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PRIMER NOTE

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

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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 (Côte-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 μ L PCR contained

0.5 U of *Taq* DNA polymerase (Promega, Madison WI, USA), 1 μ L buffer (Promega), 0.75 mM dNTPs, 1.8 mM MgCl₂ and 30–50 ng template DNA. Right before the PCR, primer (Forward) was labelled radioactively with the ³³P using 1 U of polynucleotide-kinase T4 (Eurogenetic, Liège, Belgium, 10 U/ μ L) in a reaction mixture contained 0.3 μ M of the primer and 0.4 mCi/mL of [³³P] 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 μ L 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 μ L 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

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	TTGACCTCACCTTGAAGCGCCG
			Gh2F	AGCGTTTCTCCATGCGGTACGC
Growth hormone	intron 2	Gh2	Gh3R	TCTTGTGAGTTGACGCTGGTCC
			Gh5F	AGGCCAATCAGGACGGAGC
	intron 5	Gh5	Gh6R	TGCCACTGTCAGATAAGTCTCC
			Aldoc1F	CCTGGCTGGGACGAGTCTGTGGG
Aldolase C	intron 1	Aldoc1	Aldoc2R	GGCGGTACTGTCTGCGGTTCTCC
Aldolase B	intron 1	Aldob1	Aldob1-1F	GCTCCAGGAAAGGAAATCCTGGC
			Aldob1-2R	CTCGTGGAGAAGATGATCCCGCC
	intron 2	Aldob2	Aldob2F	TCAGGGCATGTGTCGTCGG
			Aldexon3R	CTGTTCCATTTAGACCAGC
	intron 4	Aldob4	Aldo5F	GCCAGATATGCCAGCATCTGCC
Gonadotropin-releasing hormone 3	intron 1	GnRH3-1	Aldo3.1R	GGGTTCATCAGGACGATCTCTGGC
			Aldo3F	TCCTGCCTGATGGAACCC
	intron 2	GnRH3-2	Aldo2R	CAGGTACACATGGTGGTC
			GnRH1F	AATGCACCACATGCTAACAAGGC
	intron 3	GnRH3-3	GnRH1R	CGCACCATCACTCTGCTGTTCGC
GnRH2F			AGAAGTGTGGGAGAGCTAGAGGC	
GnRH2R			AGAGACACCACTTCTCCTGTACCC	
Alpha amylase	intron 1	Am2B-1	GnRH3F	GCCCAAACCAAGAGAGACTTAGACC
			GnRH3R	TTCCGGTCAAAATGACTGGAATCATC
	intron 2	Am2B-2	Am2b1F	CCTTCATCTTCCAGGAGGTAC
			Am2b2R	TTCACCTCCCAGATCAATAAC
	intron 3	Am2B-3	Am2b2F	GGCGATAAGTTGTCTTACACC
Am2b3R			AGCCCTCTCCCCAGTTCTCTGC	
Am2b3F			TGGAACCGAAACATTGTGAAC	
Alpha tropomyosin	intron 1	Tr1	Am2b4R	CCCATCCAGTCATCTGATCC
			Tr1F	AGGGAACAGAGGATGAGCTGGAC
			Tr1R	TCTCAGCTTCTCCAGCTTGGTG
Major histocompatibility 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	<i>Siganus rivulatus</i> (Siganidae)	30	0	54	—	—	—	—	—	—	
	<i>Siganus luridus</i> (Siganidae)	30	0	54	—	—	—	—	—	—	
	<i>Thunnus obesus</i> (Scombridae)	100	1	45	<i>ToCk7</i>	Yes	3	—	—	350	
	<i>Lates niloticus</i> (Centropomidae)	30	1	52	<i>LnCk7</i>	Yes	—	—	—	—	
	<i>Chaetodon citrinellus</i> (Chaetodontidae)	50	multilocus	54	—	—	—	—	—	—	
	<i>Chaetodon quadrimaculatus</i> (=)	50	multilocus	54	—	—	—	—	—	—	
Gpd2	<i>Forcipiger flavissimus</i> (=)	50	multilocus	54	—	—	—	—	—	—	
	<i>Siganus rivulatus</i>	137	2	50	<i>SrGPD2-1</i>	Yes	6	0.55	n.s.	250	
					<i>SrGPD2-2</i>	Yes	5	0.32	n.s.	360	
	<i>Siganus luridus</i>	126	1	53	<i>SlGPD2</i>	Yes	3	0.17	n.s.	175	
	<i>Ethmalosa fimbriata</i> (Clupeidae)	170	2	52	<i>EfGPD2-1</i>	Yes	14	0.80	n.s.	200	
					<i>EfGPD2-2</i>	No	—	—	—	—	
	<i>Chaetodon citrinellus</i>	50	1	54	<i>CcGPD2</i>	No	—	—	—	350	
	<i>Chaetodon quadrimaculatus</i>	60	1	54	<i>CqGPD2</i>	No	—	—	—	350	
	<i>Forcipiger flavissimus</i>	60	1	54	<i>FfGPD2</i>	No	—	—	—	350	
	<i>Dascyllus aruanus</i> (Pomacentridae)	60	1	54	<i>DaGPD2</i>	No	—	—	—	350	
	<i>Pomacentrus pavo</i> (=)	60	1	54	<i>PpGPD2</i>	No	—	—	—	350	
	<i>Plectrogliphidodon dickii</i> (=)	40	1	54	<i>PdGPD2</i>	No	—	—	—	350	
	GH2	<i>Siganus rivulatus</i>	137	2	54	<i>SrGH2-1</i>	Yes	2	0.23	n.s.	250
						<i>SrGH2-2</i>	Yes	4	0.61	n.s.	500
<i>Siganus luridus</i>		126	0	54	—	—	—	—	—	—	
<i>Lates niloticus</i>		30	1	54	<i>LnGH2</i>	Yes	—	—	—	—	
<i>Chaetodon citrinellus</i>		50	1	54	<i>CcGH2</i>	No	—	—	—	150	
<i>Chaetodon quadrimaculatus</i>		60	1	54	<i>CqGH2</i>	No	—	—	—	150	
<i>Forcipiger flavissimus</i>		234	1	54	<i>FfGH2</i>	Yes	12	0.72	n.s.	250	
<i>Dascyllus aruanus</i>		60	multilocus	54	—	—	—	—	—	—	
<i>Pomacentrus pavo</i>		60	multilocus	54	—	—	—	—	—	—	
<i>Plectrogliphidodon dickii</i>		40	multilocus	54	—	—	—	—	—	—	
GH5	<i>Siganus rivulatus</i>	137	1	52	<i>SrGH5</i>	No	—	—	—	350	
	<i>Siganus luridus</i>	126	1	52	<i>SlGH5</i>	Yes	4	0.34	n.s.	350	
	<i>Thunnus obesus</i>	20	1	52	<i>ToGH5</i>	Yes	2	—	—	—	
	<i>Lates niloticus</i>	30	multilocus	52	—	Yes	—	—	—	—	
	<i>Chaetodon citrinellus</i>	198	1	54	—	No	—	—	—	300	
	<i>Chaetodon quadrimaculatus</i>	233	1	54	—	No	—	—	—	300	
	<i>Forcipiger flavissimus</i>	234	1	54	—	—	—	—	—	—	
	<i>Dascyllus aruanus</i>	250	1	54	—	—	—	—	—	—	
	<i>Pomacentrus pavo</i>	167	1	54	<i>PpGH5</i>	Yes	2	0.31	n.s.	300	
	<i>Plectrogliphidodon dickii</i>	79	2	54	<i>PdGH5-1</i>	No	—	—	—	300	

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	<i>Siganus rivulatus</i>	137	1	53	<i>SrAldoC1</i>	Yes	4	0.12	n.s.	230
	<i>Siganus luridus</i>	126	1	54	<i>SlAldoC1</i>	Yes	2	0.19	n.s.	240
	<i>Dicentrarchus labrax</i> (Moronidae)	951	1	60	<i>DlAldoC1</i>	Yes	3	0.78	n.s.	325
AldoB1‡	<i>Siganus rivulatus</i>	137	1	49	<i>SrAldoB1</i>	Yes	2	0.11	n.s.	320
	<i>Siganus luridus</i>	126	3	52	<i>SlAldoB1-1</i>	Yes	2	0.33	n.s.	175
					<i>SlAldoB1-2</i>	Yes	2	0.19	n.s.	200
					<i>SlAldoB1-3</i>	Yes	3	0.62	n.s.	400
	<i>Chaetodon citrinellus</i>	36	1	54	<i>CcAldoB1</i>	No	—	—	—	—
	<i>Chaetodon quadrimaculatus</i>	10	1	54	<i>CqAldoB1</i>	No	—	—	—	—
	<i>Forcipiger flavissimus</i>	20	1	54	<i>FfAldoB1</i>	No	—	—	—	—
	<i>Dascyllus aruanus</i>	10	1	54	<i>DaAldoB1</i>	Yes	—	—	—	300
	<i>Pomacentrus pavo</i>	10	1	54	<i>PpAldoB1</i>	Yes	—	—	—	300
	<i>Plectroglyphidodon dickii</i>	10	1	54	<i>PdAldoB1</i>	Yes	—	—	—	300
AldoB2	<i>Dicentrarchus labrax</i>	100	1	52	<i>DlAldoB1</i>	No	—	—	—	262
	<i>Decapterus russelli</i> (Carangidae)	458	1	52	<i>DrAldoB1</i>	Yes	7	0.31	n.s.	265
	<i>Siganus rivulatus</i>	20	multilocus	52	—	—	—	—	—	—
	<i>Siganus luridus</i>	20	multilocus	52	—	—	—	—	—	—
	<i>Sargocentron rubrum</i> (Holocentridae)	20	multilocus	50	—	—	—	—	—	—
	<i>Upeneus moluccensis</i> (Mullidae)	20	multilocus	50	—	—	—	—	—	—
	<i>Chaetodon citrinellus</i>	198	2	54	<i>CcAldoB2-1</i>	Yes	4	0.10	n.s.	180
					<i>CcAldoB2-2</i>	Yes	—	—	—	300
	<i>Chaetodon quadrimaculatus</i>	10	2	54	<i>CqAldoB2-1</i>	No	—	—	—	180
					<i>CqAldoB2-2</i>	No	—	—	—	300
	<i>Forcipiger flavissimus</i>	20	2	54	<i>FfAldoB2-1</i>	No	—	—	—	280
					<i>FfAldoB2-2</i>	No	—	—	—	350
	<i>Dascyllus aruanus</i>	250	1	54	<i>DaAldoB2</i>	Yes	3	0.15	n.s.	200
AldoB4	<i>Pomacentrus pavo</i>	10	1	54	<i>PpAldoB2</i>	No	—	—	—	200
	<i>Plectroglyphidodon dickii</i>	10	1	54	<i>PdAldoB2</i>	No	—	—	—	200
	<i>Chaetodon citrinellus</i>	36	1	54	<i>CcAldoB4</i>	No	—	—	—	200
	<i>Chaetodon quadrimaculatus</i>	10	1	54	<i>CqAldoB4</i>	No	—	—	—	200
	<i>Forcipiger flavissimus</i>	20	1	54	<i>FfAldoB4</i>	No	—	—	—	200
	<i>Dascyllus aruanus</i>	255	2	54	<i>DaAldoB4-1</i>	Yes	2	0.41	n.s.	200
					<i>DaAldoB4-2</i>	No	—	—	—	200
	<i>Pomacentrus pavo</i>	167	2	54	<i>PpAldoB4-1</i>	Yes	2	0.48	n.s.	200
					<i>PpAldoB4-2</i>	No	—	—	—	200
	<i>Plectroglyphidodon dickii</i>	10	2	54	<i>PdAldoB4-1</i>	No	—	—	—	200
				<i>PdAldoB4-2</i>	No	—	—	—	200	
<i>Ethmalosa fimbriata</i>	68	1	50	<i>EfAldoB4</i>	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	<i>Siganus rivulatus</i>	20	0	58	—	—	—	—	—	—	
	<i>Siganus luridus</i>	20	0	58	—	—	—	—	—	—	
	<i>Sargocentron rubrum</i>	20	0	58	—	—	—	—	—	—	
	<i>Upeneus moluccensis</i>	20	0	58	—	—	—	—	—	—	
	<i>Chaetodon citrinellus</i>	36	1	54	CcAldoB5	No	—	—	—	200	
	<i>Chaetodon quadrimaculatus</i>	10	1	54	CqAldoB5	No	—	—	—	200	
	<i>Forcipiger flavissimus</i>	20	1	54	FfAldoB5	No	—	—	—	200	
GnRH3-1	<i>Siganus rivulatus</i>	136	2	48	SrGnRH3-1-1	Yes	2	0.41	n.s.	250	
					SrGnRH3-1-2	Yes	9	0.77	n.s.	400	
	<i>Siganus luridus</i>	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	
	<i>Chaetodon citrinellus</i>	198	1	54	CcGnRH3-1	Yes	8	0.67	0.43	280	
	<i>Chaetodon quadrimaculatus</i>	233	1	54	CqGnRH3-1	Yes	6	0.61	n.s.	280	
	<i>Forcipiger flavissimus</i>	50	1	54	FfGnRH3-1	No	—	—	—	280	
	<i>Dascyllus aruanus</i>	20	1	54	DaGnRH3-1	No	—	—	—	250	
	<i>Pomacentrus pavo</i>	20	1	54	PpGnRH3-1	No	—	—	—	250	
	<i>Plectroglyphidodon dickii</i>	20	0	54	—	—	—	—	—	—	
	GnRH3-2	<i>Siganus rivulatus</i>	137	1	56	SrGnRH3-2	No	—	—	—	230
		<i>Siganus luridus</i>	126	1	56	SlGnRH3-2	No	—	—	—	230
<i>Decapterus russelli</i>		458	1	52	DrGnRH3-2	Yes	7	0.71	n.s.	300	
<i>Chaetodon citrinellus</i>		198	1	54	CcGnRH3-2	Yes	4	0.52	0.21	280	
<i>Chaetodon quadrimaculatus</i>		233	1	54	CqGnRH3-2	Yes	4	0.53	n.s.	280	
<i>Forcipiger flavissimus</i>		234	1	54	FfGnRH3-2	Yes	7	0.36	n.s.	300	
<i>Dascyllus aruanus</i>		20	1	54	DaGnRH3-2	No	—	—	—	300	
<i>Pomacentrus pavo</i>		20	1	54	PpGnRH3-2	No	—	—	—	300	
<i>Plectroglyphidodon dickii</i>		79	1	50	PdGnRH3-2	Yes	13	0.69	n.s.	300	
GnRH3-3		<i>Siganus rivulatus</i>	137	1	52	SrGnRH3-3	Yes	4	0.40	n.s.	325
	<i>Siganus luridus</i>	126	1	53	SlGnRH3-3	Yes	4	0.48	n.s.	375	
	<i>Thunnus obesus</i>	20	1	52	ToGnRH3-3	No	—	—	—	—	
	<i>Chaetodon citrinellus</i>	50	1	54	CcGnRH3-3	No	—	—	—	400	
	<i>Chaetodon quadrimaculatus</i>	550	1	54	CqGnRH3-3	No	—	—	—	400	
	<i>Forcipiger flavissimus</i>	50	1	54	FfGnRH3-3	No	—	—	—	400	
	<i>Dascyllus aruanus</i>	20	1	54	DaGnRH3-3	Yes	—	—	—	400	
	<i>Pomacentrus pavo</i>	20	1	54	PpGnRH3-3	No	—	—	—	400	
	<i>Plectroglyphidodon dickii</i>	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	<i>Siganus rivulatus</i>	137	3	59	<i>SrAm2B-1-1</i>	Yes	13	0.79	0.48	250
					<i>SrAm2B-1-2</i>	No	—	—	—	275
					<i>SrAm2B-1-3</i>	Yes	4	0.37	n.s.	400
	<i>Siganus luridus</i>	126	2	59	<i>SlAm2B-1-1</i>	No	—	—	—	275
					<i>SlAm2B-1-2</i>	Yes	4	0.46	n.s.	400
					<i>ToAm2B-1</i>	Yes	2	—	—	—
	<i>Thunnus obesus</i>	20	1	48	<i>ToAm2B-1</i>	Yes	2	—	—	—
	<i>Lates niloticus</i>	30	1	52	<i>LnAm2B-2</i>	Yes	—	—	—	—
Am2B-2	<i>Siganus rivulatus</i>	137	2	57	<i>SrAm2B-2-1</i>	Yes	2	0.46	n.s.	350
					<i>SrAm2B-2-2</i>	Yes	4	0.54	n.s.	375
Am2B-3	<i>Siganus luridus</i>	126	multilocus	57	—	—	—	—	—	—
	<i>Siganus rivulatus</i>	137	0	58	—	—	—	—	—	—
	<i>Siganus luridus</i>	126	0	58	—	—	—	—	—	—
Tr1	<i>Siganus rivulatus</i>	137	1	50	<i>SrTr1</i>	Yes	2	0.19	n.s.	250
	<i>Siganus luridus</i>	126	1	50	<i>SITr1</i>	Yes	7	0.72	n.s.	300
	<i>Ethmalosa fimbriata</i>	20	multilocus	45	—	—	—	—	—	—
MhcII	<i>Siganus rivulatus</i>	20	multilocus	55	—	Yes	—	—	—	—
	<i>Siganus luridus</i>	20	0	55	—	—	—	—	—	—
	<i>Thunnus obesus</i>	20	multilocus	52	—	—	—	—	—	—
	<i>Ethmalosa fimbriata</i>	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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