Molecular characterization of lipoprotein lipase, hepatic lipase and pancreatic lipase genes and effects of fasting and refeeding on their gene expression in red sea bream *Pagrus major*

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Running title: Nutritional regulation of lipase genes in fish.

Abstract

To investigate the nutritional regulation of lipid metabolism in fish, molecular characterization of lipases was conducted in red sea bream *Pagrus major*, and the effects of fasting and refeeding on their gene expression was examined. Taken together with our previous study (LPL2: Oku et al. 2002, Comp. Biochem. Physiol. 131B, 775-783), four lipase genes were identified and characterized as lipoprotein lipase (LPL), hepatic lipase (HL) and pancreatic lipase (PL). These four lipase genes, termed LPL1, LPL2, HL and PL, share a high degree of similarity with each other. Both LPL1 and LPL2 genes were expressed in various tissues including adipose tissue, gill, heart and hepatopancreas. HL gene was exclusively expressed in hepatopancreas. PL gene expression was detected in hepatopancreas and adipose tissue. Red sea bream LPL1 and LPL2 gene expression levels in hepatopancreas were increased during 48 hr fasting and decreased after refeeding, whereas no significant change in the expression levels of LPL1 and LPL2 was observed in adipose tissue, indicating that LPL1 and LPL2 gene expression is regulated in a tissue-specific manner in response to the nutritional state of fish. HL and PL gene expression was not affected by fasting and refeeding. The results of this study suggested that LPL, HL and PL gene expression is under different

regulatory mechanisms in red sea bream with respect to the tissue-specificities and their nutritional regulation.

Key words: teleost, red sea bream, *Pagrus major*, LPL, HL, PL, nutritional regulation, molecular cloning

1. Introduction

Dietary triglycerides (TGs) are hydrolyzed to a monoglyceride and two free fatty acids (FFAs) for absorption in the digestive tract. The absorbed FFAs are re-esterified to TGs and incorporated into plasma lipoproteins. TGs present in plasma lipoproteins are hydrolyzed by various kinds of endogenous lipases to provide FFAs for storage in adipose tissue or for oxidation in other tissues.

Lipoprotein lipase (LPL), hepatic lipase (HL), pancreatic lipase (PL) and endothelial lipase (EL) belong to the lipase gene family (Hide et al., 1992; Wong and Schotz, 2002). These lipases share high degrees of structural similarity with each other but play different roles in lipid metabolism. LPL participates in the cellular uptake of plasma chylomicron and very low density lipoprotein in various kinds of extrahepatic tissues (Nilsson-Ehle et al., 1980; Mead et al., 2002). HL is primarily synthesized in liver and involved in chylomicron-remnant and high density lipoprotein metabolism (Santamarina-Fojo et al., 1998). PL is a digestive enzyme which plays a central role in dietary TGs digestion. Endothelial lipase shows a phospholipase A1-like activity and participates in high density lipoprotein metabolism (Hirata et al., 1999; Jaye et al., 1999). The activities of these lipases are regulated in part at a transcriptional level in response to the physiological state of animals (Semb and Olivecrona, 1989; Staels et al.,

1990; Wicker and Puigserver, 1990; Benhizia et al., 1994; Bonnet et al., 1999; Ruge et al., 2004).

In fish, studies on the regulation of lipase activity and gene expression have been conducted extensively in LPL. The changes in LPL and salt-resistant lipase (HL) activity and the developmental regulation of LPL gene during oogenesis and embryogenesis have been reported in rainbow trout (Black and Skinner, 1987; Kwon et al., 2001). Rainbow trout lipoprotein lipase and salt-resistant lipase activities increase in the ovaries but decrease in the adipose tissue of female trout in the months leading up to spawning (Black and Skinner, 1987). Rainbow trout LPL gene is expressed in ovary during oogenesis but not in embryos (Kwon et al., 2001). Furthermore, nutritional regulation of LPL has been studied in several species. In rainbow trout, the LPL activity in adipose tissue is increased during postprandial period and decreased with fasting, and the activity is regulated by insulin (Albalat et al., 2006). In gilthead sea bream, the effects of season and dietary composition on the LPL gene expression in fat storing tissues including adipose tissue and liver (hepatopancreas) have been studied (Saera-Vila et al., 2005). In our previous studies, the effects of the feeding schedule, dietary lipid level and dietary fatty acid supplementation were investigated in red sea bream (Liang et al., 2002a,b). These studies have indicated that fish LPL is regulated in

response to the physiological state of fish.

In the present study, to extend the previous research on fish lipases, the nutritional regulation of LPL, HL and PL was investigated in a marine teleost, red sea bream *Pagrus major*. We first cloned red sea bream LPL, HL and PL genes and determined the primary structures and tissue distributions. Subsequently, the effects of fasting and refeeding on the gene expression levels were examined.

2. Materials and Methods

2.1 Fish

The experimental fish (*P. major*) were purchased from a local hatchery station (Nissin Marine Tech, Aichi, Japan) and grown in our institute (Natl. Res. Inst. Aquaculture, Minami-ise, Mie, Japan) by feeding with a commercial diet (Higashimaru Co. Ltd, Kagoshima, Japan) under natural photoperiod and temperature conditions. The body weights of fish used for the cloning, RACE and RT-PCR were 290g, 80g, and 166g, respectively. For the feeding experiments, fish weighing approximately 40g were used. The mature ovary and testis of adult fish, used for RT-PCR, were provided by Dr. Koichiro Gen (Natl. Res. Inst. Aquaculture, Minami-ise, Mie, Japan).

2.2 Partial cloning of lipase genes

The total RNA was extracted from hepatopancreas of red sea bream by the method of Chomczynski and Sacchi (1987) and the 1 μg of total RNA was used for the cDNA synthesis. The cDNA was synthesized in 15 μl reaction volume with oligo-dT primer and First-Strand cDNA Synthesis Kit (Amersham, Buckinghamshire, UK) according to the manufacturer's instructions.

The 0.5 μl of synthesized cDNA was subjected to the polymerase chain reaction (PCR). The partial fragments of lipase genes were amplified by PCR with three sets of degenerated primers (LPL01Fb and LPL06Ra for LP1, LPL02Fa and LPL06Ra for LP2, and RSPL2S and RSPL2A for LP3). The PCR was carried out in 25 μl of reaction volume containing 0.5U Taq polymerase (Takara, Tokyo, Japan), 200 μM of dNTP, 10mM Tris-HCl pH 8.3, 50mM KCl, 1.5mM MgCl and 20 pmoles of oligonucleotide primers. Forty cycles of amplification were carried out for each PCR. Each cycle consisted of a denaturation step at 94ºC for 0.5 min, an annealing step at 50°C for 1 min and an extension step at 72ºC for 1 min. The final extension step was followed by a 3 min extension reaction at 72°C. The amplified fragments (LP1, LP2 and LP3) were cloned into a plasmid vector (pCR2.1, Invitrogen, Carlsbad, CA, USA) and sequenced. The cloning and sequence determination were carried out as described previously (Oku

et al., 2002). In addition, a cDNA fragment containing red sea bream β actin (GenBank accession no. AB252854) was amplified by PCR with the oligonucleotides BACT01F and BACT02R (Table 1), and the PCR, cloning and sequence determination of β actin fragment were carried out as described above. The sequences of oligonucleotides used in this study are detailed in Table 1.

2.3 3'and 5' rapid amplification of cDNA end (RACE)

The complete nucleotide sequences of the cloned lipase genes were determined by the 3' and 5' RACE method. For 3' RACE, the cDNA synthesized in *2.2 Partial cloning of lipase genes* was used as the template. The cDNA template for 5'RACE was prepared from mRNA extracted from hepatopancreas with Quick Prep Micro mRNA Purification Kit (Amersham). The cDNA with an adapter sequence at the 5' end was synthesized with SMART cDNA Library Construction Kit and SMART IV oligonucleotide (Clontech, Palo Alto, CA, USA).

The 3' and 5' RACE fragments were amplified by the first and nested PCR. The first PCR was carried out with universal (Not I-01 and 5'R-A3 for 3' and 5' RACE) and gene specific (PMHL05F and PMHL04R for 3' and 5' RACE of LP1, PMPL01F and PMPL04R for 3' and 5' RACE of LP2, and PanL01F and PanL03R for 3' and 5' RACE

of LP3) primers. The PCR experiments were performed as described in *2.2 Partial cloning of lipase genes*. After the first PCR, the reaction mixture was diluted to 1:100 and 0.5 μl of the diluted products were subject to the nested PCR in 25 μl reaction volume. The nested PCR were carried out with universal (Not I-02 and 5'R-B3 for 3' and 5' RACE) and gene specific (PMHL06F and PMHL03R for 3' and 5' RACE of LP1, PMPL03R for 5' RACE of LP2, and PanL05F for 3' RACE of LP3) primers under the same conditions as in the first PCR except the annealing temperature was 55ºC. The 3'RACE for LP2 and 5'RACE for LP3 did not require the nested PCR. The amplified fragments were cloned and sequenced as described previously (Oku et al. 2002). The oligonucleotide sequences used for 3' and 5' RACE are given in Table 1.

2.4 Phylogenetic analysis

Phylogenetic analysis of amino acid sequences was carried out using the neighbor-joining method. Analysis with 1,000 bootstrap replicates was conducted using the software DNASIS Pro version 2.06 (Hitachi, Tokyo, Japan).

2.5 RT-PCR

Visceral adipose tissue, gill, heart, hepatopancreas, immature gonad and dorsal white

muscle of young fish (body weight 166g), and mature ovary and testis of adult fish (body weight 1.8kg) were subjected to the RT-PCR. Total RNA was prepared by the method of Chomczynski and Sacchi (1987). One μg of the total RNA was used for the cDNA synthesis. Single strand cDNA was synthesized in 15μl reaction volume with random hexamer primer and First-Strand cDNA Synthesis kit (Amersham). 0.5 μl of the reaction mixture was subject to PCR in 25 μl reaction volume. Thirty cycles of amplifications were carried out for each PCR. Each cycle consisted of a denaturation step at 94ºC for 0.5 min, an annealing step at 55ºC for 0.5 min and an extension step at 72ºC for 0.5 min. The final extension step was followed by a 3 min extension at 72°C. 5 μl aliquots of the products were electrophoresed through 3% agarose gel containing ethidium bromide. The nucleotide sequence primers for the specific amplification of each gene are shown in Table 1.

2.6 Feeding procedure

For the feeding experiments, 50 fish (approx. 40g in body weight) were kept in a 1000 L indoor tank in our wet laboratory (Natl. Res. Inst. Aquaculture, Minami-ise, Mie, Japan). The fish were acclimatized for 6 weeks under natural photoperiod and temperature conditions (September-November, 22-25°C), and were fed a commercial

diet (crude fat 11.8% and crude protein 58%, Higashimaru Co. Ltd, Kagoshima, Japan) every 24 hr at 9:00 am. During the acclimation period, the fish grew to 53-80g.

After the acclimation, fish were not fed for 48 hr then refed. Four fish were randomly sampled at 0, 6, 12, 24, and 48 hr after the start of fasting and at 6 hr after the refeeding following the 48hr fasting. At each sampling, the visceral adipose tissue and hepatopancreas were dissected out and stored in -80°C until use. RNA extraction and cDNA synthesis were carried out as described in *2.5 RT-PCR*.

2.7 Real time PCR

The measurements of the gene transcripts were made by means of real time PCR with iCycler iQ real-time PCR detection system (BioRad, Hercules, CA, USA). Aliquots of 20 μl of the reaction mixture were prepared with iQ SYBR Green Supermix (BioRad) and the specific primers (PMHL07F and PMHL10R for LP1, PMPL12F and PMPL10R for LP2, PMPanL07F and PMPanL11R for LP3, and PMBACT01F and PMBACT02R for β actin) at a final concentration of 1 μM. The real time PCR protocol consisted of 3 min at 95ºC followed by 40 cycles of 0.5 min at 95°C, 0.5 min at 50ºC and 0.5 min at 72°C. Reactions were carried out in triplicate and each transcript level was calculated as copies / μg total RNA. The experimental levels of lipases were further normalized to

beta actin and shown as the ratio between the quantity of the target gene and the β actin gene. The oligonucleotide sequences used in the real-time PCR are given in Table 1.

2.8 Statistic analyses

Statistical analyses of differences among treatment means were carried out by ANOVA and Fisher's Protected Least Significant Difference (StatView 4.51, Abacus Concepts, Berkeley, CA, USA). Differences were considered significant if $P<0.05$.

3. Results

3.1 Cloning and sequence analysis

Three kinds of lipase genes (LP1, LP2 and LP3) were identified in red sea bream by cloning and sequence analyses and the entire nucleotide sequences were determined by the 3' and 5' RACE method. The phylogenetic analysis revealed that LP1, LP2 and LP3 of red sea bream correspond to LPL, HL and PL of mammals, respectively (Fig. 1). The LPL-like gene (GenBank accession no. AB054062) identified in our previous study (Oku et al., 2002) phylogenetically clustered closer to LPL than other members of lipase gene family (Fig. 1). Therefore, we have tentatively termed it red sea bream LPL2 whereas LP1, LP2 and LP3 were termed red sea bream LPL1, HL and PL, respectively.

The obtained 2,256 bp sequence of LPL1 contained 1,569 bp of an open reading frame (ORF) that is translated into 523 amino acids, with 142 bp of 5' and 542 bp of 3' untranslated region (Fig. 2). The sequence had a polyadenylation signal-like sequence (ATAAA) in place of the common signal sequences (AATAAA) (Fig. 2). The 2,261 bp sequence of HL contained 1,491 bp of an ORF that is translated into 497 amino acids, with 170 bp of 5' and 597 bp of 3' untranslated region (Fig. 3). Furthermore the 1,500 bp sequence of PL contained 1,356 bp of an ORF that is translated into 452 amino acids, with 72 bp of 5' and 69 bp of 3' untranslated region (Fig. 4).

The red sea bream LPL1, LPL2, HL and PL share a high degree of similarity with each other (18%~47% amino acid identities) with the conservation of functional domain including catalytic triads and lipid binding region (Fig. 5).

3.2 Tissue-specificities

The tissue-specificities of red sea bream LPL1, LPL2, HL and PL were determined by the RT-PCR method (Fig. 6). Red sea bream LPL1 and LPL2 were expressed in all examined tissues (adipose tissue, gill, heart, hepatopancreas, immature gonad, muscle, mature ovary and mature testis), although LPL1 gene expression was low in muscle. Red sea bream HL was exclusively expressed in hepatopancreas. The gene expression

of red sea bream PL was detected in adipose tissue and hepatopancreas (Fig. 6).

3.3 The effects of fasting and refeeding on the gene expression levels

To investigate the nutritional regulation of the lipase genes, the effects of fasting and refeeding on the gene expression levels of LPL1, LPL2, HL and PL in adipose tissue and hepatopancreas of red sea bream were analyzed. In the adipose tissue the gene expression levels of LPL1 and LPL2 were not affected by fasting and refeeding but those in the hepatopancreas were increased by fasting and decreased by refeeding. In the hepatopancreas the expression levels of LPL1 and LPL2 at 48 hr post-feeding were 3.9 fold and 3.3 fold higher, respectively in comparison with those of the 0 hr-post feeding (Fig. 7). Furthermore in the hepatopancreas the expression levels of LPL1 and LPL2 at 6 hr post-refeeding were 5.9 fold and 5.1 fold lower, respectively than those of the 0 hr post-refeeding (48 hr post-feeding) (Fig. 7). The expression levels of the LPL1 gene in adipose tissue and hepatopancreas was approximate 10 fold higher than that of the LPL2 gene (Fig. 7). During fasting, the expression level of LPL1 in adipose tissue and hepatopancreas was 30.5 - 60.7 % and 16.0 – 75.3 % of β actin, respectively, whereas that of LPL2 was 2.1 – 3.0 % and 1.3 – 4.9 %, respectively (Fig. 7). The expression levels of HL and PL genes did not change significantly during the fasting and refeeding

process (Fig. 7).

4. Discussion

In this study, we identified three lipase genes (LPL1, HL and PL) in red sea bream and determined the primary structures. Taken together with red sea bream LPL2 genes, which has been identified in our previous study (Oku et al. 2002), the tissue specific gene expression and the effects of feeding on the gene expression were investigated. The results suggest that LPL, HL and PL gene expression is under different regulatory mechanisms in red sea bream.

In red sea bream, two kinds of LPL-like genes (LPL1 and LPL2) were identified. The red sea bream LPL1 shows higher similarities to LPLs of other fish species than LPL2, and the expression levels of the LPL1 gene in adipose tissue and hepatopancreas was approximately 10 fold higher than that of the LPL2 gene (Fig. 7). These results suggest that LPL1 is the major LPL in red sea bream and that LPL2, which has been identified in our previous study (Oku et al. 2002), is a variant of LPL.

The expression of red sea bream LPL1 and LPL2 was detected in all the examined tissues (Fig. 6), suggesting that these two lipases are involved in lipid metabolism in various tissues. As reported in rainbow trout and gilthead sea bream (Lindberg and

Olivecrona, 2002; Saera-Vila et al., 2005), unlike mammalian LPL, fish LPL gene is expressed in the liver (hepatopancreas). The tissue specificities of red sea bream HL and PL were similar but differ in several respects from their mammalian counterparts. The expression of HL gene in ovary and testis, which has been reported in rat and human (Verhoeven and Jansen 1994), was not observed in red sea bream (Fig. 6). The expression of HL in ovary and testis of red sea bream may be lower in comparison with that of mammals. Red sea bream PL gene expression was detected in not only hepatopancreas but also in adipose tissue (Fig. 6). The observation in the early development of red sea bream has indicated that visceral adipose tissue develops around the pancreas tissue (Umino et al. 1996). The PL mRNA in red sea bream adipose tissue detected by RT-PCR (Fig. 6) may be derived from pancreatic cells distributed in fish adipose tissues.

During the fasting and refeeding process, red sea bream LPL1 and LPL2 were regulated in a tissue-specific manner. In the hepatopancreas, the LPL1 and LPL2 gene expression levels increased gradually between 0 hr and 48 hr post-feeding, whereas no significant change was observed in adipose tissue. In gilthead sea bream, the expression of LPL gene in the adipose tissue and the hepatopancreas is regulated coordinately in response to the dietary composition (Saera-Vila et al., 2005). Hepatic LPL expression is

up-regulated whereas an opposite trend is found in the adipose tissue in gilthead sea bream which were fed a plant-protein based diet (Saera-Vila et al., 2005). However, in red sea bream, hepatic and adipose LPL gene expression was not regulated coordinately during fasting and refeeding process (Fig. 7). The increase in hepatic LPL gene expression level at 48 hr post-feeding (Fig. 7) suggests that lipid utilization increases in the hepatopancreas during fasting. In adipose tissue, LPL activity is regulated for fat storage in response to the nutritional state of animals (Eckel, 1987) although in this study the adipose LPL1 and LPL2 gene expression was not affected by fasting and refeeding in red sea bream. The adipose LPL gene in red sea bream may be regulated mainly at translational and post translational levels. Unlike the present study, our previous study found that the LPL2 gene expression level in red sea bream adipose tissue was down-regulated by 48 hr fasting (Liang et al. 2002a), but no significant change was observed in the present study (Fig. 7). The differences in the experimental conditions between our previous and present studies were the size of experimental fish (152-154g vs 53-80g), the length and season of the rearing period (Feb-Jun, 14 weeks vs Sep-Nov, 6 weeks), and water temperature (15-21°C vs 22-25°C). These differences in experimental conditions may affect the results since LPL gene expression is mediated not only by the nutritional state but also by other conditions including the season and

reproduction cycles (Black and Skinner, 1987; Saera-Vila et al., 2005). Mammalian HL and PL activities are affected by the feeding conditions (Crass et al., 1987; Galan et al., 1994), but red sea bream HL and PL gene expression levels did not show a significant change during fasting and refeeding process (Fig. 7). As reported in mammals, the activities of HL and PL are regulated in response to the nutritional state of animals not only at transcriptional but also at post-transcriptional levels (Wicker and Puigserver 1990; Warren et al. 1991; Birk and Brannon 2004). Translational and post-translational regulation may be more important in nutritional regulation of HL and PL than transcriptional regulation in red sea bream.

In this study, we could not identify the EL gene in red sea bream. EL, which is expressed in liver, lung, kidney and placenta, shows phospholipase A1-like activity and modulates high density lipoprotein metabolism (Jaye et al., 1999; Hirata et al., 1999). For a better understanding of lipid metabolism in fish, the EL gene should be characterized in this species.

In summary, taken together with our previous study, four kinds of lipase genes (LPL1, LPL2, HL and PL) were identified in red sea bream. These genes share a sequence similarity but have distinct tissue specificities. During the fasting and refeeding process, LPL1 and LPL2 genes were regulated in a tissue-specific manner whereas HL and PL

genes showed no significant change in the expression levels in adipose tissue and hepatopancreas, suggesting that the LPL, HL and PL gene expression is under different regulatory mechanisms in red sea bream with respect to the tissue-specificities and their nutritional regulation. We anticipate that the results of this study will facilitate further investigation of lipid metabolism in fish.

Acknowledgements

The authors would like to thank Ms. Izumi Okai for her helpful assistance and to Dr. Koichiro Gen for kindly providing the mature ovary and testis of adult fish. This work was financially supported in part by the Biodesign research program of Fisheries Research Agency of Japan.

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Figure legends

Figure 1.

Phylogenetic analysis of LPL, EL, HL and PL.

The phylogenetic tree was constructed using DNASIS Pro version 2.06 software (Hitachi, Tokyo, Japan). The sequences of red sea bream lipases are underlined. The GenBank accession numbers used in this analysis were as follows; red sea bream LPL1(AB243791), red sea bream LPL2(AB054062), red sea bream HL(AB252855), red sea bream PL(AB252856), mouse LPL(NM008509), human LPL(NM000237), bovine LPL(XM616349), pig LPL(X62984), guinea pig LPL(M15483), chicken LPL(NM205282), gilthead sea bream LPL(AY495672), zebrafish LPL(BC064296),

rainbow trout LPL(AJ224693), human EL(NM006033), mouse EL(NM010720), zebrafish EL(BC044146), human HL(NM000236), mouse HL(NM008280), zebrafish HL(NM000936), human PL(000936), mouse PL(NM026925), horse PL(X66218), and dog PL(M35302).

Figure 2.

The nucleotide sequence of red sea bream LPL1 (LP1).

The polyadenylation signal is underlined. The sequence of red sea bream LPL1 was submitted under the GenBank accession number AB243791.

Figure 3.

The nucleotide sequence of red sea bream HL (LP2).

The polyadenylation signal is underlined. The sequence of red sea bream HL was submitted under the GenBank accession number AB252855.

Figure 4.

The nucleotide sequence of red sea bream PL (LP3).

The polyadenylation signal is underlined. The sequence of red sea bream PL was

submitted under the GenBank accession number AB252856.

Figure 5.

Comparison of the deduced amino acid sequences of red sea bream LPL1, LPL2, HL and PL.

Gaps (-) were introduced to maximize sequence identities. Catalytic triads are boxed and lipid binding region is underlined. Asterisks (*) indicate conserved amino acid residues.

Figure 6.

Tissue-specificities of red sea bream LPL1, LPL2, HL and PL.

The expression of each gene was detected by RT-PCR. The RNA samples were extracted from 1) adipose tissue, 2) gill, 3) heart, 4) hepatopancreas, 5) gonad, 6) muscle, 7) testis, and 8) ovary. The cDNA samples were prepared with reverse transcription (RT+) and negative controls for contamination of genomic DNA were run without reverse transcription (RT-).

Figure 7.

Changes in the expression levels of red sea bream LPL1, LPL2, HL and PL in adipose tissue and hepatopancreas during 48 hr fasting and the refeeding process.

The expression levels are indicated as % β actin. The bars represent standard error. The arrow heads indicate the time of feeding (0 hr) or refeeding (48 hr). Means (n=4) not sharing a common superscripts are significantly different ($P<0.05$).

Table.1

Oligonucleotide sequences used in this study.

Table 1.

2.2 Partial cloning of lipase genes

(Oligo-dT, Amersham)	5' -AACTGGAATTCGCGGCCGCAGGAA(T)$_{18}$		
	LPL01Fb	5'- TCCAAAACCTTGTGGTCATACACGGGTGG-3'	(res.46-55 in human LPL numbering)
	LPL02Fa	5'- GATAGCAACGTIAT(TCA)GTIGTIGA(TC)TGG-3'	(res.77-86 in human LPL numbering)
	LPL06Ra	5'-GGTGTGAAGGACGTCGACAAAGTCAGCGTCGTC-3'	(res.206-216 in human LPL numbering)
	RSPL2S	5'- TTAACTGCATAGTTGTCGA(TC)TGG-3'	(res.100-107 in human PL numbering)
	RSPL2A	5'-AAACCTGTCTGCATAATGGCCCAT-3'	(res.308-315 in human PL numbering)
	BACT01F	5'-CTGCGTCGCCTTGGACTTCGAGCA(AG)GA(GA)ATG-3'	(res.220-227 in human beta actin numbering)
	BACT02R	5'-CTCGTCGTACTCCTGCTTGGA(GAT)ATCCACAT-3'	(res.355-362 in human beta actin numbering)

2.3 3' and 5' RACE

3'RACE

Antisense	(First PCR)	NotI-01	5'-AACTGGAAGAATTCGCG-3	
	(Nested PCR)	NotI-02	5'-AATTCGCGGCCGCAGGAA-3	
Sense (gene specific)				
LP1 (LPL1)	(First PCR)	PMHL05F	5'-TCCCACCTTCGAGAACG-3'	(res.213-219)
	(Nested PCR)	PMHL06F	5'-CAGACAACCAGAACACC-3'	(res.219-224)
LP2 (HL)		PMPL01F	5'-GTCAACGTGGTGATTACA-3'	(res.107-112)
LP3 (PL)	(First PCR)	PanL01F	5'-CGCCAGGGTTGTGGC-3'	(res.121-126)
	(Nested PCR)	PanL05F	5'-AACTACAAGCAGAAGGC-3'	(res.139-144)

5'RACE

5' Adaptor (SMART IV, Clontech)	5'-AAGCAGTGGTATCAACGCAGAGTGGCCATTACGGCCGGG-3'			
Sense	(First PCR)	5'R-A3	5'-GCAGTGGTATCAACGCAGAGTGGCCA-3'	
	(Nested PCR)	5'R-B3	5'-GGTATCAACGCAGAGTGGCCATTACG-3'	
Antisense (gene specific)				
LP1 (LPL1)	(First PCR)	PMHL04R	5'-GTGGCCCACGGGTCT-3'	(res.252-256)
	(Nested PCR)	PMHL03R	5'-GGGGAGCCTCTGGTGTT-3 '	(res.239-244)
LP2 (HL)	(First PCR)	PMPL04R	5'-TGAGGCCCATACGTTCAT-3'	(res.217-223)
	(Nested PCR)	PMPL03R	5'-CAGTCTATCCGTGGGAG-3'	(res.196-201)
LP3 (PL)		PanL03R	5'-AGCCTTGGGGTTTAACCAT-3'	(res.273-279)

2.5 RT-PCR and 2.7 Real time PCR

LP1 (LPL1)	PMHL07F	5'-CTCAAGACCCGCGAGAT-3'	(res.360-365)
	PMHL10R	5'-AAGCGTCGCTCTGACC-3'	(res.519-524)
LP2 (HL)	PMPL12F	5'-ACAGTCCCTGAAACAATTTG-3'	(res.380-354)
	PMPL10R	5'-GACTAAGGCTCCCTCAC-3'	(res.494-499)
LP3 (PL)	PMPanL07F	5'-GCTGGCCCAATCCAGG-3'	(res.349-354)
	PMPanL11R	5'-TTAAACTTGGCATGGTAGTAC-3'	(res.447-453)
(LPL2)	PMLPL18F	5'-ATTCATTCCTGCTGGTGAC-3'	(res.406-412)
	PMLPL05R	5'-TCAGTGCTTCTCCAGAGTTAC-3'	(res.506-512)
Beta actin	PMBACT01F	5'-GGCACTGCTGCCTCCTC-3'	(res.228-233 in human beta actin numbering)
	PMBACT02R	5'-GCCAGGATGGAGCCTCC-3'	(res.342-347 in human beta actin numbering)

Fig.1

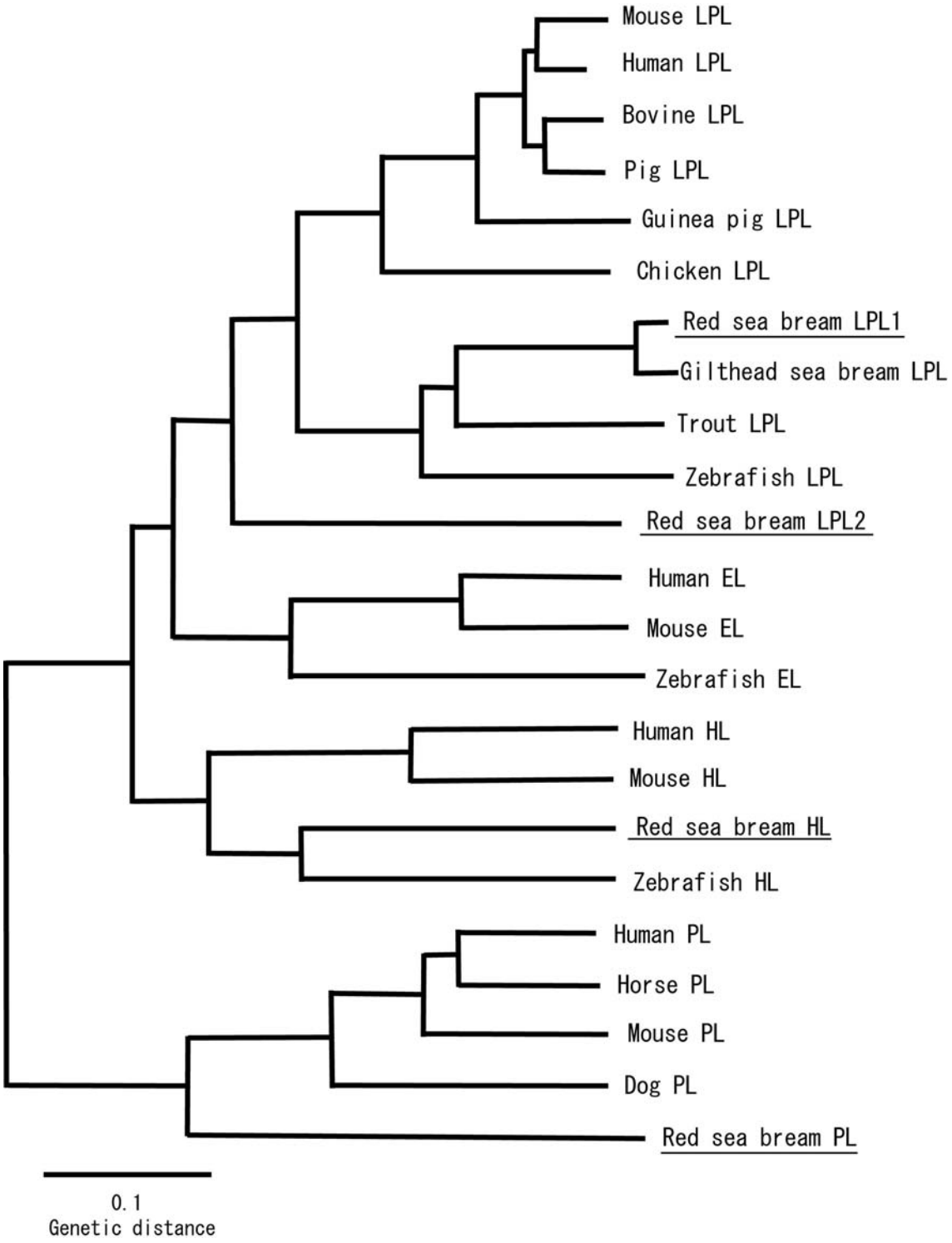
Mouse LPL
Human LPL
Bovine LPL
Pig LPL
Guinea pig LPL
Chicken LPL
Red sea bream LPL1
Gilthead sea bream LPL
Trout LPL
Zebrafish LPL
Red sea bream LPL2
Human EL
Mouse EL
Zebrafish EL
Human HL
Mouse HL
Red sea bream HL
Zebrafish HL
Human PL
Horse PL
Mouse PL
Dog PL
Red sea bream PL
0.1
Genetic distance

Fig.2

Red sea bream LPL1(LP1)

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5' ACTATAAGAAACTTTACTCCCAAACCGCAAGTCCACACCACCTTCTGACTGAAAACACACGATTGGATACACACCCGACATCCAGAGAGCTTTTTCTTTTTTGATTCAGGATACTTT
AAGAAAAAAAAACTCCTCAATAAT

ATG GGA AAG GAA AAT ATC AGT TTT TTG ACA GTT TGG ATA ATT TTG GGA AAA ATC TTT GCA ACT TTT TCT TCT GAC CCT GAA CCC ACC ACG     90
 M  G  K  E  N  I  S  F  L  T  V  W  I  I  L  G  K  I  F  A  T  F  S  S  D  P  E  P  T  T     30

ACC AGC AGC AGC AGC AGC AGC AGC ACC GTG TTC GTA AAC ACC ACC ATC ACT GCT ACT CCC CTG CCC ACC ACT ACT GAA TGG ATC ACA GAC    180
 T  S  S  S  S  S  S  S  T  V  F  V  N  T  T  I  T  A  T  P  L  P  T  T  T  E  W  I  T  D     60

TAC ACT GAC ATT GTG TCC AAG TTC TCC TTG CGC ACA GCC GAC ATC CCA GAT GAT GAC ATG TGC TAC ATT GTC GCC GGC AGT CCG GAC ACC    270
 Y  T  D  I  V  S  K  F  S  L  R  T  A  D  I  P  D  D  D  M  C  Y  I  V  A  G  S  P  D  T     90

ATC GAA GAC TGT GAA TTC AAC CCA GAA ACA CAA ACT TTC ATC GTG ATA CAT GGC TGG ACG GTA ACA GGG ATG TTC GAG AGC TGG GTG CCC    360
 I  E  D  C  E  F  N  P  E  T  Q  T  F  I  V  I  H  G  W  T  V  T  G  M  F  E  S  W  V  P    120

AAG CTG GTG TCG GCC CTC TAC GAG CGA GAG CCG AGT GCC AAC GTG ATT GTG GTG GAC TGG CTG ACC CGC GCC AAC CAG CAC TAC CCC ACA    450
 K  L  V  S  A  L  Y  E  R  E  P  S  A  N  V  I  V  V  D  W  L  T  R  A  N  Q  H  Y  P  T    150

TCT GCA GCC TAC ACC AAA CTA GTG GGC CGG GAT GTT GCT AAG TTT GTG ACC TGG ATC CAA AAA GAG CTG CAT TTG CCC TGG GAC AGG ATT    540
 S  A  A  Y  T  K  L  V  G  R  D  V  A  K  F  V  T  W  I  Q  K  E  L  H  L  P  W  D  R  I    180

CAT CTG CTG GGT TAC AGT TTG GGA GCA CAT GTG GCC GGG ATC GCG GGA GAC CTC ACT GAG CAT AAA ATC AGC AGG ATC ACA GGT CTG GAT    630
 H  L  L  G  Y  S  L  G  A  H  V  A  G  I  A  G  D  L  T  E  H  K  I  S  R  I  T  G  L  D    210

CCT GCT GGT CCC ACC TTC GAG AAC GCA GAC AAC CAG AAC ACC CTG TCC CCA GAC GAC GCC CAG TTT GTG GAC GTG CTG CAC ACC AAC ACC    720
 P  A  G  P  T  F  E  N  A  D  N  Q  N  T  L  S  P  D  D  A  Q  F  V  D  V  L  H  T  N  T    240

AGA GGC TCC CCG GAC CGC AGC ATT GGC ATC CAG AGA CCC GTG GGC CAC ATC GAC ATC TAC CCC AAC GGA GGC ACT TTC CAG CCA GGC TGC    810
 R  G  S  P  D  R  S  I  G  I  Q  R  P  V  G  H  I  D  I  Y  P  N  G  G  T  F  Q  P  G  C    270

GAC ATC CAG AGC ACA CTG CTC GGG ATC GCG TTA GAA GGC ATC AAG GGC CTC CAA AAT ATG GAC CAG CTC GTC AAA TGT TCC CAC GAG CGC    900
 D  I  Q  S  T  L  L  G  I  A  L  E  G  I  K  G  L  Q  N  M  D  Q  L  V  K  C  S  H  E  R    300

TCC ATC CAC CTG TTC ATC GAC TCT CTG CTG AAC ACC GAG CAG CAA AGC ATG GCT TAC CGC TGC AAC TCG AAA GAG GCC TTC AAC AAG GGC    990
 S  I  H  L  F  I  D  S  L  L  N  T  E  Q  Q  S  M  A  Y  R  C  N  S  K  E  A  F  N  K  G    330

CTG TGC CTC AGC TGC AGG AAG AAC CGC TGC AAC AAG CTA GGC TAC AAC ATC AAC AAG GTC CGC AGG ACC CGC AGT ACC AAG ATG TAC CTC   1080
 L  C  L  S  C  R  K  N  R  C  N  K  L  G  Y  N  I  N  K  V  R  R  T  R  S  T  K  M  Y  L    360

AAG ACC CGC GAG ATG ATG CCT TAC AAA GTT TTC CAC TAC CAA GTG AAG GTG CAT TTC TTC AGC AAG GAC CCA CTG AGC TTC ACG GAT CAG   1170
 K  T  R  E  M  M  P  Y  K  V  F  H  Y  Q  V  K  V  H  F  F  S  K  D  P  L  S  F  T  D  Q    390

CCA ATG AAG ATT TCT CTG TAT GGA ACC CAC GGA GAG AAG GAG GAC ATT CCC CAC GTC CTG CCA GTC ATG AAA GGT AAC ACC ACT CTG TCC   1260
 P  M  K  I  S  L  Y  G  T  H  G  E  K  E  D  I  P  H  V  L  P  V  M  K  G  N  T  T  L  S    420

TTC CTC ATC ACC ACC GAC GTG GAC ATC GGA GAC CTG ATG ATC GTG AAG CTG CGC TGG GAG AAG GAC ACG ATC ATC AGC TGG TCA GAC TGG   1350
 F  L  I  T  T  D  V  D  I  G  D  L  M  I  V  K  L  R  W  E  K  D  T  I  I  S  W  S  D  W    450

TGG GGC AGC AGC AAG TTC CAC ATC CGC AAA CTG CGC ATC AAG TCT GGG GAG ACC CAG TCC AAG GTG ATC TTC AGC GCA AAG GAA GGA GAG   1440
 W  G  S  S  K  F  H  I  R  K  L  R  I  K  S  G  E  T  Q  S  K  V  I  F  S  A  K  E  G  E    480

TTT GTC TAC CTT GTC AGG GGA GGA GAA GAT GGA GTC TTT GTC AAG TCA AAG GAA GAC AAC CTG AGC CGT AAA GAG AAA TTG ATG CAC AAG   1530
 F  V  Y  L  V  R  G  G  E  D  G  V  F  V  K  S  K  E  D  N  L  S  R  K  E  K  L  M  H  K    510

CTG AAG AAG CAG GGC AGT CTT TTT GGT CAG AGC GAC GCT TGA                                        1572
 L  K  K  Q  G  S  L  F  G  Q  S  D  A  *                                                      523

AGTTGCACTTGGACGCGCCCCTCCCACCCCACACCCCCACCCATCATGCACATCATCCATCTCTACATACATAGCCAAAGATCGACAGCACACAGCAACACAACACTGGGAAAGCCTTT
GCAAGCACCTGGACTTATACTGAATGCTGCGTTTTTCTCATCTTTTTCTTCCTGTTGTGCCTCCTGACTTTTAACTCCCAGTGCAAACCAACACAACCTCAACCACTGTGCTGGAAATC
AGTCTGCTCACGAACTTTCTCCTGTCTCTGCTGCAAATTCTGCAGCTGAGAAATGTTACAGATTTCTTTGTGATGATGTGCTCACTTTTGACAAATTTACACTTTCAGTAGCTTTCGCT
GCATATCTTTGTAATCATTTGTTTATGCTTTTGTAGCCTATATGTATTGTTTATACTGGTTTGGATTTGTTTTTTGAGCTGGTGTGTTTTCTTTGCCTATGTGAACTTGGGATTATTGT
AAATGTATAAATAATTCAGAGTGAAAGGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 3'
```

Fig.3

Red sea bream HL(LP2)

5' CTGTCTGTACATTACAGTACAATGTGTTTCACACTCTGATTTCAAGCCTCCGGACAGTAATGAGGCTTCTTTAACTCACCTTCAGACTCTTCATTAACTGCATCCCCTTCAAAGATTT
TAAGGCTTTCTCTTCACCTGTTGGAGAAGTAGTGACACAGAGAACTAATA

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ATG TCT GTG GTC AAA ATC CTT TGC TAT TTG CTG TTA ACA TAT CAC CTC AAT GAG GCG AAG AAA ACC AAA GGA AGC AGA GCA GCG GAC GAA     90
 M  S  V  V  K  I  L  C  Y  L  L  L  T  Y  H  L  N  E  A  K  K  T  K  G  S  R  A  A  D  E     30

GAG CAG AGG GGA GTC CTG AAG CCG CAT GTT AGC AGT TCA GTC TTC GGC CTG TTT GTA GAA GGT GAG GAA AAC TGC GCG CTG GAC CCT CTG    180
 E  Q  R  G  V  L  K  P  H  V  S  S  S  V  F  G  L  F  V  E  G  E  E  N  C  A  L  D  P  L     60

CAG CTG CAC ACT CTC ACC TCC TGT GGC TTC AAC AGC AGT AAT CCC CTC ATC ATC ATC ACT CAC GGG TGG TCG GTG GAT GGT ATG ATG GAG    270
 Q  L  H  T  L  T  S  C  G  F  N  S  S  N  P  L  I  I  I  T  H  G  W  S  V  D  G  M  M  E     90

AGC TGG GTG CAC AGG TTA GCC ACA ACC CTG AAG ACA CAT CTA ATA GAC GTC AAC GTG GTG ATT ACA GAC TGG CTG CTC CTG GCT CAC CAG    360
 S  W  V  H  R  L  A  T  T  L  K  T  H  L  I  D  V  N  V  V  I  T  D  W  L  L  L  A  H  Q    120

CAC TAC CCC ACA GCT GCA CAG AGC ACT CGC ACT GTT GGA AAA GAC ATA GCT CAC TTG CTG CAG TCA CTT CAG GTA CAC TAC CGG TTC CAA    450
 H  Y  P  T  A  A  Q  S  T  R  T  V  G  K  D  I  A  H  L  L  Q  S  L  Q  V  H  Y  R  F  Q    150

CTT AGA AAG GCT CAT TTG ATT GGC TAC AGC CTT GGG GCT CAC ATC TCT GGA TTT GCT GGA AGC TAT CTG GAA GGT TCG GAG AAG ATT GGA    540
 L  R  K  A  H  L  I  G  Y  S  L  G  A  H  I  S  G  F  A  G  S  Y  L  E  G  S  E  K  I  G    180

AGA ATT ACT GGT CTT GAT CCG GCG GGG CCC CTG TTT GAA GGC ATG TCT CCC ACG GAT AGA CTG TCT CCC GAT GAC GCT GAA TTT GTG GAC    630
 R  I  T  G  L  D  P  A  G  P  L  F  E  G  M  S  P  T  D  R  L  S  P  D  D  A  E  F  V  D    210

GCC ATC CAC ACC TTC ACC CAT GAA CGT ATG GGC CTC AGT GTG GGC ATC AAG CAA GCT GTG GCA CAT TAT GAC TTT TAC CCC AAC GGA GGA    720
 A  I  H  T  F  T  H  E  R  M  G  L  S  V  G  I  K  Q  A  V  A  H  Y  D  F  Y  P  N  G  G    240

GAC TTC CAG CCA GGA TGC GAC CTG CAA AAC ATT TAT GAG CAC ATA GCC CAG TAC GGG CTC CTT GGT TTC GAG CAG ACG GTG AAA TGT GCA    810
 D  F  Q  P  G  C  D  L  Q  N  I  Y  E  H  I  A  Q  Y  G  L  L  G  F  E  Q  T  V  K  C  A    270

CAT GAG CGG TCT GTG CAT CTG TTC ATT GAC TCT CTG CTG AAT GAA GAC AAG CAG AGC ATG GCC TAC AGG TGC AGC GAC AAC AGC GCT TTT    900
 H  E  R  S  V  H  L  F  I  D  S  L  L  N  E  D  K  Q  S  M  A  Y  R  C  S  D  N  S  A  F    300

GTC AAA GGC GTC TGT CTG GAC TGT CGG AAG AAT CGC TGC AAC ACG CTG GGC TAC AAT ATA AGG AAG GTC CGC AGC GGC GCC AGC AAG AGG    990
 V  K  G  V  C  L  D  C  R  K  N  R  C  N  T  L  G  Y  N  I  R  K  V  R  S  G  A  S  K  R    330

CTG TAC CTG AAA ACA CGC TCT CGG ATG CCT TAC AAA CTT CAT CAT TAC CAG TTC AGG ATT CAG TTT GTC AAC CAG ATG GAG AGC ATC GAG   1080
 L  Y  L  K  T  R  S  R  M  P  Y  K  L  H  H  Y  Q  F  R  I  Q  F  V  N  Q  M  E  S  I  E    360

CCC TCT CTC ACC ATC TCC CTC TCT GGA ACA AAG GAG GAG AGC GGC GAC CTC TCC ATC ACA GTC CCT GAA ACA ATT TGG GGT AAT AAA ACC   1170
 P  S  L  T  I  S  L  S  G  T  K  E  E  S  G  D  L  S  I  T  V  P  E  T  I  W  G  N  K  T    390

TTC ACC TTC CTG ATC ACC CTG GAC AAA GAC TTA GGG GAT CTG ATG TTG CTC AAG TTG CAC TGG GAG GGA TCG GCT ATG TGG AAG AAT GTG   1260
 F  T  F  L  I  T  L  D  K  D  L  G  D  L  M  L  L  K  L  H  W  E  G  S  A  M  W  K  N  V    420

TGG AAC CGG GTG CAG ACC ATT ATT CCC TGG GGC AGC CGG AGG ATG AAA CCA CTG CTG AGC GTG GGC AAG ATC AGC GTC AAA GCA GGC GAG   1350
 W  N  R  V  Q  T  I  I  P  W  G  S  R  R  M  K  P  L  L  S  V  G  K  I  S  V  K  A  G  E    450

ACG CAG GAG AGG ACA TCT TTT TGT GCC ATG ACA AAT GAG GAC CAA CAA GTT GAA GTG TCG CAA GAT AAA GTG TAC GTG CGC TGC AAA GAA   1440
 T  Q  E  R  T  S  F  C  A  M  T  N  E  D  Q  Q  V  E  V  S  Q  D  K  V  Y  V  R  C  K  E    480

GAA ACA CAG AAA CAG CGG AGA AGG AAG CAC AAC AGA CTT GTG AGG GAG CCT TAG                        1494
 E  T  Q  K  Q  R  R  R  K  H  N  R  L  V  R  E  P  *                                        497
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TCTGTTCAACACAAGATCTCTGATGGACGCCCTGCAAGTTCACAACAACTCCTGAGAGTGAGGCTGTGCAGATTCAGGGAGTAAATTAAGAATTATTAATTCTCTCAAAGAATTGGCAC
TGGAGAAATGCTGAATGCTAAAGTTTATTAGGAGAATTGAATCCTGGGAAACTGAAGCTAGAGAACAACTAGCACCTATTGTAAACTTATTTAATTTAAACATGTTTAAATTTGATTAT
GTGATAAAATTATAAATACGTATTGCATGTGACCTTTGTACCAGACACTTTGTTGACCAGAGCCACACAGTGTAGTTTAAAGAGCCAGGCAGATTTACATTACGGTCTGTCTCTCAGGG
GGATATAAACAGCTGGGCATTTGCCAATTCAAACATTAACCAGACAGTGAACACGAAGTCACTGACCTTTTGATGACGTTAGTTCCAAAGTATGTAAAGTTTTTTTTTTCTTATATAAA
ATGATCAATCTCATCATTAAAACCTGCATTACTCCTTCAAATAACATCATCTCCTAATCAAACTCAAACCAGAGCAATAAATATGCACTCTGTTCTTTTCTAAAAAAAAAAAAAAAAAA
AA 3'
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Fig.4

Red sea bream PL(LP3)

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5' GCTTCTTGTCTGGCTTCAAGATGTCACAGATGTGGAGATTGTGTCTGCTCTGCTCACTCATTAGTGCCTCAT

ATG CGG CTG AGG TCT GTT TTA ATG AGC TGG GCT GCT TTG ATG ACC TGC CTC CCC TGG GGG GGC ACT GCT CAG AGG CCA GCA TCT GTC CTG     90
 M  R  L  R  S  V  L  M  S  W  A  A  L  M  T  C  L  P  W  G  G  T  A  Q  R  P  A  S  V  L     30

CCC TGG CAC CTT GAG GAG ATT GGC ACC CGC TTC CTC CTC TTC ACC CAG AAG AAC CGC TAC TAC CAG GAG ATC AAG ACT GAT CAA AAC ATC    180
 P  W  H  L  E  E  I  G  T  R  F  L  L  F  T  Q  K  N  R  Y  Y  Q  E  I  K  T  D  Q  N  I     60

CAG GCA TCA AAC TAC AGC GGG ATG AGA AAG ACT CGA TTC ATC ATT CCT GGT TAT TTG GAA AAA GGA GAT GAG GAC TGG CCG CAG GAC ATG    270
 Q  A  S  N  Y  S  G  M  R  K  T  R  F  I  I  P  G  Y  L  E  K  G  D  E  D  W  P  Q  D  M     90

TGT AAG GTC ATG CTG AAG TGG GAG AAT GTG AAC TGC ATC GCT GTG GAG TGG AAG AAA GGT GTG AAG ACT CAG TAT GCC CAG GCT GCC AAT    360
 C  K  V  M  L  K  W  E  N  V  N  C  I  A  V  E  W  K  K  G  V  K  T  Q  Y  A  Q  A  A  N    120

AAC GCC AGG GTT GTG GCT GCC CAG GTG GCA TCC ATG ATC ACA TTC CTC ATG GGC AAC TAC AAG CAG AAG GCT GAT AAG TTC CAT ATT ATC    450
 N  A  R  V  V  A  A  Q  V  A  S  M  I  T  F  L  M  G  N  Y  K  Q  K  A  D  K  F  H  I  I    150

GGA CAC AGC CTG GGA GCT CAC GCT GCA GGA GAC ACT GGT AGC AGG ATC CCT GGC CTT GCA CGC ATC ACA GGA TTG GAC CCA GTT GAA CCT    540
 G  H  S  L  G  A  H  A  A  G  D  T  G  S  R  I  P  G  L  A  R  I  T  G  L  D  P  V  E  P    180

TAC TTC CAG GAC ACC GAT GCC TCT GTA CGT CTA GAT ACC AAT GAC GCC ATC TTT GTG GAT GTC ATT CAC ACT GAT GGG CTT CCT TTC GAC    630
 Y  F  Q  D  T  D  A  S  V  R  L  D  T  N  D  A  I  F  V  D  V  I  H  T  D  G  L  P  F  D    210

TCC AAA CTT GGT CTG GGG ATG TCA CAT TCT GTG GGC CAT ATT GAC TTC TAC CCC AAT GGA GGA GAG CTG ATG CCT GGC TGC TCC ACT AAC    720
 S  K  L  G  L  G  M  S  H  S  V  G  H  I  D  F  Y  P  N  G  G  E  L  M  P  G  C  S  T  N    240

AGA GGC CCG CCC ACT GAC CTG GAT GCC ATC TGG GAG GGT ACC AAG AAG TTT GAT GCC TGC AAC CAT GTT CGA GCA TAC CAG TAC TAC AGT    810
 R  G  P  P  T  D  L  D  A  I  W  E  G  T  K  K  F  D  A  C  N  H  V  R  A  Y  Q  Y  Y  S    270

GAG AGC ATG GTT AAA CCC CAA GGC TTT GTA GGC TTC CCC TGT TCT GAC AAA GGC AGT TTT GCT GCT GGA AAG TGT TTC CCA TGT GCA CAC    900
 E  S  M  V  K  P  Q  G  F  V  G  F  P  C  S  D  K  G  S  F  A  A  G  K  C  F  P  C  A  H    300

GAC AAC TGT CCT CTG ATG GGC CAC GAT GCT GAC AGG TTC ACT GTG ACT GAT GAC GTT TTG AAG ATG AAG TAC TTC CTC AAC ACA GGC AGA    990
 D  N  C  P  L  M  G  H  D  A  D  R  F  T  V  T  D  D  V  L  K  M  K  Y  F  L  N  T  G  R    330

TCA GAG CCC TTT GGC CGT TAC AGT TAC AGA GTA ACA GTT ACC CTG GAT GGT CCC AGC TGG CCC AAT CCA GGC TTC CTT TAT GTG GCA CTC   1080
 S  E  P  F  G  R  Y  S  Y  R  V  T  V  T  L  D  G  P  S  W  P  N  P  G  F  L  Y  V  A  L    360

GCT GGA GAC AAT GAC AAC ACC GAA GAG TAC CAG CTT CAT GTG GGT ACA CTG ATG CCC GGA CGG ACC TAC GAG CTG CTG ATT GAT GCT GAG   1170
 A  G  D  N  D  N  T  E  E  Y  Q  L  H  V  G  T  L  M  P  G  R  T  Y  E  L  L  I  D  A  E    390

GTG AAC GTG GGT AGT GTG ACA GAG GTG AAG TTT CGG TGG AAC AAC CAT ATC CTC AAC CCC TTG AAT CCC AAG TAT GGT GCC TCC AAG GTG   1260
 V  N  V  G  S  V  T  E  V  K  F  R  W  N  N  H  I  L  N  P  L  N  P  K  Y  G  A  S  K  V    420

GCG CTG CAG AGA GGA AAA GAC AAG ATG ATC ATC TTC TTC TGT GGA ACA CAG AAG GTG GAG GAG AAT GAG ATC CAG TCT GTA CTA CCA TGC   1350
 A  L  Q  R  G  K  D  K  M  I  I  F  F  C  G  T  Q  K  V  E  E  N  E  I  Q  S  V  L  P  C    450

CAA GTT TAA                                                                                                           1359
 Q  V  *                                                                                                               452

CCAGCCACAATCCCCATGTCACTCAGGTCTTTCAATAAAGAGTTTGCATTCCAAAAAAAAAAAAAAAAA 3'
```
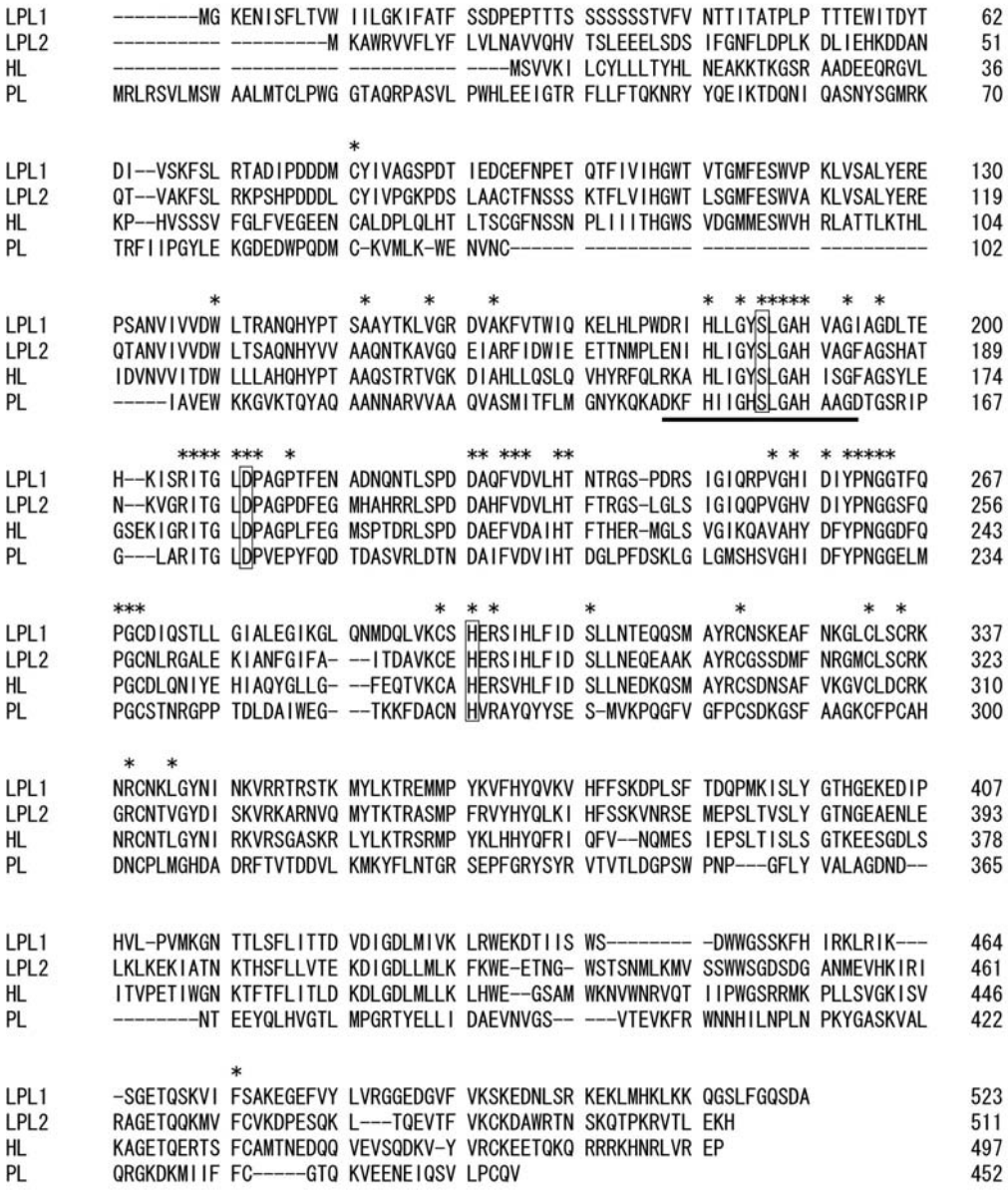

Fig.5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LPL2 ---------- ---------M KAWRVVFLYF LVLNAVVQHV TSLEEELSDS IFGNFLDPLK DLIEHKDDAN 51
HL ---------- ---------- ---------- ----MSVVKI LCYLLLTYHL NEAKKTKGSR AADEEQRGVL 36
PL MRLRSVLMSW AALMTCLPWG GTAQRPASVL PWHLEEIGTR FLLFTQKNRY YQEIKTDQNI QASNYSGMRK 70
LPL1 DI--VSKFSL RTADIPDDDM CYIVAGSPDT IEDCEFNPET QTFIVIHGWT VTGMFESWVP KLVSALYERE 130
LPL2 QT--VAKFSL RKPSHPDDDL CYIVPGKPDS LAACTFNSSS KTFLVIHGWT LSGMFESWVA KLVSALYERE 119
HL KP--HVSSSV FGLFVEGEEN CALDPLQLHT LTSCGFNSSN PLIIITHGWS VDGMMESWVH RLATTLKTHL 104
PL TRFIIPGYLE KGDEDWPQDM C-KVMLK-WE NVNC------ ---------- ---------- ---------- 102
LPL1 PSANVIVVDW LTRANQHYPT SAAYTKLVGR DVAKFVTWIQ KELHLPWDRI HLLGYSLGAH VAGIAGDLTE 200
LPL2 QTANVIVVDW LTSAQNHYVV AAQNTKAVGQ EIARFIDWIE ETTNMPLENI HLIGYSLGAH VAGFAGSHAT 189
HL IDVNVVITDW LLLAHQHYPT AAQSTRTVGK DIAHLLQSLQ VHYRFQLRKA HLIGYSLGAH ISGFAGSYLE 174
PL -----IAVEW KKGVKTQYAQ AANNARVVAA QVASMITFLM GNYKQKADKF HIIGHSLGAH AAGDTGSRIP 167
LPL1 H--KISRITG LDPAGPTFEN ADNQNTLSPD DAQFVDVLHT NTRGS-PDRS IGIQRPVGHI DIYPNGGTFQ 267
LPL2 N--KVGRITG LDPAGPDFEG MHAHRRLSPD DAHFVDVLHT FTRGS-LGLS IGIQQPVGHV DIYPNGGSFQ 256
HL GSEKIGRITG LDPAGPLFEG MSPTDRLSPD DAEFVDAIHT FTHER-MGLS VGIKQAVAHY DFYPNGGDFQ 243
PL G---LARITG LDPVEPYFQD TDASVRLDTN DAIFVDVIHT DGLPFDSKLG LGMSHSVGHI DFYPNGGELM 234
LPL1 PGCDIQSTLL GIALEGIKGL QNMDQLVKCS HERSIHLFID SLLNTEQQSM AYRCNSKEAF NKGLCLSCRK 337
LPL2 PGCNLRGALE KIANFGIFA- --ITDAVKCE HERSIHLFID SLLNEQEAAK AYRCGSSDMF NRGMCLSCRK 323
HL PGCDLQNIYE HIAQYGLLG- --FEQTVKCA HERSVHLFID SLLNEDKQSM AYRCSDNSAF VKGVCLDCRK 310
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HL NRCNTLGYNI RKVRSGASKR LYLKTRSRMP YKLHHYQFRI QFV--NQMES IEPSLTISLS GTKEESGDLS 378
PL DNCPLMGHDA DRFTVTDDVL KMKYFLNTGR SEPFGRYSYR VTVTLDGPSW PNP---GFLY VALAGDND-- 365
LPL1 HVL-PVMKGN TTLSFLITTD VDIGDLMIVK LRWEKDTIIS WS-------- -DWWGSSKFH IRKLRIK--- 464
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HL ITVPETIWGN KTFTFLITLD KDLGDLMLLK LHWE--GSAM WKNVWNRVQT IIPWGSRRMK PLLSVGKISV 446
PL --------NT EEYQLHVGTL MPGRTYELLI DAEVNVGS-- ---VTEVKFR WNNHILNPLN PKYGASKVAL 422
LPL1 -SGETQSKVI FSAKEGEFVY LVRGGEDGVF VKSKEDNLSR KEKLMHKLKK QGSLFGQSDA 523
LPL2 RAGETQQKMV FCVKDPESQK L---TQEVTF VKCKDAWRTN SKQTPKRVTL EKH 511
HL KAGETQERTS FCAMTNEDQQ VEVSQDKV-Y VRCKEETQKQ RRRKHNRLVR EP 497
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Fig.6

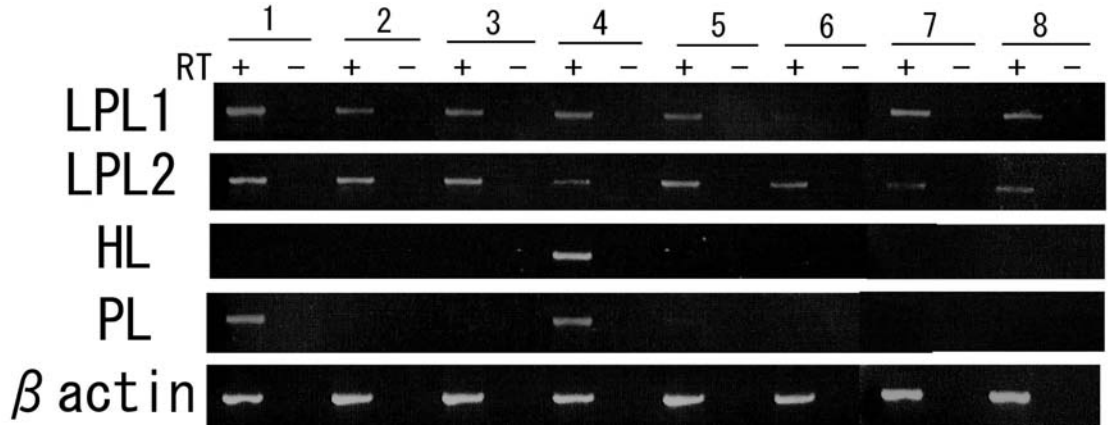
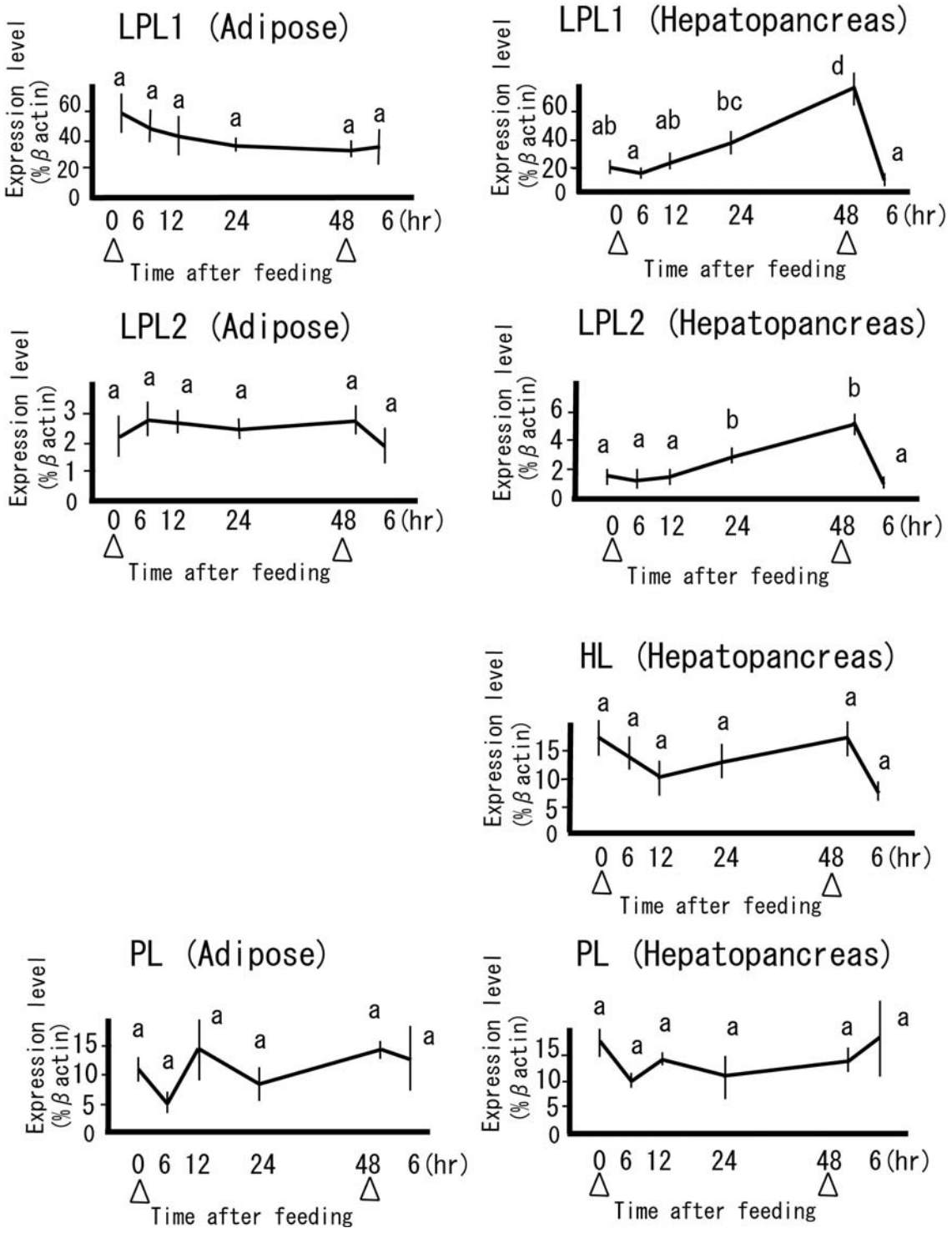


Fig.7


LPL1 (Adipose)
LPL1 (Hepatopancreas)
LPL2 (Adipose)
LPL2 (Hepatopancreas)
HL (Hepatopancreas)
PL (Adipose)
PL (Hepatopancreas)
Expression level
(%β actin)
Time after feeding
0 6 12 24 48 6(hr)