCAGCTTCC	60	124
KCNE1	BE486735	CTTCTTCACCCTGGGCATCATG	CAGCCAGCTGGTTCTCAATGAC	60	179
DSCR1	BF041330	CCCTCTTTAGGACTTATGAC	CAGTCTTATGTAGCTGGAG	60	128
CLIC6	CB456208	CCGAGCATATGCATTGTTCAAG	TCACGAGGACCATCTGTGATG	59	320

contig (Figure 1) corresponds exactly to the gene order of the NCBI HSA 21q22 map (**http://www.ncbi.nlm.nih.gov/mapview/**; build 34.3), which lists 50 gene loci in the interval between *KRTAP8P1* and *CLIC6*. Of these 50 loci, seven represent computer predicted hypothetical genes and five are pseudogenes, while 38 genes have at least some experimental evidence. The physical size of the investigated region and the distances between the mapped genes seems to be conserved between human and cattle. A high degree of gene order conservation can also be observed with respect to annotated murine genes. Some of the mapped bovine genes are assigned to the linkage map of mouse chromosome (MMU) 16. The current NCBI sequence map of MMU 16 (**http://www.ncbi.nlm.nih.gov/mapview/**; build 32.1) lists 19 of the 31 analysed genes in a similar order as in cattle or human.

Discussion

Here we describe a ~4 Mb single BAC contig that is predicted to contain the putative bovine polled gene. It establishes the physical order of the genetic microsatellite markers from different linkage maps that define the linked region and enables an exact determination of the candidate interval size. The physical map described here has a higher resolution and accuracy than other currently available maps, which often have conflicting data with respect to marker order (Rexroad *et al.*, 1999, 2000; Drögemüller *et al.*, 2002). The recombination frequency could not be reliably estimated in the investigated region, as there were inconsistencies between the different genetic maps of the BTA 1 centromere (Taylor *et al.*, 1998). The markers *TGLA49* and *BM6438* that are separated by 0.3 cM on the current MARC cattle linkage map (**http://www.marc.usda.gov**) are separated by roughly 1.4 Mb and the recombination frequency would be approximately 0.2 cM/Mb. This low value for the recombination frequency seems reasonable, considering that the investigated region is located close to the centromere, where low recombination frequencies have to be expected. The precise physical assignment of the linked microsatellites will benefit future efforts towards

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Table 4. Significant (e-value $<10^{-5}$) and unique BLAST matches of bovine RPCI-42 BAC clone end sequences against human genomic sequences (build 34.3)

Query	HSA	Human gene symbol	Alignment start	Strand	E-value	Bitscore
496H4-T7	21		31 061 111	+	7e-16	91.7
496H4-SP6	21		31 184 614	−	1e-41	176
386F4-SP6	21	*TIAM1*	31 412 535	−	9e-24	117
506K17-T7	21	*TIAM1*	31 514 415	−	1e-07	63.9
311D23-SP6	21	*TIAM1*	31 773 134	−	2e-40	172
301M9-T7	21	*TIAM1*	31 813 620	+	4e-32	145
374D19-T7	21		31 950 932	+	7e-09	67.9
374D19-SP6	21	*SFRS15*	31 978 365	−	0	769
447G24-SP6	21	*SFRS15*	31 978 365	−	0	777
266O23-SP6	21		32 067 314	+	1e-14	87.7
46I17-SP6	21		32 139 591	+	8e-06	58
213N17-SP6	21		32 139 591	+	7e-06	58
213N17-T7	21	*HUNK*	32 294 724	−	1e-23	117
46I17-T7	21		32 319 376	−	6e-16	91.7
44B5-T7	21		32 533 995	+	5e-14	85,7
249E18-SP6	21		32 702 372	−	3e-08	65.9
68K7-SP6	21		32 702 372	−	4e-08	65.9
569F23-T7	21	*SYNJ1*	32 978 627	−	1e-79	303
161B10-SP6	21		33 022 790	+	1e-07	63.9
518G6-T7	21	*C21orf66*	33 053 865	−	6e-31	141
76J4-T7	21	*C21orf62*	33 095 281	+	3e-17	95.6
21K5-T7	21		33 399 040	+	3e-14	85.7
564N14-T7	21	*GART*	33 796 824	+	1e-60	240
241F8-SP6	21	*GART*	33 804 431	−	2e-06	60
534N15-T7	21	*ITSN1*	34 053 681	+	8e-15	87.7
543J10-T7	21	*ITSN1*	34 117 826	−	2e-06	60
79M3-SP6	21	*ITSN1*	34 118 696	−	2e-09	69.9
372L18-T7	21		34 297 245	+	1e-57	230
204M10-SP6	21		34 316 613	+	2e-09	69.9
221H19-SP6	21		34 316 613	+	2e-09	69.9
204M10-T7	21		34 481 575	−	6e-07	61.9
400B6-T7	21		34 629 490	+	1e-54	220
400D6-T7	21		34 629 496	+	4e-51	208
400B6-SP6	21	*DSCR1*	34 846 334	−	5e-94	351
400D6-SP6	21	*DSCR1*	34 846 334	−	5e-94	351
543J23-SP6	21	*DSCR1*	34 899 686	+	2e-16	93.7
37H23-SP6	21	*CLIC6*	35 009 095	−	2e-29	135
380C19-SP6	13		57 544 793	+	4e-09	67.9

the positional cloning of the bovine polled gene, as the precise marker position with respect to coding genes is now available. The BAC contig we have generated also represents a resource for the isolation of additional polymorphic markers for fine mapping efforts.

In this study three techniques were used to localize bovine genes on the contig. During the first phase of contig construction we applied a comparative approach. The recent availability of the complete sequence and gene catalogue of the long arm of HSA 21 (Hattori *et al.*, 2000) has facilitated the procedure, using appropriate human heterologous screening probes to isolate bovine BAC clones. In the second phase of contig construction we increased the marker density by exploiting the available bovine EST resources that allowed the generation of bovine gene-specific primers for bovine orthologues of human genes. To develop these primers we used the rapidly growing bovine EST sequence information in combination with data on exon/intron boundaries from the human genome. Finally, in some cases genes could be localized on the contig *in silico*

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according to the BLAST search results of BAC end sequences. Using these three approaches, 31 genes could be assigned to the BAC contig, of which the following 15 gene loci had previously been mapped to cattle chromosome 1 with low precision: *KRTAP8P1* (Harlizius *et al.*, 1997); *SOD1, IFNAR1, IFNAR2* (Threadgill *et al.*, 1991); *GART* (Chowdhary *et al.*, 1996); *ATP50* (Smith *et al.*, 2001); *SLC5A3* (Rexroad *et al.*, 1999); *TIAM1, HUNK, SYNJ1, OLIG2, IL10RB, KCNE2* (Drögemüller *et al.*, 2002); *ITSN1* (Laurent *et al.*, 2000);. *CRYZL1* (Stone *et al.*, 2002), respectively.

This bovine–human comparative map provides the highest resolution comparative map of HSA 21q22 with the centromeric region of BTA 1 reported to date. The analysis of gene content of the investigated genomic region on BTA 1q12 revealed perfect synteny conservation between cattle and human. In contrast to the current bovine RH maps (Rexroad *et al.*, 1999, 2000; Drögemüller *et al.*, 2002), we found no evidence for the existence of chromosomal rearrangements in cattle, which is in part due to recent changes in the human genome assembly. High overall gene order conservation can also be observed with respect to the mouse. In other studies different gene orders within conserved synteny groups were observed across mammalian species (Schibler *et al.*, 1998). One possible explanation for the strong conservation observed here could be that the high gene content of BTA 1q12 interfered with major chromosome rearrangements during mammalian evolution.

In conclusion, the BAC contig we have constructed is an essential preliminary step toward the targeted positional cloning of the bovine polled gene. The mapping information that we present here will facilitate the accurate assembly of whole-genome shotgun DNA sequences of this region during the upcoming cattle genome project.

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