Two polymorphic microsatellite markers in the European seabass, *Dicentrarchus labrax* (L.)

R Castilho[1,2], B McAndrew[1]

[1]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK; [2]UCTRA, Universidade do Algarve, Campus de Gambelas, 8000 FARO, Portugal

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Source/description: A size-selected genomic library (300–600 bp) was created by partially digesting DNA isolated from seabass muscle with *Sau*3AI. The DNA was ligated to PUC 18 plasmid (*Bam*HI/BAP, Pharmacia) and transformed into competent *E. coli* cells (strain DH5; BRL Gibco). Replica nylon filters (Hybond-N-Amersham) were pre-hybridized (5× SSPE, 5× Denhardt's, 0·5% SDS, 100 μg ml^{-1} RNA) for 1 h at 65 °C prior to overnight hybridization at 65 °C to $(GT)_{15}$ synthetic oligonucleotides radio-labelled with $[\gamma^{32}P]$ATP using T4 polynucleotide kinase. Filters were washed twice with 2× SSC, 0·2% SDS at room temperature and with 0·2× SSC, 0·2% SDS at 65 °C, and were exposed to X-ray film (Kodak XAR-5) with two intensifying screens at −80 °C for 6 h. Positive clones were picked off the plates and grown in LB-ampicillin for 48 h. The plasmid DNA was extracted with phenol-chloroform and minipreparations and followed the classic alkaline lysis. Sequencing was performed with the T7 sequencing Kit (Pharmacia), according to the manufactures instructions, using ^{35}S label. Eight per cent acrylamide gels were run for 3·5 h at 60 mA. The gel was fixed for an hour and then was dried in a Biorad gel dryer for 2 h before being exposed overnight to Kodak X-omat R film. The sequences of *Dla 6* and *Dla 11* have been submitted to the GenBank/EMBL database and have accession numbers Y13158 and Y13159, respectively.

Primer sequences: Primer sequences were designed with the program OSP[1]:

Dla 6: F-5'-AATACGGTGGTGAATCAGTG
R-5'-GCTGTTGTCTTGCTGCATAG

Dla 11: F-5'-CACCTCTAATGCTTCCATGC
R-5'-CGAATGCGCTACAAATCTGC

PCR conditions: A 10-μl PCR reaction contained 0·6 μM of non-labelled forward and reverse primers, 0·06 μM $[\gamma^{32}P]$ATP-labelled forward primer, 200 μM of each dNTP, 1× PCR buffer containing 50 mM KCl, 10 mm Tris-HCl pH 8·3 (supplied by AB Technologies), 1·5 mM $MgCl_2$, 0·05 U of *Taq* Red hot DNA polymerase (AB Technologies) and 10–20 ng genomic DNA. A Hybaid thermocycler was programmed to run 94 °C for 3 min; 30 cycles at 94 °C for 2 min; 58 °C for 45 s and 72 °C for 45 s. PCR products were separated on 6% denaturing polyacrylamide gels together with an M13 control sequence in order to size the alleles. Gels were exposed to X-ray films (Kodak) for product detection.

Mendelian inheritance: Mendelian inheritance could not be established as no breeding populations were available at either Institution.

Table 1. Number of individuals (*n*), number of alleles (*na*), and observed and expected heterozygosity for two microsatellite loci in seabass from Portugal

Locus	Repeat structure	Number of individuals, number of alleles, and observed and expected heterozygosity[2] Foz	Aveiro	Óbidos	Milfontes	Faro	Total
Dla 6	$(AC)_{26}$	*n* = 91, *na* = 15	*n* = 59, *na* = 18	*n* = 78, *na* = 17	*n* = 111, *na* = 19	*n* = 17, *na* = 8	*na* = 28
	55–115	0·36/0·68	0·54/0·75	0·40/0·74	0·42/0·71	0·53/0·77	0·45/0·73
Dla 11	$(GT)_{16}$	*n* = 80, *na* = 15	*n* = 69, *na* = 14	*n* = 56, *na* = 11	*n* = 32, *na* = 8	–	*na* = 20
	99–141	0·90/0·86	0·80/0·86	0·88/0·85	0·78/0·82	–	0·84/0·85

Polymorphism: The number of alleles per locus, and the observed and expected heterozygosity were established (Table 1).

Chromosomal location: Unknown for both markers.

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Correspondence: R Castilho

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