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M. Hassan, C. Lemaire, Cécile Fauvelot, F. Bonhomme. Seventeen new exon-primed intron-crossing polymerase chain reaction amplifiable introns in fish. *Molecular Ecology Notes*, 2002, 10.1046/j.1471-8286 . ird-03044170

HAL Id: ird-03044170

<https://ird.hal.science/ird-03044170>

Submitted on 7 Dec 2020

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PRIMER NOTE

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

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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

Received 27 February 2002; revision received 10 April 2002; accepted 10 April 2002

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 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/µL) in a reaction mixture contained 0.3 µM of the primer and 0.4 mCi/mL of [γ 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 µL of formamide dye (95% formamide, 20 mM EDTA, 0.05% xylene cyanol and 0.05% bromophenol blue), denatured 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

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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 Ck8R	AAGAGGGTCTTGACAGGTTCTGC TTCTCCTGGATCAGACGCTCCACC
Glyceraldehyde-3-phosphate dehydrogenase	intron 2	Gpd2	Gpd2F Gpd3R	GCCATCAATGACCCCTTCATCG TTGACCTCACCCATTGCCGTGAGC
Growth hormone	intron 2	Gh2	Gh2F Gh3R	AGCGTTCTCCATTGCCGTGAGC TCTTGTGAGTTGACGCTGGTCC
	intron 5	Gh5	Gh5F Gh6R	AGGCCAATCAGGACGGAGC TGCCACTGTCAGATAAGTCTCC
Aldolase C	intron 1	AldoC1	Aldoc1F Aldoc2R	CCTGGCTGCGGACGAGTCTGAGG GGCGGTACTGTCTGCCGTCTCC
Aldolase B	intron 1	AldoB1	Aldob1-1F Aldob1-2R	GCTCCAGGAAGGGAACTCTGGC CTCGTGGAAAGAAGATGATCCC
	intron 2	AldoB2	Aldob2F Aldexon3R	TCAGGGCAATTGTCGTCGG CTGTTCCATTAGACCGC
	intron 4	AldoB4	Aldo5F Aldo3.1R	GCCAGATATGCCAGCATCTGCC GGGTTCCATCAGGCAGGATCTCTGGC
	intron 5	AldoB5	Aldo3F Aldo2R	TCC TGCTGATGGAACCC CAGGTACACATGGTGGTC
Gonadotropin-releasing hormone 3	intron 1	GnRH3-1	GnRH1F GnRH1R	AATGCACCATGCTAACAGGC CGCACCATCACTCTGCTGTTGC
	intron 2	GnRH3-2	GnRH2F GnRH2R	AGAAGTGTGGAGAGCTAGAGGC AGAGACACCACTTCTCTGTACCC
	intron 3	GnRH3-3	GnRH3F GnRH3R	GCCCCAACCAAGAGAGACTTAGACC TTCGGTCAAAATGACTGGAATCATC
Alpha amylase	intron 1	Am2B-1	Am2b1F Am2b2R	CCTTCATCTCCAGGAGGTAC TTCACCTCCCAGATCAATAAC
	intron 2	Am2B-2	Am2b2F Am2b3R	GGCGATAAGTTGTCTTACACC AGCCCTCTCCCCAGTTCTGC
	intron 3	Am2B-3	Am2b3F Am2b4R	TGGAACCGAACATTGTAAC CCCATCCAGTCATTCTGATCC
Alpha tropomyosin	intron 1	Tr1	Tr1F Tr1R	AGGGAACAGAGGATGAGCTGGAC TCTCAGCTTCCAGCTTGGTG
Major histocompatibility complex class II antigen	intron 1	MhcII	Mhc1F Mhc2R	ACTCTAACTGGAGTACATGC CAGGAGATCTCTCTCCAGGCC

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	ToCk7	Yes	3	—	—	350
	<i>Lates niloticus</i> (Centropomidae)	30	1	52	LnCk7	Yes	—	—	—	—
	<i>Chaetodon citrinellus</i> (Chaetodontidae)	50	multilocus	54	—	—	—	—	—	—
	<i>Chaetodon quadrimaculatus</i> (=)	50	multilocus	54	—	—	—	—	—	—
	<i>Forcipiger flavissimus</i> (=)	50	multilocus	54	—	—	—	—	—	—
Gpd2	<i>Siganus rivulatus</i>	137	2	50	SrGPD2-1	Yes	6	0.55	n.s.	250
					SrGPD2-2	Yes	5	0.32	n.s.	360
	<i>Siganus luridus</i>	126	1	53	SlGPD2	Yes	3	0.17	n.s.	175
	<i>Ethmalosa fimbriata</i> (Clupeidae)	170	2	52	EfGPD2-1	Yes	14	0.80	n.s.	200
					EfGPD2-2	No	—	—	—	—
	<i>Chaetodon citrinellus</i>	50	1	54	CcGPD2	No	—	—	—	350
	<i>Chaetodon quadrimaculatus</i>	60	1	54	CqGPD2	No	—	—	—	350
	<i>Forcipiger flavissimus</i>	60	1	54	FfGPD2	No	—	—	—	350
	<i>Dascyllus aruanus</i> (Pomacentridae)	60	1	54	DaGPD2	No	—	—	—	350
	<i>Pomacentrus pavo</i> (=)	60	1	54	PpGPD2	No	—	—	—	350
GH2	<i>Plectroglyphidodon dickii</i> (=)	40	1	54	PdGPD2	No	—	—	—	350
	<i>Siganus rivulatus</i>	137	2	54	SrGH2-1	Yes	2	0.23	n.s.	250
					SrGH2-2	Yes	4	0.61	n.s.	500
	<i>Siganus luridus</i>	126	0	54	—	—	—	—	—	—
	<i>Lates niloticus</i>	30	1	54	LnGH2	Yes	—	—	—	—
	<i>Chaetodon citrinellus</i>	50	1	54	CcGH2	No	—	—	—	150
	<i>Chaetodon quadrimaculatus</i>	60	1	54	CqGH2	No	—	—	—	150
	<i>Forcipiger flavissimus</i>	234	1	54	FfGH2	Yes	12	0.72	n.s.	250
	<i>Dascyllus aruanus</i>	60	multilocus	54	—	—	—	—	—	—
	<i>Pomacentrus pavo</i>	60	multilocus	54	—	—	—	—	—	—
GH5	<i>Plectroglyphidodon dickii</i>	40	multilocus	54	—	—	—	—	—	—
	<i>Siganus rivulatus</i>	137	1	52	SrGH5	No	—	—	—	350
	<i>Siganus luridus</i>	126	1	52	SlGH5	Yes	4	0.34	n.s.	350
	<i>Thunnus obesus</i>	20	1	52	ToGH5	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	PpGH5	Yes	2	0.31	n.s.	300
	<i>Plectroglyphidodon dickii</i>	79	2	54	PdGH5-1	No	—	—	—	300

DATA FILE 2

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
	<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
AldoB1‡					<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
	<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
AldoB2	<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
AldoB4	<i>Dascyllus aruanus</i>	250	1	54	<i>DaAldoB2</i>	Yes	3	0.15	n.s.	200
	<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	<i>CcAldoB5</i>	No	—	—	—	200
	<i>Chaetodon quadrimaculatus</i>	10	1	54	<i>CqAldoB5</i>	No	—	—	—	200
	<i>Forcipiger flavissimus</i>	20	1	54	<i>FfAldoB5</i>	No	—	—	—	200
GnRH3-1	<i>Siganus rivulatus</i>	136	2	48	<i>SrGnRH3-1-1</i>	Yes	2	0.41	n.s.	250
					<i>SrGnRH3-1-2</i>	Yes	9	0.77	n.s.	400
	<i>Siganus luridus</i>	126	3	48	<i>SIgnRH3-1-1</i>	Yes	5	0.53	n.s.	275
					<i>SIgnRH3-1-2</i>	Yes	4	0.15	n.s.	325
					<i>SIgnRH3-1-3</i>	Yes	8	0.74	n.s.	375
	<i>Chaetodon citrinellus</i>	198	1	54	<i>CcGnRH3-1</i>	Yes	8	0.67	0.43	280
	<i>Chaetodon quadrimaculatus</i>	233	1	54	<i>CqGnRH3-1</i>	Yes	6	0.61	n.s.	280
GnRH3-2	<i>Forcipiger flavissimus</i>	50	1	54	<i>FfGnRH3-1</i>	No	—	—	—	280
	<i>Dascyllus aruanus</i>	20	1	54	<i>DaGnRH3-1</i>	No	—	—	—	250
	<i>Pomacentrus pavo</i>	20	1	54	<i>PpGnRH3-1</i>	No	—	—	—	250
	<i>Plectroglyphidodon dickii</i>	20	0	54	—	—	—	—	—	—
	<i>Siganus rivulatus</i>	137	1	56	<i>SrGnRH3-2</i>	No	—	—	—	230
	<i>Siganus luridus</i>	126	1	56	<i>SIgnRH3-2</i>	No	—	—	—	230
	<i>Decapterus russelli</i>	458	1	52	<i>DrGnRH3-2</i>	Yes	7	0.71	n.s.	300
GnRH3-3	<i>Chaetodon citrinellus</i>	198	1	54	<i>CcGnRH3-2</i>	Yes	4	0.52	0.21	280
	<i>Chaetodon quadrimaculatus</i>	233	1	54	<i>CqGnRH3-2</i>	Yes	4	0.53	n.s.	280
	<i>Forcipiger flavissimus</i>	234	1	54	<i>FfGnRH3-2</i>	Yes	7	0.36	n.s.	300
	<i>Dascyllus aruanus</i>	20	1	54	<i>DaGnRH3-2</i>	No	—	—	—	300
	<i>Pomacentrus pavo</i>	20	1	54	<i>PpGnRH3-2</i>	No	—	—	—	300
	<i>Plectroglyphidodon dickii</i>	79	1	50	<i>PdGnRH3-2</i>	Yes	13	0.69	n.s.	300
	<i>Siganus rivulatus</i>	137	1	52	<i>SrGnRH3-3</i>	Yes	4	0.40	n.s.	325
	<i>Siganus luridus</i>	126	1	53	<i>SIgnRH3-3</i>	Yes	4	0.48	n.s.	375
	<i>Thunnus obesus</i>	20	1	52	<i>ToGnRH3-3</i>	No	—	—	—	—
	<i>Chaetodon citrinellus</i>	50	1	54	<i>CcGnRH3-3</i>	No	—	—	—	400
	<i>Chaetodon quadrimaculatus</i>	550	1	54	<i>CqGnRH3-3</i>	No	—	—	—	400
	<i>Forcipiger flavissimus</i>	50	1	54	<i>FfGnRH3-3</i>	No	—	—	—	400
	<i>Dascyllus aruanus</i>	20	1	54	<i>DaGnRH3-3</i>	Yes	—	—	—	400
	<i>Pomacentrus pavo</i>	20	1	54	<i>PpGnRH3-3</i>	No	—	—	—	400
	<i>Plectroglyphidodon dickii</i>	20	1	54	<i>PdGnRH3-3</i>	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
Am2B-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	—	—	—	—
	<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>SlTr1</i>	Yes	7	0.72	n.s.	300
MhcII	<i>Ethmalosa fimbriata</i>	20	multilocus	45	—	—	—	—	—	—
	<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.

Acknowledgements

We are grateful to N. Bierne, J.D. Durand, A. Rohfritsch, and J.F. Agnese for their help.

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