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Application of primed *in situ* DNA synthesis (PRINS) with telomere human commercial kit in molecular cytogenetics of *Equus caballus* and *Sus scrofa scrofa*

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Abstract: Recently, molecular techniques have become an indispensable tools for cytogenetic research. Especially, development of *in situ* techniques made possible detection at the chromosomal level, genes as well as repetitive sequences like telomeres or the DNA component of telomeres. One of these methods is primed *in situ* DNA synthesis (PRINS) using an oligonucleotide primer complementary to the specific DNA sequence. In this report we described application of PRINS technique with telomere human commercial kit to telomere sequences identification. This commercial kit may be use to visualization of interstitial telomeric signal in pig genome. PRINS is attractive complement to FISH for detection of DNA repetitive sequences and displays lower level of non-specific hybridization than conventional FISH.

Key words: PRINS - Telomere - Pig - Horse

Introduction

Development of the molecular cytogenetic techniques results in utilization of the fluorescence *in situ* hybrizdiation (FISH) technique in cytogenetic research [20].

Alternative method of chromosome investigations is primed *in situ* DNA synthesis (PRINS) technique. In this method unlabeled oligonucleotides that are annealed *in situ* to complementary sequences in metaphase chromosomes or interphase nuclei and extended with Taq polymerase. The major advantages of PRINS reaction are rapidity and the high sensitivity as the PCR which makes possible the chromosomal localization of specific DNA sequences [10,29].

PRINS can be applied especially in detection of repetitive sequences like: alphoid sequences, satellite sequences, Alu-dispersed sequences, rDNA sequences, heterochromatin or telomeres [6,10,14,16,34].

PRINS technique can be used as a gene mapping technique, anueploidy diagnostic method as well as in studies concerning evolutional analysis or evaluation of DNA damage [6,12,29-31].

Moreover PRINS can be applied in flow cytometry and sorting chromosomes (flow cytogenetics) [8,9,18,22,23].

In this paper, we have shown that PRINS technique with telomere human commercial kit may be applied in cytogenetic research of economically important group of mammals.

Material and methods

Chromosome preparation. Metaphase spreads of pig and horse chromosomes were obtained after routine lymphocytes culture. Preparations were aged for at least 1 week at 37°C.

Oligonucleotide - primed *in situ* DNA synthesis. We applied the standard protocol according to the manufacture instruction with minor modifications. Metaphase spreads were treated with RNase (1 h at 37°C in humid chamber) and next with pepsin solution (at 37°C for 30 min) for better results. After pepsin digestion slides were washed twice in PBS (pH 7.4) and passed through the ethanol series (70, 80 and 95 %). Before air-drying, the reaction mix was warmed to 37°C for 5 min and mixed. 25 µl of reaction mixture containing PRINS Kit (Prins-Probe 1409-T, Cambio Ltd) 0.05 mM biotin-11-dUTP and 2 U Taq polymerase (Eppendorf) was added to air-dried slide. Reaction mixture was put on the 20 × 40 mm^2 coverslip, was placed on the slide and quickly sealed with fixogum. The slide was placed on preheated block at 94°C for 5 min and next

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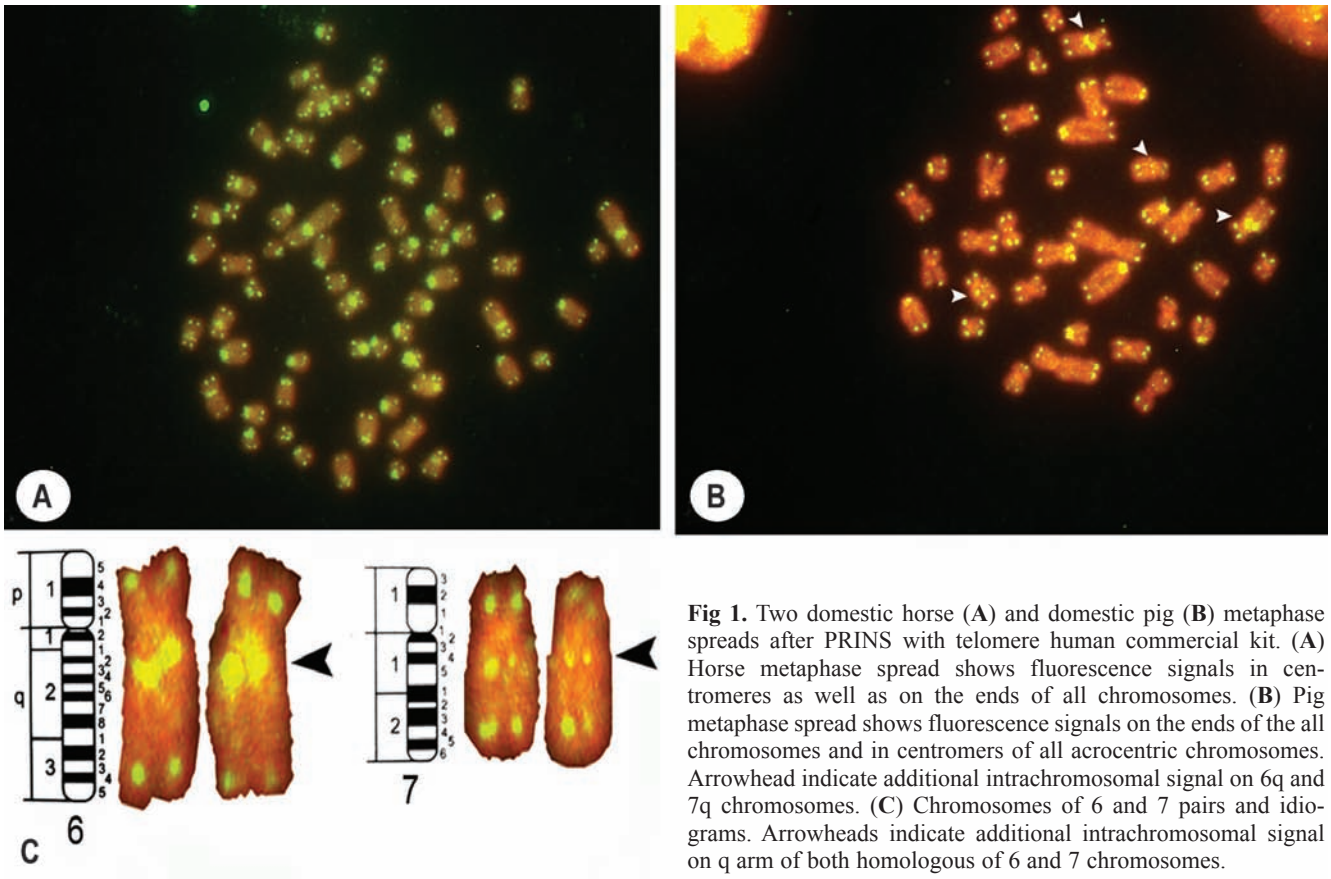

Fig 1. Two domestic horse (**A**) and domestic pig (**B**) metaphase spreads after PRINS with telomere human commercial kit. (**A**) Horse metaphase spread shows fluorescence signals in centromeres as well as on the ends of all chromosomes. (**B**) Pig metaphase spread shows fluorescence signals on the ends of the all chromosomes and in centromers of all acrocentric chromosomes. Arrowhead indicate additional intrachromosomal signal on 6q and 7q chromosomes. (**C**) Chromosomes of 6 and 7 pairs and idiograms. Arrowheads indicate additional intrachromosomal signal on q arm of both homologous of 6 and 7 chromosomes.

at 58°C for 30 min. The reaction was stopped by transferring the slide to stop buffer including 50 mM NaCl and 50 mM EDTA (pH 8.0) at 58°C for 5 min and next to stop buffer (50 mM NaCl and 50 mM EDTA pH=8.0) at room temperature for 7 min. Before detection, the slide was dehydrated through the ethanol series (70, 80 and 95%).

Detection with amplification. The slide was placed in blocking solution (3% BSA, 4x SSC, 0.05% Tween 20) and incubated at 37°C for 30 min. Biotin-labeled nucleotide was detected by incubation of the slide with the avidin-FITC, next anti-avidin antibody and once again avidin-FITC. Each incubation was performed in humid chamber (at 37°C for 45 min) and separated by washing three times at 45°C for 3 min in 4×SSC, 0.05% Tween 20. After final wash, the slide was air-dried, counterstained with solution of propidium iodide at room temperature for 2 min and washed twice in distilled water. Noteworthy, all steps of this procedure were done in the dark room. The air-dried and mounted of the slide with antifade was analysed in fluorescence microscope equipped with CDD camera and Lucia software.

Results and discussion

In the present paper, we paid attention on telomeres because telomeres as physical ends of eukaryotic chromosomes playing an important role in replication of chromosomal DNA [5], in identification of naturally occurring chromosome ends from "broken" DNA ends by the cell's DNA repair machinery [21], cellular aging, tumor progression, karyotype evolution [4,26].

The telomeric repeats can be detected either by using fluorescence *in situ* hybridization (FISH) technique with a DNA probe or by primed *in situ* labeling (PRINS) reaction using an oligonucleotide primer complementary to the telomeric DNA repeated sequence.

In this examination, application of primed *in situ* DNA synthesis (PRINS) method with telomeric oligonucleotide in pig and horse metaphase spreads gave fluorescence signals on both ends of all chromosomes (Fig. 1A and 1B). These results are consistent with previous study carried out on both species using FISH with telomeric DNA probe [7,19]. However, our results proved that PRINS technique is fast, attractive and alternative method than conventional FISH for *in situ* detection of telomeric sequences [7].

Moreover, the telomere - specific oligonucleotide revealed centromeric fluorescence signals on all horse chromosomes (Fig. 1A). This finding indicated that it is some similarity between both repetitive sequences.

In pig, PRINS method did not give any signals in centromers of metacentric chromosomes of pairs 1 to 12 as well as X (Fig. 1B). In parallel, PRINS revealed fluorescence signals in all acrocentric chromosomes (pairs 13 to 18). These findings confirm that pigs have two distinct centromeric DNA satellite families, Mc1 and Ac2 [15]. The centromeres of Mc1 family are

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comprised of divergent ~340 bp monomer units [15,27,33]. The centromeres of Ac2 family are composed of 14 bp monomer units [32].

Additionally, we showed strong interstitial telomeric signal (ITS) on both homologues of chromosome pair 6q (Fig. 1B and 1C). This data confirm previous observation using FISH with telomeric sequences [7]. Also, we observed another class of telomeric fluorescence signal on 7q chromosomes (Fig. 1B and 1C). However, both signals on 7q chromosomes were weaker than on both homologues of chromosome pair 6q and they could be related to interstitial CG-rich sequences [2].

The intrachromosomal telomeric repeats have been described in a variety of vertebrates ranging from mammals [24,35], birds [28], amphibian [36] to fishes [1]. The (TTAGGG)n sequence located between centromere and telomere can be the result of tandem chromosome fusion during evolution as well as the insertion of telomereic DNA within unstable sites during the repair of interstitial double-strand breaks [3,6,11,13]. Moreover, these sequences can be detected in many species at various nontelomeric sites, predominantly in pericentromeric regions and other regions rich in constitutive heterochromatin [25,35]. The heterochromatic nature of mammalian telomeres is responsible for transcriptional silencing of nearby genes as well as the suppression of recombination [17].

In this report, we demonstrated the application of PRINS technique with telomere human commercial kit to detect the telomeres and centromeres in horse karyotype. Additionally, the same PRINS kit can be used to labeling both the telomeric, interstitial telomeric signals (ITS) and centromers of acrocentric chromosomes in pig karyotype.

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