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**Eating behaviour regulation in Zebrafish (*Danio rerio*):  
a comparative analysis**

A thesis  
submitted in partial fulfilment  
of the requirements for the degree

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## **Abstract**

The structure, function, and tissue specific expression profiles of neuropeptides involved in regulating eating behaviour are well researched in mammals, the corresponding literature on fish homologs however is scarce. The work presented here endeavoured to characterise the full coding sequences of peptide homologs in zebrafish as well as the expression profiles of said peptides under different energy states to elucidate their function in fish. Zebrafish were chosen as a model to represent fish species as they are amenable to molecular study. Conducting a literature and TBLASTn search, genes were identified in zebrafish that are known to be involved in affecting appetite and satiety in other vertebrates. Comparison of the translated sequences of these genes against fish and other vertebrates provided confidence in the identity of AGRP, GHRL, POMCb, CART1, CART3, and CART4 and all genes showed homology against mammalian transcripts through structural and syntenic comparison. RACE synthesis of the full coding sequence of these genes was attempted from isolated brain mRNA but only resulted in the production of a partial 3' sequence for CART1 and CART4 which showed high identity against the predicted nucleotide but not the translated sequence. To observe the functional properties of a suite of genes, a qPCR analysis of the expression profiles of AGRP, GHRL, POMCa, POMCb, CART1, CART2, CART3, CART4, OXT, NPY, and AVP was undertaken in 24 hour fasted zebrafish. The results were comparable to both other fish and mammals as AGRP was orexigenically upregulated in fasted zebrafish, while POMCb and OXT were anorexigenically downregulated. This is the first time that an expression study has been conducted across all of these genes together in a single species and, while the results are preliminary, they outline that the simultaneous analysis of the many genes thought to be involved in eating behaviour is a viable approach to discovering the functional roles of the range of genes, their interaction with each other, and how that relates to eating behaviour and provides some confidence in use of zebrafish as a model to highlight this.

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## List of abbreviations

Agouti-Related protein	AGRP
Apelin	APLN
Amylin	IAPP
Arcuate nucleus	ARC
Central nervous system	CNS
Cholecystokinin	CCK
Cocaine- and Amphetamine-Regulated Transcript	CART
Dorsomedial hypothalamic nuclei	DMH
Fat mass and obesity-associated transcript	FTO
Neuropeptide Y	NPY
Paraventricular nucleus	PVN
Peptide YY	PYY
Pro-opiomelanocortin	POMC
Ghrelin	GHRL
Intracerebroventricular	ICV
Intraperitoneal	IP
Lateral hypothalamic area	LHA
Leptins	LEP
Melanocortin receptor	MCR
Oxytocin	OXT
Vasopressin	AVP

Ventromedial hypothalamic nuclei	VMH
Whole genome duplication	WGD
Zebrafish	Zf

# **1. Introduction and literature review**

## **1.1 Introduction**

Research into the regulation of eating behaviour and food reward in animals will give us much needed insights into how energy balance is maintained. It will allow us to understand what tissues and molecular components are involved, how changes in diet and food availability affect changes in eating behaviour and how disruption of healthy eating behaviour patterns (or the processes/mechanisms which underly them) can lead to pathologies in humans such as obesity and anorexia nervosa. All of this will potentially allow us to prevent certain conditions arising or uncover possible therapeutic targets for treatments. To achieve this, a number of animal models have been employed to study the physiological underpinnings of appetite, satiety, and food reward and include the complex interactions of multiple genes and their transcribed products affecting eating behaviour in different phenotypes under different conditions. However, due to an increased pressure to provide food for an increasing population, there is increasing interest in understanding these mechanisms in other organisms that are used in farming, to improve feeding efficiency and increase sustainability. This means that it is not only mammals that are of interest to study, but also fish that are used in aquaculture, where it is important to improve feeding efficiency and diets.

The underlying physiological pathways and molecular mechanisms which contribute to feeding behaviours and hedonic drives for food consumption are well documented in mammals (Blundell, 2010; Broberger, 2005; Morrison & Berthoud, 2007; Rolls, 2011) Many studies have been undertaken to improve our understanding into the motivators of food consumption and have attempted to understand and combat pathologies associated with the disruption of healthy feeding patterns such as obesity (Benelam, 2009; Harrold, Dovey, Blundell, & Halford, 2012; Suzuki, Jayasena, & Bloom, 2011, 2012). Using mice as a model, research has begun to uncover the neurobiological, gastrointestinal, and molecular underpinnings of eating behaviour.

## **1.2 Role of the Central Nervous System (CNS) in Feeding Behaviour**

Regulation of food intake is under the control of the brain which receives input from the body regarding energy requirements, the nutritional quality, and the quantity of ingested meals via circulating peptides and hormones, and vagal signals from the brain stem. The brain integrates this information and affects an appropriate behavioural response by increasing satiety or appetite and driving feeding behaviours. This response is to prevent changes in energy balance, as the CNS is most sensitive to negative energy balance (weight loss) and thereby maintaining energy homeostasis (M. H. A. G. Gorissen, Flik, G., and Huising, M. O., 2006; Harrold et al., 2012; Parker & Bloom, 2012; Smith, 2008). There are several regions of the CNS in mammals, involved in maintaining energy homeostasis whose neuronal populations recognise specific signals from the brain and periphery and integrate these signals to determine the energy input and output of the body. The hypothalamus is a key region of the brain involved in energy balance; it contains discrete neuronal circuits which interact with each other and communicate with the brain-stem to monitor and respond to changes in metabolic status by regulating eating behaviour and energy expenditure. Hypothalamic sites containing discrete neuronal populations have been identified as having important roles in maintaining energy balance and include the arcuate nucleus (ARC), the dorsomedial hypothalamic nuclei (DMH), the lateral hypothalamic area (LHA), the ventromedial hypothalamic nucleus (VMH) and the paraventricular nucleus (PVN)(Harrold et al., 2012; Schloegl, Percik, Horstmann, Villringer, & Stumvoll, 2011; Smith, 2008). For example, the arcuate nucleus, has been shown to be responsible for sensing and responding to changes in food availability and energy reserves (Broberger, 2005; Jovanovic & Yeo, 2010; Konner, Klockener, & Bruning, 2009; Parker & Bloom, 2012; Shan & Yeo, 2011).

The hypothalamus receives input from the periphery, such as the gastrointestinal tract, mainly through the vagus nerve of the brain stem, and is also known to interact with other areas of the CNS such as the pituitary and nucleus accumbens involved in endocrine regulation and reward, respectively. A wide range of molecular components involved in these pathways have been identified

including genes, and their proteins and corresponding receptors, that show discrete functions in driving appetite or satiety. These proteins are known to interact with each other to inhibit or stimulate their respective orexigenic or anorexigenic properties. In the hypothalamus, neuropeptide Y (NPY) and agouti-related peptide (AGRP) have been identified as potent appetite stimulators (orexigens), they have an opposing effect to cocaine- and amphetamine-regulated transcript (CART) and pro-opiomelanocortin (POMC) which are potent appetite suppressors (anorexigens). Both NPY and AGRP, and POMC and CART are co-localized in their respective pairs in neurons of the arcuate-nucleus (ARC) with the expression of one pair of proteins having an inhibitory effect on the other pair (Arora & Anubhuti, 2006; Gorissen, Flik, G., and Huising, M. O., 2006; Harrold et al., 2012; Parker & Bloom, 2012; Rolls, 2011). These brain peptides are influenced by the peripheral molecules leptin and ghrelin. Leptin is an anorexigenic peptide produced in white adipose tissue which stimulates POMC and CART neurons and inhibits NPY and AGRP neurons in response to the state of energy reserves (Arora & Anubhuti, 2006; Figlewicz & Sipols, 2010; M. H. A. G. Gorissen, Flik, G., and Huising, M. O., 2006; Harrold et al., 2012; Parker & Bloom, 2012; Rolls, 2011; Williams, Cai, Elliott, & Harrold, 2004) Ghrelin is an orexigenic peptide produced primarily by the stomach which acts in contrast to leptin by stimulating NPY and AGRP neurons and inhibiting POMC and CART neurons in response to nutritional status (Anderson et al., 2005; Arora & Anubhuti, 2006; Broberger, 2005; Chaudhri, Small, & Bloom, 2006; Chen et al., 2009; Cummings, 2006; Figlewicz & Sipols, 2010; M. H. A. G. Gorissen, Flik, G., and Huising, M. O., 2006). This interaction between the CNS and periphery underlies the regulation of eating behaviour in mammals. To date, homologs of some of these brain regions and molecular components have also been identified in fish, however, they are relatively understudied.

### **1.2.1 The Fish CNS and Feeding Behaviour**

In fish, the neuronal pathways which regulate food intake are not well understood, however, brain areas which are thought to be homologues to mammalian neural regions involved in control of eating behaviour have been

identified (Cerdá-Reverter, Ling, Schiöth, & Peter, 2003; Cerda-Reverter & Peter, 2003). These areas include hypothalamic regions, areas of the dorsal and ventral telencephalon, the olfactory bulb, and the pituitary. The lateral tuberal nucleus of the hypothalamus has been identified in teleosts as a possible homologue to the arcuate nucleus of mammals, the vagal lobe of teleosts shows homology to the nucleus of the tractus solitarius, and the ventral telecephalic nuclei and preoptic nucleus may be homologs to the mammalian paraventricular nucleus (Berthoud & Morrison, 2008; Cerda-Reverter & Peter, 2003). In addition, expression of key feeding signals have been mapped to these regions in multiple teleostean species indicating these areas as an important part of the neural network which drives eating behaviours in fish. The comparative homology between mammalian and fish brain regions points towards a similar function in the control of appetite and energy balance in fish as in mammals and strong evidence is starting to be gathered, that supports this, from studies into feeding signals in the fish brain.

In several fish species orexigenic and anorexigenic neurons have been found in areas of the hypothalamus, thalamus, dorsal and ventral telencephalon, olfactory bulb, inferior lobe, optic tectum, cerebellum, amygdala, and pituitary (Forlano & Cone, 2007; Singru et al., 2008). In goldfish, expression of NPY and CART have been identified in the hypothalamus, pituitary, the olfactory bulb, optic tectum, and telencephalon, while AGRP (orexigen), POMC and  $\alpha$ -MSH (derived from anorexigenic POMC) have been found to be centrally expressed in the lateral tuberal nucleus of the hypothalamus with expression also demonstrated in the lateral recess nucleus, and the pituitary (Cerdá-Reverter, Ling, et al., 2003; Cerda-Reverter & Peter, 2003; Cerdá-Reverter, Schiöth, & Peter, 2003; Cottone et al., 2009; Kojima et al., 2010; a. P. Volkoff, 2000, 2001). In zebrafish NPY and AGRP activity has been characterised in the hypothalamus, dorsal and ventral telencephalon, olfactory bulb, optic tectum, and pituitary with overlapping expression of  $\alpha$ -MSH and AGRP in the hypothalamus, dorsal and ventral telencephalon, lateral septal, and amygdala (Forlano & Cone, 2007; Matsuda, Kang, Sakashita, Yahashi, & Vaudry, 2011; Matsuda, Sakashita, Yokobori, & Azuma, 2012; Yokobori et al., 2012). The localization of these neuropeptides to areas of the brain such as the hypothalamus and olfactory bulb, which play an

important role in feeding regulation in mammals, as well as in areas of neuroendocrine control such as the pituitary indicates these areas as similarly important brain regions for the control of feeding behaviour in fish (Arora & Anubhuti, 2006; Dietrich & Horvath, 2009; Harrold et al., 2012; Schloegl et al., 2011; Smith & Ferguson, 2008).

### **1.3 Developing a Fish Model for Feeding Behaviour**

Traditionally, animal models such as the mouse and rat, and studies in humans have been utilised to deduce the physiological, genetic, and biochemical underpinnings of appetite and food reward (Benelam, 2009; Breen, Yang, & Lam, 2011; Broberger, 2005; Morrison & Berthoud, 2007; Owyang & Heldsinger, 2011). While these models have produced a wealth of information regarding feeding motivation and behaviour, an often overlooked model for this type of research is that of fish. Understanding these physiological pathways and molecular components in fish has implications for research into human diseases such as obesity as they can be used as models to investigate the mechanisms by which feeding is motivated at a conserved evolutionary level. Obesity can be described as an excess of body fat storage due to reduced energy expenditure and increased energy intake which predisposes an individual to disease (Arora & Anubhuti, 2006; Harrold et al., 2012; Morrison & Berthoud, 2007). Obesity is a growing problem in first world nations and is one of the leading causes of morbidities such as cardiovascular disease, hypertension, and diabetes mellitus and also contributes to gall bladder disease, and cancer (Arora & Anubhuti, 2006; Meister, 2007; Suzuki et al., 2012). Obesity has many risk factors involved including genetic, endocrine, and cognitive factors which create an energy imbalance that gradually develops into an obese phenotype (Suzuki et al., 2012). The potential of hypothalamic and peripheral peptides and their receptors for use for or as targets of therapy for obesity as modulators of energy consumption has great potential but requires elucidation of the complex interactions of these peptides and the pathways they are involved in. However, having a fish model would also benefit the aquaculture industry, which supplies as much as 50% of the world's fish produce, but limitations persist in optimising the quality and quantity

of feed consumed, to insure optimal growth and yield without overfeeding, while minimising wasted feed, which is expensive (feed costs account for more than half of aquaculture budgets) and exacerbates water quality degradation to the detriment of stock health (Ali, 2010; Hormiga, Almansa, Sykes, & Torres, 2010). Understanding how feeding is regulated in fish will allow us to cater a diet specific to the needs of different fish species under different conditions (feeding, fasted, stressed, temperature variations, etc.), improving costs, stock health, and product yield. Progress towards aquacultural and obesity research using fish will require improvement of the fundamental knowledge of the mechanisms driving feeding and reward and while homologous neuropeptides have been identified in fish, they have yet to be comprehensively studied to the extent which they have been in mammals.

Fish, such as zebrafish (*Danio rerio*) and goldfish (*Carassius auratus*) are emerging as promising new models for studying eating behaviour and food reward. Fish homologs of mammalian genes, peptides, and neurological regions involved in appetite and satiety have been identified and are under investigation (Cerdá-Reverter et al., 2011; Cottone et al., 2009; H. Volkoff, 2006; H. Volkoff et al., 2005). Zebrafish (*Danio rerio*) are ideal models for laboratory research as they have several major advantages over other fish and mammalian models. At their maximum growth they reach only a length of about 6cm, meaning they can be kept in higher densities, require less feed per capita, and can even be kept in a simple aquarium set up with little maintenance (Giacomotto & Segalat, 2010; Lohr & Hammerschmidt, 2011; Rinkwitz, Mourrain, & Becker, 2011). A single female can regularly spawn over 100 synchronously developing embryos weekly which develop rapidly; the major organs of a developing zebrafish are usually recognizable within 24 hours and fully formed within a week, while sexual maturation can take 3 months (Giacomotto & Segalat, 2010; Lohr & Hammerschmidt, 2011; Rinkwitz et al., 2011). The full zebrafish genome has been sequenced and over 24, 000 genes have been identified, with the sequence showing a >70% identity to that of the human genome (Giacomotto & Segalat, 2010; Rinkwitz et al., 2011). The similarities that the zebrafish have to humans both genetically and neurophysiologically, as well as its exceptional qualities as a

laboratory animal, highlights it as an ideal candidate for research into eating behaviour with implications in aquaculture and obesity research.

## **1.4 Orexigenic and Anorexigenic Proteins in Fish**

### **1.4.1 Neuropeptide Y (NPY)**

In mammals NPY is one of the most potent stimulators of appetite, being shown to stimulate dose dependent, short term increases in food intake and delay the onset of satiety in rats. NPY is highly dispersed throughout the mammalian brain (particularly found within NPY/AGRP neurons of the ARC of the hypothalamus) and its action is mediated by G-protein coupled receptors of which there are five sub-types identified (Y1, Y2, Y4, Y5, and Y6). Of these receptors Y1 and Y5 are thought to be most important to driving appetite. (Arora & Anubhuti, 2006; Harrold et al., 2012; Parker & Bloom, 2012). Peptides of the NPY family (NPY, peptide YY, and peptide Y) have been identified and their cDNA sequences characterised in several fish species including zebrafish, in which the full gene sequence has been elucidated (Cottone et al., 2009; M. H. A. G. Gorissen, Flik, G., and Huising, M. O., 2006; Kehoe & Volkoff, 2007; MacDonald & Volkoff, 2009; H. Volkoff, 2006; H. Volkoff et al., 2005; Zhou et al., 2013). Additionally, six NPY receptor sub-types have been discovered: Y1, Y2, Y4, Y7, Y8a, and Y8b – activation of the Y1 receptor demonstrates an orexigenic effect, however, the full functions of each receptor are still unknown (Matsuda et al., 2011). Administration of both mammalian and fish NPY, and Y receptor agonists has been shown to produce dose dependent, short term increases in food intake in fish (including zebrafish), while administration of Y receptor antagonists decrease food intake; this is similar to what has been observed in mammals (Cottone et al., 2009; M. H. A. G. Gorissen, Flik, G., and Huising, M. O., 2006; Narnaware, 2002; H. Volkoff, 2006; Yokobori et al., 2012). In addition, feeding studies show that extended periods of starvation (several days to several weeks) significantly increase NPY mRNA expression levels in the hypothalamus of fish, with this effect being reversed upon refeeding (M. H. A. G. Gorissen, Flik, G., and Huising, M. O., 2006; MacDonald & Volkoff, 2009; H. Volkoff, 2006; H. Volkoff et al., 2005; Yokobori et al., 2012).

### 1.4.2 Agouti-Related Peptide (AGRP)

In mammals AGRP is an orexigenic peptide and endogenous antagonist (co-expressed with NPY in ARC neurons) which acts to stimulate feeding through the competitive antagonism of the melanocortins (involved in satiety) by selectively binding to MC-3 and MC-4 receptors. Central administration of AGRP to rodents has been shown to produce prolonged food intake - on a longer term (up to 1 week) than NPY (Arora & Anubhuti, 2006; Parker & Bloom, 2012). Compared to NPY, which is a relatively well studied neuropeptide in fish, its partner AGRP is comparatively understudied. Two copies of AGRP (AGRP1 and AGRP2) have been identified in several fish species, AGRP1 is primarily expressed in the brain in the lateral tuberal nucleus while AGRP2 is primarily expressed in the pineal gland (Cerda-Reverter et al., 2011; Moen, Murashita, & Finn, 2010; Murashita, Kurokawa, Ebbesson, Stefansson, & Ronnestad, 2009; Wan et al., 2012). In mammals AGRP exerts its orexigenic effect through interaction with the melanocortin receptors MC3 and MC4 (mammals have five melanocortin receptors: MC1- MC5) where it competitively antagonises anorexigenic melanocortins (Arora & Anubhuti, 2006; Parker & Bloom, 2012). Zebrafish have six melanocortin receptors (they have a duplicate MC5 receptor), however, interaction of AGRP with melanocortin receptors has only been demonstrated in sea bass where AGRP interacts with MC1 and MC4 receptors competitively antagonising melanocortins such as POMC, which binds to MC4 receptors (Cerda-Reverter et al., 2011; M. H. A. G. Gorissen, Flik, G., and Huising, M. O., 2006; Wan et al., 2012). Studies in zebrafish, goldfish (*Carassius auratus*), salmon (*Salmo salar*), and common carp (*Cyprinus carpio*) have shown that fasting up regulates hypothalamic AGRP mRNA expression in the hypothalamus, and transgenic zebrafish overexpressing AGRP produce obese phenotypes (Murashita, Kurokawa, Ebbesson, et al., 2009; Wan et al., 2012). Research into AGRP in fish is sorely lacking, with few studies representing AGRP expression levels under different feeding conditions and limited insight into the interactions of AGRP with the melanocortin system or in fact with peripheral signals of nutritional or energy status (Lohr & Hammerschmidt, 2011).

### 1.4.3 Pro-opiomelanocortin (POMC)

Mammal and avian species contain only one copy of the POMC gene however a duplication of POMC has resulted in multiple copies of this gene in fish, in fish two variants of POMC have been identified, POMCa and POMCb (Cardoso et al., 2011; Wei, Yuan, Zhou, et al., 2013; Zhang, Forlano, & Cone, 2012). POMC is a key component of the melanocortin system in mammals and undergoes post-translational processing to produce several peptides including adrenocorticotrophic hormone (ACTH), corticotropin intermediate peptide (CLIP),  $\beta$ -endorphin ( $\beta$ -END), and the melanocyte-stimulating hormones ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -MSH), of which  $\alpha$ -MSH is a potent anorexigen (Ager-Wick et al., 2013; Cardoso et al., 2011; Cerda-Reverter et al., 2011; H. Volkoff et al., 2005). Of these features, ACTH, CLIP, and  $\beta$ -END are conserved across fish while the MSH domains vary between species;  $\alpha$ - and  $\beta$ -MSH are present in both bony and cartilaginous fish,  $\gamma$ -MSH is deleted in euteleosts, and  $\delta$ -MSH is an addition to cartilaginous fish (Cardoso et al., 2011; Cerda-Reverter et al., 2011; Dores & Baron, 2011; Murashita & Kurokawa, 2011; Murashita, Kurokawa, Ebbesson, et al., 2009; Takahashi, Kobayashi, & Mizusawa, 2013; H. Volkoff et al., 2005). Few feeding studies have been conducted for POMC with contrary findings, in Atlantic salmon and rainbow trout fasting produced increases of POMC mRNA expression, whereas in goldfish no such increase was observed (Aguilar, Conde-Sieira, Polakof, Miguez, & Soengas, 2010; Conde-Sieira, Aguilar, Lopez-Patino, Miguez, & Soengas, 2010; Moen et al., 2010; Valen, Jordal, Murashita, & Ronnestad, 2011). There are similarly few studies on the interactions of POMC with peripheral peptides, although intraperitoneal administration of leptin to rainbow trout and atlantic salmon produces increased POMC mRNA expression, decreased NPY mRNA expression (only in the salmon), and decreased food intake (for up to 20 days of treatment); these studies have not been replicated in other fish species (Aguilar et al., 2010; Murashita & Kurokawa, 2011; Murashita, Kurokawa, Ebbesson, et al., 2009; Murashita, Uji, Yamamoto, Ronnestad, & Kurokawa, 2008). POMC, ACTH, and the MSHs have been shown to interact with the melanocortin receptors in fish, however, whether these interactions are responsible for the anorexigenic action of POMC are not clearly defined (Cerda-Reverter et al., 2011; Lohr & Hammerschmidt, 2011; Schioth et al., 2005; Selz et al., 2007; Takahashi et al., 2013; H. Volkoff et al., 2005; Wei, Yuan, Zhou, et al.,

2013). However, ICV administration of melanocortin receptor agonists and antagonists do demonstrate that the melanocortin system has an active role in regulating energy consumption in fish. In rainbow trout and goldfish, ICV administration of the melanocortin agonist NDP-MSH dose dependently reduces food intake, whereas administration of the antagonist HS024 and SHU9119 increases food intake in goldfish and rainbow trout, respectively (Cerdeira-Reverte et al., 2011; Schjolden, Schioth, Larhammar, Winberg, & Larson, 2009; Valen et al., 2011). Both HS024 and SHU9119 have been shown to competitively antagonise the MC4 receptor, the MC4 receptor is important in regulation of food intake in mammals and its role may be just as crucial in energy balance in fish (Cerdeira-Reverte et al., 2011; Schjolden et al., 2009).

#### **1.4.4 Cocaine- and Amphetamine-Regulated Transcript (CART)**

Mammalian CART is an anorexigen (expressed in neurons of the ARC and co-localised with POMC neurons) whose central infusion into rat brains affects decreases in food intake through inhibiting the action of NPY (Dietrich & Horvath, 2009; Harrold et al., 2012). CART has also been demonstrated to interact with reward circuitry (Harrold et al., 2012). The number of CART transcripts varies between fish species, for instance Atlantic cod (*Gadus morhua*) has a single CART transcript, goldfish and zebrafish have two CART transcripts (CART1 and CART2), and medaka (*Oryzias latipes*) have six (CART 3, 4, 6, 9, 11, and 22) (Hoskins & Volkoff, 2012; Lohr & Hammerschmidt, 2011; Murashita & Kurokawa, 2011; Wan et al., 2012). Feeding studies monitoring the expression of CART have been conducted in a range of fish species, including zebrafish, showing a decreased expression of CART mRNA or neuronal immunoreactivity during fasting periods (of up to 30 days), with the expression levels being reversed upon refeeding (Kehoe & Volkoff, 2007; Kobayashi, Peterson, & Waldbieser, 2008; Nishio et al., 2012; Subhedar, Barsagade, Singru, Thim, & Clausen, 2011; Valen et al., 2011; a. P. Volkoff, 2000, 2001; H. Volkoff, 2013; Wan et al., 2012). In addition intracerebroventricular administration of human CART has been demonstrated increasing satiety in goldfish while inhibiting NPY (Subhedar et al., 2011; Valen et al., 2011; a. P. Volkoff, 2000). However, despite these results, the neurophysiological mechanism behind the action of CART are

still poorly understood (Subhedar et al., 2011; Valen et al., 2011). Intracranial administration of leptin to a few fish species has had mixed results, as significantly increased expression of CART, decreased expression of NPY, and decreased food intake have been observed in goldfish while catfish (*Clarias gariepinus*), coho salmon (*Oncorhynchus kisutch*), and green sunfish (*Lepomis cyanellus*) showed no changes in food intake following leptin administration (Hoskins & Volkoff, 2012; Subhedar et al., 2011; Valen et al., 2011; Helene Volkoff, Joy Eykelbosh, & Ector Peter, 2003; Zhou et al., 2013). While fish CART transcripts exhibit similar effects to mammalian homologs, their interaction with peripheral signals of appetite and satiety remain confounded requiring further study, furthermore, the actions of the variable transcripts in some fish species remain undetermined.

#### **1.4.5 Ghrelin**

In mammals, ghrelin is the only known orexigenic peptide of the gut. It has the unique feature of being acylated by post-translational modification of the Serine-3 or Threonine-3 residues, and it is the N-terminal region containing these residues which comprise the active region of ghrelin responsible for receptor binding. The gene and peptide sequences of ghrelin have been identified in several fish species, and similar modifications of residues of the highly conserved N-terminal region have been demonstrated in many of these species; in zebrafish the second amino acid (a Serine) of the N-terminus of ghrelin is substituted for a Threonine (Jonsson, 2013; Kaiya et al., 2003; Kaiya, Miyazato, Kangawa, Peter, & Unniappan, 2008; Kaiya et al., 2005; Kawakoshi et al., 2007; Suda et al., 2012). Feeding studies have shown converse results, with ghrelin expression levels being increased during fasting and reduced after feeding in zebrafish, goldfish, atlantic salmon, and black seabram (*Acanthopagrus schlegelii*) but unchanged in rainbow trout, Nile tilapia (*Oreochromis niloticus*), and channel catfish (*Ictalurus punctatus*) under similar feeding conditions (Eom, Hong, Cone, & Song, 2013; Jonsson, 2013; Kaiya, Miura, Matsuda, Miyazato, & Kangawa, 2010; Kaiya et al., 2008; Kang, Yahashi, & Matsuda, 2011a, 2011b; Ma, Zheng, Mao, Zhou, & Wang, 2013; Murashita, Kurokawa, Nilsen, & Ronnestad, 2009; Parhar, Sato, & Sakuma, 2003; Peterson et al., 2012; Polakof, Miguez, & Soengas, 2011). Similarly, studies of the

direct action of ghrelin on food intake show species specific results. In goldfish, tilapia, and brown trout (*Salmo trutta*) central, ICV, or intraperitoneal (IP) administration of mammalian or fish acyl-ghrelin stimulate increases in food intake while in rainbow trout the opposite effect is observed (this is more similar to birds in which ghrelin is an anorexigen) (Jonsson, 2013; Kaiya et al., 2010; Kaiya et al., 2008; Kang et al., 2011b; Matsuda, Miura, Kaiya, Maruyama, Shimakura, et al., 2006; Matsuda, Miura, Kaiya, Maruyama, Uchiyama, et al., 2006; Unniappan & Peter, 2005; Yahashi, Kang, Kaiya, & Matsuda, 2012). In mammals ghrelin acts via the growth hormone secretagogue receptor (GHSR). Several GHSR transcripts have been identified in fish, including zebrafish, but whether these receptors mediate the action of ghrelin is unknown (Jonsson, 2013; Kaiya et al., 2010; Kaiya et al., 2008; Suda et al., 2012; Yahashi et al., 2012). In goldfish, black seabram (*Acanthopagrus schlegelii*) and grouper (*Epinephelus coioides*) the effect of acyl-ghrelin may be mediated by NPY which shows significantly increased expression when stimulated by ghrelin (Gao et al., 2012; Jonsson, 2013; Ma et al., 2013). Ghrelin shows promise for research as the sequence and modifications of the peptide are highly conserved in comparison to other vertebrates, however, ghrelin does not demonstrate consistent orexigenic action across species as it does in mammals. Further study is required in a wider range of fish species to elucidate whether the action of ghrelin is in fact species specific or determined by other factors (variations in physiological or environmental conditions).

#### **1.4.6 Leptins**

Multiple leptin genes have been isolated in fish: zebrafish, and japanese medaka (*Oryzias latipes*) have two leptin genes (lepA and lepB) which are thought to have arisen via gene duplication, while in arctic charr (*Salvelinus alpinus*), atlantic salmon, common carp, goldfish, silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idellus*) and pufferfish only a single copy of leptin, or multiple copies of the lepA gene have been identified (Denver, Bonett, & Boorse, 2011; Froiland, Murashita, Jorgensen, & Kurokawa, 2010; M. Gorissen, Bernier, Nabuurs, Flik, & Huising, 2009; Kurokawa & Murashita, 2009; Trombley, Maugars, Kling, Bjornsson, & Schmitz,

2012). In addition the leptin receptor, which mediates the actions of leptin, has been identified in multiple fish species (Ronnestad et al., 2010; Tinoco, Nisembaum, Isorna, Delgado, & de Pedro, 2012; Trombley et al., 2012). The protein sequences of leptin show low similarity (below 30%) between fish and in comparison to mammalian species, however, the tertiary structures of leptin across vertebrates are highly similar which may represent some conservation of function (Denver et al., 2011; Hoskins & Volkoff, 2012; Kurokawa & Murashita, 2009; Kurokawa, Murashita, Suzuki, & Uji, 2008; Ronnestad et al., 2010; Tinoco et al., 2012; Trombley et al., 2012). Feeding studies have shown species specific-like results and in some cases leptin expression contrasts what is observed in mammals. Burbot (*Lota lota*), goldfish and the striped bass (*Morone saxatilis*) show decreases in leptin expression during fasting and acute increases in leptin expression following feeding (similar to mammals); in comparison, sea bass, rainbow trout, and atlantic salmon show increases in leptin expression when fasted, while no effect on leptin expression has been observed in common carp and catfish (Gambardella et al., 2012; Huising et al., 2006; Kling et al., 2009; Kobayashi, Quiniou, Booth, & Peterson, 2011; Moen & Finn, 2013; Ronnestad et al., 2010; Tinoco et al., 2012; Trombley et al., 2012; Won, Baltzegar, Picha, & Borski, 2012). In zebrafish Gorissen, 2009 have shown that chronic fasting produces a decrease in lepb gene expression in the liver while lepa expression remains unchanged. Administration of leptin (IP, ICV, etc.) has produced similarly conflicting results (M. Gorissen et al., 2009). Mammalian leptin reduces food intake when administered to goldfish and striped bass but has no effect on green sunfish (*Lepomis cyanellus*) or coho salmon (*Oncorhynchus kisutch*) (Baker, Larsen, Swanson, & Dickhoff, 2000; de Pedro, Martinez-Alvarez, & Delgado, 2006; Kobayashi et al., 2011; Tinoco et al., 2012; Vivas et al., 2011; Helene Volkoff et al., 2003; Won et al., 2012). Administration of species specific leptin results in short term but not long term decreases in food intake in rainbow trout, grass carp, and atlantic salmon (Li et al., 2010; Murashita et al., 2008; Trombley et al., 2012). In these studies specifically the anorexigenic action of leptin seems to be mediated through hypothalamic pathways as POMC expression in atlantic salmon and rainbow trout is increased with administration of leptin, and in rainbow trout and grass carp NPY gene expression is decreased (Li et al., 2010; Murashita et al., 2011; Murashita et al., 2008) These results are more consistent

with the role of leptin in eating behaviour observed in mammals, however, like ghrelin, leptin also seems to have a species specific effect and its functions are still unclear.

### **1.5 Aims of this study**

Homologous regions of the CNS including the hypothalamus, and pituitary are conserved in zebrafish, and neuropeptide homologs have been mapped to regions of the hypothalamus. AGRP, and POMC neurons have been located in the lateral tuberal nucleus, NPY-like immunoreactivity has been characterised in the periventricular nucleus and the posterior tuberal nucleus, and CART immunoreactivity has been observed in the preoptic area and lateral hypothalamus (Cerdeira-Reverte et al., 2011; Forlano & Cone, 2007; Matsuda et al., 2012; Mukherjee, Subhedar, & Ghose, 2012; Yokobori et al., 2012). In addition the peripheral peptides leptin and ghrelin have been discovered in *D. rerio* located primarily in the liver and the gut respectively, although both show expression in the brain as well (Amole & Unniappan, 2009; Denver et al., 2011; Eom et al., 2013; M. H. A. G. Gorissen, Flik, G., and Huising, M. O., 2006; Liu et al., 2012). While these neuropeptides show functions in regulating feeding and energy status in the fish, the full picture is still to be elucidated. Many of the gene sequences are incomplete or not known, the interactions of the neuropeptides with each other and their receptors not fully understood, and the tendency for gene duplication in the fish genome means that there may be key gene variants still undiscovered. To build a comprehensive picture of the regulation of eating behaviour in Zebrafish and develop it as a more robust model this study will:

- 1) Use bioinformatic approaches to search the zebrafish genome and gene databases to identify neuropeptides involved in eating behaviour and confirm their identity.
- 2) Use 3' and 5' RACE to obtain the full length gene sequence of genes that are currently uncharacterised or unfinished.
- 3) Analyse the expression profiles of these genes under different feeding conditions .

## **2. Methods and Materials**

### **2.1 Animal husbandry**

Adult zebrafish (zf), *Danio rerio*, were obtained from a local pet store, and maintained in a recirculation system in the University of Waikato at 28°C. Fish were fed with tropical fish flake twice daily. Fish were given at least 1 week for acclimatization prior to treatment. Animals to be sacrificed were first anaesthetised in AQUI-S, after which their spinal cord was cut.

### **2.2 Gene candidate selection**

An initial literature search for published articles on mammalian genes, shown to be involved in regulating eating behaviour and/or eating reward was carried out. From this a list of both anorexogenic and orexigenic genes was chosen as genes of interest to look for with regards fish eating behaviour. Some of these, such as Oxytocin (Oxy), vasopressin (AVP), Cocaine- and amphetamine-regulated transcript (CART), Pro-opiomelanocortin (POMC) and Neuropeptide Y (NPY) already had homologues identified in zebrafish and published on. However, there were a large number of genes that remained uncharacterized, which included Islet Amyloid Polypeptide (IAPP), Apelin (APLN), Cholecystokinin (CCK), Fat mass and obesity-associated transcript (FTO), Leptin (LEP), Peptide YY (PYY), Ghrelin (GHRL) and Agouti-related peptide (AgRP). Zebrafish homologues for these genes were searched for using the NCBI gene database and the zebrafish genome. Basic local alignment similarity tool (BLAST; Altschul, ) was initially used to search the database and genome, using amino acid sequences of known fish and mammalian molecules. Any regions of interest highlighted from the search within the zebrafish genome was subsequently analysed for possible coding regions using Genscan (Burge and Karlin) which identified the potential eating behaviour genes. This approach along with sequence information identified from available expressed sequence tags (EST's) allowed the construction of partial coding regions for Islet Amyloid Polypeptide (IAPP), Apelin (APLN), Cholecystokinin (CCK), Fat mass and obesity-associated transcript (FTO), Leptin (LEP), Peptide YY (PYY), Ghrelin (GHRL) and Agouti-related peptide (AgRP). In addition, on searching additional copies of POMC (termed POMCb) and three other copies of

CART (termed CART 1, 3 and 4) were discovered. Due to the amount of genes discovered, it was decided to focus on a selection of these for further investigation and included GHRL, AgRP, POMCb, CART 1, CART 3 and CART 4.

### **2.3 Sequence analysis**

To help in the further identification of the zebrafish GHRL, AgRP, POMCb, CART 1, CART 3 and CART 4, the predicted sequence was analyzed for similarity to other known vertebrate nucleotide and protein sequences using the BLAST suite of programs (Altschul, 1990). Multiple sequence alignments were generated using ClustalW v1.60 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) and phylogenetic relationships were established using the neighbour-joining method using the Geneious (Biomatters Ltd.) software (Kearse et al., 2012). Protein sequences were first aligned by ClustalW using the BLOSUM cost matrix, and trees constructed using the Geneious Tree Builder. Genscan [Burge and Karlin] and BLAST (Altschul, 1990) were used to predict genes within around the the locus of the genes of interest to examine the synteny that exists between them and the same locus within the human genome. In addition, the Expert Protein Analysis System (ExPASy) proteomics server of the Swiss Institute of Bioinformatics (SIB) (31) was used to analyze the predicted aa sequences obtained using Simple Modular Architecture Research Tool (SMART) and the TMpred programme to confirm putative protein structure. Once satisfied the best prediction of these gene sequences had been obtained, primers were designed to allow the amplification of the full length cDNA using rapid amplification of cDNA ends (RACE).

### **2.4 Primer design**

Using Primer3 (Untergasser et al., 2012) two pairs of primers were designed for GHRL, AGRP, POMCb, CART1, CART3, and CART4 (Table 1) for use in 3'-RACE PCR (F1 and F2) and 5'RACE PCR (R1 and R2) . Primers were designed with a: melting temperature (55 - 60°C), GC content (40 - 60%), 3' GC clamp (1-2bp). Primer pairs were checked for self complementarity and complementarity between each primer using OligoCalc (Kibbe WA. (2007).

Primer Name	Sequence (5' > 3')
<b>CART1 F1</b>	TCAAGAAGTTCTGGAAAAGCTGAG
<b>CART1 F2</b>	CTGCTGTGGAGAAGAAGCTC
<b>CART1 R1</b>	ACTGGAGCATTATGAGAGACTGAG
<b>CART1 R2</b>	CTGGACAACCTGCACAACCTCC
<b>CART3 F1</b>	CAAACAACCTCCACAAACCGG
<b>CART3 F2</b>	GTTGAGCCTACTTCCTTCGTG
<b>CART3 R1</b>	AATGGCATGATGTTCCCTTGTGG
<b>CART3 R2</b>	TCTTGACCTTTACGTATTGCAC
<b>CART4 F1</b>	GATGAACTTCTGGATGGAGAACAG
<b>CART4 F2</b>	AAAAGCAAGCGTCATTCCCAG
<b>CART4 R1</b>	CAGGAAGAAAGTGTTGCAGG
<b>CART4 R2</b>	CCTCGCATAACAGTCACACAG
<b>GHRL F1</b>	TTCCTTGTGTCTCGAGTCTGTG
<b>GHRL F2</b>	CTCAGTCCGACTCAGAAACC
<b>GHRL R1</b>	CTTCTTTGATCACTGGTATCTCTGG
<b>GHRL R2</b>	CTTCTCTTCTGCCACTCTTG
<b>AGRP F1</b>	AGATACTAGAAGACCTTGAAGCCTATG
<b>AGRP F2</b>	TGTATCCCTCATCAACAGTCCTG
<b>AGRP R1</b>	TTTTGCAGGTGTTGTCCATG
<b>AGRP R2</b>	AGTAACAGAAGGCCTTAAAGAAGC
<b>POMCb F1</b>	GAAGAACAGGTAGAGGAGCAGAG
<b>POMCb F2</b>	GCAGACCGGTAAAAGTGTTGAG
<b>POMCb R1</b>	AGTCCTCCGTATCGTTTGTCTG
<b>POMCb R2</b>	TTTGACGTTGTTCCCTGTGTTG

**Table 1.** PCR primers for use in RACE PCR.

## 2.5 Preparing tissue samples

Zebrafish were removed from their tank and kept in a lidded container for transport to the lab area, keeping time between transport and dissection to a minimum to limit stress. Zebrafish brains were dissected in an environment that was as RNase free as possible with tools that had been treated with DEPC. Briefly, scissors were used to sever the head from the fish, after which the top of the severed head above the eyes, was removed with a scalpel revealing the whole brain which could be extracted with a pair of fine tweezers. Three brains were dissected at a time and placed in 500µL of Lysis/Binding Buffer (from Dynabeads® mRNA DIRECT™ Kit) within a 2ml screw top tube. The brains were immediately used for mRNA extraction to minimise RNA degradation.

## **2.6 mRNA extraction**

To ensure the brain tissue was completely homogenised in the lysis buffer, 0.5mm glass and 0.1mm silica/zirconia beads (dnature) were added and shaken vigorously using a mini-beadbeater (Alphatech) until no visible pieces of brain tissue remained. The mRNA was then extracted using the Dynabeads® mRNA DIRECT™ Kit (Life Technologies), following the manufacturer's protocol. Briefly, mRNA was extracted from 300µL of the sample lysate (the minimum volume for this protocol due to the small total mass of the tissue available). Dynabeads® are magnetic beads bearing oligo(dT)<sub>25</sub> residues which specifically bind the 3' polyA tail of mRNA allowing isolation of solely mRNA from crude lysate and total RNA. Dynabeads® were added to the sample lysate and incubated at room temperature (21°C) in a thermomixer (Eppendorf) shaking at 900 rpm, for 3 minutes in to allow the mRNA to hybridise to the beads. Dynabeads® are magnetic beads bearing oligo(dT)<sub>25</sub> residues which specifically bind the 3' polyA tail of mRNA allowing isolation of solely mRNA from crude lysate and total RNA. The sample was then placed into a DynaMag™-2 magnet (which draws the magnetic beads to one side), allowing the supernatant to be removed without disturbing the mRNA bearing beads. The beads then underwent two washes, using Washing Buffer A and B, to remove any unbound material such as rRNAs which are unwanted contaminants in cDNA synthesis (supernatant was removed using the magnet after each wash). Finally, the beads were incubated at 70°C for 2 minutes with 10µL of DEPC water to elute the mRNA from the beads, then using the magnet, the supernatant containing the mRNA was transferred to a new tube. The extracted mRNA was used immediately for construction of the 5'- and 3'- RACE libraries using the FirstChoice® RLM-RACE Kit (Life Technologies).

## **2.7 RACE library construction**

The FirstChoice® RLM-RACE Kit (Life Technologies) allows construction of a 3' and 5' RACE cDNA library only from intact full length mRNA bearing the 5'-cap, this ensures synthesis of a high quality cDNA library. The RACE library was constructed following the manufacturers instructions. Briefly, for the 3' RACE library, 2µL of extracted mRNA was reverse transcribed using a 3' RACE Adapter oligonucleotide (5'-GCGAGCACAGAATTAATACGACTCACTATAGGT<sub>12</sub>VN-

3') and M-MLV Reverse Transcriptase with an incubation of one hour at 42°C. The cDNA was then stored at -20°C until it was needed for 3' RACE PCR. For the 5'RACE library, 2µL of extracted mRNA was treated with Calf Intestine Phosphatase (CIP) to remove the free 5'-phosphates of fragmented mRNAs (and any contaminating DNA or other RNA molecules) and incubated for 1 hour at 37°C. Next, 15 µL Ammonium Acetate solution, 115 µL Nuclease-free Water and 150 µL acid phenol:chloroform were added, mixed thoroughly and centrifuged  $\geq 10,000 \times g$  for 5 mins at room temperature. The aqueous phase (top layer) was transferred to a new tube, 150 µL chloroform added, mixed thoroughly and centrifuged  $\geq 10,000 \times g$  for 5 mins at room temperature. The aqueous phase (top layer) was transferred to a new tube, 150 µL isopropanol added, mixed thoroughly and chilled on ice for 10 mins to precipitate the mRNA out of solution. After centrifugation  $\geq 10,000 \times g$  at room temperature for 20 minutes the pellet was rinsed with 0.5 mL cold 70% ethanol (to remove salts, and contaminants), centrifuged for a further 5 minutes at maximum speed, the ethanol removed and the pellet left to air dry. The pellet was resuspended in 11µL of DEPC water. In a fresh tube, 1 µL 10X TAP buffer, 2 µL Tobacco Acid Pyrophosphatase, 2 µL Nuclease-free Water was added to 5 µL of the previously CIP'd RNA and incubate at 37°C for one hour, to remove the 5' cap from the full length mRNA leaving open the 5' monophosphate. Finally, in a fresh tube, 1 µL 5' RACE Adapter, 2 µL T4 RNA Ligase (2.5 U/µL), 4 µL Nuclease-free Water and 1 µL 10X RNA Ligase Buffer were added to 5 µL CIP/TAP-treated RNA and incubate at 37°C for one hour to allow the ligation of a 45bp 5' RACE Adapter oligonucleotide (5'-GCUGAUGGCGAUGAAUGAACACUGCGUUUGCUGGCUUUGAUGAAA-3') to the free 5' monophosphate. mRNA was reverse transcribed into cDNA using Moloney murine leukemia virus (M-MLV) Reverse Transcriptase and Random Decamers with an incubation of one hour at 42°C. The cDNA was then stored at -20°C until it was needed for 5' RACE PCR.

## 2.8 3' RACE PCR

Using 3' RACE allows for amplification of the full 3' region of the genes of interest, coupled with 5' RACE the full sequence of a gene can be amplified. A nested PCR approach was used to lessen the chance of amplification of non-specific products., Nested PCR involves two successive rounds of amplification in which the second round primers are designed to bind within the sequence of the amplified first round product. This reduces non-specific amplification of unwanted sequences due to erroneous primer binding as untargeted first round products will not contain the target sequence for second round amplification. The forward primers previously designed were used in conjunction with the 3' RACE Outer Primer (5'-GCGAGCACAGAATTAATACGACT-3') and 3' RACE Inner Primer (5'-CGCGGATCCGAATTAATACGACTCACTATAGG-3') provided with the kit. 3' RACE PCR was set up in using the following components, which included HOT FIREPol® DNA Polymerase (Solis BioDyne), and reaction conditions for first and second round PCR:

### *First Round PCR components:*

#### *conditions:*

2µL 10X B1 Buffer	HotStart	94°C, 15 minutes
1.5µL 25mM MgCl	Denaturation	94°C, 30 seconds
0.8 µL 20mM dNTPs	Annealing	56°C, 30 seconds
0.5 µL 3' RACE library	Extension	72°C, 45 seconds
0.2 µL HOT FIREPol® DNA Polymerase	Final Extension	72°C, 10 minutes
2 µL 10µM 3'RACE outer adapter primer		
2 µL 10µM 3' Forward primer (F1)		
11.0 µL DEPC water		

### *First Round reaction*

### *Second Round PCR components:*

2µL 10X B1 Buffer  
1.5µL 25mM MgCl  
0.8 µL 20mM dNTPs

### *Second Round reaction conditions:*

HotStart 94°C, 15 minutes  
Denaturation 94°C, 30 seconds  
Annealing 58°C, 30 seconds

0.5 $\mu$ L first round product	Extension	72°C, 45 seconds
0.2 $\mu$ L HOT FIREPol® DNA Polymerase	Final Extension	72°C, 10 minutes
2 $\mu$ L 10 $\mu$ M 3'RACE inner adapter primer		
2 $\mu$ L 3' 10 $\mu$ M Forward primer (F2)		
11.0 $\mu$ L DEPC water		

The annealing temperature was increased in the second round to further increase the specificity of primer annealing to prevent non-specific amplification of untargeted sequences. Electrophoresis of the products of each round of PCR amplification were run on a 1% TAE gel (ethidium bromide stain) using the EC250-90 (E-C Apparatus Corporation), at 90 volts for 1 hour alongside a 100bp ladder and visualized under UV light (TFX-35M - Life Technologies) to estimate product size and compare it to that of the predicted sequence sizes. Products of correct sizes were subsequently used for ligation into a cloning vector.

## 2.9 5' RACE PCR

Using 5' RACE allows for amplification of the full 5' region of the genes of interest. The reverse primers previously designed were used in conjunction with the 5' RACE Outer Primer (5'-GCTGATGGCGATGAATGAACACTG-3') and 5' RACE Inner Primer (5'-CGCGGATCCGAACACTGCGTTTGCTGGCTTTGATG-3') provided with the kit.

5' RACE PCR was set up in using the following components, which included HOT FIREPol® DNA Polymerase (Solis BioDyne), and reaction conditions for first and second round PCR:

<i>First Round PCR components:</i>	<i>First Round reaction</i>	
<i>conditions:</i>		
2 $\mu$ L 10X B1 Buffer	HotStart	94°C, 15 minutes
1.5 $\mu$ L 25mM MgCl	Denaturation	94°C, 30 seconds
0.8 $\mu$ L 20mM dNTPs	Annealing	56°C, 30 seconds
0.5 $\mu$ L 5' RACE library	Extension	72°C, 45 seconds

0.2  $\mu$ L HOT FIREPol® DNA Polymerase      Final Extension 72°C, 10 minutes  
 2  $\mu$ L 10 $\mu$ M 5'RACE outer adapter primer  
 2  $\mu$ L 10 $\mu$ M 5'Reverse primer (R1)  
 11.0  $\mu$ L DEPC water

*Second Round PCR components:*

2 $\mu$ L 10X B1 Buffer  
 1.5 $\mu$ L 25mM MgCl  
 0.8  $\mu$ L 20mM dNTPs  
 0.5  $\mu$ L First round product  
 0.2  $\mu$ L HOT FIREPol® DNA Polymerase  
 2  $\mu$ L 10 $\mu$ M 5'RACE inner adapter primer  
 2  $\mu$ L 10 $\mu$ M 5' Reverse primer (R2)  
 13.0  $\mu$ L DEPC water

*Second Round reaction conditions:*

HotStart      94°C, 15 minutes  
 Denaturation      94°C, 30 seconds  
 Annealing      58°C, 30 seconds  
 Extension      72°C, 45 seconds  
 Final Extension 72°C, 10 minutes

As for 3'RACE, the annealing temperature was increased in the second round to further increase the specificity of primer annealing to prevent non-specific amplification of untargeted sequences.

Due to 5'RACE not amplifying correctly sized products, the following reaction conditions were tested to improve 5' RACE PCR product yield (reaction components used and their volumes, remained unchanged):

	<i>First Round</i>	<i>Second Round</i>
HotStart	94°C, 15 minutes	94°C, 15 minutes
Denaturation	94°C, 30 seconds	94°C, 30 seconds
Annealing	*58°C, 30 seconds	*60°C, 30 seconds
Extension	72°C, 45 seconds	72°C, 45 seconds
Final Extension	72°C, 10 minutes	72°C, 10 minutes

\* Annealing temperatures were changed in the first and second round to increase specificity

### *First Round*

HotStart	94°C, 15 minutes		
Denaturation	94°C, 30 seconds	Denaturation	94°C, 30 seconds
Annealing	*62°C, 30 seconds	Annealing	*54°C, 30 seconds
Extension	72°C, 30 seconds	Extension	72°C, 30 seconds
		Final extension	72°C, 10 minutes

\*The first round used a type of touchdown PCR where the first four cycles were run at a high temperature, which was dropped for the following 30 cycles. The second round proceeded under standard conditions.

In addition, due to these conditions not working and a small product being found in all PCR's run, the above PCR's were also carried out with negative controls which had either 1) all components minus the 5' RACE library DNA or 2) all components minus the 5' inner adapter primer. to check for contamination in both first and second round reactions (standard reaction conditions were used).

Electrophoresis of the products of each round of PCR amplification were run on a 1% TAE gel (ethidium bromide stain) using the EC250-90 (E-C Apparatus Corporation), at 90 volts for 1 hour alongside a 100bp ladder and visualized under UV light (TFX-35M - Life Technologies) to estimate product size and compare it to that of the predicted sequence sizes. Products of correct sizes were subsequently used for ligation into a cloning vector.

## **2.10 Cloning of PCR products**

### **2.10.1 Media preparation**

Lysogeny Broth (LB) and LB agar ampicillin (LB+) plates were prepared prior to vector ligation of PCR products to grow transformed bacterial colonies. LB was prepared by adding 7.5 of LB agar, 5g NaCl, 5g Tryptone, and 2.5g yeast extract to 500mL of Milli-Q™ water (a highly purified, sterile water), gently mixing. The media was autoclaved in an Astell benchtop autoclave (Acron scientific) under the media setting, allowed to cool and stored at room temperature. LB+plates were

prepared by adding 7.5 of LB agar, 5g NaCl, 5g Tryptone, and 2.5g yeast extract to 500mL of Milli-Q™ water (a highly purified, sterile water), gently mixing. The media was autoclaved in an Astell benchtop autoclave (Acron scientific) under the media setting. It was then cooled in a Julabo 12B waterbath (Life Technologies) set at 55°C, after which 500µL of ampicillin (50µg/µL) was added, gently mixing. Finally, the media was carefully poured into sterile petri dishes (90mm), filling no more than half the volume of each dish, and allowed to cool until set. The plates were then stored at 4°C.

### **2.10.2 Ligation of PCR product**

Two cloning vector kits were used for ligation of 3' and 5' RACE PCR products, TA cloning® Kit with pCR® 2.1 (Life Technologies) and pLUG-PRIME® TA-Cloning Vector Kit (iNtRON Biotechnology, Inc.). Each kit uses TA cloning, where PCR products that have A overhangs are inserted into the target vector by ligating to T (deoxythymidine) overhangs present in the linearized cloning vectors, removing the need for restriction enzymes. The TA cloning® Kit required a single reaction to which was added 4µL of 3' or 5' PCR product, 2µL linearized pCR2.1 vector (25ng/µL), 1µL 10X T4 DNA Ligase Reaction Buffer, 1µL ExpressLink™ T4 DNA Ligase, and 2µL DEPC water, with an overnight incubation at 4°C. The pLUG-Prime® TA-Cloning Vector Kit required a single reaction to which was added 2µL 3' or 5' PCR product, 2µL linearized TA-Cloning Vector (25ng/µL), 1µL of 10X Ligation buffer A, 1µL of 10X Ligation buffer B, 1µL T4 DNA ligase, and 3µL of DEPC water, with an overnight incubation at 4°C. Where PCR products were not freshly used, an extra step of incubating the product with HOT FIREPol® Taq polymerase (Solis BioDyne) was incorporated which adds A (deoxyadenosine) overhangs to the 3' end of PCR products..

The ligation was then used for bacterial transformation.

HOT FIREPol® Taq polymerase adds A (deoxyadenosine) overhangs to the 3' end of PCR products, using either cloning kit PCR products are inserted into the target vector by ligating the A overhangs to T (deoxythymidine) overhangs present in the linearized cloning vectors - removing the need for restriction enzyme.

### 2.10.3 Transformation of chemically competent *E. coli*

*E.cloni*® 10G & 10GF' Chemically Competent Cells (Lucigen) provide high yields of high quality plasmid DNA without deletions or rearrangements of the inserts, so are ideally suited for plasmid cloning for sequencing. In preparation for use with the transformed bacterial cells, 50µL X-gal (Sigma) and 50µL IPTG (Sigma) was spread on the previously prepared LB+ plates which were then warmed at 37°C in a Precision Economy Incubator (Precision Scientific) till required. Both vectors used for transformation contain the LacZ gene which encodes β-galactosidase, the vectors containing the PCR insert have this gene, and its function, disrupted. IPTG stimulates expression of LacZ and X-gal is a substrate which is hydrolysed by β-galactosidase producing a blue colour in transformed colonies without the insert which streamlines colony selection as transformed colonies with the insert are white in colour. *E.cloni*® cells were transformed following the manufacturer's instructions. Briefly, *E.cloni*® cells (kept at -80°C) were thawed slowly on ice for 15 minutes, during which time 4µL of the ligation reaction was heat inactivated at 70°C (to prevent transformation failure). Following thawing, 5µL of the heat inactivated ligation reaction was added to the *E.cloni*® cells and they were incubated on ice for 45 minutes (to allow plasmid to interact with the bacterial membrane) followed by a 45 sec heat shock at 42°C (to allow entry of the plasmid into the bacterial cell) and another 2 minutes on ice (to allow the bacterial membrane to stabilise). 160 µL of room temperature recovery media was then added and the transformed cells were incubated for 2 hours at 37°C in a Orbital Mixer Incubator (Ratek) on a rotation setting of 9. This allows the transformed cells time for the ampicillin resistance gene to express its product. Finally, the cells were spread on the LB+ plates and incubated overnight at 37°C in a Precision Economy Incubator (Precision Scientific). Plates with white colony growth were subsequently subsequently stored at 4°C until they were screened using PCR. Two vectors were used as despite multiple attempts and an extension of incubation time to 48 hours, few colonies were obtained using the TA cloning® Kit with pCR® 2.1, , hence the switch to pLUG-Prime® TA-Cloning Vector which provided better results.

#### 2.10.4 Colony screening

Up to ten colonies per plate which had successfully been transformed (white in colour) were selected for screening by picking them from the plate using a pipette tip and transferring it to 2 $\mu$ L of DEPC water (transferring agarose was avoided as it would inhibit PCR reactions). This was then used as a template for PCR using primers M13 forward (5'-TGTAACGACGGCCAGT-3') and M13 reverse (5'-CAGGAAACAGCTATGAC-3'), which are found within the vector either side of the ligated PCR product. A PCR was set up for each colony sample using the following reaction conditions and components for cells transformed with pLUG-Prime<sup>®</sup> TA-Cloning Vector:

<i>PCR components:</i>	<i>PCR reaction conditions:</i>
2 $\mu$ L 10x B1 Buffer	HotStart 94 $^{\circ}$ C, 5 minutes
1.5 $\mu$ L 25mM MgCl	Denaturation 94 $^{\circ}$ C, 30 seconds
0.8 $\mu$ L 20mM dNTPs	Annealing 55 $^{\circ}$ C, 30 seconds <sup>2</sup>
$\mu$ L colony DNA	Extension 72 $^{\circ}$ C, 30 seconds
0.2 $\mu$ L HOT FIREPol <sup>®</sup> DNA Polymerase	Final Extension 72 $^{\circ}$ C, 7 minutes
2 $\mu$ L M13 forward primer	
2 $\mu$ L M13 reverse primer	
11.5.0 $\mu$ L DEPC water	

Electrophoresis of the products of each round of PCR amplification were run on a 1% TAE gel (ethidium bromide stain) using the EC250-90 (E-C Apparatus Corporation), at 90 volts for 1 hour alongside a 100bp ladder and visualized under UV light (TFX-35M - Life Technologies) to estimate product size and compare it to that of the predicted vector plus insert sizes. Products of correct sizes were subsequently used for isolation of plasmid.

Products of correct sizes were noted and their corresponding colonies had samples transferred to 5mL of LB media, to which 0.5mL of ampicillin (50 $\mu$ g/ $\mu$ L) was added, and then incubated overnight at at 37 $^{\circ}$ C in the Orbital Mixer Incubator (Ratek) on a rotation setting of 9. Media which had turned cloudy with cell

growth were immediately moved to the plasmid purification step.

### **2.10.5 Plasmid purification**

Transformed *E.coloni*® cells containing products of the correct sizes were again picked from the plate, using a pipette tip and directly transferred to 5mL of LB media, containing 0.5mL of ampicillin (50µg/µL) and incubated overnight at 37°C in the Orbital Mixer Incubator (Ratek) on a rotation setting of 9. The next day, media which had turned cloudy with cell growth immediately underwent plasmid purification. Plasmids were purified from the successfully transformed *E.coloni*® cells using the QIAprep® Miniprep Kit (QIAGEN) following the manufacturer's protocol. The QIAprep® Miniprep Kit uses silica-gel-membrane-filtered QIAprep spin columns to specifically purify plasmid DNA from cell lysate without having to do a phenol-chloroform extraction and an isopropanol precipitation and ethanol wash step, as phenol and ethanol contamination can inhibit downstream enzymatic applications. Briefly, 1mL of overnight LB media culture was transferred to a 1.5mL Eppendorf tube and centrifuged at full speed for 1 min in a 5415R centrifuge (Life Technologies), the supernatant was discarded, and the process repeated once more by adding another 1 mL of bacterial suspension, to guarantee enough plasmid for the extraction. The remaining culture was stored at 4°C and the pelleted bacterial cells were resuspended in 250µL Buffer P1 containing RNase A which removes RNA molecules released during alkaline lysis. 250µL of Buffer P2 was subsequently added, which is the lysis buffer to release the cells contents and mixed thoroughly by inverting the tube 6 times. Soon after 350 µl Buffer N3 was added and mixed thoroughly by inverting the tube 6 times, neutralising the lysate and adjusting it to high-salt binding conditions which causes cell debris (including proteins and chromosomal DNA) to precipitate out of solution leaving only plasmid DNA in solution. The tube was centrifuged at full speed for one 1 minute in a 5415R centrifuge (Life Technologies), where a compact white pellet forms. The supernatant is poured directly into a QIAprep spin column and centrifuged at full speed for one 1 minute in a 5415R centrifuge (Life Technologies), allowing the supernatant to move through the silica-gel-membrane, where the DNA is adsorbed.

The liquid is discarded and 0.5 ml PB buffer is added to the column and centrifuged at full speed for one 1 minute in a 5415R centrifuge (Life Technologies), to remove trace nuclease activity. The liquid is again discarded and 0.75 ml of Buffer PE is added to the column and centrifuged at full speed for one 1 minute in a 5415R centrifuge (Life Technologies), to remove salts. Once the liquid was discarded, the column was spun again to make sure as much wash as possible was removed as it contains ethanol. Finally, the plasmid DNA was eluted from the column into a new tube by adding 50 $\mu$ L of DEPC water and a final centrifugation step at full speed for one 1 minute in a 5415R centrifuge (Life Technologies). The concentration of plasmid DNA purified was measured using the Nanodrop 2000 (Thermo Scientific). All samples with a significantly high concentration of DNA ( $\geq 1\mu\text{g}/\mu\text{L}$ ), with a preferred 260/280 absorbance ratio of approximately  $\geq 1.8$ , then underwent restriction digest to confirm they contained the PCR insert before they were sequenced.

#### **2.10.6 Restriction digest of plasmid**

To check the PCR products were still inserted in the purified plasmid, a restriction endonuclease *Hind* III (Roche) was used as it creates cuts at both ends of the PCR insert region of the pLUG-Prime<sup>®</sup> TA-Cloning Vector. Following the *Hind* III protocol, a single reaction was set up using x $\mu$ L (equal to 1 $\mu$ g) of DNA, 2.5 $\mu$ L of 10X SuRE/Cut Buffer B, and 1 $\mu$ L of *Hind* III (10U/ $\mu$ L) made up to 25 $\mu$ L with DEPC water and incubated at 37 $^{\circ}$ C overnight. Electrophoresis of the restricted products were run on a 1% TAE gel (ethidium bromide stain) using the EC250-90 (E-C Apparatus Corporation), at 90 volts for 1 hour alongside a 100bp ladder and visualized under UV light (TFX-35M - Life Technologies) to visualise the vector and the restricted PCR product. Plasmids containing the correct size PCR product were subsequently used for sequencing.

## 2.11 Gel excision of RACE PCR products

Due to problems with bacterial transformation and confirmation of gene insert, as an alternative RACE PCR products were gel purified after electrophoresis using the QIAquick® Spin Kit (QIAGEN), and directly sequenced. In addition, gel excision of selected first round 5' RACE PCR product was also carried out in an attempt to remove contamination problems and improve second round product yield. Similar to the QIAprep® Miniprep Kit, the QIAquick® Spin Kit uses silica-membrane-filtered columns for purification of up to 10µg of DNA from agarose gel. The kit provides efficient recovery of DNA while removing contaminants that would inhibit sequencing reactions.

To maximise band separation, RACE PCR products were run on a 2% TAE gel (ethidium bromide stain) using the EC250-90 (E-C Apparatus Corporation), at 70 volts for 2 hour alongside a 100bp ladder and visualized under UV light (TFX-35M - Life Technologies). Selected bands of correct sizes were cut from the gel with a sterile scalpel and transferred to an Eppendorf tube, with exposure to UV light minimised to limit DNA crosslinking. The excised gel was weighed with CTX-600 (AND) and then 3 volumes of Buffer QG to 1 volume of gel (100 mg ~ 100 µl) and incubated at 50°C for 15 minutes at 800 rpm in a thermomixer (Eppendorf). Buffer QG contains a pH indicator which colours the solution yellow at optimal pH ( $\leq 7.5$ ), adsorption of the DNA to the silica-membrane is highest (approximately equal to 95%) under these pH conditions. 1 gel volume of isopropanol was added to the sample and mixed, to increase DNA yield of fragments  $<500$  bp and  $>4$  kb. The sample was transferred to a QIAquick® spin column, centrifuged at full speed for one 1 minute in a 5415R centrifuge (Life Technologies) and the supernatant discarded. During this step only the DNA adsorbs to the silica-membrane while other components from the PCR and gel electrophoresis steps (ethidium bromide, primers, agarose, etc.) flow through the column. To remove residual agarose, 0.5mL of Buffer QG was added to the column, followed by centrifugation at full speed for one 1 minute in a 5415R centrifuge (Life Technologies) and supernatant removed. Subsequently, the column was washed with 0.75mL of Buffer PE, to remove salts and underwent centrifugation at full speed for one 1 minute in a 5415R centrifuge (Life Technologies) twice to collect and remove as much wash as possible as it contains

ethanol. Finally, the DNA was eluted from the column into a new tube with 30 $\mu$ L of DEPC water and a final centrifugation step at full speed for one 1 minute in a 5415R centrifuge (Life Technologies). The concentration of purified DNA was measured using the Nanodrop 2000 (Thermo Scientific). All samples with a significantly high concentration of DNA ( $\geq 1\mu\text{g}/\mu\text{L}$ ), with a preferred 260/280 absorbance ratio of approximately  $\geq 1.8$  were sequenced.

## 2.12 Sequencing and analysis

Multiple copies of DNA samples were sequenced in one direction using the M13 Forward primer or a specific primer if the PCR product had been sent for sequencing. Samples were sent to the University of Waikato Sequencing facility, where they underwent preparation using the BigDye® Terminator v3.1 Cycle Sequencing kit (Life Technologies) and were run on a 3130xl Genetic Analyser System (Life Technologies). Sequences obtained were analysed using Geneious (Biomatters Ltd.) software (Kearse et al., 2012) and then compared against the predicted sequences using ClustalW v1.60 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). Where possible a consensus sequence was built using multiple sequences amplified by the same primer pairs.

## 2.13 Realtime expression of eating behaviour genes in zebrafish

### 2.13.1 Primer design

PCR primers were designed (Table 2) so that at least one primer in each pair straddled a predicted splicing sites, and the suitability of each primer pair in real-time PCR assays was tested by conventional PCR using cDNA and genomic. In addition the  $T_m$  temperature difference between the forward and reverse primer was within 1 $^{\circ}$ C to ensure efficient amplification.

Primer Name	Sequence (5' > 3')
zfCART1-rtF1	GTTCCATCGTGTGATGCAG
zfCART1-rtR1	ACAAGCACTTGAGGATGGAG
zfCART2-rtF1	GACCCAAACCTGAACAGCG
zfCART2-rtR1	TGCTCGCCCAAATCACAC
zfCART3-rtF1	TGAGCCTACTTCCTTCGTG

<b>zfCART3-rtR1</b>	TGGCATGATGTTTCCTTGTG
<b>zfCART4-rtF1</b>	GTCATTCCCAGGTGTGACG
<b>zfCART4-rtR1</b>	GCAGGAAGAAAGTGTTCAG
<b>zfGHRL-rtF1</b>	GAAGACGATCGGTTTATGATGAG
<b>zfGHRL-rtR1</b>	CTCCAGAAGATTCTGAAGCAC
<b>zfAGRP-rtF1</b>	TGAAGCCTATGATGAGGATCTG
<b>zfAGRP-rtR1</b>	GATGATGACCCAGACAGGAC
<b>zfPOMCa-rtF1</b>	CAGAGGAGAACATCTTGGAAATGC
<b>zfPOMCa-rtR1</b>	TCCGGCTCTATCTGTTTCAGG
<b>zfPOMCb-F1</b>	GAGCAAATTGCAGTGTCTC
<b>zfPOMCb-R1</b>	GCTCCTCTACCTGTTCTTCAC
<b>zfNPY-rtF1</b>	GGAGCTCGCCAAGTATTATTCAG
<b>zfNPY-rtR1</b>	GCCAAATGATCCTCATATCTGGTC
<b>zfOXY-rtF1</b>	CCCATTTCGACAGTGTATGCC
<b>zfOXY-rtR1</b>	TTGATTGACAGCTGCAGCG
<b>zfAVP-rtF1</b>	GCATCTGCTGCGATTTCAGAG
<b>zfAVP-rtR1</b>	GGTTGCCAGATTGAGCAGAC
<b>zfBACTIN-rtF</b>	CGAGCAGGAGATGGGAACC
<b>zfBACTIN-rtR</b>	CAACGGAAACGCTCATTGC
<b>zfGAPDH-rtF</b>	CGCTGGCATCTCCCTCAA
<b>zfGAPDH-rtR</b>	TCAGCAACACGATGGCTGTAG
<b>POMCb R1</b>	AGTCCTCCGTATCGTTTGTCTG
<b>POMCb R2</b>	TTTGACGTTGTTCTGTGTTG

**Table 2.** PCR primers for use in realtime PCR.

### 2.13.2 Zebrafish feeding experiment

An experiment was conducted to look at the roles of a selection of genes in the feeding behaviour of zebrafish. Two separate groups of five fish were kept on a constant feeding regime, being fed twice a day for one week, after which time one group was starved for 24 hours while the other was fed as normal. Fish were sacrificed and whole brains were extracted from all fish as described in 'Preparing Tissue Samples'. Each brain was placed in a 2ml screw top tube containing 500µL TRIzol® reagent (Life Technologies) and 0.5mm glass and 0.1mm silica/zirconia and the total RNA was subsequently isolated and converted to cDNA.

### 2.13.3 TRIzol® mRNA extraction

TRIzol® reagent allows the isolation of high quality RNA from tissue lysate. Extraction of total RNA from whole brain tissue was carried out following the TRIzol® reagent protocol, following the manufacturer's instructions. Briefly, the

brains sample were homogenised in the TRIzol® reagent using a mini-beadbeater (Alphatech) until no visible pieces of brain tissue remained. Chloroform (0.1mL) was added, the tube shaken vigorously by hand for 15 seconds and incubated for 2–3 minutes at room temperature. The mixture separates into a lower red phenol-chloroform phase and a white interphase (contain DNA and proteins) and a colorless upper aqueous phase (contains RNA). The upper aqueous layer was transferred into a new 1.0mL Eppendorf tube being careful not to transfer any of the lower layer and interphase as it contains phenol which would inhibit cDNA synthesis. Next, 100% Isopropanol (0.25mL) was added to the aqueous phase and incubated at room temperature for 10 minutes, allowing the RNA to precipitate out. The RNA was pelleted by centrifugation at  $12,000 \times g$  for 10 minutes at 4°C (Eppendorf). At this stage the RNA is often visible forming a gel-like pellet on the side and bottom of the tube. The supernatant was removed from the tube and the pellet washed with 1 mL of 75% ethanol by vortexing the tube and then underwent centrifugation at  $7,500 \times g$  for 5 minutes at 4°C. The ethanol was carefully removed and any residual ethanol was evaporated off by air drying for 5 minutes. The washed RNA pellet was then resuspended in 20µL (the lowest possible amount to increase the total RNA concentration) of DEPC water and incubated for 10 minutes at 60°C. The concentration of total RNA purified was measured using the Nanodrop 2000 (Thermo Scientific) and the 260/280 absorbance ratio measured to ensure purity was approximately  $\geq 1.8$ . The tRNA was then used immediately for cDNA synthesis to minimise RNA degradation.

#### **2.13.4 qScript™ flex cDNA synthesis**

The qScript™ flex cDNA synthesis kit synthesises first strand cDNA with high efficiency from extracted RNA. A concentration of 1µg of RNA was used for cDNA synthesis. Using the qScript™ flex cDNA synthesis kit, cDNA was synthesized according to the manufacturer's instructions. Briefly, XµL RNA (1µg conc.), XµL RNase free water, 2µL Oligo dT and 2µL random primer were added together in a tube giving a final volume of 15 µL. The contents were mixed and incubated at 65°C for 5 minutes, then snap chilled on ice. Next, 4 µL qscript Flex Reaction Mix (5x) and 1 µL qScript Reverse Transcriptase were added, the contents mixed and incubated at different temperatures using the following

protocol: 25°C, for 10 minutes, 42°C for 45 minutes, 85°C, 5 minutes and held at 4°C. cDNA was subsequently stored at 4°C until they were used for realtime.

#### **2.13.4 Realtime PCR**

Real-time amplification was performed using the primers designed above and q•EvaGreen® qPCR Mix (Quarta), following the manufacturer's instruction. Briefly, 4 µL q•EvaGreen®, 2 µL of forward primer (10 µM), 2 µL of reverse primer (10 µM), 0.5 µL of cDNA and nuclease free water up to a volume of 20 µL. Tubes were placed into a Rotor-Gene 6000 (Corbett) and the following programme was carried out:

Initial denature	94°C for 15 minutes
Denaturation	94°C for 15 seconds
Annealing	52-58°C for 20 seconds
Extension	72°C for 20 seconds

In addition, a melting curve for each PCR was performed between 72 and 94 C to ensure that only a single product had been amplified. Expression levels of each of the eating behaviour genes were normalized to two housekeeping genes, gapdh and b-actin. Results were entered into the relative Expression Software Tool (REST; Pfaffl et al., 2002) which analysed the data using the delta delta CT method (Livak and Schmittgen, 2001) and results expressed as the fold change in the unfed fish compared with the expression level in the fed control fish using the delta delta CT and plotted as a box and whisker plot. Subsequently the expression ratio results of the investigated transcripts were tested for significance by a Pair Wise Fixed Reallocation Randomisation Test © and plotted using standard error (SE) estimation via a complex Taylor algorithm.

## 3. Results

### 3.1 Identification of zebrafish eating behaviour genes

Initial investigations into fish eating behaviour involved the identification of genes that had not currently been characterised within zebrafish, that had been shown in mammals to have important roles in regulating eating behaviour. A literature search indicated that genes such as; Cocaine- and amphetamine-regulated transcript (CART), Pro-opiomelanocortin (POMC), Neuropeptide Y (NPY), Oxytocin (OXT) and Vasopressin (AVP) had already been identified. Using available sequence databases and the zebrafish genome, additional eating behaviour genes within the zebrafish were searched for and found, which includes; Islet Amyloid Polypeptide (IAPP), Apelin (APLN), Cholecystokinin (CCK), Fat mass and obesity-associated transcript (FTO), Leptin (LEP), Peptide YY (PYY), Ghrelin (GHRL) and Agouti-related peptide (AgRP) as well as an additional copy of POMC (termed POMCb) and three other copies of CART (termed CART 1, 3 and 4). For the purpose of this study, Ghrelin (GHRL), Agouti-related peptide (AgRP), POMCb, CART 1, CART 3 and CART 4 were chosen to be characterised further and analysis of the initial sequences found for these genes are described below. Analysis of what was found for Islet Amyloid Polypeptide (IAPP), Apelin (APLN), Cholecystokinin (CCK), Fat mass and obesity-associated transcript (FTO), Leptin (LEP) and Peptide YY (PYY) can be found in the Appendix 5.1 (**Supplementary Figures 1-26**).

#### 3.1.1 AgRP

Searching the available genome using TBLASTN with a known fish AgRP protein, identified a region of chromosome 7 that contained a potential zebrafish AgRP sequence. Using gene prediction software with this region provided a nucleotide sequence that was aligned against AgRP nucleotide sequences already identified in other fish (**Supplementary Fig. 27**) and in selected mammals (**Supplementary Fig. 28**) to identify regions of similarity. The sequence was also translated and the predicted protein was aligned against known AgRP protein

sequences already identified in other fish (**Fig. 1**) and in selected mammals (**Fig. 2**). In both alignments there was clear regions of high similarity indicating that this sequence was a likely candidate for the zebrafish AgRP gene. Phylogentic analysis of the zebrafish AgRP protein sequence with known vertebrate AgRP genes grouped the gene very clearly with the known fish AgRP protein sequences and very closely to other cyprinid species (**Fig. 3**).

```

Danio      --MMLNTVIFGWFLVNVVVMASHPHLRRRENSFILTSDDSLPEMEHLEINSAEEKILED 58
Carassius MIMMLNIAIISWFLMNVMMASHPNLRRSENFALS-DTDLPLGVENFEINSVEEKLEEE 59
Cyprinus  -----
Salmo     --MLGKHFLLWVCLLSVSIPLCFEAEDLKR-----DAHIQHENDSVWSQEKPGRLFAR 49

Danio      LEAYDEDL GKAVHLQRRGTRSPSRCIPHQQSCLGHHLPCCNPCDTCYCRFFKAFICYCRSM 118
Carassius LGSYDEDL GKAVQLQRRGTRSPSRCIPHQQSCLGHHLPCCNPCDTCYCRFFKAFICYCRSM 119
Cyprinus  -----NTFPQPCIPHQQSCLGHHLPCCNPCDTCYCRFFKAFICYCRSM 42
Salmo     RRILPHQGHVAFQKHEFAAPARRCGRLMDSAPHTPCCDPCASCRCRLFNTICHCWRL 109
          *      :  . . . *  ***:*  :*  **:*:.*:.*  :

Danio      DNTCKNEYA----- 127
Carassius DHTCKHEYA----- 128
Cyprinus  DHTCKHDVCI EPNSTLK 59
Salmo     GPHCPKKT----- 117
          .  *  :.

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**Figure 1.** ClustalW alignment of *Danio rerio* AgRP protein sequence against *Carassius auratus*, *Salmo salar*, and *Cyprinus carpio* AGRP sequences. (\*) identical sequence and (: ) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 42.37% sequence identity with the fish sequences.

```

Chinchilla -MLTTMLLSCALLLALPATQG/AQMGLPPLLEGIRRPDQVLLPEFPGLGLHVS LKRTTAEQA 59
Heterocephalus -MLTTVLLSCALLLALPETQG/AQMGLAPLEGIRRPDQVLFPEFP--GLHAPLKKT-PDKA 56
Mus -MLTAMLLSCLVLLALPPTLG/VQMGLVAPLKGIRRPDQALFPEFPGLSLNG-LKKTADRA 58
Rattus -MLTAMLLSCLVLLALPPTLG/VHMGLVAPLKGIRRSQALFPEFSGLSL----KKTADRA 55
Microtus -MLMVMLLSCAMLLALPPTLG/VQMGLAPLEGIRRLGQALFPEFPGLSLNG-PKRTTADRT 58
Pygmy -MLTAAVLLSCLLALPATRG/AQMGLAPMEGIRRPDQALLPELPGLGLRAPLKKTTAEQA 59
Pan -MLTAAVLLSCLLALPATRG/AQMGLAPMEGIRRPDQALLPELPGLGLRAPLKKTTAEQA 59
Homo -MLTAAVLLSCLLALPATRG/AQMGLAPMEGIRRPDQALLPELPGLGLRAPLKKTTAEQA 59
Macaca -MLTAAVLLSCLLALPATRG/AQMGLAPLEGIRRPDQALFPELPGLGLRAPLKKTTAEQA 59
Mustela -MLPAVLLSCLLALPMPQG/AGIGLAPLEGIRSPDQALFPELPGLGLQSS LKRTAAEQS 59
Danio      MMLNTVIFGWFLVNVVVMAS/PHLRRRENSFILTSDDSLPEMEHLEIN-----SAEEKI 55
          ** . :. : : : : : . * . :*: : : : :

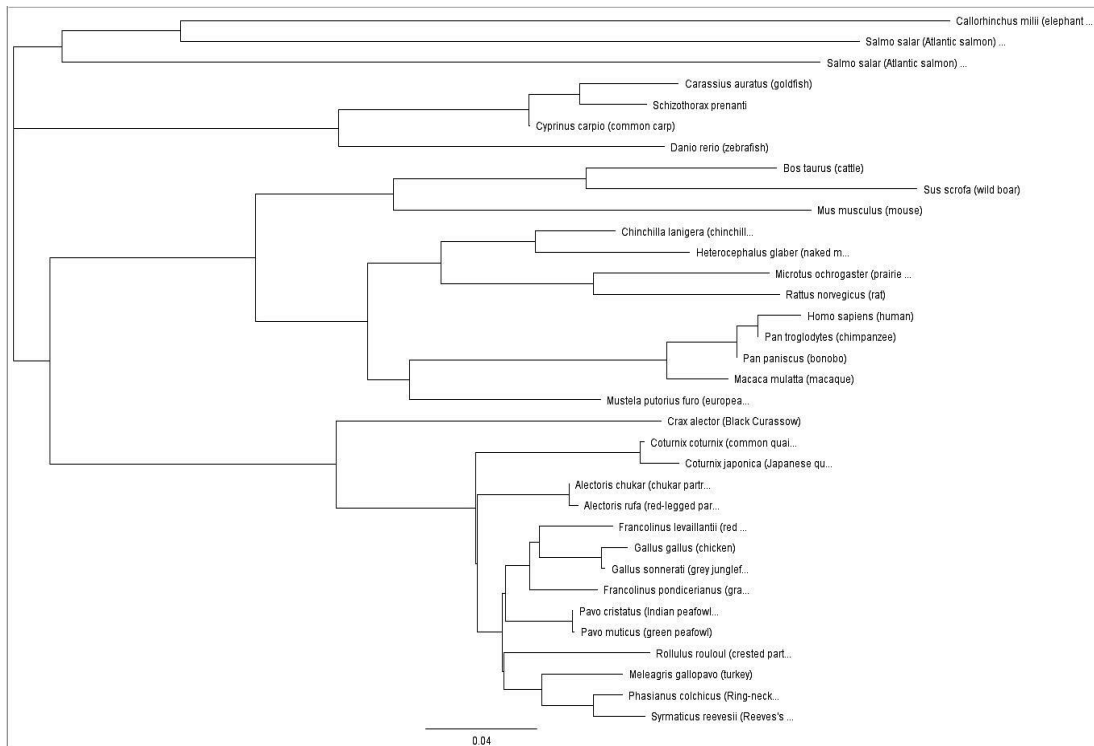
Chinchilla EEALLQKAEALAEVLDPQNRPTLR CVRLHESCLGQQVPCCDPCATCYCRFFNAFCYC 119
Heterocephalus EEALLQKAEALAEVLDAQSREPRTPR CVRVHESCLGQQVPCCDPCATCYCRFFNAFCYC 116
Mus EEVLLQKAEALAEVLDPQNRERSRPR CVRLHESCLGQQVPCCDPCATCYCRFFNAFCYC 118
Rattus EDVL-QKAEALAEVLDPQNRERSRPR CVRLHESCLGQQVPCCDL CATCYCRFFNAFCYC 114
Microtus EEALLRKAERALAKVLDPQNRERSRPR CVRLHESCLGQQVPCCDPCATCYCRFFNAFCYC 118
Pygmy EEDLLQEAQALAEVLDLQDREPRSSR CVRLHESCLGQQVPCCDPCATCYCRFFNAFCYC 119
Pan EEDLLQEAQALAEVLDLQDREPRSSR CVRLHESCLGQQVPCCDPCATCYCRFFNAFCYC 119
Homo EEDLLQEAQALAEVLDSQDREPRSSR CVRLHESCLGQQVPCCDPCATCYCRFFNAFCYC 119
Macaca EEDLLQEAQALAEVLDSQDREPRSSR CVRLHESCLGQQVPCCDPCATCYCRFFNAFCYC 119
Mustela GEALLQEAALAEVLDPQNRERSRPR CVRLHESCLGHQVPCCDPCATCYCRFFNAFCYC 119
Danio      LEDLEAYDEDL GKAVHLQRRGTRSPSRCIPHQQSCLGHHLPCCNPCDTCYCRFFKAFICYCR 115
          : * : * : . . : * : * : * : * : * : * : * : * : * : * : * : *

Chinchilla RKLG T-GTNP CSRT 132

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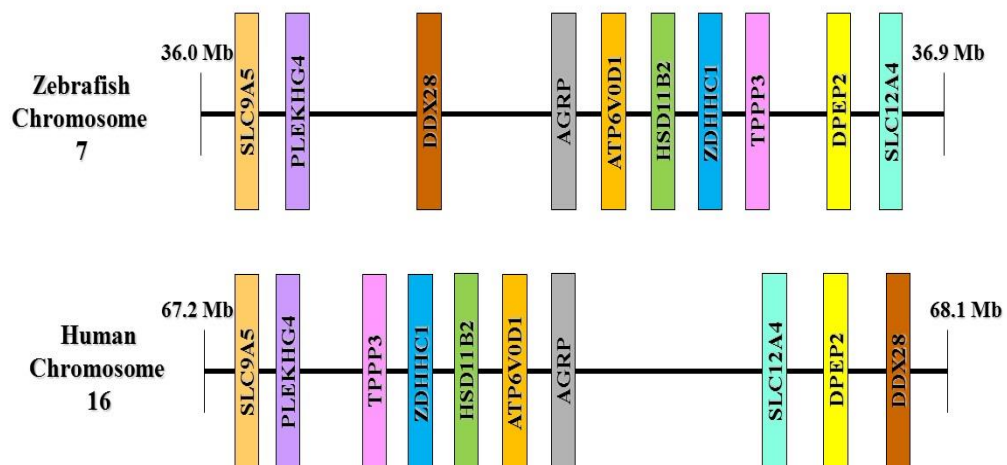
Heterocephalus	RKLGT-ATN <b>C</b> SRT	129
Mus	RKLGT-ATN <b>L</b> C <b>S</b> R <b>T</b>	131
Rattus	RKLGTGTTN <b>L</b> C <b>S</b> R <b>P</b>	128
Microtus	RKLGT-ATN <b>L</b> C <b>S</b> R <b>T</b>	131
Pygmy	RKLGT-AMN <b>P</b> C <b>S</b> R <b>T</b>	132
Pan	RKLGT-AMN <b>P</b> C <b>S</b> R <b>T</b>	132
Homo	RKLGT-AMN <b>P</b> C <b>S</b> R <b>T</b>	132
Macaca	RKLGT-AMN <b>P</b> C <b>S</b> R <b>T</b>	132
Mustela	RKLGT-AMN <b>P</b> C <b>S</b> R <b>T</b>	132
Danio	R <b>S</b> M <b>D</b> N <b>T</b> C <b>K</b> N <b>E</b> Y <b>A</b> --	127
	*.:. . . * :	

**Figure 2.** ClustalW alignment of Danio rerio AgRP protein sequence against Homo sapiens, Rattus norvegicus, Mus musculus, Pan troglodytes, Pan paniscus, Macaca mulatta, Mustela putorius furo, Heterocephalus glaber, Microtus ochrogaster, and Chinchilla lanigera AgRP sequences. (\*) identical sequence and (:) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 33.42% sequence identity with these species and 33.60% similarity to Homo sapiens. Highlighted in colour are conserved domains of protein sequence deduced from comparison against Homo sapiens protein structure: Signal sequence (dark grey/), active core (light blue), conserved cysteines (red font), agouti domain (light grey).



**Figure 3.** Phylogenetic tree, constructed using the neighbour joining method alignment, showing the relationship between zebrafish AgRP and known vertebrate AgRP sequences.

Analysis of the region within the zebrafish genome around the predicted AgRP gene on chromosome 7 allowed a gene map to be constructed that showed that there was conservation of synteny between the human and zebrafish genomes (**Fig. 4**), giving further evidence that this gene was a homologue of the human AgRP gene.



**Figure 4.** Comparison of the co-localization of genes surrounding AGRP on Human chromosome 16 and Zebrafish chromosome 7.

### 3.1.2 CART

Searching the available genome using TBLASTN with a known fish CART protein, identified multiple regions within the genome. On further investigation multiple CART genes were found within the zebrafish. One CART gene has already been characterised in zebrafish (REF) which has been named CART2.

#### 3.1.2.1 CART1

A region on chromosome 22 contained a potential zebrafish CART sequence. Using gene prediction software with this region provided nucleotide sequences that was aligned against CART nucleotide sequences already identified

in other fish (**Supplementary Fig. 29**) and in selected mammals (**Supplementary Fig. 30**) to identify regions of similarity. The sequence was also translated and the predicted protein was aligned against known CART protein sequences already identified in other fish (**Fig. 5**) and in selected mammals (**Fig. 6**). In both alignments there was clear regions of high similarity indicating that this sequence was related to known CART genes and given the name zebrafish CART1.

Tautogolabrus	-----	
Xiphophorus	-----	
Carassius	-----	
Salmo	-----	
Silurus	-----	
Leucoraja	-----	
Oryzias	-----	
Danio	MNALMFEVHRSGVSI TMMTFFAGLLTFALALEVLRGHRQH LIWNGVVGRYIKSWDSALLL	60
Pundamilia	-----MVRREVEGESPVDLLRHCAQPPLICCLPLGP	30
Oreochromis	-----MVRREVEGESPVDLLRHSAQPPLICCLPLGP	30
Haplochromis	-----MESKRAVVYLS-----	11
Maylandia	-----MESKRAVVYLS-----	11
Tautogolabrus	---MRS-TGSTRSCRL---LCVLLS--GSTGADLTENNSLTSEDELSPRVLRHFYSK	50
Xiphophorus	---MSPRTGTMQSSRMLSGALLCALLLSAGAAGAGAQGLLDAESEEELS PRALRDFYPK	57
Carassius	-----MESSKLTWTAMACAVLVSCIQGAEMDF-----DNESDLETRALREFYPK	44
Salmo	-----MESSRLWTRAVVCAVLLSIVLSAEIDYS-----DSELDLDTRSVRDFYPK	45
Silurus	-----MESSIAARALLLLVLMMLRSCARGDNE-----DTEVDLDTRAIRDFYPK	45
Leucoraja	-----MVSDRLLLAVYFCVLFMAVGAE-----NSDLEPRALRDFYSK	38
Oryzias	-----MVSARTLLLVTCSCTYLWLSRAE-----DSLETRSLDFTL--	37
Danio	FRGESCFLDTMVGERIFLLTVTCS--VLMLLVCC-----DEVLQIRSPPEED---	
104		
Pundamilia	PPFRFTATSTMDSSGMLRGLLLVGLLSVLCHGQA-----SREVSADFGEK	76
Oreochromis	PPSRFTANSTMDSSGMLRGLLLVGLLSVLCHGQA-----SREVSADFGEK	76
Haplochromis	-----VCLSVLTSLCQGQR-----SGNSHLLSAPDD	37
Maylandia	-----VCLSVLTSLCQGQR-----SGNSHLLSAPDD	37
Tautogolabrus	GPNLTSEKQ---LSGALQEVLEKQLQAKKTSWEKKFGQVPRCDVGEQCAVRKGSRIGRMC	107
Xiphophorus	GPNLTSEKQ---LLGALQEVLEKQLQAKRLPSWEKKFGQVPTCDVGEQCAVRKGARIKMC	114
Carassius	DPNLTNEKQLVSI LGALHDVLEKQLQSKRISLWEKKFGRVPTCDVGEQCAIRKGSRIGKMC	104
Salmo	DPNLTNEKQ---LLGALHDVLEKQLQTKRLPFWEKKFGQVPTCDVGEQCAVRKGARIKMC	102
Silurus	DPNLTSEKQ---LLGALHDVLEKQLQMKRIPPWEKKFGQVPMCDVGEQCAIRKGSRIGKMC	102
Leucoraja	NYYPGSEKE---LLGALHEVLKKLQTKRLPTWEKKYQGGPQCNI GDMCAVRKGPRIWRTC	95
Oryzias	--KAQQEKD---LIDALQGVLEKLRNKEMP--LEKKLGWLPSCDAGEPCAVRKGARIGTLC	91
Danio	--KAQEEKE---LIEALQEVLEKLRNKQIPAVEKKLGWVPSCDAGEQCAVRKGSRFGKLC	159
Pundamilia	KQEAADVDRD---LLEALDVLLGRNHN--QVSSPEKRGSIPLCGLGNRCAMKYGPRIKLC	131
Oreochromis	KQEAADVDRD---LLEALDVLLGRNHN--QVSSPEKRGSIPLCGLGNRCAMKYGPRIKLC	131
Haplochromis	PTLGLTTSE---LAEALQGLLDEADSSAGLSVEKKASVIPRCDVGERCAMKHGPRIGRLC	94
Maylandia	PTLGLTTSE---LAEALQGLLDEADSSAGLSVEKKASVIPRCDVGERCAMKHGPRIGRLC	94
	: : **.* . :* . * * . * : ** : * . *	
Tautogolabrus	DCPRRAFNCNFCLLKCL	123
Xiphophorus	DCPRGAFCNFFLLKCL	130
Carassius	DCPRGAFCNFFLLKCL	120
Salmo	DCPRGAFCNFYLLKCL	118
Silurus	DCPRGAFCNFFLLKCL	118
Leucoraja	NCLS-SKCNFYLFKCV	110
Oryzias	GCPRTGTCNFFYLLKCL	107
Danio	SCPGGTACSFYLLKCL	175
Pundamilia	DCGRGANCSYLLKCI	147
Oreochromis	DCGRGANCSYLLKCI	147
Haplochromis	DCLRGTACNTFFLRCY	110
Maylandia	DCLRGTACNTFFLRCY	110
	.* : * . . : *	



Homo	NSFLLKCL	116
Pygmy	NSFLLKCL	116
Pan	NSFLLKCL	116
Pongo	NSFLLKCL	116
Gorilla	NSFLLKCL	116
Nomascus	NSFLLKCL	116
Macaca	NSFLLKCL	116
Crab-eating	NSFLLKCL	116
Papio	NSFLLKCL	116
Saimiri	NSFLLKCL	116
Rattus	NSFLLKCL	129
Mesocricetus	NSFLLKCL	116
Mus	NSFLLKCL	129
Cavia	NSFLLKCL	116
Ailuropoda	NSFLLKCL	116
Sus	NSFLLKCL	116
Ovis	NSFLLKCL	90
Danio	SFSILKCL	175
.	:****	

**Figure 6.** ClustalW alignment of Danio rerio CART1 protein sequence against Homo sapiens, Rattus norvegicus, Mus musculus, Sus scrofa, Macaca mulatta, Ovis aries, Cavia porcellus, Ailuropoda melanoleuca, Pongo abelii, Macaca fascicularis, Saimiri boliviensis boliviensis, Pan troglodytes, Pan paniscus, Gorilla gorilla gorilla, Nomascus leucogenys, Papio anubis, and Mesocricetus auratus CART sequences. (\*) identical sequence and (: ) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 35.88% sequence identity with these species and 22.90% similarity to Homo sapiens. Highlighted in colour are conserved domains of protein sequence deduced from comparison against Homo sapiens protein structure: Signal sequence (dark grey/), convertase processing sites (orange font), conserved cysteines (red font), CART 55-102 (light grey).

### 3.1.2.2 CART3

A region on chromosome ? contained a potential zebrafish CART sequence. Using gene prediction software with this region provided nucleotide sequences that was aligned against CART nucleotide sequences already identified in other fish (**Supplementary Fig. 31**) and in selected mammals (**Supplementary Fig. 32**) to identify regions of similarity. The sequence was also translated and the predicted protein was aligned against known CART protein sequences already identified in other fish (**Fig. 7**) and in selected mammals (**Fig. 8**). In both alignments there was clear regions of high similarity indicating that this sequence was related to known CART genes and given the name zebrafish CART3.

Danio	-----	
Oryzias	-----MVSARTLLLVVTCSTYLWLSRAE-----	24
Xiphophorus	MSPRTGTMQSSRMLSGALLCALLLSAGAAGAGAQG-----LLD	39

```

Tautogolabrus      MRS-TGSTRSCRL-----LCVLLS--GSTGADLTE-----NNS 32
Salmo              -----MESSRLWTRAVVCAVLLSIVLSAEIDYS----- 28
Carassius          -----MESSKLWTTAMACAVLVSCIQGAEMDF----- 27
Silurus            -----MESSSIAARALLLLVLMRLRSCARGDNE----- 28
Leucoraja         -----MVSDRLLLAVYFCVLFMSAVGAE----- 23
Oreochromis       --MVRVVEGESPVDLLRHSAPOPLICCLPLGPPSRFTANSTMDSSGMLRGLLVGLS 57
Pundamilia        --MVRVVEGESPVDLLRHSAPOPLICCLPLGPPFRFTATSTMDSSGMLRGLLVGLS 57
Maylandia         -----MESKRAVVYLS-----VCLSVLT 18
Haplochromis     -----MESKRAVVYLS-----VCLSVLT 18

Danio              -----IEALQEVLEKLNKQLPQTGKKLSLLPSC 29
Oryzias           ---DSSLETRSL-DFTLK---AQQEKD---LIDALQGVLEKLRNKEMPLE-KKLGWLPSC 73
Xiphophorus       AESEELSPRALRDFYKPKGNLTSEKQ---LLGALQEVLEKLOAKRLPSWEKKFGQVPTC 96
Tautogolabrus     LTSEDELSPRVLRFYKSGKNLTSEKQ---LSGALQEVLEKLOAKTSSWEKKFGQVPRC 89
Salmo             -DSELDLDTRSVRDFYKPKDNLNTEKQ---LLGALHDVLEKLOTKRPFWEKKFGQVPTC 84
Carassius         -DNESDLETRALREFYKPKDNLNTEKQLVLSILGALHDVLEKLOSKRISLWEKKFGRVPTC 86
Silurus           -DTEVDLDTRAIRDYKPKDNLNTEKQ---LLGALHDVLEKLOMKRIPWEKKFGQVPMC 84
Leucoraja        ---NSDLEPRALRDFYKSNYYPGSEKE---LLGALHEVLEKLOTKRLLPTWEKKYQGPQC 77
Oreochromis      VLCHGQASREVSADFGEKQEAADVDR--DLLEALDVLLGRNHN--QVSSPEKRGSIPLC
113
Pundamilia       VLCHGQASREVSADFGEKQEAADVDR--DLLEALDVLLGRNHN--QVSSPEKRGSIPLC
113
Maylandia        SLCQQRSGNSHLLSAPDDPTLGLTTS--ELAEALQGLLDEADSSAGLSVEKKASVIPC 76
Haplochromis     SLCQQRSGNSHLLSAPDDPTLGLTTS--ELAEALQGLLDEADSSAGLSVEKKASVIPC 76
                                     ** . :* .          :* . **

Danio             DAGEQCAIRKGARVG-LCSCPQGTSCFFILKCL 62
Oryzias          DAGEPCAVRKGARIGTLCCGPRGTCNFFYVLKCL 107
Xiphophorus     DVGEQCAVRKGARIGKMCDCPRGAFCNFFLLKCL 130
Tautogolabrus   DVGEQCAVRKGSRIGRMCDCPRRAFCNFFLLKCL 123
Salmo           DVGEQCAVRKGARIGKMCDCPRGAFCNFFLLKCL 118
Carassius       DVGEQCAIRKGSRIGRMCDCPRGAFCNFFLLKCL 120
Silurus         DVGEQCAIRKGSRIGRMCDCPRGAFCNFFLLKCL 118
Leucoraja       NIGDMCAVRKGPRIWRTCNLS-SKCNFFLFCV 110
Oreochromis     GLGNRCAMKYGPRIKLCDCGRGANCSYLLKCI 147
Pundamilia      GLGNRCAMKYGPRIKLCDCGRGANCSYLLKCI 147
Maylandia       DVGERCAMKHGPRIGRLCDLGRGTACNTFFLRCY 110
Haplochromis    DVGERCAMKHGPRIGRLCDLGRGTACNTFFLRCY 110
. *: **:: *: * : * . * : * : *

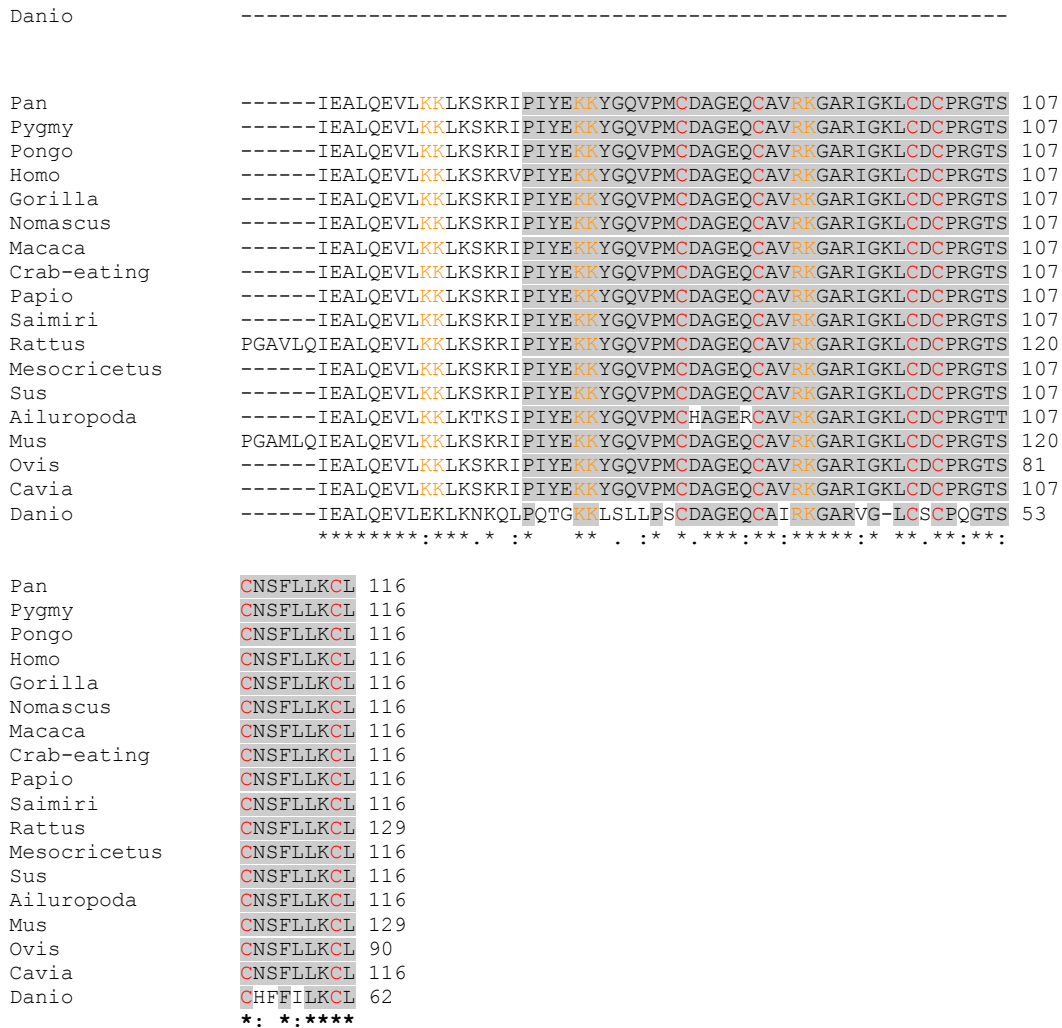
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**Figure 7.** ClustalW alignment of *Danio rerio* CART3 protein sequence against *Oryzias latipes*, *Salmo salar*, *Oreochromis niloticus*, *Maylandia zebra*, *Haplochromis burtoni*, *Pundamilia nyererei*, *Xiphophorus maculatus*, *Carassius auratus*, *Leucoraja ocellata*, *Tautogolabrus adspersus* and *Silurus meridionalis* CART sequences. (\*) identical sequence and (:) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 49.72% sequence identity with the fish sequences.

```

Pan              MESSRVRLPLLGAAALLMLPLLGTRAQ/EDAELQPRALDIYSAVDDASHEKEL----- 53
Pygmy           MESSRVRLPLLGAAALLMLPLLGTRAQ/EDAELQPRALDIYSAVDDASHEKEL----- 53
Pongo           MESSRVRLPLLGAAALLMLPLLGTRAQ/EDAELQPRALDIYSAVDDASHEKEL----- 53
Homo            MESSRVRLPLLGAAALLMLPLLGTRAQ/EDAELQPRALDIYSAVDDASHEKEL----- 53
Gorilla         MESARVRLPLLGAAALLMLPLLGTRAQ/EDAELQPRALDIYSAVDDVSHEKEL----- 53
Nomascus        MESSRVRLPLLGAAALLMLPLLGTRAQ/EDAELQPRALDIYSAVDDASHEKEL----- 53
Macaca          MESSRVRLPLLGAAALLMLPLLGTRAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Crab-eating     MESSRVRLPLLGAAALLMLPLLGTRAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Papio           MESSRVRLPLLGAAALLMLPLLGTRAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Saimiri         MESSRVRLPLLGVSLLLLPLLGTRAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Rattus          MESSRLRLLPLVGAALLLLPLLGAGAQ/EDAELQPRALDIYSAVDDASHEKELPRRQLRA 60
Mesocricetus    MESSRLRLLPLVGAALLLLPLLGAGAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Sus             MESPRLRLLPLVGAALLLLPLLGALAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Ailuropoda      MESPRLRPLTLVGAALLLLPLLGARAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Mus             MESSRLRLLPLVGAALLLLPLLGARAQ/EDAELQPRALDIYSAVDDASHEKELPRRQLRA 60
Ovis            MESH-----AELQPRALDIYSAVEDASHEKEL----- 27
Cavia           MESARLRLPLVGAALLMLPLLGARAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53

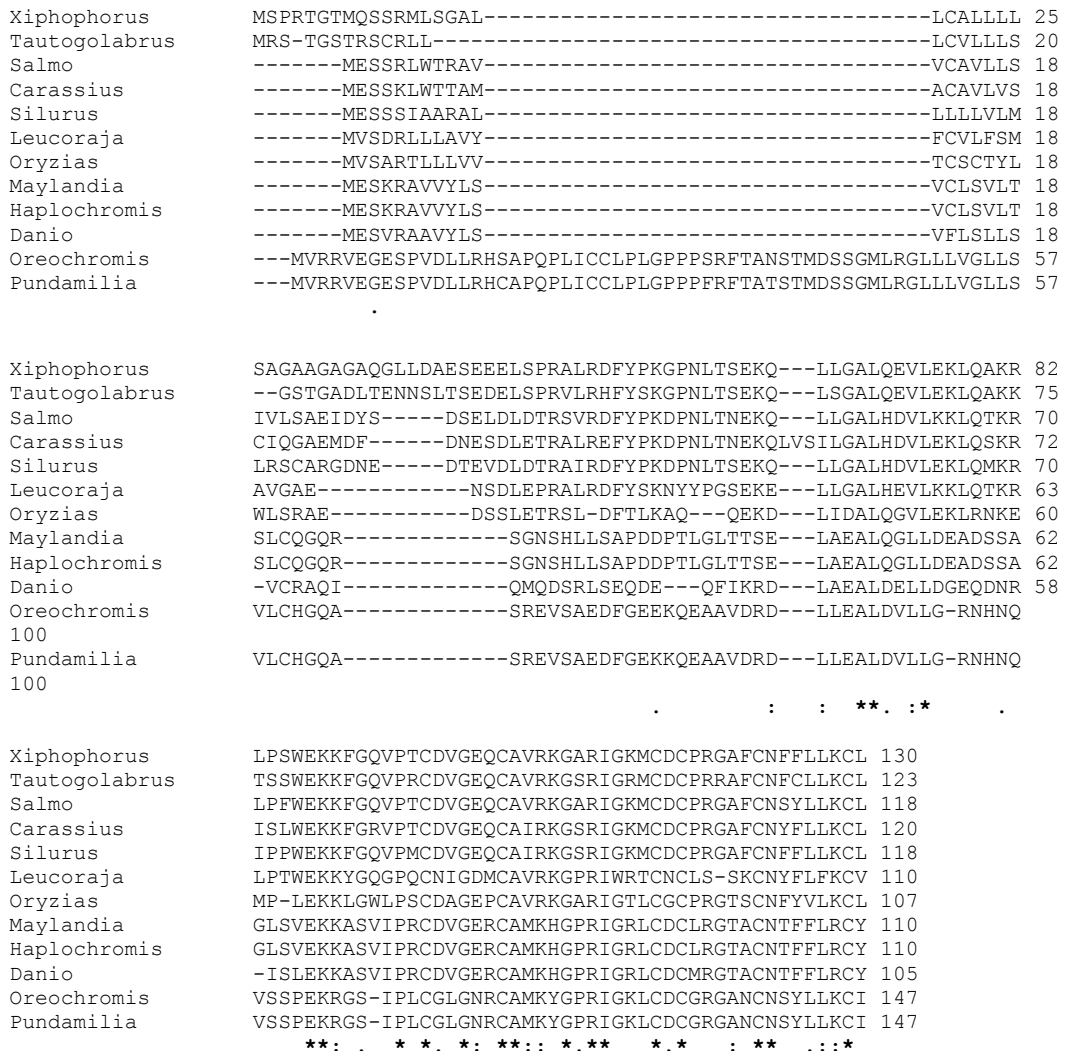
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**Figure 8.** ClustalW alignment of Danio rerio CART3 protein sequence against Homo sapiens, Rattus norvegicus, Mus musculus, Sus scrofa, Macaca mulatta, Ovis aries, Cavia porcellus, Ailuropoda melanoleuca, Pongo abelii, Macaca fascicularis, Saimiri boliviensis boliviensis, Pan troglodytes, Pan paniscus, Gorilla gorilla gorilla, Nomascus leucogenys, Papio anubis and Mesocricetus auratus CART sequences. (\*) identical sequence and (:) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 66.52% sequence identity with these species and 42.90% similarity to Homo sapiens. Highlighted in colour are conserved domains of protein sequence deduced from comparison against Homo sapiens protein structure: Signal sequence (dark grey/), convertase processing sites (orange font), conserved cysteines (red font), CART 55-102 (light grey).

### 3.1.2.3 CART4

A region on chromosome 19 contained a potential zebrafish CART sequence. Using gene prediction software with this region provided nucleotide sequences that was aligned against CART nucleotide sequences already identified in other fish (**Supplementary Fig. 33**) and in selected mammals (**Supplementary Fig. 34**) to identify regions of similarity. The sequence was also translated and the predicted protein was aligned against known CART protein sequences already identified in other fish (**Fig. 9**) and in selected mammals (**Fig. 10**). In both alignments there was clear regions of high similarity indicating that this sequence was related to known CART genes and given the name zebrafish CART4.



**Figure 9.** ClustalW pairwise alignment of Danio rerio CART4 protein sequence against Oryzias latipes, Salmo salar, Oreochromis niloticus, Maylandia zebra, Haplochromis burtoni, Pundamilia nyererei, Xiphophorus maculatus, Carassius auratus, Leucoraja

ocelatta, *Tautogolabrus adspersus* and *Silurus meridionalis* CART sequences. (\*) identical sequence and (:) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 35.51% sequence identity with the fish sequences.

```

Homo          MESSRVRLPPLLGAALLMLPLLGTAAQ/EDAELQPRALDIYSAVDDASHEKEL----- 53
Pygmy        MESSRVRLPPLLGAALLMLPLLGTAAQ/EDAELQPRALDIYSAVDDASHEKEL----- 53
Pan          MESSRVRLPPLLGAALLMLPLLGTAAQ/EDAELQPRALDIYSAVDDASHEKEL----- 53
Pongo       MESSRVRLPPLLGAALLMLPLLGTAAQ/EDAELQPRALDIYSAVDDASHEKEL----- 53
Gorilla     MESARVRLPPLLGAALLMLPLLGTAAQ/EDAELQPRALDIYSAVDDVSHEKEL----- 53
Nomascus    MESSRVRLPPLLGAALLMLPLLGTAAQ/EDAELQPRALDIYSAVDDASHEKEL----- 53
Macaca      MESSRVRLPPLLGAALLMLPLLGTAAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Crab-eating MESSRVRLPPLLGAALLMLPLLGTAAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Papio       MESSRVRLPPLLGAALLMLPLLGTAAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Saimiri     MESSRVRLPPLLGVSLLLLPLLGTAAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Rattus      MESSRLRLLPVLGAALLLPLLGLGAAQ/EDAELQPRALDIYSAVDDASHEKELPRRQLRA 60
Mesocricetus MESSRLRLLPPLLGAALLLPLLGLGAAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Sus         MESPRLRLLPPLLGAALLLPLLGLGAAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Ailuropoda MESPRLRPLTLLGAALLLPLLGLGAAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Mus         MESSRLRLLPPLLGAALLLPLLGLGAAQ/EDAELQPRALDIYSAVDDASHEKELPRRQLRA 60
Cavia      MESARLRLPPLLGAAVFLMLPLLGLGAAQ/EDAELQPRALDIYSAVEDASHEKEL----- 53
Ovis       MES-----HAEHQPRALDIYSAVEDASHEKEL----- 27
Danio      MESVR-----AAVYLSVFISLISVCRAQ/IQMDD---SRLSEQDEQFIKRDLE----- 43
***          ::* . * :: :*:

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Homo          -----IEALQEVLLKLSKRVPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Pygmy        -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Pan          -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Pongo       -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Gorilla     -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Nomascus    -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Macaca      -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Crab-eating -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Papio       -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Saimiri     -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Rattus      PGAVLQIEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 120
Mesocricetus -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Sus         -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Ailuropoda -----IEALQEVLLKLTKSIPIYEKKYGVPMCHAGERCAVKKGARIGKLCDCPRGTT 107
Mus         PGAMLQIEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 120
Cavia      -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 107
Ovis       -----IEALQEVLLKLSKRIPPIYEKKYGVPMCDAGEQCAVKKGARIGKLCDCPRGTS 81
Danio      -----AEALDELDDGEQDNRISL-EKKASVIERCDVGERCAMKHGPRIGRLCDMRTGA 96
***:*:* . : : : * * . : * * . * * : * * : * * : * * : * * :

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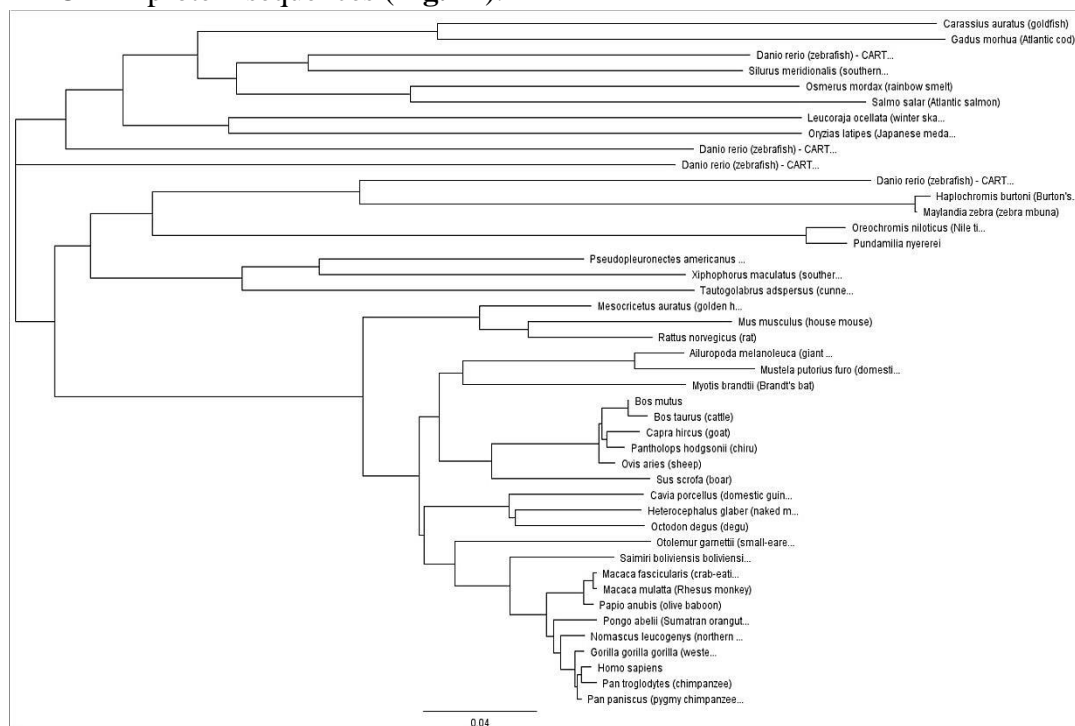
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Homo          CNSFLLKCL 116
Pygmy        CNSFLLKCL 116
Pan          CNSFLLKCL 116
Pongo       CNSFLLKCL 116
Gorilla     CNSFLLKCL 116
Nomascus    CNSFLLKCL 116
Macaca      CNSFLLKCL 116
Crab-eating CNSFLLKCL 116
Papio       CNSFLLKCL 116
Saimiri     CNSFLLKCL 116
Rattus      CNSFLLKCL 129
Mesocricetus CNSFLLKCL 116
Sus         CNSFLLKCL 116
Ailuropoda CNSFLLKCL 116
Mus         CNSFLLKCL 129
Cavia      CNSFLLKCL 116
Ovis       CNSFLLKCL 90
Danio      CNTFFLRKY 105
**:*:*:*

```

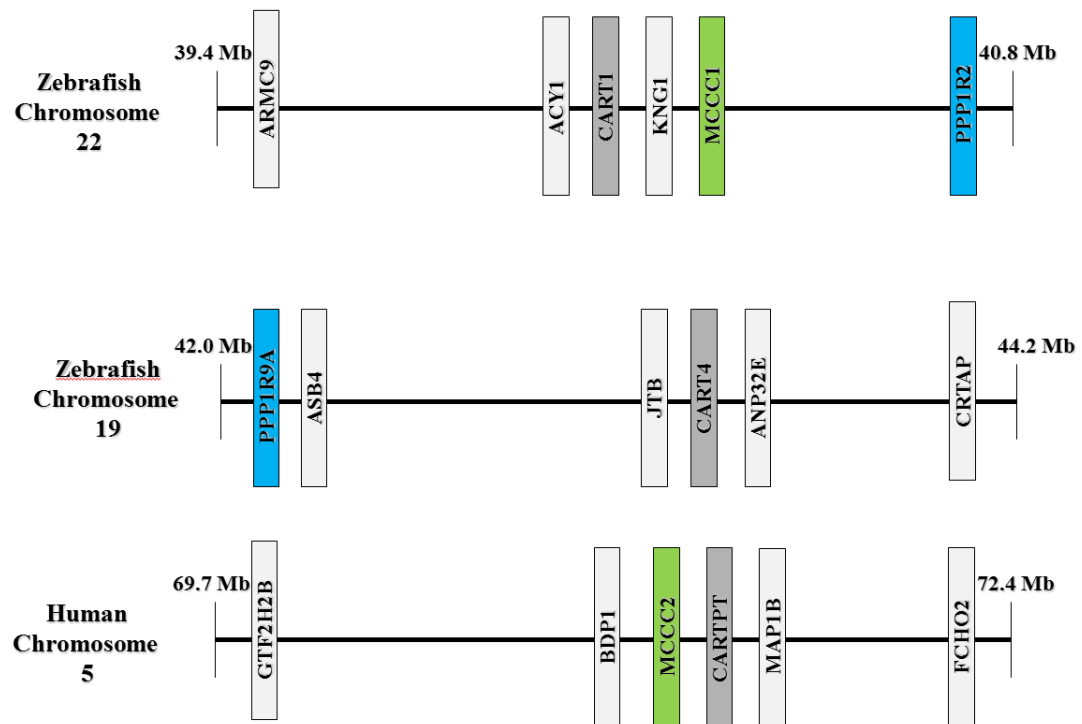
**Figure 10.** ClustalW alignment of *Danio rerio* CART4 protein sequence against *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, *Sus scrofa*, *Macaca mulatta*, *Ovis aries*, *Cavia porcellus*, *Ailuropoda melanoleuca*, *Pongo abelii*, *Macaca fascicularis*, *Saimiri boliviensis boliviensis*, *Pan troglodytes*, *Pan paniscus*, *Gorilla gorilla gorilla*, *Nomascus leucogenys*, *Papio anubis* and *Mesocricetus auratus* CART sequences. (\*) identical sequence and (:) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 37.80% sequence identity with these species and 37.90% similarity to *Homo sapiens*. Highlighted in colour are conserved domains of protein sequence deduced from comparison against *Homo sapiens* protein structure: Signal sequence (dark grey/), convertase processing sites (orange font), conserved cysteines (red font), CART 55-102 (light grey).

Phylogenetic analysis of the zebrafish CART protein sequences with known vertebrate CART genes grouped these gene very clearly with the known fish CART protein sequences (**Fig. 11**).



**Figure 11.** Phylogenetic tree, constructed using the neighbour joining method alignment, showing the relationship between zebrafish CART genes and known vertebrate CART sequences.

Analysis of the region within the zebrafish genome around the predicted CART genes on chromosome 19, 22 and 2 allowed a gene map to be constructed that showed that there was some conservation of synteny between the human and zebrafish genomes for CART1 (**Fig. 12**), giving further evidence that this gene was a homologue of the human CART gene. However, the region around CART4 only shows synteny with one gene in location of the zebrafish CART1 and nothing with the human genome and CART3 shows no synteny (data not shown) with either the human or zebrafish genomes.



**Figure 12.** Comparison of the co-localization of genes surrounding CART on human chromosome 5 and Zebrafish chromosome 19 (CART1) and 22 (CART4). The predicted CART3 sequence is not mapped.

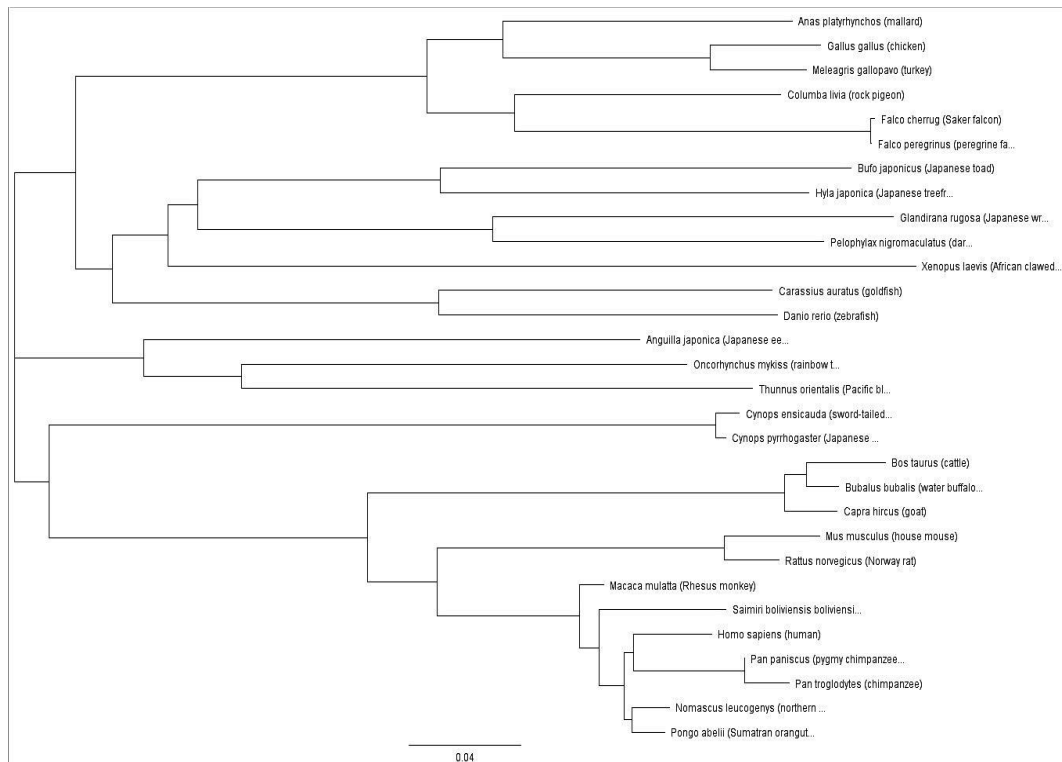
### 3.1.3 GHRL

Searching the available genome using TBLASTN with a known fish GHRL protein, identified a region of chromosome 6 that contained a potential zebrafish GHRL sequence. Using gene prediction software with this region provided a nucleotide sequence that was aligned against GHRL nucleotide sequences already identified in other fish (**Supplementary Fig. 35**) and in selected mammals (**Supplementary Fig. 36**) to identify regions of similarity. The



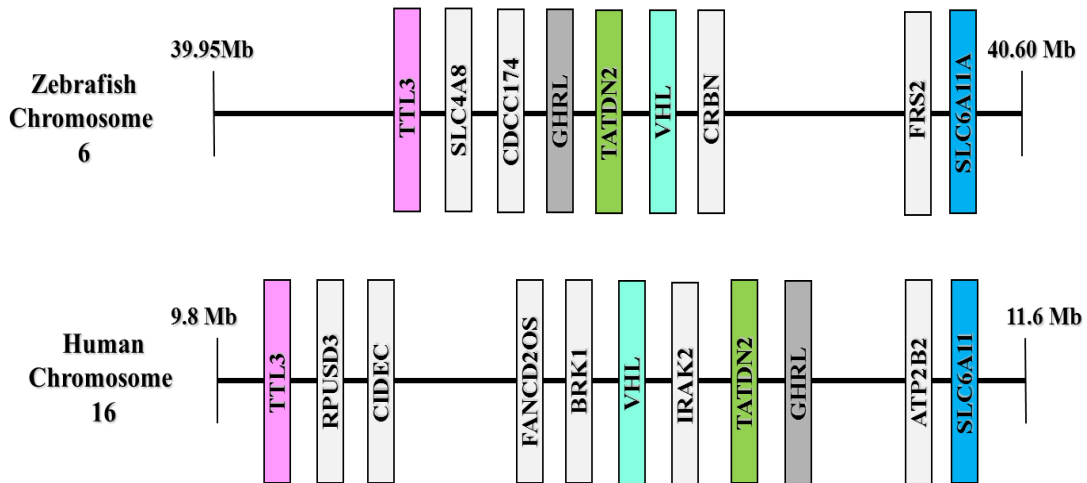
Homo	K-----	117
Pan	K-----	117
Nomascus	K-----	117
Macaca	K-----	117
Pongo	K-----	117
Saimiri	K-----	117
Mus	K-----	117
Bos	PLWKGSQTGGQREESRHS	133
Bubalus	E-----	116
Capra	E-----	116
Danio	-----	

**Figure 14.** ClustalW alignment of *Danio rerio* GHRL protein sequence against *Homo sapiens*, *Mus musculus*, *Bos taurus*, *Capra hircus*, *Bubalus bubalis*, *Pan paniscus*, *Pongo abelii*, *Macaca mulatta*, *Nomascus leucogenys* and *Saimiri boliviensis boliviensis* GHRL sequences. (\*) identical sequence and (:) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 28.90% sequence identity with these species and 29.00% similarity to *Homo sapiens*. Highlighted in colour are conserved domains of protein sequence deduced from comparison against *Homo sapiens* protein structure: Signal sequence (dark grey/), active core (light blue), mature ghrelin (red font).



**Figure 15.** Phylogenetic tree, constructed using the neighbour joining method alignment, showing the relationship between zebrafish GHRL and known vertebrate GHRL sequences.

Analysis of the region within the zebrafish genome around the predicted GHRL gene on chromosome 6 allowed a gene map to be constructed that showed that there was conservation of synteny between the human and zebrafish genomes (Fig. 16), giving further evidence that this gene was a homologue of the human GHRL gene.



**Figure 16.** Comparison of the co-localization of genes surrounding GHRL on Human chromosome 16 and Zebrafish chromosome 6.

### 3.1.4 POMCb

Searching the available genome using TBLASTN with a known fish POMCb protein, identified a region of chromosome 7 that contained a potential zebrafish POMCb sequence. Using gene prediction software with this region provided a nucleotide sequence that was aligned against POMCb nucleotide sequences already identified in other fish (Supplementary Fig. 37) and in selected mammals (Supplementary Fig. 38) to identify regions of similarity. The sequence was also translated and the predicted protein was aligned against known POMCb protein sequences already identified in other fish (Fig. 17) and in selected mammals (Fig. 18). In both alignments there was clear regions of high similarity indicating that this sequence was a likely candidate for the zebrafish POMCb gene. Phylogentic analysis of the zebrafish POMCb protein sequences with known vertebrate POMC genes grouped the gene very clearly with the known fish, amphibian and bird GHRL protein sequences and the POMCa very closely to other cyprinid species (Fig. 19). It was very clear from the phylogenetic

tree that POMCa and POMCb were quite different to each other as they branched in different positions.

```

Danio          -----MFCPSW--LLAAAVLCFHSPHVDGGRCISGLI-DCMDLESNEHKLQCLRKCRSDQ 51
Amatitlania   -----MVCLCW--LLVVVMAFVCI PGFES-VCWDNS-ICKDLSSKARILDCIHLCMSVI 50
Solea          -----MCPVW--LLVAATVLGVARGSVTNQCWEHP-SCQEVNDRSMMECVRLCRSDL 50
Common        -----MCPVW--LLVAATVLGVARGSVTNQCWEHL-SCQEVNDRSMMECVRLCRSDL 50
Sparus        -----MCPVW--LLVAVVVVGVAKGAAS-QCWEHP-SCQELNSESVMMECIQLCHSDL 49
Acanthopagrus -----MCPVW--LLVAVVVVGVAKGAAS-QCWEHP-SCQELNSESVMMECIQLCHSDL 49
Sparidentex   -----MCPAW--LLVAVVVVGVAKGAAS-QCWEHP-SCQELNSRDSVMMECIQLCHSDL 49
Black         -----MCPAW--LLVAVVVVGVAKGAAS-QCWEHP-SCQELNSESVMMECIQLCHSDL 49
Monopterus    -----MCPVW--LLVAVVVVGVTRGAVS-QCWEHP-SCQELNSESVMDCIQLCHSDL 49
Oreochromis   -----MCPVW--LFVALVVVGGAREAVS-QCWEHP-SCQELNSESVMMECIQLCHSDL 49
Dicentrarchus -----MCPVW--LLVAVALAGVARGAVG-QCLEHP-SCQELNSESVMMECIQLCHSDL 49
Epinephelus   -----MCPAW--LLVAVVVVGVTRGAVS-QCWEHP-SCQDVKSEGSMMMECIQLCHSDL 49
Gobiocypris   MVRGVRMLCPAW--LLALAVLSA-GGSEVRAQCWENT-RCRDLTTEESILERIQLCHSDL 56
Carassius     MVRGVRMLCPAW--LLALAVLSA-GGSEVRAQCWEDA-RCRDLTTEESILERIQLCHSDL 56
Ictalurus     MVKEARMGCPVW--MLALALLCA-SGSEVRAQCWENS-GCRDLSSDRNILECIRLCKSEL 56
Japanese      -----MLCPAW--MLALAVLSA-FCSEVSGQCWAHS-HCKDLSSSENMLECIRLCKSEL 50
European      -----MLCPAW--MLALAVLSA-FCSEVSGQCWAHS-HCKDLSSSENMLECIRLCKSEL 50
Anguilla      -----MLCPAW--MLALAVVVRVQKVSQCWAHS-HCKDLSSSENMLECIRLCKSEL 51
Acipenser     -----MLRSVWGCVVVVLGVLWFYSSGVQSCSEHS-QCRDLSSSENILECIRLCKVNL 53
Polyodon      -----MLRPVWGCVVVVLGVLWFYSSGVQSCSEHS-QCRDLSSSENILECIRLCKVNL 53
Lepisosteus   -----MLRSVW---VYSLGLAVLLQQSGREQCWEHS-QCRDLSSSENILECIRLCKVNL 50
Protopterus   -----MLKPVWRHLFVLSAVLMIYGTGVHSQCWETS-KCRDLSTESNLLECIKCKSDL 53
Neoceratodus  -----MLKPFWSHLFALSGLVWVYSSGVQSCSEHS-KCTDLSTELNLECIKCKFDL 53
Heterodontus  -----MMQQSMWKGNLVVLMSVWALTSGQLQDCLDHG-KCRELSSAPKLMECTEACKVEK 54
Horn          -----MMQQSMWGRNLVVLMSVWALTSGQLQDCLDHS-KCRELSSAPKLMECTEACKVEK 54
Verasper      -----MMQLLMCRGLLVVLMMWTLTSCQLEECWDQN-KKELISTPKLMECTEACKVVK 54
Chimaera      -----MELWMSRAVLLVALLWPDFAVAQGCQGDQDPDCHLNNLDTNLLNECNETVCSNGQ 54

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Danio          ESSRNIRVSSSEHQSSSEEQVEE-----QSLSLGLLLSALSPDS----IELQNPTAE 98
Amatitlania   QTEIPELSALAFNVNSDADADDGSGGDDYNDSDLLLRILATLASDDKMPESDLKARSD 110
Solea          SAEELVPGDGHLPPLS-----SLALSSSSSSSPQ----- 82
Common        SAEELVPGDGHLPPLS-----SLALSSSSSSSPQ----- 82
Sparus        TAETPVI PGNAHLQPPPPS-----DPSSFTLPSSSSSPQ----- 83
Acanthopagrus TAETPVI PGNAHLQPPPPS-----DPSSFTLPSSSSSPQ----- 83
Sparidentex   TAETPVI PGS AHLQPPPPS-----DPSSFTLPSSSSSPQ----- 83
Black         TAETPVI PGS AHLQPPPPS-----DPSSFTLPSSSSSPQ----- 83
Monopterus    TAETPLI PGR AHLQPPPPS-----DSSSILPSSSSTTPQ----- 82
Oreochromis   TAETPVI PGNAHLQPAVPS-----DAS-----SPSSQ----- 76
Dicentrarchus TAETPI I PGDGHLPPLS--E-----SLPPLSLFSSSSSPQ----- 84
Epinephelus   TAETPVI PGDNHLQVPVPSDL-----SLPPLPLSSPSSSPQ----- 86
Gobiocypris   TDETPVYLGESHLPPEPEQN-----EVIAPLSPVALATAEQM----- 95
Carassius     TDETPVYLGESHLPPEPEQN-----EVLPLSPALALAEQM----- 95
Ictalurus     TAESPLYPGDGHLPVLLIPSE-----ITTARLS---TPQED----- 91
Japanese      TAGR---RASSTMETE GEEGML-----GFLPMVSSMQEGPEVD----- 86
European      TAGR---RASSTMETE GEEGML-----GFLPMVSSMQEGPEAD----- 86
Anguilla      TA-----APWQTEGEEGML-----GFLPMVSSMQEGPEVD----- 82
Acipenser     SAKSPILPGNEHLQPTPEDIQN-----YIISHFRWNTFGQRMNGTPGGSKGESASTALN 107
Polyodon      TAESPLFPNGNEHLQPTSEEIQN-----YVMGFHWNTFGQRMNGTPGGSKRESASNALN 107
Lepisosteus   TAESPVYFGNGHLPPEADRN-----YAKSHFRSTALGRRTNGSVGSSKQAGENAALS 104
Protopterus   SAESPVYFGNGHMQPLSEDIRK-----YMMTHFRWDKFGRRNETGNKREDGYKTPLLS 107
Neoceratodus  SAESPVYFGDGHLPVLLIPSEDIRN-----YMMTHFRWDKFGRRNETGNKQEGGSDTFLFN 107
Heterodontus  TLESPIYFGNGHLPVLLIPSEDIRN-----YVMGFHWNTFGQRMNGTPGGSKRESASNALN 108
Horn          TLESPIYFGNGHLPVLLIPSEDIRN-----YVMGFHWNTFGQRMNGTPGGSKRESASNALN 108
Verasper      TLESPIYFGNGHLPVLLIPSEDIRN-----YVMGFHWNTFGQRMNGTPGGSKRESASNALN 108
Chimaera      SLEVLFPNGHRLMPVFEALRS-----YVMGFHWNTFGQRMNGTPGGSKRESASNALN 103

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Danio          AP-----HGDERRSYSMEHFRWGKPMGRKRRPVKVLNSGAL 134
Amatitlania   RRSYTMHFRWGKPSGLKMRESKLRARSDEERSYSMEHFRWGKPSGRKRRPVKVFASSLE 170
Solea          -----AKRAYSMEHFRWGKPVGKRRRPI TVYSSNSV 113
Common        -----AKRAYSMEHFRWGKPVGKRRRPI TVYSSNSV 113
Sparus        -----AKRAYSMEHFRWGKPVGKRRRPI TVYSSNSV 114
Acanthopagrus -----AKRAYSMEHFRWGKPVGKRRRPI TVYSSNSV 114
Sparidentex   -----AKRAYSMEHFRWGKPVGKRRRPI TVYSSNSV 114
Black         -----AKRAYSMEHFRWGKPVGKRRRPI TVYSSNSV 114
Monopterus    -----AKRAYSMEHFRWGKPVGKRRRPI TVYSSNSV 113
Oreochromis   -----AKRAYSMEHFRWGKPVGKRRRPI TVYSSNSV 107

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Dicentrarchus -----AKRSYSMEHFRWGKPVGRKRRPVKVFTSNGV 115  
 Epinephelus -----AKRSYSMEHFRWGKPVGRKRRPVKVYTSNGV 117  
 Gobiocypris -----DPDFSTRHEHKRSYSMEHFRWGKPVGRKRRPIKVYTNV 134  
 Carassius -----DPGSSPRHELKRSYSMEHFRWGKPVGRKRRPIKVYTNV 134  
 Ictalurus -----APESGPQHEEKRSYSMEHFRWGKPVGRKRRPIKVYANGV 130  
 Japanese -----EQGREPRHEDKRSYSMEHFRWGKPVGRKRRPIKVFPSPG-M 125  
 European -----EQGREPRHEDKRSYSMEHFRWGKPVGRKRRPIKVFPSPG-M 125  
 Anguilla -----EQGREPRHEHKRSYSMEHFRWGKPVGRKRRPIKVFPSPG-M 121  
 Acipenser ILLAALSQPTDEVERESEEAEGQQHRRDDKRSYSMEHFRWGKPVGRKRRPVKVPNGV 166  
 Polyodon VLLAVLSQPRDKVERDSEEEEGQQHRRDEKRSYSMEHFRWGKPVGRKRRPVKVPNGV 166  
 Lepisosteus ILFAALAPP--QAEEEMEESESSQQRRREDKRSYSMEHFRWGKPVGRKRRPVKVPNGV 161  
 Protopterus IIPALESHNNLDMEEDS-----LSRQDDRRSYSMEHFRWGKPVGKRRRPIKVYPNG-M 159  
 Neoceratodus ILPALESHTDLVAGEP-----LQRQDDKRSYSMEHFRWGKPVGKRRRPIKVYPNG-M 159  
 Heterodontus AFLNHLPAVEPHTSQMENEEMETLFPREDDKRSYSMEHFRWGKPMGMKRKPKVYPNN-F 167  
 Horn AFLNHLPAVEPQTSQMENEEMETLFPREDDKRSYSMEHFRWGKPMGMKRKPKVYPNN-F 167  
 Verasper VFLNQLPAVESQASQMENEEMETLLPREDEKRSYSMEHFRWGKPMGKRRKAIKVPYNS-F 167  
 Chimaera -----PRWEPKGPPTLRE-----GKRSYSMEHFRWGKPMGKRRRPIKVYPNE-E 146

:\*:\*\*\*\*\* \* \*\*:: \* ..

Danio EEEPEESESVRVERG-----QS-----GTLEVQ----- 158  
 Amatitlania GGSSSEGRFPQARRQ-----LSSSEDEAEGDVGGSNENQ----- 206  
 Solea EEEFPEVLRQELG----- 126  
 Common EEEFPEVLRQELG----- 126  
 Sparus EESAEVFPGEIRRE-----LASELLAAAAVAQEIEEKAVQEVMQE----- 156  
 Acanthopagrus EESAEVFPGEIRRE-----LASELLAAAAVAQEIEEKAVRKMVQE----- 156  
 Sparidentex EESAEVFPGEIRRE-----LASELLAAAAVAQEIEEKA----- 149  
 Black EESAEVFPGEIRRE-----LASELLAAAAVAQEIEEKA----- 149  
 Monopterus EESAEVFPGEMRRE-----LANELLAAAA-----EIEEKA----- 145  
 Oreochromis AESAEVFPPEMRRE-----LTNELLAEEG-----EKA----- 136  
 Dicentrarchus EESAEVFPPEMRRE-----LASEIIAAED-----EIEEKA----- 145  
 Epinephelus EESAEVFPGEMRRE-----LTSKLLAAKE-----KEKA----- 147  
 Gobiocypris EESAEVFPGEMRREL-----ATNEVDY----- 157  
 Carassius EESAEVFPGEMRREL-----ATNEVDY----- 157  
 Ictalurus EESSEALPAEMRREL-----G----- 147  
 Japanese EESSEAYPAEMRREL-----LADWDYA----- 148  
 European EESSEAYPAEMRREL-----LGDWDYA----- 148  
 Anguilla EESSEAYPAEMRREL-----LGDWDYA----- 144  
 Acipenser EESAEVFPPEMRREL-----SLKLDYPPQGEIEEVEVFGGE----- 201  
 Polyodon EESAEVFPPEMRREL-----SLKLDYPPQGEIEEVEVFGGE----- 201  
 Lepisosteus EESAEVFPPEMRREL-----MSLDYPLLEEVEEELGGE----- 196  
 Protopterus EDESAEVFPPEMRREL-----PMEFDYP--EPSSEKSEE----- 192  
 Neoceratodus EDESAEVFPPEMRREL-----TMEIDYP--ESSSEERSEE----- 192  
 Heterodontus EDGSVENMGPRLKREA-----SVGFDY-PVET--SEAGEEMLEDAK----- 206  
 Horn EDGSVENMGPRLKREA-----SVGFDY-PVET--SEAGEEMLEDAK----- 206  
 Verasper DDDSMHEMGPPELKREA-----SVDFDY-PVEQ--SEVGEEMLEDAK----- 206  
 Chimaera PETSAGLGDQFKRQIPPEAWGPSEPVWPVEQVWGPVEQVTSWGGEAWSPEQAAGPEEE 206

\*

Danio -----HRNNVKNNKG- 168  
 Amatitlania -----GWLRRDSTKS 217  
 Solea -----EADVEQH 133  
 Common -----EADVEQH 133  
 Sparus -----EKAQEVMEIEEIEEQ 171  
 Acanthopagrus -----EKAQEVMEIEEIEEQ 171  
 Sparidentex -----AQEVMEIEEIEEQ 162  
 Black -----AQEVMEIEEIEEQ 162  
 Monopterus -----QEVMEIEEIEEQ 154  
 Oreochromis -----QEMVEGAEIEEQ 147  
 Dicentrarchus -----QEVMEIEEIEEQ 153  
 Epinephelus -----QEVAEDEQE 156  
 Gobiocypris -----PQEESA--- 163  
 Carassius -----PQEEGA--- 163  
 Ictalurus -----AVEDST--- 153  
 Japanese -----PEEEGVAAE 157  
 European -----PEEEGVAAE 157  
 Anguilla -----PEEEGVAAE 153  
 Acipenser -----NDLLNLQ- 208  
 Polyodon -----NDLLNLQ- 208  
 Lepisosteus -----NEVLNLQ- 203  
 Protopterus -----NDIMNLL- 199  
 Neoceratodus -----NDILSLL- 199  
 Heterodontus ---KKGKVKYKMAHFRWGRGPKGPAKNWGPDMRTQPLQFPNLEDVQLQESIDNGLPEEE- 262  
 Horn ---KKGKVKYKMAHFRWGRGPKGPAKNWGPDMRTQPLQFPNLEDVQLQESIDNGLPEEE- 262  
 Verasper ---KKGKVIYKMTFRWGRGPK---HLGPDRIQSLQFPNLEDVQLQERMNDNLPPEE- 258  
 Chimaera PEQKKGKLYKMTFRWSEGSR---DGLRQAGQRYVLP I FPKTPEASQPQGDGNQAEVEG 263

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Danio -----YRMTHFRWNAPPD-KRYGGLMRPYLDESHKPLITLIRNAIGNGQQFT 214
Amatitlania QSPLSIQERKDRITYKMSHFRWGSPPASKRNGGFACLWEENPQGQLAKFFRNILVKDVERI 277
Solea KFHN-VHEKKDGAYKMKHFRWSDPPASKRYDELTERRGDGE-----AAQRGGGAGG 184
Common RFHN-VHEKKDGAYKMKHFRWSDPPTSKRYDELTERRGDAE-----AAQRGGGAGG 184
Sparus QLLGDVQEKKGGLYKMKHFRWGSPPASKRYGGFMKSWDERSQRPLTLFKNVINKDGQQQ 231
Acanthopagrus QLLGDVQEKKGGLYKMKHFRWGSPPASKRYGGFMKSWDERSQKPLTLFKNVINKDGQQQ 231
Sparidentex QLLGDVQEKKGGLYKMKHFRWGSPPASKRYGGFMKSWDERSQRPLTLFKNVINKDGQQQ 222
Black QLLGDVQEKKGGLYKMKHFRWGSPPASKRYGGFMKSWDERSQRPLTLFKNVINKDGQQQ 222
Monopterus ELLNSIQEKKGGLYKMKHFRWGSPPASKRYGGFMKSWDEGSQRPLTLFKNVINKEGQQQ 214
Oreochromis QLLNGVQEKKGGLYKMKHFRWGSPPASKRYGGFMKSWDERSQKPLTLFKNVINKEGQQQ 207
Dicentrarchus -----LHEKKDGTYKMKHFRWGGSPASKRYGGFMKSWDERSQRPLTLFKNVINKDGQEQ 208
Epinephelus QLPGDIHEKKDGGYKMKHFRWGGPPASKRYGGFMKSWDERSQRPLTLFKNVINKDGQ-Q 215
Gobiocypris ---LNQQ-KKDGSYKMKHFRWSSPPASKRYGGFMKSWDERSQKPLTLFKNVINKENQRK 219
Carassius ---LNQQDKKDGSYKMNHFRWSSPPASKRYGGFMKSWDERSQKPLTLFKNVINKEHQK 220
Ictalurus ---VSQQEKKGGLYKMKHFRWSSPPASKRYGGFMKSWDEHSQKPLTLFKNVINKDGHQK 210
Japanese HPQLTLQTKKDYKMKHFRWNGPPASKRYGGFMKSWDERSQKPLTLFKNVINIKDGQQK 217
European HPQLTLQTKKDYKMKHFRWNGPPASKRYGGFMKSWDERSQKPLTLFKNVINIKDGQQK 217
Anguilla HPQLTLQTKKDYKMKHFRWNGPPASKRYGGFMKSWDERSQKPLTLFKNVINIKDGQQK 213
Acipenser -----EKNNGSYKMHFRWSSPPDKRYGGFMKSWDERSQKPLTLFKNVINIKDGHEK 261
Polyodon -----DKKEGSYKMNHFRWSSPPDKRYGGFIKSWDERSQQPLTLFKNVINIKDVHEK 261
Lepisosteus -----EKKDGSYKMHFRWSSPPDKRYGGFMKSWDERSQKPLTLFKNVINIKDGHEK 256
Protopterus -----EKKDRNYRMQHFRWSSPPDKRYGGFMKSWDERSQKPLMTLFRNVIKDAYEK 252
Neoceratodus -----EKKDRAYRMQHFRWSSPPDKRYGGFMKSWDERSQKPLMTLFRNVMKDAYEK 252
Heterodontus -----VKKDGEDYKFGHFRWSSVPLKDKRYGGFMKSWDERGQKPLTLFRNVIKDGHEK 316
Horn -----VKKDGEDYKFGHFRWSSVPLKDKRYGGFMKSWDERGQKPLTLFRNVIKDGHEK 316
Verasper -----VKKDGEDYKFGHFRWSSVPLKDKRYGGFMKSWDERGQKPLTLFRNVIKDGHEK 312
Chimaera EEKELGPPEEKREYKIGHFRWSSPLKDKRYGGFMNSWD---HKPLTLFRNVMGKEGPGG 320

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Danio D---- 215
Amatitlania MG--- 279
Solea GR--- 186
Common GR--- 186
Sparus Q---- 232
Acanthopagrus K---- 232
Sparidentex K---- 223
Black K---- 223
Monopterus E---- 215
Oreochromis K---- 208
Dicentrarchus KREQ- 212
Epinephelus EGEQ- 219
Gobiocypris DQ--- 221
Carassius DQ--- 222
Ictalurus DH--- 212
Japanese KAQ-- 220
European KAQ-- 220
Anguilla KAQ-- 216
Acipenser KGQ-- 264
Polyodon KGQ-- 264
Lepisosteus KGQ-- 259
Protopterus KGQ-- 255
Neoceratodus KGQ-- 255
Heterodontus KTQS- 320
Horn KTQS- 320
Verasper KIQNQ 317
Chimaera RALAQ 325

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**Figure 17.** ClustalW alignment of *Danio rerio* POMCb protein sequence against *Acipenser sinensis*, *Polyodon spathula*, *Solea senegalensis*, *Sparus aurata*, *Monopterus albus*, *Lepisosteus osseus*, *Gobiocypris rarus*, *Ictalurus punctatus*, *Dicentrarchus labrax*, *Sparidentex hasta*, *Acanthopagrus schlegelii*, *Acanthopagrus latus*, *Epinephelus coioides*, *Oreochromis mossambicus*, *Amatitlania nigrofasciata*, *Anguilla japonica*, *Solea solea*, *Verasper moseri*, *Carassius auratus*, *Protopterus annectens*, *Neoceratodus forsteri*, *Chimaera phantasma*, *Heterodontus portus jacksoni*, *Heterodontus francisci*, *Anguilla rostrata* and *Anguilla anguilla* POMC sequences. (\*) identical sequence and (:) highly

similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 26.55% sequence identity with the fish sequences.

Ovis	MPRL	SSRSGALLLVLLLQA	----	SMEVRG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Pantholops	MPRL	SSRSGALLLALLLQA	----	SMEVRG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Bos	MPRL	SSRSGALLLALLLQA	----	SMEVRG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Sus	MPRL	GSRSRSGALLTLLLQA	----	SMGVRG	/WCLESSQCQDLSTESNLLACIRACKPDLS	55
Ceratotherium	MPRQR	GSRSRSGALLLALLLQA	----	SVEVRG	/WCLETSQCQDLTTESNLLACIRACKPDLS	55
Felis	MPRS	CCSRPGALLLALLLQA	----	SMEVSG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Myotis	MPRS	CCSRSGALLLALLLQA	----	FMEVRG	/WCLESSQCQDLTSESNLLACIRACKPDLS	55
Pan	MPRS	CCSRSGALLLALLLQA	----	SMEVRG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Gorilla	MPRS	CCSRSGALLLALLLQA	----	SMEVRG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Pygmy	MPRS	CCSRSGALLLALLLQA	----	SMEVRG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Homo	MPRS	CCSRSGALLLALLLQA	----	SMEVRG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Macaca	MPRS	CCSRSGALLLALLLQA	----	SMEVRG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Crab-eating	MPRS	CCSRSGALLLALLLQA	----	SMEVRG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Papio	MPRS	CFSRSGALLLALLLQA	----	SMEVRG	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Saimiri	MLRSC	CSRSGALLLALLLQA	----	SMEVRG	/WCLESSQCQDLTTESHLLACIRACKPDLS	55
Chinchilla	MPRL	YCSGALLLALLLQA	----	SMEVCG	/WCLESSQCQDLTTERNLLACIRACKPDLS	55
Mus	MPRF	YSRSGALLLALLLQT	----	SIDVWS	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Rattus	MPRF	YSRSGALLLALLLQT	----	SIDVWS	/WCLESSQCQDLTTESNLLACIRACKPDLS	55
Mesocricetus	MPRS	FYSRSGALLLALLIQT	----	SVEVWG	/WCLESRCQDLTTESNLLACIRACKPDLS	55
Microtus	MPRS	CHSRSGALLLALLLQT	----	SMEVWG	/WCLESSQCQDLTTESNLLACIRACKLELS	55
Condylura	MPRS	CCSRSGALLLVLLLQASLLQA	SMEVPG	/WCLESSQCQDLTTESNLLACIRACKWPLDS	60	
Sorex	MPRQ	RGHPGALLLVLLLHA	----	SVEVHG	/WCLESGRCQDLTTESDLLACIRACKQAALT	55
Danio	MF	CPSWLLAAVLCFHS	PHVD	----	GG/RCSGLIDCMDLESNEHKLQCLRKCRSDQE	52

\* . . \* : \* : . \* \* \* : : . \* \* : \* \*

Ovis	AETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNGSSSFGAGG	---AAQKR	-----	105
Pantholops	AETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNGSSSFGAGG	---AAQKR	-----	105
Bos	AETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNGSSSFGVGG	---AAQKR	-----	105
Sus	AETPVFPNGGDAQPLTENPRK	YVMGHFRWDRF	GRRNGSSSGGGGGGAGQKR	-----	108	
Ceratotherium	AETPVFPNGGDEQSLTENPRK	YVMGHFRWDRF	GRRNSS	---GGGG	---AGQKR	102
Felis	AETPVLFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNGS	-----	---AGQKR	98
Myotis	AETPVFPNGGEEQLLAENPRK	YVMGHFRWDRF	GRGNDSG	---GAG	---AGPKR	102
Pan	AETPMFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNSSSSSS	-GS-	-GAGQKRED-	VSA 110
Gorilla	AETPMFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNSSSSSS	-GS-	-GAGQKRED-	VSA 109
Pygmy	AETPMFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNSSSSSS	-GS-	-GAGQKRED-	VSA 110
Homo	AETPMFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNSSSSG	-SS-	-GAGQKRED-	VSA 109
Macaca	AETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNSSSGS	-----	-GAAQKRED-	VAA 107
Crab-eating	AETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNSSSGS	-----	-GAAQKRED-	VAA 107
Papio	AETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNSSSGS	-----	-GAAQKRED-	VAA 107
Saimiri	AETPVFPNGGDEQPLTENPRK	YVMSHFRWDRF	GRRNSSSGGGGG	---	-GAGQKRED-	FAA 111
Chinchilla	AETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNSSGGG	-----	-GASRKREEE-	VAA 108
Mus	LETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GPRNSSSAG	-----	-SAAQRR-	102
Rattus	AETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GPRNSSSAG	-----	-GSAQRR-	102
Mesocricetus	AETPVFPNGGDEQTLTENPRK	YVMGHFRWDRF	GPRNSS	-----	-GAAQQRRAEEEEE	106
Microtus	AETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GPRNSS	-----	-GTAQRR-	AEE 102
Condylura	AETPVFPNGGDEQPLTENPRK	YVMGHFRWDRF	GRRNSSV	-----	-AGQKRD-	106
Sorex	AETPVFPNGGDEQPLAEDARK	YVMGHFRWERF	GHRN	-----	-----	91
Danio	SSRNIRVSSSEHQSSSEQV	EQSLSLG	-----	-----	-----	79

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Ovis	EEE-VAVGEG-PGPRGDGAETGPREDKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	163		
Pantholops	EEE-VAVGEG-PGPGDGAETGPREDKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	163		
Bos	EEE-VAVGEG-PGPRGDGAETGPREDKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	163		
Sus	EEEEVAAGEG-PGPRGDGVAAPGPRQDKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	167		
Ceratotherium	EEEEAVLVGR-PAPRGDGGEPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	161		
Felis	EEEEVAAGDGGPGPRGDGGEGLGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESV	158		
Myotis	TEEAAAAGDGGGLGPRGDGAGPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESP	162		
Pan	GEDRGPLPEGGPEPRSDGAKPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	170		
Gorilla	GEDRGPLPEGGPEPRSDGAKPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	169		
Pygmy	GEDRGPLPEGGPEPRSDGAKPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	170		
Homo	GEDCGPLPEGGPEPRSDGAKPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	169		
Macaca	GEDSGLLPEGGPKPRGDGAEPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	167		
Crab-eating	GEDSGLLPEGGPKPRGDGAEPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	167		
Papio	GEDRGLLPEGGPEPRSDGAKPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	167		
Saimiri	GEDRGLLPEGGPEPRSDGAKPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNSAENESA	171		
Chinchilla	GEGRVPLPAGDPEFRGDGAEPGREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNGAEDESA	168		
Mus	AEAEAVWGDGSPE	-----	PSPREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNVAENESA	155
Rattus	AEETAGDGRPE	-----	PSPREGKR	YSMEHFRWGKPV	GKKRR	PVKVYPNVAENESA	155

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Mesocricetus      EEDAVAVEDARAE-----PGLREGKR SYSMEHFRWGKPV GKKRR PVKVY SV AE NSA 159
Microtus          EEDAAVDDGRAE-----PGLREGKR SYSMEHFRWGKPV GKKRR PVKVY PN VA ENESA 155
Condylura        -EAAAAGHGLPEALAYGTEPGPREGKR SYSMEHFRWGKPV GKKRR PVKVY PNGA ED EST 165
Sorex            -----GSG----- HFRWGKPV GKKRR PVKVY PNGA ED DSA 121
Danio            -LLLSALSPDSIELQNPTAEAPHGDERR SYSMEHFRWGKPV GKKRR PVKVLS NG AL EEEP 138
                *****:*:***** .. * ::

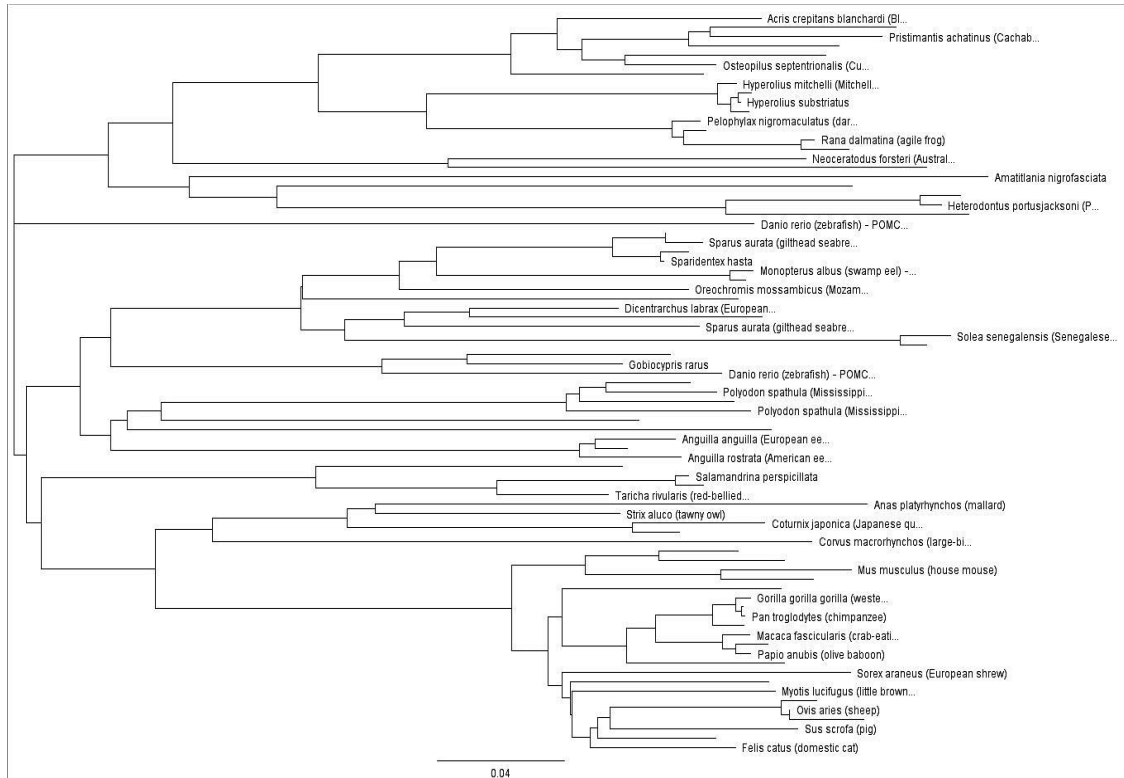
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Pantholops       QAFFLEFKRELTGERLEQARGPEAQAE-SAAARAELEYGLV--AEAEAAAKDSGPYKME 220
Bos              QAFFLEFKRELTGERLEQARGPEAQAE-SAAARAELEYGLVAEAEAAEAAEKDSGPYKME 222
Sus              EAFFLEFRRELAGAPPEPARDPEAPAE-GAAARAELEYGLV--AEAEAAEKKDEGPYKME 224
Ceratotherium   EAFFLEFKRELAGERPEPAAAP-----AGLEYSLV--AEAEAAEKKDEGPYKME 208
Felis           ETFPLEFKRELAGERPGPALGPEGPAD-GAAALPDLEYGLVAEAEV--AEKKDEGPYKME 215
Myotis          EAFFVEFKRE----RPEPALRPEGAAQ-GEAAPADLEDDLGAEEAAPSALKKDEGPYRME 217
Pan             EAFFLEFKRELTGQRPREGDGPDPADDGAGAQADLEHSLL-----VAAEKKDEGPYRME 225
Gorilla         EAFFLEFKRELTGQRPREGDGPDPADDGAGAQADLEHSLL-----VAAEKKDEGPYRME 224
Pygmy           EAFFLEFKRELTGQRPREGDGPDPADDGAGAQADLEHSLL-----VAAEKKDEGPYRME 225
Homo            EAFFLEFKRELTGQRLREGDGPDPADDGAGAQADLEHSLL-----VAAEKKDEGPYRME 224
Macaca          EAFFLEFKRELTGQRPREGDGPDPADDGAGPRADLEHSLL-----VAAEKKDEGPYRME 222
Crab-eating     EAFFLEFKRELTGQRPREGDGPDPADDGAGPRADLEHSLL-----VAAEKKDEGPYRME 222
Papio           EAFFLEFKRELTGQRPREGDGPDPADDGAGPRADLEHSLL-----VAAEKKDEGPYRME 222
Saimiri         EAFFLEFKRELADQRPLAGDGPDSPTDGGAGAEADPEHGLL-----VAAEKKDEGPYRME 226
Chinchilla      EAFFLEFKRELAGERPAAAPGD-----ELELGEA-----AAEKKDEGPYRME 212
Mus             EAFFLEFKRELEGERPLG-----LEQVLE-----SD-AEKDDEGPYRVE 192
Rattus          EAFFLEFKRELEGEQPDG-----LEHVLE-----PD-TEKADEGPYRVE 192
Mesocricetus    EAFFLEFRRELAGERPDG-----LERVLE-----AD-AEKDDEGPYRVE 196
Microtus        EAFFLEFRRELAGERPDG-----LEHILE-----PDSAEKDDEGPYRME 193
Condylura       EAFFVEFKRGLSAEHPEDPAGSA-----AGLEYGLVAET--EALEKKDDCAYQMK 213
Sorex           EAFFGEFRRDLAAAPEPALDGP-----DGLEPGLLAP----AAAKKDGTPYKME 167
Danio           EESESSVRVERGQSGTLEVQHRN-----NVKNNKGKYRMT 172
:               : ..:                               *   *:::

Ovis              HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAHKKGQ 263
Pantholops       HFRWGSPPKDKRYGGVKS--SEKSQTPLVTLFRNAIKNAHKKGQ 263
Bos              HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAHKKGQ 265
Sus              HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIVKNAHKKGQ 267
Ceratotherium   HFRWGSPPKDKRYGGFMS--SEKSQTPLVTLFKNAIKNAHKKGQ 250
Felis           HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAHKKGQ 258
Myotis          HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAHKKGQ 260
Pan             HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAYKKGE 268
Gorilla         HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAYKKGE 267
Pygmy           HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAYKKGE 268
Homo            HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAYKKGE 267
Macaca          HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAYKKGQ 265
Crab-eating     HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAYKKGQ 265
Papio           HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAYKKGQ 265
Saimiri         HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAYKKGQ 269
Chinchilla      HFRWGTPRKDKRYGGFMS--SEKSQTPLVTLFKNAIKNAHKKGQ 255
Mus             HFRWSNPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAHKKGQ 235
Rattus          HFRWNPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAHKKGQ 235
Mesocricetus    RFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIVKNAHKKGQ 239
Microtus        RFRWGSTPKDKRYGGFMT--SEKSQTPLVTLFKNAIVKNAHKKGQ 236
Condylura       HFRWGSPPKDKRYGGFMT--SEKSQTPLVTLFKNAIKNAHKKGQ 256
Sorex           HFRWGSPPKDKRYGGFMS--SEKSQTPLVTLFRNAIKNAHKKGQ 210
Danio           HFRWNAPPDKRYGGLMRPYLDESHKPLITIRNAIGNGQQFTD- 215
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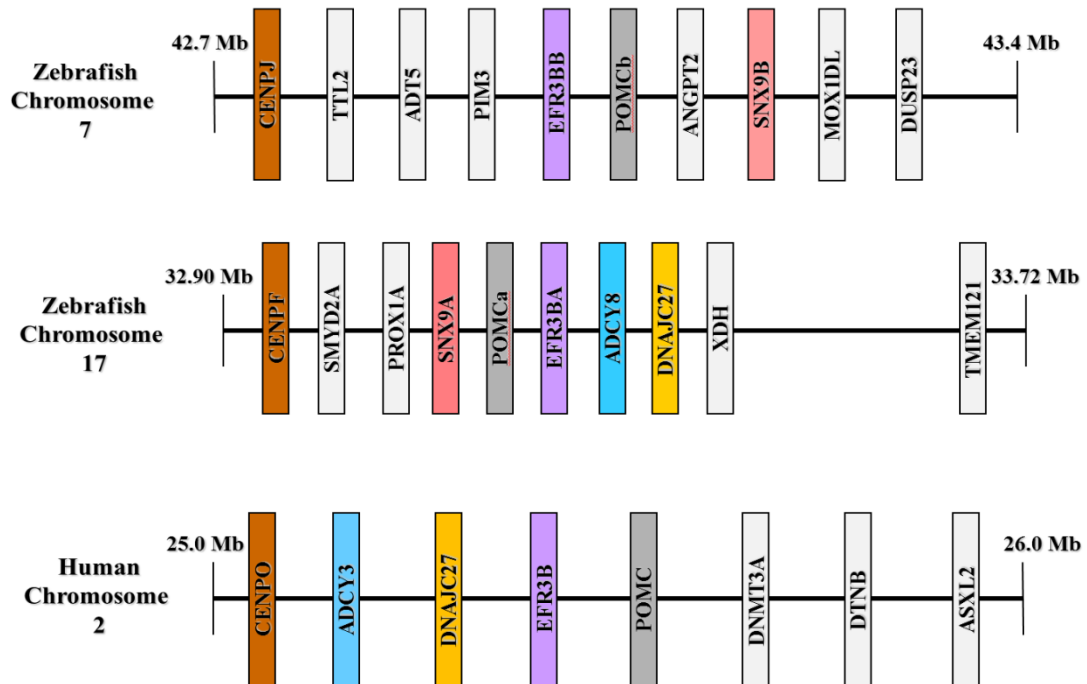
**Figure 18.** ClustalW alignment of Danio rerio POMCb protein sequence against Homo sapiens, Mus musculus, Rattus norvegicus, Sus scrofa, Bos taurus, Ovis aries, Pan troglodytes, Pan paniscus, Gorilla gorilla gorilla, Papio anubis, Macaca mulatta, Macaca fascicularis, Saimiri boliviensis boliviensis, Pantholops hodgsonii, Mesocricetus auratus, Microtus ochrogaster, Chinchilla lanigera, Felis catus, Condylura cristata, Sorex araneus, Myotis lucifugus and Ceratotherium simum simum POMC sequences. (\*) identical sequence and (:) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 26.99% sequence identity with these species and 26.40% similarity to Homo sapiens. Highlighted in colour are conserved domains of protein

sequence deduced from comparison against *Homo sapiens* protein structure: Signal sequence (dark grey/), conserved cysteines (red font), gamma-MSH (light blue), alpha MSH (green), CLIP (yellow), ACTH (blue font), delta MSH (orange), met-enkephalin (white font), beta endorphin (purple).



**Figure 19.** Phylogenetic tree, constructed using the neighbour joining method alignment, showing the relationship between zebrafish POMCb and known vertebrate POMC sequences.

Analysis of the region within the zebrafish genome around the predicted POMCb gene on chromosome 7 allowed a gene map to be constructed that showed that there was conservation of synteny between the human and zebrafish genomes (**Fig. 20**), giving further evidence that this gene was a homologue of the human POMCb gene.



**Figure 20.** Comparison of the co-localization of genes surrounding POMC on Human chromosome 7 and Zebrafish chromosomes 7 (POMCb) and 17 (POMCa).

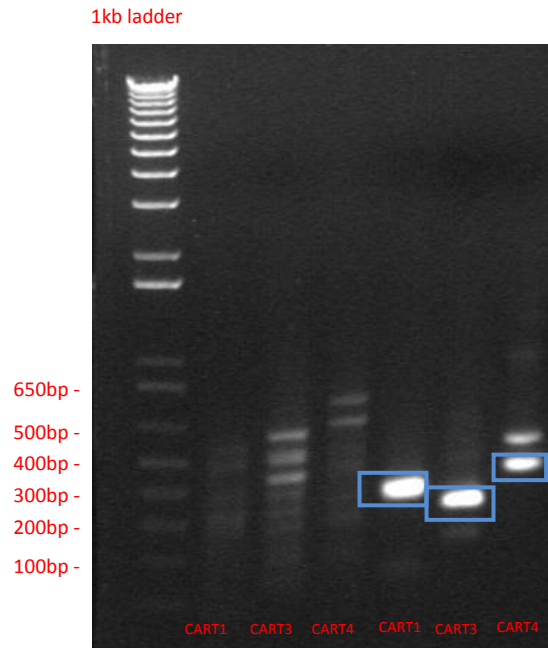
### 3.2 Confirmation of zebrafish eating behaviour gene cDNA sequence using RACE-PCR

Using the predicted sequences obtained from the genome, primers were designed for amplification of the 3' and 5' region of the previously selected eating behaviour genes; GHRL, AgRP, POMCb, CART1, CART3 and CART4 using RACE-PCR. 3'-RACE amplified bands of a reasonable size which were cloned, whereas 5'RACE did not produce anything that could be investigated further.

#### 3.2.1 3'-RACE PCR

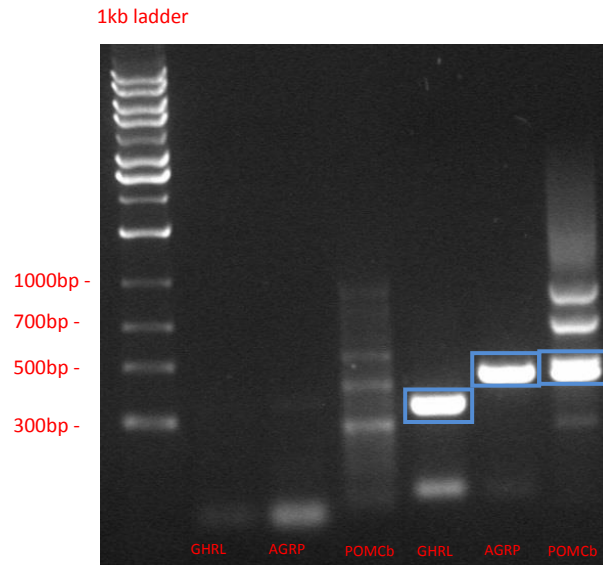
First and second round products of CART1, CART3, and CART4 were run side by side on a gel and compared against a 100 or 1000bp ladder. First round products tended to show numerous dim bands, whereas second round products, with the exception of CART4, produced a single bright band of a reasonable size (**Fig. 21**). The number of second round PCR cycles was reduced from 34 to 32

cycles to reduce non-specific band intensity and the strongest bands were gel extracted, allowing removal of the band of interest without removing excess gel or untargeted DNA.



**Figure 21.** Gel analysis of first (left) and second (right) round products of CART1, CART3, and CART4 genes against a 1kb ladder. First round products showed multiple faint bands for each gene where second round products show individual bands of approximately 400bp (CART1), 300bp (CART3) and 500bp (CART4) in length. Those in blue were selected for gel purification and ligation.

First and second round products of GHRL, AGRP, and POMCb were run side by side on a gel and compared against a 100 or 1000bp ladder. First round products tended to produce faint bands, second round products produced bright single bands, apart from POMCb which showed multiple bands. All products were of a reasonable size and this was shown to be consistent over several repeats of 3'-RACE PCR amplification of these genes with limited smearing and primer dimer formation (**Fig. 22**). The number of second round PCR cycles was reduced from 34 to 32 cycles to reduce band intensity for gel excision, allowing removal of the band of interest without removing excess gel or untargeted DNA.



**Figure 22.** Gel analysis of first (left) and second (right) round products of GHRL, AGRP and POMCb genes against a 1kb ladder. First round products showed multiple faint bands for each gene where second round products show individual bands of approximately 300bp (GHRL), 500bp (AGRP), and 500, 700, and 900bp (POMCb) in length. Those in blue were selected for gel purification and ligation.

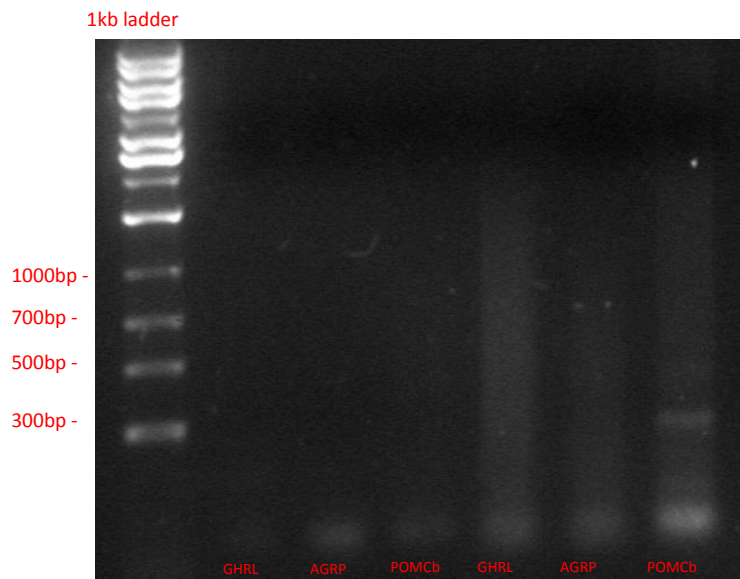
### 3.2.2 5'-RACE PCR

First and second round products of CART1, CART3 and CART4 were run side by side on a gel and compared against a 100 or 1000bp ladder. Only very faint bands were visualised for both sets of reactions (**Fig. 23**). Reaction conditions were altered for both rounds of reactions, as discussed in the methods, however results were unchanged even when a different thermocycler was used (Biorad T-100 to a MJ Research PTC200 Thermal cycler).



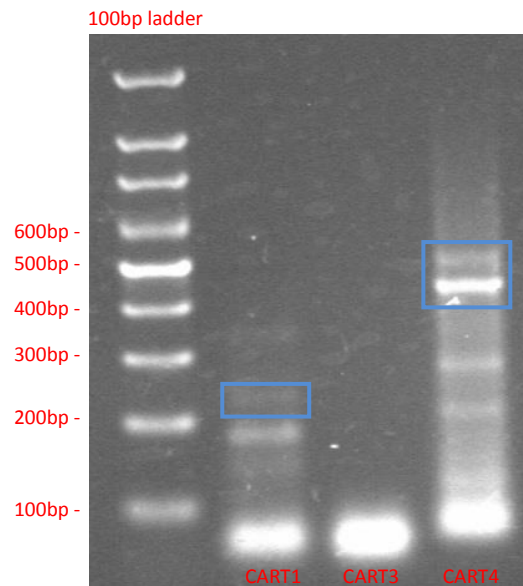
**Figure 23.** Gel analysis of first (left) and second (right) round products of CART1, CART3, and CART4 genes against a 100bp ladder. Both rounds of products showed only very faint bands.

First and second round products of GHRL, AGRP and POMCb were run side by side on a gel and compared against a 100 or 1000bp ladder. Only very faint bands were visualised for both sets of reactions (**Fig. 24**). Reaction conditions were altered for both rounds of reactions, as discussed in the methods, however results were unchanged even when a different thermocycler was used (Biorad T-100 to a MJ Research PTC200 Thermal cycler).

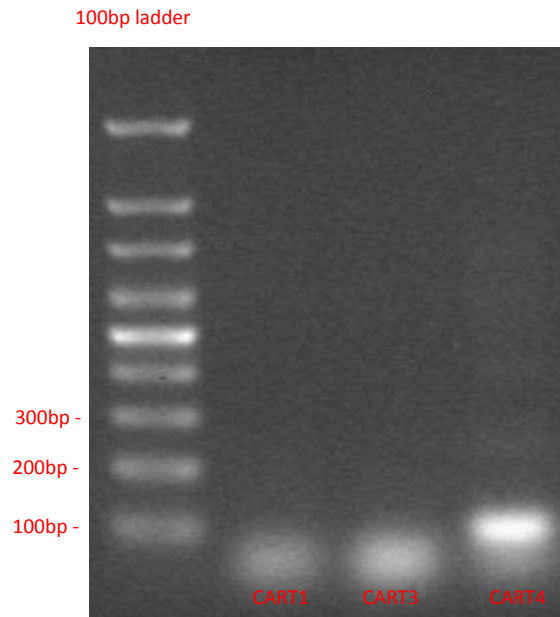


**Figure 24.** Gel analysis of first (left) and second (right) round products of GHRL, AGRP, and POMCb genes against a 1kb ladder. Both rounds of products showed only very faint bands.

To confirm the RACE library was not the problem a new one was constructed and 5'-RACE PCR run again for all genes. First round CART1, CART3 and CART4 products did produce bands for each (**Fig. 25**) this time. However, second round products for CART1, CART3 and CART4 consistently produced bands of a low product size despite alteration of reaction conditions (**Fig. 26**). For GHRL, AGRP and POMCb no products were obtained in the first or second round of PCR (data not shown).

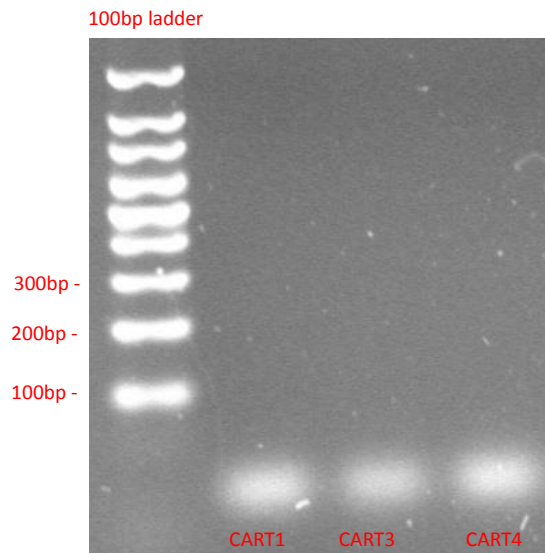


**Figure 25.** Gel analysis of first round products of CART1, CART3, and CART4 genes against a 100bp ladder. First round products showed distinct but faint bands for CART1 and CART4 with very bright small product bands for all genes. Those in blue were selected for gel purification.



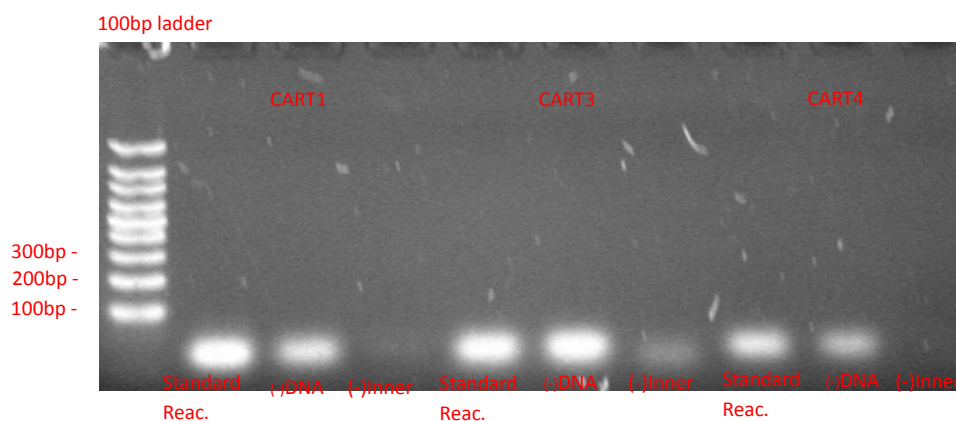
**Figure 26.** Gel analysis of second round products of CART1, CART3, and CART4 genes against a 100bp ladder. Only bright bands of a very low product size (below 100bp) and very faint bands for CART4 were identified.

In an attempt to overcome this, particular bands for CART1, CART3 and CART4 were excised from the first round gel (**Fig. 26**) and DNA was purified from the gel as described in the methods. Purified DNA (1  $\mu$ L) from the first round reactions was then used in the second round 5'-RACE PCR. However, second round products for CART1, CART3 and CART4 also produced bands of a low product size (**Fig. 27**).



**Figure 27.** Gel analysis of second round products of CART1, CART3, and CART4 synthesized from gel purified first round DNA against a 100bp ladder. Only bright bands of low product size (below 100bp) were identified.

Because small bands of high intensity were seen consistently in all gels, there was a concern of contamination within the PCR reagents. To check for this a second round reaction was run, where all reaction components were replaced apart from the 5' RACE inner adapter primer and the first round DNA template. Low product size bands were still identified within the negative control reactions (**Fig. 28**) indicating contamination. Due to time restrictions this was never resolved and products obtained from the 3' RACE were focussed on.

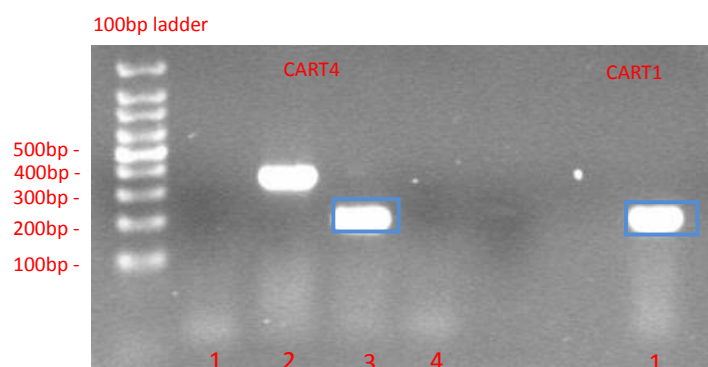


**Figure 28.** Gel analysis of second round products of CART1, CART3, and CART4 with a standard reaction, minus DNA negative control and a minus inner 5' RACE adapter primer control against a 100bp ladder. Bright bands of low product size (below 100bp)

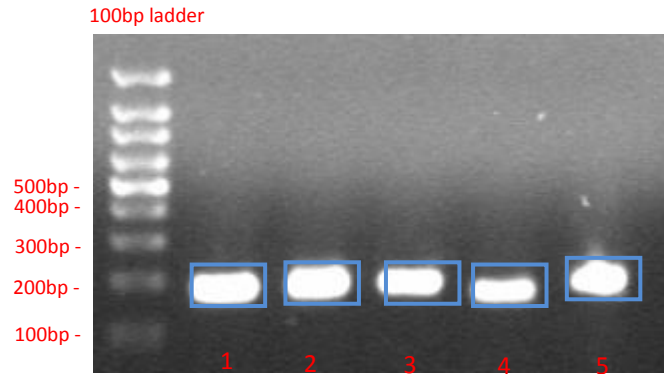
were identified in the standard reaction as well as in the DNA minus control, in addition faint bands are visible in the inner adapter minus control.

### 3.2.3 *E.coli* transformation and colony screening of PCR products

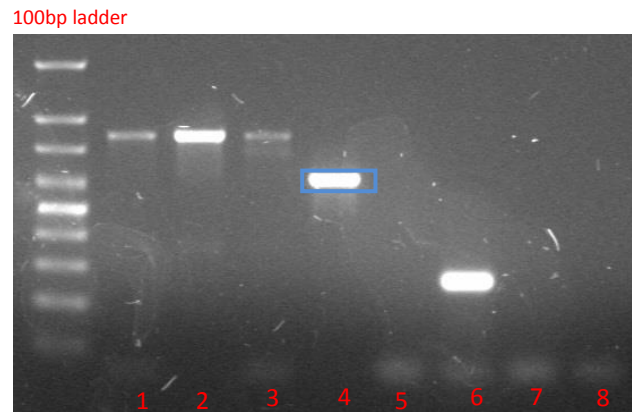
Second round products of 3' RACE GHRL, AGRP, POMCb, CART1, CART3 and CART4 were ligated into pCR® 2.1 vector and transformed into *E.coli* cells which were cultured on LB + ampicillin plates as described in the methods. Plates grew few colonies and none carrying insert (all blue colonies) despite changing pipette tips used, alternating working area, increasing cell thawing time to 20 mins, warming plates before use, and extending incubation time to 48 hours. Vector was changed to pLUG-PRIME® TA-Cloning vector which produced white colonies for CART1, CART3, CART4 and POMCb. These colonies were screened using PCR (Fig. 29-31), and selected colonies were grown overnight and had their plasmids purified, as detailed in the methods.



**Figure 29.** Gel analysis of colonies visually tested positive for containing CART1 and CART4 gene insert against a 100bp ladder. CART4 products present two bright bands of approximate product sizes 450 and 250 bp and CART1 shows a single bright band of an approximate 300bp product size. Those in blue were selected for culture and plasmid purification.



**Figure 30.** Gel analysis of colonies visually tested positive for containing CART3 gene insert against a 100bp ladder. CART3 products present 5 bright bands of approximately 200bp product size. Those in blue were selected for culture and plasmid purification.

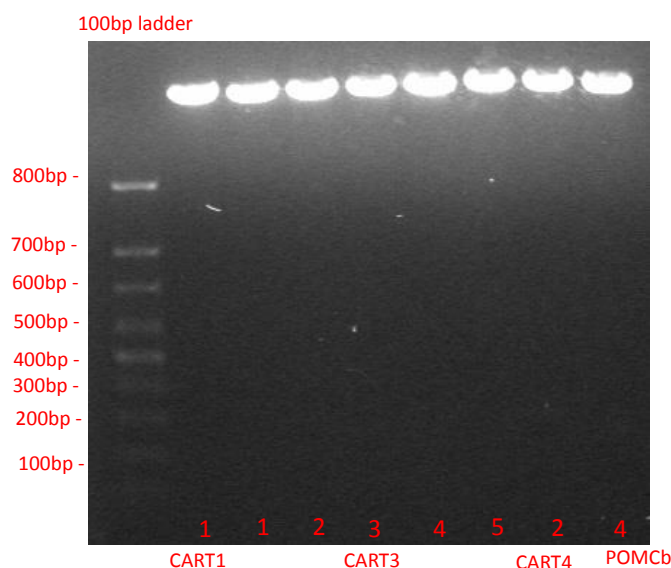


**Figure 31.** Gel analysis of colonies visually tested positive for containing POMCb gene insert against a 100bp ladder. POMCb products present 5 bright bands of approximate product sizes of 750bp, 600bp, and 300bp. Those in blue were selected for culture and plasmid purification.

### 3.2.4 Plasmid purification and gene insert identification

Selected colonies had their plasmids purified and their concentrations measured using the Nanodrop 2000 (Thermo Scientific), at least  $1\mu\text{g}/\mu\text{L}$  of plasmid DNA was isolated from selected CART1, CART3, CART4 and POMCb colonies. To confirm that the gene insert remained within the purified plasmid DNA, the plasmids were incubated with restriction enzyme to cut the insert from the plasmid as detailed in the methods. However, *HindIII* could not cut the

plasmid and only single large bands could be identified on the gel (**Fig. 32**). This was tried a few times, until due to time constraints it was decided to sequence the PCR product directly instead of cloning.



**Figure 32.** Gel analysis to confirm gene insert in selected CART1, CART3, CART4 and POMCb colonies comparing against a 100bp ladder. Only single bright bands of large product size (>800bp) were produced showing that the plasmids had not been cut or contained no insert.

### 3.2.5 Sequencing and alignment

Second round 3' RACE PCR product for CART1, CART3, CART4, GHRL, AGRP and POMCb were gel purified from 4 replicate reactions, their concentrations checked, and the DNA sequenced, as described in the methods. CART3, GHRL, AGRP and POMCb had products sequenced, but none of them were specific for the genes. Only CART1 and CART4 gave specific high quality sequence from which a consensus sequence could be built. The consensus sequences were translated using the ExPASy translate tool and aligned against fish and mammalian sequences to confirm homology.

### 3.2.5.1 CART1

The 3' end of the CART1 gene was amplified, sequenced and a consensus sequence was determined. The CART1 predicted and consensus sequence were aligned against each other using the Geneious clustal tool and showed a 99.2% sequence similarity (**Fig. 33**). The consensus sequence was then translated (**Fig. 34**) producing a 78 amino acid sequence which was then aligned against known fish and (**Fig. 35**) and mammalian (**Fig.36**) protein sequences.

```

Consensus -----
Predicted  CGATGGTCGGAGAGCGGATCTTTCTCCTGACCGTCACCTGCTCTGTTTTAATGCTGCTGG 60

Consensus -----
Predicted  TTTGCTGTGATGAAGTTCTGCAGATCCGCTCGCCTGAGGAGGATAAAGGCTCAGGAGGAGA 120

Consensus -----
Predicted  AAGAGCTGATTGAAGCTCTTCAAGAAGTTCTGGAAAAGCTGAGAAATAACAGATTCCTG 180

Consensus -----AGCAGGCGAGCAGTGTGCGGTGC 23
Predicted  CTGTGGAGAAGAAGCTCGGCTGGGTCCATCGTGTGATGCAGGCGAGCAGTGTGCGGTGC 240

*****

Consensus  GGAA-GGCTCCCCTTCGGGAAGTTGTGCAGTTGTCCAGGAGGAACCGCTGCAGCTTCT 82
Predicted  GGAAAGGCTCCCCTTCGGGAAGTTGTGCAGTTGTCCAGGAGGAACCGCTGCAGCTTCT 300
**** *****

Consensus  CCATCCTCAAGTGCTTGTGAGAGGGAAAAACAGCCTCAGTCTCTCATAATGCTCCAGTG 142
Predicted  CCATCCTCAAGTGCTTGTGAGAGGGAAAAACAGCCTCAGTCTCTCATAATGCTCCAGTG 360
*****

Consensus  TGTGTTTACCATCATCAAATCTGTAACATGTTTATCAGTACATACAGACGAATGTATAA 202
Predicted  TGTGTTTACCATCATCAAATCTGTAACATGTTTATCAGTACATACAGACGAATGTATAA 420
*****

```

```

Consensus      ATGCATTCAAATCAAATAAATGCATGCAGTTTTGTATA- 240
Predicted      ATGCATTCAAATCAAATAAATGCATGCAGTTTTGTATAT 459
*****

```

**Figure 33.** ClustalW alignment of zebrafish CART1 predicted sequence against sequenced CART1 consensus sequence.

```

SRRAVCGAEGSRFGKLCSCPGGTACSFSSILKCLEGKNSLSLSCSSVLFTI IKSVTCLS SVHTDECIN
AFKSNKCMQFCI

```

**Figure 34.** Partial amino acid sequence of CART1 in *Danio rerio* translated from CART4 consensus sequence using the Expasy translate tool.

```

Xiphophorus      MSPRTGTMQSSRMLSGALLCALLLSAGAAGAGAQG-----LLD 39
Tautogolabrus    MRS-TGSTRSCRLL----LCVLLLS--GSTGADLTE-----NNS 32
Salmo             -----MESSRLWTRAVVCAVLLSIVLSAEIDYS----- 28
Carassius        -----MESSKLWTTAMACAVLVSCIQGAEMDF----- 27
Silurus          -----MESSIAARALLLLLVMLRSCARGDNE----- 28
Leucoraja        -----MVS DRLLLAVYFCVLFMSAVGAE----- 23
Oryzias          -----MVSARTLLLVVTCSTYLWLSRAE----- 24
Oreochromis      ---MVRRVEGESPVDLLRHSAPQPLICCLPLGPPSRFTANSTMDSSGMLRGLLVGLLS 57
Pundamilia       ---MVRRVEGESPVDLLRHCAPQPLICCLPLGPPFRFTATSTMDSSGMLRGLLVGLLS 57
Maylandia        -----MESKRAVVYLS-----VCLSVLT 18
Haplochromis     -----MESKRAVVYLS-----VCLSVLT 18
Danio             -----

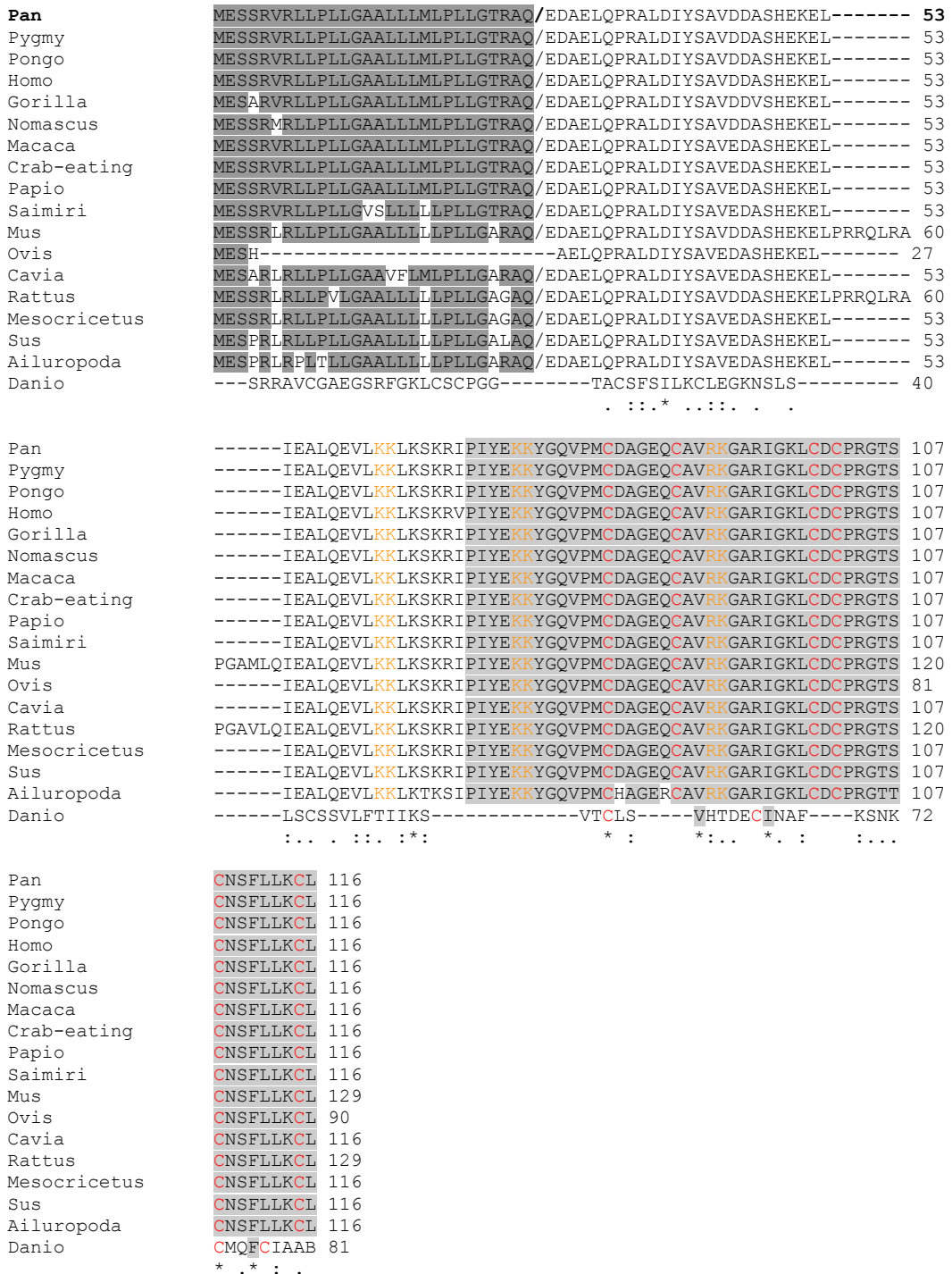
Xiphophorus      AESEELSPRALRDFYPKGPNLTSEKQ---LLGALQEVLEKLQAKRLPSWEKKFGQVPTC 96
Tautogolabrus    LTSEDELSRVLRHFYSKGNLTSEKQ---LSGALQEVLEKLQAKRTSSWEKKFGQVPRC 89
Salmo             -DSELDLDRSVRDFYPKDPNLTNEKQ---LLGALHDVLEKLLQTKRPFWEKKFGQVPTC 84
Carassius        -DNESDLETRALREFYPKDPNLTNEKQLVLSILGALHDVLEKLLQSKRISLWEKKFGRVPTC 86
Silurus          -DTEVDLDRALRDFYPKDPNLTSEKQ---LLGALHDVLEKLLQMKRIPPWEKKFGQVPMC 84
Leucoraja        ---NSDLEPRALRDFYSKNYYPGSEKE---LLGALHEVLEKLLQTKRPTWEKKYGGQVPC 77
Oryzias          ---DSSLETRS L-DFTLKAQ---QEKD---LIDALQGVLEKLRNKEMP-LEKLLGWLPSC 73
Oreochromis      VLCHGQASREVS AEDFGEKQEAADVDR--DLLEALDVLLGRNHN--QVSSPEKRGSIPLC
113
Pundamilia       VLCHGQASREVS AEDFGEKQEAADVDR--DLLEALDVLLGRNHN--QVSSPEKRGSIPLC
113
Maylandia        SLCQGQRSGNSHLLSAPDDPTLGLTTS--ELAEALQGLLDEADSSAGLSVEKKASVIPC 76
Haplochromis     SLCQGQRSGNSHLLSAPDDPTLGLTTS--ELAEALQGLLDEADSSAGLSVEKKASVIPC 76
Danio             -----SRRAV--CGAEGSRFGKLCSC---CPGGTACSFSSILK-----LEGKNSLSLSC 43
                . . . . . : * . *

Xiphophorus      DVGEQCAVRKGARIG-KMDCPRGAFCNFFLLKCL 130
Tautogolabrus    DVGEQCAVRKGSRIG-RMDCPRRAFNCFLKCL 123
Salmo             DVGEQCAVRKGARIG-KMDCPRGAFCNFLLKCL 118
Carassius        DVGEQCAIRKGSRIG-KMDCPRGAFCNFFLLKCL 120
Silurus          DVGEQCAIRKGSRIG-KMDCPRGAFCNFFLLKCL 118
Leucoraja        NIGDMCAVRKGPRIG-RTCNCLS-SKCNFVLFKCV 110
Oryzias          DAGEPCAVRKGARIG-TLCGCPRGTS CNFYVLKCL 107
Oreochromis      GLGNRCAMKYGPRIG-KLCDCGRGANCNSYLLKCI 147
Pundamilia       GLGNRCAMKYGPRIG-KLCDCGRGANCNSYLLKCI 147
Maylandia        DVGERCAMKHGPRIG-RLCDCLRG TACNTFFLR CY 110
Haplochromis     DVGERCAMKHGPRIG-RLCDCLRG TACNTFFLR CY 110
Danio             SSVLFTI IKSVTCLS SVHTDECINAFKSNKCMQFCI 78
                . :: . : * . *

```

**Figure 35.** ClustalW alignment of *Danio rerio* CART1 protein sequence against *Oreochromis niloticus*, *Maylandia zebra*, *Haplochromis burtoni*, *Pinamilia nyererei*, *Xiphophorus maclutas*, *Carassius auratus*, *Leucoraja ocellata*, *Tautogolabrus adspersus*

and *Salmo salar* CART sequences. (\*) identical sequence and (:) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 14.36% sequence identity with the fish sequences.



**Figure 36.** ClustalW alignment of *Danio rerio* CART1 protein sequence against *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, *Sus scrofa*, *Macaca mulatta*, *Ovis aries*, *Cavia*

porcellus, Ailuropoda melanoleuca, Pongo abelii, Macaca fascicularis, Saimiri boliviensis boliviensis, Pan troglodytes, Pan paniscus, Gorilla gorilla gorilla, Nomascus leucogenys, Papio anubis, and Mesocricetus auratus CART sequences. (\*) identical sequence and (:) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 15.48% sequence identity with these species and 22.90% similarity to Homo sapiens. Highlighted in colour are conserved domains of protein sequence deduced from comparison against Homo sapiens protein structure: Signal sequence (dark grey/), convertase processing sites (orange font), conserved cysteines (red font), CART 55-102 (light grey).

### 3.2.5.2 CART4

The 3' end of the CART4 gene was amplified, sequenced and a consensus sequence was determined. The CART4 predicted and consensus sequence were aligned against each other using the geneious clustal tool and showed a 92.9% sequence similarity (Fig. 37). The consensus sequence was then translated (Fig. 38) producing a 123 amino acid sequence which was then aligned against known fish and (Fig. 39) and mammalian (Fig.40) protein sequences.

```

Consensus -----
Predicted  AGCAAGCGGCAGCAAGCACACAAGAGCTGGGAAACGCTTAGAAAATAGTAGATAGCAGACT 60

Consensus -----
Predicted  TTTTACTTTAAATCTTCAGTTTGAAGTTAGACTAACGCCAAAGCATTTCAGCACCATGGAG 120

Consensus -----
Predicted  AGCGTCCGAGCCGCGGTTTACCTGAGCGTGTTCCTGTCTGCTGAGCGTCTGCAGGGCG 180

Consensus -----
Predicted  CAGATTCAGATGCAGGACAGCCGACTGAGCGAGCAGGACGAGCAGTTCATCAAGAGAGAC 240

Consensus -----

```

Predicted CTGGCTGAGGCACTCGATGAACTTCTGGATGGAGAACAGGATAATCGGATATCTCTGGAG 300

Consensus -----GGTT-----GGGGCGCTNCGCCATGAA-CACGGA 28

Predicted AAAAAAGCAAGCGTCATTCCCAGGTGTGACGTAGGGGAGCGCTGCGCCATGAAACACGGA 360

\*\*\* \*\* \*\*\*\*\* \*\*\*\*\* \*\*\*\*\*

Consensus CCACGCATCGGACGCCTGTGTGACTGTATGCGAGGAACCGCCTGCAACACTTTCTTCCTG 88

Predicted CCACGCATCGGACGCCTGTGTGACTGTATGCGAGGAACCGCCTGCAACACTTTCTTCCTG 420

\*\*\*\*\*

Consensus CGCTGCTACTGATGGAGCTGGCTGAAATCATTGATTCTTCTCTCTCAGGCTAAACTCTT 148

Predicted CGCTGCTACTGATGGAGCTGGCTGAAATCATTGATTCTTCTCTCTCAGGCTAAACTCTT 480

\*\*\*\*\*

Consensus GACAGCAACGCTTGAGACTGACACCTCATTGTTAACAGTTTATGTGTGAATTTTCATG 208

Predicted GACAGCAACGCTTGAGACTGACACCTCATTGTTAACAGTTTATGTGTGAATTTTCATG 540

\*\*\*\*\*

Consensus GGTGACCTACTACTGACTCTGTTGTATTCTTCTTGCTAAACTGCTCTGTATATGATTAAA 268

Predicted GGTGACCTACTACTGACTCTGTTGTATTCTTCTTGCTAAACTGCTCTGTATATGATTAAA 600

\*\*\*\*\*

Consensus CCTGTTTATTTTCAGTTAATAAAATGCTCTTTATAAGGCTCAAAAAAAAAAACMTTAWMK 328

Predicted CCTGTTTATTTTCAGTTAATAAAATGCTCTTTATAAGGCTCACCCATTCTGAAAATTGTCT 660

\*\*\*\*\* \* \*\* \*\*

Consensus AATTGAAAATAATTTCAATCCCCSTGTGGGTTTATCATTTAAATGTGTTAATAAAACC 388

Predicted AATTGAAAGGTATGTCTATGTTCTGTGGGTTTATCATTTAAATGTGTTAATAAAACC 720

\*\*\*\*\* \*\* \*\* \* \*\*\*\*\*

Consensus TGTAAACACAAAAAAAAAAAA 409

Predicted TGTAAAA----- 727

\*\*\*\*\*

**Figure 37.** ClustalW pairwise alignment of zebrafish CART4 predicted sequence against CART4 consensus sequence.

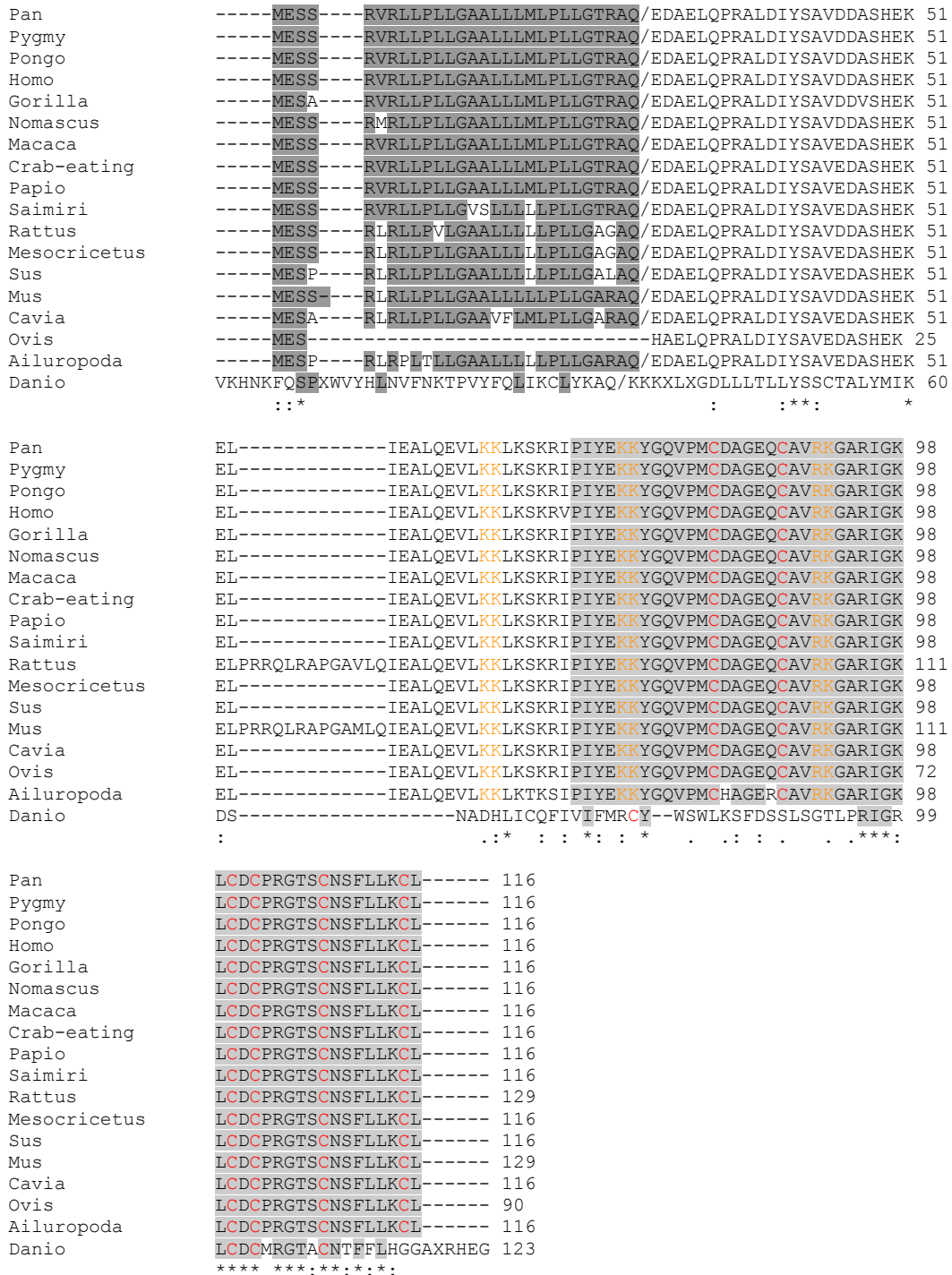
```
VKHNKFQSPXWVYHLNVFNKTPVYFQLIKCLYKAQKKKXIXGDLTLLYSSCTALYMIKDSNADH
LICQFIVIFMRCYWSWLKSFDSLSGTLPRIGRLCDCMRGTACNTFFLHGGAXRHEG
```

**Figure 38.** Partial amino acid sequence of CART4 in *Danio rerio* translated from CART4 consensus sequence using the ExpASY translate tool.

Xiphophorus	MSPRTGTMQSSRMLSGALLCALLLLSAGAAGAGAQG-----LLD	39
Tautogolabrus	MRS-TGSTRSCRLL----LCVLLLS--GSTGADLTE-----NNS	32
Salmo	-----MESSRLWTRAVVCAVLLSIVLSAEIDYS-----	28
Carassius	-----MESSKLWTTAMACAVLVSCIQGAEMDF-----	27
Silurus	-----MESSSIAARALLLLLVLMRLSCARGDNE-----	28
Leucoraja	-----MVSDRLLLAVYFCVLFMSAVGAE-----	23
Oryzias	-----MVSARTLLLVVTCSTYWLWSRAE-----	24
Oreochromis	---MVRVVEGESPVDLLRHSAPQPLICCLPLGPPPSRFTANSTMDSSGMLRGLLLVGLLS	57
Pundamilia	---MVRVVEGESPVDLLRHCAPOPLICCLPLGPPPFRTATSTMDSSGMLRGLLLVGLLS	57
Maylandia	-----MESKRAVVYLS-----VCLSVLT	18
Haplochromis	-----MESKRAVVYLS-----VCLSVLT	18
Danio	-----VKHNKFQSPXWVYHLNVFNKTPVY-----	24
Xiphophorus	AESEEEELSPRALRDFYFKGPNLTSEKQ---LLGALQEVLEKLOAKRLPSWEKKFGQVPTC	96
Tautogolabrus	LTSEDELSPRVLRFYFKGPNLTSEKQ---LSGALQEVLEKLOAKRTSSWEKKFGQVPRC	89
Salmo	-DSELDLDRSVRDFYFKDPNLTNEKQ---LLGALHDVLEKLOAKRLPFWEKKFGQVPTC	84
Carassius	-DNESDLETRALREFYFKDPNLTNEKQQLVLSILGALHDVLEKLOAKRLPSWEKKFGQVPTC	86
Silurus	-DTEVDLDRAIRDFYFKDPNLTSEKQ---LLGALHDVLEKLOAKRLPFWEKKFGQVPMC	84
Leucoraja	---NSDLEPRALRDFYFKNYYPGSEKE---LLGALHEVLEKLOAKRLPTWEKKYGGQVPTC	77
Oryzias	---DSSLETRSL-DFTLKAQ---QEKD---LIDALQGVLEKLRNKEMP-LEKKLGLWLPSC	73
Oreochromis	VLCHGQASREVS AEDFGEKQEAADVDR--DLLEALDVLLGRNHN--QVSSPEKRGSIPLC	113
Pundamilia	VLCHGQASREVS AEDFGEKQEAADVDR--DLLEALDVLLGRNHN--QVSSPEKRGSIPLC	113
Maylandia	SLCQQRSGNSHLLSAPDDPTLGLTTS--ELAEALQGLLDEADSSAGLSVEKKASVIPC	76
Haplochromis	SLCQQRSGNSHLLSAPDDPTLGLTTS--ELAEALQGLLDEADSSAGLSVEKKASVIPC	76
Danio	FQLIKCLYKAQKKKXIXGDLTLLYSSCTALYMIKDSNADHLICQFIVIFMRCYWS	81
	** :	
Xiphophorus	DVGEQCAVRKG--ARIGKMCDCPRGAFCNFFLLKCL-----	130
Tautogolabrus	DVGEQCAVRKG--SRIGRMCDCPRRAFCNFCLL-----	120
Salmo	DVGEQCAVRKG--ARIGKMCDCPRGAFCNFYLLKCL-----	118
Carassius	DVGEQCAIRKG--SRIGKMCDCPRGAFCNFYLLKCL-----	120
Silurus	DVGEQCAIRKG--SRIGKMCDCPRGAFCNFFLLKCL-----	118
Leucoraja	NIGDMCAVRKG--PRIWRTCNCLS-SKCNFYFLFKCV-----	110
Oryzias	DAGEPCAVRKG--ARIGTLCGCPRTSCNFYVLLKCL-----	107
Oreochromis	GLGNRCAMKYG--PRIGKLCDCGRGANCNSYLLKCI-----	147
Pundamilia	GLGNRCAMKYG--PRIGKLCDCGRGANCNSYLLKCI-----	147
Maylandia	DVGERCAMKHG--PRIGRLCDCLRGTCACNTFFLRCY-----	110
Haplochromis	DVGERCAMKHG--PRIGRLCDCLRGTCACNTFFLRCY-----	110
Danio	WLKSFDSLSGTLPRIGRLCDCMRGTACNTFFLHGGAXRHEG	123
	. : * .** *.* : ** .:	

**Figure 39.** ClustalW alignment of *Danio rerio* CART4 protein sequence against *Oreochromis niloticus*, *Maylandia zebra*, *Haplochromis burtoni*, *Pinamilia nyererei*, *Xiphophorus maclutas*, *Carassius auratus*, *Leucoraja ocellata*, *Tautogolabrus adspersus* and *Salmo salar* CART sequences. (\*) identical sequence and (:) highly similar sequence

or (.) similar sequences are indicated. The zebrafish sequence has an average 18.94% sequence identity with the fish sequences.

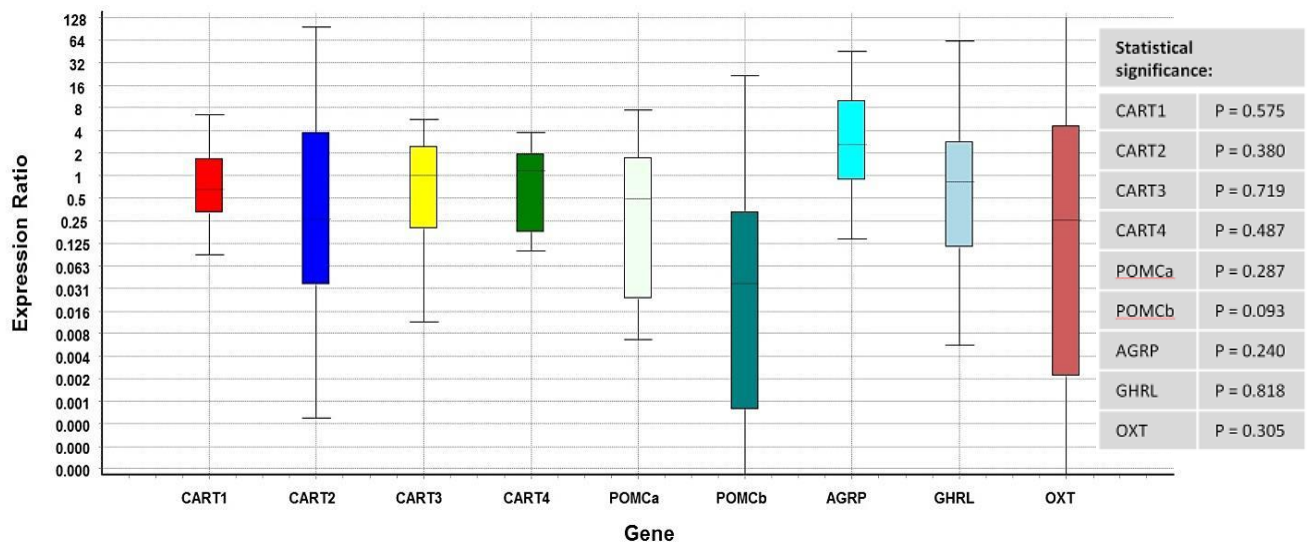


**Figure 40.** ClustalW alignment of *Danio rerio* CART4 protein sequence against *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, *Sus scrofa*, *Macaca mulatta*, *Ovis aries*, *Cavia porcellus*, *Ailuropoda melanoleuca*, *Pongo abelii*, *Macaca fascicularis*, *Saimiri boliviensis*

boliviensis, Pan troglodytes, Pan paniscus, Gorilla gorilla gorilla, Nomascus leucogenys, Papio anubis, and Mesocricetus auratus CART sequences. (\*) identical sequence and (:) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 23.25% sequence identity with these species and 22.90% similarity to Homo sapiens. Highlighted in colour are conserved domains of protein sequence deduced from comparison against Homo sapiens protein structure: Signal sequence (dark grey/), convertase processing sites (orange font), conserved cysteines (red font), CART 55-102 (light grey).

### 3.3 Realtime PCR

A real-time analysis was conducted for a suite of genes (Table 3) to elucidate fold changes (delta delta CT) in gene expression in the brain of 24 hour fasted zebrafish versus fed controls. Expression levels were normalised against GAPDH and  $\beta$ -actin. A 3 fold increase in the expression of AGRP, a 16 fold decrease in POMCb and a 3 fold decrease in OXT expression over the fasting period (Figure 40); although none of the results were statistically significant ( $p = >0.05$ ).



**Figure 41.** Realtime PCR analysis of brain CART1, CART2, CART3, CART4, POMCa, POMCb, AGRP, GHRL, and OXT expression in fasted fish over a 24 hour period, represented by ratio of fed vs fasted expression.

## 4. Discussion

The similarities between zebrafish and humans both genetically and neurophysiologically, as well as its increasing use as an alternative model to mice, make it an ideal choice for research into eating behaviour that will benefit both the aquaculture industry and obesity research. Obesity has become a growing problem in first world nations and a leading cause of morbidities. Understanding the role of hypothalamic and peripheral peptides and their receptors involved in eating behaviour, could lead to therapeutic targets for modulation of obesity. In addition, little is understood about eating behaviour in fish and a growing aquaculture industry worldwide, would benefit as studies would help to optimise the quality and quantity of feed consumed. This would insure optimal growth and yield without overfeeding and minimise wasted feed, which is expensive and lowers water quality to the detriment of wild and stock fish. The underlying physiological pathways and molecular mechanisms which contribute to feeding behaviours and hedonic drives for food consumption are well documented in mammals. Currently, very little is understood about these physiological pathways and molecular mechanisms in fish, with very few genes having been characterised within the zebrafish. Many of the genes available are incomplete or not known, the interactions of the neuropeptides with each other and their receptors have not been understood, and the tendency for gene duplication in the fish genome means that there may be key gene variants still undiscovered. Progress towards high quality aquacultural and obesity research using this model, desperately needs a better characterisation of the neuropeptides that drive feeding and reward and the role they play and while some homologous neuropeptides have been identified in fish, the studies about their functions are within their infancy and have yet to be comprehensively investigated to the same extent as they have in mammals. This study aimed to better characterise the neuropeptides that could be identified in the zebrafish so they could be used in future informative studies.

#### 4.1 Gene candidate analysis

Searching the NCBI database and the available zebrafish genome, identified thirteen candidate genes in zebrafish likely to be involved in regulating eating behaviour, based on what has been discovered in mammals. To confirm the identity of these sequences, comparison of the nucleotide and protein sequences against known sequences in other fish, mammals, amphibians and birds was carried out. In addition, the position that these genes occupied within the zebrafish genome and the genes around them was also characterised and compared to the human genome, where synteny could be used to support the identity of the feeding behaviour gene. From this analysis, a number of potential candidates including IAPP, APLN, CCK, FTO, PYY, GHRL, AgRP, two copies of LEP (termed LEPa and LEPb), an additional copy of POMC (termed POMCb) and three more copies of CART (termed CART 1, 3 and 4) were found (Table 3). The presence of multiple copies of CART, POMC, and LEP in zebrafish in comparison to mammals, is likely the result of a teleostean whole genome duplication (WGD) event. This occurred posterior to the divergence of the ray-finned fish lineage from their shared common ancestor with mammals approximately 350 million years ago and was followed by a massive loss of duplicated genes (Alsop & Vijayan, 2009; Jaiilon et al., 2005, Ravi & Venkatesh, 2008). The retention of duplicated genes, as observed in zebrafish, can be due to multiple factors. It is possible that the daughter genes provide a totally new function to that of the parent (which retains the ancestral function), or a complementary function to that of the parent due to sub functionalization of the ancestral gene; the comparative functions could be specific to certain tissues, developmental stages, or stress conditions (He & Zhang, 2005; Leder & Silverstein 2006; Ravi & Venkatesh, 2008). However, more often duplicate genes are retained as pseudogenes. For the purpose of this study, GHRL, AgRP, POMCb, CART 1, CART 3 and CART 4 were investigated further with particular interest in the duplicated CART and POMC genes due to possible novelty of function.

Gene	Tissue expression	Characterization	Putative function	Gene sequence similarity	Protein sequence similarity	Conservation of synteny
------	-------------------	------------------	-------------------	--------------------------	-----------------------------	-------------------------

<b>AGRP</b>	Brain	Predicted	Orexigen	33.13%	33.42%	High
<b>IAPP</b>	Pancreas	Predicted	Anorexigen	48.92%	33.94%	Low
<b>APLN</b>	Brain	Incomplete cds	Unknown	38.75%	36.94%	Low
<b>CART1</b>	Brain	Predicted	Anorexigen	52.19%	35.88%	Low
<b>CART2</b>	Brain	Complete cds	Anorexigen	49.48%	54.14%	None
<b>CART3</b>	Brain	Predicted	Anorexigen	64.72%	66.52%	None
<b>CART4</b>	Brain	Incomplete cds	Anorexigen	40.45%	37.80%	Low
<b>CCK</b>	Gut	Predicted	Anorexigen	40.01%	38.71%	Low
<b>FTO</b>	Brain	Incomplete cds	Orexigen	39.43%	15.44%	Very high
<b>GHRL</b>	Gut	Incomplete cds	Orexigen	40.93%	20.06%	Low
<b>LEPa</b>	Liver	Incomplete cds	Anorexigen	43.94%	22.10%	High
<b>LEPb</b>	Liver	Incomplete cds	Anorexigen	40.02%	19.30%	High
<b>NPY</b>	Brain	Complete cds	Orexigen	49.46%	64.72%	Very high
<b>PYY</b>	Brain	Incomplete cds	Anorexigen	48.60%	51.24%	Very low
<b>POMCa</b>	Brain	Complete cds	Anorexigen	44.70%	37.81%	Moderate
<b>POMCb</b>	Brain	Incomplete cds	Anorexigen	39.97%	26.99%	Moderate
<b>OXT</b>	Brain	Complete cds	Unknown	50.18%	51.04%	None
<b>AVP</b>	Brain	Complete cds	Unknown	62.60%	64.94%	None

**Table 3.** Table summary of literature and bioinformatics review of zebrafish genes potentially involved in eating behaviour. Included in this table are genes that have been characterised and published on (orange) and those that have been identified through this study (blue and white). Those highlighted in blue were chosen for further characterisation and their predicted sequences were used to design forward and reverse primers (Table 1), for use in RACE PCR. Genes were categorised based on identified tissue expression either in fish or mammalian models, extent of sequence characterization in zebrafish, putative function in fish, average zebrafish gene and protein sequence similarity to that of mammalian genes, and conservation of synteny of zebrafish gene loci against that of humans.

#### **4.1.1 AGRP**

ZfAGRP alignments against other fish showed a high average sequence identity of nucleotide and protein sequence, 42.37% and 58.28% respectively. Conservation of the protein sequence was strongest across fish species in the C terminal (3') region, particularly of the 10 cysteine residues which are involved in disulfide bond formation important for maintenance of the protein structure and therefore function (Murashita, Kurokawa, Ebbesson, et al., 2009; Song, 2003). In addition, all but *Salmo salar*, which had an L → R substitution at the second residue, maintained the RFF motif which forms the active core of AGRP shown to be key to the antagonistic role of AGRP in MC4R binding in mammals (Song, 2003). Comparing zfAGRP protein sequence against mammals, using human AGRP protein structure as a benchmark, conservation of both the RFF motif and cysteine residues in the C terminal agouti domain was demonstrated across species, although the terminal cysteine of zfAGRP was spatially displaced. Syntenic comparison of gene loci across zebrafish and human chromosomes provided further evidence for homology as there was a high conservation of mapped genes between the species. These results provide evidence not only for the identity of zfAGRP but also for its function as, despite a relatively low average sequence identity to mammals (33.42%), the protein sequence is highly conserved in key areas of structure and function from which can be inferred an orexigenic role of zfAGRP similar to that in mammals (Gorissen, Flik, G., and Huising, M. O., 2006; Lohr & Hammerschmidt, 2011). Grouping of zfAGRP with other cyprinids in the phylogenetic analysis indicates that these species likely share the closest functional relationships of AGRP.

#### **4.1.2 CART1, CART3, and CART4**

Zebrafish CART (zfCART)-1, -3, and -4 were analysed in this study. However, for zfCART1 and 4, the 3' end nucleotide sequence was amplified and sequenced and compared against the predicted sequence showing high identity (>90%). These partial sequences were translated and analysed. Translation and alignment of zfCART1 against fish and mammalian sequence showed highest similarity in the C terminal region, however, only 4 of the cysteines in the CART

(55-102) region were conserved in zfCART1 and only two of these spatially. The translated partial zfCART4 also showed similar results but with stronger spatial conservation of three terminal cysteines. Neither zfCART1 nor zfCART4 partial protein sequence showed conservation of the proteolytic cleavage sites. These results do not reflect what is seen in the predicted data sets in due to the lack of a complete coding sequence from which full and accurate comparisons can be made. These confirmed sequences were combined with the predicted sequences allowing a full characterisation of key protein features. ZfCART1, 3, and 4 alignments against other fish showed a high average sequence identity of 43.34% (CART1), 56.02% (CART3), and 40.73% (CART4) for nucleotide sequences, and 34.24% (CART1), 49.72% (CART3), and 35.51% (CART4) for protein sequences. Conservation of the protein sequences across CART transcripts and across fish species was highest in the C terminal region where all 6 cysteine residues involved in disulfide bond formation were highly conserved (Murashita, Kurokawa, Ebbesson, et al., 2009). The conservation of the cysteines in this region is particularly important because it contains the active portion of CART known as CART(55-102), and this was found to be highly conserved across transcripts and across species. Disruption of the C-C disulfide bonds in this region and the concurrent structural unsettling has been shown to cause loss of function in CART peptides (Kehoe & Volkoff, 2007; Kobayashi et al., 2008). ZfCART1, 3 and 4 alignments against mammals showed similar average sequence identities of 35.88% (CART1), 66.52% (CART3), and 37.80% (CART4) for the nucleotide alignments and 52.19% (CART1), 64.72% (CART3), and 40.45% (CART4) for the protein alignments. Comparing zfCART protein sequences against mammals, using human CART protein structure as a benchmark, showed zebrafish CART1 and CART3 retained all but the initial proteolytic cleavage sites apparent in all mammals analysed, where CART4 only retained the second KK site. These sites are processed by convertases to produce various different CART lengths which are biologically active in mammals (Kehoe & Volkoff, 2007; Murashita, Kurokawa, Ebbesson, et al., 2009). Their conservation in zfCART transcripts indicates that zfCART peptides likely undergo similar processing, however, the loss of an additional processing site in CART4 suggests this peptide specifically may have a divergent function. These results indicate homology of CART structure across transcripts and across species, however, the existence of

four CART transcripts in zebrafish indicates that one or more of the genes may have different functional roles which could manifest as different preprandial/postprandial or fasted expression times, differential tissue expression, or converse orexigenic/anorexigenic functions. An indication of the possible difference in function/regulation of these genes is that very little synteny for CART1 could be seen against human CART, with CART4 showing some synteny around the region of the zfCART1 gene and the region around CART3 having no synteny with either genome. In addition, although the zfCART protein sequences group with other fish in the phylogenetic analysis, the distance between them does also suggest these genes may have different functions.

#### **4.1.3 GHRL**

ZfGHRL alignments against other fish showed a high average sequence identity of nucleotide and protein sequence, 54.93% and 49.55% respectively. Conservation of protein sequence across species was strong across the length of the transcripts and was highest in the biologically active N terminal region which contains the mature form of ghrelin (zf: GTSFLSPTQKPQGRPPRV) (Terova, Rimoldi, Bernardini, Gornati, & Saroglia, 2008). Within this region the GSSF motif which forms ghrelins active core, responsible for receptor binding, was highly conserved across species, with only a S → T substitution of the second serine in zebrafish and goldfish (Amole & Unniappan, 2009; Peddu, Breves, Kaiya, Gordon Grau, & Riley, 2009; Terova et al., 2008). Similarly a comparison of zfGHRL protein sequence against mammals, using human GHRL protein structure as a benchmark, showed conservation across species was highest in the region containing the active core. This indicates homology of protein structure and function of mammalian GHRL to that of fish, further evidence for this in zebrafish is provided by syntenic comparison which showed syntenic conservation of gene loci surrounding GHRL across human and zebrafish chromosomes. Phylogenetic analysis of protein sequence indicates that zfGHRL likely shares the closest functional relationship with goldfish (Figure 15).

#### 4.1.4 POMCb

ZfPOMCb was aligned against other fish and demonstrated an average nucleotide and protein sequence similarity of 39.91% and 26.55% respectively. Alignment of the protein sequences showed a high conservation of several key features. The six cysteine residues of the N terminus were largely conserved across fish species, the lowest conservation being seen at the second cysteine. These cysteines, as has been described in AGRP and CART, are involved in disulfide bond formation which is crucial to the structure and function of POMC (Gonzalez Nunez, 2003). Areas encoding ACTH, CLIP, Beta endorphin, and Met enkephalin were all well conserved in fish (Dores & Baron, 2011), with the highest conservation seen in areas encoding alpha-MSH, and beta-MSH, in particular with their HFRW motifs responsible for receptor binding (Song, 2003). Conservation of alpha-MSH in particular is important as it has been shown to be a potent anorexigen in mammals and may serve a similar function in fish. Comparison of zfPOMCb protein sequence against mammals, using human POMC protein structure as a benchmark, also showed high conservation in areas encoding the products of POMC proteolytic processing (Gonzalez Nunez, 2003) as well in the 6 cysteine residues of the N terminus, although the first two cysteines were spatially displaced. Again there was excellent conservation of within the areas encoding alpha-MSH, beta-MSH, and met-enkephalin, including in the HFRW motifs of the MSHs. However only moderate conservation was seen in the 5' regions of zfPOMCb ACTH, CLIP, and beta-enkephalin, but unlike in mammals no gamma-MSH was identified. These results also suggest that MSHs produced from zfPOMCb may serve anorexigenic functions. Increased confidence in homology provided from syntenic comparisons supports the identity of these genes as POMC homologues and that may have anorexigenic function within zebrafish as has been described in mammals (Gorissen, Flik, G., and Huising, M. O., 2006; Lohr & Hammerschmidt, 2011). Interestingly the presence of two POMC transcripts in zebrafish could suggest the function of one of these genes could be divergent to that of mammals, particularly since zfPOMCa shows higher sequence similarity and synteny to that of zfPOMCb.

A number of other zebrafish eating behaviour genes were discovered when searching for potential candidates, which included. IAPP, APLN, CCK, PYY, LEPa, LEPb and FTO.

#### **4.1.5 IAPP**

Zebrafish IAPP (ZfIAPP) was aligned against *Carassius auratus* and mammals and showed protein sequence similarities of 78.0% and 33.94% (mean), respectively. Phylogenetic analysis of these protein sequences grouped ZfIAPP clearly with the known *C. auratus* sequence, which together with the high sequence similarity against the cyprinid gives credence to the identity of zfAGRP. Homology to mammals was supported by some synteny observed between zebrafish and humans, and also due to the high conservation of sequence observed across species in the protein alignment throughout the length of the mammalian transcripts.

#### **4.1.6 APLN**

Zebrafish APLN (ZfAPLN) was aligned against *Carassius auratus* and mammals and shared protein sequence similarities of 97.4% and 36.94% (mean), respectively. The alignment shows a nearly identical sequence between fish which is strongly suggestive of the identity of the predicted sequence in zebrafish as APLN, this is supported by phylogenetic analysis. ZfAPLN appears to be homologous to mammals as there is strong sequence conservation across species in the C-terminal region (unknown importance), as well as shared synteny with human APLN.

#### **4.1.7 CCK**

Zebrafish CCK (ZfCCK) was aligned against other fish and mammals and showed an average 51.38% and 38.71% protein sequence similarity, respectively. The fish protein sequence was highly conserved in the C-terminal region which

contains the biologically active mature forms of CCK peptide (Feng et al., 2012 ; MacDonald & Volkoff, 2009) In particular the sequence for the dominant CCK8 peptide (DYLGWMDF) was conserved in zebrafish. Coupled with the phylogenetic analysis which distinctly grouped zebrafish with the cyprinids fish, these analyses corroborate the identity of zfCCK. While alignment against mammals did not show high conservation in any one area, a high (63.20%) sequence identity and synteny to humans provide confidence in the homology of zfCCK sequence to mammalian copies.

#### **4.1.8 PYY**

Zebrafish PYY (ZfPYY) translated sequence was aligned against other fish and mammals and demonstrated an average 71.21% and 51.24% sequence similarity, respectively. In fish, conservation was highest in the domain encoding the biologically active mature PYY form PYY (1-36), particularly the C-terminal portion of this domain including the GKR amidation site ((Gonzalez & Unniappan, 2010). Alignment against mammals showed similar identity in these same regions, as well the conservation of the N-terminal YP across species which is cleaved by dipeptidyl peptidase 4 to form the biologically active truncated PYY (3-36) (Ballantyne, 2006). This provides good preliminary evidence as to the identity of zfPYY and its homology to PYY in mammals.

#### **4.1.9 LEPa, LEPb and FTO**

Unlike zfiAPP, zfAPLN, zfCCKPYY the zf, Zebrafish LEPa and LEPb (ZfLEPa and ZLEPb) and FTO identity was not so clear. ZfLEPa and ZLEPb protein sequences show an average 34.09% and 24.95% similarity to other fish, respectively. This initial result shows that even between fish the sequence identity is low as in LEPb. Between zebrafish and mammals sequence divergence is more noticeable as LEPa and LEPb only share an average protein sequence similarity of 22.10% and 19.30%, respectively. However, both zfLEPa and zfLEPb shared good synteny with human LEP. Zebrafish FTO (zfFTO) aligned with other fish species FTO, showed good conservation across (average 33.63% in zebrafish) and

zebrafish cluster with the other fish in phylogenetic analysis. While alignments against mammals show low protein sequence identity (15.44%), there was high conservation of synteny with the human genome. Some of this difference in homology between zebrafish and mammals could be due to the predicted sequence not being entirely correct. However, this finding could also represent an increased rate of evolution of protein-coding sequence in teleosts following WGD, resulting in divergence of protein sequence from mammals (Jailon et al. 2005; Ravi & Venkatesh, 2008). It is likely then that despite conservation of gene linkage *zfLEPa*, *zfLEPb*, and *zfFTO* could have diverged from mammalian copies at such a rate as to no longer bear comparable functions and have instead become neofunctionalised over a prolonged time period.

Due to time constraints only six of the genes discovered were further characterised. While a number of these genes were not chosen for study in this investigation, their high sequence identity against other fish, and homology to mammalian copies, presents a case for a future broader study entailing their full sequence characterisation and confirmation of protein domains of structural and functional importance as has been attempted for the target genes. For all the genes discovered, where there was a particularly low syntenic conservation, this can be explained by the higher rates of interchromosomal rearrangements which have occurred in teleosts following WGD (Jailon et al., 2005 Ravi & Venkatesh, 2008).

#### **4.2 Fed/fasted gene expression**

Despite being unable to fully confirm the sequence identity of the target genes, it was felt that the predicted sequences gave accurate enough approximations of sequence identity from which could be designed primers for use in qPCR. Therefore, expression studies were conducted for a suite of genes (GHRL, AGRP, POMCa, POMCb, CART1, CART2, CART3, CART4, NPY, AVP, and OXT), and of these genes significant expression was demonstrated for all but NPY and AVP. This is the first time a fasting study has been conducted looking at

the effect of starvation on the expression of an array of genes considered as regulators of eating behaviour in fish.

#### **4.2.1 AGRP**

Previous short-term feeding studies in atlantic salmon, and *Schizothorax prenanti* have identified different expression patterns of AGRP. Analysis of short-term fasting effects in *Schizothorax prenanti* hypothalamus over a 24 hour period, identified increases in AGRP expression in fasted fish which were highest at 3 and 4 hours following feeding (Wei, Yuan, Wang, et al., 2013). A study in Atlantic salmon, however, provides contrary evidence for the role of AGRP in the brain as qPCR analysis of AGRP-1 and AGRP-2 transcripts at 0.5, 1.5, 3, 6, 9, 12, and 24 hours postprandially showed an increase in AGRP-1 expression 3 hours post feeding, while AGRP-2 levels rose after 1.5 hours (Valen et al., 2011). Long-term fasting studies show similarly contrary results in *S. prenanti*, zebrafish, goldfish, atlantic salmon, and common carp. Analysis of a 14 day fast in *S. prenanti* showed no change in hypothalamic AGRP expression (Wei, Yuan, Wang, et al., 2013). Similarly in Atlantic salmon, no changes were seen in AGRP-2 levels after a 6 days fast, however, AGRP-1 expression showed a decrease in expression (Murashita, Kurokawa, Ebbesson, et al., 2009). An anorexigenic role for brain AGRP is also seen in AGRP-1 and AGRP-2 transcripts of common carp fasted over 7 days, over which time both transcripts show a reduced expression which only recovers to control levels after refeeding, with high induction levels observed within 24 hours (Wan et al., 2012). Contrary to these studies, but in line with the observed effect of AGRP in mammals, goldfish and zebrafish show increases in AGRP expression during fasting periods. A one week fasting period in goldfish, produced a significant increase in hypothalamic AGRP expression maximal at 5 days (Cerdeira-Reverter & Peter, 2003). In zebrafish, an orexigenic effect of AGRP was observed in adult zebrafish (5-9 months old) fasted for 2-, 5-, 10-, and 15-days, with expression increasing from 2 days onwards and peaking at 15 days (Song, 2003). These results indicate that AGRP has a species specific role and in particular multiple AGRP transcripts demonstrate different expression patterns, these effects are seen both in the long term following extended starvation periods

as well as in short term postprandial studies. Of the species studied only *S. prenanti*, zebrafish, and goldfish seem to have AGRP functioning in orexigenic roles as described in mammals. The work presented here is in agreement with these studies. A 24 hour fasting period produced a significant increase in AGRP expression in zebrafish brain indicating an orexigenic role of AGRP in zebrafish. This compares well with the starvation study conducted in zebrafish by Song 2003, furthermore, this work shows that AGRP expression is upregulated as soon as 24 hours following fasting which provides information which was absent from their study. This provides further evidence that AGRP has an orexigenic effect in zebrafish. Additional work looking at the expression of AGRP at time points across the 24 hours period (eg. 1, 3, 6, 12 and 24 hours postprandial) would provide insight into how early AGRP expression is affected including whether levels are downregulated in fed fish following a meal. This would bolster what is known of the role of AGRP in zebrafish.

#### **4.2.2 CART**

Expression of multiple CART transcripts have previously been studied in a range of fish species producing species specific results. Atlantic salmon fed a single meal, with samples taken 0.5, 1.5, 3, 6, 9, 12, and 24 hours postprandially, showed a sharp increase in expression 1.5 hours post feeding in comparison to unfed controls, however, the expression level dropped to control levels from 3 hours onwards (Valen et al., 2011). This effect was also seen in Atlantic salmon starved over a 6 day period, during which time CART levels decreased in unfed fish (Murashita, Kurokawa, Ebbesson, et al., 2009). As in AGRP, Atlantic salmon CART expression shows activity contrary to that shown in mammals. Similarly, evaluation of preprandial (1 and 2 hours before meal) and postprandial (0.5, 1, 2, and 4 hours after feeding) expression in channel catfish showed an increase in CART levels in fed fish from 0.5 up to 4 hours post feeding (Peterson et al., 2012). More consistent with what has been shown in mammals, Volkoff 2001 showed that 2h and 6h post feeding goldfish transcript CARTI was significantly increased in the hypothalamus and olfactory bulbs, CARTII showed no significant changes. Extending the starvation period (4 days) resulted in a decrease in CARTI

expression in brain regions including the hypothalamus and olfactory bulbs, while CARTII expression showed a decrease solely in the olfactory bulbs. A more recent study corroborates the work by Volkoff and shows that *C. gariepinus* fasted for 48 hours have significantly reduced CART levels in the brain of unfed fish (Subhedar et al., 2011). These studies show that CART transcripts are likely to have multiple functions and that the orexigenic effect seen in other fish may be explained through the characterisation of unidentified CART forms. This is highlighted in a study by Kehoe & Volkoff 2007 in which CART levels showed reduction in fed Atlantic cod during feeding and 2 hours afterwards, but showed a consistent decrease in CART levels in fish starved over one week. Their contrary findings could be due to the different functions of multiple CART peptides which are time dependently expressed. A study by Murashita & Kurokawa 2011 highlights this point clearly, as they investigated the long term expression of six CART cDNAs (ch3, ch4, ch6, ch9, ch11, and ch22) in the brains of Japanese medaka over a 17 day fasting period, but only ch3 showed a response, decreasing significantly after 17 days of starvation and recovering after refeeding.

In the work done here, I expected to find variation between the expression levels of the four CART transcripts studied identifying different roles for the transcripts as has been previously described in the above studies. It was also expected that this response would be significant over a 24 hour period. What was observed was no significant change in the brain expression levels of any of the transcripts in fish fasted over a 24 hour period. This could be explained by CART having a transient response to fed/fasted states in zebrafish, or in fact significant expression may only result from extended starvation periods which could not have been picked up by the single time point ( $n = 5$ ) sampling that was conducted. Taking samples ( $n = 3-5$ ) at 1, 3, 6, 12, and 24 hour time points for a postprandial experiment, and 3, 5, and 7 days for an extended starvation experiment would likely have provided a clearer representation of the functions of CART in zebrafish, and it is something to consider for future studies.

### 4.2.3 GHRL

Ghrelin expression has been extensively studied in multiple fish species under short and long term regimes of feeding and fasting; however, the results point to species specific actions of ghrelin within fish. This can be seen in a series of studies in Atlantic cod, rainbow trout, goldfish, and mozambique tilapia demonstrating brain, plasma and gut ghrelin expression patterns under short term fed and long term fasted conditions. Studies of Atlantic cod gut ghrelin expression under periprandial and extended fasting/refeeding regimes demonstrated that ghrelin expression was highest during feeding in the fed group as compared to levels before and after feeding; with no effect on ghrelin levels observed over 30 days fasting and 5 days refeeding (Xu & Volkoff, 2009). A study in rainbow trout showed no changes in postprandial plasma ghrelin levels in unfed fish, but 1 week of fasting produced reduction in plasma ghrelin; suggesting a similar function of ghrelin to that of Atlantic cod but showing a different expression pattern (Jonsson et al., 2007). These results bare closer resemblance to what is observed in birds, in which ghrelin is an anorexigen, rather than mammals. In contrast, goldfish gut, plasma, and hypothalamic ghrelin showed significant decreases in expression postprandially in fed fish, and a significant increase in plasma ghrelin levels in unfed fish was observed after 3 days of starvation, declining rapidly afterwards. Only after 7 days of starvation were hypothalamic and gut ghrelin levels shown to increase in fasted fish (Unniappan & Peter, 2005). A study in mozambique tilapia showed no changes in postprandial or intermediate (<1 week) expression of gut and plasma ghrelin in fasted fish, however in a similar vein to what was discovered in goldfish, after 2 and 4 weeks of fasting showed significant increases in plasma but not stomach levels (Fox, Breves, Hirano, & Grau, 2009). However, another study in mozambique tilapia shows an increase in postprandial plasma ghrelin, and a transient increase in brain ghrelin, in fed fish while fasted fish only showed an increase in stomach ghrelin and only after 3 hours leaving the role of ghrelin in this species uncertain (Peddu et al., 2009). These studies example the species specific variation observed in the action of ghrelin not only in the levels and length of expression in fed/fasted states, but also in the tissue specific expression profiles. Based on these observations the effects of ghrelin must be

considered on a species by species basis as the literature describes large variability in the expression profiles between tissues, and across feeding states.

Therefore, concentrating on what has been discovered in previous work in zebrafish three studies are highlighted. Amole & Unniappan 2009 demonstrated that a 7 day fast significantly increased preproghrelin expression in both the gut and brain of fasted fish and that these expression levels decreased significantly 4 hours after refeeding. This work suggests that zfGHRL has an orexigenic role and is supported by Eom, Hong, Cone, & Song, 2013 who investigated the effects of short and long term fasting on the expression of ghrelin in the pancreas and brain of adult zebrafish (5-19 months old). Fasting over a 16 hour period caused no change in either brain or pancreatic expression levels, but a 15 day fast demonstrated a significant increase in pancreatic ghrelin which recovered to fed control levels following 5 hours of refeeding. It appears that there may be a transient response to fasting in the brain, and an extended response in the gut in respect to expression levels of ghrelin in zebrafish. In addition, the expression profiles show an orexigenic pattern in zebrafish similar to what has been observed in mammals. An exception to this is the study by Koven & Schulte 2012 which showed a transient decline of stomach ghrelin following 3 days of fasting in fasted fish, which increased significantly after refeeding. This could be explained by multiple transcripts existing in zebrafish as is described in Atlantic salmon (Murashita, Kurokawa, Nilsen, et al., 2009). In comparison to what has been described here, qPCR analysis showed no effect of a 24 hour fast on the expression of ghrelin in the brain of zebrafish which is consistent with what has been described previously. This superficially suggests that ghrelin does not function in regulating eating behaviour in zebrafish, but it would be required to replicate this study with a larger sample size using a wider range of time points to draw any conclusions. In addition, analysis of expression levels in the gut as well as the brain would provide a better picture of the global activity of ghrelin in zebrafish. However, it is uncertain whether any significant expression be seen in the gut either as the expression levels of ghrelin across tissues and across species is demonstrably unpredictable and even the results of studies in zebrafish are relatively ambiguous.

#### 4.2.4 POMC

Few POMC expression studies have been conducted in fish and they largely represent no anorexigenic effect of brain POMC. This is demonstrated strongly in long term starvation studies. A one week fasting study of POMC expression levels in goldfish showed no change in POMC expression levels in the hypothalamus (Cerdá-Reverter, Schiöth, et al., 2003). The same was seen in fourteen day fasting studies in zebrafish (Song, 2003), and barfin flounder (Takahashi et al., 2005), where no significant change in POMCa or POMC-C expression, respectively, was observed in brain tissues at any time point. Only in a study by Leder & Silverstein 2006 was a change seen in POMC expression over a long term fasting period. In their study, qPCR analysis of four POMC transcripts (POMCa1, POMCa2, POMCa2s, and POMCb) isolated from the hypothalamus of rainbow trout starved for 28 days showed a significant reduction of POMCa1 by 50% at 28 days compared to fed controls. No significant changes were observed in the expression of the other transcripts (Leder & Silverstein, 2006). The Leder 2006 study, however, did not show short term effects of starvation on the expression of the multiple transcripts. This gap in information is filled by a study by Valen et al. 2011, who demonstrated the postprandial changes in expression of four POMC transcripts (POMCa1, POMCa2, POMCa2s, and POMCb) in Atlantic salmon 0.5, 1.5, 3, 6, 9, 12, and 24 hours after being fed a single meal (unfed control group). Analysis by qPCR revealed a transient but significant increase in POMCa1 expression at 3hrs post feeding, recovering to control from 6hrs onwards. POMCb showed a slight but significant increase of expression at 0.5hours and from 6 hours onwards compared to controls. POMCa2 and POMCa2s showed no significant change in expression levels over the time course. The importance of these studies is that they evaluated multiple transcripts indicating there is different functions for each of the transcripts and that these functions are time dependent. This has not been demonstrated in other studies, and characterisation of multiple transcripts and their short and long term expression profiles in other species may help explain the findings observed previously. This was what was endeavoured in the characterisation of POMCa and POMCb expression in the brains of fasted zebrafish.

In this work, fish (n = 5) fasted for 24 hours showed no significant changes in POMCa expression which is in line with what was demonstrated in the previous zebrafish (Song, 2003). It could be that POMCa in zebrafish does not function in regulating appetite, it could also be that POMCa shows transient expression as has been shown by Valen et al. 2011, or expression over an extended fast as has been shown in POMCa in Leder & Silverstein 2006, which could not be picked up by this study as samples were only taken from a single 24 hour time point. Alternatively, POMCb showed a significant down regulation after 24 hours fasting which indicates that it serves a role as an anorexigen, its down regulation likely plays a part in facilitating increased food intake. This result is corroborated by what has been observed by Valen et al. 2011. Further study using a larger group of fish, sampled over multiple time points would be able to validate what has been demonstrated here, but the preliminary work indicates that POMCb drives satiety in zebrafish.

#### **4.2.5 OXT**

To date the only study known to relate fish oxytocin homologues (eg. isotocin) to feeding behaviour was conducted by Mancera et al. 2007 in gilthead sea bream who analysed 14 day fasting effects on isotocin (IT) expression in the pituitary and plasma. IT in the pituitary was upregulated in unfed fish over the fasting period while plasma IT showed no significant change (Mancera et al., 2008). This is contrary to what is observed in mammals, in which oxytocin (produced mainly in the hypothalamus) affects hypothalamic responses to food (Olszweski et al. 2010). The expression study results imply an anorexigenic role for oxytocin in zebrafish, as oxytocin levels were decreased in the brains of 24 hour fasted fish. Replicate studies would need to be conducted to confirm these results.

#### **4.3 Conclusions and future work**

In conclusion, this investigation identified genes involved in zebrafish eating behaviour, that had not been previously characterised, which showed good

conservation of identity when aligned against other fish and mammals and in particular maintained areas of high similarity in their translated sequence in regions of structural and functional importance. In addition, the synteny that existed for these genes between the human and the zebrafish genomes, also helped confirm the identity of these genes. Unfortunately, because of issues with the cloning of PCR products within this investigation, a lot of time was wasted trying to get this to work, Hence only the 3' region of CART1 and CART4 were able to be confirmed within this investigation, these showing high identity to the 3' regions of the corresponding predicted nucleotide sequences. This in itself, showed that the time taken to make the initial predictions was worth it as they were good, however, It will be of primary importance to complete the synthesis of the 5' region of these genes to validate the identity of translated sequence. Expanding this work into the remainder of the genes targeted in this study will also be important as no study in fish has tried to look at a significant number of genes in one study, to determine the role these genes play. This will provide a clearer picture of how these genes are involved in regulating feeding in zebrafish, and in particular the role of the multiple copies of CART and POMC which are currently poorly understood. this is just the beginning of this work and a lot more needs to be done to understand the function roles of these genes in the eating behaviour of the zebrafish. In this respect, there are multiple paths of inquiry for future studies which would be of great value.

Direct intracerebroventricular (ICV) infusion of agonists and antagonists of melanocortin receptors would provide some insight as to the role of the melanocortin system in zebrafish. In goldfish, the universal melanocortin agonist [Nle<sup>4</sup>, D-Phe<sup>7</sup>]- $\alpha$ -MSH and the MC4R antagonist HS024 respectively produce dose dependent decreases and increases in food intake which indicate a role for the melanocortin system in this species as anorexigenic (Cerdá-Reverter, Ling, Schiöth, & Peter, 2003; Cerda-Reverter et al., 2011). It would be advisable to use these same agonists and antagonists in further work in zebrafish. This would provide preliminary information on the role of the melanocortin system in zebrafish, in particular the role of MC4R which is key to the anorexigenic effect of POMC in mammals and is the antagonistic target of AGRP; this has been demonstrated to some extent in fish (Forlano & Cone, 2007; Sanchez, Rubio, &

Cerda-Reverter, 2009). Knocking down gene expression could also be another way to study gene function, using morpholinos against AGRP and looking at the effect this has on POMCa and POMCb expression during a short term fasting period compared to unaffected fasted fish would provide an insight into the role of these genes. This could be extended into a study in a mutant zebrafish line, where a gene has become inactive, which would also have the added benefit of demonstrating any alterations to growth rate, and body size/weight of mutant vs wild type fish. This would complement a study which demonstrated that transgenic zebrafish overexpressing AGRP could developed an obese phenotype (Sonf & Cone, 2007) Alternatively, individual ICV infusion of recombinant POMCa and POMCb proteins in two different group of fish (unaffected controls) could be used to detect the separate effects of these peptides, whether they play an anorexigenic role, and whether they impact the levels of putative orexigens AGRP, NPY and GHRL. In a similar vein, creating a transgenic line of zebrafish to overexpress GHRL may produce valuable information of its function which is thus far variable and inconsistent in expression studies; even between studies in zebrafish. Increasing GHRL expression and monitoring the effects this has on food intake and body phenotypes of fish could alleviate some of this ambiguity. In addition it would be interesting to note any effects GHRL up regulation has on expression of AGRP, NPY, and the POMC and CART genes as GHRL in mammals is known to stimulate orexigen and inhibit anorexigen expression (Gorissen, Flik, G., and Huising, M. O., 2006). Any comparable effects seen in such a study would provide insight into the roles of GHRL as well as the targets of GHRL action in zebrafish. Of particular interest would be any discrete alterations in the levels of the different zfCART transcripts. zfCART is a special case in that it presents four transcripts of which the functions are as yet not comprehensively understood. Furthermore they are unamenable to studies using agonists or antagonists as, apart from one study which showed a link between CB1 and CART3 (Nishio et al., 2012), the receptors responsible for CART action in zebrafish are unknown. In this case, the best approach would be to infuse separate groups of zebrafish with recombinants of each of the CART proteins and evaluate the dose dependent responses on food intake, and on expression of the other orexigens and anorexigens. Using the techniques outlined here it would be possible to more thoroughly understand the functional roles of AGRP, POMCa,

POMCb, GHRL, CART1, CART2, CART3, CART4, and their interactions with each other, in zebrafish to an extent which bioinformatic analysis and expression studies alone do not permit

As well as the functional aspects, expression studies also give important insight into the possible role eating behaviour genes have in the zebrafish. AGRP, POMCb and OXT showed some change in expression profiles in fasted zebrafish that are representative of what has been previously observed in other fish species, as well as mammals: AGRP as an orexigen, and POMCb and OXT as anorexigens. However, due to the high variation between individuals in the expression levels of these genes, nothing was statistically significant. This also led to the observation that no change in expression was seen with CART1, CART2, CART3, CART4, GHRL and POMCa in zebrafish. As has been mentioned, future expression studies would benefit from several alterations to what has been conducted herein in this study. Essential modifications would consist of increasing the period of fasting to several weeks and, in conjunction with this, increasing the sample sizes to at least 60 fish per fed (control) and fasted group. This would enable 10 samples to be taken from each group across a range of time points (eg. 1, 3, 5, 7, 12, 14 days), providing a wider view of gene expression that would be more statistically significant and able to capture changes in expression that may only occur over longer periods of starvation. Conducting a postprandial study alongside this, sampling at 0.5, 3, 6, 12, and 24 hours from a fed and a fasted (control) group, would add additional stringency to the data in terms of capturing transient changes in mRNA levels. With regards to GHRL it should also be noted that only brain tissue was used in the study, ghrelin is known to be primarily expressed in peripheral tissues (Arora & Anubhuti, 2006; Lohr & Hammerschmidt, 2011), sampling of gut tissues for study also needs to be considered in future. However, real-time PCR is not the only approach to look at gene expression and brain is not the only tissue that could be considered this can limit observations of discrete differences in tissue expression. The next step would be to evaluate changes in gene expression in different regions of the brain using in situ hybridisation of histological sections, comparing between fed and fasted fish. Using multiple fluorescent stains would prove a particularly powerful technique as it would allow resolution of alternate expression patterns of genes such as AGRP and POMC

which share an antagonistic relationship, resolving any overlaps would provide information as to their interaction and role in zebrafish (Arora & Anubhuti, 2006; Gorissen, Flik, G., and Huising, M. O., 2006; Lohr & Hammerschmidt, 2011). ). It would also be used to discern region specific expression of the multiple CART and POMC copies. It would be expected that expression would be contained within areas of energy regulation (hypothalamus) or endocrine control (pituitary), discovery of expression outside of these regions, or even in separate areas within these regions, would provide some insight into the discrepancies discovered in mRNA expression levels seen in this and other studies (Leder & Silverstein 2006; Takahashi et al., 2005). Supplementary to, and of great benefit to this work, would be the development of antibodies specific to zebrafish protein epitopes. This would facilitate proteomic analysis of the expression patterns of possible feeding regulators in zebrafish under different fed/fast states. As mRNA expression does not necessarily accurately reflect the proportion of protein product, analysing protein expression would add robustness to insights garnered from in situ hybridisation analyses (Baianu, 2011). These antibodies would be used in immunohistochemical characterisation of protein expression in sections of brain tissues in those regions previously shown to express the genes of interest in in-situ analyses. The combination of these techniques would provide more comprehensive evidence regarding the roles of the target genes of this study, including in which brain regions and under what conditions they are expressed which in turn would provide evidence for the responsibilities of those regions.

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## 5. Appendix

### 5.1 Analysis relating to genes identified but not characterised further in this investigation.

#### IAPP

Searching the available genome using TBLASTN with a known fish IAPP protein, identified a region of chromosome 5 that contained a potential zebrafish IAPP sequence. Using gene prediction software with this region provided a nucleotide sequence which was translated and the predicted protein was aligned against known IAPP protein sequences already identified in other fish (**Supplementary Fig. 1**) and in selected mammals (**Supplementary Fig. 2**).

```

Danio          MYHLKLSQILIFFVMLHCVATVPHNRYSLPSIERPDALGEADGWLLTDLSDNPFLSLTR 60
Carassius      MY---LPSQILIFLVMQLQCVATVPYNRYSLSSNDKPDASREVNGWLVTDLSDNPFVSETR 57
               **   * .*****:***:*****:*****.*  ::*   *.:***:*****:*.**

Danio          PRPPRGL---NSHHVEKRKCNTATCVTQRLADFLIRSSNTIGTVYAPTNVGSNTYGKRD 117
Carassius      PRPPWGLPAVNSHYMEKRKCNTATCVTQRLADFLVRSSNTRGTVYAPTNVGANTYGKRD 117
               **** *   ***:*****:*****:***** *****:*****

Danio          LQSPVYL-- 124
Carassius      LQSPIYLP 126
               ****:***

```

**Supplementary Figure 1.** ClustalW alignment of *Danio rerio* IAPP protein sequence against *Carassius auratus* IAPP. (\*) identical sequence and (:) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 78.0% sequence identity with the fish sequences.

```

Homo          -MGILKLQVFLIVLSVALNHLKAT----- 23
Canis         -MCLKLPVVLII LSVL NHLKAT----- 23
Rattus       MRCISRLPAVLLI LSVL NGLHRAT----- 24
Mus          MMCISKLPVLLI LSVL NHLRAT----- 24
Mesocricetus -MHISKLPAALLI FSVL NHLKAT----- 23
Danio        -MYHLKLSQILIFFVMLHCVATVPHNRYSLPSIERPDALGEADGWLLTDLSDNPFLSLT 59
               :*   ::: * *   : .

Homo          ---PIES---HQVEKRKCNTATCATQRLANFLVHSSNFGAILSSNTVGSNTYGKRNAVE 77
Canis         ---PIKS---HQMEKRKCNTATCATQRLANFLVRTSNLGA ILSPTNVGSNTYGKRNTIE 77
Rattus       ---PVGSGTNPQVDRKCNTATCATQRLANFLVRSSNNGPVL PPTNVGSNTYGKRNVAE 81
Mus          ---PVRSGSNPQMDKRKCNTATCATQRLANFLVRSSNNGPVL PPTNVGSNTYGKRNAAG 81

```

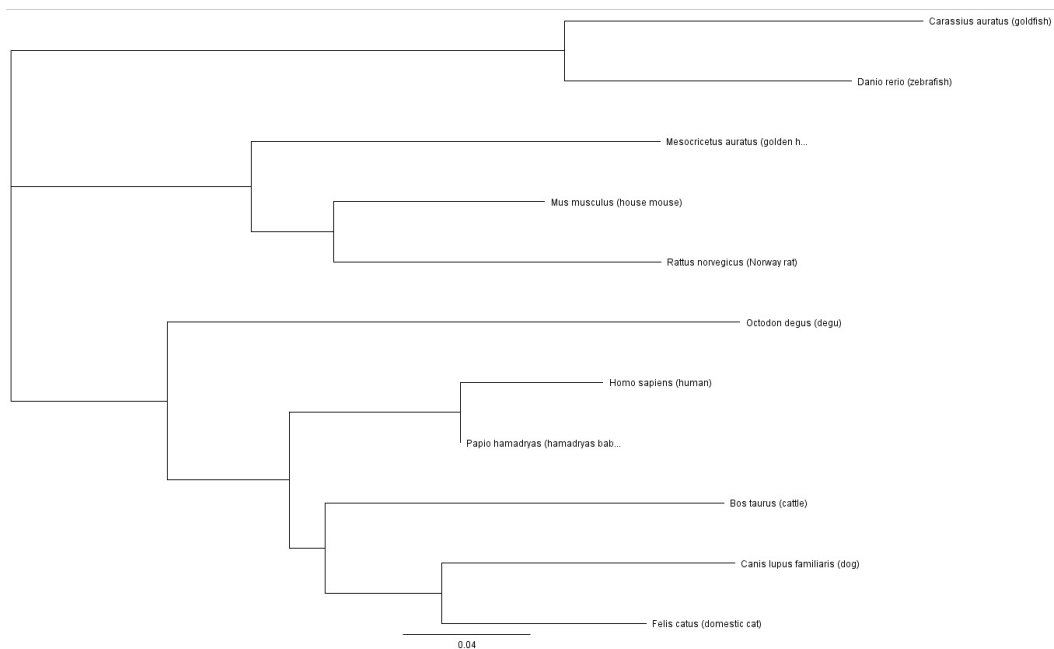
```

Mesocricetus    ---PVRSGTNHQMDKRCNTATCATQRLANFLVHSNNNLGPVLSPTNVGSNTYGKRSAAE 80
Danio           RPRPPRGLNSHHVEKRCNTATCVTQRLADFLIRSSNTIGTVYAPTNVGSNTYGKRDLLQ 119
                * .      ::*****.*****:**:::.*.:*.: .*****.
Homo            VLKREPLNYLPL 89
Canis           ILNRGPLNYLPL 89
Rattus         DENRESLDLFL 93
Mus            DPNRESLDLFLV 93
Mesocricetus   IPDGSLDLFL 92
Danio          SP-----VYL-- 124
                :

```

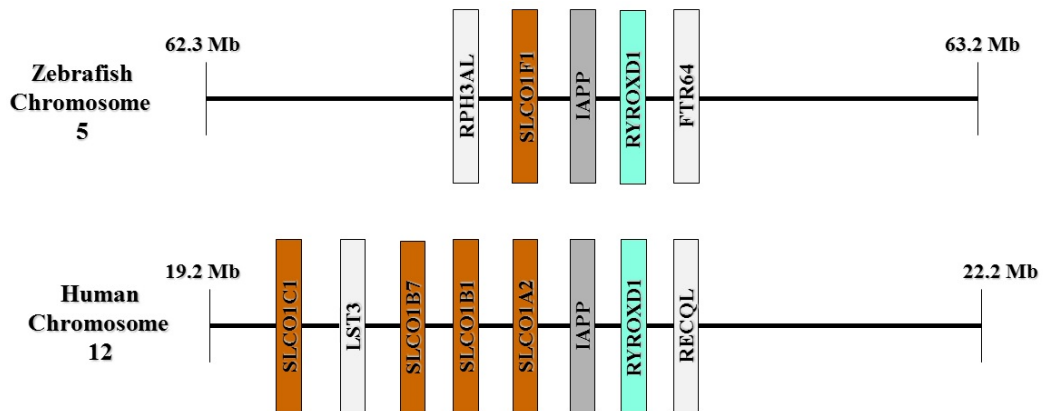
**Supplementary Figure 2** ClustalW alignment of *Danio rerio* IAPP protein sequence against *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, *Canis lupis*, and *Mesocricetus auratus* IAPP sequences. (\*) identical sequence and (: ) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 33.94% sequence identity with these species and 33.30% similarity to *Homo sapiens*.

Phylogenetic analysis of the zebrafish IAPP protein sequence with known vertebrate IAPP genes grouped the gene very clearly with the known cyprinid fish IAPP protein sequence (**Supplementary Fig. 3**). Analysis of the region within the zebrafish genome around the predicted IAPP gene on chromosome 5 allowed a gene map to be constructed that showed that there was conservation of synteny between the human and zebrafish genomes (**Supplementary Fig. 4**), giving further evidence that this gene was a homologue of the human IAPP gene.



**Supplementary Figure 3.** Phylogenetic tree, constructed using the neighbour joining

method alignment, showing the relationship between zebrafish IAPP and known vertebrate IAPP sequences.



**Supplementary Figure 4.** Comparison of the co-localization of genes surrounding IAPP on Human chromosome 12 and Zebrafish chromosome 5.

## APLN

Searching the available genome using TBLASTN with a known fish APLN protein, identified a region of chromosome 5 that contained a potential zebrafish APLN sequence. Using gene prediction software with this region provided a nucleotide sequence which was translated and the predicted protein was aligned against known APLN protein sequences already identified in other fish (**Supplementary Fig. 5**) and in selected mammals (**Supplementary Fig. 6**).

```

Carassius      MNVKILTLVIVLVVSVLLCSASAGPMAS TEHSKELEEVGSMRTPLRQNPARAGRSQRPSGW 60
Danio          MNVKILTLVIVLVVSVLLCSASAGPMAS TEHSKEIEEVGSMRTPLRQNPARAGRSQRPAGW 60
*****:*****:*****
Carassius      RRRRPRPRLSHKGMPF 77
Danio          RRRRPRPRLSHKGMPF 77
*****

```

**Supplementary Figure 5.** ClustalW alignment of *Danio rerio* APLN protein sequence against *Carassius auratus* APLN. (\*) identical sequence and (:) highly similar sequence or (.)

similar sequences are indicated. The zebrafish sequence has an average 97.4% sequence identity with the fish sequences.

```

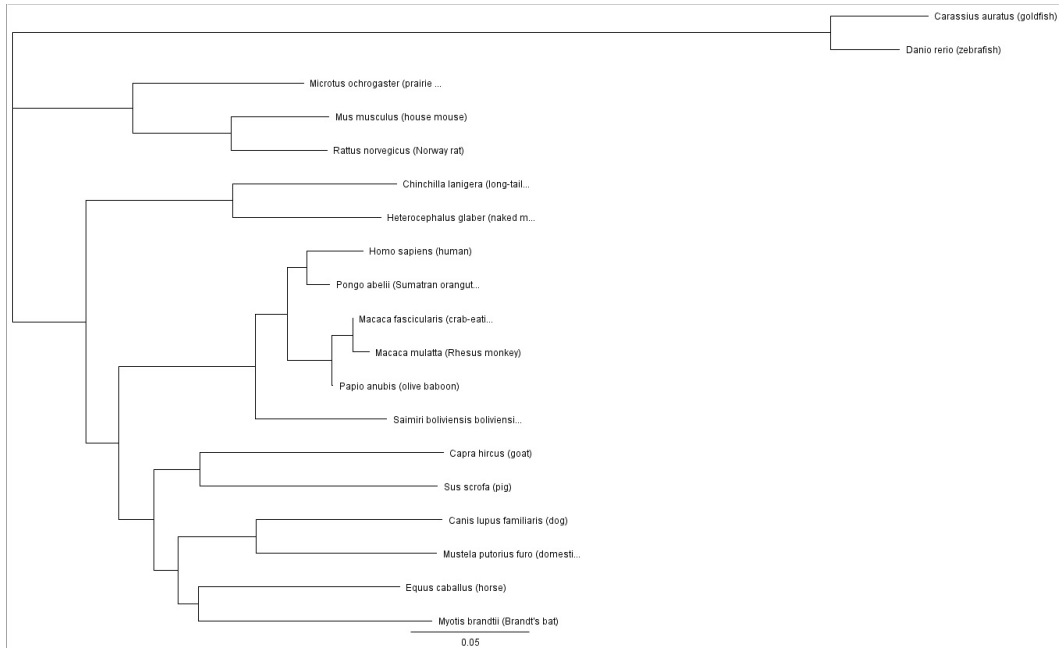
Papio      MNLRLCVQALLL----LWLSLTAVCG-GPLMQLPYGNGL EE-GNVRHLVQPRGSRNGP GP 54
Macaca     MNLRLCVQALLL----LWLSLTAVCG-GPLMQLPYGNGL EE-GNVRHLVQPRGSRNGP GP 54
Pongo      MNLRLCVQALLL----LWLSLTAVCG-GSLMPLPDGNGL EE-GNVRHLVQPRGSRNGP GP 54
Homo       MNLRLCVQALLL----LWLSLTAVCG-GSLMPLPDGNGL ED-GNVRHLVQPRGSRNGP GP 54
Rattus     MNLSFCVQALLL----LWLSLTAVCG-VPLMLPPDGKGL EE-GNMRYLVKPRTSRTGP GA 54
Mus        MNLRLCVQALLL----LWLSLTAVCG-VPLMLPPDGTGL EE-GSMRYLVKPRTSRTGP GA 54
Sus        MNLRLCVQALLL----LWLSLSAVCG-GPLLQTSGGKGM EE-GNVRYL VQPRGPRSGP GP 54
Capra      MG-ELA AKGR LYGNC ELVVETPAWRETGPLLQTS D GKEMEE-GTIRYL VQPRGARSGP GP 58
Danio      MNVKILTIVIVL-----VVSLLCSASAGPMAS TEHSKEIEEVGSMRTPLRQNP ARAGRSQ 55
          * . : . : . . : . . : * : * * : . . * * .

Papio      WQGGRRKFR RQR PRLSHKGMP PF 77
Macaca     WQGGRRKFR RQR PRLSHKGMP PF 77
Pongo      WQGGRRKFR RQR PRLSHKGMP PF 77
Homo       WQGGRRKFR RQR PRLSHKGMP PF 77
Rattus     WQGGRRKFR RQR PRLSHKGMP PF 77
Mus        WQGGRRKFR RQR PRLSHKGMP PF 77
Sus        WQGGRRKFR RQR PRLSHKGMP PF 77
Capra      WQGGRRKFR RQR PRLSHKGMP PF 81
Danio      RPAGWRR-RRPR PRLSHKGMP PF 77
          . * * : * * * * * * * * * *

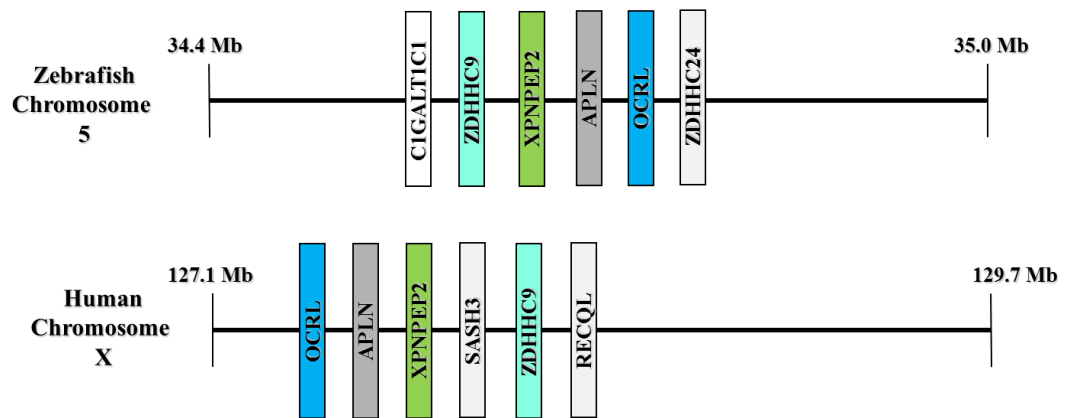
```

**Supplementary Figure 6.** ClustalW alignment of *Danio rerio* APLN protein sequence against *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, *Sus scrofa*, *Capra hircus*, *Papio anubis*, *Pongo abelii* and *Macaca mulatta* APLN sequences. (\*) identical sequence and (: ) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 36.94% sequence identity with these species and 34.20% similarity to *Homo sapiens*.

Phylogenetic analysis of the zebrafish APLN protein sequence with known vertebrate APLN genes grouped the gene very clearly with the known cyprinid fish APLN protein sequence (**Supplementary Fig. 7**). Analysis of the region within the zebrafish genome around the predicted APLN gene on chromosome 5 allowed a gene map to be constructed that showed that there was conservation of synteny between the human and zebrafish genomes (**Supplementary Fig. 8**), giving further evidence that this gene was a homologue of the human APLN gene.



**Supplementary Figure 7.** Phylogenetic tree, constructed using the neighbour joining method alignment, showing the relationship between zebrafish APLN and known vertebrate APLN sequences.



**Supplementary Figure 8.** Comparison of the co-localization of genes surrounding APLN on Human chromosome X and Zebrafish chromosome 5.

## CCK

Searching the available genome using TBLASTN with a known fish CCK protein, identified a region of chromosome 16 that contained a potential zebrafish CCK sequence. Using gene prediction software with this region provided a nucleotide sequence which was translated and the predicted protein was aligned

against known CCK protein sequences already identified in other fish (Supplementary Fig. 9) and in selected mammals (Supplementary Fig. 10).

```

Larimichthys      MTAGLCVVCVLAVALCTS-CLGLPFFSSQLLDEGQRSISAPSEALLEAGHTHTLGEPNLQHS 59
Oncorhynchus     MTAGLCVVCVLLVVLSTS-CLGRPQSSPPLQEGGPAMPSPSEARLEAYAHFLSKPRLRQTR 59
Anguilla          MNGGICVVCVLLAALSTS-CLGRP-SSNTQDE-----SRAAQSQVDTVLSE-HMREAR 49
Astyanax          -----EHTRHTR 7
Danio             MNAGLCVCALLAALSTS SCLS L P V H S E D G V Q S -----NVGSATGHTRHTR 45
                  . : : : :

Larimichthys      SAPQLRALP-LAEEDADS-----RANLSELLARLISSRK-----GSVRRNSTANSRNG 107
Oncorhynchus     SAPLDNTVPYTAEDGDS-----RANLSELLARLISSRK-----GSLRKNSTVNSRAG 108
Anguilla          STPLSDQQKPKAEEGVDS-----RASLSELLARLISSRK-----GNTRRNSTINSRAG 97
Astyanax          AVPLSGQITLLSKAQEEVEGDPARKLNELLARLISRKEDFPYLSGSYRRSPAS--RSAN 65
Danio             AAPPAGQINLLTKPEDDE---EPRSSLTELLARIISTK-----GSYRRSPAANSRTMG 95
                  :.*      : : .      .*.*****:* :      *. * : : : * .

Larimichthys      LSAHRIADRDYVGVWDFGRRSAGEYEYSS 137
Oncorhynchus     LSAHRIKDRDYNWDFGRRSAGEYEYSS 138
Anguilla          LSAHRIKDRDYLWDFGRRSAGEYEYSS 127
Astyanax          VN--HRIKDRDYLWDFGRRSAGEYEYSS 93
Danio             AS--HRIKDRDYLWDFGRRSAGEYEYSS 123
                  .   ***   ***   *****   *****

```

**Supplementary Figure 9.** ClustalW alignment of *Danio rerio* CCK protein sequence against *Astyanax mexicanus*, *Larimichthys crocea*, *Anguilla japonica* and *Oncorhynchus mykiss* CCK sequences. (\*) identical sequence and (:) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 51.38% sequence identity with the fish sequences.

```

Pygmy             MNSGVCLCVLMAVLAAG-ALTQPVPPAD-----PAGSGLQRAEEAPRRQLRVSQRTD 51
Pan               MNSGVCLCVLMAVLAAG-ALTQPVPPAD-----PAGSGLQRAEEAPRRQLRVSQRTD 51
Homo              MNSGVCLCVLMAVLAAG-ALTQPVPPAD-----PAGSGLQRAEEAPRRQLRVSQRTD 51
Nomascus          MNSGVCLCVLMAVLAAG-ALTQPVPPAD-----PASSGLQRAEEAPRRQLRVAQRTD 51
Pongo             MNSGVCLCVLMAVLAAG-ALTQPVPPAD-----PAGSRLQRAEEAPRRQLRVAQRTD 51
Callithrix        MNRGVCLCVLMAVLAAG-ALTQPVPPGE-----PAGSGLQRAEEAPRRQLRVAQRTD 51
Saimiri           MNRGVCLCVLMAVLAAG-ALTQPVPPGE-----PAGSGLQRAEEAPRRQLRVAQRTD 51
Macaca            MNSGVRLCVLMAVLAAG-ALTQPVPPAE-----PAGSGLQRAEEAPRRQLRVAQRTD 51
Papio             MNSGVRLCVLMAVLAAG-ALTQPVPPAE-----PAGSGLQRAEEAPRRQLRVAQRTD 51
Otolemur          MNRGVCLCVLMAVLAAG-TLTQPVSPAD-----PAGSGVPRPEEAPRRQLRVAQRTD 51
Cattle            MNRGVCLCVLMAVLAAG-ALAQPMPHAD-----PTGPRAQQAEEAPRRQLRVAQRTD 51
Bos               MNRGVCLCVLMAVLAAG-ALAQPMPHAD-----PTGPRAQQAEEAPRRQLRVAQRTD 51
Ovis              MNRGARLCLLMAVLAAG-ALAQPMPHAD-----PTGPRAQQAEEAPRRQLRVAQRTD 51
Capra             MNRGARLCLLMAVLAAG-ALAQPMPHAD-----PTGPRAQQAEEAPRRQLRVAQRTD 51
Sus               MNGGLCLCVLMAVLAAG-TLAQPVPPAD-----SAVPGAQ-EEEEHRRQLRVAQKVD 50
Chinchilla        MNGGLCLCVLMAVLAAG-AWTQPLSPAG-----PLGPATLGAEDAPRRQLR--TRTG 49
Mus               MKSGVCLCVVMAVLAAG-ALAQPVVPAE-----ATDPEVQRAEEAPRRQLRVAQRTD 51
Rattus            MKCGVCLCVVMAVLAAG-ALAQPVVPE-----AVDPMEQRAEEAPRRQLRVAQRTD 51
Danio             MNAGLCVCALLAALSTS SCLS L P V H S E D G V Q S NVGSATGHTRHTRAPAGQINLLTKPE 60
                  *: *  :*  :*:*. : . : * :      .      * : : :

Pygmy             GE--SRAHLGALLARYIQQARKAP----SGRMSVVKNLQNLDP----SHRISDRDYMGMW 101
Pan               GE--SRAHLGALLARYIQQARKAP----SGRMSVVKNLQNLDP----SHRISDRDYMGMW 101
Homo              GE--SRAHLGALLARYIQQARKAP----SGRMSIVKNLQNLDP----SHRISDRDYMGMW 101
Nomascus          GE--SRAHLGALLARYIQQARKAP----SGRMSIVKNLQNLDP----SHRISDRDYMGMW 101
Pongo             GE--SRAHLGALLARYIQQARKAP----SGRMSIVKNLQNLDP----SHRISDRDYMGMW 101
Callithrix        GE--SRAHLGALLARYIQQARKAP----SGRMSVVKNLQNLDP----SHRISDRDYMGMW 101
Saimiri           GE--SRAHLGALLARYIQQARKAP----SGRTPVIKNLQNLDP----SHRISDRDYMGMW 101
Macaca            GE--SRAHLGALLARYIQQARKAP----SGRMSIIKNLQNLDP----SHRISDRDYMGMW 101
Papio             GE--SRAHLGALLARYIQQARKAP----SGRMSIIKNLQNLDP----SHRISDRDYMGMW 101
Otolemur          GE--SRAQLGALLARYIQQARKAP----SGRNSIIKNLQSLDP----SHRISDRDYMGMW 101
Cattle            DE--PRAQLGALLARYIQQARKAP----SGRMSVIKNLQSLDP----SHRISDRDYMGMW 101

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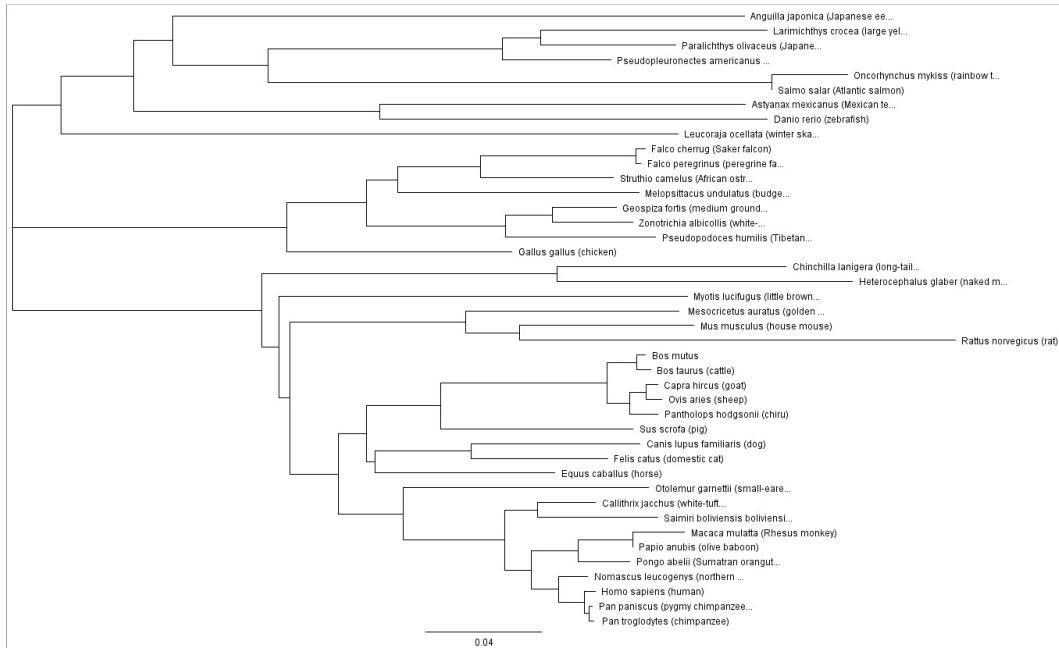
Bos          DE--PRAHLGALLARYIQQARKAP----SGRMSVIKNLQSLDP----SHRISDRDYMGMW 101
Ovis        DE--SRAHLGALLARYIQQARKAP----SGRMSVIKNLQSLDP----SHRISDRDYMGMW 101
Capra       DE--SRAHLGALLARYIQQARKAP----SGRMSVIKNLQSLDP----SHRISDRDYMGMW 101
Sus         GE--SRAHLGALLARYIQQARKAP----SGRVSMIKNLQSLDP----SHRISDRDYMGMW 100
Chinchilla  AE--PRARLGALLARYIQQARKAP----SGRMAMVKSLQGLDP----NHRISDRDYVGWM 99
Mus         GE--PRARLGALLARYIQQVRKAVAMVMTSGWVLTWTSRAGLKHRRWASFLWSSRTQFFLP 109
Rattus      SE--PRARLGALLARYIQQVRKAP----SGRMSVLKNLQGLDP----SHRISDRDYMGMW 101
Danio       DDEEPRSSLTELLARIISTKGSYR-----RSPAANSRTMGA----SHRIKDRDYLGMW 109
           : .*: * **** *. . . : .. .* .

Pygmy       DFGRRSA-----EEYEYPS---- 115
Pan         DFGRRSA-----EEYEYPS---- 115
Homo        DFGRRSA-----EEYEYPS---- 115
Nomascus    DFGRRSA-----EEYEYPS---- 115
Pongo       DFGRRSA-----EEYEYPS---- 115
Callithrix  DFGRRSA-----EEYEYPS---- 115
Saimiri     DFGRRSA-----EEYEYPS---- 115
Macaca      DFGRRSA-----EEYEYPS---- 115
Papio       DFGRRSA-----EEYEYPS---- 115
Otolemur    DFGRRSA-----EEYEYPS---- 115
Cattle      DFGRRSA-----EEFEYTS---- 115
Bos         DFGRRSA-----EEFEYTS---- 115
Ovis        DFGRRSA-----EEFEYTS---- 115
Capra       DFGRRSA-----EEFEYTS---- 115
Sus         DFGRRSA-----EEYEYTS---- 114
Chinchilla  DFGRRSAEG-----EEYEYPA---- 115
Mus         AFEQPMACRPVCIWLDCSFWPHVRS 134
Rattus      DFGRRSAE-----DYEYPS---- 115
Danio       DFGRRSA-----EEYEYSS---- 123
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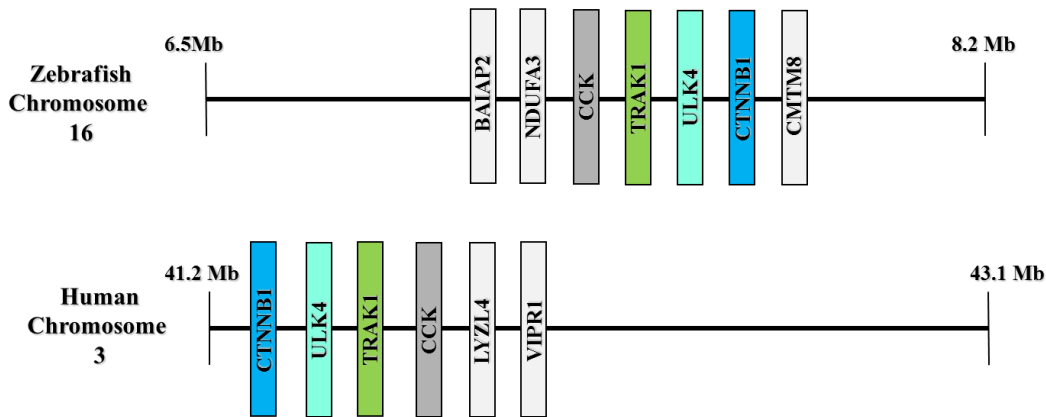
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**Supplementary Figure 10.** ClustalW alignment of *Danio rerio* CCK protein sequence against *Homo sapiens*, *Rattus norvegicus*, *Sus scrofa*, *Bos Taurus*, *Callithrix jacchus*, *Macaca mulatta*, *Otolemur garnettii*, *Saimiri boliviensis boliviensis*, *Bos mutus*, *Ovis aries*, *Capra hircus*, *Chinchilla lanígera*, *Papio anubis*, *Nomascus leucogenys*, *Pan paniscus* and *Pan troglodytes* CCK sequences. (\*) identical sequence and (: ) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 38.71% sequence identity with these species and 63.20% similarity to *Homo sapiens*.

Phylogenetic analysis of the zebrafish CCK protein sequence with known vertebrate CCK genes grouped the gene very clearly with the known cyprinid fish CCK protein sequence (**Supplementary Fig. 11**). Analysis of the region within the zebrafish genome around the predicted CCK gene on chromosome 5 allowed a gene map to be constructed that showed that there was conservation of synteny between the human and zebrafish genomes (**Supplementary Fig. 12**), giving further evidence that this gene was a homologue of the human APLN gene.



**Supplementary Figure 11.** Phylogenetic tree, constructed using the neighbour joining method alignment, showing the relationship between zebrafish CCK and known vertebrate CCK sequences.



**Supplementary Figure 11.** Comparison of the co-localization of genes surrounding CCK on Human chromosome 3 and Zebrafish chromosome 16.

## FTO

Searching the available genome using TBLASTN with a known fish FTO protein, identified a region of chromosome 7 that contained a potential zebrafish FTO sequence. Using gene prediction software with this region provided a

nucleotide sequence which was translated and the predicted protein was aligned against known FTO protein sequences already identified in other fish (Supplementary Fig. 13) and in selected mammals (Supplementary Fig. 14).

```

Takifugu      ----MCCFAYGKDS-----ALKRRRLKELGDQKI PFLGSPDRGFQQLWDSS 43
Oryzias      MSPQMKAQCKHNSRNMKRSQDSEGEKTKRRRLLRELGDHKIPYLGPSRRGFQQLWDSS 60
Danio        ----MKPRQRKQYFRNMKRSDDSEREKRRERRRLLQELGEQRI PYLSPDTPGFQDLWDSS 56
Latimeria    -----MNYSFTDCNMKRRNDVDAEKEIKKQKLLKELGDKPLQYFTPKDAEFQHLWKTH 53
                ::::**::**::: : : : *.. **.*:

Takifugu      YSGLVLKRSCSLPAELHARVQAALLTLRKGCLLRDLVVRDRDVFTNVSRRALLGDQGH 103
Oryzias      YSGLVFRRSCSLPTELHCRVQAALLTLRKGCLLKDVLVVRERDVFTAVSRALFGEPGHT 120
Danio        YAGLALRQSGTLP EGLHEKVQSALLTLQRHGCLLRDLVVRDRDVFTAVSRALVGPQGYT 116
Latimeria    YSKLLFKEANKTPHDLHEQVQSALLALLDHGCFQDLVRMKGKDI FTPI SRLLIGIPGCT 113
                *: * :.: . * ** :**::**:* :***::***: : *:* * ** * * *

Takifugu      YRYLDTRLFAIPWHNEDSE----VKGQNCDDPLRAACKALWELNFFCSDVAQLKEGDR 159
Oryzias      YRYLETRLFALPWHSEDE----VKGHNCCDPLRAACKALWELNFFCSDVSLKEGDK 176
Danio        YRYLDTRLFTIPWHCEGEGQKDEKQCCDLDLRDACKALWELNFFCSDVQKQTN--- 173
Latimeria    YKYLNTRLFAIPWPTSGKV-----VRYSDQIFKACQALLKLNLDYLCETETVKALQ--- 163
                *:*:*:*:*:*:* * .. . * : : **:* ** :***:~*~: : :

Takifugu      LAQCAKGETETKQAEQGDTESEKHSSEDSKISEGGDSESQKSEEGDTESEKHSSEWDVESKQS 219
Oryzias      LTQCVK--AEAKLGEI DTESEKHSSEDSKNSDGGDSESQKSEEGDTESEKHSSEWDIESKHS 234
Danio        -----ARGVKRTRSDTENSEDAP--GEGMCEEESVKDRILVEEKTIEE---EEEDS 218
Latimeria    -----EQSLAQAK-----EVEVTEKAATLSMLQDSVGFTKGRS 196
                :. :::: * . :. . . :. . *

Takifugu      EEGCSGKVEGDWETGSRHSSEEGESSLVKPSSETCVAGESEHSEKGLPGWQAK----TSL 275
Oryzias      DEGCSVSK-QGDWEAELKPGSEEGESSVKQSESTAPVSEHREGKCPWADEPISGKEM 293
Danio        GQGCSSHSS-----PPSS----- 230
Latimeria    PVVGDQTN----- 204
                . :.

Takifugu      KTESVGPQTQGAQQKHSS----EDKEEQVSRQGCSPSPQVCTDHARFNVTLNLYMD 330
Oryzias      KRVEIEAQERPVAQLKHSLSLDYHIQDVEDPVSGQGCSTPPQVCTGPAKFNVTLNLYMD 353
Danio        -----TPPAAQASLVQFNVTLINLYMN 251
Latimeria    -----CSFELENRASFNVTLLNLYMD 224
                . . . *****:***:

Takifugu      PSSMSQLKEEPY YGMGKMAVGWHHDENLITHSPVAVYSYSCHDDK-ESSEGS----GSE 384
Oryzias      PAKTSHLKEEPY YGMGKMAVGWHHDENLISNSPVAVYNYSYHNDKGESSEGG----TGE 408
Danio        PAAMTQLKEEPY YGMGKMAVGWHHDENLVPLSPVAVYSYSCPAEP--KNEG----- 300
Latimeria    PLKMVHLKDEPY YGMGKMAVSWHHDENLVERSTVAVYSYCKDSSLASDMKDRNEGRD 284
                * :**::**:*:*:*:*:*:*:* * :***.* * . :.

Takifugu      KACWRIGLKVAVDIHTPGLMLPLESGDCYMRDDLNSTHQHCVLAGDTARFSSTHRVAEC 444
Oryzias      KTCWRIGLKVAVDIHTPGLMPLVLESGDCYMRDNLNSTHQHCVLAGETARFSSTHRVAEC 468
Danio        -----VTEKDGEKSKKKKKK----- 316
Latimeria    PVLWHVGLKRAWDIQTPLGLAVPLPGDCYFMLDDLNMTHQHCVLAGLQPRFSSTHRVAEC 344
                :. * : . :

Takifugu      SNGTLYIQSKCQEALSNLHTDPETGSHSLALLPTTLQRCIEIHNEVEFEWLRQYWFQG 504
Oryzias      SRGTLYIQSRCQEALSNLQTDPETGSHSLLTVPITLQNCIEIHNEVEFEWLRQYWFQG 528
Danio        ----- 404
Latimeria    STGTLYIFGRCQIALNNLRKDPSSGTLASFDLPVLKQTEIEIHNEVEFEWLRQYWFQG 404

Takifugu      QRYTRFCSWWSKPMQLEKDWKLMEMMVSSVWCHS----- 539
Oryzias      QRYTRFCSWWSGPMQLEKDWRLMEKMSKSLCSR----- 563
Danio        -----
Latimeria    KRYGICSDWWEYPMAKMEDLWREMEIMTNLALCEVRNEEWPVEBKQELINCLLTLIMERQ 464

Takifugu      -----SLAQTLPQEA PVDPRPFVGVDDPGMPLPFDLADI INRVESLLWSEATN 587
Oryzias      -----AYAE---RKQAVFEQ---LGSGSSRGHSCTTVAAAGERSCSPLPPSLSP 606
Danio        -----
Latimeria    ELRKDWVARCNSRLALS LPSDQQPECHPYWTDLSDMPLPFNLEQVISDLQAMLD FED-Q 523

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Takifugu	GLQAVKQISCOMTHKSKA-	605
Oryzias	RTPAASGSSSSLSVPSAEQ	625
Danio	-----	
Latimeria	AVRDVPRSSCSVWEGQKV-	541

**Supplementary Figure 13** ClustalW alignment of *Danio rerio* FTO protein sequence against *Takifugu rubripes*, *Oryzias latipes* and *Latimeria chalumnae* FTO sequences. (\*) identical sequence and (:) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 33.63% sequence identity with the fish sequences.

Homo	-----	
Macaca	MAPDGSRTLRELHAGGGVQEGGIYAACGGEGGFSGSMKRTPTAEERER-----	48
Crab-eating	MAPDGSRTLRELHAGGGVQEGGIYAACGGEGGFSGSMKRTPTAEERER-----	48
Pongo	-----MKRTPPTAEERER-----	12
Gorilla	-----MKRTPPTAEERER-----	12
Nomascus	-----MKRTPPTAEERER-----	12
Pan	-----MKRTPPTAEERER-----	12
Rattus	-----MKRVQTAEERER-----	12
Mus	-----MKRVQTAEERER-----	12
Heterocephalus	-----MKRTQTSEEQER-----	12
Chinchilla	-----MKRTQTAEERER-----	12
Oryctolagus	-----MKRTQTAEERER-----	12
Bos	-----MKRTPPTAEERER-----	12
Capra	-----MTHTMNQIFHDRLVRNKIISLFL	24
Sus	-----MKRTPPTAEERER-----	12
Pygmy	-----MKRTPPTAEERER-----	12
Danio	-----MKPRQRKQYFRNMKRSDDSEREK	24

Homo	-----	
Macaca	--EAK-----WQLKYPKLILREASSVSEELHKEVQEAF	80
Crab-eating	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREASSVSEELHKEVQEAF	106
Pongo	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREASSVSEELHKEVQEAF	70
Gorilla	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREASSVSEELHKEVQEAF	70
Nomascus	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREASSVSEELHKEVQEAF	70
Pan	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREASSVSEELHKEVQEAF	70
Rattus	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLVFREAGSIPEELHKEVPEAF	70
Mus	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLVFREAGSIPEELHKEVPEAF	70
Heterocephalus	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLVFREAGSVSEELHKEVQEAF	70
Chinchilla	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREAGGSAELHKEVQEAF	70
Oryctolagus	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREAGSVSEELHKEVQEAF	70
Bos	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREAAVPELLHKEVQQAFL	70
Capra	LLKIKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREAAVPELLHKEVQQAFL	84
Sus	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREACSVPEGLHKEVQEAF	70
Pygmy	--EAKKLRLLLEELEDTWLPYLTPKDDEFYQQWQLKYPKLILREASSVSEELHKEVQEAF	70
Danio	---RERRRLLQELGEQRIPYLSPTDPGFQDLWSSYAGLALRQSGTLPEGLHKEVQSALL	81

Homo	-----	
Macaca	TLHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSNIKYPEA	140
Crab-eating	TLHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSNIKYPEA	166
Pongo	TMHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSNIKHTEA	130
Gorilla	TLHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSNIKHTEA	130
Nomascus	TLHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSNIKHTEA	130
Pan	TLHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSNIKHTEA	130
Rattus	TLHHKGCLFRDLVRIQGDVLTTPVSRILIGDPGCTYKYLNTRLFTVPWPVKGCTINYTEA	130
Mus	TLHHKGCLFRDLVRIQGDVLTTPVSRILIGDPGCTYKYLNTRLFTVPWPVKGCTINYTEA	130
Heterocephalus	TLHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSNVSYTEA	130
Chinchilla	TLRKHGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSNVSYTEA	130
Oryctolagus	TLHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSTINYTEA	130
Bos	TLHHKGCLFRDLVRIQGDVLTTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSDAKYNEA	130
Capra	TLHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSDAKYNEA	144
Sus	ALHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSDAKYNEA	130
Pygmy	TLHHKGCLFRDLVRIQGDLLTPVSRILIGNPGCTYKYLNTRLFTVPWPVKGSNIKHTEA	130
Danio	TLQRHGCLLRDLVRRDRDVFATVSRALVQPGYTYRYLDTRLFTIIPWHCEG-----	133

Homo -----  
 Macaca EIAAACETFLKLNNDYLIQIETIQALEELAAKEKANEDAVPLCMSADFFPRVGMGSSYDGGDE 200  
 Crab-eating EIAAACETFLKLNNDYLIQIETIQALEELAAKEKANEDAVPLCMSADFFPRVGMGSSYDGGDE 226  
 Pongo EIAAACETFLKLNNDYLIQIETIQALEELAAKEKANEDTVPLCMSADFFPRVGMGSSYNGQDE 190  
 Gorilla EIAAACETFLKLNNDYLIQIETIQALEELAAKEKANEDAVPLCMSADFFPRVGMGSSYNGQDE 190  
 Nomascus EIAAACETFLKLNNDYLIQIETIQALEELAAKEKANEDAVPLCMSADFFPRVGMGSSYNGQDE 190  
 Pan EIAAACETFLKLNNDYLIQIETIQALEELAAKEKANEDAVPLCMSADFFPRVGMGSSYNGQDE 190  
 Rattus EIAAACQTFLLKLNNDYLQVETIQALEELAIKEKANEDAVPLCM-AEFPRAGVGP--SCDDE 187  
 Mus EIAAACQTFLLKLNNDYLQVETIQALEELAVREKANEDAVPLCM-AEFPRAGVGP--SCDDE 187  
 Heterocephalus EIAAACQTFLLKLNNDYLQIETTQALEELAVKEKANEDAVPVCM-SEFPVSGMG---SSSDE 186  
 Chinchilla EIAAACQTFLLKLNNDYLQIETIQALEELAVKEKAKEDAVPVFM-TEFPRVGMG---SSHDE 186  
 Oryctolagus EIGAACQTFLLKLNNDYLQIETIQALEELAVKEKEDAVPVCM-AEFPRVGMGSSYDGGDE 189  
 Bos EIAAACQTFLLKLNNSYLQVETIQALEELAAKEKANIDAVPVCIGPDFPRVGMGSSFDGHDE 190  
 Capra EIAVACQTFLLKLNNSYLQVETIQALEELAAKEKANIDAVPVCIGPDFPRVGMGSSFDGHDE 204  
 Sus EIGAACQTFLLKLNNDYLQIETIQALEELAAKEKANIDTVPVCIGPDFPRVGMGSSFDGHDE 190  
 Pygmy EIAAACETFLKLNNDYLIQIETIQALEELAAKEKANEDAVPLCMSADFFPRVGMGSSYNGQDE 190  
 Danio -----

Homo -----  
 Macaca VDIKSRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYSYSC 260  
 Crab-eating VDIKSRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYSYSC 286  
 Pongo VDIKSRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYSYSC 250  
 Gorilla VDIKSRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYSYSC 250  
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 Rattus VDLKSRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYSYSC 247  
 Mus VDLKSRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYSYSC 247  
 Heterocephalus VDMKSRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYNSYSC 246  
 Chinchilla VDMKSRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYNSYSC 246  
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 Bos IDMKNRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYSYSC 250  
 Capra IDMKNRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYSYSC 264  
 Sus VDRKSRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYNSYSC 250  
 Pygmy VDIKSRAAYNVTLNFMNDPQKMPYLKEEYPYFGMGKMAVSWHHDENLVDRSAVAVYSYSC 250  
 Danio -----

Homo -----  
 Macaca GPEEESDDDSHFEGRDPDIWHVGFKISWDIETPGLAIPHLHQGDCYFMLDDLNATHQHCVL 320  
 Crab-eating GPEEESDDDSHFEGRDPDIWHVGFKISWDIETPGLAIPHLHQGDCYFMLDDLNATHQHCVL 346  
 Pongo GPEEESDDDSHFEGRDPDIWHVGFKISWDIETPGLAIPHLHQGDCYFMLDDLNATHQHCVL 310  
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 Pan GPEEESDDDSHFEGRDPDIWHVGFKISWDIETPGLAIPHLHQGDCYFMLDDLNATHQHCVL 310  
 Rattus GSEDESDESSFEGRDPDTWHVGFKISWDIETPGLTIPLHQGDCYFMLDDLNATHQHCVL 307  
 Mus GSEDESDESSFEGRDPDTWHVGFKISWDIETPGLTIPLHQGDCYFMLDDLNATHQHCVL 307  
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 Chinchilla DPEEESSEESHLEGRDPDTWHVGFKISWDIETPGLAIPHLHQGDCYFMLDDLNATHQHCVL 306  
 Oryctolagus GPDDSEDDDSNLEGRDPDIWHVGFKISWDIETPGLAIPHLHQGDCYFMLDDLNATHQHCVL 309  
 Bos GPEEESDDDPQLEGRDPDIWHVGFKISWDIETPGLAIPHLHQGDCYFMLDDLNATHQHCVL 310  
 Capra GPEEESDDDPQLEGRDPDIWHVGFKISWDIETPGLAIPHLHQGDCYFMLDDLNATHQHCVL 324  
 Sus GPEEESDDDPQLEGRDPDVWHVGFKISWDIETPGLAIPHLHQGDCYFMLDDLNATHQHCVL 310  
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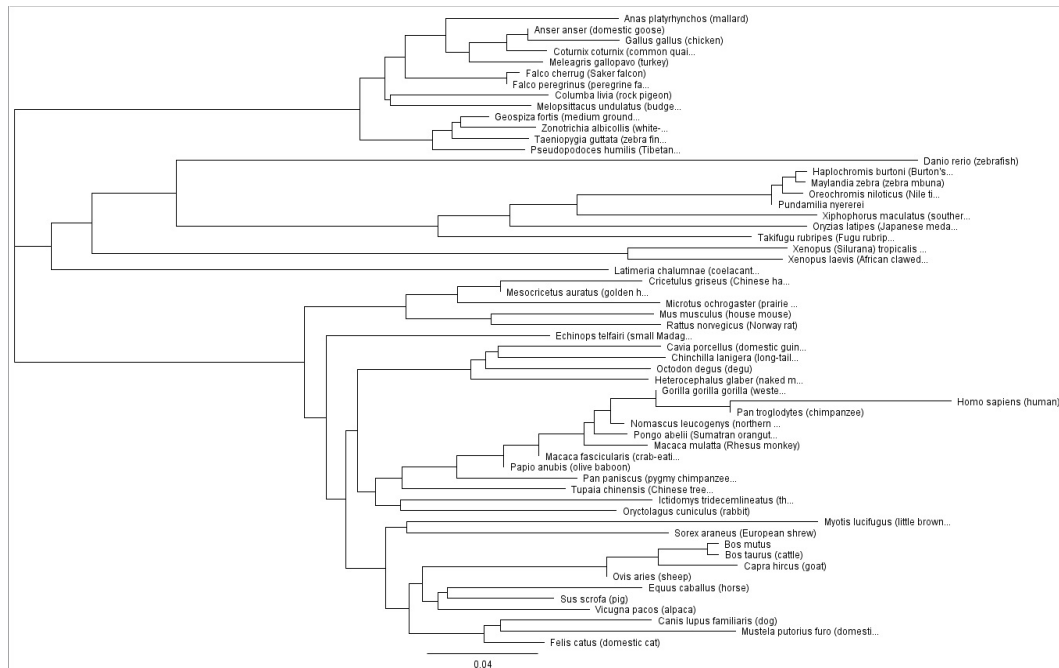
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 Crab-eating AGSQPRFSSTHRVAECSTGTLDYILQRCQLALQNVRRDDVDNGDVSLKSFEPAVLKQGEI 406  
 Pongo AGSQPRFSSTHRVAECSTGTLDYILQRCQLAPQNVRRDDVDNDVSLKSFEPAVLKQGEI 370  
 Gorilla AGSQPRFSSTHRVAEVSVN-----KNLG-----VEFEWLRQFWFQG--- 346  
 Nomascus AGSQPRFSSTHRVAECSTGTLDYILQRCQLALQNIHDNVNDNDVSLKSFEPAVLKQGEI 370  
 Pan AGSQPRFSSTHRVAECSTGTLDYILQRCQLALQNVCDVDNDNDVSLKSFEPAVLKQGEI 370  
 Rattus AGSQPRFSSTHRVAECSTGTLDYILQRCQLALQNVLNDSDNGDVSLKSFEPAVLKQGEI 367  
 Mus AGSQPRFSSTHRVAECSTGTLDYILERCQLALQNVLNDSDNGDVSLKSFDPVAVLKQGEI 367  
 Heterocephalus AGSQPRFSSTHRVAECSSGTLDYILHRCQLAVQNVHDNDVDNGDVSLKSFEPAVLKQGEI 366  
 Chinchilla AGSQPRFSSTHRVAECSTGTLDYILQRCQLALQNVRRDDVDNDVDNGDVSLKSFEPAVLKQGEI 366  
 Oryctolagus AGSQPRFSSTHRVAECSTGTLDYILQRCQLALQNVRRDDADGTAVTLSKSFEPAVLKQGEI 369  
 Bos AGLPPRFSSSTHRVAECSTGTLEYILQRCQVALQNVREEADNGEISLSKLESVVLKQGEI 370  
 Capra AGLPPRFSSSTHRVAECSTGTLEYILQRCQVALQNVREEADNGEISLSKLESVVLKQGEI 384  
 Sus AGLPPRFSSSTHRVAECSTGTLDYILQRCQLALQNVRRDEADSEVSLKSFEPAVLKQGEI 370  
 Pygmy AGSQPRFSSTHRVAECSTGTLDYILQRCQLALQNVCDVDNDVDVSLKSFEPAVLKQGEI 370  
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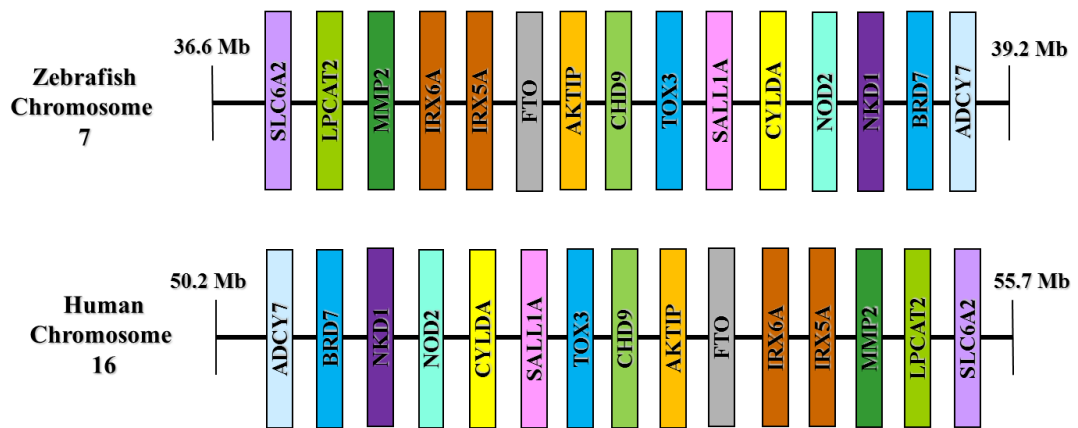
Homo -----KNFSRMTNAVLHEVKREGLPVEQ 52



Phylogenetic analysis of the zebrafish FTO protein sequence with known vertebrate FTO genes grouped the gene very clearly with the known fish FTO protein sequences (**Supplementary Fig. 15**). Analysis of the region within the zebrafish genome around the predicted FTO gene on chromosome 7 allowed a gene map to be constructed that showed that there was conservation of synteny between the human and zebrafish genomes (**Supplementary Fig. 16**), giving further evidence that this gene was a homologue of the human FTO gene.



**Supplementary Figure 15.** Phylogenetic tree, constructed using the neighbour joining method alignment, showing the relationship between zebrafish FTO and known vertebrate FTO sequences.



**Supplementary Figure 16.** Comparison of the co-localization of genes surrounding FTO on Human chromosome 16 and Zebrafish chromosome 7.

## LEP

Two LEP genes were found for zebrafish. Searching the available genome using TBLASTN with a known fish LEP protein, identified a region of chromosome 18 that contained a potential zebrafish LEPa sequence and a region of chromosome 4 that contained a potential zebrafish LEPb sequence. Using gene prediction software with this region provided a nucleotide sequence which was translated and the predicted proteins were aligned against known LEP protein sequences already identified in other fish (**Supplementary Fig. 17 & 19**) and in selected mammals (**Supplementary Fig. 18 & 20**).

### *LEPa*

Cyprinus	-MYFSALLYPCILAMLSLVHG-----IPIHSDSLKNLVKLQADTIIIRIKDHNAELKLYP	54
Carassius	-MYFPALLYPCILGMLSLVHG-----IPVHPDRLKNMVKLQADTIIIRIKDHNEKLYP	54
Hypophthalmichthys	-MYFPVLLYTCFLSILGLIDGRS---IPFHPESLKSL-KQQADTIIHRIKEHNEKLYP	55
Ctenopharyngodon	-MYSVLLYTCFLSILGMIDGRS---IPIHQDNLKNLVKLQADTIIHRIKEHNEKLYP	56
Danio	-MRFPALRSTCILSMLSLIHC-----IPVHQHDRKNV-KLQAKTIIVRIREHIDGQNLIP	53
Tachysurus	MAVYPALFCSCVVTVLTLTNG-----RALPTDSLKNSVKLQVENIISRIQKHKEFPILH	55
Oncorhynchus	MDCSMALLSSLLALFSVGAG-----ASLSLHVVRTKVKDLAQTVMVIR----IKKLDISP	51

Salmo MDCSMALLSSLLALFSVGAG-----ASLSLHVVRTKVKDLAQTMOVIR----INKLDISP 51  
 Salvelinus MDCSMAPLVSFLLAHFMSGAG-----ASLSLDIVRTKVKFLAQTMOVYRTQMEIKKLPSSS 55  
 Oreochromis -MDYGLVLLFSLFQALSMGTAAP---LPVEVVTKMSKVKWMAEQLVVR---LDKDVQVPV 53  
 Epinephelus -MDYTLALLFSLHVFVSVGTAAP---LPVEVVTKMSKVKWMAEQLVVR---LNKDFQVPP 53  
 Takifugu -MDHILALVLALLP-LSLCVALPGALDAMDVEKMSKVTWKAQGLVAR---IDKHF---P 52  
 Oryzias -MDSALVLF AFLFHCLNVATAAP---VNPELQEMKSNVIDIAKELSLR---LESIIQTSI 53

.. : : :. . : \*

Cyprinus KLLIGDPE-LYPEVPADKPIQGLGSIMDTITTFQKVLQRLPKGRVSIHIDLSTLLGHLK 113  
 Carassius KLLIGDPE-LYPEVPADRHIQGLGSIMDTLTFQKVLQRLPKGHVSQIRSDLSTLLGYLK 113  
 Hypophthalmichthys KILIGDSE-LYPEVPADKPIQGLGSIIDTLTTFQKVLQTLPKGHVSQLHSDVSTLLDYFK 114  
 Ctenopharyngodon KILIGDSE-LYPEVPADKPIQGLGSIVDTLTFQKILQTLPKGHVSQLHNDMSTLLEYFK 115  
 Danio TLIIGDPG-HYPEIPADKPIQGLGSIMETINTFHKVLQKLPKNHVDQIRRDLSTLLGYLE 112  
 Tachysurus KMILDSPE-LLPELQSDKPIEGLSSMVEMLNNFQRVLHSLPKGHMSQLHSDVSTLQHYLE 114  
 Oncorhynchus N-LIEGMDPFLPAAAVDQHIESLPSIVETMGFYQDLLLVLDWADLKQLVEDTSTMRGLLE 110  
 Salmo N-LIEGMDPFLPAAAVDQHIESLPSIMETMGFYQDMLFLDWADLKQLVEDTSTMRGLLE 110  
 Salvelinus NLVIDGLELFFPAAAGDQPIEGLPSIVETMGFYQDLLISLDMADLKQLVEDASTMRGQLE 115  
 Oreochromis NWTLNPPA-----DDLDTSSIVTVLNGYNSLIPDT-FKGVSVQIKYDISSLTGYIH 103  
 Epinephelus GLTSLPPA-----DILDGPSSIVTVLDGYNSLISDT-FNGVSVQKFDISSLTGYIG 103  
 Takifugu DRGLRFDT-----DKVEGSTSVVASLESYNNLISDR-FGGVSVQIKTEISSLAGYLN 102  
 Oryzias GPKFSPPS-----DELNGLSSIMAVLDECTNQISDN-FDEAKKIKVDISSLMDSMS 103

: :. \*:: : : :. : \*:: :

Cyprinus ERMTS--MHCTSKEPANG-RALDAFLEDNATHHHITVRYLALDRLKQFMQKLIIVNLDQLKS 170  
 Carassius ERTTS--MHCILKEPANG-RSLDAFLEENATHHHITLGYLALDRLKQFMQKLIIVNLDQLKS 170  
 Hypophthalmichthys VWMTF--MRCTPKEPANG-KSLDTFIEKNATHHHITFGYMALDRLKQFMQKLIANLDQVKS 171  
 Ctenopharyngodon DRMTF--MRCTLKEPANG-KSLDTFIEKNATHHHITFGYMALDRLKQFMQKLIANLDQLKS 172  
 Danio G-----MDCTLKESTNG-KALDAFLEDSASYPFTLEYMTLNRLKQFMQKLIDNLDQLKI 165  
 Tachysurus DRMSS--LQCTHRITGTE-KNLEDFPKNHSMYIITVRHVALDRLQKYIQRNLNHNLEQLRT 171  
 Oncorhynchus NWMTSRCPARQQKQTGEG-GLSEALKDTRRKYGLSVGPVALNRLKGYLGRLLLNLNDQLNY 169  
 Salmo NWMISRCPGRQQKQTGEG-RLSEALKDTRRKYGLSVGPVALNRLKGYLGRLLLNLNDQLNY 169  
 Salvelinus NWMMSRCPGRQQKQTGEG-GLEEALKDSVRKFGLSVCPVALNRLKGYLDRLLLNLNDQLRY 174  
 Oreochromis LWRQG---HCSEQRPKPEVPGPLQELQSHKEFIQTVGIEALMRVKEFLNLLLKNLDQLET 160  
 Epinephelus QWRQG---HCTEQRPKPSVPGPLQELQSRKEFIHTVSI EALMRVKEFLNLLLKNLDHLET 160

```

Takifugu      HWREG---NCQEQQPK-----VWPRRNIFNHTVSLEALMRVREFLKLLQKNVDLLER 151
Oryzias      EWSDK---HCGEQPST-----QAENQTSRRFSITESMQAVTRLKHFLLLLQNNSDQLEI 154
              : .                . :      :: *:: :: * * : :.

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Cyprinus      C- 171
Carassius     C- 171
Hypophthalmichthys C- 172
Ctenopharyngodon C- 173
Danio         C- 166
Tachysurus   C- 172
Oncorhynchus CY 171
Salmo        CY 171
Salvelinus   C- 175
Oreochromis  C- 161
Epinephelus  C- 161
Takifugu     C- 152
Oryzias      C- 155

```

\*

**Supplementary Figure 17.** ClustalW alignment of *Danio rerio* LEPa protein sequence against *Ctenopharyngodon idella*, *Salmo salar*, *Oncorhynchus mykiss*, *Oryzias latipes*, *Takifugu rubripes*, *Carassius auratus*, *Cyprinus carpio*, *Salvelinus alpinus*, *Epinephelus coioides*, *Tachysurus fulvidraco*, *Hypophthalmichthys molitrix* and *Oreochromis mossambicus* LEP sequences. (\*) identical sequence and (:) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 34.09% sequence identity with the fish sequences.

```

Chinchilla    MRHRVLHPIRSSLPSLECCAGTCTPAVAQVALWSYKRGWQAWSSTGILLPATARPKKLILR 60
Heterocephalus -----
Pongo        -----
Homo         -----
Gorilla      -----
Pan          -----
Nomascus     -----

```

Crab-eating -----  
 Macaca -----  
 Canis -----  
 Mustela -----  
 Felis -----  
 Pantholops -----  
 Sus -----  
 Rattus -----  
 Mus -----  
 Callithrix -----  
 Danio -----

Chinchilla RKMRCGPLCQFLWLWCLSYIQAVPIQKVQD-DAKTLVKTIISRINDISHMQSVSSKHKV 119  
 Heterocephalus --MRCGPLCLFLWLWLYLSYIQAVPIQKVQD-DTKTLIKTIVSRINDISHMQSVSSKHKV 57  
 Pongo --MHWGTLGFLWLWPYLFYVQAVPIQKVQD-DTKTLIKTVITRINDISHTQSVSSKQKV 57  
 Homo --MHWGTLGFLWLWPYLFYVQAVPIQKVQD-DTKTLIKTIVTRINDISHTQSVSSKQKV 57  
 Gorilla --MHWGTLGFLWLWPYLFYIQAVPIQKVQD-DTKTLIKTIVTRISDISHTQSVSSKQKV 57  
 Pan --MHWGTLGFLWLWPYLFYVQAVPIQKVQD-DTKTLIKTIVTRINDISHTQSVSSKQKV 57  
 Nomascus --MHWGTLGFLWLWPYLFYVQAVPIQKVQD-DTKTLIKTIVTRINDISHMQSVSSKQKV 57  
 Crab-eating --MYWRTLWGFLWLWPYLFYIQAVPIQKVQS-DTKTLIKTIVTRINDISHT-SVSSKQRV 56  
 Macaca --MYWRTLWGFLWLWPYLFYIQAVPIQKVQS-DTKTLIKTIVTRINDISHTQSVSSKQRV 57  
 Canis --MRCGPLCRFLWLWPYLSYVEAVPIRQVQD-DTKTLIKTIVARINDISHTQSVSSKQRV 57  
 Mustela --MRCGPLCRFLWLWPYLSYVEAVPIRQVQD-DTKTLIKTIVTRISDISHT-AVSSKQRV 56  
 Felis --MLCGPLCRFLWLWPYLSYVEAVPIRQVQD-DTKTLIKTIVTRINDISHTQSVSSKQRV 57  
 Pantholops --MRCGPLYRFLWLWPYLSYVEAVPIRRVQD-DTKTLIKTIVTRINDISHTQSVSSKQRV 57  
 Sus --MRCGPLCRFLWLWPYLSYVEAVPIRQVQD-DTKTLIKTIVTRISDISHTQSVSSKQRV 57  
 Rattus --MCWRPLCRFLWLWSYLSYVQAVPIHKVQD-DTKTLIKTIVTRINDISHTQSVSARQRV 57  
 Mus --MCWRPLCRFLWLWSYLSYVQAVPIQKVQD-DTKTLIKTIVTRINDISHTQSVSAKQRV 57  
 Callithrix --MRWGCLCRFLWLWACLSYQAVPIQRVQD-DTKTLIKTIARINDLSHTQSVSPRQRV 57  
 Danio --MRFPALR-STCILSMLSLIHCIQVPHQHDRKNVQLQAKTIIVRIREHIDGQNLPLPLII 57

\* \* : \* ..\*: : : :.\* \*\*: : \*\* : . : . :

Chinchilla SG---FDFIP-RNPDL SFLKMDKTLAVYQEIIINLSSR-NTGQISNDLENLRLNIRMLAS 174



```

Mus          SKSCSLPQTSGLQKPESLDGVLEASLYSTEVEALSRLQGSLQDILQQLDVVSPEC 167
Callithrix   SKSCHLPWASGLENLANLGGVLEVSLYSTEVVALSRLRGTILKDILQQLDLGPAC 167
Danio        ---CTLKESTNGKALDAFLEDSASYPFTLEYMTLNRLKQFMQKLDLNDLQKIC 166
              * *      :      :      :: *  :*.** : : : : .**  *

```

**Supplementary Figure 18.** ClustalW alignment of *Danio rerio* LEPa protein sequence against *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Sus scrofa*, *Mustela putorius furo*, *Pan troglodytes*, *Macaca mulatta*, *Canis lupus familiaris*, *Heterocephalus glaber*, *Callithrix jacchus*, *Macaca fascicularis*, *Nomascus leucogenys*, *Gorilla gorilla gorilla*, *Panholops hodgsonii*, *Pongo abelii*, *Felis catus* and *Chinchilla lanigera* LEP sequences(\*) identical sequence and (:) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 22.10 % sequence identity with these species and 22.20% similarity to *Homo sapiens*.

### *LEPb*

```

Oryzias      MDSALVLF-FLFHCLNVATAAP---VNPELQEMKSNVIDIAKELSLR-----LE 46
Oreochromis MDYGLVLLF-SLFQALSMGTAAP---LPVEVVTMKSQVWMAEQLVVR-----LD 46
Takifugu     MDHILALVL-ALLP-LSLCVALPGALDAMDVEKMSKVTWKAQGLVAR-----ID 48
Salmo        MDCSMALLSLLALFVSVGAGAS-----LSLHVVRTKVKDLAQTVMVIR----INKLDISP 51
Oncorhynchus MDCSMALLSLLALFVSVGAGAS-----LSLHVVRTKVKDLAQTVMVIR----IKKLDISP 51
Salvelinus   MDCSMAPLVSFLLAHFVSMGAGAS-----LSLDIVRTKVKFLAQTVMVYRTQMEIKKLPSS 55
Ctenopharyngodon -MYSFVLLYTCFLSILGMIDGRS---IPIHQDNLKLVKLAQDTIIHRIKEHNEKLLKSP 56
Hypophthalmichthys -MYFPVLLYTCFLSILGLIDGRS---IPFHPESLKS-LKQADTIHRIKEHNEKLLKSP 55
Carassius    -MYFPALLYPCILGMLSLVHG-----IPVHPDRLKNMVKLAQDTIILRIKDHNEKLLKLYP 54
Tachysurus   MAVYPALFCSCVVTVLTLTNGR-----ALPTDSLKNSVKLQVENIISRIQKHKDEFPILH 55
Danio        -MKSSMIFCLLISSLVAVSISR-----TAPEDRIRIARTTISR-----IKKIKD 45
Cyprinus     -MKSSMIFCYLITALVAVSISR-----TATEERIRIMARTIITR-----IKKIKD 45
Epinephelus  ---MHIFRALVYVSLVAVPGCS-----SLPTKGDSIRNTIHSIINIAQITLVHIKKLR 50
              . . . : .

Oryzias      SIIQTSIGPKFSPPS--DELNGLSSIMAVLDECTN-QISDNFDEAKKIKVDISSLMSMS 103
Oreochromis  KDVQVPVNWTLNPPA--DDLDTSSIVTVLNGYNS-LIPDTFKGVSQIKYDISSLTGYIH 103
Takifugu     KHFPD---RGLRFDT--DKVEGSTSVVASLESYNN-LISDRFGGVSQIKTEISSLAGYLN 102

```

Salmo N-LIEGMDPFLPAAAVDQHIESLPSIMETMGFYQDLMFLDWADLKQLVEDTSTMRGLLE 110  
Oncorhynchus N-LIEGMDPFLPAAAVDQHIESLPSIVETMGFYQDLLLLVLDWADLKQLVEDTSTMRGLLE 110  
Salvelinus NLVIDGLELFFPAAAGDQPIEGLPSIVETMGFYQDLLISLDMADLKQLVEDASTMRGQLE 115  
Ctenopharyngodon KILIGDSELYPEVPA-DKPIQGLGSIVDTLTLTFQKILQTLPKGHVSQLHNDMSTLLEYFK 115  
Hypophthalmichth KILIGDSELYPEVPA-DKPIQGLGSIIDTLTLTFQKVLQTLPKGHVSQLHSDVSTLLDYFK 114  
Carassius KLLIGDPELYPEVPA-DRHIQGLGSIMDTLTIQKVLQRLPKGHVSQLRSDLSTLLGYLK 113  
Tachysurus KMILDPELPELQS-DKPIEGLSSMVEMLNNFQVRLHSLPKGHMSQLHSDVSTLQHYLE 114  
Danio EHFQMSPEIDFGPDI-DNPIDGLSSVLSYLSYLQRLRHVPPAHLQVQVQIDLETLLRTLE 104  
Cyprinus EHFQMSPEIDFGTDI-DTPTDGLTSVHVLSYLQRLRVPPALHLQVQVQVQIDLETLLRTLE 104  
Epinephelus TRLPAAPQIEPSTPS----IDGLTSITQDLGLLDNELQNPVTELLSQIQADVSSLEGRVR 106  
. :. \*: : :. : :. .

Oryzias EWS-DKHCGEQ-----PSTQAENQTS--RRFSITESMQAVTRLKHFFLLLLQNSDQLEI 154  
Oreochromis LWR-QGHCSEQRPKPEVPGPLQELQSH--KEFIQTVGIEALMRVKEFLNLLLKNLDQLET 160  
Taki fugu HWR-EGNCQEQ-----QPKVWPRR--NIFNHTVSLEALMRVREFLKLQKNVDLLER 151  
Salmo NWM-ISRCPGRQKQKTGEGRLSEALKDTRRKYGLSVGPVALNRLKGYLGRLLLNLDQLNY 169  
Oncorhynchus NWM-TSRCPARQKQKTGEGGLSEALKDTRRKYGLSVGPVALNRLKGYLGRLLLNLDQLNY 169  
Salvelinus NWM-MSRCPGRQKQKTGEGGLEEALKDSVRKFGLSVCPVALNRLKGYLDRLLLNLDQLRY 174  
Ctenopharyngodon DRMTFMRCTLKEP---ANGKSLDTFIEKNATHHITFGYMALDRLKQFMQKLIANLDQLKS 172  
Hypophthalmichthy VWMTFMRCTPKEP---ANGKSLDTFIQKNATHHVTFGYMALDRLKQFMQKLIANLDQVKS 171  
Carassius ERTTSMHCILKEP---ANGRSLDAFLEENATHHITLGYLALDRLKQFMQKLIVNLDQLKS 170  
Tachysurus DRMSSLQCTHRIT---GTEKNLEDFPKNHSMYYITVRHVALDRLQKYIQRNHNLEQLRT 171  
Danio ELAVSQGCPLPNP-----ETPVHKEETAFFVTSNYLHLLLELQRFLEKLCNIDKLY 156  
Cyprinus GLAVSQGCPLPNP-----ETPVHKDETAFFVTSNYLYLLELQRYLEKLCNMDKLY 156  
Epinephelus SFALTMDCPLQAR-----PSGATSDSVFPDSQLHLTLTKVQRYLEKLFILHKDKLV 157  
\* . : : :. : :. .

Oryzias C----- 155  
Oreochromis C----- 161  
Taki fugu C----- 152  
Salmo CY----- 171  
Oncorhynchus CY----- 171  
Salvelinus C----- 175  
Ctenopharyngodon C----- 173

Hypophthalmichthys	C-----	172
Carassius	C-----	171
Tachysurus	C-----	172
Danio	CKDTDVAETFIL	168
Cyprinus	CKDTDVAETFIL	168
Epinephelus	C-----	158
	*	

**Supplementary Figure 19.** ClustalW alignment of *Danio rerio* LEPb protein sequence against *Cyprinus carpio*, *Epinephelus coioides*, *Ctenopharyngodon idella*, *Salmo salar*, *Oncorhynchus mykiss*, *Oryzias latipes*, *Takifugu rubripes*, *Carassius auratus*, *Salvelinus alpinus*, *Tachysurus fulvidraco* and *Hypophthalmichthys molitrix* LEP sequences. (\*) identical sequence and (:) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 24.95% sequence identity with the fish sequences.

Homo	-----
Pongo	-----
Pan	-----
Gorilla	-----
Nomascus	-----
Macaca	-----
Crab-eating	-----
Sus	-----
Panholops	-----
Mustela	-----
Canis	-----
Felis	-----
Mus	-----
Rattus	-----
Callithrix	-----
Heterocephalus	-----
Chinchilla	MRHRVLHPIRSSLPSLECCAGTCPAVAQVALWSYKRGWQAWSSTGILLPATARPKKLILR 60
Danio	-----

Homo --MHWGTLCGFLWLWPYLFYVQAVPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVT 58  
Pongo --MHWGTLCGFLWLWPYLFYVQAVPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVT 58  
Pan --MHWGTLCGFLWLWPYLFYVQAVPIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVT 58  
Gorilla --MHWGTLCGFLWLWPYLFYIQAAPVIQKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVT 58  
Nomascus --MHWGTLCGLLWLWPYLFYVQAVPIQKVQDDTKTLIKTIVTRINDISHMQSVSSKQKVT 58  
Macaca --MYWRTLWGFLWLWPYLFYIQAAPVIQKVQSDTKTLIKTIVTRINDISHTQSVSSKQKVT 58  
Crab-eating --MYWRTLWGFLWLWPYLFYIQAAPVIQKVQSDTKTLIKTIVTRINDISHT-SVSSKQKVT 57  
Sus --MRCGFLCRFLWLWPYLSYVEAVPIWRVQDDTKTLIKTIVTRINDISHMQSVSSKQKVT 58  
Pantholops --MRCGPLYRFLWLWPYLSYVEAVPIRRVQDDTKTLIKTIVTRINDISHTQSVSSKQKVT 58  
Mustela --MRCGFLCRFLWLWPYLSYVEAVPIRKVQDDTKTLIKTIVTRINDISHT-AVSSKQKVA 57  
Canis --MRCGFLCRFLWLWPYLSYVEAVPIRKVQDDTKTLIKTIVARINDISHTQSVSSKQKVA 58  
Felis --MLCGFLCRFLWLWPYLSYVEAVPIRKVQDDTKTLIKTIVTRINDISHTQSVSSKQKVA 58  
Mus --MCWRPLCRFLWLWSYLSYVQAVPIQKVQDDTKTLIKTIVTRINDISHTQSVSAKQKVT 58  
Rattus --MCWRPLCRFLWLWSYLSYVQAVPIHKVQDDTKTLIKTIVTRINDISHTQSVSARQKVT 58  
Callithrix --MRWGCLCRFLWLWACLSTYQAVPIQRVQDDTKTLIKTIIARINDLSHTQSVSPRQKVT 58  
Heterocephalus --MRCGFLCLFLWLWLYLSYIQAAPVIQKVQDDTKTLIKTIVSRINDISHMQSVSSKHKVT 58  
Chinchilla RKMRCGFLCQFLWLWCLSYIQAAPVIQKVQDDAKTLVKTIISRINDISHMQSVSSKHKVS 120  
Danio ----MKSSMIFCLLISSLVAVSISRPTAPEDRIRIIARTTISRICKIKDEHFQMSP---- 52

: \* \* . :. : : \* : \*\* . . . . .

Homo GLDFIPGLHPILTLKMDQTLAVYQQILTSMPSPR-NVIQISNDLENLRDLLHVLAFSKSC 117  
Pongo GLDFIPGLHPILTLKMDQTLAVYQQILTSMPSPR-NVIQISNDLENLRDLLHVLAFSKSC 117  
Pan GLDFIPGLHPILTLKMDQTLAVYQQILTSMPSPR-NMIQISNDLENLRDLLHVLAFSKSC 117  
Gorilla GLDFIPGLHPILTLKMDQTLAVYQQILTSMPSPR-NMIQISNDLENLRDLLHVLAFSKSC 117  
Nomascus GLDFIPGLHPVLTLSKMDQILAVYQQILTSLSR-NVIQISNDLENLRDLLHVLAFSKSC 117  
Macaca GLDFIPGLHPVLTLSQMDQTLAIYQQILINLPSR-NVIQISNDLENLRDLLHLLAFSKSC 117  
Crab-eating GLDFIPGLHPVLTLSQMDQTLAVYQQILINLPSR-NVIQISNDLENLRDLLHLLAFSKSC 116  
Sus GLDFIPGLHPVLSLSKMDQTLAIYQQILTSLSR-NVIQISNDLENLRDLLHLLASSKSC 117  
Pantholops GLDFIPGLHPVLSLSKMDQTLAIYQQILTSLSR-NVIQISNDLENLRDLLHLLAASKSC 117  
Mustela GLDFIPGLHPVLSLSRMDQTLAIYQQILTSLSR-NVIQISNDLENLRDLLRLLASSKSC 116  
Canis GLDFIPGLQPVLSLSRMDQTLAIYQQILNSLSR-NVVQISNDLENLRDLLHLLASSKSC 117  
Felis GLDFIPGLHPVLSLSKMDQTLAIYQQILTGLPSR-NVVQISNDLENLRDLLHLLASSKNC 117  
Mus GLDFIPGLHPILSLSKMDQTLAVYQQVLTSLPSQ-NVLQIANDLENLRDLLHLLAFSKSC 117

```

Rattus          GLDFIPGLHPILSLSKMDQTLAVYQQIILTSLSLPSQ-NVLQIAHDLENLRDLLHLLAFSKSC 117
Callithrix     GLEFIPGFHSDLSFSKMDDEILATYQQIVISLPSG-NMIQISNDLENLRALLHLLAASKSC 117
Heterocephalus GLDFIP-LYPDLSFSKMDQTLAVYQQIISNLPSSRRNMVQISNDLDNLQNLVHMLASSKGC 117
Chinchilla     GFDFIP-RNPDLNFLKMDKTLAVYQEIINLSSR-NTGQISNDLENLRNLIRMLASSKGC 178
Danio          EIDFGPDIDNPIDGLSSVLSYLSYLQLRLHVPQAHLQQVQIDLETLLRTLEELAVSQGC 112
                :::* *      :          * ::  : .  :  *:  **:. *  :.  ** *:. *

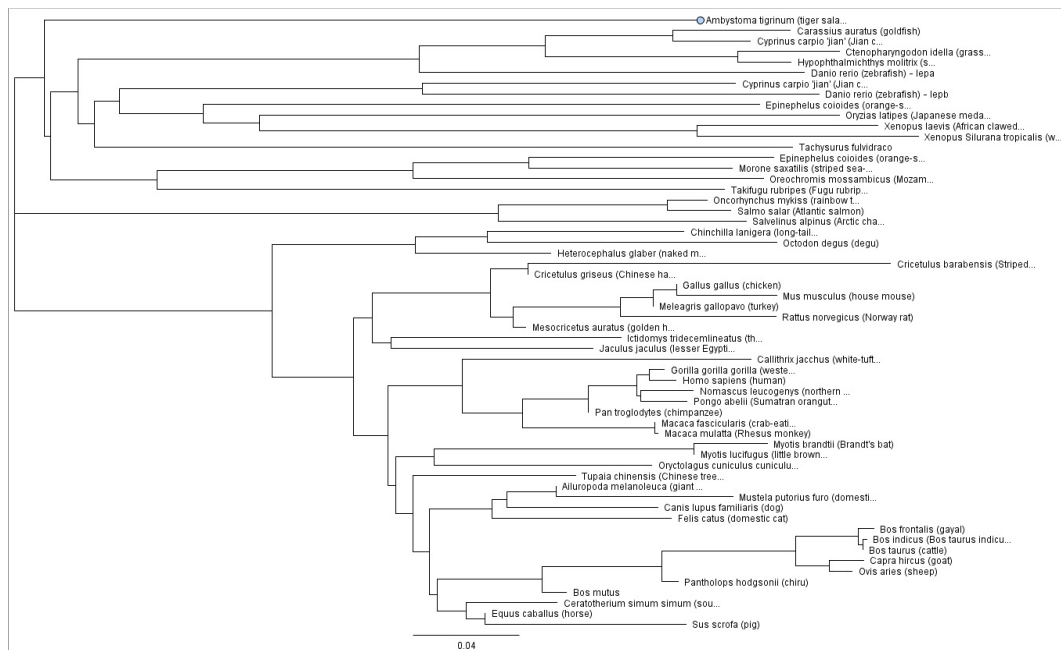
Homo           HLPWASGLETLDLGGVLEASGYSTEVALSRLQGSLQDMLWQLDLSPGC----- 167
Pongo          HLPWASGLETLDLGGVLEASGYSTEVALSRLQRLQDMLWQLDLSPGC----- 167
Pan            HLPWASGLETLDLGGVLEASGYSTEVALSRLQGSLQDMLWQLDLSPGC----- 167
Gorilla        HLPWASGLETLDLGGVLEASGYSTEVALSRLQGSLQDMLWQLDLSPGC----- 167
Nomascus       HLPWASGMETLASLGGVLEASGYSMEEVALSRLQGSLQDMLWQLDLSPGC----- 167
Macaca         HLPWASGLETLES LGDVLEASLYSTEVALSRLQGSLQDMLWQLDLSPGC----- 167
Crab-eating    HLPWASGLETLES LGDVLEASLYSTEVALSRLQGSLQDMLWQLDLSPGC----- 166
Sus            PLPQARALETLES LGGVLEASLYSTEVALSRLQALQDMLRQLDLSPGC----- 167
Pantholops     PLPQFRALESLES LGVVLEASLYSTEVALSRLQGSLQDMLRQLDLSPGC----- 167
Mustela        PLPRARGLESFESLGGILEASLYSTEVALSRLQAALQDMLGRDLSPGC----- 166
Canis          PLPRARGLETLES LGGVLEASLYSTEVALNRLQAALQDMLRRLDLSPGC----- 167
Felis          PLPRARGLETLES LGGALEASLYSTEVALSRLQASLQDMLWRDLSPGC----- 167
Mus            SLPQTSGLQKPESLDGVLEASLYSTEVEALSRLQGSLQDILQQLDVSDPEC----- 167
Rattus         SLPQTRGLQKPESLDGVLEASLYSTEVALSRLQGSLQDILQQLDLSDPEC----- 167
Callithrix     HLPWASGLENLANLGGVLEVSLSYSTEVALSRLRGTLDKILQQDLGPAC----- 167
Heterocephalus PLPQTRGPELLENLGGALKESIYSTEVALSRLQRFLQEMLLQDLHNTMC----- 167
Chinchilla     PLPYTRGPEALRNLDGALKESVSYSTEVALSRLQRFLKEMLRQLDLGTMC----- 228
Danio          PLPNPE--TPVHKEETAFPVTSNYLHLLLELQRFLEKLCLNIDKLYCKDQDVAETFIL 168
                **          :  :  .:  *.*:  *  :  :*.

```

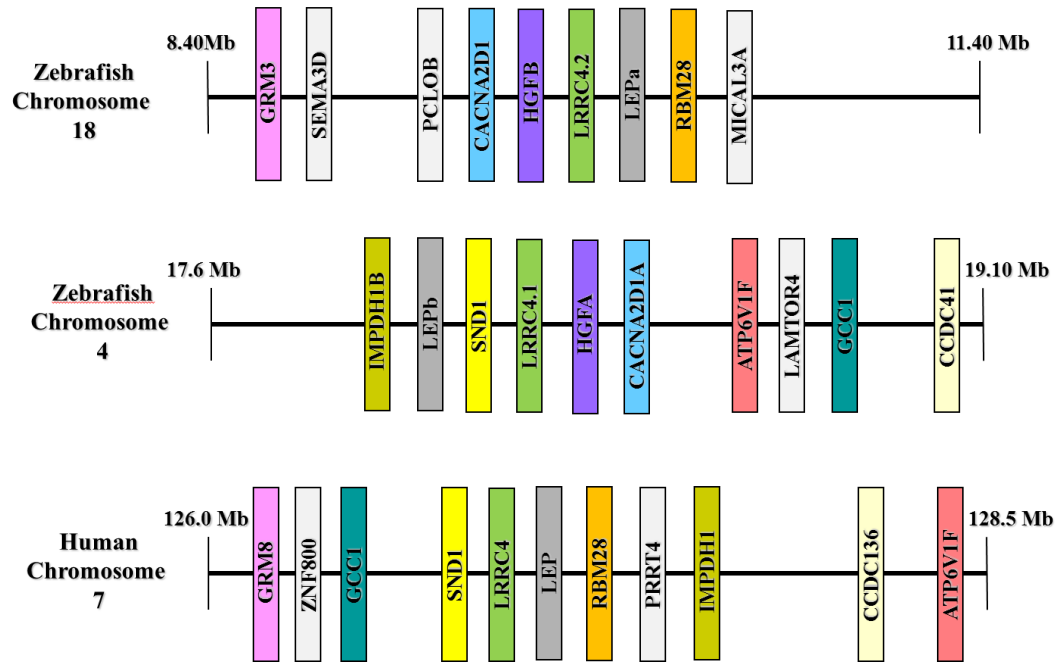
**Supplementary Figure 20.** ClustalW alignment of *Danio rerio* LEPb protein sequence against *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Sus scrofa*, *Mustela putorius furo*, *Pan troglodytes*, *Macaca mulatta*, *Canis lupus familiaris*, *Heterocephalus glaber*, *Callithrix jacchus*, *Macaca fascicularis*, *Nomascus leucogenys*, *Gorilla gorilla gorilla*, *Pantholops hodgsonii*, *Pongo abelii*, *Felis catus*, *Chinchilla lanigera*, *Mus musculus*, *Rattus norvegicus*, *Sus scrofa*, *Mustela putorius furo*, *Pan troglodytes*, *Macaca mulatta*, *Canis lupus familiaris*, *Heterocephalus glaber*, *Callithrix jacchus*, *Macaca fascicularis*, *Nomascus leucogenys*, *Gorilla gorilla gorilla*, *Pantholops hodgsonii*, *Pongo abelii*, *Felis*

*catus* and *Chinchilla lanigera* LEP sequences. (\*) identical sequence and (:) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 19.30% sequence identity with these species and 19.30% similarity to *Homo sapiens*.

Phylogenetic analysis of the zebrafish LEPa & LEPb protein sequences with known vertebrate LEP genes grouped the gene with known fish and amphibian LEP proteins (**Supplementary Fig. 21**). Analysis of the regions within the zebrafish genome around the predicted LEPa gene on chromosome 18 and LEPb gene on chromosome 4, allowed gene maps to be constructed showing there was conservation of synteny between the human and zebrafish genomes (**Supplementary Fig. 22**), providing evidence these gene were homologues of the human LEP gene.



**Supplementary Figure 21.** Phylogenetic tree, constructed using the neighbour joining method alignment, showing the relationship between zebrafish LEP genes and known vertebrate LEP sequences.



**Supplementary Figure 22.** Comparison of the co-localization of genes surrounding LEP on Human chromosome 7 and Zebrafish chromosomes 18 (LEPa) and 4 (LEPb).

## PYY

Searching the available genome using TBLASTN with a known fish PYY protein, identified a region of chromosome 3 that contained a potential zebrafish PYY sequence. Using gene prediction software with this region provided a nucleotide sequence which was translated and the predicted protein was aligned against known PYY protein sequences already identified in other fish (**Supplementary Fig. 23**) and in selected mammals (**Supplementary Fig. 24**).

```

Ctenopharyngodon -----MAVMLKPWT 9
Carassius -----MAVMMKPWT 9
Danio -----MAVMLKPWT 9
Silurus -----MSAALKSWT 9
Maylandia -----MVVILKPWT 9
Pundamilia -----MVVILKPWT 9
Xiphophorus -----MAAMLKPWT 9
Salmo -----MAMILKPWT 9
Anguilla -----MAVVLKPWT 9
Latimeria -----MVASMKPWT 9
Acipenser -----MVSCLKPWS 9
Astyanax -----MSSSMRSWT 9
Oreochromis MWSFLLYQPLVIKNGITPHPSPHGTSCIKHGQCRESDRRALNSRKSNYSRTKVTCNAVM 60
Dicentrarchus -----MIHSGAVM 8
Paralichthys -----MIRSSTVM 8

```

```

Ctenopharyngodon VIATLLFCVLLCLGTFVDAYPPKPENPGDDAPPEELAKYYTALRHYINLITRQRYGKRST 69
Carassius VIATLLFCVLLCLGTFVDAYPPKPENPGDDAPPEELAKYYTALRHYINLITRQRYGKRAT 69
Danio VVATVLCVLLCLGTFVDAYPPKPENPGDDAAPEELAKYYTALRHYINLITRQRYGKRST 69
Silurus VVFSLFCVLLYLGTVDAYPLKPENPGEDAAPEELAKYYTALRHYINLITRQRYGKRST 69
Maylandia VLVALVLCVLCVCLGTLADAYPPKPENPGEDAPPEELAKYYTALRHYINLITRQRYGKRSA 69
Pundamilia VLVALVLCVLCVCLGTLADAYPPKPENPGEDAPPEELAKYYTALRHYINLITRQRYGKRSA 69
Xiphophorus MLVAAVLCVLCVCLGALADAYPPKPENPGEDAPPEELAKYYTALRHYINLITRQRYGKRSA 69

```

Salmo	VLVALVLCVLVCLGTFVDAYPPKPENPGEDAPPEELAKYYTALRHYINLITRQRYGKRSA	69
Anguilla	VLVALVLCLLVCLGTFVDAYPPKPENPGEDASPEEQAKYYTALRHYINLITRQRYGKRSS	69
Latimeria	TMVAIIFCVLVCLGTFVDAYPPKPENPGEDAPPEELAKYYTALRHYINLITRQRYGKRSS	69
Acipenser	VMLALVLCVLVCLGTFVDAFPPKPEHPGDDAPAEDVAKYYTALRHYINLITRQRYGKRSN	69
Astyanax	ALLALVLCVLVCLSSLAAYPPKPEPPAGDVGPEEMAKYHTALRHYINLITRQRYGKRSG	69
Oreochromis	SLSILALCLLVCIHSGINAYPAKPASPRQGAPPEELAKYYTALRHYINLITRQRYGKRDS	120
Dicentrarchus	SMSILAFCLLACIHSGINAYPAKPASPRDGAPPEELAKYYTALRHYINLITRQRYGKRDT	68
Paralichthys	SPSVLALCLLACIHSGINAYPVKPTIPREGATPEDLAKYYTALRHYINLITRQRYGKRDT	68

:\*:\* : : ::\* \*\* \* .. .\*: \*\*\*:\*\*\*\*\*

Ctenopharyngodon	SEDVMAELLFGDDTEHKQRS---RYDDSFMW	97
Carassius	SEDVMAELLFGDDTEHKQRS---RYDDSYLW	97
Danio	SEDVMAELLFGDDTEHKQRS---RYDDSFMW	97
Silurus	QEGSLADLLFGDNNEHNQRS---RYDELYMW	97
Maylandia	QEDVVAELLFGGDNNRDQRS---RYDDSYMW	97
Pundamilia	QEDVVAELLFGGDNNRDQRS---RYDDSYMW	97
Xiphophorus	QEDVVEELLFGGDSSRDQRS---RYDDSFMW	97
Salmo	QEGVMSELLFGNNAERDQRS---RYDDSYMW	97
Anguilla	PEGVMSELLFGDNSEHNQRS---RYDDSYMW	97
Latimeria	PEAMISDLLFGDNSDHNGRS---RFEDSYMW	97
Acipenser	PDSLISELLFGDNSENNARS---RYDDSYVW	97
Astyanax	PEAAMAELLFGDD-EQDVRP---RADDRLAW	96
Oreochromis	PDTVFSVDLVRESTESVPGSSYIRYDGLPLW	151
Dicentrarchus	PDTVFSVDVLMRESTESIPGSNYVRYDGLPLW	99
Paralichthys	PDTVFSVDLVRESAENIPGYNYIRYDGSPLW	99

: . ::\* . . \* : \*

**Supplementary Figure 23.** ClustalW alignment of *Danio rerio* PYY protein sequence against *Salmo salar*, *Ctenopharyngodon idella*, *Oreochromis niloticus*, *Dicentrarchus labrax*, *Maylandia zebra*, *Pundamilia nyererei*, *Paralichthys olivaceus*, *Xiphophorus maculatus*, *Carassius auratus*, *Anguilla japonica*, *Latimeria chalumnae*, *Silurus meridionalis*, *Astyanax mexicanus* and *Acipenser sinensis* PYY sequences. (\*) identical sequence and (:) highly similar sequence or (.) similar sequences are indicated. The zebrafish sequence has an average 71.21% sequence identity with the fish sequences.

Pan -----  
 Homo -----  
 Gorilla -----  
 Pygmy -----  
 Nomascus -----  
 Crab-eating -----  
 Macaca -----  
 Papio -----  
 Saimiri -----  
 Odobenus -----  
 Canis -----  
 Mustela MPILERSLNLGIAPGHRYLSPRFPEGPRTCGRDISPAGKALSRRGRHSPTCSSPALGTF 60  
 Dasypus -----  
 Pantholops -----  
 Bos -----  
 Sus -----  
 Rattus -----  
 Mus -----  
 Heterocephalus -----MG 2  
 Monodelphis -----  
 Danio -----

Pan -----MVFVRRPWPALTTVLLALLVCLGALVDAYPIKPEAPGEDASPEELNR 47  
 Homo -----MVFVRRPWPALTTVLLALLVCLGALVDAYPIKPEAPGEDASPEELNR 47  
 Gorilla -----MVFVRRPWPALTTVLLALLVCLGALVDAYPIKPEAPGEDASPEELNR 47  
 Pygmy -----MVFVRRPWPALTTVLLALLVCLGALVDAYPIKPEAPGEDASPEELNR 47  
 Nomascus -----MVFVRRPWPALATVLLALLVCLGALVDAYPIKPEAPGEDASPEELSR 47  
 Crab-eating -----MVLVRRPWPALATVILALLVCLGALVDAYPIKPEAPGEDASPEELSR 47  
 Macaca -----MVLVRRPWPALATVILALLVCLGALVDAYPIKPEAPGEDASPEELSR 47  
 Papio -----MVLVRRPWPALATVILALLVCLGALVDAYPIKPEAPGEDASPEELSR 47  
 Saimiri -----MVLVRRPWLALVTVLLALLVCLGALVDAYPIKPEAPGEDASPEELSR 47  
 Odobenus -----MVTVRRPWPAMATVLLALLVCLGALVHAYPAKPEAPGEDASPEELSR 47  
 Canis -----MVTVRRPWPAVATVLLALLVCLGALVHAYPAKPEAPGEDASPEELSR 47

Mustela AGHLLIPASGAVAMVTVRRPWPAVATVLLALLACLGLVQAYPSKPEAPGEDASPEELSR 120  
 Dasypus -----MVTARRPWPAMAAARLLALLACLGLVDAYPAPKEAPGEDASPEDLNR 47  
 Pantholops -----MMAGRRSWPAMATVLLTLLVCLGELVDAYPAPKQAPGEHASPD-VNR 46  
 Bos -----MMSGRRSWPAMATVLLTLLVCLGELVDAYPAPKQAPGEHASPELNR 47  
 Sus -----MVTVRRPWPAMATVLLGLLICLGLTVDAYPAPKEAPGEDASPEELSR 47  
 Rattus -----MVAVRRPWPVMVAMLLVLLACLGLVDAYPAPKEAPGEDASPEELSR 47  
 Mus -----MVAVRRPWPVTVAMLLILLACLGLVDAYPAPKEAPGEDASPEELSR 47  
 Heterocephalus KLRPGRHAPRPQMEPMRRSRPAVAVLLALLAGLGLVDAYPIKPEAPGSDASPEELSR 62  
 Monodelphis -----MTILRPWTMSAAVLLALLACLGLVEGYPSKPEAPSADASPEELSR 46  
 Danio -----MAVMLKWPVTVATVLLICVLLCLGTFVDAYPPKPENPGDDAAPEELAK 47

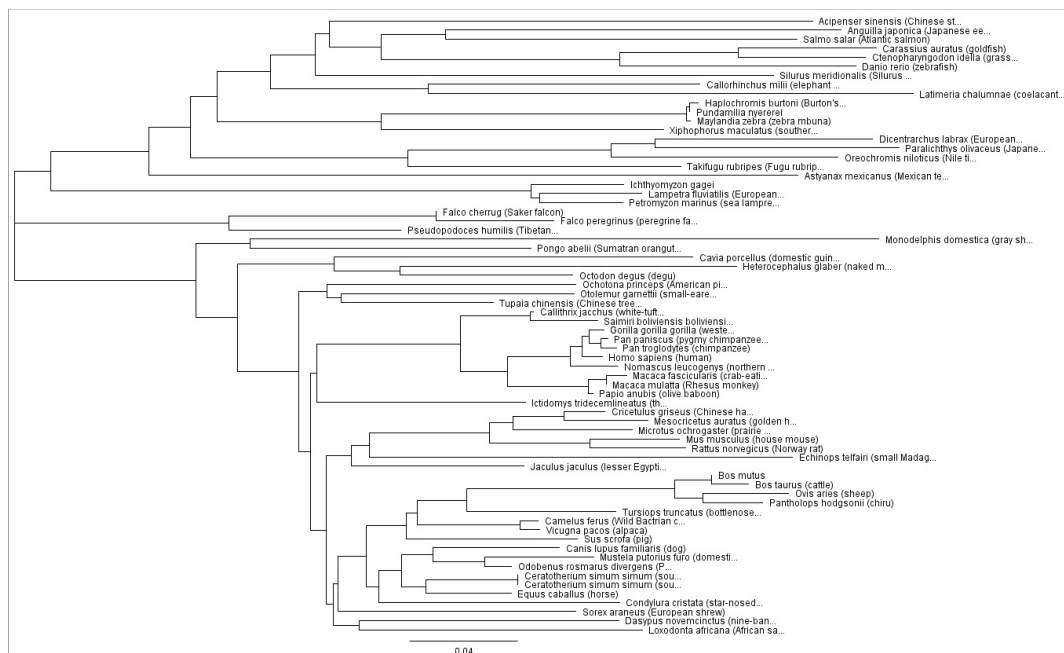
:. .: :: :\* \*\* :\*.\*\* \*\*: \*. .\*:\*: : :

Pan YYASLRHYLNLVTRQRYGKRDRPDPLLSKSFPPDGE---DRPVRSRSEGPDLW 97  
 Homo YYASLRHYLNLVTRQRYGKRDPDRLLSKTFFPDGE---DRPVRSRSEGPDLW 97  
 Gorilla YYASLRHYLNLVTRQRYGKRDPDTLLSKTFFPDGE---DRPVRSRSEGPDLW 97  
 Pygmy YYASLRHYLNLVTRQRYGKRDPDTLLSKTFFPDGE---DRPVRSRSEGPDLW 97  
 Nomascus YYASLRHYLNLITRQRYGKRDPDTLLSKTFFPDGE---DRPVRSRSEGPDLW 97  
 Crab-eating YYASLRHYLNLVTRQRYGKRDPDALLSKTFFPDGE---NRPIRSRSEVPDLW 97  
 Macaca YYASLRHYLNLVTRQRYGKRDPDALLSKTFFPDGE---NRPIRSRSEVPDLW 97  
 Papio YYASLRHYLNLVTRQRYGKRDPDALLSKTFFPDGE---NRPIRSRSEVPDLW 97  
 Saimiri YYASLRHYLNLVTRQRYGKRDSPEALLAKTFFPDGE---DRHVRPRSEGADQW 97  
 Odobenus YYASLRHYLNLVTRQRYGKRDSPEAVLSKLLFPDGE---DRRVKSRPEGAYMW 97  
 Canis YYASLRHYLNLVTRQRYGKRDSPEAVLSKLLFPDGE---DRRAKSWPGGEYMW 97  
 Mustela YYASLRHYLNLVTRQRYGKRDSPEAVLAKLLFPDDE---DRRVKTRPEDPYMW 170  
 Dasypus YYASLRHYLNLVTRQRYGKRDSAEAEPLKLPFPDGE---DRPVRSRSEGAYMW 97  
 Pantholops YYTSLRHYLNLVTRQRFGRDFSEALLSILLFPDRE---DRPVKSRPEGAYLW 96  
 Bos YYTSLRHYLNLVTRQRFGRDFSEALLSILLFPDRE---DPPVKSREPEGAYLW 97  
 Sus YYASLRHYLNLVTRQRYGKRDSPDALLSKLLFPEGE---DRPVKSWPEGAYLW 97  
 Rattus YYASLRHYLNLVTRQRYGKREVPAAALFSKLLFTDDS--ENLPFRSRPEGVDQW 98  
 Mus YYASLRHYLNLVTRQRYGKRDPAAALFSKLLFTDDSSENLPFR--PEGLDQW 98  
 Heterocephalus YYASLRHYLNLVTRQRYGKRAGPEAALPSLLSPD----- 96  
 Monodelphis YYASLRHYLNLVTRQRYGKRDSPEAGLAEKLPDDE--GNQNSKSGSEEPYV- 96  
 Danio YYTALRHYLNLVTRQRYGKRSTSEDMVAELLFGDDT---EHKQRSRYDDSFMW 97

\*\*\*:\*\*\*\*:\*\*\*:\*\*\*\*:\*\*\* . :. :

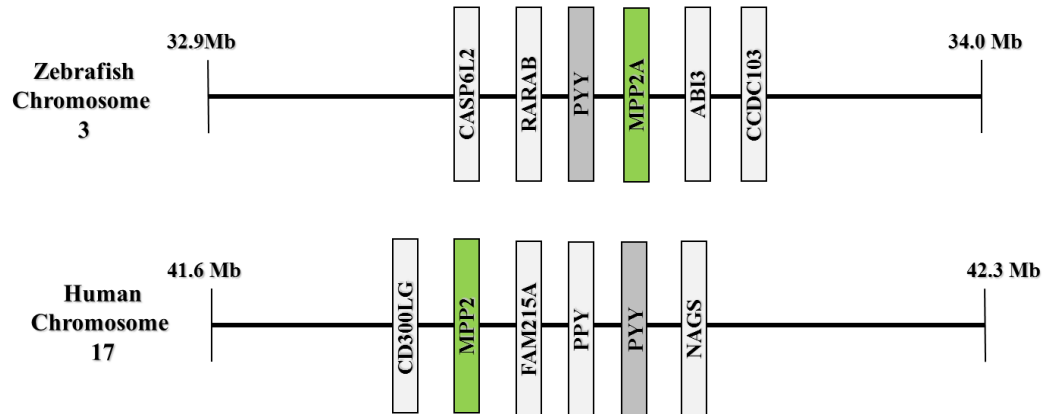
**Supplementary Figure 24.** ClustalW alignment of *Danio rerio* PYY protein sequence against *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Sus scrofa*, *Bos taurus*, *Macaca mulatta*, *Macaca fascicularis*, *Heterocephalus glaber*, *Canis lupus familiaris*, *Mustela putorius furo*, *Pan troglodytes*, *Pan paniscus*, *Gorilla gorilla gorilla*, *Papio anubis*, *Nomascus leucogenys*, *Saimiri boliviensis boliviensis*, *Panthalops hodgsonii*, *Odobenus rosmarus divergens*, *Dasyus novemcinctus* and *Monodelphis domestica* PYY sequences. (\*) identical sequence and (: ) highly similar or (.) similar sequences are indicated. The zebrafish sequence has an average 51.24% sequence identity with these species and 50.00% similarity to *Homo sapiens*.

Phylogenetic analysis of the zebrafish PYY protein sequence with known vertebrate PYY genes grouped the gene very clearly with the known fish PYY protein sequences (**Supplementary Fig. 25**). Analysis of the region within the zebrafish genome around the predicted PYY gene on chromosome 7 allowed a gene map to be constructed that showed that there was some conservation of synteny between the human and zebrafish genomes (**Supplementary Fig. 26**), giving further evidence that this gene was a homologue of the human PYY gene.



**Supplementary Figure 25.** Phylogenetic tree, constructed using the neighbour joining

method alignment, showing the relationship between zebrafish PYY and known vertebrate PYY sequences.



**Supplementary Figure 26.** Phylogenetic tree, constructed using the neighbour joining method alignment, showing the relationship between zebrafish PYY and known vertebrate PYY sequences.

## 5.2 Supplementary analysis relating to genes characterised in this investigation

### AgRP

```

Danio      -AGCCTGGGACGT-GAGCACTACAGT-----CTG----- 27
Carassius  CAGCCTTGACACAGAACCCCTGCAGTGCATACAACCTGGATCACCTAAACTGTGGCTGAAG 60
Cyprinus   -----
Salmo      --GACAAGAGCG-AGAACATTCTGAGCTGCGATATTGATATGC----- 40

Danio      -----AGTGATTATGATGCTGAACACAGTAATCTTCGGCTGGTT 66
Carassius  TTTATCTTGTTTTGGGGTTTCAATGATTATGATGCTGAACATAGCGATCATCAGCTGGTT 120
Cyprinus   -----
Salmo      -----TACCAT-ATTAAGACATTTAAAATAAAAAAATATATTCATT 81

Danio      TTT----GGTGAATGTTGTGGTGA--TGGCATCTCATCCACACCTGAGACGCAGAGAAAA 120
Carassius  TTT----GATGAATGTTCATGGCGA--TGGCATCACATCCAAACCTGAGACGTAGTGAAAA 174
Cyprinus   -----
Salmo      TCCCCAGAGTGGATAA-AAAGTAACCTGTTACAAGATTGGAAGTGGAGATGTTGGGAAAA 140

Danio      C----TCATTCATTCTGACATCTGACACAGACT----CACTGCCTGAAATGGAGCACCTT 172
Carassius  C----TCCTTTGCTCT---ATCTGACACTGACT----TACTACCTGGAGTGGAAAACCTT 223
Cyprinus   -----
Salmo      CATTTTCTCTGGGTTTGTCTCCTTTCGGTGTCTATTCCGTTATGTTTCGCCGAAGACCTG 200

Danio      GAAATAAACTCA-GCAGAAGAAAAGATACTAGAAGACCTTGAAGCCTATGATGAGGATCT 231
Carassius  GAAATAAACTCA-GTAGAAGAAAACCTGCTAGAAGAGCTTGGATCCTATGATGAGGATCT 282
Cyprinus   -----

```

```

Salmo      AAGAGGGATGCGCATATACAGCATGAAAATGATTCAGTTGGAGCCAA----GAGAAACC 256

Danio      GGGCAAAGCTGTCCACCTGCAGAGAAGAGGCACACGCTCCCCGAGC-----C 278
Carassius  GGGTAAAGCTGTACAGCTGCAGAGAAGAGGCACACGTTCCCCCAGC-----C 329
Cyprinus   -----GAACACGTTCCCCCAGC-----C 18
Salmo      CGG-AAGACTGTTTGCC-----AGGAGACGGATTTTGCCACATCAGGGACAGCACCAC 308
                * * ** * * *

Danio      GCTGTATCCCT---CATCAAC---AGTCC-TGTCTGG---GTCATCATCTG----- 319
Carassius  GCTGCATCCCT---CATCAGC---AGTCC-TGCTTGG---GTCATCACCTG----- 370
Cyprinus   G-TGCATCCCT---CATCAGC---AGTCC-TGCTTGG---GTCATCACCTG----- 58
Salmo      GTTGCATTTAGAAACATGAGCCTGCAGCCCTGCCCGCGGTGTGGTCTGTATGGAT 368
                * ** * * * ** * * ** * * **

Danio      -----CCCTGCTGCAACCCCTGCGACACCTGCTACTGCCGCTTCTTT 361
Carassius  -----CCCTGCTGCAACCCCTGTGATACGTGTACTGTCTGTTCTTT 412
Cyprinus   -----CCCTGTTGCAACCCCTGCGATACGTGTACTGTCTGTTCTTT 100
Salmo      AGCTGTGCCCTCACACCCCTGCTGTGACCCCTGTGCCTCCTGCCGCTGTGCGCTTTC 428
                ***** ** ***** * * ** *** ** *****

Danio      AAGGCCTTCTGTACTGCCGCAGCATGGACA-ACAC--CTGCAAAAACGA-ATATGCATA 417
Carassius  AAGGCCTTCTGTACTGCCGTAGCATGGATC-ACAC--CTGCAAAACATGA-GTATGCATA 468
Cyprinus   AAGGCCTTCTGTACTGCCGTAGCATGGATC-ACAC--CTGCAAAACATGATGTATGCATA 157
Salmo      AACACCATCTGCCACTGTGGAGACTGGGCCACACTGCCCAAGAA-GA-----CCTA 481
                ** ** ***** ***** * ** *** ***** * *** * ** * **

Danio      GAGACAAGTTCTGCCTTCAGATAGCAAATGGTAGCATTATATTTACAGTTTAAAGAGCTA 477
Carassius  GAGCCTATTTCTACACTGAAATAACTGATTTGTGTCTTTACATTTACAGTTT-GTAACTC 526
Cyprinus   GAGCCAAATTTCTACCCTGAAATGACTAATTTGTGGCTTTACATTTACAGTTT-ACAGCTC 216
Salmo      GA--CAAAC---ACACACACACA-----CACACACACA-----CAC 512
                ** * * * * * * * * * * * * * * *

Danio      CTGAATGTATTTGTGGACAGTGCAATCATAAACTCGCACAGAGAATCCACAGAGAAGTTT 537
Carassius  TAGAATGTATATGTGGATAGTGTAAATATACAGCCTCACACT--ATCCATAGGGAAGT-- 582
Cyprinus   TAGAATGTATATGTGGATAGTGTAAATATACAGCCTCACACT--ATCCACAGGGAAGTTT 274
Salmo      ACACACACACACACACAAA-ACAAATACACACACACACA-----CACAGAGAG---- 562
                * * * * * * * * * * * * * * *

Danio      TT-----TGTT--ATAATAGAGAAGCACAAAGCATA 566
Carassius  -T-----TGTT--ACAATATA-----ACAAA---TA 602
Cyprinus   GTAACAATATAACAAATATAATGGTTAATAATGTTTAAATAATAAAGCTGCACAAAGCATA 334
Salmo      -----AGAACAATACACACACAG-AGA 584
                * * * * * * * * * *

Danio      --GAGTGCACC-----ATTG---GCTGCGGTTTTAAGGTGTAAT 600
Carassius  --TAATG-----GTTG---ATAATG--TTTAA---TAAT 626
Cyprinus   AAGAATGCACCTTGGCTCTTAAGATTTTACATTTGTATTGCTGCAATTTTATGGTGAAT 394
Salmo      GAGAACA-----AATACAACACACAGAG-AGA 611
                * * * * *

Danio      TTAATTGCAATATTTTACTCAAATGTTG-TCTTAAATATTTTGTATATAT---GCAT 655
Carassius  AAAGCTGCA-----CAAA-----GCAT 643
Cyprinus   TTATTGCAATTTTAATG--TCAAAATCTACTGTCATATATTGTATATATATATAGGCAT 452
Salmo      ACAAATACA-----CACA-----CAC 627
                * ** * * * ** * **

Danio      TCAATAAACTCTGATTATTC----- 676
Carassius  -----AAA---GAGTG----- 651
Cyprinus   TCAATAAAATCTAATTATGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 499
Salmo      ACAAAAAA-----AAAAAAAAAAAAAAAAAAAAAAAAAAAA-- 659
                ***

```

**Supplementary Figure 27.** ClustalW pairwise alignment of *Danio rerio* AgRP nucleotide sequence against *Carassius auratus*, *Salmo salar*, and *Cyprinus carpio* AgRP sequences. (\*) identical nucleotide sequences are indicated. The zebrafish sequence has an average 59.80% sequence identity with the fish sequences.

chimpanzee AGCTCCTAGGTCCCTGTCCTGTGGAAATTTGTGGACCCTGGGCACCCTCTCTTGCTCCCA 60  
 bonobo AGCTCCTAGGTCCCTGTCCTGTGGAAATTTGTGGACCCTGGGCACCCTCTCTTGCTCCCA 60  
 macaca -GCTCCTAGGTCCCTGTCCCATGGACATTTGGGGACCCTGGGCACCCTCTCTGGCTCCCA 59  
 homo -----CCTGTCCTGTGGAAATTTGTGGACCCTGGGCACCCTCTCTTGCTCCCA 48  
 mustela -----  
 rattus -----CAGCCTGAAAGCTTT-----T TACTCTGA 24  
 microtus -----AGACGGGTTGAAAGCTTT-----T TACTC-AA 26  
 heterocephalus -----  
 chinchilla -----  
 mus -----A 1  
 Danio -----AGCCTGGGACGTGA-----GCACTAC 21

chimpanzee AATTTTAATCGGCTCCTGGAAACCTCACCCCAAATTTGGAGATAG-GCACTCCTCTTGTAG 119  
 bonobo AATTTTAATCGGCTCCTGGAAACCTCACCCCAAATTTGGAGATAG-GCACTCCTCTTGTAG 119  
 macaca AATTTTAATCGGCTCCTGGAAACCTCACCCCAAATTTGGAGATAG-GCACTCCTCTTGTAG 118  
 homo AATTTTAATCGGCTCCTGGAAACCTCACCCCAAATTTGGAGATAG-GCACTCCTCTTGTAG 107  
 mustela -----  
 rattus AGCT-----GAATGCCACCC-----TGGGAACAGTGTGTTCTGCTCT--- 62  
 microtus AGCT-----GTGTGCCACCC-----TGGGAACAGTGTGTTCTGCTCT--- 64  
 heterocephalus -----  
 chinchilla -----  
 mus GACT-----ATACAG----- 11  
 Danio AGTCT-----GAGTGATTATG--ATGCTGAACACAGTAATCTTCG----- 59

chimpanzee AACAAAAGGCTCAGGTTTCAGGGAGTGAGGGCCTGAACTGTGCCCCACCCTCCAGGAAGG 179  
 bonobo AACAAAAGGCTCAGGTTTCAGGGAGTGAGGGCCTGAACTGTGCCCCACCCTCCAGGAAGG 179  
 macaca AACAAAAGGCTCAGGTTTCAGGGAGTGAGGGTCTGAACTGTGCCCCACCCTCCAGGAAGG 178  
 homo AACAAAAGGCTCAGGTTTCAGGGAGTGAGGGCCTGAACTGTGCCCCACCCTCCAGGAAGG 167  
 mustela -----  
 rattus -----CATGGTTTCAGGAA-----CCCAAGGGAGG 88  
 microtus -----CTGGTTTACAGGAG-----CCCTAGGGAGG 90  
 heterocephalus -----  
 chinchilla -----  
 mus -----GAATT--GGGA-----CTTCTGGGAGC 32  
 Danio -----GCTGGTTTTGGTGAATG-----TTGTGGTGTAG 88

chimpanzee G----TCCTTCACGGCCTGGCTGCAGGGATCAGTCACGTGT-GGCCCTTCATTAGGCCCT 234  
 bonobo G----TCCTTCACGGCCTGGCTGCAGGGATCAGTCACGTGT-GGCCCTTCATTAGGCCCT 234  
 macaca G----CCCTTCACAGCCTGGCTGCAGGGATCAGTCACGTGT-GGCCCTTTATTAGGCCCT 233  
 homo G----TCCTTCACGGCCTGGCTGCAGGGATCAGTCACGTGT-GGCCCTTCATTAGGCCCT 222  
 mustela -----  
 rattus -----CCCCTCATGCCCTAGTACAGGAAGTAGTCACGTGTGGGCCCTTTATTAGACCCT 143  
 microtus G----CCCTTCAGGCCCTGGCTGCAGAAATCAGTCACGTGCAGGCCCTTTATTAGACTCT 146  
 heterocephalus -----CATTAGACCCT 11  
 chinchilla -----  
 mus A----TCTCTCTCAGC--GCTGGTAGGGT-----ACCT 60  
 Danio GCATCTCATCCACCTGAGACGCAGAGAAAACCTCATTATC--TCTGACATCTGACACA 145

chimpanzee GCCATATAAGCCAAGGGCACGGGTGGCCGGAACTCTCTAGGCAAGAATCCCGGAGGCA 294  
 bonobo GCCATATAAGCCAAGGGCACGGGTGGCCGGAACTCTCTAGGCAAGAATCCCGGAGGCA 294  
 macaca GCCATATAAGCCGAGGGCACGGGTGGCCAGGAATCTCTAGGCAAGAATCCCGGAGGCA 293  
 homo GCCATATAAGCCAAGGGCACGGGTGGCCGGAACTCTCTAGGCAAGAATCCCGGAGGCA 282  
 mustela -----GCAAG-----CA 7  
 rattus GCCATATAAGCTCAGGGCACAG-TGGCCAGGAACTCCTTAGGCAAGGATCA-ACAAGCA 201  
 microtus GCCATATAAGCTCCAGGCACAAG-TGGCCAGGAACTCCCGGACGAGGATCA-GCAAGCA 204  
 heterocephalus ACCATATAAG-TGAGGGCACAGG-TAGCCAGGAACTCGCTAGGCAAGGA-CGAGCAAGCA 68  
 chinchilla -----  
 mus A-----AGGATGAGG--AGAGACTAAATGGGGTTTCTCTGCTGAGC-----CA 102  
 Danio GACTCACTGCCTGAA--ATGGAGCACCTTGAATAAACTCAGCAGAAGAAAAGATACTA 202

chimpanzee GAGG----- 298  
 bonobo GAGG----- 298  
 macaca GAGG----- 297  
 homo GAGGTGAGTCCTCAGGTTGGGCAGGGACTCCTCCTCTCTGTGGGTCTCTATCTGGGCAC 342  
 mustela GAGG----- 11  
 rattus AAGG----- 205  
 microtus AAGG----- 208  
 heterocephalus GAGG----- 72  
 chinchilla -----  
 mus GG----- 104  
 Danio GAAG----- 206

chimpanzee -----C 299  
 bonobo -----C 299  
 macaca -----C 298  
 homo CTAGAGGGGACTCCAAGGATAAGGAGGGACTAAGTGGTACATCTTCCTGCTGAGCCAGGC 402  
 mustela -----T 12  
 rattus -----C 206  
 microtus -----C 209  
 heterocephalus -----C 73  
 chinchilla -----GC 2  
 mus -----C 105  
 Danio -----AC 208

chimpanzee CATGCTGACCCGACGC-----GTG--CTGAGCTGTGC-CCTGCTGCT---GGCAC-TGC 346  
 bonobo CATGCTGACCCGACGC-----GTG--CTGAGCTGTGC-CCTGCTGCT---GGCAC-TGC 346  
 macaca CATGCTGACTGCAGCA-----CTG--CTGAGCTGTGC-CCTGCTGCT---GGCAC-TGC 345  
 homo CATGCTGACCCGACGC-----GTG--CTGAGCTGTGC-CCTGCTGCT---GGCAC-TGC 449  
 mustela CATGCTGCCTGCAGTG-----CTG--CTGAGCTGTGC-CCTGCTGCT---GGCAC-TGC 59  
 rattus CATGCTGACTGCAATG-----TTG--CTGAGCTGTGT-CCTGCTGCT---GGCAC-TGC 253  
 microtus CATGCTGATGGTAATG-----CTG--CTGAGCTGTGC-CATGCTGCT---GGCAC-TGC 256  
 heterocephalus CATGCTGACCACAGTG-----CTG--CTGAGTTGTGC-CCTGCTGCT---AGCAT-TGC 120  
 chinchilla CATGCTGACTACAATG-----CTG--CTGAGTTGTGC-CTTACTGCT---GGCAT-TGC 49  
 mus CATGCTGACTGCAATG-----TTG--CTGAGTTGTGT-TCTGCTGTT---GGCAC-TGC 152  
 Danio CTTGAAGCCTATGATGAGGATCTGGGCAAAGCTGTCCACCTGCAGAGAAGAGGCACACGC 268  
 \* \*\* \* \*\* \* \*\* \* \*\* \* \*\* \* \*\*

chimpanzee CTGCCACGCGA--GGAGCCCAGATGGGCTTGGCCCCCATGGAGGGCATCAGAAGGCCTG- 403  
 bonobo CTGCCACGCGA--GGAGCCCAGATGGGCTTGGCCCCCATGGAGGGCATCAGAAGGCCTG- 403  
 macaca CTGCCACGCAA--GGAGCCCAGATGGGGCTGGCCCCCTGGAGGGCATCAGAAGGCCTG- 402  
 homo CTGCCACGCGA--GGAGCCCAGATGGGCTTGGCCCCCATGGAGGGCATCAGAAGGCCTG- 506  
 mustela CCCCCATGCAG--GGGGCCGCATCGGCCTGGCCCCACTGGAAGGCATCAGAAGTCCTG- 116  
 rattus CTCCCACATTG--GGGTCCATATGGGCGTGGCACCCTGAAGGGCATCAGAAGGTCTG- 310  
 microtus CTCCCACGCTG--GGGTCCAGATGGGCTTGGCACCCTGGAAGGCATCAGAAGGCCTG- 313  
 heterocephalus CTGAAACGCAG--GGGGCCCAGATGGGCTTGGCCCCCTGGAGGGCATCAGAAGGCCTG- 177  
 chinchilla CTGCAACACAG--GGGGCCCAGATGGGCTTGGCCCCCTGGAGGGCATCAGAAGGCCTG- 106  
 mus CTCCCACACTG--GGGTCCAGATGGGCGTGGCTCCACTGAAGGGCATCAGAAGGCCTG- 209  
 Danio TCCCCGAGCCGCTGTATCCCTCATCAACA-GTCCTGTCTG--GGTCATCATCTGCCCTGC 325  
 \* \* \*\* \* \* \*\* \* \*\* \* \*\* \* \*\*

chimpanzee ----ACCA----GGC-CCTGCTCCCAGAGCTCCC---AGG----- 431  
 bonobo ----ACCA----GGC-CCTGCTCCCAGAGCTCCC---AGG----- 431  
 macaca ----ACCA----GGC-CCTGTTCCCAGAGCTCCC---AGG----- 430  
 homo ----ACCA----GGC-CCTGCTCCCAGAGCTCCC---AGGTGAGTGTGAGCAAGGGTGGG 554  
 mustela ----ACCA----GGC-CCTGTTCCCAGAGCTCCC---AGG----- 144  
 rattus ----ACCA----GGC-CCTGTTCCCAGAGTTCTC---AGG----- 338  
 microtus ----GCCA----GGC-CCTGTTCCCAGAGTTCCC---AGG----- 341  
 heterocephalus ----ACCA----GGT-CCTGTTCCCAGAGTTCCC---AGG----- 205  
 chinchilla ----ACCA----GGT-CCTGTTGCCAGAGTTCCC---AGG----- 134  
 mus ----ACCA----GGC-TCTGTTCCCAGAGTTCCC---AGGTGAGTATGGTCAGGTTGGGG 257  
 Danio TGCAACCCCTGCCACACCTGCTACTGCCCTTCTTTAAGG----- 365  
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chimpanzee -----  
 bonobo -----  
 macaca -----  
 homo ACTGGGCGGGGCTGAATACCCTCTGGCCACAAATAGTCTCCCTGGCATAAACCTCTT 614  
 mustela -----  
 rattus -----  
 microtus -----  
 heterocephalus -----  
 chinchilla -----  
 mus ATATGTGGGGCAACGACCATTGCTGGCCACAGACCTGCCCGCCAGGCTTAGACCTCCTT 317  
 Danio -----

chimpanzee -----  
 bonobo -----  
 macaca -----  
 homo TCTCCCTTCCCAAACCTCCCTGGGAGGTGGGTGCTTTGTGCATGGGGTTCTGTCCT 674  
 mustela -----  
 rattus -----  
 microtus -----  
 heterocephalus -----  
 chinchilla -----  
 mus CCCCAATCCCAATCCCAACCTAGGGAGGTGGGTACTTGGTGCATGGTGGGTGTGGCCCTC 377  
 Danio -----

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chimpanzee -----CCTGGGCCTG--CGGGCCCCACTGAAGAAGACAACCTGCA--- 468
bonobo -----CCTGGGCCTG--CGGGCCCCACTGAAGAAGACAACCTGCA--- 468
macaca -----CCTGGGCCTG--CGGGCCCCACTGAAGAAGACAACCTGCA--- 467
homo CACATCCTCTGCCCCAGGCCTGGGCCTG--CGGGCCCCACTGAAGAAGACAACCTGCA--- 729
mustela -----CCTGGGCCTG--CAGTCCCTCGCTGAAAAGGACAGCTGCA--- 181
rattus -----TCTAAGTCTG--AA-----GAAGACAGCAGCA--- 363
microtus -----TCTAAGTCTG--AATGGCCCA--AAGAGGACAACCTGCA--- 375
heterocephalus -----TCTG--CATGCTCCGCT--GAAGAAAACCTCCA--- 233
chinchilla -----CCTAGGCCTG--CATGTTTCGCTGAAGAGGACAACCTGCA--- 171
mus ACATCTTCTTCCCCAGGTCTAAGTCTG--AATGGCC--TCAAGAAGACAACCTGCA--- 429
Danio -----CCT--TCTGTTACTGCCGAGCATGGACAACACCTGCAAAA 404

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chimpanzee --GAACAGGCA--GAAGAGGATCTGT-TGCAGGAGGCTCAGGCCTTGGCAGAGGTA--- 519
bonobo --GAACAGGCA--GAAGAGGATCTGT-TGCAGGAGGCTCAGGCCTTGGCAGAGGTA--- 519
macaca --GAACAGGCA--GAAGAGGATCTGT-TGCAGGAGGCTCAGGCCTTGGCAGAGGTA--- 518
homo --GAACAGGCA--GAAGAGGATCTGT-TGCAGGAGGCTCAGGCCTTGGCAGAGGTA--- 783
mustela --GAACAGTCA--GGAGAGGCCCTGC-TGCAGGAGGCTGAGGCCTTGTGAGAGGTA--- 232
rattus --GACCGAGCA--GAAGATGTTCTGC-TGCAGAAGGCAGAGCTTTGGCAGAGGTG--- 414
microtus --GACCGAACA--GAAGAGGCTCTGC-TGCAGAAGGCAGAGCTTTGGCAGAGGTG--- 426
heterocephalus --GACAAAGCA--GAAGAGGCTCTGC-TGCAGAAGGCAGAGCTTTGGCAGAGGTG--- 284
chinchilla --GAACAAGCA--GAAGAGGCTCTGC-TGCAGAAGGCAGAGCTTTGGCAGAGGTG--- 222
mus --GACCGAGCA--GAAGAAGTCTGC-TGCAGAAGGCAGAGCTTTGGCAGAGGTA--- 483
Danio ACGAATATGCATAGAGACAAGTCTGCTTCAGATAGCAAA-----TGGTAGCA----- 453

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chimpanzee -----
bonobo -----
macaca -----
homo GCTCAGGGAAAAGGGTAAGGTGGTGGCCCTTGGGAGGGGGCATTGGGTATTAGCTCCTCT 843
mustela -----
rattus -----
microtus -----
heterocephalus -----
chinchilla -----
mus CATTAGGGAAAAGGGATAAAGTAGAAGGTAGGGCGCATCAGATACCATCATCTCTCCCCAC 543
Danio -----

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```

chimpanzee -----
bonobo -----
macaca -----
homo CCCCAGCTCCAACTCCCTCACCAGCGACGACACTACCGACCACCCCTTCCATGCTCCA 903
mustela -----
rattus -----
microtus -----
heterocephalus -----
chinchilla -----
mus TTCCGGATTACCCAACCTGGGCAGAACTGCAGCCCTCCCTGACCTCAGTCCACTGCCAC 603
Danio -----

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chimpanzee -----
bonobo -----
macaca -----
homo CTGCCATCCTGCACAGGTTGGGACAGGTAAGATCCCTGGATCTGTCTTTAGAGGCCTGT 963
mustela -----
rattus -----
microtus -----
heterocephalus -----
chinchilla -----
mus CCTACTGGGGTCGGGGTTTGAGAGTTTCTGAACTTAT----- 642
Danio -----

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chimpanzee -----CTAGACCTGCAGGACCGGAGCCCCGCTCCTCACGT 555
bonobo -----CTAGACCTGCAGGACCGGAGCCCCGCTCCTCACGT 555
macaca -----CTAGACTCGCAGGACCGGAGCCCCGCTCCTCACGT 554
homo GCTGGTTCCCCACCCCTGCAGGTAAGTACTAGACCTGCAGGACCGGAGCCCCGCTCCTCACGT 1023
mustela -----CTAGATCCGGAGGGCCGAGAGTACGCTCCCCACGT 268
rattus -----CTAGATCCACAGAACCAGGAGTCTCGTTCTCCGCGT 450
microtus -----CTAGATCCACAGAATCGGAGTCTCGTTCTCCGCGT 462
heterocephalus -----CTAGACGCACAGAGCCGCGAGCCCCGCACCCACGC 320
chinchilla -----CTAGACCCGAGAAACCAGGAGCCCCGCACCCACGC 258
mus -----TCCCCTACGAATGCAGGTGCTAGATCCACAGAACCAGGAGTCTCGTTCTCCGCGT 697

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Danio -----TTATATTTACAGTTTAAAGAGC-----TACTGAATGT 485
                ** *      **      ***      *      *

chimpanzee CGCTGCGTAAGGCTGCA----TGAGTCCTGCCTGGG-----ACAGC-AGGTGCCTTGCT 604
bonobo      CGCTGCGTAAGGCTGCA----TGAGTCCTGCCTGGG-----ACAGC-AGGTGCCTTGCT 604
macaca      CGCTGCGTAAGGCTGCA----TGAGTCCTGCCTGGG-----ACAGC-AGGTGCCTTGCT 603
homo        CGCTGCGTAAGGCTGCA----TGAGTCCTGCCTGGG-----ACAGC-AGGTGCCTTGCT 1072
mustela     CGCTGCGTAAGGCTGCA----TGAATCCTGTCTGGG-----ACACC-AGGTACCATGTT 317
rattus      CGCTGTGTAAGGCTGCA----CGAGTCCTGCTTGGG-----ACAAC-AGGTACCTTGCT 499
microtus    CGCTGTGTAAGGCTGCA----TGAGTCCTGCCTGGG-----ACAGC-AGGTTCCTTGCT 511
heterocephalus CGCTGTGTAAGGCTGCA----CGAGTCCTGCTTGGG-----GCAGC-AAGTACCATGCT 369
chinchilla  CGCTGTGTAAGGCTGCA----CGAGTCCTGCCTGGG-----GCAGC-AGGTACCGTGCT 307
mus         CGCTGTGTAAGGCTGCA----CGAGTCCTGCTTGGG-----ACAGC-AGGTACCTTGCT 746
Danio       ATTTGTGGACAG-TGCAATCATAAA-CTCGCACAGAGAATCCACAGAGAAGTTTTTTGTT 543
                ** * * * * **      * * *      *      ** * ** ** *

chimpanzee GTGACCCATGTGCCACGTGCTACTGCCGCTTCTTCAATGCCTTCTGCTACTGCCGAAGC 664
bonobo      GTGACCCATGTGCCACGTGCTACTGCCGCTTCTTCAATGCCTTCTGCTACTGCCGAAGC 664
macaca      GTGACCCCTGTGCCACATGCTACTGCCGCTTCTTCAACGCCTTCTGCTACTGCCGAAGC 663
homo        GTGACCCATGTGCCACGTGCTACTGCCGCTTCTTCAATGCCTTCTGCTACTGCCGAAGC 1132
mustela     GCGACCCATGTGCCACGTGCTACTGCCGCTTCTTCAACGCCTTCTGCTACTGCCGAAGC 377
rattus      GCGACCTGTGCCACGTGCTACTGCCGCTTCTTCAATACCTTTTGCTACTGCCGAAGC 559
microtus    GCGACCCGTGCCACATGCTACTGCCGCTTCTTCAACGCCTTTTGCCTACTGTCGCAAGC 571
heterocephalus GTGACCCGTGCCACATGCTACTGCCGCTTCTTCAACGCCTTCTGCTACTGCCGAAGC 429
chinchilla  GCGACCCGTGTGCCACCTGCTACTGCCGCTTCTTCAACGCCTTCTGCTACTGCCGAAGC 367
mus         GCGACCCGTGCCCTACGTGCTACTGCCGCTTCTTCAATGCCTTTTGCTACTGCCGAAGC 806
Danio       ATAATA-GAGAAGCACAAAGCATAGA-GT-----GCACCATTGGCTGCGGTTTTAAGG 594
                *      *      **      * * *      * * *      * * ** * **

chimpanzee TGGGTA CTG---CCATGAACCCCTGCAGCCGCACCTAGCTGGCCAACGTCAGGGTCGGGG 721
bonobo      TGGGTA CTG---CCATGAACCCCTGCAGCCGCACCTAGCTGGCCAACGTCAGGGTCGGGG 721
macaca      TGGGTA CTG---CCATGAACCCCTGCAGCCGCACCTAGCTGGCCAACGTCAGGGTCAGGG 720
homo        TGGGTA CTG---CCATGAATCCCTGCAGCCGCACCTAGCTGGCCAACGTCAGGGTCGGGG 1189
mustela     TGGGTA CTG---CCATGAACCCCTGCAGCCGTACCTAGTGGGCGAACGTCGGGGCCGGGG 434
rattus      TAGGTA CTGGCACCAGAACCTCTGCAGCCGCCCTAGCCAACGGATGTT-----GGG 612
microtus    TGGGTA CTG---CCACGAATCTCTGCAGCCGCACCTAGCCAACGGATGCT-----GGG 621
heterocephalus TGGGTA CTG---CTACGAATCCCTGCAGTCGCACCTAACC GGCCAACATCT-----GGA 480
chinchilla  TGGGTA CTGG---TACGAACCCCTGCAGCCGCACCTAACC GGCCGACATCT-----GGA 418
mus         TGGGTA CCG---CCACGAACCTCTGTAGTCGCACCTAGCCAATGGATGTTG---TTTGGG 860
Danio       T--GTAATTTAATTGCAATATATTTTACTCAATGTTGTCT-TAAATATTT-----TGTA 646
                *      ***      *      * * *      *      *      *

chimpanzee CTAGGGTAGGGGCAAGGAAACTCGAATAAAGGATGGGACCAAC----- 764
bonobo      CTAGGGTAGGGGCAAGGAAACTCGAATAAAGGATGGGACCAAC----- 764
macaca      CTAGCGTAGGGGCAAGCAAATGCGAATAAAGGATGGGACCAAC----- 763
homo        CTAGGGTAGGGGCA----- 1203
mustela     CTCTACATGGGGTAG-----TGAATAAAG----- 458
rattus      CAAAGGCAGGG-----ACGCGAATAAACGATGGGACTAAC----- 648
microtus    CAAAGGCAGGG-----ACGTGAATAAAGGATGGGAC----- 652
heterocephalus CAGAGACGGGAG-----ACGCAATAAAGGA----- 506
chinchilla  CAGAAACGGG----- 428
mus         -AAAGGCAGGG-----ATGAGAATAAAGGATCGGGACGGTTAACCTTAAAGCTGTG 912
Danio       TATATGCA-----TTCAATAAACTCTGATTATTGC----- 676

chimpanzee -----
bonobo      -----
macaca      -----
homo        -----
mustela     -----
rattus      -----
microtus    -----
heterocephalus -----
chinchilla  -----
mus         GTTATTTCTTT 923
Danio       -----

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**Supplementary Figure 28.** ClustalW alignment of *Danio rerio* AgRP nucleotide sequence against *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, *Pan troglodytes*, *Pan paniscus*, *Macaca mulatta*, *Mustela putorius furo*, *Heterocephalus glaber*, *Microtus ochrogaster*, and *Chinchilla lanigera* AGRP sequences. (\*) identical sequences are

indicated. The zebrafish sequence has an average 33.13% sequence identity with these species and 28.50% similarity to Homo sapiens.

**CART1**

Pundamilia -----GCGGCCTCCCGG----- 13  
 Oreochromis CTTCTGCCCCCTTTTCAGTACTTTCCGGCACGGTAGTCTCGCAGCGCCTCCCGG----- 55  
 Tautogolabrus -----  
 Xiphophorus -----  
 Carassius -----CTCGAGTGTTTC 11  
 Danio -----CGA----- 3  
 Leucoraja -----CAATCCCAA-----CC 11  
 Oryzias -----TCCA----- 4  
 Salmo -----ACACAG-----AT 8  
 Silurus -----ACGCGGGGAAT 12  
 Haplochromis -----GAGGAGACAGACAAA----- 15  
 Maylandia -----AGACAGACAAA----- 11

Pundamilia CGGGGCTGTGTGAACC-----GGTATAAGAGG 40  
 Oreochromis CGGGGCTGTGTGAACC-----GGTATAAGAGG 82  
 Tautogolabrus -----AGCACAGGGA-----CGATG 15  
 Xiphophorus -----GCCACA-----TGTCG 11  
 Carassius ACTTGAATCTCAGTGC-----AGAGG 32  
 Danio ---TGG---TCGGAG-----AGCGG 17  
 Leucoraja CCGGTGTGTGTGGT-----TCG-----GCCCT---G 35  
 Oryzias -CAGGACGCGTGAT-----TCCCAATT-----GTCTCCAAAG 35  
 Salmo AAAAGGTGTTTGA-----TTGCGGTAAGTGGAGTATTAGAACGGGACCTTTTGG 58  
 Silurus CCGAGGCGCGCAATAAAAAGCCCGTGCATCCTGAGCGC-----GGTGTGTTGGAG 63  
 Haplochromis CCAACACTCATGAGGA-----GGAAGGAGAGG 42  
 Maylandia CCAACACTCATGAGGA-----GGAAGGAGAGG 38  
 \*

Pundamilia GGACATGG-----TCCGGAGGGTGGAGGGAGAGTCACT 74  
 Oreochromis GGACATGG-----TCCGGAGGGTGGAGGGAGAGTCACT 116  
 Tautogolabrus AGGAGCA-----CA-----GGGAGCACGAGGAG---CT 40  
 Xiphophorus CCGCGCA-----CC-----GGGACCATGCAGAG---CT 36  
 Carassius ACGCTTG-----CT-----GTGACCATGGAGAG---CT 57  
 Danio ATCTTTC-----TC-----CTGACCGTC---AC---CT 39  
 Leucoraja AGTCGC-----TTC-----AGCACCATGGTCAG---CG 60  
 Oryzias AGTTGCAGAAACAAGAGTTTTTGG-----TTTTTC-----AGAACCATGGTCAG---CG 80  
 Salmo AGACGAAAGATATCTTCTACTGTGCGCAATCTCTTA-----AGGACCATGGAGAG---CT 110  
 Silurus AGTCGCGCGCCCTTCGTGCACAAAAGCCCGTTTCGATCAGCACCATGGAGAG---CT 119  
 Haplochromis GAGAGGA-----AAAGCTAAAGAGAAACAAC 69  
 Maylandia GAGAGGA-----AAAGCTAAAGAGAAACAAC 65  
 \* \*

Pundamilia GTGGATCTG---CTGCGACACTGTGCTCCTCAGCCTCTAATCTGCTGCCT-----CCCAC 126  
 Oreochromis GTGGACCTG---CTGCGACACTGTGCTCCTCAGCCTCTAATCTGCTGCCT-----CCCC 168  
 Tautogolabrus G-CAGGCTG---CTG-----CTCTGCGTGTGCTGCT-----CTCAG 73  
 Xiphophorus C-CAGGATG---CTGAGCGGC-GCGCTC---CTCTGCGCGTGTGCTG---CTC--- 80  
 Carassius C-CAAACT---CTGGACCACAGCGATG---GCATGCGCGGTGCTGGTG---TCCTG 103  
 Danio ---GCT---CTGTTTTA-----ATGC---TGCTGGTT---TGCTG 67  
 Leucoraja -ACAGGCT---C---CTGCTT-GCTGTCT---ACTTTTG-CGTGTTGTTC---TCCA- 102  
 Oryzias CGCGGACT---CTCCTCCTC-GTGGTC---ACTTGCA-GCTGCACCTA---CCTT- 124  
 Salmo C-CAGGCT---ATGGACCAGAGCGGTG---GTTTGC GCGGTGTTGCTG---TCCA- 155  
 Silurus --CGAGTA---TCGCCGCGC-GCGCTCT---GCTGCTC---CTGCTCGTA---TTAA- 161  
 Haplochromis ACCTGACTGAAACCAGACACCACAGAAGAAGAGTTTGTGACACCTTCTGGAGGACCTT- 128  
 Maylandia ACCTGACTGAAACCAGACACCACAGAAGAAGAGTTTGTGACACCTTCTGGAGGACCTT- 124

Pundamilia TG-----GGACCTCCGCCCTTCCGCTTACC GCCACCAGCACCATGGATA-----GC 173  
 Oreochromis TG-----GGACCTCCGCCCTTCCGCTTACC GCCAACAGCACCATGGATA-----GC 215  
 Tautogolabrus GA-----TC-----CACCGGAGCAGA-----CCTGA----- 94  
 Xiphophorus -----TC---CGCCGGCGCCG-----CCGGAG----- 99  
 Carassius CATT CAGGGTCCCGAGATGGACTTTGACAACGAATCCGA-----CTTAGAAACGAGAGC 157  
 Danio T-----GATGAAGTT---CTGCAGATCCG-----CTCG----- 92  
 Leucoraja -----TG-----GCTGT-----TG-GAG----- 114  
 Oryzias -----TG-----GCTTT-----CCCGAG----- 137  
 Salmo -----TC-----GTCT-----TA-GTG----- 167  
 Silurus -----TGC---TGCGGAGTTGTG---CGCGAG----- 182  
 Haplochromis -----TATTAGTTTAAAGTTT-----TAAAGGTGC---TT 156  
 Maylandia -----TATTAGTTTAAAGTTT-----TAAAGGTGC---TT 152  
 \*

Pundamilia TCCGGGAT-----GCTCCGCGGGCTGCTGCTCGTCGGCCTGCTGTCCGTCCTG 221  
Oreochromis TCCGGGAT-----GCTCCGCGGGCTGCTGCTCGTCGGCCTGCTGTCCGTCCTG 263  
Tautogolabrus -CGGAGAA-----CAA---CTCCCTGAC-CAG---TGAGGACGAGC-----TG 129  
Xiphophorus CCGGAGCG-----CAGGGGCTGCTGGATGCGGAGTCCGAGGAGGAGC-----TG 143  
Carassius CCTGAGAGAGTTTTACCCAAAGGACCCGAATCTGACCAACGAGAAGCAGCT-----CGTA 212  
Danio CCTGAG-----GAGGA-----GAGGA-----TA 105  
Leucoraja -CAGA-----GAACTC-----GGACC-----TG 131  
Oryzias -CCGA-----GGACTC-----TTCCT-----TG 154  
Salmo -CTGAAATT-----GACTACTCGGATTCGGAA---TTGGACC-----TC 202  
Silurus -GAGACAAC-----GA-----GGACACCGAA---GTGGACT-----TG 211  
Haplochromis TTGGAGATG-----GAGAGCAAACGAGCCGTGGTCTATCTGTCCGTCTGTCTGTCTG 208  
Maylandia TTGGAGATG-----GAGAGCAAACGAGCCGTGGTCTATCTGTCCGTCTGTCTGTCTG 204

\* \* \*

Pundamilia TGCC-----ACGCCAGGCATC---ACGGGAAGTCTCCG-----CA 254  
Oreochromis TGCC-----ACGCCAGGCATC---ACGGGAAGTCTCCG-----CA 296  
Tautogolabrus AGC-----CCCAGAGTGC---TGCGCCACTTCTACT-----CC 159  
Xiphophorus AGC-----CCCAGAGCGC---TGCGGGACTTCTACC-----CG 173  
Carassius AGTATTGCGCACATTTCTCGGCGCATAGAAAACAATGACGTTCCCTTGTT-----TA 264  
Danio AG-----GCTCAGGAGG-----A 118  
Leucoraja GAG-----CCAAGAGCCC---TGCGAGACTTCTACT-----CC 161  
Oryzias GAA-----AC---GCGCTC---TCTGGA---TTTTACT-----CT 180  
Salmo GAC-----ACAAGAAGTG---TGAGAGACTTCTACC-----CT 232  
Silurus GAT-----ACAAGAGCCA---TCCGAGACTTCTATC-----CG 241  
Haplochromis TGCTG-ACATCTCTCTGTCAAGGCCAAAGGTCAGGCAACAGCCACCTGCTGTCCGCGCCT 267  
Maylandia TGCTG-ACATCTCTCTGTCAAGGCCAAAGGTCAGGCAACAGCCACCTGCTGTCCGCGCCC 263

Pundamilia GAAGACTT---TGGAGAGAAGA---AACAAG-AAGCAGCTGT---AGACAGAGACCTGC 303  
Oreochromis GAAGACTT---TGGAGAGGAGA---AACAAG-AAGCAGCTGT---AGACAGAGACCTGC 345  
Tautogolabrus AAAGGACC---GAACCTGACC---TCAGAG-AAACAGCT---GTCCGGAGCTCTGC 205  
Xiphophorus AAAGGACC---GAACCTGACC---AGTGAA-AAGCAGCT---GCTTGGAGCTCTCC 219  
Carassius AAAAGATTTTAGTGATACTTACCTGCATTGATTGAACATTTTACAGCTCGGGGCTTTAC 324  
Danio GAAAGA-----GCTGATTGAA-----GCTCTTC 141  
Leucoraja AAAAACTA-----TTATCCCAGC---AGCGAG-AAGGAACT-----GCTCGGGGCTCTAC 207  
Oryzias GAAAGCT-----CAGC---AA-GAA-AAGGATCT---GATCGACGCCTTGC 218  
Salmo AAAGATCC---GAATTTGACC---AACGAA-AAGCAACT---GCTTGGAGCACTAC 278  
Silurus AAAGACCC---CAATCTGACC---AGCGAA-AAACAGCT---GCTTGGCGCTTTGC 287  
Haplochromis GATGACCC---AACCTCGGACTCACAACCAGTGAGCT---GGCTGAAGCTCTGC 316  
Maylandia GATGACCC---AACCTCGGACTCACAACCAGTGAGCT---GGCTGAAGCTCTGC 312

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Pundamilia TAGAGGCACTGGA-CGTGCTCCTGGGCAGAAACCATAATCAAGTCTCCTCCCCTGAGAAA 362  
Oreochromis TAGAGGCACTGGA-TGTGCTCCTGGGCAGAAATCATAATCAAGTCTCCTCCCCTGAGAAA 404  
Tautogolabrus AGGAAGTTCTGGA-GAAACT-----TCAGGC---GAAGAAAACGTCTTCATGGGAGAAA 255  
Xiphophorus AAGAAGTTCTTGA-AAAGCT-----GCAGGC---TAAACGACTTCCATCGTGGGAGAA 269  
Carassius ATGACGTTCTGGA-GAAACT---GCAGAG---TAAACGCATTTCACTTTGGGAAAAG 374  
Danio AAGAAGTTCTGGA-AAAGCT---GAGAAA---TAAACGATTTCTGCTGTGGAGAAG 191  
Leucoraja ACGAGGTGCTGAA-GAAACT---GCAGAC---TAAGCGGCTTCCAACCTGGGAGAAG 257  
Oryzias AAGGAGTTTGA-AAAGCT---GAGAAA---CAAAGAAATGCCA---CTGG-AGAAG 265  
Salmo ATGACGTTCTTAA-AAAGCT---GCAGAC---CAAGAGACTTCCATTTGGGAAAAG 328  
Silurus ATGATGTTCTGGA-AAAGCT---TCAGAT---GAAAAGAATTCTCCTTGGGAGAAG 337  
Haplochromis AGGGTCTTCTGGATGAAGCT---GACAGCT---CAGCCGGTCTCTCTGTGG-AGAAA 366  
Maylandia AGGGTCTTCTGGATGAAGCT---GACAGCT---CAGCCGGTCTCTCTGTGG-AGAAA 362

\* \* \* \* \*

Pundamilia AGA---GGAAGCATCCCCT----- 379  
Oreochromis AGA---GGAAGCATCCCCT----- 421  
Tautogolabrus AAGTTTGGCCAAGTCCCCTAG----- 275  
Xiphophorus AAATTTGGTCAAGTCCCAAC----- 289  
Carassius AAGTTTGGGCGGTTCTTACAGTGAGTAGCGCAAACAGATTTTGGAGTAAATAGTGA 434  
Danio AAGCTCGGCTGGGTTCC----- 208  
Leucoraja AAGTATGGACAGGGGCTCA----- 277  
Oryzias AAGCTCGGATGGTTGCCTTC----- 285  
Salmo AAATTCGGCCAAGTCCCTAC----- 348  
Silurus AAATTTGGGCAAGTCCCTAT----- 357  
Haplochromis AAAGCCAGCGTGATCCCCTG----- 386  
Maylandia AAAGCCAGCGTGATCCCCTG----- 382

\* \* \*

Pundamilia -----  
Oreochromis -----  
Tautogolabrus -----  
Xiphophorus -----  
Carassius AAATATTGTAATGCCTAGATTAAATCACAATTTACTATTAACAAAACGCTGACAGTGA 494  
Danio -----ATC----- 211  
Leucoraja -----

Oryzias -----  
Salmo -----  
Silurus -----  
Haplochromis -----  
Maylandia -----

Pundamilia -----GTGCGGGTTGGGGAACCGGTGTGCCATG 407  
Oreochromis -----GTGCGGGTTGGGGAACCGGTGTGCCATG 449  
Tautogolabrus -----GTGTGACGTGCGGGAGCAGTGTGCAGTG 303  
Xiphophorus -----GTGTGACGTGAGAGCAGTGCAGTGCAGTG 317  
Carassius GTTATCTAACACTGACACTCCGCTTCACATCAGTGCAGCTCGGGAGCAGTGCGCCATC 554  
Danio -----GTGTGATGCAGGCGAGCAGTGTGCAGTG 239  
Leucoraja -----GTGTAACATCGGAGACATGTGTGCAGTG 305  
Oryzias -----ATGTGACGCAGGAGACCGTGCAGTG 313  
Salmo -----GTGCGACGTGAGAGCAGTGCAGTG 376  
Silurus -----GTGCGACGTGGGAGAGCAGTGCAGTG 385  
Haplochromis -----GTGTGATGTGGGTGAGCGGTGTGCAGTG 414  
Maylandia -----GTGTGATGTGGGTGAGCGGTGTGCAGTG 410  
\*\* \*\* \* \*\*\* \*\* \*

Pundamilia AAGTACGG-GCCTCGCATCGGGAAGCTCTGCGACTGTGGAAGAGGAGCCAA---CTGCAA 463  
Oreochromis AAGTACGG-GCCTCGCATCGGGAAGCTCTGCGACTGTGGAAGAGGAGCCAA---CTGCAA 505  
Tautogolabrus AGAAAAGG-CTCTCGGATCGGCAGGATGTGTGACTGTCTCGTAGAGCGTT---CTGTAA 359  
Xiphophorus AGGAAAAGG-CGCACGAATCGGGAAGATGTGCGACTGTCCCGGGGAGCTTT---CTGCAA 373  
Carassius AGGAAAAGG-GTCAAGAATCGGCAAAATGTGCGACTGTCCACGTGGAGCGTT---TTGCAA 610  
Danio CGGAAAAGG-CTCCCGCTTCGGGAAGTGTGCGAGTTGTCCAGGAGAACCGC---CTGCAG 295  
Leucoraja AGAAAAGGACCCAGGATTTGGAGGACC-TGTAACCTGCCT-----GAGTTCGAAAATGTAA 358  
Oryzias CGTAAAGG-TGCGCGCATCGGAACACTGTGCGGCTGTCTCGAGGGACTTC---ATGTAA 369  
Salmo AGAAAAGG-CGCGAGAATCGGCAAAATGTGCGACTGTCTCGCGGAGCTTT---CTGCAA 432  
Silurus CGTAAAGG-CTCCAGAATTTGAAAATGTGCGACTGTCCACGAGGAGCCTT---CTGCAA 441  
Haplochromis AAACACGG-ACCTCGGATTGGTCGGCTCTGCGACTGTCTGAGAGGAACAGC---CTGCAA 470  
Maylandia AAGCACGG-ACCTCGGATTGGTCGGCTCTGCGACTGTCTGAGAGGAACAGC---CTGCAA 466  
\* \*\* \* \* \*\* \*\* \*\* \*\* \*\*

Pundamilia CTCCTACCTCCTCAAGTGCATCTAAACC-----GCCCA 496  
Oreochromis CTCCTACCTCCTCAAGTGCATCTAAACC-----GCCCA 538  
Tautogolabrus CTTCTGCCTGTGAAGTGTATTATGAG-----AAACACACACAC---ACA 400  
Xiphophorus CTTCTTCTGTGAAGTGTATTATGAGCCTCTCCGGATCGGAATGTAGCTTCGGGAAGAGA 433  
Carassius TTACTTTTGTGAAGTGTATTATGAG-----T 637  
Danio CTTCTCCATCCTCAAGTGTGTGAG-----A 322  
Leucoraja TTACTTTCTGTTAAATGTGTATAG-----AAATC 388  
Oryzias CTTCTACGTTCTCAAATGTTTATAG-----GAGTA 399  
Salmo CTCTTATCTGCTCAAGTGTGTGAATT-----GAATT 465  
Silurus CTTTTCTCTGAAATGTGTGTGAG-----GAGGA 472  
Haplochromis CACCTTCTTCTGCGCTGCTACTGACC-----ACTC 501  
Maylandia CACCTTCTTCTGCGCTGCTACTGACC-----ACTC 497  
\* \* \* \*\* \*

Pundamilia GGGCTCT-----CCTTTAATTTGTACCTC----- 521  
Oreochromis GGGCTTT-----CCTTTAGTTTGTACCTC----- 563  
Tautogolabrus AGTCTTTTATTAAA-----GGTACATTT---AATGAGAAC-CAGCTGA 440  
Xiphophorus AGACTTTCACTGAACCCCTTTGTAATTACGCTCAGATTTTTTTTACTGCACAGCTCA 493  
Carassius GGATTGT---GAAT-----TGGAAT-----A 656  
Danio GG-----GAA-----AAAAC-----A 333  
Leucoraja AGACA-----AAAT-----CATTTCTATTTTC-ATCG--GCTA 418  
Oryzias AGAAA-----AGA-----GTTTATTTTAGA-GTTG--G--A 425  
Salmo GGTCT-----GAA-----TGTAAGTTAGAAGTTGATGGCA 495  
Silurus AGGCA-----A-----CTTCTGTAGCGGCTC-----A 495  
Haplochromis GCACACA-----TT-----TTTTCTCACGAGGACCT-----A 527  
Maylandia GCACACA-----TT-----TTTTCTCATGAGGACCT-----A 523

Pundamilia TGCTATGCCAA-----ACAC-ATCCTTCA-----GCACAG 550  
Oreochromis TGCAGGGCCAA-----ACAC-ATTCTTCA-----GCACAG 592  
Tautogolabrus GGAGAATGCTC-AGTGACCGTC-----TGCAACAC-ACGAGAAG---AACTACATGG 488  
Xiphophorus GGGCGCA-GCTGGAGTACTGTCCAACACTTACAACAA-CTGTAGAA----ATTGTATTA 546  
Carassius TACACATGATA-----TT-----ACAG-ATATTTATA---CATTATGTGA 692  
Danio GCCTCAGTCTC-----TC-----ATA--ATGCTC-----CAGTGTGT-- 363  
Leucoraja AATGAAGACAG-----T--T-----TAA-AG-ATCTGGT-----GGATATA- 450  
Oryzias AATTATGACAA-----TAAT-----GAATAA-GTATGGCATC--AAGGGTGCAA 466  
Salmo AACTAAAACGT-----TTACA---TCAGAACGACATGTGATAT---AGGAATATTG 540  
Silurus AATTGATGTAT-----T-----CAATTT-ATTTG-TAT----AGATATTT 529  
Haplochromis AACTCGGTCTG----GGCGCTCACAC-----AGACAGTGTCTCAGAAAGACAGAAGTGCA- 577  
Maylandia AACTCGGTCTG----GGCGCTCACAC-----AGACAGTGTCTCAGAAAGACAGAAGTGCA- 573  
\*

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Pundamilia      C-----TCCC-----ATAAACCAAC----CCTT----TC 571
Oreochromis    C-----TCCC-----ATAAACCAACTCAACCCTGTCATC 621
Tautogolabrus T---GTTTAAACAGAGCTGTGTGAGGAT-TGTTGTGATGAGATG--GTGAGTTCAAAATT 542
Xiphophorus   TTAGGAGTAAACATTCGGTGTTA----TTTTACAACAAAAA--ATGACATGAGGATG 599
Carassius     T-----TTCTATTTT-----TATGATTATGAAGCGCTGTGAGAGAACA-- 731
Danio         -----TGTT-----TACCATCATCAAAT-CTGTAACATGTTTATC 397
Leucoraja     -----TTATAAATAA-----ACAAAGCATT--GTCA--ACATT 479
Oryzias       A-----TCATAAGTAATTTTCTGTCTCATAAATCATTCAAATCC--AAATC 511
Salmo        -----TTATG-GTACTACA-----ATATACGATT--ATCCGGAGAGT 574
Silurus      -----TTTTAAGTGCAAGA-----ACACGCTGAAGAGATGTGTGTGTG 567
Haplochromis -----CTCT-----TCGCCAATCGTCTCGCTCCATATTTT 607
Maylandia    -----CTCT-----TCACCAATCGTCTCGCTCCATATTTT 603

Pundamilia    ATT-----TATAG-----TCATAGCTGTGTTAAA-----CAGTTGTCGCCTGA 610
Oreochromis  ATT-----TATTG-----CCATAGCTGTGTTAAA-----CAGTTGTCGCCTGA 660
Tautogolabrus GTG-----ATATCA-----TGTCACAA--ACAAA-----TATTTGAGTCTAAT 579
Xiphophorus  AGA-----ATATCA-----TAA-AACGTTTCTGTAAA-----TATTT-ACTTCAAC 638
Carassius    GGG-----GAATT-----TGA-----AAGAT-----AT 758
Danio       AGT-----ACATA-----CAG-----ACGAA-----TGTATAA----- 420
Leucoraja    CCT--ACTACATC--G---AACAAATC--CTGTGCAAT-----TTATCCTGTT-CTGT 522
Oryzias     AAT--GCATCATT--GGCAAATCAGTTCAGCTGGAAAATGTGGGGTCAATTTGACC-CTAA 566
Salmo       GACAGGTTGCATTCTGAGAGATTGTCCAAACCCCAAAAAG--GCCTATTTGTAGTTAT 631
Silurus     AGT---CTACAGT-----CCTGTTTCATCTGTTTCATG-----CACTGCACGCCTAT 609
Haplochromis AGT-----CTTTG-----TTTTAAAG-----TTTTTGGTGTTTGT 637
Maylandia   AGT-----CTTTG-----TTTTAAAG-----TTTTTGGTGTTTGT 633

Pundamilia    GGATAAGCA--TGGATTTTGTACTGCTGA-----CAGTACTGAATATGTTG 654
Oreochromis  GGATAAGCA--TGGATTTTGTACTGCTGA-----CAGTACTGAATATGTTG 704
Tautogolabrus GTCGG---AGTCTGTCTGTC--ATTATAA-----TAAT-TCATATTCATAA 619
Xiphophorus  TTCAATAAACTCAACCCGTTGGACCATAAGCA-----CACTGTAATGTCAAACCAGCAA 692
Carassius    TT-----GCTGCTCTTGA--TGTG-----TAAT--TAAATTGG-AA 789
Danio       -----ATG-----CATT--CAAATCA--AA 436
Leucoraja    GTGT--ATA-----TCCAG-----TG-----CCCCATGG--AAAAATTCATTA 557
Oryzias     AAATT-ATAGCCTAATTTAAG----TAAAAGGGGACTCTCTTTAG--GAAATTTT-TG 616
Salmo       GAA---ATATTTTATTTTCAAC---TGAATG-----TTTCTGTAACCAAAAATGAGTTG 679
Silurus     A----ATA-----ATGTCA-----TGAA-----TATGTAAC--ATGTCTGTTA 641
Haplochromis -----CTTTCA-----GAT--TAATTTATTTT 657
Maylandia   -----CTTTCA-----GAT--TAATTTATTTT 653

Pundamilia    TGT---TTAATGTAAACGTTTC-----ATCTT----- 678
Oreochromis  TGT---TTAATGTAAATGTTTC-----ATCTT----- 728
Tautogolabrus TA---AA--GTGCGGATCAT-----GTTTT----- 639
Xiphophorus  TA---TT--ATGCCCTTCATCCGCTGTGTTTTGTTTC----- 724
Carassius    TA---AATTGTGTGGACTTT-----TGTTTT----- 812
Danio       TA---AAT-GCATGCAGTTT-----TGATA----- 458
Leucoraja    T----TAAATAT--GCTATC----CT-ATTTCTT----- 580
Oryzias     T----TAAGTATCAAACCTTTC-----CTTATTTTTTATTTGAGAAATCCGTAGTCACTT 666
Salmo       TGC-TATGAATAAACAGCATTTCT--ATCTTGTTTTTT----- 713
Silurus     TGTGTATTAAGTGCAACGTAC-----ATTTTTT----- 670
Haplochromis TG---TTAATAAAGA-----TTCTT----- 674
Maylandia   TG---TTAATAAAGA-----TTCTT----- 670
* * * * *

Pundamilia    -----CAATAAACAC----- 688
Oreochromis  -----CAATAAACACGTCACATATAA----- 750
Tautogolabrus -----AAAAAA----- 645
Xiphophorus  -----AGCTGA----- 730
Carassius    -----T----- 459
Danio       -----CTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 620
Leucoraja    AGTTGAATGGAATGCCAACCAATAAAGTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 725
Oryzias     -----AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA----- 742
Salmo       -----TGTAAAAAAAAAAAAAAAAA----- 688
Silurus     -----CATAAAGCTGCACGGCTCCAAAG----- 697
Haplochromis -----
Maylandia   -----

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**Supplementary Figure 29.** ClustalW alignment of *Danio rerio* CART1 nucleotide sequence against *Oreochromis niloticus*, *Maylandia zebra*, *Haplochromis burtoni*, *Pinamilia nyererei*, *Xiphophorus maclutas*, *Carassius auratus*, *Leucoraja ocellata*,

*Tautogolabrus adspersus* and *Salmo salar* CART sequences. (\*) identical sequences are indicated. The zebrafish sequence has an average 43.34% sequence identity with the fish sequences.

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Homo -----
Chimpanzee AGACGGTTTGACCGGGCCCTCCNTCACACCCC-TTNCCTTCTCANNCTCTCCCTCTTTC 59
Pygmy AGACGGTT-GACCCGGGCCCTCTCCACACCCCCTTCTTCTCACCTCCTCCCTCTTTC 59
Gorilla AGACGGTT-GACCTGGGCCCTCTCCACACCCCCTTCTTCTTCGCCTCCTCCCTCTTTC 59
Nomascus AGACGGTT-GACCCGGGCCCTCTCCACACCCCCTTCTTCTTCGCCTCCTCCCTCTTTC 59
Rhesus -----
Macaca -----GTT-GACCCGGGCCCTCTCCACACCCCCTTCTTCTTCGCCTCCTCCCTCTTTC 54
Papio AGACGGTT-GACCCGGGCCCTCTCCACACCCCCTTCTTCTTCGCCTCCTCCCTCTTTC 59
Pongo AGATGGTT-GACCCGGGCCCTCTCCACACCCCCTTCTTCTTCGCCTCCTCCCTCTTTC 59
Ailuropoda -----TGGACCTCTCCACACCCAATTCTTCGTCGCCCCCTCCCTCTTGC 47
Saimiri -AGACGGTTGACCCGGGCCCTCTCCACACCCCCTTCTTCTTCGCCCCCTCCCTCTTTC 59
Cavia -----
Sus -----
Ovis -----
Rattus -----
Mus -----
Mesocricetus -----
Danio -----

Homo -----AACGACG 7
Chimpanzee CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGCGTGGTGCCCCAGCAACGACG 119
Pygmy CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGCGTGGTGCCCCAGCAACGACG 119
Gorilla CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGCGTGGTGCCCCAGCAACGACG 119
Nomascus CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGACG 119
Rhesus -----GCGCGGGGTGCCCCAGCAACGAAG 24
Macaca CTGCACGGGGGCTTGGGCTCACTATAAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGAAG 114
Papio CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGAAG 119
Pongo CTGCACGGGGGCTCGGGCTCACTATAAAAACGTGGGAGCGCGGGGTGCCCCAGCAACGACG 119
Ailuropoda CTGTACTCGGGCTCTGGCTCTCTATAAAAAGGCGGGAGCGTGGGATGCCACAGCAGCTCGG 107
Saimiri CTGCACGGGGGCTCTGGCTCACTATAAAAACGTGGGAGCGCGGGGTGCCCCAGCAACGACG 119
Cavia -----CCGGGGCTCGGGCTCGCTATAAAAAGGCGGGAGAGAGGGGTGCCCGAGCAGCGACG 55
Sus -----
Ovis -----
Rattus -----AGCGAGG 7
Mus -----
Mesocricetus -----AGCGGGAGAGCGGGTGCCCCAGCAGCGACG 31
Danio -----CG 2

Homo AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 67
Chimpanzee AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179
Pygmy AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179
Gorilla AGTTTCAGAACCATGGAGAGCGCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179
Nomascus AGTTTCAGCACCATGGAGAGCTCCCGCATGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179
Rhesus AGTTTCAGCACCATGGAGAGCTCCCGCTGCGGCTGCTGCCCTCCTGGGCGCCGCCCTG 84
Macaca AGTTTCAGCACCATGGAGAGCTCCCGCTGCGGCTGCTGCCCTCCTGGGCGCCGCCCTG 174
Papio AGTTTCAGCACCATGGAGAGCTCCCGCTGCGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179
Pongo AGTTTCAGAACCATGGAGAGCTCCCGCTGCGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179
Ailuropoda AGTTTCAGCACCATGGAGAGCCCCGCTTTCGGCCGCTGACCCTCTTGGGTGCCGCCCTG 167
Saimiri AGTTTCAGAACCATGGAGAGCTCCCGCTGCGGCTGCTGCCCTCCTGGGCGCTCTCCCTG 179
Cavia GATTTTCAGAACCATGGAGAGCGCCCGCTTCGGCTGCTGCCCTCCTGGGCGCCGCCCTG 115
Sus --TTTCATCACCATGGAGAGCCCCGCTGCGGCTGCTGCCCTGCTAGTGGCCGCCCTG 58
Ovis -----ATGGAGAGCC----- 10
Rattus AAGTCCAGCACCATGGAGAGCTCCCGCTGCGGCTGCTACCCCTCCTGGGCGCCGCCCTA 67
Mus -----CAGAACCATGGAGAGCTCCCGCTGCGGCTGCTACCCCTCCTGGGCGCCGCCCTG 55
Mesocricetus AGGTCCAGCACCATGGAGAGCTCCCGCTGCGGCTGCTACCCCTCCTGGGCGCCGCCCTG 91
Danio ATGGTC-----GGAGAGC-----GGATCTT---TCTCTGACCGTACC-TG 40
*****

Homo CTGCTGATGTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCAGCTCCAGCCCCGA 127
Chimpanzee CTGCTGATGTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCAGCTCCAGCCCCGA 239
Pygmy CTGCTGATGTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCAGCTCCAGCCCCGA 239
Gorilla CTGCTGATGTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCAGCTCCAGCCCCGA 239
Nomascus CTGCTGATGTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCAGCTCCAGCCCCGA 239
Rhesus CTGCTGATGTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCAGCTCCAGCCCCGA 144
Macaca CTGCTGATGTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCAGCTCCAGCCCCGA 234

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Papio CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCAGCTCCAGCCCCGA 239  
Pongo CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCAGCTCCAGCCCCGA 239  
Ailuropoda CTTTTGCTGCTACCTCTGCTGGGTGCCCGTGCCAGGAGACGCTGAGCTCCAGCCCCGA 227  
Saimiri CTGCTGCTGCTACCTCTGCTGGGTACCCGTGCCAGGAGGACGCCAGCTCCAGCCCCGA 239  
Cavia TTCCTGATGCTACCTCTGCTGGGCGCCCGCGCAGGAGGACGCTGAGCTCCAGCCCCGA 175  
Sus CTGCTGCTGCTACCTCTGCTGGGCGCCCTTGCCAGGAGGATGCCAGCTCCAGCCGCGA 118  
Ovis -----ACGCCGAGCTCCAGCCGCGA 30  
Rattus CTGCTGCTGCTACCTTTGCTGGGTGCCGGTGCCAGGAGGATGCCAGCTGCAGCCCCGA 127  
Mus CTGCTACTGCTACCTTTGCTGGGTGCCCGTGCCAGGAGGACGCCAGCTGCAGCCCCGA 115  
Mesocricetus CTGCTCCTGCTACCTTTGCTGGGTGCCGGTGCCAGGAGGATGCCAGCTGCAGCCCCGA 151  
Danio CT-CTGTT-TTAATGCTGCTGTT-----TGCTGTGATG-----AA 74  
\*\*\* \*\* \*

Homo GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAG---AAGGAGCTG----- 178  
Chimpanzee GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAG---AAGGAGCTG----- 290  
Pygmy GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAG---AAGGAGCTG----- 290  
Gorilla GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAG---AAGGAGCTG----- 290  
Nomascus GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAG---AAGGAGCTG----- 290  
Rhesus GCCCTGGACATCTACTCTGCCGTGGAGGATGCTCCACGAA---AAGGAGCTG----- 195  
Macaca GCCCTGGACATCTACTCTGCCGTGGAGGATGCTCCATGAA---AAGGAGCTG----- 285  
Papio GCCCTGGACATCTACTCTGCCGTGGAGGATGCTCCACGAA---AAGGAGCTG----- 290  
Pongo GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAG---AAGGAGCTG----- 290  
Ailuropoda GCCCTGGACATCTACTCTGCCGTGGAGGATGCTCCATGAG---AAAGAGCTG----- 278  
Saimiri GCCCTGGACATCTACTCTGCCGTGGAGGATGCTCCATGAG---AAGGAGCTG----- 290  
Cavia GCCCTGGACATCTATTCGGCCGTGGAGGATGCTCCACGAG---AAGGAGCTG----- 226  
Sus GCCCTGGACATCTACTCTGCCGTGGAGGATGCTCCATGAG---AAGGAGCTG----- 169  
Ovis GCCCTGGACATCTACTCCGCCGTGGAGGATGCTCCATGAG---AAGGAGCTG----- 81  
Rattus GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCATGAG---AAGGAGCTGCCAAGG 184  
Mus GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAG---AAGGAGCTGCCAAGG 172  
Mesocricetus GCCCTGGACATCTACTCTGCCGTGGAGGACGCTCCACGAG---AAGGAGTTG----- 202  
Danio GTTCTGCAGATCCGCTCGCCTGAGGAGGATAAGGCTCAGGAGGAGAAAGAGCTG----- 128  
\* \*\*\* \* \*\*\* \*\* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*

Homo -----ATCGAAGCGCTGCAAGAAGTCTTGAAG 205  
Chimpanzee -----ATCGAAGCGCTGCAAGAAGTCTTGAAG 317  
Pygmy -----ATCGAAGCGCTGCAAGAAGTCTTGAAG 317  
Gorilla -----ATCGAAGCGCTGCAAGAAGTCTTGAAG 317  
Nomascus -----ATCGAAGCGCTGCAAGAAGTCTTGAAG 317  
Rhesus -----ATCGAAGCGCTACAGGAAGTCTTGAAG 222  
Macaca -----ATCGAAGCGCTGCAAGAAGTCTTGAAG 312  
Papio -----ATCGAAGCGCTGCAAGAAGTCTTGAAG 317  
Pongo -----ATCGAAGCGCTGCAAGAAGTCTTGAAG 317  
Ailuropoda -----ATCGAAGCGCTACAGGAAGTCTTGAAG 305  
Saimiri -----ATCGAAGCGCTGCAAGAAGTCTTGAAG 317  
Cavia -----ATTGAAGCGCTGCAAGAAGTCTTGAAG 253  
Sus -----ATTGAAGCGCTGCAAGAAGTCTTGAAG 196  
Ovis -----ATTGAAGCGCTGCAAGAAGTCTTGAAG 108  
Rattus CGGCAACTTCGGGCTCCGGCGCTGTGTTGCAGATTGAAGCGCTGCAAGAAGTCTTGAAG 244  
Mus CGGCAACTTCGGGCTCCGGCGCTATGTTGCAGATCGAAGCGTTGCAAGAAGTCTTGAAG 232  
Mesocricetus -----ATTGAAGCGCTGCAAGAAGTCTTGAAG 229  
Danio -----ATTGAAGCTCTTCAAGAAGTCTTGAAG 155  
\*\* \*\*\*\*\* \* \*\* \*\*\*\*\* \*\* \*

Homo AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 264  
Chimpanzee AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 376  
Pygmy AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 376  
Gorilla AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 376  
Nomascus AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 376  
Rhesus AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 281  
Macaca AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 371  
Papio AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 376  
Pongo AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 376  
Ailuropoda AAGCTCAAGACTAAAAGTATTCCTATCTATG-AGAAGAAGTACGGCCAAGTCCCCATGTG 364  
Saimiri AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 376  
Cavia AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 312  
Sus AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 255  
Ovis AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTATGGCCAAGTCCCCATGTG 167  
Rattus AAGCTCAAGAGTAAACGTATTCCTATCTATG-AGAAGAAGTACGGCCAAGTCCCCATGTG 303  
Mus AAGCTCAAGAGTAAACGTATTCCTATCTACG-AGAAGAAGTACGGCCAAGTCCCCATGTG 291  
Mesocricetus AAGCTCAAGAGTAAACGTATTCCTATCTACG-AGAAGAAGTACGGCCAAGTCCCCATGTG 288  
Danio AAGCTGAGAAATAAACAGATTCTGTG-CTGTGGAGAAAGAGCTCGGGTGGTTCCTGTG 214  
\*\*\*\*\* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \* \*

Homo TGACGCCGGTGAGCAGTGTGAGTGTGAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 324  
Chimpanzee TGACGCCGGTGAGCAGTGTGAGTGTGAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 436  
Pygmy TGACGCCGGTGAGCAGTGTGAGTGTGAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 436

Gorilla TGACGCCGGT GAGCAGTGTGCAGT GAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 436  
 Nomascus TGATGCCGGT GAGCAGTGTGCAGT GAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 436  
 Rhesus TGACGCTGGT GAGCAGTGTGCAGT GAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 341  
 Macaca TGACGCTGGT GAGCAGTGTGCAGT GAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 431  
 Papio TGACGCCGGT GAGCAGTGTGCAGT GAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 436  
 Pongo TGACGCCGGT GAGCAGTGTGCAGT GAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 436  
 Ailuropoda CCACGCTGGG GAGCGTGTGCAGT GAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 424  
 Saimiri CGACGCCGGT GAGCAATGTGCAGT GAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 436  
 Cavia TGACGCTGG GAGAACATGCGCAGT GCGAAAAGGAGCCAGGATCGGGAAGCTGTGTGACTG 372  
 Sus TGACGCAGG GAGCAGTGTGCAGT GCGAAAAGGAGCTAGAATCGGGAAGCTGTGTGACTG 315  
 Ovis TGACGCGGG GAGCAGTGTGCAGT GCGAAAAGGAGCCAGGATCGGGAAGCTGTGTGACTG 227  
 Rattus TGACGCTGG GAGCAGTGTGCAGT GCGAAAAGGGGCCAGGATCGGGAAGCTGTGTGACTG 363  
 Mus TGACGCTGG GAGCAGTGTGCAGT GAGGAAAGGGGCAAGGATCGGGAAGCTGTGTGACTG 351  
 Mesocricetus CGATGCTGG GAGCAGTGTGCAGT GCGAAAAGGGGCCAGGATCGGGAAGCTGTGTGACTG 348  
 Danio TGATGCAGG CAGCAGTGTGCAGT GCGAAAAGGCTCCCGCTTCGCGAGTTG 274  
 \* \* \* \* \*

Homo TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 384  
 Chimpanzee TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 496  
 Pygmy TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 496  
 Gorilla TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 496  
 Nomascus TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 496  
 Rhesus TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 401  
 Macaca TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 491  
 Papio TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 496  
 Pongo TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 496  
 Ailuropoda TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 481  
 Saimiri TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 496  
 Cavia TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 432  
 Sus TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 372  
 Ovis TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 284  
 Rattus TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 422  
 Mus TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 410  
 Mesocricetus TCCCCGAGG AACCTCCTGCAATTCCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTC 405  
 Danio TCCAGGAGG AACCCGCTGCAGTTCCTCCATCCTCAAGTGCTTGTGAGAGGG----- 325  
 \* \* \* \* \*

Homo TCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGAGGACCACA 428  
 Chimpanzee TCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGAGGACCACA 540  
 Pygmy TCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGAGGACCACA 540  
 Gorilla TCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGAGGACCACA 540  
 Nomascus TCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGAGGACCACA 540  
 Rhesus TCCTCCATACATCCCCA--TCCC-----TCTGCTTTCCCC-AGAAGACCATA 445  
 Macaca TCCTCCATACATCCCCA--TCCC-----TCTGCTTTCCCC-AGAAGACCATA 535  
 Papio TCCTCCATACATCCCCA--TCCC-----TCTGCTTTCCCC-AGAAGACCATA 540  
 Pongo TCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-GGAGGACCACA 540  
 Ailuropoda GGGTCCATAAATCCCCAC-TGCC-----CCTACTTTCCCCGAGAGGACCACA 527  
 Saimiri TCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGATGACCACA 540  
 Cavia TCCTGCATAT-TCCCCAT-TTCC-----CGTACTC-CACTGAGAGGGTCCACA 476  
 Sus TTCTCCTTAAGTACCCCC-CCCCAACACACACACACACACACCTTTCCCTC-AGACGACCATA 430  
 Ovis T-CTCCATAAG--CCCC-TCCC-----CACTTTCCCC-AGGGGACCACA 324  
 Rattus TCC---TTCGGTTCCCATATTC-----CTCTTTCCCCAAGAGGCGCT 464  
 Mus GCCACCTTCGGTTCCCATATTC-----CTCTTTCCCCAAGAGGCGCT 455  
 Mesocricetus TCCCCCTTCAGTTCCACATTC-----CTCTTTCCCC-AAAGGAGCACT 449  
 Danio -----AAAAA---CA 333

Homo CCTTCCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCTCTGAAGGG- 487  
 Chimpanzee CCTTCCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCTCTGAAGGG- 599  
 Pygmy CCTTCCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCTCTGAAGGG- 599  
 Gorilla CCTTCCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCTCTGAAGGG- 599  
 Nomascus CCTTCATCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCTCTGAAGGG- 599  
 Rhesus CCTTCATCCCTGGAGTTTGGCTTTAGCAACAGATAAAGTTTTATTTTCTCTGAAGGG- 504  
 Macaca CCTTCATCCCTGGAGTTTGGCTTTAGCAACAGATAAAGTTTTATTTTCTCTGAAGGG- 594  
 Papio CCTTCATCCCTGGAGTTTGGCTTTAGCAACAGATAAAGTTTTATTTTCTCTGAAGGG- 599  
 Pongo CCTTCATCCCTGAAGTTTGGCTTTAGCAACAGATAAAGTTTTATTTTCTCTGAAGGG- 599  
 Ailuropoda CTTTCATCCCTGGAGTTTGGCTTTAGCAACA---AAGTTTGCAATTTCCCTCTGAGGGTT 583  
 Saimiri TCTTCATCCCTGGAGTTTGGCTTTAGCAACA---AAGTTTGCAATTTCCCTCTGAAGGG- 599  
 Cavia CATTTCATCG-TGGAGTTTGGCTTTAGCAACAATAAAGTTTGCAATTTTCTCTGAGGGTG 535  
 Sus CTTTCATCCCTGG-----CTTTAGCAACA---AAGTTTGCAATTTTCTCTGAGAAAA 479  
 Ovis CTTTTATCCCTGGAGTCTGGCTTTAGCAACA---AAGTTTGCAATTTCCCTCTAAGGAAA 380  
 Rattus CTTTTGTCCTGGAGCC--GCTTTAACAACA-ATAAAGTTTGGCTTTCCCTCAGAGAGTG 521  
 Mus CCATTATCCCTGGAGCTGGCTTTAGCAACA-ATAAAGTTTGGCTTTCCCTCAGAGAGCG 514  
 Mesocricetus CTTTTATCCCTGGAGTCTGGCTTTAGCAACA-ATAAAGTTTGCATTTCCCTCCGAGGGTG 508  
 Danio GCCTCA-----GTCTCTCATAATG-----CTCC----- 356  
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Homo AAAGGGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTG 545  
Chimpanzee AAAGGGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTG 657  
Pygmy AAAGGGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTG 657  
Gorilla AAAGGGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTG 657  
Nomascus AAAGGGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTG 657  
Rhesus AAAGGGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTG 562  
Macaca AAAGGGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTG 652  
Papio AAAGGGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTG 657  
Pongo AAAGGGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTG 657  
Ailuropoda AAGGGGCTTTTT-CCTG-CTGTTCCAAA-T-AAAAGAACACATTAGATGTTACTGTGTG 639  
Saimiri AAGGGGCTTTTTACCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTG 655  
Cavia AAGGGGCTTTTTCTCTG-CTGTTTTAAAAATAAAAAGAACACATTTGATGTTACTGTGTG 594  
Sus AGGGG-CTCTTTTCCTGGCTGTTCCAAA-TAAAA-GAACACATTAGACGTTTCTGTGTG 536  
Ovis AGGGGGCTCTCTTCTG-CTGTTTCCAAA-TAAAAAGAACACATTCGATGTTACTGTGTG 438  
Rattus GATGGGCTCTTTCCCTG-CTGTTTCAAAA-T-AAAAGA----TTTGATGTTACTGTGTG 573  
Mus GATGGGCTCTTTCCCTG-TTGCTTCAAAA-T-AAAAGA----TTTGACATCA----- 559  
Mesocricetus GATGGGCTCTTTCCCTG-CTGTTTCAAAA-T-AAAAGAACACATTTGACGTTACTGTGTG 565  
Danio AGTGTGTTGTTTACCAT----CATCAAACT-----GTAACATGTT---TATCAGTACATA 405

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Homo AAGAATAATGCCT-GTATGGTGTGAT-ACGTGTGTGAAGTATC--TA-----TTTA--T 594  
Chimpanzee AAGAATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTCTTA-----TTTTATT 711  
Pygmy AAGAATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTCTTA-----TTTTATT 711  
Gorilla AAGAATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTCTTA-----TTTTATT 711  
Nomascus AAGAATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTCTGA-----TTTTATT 711  
Rhesus AAGGATAATGCCTTGTGTGGTGTGAT-ACGTGTGTGAAGTATTCTTA-----TTTTATT 616  
Macaca AAGGATAATGCCTTGTGTGGTGTGAT-ACGTGTGTGAAGTATTCTTA-----TTTTATT 706  
Papio AAGGATAATGCCTTGTGTGGTGTGAT-ACGTGTGTGAAGTATTCTTA-----TTTTATT 711  
Pongo AAGAATAATGCCTTGTATGGTGTGAT-AAGCGTGTGAAGTATTCTTA-----TTTTATT 711  
Ailuropoda AAGGATAATGCCTTGTATGGTGTGAT-ACATGTGCAAAGTATTCTTT-----TCTTATT 693  
Saimiri AAGGATAATGCCTTGTATGGTGTGAT-ATGTGTGCAAAGTATTCTTA-----TTTTATT 709  
Cavia AAGGGTAATGTTATGT-TGGGGTTGAT-AGGTGTGCAAAGTATTCTTCTTCTGTTTTATT 652  
Sus AACAGTAATGCCTTGTATGGTGTGAT-ATGTGTGCAAAGCATTCT-----TATTTTATC 590  
Ovis AAGGGTAATGCCTTGTATGGTGTGCT-ATGTGTACAAAATATTCTATCTTTTTTTCTC 497  
Rattus AAGGACAATACCTTGAATGGTGTGGT-ATGTGTGCAAAGTATTCTTCTCTGTTTTATC 632  
Mus -----AAA----- 562  
Mesocricetus AAGGACATTGCCTTGCATGGTGTGGTATGTGTGCAAAGTATT-TTTTCTGTTTTATT 624  
Danio CAGACGAAT-----GTATAA-----ATGCATTCAAA----- 431

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Homo TGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGAAGCTTTG-TTTGAAAA 649  
Chimpanzee TGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGAAGCTTTG-TTTGAAAA 766  
Pygmy TGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGAAGCTTTG-TTTGAAAA 766  
Gorilla TGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGAAGCTTTG-TTTGAAAA 766  
Nomascus TGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGAAGCTTTG-TTTGAAAA 766  
Rhesus TGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGAAGCTTTGCTTTGAAAA 672  
Macaca TGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGAAGCTTTGCTTTGAAAA 762  
Papio TGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGAAGCTTTGCTTTGAAAA 767  
Pongo TGTCTGATAAACTCTT----GTGTACATTTGTGTAAAGAAGGAAGCTTTGCTTTGAAAA 767  
Ailuropoda CATCTGACACACTCTT----ATGTACTTTGTGTA----- 724  
Saimiri TGTCTGATAAACTCTT----GTGTACCTTTGTGTAAAGAAGGAAGCTTTGCTTTGAAAA 765  
Cavia TGTCTGATAAACTCTT----GTGTACTTTTGGGTAAGAAGGAAGAAATTTGCTTTGAAAA 708  
Sus TGCCTGACAAACTTTT----GTGTATATTTGAGTAAAGAAGGGGAACCTTCACTCAAAT 646  
Ovis TGTCTGACAAACTTTTAAAGTGGATATTTAAGCAAAGAGGAAGAACTTCACTTTGAAAA 557  
Rattus CACCTGACACATTCTT----GTG-ACCTTTCTGGGAAGAAGAGGACTTTGCTTTAAAA 687  
Mus -----AAAAA----- 577  
Mesocricetus CATCTGACACATTCTT----GTG-AATCTTTC-----TGGGAAGAA-TTTTGTCTTTAAAA 673  
Danio --TCAAATAAATGC-----ATGCAGTTTTGTATAT----- 459

Homo TTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGAT 708  
Chimpanzee TTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGAT 825  
Pygmy TTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGAT 825  
Gorilla TTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGAT 825  
Nomascus TTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTTGGTAGAT 825  
Rhesus TTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGG-CTAGCTAA-TCTTGGTAGAT 730  
Macaca TTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGG-CTAGCTAA-TCTTGGTAGAT 820  
Papio TTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGG-CTAGCTAA-TCTTGGTAGAT 825  
Pongo TTGTATTTTTGTGTGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTTGGTAGAT 826  
Ailuropoda -----  
Saimiri TTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TGTGGTAGAT 824  
Cavia TTGTATTTTTGTATGTGGCATGGCAGAGTAAAATTAGATCTAGTTAA-ACCTGG----T 763  
Sus TTGTATTTTTGTAGGTGGCATGGCAGGATGAAA-CTAGATCTAGTAA-TCTTGGTAGAT 704  
Ovis TTGTATTTTTGTAGGTGGCATGGCAGGATGAAAATTAGATCAGGTAATACTTGCACAGAT 617  
Rattus CTGTATTTTTGTATGTGGCGGTCACAAATGAAGATTAGACCTAGTTAA-TTTTGGCAGAT 746  
Mus -----  
Mesocricetus CTGTATTTTTATATGTGGTGTGTCAGAATGAAAATTAGATCTAGTTAA-TTTTGGTAGAT 732

Danio	-----	
Homo	GTCATTACAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAAT	767
Chimpanzee	GTCATTACAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAAT	884
Pygmy	GTCATTACAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAAT	884
Gorilla	GTCATTACAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAAT	884
Nomascus	GTCATTACAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAAT	884
Rhesus	GTCATTGCAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAAT	789
Macaca	GTCATTGCAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAAT	879
Papio	GTCATTGCAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAAT	884
Pongo	GTCATTATAACCTGGAAAATAAATCACCC-TATGTGACACAAATTGAAGCATGTACAAAT	885
Ailuropoda	-----	
Saimiri	GATATTACAATCTAAAAACAATCACCC-TAAGTAACACAACTGAAGCATGTACAAAT	883
Cavia	GACATCACAATCTGGAAAAT-AATCACCC-TAAGTAACACAAA-----	804
Sus	GACATCACAACCTGGAAAATAAATTACCC-CAAGCATCACAAATTGAAGCATGTAAAAAC	763
Ovis	GACATCACAACCTGGAAAATAAATCACCC--AAGTATCACAAATTGAAGCATGTACAAAT	675
Rattus	GACATCATAACCCGGAAAACAATCACCCCAAAGCAACACAAATTGAAGCATGTGCAAT	806
Mus	-----AAAAAA-----AAAAAA-----AAA	601
Mesocricetus	GACATCACAACCTGGGAGACAATTACCC-TAAGCAACACAAATTGAAGCATGTACAAAT	791
Danio	-----	
Homo	TATACATAATAAAGTGTTTTTAATAATTA-----	796
Chimpanzee	TATACATAATAAAGTGTTTTTAATAATTGCCATA	919
Pygmy	TATACATAATAAAGTGTTTTTAATAATTGCC---	916
Gorilla	TATACATAATAAAGTGTTTTTAATAATTGCC---	916
Nomascus	TATACATAATAAAGTGTTTTTAATAATTGCC---	916
Rhesus	TATACATAATAAAGTGTTTTTAAT-----	813
Macaca	TATACATAATAAAGTGTTTTTAATAATTGCC----	910
Papio	TATACATAATAAAGTGTTTTTAATAATTGCCCA--	917
Pongo	TATATATAATAAATGTTTTTAATAATTGCC---	917
Ailuropoda	-----	
Saimiri	TATACATAATAAATGTTTTTATTATTA-----	910
Cavia	-----	
Sus	TATACATAATAAAGTGTTTTTCATAATTGCCCTA-	797
Ovis	TATACATAATAAAGTGTTTTTCATAATT-----	703
Rattus	TACACCAATAAAGCATTTTTGATAATTGCTCAC-	840
Mus	-----AAAAA-----	607
Mesocricetus	TATATATAATAAACGTTTTTAATAATTGCTCAC-	825
Danio	-----	

**Supplementary Figure 30.** ClustalW alignment of *Danio rerio* CART1 nucleotide sequence against *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, *Sus scrofa*, *Macaca mulatta*, *Ovis aries*, *Cavia porcellus*, *Ailuropoda melanoleuca*, *Pongo abelii*, *Macaca fascicularis*, *Saimiri boliviensis boliviensis*, *Pan troglodytes*, *Pan paniscus*, *Gorilla gorilla gorilla*, *Nomascus leucogenys*, *Papio anubis*, and *Mesocricetus auratus* CART sequences. (\*) identical sequences are indicated. The zebrafish sequence has an average 52.19% sequence identity with these species and 52.50% similarity to *Homo sapiens*.

# CART3

Pundamilia	-----GCGGCCCTCCCGGGCGGGG	18
Oreochromis	CTTCTGCCCCCTTTTCAGTACTTTCCGGCACGGTAGTCTCGCAGCGGCCCTCCCGGGCGGGG	60
Carassius	-----CTCGA	5
Danio	-----	
Tautogolabrus	-----A	1
Xiphophorus	-----G	1
Salmo	-----ACACAGATAAA-----AG	13
Silurus	-----ACGCGGGGGAATCCGAG	17
Leucoraja	-----CAATCCCAACCCCG	14
Oryzias	-----TCCA-----CA	6
Haplochromis	-----GAGGAGACAGACAAACCAACA	21
Maylandia	-----AGACAGACAAACCAACA	17
Pundamilia	CT-GTGTGAACCGGTATAAGAGGGGACATGGTCCGGAGG----GTGGAGGGAGAGTCACC	73
Oreochromis	CT-GTGTGAACCGGTATAAGAGGGGACATGGTCCGGAGG----GTGGAGGGAGAGTCACC	115
Carassius	GT-GTTCAC-----TTGATCT-----CAGTGCAGAGGACGCT	37
Danio	-----	
Tautogolabrus	GC-ACAGGG-----ACGAT-----GAGGAGCA	22
Xiphophorus	CC-ACA-----TGTC-----GCCGCGCA	18
Salmo	GT-GTTTGG-----ATTGCGGTAACCTGGAGTATTAGAACGGGACCTTTTG-GAGACGAA	65
Silurus	GC-GCGCAAT-----AAAAAGCCCGTGCATCCT-GAGCGCGGTGTTGGAGAGTCGCG	70
Leucoraja	GT-GTGT-----GTGGTTCG-----GCCCT--GAGTCGC-	41
Oryzias	GG-ACGC-----GTGATTCCTCAATT--GTCTCCAAAGAGTTGCA	42
Haplochromis	CTCATGAGG-----AGGAAGGAGAGGGAGAGGAAAAGCTAAAGAGAAAACA	66
Maylandia	CTCATGAGG-----AGGAAGGAGAGGGAGAGGAAAAGCTAAAGAGAAAACA	62
Pundamilia	TG-----TGGATCTGCTGCGACACTGTGCTCCTCAGC	105
Oreochromis	TG-----TGGACCTGCTGCGACACTGTGCTCCTCAGC	147
Carassius	TG-----CTGTGACCATGGAG---AGC	56
Danio	-----	
Tautogolabrus	-----CAGGGAGCACGAGG---AGC	39
Xiphophorus	-----CCGGGACCATGCAG---AGC	35
Salmo	AGATATCTTCTACTGTGCGCAA-----TCTCT-----TAAGGACCATGGAG---AGC	109
Silurus	CG-CGCCTTC---GTGCACAAAAGCCCGTTTCGA---TCAGCACCATGGAG---AGC	118
Leucoraja	-----TTCAGCACCATGGTC---AGC	59
Oryzias	GA-----AACAAAG---GTTTTGGTTTTTCAGAACCATGGTC---AGC	79
Haplochromis	AC-----TACCTGACTGAAACCAGACACCACAGAAGA-AGA	101
Maylandia	AC-----TACCTGACTGAAACCAGACACCACAGAAGA-AGA	97
Pundamilia	CTCTAATCTGTGCTGCCCTCCCACTGGGACCTCCGCCCTTCC-GCTTCAC---CGCCACCAGC	161
Oreochromis	CTCTAATCTGTGCTGCCCTCCCACTGGGACCTCCGCCCTTCC-GCTTCAC---CGCCAACAGC	203
Carassius	-TCCAAACTCTGGACCACAGCGATGGCATGCGCGGTGCTGGTG-----TCCTGC	104
Danio	-----	
Tautogolabrus	-TGCAGGCTGCTG-----CTCTGCGTGCTGCT-GCT-CTCAGGATCCACCAGG	84
Xiphophorus	-TCCAGGATGCTGAGCGGCGCGCTCCTCTGCGCGTGTGCT-GCTGCTC---TCCGCCGGC	89
Salmo	-TCCAGGCTATGGACCAGAGCGGTGTTGCGCGGTGTT-GCT-----GTCCATCGT	159
Silurus	-TCGAG--TATCGCC---GCG---CGCGCTCTGCT-GCT-----CCTGCTCGT	156
Leucoraja	-G-----ACAGGCT---C-----CTGCTGCTGTCTACT-----TTGCGTGT	93
Oryzias	-GC-----GCGGACT---CT---CCTCCTCGTGGTC-ACT-----TGCAGCTGC	115
Haplochromis	GTTTGTGACACCTTCCTG-GAGGACCTTTATTAGTTTAAAGTT-----TTTAAAGGT	152
Maylandia	GTTTGTGACACCTTCCTG-GAGGACCTTTATTAGTTTAAAGTT-----TTTAAAGGT	148
Pundamilia	ACCATGGATAGCTCCGGGATGCTCCGCG-----GGCTGCTGCTCG-----TCGGCCT	208
Oreochromis	ACCATGGATAGCTCCGGGATGCTCCGCG-----GGCTGCTGCTCG-----TCGGCCT	250
Carassius	AT--TCAGGG---TGCCGAGATGG-----ACTTTGACA-----ACG---135	
Danio	-----	
Tautogolabrus	GC--AGACCTGA--CGGAGAACAA-----CTCCCTGAC-----CAG----116	
Xiphophorus	GC--CG-CCGGAGCCGGAGCGCAGG-----GGCTGCTGGATG-----CGGAGTC	130
Salmo	TC--TTAGTG---CTG--AAATG-----ACTACTCGGATT-----CCG---191	
Silurus	A---TTAATG---CTGCGGAGTGTGCGCGAGGAGACAACGAGGACA-----CCG---200	
Leucoraja	TG--TTCTCC---ATG---GCTGT-----TG-GAGCA-----GAG---119	
Oryzias	AC--CTACCT---TTG---GCTTT-----CCCGAGCC-----GAG---142	
Haplochromis	GCTTTTGGAGA---TGGAGAGCAAA-----CGAGCCGTGGTCTATCTGTCCGTCGTG	200
Maylandia	GCTTTTGGAGA---TGGAGAGCAAA-----CGAGCCGTGGTCTATCTGTCCGTCGTG	196
Pundamilia	GCTGTCCGTCCTGT-----GCCACGGCCAGGCATCACGGGAAGTCTCCG-----252	
Oreochromis	GCTGTCCGTCCTGT-----GCCACGGCCAGGCATCACGGGAAGTCTCCG-----294	

Carassius	--AATCCGACTTAG-----AAACGAG---AGCCCTGAGAGAGTTTTACC-----	174
Danio	-----	
Tautogolabrus	TGAGGACGAGCTGA-----GCCCCAG---AGTGTGCGCCACTTCTACT-----	157
Xiphophorus	CGAGGAGGAGCTGA-----GCCCCAG---AGCGCTGCGGGACTTCTACC-----	171
Salmo	-AATT-GGACCTCG-----ACACAAG---AAGTGTGAGAGACTTCTACC-----	230
Silurus	-AAGT-GGACTTGG-----ATACAAG---AGCCATCCGAGACTTCTATC-----	239
Leucoraja	-AACTCGGACCTGG-----AGCCAAG---AGCCCTGCGAGACTTCTACT-----	159
Oryzias	-GACTCTTCCCTGG-----AAAC--G---CGCTCTCTGGA--TTTTACT-----	178
Haplochromis	TCTGTCTGTGCTGACATCTCTGTCAAGGCCAAAGGTCAGGCAACAGCCACCTGTGTGTC	260
Maylandia	TCTGTCTGTGCTGACATCTCTGTCAAGGCCAAAGGTCAGGCAACAGCCACCTGTGTGTC	256
Pundamilia	-----CAGAAGACTTTGGAGAG---AAGAAACAAGAAGC-AGCTGT-----	289
Oreochromis	-----CAGAAGACTTTGGAGAG---GAGAAACAAGAAGC-AGCTGT-----	331
Carassius	-----CAAAGGACCC-GAATCT---GACCAACGAGAAGC-AGCTCGTAAGTATTGCGCAC	224
Danio	-----	
Tautogolabrus	-----CCAAAGGACC-GAACCT---GACCTCAGAGAAAC-AGCT-----	191
Xiphophorus	-----CGAAAGGACC-GAACCT---GACCAGTGAAAAGC-AGCT-----	205
Salmo	-----CCAAAGATCC-GAATTT---GACCAACGAAAAGC-AACT-----	264
Silurus	-----CGAAAGACCC-CAATCT---GACCAGCGAAAAC-AGCT-----	273
Leucoraja	-----CCAAAACCTA-TTATCC---CGCGAGCGAGAAGG-AACT-----	193
Oryzias	-----CTGAAAGCT-----CAGCAA-GAAAAGG-ATCT-----	204
Haplochromis	CGCGCCTGATGACCC--AACCTCGGACTCACAACCAGTGAGCT-----	302
Maylandia	CGCGCCCGATGACCC--AACCTCGGACTCACAACCAGTGAGCT-----	298
Pundamilia	-----	
Oreochromis	-----	
Carassius	ATTCTCGGCGCATAGAAAACAATGACGTTCCCTTGTTTAAAAAGATTTTAGTGATACTT	284
Danio	-----ATAGA-----	5
Tautogolabrus	-----	
Xiphophorus	-----	
Salmo	-----	
Silurus	-----	
Leucoraja	-----	
Oryzias	-----	
Haplochromis	-----	
Maylandia	-----	
Pundamilia	-----AGACAGAGACCTGCTAGAGGCACTGGA-CGTGCT	322
Oreochromis	-----AGACAGAGACCTGCTAGAGGCACTGGA-TGTGCT	364
Carassius	ACCTGCATTGATTGAACATTTTACAGCTCGGGCTTTACATGACGTTCTGGA-GAAACT	343
Danio	-----GGCATTGCAAGAGGTTCTTGA-GAAGTT	32
Tautogolabrus	-----GTCCGGAGCTCTGCAGGAAGTTCTGGA-GAAACT	224
Xiphophorus	-----GCTTGGAGCTCTCCAAGAAGTTCTTGA-AAAGCT	238
Salmo	-----GCTTGGAGCACTACATGACGTTCTTAA-AAAGCT	297
Silurus	-----GCTTGGCGCTTTGCAATGATGTTCTTGA-AAAGCT	306
Leucoraja	-----GCTCGGGGCTCTACACGAGGTGCTGAA-GAAACT	226
Oryzias	-----GATCGACGCCTTGCAAGGAGTTTTAGA-AAAGCT	237
Haplochromis	-----GGCTGAAGCTCTGCAGGGTCTTCTGGATGAAGCT	336
Maylandia	-----GGCTGAAGCTCTGCAGGGTCTTCTGGATGAAGCT	332
	* * * * *	
Pundamilia	CCTGGGCAGAAACATAATCAAGTCTCCTCCCCTGAGAAAAGAGG---AAGCA---TCCC	376
Oreochromis	CCTGGGCAGAAACATAATCAAGTCTCCTCCCCTGAGAAAAGAGG---AAGCA---TCCC	418
Carassius	-----GCAGAG---TAA--ACGCATTTCACTTTGGGAAAAGAAGTTTGGGCGCGTTCCT	392
Danio	-----AAAGAA---CAA--ACA-ACTTC-----	49
Tautogolabrus	-----TCAGGC---GAA--GAAAACGTCTTCATGGGAGAAAAGTTTGGCCAAGTCCCC	273
Xiphophorus	-----GCAGGC---TAA--ACGACTTCCATCGTGGGAGAGAAGAAATTTGGTCAAGTCCCA	287
Salmo	-----GCAGAC---CAA--GAGACTTCCATTTGGGAAAAGAATTTGGCCAAGTCCCT	346
Silurus	-----TCAGAT---GAA--AAGAATTCCTCCTTGGGAGAGAAGAAATTTGGGCAAGTTCCT	355
Leucoraja	-----GCAGAC---TAA--GCGGCTTCCAACCTGGGAGAGAAGTATGGACAGGGGCCT	275
Oryzias	-----GAGAAA---CAA--AGAAATGCCA--CTGG--AGAAGAAGCTCGGATGGTTGCCT	283
Haplochromis	----GACAGCT---CAG---CCGGTCTCTCTGTGGAGAAAAGGCC--AGCGTGATCCC	383
Maylandia	----GACAGCT---CAG---CCGGTCTCTCTGTGGAGAAAAGGCC--AGCGTGATCCC	379
	*	
Pundamilia	ACTGTG--CGGGTTGGGGAACCGGT---GTGCCATGAAGTACGG---G---CCTCGC	422
Oreochromis	ACTGTG--CGGGTTGGGGAACCGGT---GTGCCATGAAGTACGG---G---CCTCGC	464
Carassius	ACAGTGAGTAGCGCAAAACAGATTTTGGAGTAAATAGTGTAAAATATTGTAATGCCTAG	452
Danio	-----CACAAACCGGA-----AAAAAGTTGA---GCCTA-75	
Tautogolabrus	AG-GTG--TGACGTCGGGAGCAGT---GTGCAGT-GAGAAAAG---G---CTCTCGG	318
Xiphophorus	AC-GTG--TGACGTTGGAGAGCAGT---GC GCGGT-GAGGAAAG---G---CGCACGA	332
Salmo	AC-GTG--CGACGTTGGAGAGCAGT---GCGCAGT-GAGAAAAG---G---CGCGAGA	391
Silurus	AT-GTG--CGACGTTGGAGAGCAGT---GCGCAAT-CCGTAAG---G---CTCCAGA	400
Leucoraja	CA-GTG--TAACATCGGAGACATGT---GTGCAGT-GAGAAAAG---GA--CCCAGGA	321

Oryzias	TC-ATG--TGACGCAGGAGAGCCGT----GCGCAGT-GCGTAAAG----G---TGCGCGC	328
Haplochromis	GCGGTG--TGATGTGGGTGAGCGGT----GTGCGATGAAACACGGA-----CCTCGG	429
Maylandia	GCGGTG--TGATGTGGGTGAGCGGT----GTGCGATGAAGCACGGA-----CCTCGG	425
	* * *	
Pundamilia	ATCGGGA----AGCTCTGCGACT-----GTGGAAGAGGAGCCAA-----CTGCAAC	464
Oreochromis	ATCGGGA----AGCTCTGCGACT-----GTGGAAGAGGAGCCAA-----CTGCAAC	506
Carassius	ATTAAATCACACATTTACTATTAAACAAAACGCTGACAGTGAGTTATCTAACACTGACAC	512
Danio	-----C	76
Tautogolabrus	ATCGGCA----GGATGTGTGACT-----GTCCTCGTAGAGCGTT-----CTGTAAC	360
Xiphophorus	ATCGGGA----AGATGTGCGACT-----GTCCCGGGGAGCTTT-----CTGCAAC	374
Salmo	ATCGGCA----AAATGTGCGACT-----GTCCTCGGGAGCTTT-----CTGCAAC	433
Silurus	ATTGGAA----AAATGTGCGACT-----GCCACGAGGAGCCTT-----CTGCAAC	442
Leucoraja	TTTGGAG----GACC-TGTAAC-----GCCT-----GAGTTCGAA---ATGTAAT	359
Oryzias	ATCGGAA----CACTGTGCGCT-----GTCCTCGAGGACTTC-----ATGTAAC	370
Haplochromis	ATTGGTC----GGCTCTGCGACT-----GTCTGAGAGGAACAGC-----CTGCAAC	471
Maylandia	ATTGGTC----GGCTCTGCGACT-----GTCTGAGAGGAACAGC-----CTGCAAC	467
Pundamilia	TCCTACCTCCTCAAGTGCA-TCTAAACC-----GCCAGGGCTCT-----	503
Oreochromis	TCCTACCTCCTCAAGTGCA-TCTAAACC-----GCCAGGGCTTT-----	545
Carassius	TCCGCTTCACATCAGTGCGACGTCGGG-----GAGCAGTGCGCC-----	551
Danio	TTC-CTC-----GTGCGATGCGAGT-----GAGCAGTGTGCA-----	108
Tautogolabrus	TTCTGCCTGCTGAAGTGTT-TATGAG-----AAACACACACAC-----ACA	400
Xiphophorus	TTCTTCTGCTGAAGTGCT-TATGAGCCTCTCCGGATCGGAATGTAGCTTCGGGAAGAGA	433
Salmo	TCTTATCTGCTCAAGTGCT-TGT-GAATTGAATTGGTCTGAATGTAAGTTA-----G	483
Silurus	TTTTTCTTCTGAAAATGTC-TGT-GAG--GAG-----GAAGCAACTT-----	481
Leucoraja	TACTTTCTGTTAAATGTG-TATAGAAATCAGA-----CAAATCATTT-----	402
Oryzias	TTCTACGTTCCTCAAAATGTT-TATAGGAGTAAGA-----AAAGA--GTTT-----	411
Haplochromis	ACCTTCTTCTGCGCTGCT--ACTGACCACTC-----GCACACATTTTC-----	513
Maylandia	ACCTTCTTCTGCGCTGCT--ACTGACCACTC-----GCACACATTTTC-----	509
	**	
Pundamilia	---CCTTTAATTTGTC-----ACCTCTGCTATGCCAAACACATCCTTCAGCACA	549
Oreochromis	---CCTTTAGTTTGT-----AGCTCTGCAGGGCCAAACACATTTCTTCAGCACA	591
Carassius	---ATCAGGAAAGGGTC-----AAGAATCGGCAAAATGTGCGACTGTCCACGT-----	596
Danio	---ATACGTAAGGTGC-----AAGAGTTGGCAAGCTATGCAGCTGTCCACAA-----	153
Tautogolabrus	AGTCTTTTATTAATA-----GGTCACATTT-----AATGAGAAC-CAGCTGA	440
Xiphophorus	AGACTTTCACCTGAACCCCTTTGTAATTACGCTCAGATTTTTTTTATTTACTGCACAGCTCA	493
Salmo	AAGTTGATGGCAAACTA-----AA--ACTGTTACATCA---GAACGACATG-TGATATA	531
Silurus	---CTGTAGCGG-CTC-----AA--ATTGATGTATTC---AATTTATTTG-T---ATA	522
Leucoraja	-CTATTTTCATCGGCT-----AA--ATGAAGACAGT--TTAA-AGATCTGGT-----G	444
Oryzias	-ATTTTAGAGTTGG-----AA--ATTATGACAATAATGAATAAGTATGGCATCAAG	459
Haplochromis	---CTCACGAGGA-----CCTAAACTC-----GGTCTG-----	538
Maylandia	---CTCATGAGGA-----CCTAAACTC-----GGTCTG-----	534
Pundamilia	GCTCC----CATAAACCAC-----CCTT-----TCATT-----	574
Oreochromis	GCTCC----CATAAACCAC-----CAACCCTGTCA---TCATT-----	624
Carassius	GGAGCGT--TTTGCAATTACTTTTTG----CTGAAATGTTTGTGAATGGATTGTG--AAT	648
Danio	GGA-----	156
Tautogolabrus	GGAGAAT--GCTC-AGTGACCGTC-----TGCAACACACGAAGAAGAACTACA-TGGT	489
Xiphophorus	GGCGCA---GCTGGAGTGACTGTCCAACACTTACAACAACACTGTAGAA--ATTGTA-TTAT	547
Salmo	GGAATAT-TGTTATGGTAC-----TACAATATAC-GATTA--TCCGGA-GAGT	574
Silurus	GATATTT-TTTA-AGTGC-----AAGAACACGCTGAAGA--GATGT--GTG	564
Leucoraja	GATATA--TTATA-AAATA-----ACAA--AGCATT--GT	472
Oryzias	GGTGCAAAATCATA-AGTAA-----TTTTCTGTCTCATAA--ATCATTCAAAT	504
Haplochromis	GGCGC---TCACACAGACA-----GTGCTCAGAAAGA-----	567
Maylandia	GGCGC---TCACACAGACA-----GTGCTCAGAAAGA-----	563
	*	
Pundamilia	---TATAGTCATAGC-TGTTGTTA-AACAGTTGTCGCTGAGGATAAGCATGGATTT-TG	628
Oreochromis	---TATTGCCATAGC-TGTTGTTA-AACAGTTGTCGCTGAGGATAAGCATGGATTT-TG	678
Carassius	TGGAAATA-TACA-CATGATATTACAGATATTATACATTTATG-----TGATTTCTA	698
Danio	-----ACATCATG-----CCATTT---	170
Tautogolabrus	---GTTAACAGAGTCTG-TGTGAGGATTGTT--GTGATGAGAT-----GGTGAGTT-CA	537
Xiphophorus	TAGGAGTAAAACATTTCCG-TGTTA---TTTT--ACAACAAAA---AATGACAT-GA	594
Salmo	GACAGGT--TGCA-TTC--TGAGA-GATTGTCCAAACCCCAAAAAG---GCCATTT-TG	624
Silurus	GTGAGTC--TACAGTCC--TGTTT-ATCTGTTTCACTGCACACTGCAC-----GCCTATAA-TA	613
Leucoraja	CAACATTCCTACTACAT--CG---AACAAATC---CTGTGCAAT-----TTATCC-TG	516
Oryzias	CCAAATCAATGCATCAT--TGGCAAATCAGTTCA-GCTGGAAAATGTGGGGTCATTT-GA	560
Haplochromis	---CAGAAGTGCACTCTT-CGCCA--ATCGTCT-CGCTCCATATT-----TTAGTCT--	612
Maylandia	---CAGAAGTGCACTCTT-CACCA--ATCGTCT-CGCTCCATATT-----TTAGTCT--	608
Pundamilia	TACTGCTGACAGTACTG-----AATA-----TGTTGTG-----	656
Oreochromis	TACTGCTGACAGTACTG-----AATA-----TGTTGTG-----	706

Carassius	TTTTTATGA--TTATGA-----AGCG-----CTGTGAGAAGAACAGGG	734
Danio	-TTTTAT-----	176
Tautogolabrus	AAGTTGTGATATCATGTG-----AACAA-----A-----ACAAATATTTG--	571
Xiphophorus	GGATGAGAATATCATAA-----AACG-----TTTCTGTAATATTT--	630
Salmo	TAGTTATGA-AATATTTTATTTCAACTGAATG-----TTTCTGTAACCAAAAATG	674
Silurus	ATGTCATGA-A-TATGT-----AACAA-----TGTCIGTTA-----	641
Leucoraja	TT-CTGTGT-GT-ATA-----TTCCAGTG-----CCCATGGAAAA-----	549
Oryzias	CC-CTAAAA-ATTATAGC---CTAATTTAAGTAAAAGGGGACTCTCTTTAGGAAA-----	610
Haplochromis	TTGTTTAAAGTTTTTG-----GTG-----TTTGTC-----	638
Maylandia	TTGTTTAAAGTTTTTG-----GTG-----TTTGTC-----	634
Pundamilia	---TTTAATG-----TAAA-----CGTTC-ATCTTC-----	679
Oreochromis	---TTTAATG-----TAAA-----TGTTTC-ATCTTC-----	729
Carassius	GAATTGAAAGATTGTTGATTATTTGCTGCTTGATGTGT-----	776
Danio	---TTTAAAA-----TGCCTT-----	189
Tautogolabrus	-AGTCTAATG--TCGG--AG-TCTGTCTGTGTC--ATTATAA-----	603
Xiphophorus	-ACTTCAACT--TCAATAAAC-TCAACCCGTGGG-ACCATAAGCAC-----	671
Salmo	AGTTGTGCTA--TGAATAAACAGCATCTATCTT-GTTTTTT-----	713
Silurus	---TGTC-TA--T---TAAAGTGCAACGTACATT-TTTTTGT-----	673
Leucoraja	--ATTCATTA--T---TAAATAT---GCTATCCT-ATTTCTT-----	580
Oryzias	--TTTC--TG--T---TAAGTATCAAACTTTCTTATTTTTTATTTGAGAAATCCGTAGT	661
Haplochromis	---TTTCAGA---TAAAT-----TTATTTTTGTT-----	661
Maylandia	---TTTCAGA---TAAAT-----TTATTTTTGTT-----	657
	*	
Pundamilia	-----AATAAACAC-----	688
Oreochromis	-----AATAAACACGTCAACATATAA-----	750
Carassius	-----AATTAAATGGAATAAATGTGTGGACTTTT	807
Danio	-----	
Tautogolabrus	-----TAAT-TCATATTCATA-ATAAAGTGC GGATCAT-	634
Xiphophorus	-----ACTGTAATGTCAAACCAGCA-ATATTATGCCCTTCATC	708
Salmo	-----AAAAAAAA-AAAAAAAAAAAAAAAA--	737
Silurus	-----AAAAAAAA-AAAAA--	688
Leucoraja	-----CTAAAAAAAAAAAAAAAA-AAAAAAAAAAAAAAAA--	614
Oryzias	CACTTAGTTGAATGGAATGCCAACCAATAAAGTAAAAAAAA-AAAAAAAAAAAAAAAA--	718
Haplochromis	-----AATAAAGATTCTTCATAAAGCTGCAC	687
Maylandia	-----AATAAAGATTCTT-----	670
Pundamilia	-----	
Oreochromis	-----	
Carassius	GTTTT-----	812
Danio	-----	
Tautogolabrus	-----GTTTTAAAAA-	645
Xiphophorus	CGCTGTGTTTTGTTTTAGCTGA-	730
Salmo	-----AAAAA--	742
Silurus	-----	
Leucoraja	-----AAAAA-	620
Oryzias	-----AAAAAAA	725
Haplochromis	G-----GCTCCAAAG--	697
Maylandia	-----	

**Supplementary Figure 31.** ClustalW alignment of *Danio rerio* CART3 nucleotide sequence against *Oryzias latipes*, *Salmo salar*, *Oreochromis niloticus*, *Maylandia zebra*, *Haplochromis burtoni*, *Pundamilia nyererei*, *Xiphophorus maculatus*, *Carassius auratus*, *Leucoraja ocelatta*, *Tautogolabrus adspersus* and *Silurus meridionalis* CART sequences. (\*) identical sequences are indicated. The zebrafish sequence has an average 56.02% sequence identity with the fish sequences.

Homo	-----	
Chimpanzee	AGACGGTTTGACCGGGCCCTCNCACACCCC-TTNCTTCTTCANNCTCTCCCTCTTTC	59
Pygmy	AGACGGTT-GACCCGGGCCCTCCTCCACACCCCTTCTTCTTCACCTCCTCCCTCTTTC	59
Gorilla	AGACGGTT-GACCTGGGCCCTCCTCCACACCCCTTCTTCTTCGCCTCCTCCCTCTTTC	59
Nomascus	AGACGGTT-GACCCGGGCCCTCCTCCACACCCCTTCTTCTTCGCCTCCTCCCTCTTTC	59
Rhesus	-----	

Macaca -----GTT-GACCCGGGCCCCCTCCACACCCCTTCTTCTTCGCTCCTCCCTCTTTC 54  
Papio AGACGGTT-GACCCGGGCCCCCTCCACACCCCTTCTTCTTCGCTCCTCCCTCTTTC 59  
Pongo AGATGGTT-GACCCGGGCCCCCTCCACACCCCTTCTTCTTCGCTCCTCCCTCTTTC 59  
Cavia -----  
Ailuropoda -----TGGACCCCTCCACACCCACTTCTTCTTCGCTCGCCCCCTCCCTCTTTC 47  
Saimiri -AGACGGTTGACCCGGGCCCCCTCCACACCCCTTCTTCTTCGCCCCCTCCCTCTTTC 59  
Sus -----  
Ovis -----  
Rattus -----  
Mus -----  
Mesocricetus -----  
Danio -----

Homo -----AACGAGC 7  
Chimpanzee CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGCGTGGTGCCCCAGCAACGACG 119  
Pygmy CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGCGTGGTGCCCCAGCAACGACG 119  
Gorilla CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGCGTGGTGCCCCAGCAACGACG 119  
Nomascus CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGACG 119  
Rhesus -----GCGCGGGGTGCCCCAGCAACGAG 24  
Macaca CTGCACGGGGGCTTGGGCTCACTATAAAAAGGTGGGAGCGGGGTGCCCCAGCAACGAG 114  
Papio CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGGGGTGCCCCAGCAACGAG 119  
Pongo CTGCACGGGGGCTCGGGCTCACTATAAAAAGGTGGGAGCGGGGTGCCCCAGCAACGACG 119  
Cavia -----CCGGGCTCGGGCTCGCTATAAAAAGCGGGAGAGCAGGGTGCCCGAGCAGCAGC 55  
Ailuropoda CTGTACTCGGGCTCTGGCTCTCTATAAAAAGCGGGAGCGTGGGATGCCACAGCAGCTGCG 107  
Saimiri CTGCACGGGGGCTCTGGCTCACTATAAAAAGGTGGGAGCGGGGTGCCCCAGCAACGACG 119  
Sus -----  
Ovis -----  
Rattus -----AGCGAGG 7  
Mus -----  
Mesocricetus -----AGCGGGAGAGCGGGGTGCCCCAGCAGCAGCAGC 31  
Danio -----

Homo AGTTTCAGAACGATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 67  
Chimpanzee AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
Pygmy AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
Gorilla AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
Nomascus AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
Rhesus AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 84  
Macaca AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 174  
Papio AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
Pongo AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
Cavia GATTTTCAGAACCATGGAGAGCGCCGCCCTTGGCTGCTGCCCTCCTGGGCGCCGCCCTG 115  
Ailuropoda AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 167  
Saimiri AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCTCTCCCTG 179  
Sus --TTTCATCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 58  
Ovis -----ATGGAGAGC----- 10  
Rattus AAGTCCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTA 67  
Mus -----CAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 55  
Mesocricetus AGGTCCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 91  
Danio -----

Homo CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 127  
Chimpanzee CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 239  
Pygmy CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 239  
Gorilla CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 239  
Nomascus CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 239  
Rhesus CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 144  
Macaca CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 234  
Papio CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 239  
Pongo CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 239  
Cavia TTCCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 175  
Ailuropoda CTTTTCTGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 227  
Saimiri CTGCTGCTGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 239  
Sus CTGCTGCTGCTACCTCTGTTGGGTACCCGTGCCAGGAGATGCCGAGCTCCAGCCCCGA 118  
Ovis -----ACGCCGAGCTCCAGCCCCGA 30  
Rattus CTGCTGCTGCTACCTCTGTTGGGTACCCGTGCCAGGAGATGCCGAGCTCCAGCCCCGA 127  
Mus CTGCTACTGCTACCTCTGTTGGGTACCCGTGCCAGGAGACGCCGAGCTCCAGCCCCGA 115  
Mesocricetus CTGCTCCTGCTACCTCTGTTGGGTACCCGTGCCAGGAGATGCCGAGCTCCAGCCCCGA 151  
Danio -----

Homo GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAGAAGGAGCTG----- 178  
Chimpanzee GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAGAAGGAGCTG----- 290  
Pygmy GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAGAAGGAGCTG----- 290  
Gorilla GCCCTGGACATCTACTCTGCCGTGGATGATGCTCCACGAGAAGGAGCTG----- 290

Nomascus GCCCTGGACATCTACTCTGCAGTGGATGATGCCTCCCACGAGAAGGAGCTG----- 290  
Rhesus GCCCTGGACATCTACTCTGCCGTGGAGGATGCCTCCCACGAAAAGGAGCTG----- 195  
Macaca GCCCTGGACATCTACTCTGCCGTGGAGGATGCCTCCCATGAAAAGGAGCTG----- 285  
Papio GCCCTGGACATCTACTCTGCCGTGGAGGATGCCTCCCACGAAAAGGAGCTG----- 290  
Pongo GCCCTGGACATCTACTCTGCCGTGGATGATGCCTCCCACGAGAAGGAGCTG----- 290  
Cavia GCCCTGGACATCTATTCGGCCGTGGAGGATGCCTCCCACGAGAAGGAGCTG----- 226  
Ailuropoda GCCCTGGACATCTACTCTGCCGTGGAGGATGCCTCTCATGAGAAAAGAGCTG----- 278  
Saimiri GCCCTGGACATCTACTCTGCCGTGGAGGATGCCTCCCATGAGAAGGAGCTG----- 290  
Sus GCCCTGGACATCTACTCTGCCGTGGAGGATGCCTCCCATGAGAAGGAGCTG----- 169  
Ovis GCCCTGGACATCTACTCCGCCGTGGAGGATGCCTCCCATGAGAAGGAGCTG----- 81  
Rattus GCCCTGGACATCTACTCTGCCGTGGATGATGCGTCCCATGAGAAGGAGCTGCCAAGGCGG 187  
Mus GCCCTGGACATCTACTCTGCCGTGGATGATGCGTCCCACGAGAAGGAGCTGCCAAGGCGG 175  
Mesocricetus GCCCTGGACATCTACTCTGCCGTGGAGGACGCGTCCCACGAGAAGGAGTTG----- 202  
Danio -----

Homo -----ATCGAAGCGCTGCAAGAAGTCTTGAAGAAG 208  
Chimpanzee -----ATCGAAGCGCTGCAAGAAGTCTTGAAGAAG 320  
Pygmy -----ATCGAAGCGCTGCAAGAAGTCTTGAAGAAG 320  
Gorilla -----ATCGAAGCGCTGCAAGAAGTCTTGAAGAAG 320  
Nomascus -----ATCGAAGCGCTGCAGGAAGTCTTGAAGAAG 320  
Rhesus -----ATCGAAGCGCTACAGGAAGTCTTGAAGAAG 225  
Macaca -----ATCGAAGCGCTGCAGGAAGTCTTGAAGAAG 315  
Papio -----ATCGAAGCGCTGCAGGAAGTCTTGAAGAAG 320  
Pongo -----ATCGAAGCGCTGCAGGAAGTCTTGAAGAAG 320  
Cavia -----ATTGAAGCGCTGCAGGAAGTCTTGAAGAAG 256  
Ailuropoda -----ATCGAAGCGCTACAGGAAGTCTTGAAGAAG 308  
Saimiri -----ATCGAAGCGCTGCAGGAAGTCTTGAAGAAG 320  
Sus -----ATTGAAGCGCTGCAGGAAGTCTTGAAGAAG 199  
Ovis -----ATTGAAGCGCTGCAGGAAGTCTTGAAGAAG 111  
Rattus CAACTTCGGGGTCCCAGCGCTGTTGTCAGATTGAAGCGCTGCAGGAAGTCTTGAAGAAG 247  
Mus CAACTTCGGGGTCCCAGCGCTGTTGTCAGATTGAAGCGCTGCAGGAAGTCTTGAAGAAG 235  
Mesocricetus -----ATTGAAGCGCTGCAGGAAGTCTTGAAGAAG 232  
Danio -----ATAGAGGCATTGCAAGAGTTCTTGAAGAAG 30  
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Homo CTCAAGAGTAAACGTGTTCCCATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 267  
Chimpanzee CTCAAGAGTAAACGTATTTCCCATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 379  
Pygmy CTCAAGAGTAAACGTATTTCCCATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 379  
Gorilla CTCAAGAGTAAACGTATTTCCCATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 379  
Nomascus CTCAAGAGTAAACGTATTTCCCATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 379  
Rhesus CTCAAGAGTAAACGTATTTCCCATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 284  
Macaca CTCAAGAGTAAACGTATTTCCCATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 374  
Papio CTCAAGAGTAAACGTATTTCCCATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 379  
Pongo CTCAAGAGTAAACGTATTTCCCATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 379  
Cavia CTCAAGAGTAAACGCATTCCGATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 315  
Ailuropoda CTCAAGACTAAAAGTATTTCAATCTAT-GAGAAGAAGTACGGCCAAGTCCCATGTGTGA 367  
Saimiri CTCAAGAGTAAACGCATTCCCATCTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 379  
Sus CTCAAGAGTAAACGTATTTCCGATTTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 258  
Ovis CTCAAGAGTAAACGCATTCCAATTTAT-GAGAAGAAGTATGGCCAAGTCCCATGTGTGA 170  
Rattus CTCAAGAGTAAACGCATTCCGATCTAT-GAGAAGAAGTACGGCCAAGTCCCATGTGTGA 306  
Mus CTCAAGAGTAAACGCATTCCGATCTAC-GAGAAGAAGTACGGCCAAGTCCCATGTGTGA 294  
Mesocricetus CTCAAGAGTAAACGCATTCCGATCTAC-GAGAAGAAGTACGGCCAAGTCCCATGTGTGA 291  
Danio TTAAAGAACAAACA-ACTTCCACAACCGGAAAAGTTGAGCCTACTTCTCGTGTGCGA 89  
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Homo CGCCGGTGAGCAGTGTGCAGTGAGGAAAAGGGCAAGGATCGGGAAGCTGTGTGACTGTCC 327  
Chimpanzee CGCCGGTGAGCAGTGTGCAGTGAGGAAAAGGGCAAGGATCGGGAAGCTGTGTGACTGTCC 439  
Pygmy CGCCGGTGAGCAGTGTGCAGTGAGGAAAAGGGCAAGGATCGGGAAGCTGTGTGACTGTCC 439  
Gorilla CGCCGGTGAGCAGTGTGCAGTGAGGAAAAGGGCAAGGATCGGGAAGCTGTGTGACTGTCC 439  
Nomascus TGCCGGTGAGCAGTGTGCAGTGAGGAAAAGGGCAAGGATCGGGAAGCTGTGTGACTGTCC 439  
Rhesus CGCTGGTGAGCAGTGTGCAGTGAGGAAAAGGGCAAGGATCGGGAAGCTGTGTGACTGTCC 344  
Macaca CGCTGGTGAGCAGTGTGCAGTGAGGAAAAGGGCAAGGATCGGGAAGCTGTGTGACTGTCC 434  
Papio CGCCGGTGAGCAGTGTGCAGTGAGGAAAAGGGCAAGGATCGGGAAGCTGTGTGACTGTCC 439  
Pongo CGCCGGTGAGCAGTGTGCAGTGAGGAAAAGGGCAAGGATCGGGAAGCTGTGTGACTGTCC 439  
Cavia CGCTGGAGAACAAATGCGCAGTGCGAAAAGGAGCCAGGATCGGGAAGCTGTGTGACTGTCC 375  
Ailuropoda CGCTGGGGAGCGGTGCGCAGTGCGAAAAGGAGCCAGGATTTGGGAAGCTGTGTGACTGTCC 427  
Saimiri CGCCGGTGAGCAATGTGCAGTGAGGAAAAGGGCAAGGATCGGGAAGCTGTGTGACTGTCC 439  
Sus CGCAGGAGAGCAGTGCAGTGCAGTGCAGAAAAGGAGCTAGAATCGGGAAGCTGTGTGACTGTCC 318  
Ovis CGCGGGAGAGCAGTGCAGTGCAGTGCAGAAAAGGAGCCAGGATTTGGGAAGCTGTGTGACTGTCC 230  
Rattus CGCTGGAGAGCAGTGCAGTGCAGTGCAGAAAAGGGCCAGGATCGGGAAGCTGTGTGACTGTCC 366  
Mus CGCTGGAGAGCAGTGCAGTGCAGTGCAGAAAAGGGCCAGGATCGGGAAGCTGTGTGACTGTCC 354  
Mesocricetus TGCTGGAGAGCAGTGCAGTGCAGTGCAGAAAAGGGCCCGGATCGGGAAGCTGTGTGACTGTCC 351  
Danio TGCAGTGTGAGCAGTGTGCAATACGTAAGGTTGCAAGAGTTGGCAAGCTATGCAAGTGTCC 149  
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Homo CCGAGGAACCTCCTGCAATTCCTTCTCCTGAAAGTCTTATGAAGGGGCGTCCATTCTCC 387

Chimpanzee	CCGAGGGACCTCCTGCAATTCCTTCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTCTCC	499
Pygmy	CCGAGGGACCTCCTGCAATTCCTTCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTCTCC	499
Gorilla	CCGAGGAACCTCCTGCAATTCCTTCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTCTCC	499
Nomascus	CCGAGGAACCTCCTGCAATTCCTTCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTCTCC	499
Rhesus	CCGAGGAACCTCCTGCAATTCCTTCCTACTGAAGTGCTTATGAAGGGGCGTCCATTCTCC	404
Macaca	CCGAGGAACCTCCTGCAATTCCTTCCTACTGAAGTGCTTATGAAGGGGCGTCCATTCTCC	494
Papio	CCGAGGAACCTCCTGCAATTCCTTCCTACTGAAGTGCTTATGAAGGGGCGTCCATTCTCC	499
Pongo	CCGAGGAACCTCCTGCAATTCCTTCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTCTCC	499
Cavia	CCGAGGAACCTCCTGCAATTCCTTCCTCTTGAAGTGCTTATGAGGGGCGTCCATTCTCC	435
Ailuropoda	CCGAGGAACCACGTGCAATTCCTTCCTTTTGAAGTGCTTGTGAGG---CACCCAACCGGG	484
Saimiri	CCGAGGGACCTCCTGCAATTCCTTCCTCCTGAAGTGCTTATGAAGGGGCGTCCATTCTCC	499
Sus	CCGAGGAACCTCCTGCAATTCCTTCCTCCTGAAGTGCTTATGAGA---CGTCCACCCTTC	375
Ovis	CCGAGGGACCTCCTGCAATTCCTTCCTCCTGAAGTGCTTATGAGG---CACCCACGCT-C	286
Rattus	CCGAGGAACCTCCTGCAATTCCTTCCTCCTGAAGTGCTTGTGAAGGGGTGAC-AGCCTCC	425
Mus	CCGAGGAACCTCCTGCAATTCCTTCCTCCTGAAGTGCTTGTGAAGGGGAC-AGCCGCC	413
Mesocricetus	CCGAGGAACCTCCTGCAACTCCTTCCTCCTGAAGTGCTTATGAAGGG--GAC-AGCCTCC	408
Danio	ACAAGGAACATCATGCCATTTTATTTTAAATGCCTT-----	189
	* * * * *	
Homo	TCCATACATCCCCATC-CC-----TCTACTTTCCCC-AGAGGACCACACCTT	432
Chimpanzee	TCCATACATCCCCATC-CC-----TCTACTTTCCCC-AGAGGACCACACCTT	544
Pygmy	TCCATACATCCCCATC-CC-----TCTACTTTCCCC-AGAGGACCACACCTT	544
Gorilla	TCCATACATCCCCATC-CC-----TCTACTTTCCCC-AGAGGACCACACCTT	544
Nomascus	TCCATACATCCCCATC-CC-----TCTACTTTCCCC-AGAGGACCACACCTT	544
Rhesus	TCCATACATCCCCATC-CC-----TCTGCTTTCCCC-AGAAGACCATACCTT	449
Macaca	TCCATACATCCCCATC-CC-----TCTGCTTTCCCC-AGAAGACCATACCTT	539
Papio	TCCATACATCCCCATC-CC-----TCTGCTTTCCCC-AGAAGACCATACCTT	544
Pongo	TCCATACATCCCCATC-CC-----TCTACTTTCCCC-GGAGGACCACACCTT	544
Cavia	TGCATAT-TCCCCATTTC-----CGTACTC-CACTGAGAGGGTACACATT	480
Ailuropoda	TCCATAAATCCCCACTGCT-----CCTACTTTCCCTGAGAGGACCACACTTT	531
Saimiri	TCCATACATCCCTATC-CC-----TCTACTTTCTCC-AGATGACCACATCTT	544
Sus	TCCTTAAGTACCCCCCCCCAACACACACACACCCCACTTTCTC-AGACGACCATACTTT	434
Ovis	TCCATAAG--CCCCCTCC-----CACTTTCCCC-AGGGGACCACACCTT	328
Rattus	---TTCGGTTCCCATATTT-----CCTCTTTCCCTAAAGGAGCGCTCTTT	468
Mus	ACCTTCGGTTCCCATATTC-----CCTCTTTCCCCAAAGGAGCCGCTCAT	459
Mesocricetus	CCCTTCAGTTCCACATTC-----CCTCTTTCCCC-AAAGGAGCACTCTTT	453
Danio	-----	
Homo	CCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCCTCTGAAGG-GAAAG	491
Chimpanzee	CCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCCTCTGAAGG-GAAAG	603
Pygmy	CCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCCTCTGAAGG-GAAAG	603
Gorilla	CCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCCTCTGAAGG-GAAAG	603
Nomascus	CATCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCCTCTGAAGG-GAAAG	603
Rhesus	CATCCCTGGAGTTTGGCTTTAGCAACAGATAAAGTTTTATTTTCCTCTGAAGG-GAAAG	508
Macaca	CATCCCTGGAGTTTGGCTTTAGCAACAGATAAAGTTTTATTTTCCTCTGAAGG-GAAAG	598
Papio	CATCCCTGGAGTTTGGCTTTAGCAACAGATAAAGTTTTATTTTCCTCTGAAGG-GAAAG	603
Pongo	CATTCCTGAAGTTTGGCTTAAGCAACAGATAAAGTTTTATTTTCCTCTGAAGG-GAAAG	603
Cavia	CATCG-TGGAGTTTGGCTTTAGCAACAATAAAGTTTGCATTTTCCTCTGAGGGTGAAGG	539
Ailuropoda	CATCCCTGGAGTTTCGCTTTAGCAACAAA---GTTTGCATTTCCCTCTGAGGGTTAAGG	587
Saimiri	CATCGCTGGAGTTTGGCTTTAGTAACAGATAAAGTTTTCATTTTCCTCTGAAGG-GAAGG	603
Sus	CATCCCTGG-----CTTTAGCAACAAA---GTTTGCATTTTCCTCTGAGAAAAGGG	483
Ovis	TATCCCTGGAGTCTGGCTTTAGCAACAAA---GTTTGCATTTCCCTCTAAGGAAAAGGG	384
Rattus	TGTCCTGGAGCC--GCTTTAACAACA-ATAAAGTTTGCCTTCCCCCAGAGAGTGGATG	525
Mus	TATCCCTGGAGCCTGGCTTTAGCAACA-ATAAAGTTTGCCTTCCCCCAGAGAGCGGATG	518
Mesocricetus	TATCCCTGGAGTCTGGCTTTAGCAACA-ATAAAGTTTGCATTTCCCTTCCGAGGGTGGATG	512
Danio	-----	
Homo	GGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTGAAGA	549
Chimpanzee	GGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTGAAGA	661
Pygmy	GGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTGAAGA	661
Gorilla	GGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTGAAGA	661
Nomascus	GGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAACAACATATTAGATGTTACTGTGTGAAGA	661
Rhesus	GGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTGAAGG	566
Macaca	GGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTGAAGG	656
Papio	GGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTGAAGG	661
Pongo	GGCTCTTTTCCTG-CTGTTTCAAAAAT-AAAAGAACACATTAGATGTTACTGTGTGAAGA	661
Cavia	GGCTCTTTTCCTG-CTGTTTAAAAATAAAAAGAACACATTGATGTTACTGTGTGAAGG	598
Ailuropoda	GGCTTTTTTC-CTG-CTGTTCCAAAAT--AAAAGAACACATTAGATGTTACTGTGTGAAGG	643
Saimiri	GGCTTTTTACCTG-CTGTTTCAAAA--ATAAAAACACATTAGATATTACTGTGTGAAGG	659
Sus	G-CTCTTTTCCTGGCTGTTTCCAAAATA-AAA-GAACACATTAGACGTTCTGTGTGAACA	540
Ovis	GGCTCTTTTCCTG-CTGTTTCAAAAATA-AAAAGAACACATTGATGTTACTGTGTGAAGG	442
Rattus	GGCTCTTTCCCTG-CTGCTTCAAAAAT--AAAAGA-----TTTGATGTTATTGTGTGAAGG	577
Mus	GGCTCTTTCCCTG-TTGCTTCAAAAAT--AAAAGA-----TTTGACATCA-----	559
Mesocricetus	GGCTCTTTCCCTG-CTGTTTCAAAAAT--AAAAGAACACATTGACGTTACTGTGTGAAGG	569
Danio	-----	

Homo ATAATGCCT-GTATGGTGTGAT-ACGTGTGGAAGTATC--TA-----TTTA--TTGTC 598  
Chimpanzee ATAATGCCTTGTATGGTGTGAT-ACGTGTGGAAGTATTCCTA-----TTTTATTTGTC 715  
Pygmy ATAATGCCTTGTATGGTGTGAT-ACGTGTGGAAGTATTCCTA-----TTTTATTTGTC 715  
Gorilla ATAATGCCTTGTATGGTGTGAT-ACGTGTGGAAGTATTCCTA-----TTTTATTTGTC 715  
Nomascus ATAATGCCTTGTATGGTGTGAT-ACGTGTGGAAGTATTCCTA-----TTTTATTTGTC 715  
Rhesus ATAATGCCTTGTATGGTGTGAT-ACGTGTGGAAGTATTCCTA-----TTTTATTTGTC 620  
Macaca ATAATGCCTTGTATGGTGTGAT-ACGTGTGGAAGTATTCCTA-----TTTTATTTGTC 710  
Papio ATAATGCCTTGTATGGTGTGAT-ACGTGTGGAAGTATTCCTA-----TTTTATTTGTC 715  
Pongo ATAATGCCTTGTATGGTGTGAT-ACGTGTGGAAGTATTCCTA-----TTTTATTTGTC 715  
Cavia GTAATGTTATGT-TGGGGTTGAT-AGGTGTGCAAAGTATTCCTTTCCCTGTTTTATTTGTC 656  
Ailuropoda ATAATGCCTTGTATGGTGTGAT-ACATGTGCAAAGTATTCCTTT-----TCTTATTCATC 697  
Saimiri ATAATGCCTTGTATGGTGTGAT-ATGTGTGCAAAGTATTCCTA-----TTTTATTTGTC 713  
Sus GTAATGCCTTGTATGGTGTGAT-ATGTGTGCAAAGTATTCCT-----TATTTATTCGCC 594  
Ovis GTAATGCCTTGTATGGTGTGCT-ATGTGTACAAAATATTCCTATTCCTTTTTTCTCTGTC 501  
Rattus ACAATACCTTGAATGGTGTGAT-ATGTGTGCAAAGTATTCCTCTCTCGTTTTATCCACC 636  
Mus -----AAA----- 562  
Mesocricetus ACATTGCCTTGCATGGTGTGTTGTTATGTGTGCAAAGTATT-TTTTCTCGTTTTATTCATC 628  
Danio -----

Homo TGACAAACTCTT---GTGTACCTTTGTGTAAGAAGGGAAGCTTTG-TTTGAAAATTGT 653  
Chimpanzee TGACAAACTCTT---GTGTACCTTTGTGTAAGAAGGGAAGCTTTG-TTTGAAAATTGT 770  
Pygmy TGACAAACTCTT---GTGTACCTTTGTGTAAGAAGGGAAGCTTTG-TTTGAAAATTGT 770  
Gorilla TGACAAACTCTT---GTGTACCTTTGTGTAAGAAGGGAAGCTTTG-TTTGAAAATTGT 770  
Nomascus TGACAAACTCTT---GTGTACCTTTGTGTAAGAAGGGAAGCTTTG-TTTGAAAATTGT 770  
Rhesus TGACAAACTCTT---GTGTACCTTTGTGTAAGAAGGGAAGCTTTGCTTTGAAAATTGT 676  
Macaca TGACAAACTCTT---GTGTACCTTTGTGTAAGAAGGGAAGCTTTGCTTTGAAAATTGT 766  
Papio TGACAAACTCTT---GTGTACCTTTGTGTAAGAAGGGAAGCTTTGCTTTGAAAATTGT 771  
Pongo TGATAAACTCTT---GTGTACATTTGTGTAAGAAGGGAAGCTTTGCTTTGAAAATTGT 771  
Cavia TGATGAACTCTT---GTGTATCTTTGGGTAAGAAGAGGAAATTTGCTTTGAAAATTGT 712  
Ailuropoda TGACACACTCTT---ATGTATCTTTGTGTA----- 724  
Saimiri TGATAAACTCTT---GTGTACCTTTGTGTAAGAAGAGAAGCTTTGCTTTGAAAATTGT 769  
Sus TGACAAACTTTT---GTGTATATTTGAGTAAGAAGGGAAGCTTTCACCTCAAATTTGT 650  
Ovis TGACAAACTTTTAAAAGTGGATATTTAAGCAAAGAGGAAAGACTTCACTTTGAAAATTGT 561  
Rattus TGACACATCTT---GTG-ACCTTTCTGGGAAGAAGAGGACTTTGCTTTAAAAGTGT 691  
Mus -----AAAAAAAA-----AAAA----- 577  
Mesocricetus TGACACATCTT---GTG-AATCTTTC-----TGGGAAGAA-TTTTGCTTTAAAAGTGT 677  
Danio -----

Homo ATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGATGTCA 712  
Chimpanzee ATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGATGTCA 829  
Pygmy ATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGATGTCA 829  
Gorilla ATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGATGTCA 829  
Nomascus ATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTTGGTAGATGTCA 829  
Rhesus ATTTTTGTATGTGGCATGGCAGAATGAAAATTAGG-CTAGCTAA-TCTTGGTAGATGTCA 734  
Macaca ATTTTTGTATGTGGCATGGCAGAATGAAAATTAGG-CTAGCTAA-TCTTGGTAGATGTCA 824  
Papio ATTTTTGTATGTGGCATGGCAGAATGAAAATTAGG-CTAGCTAA-TCTTGGTAGATGTCA 829  
Pongo ATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTTGGTAGATGTCA 830  
Cavia ATTTTTGTATGTGGCATGACAGAGTAAAAATTAGATCTAGTTAA-ACCTGGT----GACA 767  
Ailuropoda -----  
Saimiri ATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TGTTGGTAGATGATA 828  
Sus ATTTTTGTAGGTGGCATGGCAGGATGAAA-CTAGATCTAGTGAA-TCTTGGTAGATGACA 708  
Ovis ATTTTTGTAGGTGGCATGACAGGATGAAAATTAGATCAGGTAAAAATTGACAGATGACA 621  
Rattus ATTTTTGTATGTGGCGGTCACAATGAAGATTAGACCTAGTTAA-TTTTGGCAGATGACA 750  
Mus -----  
Mesocricetus ATTTTTATATGTGGTGTGTCAGAATGAAAATTAGATCTAGTTAA-TTTTGGTAGATGACA 736  
Danio -----

Homo TTACAACCTGGAAAAATAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAATTATA 771  
Chimpanzee TTACAACCTGGAAAAATAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAATTATA 888  
Pygmy TTACAACCTGGAAAAATAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAATTATA 888  
Gorilla TTACAACCTGGAAAAATAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAATTATA 888  
Nomascus TTACAACCTGGAAAAATAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAATTATA 888  
Rhesus TTGCAACCTGGAAAAATAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAATTATA 793  
Macaca TTGCAACCTGGAAAAATAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAATTATA 883  
Papio TTGCAACCTGGAAAAATAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAATTATA 888  
Pongo TTATAACCTGGAAAAATAATCACCC-TATGTGACACAAATTGAAGCATGTACAAATTATA 889  
Cavia TCACAATCTGGAAAAATAATCACCC-TAAGTAACACAAA----- 804  
Ailuropoda -----  
Saimiri TTACAATCTAAAAACAAATCACCC-TAAGTAACACAAATTGAAGCATGTACAAATTATA 887  
Sus TCACAACCTGGAACTAAATCACCC-CAAGCATCACAAATTGAAGCATGTAAAACTATA 767  
Ovis TCACAACCTGGAAAAATAATCACCC--AAGTATCACAAATTGAAGCATGTACAAATTATA 679  
Rattus TCATAACCTGGAAAAATAATCACCCCAAAGCAACACAAATTGAAGCATGTGCAAAATTACA 810

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Mus -----AAAAAAAA-----AAAAAAAAAAAAA-----AAA----- 601
Mesocricetus TCACAACCTGGGAGACAAATTACCC-TAAGCAACACAAATTGAAGCATGTACAAATTATA 795
Danio -----

Homo CATAATAAAGTGTTTTTAATAATTA----- 796
Chimpanzee CATAATAAAGTGTTTTTAATAATTGCCATA 919
Pygmy CATAATAAAGTGTTTTTAATAATTGCC--- 916
Gorilla CATAATAAAGTGTTTTTAATAATTGCC--- 916
Nomascus CATAATAAAGTGTTTTTAATAATTGCC--- 916
Rhesus CATAATAAAGTGTTTTTAAT----- 813
Macaca CATAATAAAGTGTTTTTAATAATTGCC--- 910
Papio CATAATAAAGTGTTTTTAATAATTGCCA-- 917
Pongo TATAATAAAATGTTTTTAATAATTGCC--- 917
Cavia -----
Ailuropoda -----
Saimiri CATAATAAATGTTTTTATTATTA----- 910
Sus CATAATAAAGTGTTTTCATAATTGCCCTA- 797
Ovis CATAATAAAGTGTTTTCATAATT----- 703
Rattus CCCAATAAAGCATTTTGTATAATTGCTCAC- 840
Mus ---AAAAA----- 607
Mesocricetus TATAATAAAACGTTTTAAATAATTGCTCAC- 825
Danio -----

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**Supplementary Figure 32.** ClustalW alignment of *Danio rerio* CART3 nucleotide sequence against *Rattus norvegicus*, *Mus musculus*, *Sus scrofa*, *Macaca mulatta*, *Ovis aries*, *Cavia porcellus*, *Ailuropoda melanoleuca*, *Pongo abelii*, *Macaca fascicularis*, *Saimiri boliviensis boliviensis*, *Pan troglodytes*, *Pan paniscus*, *Gorilla gorilla gorilla*, *Nomascus leucogenys*, *Papio anubis* and *Mesocricetus auratus* CART sequences. (\*) identical sequences are indicated. The zebrafish sequence has an average 64.72% sequence identity with these species and 65.10% similarity to *Homo sapiens*.

## CART4

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Tautogolabrus -----AGCACAG-----GG 9
Xiphophorus -----GCCACA----- 6
Leucoraja -----CAATCCCAA-----CCCCGGT 16
Oryzias -----TCCA-----CAGG 8
Salmo -----ACACAG-----ATAAAAG 13
Silurus -----ACGCGGGGG---AATCCGAG 17
Carassius -----CTCGA-----GTGT 9
Pundamilia -----GCGGCCTCCCGGGCGGGG 18
Oreochromis CTTCTGCCCCCTTTTCAGTACTTTCCGGCACGGTAGTCTCGCAGCGGCCTCCCGGGCGGGG 60
Haplochromis -----GAGGAGACAGACA---AACCAAC 20
Maylandia -----AGACAGACA---AACCAAC 16
Danio -----AGCAAGCGGC---AGCAAGC 17
          *

Tautogolabrus ACGAT-----GAGGAG 20
Xiphophorus -TGTC-----GCCGCG 16
Leucoraja GTGTGTGGT-----TCG-----GCCCT---GAGTCG 40
Oryzias ACGCGTGAT-----TCCCAATT-----GTCTCCAAAGAGTTG 40
Salmo GTGTTTGA-----TTGCGGTAAGAGGGGACATTAGAACGGGACCTTTGGAGACG 63
Silurus GCGCGCAATAAAAAGCCCGCTGCGATCTCTGAGCGC-----GGTGTGGAGAGTCG 68
Carassius TCACTGAA-----TCTCA-----GTGC---AGAGGACG 35
Pundamilia CTGTGTGAA-----CCGGTATAAGAGGGGACATGGTCCGGAGGGTGGAGGGAGAG 68
Oreochromis CTGTGTGAA-----CCGGTATAAGAGGGGACATGGTCCGGAGGGTGGAGGGAGAG 110
Haplochromis ACTCATGAG-----GAGGAAGGAGAGGG---AGAG---GAAAAGCTAAAGAGAAA 64
Maylandia ACTCATGAG-----GAGGAAGGAGAGGG---AGAG---GAAAAGCTAAAGAGAAA 60
Danio ACACA-----AGAG---CTGG---GAAACGCTTAGAAATAG 47

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Tautogolabrus CA-----C---AG 25  
Xiphophorus CA-----C---CG 21  
Leucoraja C-----TTC---A 45  
Oryzias CAGAAACAAGA-----GTTTTTGG-----TTTTC---A 65  
Salmo AAAGATATCTT-----CTACTGTGCGCAATCTCTTA---A 95  
Silurus CGCGCGCCTTC-----GTGCACAAAAGCCCGTTTCGATCA 104  
Carassius C-----TGC---TG 42  
Pundamilia TCACCTGTGGATCTGCTGCGACACTGTGCTCCTCAGCCTCTAATCTGCTGCCTCCCACTG 128  
Oreochromis TCACCTGTGGACCTGCTGCGACACTGTGCTCCTCAGCCTCTAATCTGCTGCCTCCCTG 170  
Haplochromis CAACTACCTGA-CTGAAACCAGACACCACAGAAGAAGAGTT--TGTGACACCTTCCTGGA 121  
Maylandia CAACTACCTGA-CTGAAACCAGACACCACAGAAGAAGAGTT--TGTGACACCTTCCTGGA 117  
Danio TAGATAGCAGA-CT-----TTTTAC-----TT--T---AAATCTTCAGTTT 82

Tautogolabrus GGAGCACGAGGA----GCTG-CAGGTGCTG-----CTCTGCG 58  
Xiphophorus GGACCATGCAGA----GCTC-CAGGATGCTGA--GCGGC-----GCGCTC-CTCTGCG 66  
Leucoraja GCACCATGGTCA----GCG-ACAGGCTC--CT--GCTT-----GCTGTACTTTTG 88  
Oryzias GAACCATGGTCA----GCGCGCGGACTCTCCT--CCTC-----GTGGTC-ACTTGCA 110  
Salmo GGACCATGGAGA----GCTC-CAGGTATGGA--CCAGA-----GCGGTG-GTTTTGCG 140  
Silurus GCACCATGGAGA----GCT--CGAGTATCGCC--GCGC-----GCGCTGCTGCTC 148  
Carassius TGACCATGGAGA----GCTC-CAAACCTCTGGA--CCACA-----GCGATG-GCATGCG 87  
Pundamilia GGACCTCCGCCCTTCCGCTTACCAGCCACCA--GCACC-----ATGGATAGC-TCCG 177  
Oreochromis GGACCTCCGCCCTTCCGCTTACCAGCCACCA--GCACC-----ATGGATAGC-TCCG 219  
Haplochromis GGACCTTTATTA----GT-TTAAAGTTTTTAAAGGTGCTTTTGGAGATGGAGAGCAAAC 176  
Maylandia GGACCTTTATTA----GT-TTAAAGTTTTTAAAGGTGCTTTTGGAGATGGAGAGCAAAC 172  
Danio GAACTTAGACTA----ACGCCAAAGCATTCA--GCACC-----ATGGAGAGCGTCCG 128

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Tautogolabrus TGCTG--CTGCT-CTCAGGATCCACGGAGCAGA-CCTGA--CGGAGAACAA---CTCCC 109  
Xiphophorus CGCTG--CTGTGCTC----TCCGCCGGCGCCG--CCGGAGCCGGAGCGCAGGGGCTGCT 118  
Leucoraja -CGTG--TTGTCTCCA---TG-----GCTGT-TG-GAGCAGA----- 118  
Oryzias -GCTG--CACCTACCTT---TG-----GCTTT-CCCAGCCGA----- 141  
Salmo CGGTG--TTGCTGTCCA---TC-----GTCT-TA-GTGCTGAAATTGAC---TACTC 183  
Silurus --CTG--CTCGTATTAA---TGCTGCGGAGTTGTGCGGAGGAGACAACGA----- 192  
Carassius CGGTG--CTGTGTCTC-----GCATTCAGGGTCCGAGAT----- 121  
Pundamilia GGATGCTCCGCGGGCTG--CTGCTCGTCCGCTG--CTGT-CCG----- 216  
Oreochromis GGATGCTCCGCGGGCTG--CTGCTCGTCCGCTG--CTGT-CCG----- 258  
Haplochromis AG-----CCGTGGTCTAT-CTGTCCGCTGTCTG-TCTGTGCTGACAT----- 217  
Maylandia AG-----CCGTGGTCTAT-CTGTCCGCTGTCTG-TCTGTGCTGACAT----- 213  
Danio AG-----CCGCGGTTTAC-CTGAGCGTGTTCCTG-TGCTGTGCTGA----- 166

\*

Tautogolabrus TGAC-CAG----TGAGGACGAGCTGAGCCCCAGAGTGTGCGCCACTTCTACT----- 157  
Xiphophorus GGATGCGGAGTCCGAGGAGGAGCTGAGCCCCAGAGCGCTGCGGGACTTCTACC----- 171  
Leucoraja GAACTC-----GGACCTGGAGCCAAGAGCCCTGCGAGACTTCTACT----- 159  
Oryzias GGACTC-----TTCTTGGAAAC--GCGCTCTCTGGA--TTTTACT----- 178  
Salmo GGATTCGA-----ATTGGACCTCGACACAAGAAGTGTGAGAGACTTCTACC----- 230  
Silurus GGACACCGA-----AGTGGACTTGATACAGAGCCATCCGAGACTTCTATC----- 239  
Carassius GGACTTTGACAACGAATCCGACTTAGAAACGAGAGCCCTGAGAGAGTTTTTACC----- 174  
Pundamilia -TCCTGTG-----CACGCCAGGCATCACGGGAAGTCTCCGAGAAGACT--TTGGA 266  
Oreochromis -TCCTGTG-----CACGCCAGGCATCACGGGAAGTCTCCGAGAAGACT--TTGGA 308  
Haplochromis -CTCTCTGT-----CAAGGCCAAAGGTCAG--GCA--ACAGCCACCTGCTGTCCGCG 264  
Maylandia -CTCTCTGT-----CAAGGCCAAAGGTCAG--GCA--ACAGCCACCTGCTGTCCGCG 260  
Danio -GCGTCTG-----CAGGCGCAGATTCAGATGCAGG-ACAGCCG--ACTGAGCGAG 213

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Tautogolabrus -CCAAAGGACCGAACCT---GACCTCAGAGAAACAGCT----- 191  
Xiphophorus -CGAAAGGACCGAACCT---GACCAGTGAAAAGCAGCT----- 205  
Leucoraja -CCAAAACCTATTATCC---CGGCAGCGAGAAGGAACT----- 193  
Oryzias -CTGAAAGCT-----CAGCAA-GAAAAGGATCT----- 204  
Salmo -CCAAAGATCCGAATTT---GACCAACGAAAAGCAACT----- 264  
Silurus -CGAAAGACCCCAATCT---GACCAGGAAAAACAGCT----- 273  
Carassius -CAAAGGACCCGAATCT---GACCAACGAGAAGCAGCTCGTAAGTATTGCGCACATTTCTC 230  
Pundamilia GAGAAGAAACAAGAAGC--AGCT-GTAGACAGAGACCT----- 301  
Oreochromis GAGGAGAAACAAGAAGC--AGCT-GTAGACAGAGACCT----- 343  
Haplochromis CCTGATGACCCAACCCCTCGGACTCACAACCAGTGAGCT----- 302  
Maylandia CCCGATGACCCAACCCCTCGGACTCACAACCAGTGAGCT----- 298  
Danio CAGGACGAGC-----AGTTCATCAAGAGAGACCT----- 242

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Tautogolabrus -----  
Xiphophorus -----  
Leucoraja -----  
Oryzias -----  
Salmo -----

Silurus -----  
Carassius GGCGCATAGAAAACAATGACGTTCCCTTGTTTAAAAAGATTTTAGTGATACTTACCTGC 290  
Pundamilia -----  
Oreochromis -----  
Haplochromis -----  
Maylandia -----  
Danio -----

Tautogolabrus -----GTCCGGAGCTCTGCAGGAAGTTCTGGA-GAAACTTCAGGC 230  
Xiphophorus -----GCTTGGAGCTCTCCAAGAAGTTCTTGA-AAAGCTGCAGGC 244  
Leucoraja -----GCTCGGGGCTCTACACGAGGTGCTGAA-GAAACTGCAGAC 232  
Oryzias -----GATCGACGCCCTTGCAAGGAGTTTGA- AAAGCTGAGAAA 243  
Salmo -----GCTTGGAGCACTACATGACGTTCTTAA-AAAGCTGCAGAC 303  
Silurus -----GCTTGGCGCTTGCATGATGTTCTGGA-AAAGCTTCAGAT 312  
Carassius ATTGATTGAACATTTTACAGCTCGGGGCTTTACATGACGTTCTGGA-GAAACTGCAGAG 349  
Pundamilia -----GCTAGAGGCACTGGACGTGCTCCTGGGCAGAAACCATAAT 341  
Oreochromis -----GCTAGAGGCACTGGATGTGCTCCTGGGCAGAAATCATAAT 383  
Haplochromis -----GGCTGAAGCTCTGCAGGGTCTTCTGGATGAAGCTGACAGC 342  
Maylandia -----GGCTGAAGCTCTGCAGGGTCTTCTGGATGAAGCTGACAGC 338  
Danio -----GGCTGAGGCACTCGATGAACTTCTGGATGGAG--AACAGG 280  
\* \* \*\* \* \* \* \* \*

Tautogolabrus GAAGAAAACGTCTTCATGGGAGAAAAAGTTTGGCCAAGTCCCAG---GTGTGACGTCGG 287  
Xiphophorus TAAACGACTTCCATCGTGGGAGAAGAAATTTGGTCAAGTCCCAC---GTGTGACGTTGG 301  
Leucoraja TAAGCGGCTTCCAACCTGGGAGAAGAAGTATGGACAGGGGCCCTCA---GTGTAACATCGG 289  
Oryzias CAAAGAAATGCCA--CTGG-AGAAAGAGCTCGGATGGTTGCCTC---ATGTGACGACAG 297  
Salmo CAAGAGACTTCCATTCTGGGAAAAGAAATTCGGCCAAGTCCCTAC---GTGCGACGTTGG 360  
Silurus GAAAAGAAATTCCTCCTGGGAGAAGAAATTTGGGCAGGTTCCTAT---GTGCGACGTTGG 369  
Carassius TAAACGCATTTCACTTGGGAAAAGAAATTTGGGCGCGTTCCTACAGTGAGTAGCGCAA 409  
Pundamilia CAAGTC---TCCTCCCCTG-AGAAAAGAGGAAGC---ATCCCACT---GTGCGGGTTGGG 391  
Oreochromis CAAGTC---TCCTCCCCTG-AGAAAAGAGGAAGC---ATCCCACT---GTGCGGGTTGGG 433  
Haplochromis TCAGCCGGTCTCTCTGTGG-AGAAAAAGCCAGCGTGATCCCAGC---GTGTGATGTGGG 398  
Maylandia TCAGCCGGTCTCTCTGTGG-AGAAAAAGCCAGCGTGATCCCAGC---GTGTGATGTGGG 394  
Danio ATAATCGGA-TATCTCTGG-AGAAAAAGCAAGCGTCATTCCCAG---GTGTGACGTAGG 335  
\* \* \* \* \* \*\* \*

Tautogolabrus GGAG---CAGTGTGCAGT-GAGAAAAG-----G-CTCTCGGATCGGCAG----- 326  
Xiphophorus AGAG---CAGTGCAGGCT-GAGGAAAG-----G-CGCACGAATCGGAA----- 340  
Leucoraja AGAC---ATGTGTGCAGT-GAGAAAAG-----GACCCAGGATTTGGAGG----- 329  
Oryzias AGAG---CGGTGCGCAGT-GCGTAAAG-----G-TGCGCGCATCGGAAC----- 336  
Salmo AGAG---CAGTGCAGCAGT-GAGAAAAG-----G-CGCGAGAATCGGCAA----- 399  
Silurus AGAG---CAGTGCAGCAAT-CCGTAAG-----G-CTCCAGAATTGGAAA----- 408  
Carassius ACAGATTTTTGAGTAAAATAGTGTAAAATATTTGTAATGCCTAGATTAATAATCACACATTT 469  
Pundamilia GAAC---CGGTGTGCCATGAAGTACGG-----GCCTCGCATCGGGAA----- 430  
Oreochromis GAAC---CGGTGTGCCATGAAGTACGG-----GCCTCGCATCGGGAA----- 472  
Haplochromis TGAG---CGGTGTGCGATGAAACACGG-----ACCTCGGATTTGGTCG----- 437  
Maylandia TGAG---CGGTGTGCGATGAAACACGG-----ACCTCGGATTTGGTCG----- 433  
Danio GGAG---CGGTGCGCATGAAACACGG-----ACCACGCATCGGACG----- 374  
\* \* \* \* \* \*\* \*

Tautogolabrus GATGT--GTGA----CTGTCTCGTAGAGCGTT---CTGTAACCTTCTGCTGCTGAAGTG 377  
Xiphophorus GATGT--GCGA----CTGTCCCCTGGGAGCTTT---CTGCAACTTCTTCTGCTGAAGTG 391  
Leucoraja ACC-T--GTAA----CTGCCT-----GAGTTCGAAATGTAATTAATTTCTGTTTAAATG 376  
Oryzias ACTGT--GCGG----CTGTCTCGAGGGACTTC---ATGTAACCTTCTACGTTCTCAAATG 387  
Salmo AATGT--GCGA----CTGTCTCGCGGAGCTTT---CTGCAACTCTTATCTGCTCAAGTG 450  
Silurus AATGT--GCGA----CTGCCCACGAGGAGCCTT---CTGCAACTTTTTCTTCTGAAATG 459  
Carassius ACTATTAACAAAACGCTGACAGTGAGTTATCTAACACTGACACTCCGCTTCACATCAGTG 529  
Pundamilia GCTCT--GCGA----CTGTGGAAGAGGAGCCAA---CTGCAACTCCTACCTCCTCAAGTG 481  
Oreochromis GCTCT--GCGA----CTGTGGAAGAGGAGCCAA---CTGCAACTCCTACCTCCTCAAGTG 523  
Haplochromis GCTCT--GCGA----CTGTCTGAGAGGAACAGC---CTGCAACACCTTCTTCTGCGCTG 488  
Maylandia GCTCT--GCGA----CTGTCTGAGAGGAACAGC---CTGCAACACCTTCTTCTGCGCTG 484  
Danio CCTGT--GTGA----CTGTATGCGAGGAACCGC---CTGCAACACTTTCTTCTGCGCTG 425  
\* \* \* \* \* \*\* \*

Tautogolabrus T----TTATGAG-----AAACACACACAC-----ACAAGT---CTTTTATT 411  
Xiphophorus C----TTATGAGCCTCTCCGGATCGGAATGTAGCTTCGGGAAGAGAAGA---CTTTTACT 444  
Leucoraja T----GTATAG-----AA-----ATC-----AGACAAAATCATTTCTATT 407  
Oryzias T----TTATAG-----GA-----GTA---AGAAAAGA--GTTTATTTT 416  
Salmo C----TTGTGAATT-----GA-----AT---GGTCTGAA---TGTAAGTT 481  
Silurus T----CTGTGAG-----GA-----GGA---AGGCAA-----CTTCTTGT 486  
Carassius CGACGTCGGGGAGC-----AGTGCCCATC---AGGAAAGG-----GTC 565  
Pundamilia C----ATCTAA-----ACC-----GCCAGG--GCTCTCCTT 507  
Oreochromis C----ATCTAA-----ACC-----GCCAGG--GCTTTCTT 549  
Haplochromis C----TACTGA-----CC-----ACTC-----GCACACATT 510  
Maylandia C----TACTGA-----CC-----ACTC-----GCACACATT 506  
Danio C----TACTGATGG-----AGCT-----GGCT-----GAAATCATT 452

Tautogolabrus AAA-----GGTCACATTT----AATGAGAAC-CAGCTGAGGAGAATGCTC 451  
Xiphophorus GAACCCCTTTGTAATTACGCTCAGATTTTTTTTACTGCACAGCTCAGGCGCA-GCTG 503  
Leucoraja TTC-----ATCG--GCTAAAT-G-----AAGACAGT--T----TAA-AG-ATCTG 441  
Oryzias AGA-----GTTG--G--AAAT-T-----ATGACAATAAT----GAATAA-GTATG 451  
Salmo AGAA-----GTTGATGGCAAAC-T-----AAAAGTGTACATCAGAACGACATGTG 526  
Silurus AGCG-----GCTC-----AAAT-T-----GATGTATT-----CAATTT-ATTTG 518  
Carassius AAGA-----ATCG--GCAAAATGT-----GCGACTGTCCACGTGGAGCGT--TTTG 607  
Pundamilia TAAT-----TTG--TCACCTCT-----GCTATGCC-----AAACACATCT- 541  
Oreochromis TAGT-----TTG--TCAGCTCT-----GCAGGGCC-----AAACACATCT- 583  
Haplochromis T-----TC-CTCAC-----GAGGACCT-----AAAC----TC-- 532  
Maylandia T-----TC-CTCAT-----GAGGACCT-----AAAC----TC-- 528  
Danio TGAT-----TCT--TC-TCTCT-----CAGG--CT-----AAAC----TCT- 479

Tautogolabrus -AGT---GACCGTC-----TGC---AACACACGAAGAAGA-ACTACATGGT-----G 490  
Xiphophorus GAGT---GACTGTCCAACACTTAC---AACAACTGTAGAA---ATTGTATTATT---AGG 551  
Leucoraja GT----GGATATA-----TTAT--AAATAA-----ACAAAGCATT---GTC 473  
Oryzias GCATCAAGGGTGCAAA---TCAT--AAGTAATTTTCTGTCCTCATAAATCATTCAAATC 505  
Salmo ATAT-AGGAATATTG-----TTAT--G-GTACTACA-----ATATACGATT---ATC 566  
Silurus -TAT---AGATATTT-----TTTT--AAGTGCAAGA-----ACACGCTGAAGAGATG 559  
Carassius CAAT-----TACTTT-----TTGCTGAAATGTTGTG-----AATGGATTGTG---AAT 648  
Pundamilia -----TCAGCACAGCTCCCAT-----AAACCAAC---CC 567  
Oreochromis -----TCAGCACAGCTCCCAT-----AAACCAACTCAACC 613  
Haplochromis -----GGTCTGGGCGCTC-----ACACAGAC--AGTG 557  
Maylandia -----GGTCTGGGCGCTC-----ACACAGAC--AGTG 553  
Danio -----TGACAGCAACGCTTG-----AGACTGAC--A--C 504

Tautogolabrus TTAAACAGAGT---CTGTGTGAGG---ATTGTTGTGATGAGATGGTGAAGTTCAAAGTTG 543  
Xiphophorus AGTAAACATT---CCGTGTTA-----TTTTACAACAAAAAATGACATGAGGATGA 600  
Leucoraja A--ACATTCT--ACTACATC--G---AACAAATC--CTGTGCAAT-----TTA 511  
Oryzias C--AAATCAAT--GCATCATT--GGCAAATCAGTTCAGCTGGAAAAATGTGG---GGTCA 555  
Salmo CGGAGAGTGACAGGTTGCATTCTGAGAGATTGTCCAAACCCCAAAAAGGCC----TATTT 622  
Silurus TGTGTGTGAGT---CTACAGT-----CCTGTTTCTCTGT-----TCA 593  
Carassius TGGAAATATACACATGATATTACAG-ATATTTATACA-TTATGTGATTTCT---ATTTT 702  
Pundamilia TT----TCATTT---ATAGTC-----ATAGCTGTGTTAAACAGT-----TGTCG 605  
Oreochromis CTGTCTCATTT---ATTGCC-----ATAGCTGTGTTAAACAGT-----TGTCG 655  
Haplochromis CT----CA-----GAAAGAC-----AGAAGTGC-ACTCTTCGCC-----AATCG 591  
Maylandia CT----CA-----GAAAGAC-----AGAAGTGC-ACTCTTCACC-----AATCG 587  
Danio CT----CATTTGTTAAACAGTT-----TATTGTGTGAATTTTCATG-----GGTGA 545

\*

Tautogolabrus TGATATCATGTCAACAA---ACAAAT--ATTTGAGTCTA-----ATGTCGG--- 584  
Xiphophorus GAATATCATAA-AACGTTTCTGTAAT--ATTT-ACTTCA-----ACTTCAATAA 646  
Leucoraja TCCTGTCTGT-GT-GT---ATA-----TTCCAGTG-----CCCATGG--AA 547  
Oryzias TTTGACCCTAA-AA-ATT---ATAGCCTAATTTAAGTAAAAGGGGACTCTCTTAG--GA 608  
Salmo TGTAGTTATGA-AATATT---TTATT---TTCAACTGAATG-----TTTCGTAAACCA 669  
Silurus T---GCACTGC-ACGCCT---ATAAT--AATGTCA-TGAA-----TATGTAAC--- 631  
Carassius TATGATATGA-AGCGCT---GTGAGA-AGAACAGGGGAA-----TTTGA--A 743  
Pundamilia CCTGAGGATAA--GCATG-----GATTTTGTACTGCTGA----CAGTACTG--- 645  
Oreochromis CCTGAGGATAA--GCATG-----GATTTTGTACTGCTGA----CAGTACTG--- 695  
Haplochromis TCT--CGCT---CCAT-----ATTTTAGTCTTT-----GTTTTA--- 620  
Maylandia TCT--CGCT---CCAT-----ATTTTAGTCTTT-----GTTTTA--- 616  
Danio CCTACTACTGA--CTCT-----GTTGTATTCTTC-----TTGCTA--- 578

\*

Tautogolabrus AGTCTGTCTGTC-----ATTATA-A-----TAAT-TCATATT----- 614  
Xiphophorus ACTCAACCCGTTG---GACCATA-AGCACACTG--TAATGTCAAACC----- 687  
Leucoraja AAATTCATTAT-----TAAATAT--GCTATC--CT-ATTTCTT----- 580  
Oryzias AATTTCT--TGT-----TAAGTATCAAACTTTC--CTATTTTTTATTGAGAAATCCGT 658  
Salmo AAATGAGTTGTGC-TATGAATAAACAGCATTCTATCTGTTTTTTT----- 713  
Silurus ATGCTGTTATGTGTATTAAGTGAACGTAC-----ATTTTTT----- 670  
Carassius AGAT---TGTT---TGATTATTTGCTGCTC--TTGATGTGTA----- 777  
Pundamilia AA-TATGTTGTGT---TAAATGT--AAACGT---TTCATCTTC----- 679  
Oreochromis AA-TATGTTGTGT---TAAATGT--AAATGT---TTCATCTTC----- 729  
Haplochromis AAGTTTTGGTGT---TTGCTTTTCAGATTAA---TTATTTTTT----- 658  
Maylandia AAGTTTTGGTGT---TTGCTTTTCAGATTAA---TTATTTTTT----- 654  
Danio AACTGCTCTGTAT--ATGATT--AAACCTG--TTATTTTTTCA----- 614

\* \* \* \* \*

Tautogolabrus -----CATAATAAAGTGCAGATCAT----- 634  
Xiphophorus -----AGCAATATTATGCCCTTCATCCGCTGT 714  
Leucoraja -----CTAAAAAAAAAAAAAAAAAAAAAAAAAAAA 607  
Oryzias AGTCACTTAGTTGAATGGAATGCCAACCAATAAAGTAAAAAAAAAAAAAAAAAAAA 711  
Salmo -----AAAAAAAAAAAAAAAAAAAA 730

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Silurus -----TGTAATAAAAAAAAA----- 688
Carassius -----ATTAAATTGGAATAAAT----- 794
Pundamilia -----AATAAACAC----- 688
Oreochromis -----AATAAACACGTCAACATATAA--- 750
Haplochromis -----GTTAATAAAA-GATTCTTCATAAAGCTG 684
Maylandia -----GTTAATAAAA-GATTCTT----- 670
Danio -----GTTAATAAAA-ATGTCTTTATAAGGCTC 640
                                     *

Tautogolabrus -----GTTTTAAAAA----- 645
Xiphophorus GTTTTGTTTCAGCTGA----- 730
Leucoraja -----AAAAAAAAAAAA----- 620
Oryzias -----AAAAAAAAAAAA----- 725
Salmo -----AAAAAAAAAAAA----- 742
Silurus -----
Carassius -----TGTGTGGACTTTTGTTTT----- 812
Pundamilia -----
Oreochromis -----
Haplochromis CACGGCTCCAAG----- 697
Maylandia -----
Danio ACCCATCTGAAAATTGTCTAATTGAAAGGTATGTCTATGTCTCTGTGGGTTTATCATT 700

Tautogolabrus -----
Xiphophorus -----
Leucoraja -----
Oryzias -----
Salmo -----
Silurus -----
Carassius -----
Pundamilia -----
Oreochromis -----
Haplochromis -----
Maylandia -----
Danio TAAATGTGTTTAATAAAAACCTGTAAAA 727

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**Supplementary Figure 33.** ClustalW alignment of *Danio rerio* CART4 nucleotide sequence against *Oryzias latipes*, *Salmo salar*, *Oreochromis niloticus*, *Maylandia zebra*, *Haplochromis burtoni*, *Pundamilia nyererei*, *Xiphophorus maculatus*, *Carassius auratus*, *Leucoraja ocelatta*, *Tautogolabrus adspersus* and *Silurus meridionalis* CART sequences. (\*) identical sequences are indicated. The zebrafish sequence has an average 40.73% sequence identity with the fish sequences.

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Homo -----
Chimpanzee AGACGGTTTGACCGGGGCCCTCCNTCACACCCCTTNCCTTCTTCANNCTCTCCCTCTTTCC 60
Pygmy AGACGGTTGACCGGGGCCCTCCTCCACACCCCTTCCTTCTTCACCTCCTCCCTCTTTCC 60
Gorilla AGACGGTTGACCTGGGCCCTCCTCCACACCCCTTCCTTCTTCGCTCCTCCCTCTTTCC 60
Nomascus AGACGGTTGACCGGGGCCCTCCTCCACACCCCTTCCTTCTTCGCTCCTCCCTCTTTCC 60
Rhesus -----
Macaca -----GTTGACCGGGGCCCTCCTCCACACCCCTTCCTTCTTCGCTCCTCCCTCTTTCC 55
Papio AGACGGTTGACCGGGGCCCTCCTCCACACCCCTTCCTTCTTCGCTCCTCCCTCTTTCC 60
Pongo AGATGGTTGACCGGGGCCCTCCTCCACACCCCTTCCTTCTTCGCTCCTCCCTCTTTCC 60
Ailuropoda -----TGGACCCTCCTCCACACCCACTTCCTTCGTCGCCCCCTCCCTCTTGCC 48
Saimiri AGACGGTTGACCGGGGCCCTCCTCCACACCCCTTCCTTCTTCGCCCCCTCCCTCTTTCC 60
Sus -----
Ovis -----
Cavia -----
Rattus -----
Mus -----
Mesocricetus -----
Danio -----AGCAAGCGGCAGCAAGCACACA-----AGAGCTGGGAAACGCTTAGA 42

Homo -----AACGACG 7
Chimpanzee TGCACGGGGGCTCGGGCTCACTA-TAAAAGGTGGGAGCGCGTGGTGCCCCAGCAACGACG 119
Pygmy TGCACGGGGGCTCGGGCTCACTA-TAAAAGGTGGGAGCGCGTGGTGCCCCAGCAACGACG 119

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Gorilla TGCACGGGGGCTCGGGCTCACTA-TAAAAGGTGGGAGCGCGTGGTGCCCCAGCAACGACG 119  
 Nomascus TGCACGGGGGCTCGGGCTCACTA-TAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGACG 119  
 Rhesus -----GCGCGGGGTGCCCCAGCAACGAAAG 24  
 Macaca TGCACGGGGGCTTGGGCTCACTA-TAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGAAAG 114  
 Papio TGCACGGGGGCTCGGGCTCACTA-TAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGAAAG 119  
 Pongo TGCACGGGGGCTCGGGCTCACTA-TAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGACG 119  
 Ailuropoda TGTACTCGGGCTCGGGCTCACTA-TAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGACG 107  
 Saimiri TGCACGGGGGCTCGGGCTCACTA-TAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGACG 119  
 Sus -----  
 Ovis -----  
 Cavia ----CCGGGGCTCGGGCTCGCTA-TAAAAGGTGGGAGCGCGGGGTGCCCCAGCAACGACG 55  
 Rattus -----AGCGAGG 7  
 Mus -----  
 Mesocricetus -----AGCGGGAGAGCGCGGTGCCCGAGCAGCGACG 31  
 Danio AATAGTAGATAGCAGACTTTTACTTTAAATCTTCAGTTTGAACCTAGACTAACGCCAAA 102

Homo AGTTTCAGAACGATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 67  
 Chimpanzee AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
 Pygmy AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
 Gorilla AGTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
 Nomascus AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
 Rhesus AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 84  
 Macaca AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 174  
 Papio AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
 Pongo AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 179  
 Ailuropoda AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 167  
 Saimiri AGTTTCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCTTCCCTG 179  
 Sus --TTTCATCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 58  
 Ovis -----ATGGAGAGC----- 10  
 Cavia GATTTTCAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 115  
 Rattus AAGTCCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTA 67  
 Mus -----CAGAACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 55  
 Mesocricetus AGGTCCAGCACCATGGAGAGCTCCCGCTGAGGCTGCTGCCCTCCTGGGCGCCGCCCTG 91  
 Danio GCATTCAGCACCATGGAGAGCTCCG-----AGCCGC-GGTTTACCTGAGCG-----TG 150

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Homo CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 127  
 Chimpanzee CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 239  
 Pygmy CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 239  
 Gorilla CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 239  
 Nomascus CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 239  
 Rhesus CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 144  
 Macaca CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 234  
 Papio CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 239  
 Pongo CTGCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 239  
 Ailuropoda CTTTTGCTGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCTGAGCTCCAGCCCCGA 227  
 Saimiri CTGCTGCTGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 239  
 Sus CTGCTGCTGCTACCTCTGTTGGGTACCCGTGCCAGGAGGATGCCGAGCTCCAGCCCCGA 118  
 Ovis -----ACGCCGAGCTCCAGCCCCGA 30  
 Cavia TTCCTGATGCTACCTCTGTTGGGTACCCGTGCCAGGAGGACGCTGAGCTCCAGCCCCGA 175  
 Rattus CTGCTGCTGCTACCTTTGCTGGGTGCCGCTGCCAGGAGGATGCCGAGCTCCAGCCCCGA 127  
 Mus CTGCTACTGCTACCTTTGCTGGGTGCCGCTGCCAGGAGGACGCCGAGCTCCAGCCCCGA 115  
 Mesocricetus CTGCTCCTGCTACCTTTGCTGGGTGCCGCTGCCAGGAGGATGCCGAGCTCCAGCCCCGA 151  
 Danio TTCCTGCTGCT-----GCTGAGCGTCTGC-----AGGGCG--AGATTTCAGATGCA- 194

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Homo GCCCTGGACATCTACTCTGCCGTGGATGATG--CCTCCAC-GAGAAGGAGCTG----- 178  
 Chimpanzee GCCCTGGACATCTACTCTGCCGTGGATGATG--CCTCCAC-GAGAAGGAGCTG----- 290  
 Pygmy GCCCTGGACATCTACTCTGCCGTGGATGATG--CCTCCAC-GAGAAGGAGCTG----- 290  
 Gorilla GCCCTGGACATCTACTCTGCCGTGGATGATG--TCTCCAC-GAGAAGGAGCTG----- 290  
 Nomascus GCCCTGGACATCTACTCTGCCGTGGATGATG--CCTCCAC-GAGAAGGAGCTG----- 290  
 Rhesus GCCCTGGACATCTACTCTGCCGTGGAGGATG--CCTCCAC-GAAAAGGAGCTG----- 195  
 Macaca GCCCTGGACATCTACTCTGCCGTGGAGGATG--CCTCCAT-GAAAAGGAGCTG----- 285  
 Papio GCCCTGGACATCTACTCTGCCGTGGAGGATG--CCTCCAC-GAAAAGGAGCTG----- 290  
 Pongo GCCCTGGACATCTACTCTGCCGTGGATGATG--CCTCCAC-GAGAAGGAGCTG----- 290  
 Ailuropoda GCCCTGGACATCTACTCTGCCGTGGAGGATG--CCTCAT-GAGAAGGAGCTG----- 278  
 Saimiri GCCCTGGACATCTACTCTGCCGTGGAGGATG--CCTCCAT-GAGAAGGAGCTG----- 290  
 Sus GCCCTGGACATCTACTCTGCCGTGGAGGATG--CCTCCAT-GAGAAGGAGCTG----- 169  
 Ovis GCCCTGGACATCTACTCCGCGTGGAGGATG--CCTCCAT-GAGAAGGAGCTG----- 81  
 Cavia GCCCTGGACATCTATTCGGCCGTGGAGGATG--CCTCCAC-GAGAAGGAGCTG----- 226  
 Rattus GCCCTGGACATCTACTCTGCCGTGGATGATG--CGTCCAT-GAGAAGGAGCTGCCAAGG 184  
 Mus GCCCTGGACATCTACTCTGCCGTGGATGATG--CGTCCAC-GAGAAGGAGCTGCCAAGG 172  
 Mesocricetus GCCCTGGACATCTACTCTGCCGTGGAGGACG--CGTCCAC-GAGAAGGAGTGTG----- 202  
 Danio -----GGACAGCCGACTGAGCGAGGACGAGCAGTTTCATCAAGAGAGACCTG----- 243

\*\*\*\*\* \* \* \* \* \*



Mesocricetus GTCCCCGAGGAACCTTCTGCAACTCCTTCTTGAAGTGCTTATGAAGGG--GAC-AGC 404  
Danio GTATGCGAGGAACCGCTGCAACACTTCTTCTGCGCTGCTACTGATGGAG---CTGGC 442  
\*\* \*\*\*\*\* \*\* \* \*\*\*\*\* \* \*\*\* \* \*\* \*\*\*\*\* \*\* \*

Homo CTCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGAGGACCAC 427  
Chimpanzee CTCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGAGGACCAC 539  
Pygmy CTCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGAGGACCAC 539  
Gorilla CTCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGAGGACCAC 539  
Nomascus CTCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-AGAGGACCAC 539  
Rhesus CTCCTCCATACATCCCCA--TCCC-----TCTGCTTTCCCC-AGAAGACCAT 444  
Macaca CTCCTCCATACATCCCCA--TCCC-----TCTGCTTTCCCC-AGAAGACCAT 534  
Papio CTCCTCCATACATCCCCA--TCCG-----TCTGCTTTCCCC-AGAAGACCAT 539  
Pongo CTCCTCCATACATCCCCA--TCCC-----TCTACTTTCCCC-GGAGGACCAC 539  
Ailuropoda CGGGTCCATAAATCCCCAC-TGCC-----CCTACTTTCCCTGAGAGGACCAC 526  
Saimiri CTCCTCCATACATCCCTA--TCCC-----TCTACTTTCCCC-AGATGACCAC 539  
Sus CTTCTCCTTAAGTACCCCC-CCCCAACACACACACACCCCACTTTCTC-AGACGACCAT 429  
Ovis CT-CTCCATAAG--CCCC-TCCC-----CACTTTCCCC-AGGGGACCAC 323  
Cavia CTCCTGCATAT-TCCCAT-TTCC-----CGTACTC-CACTGAGAGGGTCA 475  
Rattus CTC---TTGCGTTCCCATATTTT-----CTCTTTCCCCAAAGGAGCGC 463  
Mus CGCCACCTTCGGTTCCCATATTTCC-----CTCTTTCCCCAAAGGAGCGC 454  
Mesocricetus CTCCCCTTCAGTTCCACATTTCC-----CTCTTTCCCC-AAAGGAGCAC 448  
Danio TG-----AAATCATTTGATTT-----TCTCTCTC-----AGGCTAA 474  
\*\* \* \*

Homo ACCTTCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTTATTTTCCCTGAAGGG 487  
Chimpanzee ACCTTCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTTATTTTCCCTGAAGGG 599  
Pygmy ACCTTCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTTATTTTCCCTGAAGGG 599  
Gorilla ACCTTCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTTATTTTCCCTGAAGGG 599  
Nomascus ACCTTCTCCCTGGAGTTTGGCTTAAGCAACAGATAAAGTTTTTATTTTCCCTGAAGGG 599  
Rhesus ACCTTCTCCCTGGAGTTTGGCTTTAGCAACAGATAAAGTTTTTATTTTCCCTGAAGGG 504  
Macaca ACCTTCTCCCTGGAGTTTGGCTTTAGCAACAGATAAAGTTTTTATTTTCCCTGAAGGG 594  
Papio ACCTTCTCCCTGGAGTTTGGCTTTAGCAACAGATAAAGTTTTTATTTTCCCTGAAGGG 599  
Pongo ACCTTCTCCCTGAAGTTTGGCTTAAGCAACAGATAAAGTTTTTATTTTCCCTGAAGGG 599  
Ailuropoda ACTTTCTCCCTGGAGTTGCGCTTTAGCAACA----AAGTTTGCATTTCCCTGAAGGG 582  
Saimiri ATCTTCTCCCTGGAGTTTGGCTTTAGCAACAGATAAAGTTTTTATTTTCCCTGAAGGG 599  
Sus ACTTTCTCCCTGG-----CTTTAGCAACA----AAGTTTGTATTTTCCCTGAAGAAA 478  
Ovis ACCTTTATCCCTGGAGTCTGGCTTTAGCAACA----AAGTTTGCATTTCCCTGAAGAAA 379  
Cavia ACATTTCTCG-TGGAGTTTGGCTTTAGCAACAATAAAGTTTGCATTTCCCTGAAGGGT 534  
Rattus TCTTTTGTCCCTGGAGCC--GCTTTAGCAACA-ATAAAGTTTGGCTTTCCCTCAGAGAGT 520  
Mus TCCATTTATCCCTGGAGCTGGCTTTAGCAACA-ATAAAGTTTGGCTTTCCCTCAGAGAGC 513  
Mesocricetus TCTTTTATCCCTGGAGTCTGGCTTTAGCAACA-ATAAAGTTTGCATTTCCCTCCGAGGGT 507  
Danio ACTCTTGACAGCAACGCTTGAGACTGACACCTCAT-TTGTTAACAGTTTATTTGTGTGAA- 532  
\* \* \* \*

Homo -AAAGGGCTCTTTTCCCTG-CTGTTTCAAAAATAAAA-GAACACATTAGATGTTACTGTGT 544  
Chimpanzee -AAAGGGCTCTTTTCCCTG-CTGTTTCAAAAATAAAA-GAACACATTAGATGTTACTGTGT 656  
Pygmy -AAAGGGCTCTTTTCCCTG-CTGTTTCAAAAATAAAA-GAACACATTAGATGTTACTGTGT 656  
Gorilla -AAAGGGCTCTTTTCCCTG-CTGTTTCAAAAATAAAA-GAACACATTAGATGTTACTGTGT 656  
Nomascus -AAAGGGCTCTTTTCCGG-CTGTTTCAAAAATAAAA-CAACATATTAGATGTTACTGTGT 656  
Rhesus -AAAGGGCTCTTTTCCCTG-CTGTTTCAAAAATAAAA-GAACACATTAGATGTTACTGTGT 561  
Macaca -AAAGGGCTCTTTTCCCTG-CTGTTTCAAAAATAAAA-GAACACATTAGATGTTACTGTGT 651  
Papio -AAAGGGCTCTTTTCCCTG-CTGTTTCAAAAATAAAA-GAACACATTAGATGTTACTGTGT 656  
Pongo -AAAGGGCTCTTTTCCCTG-CTGTTTCAAAAATAAAA-GAACACATTAGATGTTACTGTGT 656  
Ailuropoda TAAGGGCTCTTTT-CCTG-CTGTTTCCAAA-TAAAA-GAACACATTAGATGTTACTGTGT 638  
Saimiri -AAGGGCTCTTTTACCTG-CTGTTTCAAAA--ATAA-AAACACATTAGATGTTACTGTGT 654  
Sus AAGGGG-CTCTTTTCCCTGGCTTTTCCAAAATAAAA--GAACACATTAGATGTTACTGTGT 535  
Ovis AAGGGGGCTCTTTTCCCTG-CTGTTTCCAAAATAAAA-GAACACATTAGATGTTACTGTGT 437  
Cavia GAAGGGCTCTTTTCCCTG-CTGTTTCAAAAATAAAAAGAACACATTGATGTTACTGTGT 593  
Rattus GGATGGGCTCTTTTCCCTG-CTGTTTCAAAA-TAAAA-GA-----TTTGATGTTATTTGT 572  
Mus GGATGGGCTCTTTTCCCTG-TTGCTTCAAAA-TAAAA-GA-----TTTGACATCA----- 559  
Mesocricetus GGATGGGCTCTTTTCCCTG-CTGTTTCAAAA-TAAAA-GAACACATTGACGTTACTGTGT 564  
Danio -----TTTTTATGGGTGACCTACTACT-----GACTCTGTTGTTATTTCTT-TGC 576  
\* \* \* \* \*

Homo GAAGAATAATGCCT-GTATGGTGTGAT-ACGTGTGTGAAGTATC-----TATTTA-- 593  
Chimpanzee GAAGAATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTTCT-----TATTTTAT 710  
Pygmy GAAGAATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTTCT-----TATTTTAT 710  
Gorilla GAAGAATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTTCT-----TATTTTAT 710  
Nomascus GAAGAATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTTCT-----GATTTTAT 710  
Rhesus GAAGGATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTTCT-----TATTTTAT 615  
Macaca GAAGGATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTTCT-----TATTTTAT 705  
Papio GAAGGATAATGCCTTGTATGGTGTGAT-ACGTGTGTGAAGTATTTCT-----TATTTTAT 710  
Pongo GAAGAATAATGCCTTGTATGGTGTGAT-AAGCGTGTGAAGTATTTCT-----TATTTTAT 710  
Ailuropoda GAAGGATAATGCCTTGTATGGTGTGAT-ACATGTGCAAAAGTATTTCT-----TTTCTTAT 692  
Saimiri GAAGGATAATGCCTTGTATGGTGTGAT-ATGTGTGCAAAAGTATTTCT-----TATTTTAT 708  
Sus GAACAGTAATGCCTTGTATGGTGTGAT-ATGTGTGCAAAAGTATTTCT-----TATTTTAT 589  
Ovis GAAGGGTAATGCCTTGTATGGTGTGAT-ATGTGTACAAAATAATTTCTTTTCTTTTCT 496

Cavia GAAGGGTAATGTTATGT-TGGGGTTGAT-AGGTGTGCAAAGTATTCTTTCCTTGTTTTAT 651  
Rattus GAAGGACAATACCTTGAATGGTGGT-ATGTGTGCAAAGTATTCTTCTCTCGTTTTAT 631  
Mus -----AAA----- 562  
Mesocricetus GAAGGACATTGCCTTGCATGGTGGTTATGTGTGCAAAGTATT-TTTTCTCGTTTTAT 623  
Danio TAA----ACTGCTCTGTATA----TGAT-----TAAACCTGTTT-----ATTTT-- 612

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Homo TTGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGGAAGCTTTG-TTTGAAA 648  
Chimpanzee TTGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGGAAGCTTTG-TTTGAAA 765  
Pygmy TTGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGGAAGCTTTG-TTTGAAA 765  
Gorilla TTGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGGAAGCTTTG-TTTGAAA 765  
Nomascus TTGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGGAAGCTTTG-TTTGAAA 765  
Rhesus TTGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGGAAGCTTTGCTTTGAAA 671  
Macaca TTGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGGAAGCTTTGCTTTGAAA 761  
Papio TTGTCTGACAAACTCTT----GTGTACCTTTGTGTAAAGAAGGGAAGCTTTGCTTTGAAA 766  
Pongo TTGTCTGATAAACTCTT----GTGTACATTTGTGTAAAGAAGGGAAGCTTTGCTTTGAAA 766  
Ailuropoda TCATCTGACACACTCTT----ATGTATCTTTGTGTA----- 724  
Saimiri TTGTCTGATAAACTCTT----GTGTACCTTTGTGTAAAGAAGGGAAGCTTTGCTTTGAAA 764  
Sus CTGCCCTGACAAACTTTT----GTGTATATTTGAGTAAAGAAGGGAAGCTTCCCTCAAAA 645  
Ovis CTGTCTGACAAACTTTTAAAGTGGATATTTAAGCAAAGAGGAAGAAGCTTCACTTTGAAA 556  
Cavia TTGTCTGATGAACCTCTT----GTGTATCTTTGGGTAAGAAGGGAAGCTTTGCTTTGAAA 707  
Rattus CCACCTGACACATTCTT----GTG-ACCTTTCTGGGAAGAAGGGGACTTTGCTTTTAAA 686  
Mus -----AAAAAAAA-----AAA 576  
Mesocricetus TCATCTGACACATTCTT----GTG-AATCTTTC-----TGGGAAGAA-TTTTGTCTTTAAA 672  
Danio -CAGTTAATAAAATGTC-----TTTAT-----AAGGCTCACC-CATTCTGAAA 653

Homo ATTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGA 707  
Chimpanzee ATTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGA 824  
Pygmy ATTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGA 824  
Gorilla ATTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGA 824  
Nomascus ATTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTCGGTAGA 824  
Rhesus ATTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGG-CTAGCTAA-TCTTGGTAGA 729  
Macaca ATTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGG-CTAGCTAA-TCTTGGTAGA 819  
Papio ATTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGG-CTAGCTAA-TCTTGGTAGA 824  
Pongo ATTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TCTTGGTAGA 825  
Ailuropoda -----  
Saimiri ATTGTATTTTTGTATGTGGCATGGCAGAATGAAAATTAGATCTAGCTAA-TGTGGTAGA 823  
Sus TTTGTATTTTTGTAGGTGGCATGGCAGGATGAAA-CTAGATCTAGTAA-TCTTGGTAGA 703  
Ovis ATTGTATTTTTGTAGGTGGCATGACAGGATGAAAATTAGATCAGGTAATACTTGACAGA 616  
Cavia ATTGTATTTTTGTATGTGGCATGACAGAGTAAAATTAGATCTAGTTAA-ACTTGG---- 762  
Rattus ACTGTATTTTTGTATGTGGCGGGTCACAATGAAGATTAGACCTAGTTAA-TTTTGGCAGA 745  
Mus A----- 577  
Mesocricetus ACTGTATTTTTATATGTGGTGTGTCAGAATGAAAATTAGATCTAGTTAA-TTTTGGTAGA 731  
Danio ATTGTCT-----AATTGAAAGGTATGTCTATGT---TCTCTGTGGG 691

Homo TGTCATTACAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAA 766  
Chimpanzee TGTCATTACAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAA 883  
Pygmy TGTCATTACAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAA 883  
Gorilla TGTCATTACAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAA 883  
Nomascus TGTCATTACAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAA 883  
Rhesus TGTCATTGCAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAA 788  
Macaca TGTCATTGCAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAA 878  
Papio TGTCATTGCAACCTGGAAAATAAATCACCC-TAAGTGACACAAATTGAAGCATGTACAAA 883  
Pongo TGTCATTATAACCTGGAAAATAAATCACCC-TATGTGACACAAATTGAAGCATGTACAAA 884  
Ailuropoda -----  
Saimiri TGATATTACAATCTAAAAACAATCACCC-TAAGTAACACAAACTGAAGCATGTACAAA 882  
Sus TGACATCACAACCTGGAAACTAAATTACCC-CAAGCATCACAAATTGAAGCATGTAAAA 762  
Ovis TGACATCACAACCTGGAAAATAAATCACCC--AAGTATCACAAATTGAAGCATGTACAAA 674  
Cavia TGACATCACAATCTGGAAAAT-AATCACCC-TAAGTAACACAAA----- 804  
Rattus TGACATCATAACCCGGAAAACAATCACCCCAAAGCAACACAAATTGAAGCATGTGCAAA 805  
Mus -----AAAAAAAA-----AAAAAAAA-----AAA 601  
Mesocricetus TGACATCACAACCTGGGAGACAAATTACCC-TAAGCAACACAAATTGAAGCATGTACAAA 790  
Danio T----TTATCATTT---AAATGTGTT-----TAATAAAA-----CCTGTAAAA- 727

Homo TTATACATAATAAAGTGTTTTTAATAATTA----- 796  
Chimpanzee TTATACATAATAAAGTGTTTTTAATAATTGCCATA 919  
Pygmy TTATACATAATAAAGTGTTTTTAATAATTGCC--- 916  
Gorilla TTATACATAATAAAGTGTTTTTAATAATTGCC--- 916  
Nomascus TTATACATAATAAAGTGTTTTTAATAATTGCC--- 916  
Rhesus TTATACATAATAAAGTGTTTTTAAT----- 813  
Macaca TTATACATAATAAAGTGTTTTTAATAATTGCC--- 910  
Papio TTATACATAATAAAGTGTTTTTAATAATTGCCA-- 917  
Pongo TTATATATAATAAAGTGTTTTTAATAATTGCC--- 917  
Ailuropoda -----

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Saimiri      TTATACATAATAAATGTTTTTATTATTA----- 910
Sus          CTATACATAATAAAGTGTTCATAATTGCCCTA- 797
Ovis        TTATACATAATAAAGTGTTCATAATT----- 703
Cavia       -----
Rattus      TTACACCCAATAAAGCATTTTGGATAATTGCTCAC- 840
Mus         -----AAAAAA----- 607
Mesocricetus TTATATATAATAAAAACGTTTTAAATAATTGCTCAC- 825
Danio       -----

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**Supplementary Figure 34.** ClustalW alignment of *Danio rerio* CART4 nucleotide sequence against *Homo sapiens*, *Rattus norvegicus*, *Mus musculus*, *Sus scrofa*, *Macaca mulatta*, *Ovis aries*, *Cavia porcellus*, *Ailuropoda melanoleuca*, *Pongo abelii*, *Macaca fascicularis*, *Saimiri boliviensis boliviensis*, *Pan troglodytes*, *Pan paniscus*, *Gorilla gorilla gorilla*, *Nomascus leucogenys*, *Papio anubis* and *Mesocricetus auratus* CART sequences. (\*) identical sequences are indicated. The zebrafish sequence has an average 40.45% sequence identity with these species and 44.90% similarity to *Homo sapiens*.

## GHRL

```

oncorhynchus  -----GATATCAATGTCCAAGGTATA-----TCTGT-----TCTCTCT 33
Thunnus      -----TACCAACACTGTAAGTACACATCATTGCGTGTGTAAGTACTAGATCTCTCT 48
Anguilla     -----TCCAA-----GAGGCAC-----TGGGT-----TTCCTCT 24
Danio        -----
Carassius    CTGTGCATTCTGCATACATATTTGAGACTTTTAAAGTGCAGCCATTCA--GAGTGTGTGTC 58

oncorhynchus  ACAAAGCGCAA---CAGCCAT-----CCAT-CATGCCACTGAAGAGAAACACAGGTCTC 84
Thunnus      G-GCAGAGTAGA---TTTCTTTTGTTCAC-CATGTTTTGAAGAGAAATACATTCTCTG 103
Anguilla     T-AAAGTGCAAACACTCCACTGTGAGCTTCAGACATGAGGCAGATGAAACGCACCGCATA 83
Danio        -----ATGCCTCTGAGGTGCCGTGCCAGCAGC 27
Carassius    GTAAAACAGAACTAAACCAGGTGACTTCCCAGGATGCCTCTGCGTCGTCGTGCCAGCCAC 118

***      *      *

oncorhynchus  ATGATACTGATGCTGTGTA---CTCTGGCTCTGTGGCCAAGTCAGTCAGTCTGGCTCC 141
Thunnus      CTGGTCTTTCTGTGTGTGCT---CTCTGGCCTTGTGGTGAAGTCGATCAGTCTGGCTCC 160
Anguilla     ATCATCTGCTGGTCTGCG---TCCTGGCGCTGTGGATGGACTCTGTCCAGGCTGGCTCC 140
Danio        ATGTTTCTGCTCCTGTGTGTTTCTCTTTCTTCTGTGTCTCGAGTCTGTGAGCGGTGGCACC 87
Carassius    ATGTTTGTGCTCTTATGTG---CTCTTCTCTGTGTGTGAGTCTGTGAAAGGTGGCACC 175

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* * * * * ** * **** * ** * * **** **
oncorhynchus AGCTTCCTCAGCCCCCTCCAGAAACCACAGGTAAGACAGGGTAAAGGGAAGCCCCCTCGA 201
Thunnus AGCTTTCTCAGCCCCCTCTCATAAACCTCAGA----ACAG-----AGGGAATCATCCAGA 211
Anguilla AGCTTCCTCAGCCCCCTCACAGAGACCGCAGG---GGAAGGAT--AAGAAGCCTCCCAGG 194
Danio AGCTTCCTCAGTCCGACTCAGAAACCGCAGG----GTCG-----AAGGCCACCAAGA 135
Carassius AGCTTCCTCAGCCCTGCTCAGAAACCACAGG----GTCG-----AAGGCCACCCCGG 223

***** ** * ** * ** * * * * * * * * * *
oncorhynchus GTTGGTCGGCGAGACATTGA-GAGCTTGCTGAGCTGTTTGAGGGTCCCC---TTCACCA 257
Thunnus GTCGGCCGCCAAGTCATGGA-GGAGTCCGGTCAAC-----CTCC---T----- 250
Anguilla GTTGGCAGACGAGACTCAGATGGGATCCTG-GACCTGTTTATGAGGCCCCCATTCAGGA 253
Danio GTGGGCAGAAGAGAAGCTGC--TGATCCAGAGA-----TACCAGTGATCAA 179
Carassius ATGGGCAGAAGAGATGTTGC--AGAGCCAGAGA-----TCCCAGTGATTAA 267

* ** * ** * * * * * * * * * *
oncorhynchus GGAAGACA---AACACAATACGATCAAGGCTCCTTTTGAGATGGGCATCACCATGAGTGA 314
Thunnus -GAGGACG---ACCACATCACAATAAGTGCCCCATTTGAAATGGCATCACCATGAGTGA 306
Anguilla TGAAGACATCAGACACATTACGTTTAACACTCCTTTTGAGATCGGGATCACCATGACTGA 313
Danio AGAAGACG---ATCGGTTTATGATGAGCGCTCCATTTGAACTGTCCATGTCTCTGAGCGA 236
Carassius AGAGGATG---ACCAGTTCATGATGAGTGCTCCGTTGAACTGTCTGTGTCTCTGAGCGA 324

** ** * * * * * ** ** * * ** **
oncorhynchus GGAGGAGTTCAGGAGTATGGTGCCGTGCTGCAGAAGATCCTGCAGGACGCTCTGGGAGA 374
Thunnus AGAGGACTTTGAGGAGTATGGCGACATGATGCAGGAGGTCATTCAGCGTCTGCTGGGAAA 366
Anguilla GGAGCTGTTCCAGCAATATGGAGAAGTGATGCAGAAGATCATGCAGGATTTGCTGATGGA 373
Danio AGCTGAATATGAGAAATATGGTCCCCTGCTTCAGAATCTTCTGGAGGATCTTCTTAGAGA 296
Carassius GGCGGAGTATGAGAAATATGGTCCCTGTCTGCAGAAGGTTTGGTCAATCTTCTGGCGA 384

* * ** * **** * * ** * * * * *
oncorhynchus CACTGCCACTGCAGA---ATGATCACAACCTGGCATAGACACGGAATACAAAGAACCTCC 431
Thunnus CACAAAGACTGCAGAGAGATCATCACAACCTTGA--AGATAATGGAT---GAGA----- 415
Anguilla CACACCTGCCAAAGA---GTGACAAGAG--TGGATATGATCTGGACTTCATAAAACC--- 425
Danio CTCTTCTTTGAGT---CTGACAAGAGTCTTACAAAGTCTCTCTTATAAGCAATTGAC 353

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Carassius      TTCGCCACTTGAATT---CTGACAAGAG--CTA-AAAGTTC-----TACAAG--ATTCA- 430
                *                * * * *                *                *

oncorhynchus   ATTCCCTGTTCTCCAAC--TTCC-TTTCTCAACTTGCTTTATACCCAATGTACTGTGTGA 489

Thunnus        -----TCTTCAAAT--TTGC-AGTCTCAA--TGCCTT---CCAA-----TTTCA 451

Anguilla       -----CTGCGTCCCATATATTCC-TGCATTAT--TGCATG---CATAA-----TTCA 466

Danio          ----AATATTCACAATTTATTAATGATGTCAT-TTATGGGTTTAACAA-----ATAA 400

Carassius      ----GCTCCTTATAAACCATTA--AATGT----TTGTA-----AA-----GTAG 465
                *      **      *      *      **      *

oncorhynchus   AC--ATCGTTTGAATTGTAAGATGAATAA-----AATAAC 524

Thunnus        AC--ATCATT-----AGAT-----AGTGAT 469

Anguilla       ACCAATTGTT-----AAAC--ATTT-----AATAAA 490

Danio          AG-AATGATAAT-----AAATTATTCTCTATTCTATGTTCTTTATTCTGTAGCAA 450

Carassius      A--AATGATG-----TAATAATCTTC-----CAA 488
                *  ** *                *                *

oncorhynchus   ACTGCTTCCTT----- 535

Thunnus        A----- 470

Anguilla       ATTTTGCAAACGC----- 503

Danio          GTGGGTGCATTGTTACATTGTT 472

Carassius      AC----- 490

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**Supplementary Figure 35.** ClustalW alignment of *Danio rerio* GHRL nucleotide sequence against *Oncorhynchus mykiss*, *Thunnus orientalis*, *Carassius auratus* and *Anguilla japonica* GHRL sequences. (\*) identical sequences are indicated. The zebrafish sequence has an average 54.93% sequence identity with the fish sequences.

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Homo           -----GGAAGTGCAGGCCACCTGTCTGCAACCCAGCTGAGGCCATGCCCTCCC 49
Macaca         -----ATGCCCTCCC 10
Pan            -----AGTTCCC 7
Pygmy         -----C 1
Pongo         -----C 1

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Nomascus -----C 1  
Saimiri -----C 1  
Mus -----CCCAGGCATTCCAGGT-CATCTGTCTCA---CCACCAAGACCATGCTGTCTT 49  
Rattus -----CCAGAT-CATCTGTCTCA---CCACCAAGGCCATGGTGTCTT 39  
bos GACCTCCGCCCGGAACCCAGGTCCATCTGCCTCCAG--CCAGGGA-GCCATGCCCGCCC 57  
Bubalus -----TTCATCTGCCTCCAG--CCAGGGAAGCCATGCCCGCCC 36  
Capra --CCTCCGCCCGGAACCCAGGTCCATCTGCCTCCAG--CCAGAGAAGCCATGCCCGCCC 56  
Danio -----ATGCC--TCT 8

Homo CAGGGACCGTCT--GCAGCCTCCTGCTCCTCG-GCATGCT-----CTGGCTGGA---CTT 98  
Macaca CAGGGACCGTCT--GTAGCCTCCTACTCCTCG-GCATGCT-----CTGGCTGGA---CTT 59  
Pan CAAAGATAAC----ACAGCTT--TGCACAGTG-G-ATGTTTA---CTTGCTGGTGGTCTT 56  
Pygmy CAAAGATAAC----ACAGCTT--TGCACAGTG-G-ATGTTTA---CTTGCTGGTGGTCTT 50  
Pongo CAAAGATAAC----ACAGCTT--TGCGCAGTG-G-GTGTFTA---CTTGCTGGTGGTCTT 50  
Nomascus CAAAGATAAC----ACAGCTT--TGCGCAGTG-G-GTGTFTA---CTTGCTGGTGGTCTT 50  
Saimiri CAAAGATAAC----ACAGCT----CGAAGTG-G-GTGTFTA---CTCGCTGGTGGTCTT 47  
Mus CAGGCACCATCT--GCAGTTTGCTGCTACTCA-GCATGCT-----CTGGATGGA---CAT 98  
Rattus CAGCGACTATCT--GCAGTTTGCTACTCCTCA-GCATGCT-----CTGGATGGA---CAT 88  
bos CGTGGACCATCT--GCAGCCTGCTGCTGCTCA-GCGTGCT-----CTGCATGGA---CTT 106  
Bubalus CGTGGACCATCT--GCAGCCTGCTGCTGCTCA-GTGTGCT-----CTGCATGGA---CTT 85  
Capra CGCGGACCATCT--GCAGCCTGCTGCTGCTCA-GCATGCT-----CTGGATGGA---CTT 105  
Danio GAGGTGCCGTGCCAGCAGCATGTTTCTGCTCCTGTGTGTTTCTTTCTTCTGTGTCTCGA 68

\*\* \* \* \*\* \* \*\* \*

Homo GGCCATG-GCAG--GCTCCAGCTTCCTGAGCCCTGAACA--CCAGAGAGTCCAG----- 147  
Macaca GGCCATG-GCAG--GCTCCAGCTTCCTGAGCCCTGAACA--CCAGAGAGCCCAG----- 108  
Pan ATCTAAG-ATCA--ACATTGGCAGC-TGTGCCCGGAGAGGCCTCCAGGGTCCAG----- 106  
Pygmy ATCTAAG-ATCA--ACATTGGCAGC-TGTGCCCGGAGAGGCCTCCAGGGTCCAGGCCAC 106  
Pongo ATCTAAG-ATCA--ACACTGGCAGC-TGTGCCCGGAAAGGCCTCCAGGGTCCAGGCCAC 106  
Nomascus ATCTAAG-ATCA--ACACTGGCAGC-TGTGCCCGGAGAGGTCTCCAGGGTCCAGGCCAC 106  
Saimiri ATCTAAG-ATCA--ACACTGGCAGC-TCTGCCTGGAGAAGCCTCTGGGGTCCAGGCCAC 103  
Mus GGCCATG-GCAG--GCTCCAGCTTCCTGAGCCAGAGCA--CCAGAAAGCCCAG----- 147  
Rattus GGCCATG-GCAG--GTTCCAGCTTCCTGAGCCAGAGCA--CCAGAAAGCCCAG----- 137

bos GGCCATG-GCGG--GCTCCAGCTTTCTGAGCCCCGAACA--TCAGAAA---CTG----- 152  
 Bubalus GGCCATG-GCGG--GCTCCAGCTTTCTGAGCCCTGACCA--TCAGAAA---CTG----- 131  
 Capra GGCCATG-GCGG--GCTCCAGCTTTCTGAGCCCCGAACA--TCAGAAA---CTG----- 151  
 Danio GTCTGTGAGCGGTGGCACCAGCTTCCTCAGTCC-GA----CTCAGAAA---CCG----- 114

\* \*                    \*\* \* \* \* \*                    \* \*

Homo -----

Macaca -----

Pan -----

Pygmy CTGTCTGCAACCCAGCTGAGGCCATGCCCTCCCCAGGGACCGTCTGCAGCCTCCTGCTCC 166

Pongo CTGTCTGCAACCCAGCTGAGGCCATGCCCTCCCCAGGTACCATCTGCAGCCTCCTGCTCC 166

Nomascus CTGTCTGCAACCCAGCTGAGGCCATGCCCTCCCCAGGGACTGTCTGCAGCCTCCTGCTCC 166

Saimiri CTGTCTGCAACCCAGCCGAGGCCATGCCCTCCCCAAAGACCATCTGCAGCCTCCTGCTCC 163

Mus -----

Rattus -----

bos -----

Bubalus -----

Capra -----

Danio -----

Homo -----

Macaca -----

Pan -----

Pygmy TCGGCATGCTCTGGTTGGACTTGGCCATGGCAGGCTCCAGCTTCCTGAGCCCTGAACACC 226

Pongo TCGGCATGCTCTGGCTGAACTTGGCCATGGCAGGCTCCAGCTTCCTGAGCCCTGAACACC 226

Nomascus TCGGCATGCTCTGGCTGGACTTGGCCATGGCAGGCTCCAGCTTCCTGAGCCCTGAACATC 226

Saimiri TGTGCGTGCTCTGGCTGGACTTGGCCATGGGAGGCTCCAGCTTCCTGAGCCCTGAACACC 223

Mus -----

Rattus -----

bos -----

Bubalus -----

Capra -----

Danio -----

Homo -----CAGAGAAAGGAGTCAAGAAGCCACCAGCCAAGCTGCAGCCCCGAGCTC 196  
Macaca -----CAGAGAAAGGAGTCCAAGAAGCCACCAGCCAAGCTGCAGCCCCGAGCTC 157  
Pan -----CAGAGAAAGGAGTCAAGAAGCCACCAGCCAAGCTGCAGCCCCGAGCTC 155  
Pygmy AGAGAGTCCAGCAGAGAAAGGAGTCAAGAAGCCACCAGCCAAGCTGCAGCCCCGAGCTC 286  
Pongo AGAGAGTCCAGCAGAGAAAGGAGTCCAAGAAGCCACCAGCCAAGCTGCAGCCCCGAGCTC 286  
Nomascus AGAGAGTCCAGCAGAGAAAGGAGTCCAAGAAGCCACCAGCCAAGCTGCAGCCCCGAGCTC 286  
Saimiri AGAGAATCCAGCAGAGAAAGGAGTCCAAGAAGCCACCAGCCAAGCTGCAGCCCCGAGCTC 283  
Mus -----CAGAGAAAGGAATCCAAGAAGCCACCAGCTAAACTGCAGCCACGAGCTC 196  
Rattus -----CAGAGAAAGGAATCCAAGAAGCCACCAGCTAAACTGCAGCCACGAGCTC 186  
bos -----CAGAGAAAGGAAGCTAAGAAGCCATCAGGCAGACTGAAGCCCCGGACCC 201  
Bubalus -----CAGAGAAAGGAACCTAAGAAGCCATCAGGCAGACTGAAGCCCCGGGCC 180  
Capra -----CAGAGAAAGGAACCTAAGAAGCCGTCAGGCAGACTGAAGCCCCGGGCC 200  
Danio -----CAGGG-----TCGAAG--GCCACCA---AGAGTG--GGC--AGAA--- 145

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Homo TAGCAGGCTGGCTCCGCCCGGAAGA--TGGAGGTCAAGCAGAAGGGGCAG---AGGATGA 251  
Macaca TAGGAGGCTGGCTCCGCCCGGAAGA--TGGAGATCAGGCAGAAGGGGCGG---AGGATGA 212  
Pan TAGCAGGCTGGCTCCGCCCGGAAGA--TGGAGGTCAAGCAGAAGGGGCAG---AGGATGA 210  
Pygmy TAGCAGGCTGGCTCCGCCCGGAAGA--TGGAGGTCAAGCAGAAGGGGCAG---AGGATGA 341  
Pongo TAGAAGGCTGGCTCCGCCCGGAAGA--TGGAGGTCAGGCAGAAGGGGCAG---AGGATGA 341  
Nomascus TAGAAGGCTGGCTCCGCCCGGAAGA--TGGAGGTCAGGCAGAAGGGGCAG---AGGATGA 341  
Saimiri TGGAAGCTGGCTCCACCCGGAAGA--TGGAGGCCAGGCAGAAGGGGCAG---AGGATGA 338  
Mus TGGAAGGCTGGCTCCACCCAGAGGA--CAGAGGACAAGCAGAAGAGACAG---AGGAGGA 251  
Rattus TGGAAGGCTGGCTCCACCCAGAGGA--CAGAGGACAAGCAGAAGAGGCAG---AGGAGGA 241  
bos TGGAAGGCCAGTTTGACCCGAGGT--GGGAAGTCAGGCGGAAGGTGCAG---AGGACGA 256  
Bubalus TGGAAGGCCAGTTTGACCCGAGGT--GGGAAGTCAGACGGAAGGTGCAG---AGGACGA 235  
Capra TGGAAGGCCAGTTTGACCCGATGT--GGGAAGTCAGGAGGAAGGTGCAG---AGGACGA 255  
Danio -GAGAAGCTG--CTGATCCAGAGATACCAGTGATCAAAGAAGACGATCGGTTTATGATGA 202

\* \* \* \* \* \* \* \* \* \*

Homo AATGGAAGTCCGGTTCAACGCCCCCTTTGATGTTGGAATCAAGCTG----TCAGGGGTTTC 307  
Macaca ACTGGAATCCAGTTCAACGCCCCCTTTGATGTTGGAATCAAGCTG----TCAGGGGTTTC 268

Pan ACTGGAAATCCGGTTCAACGCCCCCTTTGATGTTGGAATCAAGCTG----TCAGGGGTTTC 266  
 Pygmy ACTGGAAATCCGGTTCAACGCCCCCTTTGATGTTGGAATCAAGCTG----TCAGGGGTTTC 397  
 Pongo ACTGGAAATCCGGTTCAACGCCCCCTTTGATGTTGGAATCAAGCTG----TCAGGGGTTTC 397  
 Nomascus ACTGGAAATCCGGTTCAACGCCCCCTTTGATGTTGGAATCAAGCTG----TCAGGGGTTTC 397  
 Saimiri ACTGGAAATCCAGTTCAACGCACCCTTTGATGTTGGAATCAAGCTG----TCAGGGGTTTC 394  
 Mus GCTGGAGATCAGGTTCAATGCTCCCTTCGATGTTGGCATCAAGCTG----TCAGGAGCTC 307  
 Rattus GCTGGAAATCAGGTTCAATGCTCCCTTCGATGTTGGCATCAAGCTG----TCAGGAGCTC 297  
 bos GCTGGAAATCCGGTTCAACGCCCCCTTTAACATTGGGATCAAGCTA----GCAGGGGCTC 312  
 Bubalus GCTGGAAATCCGGTTCAACGCCCCCTTTAACATTGGGATCAAGCTG----TCAGGGGCTC 291  
 Capra GCTGGAAATCCGGTTCAACGCCCCCTTTAACATTGGGATCAAGCTG----TCAGGGGCTC 311  
 Danio GC----GCTCCATTTGAACTGTCCAT---GTCTCTGAGCGAAGCTGAATATGAGAAATAT 255

\*\*\* \*\* \*\* \*\* \* \* \*\*\*\*\* \*\*

Homo AGTACC-AGCAGCACAGCCAGGCCCTGGGGAAGTTTCTTCAGGACA--TCCTCTGGG--- 361  
 Macaca AGTACC-AGCAGCACAGCCAGGCCCTGGGGAAGTTTCTTCAGGACA--TCCTCTGGG--- 322  
 Pan AGTACC-AGCAGCACAGCCAGGCCCTGGGGAAGTTTCTTCAGGACA--TCCTCTGGG--- 320  
 Pygmy AGTACC-AGCAGCACAGCCAGGCCCTGGGGAAGTTTCTTCAGGACA--TCCTCTGGG--- 451  
 Pongo AGTACC-AGCAGCACAGCCAGGCCCTGGGGAAGTTTCTTCAGGACA--TCCTCTGGG--- 451  
 Nomascus AGTACC-AGCAGCACAGCCAGGCCCTGGGGAAGTTTCTTCAAGACA--TCCTCTGGG--- 451  
 Saimiri AGTACC-AGCAGCACAGCCAGGCCCTGGGGAAGTTTCTTCAGGACA--TCCTCTGGG--- 448  
 Mus AGTATC-AGCAGCATGGCCGGGCCCTGGGGAAGTTTCTTCAGGATA--TCCTCTGGG--- 361  
 Rattus AGTACC-AGCAGCATGGCCGGGCCCTGGGAAAGTTTCTTCAGGATA--TCCTCTGGG--- 351  
 bos AGTCCC-TCCAGCATGGCCAGACGTTGGGGAAGTTTCTTCAGGACA--TCCTTTGGG--- 366  
 Bubalus AGTCCC-TCCAGCACGGCCAGACGCTGGGGAAGTTTCTTCAGGACA--TCCTTTGGG--- 345  
 Capra AGTCCC-TCCAGCATGGCCAGACGCTGGGGAAGTTTCTTCAGGACA--TCCTTTGGG--- 365  
 Danio GGTCCCGTGCTTCAGAATC---TTCTGGAGGATCTTCTTAGAGACTCTTCTTTGAGTTTC 312

\*\* \* \* \*\* \* \*\*\* \* \*\*\*\*\* \*\* \*\* \* \* \*

Homo ----AAGAGGCCAAAG----- 373  
 Macaca ----AAGAGGCCAAAG----- 334  
 Pan ----AAGAGGCCAAAG----- 332  
 Pygmy ----AAGAGGCCAAAG----- 463  
 Pongo ----AAAAGGCCAAAG----- 463

Nomascus ----AAGAGGCCAAAG----- 463  
Saimiri ----AAGAGGCCAAAG----- 460  
Mus ----AAGAGGTCAAAG----- 373  
Rattus ----AAGAGGTCAAAG----- 363  
bos ----AAGAAGCTGAAGCACATCTCCTCCGCCCTGTGGAAGGGAAGCCAAACCGGAGGG 422  
Bubalus ----AAGAAGCTGAAG----- 357  
Capra ----AAGAAGCCGAAG----- 377  
Danio TGACAAGAGTCTTACA----- 328

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Homo -----AGGCCCCAGCCGACAAGTGATCGCCCAAGC 405  
Macaca -----AGGCCCCAGCCGACAAGTGA----- 354  
Pan -----AGGCCCCAGCTGACAAGTGATCGCCCAAGC 364  
Pygmy -----AGGCCCCAGCTGACAAGTGATCGCCCAAGC 495  
Pongo -----AGGCCCCAGCTGACAAGTGATCGCCCAAGC 495  
Nomascus -----AGGCCCCAGCTGACAAGTGATCGCCCAAGC 495  
Saimiri -----AGGCCCCAGCTGACAAGTGATCGCCCAAGC 492  
Mus -----AGGCGCCAGCTGACAAGTAACCACGGACAGGC 405  
Rattus -----AGGCGCCAGCTAACAAGTAACCACTGACAGGA 395  
bos CAACGGGAGGAGAGCAGACACAGTTGAGAAACCCTGGCTAACGAGTGAGTGGCCCTGGGA 482  
Bubalus -----AAACCCTGGCTGATGAGTGAGCGGCCCTGGGA 389  
Capra -----AAACCCTGGCTGACGAGTGACCGGCCCTGGGA 409  
Danio -----AAGTTCCTCCTTATAAGCAATTGACAATATTC 360

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Homo CTTACTCACC-----T---CTCTCT-----AAGTTTAGAAGCGCTCATCTGGCTTTT- 449  
Macaca -----  
Pan CTTACTCACC-----T---CTCTCT-----AAGTTTAGAAGCGCTCATCTGGCTTTT- 408  
Pygmy CTTACTCACC-----T---CTCTCT-----AAGTTTAGAAGCGCTCATCTGGCTTTT- 539  
Pongo CTTACTCACC-----T---CTCTCTTCCCTCCTAAGCTTAGAAGCGCTCATCTGGCTTTT- 546  
Nomascus CTTACTCACC-----T---CTCTTC--CTCCTAAGCTTAGAAGCGCTCATCTGGCTTTT- 544  
Saimiri CTCACCTACC-----T---CTCTCTTCCCTCCAACTTAGAAGCGCTCATCTGGCTTTT 544  
Mus CTGAC-C-CC-----CGTGCTTTCTCTCTCTGAG--CAAGAACTCACATCC-GCCTCA- 454  
Rattus CTGGT-C-CC-----TGTACTTTCT--CCTAAG--CAAGAACTCACATCCAGCTTCT- 442

bos CCAAC-CACC-----TGTCGGTTCTCCACCCCT----CAGAAGCTCTCACCTGGCTTCC- 531  
 Bubalus CCAAC-CACC-----TGTCGGTTCTCCACCCCT----CAGAAGCTCTCACCTGGCTTCC- 438  
 Capra CCAAC-CACC-----TGTCGGTTCTCCCGCCCT----CAGAAGCTCTCACCTGGCTTCC- 458  
 Danio ACAATTTATTAATGATGTCATTTATGGGTTTAACAAATAAAGAATGATAATAAATTATT- 419  
  
 Homo CGCTTGCTTCTGCAGCAACTCCCACGACTGTTG--TACAAGCTCAGGAGGCGAATAAATG 507  
 Macaca -----  
 Pan CGCTTGCTTCTGCAGCAACTCCCACGACTGCTG--TACAAGCTCAGGAGGCGAATAAATG 466  
 Pygmy CGCTTGCTTCTGCAGCAACTCCCACGACTGCTG--TACAAGCTCAGGAGGCGAATAAATG 597  
 Pongo CGGTTGCTTCTGCAGCAACTCCCACGACTGTTG--TACAAGCTCAGGAAGCAAATAAATG 604  
 Nomascus CGCTTGCTTCTGCAGCAACTCCCATGATTGTTG--TACAAGCTCAGGAGGCGAATAAATG 602  
 Saimiri CGCTCGCTTCGGCAGCAACTCCCACAACGTTG--TATGAGCTCAGGAGGTGAATAAATG 602  
 Mus G--CCTCCTCGGCAACTCCCAGCA-CTCTCC----- 482  
 Rattus G--CCTCCTCTGCAACTCCCAGCA-CTCTCCTGCTGACTTA-----CAAATAAATG 490  
 bos GGGACACTTCCGAGACCACGTGGGGCTCTGAGGGTACTAGAGTAGGCAGTGAATAAATG 591  
 Bubalus GGGACACTTCCGAGACCACAGGCAGCTCTGA----- 469  
 Capra GGGACACTTCCGAGACCACAGGCAGCTCTGAGGGTACTAGCCTAGGAGGTGAATAAATA 518  
 Danio --CTCTATTCTATGTTCTTTA----TTCTGTAGC-----AAAGTGGGTGCATT 461  
  
 Homo TTCAAACGTGA-----AAAAAAAAA----- 529  
 Macaca -----  
 Pan TTCAAACGTATGCTGATGTTCCAAATGGGAATTTATTTCAAAGAGGAAAAGTTAGTATT 526  
 Pygmy TTCAAACGTATGCTGATGTTCCAAATGGGAATTTATTTCAAAGAGGAAAAGTTAGTATT 657  
 Pongo TTCAAACGTATGCTGATGTTCCAAATGGGAATTTATTTCAAAGAGGAAAAGTTAACATT 664  
 Nomascus TTCAAACGTATGCTGATGTTCCAAATGGGAATGTATTTCAAAGAGGAAAAGTTAACATT 662  
 Saimiri TTCAAACGTATGCTGATGTTCCAAATGGGAATTTATTTCAAAGATGAAAAGTTAACATT 662  
 Mus -----  
 Rattus TTCAAGCTGT----- 500  
 bos CTCAGATGGA-----TGCAAAAAAAAAAAAAAAAA----- 620  
 Bubalus -----  
 Capra CTCAAACGG----- 527  
 Danio GTTACATTGTT----- 472

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Homo          -----AAAAAAAAAAAA----- 542
Macaca        -----
Pan           TCACTTTAAAAAAATCAAATAATAC 552
Pygmy        TCACTTTAAAAAAATCAAATAATAC 683
Pongo        TCACTTTAAAAAAATCAAATAATAC 690
Nomascus     TCACTTTAAAAAAATCAAATAATAC 688
Saimiri      TTACTTAAAAAAAAGTCAA----- 682
Mus          -----
Rattus       -----
bos          -----
Bubalus      -----
Capra        -----
Danio        -----

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**Supplementary Figure 36.** ClustalW alignment of *Danio rerio* GHRL nucleotide sequence against *Homo sapiens*, *Mus musculus*, *Bos taurus*, *Capra hircus*, *Bubalus bubalis*, *Pan troglodytes*, *Pan paniscus*, *Pongo abelii*, *Macaca mulatta*, *Nomascus leucogenys* and *Saimiri boliviensis boliviensis* GHRL sequences. (\*) identical sequences are indicated. The zebrafish sequence has an average 40.93% sequence identity with these species and 41.60% similarity to *Homo sapiens*.

## POMCb

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Heterodontus -----GAGGTAGAGGGAGAG-----AGTTGCGCTA----- 26
Horn          -----GAGGTAGAGGGAGAG-----AGTTGCGCTA----- 26
Verasper     -----GGTAGAG-----AGCTGAACC----- 17
Danio        -----AT-----GTTCTGTCC----- 11
Protopterus  -----CCC--ACA--TCAGCACCTTC--AACTGCGAGT-----TGG 30
Neoceratodus -----GACTCTCGGCGGCTCGGACAGAGTCAGAAGCAACGGAACCTGCAACGGCACC GG 54
Acipenser    -----GGAATAAGAAGG-----AAGCAGTGAT-----CAG 25
Polyodon     -----GGAATAAGAAGG-----AAGCAGTGAT-----CAG 25
Japanese     -----AGTCGAGTCACACA--AACAGAG-----ACAAC T--GTT-----CAG 33
European     ---GAAGCAGGCAGTCGAGTCACACA--AACAGAG-----ACAAC T--GTT-----CAG 42
Anguilla     -----AGTCGCGTCACACACAAACAGAAG--AGCACAACTCGTTGT-----CAG 42
Lepisosteus -----GT-GCTTTGAAGAT-AACAAGA-----ATACCT-GCAG-----AG 32
Gobiocypris  -----
Carassius    -----AAAGAATTCTGGC---ACGAGCGCACA-----CAT 26
Ictalurus   CACGAACATACATAGTGGGACGTCGAGTGAAGAAG---TTGAGTCACT-----TAC 50
Solea       -----CTTT-----AATCTCTG----- 12
Common      -----AGGAGCGACTTT-----AATCTCTG----- 20

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Sparus -----GAGA--AACGAC----AGAAATCTCCA----- 21  
 Yellowfin -----GGGAGATGAGGAGAGA--AACGAC----AGAAATCTCCA----- 33  
 Sparidentex -----GCCGGACGAGGAGAGA--AACGAC----TGAAATCTCCA----- 33  
 Acanthopagrus -----GAGAAGAGGAGAGAGCAACGGCT----AAAACCTCCA----- 10  
 Oreochromis -----GAAAAACAGAAAAGGAGAGG--AACAAAC----AGGAAGCTCCA----- 34  
 Monopterus -----GAAAAACAGAAAAGGAGAGG--AACAAAC----AGGAAGCTCCA----- 38  
 Dicentrarchus -----GAAGTGGAGGGGAC----AGAAATCTTTG----- 25  
 Epinephelus -----ATC--TG----- 5  
 Amatitlania -----AGAGTCGCCAGAGTGAAGGATCAGTGGAC-----AAACGGAG----- 37  
 Chimaera -----GAGAGCGAGCGGAGAGCAAGCTAGCGAGAG-CACGAGAGGTGTGAAGAGAGAGG 53

Heterodontus -----TAGGAGTGAC-CTT-----CA-----AC 43  
 Horn -----TAGGAGTGACACTT-----CA-----AC 44  
 Verasper -----AGGACTGATACTT-----CA-----AC 34  
 Danio -----GTCTTGGC-TTT-----TG----GC 26  
 Protopterus -----TT-----A-----TCAGCAT-----C 41  
 Neoceratodus -----TCTCAACA-----TCAGCATTAGCGTCC-----TC----CTC 82  
 Acipenser AAA-TCTGAGCAG-----TCAGTAAGGAGAAGG-----C-----C 54  
 Polyodon AAA-TCAGAGGAGCAG-----AGTCAGTGAGGAGAAGG-----CA-----C 60  
 Japanese A---GCGAAAAA-----AGAGAGTGGAAAAAGAC-----CA-GTA-GC 66  
 European A---GCGAAAAA-----AGAGAGTGGAAAAAGAC-----CA-GTG-GC 75  
 Anguilla AAGCGCAGAAAATGA-----AGAGTGTGAGAAAAAGAC-----CACGTGTGC 83  
 Lepisosteus AAGGACTGAAGACCT-----GTTGAGTGTGACAGAC-----TG---AAGC 70  
 Gobiocypris -----  
 Carassius AACCAGACAGATCAGA-----GATCATCAGAGAGGAAA-----AACTTCGGT 68  
 Ictalurus GCTGTTGCAGAGGGAG-----AATTCGCAACAGGGGA-----AGT----GC 88  
 Solea -----TCCATCGAC-----CGAACAAACAAGAAAA-----CA-----AC 40  
 Common -----TCCATCGAC-----CGAACAAACAAGAAAA-----CA-----AC 48  
 Sparus -----TCCAACGGC-----CGAACGACAGGAAAA-----CA-----CC 49  
 Yellowfin -----TCCAACGGC-----CGAACGACAGGAAAA-----CA-----CC 61  
 Sparidentex -----TCCAACGGC-----CGAACGACAGGAAAA-----CA-----CC 61  
 Acanthopagrus -----TCCAACGGC-----CGAACGACAGGAAAA-----CA-----CC 38  
 Oreochromis -----TCCAGTGGC-----CGAGTGACGAGAAAA-----CA-----CC 62  
 Monopterus -----TCCGACAGC-----CTC-TGCCGCCAAAA-----CA-----CC 65  
 Dicentrarchus -----TCCAACAGC-----TGAACAACAAGAAAA-----CA----- 51  
 Epinephelus -----TCCAACAGC-----CAAACGACGAGAAAA-----CA-----TT 33  
 Amatitlania -----TGAGAAAAAG-----CAGGAGAAGAGAAAA-----CG-----TC 65  
 Chimaera AGCCAAGAGAAACGAGTGAAGAGCAAGTGAAGAGAAAAGACTCACACACTTTCAGGGCAGT  
 113

Heterodontus A-GTACAGGAGTTGT-TGACCCTG-----AGGCCTGG 73  
 Horn AAGTACAGGAGTTCT-TGATCCTG-----AGGCCTGG 75  
 Verasper TAGAACAGGAGTTGC-TG-TACTG-----AGGGGTGC 64  
 Danio ---TGACGCGGTCTTTGTGTTTTCA-----CAGCCCA 55  
 Protopterus TTCATCACTACCA-CA--AGCAAA-----ATCAAG--C 69  
 Neoceratodus TTCGTTACCATCAACAGCAGCAGG-----CTCAGA--C 113  
 Acipenser TGGACCAAGTGACACTGCAGCAGA-----TCATTTCTGAAGTTAGGT-AC 98  
 Polyodon TGGACCAAGTGACACTGCAGCAGA-----TTGTTTCAGAAGTTAGGT-AT 104  
 Japanese AGGAAGAGCAGAGAGAAGAGGAGTGCAGACGCTTGGCATCCGTGACT---GCAGAG-TG 121  
 European AGGAAGAGAAGAGAGAAGAGGAGTGCAGACGCTTGGCATCAGTGACT---GCAGAG-TG 130  
 Anguilla AGGAAGAGAAGAGAGAAGAGGA----- 105  
 Lepisosteus --GAAACACTGAGAGG-CAGG-----CCATTCTT---TCAG---TT 102  
 Gobiocypris -----GACATGGTGAGGGGAG 16  
 Carassius CGGCTCACTTCTTTTTGCACAACA-----GACATGGTGAGGGGAG 108  
 Ictalurus AGACACCTTTCTTCTTCCATCACAACAT-----TTCCTTTGCAATGGTTAAAGAAG 136  
 Solea AG-----AGAGCAAGAGAGAG-----AGGGCAG 63  
 Common AG-----AGAGCAAGAGAGAG-----AGGGCAG 71  
 Sparus AGCAATAAGAGAAAAGAAGACGAG-----AGGAAGA 79  
 Yellowfin AGCAATAAGAGAAAAGAAGACGAG-----AGGAAGA 91  
 Sparidentex AGCA-TAAGAGACAGAAGCTGAG-----AGGACGG 90  
 Acanthopagrus AGCAATAAGAGACAGAAGCTGAG-----AGGACGG 68  
 Oreochromis AGCAATAAGAGAGCAGAAGAGTGC-----AGAAAAG 93  
 Monopterus AGCAATAAGAGCAAGAAGAAGAGAG-----ACGGGACAA 99  
 Dicentrarchus ---ACA-GAGAAAGA-----C-----AGGACAG 70  
 Epinephelus AG--ACA-GAGAGAGACAGAC-----AGGACAG 60  
 Amatitlania TGAAAAAAGCTCCATCCAGTCGAG-----TTTGTGCTTGTGCACCCGGC 111  
 Chimaera AGGTAAGGGAGCAAGTGGGTGAAG-----AGCGAGGGAGCAGGGCAGAGGAC 161

Heterodontus A-TATGATGCAGCAGTCGATGTGGAAAGGCAACTTGGT----AGTGTTAAGCATGGTATG 128  
 Horn A-TATGATGCAGCAGTCGATGTGGAGAGGCAACTTGGT----AGTGTTAAGCATGGTATG 130  
 Verasper C-CATGATGCAGCTGTTGATGTGCAGAGGTCCTTGGT----GGTGTAAAGCATGATGTG 119  
 Danio A-TGT-AGACGGTGGCAGATGCTCGGG-----TTTGAT----AG-----ACTGCATG 96  
 Protopterus C-AAAGATGCTGAAGCCAGTTTGA-----GGCATCTCTTTGACTTTCTGCTGTGCT 121  
 Neoceratodus A-GAAGATGTTGAAGCCATTTTGA-----GCCATCTCTTTGACTTTCTGGGGTGTCT 165

Acipenser T-GAAGATGCTGCGTTTCAGTTTGGGGCTG--TGTTGTGGTTGTGCTGGGGGTGCTGTGGT 155  
Polyodon T-GAAGATGCTGCGTCCAGTTTGGGGCTG--TGTTGTGGTTGTGCTGGGGGTGCTATGGT 161  
Japanese T-GAAGATGCTGTGTCCGGCTGGA-----TGCTGGCACT--GGCTGT-GCTGT-GTGCG 171  
European T-GAAGATGCTGTGTCCGGCTGGA-----TGCTGGCACT--GGCTGT-GCTGT-GTGCG 180  
Anguilla -----TGCTGTGTCCGGCTGGA-----TGCTGGCACT--GGCTGTGTGCTGTGGT 151  
Lepisosteus T-GAAGATGCTGCGTTTCAGTCTGGG-----TGACTCTCTCGGCCTT-GCTGT---ACT 151  
Gobiocypris T-GAGGATGTTGTGTCTGCTGGC-----TCTTGGCTCT--GGCTGT-ACTGT-CCGCG 66  
Carassius T-GAGGATGTTGTGTCCGGCTGGC-----TCTTGGCTCT--GGCTGT-TCTGT-GTGCG 158  
Ictalurus C-GAGAATGGGGTGTCTGTGTGGA-----TGTTAGCTCT--GGCTTT-GCTGT-GTGCA 186  
Solea T-GTGGAGAATGTGTCTGTGTGGC-----TATTGGT---TGCTGCGACGGTGTGGG 112  
Common T-GTGGAGAATGTGTCTGTGTGGC-----TATTGGT---TGCTGCGACGGTGTGGG 120  
Sparus A-GCGGAGAATGTGTCTGTGTGGC-----TATTGGT---GGCTGTGGTGGTGTGGG 128  
Yellowfin A-GCGGAGAATGTGTCTGTGTGGC-----TATTGGT---GGCTGTGGTGGTGTGGG 140  
Sparidentex A-GCGGAGAATGTGTCTGTGTGGC-----TATTGGT---GGCTGTGGTGGTGTGGG 139  
Acanthopagrus A-GCGGAGAATGTGTCTGTGTGGC-----TATTGGT---GGCTGTGGTGGTGTGGG 117  
Oreochromis T-GTGGAGGATGTGTCTGTGTGGC-----TTTTTGT---GGCATTAGTGGTAGTGGG 142  
Monopterus C-GCGGACAATGTGTCTGTGTGGC-----TGTTGGT---GGCTGTGGTAGTGTGGG 148  
Dicentrarchus C-GTGGGAAATGTGTCTGTGTGGT-----TATTGGT---GGCTGTGGCCTGGCGGG 119  
Epinephelus C-GTGGGAAATGTGTCTGTGTGGT-----TTTTGGT---GGCTGTGGCCTGGCGGG 109  
Amatitlania T-GAAGATGGTGTGTCTGTGTGGT-----TGTTGGTGGT--GGTGTATGGCATTTGTGTG 163  
Chimaera AAGGAGATGGAGCTGTGGATGTGGAGCCGAGCTGTGCT---GCTGGTGGCTTTGCTGTG 217

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Heterodontus G-----GCTCTGACCTCCGGCCAGCTGCAGG---ACTG--CTTGGACC----ACGGCAAG 174  
Horn G-----GCTCTGACCTCCGGCCAGCTGCAGG---ACTG--CTTGGACC----ACAGCAAG 176  
Verasper G-----ACACTGACATCCTGTTCAGCTGGAGG---AGTG--CTGGGACC----AAAACAAG 165  
Danio G-----ACCTTGA-ATCCAATGAGC-ACAA----ATTG-C-AGTGT----TCAGAAG 138  
Protopterus CATGATATATGG-TACTGGA---GTTACAG-CCAGTG--CTGGGAAA----CAAGCAA 170  
Neoceratodus CTGGGTGTACAG-TATGGGG---GTTGACAG-CCAGTG--CTGGGAGA----TCAGCAAG 214  
Acipenser -----TCTACAG-CAGTGGG---GTGACAG-TCAGTG--TTCCGGAGC----ACAGTCAA 199  
Polyodon -----TCTACAG-CAGTGGG---GTTACAG-TCATG--TTACAGAGC----ACAGTCAA 205  
Japanese -----TTTTGTT-CAG--A---GGTGAGCGGTTCAGTG--CTGGGCAC----ACTCACAT 213  
European -----TTTTGTT-CAG--A---GGTGAGCGGTTCAGTG--CTGGGCAC----ACTCACAT 222  
Anguilla -----TTTTGTT-CAG--AA---GGTGAGCGGTCAATG--CTGGGCAC----ACTCACAT 194  
Lepisosteus -----TCTACAG-CAGTCAG---GTCGAGAG--CAGTG--TTGGGAAC----ACTCTCAA 194  
Gobiocypris -----GGCGG-CTCTGAA---GTC-AGAGCTCAGTG--CTGGGAAA----ACACTCGA 108  
Carassius -----GGTGG-ATCTGAA---GTC-AGAGCTCAGTG--TTGGGAGG----ACGCCGCG 200  
Ictalurus -----AGTGG-CTCAGAG---GTT-AGAGCCAGTG--CTGGGAGA----ACAGTGGC 228  
Solea -----CGTGGCCAGAGGATCAGTACCAA-CCAGTG--CTGGGAGC----ATCCAAG 158  
Common -----CGTGGCCAGAGGATCAGTACCAA-CCAGTG--CTGGGAGC----ATCTGAGC 166  
Sparus -----CGTGGCCAAAGGA---GCTGCCAG-TCAGTG--CTGGGAGC----ATCCGAGC 171  
Yellowfin -----CGTGGCCAAAGGA---GCTGCCAG-TCAGTG--CTGGGAGC----ATCCGAGC 183  
Sparidentex -----CGTGGCCAAAGGA---GCTGCCAG-TCAGTG--CTGGGAGC----ATCCGAGC 182  
Acanthopagrus -----CGTGGCCAAAGGA---GCTGCCAG-TCAGTG--CTGGGAGC----ATCCGAGC 160  
Oreochromis -----CGGGCCAGAGAA---GCAGTCAG-CCAGTG--CTGGGAGC----ATCCAAGC 185  
Monopterus -----CGTGACCAGAGGA---GCTGTTCAG-TCAGTG--CTGGGAGC----ACCCGAGC 191  
Dicentrarchus -----CGTGGCCAGAGGA---GCTGTTGG-TCAGTG--TTTGGAAAC----ATCCCAGC 162  
Epinephelus -----CGTGGTCAGAGGA---GCTGTTCAG-TCAGTG--CTGGGAAAC----ATCCGAGC 152  
Amatitlania -----CAT---CCCTGGG-----TTTTGAG-TCAGTGTGCTGGGACA----ACTCAATC 203  
Chimaera G-----CCCAGCTTTGCGGT--GGCTCAGGG-CCAGTG---TGGGGACCAAGACCCTGAC 266

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Heterodontus TGCAGGGAGCTGTCC--TCAGCTCC-AAAACATGATGGAATGTACTGAAGCCTGCAAAGTG 231  
Horn TGCAGGGAGCTGTCC--TCAGCTCC-AAAACATGATGGAATGTACTGAAGCCTGCAAAGTG 233  
Verasper TGCAAGGAACCTGATT--TCAACTCC-AAAACATGATGGAATGTATTGAAACTTGCAAAGTG 222  
Danio TGCAGA-----TCGGA-CC-AA-----GAATCTTCCAGAA-- 166  
Protopterus TGTAGAGACTTGTCC--ACAGAAAG-TAATCTCCTGGAATGCATCAAGTCTTGCAAATCT 227  
Neoceratodus TGCACAGACCTGTCC--ACAGAACT-CAATCTACTGGAATGCATCAAGCCTGTAAATTT 271  
Acipenser TGCAGAGACCTTTCC--TCAGAGGA-AAACATACTGGAATGTATACAAGCCTGTAAAGTG 256  
Polyodon TGCAGAGACCTTTCC--TTGAGAGG-AAACATACTGGAATGTATACAAGCCTGTAAAGTT 262  
Japanese TGCAAAGATCTCAGC--TCTGAGGA-GAACATGCTGGAATGCATAC--GCCTGT----- 262  
European TGCAAAGATCTCAGC--TCTGAGGA-GAACATGTTGGAATGCATAC--GCCTGT----- 271  
Anguilla TGCAAAGATCTTAGC--TCTGAGGA-GAACATGCTGGAATGCATAC--GGCTGT----- 243  
Lepisosteus TGCAGAGACCTCAGC--TCGGAGGA-AAACATCTTGGAGTGTATACAAGCCTGCAATTCG 251  
Gobiocypris TGCAGAGACCTCACC--ACTGAGGA-GAGCATCTTGGAAACGCATACAGCTATGCAAGTCT 165  
Carassius TGCAGAGACCTCACC--ACTGACGA-GAACATCTTGGACTGCATACAGCTATGCAAGTCT 257  
Ictalurus TGCAGAGATCTAAGC--TCTGACAG-AAATATCCTGGAATGCATCAGGTTGTGCAGATCT 285  
Solea TGTCAAGGAAGTCAAC--TGTGAGGA-GAGCATGATGGAGTGTGTCCGGCTGTGTCCGCTCT 215  
Common TGTCAAGGAGTCACT--GATGAGAG-GAGCATGATGGAGTGTGTCCGGCTGTGTCCGCTCT 223  
Sparus TGTCAAGGAGTCAAC--TCTGAGAG-CAGCGTGTGAGTGCATCCAGCTTTGTCACTCC 228  
Yellowfin TGTCAAGGAGTCAAC--TCTGAGAG-CAGCGTGTGAGTGCATCCAGCTTTGTCACTCC 240  
Sparidentex TGTCAAGGAGTCAAC--TC-GAGAGACAGCGTGTGAGTGCATCCAGCTTTGTCACTCC 239  
Acanthopagrus TGTCAAGGAGTCAAC--TCTGAGAG-CAGCATGATGGAGTGCATCCAGCTTTGTCACTCC 217  
Oreochromis TGCCAGGAGCTCAGC--TCTGAGAG-CAACATGATGGAGTGCATCCAGCTCTGTCACTCT 242  
Monopterus TGCCAGGAGCTCAAC--TCTGAG-GCCAGCGTGTGAGTGTATCCAGCTCTGTCACTCC 248  
Dicentrarchus TGTCAAGGAGTCAAC--TCTGAGAG-CAATATGCTGAGTGTATCCAGCTCTGTCCGCTCC 219  
Epinephelus TGTCAAGGAGTCAAG--TCTGAGGG-CAGCATGATGGAATGTATCCAGCTCTGTCCGCTCC 209

Amatitlania	TGCAAGGACCTGAGC--AGCAAGGC--AAGGATACTGGACTGCATTACCTCTGCATGTCT	260
Chimaera	TGTC--ACCTCAACAACCTGGATACCAACCTCCTGCCCTGTAATGAGGTGTGTAGCAAT	323
	** * *	
Heterodontus	GAGAAGACACTTGAATCCCCAA-----TCTACCC-----	260
Horn	GAGAAGACACTTGAATCCCCAA-----TCTATCC-----	262
Verasper	GACAAGACACTTGAATCCCCAA-----TCTACCC-----	251
Danio	-----ACATCAGAGTTTCT-----	180
Protopterus	GATCTTTCAGCTGAATCTCCAG-----TTTATCC-----	256
Neoceratodus	GACCTGTCAGCTGAGTCTCCAG-----TATAACC-----	300
Acipenser	AACCTCTCTGCTAAGTCCCCCA-----TCTTACC-----	285
Polyodon	GACCTTACTGCAGAGTCCCCC-----TTTTTCC-----	291
Japanese	-----GCA-AGTCT-----	270
European	-----GCA-AGTCT-----	279
Anguilla	-----GCA-AGTCT-----	251
Lepisosteus	GACCTCACCGCAGAATCTCCCA-----TTTTTCC-----	280
Gobiocypris	GATCTGACAGATGAAACCCCG-----TATAACC-----	194
Carassius	GATTGACAGATGAAACCCCTG-----TCTACCT-----	286
Ictalurus	GAGCTTACAGCAGAATCTCCTC-----TCTACCC-----	314
Solea	GACCTCAGCGCCGAGGAGCCGC-----TGGTCCC-----	244
Common	GACCTCAGCGCCGAGGAGCCGC-----TGGTCCC-----	252
Sparus	GACCTCACCGCGGAGACCCCG-----TCATCCC-----	257
Yellowfin	GACCTCACCGCGGAGACCCCG-----TCATCCC-----	269
Sparidentex	GACCTCACCGCGGAGACGCCCC-----TCATCCC-----	268
Acanthopagrus	GACCTCACCGCGGAGACGCCCC-----TCATCCC-----	246
Oreochromis	GACCTCACCGCGGAGACCCCG-----TCATCCC-----	271
Monopterus	GACCTCACCGCGGAGACGCCCC-----TCATCCC-----	277
Dicentrarchus	GACCTCACCGCAGAGACCCGA-----TCATCCC-----	248
Epinephelus	GACCTCACCGCAGAGACCCGT-----TCATCCC-----	238
Amatitlania	GTGATCCAGACTGAAATCCCAGAGCTCAGTGCACCTGGCCCCGAATGTTAACAGTGATGCT	320
Chimaera	GGGCAGAGCCTGGAGGTCCCC-----TGTCCC-----	352
	*	
Heterodontus	-----TGGCAATGGCCAC-----CTGCAGCCAATTG-----CA	288
Horn	-----TGGCAATGGCCAC-----CTGCAGCCAATTG-----CA	290
Verasper	-----TGGCAACAATCAC-----CTGCAGCCAATTG-----CA	279
Danio	-----TCAT-----CT-----	186
Protopterus	-----TGGCAATGGACAC-----ATGCAGCCATTGT-----CAG	285
Neoceratodus	-----TGGAGATGGTCAC-----TTGCAACCCTTGT-----CAG	329
Acipenser	-----TGGAAATGAGCAT-----CTCCAGCCTACCC-----CCG	314
Polyodon	-----TGGAAATGAGCAT-----CTCCAGCCTACCT-----CCG	320
Japanese	-----GAA-----CTG	276
European	-----GAA-----CTG	285
Anguilla	-----GAA-----CTG	257
Lepisosteus	-----TGGGAATGGGCAC-----CTCCAGCCTCCTTCAGAGGCTG	315
Gobiocypris	-----CGGAGAAAGCCAT-----TTGCAGCCTCCCT-----CCG	223
Carassius	-----TGGAGAAAGCCAT-----TTCCAGCCTCCCT-----CTG	315
Ictalurus	-----TGGAGATGGCCAC-----CTCCAGTCCGTCT-----T--	341
Solea	-----GGGTGACGGTCAC-----CTCCAGCCTCC-----	268
Common	-----GGGTGACGGTCAC-----CTGCAGCCTCC-----	276
Sparus	-----AGGCAATGCCCAC-----CTCCAACCTCC-----	281
Yellowfin	-----AGGCAATGCCCAC-----CTCCAACCTCC-----	293
Sparidentex	-----AGGCAGCGCCCAC-----CTCCAGCCTCC-----	292
Acanthopagrus	-----AGGCAGCGCCCAC-----CTCCAGCCTCC-----	270
Oreochromis	-----CGGCAACGCCCAC-----CTCCAGCCCGC-----	295
Monopterus	-----AGGAAGAGCGCAC-----CTCCAACCTCA-----	301
Dicentrarchus	-----GGGCGATGGCCAC-----CTCCAACCTCT-----	272
Epinephelus	-----AGGCAGCAACCAC-----CTCCAACCTGT-----	262
Amatitlania	GACGCTGATGACAGCGGTGGTGGTGTGACTATAATGATGACAGCCTTACT----	CAG 376
Chimaera	-----TGGGAATGGTCGG-----CTGATGCCGGTGT-----TCG	381
Heterodontus	GAGAGCATCAGGAATTATGTCATGGGTCAATTTCCGCTGGAACAAGTTTGGGAAAAAAGA	348
Horn	GAGAGCATCAGGAATTATGTCATGGGTCAATTTCCGCTGGAACAAGTTTGGGAAAAAAGA	350
Verasper	GAGAGCATCAGGAATTATGTCATGGGTCAATTTCCGCTGGAATAAATTTGGGAAAAAGAGA	339
Danio	--GAACATCAGA-----GCAGTGAA-----GAACAGGT---AGAGGAGCAGA	223
Protopterus	AAGATAT--TCGCAATATATGATGACTCACTTTTCGATGGGACAAGTTTGGCAGGCCGAAAC	344
Neoceratodus	AGGACAT--CCGTAACATACATGATGACTCACTTCCACTGGGACAAGTTTGGGAGGAGGAAC	388
Acipenser	AGGATATTCAGAA--CTACATCATTAGCCACTTTTCGCTGGAACACATTCGGTCAGAGAATG	373
Polyodon	AGGAGATTCAGAA--CTACGTCATGGGTCACTTCCACTGGAACACATTCGGTCAGAGAATG	379
Japanese	A-----CAGCA-----GGCCGGCGTGCC	294
European	A-----CAGCA-----GGTCGGCGTGCC	303
Anguilla	A-----CAGCA-----G-----	264
Lepisosteus	A-----CCGCAACTACGCGAAGAGTCACTTCCGCTCGACTGCCTTGGGGCGGCGCACC	368
Gobiocypris	A-----	224
Carassius	A-----	316
Ictalurus	-----	

Solea -----  
Common -----  
Sparus -----  
Yellowfin -----  
Sparidentex -----  
Acanthopagrus -----  
Oreochromis -----  
Monopterus -----  
Dicentrarchus -----  
Epinephelus -----  
Amatitlania AATCATTCTGGCC-----ACACTGGCTTCTGATGA 406  
Chimaera AGGCCCT----- 388

Heterodontus GGCAACAACACTGGGTTTTTCAGGCAACAAGCGAG-----ATGATGAACCTGTCA 397  
Horn GGCAACAACACTGGGTTTTTCAGGCAACAAGCGAG-----ATGATGAACCTGTCA 399  
Verasper GGCAACAACACTGGGTTTTTCAGGCAACAAGCGAG-----AAGATGAACCTGTCA 388  
Danio GTC--TGAGCTTGGGTTTGCTTTTATCA-GC-----TCTGTC- 257  
Protopterus AATG-----AAACAGGTAATAAGCGAG-----AAGATGGGTAC----AAAA 381  
Neoceratodus AATG-----AAACAGGTAACAACAGG-----AAGGTGGGTCA----GACA 425  
Acipenser AACG-----GCACACCTGG-AGGCAGC-----AAGGGGAGAGTGCCAGTA 413  
Polyodon AATG-----GCACACCTGG-AGGCAGC-----AAGAGAGAGAGTGCCAGCA 419  
Japanese TCCA-----GCAC-CATGG-AAACGG-----AGGGGGAAGAG---GGCA 328  
European TCCA-----GCAC-CATGG-AAACGG-----AGGGGGAAGAG---GGCA 337  
Anguilla -----CAC-CATGGCAAACGG-----AGGGGGAAGAG---GGCA 294  
Lepisosteus AACG-----GCTC-TGTGGGAAGCAGCAAG---CAAGCCGGAGAG---AACG 408  
Gobiocypris -----GCCGGAGCAAAATGA-----GGTCATCGCACCA 252  
Carassius -----GCTGGAGCAAACCGA-----GGTCCTCCCACCC 344  
Ictalurus -----GCTCATACCCAGTGA-----AATC----ACGA 364  
Solea -----TCCTCTGT----- 276  
Common -----TCCTCTGT----- 284  
Sparus -----GCCTCCATCAGA-----CC 295  
Yellowfin -----GCCTCCATCAGA-----CC 307  
Sparidentex -----GCCTCCGTTAGA-----CC 306  
Acanthopagrus -----GCCTCCGTCAGA-----CC 284  
Oreochromis -----TGTCATCAGA-----CG 309  
Monopterus -----GCCTCC---AGA-----CT 312  
Dicentrarchus -----TCCTCCGTCAGAG-----TCT 288  
Epinephelus -----TCCTCCATCAGATCTC-----GATCT 284  
Amatitlania CAAA-----ATGCCGAGTCAGACCTGAAA---GCCCGCAGCGACGAGCGCG 451  
Chimaera -----GCGCAGTTATGTGATGGGACATTTCCGCTGGAACAAGTTTGGGA 432

Heterodontus GGGCATTCCTAAACCACCTCCCTGCTGTAGAA-----CCCCATAC 437  
Horn GGGCATTCCTAAACCACCTCCCTGCTGTAGAG-----CCCCAAC 439  
Verasper GGGTATTCCTGAACCAGCTCCCTGCTGTAGAG-----TCCCAAGC 428  
Danio -----TCCCGA-----CTCC-----ATCGAG-----CTCCAAA 281  
Protopterus CTC---CTTTACTCAGCATCATTCCTGCTTTG-----GAATCTCATAAT 422  
Neoceratodus CTT---TCTTATTCAACATCCTGCCTTG-----GAATCTCACAGT 466  
Acipenser CCG---CCCTTAACATTCCTTAGCAGCGTTA-TCCCAACCCACAGATGAAGTTGAGAG 469  
Polyodon ATG---CCCTTAATGTTCTCCTAGCAGTGTTA-TCCCAACCCAGAGATAAAGTTGAGAG 475  
Japanese TG-----CTGGGATTCCTGCTCCCTATGGTG--TCCAGC-----ATGC--AGGAG 369  
European TG-----CTGGGATTCCTGCTCCCTATGGTG--TCCAGC-----ATGC--AGGAG 378  
Anguilla TG-----CTGGGATTCCTGCTCCCTATGGTG--TCCAGC-----ATGC--AGGAG 335  
Lepisosteus CGG---CTCTGAGCATCCTCTTTGCCGCCCTGGCTCCACCCC-----AGGCCGAAGAA 458  
Gobiocypris CTGT---CCCCAGTTGCCCTCGGACTGCT-----GAGCAAATGGA 290  
Carassius CTGT---CCCCAGCGGCCCTCGCTCTTGCT-----GAGCAAATGGA 382  
Ictalurus CTG-----CCAG--ACTTTCACCCAC-----AAGAGGATGAA 396  
Solea CTT---CCCTGGCGCTCTCCTCCTCCTCCTCC----- 305  
Common CTT---CCCTCGCGCTCTCCTCCTCCTCCTCC----- 313  
Sparus CCT---CCTCCTTACCCCTCCCTTCCCTCGTCC----- 324  
Yellowfin CCT---CCTCCTTACCCCTCCCTTCCCTCGTCC----- 336  
Sparidentex CCT---CCTCCTTCCGCCCTCCCTTCCCTCGTCC----- 335  
Acanthopagrus CCT---CCTCCTTACCCCTCCCTTCCCTCGTCC----- 313  
Oreochromis CCT---CCTC---ACCTTC----- 322  
Monopterus CCT---CCTCC---ATCCTCCGTCCTCCTCC----- 338  
Dicentrarchus CTG---CCTCC---CCTCCTCCTGTTCTCTTCC----- 315  
Epinephelus CTG---CCTCC---CCTCCTCCTTTTCTCTTCC----- 311  
Amatitlania CTCCTACACAATGGAGCATTTCCGCTGGGGCAAACCTCA-----GGACTTAAGAT 502  
Chimaera AGAAAGGTGGGAACAGCTCCAGGTCAATGAAA-----GGCGGGGCGG 474

Heterodontus CTCTCAGATGGAAAATGAAGAGATGGAGACTCTGTTTCCAAGAGAAGATGATAAAAAGATC 497  
Horn CTCTCAGATGGAAAATGAAGAGATGGAGACTCTGTTTCCAAGAGAAGATGATAAAAAGATC 499  
Verasper GTCTCAGATGGAAAATGAGGAGATGGAAACTCTGCTTCCAAGGGAAGATGAAAAGAGATC 488  
Danio CCCC---ACGGCA-----GAAGCTC---CTCACGGG--GACGAGAGGCGGTC 320  
Protopterus AACCTTGACATGGAAGA-----GGATTCTTTATCG--AGGCAAGATGACAGGCGATC 472

Neoceratodus GACCTTGATGTGGCAGG-----GGAACCCCTCCAG--AGACAAGATGACAAGAGGTC 516  
 Acipenser GAATCTGAGGAGGCAGA-----AGGGCTGCAGCAGCACAGGCGGGATGACAAGAGGTC 522  
 Polyodon GATTCGAGGAGGAAGA-----AGGTCTGCAGCAGCACAGACGGGATGAAAAGAGGTC 528  
 Japanese GGCCCCGAGGTGGACGA-----GCAGGGGCGTGAGCCTCGGCACGAGGACAAGCGCTC 422  
 European GGCCCCGAGGCGGACGA-----GCAGGGGCGCGAGCCTCGGCACGAGGACAAGCGCTC 431  
 Anguilla GGCCCCGAGGTGGACGA-----GCAGGGGCGCGAGCCTCGGCACGAGCACAAGCGCTC 388  
 Lepisosteus GAGATGGAGGAGAGTGA-----GAGCTCGCAGCAGCAGAGGCGTGAGGACAAGCGCTC 511  
 Gobiocypris CCCC--GATTTCA-----GCACTC-----GGCACGAGCACAAGCGCTC 326  
 Carassius CCCC--GGGTCCA-----GCCCTC-----GGCACGAGCTCAAGCGCTC 418  
 Ictalurus GCTCCAGAGTCTG-----GTCCAC-----AACACGAAGAAAAGCGCTC 434  
 Solea -TCCTC-----TCCTC-----AGGCCAAGCGCGC 328  
 Common -TCCTC-----TCCTC-----AGGCCAAGCGCGC 336  
 Sparus -TCCTC-----TCCTC-----AGGCCAAGCGCTC 347  
 Yellowfin -TCCTC-----TCCTC-----AGGCCAAGCGCTC 359  
 Sparidentex -TCCTC-----CCCTC-----AGGCCAAGCGCTC 358  
 Acanthopagrus -TCCTC-----CCCTC-----AGGCCAAGCGCTC 336  
 Oreochromis -----TTCTC-----AGGCCAAGCGCTC 340  
 Monopterus -TCCACTAC-----TCCTC-----AGGCCAAGCGGTC 364  
 Dicentrarchus -TCCTCCTC-----TCCAC-----AGGCCAAGCGCTC 341  
 Epinephelus -CCCTCCTC-----TCCTC-----AGGCCAAGCGCTC 337  
 Amatitlania GCGAGAGTCAAAGCTGA-----GGGCCCGCA-----GCATGAGAGACGCTC 544  
 Chimaera GCCCTCGTGGGAACCCAA-----GGGGCTCTACTCT--GCGGGAAGGCAAACGTT 526

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Heterodontus CTACTCTATGGAACATTTCCGATGGGGGAAGCCCATGGGCATGAAGAGGAAGCCTGTAA 557  
 Horn CTACTCTATGGAACATTTCCGATGGGGGAAGCCCATGGGCATGAAGAGGAAGCCTGTAA 559  
 Verasper CTACTCCATGGAACATTTCCGATGGGGGAAGCCCATGGGCATGAAGAGGAAGCCTGTAA 548  
 Danio ATACTCCATGGAGCACTTCAGATGGGGCAAACCCATGGGC-CGCAAACGCAGACCGGTAA 379  
 Protopterus TTACTCCATGGAGCACTTCGCTGGGGTAAACCCATGGGC-AGCAAACGCAGACCGGTAA 532  
 Neoceratodus CTATTCCATGGAGCACTTCGCTGGGGGAAGCCAGTAGGCAAGAAGAGGAGCCAGTCAA 576  
 Acipenser CTATTCCATGGAGCACTTCGCTGGGGCAAACCTGTGGGCCGCAAGCGCCGTCCGGTAA 582  
 Polyodon CTATTCCATGGAGCACTTCGCTGGGGCAAACCTGTGGGCCGCAAGCGCCGTCCAGTAA 588  
 Japanese CTACTCCATGGAGCACTTCGCTGGGGAAAGCCGTCGGGCCGCAAGCGCCGCAATCAA 482  
 European CTACTCCATGGAGCACTTCGCTGGGGAAAGCCGTCGGGCCGCAAGCGCCGCAATCAA 491  
 Anguilla CTACTCCATGGAGCACTTCGCTGGGGAAAGCCGTCGGGCCGCAAGCGCCGCAATCAA 448  
 Lepisosteus CTATTCCATGGAGCACTTCGCTGGGGGAAGCCGTCGGGCCGCAAGCGCCGTCTGTAA 571  
 Gobiocypris TTACTCCATGGAGCACTTCGCTGGGGAAAGCCGTCGGGCCGCAAGCGCCGCAATCAA 386  
 Carassius CTACTCCATGGAGCACTTCGCTGGGGAAAGCCAGTAGGTCGCAAGCGCACGCCATCAA 478  
 Ictalurus TTACTCCATGGAGCACTTCGCTGGGGTAAAGCCGTGGGTGCGCAAGCGCCGCAATCAA 494  
 Solea CTACTCCATGGAGCACTTCGCTGGGGGAAGCCGTGTCGGTAAGAAAAGCGCCGCAATCAA 388  
 Common CTACTCCATGGAGCACTTCGCTGGGGGAAGCCGTGTCGGTAAGAAAAGCGCCGCAATCAA 396  
 Sparus CTACTCCATGGAGCACTTCGCTGGGGGAAGCCAGTCGGCCGAAAACGTCGCCCCGTCAA 407  
 Yellowfin CTACTCCATGGAGCACTTCGCTGGGGGAAGCCAGTCGGCCGAAAACGTCGCCCCGTCAA 419  
 Sparidentex CTACTCCATGGAGCACTTCGCTGGGGGAAGCCAGTCGGCCGAAAACGTCGCCCCGTCAA 418  
 Acanthopagrus CTACTCCATGGAGCACTTCGCTGGGGGAAGCCAGTCGGCCGAAAACGTCGCCCCGTCAA 396  
 Oreochromis CTACTCAATGGAGCACTTCGCTGGGGGAAGCCGTCGGCCGAAAACGTCGCCCCGTCAA 400  
 Monopterus CTACTCCATGGAGCACTTCGCTGGGGGAAGCCGTCGGTCGAAAACGTCGCCCCGTCAA 424  
 Dicentrarchus CTACTCCATGGAGCACTTCGCTGGGGGAAGCCGTGTTGGACGAAAAGCGCCGCAATCAA 401  
 Epinephelus CTACTCCATGGAGCACTTCGCTGGGGGAAGCCGTGTTGGACGAAAAGCGCCGCCCCGTCAA 397  
 Amatitlania CTATTCCATGGAGCACTTCGCTGGGGGAAGCCCTCAGGCCGTAACGTCGACCCGTAA 604  
 Chimaera CTACTCCATGGAGCACTTCGCTGGGGGAAGCCCATGGGCAGGAAGGAGACCCATCAA 586

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Heterodontus GGTCTAC---CCAAATAACTTTGAAGATGGGTGAGTGGAGAACATGGGACCTAGACTCAA 614  
 Horn GGTCTAC---CCAAATAACTTTGAAGATGGGTGAGTGGAGAACATGGGACCTAGACTCAA 616  
 Verasper GGTGTAC---CCAAACAGCTTTGATGATGATTCAATGGAACATATGGGACCTGAACCTCAA 605  
 Danio -----AAGT-----GTTGAGCA-ACGGGGC----- 398  
 Protopterus GGTATAC---CCAAATGGC-----ATGGAGGA---TGAA----- 560  
 Neoceratodus GGTGTAC---CCAAATGGC-----ATGGAGGA---TGAA----- 604  
 Acipenser GGTGTAC---CCCAATGGA-----GTGGAGGA---GGAG----- 610  
 Polyodon GGTGTAC---CCCAATGGA-----GTGGAGGA---GGAG----- 616  
 Japanese GGTCTTC---CCCAGTGGC-----ATGGAGGA---GGAG----- 510  
 European GGTCTTC---CCCAGTGGC-----ATGGAGGA---GGAG----- 519  
 Anguilla GGTCTTC---CCCAGTGGC-----ATGGAGGA---GGAG----- 476  
 Lepisosteus GGTGTAC---CCCAACGGG-----GTGGAAGA---GGAA----- 599  
 Gobiocypris GGTGTAC---ACAAACGGC-----GTGGAGGA---GGAA----- 414  
 Carassius GGTGTAC---ACCAACGGC-----GTGGAGGA---GGAA----- 506  
 Ictalurus GGTGTAC---GCCAATGGG-----GTGGAGGA---AGAG----- 522  
 Solea AGTCTACAGCTCCAACAGT-----GTGGAGGA---AGAA----- 419  
 Common AGTCTACAGCTCCAACAGT-----GTGGAGGA---AGAA----- 427  
 Sparus AGTCTACACCTCCAACGGC-----GTGGAGGA---GGAG----- 438  
 Yellowfin AGTCTACACCTCCAACGGC-----GTGGAGGA---GGAG----- 450  
 Sparidentex AGTCTACACCTCCAACGGC-----GTGGAGGA---GGAG----- 449  
 Acanthopagrus AGTCTACACCTCCAACGGC-----GTGGAGGA---GGAG----- 427  
 Oreochromis AGTGTACACCTCCAACGGC-----GTGGCGGA---GGAG----- 431  
 Monopterus AGTCTACGCCCTCCAATGGT-----GTGGAGGA---GGAG----- 455  
 Dicentrarchus AGTCTTACCTCCAACGGC-----GTGGAGGA---GGAG----- 432

Epinephelus	AGTCTACACCTCAAACGGC-----GTAGAAGA--AGAG-----	428
Amatitlania	AGTCTTT--GCCTCTTC-----CTGGAAGG--AGGGAGC-----	635
Chimaera	GGTTTAC---CCTAATGAGGAGCCAGAAACCTCAGCTGAGGGCCTGGGGATCAGTTCAA	643
	* *	
Heterodontus	GCGTGAAGCATCTG-----TAGGTTTTGACTA-----	641
Horn	GCGTGAAGCATCTG-----TAGGTTTTGACTA-----	643
Verasper	GCGAGAAGCTTCTG-----TTGATTTTGACTA-----	632
Danio	-----TCTG-----	402
Protopterus	-----TCAG-----CTGAAAGC--TA-----	574
Neoceratodus	-----TCAG-----CTGAGAGC--TT-----	618
Acipenser	-----TCTG-----CTGAGAGC--TA-----	624
Polyodon	-----TCTG-----CTGAGAGC--TA-----	630
Japanese	-----TCGT-----CCGAGGCC--TA-----	524
European	-----TCGT-----CCGAGGCC--TA-----	533
Anguilla	-----TCGT-----CCGAGGCC--TA-----	490
Lepisosteus	-----TCGG-----CCGAGGCC--TA-----	613
Gobiocypris	-----TCCG-----CTGAGACT--CT-----	428
Carassius	-----TCCG-----CCGAGACT--CT-----	520
Ictalurus	-----TCAT-----CTGAGGCC--CT-----	536
Solea	-----TTCC-----CCGAGGTT--TT-----	433
Common	-----TTCC-----CCGAGGTT--TT-----	441
Sparus	-----TCCG-----CCGAGGTC--TT-----	452
Yellowfin	-----TCCG-----CCGAGGTC--TT-----	464
Sparidentex	-----TCAG-----CCGAGGTC--TT-----	463
Acanthopagrus	-----TCAG-----CCGAGGTC--TT-----	441
Oreochromis	-----TCTG-----CTGAAGTT--TT-----	445
Monopterus	-----TCCG-----CTGAAGTT--TT-----	469
Dicentrarchus	-----TCAG-----CCGAGGTT--TT-----	446
Epinephelus	-----TCAG-----CCGAGGTT--TT-----	442
Amatitlania	-----TCTT-----CTGAGGGCCGTT-----	652
Chimaera	ACGTCAGATCCCCGAAGAAGCCTGGGGTCTAGTGAACCCTGGGTCCCTGTAGAGCAGGT	703

Heterodontus	-----CCCAGTAGAGACATCTGAAGCAGGTGAAGAGGAGATGCTGGAAGA-TGCCAAGA	694
Horn	-----CCCAGTAGAGACATCTGAAGCAGGTGAAGAGGAGATGCTGGAAGA-TGCCAAGA	696
Verasper	-----TCCAGTCGAGCAATCTGAGGTCCGGTGAAGAGGAGATGCTGGAAGA-TGCCAAGA	685
Danio	-----GAAGAAGA---GCCGGAGGA-GTCGGAGG	427
Protopterus	-----CTCCCTGA-----CCT---CAGACGT--GACA--TGCCATAG	605
Neoceratodus	-----CTCCCTGA-----GTT---CAGGCGG--GACA--TGACCATG	649
Acipenser	-----CCCTGCCGA-----GAT---CCGCCGG--GACT--TGTCACATC	655
Polyodon	-----CCCTGCTGA-----GAT---CCGCCGG--GACT--TGTCACATC	661
Japanese	-----CCC GCCGA-----GAT---GAGGCGA--GAGC--TCCTGGCC	555
European	-----CCC GCCGA-----GAT---GAGGCGA--GAGC--TCCTGGGC	564
Anguilla	-----CCC GCCGA-----GAT---GAGGCGA--GAGC--TCCTGGGC	521
Lepisosteus	-----TCCCACAGA-----GAT---GCGGCGC--GACC--TCATGAGC	644
Gobiocypris	-----CCC GGTTGA-----GAT---GAGACGT--GAGC--TGGCAACC	459
Carassius	-----CCCAGCTGA-----GAT---GAGGCGC--GAGC--TGGCCACC	551
Ictalurus	-----GCCAGCAGA-----GAT---GAGGCGT--GAGC--T-----	560
Solea	-----GAGGCAG--GAGC--TC-----	446
Common	-----GAGGCAG--GAGC--TC-----	454
Sparus	-----CCCTGGAGA-----GATCAGGAGGCGT--GAGC--TCGCCAGT	486
Yellowfin	-----CCCTGGAGA-----GATCAGGAGGCGT--GAGC--TCGCCAGT	498
Sparidentex	-----CCCCGGAGA-----GATCCGGAGGCGT--GAGC--TCGCCAGT	497
Acanthopagrus	-----CCCCGGAGA-----GATCCGGAGGCGT--GAGC--TCGCCAGT	475
Oreochromis	-----CCCCGAAGA-----GATGAGGAGACGA--GAGC--TTACCAAT	479
Monopterus	-----CCCCGGAGA-----GATGAGGAGGCGG--GAGC--TGGCCAAC	503
Dicentrarchus	-----CCCTGAAGA-----GATGAGAAGGCGA--GAGC--TTGCCAGC	480
Epinephelus	-----CCCCGGAAA-----GATGAGGAGGCGG--GAGC--TCACCAGC	476
Amatitlania	-----TCCTTTTCA-----GGCTCGCAGGCAGCTGAGCAGTAGTGAAG	690
Chimaera	CTGGGGCCCTGTAGAGCAGACCTGGAGCCAGGGTGGTGAGGCAT--GGAGCCCTGAACAG-	761
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Heterodontus	AGAAAGATGGGAAGGTTTACAAAATGGC---CCACTTCAGGTGGGGCAGGGGA-----	744
Horn	AGAAAGATGGGAAGGTTTACAAAATGGC---CCACTTCAGGTGGGGCAGGGGA-----	746
Verasper	AGAAAGATGGGAAGATTTACAAAATGAC---CCATTCAGGTGGGGCAGGGGA-----	735
Danio	AGAGCG-----TCCGTGTGGAACGAGGA-----	450
Protopterus	---GAATTTGATTACCTGAACC-----TAGTCTGAGGAGAAG-----	641
Neoceratodus	---GAGATTGACTATCCTGAGTC-----TAGCTCTGAAGAGAGA-----	685
Acipenser	---AAGCTTGATTACCCCCAGGGA-----GAGGAGCTGGAGGAAGTGT-----	695
Polyodon	---AAGCTTCATTACCCCCAGGGA-----GAGGAGCTGGAGGAAGTGT-----	701
Japanese	---GACTGGGACTACGCCCCG-----GAAGAAGAGGGCGTGG-----	589
European	---GACTGGGACTACGCTCCG-----GAAGAAGAGGGCGTGG-----	598
Anguilla	---GACTGGGACTACGCTCCG-----GAAGAAGAGGGCGTGG-----	555
Lepisosteus	---GACCTTGACTACCCCCCTCTC-----GAAGAAGTGGAGGAGGAGC-----	684
Gobiocypris	AACGAGGTCGACTATCCCCAG-----	480
Carassius	AACGAGGTCGACTATCCTCAA-----	572

Ictalurus ---AGGT--GCTGTT----- 570  
Solea -----GGCG----- 450  
Common -----GGCG----- 458  
Sparus ---GAGCT--GTTGGCAGCAGCAGCTGTGGCGCAGGAGGAGGAGGAGAAGGCGGTGCAGG 541  
Yellowfin ---GAGCT--GTTGGCAGCAGCAGCTGTGGCGCAGGAGGAGGAGGAGAAGGCGGTGCGAA 553  
Sparidentex ---GAGCT--GCTGGCAGCAGCGGCTGCAGCGCAAGAGGAGGAGGAGAAGGCG----- 545  
Acanthopagrus ---GAGCT--GCTGGCAGCAGCAGCTGCAGCGCAGGAGGAGGAGGAGAAGGCG----- 523  
Oreochromis ---GAGCT--GCTGGCAG-----AGGAAGGAGAGAAGGCT----- 509  
Monopterus ---GAGCT--GTTGGCAGCGGCAGC-----AGAGGAGGAGGAGAAGGCA----- 542  
Dicentrarchus ---GAGAT--TATAGCGGCAG-----AGGACGAGGAGAAGGCA----- 513  
Epinephelus ---AAGCT--GCTTGCAGCAA-----AGGAAAAGGAGAAGGCG----- 509  
Amatitlania ATGAAGCAGAGGGGGACGTTGATG-----GAGGAAGTAATGAAAATCA----- 733  
Chimaera ---GCGGCCTGGGGCCAGAGGAGGAGCCTGAGCAGAAGAAGGATGGGAAGTTATAT--- 815

Heterodontus -----CCAAAGGGCCCCGCAAAGA 763  
Horn -----CCAAAGGGCCCCGCAAAGA 765  
Verasper -----CCAAAACACCT----- 746  
Danio -----CAGAGCG----- 457  
Protopterus -----TCAGA----- 646  
Neoceratodus -----TCAGA----- 690  
Acipenser -----TCGGAGG----- 702  
Polyodon -----TTGGAGG----- 708  
Japanese -----CTGAGG----- 595  
European -----CTGAGG----- 604  
Anguilla -----CTGAGG----- 561  
Lepisosteus -----TCGGAGG----- 691  
Gobiocypris -----GAGG----- 484  
Carassius -----GAGG----- 576  
Ictalurus -----GAGG----- 574  
Solea -----AGG-----CGGACG----- 459  
Common -----AGG-----CGGACG----- 467  
Sparus AAGTGATGCAGGAGGAGAAGGCGCAGGAGGTGATGGAGGAGGCGGAGG----- 589  
Yellowfin AAGTGATGCAGGAGGAGAAGGCGCAGGAGGTGATGGAGGAGGCGGAGG----- 601  
Sparidentex -----GCACAGGAGGTGATGCAGGAGGCGGAGG----- 573  
Acanthopagrus -----GCGCAGGAGGTGATGCAGGAGGCGGAGG----- 551  
Oreochromis -----CAGGAGATGGTCGAGGGGGCGGAGG----- 534  
Monopterus -----CAGGAGGTGAT-----GGAGG----- 558  
Dicentrarchus -----CAGGAGGTGG-----CGGAAG----- 529  
Epinephelus -----CAGGAGGTGG-----CTGAAG----- 525  
Amatitlania -----GGGATGGCTGAGGGCC----- 749  
Chimaera -----AAAATGACCCACTTCAGGTGGTCCGAGGGCTCCAGGGACG 855

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Heterodontus ACT-----GGGGTCCTGACCACATGCGCACTCAGCCATTGCAGTTTCC----- 806  
Horn ACT-----GGGGTCCTGACCAGCATGCGCACTCAGCCATTGCAGTTTCC----- 808  
Verasper -----GGGCCCTGACCCGATACGTATTCAGTCATTGCAGTTTCC----- 785  
Danio -----GCACCC-----TGGAGGTTTC----- 472  
Protopterus -----A-GAGAAATGACATTATG----- 662  
Neoceratodus -----A-GAGAAATGACATTTTG----- 706  
Acipenser -----G-GAGAAATGACCTCCTT----- 718  
Polyodon -----G-GAGAAATGACCTCCTT----- 724  
Japanese -----A-ACACCCACAG--CTC----- 609  
European -----A-ACACCCACAG--CTC----- 618  
Anguilla -----A-ACACCCACAG--CTC----- 575  
Lepisosteus -----A-GAGAACGAGGTCTCTC----- 707  
Gobiocypris -----A-GAGCGC-----TTTA----- 495  
Carassius -----A-GGGCGC-----TTTA----- 587  
Ictalurus -----A-CAGCAC-----CGTC----- 585  
Solea -----TCGAGCAACACAAGTTCC---AC----- 479  
Common -----TCGAGCAACACAGTTCC---AC----- 487  
Sparus -----AGGAGCAGCAGCAGCTTCTGGGA----- 612  
Yellowfin -----AGGAGCAGCAGCAGCTTCTGGGA----- 624  
Sparidentex -----AGGAGCAGCAGCAGCTCCTGGGA----- 596  
Acanthopagrus -----AGGAGCAGCAGCAGCTCCTGGGA----- 574  
Oreochromis -----AGGAGCAGCAGC--TCCTGAAC----- 554  
Monopterus -----AGGAGCAGCGGGAGCTCCTGAAT----- 581  
Dicentrarchus -----A-----GGAGCAGCTCC----- 541  
Epinephelus -----ACGAGCAGGAGCAGCTCCAGGT----- 548  
Amatitlania -----AGGGACAGCACCAATCACAAAGCCCCCT----- 777  
Chimaera GCCTGCGGCAGGCTGGGCAAAGATACGTGTTGCCGATCTTTCCAAAGACCCCGAGGCCA 915

Heterodontus --AAACCTTGAAGATGTGCT--CCAGGAAAGCATCGACAATGGCCTGCCTGAAGAAGAGG 862  
Horn --AAACCTTGAAGATGTGCT--CCAGGAAAGCATCGACAATGGCCTGCCTGAAGAAGAGG 864  
Verasper --AAACATTGAAGATGTGCT--CCAAGAACGCATGGACAATGACTTGCCTGAAGAGGAGG 841  
Danio ---AAC--A-----CAGGAA-----CAACGTC----- 489

Protopterus ---AATCT-----TCTAGAG----- 674  
 Neoceratodus ---AGCCT-----GCTGGAG----- 718  
 Acipenser ---AATCT-----CCAGGAG----- 730  
 Polyodon ---AACCT-----CCAGGAC----- 736  
 Japanese ---ACCT-----CCAGACC----- 621  
 European ---ACCT-----CCAGACC----- 630  
 Anguilla ---ACCT-----CCAGACC----- 587  
 Lepisosteus ---AACCT-----GCAGGAG----- 719  
 Gobiocypris ---AATCA-----GCAG----- 504  
 Carassius ---AACCA-----GCAGGAT----- 599  
 Ictalurus ---AGCCA-----GCAAGAG----- 597  
 Solea ---AACGT-----CCACGAG----- 491  
 Common ---AACGT-----CCACGAG----- 499  
 Sparus ---GACGT-----CCAGGAG----- 624  
 Yellowfin ---GACGT-----CCAGGAG----- 636  
 Sparidentex ---GACGT-----CCAGGAG----- 608  
 Acanthopagrus ---GACGT-----CCAGGAG----- 586  
 Oreochromis ---GGGGT-----GCAGGAG----- 566  
 Monopterus ---AGCAT-----CCAGGAG----- 593  
 Dicentrarchus --------ACGAG----- 546  
 Epinephelus ---GACAT-----CCACGAA----- 560  
 Amatitlania -TGAGCTT-----GCAGGAG----- 791  
 Chimaera GTCAGCCTCAGGGGATGGCAACCAGGCTGAAGTGGAGGGGAGGAGAAGGAGCTGGGGC 975

Heterodontus TGAAGAAAGATGGGGAAGATTACAAATTCGGCCATTTCCGATGGAGTGTACCATTGAAAG 922  
 Horn TGAAGAAAGATGGGGAAGATTACAAATTCGGCCATTTCCGATGGAGTGTACCATTGAAAG 924  
 Verasper TGAAGAAAGATGGGGAAGATTACAAATTCGGCCATTTCCGATGGAGTGTACCATTGAAAG 901  
 Danio --AAAAACAACGGGAAGTATCGCA---TGACCACCTTCGGCTGGAATGCACCACC---AG 541  
 Protopterus --AAAAAGGACAGA---AACTACAGGATGCAGCATTTCCGCTGGAACAGCCCACCTAAAG 729  
 Neoceratodus --AAAAAGGACAGA---GCCTACAGAATGCAGCATTTCCGCTGGAACAGCCCCTCTAAGG 773  
 Acipenser --AAGAATGGTGGC---TCCTACAAGATGCATCACTTCGGTTGGAGTGGCCCACCCAAGG 785  
 Polyodon --AAGAAAGAAGGC---TCCTACAAGATGAACCATTTCCGTTGGAGTGGCCCACCCAAGG 791  
 Japanese --AAGAAGGACAGC---ACCTACAAGATGAAGCACTTCGGCTGGAACGGGCCGCTGCCA 676  
 European --AAGAAGGACAGC---ACCTACAAGATGAAGCACTTCGGCTGGAACGGGCCGCTGCCA 685  
 Anguilla --AAGAAGGACAGC---ACCTACAAGATGAAGCACTTCGGCTGGAACGGGCCGCTGCCA 642  
 Lepisosteus --AAGAAAGATGGC---TCCTACAAAATGCACCATTTCCGCTGGAGCGCCCGCCAAGG 774  
 Gobiocypris --AAGAAGGATGGG---TCCTACAAAATGAAACACTTCGGCTGGAGCAGCCCCTGTGTA 559  
 Carassius --AAGAAGGATGGC---TCCTACAAGATGAACCATTTCCGCTGGAGTGGGCCGCTGCTA 654  
 Ictalurus --AAGAAAGATGGT---TCCTATAAGATGAATCACTTCGGCTGGAGTGGCCCACCTACCA 652  
 Solea --AAAAAGGACGGC---GCGTACAAGATGAAGCACTTCGGCTGGAGCGACCCGCGGCCA 546  
 Common --AAAAAGGACGGC---GCGTACAAGATGAAGCACTTCGGCTGGAGCGACCCGCCCCA 554  
 Sparus --AAGAAAGATGGT---TTGTACAAGATGAAGCACTTCGGCTGGAGCGCCCGCCGCCA 679  
 Yellowfin --AAGAAAGATGGT---TTGTACAAGATGAAGCACTTCGGCTGGAGCGGTCGGCCGCCA 691  
 Sparidentex --AAGAAAGATGGT---TTGTACAAGATGAAGCACTTCGGCTGGAGCGCCCGCCGCCA 663  
 Acanthopagrus --AAGAAAGATGGT---TTGTACAAGATGAAGCACTTCGGCTGGAGCGCCCGCCGCCA 641  
 Oreochromis --AAGAAAGACGGC---TCGTATAAGATGAAGCACTTCGGCTGGAGCGCCCGCCGCCA 621  
 Monopterus --AAGAAAGATGGC---CCCTACAAGATGAAGCACTTCGGCTGGAGCGGTCGGCCAAACCG 648  
 Dicentrarchus --AAGAAAGATGGC---ACGTACAAGATGAAGCACTTCGGCTGGGGCGGGTCGCGGCCA 601  
 Epinephelus --AAGAAAGACGGC---GGGTACAAGATGAAGCACTTCGGCTGGGGTGGCCCGCCGCCA 615  
 Amatitlania --AGGAAAGATAGG---ACCTATAAGATGAGTCACTTCAGATGGGGCAGCCACCTGCAT 846  
 Chimaera CCGAGGAGAAGAGAGGAATACAAAATTCGGTCACTTTAGGTGGGGTTCCCCACTGAAGG 1035  
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Heterodontus ACAAGCGCTACGGGGTTTCATGAAGTC---TTGGGATGAGAGAGGTGAGAAGCCACTTC 979  
 Horn ACAAGCGCTACGGGGTTTCATGAAATC---TTGGGATGAGAGAGGTGAGAAGCCACTTC 981  
 Verasper ACAACCGCTATGGAGTTTCATGAAATC---TTGGGATGAGAGAGGTGAGAAGCCACTTC 958  
 Danio ACAACCGCTATGGAGTTTCATGAAATC---TTGGGATGAGAGAGGTGAGAAGCCACTTC 598  
 Protopterus ATAAACGTTATGGTGGATTTCATGAAATC---TTGGGATGAGAGAGGTGAGAAGCCACTTC 786  
 Neoceratodus ACAAAAAGATATGGAGGTTTCATGAAATC---TTGGGATGAGAGAGGTGAGAAGCCACTTC 830  
 Acipenser ACAAGCGCTATGGGGTTTCATGAAGTC---TTGGGATGAGAGAGGTGAGAAGCCACTTC 842  
 Polyodon ACAAGCGCTATGGGGTTTCATGAAATC---TTGGGATGAGAGAGGTGAGAAGCCACTTC 848  
 Japanese GCAAACGCTACGGTGGCTTCATGAAGCC---CTGGGGCGAGCGAAGCCAAAACCCCTGC 733  
 European GCAAACGCTACGGTGGCTTCATGAAGCC---CTGGGGCGAGCGAAGCCAAAACCCCTGC 742  
 Anguilla GCAAACGCTACGGTGGCTTCATGAAGCC---CTGGGGCGAGCGAAGCCAAAACCCCTGC 699  
 Lepisosteus ACAAGCGGTACGGGGTTTCATGAAGTC---CTGGGACGAGCGCAGCCAGAAGCCACTTC 831  
 Gobiocypris GCAAGCGCTACGGAGGCTTCATGAAGTC---CTGGGATGAGCGCAGTCAGAAAACCCCTTC 616  
 Carassius GCAAGCGCTACGGAGGCTTCATGAAGTC---CTGGGACGAGCGCAGCCAGAAGCCACTTC 711  
 Ictalurus GCAAACGCTATGGAGTTTCATGAAGTC---TTGGGATGAGCAGCCAAAAGCCACTTC 709  
 Solea GCAAACGCTACGACGAGCTGACGGAGAG---GCGGGGCGACGCCGA--GGAGGCGGCGGC 601  
 Common GCAAACGCTACGACGAGCTGACGGAGAG---GCGGGGCGACGCCGA--GGAGGCGGCGGC 609  
 Sparus GCAAACGCTACGGCGGCTTCATGAAGAG---CTGGGACGAGCGCAGCCAGAAGCCACTTC 736  
 Yellowfin GCAAACGCTACGGCGGCTTCATGAAGAG---CTGGGACGAGCGCAGCCAGAAGCCACTTC 748  
 Sparidentex GCAAACGCTACGGCGGCTTCATGAAGAG---CTGGGACGAGCGCAGCCAGAAGCCACTTC 720  
 Acanthopagrus GCAAACGCTACGGCGGCTTCATGAAGAG---CTGGGACGAGCGCAGCCAGAAGCCACTTC 698  
 Oreochromis GCAAACGCTACGGCGGCTTCATGAAGAG---CTGGGACGAGCGCAGCCAGAAGCCACTTC 678  
 Monopterus GCAAACGCTACGGCGGCTTCATGAAGAG---CTGGGATGAGGCGAGCCAGACCCTTC 705

Dicentrarchus GCAAACGCTACGGCGGCTTCATGAAGAG---CTGGGACGAGCGCAGCCAGAGGCCCTGC 658  
 Epinephelus GCAAACGCTACGGTGGGTTCATGAAAAG---CTGGGATGAACGCAGCCAAAGGCCGCTGC 672  
 Amatitlania CTAAACGTAACGGCGGCTTTGGGAAGCT---GTGGGAGGAGAACCCTCAGGGCAGCTGG 903  
 Chimaera ACAAGCGCTATGGGGCTTCATGAACTC---CTGGGA-----CCACAAACCCTGC 1083

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Heterodontus TGACTCTCTTCAGGAATGTCATGTGCAA-GGATGGTCATGAGAAGAAAACCTCAAAGC--- 1035  
 Horn TGACTCTCTTCAGGAATGTCATGTGCAA-AGATGGTCATGAGAAGAAAACCTCAAAGC--- 1037  
 Verasper TGACCCCTCTTCAGGAACGTCATATCAA-GGATGGTCATGAGAAGAAATTCAAAATCAA 1017  
 Danio TCACACTCATAACGAAACGCCATCGGCCA----TGGACA----- 632  
 Protopterus TGACACTTTTCAAGAATGTTATGATTAA-AGATGCTTATGAGAAGAAAAGGACAGTAGAAA 845  
 Neoceratodus TGACTCTTTTCAAGAATGTTATGGTTAA-AGATGCTTATGAGAAGAAAAGGGCAATAGAAA 889  
 Acipenser TGACTCTCTTCAAAAACGTCATGATCAA-GGATGGCCATGAGAAAAAGGGCCAGTAAAGG 901  
 Polyodon TGACTCTCTTCAAAAACATCATGATCAA-GGATGTCCATGAGAAAAAGGGCCAGTAAAGG 907  
 Japanese TGACTCTTTTCAAAAATGTCATCATCAA-GGACGGCCAACAGAAGAAAAGCTCAGTGAAGA 792  
 European TGACTCTCTTTAAGAACGTCATCATCAA-GGACGGCCAACAGAAGAAAAGCTCAGTGAAGA 801  
 Anguilla TGACTCTCTTTAAAAACGTCATCATCAA-GGACGGCCAACAGAAGAAAAGCTCAGTGAAGA 758  
 Lepisosteus TGACGCTCTTTAAGAACGTCATCATTAA-GGACGGCCATCAGAAAAAGGGCCAGTAAAGG 890  
 Gobiocypris TCACACTTTTCAAAAACGTCATAAACAA-AGAGAACCAGAGGAAGGA---CCAGTGAGGG 672  
 Carassius TCACGCTCTTCAAAAACGTCATAAACAA-AGAGCACCAGAAGGA---CCAGTGAGGG 767  
 Ictalurus TCACACTTTTCAAGAACATCATCAACAA-AGACGGACACCAGAAGGA---CCACTGAAGA 765  
 Solea GCA-GCGG---GGAGGAGCCGGAGGCGGGAGATAGATGGAAGT-----CAAGAGAGA 649  
 Common GCA-GCGG---GGAGGAGCCGGAGGCGGGAGATAGATGGAAGT-----CAGGAGAGA 657  
 Sparus TCACGCTGTTCAAGAACGTCATCAACAA-AGACGGACAGCAG-----CAGCAGTGA 786  
 Yellowfin TCACGCTGTTCAAGAACGTCATCAACAA-AGACGGACAGCAG-----CAGAAGTGA 798  
 Sparidentex TCACGCTGTTCAAGAACGTCATCAACAA-AGACGGACAGCAG-----CAGAAGTGA 770  
 Acanthopagrus TCACGCTGTTCAAGAACGTCATCAACAA-AGACGGACAGCAG-----CAGAAGTGA 748  
 Oreochromis TGACGCTCTTCAAGAACGTCATCAACAA-AGAAGGACAGCAG-----CAGAAGTGA 728  
 Monopterus TCACCCCTCTTCAAAAACGTCATCCACAA-AGAGGGACAGCAG-----CAGGAATGA 755  
 Dicentrarchus TCACGCTCTTCAAAAATGTCATCAACAA-AGACGGACAGGAG-----CAGAAGAGA 708  
 Epinephelus TCACGCTTTTCAAAAACGTCATCAACAA-AGACGGACA---G-----CAGGAGGA 719  
 Amatitlania CCAAGTCTTTCAGGAACATTTTAGTCAA-GGATGTGGAGAGGA-----TAATGGGAT 954  
 Chimaera TTACCCCTCTTCAGGAATGTAATGGGGAAGGAGGTCAGGTGGAAGGCCCTGCCACAGT 1143

\*

Heterodontus -----T 1036  
 Horn -----T 1038  
 Verasper TAGATAGATCACCTTCAATAAAGATTCTTAGCTGTGA----CAGATAGGAGCAGTCCAT 1072  
 Danio -----  
 Protopterus GAAG-----AATA----TACGTACAACATCTATAT 871  
 Neoceratodus TGAG-----AAT-----TA-ATAAACATGTCCTT 913  
 Acipenser -----TCTTTT----CTCTCTTT-TCTGTTCTT 924  
 Polyodon GCGG-----TACACTTTGTTT----CTCTCTCTCTCTCTCT 941  
 Japanese -----CACTC----CA-----TT 801  
 European -----CACTC----CA-----GT 810  
 Anguilla -----CACTC----CA-----GT 767  
 Lepisosteus GTGTGGAGAAGCAATAAAATAGCCCCAACCTCGCTT----TACTTCTCCTTCATCTT 945  
 Gobiocypris G-----GATT----TTAATGG-----GAT 687  
 Carassius G-----G-TT----TTAATGG-----GAG 781  
 Ictalurus ACTC-----TTGATTAACATTAACAATTAATATTCATT 798  
 Solea CGACC-----AAT----- 657  
 Common CG----- 659  
 Sparus G----- 787  
 Yellowfin G----- 799  
 Sparidentex G----- 771  
 Acanthopagrus G----- 749  
 Oreochromis GGCCA-----GAC 736  
 Monopterus GTCT----- 759  
 Dicentrarchus G----- 709  
 Epinephelus G----- 720  
 Amatitlania G----- 955  
 Chimaera G----- 1144

Heterodontus AGATCTATGATAGCA-----ATTGATAAGAAGCTGTT-ATTGAAAC 1076  
 Horn A-----ACTGATAGACAGCCCTCCATGGAGA- 1064  
 Verasper AGATCTATGATAGC-----ATTGATAAGAAGTGTGTT-GTTGAAGC 1111  
 Danio -----  
 Protopterus GAT-A-AATATAGCTGAT-----AGTCTGTTAAACATTTTCATATACT 911  
 Neoceratodus GTTTA-AAAATAGCTTAA-----GGCATGTTAGCTTTTCCATATAGA 954  
 Acipenser CGCTACCCCGGATATCAT-----AGGTAGTCAGCAGGTTAGCAGG-- 964  
 Polyodon CGTTGCACCA-ATCTCAT-----AGTTAGTCAGCAG-TTAGCAAG-- 979  
 Japanese GGTTG-AAAGGG-----GGGAAGGAAGAAAGGAAGGAAGGA 836  
 European GGGTG-AGAGGG-----AGGGAGGGAGGAAGGAAGGAAGGA 845  
 Anguilla GGGTG-ATGGAA-----AGGGAGATTTAAA-----ATGGA 796  
 Lepisosteus GGCAA-GCAAAAATTT--T-----AGGTAGTTGGCATTTCTGGTAAAGA 984  
 Gobiocypris GGGTGAGAGGAG-----T-----TGAGTGAG-----AG-- 711

Carassius AAGTGGATAGGGGATTGTTT-----TTAATATATTTTCTTCCCAGC- 822  
 Ictalurus ACGTAGAAATATATTTATTTGTTCTTATTTTCCCCACATAAATTCATATTTCTTTGAAGTG 858  
 Solea CGACACACGTTATGTT-----CGACGACGAGCGACCCCAAAAAA 696  
 Common CG-----GTTATGTT-----CAGCGACGAGCGACC--AAAAAA- 690  
 Sparus -AGGAC-GAGGATG-----GA-GAGG-----CAGGAG-- 811  
 Yellowfin -AGGA---GGATG-----GA-GAGG-----CAGGAG-- 820  
 Sparidentex -AGAA---GGATG-----GA-GAGG-----CAGGAG-- 792  
 Acanthopagrus -AGGA---GGATG-----GA-GAGG-----CAGGAG-- 770  
 Oreochromis GAGAA--GAGGATGAGGCAG-----CGGAGA-GAGGAGTGACAGGAGGA 777  
 Monopterus ---GA---GGAAG-----GA-GAG-----AGGAG-- 776  
 Dicentrarchus -AGCA-----GTGA-----GG-GAG----- 722  
 Epinephelus -AGCA-----GTGA-----GG-GAGT-----AAAAGG- 740  
 Amatitlania ---AACGGAGGACC-----GAGCAGG-----AGAGGCA 980  
 Chimaera ---AGGCCCCACCT-----CACTGGGCTTCTGTGTAGACC 1176

Heterodontus ---AAAAATTTCTTTCAGATGGAACCTGTGCAATGCACCTTGTCTGG----- 1120  
 Horn -----TTCTT--AGGTGCAACA-----AAT----- 1082  
 Verasper ---AAAAGATTTTTCAAATGGAACCTGTACAATGCACCTTGTTTGG----- 1155  
 Danio -----  
 Protopterus --TAATGAT-TCATTA----TAACCT-AAGTATGGGTG----- 941  
 Neoceratodus --TAAAAATATATTGG---TGACAT-AATAAGGGGCA----- 986  
 Acipenser ---AGGATCCATATTTG---AAACA--AAGGCCTTCA----- 994  
 Polyodon ---AGGATCCATGTTGG---AAACA--AAG--CCTTCA----- 1007  
 Japanese --AGGGAGGAA-----GG--AAGGATGTT----- 857  
 European --AGGAAGGAAAGGAGG---GAGGG--AGGGATGGTTACGTGAGATTGAAATGCTGAAA 897  
 Anguilla --AAGCA-----TT----- 803  
 Lepisosteus --AAGGGGCTCAGTCTG----AAGG--TAGCTCGGTTGTATG----- 1018  
 Gobiocypris ---GGTCTTATCATGCA--GTCAAAC--AAAAGCGGGTGGGAG----- 747  
 Carassius ---AATCTTATCATGCATAGTAAACA--AAAAGAGGGCGGGTG----- 860  
 Ictalurus --TAAGCCTACTGTACACAATAAACGTTAGATTAAGATGAAACTTA----- 902  
 Solea ---AACCTGTCAAGT----- 708  
 Common ---AACCTGTCAAGT----- 701  
 Sparus ---ACGCTCTC-----ATTGGCCAGGGGAG----- 833  
 Yellowfin ---ACGCTCGC-----A-----G----- 830  
 Sparidentex ---ACGCTCGC-----A-----G----- 802  
 Acanthopagrus ---ACGCTCGC-----A-----G----- 780  
 Oreochromis GGAAACGTTTTCCAAGG---CAATCAGGCACCAAGAGTT----- 814  
 Monopterus -----  
 Dicentrarchus -----  
 Epinephelus -----  
 Amatitlania -----  
 Chimaera -----

Heterodontus -----TTT 1123  
 Horn -----  
 Verasper -----TTT 1158  
 Danio -----  
 Protopterus -----CTC 944  
 Neoceratodus -----CTC 989  
 Acipenser -----TT 996  
 Polyodon -----TT 1009  
 Japanese -----TCC-----TTC 863  
 European TCAAAGTCTGCCACCTCTGCCTCCACTTCTGTCTTTACCCATCAGGTAGTCAACTTTC 957  
 Anguilla -----TTT 806  
 Lepisosteus -----TT 1020  
 Gobiocypris -----A 748  
 Carassius -----G 861  
 Ictalurus -----TA 904  
 Solea -----  
 Common -----  
 Sparus -----  
 Yellowfin -----  
 Sparidentex -----  
 Acanthopagrus -----  
 Oreochromis -----  
 Monopterus -----  
 Dicentrarchus -----  
 Epinephelus -----  
 Amatitlania -----  
 Chimaera -----

Heterodontus ACTGTATGTAGCAATCAATAAAGG-----AGTGAATGTTGTAC 1161  
 Horn -----TGGAGCAGTCAATAGA-----TCTATG----- 1104  
 Verasper ACTGTATCTAGCAATCAATAAAGG-----AGTTAACATTGTAC 1196

Danio	-----GCAGTTCACAGA-----	644
Protopterus	TGGGTGACATTTT-CTCACAG-GGCTTCTTTCTAAGTGTA----TAATAAATAA--AAT	995
Neoceratodus	TGGGTGTACATG-CTTACGCTGACCCCTCCCAAATGCA-----CATTAAATAA--A--	1039
Acipenser	CA----TCATTT-CTGAAAAATCCTTGTTCGATT--AGTC--CTATAAGTTAT-AAT	1045
Polyodon	CA----CCATTT-CTGACAAAATCCCTGTTTGAATGTAGTCGCCAATAAATGATGAAT	1063
Japanese	TA-----TT--TGAAA-----	872
European	TGATT--TGTTT--TGAGAAAACACTTTGCTCAAAGGAGATGCTTTATACCAGCAACT	1012
Anguilla	TA-----TTT--TG-----	813
Lepisosteus	TGTTT--TCTCTG-CTGCCAAAGCCCTGTTTACAATGTAGTCGCCCGTAAAAATCTGAAA	1077
Gobiocypris	TG-----TTA-CCAATAGCACTTAATCTTG-----ATGCTTT-----	780
Carassius	TG-----TTA-CCAGTAGCACTTAATCTTG-----ATGCTT-----	892
Ictalurus	TG-----TTAATAATAGTACTTTGTTCTAGTAGCCAG---CAGTACTTTCACCAT	952
Solea	-----TTTGTGTTGAAGGGTT-----AAAAATGAAAAATCGAC	740
Common	-----TTTTTTGAAGGGTT-----AAAAATGAAAAATCAAC	732
Sparus	-----CTCGTTAGCAGA-----CCAATAAAAA-GCTTT	860
Yellowfin	-----CCTGTTAGCAGA-----CCAATAAAAA-----	852
Sparidentex	-----CCTGTTAGCAGA-----CCAATAAAAA-----	824
Acanthopagrus	-----CCTGTTAGCAGA-----CCAATAAAAA-GCTTT	807
Oreochromis	-----CCATTAAACAGATG-----ACCAATAAAAAAGCTCT	846
Monopterus	-----TTAA-AGA-----GGGAGATAACTTTT	797
Dicentrarchus	-----GAGAGG-----GAGGAAAAAGCTCG	760
Epinephelus	-----ACTAGCTGAAAGAGTC-----AAATAGCCATT	1007
Amatitlania	-----TGCTGTCCCCAGCACC-----CCACCCCGCCT	1206
Chimaera		
Heterodontus	ATAA-----GGCTTCAATGAATCTGATATCCAGTCATGGGCT-----	1198
Horn	ATA-----GCATTGATAGG-----AGCCTGTTATGAA-----	1131
Verasper	ATAAA-----GGCTTCAATGAGTCCAATACCCAGTCATGGGCT-----	1234
Danio	-----TTAA-----	648
Protopterus	AAAAAAAA-ATAAAATAAAAATCATGAAGTGATATAATGGGAAATGCTTCAACGAGTTA	1054
Neoceratodus		
Acipenser	ATTGTT-----ATCCGATTCCAAGAAAACATATGATATACATC--TGCCATACATACTA	1097
Polyodon	ATTGT-----AT---ATACAAAATAAATGCAT-ATATGAGTGAGTGCC-TA-GTTTTG	1110
Japanese		
European	ACTTTGCT-TTTACAACGTAGTGACACATAACTT-CTGAATAGTGATACATAAACCTGCTT	1070
Anguilla		
Lepisosteus	ACTGT-----ATATAGAAAATAAATGCTT-TTGTGAGTTAGAGTTAGAGCTGCAA	1126
Gobiocypris		
Carassius		
Ictalurus	ATAATC-----TATAATAAATGCAGACTAGAAGTAGTATATTGATTAAATA	998
Solea	ATA-----TATATATATATATATTTATTTTGTG-----	768
Common	ACA-----TAT-----	738
Sparus	GAAAT-----CATGATTCTGAGTGACTGAGAGAGA-----	890
Yellowfin		
Sparidentex		
Acanthopagrus	GAAAT-----CATGATTCTGAGTGACTGAGAGAGA-----	837
Oreochromis	GAAACTGCCATCTGTTACATGATCCTGATTGAGTGATGGAGG-----	888
Monopterus	-----TATGCAGCTAACTGGTTGGTAGTGA-----	822
Dicentrarchus	-----GACA-----	726
Epinephelus	AAAA-----GGAGCAA-----GACATCGG-----	780
Amatitlania	GCA-----TGTTAGTAACCACATGTCTTAATTT-----	1035
Chimaera	CCACC-----CTACCCACCACAGCCC-----	1227
Heterodontus	-----GTGCAAAG	1206
Horn	-----ACAAA-	1136
Verasper	-----GTGCAAAG	1242
Danio		
Protopterus	ATATTT-----AGTTATGATCGATTGCCACAGAGATATTTTCATAT	1095
Neoceratodus		
Acipenser	CCAAAA-----AAGTTTCTCTCGGGT---GTAATCCCACGTGCTTAT	1136
Polyodon	GAAGGG-----AAGCTATACATAAAAAAGGAAGACTATTTTCTTAT	1152
Japanese		
European	TTAACTGGGGAAGGTGGTGAGG--AAAGGGAGATTTAAAATGGAAAGCATTTTTTATTTT	1128
Anguilla		
Lepisosteus	AAGGGAGGGGAGGGAGAGAAATCAACTTAAATTAATAGTGAAAGTTATTTTCTTAT	1186
Gobiocypris	-----AGAGT-----	785
Carassius	-----AGAGT-----	897
Ictalurus	TTGATT-----AGAGTCTGATGGTCAA-----ACCATTTTCAT	1031
Solea	-----ATTGTTTATTTGATAAGTTTGTGTTTTTCTAAACAC	804
Common		
Sparus	-----TGAAGAG-----AGAGTGTTTTGATATTTAT	916
Yellowfin		
Sparidentex		
Acanthopagrus	-----CGACGAG-----AGAGTGTTTTGATATTTAT	863
Oreochromis	-----AGGGGAAGAGTCTGAGAAAGATGTTTTGATATGTAT	924

Monopterus	-----AGAG-----TCCTCAC	833
Dicentrarchus	-----	
Epinephelus	-----CCAACAA-----ATCCTCCA	795
Amatitlania	-----TAAATTACA-----CAAAC	1049
Chimaera	-----CTCCTAT	1234
Heterodontus	ATATTTTCCTATTTATTGAGCTGTACAATATGTAAA-----	1242
Horn	ATTTTTTCT-----	1145
Verasper	ATATTTTCCTATTTATTGAGCTGTACAATATGTAAA-----	1278
Danio	-----	
Protopterus	TTTCTC--AGCTGTA--AAAATGTAAATTACTAATATA-----T	1130
Neoceratodus	-----	
Acipenser	TTGTTG--AGCTGTA--ACTATACTGTAAATGA-----	1164
Polyodon	TTGTTG--AGCTG--ACTATACTGTAAATG-----	1178
Japanese	-----	
European	GTATTG--ACATGTA--ACTATACTGTAAATG-----	1155
Anguilla	-TATTG--ACATGTA--ACTATACTGTAAATG-----	839
Lepisosteus	TTATTG--TGATGATCTATAATGTAAAT-----	1213
Gobiocypris	TTATT-----CACATTGTA-----T	800
Carassius	TTATT-----CACATTGTA-----T	912
Ictalurus	TTATTA--AGATGTA--ACTATACTGTAAATACGAAAATGTTAACTATAAAATAATTGTATT	1089
Solea	ACACAC--ACGTTTATCATGTGACAGAAACAA-----	834
Common	-----	
Sparus	TGATTT--ATGTAAATAACTGTAAATGATATG-----	946
Yellowfin	-----	
Sparidentex	-----	
Acanthopagrus	TGATTT--ATGTAAATAACTGTAAATGATATG-----	893
Oreochromis	TGATAT--ATGTAAATAACTTTAATTATTA--	954
Monopterus	TGGC-C--AGGAAAGCTTGTGACGATTTCAG-----	862
Dicentrarchus	-----	
Epinephelus	AAATAA--AAACAAAATATGATGTTTGTCCC-----	824
Amatitlania	TTGTTGCTATCTTTAGCATGTTGTTGATGTC-----T	1082
Chimaera	TTATTGCCTTGTA--TGAAGTGTAAATGA-----	1261
Heterodontus	-TGAAGATGAAAAAT-----ACACTCTTTTTTTGCAAGCT-----	1277
Horn	-----AGATTGGA-----CCT-----	1156
Verasper	-TGAAGGATGAAAA-----ATACATTCTATTGCAAGCT-----	1312
Danio	-----	
Protopterus	TTAA---AAAGCAAAAGAAATATACGCTTTTTT-TTGCAAGC-----	1168
Neoceratodus	-----	
Acipenser	-TAA---TTAAACA-----AAAATGCAAGCTAAAAAA-----	1192
Polyodon	--AA---TTAAACA-----AAAAT-----TGAAGG-----	1198
Japanese	-----	
European	--AA---TGAAACATTTAAAAGATGGTACTTTG--TTGCAAA-----	1189
Anguilla	--AA---TGAAACATTTAAAAGATGGTACTTTG--TTGCAAAA-----	874
Lepisosteus	--AA---ATAAA-----ATACTTT--TTGCAAG-----	1234
Gobiocypris	TCAA-CAATAAAT-TC-----TGA--ATTCTGTACATAAA-----	831
Carassius	TCAA-CAATAAAT-TC-----TGA--ATTCTGTA--AAA-----	940
Ictalurus	TCAAGCAGTAAATGTCA--AGGTGATTATTTGAAATGAAAACCCAGTAATGCCATTAAAA	1147
Solea	-AGAAACAATAAAAC-----CAAAGTTCAATC-----	860
Common	-----	
Sparus	--AAAGCATAAAGAG-----TTATTTGCAAGC-----	971
Yellowfin	-----	
Sparidentex	-----	
Acanthopagrus	--AAAGCATAAAGAG-----TTATTTGCAAGC-----	918
Oreochromis	-TGAATTATAAAAAG-----CTATTTGTAAGA-----	980
Monopterus	-TGACCAATAAAAAC-----CT--CTGAAACA-----	886
Dicentrarchus	-----AAAAA-----AAAA-----	735
Epinephelus	-TGATTTTAAAAA-----AAAA-----	841
Amatitlania	TTTATGGCCAATGTT-----CCTCTCAATAAAACC-----	1113
Chimaera	-TGAATGATGATAATT-----ACACATGTCTTTGTGGCT-----	1294
Heterodontus	-----	
Horn	-----	
Verasper	-----	
Danio	-----	
Protopterus	-----	
Neoceratodus	-----	
Acipenser	-----AAAAAAAAAAAAAAAAAAAAAAAA-----	1216
Polyodon	-----	
Japanese	-----	
European	-----CTAAAAAATATGT-----	1202
Anguilla	-----CTAAAAAATAAA-----	888
Lepisosteus	-----CT-----	1236

Gobiocypris	-----AAAAAAAAAAAAA-----	846
Carassius	-----AAAAAAAAAAAAA-----	954
Ictalurus	ATATAAGCACACAAACCAAAAAAAAAAAAAA-----	1180
Solea	-----ACAAAAAAAAAAAAA-----	878
Common	-----	
Sparus	-----AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-----	1009
Yellowfin	-----	
Sparidentex	-----	
Acanthopagrus	-----AAAAAAAAAAAAA-----	933
Oreochromis	-----AAAAAA-----	987
Monopterus	-----AAAAAAAAAAAAA-----	897
Dicentrarchus	-----AA-----	737
Epinephelus	-----AAAAAAAAAAAAAAAAAAAAA-----	863
Amatitlania	-----TAACTAGACAGTGTTA-----	1130
<b>Chimaera</b>	-----	

**Supplementary Figure 37.** ClustalW alignment of *Danio rerio* POMCb nucleotide sequence against *Acipenser sinensis*, *Polyodon spathula*, *Solea senegalensis*, *Sparus aurata*, *Monopterus albus*, *Lepisosteus osseus*, *Gobiocypris rarus*, *Ictalurus punctatus*, *Dicentrarchus labrax*, *Sparidentex hasta*, *Acanthopagrus schlegelii*, *Acanthopagrus latus*, *Epinephelus coioides*, *Oreochromis mossambicus*, *Amatitlania nigrofasciata*, *Anguilla japonica*, *Solea solea*, *Verasper moseri*, *Carassius auratus*, *Protopterus annectens*, *Neoceratodus forsteri*, *Chimaera phantasma*, *Heterodontus portus jacksoni*, *Heterodontus francisci*, *Anguilla rostrata* and *Anguilla anguilla* POMC sequences. (\*) identical sequences are indicated. The zebrafish sequence has an average 39.97% % sequence identity with the fish sequences.

Condylura	-----	
Myotis	-----	
Sorex	-----	
Felis	-----	
Ceratotherium	-----	
Sus	-----AAGGCAGCGAGAGGGGAAGAGCAAGAG	26
Ovis	-----	
Pantholops	-----GAAGAGAACGAA	12
Bos	-----	
Pan	-----CCTGGGAGGGGAAGA-----AGAGCCGCG--	24
Pygmy	-----CCTGGGAGGGGAAGA-----AGAGCCGCG--	24
Gorilla	-----CCTGGGAGCGGAAGA-----AGAGCCGCG--	24
Homo	-----GCGTCCCCGCCCTCAGAGA-----GCAGCCTC--	27
Macaca	-----	
Crab-eating	ACAGAGGAGCGCGGACCAAGAGACGGCGAAGGAGGGA-----TAGAAGAGCAGCG--	51
Papio	-TAGAAGAGCA-GCGACCGAGAGAGCCGCGAGCGTCCCCGCCCTCAGAGAGCAGCCTC	58
Saimiri	-----AAGG-----AGAGCAGCG--	13
Chinchilla	-----	
Mus	-----GGGACCAAACGGGAGGCGACGGGAAGAGAAAAGAGGTT	37
Rattus	-----GGGGAT--AACGGGAGGCGACGGAGGAGAAAAGAGGTT	36
Microtus	-----CGAAGGAGGAGAAA-GAGGTT	20
Mesocricetus	-----	
Danio	-----	
Condylura	-----	
Myotis	-----GCGTCTTGTTTAGCT	15
Sorex	-----	
Felis	-----	
Ceratotherium	-----	
Sus	GGGAAGAAGAGTGACCAAGAAACCGCGAGTTTCCT-GCCTCGGCGCAGCGGAGTCGCC	85
Ovis	-----	
Pantholops	AGGAAGAAAAGTGACCGAGAGCCGCTGAACATCCTCGCCCCGGCGCAGCGGAGCCGCC	72
Bos	-----	

Pan -----ACCAAGAGAGGCCGCGAGCGTCCCCGCCCTCAGAGAGCAGCC----- 67  
 Pygmy -----ACCGAGAGAGGCCGCGAGCGTCCCCGCCCTCAGAGAGCAGCC----- 67  
 Gorilla -----ACCGACAGAGGCCGCGAGCGTCCCCGCCCTCAGAGAGCAGCC----- 67  
 Homo -----CCGAGACAGGGGTCCACCAATCTTGTTT-----GCT----- 59  
 Macaca -----ACTTCTGGATTCTTCAAAGTATCTGC-----AGTGCT----- 35  
 Crab-eating -----ACCGAGAGAGGCCGCGAACGTCCCCGCCCTCAGAGAGCAGCCTCCCG 100  
 Papio CCGGGACAGGCATTTCTGGATTCTTCAAAGTATCTGC-----AGTGCTGTTCCA 110  
 Saimiri -----ATCAAGAGAAGCTGCGGAGCGTCTCGCACGCAGAGAGCAGCCTCCCG 62  
 Chinchilla -----  
 Mus AAG-AGCAGTGACTAAGAGAGGCCACTGAACATCTTTGTCCCAGAGAGCTGCCTTTCCG 96  
 Rattus AAGGAGCAGTGACTAAGAGAGGCCACTGAACATCTTCTCGTCCCTCAGAGAGCTGCCTTTCCG 96  
 Microtus AAAGAGCGGTGAGCAAGAGAGGACACCAACACTTTCTCGTCCCCGAGAGCTGCCTTTCCG 80  
 Mesocricetus -----  
 Danio -----

Condylura -----  
 Myotis CACAGAGAACTCACC-----TGCACATAGCCTCTG 45  
 Sorex -----  
 Felis -----  
 Ceratotherium -----  
 Sus CCGAGAGCGGCCTCC-----CCGCGACAGAGCCTCAG 117  
 Ovis -----  
 Pantholops CGAGGCAGCTTCCCCGTGACAGGGTCCCACTCATCTTGTTTGCTCTGCAGAGCTTCAG 132  
 Bos -----  
 Pan -----TCCCGAGA-----CAGAGCCTCAG 86  
 Pygmy -----TCCCGAGA-----CAGAGCCTCAG 86  
 Gorilla -----TCCCGAGA-----CAGAGCCTCAG 86  
 Homo -----TCT---G-----CAGAGCCTCAG 74  
 Macaca -----GTTCACCA-----GGAGTGCCTCAG 56  
 Crab-eating C--ACAGGGGTCCCACCAA-----TCTGTTTGCTTCTGCAGTGCCTCAG 143  
 Papio CCAGGAGGGGTCCCACCAA-----TCTGTTTGCTTCTGCAGTGCCTCAG 155  
 Saimiri G--ACAGGGGTCCCACCAA-----TCTGTTTGCTTCTGAAGAGGCTCAG 105  
 Chinchilla -----  
 Mus CGACAGGGGTCCCTCCAAT-----CTGTTTGCTTCTGCAGAGACTAGG 140  
 Rattus CGACAGAG-----CCTCAGC----- 111  
 Microtus CAATAGAA-----CTTCAGC----- 95  
 Mesocricetus -----  
 Danio -----

Condylura -----ATGCCGAGATCATGCTGCAGCCGCTCAGGGGCCCTGCTGCTGGTC 45  
 Myotis CCTG---CCTGAAAATGCCTAGATCATGCTGCAGCCGCTCGGGGGCCCTCCTGCTTGCC 102  
 Sorex -----ATGCCGAGACAGTGCCGTGGCCACCCGGGGGCCCTGCTGCTGGTC 45  
 Felis -----ATGCCGAGATCGTGCAGCCGCCAGGGGCCCTGCTGCTGGCC 45  
 Ceratotherium -----ATGCCGAGACAACCGCGCAGCCGCTCGGGGGCCCTGCTGCTGGCC 45  
 Sus CCTG---CGTGGGAGATGCCGAGATTGTGCGGCAGTGCCTCGGGGGCCCTGCTGCTGACC 174  
 Ovis -----ATGCCGAGACTGTGCAGCAGTCTGTTCCGGGCGCCCTGCTGCTGGTC 45  
 Pantholops CCTG---CCTGGAAGATGCCGAGATTGTGCAGCAGTCTGTTCCGGGCGCCCTGCTGCTGGCC 189  
 Bos --GG---CCTGTAAGATGCCGAGACTGTGCAGCAGTCTGTTCCGGGCGCCCTGCTGCTGGCC 55  
 Pan CCTG---CCTGGAAGATGCCGAGATCGTGCAGCCGCTCGGGGGCCCTGTTGCTGGCC 143  
 Pygmy CCTG---CCTGGAAGATGCCGAGATCGTGCAGCCGCTCGGGGGCCCTGTTGCTGGCC 143  
 Gorilla CCTG---CCTGGAAGATGCCGAGATCGTGCAGCCGCTCGGGGGCCCTGTTGCTGGCC 143  
 Homo CCTG---CCTGGAAGATGCCGAGATCGTGCAGCCGCTCGGGGGCCCTGTTGCTGGCC 131  
 Macaca CCTG---CCTGGAAGATGCCGAGATCGTGCAGCCGCTCAGGGGCCCTGTTGCTGGCC 113  
 Crab-eating CCTG---CCTGGAAGATGCCGAGATCGTGCAGCCGCTCGGGGGCCCTGTTGCTGGCC 200  
 Papio CCTG---CCTGGAAGATGCCGAGATCGTGCAGCCGCTCGGGGGCCCTGTTGCTGGCC 212  
 Saimiri CCTG---CCTGGAAGATGCTGAGATCGTGCAGCCGCTCAGGGGCCCTGTTGCTGGCC 162  
 Chinchilla -----ATGCCGAGATTGTGCTACAGTCTGTTCCGGGCGCCCTGCTGCTGGCC 45  
 Mus CCTGACACGTGGAAGATGCCGAGATTCTGCTACAGTCTGCTCAGGGGCCCTGTTGCTGGCC 200  
 Rattus -----CACCTGGAAGATGCCGAGATTCTGCTACAGTCTGCTCAGGGGCCCTGCTGCTGGCC 166  
 Microtus -CTGTACCTGGAAGATGCCGAGATCCTGCCACAGTCTGCTCAGGGGCCCTGCTGCTGGCC 154  
 Mesocricetus -----ATGCCGAGATCCTTCTACAGTCTGTTCCAGGGGCCCTGCTACTGGCC 45  
 Danio -----ATGTTCTGTCCGTCTTG-----GCTTTTGG---CTGCAGC-GGTC 36

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Condylura TTGCTGCTTCCAGGCCCTCCTGCTTCCAGGCCCTCCATGGAGGTGCCCGGCTGGTGTCTGGAG 105  
 Myotis CTGCTGCTTCAG-----GCCTTCATGGAAGTGCCTGGCTGGTGTCTGGAG 147  
 Sorex TTGCTGCTGCAC-----GCCTCCGTGGAAGTGCATGGCTGGTGCCTGGAG 90  
 Felis TTGCTGCTTCAG-----GCCTCCATGGAAGTGCATGGCTGGTGCCTGGAG 90  
 Ceratotherium CTGCTGCTTCAG-----GCCTCCGTGGAAGTGCCTGGCTGGTGCCTGGAG 90  
 Sus TTGCTGCTCCAG-----GCCTCCATGGGAGTGCCTGGCTGGTGCCTGGAG 219  
 Ovis TTGCTGCTTCAG-----GCCTCCATGGAAGTGCCTGGTGGTGCCTGGAG 90  
 Pantholops TTGCTGCTTCAG-----GCCTCCATGGAAGTGCCTGGTGGTGCCTGGAG 234  
 Bos TTGCTGCTTCAG-----GCCTCCATGGAAGTGCCTGGTGGTGCCTGGAG 100  
 Pan TTGCTGCTTCAG-----GCCTCCATGGAAGTGCCTGGTGGTGCCTGGAG 188  
 Pygmy TTGCTGCTTCAG-----GCCTCCATGGAAGTGCCTGGTGGTGCCTGGAG 188

Gorilla TTGCTGCTTCAG-----GCCTCCATGGAAGTGCCTGGCTGGTGCCTGGAG 188  
Homo TTGCTGCTTCAG-----GCCTCCATGGAAGTGCCTGGCTGGTGCCTGGAG 176  
Macaca TTGCTGCTTCAG-----GCCTCCATGGAAGTGCCTGGCTGGTGCCTGGAG 158  
Crab-eating TTGCTGCTTCAG-----GCCTCCATGGAAGTGCCTGGCTGGTGCCTGGAG 245  
Papio TTGCTGCTTCAG-----GCCTCCATGGAAGTGCCTGGCTGGTGCCTGGAG 257  
Saimiri TTGCTGCTTCAG-----GCTTCCATGGAAGTGCCTGGCTGGTGCCTGGAG 207  
Chinchilla TTGCTACTTCAG-----GCCTCCATGGAAGTGCCTGGCTGGTGCCTGGAA 90  
Mus CTCCTGCTTCAG-----ACCTCCATAGATGTGTGGAGCTGGTGCCTGGAG 245  
Rattus CTCCTGCTTCAG-----ACCTCCATAGACGTGTGGAGCTGGTGCCTGGAG 211  
Microtus TTGCTGCTTCAG-----ACCTCCATGGAAGTGTGGGGCTGGTGCCTGGAG 199  
Mesocricetus TTGCTGATTCAG-----ACCTCCGTTGGAAGTGTGGGGCTGGTGCCTGGAG 90  
Danio TTGTGTTTTTCCACA-----GCCACATGTAGACG-GTGGCAGATGCTCGGGT 81  
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Condylura --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGTAACTGCTGGCCTGCCTGGGG 160  
Myotis --AGCAGCCAGTGTTCAGGACCTCA---CCTCGAAAGTAACTGCTGGCCTGCATTGGG 202  
Sorex --AGTGGCCGCTGCCAGGACCTCA---CCACAGAAAGTACCTGCTGGCCTGCCTCCGCG 145  
Felis --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGTAACTGCTGGCCTGCATCCGG 145  
Ceratotherium --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGTAACTGCTGGCCTGCATCCGG 145  
Sus --AGCAGCCAGTGTTCAGGACCTCT---CCACGAAAGTAACTGCTGGCCTGCATCCGG 274  
Ovis --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGTAACTGCTGGCCTGCATCCGG 145  
Pantholops --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGTAACTGCTGGCCTGCATCCGG 289  
Bos --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGTAACTGCTGGCCTGCATCCGG 155  
Pan --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGCAACTGCTGGAGTGCATCCGG 243  
Pygmy --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGCAACTGCTGGAGTGCATCCGG 243  
Gorilla --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGCAACTGCTGGAGTGCATCCGG 243  
Homo --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGCAACTGCTGGAGTGCATCCGG 231  
Macaca --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGCAACTGCTGGAGTGCATCCGG 213  
Crab-eating --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGCAACTGCTGGAGTGCATCCGG 300  
Papio --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGCAACTGCTGGAGTGCATCCGG 312  
Saimiri --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGCAACTGCTGGAGTGCATCCGG 262  
Chinchilla --AGCAGCCAGTGTTCAGGACCTCA---CCACAGAAAGAACTGCTGGAGTGCATCCGG 145  
Mus --AGCAGCCAGTGTTCAGGACCTCA---CCACGAGAGCAACTGCTGGCTGCATCCGG 300  
Rattus --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGCAACTGCTGGCTGCATCCGG 266  
Microtus --AGCAGCCAGTGTTCAGGACCTCA---CCACGAAAGCAACTGCTGGCTGCATCCGG 254  
Mesocricetus --AGCAGCCAGTGTTCAGGACCTCA---CCACTGAAAGCAACTGCTGGCTGCATCCGG 145  
Danio TTGATAGACTGCAT--GGACCTTGAATCCAATGAGCACAATTTGCAG---TGTCTCAGAA 136  
\* \* \* \* \*

Condylura CCTGTGGCCGGACC-----TCTCTGCCGAGACGCTGTGTTCCTCCGGC---AACGGCGA 212  
Myotis CCTGCAAGCCCGACC-----TCTCGCCGAGACGCTGTGTTCCTCCGGG---AACGGCGA 254  
Sorex CCTGCAAGCCCGACC-----TGACGCCGAGACGCTGTGTTCCTCCGGG---AACGGCGA 197  
Felis CGTGCAGCCCGACC-----TCTCGCCGAGACGCTGTGTTCCTCCGGC---AACGGCGA 197  
Ceratotherium CCTGCAAGCCCGACC-----TCTCGCCGAGACGCTGTGTTCCTCCGGC---AACGGCGA 197  
Sus CCTGCAAGCCCGACC-----TCTCTGCCGAGACGCTGTGTTCCTCCGGC---AACGGCGA 326  
Ovis CCTGCAAGCCCGACC-----TCTCGCCGAGACGCTGTGTTCCTCCGGC---AACGGCGA 197  
Pantholops CCTGCAAGCCCGACC-----TCTCGCTGAGACGCTGTGTTCCTCCGGC---AACGGCGA 341  
Bos CCTGCAAGCCCGACC-----TCTCGCCGAGACGCTGTGTTCCTCCGGC---AACGGCGA 207  
Pan CCTGCAAGCCCGACC-----TCTCGCCGAGACTCCCATGTTCCTCCGGC---AATGGCGA 295  
Pygmy CCTGCAAGCCCGACC-----TCTCGCCGAGACTCCCATGTTCCTCCGGC---AATGGCGA 295  
Gorilla CCTGCAAGCCCGACC-----TCTCGCCGAGACTCCCATGTTCCTCCGGC---AATGGCGA 295  
Homo CCTGCAAGCCCGACC-----TCTCGCCGAGACTCCCATGTTCCTCCGGG---AATGGCGA 283  
Macaca CCTGCAAGCCCGACC-----TCTCGCCGAGACTCCCGTGTTCCTCCGGC---AATGGCGA 265  
Crab-eating CCTGCAAGCCCGACC-----TCTCGCCGAGACTCCCGTGTTCCTCCGGC---AATGGCGA 352  
Papio CCTGCAAGCCCGACC-----TCTCGCCGAGACTCCCGTGTTCCTCCGGC---AATGGCGA 364  
Saimiri CCTGCAAGCCCGACC-----TCTCGCCGAGACTCCCGTGTTCCTCCGGC---AATGGCGA 314  
Chinchilla CCTGCAAGCCCGACC-----TCTCGCCGAGACTCCCATGTTCCTCCGGG---AACGGCGA 197  
Mus CTTGCAAACTCGACC-----TCTCGCTGAGACGCTGTGTTCCTCCGGC---AACGGGGA 352  
Rattus CTTGCAAACTCGACC-----TCTCGCCGAGACTCCCGTGTTCCTCCGGC---AACGGGGA 318  
Microtus CTTGCAAACTCGAAC-----TCTCGCCGAGACTCCCGTGTTCCTCCGGC---AATGGGGA 306  
Mesocricetus CTTGCAAACTCGACC-----TCTCGCCGAGACTCCCGTGTTCCTCCGGC---AACGGGGA 197  
Danio AGTGCAAGTCCGACCAAGAAATCTTC-CAGAAACATCAGAGTTTCTTCATCTGAACATCA- 194  
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Condylura CGAGCAGCCGCTGACCGAGAACCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG 268  
Myotis GGAACAGCTGTGGCCGAGAACCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG 310  
Sorex CGAGCAGCCGCTGACCGAGAACCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG 253  
Felis CGAGCAGCCGCTGACCGAGAACCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG 253  
Ceratotherium CGAGCAGCTGCTGACCGAGAACCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG 253  
Sus CGCGCAACCGCTGACCGAGAACCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG 382  
Ovis TGAGCAGCCGCTGACTGAGAACCCCGGAAGTAC---GTCATGGGCC-ATTTCCGCTGGG 253  
Pantholops TGAGCAGCCGCTGACTGAGAACCCCGGAAGTAC---GTCATGGGCC-ATTTCCGCTGGG 397  
Bos TGAGCAGCCGCTGACTGAGAACCCCGGAAGTAC---GTCATGGGCC-ATTTCCGCTGGG 263  
Pan CGAGCAGCCTCTGACCGAGAACCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG 351  
Pygmy CGAGCAGCCTCTGACCGAGAACCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG 351  
Gorilla CGAGCAGCCTCTGACCGAGAACCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG 351  
Homo CGAGCAGCCTCTGACCGAGAACCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG 339

Macaca	CGAGCAGCCTCTGACCGAGAACCCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG	321
Crab-eating	CGAGCAGCCTCTGACCGAGAACCCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG	408
Papio	CGAGCAGCCTCTGACCGAGAACCCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG	420
Saimiri	CGAGCAGCCACTGACCGAGAACCCCCGGAAGTAC---GTCATGAGCC-ACTTCCGCTGGG	370
Chinchilla	CGAGCAACCGCTGACAGAGAACCCCCGGAAGTAC---GTCATGGGCC-ACTTCCGCTGGG	253
Mus	TGAACAGCCCCCTGACTGAAAACCCCCGGAAGTAC---GTCATGGGTC-ACTTCCGCTGGG	408
Rattus	TGAACAGCCCCCTGACTGAAAATCCCCGGAAGTAC---GTCATGGGTC-ACTTCCGCTGGG	374
Microtus	CGAGCAGCCGCTGACCGAGAACCCCCGGAAGTAC---GTCATGGGTC-ACTTCCGCTGGG	362
Mesocricetus	CGAGCAGACGCTGACCGAGAACCCCCGGAAGTAC---GTCATGGGTC-ACTTCCGCTGGG	253
Danio	-GAGCAG---TGA---AGAACAGGTAGAGGAGCAGAGTC-TGAGCTTGGGTTTGCTTTT	245
	* * * * *	
Condylura	ACCGCTTCGGCCGCGGGAACAGCAGCGTT-----GCGGGCCAGAAGCGC	312
Myotis	ACCGCTTCGGCCGCGGGAACGACAGCG-----GCGGGCCGCGCGCG	352
Sorex	AGCGCTTCGGCCACCGGAACGCGCAGG-----	280
Felis	ACCGGTTTCGGCCCGGGAATGGCAGC-----G-----CGG	283
Ceratotherium	ACCGCTTCGGCCGCGGGAACAGCAGC-----GGCGGCGCGCGCGCG	295
Sus	ACCGCTTCGGCCGCGGGAATGGCAGCAGCAGCGCGCGGTTGGCGGTGGCGGCGCGCG	442
Ovis	ACCGCTTCGGCCGCGGGAATGGTAGCAGCAGCTTC-----GGAGCTGGGGCGCGCG	304
Pantholops	ACCGCTTCGGCCGCGGGAATGGTAGCAGCAGCTTC-----GGAGCTGGGGCGCGCG	448
Bos	ACCGCTTCGGCCGCGGGAATGGTAGCAGCAGCAGC-----GGAGTTGGGGCGCGCG	314
Pan	ACCGATTTCGGCCGCGGCAACAGCAGCAGCAGCAGCAG-----CGGCAGCGCGCGCAG	402
Pygmy	ACCGATTTCGGCCGCGGCAACAGCAGCAGCAGCAGCAG-----CGGCAGCGCGCGCAG	402
Gorilla	ACCGATTTCGGCCGCGGCAACAGCAGCAGCAGCAGCAG-----CGGCAGCGCGCGCAG	399
Homo	ACCGATTTCGGCCGCGGCAACAGCAGCAGCAGCAGCG-----CAGCAGCGCGCGCAG	387
Macaca	ACCGATTTCGGCCGCGGCAACAGTAGCAGC-----GGCAGCGCGCGCAG	363
Crab-eating	ACCGATTTCGGCCGCGGCAACAGTAGCAGC-----GGCAGCGCGCGCAG	450
Papio	ACCGATTTCGGCCGCGGCAACAGTAGCAGC-----GGCAGCGCGCGCAG	462
Saimiri	ACCGCTTCGGTCGCCCGCAATAGCAGCAGCAGCGCGCG-----GGCGGCGCGCGCGCAG	424
Chinchilla	ACCGCTTCGGCCGCGGGAACAGCAGCGGC-----GGCGGCGGTGCGA	295
Mus	ACCGCTTCGGCCCGAGGAACAGCAGCAGT-----GCTGGCAGCGCGCG	450
Rattus	ACCGCTTCGGCCCCAGAAACAGCAGCAGT-----GCTGGCAGCGCGCAG	416
Microtus	ACCGCTTCGGCCCGAGGAACAGCAGC-----GGCAGCG	395
Mesocricetus	ACCGCTTCGGCCCGAGGAACAGCAGC-----GGCAGCG	286
Danio	ATCAGCTCTGTCTCCCGA-CTCCATC-----	270
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Condylura	GACCC-----GGA-----GGAGGCGGCGCGCGGGC-----CATG	343
Myotis	GCCCC---AAACGCAC---GGA-----GGAGGCGGCGGCTGCGGGC-----GACG	391
Sorex	-----	
Felis	GCCAG---AAGCGCGA---GGA-----GGAAGAGGTGCGCGGGGC-----GACG	322
Ceratotherium	GCCAG---AAGCGCGA---GGA-----GGAGGCGGTGCTG-CTGGT-----G--G	331
Sus	GCCAG---AAGCGCGA---GGA-----GGAGGAGGTGGCGGGGGC-----G---	478
Ovis	CCCAG---AAGCGCGA---GGA-----GGAAG---TGCGGTTGGGC-----G---	337
Pantholops	CCCAG---AAGCGCGA---GGA-----GGAAG---TGCGGTTGGGC-----G---	481
Bos	CCCAG---AAGCGCGA---GGA-----GGAAG---TGCGGTTGGGC-----G---	347
Pan	GGCAG---AAGCGCGA---GGACGTCTCAGCGGGCGAAGACCAGCGGCGCGCTGCCTGAGG	456
Pygmy	GGCAG---AAGCGCGA---GGACGTCTCAGCGGGCGAAGACCAGCGGCGCGCTGCCTGAGG	456
Gorilla	GGCAG---AAGCGCGA---GGATGTCTCAGCGGGCGAAGACCAGCGGCGCGCTGCCTGAGG	453
Homo	GGCAG---AAGCGCGA---GGACGTCTCAGCGGGCGAAGACTGCGGCGCGCTGCCTGAGG	441
Macaca	CGCAG---AAGCGCGA---GGACGTCTCAGCGGGCGAAGACCAGCGGCGCTGCCTGAGG	417
Crab-eating	CGCAG---AAGCGCGA---GGACGTCTCAGCGGGCGAAGACCAGCGGCGCTGCCTGAGG	504
Papio	CGCAG---AAGCGCGA---GGACGTCTCAGCGGGCGAAGACCAGCGGCGCTGCCTGAGG	516
Saimiri	GGCAG---AAGCGCGA---GGACTTCGCGGGGGCGAAGACCAGCGGCGCTGCCTGAGG	478
Chinchilla	GCCGG---AAGCGCGAAGAGAGGTTGCGGGCGGCGAAGGCCCGCTGCCGCTGCCCGCTG	352
Mus	CGCAG---AGGCGTGC---GGA-----GGAAGAGG-----	474
Rattus	CGCAG---AGGCGTGC---GGA-----GGAAGAGA-----	440
Microtus	CGCAG---AGGCGCGC---GGA-----GGAGGAGGAGGA-----TGCC	427
Mesocricetus	CGCAGCAGAGGCGCGC---GGA-----GGAGGAGGAGGAGGAGGAGGA---TGCT	330
Danio	-----GA-----	272
Condylura	GCCTCCCGGAGGCCCTGGCCATATGGTACCAGCCGGGCCACGCGAGGGCAAGCGCTCCT	403
Myotis	GCGGCCTCGGGCCCCGCGGATGGCGCCGGGCCGGGCCCGCGGAGGGCAAGCGCTCCT	451
Sorex	-----GCC-----	283
Felis	GCGGCCCGGGCCCCGCGGCGATGGCGGGGAGCTGGGCTTGCGCAGGGCAAGCGCTCCT	382
Ceratotherium	GCGGCCCGGGCCCCGCGGCGATGGCGGGGAGCCGGGCCCGCGGAGGGCAAGCGCTCCT	391
Sus	AAGGCCCGGGCCCCGCGGATGGCGTCCGCGGGGCCCGCGGAGGGCAAGCGCTCCT	538
Ovis	AAGGCCCGGGCCCCGCGGCGATGGCGCCGAGACGGGTCCGCGAGAGGACAAGCGTTCTT	397
Pantholops	AAGGCCCGGGCCCCGCGGCGATGGCGCCGAGACGGGTCCGCGAGAGGACAAGCGTTCTT	541
Bos	AAGGCCCGGGCCCCGCGGCGATGGCGCCGAGACGGGTCCGCGAGAGGACAAGCGTTCTT	407
Pan	GCGGCCCGGAGCCCCGCGGCGATGGTGCAGCCGGGCCCGCGGAGGGCAAGCGCTCCT	516
Pygmy	GCGGCCCGGAGCCCCGCGGCGATGGTGCAGCCGGGCCCGCGGAGGGCAAGCGCTCCT	516
Gorilla	GCGGCCCGGAGCCCCGCGGCGATGGTGCAGCCGGGCCCGCGGAGGGCAAGCGCTCCT	513
Homo	GCGGCCCGGAGCCCCGCGGCGATGGTGCAGCCGGGCCCGCGGAGGGCAAGCGCTCCT	501
Macaca	GCGGCCCGGAGCCCCGCGGCGATGGCGCCGAGCCGGGCCCGCGGAGGGCAAGCGCTCCT	477
Crab-eating	GCGGCCCGGAGCCCCGCGGCGATGGCGCCGAGCCGGGCCCGCGGAGGGCAAGCGCTCCT	564

Papio GCGGCCCCGAGCCCCGTGGCGATGGCGCCGAGCCGGGCCCGCGCAGGGCAAGCGGTCT 576  
Saimiri GCGGCCCCGAGCCCCGCGCGATGGCGCCGAGCCGGGCCCGCGCAAGGCAAGCGTCT 538  
Chinchilla GCGATCCTGAGTTCCGCGCGATGGCGCCGAGCCGGGCCCTCACGAGCCAAAGCGTCT 412  
Mus -CGGTGTGGG-----AGATGGCAGTCCAGAGCCGAGTCCACGCGAGGGCAAGCGTCT 528  
Rattus -CGGCCGGGG-----AGATGGCCGTCGGAGCCAAGTCCACGGGAGGGCAAGCGTCT 494  
Microtus GCGGCAGTGA-----CGATGGTCGCGCTGAGCCCGCCTGCGCGAGGGCAAGCGTCT 482  
Mesocricetus GTGGCGGTGGA-----AGATGCTCGCGCCGAGCCCGCCTGCGCGAGGGCAAGCGTCT 385  
Danio --GCTCCAAAACCCACGGCAGAAGCTCTCACGGG-----ACGAG--AGGCGTCT 322

Condylura ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 463  
Myotis ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 511  
Sorex -----ACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 331  
Felis ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 442  
Ceratotherium ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 451  
Sus ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 598  
Ovis ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 457  
Pantholops ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 601  
Bos ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 467  
Pan ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 576  
Pygmy ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 576  
Gorilla ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 573  
Homo ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 561  
Macaca ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 537  
Crab-eating ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 624  
Papio ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 636  
Saimiri ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 598  
Chinchilla ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 472  
Mus ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 588  
Rattus ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 554  
Microtus ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 542  
Mesocricetus ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 445  
Danio ACTCCATGGAGCACTTCCGCTGGGGCAAGCCGGTGGGCAAGAAGCGGCGCCCCGTGAAG 382

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Condylura TGTACCCCAATGGCGC-----GAG---GACGAGTCGACCGAGGCTTCCCCGTCG 511  
Myotis TGTACCCCAACGGCGC-----GAG---GACGAGTCGCCCAGGCTTCCCCGTGG 559  
Sorex TGTACCCCAACGGCGC-----GAG---GACGACTCGGCCGAGGCTTCCCCGTCG 379  
Felis TGTACCCCAACGGCGC-----GAG---GACGAGTCGTCGAGCTTCCCCCTCG 490  
Ceratotherium TGTACCCCAACGGCGC-----GAG---GACGAGTCGCCCAGGCTTCCCCCTGG 499  
Sus TGTATCCCAACGGCGC-----GAG---GACGAGTTGGCCGAGGCTTCCCCCTCG 646  
Ovis TGTACCCCAACGGCGC-----GAG---GACGAGTCGCCCCAGGCTTCCCCCTCG 505  
Pantholops TGTACCCCAACGGCGC-----GAG---GACGAGTCGCCCAGGCTTCCCCCTGG 649  
Bos TGTACCCCAACGGCGC-----GAG---GACGAGTCGCCCCAGGCTTCCCCCTCG 515  
Pan TGTACCCCAACGGCGC-----GAG---GACGAGTCGCCCAGGCTTCCCCCTGG 624  
Pygmy TGTACCCCAACGGCGC-----GAG---GACGAGTCGCCCAGGCTTCCCCCTGG 624  
Gorilla TGTACCCCAACGGCGC-----GAG---GACGAGTCGCCCAGGCTTCCCCCTGG 621  
Homo TGTACCCCAACGGCGC-----GAG---GACGAGTCGCCCAGGCTTCCCCCTGG 609  
Macaca TGTACCCCAATGGCGC-----GAG---GACGAGTCGCCCAGGCTTCCCCCTGG 585  
Crab-eating TGTACCCCAATGGGGC-----GAG---GACGAGTCGCCCAGGCTTCCCCCTGG 672  
Papio TGTACCCCAATGGCGC-----GAG---GACGAGTCGCCCAGGCTTCCCCCTGG 684  
Saimiri TGTACCCCAACGGCGC-----GAG---AACGAGTCGCCCAGGCTTCCCCCTGG 646  
Chinchilla TGTACCCCAACGGCGC-----GAG---GACGAGTCGCCCAGGCTTCCCCCTGG 520  
Mus TGTACCCCAACGTTGCT-----GAG---AACGAGTCGCCCAGGCTTCCCCCTAG 636  
Rattus TGTACCCCAATGTCGCC-----GAG---AACGAGTCGCCCAGGCTTCCCCCTAG 602  
Microtus TGTACCCCAACGTCGCC-----GAG---AACGAGTCGCCCAGGCTTCCCCCTGG 590  
Mesocricetus TGTACCCCAACGTCGCC-----GAG---AACGAGTCGCCCAGGCTTCCCCCTGG 493  
Danio TGTTGAGCAACGGGGCTTGGAGAAGAGCCGGAGGAGTCGGAGGAGAGCGTCCGTG 442

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Condylura AGTTCAAGAGGGGGCTG-TCGG-----CCGAG---CACCTGAGGACCCGG 553  
Myotis AGTTCAAGCGGGAGCGG-CCGGAG-----CCGCGCTCCGCCCGAGGGCGCGG 607  
Sorex AGTTCCGGAGGGACCTG-GCGG-----CCGCCG---CGCCC---GAGCCCG 419  
Felis AGTTCAAGAGGGAGCTGGCCGGGAGCGGCCGGGGCCGCGCTCGGCCCGAGGCCCGG 550  
Ceratotherium AGTTCAAGCGGGAGCTGGCCGGGAGCGGCCCGAGCCCGCG----- 540  
Sus AGTTCAAGAGGGAGCTGGCCGGGCGCCCCCGAGCCGGCACGGGACCCCGAGGCCCGG 706  
Ovis AATTCAAGAGGGAGCTGACCGGGGAGAGGCTCGAGCAGGCGCGCNGCCCGAGGCCCGG 565  
Pantholops AATTCAAGAGGGAGCTGACCGGGGAGAGGCTCGAGCAGGCGCGGCCCGAGGCCCGG 709  
Bos AATTCAAGAGGGAGCTGACCGGGGAGAGGCTCGAGCAGGCGCGGCCCGAGGCCCGG 575  
Pan AGTTCAAGAGGGAGCTGACTGGCCAGCGACCCCGGGAGGGAGATGGCCCGACGGCCCTG 684  
Pygmy AGTTCAAGAGGGAGCTGACTGGCCAGCGACCCCGGGAGGGAGATGGCCCGACGGCCCTG 684  
Gorilla AGTTCAAGAGGGAGCTGACTGGCCAGCGACTCCGGGAGGGAGATGGCCCGACGGCCCTG 681  
Homo AGTTCAAGAGGGAGCTGACTGGCCAGCGACTCCGGGAGGGAGATGGCCCGACGGCCCTG 669  
Macaca AGTTCAAGAGGGAGCTGACCGGCCAGCGGCCCGGGCGGGGATGGCCCGATGGCCCTG 645  
Crab-eating AGTTCAAGAGGGAGCTGACCGGCCAGCGGCCCGGGCGGGGATGGCCCGATGGCCCTG 732  
Papio AGTTCAAGAGGGAGCTGACCGGCCAGCGGCCCGGGCGGGGATGGCCCGACGGCCCTG 744  
Saimiri AGTTCAAGAGGGAGTTGGCCGACAGCGGCCCTGGCGGGGAGCGGCCCGACAGCCCA 706

Chinchilla AGTTCAAGAGGGAGCTGGCCGGGAGCGGCCCGCGGCGCCCGGCCCGA----- 572  
Mus AGTTCAAGAGGGAGCTG-----GAAGGCGAGCGGCCA----- 668  
Rattus AGTTCAAGAGGGAGCTG-----GAAGGCGAGCAGCT----- 634  
Microtus AGTTCAGGAGGGAGCTG-----GCGGGCGAGCGCCCC----- 622  
Mesocricetus AGTTCGAGGGAGCTG-----GCGGGCGAGCGGCC----- 525  
Danio AACG-AGGACAGAGCGG----- 458

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Condylura C---AGGCAGCGCGG---CC-----GGCCTGGAA-TACGGCTGGTGGCGGAA---AC- 596  
Myotis CCCAGGGCGAGGGCGG---CCCCCGCCGACCTGGAG-GACGACCTGGGGGCGGAG---GC- 659  
Sorex C-----CTCGACGG---CCCC-GACGGCCTGGAG-CCCGCCTGCTGGCC-----CC- 461  
Felis CCGACGGCGCCCGCGG---CCCTGCCCGACCTGGAG-TACGGCTGGTGGCAGAG---GC- 602  
Ceratotherium -----GCGG---CCCCGGCCGGCCTGGAG-TACAGCTGGTAGCGGAG---GC- 581  
Sus CCGAGGGCGCGGGCGG---CCCGGGCCGAGCTGGAG-TACGGCTGGTGGCCGAG---GC- 758  
Ovis CTGAGAGCGCGGGCGG---CCCGGGCTGAGCTGGAG-TATGGCTGGTGGCAGAG---GC- 617  
Pantholops CCGAGAGCGCGGGCGG---CCCGGGCTGAGCTGGAG-TATGGCTGGTGGCGGAG---GC- 761  
Bos CTGAGAGTGCGGCGG---CCCGGGCTGAGCTGGAG-TATGGCTGGTGGCGGAG---GCG 628  
Pan CCGATGACGGCGCGGGGGCCAGGCCGACCTGGAG-CACAGCTGTGGTGGCG---GC- 739  
Pygmy CCGATGACGGCGCGGGGGCCAGGCCGACCTGGAG-CACAGCTGTGGTGGCG---GC- 739  
Gorilla CCGATGACGGCGCGGGGGCCAGGCCGACCTGGAG-CATAGCTGTGGTGGCG---GC- 736  
Homo CCGATGACGGCGCAGGGGGCCAGGCCGACCTGGAG-CACAGCTGTGGTGGCG---GC- 724  
Macaca CCGACGACGGCGCGGGGGCCCGGGCCGACCTGGAG-CACAGCTGTGGTGGCG---GC- 700  
Crab-eating CCGACGACGGCGCGGGGGCCCGGGCCGACCTGGAG-CACAGCTGTGGTGGCG---GC- 787  
Papio CCGACGACGGCGCGGGGGCCCGGGCCGACCTGGAG-CACAGCTGTGGTGGCG---GC- 799  
Saimiri CCGACGGGGGCGCGGGGGCCGAGGCCAGCCGGAG-CACGGCTGTGGTGGCG---GC- 761  
Chinchilla -----CGAGCTGGAG-CTCGGCGAGGCGGCCGCG---GC- 602  
Mus -----TTAGGCTTGGAG-CAGGTCTGGAGTCCGAC---GC- 700  
Rattus -----GATGGCTTGGAG-CACGTCTGGAGCCGGAT---AC- 666  
Microtus -----GACGGCTTGGAG-CACATCTGGAGCCCGACTCCGC- 657  
Mesocricetus -----GACGGCTTGGAG-CGCGTCTGGAGGCGGAC---GC- 557  
Danio -----CACCTGGAGTTCAACACAGGAACAACG---TC- 489

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Condylura -----CGAGGCGCTG--GA---GAAGAAGGACGACGGGGCCCTACCAGATGAAGCATTTTC 645  
Myotis -----CGAGGCCCGTCCGGCTGAAGAAGGACGAGGGGCCCTACCGGATGGAGCACTTC 714  
Sorex -----CGCGGCGGCC-----AAGAAGGACGGGACGCCCTACAAGATGGAGCACTTC 507  
Felis -----CGAGGTGGCC--GA---GAAGAAGGACGAGGGGCCCTATAAGATGGAGCATTTTC 651  
Ceratotherium -----CGAGGCGGCC--GA---GAAGAAGGACGAGGGGCCCTATAAGATGGAGCATTTTC 630  
Sus -----CGAGGCGGCC--GA---GAAGAAGGACGAGGGGCCCTATAAGATGGAGCACTTC 807  
Ovis -----TGAGGCGGCC--GA---GAAGAAGGACTCGGGGCCCTATAAGATGGAACACTTC 666  
Pantholops -----TGAGGCGGCC--GC---GAAGAAGGACTCGGGGCCCTACAAGATGGAACACTTC 810  
Bos GAGGCTGAGGCGGCC--GA---GAAGAAGGACTCGGGGCCCTATAAGATGGAACACTTC 682  
Pan -----CGAG-----AAGAAGGACGAGGGGCCCTACAGGATGGAGCACTTC 779  
Pygmy -----CGAG-----AAGAAGGACGAGGGGCCCTACAGGATGGAGCACTTC 779  
Gorilla -----CGAG-----AAGAAGGACGAGGGGCCCTACAGGATGGAGCACTTC 776  
Homo -----CGAG-----AAGAAGGACGAGGGGCCCTACAGGATGGAGCACTTC 764  
Macaca -----CGAG-----AAGAAGGATGAGGGGCCCTACAGGATGGAGCACTTC 740  
Crab-eating -----CGAG-----AAGAAGGATGAGGGGCCCTACAGGATGGAGCACTTC 827  
Papio -----CGAG-----AAGAAGGATGAGGGGCCCTACAGGATGGAGCACTTC 839  
Saimiri -----CGAG-----AAGAAGGACGAGGGGCCCTACAGGATGGAGCACTTC 801  
Chinchilla -----CGAG-----AAGAAGGACGAGGGGCCCTATCGCATGGAGCACTTC 642  
Mus -----GGAG-----AAGGACGAGGGGCCCTACCGGTTGGAGCACTTC 737  
Rattus -----CGAG-----AAGGCCGACGGGCCCTATCGGGTGGAGCACTTC 703  
Microtus -----CGAG-----AAGGAAGATGGGCCCTACCGGATGGAGCGCTTC 694  
Mesocricetus -----CGAG-----AAGGAAGACGGGCCCTATCGGGTGGAGCGCTTC 594  
Danio -----AAAAACAACGGGAAGTATCGCATGACCCACTTC 522

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Condylura CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----GAA 701  
Myotis CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----GAA 770  
Sorex CGCTGGGGCAGCCCGCCCAAGGACAAGCGGTACGGCGGCTTCATGTCTCCGA----GAA 563  
Felis CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----GAA 707  
Ceratotherium CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGAGCTCCGA----GAA 686  
Sus CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----GAA 863  
Ovis CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----GAA 722  
Pantholops CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGGTTCATGACCTCCGA----GAA 866  
Bos CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGGTTCATGACCTCCGA----GAA 738  
Pan CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGTTTCATGACCTCCGA----GAA 835  
Pygmy CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGTTTCATGACCTCCGA----GAA 835  
Gorilla CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGTTTCATGACCTCCGA----GAA 832  
Homo CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGTTTCATGACCTCCGA----GAA 820  
Macaca CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----GAA 796  
Crab-eating CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----GAA 883  
Papio CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----GAA 895  
Saimiri CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----GAA 857  
Chinchilla CGCTGGGGCAGCCCGCCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----GAA 698  
Mus CGCTGGAGCAACCCCGCCCAAGGACAAGCGTTACGGTGGCTTCATGACCTCCGA----GAA 793

Rattus	CGCTGGGGCAACCCGCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----	GAA	759
Microtus	CGCTGGGGCAGCAGCCCAAGGACAAGCGCTATGGCGGCTTCATGACCTCCGA----	GAA	750
Mesocricetus	CGCTGGGGCAGCCGCCAAGGACAAGCGCTACGGCGGCTTCATGACCTCCGA----	GAA	650
Danio	CGCTGGAATGCACCACC--AGACAAACGATACGGAGGACTCATGAGGCCGTATTTAGAC	579	
	***** * * ***** ** * * * * * * * * * * * * * * * * *		
Condylura	GAG--CCAGACGCCACTCGTGACGCTGTTCAAAAATGCCATCATCAA-GAAC----	GCCC	754
Myotis	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAAAACGCCATCATCAA-GAAC----	GCCC	823
Sorex	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAAAACGCCATCATCAA-GAAC----	GCGC	616
Felis	GAG--CCAGACGCCTCTGGTGACGCTGTTCAAAAACGCCATCATCAA-GAAC----	GCCC	760
Ceratotherium	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAAAACGCCATCA--A-GAAC----	GCCC	736
Sus	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAAAACGCCATCGTCAA-GAAC----	GCCC	916
Ovis	GAG--CCAAAACGCCCCCTGTGACGCTGTTCAAAAACGCCATCATCAA-GAAC----	GCCC	775
Pantholops	GAG--CCAAAACGCCCCCTGTGACGCTGTTCAAAAACGCCATCATCAA-GAAC----	GCCC	919
Bos	GAG--CCAAAACGCCCCCTGTGACGCTGTTCAAAAACGCCATCATCAA-GAAC----	GCCC	791
Pan	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAAAACGCCATCATCAA-GAAC----	GCCT	888
Pygmy	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAAAACGCCATCATCAA-GAAC----	GCCT	888
Gorilla	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAAAACGCCATCATCAA-GAAC----	GCCT	885
Homo	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAAAACGCCATCATCAA-GAAC----	GCCT	873
Macaca	GAG--CCAGACTCCCCCTGGTGACACTGTTCAAAAACGCCATCATCAA-GAAC----	GCCT	849
Crab-eating	GAG--CCAGACTCCCCCTGGTGACACTGTTCAAAAACGCCATCATCAA-GAAC----	GCCT	936
Papio	GAG--CCAGACTCCCCCTGGTGACACTGTTCAAAAACGCCATCATCAA-GAAC----	GCCT	948
Saimiri	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAAAACGCCATTATCAA-GAAC----	GCCT	910
Chinchilla	GAG--CCAGACGCGCTGGTGACGCTGTTCAAGAACGCCATCATCAA-GAAC----	GCCC	751
Mus	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAGAACGCCATCATCAA-GAAC----	GCGC	846
Rattus	GAG--CCAGACGCCCCCTGGTGACGCTGTTCAAGAACGCCATCATCAA-GAAC----	GCGC	812
Microtus	GAG--CCAGACGCCCCCTGGTGACACTGTTTAAAGAACGCCATCGTCAA-GAAC----	GCTC	803
Mesocricetus	GAG--CCAGACGCCCCCTGGTGACACTGTTTAAAGAATGCCATCGTCAA-GAAC----	GCGC	703
Danio	GAATCCCAACAACCCCTGATCACACTCATACGAAACGCCATCGGCAATGGACAGCAGTTC	639	
	** *		
Condylura	ACAAGAAGGGCCAGTAG-----	771	
Myotis	ACCGGAAGGGCCAGTGAGGGCGCCGCGGG-----GCCGGCCTCTCCCC-GGAAGGCGGC	877	
Sorex	ACAAGAAGGGCCAGTGA-----	633	
Felis	ACAAGAAGGGCCAGTGACG-TGCAGCGGGG-CCAGAGGCTTCTCTTCCC-AGGAAGTCGA	817	
Ceratotherium	ACAAGAAGGGCCAGTGAGGGCGCAGCGGG--CGAGGGGCTTCTCTTCCC-GGGGAGTCGG	793	
Sus	ACAAGAAGGGCCAGTGAGGGTTGAGGGGG--CAGGGCCTCTCACCCC--GGAAGCCGA	971	
Ovis	ACAAGAAGGGCCAGTGA-----	792	
Pantholops	ACAAGAAGGGCCAGTGAGGGCGCAGCGGG--CAGGGCCTCTCTCCGC-GGAAGTTGA	975	
Bos	ACAAGAAGGGCCAGTGAGGGCGCAGCGGG--CAGGGCCTCTCTCCGC-GGAAGTTGA	847	
Pan	ACAAGAAGGGCCAGTGAGGGCCACAGCGGGCCCGGGGCTACCTCCCCAGGAGGTGCGA	948	
Pygmy	ACAAGAAGGGCCAGTGAGGGCCACAGCGGGCCCGGGGCTACCTCCCCAGGAGGTGCGA	948	
Gorilla	ACAAGAAGGGCCAGTGAGGGCCACAGAGGGGCCCGGGGCTACCTCCCCAGGAGGTGCGA	945	
Homo	ACAAGAAGGGCCAGTGAGGGCCACAGCGGGGCCCGGGGCTACCTCCCCAGGAGGTGCGA	933	
Macaca	ACAAGAAGGGCCAGTGAGGGCCACATCAGGGCCCTGGGGCTACCTCCCCAGGAGGTGCGA	909	
Crab-eating	ACAAGAAGGGCCAGTGAGGGCCACATCAGGGCCCTGGGGCTACCTCCCCAGGAGGTGCGA	996	
Papio	ACAAGAAGGGCCAGTGAGGGCCACAGCGGGGCCCTGGGGCTACCTCCCCAGGAGGTGCGA	1008	
Saimiri	ACAAGAAGGGCCAGTGAAGGGCCACAGCGAGACCCCGGGGCTTCTCTCCCCAGGAGGTGCGA	970	
Chinchilla	ACAAGAAGGGCCAGTGA-GGCGC-----GGGCCCGGGCTGCCCGCC-----GCCCG	798	
Mus	ACAAGAAGGGCCAGTGAGGGTGCAG-----GGGTCTTCTCATTC-----886		
Rattus	ACAAGAAGGGCCAGTGAGGGTGCAG-----GGGTCTTCTCATTC-----852		
Microtus	ACAAGAAGGGCCAGTGAGGGTGCAG-----GGGTCTTCTCACCC-----843		
Mesocricetus	ACAAGAAGGGCCAGTGA-----720		
Danio	ACAG-----ATTAA-----648		
	** *		
Condylura	-----		
Myotis	CCCT-----881		
Sorex	-----		
Felis	CCCTAAGGCCCTTCTCTCTCCCTGCCCTCCTGCCGCTCCAGCCTGGGTGTGCTACTC	877	
Ceratotherium	CCCTACG--CCCCGCTCTCGCTGCC-TCTGCGCCTCGCAGCC-----836		
Sus	CCCCAAAGCCCCCTCTCTCGCTGCCCTCCTGC--CCCCCAGCCCGGTA-----C	1022	
Ovis	-----		
Pantholops	CCCCGAAGGCCCTCTCTCT-----GTCTCTGTCGCTCGCAGCCCGGGTGAGG-ACTT	1029	
Bos	CCCTGAAGGCCCTCTCTCT-----GCCCTCTACCGCTCGCAGCCTGGGTGAGG-ATTC	901	
Pan	CCCCAAAGCCCCCTTGTCTCCCTGCCCTGCTGCCGCTCCAGCCTGGG-----998		
Pygmy	CCCCAAAGCCCCCTTGTCTCCCTGCCCTGCTGCCGCTCCAGCCTGGG-----998		
Gorilla	CCCCAAAGCCCCCTTGTCTCCCTGCCCTGCTGCCGCTCCAGCCTGGG-----995		
Homo	CCCCAAAGCCCCCTTGTCTCCCTGCCCTGCTGCCGCTCCAGCCTGGG-----983		
Macaca	CCCCAAAGCCCCCTTGTCTCCCTGCCCTGCTGC--CTCCAGCCTGGG-----956		
Crab-eating	CCCCAAAGCCCCCTTGTCTCCCTGCCCTGCTGC--CTCCAGCCTGGG-----1043		
Papio	CCCCAAAGCCCCCTTGTCTCCCTGCCCTGCTGC--CGCCAGCCTGGG-----1055		
Saimiri	CCCCAAAGCCCCCTTGTCTCCCTGCCCTGCCGCGCTCCAGCCGGG-----1020		
Chinchilla	CCCGAGAGGCAGT--CCGCCCGAGCC--CTGC--CCGCCAGCC-----838		
Mus	-----AAGGCCCTCCTGCATGGGCGAGCTGA-----TGACCT-----921		
Rattus	-----AAGGCCCTCCTGCATGGGCGAGAGCACC-----TGACCT-----889		
Microtus	-----GAG-CCCCCTCCTGCATGGGCGAGCTACTCGCCCGCAGCT-----884		

Mesocricetus -----  
 Danio -----

Condylura -----  
 Myotis -----  
 Sorex -----  
 Felis GCTCAGGCCGGGTATAGCCCCAGATAGTCAGCCTCTTAAAGCTG-CCTGTAGTT----- 930  
 Ceratotherium -----  
 Sus GCTCAGGCTGTGTG-GGCGCCAGATATCCCGCCTCTTA-----CCTGTAGTTAGGAAA 1074  
 Ovis -----  
 Pantholops GCCCAGGCAGTG-ATGGCGCCAGGTATCCCGACTCTTAAAGCTG-TCTGTAGTTAAGAAA 1087  
 Bos GCCCAGGCAGTG-ATGGCGCCAGGTATCCCGACTCTTAAAGCTG-TCTGTAGTTAAGAAA 959  
 Pan -----GG-----GTCGTGGCAGATAATCAGCCTCTTAAAGCTG-CCTGTAGTTAGGAAA 1046  
 Pygmy -----GG-----GTCGTGGCAGATAATCAGCCTCTTAAAGCTG-CCTGTAGTTAGGAAA 1046  
 Gorilla -----GG-----GTCGTGGCAGATAATCAGCCTCTTAAAGCTG-CCTGTAGTTAGGAAA 1043  
 Homo -----GG-----GTCGTGGCAGATAATCAGCCTCTTAAAGCTG-CCTGTAGTTAGGAAA 1031  
 Macaca -----GGCGG-TGGTCGCGGCAGATATTCAGCCTCTTAAAGCTA-TCTGTAGTCAGGAAA 1009  
 Crab-eating -----GGCGG-TGGTCGCGGCAGATATTCAGCCTCTTAAAGCTG-TCTGTAGTCAGGAAA 1096  
 Papio -----GGCGG-TGGTCGCGGCAGATATTCAGCCTCTTAAAGCTG-TCTGTAGTCAGGAAA 1108  
 Saimiri -----GGCAGATGGTCGAGGCAGATATTCAGCCTCTTAAAGCTG-TCTATAGTTAGGAAA 1074  
 Chinchilla -----TCCATCGCC---TTCTGGTCACTGAAGCTG-CCTGTAGTTAGGAAA 880  
 Mus -----CTAGCCTCTTAGAGTTA-CCTGT-GTTAGGAAA 952  
 Rattus -----CCAGCCTCTTAGAGTTA-CCTGTAGTTAGGAAA 921  
 Microtus -----CAAGCCTCC-ATAGTTAGCCTGTAGTTAGGAAA 916  
 Mesocricetus -----  
 Danio -----

Condylura -----  
 Myotis -----  
 Sorex -----  
 Felis -----  
 Ceratotherium -----  
 Sus TAAAACCTTTCAAGTTTCAAAAA----- 1097  
 Ovis -----  
 Pantholops TAAAACCTCTCAAGTTTCACGAA----- 1110  
 Bos TAAAACCTTTCAAGTTTCACGAAAAAAAAAAAAAAAA----- 996  
 Pan TAAAACCTTTCAAATTTACATCCACCTCTGACTTTGAATGTAACTGTGTGAATAAAGT 1106  
 Pygmy TAAAACCTTTCAAATTTACATCCACCTCTGACTTTGAATGTAACTGTGTGAATAAAGT 1106  
 Gorilla TAAAACCTTTCAAATTTACATCCACCTCTGACTTTGAATGTAACTGTGTGAATAAAGT 1103  
 Homo TAAAACCTTTCAAATTTCA-----AAAAAAAA-----AAAAAAAA 1067  
 Macaca TAAAACCTTTCAAATTTCA----- 1028  
 Crab-eating TAAAACCTTTCAAATTTACATCCA----- 1121  
 Papio TAAAACCTTTCAAATTTACATCCGCCTCTGACTTTGAATGTAACTGTGTGAATGAAAT 1168  
 Saimiri TAAAACCTTTCAAATTTACATCCGCCTCTGACTTTGAATGTA--CCCTGTGAATAAAT 1132  
 Chinchilla TAAAGCCTGTCAAATTTCA----- 898  
 Mus TAAAACCTTTCAGATTTACAGTCCGCTCTGATCTTCAATAAAAACTGCGTAAATAAAGT 1012  
 Rattus TAAAACCTTTCAGACTTAA-----AAAAAAAA-----AAAAAAAA 957  
 Microtus TAAAACCTTTCAGATTTACAGATGTCTCTGACCTTCAATATAGCGCGTGTGAATAAAGT 976  
 Mesocricetus -----  
 Danio -----

Condylura -----  
 Myotis -----  
 Sorex -----  
 Felis -----  
 Ceratotherium -----  
 Sus -----  
 Ovis -----  
 Pantholops -----  
 Bos -----  
 Pan AAAAA-TACGTAGCCGTCAAATAACAGC 1133  
 Pygmy AAAAA-TACGTAGCCGTCAAATAACAGC 1133  
 Gorilla AAAAA-TACGTAGCCGTCAAATAACAGC 1130  
 Homo AAAAA-----AAAAA---- 1078  
 Macaca -----  
 Crab-eating -----  
 Papio AAAAA-TACGTAGCCGTCAAATAACAGC 1195  
 Saimiri AAAAAACACATAGCCATCAAATAACAGC 1160  
 Chinchilla -----  
 Mus CAAAACACAACCTGTCCAGTTACACTA-- 1038  
 Rattus AAAAAAAAA----- 967  
 Microtus CAA----- 979  
 Mesocricetus -----  
 Danio -----

**Supplementary Figure 38** ClustalW alignment of *Danio rerio* POMCb nucleotide sequence against *Homo sapiens*, *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Sus scrofa*, *Bos taurus*, *Ovis aries*, *Pan troglodytes*, *Pan paniscus*, *Gorilla gorilla gorilla*, *Papio anubis*, *Macaca mulatta*, *Macaca fascicularis*, *Saimiri boliviensis boliviensis*, *Panholops hodgsonii*, *Mesocricetus auratus*, *Microtus ochrogaster*, *Chinchilla lanigera*, *Felis catus*, *Condylura cristata*, *Sorex araneus*, *Myotis lucifugus* and *Ceratotherium simum simum* POMC sequences. (\*) identical sequences are indicated. The zebrafish sequence has an average 41.14% sequence identity with these species and 39.90% similarity to *Homo sapiens*.