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L. Ordovas, R. Roy, P. Zaragoza, Hélène Hayes, Andre A. Eggen, et al.. Assignment of the solute carrier family 27 member 1 (SLC27A1)gene to bovine chromosome 7. *Animal Genetics*, 2005, 36, pp.352-353. hal-02676420

HAL Id: hal-02676420

<https://hal.inrae.fr/hal-02676420>

Submitted on 31 May 2020

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Assignment of the *solute carrier family 27 member 1 (SLC27A1)* gene to bovine chromosome 7

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Accepted for publication 28 February 2005

Source/description: Long chain fatty acids (LCFAs) are an important source of energy for most organisms and are also involved in different cellular processes such as membrane synthesis, intracellular signalling, protein modification and transcriptional regulation. SLC27A1 is a membrane protein that facilitates LCFAs transport across the plasma membrane.¹ It may play a key role in regulating available LCFAs substrates from exogenous sources in tissues undergoing high levels of β -oxidation or triglycerides synthesis. SLC27A1 protein is present in several tissues.² Skeletal and heart muscle and adipose tissue are the tissues with the highest levels of SLC27A1 mRNA in man.² SLC27A1 has been cloned in man,² rat¹ and mouse.³

Primer sequences: In order to obtain a bovine SLC27A1 DNA fragment, PCR was performed using the following set of primers:

Forward: 5'-CTG CTC AAC GTG AAC CTG C-3'

Reverse: 5'-GGT CCC CGA CGT GTA GAT G-3'

Primers were designed from the human SLC27A1 mRNA sequence (GenBank no. NM_198580) and amplified a 505 bp specific-bovine fragment (GenBank no. AY738458).

PCR conditions: The PCR reaction was performed in 25 μ l using 50 ng bovine genomic DNA, standard PCR buffer, 1.5 mM MgCl₂, 100 μ M each dNTP, 240 nM each primer and 1.25 U Taq DNA polymerase (Invitrogen, Barcelona, Spain). The PCR profile included an initial denaturation step of 95 °C for 5 min and a final extension step of 72 °C for 5 min. Cycling conditions were 94 °C for 30 s, 58 °C for 30 s and 72 °C for 30 s for 35 cycles. The PCR fragment was sequenced and showed a high level of sequence identity (91 and 80%) with coding regions (exons 2 and 3 respectively) of the orthologous human gene.

Screening of a bovine BAC library: A bovine bacterial artificial chromosome (BAC) library⁴ was screened by PCR and a BAC clone containing the bovine SLC27A1 gene was identified. The positive BAC clone (927A3) was partially sequenced and the presence of SLC27A1 gene was confirmed.

Fluorescent in situ hybridization: Fluorescent *in situ* hybridization was performed on bovine, ovine and caprine chromosome spreads prepared from fibroblast cell cultures from normal embryos.⁵ The BAC clone, 927A3, containing the bovine SLC27A1 gene was used as a probe. The R-banded chromosome spreads were obtained by synchronizing cell cultures with an excess of thymidine and by treating them with 5-bromode-

oxyuridine during the second half of S phase. The BAC DNA extracts were prepared according to standard protocols and the probes were nick-translated in the presence of biotiny-14-dATP with the BioNick labelling system (Invitrogen) and prepared for hybridization.⁶ Chromosome and band numbering followed the ISCNDB 2000 bovine nomenclature.⁷

Chromosomal location: Clear and consistent hybridization signals were obtained in the three species (Fig. 1). The SLC27A1

Figure 1 Chromosome mapping of SLC27A1 by fluorescent *in situ* hybridization. A specific signal is observed on chromosomes 7q11-q12 in cattle (a), 7q11-q12 in goat (b) and 5q11-q12 in sheep (c).

gene is located on the homologous bovine and caprine chromosomes 7q11-q12 and ovine chromosome 5q11-q12, which is in agreement with comparative mapping results in man (HSA19p13.12), mouse (MMU8B3.3) and rat (RNO16p14), as these segments are conserved with bovine chromosome 7.⁸

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doi:10.1111/j.1365-2052.2005.01304.x

Localization of *Xba* repetitive elements to channel catfish (*Ictalurus punctatus*) centromeres via fluorescence *in situ* hybridization.

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Accepted for publication 31 March 2005

Source/description: To date, four classes of repetitive DNA have been identified in the channel catfish (*Ictalurus punctatus*) genome. The most abundant repeat class is approximately 325 bp A/T-rich *Xba* element with approximately 150 000 copies in the catfish genome,¹ but these elements have not been localized in catfish. Approximately 32 000 copies of *Tipnon* a 535 bp *Tc1*-like transposable element, are dispersed throughout the genome.² The catfish genome also contains approximately 9000 copies of the 57 bp *Mermaid* and approximately 1200 copies of the 64 bp *Merman* elements, which are short interspersed repetitive elements found in many bony fish.³ An all-human telomere probe (TTAGGG)_n have been previously localized to the telomeres of catfish metaphase chromosomes by fluorescence *in situ* hybridization (FISH).⁴ Because the *Xba* elements are the largest repeat class characterized to date in catfish (5–6% of the genome), this study was designed to determine the genomic distribution of these repeats using FISH.

Primer development: Primers were designed to highly conserved regions on each end of seven *Xba* elements (GenBank accession nos AF112193–AF112199).³ The forward primer (5'-CTAG-AAAGTAAGTATTGAACAAAAGTAC-3') perfectly matched to

elements *Xba*-1 to -5, and contained three mismatches to elements *Xba*-6 and -7 at positions 14, 17 and 19. The reverse primer (5'-GTCACCTTTTTCACCTTATCATAC-3') was perfectly matched to elements *Xba*-1, -2, -3 and -6, and contained mismatches at nucleotide positions 11 and 18 to *Xba*-4 and 5, and a mismatch at position 3 to *Xba*-7. Because of the sequence similarity between the seven *Xba* element sequences (>95%), we expected the fragment amplified with these primers to hybridize to all elements.

PCR conditions: The hybridization probe was produced by PCR amplification from genomic DNA isolated from the T-cell line G14D.⁵ The 50-μl PCR reaction contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 800 nM of each primer, 200 μM of each deoxynucleotides, 2% glycerol, 100 μM digoxigenin-11-dUTP, 1 μl Accuprime Taq polymerase and 30 ng genomic DNA. The PCR cycling protocol was 95 °C for 5 min, 45 cycles of 95 °C for 15 s, 50 °C for 15 s, 72 °C for 30 s and a final extension at 72 °C for 5 min. The PCR fragments were purified on micro Bio-spin 30 chromatography columns (Bio-Rad, Hercules, CA, USA) by centrifugation at 1000 g.

Chromosomal localization by FISH: The *Xba* elements were hybridized to channel catfish metaphase chromosomes using 25 ng of digoxigenin-labelled *Xba* probe in 30-μl hybridization buffer. Hybridization and one-step detection with FITC-labelled anti-digoxigenin was performed as previously described.⁶ Ten metaphase chromosome spreads were analysed in each of four replicate experiments. Signal was detected at the centromere of every chromosome pair with variability in signal intensity between chromosome pairs (Fig. 1).

Comments: A recent BLAST search of catfish *Xba* elements against mammalian, chicken, zebrafish, and fugu genomic sequences revealed no significant matches. However, the *Xba* element sequences identified within this study will help researchers identify genomic clones and contigs containing centromeric DNA. This information will be useful in the assembly of catfish genetic maps, the integration of genetic and

Figure 1 Fluorescence *in situ* hybridization of A/T-rich *Xba* repetitive elements labelled with digoxigenin and detected with FITC-labelled anti-digoxigenin on channel catfish metaphase chromosomes (2n = 58).