Seventeen new exon-primed intron-crossing polymerase chain reaction amplifiable introns in fish

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PRIMER NOTE

Seventeen new exon-primed intron-crossing polymerase chain reaction amplifiable introns in fish

M. HASSAN,* C. LEMAIRE,* C. FAUVELOT*† and F. BONHOMME*

**Laboratoire Génome, Populations, Interactions Cnrs Umr 5000, SMEL 1, Quai de la Daurade, 34200 Sète, France; †Ecole Pratique, Des Hautes Etudes, Université de Perpignan, Perpignan, France*

Abstract

We used exon-primed, intron-crossing polymerase chain reaction (EPIC-PCR) amplification to assay variation in nuclear loci in some teleost fishes (Carangidae, Centropomidae, Chaetodontidae, Clupeidae, Holocentridae, Moronidae, Mullidae, Pomacentridae, Scombridae, Siganidae). We designed primers in the conserved regions flanking splice sites of consecutive exons of different genes, allowing the amplification of 17 putative introns. Among the satisfactory amplified systems, 14 showed length polymorphism with 2–14 alleles.

Keywords: EPIC-PCR, length polymorphism, teleost fishes

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Introns range in size from ≈ 80 to 10 000 or more nucleotides. Unlike exons, the nucleotide sequence of introns seems to be little constrained; it is often possible to alter most of their sequence without greatly affecting gene function. Introns accumulate mutations rapidly and often display a high genetic variability, including length polymorphism (Palumbi 1995). Exon-primed, intron-crossing polymerase chain reaction (EPIC-PCR) amplification provides a widely applicable strategy for finding DNA polymorphism in eukaryotic genomes. Thus, introns can be targeted as markers of population variation and subdivision (Lessa 1992). They constitute suitable markers for analysing population structure within a species as well as for reconstructing relationships among closely related species (He & Haymer 1997). Intron variation allows high-resolution genetic characterization of invading populations in both natural and managed systems (Villablanca *et al*. 1998). This method was used to detect and rapidly assess allelic variation at the nucleotide level in mammals (Palumbi & Baker 1994), birds (Heslewood *et al*. 1998), insects (Gomulski *et al*. 1998; He & Haymer 1999), crustaceans (Bierne *et al*. 2000) and mussels (Côrte-Real *et al*. 1994; Daguin *et al*. 2001).

Using the same approach, we designed primers in the conserved regions of consecutive exons of different genes, after a search of exonic sequences published to date (Table 1) in different species of teleost fishes. Each 10 μL PCR contained 0.5 U of *Taq* DNA polymerase (Promega, Madison WI, USA), 1 μL buffer (Promega), 0.75 mM dNTPs, 1.8 mM $MgCl_2$ and 30–50 ng template DNA. Right before the PCR, primer (Forward) was labelled radioactively with the ^{33}P using 1 U of polynucleotide-kinase T4 (Eurogenetic, Liège, Belgium, 10 U/μL) in a reaction mixture contained 0.3 μM of the primer and 0.4 mCi/mL of [$\gamma^{33}P$] ATP (Amersham Pharmacia Biotech, UK). This mixture was incubated 30 min at 37 °C. PCR was performed in a Crocodile III Thermocycler (Appligène, Strasbourg, France), using the following cycle profile: one initial denaturation step at 95 °C for 3 min, followed by 35 cycles of amplification [denaturation at 94 °C for 12 s, annealing at (see Table 2) for 12 s and extension at 72 °C for 20 s] and a final extension at 72 °C for 5 min. PCR products were mixed with 5 μL of formamide dye (95% formamide, 20 mM EDTA, 0.05% xylene cyanol and 0.05% bromophenol blue), denaturated at 95 °C for 5 min and 4 μL were loaded on a 6% polyacrylamide gel in TBE buffer. The gels were run for 3 h at 50 W. After drying, the gels were exposed against films of Biomax autoradiograph.

Table 2 presents the principal results of the 17 EPIC-PCR on the tested species. The simultaneous amplification of two or three loci can reveal either the presence of another gene, or of a pseudogene in the species that show these multiple amplifications. It does not seem that these simultaneous amplifications disturb the quality of PCRs and the reading of the genotypes because: (i) the supplementary profiles do not present parasitic bands, (ii) the variation

Correspondence: M. Hassan. Fax: +33 4 67 46 33 99; E-mail: m-hassan@crit.univ-montp2.fr

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Table 1 Designed primers for introns amplifications

Gene	Intron number	Intron name	Primer name	Primer sequence (5′–3′)
Creatin kinase (mitochondrial)	intron 7	Ck7	Ck7F	AAGAGGGTCTTTGACAGGTTCTGC
			Ck8R	TTCTCCTGGATCAGACGCTCCACC
Glyceraldehyde-3-phosphate dehydrogenase	intron 2	Gpd2	Gpd2F	GCCATCAATGACCCCTTCATCG
			Gpd3R	TTGACCTCACCCTTGAAGCGGCCG
Growth hormone	intron 2	Gh2	Gh2F	AGCGTTTCTCCATTGCCGTCAGC
			Gh3R	TCTTGTTGAGTTGACGCTGGTCC
	intron 5	Gh5	Gh5F	AGGCCAATCAGGACGGAGC
			Gh6R	TGCCACTGTCAGATAAGTCTCC
Aldolase C	intron 1	AldoC1	Aldoc1F	CCTGGCTGCGGACGAGTCTGTGGG
			Aldoc2R	GGCGGTACTGTCTGCGGTTCTCC
Aldolase B	intron 1	AldoB1	Aldob1-1F	GCTCCAGGAAAGGGAATCCTGGC
			Aldob1-2R	CTCGTGGAAGAAGATGATCCCGCC
	intron 2	AldoB2	Aldob2F	TCAGGGCATTGTCGTCGG
			Aldexon3R	CTGTTCCATTTAGACCAGC
	intron 4	AldoB4	Aldo5F	GCCAGATATGCCAGCATCTGCC
			Aldo3.1R	GGGTTCCATCAGGCAGGATCTCTGGC
	intron 5	AldoB5	Aldo3F	TCCTGCCTGATGGAACCC
			Aldo2R	CAGGTACACATGGTGGTC
Gonadotropin-releasing hormone 3	intron 1	GnRH3-1	GnRH1F	AATGCACCACATGCTAACAAGGC
			GnRH1R	CGCACCATCACTCTGCTGTTCGC
	intron 2	GnRH3-2	GnRH2F	AGAAGTGTGGGAGAGCTAGAGGC
			GnRH2R	AGAGACACCACTTCTCCTGTACCC
	intron 3	GnRH3-3	GnRH3F	GCCCAAACCCAAGAGAGACTTAGACC
			GnRH3R	TTCGGTCAAAATGACTGGAATCATC
Alpha amylase	intron 1	Am2B-1	Am2b1F	CCTTCATCTTCCAGGAGGTAC
			Am2b2R	TTCACCTCCCAGATCAATAAC
	intron 2	Am2B-2	Am2b2F	GGCGATAAGTTGTCTTACACC
			Am2b3R	AGCCCTCTCCCCAGTTCCTGC
	intron 3	Am2B-3	Am2b3F	TGGAACCGAAACATTGTGAAC
			Am2b4R	CCCATCCAGTCATTCTGATCC
Alpha tropomyosin	intron 1	Tr1	Tr1F	AGGGAACAGAGGATGAGCTGGAC
			Tr1R	TCTCAGCTTCCTCCAGCTTGGTG
Major histocomptability complex class II antigen	intron 1	MhcII	Mhc1F	ACTCTAATCTGGAGTACATGC
			Mhc2R	CAGGAGATCTTCTCTCCAGCC

Table 2 Details of general results

Intron name	Species name	Sample size (ind.)	No. of amplified loci	Annealing temp. (°C)	Name of amplified loci	Length polymorphism	No. of alleles at each locus	H_E	F^*_{IS}	Size of focal intron (bp)†
Ck7	*Siganus rivulatus* (Siganidae)	30	0	54	—	—	—	—	—	—
	Siganus luridus (Siganidae)	30	0	54	—	—	—	—	—	—
	Thunnus obesus (Scombridae)	100	1	45	*ToCk7*	Yes	3	—	—	350
	Lates niloticus (Centropomidae)	30	1	52	*LnCk7*	Yes	—	—	—	—
	Chaetodon citrinellus (Chaetodontidae)	50	multilocus	54	—	—	—	—	—	—
	Chaetodon quadrimaculatus (=)	50	multilocus	54	—	—	—	—	—	—
	Forcipiger flavissimus (=)	50	multilocus	54	—	—	—	—	—	—
Gpd2	*Siganus rivulatus*	137	2	50	*SrGPD2-1*	Yes	6	0.55	n.s.	250
					SrGPD2-2	Yes	5	0.32	n.s.	360
	Siganus luridus	126	1	53	*SlGPD2*	Yes	3	0.17	n.s.	175
	Ethmalosa fimbriata (Clupeidae)	170	2	52	*EfGPD2-1*	Yes	14	0.80	n.s.	200
					EfGPD2-2	No	—	—	—	—
	Chaetodon citrinellus	50	1	54	*CcGPD2*	No	—	—	—	350
	Chaetodon quadrimaculatus	60	1	54	*CqGPD2*	No	—	—	—	350
	Forcipiger flavissimus	60	1	54	*FfGPD2*	No	—	—	—	350
	Dascyllus aruanus (Pomacentridae)	60	1	54	*DaGPD2*	No	—	—	—	350
	Pomacentrus pavo (=)	60	1	54	*PpGPD2*	No	—	—	—	350
	Plectroglyphidodon dickii (=)	40	1	54	*PdGPD2*	No	—	—	—	350
GH2	*Siganus rivulatus*	137	2	54	*SrGH2-1*	Yes	2	0.23	n.s.	250
					SrGH2-2	Yes	4	0.61	n.s.	500
	Siganus luridus	126	0	54	—	—	—	—	—	—
	Lates niloticus	30	1	54	*LnGH2*	Yes	—	—	—	—
	Chaetodon citrinellus	50	1	54	*CcGH2*	No	—	—	—	150
	Chaetodon quadrimaculatus	60	1	54	*CqGH2*	No	—	—	—	150
	Forcipiger flavissimus	234	1	54	*FfGH2*	Yes	12	0.72	n.s.	250
	Dascyllus aruanus	60	multilocus	54	—	—	—	—	—	—
	Pomacentrus pavo	60	multilocus	54	—	—	—	—	—	—
	Plectroglyphidodon dickii	40	multilocus	54	—	—	—	—	—	—
GH5	*Siganus rivulatus*	137	1	52	*SrGH5*	No	—	—	—	350
	Siganus luridus	126	1	52	*SlGH5*	Yes	4	0.34	n.s.	350
	Thunnus obesus	20	1	52	*ToGH5*	Yes	2	—	—	—
	Lates niloticus	30	multilocus	52	—	Yes	—	—	—	—
	Chaetodon citrinellus	198	1	54	—	No	—	—	—	300
	Chaetodon quadrimaculatus	233	1	54	—	No	—	—	—	300
	Forcipiger flavissimus	234	1	54	—	—	—	—	—	—
	Dascyllus aruanus	250	1	54	—	—	—	—	—	—
	Pomacentrus pavo	167	1	54	*PpGH5*	Yes	2	0.31	n.s.	300
	Plectroglyphidodon dickii	79	2	54	*PdGH5-1*	No	—	—	—	300
					PdGH5-2	No				

Table 2 *Continued*

Intron name	Species name	Sample size (ind.)	No. of amplified loci	Annealing temp. (°C)	Name of amplified loci	Length polymorphism	No. of alleles at each locus	H_E	F^*_{IS}	Size of focal intron (bp)†
AldoC1	*Siganus rivulatus*	137	1	53	*SrAldoC1*	Yes	4	0.12	n.s.	230
	Siganus luridus	126	1	54	*SlAldoC1*	Yes	2	0.19	n.s.	240
	Dicentrarchus labrax (Moronidae)	951	1	60	*DlAldoC1*	Yes	3	0.78	n.s.	325
AldoB1‡	*Siganus rivulatus*	137	1	49	*SrAldoB1*	Yes	2	0.11	n.s.	320
	Siganus luridus	126	3	52	*SlAldoB1-1*	Yes	2	0.33	n.s.	175
					SlAldoB1-2	Yes	2	0.19	n.s.	200
					SlAldoB1-3	Yes	3	0.62	n.s.	400
	Chaetodon citrinellus	36	1	54	*CcAldoB1*	No	—	—	—	—
	Chaetodon quadrimaculatus	10	1	54	*CqAldoB1*	No	—	—	—	—
	Forcipiger flavissimus	20	1	54	*FfAldoB1*	No	—	—	—	—
	Dascyllus aruanus	10	1	54	*DaAldoB1*	Yes	—	—	—	300
	Pomacentrus pavo	10	1	54	*PpAldoB1*	Yes	—	—	—	300
	Plectroglyphidodon dickii	10	1	54	*PdAldoB1*	Yes	—	—	—	300
	Dicentrarchus labrax	100	1	52	*DlAldoB1*	No	—	—	—	262
	Decapterus russelli (Carangidae)	458	1	52	*DrAldoB1*	Yes	7	0.31	n.s.	265
AldoB2	*Siganus rivulatus*	20	multilocus	52	—	—	—	—	—	—
	Siganus luridus	20	multilocus	52	—	—	—	—	—	—
	Sargocentron rubrum (Holocentridae)	20	multilocus	50	—	—	—	—	—	—
	Upeneus moluccensis (Mullidae)	20	multilocus	50	—	—	—	—	—	—
	Chaetodon citrinellus	198	2	54	*CcAldoB2-1*	Yes	4	0.10	n.s.	180
					CcAldoB2-2	Yes	—	—	—	300
	Chaetodon quadrimaculatus	10	2	54	*CqAldoB2-1*	No	—	—	—	180
					CqAldoB2-2	No	—	—	—	300
	Forcipiger flavissimus	20	2	54	*FfAldoB2-1*	No	—	—	—	280
					FfAldoB2-2	No	—	—	—	350
	Dascyllus aruanus	250	1	54	*DaAldoB2*	Yes	3	0.15	n.s.	200
	Pomacentrus pavo	10	1	54	*PpAldoB2*	No	—	—	—	200
	Plectroglyphidodon dickii	10	1	54	*PdAldoB2*	No	—	—	—	200
AldoB4	*Chaetodon citrinellus*	36	1	54	*CcAldoB4*	No	—	—	—	200
	Chaetodon quadrimaculatus	10	1	54	*CqAldoB4*	No	—	—	—	200
	Forcipiger flavissimus	20	1	54	*FfAldoB4*	No	—	—	—	200
	Dascyllus aruanus	255	2	54	*DaAldoB4-1*	Yes	2	0.41	n.s.	200
					DaAldoB4-2	No	—	—	—	200
	Pomacentrus pavo	167	2	54	*PpAldoB4-1*	Yes	2	0.48	n.s.	200
					PpAldoB4-2	No	—	—	—	200
	Plectroglyphidodon dickii	10	2	54	*PdAldoB4-1*	No	—	—	—	200
					PdAldoB4-2	No	—	—	—	200
	Ethmalosa fimbriata	68	1	50	*EfAldoB4*	Yes	12	0.87	n.s.	380

Table 2 *Continued*

Intron name	Species name	Sample size (ind.)	No. of amplified loci	Annealing temp. (°C)	Name of amplified loci	Length polymorphism	No. of alleles at each locus	H_E	F^*_{IS}	Size of focal intron (bp)†
AldoB5	*Siganus rivulatus*	20	0	58	—	—	—	—	—	—
	Siganus luridus	20	0	58	—	—	—	—	—	—
	Sargocentron rubrum	20	0	58	—	—	—	—	—	—
	Upeneus moluccensis	20	0	58	—	—	—	—	—	—
	Chaetodon citrinellus	36	1	54	*CcAldoB5*	No	—	—	—	200
	Chaetodon quadrimaculatus	10	1	54	*CqAldoB5*	No	—	—	—	200
	Forcipiger flavissimus	20	1	54	*FfAldoB5*	No	—	—	—	200
GnRH3-1	*Siganus rivulatus*	136	2	48	*SrGnRH3-1–1*	Yes	2	0.41	n.s.	250
					SrGnRH3-1–2	Yes	9	0.77	n.s.	400
	Siganus luridus	126	3	48	*SlGnRH3-1–1*	Yes	5	0.53	n.s.	275
					SlGnRH3-1–2	Yes	4	0.15	n.s.	325
					SlGnRH3-1–3	Yes	8	0.74	n.s.	375
	Chaetodon citrinellus	198	1	54	*CcGnRH3-1*	Yes	8	0.67	0.43	280
	Chaetodon quadrimaculatus	233	1	54	*CqGnRH3-1*	Yes	6	0.61	n.s.	280
	Forcipiger flavissimus	50	1	54	*FfGnRH3-1*	No	—	—	—	280
	Dascyllus aruanus	20	1	54	*DaGnRH3-1*	No	—	—	—	250
	Pomacentrus pavo	20	1	54	*PpGnRH3-1*	No	—	—	—	250
	Plectroglyphidodon dickii	20	0	54	—	—	—	—	—	—
GnRH3-2	*Siganus rivulatus*	137	1	56	*SrGnRH3-2*	No	—	—	—	230
	Siganus luridus	126	1	56	*SlGnRH3-2*	No	—	—	—	230
	Decapterus russelli	458	1	52	*DrGnRH3-2*	Yes	7	0.71	n.s.	300
	Chaetodon citrinellus	198	1	54	*CcGnRH3-2*	Yes	4	0.52	0.21	280
	Chaetodon quadrimaculatus	233	1	54	*CqGnRH3-2*	Yes	4	0.53	n.s.	280
	Forcipiger flavissimus	234	1	54	*FfGnRH3-2*	Yes	7	0.36	n.s.	300
	Dascyllus aruanus	20	1	54	*DaGnRH3-2*	No	—	—	—	300
	Pomacentrus pavo	20	1	54	*PpGnRH3-2*	No	—	—	—	300
	Plectroglyphidodon dickii	79	1	50	*PdGnRH3-2*	Yes	13	0.69	n.s.	300
GnRH3-3	*Siganus rivulatus*	137	1	52	*SrGnRH3-3*	Yes	4	0.40	n.s.	325
	Siganus luridus	126	1	53	*SlGnRH3-3*	Yes	4	0.48	n.s.	375
	Thunnus obesus	20	1	52	*ToGnRH3-3*	No	—	—	—	—
	Chaetodon citrinellus	50	1	54	*CcGnRH3-3*	No	—	—	—	400
	Chaetodon quadrimaculatus	550	1	54	*CqGnRH3-3*	No	—	—	—	400
	Forcipiger flavissimus	50	1	54	*FfGnRH3-3*	No	—	—	—	400
	Dascyllus aruanus	20	1	54	*DaGnRH3-3*	Yes	—	—	—	400
	Pomacentrus pavo	20	1	54	*PpGnRH3-3*	No	—	—	—	400
	Plectroglyphidodon dickii	20	1	54	*PdGnRH3-3*	No	—	—	—	400

Table 2 *Continued*

Intron name	Species name	Sample size (ind.)	No. of amplified loci	Annealing temp. (°C)	Name of amplified loci	Length polymorphism	No. of alleles at each locus	H_E	F^*_{IS}	Size of focal intron (bp)†
Am2B-1	*Siganus rivulatus*	137	3	59	*SrAm2B-1–1*	Yes	13	0.79	0.48	250
					SrAm2B-1–2	No	—	—	—	275
					SrAm2B-1–3	Yes	4	0.37	n.s.	400
	Siganus luridus	126	2	59	*SlAm2B-1–1*	No	—	—	—	275
					SlAm2B-1–2	Yes	4	0.46	n.s.	400
	Thunnus obesus	20	1	48	*ToAm2B-1*	Yes	2	—	—	—
	Lates niloticus	30	1	52	*LnAm2B-2*	Yes	—	—	—	—
Am2B-2	*Siganus rivulatus*	137	2	57	*SrAm2B-2–1*	Yes	2	0.46	n.s.	350
					SrAm2B-2–2	Yes	4	0.54	n.s.	375
	Siganus luridus	126	multilocus	57	—	—	—	—	—	—
Am2B-3	*Siganus rivulatus*	137	0	58	—	—	—	—	—	—
	Siganus luridus	126	0	58	—	—	—	—	—	—
Tr1	*Siganus rivulatus*	137	1	50	*SrTr1*	Yes	2	0.19	n.s.	250
	Siganus luridus	126	1	50	*SlTr1*	Yes	7	0.72	n.s.	300
	Ethmalosa fimbriata	20	multilocus	45	—	—	—	—	—	—
*Mhc*II	*Siganus rivulatus*	20	multilocus	55	—	Yes	—	—	—	—
	Siganus luridus	20	0	55	—	—	—	—	—	—
	Thunnus obesus	20	multilocus	52	—	—	—	—	—	—
	Ethmalosa fimbriata	20	0	52	—	—	—	—	—	—

*$F_{IS} = (H_E - H_O)/H_E$.

†Approximate size.

‡Additionally, this intron was proven to amplify correctly in 23 other teleost species and one chondrichthyes species; (–) incomplete data; n.s. = insignificant value.

of size between band systems was larger than the size polymorphism of each locus and, finally, (iii) we did not find significant departure from Hardy–Weinberg equilibrium in those samples in which we could analyse a sufficient number of individuals to test it properly.

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