

## NOVEL CRUSTACEAN ANDROGENIC GLAND HORMONE

The present invention relates to a novel crustacean androgenic gland hormone. More specifically, the invention relates to an androgenic gland hormone derived from *Penaeus monodon*. The invention relates further to the use of said hormone to influence the sex ration in prawn and shrimp cultures, and to set up monosex cultures. It also relates to the use of the hormone and its gene or fragments thereof in sex determination.

Shrimp and prawn cultivation and trade is a very important activity all over the world. The main species under cultivation are *Penaeus monodon* (Giant tiger prawn, Jumbo tiger prawn, Jumbo tiger shrimp, Black tiger prawn, Blue tiger prawn, Grass shrimp...), mainly cultivated in Asia, with an aquaculture production of about 600.000 tons in 2003; and *Penaeus vannamei* (Whiteleg shrimp, white shrimp), mainly cultivated in the Americas and in China and Thailand, with an aquaculture production that is comparable to *P. monodon*. For those species, aquaculture is far more important than capture.

Increasing demands for aquaculture production mean increasing pressure for the development of more efficient production systems.

As most *Penaeus* sp. are sexually dimorphic (Hansford and Hewitt, 1994), a lot of effort has been paid in setting up monosex cultures. In that respect, several groups have tried to develop reliable sex markers (Khamnamtong *et al.*, 2006). Alternatively, monosex cultures could be obtained by in vitro secretion of androgenic sex hormone from the androgenic gland, as disclosed in US6740794. Although this may be an interesting approach, it is hampered by the fact that the structure of the androgenic gland hormone, or of the gene encoding is not known. Recently Manor *et al.* (2007) described an insulin-like gene that is exclusively expressed in the androgenic gland of the male Australian Crayfish *Cherax quadricarinatus*. This may be an interesting candidate gene, possibly coding for the androgenic gland hormone, but its effect is not yet demonstrated. Moreover, no homologues in prawns or shrimps are known today. Searching for homologues sequences is hampered by the fact that only a limited number of prawn and shrimp sequences are available in the databases, and by the fact that the interspecies homology is probably low – if any.

Surprisingly, in an AFLP-based transcriptomic profiling experiment in the black tiger shrimp (*Penaeus monodon*), we identified 2 androgenic gland-specific DNA fragments homologous to isopod 'androgenic gland hormone' and crayfish 'insulin-like androgenic gland' factor. By further cDNA sequencing and RACE experiments, we identified four mRNAs, termed PmAGH1, PmAGH2, PmAGH3 and PmAGH4, most probably originating from a single gene by alternative splicing. Even more surprisingly, treatment of genetically female juvenile shrimp using recombinant Pm\_AGH1, Pm\_AGH2, PmAGH3 and/or PmAGH4, had masculinising

effects. Genetically male juvenile shrimp were feminized by dsRNA-mediated knock-down of Pm\_AGH1, Pm\_AGH2, PmAGH3 and/or PmAGH4.

## SUMMARY

In a first aspect, there is provided an isolated protein, selected from the group consisting of SEQ ID N° 3, SEQ ID N° 6, SEQ ID N° 9 and SEQ ID N° 12, or a mutant or variant thereof with at least 50% identities and retaining the biological activity of said isolated protein.

In a second aspect, there is provided a nucleic acid fragment, encoding a protein as defined above.

In a third aspect, there is provided a nucleic acid fragment, comprising SEQ ID N° 1, SEQ ID N° 4, SEQ ID N° 7 or SEQ ID N° 10, or its complement, or a functional fragment thereof specifically hybridizing with said nucleic acid.

In a fourth aspect, there is provided the use of a protein as defined above to modify the sex ratio in prawns or shrimps.

In a fifth aspect, there is provided the use of a nucleic acid fragment as defined above, or a functional fragment thereof to modify the sex ratio in prawns and shrimps.

In a sixth aspect, there is provided the use of a nucleic acid fragment as defined above, or a functional fragment thereof, to determine sex in prawns or shrimps.

In a seventh aspect, there is provided a method of setting up a monosex culture in prawns or shrimps, comprising the use of a protein as defined above.

In an eighth aspect, there is provided a method of setting up a monosex culture in prawns or shrimps, comprising the use of a nucleic acid fragment as defined above.

In a ninth aspect, there is provided a method of setting up a monosex culture in prawns or shrimps, comprising sex determination as defined above.

An embodiment of the disclosure is an isolated protein selected from the group consisting of SEQ ID N° 3, SEQ ID N° 6, SEQ ID N° 9 and SEQ ID N° 12, or a mutant or variant thereof. Preferably, said protein is isolated from a *Penaeus* species, more preferably it is isolated from *Penaeus monodon*. Mutants and variants as described here are mutants and variants with at least 50% identity, as measured in a BLASTp (Altschul *et al.*, 1997). Preferably, said mutants and variants are biologically active, as measured by their hormone

effect (masculinization; or feminization when acting as a dominant negative compound). Even more preferably, said mutants and variants are retaining the masculinization capacity of the isolated proteins with SEQ ID N° 3, SEQ ID N° 6, SEQ ID N° 9 or SEQ ID N° 12.

Another embodiment of the disclosure is a nucleic acid fragment, encoding a protein according to the disclosure. Nucleic acid fragment, as used here, can be any nucleic acid, including but not limited to messenger RNA, single stranded DNA, double stranded DNA, including genomic DNA, possible with its intron-exon organisation. In its simplest form, the nucleic acid fragment is limited to the coding sequence, but it may comprise other sequences such as, but not limited to, 5' en 3' untranslated sequences, regulatory sequences such as promoter and terminator sequences, and introns. Preferably, said nucleic acid fragment comprises SEQ ID N° 2, SEQ ID N° 5, SEQ ID N° 8 or SEQ ID N° 11(coding sequences).

Still another embodiment of the disclosure is a nucleic acid fragment, comprising SEQ ID N° 1, SEQ ID N° 4, SEQ ID N° 7 or SEQ ID N° 10 (mRNA) or SEQ ID N° 13 (gDNA), or its complement, or a functional fragment thereof. A functional fragment as used here is, as a non limiting example, a fragment that can be used as primer for detection, or a fragment that can be used as antisense RNA or as RNAi for downregulating the expression.

Another embodiment of the disclosure is the use of a protein according to the disclosure to modify the sex ration in prawns or shrimps. Preferably, said prawns or shrimps belong to the family of *Penaeidae*, even more preferably said prawn or shrimp is a *Penaeus* species, most preferably said prawn or shrimp is *Penaeus monodon*.

Still another embodiment of the disclosure is the use of a nucleic acid fragment according to the disclosure to modify the sex ration in prawns or shrimps. Preferably, said prawns or shrimps belong to the family of *Penaeidae*, even more preferably said prawn or shrimp is a *Penaeus* species, most preferably said prawn or shrimp is *Penaeus monodon*.

Yet another embodiment of the disclosure is the use of a nucleic acid sequence according to the disclosure to determine sex in prawns or shrimps. Preferably, said prawns or shrimps belong to the family of *Penaeidae*, even more preferably said prawn or shrimp is a *Penaeus* species, most preferably said prawn or shrimp is *Penaeus monodon*. Indeed, on the base of the

sequence presented, it is obvious for the person skilled in the art to develop primers that can be used to measure gene expression of the androgenic gland specific gene.

Yet another embodiment of the disclosure is a method of setting up a monosex culture in prawns or shrimps, comprising the use of a protein and/or a nucleic acid according to the disclosure. Preferably, said prawns or shrimps belong to the family of *Penaeidae*, even more preferably said prawn or shrimp is a *Penaeus* species, most preferably said prawn or shrimp is *Penaeus monodon*. Indeed, by injecting the protein into young post-larval animals, females can be converted into males. Alternatively, expression and/or injection of anti-sense RNA, dsRNA and/or RNAi will repress the expression of the androgenic gland specific protein, and feminizes the animals. As an alternative, antibodies, preferably single chain antibodies or camelid antibodies or antibody fragments such as nanobodies against the proteins of the disclosure can be used to feminize the prawns or shrimps. Using the purified protein, or fragments thereof, the generation of antibodies is a process known to the person skilled in the art.

Another embodiment of the disclosure is a method of setting up a monosex culture in prawns or shrimps, comprising sex determination using a nucleic acid according to the disclosure. Preferably, said prawns or shrimps belong to the family of *Penaeidae*, even more preferably said prawn or shrimp is a *Penaeus* species, most preferably said prawn or shrimp is *Penaeus monodon*. Alternatively, a protein according to the disclosure could be used in sex determination too, especially in combination with an antibody against the protein according to the disclosure, as described above. Such antibody can be used, as a non limiting example, in an ELISA assay to test the concentration of androgenic gland specific protein present in the animal.

## **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1: Schematic overview of the genomic DNA encoding the androgenic gland hormone, and the messenger RNAs derived from it by alternative splicing.

## **EXAMPLES**

### ***Materials and methods to the examples:***

#### **Sampling**

Tissue was dissected at the base of the fifth walking leg, where the androgenic gland is supposed to be situated, from mature males. Control tissue was dissected from the identical location in females. Muscle samples were taken from the tail of adult males and females. All samples were immediately snap frozen in liquid nitrogen and stored at -70 C until RNA preparation.

**cDNA-AFLP**

Snap frozen androgenic gland samples (n=4), control surrounding tissue samples (n=2) and muscle samples (n=6) were pulverized using mortar and pestle under liquid nitrogen. Total RNA was isolated using the TRIzol reagent (Invitrogen). RNA quality was assessed spectrophotometrically (Nanodrop) and by gel electrophoresis. High quality RNA (2.5 µg) was converted to double stranded cDNA using a biotinylated oligo-dT primer. After purification of the ds cDNA (Qiaquick PCR purification kit, Qiagen) the quality and yield were analysed by gel electrophoresis.

AFLP templates were prepared by restriction digest of the ds cDNA using *Bst*YI. The 3' end fragments were isolated using streptavidin-coated magnetic Dynabeads (Dyna) and further digested using *Mse*I. Restriction site specific adapters were ligated to the ends of the fragments.

The adapter-ligation mixture was diluted two-fold to a total volume of 100 µl. Amplification using a *Bst*YI-specific primer with either a T (*Bst*YI-T+0) or C (*Bst*YI-C+0) at its 3' end in combination with an *Mse*I-specific primer divided the set of fragments in two. Specific amplification of 5 µl of the 600-fold diluted pre-amplification mixtures was performed using the <sup>33</sup>P labelled *Bst*YI-C/T primer with one additional selective nucleotide at its 3' end in combination with the *Mse*I primer with two selective nucleotides. In this setting, 128 primer combinations were needed to screen the full set of transcript fragments.

After amplification, the samples were separated on a 5% polyacrylamide sequencing gel. A <sup>33</sup>P labelled 10 bp sizing standard was included. After running and vacuum-drying on Whatmann paper, the gel was scanned in a phosphor-imager.

**Sequence analysis of cDNA-AFLP fragments.**

When cDNA-AFLP fragments were to be cut from the gel for sequence analysis, the gel was exposed on a Biomax MR autoradiogram (Kodak). Gels were overlaid with the developed film and the relevant fragments were cut from the gel. DNA was dissolved in TE buffer (10 mM Tris, 0.1 mM EDTA) and reamplified using the selective *Bst*YI and *Mse*I primers. Amplification was checked by gel electrophoresis and fragments were directly sequenced using either the selective *Bst*YI or *Mse*I primer.

**Homology search using obtained sequences.**

The non-redundant protein sequence database and an in-house compiled DNA sequence database containing all available DNA sequences from invertebrates were searched for sequences homologous to the sequence of the cDNA-AFLP fragment. An in-house developed algorithm first searched the protein database for sequences homologous to the translated (in

all 6 possible reading frames) query sequence (blastx search). When no homology was found, the invertebrate DNA database was searched for homologous DNA sequences (blastn search). When homology was identified to a DNA sequence, a blastx search was performed with this hit. When no homology was found in the invertebrate DNA database, a tblastx search was performed, in which homology was searched in the translated DNA database using the translated query sequence.

#### **RACE analysis of putative androgenic gland hormone genes.**

Rapid Amplification of cDNA Ends (RACE) analysis was performed using the BD SMART RACE cDNA amplification kit (BD Biosciences). High-quality total RNA (1 µg) from androgenic gland tissue was converted to 5' RACE-ready cDNA and 3' RACE-ready RACE cDNA according to the manufacturer's instructions. 5' and nested 5' RACE were performed using AGHRACE\_Rev1 (5'-TATGACAGAAACCGCCAGGGGAGAC-3')

and AGHRACE\_Rev2 (5'-AGGTACCGGGTCCTCGCAATACTCC-3') primers, respectively. 3' and nested 3' RACE were performed using AGHRACE\_Fw1 (5'-CTGGCGGTTTCTGTCATATGCGATG-3') and AGHRACE\_Fw2 (5'-GTCTCCCCTGGCGGTTTCTGTCATA-3') primers, respectively. Only one distinct fragment was amplified in both the 5' and nested 5' RACE while two distinct fragments were amplified in both the 3' and 3' nested RACE. Fragments were isolated from the gel and cloned using the TOPO

TA cloning kit for sequencing (Invitrogen). Cloned fragments were sequenced using standard T7 and SP6 primers. Sequences were manually curated and assembled using the software packages phredPhrap and consed (Ewing et al, 1998; Ewing and Green, 1998; Gordon et al, 1998).

Based on the consensus cDNA sequence obtained in the RACE experiments, primers were designed for amplification of the full-length cDNA. Forward primers AGHRT\_3 (5'-ACACAGGACAGGGCAAGTTC-3') and AGHRT\_1 (5'-TCCCTCCACAAAAACCACTC-3') were used in combination with reverse primers AGHRT\_12 (5'-TCTGGGGCCTATTAATCGAA-3') and AGHRT\_16 (5'-ACGTTCGGAAAATCGAAAGAT-3'). The sizes of the PCR fragments were according to what was expected from the RACE experiments and sequencing of the fragments confirmed the sequences obtained previously by RACE.

#### **Genomic sequencing of putative androgenic gland hormone genes.**

Using primers AGHFULL2\_FW (5'-GTGCTGCCACACACAGGAC\_-3') and AGHPC3\_FW (5'-GCACGCCCTCGTCAAG-3') in combination with primer AGHFULL2\_REV (5'-TCAAGAATTACTCTGGGGCCTA-3') and primer AGH\_indel1 (5'-CCAGTACCGCCTGCATCC-3') in combination with primer AGH\_indel2 (5'-

GGCTCACCAGGTGGTCTCT-3'), we PCR amplified overlapping fragments of the AGH gene on genomic DNA. Additional sequence information was obtained using the Genome Walker Universal Kit (Clontech). Genomic DNA was isolated from several female shrimp and pooled. DNA libraries were prepared according to the manufacturer's instructions and primary 5' and 3' amplification reactions were performed using primers AGHRACE\_REV1 and AGHRACE\_FW1 (see above), respectively. Nested amplification reactions were performed using primers AGHRACE\_REV2 and AGHRACE\_FW2 (see above), respectively. A partial genomic sequence, including the complete 3' UTR region of the mRNA sequences up until the polyA-tail, was assembled using the software packages phredPhrap and consed.

#### **Cloning and recombinant protein expression putative androgenic gland hormone genes.**

The full-length coding sequence was expressed in a yeast host (*Pichia pastoris*), to ensure correct folding and glycosylation of the recombinant protein.

#### **dsRNA production.**

Using PCR primers AGH\_CDS\_T7\_Fw

(5'-TAATACGACTCACTATAGGGATGCCCCACTCAGCTGCTT3') and AGH\_CDS\_T7\_Rev (5'-TAATACGACTCACTATAGGGCTAAGGTACCGGGTCCTCG-3'), we generated a PCR fragment incorporating a T7 promoter sequence at both. This fragment was used as a for T7 RNA polymerase template for dsRNA production using the Megascript RNAi kit (Ambion).

#### **Sex reversal experiments in juvenile *P.monodon* shrimp by injection of recombinant AGH.**

Groups of young post-larval animals (PL30-PL60) are injected with 120 ng of the recombinant AGH every third day. Group 1 receives injections covering the period of PL30 to PL60. Group 2 receives injections covering the period of PL30 to PL45. Finally group 3 receives injections covering the period of PL45 to PL60. At PL150, phenotypic sex is assessed by visual inspection and histology while genotypic sex is determined at the DNA level using a PCR-based sex marker as disclosed in PCT/EP2007/054041.

#### **Sex reversal experiments in juvenile *P.monodon* shrimp by injection of AGH-specific dsRNA.**

Groups of young post-larval animals (PL30-PL60) are injected with 0.5 µg of AGH dsRNA every third day. Group 1 receives injections covering the period of PL30 to PL60. Group 2 receives injections covering the period of PL30 to PL45. Finally group 3 receives injections covering the period of PL45 to PL60. At PL150, phenotypic sex is assessed by visual

inspection and histology while genotypic sex is determined at the DNA level using a PCR-based sex marker as disclosed in PCT/EP2007/054041.

5 **Example 1: Identification of *Penaeus monodon* androgenic gland hormones.**

Transcript profiling of all possible cDNA-AFLP primer combinations (128 PC) was performed on androgenic gland tissue samples (male; n=4), surrounding tissue control samples (female; n=2) and female and male muscle samples (n=3 each). By visual inspection of the cDNA-AFLP gels, we selected 355 androgenic gland specific fragments. We obtained sequence information for 254 (71.6 %) of these fragments. Homology searches indicated that at least 47 (18.5 %) of these were identical (blastx alignment showing at least 75% identities) to bacterial sequences (*Propionibacterium acnes*). This is most likely due to a bacterial infection of the androgenic glands in the sampled animals.

Two cDNA-AFLP fragments were found to be homologous to "insulin-like androgenic gland factor" from the red-claw crayfish (*Cherax quadricarinatus*): BC4M21M263.5 and BC4M34M404.4.

RACE analysis, followed by full-length cDNA amplification and sequencing resulted in the identification of four cDNA sequences PmAGH1 (Seq ID N° 1), PmAGH2 (Seq ID N° 4), PmAGH3 (Seq ID N° 7) and PmAGH4 (Seq ID N° 10). Genomic sequencing of a part of the gene (Seq ID N° 13) indicated that all four of these cDNAs originate by alternative splicing from a single gene.

Fragment BC4M21M263.5 corresponds to a 234 bp BstYI-MseI restriction fragment originating from PmAGH1 and PmAGH3, while fragment BC4M34M404.4 corresponds to a 372 bp BstYI-MseI restriction fragment originating from PmAGH2 and PmAGH4.

Homology search with the full cDNA sequences revealed no significant homology on the nucleotide level. On the protein level, homology was again found with the crayfish "insulin-like androgenic gland factor" (32 % identity) and to a lesser extent with the androgenic gland hormones from the isopods *Armadillidium vulgare*, *Porcelio scaber* and *Porcelio dilatatus* (between 29 and 26 % identity).

**Example 2: Sex reversal in juvenile *P.monodon* using recombinant *Pm\_AGH1*, *Pm\_AGH2*, *Pm\_AGH3* and/or *Pm\_AGH4*.**

- 5 Administration of recombinant PenMon\_AGH1 and PenMon\_AGH2 to juvenile *P.monodon* is able to masculinize genetic females.

**Example 3: Sex reversal in juvenile *P.monodon* using dsRNA-mediated knock-down of the androgenic gland hormone gene.**

- 10 Administration of dsRNA targeting PenMon\_AGH1/2 is able to knock down gene expression of both variants and feminized genetically male juvenile *P.monodon*.

## REFERENCES

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20      exclusively in the androgenic gland of the male crayfish. Gen Comp. Endocrinol. 150, 326-336.

## CLAIMS

1. An isolated protein, selected from the group consisting of SEQ ID N° 3, SEQ ID N° 6, SEQ ID N° 9 and SEQ ID N° 12, or a mutant or variant thereof with at least 50% identities and retaining the biological activity of said isolated protein.
2. A nucleic acid fragment, encoding a protein according to claim 1.
3. A nucleic acid fragment according to claim 2, whereby said fragment comprises SEQ ID N° 2, SEQ ID N° 5, SEQ ID N° 8 or SEQ ID N° 11.
4. A nucleic acid fragment, comprising SEQ ID N° 1, SEQ ID N° 4, SEQ ID N° 7 or SEQ ID N° 10, or its complement, or a functional fragment thereof specifically hybridizing with said nucleic acid.
5. The use of a protein according to claim 1 to modify the sex ratio in prawns or shrimps.
6. The use of a protein according to claim 5, whereby said prawn or shrimp belongs to the family of *Penaeidae*.
7. The use of a protein according to claim 6, whereby said prawn or shrimp is *Penaeus monodon*.
8. The use of a nucleic acid fragment according to any of the claims 2 – 4, or a functional fragment thereof to modify the sex ratio in prawns and shrimps.
9. The use of a nucleic acid fragment according to any of the claims 2 - 4, or a functional fragment thereof, to determine sex in prawns or shrimps.
10. The use of a nucleic acid, or a functional fragment thereof, according to claim 8 or 9, whereby said prawn or shrimp belongs to the family of *Penaeidae*.
11. The use of a nucleic acid, or a functional fragment thereof, according to claim 10, whereby said prawn or shrimp is *Penaeus monodon*.
12. A method of setting up a monosex culture in prawns or shrimps, comprising the use of a protein according to claim 1.
13. A method of setting up a monosex culture in prawns or shrimps, comprising the use of a nucleic acid fragment according to any of the claims 2-4.
14. A method of setting up a monosex culture in prawns or shrimps, comprising sex determination according to claim 9.
15. A method of any of the claims 12-14, whereby said prawn or shrimp is *Penaeus monodon*.

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Thr | Ser | Ser | Cys | Tyr | Asn | Val | Thr | Gly | Ile | Pro | Val | Asp | Phe | Asp |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cys | Gly | Asp | Ile | Gly | Asp | Thr | Met | Ser | His | Ile | Cys | Lys | Thr | Phe | Pro |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |

Thr Ala Arg Pro His Ser Arg Val Ser Arg Ser Ala Asp Thr Asp Asp  
50 55 60

Leu Trp Gln Asp Thr Arg Ala Gly Gln Thr Thr Pro Ile Asp Leu Leu  
65 70 75 80

Pro Arg Gln Tyr Arg Leu His Pro Arg Ala Leu Asn Pro Met Arg Tyr  
85 90 95

Phe Glu Met Val Ser Gln Asp Leu Ile Arg Asp His Leu Val Ser Pro  
100 105 110

Glu Ala Ala His Ala Leu Val Lys Thr Ser Gly Arg Arg Ala Lys Arg  
115 120 125

Ser Tyr Asn Val Gln Asp Glu Cys Cys Asn His Val Ser Gln Arg Thr  
130 135 140

Cys Val Ala Glu Glu Ile Leu Glu Tyr Cys Glu Asp Pro Val Pro  
145 150 155

<210> 4  
<211> 1709  
<212> PRT  
<213> *Penaeus monodon*

<400> 4

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1 5 10 15

Cys Ala Gly Gly Ala Cys Ala Gly Gly Gly Cys Ala Ala Gly Thr Thr  
20 25 30

Cys Thr Cys Thr Gly Cys Cys Cys Gly Gly Cys Cys Thr Thr Ala Cys  
35 40 45

Cys Cys Thr Gly Ala Thr Cys Cys Ala Ala Gly Ala Ala Cys Cys Ala  
50 55 60

Thr Thr Cys Ala Ala Gly Gly Thr Thr Gly Thr Gly Cys Gly Thr Gly  
65 70 75 80

Thr Ala Cys Cys Cys Thr Cys Ala Cys Thr Cys Cys Thr Thr Cys Ala  
85 90 95

Cys Thr Thr Thr Cys Cys Cys Gly Thr Ala Thr Cys Cys Thr Cys Cys  
100 105 110

Cys Cys Ala Cys Cys Ala Thr Ala Cys Cys Cys Ala Cys Cys Ala Cys  
115 120 125

Thr Cys Cys Ala Gly Thr Thr Cys Thr Cys Cys Ala Cys Cys Thr Cys  
Page 3

130

135

Cys Ala Ala Cys Cys Cys Thr Cys Cys Thr Cys Ala Gly Ala Thr Cys  
145 150 155 160

Thr Cys Thr Cys Cys Ala Cys Cys Ala Ala Thr Cys Cys Cys Thr Cys  
165 170 175

Cys Ala Cys Ala Ala Ala Ala Cys Cys Ala Cys Thr Cys Cys Ala  
180 185 190

Thr Cys Ala Ala Cys Cys Cys Thr Ala Cys Gly Thr Ala Thr Thr Thr  
195 200 205

Gly Cys Thr Ala Ala Cys Thr Cys Cys Thr Cys Cys Ala Cys Cys Thr  
210 215 220

Cys Cys Cys Cys Thr Cys Thr Ala Thr Cys Cys Ala Cys Cys Cys Cys  
225 230 235 240

Thr Cys Cys Thr Ala Thr Cys Cys Thr Ala Thr Thr Ala Thr Cys Cys  
245 250 255

Cys Thr Cys Cys Thr Cys Cys Thr Thr Thr Thr Cys Cys Ala Cys Thr  
260 265 270

Cys Thr Thr Cys Thr Cys Ala Thr Ala Gly Ala Cys Ala Cys Ala Ala  
275 280 285

Ala Gly Ala Ala Ala Ala Ala Ala Ala Thr Gly Ala Ala Cys Cys Ala  
290 295 300

Ala Cys Thr Cys Gly Cys Thr Gly Cys Cys Thr Cys Ala Cys Gly Cys  
305 310 315 320

Ala Cys Cys Thr Ala Cys Gly Gly Cys Cys Thr Cys Gly Gly Cys Ala  
325 330 335

Thr Gly Cys Cys Cys Ala Cys Thr Cys Ala Gly Cys Thr Gly Cys Thr  
340 345 350

Thr Gly Thr Gly Gly Gly Ala Ala Thr Gly Cys Thr Gly Ala Thr Gly  
355 360 365

Gly Thr Cys Cys Thr Cys Thr Cys Cys Cys Thr Gly Ala Cys Thr Thr  
370 375 380

Cys Gly Ala Cys Gly Thr Cys Ala Ala Gly Cys Thr Gly Cys Thr Ala  
385 390 395 400

Cys Ala Ala Cys Gly Thr Cys Ala Cys Gly Gly Gly Ala Thr Thr  
Page 4

Cys Cys Thr Gly Thr Thr Gly Ala Cys Thr Thr Cys Gly Ala Cys Thr  
420 425 430

Gly Cys Gly Gly Thr Gly Ala Cys Ala Thr Cys Gly Gly Cys Gly Ala  
435 440 445

Thr Ala Cys Cys Ala Thr Gly Ala Gly Thr Cys Ala Cys Ala Thr Cys  
450 455 460

Thr Gly Cys Ala Ala Gly Ala Cys Gly Thr Thr Thr Cys Cys Cys Ala  
465 470 475 480

Cys Ala Gly Cys Cys Cys Gly Gly Cys Cys Thr Cys Ala Cys Thr Cys  
485 490 495

Gly Cys Gly Ala Gly Thr Gly Thr Cys Cys Ala Gly Gly Thr Cys Ala  
500 505 510

Gly Cys Thr Gly Ala Thr Ala Cys Cys Gly Ala Cys Gly Ala Cys Cys  
515 520 525

Thr Cys Thr Gly Gly Cys Ala Gly Gly Ala Cys Ala Cys Gly Ala Gly  
530 535 540

Ala Gly Cys Ala Gly Gly Thr Cys Ala Gly Ala Cys Cys Ala Cys Gly  
545 550 555 560

Cys Cys Cys Ala Thr Thr Gly Ala Cys Cys Thr Gly Cys Thr Thr Cys  
565 570 575

Cys Thr Cys Gly Cys Cys Ala Gly Thr Ala Cys Cys Gly Cys Cys Thr  
580 585 590

Gly Cys Ala Thr Cys Cys Cys Cys Gly Gly Gly Cys Cys Thr Thr Gly  
595 600 605

Ala Ala Thr Cys Cys Gly Ala Thr Gly Cys Gly Ala Thr Ala Thr Thr  
610 615 620

Thr Cys Gly Ala Ala Ala Thr Gly Gly Thr Thr Ala Gly Thr Cys Ala  
625 630 635 640

Gly Gly Ala Cys Cys Thr Gly Ala Thr Ala Ala Gly Ala Gly Ala Cys  
645 650 655

Cys Ala Cys Cys Thr Gly Gly Thr Gly Ala Gly Cys Cys Cys Cys Gly  
660 665 670

Ala Arg Gly Cys Cys Gly Cys Gly Cys Ala Cys Gly Cys Cys Cys Thr  
Page 5

675

680

685

Cys Gly Thr Cys Ala Ala Gly Ala Cys Ala Thr Cys Cys Arg Gly Gly  
 690 695 700

Ala Gly Gly Cys Gly Cys Gly Cys Gly Ala Ala Gly Ala Gly Ala Thr  
 705 710 715 720

Cys Cys Thr Ala Cys Ala Ala Cys Gly Thr Gly Cys Ala Gly Gly Ala  
 725 730 735

Thr Gly Ala Gly Thr Gly Cys Thr Gly Cys Ala Ala Cys Cys Ala Cys  
 740 745 750

Gly Thr Gly Ala Gly Cys Cys Ala Gly Cys Gly Gly Ala Cys Gly Thr  
 755 760 765

Gly Thr Gly Thr Gly Gly Cys Gly Gly Ala Gly Gly Ala Gly Ala Thr  
 770 775 780

Thr Cys Thr Gly Gly Ala Gly Thr Ala Thr Thr Gly Cys Gly Ala Gly  
 785 790 795 800

Gly Ala Cys Cys Cys Gly Thr Ala Cys Thr Thr Cys Thr Ala Ala Ala  
 805 810 815

Cys Cys Thr Thr Gly Ala Ala Cys Cys Thr Gly Ala Cys Cys Thr Gly  
 820 825 830

Ala Cys Cys Thr Ala Thr Gly Gly Cys Cys Thr Thr Cys Cys Thr Ala  
 835 840 845

Ala Cys Cys Thr Cys Thr Cys Thr Ala Ala Gly Thr Gly Gly Ala Gly  
 850 855 860

Thr Cys Gly Thr Cys Ala Gly Thr Gly Gly Thr Cys Cys Ala Thr Gly  
 865 870 875 880

Gly Cys Cys Cys Ala Cys Cys Cys Ala Thr Cys Cys Thr Cys Cys Thr  
 885 890 895

Thr Ala Cys Ala Ala Cys Ala Gly Thr Cys Thr Thr Cys Gly Gly Thr  
 900 905 910

Gly Ala Cys Cys Thr Gly Ala Thr Cys Ala Thr Ala Thr Gly Gly Cys  
 915 920 925

Cys Thr Cys Thr Cys Cys Ala Thr Cys Cys Thr Cys Thr Cys Ala Ala  
 930 935 940

Thr Gly Ala Cys Cys Thr Gly Ala Cys Cys Thr Cys Ala Ala Cys Cys  
 Page 6

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|         |                 |      |                 |                 |
|---------|-----------------|------|-----------------|-----------------|
| 1205    |                 | 1210 |                 | 1215            |
| Tyr Gly | Thr Ala Thr Ala | Thr  | Thr Cys Ala Gly | Arg Gly Cys Ala |
| 1220    |                 | 1225 |                 | 1230            |
| Ala Gly | Ala Ala Cys Thr | Ala  | Thr Thr Thr Thr | Cys Ala Thr Thr |
| 1235    |                 | 1240 |                 | 1245            |
| Cys Ala | Thr Gly Thr Thr | Cys  | Thr Gly Thr Cys | Ala Ala Thr Thr |
| 1250    |                 | 1255 |                 | 1260            |
| Thr Cys | Thr Thr Tyr Gly | Gly  | Cys Gly Thr Cys | Ala Thr Ala Ala |
| 1265    |                 | 1270 |                 | 1275            |
| Thr Thr | Thr Cys Thr Thr | Thr  | Cys Thr Ala Thr | Cys Thr Thr     |
| 1280    |                 | 1285 |                 | 1290            |
| Thr Thr | Ala Thr Thr Ala | Ala  | Ala Cys Ala Ala | Ala Ala Thr Ala |
| 1295    |                 | 1300 |                 | 1305            |
| Ala Ala | Thr Ala Ala Ala | Ala  | Gly Ala Thr Ala | Ala Cys Cys Ala |
| 1310    |                 | 1315 |                 | 1320            |
| Thr Ala | Cys Ala Thr Thr | Ala  | Ala Cys Ala Ala | Ala Gly Gly Thr |
| 1325    |                 | 1330 |                 | 1335            |
| Gly Thr | Gly Ala Thr Ala | Cys  | Ala Ala Ala Gly | Ala Cys Ala Thr |
| 1340    |                 | 1345 |                 | 1350            |
| Ala Ala | Ala Ala Gly Ala | Thr  | Ala Thr Cys Ala | Ala Ala Gly Thr |
| 1355    |                 | 1360 |                 | 1365            |
| Thr Cys | Ala Gly Thr Ala | Cys  | Thr Thr Thr Cys | Thr Thr Thr Cys |
| 1370    |                 | 1375 |                 | 1380            |
| Gly Cys | Thr Thr Thr Thr | Thr  | Cys Thr Ala Thr | Thr Thr Thr Thr |
| 1385    |                 | 1390 |                 | 1395            |
| Ala Thr | Cys Thr Thr Thr | Cys  | Gly Ala Thr Thr | Thr Thr Cys Cys |
| 1400    |                 | 1405 |                 | 1410            |
| Gly Ala | Ala Cys Gly Thr | Thr  | Thr Thr Ala Ala | Ala Gly Thr Thr |
| 1415    |                 | 1420 |                 | 1425            |
| Thr Cys | Thr Ala Ala Cys | Thr  | Thr Thr Thr Ala | Thr Cys Cys Cys |
| 1430    |                 | 1435 |                 | 1440            |
| Tyr Cys | Thr Gly Thr Ala | Ala  | Ala Ala Ala Thr | Ala Ala Gly     |
| 1445    |                 | 1450 |                 | 1455            |
| Thr Thr | Gly Ala Cys Ala | Gly  | Ala Cys Gly Ala | Thr Ala Ala Thr |

|         |                 |      |                 |                 |
|---------|-----------------|------|-----------------|-----------------|
| 1460    |                 | 1465 |                 | 1470            |
| Ala Ala | Ala Ala Ala Gly | Cys  | Ala Ala Thr Ala | Ala Thr Gly Thr |
| 1475    |                 | 1480 |                 | 1485            |
| Thr Thr | Thr Ala Gly Ala | Ala  | Thr Cys Thr Ala | Thr Thr Cys Cys |
| 1490    |                 | 1495 |                 | 1500            |
| Thr Thr | Thr Thr Thr Thr | Gly  | Thr Gly Ala Thr | Cys Ala Cys Cys |
| 1505    |                 | 1510 |                 | 1515            |
| Thr Gly | Ala Ala Thr Ala | Thr  | Cys Thr Thr Thr | Thr Thr Thr Ala |
| 1520    |                 | 1525 |                 | 1530            |
| Thr Ala | Thr Ala Thr Ala | Cys  | Ala Thr Thr Ala | Thr Cys Gly Thr |
| 1535    |                 | 1540 |                 | 1545            |
| Ala Thr | Thr Gly Gly Ala | Ala  | Thr Cys Ala Gly | Ala Thr Ala Gly |
| 1550    |                 | 1555 |                 | 1560            |
| Ala Thr | Cys Ala Gly Tyr | Ala  | Cys Gly Gly Tyr | Ala Thr Ala Thr |
| 1565    |                 | 1570 |                 | 1575            |
| Cys Ala | Gly Ala Ala Thr | Thr  | Ala Ala Thr Thr | Cys Gly Thr Ala |
| 1580    |                 | 1585 |                 | 1590            |
| Ala Thr | Ala Ala Ala Ala | Ala  | Ala Ala Thr Gly | Cys Ala Thr Thr |
| 1595    |                 | 1600 |                 | 1605            |
| Thr Cys | Ala Ala Thr Ala | Ala  | Cys Thr Gly Ala | Ala Thr Thr Thr |
| 1610    |                 | 1615 |                 | 1620            |
| Cys Cys | Gly Ala Thr Ala | Ala  | Ala Ala Ala Ala | Ala Thr Thr Thr |
| 1625    |                 | 1630 |                 | 1635            |
| Cys Met | Cys Cys Thr Gly | Thr  | Ala Thr Cys Thr | Gly Thr Cys Thr |
| 1640    |                 | 1645 |                 | 1650            |
| Thr Thr | Thr Gly Cys Gly | Ala  | Ala Ala Cys Ala | Ala Thr Ala Ala |
| 1655    |                 | 1660 |                 | 1665            |
| Ala Ala | Gly Cys Ala Thr | Thr  | Thr Cys Ala Ala | Ala Ala Thr Cys |
| 1670    |                 | 1675 |                 | 1680            |
| Gly Arg | Ala Ala Ala Ala | Ala  | Ala Ala Ala Ala | Ala Ala Ala Ala |
| 1685    |                 | 1690 |                 | 1695            |
| Ala Ala | Ala Ala Ala Ala | Ala  | Ala Ala Ala Ala |                 |
| 1700    |                 | 1705 |                 |                 |

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<211> 480  
<212> DNA  
<213> Penaeus monodon

<400> 5  
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tgctacaacg tcacggggat tcctgttgac ttcgactgcg gtgacatcgg cgataccatg 120  
agtcacatct gcaagacggt tcccacagcc cggcctcact cgcgagtgtc caggtcagct 180  
gataccgacg acctctggca ggacacgaga gcaggtcaga ccacgccccat tgacctgctt 240  
cctcgccagt accgcctgca tccccggggc ttgaatccga tgcgatattt cgaaatgggt 300  
agtcaggacc tgataagaga ccacctggtg agccccgarg ccgcgcacgc cctcgtcaag 360  
acatccrgga ggcgcgcgaa gagatcctac aacgtgcagg atgagtgtg caaccacgtg 420  
agccagcggg cgtgtgtggc ggaggagatt ctggagtatt gcgaggaccc gtacttctaa 480

<210> 6  
<211> 159  
<212> PRT  
<213> Penaeus monodon

<400> 6  
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1 5 10 15  
Ser Thr Ser Ser Cys Tyr Asn Val Thr Gly Ile Pro Val Asp Phe Asp  
20 25 30  
Cys Gly Asp Ile Gly Asp Thr Met Ser His Ile Cys Lys Thr Phe Pro  
35 40 45  
Thr Ala Arg Pro His Ser Arg Val Ser Arg Ser Ala Asp Thr Asp Asp  
50 55 60  
Leu Trp Gln Asp Thr Arg Ala Gly Gln Thr Thr Pro Ile Asp Leu Leu  
65 70 75 80  
Pro Arg Gln Tyr Arg Leu His Pro Arg Ala Leu Asn Pro Met Arg Tyr  
85 90 95  
Phe Glu Met Val Ser Gln Asp Leu Ile Arg Asp His Leu Val Ser Pro  
100 105 110  
Glu Ala Ala His Ala Leu Val Lys Thr Ser Gly Arg Arg Ala Lys Arg  
115 120 125  
Ser Tyr Asn Val Gln Asp Glu Cys Cys Asn His Val Ser Gln Arg Thr  
130 135 140  
Cys Val Ala Glu Glu Ile Leu Glu Tyr Cys Glu Asp Pro Tyr Phe  
145 150 155

## V258\_ST25.txt

<210> 7  
 <211> 2082  
 <212> DNA  
 <213> *Penaeus monodon*

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 accattcaag gttgtgctg taccctcact ccttcacttt cccgtatcct cccaccata 120  
 cccaccactc cagttctcca cctccaaccc tcctcagatc tctccaccaa tccctccaca 180  
 aaaaccactc catcaaccct acgtatttgc taactcctcc acctcccctc tatccacccc 240  
 tcctatccta ttatccctcc tccttttcca ctcttctcat agacacaaag aaaaaaatga 300  
 accaactcgc tgcctcacgc acctacggcc tcggcatgcc cactcagctg cttgtgggaa 360  
 tgctgatggt cctctccctg acttcgacgt caagctgcta caacgtcacg gggattcctg 420  
 ttgacttcga ctgcggtgac atcggcgata ccatgagtc catctgcaag acgtttccca 480  
 cagcccggcc tcaactcgcga gtgtccaggt cagctgatac cgacgacctc tggcaggaca 540  
 cgagagcagg tcagaccacg cccattgacc tgcttcctcg ccagtaccgc ctgcatcccc 600  
 gggccttgaa tccgatgcga tatttcgaaa tggacctgat aagagaccac ctggtgagcc 660  
 ccgargccgc gcacgccctc gtcaagacat ccrggaggcg cgcaagaga tcctacaacg 720  
 tgcaggatga gtgctgcaac cacgtgagcc agcggacgtg tgtggcggag gagattctgg 780  
 agtattgcga ggacccggta ccttagtctc ccctggcggg ttctgtcata tgcgatgttc 840  
 tctcwtattt acactttttt tgctaattgct tacagatata cagtatatgc atagcgcatt 900  
 ctgataacac atatgtatat ttatakgtg tacaatgtgt gttaaaacaa attcattgtg 960  
 ttctctctgt ttcacaagg agaatataat cctatccttt tgatagtaat ttactcgcatt 1020  
 acacgcttgt aaatcttggt ttcgtctcgt gtggttttga tctacattct tcttcatgta 1080  
 tgattttctag atgtgagagt aagtaggcaa gttatctctc gaatgaaatt cgaaatcagg 1140  
 caaatcagty attaacattt ctcccttctg tttccccagt acttctaaac cttgaacctg 1200  
 acctgacctc tggccttcct aacctctcta agtggagtcg tcagtgggtcc atggcccacc 1260  
 catcctcctt acaacagtct tcggtgacct gatcatatgg cctctccatc ctctcaatga 1320  
 cctgacctca acccaygggt ccccccttct ttaggccaag tctttgcgga cctgacccaa 1380  
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 atgtctgcc aagtagcgt tacattccat ttttcttttc gaktawtagg cccagagta 1560  
 attcttgacc tcaactttct aaaaaaaaaa aygtatatc agrgcaagaa ctattttcat 1620  
 tcatgttctg tcaatttctt yggcgtcata atttctttct atccttttat taaacaaaat 1680  
 aaataaaaga taaccataca ttaacaaagg tgtgatacaa agacataaaa gatatcaaag 1740  
 ttcagtactt tctttcgctt tttctatatt tatctttcga ttttccgaac gttttaaagt 1800  
 ttctaacttt tatcccyctg taaaaataaa gttgacagac gataataaaa agcaataatg 1860

V258\_ST25.txt

|                                                                   |      |
|-------------------------------------------------------------------|------|
| ttttagaatc tattcctttt ttgtgatcac ctgaatatct tttttatata tacattatcg | 1920 |
| tattggaatc agatagatca gyacggyata tcagaattaa ttcgtaataa aaaaatgcat | 1980 |
| ttcaataact gaatttccga taaaaaaatt tcmctgtat ctgtcttttg cgaaacaata  | 2040 |
| aaagcatttc aaaatcgraa aaaaaaaaaa aaaaaaaaaa aa                    | 2082 |

<210> 8  
 <211> 471  
 <212> DNA  
 <213> *Penaeus monodon*

|                                                                    |     |
|--------------------------------------------------------------------|-----|
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| tgctacaacg tcacggggat tcctgttgac ttcgactgcg gtgacatcgg cgataccatg  | 120 |
| agtcacatct gcaagacgtt tcccacagcc cggcctcact cgcgagtgtc caggtcagct  | 180 |
| gataccgacg acctctggca ggacacgaga gcaggtcaga ccacgccccat tgacctgctt | 240 |
| cctcgccagt accgcctgca tccccggggc ttgaatccga tgcgatattt cgaaatggac  | 300 |
| ctgataagag accacctggt gagccccgar gccgcgcacg ccctcgtaa gacatccrgg   | 360 |
| aggcgcgcga agagatccta caacgtgcag gatgagtgtc gcaaccacgt gagccagcgg  | 420 |
| acgtgtgtgg cggaggagat tctggagtat tgcgaggacc cggtagctta g           | 471 |

<210> 9  
 <211> 156  
 <212> PRT  
 <213> *Penaeus monodon*

<400> 9

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Met Pro Thr Gln Leu Leu Val Gly Met Leu Met Val Leu Ser Leu Thr |  |
| 1 5 10 15                                                       |  |
| Ser Thr Ser Ser Cys Tyr Asn Val Thr Gly Ile Pro Val Asp Phe Asp |  |
| 20 25 30                                                        |  |
| Cys Gly Asp Ile Gly Asp Thr Met Ser His Ile Cys Lys Thr Phe Pro |  |
| 35 40 45                                                        |  |
| Thr Ala Arg Pro His Ser Arg Val Ser Arg Ser Ala Asp Thr Asp Asp |  |
| 50 55 60                                                        |  |
| Leu Trp Gln Asp Thr Arg Ala Gly Gln Thr Thr Pro Ile Asp Leu Leu |  |
| 65 70 75 80                                                     |  |
| Pro Arg Gln Tyr Arg Leu His Pro Arg Ala Leu Asn Pro Met Arg Tyr |  |
| 85 90 95                                                        |  |
| Phe Glu Met Asp Leu Ile Arg Asp His Leu Val Ser Pro Glu Ala Ala |  |
| 100 105 110                                                     |  |

V258\_ST25.txt

His Ala Leu Val Lys Thr Ser Gly Arg Arg Ala Lys Arg Ser Tyr Asn  
115 120 125

Val Gln Asp Glu Cys Cys Asn His Val Ser Gln Arg Thr Cys Val Ala  
130 135 140

Glu Glu Ile Leu Glu Tyr Cys Glu Asp Pro Val Pro  
145 150 155

<210> 10  
<211> 1700  
<212> DNA  
<213> *Penaeus monodon*

<400> 10  
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accattcaag gttgtgctgt taccctcact ccttcacttt cccgtatcct cccaccata 120  
cccaccactc cagttctcca cctccaaccc tctcagatc tctccaccaa tccctccaca 180  
aaaaccactc catcaaccct acgtatttgc taactcctcc acctccccctc tatccacccc 240  
tcctatccta ttatccctcc tccttttcca ctcttctcat agacacaaaag aaaaaaatga 300  
accaactcgc tgcctcacgc acctacggcc tcggcatgcc cactcagctg cttgtgggaa 360  
tgctgatggt cctctccctg acttcgacgt caagctgcta caacgtcacg gggattcctg 420  
ttgacttcga ctgcggtgac atcggcgata ccatgagtca catctgcaag acgtttccca 480  
cagcccggcc tcaactcgca gtgtccagggt cagctgatac cgacgacctc tggcaggaca 540  
cgagagcagg tcagaccacg cccattgacc tgcttcctcg ccagtaccgc ctgcatcccc 600  
gggccttgaa tccgatgcga tatttcgaaa tggacctgat aagagaccac ctggtgagcc 660  
ccgargccgc gcacgccctc gtcaagacat ccrggaggcg cgcgaagaga tcctacaacg 720  
tgcaggatga gtgctgcaac cacgtgagcc agcggacgtg tgtggcggag gagattctgg 780  
agtattgcga ggacccgtac ttctaaacct tgaacctgac ctgacctatg gccttcctaa 840  
cctctctaag tggagtcgtc agtgggtccat ggcccacca tcctccttac aacagtcttc 900  
ggtgacctga tcatatggcc tctccatcct ctcaatgacc tgacctcaac ccaygggtcc 960  
ccccctcttt aggccaagtc tttgcggacc tgacccaaac cgaggcttct ctaaccctcc 1020  
tctgatgagt ttgtaataaa aataaccggc ctggtgaccc aagttctcat gaatttgact 1080  
taatctgcaa ttaatgaccc gttagagtcc tttcaatgat gtctgcaaaa agtagcgтта 1140  
cattccattt ttcttttcga ktawtaggcc ccagagtaat tcttgacctc aactttctaa 1200  
aaaaaaatay gtatattcag rgcaagaact attttcattc atgttctgtc aattttcttyg 1260  
gcgtcataat ttctttctat ctttttatta aacaaaataa ataaaagata accatacatt 1320  
aacaaagggtg tgatacaaag acataaaaga tatcaaagtt cagtactttc tttcgctttt 1380  
tctattttta tctttcgatt ttccgaacgt tttaaagttt ctaactttta tcccycgtga 1440  
aaaataaagt tgacagacga taataaaaag caataatgtt ttagaatcta ttcctttttt 1500

V258\_ST25.txt

|                                                                   |      |
|-------------------------------------------------------------------|------|
| gtgatcacct gaatatcttt tttatatata cattatcgta ttggaatcag atagatcagy | 1560 |
| acggyatatc agaattaatt cgtaataaaa aaatgcattt caataactga atttccgata | 1620 |
| aaaaaatttc mcctgtatct gtcttttgcg aaacaataaa agcatttcaa aatcgraaaa | 1680 |
| aaaaaaaaaa aaaaaaaaaa                                             | 1700 |

<210> 11  
 <211> 471  
 <212> DNA  
 <213> Penaeus monodon

|                                                                    |     |
|--------------------------------------------------------------------|-----|
| <400> 11                                                           |     |
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| tgctacaacg tcacggggat tcctgttgac ttcgactgcg gtgacatcgg cgataccatg  | 120 |
| agtcacatct gcaagacgtt tcccacagcc cggcctcact cgcgagtgtc caggtcagct  | 180 |
| gataccgacg acctctggca ggacacgaga gcaggtcaga ccacgcccatt tgacctgctt | 240 |
| cctcgccagt accgcctgca tccccggggc ttgaatccga tgcgatattt cgaaatggac  | 300 |
| ctgataagag accacctggt gagccccgar gccgcgcacg ccctcgtaa gacatccrgg   | 360 |
| aggcgcgca agagatccta caacgtgcag gatgagtgtc gcaaccacgt gagccagcgg   | 420 |
| acgtgtgtgg cggaggagat tctggagtat tgcgaggacc cgtacttcta a           | 471 |

<210> 12  
 <211> 156  
 <212> PRT  
 <213> Penaeus monodon

<400> 12

|                                                                 |  |
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| Met Pro Thr Gln Leu Leu Val Gly Met Leu Met Val Leu Ser Leu Thr |  |
| 1 5 10 15                                                       |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Ser Thr Ser Ser Cys Tyr Asn Val Thr Gly Ile Pro Val Asp Phe Asp |  |
| 20 25 30                                                        |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Cys Gly Asp Ile Gly Asp Thr Met Ser His Ile Cys Lys Thr Phe Pro |  |
| 35 40 45                                                        |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Thr Ala Arg Pro His Ser Arg Val Ser Arg Ser Ala Asp Thr Asp Asp |  |
| 50 55 60                                                        |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Leu Trp Gln Asp Thr Arg Ala Gly Gln Thr Thr Pro Ile Asp Leu Leu |  |
| 65 70 75 80                                                     |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Pro Arg Gln Tyr Arg Leu His Pro Arg Ala Leu Asn Pro Met Arg Tyr |  |
| 85 90 95                                                        |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| Phe Glu Met Asp Leu Ile Arg Asp His Leu Val Ser Pro Glu Ala Ala |  |
| 100 105 110                                                     |  |

|                                                                 |  |
|-----------------------------------------------------------------|--|
| His Ala Leu Val Lys Thr Ser Gly Arg Arg Ala Lys Arg Ser Tyr Asn |  |
|-----------------------------------------------------------------|--|

115

120

125

Val Gln Asp Glu Cys Cys Asn His Val Ser Gln Arg Thr Cys Val Ala  
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Glu Glu Ile Leu Glu Tyr Cys Glu Asp Pro Tyr Phe  
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&lt;210&gt; 13

&lt;211&gt; 2008

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<213> *Penaeus monodon*

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V258\_ST25.txt

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| gtaaaaataa agttgacaga cgataataaa aagcaataat gttttagaat ctattccttt    | 1800 |
| tttgtgatca cctgaatatc ttttttatat atacattatc gtattggaat cagatagatc    | 1860 |
| agyacggyat atcagaatta attcgtaata aaaaaatgca tttcaataac tgaatttccg    | 1920 |
| ataaaaaaat ttcacctgta tctgtctttt gcgaacaat aaaagcattt caaaatcgrg     | 1980 |
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