

revealed that the most significantly linked marker was *SW18* on chromosome 16 at a distance of 31 cR (LOD = 10.25). *SO105*, one of the significantly linked markers, had been previously physically localized to SSC16q23.<sup>7</sup>

**Comments:** The human *ALS* gene has been assigned to HSA16p13.3 (<http://www.ncbi.nlm.nih.gov/LocusLink>). Bi-directional painting results indicate that SSC16 corresponds in its entirety to HSA5p15.3–q13;<sup>8</sup> therefore, our mapping result is not consistent with the established conservation of synteny. However, an expressed sequence tag (EST) radiation hybrid comparative map of the porcine and human genomes reveals a break in conservation between SSC16 and HSA16.<sup>9</sup> The porcine *nucleoside-diphosphate kinase 3* EST (NME3, EST-AR079H05), *ribosomal protein S2* EST (RPS2, EST-AR073G07) and F22874 EST (EST-AR092E08) are also closely linked with *SW18* (LOD = 9.82, 8.2 and 8.72 respectively; <http://www.ag.unr.edu/ab/standard.htm>), and their human counterparts are all assigned to HSA16p13.3 (1.76–2.08 MB, NCBI Build 35; <http://www.ncbi.nlm.nih.gov/LocusLink>). Our data are in agreement with this result, supporting the synteny between SSC16q23 and HSA16p13.

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## Comparative mapping of the *fragile histidine triad (FHIT)* gene in cattle, river buffalo, sheep and goat by FISH and assignment to BTA22 by RH-mapping: a comparison with HSA3

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**Description/source:** Common fragile sites can be damaged by exposure to a variety of carcinogens. The *fragile histidine triad (FHIT)* gene, including the most active human chromosomal fragile site (*FRA3B*) at chromosome band HSA3p14.2,<sup>1</sup> has been proposed as a tumour suppressor gene for a variety of tumours.<sup>2</sup> The most common response to carcinogen exposure is deletions at the *FHIT* locus that alter the gene structure and function. In this study we assign the *FHIT* gene in cattle, river buffalo, sheep and goat chromosomes by comparative fluorescence *in situ* hybridization (FISH)-mapping. In addition, the assignment to BTA22 was confirmed by typing the marker across a bovine radiation hybrid (RH) panel.<sup>3</sup>

**Isolation of a bovine FHIT fragment:** Primers were designed from a bovine expressed sequence tag (BF076413):

F: 5'-GAACAAATCCCCAGGCATAAG-3'

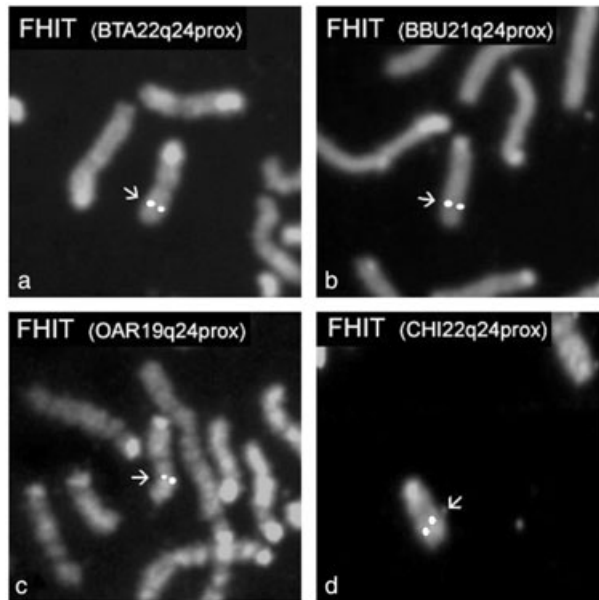
R: 5'-TTGAAACATAACCCCGTTGGC-3'

Polymerase chain reaction (PCR) was performed using 20 ng genomic DNA. Cycling conditions were for 32 cycles of 94 °C at 20 s, 60 °C at 30 s and 72 °C at 45 s. The 157-bp PCR product was sequenced and used to search for homology in GenBank with BLAST. There was 84% identity ( $E=8e-24$ ) with a human clone (AC093418) containing a portion of the *FHIT* gene.

**Screening of a bovine BAC library:** A bovine BAC library<sup>4</sup> was screened by PCR with the bovine-specific primers described above; three positives BACs were recovered. One of the BAC clones (bI0876E06), was partially sequenced to confirm the presence of bovine *FHIT* exon 9 and the sequence has been deposited in GenBank (AY862498). The clone was subsequently used as a hybridization probe to perform FISH.

**Radiation hybrid mapping:** The *FHIT* marker was analysed in the 3000 rad bovine-hamster RH-panel as described earlier.<sup>3</sup> Primers and PCR cycling conditions were as described above for the BAC library screening. *FHIT* was mapped to BTA22 and the most closely linked markers by two-point analysis were *BMS980* and *HAUT24* (LODs 10.699 and 6.127 respectively).

**Cell cultures and FISH-mapping:** The FISH experiments were performed as reported earlier.<sup>5</sup> *FHIT* maps to BTA22q24prox, BBU21q24prox, OAR19q24prox and CHI22q24prox (Fig. 1) in cattle, river buffalo, sheep and goat respectively. These chromosomes and chromosome bands are homologues, further confirming the high degree of chromosome conservation among bovid species. The FISH and RH mapping of *FHIT* in the bovine represent the first physical localization of a type I marker in a bovine chromosomal region that shares conservation of synteny with band HSA3p14.2. Figure 2 shows corresponding cytogenetic maps of the four species. The cytogenetic maps of BTA22 and CHI22 are richer than those of OAR19 and BBU21. These comparisons with HSA3 suggests complex chromosomal rearrangements differentiating bovid chromosome 22 and the human chromosome, including a centromere repositioning, as also revealed by RH-mapping.<sup>6</sup> Thus, markers which are clus-



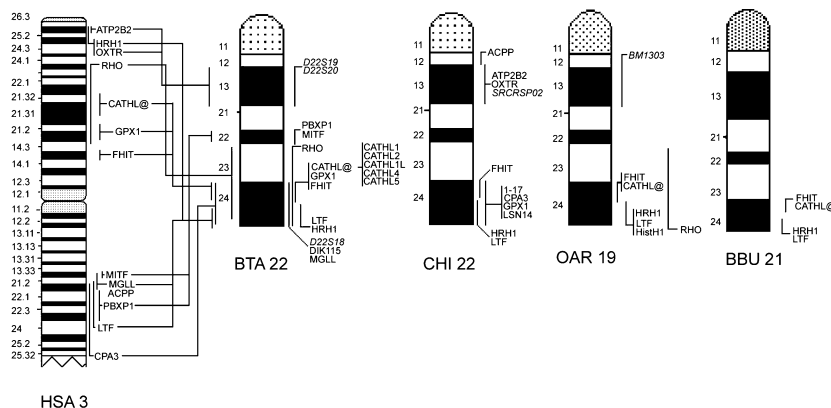
**Figure 1** Fluorescence *in situ* hybridization mapping of a bovine BAC clone containing *FHIT* to RBPI-banded BTA22q24prox (a), BBU21q24prox (b), OAR19q24prox (c) and CHI22q24prox (d). Arrows indicate the position of hybridization FITC-signals.

tered on the same chromosomal band on BTA22q24 (*CATHL@*, *GPX1*, *FHIT*, *LTF*, *HRH1*, *MGLL*) map to different chromosome arms and regions of HSA3. Specifically, *FHIT*, *GPX1* and *CATHL@* map to three different bands of HSA3p (14.2, 21.1–21.2 and 21.31–21.32 respectively), while *LTF* and *MGLL* map to HSA3q21–q22. In contrast, *HRH1* maps independently to HSAp25 together with *ATP2B2* that is localized also to CHI22q13. Furthermore, *PBXP1* and *MITF* that are assigned to BTA22q22, map to HSA3q21–q22, as does *MGLL*.

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**Figure 2** Cytogenetic maps (only fluorescence *in situ* hybridization-mapped loci) of BTA22, CHI22, OAR19 and BBU21 shown as R-banded ideograms in comparison with HSA3. Note the loci clustered on bovine band 24 and spread to different arms and bands of HSA3. For comparison with other loci see details in the text. Type I loci are shown as regular characters as opposed to type II loci that are in italics.

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## Assignment porcine *PCK1* and *PCK2* genes to SSC17 and SSC7, respectively, by radiation hybrid mapping

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**Source/description:** Phosphoenolpyruvate carboxykinase (PCK) plays a critical role in the formation of phosphoenolpyruvate from oxaloacetate during gluconeogenesis. In vertebrates, PCK has two isoforms: cytosolic PCK1 and mitochondrial PCK2. Mice that lack the PCK1 enzyme die within 3 days of birth, while mice with up to a 90% global reduction of PCK, or a liver-specific knockout of *PCK1* gene, are viable.<sup>1</sup> PCK2 is also a pivotal component in gluconeogenesis, producing phosphoenolpyruvate from lactate via the Cori cycle. *PCK2* is expressed in several human tissues including liver, kidney, pancreas, intestine and fibroblasts.<sup>2</sup> Thus, *PCK1* and *PCK2* are important genes involved in maintaining the energy balance of mammals. In this study, we mapped *PCK1* and *PCK2* to porcine chromosomes using a porcine radiation hybrid panel (IMpRH).<sup>3,4</sup>

**Primer sequences:** Human cDNA sequences of *PCK1* and *PCK2* genes (NM\_002591 and NM\_004563 respectively) were compared with all sequences in the standard BLAST (<http://www.ncbi.nlm.nih.gov/blast/>). Porcine ESTs, which shared at least 80% identity to the corresponding human cDNA, were used to design primers. A fragment of *PCK1*, containing portions of