GENERATION AND USE OF CHROMOSOME FRAGMENT SPECIFIC BOVINE DNA PROBES FOR CYTOGENETIC STUDIES IN CATTLE

GENERACIÓN Y USO DE SONDAS DE DNA BOVINO ESPÉCIFICAS PARA FRAGMENTOS CROMOSÓMICOS PARA ESTUDIOS CITOGENÉTICOS EN BOVINO

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Palabras clave adicionales

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SUMMARY

The combined use of developed techniques as chromosome (Chr) microdissection and DOP-PCR amplification of Chr fragments allows the rapid generation of Chr fragment specific DNA probes. In the present study different individual bovine Chr fragments of various size were microdissected, amplified by DOP-PCR, biotinylated with biotin-16-dUTP and used as probes in fluorescence *in situ* hybridization (FISH) to bovine Chrs. It is shown that such DNA probes could be a useful tool for the identification of individual Chrs or Chr fragments and for the characterization of chromosomal rearrangements.

RESUMEN

El uso combinado de las técnicas desarrolladas como la microdisección de cromosomas (Chr) y la amplificación de los fragmentos cromosómicos mediante reacción DOP-PCR, permite la rápida generación de sondas específicas para fragmentos cromosómicos. En el presente estudio diferentes fragmentos cromosómicos bovinos individuales fueron microdiseccionados, amplificados mediante la reacción DOP-PCR, biotinilados con biotina-16-dUTP y empleados como sondas en fluorescencia de hibridación *in situ* (FISH) para los cromosomas bovinos. Se demuestra que tales sondas DNA podrían ser una útil herramienta para la identificación de cromosomas individuales o fragmentos cromosómicos y para la caracterización de reagrupamientos cromosómicos.

INTRODUCTION

In the recent years different cytogenetic methods including FISH techniques came to the fore for the analysis and description of the genome. Also the cytogenetic analysis of the bovine genome was performed by FISH techniques ether by the direct localization of single DNA sequences, summarized in the bovine gene map (Egcen and Fries, 1995), or by the use of whole Chr painting probes (WCP's)

generated by FACS sorting (Schmitz *et al.,* 1995) or microdissection (Ponce de León, 1996). Additionally, WCP's from the human genome were applied in ZOO-FISH reactions to describe the evolution of Chrs between human and bovine (Solinas-Toldo *et al.*, 1995; Hayes, 1995; Chowdhary *et al.*, 1996). A specific variation of these cytogenetic methods is the analysis of different bovine Chr regions of interest with Chr fragment specific painting probes generated by microdissection and PCR amplification. Guan *et al.* (1993) have shown on the human genome that such DNA probes can be a useful tool to identify specific Chrs or Chr regions and to analyze structural Chr rearrangements. In this report we describe the generation of Chr fragment specific DNA probes from the bovine genome and their effective use for the identification of different bovine Chrs or Chr fragments, for structure analysis of meiotic Chrs, for identification of Chr rearrangements and for comparative studies.

MATERIALS AND METHODS

CHROMOSOME PREPARATION AND C-BANDING

Metaphase spreads were prepared from fibroblast cell cultures of *Bos taurus* and *Bos indicus* by standard cytogenetic techniques. Chrs were GTG-banded by trypsin and Giemsa stained as described by Seabright (1971) prior their use for microdissection or FISH. GTG-banded metaphase spreads were digitized and Chrs were karyotyped according to the ISCNDA (1990) for bovine GTG-banded Chrs.

CHROMOSOME MICRODISSECTION, TOPOISOMERASE I TREATMENT AND PCR AMPLIFICATION

These techniques are described in detail by Goldammer *et al.* (1996). Briefly, all Chr manipulations were observed under an inverted microscope. Microdissection of Chr fragments was performed with glass microcapillaries which were fixed and controlled by a motor driven micromanipulator. Microcapillaries were forged with an automatic pipette puller. The capillary touched the cover glass in a right angle to the Chr area to be dissected. The Chr material will be saaped by forward movement of the microcapillary. The dissected Chr material adherent to the tip of a microneedle was transferred into a PCR tube containing the DOP-PCR mix and Topoisomerase I. The Chr material was treated with Topoisomerase I and amplified by DOP-PCR It is sufficient to isolate only a single Chr fragment for the successful amplification of the chromosomal DNA. The generated PCR products were characterized by agarose gel electrophoresis.

FISH

The generated PCR products were biotinylated with biotin-16-dUTP in a second DOP-PCR and used as DNA probes for the rehybridization to bovine Chrs. The FISH reaction was performed essentially as described by Pinkel *et al.* (1988).

RESULTS

Chr fragments from different Chr regions (Iql3-24, lql2-31, 3q21-31, Sq21-

24, 6q21-31, 6q31-32, 6q32-33, 7q21-22, 12q24-ter, 29ql6-ter, Ypl2 and Yql2.1-12.6) were isolated by microdissection. The dissection of a specific fragment from Chr 1 is shown for example in **figure 1a**. The generated DNA sequences were characterized by size fractionation with the agarose gel electrophoresis **(figure 1b)** after Topoisomerase I treatment and DOP-PCR amplification of microdissected Chr fragments. Generated Chr fragment specific DNA shows a DNA smear ranging from 100 to 1000 bp with a peak at approximately 250 bp. These PCR products were biotinylated and used as painting probes firstly to proof their specificity and complexity. About 70 to 80 p. cent of all generated painting probes hybridized specific and are representative for the microdissected area. The hybridization signals correspond to the microdissected Chr region. Secondly the painting probes

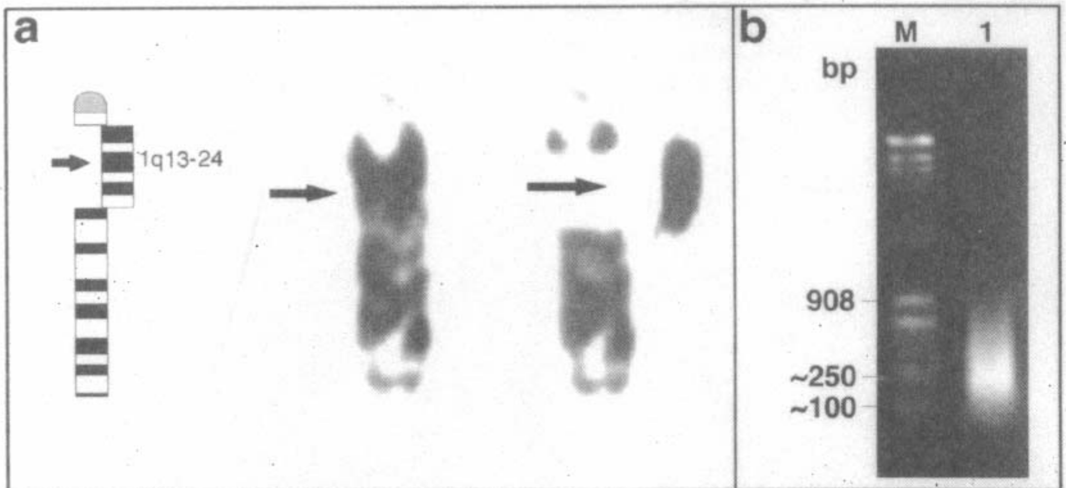

Figure 1. *(a) Microdissection of bovine Chr fragment 1q13-24. (Left) GTG-band ideogram of Chr 1; the region to be dissected is moved. (Middle). Chr 1 prior to the dissection. (Right) The same Chr after dissection. (b) Electrophoretic characterization of the DOP-PCR amplified microdissected Chr fragment 1q13-24. The size markers were pBR322* AluI/*Lambda* HindIII. (a) Microdisección del fragmento cromosómico bovino 1q13-24. Izquierda: idiograma con bandas GTG del cromosoma 1; la región que debe ser disecada es separada. Centro: el cromosoma 1 antes de la disección. Derecha: el mismo cromosoma después de la disección. b) Caracterización electroforética del fragmento cromosómico 1q13-24 microdisecado y amplificado mediante la reacción DOP-PCR. Los marcadores de tamaño fueron pBR322 AluI/Lambda HindIII).

were used for the identification and morphological analysis of specific bovine Chrs and Chr fragments as shown for the paints on Chr 3 and 29 in **figure 2**. Painting probes with positive hybridization signals on Chrs were than used for further investigations. After FISH ofthe probe Ypl2 from *B. taurus* to meiotic Chrs in metaphase I the XY-bivalent could be identified and it was shown that the p-arm of Chr Y is linked to Chr X **(figure 3a)**. A probe generated from Chr fragment 29ql6-ter hybridized specific to this region and supported the identification of a double 1/29 translocation in *B. indicus* **(figure 3b)**. The comparative hybridization of the Y-Chr probe ql2.1-12.6 of *B. indicus* resulted in a hybridization area on the same region on the Y-Chr of *B. taurus* **(figure 3c)**. The proximal part of the q-arm of the *B. indicus* Y-Chr corresponds to the q-arm of the Y-Chr from *B. taurus*.

DISCUSSION

A fast method was presented which allow the production of Chr fragment specific DNA-sequences of the bovine. The generaated probes show a high fragment specificity and are representative for the microdissected Chr region as demonstrated by FISH. The

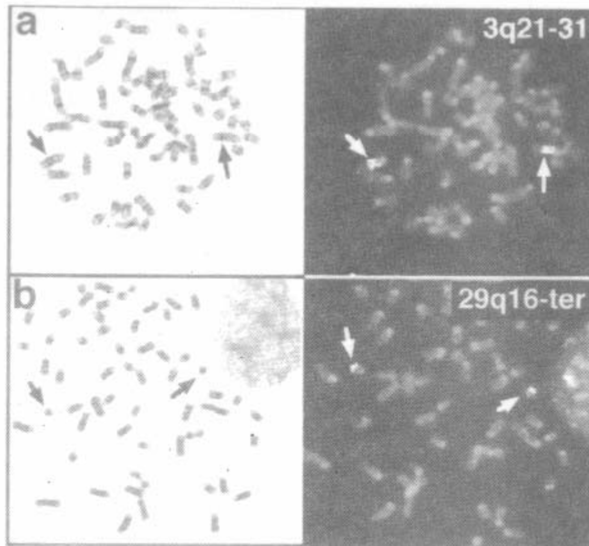

***Figure 2.** Rehybridization of amplified Chr fragments by FISH: (a) 3q21-31, (b) 29q16-ter. (Arrows indicate the specific Chrs and hybridization sites. (Left) GTG-banded metaphase spreads prior to FISH. (Right) The same metaphase spreads after FISH with biotinylated Chr fragments.*
(Rehibridación de los fragmentos cromosómicos amplificados mediante FISH: (a) 3q21-31, (b) 29q16-ter. (Las flechas indican los cromosomas específicos y los lugares de hibridación). Izquierda: desarrollo de la metafase con bandas GTG antes de FISH. Derecha: desarrollo de la misma metafase después de FISH con fragmentos cromosómicos biotinilados).

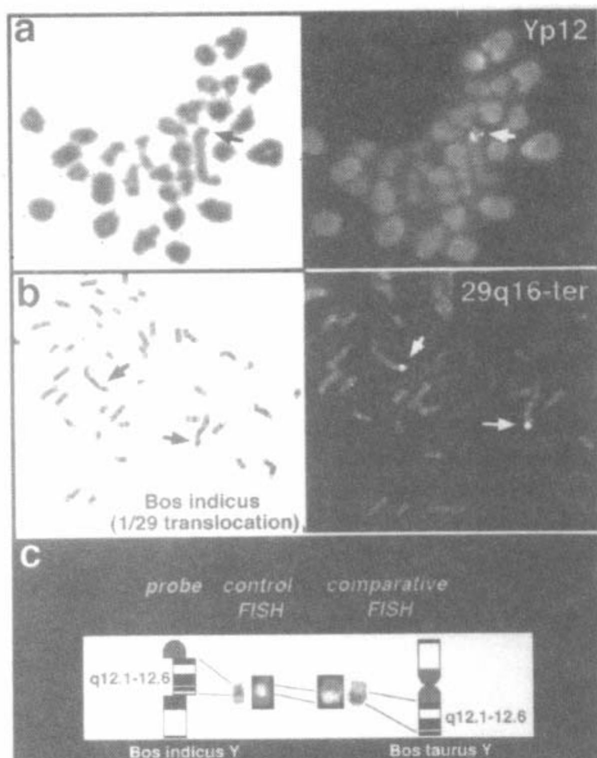

Figure 3. *Use of painting probes for the genome analysis of the bovine. (a) Identification of the XY-bivalent by FISH of the probe Yp12 to M I-Chrs of male. (b) Identification of a double 1/29 translocation in a fibroblast cell line of* B. indicus *by the painting probe 29ql6-ter (metaphase spreads before (left) and after (right) FISH). (c) FISH of the probe Yq12.1-12.6 of* B. indicus *to the* B. indicus *and* B. taurus *Y-Chr.* (Uso de las sondas colorantes para el análisis del genoma bovino. a) Identificación del bivalente XY mediante FISH de la sonda Yp12 a cromosomas M I de macho. b) identificación de una doble translocación 1/29 en una línea celular de fibroblastos de *B. indicus* mediante la sonda colorante 29q16-ter (desarrollo metafásico antes (izquierda) y después (derecha). c) FISH de la sonda yq12.1-12.6 de *B. indicus* a *B. indicus* y *B. taurus*).

Chr specific DNA-fragments are a useful tool for cytogenetic studies on cattle. Individual bovine Chrs or Chr rearrangements could be identified by Chr fragment specific painting probes. Additional, generated DNA sequences could be used for the establishment of Chr fragment specific DNA libraries. Chr fragment specific Type I and Type II DNA markers from these libraries could provide the generation of Chr fragment specific contigs by screening of large DNA fragment libraries (YAC's, BAC's). Large overlapping DNA fragments enable the precise description of a specific Chr region.

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