THESIS

CHARACTERIZATION AND EXPRESSION OF GnRH GENES IN CHANNEL (*Ictalurus punctatus*) AND BLUE CATFISH (*I. furcatus*) AND THE GENETIC DIVERSITY OF THE HATCHERY STRAINS

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GRADUATE SCHOOL, KASETSART UNIVERSITY
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THESIS

CHARACTERIZATION AND EXPRESSION OF GnRH GENES IN CHANNEL (*Ictalurus punctatus*) AND BLUE CATFISH (*I. furcatus*) AND THE GENETIC DIVERSITY OF THE HATCHERY STRAINS

THANATHIP LAMKOM

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Channel, *Ictalurus punctatus* and blue catfish, *I. furcatus* are important aquaculture species in the United States. The long term selection programs have led to many strains possess different traits. Recently the transgenic line of channel catfish has been successfully achieved and hence requires a biological containment, e.g. a knockdown of genes regulating reproduction. Therefore this study was conducted to characterize GnRH genes in channel and blue catfish and to express the channel catfish GnRH. Moreover the genetic diversity of 15 channel and 5 blue catfish strains was assessed in order to apply the information for broodstock management.

Genetic diversity of 15 strains of channel and 5 strains of blue catfish were examined utilizing 8 microsatellites loci. Genetic variation of hatchery strains was low, A = 4.00-6.88, H_o= 0.449-0.775, mean H_o=0.54 in channel catfish and A=2.88-3.38, H_o=0.354-0.504, mean H_o=0.41 in blue catfish. Four strains of channel catfish did not conform to Hardy Weinberg equilibrium while all of blue catfish strains did. F_{ST} value bootstrapped across overall loci exhibited significant population differentiation within the channel (0.2136, 95 % CI, 0.131-0.304) and blue catfish (0.2905, 95 % CI, 0.204-0.304). The variation between channel and blue catfish accounted for 15.05 % of the total genetic variation. The variation among population (population-level variation) within channel and blue catfish group were 20.42 % and 23.71 %, respectively. Genetic variation within strains was low, therefore genetic variation of the strains should be increased before further selection is performed.

GnRH (Gonadotropin releasing hormone) cDNA from a brain of channel and blue catfish was characterized by RACE-PCR (Rapid Amplification cDNA Ends-Polymerase Chain Reaction). Two GnRH types, catfish type (*caGnRH*) and chicken type II (*cGnRH II*) were identified. The catfish type GnRH encoded 4 peptides, 21 amino acid (aa), the signal peptide (SP), 10 aa catfish gonadotropin releasing hormone, 3 aa proteolytic processing site, and 46 aa GnRH-associated peptide (GAP). The chicken type II GnRH encoded the 24 aa SP, 10 aa gonadotropin releasing hormone-II, 3 aa proteolytic processing site, and 49 aa GAP. The catfish type GnRH of channel and blue catfish showed higher similarity (87 %) than between the chicken type II GnRH (67 %) in nucleotide identity. Both GnRH cDNA sequences were unique comparing to other forms of GnRH and the same forms of different species. The expression of *caGnRH* was high and restricted in brain and liver of channel catfish, while the *cGnRH II* expressed in brain, head kidney, intestine, spleen and trunk kidney. Moreover, the differential splicing GnRH transcripts were observed.

Thanathip Lamkom	Uthairat Na-Nakorn	18 Apr. 08
Student's signature	Thesis Advisor's signature	

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Thanathip Lamkom

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LIST OF ABBREVIATIONS

BAC = Bacterial artificial chromosome

caGnRH = Catfish type gonadotropin-releasing hormone

cDNA = Complementary deoxyribonucleic acid

cGnRH II = Chicken type II gonadotropin releasing hormone

CIP = Calf intestinal phosphatase

DEPC = Diethylpyrocarbonate

DNA = Deoxyribonucleic acid

GAP = GnRH associated peptide

GnRH = Gonadotropin-releasing hormone

GSP = Gene specific primer

GTH = Gonadotropin hormone

µg = Microgram

µl = Microliter

µM = Micromolar

mg = Milligram

ml = Milliliter

mM = Micromolar

mRNA = Messenger ribonucleic acid

$MgCl_2$ = Magnesium chloride

MS-222 = Tricaine methanesulfonate

ng = nanogram

PAGE = Polyacrylamide gel electrophoresis

PCR = Polymerase chain reaction

POA = Preoptic area

PPS = Proteolytic processing site

RACE = Rapid amplification cDNA ends

RNA = Ribonucleic aci

RLM-RACE = RNA ligase-mediated RACE

LIST OF ABBREVIATIONS (continued)

RT-PCR = Reverse-transcriptase

sbGnRH = Sea bream gonadotropin releasing hormone

sGnRH = Salmon gonadotropin releasing hormone

SP = Signal peptide

TAP = Tobacco acid pyrophosphatase

TBE = Tris-borate-EDTA electrophoresis buffer solution

UTR = Untranslated region

CHARACTERIZATION AND EXPRESSION OF GnRH GENES IN CHANNEL (*Ictalurus punctatus*) AND BLUE CATFISH (*I. furcatus*) AND THE GENETIC DIVERSITY OF THE HATCHERY STRAINS

INTRODUCTION

With the rate of remarkable population growth, world population may reach 6.7 billion people in 2007. Among which more than 1.1 billion people are living in extreme poverty. Thus economic growth based on agriculture and on-farm rural activities is essential to improve their livelihoods. To improve food security, agricultural products are essential to be promoted (FAO, 2002). With a source of micro-nutrients, minerals, essential fatty acids and proteins, fish makes a very significant contribution to the diet of many communities in both the developed and developing world.

Average world consumption of fish per person possibly grows from 16 kg/year in 1997 to 19-20 kg by 2030, raising total fish demand to 150-160 million tonnes. According to the marine capture fisheries estimated at no more than 100 million tonnes, aquaculture plays an important role to fullfill the requirment (FAO, 2000). Currently, aquaculture continues to grow more rapidly than all other animal food producing sectors. The sector has grown at an average rate of 8.8% per year since 1970, compared with only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period. The total fisheries production (capture and aquaculture production) has increased from 3.9% of total production by weight in 1970 to 32.4% in 2004 (FAO, 2006b).

Channel catfish, *Ictalurus punctatus* and blue catfish, *I. furcatus* blue catfish are indigenous species to the southern United States of America. The total production of channel catfish in United States of America have greatly increased from 0.5 ton in 1950 to 276,262 tonnes in 2005. The channel catfish as the economic fish was cultured the highest in USA (Figure 1). The values of channel catfish production revealed the highest, US$ 430,176,200 in 2005, among all economic aquatic organisms in USA. (FAO, 2006a).

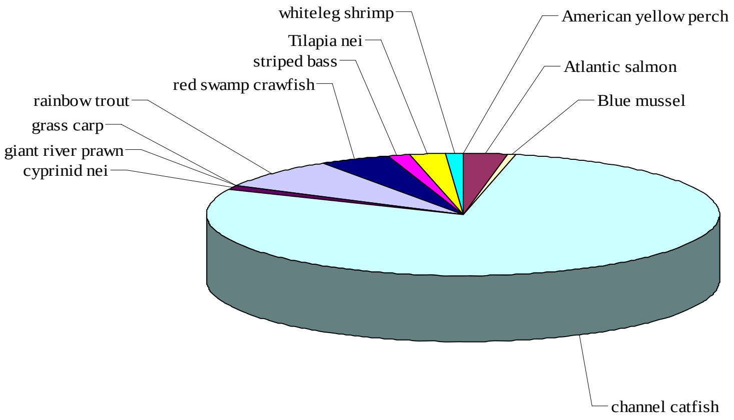

Figure 1 The annual production of channel catfish in USA (unit: tonnes)
Source: FAO (2006a)

Channel catfish is a primary species used for commercial aquaculture in major catfish producing states such as Mississippi, Alabama, Arkansas and Louisiana since 1950s. The production of channel catfish is significantly increased during the past decades (Figure 2) while the blue catfish production is still limited (FAO, 2006a). The important role of blue catfish in the catfish industry is for producing hybrids with channel catfish (Dunham, 2006).

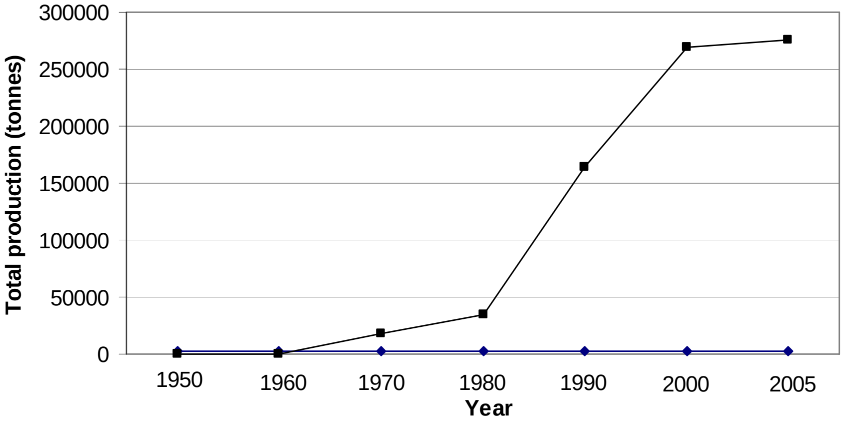

Figure 2 The channel catfish production from aquaculture in USA
Source: FAO (2006a)

With increased demand for high quality protein from fish and limited available resources, more efficient production systems are required. Genetics can greatly contribute to production efficiency, increasing production and enhancing sustainability (Dunham, 2004). Currently, cultured fish are being genetically improved for a multitude of traits, including growth rate, feed conversion efficiency, disease resistance, tolerance of low water quality, cold tolerance, stress tolerance, body shape, dress-out percentage, carcass quality, fertility and reproduction and harvestability (Gall and Neira, 2004; Charo-Karisa *et al.*, 2006; Mambrini, *et al.*, 2006; Øverli, *et al.*, 2006).

Efforts to genetically improve the aquacultural traits of channel catfish through selection are in the early generations. Domesticated channel and blue catfish exhibit significant phenotypic variation for economic traits, growth rate, disease resistance, feed conversion efficiency, environmental stress, tolerance, carcass yield, seinability, and reproduction (Dunham, 1996; Dunham and Smitherman, 1983a, 1983b; Dunham, *et al.*, 1983; Dunham *et al.*, 1985). Realized heritability and heritability estimates indicate potential for improving growth rate in channel catfish through selection (EI-Ibiary and Joyce, 1978; Bondari, 1983; Dunham and Smitherman, 1983a, 1983b; Dunham and Brummett, 1999).

Generally domesticated and/or selected strains always lose genetic variation within strains due to genetic drift and inbreeding (Jackson *et al.*, 2003). Therefore it is essential to monitor the genetic variation in order to sustain the selection response (Reed and Frankham, 2003). In this regard microsatellite DNA has been adopted as a marker of choice due to its hypervariability (Chistiakov *et al.*, 2006). In this study hatchery strains of channel and blue catfish were subjected to the study on genetic diversity aiming to obtain the base line information for further broodstock management.

In addition, a transgenic technology has been successfully applied to enhance growth of channel catfish, e.g. F1 transgenic progeny grew 26% faster than the control fish (Dunham *et al.*, 1992). However the transgenic channel catfish would never be accepted by the public due to a biosafety concern until suitable measures are established. Induction of sterility is one of the measures that will avoid spreading of the transgenes to the environment, hence may convince the positive public opinion on this issue. Therefore the GnRH genes controlling reproduction of channel and blue catfish were studied. The knowledge obtained will be used for the knockdown technology that may completely sterilize the transgenic fish.

OBJECTIVES

1. To study genetic diversity of domesticated strains of channel and blue catfish using microsatellites.

2. To characterize GnRH genes in the brain tissue of channel and blue catfish.

3. To study the expression of GnRH mRNA in the brain, gill, head kidney, intestine, liver, muscle, ovary, skin, spleen, and trunk kidney tissues of channel catfish.

LITERATURE REVIEW

1. Genetic diversity of domesticated channel and blue catfish

Channel (*Ictalurus punctatus*) and blue catfish (*I. furcatus*) are native to North America. They have been domesticated by scientists at Auburn University under a catfish genetics research program since 1969. Significant improvement of traits, such as disease resistance, growth rate, feed conversion efficiency (found highly correlated with growth), tolerance to environmental stress, carcass yield, seinability and reproduction (Dunham *et al.*, 1983, 1985, 1987, 1990; Dunham and Smitherman, 1983a, 1983b; Bondari, 1984; Dunham, 1996; Wolters *et al.*, 1996), are achieved through the application of traditional selective breeding and molecular genetics.

Growth rate and feed conversion efficiency of channel catfish have been improved by as much as 50% through selection (Bondari, 1983; Dunham and Smitherman, 1983a, 1987; Rezk 1993; Padi 1995). In Rio Grande strain (R) of blue catfish, Marion (M) and Kansas (K) strains of channel catfish, responses to one generation of selection were 63, 73, and 54 g (17%, 18%, and 12% increase in body weight), respectively, when grown in earthen ponds at 7500 fish/ha (Dunham and Smitherman, 1983a,b). After two generations of mass selection for increased body weight in channel catfish, an improvement of 12% was accomplished (Brummett, 1986; Dunham and Brummett, 1999). After three generations of mass selection, body weight of M and K strains increased by 21 % and 29 %, respectively (Rezk *et al.*, 2003).

Disease and low oxygen resistance of channel catfish has been improved primarily through interspecific hybridization with blue catfish (Dunham *et al.*, 1990; Dunham *et al.*, 1983; Argue, 1996; Dunham 1996; Wolters *et al.* 1996). The hybrid between a channel catfish female and a male blue catfish is a very desirable culture fish. It grows faster than the pure species, tolerates poor water quality, resists as many as 106 diseases and gives a better processing yield (Argue *et al.* 2003) comparing to the parental species. The hybrid showed better performances than channel catfish for tolerance to low dissolved oxygen, disease resistance, uniformity in body conformation, catchability, and dress-out percentage (Tave, *et al.*, 1981; Dunham *et al.*, 1983a, b; Ella, 1984; Brummet, 1986; Argue, *et al.*, 2003). As such, the hybrid is being promoted for commercial aquaculture in USA.

Genetic variation of domesticated stocks is always lower than that of the wild stocks. The hatchery stocks of cutthroat trout showed the 57% reduction in proportion of polymorphic loci, 29 % reduction in the average number of alleles per locus, 21 % reduction in the average heterozygosity per individual, and substantial changes in allele frequencies between age-classes when compared with the wild stocks (Allendorf and Phelps, 1980). The number of alleles per locus and allelic richness of Atlantic salmon reduced from 2-41 alleles and 11.3-12.4 in wild populations to 2-20 alleles and 6.0-7.8 in domesticated populations (Skaala *et al.*, 2004). Similarly Lundrigan *et al.* (2005) reported a reduction of genetic variation of domesticated Arctic charr (mean number of allele across loci of 4.17-15.50 and observed heterozygosity of 0.54-0.73) comparing to those of wild populations, 12-16.50 and 0.69-0.77 for mean number of alleles across loci and observed heterozygosity respectively. However, the allelic diversity of domesticated population (mean number of allele across loci = 5.43-13.42) in *Brycon opalinus* showed similarity with wild populations (13.28) (Borroso *et al.*, 2005).

Using RAPD (Random Amplified Polymorphic DNA) marker, polymorphism is low within strains of channel catfish and blue catfish, but high between channel and blue catfish (Liu *et al.* 1999a). Mickett *et al.* (2003) studied the genetic diversity of domestic channel catfish populations using AFLP (Amplified fragment length polymorphism) and reported heterozygosity of 0.135 across 16 populations which was lower than that of the wild populations (H_e = 0.16; Simmons, *et al.*, 2006).

The genetic diversity of channel catfish was studied using microsatellites and showed that the number of alleles per locus in domesticated, research, and wild populations were 1-11, 3-9, and 3-17 alleles, respectively (Waldbieser and Bosworth, 1997). The offspring of the research strains showed the average number of allele per locus and average heterozygosity overall loci of 8 and 0.712 respectively (Waldbieser *et al*., 2001). Tan *et al*. (1999) tested the microsatellite primers with 2 Auburn resource families and found the number of allele per locus and observed heterozygosity were 1-7 and 0.08-0.83.

The loss of genetic variation may occur through many processes, genetic drift, inbreeding, and selection. Genetic drift can cause changes of allele frequencies and a loss of rare allele (Tave, 1999), e.g. 50% reduction of the number of alleles per locus of Senegal sole (*Solea senegalensis*) (Porta *et al*., 2006) and a loss of approximately 26 % total number of alleles in Atlantic halibut (*Hippoglossus hippoglossus*) (Jackson *et al*., 2003) after one generation of captive breeding; 29 % decline of the number of alleles per locus and 6.36% reduction of average expected heterozygosity in Japanese flounder (*Paralichthys olivaceus*) hatchery populations (Hara and Sekino, 2003) relative to the wild population. The hatchery stocks of sea trout (*Salmo trutta*) showed the decrease of number of alleles per locus and the loss of private alleles when compared with the wild populations (Hansen *et al*., 2001; Was and Wenne, 2002).

Selection, intentionally or unintentionally occurred, causes the change of allelic diversity. The domesticated population of Atlantic salmon had been selected for economic traits, for example growth rate in sea, maturation, disease resistance and fillet pigmentation, for about 30 years and showed the reduction of the number of alleles per locus and allelic richness and the loss of rare alleles (Skaala *et al*., 2004).

Genetic diversity of economic traits is an essential component for artificial selection aiming to improve traits (Falconer and Mackay, 1996) while the overall genetic diversity determines sustainability of populations (Hamrick *et al*., 1991). Shikano and Taniguchi (2002) studied F1 performance from strain combination of guppy (*Poecilia reticulate*) and found that level of genetic diversity of parental strains considerably affect the amount of heterosis in offspring for a salinity tolerance trait. Overturf *et al*. (2003) examined five domesticated strains of rainbow trout

(*Oncorhynchus mykiss*) and showed that the genetic diversity positively correlated with feed conversion ratio (FCR) and negatively with specific growth rates (SGR) and thermal-unit growth coefficients (TGC). Heath *et al*. (2002) found that the reduced genetic diversity of chinook salmon (*Oncorhynchus tshawytscha*) affect reduction in reproductive trait, because of breeding.

Microsatellites, tandem simple sequence repeats (SSRs), disperse widely in the genome, both in protein-encoding and noncoding DNA. Microsatellites are inherited in a Mendelian fashion as codominant markers. The mutation rate is estimated 10^{-2}-10^{-6} per locus per generation (Ellegren, 2000). In channel catfish, microsatellites type I (coding gene) and II (non-coding gene) were identified through expressed sequence tags (ESTs) (Karsi *et al*., 2002; Ju *et al*., 2000) and genomic DNA library (Waldbieser and Bosworth, 1997; Waldbieser *et al*., 2001). The primers for 32 microsatellites from channel catfish genomic libraries were designed and were used to amplify genomic DNA of blue catfish. The results showed that 29 loci were highly conserved at the flanking primer regions between channel and blue catfish (Liu *et al*., 1999a, b).

Previously, the genetic variability of nine research lines and strains of channel catfish at Auburn University was studied using isozymes, the results showed that percent polymorphic loci, mean observed heterozygosity, and average heterozygosity across populations were 0-28.6 %, 0.000-0.070, and 0.0431, respectively (Hallerman *et al*., 1986). Mickett *et al*. (2003) studied the genetic diversity of 16 domesticated populations of channel catfish from fingerling suppliers and Auburn University Catfish Genetics Research Unit using AFLP markers and found that the percent of polymorphic loci, mean observed heterozygosity, and average heterozygosity across populations were 18.3-100 %, 0.0721-0.2471, and 0.135, respectively. The genetic diversity of two hundred full-sib offspring of two research lines of channel catfish were studied using microsatellites and revealed that the average number of alleles per locus and average heterozygosity loci were 8 and 0.712, respectively (Waldbieser *et al*., 2001) indicating the ability of microsatellite marker to detect the higher level of polymorphism.

2. Characterization and expression analysis of GnRH genes from channel and blue catfish

Gonadotropin-releasing hormone (GnRH=LHRH) is an important molecule controlling reproduction of all vertebrates (Millar, 2004). It is one of primary factors in the HPG (Hypothalamo-Pituitary-Gonad) axis (Amano *et al.*, 2004) and also shows a neuromodulatory function (Gopinath *et al.*, 2004). GnRH stimulates the synthesis and release of pituitary gonadotropins (GTHs) which stimulates the secretion of steroid hormones from the gonads (Sherwood *et al.*, 1993). Additionally, GnRH may act as a pheromone regulating reproductive behaviors (Temple *et al.*, 2003). In fish, GnRH can stimulate the release of other pituitary hormones including growth hormone (Klausen *et al.*, 2001), prolactin (Weber *et al.*, 1997) and somatolactin (Parhar, 1997).

GnRH1, mammalian, catfish, medaka, and seabream type of GnRH, neurons originate in the anteroventral preoptic area (Parhar, 2002) and the olfactory placode early in development and migrate to their destinations as the individual grows. *GnRH1* releases the peptide into the hypothalamo-hypophysial portal vasculature, from which it ultimately reaches the pituitary gonadotrophs (Hoffmann, 2006). *GnRH2*, chicken type II GnRH, neurons are localized in the midbrain with a few present in hypothalamic and extrahypothalamic regions (Dellovade *et al.*, 1993). *GnRH3*, salmon GnRH, neurons are found in the terminal nerve, with projections throughout the brain, which implies a neuromodulatory role (Oka and Matsushima, 1993) and their final position in the POA (preoptic area) (Lethimonier *et al.*, 2004). In barfin flounder, *Verasper moseri* (Amano *et al.*, 2002 a, b) and pejerrey fish, *Odontesthes bonariensis*, (Miranda *et al.*, 2003) *sbGnRH* (sea bream GnRH), *sGnRH* (salmon GnRH), and *cGnRH-II* (chicken type II GnRH) were found in the preoptic area, the ventromedial part of the rostral olfactory bulbs and the terminal nerve ganglion, and the midbrain tegmentum, respectively. In the zebrafish, *Danio rerio, sGnRH* and *cGnRH-II* were found in the terminal nerve and the midbrain (Gopinath *et al.*, 2004).

In general the *GnRH1* regulates the synthesis and release of LH and FSH (in mammal which equal to maturational and vitellogenic hormone in fish). *GnRH2* (*cGnRH-II*) has the function in the midbrain neurons. The *cGnRH-II* occupies a unique location in the brain compared with neurons producing other GnRH forms, suggesting a specialized and conserved function for this neuron (Vickers *et al.*, 2004). *GnRH III* (*sGnRH*) has the function in the terminal nerve or the olfactory bulb. *In situ* hybridization showed that three GnRH genes of barfin flounder, *Verasper moseri*, *sGnRH*, *cGnRH-II*, and *sbGnRH* expressed in the ventromedial olfactory bulbs and the terminal nerve ganglion, the midbrain tegmentum, and the preoptic area with axons terminating in the pituitary gland, respectively (Amano *et al.*, 2002b).

GnRH is a high conserved peptide, with multiple forms within a species that differ in up to five amino acid substitutions (Gopinath *et al.*, 2004). The number of GnRH family members in vertebrates and protochordates was 14 and 2 types respectively (Somoza *et al.*, 2002). Among vertebrates, teleost fish represent the group exhibiting the highest number of GnRH variants (8 types) (Lethimonier *et al.*, 2004), for examples, mammalian type (Matsuo *et al.*, 1971), chicken type II (King *et al.*, 1990), catfish type (Bogerd *et al.*, 1992), seabream type (Powell *et al.*, 1994), herring type (Carolsfeld *et al.*, 2000), pejerey or medaka type (Montaner *et al.*, 2001), and whitefish type (Adams *et al.*, 2002) (Table 1).

It is now well accepted that at least two GnRH forms present in the brain of one species, with modern teleosts expressing three GnRH forms, *sGnRH*, *cGnRH-II* and *sbGnRH* (Zmora *et al.*, 2002). The goldfish (*Carassius auratus*) contains at least two forms of GnRH; *sGnRH* and *cGnRH-II* (Klausen *et al.*, 2001). The brains of tilapia (*Oreochromis mossambicus*), sockeye (*Oncorhynchus nerka*), turbot (*Scophthalmus maximus*) and barfin flounder have three forms of the GnRH molecules; *cGnRH-II*, *sGnRH*, and *sbGnRH* (Parhar *et al.*, 1996; Andersson *et al.*, 2001; Amano *et al.*, 2002a).

Table 1 Amino acid sequences of the GnRH forms found in fishes

GnRH	**Group**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Mammalian	GnRH`I	pGln	His	Trp	Ser	**Tyr**	Gly	**Leu**	**Arg**	Pro	Gly-NH_2
Chicken II	GnRH II	pGln	His	Trp	Ser	His	Gly	Trp	Tyr	Pro	Gly-NH_2
Catfish	GnRH I	pGln	His	Trp	Ser	His	Gly	Leu	Asn	Pro	Gly-NH_2
Pejerrey	GnRH I	pGln	His	Trp	Ser	**Phe**	Gly	**Leu**	**Ser**	Pro	Gly-NH_2
Seabream	GnRH I	pGln	His	Trp	Ser	**Tyr**	Gly	**Leu**	**Asn**	Pro	Gly-NH_2
Salmon	GnRH III	pGlu	His	Trp	Ser	Tyr	Gly	**Trp**	**Leu**	Pro	Gly-NH_2
Herring	GnRH I	pGln	His	Trp	Ser	His	Gly	**Leu**	**Ser**	Pro	Gly-NH_2
Whitefish	GnRH I	pGlu	His	Trp	Ser	**Tyr**	Gly	**Met**	**Asn**	Pro	Gly-NH_2

Source: Dubois *et al.* (2002)

All vertebrate groups share a common structure of GnRH with three introns and four exons (Figure 3) (Suzuki *et al.*, 2000). The typical structure of the GnRH precursor protein consists of 1) 5’UTR (Untranslated region) and a signal peptide at the N-terminal [about 23 amino acids (aa) in size], 2) the GnRH decapeptide which is the bioactive peptide, followed by a 3 aa cleavage site (Gly-Lys-Arg) and 3) a GnRH-associated peptide (GAP) and 3’UTR at the C-terminal (about 60 aa in size) (Zmora *et al.*, 2002).

The amide-donating site is relatively conserved while the GAP domain associated with different GnRH forms shows only limited homology. The amino-terminus (pGlu) and carboxy-terminus (NH_2) are modified and preserved and have conserved positions at 1^{st} , 4^{th} , 9^{th} , and 10^{th} amino acid (Somoza *et al.*, 2002).

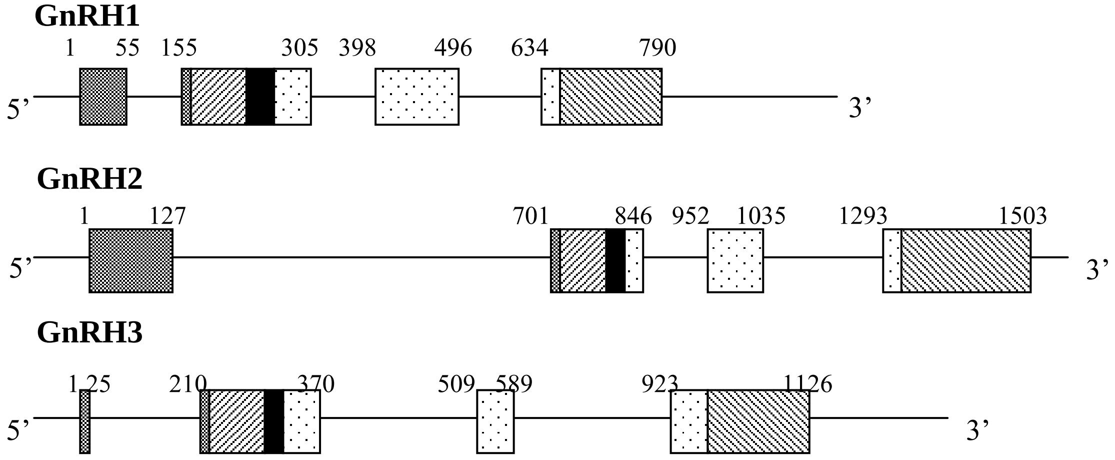

Figure 3 Schematic diagrams, showing the genomic structure of *GnRH1*, *GnRH2,* and *GnRH3* genes of Nile tilapia (*Oreochromis niloticus*)
Source: Kitahashi *et al.* (2005)

GnRH gene expression was site- and time- specific (Amano *et al.*, 2004; Andersson *et al*., 2001). In the species having two GnRH systems, it is known that the species-specific GnRH form, predominating in the hypothalamus, related with the control of the pituitary function (Table 2); and that the conserved *cGnRH-II*, expressed by midbrain neurons, with unknown function, may play a role as a neurotransmitter and/or neuromodulator (Somoza *et al.*, 2002). The primary role of *sGnRH* expressed by terminal nerve ganglia is different. First, the *sGnRH* expressing neurons which located in the terminal nerve have an endogenous rhythm and although they project widely into the brain, they do not reach the pituitary gland (Oka and Matsushima, 1993). The primary role of TNG (Terminal nerve ganglion) *sGnRH* may be to coordinate olfactory and visual input for reproduction (Kudo *et al.*, 1996; Parhar *et al.*, 1994).

Most of fishes having 3 types of GnRH demonstrated *sbGnRH* in the pituitary, *cGnRH-II* in the brain, and *sGnRH* in both the brain and pituitary. The absence of *cGnRH-II* in the pituitary indicates that it is not directly involved in the control of reproduction (Table 2). The *sbGnRH* is the dominant form, with the amount greater than those of *sGnRH* by 100-600 folds in turbot (Andersson *et al.*, 2001) and 500 folds in the gilthead seabream pituitary gland (Powell *et al.*, 1994). The increase of *sbGnRH* mRNA levels correlates with the increase in day length, water temperature and serum steroids levels, suggesting that these factors are candidates for regulators of *sbGnRH* synthesis (Okuzawa *et al.*, 2003).

Table 2 The GnRH gene expression in each tissues.

Species	**Tissue**			**Reference**
	Brain	**pituitary**	**gonad**	
Barfin flounder	sGnRH	-	-	Amano *et al*., 2004
	sbGnRH	sbGnRH	-	
	cGnRH-II	-	-	
Zebrafish	sGnRH	-	-	Gopinath *et al*., 2004
	cGnRH-II	-	-	
Red seabream	sGnRH	-	-	Okuzawa *et al*., 2003
	cGnRH-II	-	-	
	sbGnRH	sbGnRH	-	
Rainbow trout	sGnRH-1	sGnRH-2	sGnRH-1	Uzbekova *et al*., 2001
	sGnRH-2	-	sGnRH-2	
Goldfish	sGnRH	cGnRH-II	-	Klausen *et al*., 2001
Turbot	sGnRH	sGnRH	-	Andersson *et al*., 2001
	cGnRH-II	sbGnRH	-	
Gilthead seabream	sGnRH	cGnRH-II	-	Holland *et al*., 1998
	cGnRH-II	sbGnRH	-	
	sbGnRH	-	-	

The *sbGnRH* may control reproduction in the highly evolved teleosts, e.g. gilthead seabream (Holland *et al.*, 1998), striped bass, cichlid, and tilapia, in which three forms of GnRH are found in the brain. Generally the levels of *cGnRH-II* and *sGnRH* are low and unchanged, while *sbGnRH* levels fluctuate during spawning season (Rodriguez *et al.*, 2000) and testicular maturation (Amano *et al.*, 2004). Holland *et al.* (1998) studied GnRH mRNA expression in gilthead seabream and found that in recrudescent fish the pituitary contained similar levels of *sbGnRH* and cGnRH-II. In the sexually mature fish, the levels of *sbGnRH* was higher than that in recrudescent fish, while pituitary *cGnRH-II* content remained unchanged. The *sbGnRH* mRNA levels in gilthead seabream were 3- to 17- fold higher than *cGnRH-II* levels in mature fish (Holland *et al.*, 1998).

The reproductive expression of GnRH gene had been studied in many fish species. In turbot, *cGnRH-II* was detected in the brain, while *sbGnRH* and sGnRH were detected in the pituitary. Both *sGnRH* and *sbGnRH* in the pituitary showed variation during the spawning season (Andersson *et al.*, 2001). In the red seabream, the *sbGnRH* mRNA level in the brain and pituitary gland was low during the immature phase and increased until reaching a peak in the spawning season while levels of *sGnRH* mRNA showed no variation and *cGnRH-II* mRNA were elevated only slightly in spawning season (Okuzawa *et al.*, 1999). In the rainbow trout, *Oncorhynchus mykiss*, *sGnRH-1* and *sGnRH-2* forms were found in the brain and gonads. The level of *sGnRH* expression varied considerably during spermatogenesis (Uzbekova *et al.*, 2001).

At the early stages of gonad containing only some initially differentiated spermatogonia, three GnRHs involve in promoting sex differentiation. In European sea bass, *Dicentrarchus labrax* L., levels of all three GnRHs during the sex differentiation period (11.6 months) were higher than the levels found during first spawning season (24 months) (5-, 11-, and 8-fold for *sb*-, *cII*-, and *sGnRH*, respectively) (Rodriguez *et al.*, 2000). Schalburg *et al.* (1999) studied GnRH expression in sockeye salmon and found that *sGnRH* mRNA in immature sockeye salmon during the first 2 years expressed for a shorter period than mature fish did (2 months) in the second year.

The GnRH gene expression affects the growth hormone synthesis and secretion. In the goldfish, *sGnRH* and *cGnRH-II* presents in the brain and pituitary, respectively. Klausen *et al.* (2001) treated goldfish with 10^{-7}M *sGnRH* or *cGnRH-II* for 12 hours and found that gonadotropin and growth hormone gene expression was stimulated.

MATERIALS AND METHODS

Genetic diversity of domesticated channel and blue catfish

1. Sample collection

Fifteen strains of channel catfish and five strains of blue catfish were collected from across states. Channel catfish lines, Auburn Select, AR, Kansas Select, MK, Marion Select and ARMK, were selected for increased body weight for six generations. Tishomingo, and TA were selected for increased body weight for four generations. Goldkist and Goldkist albino were selected for increased body weight for 1-2 generations. Albino Forks, Kansas Random, Marion Random, S1 and S2 were randomly bred populations. All albino channel catfish have a common origin, and have been mixed into a number of strains (Dunham and Smitherman, 1984). Goldkist, AR, MK and TA were originally developed by crossing two lines. ARMK was originally established by crossing four strains. All populations except Kansas Select, Kansas Random, S1 and S2 originated from multiple rivers. Kansas lines and S1/S2 were derived from the oldest domestic strain of channel catfish. Kansas random, transferred to Auburn University in 1971 (Dunham and Smitherman, 1984) was a subsample from S1/S2. It is possible, but not certain that S1/S2 may have been contaminated with a second strain after Kansas random was transferred to Auburn University.

In the case of blue catfish, D&B is a commercial strain with a relatively long history established from multiple rivers (Dunham and Smitherman, 1983a). Rio Grande is a more recently established line from a single river. AR is a mixed strain from the Tensaw River, AL; Warrior River, AL; and Rio Grande River , TX. ARR is AR backcrossed to Rio Grande and D X R is D&B X Rio Grande.

Five hundred microliters of blood was collected from the caudal vein of each individual (Table 3) using a 1 ml syringe and was transferred to a 50-ml centrifuge tube with digestion buffer, containing 100 μg/ml proteinase K (Liu *et al*., 1998a, b).

The samples were incubated in waterbath at 55^0C for 3 hours and kept at room temperature until use.

Table 3 Details of fish samples used in this study: fish species, name of population and a sample size of each population

Species	Number	Population name	Sample size
Channel catfish	1	Tishmingo (T)	22
	2	Albino Fork (AF)	21
	3	Auburn-Rio Grande (AR1)	26
	4	Auburn Select (AS)	21
	5	Goldkist (GK)	14
	6	Goldkist Albino (GKal)	13
	7	Kansas Random (KR)	28
	8	Kansas Select (KS)	34
	9	Marion-Kansas (MK)	25
	10	Marion Select (MS)	11
	11	Tishmingo-Auburn (TA)	5
	12	Auburn-Rio grande x Marion-Kansas (ARMK)	24
	13	Marion Random (MR)	28
	14	S1 (S1)	27
	15	S2 (S2)	26
Blue catfish	16	D&B (D)	13
	17	Rio grande (R)	11
	18	1-R (AR2)	20
	19	9-L (ARR)	4
	20	197 (DxR)	30

2. DNA extraction

DNA was isolated using a Puregene® DNA isolation kit (Gentra Systems, Minneapolis, MN), following the protocol recommended by the manufacturer. Briefly, 0.3 µl of blood samples was added to 300 µl of the protein precipitation solution. The mixture was vortexed vigorously at high speed for 20 sec and centrifuged at 16,000 rpm for 3 min at room temperature. The supernatant was transferred to a new tube and 600 µl of 100% isopropanol (2-propanol) was added and thoroughly mixed by inverting of the tube for 50 times. DNA was recovered by centrifugation at 16,000 rpm for 3 min. The DNA pellet was briefly washed with 70% ethanol and centrifuged at 16,000 rpm for 4 min at room temperature. The supernatant was removed. DNA was air-dried for 10-15 min, resuspended with 100 µl of TE buffer (pH 8.0), stored at -20^0C.

3. Estimation of the amount of nucleic acid

The concentration of extracted DNA or RNA (see below) was estimated by measuring the optical density at 260 nanometre (OD_{260}). An OD_{260} of 1.0 corresponds to a concentration of 50 µg/ml double stranded DNA, 40 µg/ml single stranded RNA and 33 µg/ml oligonucleotide (Sambrook and Russell, 2001). Therefore, the concentrations of DNA/RNA are estimated in µg/ml by the following equation;

$$[\text{Nucleic acid}] = OD_{260} \text{ x dilution factors x nucleotide factor}$$

<u>Note</u> nucleotide factor = 50, 40 or 33 for DNA, RNA or oligonucleotides, respectively

The value at OD_{260} allows calculation of total nucleic acid whereas the value at OD_{280} determines the amount of proteins in the DNA and RNA solution. The ratio between OD_{260}/OD_{280} provides an estimate on the purity of extracted DNA/RNA. For the extracted DNA, a pure preparation of DNA has OD_{260}/OD_{280} ratio of 1.8 - 2.0. The ratio of approximately 2.0 indicates the good quality of the extracted RNA. The ratios that much lower than those values indicate contamination of residual proteins or phenol in the extracted DNA or RNA (Kirby, 1992).

DNA concentration can also be estimated on the basis of its direct relationships between the amount of DNA and the level of fluorescence after ethidium bromide staining. DNA was electrophoresed in 1.0% agarose gel prepared in 1xTBE buffer (89 mM Tris-HCl, 8.91 mM boric acid and 2.5 mM EDTA, pH 8.0) at 100 V. After electrophoresis, the gel was stained with ethidium bromide. DNA concentration was estimated from the intensity of the fluorescent band by comparing with that of undigested λ-DNA.

4. PCR reaction

Eight primer pairs (AU935, AU936, AU904, AU954, AU959, AU865, AU1097 and AU1081) were used for determination of microsatellite polymorphism in channel and blue catfish. PCR was performed in a 10 μl reaction containing 1x PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dATP, dTTP, dCTP and dGTP, 6 ng/ul of upper tailed (UT) and lower (L) primer (Table 4), 0.02 IRD-700 or IRD-800 dye labeled tail primer, 0.25 U of Jumpstart *Taq* polymerase, 50 ng (0.9 μl) of DNA, and 2.1 μl of sterile deionized water. PCR was carried out by predenaturation at 94^0C for 3 min, followed by 40 cycles of 94^0C for 30 sec, 53^0C or 55^0C for 30 sec (Table 4) and 72^0C for 30 sec. The final extension was carried out at 72^0C for 5 min.

After amplification, an equal volume of the formamide loading dye (1 g bromophenol blue, 44.5 ml formamide, and 0.5 deionized water) was added to 2.5 μl of the PCR product. The mixture was denatured at 95^0C for 10 min, and electrophoretically analyzed in 7% polyacrylamide gels. The $ladder_{700}$ and $ladder_{800}$ were used as reference marker for estimation of allele sizes.

Table 4 List of primers, primer sequences, repeat motif, annealing temperature and amplicon size of microsatellite primers used in this study. UT and L at the end of each primer name represent "upper tailed" primer and "lower" primer respectively. Tail sequences are underlined. AU904 is from an unknown spleen gene; AU935, AU936,AU954,AU959 are from unknown brain genes; AU865 is from an unknown skin gene, and AU1097 and AU1081 are likely from non-coding regions.

Primer name	Sequence	Base repeat	Annealing Temperature (^{0}C)	Amplicon size
AU935	GAGTTTTCCCAGTCACGACCCGTTAAGACATAATGAGTAGGACG CGTACTGCAAAACA8TCATTTTCG	$(CAA)_9$	53	118
AU936	GAGTTTTCCCAGTCACGACAACAGTATAGGGAAACCTGTTGAC GTCACACACACACACATGCA	$(ATTC)_6$	53	177
AU904	GAGTTTTCCCAGTCACGACGGACATTGTTATGGTTTAGTGC CGCTGTGTGCGTTGGCTTTGC	$(TAA)_7$	53	129
AU954	GAGTTTTCCCAGTCACGACAGCCCCTTACTCAGGGACTG GCTGTGTGCGTTGGCTTTGC	$(GT)_{12}$	53	179
AU959	GAGTTTTCCCAGTCACGACACGATTTTCAGTTGAGCCACC GCAGCGTAAAAAAGAACCGAAGC	$(GATT)_{11}$	53	177
AU865	GAGTTTTCCCAGTCACGACTGTTCTGTGTCTAAATGCTGCAC CACTGTTCGATTACAAGTCCGG	$(TA)_{13}$	53	169
AU1097	GAGTTTTCCCAGTCACGACGACAGTGCAGCGTAGTGGAG CTTCGGTCTTCTCGAAAGTGG	$(TG)_{15}$	55	141
AU1081	GAGTTTTCCCAGTCACGACTGGAGGCGACAGGCAGGTGG CATCAACTACAATATCAGCCGCAG	$(ATT)_{13}$	55	208

5. Gel electrophoresis

5.1 Agarose gel electrophoresis

The amplification products were electrophoresed through 1.2 - 1.5% agarose gels. The appropriate amount of agarose was weighed and mixed with 1x TBE (89 mM Tris-HCl, 8.91 mM boric acid and 2.5 mM EDTA, pH 8.0). The solution was boiled in a microwave to complete solubilization and left at room temperature to approximately 60^{o}C before pouring into a gel mould. The gel was left at room temperature for 30-45 min to completely solidified. When needed, the gel was placed in the electrophoretic chamber containing a sufficient amount of 1xTBE buffer covering the gel for approximately 0.5 cm and the comb was gently removed.

Prior to loading, the PCR products were mixed with one-fourth volume of a loading dye solution (0.25% bromophenol blue and 25% ficoll) and then loaded into the well. A 100 bp DNA ladder was used as a standard DNA marker. Electrophoresis was carried out at 4-5 V/cm until the tracking dye migrated about three-quartered of the gel.

5.2 Polyacrylamide gel electrophoresis

5.2.1 Preparation of the glass plate

Forty microliters of bind silane was mixed with an equal volume of 10% acetic acid. The solution was applied to the front plate where the wells are formed using the cotton swap. The cleaned glass plates were assembled with a pair of 0.25 mm spacer. The side clamps was securely implied to either side of the assembled glass plates.

Six percent denaturing polyacrylamide gel was prepared by combining 25 ml of the degassed acrylamide solution (19 : 1 acrylamide: bisacrylamide with 7 M urea in TBE buffer) with 150 µl of freshly prepared 10 % ammonium persulphate and 15 µl of TEMED. The acrylamide solution was gently swirled. The assembled plate sandwich was hold at a 45 degree angle on the bottom corner. The acrylamide solution was then gently injected into one side of the assembled plates using a 50 ml syringe. The filled plate sandwich was left in the horizontal position. The 64 well rectangular tooth comb was then inserted. The gel was left at room temperature for 1-2 hours.

5.2.2 Electrophoresis

The two upper knobs were loosened and the casting plate was removed. The other comb was taped at the back plate to position the inserted comb. The gel sandwich was placed in the vertical sequencing apparatus (Licor). The running buffer (0.8X TBE) was filled into the upper buffer tank to the maximum filling line. The lower buffer tank was also filled with the same buffer. The power cables were connected to the high voltage connector on the instrument chassis.

The DNA sequencer was pre-run until the temperature reached 50^0C. The PCR products and markers were loaded into the gel and electrophoretically fractionated for approximately 2 hours depending on the amplicon sizes. Microsatellite patterns were scored manually.

6. Data analysis

6.1 Genetic variation within populations

The effective number of alleles at each locus (Crow and Kimura, 1965) and observed and expected heterozygosity (Nei, 1987) were calculated. Allele frequencies at each locus of all population were analyzed against Hardy-Weinberg equilibrium and linkage disequilibrium implemented in GENEPOP version 3.4 (Raymond and Rousset, 1995). Allelic richness and *Fis* were estimated using FSTAT program (Goudet, 2002). Critically significant levels for the tests were adjusted using a sequential Bonferroni approach (Rice, 1989).

6.2 Interpopulational genetic differences

Genetic heterogeneity of overall samples and between pairs of populations across all loci was evaluated using F_{ST}-statistics (Weir & Cockerham, 1984; Slatkin, 1995) facilitated by FSTAT and ARLEQUIN version 3.01 (Excoffier *et al*., 2005), respectively. Analysis of molecular variance (AMOVA) was applied to detect the hierarchical genetic variance between species, among populations within species and among individuals within populations by ARLEQUIN version 3.01. The genetic distance between geographic samples was based on the Cavalli-Sforza and Edwards (1967) chord distance because for microsatellites it is among the best genetic distance measures to recover the true tree topology (Takezaki and Nei, 1996). Allele frequencies were bootstrapped 1000 times using SEQBOOT. The resulting data were used to calculate the Cavalli-Sforza and Edwards distance using GENDIST. A neighbor-joining tree (Saitou and Nei, 1987) was constructed to illustrate the relations among samples using NEIGHBOR and CONSENSE. A phylogenetic program described was routinely implemented in PHYLIP (Felsenstein, 1993).

Characterization and expression analysis of GnRH genes of *I. punctatus* and *I. furcatus*

1. Tissues for total RNA extraction

Tissues (brain, gill, head kidney, intestine, liver, muscle, ovary, skin, spleen, and trunk kidney) were collected from healthy channel and blue catfish. The fish were euthanized with tricaine methanesulfonate (MS 222) at 100 mg/l before sacrificed. Tissues were quick frozen in liquid nitrogen and kept in a -80^{0}C freezer until preparation of RNA.

2. Total RNA extraction

Total RNA was extracted from brain, gill, head kidney, intestine, liver, muscle, ovary, skin, spleen, and trunk kidney of each fish using Trizol (Invitrogen). A piece of tissue was immediately placed in mortar containing liquid nitrogen and ground to the fine powder. The tissue powder was transferred to a microcentrifuge tube containing 500 µl of Trizol (1 ml/50-100 mg tissue) and homogenized. Additional 500 µl of TRI REAGENT were added. The homogenate was left at room temperature for 5 minutes before 0.2 ml of chloroform was added. The homogenate was vortexed for at least 15 sec, left at room temperature for 2 - 15 min and centrifuged at 12000 *g* for 15 min at 4°C. The mixture was separated into the lower phenol-chloroform phase (red), the interphase, and the upper aqueous phase (colorless).

The aqueous phase (inclusively containing RNA) was carefully transferred to a new 1.5 ml microcentrifuge tube. RNA was precipitated by an addition of 0.5 ml of isopropanol and mixed thoroughly. The mixture was left at room temperature for 10 - 15 min and centrifuged at 12000 *g* for 10 min at 4 °C. The supernatant was removed. The RNA pellet was washed with 1 ml of 75% ethanol and centrifuged at 7500 *g* for 10 min at 4°C. Then the ethanol was removed. The RNA pellet was air-dried for 5-10 min and subsequently dissolved in DEPC-treated H_2O for immediate used. Alternatively, the RNA pellet was kept under absolute ethanol in a -80 °C freezer for long storage. The quality of extracted total RNA was examined by electrophoresed through 1.0% formaldehyde agarose gels. Qualification of total RNA was estimated as previously described.

3. 3' and 5' Rapid amplification of cDNA ends (RACE)-PCR

3.1 3'RACE-PCR

RACE-PCR was carried out using a 3' RACE System for Rapid Amplification of cDNA Ends Version 2.0 kit (Invitrogen). One microgram of total RNA was added into DEPC-treated water to a final volume of 11 µl and 1 µl of the AP solution (10 µM) was added. The reaction was heated at 70^0C for 10 min and chilled on ice for at least 1 min. Other components were added to the final composition of 20 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM DTT and 500 µM each dATP, dCTP, dGTP, dTTP. The mixtures were equilibrated at 42^0C for 5 min. SuperScript II RT (1 µl) was added to the reaction mixture and further incubated in a 42^0C water bath for 50 min. The reaction was terminated by incubating at 70^0C for 15 min. One microlitres of RNase H was added and incubated for 20 min at 37^0C. The first strand cDNA was stored at -20^0C until use.

PCR was carried out in a final volume of 50 µl. The reaction consisted of 1 µl of initial PCR, 1x of 10x *Taq* polymerase buffer, 1.0 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.2 µM of each primer and 1 unit of *Taq* DNA polymerase. PCR was carried out with the temperature profiles of predenaturation at 94^0C for 3 min, followed by 25 cycles of 94^0C for 30 sec, 55^0C for 30 sec, 72^0C for 1.30 min, and a final extension at

72^{0}C for 5 min. The 25 µl of the amplification product was electrophorosed on 1.2% agarose gel to check the target band.

3.2 5'RACE

The reaction components composing of 2.5 pmoles of gene specific primer (GSP), 1 µg of total RNA, and DEPC-treated water were added for a final volume of 15.5 µl. The mixtures were incubated at 70^{0}C for 10 min and chilled on ice for 1 min. The ramaining components; 2.5 µl of 10x PCR buffer, 2.5 µl of 25 mM $MgCl_2$, 1 µl of 10 mM dNTP mix and 2.5 µl of 0.1 M DTT were added and incubated at 42^{0}C for 1 min. One microlitre of SuperScript II RT was added. The reaction was incubated at 42^{0}C for 50 min. At the end of the incubation period, the reaction was terminated at 70^{0}C for 15 min. After that 1 µl of RNase mix was added and incubated at 37^{0}C for 30 min. The reaction was stored at -20^{0}C.

Synthesized cDNA was purified with S.N.A.P. column. The first strand cDNA reaction was mixed with 120 µl of binding solution (6 M NaI). The mixture was transferred to a S.N.A.P. column and centrifuged at 13,000 *g* for 20 sec. The flow through was discarded and 0.4 ml of cold 1x wash buffer was added to the spin cartridge. The column was centrifuged at 13,000 *g* for 20 sec. The flow through was discarded. This washing step was repeated 3 times. The cartridge was washed twice with 400 µl of cold 70% ethanol. The cartridge was transferred to the new tube and 50 µl of sterile distilled water (preheated to 65^{0}C) was added and centrifuged at 13,000 *g* for 20 sec.

Homopolymeric tails were added at the 3' ends of the cDNA using terminal deoxynucleotidyl transferase (TdT). The reaction components including 6.5 µl of DEPC-treated water, 5 µl of 5x tailing buffer, 2.5 µl of 2mM dCTP, and 10 µl of S.N.A.P.-purified cDNA sample, were added. The mixture was incubated at 94^{0}C for 3 min and chilled on ice for 1 min. One microlitre of TdT was added. The reaction was incubated at 37^{0}C for 10 min and inactivated at 65^{0}C for 10 min.

The tailed cDNA was amplified by PCR. The PCR recipes consisted 20 mM Tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 400 mM GSP2, 400 mM Abridged anchor primer from the kit, 200 µM each dATP, dCTP, dGTP and dTTP, 5 µl of tailed cDNA, and 2.5 units of *Taq* DNA polymerase. PCR was carried out using the amplification profile of predenaturation at 94^0C for 3 min followed by 35 cycles of 94^0C for 30 sec, 55^0C for 30 sec and 72^0C for 1.30 min and a final extension at 72^0C for 5 min. Twenty-five microlitres of the amplification product was electrophorosed in a 1.2% agarose gel at 100 volts for 40 min.

4. RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE)

4.1 Dephosphorylation

RLM-RACE was amplified using a GeneRacer kit following the manufacturer's instructions. Total RNA was dephosphorylated in 10 µl of the reaction mixture containing 2 µl of total RNA, 1 µl of 10x Calf intestinal alkaline phosphatase (CIP) buffer, 1 µl of RNase Out (40U/ul), 1 µl of CIP (10U/ul), and 5 µl of DEPC-treated water. The mixture was vortexed briefly and incubated at 50^0C for 1 hour before placing on ice for 2 min to terminate the dephosphorylation reaction .

Dephosphorylated RNA was purified by adding 90 µl of DEPC water and 100 µl of phenol:chloroform:isoamyl:alcohol (25:24:1). The mixture was mixed thoroughly and centrifuged at the maximum speed for 5 min at room temperature. The aqueous phase was transferred to a fresh tube. Two microlitres of 10 mg/ml mussel glycogen, 10 µl of 3 M sodium acetate, pH 5.2, and 220 µl absolute ethanol were added in order and briefly vortexed. The mixture was kept at -80^0C for 10 min before centrifuging at the maximum speed for 20 min at 4 ^{0}C. The supernatant was decanted. The RNA pellet was washed with 500 µl of 70% ethanol and vortexed briefly before centrifuging at the maximum speed for 2 min at 4^0C. Ethanol was removed. The tube was recentrifuged and the residual ethanol was then removed. The RNA was air-dried for 1 min at room temperature and resuspended with 8 µl of

DEPC water. An aliquot of 1 µl of RNA was electrophorosed on 1.2% agarose gel electrophoresis.

4.2 Decapping of RNA

The decapping reaction was carried out using 7 µl of dephosphorylated RNA, 1 µl of 10x TAP buffer, 1 µl of RNase Out (40U/µl), and 1µl of TAP (0.5U/µl). The mixture was briefly mixed, incubated at 37^{0}C for 1 hour, and terminated by placing on ice. The decapped RNA was purified by phenol-chloroform and recovered by ethanol precipitation as previously described. The quality of the decapped total RNA (1 µl) was examined by 1.2% agarose gel electrophoresis.

4.3 Ligation

The decapped RNA was added to the tube containing the pre-aliquoted, lyophilized GeneRacer RNA oligo (0.25 µg), mixed and centrifuged briefly. The reaction mixture was incubated at 65^{0}C for 5 min and placed on ice for 2 min. The remaining components; 1 µl of 10x ligase buffer, 1 µl of 10 mM ATP, 1 µl of RNase Out (40U/ul), and 1 µl of T4 RNA ligase (5U/µl) were added and incubated at 37^{0}C for 1 hour before placing on ice to terminate the reaction. The processed RNA was purifie by extracting with phenol-chloroform and recovering by ethanol precipitation. RNA was resuspended with 11 µl of DEPC–treated water. The quality of the decapped total RNA (1 µl) was examined by 1.2% agarose gel electrophoresis.

4.4 Reverse transcription (RT)

Reverse transcription was carried out using 10 µl of ligated RNA, 1 µl of gene specific primer, 1 µl of dNTP mix, and 1 µl of sterile water. The mixture was incubated at 65^{0}C for 5 min and placed on ice for 1 min. The remaining reagents composing of 4 µl of 5x first strand buffer, 1 µl of 0.1 M DTT, 1 µl of RNaseOut (40U/µl), and 1 µl of SuperScript III RT (200U/µl) were added and thoroughly mixed. The mixture was incubated at 55^{0}C for 1 hour. The reaction was inactivated at 70^{0}C for 15 min before placing on ice for 2 min. One microlitre of RNase H (2 U) was added and further incubated at 37^0C for 20 min.

The cDNA ends were amplified by using gene specific primer and primer provided by the kit. For 3' end, the forward gene-specific primer and GeneRacer 3' primer were used. Nested PCR was carried out using the nested forward primer and the nested 3'GeneRacer primer using the PCR product from the first amplification reaction as the template. For 5'end, the reverse gene-specific primer and the 5'GeneRacer primer were used. Nested PCR was carried out using the nested forward primer and the nested 5'GeneRacer primer.

4.5 Amplifying cDNA ends by PCR

PCR was carried out in a final volume of 50 µl composing of 1 µl of the cDNA template 1x of *Taq* polymerase buffer, 1.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM primer (Table 5) and 1 unit *Taq* DNA polymerase. PCR was carried out with the temperature profiles of predenaturation at 94^0C for 3 min, followed by 5 cycles of 94^0C for 30 sec and 72^0C for 2 min, 5 cycles of 94^0C for 30 sec and 72^0C for 1.30 min, 25 cycles of 94^0C for 30 sec, 66^0C for 30 sec and 72^0C for 1.30 min, and a final extension at 72^0C for 10 min.

Ten microlitres of the amplified products were electrophorosed on the agarose gel. The PCR product size was estimated by comparing with a 100 bp DNA marker.

Nested PCR was carried out using 1 µl of the original PCR reaction as the template. PCR was performed in a 50 µl reaction mixture containing 1x *Taq* DNA polymerase buffer, 1.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 µM of each primer (Table 5), 1 unit *Taq* DNA polymerase, and sterile water. The amplification reaction was carried out using the thermal profiles of predenatuation at 94^0C for 3 min, followed by 25 cycles of 94^0C for 30 sec, 66^0C for 30 sec and 68^0C for 2.00 min and a final extension at 68^0C for 10 min. The nested PCR product (25 µl) was electrophoretically analyzed in a 1.2% agarose gel.

Table 5 The forward and reverse primer6s used for isolating of the full length cDNA of *GnRH* using RACE-PCR.

RACE-PCR	Forward primer	Reverse primer
3'RACE		
PCR	CAGGTGAGCGCTCAGCACTGGTCTCATGG	GCTGTCAACGATACGCTACGTAACG
Nested PCR	CAGGTGAGCGCTCAGCACTGGTCTCATGG	CGCTACGTAACGGCATGACAGTG
PCR	AGCTCACCAGAGATATCTGGGGAGA	GCTGTCAACGATACGCTACGTAACG
Nested PCR	ACTGTGTGAAGCGGGAGAATGCAG	CGCTACGTAACGGCATGACAGTG
5'RACE		
PCR	CGACTGGAGCACGAGGACACTGA	GCCTGTAGAGTTTATTCCGAGGTG
Nested PCR	GGACACTGACATGGACTGAAGGAGTA	GCAACGCTGCACGCTTTCCTCCAG
PCR	CGACTGGAGCACGAGGACACTGA	CAGATATCTCTGGTGAGCTGTAAGAGT
Nested PCR	GGACACTGACATGGACTGAAGGAGTA	CAGATATCTCTGGTGAGCTGTAAGAGT

5. Cloning of PCR product

5.1 Ligation of PCR product to the TOPO vector

The ligation reaction was set up in a total volume of 10 µl containing 4 µl of the PCR product, 1 µl of the Topo cloning vector, 1 µl of ligation solution. The ligation mixture was gently mixed by pipetting and incubated at room temperature (22-23 °C) for 5 min and stored at -20^0C overnight.

5.2 Transformation

The competent cells (MAX Efficiency® DH5α™ Competent Cells, Invitrogen), were thawed on ice for 5 min. Two microlitres of the ligation mixture were added and gently mixed by pipetting. The mixtures were incubated on ice for 30 min. The reaction tube was heat-shocked in a 42°C water bath for exactly 30 sec without shaking. The reaction tube was then immediately snapped on ice for 2–3 min. Two hundred and fifty microlitre of SOC medium (2% Bacto tryptone, 0.5% Bacto yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$ and 20 mM glucose) was added to the tube. The cell suspension was incubated with vigorous shaking at 37°C for 1 hour. At the end of the incubation period, the cultured cell suspension was spreaded on a LB agar plate containing 50 μg/ml of ampicillin pre-spreaded with 20 μl of 40 mg/ml of X-gal and 4 μl of 100 mM IPTG. The plate was left until the cell suspension was absorbed and further incubated at 37°C overnight (Sambrook and Russell, 2001). The recombinant clones containing inserted DNA are white whereas those without the inserted DNA are blue.

6. Plasmid extraction

Ten white colonies were taken out and inoculated in 2 ml of (1% tryptone, 0.5% yeast extract, 1.0 % NaCl) containing 50 μg/ml of ampicillin and incubated at 37°C with constant shaking at 225 rpm overnight. The culture was transferred into 1.5 ml microcentrifuge tube and centrifuged at 14,000 rpm for 1 min. Then the supernatant was discarded. The bacterial cell pellet was collected and resuspended with 250 μl of the P1 buffer and thoroughly mixed by a vortex. The resuspended cells were lysed by the addition of 250 μl of the P2 buffer and mixed gently by inverting the tube 4-6 times. After that, 300 μl of the buffer N3 was added to neutralize the alkaline lysis step and mixed immediately by inverting the tube for 4-6 times. To separate the cell debris, the mixture was centrifuged at 14,000 rpm for 10 min. The supernatant was transferred into a new microcentrifuge tube and to the Qiaprep column and centrifuged at 6,000 *g* (8,000 rpm) for 1 min. The flow-through was discarded. The column was placed back in the collection tube. The column was washed by adding 400 μl of the W1 buffer and centrifuged at 6,000 *g* (8,000 rpm) for 1 min. After discarding the flow-through, 750 μl of the wash buffer was added and centrifuged as above. The flow-through was discarded. The spin tube was centrifuge for 1 min at full speed (14,000 rpm) to remove the residual wash buffer. The dried column was placed in a new 1.5 ml microcentrifuge tube and 50 μl of the Elution buffer was added at the center of the column to elute the extracted plasmid DNA. The column was left at room temperature for 2 min and centrifuge at 14,000 rpm for 2 min. The concentration of extracted plasmid DNA was by spectrophotometry measured.

6.1 Determination of the insert by PCR

PCR was performed in a 10 µl reaction containing 1x buffer, 1 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP and dTTP, 0.2 µM of M13-F (5'-TGT AAA ACG ACG GCC AGT-3') and M13-R (5'-TCA CAC AGG AAA CAG CTA TGA C-3') primers, and 1 unit of *Taq* DNA Polymerase. The PCR profile was predenaturing at 95°C for 4 min, followed by 40 cycles of 94°C for 45 sec, 55°C for 45 sec and 72 °C for 2 min. The final extension was carried out at 72°C for 7 min. The PCR products were electrophoretically analyzed in a 1.5 % agarose gel and visualized after ethidium bromide staining.

7. DNA sequencing

DNA sequencing was carried out using a Big Dye Terminator Sequencing kit (ABI, Foster City, CA). The sequencing reaction was prepared by combining 1 µl of the big dye terminator, 2 µl of 5x sequence buffer, 1 µl of T7 (TAA TAC GAC TCA CTA TAG GG) or SP6 (GAT TTA GGT GAC ACT ATA G) primer, 2 µl of plasmid DNA, and 4 µl of sterile water. The PCR profiles composed of 94^0C for 3 min following by 35 cycles of 94^0C for 30 sec, 55^0C for 30 sec and 72^0C for 1.30 min. The final extension was performed at 72^0C for 10 min.

The reaction was terminated and precipitated by the addition of 2.5 µl of 125 mM EDTA followed by 30 µl of absolute ethanol (prechilled at -80^0C) and inverted for 4 times. The mixture was incubated at room temperature for 15 min and centrifuged at the maximum speed of the microplate centrifuge for 40 min at 4^0C. The supernatant was removed and 30 µl of 70% ethanol was added. The reaction tube was centrifuged at 1650 *g* for 15 min at 4^0C. The supernatant was decanted and the plate was centrifuged to 200 *g* for 1 min. The pellet was resuspended by adding 8 µl of the formamide dye (Formamide loading dye (1000 X), dilute 1:1000 with deionized formamide to make 1X dye , ABI). The resuspended mixture was denatured at 95^0C for 5 min. Nucleotide sequence of each recombinant plasmid was analyzed on an ABI Prism 3130XL automatic sequencer.

8. Overgo hybridization of High-Density Filters of BAC clones

Several types of GnRH cDNA sequences including the catfish type of African catfish and the salmon type of common carp, goldfish and Nile tilapia were retrieved from the GenBank (http://ncbi.nlm.nlh.gov) and multiple aligned to screen out repeated sequences. The overgo probe for hybridization of high density filters of BAC was designed using an overgo-maker program (Figure 4). Primers were purchased from Sigma Genosys (Woodlands, Texas). High density filters of *I. punctatus* BAC library were purchased from Children's Hospital of the Oakland Research Institute (CHORI, Oakland, CA). Each set of filters contained 10-genome coverage of *I. punctatus* BAC clones (BAC library CHORI 212; http://bacpac .chori.org/library.php?id=103). The sequence of overgo primers are shown in Table 6.

Table 6 The forward and reverse overgo primer used for hybridization of BAC clones

GnRH	Forward primer	Reverse primer
Catfish type	AGGCTGAAAGATCTGCTGACCAGT	TTTCTCGCTCAGCAACACTGGTCA
Chicken type	AGCACTGGTCTCATGGCTGGTACC	TCTCTCTTTCCTCCAGGGTACCAG

a) Probe of catfish type GnRH

```
AGGCTGAAAGATCTGCTGACCAGT
                ACTGGTCACAACGACTCGCTCTTT
```

b) Probe of chicken type II GnRH

```
AGCACTGGTCTCATGGCTGGTACC
                GACCATGGGACCTCCTTTCTCTCT
```

Figure 4 Probes for hybridization of *caGnRH* and *cGnRH-II*.

For the caGnRH, many positive BAC clones were obtained from overgo hybridization. The catfish type GnRH was probed again with the more specific (longer) DNA probe synthesized from a 3’ RACE-PCR fragment (Figure 5).

```
CAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCA
GGAGACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTACGTAGATGTTTCAC
CTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGACCAGTGTTGCTGAGCGAGAAATTGGA
CAGTAAGACAAATAACAAAACTGATCAAGAGCTTCAATAAAATGCTTTGCCTTTAAAAAAAA
AAAAAAAAAA
```

Figure 5 Nucleotide sequence of the 3’ RACE-PCR fragment used for synthesis of the *caGnRH* probe.

8.1 Labeling of overlapping oligonucleotides

The labeling reaction was carried out in 40 µl reaction mixture containing 14.0 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 0.02 mM dGTP, 0.02 mM dTTP, 20 µCi [α-^{32}P] dCTP, 20 µCi [α-^{32}P] dATP (3000 Ci/mmol, Amersham, Pistacaway, NJ), 200 ng of each overgo primer, and 5 units of Klenow enzyme (Invitrogen). The labeling reaction was left at room temperature for 2 hours.

8.2 Prehybridization and hybridization

Prehybridization was performed in 18 ml of the prehybridization solution (50 ml of 1% BSA, 1 mM EDTA at pH 8.0, 7% SDS, 0.5 mM sodium phosphate, pH 7.2) at 54^0C for 4 hours. Unincorporated nucleotides were removed from the labeling reaction using a Sephadex G50 spin column. The DNA probe was denatured at 95^0C for 10 min and added to the prehybridization buffer. Hybridization was performed at 54^0C for 18 hours. The hybridized BAC filter was posthybridization washed with 2xSSC at room temperature for 15 min and 0.5x SSC/0.1%SDS at 42 ^{0}C for 10 min. The filter was wrapped and exposed to an X-ray film at -80^0C for 2 days. After autoradiography, positive clones were identified according to the clone distribution instructions and picked out from the channel catfish BAC library.

9. Growing BAC clones and purification of plasmid DNA

Putative catfish type and chicken type II GnRH was isolated from positive BAC clones using the Perfectprep® BAC 96 BAC DNA Isolation kit (Brinkmann instruments, Inc., Westbury, NY).

9.1 Preculture

The bacterial clone was cultured in 150 µl 2x YT containing 12.5 µg/ul of chloramphenicol. The plate of bacteria was covered with a piece of the adhesive tape (Airpore Tape Sheets) to allow air to circulate and incubated overnight (approximately 22 hours) at 37^0C with shaking at 325 rpm. Plasmid DNA was immediately isolated from the bacterial culture or alternatively, 100 µl of 60% glycerol was added and the preculture plate was stored at -80^0C until needed.

9.2 Bacterial culture

Each BAC clone was picked up and cultured in 1.5 ml of 2xYT containing12.5 µg/µl chloramphenicol in a Perfectprep BAC 96 Culture Plate (2.4 ml deep well culture plate). The culture plate was sealed using an Air Permeable Seal and incubated overnight (22-26 hours) at 37^0C with shaking at 325 rpm.

9.3 BAC DNA isolation

BAC DNA was isolated according to the vacuum protocol of a Prefectprep BAC 96 kit. The overnight culture was centrifuged at 1,900 *g* for 10 min. The supernatant was removed and the plate was inverted on a clean absorbent material. Then, 200 µl of solution I was added into the culture plate and vortexed for complete dispersion of the bacterial pellet. Two hundred microlitres of solution II was added, the plates were sealed and placed upside down. The Culture plate was inverted 5 times. The plate was incubated for 5 min at room temperature and 200 µl of solution III was added. The culture plate was inverted 10 times to thoroughly mix the mixture.

The neutralized bacterial culture was transferred from the culture plate to the corresponding wells of the Filter plate A. The manifold was prepared by placing a short adaptor in the manifold chamber. The filter plate BAC was positioned over the short adaptor and the filter plate A on the manifold lid. The vacuum pump (Single Vac manifold) was applied until all liquid flowed through the filter plate A to the filter plate BAC. The filter plate A was removed and discarded. The used culture plate was placed into the vacuum manifold chamber. The filter plate BAC was positioned on the manifold lid. Two hundred microlitres of diluted trapping buffer was added in the filter plate BAC. The pressure was applied to the lid with both hands and the filter plate BAC was inverted 4 times. The filter plate BAC was placed on the manifold lid and incubated for 5 min at room temperature. The vacuum was applied until all liquid flowed through the filter plate.

Six hundred microlitres of the diluted wash buffer was added to the filter plate. The vacuum was applied until all of the liquid has passed through the filter plate. The excess liquid was removed by blotting the filter plate on a clean absorbent material. The filter plate was placed over the empty manifold chamber and allowed it to air dry for 5 min. The Tall adapter plate and Collection Plate were placed inside the manifold chamber. The filter plate containing the trapped BAC DNA was placed on the manifold lid. Thirty microlitres of elution buffer was added to the filter plate BAC and incubated for 5 min. The vacuum was applied until all liquid passes through the filter plate. Additional thirty microlitres of elution buffer was added and the vacuum was applied. The collection plate was recovered from the vacuum manifold chamber. The purified BAC DNA was stored at -20^{0}C.

10. Primer walking

The full length genomic sequences of *caGnRH* and *cGnRH II* of *I. punctatus* were characterized using a primer walking approach. Primers used are illustrated in Table 7. DNA sequencing was carried out using a Big Dye Terminator Sequencing kit (ABI, Foster City, CA). The sequencing reaction was prepared by combining 1 µl of big dye terminator, 2 µl of 5x sequence buffer, 1 µl of primer, 5 µl of BAC-DNA, and 1 µl of sterile water. The PCR profiles comprised of 94^{0}C for 5 min followed by 100 cycles of 94^{0}C for 30 sec, 55^{0}C for 15 sec and 60^{0}C for 4 min.

The reaction was terminated and precipitated by the addition of 2.5 µl of 125 mM EDTA followed by 30 µl of absolute ethanol (prechilled at -80^{0}C) and inverted for 4 times. The mixture was incubated at room temperature for 15 min and centrifuged at the maximum speed of the microplate centrifuge for 40 min at 4^{0}C. The supernatant was removed and 30 µl of 70% ethanol was added. The reaction tube was centrifuged at 1650 *g* for 15 min at 4^{0}C. The supernatant was decanted and the plate was centrifuged to 200 *g* for 1 min. The pellet was resuspended by adding 8 µl of the formamide dye (Formamide loading dye (1000 X), dilute 1:1000 with deionized formamide to make 1X dye , ABI). The resuspended mixture was denatured at 95^{0}C for 5 min. Nucleotide sequence of each recombinant plasmid was analyzed on an ABI Prism 3130XL automatic sequencer.

11. PCR walking

The full length genomic sequences of *caGnRH* and *cGnRH II* of *I. punctatus* were characterized using a PCR walking approach. Primers were based on genomic sequences of *caGnRH* and *cGnRH II* of channel catfish and are illustrated in Table 7. PCR was carried out in a final volume of 50 µl composing of 1 µl of the cDNA template, 1x of *Taq* polymerase buffer, 1.0 mM of $MgCl_2$, 0.2 mM of dNTPs, 0.2 µM of primer and 1 unit of *Taq* DNA polymerase. PCR was carried out with the temperature profiles of predenaturation at 94^0C for 3 min, followed by 35 cycles of 94^0C for 30 sec, annealing temperature (up to each primer) for 30 sec and 72^0C for 1.30 min, and a final extension at 72^0C for 10 min.

Twenty five microlitres of amplified products was electrophorosed on 1.2% agarose gel. The PCR product size was estimated by comparing with a 100 bp DNA marker.

The PCR products were ligated into the TOPO vector, cloned and transformed into competent cells. The plasmid DNA was extracted using alkaline lysis method. The DNA insert was checked with M13 forward and reverse primer.

DNA sequencing was carried out using a Big Dye Terminator Sequencing kit (ABI, Foster City, CA). The sequencing reaction was prepared by combining 1 µl of big dye terminator, 2 µl of 5x sequence buffer, 1 µl of primer, 2 µl of plasmid DNA, and 4 µl of sterile water. The PCR profiles were composed of 94^0C for 3 min followed by 35 cycles of 94^0C for 30 sec, 55^0C for 30 sec and 72^0C for 1.30 min.

The reaction was terminated and precipitated by the addition of 2.5 µl of 125 mM EDTA followed by 30 µl of absolute ethanol (prechilled at -80^0C) and inverted for 4 times. The mixture was incubated at room temperature for 15 min and centrifuged at the maximum speed for 40 min at 4^0C. The supernatant was removed and 30 µl of 70% ethanol was added. The reaction tube was centrifuged at 1650 *g* for 15 min at 4^0C. The supernatant was decanted and the plate was centrifuged to 200 *g* for 1 min. The pellet was resuspended by adding 8 µl of the formamide dye (Formamide loading dye (1000 X), dilute 1:1000 with deionized formamide to make 1X dye, ABI). The resuspended mixture was denatured at 95^0C for 5 min. Nucleotide sequence of each recombinant plasmid was analyzed on an ABI Prism 3130XL automatic sequencer.

Table 7 Forward and reverse primers used for characterization of genomic sequence of GnRH of *I. punctatus* and *I. furcatus* (CA-CH=catfish type of *I. punctatus*, CA-BL=catfish type of *I. punctatus*, CH-CH=chicken type II of *I. punctatus*, CH-BL=chicken type II of *I. punctatus*)

Type	Forward primer	Reverse primer
CA-CC	F1: ATGGGTATAAAGCGAGCACTCTGG	R1: CTTTCCTCCAGGATTGAGACCATGAGACCAGTGCTGA
	F2: GAATGTTGTACTATACCATACTCAC	R2: GCAACGCTGCACGCTTTCCTCCAG
	F3: AGCCCTACTCGTTTATCAG	R3: GGATTTAGTGAGCTCTAAGTGCC
	F4: CAAGAGTGCTGCTCAGCACGG	R4: CGCGACATTTGTACGATGTG
	F5: GGAAGATTCTGGAATGAGGAC	R5: CAGTGTGATCTTGCTACTGGTTGC
CA-BL	F1: GCTCCTTGTGTGCGCTGTTC	R1: GCATAGGGAAGCACTAGATGAAG
	F2: GACGGTTTACCAAAATGCAGTCG	R2: CGCGACATTTGTACGATGTG
	F3: CAGACCCTCACTGAAAGGGCAC	R3: CTGATAAACGAGTAGGGCT
	F4: AGCCCTACTCGTTTATCAG	R4: GATCAGTCGCCCTACAGACAG
	F5: GAAGAGCAGAAGAACTGTCTG	R5: CACACACCACCATCCACCAGAG
	F6: CTCTGGTGGATGGTGGTGTGTG	R6: CGCTGCACGCTTTCCTCCAG
	F7: ACCAGTGTTGCTGAGCGAG	R7: GATGTGTATACAGACAGCTGTTG
	F8: CCAGCATGTATCATCTGAGC	R8: CACAGGCCCAGATGACATTGG
	F9: CACTCTAGATACGCCATGGAAC	F9: AGGTGGAAGCTTAAGCAAGCC
CH-CC	F1: CAGGACAGTGTCCAGGTCTG	R1: TCTCTCTTTCCTCCAGGGTACCAG
	F2: CATCTGGATTGTGTATTTGCGAC	R2: GCCTAGAACGCCTGTCTTAGG
	F3: GAAGCGTTCTACTTCTGAAC	R3: CCTGAGAGATGCTGCCCGAATAC
	F4: GAACTCAAGCCTGTTTGTGGA	R4: CACAGAGAAGGCTACGATTCGTC
	F5: GAATGTCCCATGTATTCTTTC	R5: GCCCGATATTAACACGTGCCGA
CH-BL	F1: CAGTAATATGGCGAGAGTGTTTG	R1: CACCACAGAGAAGGCTACGATTC
	F2: CGTTGATGTAGATTATACACGAG	R2: GCCTAGAACGCCTGTCTTAGG
	F3: CATCTGGATTGTGTATTTGCGAC	R3: GCTGCATTCTCCCGCTTCACACAG
	F4: GTGTTGAGGGGCACTCTGTTCTC	R4: CAGATAGCTGCGCTTGCAAACAC
	F5: CAGGACAGTGTCCAGGTCTG	R5: TCCTTATGAGCTGGATCAGCCG
	F6: CATCTGCTCATCCGTGCAGTG	R6: CAGAGCATTATGCGTGGACCG
	F7: CCAAGCTGAACATATCACACGG	R7: TGTGTGTGTGTTCAGATCAGTG

12. Sequence analysis

Nucleotide sequences were searched against data in the GenBank BLAST*N* and BLAST*X* to determine gene identities and to examine if the obtained cDNA contained a full length open reading frame (ORF). Sequences were also analyzed by the DNASTAR software package (Serapion *et al.,* 2004). Isoelectric point (pI) and molecular weight of each type of GnRH were analyzed using Protparam (http://au.expasy.org/tools/protparam.html). The cDNA sequences of each GnRH type were aligned by ClustalW program. Nucleotide divergence between pairs of sequences was calculated using the two parameter method (Kimura, 1980). The original data was bootstrapped 1000 times using Seqboot. Data were further analyzed by DNAdist, Neighbor and Consense. All phylogenetic analysis was carried out using PHYLIP (Felsenstein, 1993).

13. Reverse transcription (RT)-PCR

13.1 First strand cDNA synthesis

The first strand cDNA was synthesized by using SuperScript III RT (Invitrogen). The reaction mixture containing 1 µl of oligo $(dT)_{20}$ (50 uM), 1 µg of total RNA, 1 µl of 10 mM dNTP mix, and sterile distilled water to make up 13 µl. The mixture was heated to 65^0C for 5 min and incubated on ice for at least 1 min. Subsequently, 4 µl of 5X First strand buffer, 1 µl of 0.1 M DTT, and 1µl of SuperScript III RT (200 units/µl) were added. The components were mixed by pipetting and incubated at 50^0C for 1 hour. The reaction was inactivated by incubating at 70^0C for 15 min.

12.2 Reverse transcriptase PCR (RT-PCR)

RT-PCR of *GnRH* was carried out in a 25 µl reaction mixture containing 1x of PCR buffer, 0.2 mM dNTPs, 0.75 mM $MgCl_2$, 0.25 µM of each primer (Table 8), 2 units of Platinum *Taq* DNA polymerase and 1.5 µl of first strand cDNA. *β-actin* was included as positive control and amplified using the same component except the primer and first strand cDNA was decreased to 0.2 μM and 1 μl, respectively. RT-PCR was performed by predenaturation at 94^0C for 3 min followed by 30 cycles (GnRH) or 28 cycles (*β-actin*) of 94^0C for 30 sec, 64^0C for 30 sec and 72^0C for 1 min. The final extension was carried out at 72^0C for 5 min. The RT-PCR product was electrophoretically analyzed in a 1.2% agarose gel, stained with ethidium bromide and photographed using a Gel Documentation System.

Table 8 Forward and reverse primers used for RT-PCR of GnRH of *I. punctatus*

Type	Forward primer	Reverse primer
caGnRH	TGGTGCTGCAGGTGAGCTCTCAG	CTACGTAATCACACACGTAGCCG
cGnRH II	GTGATGGTCAGTGTGTGCAGGC	CTCTGGTGAGCTGTAAGAGTCG
β-actin	AGAGAGAAATTGTCCGTGACATC	CTCCGATCCAGACAGAGTATTTG

PLACE AND DURATION

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RESULT AND DISCUSSION

Results

Genetic diversity of channel and blue catfish strains

1. Allele frequency

Allele size ranged from 121 to 139 bp for AU935 (7 alleles), 140 to 196 bp for AU936 (8 alleles), 134 to 152 bp for AU904 (7 alleles), 191 to 233 bp for AU954 (15 alleles), 153 to 209 bp for AU959 (14 alleles), 148 to 188 bp for AU865 (17 alleles), 146 to 180 bp for AU1097 (11 alleles), and 203 to 254 bp for AU1081 (18 alleles). Allele frequencies of channel and blue catfish are shown in Table 9.

Most of the loci were polymorphic in every population except for AU1081 and AU959 (monomorphic in R and ARR strain of blue catfish respectively) and AU904 (monomorphic in ARMK, MS and MR of channel catfish). No private alleles were observed.

2. Hardy-Weinberg equilibrium

Among 15 strains of channel catfish, genotype frequency distribution of 4 strains did not conform to Hardy Weinberg equilibrium. Whereby AR, KR, KS and MR departed from the equilibrium at 7, 5, 6 and 4 loci respectively (appendix 1). The overall *Fis* indicated a departure towards homozygote excess in all but one (MR) strain (Table 11). All of blue catfish strains were in Hardy-Weinberg equilibrium.

Among 8 loci used, AU935, AU954, and AU865 exhibited significant deviations from Hardy–Weinberg equilibrium in most channel catfish populations (60%, 66.7% and 66.7% respectively). While the genotype distribution of each locus conformed to HWE in all samples of blue catfish.

3. genotype disequilibrium

The exact tests for genotype disequilibrium between loci in each population of channel and blue catfish showed 4 and 1 significant values ($P< 0.00179$; Bonferroni corrected) out of 420 and 140 tests, respectively (Table 10).

4. Allele diversity

The mean number of alleles per locus (A) was relatively similar among strains within species, ranged from 4.00±1.41 in MS and TA strain to 6.88±3.23 in MK strain of channel catfish and from 2.88±0.84 in R strain to 3.38±2.39 in DxR strain of blue catfish (Table 11). Nevertheless channel catfish strains tended to have higher A than that of blue catfish.

When the mean number of alleles per locus was adjusted with allele frequencies, the effective number of alleles per locus (A_e) showed the same trend. A_e ranged from 2.58±0.98 in MS strain to 4.39±1.10 in KR strain of channel catfish and from 1.73±0.57 in R strain to 2.42±1.44 in D strain of blue catfish (Table 11).

However, the difference in allele diversity was not shown both among strains within species and between species when the allelic richness which is the average number of alleles per locus based on the least sample size (A_r) was considered. Wherein A_r ranged from 2.96±0.98 in MS strain to 4.12±0.63 in KR strain of channel catfish and 2.75±0.79 in R strain to 3.08±0.97 for D strain of blue catfish populations (Table 11 and Figure 6).

5. Heterozygosity

The mean observed heterozygosity across loci for each population ranged from 0.45 in S1 line to 0.78 in GKal line of channel catfish and 0.35 in ARR line to 0.50 in AR2 line of blue catfish population (Table 11).

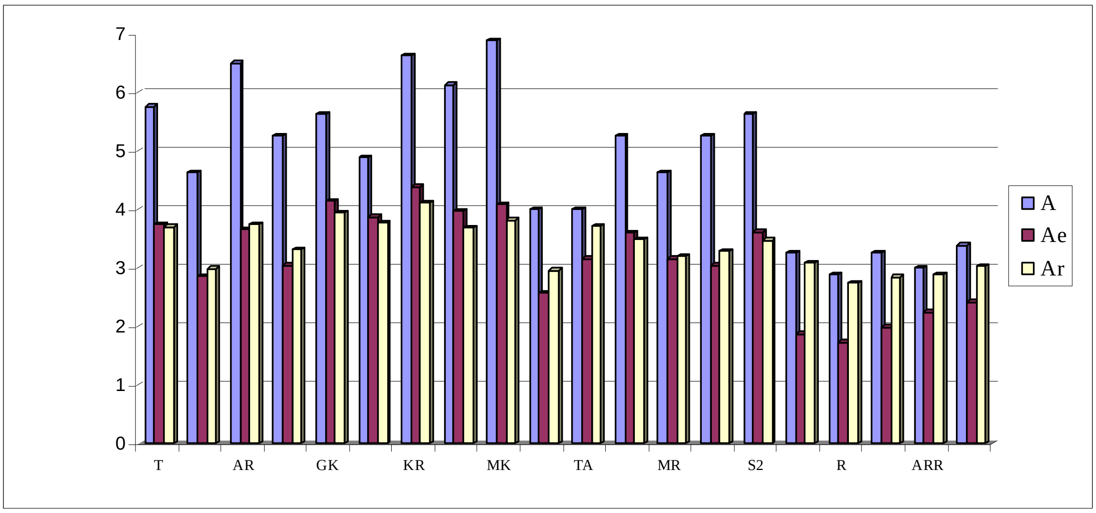


Figure 6 The allele diversity of channel and blue catfish populations (*A*=mean number of alleles per locus, *Ae*=effective number of alleles per locus, *Ar*=allelic richness)

6. Genetic variation among populations

6.1 F-coefficient

F_{ST} value bootstrapped across loci exhibited significant population differentiation within channel (0.2136, 95 % CI, 0.131-0.304) and blue catfish (0.2905, 95 % CI, 0.204-0.304).

6.2 Population differentiation

The pairwise F_{ST} showed significant differentiation between all but one pairs (AR2 and ARR in the blue catfish) of strains ($P<0.0025$ after Bonferroni correction) (Table 12). Similarly, the population differentiation test showed differences in allele frequencies between every pair of strains (Table 13). The pairwise F_{ST} between species ranged from 0.2697 (KR of channel and DxR of blue catfish) to 0.5361 (MS of channel and R of blue catfish).

Genetic distances (Cavalli-Sforza’s chord distance) among 15 channel catfish strains were between 0.0535 (GK and GKal) and 0.1963 (AS and MS) while they were between 0.0579 (D and R strains) and 0.1737 (D and DxR strains) among strains of blue catfish. The genetic distances between species were noticeably high and ranged from 0.2052 (KR of channel and R of blue catfish) to 0.2915 (MS of channel and R of blue catfish).

6.3 AMOVA (Analysis of molecular variance)

The variation between channel and blue catfish accounted for 15.05 % of the total genetic variation. The variation among population (population-level variation) within channel and blue catfish group were 20.42 % and 23.71 %, respectively. Genetic variation among individuals within population (individual-level variation) in channel (14.16 %) and blue catfish (2.13 %) was a minor component of variation (Table 14).

6.4 Phylogenetic dendrogram

A neighbor-joining dendrogram based on Cavalli-Sforza’s chord distance demonstrated clear differentiation between channel catfish and blue catfish species (Fig 1). Among channel catfish populations, it was likely that they separated into 5 main clusters 1) GK, AS, GKal; 2)S2, S1, and MR; 3) MS, TA, and ARMK; 4) T, AF, AR1 and 5) KR, MK and KS. However most of the nodes were not supported by the bootstrap values. The blue catfish strains clearly separated into 2 groups, ARR - DxR and D- R - AR2 with a bootstrap value of 99% whereas other internal nodes were not congruent.

7. Identification of individuals

Accuracy of identification of an individual blue catfish based on composite microsatellite genotypes was very accurate wherein 84.6-100.0 % of the individuals were correctly assigned to the original strains with an overall mean of 94.2% (Table 15). In the case of the 15 channel catfish strains, correctness of identification ranged from 57.1-100.0% with an overall mean of 84.3% (Table 16).

Table 9 Allele frequencies of eight microsatellite loci in channel and blue catfish strains

Locus		**Populations**																			
	allele	**T**	**AF**	**AR1**	**AS**	**GK**	**GKal**	**KR**	**KS**	**MK**	**MS**	**TA**	**ARMK**	**MR**	**S1**	**S2**	**D**	**R**	**AR2**	**ARR**	**DxR**
AU935	**121**	0.16	0.05	0.12	0.21	0.21	0.15	0.21	0.36	0.07	0.27	0.25	0.20	0.04	0.06	0.33	0.12	0.00	0.03	0.00	0.00
	124	0.14	0.00	0.18	0.04	0.04	0.00	0.14	0.08	0.30	0.27	0.00	0.04	0.21	0.39	0.04	0.04	0.00	0.03	0.00	0.00
	127	0.00	0.00	0.00	0.17	0.14	0.38	0.16	0.00	0.02	0.36	0.13	0.04	0.38	0.00	0.00	0.81	0.86	0.95	0.95	0.85
	130	0.30	0.21	0.38	0.58	0.57	0.46	0.48	0.28	0.57	0.05	0.38	0.20	0.00	0.44	0.60	0.04	0.09	0.00	0.00	0.15
	133	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.23	0.05	0.05	0.25	0.52	0.13	0.04	0.00	0.00	0.05	0.00	0.05	0.00
	136	0.41	0.74	0.32	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.04	0.00	0.00	0.00	0.00	0.00
	139	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AU936	**140**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.35	0.05	0.34	0.08	0.34
	144	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.04	0.00
	176	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	180	0.25	0.48	0.29	0.00	0.00	0.00	0.30	0.34	0.62	0.05	0.30	0.64	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	184	0.00	0.00	0.13	0.45	0.54	0.42	0.26	0.00	0.00	0.00	0.00	0.00	0.44	0.38	0.63	0.00	0.00	0.25	0.42	0.66
	188	0.43	0.33	0.52	0.00	0.00	0.00	0.24	0.59	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.58	0.82	0.34	0.00	0.00
	192	0.32	0.19	0.04	0.5	0.46	0.29	0.18	0.03	0.10	0.64	0.60	0.21	0.52	0.46	0.21	0.00	0.00	0.00	0.46	0.00
	196	0.00	0.00	0.02	0.05	0.00	0.29	0.02	0.04	0.04	0.32	0.10	0.14	0.00	0.15	0.15	0.08	0.00	0.06	0.00	0.00
AU904	**134**	0.00	0.00	0.02	0.10	0.00	0.04	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	137	0.25	0.10	0.10	0.00	0.32	0.19	0.16	0.05	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.81	0.14	0.70	0.46	0.23
	140	0.02	0.26	0.23	0.9	0.36	0.27	0.29	0.05	0.36	1.00	0.90	1.00	0.00	0.13	0.02	0.00	0.00	0.00	0.00	0.00
	143	0.68	0.64	0.65	0.00	0.32	0.50	0.39	0.91	0.64	0.00	0.00	0.00	0.00	0.02	0.04	0.04	0.00	0.03	0.04	0.00
	146	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.85	0.94	0.15	0.36	0.28	0.50	0.07
	149	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50	0.00	0.00	0.63
	152	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07

Table 9 (continued)

Locus		**Populations**																			
	allele	**T**	**AF**	**AR**	**AS**	**GK**	**GKal**	**KR**	**KS**	**MK**	**MS**	**TA**	**ARMK**	**MR**	**S1**	**S2**	**D**	**R**	**AR**	**ARR**	**DxR**
AU954	**191**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.15
	195	0.00	0.02	0.02	0.18	0.00	0.00	0.18	0.59	0.19	0.00	0.10	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.11
	197	0.09	0.52	0.29	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.20	0.46	0.07	0.00	0.08	0.08	0.05	0.00	0.05	0.11
	199	0.41	0.38	0.52	0.08	0.29	0.23	0.34	0.22	0.29	0.50	0.50	0.38	0.32	0.59	0.33	0.00	0.00	0.00	0.00	0.26
	201	0.39	0.05	0.02	0.63	0.18	0.46	0.29	0.00	0.00	0.00	0.00	0.00	0.41	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	203	0.00	0.00	0.12	0.05	0.14	0.27	0.05	0.02	0.29	0.05	0.20	0.02	0.20	0.20	0.21	0.13	0.73	0.82	0.09	0.22
	205	0.00	0.00	0.00	0.05	0.25	0.00	0.04	0.00	0.02	0.18	0.00	0.06	0.00	0.13	0.04	0.00	0.00	0.00	0.00	0.00
	207	0.00	0.00	0.00	0.00	0.04	0.00	0.07	0.14	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
	209	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.17	0.58	0.14	0.18	0.41	0.04
	211	0.05	0.02	0.02	0.00	0.11	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00
	215	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.08	0.00	0.00	0.08	0.08	0.09	0.00	0.14	0.00
	221	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	223	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	225	0.02	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
	233	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.23	0.02
AU959	**153**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.62	0.55	0.78	1.00	0.67
	161	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.38	0.14	0.20	0.00	0.00
	165	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32	0.00	0.00	0.33
	169	0.00	0.00	0.00	0.00	0.12	0.12	0.00	0.05	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	173	0.00	0.02	0.40	0.05	0.00	0.00	0.04	0.12	0.00	0.00	0.30	0.31	0.00	0.00	0.02	0.00	0.00	0.03	0.00	0.00
	177	0.23	0.00	0.02	0.07	0.12	0.00	0.15	0.17	0.02	0.05	0.00	0.00	0.23	0.07	0.13	0.00	0.00	0.00	0.00	0.00
	181	0.05	0.00	0.14	0.24	0.12	0.31	0.10	0.11	0.13	0.05	0.10	0.04	0.23	0.24	0.17	0.00	0.00	0.00	0.00	0.00
	185	0.30	0.05	0.08	0.38	0.15	0.08	0.04	0.12	0.13	0.00	0.00	0.21	0.07	0.04	0.02	0.00	0.00	0.00	0.00	0.00
	189	0.00	0.00	0.02	0.05	0.15	0.23	0.30	0.05	0.20	0.00	0.10	0.00	0.16	0.00	0.13	0.00	0.00	0.00	0.00	0.00
	193	0.00	0.43	0.10	0.05	0.19	0.15	0.26	0.33	0.22	0.23	0.20	0.00	0.25	0.20	0.08	0.00	0.00	0.00	0.00	0.00

Table 9 (continued)

Locus		**Populations**																			
	allele	**T**	**AF**	**AR**	**AS**	**GK**	**GKal**	**KR**	**KS**	**MK**	**MS**	**TA**	**ARMK**	**MR**	**S1**	**S2**	**D**	**R**	**AR**	**ARR**	**DxR**
AU959	**197**	0.00	0.26	0.15	0.17	0.12	0.08	0.07	0.06	0.24	0.41	0.10	0.27	0.00	0.19	0.13	0.00	0.00	0.00	0.00	0.00
	201	0.18	0.19	0.08	0.00	0.04	0.04	0.06	0.00	0.04	0.00	0.20	0.17	0.00	0.02	0.08	0.00	0.00	0.00	0.00	0.00
	205	0.23	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.27	0.00	0.00	0.00	0.19	0.04	0.00	0.00	0.00	0.00	0.00
	209	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.19	0.00	0.00	0.00	0.00	0.00
AU865	**148**	0.05	0.00	0.00	0.00	0.00	0.12	0.10	0.02	0.02	0.09	0.00	0.02	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00
	150	0.20	0.03	0.00	0.00	0.00	0.00	0.00	0.23	0.00	0.00	0.00	0.00	0.00	0.24	0.02	0.00	0.00	0.00	0.00	0.00
	156	0.02	0.00	0.10	0.00	0.00	0.00	0.00	0.03	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	158	0.30	0.40	0.08	0.00	0.00	0.00	0.00	0.06	0.02	0.00	0.50	0.24	0.30	0.00	0.12	0.00	0.00	0.00	0.00	0.00
	160	0.11	0.50	0.28	0.38	0.09	0.27	0.00	0.00	0.04	0.82	0.20	0.19	0.00	0.31	0.00	0.12	0.60	0.53	0.28	0.00
	164	0.25	0.03	0.30	0.05	0.27	0.12	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.62	0.30	0.10	0.44	0.12
	166	0.00	0.00	0.06	0.30	0.23	0.15	0.26	0.11	0.11	0.05	0.30	0.17	0.48	0.24	0.00	0.19	0.10	0.08	0.00	0.36
	168	0.00	0.05	0.12	0.13	0.05	0.08	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00
	170	0.00	0.00	0.02	0.05	0.09	0.00	0.08	0.02	0.02	0.00	0.00	0.00	0.18	0.00	0.29	0.00	0.00	0.00	0.00	0.00
	172	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.04	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	174	0.00	0.00	0.00	0.00	0.00	0.00	0.12	0.20	0.02	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.00
	176	0.00	0.00	0.02	0.00	0.05	0.00	0.00	0.00	0.02	0.05	0.00	0.05	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00
	178	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	180	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	182	0.00	0.00	0.00	0.00	0.09	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.00
	188	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AU1097	**146**	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.73	0.05	0.48	0.38	0.23
	152	0.16	0.00	0.02	0.00	0.04	0.23	0.02	0.15	0.32	0.55	0.10	0.38	0.13	0.00	0.14	0.00	0.00	0.00	0.00	0.00
	154	0.02	0.00	0.12	0.41	0.46	0.23	0.30	0.25	0.12	0.00	0.50	0.10	0.00	0.37	0.35	0.08	0.05	0.00	0.29	0.00
	156	0.07	0.00	0.00	0.33	0.07	0.27	0.34	0.15	0.14	0.05	0.00	0.25	0.05	0.46	0.50	0.00	0.00	0.00	0.00	0.00
	158	0.39	0.76	0.44	0.19	0.29	0.23	0.23	0.19	0.10	0.00	0.00	0.00	0.13	0.00	0.00	0.19	0.91	0.50	0.33	0.77

Table 9 (continued)

Locus		Populations																			
	allele	**T**	**AF**	**AR**	**AS**	**GK**	**GKal**	**KR**	**KS**	**MK**	**MS**	**TA**	**ARMK**	**MR**	**S1**	**S2**	**D**	**R**	**AR**	**ARR**	**DxR**
AU1097	**160**	0.09	0.24	0.25	0.02	0.07	0.00	0.05	0.00	0.04	0.18	0.30	0.08	0.48	0.09	0.00	0.00	0.00	0.00	0.00	0.00
	162	0.25	0.00	0.15	0.02	0.00	0.00	0.00	0.00	0.10	0.05	0.10	0.13	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	164	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	168	0.02	0.00	0.00	0.00	0.04	0.00	0.02	0.27	0.18	0.18	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.03	0.00	0.00
	174	0.00	0.00	0.02	0.02	0.04	0.00	0.04	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00
	180	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AU1081	**203**	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	206	0.00	0.07	0.00	0.05	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	209	0.27	0.05	0.00	0.10	0.18	0.27	0.00	0.04	0.02	0.00	0.00	0.07	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	212	0.14	0.07	0.08	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.17	0.16	0.61	0.23	0.00	0.00	0.00	0.00	0.00
	215	0.02	0.21	0.04	0.03	0.11	0.19	0.04	0.10	0.00	0.00	0.10	0.02	0.00	0.09	0.17	0.00	0.00	0.00	0.00	0.00
	218	0.00	0.00	0.08	0.08	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00
	221	0.00	0.02	0.00	0.00	0.00	0.08	0.11	0.00	0.12	0.00	0.00	0.00	0.00	0.05	0.06	0.00	0.00	0.00	0.00	0.09
	224	0.16	0.14	0.25	0.00	0.00	0.00	0.00	0.09	0.02	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00
	227	0.00	0.00	0.02	0.23	0.00	0.08	0.02	0.13	0.12	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.83	0.46
	230	0.21	0.14	0.00	0.03	0.00	0.00	0.04	0.02	0.00	0.00	0.00	0.00	0.00	0.07	0.12	0.00	0.00	0.75	0.17	0.46
	233	0.05	0.00	0.15	0.33	0.14	0.27	0.38	0.10	0.12	0.00	0.10	0.17	0.02	0.02	0.08	0.89	1.00	0.10	0.00	0.00
	236	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.12	0.02	0.25	0.10	0.09	0.21	0.07	0.00	0.12	0.00	0.00	0.00	0.00
	239	0.09	0.05	0.23	0.03	0.32	0.12	0.13	0.03	0.10	0.30	0.30	0.04	0.38	0.05	0.04	0.00	0.00	0.15	0.00	0.00
	242	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.12	0.35	0.20	0.22	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	245	0.02	0.21	0.15	0.15	0.21	0.00	0.00	0.00	0.18	0.00	0.00	0.17	0.04	0.00	0.29	0.00	0.00	0.00	0.00	0.00
	248	0.05	0.02	0.00	0.00	0.00	0.00	0.16	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	251	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.10	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	254	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 10 The test of linkage disequilibrium of channel and blue catfish populations (after Bonferroni correction; α=0.00179)

	T	AF	AR	AS	GK	Gkal	KR	KS	MK	MS	TA	ARMK	MR	S1	S2	D	R	AR	ARR	DxR
AU935 x AU936	0.7834	0.6729	0.4486	0.1796	0.2018	0.797	0.0152	0.305	0.2196	0.9108	-	0.2745	0.7858	0.4003	0.2874	0.8193	0.6233	0.535	0.3082	0.627
AU935 x AU904	0.9979	0.7982	0.4347	0.3622	0.2602	0.2073	0.7143	0.4215	0.2265	-	-	-	-	0.6488	0.0188	0.229	0.7433	1	0.6051	0.734
AU935 x AU954	0.2044	0.0769	0.1511	0.4839	0.8444	1	0.0743	0.0307	0.0098	0.1583	-	0.3476	0.0599	0.0835	0.1974	0.6012	0.1336	1	-	0.9257
AU935 x AU959	0.1638	0.0025	0.0814	0.7937	0.094	1	0.9454	0.3301	0.8786	0.7537	-	0.2687	0.0315	0.0647	0.1164	0.7063	0.4457	0.1903	-	1
AU935 x AU865	0.6334	0.3007	0.0947	0.3254	0.2933	1	0.0321	0.6196	0.419	0.7801	-	0.3667	0.7356	0.1016	0.8659	0.1522	1	0.8143	1	0.3293
AU935 x AU1097	0.1556	0.1272	0.0649	0.0913	0.3068	0.8964	0.5671	0.0831	0.0172	0.3972	-	0.9843	0.0999	0.2929	0.7949	0.1395	0.1755	0.1023	1	0.3939
AU935 x AU1081	0.3956	0.0915	0.0924	0.4437	0.0378	1	0.0132	0.1922	0.1594	0.6295	-	0.232	0.0107	0.9334	0.5433	0.6501	-	0.4543	1	0.8171
AU936 x AU904	0.3669	0.4586	0.0018	0.1121	0.6364	0.2898	0.035	0.7057	0.0514	-	1	-	-	0.3397	0.3463	0.7202	0.4114	0.0032	0.3047	0.2304
AU936 x AU954	0.236	0.4584	0.0253	0.0443	0.888	0.1289	<0.0001*	0.2313	0.4706	1	1	0.8882	0.2777	0.7363	0.995	0.387	0.4197	0.8137	0.3625	0.0011*
AU936 x AU959	0.3041	0.9986	0.4362	0.2508	0.7957	1	0.2019	0.2452	0.8597	0.4913	1	0.9579	0.0748	0.0958	0.8117	0.6183	0.1526	0.9445	-	0.0085
AU936 x AU865	0.2546	0.6083	0.2038	0.3989	1	0.5276	<0.0001*	0.1772	0.7282	0.7349	0.6935	0.0446	0.2249	0.439	0.4739	0.4518	1	0.5732	0.9141	0.1526
AU936 x AU1097	0.4603	0.213	0.0796	0.1279	0.6213	0.3416	0.3522	0.922	0.7047	0.7763	1	0.2216	0.2791	0.4108	0.9692	0.781	0.3637	0.3085	0.8745	0.2312
AU936 x AU1081	0.4186	0.231	0.9001	0.5765	0.426	0.3366	0.0219	0.0408	0.2714	1	-	1	0.1537	1	0.4009	0.1909	-	0.0183	1	0.4125
AU904 x AU954	0.8964	0.9381	0.1964	0.8682	0.1062	0.4189	0.0083	0.421	0.3078	-	1	-	-	0.4958	0.1217	0.4108	0.9464	0.5941	0.2314	0.6441
AU904 x AU959	0.835	0.8474	0.4414	0.2283	0.087	0.7929	0.9917	0.0104	0.2527	-	0.6004	-	-	0.676	0.5925	1	0.283	1	-	0.9836
AU904 x AU865	0.789	0.5038	0.2725	0.0143	0.3331	0.1588	0.2159	0.3803	0.027	-	1	-	-	0.5539	0.5658	0.5999	0.2228	0.3287	0.628	0.0157
AU904 x AU1097	0.7148	0.2181	0.8386	0.0365	0.5952	0.3497	0.5809	0.2759	0.7924	-	0.6029	-	-	0.3709	0.225	0.0026	1	1	0.76	0.3056
AU904 x AU1081	0.804	0.0637	0.7542	0.0863	0.2954	0.0308	0.5136	0.3563	0.3372	-	-	-	-	0.5764	0.359	0.4278	-	0.0271	0.5466	0.9795
AU954 x AU959	<0.0001*	0.0091	0.2852	0.1734	0.2745	1	1	0.0077	1	0.389	1	0.0184	0.9651	0.001*	0.1802	0.4113	1	0.1487	-	0.1248
AU954 x AU865	0.0521	0.2242	0.1673	0.8198	1	0.0839	0.0919	0.0642	0.805	0.2251	0.6018	0.8885	0.1467	0.5695	0.0026	0.8813	0.9099	0.7052	-	0.0396
AU954 x AU1097	0.0926	0.4283	0.8772	0.2307	0.4759	0.9625	0.3432	0.0204	0.6281	0.5751	1	0.3809	0.5676	0.0341	0.126	0.424	0.1927	1	0.7423	0.2969
AU954 x AU1081	0.2246	0.0134	0.4647	0.8584	1	0.3767	0.3096	0.8515	0.2529	0.0116	-	0.3565	0.0885	0.6203	0.4706	0.16	-	0.7638	0.9009	0.2271
AU959 x AU865	0.817	0.1552	0.7185	0.0321	1	1	0.032	0.9571	0.755	0.3162	1	0.8847	0.4778	0.0645	0.418	0.6166	0.4013	0.8625	-	0.5958
AU959 x AU1097	0.362	0.0899	0.9147	0.3308	0.7328	1	0.0252	0.2999	1	0.6762	1	0.7938	0.3254	0.8708	0.9251	1	0.6389	0.0508	-	1
AU959 x AU1081	0.3184	0.6607	0.1078	0.62	0.2576	0.3199	0.925	0.3313	1	1	-	0.1043	0.1265	0.5299	0.3644	1	-	0.1468	-	0.0799
AU865 x AU1097	0.1007	1	0.3792	0.1462	1	1	0.4969	0.0326	0.2717	0.0345	1	0.8338	0.2959	0.9862	0.2916	0.058	1	1	0.0595	0.2437
AU865 x AU1081	0.2243	0.8374	0.2801	1	1	1	0.4952	0.9376	0.8431	0.9336	-	1	0.8393	0.7946	0.5684	0.6268	-	0.324	0.4177	0.0327
AU1097 x AU1081	0.7085	0.2957	0.4148	0.3153	0.7612	0.1597	0.2244	0.4726	0.0135	1	-	0.3629	0.23	0.013	0.1547	0.4394	-	0.253	0.3092	0.8212

Table 11 Genetic variation, mean number of alleles per locus (A), effective number of allele (A_e), allelic richness (A_r), mean expected and observed heterozygosity across loci (H_e, H_o), and fixation index (F_{is}).

Population	A	A_e	A_r	H_e	H_o	F_{is}
T	5.75±1.98	3.75±1.22	3.70±0.81	0.720±0.11	0.642±0.16	0.111
AF	4.63±2.56	2.86±1.67	2.99±1.06	0.592±0.16	0.495±0.28	0.168
AR1	6.50±2.07	3.65±1.24	3.74±0.79	0.710±0.11	0.451±0.23	0.370
AS	5.25±2.25	3.04±1.24	3.31±1.01	0.623±0.20	0.528±0.26	0.158
GK	5.63±2.20	4.14±1.77	3.94±1.18	0.743±0.13	0.634±0.24	0.151
GKal	4.88±1.64	3.87±1.15	3.77±0.86	0.748±0.08	0.775±0.23	-0.025
KR	6.63±2.07	4.39±1.10	4.12±0.63	0.774±0.06	0.542±0.20	0.304
KS	6.13±2.53	3.97±2.01	3.69±1.25	0.670±0.23	0.467±0.27	0.306
MK	6.88±3.23	4.09±2.44	3.81±1.26	0.691±0.17	0.497±0.27	0.284
MS	4.00±1.41	2.58±0.98	2.96±0.98	0.557±0.27	0.564±0.31	-0.012
TA	4.00±1.41	3.16±1.32	3.71±1.23	0.692±0.22	0.675±0.28	0.026
ARMK	5.25±2.55	3.60±1.79	3.49±1.30	0.637±0.28	0.534±0.31	0.165
MR	4.63±2.13	3.16±1.22	3.20±1.10	0.618±0.26	0.642±0.33	-0.041
S1	5.25±2.19	3.04±1.30	3.29±0.86	0.628±0.17	0.449±0.22	0.289
S2	5.63±2.56	3.62±2.08	3.47±1.33	0.633±0.25	0.490±0.26	0.229
mean	**11.38±4.84**	**6.66±2.77**	**3.55**	**0.83±0.07**	**0.54±0.17**	**0.14**
D	3.25±1.04	1.87±0.46	3.08±0.97	0.452±0.15	0.360±0.26	0.211
R	2.88±0.84	1.73±0.57	2.75±0.79	0.373±0.23	0.378±0.30	0.015
AR2	3.25±0.89	1.99±0.77	2.84±0.97	0.447±0.20	0.504±0.39	-0.132
ARR	3.00±1.51	2.24±1.02	2.89±1.43	0.468±0.30	0.354±0.33	0.253
DxR	3.38±2.39	2.42±1.44	3.03±1.66	0.516±0.18	0.409±0.26	0.211
mean	**5.63±1.92**	**2.99±1.14**	**2.92**	**0.61±0.20**	**0.41±0.23**	**0.09**

Table 12 Genetic distance (Cavalli-Sforza's chord distance) (upper diagonal) and pairwise F_{ST} (Weir and Cockerham, 1984) (below diagonal) among strains of channel and blue catfish based on 8 microsatellite loci

	Population																			
	T	**AF**	**AR**	**AS**	**GK**	**GKal**	**KR**	**KS**	**MK**	**MS**	**TA**	**ARMK**	**MR**	**S1**	**S2**	**D**	**R**	**AR**	**ARR**	**DxR**
T		0.0952	0.1016	0.1445	0.1378	0.1606	0.1344	0.1411	0.1404	0.1893	0.1871	0.1451	0.1555	0.1418	0.1490	0.2607	0.2430	0.2424	0.2749	0.2480
AF	0.1196*		0.0785	0.1805	0.1548	0.1861	0.1499	0.1515	0.1414	0.1911	0.1573	0.1537	0.1943	0.1594	0.1576	0.2806	0.2619	0.2531	0.2778	0.2410
AR	0.0890*	0.0720*		0.1211	0.1132	0.1479	0.1058	0.1105	0.1040	0.1814	0.1090	0.1071	0.1549	0.1334	0.1219	0.2277	0.2129	0.2274	0.2719	0.2441
AS	0.2436*	0.3049*	0.2570*		0.0572	0.0653	0.0805	0.1308	0.1118	0.1963	0.1545	0.1370	0.1403	0.1252	0.1040	0.2352	0.2269	0.2377	0.2075	0.2125
GK	0.1398*	0.1892*	0.1438*	0.1100*		0.0535	0.0763	0.1367	0.1083	0.1608	0.1277	0.1512	0.0998	0.1171	0.0854	0.2495	0.2345	0.2365	0.2436	0.2388
GKal	0.1161*	0.1957*	0.1471*	0.1161*	0.0404*		0.0781	0.1415	0.1171	0.1768	0.1312	0.1588	0.1246	0.1339	0.1046	0.2278	0.2135	0.2258	0.2232	0.2139
KR	0.1075*	0.1467*	0.1032*	0.1257*	0.0645*	0.0466*		0.0889	0.0791	0.1597	0.1086	0.1477	0.1091	0.1145	0.1050	0.2079	0.2052	0.2177	0.2453	0.2174
KS	0.1390*	0.1755*	0.1262*	0.3076*	0.2160*	0.1867*	0.1194*		0.0762	0.1534	0.1184	0.1166	0.1555	0.1258	0.1499	0.2386	0.2379	0.2557	0.2639	0.2487
MK	0.1233*	0.1335*	0.1003*	0.2210*	0.1451*	0.1146*	0.0770*	0.0871*		0.1172	0.1021	0.1036	0.1540	0.1319	0.1256	0.2412	0.2284	0.2534	0.2612	0.2409
MS	0.2751*	0.2939*	0.2656*	0.2366*	0.2023*	0.2230*	0.2176*	0.3315*	0.2015*		0.0984	0.1289	0.1305	0.1305	0.1752	0.2663	0.2915	0.2648	0.2755	0.2795
TA	0.2185*	0.2217*	0.1645*	0.1569*	0.1023*	0.1518*	0.1195*	0.2689*	0.1399*	0.0780*		0.0841	0.1434	0.1453	0.1429	0.2479	0.2524	0.2596	0.2642	0.2725
ARMK	0.2589*	0.2411*	0.2068*	0.2021*	0.2263*	0.2316*	0.1910*	0.3021*	0.1551*	0.1366*	0.0798*		0.1696	0.1475	0.1288	0.2485	0.2582	0.2832	0.2669	0.2783
MR	0.2616*	0.3216*	0.2777*	0.2964*	0.2108*	0.2203*	0.2175*	0.3415*	0.2844*	0.3209*	0.2723*	0.3546*		0.1336	0.1544	0.2614	0.2604	0.2375	0.2480	0.2475
S1	0.2662*	0.3200*	0.2678*	0.2703*	0.1909*	0.2076*	0.1933*	0.3080*	0.2545*	0.2922*	0.2409*	0.3041*	0.1340*		0.0591	0.2749	0.2842	0.2569	0.2611	0.2492
S2	0.2783*	0.3217*	0.2713*	0.2623*	0.1813*	0.1868*	0.1923*	0.3139*	0.2521*	0.3297*	0.2702*	0.3079*	0.1468*	0.0534*		0.2549	0.2639	0.2404	0.2479	0.2378
D	0.3429*	0.4091*	0.3388*	0.4345*	0.3510*	0.3444*	0.2892*	0.3817*	0.3605*	0.4971*	0.4470*	0.4486*	0.4466*	0.4517*	0.4077*		0.0579	0.0867	0.1454	0.1737
R	0.3514*	0.3863*	0.3217*	0.4501*	0.3800*	0.3601*	0.3056*	0.3905*	0.3610*	0.5361*	0.4906*	0.4804*	0.4393*	0.4583*	0.4145*	0.3359*		0.1000	0.1644	0.1469
AR	0.3331*	0.3863*	0.3427*	0.4417*	0.3326*	0.3377*	0.3178*	0.4017*	0.3551*	0.4943*	0.4472*	0.4652*	0.4056*	0.4131*	0.3713*	0.2824*	0.3599*		0.1119	0.0942
ARR	0.3368*	0.3902*	0.3539*	0.3664*	0.2813*	0.3060*	0.2978*	0.3900*	0.3473*	0.4513*	0.3913*	0.4324*	0.3495*	0.3489*	0.3066*	0.3469*	0.4583*	0.2893*		0.0689
DxR	0.3059*	0.3236*	0.3003*	0.3682*	0.2742*	0.2896*	0.2697*	0.3675*	0.3301*	0.4515*	0.4098*	0.4171*	0.3727*	0.3762*	0.3359*	0.3929*	0.3469*	0.2494*	0.2404*	

* Highly significant

Table 13 The probability values for the population differentiation tests between strains of channel and blue catfish (α=0.000435 after Bonferroni correction)

Population	**Loci**							
	AU935	**AU936**	**AU904**	**AU954**	**AU959**	**AU865**	**AU1097**	**AU1081**
T X AF	0.03	0.1398	0.0027	<0.0001	<0.0001	0.0001	<0.0001	0.0041
T x AR	0.7980	0.0015	0.0079	0.0007	<0.0001	0.0003	<0.0001	<0.0001
T x AS	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T x GK	0.0005	<0.0001	0.001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
T x Gkal	<0.0001	<0.0001	0.0056	0.001	<0.0001	<0.0001	<0.0001	0.0002
T x KR	<0.0001	0.0062	0.0001	0.0028	<0.0001	<0.0001	<0.0001	<0.0001
T x KS	<0.0001	0.0007	0.0023	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T x MK	<0.0001	0.0006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T x MS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001
T x TA	0.0047	0.0152	0.0001	0.0085	<0.0001	0.0105	0.0018	0.0012
T x ARMK	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T x MR	<0.0001	<0.0001	<0.0001	0.0072	<0.0001	<0.0001	<0.0001	<0.0001
T x S1	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T x S2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.00016	<0.0001	<0.0001
AF x AR	0.0016	0.0046	0.9775	0.1929	<0.0001	0.0017	0.0002	<0.0001
AF x AS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AF x GK	<0.0001	<0.0001	0.0299	<0.0001	0.0002	<0.0001	<0.0001	0.0002
AF x Gkal	<0.0001	<0.0001	0.3338	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AF x KR	<0.0001	0.0092	0.0064	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AF x KS	<0.0001	0.0060	0.0068	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AF x MK	<0.0001	0.1901	0.1508	<0.0001	0.0009	<0.0001	<0.0001	<0.0001
AF x MS	<0.0001	<0.0001	<0.0001	<0.0001	0.0036	0.0039	<0.0001	<0.0001
AF x TA	0.0007	0.0054	0.0004	0.0169	0.0297	0.0605	<0.0001	0.0009
AF x ARMK	<0.0001	0.0002	<0.0001	0.0226	<0.0001	0.0002	<0.0001	<0.0001
AF x MR	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AF x S1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AF x S2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0083
AR x AS	0.0008	<0.0001	<0.0001	<0.0001	<0.0001	0.0052	<0.0001	<0.0001
AR x GK	0.0005	<0.0001	0.0257	0.0002	0.0004	0.0828	0.0007	0.0042
AR x Gkal	<0.0001	<0.0001	0.6210	<0.0001	0.00003	0.0147	<0.0001	<0.0001
AR x KR	<0.0001	0.0736	0.0205	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AR x KS	<0.0001	0.0814	0.0086	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AR x MK	0.0004	0.0011	0.0662	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AR x MS	<0.0001	<0.0001	<0.0001	0.0384	<0.0001	0.0023	<0.0001	<0.0001
AR x TA	0.0005	0.0001	0.0027	0.8710	0.8061	0.0577	0.0210	0.0018
AR x ARMK	<0.0001	<0.0001	<0.0001	0.0352	0.0128	0.0001	<0.0001	<0.0001
AR x MR	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AR x S1	0.0073	<0.0001	<0.0001	0.0011	<0.0001	<0.0001	<0.0001	<0.0001
AR x S2	0.0003	<0.0001	<0.0001	0.0011	<0.0001	<0.0001	<0.0001	<0.0001
AS x GK	1	0.5442	<0.0001	0.0003	0.0187	0.0705	0.0846	0.0042
AS x Gkal	0.4323	0.0629	<0.0001	0.0026	0.0011	0.1205	0.0027	0.0121

Table 13 (continued)

Population	**Loci**							
	AU935	**AU936**	**AU904**	**AU954**	**AU959**	**AU865**	**AU1097**	**AU1081**
AS x KR	0.7165	<0.0001	<0.0001	0.0417	<0.0001	0.0004	0.8822	<0.0001
AS x KS	0.0007	<0.0001	<0.0001	<0.0001	0.0006	<0.0001	<0.0001	<0.0001
AS x MK	0.0273	<0.0001	<0.0001	<0.0001	0.0064	<0.0001	<0.0001	0.0016
AS x MS	0.001	<0.0001	0.2909	<0.0001	<0.0001	0.0010	<0.0001	<0.0001
AS x TA	0.2966	0.0007	0.2030	0.0007	0.0025	0.0072	0.0038	0.0006
AS x ARMK	<0.0001	<0.0001	0.0840	<0.0001	<0.0001	0.0014	<0.0001	0.0002
AS x MR	<0.0001	0.2101	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
AS x S1	<0.0001	0.3535	<0.0001	<0.0001	<0.0001	0.0002	0.0014	<0.0001
AS x S2	0.0377	0.0324	<0.0001	<0.0001	<0.0001	<0.0001	0.0011	<0.0001
GK x Gkal	0.3389	0.0156	0.2471	0.0416	0.5029	0.2405	0.0088	0.0177
GK x KR	0.5011	0.0002	0.0246	0.0793	0.1914	0.0342	0.3475	<0.0001
GK x KS	0.0004	<0.0001	<0.0001	<0.0001	0.2013	<0.0001	0.0036	<0.0001
GK x MK	0.0188	<0.0001	0.0015	0.0007	0.3622	0.0003	<0.0001	<0.0001
GK x MS	0.0015	<0.0001	0.0002	0.0351	0.0011	0.0006	<0.0001	<0.0001
GK x TA	0.3120	0.0007	0.0255	0.0729	0.0958	0.0145	0.0927	0.0101
GK x ARMK	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0039	<0.0001	0.0004
GK x MR	<0.0001	0.3687	<0.0001	0.0009	0.0974	<0.0001	<0.0001	<0.0001
GK x S1	<0.0001	0.0472	<0.0001	0.0070	0.0016	0.0006	0.0003	<0.0001
GK x S2	0.0351	0.0163	<0.0001	0.0016	0.0175	<0.0001	<0.0001	<0.0001
Gkal x KR	0.1112	0.0001	0.2932	0.0327	0.0197	0.0085	0.0376	0.0006
Gkal x KS	<0.0001	<0.0001	<0.0001	<0.0001	0.0026	<0.0001	0.0122	<0.0001
Gkal x MK	0.0006	<0.0001	0.0103	<0.0001	0.1868	0.0003	0.0038	<0.0001
Gkal x MS	0.0032	0.0023	<0.0001	<0.0001	<0.0001	0.0006	<0.0001	<0.0001
Gkal x TA	0.1530	0.0026	0.0006	0.004	0.0667	0.0036	0.0029	0.0087
Gkal x ARMK	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0182	0.0003	0.0001
Gkal x MR	<0.0001	0.0003	<0.0001	0.3017	0.0100	<0.0001	<0.0001	<0.0001
Gkal x S1	<0.0001	0.3330	<0.0001	<0.0001	0.0004	0.0002	<0.0001	<0.0001
Gkal x S2	0.0001	0.2784	<0.0001	<0.0001	0.0029	<0.0001	0.0021	<0.0001
KR x KS	<0.0002	<0.0001	<0.0001	<0.0001	0.0063	<0.0001	<0.0001	<0.0001
KR x MK	0.0255	0.0018	<0.0001	0.0011	0.0709	0.0010	<0.0001	<0.0001
KR x MS	0.0057	<0.0001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	<0.0001
KR x TA	0.0923	0.0434	0.0014	0.0973	0.1678	0.0009	0.0091	0.0093
KR x ARMK	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
KR x MR	<0.0001	<0.0001	<0.0001	0.0003	0.0067	<0.0001	<0.0001	<0.0001
KR x S1	0.0003	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.0053	<0.0001
KR x S2	0.0068	<0.0001	<0.0001	<0.0001	0.0014	<0.0001	0.0008	<0.0001
KS x MK	<0.0001	0.0046	0.0010	<0.0001	0.0005	0.001	0.0051	<0.0001
KS x MS	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001
KS x TA	0.3017	<0.0001	<0.0001	0.0087	0.0482	0.0033	0.0011	0.0275
KS x ARMK	0.0096	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0052
KS x MR	<0.0001	<0.0001	<0.0001	0.0002	0.0055	<0.0001	<0.0001	<0.0001
KS x S1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
KS x S2	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MK x MS	<0.0001	<0.0001	0.0004	0.0002	0.0005	<0.0001	0.0489	0.0015
MK x TA	0.0521	0.0008	0.0057	0.2012	0.0713	0.0025	0.0117	0.2789
MK x ARMK	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.0118	0.0023	0.0016

Table 13 (continued)

Population	Loci							
	AU935	**AU936**	**AU904**	**AU954**	**AU959**	**AU865**	**AU1097**	**AU1081**
MK x MR	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MK x S1	0.2635	<0.0001	<0.0001	0.0040	0.0009	<0.0001	<0.0001	<0.0001
MK x S2	<0.0001	<0.0001	<0.0001	<0.0001	0.0031	<0.0001	<0.0001	<0.0001
MS x TA	0.0082	0.0606	0.3118	0.2195	0.0061	0.0025	0.0072	0.5140
MS x ARMK	<0.0001	<0.0001	-	0.0136	<0.0001	0.0012	0.0025	0.0002
MS x MR	0.0007	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.0009
MS x S1	<0.0001	0.0019	<0.0001	0.0224	0.1294	<0.0001	<0.0001	<0.0001
MS x S2	<0.0001	<0.0001	<0.0001	0.0057	0.0002	<0.0001	<0.0001	<0.0001
TA x ARMK	0.4587	0.1265	0.1724	0.0582	0.0144	0.6265	0.0142	0.0294
TA x MR	0.0004	<0.0001	<0.0001	0.0446	0.0003	0.0342	0.0002	0.0051
TA x S1	0.0014	0.0019	<0.0001	0.1787	0.0016	0.0056	0.0041	0.0005
TA x S2	0.0075	0.0001	<0.0001	0.2780	0.0751	0.0003	0.0032	<0.0001
ARMK x MR	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ARMK x S1	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
ARMK x S2	<0.0001	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MR x S1	<0.0001	0.0028	0.0014	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
MR x S2	<0.0001	<0.0001	0.1014	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S1 x S2	<0.0001	0.0367	0.0927	0.0009	0.0013	<0.0001	0.0042	0.0005
D x R	0.2952	0.0011	<0.0001	0.0035	0.0057	0.02864	0.0002	0.4828
D x AR	0.3412	0.0231	0.6678	<0.0001	0.1510	0.0003	<0.0001	<0.0001
D x ARR	0.2050	<0.0001	0.0597	0.6881	0.0001	0.1800	0.1147	<0.0001
D x (DxR)	0.0056	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R x AR	0.1235	0.0004	<0.0001	0.1948	0.0132	0.0089	<0.0001	<0.0001
R x ARR	0.7385	<0.0001	<0.0001	0.0025	0.0013	0.0076	0.0061	<0.0001
R x (DxR)	0.3191	<0.0001	0.0151	0.0001	0.0614	<0.0001	0.0075	<0.0001
AR2 x ARR	0.7133	<0.0001	0.2357	<0.0001	0.0551	0.0062	0.0132	<0.0001
AR2 x (DxR)	0.0019	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	<0.0001
ARR x (DxR)	0.0218	<0.0001	<0.0001	<0.0001	0.0156	0.0002	<0.0001	0.0689

Table 14 Hierarchical analysis of molecular variance (AMOVA) for channel catfish and blue catfish

Source of variation	**Variance component**	**Percentage variation**	**Fixation indices**
Among groups (channel and blue)	0.40840	15.05	F_{CT} = 0.15052
Among populations within channel or blue	0.52484	19.34	F_{SC} = 0.22772
Among individuals within populations	0.21548	7.94	
Among population within channel	0.57643	20.42	F_{ST} = 0.20425
Among individuals within channel populations	0.39966	14.16	
Among populations within blue	0.20262	23.71	F_{ST} = 0.23710
Among individuals within blue populations	0.01823	2.13	

Table 15 Accuracy of identification of individual blue catfish based on composite microsatellite genotypes utilizing the geneclass v. 2.0 program

Population	**D**	**R**	**AR**	**ARR**	**DxR**	**Sample size**	**No.correctly classified**	**%correctly classified**
D	11	2	-	-	-	13	11	84.6
R	-	11	-	-	-	11	11	100
AR	-	1	19	-	-	20	19	95
ARR	-	-	-	12	-	12	12	100
DxR	-	-	-	2	28	30	28	93.3
Total	**-**	**-**	**-**	**-**	**-**	**86**	**81**	**94.2**

Table 16 Accuracy of identification of individual channel catfish based on composite microsatellite genotypes utilizing the geneclass v. 2.0 program (values in parentheses are the adjusted values including individuals incorrectly assigned to the expected ancestral groups)

Pop	**T**	**AF**	**AR**	**AS**	**GK**	**GKal**	**KR**	**KS**	**MK**	**MS**	**TA**	**ARMK**	**MR**	**S1**	**S2**	**N**	**C**	**%C**
T	20	-	2	-	-	-	-	-	-	-	-	-	-	-	-	22	20	90.9
AF	3	17	1	-	-	-	-	-	-	-	-	-	-	-	-	21	17	80.95
AR	2	1	18	1	4	-	-	-	-	-	-	-	-	-	-	26	18	69.2
AS	-	-	-	20	-	1	-	-	-	-	-	-	-	-	-	21	20	95.2
GK	-	-	-	2	8	3	-	-	-	-	1	-	-	-	-	14	8	57.1(78.6)
GKal	-	-	-	1	4	8	-	-	-	-	-	-	-	-	-	13	8	61.54(92.3)
KR	-	-	-	1	4	3	16	2	-	-	1	1	-	-	-	28	16	57.1(64.3)
KS	-	-	-	-	-	-	-	31	1	2	-	-	-	-	-	34	31	91.17
MK	-	-	-	-	-	-	1	4	18	1	-	-	-	-	-	25	18	72(100)
MS	-	-	-	-	-	-	-	-	-	11	-	-	-	-	-	11	11	100
TA	-	-	-	-	-	-	-	-	-	-	4	1	-	-	-	5	4	80(100)
ARMK	-	-	-	-	-	-	-	-	-	-	2	22	-	-	-	24	22	91.7(100)
MR	-	-	-	-	-	-	-	-	-	-	-	-	28	-	-	28	28	100
S1	-	-	-	-	-	-	-	-	-	-	-	-	-	27	-	27	27	100
S2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26	26	26	100
Total																**325**	**274**	**84.3**

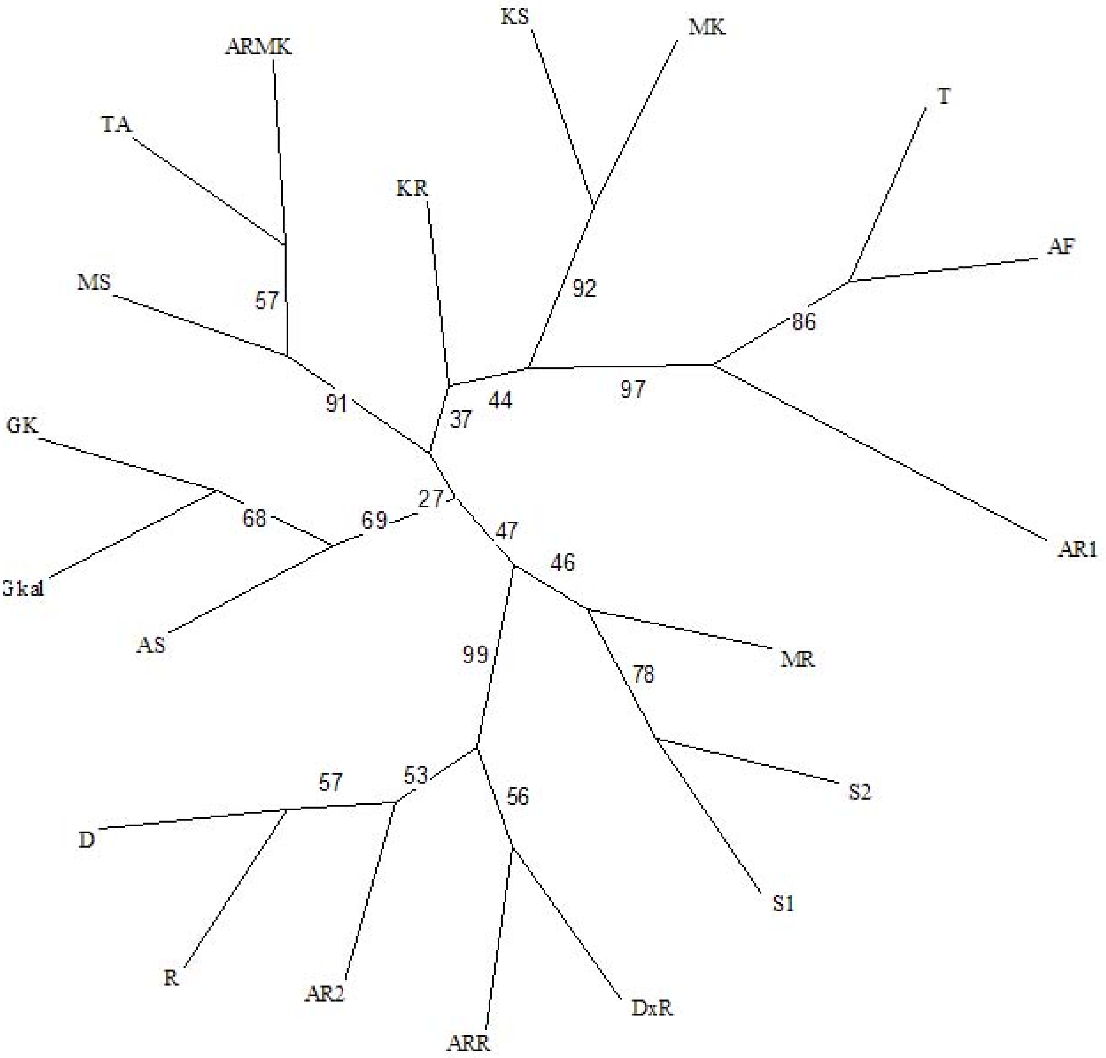


Figure 7 The neigbour-joining dendrogram based on Cavalli-Sforza’s chord distance among channel and blue populations

Characterization and expression analysis of GnRH genes of *I. punctatus* and *I. furcatus*

1. Total RNA

Total RNA from a brain of *I. punctatus* and *I. furcatus* revealed predominant discrete bands of 28S and 18S rRNA along with smeared high molecular weight RNA (Figure 8). The ratios of the extracted RNA were 1.7 - 2.0. The first strand cDNA synthesized from those total RNA covered the large products indicating the acceptable quality of the synthesized first strand cDNA.

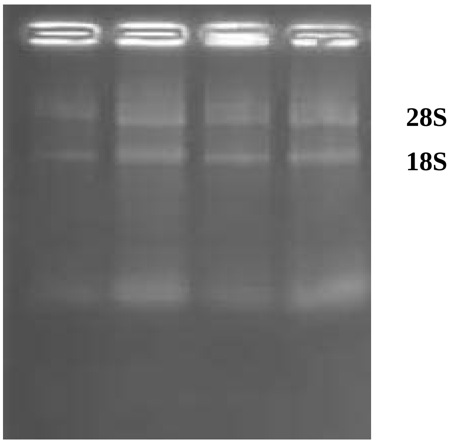


Figure 8 A 1.0% ethidium bromide-stained agarose gel showing the quality of total RNA extracted from brain of *I. punctatus* (Lane 1 - 4 = total RNA individually extracted from ovaries of each individual of channel catfish)

2. Identification of the full length cDNA of a catfish type *GnRH* (*caGnRH*) of *I. punctatus* by RACE-PCR

RACE-PCR was carried out for both directions. A 258 bp amplification product (Figure 9) was obtained using the 2 forward primer (GSP) and the Abridged universal amplification primer. The amplification fragment was cloned and sequenced. Similarity analysis using Blast*X* indicated that *GnRH-SEQ1* siginificantly matched the catfish gonadotropin-releasing hormone and the *catfish gonadotropin-releasing hormone* (*caGnRH*) of *Clarias gariepinus* (E-value = 2e-21).

```
CAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCA
GGAGACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTACGTAGATGTTTCAC
CTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGACCAGTGTTGCTGAGCGAGAAATTGGA
CAGTAAGACAAATAACAAAACTGATCAAGAGCTTCAATAAAATGCTTTGCCTTTAAAAAAAA
AAAAAAAAAA
```

Figure 9 Nucleotide sequence of *GnRH-SEQ1* (258 bp) amplified from cDNA of *I. punctatus*

However, 5' RACE-PCR was unsuccessful. The amplification conditions were further adjusted but no positive amplification product was obtained. Accordingly, the 5'end of *caGnRH* of *I. punctatus* was amplified with RLM-RACE method.

3. Identification of the full length cDNA of a catfish type GnRH (caGnRH) of *I. punctatus* using Generacer

3.1 3' RACE-PCR and nested RACE-PCR

PCR was carried out at the annealing temperature of 64^0C using primers caGnRH-F1/R1. A 294 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. A pair of primers was designed. Nested RACE-PCR was carried out using caGnRH-F2/R2 as primers and diluted primary 3'RACE-PCR as the template. A 281 bp fragment (including Generacer oligo dT primer sequence) was obtained (Figure 10). The amplification fragment was cloned and sequenced. Similarity analysis using Blast*X* indicated that *GnRH-SEQ3* (Figure 11) significantly matched the catfish gonadotropin-releasing hormone and the *catfish type gonadotropin-releasing hormone* (*caGnRH*) of *Clarias gariepinus* (E-value = 2e-21).

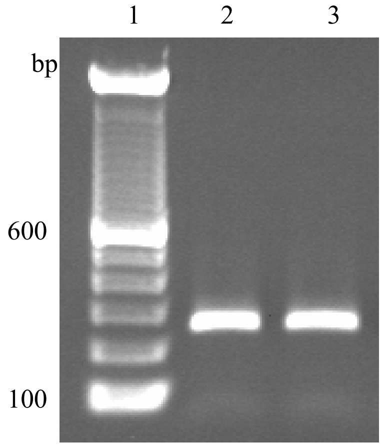


Figure 10 Agarose gel electrophoresis illustrating the product from 3’ (lane 2) and 5’RACE-PCR (lane 3) of *caGnRH* of *I. punctatus* (Lane 1 is a 100 bp Ladder)

A.

CAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCA
GGAGACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTACGTAGATGTTTCAC
CTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGACCAGTGTTGCTGAGCGAGAAATTGGA
CAGTAAGACAAATAACAAAACTGATCAAGAGCTTCAATAAAATGCTTTGCCTTTAAAAAAAA
AAAAAAAAAACACTGTCATGC***CGTTACGTAGCGTATCGTTGACAGC***

B.

CAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAG
GAGACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTACGTAGATGTTTCACCT
CGGAATAAACTCTACAGGCTGAAAGATCTGCTGACCAGTGTTGCTGAGCGAGAAATTGGACAG
TAAGACAAATAACAAAACTGATCAAGAGCTTCAATAAAATGCTTTGCCTTTAAAAAAAAAAA
AAAAAA***CACTGTCATGCCGTTACGTAGCG***

Figure 11 Nucleotide sequence of the 3’ portion *caGnRH-SEQ2* (A) and *caGnRH-SEQ3* (B) amplified from cDNA of *I. punctatus*
(The location and sequence of a forward primer (caGnRH-F1 and caGnRH-F2) and those complementary to a reverse primer (caGnRH-R1 and caGnRH-R2) are illustrated in boldface and italicized)

3.2 5’ RACE-PCR and nested RACE-PCR

A 5’ RACE-PCR was carried out using primers caGnRH-F3/R3. A 301 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. Similarity analysis using Blast*X* indicated that *GnRH-SEQ4* (Figure 12) significantly matched the catfish gonadotropin-releasing hormone and the *catfish type gonadotropin-releasing hormone* (*caGnRH*) of *C. gariepinus* (E-value = 9e-27).

A pair of primers was designed. Nested RACE-PCR was carried out using caGnRH-F4/R4 as primers and diluted primary 3'RACE-PCR as template. A 203 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. Similarity analysis using Blast*X* indicated that *GnRH-SEQ5* (Figure 12) significantly matched the catfish gonadotropin-releasing hormone and the *catfish type gonadotropin-releasing hormone* (*caGnRH*) of *C. gariepinus* (E-value = 7e-14).

A.

CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAAACAGACTGAGAGACCACA
TTGTGAAGAGCAGAAGAACTGTCTGCAGGGCGACTGATCAAGGATGGGTATAAAGCGAGCAC
TCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGCAGGTGAGCTCTCAGCACTGGTCTCATGGC
CTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGGAGACTGTTGAAGAAATGCCGAGGACCTC
CGGCTACGTGTGTGATTACGTAGATGTTT***CACCTCGGAATAAACTCTACAGGC***
B.
GGACACUGACAUGGACUGAAGGAGUAGAAAACAGACTGAGAGACCACATTGTGAAGAGCAGA
AGAACTGTCTGCAGGGCGACTGATCAAGGATGGGTATAAAGCGAGCACTCTGGTGGATGGTG
GTGTGTGTTGTGGTGCTGCAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATC***CTGGAGG***
AAAGCGTGCAGCGTTGC

Figure 12 Nucleotide sequence of *caGnRH-SEQ4* (A) and *caGnRH-SEQ5* (B) of *I. punctatus* amplified from 5' RACE-PCR (The location and sequence of a forward prime2r (caGnRH-F3 and caGnRH-F4) and those complementary to a reverse primer (caGnRH-R3 and caGnRH-R4) are illustrated in boldface and italicized.)

3.3 Identification of the full length cDNA of *caGnRH* of *I. punctatus*

Nucleotide sequences from 3' and 5' RACE-PCR of *caGnRH* of *I. punctatus* were assembled. The full length cDNA of this transcript was 370 bp (Figure 13) long with the open reading frame (ORF) of 243 bp encoding a polypeptide of 80 amino acids (aa). Deduced *caGnRH* of *I. punctatus* was composed of the signal peptide (21 aa), the active site (10 aa), the proteolytic processing site (3 aa), and the GnRH associated peptide (46 aa) (Table 16). The closest sequence to *caGnRH* of *I. punctatus* was *catfish type gonadotropin-releasing hormone* (*caGnRH*) of *C. gariepinus* (E-value = 3e-32).

```
ACAGACTGAGAGACCACATTGTGAAGAGCAGAAGAACTGTCTGCAGGGCGACTGATCAA
GGATGGGTATAAAGCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGCAGGTG
   M  G  I  K  R  A  L  W  W  M  V  V  C  V  V  V  L  Q  V

AGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGGA
 S  S  Q  H  W  S  H  G  L  N  P  G  G  K  R  A  A  L  Q  E

GACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTACGTAGATGTTTCAC
  T  V  E  E  M  P  R  T  S  G  Y  V  C  D  Y  V  D  V  S
CTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGACCAGTGTTGCTGAGCGAGAAATT
P  R  N  K  L  Y  R  L  K  D  L  L  T  S  V  A  E  R  E  I

GGACAGTAAGACAAATAACAAAACTGATCAAGAGCTTCAATAAAATGCTTTGCCTTTAA
 G  Q
AAAAAAAAAAAAAAAA
```

Figure 13 The full length cDNA sequences of *caGnRH* of *I. punctatus* (Start and stop codons are illustrated in boldface and underlined. The 5′RACE-PCR primer is underlined, boldfaced and italicized. The poly A additional signal is boldfaced and underlined. The signal peptide was underlined. The active site was boxed. The GnRH associated peptide was boldfaced.)

4. Identification of the full length cDNA of a catfish type GnRH (*caGnRH*) of *I. furcatus* using Generacer

4.1 3’ RACE-PCR and nested RACE-PCR

PCR was carried out at the annealing temperature of 67^0C using primers caGnRH-F5/R5. A 285 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. A pair of primers was designed. Nested RACE-PCR was carried out using caGnRH-F6/R6 as primers and the diluted primary 3’RACE-PCR as the template. A 272 bp fragment (including Generacer oligo dT primer sequence) was obtained (Figure 14). The amplification fragment was cloned and sequenced. Similarity analysis using Blast*X* indicated that *GnRH-SEQ8* (Figure 15) significantly matched the catfish gonadotropin-releasing hormone and the *catfish type gonadotropin-releasing hormone* (*caGnRH*) of *C. gariepinus* (E-value = 2e-20).

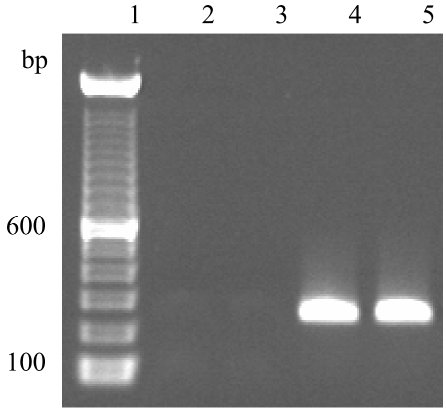


Figure 14 Agarose gel electrophoresis illustrating the product from 3’ (lane 4) and 5’RACE-PCR (lane 5) of *caGnRH* of *I. furcatus* (Lane 1 is a 100 bp ladder.)

A.
CAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCA
GGAGACTGTTGAAGAAATGCAGAGGACCTCCGGCTACGTGTGTGATTACGTAGACGTTTCAC
CTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGACCAGTGTTGCTGAGCGAGAAATTGGA
CAGTAAGACGAATAACAAAACTGATCAAGATCTTCAATAAAATGCTTCGCCTTTAAAAAAAA
ACACTGTCATGC***CGTTACGTAGCGTATCGTTGACAGC***3′
B.
CAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCA
GGAGACTGTTGAAGAAATGCAGAGGACCTCCGGCTACGTGTGTGATTACGTAGACGTTTCAC
CTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGACCAGTGTTGCTGAGCGAGAAATTGGA
CAGTAAGACGAATAACAAAACTGATCAAGATCTTCAATAAAATGCTTCGCCTTTAAAAAAAA
A***CACTGTCATGCCGTTACGTAGCG***

Figure 15 Nucleotide sequence of *caGnRH-SEQ7* (A) and *caGnRH-SEQ8* (B) of *I. furcatus* amplified from 3’ RACE-PCR (The location and sequence of a forward primer (caGnRH-F5 and caGnRH-F6) and those complementary to a reverse primer (caGnRH-R5 and caGnRH-R6) are illustrated in boldface and italicized.)

4.2 5’ RACE-PCR and nested RACE-PCR

5’ RACE-PCR was carried out using primers caGnRH-F7/R7. A 302 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. Similarity analysis using Blast*X* indicated that *GnRH-SEQ9* (Figure 16) significantly matched the catfish gonadotropin-releasing hormone and the *catfish type gonadotropin-releasing hormone* (*caGnRH*)of *C. gariepinus* (E-value = 8e-26).

A pair of primers was designed. Nested RACE-PCR was carried out using caGnRH-F8/R8 as primers and diluted primary 3’RACE-PCR as the template. A 204 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. Blast analysis indicated that *GnRH-SEQ10* (Figure 16) significantly matched the catfish gonadotropin-releasing hormone and the *catfish type gonadotropin-releasing hormone* (*caGnRH*) of *C. gariepinus* (E-value = 7e-14).

A.
CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAAATAGACTGTAGAGATCAT
CATTGTGAAGAGCAGAAGAACTGTCTGTAGGGCGACTATCAAGGATGGGTATAAAGCGAGCA
CTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGCAGGTGAGCTCTCAGCACTGGTCTCATGG
CCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGGAGACTGTTGAAGAAATGCAGAGGACCT
CCGGCTACGTGTGTGATTACGTAGACGTT***TCACCTCGGAATAAACTCTACAGGC***3′
B.
GGACACUGACAUGGACUGAAGGAGUAGAAAATAGACTGTAGAGATCATCATTGTGAAGAGCA
GAAGAACTGTCTGTAGGGCGACTATCAAGGATGGGTATAAAGCGAGCACTCTGGTGGATGGT
GGTGTGTGTTGTGGTGCTGCAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATC***CTGGAG***
GAAAGCGTGCAGCGTTGC

Figure 16 Nucleotide sequence of *caGnRH-SEQ9* (A) and *caGnRH-SEQ10* (B) of *I. furcatus* amplified from 5’ RACE-PCR (The location and sequence of a forward primer (caGnRH-F7 and caGnRH-F8) and those complementary to a reverse primer (caGnRH-R7 and caGnRH-R8) are illustrated in boldface and italicized.)

4.3 Identification of the full length cDNA of *caGnRH* of *I. punctatus*

Nucleotide sequences from 3’ and 5’ RACE-PCR of *caGnRH* of *I. punctatus* were assembled. The full length cDNA of this transcript was 362 bp (Figure 17) long with the open reading frame (ORF) of 243 bp encoding a polypeptide of 80 amino acids (aa). Deduced *caGnRH* of *I. furcatus* was composed of the signal peptide (21 aa), the active site (10 aa), the proteolytic processing site (3 aa), and the GnRH associated peptide (46 aa) (Table 17). The closest sequence to *caGnRH* of *I. punctatus* was *catfish type gonadotropin-releasing hormone* (*caGnRH*) of *C. gariepinus* (E-value = 3e-32). The closest sequence to *caGnRH* of *I. punctatus* was *catfish type gonadotropin-releasing hormone* (*caGnRH*) of *C. gariepinus* (E-value = 3e-31).

```
ATAGACTGTAGAGATCATCATTGTGAAGAGCAGAAGAACTGTCTGTAGGGCGACTATCA
AGGATGGGTATAAAGCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGCAGGT
    M  G  I  K  R  A  L  W  W  M  V  V  C  V  V  V  L  Q  V

GAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGG
  S  S  Q  H  W  S  H  G  L  N  P  G  G  K  R  A  A  L  Q

AGACTGTTGAAGAAATGCAGAGGACCTCCGGCTACGTGTGTGATTACGTAGACGTTTCA
E  T  V  E  E  M  Q  R  T  S  G  Y  V  C  D  Y  V  D  V  S

CCTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGACCAGTGTTGCTGAGCGAGAAAT
 P  R  N  K  L  Y  R  L  K  D  L  L  T  S  V  A  E  R  E  I

TGGACAGTAAGACGAATAACAAAACTGATCAAGATCTTCAATAAAATGCTTCGCCTTTA
  G  Q
AAAAAAAA
```

Figure 17 The full length cDNA sequences of *caGnRH* of *I. furcatus* (Start and stop codons are illustrated in boldface and underlined. The 5′RACE-PCR primer is underlined, boldfaced and italicized. The polyA additional signal is boldfaced and underlined. The signal peptide was underlined. The active site was boxed. The GnRH associated peptide was boldfaced.)

5. Identification of the full length cDNA of chicken gonadotropin-releasing hormone-II (*cGnRHII*) of *I. punctatus* using Generacer

5.1 3' RACE-PCR and nested RACE-PCR

PCR was carried out at the annealing temperature of 66^{0}C using primers caGnRH-F9/R9. A 393 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. A pair of primers was designed. Nested RACE-PCR was carried out using caGnRH-F10/R10 as primers and the diluted primary 3'RACE-PCR as the template. A 350 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. Similarity analysis using Blast*X* indicated that *GnRH-SEQ12* (Figure 18) significantly matched the catfish gonadotropin-releasing hormone and the *chicken gonadotropin-releasing hormone-II* (*cGnRH*) of *C. gariepinus* (E-value = 7e-9).

A.

AGCTCACCAGAGATATCTGGGGAGATCAAACTGTGTGAAGCGGGAGAATGCAGCTATCTGAG
GCCACTGAGAACCAACGTCCTGAAGAGCATCCTGCTCGATGCCCTTGTGAAAGAATTCCAAA
AGAAGAAGTGAAAACAATTCAATCATGCCCCAGCTTCCTCCAGCCTCTTTCTTCGCTGATAA
CACTTATCTGAACACACACACACACATCTGCTCATCCGTGGAGTGTGTCTTTTTCTGTTTAT
GTGGTTTTAGCGTCAAGCTTTCTGTCATCTGTGTATAAGCAGCGAAGTGAAATGCATCATGT
GATCATTCAATAAAACTTTTATTTTGGAAAAAAAAAAAAAAAAAAACACTGTCATGC***CGTTA***
CGTAGCGTATCGTTGACAGC3′
B.
ACTGTGTGAAGCGGGAGAATGCAGCTATCTGAGGCCACTGAGAACCAACGTCCTGAAGAGCA
TCCTGCTCGATGCCCTTGTGAAAGAATTCCAAAAGAAGAAGTGAAAACAATTCAATCATGCC
CCAGCTTCCTCCAGCCTCTTTCTTCGCTGATAACACTTATCTGAACACACACACACACATCT
GCTCATCCGTGGAGTGTGTCTTTTTCTGTTTATGTGGTTTTAGCGTCAAGCTTTCTGTCATC
TGTGTATAAGCAGCGAAGTGAAATGCATCATGTGATCATTCAATAAAACTTTTATTTTGGAA
AAAAAAAAAAAAAAAAA***CACTGTCATGCCGTTACGTAGCG***

Figure 18 Nucleotide sequence of *cGnRHII-SEQ12* (A) and *cGnRHII-SEQ13* (B) of *I. furcatus* amplified from 5' RACE-PCR (The location and sequence of a forward primer (caGnRH-F7 and caGnRH-F8) and those complementary to a reverse primer (caGnRH-R7 and caGnRH-R8) are illustrated in boldface and italicized.)

5.2 5' RACE-PCR and nested RACE-PCR

5' RACE-PCR was carried out using primers caGnRH-F11/R11. A 269 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. A pair of primers was designed. Nested RACE-PCR was carried out using caGnRH-F12/R12 as primers and diluted primary 3'RACE-PCR as the template. A 256 bp fragment (including Generacer oligo dT primer sequence) was obtained (Figure 19). The amplification fragment was cloned and sequenced. Blast analysis indicated that *GnRH-SEQ15* (Figure 20) significantly matched the catfish gonadotropin-releasing hormone and the *catfish type gonadotropin-releasing hormone* (*caGnRH*) of *C. gariepinus* (E-value = 7e-14).

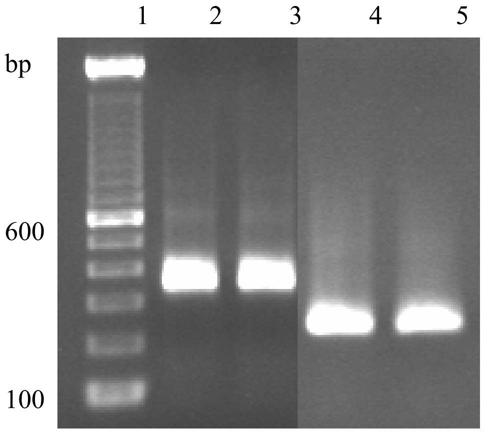


Figure 19 Agarose gel electrophoresis illustrating the product from 3' (lane 2) and 5'RACE-PCR (lane 4) of *cGnRH-II* of *I. punctatus* (Lane 1 is a 100 bp ladder.)

A.
CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAAAGGCATTGATCCCAAGAC
AAGAGCCCCTTGAGATCCACATCTAGAAGATCAAATCTGAATAAAGCACATCAAATAAGAGT
GATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTGCCTTGCTGTTGTGTTTGCAAGCGCAGC
TATCTGTTTCTCAACACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAAATCG***ACTCT***
TACAGCTCACCAGAGATATCTG
B.
GGACACUGACAUGGACUGAAGGAGUAGAAAAGGCATTGATCCCAAGACAAGAGCCCCTTGAG
ATCCACATCTAGAAGATCAAATCTGAATAAAGCACATCAAATAAGAGTGATGGTCAGTGTGT
GCAGGCTGTTGCTGGTTGCTGCCTTGCTGTTGTGTTTGCAAGCGCAGCTATCTGTTTCTCAA
CACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAAATCG***ACTCTTACAGCTCACCAGA***
GATATCTG

Figure 20 Nucleotide sequence of *cGnRHII-SEQ14* (A) and *cGnRHII-SEQ15* (B) of *I. furcatus* amplified from 5’ RACE-PCR (The location and sequence of a forward primer (caGnRH-F9 and caGnRH-F10) and those complementary to a reverse primer (caGnRH-R9 and caGnRH-R10) are illustrated in boldface and italicized.)

5.3 Identification of the full length cDNA of *caGnRH* of *I. punctatus*

Nucleotide sequences from 3’ and 5’ RACE-PCR of *caGnRH* of *I. punctatus* were assembled. The full length cDNA of this transcript was 563 bp (Figure 21) long with the open reading frame (ORF) of 261 bp encoding a polypeptide of 86 amino acids (aa). Deduced *cGnRH-II* of *I. punctatus* was composed of the signal peptide (24 aa), the active site (10 aa), the proteolytic processing site (3 aa), and the GnRH associated peptide (49 aa) (Table 16). The closest sequence to *cGnRH-II* of *I. punctatus* was *chicken-II gonadotropin-releasing hormone* (*cGnRH-II*) of *C. gariepinus* (E-value = 9e-40).

```
AGGCATTGATCCCAAGACAAGAGCCCCTTGAGATCCACATCTAGAAGATCAAATCTGAA
TAAAGCACATCAAATAAGAGTGATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTGCCT
                       M  V  S  V  C  R  L  L  L  V  A  A

TGCTGTTGTGTTTGCAAGCGCAGCTATCTGTTTCTCAACACTGGTCTCATGGCTGGTAT
L  L  L  C  L  Q  A  Q  L  S  V  S  Q  H  W  S  H  G  W  Y

CCTGGAGGAAAGAGAGAAATCGACTCTTACAGCTCACCAGAGATATCTGGGGAGATCAA
 P  G  G  K  R  E  I  D  S  Y  S  S  P  E  I  S  G  E  I  K

ACTGTGTGAAGCGGGAGAATGCAGCTATCTGAGGCCACTGAGAACCAACGTCCTGAAGA
  L  C  E  A  G  E  C  S  Y  L  R  P  L  R  T  N  V  L  K

GCATCCTGCTCGATGCCCTTGTGAAAGAATTCCAAAAGAAGAAGTGAAAACAATTCAAT
S  I  L  L  D  A  L  V  K  E  F  Q  K  K  K
CATGCCCCAGCTTCCTCCAGCCTCTTTCTTCGCTGATAACACTTATCTGAACACACACA
CACACATCTGCTCATCCGTGGAGTGTGTCTTTTTCTGTTTATGTGGTTTTAGCGTCAAG
CTTTCTGTCATCTGTGTATAAGCAGCGAAGTGAAATGCATCATGTGATCATTCAATAAA
ACTTTTATTTTGGAAAAAAAAAAAAAAAAAAA
```

Figure 21 The full length cDNA sequences of *cGnRH-II* of *I. punctatus* (Start and stop codons are illustrated in boldface and underlined. The 5′RACE-PCR primer is underlined, boldfaced and italicuzed. The polyA additional signal is boldfaced and underlined. The signal peptide was underlined. The active site was boxed. The GnRH associated peptide was boldfaced.)

6. Identification of the full length cDNA of *chicken gonadotropin-releasing hormone- II* (*cGnRHII*) of *I. furcatus* using Generacer

6.1 3’ RACE-PCR and nested RACE-PCR

PCR was carried out at the annealing temperature of 66^{0}C using primers caGnRH-F13/R13. A 392 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. Blast*X* indicated that *GnRH-SEQ17* (Figure 22) significantly matched the catfish gonadotropin-releasing hormone and the *chicken gonadotropin-releasing hormone-II* (*cGnRH-II*) of *C. gariepinus* (E-value = 1e-15).

A pair of primers was designed. Nested RACE-PCR was carried out using caGnRH-F14/R14 as primers and the diluted primary 3'RACE-PCR as the template. A 363 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment was cloned and sequenced. Similarity analysis using Blast*X* indicated that *GnRH-SEQ18* (Figure 22) significantly matched the catfish gonadotropin-releasing hormone and the *chicken gonadotropin-releasing hormone-II* (*cGnRH-II*) of *C. gariepinus* (E-value = 5e-10).

A.

AGCTCACCAGAGATATCTGGGGAGATTAAACTGTGTGAAGCGGGAGAATGCAGCTATCTGAGGCCACTG
AGAACCAACGTCCTGAAGAGCATCCTGCTCGATGCCCTTGCGAGAGAATTCCAAAAGAGGAAGTGAAAA
CAATCCAATCCTGCCCCAGCTTCCTACAGCCTCTTTCTTCGCTGATAACACTGATCTGAACACACACAC
ACATCTGCTCATCCGTGCAGTGTGTCTATTTCTGTTTATGTGGTTTTAGCATCAAGCTTTCTGTCATCT
GTGATAAGCAGCGAAGTGAAATGCATCATGTGATCATACAATAAAACTTTTATTTTGGTAAAAAAAAAA
AAAAAAAAAAACACTGTCATGC***CGTTACGTAGCGTATCGTTGACAGC***3′

B.

ACTGTGTGAAGCGGGAGAATGCAGCTATCTGAGGCCACTGAGAACCAACGTCCTGAAGAGCATCCTGCT
CGATGCCCTTGCGAGAGAATTCCAAAAGAGGAAGTGAAAACAATCCAATCCTGCCCCAGCTTCCTACAG
CCTCTTTCTTCGCTGATAACACTGATCTGAACACACACACACATCTGCTCATCCGTGCAGTGTGTCTAT
TTCTGTTTATGTGGTTTTAGCATCAAGCTTTCTGTCATCTGTGATAAGCAGCGAAGTGAAATGCATCAT
GTGATCATACAATAAAACTTTTATTTTGGTAAAAAAAAAAAAAAAAAAAAACACTGTCATGC***CGTTACG***
TAGCGTATCGTTGACAGC3′

Figure 22 Nucleotide sequence of *cGnRHII-SEQ17* (A) and *cGnRHII-SEQ18* (B) of *I. furcatus* amplified from 3' RACE-PCR (The location and sequence of a forward primer (caGnRH-F15 and caGnRH-F16) and those complementary to a reverse primer (caGnRH-R15 and caGnRH-R16) are illustrated in boldface and italicized.)

6.2 5' RACE-PCR and nested RACE-PCR

5' RACE-PCR was carried out using primers caGnRH-F11/R11. A 275 bp fragment (including Generacer oligo dT primer sequence) was obtained. The amplification fragment (*GnRH-SEQ20*) was cloned and sequenced. A pair of primers was designed. Nested RACE-PCR was carried out using caGnRH-F12/R12 as primers and diluted primary 3'RACE-PCR as the template. A 261 bp fragment (including Generacer oligo dT primer sequence) was obtained (Figure 23). The amplification fragment was cloned and sequenced (Figure 24). Blast analysis indicated that *GnRH-SEQ20* significantly matched the catfish gonadotropin-releasing hormone and the *catfish type gonadotropin-releasing hormone* (*caGnRH*) of *C. gariepinus* (E-value = 7e-14).

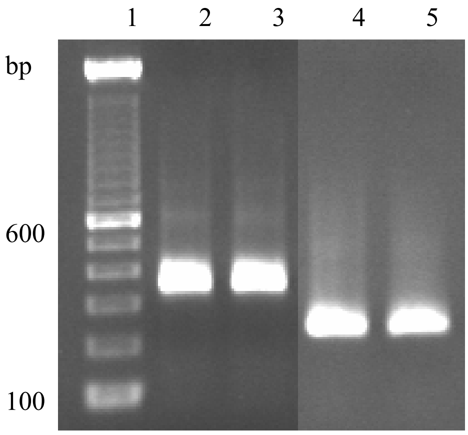


Figure 23 Agarose gel electrophoresis illustrating the product from 3' (lane 3) and 5'RACE-PCR (lane 5) of *cGnRH-II* of *I. punctatus* (Lane 1 is a 100 bp ladder.)

A.
CGACUGGAGCACGAGGACACUGACAUGGACUGAAGGAGUAGAAAAAGCATTGATACTCCAAG
ACAAGAGCTTCTCAGAGTTCCACATCTCAGAAGATTCAAATCTGAACAAAACACATCAAATA
AGAGTGATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTGCCTTGCTGTTGTGTTTGCAAGC
GCAGCTATCTGTTTCTCAACACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAAATCG
ACTCTTACAGCTCACCAGAGATATCTG
B.
GGACACUGACAUGGACUGAAGGAGUAGAAAAAGCATTGATACTCCAAGACAAGAGCTTCTCA
GAGTTCCACATCTCAGAAGATTCAAATCTGAACAAAACACATCAAATAAGAGTGATGGTCAG
TGTGTGCAGGCTGTTGCTGGTTGCTGCCTTGCTGTTGTGTTTGCAAGCGCAGCTATCTGTTT
CTCAACACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAAATCG***ACTCTTACAGCTCA***
CCAGAGATATCTG3′

Figure 24 Nucleotide sequence of *cGnRHII-SEQ19* (A) and *cGnRHII-SEQ20* (B) of *I. furcatus* amplified from 5' RACE-PCR (The location and sequence of a forward primer (caGnRH-F17 and caGnRH-F18) and those complementary to a reverse primer (caGnRH-R17 and caGnRH-R18) are illustrated in boldface and italicized.)

6.3 Identification of the full length cDNA of *caGnRH* of *I. punctatus*

Nucleotide sequences from 3’ and 5’ RACE-PCR of *caGnRH* of *I. punctatus* were assembled. The full length cDNA of this transcript was 568 bp (Figure 25) long with the open reading frame (ORF) of 261 bp encoding a polypeptide of 86 amino acids (aa). Deduced *cGnRH-II* of *I. punctatus* was composed of the signal peptide (24 aa), the active site (10 aa), the proteolytic processing site (3 aa), and the GnRH associated peptide (49 aa) (Table 16). The closest sequence to *cGnRH-II* of *I. punctatus* was *chicken-II gonadotropin-releasing hormone* (*cGnRH-II*) of *C. gariepinus* (E-value = 6e-41).

```
AAGCATTGATACTCCAAGACAAGAGCTTCTCAGAGTTCCACATCTCAGAAGATTCAAAT
CTGAACAAAACACATCAAATAAGAGTGATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGC
                            M  V  S  V  C  R  L  L  L  V  A

TGCCTTGCTGTTGTGTTTGCAAGCGCAGCTATCTGTTTCTCAACACTGGTCTCATGGCT
  A  L  L  L  C  L  Q  A  Q  L  S  V  S  Q  H  W  S  H  G

GGTATCCTGGAGGAAAGAGAGAAATCGACTCTTACAGCTCACCAGAGATATCTGGGGAG
W  Y  P  G  G  K  R  E  I  D  S  Y  S  S  P  E  I  S  G  E

ATTAAACTGTGTGAAGCGGGAGAATGCAGCTATCTGAGGCCACTGAGAACCAACGTCCT
 I  K  L  C  E  A  G  E  C  S  Y  L  R  P  L  R  T  N  V  L

GAAGAGCATCCTGCTCGATGCCCTTGCGAGAGAATTCCAAAAGAGGAAGTGAAAACAAT
  K  S  I  L  L  D  A  L  A  R  E  F  Q  K  R  K
CCAATCCTGCCCCAGCTTCCTACAGCCTCTTTCTTCGCTGATAACACTGATCTGAACAC
ACACACACATCTGCTCATCCGTGCAGTGTGTCTATTTCTGTTTATGTGGTTTTAGCATC
AAGCTTTCTGTCATCTGTGATAAGCAGCGAAGTGAAATGCATCATGTGATCATACAATA
AAACTTTTATTTTGGTAAAAAAAAAAAAAAAAAAAAA
```

Figure 25 The full length cDNA sequences of *cGnRH-II* of *I. furcatus* (Start and stop codons are illustrated in boldface and underlined. The 5′RACE-PCR primer is underlined, boldfaced and italicuzed. The poly A additional signal is boldfaced and underlined. The signal peptide was underlined. The active site was boxed. The GnRH associated peptide was boldfaced.)

The full length transcript of *GnRH* consists 5’ untranslated region (UTR), the signal peptide (SP), the active site (AS), the proteolytic processing site (PPS), the GnRH associated peptide (GAP), and 3’UTR. The length of each *caGnRH* and *cGnRH-II* gene region of *I. punctatus* and *I. furcatus* are showed in Table 17. Considering only the ORF, identical length of each region was found interspecifically for each type of *GnRH* (80 and 86 aa for *caGnRH* and *cGnRH-II*, respectively). Disregarding catfish species, 5' and 3'UTRs of *cGnRH-II* were slightly longer than those of *caGnRH* (Table 18).

Table 17 Sizes of different regions of *caGnRH* and *cGnRH-II* of *I. punctatus* and *I. furcatus*

GnRH	**GnRH cDNA**							
	5'UTR	**SP**	**AS**	**PPS**	**GAP**	**3'UTR**	**Poly A**	**total**
caGnRH								
channel catfish	61	63	30	9	138	51	18	370
blue catfish	61	63	30	9	138	51	9	362
cGnRH-II								
channel catfish	81	72	30	9	147	205	19	563
blue catfish	86	72	30	9	147	203	21	568

Table 18 The amino acid in the ORF of of *caGnRH* and *cGnRH-II* of *I. punctatus* and *I. furcatus*

GnRH	**Region**				**Total**
	SP	**AS**	**PPS**	**GAP**	
caGnRH					
channel catfish	21	10	3	46	80
blue catfish	21	10	3	46	80
cGnRH-II					
channel catfish	24	10	3	49	86
blue catfish	24	10	3	49	86

The molecular weight and isoelectric focusing point (pI) of deduced proteins were estimated (Table 19). GnRHs are basic proteins having pI of 8.7-8.8 and the molaecular mass of 91 kDa for *caGnRH* and 97 kDa for *cGnRH II*.

Table 19 Estimated molecular weight and isoelectric focusing point of *caGnRH* and *cGnRH II* of *I. punctatus* and *I. furcatus*.

Type	**Isoelectric point (PI)**	**Molecular weight (Dalton)**
caGnRH of channel catfish	8.71	9087.53
caGnRH of blue catfish	8.71	9118.54
cGnRH II of channel catfish	8.76	9647.36
cGnRH II of blue catfish	8.80	9675.53

7. Comparisons *GnRH* of *I. punctatus* and *I. furcatus* with other species

7.1 *Catfish type GnRH*

The full length cDNA of caGnRH of *I. punctatus* show the highest similarity with that of the African catfish (*C. gariepinus*). The percentage of similarity was much lower with other types of GnRH in various fish species (36-46%, Table 20). Likewise, *caGnRH* of *I. furcatus* showed the highest similarity to that of *C. gariepinus* and reduced similarity was observed when compared with other types of GnRH in other species (37-44%, Table 21).

7.2 *Chicken type II GnRH*

The full length cDNA of *cGnRH-II* of *I. punctatus* show the highest similarity with that of the African catfish (*C. gariepinus*). The percentage of similarity of *cGnRH-II* was relatively similar across different fish species (70-81%, Table 22). Likewise, *cGnRH-II* of *I. furcatus* showed the highest similarity to that of *C. gariepinus* and similar levels of similarity with the remaining species (75-81%, Table 23).

Table 20 Similarity analysis of *caGnRH* of *I. punctatus* and different types of *GnRH* in various fish species

Species	Assession number	Number of amino acid	% identity	Score (bits)	e-value
Clarias gariepinus (catfish type)	CAA54971	80	83	115	8e-25
Coregonus clupeaformis (whitefish type)	AAP57221	93	46	54.7	9e-07
Oreochromis niloticus (seabream type)	AAM90220	94	45	54.3	2e-06
Alosa sapidissima (herring type)	AAN04492	86	43	51.2	2e-05
Anguilla japonica (mGnRH)	BAA82608	91	36	48.1	1e-04
Odontesthes bonariensis (pejerrey type)	AAU94309	94	43	45.8	74-04
Oryzias latipes (medaka type)	BAC06421	91	38	42	0.011

Table 21 Similarity analysis of *caGnRH* of *I. furcatus* and different types of *GnRH* in various fish species

Species	Assession number	Number of amino acid	% identity	Score (bits)	e-value
Clarias gariepinus (catfish type)	CAA54971	80	82	112	6e-24
Coregonus clupeaformis (whitefish type)	AAP57221	93	44	52.4	8e-06
Anguilla japonica (mGnRH)	BAA82608	91	37	50.4	3e-05
Oreochromis niloticus (seabream type)	AAM90220	94	43	51.2	2e-05
Alosa sapidissima (herring type)	AAN04492	86	42	48.1	1e-04
Odontesthes bonariensis (pejerrey type)	AAU94309	94	42	42.7	0.006
Oryzias latipes (medaka type)	BAC06421	91	38	42	0.01

Table 22 Similarity analysis of *cGnRH-II* of *I. punctatus* and *cGnRH-II* in various fish species

Species	Assession number	Number of amino acid	% identity	Score (bits)	e-value
Clarias gariepinus (chicken type II)	CAA54969	86	94	113	5e-24
Coregonus clupeaformis (chicken type II)	AAP57219	86	81	99.4	9e-20
Oncorhynchus mykiss (chicken type II)	AAF08687	86	77	98.2	2e-19
Tetraodon nigroviridis (chicken type II)	BAE45691	85	81	97.1	4e-19
Danio rerio (chicken type II)	AAU43784	86	74	95.1	2e-18
Anguilla japoica (chicken type II)	BAA82609	87	73	90.9	3e-17
Cyprinus carpio (chicken type II)	AAN64351	86	70	90.5	4e-17

Table 23 Similarity analysis of *cGnRH-II* of *I. furcatus* and *cGnRH-II* in various fish species

Species	Assession number	ORF	% identity	Score (bits)	e-value
Clarias gariepinus (chicken type II)	CAA54969	86	93	127	3e-28
Coregonus clupeaformis (chicken type II)	AAP57219	86	81	113	5e-24
Oreochromis niloticus (chicken type II)	BAC56850	85	78	108	1e-22
Tetraodon nigroviridis (chicken type II)	BAE45690	85	80	108	2e-22
Cyprinus carpio (chicken type II)	AAO39753	86	77	106	6e-22
Danio rerio (chicken type II)	AAU43784	86	75	106	8e-22

8. Sequence divergence between *GnRH* of *I. punctatus* and *I. furcatus*

8.1 *Catfish type GnRH*

Nucleotide sequence of *caGnRH* in of *I. punctatus* and *I. furcatus* are highly similar (Figure 26). Disregarding the poly A tail, 9 substitutions were observed in these transcripts. These sequences revealed 97% similarity with the E-value of 2e-167. Deduced amino acid sequences revealed only one different position between sequences (P to Q in the GAP site, Figure 27).

```
CC   ACAGACTG-AGAGACCA-CATTGTGAAGAGCAGAAGAACTGTCTGCAGGGCGACTGATCA 58
BL   ATAGACTGTAGAGATCATCATTGTGAAGAGCAGAAGAACTGTCTGTAGGGCGACT-ATCA 59
     * ****** ***** ** *************************** ********* ****

CC   AGGATGGGTATAAAGCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGCAGGTG 118
BL   AGGATGGGTATAAAGCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGCAGGTG 119
     ************************************************************

CC   AGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGGAG 178
BL   AGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGGAG 179
     ************************************************************

CC   ACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTACGTAGATGTTTCACCT 238
BL   ACTGTTGAAGAAATGCAGAGGACCTCCGGCTACGTGTGTGATTACGTAGACGTTTCACCT 239
     **************** ********************************* *********

CC   CGGAATAAACTCTACAGGCTGAAAGATCTGCTGACCAGTGTTGCTGAGCGAGAAATTGGA 298
BL   CGGAATAAACTCTACAGGCTGAAAGATCTGCTGACCAGTGTTGCTGAGCGAGAAATTGGA 299
     ************************************************************

CC   CAGTAAGACAAATAACAAAACTGATCAAGAGCTTCAATAAAATGCTTTGCCTTTAAAAAA 358
BL   CAGTAAGACGAATAACAAAACTGATCAAGATCTTCAATAAAATGCTTCGCCTTTAAAAAA 359
     ********* ******************** **************** ************

CC   AAAAAAAAAAAA 370
BL   AAA--------- 362
     ***
```

Figure 26 Nucleotide sequence alignment of *caGnRH* of *I. punctatus* and *I. furcatus*

```
CC   MGIKRALWWMVVCVVVLQVSSQHWSHGLNPGGKRAALQETVEEMPRTSGYVCDYVDVSPR 60
BL   MGIKRALWWMVVCVVVLQVSSQHWSHGLNPGGKRAALQETVEEMQRTSGYVCDYVDVSPR 60
     ******************************************** ***************

CC   NKLYRLKDLLTSVAEREIGQ 80
BL   NKLYRLKDLLTSVAEREIGQ 80
     ********************
```

Figure 27 Alignment of deduced amino acid sequences of *caGnRH* of *I. punctatus* and *I. furcatus*

8.2 *Chicken type II GnRH*

Nucleotide sequence of *cGnRH-II* in of *I. punctatus* and *I. furcatus* are highly similar (Figure 28) but the similarity index was lower than that between *caGnRH* of these two species. Disregarding the poly A tail, 23 substitutions were observed in these transcripts. These sequences revealed 95% similarity with the E-value of 0. Deduced amino acid sequences revealed 3 amino acid replacement at the C terminus of deduced proteins (Figure 29).

```
CC    AGGCATTGAT--CCCAAGACAAGAGCCCCTT-GAGATCCACATCT-AGAAGAT-CAAATC 55
BL    AAGCATTGATACTCCAAGACAAGAGCTTCTCAGAGTTCCACATCTCAGAAGATTCAAATC 60
      * ********   *************  **  *** ********* ******* ******

CC    TGAATAAAGCACATCAAATAAGAGTGATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTG 115
BL    TGAACAAAACACATCAAATAAGAGTGATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTG 120
      **** *** ***************************************************

CC    CCTTGCTGTTGTGTTTGCAAGCGCAGCTATCTGTTTCTCAACACTGGTCTCATGGCTGGT 175
BL    CCTTGCTGTTGTGTTTGCAAGCGCAGCTATCTGTTTCTCAACACTGGTCTCATGGCTGGT 180
      ************************************************************

CC    ATCCTGGAGGAAAGAGAGAAATCGACTCTTACAGCTCACCAGAGATATCTGGGGAGATCA 235
BL    ATCCTGGAGGAAAGAGAGAAATCGACTCTTACAGCTCACCAGAGATATCTGGGGAGATTA 240
      ********************************************************** *

CC    AACTGTGTGAAGCGGGAGAATGCAGCTATCTGAGGCCACTGAGAACCAACGTCCTGAAGA 295
BL    AACTGTGTGAAGCGGGAGAATGCAGCTATCTGAGGCCACTGAGAACCAACGTCCTGAAGA 300
      ************************************************************

CC    GCATCCTGCTCGATGCCCTTGTGAAAGAATTCCAAAAGAAGAAGTGAAAACAATTCAATC 355
BL    GCATCCTGCTCGATGCCCTTGCGAGAGAATTCCAAAAGAGGAAGTGAAAACAATCCAATC 360
      ********************* ** ************** ************** *****

CC    ATGCCCCAGCTTCCTCCAGCCTCTTTCTTCGCTGATAACACTTATCTGAACACACACACA 415
BL    CTGCCCCAGCTTCCTACAGCCTCTTTCTTCGCTGATAACACTGATCTGAACACACACACA 420
       ************** ************************** *****************

CC    CACATCTGCTCATCCGTGGAGTGTGTCTTTTTCTGTTTATGTGGTTTTAGCGTCAAGCTT 475
BL    CA--TCTGCTCATCCGTGCAGTGTGTCTATTTCTGTTTATGTGGTTTTAGCATCAAGCTT 478
      **  ************** ********* ********************** ********

CC    TCTGTCATCTGTGTATAAGCAGCGAAGTGAAATGCATCATGTGATCATTCAATAAAACTT 535
BL    TCTGTCATCTGTG-ATAAGCAGCGAAGTGAAATGCATCATGTGATCATACAATAAAACTT 537
      ************* ********************************** ***********

CC    TTATTTTGGAAAAAAAAAAAAAAAAAAA--- 563
BL    TTATTTTGGTAAAAAAAAAAAAAAAAAAAAA 568
      ********* ******************
```

Figure 28 The nucleotide alignment of *cGnRH II* in channel and blue catfish

```
CC   MVSVCRLLLVAALLLCLQAQLSVSQHWSHGWYPGGKREIDSYSSPEISGEIKLCEAGECS 60
BL   MVSVCRLLLVAALLLCLQAQLSVSQHWSHGWYPGGKREIDSYSSPEISGEIKLCEAGECS 60
     ************************************************************

CC   YLRPLRTNVLKSILLDALVKEFQKKK 86
BL   YLRPLRTNVLKSILLDALAREFQKRK 86
     ******************.:****:*
```

Figure 29 The amino acid alignment of *cGnRH II* GnRH in channel and blue catfish

Multiple alignments of amino acid sequences of different GnRH types in various fish species was carried out (Figure 30).

```
CA-CC -MGIKRALWWMVVCV--VVLQVSSQHWSHGLNPGGKR---------AALQETVEEMPRTS 48
CA-BL -MGIKRALWWMVVCV--VVLQVSSQHWSHGLNPGGKR---------AALQETVEEMQRTS 48
CA-AF -MGIKRALWWMVVCV--VVLQVSAQHWSHGLNPGGKR---------AVMQESAEEIPRRS 48
CH-CC MVSVCRLLLVAALLLCLQAQLSVSQHWSHGWYPGGKR---EIDS-YSSPEIS-GEIKLCE 55
CH-BL MVSVCRLLLVAALLLCLQAQLSVSQHWSHGWYPGGKR---EIDS-YSSPEIS-GEIKLCE 55
CH-AF MVSVCRLLLVAALLLCLQAQLSFSQHWSHGWYPGGKR---EIDS-YSSPEIS-GEIKLCE 55
CH-CA MVHICRLFVVMGMLLCLSAQFASSQHWSHGWYPGGKR---EIDV-YDTSEVS-EEIKLCE 55
CH-GO MVHICRLFVVMGMLLCLSAQFASSQHWSHGWYPGGKR---EIDV-YDSSEVS-GEIKLCE 55
CH-NT -MCVSRLALLLGLLLCVGAQLSFAQHWSHGWYPGGKR---ELDS-FGTSEIS-EEIKLCE 54
SA-CA -MEWKGRVLVQLLMLVCVLEVSLCQHWSYGWLPGGKRSTGEVEATFRMMDPGDAVLSIPE 59
SA-GO -MEGKGRVLVQLLMLACVLEVSLCQHWSYGWLPGGKRSVGEVEATFRMMDSGDAVLSIPM 59
SA-NT -MEAGSRVIMQVLLLALVVQVTLSQHWSYGWLPGGKRSVGELEATIRMMGTG-GVVSLPD 58
SB-NT -MAAKILALWLLLAG-TVFPQGCCQHWSYGLSPGGKRD---LDNFSDTLGNMVEEFPRVE 55
SB-SB -MAPQTSNLWLLLVVMMVMSQGCCQHWSYGLSPGGKRD---LDSLSDTLGDIIERFPHAD 56
       :          :          .****:*  *****                  .

CA-CC G--Y-VCDYVDVSPRNKLYRLKDLLTSVAEREIGQ--------- 80
CA-BL G--Y-VCDYVDVSPRNKLYRLKDLLTSVAEREIGQ--------- 80
CA-AF G--Y-LCDYVAVSPRNKPFRLKDLLTPVAGREIEE--------- 80
CH-CC AG-----ECSYLRPLR-TNVLKSILLDALVKEFQKKK------- 86
CH-BL AG-----ECSYLRPLR-TNVLKSILLDALAREFQKRK------- 86
CH-AF AG-----ECSYLRPLR-TNILKSILIDTLARKFQKRK------- 86
CH-CA AG-----KCSYLRPQG-RNILKTILLDALIRDFQKRK------- 86
CH-GO AG-----KCSYLRPQG-RNILKTILLDAIIRDSQKRK------- 86
CH-NT AG-----ECSYLRPQR-RSILRNILLDALARELQKRK------- 85
SA-CA DS-----PMERLSPIRIVNEVDAEGLPLKEQRFSNRRGRV---- 94
SA-GO DS-----PMERLSPIHIVSEVDAEGLPLKEQRFPNRRGRD---- 94
SA-NT EA--NAQTQERLRPYNIIND-DSSHFDRK-KRFPNN-------- 90
SB-NT APCS-VFGCAEESPFAKMYRVKGLLASLAEGKTDTGHSRNERFL 98
SB-SB SPCS-VLGCAEEPPFPKMYRMKGFIGSGTDRDNGHRTYKKNRH- 98
                   *
```

Figure 30 Multiple alignments of different types of GnRH from various fish species. Abbreviations:CA=catfish type; CH=chicken type II; SA=salmon type; SB=sea bream type; CC=channel catfish; BL=blue catfish; AF=African catfish; CA=common carp; GO=goldfish; NT=Nile tilapia and SB=sea bream.

A bootstrapped phylogenetic tree was constructed using a neighbor-joining approach. The tree topology allocated GnRH to 4 major groups; catfish, chicken, salmon and sea bream types with statistical support from boostrapping data. GnRH of *I. punctatus* and *I. furcatus* are well allocated into the expected groups (Figure 31).

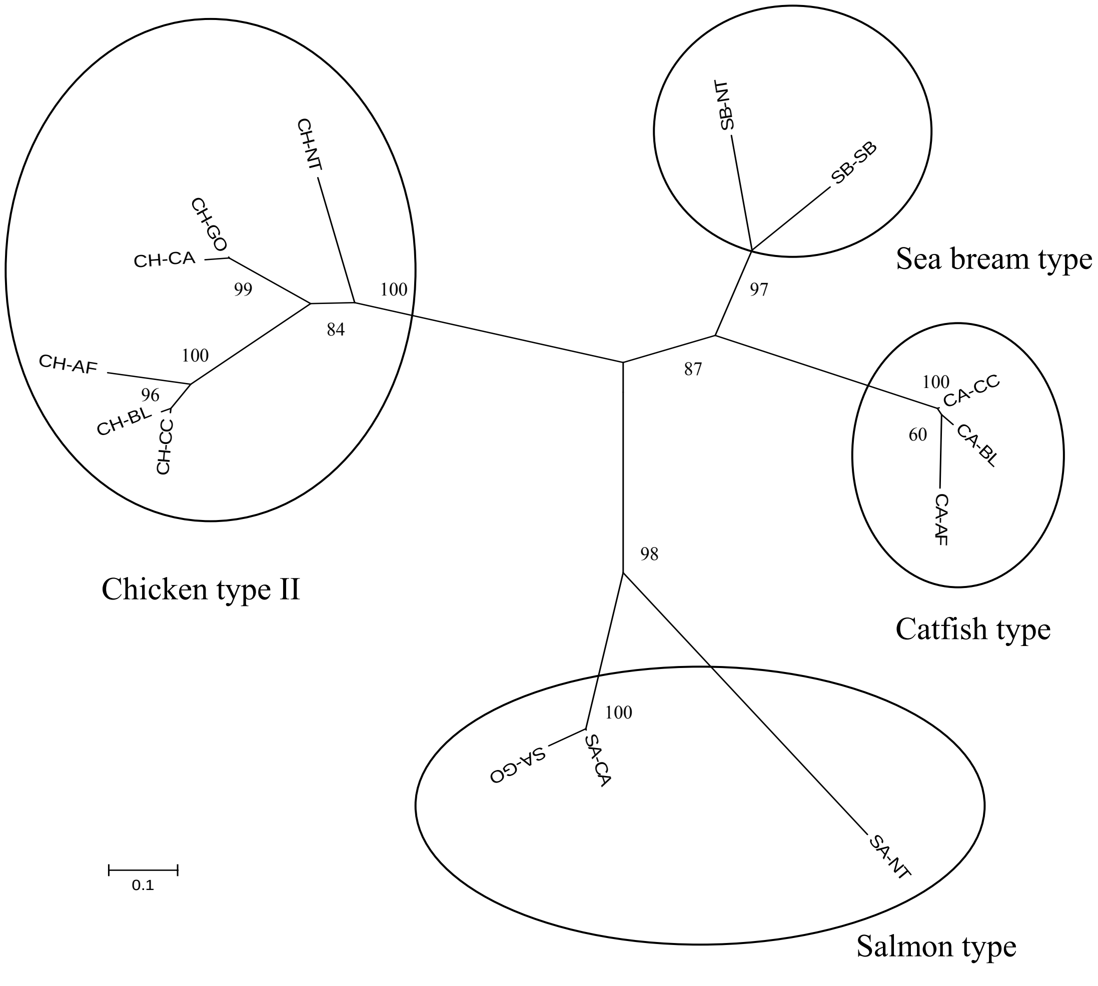


Figure 31 A bootstrapped neighbor-joining tree of *GnRH* from various fish species Abbreviations: CC=channel catfish, BC=blue catfish, AF=African catfish, CA=carp, GO=goldfish, NT=Nile tilapia, SB=sea bream, CH=chicken II, CA=catfish, and SA=salmon.

9. Characterization of genomic sequences of *caGnRH* and *cGnRH-II* of *I. punctatus* and *I. furcatus*

9.1 Catfish type GnRH

Filter hybridization was carried out for identification of BAC clones that contain *GnRH* sequences using the overgo primer. Four filters were hybridized and a total of 39 positive clones were found. BAC clones showing the positive signal are shown in Table 24.

Table 24 BAC clones containing *caGnRH* hybridized using overgo primer as a probe

Filter	Panel	Plate	Well
1	4	46	N22
		28	J8
		16	G6
		16	B13
	1	37	P19
		19	G17
	5	5	G3
		5	C17
	2	38	P16
		32	J24
		14	D23
	6	12	J23
	3	45	G15
2	1	73	D8
	2	86	A23
	6	84	B18
	3	87	P8
		93	**N10**
3	4	118	M2
		130	K14
		136	C17
	1	121	I16
	6	120	O14
		126	K21
		120	C18
3	3	129	M9
		135	I1
		117	B5
4	4	184	O2
		172	I8

Table 24 (Continued)

Filter	Panel	Plate	Well
4	1	151	E24
	5	191	C18
	2	164	J1
		146	P8
		188	B12
	6	150	L8
		150	H2
	3	177	I15
		177	B18

To obtain more specific results of hybridization, the 3'RACE-PCR product of *caGnRH* was used as the DNA probe. Only 6 positive BAC clones from different panels; 2nd panel (1 clones), 3rd panel (2 clones), and 3rd panel (3 clones) showed positive hybridization signals (Table 25).

Table 25 BAC clones containing *caGnRH* hybridized using the 3'RACE-PCR product of *caGnRH* as a probe

Filter	Panel	Plate	Well
2	3	93	N10
3	6	120	O14
	3	117	B5
4	2	164	J1
	3	171	A11
		185	J13

9.2 Chicken type II GnRH

Hybridization using the overgo hybridization provided 24 positive clones as can be seen from Table 26. A few positive clones were selected for further analysis.

Table 26 The BAC clones of *cGnRH-II* using the overgo primer as a probe

Filter	Panel	Plate	Well
1	4	28	J8
	5	5	E1
	2	20	A16
		20	K16
	6	12	H9
		48	**E22**
	3	21	K3
2	1	55	D9
		49	B9
	5	53	M17
	2	**92**	**B18**
	3	93	N10
3	4	124	N1
	6	120	O14
		126	J10
		138	E15
	3	117	B5
4	4	154	O10
	1	**151**	**H18**
	5	185	J13
	2	164	J1
		146	E8
	6	174	G5
	3	171	A11

9.3 Selection of BAC clones

Six clones containing *caGnRH* (93N10, 120O14, 117B5, 164J1, 171A11, and 185J13) and fifteen clones containing *cGnRH-II* (28J8, 5E1, 20A16, 48E22, 55D9, 53M17, 92B18, 93N10, 120O14, 117B5, 151H18, 164J1, 164J1, 146E8, and 171A11) were cultured. Recombinant BAC DNA was extracted and sequenced using the overgo primer, AU50870ova and AU50870ovb for *caGnRH* and AU50871ova and AU50871ovb for *cGnRH-II.*

Nucleotide sequences from AU50870ova and AU50870ovb did not match with any part of GnRH gene. The new primer, AU75554U corresponding to part of exon2 of *caGnRH* was used for sequencing the 93N10 clone. The sequence of 93N10 significantly matched exon3, 4 and part of exon2 of *caGnRH.* Nucleotide sequences of 48E22, 92B18, and 151H18 showed exon1 and part of exon2 of *cGnRH II*. The 48E22 clone represented more complete *cGnRH II* than 92B18 and 151H18. Accordingly, 93N10 and 48E22 clones were further used for primer walking to isolate the complete genomic sequences of *caGnRH* and *cGnRH-II*, respectively.

10. Primer and PCR walking of *caGnRH* and *cGnRH II* in channel and blue Catfish

10.1 Primer walking of *I. punctatus caGnRH*

The forward and reverse primers of each fragment were designed manually and used for sequencing. The internal forward and reverse primers were designed from the previous obtained sequences and successfully used to isolate the complete genomic sequence of *caGnRH* of *I. punctatus* (Figure 32 and 33 and appendix figure 2).

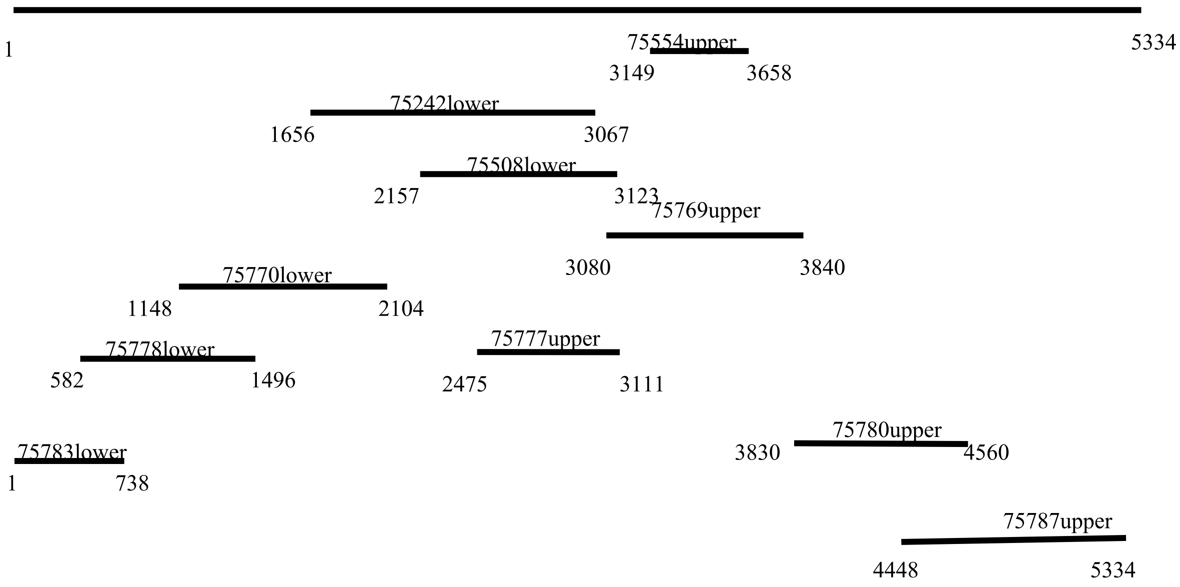
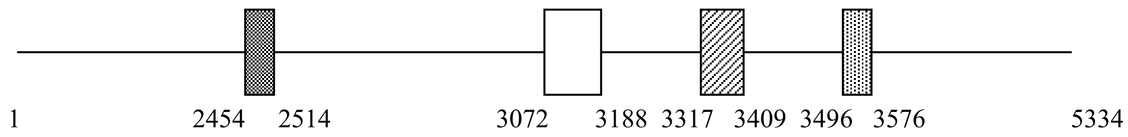


Figure 32 Schematic diagram showing primer walking of *caGnRH* of *I. punctatus*

A.



B.

```
GATTACTCAGCTCCTTAAAGTGGAAAGTGGTTTCTTCAGTCTCGGCGAACAACCAATGGGATTTTTCCA
GGCCGACACACAAAATATGAAAAGTTTCTGCTTTGGTACTTAGTGGAAATTGCTAGAGTTTGACTCTGA
AATCTGAAGGAGGAGCCATTTTTAAAGCTCTTCTGCTTTTCTTTAGCAAATTCCGTACCATATCATATA
TATTTATAATATATGCATGCTTAAAAAGACAAACGTATTGCTACCTATATCCCATATATAACAGGTGAT
AAGCTTATTGAACTATTTTAAAGTTAGGTATAAAATTACATATGCAATGTAAATGTATGTTTCTTAGCT
CCTTGTGTGCGCTGTTCAAACGGAAACAGGATTTTTCTTTCGCTTACCGATTTTTATTTCAGATCTACC
TCCCATAACAAAACTGACTTTTGCTCTCGTCAATCCTGTGGATTTCTTTCAGATCCTCATGAGAAGGCT
CGAATACCCTGCTGAAATAAACAACCACGGAAATTTGAATGGTTTCCTCTACAAATACCATTACAAACT
ATCAGAGAACCATTAAAACCATTATCATTATTGGTCTTCAATGGTATCCACCGGACATAACATGTCACC
AATAGAAAGCAACAAATTATCAGTAGAATTCCCATTAAAACCATTACAATTCCCATAAAACCATTACAA
ATTCTATGGGGTTTCTATTGGTATTCTATTTTTTTTTTTTATCTTTTTCAGCAGGGTAGATAGAAATAA
GGATGCTGAATGAAAGACGGTTTACCAAAATGCAGTCGTGCCTAAAAAAAAAAAAAAAAAAAAGATTGT
GTAGTGATGCAACCAGTAGCAAGATCACACTGGTTTGAATGCATTCATTATTCGTAATTCATTATTGTG
TGATTGTTTGTCATCAGACATCTCATGATTCCTTATTGTTGTGACATTTTTTGAAAGTTGTTTGACACC
AAAAAGGGTTAAAAGGGTGCTTTACTTCATCTAGTGCTTCCCTATGCATTAGTGTGTGTATGTATGTAT
GTATGTATGTATGTATAGTGTGTATATATAATATACACACACACAAGCTGTCGTTCCCTACTTAGCACT
TTTGTGTATAGCGTATTTGTTGTAGTACATAAGTCACCATTTCTCGTGTTGTCTGTATTCATTAAATTC
GACCAGATTAATGCTGAATGGTCAAACACTTGACTGGTTCAGTTGGAAAATAATTTTTTGACTAACAAT
ATGCTGTTATTTTCTCTTAGATGTAATCGGAGGGTACTCATTAAATGAAGGCCAATCCGAAGCACACTT
TTCAGTGCGATTCTCTGATTATCAGACCCTCACTGAAAGGGCACAGGCTATTTATTTATGTATGTAAGC
AGGCTGTATTTACGTAAGGTGTAATCGAAGAGGATTCGTCTCCTCTCCACTCACTGTTACGGAAGTGTA
TTTCCATAAACAGACTGTTATAAACAAATCAAGTTGTTCTGCGTGGTTGATTTTTGTTGTTGCATTTAT
TTCACATCGTACAAATGTCGCGAAAGAGCTGGAGACGCTGTAGCACTATAGCACGCTGACCTATTCAGG
AAATGTTTTCGCAAAATTTATGGATATTCTGTTGTCAGAGTGAAAGAGACTTGAGACAACAAATGTGAG
CCCAGGACTATCTGTGTCCTGATTGTGTGCATGTGTTGCTTCCCAAGCACCATAACTCTACCTGCTGAA
GTAGGAGGAGTTTGGAGGTCTCCCAAAATTTTTGGATTGAGTAACCTCTGTAACTGTAAAATAAATCCC
ACACCCCGTCAGTACAGAATTATTTTATCAACTTGATTTATTATTCCATTTATAATTTAATTCAAGTAG
TAGGTTTATGATTTTAACTTACGATTTAATAATATTCCGACTATTAATTAGCGCTTTTGAGGTTTTATT
TCTGATCTTTACACTAATGTAATCTATTCGAATTAATTTGACGTTATTTATAGCCCTACTCGTTTATCA
GTTATTGAGCTTTGTGATCAGTAAAACAGGCTAATAATGTATAACTAATCAATAAAGGAAAAAATAACT
TTGAAATGACTTTGATCCCACTTTGAGAACCACTATTTTAAGCTTTAGTGGACACACAGAGAGCAAACT
TTCACTTACTGGCTACCCAGGCACCAAAACAGCAGTGTGTGCCAATACTTTAATCTTTAGCTTAGCTTT
TGAAAGCAGAAGTGGCACTTAGAGCTCACTAAATCCCCTTTCTCATCTTTTTAATACGCACGCATGGCG
ATTTTCCGACTTAACAGATTAATGTTAAGTGGTAAAGATGCTCAGCATCATTCTGCTTTTAGTCTCCTG
CAGTGCAAGGTTGGACAAAGGTAAAGTAAAGTGTTTGTGTGAGAGAGATAGAAAGGGGGTGGGGTGGGG
GGAGTGCTATAAAAGGCCTGCTGCTGAGAGAAAAATGCACAGACTGAGAGACCACATTGTGAAGAGCAG
AAGAACTGTCTGCAGGGCGACTGATCAAGGTAGGTACATGCAAGCTGTGGAACATATAACTATATAATA
TATACGTATTTTCAAACTGGTGTTTGGTTTTCCGTGAGCATAAAGATTTTCAGAGAGGCAGCAGACAAG
TGCTGTGTGTAATGGAATGGCTGAGCAGTTACCTGAGTGAATGTAACTCGGTTGGATTAAATGGTGAGA
GAGTGAGAGAAAGATAGAAACAGATTAAGGCAGAAAGAGAGGAACAGAAGCAAAGAGAGGAAGAAATGA
AGAGTCCATGTGGGTTTCCTCTGGGTGCTCTGGTTTCCACCTACCTTCTGGTAAGATACCACTGTTGAA
GTGTGAATGTCTTGCGATATCCTTGTATTCCATCCAGAGTGTGTTCCCAGTATCGGCTCCAGATCCGTC
GCAACCCTGACCAAGATAAAGCTCTTAGTGAAGAAGAATAAATGAATGTTGTACTATACCATACTCACC
TCACAGCGTGTGAATCCAGGCTTACTGCACAACTTTTATTAGATAATACTATTTACATGCTTAAGGTAA
GATGGATGCTGTCTTTGTTTTCCTGTTTCTTTAGGATGGGTATAAAGCGAGCACTCTGGTGGATGGTGG
TGTGTGTTGTGGTGCTGCAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTG
CAGCGTTGCAGGAGGTAACGGCCACTAAACTAAACAATGTCTAAACTCAATGATATTGTTCTGCTAAGC
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TGAGCTACCAAACCTATACACTTTATTCGTTTTAAATGTGTGTTTGACTGACTTTTTTTTCCCCCCTCC
CGAGACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTACGTAGATGTTTCACCTCGGAA
TAAACTCTACAGGCTGAAAGATCTGCTGGTGCACAGCTCATAATTTTCTTTTTGCTCGTAAGCCAACAA
ATTGAAGGCAGTGTCTTGATGTTTATCTTTTGTCTTCTCTTATAGACCAGTGTTGCTGAGCGAGAAATT
GGACAGTAAGACAAATAACAAAACTGATCAAGAGCTTCAATAAAATGCTTTGCCTTTATTTCGTGTGGG
TTTATTTTTTTATTACCGAAACACTTTCGCTTTTTTTTTTTTTAACGTACTGCTTTTATGTTGCACATTG
TTATTCTCACAATTTATTAGGAATACAGTATTAATAAAATGTGTTATAATTAAGTAATACCACACACAC
AAGAGTGCTGCTCAGCACGGCTGGGATTTTGCCGCAGGTAATCCAGGCGGGTGTGAGCACTGGGTATCC
ATAGGACCCTTTAAAAAAGTTTTTATTCTCATGCATATGTCGTATTCATCCTAATCCTCACTCTATCTC
CATCCCCGACCTTATCCTCATCTTCAACCCCATCTCCATCCCTTTCCTCATCAACAGCATCTTCATTTT
TGTTCTCATCTCCAAAATGGCCTTTTCTGATCATACTGTATGTGACGTGAATCAAGATTCCAAGCAGCT
AAAAATGTTCACGTGATGTAAGATGTCCCTAGTTATCTTACCCCAGCATGTATCATCTGAGCCATCAAC
AGCTGTCTGTATACACATCTCCTTGTACTGAATTCCATTGGAAAAGATATGAATTTCCTGAATCTGTTA
TAATTCATGTTACATTTATAATTGAATGACATCCAAAAGTTCAAAAAATTCTGGACATATCGATAACAA
CTTCACAATAGATGCCATGGCTGAGTATTTTTTTAATTATAAAAATGATTCTATTCTATACAGTAAAGA
TTTATATAATATTAATAACATTCTTTTGCGTTTGCGAGCCTATTAAAAAATGAGAATTCATGCCATGCC
ATGATATTGTGTCATGCAGCTTAATATACATCAGTGGTTTCGTTCTGGAAGATTCTGGAATGAGGACAA
AAATGGATTGCGTTTTGCCACAAGAAAGGATTTTTTCCCCCGCTTTTTAAGATTCACATCTTACAAAAT
GCTCTTTCTTTGAAATTTTGAATTCTTCTACATGGAAGCTATGGCCAAGGAACTTAAAAGGTATGTCCA
TCTTGGTACCTCTGGTCACTCTAGATACGCCATGGAACCACAGACAAATTCTCAAATTCTAAGAAATGA
TATGATAATGTTATTCATTGAAGCAAAAAAGTTATCCAATGTCATCTGGGCCTGTGTGAAAATGTATTT
GCCCCCATAGTTACTAATTCCCCAAATCTATGAAACGGCATTCGTAATGGGGTTCAGCTGGACTAGATG
CAACCAAGCCTGATTACTGCTGGTAAAAGGTTAAAAGGTTTTACCCAGTACGACTCATCCAGGAAGCCA
CAAAAGAGCCAAGGACACCATCAAAGGAACTACAGGCCTCTCTTGCATCAATAAAGGTCACTGTTCATG
ACTCCACTCAGAAAGACACTGGGCAAAAATGGCAACCATGGAAGAGTGGTGAGGTGAAAACCACTGCTA
ACCCAGAAGAACATTAAGGTTCATCTGAATTTTGCCAAAACACACCTTGATGATCAAAATGAACACTGA
AGTCTTAGTGTTGTTTGAGATGGTTCAAATGAGCCCCATGGTCGAAAGTCTGGGACCTCAGGGTCCCAA
GAAACCAACCATGTGAGACCAACTGATTGTGACCAAGCTTCGATGCGCTCTCTTCGGCTTGCTTAAGCT
TCCACCTGCCCCCTGGCTTTCTGGAGCTCTGGAATTGACACCTTCAAGTTAAATTAGGACAGTAAAAAA
AAAAAAAAAACAGTACTTGAATGAAAATTTTTATCTGAAATGTATGTTTCTTAGCTCCTTGTGTGCGCT
GTTCAAACGGAAACAGGATTT
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Figure 33 Diagram (A) and nucleotide sequence (B) of complete *caGnRH* of *I. punctatus*

10.2 PCR walking of *I. furcatus caGnRH*

The forward and reverse primers of each fragment were designed manually and applied for sequencing. The internal forward and reverse primers were designed from the previously obtained sequences and successfully used to isolate the complete genomic sequence of *caGnRH* of *I furcatus* (Figure 34 and 35 and appendix figure 3).

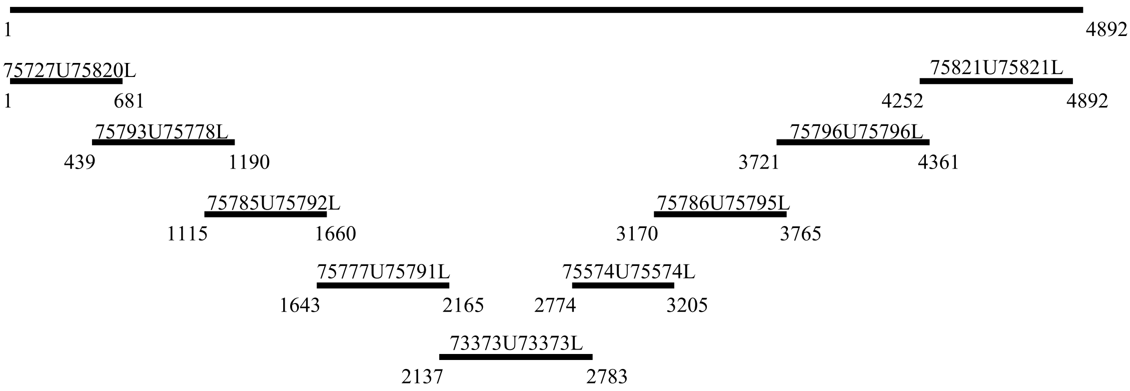
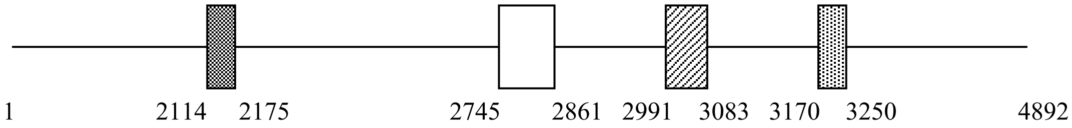


Figure 34 Schematic diagram showing primer walking of *caGnRH* of *I. furcacus*

A.



B.

GCTCCTTGTGTGCGCTGTTCAAACGGAAACAGGATTTTTCTTTCACGTACCGATTTTTATTTCAGATCT
ACCTCCCATAACAAAACTGACTTTTGCTCTCGTCAATCCTGTGGATTTCTTTCAGATCCTCATGAGAAG
GCTCAAATACCCTGCTGAAATAAACAACCACGGAAATTTGAATGGATTCCTCTACAAATACCATTACAA
ACTATCAGCGAACCATTAAAACCATTATCATTATTGGTCCTTAATGGTATCCACCGGACATAACATGAC
ACCAATAGAAAGCAACAAATTATCAGTAGAATTCCCATTATAACCATTACAATTCCCATTAAAACCATT
ACAAATTCTATGAGGGTTTCTATTGGTATTCTGTTTTTTTTTTTCTTTTATCTTTTTCAGCAGGGTAGA
TAGAAATGAGGATGCTGAATGAAAGACGGTTTACCCAAAATGCAGTCGTGCCTAAAAGAAAAAAAAAAA
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Figure 35 Diagram (A) and nucleotide sequence (B) of complete caGnRH of *I. furcatus*

10.3 *Primer walking of I. punctatus cGnRH-II*

The overgo primer, AU50871ovb, from the overgo hybridization step was used for sequencing. The internal forward and reverse primers were designed from the previously obtained sequences and successfully used to isolate the complete genomic sequence of *cGnRH-II* of *I punctatus* (Figure 36 and 37 and appendix figure 4).

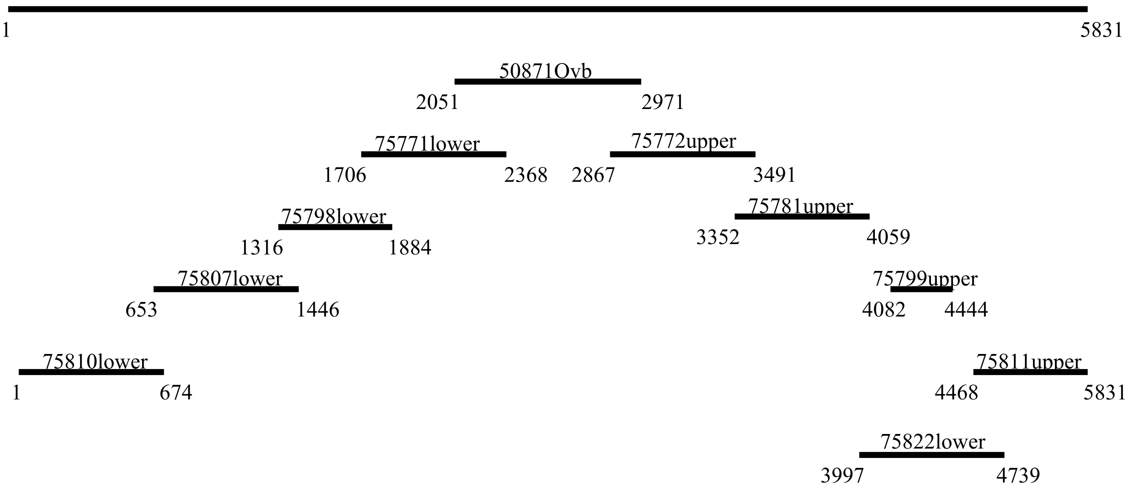
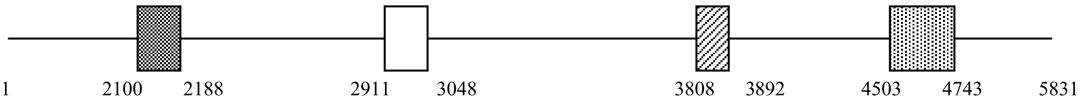


Figure 36 Schematic diagram showing primer walking of *cGnRH-II* of *I. punctatus*

A.



B.

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Figure 37 Diagram (A) and nucleotide sequence (B) of complete *cGnRH-II* of *I. punctatus*

10.4 PCR walking of *I. furcatus cGnRH II*

The complete genomic sequence of cGnRH-II in channel catfish was used to design the forward and reverse primers for sequencing of the ortologous gene in *I. furcatus*. Seven pairs of forward and reverse primer were used for primer walking and successfully identified the complete genomic sequence of *cGnRH-II* of *I. furcatus* (Figure 38 and 39 and appendix figure 5).

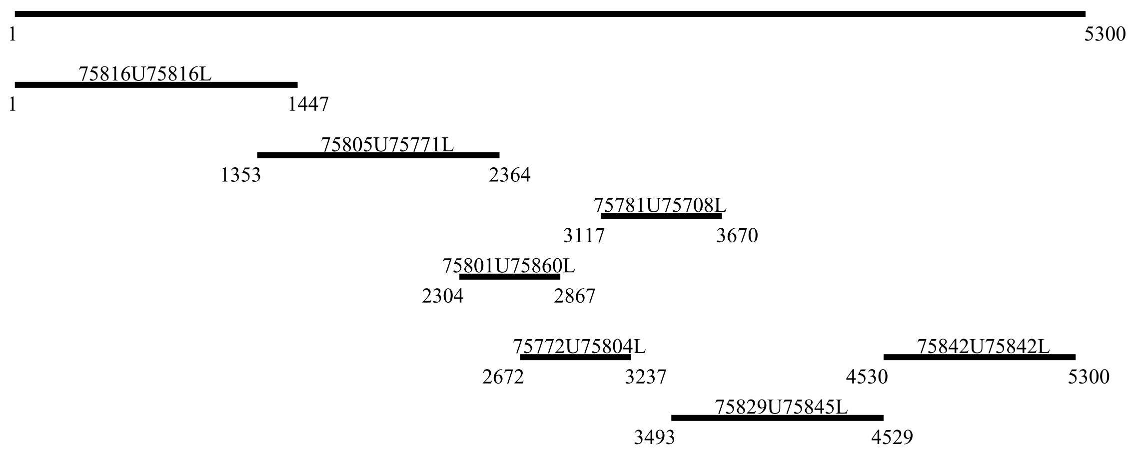

Figure 38 Schematic diagram showing primer walking of *cGnRH-II* of *I. furcatus*

A.

B.

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CCAAATATGTTCCAGTATGGCAATGCCCCTGTGCACAAAGTGAGGTCCATGAAGACATGGTTTGCTGAG
TCTGGATCAGAAGGATTCAAGTGGTCTGCACAGAGCCCTGACCTCAACCCCTTTGAACACCACTGGGAT
GAATTAGAACACCGGGCATCTGTCCGTTTGGGAGTAGATGCCTCTCTCACAGAGTCGTGTGGTTCCACA
GAGGTACTGGGGCGTGTTGGTCCCGGTGTAAGGTTACGACACATCAACCAGCTGAAGATTAGGGCGGTC
CATCTTGGCCTGAAGTGTCAGCTGACACCAATTGCCGGTCCACGCATAATGCTCTG
```

Figure 39 Diagram (A) and nucleotide sequence (B) of complete *cGnRH-II* of *I. furcatus*.

11. Comparisons of *GnRH* cDNA and genomic DNA of *I. punctatus* and *I. furcatus*

11.1 *caGnRH* of *I. punctatus*

The complete *caGnRH* genomic sequence was composed of 4 exons and 3 introns, (Figures 40 and 41). The exon1, 2, 3, and 4 of of genomic sequences were identical with those of *caGnRH* cDNA. The length of each exon and intron of *caGnRH* was illustrated by Table 25. Exon1 contained the signal peptide while exon2 comprised the active site (10 aa), the proteolytic processing site (3 aa) and the 5' portion of GAP (5 aa). Exon3 contained the middle portion of GAP (31 aa) and exon4 contained the 3' portion of GAP (10 aa) and 3'UTR.

Figure 40 Gene organization of *caGnRH* of *I. punctatus* catfish using Spidey

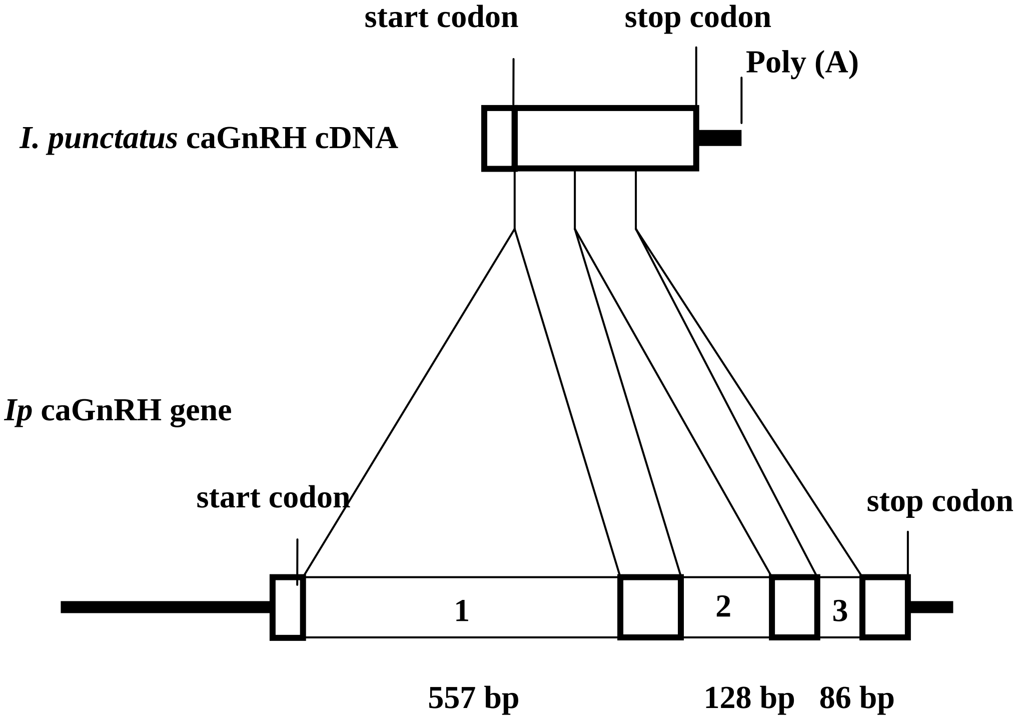


Figure 41 Schematic diagrams of *I. punctatus caGnRH* cDNA and a gene

(Introns are illustrated with numbers shaded.)

Table 27 Length of nucleotide in each part of *caGnRH* genomic sequence of *I. punctatus*

Part	Length
5'binding site - TATA box	2422
TATA box – 5'UTR	31
Exon 1 (red)	61
Intron1 (between exon 1 and 2)	557
Exon 2 (blue)	117
Intron2 (between exon 2 and 3)	128
Exon 3 (green)	93
Intron3 (between exon 3 and 4)	86
Exon 4 (pink)	81
3' binding sites	1758
Total genomic sequence	5334

11.2 *caGnRH* of *I. furcatus*

Likewise, the complete *caGnRH* genomic sequence was composed of 4 exons and 3 introns, (Figures 42 and 43). The exon1, 2, 3, and 4 of of genomic sequences were identical with those of *caGnRH* cDNA. The length of each exon and intron of *caGnRH* was illustrated by Table 28. Exon1 contained the signal peptide (21 aa) while exon2 comprised the active site (10 aa), the proteolytic processing site (3 aa) and the 5' portion of GAP (5 aa). Exon3 contained the middle portion of GAP (31 aa) and exon4 contained the 3' portion of GAP (10 aa) and 3'UTR.

Figure 42 Gene organization of *caGnRH* of *I. furcatus* catfish using Spidey program (box=exon, line=intron)

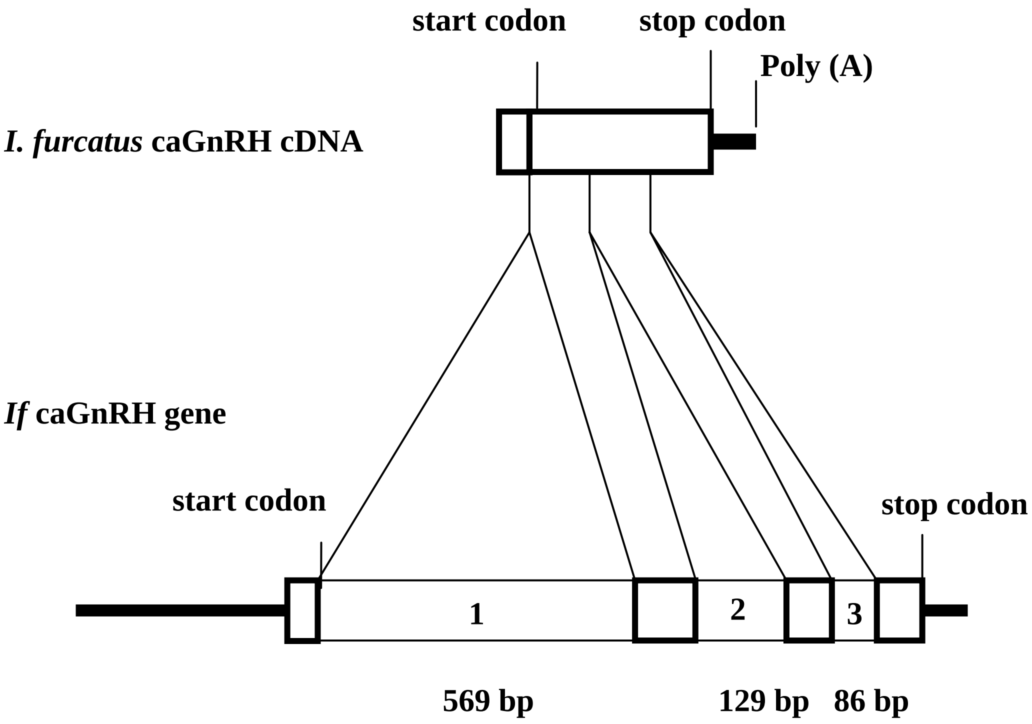


Figure 43 Schematic diagrams of *I.furcatus caGnRH* cDNA and a gene. (Introns are illustrated with numbers shaded.)

Table 28 Length of nucleotide in each part of *caGnRH* genomic sequence of *I. furcatus*

Part	Length
5’binding site - TATA box	2080
TATA box – 5’UTR	33
Exon 1 (red color)	62
Intron1 (between exon 1 and 2)	569
Exon 2 (Blue color)	117
Intron2 (between exon 2 and 3)	129
Exon 3 (green color)	93
Intron3 (between exon 3 and 4)	86
Exon 4 (pink color)	81
3’ binding sites	1642
Total genomic sequence	4892

12. Comparisons of *cGnRH-II* cDNA and genomic DNA of *I. punctatus* and *I. furcatus*

12.1 *cGnRH-II* of *I. punctatus*

The complete *cGnRH-II* genomic sequence was composed of 4 exons and 3 introns, (Figures 44 and 45). The exon1, 2, 3, and 4 of of genomic sequences were identical with those of *cGnRH-II* cDNA. The length of each exon and intron of *caGnRH* was illustrated by Table 29. Exon1 contained the signal peptide (24 aa) while exon2 comprised the active site (10 aa), the proteolytic processing site (3 aa) and the 5’ portion of GAP (9 aa). Exon3 contained the middle portion of GAP (28 aa) and exon4 contained the 3’ portion of GAP (3 aa) and 3’UTR.

Figure 44 Gene organization of *cGnRH-II* of *I. punctatus* using Spidey program (box=exon, line=intron)

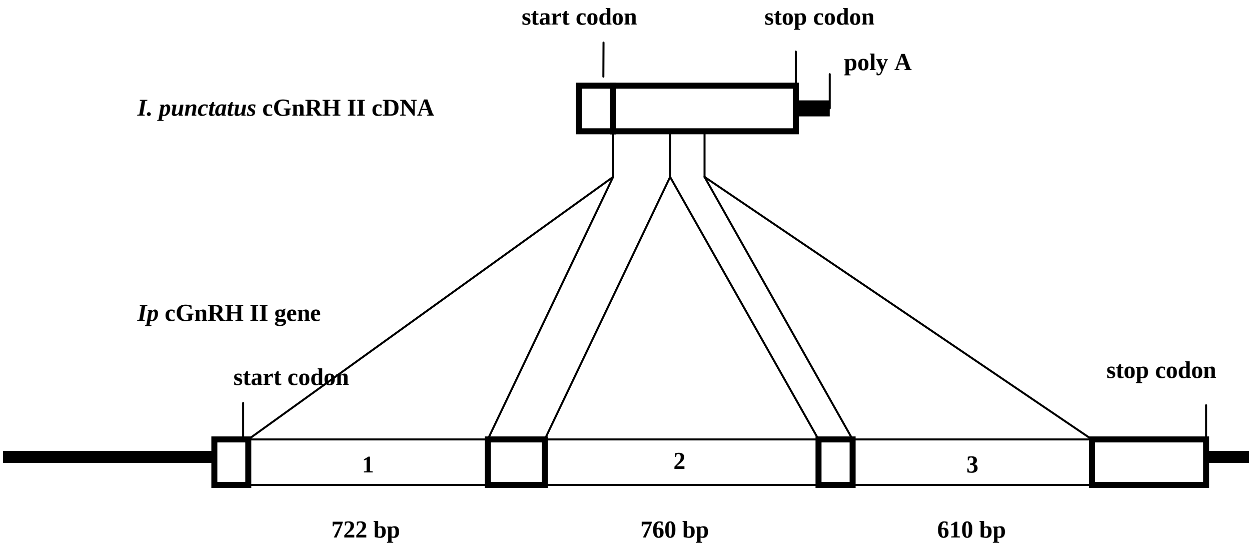


Figure 45 Schematic diagrams of *I. punctatus cGnRH-II* cDNA and a gene (Introns are illustrated with numbers shaded.)

Table 29 Length of nucleotide in each part of *cGnRH-II* genomic sequence of *I. punctatus*

Part	Length
5'binding site - TATA box	2068
TATA box – 5'UTR	31
Exon 1 (red color)	89
Intron1 (between exon 1 and 2)	722
Exon 2 (Blue color)	138
Intron2 (between exon 2 and 3)	760
Exon 3 (green color)	84
Intron3 (between exon 3 and 4)	610
Exon 4 (pink color)	241
3' binding sites	1088
Total genomic sequence	5831

12.2 *cGnRH-II of I. furcatus*

Likewise, complete *cGnRH-II* genomic sequence was composed of 4 exons and 3 introns (Figures 46 and 47). The exon 1, 2, 3, and 4 of of genomic sequences were identical with those of *cGnRH-II* cDNA. The length of each exon and intron of *caGnRH* was illustrated by Table 30. Exon1 contained the signal peptide (24 aa) while exon2 comprised the active site (10 aa), the proteolytic processing site (3 aa) and the 5' portion of GAP (9 aa). Exon3 contained the middle portion of GAP (28 aa) and exon4 contained the 3' portion of GAP (3 aa) and 3'UTR.

Figure 46 Gene organization of *caGnRH* of *I. furcatus* catfish using Spidey program (box=exon, line=intron)

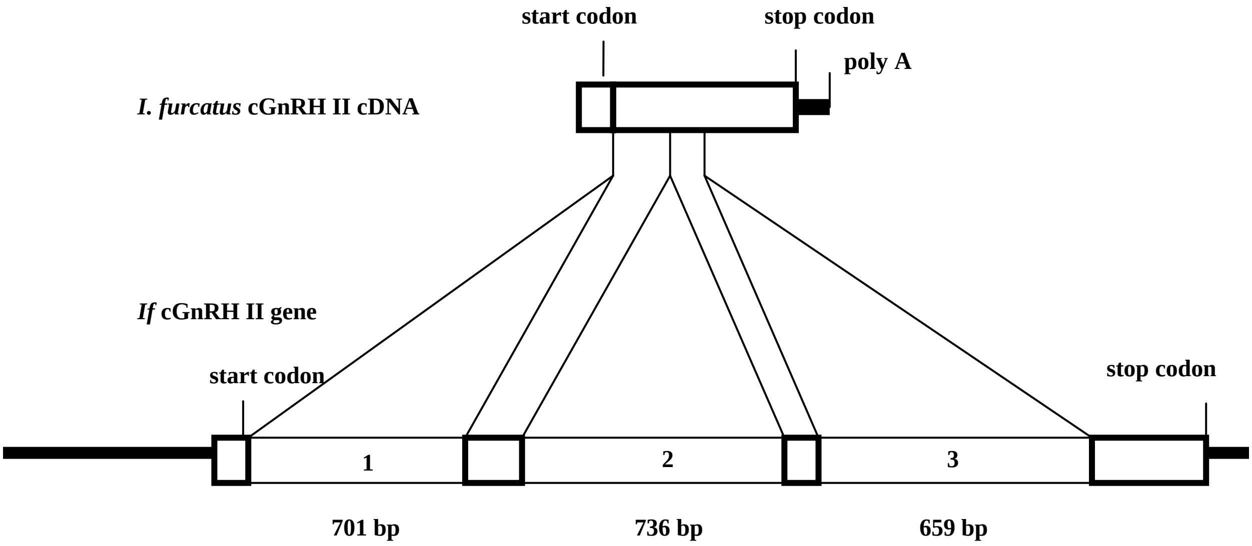


Figure 47 Schematic diagrams of *I. furcatus cGnRH-II* cDNA and a gene (Introns are illustrated with numbers are shaded.)

Table 30 Length of nucleotide in each part of *cGnRH-II* genomic sequence of *I. furcatus*

Part	Length
5'binding site - TATA box	1982
TATA box – 5'UTR	31
Exon 1 (red color)	86
Intron1 (between exon 1 and 2)	701
Exon 2 (Blue color)	138
Intron2 (between exon 2 and 3)	736
Exon 3 (green color)	84
Intron3 (between exon 3 and 4)	659
Exon 4 (pink color)	238
3' binding sites	645
Total genomic sequence	5831

GC content of exons in different types of *GnRH* of *I. punctatus* and *I. furcatus* are illustrated by Table 31. Generally, similar levels of GC content were observed for the same GnRH type except exon1 of *caGnRH* where *I. punctatus* possessed a greater percentage of GC content than did *I. furcatus*. Intron 2 of both GnRH types and intron3 of *caGnRH* followed the GT/AG rule.

Table 31 GC content of exons and 5' and 3' ends of introns of *GnRH* in *I. punctatus* and *I. furcatus*

Type	**%GC content of exon**				**Intron (5' – 3' end)**		
	exon1	**exon2**	**exon3**	**exon4**	**intron1**	**intron2**	**intron3**
I. punctatus caGnRH	50.8	55.6	47.3	37.0	TA-GG	GT-AG	GT-AG
I. furcatus caGnRH	43.6	55.6	47.3	38.3	AT-GG	GT-AG	GT-AG
I. punctatus cGnRH II	37.1	50.7	51.2	40.3	AA-GT	GT-AG	GG-AG
I. furcatus cGnRH II	37.2	50.7	50.0	42.0	AA-TG	GT-AG	GT-TG

13. Comparison of genomic sequence of *I. punctatus* and *I. furcatus GnRH*

13.1 *caGnRH*

Similarity of *caGnRH* of channel and blue catfish was examined by blast analysis. Five portion representing different regions of the *GnRH* gene were matched with high percentage of identity and highly significant E-values (Table 32 and appendix figure 6).

Table 32 Percentage of identity, score and E-value from comparing *caGnRH* of *I. punctatus* and *I. furcatus*

Part	% identity	Score (bits)	Expect value
1	96	3705 (1927)	0
2	95	2379 (1237)	0
3	95	710 (369)	0
4	98	348 (181)	1e-91
5	97	246 (128)	5e-61

13.2 *cGnRH-II*

Likewise, similarity of *caGnRH* of channel and blue catfish was examined by blast analysis. Sixteen portions representing different regions of the *GnRH* gene were matched with high percentage of identity and highly significant E-values (Table 33 and appendix figure 7).

14. Pairwise alignment of *GnRH* genomic sequence of *I. punctatus* and *I. furcatus*

14.1 Catfish type GnRH

Genomic sequence of *caGnRH* was similar interspecifically. High similarity was found in exons and *caGnRH* of both species contained the same repeats and length of microsatellites (appendix figure 7).

14.2 *cGnRH-II*

Several parts of genomic sequence of *cGnRH-II* were similar interspecifically. High similarity was found in exons of this gene. However, the same types of microsatellite repeats but different length were found between these catfish species (Table 34 and appendix figure 8).

Table 33 Percentage if identity, score and E-value from comparing *cGnRH-II* of *I punctatus* and *I. furcatus*

Part	% identity	Score (Bits)	Expect value
1	92	1383 (719)	0
2	92	1063 (553)	0
3	91	927 (482)	0
4	96	575 (299)	6e-160
5	93	408 (212)	1e-109
6	89	319 (166)	6e-83
7	100	267 (139)	2e-67
8	97	233 (121)	6e-57
9	96	189 (98)	1e-43
10	94	183 (95)	7e-42
11	97	175 (91)	1e-39
12	90	148 (77)	2e-31
13	98	117 (61)	3e-22
14	98	93 (48)	1e-14
15	97	62.2 (32)	2e-05
16	84	48.8 (25)	0.23

Table 34 Microsatellite repeats, location and the number of repeat units found in *I. punctatus* and *I. furcatus*

Microsatellites	Location	Channel	Blue
$(TG)_n$	Intron	35	16
$(GT)_n$	Intron	19	15
$(AT)_2(GT)_n$	Intron 1	28	8
$(AT)_n$	Intron 1	6	10
$(TG)_n(AG)_2$	Intron 1	9	14
$(CT)_n(CA)_{12}$	Intron 2	21	10
$(GT)_n$	Intron 3	20	21
$(AC)_n$	Exon 4	7	6

The phylogenetic tree was constructed from nucleotide sequence divergence between genomic sequences of different GnRH types of various species including *caGnRH* and *cGnRH-II* in this study and Nile tilapia (AB104862), and Japanese medaka (AB041334), salmon type of Nile tilapia (AB104863) and Japanese medaka (AB041335), and sea bream type of Nile tilapia (AB104861). The bootstrapped neighbor-joining tree indicated concordant topology with that from cDNA sequences and allocated GnRH to 5 different groups; 1) catfish type of channel and blue catfish, 2) chicken type II of channel and blue catfish, 3) salmon type of Nile tilapia and Japanese medaka, 4) sea bream type of Nile tilapia, 5) chicken type II of Nile tilapia and Japanese medaka (Figure 48).

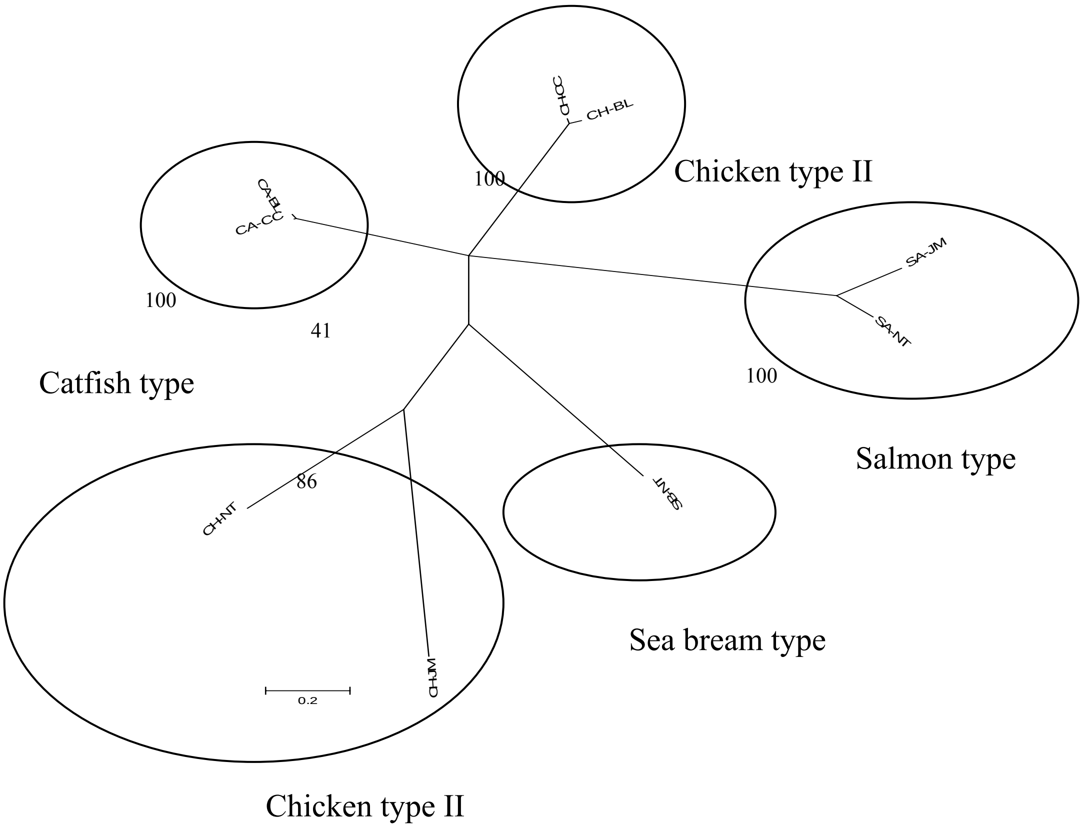


Figure 48 The bootstrapped neighbor-joining tree constructed from genomic sequences of *GnRH* (CA=catfish type; CH=chicken type II; SA=salmon; SB=sea bream; CC=channel catfish; BL=blue catfish; JM=Japanese medaka; NT=Nile tilapia)

15. Expression of *GnRH* in *I. punctatus*

15.1 Quality of total RNA

The quality of total RNA extracted from each tissue of *I. punctatus* was examined using a 1% formaldehyde gels. Two discrete bands representing 28S and 18S RNA were clearly seen across all samples indicating high quality of extracted total RNA (Figure 49).

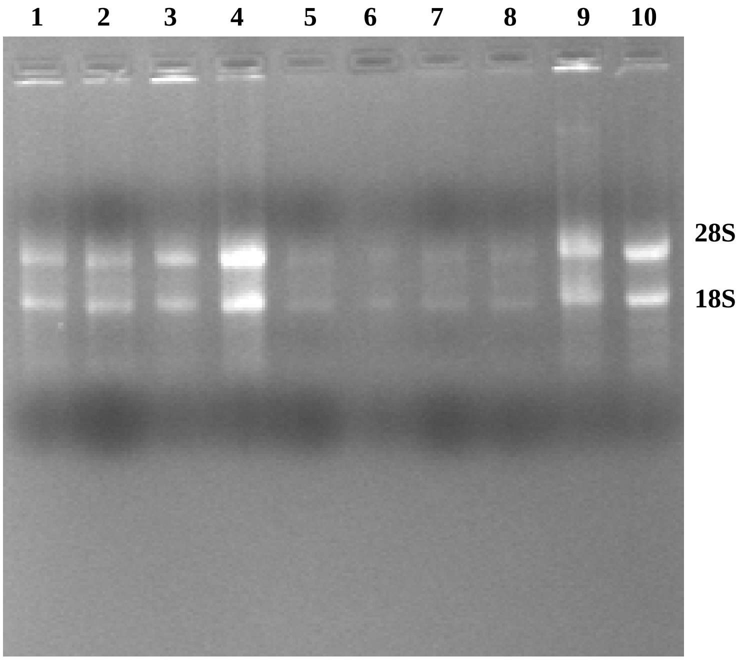


Figure 49 The quality of total RNA extracted from various tissues of channel catfish (Lanes 1-9 are RNA from brain, gill, head kidney, intestine, liver, muscle, ovary, skin, spleen and trunk kidney.)

15.2 RT-PCR

RT-PCR of *caGnRH* and *cGnRH-II* and *β-actin* (the positive control) were performed using the first strand cDNA synthesized from total RNA of various tissues as the template (Figure 50).

A 420 bp, fragment of *β-actin* was consistently amplified from all tissues. The expected size of *caGnRH* (123 bp) was abundantly expressed and only observed in brain of *I. punctatus*. A larger amplification band of 244 bp in length was differentially expressed in head kidney, intestine, spleen, liver, ovary, and trunk kidney. A 141 bp fragment of *cGnRH-II* was successfully amplified in brain, head kidney, intestine, spleen and trunk kidney. This transcript was abundantly expressed in brain and spleen of *I. punctatus*. Like *caGnRH*, a larger amplification product (251 bp) was also found in spleen. Larger bands of *caGnRH* and *cGnRH-II* in spleen were then cloned and sequenced (Figure 50).

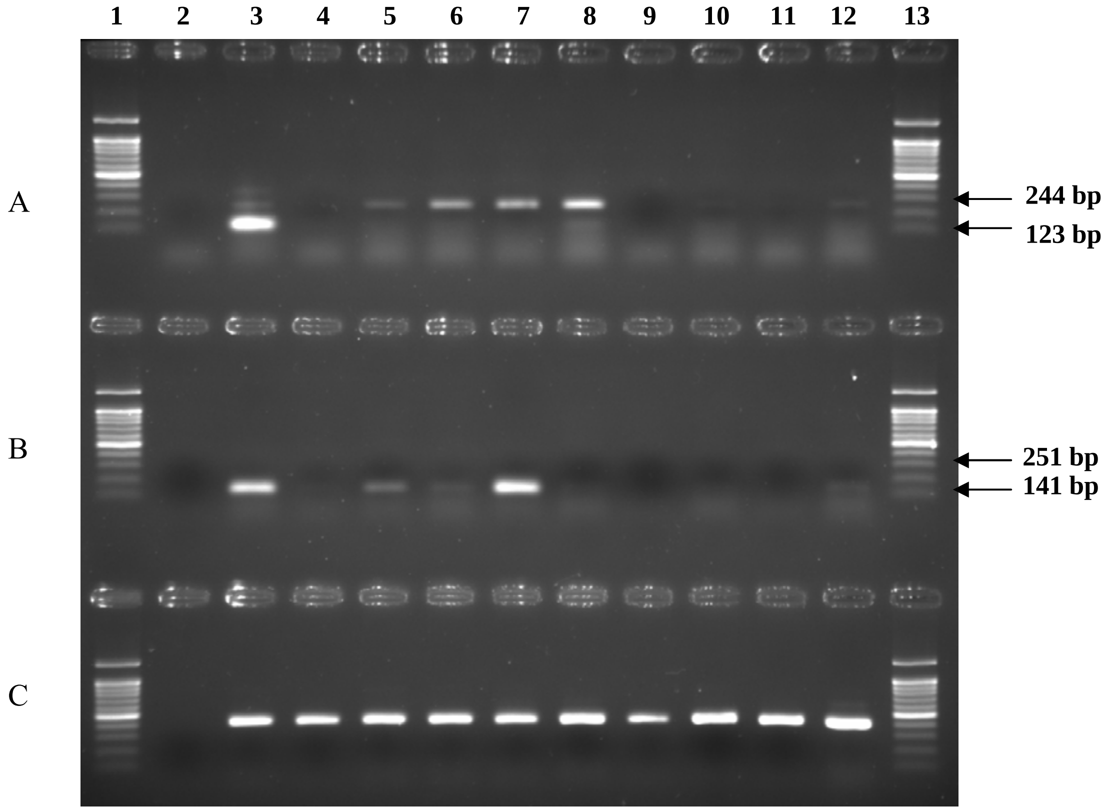
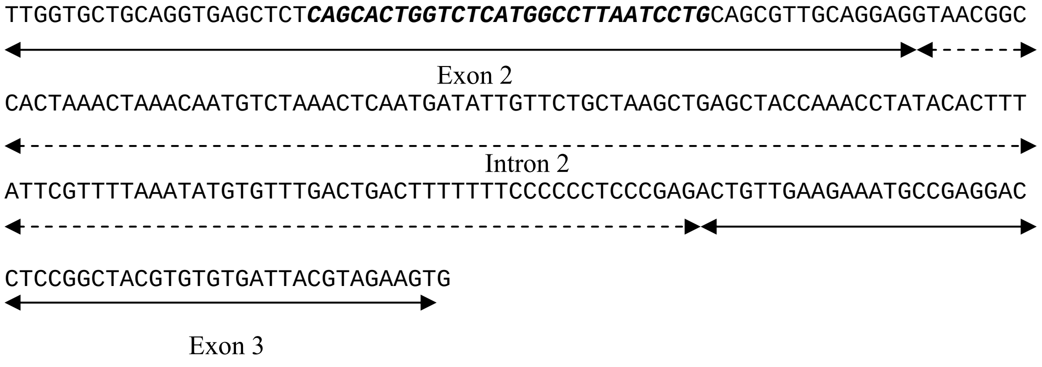


Figure 50 RT-PCR of *caGnRH* (A), *cGnRH II* (B), and *β-actin* (C) (lanes 1 and 13=100 bp DNA marker, 2=negative control; 3=brain; 4=gill; 5=head kidney; 6=intestine; 7=spleen; 8=liver; 9=muscle; 10=ovary; 11=skin; 12=trunk kidney)

15.3 Characterization of larger PCR fragments found from amplification of *caGnRH* and *cGnRH-II*

A. *caGnRH*

The length of investigated fragment was 244 bp. Nucleotide sequence this fragment was similar to part of genomic *caGnRH* (a portion of exon2, intron2, and a portion of exon3) (Figure 51). Pairwise alignments with different regions of *caGnRH* further indicated that an investigated fragment was not the non-specific amplification product but it is a variant of *caGnRH* that lacks the proteolytic processing site in exon2 (Figure 52).



TTGGTGCTGCAGGTGAGCTCT***CAGCACTGGTCTCATGGCCTTAATCCTG***CAGCGTTGCAGGAGGTAACGGC
CACTAAACTAAACAATGTCTAAACTCAATGATATTGTTCTGCTAAGCTGAGCTACCAAACCTATACACTTT
ATTCGTTTTAAATATGTGTTTGACTGACTTTTTTTCCCCCCTCCCGAGACTGTTGAAGAAATGCCGAGGAC
CTCCGGCTACGTGTGTGATTACGTAGAAGTG

Figure 51 Nucleotide sequence of *caGnRH* (The active site of GnRH is boldfaced and italicized.)

A) Exon 2

```
PL1    ------------------------------------------TTGGTGCTGCAGGTGAGC 18
exon2  ATGGGTATAAAGCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGCAGGTGAGC 60
                                                  *****************

PL1    TCTCAGCACTGGTCTCATGGCCTTAATCCTG------------CAGCGTTGCAGGAG 63
exon2  TCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGGAG 117
       *******************************            **************
```

Proteolytic processing site

B) Intron 2

```
PL1  GTAACGGCCACTAAACTAAACAATGTCTAAACTCAATGATATTGTTCTGCTAAGCTGAGC 60
intr2GTAACGGCCACTAAACTAAACAATGTCTAAACTCAATGATATTGTTCTGCTAAGCTGAGC 60
      ************************************************************
PL1  TACCAAACCTATACACTTTATTCGTTTTAAATATGTGTTTGACTGACTTTTTTT-CCCCC 119
intr2TACCAAACCTATACACTTTATTCGTTTTAAATGTGTGTTTGACTGACTTTTTTTTCCCCC 120
     ******************************** ********************* *****
PL1  CTCCCGAG 127
intr2CTCCCGAG 128
     ********
```

C) Exon 3

```
PL1    ACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTACGTAGAAGTG------ 54
exon3  ACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTACGTAGATGTTTCACCT 60
       ************************************************** **
PL1    ---------------------------------
exon3  CGGAATAAACTCTACAGGCTGAAAGATCTGCTG 93
```

Figure 52 Pairwise alignments of a larger PCR fragment from RT-PCR of *caGnRH* and part of *caGnRH* genomic sequences (Abrreviation: PL1= a larger PCR fragment. Exon 2 (A), intron 2 (B), and exon 3 (C) are regions of *GnRH* gene.)

B. *cGnRH-II*

The length of investigated fragment was 251 bp. Nucleotide sequence this fragment was similar to exon2 of *cGnRH-II* (Figure 53). Pairwise alignments with part of *caGnRH* further indicated that an investigated fragment was not the non-specific amplification product but it is a variant of caGnRH that contains the additional 64 bp at the 5'end and 46 nucleotides in exon2 (Figures 54 and 55).

```
GTAATTTGGGAATGGGCCGAGTCCATGATACTGCCGCCATGGTGGCCGCGGGAATTCGCCTGTGATGGTCA
```

Unknown portion

```
GTGGGTGCAAGGGTGAGTGCTCGGTTGCTGCCATTGATGTTGTGTTTGCAGCGCAGCTATCTGTTTCTAAC
```

Exon 2

```
ACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAAAGAAATCGACTCTTACAGTTCACCAGAGAATCACTAG

TGATTTCTCGGCCGCACTGCAGGACGACCATACTGGT
```

Unknown portion

Figure 53 Nucleotide sequence of *cGnRH-II* (Exon2 of *GnRH* is underlined. An unknown gene region was dashed.)

```
PL2  GTAATTTGGGAATGGGCCGAGTCCATGATACTGCCGCCATGGTGGCCGCGGGAATTCGCC 60
exon2------------------------------------------------------------


PL2  TGTGATGGTCAGTGGGTGCAAGGGTGAGTGCTCGGTTGCTGCCATTGATGTTGTGTTTGC 120
exon2----ATGGTCAGTGTGTGCA--GGCTGTTGCT-GGTTGCTGCC-TTGCTGTTGTGTTTGC 52
         ********** *****  **    **** ********** *** ************

PL2  A-GCGCAGCTATCTGTTTCT-AACACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAAAG 178
exon2AAGCGCAGCTATCTGTTTCTCAACACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAG 112
     * ****************** ************************************ **

PL2  AAATCGACTCTTACAGTTCACCAGAGAATCACTAGTGATTTCTCGGCCGCACTGCAGGAC 238
Exon2AAATCGACTCTTACAGCTCACCAGAG---------------------------------- 138
     **************** *********

PL2  GACCATACTGGT 250
exon2------------
```

Figure 54 Pairwise alignments of a larger PCR fragment from RT-PCR of *caGnRH* and part of *cGnRH-II* genomic sequences (Abbreviation: PL2= a larger PCR fragment. Exon 2 is part of c*GnRH-II* gene.)

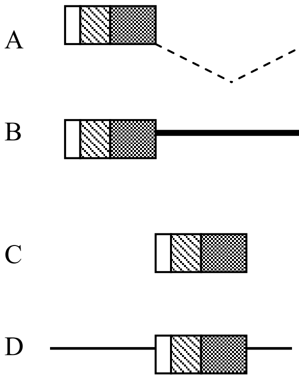


Figure 55 The caGnRH and cGnRH II cDNA transcripts found in brain of *I. punctatus* (The typical caGnRH (A) and cGnRH-II (C) are illustrated in A and B. The variants of *caGnRH* and *cGnRH-II* contain non-spiced intron2 as shown by the thick line (B) and unknown sequences of 64 bp at the 5' end and 46 bp at the 3' end, respectively.)

Discussion

Genetic diversity of domesticated channel and blue catfish

1. Genetic variation within population

Genetic variation within a population determines adaptability of the population to environmental change (Allendorf, 1986). There have been empirical evidences showing the relationship of genetic variation with fitness related traits, e.g. correlation between heterozygosity and reproductive fitness of Chinook salmon (Heath *et al*., 2002); correlation between level of gene diversity and food conversion ratio of rainbow trout (Overturf *et al*., 2003). Generally genetic variation of a strain exposed to domestication and/or selection always decline. In the present study genetic variation of hatchery strains (A = 4.00-6.88, H_o= 0.449-0.775, mean H_o=0.54) in channel catfish and blue catfish (A=2.88-3.38, H_o=0.354-0.504, mean H_o=0.41) were lower than those of a wild population of channel catfish from Missisippi River (A = 9.00, H_o= 0.74 based on 4 microsatellite loci; Waldbieser and Bosworth, 1997). They were also lower than the average A and H_o across 78 freshwater fish (7.5 and

0.46) (DeWoody and Avise, 2000). The present result was supported by Simmons *et al.* (2006) who reported the reduction of the AFLP based genetic variation of 17 hatchery strains relative to that of 14 wild strains of channel catfish.

Loss of alleles is mainly caused by founder effect, using small number of founder individuals, and genetic drift during domestication and/or selection (Lacy, 1987). Although the number of founders was not available for every strain, the history of some strain (e.g T, GK, D, and R) clearly indicated that they were founded from small number of broodstock (e.g. 6-8 pairs for Kansas; 6 pairs for Marion (Dunham and Smitherman, 1984). Generally 50 fish were recommended to retain genetic diversity of a population (Frankham *et al.*, 2004).

The reduction of allele diversity in hatchery strains is a common occurrence. For example, Atlantic salmon reduced *A* from 12.82-17.73 in wild populations to 7.18-10.09 in hatchery populations (Skaala *et al.*, 2004), while sea trout showed *A* of 7.25-8.75 in wild and 5.75-8 in hatchery populations (Was and Wenne, 2002). The substantial loss of number of alleles per locus corrected for sample size (*Ar*) was reported in Senegal sole after one generation of domestication (Porta *et al.*, 2006). The loss of alleles is a signature of genetic drift which occurrs when a small number of broodstock is used. In the selection programs high selection intensity may lead to tremendously reduced number of broodstock and eventually cause losing of genetic variation. During the early period of selection rare alleles will disappear while the heterozygosity is not much affected because low frequency alleles have small contribution to heterozygosity. The present results were supported by Appleyard and Ward (2006) who surveyed genetic diversity of oyster) *Crassostrea gigas*) in Tasmania. They showed that the populations subjected to 3 generations of selection lost some alleles as indicated by the reduction of allelic richness from 5.91-6.56 alleles/locus in wild populations to 4.15-5.76 alleles/locus in the hatchery strains. Nevertheless heterozygosity of these hatchery populations did not change much comparing to those of wild populations (H_o=0.315-0.444 and 0.31-0.445 in wild and hatchery strains respectively).

However, some domesticated populations may retain substantial level of variation [for example the *Brycon opalinus* showed a high value of *A* (13.28) comparing with that of the wild populations (5.43-15.14) (Borroso *et al*., 2005)] due to good management practices, e.g. cross breeding between two strains of channel catfish resulted in *A* of 8 (Waldbieser *et al*., 2001).

The loss of genetic variation in the selected strains is of great concern. Although the variation was determined using neutral markers, there are evidences of correlation between level of genetic variation and fitness of populations, e.g. correlation between heterozygosity and survival of guppy in salt water (Shikano and Taniguchi, 2002); correlation between heterozygosity and reproductive trait (Porta *et al*., 2006). Recently Reed and Frankham (2002) reported a result from meta-analyses based on 34 data-set that measures of genetic diversity correlates with population fitness (r=0.4323), the correlation was highly significant and explained 19% of the variation in fitness. Therefore it is recommended to increase the genetic variation within strains, probably by crossing between strains of different origins (Bakos and Gorda, 1995).

The response to selection is partly determined by genetic variation of the targeted traits (Thelen and Allendorf, 2001) which does not directly correlate with variation of genetic markers. However it is likely that the genetic variation of the traits is always lower than that of the neutral genetic markers due to natural and artificial selection. Therefore it is surmised that the populations with low marker variation may not response well to the artificial selection.

Channel catfish had about twice as many alleles as blue catfish. Blue catfish has low breeding success in captivity (Dunham and Smithnerman, 1987). Therefore it is likely that low number of broodstock were used in each generation and thus has led to a small N_e.

2. Genetic diversity among populations

The F_{ST} greater than 0 indicated that population structuring existed among strains of channel and blue catfish. The differences between strains were obvious which could be explained by different populations of origin and hatchery practices. For example GK and GKal which originated from a single population were significantly different although they have been separated and selected for only 1-2 generations. The results revealed rich gene pools of channel and blue catfish wherein the genetically different strains can be used for further genetic improvement program, e.g. for establishing of the base population, or be used for increasing genetic variation of a strain.

The neighbour joining dendrogram revealed approximately 5 groups of the channel catfish strains. The grouping of strains did not agree with their origins. This could have been the effects of genetic drift. In addition the small sample sizes (5-34 individuals in channel and 11-30 individuals in blue catfish) may be responsible for the unexpected grouping. Despite the low bootstrap values at most of the nodes the five groups should be considered whenever strain crossing is planned.

3. Proportion of genetic variation within and among strains

The AMOVA revealed that variation among populations within species of both channel and blue catfish contributed largest proportion to the overall variation (20.42% in channel and 23.71% in blue catfish) while the variation among individuals within population contributed only 14.16% and 2.13% in channel and blue catfish respectively. The results obviously reflected the effect of selection process and/or other hatchery practices that have diversified genetic background of the strains. Generally in wild populations the variation among populations would have small contribution to the total genetic variation (Bremer *et al.*, 2005). The genetic variation among individuals within strains accounted for the smallest portion of the total variation indicating the homogeneity of the members of each strain. In wild population this proportion of genetic variation would be the largest (Kochzius *et al.*, 2005).

Characterization and expression analysis of GnRH gene from the brain tissue of channel and blue catfish

1. Two types of GnRH are found in the channel and blue catfish

Gonadotropin releasing hormone (GnRH) is an evolutionarily conserved peptide that plays a crucial role in the regulation of reproduction. GnRH is functionally control the releasing of gonadotropins from a pituitary gland. Most vertebrates possess two forms of *GnRH* (hypothalamus, *GnRH1* and midbrain, *GnRH2*) (Guilgur *et al.*, 2006) while only a few species have three *GnRH* types (*GnRH1*, *GnRH2* and olfactory bulbs, *GnRH3*) (Lethimonier *et al.*, 2002) in a single brain.

Teleosts contain two or three variants of *GnRH* (GnRH1, GnRH2 and GnRH3 but the name of variants are more specifically called to mention the original species that were identified and characterized). Three types of *GnRH*; *salmon GnRH* (*sGnRH*), *chicken GnRH-II* (*cGnRH-II*), and *herring GnRH* (*hgGnRH*), were found in the Pacific herring (*Clupea harenguspallasi*) (Carolsfeld *et al.*, 2000). Likewise, the whitefish (*Coregonus clupeaformis*) was also possessed three types of *GnRH*; *sGnRH*, *cGnRH-II*, and *wfGnRH* (Adam *et al.*, 2002). In contrast, two *GnRH* (*sGnRH* and *cGnRH-II*) types were found in the rainbow trout and the masu salmon (Okuzawa *et al.*, 1990; Amano *et al.*, 1991).

The existence of both *GnRH1* (catfish type, *caGnRH*) and *GnRH2* (chicken type II, *cGnRH-II*) encoding the GnRH precursor in the channel and blue catfish is reported for the first time here. The chicken type II *GnRH* is an ancestral form found in all fish species (Sherwood and Lovejoy, 1989) coexisting with the other form (s). In the present study, isolation of different types of *GnRH* was carried out by RACE-PCR, therefore, it is premature to conclude that only 2 types of *GnRH* were existent in the channel and blue catfish. For further characterization of *GnRH* isotypes and other functionally important genes, the cDNA library should be established from brains of each catfish species. Different types of *GnRH* can then be identified by hybridization to determine whether the additional type of *GnRH* is found in these fish or not.

Data across 25 teleosts revealed that *GnRH2* was conserved across species while a remarkable variation of *GnRH1* was observed (O'Neill *et al*., 1998; Lethimonier *et al.* 2004). In the present study both *GnRH1* (*caGnRH*) and *GnRH2* (*cGnRH-II*) showed high sequence similarity between the channel and blue catfish. A lower similarity was found between the *caGnRH* of these and the African catfish (83 % and 82 % for African-channel and African-blue catfish) but greater similarity (94 % and 93 % for African-channel and African-blue catfish) was observed in the *cGn RH-II* interspecifically. This reflected close relationship between the channel and blue than the African catfish.

Interspecific length polymorphism was not found in the ORF of both GnRH types. Nevertheless, the length of 3' UTR of *cGnRH-II* was longer than that of *caGnRH*. This resulted in a longer transcript of the latter *GnRH* gene. Identity of nucleotide sequences varied between regions of *GnRH*. The *GnRH* decapeptide and the proteolytic processing site are highly conserved and showed 100% similarity between the channel and blue catfish where 94-97% sequence similarity was found when compared with *caGnRH* and *cGnRH-II* of the African catfish.

Although it is expected that gene duplication of *GnRH* arisen at approximately 500 million years ago, decapeptide and the processing site are highly conserved even in the Lamprey (Suzuki *et al*., 2000). In contrast, the *GnRH associated peptide* (*GAP*) of *caGnRH* and *cGnRH-II* was less conserved and showed the lowest similarity of 72% and 85% in the channel catfish and 70% and 91% similarity in the blue catfish when compared with the GnRH precursor of the African catfish (Bogerd *et al*., 1994).

Phylogenetic analysis allocated *GnRH* from different fish species to 4 different groups (*sGnRH*, *sbGnRH-II caGnRH* and *cGnRH-II*). Considering the *caGnRH* and *cGnRH-II* groups, sequence divergence of the former was lower than that of the latter. The tree also indicated that *sbGnRH-II* and *caGnRH* are more closely related than other types. This further suggested that *sbGnRH-II* and *caGnRH* are recently derived from the ancestral isotypes.

The basic information on numbers of exons and introns of *GnRH* was limited but resulted from the present study was concordant with those of the Nile tilapia (Kitahashi *et al*., 2005) and *Haplochromis burtoni* (White and Fernald, 1998) for which 4 exons and 3 introns were found. When nucleotide sequences of *GnRH* of the channel and blue catfish were compared with the Nile tilapia and the medaka (Okubo *et al*., 2002), length polymorphism of each intron was observed interspecifically. In addition, substantial variability of nucleotide sequences was found at the introns and untranslated (UTR) regions but limited variability was found at the protein encoding sequences. This further indicated that the coding sequence of GnRH is quite conserved.

2. Gene expression and the alternative splicing of *GnRH*

RT-PCR indicated that the expected size of *caGnRH* (123 bp) was found in brain of the channel catfish whereas a larger product was also observed in other tissues (head kidney, intestine, spleen, liver, ovary, and trunk kidney). This was due to the existence of intron 2 in the PCR product probably resulted from the alternative spicing process in the non-target tissues. In contrast, the expected amplification product of *cGnRH-II* (141 bp) was expressed in the brain and other tissues including head and trunk kidney, intestine and spleen.

Alternative splicing of *GnRH* was previously reported. In the rainbow trout, *sGnRH-II* transcripts in testis but not ovaries was undergone alternative splicing at different stages of spermatogenesis (Uzbekova *et al*., 2001). Likewise, in the lamprey *cGnRH-II* was alternative-spliced at the intron 2 (Suzuki *et al*., 2000). On the contrary, neither alternately spliced *GnRH-1* transcripts nor significant variations of *sGnRH1* expression could be detected during testis development of rainbow trout (Von Schalberg and Sherwood, 1999).

Alternative splicing leads to the production of different proteins from a single pre mRNA and functionally switch on/off during development (Miniatis, 1991). In rat, several GAP molecular forms resulted from the alternative splicing were found in the hypothalamus. Changing in their proportions and regulation by steroids according to the stages of sexual development was noticed (Ackland *et al*., 1988).

It has been reported that *cGnRH-II* does not directly stimulate gonadotropins but rather acts as a neurotransmitter regulating reproduction, e.g. by involving with a female's energetic status (King and Millar,1997; Ngamvongchon and Sherwood, 2001; Temple *et al*., 2003), influencing reproductive behavior in female mammals (Kauffman and Rissman, 2004; Gault *et al*., 2003), birds (Sharp *et al*., 1990), red seabream (Okyzawa et al., 2003) and pejerrey fish (*Odontesthes bonariensis*) (Miranda *et al*., 2003) and regulating osmoregulation of amphibians (Ford *et al*., 2003). The previously reported information and results from RT-PCR of this study implied that *caGnRH* is more functionally important in reproduction than *cGnRH-II*.

Each type of GnRH is expressed differently during gonadal development. In the red seabream, the amount of *sbGnRH* increased in brain and pituitary gland in the immature stage and reached a peak in the spawning stage while the amount of *sGnRH* and *cGnRH-II* remained low in the spawning stage, but were higher in the regressed stage (Senthikumaran *et al*., 1999; Okuzawa *et al*., 2003). Likewise, the levels of *sGnRH*, *cGnRH-II*, and *sbGnRH* mRNAs control ovulation and spawning in the female gilthead seabream and the highest expression level was found at 8 hours before spawning (Gothilf *et al*., 1997).

The higher level of *sbGnRH* in pituitary gland was found from the immature stage to the spawning stage for fish that possess 3 different types of GnRH, such as the gilthead seabream (Gothilf *et al*., 1997; Holland *et al*., 1998), the red seabream (Senthikumaran *et al*., 1999; Okuzawa *et al*., 2003), the European see bass (Rodriguez *et al*., 2000), the turbot (Andersson *et al*., 2001). During testicular maturation of the barfin flounder, a high level of *sbGnRH* was found in brain and pituitary gland but levels of *sGnRH* and *cGnRH-II* were only slightly changed (Amano *et al*., 2004).

Fish that possess two types of GnRH may show different types of *GnRH*, for example, *caGnRH* and *cGnRH-II* in the African catfish and *sGnRH* and *cGnRH-II* in the masu salmon but localization of *caGnRH* and *sGnRH* and *cGnRH-II* is found at the anterior ventral brain (Tel+POA) and midbrain, respectively (Zandbergen *et al*., 1995; Montero *et al*., 1994; Amano *et al*., 1991).

Localization of GnRH in the African catfish brain was studied using *in situ* hybridization. Cell bodies showing signals for *caGnRH* mRNA were scattered in the rostroventral part of the forebrain. They were found in the medial olfactory tract, the ventral telencephalon, the lateral pre-optic area and in the infundibular stalk close to the pituitary. In contrast, cell bodies showing signals for *cGnRH-II* mRNA were exclusively located in the midbrain tegmentum (Bogerd *et al*., 1994). It is interesting to further examine expression levels (e.g by semiquantitative RT-PCR or real-time quantitative RT-PCR) and localization (e.g. by *in situ* hybridization or immunohistochemistry) of different types of *GnRH* during the seasonal reproduction period of the channel and blue catfish.

CONCLUSIONS AND RECOMMENDATION

From the results and discussion of this study, the conclusions can be shown as follow:

1. Genetic variation within domesticated strains of the channel and blue catfish was limited and may affect the sustainability of the maintained populations and the selection responses. Therefore it is recommended to increase intrapopulational genetic variation of the target strains before selection is conducted.

2. Two types of *GnRH* genes, *caGnRH* and *cGnRH-II*, of the channel and blue catfish were identified and characterized. The full length cDNA of *caGnRH* in respective species was 370 bp and 362 bp with the identical length of ORF of 243 bp corresponding to a deduced protein of 80 amino acid residues. The full length cDNA of *cGnRH-II* in respective species was 563 bp and 568 bp with the identical length of ORF of 259 bp corresponding to a deduced protein of 86 amino acid residues.

3. Genomic organization of *caGnRH* and *cGnRH-II* of the channel and blue catfish was examined by sequencing the portion of positively hybridized BAC clones. Both GnRH isotypes contain 4 exons and 3 introns as previously reported in other fish species.

4. Expression of *caGnRH* and *cGnRH-II* was examined by RT-PCR. The expected size of *caGnRH* was found in brain of the channel catfish whereas the larger amplification product containing intron 2 which was possibly resulted from alternative splicing was also found in head kidney, intestine, spleen, liver, ovary, and trunk kidney. The expected amplification product of *cGnRH-II* was found in brain, head kidney, intestine, spleen and trunk kidney of the channel catfish.

5. RNA interference (RNAi) of GnRH should be carried out to control sexual maturation of the channel and blue catfish. This technique can be applied for production of sterile transgenic channel and blue catfish in the future.

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APPENDIX

Appendix A

Genetic diversity of domesticated channel and blue catfish

Appendix Table A1 Summary of genetic variation of channel and blue catfish strains based on 8 microsatellite loci.

Locus		**Populations**																			
	Allele	**T**	**AF**	**AR**	**AS**	**GK**	**GKal**	**KR**	**KS**	**MK**	**MS**	**TA**	**ARMK**	**MR**	**S1**	**S2**	**D**	**R**	**AR**	**ARR**	**DxR**
AU935	A	4	3	4	4	5	3	4	5	5	5	4	5	5	5	4	4	3	3	2	2
	A_r	3.453	2.231	3.443	3.059	3.232	2.785	3.385	3.651	2.932	3.637	4	3.344	3.726	3.13	2.542	3.363	2.792	1.9	1.9	1.976
	H_e	0.718	0.417	0.721	0.612	0.623	0.64	0.688	0.74	0.596	0.749	0.821	0.662	0.747	0.653	0.545	0.347	0.255	0.099	0.119	0.265
	H_o	0.455	0.095	0.44	0.417	0.357	0.231	0.357	0.688	0.364	1	1	0.696	0.75	0.63	0.731	0.385	0.182	0.1	0.1	0.308
	HW	0.0001	<0.0001	0.0003	0.3531	0.0063	0.007	<0.0001	0.0059	0.0039	0.3866	1	0.3187	<0.0001	0.3078	<0.0001	1	0.1444	1	-	1
	F_{is}	0.372*	0.776*	0.394*	0.329	0.44	0.649*	0.485*	0.072*	0.396*	-0.358	-0.263	-0.052	-0.004*	0.037	-0.349*	-0.121	0.298	-0.013	-	-0.163
AU936	A	3	3	5	3	2	3	5	4	4	3	3	3	3	3	3	3	3	4	4	2
	A_r	2.883	2.817	3.101	2.376	1.998	2.93	3.775	2.511	2.804	2.344	2.8	2.626	2.301	2.746	2.637	2.914	2.816	3.816	3.696	2
	H_e	0.665	0.641	0.64	0.562	0.516	0.685	0.767	0.545	0.558	0.515	0.6	0.533	0.547	0.627	0.589	0.563	0.325	0.72	0.634	0.456
	H_o	0.5	0.524	0.385	0.421	0.357	0.583	0.36	0.382	0.64	0.727	0.8	0.381	0.958	0.5	0.385	0.692	0.364	0.813	1	0.679
	HW	0.3647	0.0299	<0.0001	0.5691	0.3175	0.0541	<0.0001	0.0226	0.6644	0.2977	1	0.2081	<0.0001	0.0273	0.1255	0.01	1	0.0171	0.0109	0.0096
	F_{is}	0.252	0.187	0.403*	0.256	0.316	0.154	0.536*	0.301	-0.151	-0.441	-0.391	0.29	-0.781*	0.206	0.291	-0.241	-0.127	-0.134	-0.62	-0.5
AU904	A	4	3	4	2	3	4	4	3	2	1	2	1	1	3	3	3	3	3	3	4
	A_r	2.438	2.519	2.634	1.607	2.937	3.128	3.49	1.651	1.98	1	1.8	1	1	1.846	1.44	2.688	2.997	2.45	2.75	3.595
	H_e	0.481	0.521	0.52	0.185	0.69	0.665	0.725	0.172	0.47	-	0.2	-	-	0.262	0.112	0.335	0.628	0.445	0.562	0.555
	H_o	0.545	0.429	0.308	0.1	0.714	1	0.786	0.182	0	-	0.2	-	-	0.296	0.115	0.154	0.818	0.55	0.5	0.571
	HW	0.0147	0.2351	0.0016	0.1512	0.0832	0.0064	0.0672	1	<0.0001	-	-	-	-	1	1	0.0239	0.3215	0.0863	1	0.2264
	F_{is}	-0.138	0.182	0.413*	0.465	-0.036	-0.537	-0.085	-0.058	1*	-	-	-	-	-0.134	-0.027	0.551	-0.324	-0.244	0.114	-0.03
AU954	A	6	5	7	5	6	4	7	5	7	5	4	5	4	5	7	5	4	2	6	9
	A_r	3.395	2.715	3.216	3.12	4.437	3.177	4.116	2.964	4.19	3.754	3.756	3.106	3.274	3.094	4.515	4.872	3.79	1.994	5.764	6.855
	H_e	0.686	0.591	0.645	0.57	0.82	0.686	0.775	0.588	0.791	0.71	0.733	0.652	0.697	0.598	0.811	0.641	0.463	0.309	0.799	0.845
	H_o	0.682	0.905	0.115	0.421	0.357	0.846	0.25	0.063	0.417	0.455	0.6	0.417	0.429	0.222	0.577	0.417	0.364	0.351	0.455	0.741
	HW	0.0835	0.0017	<0.0001	0.1276	0.0003	0.0698	<0.0001	<0.0001	0.0008	0.0005	0.1072	0.0064	0.0003	<0.0001	0.0002	0.0852	0.1438	1	0.0042	0.0001
	F_{is}	0.006	-0.551*	0.824*	0.267	0.574*	-0.245	0.681*	0.895*	0.478*	0.371*	0.2	0.366	0.389*	0.633*	0.293*	0.36	0.223	-0.2	0.429*	0.125*
AU959	A	6	6	9	7	8	7	8	8	8	5	6	5	6	8	10	2	3	3	1	2
	A_r	4.096	3.66	4.508	4.222	5.513	4.739	4.62	4.766	4.725	3.607	5.356	3.876	4.338	4.725	5.383	2	2.997	2.446	1	2
	H_e	0.792	0.724	0.788	0.777	0.895	0.834	0.818	0.825	0.839	0.736	0.889	0.772	0.81	0.837	0.882	0.492	0.61	0.368	-	0.448
	H_o	0.955	0.619	0.808	0.857	0.846	0.769	0.704	0.697	0.609	0.818	1	0.833	0.893	0.815	0.885	0.769	0.364	0.25	-	0.103
	HW	0.0528	0.0001	0.0064	0.8525	0.0544	0.3992	0.0758	0.0001	0.0149	0.7952	0.6217	0.313	0.0259	0.1669	0.2818	0.0742	0.025	0.3135	-	0.0001
	F_{is}	-0.212	0.148*	-0.025	-0.106	0.057	0.08	0.152	0.157*	0.279	-0.118	-0.143	-0.081	-0.104	0.027	-0.003	-0.6	0.416	0.326	-	0.772*

Appendix Table A1 (continued)

Locus	Populations																				
	Allele	**T**	**AF**	**AR**	**AS**	**GK**	**GKal**	**KR**	**KS**	**MK**	**MS**	**TA**	**ARMK**	**MR**	**S1**	**S2**	**D**	**R**	**AR**	**ARR**	**DxR**
AU865	A	7	5	9	6	8	7	8	9	10	4	3	8	4	5	6	4	3	5	3	3
	A_r	4.374	2.753	4.576	3.974	5.193	4.876	5.144	4.476	3.319	2.333	2.978	4.677	3.038	3.983	3.745	3.891	2.995	4.725	3	2.943
	H_e	0.807	0.601	0.812	0.758	0.826	0.849	0.863	0.81	0.539	0.333	0.689	0.834	0.654	0.778	0.726	0.588	0.568	0.681	0.686	0.598
	H_o	0.636	0.15	0.28	0.55	0.727	0.923	0.52	0.281	0.348	0.364	0.6	0.286	0.464	0.148	0.269	0.077	0.8	0.95	0.444	0.4
	HW	0.002	<0.0001	<0.0001	0.0142	0.3073	0.6207	<0.0001	<0.0001	<0.0001	1	0.1101	<0.0001	0.0017	<0.0001	<0.0001	<0.0001	0.3606	0.0768	0.2602	0.0077
	F_{is}	0.215*	0.755*	0.66*	0.279	0.167	-0.091	0.402*	0.656*	0.36*	-0.096	0.143	0.663*	0.294*	0.813*	0.634*	0.874*	-0.44	-0.41	0.366	0.335
AU1097	A	7	2	6	6	7	5	7	5	7	5	4	6	6	4	4	3	3	3	3	2
	A_r	4.082	1.911	3.638	3.382	3.809	4.017	3.757	4.158	4.682	3.453	3.6	4.166	3.729	3.075	2.841	2.913	2.636	2.45	3	1.997
	H_e	0.766	0.372	0.718	0.704	0.714	0.797	0.747	0.799	0.826	0.662	0.711	0.776	0.707	0.654	0.624	0.44	0.177	0.537	0.692	0.364
	H_o	0.773	0.476	0.654	0.905	0.929	1	0.643	0.706	0.880	0.545	0.4	0.917	0.893	0.435	0.308	0.308	0.091	1	0.333	0.467
	HW	0.0869	0.2897	0.0038	0.0018	0.0635	0.6845	0.0005	<0.0001	0.023	0.1741	0.1404	0.0002	0.3449	0.0308	0.0018	0.187	0.0504	<0.0001	0.0129	0.2922
	F_{is}	-0.008	-0.29	0.091*	-0.295*	-0.315	-0.268	0.142*	0.119*	-0.067	0.184	0.467	-0.186*	-0.269	0.34	0.512*	0.309	0.5	-0.905*	0.529	-0.289
AU1081	A	9	10	8	9	6	6	9	10	12	4	6	9	8	9	8	2	1	3	2	3
	A_r	4.912	5.287	4.783	4.701	4.416	4.518	4.634	5.356	5.805	3.567	5.356	5.129	4.192	3.701	4.647	1.978	1	2.901	1.999	2.89
	H_e	0.846	0.872	0.839	0.822	0.815	0.825	0.805	0.877	0.905	0.753	0.889	0.865	0.778	0.614	0.825	0.212	-	0.415	0.290	0.592
	H_o	0.591	0.762	0.615	0.550	0.786	0.769	0.714	0.735	0.72	0.600	0.8	0.739	0.75	0.545	0.654	0.077	-	0	0	0
	HW	0.0021	0.0020	0.0084	0.0049	0.5308	0.0409	0.0091	0.0052	0.0344	0.2097	0.614	0.2709	0.059	0.0373	0.0073	0.119	-	<0.0001	0.0065	<0.0001
	F_{is}	-0.008*	-0.29*	0.091*	-0.295*	-0.315	-0.268	0.142	0.119*	-0.067	0.184	0.467	-0.186	-0.269	0.34	0.512	0.647	-	1*	1	1*
	Mean	5.75	4.625	6.5	5.25	5.625	4.875	6.5	6.125	6.875	4	4	5.25	4.625	5.625	5.625	3.25	2.875	3.25	3	3.375

A=total number of alleles per locus, A_r=allelic richness, H_e=expected heterozygosity, H_o=observed heterozygosity, HW=probability of departure from Hardy-Weinberg equilibrium ($\alpha = 0.05$), F_{is}=fixation index, and mean=average number of allele per locus per population (under bonferroni correction; α=0.0062)

Appendix B
Characterization and expression analysis of GnRH gene from the brain tissue of channel and blue catfish

Appendix B

AU75554upper ATGGGTATAAAGCGAGCACTCTGG (3072-3095)

GGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGGAGGTAACGGCCACTAAACTAAACAATGTCTAA
ACTCAATGATATTGTTCTGCTAAGCTGAGCTACCAAACCTATACACTTTATTCGTTTTAAATGTGTGTT
TGACTGACTTTTTTTTCCCCCCTCCCGAGACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTG
ATTACGTAGATGTTTCACCTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGGTGCACAGCTCATAAT
TTTCTTTTTGCTCGTAAGCCAACAAATTGAAGGCAGTGTCTTGATGTTTATCTTTTGTCTTCTCTTATA
GACCAGTGTTGCTGAGCGAGAAATTGGACAGTAAGACAAATAACAAAACTGATCAAGAGCTTCAATAAA
ATGCTTTGCCTTTATTTCGTGTGGGTTTATTTTTTTATTACCGAAACACTTTCGCTTTTTTTTTTTTAA
CGTACTGCTTTTATGTTGCACATTGT

AU75242lower CTTTCCTCCAGGATTGAGACCATGAGACCAGTGCTGA (3134-3170)

CCCAGGACTATCTGTGTCCTGATTGTGTGCATGTGTTGCTTCCCAAGCACCATAACTCTACCTGCTGAA
GTAGGAGGAGTTTGGAGGTCTCCCAAAATTTTTGGATTGAGTAACCTCTGTAACTGTAAAATAAATCCC
ACACCCCGTCAGTACAGAATTATTTTATCAACTTGATTTATTATTCCATTTATAATTTAATTCAAGTAG
TAGGTTTATGATTTTAACTTACGATTTAATAATATTCCGACTATTAATTAGCGCTTTTGAGGTTTTATT
TCTGATCTTTACACTAATGTAATCTATTCGAATTAATTTGACGTTATTTATAGCCCTACTCGTTTATCA
GTTATTGAGCTTTGTGATCAGTAAAACAGGCTAATAATGTATAACTAATCAATAAAGGAAAAAATAACT
TTGAAATGACTTTGATCCCACTTTGAGAACCACTATTTTAAGCTTTAGTGGACACACAGAGAGCAAACT
TTCACTTACTGGCTACCCAGGCACCAAAACAGCAGTGTGTGCCAATACTTTAATCTTTAGCTTAGCTTT
TGAAAGCAGAAGTGGCACTTAGAGCTCACTAAATCCCCTTTCTCATCTTTTTAATACGCACGCATGGCG
ATTTTCCGACTTAACAGATTAATGTTAAGTGGTAAAGATGCTCAGCATCATTCTGCTTTTAGTCTCCTG
CAGTGCAAGGTTGGACAAAGGTAAAGTAAAGTGTTTGTGTGAGAGAGATAGAAAGGGGGTGGGGTGGGG
GGAGTGCTATAAAAGGCCTGCTGCTGAGAGAAAAATGC**ACAGACTGAGAGACCACATTGTGAAGAGCAG**
AAGAACTGTCTGCAGGGCGACTGATCAAGGTAGGTACATGCAAGCTGTGGAACATATAACTATATAATA
TATACGTATTTTCAAACTGGTGTTTGGTTTTCCGTGAGCATAAAGATTTTCAGAGAGGCAGCAGACAAG
TGCTGTGTGTAATGGAATGGCTGAGCAGTTACCTGAGTGAATGTAACTCGGTTGGATTAAATGGTGAGA
GAGTGAGAGAAAGATAGAAACAGATTAAGGCAGAAAGAGAGGAACAGAAGCAAAGAGAGGAAGAAATGA
AGAGTCCATGTGGGTTTCCTCTGGGTGCTCTGGTTTCCACCTACCTTCTGGTAAGATACCACTGTTGAA
GTGTGAATGTCTTGCGATATCCTTGTATTCCATCCAGAGTGTGTTCCCAGTATCGGCTCCAGATCCGTC
GCAACCCTGACCAAGATAAAGCTCTTAGTGAAGAAGAATAAATGAATGTTGTACTATACCATACTCACC
TCACAGCGTGTGAATCCAGGCTTACTGCACAACTTTTATTAGATAATACTATTTACATGCTTAAGGTAA
GATGGATGCTGTCTTTGTTTTCCTGTTTCTT

AU75508lower GCAACGCTGCACGCTTTCCTCCAG (3159-3183)

AGGCACCAAAACAGCAGTGTGTGCCAATACTTTAATCTTTAGCTTAGCTTTTGAAAGCAGAAGTGGCAC
TTAGAGCTCACTAAATCCCCTTTCTCATCTTTTTAATACGCACGCATGGCGATTTTCCGACTTAACAGA
TTAATGTTAAGTGGTAAAGATGCTCAGCATCATTCTGCTTTTAGTCTCCTGCAGTGCAAGGTTGGACAA
AGGTAAAGTAAAGTGTTTGTGTGAGAGAGATAGAAAGGGGGTGGGGTGGGGGGAGTGCTATAAAAGGCC
TGCTGCTGAGAGAAAAATGCACAGACTGAGAGACCACATTGTGAAGAGCAGAAGAACTGTCTGCAGGGC
GACTGATCAAGGTAGGTACATGCAAGCTGTGGAACATATAACTATATAATATATACGTATTTTCAAACT
GGTGTTTGGTTTTCCGTGAGCATAAAGATTTTCAGAGAGGCAGCAGACAAGTGCTGTGTGTAATGGAAT
GGCTGAGCAGTTACCTGAGTGAATGTAACTCGGTTGGATTAAATGGTGAGAGAGTGAGAGAAAGATAGA
AACAGATTAAGGCAGAAAGAGAGGAACAGAAGCAAAGAGAGGAAGAAATGAAGAGTCCATGTGGGTTTC
CTCTGGGTGCTCTGGTTTCCACCTACCTTCTGGTAAGATACCACTGTTGAAGTGTGAATGTCTTGCGAT
ATCCTTGTATTCCATCCAGAGTGTGTTCCCAGTATCGGCTCCAGATCCGTCGCAACCCTGACCAAGATA

Appendix Figure B1 The primer walking of caGnRH genomic sequence in channel catfish

AAGCTCTTAGTGAAGAAGAATAAATGAATGTTGTACTATACCATACTCACCTCACAGCGTGTGAATCCA
GGCTTACTGCACAACTTTTATTAGATAATACTATTTACATGCTTAAGGTAAGATGGATGCTGTCTTTGT
TTTCCTGTTTCTTTAGG**ATGGGTATAAAGCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGC**

AU75769upper GAATGTTGTACTATACCATACTCAC (2942-2966)

AAGCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGCAGGTGAGCTCTCAGCACTGGTCTCAT
GGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGGAGGTAACGGCCACTAAACTAAACAATGTCTAA
ACTCAATGATATTGTTCTGCTAAGCTGAGCTACCAAACCTATACACTTTATTCGTTTTAAATGTGTGTT
TGACTGACTTTTTTTTCCCCCCTCCCGAGACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTG
ATTACGTAGATGTTTCACCTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGGTGCACAGCTCATAAT
TTTCTTTTTGCTCGTAAGCCAACAAATTGAAGGCAGTGTCTTGATGTTTATCTTTTGTCTTCTCTTATA
GACCAGTGTTGCTGAGCGAGAAATTGGACAGTAAGACAAATAACAAAACTGATCAAGAGCTTCAATAAA
ATGCTTTGCCTTTATTTCGTGTGGGTTTATTTTTTTATTACCGAAACACTTTCGCTTTTTTTTTTTTAA
CGTACTGCTTTTATGTTGCACATTGTTATTCTCACAATTTATTAGGAATACAGTATTAATAAAATGTGT
TATAATTAAGTAATACCACACACACAAGAGTGCTGCTCAGCACGGCTGGGATTTTGCCGCAGGTAATCC
AGGCGGGTGTGAGCACTGGGTATCCATAGGACCCTTTAAAAAAGTTTTTATTCTCATGCATATGTCGTA
T

AU75770lower GGATTTAGTGAGCTCTAAGTGCC (2222-2244)

CGTGTTGTCTGTATTCATTAAATTCGACCAGATTAATGCTGAATGGTCAAACACTTGACTGGTTCAGTT
GGAAAATAATTTTTTGACTAACAATATGCTGTTATTTTCTCTTAGATGTAATCGGAGGGTACTCATTAA
ATGAAGGCCAATCCGAAGCACACTTTTCAGTGCGATTCTCTGATTATCAGACCCTCACTGAAAGGGCAC
AGGCTATTTATTTATGTATGTAAGCAGGCTGTATTTACGTAAGGTGTAATCGAAGAGGATTCGTCTCCT
CTCCACTCACTGTTACGGAAGTGTATTTCCATAAACAGACTGTTATAAACAAATCAAGTTGTTCTGCGT
GGTTGATTTTTGTTGTTGCATTTATTTCACATCGTACAAATGTCGCGAAAGAGCTGGAGACGCTGTAGC
ACTATAGCACGCTGACCTATTCAGGAAATGTTTTCGCAAAATTTATGGATATTCTGTTGTCAGAGTGAA
AGAGACTTGAGACAACAAATGTGAGCCCAGGACTATCTGTGTCCTGATTGTGTGCATGTGTTGCTTCCC
AAGCACCATAACTCTACCTGCTGAAGTAGGAGGAGTTTGGAGGTCTCCCAAAATTTTTGGATTGAGTAA
CCTCTGTAACTGTAAAATAAATCCCACACCCCGTCAGTACAGAATTATTTTATCAACTTGATTTATTAT
TCCATTTATAATTTAATTCAAGTAGTAGGTTTATGATTTTAACTTACGATTTAATAATATTCCGACTAT
TAATTAGCGCTTTTGAGGTTTTATTTCTGATCTTTACACTAATGTAATCTATTCGAATTAATTTGACGT
TATTTATAGCCCTACTCGTTTATCAGTTATTGAGCTTTGTGATCAGTAAAACAGGCTAATAATGTATAA
CTAATCAATAAAGGAAAAAATAACTTTGAAATGACTTTGATCCCACTTT

AU75778lower CGCGACATTTGTACGATGTG (1521-1539)

TTGGTCTTCAATGGTATCCACCGGACATAACATGTCACCAATAGAAAGCAACAAATTATCAGTAGAATT
CCCATTAAAACCATTACAATTCCCATAAAACCATTACAAATTCTATGGGGTTTCTATTGGTATTCTATT
TTTTTTTTTTATCTTTTTCAGCAGGGTAGATAGAAATAAGGATGCTGAATGAAAGACGGTTTACCAAAA
TGCAGTCGTGCCTAAAAAAAAAAAAAAAAAAAAGATTGTGTAGTGATGCAACCAGTAGCAAGATCACAC
TGGTTTGAATGCATTCATTATTCGTAATTCATTATTGTGTGATTGTTTGTCATCAGACATCTCATGATT
CCTTATTGTTGTGACATTTTTTGAAAGTTGTTTGACACCAAAAAGGGTTAAAAGGGTGCTTTACTTCAT
CTAGTGCTTCCCTATGCATTAGTGTGTGTATGTATGTATGTATGTATGTATGTATAGTGTGTATATATA
ATATACACACACACAAGCTGTCGTTCCCTACTTAGCACTTTTGTGTATAGCGTATTTGTTGTAGTACAT
AAGTCACCATTTCTCGTGTTGTCTGTATTCATTAAATTCGACCAGATTAATGCTGAATGGTCAAACACT
TGACTGGTTCAGTTGGAAAATAATTTTTTGACTAACAATATGCTGTTATTTTCTCTTAGATGTAATCGG
AGGGTACTCATTAAATGAAGGCCAATCCGAAGCACACTTTTCAGTGCGATTCTCTGATTATCAGACCCT
CACTGAAAGGGCACAGGCTATTTATTTATGTATGTAAGCAGGCTGTATTTACGTAAGGTGTAATCGAAG
AGGATTCGTCTCCTCTCCACTCACTGTTACGGAAGTGTATTTCCATAAACAGACTGTTATAAACAAATC
AAGTTGTTCTGCGTGG

Appendix Figure B1 (Continued)

AU75777upper AGCCCTACTCGTTTATCAG (1984-2002)

TTTGTGATCAGTAAAACAGGCTAATAATGTATAACTAATCAATAAAGGAAAAAATAACTTTGAAATGAC
TTTGATCCCACTTTGAGAACCACTATTTTAAGCTTTAGTGGACACACAGAGAGCAAACTTTCACTTACT
GGCTACCCAGGCACCAAAACAGCAGTGTGTGCCAATACTTTAATCTTTAGCTTAGCTTTTGAAAGCAGA
AGTGGCACTTAGAGCTCACTAAATCCCCTTTCTCATCTTTTTAATACGCACGCATGGCGATTTTCCGAC
TTAACAGATTAATGTTAAGTGGTAAAGATGCTCAGCATCATTCTGCTTTTAGTCTCCTGCAGTGC

AU75783lower CAGTGTGATCTTGCTACTGGTTGC (836-860)

GATTACTCAGCTCCTTAAAGTGGAAAGTGGTTTCTTCAGTCTCGGCGAACAACCAATGGGATTTTTCCA
GGCCGACACACAAAATATGAAAAGTTTCTGCTTTGGTACTTAGTGGAAATTGCTAGAGTTTGACTCTGA
AATCTGAAGGAGGAGCCATTTTTAAAGCTCTTCTGCTTTTCTTTAGCAAATTCCGTACCATATCATATA
TATTTATAATATATGCATGCTTAAAAAGACAAACGTATTGCTACCTATATCCCATATATAACAGGTGAT
AAGCTTATTGAACTATTTTAAAGTTAGGTATAAAATTACATATGCAATGTAAATGTATGTTTCTTAGCT
CCTTGTGTGCGCTGTTCAAACGGAAACAGGATTTTTCTTTCGCTTACCGATTTTTATTTCAGATCTACC
TCCCATAACAAAACTGACTTTTGCTCTCGTCAATCCTGTGGATTTCTTTCAGATCCTCATGAGAAGGCT
CGAATACCCTGCTGAAATAAACAACCACGGAAATTTGAATGGTTTCCTCTACAAATACCATTACAAACT
ATCAGAGAACCATTAAAACCATTATCATTATTGGTCTTCAATGGTATCCACCGGACATAACATGTCACC
AATAGAAAGCAACAAATTATCAGTAGAATTCCCATTAAAACCATTACAATTCCCATAAAACCATTACAA
ATTCTATGGGGTTTCTATTGGTATTCTATTTTTTTTTTTTATCTTTTT

AU75780upper CAAGAGTGCTGCTCAGCACGG (3726-3746)

ATATGTCGTATTCATCCTAATCCTCACTCTATCTCCATCCCCGACCTTATCCTCATCTTCAACCCCATC
TCCATCCCTTTCCTCATCAACAGCATCTTCATTTTTGTTCTCATCTCCAAAATGGCCTTTTCTGATCAT
ACTGTATGTGACGTGAATCAAGATTCCAAGCAGCTAAAAATGTTCACGTGATGTAAGATGTCCCTAGTT
ATCTTACCCCAGCATGTATCATCTGAGCCATCAACAGCTGTCTGTATACACATCTCCTTGTACTGAATT
CCATTGGAAAAGATATGAATTTCCTGAATCTGTTATAATTCATGTTACATTTATAATTGAATGACATCC
AAAAGTTCAAAAAATTCTGGACATATCGATAACAACTTCACAATAGATGCCATGGCTGAGTATTTTTTT
AATTATAAAAATGATTCTATTCTATACAGTAAAGATTTATATAATATTAATAACATTCTTTTGCGTTTG
CGAGCCTATTAAAAAATGAGAATTCATGCCATGCCATGATATTGTGTCATGCAGCTTAATATACATCAG
TGGTTTCGTTCTGGAAGATTCTGGAATGAGGACAAAAATGGATTGCGTTTTGCCACAAGAAAGGATTTT
TTCCCCCGCTTTTTAAGATTCACATCTTACAAAATGCTCTTTCTTTGAAATTTTGAATTCTTCTACATG
GAAGCTATGGCCAAGGAACTTAAAAGGTATGTCCATCTTGG

AU75787upper GGAAGATTCTGGAATGAGGAC (4394-4414)

TTTTCCCCCGCTTTTTAAGATTCACATCTTACAAAATGCTCTTTCTTTGAAATTTTGAATTCTTCTACA
TGGAAGCTATGGCCAAGGAACTTAAAAGGTATGTCCATCTTGGTACCTCTGGTCACTCTAGATACGCCA
TGGAACCACAGACAAATTCTCAAATTCTAAGAAATGATATGATAATGTTATTCATTGAAGCAAAAAAGT
TATCCAATGTCATCTGGGCCTGTGTGAAAATGTATTTGCCCCCATAGTTACTAATTCCCCAAATCTATG
AAACGGCATTCGTAATGGGGTTCAGCTGGACTAGATGCAACCAAGCCTGATTACTGCTGGTAAAAGGTT
AAAAGGTTTTACCCAGTACGACTCATCCAGGAAGCCACAAAAGAGCCAAGGACACCATCAAAGGAACTA
CAGGCCTCTCTTGCATCAATAAAGGTCACTGTTCATGACTCCACTCAGAAAGACACTGGGCAAAAATGG
CAACCATGGAAGAGTGGTGAGGTGAAAACCACTGCTAACCCAGAAGAACATTAAGGTTCATCTGAATTT
TGCCAAAACACACCTTGATGATCAAAATGAACACTGAAGTCTTAGTGTTGTTTGAGATGGTTCAAATGA
GCCCCATGGTCGAAAGTCTGGGACCTCAGGGTCCCAAGAAACCAACCATGTGAGACCAACTGATTGTGA
CCAAGCTTCGATGCGCTCTCTTCGGCTTGCTTAAGCTTCCACCTGCCCCCTGGCTTTCTGGAGCTCTGG
AATTGACACCTTCAAGTTAAATTAGGACAGTAAAAAAAAAAAAAAAACAGTACTTGAATGAAAATTTTT
ATCTGAAATGTATGTTTCTTAGCTCCTTGTGTGCGCTGTTCAAACGGAAACAGGATTT

Appendix Figure B1 (Continued)

AU75727U GCTCCTTGTGTGCGCTGTTC (1-20)
AU75820L GCATAGGGAAGCACTAGATGAAG (658-681)

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GCTCCTTGTGTGCGCTGTTCAAACGGAAACAGGATTTTTCTTTCACGTACCGATTTTTATTTCAGATCT
ACCTCCCATAACAAAACTGACTTTTGCTCTCGTCAATCCTGTGGATTTCTTTCAGATCCTCATGAGAAG
GCTCAAATACCCTGCTGAAATAAACAACCACGGAAATTTGAATGGATTCCTCTACAAATACCATTACAA
ACTATCAGCGAACCATTAAAACCATTATCATTATTGGTCCTTAATGGTATCCACCGGACATAACATGAC
ACCAATAGAAAGCAACAAATTATCAGTAGAATTCCCATTATAACCATTACAATTCCCATTAAAACCATT
ACAAATTCTATGAGGGTTTCTATTGGTATTCTGTTTTTTTTTTTCTTTTATCTTTTTCAGCAGGGTAGA
TAGAAATGAGGATGCTGAATGAAAGACGGTTTACCCAAAATGCAGTCGTGCCTAAAAGAAAAAAAAAAA
AAAAAGATTGTGTAGTGATGCAACCAGTAGCAAGATCACACTGGTTTGAATGCATTTCATTATTCGTAA
TTCATTATTGTGTGATTGTTTGTCATCAGACATCTCATGATTCCTTATTGTTGTGACATTTTTTGAAAG
TTGTTTTGACACCAAAAAGGGTTAAAAGGGTGCTTTACTTCATCTAGTGCTTCCCTATGC
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AU75793U GACGGTTTACCAAAATGCAGTCG (439-463)
AU75778L CGCGACATTTGTACGATGTG (1183-1203)

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GACGGTTTACCCAAAATGCAGTCGTGCCTAAAAGAAAAAAAAAAAAAAAAGATTGTGTAGTGATGCAAC
CAGTAGCAAGATCACACTGGTTTGAATGCATTTCATTATTCGTAATTCATTATTGTGTGATTGTTTGTC
ATCAGACATCTCATGATTCCTTATTGTTGTGACATTTTTTGAAAGTTGTTTTGACACCAAAAAGGGTTA
AAAGGGTGCTTTACTTCATCTAGTGCTTCCCTATGCATTAGTGTGTGTATGTATGTATGTATGTATGTA
TGTATGTGTGTGTAGTATATATAATATACACACACAAGCTGTCGTTCCCTACTTAGCACTTTTGTGTAT
AGCGTATTTGTTGTAGTACATAACATAAGTCACCATTTCTCGTGTCGTCTGTATCATTAATATCGACCC
AGATTAATGCTGCGTGGTCAAACACTTGACCGGTTCAGTTGGAAATAATTTCTGACTAACAATATGCTG
TTATTTCTCTTAGATGTAATCGTAGGTACTCATTAAATGAAGGCAATCCGAAGCACACTTTTCAGTGCG
ATTCTCTGGTTATCAGACCCTCACAGAAAGGGCACAGGCTATTTATTTATGTATGTAAGCAGGCTGTGT
TTACGTAAGGTGTAATCGAAGAGGATTCGTCTCCTCTCCACTCACTGTTACGGAAGTGTTTTCCATAAC
AGACTGTTATAAACAAATCAGGTTGTTCTGCGTGGTTTATTTTGTTGCATTATTTCACATCG
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AU75785U CAGACCCTCACTGAAAGGGCAC (1115-1125)
AU75792L CTGATAAACGAGTAGGGCT (1643-1662)

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GAAAGGGCACAGGCTATTTATTTATGTATGTAAGCAGGCTGTGTTTACGTAAGGTGTAATCGAAGAGGA
TTCGTCTCCTCTCCACTCACTGTTACGGAAGTGTTTTCCATAACAGACTGTTATAAACAAATCAGGTTG
TTCTGCGTGGTTTATTTTGTTGCATTATTTCACATCGTACAAACGTCGCGAAAGAGCTGGAGACGCTGT
AGCACTACGCTGACCTGTTCAGGAAATGTTTTCGCAAAATTTATGGATATTCTGTTGTCAGAGTGAAAG
AGACTTGAGACAACAAATGTGAGCCCAGGACTATCTGTGTCCTGATTGTGTGCATGTGTTGCTTCCCAA
GCACCATAACTCTACCTGCTGAAGTAGGAGGAGTTTGGAGGTCTCCCAAAATTTTTGGATCGAGTAACC
TCTGTAACTGTAAAATAAATCCCACACCCCGTCAGTACAGAATTATTTTATCAACTTGATTTATTATTC
CATTTATAATTTAATTCAAGTAGTAGGTTTATGATTTTAACTGATGATGTAATAATATTCCGACTATTA
ATTAGCGCTTTTGAGGTTTTATTCCTGATCTTTACAACTAATGTAATCTATTCTAATTTAATTTGACGT
TATTATAGCCCTACTCGTTTATCA
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AU75777U AGCCCTACTCGTTTATCAG (1643-1662)
AU75791L GATCAGTCGCCCTACAGACAG (2151-2172)

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AGCCCTACTCGTTTATCAGTTATTGAGCTTTGTGATCAGTAAAACAGGCTAATAACGTATAACTAATCA
ATAAAGGAAAAAATAACTTTGAAATGACTTTGATCCCACTTTGAGAACCACTATTTTAAGCTTTAGTGG
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Appendix Figure B2 The primer walking of caGnRH genomic sequence in blue catfish

ACACACAGAGAGCAAGCTTTCACTTACTGGCTACCCAGGCACCAAAACAGCAAAGTGTGCCAATACTTT
AATCTTTAGCTTAGCTTTTGAAAGCAGAAGTGGCACTTAGAGCTCACTAAATCCCCTTTCTCATCTTCT
TAATACGCACGCATGGCGATTTTCCGACTTAACAGATTAATGTTAAGTGGTAAAGATGCTCAGCATCAT
TCTGCTTTTAGTCTCCTGCAGTGCAAAGTTGGACAAAGGTAAGTAAAGTGTTTGTGTGAGAGAGATAGA
AAGGGGGTGGGGTGGGGGGAGTGCTATAAAAGGCCTGCTGTTGAGAGAAAATGCACCATAGACTGTAGA
GATCATCATTGTGAAGAGCAGAAGAACTGTCTGTAGGGCG

AU73373U GAAGAGCAGAAGAACTGTCTG (2137-2158)
AU73373L CACACACCACCATCCACCAGAG (2763-2785)

GAAGAGCAGAAGAACTGTCTGTAGGGCGACTATCAAGGATGGGTACGTGCAAGTTGTGGAACATATAAC
TATATAATATAATATATACATATTTTCAGACTGGTGTTTGGTTTTCTGTGAGCATAAAGAAAGATTTTC
AGAGAGGCAGCAGACAAGTGCTGTGTGTAATGGAATGGCTGAGCAGTTACCTGAGTGAATGTAACTCGG
TTGGATTAAATGGTGAGAGAGTGAGAGAAAGAGAAACAGATTACGGCAGAAAGAGAGGAACAGAAGCAA
AGAGAGAGGAAGAAATGAAGAGTCCATGTGGGTTTCCTCCGGGTGCTCTGGTTTCCACCTACCTTCTGG
TAACATACCACTGTTAAAGTGTGAATGTCTTGCGATATCCTTGTATCCCATCCAGAGTGTGTTCCCAGT
ATCGGCTCCAGATCCATCGCAACCCTGACCAAGATAAAGCTCTTAGTGAAGAAGAATAAATGAATGTTG
TACTATACCATACTCACCTCACAGTGTGTGAATCCAGGACTTACTGCACAACTTTTATTAGATAATACT
ATTTACATTCTTAAGGTAAGTATGGAAGCTGTCTTTGTTTTCCTGTTTCTTTAGGATGGGTATAAAGCG
AGCACTCTGGTGGATGGTGGTGTGT

AU75574U CTCTGGTGGATGGTGGTGTGTG (2763-2785)
AU75574L CGCTGCACGCTTTCCTCCAG (2833-2852)

GGTGGTGTGTGTTGTGGTGCTGCAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAA
GCGTGCAGCGTTGCAGGAGGTAACGGCCACTAAACTAAACAATGTCTAAACTCAATGATATTGTTCTGC
TAAGCTGAGCTAGCAAACCTATACACTTTATTCCTTTTAAATATGTGTTTGACTGACTTTTTTTTTTCC
CCCTCCCCAGACTGTTGAAGAAATGCAGAGGACCTCCGGCTACGTGTGTGATTACGTAGACGTTTCACC
TCGGAATAAACTCTACAGGCTGAAAGATCTGCTGGTGCACAGCTCATAGTTTTCTTTTTGCTCATAAGC
CAACAAATTGAAGGCAGTGTCTTGATGTTTATCTTTTGTCTTCTCTTATAGACCAGTGTTGCTGAGCGA
GAAATTGGACAGTAAGAC

AU75786U ACCAGTGTTGCTGAGCGAG (3169-3188)
AU75795L GATGTGTATACAGACAGCTGTTG (3743-3765)

CCAGTGTTGCTGAGCGAGAAATTGGACAGTAAGACGAATAACAAAACTGATCAAGATCTTCAATAAAAT
GCTTCGCCTTTATTTCGTGTGGGTTTATTTTTTTTATTACCGAAACACTTTCGCTTTTTTTTTTTTAACA
TACTGCTTTTAAGTTGCACATTGTTATTTGCACAATTTATTAGGAATACAGTATTAATAAGATGTGTTG
TAATTAAGTAATACCGCACACACAAGAGTGCTGTTCAGCACGGCTGGGATTTTGCCGCAGGTAATCAAG
GCGGGTGTGAGCACTGGGTATCCATAGGACCCCTTTAAAAAAGTTTTTATTCTCATGCATATGTCGTAT
TCATCCTAATCCTCACTCCATCTCCATCCCCAACCTTATCCTCATCTTCAACCCCATCTCCATCCCTTT
CCTCATCAACAGCATCTTCATTTTTATTCTCATCTCCAAAATGGCCTTTCCTGATCATACTGTATGTGA
CGTGAATCAAGTTTCCAAGCAGATAAAGATGTTCACGTGATGTAAGATGTTCCTAGTTATCTTACCCCA
GCATGTATCATCTGAGCCATCAACAGCTGTCTGTATACACATC

AU75796U CCAGCATGTATCATCTGAGC (3719-3739)
AU75796L CACAGGCCCAGATGACATTGG (4341-4361)

CAGCATGTATCATCTGAGCCATCAACAGCTGTCTGTATACACATCTCCTTGTACTGAATTCCATTGGAA
AAGATACGAATGTCCTGAATCTGTTATAATTCATGTTACATTTATAATTGAATTACATCCAAAAGTTAA

Appendix Figure B2 (Continued)

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AAAAGTTCTGGACATATCGATAATAGATATGTCATCTTCATAATAGATGTCATGGCTGAGTATTTTTTT
AATTATAAAAATGATTCTATTCTATACAGTAAAGATTTATATAATATTAATAACATTCTTTTGCGTTTG
CGAGCCTATTAAAAAATGAGAATTCATGTGTCATGCCATGATATCGTGTCATGCAGCTTAATATGCATC
AGTGGTTTCGTTCTGGAAGATTCTGGAATGAGGACAAAAATGGATTGCGTTTGCCACAAGAAGGATTTT
TTTCCCCCGCTTTTTAAGATTCACATCTTACAAAATGCTCTTCTTTGAAATTTGAATTCTTCTACATGG
AGCTATGGCCAAGGAACTTAAAAGGTATGTCCATCTTGGTACCTCTGGTCACTCTAGATACGCCATGGA
ACCACACACAAATTCTCAAATTCTAAGAAATGATATGATAATGTTATTCATTGAAGCAAAAAAGTTATC
CAATGTCATCTGGGCCTGTG
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AU75821U CACTCTAGATACGCCATGGAAC (4252-4274)
AU75821L AGGTGGAAGCTTAAGCAAGCC (4872-4892)

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CACTCTAGATACGCCATGGAACCACACACAAATTCTCAAATTCTAAGAAATGATATGATAATGTTATTC
ATTGAAGCAAAAAAGTTATCCAATGTCATCTGGGCCTGTGTGAAAATGTATTTGCCCCCATAGTTACTA
ATTCCCCAAATCTATGAAATGGCATTCGTAATGGGGTTCAGCTGGACTAGATGCAACCAGGCCTGATTA
CTGCTGGTAAAAGGTTAAAAGGTTTTACCCAGTATGACTCATCCAGGAAGTCACAAAAGAGCCAAGGAT
ACCATCAAAGGAACTACAGGCCTCTCTTGCATCAATAAAGGTCACTGTTCATGACTCCACTCAGAAAGA
CACTGGGCAAAAATGGCATCCATGGAAGAGTGGTGAGGTGAAAACCACTGCTAACCCAGAAGAACATTA
AGGTTCATCTGAATTTTGCCAAAACACACCTTGATGATCAAAATGAACACTGAAATGGCCTCGAGGCAA
TTCACCTTCATTTAGTCTTAGTGTTGTTTGAGATGGTTCAAATGAGCCCCATGGTCGAAACTCTGGGAC
CCAGGGTCCCAAGAAACCAACCATGTGAGACCAACTGATTGTGACCAAGCTTCAGTGCGCTCTCTTCGG
CTTGCTTAAGCTTCCACCT
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Appendix Figure B2 (Continued)

AU50871ovb TCTCTCTTTCCTCCAGGGTACCAG (2999-3023)

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TGTGTGTGTGTGCTGGGGTATAAAAAGAGAGAAGTATGAAGATGGTCTCAGGCATTGATACTTCCAAGA
CAAAGAGCTCTTCAGAGTTCCACATCTCAGAAGAATTCAAATCTGAATAAAGCACATCAAATAAGAGTG
AAAGGGGTCATATATTTCATCATTTTCCTGATTTTTGATGTCATTTTTAGAACATTATAGTGGCATTTA
TATCTCTGTGTTAATCCTCTCCACCACATATTTTTCTCCTTTTCCGGTTTATATTGTAATCGGTTAATT
ATTATTATTATTTTTTTTTAAAGCATTGTCTTAAAATACTGTTATTGTTGTTATATGTGTGTGTGTGTG
TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTGAGGGGCACTCTGTTCTCCCTGCACTTTG
GGGGTTTCCTCCAGTTTCCTCTCCCGGTCCTAAGACAGGCGTTCTAGGCTGATTGGCATTTCCAAACTG
TCCACAGTGTGTGAATGGGTGTGTGTGCGATTGTACCCTGCATGAGTTGGCACCCCGTCCAGGGTGTCC
TCCATCTCGTGCCCTGAGTTCCCAGGGATAGGCTCCAGGCTCCCTCAGACCCTGTGTAGAATACACGGT
ACATAAAATGGATCAATGGATATTGTTGCTCTTGTTGTTGTTAAATCAGGACAGTGTCCAGGTCTGAGG
CGCAACTAAACAATAACTGTAGCTCTTCTTTTGATGTATATATATATATTCCACAAGAGGAGATTACTC
ACTTACTGTCTGACTTACTTTATTATTAAGATTATTTTATTAATTACCTTTAATATTTCATTGACTGAC
TGTAAGATCTGTGTGTGTGTGTGTGTGAGAGTATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTGCCT
TGCTGTTGTGTTTGCAAGCGCAGC
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AU75772upper CAGGACAGTGTCCAGGTCTG (2718-2737)

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CATTGACTGACTGTAAGATCTGTGTGTGTGTGTGTGAGAGTATGGTCAGTGTGTGCAGGCTGTTGCT
GGTTGCTGCCTTGCTGTTGTGTTTGCAAGCGCAGCTATCTGTTTCTCAACACTGGTCTCATGGCTGGTA
TCCTGGAGGAAAGAGAGAAATCGACTCTTACAGCTCACCAGAGGTCTGTCTCTTTCTCTCTGTCTCTCT
GTCTCTCTCTCTCTCTCTCTCTCACACACACACACACACACACACTCACGCATACATGGGCACACAT
```

Appendix Figure B3 The primer walking of cGnRH II genomic sequence in channel catfish

GCACCCATGCACACAGGCATTTCAGAACAAAGGTTTCTTTGTTATAAAATCTGGCACTTTTTCATAAAA
CAGCTGTTAACAAAAACATCTGGATTGTGTATTTGCGACACAAGCATTAAAAACATGCTTAAAAACGAT
CAGTCAAACATGAGCATAAGCTCATGTGAACGAGATAAATGTAAAGCTCCCTGTAAACGCTCTTATTAC
TCCCAAAAAATTTTTTAATTCCGTGCGATTAATTGATTGATTCAGTTACGTGTTTGAAATCTTAACACG
GCTGATCCAGCTCATAAGGACGCACGTGAAATATTCTAATCGTTTTCAGAGACCAGTTAGTGGAAAATG
GTT

AU75781upper CATCTGGATTGTGTATTTGCGAC (3228-3251)

CCAAAAAATTTTTTAATTCCGTGCGATTAATTGATTGATTCAGTTACGTGTTTGAAATCTTAACACGGC
TGATCCAGCTCATAAGGACGCACGTGAAATATTCTAATCGTTTTCAGAGACCAGTTAGTGGAAAATGGT
TCATTTATCGAGGCTATTCCAGGACCGCAAGGAACTCAAGCTATGCCACTACAGTATGTTGACTATGCT
GAACCCGGTGTGGATTCGAGTGGCTGTCATGTGCGCCGGTCTTTAAACATCACTGAAACGAGACCGTCC
AAGCTGAACATATCACACGGATCTCAATTTAAGGTTCAAACTGTATCGTACTAGATACAAGTGTGTAAC
AAACAAATATAATAGAGCAACATGAAATAGTAGGTCAGAGCAGAAATATTCACATTCCCTGCCAGTGGT
TCTGAAATCTCGCCCTCCTGCTGTGCCTCACCGCTCGGACAGATATCTGGGGAGATCAAACTGTGTGAA
GCGGGAGAATGCAGCTATCTGAGGCCACTGAGAACCAACGTCCTGAAGAGCATCCTGGGGAGAACACGC
TCGGCACATTTAATCGTTTTATTTTATTAGAAGCGTCTCTCTGAACTTAAACATACGTTTCCTGTTCCA
AACTGTATAAGGCACATGTTTATTCACAGTGACAGTGCTGTGGGAAAAGTATTTGCTTTGATTTGTGTG
TGTGTGTGTGTGTGTGT

AU75799upper GAAGCGTTCTACTTCTGAAC (4264-4283)

CATACTAAATAGTTTTAGATCTTCAAACGAAATACAACAGAAAACAAAAGCAACCTGAGTAAACACACA
ATACAGTTATTTATTTATTTATTTATTTATTGAAGGAAAAAAAAAAAAAAAAGGTTATCCAACACCTATCA
CCCATGTGAAAAACTAATTGCCCCCTTAAACTCGAAACCTTGTTGTGACATTTTATTTATGAATGTATT
ACTAAGAATAAAGAATTGAATCTCATTTTTTATATATATATATATATATCATGCTGTACTCATGCACCT
CCAAATGCATTTGAACTCAAGCCTGTTTGTGAATTGTCTTTATATGACCTTCTTTAACATCTGCGAGTA
CTTTTAATTCCTTATTCT

AU75811upper GAACTCAAGCCTGTTTGTGGA (4369-4390)

AGTTTTTTGGTGCCACTTCCTGACAATGTTGTTCCCCTTATAGCTCGATGCCCTTGTGAAAGAATTCCA
AAAGAAGAAGTGAAAACAATTCAATCATGCCCCAGCTTCCTCCAGCCTCTTTCTTCGCTGATAACACTT
ATCTGAACACACACACACACATCTGCTCATCCGTGGAGTGTGTCTTTTTCTGTTTATGTGGTTTTAGCG
TCAAGCTTTCTGTCATCTGTGTATAAGCAGCGAAGTGAAATGCATCATGTGATCATTCAATAAAACTTT
TATTTTGGTATTCGGTCTCTTTCTGAAAGAATACATGGGACATTCACTGCTTTGCCAAATCCGTTATTA
AAATCGGAGTTAACGGCTTACGTTAGCGTGTGTGATTTATCTGATTTCCTTAACCTGTGAAGCGCATTC
TGAGCACAGCTGATGCCCACCATATTAAAAACATACATTGCCTAAAGTATGTGGACACCTGACCATCAC
CCATTTGTGGTTCTTCTCCAGACTGTTGCCACGTAGTTGGAAGCACACAGTTCAGAATGCCTTTGTACT
GCGTTACAATTACACTTCACTCGAAGTAAGGGACCAGATATGTTCCAGTATGGCAATGCCTCTGTACAC
AAAGTGAGGTCCATGAAGACGTGGTTTGCTGAGTCTGGATCAGAAGGATTCAAGTGGTCTGCACAGAGC
CCTGACTTCAACCCCTTTGAACACCACTGGGATGAAATTAGAACACCGGGGCATCTGTCCGTTGGGGAG
TAGATGCCCTCTCTCACAGAGTCGTGTGGTCCACAGAGGTACTGGGGCGTGTTGGCCCGGTGTAAGGTA
CGACACATTCAACCAGCTGAAGATAGGGCGGTCC

AU75822Upper GAATGTCCCATGTATTCTTTC (4760-4781)

CACAGTGACAGTGCTGTGGGAAAAGTATTTGCTTTGATTTGTGTGTGTGTGTGTGTGTGTGTGGGTGGG
TGGGTGGGTGTGCGTCATACTAAATAGTTTTAGATCTTCAAACGAAATACAACAGAAAACAAAAGCAAC

Appendix Figure B3 (Continued)

CTGAGTAAACACACAATACAGTTATTTATTTATTTATTTATTTATTGAAGGAAAAAAAAAAAAAAGGTT
ATCCAACACCTATCACCCATGTGAAAAACTAATTGCCCCCTTAAACTCGAAACCTTGTTGTGACATTTT
ATTTATGAATGTATTACTAAGAATAAAGAATTGAATCTCATTTTTTATATATATATATATATATCATGC
TGTACTCATGCACCTCCAAATGCATTTGAACTCAAGCCTGTTTGTGAATTGTCTTTATATGACCTTCTT
TAACATCTGCGAGTACTTTTAATTCCTTATTCTTTTGATTGTTCTTTCAGTTTTTTGGTGCCACTTCCT
GACAATGTTGTTCCCCTTATAGCTCGATGCCCTTGTGAAAGAATTCCAAAAGAAGAAGTGAAAACAATT
CAATCATGCCCCAGCTTCCTCCAGCCTCTTTCTTCGCTGATAACACTTATCTGAACACACACACACACA
TCTGCTCATCCGTGGAGTGTGTCTTTTTCTGTTTATGTGGTTTTAGCGTCAAGCTTTCTGTCATCTGTG
TATAAGCAGCGAAGTGAAATGCATCATGTGATCATTCAATAAAACTTTTATT

AU75771lower GCCTAGAACGCCTGTCTTAGG (2493-2513)

CCCCCCCCATTCTTTTCTGAACTATACACTATATTCATGACGCAGGCTTAAAGATAATCCACATCTGAA
TAGTAGTAGGCCTATATCTGCAAAATGCTCATGCCTCAAAAGTCCTCGTGTTGTCGCACGCCATTCACT
TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT
GTTTGAGTGCATATATGTATTCGGGCAGCATCTCTCAGGGGGTCAGGGGTGTTTGAAGATGATAATTGG
AATCGAGCCGACGTTATGTGTCCCAGTGGAACCCCTCAGACCGTGTGTGTGTGTGTGTGTGTGTGTGTG
TGTGTGTGTGTGCTGGGGTATAAAAAGAGAGAAGTATGAAGATGGTCTCAGGCATTGATACTTCCAAGA
CAAAGAGCTCTTCAGAGTTCCACATCTCAGAAGAATTCAAATCTGAATAAAGCACATCAAATAAGAGTG
AAAGGGGTCATATATTTCATCATTTTCCTGATTTTTGATGTCATTTTTAGAACATTATAGTGGCATTTA
TATCTCTGTGTTAATCCTCTCCACCACATATTTTTCTCCTTTTCCGGTTTATATTGTAATCGGTTAATT
ATTATTATTATTTTTTTTTAAAGCATTGTCTTAAAATACTGT

AU75798lower CCTGAGAGATGCTGCCCGAATAC (1929-1952)

AACTCCAAATGGGCTTCGACTAAGCATTACTTTATTATACGTTGATGTTGATATACACGAGATTCATTT
TTCNTCACAAAAATCTGCCAGTTAAAAGTGTGTAGACTTTTTACATCCACTCTAAAAATATATATGTTC
ATTGTTAATATTTATCTAACTATTAACTTTTAAAACTCTTCGAATAAAAAAGACGAATCGTAGCCTTCT
CTGTGGTGATTATAATAATTATGATTATGGCAAAGAAATCCCTTTATATATATATATATACATATATAC
ATATTGTCATAGTTTATCATTGCATTTAGCTATGAATGCATTATTAATTATAATTATTATTTATTATCT
TCAATATAAATTATTTGTCTGTTCGAGACCTTTTGTAAACCCCTCCCCCCCCATTCTTTTCTGAACTAT
ACACTATATTCATGACGCAGGCTTAAAGATAATCCACATCTGAATAGTAGTAGGCCTATATCTGCAAAA
TGCTCATGCCTCAAAAGTCCTCGTGTTGTCGCACGCCATTCACTTGTGTGTGTGTGTGTGTGTGTGTGT
GTGTGTGTGTGTGTGT

AU75807lower CACAGAGAAGGCTACGATTCGTC (1506-1529)

AATTGCTCCCACACATTCAAAATGAATGCGTCTTTTTCTGGGGCGATCAGTTTGTCTACGTGGTACGTT
TTCGGCACGTGTTAATATCGGGCCTGTTTTGAAATGATTATAAGAGGCGACTTTTAAAGCGCGAGTCAG
TGTGACCTCGTGACTTTGTGCGCGTGTGTCAGACTGGTACTCTGAGCGGATTAGTGCTGTCGGAAGCCT
CGAGCGTGTTTAAACACCACTCGAGGGACAGGAAGTCAATCGGTGTCTCAGCCAGCCGAATCAATCAGC
CCTGTAATAGCTCGCAAGACTGTTTTCAGATTTGGGTCTTGTCAGGGTCACCGTGTGCTAGTCAAGGTT
CAAAGGTCACTGGGCGGAGTTCACCTTGGACAGCTGAGAGGAAATGAGGTGTCTTACGGGTGGCCAGAA
GAGGGCAGCCACACTTATGAAACATTCATAATACGCCCTGTTTGACCGTGATGATAAGATGGAAACGGC
TTCGACACATTAGCCAGGATACAGTGGATATAAAGAATTTTACAATGTTTTTGTGATGTAAAAAAAAGA
AAAAGAAATAAATCGAGATAAATCAGAAGTCTGAATTTTTTCCACTCTCGATGTGAAATGGCCTGAATC
AGACCAGACCTGAATCCAGACTCCAGACTGAGTGGTGTAATAAACTCCAAATGGGCTTCGACTAAGCAT
TACTTTATTATACGTTGATGTTGATATACACGAGATTCATTTTTCNTCACAAAAATCTGCCAGTTAAAA
GTGTGTAGACTTTTTACATCCACTCTAAAAATAT

Appendix Figure B3 (Continued)

AU75810lower GCCCGATATTAACACGTGCCGA (724-746)

```
TCAGGTCGAGTGCAGTAATATGGCGAGAGTGTTTGGAAAATGCGTGGAGTTTGCATGTTCTTGGGGGTT
TCCTCCTCCAGTCCAAGGACATGTTGTAGACTGATTGGCGATGGGTTGGCACCTTGTGCCTTGTGCCCC
AGTATTTGTACCCCAAATACTTTGTGATACAGGATTTTTTAAAAAAAAATTTTATTTCCATGAGCTGTA
AGCCTTAATCATCAAGAATAAAACGAACAAAAGGTTTGAAACATTTCACTTTGTGTATTGAATCTAAGN
AAATTTGAAAGTCCCACTTTTTGATTTCGTTCCAGAAAAAAAAAGAAAAAAAGAAAAGAAAAAAAAACT
TTCCCACGATATTCACTTTTTTTTTTTCTTTTCTTTTTTTTTTTTTGAGATGCACCTGTAGTGGCATCC
ATTCGAAATGGCTGAACGAGTCAGACTTCTTGTCTGTGTCAGCTACACCGTGGAGGGCGTACAGATTAG
ACTCGAACCTAACAGAGCATTCCTTTCAAGTTAGGACAGGGGCCGGGGTTTTCTTCTCGCTTTATGAAC
ATTATTTGTATGCGTGCTTCCTGCTCAGACTGGTGTGTACGGTGTGCGGGGGTTAATTTGACTGCAGCG
AGCTTGATCCTTTTGACTGTCTTTTTCACAGGAATTGCTCCCACACATTCAAA
```

Appendix Figure B3 (Continued)

AU75816U CAGTAATATGGCGAGAGTGTTTG
AU75816L CACCACAGAGAAGGCTACGATTC (1476-1498)

```
TGCGTGGAGTTGCATGTTCTTGGGGGTTTCCTCCTCCGGTCCAAAGACATGTTGTAGACTGATTGGCGA
TGGGTTGGCACCTTGTGCCTTGTGCCCCAGTATTTGTACCCCAAATATTTTGTGATACAGGATTTTTTA
TTTCCATGAGCTGTAAGCCTTAATCATCAAGAATAAAACGAACAAAAGGTTTGAAACGTTTCACTTTGT
GTATTGAATCTAGAATATATGAAAGTCCCACTTTTTGAATTCAATTACAGAAAAGAAAAAAAAAAAAAA
ACTTTCCCACGATATTCACTTTTTTTTTTTTTGAGATGCACCTGTAGTGGCATCCATTCGAAATGGCTA
AACGAGTCAGACTTCTTGTCTGTGTCAGCTACACCGTAGAGGGCGTAGAGATTAGACTCGAACATAACA
GAGCATTCCTTTCAAGTTAGGACAGGGGCTGGGGTTTTACTTGTCGCTTTATTCAACATCATTTGTATG
CGTACTTCTGCTCCGACTGGTGTGTAAGCTGTGCGTGGGTTAGATTTGGCTAGCAGACAAGGGTCGATC
CTTTTGTATGACTTTATGTACATGAAATTGCTCCCACACATTCAAAAGAATGCATCTTTTTATGGGCGG
TCAGTTTGTCTACGTGGTACGTTTTCGGCATGTGTAATATCGGGTCTGTTTTGAAATGATTATAGAGGC
GAATTTTAAAGTGCGAGTCAGTGTGACCTCGTGACTTTGTGCGCGTGTGTCAAACTGGTACTCTGAGCG
GATTAGTGCTGTCGGAAGCCTCGGGCGTGTTTAAACACCACTCGAGGGACAGGAACTCAATCGGTGTCT
CAGCCAGACGAATTAATCAGCCCTGTAATAGCTCGCGAAACTGTTTTCAGATTTGGGTCTTGTCAGGGT
CACCGTGTGCTAGTCAAGGTTCAAAGGTCACTGGGCTGAGTTCACCTTGGACAGCTGAGAGGAAATGAG
GTGTCTGAAAGGTGGCCAGAAGAGGGCAGTCACACTTATGAAACATTCATAATACGCCCCTGTTTGACC
ATGATGAATCCGTAAGATGGAGACGGCTTCGACACATGAGCCAGGATACAGTGGATATAAAGAATTTTA
CAATGTTTTTGTGATGTAAAAAAATAAATAAATAAATGAAATCGAGATAAATCAGAAGTCAGAATTTTT
TCCACTCTCGATGTGAAATGACAGCGTATAAAACGGTGTCTGTTAGTAAGTCCAATAGAAGATCAACGT
TTCTGACCAGACCTGAATCCAGACTCCAGACTGAGTGGTGTAATAAACTCCAAATGGGCTTCAACTAAG
CATTAGTTTATTATACATTGATGTTGATTATACACGAGTATTCATTTTACATCACAAAAATCTGCAGTT
AAAAGTGGGTGCTTTTACATCCACTCNAAAATCACATGTTATTTTTAATATTTAATCTAACTATTAA
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AU75805U CGTTGATGTAGATTATACACGAG (1327-1349)
AU75771L GCCTAGAACGCCTGTCTTAGG (1353-2364)

```
TCATTTTACATCACAAAAATCTGCAGTTAAAAGTGGGTGCTTTTACATCCACTCNAAAATCACATGTTA
TTTTTAATATTTAATCTAACTATTAACTTTTAAAACTGTTCGAATAGCTGATACGAATCGTAGCCTTCT
CTGTGGTGATTAGAATAATTACGATGATGGCAAAGAAATCCCTTTATATATATATATATATATACACAT
ATTGTCATAGTTTATCATTGCATTTAGCTATAAATGCATTATTAATTATAATTATTATTTATTATCTTC
AATATAAATTATTTGTCCTGTTCGAGACCTTTCGTACCCCCCCCATTCTTTTCTGATCTATACACTATA
```

Appendix Figure B4 The primer walking of cGnRH II genomic sequence in blue catfish

TTCATGACGCAGGCTTAAAAATAATCCACATTTGGAATAGTTAGTAGGCCTATATCTGCAAATGACTCA
TGCCTATGGAAGTCCTCGTGTTGTTGCACGCCATTCATGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTG
TTTGAGTGCATATATGTATTCGGGCGGCATCTCTCAGGGGGTCAGGGGTGTTGAAGATGATAATTGGGA
ATCGAGCCGACGTTATGTGTCCCAGTGGAACCCCTCAGACCGTGTGTGTGTGTGTGTGTGTGTGTGTGT
GTGCTGGGGTATAAAAAGAGAGAAGTATGAAGATGGTCTCAAGCATTGATACTCCAAGACAAGAGCTTC
TCAGAGTTCCACATCTCAGAAGATTCAAATCTGAACAAAACACATCAAATAAGAGTGAAAAGGGTCATA
TATTTCATCATTTTCCTGATTTTTGATGTCATTTTAGAACATTATAGTGGCATATATTATCTCTGTGTT
AATCCTCTCCACCACATTATTTTCTCCTTTTCTGGTTTATATTGTAATCGGTTAATTATTATTATTATT
ATTATTTTTTAAAGCATTTTCTTAAAATACTGTTATTGTTATGTGTGTGTGTGTGTGTTGAGGGGCACT
CTGTTCTCCCTGTGCTTTGGGGGTTTCCTCCAGTTTCCTCCCCCGG

AU75781U CATCTGGATTGTGTATTTGCGAC (3097-3121)
AU75708L GCTGCATTCTCCCGCTTCACACAG (3693-3717)

ACACAAGTTAAAAACACGCTAAAATCATCAGTCAAACACACTATTTCTGAACATAAGCTCACGAAACGA
GATGAATGAAAGCTCCTGTAAACGCTCGTATTACTCCCAATTTTTTTTTTTAATTCCATGCAGTTAATT
GATTGATTCAGTACATGTTTGAAATCTAATACGGCTGATCCAGCTCATAAGGATGCACGTGAATATTAA
ATCGTTTCAGAAACGAGTCAGTGGAAAACGGTTCATTTATCGAGGCTATTCCAGGACAGCAAGGAACTC
AAACTATGCTACTACAGTATGTTGACTATGTTGAACCCGGTGTGGATTCGAGTGGCTGTTATGTGCGCC
GGTCTTTAAACATTACTGAAATGAGACCGTCCAAGCTGAACATATCACACGGATCTCAGTTTAAGGTTC
AAACTGTATCGTACTAGATACAAGTGTATAACAAACAAATATAATACAGCAACAGGAAATAGTAGGTCA
GAGCAGAAATATTCACATTCCCTGCTAGTGGTTCTGAAATCTCGCCCTCCTGCTGTGCCTCACCGCTCG
G

AU75801U GTGTTGAGGGGCACTCTGTTCTC (2304-2326)
AU75760L CAGATAGCTGCGCTTGCAAACAC (2845-2867)

GTGTTGAGGGGCACTCTGTTCTCCCTGTGCTTTGGGGGTTTCCTCCAGTTTCCTCCCCCGGTCCTAAGA
CAGGCGTTCTAGGCTGATTGGCATTTCCAAATGGTCCACAGTGTGTGAATGGGTGTGTGTGCGATTGTG
CCCTGCGATGAGTTGGCACCCCGTCCAGGGTGTCCTAGATCTCGTACCCTGAGTTCCCAGGGATAGGCT
CCAGGCTCCCTCAGACCCTGTGTAGAATACACGGTACATAAAATGGATCAATGGATATTGTTGCTCTTG
TTGTTGTTAAATCAGGACAGTATCCAGGTCTGAGGCGCAACTAAACAATAACTGTAACTGTTCTTTTGA
TGTTATATATATAAATATATATATTCCACAAGAGGAGATTACTTACTATCTGACTTACTTTATTATTAA
GATTATTTTATTAATTACCTTTAATATTTCATCGACTGACTGTAAGATCTGTGTGTGTGTGTGTGTGTG
TGTGTGTGAGAGTGATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTGCCTTGCTGTTGTGTTTGCAAG
CGCAGCTATCTG

AU75772U CAGGACAGTGTCCAGGTCTG (2592-2611)
AU75804L TCCTTATGAGCTGGATCAGCCG (3286-3308)

TTCCACAAGAGGAGATTACTTACTATCTGACTTACTTTATTATTAAGATTATTTTATTAATTACCTTTA
ATATTTCATCGACTGACTGTAAGATCTGTGTGTGTGTGTGTGTGTGTGTGTGTGAGAGTGATGGTCAGT
GTGTGCAGGCTGTTGCTGGTTGCTGCCTTGCTGTTGTGTTTGCAAGCGCAGCTATCTGTTTCTCAACAC
TGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAAATCGACTCTTACAGCTCACCAGAGGTCTCTCTC
TCTCTCTCTCTCTTTCACACACACACACACACACACACTCACGCATACATGGGCACACACACACACTTG
CACCCATGCACACACGCATTTCAGACAAAGGTTTCTTTGTTATAAAATCTGGCACTTTTTCAAAAACAG
CTGTAACAAAAACATCTGGATTGTGTATTTGCACACAAGTTAAAAACACGCTAAAATCATCAGTCAAAC
ACACTATTTCTGAACATAAGCTCACGAAACGAGATGAATGAAAGCTCCTGTAAACGCTCGTATTACTCC
CAATTTTTTTTTTT

Appendix Figure B4 (Continued)

AU75842U CATCTGCTCATCCGTGCAGTG (4535-4550)
AU75842L CAGAGCATTATGCGTGGACCG (5279-5300)

```
CATCTGCTCATCCGTGCAGTGTGTCTATTTCTGTTTATGTGGTTTTAGCATCAAGCTTTCTGTCATCTG
TGATAAGCAGCGAAGTGAAATGCATCATGTGATCATACAATAAAACTTTTATTTTGGTATTCGGTCTCT
TTCTGAAGGAATACATGGAACATTCACTACTTTGCCAAATACGTTATTAAAATCGGAGTTAACGGGTCA
CATCAGCTTGTGTGATTTAGCTGATTTCCTTAACCTGTAAAGCACATTCTGAGCACTTCTTTCAGCTGA
TGCCCACCATATTAAAAACATACATTACATTGCCTAAAGTATGTGGACACCTGACCATCACCCATTTGT
GCTTCTTCTCCAAACTGTTGCCACATAGTTGGAAGCACACAGTTTAGAATGCCTTTGTACTGCGTTACA
ATTACACTTCACTCGAAGTAAGGGCCCAAATATGTTCCAGTATGGCAATGCCCCTGTGCACAAAGTGAG
GTCCATGAAGACATGGTTTGCTGAGTCTGGATCAGAAGGATTCAAGTGGTCTGCACAGAGCCCTGACCT
CAACCCCTTTGAACACCACTGGGATGAATTAGAACACCGGGCATCTGTCCGTTTGGGAGTAGATGCCTC
TCTCACAGAGTCGTGTGGTTCCACAGAGGTACTGGGGCGTGTTGGTCCCGGTGTAAGGTTACGACACAT
CAACCAGCTGAAGATTAGGGCGGTCCATCTTGGCCTGAAGTGTCAGCTGACACCAATTGCCGGTCCACG
CATAATGCTCTG
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AU75829U CCAAGCTGAACATATCACACGG (3493-3514)
AU75845L TGTGTGTGTGTTCAGATCAGTG (4508-4529)

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CCAAGCTGAACATATCACACGGATCTCAGTTTAAGGTTCAAACTGTATCGTACTAGATACAAGTGTATA
ACAAACAAATATAATACAGCAACAGGAAATAGTAGGTCAGAGCAGAAATATTCACATTCCCTGCTAGTG
GTTCTGAAATCTCGCCCTCCTGCTGTGCCTCACCGCTCGGACAGATATCTGGGGAGATTAAACTGTGTG
AAGCGGGAGAATGCAGCTATCTGAGGCCACTGAGAACCAACGTCCTGAAGAGCATCCTGGTGAGAACTC
TATCTGTATCGTTTTATTTATCCATTTTTATGAATGTATTAAGAAGCGTTCTACTTCTGAACTTAAAAT
ATAACTATTCATGTTTCCAAACTGTTACAAAGGCACATATTTAATTTCACATGTACAGTGCTGTGAAAA
ATGTATTTGCTTTGATTTGTGTGCGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTATCTCATACGAAA
TAGTTTTAGATCTTCAAACGAAATACAACAGAAAACAAAAGCAACCTGAGTAAAAAAAAGGTTATCCAA
CACCTATCACCCATGTGAAAAACTAATTGCCCCCCTTAAACTTGAAACCTTGTTGTGACATTTTATTTA
TGAATGTATTAGTAAAGAATAAAGAATTGAATCTCATTTTTTATATATATATATATATCATAAAAAATG
TGTATATATATATATATATATATATATATATATATATATATATATATATATATATATATAATCATGC
TGTACTCATGCACCTCCAAGTGCATTTTAACTCAAGCCTCTTTGTAAATTGTCTTTATATGACCTTCTT
TACATCTGCAAGTACTTTTACTTCCTTCTTTTTTTTGATTGTTCTTTCATATTTTTTTTTGTGTCATTT
CCTGACCATGTTGTTCCCCTTATACCTGCTCGATGCCCTTGCGAGAGAATTCCAAAAGAGGAAGTGAAA
ACAATCCAATCCTGCCCCAGCTTCCTACAGCCTCTTTCTTCGCTGATAACACTGATCTGAACACACACA
CA
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Appendix Figure B4 (Continued)

>part1

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CC   TCCATGTGGGTTTCCTCTGGGTGCTCTGGTTTCCACCTACCTTCTGGTAAGATACCACTG 60
BL   TCCATGTGGGTTTCCTCCGGGTGCTCTGGTTTCCACCTACCTTCTGGTAACATACCACTG 60
     ***************** ******************************** *********

CC   TTGAAGTGTGAATGTCTTGCGATATCCTTGTATTCCATCCAGAGTGTGTTCCCAGTATCG 120
BL   TTAAAGTGTGAATGTCTTGCGATATCCTTGTATCCCATCCAGAGTGTGTTCCCAGTATCG 120
     ** ****************************** **************************
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Appendix Figure B5 The comparison of caGnRH in channel and blue catfish by using blast 2 sequences program

```
CC  GCTCCAGATCCGTCGCAACCCTGACCAAGATAAAGCTCTTAGTGAAGAAGAATAAATGAA 180
BL  GCTCCAGATCCATCGCAACCCTGACCAAGATAAAGCTCTTAGTGAAGAAGAATAAATGAA 180
    *********** ************************************************

CC  TGTTGTACTATACCATACTCACCTCACAGCGTGTGAATCCAGG-CTTACTGCACAACTTT 239
BL  TGTTGTACTATACCATACTCACCTCACAGTGTGTGAATCCAGGACTTACTGCACAACTTT 240
    ***************************** ************* ****************

CC  TATTAGATAATACTATTTACATGCTTAAGGTAAG-ATGGATGCTGTCTTTGTTTTCCTGT 298
BL  TATTAGATAATACTATTTACATTCTTAAGGTAAGTATGGAAGCTGTCTTTGTTTTCCTGT 300
    ********************** *********** ***** *******************

CC  TTCTTTAGGATGGGTATAAAGCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTG 358
BL  TTCTTTAGGATGGGTATAAAGCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTG 360
    ************************************************************

CC  CAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTG 418
BL  CAGGTGAGCTCTCAGCACTGGTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTG 420
    ************************************************************

CC  CAGGAGGTAACGGCCACTAAACTAAACAATGTCTAAACTCAATGATATTGTTCTGCTAAG 478
BL  CAGGAGGTAACGGCCACTAAACTAAACAATGTCTAAACTCAATGATATTGTTCTGCTAAG 480
    ************************************************************

CC  CTGAGCTACCAAACCTATACACTTTATTCGTTTTAAATGTGTGTTTGACTGACTTTTTTT 538
BL  CTGAGCTAGCAAACCTATACACTTTATTCCTTTTAAATATGTGTTTGACTGACTTTTTTT 540
    ******** ******************** ******** *********************

CC  TC-CCCCCTCCCGAGACTGTTGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTAC 597
BL  TTTCCCCCTCCCCAGACTGTTGAAGAAATGCAGAGGACCTCCGGCTACGTGTGTGATTAC 600
    *  ********* ****************** ****************************

CC  GTAGATGTTTCACCTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGGTGCACAGCTCA 657
BL  GTAGACGTTTCACCTCGGAATAAACTCTACAGGCTGAAAGATCTGCTGGTGCACAGCTCA 660
    ***** ******************************************************

CC  TAATTTTCTTTTTGCTCGTAAGCCAACAAATTGAAGGCAGTGTCTTGATGTTTATCTTTT 717
BL  TAGTTTTCTTTTTGCTCATAAGCCAACAAATTGAAGGCAGTGTCTTGATGTTTATCTTTT 720
    ** ************** ******************************************

CC  GTCTTCTCTTATAGACCAGTGTTGCTGAGCGAGAAATTGGACAGTAAGACAAATAACAAA 777
BL  GTCTTCTCTTATAGACCAGTGTTGCTGAGCGAGAAATTGGACAGTAAGACGAATAACAAA 780
    ************************************************** *********

CC  ACTGATCAAGAGCTTCAATAAAATGCTTTGCCTTTATTTCGTGTGGGTTTATTTTTTT-A 836
BL  ACTGATCAAGATCTTCAATAAAATGCTTCGCCTTTATTTCGTGTGGGTTTATTTTTTTTA 840
    *********** **************** ***************************** *

CC  TTACCGAAACACTTTCGCTTTTTTTTTTTTTAACGTACTGCTTTTATGTTGCACATTGTA 896
BL  TTACCGAAACACTTTCGCTTTTTTTTTTTT-AACATACTGCTTTTAAGTTGCACATTGTA 899
    ****************************** *** *********** *************
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Appendix Figure B5 (Continued)

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CC  TTCTCACAATTTATTAGGAATACAGTATTAATAAAATGTGTTATAATTAAGTAATACCAC 956
BL  TTTGCACAATTTATTAGGAATACAGTATTAATAAGATGTGTTGTAATTAAGTAATACCGC 959
    **  ****************************** ******* *************** *

CC  ACACACAAGAGTGCTGCTCAGCACGGCTGGGATTTTGCCGCAGGTAATCCAGGCGGGTGT 1016
BL  ACACACAAGAGTGCTGTTCAGCACGGCTGGGATTTTGCCGCAGGTAATCAAGGCGGGTGT 1019
    **************** ******************************** **********

CC  GAGCACTGGGTATCCATAGGACCC-TTTAAAAAAGTTTTTATTCTCATGCATATGTCGTA 1075
BL  GAGCACTGGGTATCCATAGGACCCCTTTAAAAAAGTTTTTATTCTCATGCATATGTCGTA 1079
    ************************ ***********************************

CC  TTCATCCTAATCCTCACTCTATCTCCATCCCCGACCTTATCCTCATCTTCAACCCCATCT 1135
BL  TTCATCCTAATCCTCACTCCATCTCCATCCCCAACCTTATCCTCATCTTCAACCCCATCT 1139
    ******************* ************ ***************************

CC  CCATCCCTTTCCTCATCAACAGCATCTTCATTTTTGTTCTCATCTCCAAAATGGCCTTTT 1195
BL  CCATCCCTTTCCTCATCAACAGCATCTTCATTTTTATTCTCATCTCCAAAATGGCCTTTC 1199
    *********************************** ***********************

CC  CTGATCATACTGTATGTGACGTGAATCAAGATTCCAAGCAGCTAAAAATGTTCACGTGAT 1255
BL  CTGATCATACTGTATGTGACGTGAATCAAGTTTCCAAGCAGATAAAGATGTTCACGTGAT 1259
    ****************************** ********** **** *************

CC  GTAAGATGTCCCTAGTTATCTTACCCCAGCATGTATCATCTGAGCCATCAACAGCTGTCT 1315
BL  GTAAGATGTTCCTAGTTATCTTACCCCAGCATGTATCATCTGAGCCATCAACAGCTGTCT 1319
    ********* **************************************************

CC  GTATACACATCTCCTTGTACTGAATTCCATTGGAAAAGATATGAATTTCCTGAATCTGTT 1375
BL  GTATACACATCTCCTTGTACTGAATTCCATTGGAAAAGATACGAATGTCCTGAATCTGTT 1379
    ***************************************** **** *************

CC  ATAATTCATGTTACATTTATAATTGAATGACATCCAAAAGTTCAAAAAATTCTGGACATA 1435
BL  ATAATTCATGTTACATTTATAATTGAATTACATCCAAAAGTTAAAAAAGTTCTGGACATA 1439
    **************************** ************* ***** ***********

CC  TCGATAACAA---------CTTCACAATAGATGCCATGGCTGAGTATTTTTTTAATTATA 1486
BL  TCGATAATAGATATGTCATCTTCATAATAGATGTCATGGCTGAGTATTTTTTTAATTATA 1499
    ******* *          ***** ******** **************************

CC  AAAATGATTCTATTCTATACAGTAAAGATTTATATAATATTAATAACATTCTTTTGCGTT 1546
BL  AAAATGATTCTATTCTATACAGTAAAGATTTATATAATATTAATAACATTCTTTTGCGTT 1559
    ************************************************************

CC  TGCGAGCCTATTAAAAAATGAGAATTCATGC--CATGCCATGATATTGTGTCATGCAGCT 1604
BL  TGCGAGCCTATTAAAAAATGAGAATTCATGTGTCATGCCATGATATCGTGTCATGCAGCT 1619
    ******************************   ************* *************

CC  TAATATACATCAGTGGTTTCGTTCTGGAAGATTCTGGAATGAGGACAAAAATGGATTGCG 1664
BL  TAATATGCATCAGTGGTTTCGTTCTGGAAGATTCTGGAATGAGGACAAAAATGGATTGCG 1679
    ****** *****************************************************
```

Appendix Figure B5 (Continued)

```
CC  TTTTGCCACAAGAAAGGATTTTTTCCCCCGCTTTTTAAGATTCACATCTTACAAAATGCT 1724
BL  TTT-GCCACAAGAAGGATTTTTTTCCCCCGCTTTTTAAGATTCACATCTTACAAAATGCT 1738
    *** ********** *  ******************************************

CC  CTTTCTTTGAAATTTTGAATTCTTCTACATGGAAGCTATGGCCAAGGAACTTAAAAGGTA 1784
BL  CTT-CTTTGAAATTT-GAATTCTTCTACATGGA-GCTATGGCCAAGGAACTTAAAAGGTA 1795
    *** *********** ***************** **************************

CC  TGTCCATCTTGGTACCTCTGGTCACTCTAGATACGCCATGGAACCACAGACAAATTCTCA 1844
BL  TGTCCATCTTGGTACCTCTGGTCACTCTAGATACGCCATGGAACCACACACAAATTCTCA 1855
    ************************************************ ***********

CC  AATTCTAAGAAATGATATGATAATGTTATTCATTGAAGCAAAAAAGTTATCCAATGTCAT 1904
BL  AATTCTAAGAAATGATATGATAATGTTATTCATTGAAGCAAAAAAGTTATCCAATGTCAT 1915
    ************************************************************

CC  CTGGGCCTGTGTGAAAATGTATTTGCCCCCATAGTTACTAATTCCCCAAATCTATGAAAC 1964
BL  CTGGGCCTGTGTGAAAATGTATTTGCCCCCATAGTTACTAATTCCCCAAATCTATGAAAT 1975
    ***********************************************************

CC  GGCATTCGTAATGGGGTTCAGCTGGACTAGATGCAACCAAGCCTGATTACTGCTGGTAAA 2024
BL  GGCATTCGTAATGGGGTTCAGCTGGACTAGATGCAACCAGGCCTGATTACTGCTGGTAAA 2035
    *************************************** ********************

CC  AGGTTAAAAGGTTTTACCCAGTACGACTCATCCAGGAAGCCACAAAAGAGCCAAGGACAC 2084
BL  AGGTTAAAAGGTTTTACCCAGTATGACTCATCCAGGAAGTCACAAAAGAGCCAAGGATAC 2095
    *********************** *************** ***************** **

CC  CATCAAAGGAACTACAGGCCTCTCTTGCATCAATAAAGGTCACTGTTCATGACTCCACTC 2144
BL  CATCAAAGGAACTACAGGCCTCTCTTGCATCAATAAAGGTCACTGTTCATGACTCCACTC 2155
    ************************************************************

CC  AGAAAGACACTGGGCAAAAATGGCAACCATGGAAGAGTGGTGAGGTGAAAACCACTGCTA 2204
BL  AGAAAGACACTGGGCAAAAATGGCATCCATGGAAGAGTGGTGAGGTGAAAACCACTGCTA 2215
    ************************ ***********************************


CC  ACCCAGAAGAACATTAAGGTTCATCTGAATTTTGCCAAAACACACCTTGATGATCAAAAT 2264
BL  ACCCAGAAGAACATTAAGGTTCATCTGAATTTTGCCAAAACACACCTTGATGATCAAAAT 2275
    ************************************************************

CC  GAACACTGAA 2274
BL  GAACACTGAA 2285
    **********
```

>part2

```
CC  ACACACACAAGCTGTCGTTCCCTACTTAGCACTTTTGTGTATAGCGTATTTGTTGTAGTA 60
BL  ACACACACAAGCTGTCGTTCCCTACTTAGCACTTTTGTGTATAGCGTATTTGTTGTAGTA 60
    ************************************************************
```

Appendix Figure B5 (Continued)

```
CC    CATAA-----GTCACCATTTCTCGTGTTGTCTGTATTCATTAAATTCGACC-AGATTAAT 114
BL    CATAACATAAGTCACCATTTCTCGTGTCGTCTGTAT-CATTAATATCGACCCAGATTAAT 119
      *****     ***************** ******** ******  ****** ********

CC    GCTGAATGGTCAAACACTTGACTGGTTCAGTTGGAAAATAATTTTTTGACTAACAATATG 174
BL    GCTGCGTGGTCAAACACTTGACCGGTTCAGTTGGAAA-TAATTTCT-GACTAACAATATG 177
      ****  **************** ************** ****** * *************

CC    CTGTTATTTTCTCTTAGATGTAATCGGAGGGTACTCATTAAATGAAGGCCAATCCGAAGC 234
BL    CTGTTATTT-CTCTTAGATGTAATCGTAGG-TACTCATTAAATGAAGGC-AATCCGAAGC 234
      ********* **************** *** ****************** **********

CC    ACACTTTTCAGTGCGATTCTCTGATTATCAGACCCTCACTGAAAGGGCACAGGCTATTTA 294
BL    ACACTTTTCAGTGCGATTCTCTGGTTATCAGACCCTCACAGAAAGGGCACAGGCTATTTA 294
      *********************** *************** ********************

CC    TTTATGTATGTAAGCAGGCTGTATTTACGTAAGGTGTAATCGAAGAGGATTCGTCTCCTC 354
BL    TTTATGTATGTAAGCAGGCTGTGTTTACGTAAGGTGTAATCGAAGAGGATTCGTCTCCTC 354
      ********************** *************************************

CC    TCCACTCACTGTTACGGAAGTGTATTTCCATAAACAGACTGTTATAAACAAATCAAGTTG 414
BL    TCCACTCACTGTTACGGAAGTGT-TTTCCATAA-CAGACTGTTATAAACAAATCAGGTTG 412
      *********************** ********* ********************* ****

CC    TTCTGCGTGGTTGATTTTTGTTGTTGCATTTATTTCACATCGTACAAATGTCGCGAAAGA 474
BL    TTCTGCGTGGTTTATTT----TGTTGCATT-ATTTCACATCGTACAAACGTCGCGAAAGA 467
      ************ ****    ********* ***************** ***********

CC    GCTGGAGACGCTGTAGCACTATAGCACGCTGACCTATTCAGGAAATGTTTTCGCAAAATT 534
BL    GCTGGAGACGCTGTAGCACTA-----CGCTGACCTGTTCAGGAAATGTTTTCGCAAAATT 522
      *********************     ********* ************************

CC    TATGGATATTCTGTTGTCAGAGTGAAAGAGACTTGAGACAACAAATGTGAGCCCAGGACT 594
BL    TATGGATATTCTGTTGTCAGAGTGAAAGAGACTTGAGACAACAAATGTGAGCCCAGGACT 582
      ************************************************************

CC    ATCTGTGTCCTGATTGTGTGCATGTGTTGCTTCCCAAGCACCATAACTCTACCTGCTGAA 654
BL    ATCTGTGTCCTGATTGTGTGCATGTGTTGCTTCCCAAGCACCATAACTCTACCTGCTGAA 642
      ************************************************************

CC    GTAGGAGGAGTTTGGAGGTCTCCCAAAATTTTTGGATTGAGTAACCTCTGTAACTGTAAA 714
BL    GTAGGAGGAGTTTGGAGGTCTCCCAAAATTTTTGGATCGAGTAACCTCTGTAACTGTAAA 702
      ************************************* **********************

CC    ATAAATCCCACACCCCGTCAGTACAGAATTATTTTATCAACTTGATTTATTATTCCATTT 774
BL    ATAAATCCCACACCCCGTCAGTACAGAATTATTTTATCAACTTGATTTATTATTCCATTT 762
      ************************************************************

CC    ATAATTTAATTCAAGTAGTAGGTTTATGATTTTAACTTACGATTTAATAATATTCCGACT 834
BL    ATAATTTAATTCAAGTAGTAGGTTTATGATTTTAACTGATGATGTAATAATATTCCGACT 822
      ************************************* * *** ****************
```

Appendix Figure B5 (Continued)

```
CC  ATTAATTAGCGCTTTTGAGGTTTTATTTCTGATCTTTACA-CTAATGTAATCTATTCGAA 893
BL  ATTAATTAGCGCTTTTGAGGTTTTATTCCTGATCTTTACAACTAATGTAATCTATTCTAA 882
    *************************** ************ **************** **

CC  TT-AATTTGACGTTATTTATAGCCCTACTCGTTTATCAGTTATTGAGCTTTGTGATCAGT 952
BL  TTTAATTTGACGTTATT-ATAGCCCTACTCGTTTATCAGTTATTGAGCTTTGTGATCAGT 941
    ** ************** ******************************************

CC  AAAACAGGCTAATAATGTATAACTAATCAATAAAGGAAAAAATAACTTTGAAATGACTTT 1012
BL  AAAACAGGCTAATAACGTATAACTAATCAATAAAGGAAAAAATAACTTTGAAATGACTTT 1001
    *************** ********************************************

CC  GATCCCACTTTGAGAACCACTATTTTAAGCTTTAGTGGACACACAGAGAGCAAACTTTCA 1072
BL  GATCCCACTTTGAGAACCACTATTTTAAGCTTTAGTGGACACACAGAGAGCAAGCTTTCA 1061
    ***************************************************** ******

CC  CTTACTGGCTACCCAGGCACCAAAACAGCAGTGTGTGCCAATACTTTAATCTTTAGCTTA 1132
BL  CTTACTGGCTACCCAGGCACCAAAACAGCAAAGTGTGCCAATACTTTAATCTTTAGCTTA 1121
    ******************************  ****************************

CC  GCTTTTGAAAGCAGAAGTGGCACTTAGAGCTCACTAAATCCCCTTTCTCATCTTTTTAAT 1192
BL  GCTTTTGAAAGCAGAAGTGGCACTTAGAGCTCACTAAATCCCCTTTCTCATCTTCTTAAT 1181
    ****************************************************** *****

CC  ACGCACGCATGGCGATTTTCCGACTTAACAGATTAATGTTAAGTGGTAAAGATGCTCAGC 1252
BL  ACGCACGCATGGCGATTTTCCGACTTAACAGATTAATGTTAAGTGGTAAAGATGCTCAGC 1241
    ************************************************************

CC  ATCATTCTGCTTTTAGTCTCCTGCAGTGCAAGGTTGGACAAAGGTAAAGTAAAGTGTTTG 1312
BL  ATCATTCTGCTTTTAGTCTCCTGCAGTGCAAAGTTGGACAAAGGTAA-GTAAAGTGTTTG 1300
    ******************************* *************** ************

CC  TGTGAGAGAGATAGAAAGGGGGTGGGGTGGGGGGAGTGCTATAAAAGGCCTGCTGCTGAG 1372
BL  TGTGAGAGAGATAGAAAGGGGGTGGGGTGGGGGGAGTGCTATAAAAGGCCTGCTGTTGAG 1360
    ******************************************************* ****

CC  AGAAAAATGCAC---AGACTG-AGAGACCA-CATTGTGAAGAGCAGAAGAACTGTCTGCA 1427
BL  AGAAAA-TGCACCATAGACTGTAGAGATCATCATTGTGAAGAGCAGAAGAACTGTCTGTA 1419
    ****** *****   ****** ***** ** *************************** *

CC  GGGCGACTGATCAAGGTAGGTACATGCAAGCTGTGGAACATATAACTATATAATATA--- 1484
BL  GGGCGACTATCAAGGATGGGTACGTGCAAGTTGTGGAACATATAACTATATAATATAATA 1479
    ********    * * * ***** ****** **************************

CC  --TACGTATTTTCAAACTGGTGTTTGGTTTTCCGTGAGCATAAAGA----TTTTCAGAGA 1538
BL  TATACATATTTTCAGACTGGTGTTTGGTTTTCTGTGAGCATAAAGAAAGATTTTCAGAGA 1539
      *** ******** ***************** *************    **********

CC  GGCAGCAGACAAGTGCTGTGTGTAATGGAATGGCTGAGCAGTTACCTGAGTGAATGTAAC 1598
BL  GGCAGCAGACAAGTGCTGTGTGTAATGGAATGGCTGAGCAGTTACCTGAGTGAATGTAAC 1599
    ************************************************************
```

Appendix Figure B5 (Continued)

```
CC  TCGGTTGGATTAAATGGT 1616
BL  TCGGTTGGATTAAATGGT 1617
    ******************
```

>part3

```
CC  GCTCCTTGTGTGCGCTGTTCAAACGGAAACAGGATTTTTCTTTCGCTTACCGATTTTTAT 60
BL  GCTCCTTGTGTGCGCTGTTCAAACGGAAACAGGATTTTTCTTTCACGTACCGATTTTTAT 60
    ******************************************** * *************

CC  TTCAGATCTACCTCCCATAACAAAACTGACTTTTGCTCTCGTCAATCCTGTGGATTTCTT 120
BL  TTCAGATCTACCTCCCATAACAAAACTGACTTTTGCTCTCGTCAATCCTGTGGATTTCTT 120
    ************************************************************

CC  TCAGATCCTCATGAGAAGGCTCGAATACCCTGCTGAAATAAACAACCACGGAAATTTGAA 180
BL  TCAGATCCTCATGAGAAGGCTCAAATACCCTGCTGAAATAAACAACCACGGAAATTTGAA 180
    ********************** *************************************

CC  TGGTTTCCTCTACAAATACCATTACAAACTATCAGAGAACCATTAAAACCATTATCATTA 240
BL  TGGATTCCTCTACAAATACCATTACAAACTATCAGCGAACCATTAAAACCATTATCATTA 240
    *** ******************************* ************************

CC  TTGGTCTTCAATGGTATCCACCGGACATAACATGTCACCAATAGAAAGCAACAAATTATC 300
BL  TTGGTCCTTAATGGTATCCACCGGACATAACATGACACCAATAGAAAGCAACAAATTATC 300
    ****** * ************************* *************************

CC  AGTAGAATTCCCATTAAAACCATTACAATTCCCAT-AAAACCATTACAAATTCTATG-GG 358
BL  AGTAGAATTCCCATTATAACCATTACAATTCCCATTAAAACCATTACAAATTCTATGAGG 360
    **************** ****************** ********************* **

CC  GTTTCTATTGGTATTCTATTTTTTTTTTT----TATCTTTTTCAGCAGGGTAGATAGAAA 414
BL  GTTTCTATTGGTATTCTGTTTTTTTTTTTCTTTTATCTTTTTCAGCAGGGTAGATAGAAA 420
    ***************** ***********    ***************************

CC  TAAGGATGCTGAATGAAAGACGGTTTACC-AAAATGCAGTCGTGCCT 460
BL  TGAGGATGCTGAATGAAAGACGGTTTACCCAAAATGCAGTCGTGCCT 467
    * *************************** *****************
```

>part4

```
CC  GATTGTGTAGTGATGCAACCAGTAGCAAGATCACACTGGTTTGAATGCATT-CATTATTC 59
BL  GATTGTGTAGTGATGCAACCAGTAGCAAGATCACACTGGTTTGAATGCATTTCATTATTC 60
    *************************************************** ********

CC  GTAATTCATTATTGTGTGATTGTTTGTCATCAGACATCTCATGATTCCTTATTGTTGTGA 119
BL  GTAATTCATTATTGTGTGATTGTTTGTCATCAGACATCTCATGATTCCTTATTGTTGTGA 120
    ************************************************************

CC  CATTTTTTGAAAGTTGTTT-GACACCAAAAAGGGTTAAAAGGGTGCTTTACTTCATCTAG 178
BL  CATTTTTTGAAAGTTGTTTTGACACCAAAAAGGGTTAAAAGGGTGCTTTACTTCATCTAG 180
    ******************* ****************************************
```

Appendix figure B5 (Continued)

```
CC  TGCTTCCCTATGCATTA 195
BL  TGCTTCCCTATGCATTA 197
    *****************
```

>part5

```
CC  AGTCTTAGTGTTGTTTGAGATGGTTCAAATGAGCCCCATGGTCGAAAGTCTGGGACCTCA 60
BL  AGTCTTAGTGTTGTTTGAGATGGTTCAAATGAGCCCCATGGTCGAAACTCTGGGACC-CA 59
    *********************************************** ********* **

CC  GGGTCCCAAGAAACCAACCATGTGAGACCAACTGATTGTGACCAAGCTTCGATGCGCTCT 120
BL  GGGTCCCAAGAAACCAACCATGTGAGACCAACTGATTGTGACCAAGCTTCAGTGCGCTCT 119
    **************************************************  ********

CC  CTTCGGCTTGCTTAAGCTTCCACCT 145
BL  CTTCGGCTTGCTTAAGCTTCCACCT 144
    *************************
```

Appendix figure B5 (Continued)

>part1

```
CC  CATGCTGTACTCATGCACCTCCAAATGCATTTGAACTCAAGCCTGTTTGTGAATTGTCTT 60
BL  CATGCTGTACTCATGCACCTCCAAGTGCATTTTAACTCAAGCCTCTTTGTAAATTGTCTT 60
    *********************** ******** *********** ***** *********

CC  TATATGACCTTCTTTAACATCTGCGAGTACTTTTAATTCCTTATTCTTTT-GATTGTTCT 119
BL  TATATGACCTTCTTTA-CATCTGCAAGTACTTTTACTTCCTTCTTTTTTTTGATTGTTCT 119
    **************** ******* ********** ****** ** **** *********

CC  TTCAG---TTTTTTGGTGCCACTTCCTGACAATGTTGTTCCCCTTATA---GCTCGATGC 173
BL  TTCATATTTTTTTTTGTGTCATTTCCTGACCATGTTGTTCCCCTTATACCTGCTCGATGC 179
    ****    ****** *** ** ******** *****************   *********

CC  CCTTGTGAAAGAATTCCAAAAGAAGAAGTGAAAACAATTCAATCATGCCCCAGCTTCCTC 233
BL  CCTTGCGAGAGAATTCCAAAAGAGGAAGTGAAAACAATCCAATCCTGCCCCAGCTTCCTA 239
    ***** ** ************** ************** ***** **************

CC  CAGCCTCTTTCTTCGCTGATAACACTTATCTGAACACACACACACACATCTGCTCATCCG 293
BL  CAGCCTCTTTCTTCGCTGATAACACTGATCTGAACACACACACACA--TCTGCTCATCCG 297
    ************************** *******************  ************

CC  TGGAGTGTGTCTTTTTCTGTTTATGTGGTTTTAGCGTCAAGCTTTCTGTCATCTGTGTAT 353
BL  TGCAGTGTGTCTATTTCTGTTTATGTGGTTTTAGCATCAAGCTTTCTGTCATCTGTG-AT 356
    ** ********* ********************** ********************* **

CC  AAGCAGCGAAGTGAAATGCATCATGTGATCATTCAATAAAACTTTTATTTTGGTATTCGG 413
BL  AAGCAGCGAAGTGAAATGCATCATGTGATCATACAATAAAACTTTTATTTTGGTATTCGG 416
    ******************************* ****************************
```

Appendix figure B6 The comparison of cGnRH II in channel and blue catfish by using blast 2 sequences program

```
CC  TCTCTTTCTGAAAGAATACATGGGACATTCACTGCTTTGCCAAATCCGTTATTAAAATCG 473
BL  TCTCTTTCTGAAGGAATACATGGAACATTCACTACTTTGCCAAATACGTTATTAAAATCG 476
    ************ ********** ********* *********** **************

CC  GAGTTAACGGCTTACGTTAGCGTGTGTGATTTATCTGATTTCCTTAACCTGTGAAGCGCA 533
BL  GAGTTAACGGGTCACATCAGCTTGTGTGATTTAGCTGATTTCCTTAACCTGTAAAGCACA 536
    ********** * ** * *** *********** ****************** **** **

CC  TTCTGAGCAC-------AGCTGATGCCCACCATATTAAAAACATACATT-----GCCTAA 581
BL  TTCTGAGCACTTCTTTCAGCTGATGCCCACCATATTAAAAACATACATTACATTGCCTAA 596
    **********       ********************************     ******

CC  AGTATGTGGACACCTGACCATCACCCATTTGTGGTTCTTCTCCAGACTGTTGCCACGTAG 641
BL  AGTATGTGGACACCTGACCATCACCCATTTGTGCTTCTTCTCCAAACTGTTGCCACATAG 656
    ********************************* ********** *********** ***

CC  TTGGAAGCACACAGTTCAGAATGCCTTTGTACTGCGTTACAATTACACTTCACTCGAAGT 701
BL  TTGGAAGCACACAGTTTAGAATGCCTTTGTACTGCGTTACAATTACACTTCACTCGAAGT 716
    **************** *******************************************

CC  AAGGGACCAGATATGTTCCAGTATGGCAATGCCTCTGTACACAAAGTGAGGTCCATGAAG 761
BL  AAGGGCCCAAATATGTTCCAGTATGGCAATGCCCCTGTGCACAAAGTGAGGTCCATGAAG 776
    ***** *** *********************** **** *********************

CC  ACGTGGTTTGCTGAGTCTGGATCAGAAGGATTCAAGTGGTCTGCACAGAGCCCTGACTTC 821
BL  ACATGGTTTGCTGAGTCTGGATCAGAAGGATTCAAGTGGTCTGCACAGAGCCCTGACCTC 836
    ** ****************************************************** **

CC  AACCCCTTTGAACACCACTGGGATGAAATTAGAACACCGGGGCATCTGTCCGTTGGGGAG 881
BL  AACCCCTTTGAACACCACTGGGATGAA-TTAGAACACCGGG-CATCTGTCCGTTTGGGAG 894
    *************************** ************* ************ *****

CC  TAGATGCCCTCTCTCACAGAGTCGTGTGGT-CCACAGAGGTACTGGGGCGTGTTGG-CCC 939
BL  TAGATGCC-TCTCTCACAGAGTCGTGTGGTTCCACAGAGGTACTGGGGCGTGTTGGTCCC 953
    ******** ********************* ************************* ***

CC  GGTGTAAGGT-ACGACACATTCAACCAGCTGAAGAT-AGGGCGGTCCATCTTGGCCTGAA 997
BL  GGTGTAAGGTTACGACACAT-CAACCAGCTGAAGATTAGGGCGGTCCATCTTGGCCTGAA 1012
    ********** ********* *************** ***********************

CC  GTGTCAGCTGACACCAAATGCCGGTCCACGCATAATGCTCTG 1039
BL  GTGTCAGCTGACACCAATTGCCGGTCCACGCATAATGCTCTG 1054
    ***************** ************************
```

>part2

```
CC  GAGATGCACCTGTAGTGGCATCCATTCGAAATGGCTGAACGAGTCAGACTTCTTGTCTGT 60
BL  GAGATGCACCTGTAGTGGCATCCATTCGAAATGGCTAAACGAGTCAGACTTCTTGTCTGT 60
    ************************************ ***********************

CC  GTCAGCTACACCGTGGAGGGCGTACAGATTAGACTCGAACCTAACAGAGCATTCCTTTCA 120
BL  GTCAGCTACACCGTAGAGGGCGTAGAGATTAGACTCGAACATAACAGAGCATTCCTTTCA 120
    ************** ********* *************** *******************
```

Appendix Figure B6 (Continued)

```
CC    AGTTAGGACAGGGGCCGGGGTTTT-CTTCTCGCTTTATG-AACATTATTTGTATGCGTGC 178
BL    AGTTAGGACAGGGGCTGGGGTTTTACTTGTCGCTTTATTCAACATCATTTGTATGCGTAC 180
      *************** ******** *** *********  ***** ************ *

CC    TTCCTGCTCAGACTGGTGTGTACGGTGTGCGGGGGTTA-ATTTGACT-GCAGCGA--GCT 234
BL    TTC-TGCTCCGACTGGTGTGTAAGCTGTGCGTGGGTTAGATTTGGCTAGCAGACAAGGGT 239
      *** ***** ************ * ****** ****** ***** ** ****  *  * *

CC    TGATCCTTTTGACTGTCTTTTT-CACA-GGAATTGCTCCCACACATTCAAAATGAATGCG 292
BL    CGATCCTTTTGTATGACTTTATGTACATGAAATTGCTCCCACACATTCAAAA-GAATGCA 298
       **********  ** **** *  *** * ********************** ******

CC    TCTTTTTCTGGGGCGATCAGTTTGTCTACGTGGTACGTTTTCGGCACGTGTTAATATCGG 352
BL    TCTTTTTATGGG-CGGTCAGTTTGTCTACGTGGTACGTTTTCGGCATGTGT-AATATCGG 356
      ******* **** ** ****************************** **** ********

CC    GCCTGTTTTGAAATGATTATAAGAGGCGACTTTTAAAGCGCGAGTCAGTGTGACCTCGTG 412
BL    GTCTGTTTTGAAATGATTATA-GAGGCGAATTTTAAAGTGCGAGTCAGTGTGACCTCGTG 415
      * ******************* ******* ******** *********************

CC    ACTTTGTGCGCGTGTGTCAGACTGGTACTCTGAGCGGATTAGTGCTGTCGGAAGCCTCGA 472
BL    ACTTTGTGCGCGTGTGTCAAACTGGTACTCTGAGCGGATTAGTGCTGTCGGAAGCCTCGG 475
      ******************* ***************************************

CC    GCGTGTTTAAACACCACTCGAGGGACAGGAAGTCAATCGGTGTCTCAGCCAGCCGAATCA 532
BL    GCGTGTTTAAACACCACTCGAGGGACAGGAACTCAATCGGTGTCTCAGCCAGACGAATTA 535
      ******************************* ******************** ***** *

CC    ATCAGCCCTGTAATAGCTCGCAAGACTGTTTTCAGATTTGGGTCTTGTCAGGGTCACCGT 592
BL    ATCAGCCCTGTAATAGCTCGCGAAACTGTTTTCAGATTTGGGTCTTGTCAGGGTCACCGT 595
      ********************* * ************************************

CC    GTGCTAGTCAAGGTTCAAAGGTCACTGGGCGGAGTTCACCTTGGACAGCTGAGAGGAAAT 652
BL    GTGCTAGTCAAGGTTCAAAGGTCACTGGGCTGAGTTCACCTTGGACAGCTGAGAGGAAAT 655
      ****************************** *****************************

CC    GAGGTGTCTTACGGGTGGCCAGAAGAGGGCAGCCACACTTATGAAACATTCATAATACGC 712
BL    GAGGTGTCTGAAAGGTGGCCAGAAGAGGGCAGTCACACTTATGAAACATTCATAATACGC 715
      ********* *  ******************* ***************************

CC    CC-TGTTTGACCGTGATGA-----TAAGATGGAAACGGCTTCGACACATTAGCCAGGATA 766
BL    CCCTGTTTGACCATGATGAATCCGTAAGATGGAGACGGCTTCGACACATGAGCCAGGATA 775
      ** ********* ******     ********* *************** **********

CC    CAGTGGATATAAAGAATTTTACAATGTTTTTGTGATGT 804
BL    CAGTGGATATAAAGAATTTTACAATGTTTTTGTGATGT 813
      **************************************
```

>part3

```
CC    GCATTTCAGAACAAAGGTTTCTTTGTTATAAAATCTGGCACTTTTTCATAAAACAGCTGT 60
BL    GCATTTCAGA-CAAAGGTTTCTTTGTTATAAAATCTGGCACTTTTTCA-AAAACAGCTGT 58
      ********** ************************************* ***********
```

Appendix Figure B6 (Continued)

```
CC    TAACAAAAACATCTGGATTGTGTATTTGCGACACAAGCATTAAAAACATGCTTAAAAACG 120
BL    -AACAAAAACATCTGGATTGTGTATTTGC-ACACAAG--TTAAAAACACGCT--AAAATC 112
       **************************** *******  ********* ***  ****

CC    ATCAGTCAAACA---------TGAGCATAAGCTCATGTGAACGAGATAAATGTAAAGCTC 171
BL    ATCAGTCAAACACACTATTTCTGAACATAAGCTCACG-AAACGAGATGAATG-AAAGCTC 170
      ************         *** ********** *  ******** **** *******

CC    CCTGTAAACGCTCTTATTACTCCCAAAAAATTTTTT-AATTCCGTGCGATTAATTGATTG 230
BL    C-TGTAAACGCTCGTATTACTCCCAATTTTTTTTTTTAATTCCATGCAGTTAATTGATTG 229
      * *********** ************    ****** ****** ***  ***********

CC    ATTCAGTTACGTGTTTGAAATCTTAACACGGCTGATCCAGCTCATAAGGACGCACGTGAA 290
BL    ATTCAGT-ACATGTTTGAAATCT-AATACGGCTGATCCAGCTCATAAGGATGCACGTGAA 287
      ******* ** ************ ** *********************** *********

CC    ATATTCTAATCGTTTTCAGAGACCAGTTAGTGGAAAATGGTTCATTTATCGAGGCTATTC 350
BL    -TATTA-AATCGTTT-CAGAAACGAGTCAGTGGAAAACGGTTCATTTATCGAGGCTATTC 344
       ****  ******** **** ** *** ********* **********************

CC    CAGGACCGCAAGGAACTCAAGCTATGCCACTACAGTATGTTGACTATGCTGAACCCGGTG 410
BL    CAGGACAGCAAGGAACTCAAACTATGCTACTACAGTATGTTGACTATGTTGAACCCGGTG 404
      ****** ************* ****** ******************** ***********

CC    TGGATTCGAGTGGCTGTCATGTGCGCCGGTCTTTAAACATCACTGAAACGAGACCGTCCA 470
BL    TGGATTCGAGTGGCTGTTATGTGCGCCGGTCTTTAAACATTACTGAAATGAGACCGTCCA 464
      ***************** ********************** ******* ***********

CC    AGCTGAACATATCACACGGATCTCAATTTAAGGTTCAAACTGTATCGTACTAGATACAAG 530
BL    AGCTGAACATATCACACGGATCTCAGTTTAAGGTTCAAACTGTATCGTACTAGATACAAG 524
      ************************* **********************************

CC    TGTGTAACAAACAAATATAATAGAGCAACATGAAATAGTAGGTCAGAGCAGAAATATTCA 590
BL    TGTATAACAAACAAATATAATACAGCAACAGGAAATAGTAGGTCAGAGCAGAAATATTCA 584
      *** ****************** ******* *****************************

CC    CATTCCCTGCCAGTGGTTCTGAAATCTCGCCCTCCTGCTGTGCCTCACCGCTCGGACAGA 650
BL    CATTCCCTGCTAGTGGTTCTGAAATCTCGCCCTCCTGCTGTGCCTCACCGCTCGGACAGA 644
      ********** *************************************************

CC    TATCTGGGGAGATCAAACTGTGTGAAGCGGGAGAATGCAGCTATCTGAGGCCACTGAGAA 710
BL    TATCTGGGGAGATTAAACTGTGTGAAGCGGGAGAATGCAGCTATCTGAGGCCACTGAGAA 704
      ************* **********************************************

CC    CCAACGTCCTGAAGAGCATCCTGGGGAGAAC 741
BL    CCAACGTCCTGAAGAGCATCCTGGTGAGAAC 735
      ************************ ******
```

>part4

```
CC    TGAGGGGCACTCTGTTCTCCCTGCACTTTGGGGGTTTCCTCCAGTTTCCTCTCCCGGTCC 60
BL    TGAGGGGCACTCTGTTCTCCCTGTGCTTTGGGGGTTTCCTCCAGTTTCCTCCCCCGGTCC 60
      ***********************  ************************** ********
```

Appendix Figure B6 (Continued)

```
CC   TAAGACAGGCGTTCTAGGCTGATTGGCATTTCCAAACTGTCCACAGTGTGTGAATGGGTG 120
BL   TAAGACAGGCGTTCTAGGCTGATTGGCATTTCCAAATGGTCCACAGTGTGTGAATGGGTG 120
     ************************************  **********************

CC   TGTGTGCGATTGTACCCTGC-ATGAGTTGGCACCCCGTCCAGGGTGTCCTCCATCTCGTG 179
BL   TGTGTGCGATTGTGCCCTGCGATGAGTTGGCACCCCGTCCAGGGTGTCCTAGATCTCGTA 180
     ************* ****** *****************************  ******* 

CC   CCCTGAGTTCCCAGGGATAGGCTCCAGGCTCCCTCAGACCCTGTGTAGAATACACGGTAC 239
BL   CCCTGAGTTCCCAGGGATAGGCTCCAGGCTCCCTCAGACCCTGTGTAGAATACACGGTAC 240
     ************************************************************

CC   ATAAAATGGATCAATGGATATTGTTGCTCTTGTTGTTGTTAAATCAGGACAGTGTCCAGG 299
BL   ATAAAATGGATCAATGGATATTGTTGCTCTTGTTGTTGTTAAATCAGGACAGTATCCAGG 300
     ***************************************************** ******

CC   TCTGAGGCGCAACTAAACAATAACTGTAGCTCTTCTTTTGATG 342
BL   TCTGAGGCGCAACTAAACAATAACTGTAACTGTTCTTTTGATG 343
     **************************** ** ***********
```

>part5

```
CC   CTGGGGTATAAAAAGAGAGAAGTATGAAGATGGTCTCAGGCATTGATACTTCCAAGACAA 60
BL   CTGGGGTATAAAAAGAGAGAAGTATGAAGATGGTCTCAAGCATTGATACT-CCAAGACAA 59
     ************************************** *********** *********

CC   AGAGCTCTTCAGAGTTCCACATCTCAGAAGAATTCAAATCTGAATAAAGCACATCAAATA 120
BL   -GAGCTTCTCAGAGTTCCACATCTCAGAAGA-TTCAAATCTGAACAAAACACATCAAATA 117
      *****  *********************** ************ *** ***********

CC   AGAGTGAAAGGGGTCATATATTTCATCATTTTCCTGATTTTTGATGTCATTTTTAGAACA 180
BL   AGAGTGAAAAGGGTCATATATTTCATCATTTTCCTGATTTTTGATGTCATTTT-AGAACA 176
     ********* ******************************************* ******

CC   TTATAGTGGCATTTAT-ATCTCTGTGTTAATCCTCTCCACCACATATTTTTCTCCTTTTC 239
BL   TTATAGTGGCATATATTATCTCTGTGTTAATCCTCTCCACCACATTATTTTCTCCTTTTC 236
     ************ *** ****************************  *************

CC   CGGTTTATATTGTAATCGGTTAATTATTATTATTATTTTT---TTTTAAAGCATTGTCTT 296
BL   TGGTTTATATTGTAATCGGTTAATTATTATTATTATTATTATTTTTTAAAGCATTTTCTT 296
      ************************************ **    ************ ****

CC   AAAATACTGTTATTGTT 313
BL   AAAATACTGTTATTGTT 313
     *****************
```

>part6

```
CC   GACCAGACCTGAATCCAGACTCCAGACTGAGTGGTGTAATAAACTCCAAATGGGCTTCGA 60
BL   GACCAGACCTGAATCCAGACTCCAGACTGAGTGGTGTAATAAACTCCAAATGGGCTTCAA 60
     ********************************************************** *
```

Appendix Figure B6 (Continued)

```
CC  CTAAGCATTACTTTATTATACGTTGATGTTGAT-ATACACGAG-ATTCATTTTTCNTCAC 118
BL  CTAAGCATTAGTTTATTATACATTGATGTTGATTATACACGAGTATTCATTTTACATCAC 120
    ********** ********** *********** ********* ********* * ****

CC  AAAAATCTGCCAGTTAAAAGTGTGTAGACTTTTTACATCCACTCTAAAAATATATATGTT 178
BL  AAAAATCTGC-AGTTAAAAGTGGGTG---CTTTTACATCCACTCNAAAATCACATGT--- 173
    ********** *********** **     ************** ****  * ** *

CC  CATTGTTAATATTTA-TCTAACTATTAACTTTTAAAACTCTTCGAATAAAAAAGACGAAT 237
BL  TATTTTTAATATTTAATCTAACTATTAACTTTTAAAACTGTTCGAATAGCTGATACGAAT 233
     *** ********** *********************** ********    * ******

CC  CGTAGCCTTCTCTGTGGTGATTATAATAATTATGATTATGGCAAAGAAATCCCTT 292
BL  CGTAGCCTTCTCTGTGGTGATTAGAATAATTACGATGATGGCAAAGAAATCCCTT 288
    ********************** ******** *** *******************
```

>part7

```
CC  ATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTGCCTTGCTGTTGTGTTTGCAAGCGCAG 60
BL  ATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTGCCTTGCTGTTGTGTTTGCAAGCGCAG 60
    ************************************************************

CC  CTATCTGTTTCTCAACACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAAATCGAC 120
BL  CTATCTGTTTCTCAACACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAAATCGAC 120
    ************************************************************

CC  TCTTACAGCTCACCAGAGG 139
BL  TCTTACAGCTCACCAGAGG 139
    *******************
```

>part8

```
CC  TGCGTGGAGTTTGCATGTTCTTGGGGGTTTCCTCCTCCAGTCCAAGGACATGTTGTAGAC 60
BL  TGCGTGGAGTT-GCATGTTCTTGGGGGTTTCCTCCTCCGGTCCAAAGACATGTTGTAGAC 59
    *********** ************************** ****** **************

CC  TGATTGGCGATGGGTTGGCACCTTGTGCCTTGTGCCCCAGTATTTGTACCCCAAATACTT 120
BL  TGATTGGCGATGGGTTGGCACCTTGTGCCTTGTGCCCCAGTATTTGTACCCCAAATATTT 119
    ********************************************************* **

CC  TGTGATACAGGATTTTTT 138
BL  TGTGATACAGGATTTTTT 137
    ******************
```

>part9

```
CC  GGTTATCCAACACCTATCACCCATGTGAAAAACTAATTGCCCCC-TTAAACTCGAAACCT 59
BL  GGTTATCCAACACCTATCACCCATGTGAAAAACTAATTGCCCCCCTTAAACTTGAAACCT 60
    ******************************************** ******* *******
```

Appendix Figure B6 (Continued)

```
CC  TGTTGTGACATTTTATTTATGAATGTATTACTAA-GAATAAAGAATTGAATCTCATTTTT 118
BL  TGTTGTGACATTTTATTTATGAATGTATTAGTAAAGAATAAAGAATTGAATCTCATTTTT 120
    ****************************** *** *************************
```

>part10

```
CC  TTTTATTTCCATGAGCTGTAAGCCTTAATCATCAAGAATAAAACGAACAAAAGGTTTGAA 60
BL  ----ATTTCCATGAGCTGTAAGCCTTAATCATCAAGAATAAAACGAACAAAAGGTTTGAA 56
        ********************************************************

CC  ACATTTCACTTTGTGTATTGAATCTAAGNAAATTTGAAAGTCCCACTTTTTGATTTC 117
BL  ACGTTTCACTTTGTGTATTGAATCTA-GAATATATGAAAGTCCCACTTTTTGAATTC 112
    ** *********************** * * ** ******************* ***
```

>part11

```
CC  TTGAGTGCATATATGTATTCGGGCAGCATCTCTCAGGGGGTCAGGGGTGTTTGAAGATGA 60
BL  TTGAGTGCATATATGTATTCGGGCGGCATCTCTCAGGGGGTCAGGGGTGTT-GAAGATGA 59
    ************************ ************************** ********

CC  TAATTGG-AATCGAGCCGACGTTATGTGTCCCAGTGGAACCCCTCAGACC 109
BL  TAATTGGGAATCGAGCCGACGTTATGTGTCCCAGTGGAACCCCTCAGACC 109
    ******* ******************************************
```

>part12

```
CC  ATTCTTTTCTGAACTATACACTATATTCATGACGCAGGCTTAAAGATAATCCACATCTG- 59
BL  ATTCTTTTCTGATCTATACACTATATTCATGACGCAGGCTTAAAAATAATCCACATTTGG 60
    ************ ******************************* *********** **

CC  AATAGT-AGTAGGCCTATATCTGCAAAATGCTCATGCCT-CAAAAGTCCTCGTGTTGTCG 117
BL  AATAGTTAGTAGGCCTATATCTGCAAATGACTCATGCCTATGGAAGTCCTCGTGTTGTTG 120
    ****** ********************   *********    *************** *

CC  CACGCCATTCA 128
BL  CACGCCATTCA 131
    ***********
```

>part13

```
CC  TCATACTAAATAGTTTTAGATCTTCAAACGAAATACAACAGAAAACAAAAGCAACCTGAG 60
BL  TCATACGAAATAGTTTTAGATCTTCAAACGAAATACAACAGAAAACAAAAGCAACCTGAG 60
    ****** *****************************************************

CC  TAAA 64
BL  TAAA 64
    ****
```

Appendix Figure B6 (Continued)

>part14

```
CC  AAATCGAGATAAATCAGAAGTCTGAATTTTTTCCACTCTCGATGTGAAATG 51
BL  AAATCGAGATAAATCAGAAGTCAGAATTTTTTCCACTCTCGATGTGAAATG 51
    ********************** ****************************
```

>part15

```
CC  TGTCATAGTTTATCATTGCATTTAGCTATGAATGC 35
BL  TGTCATAGTTTATCATTGCATTTAGCTATAAATGC 35
    ***************************** *****
```

>part16

```
CC  AAGGCACATGTTTA--TTCACAGTGACAGTGCTGTGGGAAAAGTATTTGCTTTGATT 55
BL  AAGGCACATATTTAATTTCACATGTACAGTGCTGTGAAAAATGTATTTGCTTTGATT 57
    ********* ****  ******   ***********  *** ***************
```

Appendix Figure B6 (Continued)

```
CC  GATTACTCAGCTCCTTAAAGTGGAAAGTGGTTTCTTCAGTCTCGGCGAACAACCAATGGG 60
BL  ------------------------------------------------------------

CC  ATTTTTCCAGGCCGACACACAAAATATGAAAAGTTTCTGCTTTGGTACTTAGTGGAAATT 120
BL  ------------------------------------------------------------

CC  GCTAGAGTTTGACTCTGAAATCTGAAGGAGGAGCCATTTTTAAAGCTCTTCTGCTTTTCT 180
BL  ------------------------------------------------------------

CC  TTAGCAAATTCCGTACCATATCATATATATTTATAATATATGCATGCTTAAAAAGACAAA 240
BL  ------------------------------------------------------------

CC  CGTATTGCTACCTATATCCCATATATAACAGGTGATAAGCTTATTGAACTATTTTAAAGT 300
BL  ------------------------------------------------------------

CC  TAGGTATAAAATTACATATGCAATGTAAATGTATGTTTCTTAGCTCCTTGTGTGCGCTGT 360
BL  ------------------------------------------GCTCCTTGTGTGCGCTGT 18
                                              ******************
CC  TCAAACGGAAACAGGATTTTTCTTTCGCTTACCGATTTTTATTTCAGATCTACCTCCCAT 420
BL  TCAAACGGAAACAGGATTTTTCTTTCACGTACCGATTTTTATTTCAGATCTACCTCCCAT 78
    ************************** * *******************************

CC  AACAAAACTGACTTTTGCTCTCGTCAATCCTGTGGATTTCTTTCAGATCCTCATGAGAAG 480
BL  AACAAAACTGACTTTTGCTCTCGTCAATCCTGTGGATTTCTTTCAGATCCTCATGAGAAG 138
    ************************************************************

CC  GCTCGAATACCCTGCTGAAATAAACAACCACGGAAATTTGAATGGTTTCCTCTACAAATA 540
BL  GCTCAAATACCCTGCTGAAATAAACAACCACGGAAATTTGAATGGATTCCTCTACAAATA 198
    **** **************************************** **************
```

Appendix figure B7 The comparison of caGnRH in channel and blue catfish by using clustalW program

```
CC  CCATTACAAACTATCAGAGAACCATTAAAACCATTATCATTATTGGTCTTCAATGGTATC 600
BL  CCATTACAAACTATCAGCGAACCATTAAAACCATTATCATTATTGGTCCTTAATGGTATC 258
    ***************** ****************************** * *********

CC  CACCGGACATAACATGTCACCAATAGAAAGCAACAAATTATCAGTAGAATTCCCATTAAA 660
BL  CACCGGACATAACATGACACCAATAGAAAGCAACAAATTATCAGTAGAATTCCCATTATA 318
    *************** ****************************************** *

CC  ACCATTACAATTCCCAT-AAAACCATTACAAATTCTATG-GGGTTTCTATTGGTATTCTA 718
BL  ACCATTACAATTCCCATTAAAACCATTACAAATTCTATGAGGGTTTCTATTGGTATTCTG 378
    ***************** ********************* *******************

CC  TTTTTTTTTTT----TATCTTTTTCAGCAGGGTAGATAGAAATAAGGATGCTGAATGAAA 774
BL  TTTTTTTTTTTCTTTTATCTTTTTCAGCAGGGTAGATAGAAATGAGGATGCTGAATGAAA 438
    ***********    **************************** ****************

CC  GACGGTTTACC-AAAATGCAGTCGTGCCTAAAA-AAAAAAAAAAAAAAAAGATTGTGTAG 832
BL  GACGGTTTACCCAAAATGCAGTCGTGCCTAAAAGAAAAAAAAAAAAAAAAGATTGTGTAG 498
    *********** ********************* **************************

CC  TGATGCAACCAGTAGCAAGATCACACTGGTTTGAATGCATT-CATTATTCGTAATTCATT 891
BL  TGATGCAACCAGTAGCAAGATCACACTGGTTTGAATGCATTTCATTATTCGTAATTCATT 558
    ***************************************** ******************

CC  ATTGTGTGATTGTTTGTCATCAGACATCTCATGATTCCTTATTGTTGTGACATTTTTTGA 951
BL  ATTGTGTGATTGTTTGTCATCAGACATCTCATGATTCCTTATTGTTGTGACATTTTTTGA 618
    ************************************************************

CC  AAGTTGTTT-GACACCAAAAAGGGTTAAAAGGGTGCTTTACTTCATCTAGTGCTTCCCTA 1010
BL  AAGTTGTTTTGACACCAAAAAGGGTTAAAAGGGTGCTTTACTTCATCTAGTGCTTCCCTA 678
    ********* **************************************************

CC  TGCATTAGTGTGTGTATGTATGTATGTATGTATGTATGTATA-GTGTGTA-TATATAATA 1068
BL  TGCATTAGTGTGTGTATGTATGTATGTATGTATGTATGTATGTGTGTGTAGTATATATAA 738
    *****************************************  ******* ******  *

CC  TACACACACACAAGCTGTCGTTCCCTACTTAGCACTTTTGTGTATAGCGTATTTGTTGTA 1128
BL  TATACACACACAAGCTGTCGTTCCCTACTTAGCACTTTTGTGTATAGCGTATTTGTTGTA 798
    ** *********************************************************

CC  GTACATAA-----GTCACCATTTCTCGTGTTGTCTGTATTCATTAAATTCGACC-AGATT 1182
BL  GTACATAACATAAGTCACCATTTCTCGTGTCGTCTGTAT-CATTAATATCGACCCAGATT 857
    ********     ***************** ******** ******  ****** *****

CC  AATGCTGAATGGTCAAACACTTGACTGGTTCAGTTGGAAAATAATTTTTTGACTAACAAT 1242
BL  AATGCTGCGTGGTCAAACACTTGACCGGTTCAGTTGGAAA-TAATTTCT-GACTAACAAT 915
    *******  **************** ************** ****** * **********

CC  ATGCTGTTATTTTCTCTTAGATGTAATCGGAGGGTACTCATTAAATGAAGGCCAATCCGA 1302
BL  ATGCTGTTATTT-CTCTTAGATGTAATCGTAGG-TACTCATTAAATGAAGGC-AATCCGA 972
    ************ **************** *** ****************** *******
```

Appendix Figure B7 (Continued)

```
CC  AGCACACTTTTCAGTGCGATTCTCTGATTATCAGACCCTCACTGAAAGGGCACAGGCTAT 1362
BL  AGCACACTTTTCAGTGCGATTCTCTGGTTATCAGACCCTCACAGAAAGGGCACAGGCTAT 1032
    ************************** *************** *****************

CC  TTATTTATGTATGTAAGCAGGCTGTATTTACGTAAGGTGTAATCGAAGAGGATTCGTCTC 1422
BL  TTATTTATGTATGTAAGCAGGCTGTGTTTACGTAAGGTGTAATCGAAGAGGATTCGTCTC 1092
    ************************* **********************************

CC  CTCTCCACTCACTGTTACGGAAGTGTATTTCCATAAACAGACTGTTATAAACAAATCAAG 1482
BL  CTCTCCACTCACTGTTACGGAAGTGT-TTTCCATAA-CAGACTGTTATAAACAAATCAGG 1150
    ************************** ********* ********************* *

CC  TTGTTCTGCGTGGTTGATTTTTGTTGTTGCATTTATTTCACATCGTACAAATGTCGCGAA 1542
BL  TTGTTCTGCGTGGTTTATTTT----GTTGCATT-ATTTCACATCGTACAAACGTCGCGAA 1205
    *************** *****    ******** ***************** ********

CC  AGAGCTGGAGACGCTGTAGCACTATAGCACGCTGACCTATTCAGGAAATGTTTTCGCAAA 1602
BL  AGAGCTGGAGACGCTGTAGCACTA-----CGCTGACCTGTTCAGGAAATGTTTTCGCAAA 1260
    ************************     ********* *********************

CC  ATTTATGGATATTCTGTTGTCAGAGTGAAAGAGACTTGAGACAACAAATGTGAGCCCAGG 1662
BL  ATTTATGGATATTCTGTTGTCAGAGTGAAAGAGACTTGAGACAACAAATGTGAGCCCAGG 1320
    ************************************************************

CC  ACTATCTGTGTCCTGATTGTGTGCATGTGTTGCTTCCCAAGCACCATAACTCTACCTGCT 1722
BL  ACTATCTGTGTCCTGATTGTGTGCATGTGTTGCTTCCCAAGCACCATAACTCTACCTGCT 1380
    ************************************************************

CC  GAAGTAGGAGGAGTTTGGAGGTCTCCCAAAATTTTTGGATTGAGTAACCTCTGTAACTGT 1782
BL  GAAGTAGGAGGAGTTTGGAGGTCTCCCAAAATTTTTGGATCGAGTAACCTCTGTAACTGT 1440
    **************************************** *******************

CC  AAAATAAATCCCACACCCCGTCAGTACAGAATTATTTTATCAACTTGATTTATTATTCCA 1842
BL  AAAATAAATCCCACACCCCGTCAGTACAGAATTATTTTATCAACTTGATTTATTATTCCA 1500
    ************************************************************

CC  TTTATAATTTAATTCAAGTAGTAGGTTTATGATTTTAACTTACGATTTAATAATATTCCG 1902
BL  TTTATAATTTAATTCAAGTAGTAGGTTTATGATTTTAACTGATGATGTAATAATATTCCG 1560
    **************************************** * *** *************

CC  ACTATTAATTAGCGCTTTTGAGGTTTTATTTCTGATCTTTACA-CTAATGTAATCTATTC 1961
BL  ACTATTAATTAGCGCTTTTGAGGTTTTATTCCTGATCTTTACAACTAATGTAATCTATTC 1620
    ****************************** ************ ****************

CC  GAATT-AATTTGACGTTATTTATAGCCCTACTCGTTTATCAGTTATTGAGCTTTGTGATC 2020
BL  TAATTTAATTTGACGTTATT-ATAGCCCTACTCGTTTATCAGTTATTGAGCTTTGTGATC 1679
     **** ************** ***************************************

CC  AGTAAAACAGGCTAATAATGTATAACTAATCAATAAAGGAAAAAATAACTTTGAAATGAC 2080
BL  AGTAAAACAGGCTAATAACGTATAACTAATCAATAAAGGAAAAAATAACTTTGAAATGAC 1739
    ****************** *****************************************
```

Appendix Figure B7 (Continued)

```
CC  TTTGATCCCACTTTGAGAACCACTATTTTAAGCTTTAGTGGACACACAGAGAGCAAACTT 2140
BL  TTTGATCCCACTTTGAGAACCACTATTTTAAGCTTTAGTGGACACACAGAGAGCAAGCTT 1799
    ******************************************************** ***

CC  TCACTTACTGGCTACCCAGGCACCAAAACAGCAGTGTGTGCCAATACTTTAATCTTTAGC 2200
BL  TCACTTACTGGCTACCCAGGCACCAAAACAGCAAAGTGTGCCAATACTTTAATCTTTAGC 1859
    *********************************  *************************

CC  TTAGCTTTTGAAAGCAGAAGTGGCACTTAGAGCTCACTAAATCCCCTTTCTCATCTTTTT 2260
BL  TTAGCTTTTGAAAGCAGAAGTGGCACTTAGAGCTCACTAAATCCCCTTTCTCATCTTCTT 1919
    ********************************************************* **

CC  AATACGCACGCATGGCGATTTTCCGACTTAACAGATTAATGTTAAGTGGTAAAGATGCTC 2320
BL  AATACGCACGCATGGCGATTTTCCGACTTAACAGATTAATGTTAAGTGGTAAAGATGCTC 1979
    ************************************************************

CC  AGCATCATTCTGCTTTTAGTCTCCTGCAGTGCAAGGTTGGACAAAGGTAAAGTAAAGTGT 2380
BL  AGCATCATTCTGCTTTTAGTCTCCTGCAGTGCAAAGTTGGACAAAGGTAA-GTAAAGTGT 2038
    ********************************** *************** *********

CC  TTGTGTGAGAGAGATAGAAAGGGGGTGGGGTGGGGGGAGTGCTATAAAAGGCCTGCTGCT 2440
BL  TTGTGTGAGAGAGATAGAAAGGGGGTGGGGTGGGGGGAGTGCTATAAAAGGCCTGCTGTT 2098
      ********************************************************** *

CC  GAGAGAAAAATGCAC---AGACTG-AGAGACCA-CATTGTGAAGAGCAGAAGAACTGTCT 2495
BL  GAGAGAAAA-TGCACCATAGACTGTAGAGATCATCATTGTGAAGAGCAGAAGAACTGTCT 2157
    ********* *****   ****** ***** ** **************************

CC  GCAGGGCGACTGATCAAGGTAGGTACATGCAAGCTGTGGAACATATAACTATATAATATA 2555
BL  GTAGGGCGACTATCAAGGATGGGTACGTGCAAGTTGTGGAACATATAACTATATAATATA 2217
    * *********    * * * ***** ****** **************************

CC  -----TACGTATTTTCAAACTGGTGTTTGGTTTTCCGTGAGCATAAAGA----TTTTCAG 2606
BL  ATATATACATATTTTCAGACTGGTGTTTGGTTTTCTGTGAGCATAAAGAAAGATTTTCAG 2277
         *** ******** ***************** *************    *******

CC  AGAGGCAGCAGACAAGTGCTGTGTGTAATGGAATGGCTGAGCAGTTACCTGAGTGAATGT 2666
BL  AGAGGCAGCAGACAAGTGCTGTGTGTAATGGAATGGCTGAGCAGTTACCTGAGTGAATGT 2337
    ************************************************************

CC  AACTCGGTTGGATTAAATGGTGAGAGAGTGAGAGAAAGATAGAAACAGATTAAGGCAGAA 2726
BL  AACTCGGTTGGATTAAATGGTGAGAGAGTGAGAGAAAGA--GAAACAGATTACGGCAGAA 2395
    ***************************************  *********** *******

CC  AGAGAGGAACAGAAGCAAAGAGAG--GAAGAAATGAAGAGTCCATGTGGGTTTCCTCTGG 2784
BL  AGAGAGGAACAGAAGCAAAGAGAGAGGAAGAAATGAAGAGTCCATGTGGGTTTCCTCCGG 2455
    ************************  ******************************* **

CC  GTGCTCTGGTTTCCACCTACCTTCTGGTAAGATACCACTGTTGAAGTGTGAATGTCTTGC 2844
BL  GTGCTCTGGTTTCCACCTACCTTCTGGTAACATACCACTGTTAAAGTGTGAATGTCTTGC 2515
    ****************************** *********** *****************
```

Appendix Figure B7 (Continued)

```
CC  GATATCCTTGTATTCCATCCAGAGTGTGTTCCCAGTATCGGCTCCAGATCCGTCGCAACC 2904
BL  GATATCCTTGTATCCCATCCAGAGTGTGTTCCCAGTATCGGCTCCAGATCCATCGCAACC 2575
    ************* ************************************* ********

CC  CTGACCAAGATAAAGCTCTTAGTGAAGAAGAATAAATGAATGTTGTACTATACCATACTC 2964
BL  CTGACCAAGATAAAGCTCTTAGTGAAGAAGAATAAATGAATGTTGTACTATACCATACTC 2635
    ************************************************************

CC  ACCTCACAGCGTGTGAATCCAGG-CTTACTGCACAACTTTTATTAGATAATACTATTTAC 3023
BL  ACCTCACAGTGTGTGAATCCAGGACTTACTGCACAACTTTTATTAGATAATACTATTTAC 2695
    ********* ************* ************************************

CC  ATGCTTAAGGTAAG-ATGGATGCTGTCTTTGTTTTCCTGTTTCTTTAGGATGGGTATAAA 3082
BL  ATTCTTAAGGTAAGTATGGAAGCTGTCTTTGTTTTCCTGTTTCTTTAGGATGGGTATAAA 2755
    ** *********** ***** ***************************************

CC  GCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGCAGGTGAGCTCTCAGCACTG 3142
BL  GCGAGCACTCTGGTGGATGGTGGTGTGTGTTGTGGTGCTGCAGGTGAGCTCTCAGCACTG 2815
    ************************************************************

CC  GTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGGAGGTAACGGCCACTAA 3202
BL  GTCTCATGGCCTTAATCCTGGAGGAAAGCGTGCAGCGTTGCAGGAGGTAACGGCCACTAA 2875
    ************************************************************

CC  ACTAAACAATGTCTAAACTCAATGATATTGTTCTGCTAAGCTGAGCTACCAAACCTATAC 3262
BL  ACTAAACAATGTCTAAACTCAATGATATTGTTCTGCTAAGCTGAGCTAGCAAACCTATAC 2935
    ************************************************ ***********

CC  ACTTTATTCGTTTTAAATGTGTGTTTGACTGACTTTTTTTTC-CCCCCTCCCGAGACTGT 3321
BL  ACTTTATTCCTTTTAAATATGTGTTTGACTGACTTTTTTTTTTCCCCCTCCCCAGACTGT 2995
    ********* ******** **********************  ********* *******

CC  TGAAGAAATGCCGAGGACCTCCGGCTACGTGTGTGATTACGTAGATGTTTCACCTCGGAA 3381
BL  TGAAGAAATGCAGAGGACCTCCGGCTACGTGTGTGATTACGTAGACGTTTCACCTCGGAA 3055
    *********** ********************************* **************

CC  TAAACTCTACAGGCTGAAAGATCTGCTGGTGCACAGCTCATAATTTTCTTTTTGCTCGTA 3441
BL  TAAACTCTACAGGCTGAAAGATCTGCTGGTGCACAGCTCATAGTTTTCTTTTTGCTCATA 3115
    ****************************************** ************** **

CC  AGCCAACAAATTGAAGGCAGTGTCTTGATGTTTATCTTTTGTCTTCTCTTATAGACCAGT 3501
BL  AGCCAACAAATTGAAGGCAGTGTCTTGATGTTTATCTTTTGTCTTCTCTTATAGACCAGT 3175
    ************************************************************

CC  GTTGCTGAGCGAGAAATTGGACAGTAAGACAAATAACAAAACTGATCAAGAGCTTCAATA 3561
BL  GTTGCTGAGCGAGAAATTGGACAGTAAGACGAATAACAAAACTGATCAAGATCTTCAATA 3235
    ****************************** ******************** ********

CC  AAATGCTTTGCCTTTATTTCGTGTGGGTTTATTTTTTT-ATTACCGAAACACTTTCGCTT 3620
BL  AAATGCTTCGCCTTTATTTCGTGTGGGTTTATTTTTTTTATTACCGAAACACTTTCGCTT 3295
    ******** ***************************** *********************
```

Appendix Figure B7 (Continued)

```
CC  TTTTTTTTTTAACGTACTGCTTTTATGTTGCACATTGTTATTCTCACAATTTATTAGGAA 3680
BL  TTTTTTTTT-AACATACTGCTTTTAAGTTGCACATTGTTATTTGCACAATTTATTAGGAA 3354
    ********* *** *********** ****************  ****************

CC  TACAGTATTAATAAAATGTGTTATAATTAAGTAATACCACACACACAAGAGTGCTGCTCA 3740
BL  TACAGTATTAATAAGATGTGTTGTAATTAAGTAATACCGCACACACAAGAGTGCTGTTCA 3414
    ************** ******* *************** ***************** ***

CC  GCACGGCTGGGATTTTGCCGCAGGTAATCCAGGCGGGTGTGAGCACTGGGTATCCATAGG 3800
BL  GCACGGCTGGGATTTTGCCGCAGGTAATCAAGGCGGGTGTGAGCACTGGGTATCCATAGG 3474
    ***************************** ******************************

CC  ACCC-TTTAAAAAAGTTTTTATTCTCATGCATATGTCGTATTCATCCTAATCCTCACTCT 3859
BL  ACCCCTTTAAAAAAGTTTTTATTCTCATGCATATGTCGTATTCATCCTAATCCTCACTCC 3534
    **** ******************************************************

CC  ATCTCCATCCCCGACCTTATCCTCATCTTCAACCCCATCTCCATCCCTTTCCTCATCAAC 3919
BL  ATCTCCATCCCCAACCTTATCCTCATCTTCAACCCCATCTCCATCCCTTTCCTCATCAAC 3594
    ************ ***********************************************

CC  AGCATCTTCATTTTTGTTCTCATCTCCAAAATGGCCTTTTCTGATCATACTGTATGTGAC 3979
BL  AGCATCTTCATTTTTATTCTCATCTCCAAAATGGCCTTTCCTGATCATACTGTATGTGAC 3654
    *************** *********************** ********************

CC  GTGAATCAAGATTCCAAGCAGCTAAAAATGTTCACGTGATGTAAGATGTCCCTAGTTATC 4039
BL  GTGAATCAAGTTTCCAAGCAGATAAAGATGTTCACGTGATGTAAGATGTTCCTAGTTATC 3714
    ********** ********** **** ********************** **********

CC  TTACCCCAGCATGTATCATCTGAGCCATCAACAGCTGTCTGTATACACATCTCCTTGTAC 4099
BL  TTACCCCAGCATGTATCATCTGAGCCATCAACAGCTGTCTGTATACACATCTCCTTGTAC 3774
    ************************************************************

CC  TGAATTCCATTGGAAAAGATATGAATTTCCTGAATCTGTTATAATTCATGTTACATTTAT 4159
BL  TGAATTCCATTGGAAAAGATACGAATGTCCTGAATCTGTTATAATTCATGTTACATTTAT 3834
    ********************* **** *********************************

CC  AATTGAATGACATCCAAAAGTTCAAAAAATTCTGGACATATCGATAACAA---------C 4210
BL  AATTGAATTACATCCAAAAGTTAAAAAAGTTCTGGACATATCGATAATAGATATGTCATC 3894
    ******** ************* ***** ****************** *          *

CC  TTCACAATAGATGCCATGGCTGAGTATTTTTTTAATTATAAAAATGATTCTATTCTATAC 4270
BL  TTCATAATAGATGTCATGGCTGAGTATTTTTTTAATTATAAAAATGATTCTATTCTATAC 3954
    **** ******** **********************************************

CC  AGTAAAGATTTATATAATATTAATAACATTCTTTTGCGTTTGCGAGCCTATTAAAAAATG 4330
BL  AGTAAAGATTTATATAATATTAATAACATTCTTTTGCGTTTGCGAGCCTATTAAAAAATG 4014
    ************************************************************

CC  AGAATTCATGC--CATGCCATGATATTGTGTCATGCAGCTTAATATACATCAGTGGTTTC 4388
BL  AGAATTCATGTGTCATGCCATGATATCGTGTCATGCAGCTTAATATGCATCAGTGGTTTC 4074
    **********   ************* ******************* *************
```

Appendix Figure B7 (Continued)

```
CC  GTTCTGGAAGATTCTGGAATGAGGACAAAAATGGATTGCGTTTTGCCACAAGAAAGGATT 4448
BL  GTTCTGGAAGATTCTGGAATGAGGACAAAAATGGATTGCGTTT-GCCACAAGAAGGATTT 4133
    ******************************************* ********** *  **

CC  TTTTCCCCCGCTTTTTAAGATTCACATCTTACAAAATGCTCTTTCTTTGAAATTTTGAAT 4508
BL  TTTTCCCCCGCTTTTTAAGATTCACATCTTACAAAATGCTCTT-CTTTGAAATTT-GAAT 4191
    ******************************************* *********** ****

CC  TCTTCTACATGGAAGCTATGGCCAAGGAACTTAAAAGGTATGTCCATCTTGGTACCTCTG 4568
BL  TCTTCTACATGGA-GCTATGGCCAAGGAACTTAAAAGGTATGTCCATCTTGGTACCTCTG 4250
    ************* **********************************************

CC  GTCACTCTAGATACGCCATGGAACCACAGACAAATTCTCAAATTCTAAGAAATGATATGA 4628
BL  GTCACTCTAGATACGCCATGGAACCACACACAAATTCTCAAATTCTAAGAAATGATATGA 4310
    **************************** *******************************

CC  TAATGTTATTCATTGAAGCAAAAAAGTTATCCAATGTCATCTGGGCCTGTGTGAAAATGT 4688
BL  TAATGTTATTCATTGAAGCAAAAAAGTTATCCAATGTCATCTGGGCCTGTGTGAAAATGT 4370
    ************************************************************

CC  ATTTGCCCCCATAGTTACTAATTCCCCAAATCTATGAAACGGCATTCGTAATGGGGTTCA 4748
BL  ATTTGCCCCCATAGTTACTAATTCCCCAAATCTATGAAATGGCATTCGTAATGGGGTTCA 4430
    *************************************** ********************

CC  GCTGGACTAGATGCAACCAAGCCTGATTACTGCTGGTAAAAGGTTAAAAGGTTTTACCCA 4808
BL  GCTGGACTAGATGCAACCAGGCCTGATTACTGCTGGTAAAAGGTTAAAAGGTTTTACCCA 4490
    ******************* ****************************************

CC  GTACGACTCATCCAGGAAGCCACAAAAGAGCCAAGGACACCATCAAAGGAACTACAGGCC 4868
BL  GTATGACTCATCCAGGAAGTCACAAAAGAGCCAAGGATACCATCAAAGGAACTACAGGCC 4550
    *** *************** ***************** **********************

CC  TCTCTTGCATCAATAAAGGTCACTGTTCATGACTCCACTCAGAAAGACACTGGGCAAAAA 4928
BL  TCTCTTGCATCAATAAAGGTCACTGTTCATGACTCCACTCAGAAAGACACTGGGCAAAAA 4610
    ************************************************************

CC  TGGCAACCATGGAAGAGTGGTGAGGTGAAAACCACTGCTAACCCAGAAGAACATTAAGGT 4988
BL  TGGCATCCATGGAAGAGTGGTGAGGTGAAAACCACTGCTAACCCAGAAGAACATTAAGGT 4670
    ***** ******************************************************

CC  TCATCTGAATTTTGCCAAAACACACCTTGATGATCAAAATGAACACTGAA---------- 5038
BL  TCATCTGAATTTTGCCAAAACACACCTTGATGATCAAAATGAACACTGAAATGGCCTCGA 4730
    **************************************************

CC  -------------------GTCTTAGTGTTGTTTGAGATGGTTCAAATGAGCCCCATGGT 5079
BL  GGCAATTCACCTTCATTTAGTCTTAGTGTTGTTTGAGATGGTTCAAATGAGCCCCATGGT 4790
                       *****************************************

CC  CGAAAGTCTGGGACCTCAGGGTCCCAAGAAACCAACCATGTGAGACCAACTGATTGTGAC 5139
BL  CGAAACTCTGGGACC-CAGGGTCCCAAGAAACCAACCATGTGAGACCAACTGATTGTGAC 4849
    ***** ********* ********************************************
```

Appendix Figure B7 (Continued)

```
CC  CAAGCTTCGATGCGCTCTCTTCGGCTTGCTTAAGCTTCCACCTGCCCCCTGGCTTTCTGG 5199
BL  CAAGCTTCAGTGCGCTCTCTTCGGCTTGCTTAAGCTTCCACCT----------------- 4892
    ********  *********************************

CC  AGCTCTGGAATTGACACCTTCAAGTTAAATTAGGACAGTAAAAAAAAAAAAAAAACAGTA 5259
BL  ------------------------------------------------------------

CC  CTTGAATGAAAATTTTATCTGAAATGTATGTTTCTTAGCTCCTTGTGTGCGCTGTTCAA 5319
BL  ------------------------------------------------------------

CC  ACGGAAACAGGATTT 5334
BL  ---------------
```

Appendix Figure B7 (Continued)

```
CC  TCAGGTCGAGTGCAGTAATATGGCGAGAGTGTTTGGAAAATGCGTGGAGTTTGCATGTTC 60
BL  ----------------------------------------TGCGTGGAGTT-GCATGTTC 19
                                            *********** ********

CC  TTGGGGGTTTCCTCCTCCAGTCCAAGGACATGTTGTAGACTGATTGGCGATGGGTTGGCA 120
BL  TTGGGGGTTTCCTCCTCCGGTCCAAAGACATGTTGTAGACTGATTGGCGATGGGTTGGCA 79
    ****************** ****** **********************************

CC  CCTTGTGCCTTGTGCCCCAGTATTTGTACCCCAAATACTTTGTGATACAGGATTTTTTAA 180
BL  CCTTGTGCCTTGTGCCCCAGTATTTGTACCCCAAATATTTTGTGATACAGGATTTTTTA- 138
    ************************************* *********************

CC  AAAAAAATTTTATTTCCATGAGCTGTAAGCCTTAATCATCAAGAATAAAACGAACAAAAG 240
BL  ------------TTTCCATGAGCTGTAAGCCTTAATCATCAAGAATAAAACGAACAAAAG 186
                ************************************************

CC  GTTTGAAACATTTCACTTTGTGTATTGAATCTAAGNAAATTTGAAAGTCCCACTTTTTGA 300
BL  GTTTGAAACGTTTCACTTTGTGTATTGAATCTA-GAATATATGAAAGTCCCACTTTTTGA 245
    ********* *********************** * * ** *******************

CC  TTTCGTTCCAGAAAAAAAAAGAAAAAAAGAAAAGAAAAAAAAACTTTCCCACGATATTCA 360
BL  ATTC-----------AATTACAGAAAAGAAAAAAAAAAAAAAACTTTCCCACGATATTCA 294
     ***           **  * * ****  **** **************************

CC  CTTTTTTTTTTTCTTTCTTTTTTTTTTTTTGAGATGCACCTGTAGTGGCATCCATTCGA 420
BL  CTTTTTTTT-----------------TTTTTGAGATGCACCTGTAGTGGCATCCATTCGA 337
    *********                 **********************************

CC  AATGGCTGAACGAGTCAGACTTCTTGTCTGTGTCAGCTACACCGTGGAGGGCGTACAGAT 480
BL  AATGGCTAAACGAGTCAGACTTCTTGTCTGTGTCAGCTACACCGTAGAGGGCGTAGAGAT 397
    ******* ************************************* ********* ****

CC  TAGACTCGAACCTAACAGAGCATTCCTTTCAAGTTAGGACAGGGGCCGGGGTTTT-CTTC 539
BL  TAGACTCGAACATAACAGAGCATTCCTTTCAAGTTAGGACAGGGGCTGGGGTTTTACTTG 457
    *********** ********************************** ******** ***
```

Appendix Figure B8 The comparison of chicken type II GnRH in channel and blue catfish by using clustalW program

```
CC    TCGCTTTATG-AACATTATTTGTATGCGTGCTTCCTGCTCAGACTGGTGTGTACGGTGTG 598
BL    TCGCTTTATTCAACATCATTTGTATGCGTACTTC-TGCTCCGACTGGTGTGTAAGCTGTG 516
      *********  ***** ************ **** ***** ************ * ****

CC    CGGGGGTTA-ATTTGACT-GCAGCGA--GCTTGATCCTTTTGACTGTCTTTTT-CACA-G 652
BL    CGTGGGTTAGATTTGGCTAGCAGACAAGGGTCGATCCTTTTGTATGACTTTATGTACATG 576
      ** ****** ***** ** ****  *  * * **********  ** **** *  *** *

CC    GAATTGCTCCCACACATTCAAAATGAATGCGTCTTTTTCTGGGGCGATCAGTTTGTCTAC 712
BL    AAATTGCTCCCACACATTCAAAA-GAATGCATCTTTTTATGGG-CGGTCAGTTTGTCTAC 634
       ********************** ****** ******* **** ** *************

CC    GTGGTACGTTTTCGGCACGTGTTAATATCGGGCCTGTTTTGAAATGATTATAAGAGGCGA 772
BL    GTGGTACGTTTTCGGCATGTGT-AATATCGGGTCTGTTTTGAAATGATTATA-GAGGCGA 692
      ***************** **** ********* ******************* *******

CC    CTTTTAAAGCGCGAGTCAGTGTGACCTCGTGACTTTGTGCGCGTGTGTCAGACTGGTACT 832
BL    ATTTTAAAGTGCGAGTCAGTGTGACCTCGTGACTTTGTGCGCGTGTGTCAAACTGGTACT 752
       ******** **************************************** *********

CC    CTGAGCGGATTAGTGCTGTCGGAAGCCTCGAGCGTGTTTAAACACCACTCGAGGGACAGG 892
BL    CTGAGCGGATTAGTGCTGTCGGAAGCCTCGGGCGTGTTTAAACACCACTCGAGGGACAGG 812
      ****************************** *****************************

CC    AAGTCAATCGGTGTCTCAGCCAGCCGAATCAATCAGCCCTGTAATAGCTCGCAAGACTGT 952
BL    AACTCAATCGGTGTCTCAGCCAGACGAATTAATCAGCCCTGTAATAGCTCGCGAAACTGT 872
      ** ******************** ***** ********************** * *****

CC    TTTCAGATTTGGGTCTTGTCAGGGTCACCGTGTGCTAGTCAAGGTTCAAAGGTCACTGGG 1012
BL    TTTCAGATTTGGGTCTTGTCAGGGTCACCGTGTGCTAGTCAAGGTTCAAAGGTCACTGGG 932
      ************************************************************

CC    CGGAGTTCACCTTGGACAGCTGAGAGGAAATGAGGTGTCTTACGGGTGGCCAGAAGAGGG 1072
BL    CTGAGTTCACCTTGGACAGCTGAGAGGAAATGAGGTGTCTGAAAGGTGGCCAGAAGAGGG 992
      * ************************************** *  ****************

CC    CAGCCACACTTATGAAACATTCATAATACGCCC-TGTTTGACCGTGATGA-----TAAGA 1126
BL    CAGTCACACTTATGAAACATTCATAATACGCCCCTGTTTGACCATGATGAATCCGTAAGA 1052
      *** ***************************** ********* ******     *****

CC    TGGAAACGGCTTCGACACATTAGCCAGGATACAGTGGATATAAAGAATTTTACAATGTTT 1186
BL    TGGAGACGGCTTCGACACATGAGCCAGGATACAGTGGATATAAAGAATTTTACAATGTTT 1112
      **** *************** ***************************************

CC    TTGTGATGTAAAAAAAAGAA-AAAGAAAT-AAATCGAGATAAATCAGAAGTCTGAATTTT 1244
BL    TTGTGATGTAAAAAAATAAATAAATAAATGAAATCGAGATAAATCAGAAGTCAGAATTTT 1172
      ****************  ** *** **** ********************** *******

CC    TTCCACTCTCGATGTGAAATGGC-----------------CTG----------------- 1270
BL    TTCCACTCTCGATGTGAAATGACAGCGTATAAAACGGTGTCTGTTAGTAAGTCCAATAGA 1232
      ********************* *                 ***
```

Appendix Figure B8 (Continued)

```
CC  -AATCA--------GACCAGACCTGAATCCAGACTCCAGACTGAGTGGTGTAATAAACTC 1321
BL  AGATCAACGTTTCTGACCAGACCTGAATCCAGACTCCAGACTGAGTGGTGTAATAAACTC 1292
      ****        **********************************************

CC  CAAATGGGCTTCGACTAAGCATTACTTTATTATACGTTGATGTTGAT-ATACACGAG-AT 1379
BL  CAAATGGGCTTCAACTAAGCATTAGTTTATTATACATTGATGTTGATTATACACGAGTAT 1352
    ************ *********** ********** *********** ********* **

CC  TCATTTTTCNTCACAAAAATCTGCCAGTTAAAAGTGTGTAGACTTTTTACATCCACTCTA 1439
BL  TCATTTTACATCACAAAAATCTGC-AGTTAAAAGTGGGTG---CTTTTACATCCACTCNA 1408
    ******* * ************** *********** **     ************** *

CC  AAAATATATATGTTCATTGTTAATATTTA-TCTAACTATTAACTTTTAAAACTCTTCGAA 1498
BL  AAATCACATGT---TATTTTTAATATTTAATCTAACTATTAACTTTTAAAACTGTTCGAA 1465
    ***  * ** *    *** ********** *********************** ******

CC  TAAAAAAGACGAATCGTAGCCTTCTCTGTGGTGATTATAATAATTATGATTATGGCAAAG 1558
BL  TAGCTGATACGAATCGTAGCCTTCTCTGTGGTGATTAGAATAATTACGATGATGGCAAAG 1525
    **    * ***************************** ******** *** *********

CC  AAATCCCTTTATATATATATATATACATATATACATATTGTCATAGTTTATCATTGCATT 1618
BL  AAATCCCTTTATATATATATATAT--ATATACACATATTGTCATAGTTTATCATTGCATT 1583
      ************************  ***** ****************************

CC  TAGCTATGAATGCATTATTAATTATAATTATTATTTATTATCTTCAATATAAATTATTTG 1678
BL  TAGCTATAAATGCATTATTAATTATAATTATTATTTATTATCTTCAATATAAATTATTTG 1643
    ******* ****************************************************

CC  TC-TGTTCGAGACCTTTTGTAAACCCCTCCCCCCCCATTCTTTTCTGAACTATACACTAT 1737
BL  TCCTGTTCGAGACCTTTCGTA-------CCCCCCCCATTCTTTTCTGATCTATACACTAT 1696
    ** ************** ***       ******************** ***********

CC  ATTCATGACGCAGGCTTAAAGATAATCCACATCTG-AATAGT-AGTAGGCCTATATCTGC 1795
BL  ATTCATGACGCAGGCTTAAAAATAATCCACATTTGGAATAGTTAGTAGGCCTATATCTGC 1756
    ******************** *********** ** ****** *****************

CC  AAAATGCTCATGCCT-CAAAAGTCCTCGTGTTGTCGCACGCCATTCACTTGTGTGTGTGT 1854
BL  AAATGACTCATGCCTATGGAAGTCCTCGTGTTGTTGCACGCCATTCA------------- 1803
    ***   *********    *************** ************

CC  GTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT 1914
BL  ---------------------------TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGT 1836
                               *********************************

CC  TTGAGTGCATATATGTATTCGGGCAGCATCTCTCAGGGGGTCAGGGGTGTTTGAAGATGA 1974
BL  TTGAGTGCATATATGTATTCGGGCGGCATCTCTCAGGGGGTCAGGGGTGTT-GAAGATGA 1895
    ************************ ************************** ********

CC  TAATTGG-AATCGAGCCGACGTTATGTGTCCCAGTGGAACCCCTCAGACCGTGTGTGTGT 2033
BL  TAATTGGGAATCGAGCCGACGTTATGTGTCCCAGTGGAACCCCTCAGACCGTGTGTGTGT 1955
    ******* ****************************************************
```

Appendix Figure B8 (Continued)

```
CC  GTGTGTGTGTGTGTGTGTGTGTGTGTGTGCTGGGGTATAAAAGAGAGAAGTATGAAGAT 2093
BL  GTGTGTGTGTGTGTGTGT--------GTGCTGGGGTATAAAAGAGAGAAGTATGAAGAT 2007
    ******************        **********************************

CC  GGTCTCAGGCATTGATACTTCCAAGACAAAGAGCTCTTCAGAGTTCCACATCTCAGAAGA 2153
BL  GGTCTCAAGCATTGATACT-CCAAGACAA-GAGCTTCTCAGAGTTCCACATCTCAGAAGA 2065
    ******* *********** ********* *****  ***********************

CC  ATTCAAATCTGAATAAAGCACATCAAATAAGAGTGAAAGGGGTCATATATTTCATCATTT 2213
BL  -TTCAAATCTGAACAAAACACATCAAATAAGAGTGAAAAGGGTCATATATTTCATCATTT 2124
     ************ *** ******************** *********************

CC  TCCTGATTTTTGATGTCATTTTTAGAACATTATAGTGGCATTTAT-ATCTCTGTGTTAAT 2272
BL  TCCTGATTTTTGATGTCATTTT-AGAACATTATAGTGGCATATATTATCTCTGTGTTAAT 2183
    ********************** ****************** *** **************

CC  CCTCTCCACCACATATTTTTCTCCTTTTCCGGTTTATATTGTAATCGGTTAATTATTATT 2332
BL  CCTCTCCACCACATTATTTTCTCCTTTTCTGGTTTATATTGTAATCGGTTAATTATTATT 2243
    **************  ************* ******************************

CC  ATTATTTTT---TTTTAAAGCATTGTCTTAAAATACTGTTATTGTTGTTATATGTGTGTG 2389
BL  ATTATTATTATTTTTTAAAGCATTTTCTTAAAATACTGTTATTGTTAT------------ 2291
    ****** **   ************ ********************* *

CC  TGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTGTTGAGGGGCACTCTGT 2449
BL  -----------------------------GTGTGTGTGTGTGTGTTGAGGGGCACTCTGT 2322
                                 *******************************

CC  TCTCCCTGCACTTTGGGGGTTTCCTCCAGTTTCCTCTCCCGGTCCTAAGACAGGCGTTCT 2509
BL  TCTCCCTGTGCTTTGGGGGTTTCCTCCAGTTTCCTCCCCCGGTCCTAAGACAGGCGTTCT 2382
    ********  ************************** ***********************

CC  AGGCTGATTGGCATTTCCAAACTGTCCACAGTGTGTGAATGGGTGTGTGTGCGATTGTAC 2569
BL  AGGCTGATTGGCATTTCCAAATGGTCCACAGTGTGTGAATGGGTGTGTGTGCGATTGTGC 2442
    *********************  *********************************** *

CC  CCTGC-ATGAGTTGGCACCCCGTCCAGGGTGTCCTCCATCTCGTGCCCTGAGTTCCCAGG 2628
BL  CCTGCGATGAGTTGGCACCCCGTCCAGGGTGTCCTAGATCTCGTACCCTGAGTTCCCAGG 2502
    ***** *****************************  ******* ***************

CC  GATAGGCTCCAGGCTCCCTCAGACCCTGTGTAGAATACACGGTACATAAAATGGATCAAT 2688
BL  GATAGGCTCCAGGCTCCCTCAGACCCTGTGTAGAATACACGGTACATAAAATGGATCAAT 2562
    ************************************************************

CC  GGATATTGTTGCTCTTGTTGTTGTTAAATCAGGACAGTGTCCAGGTCTGAGGCGCAACTA 2748
BL  GGATATTGTTGCTCTTGTTGTTGTTAAATCAGGACAGTATCCAGGTCTGAGGCGCAACTA 2622
    ************************************** *********************

CC  AACAATAACTGTAGCTCTTCTTTTGATGT---------ATATATATATATTCCACAAGAG 2799
BL  AACAATAACTGTAACTGTTCTTTTGATGTTATATATATAAATATATATATTCCACAAGAG 2682
    ************* ** ************         * ********************
```

Appendix Figure B8 (Continued)

```
CC    GAGATTACTCACTTACTGTCTGACTTACTTTATTATTAAGATTATTTTATTAATTACCTT 2859
BL    GAGATTAC----TTACTATCTGACTTACTTTATTATTAAGATTATTTTATTAATTACCTT 2738
      ********    ***** ******************************************

CC    TAATATTTCATTGACTGACTGTAAGATCTGTGTGTGTGTGTGTGTG----------AGAG 2909
BL    TAATATTTCATCGACTGACTGTAAGATCTGTGTGTGTGTGTGTGTGTGTGTGTGTGAGAG 2798
      *********** **********************************          ****

CC    T-ATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTGCCTTGCTGTTGTGTTTGCAAGCGC 2968
BL    TGATGGTCAGTGTGTGCAGGCTGTTGCTGGTTGCTGCCTTGCTGTTGTGTTTGCAAGCGC 2858
      * **********************************************************

CC    AGCTATCTGTTTCTCAACACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAAATCG 3028
BL    AGCTATCTGTTTCTCAACACTGGTCTCATGGCTGGTATCCTGGAGGAAAGAGAGAAATCG 2918
      ************************************************************

CC    ACTCTTACAGCTCACCAGAGGTCTGTCTCTTTCTCTCTGTCTCTCTGTCTCTCTCTCTCT 3088
BL    ACTCTTACAGCTCACCAGAGGTCT------------------CTCTCTCTCTCTCTCTCT 2960
      ************************                  **** *************

CC    CTCTCTCTCACACACACACACACACACACACACTCACGCATACATGGGCACACATGCACC 3148
BL    TTCACACACACACACACACACACACTCACGCATACATGGGCACACACACACACTTGCACC 3020
       ** * * ***************** *** **  ** *   ***    ***** ******

CC    CATGCACACAGGCATTTCAGAACAAAGGTTTCTTTGTTATAAAATCTGGCACTTTTTCAT 3208
BL    CATGCACACACGCATTTCAGA-CAAAGGTTTCTTTGTTATAAAATCTGGCACTTTTTCA- 3078
      ********** ********** *************************************

CC    AAAACAGCTGTTAACAAAAACATCTGGATTGTGTATTTGCGACACAAGCATTAAAAACAT 3268
BL    AAAACAGCTGT-AACAAAAACATCTGGATTGTGTATTTGC-ACACAAG--TTAAAAACAC 3134
      *********** **************************** *******  *********

CC    GCTTAAAAACGATCAGTCAAACA---------TGAGCATAAGCTCATGTGAACGAGATAA 3319
BL    GCT--AAAATCATCAGTCAAACACACTATTTCTGAACATAAGCTCACG-AAACGAGATGA 3191
      ***  ****  ************         *** ********** *  ******** *

CC    ATGTAAAGCTCCCTGTAAACGCTCTTATTACTCCCAAAAAATTTTTT-AATTCCGTGCGA 3378
BL    ATG-AAAGCTCC-TGTAAACGCTCGTATTACTCCCAATTTTTTTTTTTAATTCCATGCAG 3249
      *** ******** *********** ************    ****** ****** ***

CC    TTAATTGATTGATTCAGTTACGTGTTTGAAATCTTAACACGGCTGATCCAGCTCATAAGG 3438
BL    TTAATTGATTGATTCAGT-ACATGTTTGAAATCT-AATACGGCTGATCCAGCTCATAAGG 3307
      ****************** ** ************ ** **********************

CC    ACGCACGTGAAATATTCTAATCGTTTTCAGAGACCAGTTAGTGGAAAATGGTTCATTTAT 3498
BL    ATGCACGTGAA-TATTA-AATCGTTT-CAGAAACGAGTCAGTGGAAAACGGTTCATTTAT 3364
      * ********* ****  ******** **** ** *** ********* ***********

CC    CGAGGCTATTCCAGGACCGCAAGGAACTCAAGCTATGCCACTACAGTATGTTGACTATGC 3558
BL    CGAGGCTATTCCAGGACAGCAAGGAACTCAAACTATGCTACTACAGTATGTTGACTATGT 3424
      ***************** ************* ****** ********************
```

Appendix Figure B8 (Continued)

```
CC  TGAACCCGGTGTGGATTCGAGTGGCTGTCATGTGCGCCGGTCTTTAAACATCACTGAAAC 3618
BL  TGAACCCGGTGTGGATTCGAGTGGCTGTTATGTGCGCCGGTCTTTAAACATTACTGAAAT 3484
    **************************** ********************** *******

CC  GAGACCGTCCAAGCTGAACATATCACACGGATCTCAATTTAAGGTTCAAACTGTATCGTA 3678
BL  GAGACCGTCCAAGCTGAACATATCACACGGATCTCAGTTTAAGGTTCAAACTGTATCGTA 3544
    ************************************ ***********************

CC  CTAGATACAAGTGTGTAACAAACAAATATAATAGAGCAACATGAAATAGTAGGTCAGAGC 3738
BL  CTAGATACAAGTGTATAACAAACAAATATAATACAGCAACAGGAAATAGTAGGTCAGAGC 3604
    ************** ****************** ******* ******************

CC  AGAAATATTCACATTCCCTGCCAGTGGTTCTGAAATCTCGCCCTCCTGCTGTGCCTCACC 3798
BL  AGAAATATTCACATTCCCTGCTAGTGGTTCTGAAATCTCGCCCTCCTGCTGTGCCTCACC 3664
    ********************* **************************************

CC  GCTCGGACAGATATCTGGGGAGATCAAACTGTGTGAAGCGGGAGAATGCAGCTATCTGAG 3858
BL  GCTCGGACAGATATCTGGGGAGATTAAACTGTGTGAAGCGGGAGAATGCAGCTATCTGAG 3724
    ************************ ***********************************

CC  GCCACTGAGAACCAACGTCCTGAAGAGCATCCTGGGGAGAACACGCTCGGCAC------A 3912
BL  GCCACTGAGAACCAACGTCCTGAAGAGCATCCTGGTGAGAACTCTATCTGTATCGTTTTA 3784
    *********************************** ****** *  ** * *       *

CC  TTTAATCGTTTT----ATTTTATTA-GAAGCGTCTC---TCTGAACTTAAA-CATACGT- 3962
BL  TTTATCCATTTTTATGAATGTATTAAGAAGCGTTCTACTTCTGAACTTAAAATATAACTA 3844
    ****  * ****    * * ***** *******      ************  ***  *

CC  TTCCTGTT-CCAAACTGTAT--AAGGCACATGTTTA--TTCACAGTGACAGTGCTGTGGG 4017
BL  TTCATGTTTCCAAACTGTTACAAAGGCACATATTTAATTTCACATGTACAGTGCTGTGAA 3904
    *** **** *********    ********* ****  ******   ***********

CC  AAAAGTATTTGCTTTGATTTGTGTGTGTGTGTGTGTGTGTGTGGGTGGGTGGGTGGGTGT 4077
BL  AAATGTATTTGCTTTGATTTGTGTGCGTGTGTGTGTGTGTGTGTGTGTGTG--TGTGTGT 3962
    *** ********************* ***************** *** ***  ** ****

CC  GCGTCATACTAAATAGTTTTAGATCTTCAAACGAAATACAACAGAAAACAAAAGCAACCT 4137
BL  ATCTCATACGAAATAGTTTTAGATCTTCAAACGAAATACAACAGAAAACAAAAGCAACCT 4022
       ****** **************************************************

CC  GAGTAAACACACAATACAGTTATTTATTTATTTATTTATTTATTGAAGGAAAAAAAAAAA 4197
BL  GAGTAAAAAAAA------------------------------------------------ 4034
    ******* * *

CC  AAAGGTTATCCAACACCTATCACCCATGTGAAAAACTAATTGCCCCC-TTAAACTCGAAA 4256
BL  ---GGTTATCCAACACCTATCACCCATGTGAAAAACTAATTGCCCCCCTTAAACTTGAAA 4091
       ******************************************** ******* ****

CC  CCTTGTTGTGACATTTTATTTATGAATGTATTACTAA-GAATAAAGAATTGAATCTCATT 4315
BL  CCTTGTTGTGACATTTTATTTATGAATGTATTAGTAAAGAATAAAGAATTGAATCTCATT 4151
    ********************************* *** **********************
```

Appendix Figure B8 (Continued)

```
CC  TTTTATATATATATATATATA--------------------------------------- 4336
BL  TTTTATATATATATATATATCATAAAAAATGTGTATATATATATATATATATATATATAT 4211
    ********************

CC  ----------------------------------TCATGCTGTACTCATGCACCTCCAAA 4362
BL  ATATATATATATATATATATATATATATATATAATCATGCTGTACTCATGCACCTCCAAG 4271
                                      *************************

CC  TGCATTTGAACTCAAGCCTGTTTGTGAATTGTCTTTATATGACCTTCTTTAACATCTGCG 4422
BL  TGCATTTTAACTCAAGCCTCTTTGTAAATTGTCTTTATATGACCTTCTTTA-CATCTGCA 4330
    ******* ***************** ************************* *******

CC  AGTACTTTTAATTCCTTATTCTTTT-GATTGTTCTTTCAG---TTTTTTGGTGCCACTTC 4478
BL  AGTACTTTTACTTCCTTCTTTTTTTTGATTGTTCTTTCATATTTTTTTTTGTGTCATTTC 4390
    ********** ****** ** **** *************    ****** *** ** ***

CC  CTGACAATGTTGTTCCCCTTATA---GCTCGATGCCCTTGTGAAAGAATTCCAAAAGAAG 4535
BL  CTGACCATGTTGTTCCCCTTATACCTGCTCGATGCCCTTGCGAGAGAATTCCAAAAGAGG 4450
    ***** *****************   ************** ** ************** *

CC  AAGTGAAAACAATTCAATCATGCCCCAGCTTCCTCCAGCCTCTTTCTTCGCTGATAACAC 4595
BL  AAGTGAAAACAATCCAATCCTGCCCCAGCTTCCTACAGCCTCTTTCTTCGCTGATAACAC 4510
    ************* ***** ************** *************************

CC  TTATCTGAACACACACACACACATCTGCTCATCCGTGGAGTGTGTCTTTTTCTGTTTATG 4655
BL  TGATCTGAACACACACACAC--ATCTGCTCATCCGTGCAGTGTGTCTATTTCTGTTTATG 4568
    * ******************  *************** ********* ************

CC  TGGTTTTAGCGTCAAGCTTTCTGTCATCTGTGTATAAGCAGCGAAGTGAAATGCATCATG 4715
BL  TGGTTTTAGCATCAAGCTTTCTGTCATCTGTG-ATAAGCAGCGAAGTGAAATGCATCATG 4627
    ********** ********************* ***************************

CC  TGATCATTCAATAAAACTTTTATTTTGGTATTCGGTCTCTTTCTGAAAGAATACATGGGA 4775
BL  TGATCATACAATAAAACTTTTATTTTGGTATTCGGTCTCTTTCTGAAGGAATACATGGAA 4687
    ******* *************************************** ********** *

CC  CATTCACTGCTTTGCCAAATCCGTTATTAAAATCGGAGTTAACGGCTTACGTTAGCGTGT 4835
BL  CATTCACTACTTTGCCAAATACGTTATTAAAATCGGAGTTAACGGGTCACATCAGCTTGT 4747
    ******** *********** ************************ * ** * *** ***

CC  GTGATTTATCTGATTTCCTTAACCTGTGAAGCGCATTCTGAGCAC-------AGCTGATG 4888
BL  GTGATTTAGCTGATTTCCTTAACCTGTAAAGCACATTCTGAGCACTTCTTTCAGCTGATG 4807
    ******** ****************** **** ************       ********

CC  CCCACCATATTAAAAACATACATT-----GCCTAAAGTATGTGGACACCTGACCATCACC 4943
BL  CCCACCATATTAAAAACATACATTACATTGCCTAAAGTATGTGGACACCTGACCATCACC 4867
    ************************     *******************************

CC  CATTTGTGGTTCTTCTCCAGACTGTTGCCACGTAGTTGGAAGCACACAGTTCAGAATGCC 5003
BL  CATTTGTGCTTCTTCTCCAAACTGTTGCCACATAGTTGGAAGCACACAGTTTAGAATGCC 4927
    ******** ********** *********** ******************* ********
```

Appendix Figure B8 (Continued)

```
CC   TTTGTACTGCGTTACAATTACACTTCACTCGAAGTAAGGGACCAGATATGTTCCAGTATG 5063
BL   TTTGTACTGCGTTACAATTACACTTCACTCGAAGTAAGGGCCCAAATATGTTCCAGTATG 4987
     **************************************** *** ***************

CC   GCAATGCCTCTGTACACAAAGTGAGGTCCATGAAGACGTGGTTTGCTGAGTCTGGATCAG 5123
BL   GCAATGCCCCTGTGCACAAAGTGAGGTCCATGAAGACATGGTTTGCTGAGTCTGGATCAG 5047
     ******** **** *********************** **********************

CC   AAGGATTCAAGTGGTCTGCACAGAGCCCTGACTTCAACCCCTTTGAACACCACTGGGATG 5183
BL   AAGGATTCAAGTGGTCTGCACAGAGCCCTGACCTCAACCCCTTTGAACACCACTGGGATG 5107
     ******************************** ***************************

CC   AAATTAGAACACCGGGGCATCTGTCCGTTGGGGAGTAGATGCCCTCTCTCACAGAGTCGT 5243
BL   AA-TTAGAACACCGGG-CATCTGTCCGTTTGGGAGTAGATGCC-TCTCTCACAGAGTCGT 5164
     ** ************* ************ ************* ****************

CC   GTGGT-CCACAGAGGTACTGGGGCGTGTTGG-CCCGGTGTAAGGT-ACGACACATTCAAC 5300
BL   GTGGTTCCACAGAGGTACTGGGGCGTGTTGGTCCCGGTGTAAGGTTACGACACAT-CAAC 5223
     ***** ************************* ************* ********* ****

CC   CAGCTGAAGAT-AGGGCGGTCCATCTTGGCCTGAAGTGTCAGCTGACACCAAATGCCGGT 5359
BL   CAGCTGAAGATTAGGGCGGTCCATCTTGGCCTGAAGTGTCAGCTGACACCAATTGCCGGT 5283
     *********** **************************************** *******

CC   CCACGCATAATGCTCTGACAGTCAGCTACAGTCACTTCCAAAACTATTGGAATGACAAGG 5419
BL   CCACGCATAATGCTCTG------------------------------------------- 5300
     *****************

CC   CCATTAATTTCTTTTTGCTGTAGACTGAAAACATTCAGACATATTGAGATCACGAGATCA 5479
BL   ------------------------------------------------------------

CC   AAAGAAGCAGTGGCGGTTAAGGCTTGGGTGACTGATAGAATGGGCGGGGGTCAAGCCCCA 5539
BL   ------------------------------------------------------------

CC   GCACTTGCTAAGCTGCCACTGTTGGGCCTTTGAGCAAGGCCCTTAACCCTCTCACTCCAG 5599
BL   ------------------------------------------------------------

CC   GGGGCGCTGTATCATGGTCGACCCTGTGAGAGTTCAGAATTTCAGCTTTTATTTCCTGGC 5659
BL   ------------------------------------------------------------

CC   ATTATATGGATAAATAGGTGTAAACAACATACCACATTTTGTACAGAAATCCAATTGTAG 5719
BL   ------------------------------------------------------------

CC   GTGAGCGAGCAAAAAACTCTAACATGTACCGACATCTGTTCTTTTTTACTCAGTGGCCCT 5779
BL   ------------------------------------------------------------

CC   GTAACAGATTTTTTTAAACAATAATAACTCTGAATTATCCCCTGGATTCTGT 5831
BL   ----------------------------------------------------
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Appendix Figure B8 (Continued)