

Rapid communication: Microsatellite DNA markers (BFRO010 and BFRO011) for grayling¹

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Polymorphism. Two polymorphic microsatellite loci, BFRO010 and BFRO011, were identified in genomic DNA of grayling (*Thymallus thymallus*).

Source and Description. The template DNA was isolated from a library of size-selected (200 to 800 bp) *Sau3AI* genomic restriction fragments from grayling. The DNA fragments were cloned into pBluescript II SK+ (Stratagene, La Jolla, CA) vector, previously restricted with *Bam*HI, and propagated in Epicurian Coli Competent Cells (Stratagene). Screening of the library was performed with the Chemilluminescence Quick-Light Genome Mapping Probe Kit (FMC BioProducts, Rockland, ME) applying (CA)_n and (GA)_n as probes (FMC BioProducts). The DNA sequences (GenBank Accession No. AF130409 and AF130410 for loci BFRO010 and BFRO011, respectively) were obtained with an ABI Prism 310 Genetic Analyser using a dRhodamine Terminator Cycle Sequencing Kit (PE Biosystems, Warrington, U.K.). Primers were designed to amplify regions containing repetitive motifs, which were (AC)₁₇ for BFRO010 and (GT)₁₆ for BFRO011.

Primer Sequences. Locus BFRO010: Fam-5'-GGACG-GAGCCAGCATCAC-3' and 5'-GATTCATAATCAGG-TCAATAGTCAT-3. Locus BFRO011: Hex-5'-CATGGTTGATTGTGGGGGA-3' and 5'-AACATCCT-TACGCCCTAGCA-3'.

Method of Detection. Polymerase chain reaction was carried out in a 10-μL reaction containing 50 ng of genomic DNA, .25 μM each primer, .2 mM dNTP, .5 U of *Taq* polymerase (PE Biosystems), 1.5 mM MgCl₂, 20 mM Tris-HCl, and 50 mM KCl. Thermal cycling reaction was performed in an MJ Research PTC-100 Thermal Cycler under the following conditions: 3 min at 94°C followed by 30 cycles of 94°C for 1 min, 55°C for 20 s, and 72°C for 5 s. The PCR products were mixed with formamide and GENESCAN-350 (TAMRA) Size Standard (PE Biosystems) and genotyped with an ABI

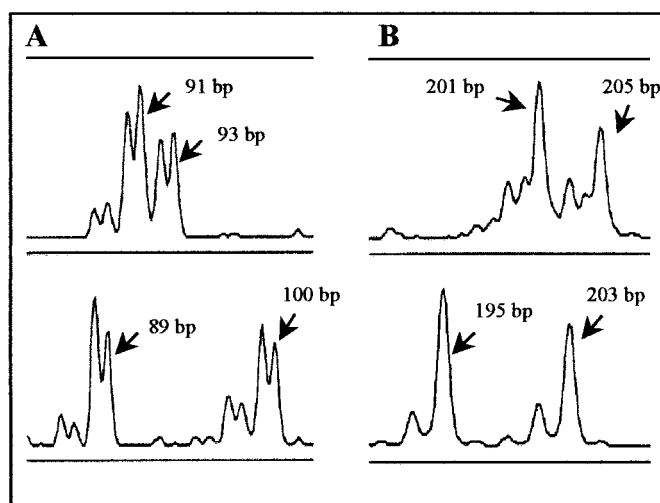


Figure 1. Electropherograms showing (A) the alleles found at locus BFRO011 and (B) the alleles found at locus BFRO010. The allele sizes are given in base pairs (bp).

Table 1. Observed allele frequencies, heterozygosity (H), and polymorphism information content (PIC) values of two microsatellite loci in the Danubian and Adriatic populations of grayling in Slovenia

Locus and allele	Population	
	Danubian (n = 49)	Adriatic (n = 34)
BFRO010		
195	.337	.206
201	.632	.500
203	.031	.000
205	.000	.294
H	.551	.588
PIC	.394	.549
BFRO011		
89	.245	.103
91	.755	.574
93	.000	.029
100	.000	.294
H	.367	.529
PIC	.302	.506

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Prism 310 Genetic Analyser using GeneScan Analysis Software 2.1 (PE Biosystems).

Description of Polymorphism. The markers BFRO010 and BFRO011 were tested on the material sampled from two geographically remote populations of grayling (i.e., Adriatic and Danubian). Despite the geographical separation of the Adriatic and Danubian river systems, the Danubian type of grayling is also present in the Adriatic river system as a consequence of recent stocking (Voljč and Ocvirk, 1982). Four alleles were found at each locus (Figure 1). The observed frequencies of these alleles, heterozygosity, and polymorphism information content (PIC) values (Botstein et al., 1980) are given in Table 1. The alleles 205 at BFRO010 and 93 and 100 at BFRO011 were found only in the Adriatic grayling population, indicating a genetic difference between the Danubian and Adriatic graylings ($\chi^2_{\text{BFRO010}} = 35.71$, $P < .001$; $\chi^2_{\text{BFRO011}} = 37.96$, $P < .001$). These results confirm our previous findings (Snoj et al., 1999).

Comments. The PCR using either of each primer pairs failed to amplify genomic DNA from brown trout (*Salmo trutta*).

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Key Words: Fishes, *Thymallus thymallus*, Microsatellites, DNA

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