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# Probe generated by Y chromosome microdissection is useful for analysing the sex chromosomes of the domestic horse*

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ABSTRACT

Microdissection of chromosomes is an invaluable tool for physical isolation of single chromosomes, chromosome arms or chromosome bands. In the present study we have generated a fluorescence *in situ* hybridization (FISH) probe from the microdissected Y chromosome of the domestic horse. For the identification of the Y chromosome, we used the CBG technique. A biotin-labelled probe in the FISH technique gave a specific signal on all Y chromosomes and an additional signal in the interstitial part of the long arm of the X chromosome. This area showed an additional C band detected by the CBG method and characteristic for this chromosome. The probe obtained can therefore be used for analysis of the Y and X chromosomes and their copies in the genome, and, in the case of the X chromosome, also for gene mapping.

KEY WORDS: horse, chromosome painting, microdissection, sex chromosomes

## INTRODUCTION

Cytogenetic investigation of horses, which has been carried out for more than 35 years, has shown that sex chromosome abnormalities are a common karyotype

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aberration in this species, causing infertility or subfertility. The majority of the horse karyotypic abnormalities identified worldwide have demonstrated X monosomy in the non-mosaic (63,X) or mosaic (63,X/64,XX) form (Power, 1990). Moreover, the sex-reversal syndrome (Power, 1990; Buoen et al., 2000) has also been diagnosed quite frequently. On the other hand, X chromosome trisomy appears to be rather rare in this species (Breen et al., 1997; Makinen et al., 1999; Wieczorek et al., 2001; Bugno et al., 2003). Additionally, 64,XX/64,XY lymphocyte chimerism has been identified not as often in horses as in cattle (Power, 1990).

For several decades chromosomes have been analysed by traditional karyotyping, which was based on the analysis of characteristic banding patterns. The major disadvantage of conventional cytogenetic banding methods is a limited resolution. Fluorescence *in situ* hybridization (FISH) now plays an essential role in clinical diagnostics as well as in experimental research. The use of chromosome painting in farm animals is mainly limited by the fact that chromosome-specific probes are not commercially available for individual animal species.

In the present work, we used the conventional microdissection to generate a domestic horse whole Y chromosome painting probe (WYCPP) that was then applied to the analysis of sex chromosomes (X and Y) in domestic horses.

## MATERIAL AND METHODS

### *Chromosome preparation and identification*

Male metaphase chromosomes of domestic horses were obtained from Pokeweed-stimulated lymphocyte cultures according to the standard protocols. Chromosome preparations for microdissection and FISH experiments were stored at -80 and -20°C, respectively. The CBG technique was used for the identification of Y chromosomes (Sumner, 1972).

### *Preparation of a horse whole Y chromosome painting probe (WYCPP)*

An aliquot of the equine metaphase chromosome culture was spread onto a coverslip and stained with Giemsa. For each microdissection experiment fifteen Y chromosome copies were dissected with glass microneedles controlled by a manipulator attached to an inverted microscope. The dissected chromosomes were transferred by breaking off the microneedle to a PCR tube containing collection drop solution.

The dissected DNA material was amplified in a DOP-PCR using degenerate oligonucleotide primes (5′-CCGACTCGAGN6ATGTGG-3′) (DOP PCR; Telenius et al., 1992).

Aliquots of the amplified DNA material were labelled by DOP PCR with biotin-16-dUTP for FISH experiment. The labelled PCR products were purified

using Nick Columns according to the manufacturer's protocol and co-precipitated with 5 µg salmon sperm DNA and 5 µg horse Cot-1 DNA.

*Fluorescence in situ hybridization*

The labelled probe was denatured at 70°C for 10 min. After RNase and pepsin digestion, target metaphase spreads were denatured in a hybridization solution containing 2xSSC and 70% formamide, at 70°C for 2.5 min. The probe was dropped onto the metaphase spread, covered, sealed with rubber cement and hybridized overnight in a moist chamber at 37°C. Post-hybridization washes were as follows: three times at 50% formamide in 2×SSC and three times in 2×SSC at 42°C. Biotin-labelled probe was detected by the avidin-FITC and anti-avidin system on propidium iodide-stained slides. Microscopic evaluation was performed under a fluorescence microscope equipped with a CCD camera and Lucia software.

## RESULTS

The horse WYCPP was hybridized onto metaphase chromosomes of the three horses demonstrating a high specificity for whole Y chromosome. In addition, an interstitial band was observed on the heterochromatic region of the X chromosome. The results are shown in Figure 1.

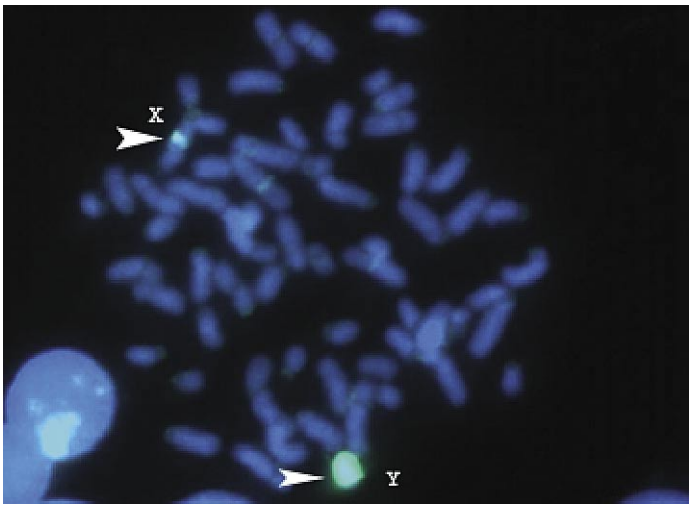


Figure 1. Metaphase spread from stallion, FISH-painted with the use of the equine Y whole chromosome specific probe. Large arrows indicate green Y chromosome and signal in the interstitial part of the q arm of the X chromosome. Small arrows indicate additional signal on centromeric areas of chromosomes pair 7

## DISCUSSION

There are various ways for the generation of chromosome-specific painting probes (reviewed by Langer et al., 2004). Chromosome-specific DNA probes can be created by flow sorting of whole chromosomes and subsequent universal amplification by DOP- PCR (Carter et al., 1992; Wienberg et al., 1994).

Flow sorting has been successfully applied to the generation of whole chromosome-specific libraries from various species, including painting probes for pigs, dogs, mice, rats, and muntjacs. Usually, several hundred chromosomes have been sorted to generate a painting probe by flow sorting (Telenius et al., 1992).

Microdissection represents an alternative method for physical isolation of single chromosomes in cases where the flow sorting technology is not available or the desired chromosomes are very similar in size to other pairs so the separation is impossible. In addition, it allows for the isolation of chromosome arms or even chromosome bands and, therefore, is an invaluable tool for studying numerical or structural chromosome aberrations. Unlike in humans, this method has rarely been applied in the field of animal genetics (Pinton et al., 2003).

To date, chromosomes of farm animals for the construction of chromosome-specific libraries or painting probes have been obtained by flow sorting (Dixon et al., 1992; Schmitz et al., 1992) or conventional microdissection (Goldammer et al., 1996; Chaudhary et al., 1998; Raudsepp and Chowdhary, 1999).

Thalhammer et al. (2004) showed the usefulness of probes obtained from single copies using microdissection to identify small interchromosomal rearrangements.

Molecular probes obtained by chromosome microdissection are a valuable tool for analysing the animal karyotype and for observing genetic polymorphism or conservatism.

In the present study, we showed that FISH with a Y-specific probe derived from the horse genome yielded strong and clear signals not only on Y chromosomes (painted in their entirety) but also on X chromosomes at the site of an additional interstitial band, which is usually visible after CBG staining. Therefore, identification of the X chromosome is also possible, and even the use of this probe for mapping genes located on the X chromosome to confirm this localization.

Analogous observations were made in the maned wolf, where a Y-specific probe derived from the canine genome painted the entire Y chromosome and the constitutive heterochromatin block on the long arm of the X chromosome. In the bat-eared fox and the fennec, however, it hybridized only in the Y chromosome centromeric area and in the X chromosome centromeric and subcentromeric area (Pieńkowska-Schelling et al., 2004).

Very interesting studies have been reported by Raudsepp and Chowdhary (1999), who performed a comparative analysis of the chromosomes of the domestic

horse (*Equus caballus*) and the domestic donkey (*Equus asinus*) using meta-/submetaspecific and Y-specific equine painting probes. The Y-specific equine probe painted the entire Y chromosome of the domestic donkey and gave a signal on the q arm of the donkey X chromosome, analogously to the signal present on the q arm of the X chromosome in the domestic horse. Additional signals were noted on the centromeres and telomeres of some domestic donkey chromosomes (Raudsepp and Chowdhary, 1999). A similar result was obtained in the present study, however, in addition to signals on the Y and X chromosomes additional signal on chromosomes pair 7 (centromeric areas) was observed.

The described application of chromosome painting probe in horses is of great importance, as sex chromosomes aberrations, characteristic for this species cause large economic losses in horse breeding. The probes will be useful for analysing the number of sex chromosome copies, as well as in physical gene mapping, and in studies of mammalian species evolution, based on karyotype relationships and similarities.

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