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Cloning, Expression and Polymorphism Analyses of PGC-1 α Gene of Schizothorax prenanti

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ABSTRACT

PGC-1 α (peroxisome proliferator-activated receptor γ coactivator-1 α) is a master regulator of lipid metabolism and a candidate of flesh quality determinant. In this study, we characterized a cDNA of *Schizothorax prenanti* PGC-1 α and investigated its tissue and developmental profiles, as well as the relationship between its developmental change and intramuscular fat (IMF) content. Cloning and sequencing analysis revealed that *S. prenanti* PGC-1 α showed high similarity to those of other vertebrates with conservation of functional domain including Peroxisome Proliferator-Activated Receptor γ (PPAR γ) binding site, RNA Recognition Motif (RRM) and serine-arginine repeats. The expression of PGC-1 α in kidney, heart and intestine was significantly higher than in other tissues studied (p<0.05). Furthermore, the PGC-1 α gene expression level in muscle increased with the growth of fish, showing higher mRNA level at 24 months than at other stages (p<0.05). PGC-1 α mRNA levels were positively correlated with intramuscular fat (IMF) content (R² = 0.714, p<0.01). Allelic variation at nucleotide positions 588 of *S. prenanti* PGC-1 α was detected by PCR-SSCP method and most samples examined were *CIC* homozygous. The results of this study will facilitate further investigation of fish PGC-1 α function and the eventual control of cultured fish quality.

Key words: $Schizothorax\ prenanti$, peroxisome proliferator-activated receptor γ coactivator- 1α , cloning, expression, polymorphism

INTRODUCTION

The peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1) family is master regulators of energy metabolism which include three members termed PGC-1 α , PGC-1 β and PGC-1 related protein (PRC) (LeMoine et al., 2010). PGC-1 α which is the first member discovered in this family, has been firstly characterized as an inducer of brown adipose tissue development in mouse (Puigserver et al., 1998). The subsequent analyses have revealed that the PGC-1 α is involved in the regulation of fiber-type switching (Lin et al., 2002), mitochondrial biogenesis (Ventura-Clapier et al., 2008), oxidative metabolism (Summermatter et al., 2011), adaptive thermogenesis (Puigserver et al., 1998), glucose/fatty-acid metabolism (Zhu et al., 2009), peripheral circadian clock in skeletal muscle (Wende et al., 2007) and heart development (Sihag et al., 2009).

PGC-1α protein is composed of four main functional regions i.e., the Activation Domain (AD), the nuclear respiratory factor-1 (NRF-1) binding domain, the myocyte-specific enhancer factor 2C

(MEF-2C) binding domain and the RNA Binding Domain (RBD) (LeMoine et al., 2010). The C-terminus and N-terminus of PGC-1 α contain the sequences that are responsible for binding of many nuclear receptors (Handschin, 2010). PGC1- α interacts with various members of the nuclear receptor superfamily, including Peroxisome Proliferator-Activated Receptors (PPARs), retinoid X receptor α (RXR α), hepatocyte nuclear factor 4α (HNF4 α), as well as many non-nuclear receptor-type transcription factors such as MEF2, forkhead box1(FOXO1) and sirtuin 1 (Sirt1) (Handschin, 2010). Moreover, the PGC-1 α transcriptional activator complex is able to displace repressor proteins such as histone deacetylase and small heterodimer partner and provides an alternative mechanism for gene activation (Puigserver et al.,1999). Among vertebrates, PGC-1 α exhibits a high degree of sequence similarity with conservation of functional domains (LeMoine et al., 2010).

PGC-1 α is a candidate of the determinant of meat quality. PGC-1 α plays a key role in fiber-type switching by controlling the formation of type II fiber from type I in skeletal muscle and thereby PGC-1 α influent meat quality such as color, juiciness and taste (Lefaucheur *et al.*, 2004; Bowker *et al.*, 2004). Besides, polymorphism analyses of PGC-1 α gene in land animals revealed that it is a functional candidate gene for determining the body lipid content (Kunej *et al.*, 2005; Wu *et al.*, 2006). Whether PGC-1 α polymorphism is present in fish and its association with fat deposition has not been reported at present. Therefore, we selected *S. prenanti* that is a unique cyprinid fish in Tibet plateau. We cloned *S. prenanti* PGC-1 α gene, analyzed its tissue and developmental age expression profile and polymorphism. Present results will be helpful for elucidating the functions of PGC-1 α gene in fish.

MATERIALS AND METHODS

Experimental fish: The experimental fish (S. prenanti) were purchased from a local dealer (Lushan farm, Yaan, China). The experimental fish were kept in our laboratory (Laboratory for genetic breeding of animals, Southwest University for Nationalities, Chengdu, China) for 1 week by feeding commercial diet. The details of the fish used in this study are presented in Table 1. This research project was conducted from 2009.10-2011.10.

Cloning and sequence analysis of S. prenanti PGC-1α gene: Total RNA was extracted from heart of S. prenanti with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the instruction manuals. cDNA was synthesized by reverse transcription from 2 μg of total RNA as described in the manufacturer's instruction (Fermentas Life Science, Hanover, MD, US). PCR amplification was performed in standard conditions: denaturation at 95°C for 5 min, then 38 cycles of amplification including 95°C for 45 sec, 62°C for 1 min and 72°C for 1.5 min. The amplification was followed by a final extension at 72°C for 10 min. The primers were designed to amplify the entire open reading frame of PGC-1α cDNA of S. prenanti using Primer Premier 5 software based

Table 1: The experimental fish

Experiments	Body weight (g)	No.	
Cloning and sequence analysis of S. prenanti	350.0	1	
Tissue distribution of PGC- 1α gene in S. prenanti	349.8±0.70	6	
The developmental changes of PGC-1 α gene in S. prenati	nti		
3 months	10.1±0.30	10	
12 months	70.6±0.60	10	
18 months	132.2±0.90	10	
24 months	351.8±1.40	10	
Polymorphism assay by SSCP analyses	129.2±13.9	60	

on the sequences of PGC-1α of *Danio rerio* (GenBank accession No. XM_002667531, AY998087, FJ710604 and DQ017637). The sequences of primers are as follows: F1: 5'-GGATGGCGTGGACA GGTGTAATC-3', R1: 5'-GCTGGGGTGGTGCTGTCTCGTT-3', F2: 5'-CTGAGCAAGGCGTCCTCCA CTATG-3', R2: 5'-TTACCTTCTCAGGCTGTACTGGG-3'.

The PCR fragments were gel purified and cloned into pMD19-T vector (TaKaRa, Dalian, China) and transformed into $E.\ coli$ DH5 α . For each fragment, five clones were sequenced in both directions by Shanghai Sangon Biological Engineering Technology (Shanghai, China). The sequence, isoelectric point and molecular weight of the deduced amino acids were analyzed using ExPASy-Tools (http://www.expasy.org/tools). The amino acid sequence multiple alignment was constructed with the BioEdit software version 5.0.6 (Hall, 2001). The phylogenetic tree was generated using Neighbour-Joining (NJ) methods (Kimura two-parameter model, 10 000 replicates, bootstrap phylogeny test) based on PGC-1 α amino acid sequences using MEGA software version 3.1 (Kumar et al., 2004).

Analysis of mRNA level of PGC-1a in tissues of S. prenanti and muscles of S. prenanti at different ages: Semi-quantitative RT-PCR was employed to reveal tissue and age differences of PGC- 1α mRNA level. Total RNA was extracted as described above from the liver, heart, kidney, muscle, adipose tissue, intestine, brain and gill of S. prenanti (n = 6) and from muscles of S. prenanti at ages 3, 12, 18 and 24 months (n = 10 for each age). A pair of primers (PGC- 1α -PF: GCTGCCTTGGTTGGTGAA, PGC-1α-PR: CCTTGCCACCTGGGTATTG) were designed to amplify a 439 bp fragment of S. prenanti PGC-1α cDNA. The primers for a reference gene β-actin (βF: GATTCGCTGGAGATGATGCT, βR: CGTTGTAGAAGGTGTGATGCC) were designed based on β-actin sequence of S. prenanti (GenBank accession No. JQ013000), the expected fragment size is 219 bp. The PCR condition was as follows: denaturation at 95°C for 5 min, then 32 cycles of amplification was performed and each cycle was consisted of denaturation step at 95°C for 30 sec, annealing step at 56.8°C (PGC-1α) or 54.5°C (β-actin) for 30 sec and extension step at 72°C for 30 sec. The amplification was followed by a final extension at 72°C for 5 min. The amplified fragments were separated by 1% agarose gel electrophoresis. The images of the RT-PCR stained with ethidium bromide were analyzed with Quantity One software (Bio-Rad, Hercules, CA, USA). The band intensity of the genes of interest was normalized to β -actin.

Intramuscular fat content assay: Intramuscular fat content in different ages fish (n = 10 for each age) muscle was measured by using Soxhlet petroleum-ether extraction.

Polymorphism assay by PCR-SSCP analyses: Genomic DNA was extracted from muscle of $S.\ prenanti$ (n = 60) by Ausubel method (Ausubel, 1992). PCR primers (SSCP-F: AAACCCCTG GAACAGCAA (Res. 185-Res. 190), SSCP-R: AGGACGATGGAGGAAGAA (Res. 247-Res.252) were designed for 206 bp amplification. This part corresponds to the exon 5 of zebrafish PGC-1 α (XM_002667531). A 50 ng of genomic DNA was subjected to PCR in 25 μ L reaction volume. The composition of PCR mixture was described above. The thermal condition was as follows: the first denaturation was carried out at 95°C for 3 min. A 32 cycles of amplification was performed and each cycle was consisted of denaturation step at 95°C for 30 sec, annealing step at 56°C for 30 sec and extension step at 72°C for 10 sec.

One microliter of PCR product was added to 20 μ L of dye solution (10% saccharose, 0.01% bromophenol blue and 0.01% xylene cyanol FF) and incubated for 2 min at 97°C. The 10 μ L of the mixture was applied to a 12% polyacrylamide gel electrophoresis. Following pre-run at 200 V for 10 min, the electrophoresis was carried out in 45 mM tris-borate (pH 8.0)/1 mM EDTA on ice at

180 V for 10 min and then 150 V for 4 h. After electrophoresis, the gel was subjected to silver staining. PCR products with different SSCP patterns were subjected to the direct sequence analyses by Shanghai Sangon Biological Engineering Technology (Shanghai, China).

Statistics: Data were expressed as Mean±SE and statistically analyzed using SPSS 13.0 for Windows Software (SPSS, Chicago, IL, USA). Differences of the IMF content and the gene expression level among tissues and different ages were analyzed by one-way ANOVA and independent-sample t-test, respectively. Significant differences were set at p<0.05.

RESULTS

Cloning and sequence analysis of *S. prenanti* PGC-1 α gene: Two fragments (approximately 1.4 kb) were amplified by RT-PCR and a 2,633 bp of *S. prenanti* PGC-1 α nucleotide sequence was obtained (GenBank accession No. JN195738). The obtained nucleotide sequence covered an entire ORF of 2,631 bp encoding 876 amino acids (Fig. 1). The predicted PGC-1 α protein has a molecular weight and isoelectric point of 96.78 kDa and 6.11, respectively.

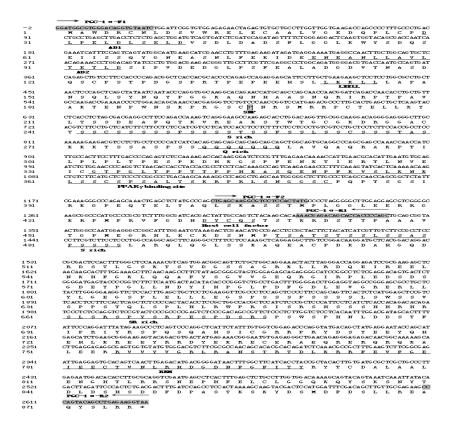


Fig. 1: The cDNA sequence of PGC-1α and the deduced amino acid sequence of S. prenanti, Grey bases: The primers were used for the cloning, *A terminal codon, AD1 and AD2: The activation domain 1 and 2, LXXLL: Motif, PPARγ: Host cell factor binding sites, RRM: RNA recognition motif, SR rich: Sequential series of serine-arginine repeats, S-rich: Fish specific serine rich sequence, Q rich: Fish specific glutamine rich sequence, SNP: Single nucleotide polymorphic site. This sequence was submitted to the NCBI GenBank with accession No. JN195738

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Phylogenetic analysis revealed that the $S.\ prenanti\ PGC-1\alpha$ was closely related to the PGC-1 α of other vertebrates (Fig. 2). The $S.\ prenanti\ PGC-1\alpha$ amino acid exhibits high degrees of sequence identities with cyprinidae but low with mammals and birds. Amino acid sequence analysis revealed that the functional domains of PGC-1 α including canonical LXXLL (a.a. 142-146) motifs, PPAR γ binding site (a.a. 333-382), RNA Recognition Motif (RRM) (a.a. 755-822) and sequential series of serine-arginine repeats (a.a. 646-677) were conserved among species (Fig. 3). In addition, the fish specific serine and glutamine rich sequences were found in $S.\ prenanti\ PGC-1\alpha$ (Fig. 1, 3).

Expression patterns of PGC-1α gene in tissues of S. prenanti: By RT-PCR, the expression of PGC-1α gene was detected in eight kinds of tissues of adult S. prenanti (Fig. 4). The expression

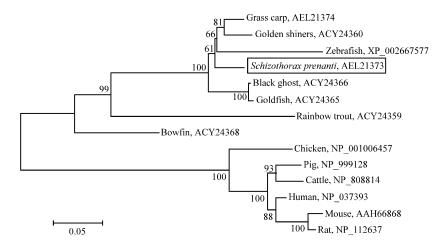


Fig. 2: Phylogenetic analysis of PGC-1α amino acid sequences, The phylogenetic tree was generated using neighbour-joining (NJ) methods (Kimura two-parameter model, 10,000 replicates, bootstrap phylogeny test) based on PGC-1α amino acid sequences using MEGA software version 3.1. Bootstrap values and genetic distance are shown

				Ac	tivation Doma	dn					
(a)											
S.prenanti	DS	UNRELECA	ALUGEDQPLC	PDLPELDLSE	LDUSDLDADS	FLGGLKWYSD	QSEIISSQYG	NEASNLFEKI	DEENEANLLA	VLTETLDSIP	98
Grass carp	MAMDRCHQDS	UMRELECA	ALVGEDQPLC	LDTLEFDT2E	TDA2DFDUD2	FLGGLKMA2D	d2E1122dAC	NEU2NTLEKI	DEENEANLLA	ULTETLOSIP	98
Zebrafish		MG	TD-P2D2UIA	ALPSPTEDE	-PANNURUD2	-AADIRUTND	GPPCRCAAH-	-KPAN2PKKI	DEENEANLLA	ULTETLOSIP	77
Golden shiner	QDS	UMRELECA	ALVGEDQPLC	POLPELOLSE	LDUSDLDADT	FLGGLKWYSD	QSEIISSQYG	NEASHLFEKI	DEENEANLLA	VLTETLDSIP	91
Goldfish	QDS	UMRELECA	ALVGEDQPLC	POLPELDLSE	LDUSDLDADI	FLGGLKWYSD	QSEIISSQYG	NETSHLFEKI	DEENEANLLA	VLTETLDIIP	91
Rainbow trout	QDS	VWRELECA	ALVGEDQPLC	POLPELOLSE	LDUSDLDADS	FLGGLKWYSD	QSEIISSQYG	NEASHLFE-I	DEENEANLLA	VLTETLDSIP	90
black ghost		A	ALVGEDQPLC	POLPELOLSE	LDUSDLDADS	FLGGLKWYSD	QSEIISSQYG	NESSNLFEKI	DEENEANLLA	VLTETLDSIP	81
bowfin		A	ALVGADQPLC	POLPELOLSE	LDUSDLDADS	FLGGLKWYSD	QSEIISNQYG	SESANLFEKI	DEENEANLLA	VLTETLDSIP	81
Human	MAWDMCHQDS	ESUWSDIECA	ALVGEDQPLC	POLPELOLSE	LOUHDLOTDS	FLGGLKWCSD	QSEIISHQYH	HEPSHIFEKI	DEENEANLLA	VLTETLDSLP	100
Mouse	MAWDMCSQDS	UWSDIECA	ALVGEDQPLC	POLPELOLSE	LDUNDLDTDS	FLGGLKWCSD	QSEIISNQYN	NEPANIFEKI	DEENEANLLA	VLTETLDSLP	98
Chicken	MAWDMCNQDS	UWSDIECA	ALVGEDQPLC	POLPELDLSE	LDUNDLDADS	FLGGLKWYSD	QSEVISSQYS	NEPANIFEKI	DEENEANLLA	VLTETLOSIP	98
				AD1						AD2	
S.prenanti	UDEDGLPSFE	ALADGDUTNA	SDQSCPSTPD	GSPRTPEPEE	HSLLKKLLLA	PANSQLSYNQ	YPGGKAQNHA	A-SHQRIRPT	PA 179		
Grass carp	VDEDGLPSFE	ALADGDUTNA	SDQSCPSTPD	GSPRTPEPEE	PSLLKKLLLA	PANSQLSYNQ	YPGGKAQNHA	A-SHQRIRPT	PA 179		
Zebrafish	UDEDBLPSFE	ALADGDUTHA	SDQSCPSTPD	GSPRTPEPEE	PSLLKKLLLA	PAHSQLSYNQ	YPGGKAQHHA	A-SHQRIRPA	PA 158		
Golden shiner	VDEDGLPSFE	ALADGDUTNA	SDQSCPSTPD	GSPRTPEPEE	PSLLKKLLLA	PANSQLSYNQ	YPGGKAQNHA	A-SHQRIRPT	PA 172		
Goldfish	UDEDGLPSFE	ALADGDUTNA	SDQSCPSTPD	GSPRTPEPEE	PSLLKKLLLA	PANSQLSYNQ	YPGGKAQNHA	A-SHLRIRPT	PA 172		
Rainbow trout	VDEDGLPSFE	ALADGDUTNA	SDQSCPCTPD	GSPRTPEPEE	PSLLKKLLLA	PANSQLSYNQ	YIGDKAQNHA	A-SDHRIRPP	PA 171		
black ghost	UDEDGLPSFE	ALADGDUTNA	data data da	GSPRTPEPEE	PSLLKKLLLA	PAY2JD2NA9	YPGGKAQNHA	A-SHLRIRHT	PA 162		
bowfin	VDEDGLPSFE	ALADGDEASA	SDHSCPSTPD	GSPPTPEAEE	PSLLKKLLLA	PANSQLSYNQ	YTGGKUQNHA	P-SHHRIRPT	PA 162		
Human	VDEDGLPSFD	ALTDGDUTTD	NEASPSSMPD	GTPPPQEAEE	PSLLKKLLLA	PANTQLSYNE	CSGLSTQNHA	N-HNHRIRTN	PA 181		
Mouse	VDEDGLPSFD	ALTDGAUTTD	NEASPSSMPD	GTPPPQEAEE	PSLLKKLLLA	PANTQLSYNE	CSGLSTQNHA	ANHTHRIRTN	PA 186		
Chicken	VDEDGLPSFD	ALTDGDUTNE	HDASPSPMPD	GTPPPQEAEE	PSLLKKLLLA	PANTQLNYNE	CSGLSTQNHA	N-THHRIRTS	PV 179		
					LXXLL						

Fig. 3(a-d): Continue

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.prenanti	CAID
	SNP UAKTENP- WASKPROSOP NRSHRR PCTELLKYLT SSDE-AFQTK UREAKSTWTG CGKORGG-AC 1/SSCSSSSSP SSSSTSSFSS LSSCSSSTAS 2:
rass carp	UAKTENP- WHSKPRGACP HRSHRR PCTELLKYLT SSDE-AFQTK AREAKSTWTG CGKDRGG-AC TSSCSSSSSP SSSSTSSFSS LSSCSSSTAS 2:
ebrafish	UAKTENP WHSKPREACP HRSHRR PCTELLKYLT SSDE-AFGIK AGEARSTWITG CGKDREG-AC ISSESSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSSS
olden shiner oldfish	UAKTENP- WHSKTRGACP HRSHRR PCTELLKYLT SSDE-AFQTK AREAKSTUTG CSKDRGGPLC TSSCSSSSSP SSSSTSSFSS LSSCSSSTAS 24 UAKTENP- WHTKPRGACP HRSURH PCTELLKYLT SSDE-AFQTK AREAKGTUTG CGKDRGG-AC TSSCSSSSSP SSSSTSSFSS LSSCSSSTS-24
ainbow trout	UNKTEIS- WHSKPRGGCP QUSRLURR PCTELLKYLI ATDDILLQTK ASDAKSANGG GGKDKGG-LL GASSSTSSSF SSSSTSSFSS LSS-SSSIAS 2
lack ghost	UAKTENP- WHTKPRGACP HRSURH PCTELLKYLT SSDE-AFQTK AREAKSANTG CGKDRGG-AC TSSCSSSSSP SSSSTSSFSS LSSCSSSTS-25
owfin	UUKTENPS WINSKORSPCP QQQPPKPARR PCAELLKYLT GSDE-TLPTK ASEPKSSS-G UGGGRSS-SS KDKTGSGAAC SASSSSTFSS SSSTSSTSPA 2
uman louse	IUKTENS- WSNKAKSICQ QQKPQRR PCSELLKYLT TNDD-PPHTK PTENRNSSRDKCT 23
hicken	UUKTENS- WSNKAKSICQ QQKPQRR PCSELLKYLT INDD-PPQTK PAENRNSSKEKCT 2:
S.prenanti	KKKTSSASPS \$\frac{0000000}{0000000} LAUQAQRAKP TILPLPLTPE SPKDHKGSPF ENKTIERTLN UEIGGTPGLT PPTTPPHKAS QENPFKUSLK HKLSSCSPSA 3
Grass carp Zebrafish	KKKTSSASPS SQQQQQQQQQ LALQAQRAKP TILPLPLTPE SPNDHKGSPF ENKTIERTLS UEIGTPGLT PPTTPPHKAS QENPFKUSLK NKLSSCSPSA 3 KKKTSSASPS SQQQQ LAUQAQRAKP TILPLPLTPE SPNDHKGSPF ENKTIERTLS UEIGTPGLT PPTTPPHKAS QENPFKUSLK NKLSSCSPSA 3
Golden shiner	RKKTSSASPS QQQQQQQQ LAQQAQAAAR TILPLETIFE SEMDINGSFF ENKILERILS UELQGEFGLT PPTTPPHKAS QENTFUSLA MALSSCSFLA S
Goldfish	KKKTPSASPS \$QQQQEQQ LAUQAQRAKP TILPLPLTPE SPNDHKGSPF ENKTTERTLS UEIGTPGLT PPTTPPHKAS QENPFKUSLK NKLSSCSPSA 3
Rainbow trout	KKKSSSSSUU SQQQQQPKP TTLPLPLTPE SPNDHKGSPF ESKSIERTLS UEISGTPGLT PPTTPPHKAS QENPFKASLK TKLSSCSSSA 3
black ghost	KKKTPSASPS SQQQQEQQ LAUQAQRAKP TILPLPLTPE SPNDHKGSPF ENKTTERTLS UELGGTPGLT PPTTPPHKAS QENPFKUSLK NKLSSCSPSA 3 SKKKPELPPH HQRAKP TILPLPLTPE SPNDPKGSPF ENKAIERTLS UELSGTAGLT PPTTPPHKAS QENPFKUSLK NKLSSCSPSA 3
bowfin Human	SKKKPELPPH HUK
Mouse	SKKKSHTQPQ SQHAQAKP TILSLPLTPE SPNDPKGSPF ENKTIERTLS UELSGTAGLT PPTTPPHKAN QDNPFKASPK LKPSCKTUUP 3
Chicken	SKRKPHLQSQ THHLQAKP TSLSLPLTPE SPNDPKGSPF ENKTIEQTLS UELSGTAGLT PPTTPPHKAN QDNPFRTSPK PKSSCKTUAP 3
S.prenanti	LTSKRPRLSH GGSGPQPTSGSIRK GPEQTELYAQ LSKASSTHPL GGLEERRGKRPHP RUFGDHPVCQ S-TSTKRDS
Grass carp	LTSKRPRLSH GGSCPQPHSGSIRK GPEQTELYAQ LSKASSTMPL GGLEERRGKRPMP RUFGDHÞYCQ YTSTKRDS4 LTSKRPRLSH GGSCPQPTSGSIRK GPEQTELYAQ LSKASSTMPQ GGLEDRRGKRPMP RUFGDHÞYCQ STSTKRDS4
Zebrafish	LTSKRPRLSN GGSCPQPTSGSIRK GPEQTELYAQ LSKASFTMPL GGLEERRGKRPMP RUFGDHÞYCQ STSTKRDS4
Golden shiner Goldfish	LTSKRPRLSN GGSCPQPTSGSIRK GPEQTELYAQ LSKASSTMPL GGLEERRGKR PMPRGKRPMP RUFGDHÞYCQ STSTKRDS 4
Rainbow trout	LACKRURLSE AGPCGPPALT PTLGGGPSRK GPEQTELYAQ LSKASTALPN SUUTATTGGC QEEP RGFSDHDYCQ SPAASTKRDA DANUANAAVA 4
black ghost	LTSKRPRLSN GGSCPQPTSGSIRK GPEQTELYAQ LSKASSTMPL GGLEERRGKRPMP RUFGDHÞYCQ S-TSTKRDS4 LTSKRPRLSN GGSCPQPTSGSIRK GPEQTELYAQ LSKASSTMPL GGLEERRGKRAHCP RUFGDHÞYCQ S-TSTKRDS4
bowfin	PPSKKPRYSE SSGTQGNNSTKK GPEQSELYAQ LSKSSULT- GGHEERKTKRPSL RLFGDHDYCQ S-INSKTEI3
Human Mouse	PPTKRARYSE CSGTQGSHSTKK GPEQSELYAQ LSKSSGLS- RGHEERKTKRPSL RLFGDHDYCQ S-LNSKTDI3
mouse Chicken	P-SKKPRYSE SSGSGORNPUKK GPEQTELYAQ LSKTTALS SGHEERKTKRPSL RLFGDH <u>bycQ s</u> UNSKSEI 3 PPARy binding site Host cell factor
nrenarti	, 0
	TIPAAA UTGP 453 TIPATA UTGP 455
ebrafish	TIPRAN UPGP 429
Golden shiner	TITATA UTGP 448
	TITAAA LTWP 452 TUTHTTTHTT PLMPNPUM 465
	TITAAA LTWP 435
oowfin	TITAAA LTWP 429
luman	LINIS QEL 484
	LINIS QEL 403 HIKIS OEL 401
	Myocyte-specific Enhancer Factor 2c (MEF-2c) Binding Domain
(c)	
	AND THE RESIDENCE OF THE PROPERTY OF THE PROPE
S.prenanti	MEGRHLECKD SHMFTSATST SSLSSASP SSSSLARQLQ GLSSKAQEAC PDKDAHGQDR DSTLGSKTSU DGS SAGRKLLRDQ EIREELNKHF
	TEGRHAECKD ZHWITZZIZI ZZFZZUZZ- FZZZFUKÓTÓ EFZЬTUÓEUC ÞAMDAHÐÓDH NZIZDZKIZA ÞZZ ZUĞKKTRDÓ ETKEFTUKHŁ
S.prenanti	TEGRHVECKO SHMLTSSTST SSLSSASP LSSSLARQLQ GLSPTAQEAC PDMDAHGQDH MSTSDSKTSU DCS SAGRKLLRDQ EIREELNKHF Tegrhvecko lhhr <mark>t</mark> stttt sslsstpp sssslarqlq glsptpqeac pdtvahuqhh dssskmtm dcs sggrkllrdq eirdelnkhf
S.prenanti Grass carp	TEGRHAECKD ZHWITZZIZI ZZFZZUZZ- FZZZFUKÓTÓ EFZЬTUÓEUC ÞAMDAHÐÓDH NZIZDZKIZA ÞZZ ZUĞKKTRDÓ ETKEFTUKHŁ
S.prenanti Grass carp Zebrafish	TEGRHVECKO SHMLTSSTST SSLSSASP LSSSLARQLQ GLSPTAQEAC PDMDAHGQDH MSTSDSKTSU DCS SAGRKLLRDQ EIREELNKHF Tegrhvecko lhhr <mark>t</mark> stttt sslsstpp sssslarqlq glsptpqeac pdtvahuqhh dssskmtm dcs sggrkllrdq eirdelnkhf
S.prenanti Grass carp Zebrafish Golden shiner	TEGRHUECKO SHMITSSTST SSLSSASP LSSSLARQLQ GLSPTAQEAG POHOHAGON HSTSDSKTSV DCS SAGRKLLROQ EIREELNKHF TEGRHUECKO LHMITSTTTT SSLSSTPP SSSLARQLQ GLSPTAQEAG POTVAHUQHH DSSSKMTM DCS SGGRKLLROQ EIRDELNKHF TEGRHUECKO SHMITSSTST SSLSSTTP LSSSLARQLQ GLSPTAQEAG LDTDAHAGON KSTGGSETSD DSS SAGRKLLROQ EIREELNKHHF
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout	TEGRHUECKO SHMITSSTST SSLSSASP LSSSLARQLQ GLSPTAQEAC PDIDAHGQDH MSTSDSKTSU DCS SAGRKLLRDQ EIREELNKHF TEGRHUECKO LHHITSTITT SSLSSTPP SSSSLARQLQ GLSPTQEAC POTVAHUQHH DSSSKMTM DCS SAGRKLLRDQ EIREELNKHF UEGRHUECKO SHMITSSTST SSLSSTTP LSSSLARQLQ GUSPKAQGAC PDHDAHGQDH KSTSGSRTSU DCS SAGRKLLRDQ EIREELNKHF UEGRHUECKO SHMITSSTST SSLSSASP SSSFLARQLQ GUSPKAQGAC PDHDAHGQDH DSTSGSRTSU DCS SAGRKLLRDQ EIREELNKHF AEDRHUKCKO SHMIPSSFSS SSSSTSST SSS-LAKQLQ GUSPVOATEA RAGBARAQDR TPTTQTRAPP PQTLDGDQHS TSRKQPLRDQ EIRAELNHHF
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost	TEGRHUECKO SHUITSSTST SSLSSASP LSSSLARQLQ GLSPTOQEOG POMOHAGODH MSTSOSKTSU DS SAGRKLLROQ EIREELNIKH TEGRHUECKO LHUITSSTTT SSLSSTPP SSSLARQLQ GLSPTOQEOG POTVAHUQHH DSS-SKMTM DS SAGRKLLROQ EIREELNIKH TEGRHUECKO SHUITSSTST SSLSSASP SSSLARQLQ GLSPTOQEOG LOTOAHAGOH KSTGGSETSD DS SAGRKLLROQ EIREELNIKH UEGRHUECKO SHUITSSAST SSLSSASP SSSLARQLQ GUSPKOQEOG POMOHAGOH DSTGGSRTSU DS SAGRKLLROQ EIREELNIKH UEGRHUECKO SHUITSSAST SSLSSASP SSSLARQLQ GUSPKOQEOG POMOHAGOH DSTGGSRTSU DS SAGRKLLROQ EIREELNIKH UEGRHUECKO SHUITSSAST SSLSSASP SSSLARQLQ GUSPKOQEOG POMOHAGOH DSTGGSRTSU DS SAGRKLLROQ EIREELNIKH
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin	TEGRHUECKO SHMITSSTST SSLSSASP LSSSLARQLQ GLSPTOGEGC POMOHAGODH MSTSOSKTSU DCS SAGRKLLROQ EIREELNIKH TEGRHUECKO LHMITSSTTT SSLSSTPP SSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGCRKLLROQ EIREELNIKH TEGRHUECKO SHMITSSAST SSLSSASP SSSLARQLQ GLSPTAQEGC LOTOAHGODH KSTSGSETSU DCS SAGRKLLROQ EIREELNIKHF MEGRHUECKO SHMITSSAST SSLSSASP SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTSGSRTSU DGS SAGRKLLROQ EIREELNIKHF MEGRHUECKO SHMITSSAST SSLSSASP SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTSGSRTSU DGS SAGRKLLROQ EIREELNIKHF UEGRHUECKO SHMITSSSAST SSLSSASP SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTSGSRTSU DGS SAGRKLLROQ EIREELNIKHF UEGRHUECKO SHMITSSSAST SSLSSASP SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTSGSRTSU DGS SAGRKLLROQ EIREELNIKHF
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human	TEGRHUECKO SHMITSSTST SSLSSASP- LSSSLARQLQ GLSPTOGEGC POMOHAGODH MSTSOSKTSU DGS SAGRKLROQ EIREELNKHF TEGRHUECKA LHMITSTTTT SSLSSTPP- SSSLARQLQ GLSPTOGEGC POMOHAGODH MSTSOSKTSU DGS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GLSPTOGEGC DOMOHAGODH MSTSGSRTSU DGS SAGRKLROQ EIREELNKHF ACDHUNCKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTGGSRTSU DGS SAGRKLROQ EIREELNKHF ACDHUNCKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTGGSRTSU DGS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTGGSRTSU DGS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTGGSRTSU DGS SAGRKLROQ EIREELNKHF -DSRQLENKO USS
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human Mouse	TEGRHUECKO SHMITSSTST SSLSSASP- LSSSLARQLQ GLSPTOQEGG POMOHAGOM MSTSOSKTSU DCS SAGRKLLROQ EIREELNIKH TEGRHUECKO LHMITSSTTT SSLSSTPP- SSSSLARQLQ GLSPTOQEGC POTVAHUQHH DSS-SKMTM DCS SAGRKLLROQ EIREELNIKH TEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GLSPTOQEGC LOTOAHAGOM KSTGSESTSD DCS SAGRKLLROQ EIREELNIKH UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKOQEGC POMOHAGOM DSTGSRTSU DCS SAGRKLLROQ EIREELNIKH UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKOQEGC POMOHAGOM DSTGSRTSU DCS SAGRKLLROQ EIREELNIKH UEGRHUECKO SHMITSSAST SSLSSAST- SSSLARQLQ GUSPKOQEGC POMOHAGOM DSTGSRTSU DCS SAGRKLLROQ EIREELNIKH UEGRHUECKO SHMITSSAST SSLSSAST- SSSLARQLQ GUSPKOQEGC POMOHAGOM DSTGSRTSU DCS SAGRKLLROQ EIREELNIKH UEGRHUECKO SHMITSSAST SSLSSAST- SSSLARQLQ GUSPKOQEGC VIRETLEASK QUSP
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Raidfish Holack ghost bowfin Human Mouse	TEGRHUECKO SHMITSSTST SSLSSASP- LSSSLARQLQ GLSPTOGEGC POMOHAGODH MSTSOSKTSU DGS SAGRKLROQ EIREELNKHF TEGRHUECKA LHMITSTTTT SSLSSTPP- SSSLARQLQ GLSPTOGEGC POMOHAGODH MSTSOSKTSU DGS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GLSPTOGEGC DOMOHAGODH MSTSGSRTSU DGS SAGRKLROQ EIREELNKHF ACDHUNCKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTGGSRTSU DGS SAGRKLROQ EIREELNKHF ACDHUNCKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTGGSRTSU DGS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTGGSRTSU DGS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAQGAC POMOHAGODH DSTGGSRTSU DGS SAGRKLROQ EIREELNKHF -DSRQLENKO USS
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Raidfish Holack ghost bowfin Human Mouse	TEGRHUECKO SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTAQEAC PDTVAHUQHH DSS-SKMTN DCS SAGRKLROQ EIREELNKHF TEGRHUECKO LHMITSSTST SSLSSARP- SSSLARQLQ GLSPTAQEAC PDTVAHUQHH DSS-SKMTN DCS SAGRKLROQ EIREELNKHF TEGRHUECKO SHMITSSTST SSLSSATP- SSSLARQLQ GLSPTAQEAC DTDAHAQDH KSTGSGETSD DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PDIDAHAQDH SSTGSGTSU DCS SAGRKLROQ EIREELNKHF HEDHUKCKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PDIDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PDIDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PONDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PONDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PONDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PONDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PONDAHAQDH DSTGSGRTSU D
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human Mouse Chicken	TEGRHUECKO SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTAQEAC PDTVAHUQHH DSS-SKMTN DCS SAGRKLROQ EIREELNKHF TEGRHUECKO LHMITSSTST SSLSSARP- SSSLARQLQ GLSPTAQEAC PDTVAHUQHH DSS-SKMTN DCS SAGRKLROQ EIREELNKHF TEGRHUECKO SHMITSSTST SSLSSATP- SSSLARQLQ GLSPTAQEAC DTDAHAQDH KSTGSGETSD DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PDIDAHAQDH SSTGSGTSU DCS SAGRKLROQ EIREELNKHF HEDHUKCKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PDIDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PDIDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PONDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PONDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PONDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PONDAHAQDH DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPVAQGAC PONDAHAQDH DSTGSGRTSU D
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human	TEGRHUCKU SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTOGEGC POMOHAGODH MSTOSMTSU DCS SGERKLEROQ EIRBELNIKHF TEGRHUCKU SHMITSSTST SSLSSARP- SSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIRBELNIKHF UEGRHUCKU SHMITSSTST SSLSSARP- SSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIRBELNIKHF UEGRHUCKU SHMITSSTST SSLSSASP- SSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTSU DCS SGERKLEROQ EIRBELNIKHF GEDRHUKKU SAHIPSS-SSSSSTSSPT SSSLARQLQ GUSPKAGGGC POMOHAGODH DSTSGSRTSU DCS SGERKLEROQ EIRBELNIKHF UEGRHUCKU SAHIPTS-SS-SSSSTSSPT SSSLARQLQ GUSPKAGGGC POMOHAGODH DSTSGSRTSU DCS SGERKLEROQ EIRBELNIKHF UEGRHUCKU SHMITS-SS-SSSSSTSSPT SSSLARQLQ GUSPKAGGGC POMOHAGODH DSTSGSRTSU DCS SAGRKLEROQ EIRBELNIKHF DSRQLENKO USSCHKPLROQ EIRBELNIKHF -DSRQLENKO USS
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Hunan House Chicken	TEGRHUECKO SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTQGGG PDMDHIGDIN MSTSOSKTSU DCS SGERKLEROQ EIREELNIKHF TEGRHUECKO LIMIFISTITT SSLSSTPP- SSSSLARQLQ GLSPTQGGG PDMDHIGDIN MSTSGSETSU DCS SGERKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSARP- SSSFLARQLQ GLSPTQGGG PDMDHIGDIN DSTSGSRTSU DCS SGERKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGG PDMDHIGDIN DSTGSGRTSU DCS SAGRKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGG PDMDHIGDIN DSTGSGRTSU DCS SAGRKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGG PDMDHIGDIN DSTGSGRTSU DCS SAGRKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGG PDMDHIGDIN DSTGSGRTSU DCS SAGRKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGG PDMDHIGDIN DSTGSGRTSU DCS SAGRKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGG PDMDHIGDIN DSTGSGRTSU DCS SAGRKLEROQ EIREELNIKHF -DSRQLERKO DUS
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Hunan House Chicken S.prenanti Grass carp Zebrafish	TEGRHUECKO SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTAGEGC POMPHAGON MSTOSKTSU DCS SGERKLEROQ EIREELNIKHF TEGRHUECKO LIMITSTTTT SSLSSTPP- SSSSLARQLQ GLSPTAGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIREELNIKHF TEGRHUECKO SHMITSSTST SSLSSTPP- LSSSLARQLQ GLSPTAGEGC LOTDAHAGON KSTGSSETSD DCS SGERKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAGGAC POMPHAGON DST-SGSRTSU DCS SAGRKLEROQ EIREELNIKHF REDRHUKCKO SHMITSSTS SSLSSASP- SSSFLARQLQ GUSPKAGGAC POMPHAGON DST-SGSRTSU DCS SAGRKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSTS SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ EIREELNIKHF UEGRHUECKO SHMITSSTS SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSSTS SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSSTS SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSSTS SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSLSSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSLSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSLSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSLSASP- SSSF- ARQLQ GUSPKAGGAC POMPHAGON TOTTOTRAPP PQTLOBOQNIS TRROQFLARQ UEGRHUECKO SHMITSST SSSTAGACACACACACACACACACA
S.prenanti Grass Carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human House Chicken S.prenanti Grass carp Zebrafish Golden shiner	TEGRHUCKO SHMITSSTST SSLSSASP- LSSSLARQLQ GLSPTOGEGC POMPHAGONH MSTSOSKTSU DCS SGERKLEROQ EIRBELNIKHF TEGRHUCKO SHMITSSTST SSLSSASP- SSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIRBELNIKHF UEGRHUCKO SHMITSSTST SSLSSASP- SSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIRBELNIKHF UEGRHUCKO SHMITSSAST SSLSSASP- SSSLARQLQ GLSPTAGGAC POMPHAGONH BSTSGSETSU DCS SGERKLEROQ EIRBELNIKHF UEGRHUCKO SHMITSSAST SSLSSASP- SSSLARQLQ GLSPKAGGAC POMPHAGONH BSTSGSETSU DCS SGERKLEROQ EIRBELNIKHF UEGRHUCKO SHMITSSAST SSLSSASP- SSSLARQLQ GSSPKOATEA ROGGARGORD TPTTQTRAPP PQTLODOQHS TSRKQPLROQ EIRBELNIKHF UEGRHUCKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAGGAC POMPHAGONH BTSTSSSKTSU DCS SAGRKLEROQ EIRBELNIKHF UEGRHUCKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAGGAC POMPHAGONH BTSTSGSSTSU DCS SAGRKLEROQ EIRBELNIKHF -BSRQLENKO USSCRKPLROQ EIRBELNIKHF -BSRQLENKO USS
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Hunan House Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish	TEGRHUECKO SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTQGGG PDMDHIGDIN MSTSOSKTSU DCS SGERKLEROQ EIREELNIKHF TEGRHUECKO LIMIFISTITT SSLSSTPP- SSSSLARQLQ GLSPTQGGG PDTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIREELNIKHF TEGRHUECKO SHMITSSTST SSLSSTP- SSSFLARQLQ GLSPTQGGG PDTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIREELNIKHF UGGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGG PDMDHIGDIN DSTGSRTSU DCS SGERKLEROQ EIREELNIKHF UGGRHUECKO SHMITSSTST SSLSSASP- SSSFLARQLQ GUSPKQGGG PDMDHIGDIN DSTGSRTSU DCS SGERKLEROQ EIREELNIKHF UGGRHUECKO SHMITSSTST SSLSSASP- SSSFLARQLQ GUSPKQGGG PDMDHIGDIN DSTGSRTSU DCS SGERKLEROQ EIREELNIKHF UGGRHUECKO SHMITSSTST SSLSSASP- SSSFLARQLQ GUSPKQGGC PDMDHIGDIN DSTGSRTSU DCS SGERKLEROQ EIREELNIKHF UGGRHUECKO SHMITSSTST SSLSSASP- SSSFLARQLQ GUSPKQGGC PDMDHIGDIN DSTGSRTSU DCS SGERKLEROQ EIREELNIKHF UGGRHUECKO SHMITSSTST SSLSSASP- SSSFLARQLQ GUSPKQGGC VIRETLEASK QUSP
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Hunan Mouse Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout	TEGRHUECKO SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTOGEGC POMPHEGOM MSTSOSKTSU DCS SGERKLEROQ EIRBELINKHF TEGRHUECKO LIMIFTSTTTT SSLSSTPP- SSSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIRBELINKHF TEGRHUECKO SHMITSSTST SSLSSATP- LSSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIRBELINKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSSLARQLQ GLSPYGAGGC POMPHAGOM DSTSGSRTSU DCS SGERKLEROQ EIRBELINKHF REDRHUKCKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKAGGGC POMPHAGOM DSTSGSRTSU DCS SGERKLEROQ EIRBELINKHF UEGRHUECKO SHMITSSTST SSLSSASP- SSSLARQLQ GUSPKAGGGC POMPHAGOM DSTSGSRTSU DCS SGERKLEROQ EIRBELINKHF UEGRHUECKO SHMITSSTST SSLSSASP- SSSLARQLQ GUSPKAGGGC POMPHAGOM DSTSGSRTSU DCS SGERKLEROQ EIRBELINKHF UEGRHUECKO SHMITSSTST SSLSSASP- SSSLARQLQ GUSPKAGGGC POMPHAGOM DSTSGSSRTSU DCS SAGRKLEROQ EIRBELINKHF UEGRHUECKO SHMITSSTST SSLSSASP- SSSLARQLQ GUSPKAGGGC POMPHAGOM DSTSGSSRTSU DCS SAGRKLEROQ EIRBELINKHF UEGRHUECKO SHMITSSTST SSLSSASP- SSSLARQLQ GUSPKAGGGC POMPHAGOM DSTSGSSRTSU D
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainhow trout black ghost bowfin Human House Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost	TEGRHUCKN SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTOGEGC POMPHAGONH MSTOSMATSU DCS SGERKLEROQ EIRBELINKHE TEGRHUCKN SHMITSSTST SSLSSARP- SSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIRBELINKHE TEGRHUCKN SHMITSSTST SSLSSARP- SSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIRBELINKHE UEGRHUCKN SHMITSSTST SSLSSASP- SSSLARQLQ GLSPTOGEGC DTOAHGODH KSTGSETSU DCS SGERKLEROQ EIRBELINKHE UEGRHUCKN SHMITSSTST SSLSSASP- SSSLARQLQ GLSPKOGAC POMPHAGODH DST-SKRTSU DCS SGERKLEROQ EIRBELINKHE UEGRHUCKN SHMITSSTST SSLSSASP- SSSLARQLQ GUSPKOGAC POMPHAGODH DST-SGSRTSU DCS SGERKLEROQ EIRBELINKHE UEGRHUCKN SHMITSSTST SSLSSASP- SSSLARQLQ GUSPKOGAC POMPHAGODH DST-SGSRTSU DCS SGERKLEROQ EIRBELINKHE UEGRHUCKN SHMITSSTST SSLSSASP- SSSLARQLQ GUSPKOGAC POMPHAGODH DST-SGSRTSU DCS SGERKLEROQ EIRBELINKHE UEGRHUCKN SHMITSSTST SSLSSASP- SSSLARQLQ GUSPKOGAC POMPHAGODH DST-SGSRTSU DCS SGERKLEROQ EIRBELINKHE UEGRHUCKN SHMITSSTST SSLSSASP- SSSLARQLQ GUSPKOGAC POMPHAGODH DST-SGSRTSU D
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Hunan House Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin	TEGRHUECKO SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTAQEGC POPUBHAGON MSTSOSKTSU DCS SGERKLEROQ EIREELNIKHF TEGRHUECKO SHMITSSTST SSLSSTPP- LSSSLARQLQ GLSPTAQEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIREELNIKHF TEGRHUECKO SHMITSSAST SSLSSARP- SSSFLARQLQ GLSPTAQEGC LOTOAHRQDH KSTGSESTSU DCS SGERKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC POHOAHRQDH DSTGSGRTSU DCS SGERKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC POHOAHRQDH DSTGGSRTSU DCS SGERKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC POHOAHRQDH DSTGGSRTSU DCS SGERKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC POHOAHRQDH DSTGGSRTSU DCS SGERKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC POHOAHRQDH DSTGGSRTSU DCS SGERKLEROQ EIREELNIKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC VIRETLEASK QUSP
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human Mouse Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human	TEGRHUECKO SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTAGGAC POTVAHUQHH DSS-SKMTM DCS SGCRKLROQ EIREELNKHF TEGRHUECKO SHMITSSTST SSLSSTPP- LSSSLARQLQ GLSPTAGGAC POTVAHUQHH DSS-SKMTM DCS SGCRKLROQ EIREELNKHF TEGRHUECKO SHMITSSAST SSLSSARP- SSSFLARQLQ GLSPTAGGAC LOTOAHGQH KSTGSGETSD DCS SGCRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSARP- SSSFLARQLQ GUSPKAGGAC POMDAHGQHD DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF REDRHUKKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAGGAC POMDAHGQHD DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSF ARQLQ GUSPKAGGAC POMDAHGQHD DSTGSGRTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSF ARQLQ GUSPKAGGAC POMDAHGQHD STGSGSTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSF ARQLQ GUSPKAGGAC POMDAHGQHD STGSGSTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSF ARQLQ GUSPKAGGAC POMDAHGQHD STGSGSTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSF ARQLQ GUSPKAGGAC POMDAHGQHD STGSGSTSU DCS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSF ARQLQ GUSPKAGGAC POMDAHGQHD STGSGSTSU DCGRKQLQQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSF ARQLQ GUSPKAGGAC POMDAHGQHD STGSGSTSU DCGRKPLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSF ARQLQ GUSPKAGGAC POMDAHGQHD STGSFSSSS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSLSSASP- SSSF ARQLQ GUSPKAGGAC POMDAHGQH STGSFSSSSS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSTAGSST SSSF ARQLQ GUSPKAGGAC POMDAHGQH STGSFSSSSS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSTAGSSST SSSF SSSF ARQLQ GUSPKAGGAC POMDAHGQH STGSFSSSSSS SAGRKLROQ EIREELNKHF UEGRHUECKO SHMITSSAST SSTAGSSST SSSF SSSF SSSF ARQLQ GUSPKAGGAC POMDAHGQA STGSFSSSSSSS SSSF SSSF SSSF SSSF SSSF S
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S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainhow trout black ghost bowfin Human House Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human House	TEGRHUECKO SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTOGEGC POMPHHODH MSTSOSKTSU DCS SGERKLEROQ EIREELNIKH TEGRHUECKO LHMITSSTST SSLSSTPP- SSSLARQLQ GLSPTOGEGC POMPHHODH MSTSOSKTSU DCS SGERKLEROQ EIREELNIKH TEGRHUECKO SHMITSSAST SSLSSARP- SSSLARQLQ GLSPTOGEGC DTORHRIQDH MSTSGSETSD DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSARP- SSSLARQLQ GLSPTOGEGC POMPHHODH DSTSGSRTSU DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGGC POMPHHODH DSTSGSRTSU DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGGC POMPHHODH DSTSGSRTSU DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGGC POMPHHODH DSTSGSRTSU DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGGC POMPHHODH DSTSGSRTSU DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGGC POMPHHODH DSTSGSRTSU DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGGC POMPHHODH DSTSGSRTSU DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGC POMPHHODH DSTSGSRTSU DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGC POMPHHODH DSTSGSRTSU DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGC POMPHHODH DSTSGSRTSU DCS SGERKLEROQ EIREELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGC PURBELNIKH UGERHUECKO SHMITSSAST SSLSSASP- SSSLARQLQ GUSPKOGGC POMPHHODH DSTSGSRTSU D
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainhow trout black ghost bowfin Human House Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human House	TEGRHUECKO SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTOGEGC POPOBHEGOPH MSTSOSKTSU DCS SGERKLEROQ EIREELNIKHE TEGRHUECKO SHMITSSTST SSLSSTPP- SSSSLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIREELNIKHE TEGRHUECKO SHMITSSTST SSLSSTPP- SSSFLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKMTM DCS SGERKLEROQ EIREELNIKHE UCGRHUECKO SHMITSSAST SSLSSARP- SSSFLARQLQ GLSPTOGEGC POTVAHUQHH DSS-SKSTSU DCS SGERKLEROQ EIREELNIKHE UCGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKOGGGC POHOBHEGOHD DSTSGSRTSU DSS SAGERKLEROQ EIREELNIKHE UCGRHUECKO SHMITSSAST SSLSSASS- SSSFLARQLQ GUSPKOGGGC POHOBHEGOHD DSTSGSRTSU DSS SAGERKLEROQ EIREELNIKHE UCGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKOGGGC POHOBHEGOHD DSTSGSRTSU DSS SAGERKLEROQ EIREELNIKHE UCGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKOGGGC POHOBHEGOHD DSTSGSRTSU DSS SAGERKLEROQ EIREELNIKHE UCGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKOGGGC POHOBHEGOHD DSTSGSRTSU DSS SAGERKLEROQ EIREELNIKHE UCGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKOGGGC POHOBHEGOHD DSTSGSRTSU DSS SAGERKLEROQ EIREELNIKHE UCGRHUECKO SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKOGGGC POHOBHEGOHD DSTSGSRTSU DSS SAGERKLEROQ EIREELNIKHE UCGRHUECKO SSMITSSAST SSLSSASSP SSSFLARQLQ GUSPKOGGGC POHOBHEGOHD DSTSGSRTSU DSS SAGERKLEROQ EIREELNIKHE UCGRHUECKO SSMITSSAST SSLSSASSP SSSFLARQLQ GUSPKOGGGC POHOBHEGOHD USSTSGSRTSU D
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S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human Huuse Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human House Chicken	TEGRHUCKK SHMITSSTST SSLSSAFP- LSSSLARQLQ GLSPTAQGGC PDTVAHUQHH DSS-SKMTM DCS SGCKKLRDQ EIRECHNUHF TEGRHUCKK SHMITSSTST SSLSSTP- SSSSLARQLQ GLSPTAQGGC DTVAHUQHH DSS-SKMTM DCS SGCKKLRDQ EIRECHNUHF TEGRHUCKK SHMITSSAST SSLSSAFP- SSSFLARQLQ GLSPTAQGGC DTVAHUQHH DSS-SKMTM DCS SGCKKLRDQ EIRECHNUHF UGGRHUCKK SHMITSSAST SSLSSASP- SSSFLARQLQ GLSPYAQGGC PDTVAHUQHH DSS-SKMTM DCS SGCKKLRDQ EIRECHNUHF UGGRHUCKK SHMITSSAST SSLSSASP- SSSFLARQLQ GLSPYAQGGC PDTVAHUQHH DSS-SSKMTS DCS SGCKKLRDQ EIRECHNUHF UGGRHUCKK SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC PDTVAHUQHH DSS-SSSTSUD DCS SGCKKLRDQ EIRECHNUHF UGGRHUCKK SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC PDTVAHUQHH DSS-SSSTSUD DCS SGCKKLRDQ EIRECHNUHF UGGRHUCKK SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC PDTVAHUQH DSS-SSSSTSUD DCS SGCKKLRDQ EIRECHNUHF UGGRHUCKK SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC PDTVAHUQH DSS-SSASTSUD DCS SGCKKLRDQ EIRECHNUHF UGGRHUCKK SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC PDTVAHUQH DSS-SSAGKKLRDQ EIRECHNUHF UGGRHUCKK SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKAQGGC PDTVAHUQH DSS-SSAGKKLRDQ EIRECHNUHF UGGRHUCK SHMITSSAST SSLSSASTS SSSSTSSTSSTS SSSSTSSTSSTSSASS SSSSTSSTSSTSS SSSSTSSTSSTSSTS SSSSSTSST
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human Mouse Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human Human Human Human Human Human Human S.prenanti Grass carp Zebrafish	TEGRHUECK SHUPTSSTST SSLSSAPP- LSSSLARQLQ GLSPTOGEGC POPUBHIGON HSTSDSKTSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSTST SSLSSTPP- SSSLARQLQ GLSPTOGEGC POPUBHIGON HSTSGSETSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSTST SSLSSTP- SSSLARQLQ GLSPTOGEGC POPUBHIGON HSTSGSETSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSTST SSLSSAPP- SSSLARQLQ GLSPTOGEGC POPUBHIGON HSTSGSETSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSAST SSLSSASP- SSSLARQLQ GLSPTOGEGC POPUBHIGON DSTSGSRTSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSAST SSLSSASP- SSSLARQLQ GUSPKOGEGC POPUBHIGON DSTSGSRTSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSAST SSLSSASP- SSSLARQLQ GUSPKOGEGC POPUBHIGON DSTSGSRTSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSAST SSLSSASP- SSSLARQLQ GUSPKOGEGC POPUBHIGON DSTSGSRTSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSAST SSLSSASP- SSSLARQLQ GUSPKOGEGC POPUBHIGON DSTSGSRTSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSAST SSLSSASP- SSSLARQLQ GUSPKOGEGC POPUBHIGON DSTSGSRTSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSAST SSLSSASP- SSSLARQLQ GUSPKOGEGC POPUBHIGON DSTSGSRTSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSAST SSLSSAST SSSLSSAST SSSLARQLQ GUSPKOGEGC POPUBHIGON DSTSGSRTSU DCS SGERKLERQ EIREELNIKH TEGRHUECK SHUPTSSAST SSLSSAST SSSLSAST SSSLARQLQ GUSPKOGEGC SSSLARQLQ GUSPKOGEGCC SSSLARQLQ GUSPKOGEGCC STRAGLUKH TEGRHUECK SHUPTSSAST SSLSSAST SSSLSAST SSSLARQLQ GUSPKOGEGCC SSSLARQLQ GUSPKOGEGCCC SSSLARQLQ GUSPKOGEGCCC SSSLARQLQ GUSPKOGEGCCC SSSLARQLQ GUSPKOGEGCCC SSSLARQLQ GUSPKOGCCC SSSLARQLQ GUSPKOGCCCC SSSLARQLQ GUSPKOGCCC SSSLARQLQ GUSPKOGCCC SSSLARQLQ GUSPKOGCCCC SSSLARQLC GUSPKOGCCCC SSSLARQLC GUSPKOGCCCC SSSLARQLC GUSPKOGCCCC SSSLARQLC GUSPKOGCCCC SSSLARQLC GUSPKOGCCCC SSSLARQLC GUSPKOGCCCC SSSLAR
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S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human Hu	TEGRHUECK SHUPTSSTST SSLSSAPP- LSSSARQLQ GLSPTQGGC PDTVAHUQHH BSS-SKMTM DCS SGRKLLROQ EIREELNKHF TEGRHUECK SHUPTSSTST SSLSSTPP- SSSFARQLQ GLSPTQGGC PDTVAHUQHH BSS-SKMTM DCS SGRKLLROQ EIREELNKHF TEGRHUECK SHUPTSSTST SSLSSTP- SSSFARQLQ GLSPTQGGC PDTVAHUQHH BSS-SKMTM DCS SGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSAPP- SSSFARQLQ GLSPTQGGC PDTVAHUQHH BSS-SKMTM DCS SAGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GLSPTQGGC PDTVAHUQHH BSS-SKRTSU DCS SAGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GUSPKQGGC PDTVAHUQH BSS-SKRTSU DCS SAGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GUSPKQGGC PDTVAHUQH BSTSGSRTSU DCS SAGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GUSPKQGGC PDTVAHUQH BSTSGSRTSU DCS SAGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GUSPKQGGC PDTVAHUQH BSTSGSRTSU DCS SAGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GUSPKQGGC PDTVAHUQH BSTSGSRTSU DCS SAGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GUSPKQGGC PDTVAHUQH BSTSGSRTSU DCS SAGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GUSPKQGGC PDTVAHUQH BSTSGSRTSU DCS SAGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GUSPKQGGC VLRETLASK QUSPCS SAGRKLLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GUSPCGC VLRETLASK QUSPCS SAGRKLROQ EIREELNKHF UGRHUECK SHUPTSSTST SSLSSASP- SSSFARQLQ GUSPCGC VLRETLASK QUSPDFG DLEUGRE-RL LYLGEGSPLE LLLEGSPSSSGRPQAFYSG
S.prenanti Grass carp Zobrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human Mouse Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human House Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human House Chicken	TEGRHUCKN SHMITSSTST SSLSSARP- LSSSLARQLQ GLSPTQGGC PDTWAHQUH BSS-SKMTN DCS SGRKLRDQ EIRELINKHF TEGRHUCKN SHMITSSTST SSLSSTP- SSSFLARQLQ GLSPTQGGC PDTWAHQUH BSS-SKMTN DCS SGRKLRDQ EIRELINKHF TEGRHUCKN SHMITSSAST SSLSSARP- SSSFLARQLQ GLSPTQGGC PDTWAHQUH BSS-SKMTN DCS SGRKLRDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GLSPTQGGC PDTWAHQUH BSS-SKMTN DCS SGRKLRDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GLSPKQGGC PDTWAHQUH BSS-SKMTN DCS SGRKLRDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GLSPKQGGC PDTWAHQUH BSTSGSRTSU DS SAGRKLLDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGC PDTWAHQUH BSTSGSRTSU DS SAGRKLLDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGC PDTWAHQUH BSTSGSRTSU DS SAGRKLLDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGC PDTWAHQUH BSTSGSRTSU DS SAGRKLLDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGC PDTWAHQUH BSTSGSRTSU DS SAGRKLLDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGC PDTWAHQUH BSTSGSRTSU DS SAGRKLLDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGC PDTWAHQUH BSTSGSRTSU DS SAGRKLLDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGC PDTWAHQUH BSTSGSRTSU DS SAGRKLLDQ EIRELINKHF UGBRUCKN SHMITSSAST SSLSSASP- SSSFLARQLQ GUSPKQGGC VHRETLASK QUSP
S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human House Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human House Chicken S.prenanti Grass carp Zebrafish Golden shiner Goldfish Rainbow trout black ghost bowfin Human	TEGRHUCKK SHMITSSTST SSLSSAFP- LSSSLARQLQ GLSPTAQEAC PDTVAHUQHH DSS-SKMTM DCS SAGRKLROQ EIRECHNUH TEGRHUCKK SHMITSSAST SSLSSTP- SSSFARQLQ GLSPTAQEAC DTVAHUQHH DSS-SKMTM DCS SAGRKLROQ EIRECHNUH TEGRHUCKK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPTAQEAC DTVAHUQHH DSS-SKMTM DCS SAGRKLROQ EIRECHNUH UEGRHUCKK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPYAQEAC PDTVAHUQHH DSS-SKMTM DCS SAGRKLROQ EIRECHNUH UEGRHUCKK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPYAQEAC PDTVAHUQHH DSS-SKMTM DCS SAGRKLROQ EIRECHNUH UEGRHUCKK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPYAQEAC PDWDAHEQHH DST-SSSATSU DCS SAGRKLROQ EIRECHNUH UEGRHUCKK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPYAQEAC PDWDAHEQHH DST-SSSATSU DCS SAGRKLROQ EIRECHNUH UEGRHUCKK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPYAQEAC PDWDAHEQHH DST-SSSATSU DCS SAGRKLROQ EIRECHNUH UEGRHUCKK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPYAQEAC UEGRHUCKK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPYAQEAC UEGRHUCK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPYAQEAC UEGRHUCK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPYAQEAC UEGRHUCK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPACHNUH UEGRHUCK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPACHNUH UEGRHUCK SHMITSSAST SSLSSASP- SSSFARQLQ GLSPACHNUH UEGRHUCK SHMITSSAST SSLSSASTSSAST SSSSASTSSAST SSSSASTAST SSSASTAST SSSASTA
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Fig. 3(a-d): Continue

(d)			RNA	A Binding Do	nain(RBD)						
S.prenanti	SISRSHS	-SSHHRHRSL	SRSPYSRPES	PDSRSPSW	SPHNLDDSTF	IPRIVESPOS	OAHSIC	GRRPRYDSYE	EYOHEHLKRE	EYRRDYEKRE	736
Grass carp	SITRSRS	-SSHYRCRSL	SRSPYSRSES	PDSRSPSR	SPHNUDDSTF	TSRIYRSPRP	QSHSIF	GRRPRYDSYE	EYQHERLKQE	EFRRDYEKRE	738
Zebrafish	SISRSRSR	SSSHHRRRSL	SRSPYSRSGS	PSSRSPSW	SPRNHDESTF	TPRICGNPQS	QSHSLF	GRRPRYDSYE	EYQHERLKRE	EYRRDYEKRE	712
Golden shiner	SISRTRL	-SSHHRYRSL	SRSPYSHSES	PDSRSPSR	SPHNHDDSTI	LPRIVKSPRS	QSNSIF	GRRPRYDSYE	EYQHERLKQE	EFRRDYEKRE	731
Goldfish	SISRSCS	-SSHHRRRSL	SRSPHSRSES	PDNCSPSW	SPHNHDNSTF	IPRIVRSPQS	QAHSIF	GRRPRYDSYE	EYQHURLKRE	EYRRDNEKRE	732
Rainbow trout	SGSPSSR	SRSRSRSSSS	HHRRRSLSSS	PDGRPSSR	SRHNHDSSTY	RSRTHKSPHS	QSRSQSRSPL	SRRPRYDSYE	EYQHERLKRE	EYRQDYQKRE	774
black ghost	SISRSCS	-SSHHRRRSL	SRSPHSRSES	PDNCSPSW	SPHNINDISTF	IPRIVRSPQS	QAHSIF	GRRPRYDSYE	EYQHURLKRE	EYRRDNEKRE	715
bowfin	SASRSRSHSR	TDAHRRRRSY	SRSPYSRSRS	RSPYSRSSSR	SHEGADSSGS	RPRAHRSPHS	HSRSHSGSPF	SRRPRYDSYE	EYQHERLKRE	EYRRDYEKRE	705
Hunan	SRSRSFS	RHRSC	SRSPYSRSRS	RSPGSRSSSR	SCYYYESSHY	RHRTHRNSPL	YURSRSRSPY	SRRPRYDSYE	EYQHERLKRE	EYRREYEKRE	658
House	SRSRSFS										
Chicken	SRSRSFP	QRR <mark>SC</mark>	SRSPYSRSRS	RSPCSRSSSR	2CHCAE22HC	RHRAHRSSPS	RARSRSRSPY	SRRPRYDSYE	EYQHERLKRE	EYRKEYEKRE	655
			SR rid	t							
S.prenanti	CERAEQRERQ										
Grass carp	CERAEQRERQ	RQKAIEERRU	UYUGRLRADS	TRTELKRRFE	VFGGIEECTV	NLRHDGDNFG	FINYRYTCDA	LAALENGHTL	RRSHEPHFEL	CLGGQKQYSK	838
Zebraf1sh	CERAEQRERQ	RQKAIEERRU	UYUGRLRADS	TRTELKRRFE	NEGELEE210	NLRHDGDNFG	FITYRYTCDA	LRALENGHTL	KK2HEHHEFT	CFCCCKGA2K	812
Golden shiner	CERAEQRERQ										
Goldfish	CERAEQREKQ										
Rainbow trout	SQRAEQRERQ										
black ghost	CERAEQREKQ										
bowfin	FERAEQRERQ										
Hunan	SERAKQRERQ										
Mouse	SERAKQRERQ										
Chicken	SERAKQRERQ	RQKAIEER <u>ru</u>	IYUGKIRPDT	TRKDLRDRFE	VFGETEECTV	NLRDDGDSYG	FITYRYTCDA	FAALENGYTL	RRSHEPDFEL	YFCGRKQFCK	755
				R	RM						
S.prenanti	SHYTDLDSHS	DDFDPASTKS	KYDSHDFDSL	LRE	869						
Grass carp	SHYTDLDSHS										
Zebrafish	SHYTDLDSHS	DDFDPASTKS	KYDSHDFDSL	LREAQYSLRR	852						
Golden shiner	SHYTDLDSHS										
Goldfish	SHYTDLDSHS										
Rainbow trout	SHYTDLDSHS										
black ghost	SHYTDLDSHS										
bowfin	SHYTDLDSHS										
Human	SHYADLDSHS										
Mouse	SHYADLDTHS										
Chicken	SHYADLDSHS	DDFDPASTKS	KYDSHDFDSL	LKEAQRSLRR	795						

Fig. 3(a-d): Multiple alignment of the PGC-1α amino acid sequences, AD1 and AD2: The activation domain 1 and 2, LXXLL: Motif, PPARγ: Host cell factor binding sites, RRM: RNA Recognition Motif, SR-rich: Sequential series of serine-arginine repeats, S rich: Fish specific serine rich sequence, Q rich: Fish specific glutamine rich sequences, SNP: A single nucleotide polymorphic site, GenBank accession number used in this analysis are Schizothorax prenanti: AEL21373, Grass carp: AEL21374, Zebrafish: XP_002667577, Golden shiners: ACY24360, Goldfish: ACY24365, Black ghost: ACY24366, Bowfin: ACY24368, Rainbow trout: ACY24359, Human: NP_037393, Mouse: AAH66868, Chicken: NP_001006457

levels of PGC-1 α in kidney and head were greater than the other tissues. However, the expression of PGC-1 α was not detected in adipose tissue and gill.

Expression level of PGC-1 α gene in muscle of *S. prenanti* at different ages: Semi-quantitative RT-PCR analyses revealed that PGC-1 α mRNA level in muscle increased with growth in *S. prenanti* (Fig. 5). The significantly higher mRNA level than those in other ages was observed at 24 months (p<0.05). PGC-1 α mRNA levels were positively correlated with IMF content (R² = 0.714, p<0.01).

Polymorphism of exon 5 of S. prenanti PGC-1 α gene: PCR-SSCP analysis revealed two SSCP patterns (C/C and CT) in exon 5 of S. prenanti PGC-1 α gene in 60 samples, originated from two alleles (C/T single nucleotide polymorphism at nt588) as shown by direct sequencing of PCR product (Fig. 6). The allelic and genotype frequencies in PGC-1 α gene are shown in Table 1. CC is the dominate genotype.

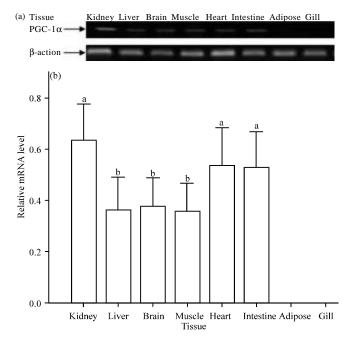


Fig. 4(a-b): Semi-quantitative RT-PCR analysis of PGC-1α mRNA level in tissues of S. prenanti, (a) Typical results of RT-PCR and (b) Relative level of PGC-1α mRNA, The values are the means of six independent experiments, Error bars represent the SE, Values without the same superscript are significantly different at p<0.05</p>

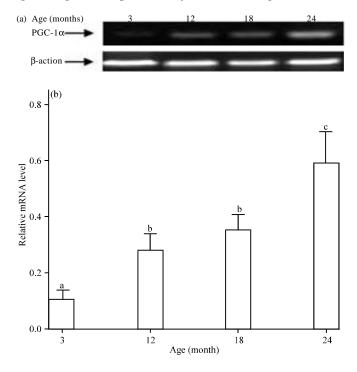


Fig. 5(a-b): Semi-quantitative RT-PCR analysis of PGC-1 α mRNA level in muscle of *S. prenanti* at different ages, (a) Typical results of RT-PCR and (b) Relative level of PGC-1 α mRNA in muscle, The values are the means of 10 independent experiments, Error bars are the SE, Values without the same superscript are significantly different at p<0.05

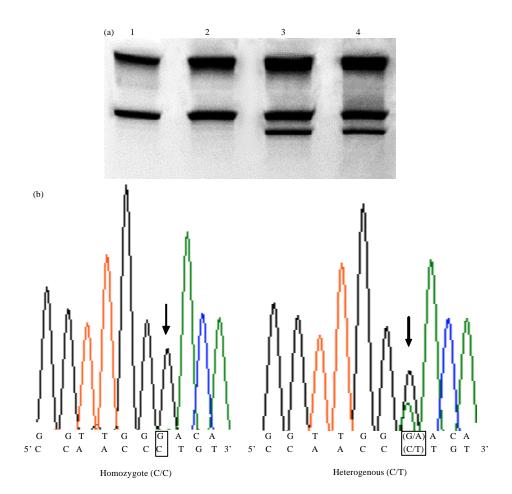


Fig. 6(a-b): Detection of allelic variation at nucleotide positions 588 of S. prenanti PGC-1α gene by PCR-SSCP, (a) Typical results of PCR-SSCP of S. prenanti PGC-1α, Two samples were electrophoresed for each genotype. Lane 1 and 2: C/C homogeneous genotype, Lane 3 and 4: C/T heterogeneous genotype, The 206 bp PCR products of PGC-1α fragment were separated by 12% PAGE, followed by silver staining and (b) Sequence analyses of two genotypes by direct sequencing at nucleotide position 588 with homogeneous and heterogeneous genotype, The 206 bp products of S. prenanti genomic PCR were subjected to the direct sequence analyses

DISCUSSION

In this study, we characterized PGC-1 α gene of S. prenanti by the sequence and expression analysis. We determined the nucleotide sequence of the most part of coding region of S. prenanti PGC-1 α (Fig. 1). The deduced amino acid sequence of S. prenanti PGC-1 α is similar to those of other vertebrates (Fig. 3) but possesses the structural features unique for fish. S. prenanti PGC-1 α contains only one Serine-arginine Repeats (SR) whereas mammalian PGC-1 α contains two (Fig. 3). Moreover, fish specific serine and glutamine rich sequences were found in S. prenanti PGC-1 α (Fig. 3). Thus the primary structure of S. prenanti PGC-1 α is similar to but differs in several aspects from those of mammals and avian.

PGC-1 α is expressed in high energy demand tissues such as mitochondria-rich tissues. The human PGC-1 α is highly expressed in heart, kidney, liver and skeletal muscle but expressed at very low level in intestine and white adipose tissue (Larrouy et al., 1999). The mouse PGC-1 α is highly expressed in brown adipose tissue, heart, kidney and brain (Puigserver et al., 1998). In this study, we determined the tissue distribution of PGC-1 α mRNA in S. prenanti (Fig. 4). As in mammals (Larrouy et al., 1999; Puigserver et al., 1998), high level of PGC-1 α mRNA was observed in kidney of S. prenanti (Fig. 4). Kidney of fresh water fish has a function as an osmoregulatory organ as well as a urinary organ (Lin, 1999). The high level of PGC-1 α mRNA in kidney of S. prenanti may reflect the high energy demand in this tissue. PGC-1 α showed high mRNA level in kidney of S. prenanti, quite different from that in mammals. The intestine of cyprinid fish (stomach less fish) is a main organ for digestion and absorption of diet (Lin, 1999). The difference in the intestinal PGC-1 α gene expression level between S. prenanti and mammals may suggest the functional difference of intestine among species. Moreover, the muscular PGC-1 α expression was increased with the growth of S. prenanti (Fig. 5). Although the detailed mechanisms remains to be elucidated, the results suggest that PGC-1 α is most likely involved in fish growth.

IMF is an important factor affecting meat flavor, tenderness and juiciness (Wang et al., 2005) and thus it is of significance to discover the candidate genes associated with IMF. This study examined the expression pattern of PGC-1 α gene in different developmental stages of Schizothorax and analyzed correlation with IMF content. The results indicate that PGC-1 α expression was increased with the growth of Schizothorax and positively correlated with IMF content, suggesting that the gene may be associated with IMF deposition but the detailed mechanism needs further study.

It has been reported that Single Nucleotide Polymorphisms (SNPs) of PGC-1 α gene is associated with metabolic disorder (Hara et al., 2002). A Gly482Ser polymorphism in the human PGC-1 α gene has been reported as a risk factor for development of type 2 diabetes (Pratley et al., 1998; Kunej et al., 2004) which is associated with obesity indices in middle-aged women (Esterbauer et al., 2002). Furthermore, in domestic animals, it has been reported that SNPs in PGC-1α gene affect economically important traits as one of the Quantitative Trait Loci (QTL). A Cys430Ser polymorphism in PGC-1α gene has been proposed as a candidate for determining breed specific phenotypes (fat and lean) in pig (Kunej et al., 2005). Weikard et al. (2005) have indicated that PGC-1 α is involved in the genetic variation of milk fat synthesis which is determined by QTL and they identified the gene locus on bovine chromosome (Weikard et al., 2005). Wu et al. (2006) have reported the existence of a SNP in exon 5 of PGC-1α gene in chicken which cause amino acid change from Asp to Asn at codon 216 and this SNP is associated with abdominal fatness (Wu et al., 2006). In the current study, a SNP (C and T at position nt588) in the open reading frame of S. prenanti PGC-1α gene was identified which causes amino acid change from Pro to Ser at codon 196 (Fig. 3). This position locates in the NRF-1 binding domain which contains PPARy and host cell factor binding sites (Fig. 1, 3). Furthermore, we found a SNP in exon 5 of S. prenanti PGC-1α gene in 60 samples, however, whether it correlates with IMF content needs further investigation.

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