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PRIMER NOTE

PCR primers for fish G6PD gene intron and characterization of intron length variation in the albacore *Thunnus alalunga*

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Abstract

Polymerase chain reaction (PCR) primers to amplify the fourth intron of glucose-6-phosphate dehydrogenase (G6PD) gene were designed. A large length variation of amplified fragment was observed in the Atlantic albacore sample with a moderate level of heterozygosity (H_E = 0.488). Nucleotide sequence analysis revealed deletion or insertion of a large nucleotide block (110 base pairs) to be responsible for the length difference. Successful amplification of single or two fragments was confirmed in the northern bluefin tuna and Pacific saury, indicating the wide cross-species applicability.

Keywords: cross-species amplification, G6PD intron, length variation, *Thunnus alalunga*

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The albacore (*Thunnus alalunga*) is one of the most important cosmopolitan tuna species, and knowledge on the stock structure is essential for its management. Mitochondrial and microsatellite DNA analyses in the albacore have revealed genetic differentiation not only between Atlantic and Pacific stocks but also between north and south Atlantic stocks (Chow & Ushiama 1995; Takagi *et al*. 2001). We have investigated the relatively less variable DNA markers. Introns may be a good source for such DNA polymorphism, and several universal primers for exon-primed intron-targeted polymerase chain reactions (PCR) have been designed and used for population genetic analysis in marine animals (Corte-Real *et al*. 1994; Chow & Takeyama 1998, 2000; Quattro & Jones 1999; Smith *et al*. 2002). We have designed a primer pair to amplify the fourth intron of the glucose-6-phosphate dehydrogenase gene (G6PD) of fish and found fragment length variation in the albacore. Here, we introduce the PCR primers, characterize the length variation, and demonstrate their applicability among distant fish species.

The DNA sequence data of the G6PD gene of mammal and fish were obtained from published data (Martini *et al*. 1986; Mason *et al*. 1995). The nucleotide sequence of the forward primer (G6PDex4F) designed at the central domain of exon 4 is 5′-GAGCAGACGTATTTTGTGGG-3′, and that of the reverse primer (G6PDex5R) near the 3′ end of exon 5 is 5′-GCCAGGTAGAAGAGGCGGTT-3′. The PCR reaction mixture (10 μL) contained 0.2 U *Taq* DNA polymerase (Perkin Elmer Cetus), 0.2 mM of each dNTP, 1 μL of the 10× buffer supplied by the manufacturer, 2 mM $MgCl_2$, 10 pmol of each primer and 10–100 ng template DNA. Amplification was carried out with an initial denaturation at 95 °C for 2 min, followed by 30 cycles of amplification (denaturation at 95 °C for 0.5 min, annealing at 55 °C for 1 min and extension at 72 °C for 1.5 min) with a final extension at 72 °C for 10 min. PCR products were electrophoresed on a 2.5% agarose gel (Biogel) in TBE buffer (50 mM Tris–HCl, 1 mM ethylenediaminetetraacetic acid, and 48.5 mM boric acid).

Length variation of the amplified fragments was observed as shown in Fig. 1. One- or two-banded phenotypes were tentatively determined as homozygote and heterozygote, where the longer and shorter fragments were designated *A* and *B* alleles, respectively. Among a total of 60 albacore juveniles caught in the Bay of Biscay (northeast Atlantic) in 1994 by the Institut de Recherche Pour le Developpment (IRD), the numbers of designated genotypes were nine *AA*, 31 *AB* and 20 *BB*. Observed and expected heterozygosities were 0.521 and 0.488, respectively, and no significant departure from the Hardy–Weinberg equilibrium was observed ($P > 0.9$), suggesting that the variation was Mendelian. To characterize the length variation, one heterozygous

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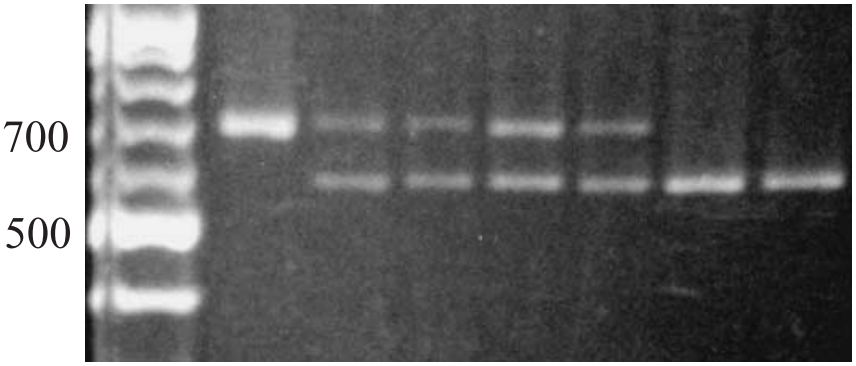

Fig. 1 An agarose gel electrophoresis profile of amplified G6PD fragments of the albacore (*Thunnus alalunga*). On the left is a 100-bp DNA ladder with size (in bp) along the left margin.

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                                                                                                  90
A   GAGCAGACGTATTTTGTGGGATTTGCTCGCTCTGACCTGACAGTTGATGCCATCCGGACATCATGCATGCCATACCTGAAGGTACAACTT
B   ..............................................................................................

A   GACCTTTTAAGTAACAGTTTTTTTTTCCTTTGTAATATAATTTTTCATTTTTAATTGAGAGGAGGTAGTAGGATTTACTGCAGATAGTAG
B   ..............................................................................G.............

A   CAAACATTATACAAACACCATGTAAATAAAAACCTTTTCAAGGCAAGAAAATAAATATAGAAATAAATGAAAGAAAGAAACAAAACAAAA
B   ..............................................................A...............................

A   CAATAAAAAATCCAACTGAACTTAATCTCAAGGTGATAGTTATCAAATAAATGAGTCATAACACAAATGCATACCATTTAAAAAAAAAAA
B   ..............................................................................................

A   AAAAAAAAAAAAGATAATAAATATACTCACTTTTAACTTTTCCAGTGTGAATTTTTCTGCTCTAAATTAAAACTTTCCGTATGAATTCTC
B   .....----------------------------------------------------------------------------------------

A   TTTTCACTTATTTTAAAGCGATTTTCTTGTCAATAGAATTTTTTT-CACACAATTTTTAATGATCCTAGTTAAATCTATGCGTGCTCAAA
B   ----------------------------.A.................T......G.....................C....A.........

A   TGTTTTCACTCTCTGGCCCAGGTGACAGACTCAGAAGCAGATCGGTTGACAGCCTTCTTCAGCAGGAACACCTACATCAGTGGGAAATAT
B   ....................:.........................................................................

A   GCAGAAGAAGGTTCCTTCTCCAAGCTCAACACACATCCACACTCTGCCCGGAGGAACCGAGGCAAACCGCCTCTTCTACCTGGC
B   ...............................................................................
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Fig. 2 Nucleotide sequence alignment of *A* and *B* alleles. Primer regions are boxed and exons are underlined. The same nucleotides as those of the top sequence are shown by dots and deletions are denoted by dashes. The sequences without primer regions are available in DNA Data Bank of Japan (DDBJ) under the accession numbers of AB159515 and AB159516.

individual was chosen and subjected to nucleotide sequence analysis. Cloning of PCR products was carried out using a TA cloning vector, PUCII (3.9 kilobase pairs; Invitrogen) without further purification. A transformed bacterial colony was screened using direct PCR with the G6PD primers, followed by gel electrophoresis to observe the size of the inserted fragment. Colonies found to have longer or shorter inserts were re-amplified using M13 reverse and M13 (–20) forward primers. The nucleotide sequences of the amplified fragments were determined by automated sequencer (Applied Biosystems model 373A) using a *Taq* dye terminator cycle sequencing kit (Perkin-Elmer Cetus) with M13 and G6PD primers. Sequence alignment of the *A* [715 base pairs (bp)] and *B* (605 bp) alleles (accession numbers AB159515 and AB159516) is shown in Fig. 2. An investigation of the homology between the G6PD genes of humans (Martini *et al*. 1986) and pufferfish (Mason *et al*. 1995) indicated that the fragments consisted of partial sequences of exons 4 and 5 and the intervening intron 4. A large deletion or insertion of nucleotides (110 bp) was observed in the intron.

Using the same PCR conditions, successful amplification was observed in the closely related bluefin tuna (*Thunnus thynnus thynnus*) and the distantly related Pacific saury (*Cololabis saira*). The agarose gel electrophoresis results of the bluefin tuna and albacore indicated that they share the same length variation, indicating amplification of a homologous gene locus. The numbers of genotypes in a bluefin tuna sample obtained from the Mediterranean sea were five *AA*, 11 *AB* and five *BB* with no significant departure from Hardy–Weinberg equilibrium. The size of a single fragment amplified in the Pacific saury was estimated to be *c*. 400 bp and no length variation was observed among the four individuals examined.

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