

Rapid Communication: The First Microsatellite DNA Marker for Marble Trout, BFRO 001¹

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Polymorphism. A polymorphic locus, characterized by multiple dinucleotide repeats (TG- and AG-microsatellites), was identified in genomic DNA of marble trout (*Salmo marmoratus* Cuvier 1817) and brown trout (*Salmo trutta* Linnaeus 1758) by PCR and designated as BFRO 001.

Source and Description. The template DNA was isolated from a library of size selected (100 to 600 bp) *Sau3AI* genomic restriction fragments from marble trout cloned into pBluescript II SK+ (Stratagene) 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) applying (CA)_n and (GA)_n as probes. The DNA sequence (GenBank Accession No. U90327) was obtained with the conventional dideoxy method using DIG *Taq* DNA Sequencing Kit (Boehringer Mannheim). Primers were designed to amplify a region containing a cluster of repetitive motifs: 5'-(TG)₁₃(AG)₄(TG)₂CAT-GTGCAC(TG)₁₂-3'.

Method of Detection. Polymerase chain reaction was carried out using primers SM 10/9 F: 5'-TTT GGA ATG ATA TGG ATA TGG -3' (CA/CT strand) and 5'-digoxigenin labeled SM 10/9 R: 5'-CTT ACA GCC ACC TTT ATG CG-3'. Each reaction (10 μL final volume) contained 50 ng of genomic DNA, .75 μM each primer, .2 mM dNTP, .5 U of *Taq* polymerase (Gibco), 1.5 mM MgCl₂, 20 mM Tris-HCl, and 50 mM KCl. Thermal cycling reaction was performed in a Perkin Elmer 2400 Cycler under the following conditions: 3 min at 94°C followed by 29 cycles of 94°C for 45 s and 60°C for 25 s. The PCR products were run on a direct blotting electrophoresis device GATC 1500 (Gesellschaft für Analyse-Technik und Consulting mbH) using 4% denaturing polyacrylamide gel and detected by color reaction using alkaline phosphatase conjugated anti-digoxigenine antibodies.

Description of Polymorphism. In the test material, 12 alleles were detected in 57 marble trout, originat-

ing from two geographically remote wild populations, and in 24 brown trout, originating from three different river basins. The observed allele sizes were as follows: 202, 204, 206, 212, 216, 218, 220, 224, 228, 232, 236, and 238 bp. The alleles 478, 480, and 488 were characteristic for marble trout. The observed frequencies of these three alleles differed between the two sampling locations and were .18₂₀₂, .07₂₀₄, and .75₂₁₂ and .19₂₀₂, .78₂₀₄, and .03₂₁₂, respectively ($\chi^2 = 63.9$, $P < .001$). The other alleles were evenly distributed among the brown trout populations except for the alleles 218, 228, and 232, which were restricted only to a certain geographic location. The results from 16 specimens, indicating all the alleles observed, are shown in Figure 1.

Comments. The PCR with SM 10/9 F and SM 10/9 R primers failed to amplify genomic DNA from rainbow trout (*Oncorhynchus mykiss*) and grayling (*Thymallus thymallus*).

Key Words: Fishes, Brown Trout, Microsatellites, DNA, Polymorphism

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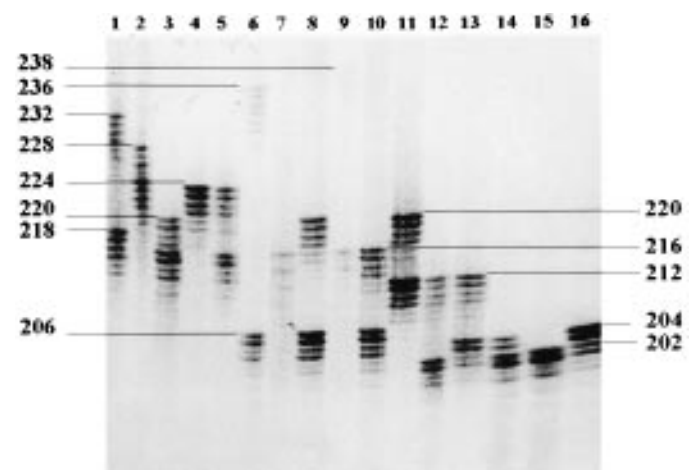


Figure 1. Twelve alleles representing 16 different genotypes. Lanes 1 to 2: brown trout from the Ohrid lake; lanes 3 to 10: the Danubian and Atlantic brown trout; lane 11: hybrid between marble and brown trout; lanes 12 to 16: marble trout. Allele sizes in base pairs are indicated on the margins.

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