

Microdissected Bovine X Chromosome Segment Delineates Homologous Chromosomal Regions in Sheep and Goat*

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In this study we microdissected the proximal part band Xq24 of the bovine X chromosome long arm, in which the *Xist* gene is located in cattle. The obtained DNA fragment was PCR amplified, labelled and used as a probe for cattle, sheep and goat chromosomes. In cattle, as expected, distinct hybridization signals were observed on Xq24. The painting signals were also observed on Xq24 in sheep and goat. The chromosome painting probes can be used for comparative mapping and searching for internal X chromosome rearrangements in Bovidea and may contribute to the understanding of mammalian sex chromosome evolution.

Key words: Bovidae, chromosome microdissection, comparative painting.

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Chromosome microdissection and the generation of chromosome painting probes can be used as a tool in physical gene mapping and in studies of the evolution of mammalian species. It has been shown, initially for humans and later for other species including cattle, that this is a valuable and accurate tool in the cytogenetic and comparative analysis of genomes (e.g. IANNUZZI & DE MEO 1995; HASSANANE *et al.* 1998; XIAO *et al.* 1998; KUBICKOVA *et al.* 2002; RUBES *et al.* 2005). Comparative cytogenetics and gene mapping have successfully enabled the detection of conserved synteny between evolutionarily close as well as distant genomes. The X chromosome linkage group is known to be conserved in placental mammals. However, X chromosome morphology is different in the Bovidae family, being submetacentric in cattle and acrocentric in sheep and goats. In this present study, a painting probe specific for cattle chromosome band Xq24 was generated by basic manual microdissection and used for Zoo-FISH on goat and sheep chromosomes in order to localize the chromosome fragment comprising the *Xist* gene.

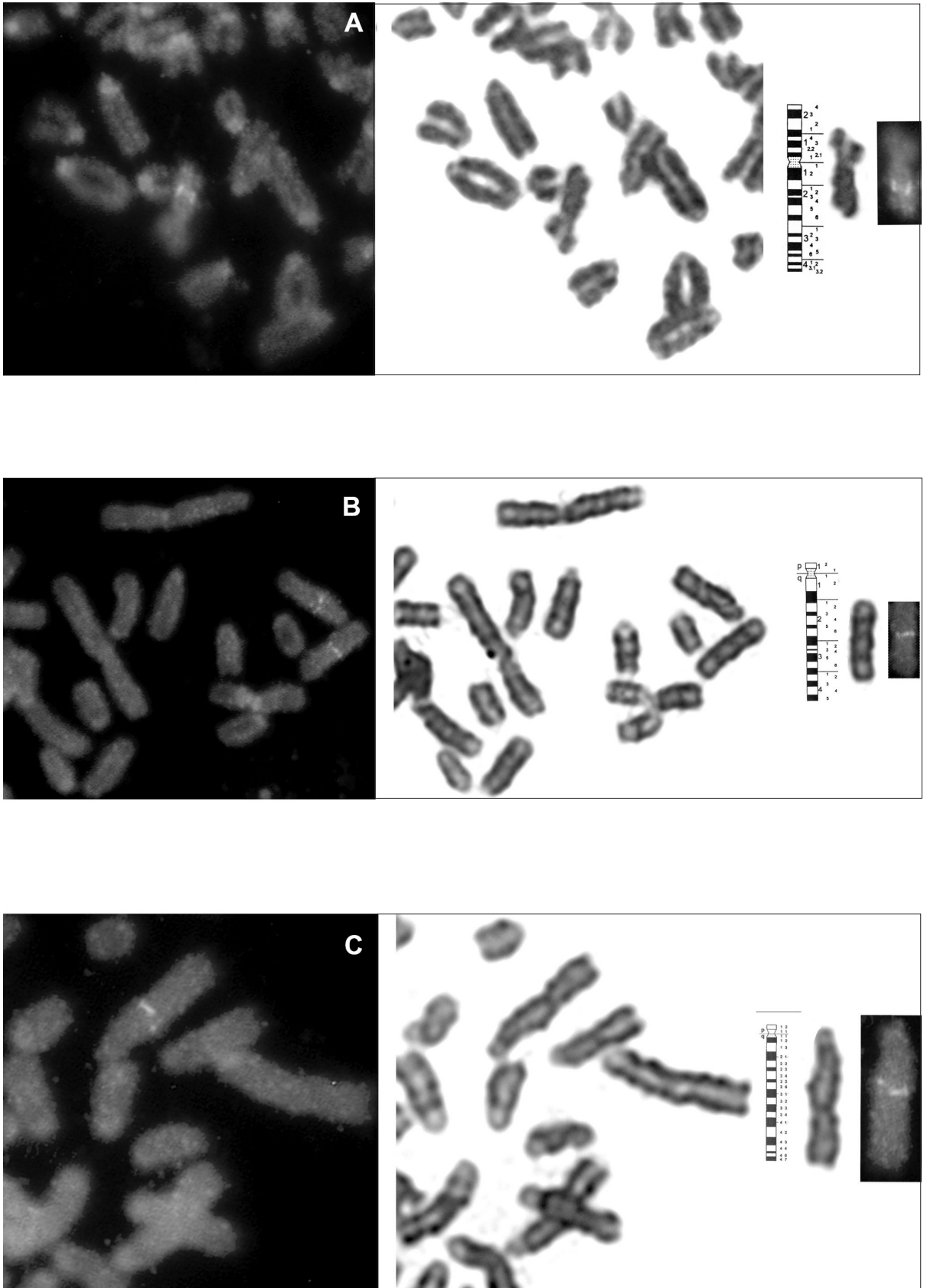
Material and Methods

Metaphase spreads were prepared after cattle lymphocyte culture by standard cytogenetic tech-

niques. The detection of the Xq24 chromosome band was carried out by fluorescence *in situ* hybridisation (FISH) with the use of a bovine Xq24 PCP (Partial Chromosome Painting) probe. An aliquot of the cattle metaphase chromosome suspension was spread onto a coverslip and stained with the GTG banding technique. For painting probe acquisition, five copies of the Xq24 chromosome band were dissected with glass microneedles controlled by a micromanipulator attached to an inverted microscope. The dissected fragments were transferred by breaking off the microneedle into a PCR tube containing a collection drop solution. The dissected DNA material was amplified by DOP-PCR using degenerate oligonucleotide primes (5'-CCGACTCGAGN6ATGTGG-3') (DOP PCR; TELENUS *et al.*, 1992). Aliquots of the amplified DNA were labelled by DOP PCR with biotin-16-dUTP for the FISH experiment. The labelled PCR products were purified using Nick Columns according to the manufacturer's protocol and co-precipitated with 5 µg of salmon sperm DNA.

The biotin-labelled probe in a hybridisation mix (50% formamide, 10% dextran sulphate, 10% 20xSSC, 1% Tween 20 and 29% H₂O) was applied to the chromosome preparations. Briefly, the chromosome preparations were denatured in 70% for-

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mamide in saline-sodium citrate buffer (2xSSC) for 2.5 min at 70°C. The probe was denatured at 70°C for 10 min. Hybridisation was carried out in 37°C for three days. Post-hybridisation washes were as follows: three times in 50% formamide in 2xSSC and three times in 2xSSC at 42°C. Hybridisation signals were detected by avidin – FITC (fluorescein isothiocyanate) and anti-avidin system on propidium iodide stained slides. The identification of PCP probe signals was conducted on G-banded cattle, sheep and goat chromosomes (ISCANDB 2000, 2001) Microscopic evaluation was performed under an Opton Axiophot fluorescence microscope equipped with a camera and Lucia software.

Results

A bovine PCP probe was successfully used to identify a homologous chromosome segment in cattle, sheep and goat karyotypes. Hybridization to cattle chromosomes gave a strong painting signal in the Xq24 segment (Fig. 1A), which confirmed its specificity. No cross-hybridization to other chromosomes was observed. On sheep and goat chromosomes, the microdissected probe demonstrated a distinct hybridization signal on the proximal part of the long arm of the X chromosome on the q24 band (Figs 1B & 1C). Other chromosomes did not show non-specific hybridization with the microdissected cattle X chromosome fragment.

Discussion

Cattle, sheep and goat included in the Bovidae family are economically important farm animals. Numerous cross-species painting experiments have demonstrated the usefulness of the Zoo-FISH technique in localization of homologous chromosomal segments between different species (SCHERTHAN *et al.* 1994; FRÖNICKE *et al.* 1995; HAYES 1995; RETTENBERGER *et al.* 1995; SOLINAS-TOLDO *et al.* 1995; CHOWDHARY *et al.* 1996; HASSANANE *et al.* 1998; RUBES *et al.* 2005).

The cattle genome map is fully developed in comparison with the sheep or goat map. Based on the cattle physical genome map, the region of the X chromosome in which the *Xist* gene is localized was chosen. Using the Zoo-FISH technique this chromosome fragment was localized on the proximal regions of sheep and goat X chromosomes.

Our Zoo-FISH experiments, involving painting probes from the Xq24 segment in cattle, clearly

show that this region corresponds to the proximal region of sheep and goat X chromosomes.

According to the physical map of cattle, the *Xist* gene is localized in the region Xq24 (GOLDAMER *et al.* 2003). This region shows homology to Xp24 in sheep and goats, suggesting that the *Xist* gene is localized in the same region in sheep and goats.

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