

Universal PCR primers for S7 ribosomal protein gene introns in fish

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Lessa (1992) introduced intron-targeted PCR, in which a non-coding intron was amplified using primers designed from highly conserved exon sequences. Introns appear to harbour a much greater degree of genetic polymorphism within and between species than exons. On the other hand, length and nucleotide sequence of exons, and exon-intron arrangement can be highly conserved between considerably distant animal taxa. These characteristics may allow us to design sets of primers based on exon sequences to amplify flanking intron regions. Such sets of primers might function in very distant species. This study introduces two pairs of primer sets which were designed for amplifying the 1st and 2nd introns of the S7 ribosomal protein gene in fish. These primers were applied to distant fish species in order to determine their universality, and polymorphism in the amplified fragments was investigated.

The DNA sequence data of the S7 ribosomal protein gene of puffer fish (*Fugu rubripes*), frog (*Xenopus laevis*) and human were derived from Cecconi *et al.* (1996), Mariottini *et al.* (1993) and Annilo *et al.* (1995), respectively. Exons 1, 2 and 3 of these species were aligned to determine conserved sequence regions. Because exon 1 of humans showed very poor homology with exon 1 of other species, data from puffer fish and frog were used for aligning exon 1. By contrast, highly conserved regions among these distant species were observed in exons 2 and 3. Two sets of primers were designed from the conserved sequence regions. The primer sequences to amplify the 1st intron (RP1) were 5'-TGGCC-TCTTCCTTGGCCGTC-3' (S7RPEX1F) and 5'-AACTCGTCTGGCTTTTCGCC-3' (S7RPEX2R), and those for the 2nd intron (RP2) were 5'-AGCGCCAAAATAGTGAAGCC-3' (S7RPEX2F) and 5'-GCCTTCAGGTCAGAGTTCAT-3' (S7RPEX3R). The PCR reaction mixture contained 0.2 U of *Taq* DNA polymerase (Perkin Elmer Cetus), 0.2 mM of each dNTP, 1 μ L of the manufacturer's supplied 10 \times buffer, 2 mM MgCl₂, 10 pmol of each primer and 10–50 ng of template DNA, in a final volume of 10 μ L. Amplification was carried out with an initial denaturation at 95 °C for 1 min, followed by 30 cycles of amplification (denaturation at 95 °C for 30 s, annealing at 60 °C for 1 min and extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min). PCR products and those digested by endonuclease were electrophoresed on a 2.5% agarose gel (Biogel) in TBE buffer (50 mM Tris, 1 mM EDTA, and 48.5 mM boric acid).

Using the standard phenol–chloroform method, crude DNA was extracted from frozen or ethanol-preserved muscles of chum salmon (*Onchorhynchus keta*), tuna (*Thunnus* spp.)

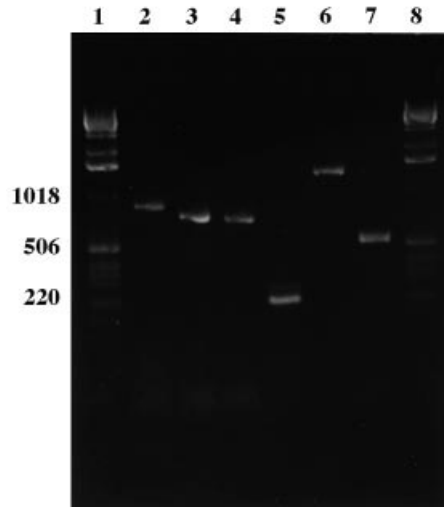


Fig. 1 Result of PCR amplification targeting the 1st (lanes 2–4) and 2nd introns (lanes 5–7) of the S7 ribosomal protein gene of three distant fish species. Lanes: 1 and 8, molecular weight marker (Gibco BRL 1 kb ladder); 2 and 5, chum salmon (*Onchorhynchus keta*); 3 and 6, yellowfin tuna (*Thunnus albacares*); 4 and 7, puffer fish (*Fugu rubripes*).

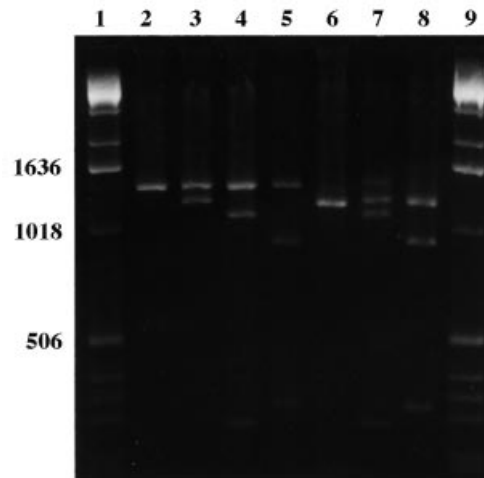


Fig. 2 *Hha*I restriction profiles observed in the 2nd intron of the S7 ribosomal protein gene of yellowfin tuna. Lanes 1 and 9, molecular weight marker (Gibco BRL 1 kb ladder). Deduced genotypes are: lane 2 AA; lane 3, AB; lane 4, AC; lane 5, AD; lane 6, BB; lane 7, BC; and lane 8, BD.

and puffer fish (*Fugu rubripes*), each of which belonged to a different order. Results from PCR amplifications of RP1 and RP2 are shown in Fig. 1, where amplification of a single fragment was eminent in all species. Amplified fragments of salmon, tuna and puffer fish were all different in length with respect to each other, while no length difference was observed among eight tuna species (data not shown).

A battery of 4-bp cutter endonucleases was applied to PCR products of yellowfin tuna (*Thunnus albacares*) in order to investigate intraspecific restriction site polymorphism.