

## Physical assignment of six type I anchor loci to bovine chromosome 19 by fluorescence *in situ* hybridization

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### Summary

A bovine bacterial artificial chromosome (BAC) library was screened for the presence of eight type I anchor loci previously used within hybrid somatic cells and an interspecies hybrid backcross to construct a genome map of bovine chromosome 19 (BTA19). Six out of eight loci were identified in the BAC library (*NF1*, *CRYB1*, *CHRNB1*, *TP53*, *GH1* and *P4HB*). The BACs were then used in single-colour fluorescence *in situ* hybridization (FISH) to assign these genes to BTA19 band locations. Gene order was determined by single-colour FISH, and was confirmed by dual-colour FISH to mitotic and meiotic chromosomes. The order, centromere-*NF1-CRYB1-CHRNB1-TP53-GH1-P4HB*, was in agreement with the order determined by linkage analyses. In addition, the order of *CHRNB1* and *TP53*, previously unresolved by linkage analyses, was established. These data provide high-resolution cytogenetic anchorage of the BTA19 genome map from chromosome bands 14–22.

**Keywords:** bovine, cattle, fluorescence *in situ* hybridization, chromosomes, physical gene mapping, bovine bacterial artificial chromosome library

Genome mapping in the bovine began with comparative mapping of type I loci by analysis of hybrid somatic cells. This has resulted in the identification of extensive conservation of synteny with man and mouse, but has provided little information regarding gene order within conserved chromosomal segments (Womack & Moll 1986). Comparative mapping efforts in bovine are now focused on resolving internal rearrangements within conserved syntenies (Womack & Kata 1995). Bovine chromosome 19 (BTA19) has earned particular attention, since comparative chromosome painting studies

have indicated that BTA19, domestic pig chromosome 12, lesser Malay chevrotain chromosome 15 and human chromosome 17 (HSA17) are representative of an ancient autosomal synteny that apparently existed prior to the evolutionary divergence of the artiodactyl and primate lineages (Rettenberger *et al.* 1995; Solinas-Toldo *et al.* 1995; Gallagher *et al.* 1996).

Yang & Womack (1995) have shown by somatic cell analysis that HSA17 genes map exclusively to BTA19, consistent with the comparative chromosome painting data. More recently, Yang & Womack (1997) constructed a BTA19 linkage map using an interspecies hybrid backcross and type I anchor loci for the purpose of examining conservation of gene order among bovine, human and mouse within this highly conserved autosomal synteny. The linkage map of eight polymorphic genes [neurofibromatosis (*NF1*); crystallin  $\beta$ -polypeptide I (*CRYB1*); cholinergic receptor, nicotinic,  $\beta$ -polypeptide (*CHRNB1*); tumour protein p53 (*TP53*); myosin light polypeptide 4, alkali, atrial, embryonic (*MYL4*); growth hormone 1 (*GH1*); procollagen-proline, 2-oxoglutarate 4-dioxygenase,  $\beta$ -polypeptide (*P4HB*); and thyroid hormone receptor (*THRA1*)] demonstrated that gene orders were not conserved among the three taxa [see Yang & Womack (1995, 1997) for comparisons of gene order among the three taxa]. However, the order of some of the genes remained unresolved in bovine and most of these type I loci were not cytogenetically mapped in bovine. Herein, the present authors report chromosomal fluorescence *in situ* hybridization (FISH) results that physically assign six of the genes to BTA19 band locations.

The strategy which the present authors employed was to first screen a bovine bacterial artificial chromosome (BAC) library (Cai *et al.* 1995) by PCR using amplification conditions and primer sequences specific to the eight genes previously mapped to BTA19 by somatic cell (Yang & Womack 1995) and linkage analysis (Yang & Womack 1997). Six out of the eight genes were present in the BAC library, which was consistent with the estimated 75% prob-

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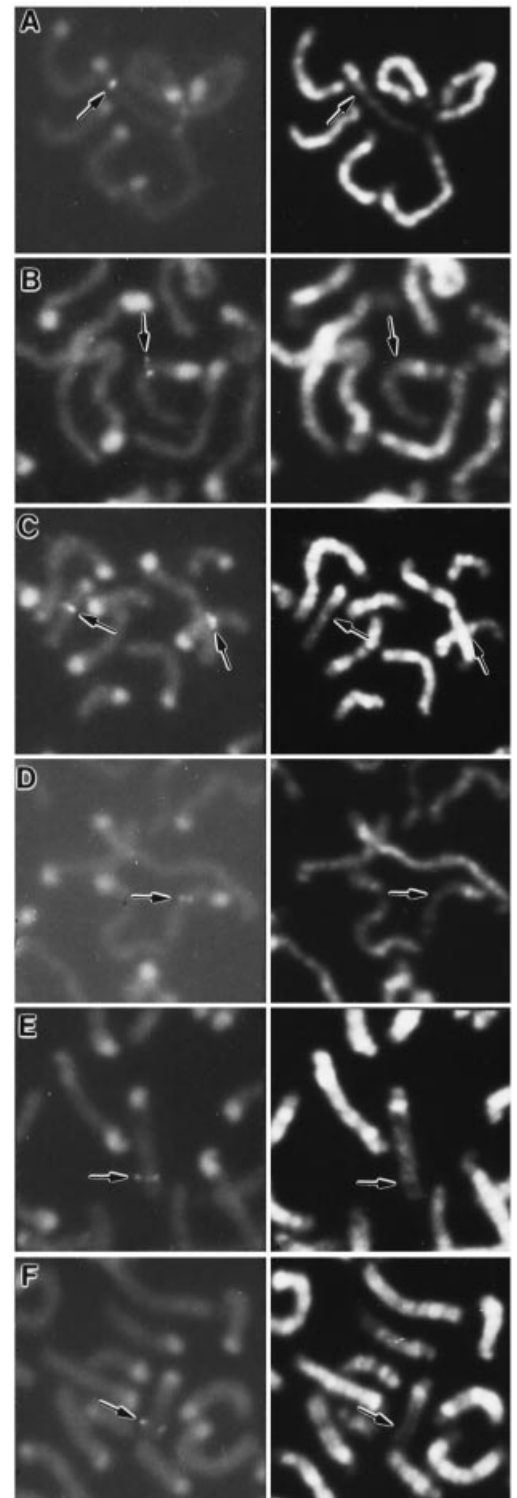
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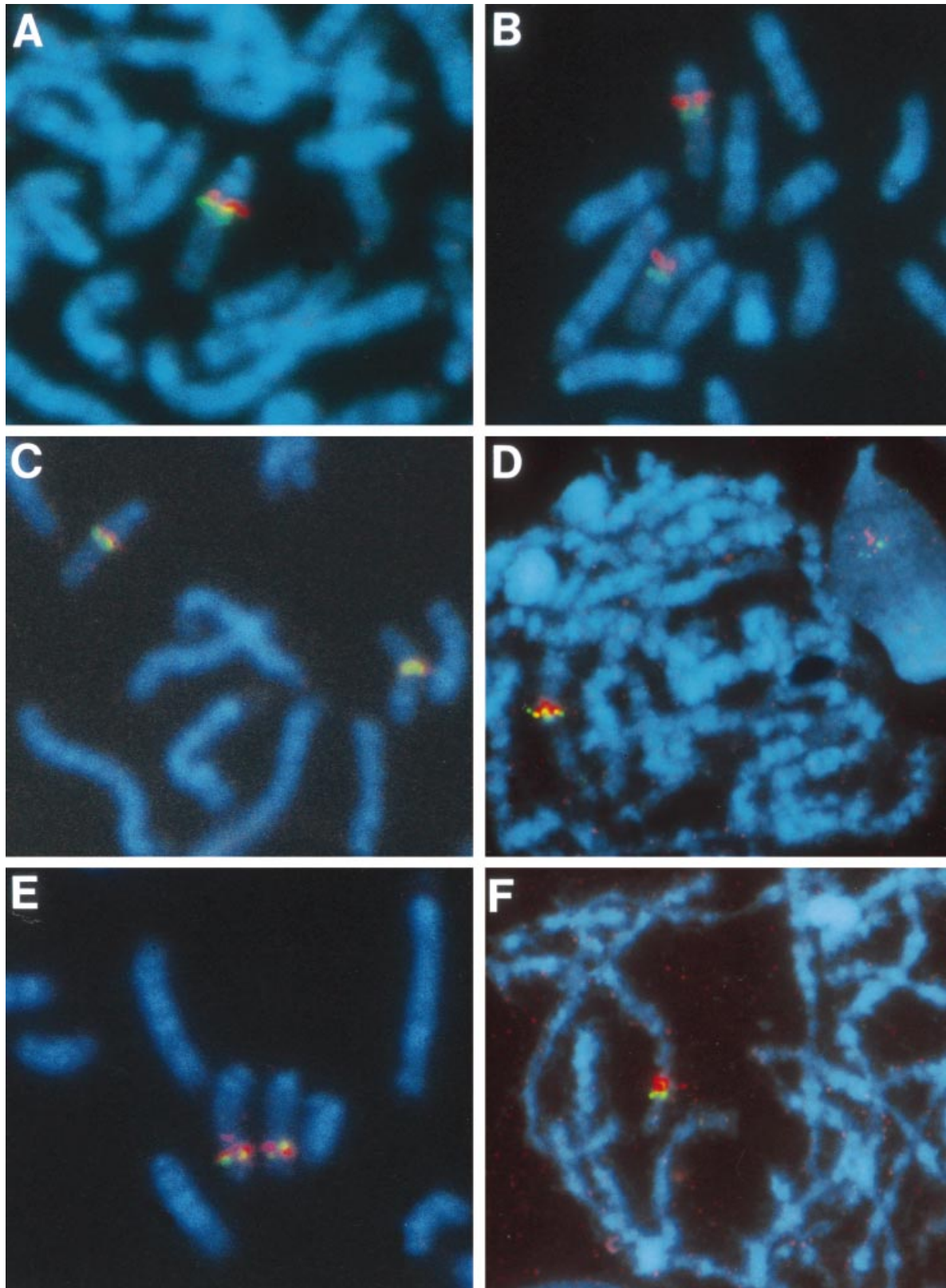
ability of the library containing a unique sequence at the time of screening. The six genes are listed here by gene symbols along with their BAC clone designations: *NF1*, BAC55R3C6; *CRYB1*, BAC66R7C5 and BAC29R3C12; *CHRNB1*, BAC112R2C11; *TP53*, BAC209R6C10; *GH1*, BAC110R2C3; and *P4HB*, BAC166R1C1. Prior to chromosomal FISH, DNA from the seven BACs was isolated and purified using a commercially available kit (Qiagen Inc., Valencia, CA), and gene identities were confirmed through comparison of restriction fragment length patterns of these BAC clones with those previously established for bovine total genomic DNA (Y.-P. Yang & J. E. Womack, unpublished data).

In turn, the identified BACs were used in FISH to assign the genes to chromosome band locations. The FISH procedure followed standard protocols (Pinkel *et al.* 1986) with slight modification as previously reported (Yeh *et al.* 1995). Mitotic and meiotic chromosomes were prepared following standard cytogenetic methods, and stored at  $-20^{\circ}\text{C}$  until needed. Purified BAC DNAs (insert and vector) were labelled with both biotin (Life Technologies Inc., Rockville, MD) and digoxigenin (Boehringer Mannheim, Indianapolis, IN) by nick translation, and then purified by ethanol precipitation in the presence of 100X bovine Cot-1 DNA (Applied Genetics Laboratories, Alachua, FL). Single and dual-colour FISH were performed in 10- $\mu\text{l}$  volumes containing either 200 or 400 ng of labelled probe, respectively. Biotinylated probes were detected with FITC (Vector Laboratories, Burlingame, CA) or Cy3 (Jackson ImmunoResearch Laboratories, West Grove, PA) conjugated to avidin, whereas digoxigenin-labelled probes were detected with FITC-conjugated antidigoxigenin (Oncor Inc., Gaithersburg, MD). The FISH preps were mounted in antifade solution containing propidium iodide (PI) and/or Hoechst 33258, each at 500–750 ng  $\text{ml}^{-1}$ . Photographs were taken on colour or high-contrast black and white print film, and bovine chromosomes were identified according to domestic cattle standard nomenclature (Reading Conference 1980; ISCNDA 1990; Popescu *et al.* 1996).

The seven BACs were first assigned to a bovine chromosome band location by single-colour FISH, and all were shown to hybridize exclusively to BTA19 under hybridization suppression conditions (Fig. 1). Both BAC66R7C5 and BAC29R3C12 appeared to hybridize to the same band location; however, BAC66R7C5 was used to assign *CRYB1* to a chromosome band since the signal was less intense and could be more



**Fig. 1.** Single-colour fluorescence *in situ* hybridization results for bovine chromosome 19 bacterial artificial chromosomes: (A) *NF1*; (B) *CRYB1*; (C) *CHRNB1*; (D) *TP53*; (E) *GH1*; and (F) *P4HB*. Photographs in the left-hand column show the FITC probe signal relative to propidium iodide (PI)-stained chromosomes, while those in the right-hand column are the same chromosomes after QFH-banding. Note that the heterochromatic pericentromeric regions are stained intensely with PI.



**Fig. 2.** Dual-colour fluorescence *in situ* hybridization of bovine chromosome 19 bacterial artificial chromosomes where red and green colour results from avidin-Cy3 and antidigoxigenin-FITC fluorescence detection of biotin- and digoxigenin-labelled probes, respectively. Hybridization is to the mitotic chromosomes unless otherwise indicated: (A) *NF1* (red) and *CRYB1* (green); (B) *CRYB1* (red) and *CHRNB1* (green); (C) *CHRNB1* (red) and *TP53* (green); (D) *CHRNB1* (red) and *TP53* (green) to pachytene chromosomes; (E) *GH1* (red) and *P4HB* (green); (F) *GH1* (red) and *P4HB* (green) to pachytene chromosomes. Note that labelled chromosomes are oriented with telomeric ends downward with the exception of the chromosome positioned to the right in (C).

precisely placed. (Further references to FISH results are by gene symbol.) By shifting between excitation of FITC/PI and Hoechst, probe signal was scored relative to QFH-banded chromosomes for a minimum of 10 cells per BAC clone and then plotted along the BTA19 ideogram. This analysis was conducted at the microscope and from high-contrast black and white photographs, and resulted in the assignment of *NF1* to band 14 (Fig. 1A), *CRYB1* and *CHRN1* to band 15 (Fig. 1B,C), *TP53* to the boundary between bands 15 and 16 (Fig. 1D), and *GH1* and *P4HB* to band 22 (Fig. 1E,F). The present authors tentatively placed *CRYB1* centromeric to *CHRN1*, and *GH1* centromeric to *P4HB*.

Because it was evident from the single-colour FISH analysis that the genes were clustered in two tight groups, the present authors used dual-colour FISH to mitotic and pachytene chromosomes (Fig. 2) to confirm the gene order deduced from the single-colour analysis. It was readily evident from the dual-colour analysis of mitotic chromosomes that the gene order centromere-*NF1-CRYB1-CHRN1* was correct (Fig. 2A,B), but that the order centromere-*CHRN1-TP53* (Fig. 2C) and centromere-*GH1-P4HB* (Fig. 2E) remained equivocal because of the overlap in probe signals. Hybridization to pachytene chromosomes provided consistently better separation of probe signal, and confirmed that *CHRN1* mapped centromeric to *TP53* (Fig. 1D), and that *GH1* was centromeric to *P4HB* (Fig. 1F).

The gene order that the present authors have arrived at through chromosomal FISH analysis is in agreement with the BTA19 linkage map of Yang & Womack (1997), i.e. centromere-*NF1*-4.0 cM-*CRYB1*-11.2 cM-(*CHRN1*, *TP53*)-4.0 cM-(*GH1*, *MYL4*, *THRA1*)-14.4 cM-*P4HB*. In addition, the present authors have resolved the order of *CHRN1* and *TP53* on pachytene chromosomes. The interspecies hybrid backcross map of Yang & Womack (1997) provides integration between the BTA19 comparative map and linkage map because it incorporates microsatellite markers found in second-generation bovine linkage maps (Barendse *et al.* 1997; Kappes *et al.* 1997) with the eight type I anchor loci previously mentioned. Since these chromosomal FISH results assign six out of the eight type I anchor loci to chromosome band locations, the present authors indirectly provided additional cytogenetic anchorage for the higher resolution linkage maps of Barendse *et al.* (1997) and Kappes *et al.* (1997).

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