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A new set of microsatellite markers for grayling: *BFRO014*, *BFRO015*, *BFRO016*, *BFRO017* and *BFRO018*

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Accepted 31 August 1999

Source/description: A grayling (*Thymallus thymallus*) genomic library containing size-selected (200–800 bp) *Sau3AI* fragments was constructed in *Bam*HI-restricted pBluescript SK+ (Stratagene, La Jolla, CA) vector and propagated in Epicurian Coli Competent Cells (Stratagene). The screening of the library was performed by Chemilluminescence Quick-light Genome Mapping Probe Kit (FMC, Bioproducts, ME) using (CA)*n* and (GA)*n* oligonucleotide as a probe. After sequencing of positive clones, which was performed on ABI Prism 310 Genetic Analyser (PE Applied Biosystems, Warrington UK), primers for amplification of informative microsatellites were designed.

Primer sequences:

BFRO014 (AF175248)

F: 5'-ACTACATTACTACATTCTCTCGCA-3' (5' HEX labelled)

R: 5'-CAAACCTCCACTTCTCTATCTCAG-3'

BFRO015 (AF175249)

F: 5'-GACTCAGTGAAGAATAAGTACA-3' (5' TET labelled)

R: 5'-GAAAAGTTATGAAGGTCAACCC-3'

BFRO016 (AF175250)

F: 5'-GTAGAGGCAGGGTTTCAGGCA-3' (5' TET labelled)

R: 5'-ATCAGCCCAAGGTTGTAACA-3'

BFRO017 (AF175251)

F: 5'-GCCCTCTGCTAAACACAC-3' (5' FAM labelled)

R: 5'-TCTATTGGGTTGAGGTCTGG-3'

BFRO018 (AF175252)

F: 5'-AGAGGGTCCAGCAACATCA-3' (5' FAM labelled)

R: 5'-GGGAACAGTCTAAAGCCT-3'

PCR conditions: PCR reaction was performed in MJ Research PTC-100 Thermal cycler with the following conditions: 3 min at 94 °C followed by 30 cycles of 45 s at 94 °C, 15 s at 60 °C (*BFRO015*, *BFRO016*, *BFRO017* and *BFRO018*) or 55 °C (*BFRO014*) and 5 s at 72 °C. Reaction mix of 10 µl contained 50 ng of template DNA, 0·5 µM of each primer, 0·2 mM dNTP, 1·5 mM MgCl₂, 20 mM Tris–

Table 1. Allele frequencies, observed heterozygosity (H) and PIC values at *BFRO014*, *BFRO015*, *BFRO016*, *BFRO017* and *BFRO018*, found in the Adriatic and Danubian type of grayling in Slovenia

Locus	Allele (bp)	Type of grayling	
		Adriatic	Danubian
<i>BFRO014</i>	144	0·12	0·13
	146	0·51	0·87
	155	0·37	0·00
	H	0·53	0·22
	PIC		0·39
	<i>BFRO015</i>	140	0·06
<i>BFRO016</i>	144	0·16	0·33
	148	0·06	0·03
	150	0·21	0·25
	154	0·16	0·39
	162	0·35	0·00
	H	0·74	0·78
	PIC		0·73
<i>BFRO017</i>	231	0·03	0·30
	233	0·24	0·28
	235	0·26	0·42
	237	0·43	0·00
	239	0·04	0·00
	H	0·71	0·62
<i>BFRO018</i>	109	0·41	0·00
	111	0·59	1·00
<i>BFRO018</i>	H	0·59	/
	PIC		0·25
	178	0·19	0·42
	182	0·57	0·36
<i>BFRO018</i>	184	0·12	0·02
	186	0·12	0·20
	H	0·62	0·64
PIC		0·60	

HCl, 50 mM KCl and 0·5 U of *Taq* polymerase (PE Applied Biosystems). Amplification products of five loci were run together on ABI Prism 310 Genetic Analyzer and genotyped with GENESCAN Software 2·1.

Test material and polymorphism: Grayling originating from the Danubian and Adriatic river system in Slovenia were genotyped. The Danubian type was represented by 50 animals and the Adriatic type by 34. The observed allele sizes, heterozygosities and PIC values¹ are shown in Table 1. All five loci indicate genetic dissimilarity between the two types of grayling ($\chi^2_{BFRO014} = 43·85$, $P < 0·001$; $\chi^2_{BFRO015} = 53·79$, $P < 0·001$; $\chi^2_{BFRO016} = 65·64$, $P < 0·001$; $\chi^2_{BFRO017} = 49·41$, $P < 0·001$; $\chi^2_{BFRO018} = 18·79$, $P < 0·001$). At four loci alleles being private for the Adriatic type were found.

Acknowledgements: We thank Dušan Ulčar, Simon Pleško and Dušan Jesenšek for providing samples. This work was supported by the Ministry of Science and Technology of the Republic of Slovenia (Grant No. J4–559–402).

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