

Isolation and physical localization of new chromosome-specific centromeric repeats in farm animals

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ABSTRACT: In this study, new classes of tandemly repeated DNA sequences in the centromeric regions of three farm animal species are reported. Pericentromeric regions of bovine chromosome X, porcine chromosome 14 and equine chromosome 1 were microdissected by laser and amplified by DOP-PCR. Painting probes showed strong hybridization signals on their corresponding centromeres. Specific DNA fragments were isolated by cloning and sequenced. For each species, high homology level was found among the sequences. The presence of specific repeats within each sequence allows us to consider them as monomeric units or parts of longer tandemly repeated monomeric units that generate a specific higher-order repeat. In this respect, the isolation of new centromeric sequences enriches the genetic map of these three species and represents a useful tool for FISH analysis, thus expanding our knowledge on centromeres of farm animal species.

Keywords: *Bos taurus*; *Sus scrofa*; *Equus caballus*; cattle; pig; horse; centromeres; tandem repeats; chromosomes; FISH

Centromeres are an essential structural domain of chromosomes and play an essential role accurate chromosomal segregation during mitosis and meiosis. In humans, centromeric regions of all chromosomes contain a huge repetitive sequence, known as alphoid DNA that constitutes as much as 3–4% of chromosomal DNA (Mitchell et al., 1985; Alexandrov et al., 2001). Alphoid sequences consist of tandem repeats of an AT-rich 171-bp alphoid monomer unit, organized into higher order repeats (HORs). These monomers appear to be conserved among all human chromosomes, but the nucleotide sequence and the organization of the monomeric units are known to vary from one chromosome to another, showing substantial chromosome-specific variation which can be used as target for the construction of chromosome-specific probes (Willard, 1985; Willard and Wayne, 1987).

The isolation and the analysis of satellite DNA sequences and their hybridization on individual chromosomes have opened new diagnostic pos-

sibilities in the cytogenetic fields, especially in humans. Alphoid probes which hybridize to repetitive DNA sequences on specific chromosomes have been used to identify chromosome abnormalities in interphase nuclei of tumoral cells and tissues including cells of breast (Devilee et al., 1988), bladder and buccal mucosa (Moore et al., 1993), bone marrow (Kolluri et al., 1990) and ovarian epithelium (Huang et al., 2002).

Specific alphoid DNA probes have been developed for most of the human chromosomes (Burke et al., 1985; Moyzis et al., 1987; Willard and Wayne, 1987; Pellestor et al., 1996), but little information is available on high-order specific repeats in non-human species, because they have not been adequately investigated yet. At present, alpha satellite DNA probe, specific for the centromere of chimpanzee chromosome 4, has been obtained by using oligonucleotide primers specific for a conserved region of human satellite DNA (Haaf and Willard, 1997). High-order repeats probes that hybridize

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specifically to the centromere of metaphase chromosome 9 (Janzen et al., 1999), chromosome 11 (Riquet et al., 1996), and chromosome 1 (Jantsch et al., 1990) have been produced in pigs.

On the contrary, unspecific repetitive probes have been obtained for the centromeric regions of many chromosomes of species belonging to Bovidae (Nijman and Lenstra, 2001) and Caprinae subfamilies (Chaves et al., 2000). Most of the pig acrocentric and metacentric chromosomes have been hybridized by using centromeric DNA probes (Rogel-Gaillard et al., 1997a, b). In the horse chromosomes, tandem repeat elements have been localized only in the centromeres of the acrocentrics, not in the metacentrics (Broad et al., 1995). Centromeric satellite probes have been obtained for many other species including rabbit (Ekes et al., 2004), roe deer (Buntjer et al., 1998) and caribou (Lee et al., 1994).

In order to extend our knowledge on the centromeric region of farm animals, we detected and characterized new chromosome-specific repeats for cattle (*Bos taurus*), pig (*Sus scrofa domestica*) and horse (*Equus caballus*) by using chromosome microdissection of the pericentromeric area of chromosomes X, 14 and 1, respectively, FISH and sequencing.

MATERIAL AND METHODS

Chromosome preparation and PCR amplification. Metaphase spreads were prepared from bovine, porcine and equine lymphocytes by standard cytogenetic techniques. To identify the chromosome of interest, GTG-banding was performed.

The PALM[®] MicroLaser system (P.A.L.M. GmbH, Bernried, Germany) was used for dissection and collection of pericentromeric part of chromosomes (Bos X, Sus 14 and Equus 1). The microdissected DNA was amplified as described earlier (Kubickova et al., 2002). Briefly, after collection, parts of chromosomes were transferred to 20 µl of 10mM Tris-HCl pH 8.8 and the DOP-PCR was performed without any pretreatment in a PTC-200 thermocycler (MJ Research, Watertown, USA). The reaction mixture contained 60mM Tris-HCl pH 8.8, 15mM (NH₄)₂SO₄, 3.5mM MgCl₂, 0.2mM each dNTPs, 1.6µM DOP primer (5'-CCG ACT CGA GNN NNN NAT GTG G-3', Telenius, 1992), 0.05% W-1 (Invitrogen Life Technologies, Carlsbad, CA, USA), 2U Taq polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA) in final volume of 40 µl. After

the initial denaturation step at 96°C for 3 min, eight cycles were performed at 96°C for 1 min, at 30°C for 1 min with a 2 min transition from 30°C to 72°C, and at 72°C for 2 min. This was followed by 35 cycles of 1 min at 94°C, 1 min at 56°C, and 2 min at 72°C. The final extension was carried out at 72°C for 5 min. PCR products (5µl) were analyzed by electrophoresis using 1% agarose gel.

Cloning and hybridization analysis. The remaining primary DOP-PCR products were reamplified under the same conditions and then purified using the High Pure PCR Product Purification kit (Roche Diagnostics GmbH, Mannheim, Germany). The pDrive vector (QIAGEN) was used for cloning. The ligation products were transformed into QIAGEN EZ Competent cells following the manufacturers' guidelines (QIAGEN PCR Cloning ^{plus} Kit). Screening of the recombinant clones was performed by PCR using the vector primers M 13. A small portion of the product was run on a 1% TBE agarose gel to determine the size of the insert.

Amplification products (1.5 µl) were spotted onto two copies of nylon filters and hybridized with digoxigenin-11-dUTP probes (Roche Diagnostics GmbH, Mannheim, Germany) under standard conditions (Maniatis et al., 1982). One filter was hybridized with specific primary DOP-PCR and the second one with genomic DNA at 42°C for 16 hours. Anti-DIG antibody coupled to alkaline phosphatase (anti-DIG-AP; Roche Diagnostics GmbH, Mannheim, Germany) and BM purple AP substrate (Roche Diagnostics GmbH, Mannheim, Germany) were used for DIG signal detection. In order to estimate the amount of specific repeats present in each clone, a comparison of the intensity of the hybridization signals was performed. The clones showing a higher hybridization signal with specific probe were considered positive. Chosen Dot Blot positive clones were labelled by PCR with Spectrum Orange-dUTP (Vysis, Richmond, UK) and used as probes in FISH.

Fluorescence *in situ* hybridization. Metaphase spreads for FISH were prepared from lymphocytes using standard methanol:acetic acid (3:1) fixation.

For hybridization with a single painting probe, 1 µl of probe was placed in 10 µl of a hybridization mixture containing 50% formamide, 2× SSC, 10% dextran sulphate, and 7 µg of salmon sperm DNA. The hybridization mixture was denatured at 72°C for 10 min. Slides were denatured in 70% formamide, 2× SSC (pH 7.0) at 72°C for 2 min,

dehydrated and hybridized overnight in a moist chamber at 37°C with the probe. Slides were then washed twice in 50% formamide 2× SSC (pH 7.0) at 42°C for 5 min, in 0.1× SSC (pH 7.0) at 42°C for 5 min, followed by one wash in Tris-NaCl-Tween 20 buffer at 42°C for 5 min. Slides were counterstained with 4, 6-diamino-2-phenolindole (DAPI) and mounted in vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA). Probe signals on metaphase spreads were visualized under an Olympus BX60 fluorescent microscope. Digital images were captured using the ISIS 3 software (MetaSystems, Altlussheim, Germany).

Sequencing. Plasmid DNA from FISH positive clones was isolated using Wizard[®] Plus SV Minipreps DNA Purification System (Promega, Madison WI, USA) and sequenced using an ALFexpress Sequencing System (Pharmacia, Biotech). Sequences of the inserts have been deposited in EMBL database. DNAsis-Pro software (Hitachi) was used to analyze the global structure of DNA sequences and create Dot Plot graphs useful to show the degree of similarity, deletions, insertions, direct and indirect repeats. For Dot Plot analysis, a window of 8 bases was used with a stringency of 6.

RESULTS

Isolation and physical localization of bovine X, porcine 14 and equine 1 chromosome-specific repeats

DNA specific probes, generated by laser microdissection, targeted recombinant clones containing repetitive DNA sequences from the centromeric regions of bovine X, porcine 14 and equine 1 chromosomes. Most of the recombinant clones contained inserts ranging approximately from 300 bp to 500 bp. To examine the insert of the recombinant clones contained chromosome-specific repeats, a Dot Blot assay was performed on the corresponding PCR products. Clones that showed a higher hybridization signal with specific probe were considered positive. Fluorescence *in situ* hybridization was performed in order to confirm their position on species specific metaphase spreads. The G-like pattern generated by DAPI staining was used for chromosome identification.

In cattle, four clones showed strong fluorescent signal on metaphase chromosomes, specifically

located in the centromeric region of the X chromosome. The signal was also clearly visible in interphase nuclei (Figure 1a), showing the ability of these clones to detect specific chromosomal domains. The same clones revealed positive hybridization signals on mitotic chromosomes of river buffalo, sheep and goat as demonstrated in Figure 2. In the pig, specific hybridization signals were generated from 11 single probes on the centromeric region of chromosomes 14; however, the signal intensity of the single clone was not satisfactory in interphase nuclei (Figure 1b). On the contrary, when the probes were mixed, a higher intensity of the hybridization signal was observed also in interphase nuclei (Figure 1c). In the horse, two probes showed a discrete fluorescent signal specifically located on the centromeric region of chromosome 1, but the intensity of hybridization was not high to show the localization of chromosomes in interphase nuclei (Figure 1d).

DNA sequencing and Dot Plot analysis

DNA sequencing was performed on two bovine recombinant clones with the strongest FISH signal and on all specific positive FISH inserts from the porcine and equine clones. In cattle, a significant homology (98.3%) was found among the insert sequences. The centromeric sequence corresponding to the highest molecular weight (without vector- and DOP-primer sequences) was deposited in the EMBL database under accession number AJ884576. In the pig, sequence analysis also indicated high homology level among the 11 clones examined. The molecular data allowed a subdivision of the clones in 2 groups: the first one, composed 6 clones with average homology of 95.6% (Accession numbers AJ889249, AJ937272-AJ937276); the second, composed 2 clones with a similarity of 99.6% (AJ920051-AJ920052). No homology was found among these two groups and the remaining 3 clones (Accession numbers AJ920048-AJ920050). BLAST analysis confirmed that the isolated DNA inserts did not have any homology with other centromeric repeated regions. In the horse, the nucleotide sequence of both investigated clones showed 98.3% homology. The sequences were deposited in the EMBL database under accession numbers AJ889250 and AJ937277. A summary of microdissected chromosomal regions and positive clones subjected to different assays is presented in Table 1. Dot Plot

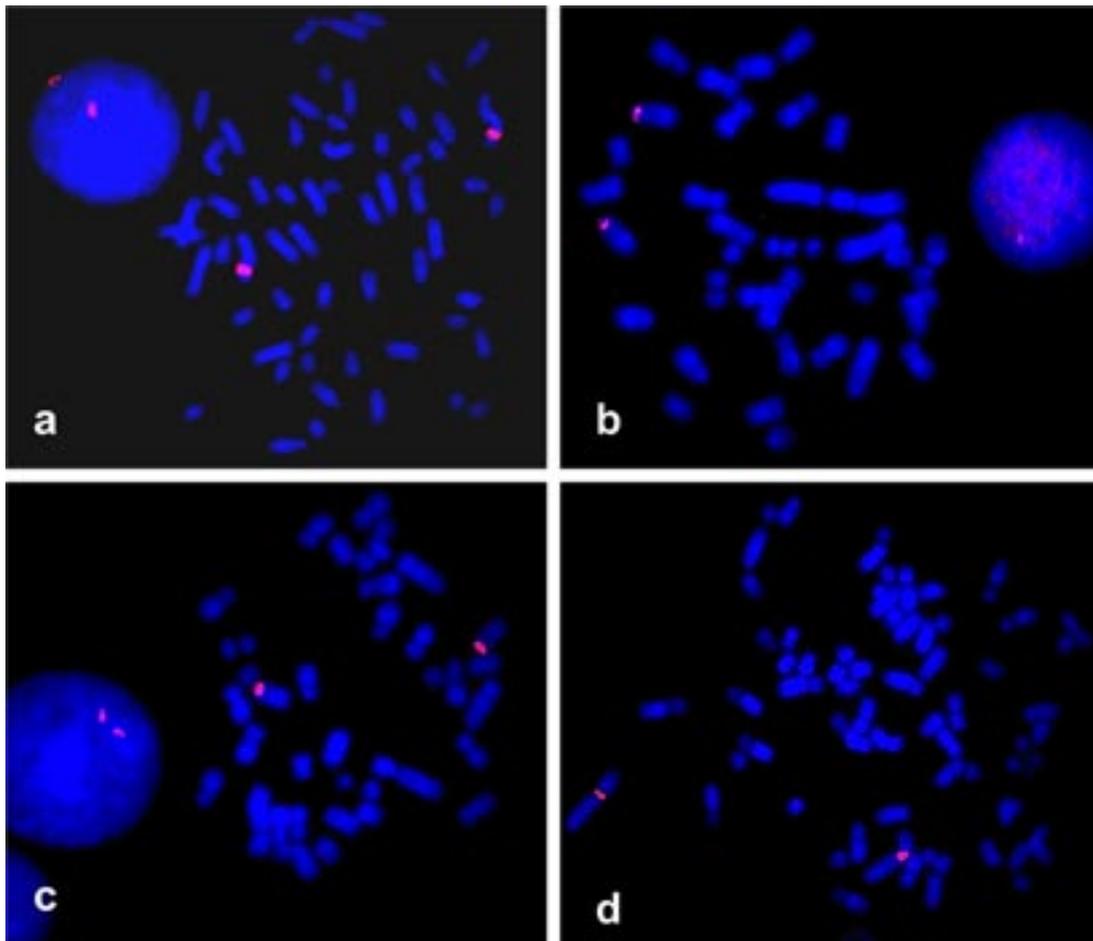


Figure 1. (a) Hybridization signals shown from four mixed bovine probes specifically linked just above the centromere of chromosome X (counterstained blue with DAPI). The signal is clearly visible in interphase nuclei, too. (b) *In situ* hybridization signal obtained from a single porcine clone to the centromeric region of pig metaphase chromosomes 14. (c) A higher intensity of signal was observed when FISH was performed with mix of all porcine clones. (d) Results of fluorescence *in situ* hybridization experiment with two probes generated from equine positive clones. A discrete fluorescent signal was specifically located on the centromeric region of equine chromosome 1

analysis showed presence of various short repeated elements in the investigated clones. The results are summarized in Table 2. In the pig, a consensus sequence was generated by multiple alignments of the inserts (Figure 3) in order to facilitate the Dot Plot analysis of the clones belonging to the first homology group.

DISCUSSION

In this study we report on new classes of tandemly repeated DNA sequences in the centromeric regions of three species of farm animals detected by laser microdissection and DOP-PCR procedures.

Table 1. Summary of positive clones subjected to different assays and homology shown from sequenced probes

Species and chromosome	Dot Blot	FISH	Sequencing	Clone names	Homology (%)	
<i>Bos taurus</i>	X	9	4	2	B3, B11	98.3
				6	S35, S37, S50, S69, S74, S76	95.6
<i>Sus scrofa</i>	14	34	11	11	S26, S38	99.6
				3	S16, S45, S46	0
<i>Equus caballus</i>	1	19	5	2	H2, H12	98.3

In cattle FISH experiments, strong hybridization signals were shown from four bovine probes isolated from the pericentromeric region of chromosome X and specifically linked just above the centromere of the same chromosome. The signal obtained from the single clone appeared like an extensive spot, thus confirming the presence at least one specific repeated region. When the probes were mixed and hybridized together, the signal was also clearly visible in interphase nuclei showing the ability of these clones to detect specific chromosomal domains. When the isolated clones were hybridized to the acrocentric X chromosomes of water buffalo (*Bubalus bubalis*), sheep (*Ovis aries*) and goat (*Capra hircus*), strong signals were clearly detected below the centromere (water buffalo) and in the middle of the chromosome (sheep and goat) (Figure 2), confirming that the cross-hybridization of bovine probes to other species is possible (Di Berardino et al., 2004). As demonstrated by comparative FISH-mapping (Iannuzzi et al., 2000) with using 17 markers Bovinae (cattle and river buffalo) and Caprinae (goat and sheep) X-chromosomes were complex rebuilt. These rearrangements required at least four transpositions because at least 5 chromosome regions with the same gene order within each segment were found. While, cattle and river buffalo X-chromosomes differ only for one centromeric transposition with loss (cattle) or acquisition (river buffalo) of constitutive heterochromatin because the two chromosomes showed the same gene order. The signals found in the present study in the pericentromeric region of cattle p arms, proximal part of river buffalo q arms and distal part of ovine q arms of chromosomes X (Figure 2) are in agreement with the study (Iannuzzi et al., 2000).

DNA sequencing, performed on two bovine recombinant clones with the highest FISH signal, revealed high level of homology (98.3%) between the two; so, we assume that both sequences could be considered as the same monomeric unit, or a part of a longer tandemly repeated monomeric unit, that generates a specific higher-order repeat. BLAST analysis confirmed that the isolated DNA inserts did not reveal any homology with the known bovine satellites (Plucienniczak et al., 1982; Buckland, 1985; Jobse et al., 1995), and no information about satellite DNA in sub-metacentric chromosome X is available at present (Chaves et al., 2003). We believe that these sequences can be considered as a new class of specific satellite DNA located on the bovine X chromosome. The porcine and horse

clones were also tested on bovine metaphases to establish whether the FISH signal is visible in species belonging to different families. However, no hybridization signal was detected, indicating that these clones could be species-specific.

All the positive porcine clones were subject to sequencing. The molecular data revealed a strong similarity level, allowing subdivision of the clones into 2 groups. The first one includes 6 clones (S35; S37; S50; S69; S74 and S76) with an average homology of 95.6%. In particular, taking the longest sequence (S69 clone) as reference, the comparison showed similarities of 96.4%; 96.3% and 94.9% for S35; S37 and S50, respectively. The last two clones of the first group showed homology of 94.0% and 96.4%, respectively, with complementary sequence of the reference clone. The second group includes 2 clones (S26 and S38), with a similarity of 99.6%. No homology was found for these clones either within these groups or between these groups and the remaining 3 clones. The analysis of our sequences showed that the clones are characterized by a variable AT content, from 42.5% (clone S45) to 55.5% (clone S38), with an average AT content of 50.4%. The results confirm that the repeated regions of porcine chromosome 14 are characterized by equilibrium in the base content, according to the data already shown Jantsch et al. (1990) on Ac2 porcine centromeric repeated family. On the contrary, the metacentric repeated regions are characterized by a lower AT content: 36.6%, 42.1% and 31.7% for chromosome 1 (Jantsch et al., 1990), chromosome 9 (Janzen et al., 1999) and chromosome 11 (Riquet et al., 1996), thus suggesting a different structure and organization of the centromeres in the metacentric and acrocentric chromosomes of this species.

BLAST analysis of all the sequences indicated that the isolated DNA inserts did not share any homology with the already known family (Mc1, Ac2 and Mc2) of porcine centromeric satellite DNA. At present, only unspecific repetitive sequences are known for all the porcine acrocentric chromosomes (Jantsch et al., 1990; Rogel-Gaillard et al., 1997a,b). On the basis of these observations, we suggest the existence of new classes of specific satellite DNA located on the centromere of porcine chromosome 14.

The same analysis was applied to the horse positive clones. Two probes showed a discrete fluorescent signal specifically hybridized on the centromeric region of chromosome 1, as shown in Figure 1d. The same probes were also hybridized on donkey (*Equus asinus*) and zebra (*Equus zebra*) metaphase

chromosomes. Although these two species are taxonomically close to the investigated one, no FISH signal was detected, thus indicating that these sequences represent a species-specific target for the centromeric region of equine chromosome 1. DNA sequencing performed on both positive probes revealed 98.3% of similarity. So far, Sakagami et al. (1994) found satellite sequences localized on centromeres of all chromosomes, except for chromosomes 2, 9 and 11. Broad et al. (1995) reported the existence of two DNA repetitive elements, known as Sau-like families, frequently present in tandem and unspecifically hybridized to acrocentric horse chromosomes. Recently, Gallagher et al. (2000) submitted unspecific centromeric sequence for many horse acrocentric, metacentric and submetacentric chromosomes (Accession number: AF324422), but at present no information is available on specific probes for this species. Here we report on the first class of specific satellite DNA localized on the centromere of equine submetacentric chromosome 1. BLAST analysis of the new sequences confirmed FISH hybridization data. No homologies were found to the known equine satellites.

Presence of several types of short repetitive elements was detected by Dot Plot analysis; however, specific for a particular species. All of the detected repeated elements are extremely short and none of the clones in our study is formed by complete or incomplete repeats of any longer basic sequence. Accordingly, we can suppose that none of the detected short repeated elements is essential for the structure of our probes.

At present, very little is known about the organization of centromeric DNA repeats in farm animals. The isolation and identification of new sequences suggest that these repeats are present in a relatively small proportion of the genome and each of these is specific for only one pair of chromosomes. According to Janzen et al. (1999), a great benefit can result from the construction of a catalog of species-specific centromeric probes and in that respect our findings extend knowledge on farm animal centromeres. Moreover, the specific hybridization of these probes on metaphases of different species could represent a powerful tool for Zoo-FISH analysis and could facilitate routine cytogenetic analysis in farm animals.

In conclusion, the characteristic repeated sequences found in the centromeric region of bovine chromosome X, porcine chromosome 14 and equine chromosome 1 might enrich the genetic

map of these three species in a region that has not received adequate attention.

REFERENCES

- Alexandrov I., Kazakov A., Tumeneva I., Shepelev V., Yurov Y. (2001): α -Satellite DNA of primates: old and new families. *Chromosoma*, 110, 253–266.
- Broad T.E., Forrest J.W., Lewis P.E., Pearce P.D., Phua S.H., Pugh P.A., Stewart-Scott I.A. (1995): Cloning of a DNA repeat element from horse: DNA sequence and chromosomal localization. *Genome*, 38, 1132–1138.
- Buckland R.A. (1985): Sequence and evolution of related bovine and caprine satellite DNAs. Identification of a short DNA sequence potentially involved in satellite DNA amplification. *Journal of Molecular Biology*, 186, 25–30.
- Buntjer J.B., Nijman I.J., Zijlstra C., Lenstra J.A. (1998): A satellite DNA element specific for roe deer (*Capreolus capreolus*). *Chromosoma*, 107, 1–5.
- Burke R.D., Szabo P., O'Brien S., Nash W.G., Yu L., Smith K.D. (1985): Organization and chromosomal specificity of autosomal homologs of human Y chromosome repeated DNA. *Chromosoma*, 92, 225–233.
- Chaves R., Guedes-Pinto H., Heslop-Harrison J., Schwarzbacher T. (2000): The species and chromosomal distribution of the centromeric alpha-satellite I sequence from sheep in the tribe Caprini and other Bovidae. *Cytogenetics and Cell Genetics*, 91, 62–66.
- Chaves R., Adegas F., Heslop-Harrison J.S., Guedes-Pinto H., Wienberg J. (2003): Complex satellite DNA reshuffling in the polymorphic t(1;29) Robertsonian translocation and evolutionarily derived chromosomes in cattle. *Chromosome Research*, 11, 641–648.
- Devilee P., Thierry R.F., Kievits T., Kolluri R., Hopman A.H., Willard H.F., Pearson P.L., Cornelisse C.J. (1988): Detection of chromosome aneuploidy in interphase nuclei from human primary breast tumors using chromosome specific repetitive DNA probes. *Cancer Research*, 48, 5825–5830.
- Di Berardino D., Vozdova M., Kubickova S., Cernohorska H., Coppola G., Coppola G.F., Enne G., Rubes J. (2004): Sexing river buffalo (*Bubalus bubalis* L.), sheep (*Ovis aries* L.), goat (*Capra hircus* L.), and cattle spermatozoa by double color FISH using bovine (*Bos taurus* L.) X- and Y-painting probes. *Molecular Reproduction and Development*, 67, 108–115.
- Ekes C., Csonka E., Hadlaczky G., Cserpan I. (2004): Isolation, cloning and characterization of two major satellite DNA families of rabbit (*Oryctolagus cuniculus*). *Gene*, 343, 271–279.

- Gallagher P.C., Lear T.L., Bailey E. (2000): Sequence characteristics and chromosomal distribution of a DNA satellite, ES22, in genomes of the horse and other equids. <http://www.ncbi.nlm.nih.gov> (unpublished).
- Haaf T., Willard H.F. (1997): Chromosome-specific alpha satellite DNA from the centromere of chimpanzee chromosome 4. *Chromosoma*, 106, 226–232.
- Huang N.F., Gupta M., Varghese S., Rao S., Luke S. (2002): Detection of numerical chromosomal abnormalities in epithelial ovarian neoplasms by fluorescence *in situ* hybridization (FISH) and a review of the current literature. *Applied Immunohistochemistry and Molecular Morphology*, 10, 187–193.
- Iannuzzi L., Di Meo G.P., Perucatti A., Incarnato D., Schibler L., Crihiu E.P. (2000): Comparative FISH mapping of bovid X chromosomes reveals homologies and divergences between the subfamilies Bovinae and Caprinae. *Cytogenetics and Cell Genetics*, 89, 171–176.
- Jantsch M., Hamilton B., Mayr B., Schweizer D. (1990): Meiotic chromosome behaviour reflects levels of sequence divergence in *Sus scrofa domestica* satellite DNA. *Chromosoma*, 99, 330–335.
- Janzen M.A., Buoen L.B., Zhao F., Louis C.F. (1999): Characterization of a swine chromosome-specific centromeric high-order repeat. *Mammalian Genome*, 10, 579–584.
- Jobse C., Buntjer J.B., Haagsma N., Breukelman H.J., Beintema J.J., Lenstra J.A. (1995): Evolution and recombination of bovine DNA repeats. *Journal of Molecular Evolution*, 41, 277–283.
- Kolluri R.V., Manuelidis L., Cremer T., Sait S., Gezer S., Raza A. (1990): Detection of monosomy 7 in interphase cells of patients with myeloid disorders. *American Journal of Hematology*, 33, 117–122.
- Kubickova S., Cernohorska H., Musilova P., Rubes J. (2002): The use of laser microdissection for the preparation of chromosome-specific painting probes in farm animals. *Chromosome Research*, 10, 571–577.
- Lee C., Ritchie D.B., Lin C.C. (1994): A tandemly repetitive, centromeric DNA sequence from the Canadian woodland caribou (*Rangifer tarandus caribou*): its conservation and evolution in several deer species. *Chromosome Research*, 2, 293–306.
- Maniatis T., Fritsch E.F., Sambrook J. (1982): *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Mitchell A.R., Gosden J.R., Miller D.A., (1985): p82H: a cloned sequence of the alphoid repeated DNA family found at the centromeres of all human chromosomes. *Chromosoma*, 92, 369–377.
- Moore L.E., Titenko-Holland N., Smith M.T. (1993): Use of fluorescent *in situ* hybridization to detect chromosome specific changes in exfoliated human bladder and oral mucosa cells. *Environmental and Molecular Mutagenesis*, 22, 130–137.
- Moyzis R.K., Albright K.L., Bertholdi M.F., Cram L.S., Deaven L.L., Hildebrand C.E., Joste N.E., Longmire J.L., Meyne J., Schwarzacherrobinson T. (1987): Human chromosome-specific repetitive DNA sequences: novel markers for genetic analysis. *Chromosoma*, 95, 375–386.
- Nijman I.J., Lenstra J.A. (2001): Mutation and recombination in cattle satellite DNA: a feedback model for the evolution of satellite DNA repeats. *Journal of Molecular Evolution*, 52, 361–371.
- Pellestor F., Girardet A., Coignet L., Andreo B., Charliou J.P. (1996): Assessment of aneuploidy for chromosomes 8, 9, 13, 16, and 21 in human sperm by using primed *in situ* labeling technique. *American Journal of Human Genetics*, 58, 797–802.
- Plucienniczak A., Skowronski J., Jaworski J. (1982): Nucleotide sequence of bovine 1.715 satellite DNA and its relation to other bovine satellite sequences. *Journal of Molecular Biology*, 158, 293–304.
- Riquet J., Mulsant P., Yerle M., San Cristobal-Gaudy M., Le Tissier P., Milan D., Gellin J. (1996): Sequence analysis and genetic mapping of porcine chromosome 11 centromeric S0048 marker. *Cytogenetics and Cell Genetics*, 74, 127–132.
- Rogel-Gaillard C., Hayes H., Coullin P., Chardon P., Vaiman M. (1997a): Swine centromeric DNA repeats revealed by primed *in situ* (PRINS) labeling. *Cytogenetics and Cell Genetics*, 79, 79–84.
- Rogel-Gaillard C., Bourgeaux N., Save J.C., Renard C., Coullin P., Pinton P., Yerle M., Vaiman M., Chardon P. (1997b): Construction of a swine YAC library allowing an efficient recovery of unique and centromeric repeated sequences. *Mammalian Genome*, 8, 186–192.
- Sakagami M., Hirota K., Awata T., Yasue H. (1994): Molecular cloning of an equine satellite-type DNA sequence and its chromosomal localization. *Cytogenetics and Cell Genetics*, 66, 27–30.
- Willard H.F. (1985): Chromosome-specific organization of human α -satellite DNA. *American Journal of Human Genetics*, 37, 524–532.
- Willard H.F., Wayne J.S. (1987): Hierarchical order in chromosome-specific human alpha satellite DNA. *Trends in genetics*, 3, 192–198.

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