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## Note

# Cross-species amplification of *Catla Catla* microsatellite locus in *Labeo rohita*.

VINDHYA MOHINDRA, LAVIE KHUBE, KULDEEP K. LAL AND A.G. PONNIAH

*National Bureau of Fish Genetic Resources, Canal Ring Road, P.O. Dilkhusha, Telibagh, Lucknow, U.P., India - 226 002.*

## ABSTRACT

The DNA sequence of G1 locus in *Labeo rohita* obtained from cross-species amplification of microsatellite primers from *Catla catla* is reported. This locus in *L. rohita* consist of tetranucleotide and trinucleotide repeats and has 79% homology with that of *Catla catla*.

Microsatellites are DNA sequences that contain short tandem repeats of 2-5 bp and are highly variable in repeat number even among individuals of same population (Tautz, 1989). Because of high degree of polymorphism, microsatellites are widely used in population level evolutionary context, such as indicators of kinship, geneflow and population structure (O'Connell *et al.*, 1998). Flanking sequences of microsatellite loci have been reported to be conserved across the related taxa (Presa and Guyomard, 1996) and this fact has been exploited to amplify homologous regions in the related species. Wherever successful, this approach alleviates need for molecular work prerequisite to develop species specific PCR primers and can catalyze widespread application of single locus microsatellite markers (Zardoya *et al.*, 1996). In our earlier communication (Mohindra *et al.*, 2001) successful amplification of homologous locus in *Labeo rohita* using *Catla catla* microsatellite *CcatG1* locus primers (Naish and Skibinski, 1998) was demonstrated and proved to be useful as a marker for population differentiation in *L. rohita*. In continuation, the present report further expands the scope of results through direct sequencing of PCR amplified product in *L. rohita* with an aim to confirm its nature and explore homology with that of *C. catla*.

The genomic DNA of *L. rohita* was subjected to PCR with *Ccat* G1 locus primers, identified in *C. catla* (Naish & Skibinski, 1998), as described in Mohindra *et al.* (2001). Primer sequences are as follows:

Forward primer

5' AGCAGGTTGATCATTTCTCC 3'

Reverse primer

5' TGCTGTGTTTCAAATGTTCC 3'

The most common allele of PCR am-

plified locus, named as *Lroh* G1 locus, was directly sequenced with forward and reverse primers using Applied Biosystems 373. Alignment of the sequences was done using the software DNASIS v. 2.6 (Hitachi Software Engineering Co., Ltd.).

The sequence of *Lroh* G1 locus, excluding the primer sequences (submitted to Genebank, accession No. AF415207) in 5' to 3' is as follows:

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       10         20         30         40         50
TGAATTGCAT CCATCTATCT ATCCATCTAT CTATTCCTTT CCTGTGGTGT
       60         70         80
TGTTGCTGCA ACTGTTCTTT TTCCCAAATA  TTCTTT
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The microsatellite *Lroh* G1 locus in *L. rohita* consisted of 86 bp (excluding the primer sequences) and can be classified as compound and imperfect in nature as it contains tetranucleotide (CTAT) and trinucleotide (GTT) repeats. Major portion of this sequence was found to be similar when compared with 88 bp microsatellite sequence of *Ccat* G1 (excluding primer sequence) of *C. catla* (Genebank accession no. AF045381, Naish and Skibinski, 1998). Alignment revealed significant homology i.e., 79%, although substitutions, deletions and insertion mutations were observed (Fig. 1). The number of repeats observed in *L. rohita* (CTAT-4, GTT-3) was lower than that of *C. catla* (CTAT-7, GTT-4) as these mutations were at the repeat regions itself.

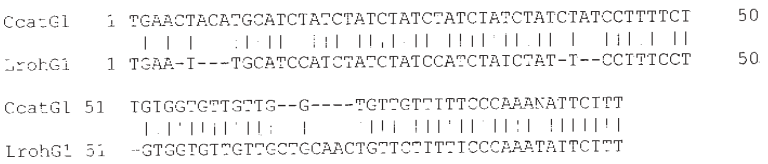

Fig.1. Sequence alignment of microsatellite locus in *Catla catla (Ccat* G1*)* and *Labeo rohita (Lroh* G1*).* Vertical lines denote sequence homology, letters without vertical lines indicate point mutations and dashes indicate deletions. The sequences presented 5' to 3'.

This study substantiates the fact that the primers of G1 locus from *C. catla* is amplifying the homologous microsatellite sequence in *L. rohita*, and is conserved between two species belonging to two different genera of subfamily cyprininae.

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