

## Fluorescent *in situ* hybridization (FISH) on the veterinary diagnostic field

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Proceeding deals with of genomic changes detectable by FISH. The DSD syndrom in Yorkshire terrier 78, XY t (Y-;6p+) was observed by the use of X and Y FISH WCP probes. Following results indicated numerous genomic changes in cancers. Using comparative genomic hybridization numerous chromosomal rearrangements were detected, which indicated the heterogeneity in tumour growth. In Bernese Mountain Dog bitch, 8 losses on chromosomes and gains on 18 different of chromosomes were detected. The last study was focused on chromosomal position and nucleotide sequencing of the *LCA5L* exons. Those were analysed in cattle of BTA1q44, sheep OAR1q43 and of CHI1q44 in the goats.

**Keywords:** fluorescent *in situ* hybridization, FISH, veterinary diagnostics

### 1 Introduction

Fluorescence *in situ* hybridization (FISH) is a cytogenetic technique that uses fluorescent probes that bind to only those parts of the chromosome with a high degree of sequence complementarity. Diagnostic of diseases based on FISH technique is applied in different areas i.e. fertility errors, developmental defects, genotoxic effects (mutagenicity and/or carcinogenicity) and at the gene positional mapping, as well.

Numerous disorders of sexual development (DSD) are classified as a hereditary and chromosomal dependent defects in human and animals, including dog (Alarm et al., 2007).

Lymphomas (malignant lymphomas or lymphosarcomas) are among the most frequently described tumours in mammals; it is known that they are a result of malignant transformation of developed lymphocytes. Numerous chromosomal aberrations were documented in human lymphomas. Using comparative genomic hybridization according to D'Haese et al. (2005) recorded partly chromosomal imbalances and partly the translocation t(14;18) after sequencing in *BCL2* gene in patients with follicular non-Hodgkin lymphomas (Streubel et al., 2004).

FISH method allows the detection of the position of the gene on the chromosome and arrangement the conserved segments among species (Di Meo, 2006; Schibler, 2009). Leber congenital amaurosis 5-like has been studied in humans. The clinical symptoms of LCA have not yet been described in ruminants. Similar clinical symptoms of the

disease resulting from *RPE65* gene mutation have been described in dogs and humans i.e. night blindness, visual deficits and nystagmus (Nafström, 2007).

### 2 Material and methods

#### 2.1 Disorder of sexual development in dog

A 9-month-old female like Yorkshire terrier was examined due to references about abnormal rutting like behavior and visible clitoral hypertrophy. Peripheral blood was collected for the detection of sex hormones concentration  $\beta$ -estradiol, testosterone and progesterone by standard RIA tests (Beckman Coulter, ISO 9001); and analysis of sex chromosomes constitution. Second examination one month later was completed with laparotomy to obtain the material for standard histopathological examination of gonads and genital ridges. For FISH the orange-red labelled whole chromosome painting (WCP) probe, specific for canine chromosome X (CFA X) and green labelled whole chromosome painting probe, specific for canine chromosome Y, Veterinary Research Institute (VRI) Brno, were used for precise detection of sex chromosomes.

#### 2.2 Comparative genomic hybridization in malignant lymphoma

Tested genomic DNA was isolated from tumor tissue (lymphoma) of two dogs 10 and 12 years old bitches, and reference DNA from whole blood of healthy 7 years old male dog using DNeasy® Blood & Tissue Kit (Qiagen). Tested DNA was labeled by BioPrime® Array

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CGH Genomic Labeling System (Invitrogen) with Cyanine 3-dUTP (Enzo Life Sciences, red color) fluorescent dye; reference DNA was labeled with Green 500-dUTP (Enzo Life Sciences, green color). Comparative genomic hybridization has been performed, subsequently. After CGH chromosome metaphases were evaluated using an Olympus BX 60 fluorescence microscope and captured image was analyzed by CGH-ISIS software. Nine marker chromosomes (8–10 metaphases) were karyotyped and used for average red:green ratio calculation. A ratio of >1.25 : 1 indicated gain of genetic material, and ratio <0.75 : 1 loss.

### 2.3 FISH in comparative mapping of *LCA5L* gene in bovine family

Peripheral blood samples from two calves (Slovak indigenous cattle), two sheep (Walachian sheep) and two goats (White goats) were collected and cultured in a medium for 72 h at 37 °C. Chromosome preparations were prepared by standard cytogenetic method. BAC clone (BAC CH240-118J20) containing a partial sequence of *LCA5L* gene (VRI, Brno) was used for comparative FISH mapping. The digoxigenin labelled BAC probe was detected by sheep Anti-digoxigenin-rhodamine and slides were counterstained with DAPI/Antifade. Chromosomes with mapped loci were identified by computer DAPI reversed banding according to standard karyotype.

## 3 Results and discussion

### 3.1 DSD syndrom

External genitalia i. e. a vagina and a vulva were stated in a normal configuration, except of the clitoral hypertrophy (2.5 cm in length). Hormonal profile indicated the low level of 17 bestradiol (<10.0 pg ml<sup>-1</sup>) contrary to blood testosterone with concentration of 9.1 ng ml<sup>-1</sup> corresponding to the normal male values (ranges 3.0–10.0 ng ml<sup>-1</sup>).

Histopathological examination confirmed that the primary gonads were testicles. The seminiferous tubules were well developed, but only Sertoli cells were present next to the baseline membranes. Significant accumulation of Leydig cells was detectable in interstitial space. Well developed epididymis free of spermatozoa were visible in the cross-section of its head. Bilateral deferent ducts were late up to next tubular structure. All of layers of the uterine wall were developed. Endometrium was hypoplastic, lacking corresponding glands. The rest parts of genital tract i.e. cervix uteri, vagina and vulva were classified as a standard female organs except the clitoral hypertrophy, as described above.

Conventional cytogenetic analysis (Giemsa and GTG) showed that the studied Yorkshire dog had a male

chromosomal complement 78, XY in all metaphases, with normal size and morphology of both sex chromosomes. The results were confirmed by FISH with WCP probes specific for chromosome X and Y and by PCR amplification of the Y-linked fragment of SRY gene (271 bp). Telomeric parts of presumed autosome 6 (78, XY t (Y-; 6p+), as deduced from GTG banding pattern) were recognised by CFA Y probe as an additional mark, only.

### 3.2 Comparative genomic hybridization in malignant lymphoma

DNA copy number changes from lymphoma tissues of both dogs were detected.

Deletion of the chromosome 9q26.2, and duplications of the chromosomes 5q35-36; 8q33.3 and 17q23-24 were recorded in lymphomas of cross breed bitch.

Numerous partial genetic imbalances, such as losses of genetic material of the chromosome 1q14; 5q33-34; 8q13-31; 12q14-23; 18q22.1; 22q12.3-23; 27q12-14; 29q13-15 and gains of genetic material of the chromosomes 1q34-38; 2q11,32-35; 9q11-12,25; 11q23; 15q11,26; 16q25.2; 18q25.3; 20q16-17; 23q24; 24q25; 25q24; 28q11; 29q23.1-23.2; 30q15.1-15.2; 34q17; 36q15; 37q17, 38q11 were detected in sample second of patient (Bernese Mountain Dog bitch).

### 3.3 FISH in comparative mapping of *LCA5L* gene in bovine family

Positional mapping of *LCA5L* gene in Bovids has not yet been made. In our study BAC clone CH240-118J20 containing the *LCA5L* gene was used to determine the chromosomal localization in *Bos taurus*, *Ovis aries* and *Capra hircus*. In comparison with standard karyotypes the FISH-mapped locus was assigned to BTA1q44 in cattle, OAR1q43 in sheep, and CHI1q44 in goats. Our results confirmed the high conservation of autosomal chromosomes within the Bovids and the FISH locations of *LCA5L* approximate the positions reported in the sequence map.

## 4 Conclusions

As to our results we can conclude the fluorescence *in situ* hybridisation (FISH) method allows for: the detection of the position of the gene on the chromosome, identifying conserved segments among species; and defining the chromosomal rearrangements or abnormalities that may cause an abnormal phenotype.

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